A Genetically Amenable Platensimycin- and Platencin- Overproducer as a Platform for Biosynthetic Explorations: a Showcase of PtmO4, a Long-Chain Acyl-CoA Dehydrogenase

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Supplementary Information (SI)

Table S1. Strains used in this study.

Strain	Genotype, Description	Source (Reference)
E. coli DH5α	*E. coli* host for general cloning	Life Technologies
E. coli BW25113/pIJ790	*E. coli* host for λRED-mediated PCR targeting	1
E. coli DH5α/BT340	*E. coli* host for FLP-mediated excision of disruption cassette	2
E. coli ET12567/pUZ8002	Methylation-deficient *E. coli* host for intergeneric conjugation	3
S. platensis CB00739	Wild-type PTM/PTN producer	4
S. platensis SB12029	CB00739 Δ*ptmR1* (markerless)	This study
S. platensis SB12030	SB12029 Δ*ptmO4::aac(3)IV*	This study
S. platensis SB12031	SB12030 harboring pBS12041	This study
S. lividans SB12606	Heterologous PTN producer	5
S. lividans SB12608	Heterologous PTN producer	5
S. lividans SB12614	SB12606 harboring pBS12042	This study
S. lividans SB12615	SB12608 harboring pBS12042	This study
S. aureus ATCC25923	Methicillin sensitive strain for antibacterial assay	ATCC
M. luteus ATCC9431	Strain for antibacterial assay	ATCC

Table S2. Plasmids and cosmids used in this study.

Plasmid	Description	Source (Reference)
pIJ773	Plasmid containing the apramycin resistance cassette (*aac(3)IV+oriT*)	1
pIJ778	Plasmid containing the spectinomycin resistance cassette (*aadA+oriT*)	1
pJTU2170	*E. coli-Streptomyces* expression shuttle vector	6
pUWL201PW	*E. coli-Streptomyces* expression shuttle vector	7
pUWL201PWT	*E. coli-Streptomyces* expression shuttle vector harboring *oriT* (cloned into the *Pst*I site)	This study
pBS12034	Cosmid containing partial *ptm* gene cluster and Δ*ptmR1::aac(3)IV*	4
pBS12037	pBS12034 Δ*ptmR1* (markerless)	This study
pBS12038	pBS12037 with *aadA-oriT* inserted in cosmid backbone	This study
pBS12039	Cosmid 2F2 containing a partial *ptm* gene cluster	This study
pBS12040	pBS12039 Δ*ptmO4::aac(3)IV*	This study
pBS12041	pJTU2170 harboring *ptmO4* (cloned into the *Nde*I and *Xba*I)	This study
pBS12042	pUWL201PW harboring *ptmO4* (cloned into the *Nde*I and *Bam*HI sites)	This study

Table S3. Primers used in this study. *Nde*I, *Xba*I, and *Bam*HI restriction sites are underlined.

Primer	Nucleotide Sequence (5'-3')	Function
aadA-oriT_F	AGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTGAAGTTCCCGCCAGCCTCG	PCR targeting for *aadA-oriT* insertion
aadA-oriT_R	GTGTATTTTAGATTCCAACCTATGGAACTGATGAATGGGGAGCTCAGCCAATCGACTGGC	PCR targeting for *aadA-oriT* insertion
oriT_ID_F	GTCCTCAACGACAGGAGCAC	PCR confirmation
oriT_ID_R	ATGAAAGGTTGGGCTTCGGA	PCR confirmation
739R1ID_F	CCTGATGGAGCAGTTCGG	PCR confirmation
739R1ID_R	GGAGTTCCAGACGGGTATTG	PCR confirmation
739R1south_F	GATGACCGAGGATCTGAGCG	Southern probe R1
739R1south_R	GCCAATACCCGTCTGGAACT	Southern probe R1
739O4KO_F	CGCCGAGGACAAGGCCACCGCGTAATGAGGCGCACGCTCATTCCGGGGATCCGTCGACC	PCR targeting for replacement of *ptmO4*
739O4KO_R	GTAAGTGGGAGACGAGCTTGTTGTTCATTCCTGCTTCCTTGTAGGCTGGAGCTGCTTC	PCR targeting for replacement of *ptmO4*
739O4ID_F	AAGGCCACCGCGTAATGAG	PCR confirmation
739O4ID_R	AGTGGGAGACGAGCTTGTTG	PCR confirmation
739O4south_F	CGGGTGATCGCCGTACTC	Southern probe O4
739O4south_R	ACGTTCAAGGTGCCCTCTTC	Southern probe O4
739O4comp_F	GGAATTCCATATGAGCATTCCTGGCGGC	*ptmO4* amplification
739O4comp_R	GCTCTAGAGGTCTCGCCCAGTGGC	*ptmO4* amplification
739O4compF2	GGAATTCCATATGACAAGGCCACCGCGTAAT	*ptmO4* amplification
739O4compR2	CGGGATCCGGTCTCGCCCAGTGGC	*ptmO4* amplification

Figure S1. Inactivation of *ptmR1* affording the in-frame Δ*ptmR1* markerless mutant *S. platensis* SB12029. (A) Schematic representation for the deletion of *ptmR1* in *S. platensis* CB00739 by insertion of a 81 bp scar originating from an apramycin resistance cassette [*aac(3)IV* + *oriT*]. (B) PCR verification of wild-type (608 bp) and double crossover (144 bp) mutant genotypes using the primers 739R1ID_F and 739R1ID_R. Lanes 1–3, *S. platensis* SB12029 isolates; lane 4, *S. platensis* CB00739; lane 5, 1 kb DNA ladder (NEB).

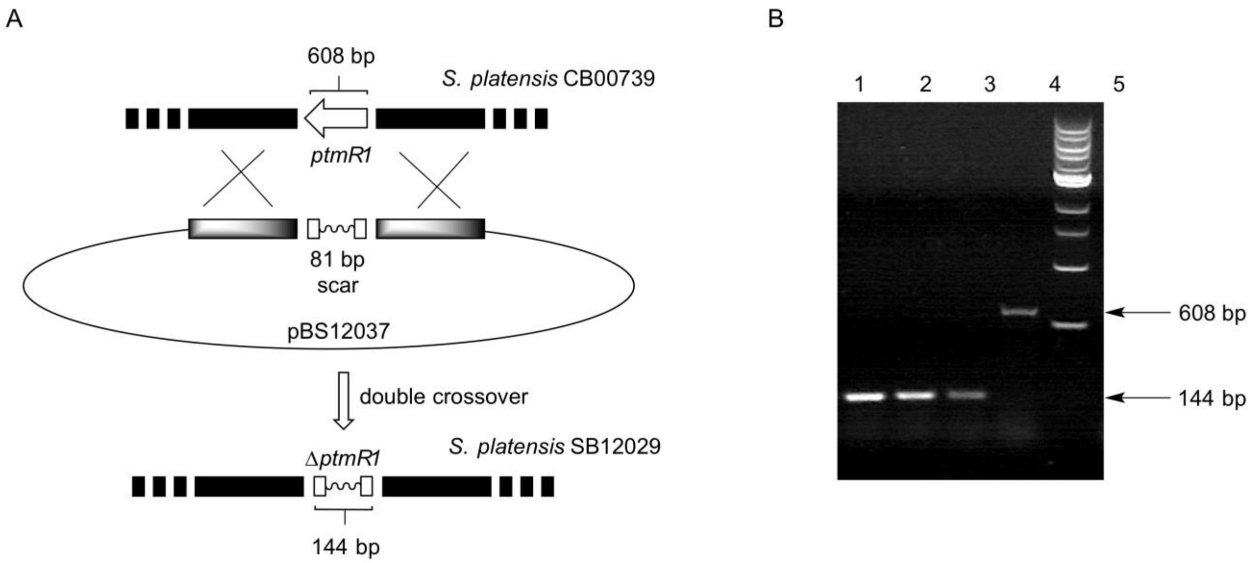

Figure S2. Southern analysis of the in-frame Δ*ptmR1* markerless mutant *S. platensis* SB12029. (A) Schematic representation for the deletion of *ptmR1* in *S. platensis* CB00739 by insertion of an 81 bp scar. Two probes, one for Δ*ptmR1* (351 bp) and one for *ptmO4* (422 bp), were amplified using genomic DNAs as the template. (B) Southern blot verification of wild-type *ptmR1* (2223 bp) and double crossover Δ*ptmR1* markerless mutant (1759 bp) genotypes. In SB12029, *ptmO4* is unmodified (8298 bp). Lane 1, DNA marker VII, DIG-labeled (Roche); lane 2, *S. platensis* SB12029; lane 3, *S. platensis* CB00739; lane 4, DNA marker III, DIG-labeled (Roche).

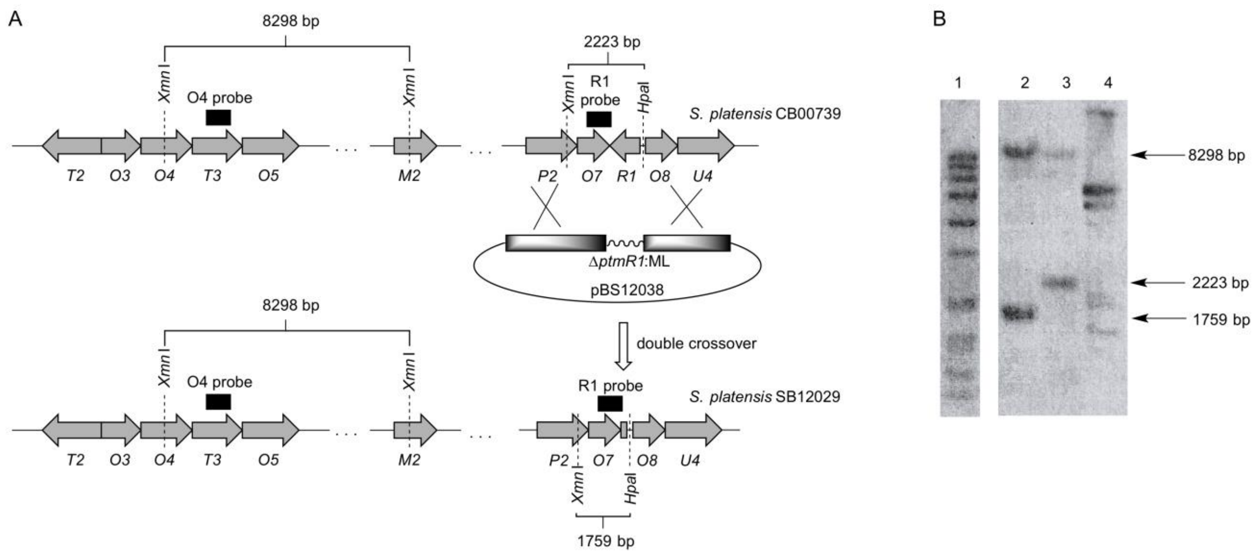

Figure S3. Inactivation of *ptmO4* in the PTM-PTN overproducer *S. platensis* SB12029 affording *S. platensis* SB12030. (A) Schematic representation for the deletion of *ptmO4* in *S. platensis* SB12029 by insertion of an apramycin resistance cassette [*aac(3)IV* + *oriT*]. (B) PCR verification of wild-type (1202 bp) and double crossover (1447 bp) mutant genotypes using the primers 739O4ID_F and 739O4ID_R. Lane 1, 1 kb DNA ladder (NEB); lane 2, *S. platensis* SB12029; lane 3, *S. platensis* SB12030.

