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Maklamicin, an Antibacterial Polyketide from an Endophytic *Micromonospora* sp.

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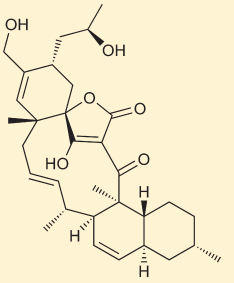
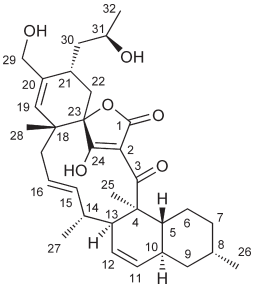
Supporting Information

ABSTRACT: A new spirotetronate-class polyketide, maklamicin (**1**), was isolated from the culture extract of an endophytic actinomycete of the genus *Micromonospora*. The structure and relative configuration of **1** were elucidated by interpretation of NMR and other spectroscopic data, and the absolute configuration was determined using the modified Mosher method. Maklamicin (**1**) showed strong to modest antimicrobial activity against Gram-positive bacteria.



Actinomycetes are Gram-positive bacteria, which have served as a significant source for drug discovery for more than five decades and have yielded more than 10 000 bioactive compounds. Of these, approximately 75% are produced by *Streptomyces* species, the dominant genus in terrestrial environments such as soil and leaf litter, while 25% are the products of non-*Streptomyces* species traditionally termed "rare actinomycetes".[1] Within this group, *Micromonospora* is the most prolific in producing metabolites, accounting for more than 700 compounds to date including pharmaceutically important chemical classes such as calicheamicin and gentamicin.[1] Wide distribution of this genus in various aquatic and terrestrial environments has been demonstrated, but its association with plants is not fully recognized.[2] Recently, members of this genus were recovered from nitrogen-fixing root nodules of diverse legumes in significant numbers.[3] In addition, some of these endophytic actinomycetes were suggested to have nitrogen fixation ability, and they were speculated to promote the growth of host plants.[3d,4] Although evidence is still lacking, their phytoprotective properties through antibiotic production is also possible.[5] Intrigued by their potential as a source of medicinally useful molecules, we examined the metabolites of *Micromonospora lupini* isolated from a root nodule of the legume *Lupinus angustifolius* and found new anthraquinones possessing anti-invasive activity.[6] As part of our continuing chemical investigation on plant-associated actinomycetes,[7] we now report on the isolation of a new tetronate-class polyketide, maklamicin (**1**), from the culture extract of *Micromonospora* sp. GMKU326 isolated from the root of a leguminous plant, Maklam phueak (*Abrus pulchellus* Wall. Ex Thwaites subsp. *pulchellus*), collected in Thailand.



Maklamicin (**1**)

RESULTS AND DISCUSSION

The producing strain GMKU326 was cultured in A-11 M medium at 30 °C for 6 days, and the whole culture broth was extracted with 1-butanol. The extract was consecutively fractionated by normal- and reversed-phase column chromatographies, followed by HPLC purification on a C18 column, to yield maklamicin (**1**) as an optically active, amorphous solid ($[\alpha]_D$ −110, $CHCl_3$). A molecular formula of $C_{32}H_{44}O_6$ was confirmed by high-resolution ESITOFMS data showing a pseudomolecular ion at m/z 547.3034 $[M + Na]^+$, which was corroborated by the interpretation of NMR data. The IR spectrum indicated the presence of hydroxyl (3330 cm^{-1}) and carbonyl (1755 cm^{-1}) groups. ^1H and ^{13}C NMR data in combination with the HSQC analysis revealed the presence of 32 carbons

