Identification of a New Antimicrobial, Desertomycin H, Utilizing a Modified Crowded Plate Technique

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1. Comparison of antibiotic discovery methods

Table S1 Efficiency of the modified crowded plate technique (mCPT)**.** Comparison of isolation techniques for identifying antibiotic producers effective against *B. subtilis* using the spread-patch assay: random selection, selection of *Streptomyces* sp., Waksman's crowded plate technique (CPT), and modified crowded plate technique (mCPT).

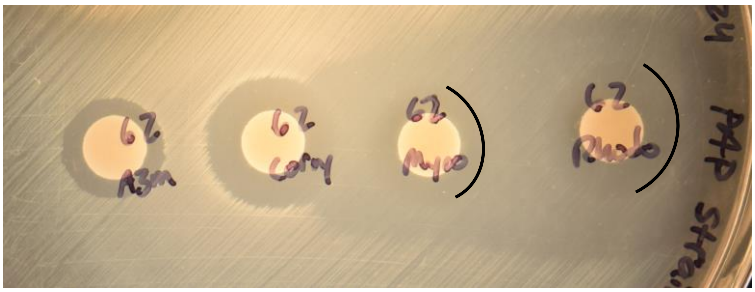
Screening method	Number screened	Number of producers	Efficiency (%)
Random	210	13	6.2%
Streptomycete isolation	100	18	18%
CPT	250	62	25%
mCPT (*B. subtilis*)	100	75	75%

2. Comparison of media type for the mCPT method

Table S2. Effect of media type on the mCPT. Representative experiment of the number of zones of inhibition observed over time on various types of media using D-alanine auxotrophic strains of *E. coli* or *B. subtilis* as target organisms over 2 agar plates.

		Time				
Medium	Target organism	Day 1	Week1	Week2	Week 3	Week 4
TY	*E. coli*	0	0	0	0	1
	B. subtilis	0	0	0	0	0
10% TSA	*E. coli*	0	0	0	0	0
	B. subtilis	0	2	2	2	2
MYM	*E. coli*	0	4	4	4	5
	B. subtilis	0	0	0	2	3
R2A	*E. coli*	0	2	4	4	4
	B. subtilis	0	0	0	3	3
TYME	*E. coli*	0	8	9	12	13
	B. subtilis	0	3	4	8	8

3. Secondary Zone of Inhibition for *Streptomyces* sp. PAP62



Target organism: *S. aureus*

Figure S1. Natural Product Extract (NPE) testing for *Streptomyces* sp. PAP62 using a drug-sensitive strain of *S. aureus*. (Left to Right) NPEs for *Streptomyces* sp. PAP62 grown in A3M (62-ASM) alone or with *C. glutamicum* (62-Corny), *M. smegmatis* (62-Myco), or *R. erythropolis* (62-Rhodo). The primary zone of inhibition on day one is denoted by the black lines. TYME plates were incubated at 37 ℃ and imaged at 3 days post-inoculation.

4. Spectroscopic characterisation of desertomycin A (1)

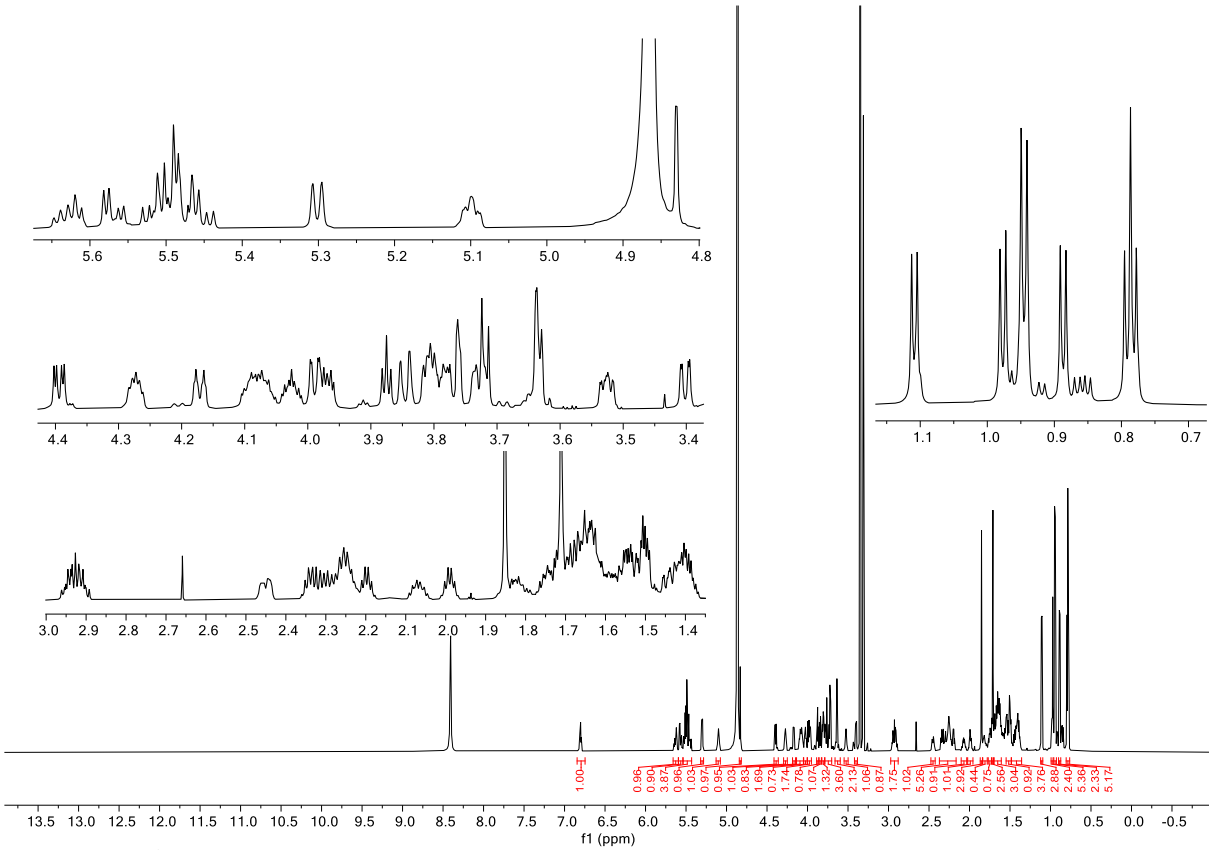


Figure S2. ^{1}H NMR (800 MHz, methanol-d_4) spectrum for desertomycin A (**1**)

