

**Supplementary: Heterologous Biosynthetic Crosstalk with the Native Mansouramycin Cluster
in *Streptomyces albus* Del14 Reveals Unexpected Metabolites**

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Table S1: Bacterial strains used in this study.

Strain	Description	Source
<i>S. lividans</i> Del8	Cluster free derivative of <i>S. lividans</i> TK24	Yousra A., et al. ¹
<i>S. albus</i> Del14	Cluster free derivative of <i>S. albus</i> J1074	Myronovskyi, M., et al. ²
<i>S. albus</i> Del15	Derivative of <i>S. albus</i> Del14 with the deletion of mansouramycin biosynthetic pathway	Shuai, H., et al. ³
<i>Escherichia coli</i> ET12567 pUB307	Donor strain for intergeneric conjugation	Flett, F. et al. ⁴
<i>Escherichia coli</i> DH10 β	General cloning strain	Grant, S.G., et al. ⁵
<i>Escherichia coli</i> GB05-red	<i>E. coli</i> strain used for the Red/ET recombination	Zhang et al. ⁶
<i>Saccharomyces cerevisiae</i> BY4742	Auxotrophic strain used for homologous recombination in yeast	Baker Brachmann, C., et al. ⁷
<i>S. albus</i> Del14_cluster3	Derivative of <i>S. albus</i> Del14 harboring the cluster 3	this study
<i>S. albus</i> Del15_cluster3	Derivative of <i>S. albus</i> Del15 harboring cluster 3	this study
<i>S. albus</i> Del14_cluster3_delOrf22	Derivative of <i>S. albus</i> Del14 harboring the cluster 3 with deletion of orf22 gene	this study
<i>S. albus</i> Del15_C16	Derivative of <i>S. albus</i> Del15 harboring cluster 16	this study
<i>S. albus</i> Del14_C16	Derivative of <i>S. albus</i> Del14 harboring cluster 16	this study
<i>S. albus</i> Del14_C16h7	Derivative of <i>S. albus</i> Del14 harboring the cluster 16 and pRT801_ampery-AfsR16 plasmid	this study
<i>Streptomyces</i> LV45-129	The wild-type strain; the source of cluster 16	this study
<i>S. albus</i> Del14_C1.8	Derivative of <i>S. albus</i> Del14 harboring BAC_C1.8	this study
<i>S. albus</i> Del14_C1.8_pRT-Dnrl	Derivative of <i>S. albus</i> Del14 harboring BAC_C1.8 and pRT801_cat_ampery_Sk1.8_Dnrl	this study
<i>S. albus</i> Del14_cluster1.7	Derivative of <i>S. albus</i> Del14 harboring BAC1.7	this study
<i>S. albus</i> Del14_cluster1.7-delRebH	Derivative of <i>S. albus</i> Del14 harboring BAC1.7 with RebH deletion	this study
<i>S. albus</i> Del15_cluster1.7	Derivative of <i>S. albus</i> Del15 harboring BAC1.7	this study

Table S2: Plasmids and BACs used in this study.

Name	Description	Source
cluster 3	cluster originating from a COSMID library created for <i>Streptomyces kitasatoensis</i>	this study
cluster 3_Delorf22	cluster 3 with deletion of NRPS gene orf22	this study
pCLY10	Shuttle vector for <i>E. coli</i> -yeast-actinomycetes	Bilyk O., et al. ⁸
cluster 16	cluster originating from a COSMID library created for <i>Streptomyces strain</i> LV45-129	Oberhäuser P., et al. ⁹
pRT801	Plasmid; BT1 integrative vector for <i>Streptomyces</i> strains; apramycin resistance	Gregory, M.A. ¹⁰
pRT801_cat_ampery	Derivative of pRT801 containing ampicillin-erythromycin resistance cassette and strong synthetic promoter TS81	Shuai, H., et al. ³
pRT801_ampery_AfsR16	Derivative of pRT801_cat_ampery, catalase gene was replaced with the SARP gene from cluster 16	this study
BAC_C1.8	Cluster originating from a COSMID library created for <i>Streptomyces</i>	this study

	<i>kitasatoensis</i>	
pRT801_cat_ampery _Sk1.8_Dnrl	Derivative of BT1 integrative vector pRT801_cat_ampery, catalase gene was replaced with the SARP gene from cluster 1.8	this study
BAC1.7	cluster 1.7	this study
BAC1.7-delRebH-delAp	cluster 1.7 with in-frame deletion of RebH halogenase gene	this study

Table S3: Primers used for deletion of genes.

Primer	Sequence	Description
Cluster3Reddel NRPS_F	TTCCAGATCCGCCGCGGGTGCTTCGGTGCCCCGAGGCCGCGAGGTCG TGGACGAAGGCGGCCAGCAGCTCAACGGGAATCCTGCTCTGCGAG	Forward primer for the deletion of the NRPS-1 gene in the cluster 3.
Cluster3Reddel NRPS_R	CTGATGACCGACGCGACGCCCCGGCAGACGACGATCGAGGAGAAGCGG CGCGCGCTGCTGGCCCTGCGGCTGTCTTGTAGGCTGGAGCTGCTTC	Reverse primer for the deletion of the NRPS-1 gene in the cluster 3.
Cluster3checkF	ATCAGACCGGTGCGCTCCAGG	Forward primer for checking the deletion of the NRPS-1 gene.
Cluster3checkR	ACCGACTGCTGAGTGAGGAAG	Reverse primer for checking the deletion of the NRPS-1 gene.
delRebH-f	CTCAAGGCGGCCTACGGTGACCGCATCAACGCTACTCTGGTGGATCCG AGTTTAAACAGCTGTTCCGGGGATCCGTC	Amplification of ampicilin cassette for in frame deletion of RebH, forward.
delRebH-r	CGCCCGGAGCCGCGCGAGCTCGGCGCGCGCTTCGTCGGGTCGATGTG CGGTTTAAACTGTAGGCTGGAGCTGCTTCG	Amplification of ampicilin cassette for in frame deletion of RebH, reverse.
RebH-chk-F	ATGGCGCGAAGAATCTGCCG	Sequencing of deletion construct for verification, forward.
RebH-chk-R	GAAGGGTGGCCATGGTGAGG	Sequencing of deletion construct for verification, reverse.

Table S4: Primers used for SARP overexpression.

Primer	Sequence	Description
AsfR16_Terp_For	CATATGGTCGAGCAACGGAGGTACGGACGTGGAGTTCC GGCTGCTCGG	AsfR amplification primer including rbs and NdeI restriction side
AsfR16_Terp_Rev	GATATCTCAGGTCCGCGCCATGAGCCGGACCAT	AsfR amplification primer including EcoRV restriction side
pRT_chk_For	TAGTTCCTTCGTCACCACAG	Primers for sequencing from pRT801 region
pRT_chk_Rev	TCACTCATTAGGCACCCAG	Primers for sequencing from pRT801 region
AfsR16_chk1	CTCGACTGGTTCCACCGC	Primers for sequencing from AsfR Region
AfsR16_chk2	GTTTCAGCGACACGGGAGA	Primers for sequencing from AsfR Region
AfsR16_chk3	CGAACTGTCCGACGAGTCAC	Primers for sequencing from AsfR Region
AfsR16_chk4	CAGGACCTGGCGGATCATC	Primers for sequencing from AsfR Region
C1.8_Dnrl_act-ML-f	AAAACATATGGTCGAGCAACGGAGGTACGGACATGCAC GCACTCCGGACG	Dnrl amplification primer including rbs and NdeI restriction side
C1.8_Dnrl_act-r	TTTTGATATCTCAGCACCCCTCAGCAC	Dnrl amplification primer including EcoRV restriction side
pRT801_cat- exchange_chk_f	TGAAGGAGGAAGACGAAGCG	Primers for sequencing from pRT801 region

prt801_cat-exchange_chk_r	AGTCAGTGAGCGAGGAAGC	Primers for sequencing from pRT801 region
Sk1.8_Dnrl_seq-1	TGGAGTCCTACGCCGAGG	Primers for sequencing from Dnrl Region
Sk1.8_Dnrl_seq-2	TTACTGATGGAGCTCGCC	Primers for sequencing from Dnrl Region
Sk1.8_Dnrl_seq-3	TACTTCGTCATGCAGCTCCT	Primers for sequencing from Dnrl Region
Sk1.8_Dnrl_seq-4	TGCACATCCAGCTGGGGC	Primers for sequencing from Dnrl Region

Table S5: Proposed function of the genes from cluster 3 and similarity comparison to the *vzb* gene cluster.

<i>cluster 3</i>		<i>vzb cluster</i>	
orf	proposed function	corresponding genes	% Similarity to cluster 3
1	ATP-dependent protease HslVU (ClpYQ), peptidase subunit		
2	putative protein		
3	autoinducer-binding transcriptional regulator		
4	SARP		
5	SARP		
6	acyl-CoA_dehydrogenase	vzb5	49
7	Cyclohexane-1-carbonyl-CoA dehydrogenase	vzb6	66
8	Thioesterase	vzb14	58
9	sulfate adenyl transferase	vzb18	77
10	Sulfate adenyltransferase subunit 2	vzb19	86
11	Adenyl-sulfate kinase	vzb20	73
12	Sulfotransferase family protein	vzb21	65
13	Alpha-aminoadipate carrier protein LysW, vzb22	vzb22	67
14	acetyl-CoA_carboxylase_biotin_carboxylase, vzb23	vzb23	70
15	N-acetyl-gamma-glutamyl-phosphate_reductase, vzb24	vzb24	75
16	acetylglutamate kinase-like protein LysZ, vzb25	vzb25	72
17	acetylornithine_deacetylase, vzb26	vzb26	69
18	transketolase, vzb27	vzb27	62
19	1-deoxy-D-xylulose-5-phosphate_synthase	vzb28	70
20	Glyoxalase/bleomycin resistance protein/dioxygenase		
21	major_facilitator_transporter		
22	NRPS-1		
23	NRPS-2		
24	Argininosuccinate lyase/adenylosuccinate lyase	vzb2	57
25	PCP	vzb8	50
26	Aminotransferase, vzb9	vzb9	70
27	Butirosin biosynthesis protein H, N-terminal, Azi29	vzb10	69
28	Azi28	vzb11	69
29	Acetyltransferase (GNAT) family protein (there is an acetyl group in the product)	vzb12	49
30	Succinyl-diaminopimelate desuccinylase	vzb13	69
31	cytochrome_P450		
32	NRPS-3	vzb15	59
33	mbtH-like protein	vzb16	49
34	Putative protein		
35	LuxR family DNA-binding response regulator	vzb17	50
36	Putative protein	vzb0	14

37	Helix-turn-helix domain protein		
38	Hypothetical protein		
39	LysR family transcriptional regulator		
40	2-amino-3-ketobutyrate coenzyme A ligase		
41	Alcohol dehydrogenase		
42	AsnC family transcriptional regulator		
43	L-asparaginase		
44	Aspartate ammonia-lyase		
45	NAD-dependent malic enzyme		
46	TetR_family_transcriptional_regulator		
47	GCN5-related_N-acetyltransferase		
48	Putative membrane protein		
49	Protease HtpX		
50	Acyl-CoA dehydrogenase		

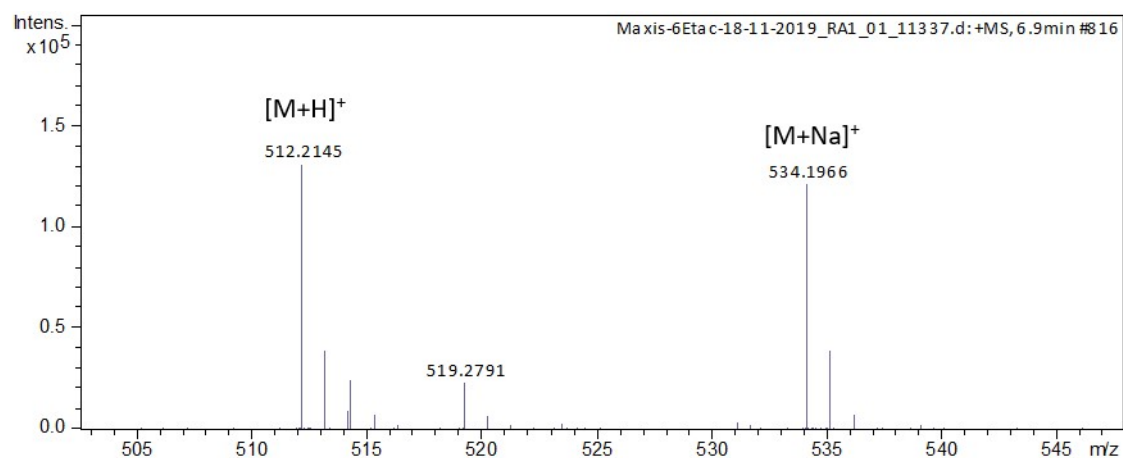


Figure S1: HRMS spectrum of malevonin.

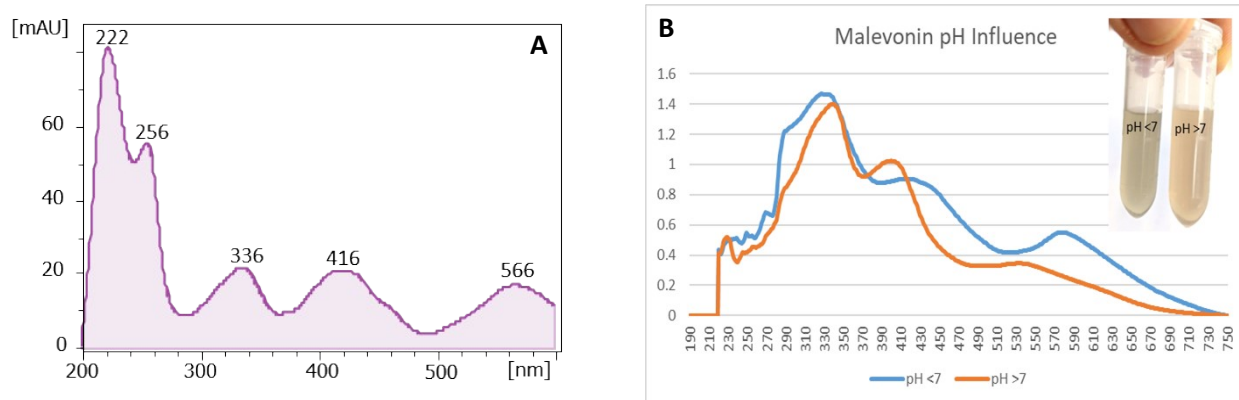
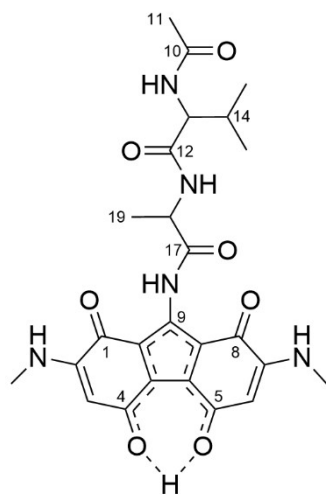


Figure S2: UV/VIS spectrum of malevonin extracted from the LC-MS run (A) and UV/VIS measurement of malevonin in DMSO/water (50/50) at high and low pH (B).