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Table 1. ^{1}H and ^{13}C NMR Data for Maklamicin (1) in $CDCl_3$

no.	δ_C[a]	δ_H mult (J in Hz)[b]	HMBC[b,c]
1	167.0, qC		
2	107.2, qC		
3	201.9, qC		
4	50.6, qC		
5	42.2, CH	1.47, dd (10.8, 10.8)	3, 4, 7, 9, 25
6α	23.1, CH_2	1.23, m	
6β		2.00, m	
7α	32.71, CH_2	1.57, m	
7β		1.73, m	
8	27.7, CH	2.08, m	
9α	39.5, CH_2	1.59, m	
9β		1.40, ddd (12.9, 12.5, 5.0)	5, 8, 10, 26
10	32.68, CH	1.98, m	
11	130.38,[d] CH	5.38, d (10.1)	5, 10
12	125.0, CH	5.46, ddd (10.1, 5.8, 2.4)	4, 10, 13
13	41.0, CH	2.83, br dd (5.8, 5.8)	4, 5, 11, 12, 14, 15, 25
14	39.8, CH	1.85, m	4, 15, 16, 27
15	144.4, CH	5.49, ddd (15.5, 9.6, 2.0)	17
16	120.7, CH	5.01, ddd (15.5, 11.2, 2.5)	14
17a	42.0, CH_2	1.93, br d (14.2)	15, 16, 18, 19, 28
17b		2.37, dd (14.2, 11.2)	15, 16, 18, 23
18	39.1, C		
19	130.41,[d] CH	5.22, s	17, 18, 21, 23, 29
20	137.0, C		
21	28.6, CH	2.74, br dd (8.9, 8.0)	20
22α	31.2, CH_2	1.72, d (14.7)	18, 20, 21, 23, 24
22β		2.36, dd (14.7, 8.0)	21, 30
23	86.1, qC		
24	204.3, qC		
25	15.8, CH_3	1.57, s	3, 4, 5, 13
26	18.9, CH_3	1.03, d (7.5)	7, 8, 9
27	20.7, CH_3	0.86, d (7.5)	13, 14, 15
28	23.4, CH_3	1.25, s	17, 18, 19, 23
29a	65.0, CH_2	3.99, d (13.1)	19, 20, 21
29b		4.34, d (13.1)	19, 20, 21
30a	41.6, CH_2	1.67, dd (14.8, 10.6)	20
30b		1.80, dd (14.8, 8.9)	20, 31
31	66.6	3.82, dq (10.6, 6.4)	
32	24.6	1.21, d (6.4)	30, 31

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbon. [d] Interchangeable.

attributable to three oxygen-bearing quaternary sp^2 carbons, seven sp^2 carbons (five are proton-bearing), three quaternary sp^3 carbons (one is oxygen-bearing), seven sp^3 methylenes (one is oxygen-bearing), seven sp^3 methines (one is oxygen-bearing), and five methyl groups. These data accounted for all of the observed NMR resonances except for three exchangeable protons. The ^{13}C NMR spectrum of **1** showed distinctive resonances of sp^2 quaternary carbons at δ 204.3, 201.9, 167.0, and 107.2, characteristic of tetronic acid carbons,[8,9] although its UV absorption maxima λ_{max} at 246 and 291 nm were obviously different from the reported values for tetronic acids such as tetrocarcins (λ_{max} 250, 270 nm)[8] and abyssomicins (λ_{max} 215, 255 nm).[9]

The full planar structure of **1** was assigned through interpretation of 1D and 2D NMR spectroscopic data recorded in $CDCl_3$ (Table 1). From the ^{1}H−^{1}H COSY spectrum, two fragments were established (Figure 1). The first fragment, constructed on the basis of a series of correlations from H-5 to H-10, was a substituted cyclohexane ring bearing a secondary methyl group at C-8. This unit was expanded to include an eight-carbon fragment containing two double bonds (C-11/C-12 and C-15/C-16) and a secondary methyl group (H_3-27) at C-14 on the basis of a series of COSY correlations from H-10 to H_2-17. The geometries of the C-11−C-12 and C-15−C-16 double bonds were assigned as *Z* and *E*, respectively, on the basis of $^3J_{HH}$ vicinal coupling