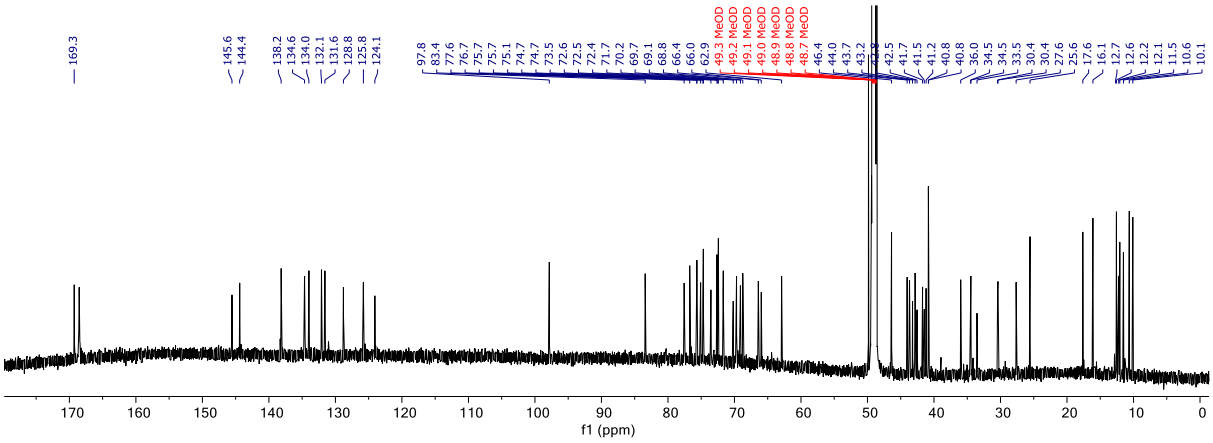


Figure S3. ^{13}C NMR (200 MHz, methanol-d_4) spectrum for desertomycin A (**1**)

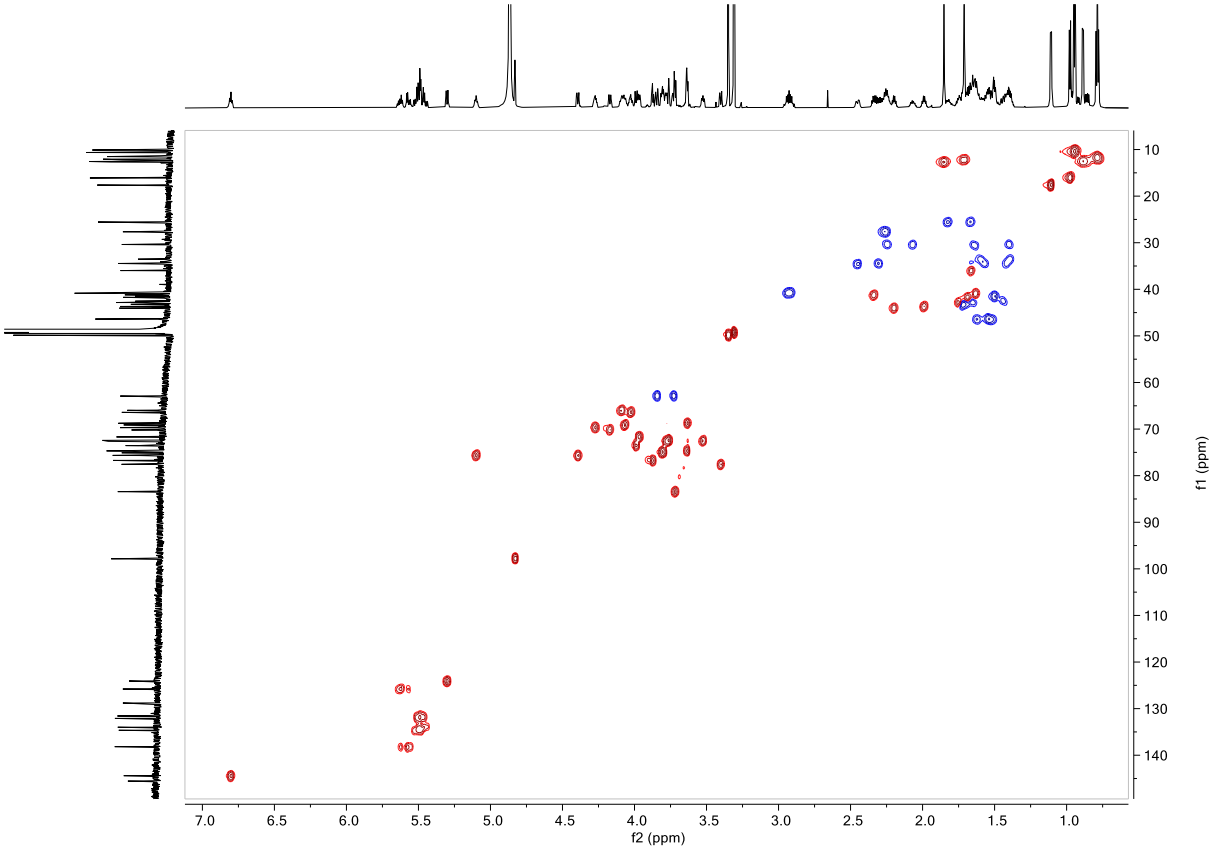


Figure S4. 2D NMR HSQC spectrum (methanol-d_4) for desertomycin A (**1**)

