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Identification of 4,5-Dihydro-4-hydroxygeldanamycins As Shunt Products of Geldanamycin Biosynthesis

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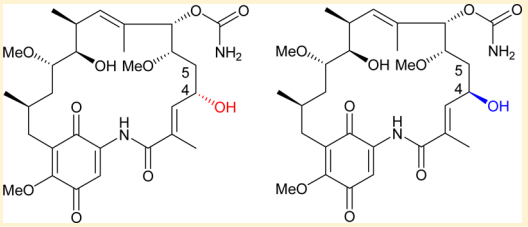
Supporting Information

ABSTRACT: Two new geldanamycin (GDM) analogues, (4*S*)-4,5-dihydro-4-hydroxygeldanamycin (**1**) and (4*R*)-4,5-dihydro-4-hydroxygeldanamycin (**2**), were identified from *Streptomyces hygroscopicus* 17997. Compounds **1** and **2** were not normal intermediates of GDM biosynthesis but shunt products of C-4,5 oxidation catalyzed by GdmP, a cytochrome P450 oxidase acting as a desaturase in GDM biosynthesis. Preliminary assays implied that, compared with GDM, **1** and **2** exhibited decreased cytotoxicity.



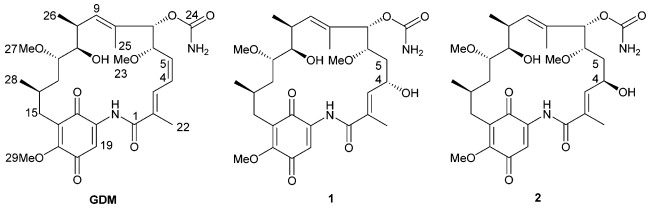
Geldanamycin (GDM) is a benzoquinone ansamycin produced by *Streptomyces hygroscopicus*.[1−3] It binds to heat shock protein 90 and inhibits its function.[4] As GDM shows potent antitumor potential but also presents drawbacks such as severe hepatotoxicity and poor water solubility, various approaches to prepare novel GDM analogues have been explored to obtain better clinical candidate(s).[5−8]

We are interested in discovering novel natural/biosynthetic GDM analogues with improved pharmacological profiles. In our previous studies, we reported thiazinogeldanamycin and 19-*S*-methylgeldanamycin, two novel natural GDM analogues from *S. hygroscopicus* 17997 with improved water solubility but decreased antitumor cell activity.[9,10]

An understanding of GDM biosynthesis may help us obtain novel biosynthetic GDM analogues. GDM is biosynthesized by type I polyketide synthase (PKS). The PKS is first loaded with 3-amino-5-hydroxybenzoic acid and then utilizes malonyl-CoA, methylmalonyl-CoA, and methoxymalonyl-CoA to generate the precursor molecule, progeldanamycin. Progeldanamycin undergoes further post-PKS tailoring modifications to produce GDM, which include C-17 hydroxylation, C-17-O-methylation, C-21 hydroxylation, C-7 carbamoylation, and C-4,5 oxidation. Cloning and bioinformatics analysis of the GDM biosynthetic gene cluster indicates a continuous DNA fragment of over 60 kb encoding several putative post-PKS tailoring genes (*gdmN*, *gdmM*, *gdmP*, etc.), together with type I PKS genes.[11−13] Among them, *gdmP*, which encodes a cytochrome P450 oxidase, is confirmed to be essential in C-4,5 oxidation (desaturation) of GDM biosynthesis.[13]

Most cytochrome P450 oxidases for secondary metabolite biosynthesis in actinomycetes function as hydroxylases/monooxygenases (cytochrome P450 oxidases acting as desaturases are rare).[14] In the few known desaturation reactions catalyzed by cytochrome P450 oxidases, substrate hydroxylation (and/or epoxidation) may also take place.[15] Blast comparison of amino acid sequences of GdmP (encoded by *P* gene, i.e., *gdmP*, of geldanamycin biosynthetic gene cluster from *S. hygroscopicus* 17997) and cytochrome P450 oxidases such as NikF (a hydroxylase encoded by *F* gene, i.e., *nikF*, of nikkomycin biosynthetic gene cluster from *Streptomyces tendae*) showed >40% identities.