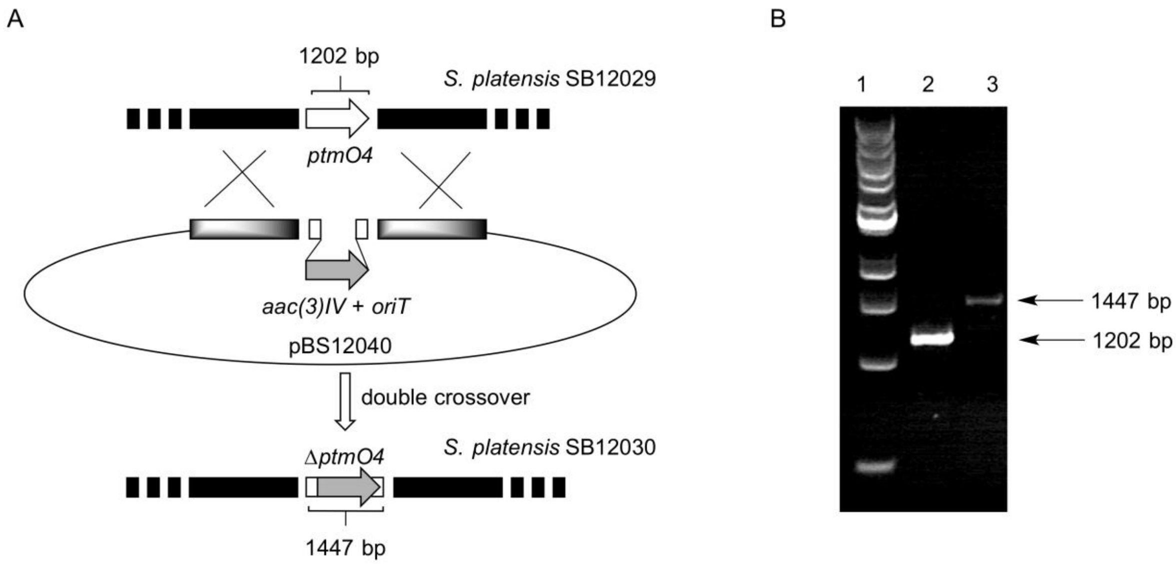

Figure S4. Southern analysis of the Δ*ptmO4* Δ*ptmR1* double mutant *S. platensis* SB12030. (A) Schematic representation for the deletion of *ptmO4* in *S. platensis* SB12029 by insertion of an *aac(3)IV* + *oriT* cassette. Two probes, one for Δ*ptmR1* (351 bp) and one for *ptmO4* (422 bp), were amplified using genomic DNAs as the template. (B) Southern blot verification of wild-type *ptmO4* (2657 bp) and *ptmR1* (3776 bp) and double crossover Δ*ptmO4* (4185 bp) Δ*ptmR1* (3312 bp) mutant genotypes. Lane 1, DNA marker VII, DIG-labeled (Roche); lane 2, *S. platensis* SB12030; lane 3, *S. platensis* CB00739.

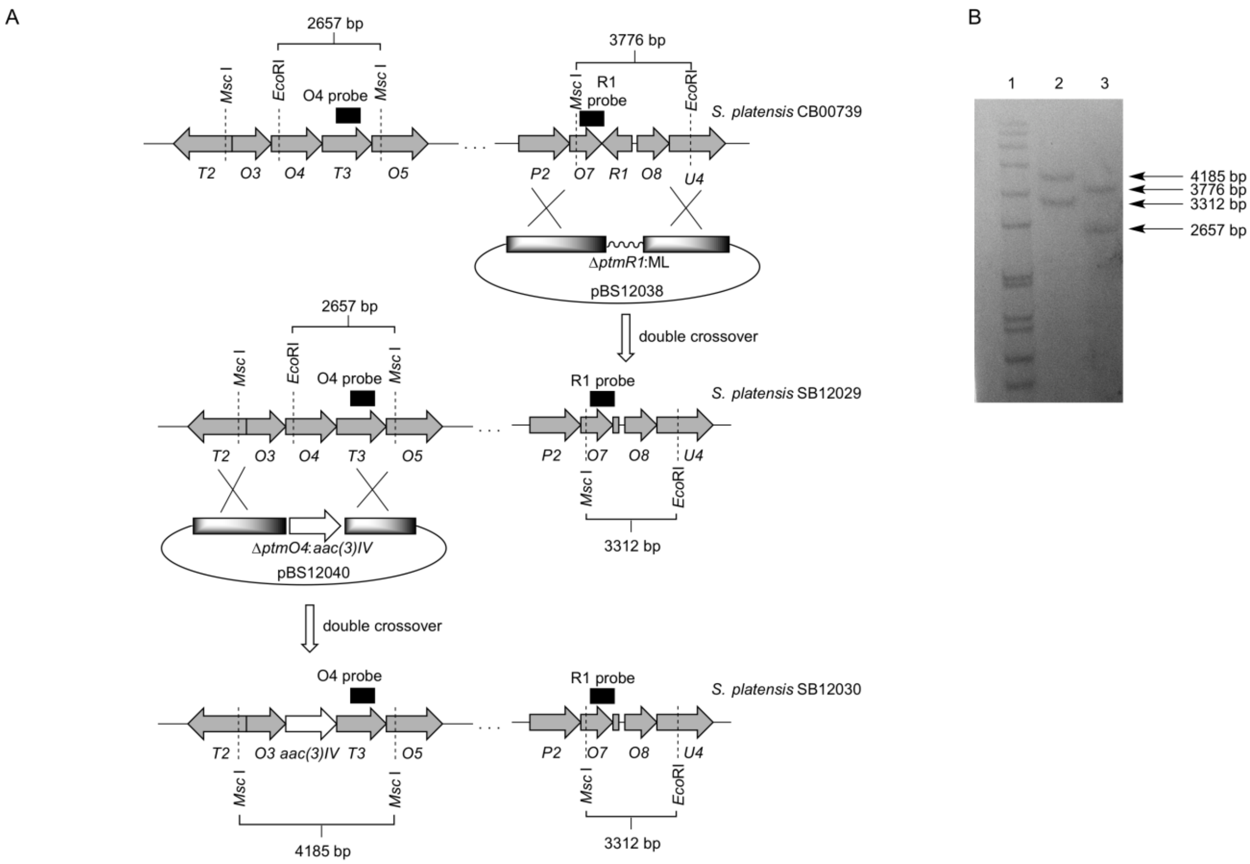

Table S4. Summary of ^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) data for compounds **3–5** in pyridine-d_5 (δ in ppm, J in Hz)[a]

No.	**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1a	36.8, t	2.05, ddd (13.3, 11.9, 2.8)	37.3, t	2.18, ddd (12.6, 12.6, 3.5)	37.0, t	2.13, dd (11.9, 10.5)
1b		1.40, ddd (13.3, 11.9, 3.5)		1.49, m		1.44, m
2a	23.1, t	1.53, overlapped	23.3, t	1.63, m	23.3, t	1.64, m
2b		1.34, m		1.45, m		1.44, m
3a	35.2, t	1.85, d (11.2)	35.4, t	1.94, m	35.3, t	1.91, m
3b		1.53, overlapped		1.61, m		1.58, m
4	40.2, d	2.53, m	40.4, d	2.61, m	40.4, d	2.60, m
5	203.7, s		203.5, s		203.4, s	
6	127.7, d	5.93, d (10.5)	128.1, d	6.13, d (10.2)	128.4, d	6.13, d (10.0)
7	154.1, d	6.39, d (10.5)	151.5, d	7.28, d (10.2)	153.3, d	7.08, d (10.0)
8	46.3, s		51.1, s		52.1, s	
9	46.5, d	2.50, br s	47.4, d	2.67, br s	49.2, d	2.74, br s
10	47.3, s		47.2, s		47.3, s	
11	76.9, d	4.38, br s	76.8, d	4.45, br s	76.7, d	4.41, br s
12a	41.0, t	1.93, m	41.2, t	2.02, m	40.6, t	2.06, 2H, m
12b		1.85, d (11.2)		1.98, d (11.2)		
13	45.3, d	2.21, dd (6.3, 6.3)	44.6, d	2.33, dd (6.3, 6.3)	54.6, d	2.45, br d (6.3)
14a	43.3, t	1.82, dd (11.2, 2.8)	39.3, t	2.69, dd (11.2, 6.3)	82.5, d	4.64, s
14b		1.59, dd (11.9, 7.0)		1.87, br d (11.2)		
15a	55.2, t	1.70, dd (10.5, 3.2)	85.4, d	3.96, s	49.7, t	2.85, d (11.2)
15b		1.48, d (10.5)				1.71, d (11.2)
16	87.1, s		90.4, s		87.2, s	
17	23.5, q	1.37, s	19.5, q	1.65, s	24.2, q	1.54, s
18	17.9, q	1.20, d (7.0)	18.0, q	1.26, d (7.0)	17.9, q	1.25, d (7.0)
19	179.2, s		179.4, s		179.4, s	
20	25.1, q	1.10, s	25.7, q	1.23, s	26.0, q	1.15, s

[a] Assignments are based on 1D and 2D NMR experiments.

Table S5. Summary of ^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) data for compounds **6–8** in pyridine-d_5 (δ in ppm, J in Hz)[a]

	6		**7**		**8**	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1a	36.8, t	2.02, dd (11.2, 11.2)	36.8, t	2.02, dd (10.5, 10.5)	37.8, t	2.00, ddd (14.0, 12.6, 3.5)
1b		1.38, overlapped		1.37, overlapped		1.32, m
2a	23.2, t	1.56, overlapped	23.1, t	1.55, overlapped	22.7, t	1.51, 2H, m
2b		1.39, overlapped		1.39, overlapped		
3a	35.8, t	1.96, m	35.8, t	1.95, overlapped	35.4, t	1.90, m
3b		1.56, overlapped		1.56, overlapped		1.60, m
4	41.4, d	2.59, m	41.1, d	2.64, m	40.5, d	2.63, m
5	204.1, s		204.0, s		209.2, s	
6	127.8, d	5.98, d (9.8)	127.8, d	5.97, d (9.8)	56.1, d	3.40, d (3.5)
7	154.2, d	6.37, d (9.8)	154.1, d	6.37, d (9.8)	64.4, d	3.29, d (3.5)
8	46.4, s		46.3, s		43.7, s	
9	46.4, d	2.55, br s	46.4, d	2.54, br s	49.0, d	2.30, br s
10	47.4, s		47.3, s		48.7, s	
11	77.0, d	4.40, br s	76.9, d	4.37, br s	77.4, d	4.33, br s
12a	41.1, t	1.96, m	41.1, t	1.96, overlapped	41.4, t	1.91, m
12b		1.84, d (11.2)		1.84, d (11.2)		1.82, d (11.2)
13	45.4, d	2.20, dd (7.0, 6.3)	45.3, d	2.20, dd (6.3, 6.3)	44.4, d	2.17, dd (7.0, 6.3)
14a	43.3, t	1.81, dd (11.9, 3.5)	43.3, t	1.80, dd (11.9, 3.5)	42.6, t	2.08, dd (11.9, 3.5)
14b		1.56, overlapped		1.56, overlapped		1.85, dd (11.9, 4.9)
15a	55.2, t	1.76, dd (10.5, 3.2)	55.2, t	1.75, dd (11.2, 3.2)	53.2, t	1.87, dd (11.2, 3.5)
15b		1.46, d (10.5)		1.45, d (11.2)		1.55, d (11.2)
16	87.2, s		87.1, s		87.6, s	
17	23.5, q	1.39, s	23.5, q	1.37, s	23.6, q	1.43, s
18	18.4, q	1.25, d (7.0)	18.2, q	1.21, d (6.3)	18.0, q	1.29, d (7.0)
19	177.4, s		177.3, s		179.3, s	
20	25.2, q	1.09, s	25.1, q	1.08, s	24.2, q	1.33, s
1′	175.7, s		174.3, s			
2′	53.6, d	5.17, m	56.5, d	5.28, m		

Table S5 cont.

	6		7		8	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3′a	29.0, t	2.83, m	63.4, t	4.53, dd (11.2, 4.2)		
3′b		2.59, m		4.40, dd (11.2, 4.2)		
4′	33.2, t	2.83, 2H, m				
5′	175.6, s					
-CONH		8.76, d (7.0)		8.57, d (7.7)		

[a] Assignments are based on 1D and 2D NMR experiments.

Table S6. Summary of ^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) data for compounds **9** and **10** in pyridine-d_5 (δ in ppm, J in Hz)[a]

	9		10	
No.	δ_C	δ_H	δ_C	δ_H
1a	39.6, t	1.83, m	39.8, t	1.75, m
1b		1.52, m		1.42, m
2a	23.4, t	1.53, 2H, m	23.3, t	1.54, overlapped
2b				1.40, m
3a	35.4, t	1.93, m	35.9, t	1.98, m
3b		1.59, m		1.54, overlapped
4	40.3, d	2.65, m	41.1, d	2.72, m
5	214.9, s		215.3, s	
6a	36.8, t	2.53, ddd (15.4, 13.3, 6.3)	36.8, t	2.49, ddd (14.7, 13.3, 6.3)
6b		2.31, ddd (15.4, 5.6, 2.8)		2.33, ddd (15.4, 5.6, 2.8)
7a	34.0, d	1.78, ddd (13.3, 13.3, 6.3)	33.7, d	1.83, overlapped
7b		1.43, m		1.40, m
8	44.9, s		44.9, s	
9	50.9, d	2.25, br s	50.8, d	2.26, br s
10	50.0, s		50.0, s	
11a	77.6, d	4.39, br s	77.7, d	4.38, br s
11b				
12a	41.4, t	1.93, m	41.4, t	1.92, m
12b		1.85, overlapped		1.83, overlapped
13	45.5, d	2.15, dd (6.3, 6.3)	45.4, d	2.13, dd (7.0, 6.3)
14a	43.0, t	1.85, br d (11.2)	42.9, t	1.79, dd (11.9, 3.5)
14b		1.29, overlapped		1.26, overlapped
15a	55.6, t	1.61, dd (10.5, 3.5)	55.4, t	1.65, dd (10.5, 3.5)
15b		1.32, br d (11.2)		1.29, br d (11.2)
16	86.5, s		86.5, s	
17	23.7, q	1.40, s	23.7, q	1.38, s
18	18.0, q	1.30, d (7.0)	18.4, q	1.28, d (6.3)
19	179.4, s		177.3, s	
20	23.6, q	1.13, s	23.4, q	1.12, s
1′			174.5, s	
2′			56.5, d	5.36, m
3′a			63.6, t	4.58, dd (11.2, 4.2)
3′b				4.43, dd (11.2, 4.2)
-CONH				8.69, d (8.4)

[a] Assignments are based on 1D and 2D NMR experiments.