Table S6: NMR data (500 MHz, DMSO-d₆) of malevonin.

residue	no	$\delta(^{13}\text{C})$ [ppm], type	$\delta(^1\text{H})$ [ppm], mult (J)	COSY (H-)	HMBC (C-)
AHMFT*	1/8	172.3, 2xC			3,6
	1a/8a	115.1, 2xC			9-NH, 3/6
	2/7	157.7, 2xC			3/6, 2/7-NMe
	2/7-NH		8.94, 2	2/7-NMe	
	2/7-NMe	30.8 2xCH ₃	2.93, s	2/7-NH	
	3/6	94.5, 2xCH	5.53, s	2/7-NMe	
	4/5	178.2, 2xC			3/6
	4a/5a	124.8, 2xC			3/6
	9	138.5, C			9-NH
	9-NH		9.89, s		
	OH		18.47, bs		
Ac	10	170.1, C			13-NH, 13, 11
	11	23.3, CH ₃	1.87, s		-
Val	12	171.7, C			18-NH, 13,14,18
	13	58.2, CH	4.22, t (7.9)	13-NH,14	13-NH, 14, 15, 16
	13-NH		7.91, d (8.9)	13	
	14	31.3, CH	1.94, m (6.7)	13, 15, 16	13-NH, 13, 15, 16
	15	20.1, CH ₃	0.84, d (6.7)	14	12, 13, 16
	16	19.0, CH ₃	0.81, d (6.7)	14	12, 13, 15
Ala	17	170.2, C			9-NH, 18, 18-NH, 19
	18	49.6, CH	4.53, p (6.9)	18-NH, 19	18-NH, 19
	18-NH		8.16, d (7.0)	18	
	19	18.6, CH ₃	1.38, d (7.0)	18	18-NH, 18

*9-amino-5-hydroxy-2,7-bis(methylamino)-1H-fluorene-1,4,8-trione (AHMFT)



malevonin (1)

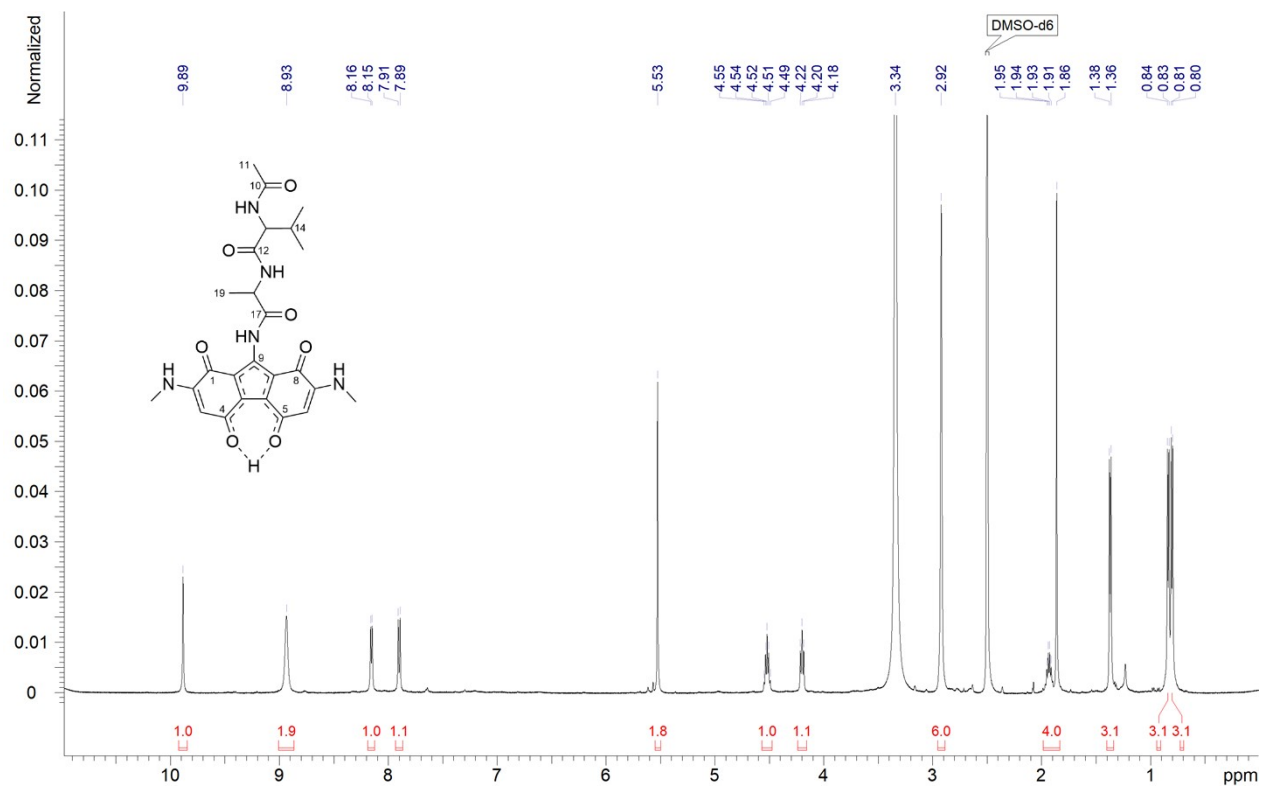


Figure S3. ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of malevonin.

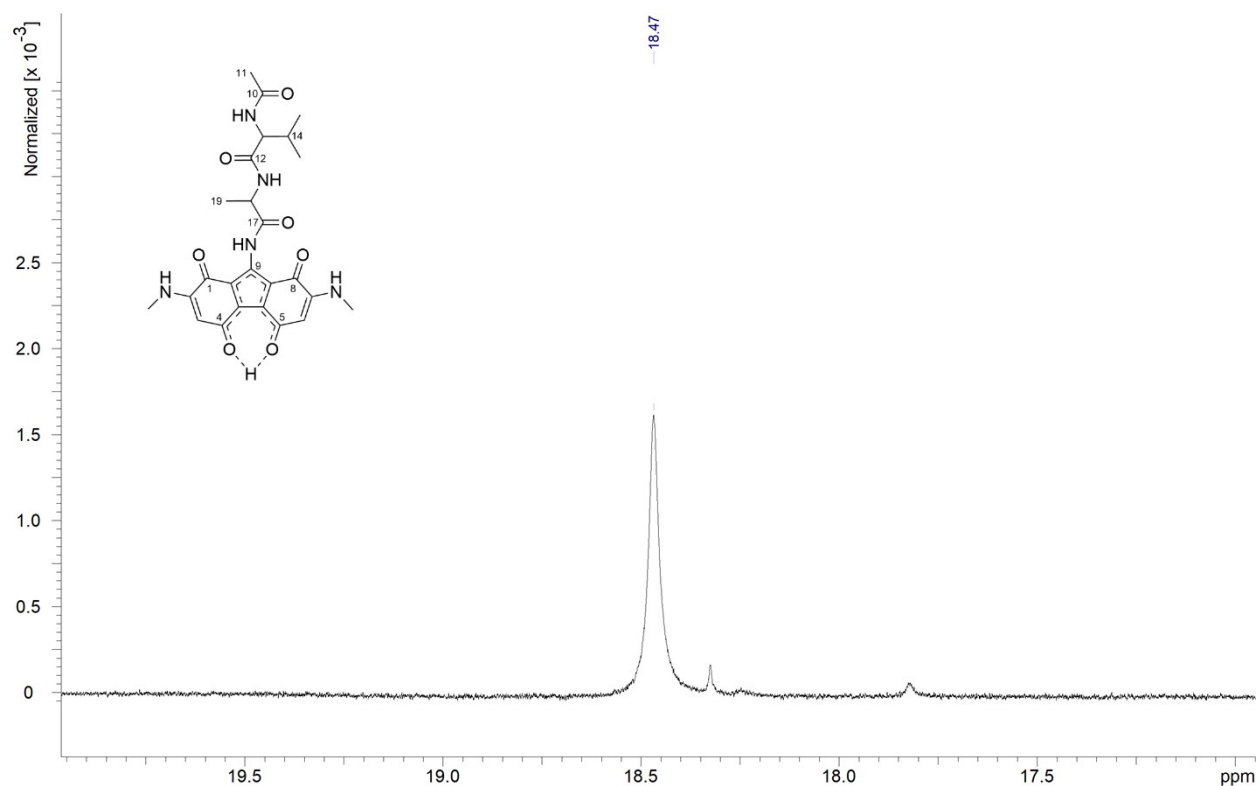


Figure S4: ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of malevonin showing the hydrogen bridge stabilized proton signal in the downfield.

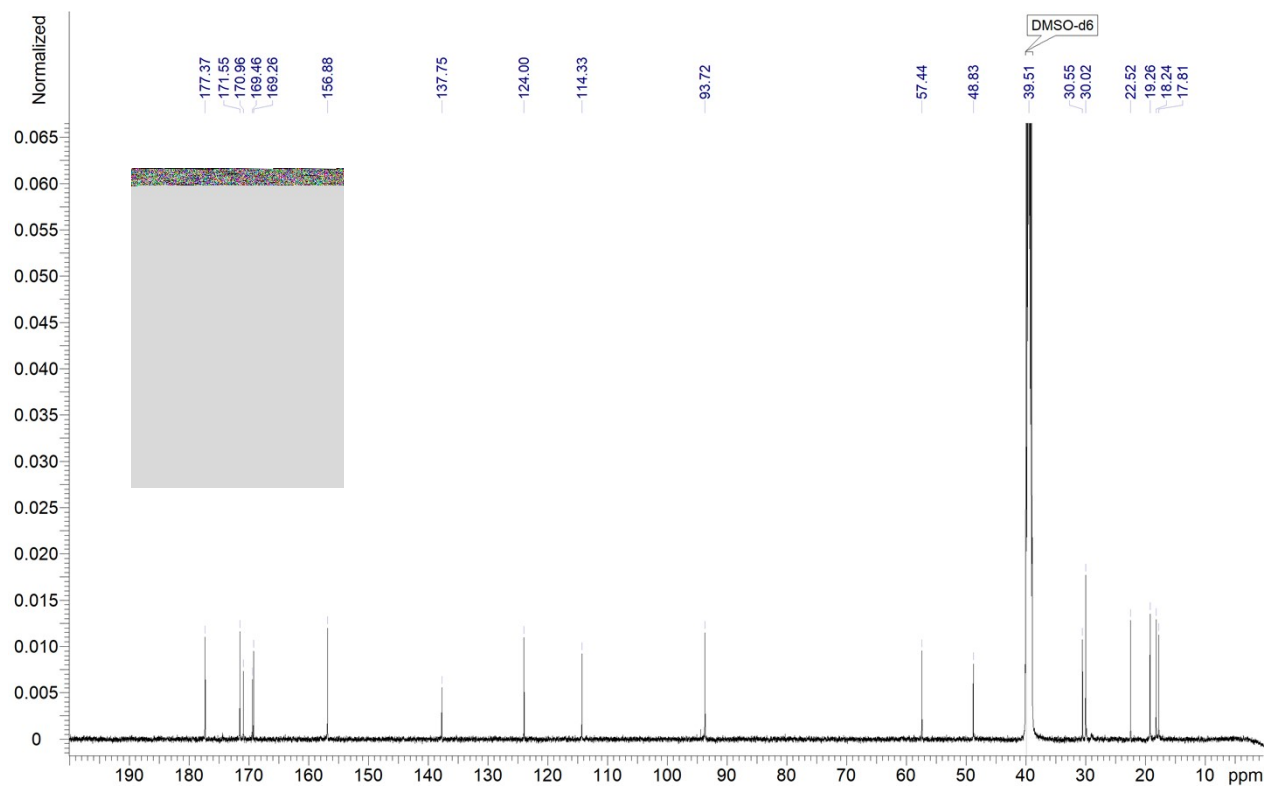


Figure S5: ^{13}H NMR spectrum (125 MHz, $\text{DMSO-}d_6$) of malevonin.

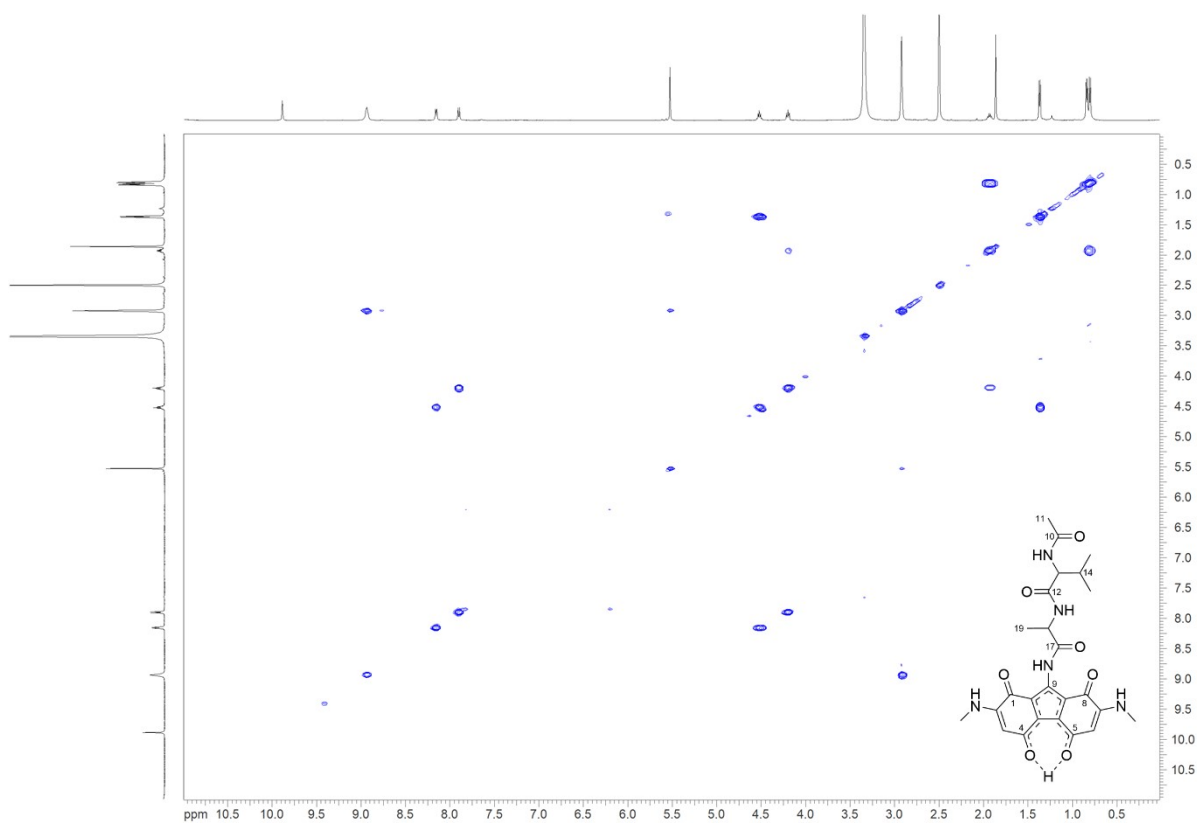


Figure S6: COSY spectrum ($\text{DMSO-}d_6$) of malevonin.