We believe GdmP also catalyzes C-4/5 hydroxylation in the C-4,5 oxidation (desaturation) of GDM biosynthesis. Therefore, C-4/5 hydroxy GDM analogues should exist in *S. hygroscopicus* 17997. This paper reports the identification of (4*S*)-4,5-dihydro-4-hydroxygeldanamycin (**1**) and (4*R*)-4,5-dihydro-4-hydroxygeldanamycin (**2**) from *S. hygroscopicus* 17997.



A silica gel TLC comparison of metabolites from *S. hygroscopicus* 17997 and its *gdmP* gene disruption mutant (*gdmP*$^-$) showed a band with a very small R_f value (Supporting Information, Figure S1). It appeared only in *S. hygroscopicus* 17997 and not in *gdmP*$^-$,[16] suggesting a close relation to the *gdmP* gene. The band changed to blue upon spraying with $FeCl_3$ (10%), suggesting the existence of a phenolic moiety. The band displayed two principal ions at *m*/*z* 601 $[M + Na]^+$ and 596 $[M + NH_4]^+$ and a minor ion at *m*/*z* 603 (putative hydroquinone form of *m*/*z* 601) by ESIMS analysis. MS^2 of *m*/*z* 601 exhibited a typical fragment pattern of GDM and its

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analogues (601 $[M + Na]^+ \rightarrow 557 [M + Na - CO_2]^+$, 540 $[M + Na - HOCONH_2]^+$) (Supporting Information, Figures S2 and S3).[17] On the basis of these preliminary results, we identified the putative C-4/5 hydroxy GDM analogue of GDM biosynthesis.

From 8.0 L of *S. hygroscopicus* 17997 cultured on ISPII medium plates, we obtained two pure compounds (yellow, amorphous powder), **1** (14.7 mg) and **2** (6.5 mg), as putative C-4/5 hydroxy GDM analogues.

Compound **1** had the molecular formula $C_{29}H_{42}N_2O_{10}$, as indicated by HRESIMS and NMR data. Compound **1** lost GDM's characteristic UV absorption maxima at 254 nm, indicating that it lacked the dienamide chromophore. The IR spectrum suggested the presence of hydroxy or amine groups (3476, 3376, 3286, and 3191 cm^{-1}) and carbonyl groups (1724, 1701, and 1654 cm^{-1}). The NMR spectra of **1** (Table 1) showed resonances common to GDM.[12] These resonances comprised four methyl groups (C-22, 25, 26, and 28), three methoxyl groups (C-23, 27, and 29), two methylene groups (C-13 and 15), nine methine groups (C-3, 6, 7, 9, 10, 11, 12, 14, and 19), nine quaternary carbons (C-1, 2, 8, 16, 17, 18, 20, 21, and 24), and two recognizable active hydrogens (*NH*-1 and *OH*-11). Resonances of H-3 and H-6 in **1** were shielded by $\Delta\delta_H$ −0.92 and −0.53 ppm, respectively. The C-3 in **1** was deshielded by $\Delta\delta_C$ 8.0 ppm, and C-6 was shielded by $\Delta\delta_C$ −3.5 ppm. The resonance of H-7 emerged as a doublet (J = 4.2 Hz) in **1** rather than a singlet as found in GDM, probably because the rigid dienamide system of GDM kept the dihedral angle between H-6 and H-7 close to 60°, and the electronegative *O*-6 and *O*-7 were antiperiplanar to H-7 and H-6, respectively. This implied that some changes had happened to the moiety of C-3 to C-6. Compared to GDM, the NMR spectra of **1** showed the loss of olefinic CH-4 and CH-5 and at the same time the gain of a methine group CH-X [δ_H 4,71 (1H, m) and δ_C 65.6] and a methylene group CH_2-Y [δ_H 1.88 (2H, m) and δ_C 38.7]. The COSY spectrum showed sequential correlations among H-3, H-X, H-Y, and H-6, and the HMBC spectrum exhibited correlations from H-3 to C-Y, from H-Y to C-6, C-7, and C-X, and from H-6 to C-X, which revealed that CH-X was CH-4 and CH_2-Y was CH_2-5 in **1**. The obvious downfield chemical shifts of C-4 and its associated hydrogen, together with HRMS data, showed the presence of a hydroxy group. The broadening quartet of H-5a and H-5b at 1.88 ppm indicated that the two hydrogens had slightly different chemical shifts with geminal coupling. The other coupling constants, which were all very similar to GDM, plus the consideration from a biosynthetic aspect, suggested that **1** shared the same configuration as GDM in other positions of the molecule. Thus, the structure of **1** was determined as 4,5-dihydro-4-hydroxygeldanamycin.