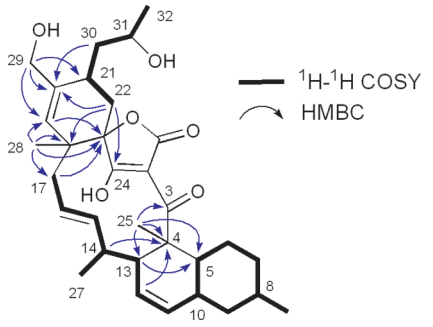

Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations for **1**.

constants (10.1 and 15.5 Hz, respectively). For the second fragment, a cross-peak was observed between the methyl protons H_3-32 and an oxymethine proton H-31, which in turn showed correlation to H_2-30. Cross-peaks were also observed for H_2-30/H-21 and H-21/H_2-22, and thus a five-carbon fragment bearing an oxygen substitution at C-31 was established.

Interpretation of HMBC NMR data allowed the ^{1}H−^{1}H COSY-defined fragments to be joined as shown in Figure 1. HMBC correlations from the singlet methyl protons H_3-25 to C-3, C-4, C-5, and C-13 connected a three-carbon fragment (C-3/C-4/C-25) to the first fragment, thereby establishing the decalin unit possessing a keto group at C-3 and a linear chain at C-13. The second COSY-defined fragment was connected to a three-carbon unit consisting of two sp^2 carbons C-19 and C-20 and an oxygenated carbon C-29 on the basis of a series of HMBC correlations from H_2-30, H-21, and H_2-22 to C-20 and from H_2-29 to C-19, C-20, and C-21. This fragment was expanded to include two sp^3 quaternary carbons (C-18 and C-23) on the basis of HMBC correlations from H-19 and H_2-22 to these carbons, providing a cyclohexene ring unit. This unit and the decalin unit were joined between C-17 and C-18 on the basis of HMBC correlations from H_3-28 to C-17 and from H_2-17 to C-18, C-19, and C-23. Furthermore, an HMBC correlation from H_2-22 to an oxygenated olefin carbon C-24 at δ_C 204.3 connected this carbon to C-23. The molecular formula of **1** indicated that the remaining part had to account for C_2H_3O, which could be attributed to one carbonyl (δ_C 167.1), one quaternary sp^2 carbon (δ_C 107.2), and three exchangeable protons. Two protons were assigned to the hydroxyl protons at C-29 and C-31; thus the remaining atoms had to be connected to either O-23, C-24, or C-3, and two more rings were required by the unsaturation degree. These requirements gave only the tetronic acid substructure, as suggested by the aforementioned chemical shift similarity of C-1, C-2, C-3, and C-24 to the tetronic acid carbons in the known compounds.[8,9]

The relative configuration of **1** was assigned by analysis of NOESY and ROESY correlations and vicinal ^{1}H−^{1}H and long-range ^{1}H−^{13}C coupling constants. An NOE between H-5 and H-7β and large scalar couplings ($^3J_{HH}$ > 10 Hz) shown by H-5/H-6α, H-5/H-10, and H-9β/H-10 indicated the 1,3-diaxial relationships for H-5, H-7β, and H-9β and a *trans* ring fusion in the decalin unit. An NOE between H_3-26 and H-10 placed this methyl group in axial orientation at C-8. The pseudoaxial orientation of the side chain at C-13 and the axial orientation of the methyl group at C-4 were established on the basis of NOEs between H-5 and H-14 and between H-6β and H_3-25. The configuration at C-14 was determined by an NOE between H-12 and H_3-27. A zigzag conformation of the C-13 to C-18 chain was suggested by ROESY correlations for H-13/H-15, H-14/H-16, H-15/H-17b, and H-17a/H-19. This was consistent with the large coupling constants between H-14 and H-15 (J = 9.6 Hz) and between H-16 and H-17b (J = 11.2 Hz). Furthermore, NOEs between H-21 and H-22β and between H-22β and H_3-28 placed these protons on the same side of the cyclohexene ring (Figure 2). Conformation of the side chain at C-21 was deduced from the large

