

In the format provided by the authors and unedited.

A genetics-free method for high-throughput discovery of cryptic microbial metabolites

Fei Xu ^{1,3}, Yihan Wu^{1,3}, Chen Zhang¹, Katherine M. Davis ¹, Kyuho Moon¹, Leah B. Bushin¹ and Mohammad R. Seyedsayamdost ^{1,2*}

¹Department of Chemistry, Princeton University, Princeton, NJ, USA. ²Department of Molecular Biology, Princeton University, Princeton, NJ, USA.

³These authors contributed equally: Fei Xu, Yihan Wu. *e-mail: mrseyed@princeton.edu

Supplementary Table 1. HR-MS data for orfamides, canucins, keratinimicins, and keratinicyclins.^a

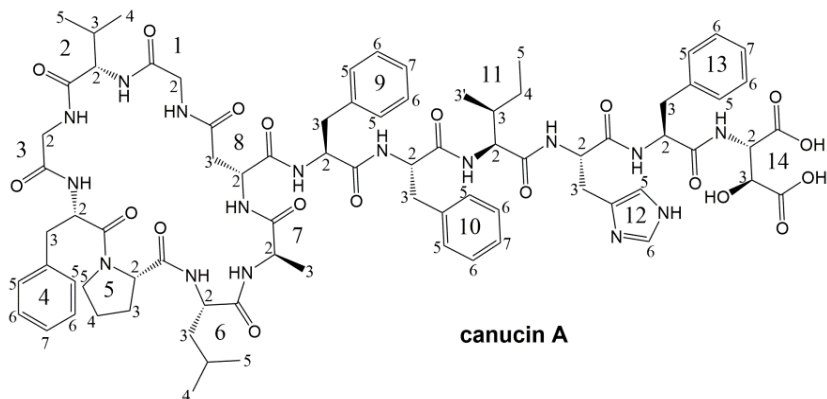
Orfamide	Formula	[M+H] ⁺ _{calc}	[M+H] ⁺ _{obs}	Δppm
A	C ₆₄ H ₁₁₄ N ₁₀ O ₁₇	1295.84362	1295.84380	0.1
B	C ₆₃ H ₁₁₂ N ₁₀ O ₁₇	1281.82797	1281.83038	1.9
Canucin	Formula	[M+H] ⁺ _{calc}	[M+H] ⁺ _{obs}	Δppm
A	C ₇₉ H ₁₀₂ N ₁₆ O ₁₉	1579.75799	1579.75592	1.3
B	C ₇₉ H ₁₀₂ N ₁₆ O ₁₈	1563.76308	1563.76242	0.4
Keratinimicin		[M+H] ⁺ _{calc}	[M+H] ⁺ _{obs}	Δppm
A	C ₈₄ H ₉₂ Cl ₂ N ₈ O ₃₃	1811.52166	1811.52091	0.4
B	C ₇₈ H ₈₃ Cl ₂ N ₉ O ₂₆	1632.48991	1632.49397	2.5
C	C ₈₄ H ₉₀ Cl ₂ N ₈ O ₃₂	1793.51109	1793.50422	3.8
D	C ₈₄ H ₈₉ Cl ₂ N ₇ O ₃₄	1810.49002	1810.48720	1.6
Keratinicyclin		[M+H] ⁺ _{calc}	[M+H] ⁺ _{obs}	Δppm
A	C ₇₇ H ₈₄ CIN ₇ O ₃₂	1654.49222	1654.49089	0.8
B	C ₇₇ H ₈₅ CIN ₈ O ₃₀	1637.51329	1637.51130	1.2
C	C ₇₁ H ₇₅ CIN ₈ O ₂₅	1475.46046	1475.45782	1.8

^a[M+H]⁺_{calc} and [M+H]⁺_{obs} denote calculated and observed [M+H]⁺, respectively. Δppm denotes the mass error in parts per million.

Supplementary Table 2. NMR assignments for canucin A in CD₃OH. The numbering scheme for canucin A is shown below the table.

Residue		δH^a	δC^b	Residue		δH^a	δC^b
Gly 1	1	-	169.9	Phe 9	1	-	ND
	2	4.35, 3.66	42.5		2	3.42	58.1
Val 2	NH	8.31	-		3	3.54, 3.11	34.7
	1	-	171.4		4	-	139.7
	2	4.09	56.7		5, 9	7.44	129.5
	3	2.07	33.3		6, 8	7.29	128.0
	4	0.88	18.3		7	7.20	126.0
Gly 3	5	0.90	16.0	Phe 10	NH	8.67	-
	NH	7.96	-		1	-	ND
	1	-	ND		2	3.84	61.2
	2	4.60, 3.756	42.3		3	3.70, 3.04	35.4
	NH	9.02	-		4	-	138.9
Phe 4	1	-	171.6		5, 9	7.18	126.4
	2	4.84	55.3		6, 8	7.25	128.2
	3	3.39, 2.88	35.2		7	7.33	126.5
	4	-	137.3		NH	7.80	-
	5, 9	7.37	128.5	Ile 11	1	-	171.5
	6, 8	7.33	128.3		2	4.31	58.7
	7	7.25	126.6		3	1.85	37.1
Pro 5	NH	8.75	-		3'	0.93	15.0
	1	-	ND		4	1.13, 0.70	23.1
	2	3.46	63.2		5	0.80	11.2
	3	2.54, 1.91	29.7		NH	8.59	-
	4	2.03, 1.92	24.9	His 12	1	-	172.1
Leu 6	5	3.88, 3.50	47.6		2	5.40	50.8
	1	-	173.0		3	2.92, 2.75	27.6
	2	4.10	57.7		4	-	ND
	3	1.70, 1.82	39.2		5	7.24	119.1
	4	1.24	25.4		6	8.67	133.3
	5	0.44	22.5	Phe 13	NH	8.47	-
	6	0.90	20.24		1	-	ND
Ala 7	NH	8.02	-		2	4.74	60.7
	1	-	172.1		3	4.26, 3.56	38.6
	2	4.51	50.0		4	-	137.0
	3	1.34	17.2		5, 9	7.45	130.0
Asp 8	NH	8.33	-		6, 8	7.25	130.1
	1	-	172.8		7	7.17	126.4
	2	4.56	48.0	β -OH-Asp 14	NH	8.26	-
	3	2.32	36.7		1	-	ND
	4	-	ND		2	4.73	58.1
	NH	7.35	-		3	4.35	71.3
					4	-	ND
					NH	8.00	-

^a800 MHz, ^bdetermined by edited HSQC and HMBC, ND: not detected.



Supplementary Table 3. Summary of CYANA-derived parameters from calculations of *R* and *S* stereoisomers at the C β of Asp14 in canucin A.^a

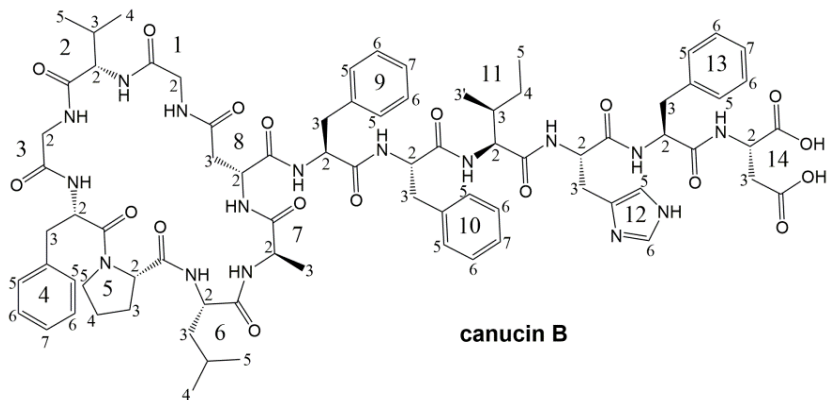
Parameter	R	S
# of distance constraints used for final structure calculation	189	183
average target function (<i>f</i>)	0.23 $\pm$ 0.00	0.17 $\pm$ 8.47E-05
average backbone RMSD to mean	0.03 Å $\pm$ 0.01	0.01 Å $\pm$ 0.00
average heavy atom RMSD to mean	0.39 Å $\pm$ 0.04	0.46 Å $\pm$ 0.05
# of violated distance constraints	3	3
# of violated van der Waals constraints	0	0

^aThe mean and standard deviation are listed for the parameters indicated.

Supplementary Table 4. NMR assignments for canucin B in CD₃OH. The numbering scheme for canucin B is shown below the table.