Compound **2** had the same molecular formula as **1** by its HRESIMS data and NMR spectra. Compound **2** showed nearly identical UV and IR absorption profiles to **1**. The superimposable ^1H NMR and ^{13}C NMR spectra of **1** and **2** revealed that **2** shared the same planar structure as **1**; C-4 was also a methine group [δ_H 4.74 (1H, m) and δ_C 65.9] carrying a hydroxy functionality, and C-5 was also a methylene group [δ_H 1.97 (1H, m), 1.80 (1H, overlap), and δ_C 38.5]. Compounds **1** and **2** are therefore a pair of C-4 epimers.

The absolute configuration of the *Sec*-OH at C-4 was determined by the modified Mosher's method.[18,19] MPA, which provides an improved long-range anisotropic effect compared to MTPA, was used as an auxiliary reagent to obtain the $\Delta\delta^{RS}$ values. To inhibit the racemization of MPA in the esterification process, the method established by Hessner et al. involving the reagents of DCC and DMAP was applied.[20] Separate esterifications of **1** with *R*- or *S*-MPA were performed. Two MPA groups were expected to couple to **1**, one at C-4 and the other at C-11 (another *Sec*-OH in the molecule). Considering the racemization of MPA, four double-esterified products were expected and were detected by HPLC and MS. The desired **1*R*** and **1*S*** constituted >90% (based on UV area at 307 nm) of all the double-esterified products. To reduce line broadening and enhance resolution, the ^1H NMR spectra of **1*R***, **1*S***, and **1** were recorded at 70 °C in DMSO-d_6. The $\Delta\delta^{RS}$ values of the **1*R*** and **1*S*** protons attached to flank L1 are all positive, while those located on flank L2 are all negative (Figure 1). Therefore, the absolute configuration of the *Sec*-OH at C-4 in **1** is *S*. The absolute configuration of the *Sec*-OH at C-11 in **1** was also determined to be *R* (same as in GDM) according to $\Delta\delta^{RS}$

Table 1. The ^1H and ^{13}C NMR Data of 1 and 2 (in $CDCl_3$)[a]

	1		2	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	166.8, C		167.7, C	
2	132.5, C		134.5, C	
3	135.3, CH	6.02, brd (9.0, 1.2)	138.4, CH	6.18, brd (8.4, 1.2)
4	65.6, CH	4.71, m	65.9, CH	4.74, m
5	38.7, CH_2	1.88, m	38.5, CH_2	1.97, m
				1.80, overlap
6	77.8, CH	3.78, m	80.9, CH	3.36, m
7	80.2, CH	5.23, d (4.2)	82.5, CH	5.25, d (5.4)
8	128.5, C		130.6, CH	
9	130.3, CH	5.60, d (10.2)	135.4, CH	5.75, d (9.6)
10	34.4, CH	2.69, m	32.5, CH	2.73, m
11	73.8, CH	3.58, m	74.3, CH	3.54, m
12	81.3, CH	3.43, m	80.6, CH	3.02, dd (5.4, 2.4)
13	36.3, CH_2	1.76, dt, (15.0, 4.8)	36.6, CH_2	1.82, overlap
		1.53, m		1.60, overlap
14	30.9, CH	1.59, m	28.9, CH	1.58, m
15	31.5, CH_2	2.45, dd, (13.2, 3.6)	31.8, CH_2	2.49, dd, (12.6, 8.4)
		2.38, dd, (13.2, 9.6)		2.42, dd, (12.6, 4.2)
16	126.3, C		127.6, C	
17	154.4, C		156.8, C	
18	180.9,[b] C		184.0,[c] C	
19	110.5, CH	7.15, s	111.5, CH	7.12, s
20	136.6, C		138.2, C	
21	181.2,[b] C		184.9,[c] C	
22	14.8, CH_3	1.97, d (1.8)	12.8, CH_3	2.00, s
23	59.2, CH_3	3.45, s	59.1, CH_3	3.39, s
24	153.8, C		156.5, C	
25	15.2, CH_3	1.65, s	12.2, CH_3	1.61, s
26	15.9, CH_3	1.0, d (7.2)	12.6, CH_3	0.99, d (7.2)
27	57.1, CH_3	3.34, s	57.7, CH_3	3.36, s
28	22.7, CH_3	0.92, d (6.6)	22.1, CH_3	0.97, d (6.6)
29	62.0, CH_3	4.08, s	61.6, CH_3	4.11, s
1-*NH*-		8.92, s		8.81, s
4-*OH*				
24-NH_2				
11-*OH*		2.39, overlap		3.02, d (6.6)