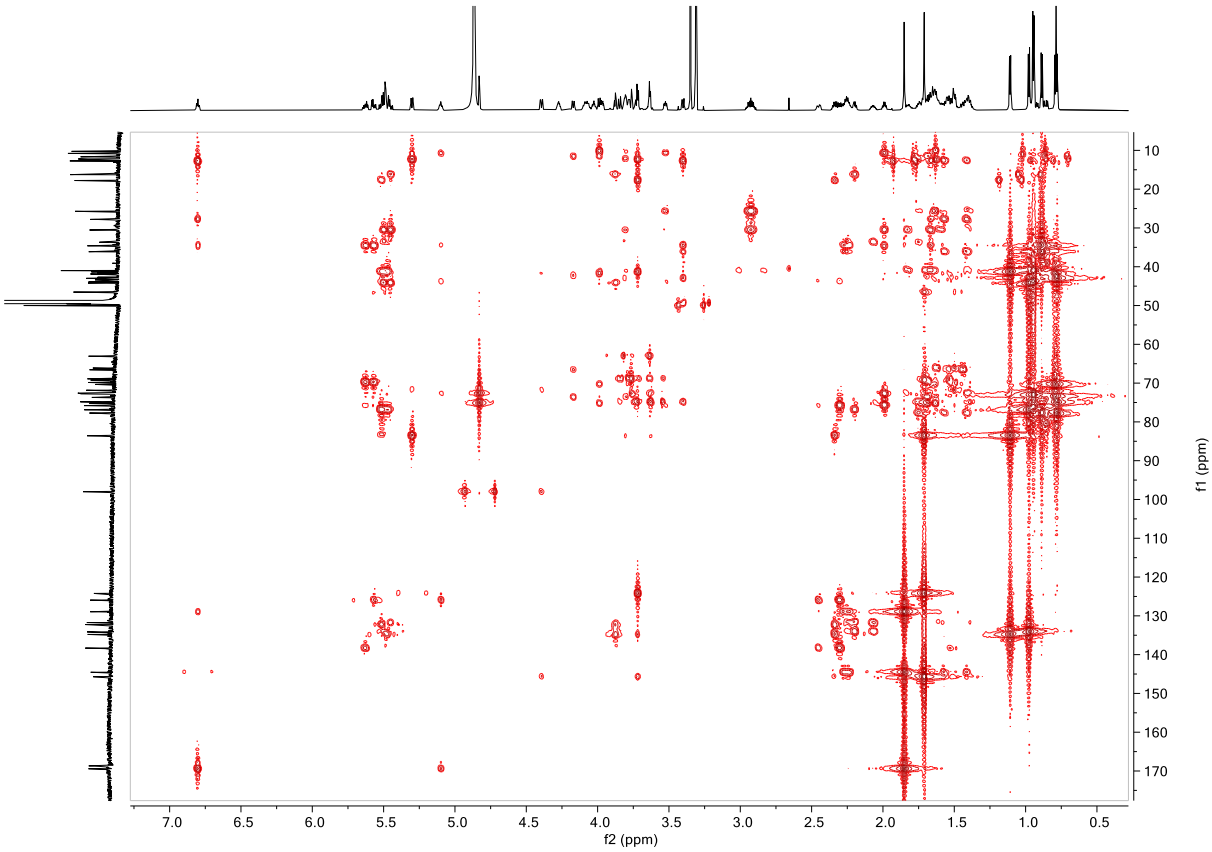


Figure S5. 2D NMR HMBC spectrum (methanol-d_4) for desertomycin A (**1**)