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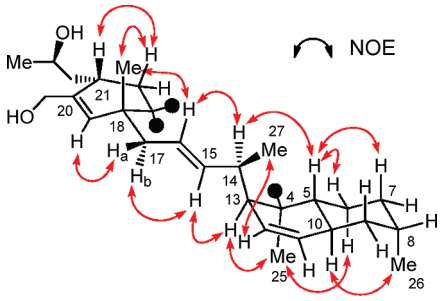

Figure 2. Conformation and configuration of the decalin and cyclohexene moieties of **1** determined by NOESY and ROESY data and *J*-based analysis.

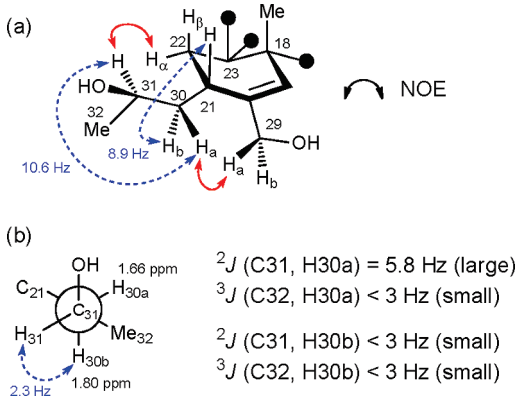

Figure 3. Configuration at C-31 determined on the basis of (a) NOE and $^3J_{HH}$ value analyses and (b) $^2J_{CH}$ and $^3J_{CH}$ values of **1**.

coupling constants between H-21 and H-30b and between H-30a and H-31 along with ROESY correlations for H-30a/H-29a and H-22α/H-31 (Figure 3a). Within this side chain, small $^3J_{CH}$ coupling constants for C-32/H-30a and C-32/H-30b indicated a *gauche* relationship of H-32 methyl to H-30a and H-30b, and a large $^2J_{CH}$ value for C-31/H-30a and a small $^2J_{CH}$ value for C-31/H-30b indicated an *anti* relationship of the 31-OH group to H-30b, thereby establishing the configuration at C-31 (Figure 3b).[10] Finally, the configuration at the spirocarbon C-23 was determined on the basis of a small coupling constant between H-22β and C-23 ($^2J_{CH}$ < 3 Hz) and a large coupling constant between H-22α and C-23 ($^2J_{CH}$ = 4.8 Hz) that allowed an *anti* orientation of H-22β to the oxygen atom at C-23 for the C-22−C-23 bond (Figure 4).

The absolute stereochemistry of **1** was determined by applying the modified Mosher method[11] to the secondary hydroxyl group at C-31. Prior to the esterification, the enolic hydroxy group at C-24 was protected as a methyl ether by treating **1** with $TMSCHN_2$ in $CHCl_3$/MeOH. The methylated derivative **2** was then treated with (*S*)- and (*R*)-MTPA chloride, yielding the bis-(*R*)- and (*S*)-MTPA esters (**3** and **4**), respectively. In the ^1H NMR spectra of **3** and **4**, positive $\Delta\delta_{S-R}$ ($\delta_S - \delta_R$) values were observed for the protons for H_3-32, while negative $\Delta\delta_{S-R}$ values were observed for H_2-30, H-21, H_2-22, H_2-29, H-19, H_3-28, and H_2-17 (Figure 5). These data allowed assignment of the absolute configuration of C-31 as *R*. The absolute configurations of the polycyclic stereogenic centers of **1** were identical with those of the previously reported spirotetronate antibiotics.[8b,c]

Maklamicin (**1**) is a new member of spirotetronic acids comprising a *trans*-decalin unit and a tetronic acid moiety spiro-linked with a cyclohexene ring. Although over 50 related spirotetronates have been reported from actinomycetes, two structural features differentiate this new compound from others.[12] First, the presence of a stereogenic center (C-31) on the substituent of the cyclohexene unit has not been reported.