[a]Resonances of 4-*OH* and 24-NH_2 were missed. [b,c]Signals with the same superscript are interchangeable.

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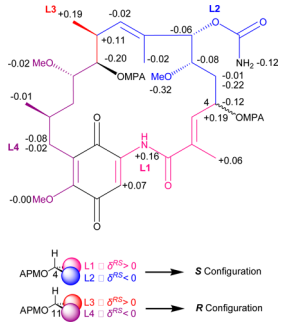

Figure 1. $\Delta\delta^{RS}$ values of **1***R* and **1***S* from modified Mosher's reactions and the absolute configuration of *Sec*-OH at C-4 in **1**.

values, and the absolute configuration of the *Sec*-OH at C-4 in **2** should therefore be *R*.

The bioconversion of **1** or **2** to GDM was investigated using GDM-*pks*$^-$, a GDM polyketide synthase gene disruption mutant of *S. hygroscopicus* 17997, which had a full complement of post-PKS tailoring genes for GDM biosynthesis but had lost the ability to produce GDM.[21] As indicated in Figure 2, both **1** and **2** were not bioconverted to GDM by GDM-*pks*$^-$ and are probably not normal intermediates of GDM biosynthesis but are shunt products of GdmP-catalyzed C-4,5 oxidation (**1** or **2** was not detected in *gdmP*$^-$).

Previous LCMS analyses of GDM revealed the existence of trace amounts of a putative GDM analogue with *m*/*z* 601 (possibly corresponding to **1** or **2**),[17,22] but it was difficult to isolate sufficient amounts for structure confirmation by NMR. In this study, EtOAc extraction of a fresh culture of *S. hygroscopicus* 17997 on ISPII medium disrupted the ongoing C-4,5 oxidation of GDM biosynthesis and induced higher levels of abnormal C-4,5 oxidation (or dissociation of 4,5-dihydrogeldanamycin-GdmP-O_2 complex); increased amounts of **1** and **2** were therefore produced for subsequent isolation and characterization.

On the basis of the above results, we propose a scheme for GdmP-catalyzed C-4,5 oxidation (Supporting Information, Figure S28), similar to the scheme proposed by Rettie et al. for cytochrome P450 oxidase-catalyzed desaturation of valproic acid.[15] In both schemes, a close mechanistic link exists between hydroxylation and desaturation in the cytochrome P450 oxidase-catalyzed oxidations.

Buchanan et al. reported an unexpected minor product, 4,5-epoxy-8-demethylgeldanamycin, from an AT4-engineered *S. hygroscopicus* var. *geldanus* and suggested that the epoxide moiety of this GDM analogue is quite likely introduced by GdmP.[23] We detected traces of a GDM analogue (*m*/*z* 599, [M + Na]$^+$, Supporting Information, Figure S29) from *S. hygroscopicus* 17997 by LCMS, having the same molecular mass as 4,5-epoxygeldanamycin ($C_{29}H_{40}N_2O_{10}Na$, 599).

Both **1** and **2** degraded to a red compound at room temperature in water−methanol mixtures. The two red compounds showed the same UV absorption profile and identical molecular formula, $C_{30}H_{46}N_2O_{11}Na$ (exact mass 633.29938), which is CH_4O more than **1** or **2**. According to Sasaki et al. and Hu et al., GDM could degrade into methyl geldanamycinate,[24,25] and it is possible the degradation products from **1** and **2** are methyl (4*S*/*R*)-4,5-dihydro-4-hydroxygeldanamycinate (Supporting Information, Figure S30).

An early structure−activity study indicated that the C-4,5 olefinic bond of GDM was not crucial for its cytotoxic activity.[26] We performed a preliminary cytotoxicity evaluation of **1** or **2** against HepG2 cells and determined an IC_{50} of 10.5 μM for **1** and 10.8 μM for **2** (0.37 μM for GDM).