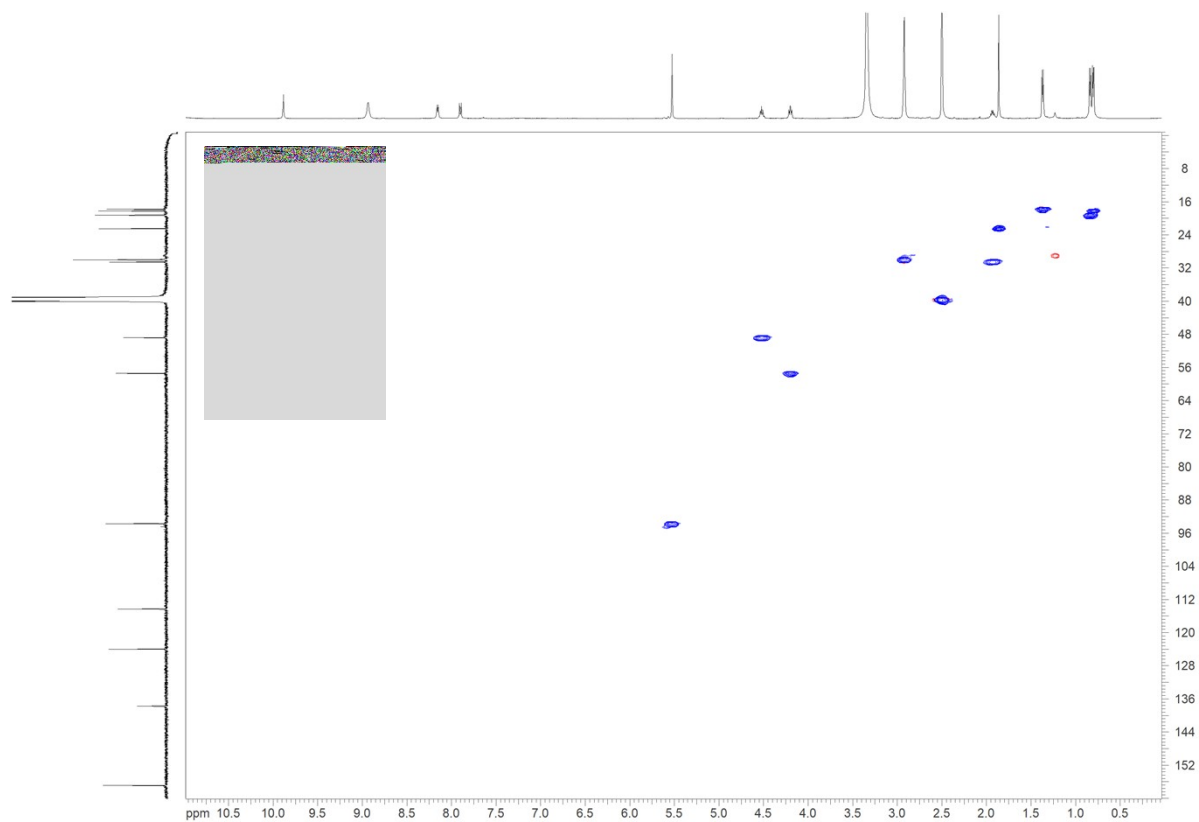


Figure S7: Edited HSQC spectrum (DMSO-*d*₆) of malevonin.

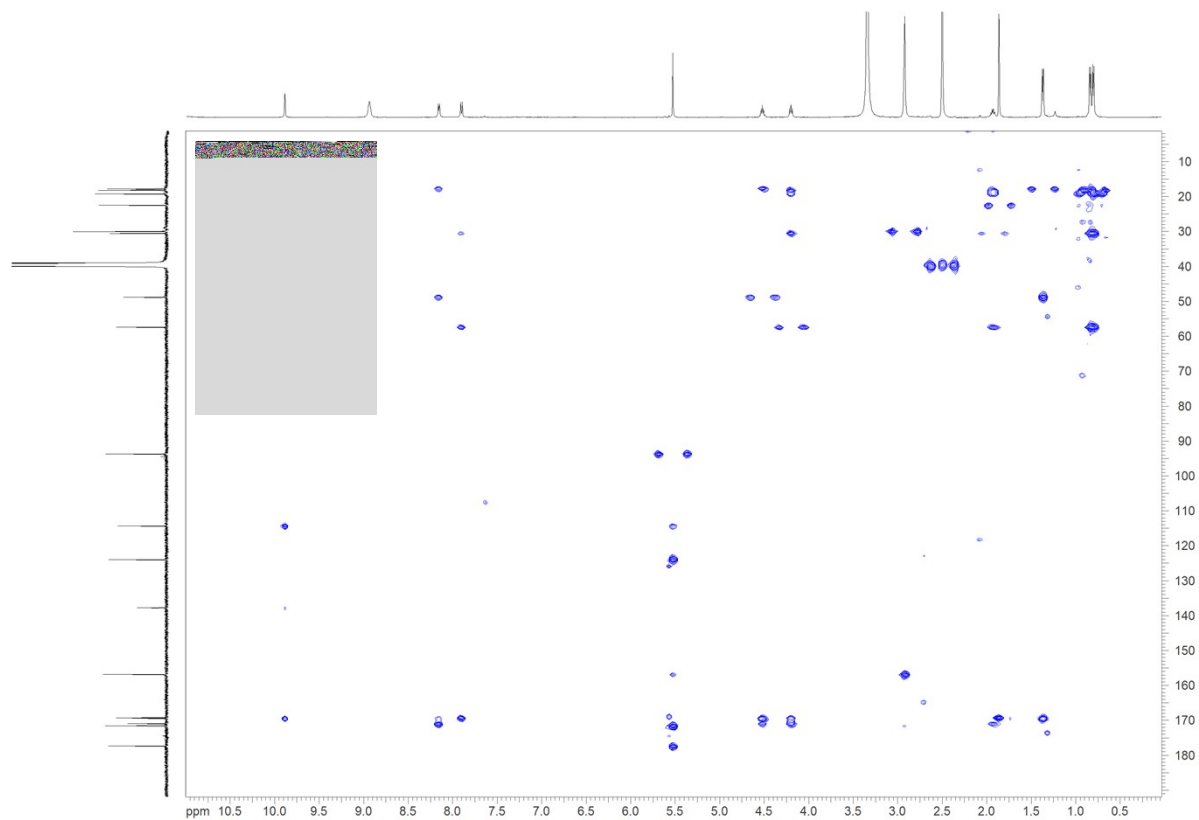


Figure S8: HMBC spectrum (DMSO-*d*₆) of malevonin.

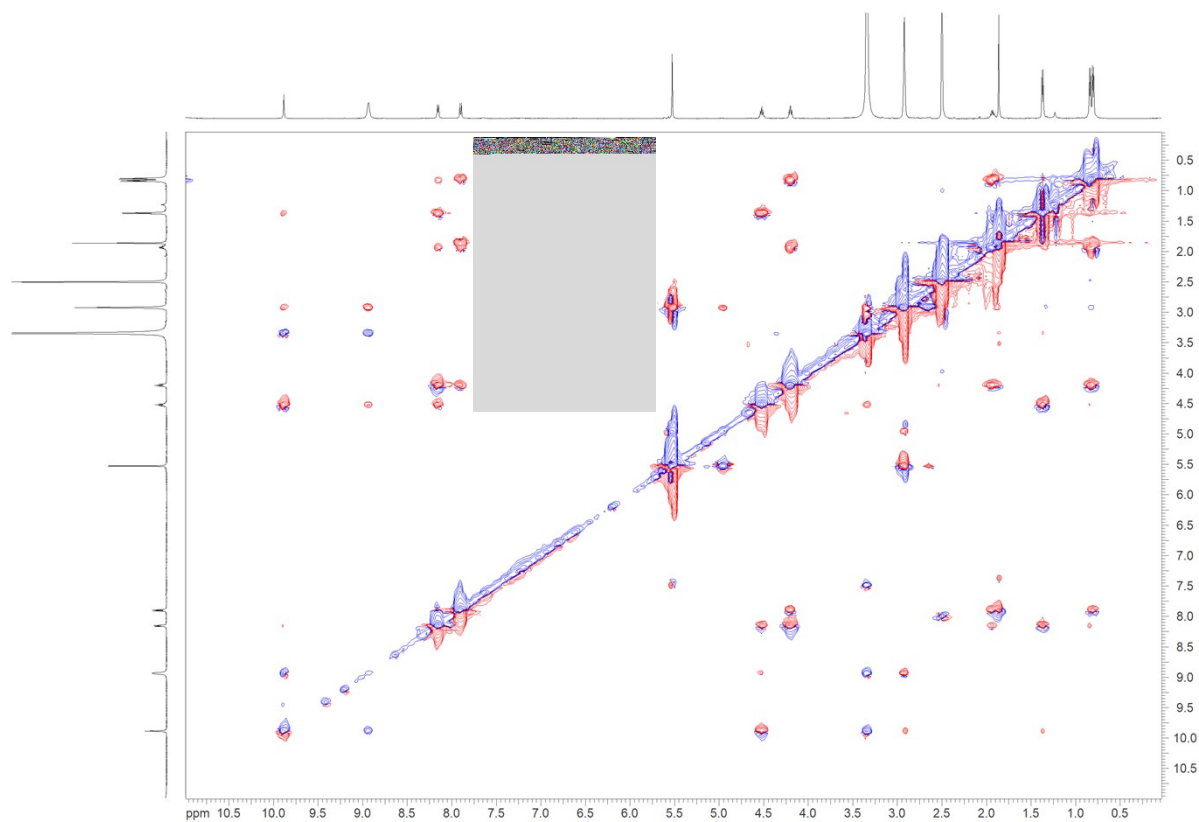


Figure S9: ROESY spectrum (DMSO-*d*₆) of malevonin.

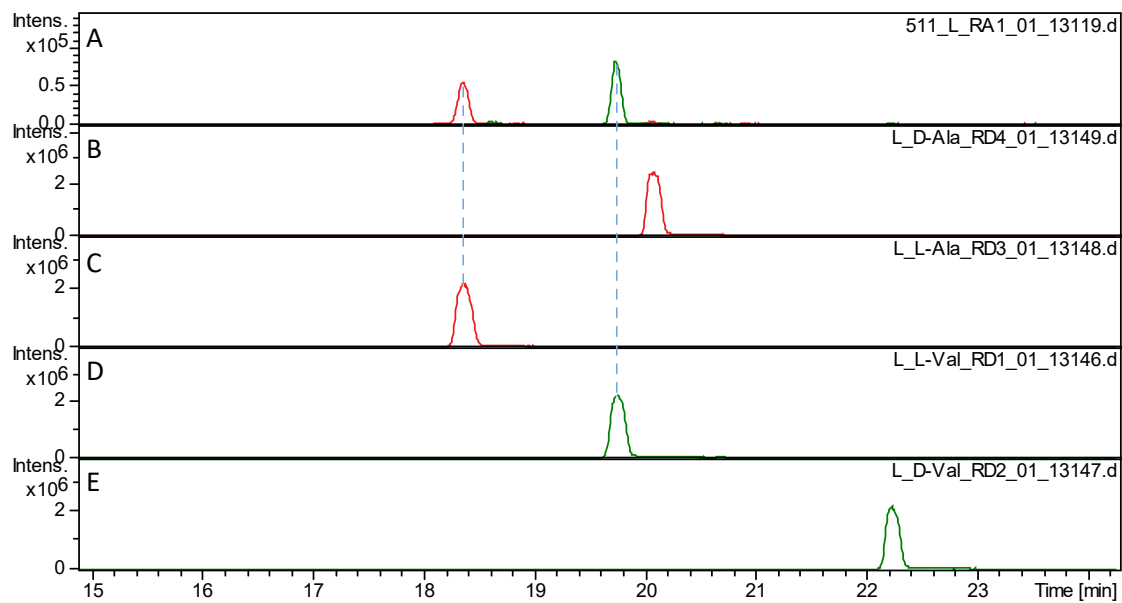


Figure S10: Determination of the absolute stereochemistry by Marfey's method using L-FDLA Derivatization agent with (A) malevonin hydrolyzed with 6M HCl and the standards (B, C) D/L-alanine and (D, E) L/D-valine.

