Supplementary Online Material for

Dentigerumycin: a bacterial mediator of an ant-fungus symbiosis

Dong-Chan Oh[1,3], Michael Poulsen[2,3], Cameron R. Currie[2], and Jon Clardy[1]

[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave., Boston, Massachusetts 02115, USA.

[2]Department of Bacteriology, University of Wisconsin-Madison, 1550 Linden Dr., Madison, Wisconsin 53706, USA.

[3]These authors contributed equally to this work

Correspondence should be addressed to C.R.C. (currie@bact.wisc.edu) or J.C. (jon_clardy@hms.harvard.edu).

Supplementary Figures

Fig. 1. An example of pairwise bioassay challenges of the fungal cultivar (left) to *Pseudonocardia* sp. (center).

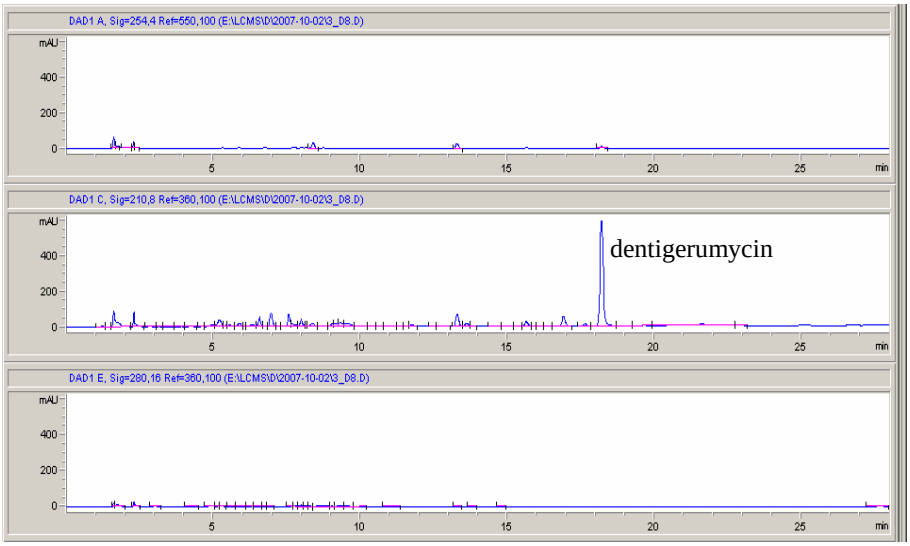
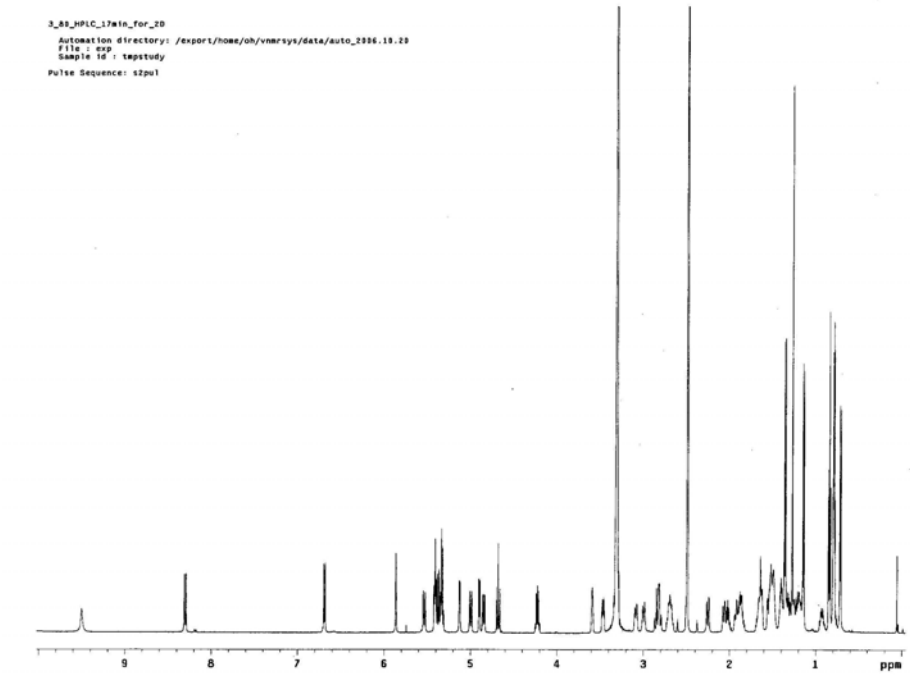
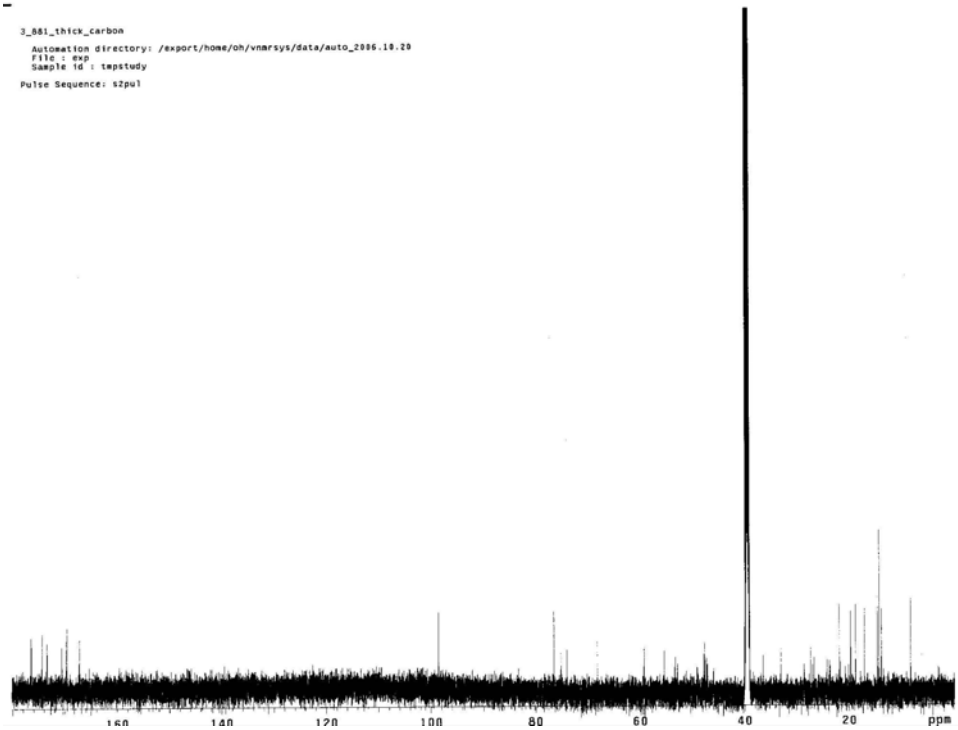


Fig. 2. The HPLC trace of the culture of the *Pseudonocardia* strain CC011120-4 showing only one major secondary metabolite peak, dentigerumycin (**1**), at 18.3 min.

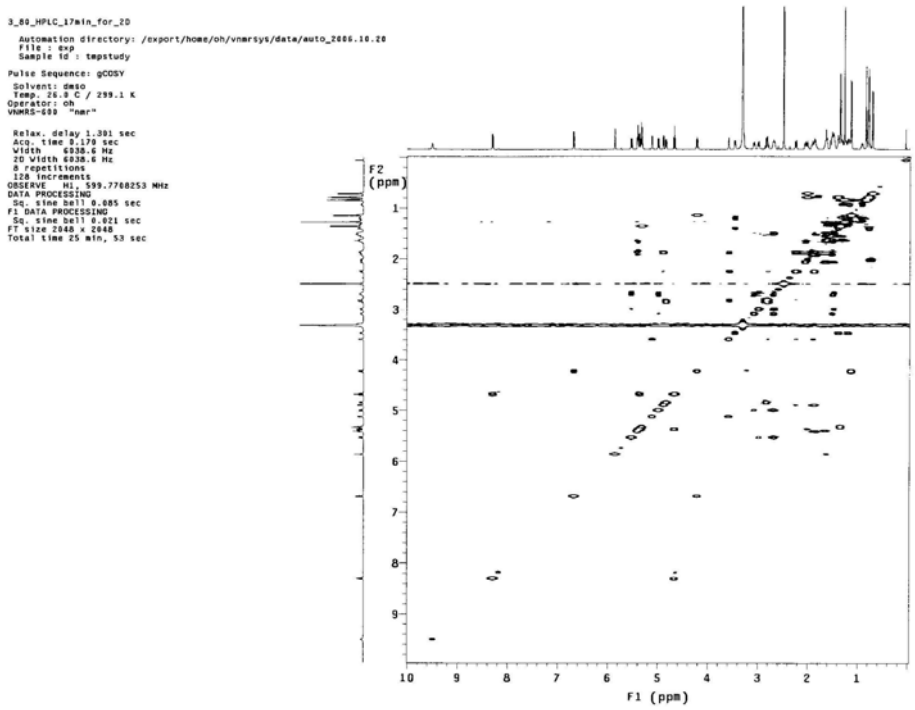
Fig. 3. NMR spectra of **1, 6, 7,** and **8**.



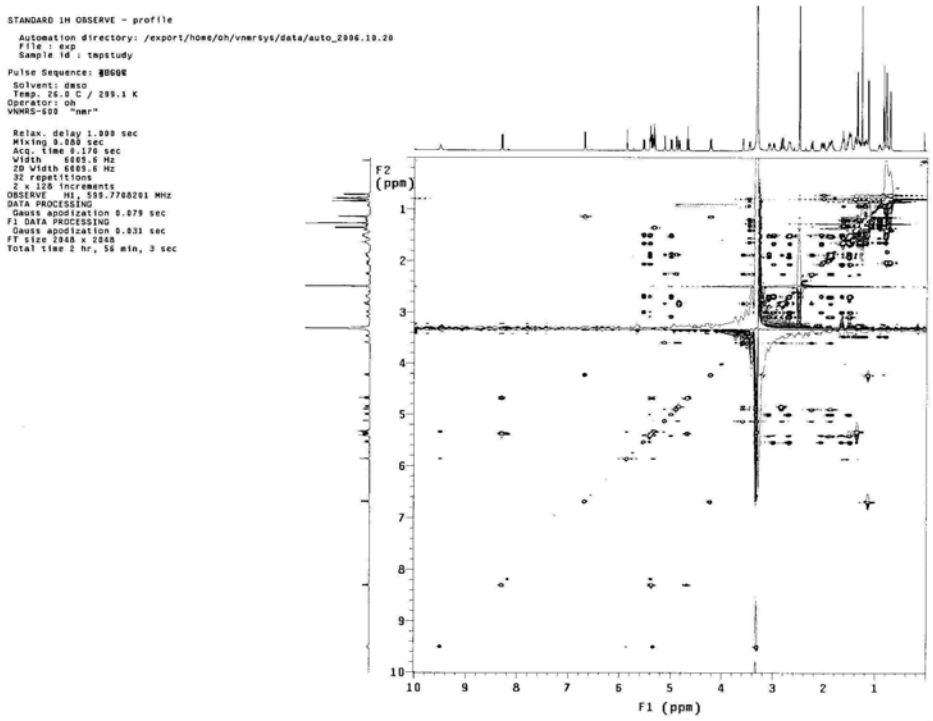
(a) ^{1}H NMR spectrum of dentigerumycin (**1**).