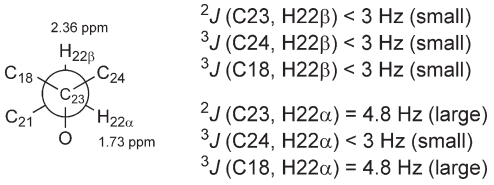

Figure 4. Configuration at C-23 determined on the basis of $^2J_{CH}$ and $^3J_{CH}$ values of **1**.

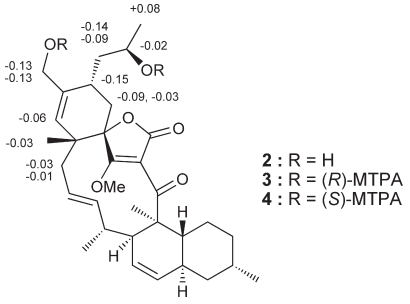

Figure 5. $\Delta\delta_{S-R}$ values for MTPA esters (**3** and **4**) of **2**.

The other spirotetronates have an achiral substituent at the same position such as methyl,[8c] formyl,[8a] ethyl,[8b,13] and *n*-propyl groups.[13] Second, the carbon chain connecting the decalin and the cyclohexene units in **1** is the shortest ever reported. While the carbon chain length between the two ring systems in the known spirotetronates is six or 10, constructing a 13- or 17-membered ring, **1** contains an 11-membered ring closed by a four-carbon chain linker. Biosynthetic gene analysis of spirotetronic acids suggests that the ring size is dependent on the location of the 1,3-diene in the polyketide chain that undergoes Diels−Alder cycloaddition with the dienophile part in the tetronic acid moiety.[14]

Maklamicin (**1**) showed strong to modest antimicrobial activities against Gram-positive bacteria *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Enterococcus faecalis* with MIC values of 0.2, 1.7, 6.5, 13, and 13 μg/mL, respectively, while it was inactive against *Escherichia coli* (MIC > 50 μg/mL) and slightly active against *Candida albicans* (MIC = 50 μg/mL). **1** displayed moderate cancer cell cytotoxicity against HeLa human cervical cancer cells and MCF7 human breast cancer cells with IC_{50} values of 17 and 34 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-3000 polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were measured on a Perkin-Elmer Spectrum 100. NMR spectra were obtained on a Bruker AVANCE 400 or a Bruker AVANCE 500 spectrometer and referenced to residual solvent signals (δ_H 7.26, δ_C 77.0). *J*-resolved HMBC experiments[15] were performed on a Varian INOVA-500 spectrometer. HRESITOFMS were recorded on a Bruker microTOF focus. Cosmosil 75C18-PREP (Nacalai Tesque, Inc., 75 μm) was used for ODS column chromatography. HPLC separation was performed using an XTerra Prep RP18 (Waters, 19 × 300 mm) with a photodiode array detector.

Microorganism. Strain GMKU326 was isolated from a root of Maklam phueak (*Abrus pulchellus* Wall. Ex Thwaites subsp. *pulchellus*) collected at Eastern Botanical Garden (Khao Hin Son), Chachoengsao Province, Thailand, according to the reported protocol.[16] The strain was identified as a member of the genus *Micromonospora* on the basis of

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99.3% 16S rRNA gene sequence identity (1368 nucleotides; GenBank accession number GU45831) with the *Micromonospora auratinigra* TT1-11^T type strain (accession number AB159779).