Residue		δH^a	δC^b	Residue		δH^a	δC^b
Gly	1	-	169.9	Phe	1	-	ND
	2	4.36, 3.66	42.5		2	3.42	58.1
Val	NH	8.44	-		3	3.54, 3.11	34.8
	1	-	174.1		4	-	139.8
	2	4.09	56.7		5, 9	7.44	129.5
	3	2.07	33.3		6, 8	7.29	128.1
	4	0.93	16.1		7	7.20	126.0
Gly	5	0.90	18.3	Phe	NH	8.66	-
	NH	8.02	-		1	-	ND
	1	-	171.5		2	3.82	61.3
	2	4.57, 3.72	42.3		3	3.70, 3.03	35.4
Phe	NH	9.00	-		4	-	138.1
	1	-	ND		5, 9	7.18	126.9
	2	4.84	55.3		6, 8	7.25	128.1
	3	3.37, 2.85	35.2		7	7.30	128.12
	4	-	137.3		NH	7.82	-
	5, 9	7.35	128.4	Ile	1	-	ND
	6, 8	7.31	128.3		2	4.31	58.7
Pro	7	7.23	126.6		3	1.86	37.0
	NH	8.68	-		3'	0.93	15.8
	1	-	171.5		4	1.11, 0.69	23.0
	2	3.50	63.2		5	0.79	11.2
	3	2.54, 1.91	29.7	His	NH	8.61	-
Leu	4	2.02	24.9		1	-	175.3
	5	3.88, 3.50	47.6		2	5.37	50.9
	1	-	172.1		3	2.92, 2.76	27.6
	2	4.13	57.7		4	-	ND
	3	1.75, 1.87	39.6		5	7.15	119.8
	4	1.28	25.4	Phe	6	8.53	133.3
Ala	5	0.50	22.6		NH	8.49	-
	6	0.91	20.2		1	-	173.9
	NH	8.08	-		2	4.76	60.5
	1	-	172.0		3	4.27, 3.50	38.2
Asp	2	4.55	50.0		4	-	136.9
	3	1.33	17.1		5, 9	7.31	129.9
	NH	8.38	-		6, 8	7.23	130.0
	1	-	ND		7	7.17	126.4
Asp	2	4.58	48.1	Asp	NH	8.28	-
	3	2.31	36.7		1	-	ND
	4	-	ND		2	4.29	50.9
	NH	7.34	-		3	2.94, 2.69	36.2
					4	-	ND
					NH	8.04	-

^a800 MHz, ^bdetermined by edited HSQC and HMBC, ND: not detected.



Supplementary Table 5. Annotation of the canucin biosynthetic gene cluster (*can*).^a

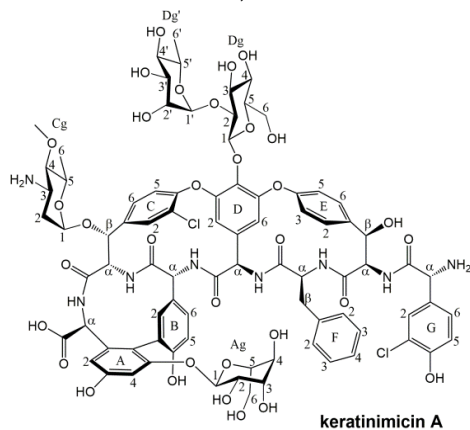
Gene	Length (AA)	Homology-Based Predicted Function
<i>canA</i>	40	leader/core peptide, putative Class II
<i>canB</i>	625	asparagine synthetase
<i>canC</i>	85	PqqD family protein
<i>canD</i>	137	transglutaminase-like superfamily protein
<i>canE</i>	283	TauD/TfdA family dioxygenase

^aGene name, the corresponding protein amino acid length, and a homology-predicted function are listed.

Supplementary Table 6. NMR assignments for keratinimicin A in DMSO-*d*₆. The numbering scheme for keratinimicin A is shown below the table.

C/H	δH^a	Multiplicity (Hz)	δC^b	C/H	δH^a	Multiplicity (Hz)	δC^b
NH	8.54	UM	-	NH	7.42	UM	-
COOH	-	-	173.2	COOH	-	-	169.5
α	4.42	d (6.1)	57.6	α	4.45	q (9.8)	56.4
1	-	-	137.7	β	2.69, 2.55	d (7.0, 9.7)	39.4
2	6.57	s	109.1	1	-	-	137.0
3	-	-	157.7	2	6.87	d (7.5)	129.5
4	6.69	s	102.0	3	6.91	t (7.4)	128.5
5	-	-	155.2	4	7.03	t (7.3)	126.8
6	-	-	120.8	NH	ND	-	-
NH	-	-	-	COOH	-	-	174.3
COOH	-	-	173.2	α	4.41	d (3.9)	59.0
α	4.48	d (3.9)	54.2	1	-	-	ND
1	-	-	ND	2	7.44	s	129.3
2	7.18	s	136.2	3	-	-	119.2
3	-	-	120.8	4	-	-	152.9
4	-	-	155.8	5	6.94	d (8.0)	116.7
5	6.80	UM	116.2	6	7.21	UM	127.8
6	6.80	UM	126.2	1	5.18	UM	98.1
NH	6.92	-	-	2	3.19	t (4.1)	70.4
COOH	-	-	167.0	3	3.22	t (8.9)	70.7
α	4.23	d (11.4)	61.5	4	3.39	t (9.2)	66.9
β	5.11	UM	74.8	5	3.49	UM	74.2
1	-	-	138.8	6	3.49, 3.45	UM	61.2
2	7.89	s	129.3	1	4.67	UM	94.2
3	-	-	127.5	2	2.09, 1.48	UM	37.2
4	-	-	150.0	3	3.23	m	47.2
5	7.32	d (8.3)	124.2	3-NH ₂	ND	-	-
6	7.36	d (8.3)	127.8	4	2.57	t (9.6)	86.5
NH	8.19	UM	-	4-OMe	3.33	s	58.
COOH	-	-	170.3	5	3.64	m	67.3
α	5.62	d (8.5)	54.6	6	1.17	d (5.7)	18.7
1	-	-	133.7	1	5.67	d (7.4)	99.8
2	5.17	s	104.9	2	3.62	t (8.2)	77.1
3	-	-	151.4	3	3.46	t (8.6)	78.2
4	-	-	132.4	4	3.22	t (8.9)	70.8
5	-	-	153.1	5	3.29	m (4.6, 10.2)	78.1
6	5.65	s	108.4	6	3.72	d (10.2)	61.4
NH	ND	-	-	1'	5.18	UM	100.6
COOH	-	-	168.5	2'	3.71	t (3.2)	71.1
α	4.52	t (3.8)	59.7	3'	3.5	t (10.2)	71.1
β	5.2	d (4.1)	71.5	4'	3.2	t (10.2)	72.2
1	-	-	137.6	5'	4.12	m (6.2)	68.8
2	7.78	d (8.3)	128.2	6'	1.08	d (6.0)	18.5
3	7.22	d (6.3)	122.9				
4	-	-	155.2				
5	7.13	d (8.4)	124.1				
6	7.05	d (8.4)	128.6				

^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.



Supplementary Table 7. Annotation of the keratinimicin biosynthetic gene cluster (*ker*).