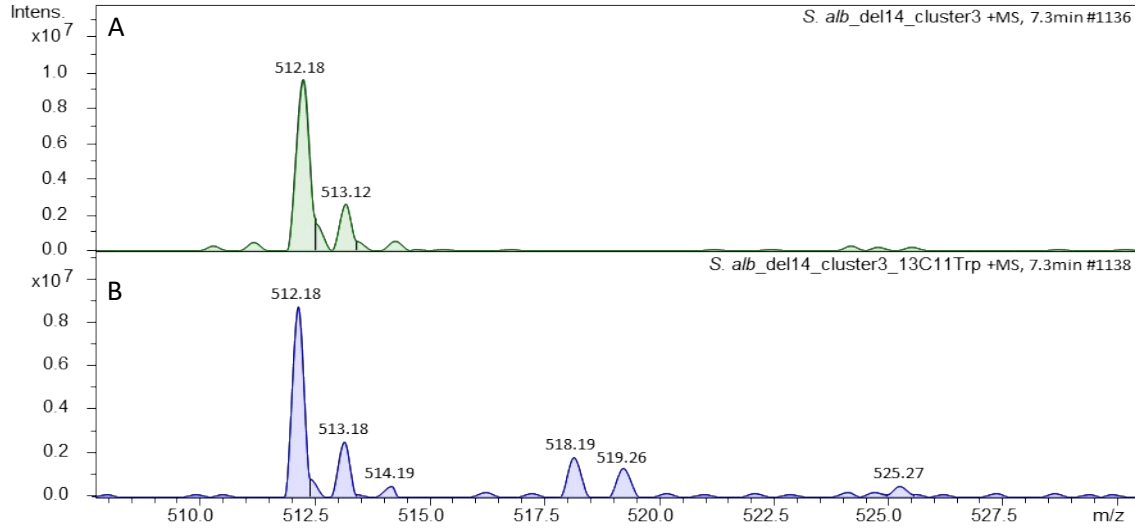


Figure S11: Feeding of *S. albus* Del14 with ¹³C₁₁ Trp (**2**) revealed (B) a mass of 518.18, 519.26 and 525.27 corresponding to +6, +7 and +13 Da compared to malevonin without labeling (A).

Score	Expect	Method	Identities	Positives	Gaps
699 bits(1805)	0.0	Compositional matrix adjust.	359/404(89%)	369/404(91%)	4/404(0%)
Orf24	1	SYGELDTTSTRLAYVLRATFGVGPDAVAGVLLERGLDLPVAQLGVLKAGGAWLPLDPQHP	60		
		SYGELD T+TRLA+VLR FGVGPDAV GVLL+RGLDLPVAQLGVLKAGGAWLPLDPQHP			
Orf25	1	SYGELDATATRLAHVLRADFGVGPDAVVGVLDRGLDLPVAQLGVLKAGGAWLPLDPQHP	60		
Orf24	61	AERLTGQLADAGARVVTTTRALATALPADMPRLCLDDPTTTHLLDRTPDTPLPHSGTKPE	120		
		ERL L+DA A VVTRALA ALPAD+PRL LDDP L RTP+TPL T P+			
Orf25	61	TERLASYLSDARAAAVTTTRALAAALPADVPRLVLDDPAPRERLARTPETPL-TGNTDPD	119		
Orf24	121	NLAYLIYTSGSTGAPKGMVSHGAANFVLNARELFGIRPGDRLLQFANPAFDVSVDFY	180		
		+LAY+IYTSGSTGAPKGMVSHGAANFVLNARELFGIRPGDRLLQFANPAFDVSVDFY			
Orf25	120	HLAYIIYTSGSTGAPKGMVSHGAANFVLNARELFGIRPGDRLLQFANPAFDVSVDFY	179		
Orf24	181	GALGSGAAVVGASREVLLDPDALQELLVRERVSVD---VPPAVLRLLDPGSLPDLRALF	237		
		GALGSGAAVVGASRE LLDPDALQELLVRERVSVD VPPAVLRLLDPGSLPDLRALF			
Orf25	180	GALGSGAAVVGASRETLDPDALQELLVRERVSVDORFVPPAVLRLLDPGSLPDLRALF	239		
Orf24	238	VGLEAFPAELVNRWSSEKREFHNGYGPTATVACVDYLCPPGGLSASPPIGRAMANHRAY	297		
		VGLEAFPAELVNRWSSEKREFHNGYGPTATVACVDYLCPPGGLSASPPIGRAMANHRAY			
Orf25	240	VGLEAFPAELVNRWSSEKREFHNGYGPTATVACVDYLCPPGGLSASPPIGRAMANHRAY	299		
Orf24	298	VLNAETFEPVPVGPGEFVAGAGLARGYLNRPDLTAERFVPDPFSGSGERMYRTGDVVR	357		
		VLNAETFEPVPVGPGEFVAGAGLARGYLNRPDLTAERFVPDPFSGSGERMYRTGDVVR			
Orf25	300	VLNAETFEPVPVGPGEFVAGAGLARGYLNRPDLTAERFVPDPFSGSGERMYRTGDVVR	359		
Orf24	358	WREDGNLEFLGRADRQVKIRGLRIEPGEIEHALAGCEGVRQGTV	401		
		WREDGNLEFLGRADRQ+KIRGLRIEPGEIEHAL GV Q V			
Orf25	360	WREDGNLEFLGRADRQIKIRGLRIEPGEIEHALTTSPGVAQAVV	403		

Figure S12: Alignment of Orf24 and Orf25 from cluster 3 shows 90% similarity.

Table S7: Proposed functions of genes within cluster 16.

Orf	Proposed function	LocusTag in <i>S. alboniger</i> (CP023695.1)	GeneBank homologue
1	VOC family protein	CP975_33055	WP_150477657.1
2	NAD(P)/FAD-dependent oxidoreductase	CP975_33060	WP_150477658.1
3	CPBP family glutamic-type intramembrane protease	CP975_33065	WP_150477659.1
4	helix-turn-helix domain-containing protein	CP975_33070	WP_150478040.1
5	M64 family metallopeptidase	CP975_33075	WP_055536098.1
6	LacI family DNA-binding transcriptional regulator	CP975_33080	WP_150477660.1
7	nucleoside hydrolase	CP975_33085	WP_055536099.1
8	MFS transporter	CP975_33090	WP_055536100.1
9	terpene synthase family protein	CP975_33095	WP_055536101.1
10	family 2B encapsulin nanocompartment shell protein	CP975_33100	WP_055536102.1
11	hypothetical protein	CP975_33105	WP_055536103.1
12	MBL fold metallo-hydrolase	CP975_33110	WP_055536123.1
13	DUF4236 domain-containing protein	CP975_33115	WP_030775935.1
14	hypothetical protein	CP975_33120	WP_167532771.1
15	hypothetical protein	CP975_33125	WP_055536104.1
17	AfsR/SARP family transcriptional regulator	CP975_33130	WP_055536106.1
18	cytochrome P450	CP975_33135	WP_055536124.1
19	HAD family hydrolase	CP975_33140	WP_055536107.1
20	SigB/SigF/SigG family RNA polymerase sigma factor	CP975_33145	WP_055536108.1
21	aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme	CP975_33150	WP_055536109.1
22	hypothetical protein	CP975_33155	WP_246201713.1
23	PHP domain-containing protein	CP975_33160	WP_055536110.1
24	xanthine dehydrogenase family protein molybdopterin-binding subunit	CP975_33165	WP_055536111.1
25	FAD binding domain-containing protein	CP975_33170	WP_055536112.1
26	2Fe-2S iron-sulfur cluster-binding protein	CP975_33175	WP_055536113.1
27	hypothetical protein	CP975_33180	WP_055536114.1
28	alpha/beta hydrolase	CP975_33185	WP_055536115.1
29	ATP-binding protein	CP975_33190	WP_150477663.1
30	ribonuclease H family protein	CP975_33195	WP_055536116.1
31	MarR family winged helix-turn-helix transcriptional regulator	CP975_33200	WP_150478041.1
32	MFS transporter	CP975_33205	WP_055536118.1
33	iron-containing redox enzyme family protein	CP975_33210	WP_055536119.1
34	DUF6131 family protein	CP975_33215	WP_167532773.1
35	DUF5133 domain-containing protein	CP975_33220	WP_055536120.1
36	catalase	CP975_33225	WP_055536121.1
37	hypothetical protein	CP975_33230	WP_167532774.1

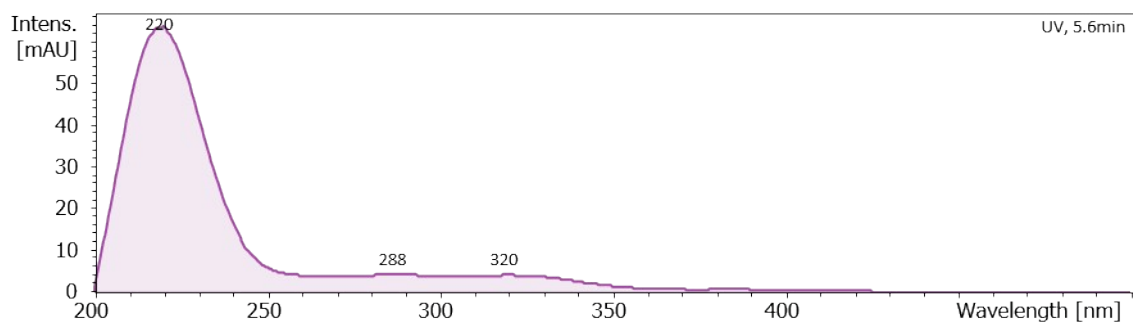
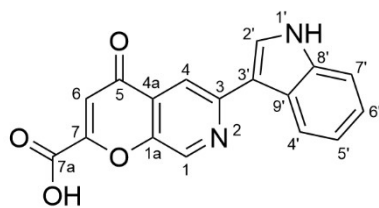


Figure S13: UV/VIS spectrum of mansevorone.

Table S8: NMR data (500 MHz, CD₃OD) of mansevorone.

no	$\delta(^{13}\text{C}, ^{15}\text{N})$ [ppm], type	$\delta(^1\text{H})$ [ppm], mult (J)	COSY (H-)	HMBC (H-)	NOESY (H-)
1	143.8, CH	9.14, d (0.5)	4 (^4J)	1a, 3, 4, 4a, 5	
1a	150.5, C	-	-	-	
2-N	319.0, N	-	-	-	
3	153.8, C	-	-	-	
4	112.8, CH	8.23, d (0.5)	1 (^4J)	1a, 2-N, 3, 3', 5	2'
4a	130.5, C	-	-	-	
5	181.3, C	-	-	-	
6	112.8, CH	7.04, s	-	4a, 7, 7a	
7	165.7, C	-	-	-	
7a	162.2, C	-	-	-	
1'-NH	133.6, NH	-	-	-	
2'	127.1, CH	7.97, s	-	1'-NH, 3, 3', 8', 9'	4
3'	116.5, C	-	-	-	
4'	121.7, CH	8.30, m	5', 7' (^4J)	5', 8', 9'	
5'	121.7, CH	7.19, m	4', 6'	6', 7', 9'	
6'	123.4, CH	7.20, m	5', 7'	4', 8'	
7'	112.9, CH	7.46, m	6', 4' (^4J)	5', 9'	
8'	139.0, C	-	-	-	
9'	126.6, C	-	-	-	



mansevorone (**2**)

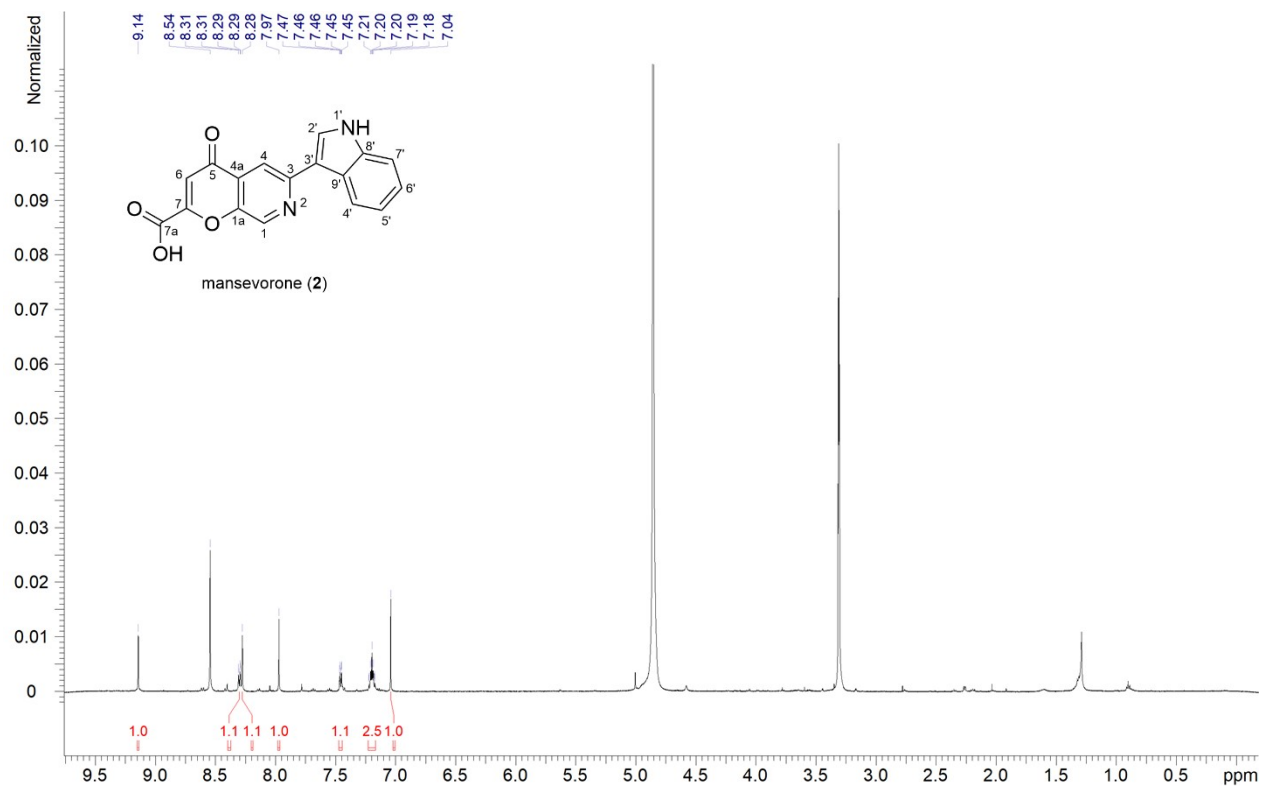


Figure S14. ^1H NMR spectrum (500 MHz, CD_3OD) of mansevorone.