Fermentation. Strain GMKU326 cultured on a slant agar medium consisting of soluble starch 0.5%, glucose 0.5%, meat extract (Kyokuto Pharmaceutical Industrial Co., Ltd.) 0.1%, yeast extract (Difco Laboratories) 0.1%, NZ-case (Wako Pure Chemical Industries, Ltd.) 0.2%, NaCl 0.2%, $CaCO_3$ 0.1%, and agar 1.5% was inoculated into 500 mL K-1 flasks each containing 100 mL of the V-22 seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone (Difco Laboratories) 0.5%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.3% (pH 7.0). The cultures were incubated on a rotary shaker (200 rpm) at 30 °C for 4 days. The seed culture (3 mL) was transferred into 500 mL K-1 flasks each containing 100 mL of the A-11 M production medium consisting of glucose 0.2%, soluble starch 2.5%, yeast extract 0.5%, polypeptone (Wako Pure Chemical Industries, Ltd.) 0.5%, NZ-amine (Wako Pure Chemical Industries, Ltd.) 0.5%, $CaCO_3$ 0.3%, and Diaion HP-20 (Mitsubishi Chemical Co.) 1%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were placed on a rotary shaker (200 rpm) at 30 °C for 6 days.

Extraction and Isolation. At the end of the fermentation period, 100 mL of 1-butanol was added to each flask, and they were allowed to shake for one hour. The mixture was centrifuged at 6000 rpm for 10 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent gave 7.5 g of extract from 5 L of culture. The crude extract (7.5 g) was subjected to silica gel column chromatography with a step gradient of $CHCl_3$/MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1, and 0:1 v/v). Fractions 6 and 7 were combined and concentrated to provide 3.0 g of brown oil, which was repeatedly purified by reversed-phase ODS column chromatography with a gradient of MeCN/0.1% HCO_2H (2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2 v/v). Fraction 7 was evaporated, and the remaining aqueous solution was extracted with EtOAc. The organic layer was then concentrated to give a brown solid (0.8 g). Final purification was achieved by preparative C-18 HPLC using a gradient of MeCN/0.1% HCO_2H (MeCN concentration: 50% for 0−5 min; 50−60% for 5−10 min; 60% for 10−20 min; 60−80% for 20−30 min) at 15 mL/min, yielding maklamicin (**1**, 60 mg) as a colorless, amorphous solid with a retention time of 22.5 min.

*Maklamicin (**1**):* colorless, amorphous solid; $[\alpha]^{25}_D$ −110 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 246 (4.06), 291 (3.90) nm; (MeOH/0.01 N HCl) 222 (4.50), 308 (3.96); (MeOH/0.01 N NaOH) 248 (4.30), 290 (4.17); IR (ATR) ν_{max} 3330, 1755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESITOFMS $[M + Na]^+$ 547.3034 (calcd for $C_{32}H_{44}O_6Na$, 547.3030).