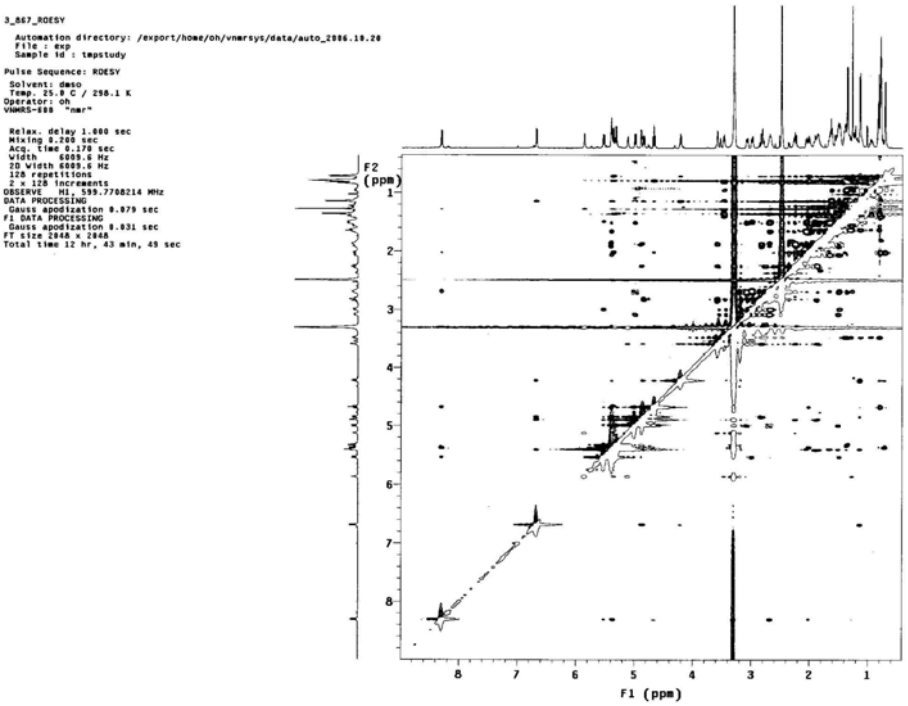
(b) ^{13}C NMR spectrum of dentigerumycin (**1**).



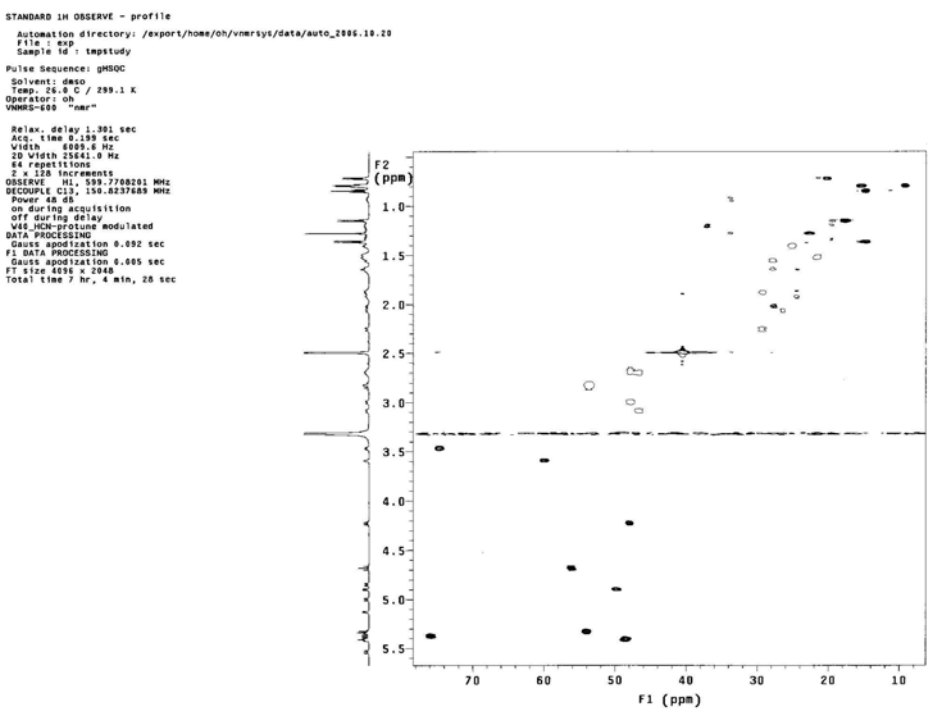
(c) gCOSY NMR spectrum of dentigerumycin (**1**).



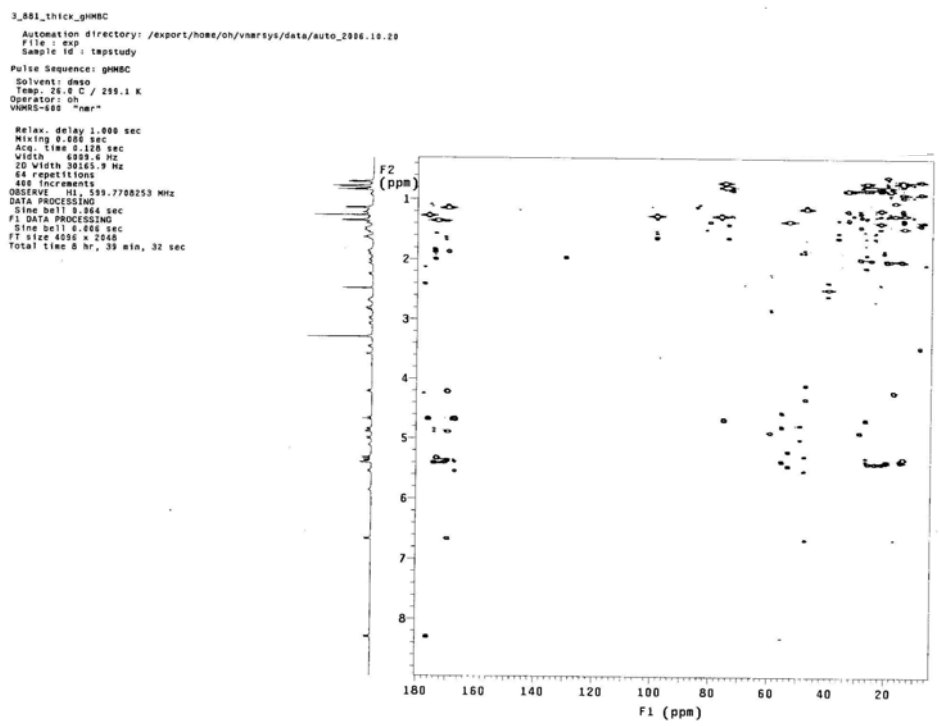
(d) TOCSY NMR spectrum of dentigerumycin (**1**).



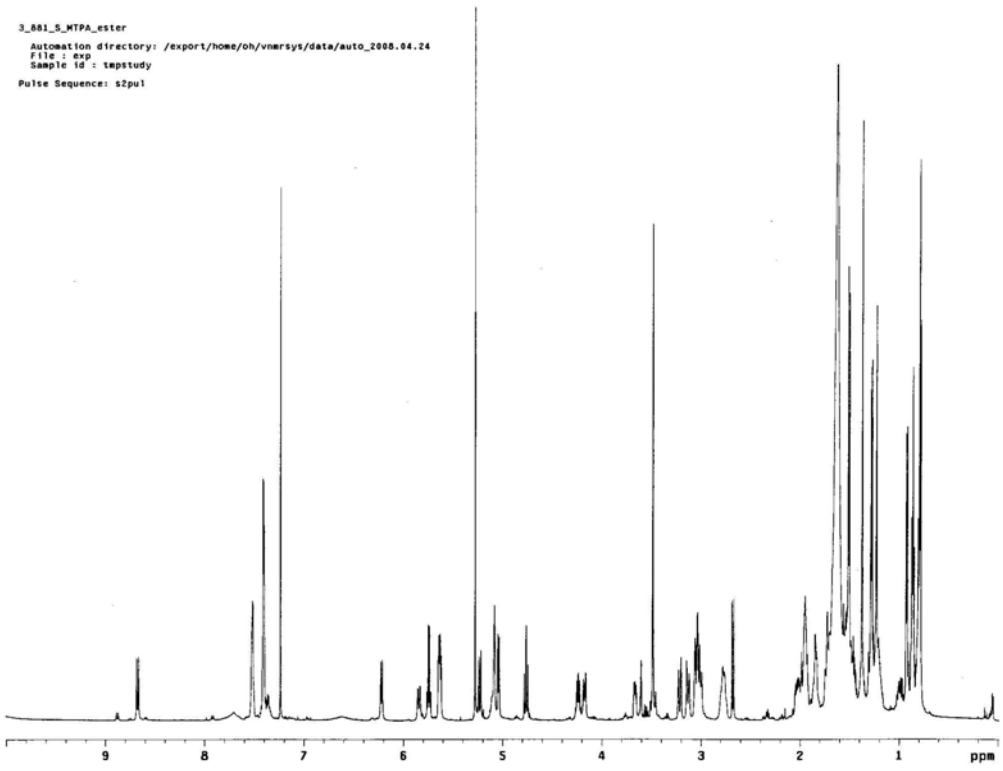
(e) ROESY NMR spectrum of dentigerumycin (**1**).