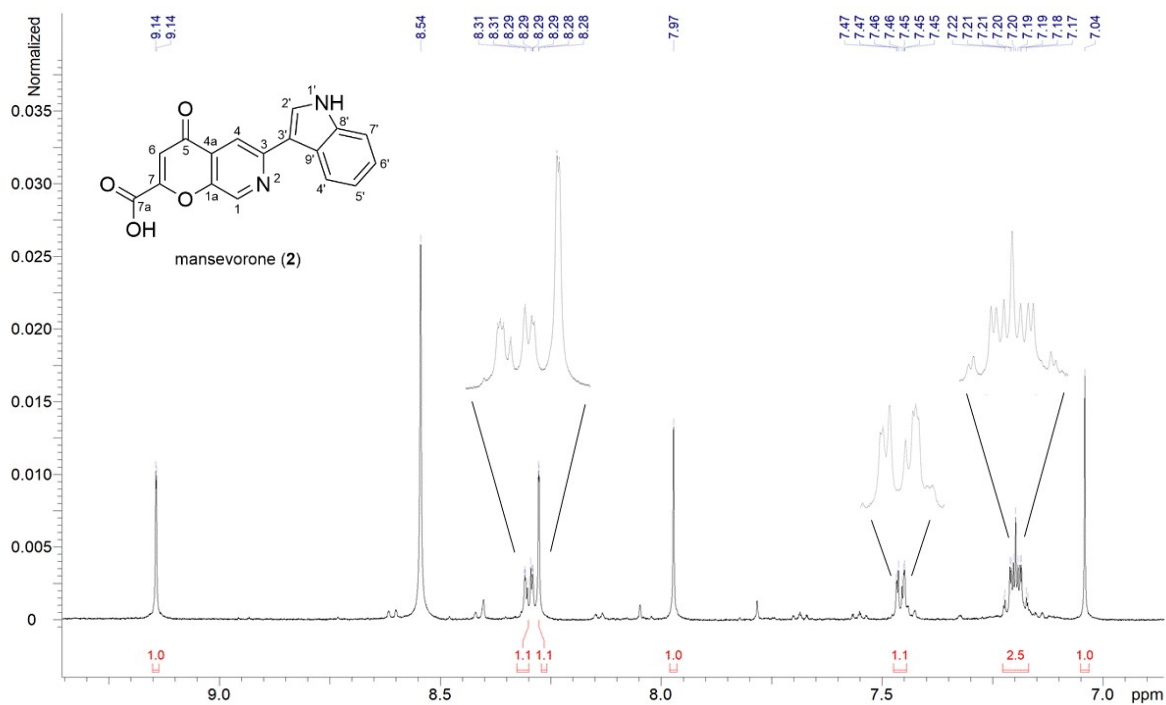


Figure S15. ^1H NMR spectrum (500 MHz, CD_3OD) of mansevorone zoomed in on the relevant peaks.

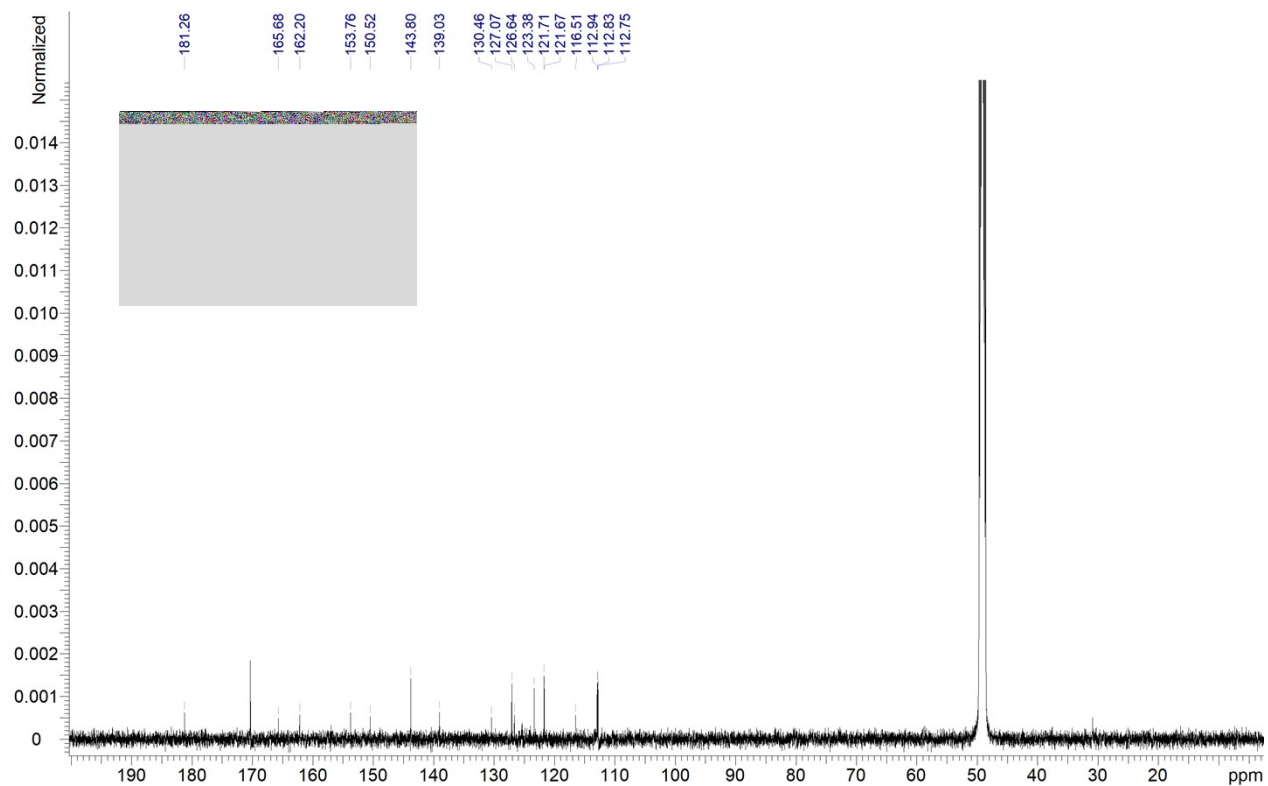


Figure S16: ^{13}C NMR spectrum (125MHz, CD_3OD) of mansevorone

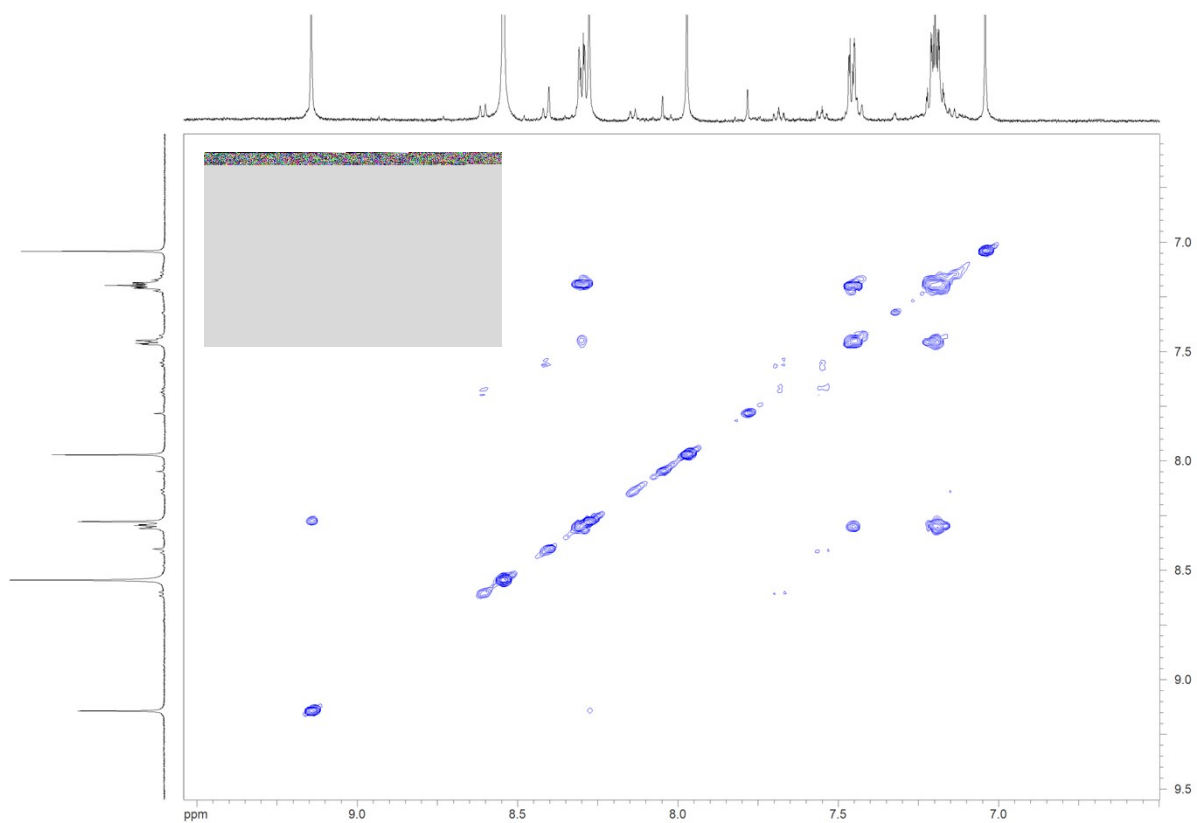


Figure S17: COSY spectrum (CD_3OD) of mansevorone.

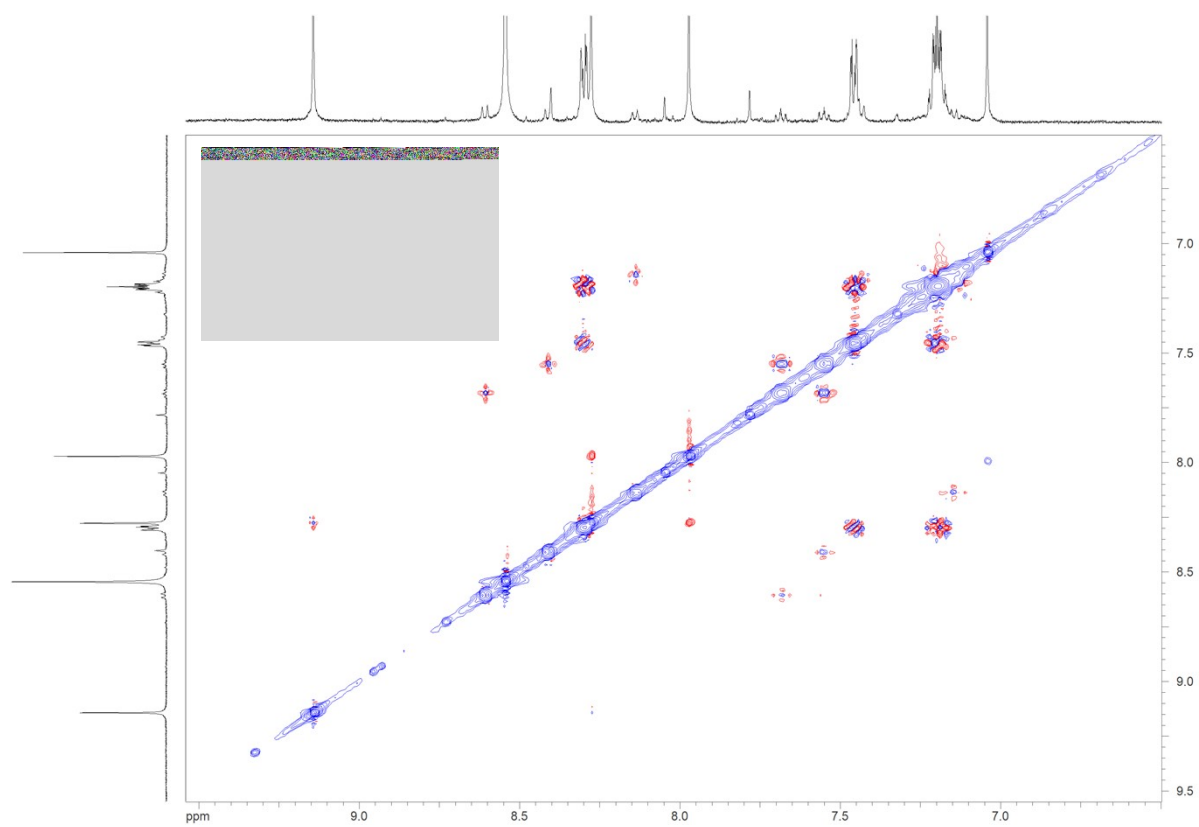


Figure S18: NOESY spectrum (CD₃OD) of mansevorone.

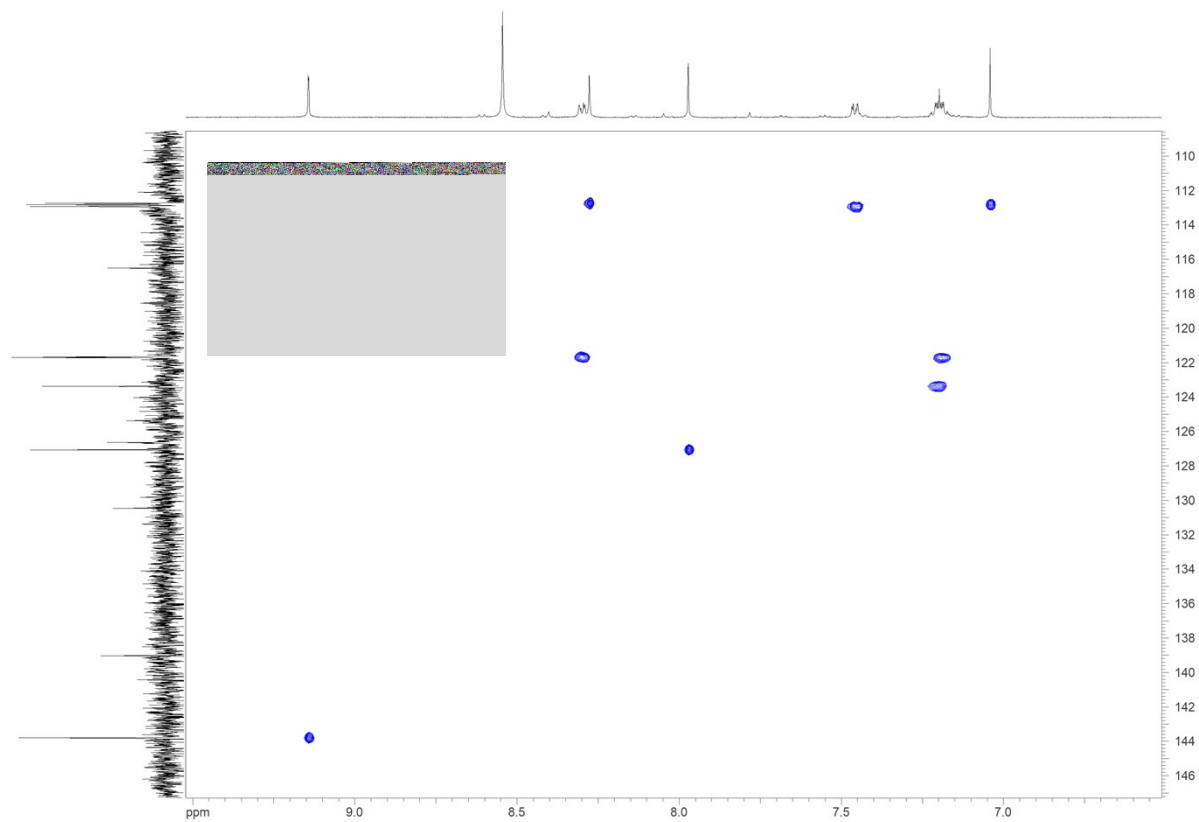


Figure S19: Edited HSQC spectrum (CD₃OD) of mansevorone.