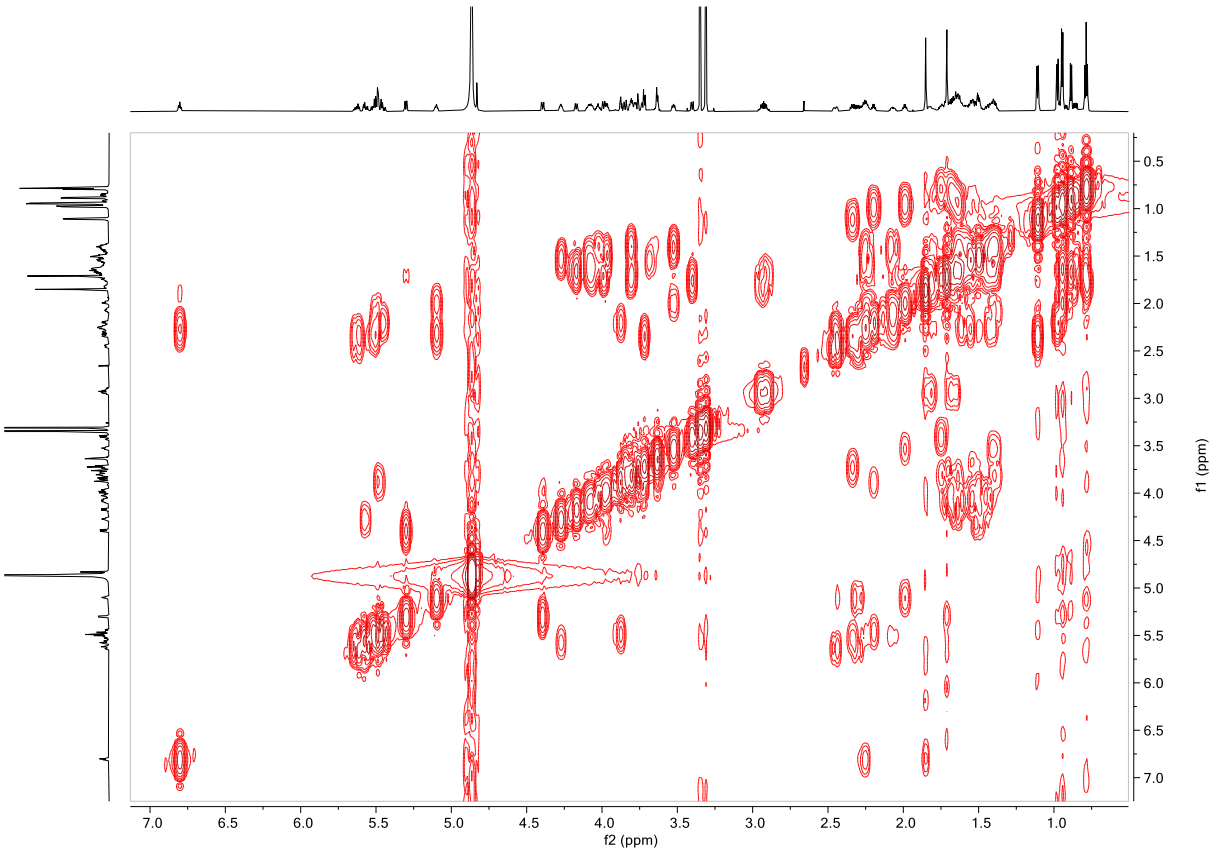


Figure S6. 2D NMR COSY spectrum (methanol-d_4) for desertomycin A (**1**)

Table S3. 1D and 2D NMR (800 MHz, methanol-d_4) data for desertomycin A (**1**)

Pos.	δ_H, m (*J* Hz)	δ_C	*COSY*	*HMBC*	Pos.	δ_H, m (*J* Hz)	δ_C	*COSY*	*HMBC*
1	---	169.3			33	4.17, td (*10.0*, *2.3*)	70.2	32, 34	
2	---	128.8			34	1.69[b]	43.2	33	
3	6.80, m	144.4	4	1, 2, 4, 5	35	4.02, m	66.5	34, 36	
4	2.26[a], m	27.6	3	2, 3, 5, 6	36	1.53[g]	46.4	35, 37	
5	a 1.42, m	34.5	4, 6	3, 4, 6, 7	37	4.27, m	69.7	36, 37	34, 35, 38, 39
	b 1.57, m				38	5.58, dd (*15.4*, *5.5*)	138.2	37, 39	36, 37, 39, 40
6	1.67[b], m	36.0	48		39	5.62, dd (*15.4*, *6.7*, *0.9*)	125.8	38, 40a	37, 38, 40, 41
7	3.40, dd (*9.6*, *1.7*)	77.5	8		40	a 2.30, dd (*13.4*, *6.7*)	34.5	39, 40b	
8	1.75, m	42.8	49			b 2.45, dd (*13.4*, *5.3*)		39, 40b, 41	
9	3.80[c]	74.7[k]	10a, 10b		41	5.10, m	75.6[l]	40b, 42	
10	a 1.40[d]		9		42	1.99, q (*6.9*)	43.7	41, 43, 55	
	b 1.60[e]		9		43	3.52, ddd (*9.8*, *5.6*, *1.9*)	72.6	42, 44a, 44b	
11	a 2.07, m	30.40	10a, 11b		44	a 1.40[d]	30.44	43, 44b, 45	
	b 2.24[a]		10a, 10b, 11a			b 1.63[e]		43, 44a, 45	
12	5.49[f]	131.6	11a, 11b, 13	10, 11, 13, 14	45	a 1.67[b]	25.6	44a, 44b, 46	
13	5.45[f]	134.0	12, 14	11, 12, 14, 15		b 1.82, m			
14	2.19, q (*6.7*)	44.1			46	2.93, m	40.8	45	44, 45
15	3.87, dd (*6.7*, *5.5*)	76.7	14		47	1.85, s	12.7	3	1, 2, 3
16	5.49[f]	132.1	15, 17	14, 15, 17, 18	48	0.88, d (*6.8*)	12.6	6	5, 6, 7
17	5.50[f]	134.7	16, 18	14, 15, 18, 19, 51	49	0.78, d (*6.9*)	12.0	8	7, 8, 9
18	2.34, m	41.2	19, 51		50	0.97, d (*6.8*)	16.1	14	13, 14, 15
19	3.72, d (*8.5*)	83.4	18		51	1.11, d (*6.9*)	17.6	18	17, 18, 19
20	---	145.5			52	1.71, s	12.2		19, 20, 21
21	5.30, d (*9.6*)	124.1	22		53	0.94[h], d (*6.9*)	10.1	30	29, 30, 31
22	4.39, dd (*9.6*, *3.5*)	75.6[l]	21		54	0.79, d (*7.0*)	11.5	32	31, 32, 33
23	3.96, dd (*8.3*, *3.5*)	71.7	24		55	0.94[h], d (*6.9*)	10.6	42	41, 42, 43
24	1.49, m	41.5	23		1’	4.83, d (*1.5*)	97.8		22, 2’, 3’, 5’
25	4.08, m	66.0	26a, 26b		2’	3.76[i]	72.4*		
26	a 1.53[g]	46.4	25		3’	3.76[i]	72.5*		
	b 1.62[e]		25		4’	3.63[j]	68.7		
27	4.06, m	69.1	26, 28		5’	3.63[j]	74.7[k]		
28	1.44, m	42.5	27		6’	a 3.72, dd (*11.5*, *3.6*)	62.9		
29	3.80[c]	75.1	28, 30			b 3.84, dd (*11.5*, *1.3*)			
30	1.63[e]	40.8	31, 53						
31	3.98, dd (*9.5*, *1.7*)	73.5	30						
32	1.69[b]	41.75	54						

[a-l] overlapping signals within the same superscript letter, * interchangeable assignments

Table S4. ^{13}C NMR (DMSO-d_6) data comparison of desertomycin A (**1**) with published data [1]