EXPERIMENTAL SECTION

General Experimental Procedures. IR spectra were recorded on a Nicolet 5700 FTIR microscope spectrometer. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) of **1** and **2** were recorded in $CDCl_3$ in a Norell 3 mm nuclear tube with a Varian VNS-600 spectrometer. For the elucidation of the *Sec*-OH in **1**, the ^{1}H NMR (600 MHz) of **1**, **1***R*, and **1***S* were determined in DMSO-d_6 in a Norell 3 mm nuclear tube at 70 °C on a Varian VNS-600 spectrometer. Chemical shifts given in ppm were referenced to the solvent signals: δ H/C 7.26/77.0 and δ H/C 2.50/39.5 for $CDCl_3$ and DMSO-d_6, respectively. ESIMS was acquired with an LTQ XL from Thermo Fisher Scientific. HRMS were analyzed using an LTQ Orbitrap XL from Thermo Fisher Scientific. Flash chromatography was performed on an Ez Purifier from Lisure Science (Suzhou) Co., Ltd., with a UV detector. Analytical and semipreparative HPLC were conducted on a Shimadzu LC-20AT or HPLC system with a photodiode array detector. The primary preparative HPLC separation was accomplished on a Shimadzu Prominence preparative HPLC system with an LC-20AP pump and SPD-20AV UV−vis detector.

Fermentation. *Streptomyces hygroscopicus* 17997, a wild-type GDM producer, was isolated from Chinese soil at the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences. Frozen stock spores of *S. hygroscopicus* 17997 were thawed, spread onto four ISPII medium plates (diameter 8.5 cm; medium composition: 0.4% yeast extract, 1.0% malt extract, 0.4% glucose, and 1.5% agar powder), and incubated at 28 °C for 8−10 days for sporulation to occur. Fresh

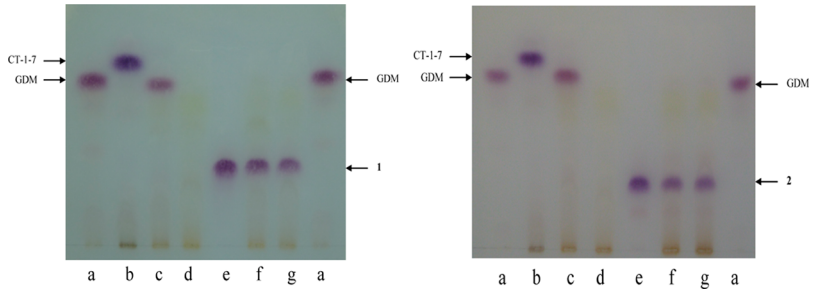

Figure 2. Bioconversion of **1** and **2** in GDM-*pks*$^-$. Compounds **1** and **2** stayed unchanged in GDM-*pks*$^-$. Compound 4,5-dihydro-7-descarbamoyl-7-hydroxygeldanamycin (CT-1-7), a known normal intermediate of GDM biosynthesis as positive control,[12] was converted to GDM by GDM-*pks*$^-$. Left: Bioconversion of **1** in GDM-*pks*$^-$. a, GDM; b, CT-1-7; c, CT-1-7+GDM-*pks*$^-$ (incubation time, 1 d); d, GDM-*pks*$^-$ (blank); e, **1**; f, **1**+GDM-*pks*$^-$ (incubation time, 1 d); g, **1**+GDM-*pks*$^-$ (incubation time, 2 d). Right: Bioconversion of **2** in GDM-*pks*$^-$. a, GDM; b, CT-1-7; c, CT-1-7+GDM-*pks*$^-$ (incubation time, 1 d); d, GDM-*pks*$^-$ (blank); e, **2**; f, **2**+GDM-*pks*$^-$ (incubation time, 1 d); g, **2**+GDM-*pks*$^-$ (incubation time, 2 d).

spores from the ISPII medium plates were recovered by washing with sterile water and used to inoculate ISPII medium plates (~400). Plates were incubated at 28 °C for 4 days for the production of geldanamycins.

The *gdmP*$^-$ mutant strain of *S. hygroscopicus* 17997 was cultured the same way as the wild-type strain.