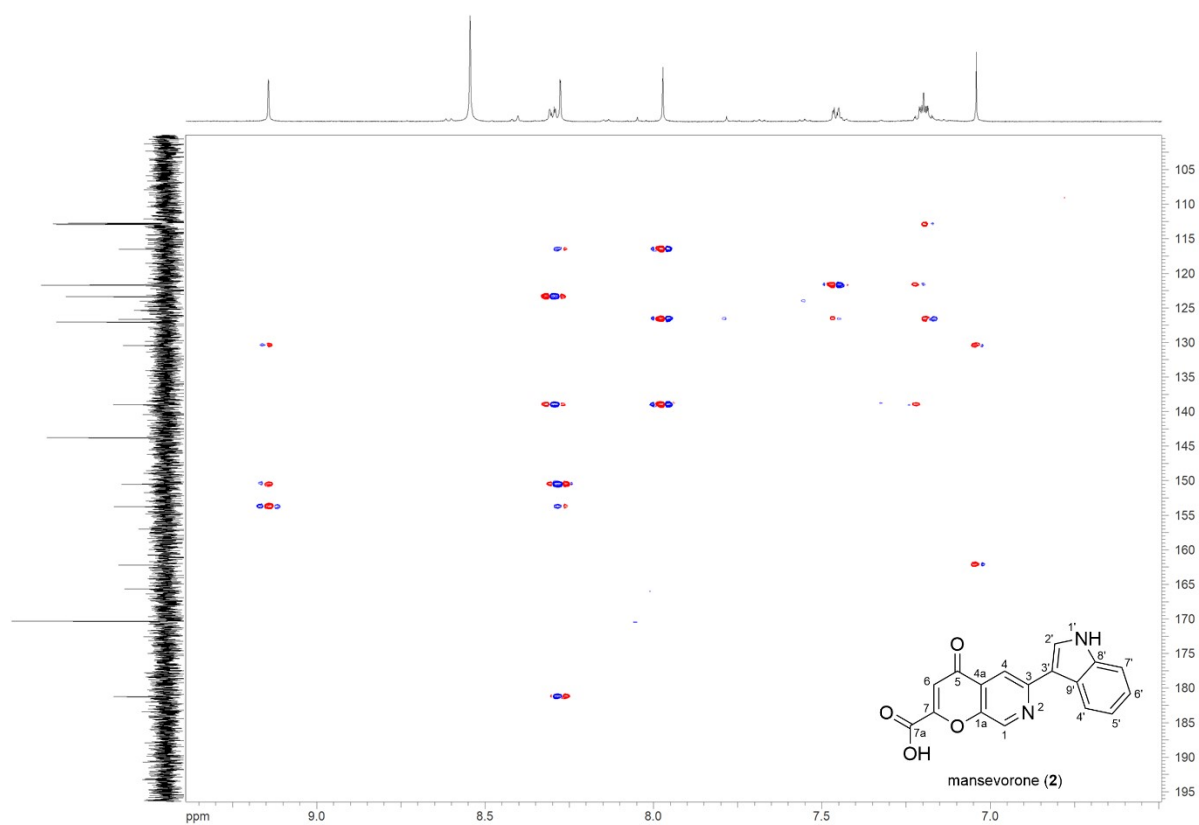


Figure S20: HMBC spectrum (CD3OD) of mansevorone.

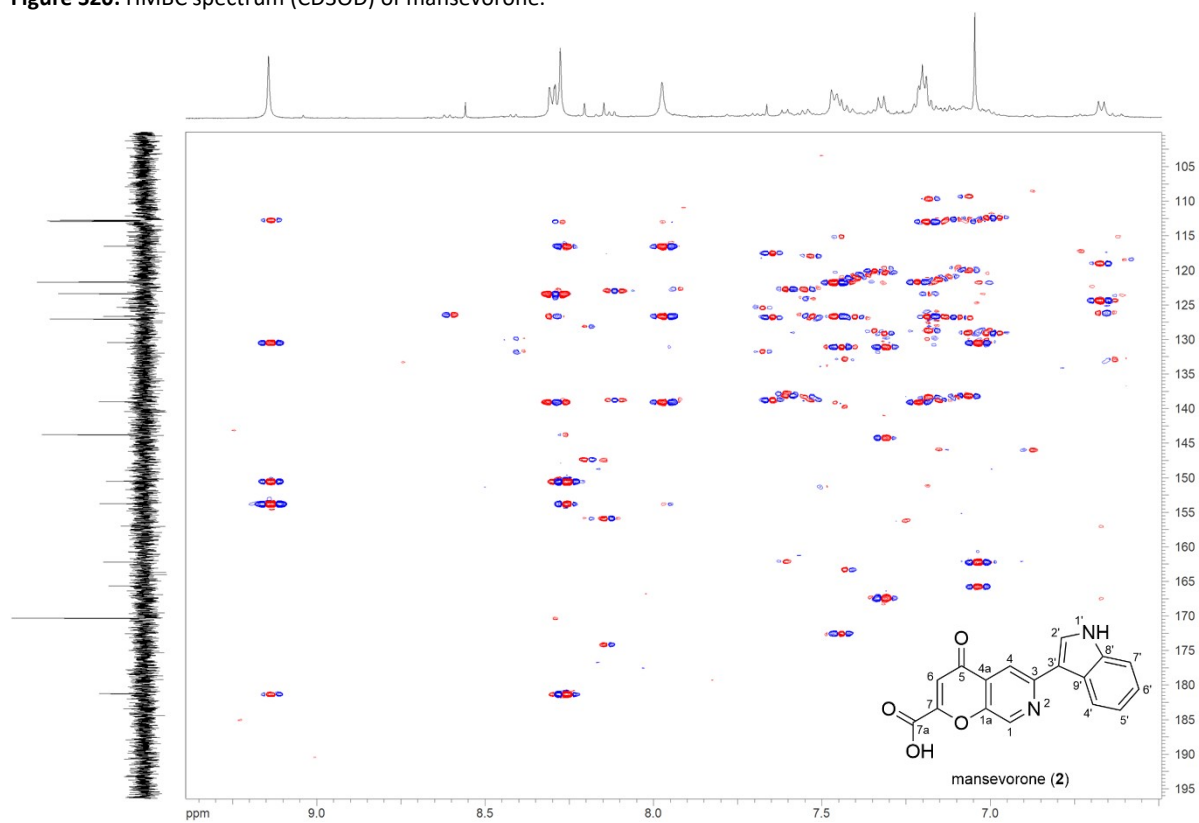


Figure S21: HMBC spectrum (CD3OD) of mansevorone before final HPLC purification.

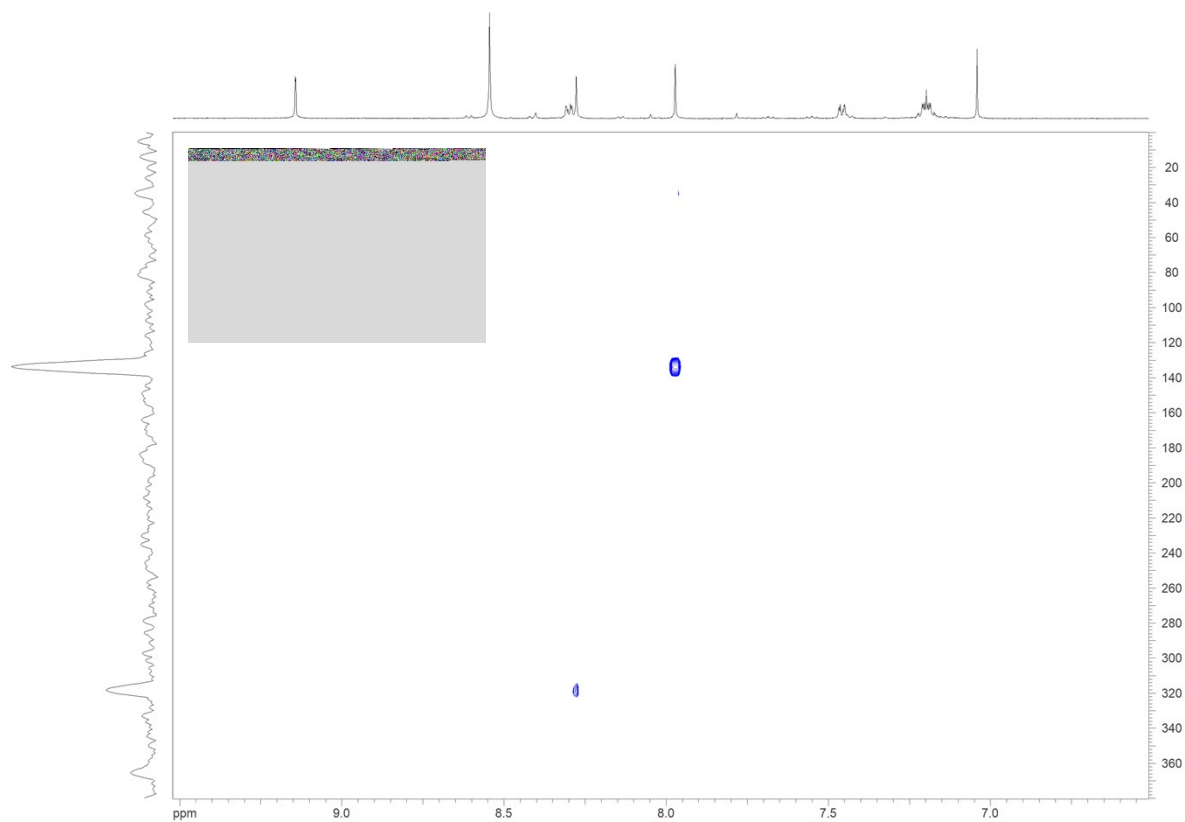


Figure S22: N-HMBC spectrum (CD3OD) of mansevorone.

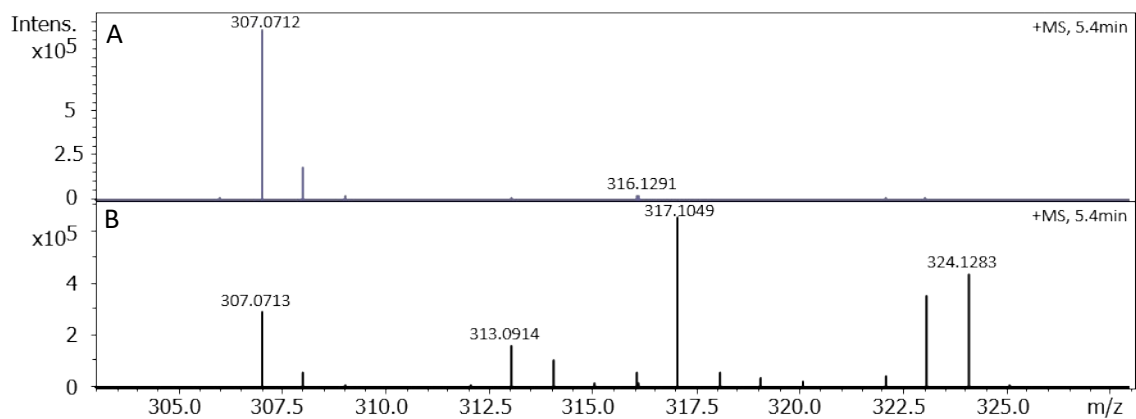


Figure S23: Comparison of MS spectra for mansevorone between (A) a culture of *S. albus* C16h7 and (B) a culture of *S. albus* C16h7 supplemented with $^{13}\text{C}_{11}$ -labeled tryptophan.

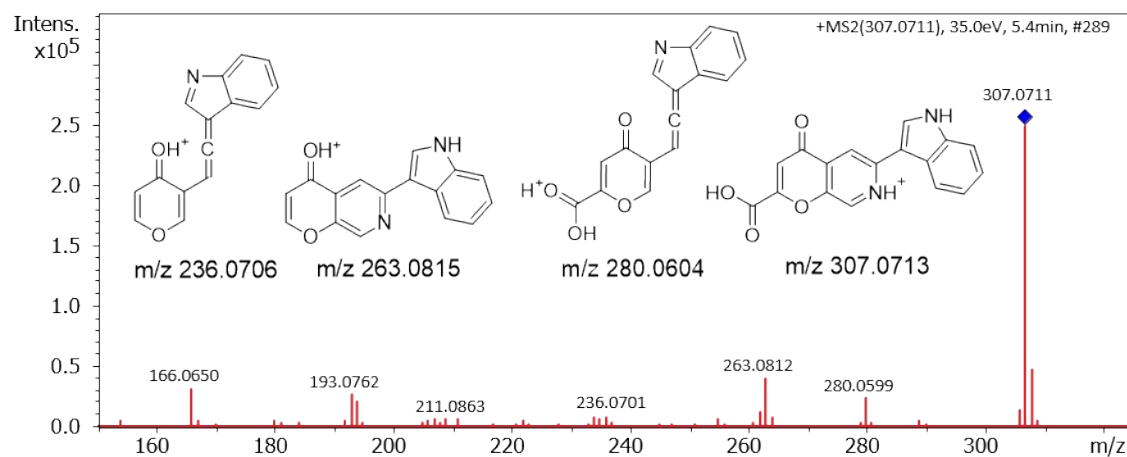


Figure S24: MS/MS fragmentation spectrum of mansevorone from cluster 16.