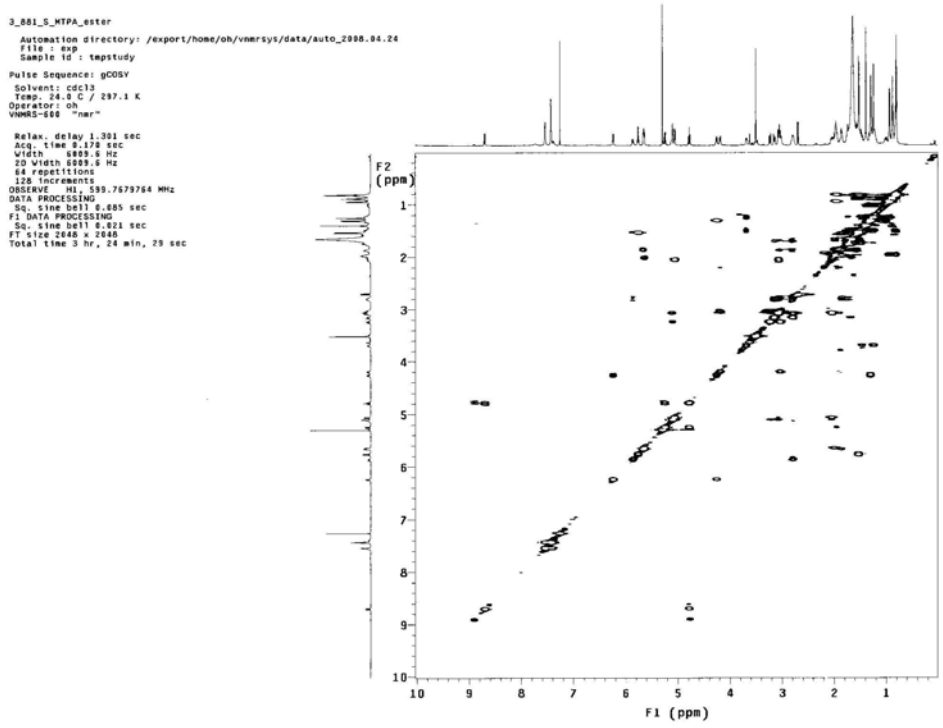
(f) gHSQC NMR spectrum of dentigerumycin (**1**).



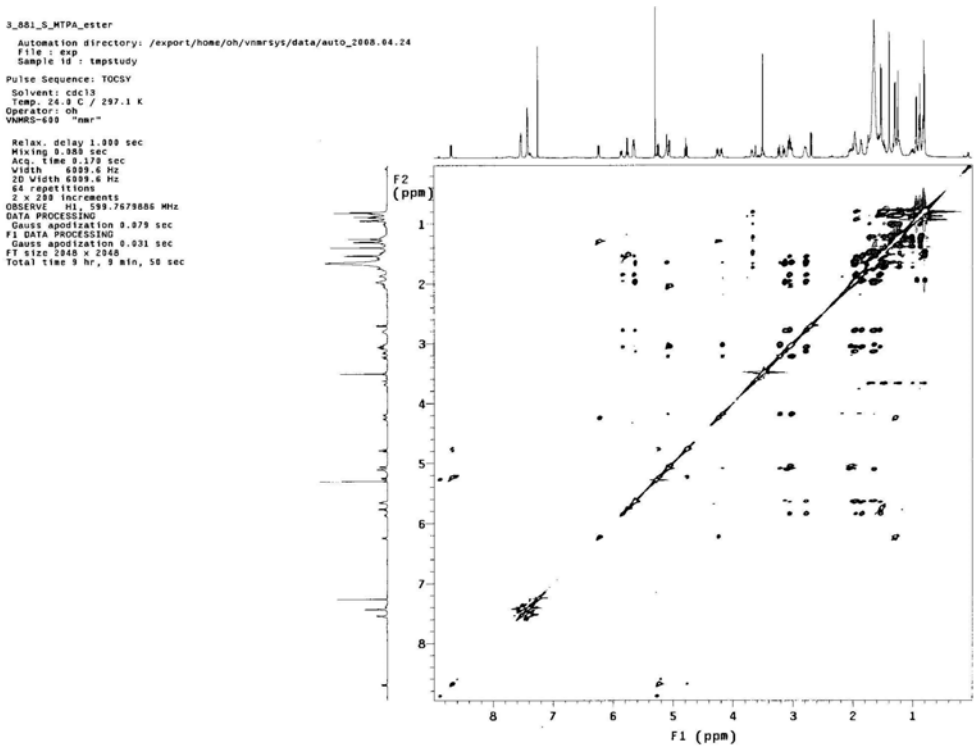
(g) gHMBC NMR spectrum of dentigerumycin (**1**).



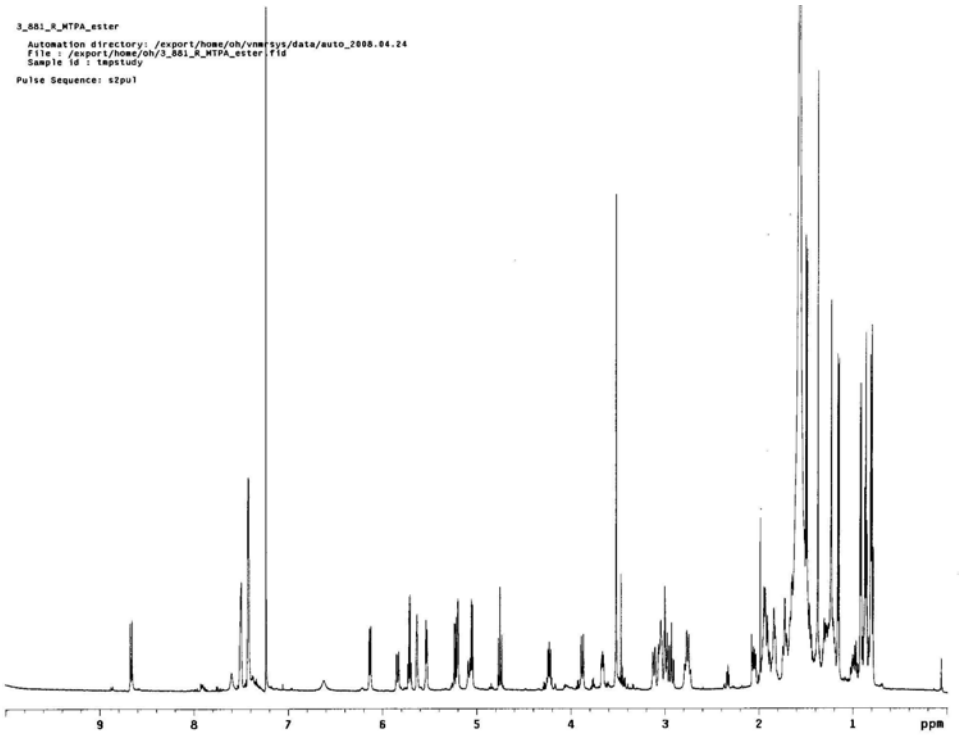
(h) ^{1}H NMR spectrum of *S*-MTPA ester (**6**) of dentigerumycin.



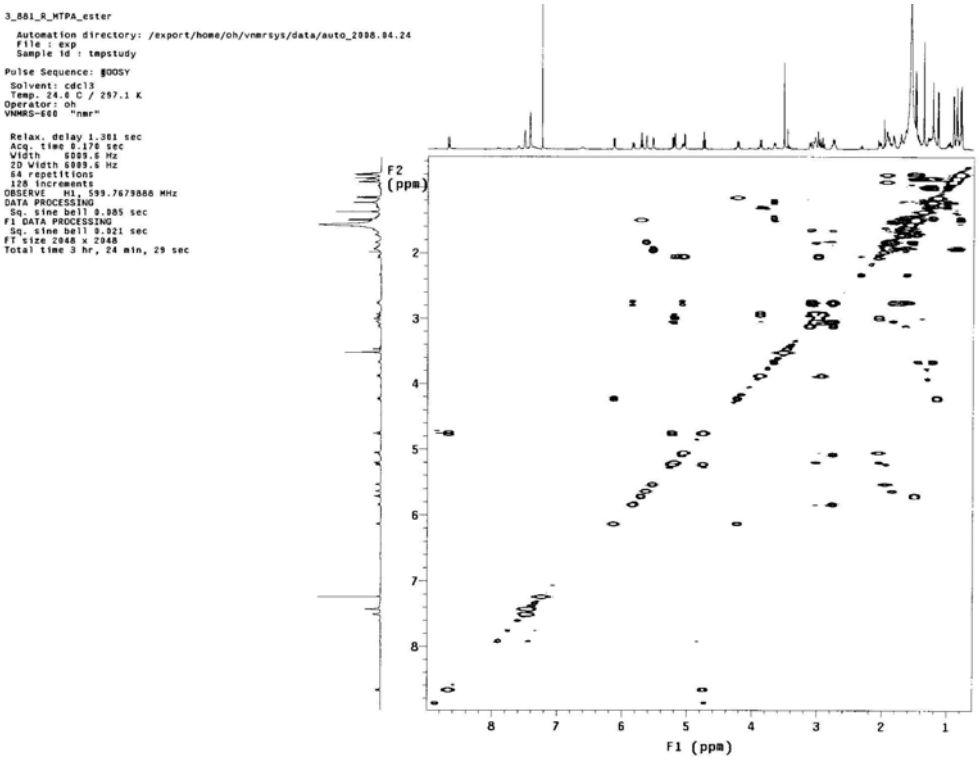
(i) gCOSY NMR spectrum of *S*-MTPA ester (**6**) of dentigerumycin.



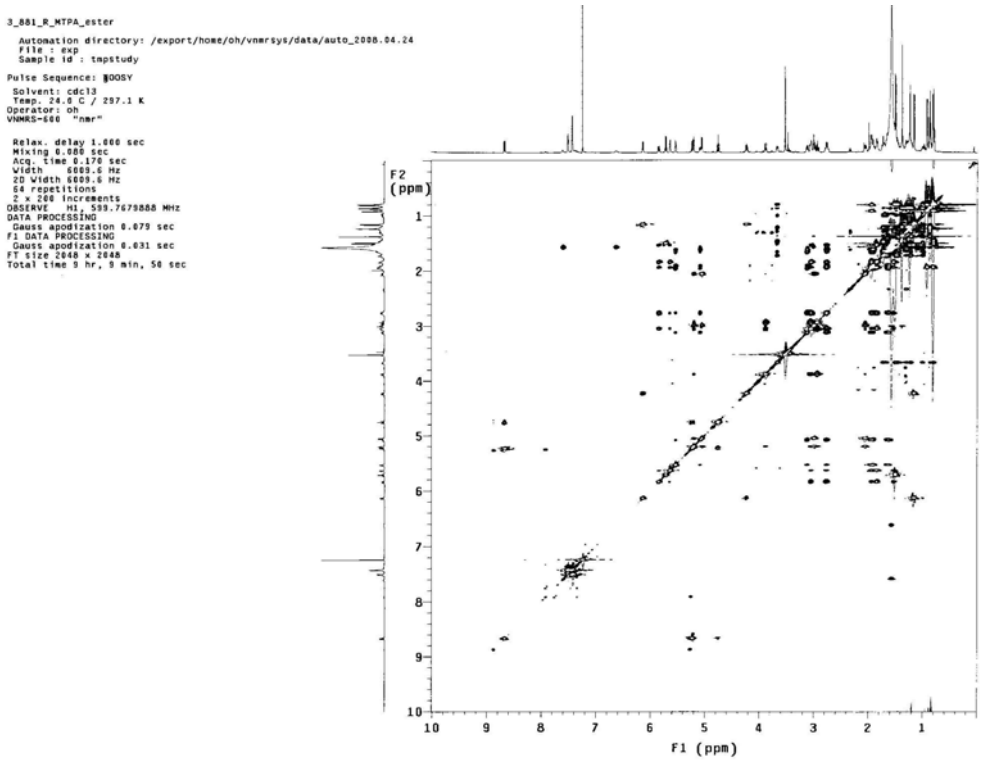
(j) TOCSY NMR spectrum of *S*-MTPA ester (**6**) of dentigerumycin.