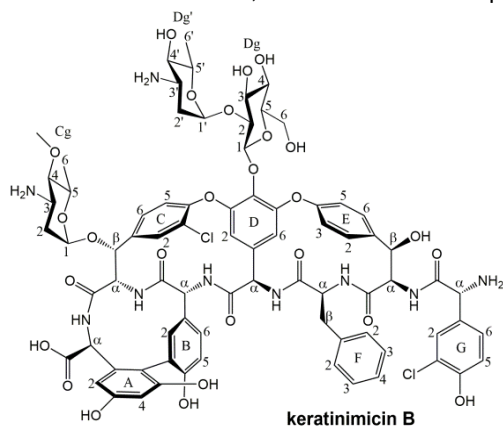
Gene	Length (AA)	Homology-Based Predicted Function
<i>vanH</i>	291	D-lactate dehydrogenase
<i>vanA</i>	348	D-alanine--(R)-lactate ligase
<i>vanX</i>	202	D-Ala-D-Ala dipeptidase VanX
<i>ajrR</i>	340	StrR-like transcriptional regulator
<i>pdh</i>	303	prephenate dehydrogenase
<i>tri</i>	644	ABC transporter
<i>kerA</i>	2082	NRPS (A-T-C-A-T-E)
<i>kerB</i>	1055	NRPS (C-A-T)
<i>kerC</i>	4059	NRPS (C-A-T-E-C-A-T-E-C-A-T)
<i>kerD</i>	1864	NRPS (C-A-T-X-TE)
<i>mbtH</i>	69	MbtH protein
<i>oxyA</i>	401	cytochrome P450
<i>ker1</i>	61	hypothetical protein
<i>oxyB</i>	398	cytochrome P450
<i>oxyC</i>	406	cytochrome P450
<i>khal</i>	491	Halogenase
<i>gtfA</i>	363	Glycosyltransferase
<i>gtfB</i>	408	Glycosyltransferase
<i>kerM</i>	309	O-methyltransferase
<i>gtfC</i>	408	Glycosyltransferase
<i>ker2</i>	270	PIG-L family deacetylase
<i>gtfD</i>	623	Mannosyltransferase
<i>hpgT</i>	434	Aminotransferase
<i>kph</i>	276	α/β fold hydrolase
<i>kerE</i>	577	AMP-dependent synthetase and ligase
<i>oxyD</i>	396	cytochrome P450
<i>hmaS</i>	350	4-hydroxyphenylpyruvate dioxygenase
<i>hmo</i>	358	4-hydroxymandelate oxidase
<i>ker3</i>	448	cation/H(+) antiporter
<i>kraA</i>	476	NDP-hexose 2,3-dehydratase
<i>kraB</i>	325	NAD-dependent epimerase/dehydratase
<i>kraC</i>	369	Aminotransferase
<i>kraD</i>	205	dTDP-4-keto-6-deoxy-D-glucose epimerase
<i>dpgA</i>	366	type III polyketide synthase
<i>dpgB</i>	209	enoyl-CoA hydratase
<i>dpgC</i>	404	enoyl-CoA hydratase
<i>dpgD</i>	263	enoyl-CoA hydratase
<i>dahp</i>	358	3-deoxy-7-phosphoheptulonate synthase
<i>ker4</i>	188	hypothetical protein

^aGene name, the corresponding protein amino acid length, and a homology-predicted function are listed.

Supplementary Table 8. NMR assignments for keratinimicin B in DMSO-*d*6. The numbering scheme for keratinimicin B is shown below the table.

C/H	δH^a	Multiplicity (Hz)	δC^b	C/H	δH^a	Multiplicity (Hz)	δC^b
A	NH	8.57	UM	-	NH	7.58	d (8.6)
	COOH	-	-	-	COOH	-	-
	α	4.46	d (4.7)	-	α	4.33	q (8.0)
	1	-	-	-	β	2.75, 2.56	d (8.4, 7.8)
	2	6.29	s	-	1	-	-
	3	-	-	-	2	6.83	UM
	4	6.42	s	-	3	6.83	UM
	5	-	-	-	4	6.93	t (6.8)
	6	-	-	-	NH	7.52	UM
	NH	8.51	UM	-	COOH	-	-
B	COOH	-	-	-	α	4.82	UM
	α	4.51	d (3.4)	-	1	-	-
	1	-	-	-	2	7.52	s
	2	7.23	S	-	3	-	-
	3	-	-	-	4	-	-
	4	-	-	-	5	7.04	d (9.0)
	5	6.78	UM	-	6	7.34	UM
	6	6.77	UM	-	1	4.75	UM
	NH	7.32	d (8.6)	-	2	2.24, 1.76	t (3.2)
	COOH	-	-	-	3	3.57	m
C	α	4.27	t (9.0)	-	3-NH ₂	ND	-
	β	5.1	U.S.	-	4	2.98	t (7.4)
	1	-	-	-	4-OMe	3.35	s
	2	7.91	s	-	5	3.67	m
	3	-	-	-	6	1.19	d (5.4)
	4	-	-	-	1	5.67	d (7.4)
	5	7.32	d (8.6)	-	2	3.61	t (7.9)
	6	7.34	UM	-	3	3.51	t (6.1)
	NH	8.06	d (7.5)	-	4	3.25	t (9.0)
	COOH	-	-	-	5	3.31	m
D	α	5.62	d (8.4)	-	6	3.72	d (10.8)
	1	-	-	-	1'	5.41	UM.
	2	5.15	s	-	2'	2.19, 1.76	d (3.6)
	3	-	-	-	3'	3.21	UM
	4	-	-	-	3'-NH ₂	ND	-
	5	-	-	-	4'	3.16	t (9.2)
	6	5.67	s	-	5'	4.28	M
	NH	ND	-	-	6'	1.14	d (6.0)
	COOH	-	-	-			
	α	4.73	t (3.4)	-			
E	β	5.09	UM	-			
	1	-	-	-			
	2	7.79	d (8.2)	-			
	3	7.21	UM	-			
	4	-	-	-			
	5	7.11	d (7.8)	-			
	6	7.02	d (8.8)	-			

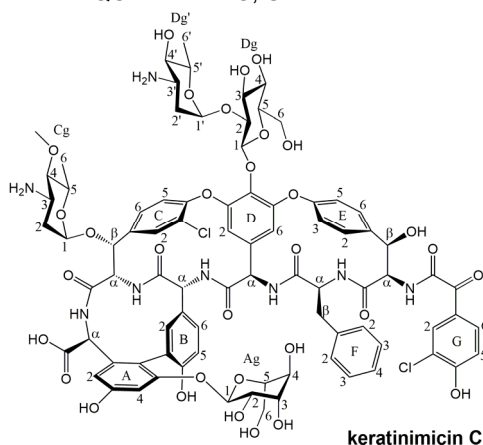
^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.



Supplementary Table 9. NMR assignments for keratinimicin C in DMSO-*d*₆. The numbering scheme for keratinimicin C is shown below the table.

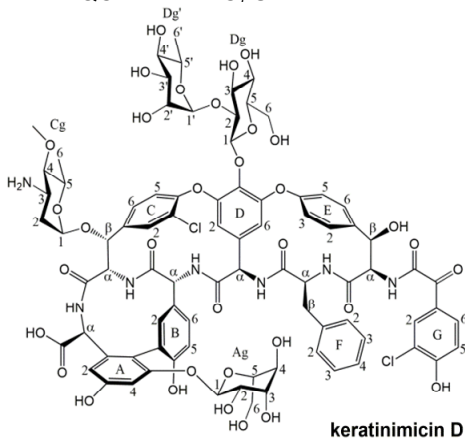
C/H	δH^a	Multiplicity (Hz)	δC^b	C/H	δH^a	Multiplicity (Hz)	δC^b
NH	8.47	UM	-	NH	7.3	-	-
COOH	-	-	173.3	COOH	-	-	169.5
α	4.42	d (6.1)	57.6	α	4.5	q (9.2)	55.5
1	-	-	137.7	β	2.61, 2.57	d (6.9, 7.0)	40.0
2	6.53	s	109.0	1	-	-	136.3
3	-	-	157.6	2	6.79	d (7.7)	129.1
4	6.66	s	102.7	3	6.86	t (7.3)	128.3
5	-	-	155.1	4	6.99	t (7.2)	126.4
6	-	-	121.2	COOH	-	-	172.0
NH	8.5	UM	-	CO	-	-	183.2
COOH	-	-	174.5	1	-	-	124.5
α	4.45	UM	54.1	2	7.98	s	131.9
1	-	-	ND	3	-	-	115.6
2	7.22	s	136.2	4	-	-	152.9
3	-	-	121.5	5	6.34	d (6.6)	119.6
4	-	-	155.8	6	7.69	d (8.5)	132.9
5	6.82	d (8.2)	115.9	1	5.15	UM	98.0
6	6.8	UM	125.8	2	3.11	UM	70.0
NH	7.19	UM	-	3	3.16	t (8.2)	70.6
COOH	-	-	167.2	4	3.36	t (9.4)	66.9
α	4.23	t (7.5)	61.23	5	3.49	t (7.7)	74.1
β	5.07	UM	74.8	6	3.45, 3.49	UM	61.2
1	-	-	138.7	1	4.72	UM	93.4
2	7.86	s	129.1	2	2.16, 1.63	UM	35.0
3	-	-	127.7	3	3.5	t (8.8)	46.9
4	-	-	149.9	3-NH ₂	ND	-	-
5	7.3	d (7.8)	123.9	4	2.78	UM	83.5
6	7.32	d (10.5)	127.3	4-OMe	3.33	s	58.1
NH	7.79	d (7.6)	-	5	3.67	UM	67.0
COOH	-	-	170.5	6	1.18	d (5.7)	18.3
α	5.61	d (9.4)	54.1	1	5.63	d (7.5)	99.6
1	-	-	133.5	2	3.59	t (8.2)	77.3
2	5.1	s	104.3	3	3.48	t (7.7)	77.5
3	-	-	151.6	4	3.21	t (8.7)	70.4
4	-	-	132.2	5	3.31	UM	77.8
5	-	-	153.2	6	3.72, 3.45	d (10.5)	61.3
6	5.58	s	108.1	1'	5.39	UM	95.5
NH	7.62	d (7.9)	-	2'	2.14, 1.67	UM	34.3
COOH	-	-	167.8	3'	3.16	m (8.2)	49.5
α	4.77	t (3.8)	59.3	3'-NH ₂	ND	-	-
β	5.24	d (2.6)	71.2	4'	3.06	UM	73.1
1	-	-	137.3	5'	4.23	UM	68.4
2	7.62	d (7.9)	128.1	6'	1.08	d (6.0)	18.5
3	7.2	d (7.8)	122.7				
4	-	-	155.2				
5	7.13	d (7.9)	123.9				
6	7.07	d (8.1)	128.4				

^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.