*24-O-Methyl Ether (**2**).* To a solution of **1** (5.0 mg, 0.0095 mmol) in $CHCl_3$/MeOH (0.25 mL each) was added a solution of $TMSCHN_2$ in Et_2O (2.0 M, 0.25 mL, 0.50 mmol) at room temperature. After stirring for 15 min, the reaction mixture was concentrated to dryness. The residue was purified on a silica gel column (hexane/EtOAc, 20:1−1:1) to give **2** (3.2 mg, 62% yield) as a colorless, amorphous solid: $[\alpha]^{24}_D$ −33 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, d, *J* = 7.1 Hz, H-27), 1.01 (3H, d, *J* = 7.2 Hz, H-26), 1.08 (3H, s, H-28), 1.12 (1H, m, H-6α), 1.23 (3H, d, *J* = 6.1 Hz, H-32), 1.38 (1H, ddd, *J* = 12.8, 12.8, 4.7 Hz, H-9β), 1.42 (1H, br d, *J* = 13.6 Hz, H-6β), 1.50 (3H, s, H-25), 1.51 (1H, m, H-7α), 1.52 (1H, m, H-5), 1.57 (1H, m, H-9α), 1.63 (1H, m, H-30a), 1.73 (1H, m, H-7β), 1.73 (1H, d, *J* = 14.6 Hz, H-22α), 1.88 (1H, d, *J* = 14.1 Hz, H-17a), 1.90 (1H, m, H-30b), 1.94 (1H, m, H-10), 1.98 (1H, m, H-14), 2.06 (1H, m, H-8), 2.31 (1H, dd, *J* = 14.6, 7.0 Hz, H-22β), 2.39 (1H, dd, *J* = 14.1, 11.4 Hz, H-17b), 2.72 (1H, dd, *J* = 8.9, 7.0 Hz, H-21), 2.77 (1H, dd, *J* = 6.2, 5.7 Hz, H-13), 3.87 (1H, m, H-31), 4.02 (1H, d, *J* = 13.2 Hz, H-29b), 4.06 (3H, s, 24-OC*H*$_3$), 4.32 (1H, d, *J* = 13.2 Hz, H-29a), 5.01 (1H, ddd, *J* = 15.2, 11.4, 2.1 Hz, H-16), 5.12 (1H, s, H-19), 5.14 (1H, ddd, *J* = 15.2, 9.6, 1.8 Hz, H-15), 5.37 (1H, d, *J* = 10.1 Hz, H-11), 5.48 (1H, ddd, *J* = 10.1, 5.7, 2.1 Hz, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3 (C-25), 18.7 (C-26), 20.2 (C-27), 22.2 (C-6), 23.3 (C-28), 24.6 (C-32), 27.8 (C-8), 28.8 (C-21), 31.4 (C-22), 31.8 (C-10), 32.6 (C-7), 39.0 (C-18), 39.3 (C-9), 39.6 (C-14), 41.2 (C-13), 41.4 (C-30), 42.7 (C-5), 43.2 (C-17), 52.7 (C-4), 65.0 (C-29), 65.7 (24-OCH$_3$), 66.8 (C-31), 85.8 (C-23), 112.3 (C-2), 124.4 (C-16), 126.2 (C-12), 130.6 (C-11), 130.8 (C-19), 137.1 (C-20), 139.1 (C-15), 168.1 (C-1), 192.5 (C-24), 197.7 (C-3); HRESITOFMS $[M + Na]^+$ 561.3189 (calcd for $C_{33}H_{46}O_6Na$, 561.3187).

*Bis-(R)-MTPA Ester of **2** (**3**).* (*S*)-MTPA chloride (9.5 mg, 0.037 mmol) was added to a solution of **2** (5.0 mg, 0.0093 mmol) in dry pyridine (1 mL) at room temperature. After 18 h, the reaction mixture was concentrated to dryness and purified by HPLC using a Nacalai Cosmosil πNAP column (4.6 × 150 mm) with a MeCN/H_2O gradient (55−100% MeCN in H_2O over 21 min; 100% MeCN for 20 min, 1 mL/min) to afford **3** (2.5 mg, t_R 34.2 min, 28% yield): colorless, amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04 (3H, s, H-28), 1.21 (3H, d, *J* = 6.0 Hz, H-32), 1.73 (1H, d, *J* = 15.0 Hz, H-22β), 1.85 (1H, d, *J* = 14.5 Hz, H-17a), 1.85 (1H, m, H-30a), 2.07 (1H, m, H-30b), 2.08 (1H, d, *J* = 15.0, 6.5 Hz, H-22α), 2.24 (1H, m, H-21), 2.35 (1H, dd, *J* = 12.5, 11.5 Hz, H-17b), 4.67 (1H, d, *J* = 12.5 Hz, H-29a), 4.75 (1H, d, *J* = 12.5 Hz, H-29b), 5.19 (1H, ddd, *J* = 9.9, 6.0, 2.1 Hz, H-31), 5.33 (1H, s, H-19); HRESITOFMS *m/z* 993.3987 $[M + Na]^+$ (calc for $C_{53}H_{60}F_6O_{10}Na$ 993.3983).