Extraction and Isolation. Fermentation cultures (8 L, 400 ISPII medium plates) of *S. hygroscopicus* 17997 were collected and extracted twice with ethyl acetate (EtOAc). The EtOAc extracts were combined and evaporated under reduced pressure to give a crude solid product (8.5 g). The solid residue was resuspended in 200 mL of EtOAc and mixed with 200 mL of $FeCl_3$ (10%) for 3 h to convert the hydroquinone form of C-4/5 hydroxy GDM analogue(s) to the quinone form for subsequent purification.[27] The upper EtOAc layer was centrifuged (10 000 rpm, 20 min) to remove insoluble residues, predominantly GDM. The clear EtOAc solution was evaporated at 35 °C to give 5.0 g of extract, which was fractionated by normal-phase flash column chromatography with EtOAc–petroleum ether (PE) (silica gel column: 40 × 250 mm; EtOAc–PE, 0–100% in 120 min, 30 mL/min; compounds **1** and **2** were eluted with ~90% EtOAc). Fractions containing **1** and **2** (yellow) were pooled and dried (210 mg) for preparative HPLC (Agilent Zorbax SB C_{18} column: 21.4 × 150 mm, MeOH–H_2O, 40–100% in 30 min, 21 mL/min; compounds **1** and **2** were eluted out at ~18 and ~21 min, respectively). Semipreparative HPLC separation was applied to refine the products (Agilent Zorbax C_{18} column: 10.0 × 250 mm, MeOH–H_2O, 66:34; compounds **1** and **2** were eluted out at 13.5 and 15.5 min, respectively), which finally yielded pure preparations of **1** (14.7 mg, purity >97%) and **2** (6.5 mg, purity >93%) as putative C-4/5 hydroxy GDM analogues. Compounds **1** and **2** showed good resolution in reversed-phase HPLC (Supporting Information, Figure S4).

Compound **1**: IR (KBr) ν_{max} 3476, 3376, 3286, 3191, 2933, 1724, 1701, 1654, 1610, 1507 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 596.1 $[M + NH_4]^+$, 601.4 $[M + Na]^+$; HRMS *m/z* 596.31967 $[M + NH_4]^+$ (calcd for $C_{29}H_{46}O_{10}N_3$, 596.31777).

Compound **2**: IR (KBr) ν_{max} 3459, 3362, 2952, 2833, 1706, 1652, 1604, 1504 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 596.0 $[M + NH_4]^+$, 601.3 $[M + Na]^+$; HRMS *m/z* 596.31958 $[M + NH_4]^+$ (calcd for $C_{29}H_{46}O_{10}N_3$, 596.31777).

MPA Esters 1*R* and 1*S*. To a solution of **1** (5.78 mg, 0.01 mmol) and methoxylphenylacetic acid (MPA) (9.96 mg, 0.06 mmol) in CH_2Cl_2 (2.0 mL) were added *N,N′*-dicyclohexylcarbodiimide (DCC) (12.38 mg, 0.06 mmol) and 4-(dimethylamino) pyridine (DMAP) (7.33 mg, 0.06 mmol), and the resultant mixture was stirred at room temperature under nitrogen protection for 24 h. It was then separated by normal-phase silica solid phase extraction and further purified by semipreparative HPLC [Agilent Zorbax SB C_{18} 250 × 10 mm, CH_3CN–H_2O (77:23), obtaining **1*R*** (2.8 mg) or **1*S*** (3.0 mg].

*Compound **1**:* yellow, amorphous powder; ESIMS *m/z* 601.3 $[M + Na]^+$; ^{1}H NMR (600 MHz, DMSO-d_6, 70 °C) δ 8.96 (1H, s, *HN*-1), 6.90 (1H, s, H-19), 6.29 (2H, brs, NH_2-24), 6.03 (1H, brd, *J* = 9.0, 1.2 Hz, H-3), 5.36 (1H, brd, *J* = 9.0, 1.2 Hz, H-9), 4.88 (1H, d, *J* = 5.4 Hz, H-7), 4.48 (1H, m, H-4), 3.95 (3H, s, H-29), 3.95 (1H, overlap, H-11), 3.55 (1H, q, *J* = 5.4 Hz, H-6), 3.33 (3H, s, H-23), 3.24 (3H, s, H-27), 3.08 (1H, m, H-12), 2.53 (1H, m, H-10), 2.45 (1H, dd, *J* = 13.2, 7.2 Hz, H-15a), 2.25 (1H, dd, *J* = 13.2, 6.0 Hz, H-15b), 1.85 (3H, d, *J* = 1.2 Hz, H-22), 1.84 (1H, overlap, H-14), 1.76 (1H, m, H-5a), 1.58 (1H, m, H-5b), 1.54 (3H, d, *J* = 1.2 Hz, H-25), 1.41(1H, m, H-13a), 1.36 (1H, m, H-13b), 0.91 (3H, d, *J* = 6.6 Hz, H-28), 0.79 (3H, d, *J* = 7.2 Hz, H-26).