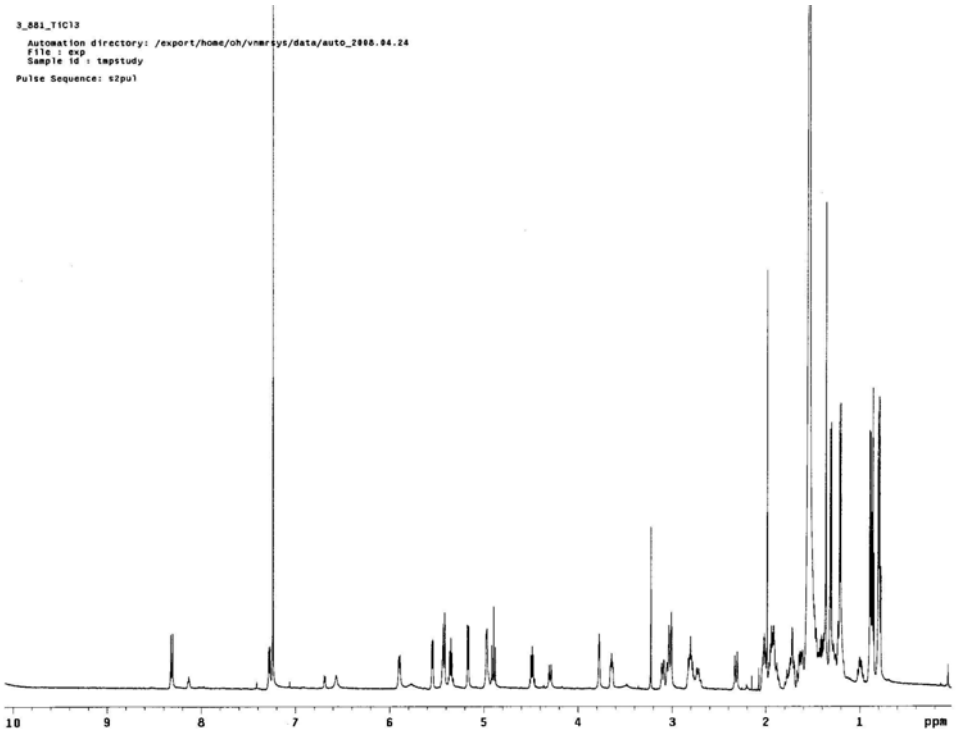
(k) ^{1}H NMR spectrum of *R*-MTPA ester (**7**) of dentigerumycin.



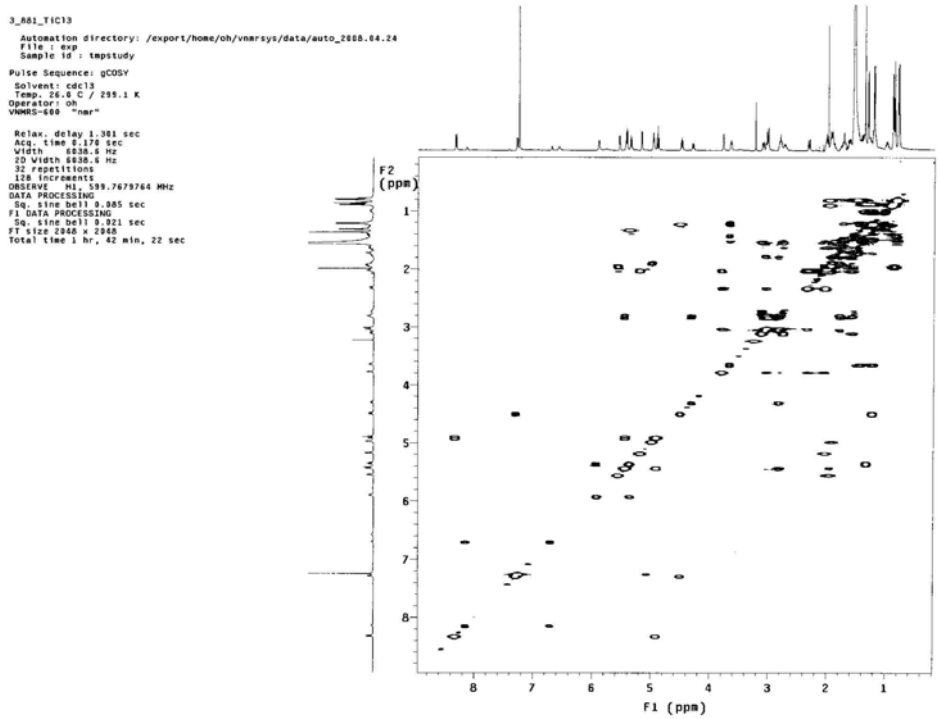
(l) gCOSY NMR spectrum of *R*-MTPA ester (**7**) of dentigerumycin.



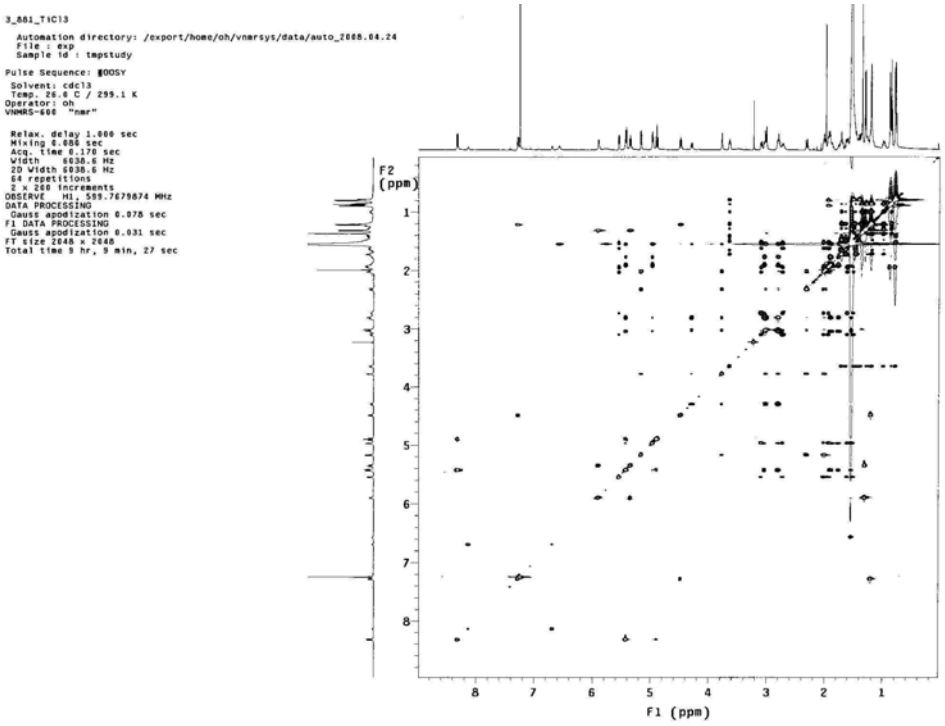
(m) TOCSY NMR spectrum of *R*-MTPA ester (**7**) of dentigerumycin.



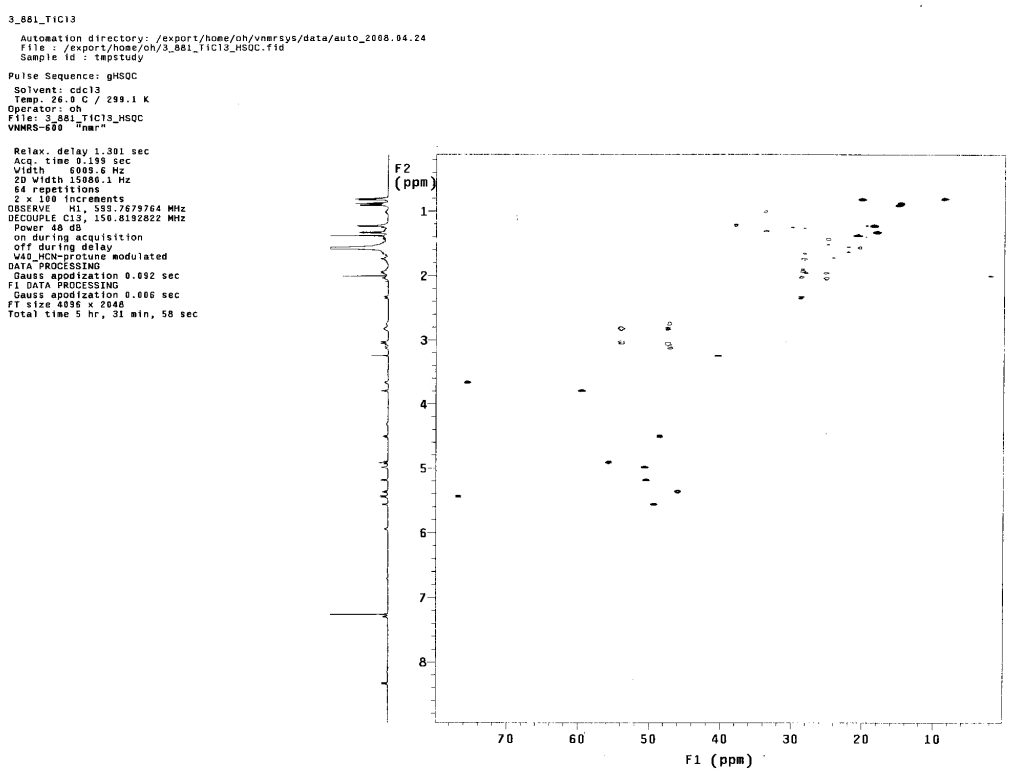
(n) ^{1}H NMR spectrum of 15-*N*-deoxydentigerumycin (**8**) of dentigerumycin.