Table S7. Summary of ^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) data for compounds **12** and **13** in pyridine-d_5 (δ in ppm, J in Hz)[a]

	12		13	
No.	δ_C	δ_H	δ_C	δ_H
1a	36.2, t	2.04, m	36.0, t	1.98, m
1b		1.39, m		1.20, m
2a	23.1, t	1.47, 2H, m	23.1, t	1.45, 2H, m
2b				
3a	35.5, t	1.95, m	35.4, t	1.92, m
3b		1.60, m		1.57, m
4	40.4, d	2.63, m	40.3, d	2.61, br s
5	204.2, s		204.2, s	
6	127.3, d	6.11, d (10.5)	127.3, d	6.14, d (10.5)
7	153.6, d	7.22, d (10.5)	154.6, d	7.14, d (10.5)
8	40.9, s		42.0, s	
9	38.2, d	2.08, dd (9.8, 9.8)	40.3, d	2.32, dd (9.8, 9.8)
10	48.3, s		48.0, s	
11a	28.3, t	1.63, overlapped	28.4, t	1.45, m
11b		1.50, overlapped		1.41, dd (11.2, 11.2)
12	36.6, d	2.45, br s	37.6, d	2.41, m
13a	26.6, t	1.89, m	38.9, t	2.29, ddd (13.3, 9.8, 3.5)
13b		1.62, overlapped		1.84, m
14a	20.7, d	2.28, ddd (11.9, 11.9, 2.8)	67.1, d	4.68, ddd (9.8, 4.4, 1.4)
14b		1.76, ddd (11.9, 11.9, 4.9)		
15a	76.1, d	4.00, s	37.0, t	3.31, d (16.8)
15b				1.79, dd (16.1, 1.4)
16	155.3, s		150.4, s	
17a	111.2, t	5.24, s	106.9, t	4.86, dd (4.2, 2.1)
17b		5.12, s		4.69, dd (4.2, 2.1)
18	18.0, q	1.27, d (6.3)	17.9, q	1.25, d (6.3)
19	179.5, s		179.7, s	
20	22.4, q	1.19, s	23.0, q	1.08, s

[a] Assignments are based on 1D and 2D NMR experiments.

Table S8. Summary of [1]H NMR (700 MHz) and [13]C NMR (175 MHz) data for compounds **3a** and **3b** in $CDCl_3$ (δ in ppm, J in Hz)[a]

	3a		**3b**	
No.	δ_C	δ_H	δ_C	δ_H
a	36.3, t	1.78, overlapped	36.5, t	1.73, overlapped
1b		1.21, overlapped		1.18, ddd (11.2, 11.2, 2.8)
2a	22.3, t	1.12, m	22.3, t	1.01, m
2b		0.92, m		0.91, m
3a	34.1, t	1.33, m	34.0, t	1.15, m
3b		1.15, m		1.01, m
4	39.5, d	2.07, m	39.9, d	2.04, m
5	204.0, s		204.0, s	
6	127.7, d	5.83, d (9.8)	127.7, d	5.83, d (9.8)
7	153.5, d	6.41, d (9.8)	153.5, d	6.41, d (9.8)
8	46.1, s		46.1, s	
9	45.9, d	2.35, br s	46.0, d	2.34, br s
10	47.1, s		47.0, s	
11	76.8, d	4.26, br s	76.8, d	4.26, br s
12a	40.8, t	2.03, m	40.8, t	2.03, overlapped
12b		1.96, d (11.9)		1.97, overlapped
13	44.9, d	2.36, overlapped	44.9, d	2.37, dd (7.0, 6.3)
14a	43.4, t	1.96, br d (11.9)	43.4, t	1.96, overlapped
14b		1.71, dd (11.9, 7.0)		1.72, overlapped
15a	55.1, t	1.81, br d (11.2)	55.1, t	1.83, dd (10.5, 2.8)
15b		1.56, d (11.2)		1.57, d (11.2)
16	87.1, s		87.1, s	
17	23.3, q	1.41, s	23.3, q	1.42, s
18	16.8, q	0.66, d (7.0)	16.5, q	0.76, d (7.0)
19	176.2, s		176.1, s	
20	25.1, q	1.11, s	25.1, q	1.11, s
1′	75.7, d	4.93, ddd (11.2, 10.5, 4.2)	75.7, d	4.92, ddd (11.2, 10.5, 4.2)
2′	50.1, d	2.64, ddd (11.2, 11.2, 2.8)	50.2, d	2.63, ddd (11.2, 11.2, 2.8)
3′a	34.1, t	1.90, br d (13.3)	34.2, t	1.90, br d (13.3)
3′b		1.53, dd (13.3, 2.1)		1.53, dd (13.3, 2.8)
4′a	26.1, t	1.77, m	26.1, t	1.76, m
4′b		1.32, m		1.33, m
5′a	25.0, t	1.82, m	25.0, t	1.83, m
5′b		1.49, m		1.49, m
6′a	32.6, t	2.09, m	32.5, t	2.07, m
6′b		1.40, m		1.39, m
7′	143.4, s		143.4, s	
8′/12′	127.8, d	7.16, br d (7.7)	127.8, d	7.15, br d (7.7)
9′/11′	128.4, d	7.22, dd (7.7, 7.2)	128.4, d	7.23, dd (7.7, 7.2)
10′	126.5, d	7.13, dd (7.7, 7.2)	126.6, d	7.13, dd (7.7, 7.2)

[a] Assignments are based on 1D and 2D NMR experiments.

Figure S5. ^{1}H NMR spectrum of platensimycin ML1 (**3**) in pyridine-d_5 (700 MHz).

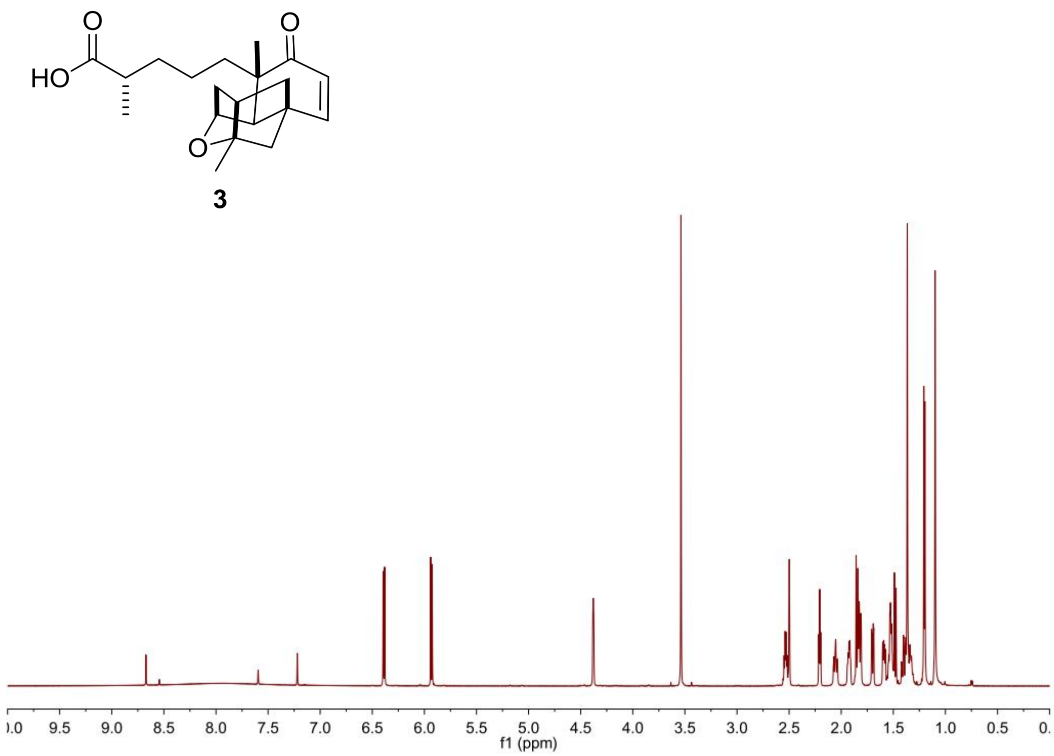

Figure S6. ^{13}C NMR spectrum of platensimycin ML1 (**3**) in pyridine-d_5 (175 MHz).

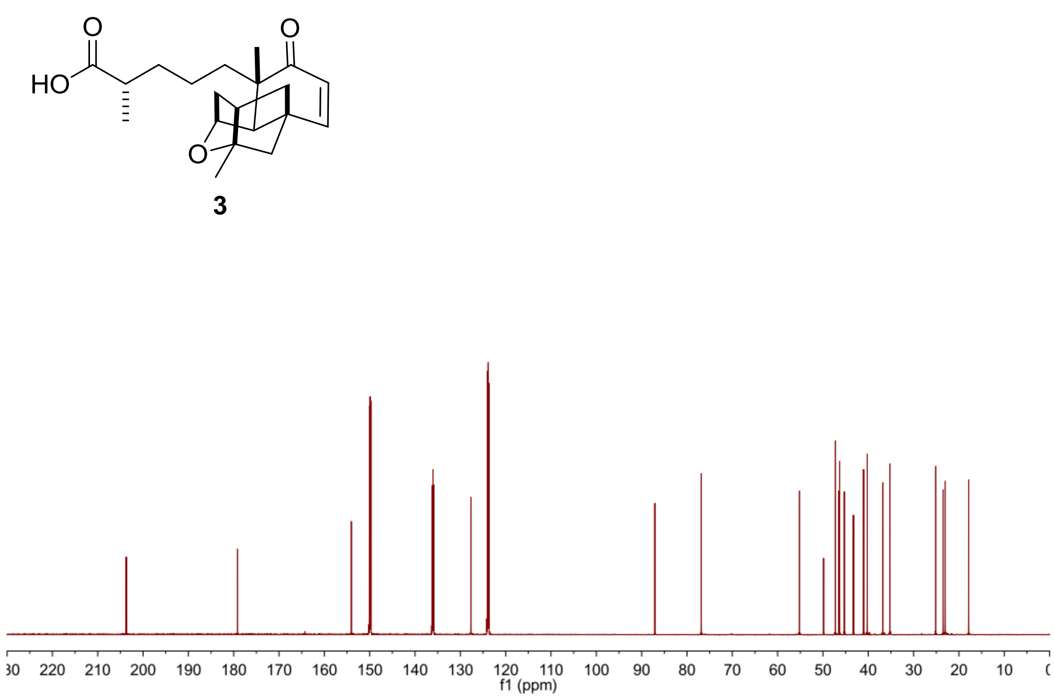

Figure S7. ^{1}H NMR spectrum of platensimycin ML2 (**4**) in pyridine-d_5 (700 MHz).

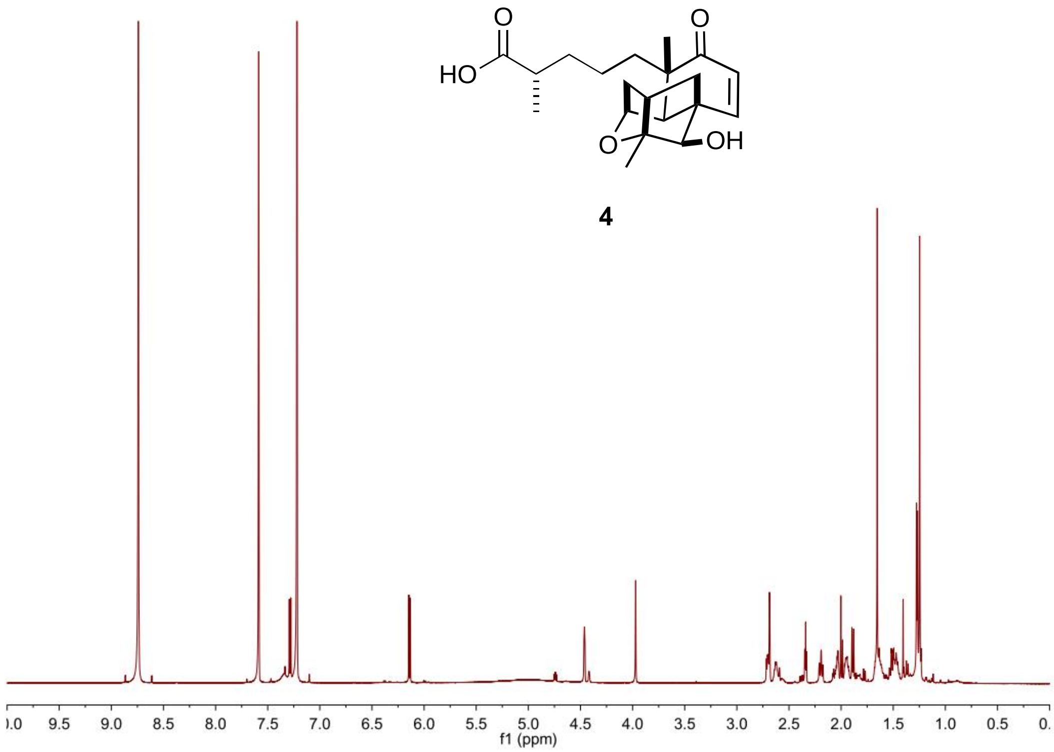

Figure S8. ^{13}C NMR spectrum of platensimycin ML2 (**4**) in pyridine-d_5 (175 MHz).