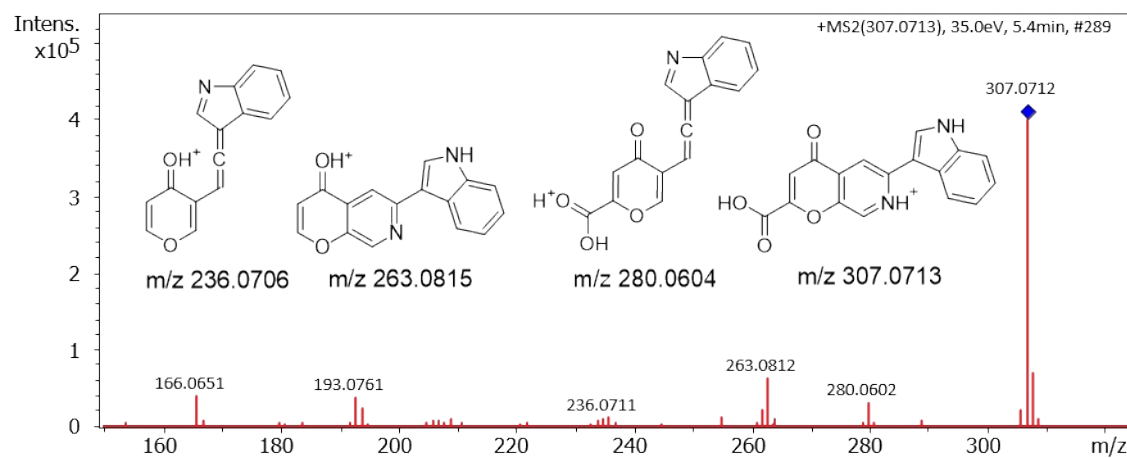


Figure S25: MS/MS fragmentation spectrum of mansevorone from cluster C1.8.

>AsfR sequence

MEFRLLGAVSVATEVGVLPPLGPAKRSLAALLRPNHVPVDRDLTAALWDQEPARARGVIQGHVSQLRVLLTEAEAG
MFGVELVTQGTAYVLRMPESLLDAHRFEELVTARGQRAPADAVAMYQEALSLWQGPALTGAYPSQPLQAAAQALEE
LRLASVESLAGAYTRMGEHARAAVLRAEAGAHPLRESLSAELMRALQRAGRRTALDWFHRTRRVLDELGVDPGRE
LADAYAAALRGAGDEADGAGGGASVADVPPSGGPVALAAPPASAPASLPAAAPAGGSVPAAPVASPASGPVLPPA
AIDLLPRMPRGFHRGAGELTALSRAAAGEAPVCLVTGPAGVGKTALVTHWAHRNREHFPGGLLYADLRGFSDTGEPAL
LEVLRFLALGVAPRRIPESAGAASALFRSLSADRQLLVLDNARASDQVRELLPGGARCVTVTSRYRLRGLIASDAARP
VPVDVLEPEDSTALLAAVLGTDVRFAEEVAARRLAELCGGLPLALRVAAARLADQPESLSTMCAELSDSRRLGLLDVED
TGVRAALRLTVRRLPADAARQFAHLGRHPGMYIDLYAAAAALADTDPATAEAALDKLTAHLVMRAGPDRWTLHDLVR
LYARDLDAEADALLRVLDLTVATALAAADAAEPGDESCFTLPADFRPPWAAREFADREQAMAWYAAERDDLTAATA
ADSAGLHDTWRILGMWPQIVWRVQDGWAPLLKTALEATRADGDARAESRVLALLGWVLTEEGRIDEALGHLEAAP
LLAARAGDTRGEATALMNLSLAQAALGSPDEAAEGCALAAELAHVSGDRHTERLALCHLARHRLDAREWQAAHETAIA
ALDLVGPSEASAAARVLLLTALIGEALLGMGDETEGIRRENAALAEACGYDDGAVRALGALLRVSDAGLQARYDTAM
VRLMART*

Figure S26: Sequence of SARP regulator AsfR16 from cluster C16.

>DnrI sequence

MHALRTPGSPERTDPAGPLLRVLGPMSARFDGQDLPLGPPRRRALLALLLIRLGRVVPTELIVDELWGDEPPRQPVATL
QSHVSHLRRLVDPTAGPGAASVLRYPAGYVLRRLAPEQVDFCRFEDLVSTGRRSLEQRDPLAARDRLAEALELWHGSPY
TEFSAHPPLADETARLEQVRLAALESYAEARLTGAAAEEVAADLDREVRNHPMRERLVGHLM TALTRLGRQAEALEVYE
RTRSHLVEEFGVDTAVELQVRVTAIRLQELGDGGPGGATAAPAVSAPSAPLTPPARPTRSPASAVPAPYGGTGRPARTP
VPAPAAAPARAEDPGTDVPDPSAPWPFTGRDQELHRLAAAAAGALTGHGHVSVLGPAGVGKTRLLMELAPRLETAD
EHLEVWWSHCFFGEGVPPYWLWTQVLRRLSATRPDAFRAATAPFGALLGPLMPERSAGPGGAPGPEVDWAQARFLT
HDAVCEVLLALAAESPLVLLLEDLHWADTASLDLLRLLGVRRLGHPLSIVLTARDFEIESDATMRRLLAEVVRGPRSETLRL
DGLPRQAVATLVEAQAGPGVGAEEVVEALHRRSKGNPYFVMQLLSLLGDVRHLHDPGASAVLLAQVPTGVREALRQRF
SALPEPVLRLVRLCAVIGTEVDTDLLHRTADGDEPVAAEALSAIRAGLLGEDPHHPGRLHFAHALVQETLAEPPAREERHR
LHARVAEALCARGRGQMGEDEIERVAHHSWHAKDALAPGETLPRLRAAERAHHLAYEQVETWLRRAVHLAGFLPA
DDPSAPGLEQRLHIQLGQVLATIRGYGDAEAEALGRGRALSAVTHSPEDPSVLWALCAALLVTGRYDDSRQFSGLLRD
LAGRTRQPVAVLGAAYGEGIVLHVRGRLPEALAELEHGVDMA DRFAREGHSLARTFQHDPRVSCRSYDTFTHWLLGDR
RTAAERRDQLRLTDYESRPSDRAFALYVDVVAWEGDVGTALASGGEGARVAGEHGLLYWKAMLSLPAGWALTHS
GREEEGLALMRTSLDELCPSTRHLRLPLHLGLLQQAQFHAGRREEATDTLRRMLSVERREYVYLDPALPATRLLHELV
GREAAEAVLRGC*

Figure S27: Sequence of SARP regulator DnrI from cluster C1.8.

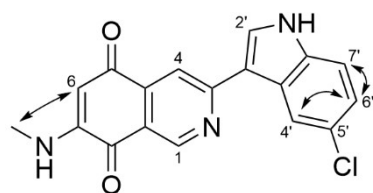
Score	Expect	Method	Identities	Positives	Gaps
144 bits(364)	4e-39	Compositional matrix adjust.	144/372(39%)	178/372(47%)	13/372(3%)
DnrI	21	LRVLGPMSARFDGQDLPLGPPRRRALLALLLIRLGRVVPTELIVDELWGDEPPRQPVATL	80		
AsfR	3	R+LG +S + LPLGP +RR+LLA LL+R VP + + LW EPP + + FRLLGAVSVATEVGVLPPLGPAKRRSLLAALLLRPNHPVPVDRLTAALWDQEPPARARGVI	62		
DnrI	81	QSHVSHLRRLVDPTAGPGAASVLRYPAPGYVLRRLAPEQVDFCRFEDLVSTGRRSLEQRDP	140		
AsfR	63	Q HVS LR +L L + YVLR+ +D RFE+LV+ R QR P QGHVSQLRVLLTEAEAGMFGVELVTQGTAYVLRMPESLLDAHRFEELVTLAR---GQRAP	119		
DnrI	141	LAARDRLAEALELWHGSPYTEFSAHPPLADETARLEQVRLAALESYAEARLTLGAAAEVA	200		
AsfR	120	A EAL LW G T PL LE++RLA++ES A A +G A A ADAVAMYQEALSLWQGPALTGAYPSQPLQAAAQALEELRLASVESLAGAYTRMGEHARAA	179		
DnrI	201	ADLDREVRNHPMRERLVGHMLTALTRLGRQAEALEVYERTRSHLVEEFGVDTAVELQQRVR	260		
AsfR	180	A L E HP+RE L LM AL R GR+ EAL+ + RTR L +E GVD EL AVLRAEAGAHPLRESLSAELMRALQRAGRRTEALDWFHRTTRVLADELGVDPGRELADAY	239		
DnrI	261	TAILRQELGDGGPGGATAAPAVSAPSA---PLTPPARPTRSPASAVPAPYGGTGRPARTP	317		
AsfR	240	A LR + G A+ A PS L P + A PA GG+ PA P AAALRGAGDEADGAGGGASVADVPPSGGPVALAAPPASAPASLPAPAAPGGSVVPA-AP	298		
DnrI	318	VPAPAAAPARAEDPGTDVDPSPAPWPFTGRDQELHRLAAAAAGALTGHGHVSVVLGPAGV	377		
AsfR	299	V +PA+ P P P P F GR EL L+ AAA G V V GPAGV VASPASGPVL--PPAAIDLLPRMPRGFHRAGELTALSRAA----GEAPVCLVTGPAGV	352		
DnrI	378	GKTRLLMELAPR 389			
AsfR	353	GKT L+ A R GKTALVTHWAHR 364			

Figure S28: Alignmet of SARP regulators DnrI from cluster 1.8 and AsfR from cluster 16.

Table S9: NMR data of 5'-Cl-Mansouramycin D (500MHz, CD3OD) and lit. data of mansouramycin D.

5'-Cl-Mansouramycin D (CD3OD)					Mansouramycin D (DMSO- <i>d</i> ₆)**	
No	$\delta(^{13}\text{C})$ [ppm], type	$\delta(^{13}\text{C})$ [ppm], predicted*	$\delta(^1\text{H})$ [ppm], mult. (J)	COSY (H-)	$\delta(^{13}\text{C})$ [ppm]	$\delta(^1\text{H})$ [ppm], mult. (J)
1	147.9, CH	149.0	9.20, s		147.6	9.11, d (0.7)
3						
4	113.6, CH	114.6	8.22, s		113.0	8.18, d (0.7)
5						
6	98.9, CH	99.6	5.79, s	N-Me	99.4	5.71, d (0.4)
7						
7-NH						
N-Me	27.8, CH	29.4	2.95, s	6	28.6	2.82 d (5.0)
8						
9						
10						
1'-NH						
2'	129.6, CH	131.6	8.25, s		129.8	8.53, br s
3'						
4'	121.1, CH	119.07	8.55, s	6'	121.7	8.52, dd (1.9, 6.0)
5'					122.2	7.22, m
6'	122.7, CH	123.85	7.22, dd (2.0, 8.6)	7', 4'	120.8	7.20, m
7'	112.7, CH	113.13	7.45, d (8.5)	6'	111.9	7.48, m
8'						
9'						

* predicted by ACD Labs ** ACS Chem. Biol. 2022, 17, 3, 598–608



5'-Cl mansouramycin D (**3**)

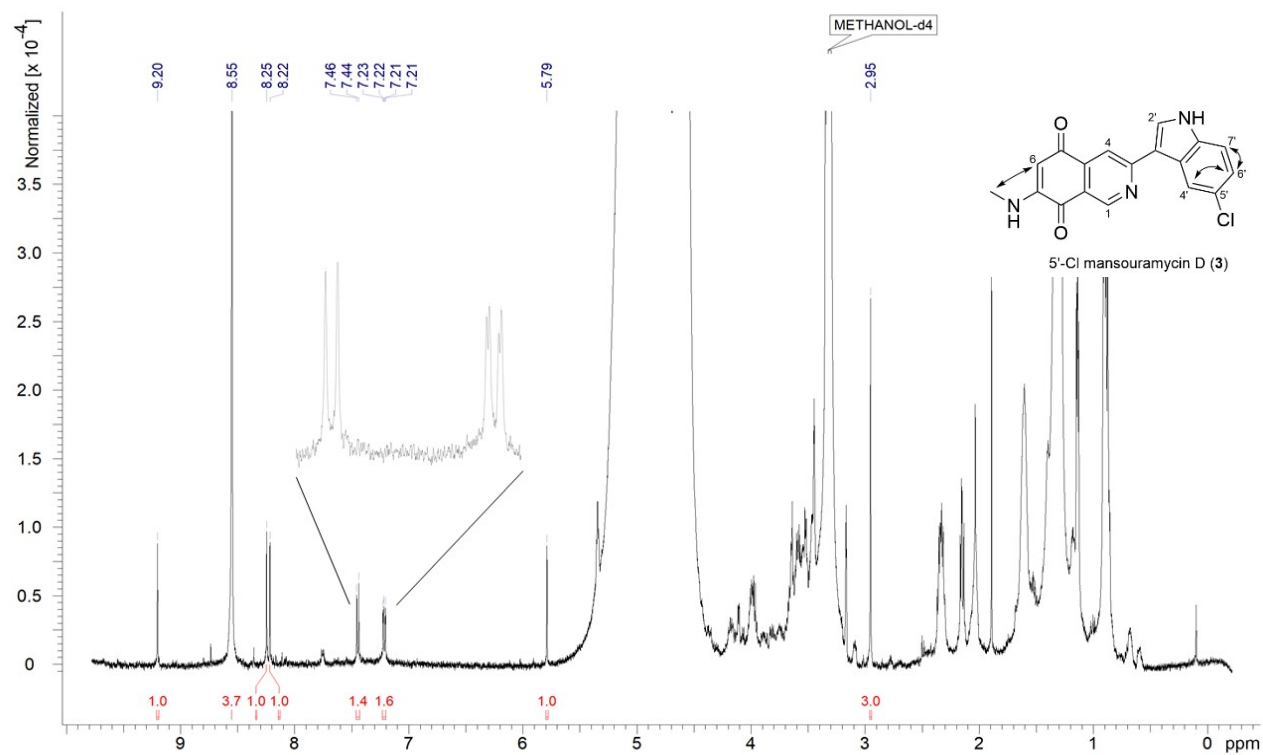


Figure S29: ^1H NMR spectrum (500 MHz, CD_3OD) of 5'-Cl-mansouramycin D.