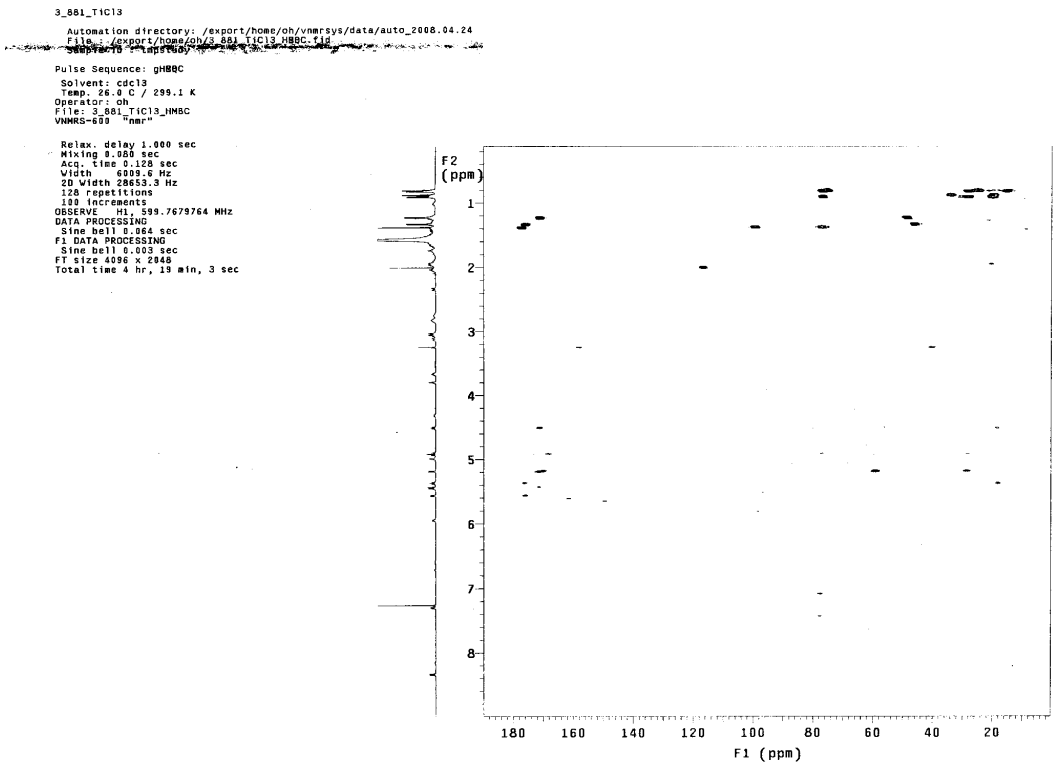
(o) gCOSY NMR spectrum of 15-*N*-deoxydentigerumycin (**8**) of dentigerumycin.



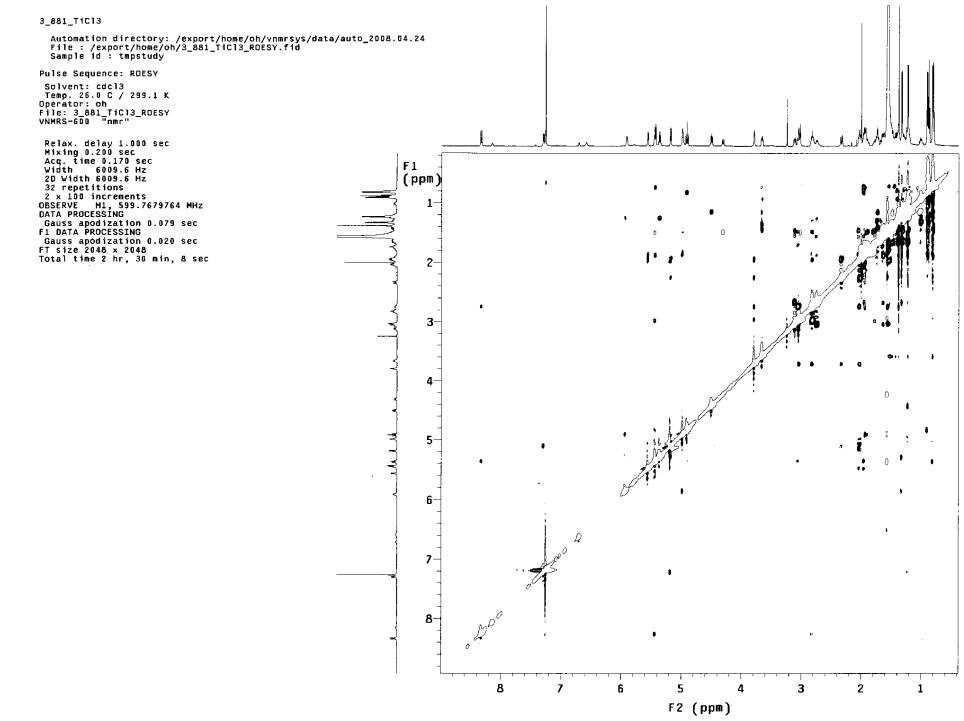
(p) TOCSY NMR spectrum of 15-*N*-deoxydentigerumycin (**8**) of dentigerumycin.



(q) gHSQC NMR spectrum of 15-*N*-deoxydentigerumycin (**8**) of dentigerumycin.



(r) gHMBC NMR spectrum of 15-*N*-deoxydentigerumycin (**8**) of dentigerumycin.



(s) ROESY NMR spectrum of 15-*N*-deoxydentigerumycin (**8**) of dentigerumycin.

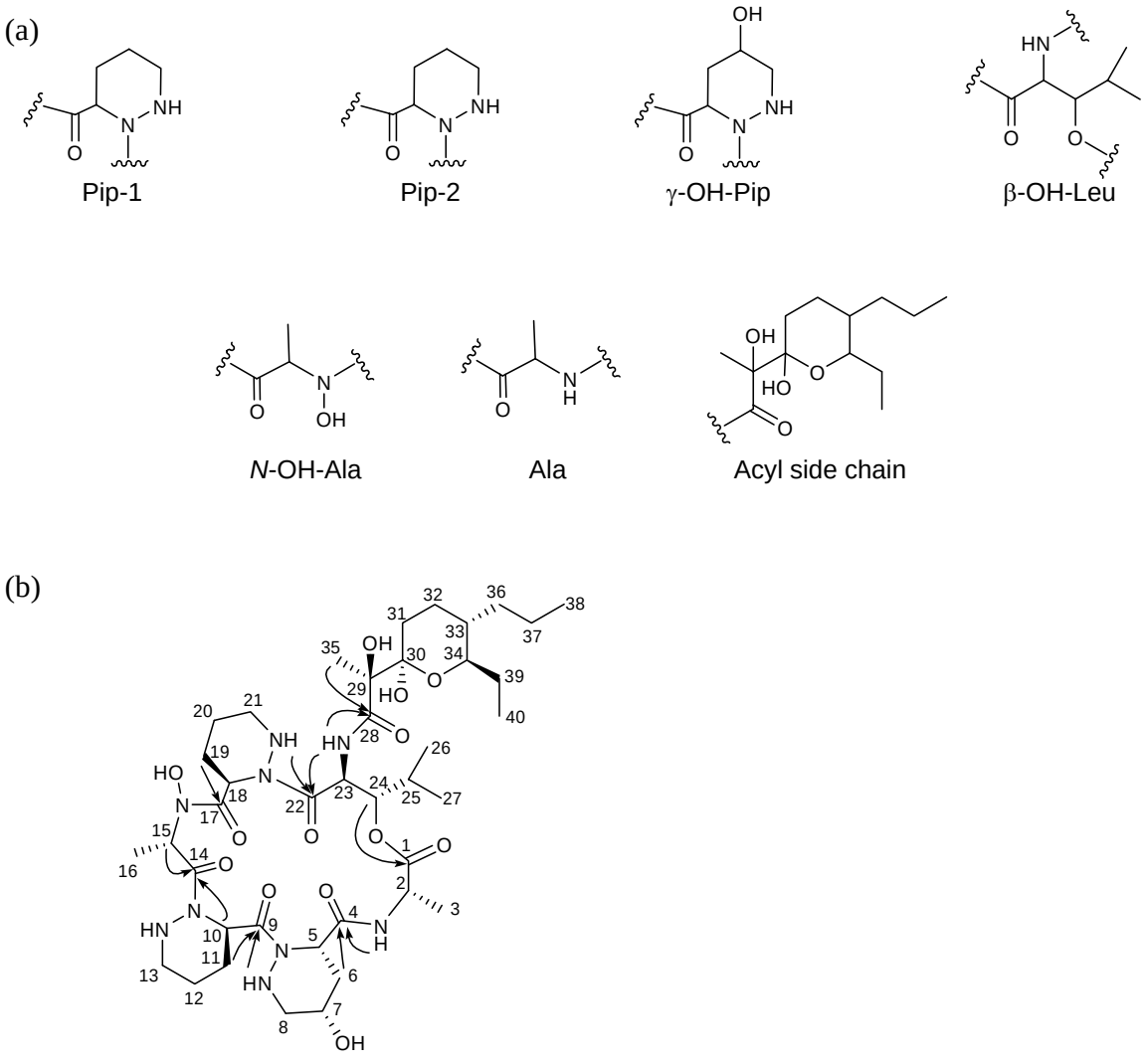


Fig. 4. Determination of the planar structure of dentigerumycin (**1**). (a) The partial structures of dentigerumycin (**1**) identified by gCOSY, TOCSY, HSQC, and HMBC NMR spectral analysis. (b) Key HMBC correlations (arrows) in dentigerumycin (**1**) secure the connectivity of these partial structures.