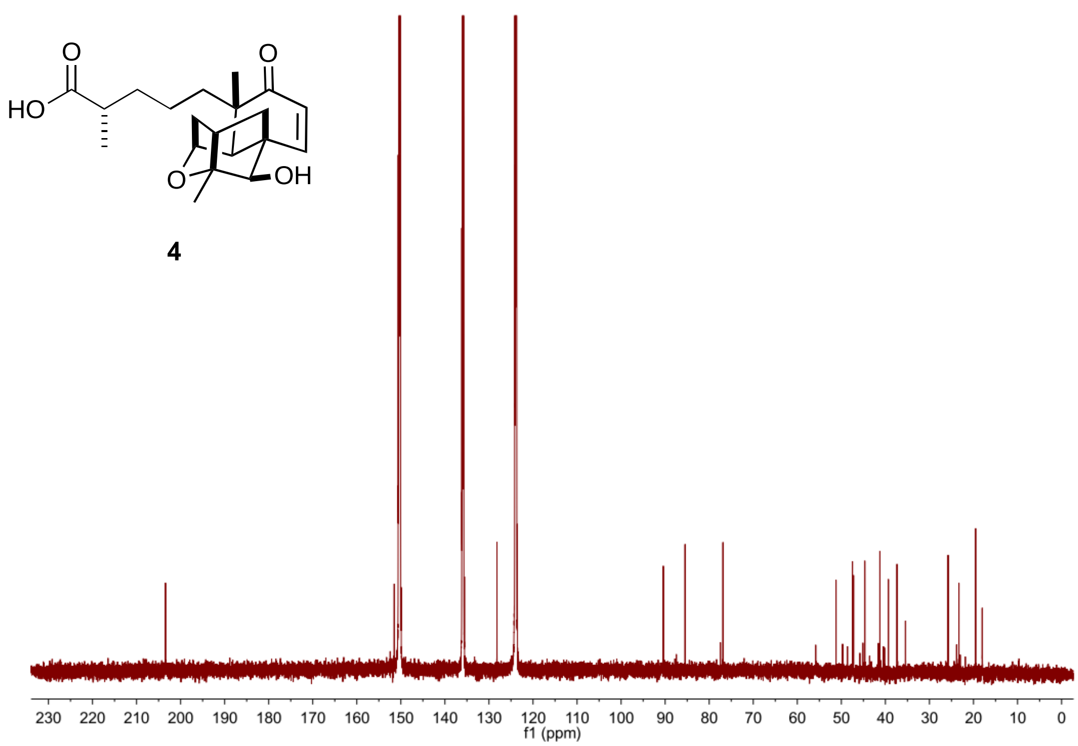

Figure S9. ^{1}H NMR spectrum of platensimycin ML3 (**5**) in pyridine-d_5 (700 MHz).

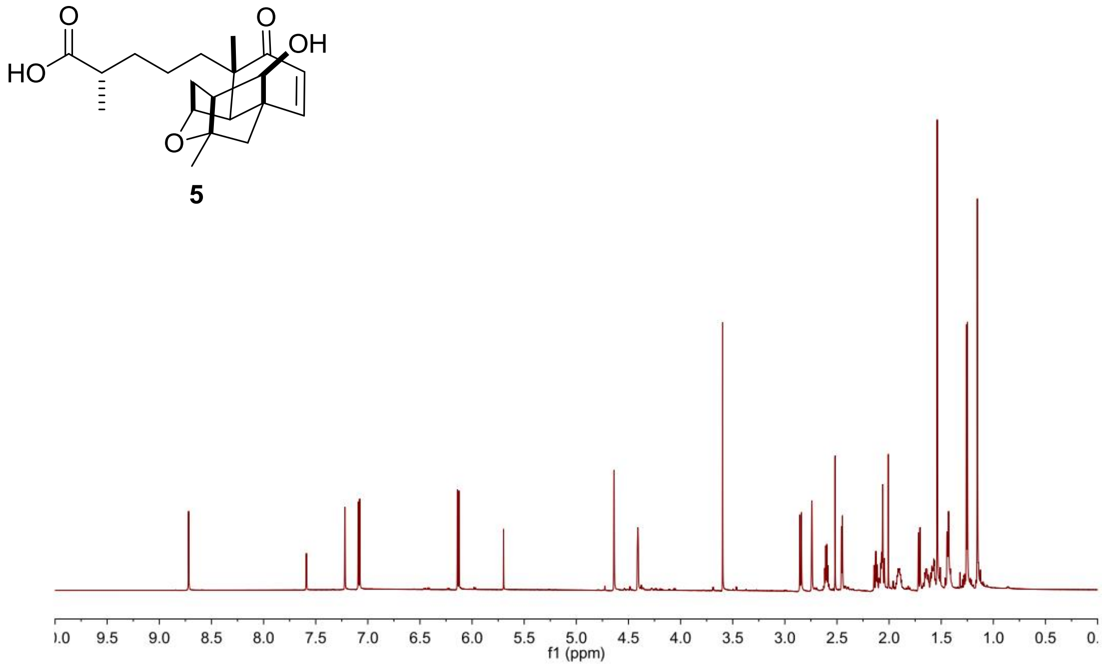

Figure S10. ^{13}C NMR spectrum of platensimycin ML3 (**5**) in pyridine-d_5 (175 MHz).

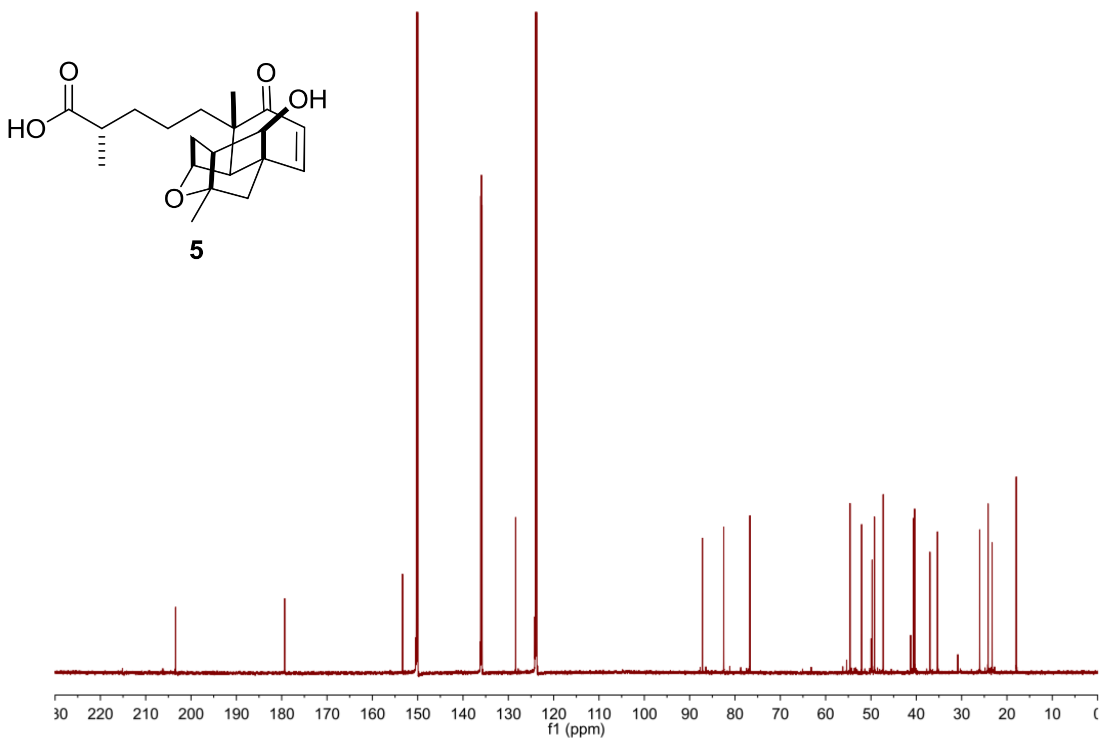

Figure S11. ^{1}H NMR spectrum of platensimycin ML4 (**6**) in pyridine-d_5 (700 MHz).

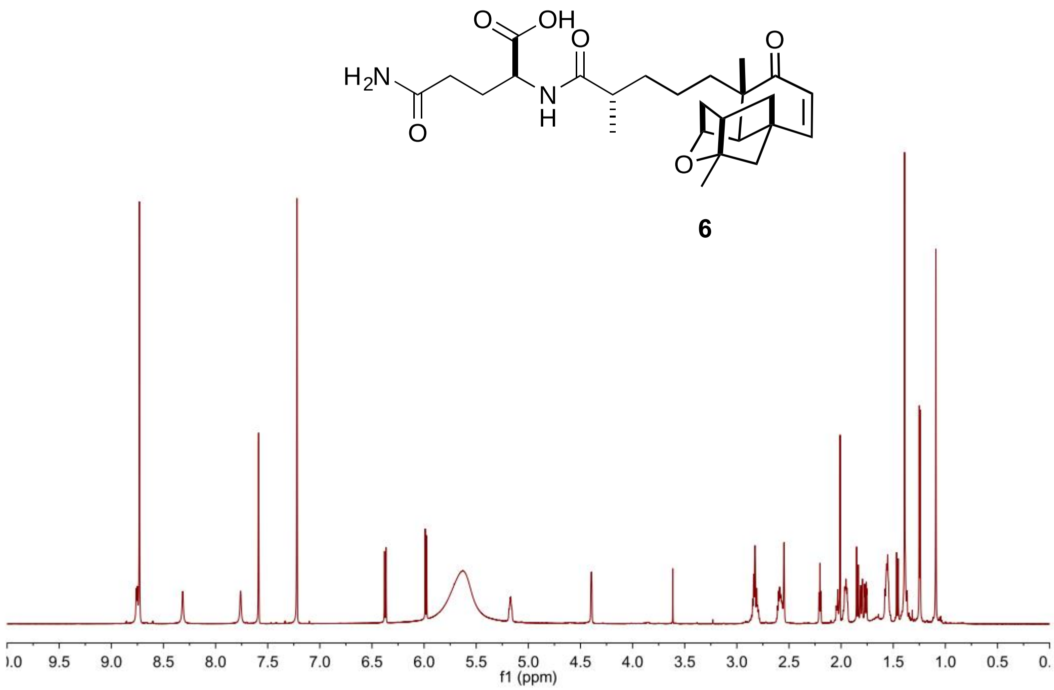

Figure S12. ^{13}C NMR spectrum of platensimycin ML4 (**6**) in pyridine-d_5 (175 MHz).