*Bis-(S)-MTPA Ester of **2** (**4**).* In the same manner as described for **3**, **2** (5.0 mg, 0.0093 mmol) was reacted with (*R*)-MTPA chloride to give **4** (2.6 mg, t_R 33.2 min, 29% yield): colorless, amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, s, H-28), 1.29 (3H, d, *J* = 6.0 Hz, H-32), 1.64 (1H, d, *J* = 15.0 Hz, H-22β), 1.76 (1H, m, H-30a), 1.82 (1H, d, *J* = 14.5 Hz, H-17a), 1.93 (1H, m, H-30b), 2.05 (1H, d, *J* = 15.0, 8.0 Hz, H-22α), 2.09 (1H, m, H-21), 2.34 (1H, dd, *J* = 12.5, 11.5 Hz, H-17b), 4.54 (1H, d, *J* = 12.5 Hz, H-29a), 4.62 (1H, d, *J* = 12.5 Hz, H-29b), 5.17 (1H, ddd, *J* = 10.1, 6.0, 2.0 Hz, H-31), 5.27 (1H, s, H-19); HRESITOFMS *m/z* 993.3989 $[M + Na]^+$ (calc for $C_{53}H_{60}F_6O_{10}Na$ 993.3983).

Biological Assays. Antimicrobial assays were carried out using *Escherichia coli* NIH-JC2, *Micrococcus luteus* ATCC9343, *Bacillus subtilis* PCI219, *Bacillus cereus* NBRC15305, *Staphylococcus aureus* IFO12732, *Enterococcus faecalis* NBRC100480, and *Candida albicans* IFO1594. Mueller Hinton broth (DIFCO Laboratories) was used for bacteria, and Yeast Nitrogen Base (DIFCO Laboratories) supplemented with 2% glucose was used for *C. albicans*. Test microorganisms were inoculated into a 32 mL test tube containing 8 mL of the liquid medium. After incubation on a reciprocal shaker for 20 h at 30 °C, the cells were collected by centrifugation (3000 rpm, 5 min), and the cell suspension (1 × 10^5 cells/mL) was prepared in saline. Then, the liquid medium (135 μL), the cell suspension (15 μL), and the sample solution in DMSO (0.5 to 1 μL) were added into the wells of a 96-well culture plate, and the plate was agitated gently to mix the solution. After incubation for 20 h at 37 °C (*E. coli*) or 30 °C (other bacteria and *C. albicans*), the absorbance at 650 nm was measured using a microplate reader. MIC values of the standard antibiotic tetracycline hydrochloride (Sigma-Aldrich Co.) against the above-described bacteria *E. coli*, *M. luteus*, *B. subtilis*, *B. cereus*, *S. aureus*, and *E. faecalis* were 0.8, 0.1, 0.05, 1.5, 0.05, and 0.2 μg/mL, respectively.

Cytotoxic assay was carried out using HeLa human cervical cancer cells and MCF7 human breast cancer cells. Cancer cells were suspended in RPMI medium containing 10% FBS (Sigma-Aldrich Co.) and 2 mM L-glutamine and seeded into the wells of a 96-well culture plate (1 × 10^4 cells/50 μL/well). Then, test compounds at various concentrations in DMSO/RPMI medium (0.8:92.2 v/v, 50 μL) were added to the wells. After incubation for 48 h in a humidified 5% CO_2 incubator at 37 °C, MTT (0.25 mg, Sigma-Aldrich Co.) in PBS (−) (50 μL) was added to each well, and the plates were placed in the incubator 37 °C for 4 h. Medium in the wells was removed by suction, and DMSO (100 μL) was added to each well. After 10 min, the absorbance at 570 nm was read by a

microplate reader. Staurosporin (Wako Pure Chemical Industries, Ltd.) was used as a positive control. Its IC_{50} values against HeLa and MCF7 cell lines were 4 pM and 50 nM, respectively.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR spectra of maklamicin (**1**) and ^{1}H NMR spectra of **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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