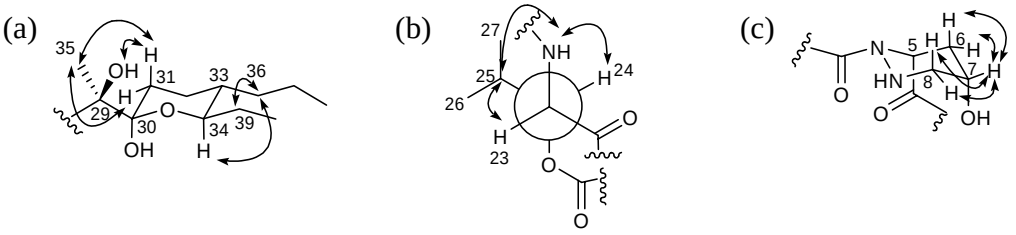


Fig. 5. Determination of relative configurations of dentigerumycin (**1**). (a) Key ROESY correlations in the acyl side chain. (b) C-23-C-24 rotamer with key ROESY correlations. The anti-relationship between H-23 and H-24 was established by J_{H23H24} (11.5 Hz)[2] (c) Key ROESY correlations in γ-OH-Pip. The ^{1}H-^{1}H coupling constants of the γ-OH-Pip unit were compared with the literature values.[3]

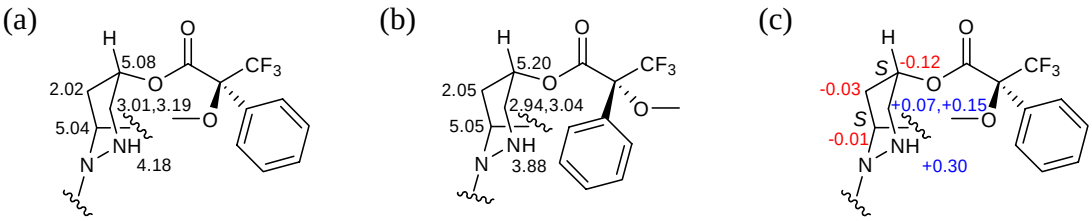


Fig. 6. The modified Mosher method analysis of **1**. ^{1}H chemical shifts of γ-hydroxy piperazic acid (a) in *S*-MTPA ester (**6**) and (b) in *R*-MTPA ester (**7**) in $CDCl_3$. (c) $\Delta\delta_{S-R}$ values in ppm of γ-hydroxy piperazic acid ppm for the *S*- and *R*-MTPA esters of dentigerumycin in $CDCl_3$, determining *S* configuration of the secondary alcohol.

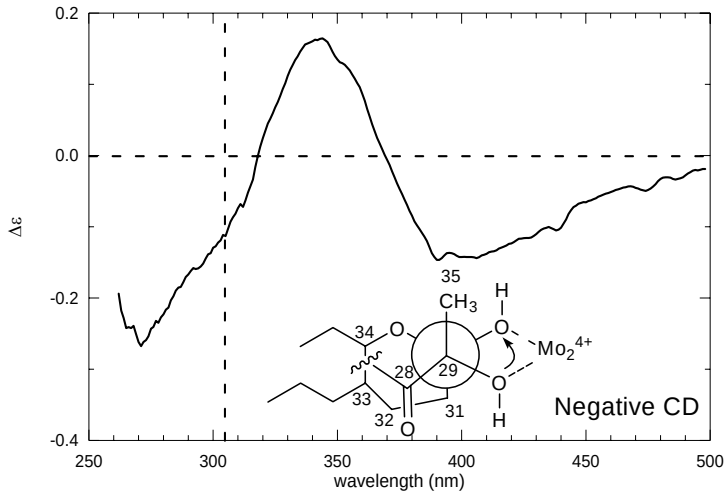


Fig. 7. Induced CD spectrum by dentigerumycin and $Mo_2(OAc)_4$ complex. The negative sign at 305 nm determines the absolute configurations of 29*S* and 30*R* based on the preferred conformation of the complex of the diol and the molybdenum ions shown with the spectrum.

(a)

(b)

(c)

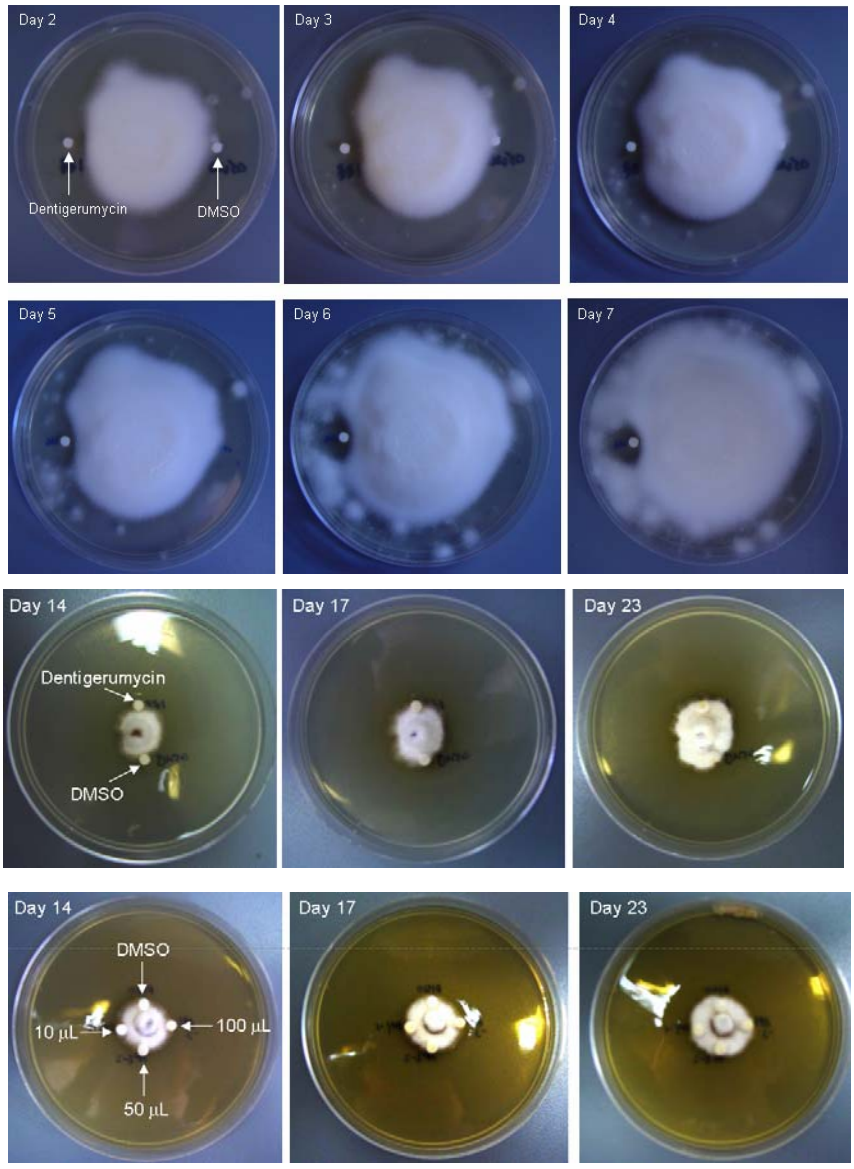


Fig. 8. Plate antifungal assays. (a) An example of a plate antifungal assay progress against *Escovopsis* CC011120-4. 10 μL of 5 mg/mL pure dentigerumycin and DMSO solution were applied to each disk. Dentigerumycin clearly inhibits the growth of *Escovopsis* CC011120-4. (b) An example of a plate antifungal assay progress against the cultivar fungus CC011120-4. 10 μL of 5 mg/mL pure dentigerumycin and DMSO solution were applied to each disk. Dentigerumycin does not inhibit the growth of the cultivar fungus CC011120-4. (c) An example of a plate antifungal assay progress against the cultivar fungus CC011120-4 with different doses. 10, 50, and 100 μL of 5 mg/mL (in DMSO) pure dentigerumycin (**1**) and 100 μL of DMSO were applied to each disk. Dentigerumycin does not inhibit the growth of the cultivar fungus CC011120-4 even at higher doses.

Supplementary Tables

Table 1. NMR Spectral Data for dentigerumycin (**1**) in DMSO-d_6.

C/H	δ_H	mult (*J* in Hz)	δ_C	
1			169.8	C
2	4.22	m	47.7	CH
2-NH	6.69	d (8.5)		
3	1.15	d (7.5)	17.1	CH_3
4			169.6	C
5	4.90	dd (7.5, 1.5)	49.1	CH
6a	2.26	br. d (13.0)	28.6	CH_2
6b	1.88	m		
7	3.60	br. m	59.3	CH
7-OH	5.12	d (5.0)		
8	2.84	m	52.8	CH_2
8-NH	4.85	dd (12.0, 2.5)		
9			174.4	C
10	5.42	dd (6.5, 3.0)	47.7	CH
11a	1.92	m	23.7	CH_2
11b	1.85	m		
12	1.51	m	20.7	CH_2
13a	3.09	br. d (13.0)	46.0	CH_2
13b	2.70	m		
13-NH	5.00	dd (12.5, 1.5)		
14			173.4	C
15	5.33	q (7.5)	53.2	CH
15-N-OH	9.50	s		
16	1.37	d (7.5)	13.8	CH_3
17			170.6	C
18	5.40	dd (5.5, 1.5)	47.8	CH
19a	2.07	br. d (12.5)	25.7	CH_2
19b	1.66	m		
20	1.50	m	20.1	CH_2
21a	3.00	br. d (12.5)	47.3	CH_2
21b	2.68	m		
21-NH	5.53	dd (12.5, 1.5)		
22			167.3	C
23	4.68	dd (11.0, 1.0)	55.4	CH
23-NH	8.30	d (11.0)		
24	5.37	dd (11.0, 1.5)	75.2	CH
25	2.03	m	26.7	CH
26	0.80	d (7.0)	14.6	CH_3
27	0.72	d (7.0)	19.7	CH_3
28			176.4	C
29			76.5	C
29-OH	5.34	s		
30			98.6	C
30-OH	5.86	s		
31a	1.64	m	27.0	CH_2
31b	1.55	m		
32	1.63	m	23.8	CH_2
33	1.20	m	36.5	CH
34	3.47	m	74.0	CH
35	1.27	s	21.9	CH_3
36a	1.23	m	33.1	CH_2
36b	0.94	m		
37a	1.33	m	19.7	CH_2
37b	1.17	m		
38	0.85	t (7.0)	14.3	CH_3
39	1.40	m	24.3	CH_2
40	0.80	t (7.5)	8.2	CH_3

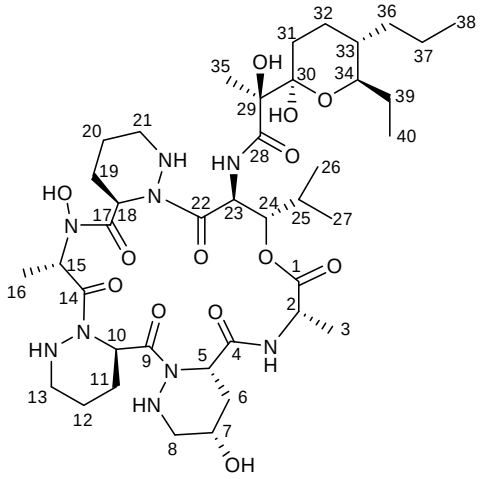
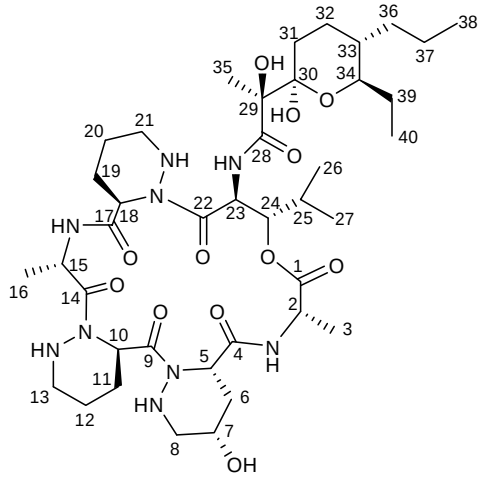


Table 2. NMR Spectral Data for reduction product (15-*N*-deoxydentigerumycin, **8**) of dentigerumycin in $CDCl_3$.