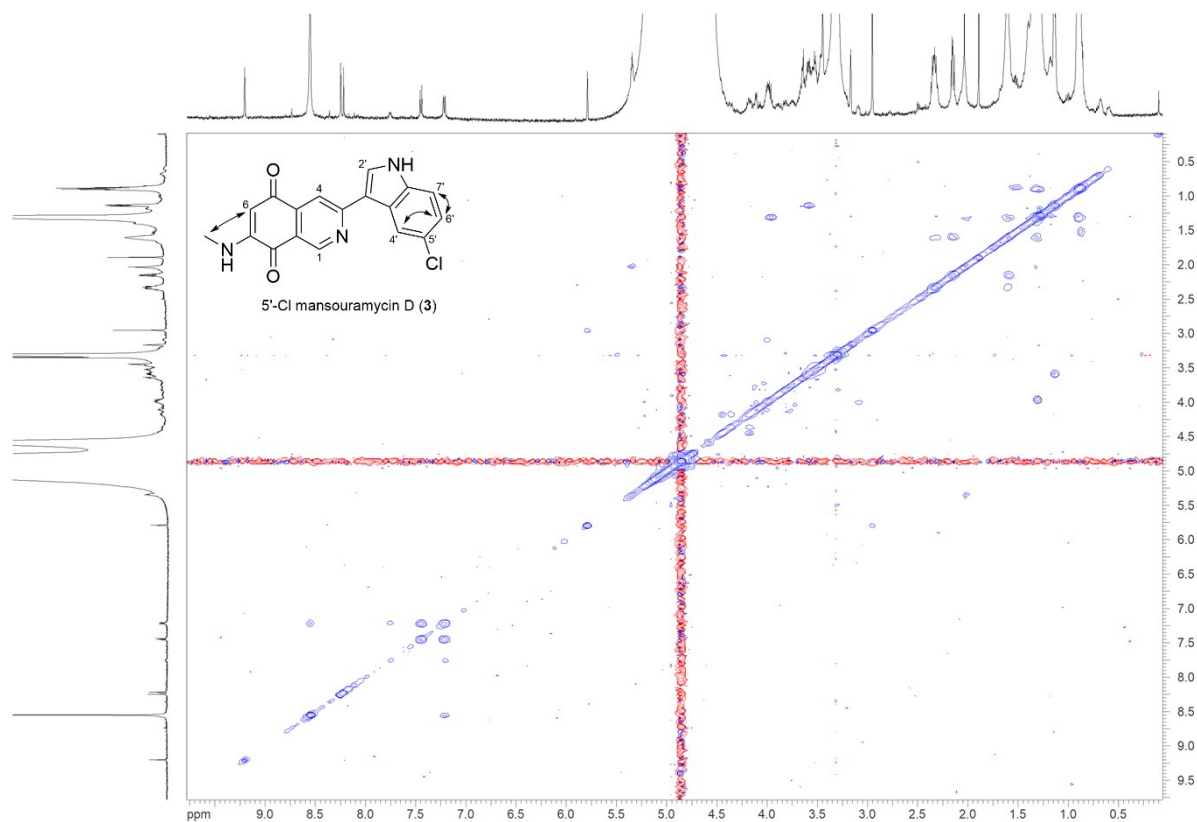


Figure S30: COSY spectrum (CD_3OD) of 5'-Cl-mansouramycin D.

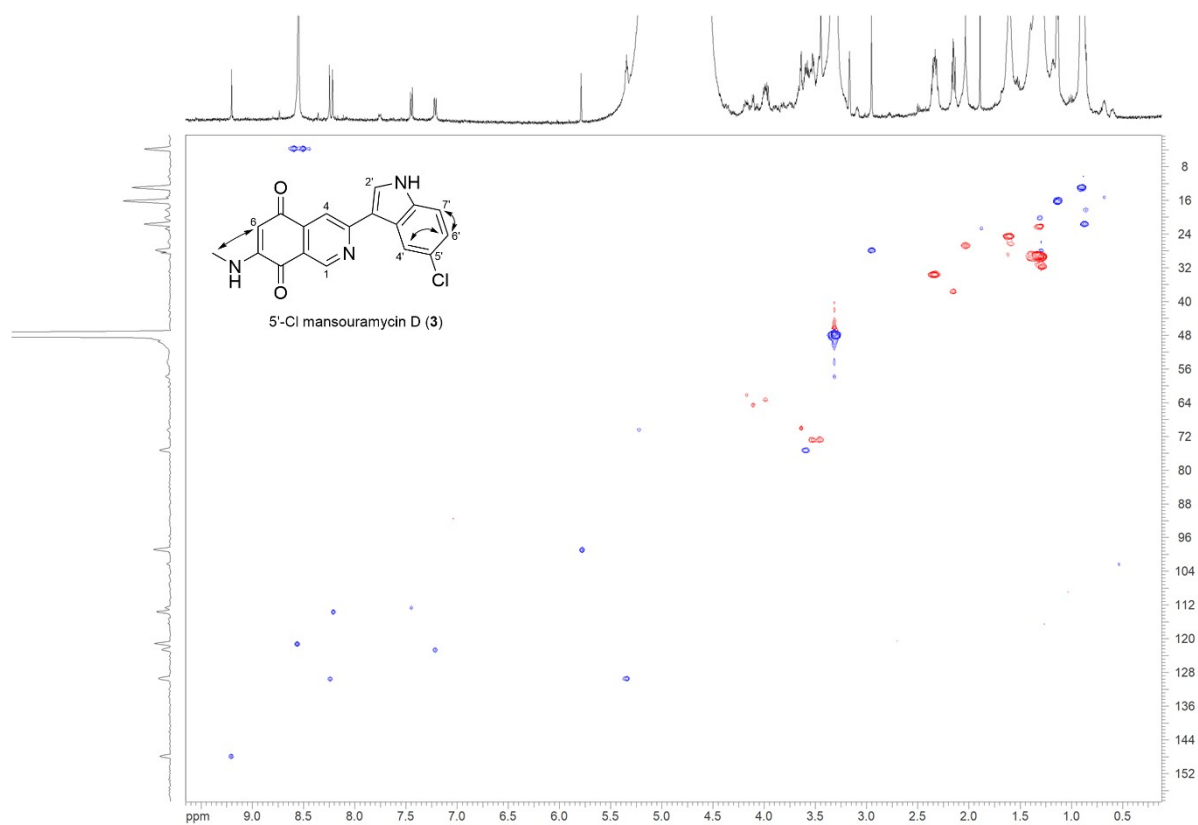


Figure S31: Edited HSQC spectrum (CD3OD) of 5'-Cl-mansouramycin D.

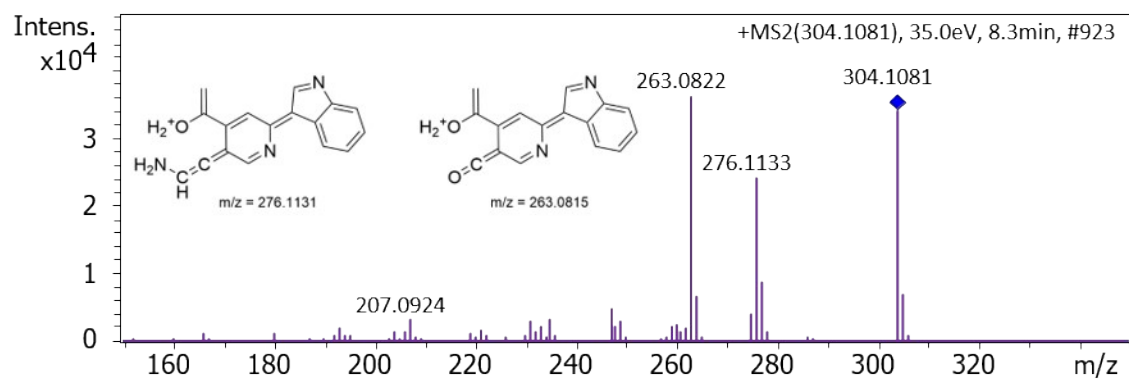


Figure S32: MS/MS fragmentation spectrum of mansouramycin D.

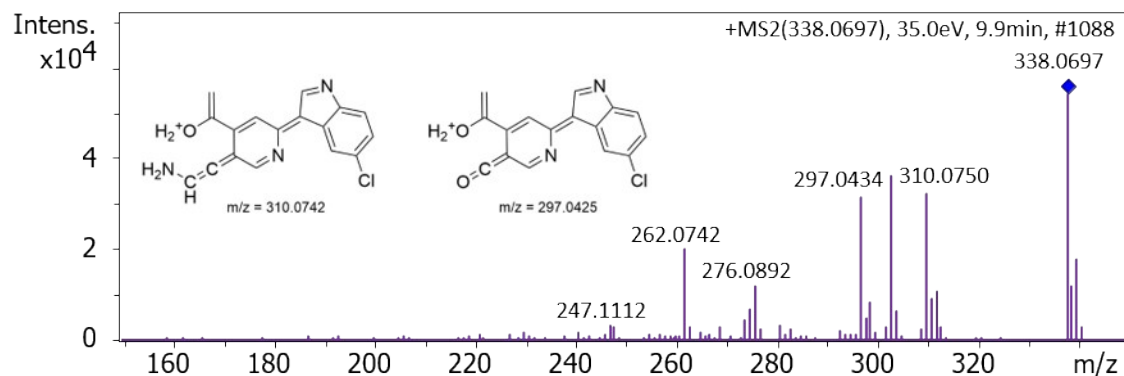


Figure S33: MS/MS fragmentation spectrum of 5'-Cl-mansouramycin D.

>RebH sequence

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GTGATTAATTCAAGTGGTCATTGTGCGCGGCGGCACTGCCGGATGGATGAGCGCCTCTACCTCAAGGCGGCCTAC
GGTGACCGCATCAACGTCACTCTGGTGGAATCCGACCGCGTCGCGACCATCGGCGTCGGTGAAGCGACCTTCAGC
ACGGTCCGGCATTCTTCGACTATCTCGGCCTGGACGAGCGCGAATGGATGCCGGAGTGCTCCGGTTCGTACAAGC
TGGGCATCCGGTTCGAGAACTGGCGGGAGCCGGGGCAGCACTTCTACCACCCCTTCGAGCGGCTGCGGACCTCCG
ACGGGTACACCCTGGCCGACTGGTGGCTTCAGGAGGGCGACCGCAGCGAGCCGTTGACCGTTCCTGCTTCATCA
CGCCGGCGCTGTGCGAGGCCAAGCGCTCGCCGCGGCTGCTCGACGGCTCCCTGTTGCGGGGCGGTCTCGACGGCT
CGCTGGGCGGCTCGACGCTGGAGGAGCAGCGGTCCAGTTCCTCGTACGCGTACCACTTCGACGCGGCCCTGCTGG
CGAAGTTCCTGACCAAGTACGGCACCGACCGCGGCGTCCGCCATGTCGTGACGACGTGACCGAGGTGGGCCGCG
ACGAGCGCGGCTGGATCAGCCATGTGCCACCCGTGAGCACGGTGACCTCACCGGTGACCTGTTTCATCGACTGCAC
CGGCTTCAAGGGCATGCTGATCAACGAGACCCTGGAGGAGCCGTTTCGAGTCCTTCAGGACGTGCTGCCGAACAA
CCGCGCGGCTCGCGCTCGCGTCCCGCAGGACGACAGGCCACCGGGATGAACCCGTACACCACGGCGACCGC
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CTGCTCCCCGGAGGAGGCGGAGCGCACCCCTGCGGAACACGTGCCCCCGGCCGGACGACCTGGAGGCCAACC
ACATCCGGATGCGCATCGGGCGCAACCGCGCTCGTGGGTCAACAACGTGTGTGGCCATCGGCCTGTCCAGCGCCTT
CGTCGAGCCGCTGGAGTCCACCGGCATCTTCTCATCCAGCACGGCATCGAGCAGCTGGTGAAGAACTTCCCGGAC
GAGCGCTGGGACCCGGCCCTGGCCGACGACTACAACAACCGGGTCGCCGAGGTCCTGGACGGCGTCAAGGAGTT
CCTGGTCCTGCACTACAAGGCGGCGCAGCGCAGGACACCCGTAAGGAGGAGGCCAAGACCCGTGCCCTGCC
CGACGGGCTCGCCGAGCGCCTCGCCATCGGCACCTCGCACCTGCTCGACGAGCGCACCATCTACCAGCCGTACCAC
GGCTTCGAGCAGTACTCCTGGATCACGATGATGCTGGGCTCGGCCATGAGCCGGAGCGGCCGCGCCCTCCCTC
GCGCACATCGACCCGACGAACGCGCGCGCCGAGCTCGCGCGGCTCCGGGCGGACGCGGACGAGCTGGTCGCCGC
CCTGCCAGCTGCTACGAGTACATCGCCTCGCTCAACAGCTGA

```

Figure S34: DNA sequence of the tryptophan halogenase enzyme RebH.

References

- 1 Ahmed, Y.; Rebets, Y.; Estévez, M. R.; Zapp, J.; Myronovskyi, M.; Luzhetskyy, A., *Microbial cell factories* 2020, **19**, 1-16.
- 2 Myronovskyi, M.; Rosenkränzer, B.; Nadmid, S.; Pujic, P.; Normand, P.; Luzhetskyy, A., *Metabolic engineering* 2018, **49**, 316-324.
- 3 Shuai, H.; Myronovskyi, M.; Rosenkränzer, B.; Paulus, C.; Nadmid, S.; Stierhof, M.; Kolling, D.; Luzhetskyy, A., *ACS Chemical Biology* 2022, **17**, 598-608.
- 4 Flett, F.; Mersinias, V.; Smith, C. P., *FEMS microbiology letters* 1997, **155**, 223-229.
- 5 Grant, S. G.; Jessee, J.; Bloom, F. R.; Hanahan, D., *Proceedings of the National Academy of Sciences* 1990, **87**, 4645-4649.
- 6 Zhang, Y.; Muylers, J. P.; Testa, G.; Stewart, A. F., *Nature biotechnology* 2000, **18**, 1314-1317.
- 7 Baker Brachmann, C.; Davies, A.; Cost, G. J.; Caputo, E.; Li, J.; Hieter, P.; Boeke, J. D., *Yeast* 1998, **14**, 115-132.
- 8 Bilyk, O.; Sekurova, O. N.; Zotchev, S. B.; Luzhetskyy, A., *PloS one* 2016, **11**, e0158682.
- 9 Oberhäuser, P.; Myronovskyi, M.; Stierhof, M.; Gromyko, O.; Luzhetskyy, A., *Microbial Cell Factories* 2025, **24**, 1-11.
- 10 Gregory, M. A.; Till, R.; Smith, M. C., *Journal of bacteriology* 2003, **185**, 5320-5323.