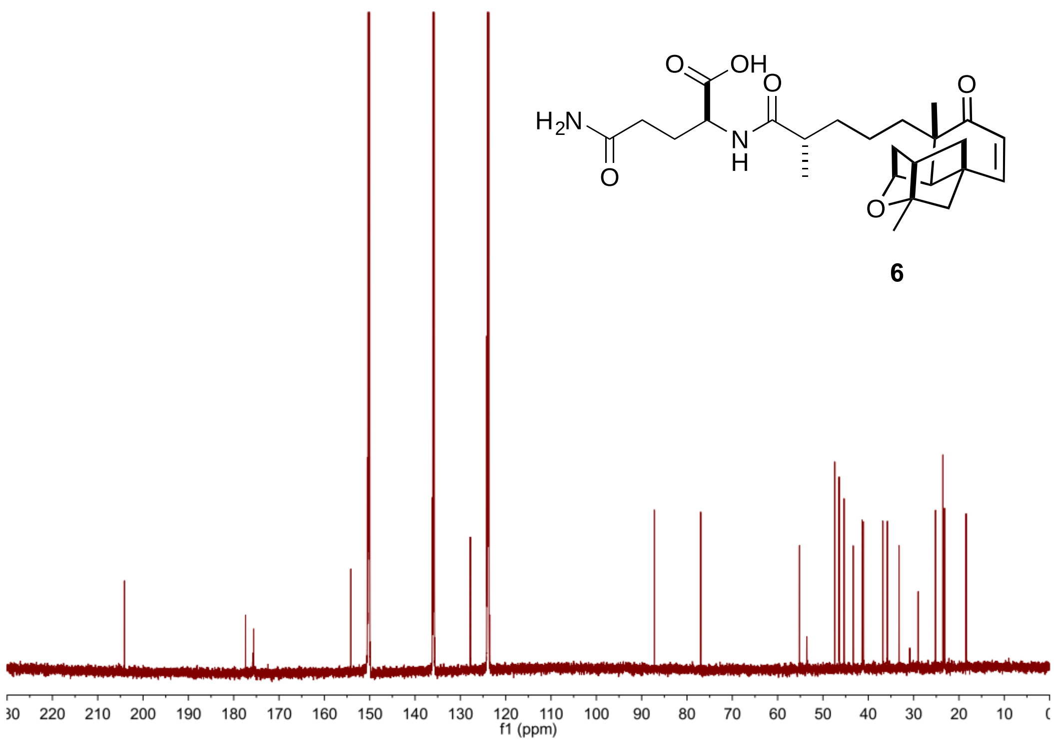

Figure S13. ^{1}H NMR spectrum of platensimycin ML5 (**7**) in pyridine-d_5 (700 MHz).

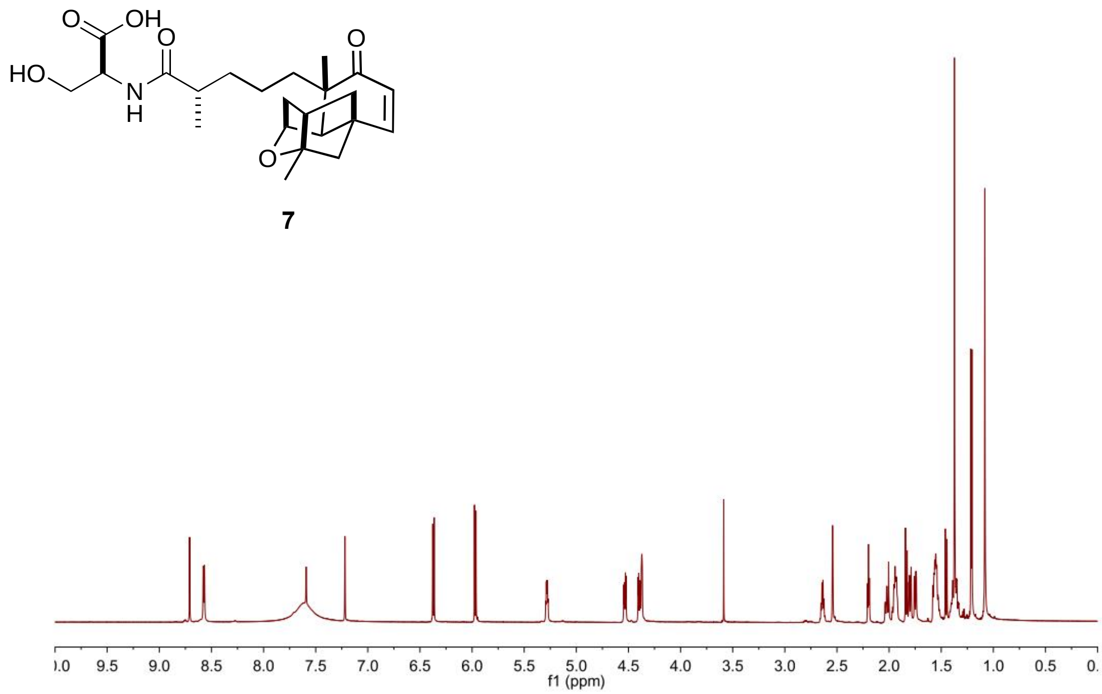

Figure S14. ^{13}C NMR spectrum of platensimycin ML5 (**7**) in pyridine-d_5 (175 MHz).

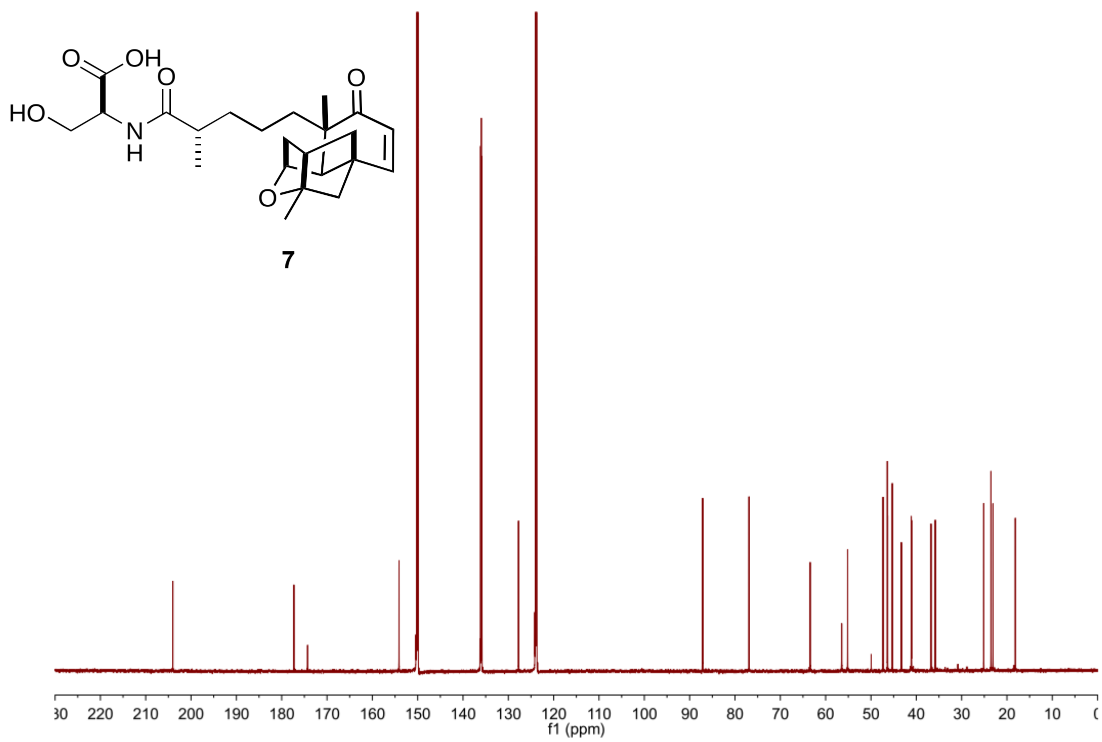

Figure S15. ^{1}H NMR spectrum of platensimycin ML6 (**8**) in pyridine-d_5 (700 MHz).

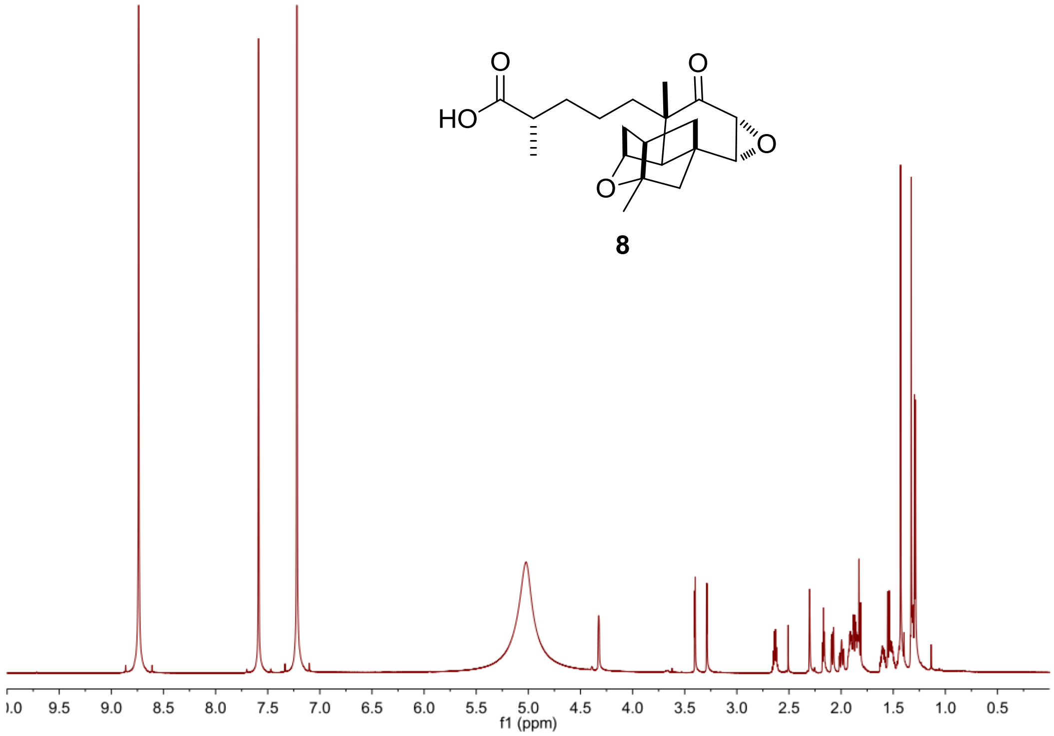

Figure S16. ^{13}C NMR spectrum of platensimycin ML6 (**8**) in pyridine-d_5 (175 MHz).

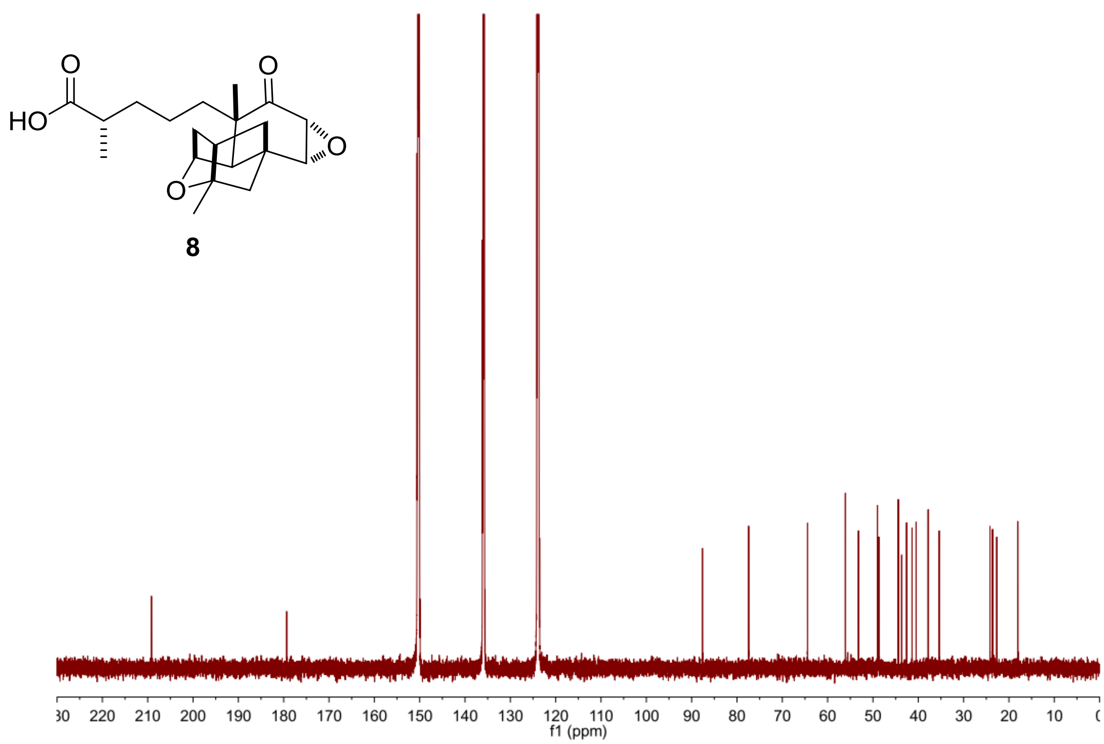

Figure S17. ^{1}H NMR spectrum of platensimycin ML7 (**9**) in pyridine-d_5 (700 MHz).