*Compound **1R**:* yellow, amorphous powder; ESIMS *m/z* 891.8 $[M + NH_4]^+$; ^{1}H NMR (600 MHz, DMSO-d_6, 70 °C) δ 9.24 (1H, brs, *NH*-1), 6.85 (1H, s, H-19), 6.26 (2H, brs, NH_2-24), 6.02 (1H, brd, *J* = 9.0, 1.2 Hz, H-3), 5.37 (1H, m, H-4), 4.86 (1H, brd, 8.4 Hz, H-9), 4.75 (1H, brs, H-7), 4.51 (1H, brs, H-11), 3.95 (3H, s, H-29), 3.33 (1H, overlap, H-6), 3.32 (3H, s, H-27), 2.94 (1H, m, H-12), 2.74 (3H, s, H-23), 2.68 (1H, m, H-10), 2.33 (1H, dd, *J* = 12.6, 5.4 Hz, H-15a), 2.18 (1H, dd, *J* = 12.6, 5.4 Hz, H-15b), 1.89 (3H, d, *J* = 1.2 Hz, H-22), 1.69 (1H, m, H-5a), 1.38 (3H, s, H-25), 1.30 (1H, m, H-5b), 1.24 (1H, m, H-14), 0.95 (1H, dt, *J* = 15.0, 5.4 Hz, H-13a), 0.86 (3H, d, *J* = 6.0 Hz, H-28), 0.84 (3H, d, *J* = 6.6 Hz, H-26), 0.79 (1H, m, H-13b).

*Compound **1S**:* yellow, amorphous powder,; ESIMS *m/z* 897.1 $[M + Na]^+$; ^{1}H NMR (600 MHz, DMSO-d_6, 70 °C) δ 9.08 (1H, brs, *NH*-1), 6.78 (1H, brs, H-19), 6.38 (2H, brs, NH_2-24), 5.83 (1H, brd, *J* = 9.6 Hz, H-3), 5.49 (1H, m, H-4), 4.88 (1H, overlap, H-9), 4.81 (1H, overlap, H-7), 4.71 (1H, brs, H-11), 3.95 (3H, s, H-29), 3.41 (1H, m, H-6), 3.34 (3H, s, H-27), 3.32 (1H, m, H-12), 3.06 (3H, s, H-23), 2.57 (1H, m, H-10), 2.41 (1H, dd, *J* = 13.2, 4.8 Hz, H-15a), 2.20 (1H, dd, *J* = 13.2, 4.8 Hz, H-15b), 1.83 (3H, s, H-22), 1.70 (1H, m, H-5a), 1.52 (1H, m, H-5b), 1.39 (3H, s, H-25), 1.33 (1H, m, H-13a), 1.24 (1H, m, H-14), 1.16 (1H, m, H-13b), 0.87 (3H, d, *J* = 6.6 Hz, H-28), 0.65 (3H, d, *J* = 6.6 Hz, H-26).

Bioconversion Experiment. Three ISPII medium plates were inoculated with spores of GDM-*pks*$^-$ and incubated at 28 °C for 3–4 d. One plate was overlaid with 1.0–2.0 mg of **1** or **2** (in 100 μL of dimethylformamide + 200 μL of H_2O), and another plate with about 3.0 mg of CT-1-7 (in 100 μL of dimethylformamide + 200 μL of H_2O); a third plate was used as a control (without any compound added). These plates were incubated at 28 °C for another 2 d; for each day, half of each plate culture was cut out for EtOAc extraction (24 h). Each EtOAc extraction was poured out and air-concentrated to a final volume of about 200 μL. Samples of 15–20 μL were used for silica gel TLC analysis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MS, IR, and NMR spectra of **1** and **2**. This material can be accessed free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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