Pos.	δ_C, experimental	δ_C literature	Pos.	δ_C, experimental	δ_C literature
1	166.5	166.5	32	40.5	40.3
2	127.1	127.1	33	66.7	66.7
3	142.1	142.1	34	42.2	41.9
4	26.1	26.1	35	63.7	63.7
5	33.0	32.5	36	46.0	45.9
6	34.4	34.4	37	66.7	66.8
7	74.6	74.9	38	138.0	137.6
8	41.4	41.4	39	122.8	122.9
9	72.0	72.1	40	32.1	32.1
10	32.5	32.4	41	73.6	73.5
11	29.1	29.0	42	41.8	41.7
12	129.6	129.5	43	70.2	69.4
13	132.6	132.6	44	30.0	29.9
14	42.2	42.1	45	25.0*	24.0
15	74.4	74.4	46	39.2	39
16	131.1	130.9	47	12.3	12.0
17	132.7	132.7	48	12.3	12.2
18	39.2	39.2	49	11.2	11.6
19	80.9	80.9	50	15.3	15.3
20	143.3	143.3	51	16.8	16.8
21	122.3	122.3	52	11.6	11.6
22	73.6	73.6	53	9.7	9.7
23	69.4	69.5	54	10.5	11.2
24	40.9	40.6	55	10.4	11.2
25	63.3	63.3	1’	95.9	95.9
26	45.8	45.7	2’	70.5	70.1
27	66.5	66.4	3’	71.0	70.6
28	42.4	42.1	4’	67.2	67.2
29	72.8	72.8	5’	73.5	73.5
30	39.3	39.3	6’	61.3	61.3
31	70.6	70.6			

*signal determined by HSQC

5. Spectroscopic characterisation of desertomycin H (2)

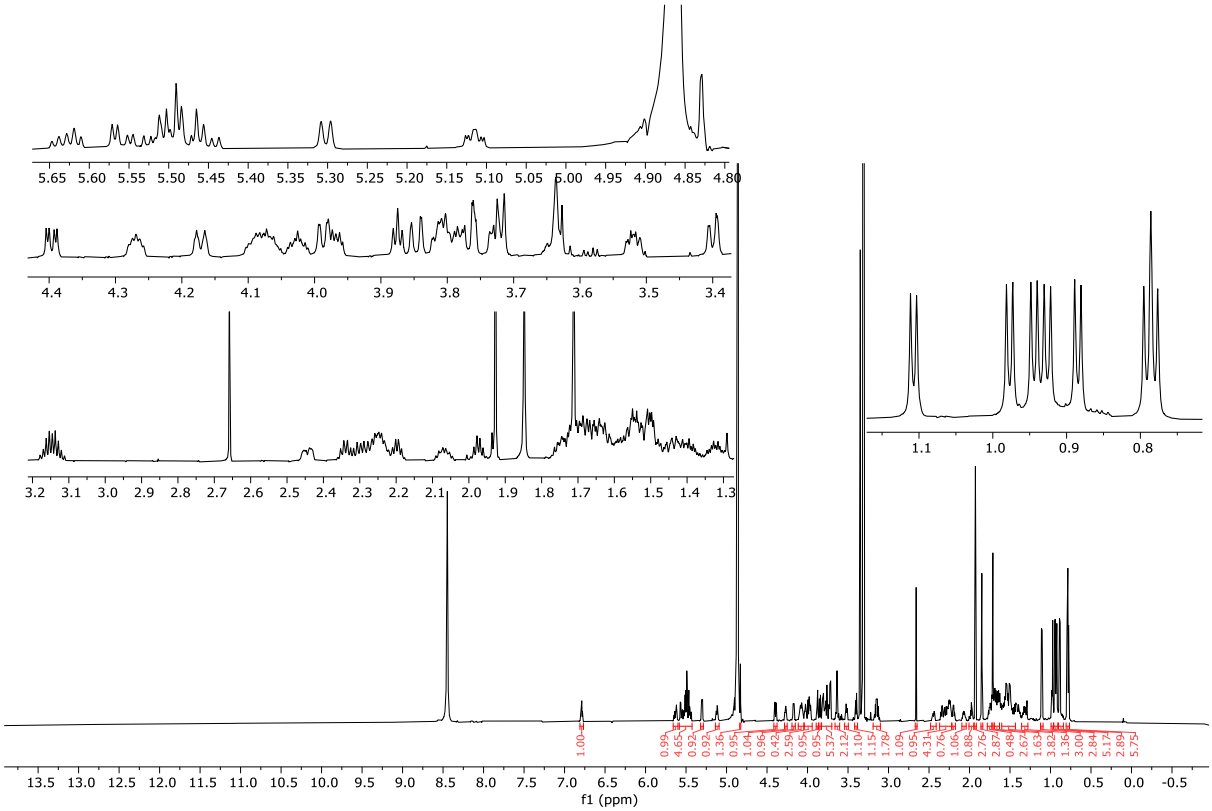


Figure S7. ^{1}H NMR (800 MHz, methanol-d_4) spectrum for desertomycin H (**2**)

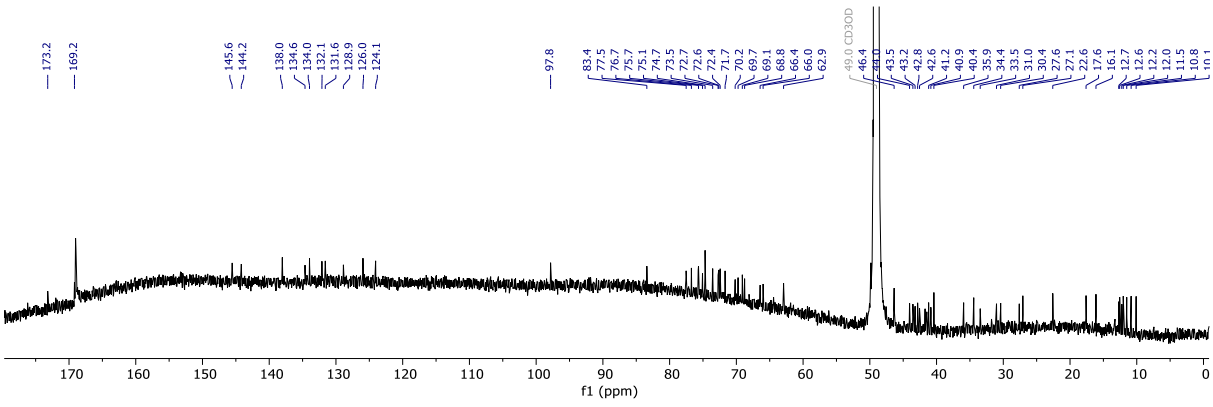


Figure S8. ^{13}C NMR (200 MHz, methanol-d_4) spectrum for desertomycin H (**2**)