C/H	δ_H	mult (*J* in Hz)	δ_C	
1			171.0	C
2	4.49	dq (8.5, 7.0)	48.5	CH
2-NH	7.28	d (8.5)		
3	1.21	d (7.0)	18.4	CH_3
4			171.0	C
5	5.17	br. d (7.0)	50.4	CH
6a	2.32	br. d (15.0)	28.5	CH_2
6b	2.01	m		
7	3.78	br. m	59.4	CH
8a	3.02	m	53.9	CH_2
8b	2.80	m		
8-NH	4.30	br. d (13.0)		
9			175.9	C
10	5.54	dd (7.0, 1.5)	49.3	CH
11a	2.02	m	25.1	CH_2
11b	1.97	m		
12a	1.61	m	22.0	CH_2
12b	1.52	m		
13a	3.10	br. d (13.0)	47.0	CH_2
13b	2.73	m		
13-NH	4.96	m		
14			171.7	C
15	5.35	dq (8.0, 7.5)	45.9	CH
15-NH	5.92	d (8.0)		
16	1.31	d (7.5)	18.0	CH_3
17			171.4	C
18	4.97	m	50.6	CH
19	1.90	m	28.4	CH_2
20	1.72	m	24.1	CH_2
21a	3.04	m	47.4	CH_2
21b	2.81	m		
21-NH	5.42	m		
22			168.4	C
23	4.90	dd (11.0, 1.0)	55.6	CH
23-NH	8.31	d (11.0)		
24	5.42	m	76.7	CH
25	1.93	m	27.9	CH
26	0.89	d (6.5)	14.8	CH_3
27	0.80	d (6.5)	20.9	CH_3
28			177.0	C
29			76.9	C
30			99.2	C
31a	1.72	m	28.2	CH_2
31b	1.64	m		
32	1.70	m	24.1	CH_2
33	1.22	m	37.8	CH
34	3.64	m	75.4	CH
35	1.36	s	20.6	CH_3
36a	1.28	m	33.6	CH_2
36b	0.99	m		
37a	1.38	m	19.4	CH_2
37b	1.20	m		
38	0.86	t (7.0)	14.6	CH_3
39	1.50	m	24.8	CH_2
	1.43	m		
40	0.79	t (7.5)	8.6	CH_3



Supplementary Methods

General chemical analysis procedures

Optical rotation was measured on a Jasco P-2000 polarimeter with a 5 cm cell. IR spectra were obtained in a Perkin-Elmer 1600 FT-IR spectrometer. CD spectra were collected in an AVIV model 202 CD spectrometer with a 0.5 cm long cuvette. ^{1}H and two dimensional NMR spectra were acquired in a Varian Inova 600 MHz spectrometer. ^{13}C NMR spectra were collected on a Varian Inova 150 MHz spectrometer. LC/MS analysis was performed on an Agilent 1200 Series HPLC / 6130 Series mass spectrometer. High resolution mass spectra were obtained on a Waters Micromass Q-Tof Ultima ESI-TOF mass spectrometer.

Isolations of microbial symbionts from *Apterostigma dentigerum* colony CC011120-04

The colony was collected in 2001 in Gamboa, Panama, and isolates of the mutualistic fungus, the *Escovopsis* parasite, and the *Pseudonocardia* actinobacterium were obtained. The mutualistic fungus was isolated by applying small tufts of fungus mycelium on Potato Dextrose Agar (PDA; 39 g/L) and subculturing until a pure culture of the symbiont was obtained. *Escovopsis* was obtained by placing larger fungus garden fragments on PDA allowing the parasite to grow and sporulate from the garden piece. Pure cultures were obtained by transferring *Escovopsis* spores to new plates containing PDA. The *Pseudonocardia* isolate was obtained by scraping bacteria from the ant cuticle onto chitin agar plates containing antifungals (nystatin 10,000 units/mL and cycloheximide 5% w/v), and subsequently sub-cultured onto yeast malt extract agar (YMEA; 10 g yeast extract, 4 g dextrose, 10 g malt extract and 20 g agar per liter of medium) with antifungals (concentrations as above).[1]

Cultivation of *Pseudonocardia* sp. CC011120-4

The strain CC011120-4 was initially cultured on the medium YMEA (4 g yeast extract, 10 g malt extract, 4 g glucose, and 20 g agar per 1 L of deionized water) agar plates for 7 days. The bacterial cells were scraped from the plate and transferred to a 125 mL Erlenmeyer flask containing 25 mL of YMEA liquid medium (4 g yeast extract, 10 g malt extract, and 4 g glucose per 1 L of deionized water). The liquid culture was incubated at 30 °C with shaking at 250 rpm for 7 days. The 20 mL of the YMEA culture was inoculated to 200 mL of YMEA in 500 mL Erlenmeyer flask. After incubating the 200 mL culture at 30 °C with shaking at 250 rpm for 5 days, 25 mL of the culture was transferred to 1 L of YMEA medium in 4 L Erlenmeyer flask (8 L). The 8 L of YMEA culture was also incubated at 30 °C with shaking at 250 rpm for 14 days.

Extraction and isolation of dentigerumycin (1)

The whole culture (8 L) was extracted with 12 L of ethyl acetate (EtOAc) twice. The EtOAc layer was separated from the aqueous phase with a fractionating funnel. After fractionation, residual amount of water in the organic layer was removed by adding sodium sulfate anhydrous. The EtOAc was removed by rotavap and dry crude extract material was obtained. The crude material was resuspended in dichloromethane (DCM) / methanol (MeOH) 1:1 solvent and celite was added to the mixture. The celite-extract mixture was dried *in vacuo* and the dry material was loaded on 2 g of a pre-packed C_{18} Sepak resin. The crude extract was fractionated by eluting with a step gradient of water, MeOH, and DCM combinations. The 80% MeOH fraction was most active in inhibition of the parasitic fungus *Escovopsis* strain (CC011120-4). The fraction was subsequently purified by reversed-phase HPLC (Agilent 1100 Series HPLC system, Alltech semipreparative column C_{18}, 10 mm × 250 mm, 2 mL/min, UV 210 nm detection) with the isocratic solvent mixture 80% CH_3CN in water. Dentigerumycin (**1**) was eluted at 16.4 min. The overall yield of the pure dentigerumycin through extraction and isolation is approximately 3 mg / L.

Dentigerumycin (1): $[\alpha]_D^{24}$ -15 (c 0.59, $CHCl_3$) ; IR (neat) ν_{max} 3372, 2936, 1752, 1648, 1503, 1452, 1322, 1249, 1192 cm^{-1}; NMR spectral data, see **Supplementary Table 1**; HR-ESI-TOFMS $[M+Na]^+$ *m*/*z* at 904.4775, calcd $[M+Na]^+$ at 904.4756.

Determination of the absolute configuration of the amino acid units in dentigerumycin

1.2 mg of dentigerumycin was hydrolyzed in 0.5 mL of 6 N HCl at 115 °C for 1 h, the HCl was removed under vacuum. The dry material was resuspended in 0.5 mL of water and dried three times to remove residual acid. The hydrolysate was purified by column chromatography using a C_{18} Sepak column (0.5 g). The free amino acids were eluted with 10 % CH_3CN in water. The purified hydrolysate with the free amino acids was divided into two portions and dissolved in 1 N $NaHCO_3$ (100 μL). 50 μL of 10 mg/mL L-FDLA (**2**) and D-FDLA (**3**)[4] were added to the solution and the reaction mixtures were heated at 80 °C for 3 min. The reaction was quenched by neutralization with 50 μL of 2 N HCl. Aqueous 50 % CH_3CN (300 μL) was added to the solution to dissolve the products, which were analyzed by LC/MS with a gradient solvent system from 20 % to 70 % CH_3CN (0.1% formic acid) over 50 min (Agilent 1200 Series HPLC / 6130 Series mass spectrometer, Phenomenex C_{18}(2) 5 μm, 4.6 mm × 100 mm, 0.7 mL/min flow rate). The L-FDLA and D-FDLA derivatives of two piperazic acid units were eluted at retention time 22.0 and 24.8 min, respectively. Both piperazic acid units in **1** were determined to have *R* configuration based on the retention times of the L-FDLA derivatives of synthetic *R*- and *S*-piperazic acids (**4** and **5**) (*R*: 22.3 min and *S*: 25.0 min). Alanine was eluted at 23.5 and 27.4 min with L-FDLA and

D-FDLA derivatizations, respectively, establishing its *S* configuration. The products derived from β-hydroxy leucine unit were detected at 23.2 and 29.5 min for L and D-FDLA derivatization, determining *S* configuration at its α-carbon chiral center. The L-FDLA and D-FDLA derivates of γ-hydroxy piperazic acid unit were eluted at 19.6 min and 18.1 min but the absolute configuration could not be established only with the Marfey analysis. The products originated from *N*-hydroxy alanine were not detected.