Supplementary Table 10. NMR assignments for keratinimicin D in DMSO-*d*₆. The numbering scheme for keratinimicin D is shown below the table.

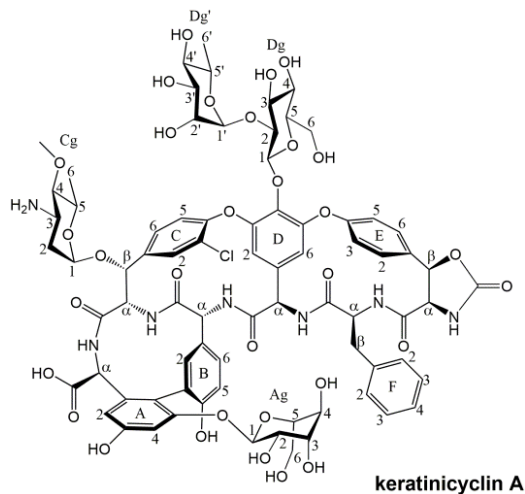
	C/H	δH^a	Multiplicity (Hz)	δC^b		C/H	δH^a	Multiplicity (Hz)	δC^b
A	NH	8.32	UM	-	F	NH	ND	-	-
	COOH	-	-	ND		COOH	-	-	ND
	α	4.43	d (5.1)	57.3		α	4.5	q (8.2)	55.8
	1	-	-			β	2.59	d (6.3)	40.3
	2	6.48	s	108.6		1	-	-	ND
	3	-	-			2	6.78	d (7.6)	129.3
	4	6.69	s	103.2		3	6.84	t (7.5)	128.5
B	5	-	-	ND	4	6.98	t (7.4)	126.6	
	6	-	-	ND	COOH	-	-	ND	
	NH	8.55	UM	-	CO	-	-	ND	
	COOH	-	-	ND	1	-	-	ND	
	α	4.44	UM	54.3	2	7.96	s	131.4	
	1	-	-	-	3	-	-	ND	
	2	7.23	s	136.2	4	-	-	ND	
C	3	-	-	ND	5	6.3	d (6.3)	119.8	
	4	-	-	ND	6	7.67	d (8.6)	128.3	
	5	6.83	d (7.5)	116.2	1	5.16	UM	98.3	
	6	6.81	d (8.8)	126.2	2	3.12	UM	70.2	
	NH	ND	-	-	3	3.2	t (8.7)	70.8	
	COOH	-	-	ND	4	3.37	UM	67.1	
	α	4.25	d (11.5)	61.5	5	3.48	t (7.7)	74.3	
D	β	5.07	UM	74.4	6	3.47	UM	61.4	
	1	-	-	ND	1	4.73	UM	93.7	
	2	7.86	s	129.3	2	2.16, 1.61	UM	35.3	
	3	-	-	ND	3	3.49	UM	47.3	
	4	-	-	ND	3-NH ₂	ND	-	-	
	5	7.28	d (8.3)	124.1	4	2.55	UM	86.7	
	6	7.32	d (8.4)	127.5	4-OMe	3.34	s	58.5	
E	NH	ND	-	-	5	3.65	m	67.3	
	COOH	-	-	ND	6	1.19	d (5.6)	18.5	
	α	5.59	d (9.0)	54.3	1	5.63	d (7.4)	99.8	
	1	-	-	ND	2	3.59	t (8.3)	77.6	
	2	5.09	s	104.4	3	3.45	UM	77.8	
	3	-	-	ND	4	3.15	d (9.1)	70.9	
	4	-	-	ND	5	3.31	m	78.1	
F	5	-	-	ND	6	3.73, 3.44	UM	61.5	
	6	5.55	s	108.3	1'	5.13	UM	100.8	
	NH	7.83	d (8.5)	-	2'	3.72	UM	71.0	
	COOH	-	-	ND	3'	3.48	UM	71.1	
	α	4.77	dd (8.4,4.6)	59.6	4'	3.21	t (9.4)	72.4	
	β	5.23	d (3.3)	71.4	5'	4.08	m	68.8	
	1	-	-	ND	6'	1.08	d (6.1)	18.4	
G	2	7.61	d (8.5)	128.3					
	3	7.21	dd (7.8,1.6)	123.0					
	4	-	-	ND					
	5	7.13	dd (7.8,1.7)	124.1					
	6	7.06	d (8.3)	128.7					

^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.

Supplementary Table 11. NMR assignments for keratinicyclin A in DMSO-*d*₆. The numbering scheme for keratinicyclin A is shown below the table.

C/H	δH^a	Multiplicity (Hz)	δC^b	C/H	δH^a	Multiplicity (Hz)	δC^b
NH	8.7	d (4.4)	-	NH	8.16	UM	-
COOH	-	-	173.2	COOH	-	-	166.5
α	4.49	d (5.8)	57.3	α	4.74	UM	62.8
1	-	-	137.3	β	5.93	d (8.4)	77.1
2	6.52	s	108.9	1	-	-	137.6
3	-	-	158.0	2	7.29	d (8.5)	127.5
4	6.71	s	102.7	3	7.00	d (8.8)	123.5
5	-	-	155.6	4	-	-	158.7
6	-	-	121.5	5	7.22	d (8.0)	122.0
NH	8.65	d (3.5)	-	6	7.12	d (8.1)	128.8
COOH	-	-	ND	NHCO	-	-	160.1
α	4.59	d (3.9)	54.2	NH	7.84	d (9.2)	-
1	-	-	132.8	COOH	-	-	169.5
2	7.23	s	136.4	α	4.34	q (7.8, 9.2)	57.6
3	-	-	119.2	β	2.75, 2.58	d (10.7)	39.9
4	-	-	156.0	1	-	-	137.0
5	6.84	d (8.1)	116.2	2	6.85	d (7.4)	129.9
6	6.82	UM	126.3	3	6.8	t (7.4)	128.5
NH	7.2	-	-	4	6.97	t (7.2)	127.1
COOH	-	-	167.7	1	5.2	UM	98.3
α	4.29	d (11.3)	61.6	2	3.18	t (3.1)	70.4
β	5.11	UM	75.2	3	3.24	t (3.6, 9.7)	71.0
1	-	-	138.8	4	3.39	t (9.7)	67.1
2	7.89	s	129.5	5	3.48	UM	74.4
3	-	-	127.5	6	3.49, 3.45	d (7.5)	61.2
4	-	-	150.0	1	4.76	t (4.0)	93.9
5	7.26	d (8.2)	124.0	2	2.17, 1.65	UM	35.2
6	7.35	d (8.0)	128.1	3	3.52	m (8.3)	47.3
NH	8.13	d (8.5)	-	3-NH ₂	ND	-	-
COOH	-	-	170.2	4	2.8	t (9.9)	83.7
α	5.56	d (8.6)	54.1	4-OMe	3.35	s	58.6
1	-	-	133.7	5	3.68	m (6.0)	67.3
2	5.11	s	105.0	6	1.2	d (5.5)	18.8
3	-	-	151.4	1	5.63	d (7.5)	99.5
4	-	-	132.4	2	3.59	t (8.4)	77.7
5	-	-	153.1	3	3.41	t (9.0)	78.1
6	5.51	s	112.6	4	3.14	t (9.0)	71.1
				5	3.22	m (8.7)	78.7
				6	3.72, 3.37	d (9.4, 6.2)	62.0
				1'	5.13	UM	101.2
				2'	3.71	t (3.3)	71.0
				3'	3.49	t (9.2)	71.4
				4'	3.2	t (9.4)	72.5

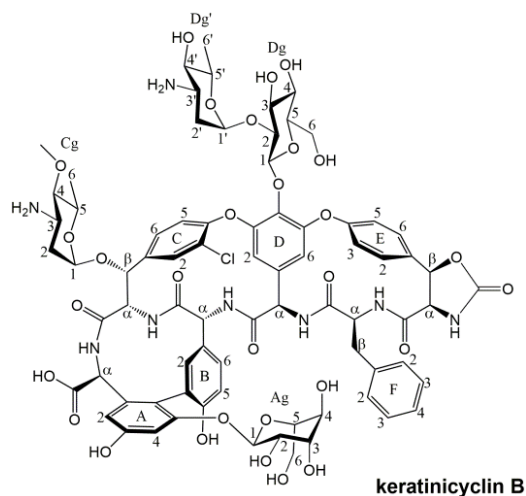
^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.