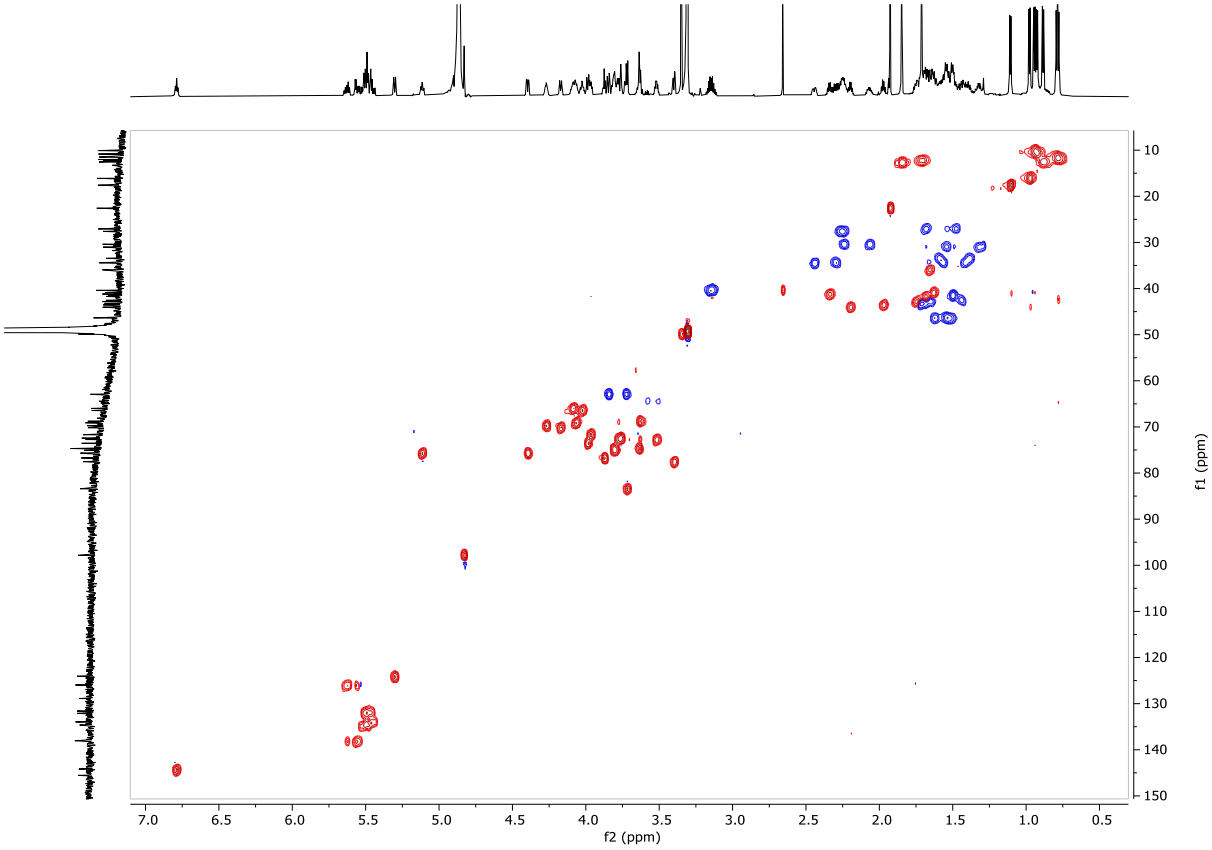


Figure S9. 2D NMR HSQC spectrum (methanol-d_4) for desertomycin H (**2**)