_S_- and _R_-MTPA esters (6 and 7) of dentigerumycin

Dry dentigerumycin (0.8 mg) was prepared in two separate vials and several dry crystals of dimethylaminopyridine were added to the two reaction vials. The mixture was dissolved in freshly distilled dry pyridine (2 mL) and stirred for 15 min under argon at room temperature. 50 μL of *R*- and *S*-MTPA chloride (5.36 μmol/μL) were added respectively to the separate reaction vials and the reaction mixtures were stirred at room temperature for 1 h. Then the reaction vials were heated at 65 °C for 3 h with monitoring the reaction by LC/MS. After 3 hours, 100 μL of MeOH were added and the acylation products were purified by reversed-phase HPLC (Agilent HPLC 1100 Series, Alltech C_{18} semipreparative column, 10 mm × 250 mm, 2 mL/min, gradient 0-10 min: 20% aqueous CH_3CN / 10-50 min: 20-100% aqueous CH_3CN). *S*-MTPA ester (**6**) and *R*-MTPA ester (**7**) were eluted at 43.3 and 43.5 min respectively. The molecular formulas for *S*- and *R*-MTPA esters were confirmed as $C_{50}H_{74}F_3N_9O_{15}$ by ESI-HRMS analysis ($[M\text{-}H_2O+H]^+$ *m*/*z* at 1080.5253 for *S*-MTPA ester and at 1080.5255 for *R*-MTPA ester, calculated at 1080.5229). The ^1H chemical shifts of *S*- and *R*-MTPA esters were assigned by ^1H, gCOSY, and TOCSY NMR spectral analysis. The Δδ values in Fig. 5 determined the absolute configuration of the secondary alcohol at C-7 as *S*.[5]

_S_-MTPA ester (7-_S_-methoxy-trifluoromethyl-phenyl acetyl dentigerumycin ester, 6):

^1H NMR (600 MHz, $CDCl_3$) δ 0.79 (3H, d, *J* = 7.0 Hz) 0.80 (3H, t, *J* = 7.5 Hz), 0.87 (3H, t, *J* = 7.5 Hz), 0.93 (3H, d, *J* = 7.0 Hz), 0.99 (2H, m), 1.22 (1H, m), 1.28 (3H, d, *J* = 7.5 Hz), 1.37 (3H, s), 1.38 (2H, m), 1.48 (2H, m), 1.51 (3H, d, *J* = 7.0 Hz), 1.53 (2H, m), 1.61 (2H, m), 1.71 (4H, m), 1.82 (1H, m), 1.94 (2H, m), 1.95 (1H, m), 1.96 (1H, m), 2.02 (2H, m), 2.76 (1H, m), 2.78 (1H, m), 3.02 (1H, m), 3.04 (1H, m), 3.13 (1H, d, *J* = 12.0 Hz), 3.22 (1H, d, *J* = 12.0 Hz), 3.49 (3H, s), 3.66 (1H, m), 4.18 (1H, d, *J* = 12.5 Hz), 4.24 (1H, m), 4.76 (1H, dd, *J* = 11.0, 11.0 Hz), 5.04 (1H, br. d, *J* = 7.5 Hz), 5.08 (1H, m), 5.10 (1H, m), 5.23 (1H, dd, *J* = 11.0, 1.5 Hz), 5.63 (1H, m), 5.64 (1H, m), 5.74 (1H, q, *J* = 7.0), 5.84 (1H, d, *J* = 12.0 Hz), 6.22 (1H, d, *J* = 9.0 Hz), 7.41 (3H, m), 7.52 (2H, m), 8.68 (1H, d, *J* = 11.0 Hz).

***R*-MTPA ester (7-*R*-methoxy-trifluoromethyl-phenyl acetyl dentigerumycin ester, 7):**
^{1}H NMR (600 MHz, $CDCl_3$) δ 0.80 (3H, t, *J* = 7.5 Hz), 0.81 (3H, d, *J* = 7.0 Hz), 0.87 (3H, t, *J* = 7.5 Hz), 0.91 (3H, d, *J* = 7.0 Hz), 0.99 (2H, m), 1.17 (3H, d, *J* = 7.5 Hz), 1.22 (1H, m), 1.37 (3H, s), 1.38 (2H, m), 1.48 (2H, m), 1.50 (3H, d, *J* = 7.0 Hz), 1.52 (2H, m), 1.61 (2H, m), 1.72 (4H, m), 1.83 (1H, m), 1.91 (1H, m), 1.92 (2H, m), 1.93 (1H, m), 2.05 (2H, m), 2.74 (1H, m), 2.78 (1H, m), 2.93 (1H, m), 3.00 (1H, m), 3.06 (1H, m), 3.12 (1H, d, *J* = 14.0 Hz), 3.52 (3H, s), 3.66 (1H, m), 3.88 (1H, d, *J* = 12.5 Hz), 4.23(1H, m), 4.75 (1H, dd, *J* = 11.0, 11.0 Hz), 5.05 (1H, br. d, *J* = 7.5 Hz), 5.08 (1H, br. d, *J* = 13.0 Hz), 5.20 (1H, m), 5.23 (1H, dd, *J* = 11.0, 1.5 Hz), 5.53 (1H, dd, *J* = 6.5, 1.5 Hz), 5.63 (1H, m), 5.71 (1H, q, *J* = 7.0 Hz), 5.84 (1H, d, J = 12.0 Hz), 6.13 (1H, d, *J* = 9.0 Hz), 7.43 (3H, m), 7.51 (2H, m), 8.67 (1H, d, *J* = 11.0 Hz).

Reduction of *N*-OH in dentigerumycin and determination of the absolute configuration of *N*-hydroxy alanine

Dentigerumycin (1.0 mg) was dissolved in 2 mL of tetrahydrofuran (THF) under argon. 1 mL of 4.5 M aqueous ammonium acetate and 0.5 mL of a 10 % $TiCl_3$ solution in 20-30 wt. % HCl were added to the solution in sequence. The mixture was stirred at room temperature for 2 h. The product was extracted with EtOAc (10 mL) and the organic layer was washed with saturated $NaHCO_3$ (15 mL) and NaCl (15 mL) solutions.[6] The reduction product (15-*N*-deoxydentigerumycin, **8**) was purified at 46.7 min by reversed-phase HPLC (Agilent HPLC 1100 Series, Alltech C_{18} semipreparative column, 10 mm × 250 mm, 2 mL/min, gradient 0-10 min: 30% aqueous CH_3CN / 10-50 min: 30-90% aqueous CH_3CN). The molecular formula was confirmed as $C_{40}H_{67}N_9O_{12}$ by ESI-HRMS analysis ($[M+Na]^+$ *m*/*z* at 888.4825, calculated at 888.4807). The ^{1}H and ^{13}C chemical shifts of 15-*N*-deoxydentigerumycin (**8**) were completely assigned by ^{1}H, gCOSY, TOCSY, gHSQC, gHMBC, and ROESY NMR spectral analysis (**Table 2**). As the result of reduction, two alanine units were observed at (1) C-1, C-2, C-3, 2-NH and (2) C-14, C-15, C-16, 15-NH.

15-*N*-deoxydentigerumycin (**8**) was hydrolyzed in 6 N HCl at 115 °C for 1 h, derivatized with L-FDLA, and the L-FDLA derivatives were analyzed by LC/MS as described above. The single L-FDLA derivative originated from two alanine units eluted at 23.9 min, determining that the alanine unit derived from *N*-hydroxy alanine possesses *S* configuration.

Determination of the 29, 30-diol moiety using CD spectroscopy

Dentigerumycin (5.8 mg) was dissolved in 1.50 mL of anhydrous dimethyl sulfoxide (DMSO) and the solution was divided exactly to two 0.75 mL aliquots. 4.38 mM DMSO solution of $Mo_2(OAc)_4$ was prepared and 0.75 mL of the solution was added to 0.75 mL of prepared dentigerumycin solution to make 2.19 mM dentigerumycin and $Mo_2(OAc)_4$ mixture solution.

After 40 min, a CD spectrum was recorded for induced CD. The other 0.75 mL of dentigerumycin solution was diluted to 1.50 mL with 0.75 mL of anhydrous DMSO and an inherent CD spectrum was obtained. The inherent molar CD spectrum was subtracted from the induced molar CD spectrum. The negative sign of the diagnostic band at 305 nm is correlated to the absolute configuration of the 29, 30-diol moiety, determining 29*S* and 30*R* absolute configurations based on the preferred conformation of the complex.[7]

Plate antifungal assay

The parasitic fungal strain *Escovopsis* CC011120-4 was plated on YMEA agar plates. The plate cultures were incubated for 2 days at 30 °C. Sterile paper disks (2.5 mm diameter) were placed on the agar plates, not touching the fungal culture. Ethyl acetate extracts and fractions of *Pseudonocardia* sp. CC011120-4 cultures and pure dentigerumycin (**1**) were dissolved in DMSO at various concentrations and applied to the paper disks on the plates. For negative control, pure DMSO was also applied to a disk on each assay plate. The cultures were incubated at 30 °C and monitored for inhibition. The fungal cultivar CC01120-4 was cultivated from the center of YMEA plates. Since the fungal cultivar grows much slowly, the plates were incubated at 30 °C for 14 days before dentigerumycin was applied on paper disks. After applying dentigerumycin, the cultures were placed in a 30 °C incubator and monitored for inhibition.

Liquid antifungal assay

The *Escovopsis* strain CC011120-4 was cultivated on potato dextrose agar plates. The mycelia were collected and homogenized in potato dextrose broth medium. The suspension was transferred to the wells in 96-well plates and various concentrations of pure dentigerumycin were applied. The plates were incubated at 37 °C for 48 h and alamarBlue (Soretec Ltd.) was added. The fluorescence was measured with excitation at 540 nm and emission at 590 nm by Wallac Vector 2 plate reader after 15 -18 h. A liquid antifungal assay was not applicable to the mutualistic fungus because it grows extremely slow in liquid culture. Bioassays against *Candida albicans* strains (wild type, ATCC10231, ATCC200955) were performed as described above but without cell homogenization.

Supplementary References

1. Cafaro, M.J. & Currie, C.R. *Can. J. Microbiol.* **51**, 441-446 (2005).
2. Matsumori, N., Kaneno, D., Murata, M., Nakamura, H. & Tachibana, K. *J. Org. Chem.* **64**, 866-876 (1999).
3. Miller, E.D., Kauffman, C.A., Jensen, P.R. & Fenical, W. *J. Org. Chem*. **72**, 323-330 (2007).

4. Fujii, I.K., Ikai, Y., Oka, H., Suzuki, M. & Harada, K. *Anal. Chem.* **69**, 5146-5151 (1997).
5. Séco, J.M., Quiñoa, E. & Riguera, R. *Tetrahedron: Asymmetry* **12**, 2915-2925 (2001).
6. Pennings, M.L.M., Reinhoudt, D.N., Harkema, S. & van Hummel, G.J. *J. Org. Chem*. **48**, 486-491 (1983).
7. Snatzke, G., Wagner, U. & Wolff, H.P. *Tetrahedron* **37**, 349-361 (1981).