Supplementary Table 12. NMR assignments for keratinicyclin B in DMSO-*d*₆. The numbering scheme for keratinicyclin B is shown below the table.

C/H	δH^a	Multiplicity (Hz)	δC^b	C/H	δH^a	Multiplicity (Hz)	δC^b
A	NH	8.67	d (4.8)	-	NH	8.19	UM
	COOH	-	-	-	COOH	-	-
	α	4.49	d (5.8)	-	α	4.75	UM
	1	-	-	-	β	5.93	d (8.4)
	2	6.29	s	-	1	-	-
	3	-	-	-	2	7.3	dd (1.5,9.1)
B	4	6.42	s	-	3	7.00	dd (1.6,9.1)
	5	-	-	-	4	-	-
	6	-	-	-	5	7.22	d (7.1)
	NH	8.64	d (3.2)	-	6	7.14	dd (1.4,7.4)
	COOH	-	-	-	NHCO	-	-
	α	4.59	d (3.4)	-	NH	7.85	d (9.1)
C	1	-	-	-	COOH	-	-
	2	7.23	s	-	α	4.35	q (8.7)
	3	-	-	-	β	2.76, 2.6	t (10.3), d (7.1)
	4	-	-	-	1	-	-
	5	6.84	d (8.9)	-	2	6.86	d (7.7)
	6	6.82	dd (2.8,9.0)	-	3	6.8	t (7.3)
D	NH	ND	-	-	4	6.98	t (7.2)
	COOH	-	-	-	1	5.19	UM
	α	4.29	d (10.6)	-	2	3.18	t (3.0)
	β	5.13	UM	-	3	3.25	t (9.0)
	1	-	-	-	4	3.4	t (9.3)
	2	7.9	s	-	5	3.5	m
E	3	-	-	-	6	3.48, 3.46	UM
	4	-	-	-	1	4.75	UM
	5	7.28	d (8.2)	-	2	2.19, 1.66	UM
	6	7.35	d (7.4)	-	3	3.53	m
	NH	8.13	d (8.6)	-	3-NH ₂	ND	-
	COOH	-	-	-	4	2.83	t (9.6)
F	α	5.57	d (8.6)	-	4-OMe	3.34	s
	1	-	-	-	5	3.69	m
	2	5.13	s	-	6	1.2	d (5.3)
	3	-	-	-	1	5.64	d (7.4)
	4	-	-	-	2	3.59	t (8.4)
	5	-	-	-	3	3.43	t (9.4)
G	6	5.53	s	-	4	3.16	t (9.0)
				-	5	3.23	m
				-	6	3.72, 3.39	d (10.8)
				-	1'	5.37	UM
				-	2'	2.14, 1.69	UM
				-	3'	3.2	m
H				-	3'-NH ₂	ND	-
				-	4'	3.06	t (8.4)
				-			
				-			
				-			
				-			

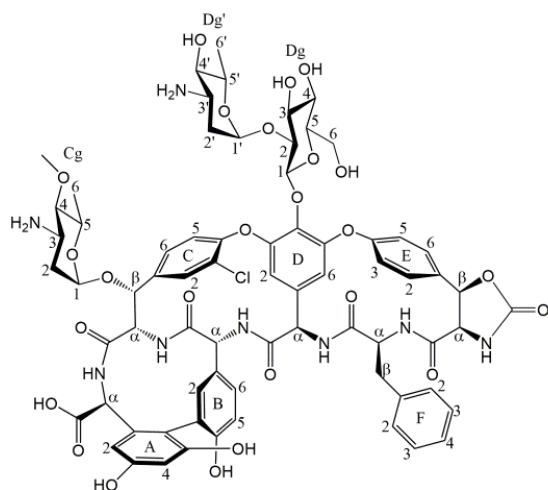
^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.



Supplementary Table 13. NMR assignments for keratinicyclin C in DMSO-*d*₆. The numbering scheme for keratinicyclin C is shown below the table.

C/H	δH^a	Multiplicity (Hz)	δC^b	C/H	δH^a	Multiplicity (Hz)	δC^b
NH	8.52	UM	-	NH	8.14	UM	-
COOH	-	-	173.3	COOH	-	-	166.2
α	4.44	d (5.8)	57.7	α	4.75	d (9.0)	62.6
1	-	-	137.3	β	5.93	d (8.2)	76.8
2	6.34	s	106.6	1	-	-	132.4
3	-	-	157.5	2	7.31	dd (2.1,8.5)	127.5
4	6.39	s	102.7	3	7.02	d (3.1,9.0)	123.4
5	-	-	ND	4	-	-	158.7
6	-	-	118.6	5	7.22	dd (2.3)	121.8
NH	8.75	d (3.4)	-	6	7.12	dd (1.7,8.2)	128.6
COOH	-	-	ND	NHCO	-	-	159.7
α	4.58	d (4.3)	54.1	NH	7.84	d (9.8)	-
1	-	-	126.4	COOH	-	-	169.5
2	7.27	s	136.6	α	4.31	q (7.8, 9.2)	57.6
3	-	-	122.4	β	2.76, 2.60	d (10.6)	39.9
4	-	-	155.9	1	-	-	137.0
5	6.76	d (8.6)	116.5	2	6.88	d (7.5)	129.6
6	6.82	dd (1.8,8.6)	125.7	3	6.94	t (7.6)	128.5
NH	6.91	-	-	4	7.02	t (7.4)	126.7
COOH	-	-	ND	1	4.69	UM	94.0
α	4.32	t (8.7)	61.5	2	2.12, 1.54	UM	36.7
β	5.13	UM	74.8	3	3.31	dd (10.2)	47.3
1	-	-	138.8	3-NH2	ND	-	-
2	7.93	s	129.4	4	2.65	t (9.7)	85.7
3	-	-	127.5	4-OMe	3.34	s	58.3
4	-	-	149.9	5	3.65	UM	67.8
5	7.26	d (8.6)	124.1	6	1.18	d (5.8)	18.7
6	7.38	dd (1.7,8.0)	127.9	1	5.65	d (7.6)	99.2
NH	8.24	d (8.7)	-	2	3.59	t (8.1)	77.5
COOH	-	-	170.0	3	3.43	UM	78.0
α	5.64	d (8.9)	54.2	4	3.15	t (9.5)	70.8
1	-	-	134.5	5	3.23	m (6.0)	78.5
2	5.17	s	104.8	6	3.73, 3.38	d (10.3)	61.9
3	-	-	ND	1'	5.35	UM	96.1
4	-	-	133.1	2'	2.1, 1.6	UM	35.4
5	-	-	ND	3'	3.11	UM	49.8
6	5.52	s	112.4	3'-NH2	ND	-	-
				4'	2.94	t (10.1)	74.5
				5'	4.17	m (6.4,8.8)	68.5

^a800 MHz, ^bdetermined by edited HSQC and HMBC, UM: unresolved multiplicity, ND: not detected.



keratinicyclin C

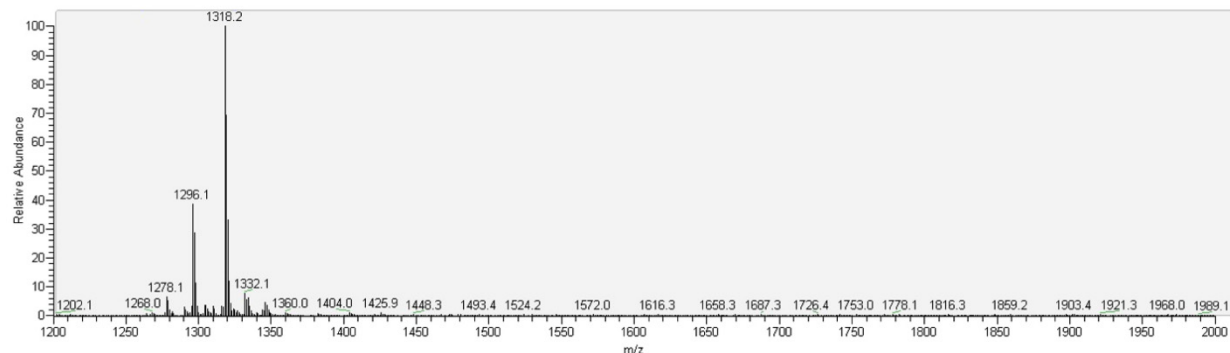
Supplementary Table 14. MIC values (in μM) for keratinimicins and keratinicyclin B against select pathogenic Gram-negative bacteria and viruses.