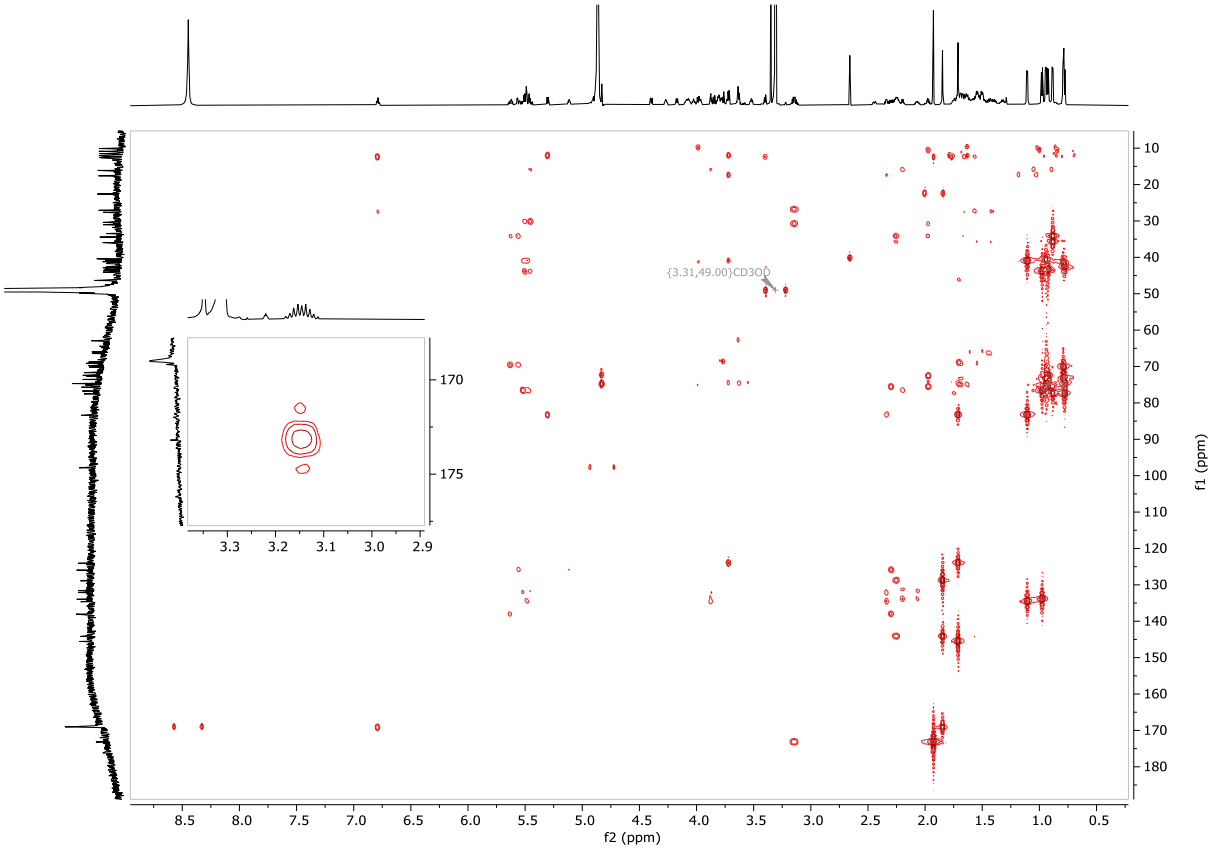


Figure S10. 2D NMR HMBC spectrum (methanol-d_4) for desertomycin H (**2**)

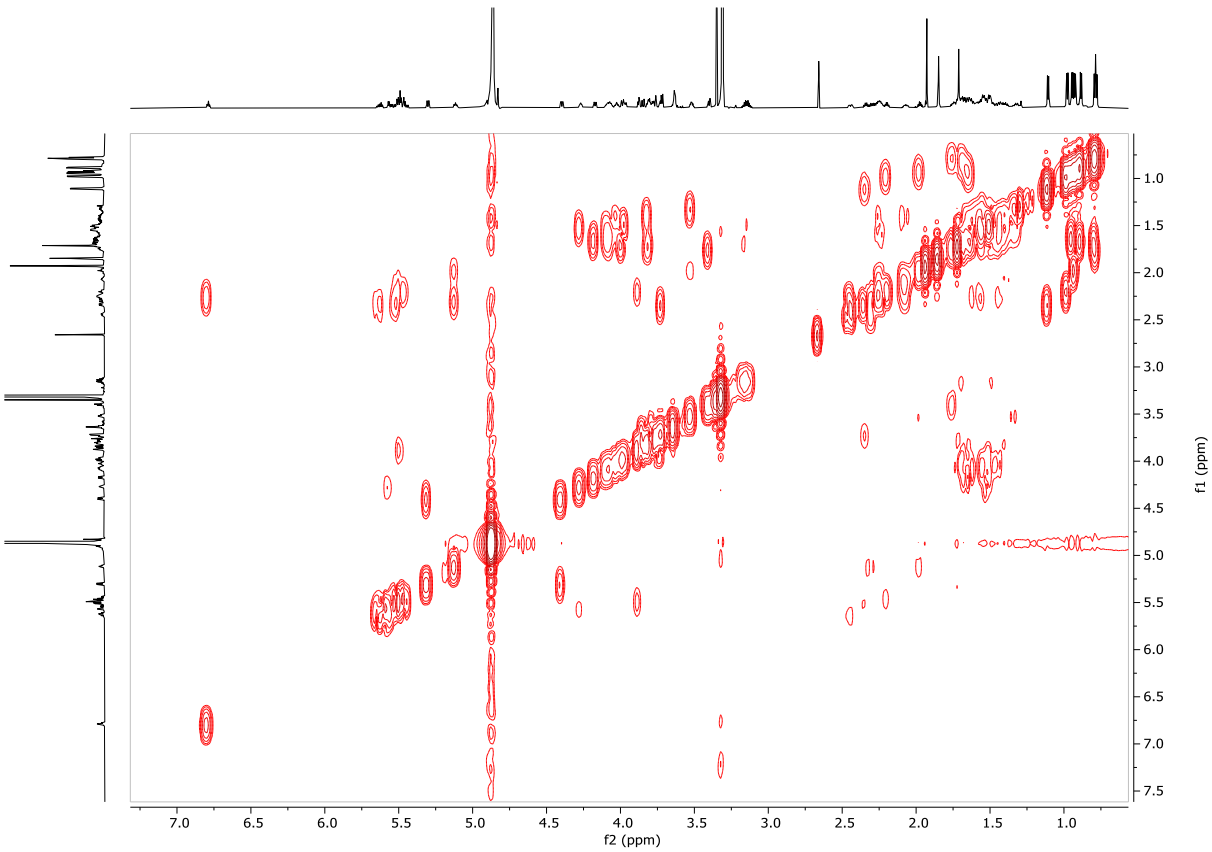


Figure S11. 2D NMR COSY spectrum (methanol-d_4) for desertomycin H (**2**)

Table S5. 1D and 2D NMR (800 MHz, methanol-d_4) data for desertomycin H (**2**)