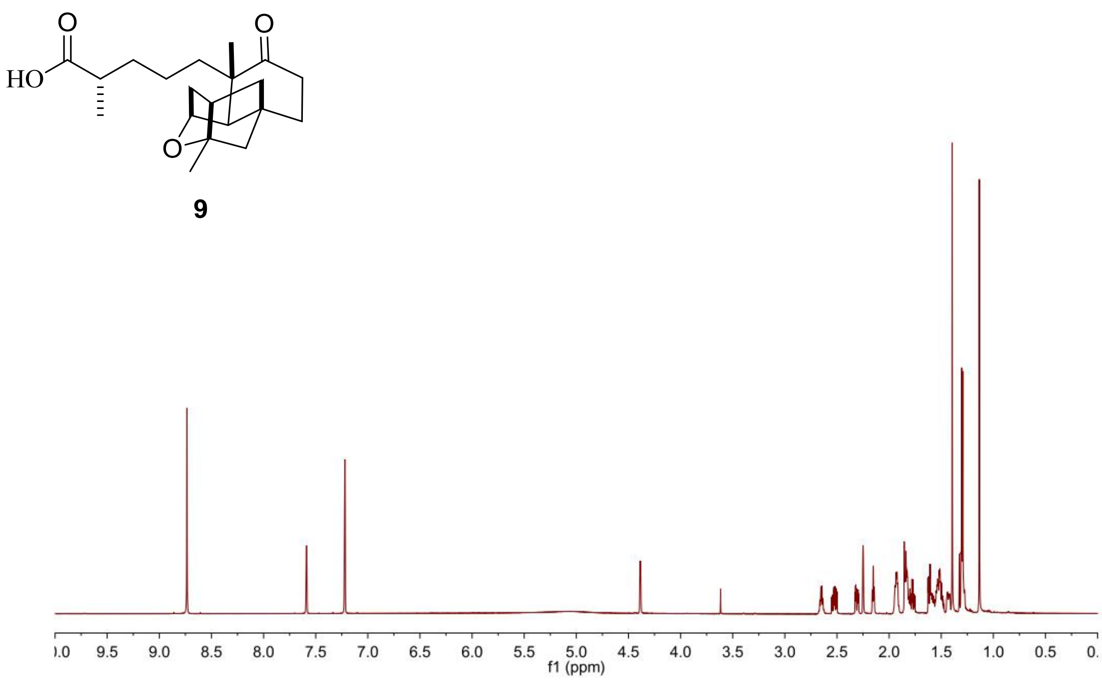

Figure S18. ^{13}C NMR spectrum of platensimycin ML7 (**9**) in pyridine-d_5 (175 MHz).

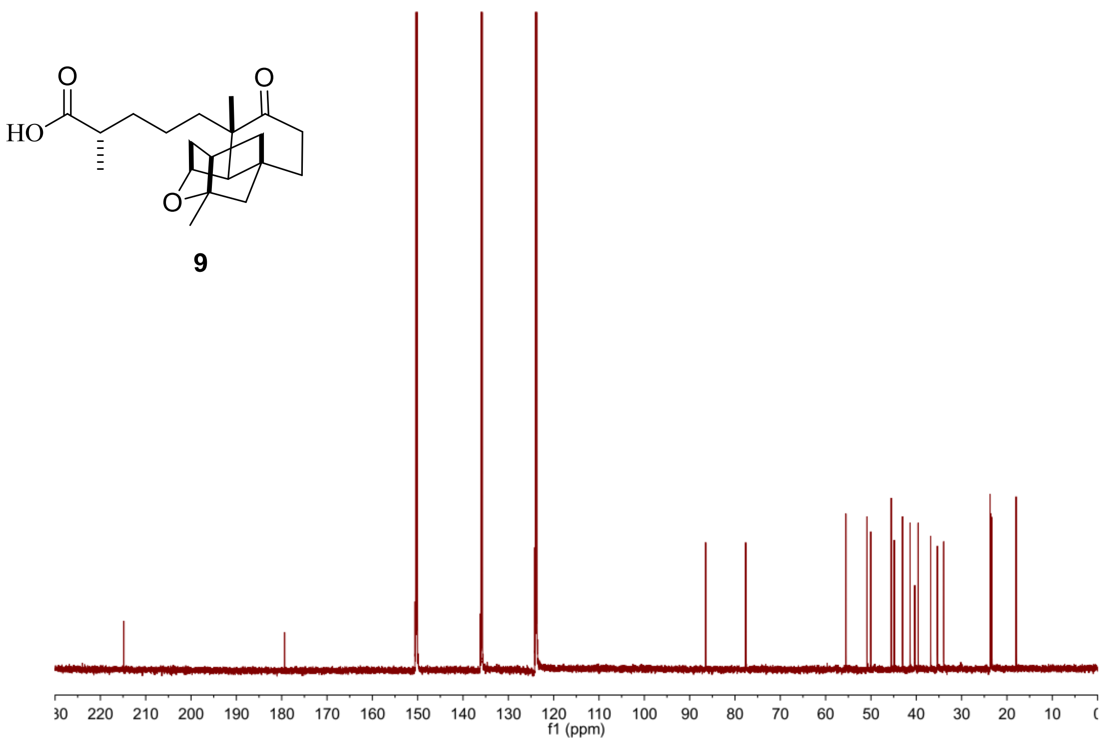

Figure S19. ^{1}H NMR spectrum of platensimycin ML8 (**10**) in pyridine-d_5 (700 MHz).

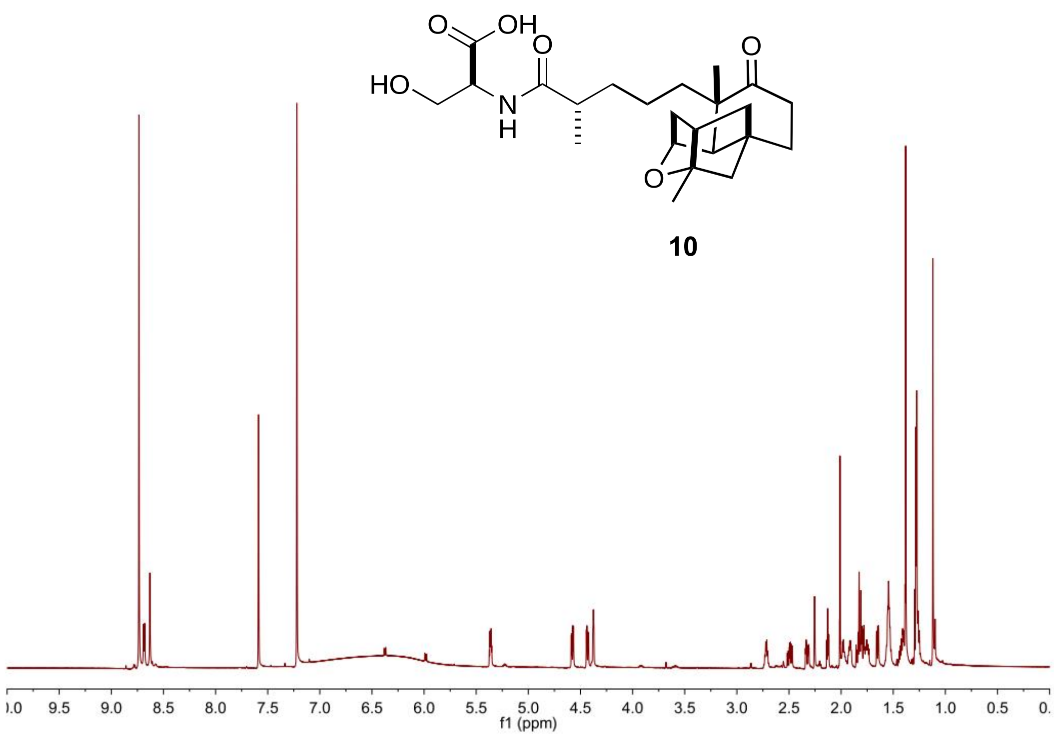

Figure S20. ^{13}C NMR spectrum of platensimycin ML8 (**10**) in pyridine-d_5 (175 MHz).

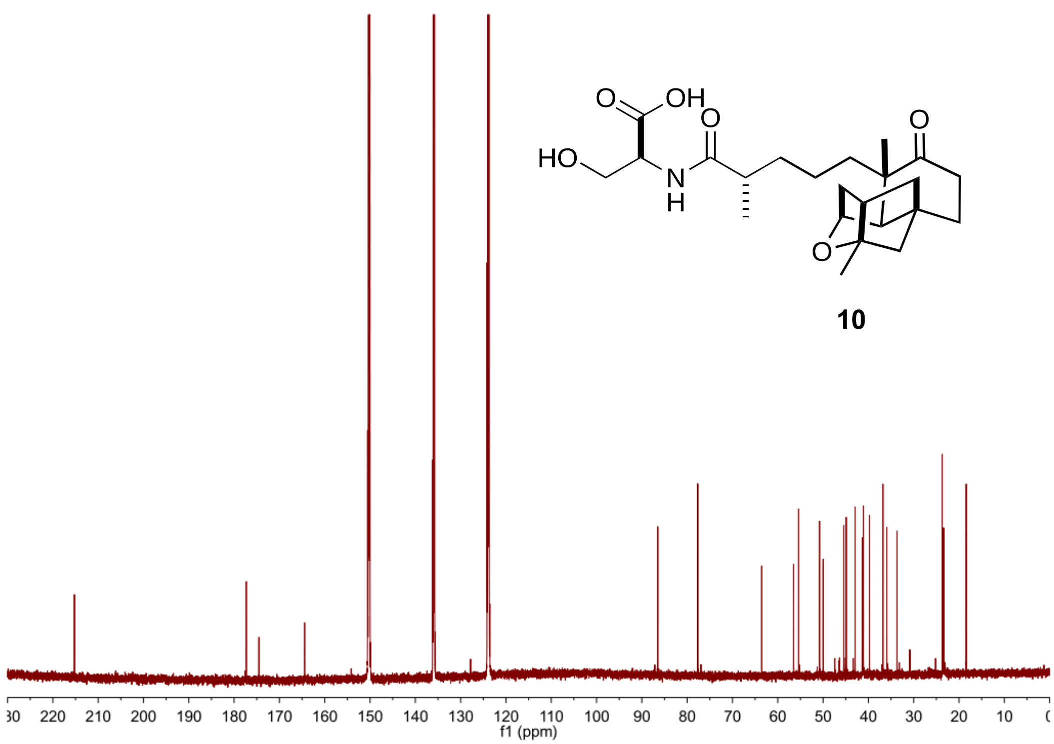

Figure S21. ^{1}H NMR spectrum of platencin ML1 (**12**) in pyridine-d_5 (700 MHz).

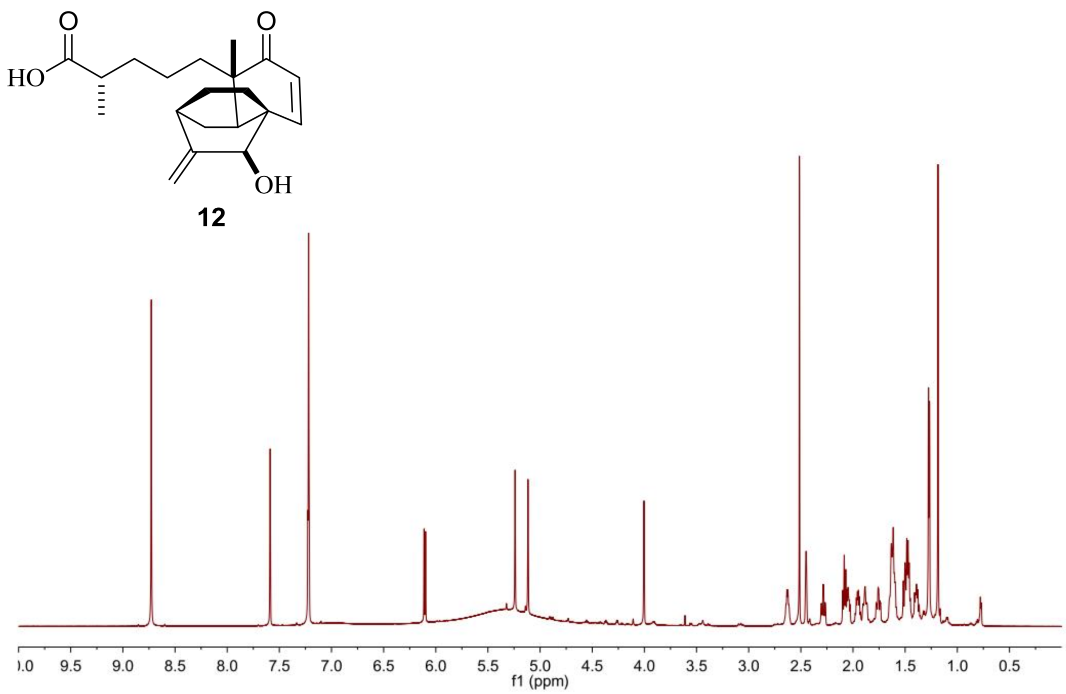

Figure S22. ^{13}C NMR spectrum of platencin ML1 (**12**) in pyridine-d_5 (175 MHz).