Strain	Keratini- micin A	Keratini- micin C	Keratini- cyclin B	Control drug ^a
<u>Bacterium</u>				
<i>E. coli</i>	>35	>35	>39	>44 (V), 0.1 (C)
<i>K. pneumoniae</i>	>35	>35	>39	>44 (V), 0.1 (C)
<i>E. cloacae</i>	>35	>35	>39	>44 (V), 0.1 (C)
<i>P. aeruginosa</i>	>35	>35	>39	>44 (V), 3.0 (C)
<i>A. baumannii</i>	>35	>35	>39	>44 (V), 3.0 (C)
<i>V. cholerae</i>	>35	>35	>39	>44 (V), 0.8 (C)
<i>B. fragilis</i>	>35	>35	>39	11.7 (M)
<u>Virus</u>				
<i>Influenza B</i>	— ^b	—	>92	6.1 (O)
<i>HSV-1</i>	—	—	>92	97 (A)
<i>HSV-2</i>	—	—	>92	32 (A)
<i>Vaccinia</i>	—	—	>92	—
<i>Rhinovirus</i>	—	—	>92	204 (R)

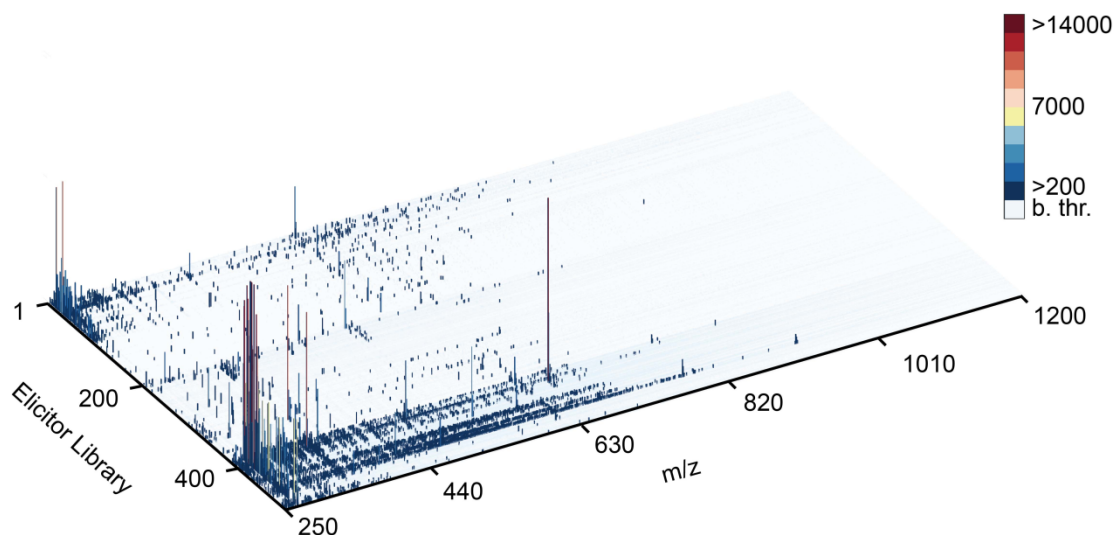
^aControl drugs are abbreviated as follows: V, vancomycin; C, ciprofloxacin, M, metronidazole; O, oseltamivir; A, acyclovir; R, ribavirin.

^bNot determined.

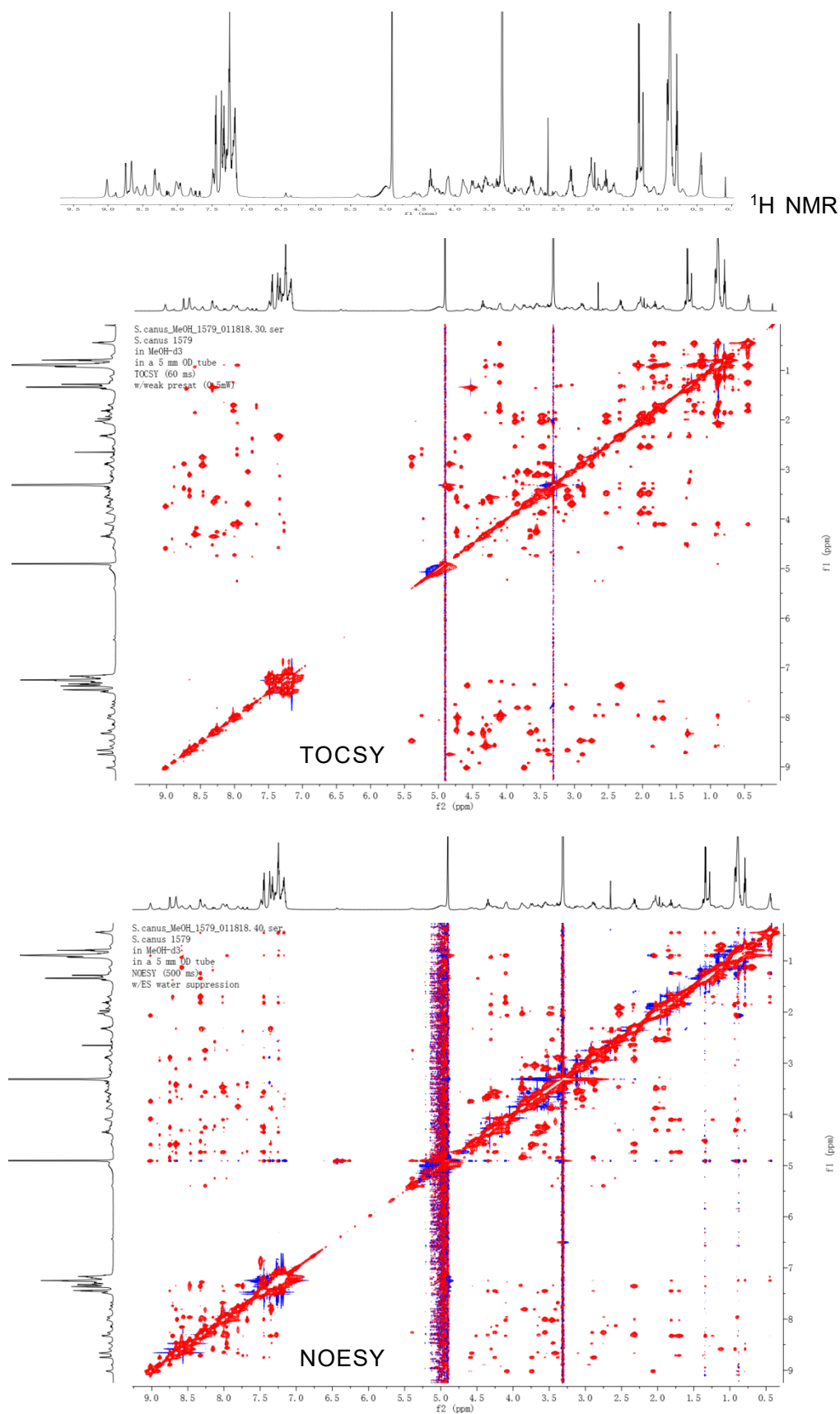
Supplementary Fig. 1. Direct LAESI-IMS output from a well containing orfamide. For each strain, 502 such spectra were compiled (one per elicitor), the observed signals extracted using GMSU-LAESI software, which gave all m/z values and the corresponding intensities per well, and the three-dimensional dataset (elicitor vs. m/z vs. intensity) then plotted in MatLab (see Supplementary Methods for details).