Pos.	δ_H, m (*J* Hz)	δ_C	*COSY*	*HMBC*	Pos.	δ_H, m (*J* Hz)	δ_C	*COSY*	*HMBC*
1	---	169.2			33	4.17, td (*10.0, 2.0*)	70.2	32, 34	
2	---	128.9			34	1.70[b]	43.2	33	
3	6.79, m	144.2	4	1, 2, 4, 5	35	4.01, m	66.4	34, 36	
4	2.25[a], m	27.6	3	2, 3, 5, 6	36	1.52[f]	46.3[l]	35, 37	
5	a 1.41, m	34.4[i]	4, 6	3, 4, 6, 7	37	4.27, m	69.7	36, 37	34, 35, 38, 39
	b 1.56, m				38	5.56, dd (*15.5, 5.7*)	138.0	37, 39	36, 37, 39, 40
6	1.65[b], m	35.9	48		39	5.62, dd (*15.0, 7.2*)	125.9	38, 40a	37, 38, 40, 41
7	3.39, dd (	77.5	8		40	a 2.29, dd (*13.5, 7.2*)	34.4[i]	39, 40b	
8	1.74, m	42.8	49			b 2.44, dd (*13.5, 5.1*)		39, 40b, 41	
9	3.80[c]	74.7[j]	10a, 10b		41	5.11, m	75.7[k]	40b, 42	
10	a 1.38	33.5	9		42	1.97, q (*6.8*)	43.5	41, 43, 55	
	b 1.59[d]		9		43	3.50, ddd (*8.4, 5.5, 1.8*)	72.7	42, 44a, 44b	
11	a 2.07, m	30.4	10a, 11b		44	a 1.31	31.0	43, 44b, 45	
	b 2.24[a]		10a, 10b, 11a			b 1.54[f]		43, 44a, 45	
12	5.49[e]	131.6	11a, 11b, 13	10, 11, 13, 14	45	a 1.48[f]	27.1	44a, 44b, 46	
13	5.46[e]	134.0	12, 14	11, 12, 14, 15		b 1.68[b]			
14	2.19, q (*6.6*)	44.0			46	3.15, m	40.4	45	44, 45, 46-NHCOCH$_3$
15	3.87, dd (6.6, 5.3)	76.7	14		46-NHCOCH$_3$	---	173.2		
16	5.46[e]	132.1	15, 17	14, 15, 17, 18	46-NHCOCH$_3$	1.92, s	22.6		46, 46-NHCOCH
17	5.49[e]	134.6	16, 18	14, 15, 18, 19, 51	47	1.85, s	12.7	3	1, 2, 3
18	2.33, m	41.2	19, 51		48	0.88, d (*6.8*)	12.5	6	5, 6, 7
19	3.71, d	83.4	18		49	0.78, d (*7.5*)	12.0	8	7, 8, 9
20	---	145.6			50	0.98, d (*6.9*)	16.1	14	13, 14, 15
21	5.30, d (*9.3*)	124.0	22		51	1.11, d (*6.6*)	17.6	18	17, 18, 19
22	4.39, dd (*9.6, 3.6*)	75.7[k]	21		52	1.71, s	12.2		19, 20, 21
23	3.96, dd (*8.0, 3.4*)	71.7	24		53	0.94, d (*6.9*)	10.1	30	29, 30, 31
24	1.49, m	41.46	23		54	0.79, d (*7.5*)	11.5	32	31, 32, 33
25	4.08, m	66.0	26a, 26b		55	0.92, d (*6.9*)	10.8	42	41, 42, 43
26	a 1.53[f]	46.3[l]	25		1’	4.83, d (*1.3*)	97.8		22, 2’, 3’, 5’
	b 1.62[d]		25		2’	3.77[g]	72.4*		
27	4.07, m	69.1	26, 28		3’	3.75[g]	72.6*		
28	1.44, m	42.55	27		4’	3.63[h]	68.7		
29	3.80[c]	75.1	28, 30		5’	3.63[h]	74.7[j]		
30			31, 53		6’	a 3.72	62.9		
31	3.98, dd (*9.9, 1.7*)	73.5	30			b 3.84			
32	1.68[b]	41.7	54						

[a-l] overlapping signals within the same superscript letter, * interchangeable assignments

6. Desertomycin A and desertomycin H bioactivity

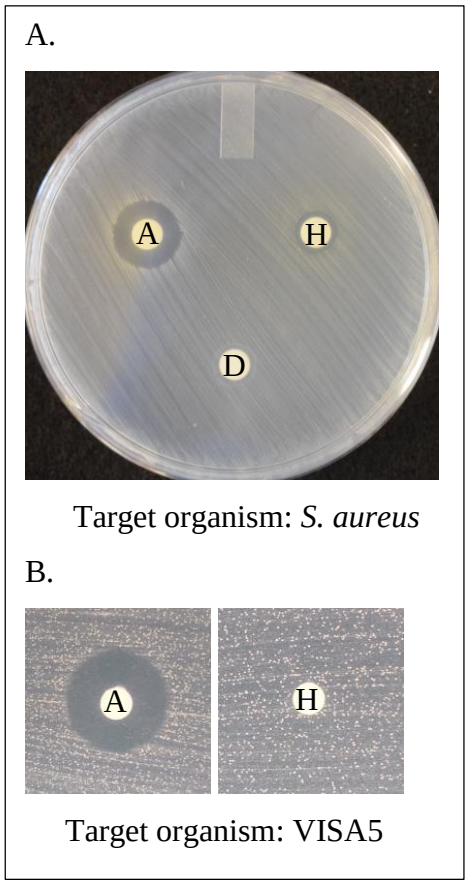


Figure S12. Desertomycin A and H bioactivity testing. (A) 30 μg of desertomycin A (A) and H (H) were tested for activity using a drug-sensitive strain of *S. aureus* and (B) a vancomycin intermediate-sensitive *S. aureus* (VISA5). DMSO (D) was used a solvent control. TYME plates were incubated at 37 ℃ and imaged at 24 hours post-inoculation.

7. References:

1. Ivanova, V. New Macrolactone of the Desertomycin Family from Sttreptomyces Spectabilis. *Preparative biochemistry & biotechnology* **1997**, *27*, 19-38.