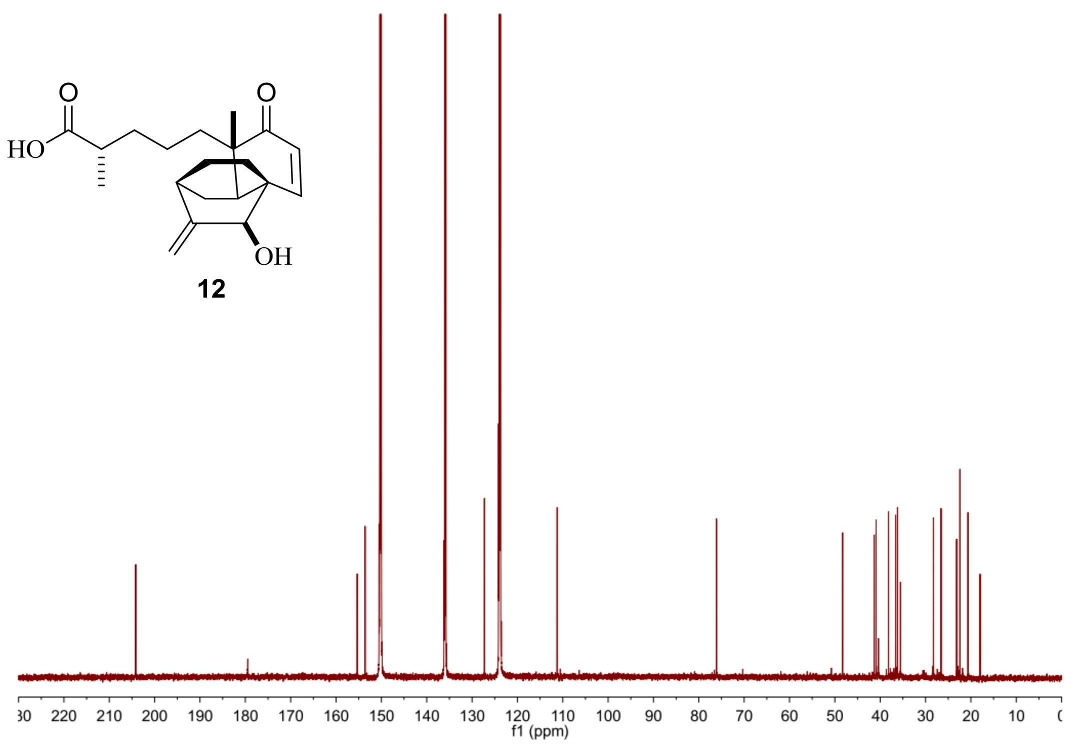

Figure S21. ^{1}H NMR spectrum of platencin ML2 (**13**) in pyridine-d_5 (700 MHz).

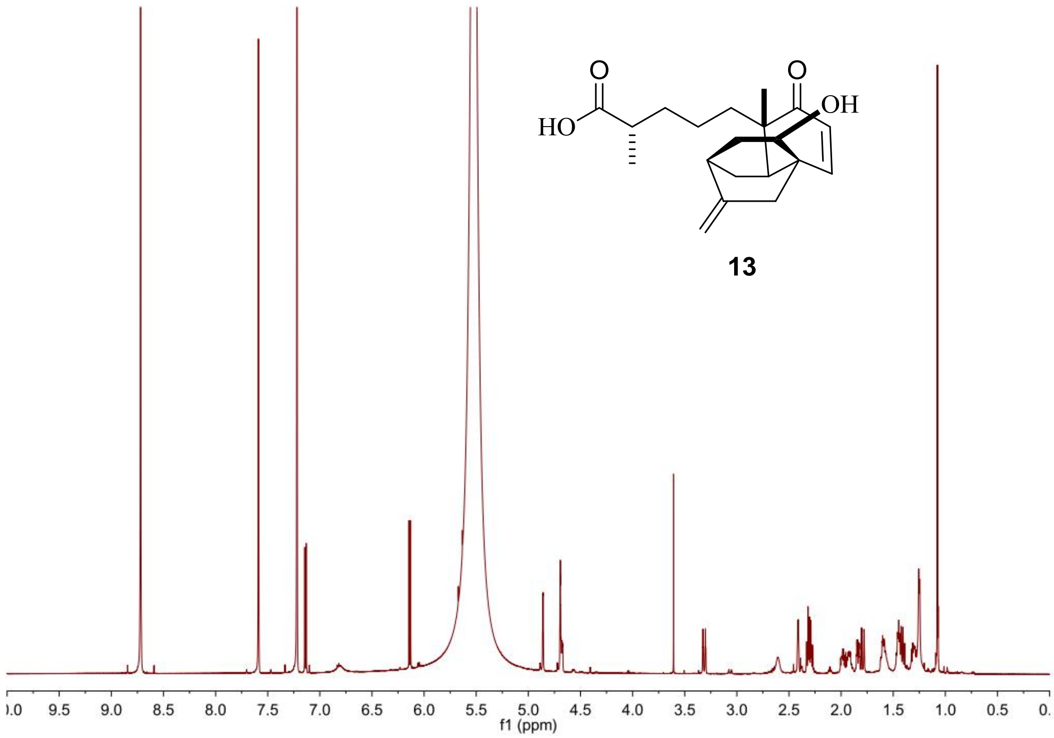

Figure S22. ^{13}C NMR spectrum of platencin ML2 (**13**) in pyridine-d_5 (175 MHz).

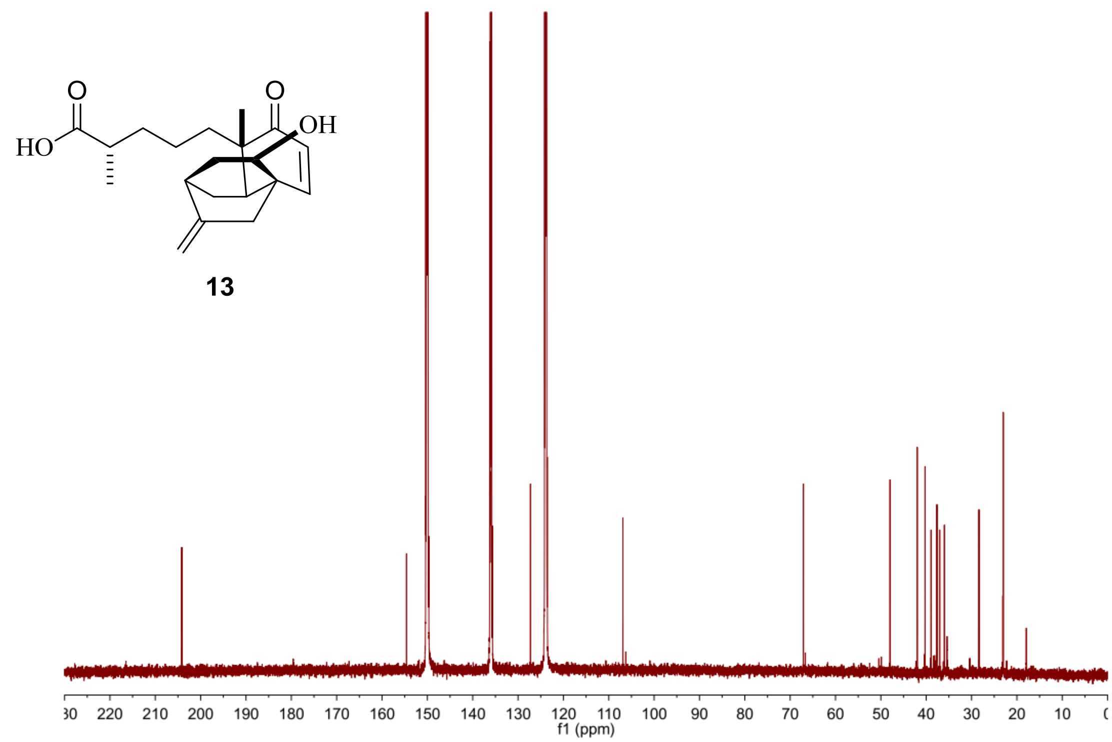

Figure S23. ^{1}H NMR spectrum of **3a** in $CDCl_3$ (700 MHz).

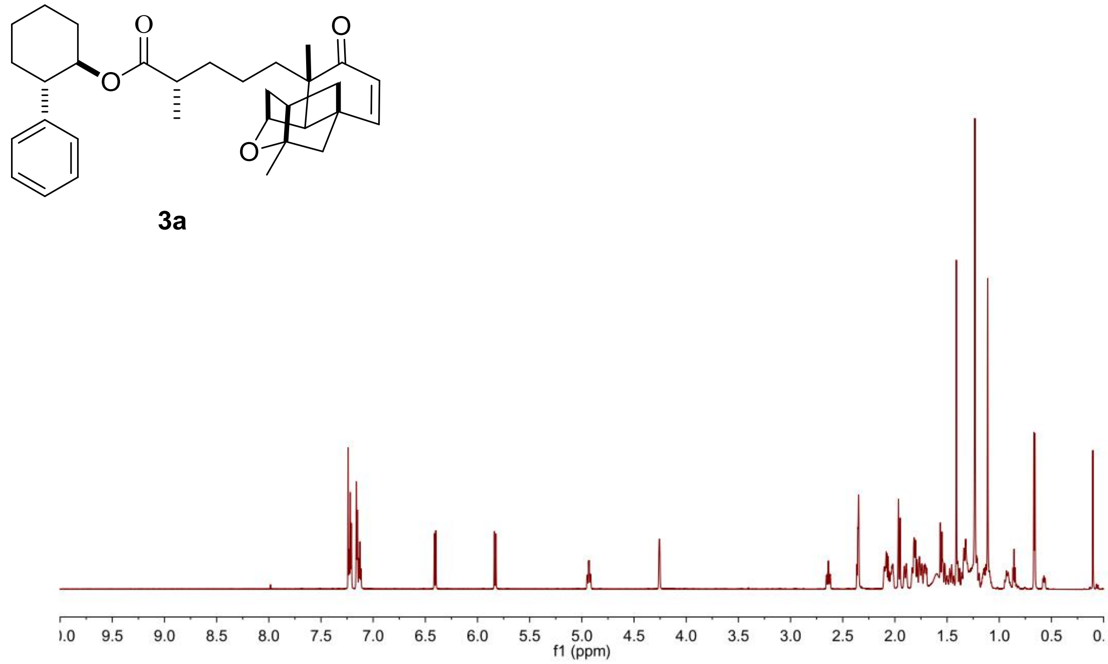

Figure S24. ^{13}C NMR spectrum of **3a** in $CDCl_3$ (175 MHz).

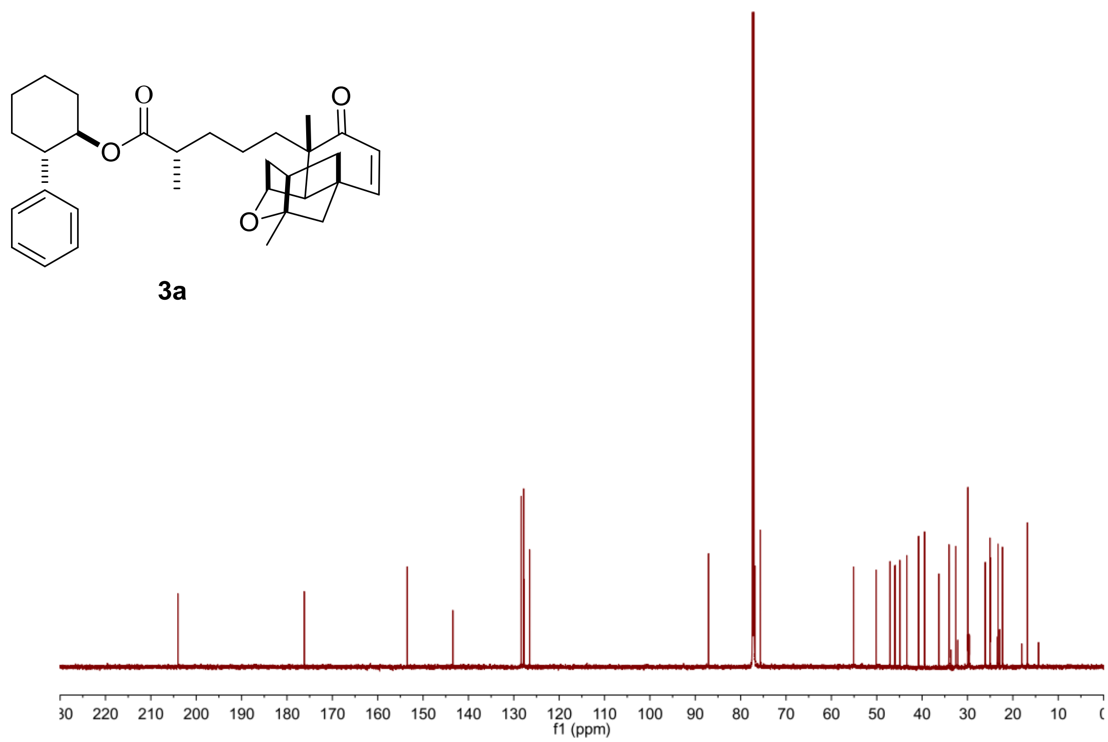

Figure S25. ^{1}H NMR spectrum of **3b** in $CDCl_3$ (700 MHz).