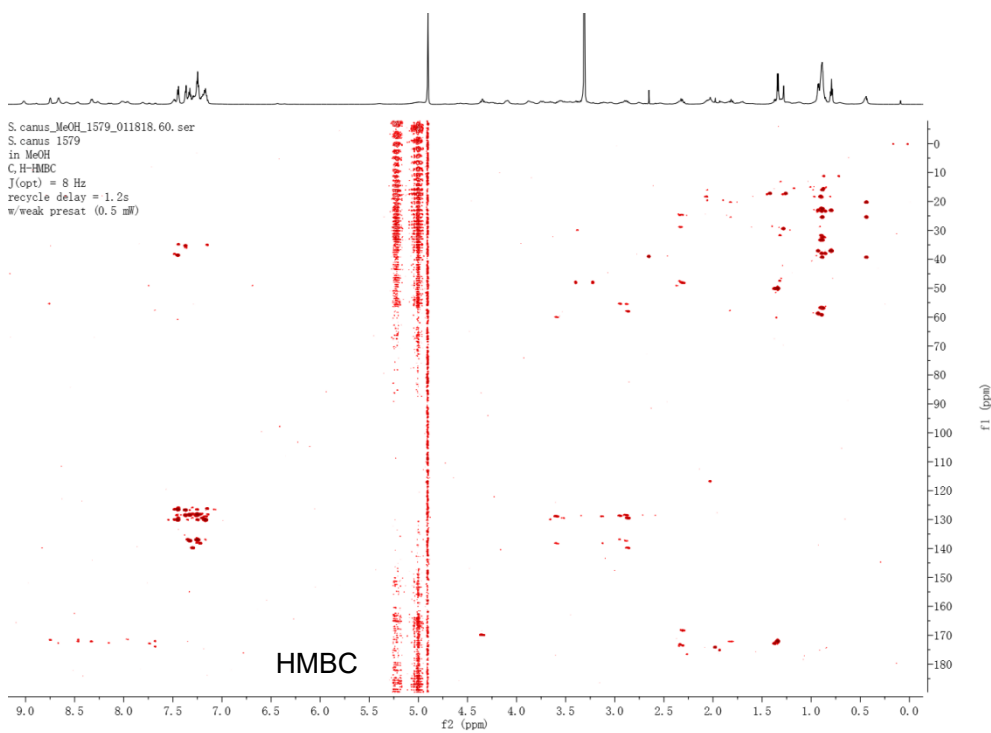
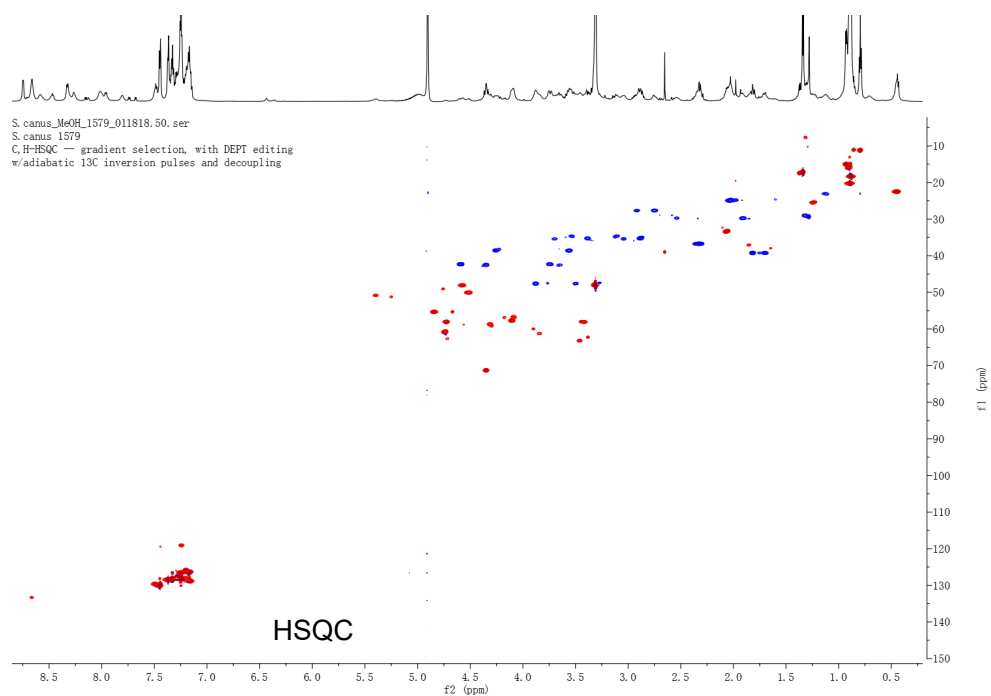


Supplementary Fig. 2. Secondary metabolome of *S. canus*, as detected by LAESI-MS in response to 502 elicitors in the m/z range of 250-1200. b. thr. designates below threshold of detection. The HiTES-IMS screen was carried out in a single replicate.

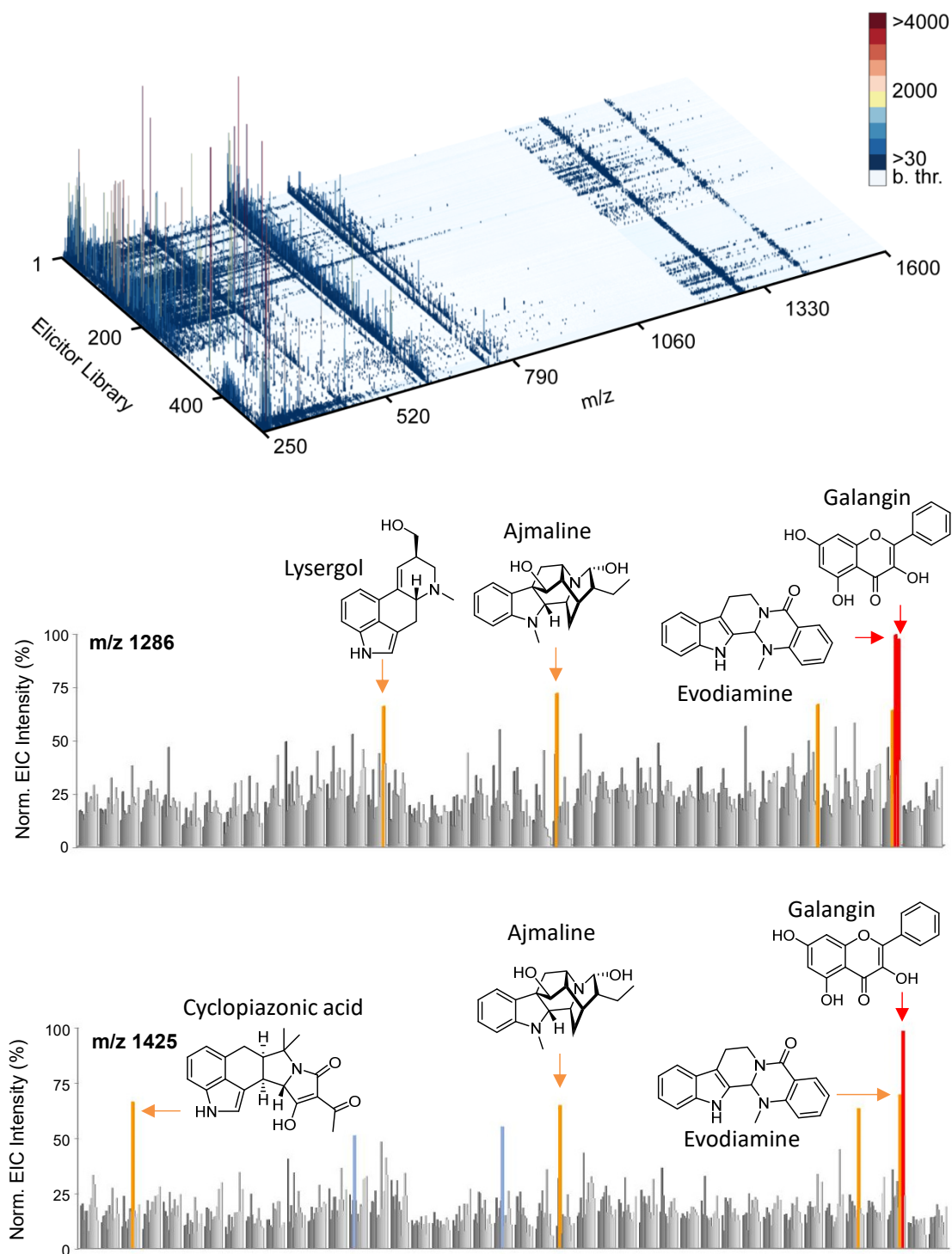


Supplementary Fig. 3. NMR spectra of canucin A in CD₃OH. Full 1D/2D NMR datasets on canucins were collected once.

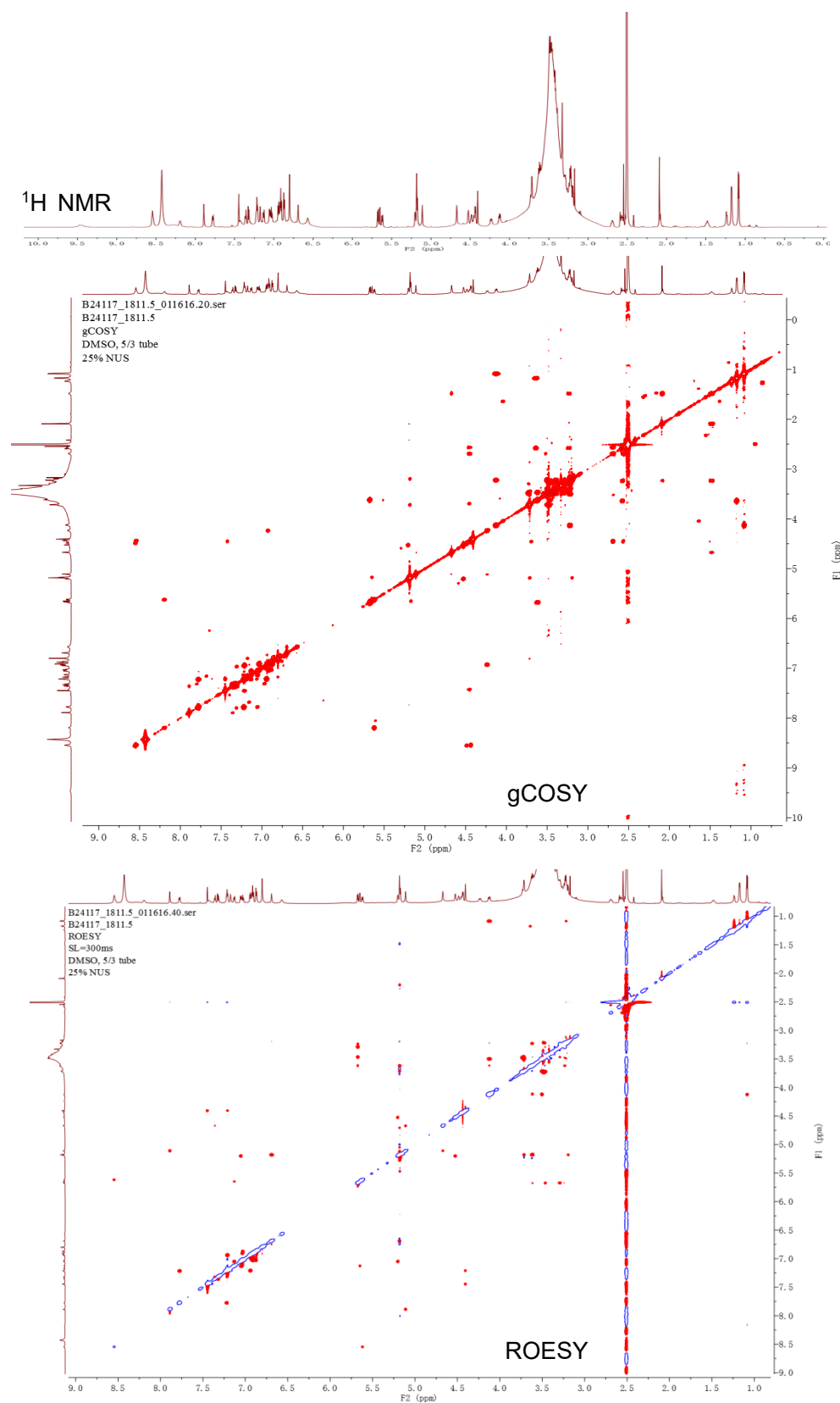


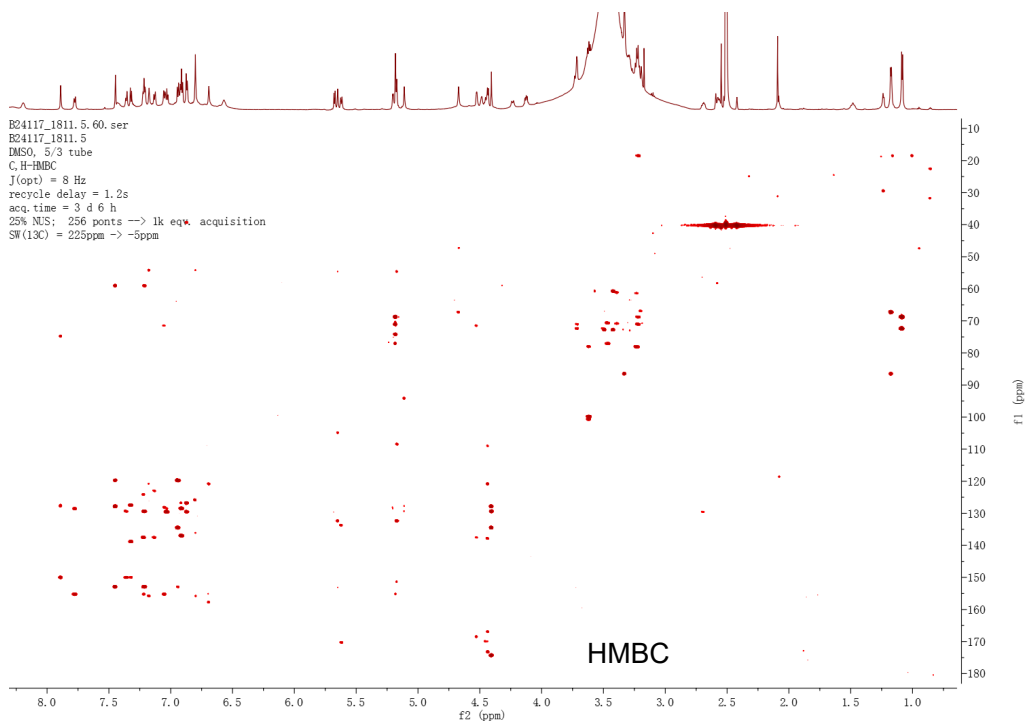
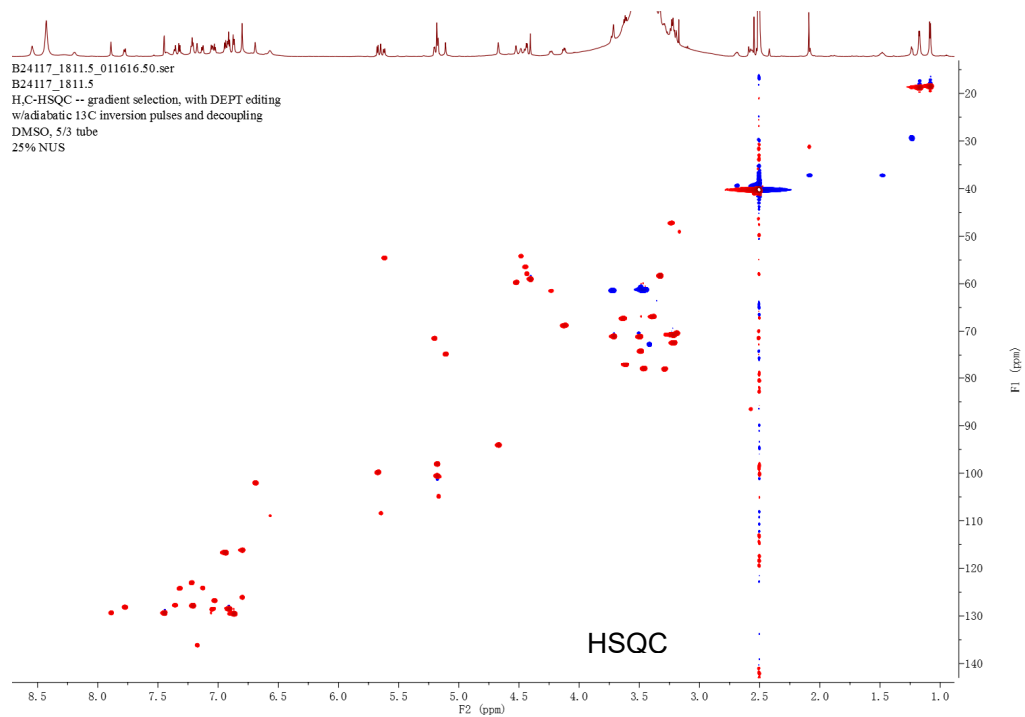


Supplementary Fig. 4. Secondary metabolome of *A. keratiniphila* as detected by LAESI-MS in response to 502 elicitors. (Top) Shown is the m/z range 250-1600; b. thr. designates below threshold of detection. Note that a lower threshold was used for signals in the range of m/z 1100-1500 (as described in Supplementary Methods) to highlight production of two cryptic metabolites with m/z 1286 and 1425). 2D components of the 3D plot focusing on m/z 1286 (middle) and 1425 (bottom) are also shown along with the best elicitors. The HiTES-IMS screen was carried out in a single replicate.



Supplementary Fig. 5. NMR spectra of keratinimicin A in DMSO-*d*₆. Full 1D/2D NMR datasets on keratinimicins were collected once.





Supplementary Fig. 6. NMR spectra of keratinicyclin A in DMSO-*d*₆. Full 1D/2D NMR datasets on keratinicyclins were collected once.