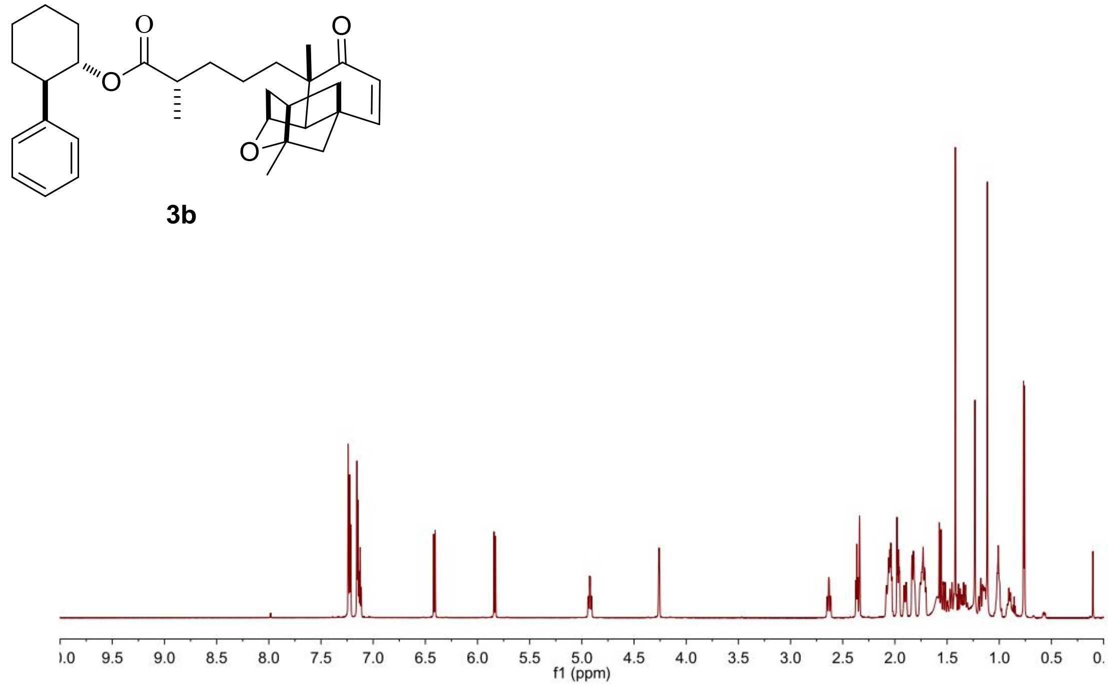

Figure S26. ^{13}C NMR spectrum of **3b** in $CDCl_3$ (175 MHz).

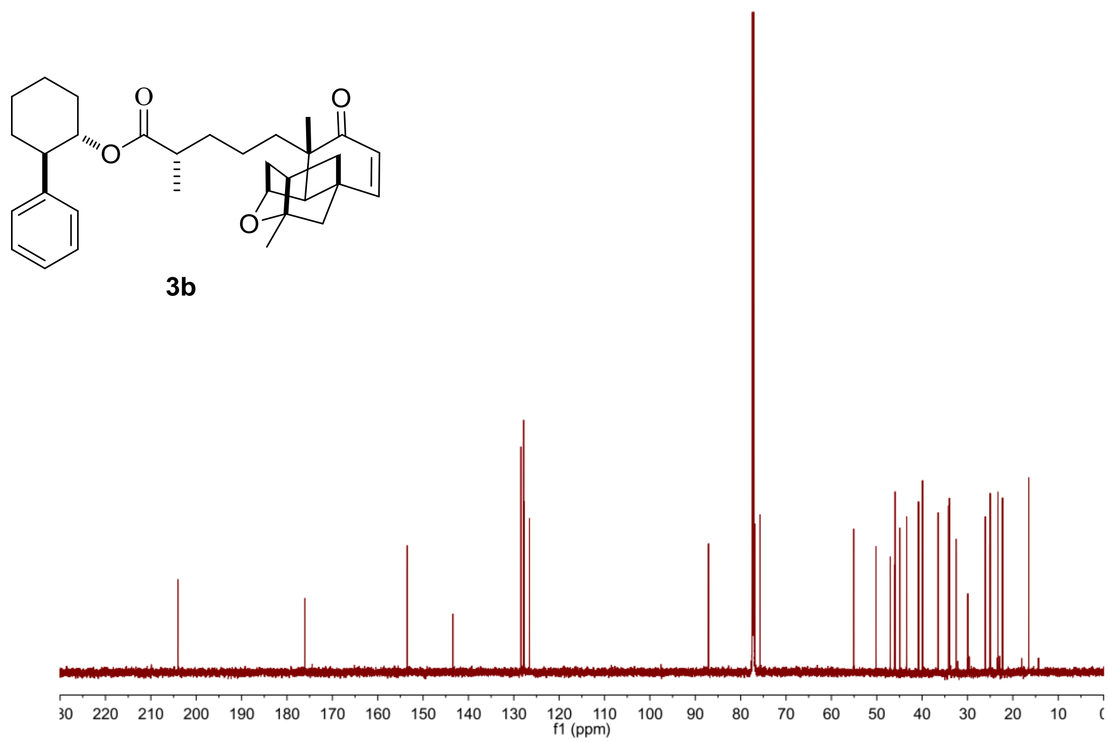

Figure S27. Determination of the absolute configuration at C4 of **3** by measuring $\Delta\delta^{RS}$ values of the C20 methyl protons of **3a** and **3b**, (1*R*,2*S*)- and (1*S*,2*R*)-2-phenyl-1-cyclohexanol esters of **3**.

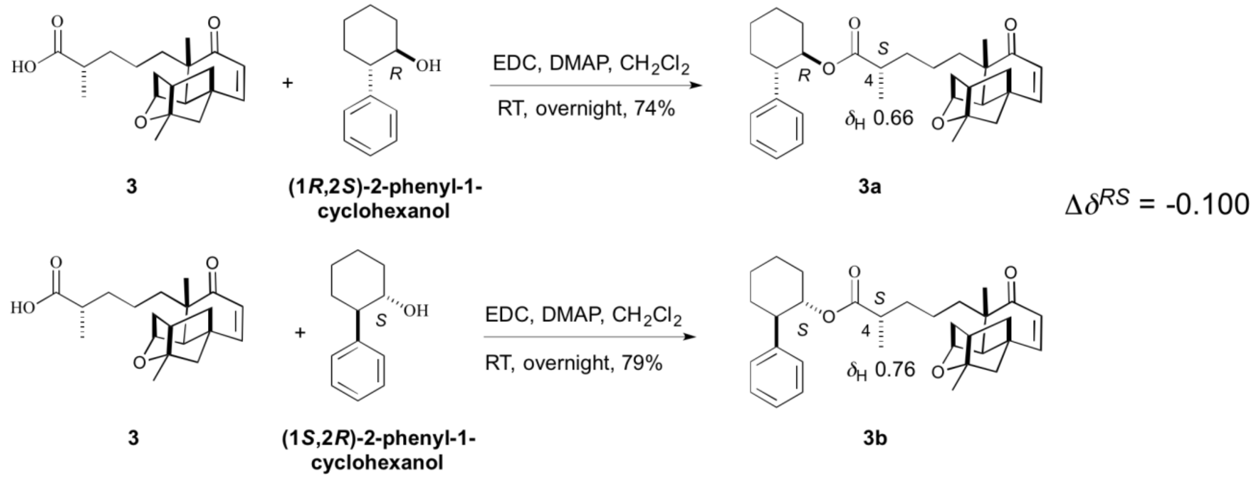

Figure S28. Key COSY (bold lines), HMBC (blue single arrows), and ROESY (red double arrows) correlations supporting the structures of compounds **3 – 5, 8, 12,** and **13**.

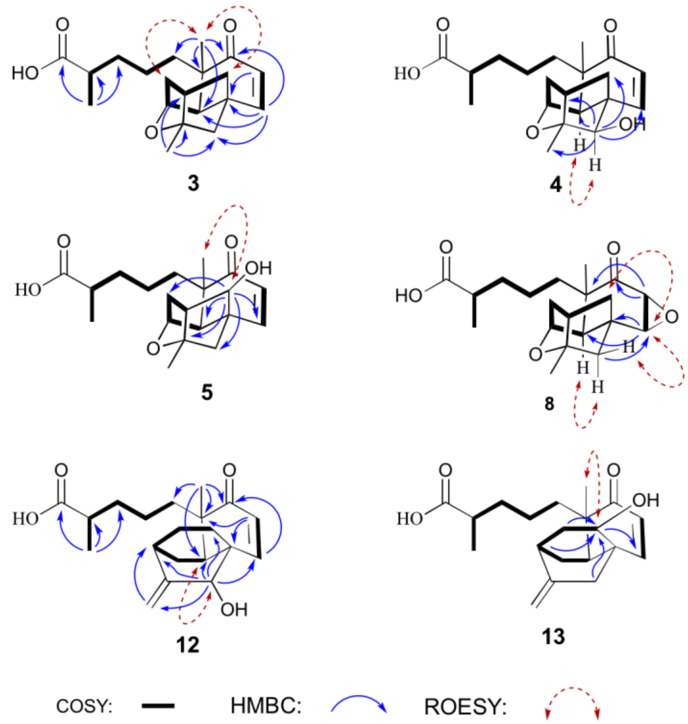

Table S9. Genes with high homology to *ptmO4* found within the *S. platensis* CB00739 genome. A BLAST[8] search of the *S. platensis* CB00739 genome using *ptmO4* as the query sequence revealed putative long-chain acyl-CoA dehydrogenases that may be responsible for limited processing of PTM and PTN in the absence of *ptmO4*. The top 17 hits with >90% coverage are listed below; the top hit is *ptmO4*.

Contig	Sequence	Coverage (%)	Identity (%)	Positives (%)	Gaps (%)
6	585876-587042	100	100	100	0
1	832033-834468	97	53	69	0
3	1032651-1033808	97	47	63	1
7	662602-663753	97	37	53	1
3	1040826-1041995	95	41	53	4
8	482090-483265	96	39	54	3
1	650841-652046	93	35	54	4
1	1549974-1551146	96	36	51	0
1	2169130-2170407	96	33	47	9
1	1738158-1739384	95	34	49	7
1	1828781-1829953	95	33	47	5
14	71912-73120	96	32	47	2
4	534421-535659	94	31	48	11
8	66473-68392	94	31	47	5
5	86803-87966	96	33	47	5
1	910841-912166	94	28	42	1
3	390764-392029	94	30	42	2

Supplementary References

1. B. Gust, G. L. Challis, K. Fowler, T. Kieser and K. F. Chater, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 1541-1546.
2. P. P. Cherepanov and W. Wackernagel, *Gene*, 1995, **158**, 9-14.
3. D. J. MacNeil, K. M. Gewain, C. L. Ruby, G. Dezeny, P. H. Gibbons and T. MacNeil, *Gene*, 1992, **111**, 61-68.
4. Hindra, T. Huang, D. Yang, J. D. Rudolf, P. Xie, G. Xie, Q. Teng, J. R. Lohman, X. Zhu, Y. Huang, L.-X. Zhao, Y. Jiang, Y. Duan and B. Shen, *J. Nat. Prod.*, 2014, **77**, 2296-2303.
5. M. J. Smanski, J. Casper, R. M. Peterson, Z. Yu, S. R. Rajski and B. Shen, *J. Nat. Prod.*, 2012, **75**, 2158-2167.
6. W. Chen, D. Dai, C. Wang, T. Huang, L. Zhai and Z. Deng, *Microb. Cell Factories*, 2013, **12**, 121.
7. M. Doumith, P. Weingarten, U. F. Wehmeier, K. Salah-Bey, B. Benhamou, C. Capdevila, J. M. Michel, W. Piepersberg and M. C. Raynal, *Mol. Gen. Genetics*, 2000, **264**, 477-485.
8. S. F. Altschul, W. Gish, W. Miller, E. W. Myers and D. J. Lipman, *J. Mol. Biol.*, 1990, **215**, 403-410.