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ORIGINAL ARTICLE

Nomimicin, a new spirotetronate-class polyketide from an actinomycete of the genus *Actinomadura*

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Nomimicin (1), a new spirotetronate-class polyketide, was isolated from the culture broth of an actinomycete of the genus *Actinomadura*. Its structure was established by spectroscopic methods, and the absolute configuration was determined by a combination of NOESY experiment, *J*-based configuration analysis and the modified Mosher method. Nomimicin (1) showed antimicrobial activity against *Micrococcus luteus*, *Candida albincans* and *Kluyveromyces fragilis*.
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INTRODUCTION

Microbial secondary metabolites have been the most productive source of small molecules for the development of drugs.[1] Almost a half of the known microbial bioactive compounds are derived from actinomycetes, and specifically *Streptomyces* accounts for more than 70% of the actinomycete-derived metabolites.[2] Among the non-*Streptomyces* species, *Micromonospora* is the leading producer of secondary metabolites, including polyketides, peptides and glycosides, whereas *Actinomadura* is known as a producer of chemically and biologically unique polyketides such as antitumor enediynes,[3] mannose-binding quinone glycosides with antifungal and anti-HIV activities[4] and anticoccidial polyethers,[5] accounting for up to 350 compounds reported as of 2005.[2] During our continuing investigation on the metabolites from actinomycetes of non-*Streptomyces* group,[6,7] a new spirotetronate antibiotic of polyketide origin, nomimicin (**1**, Figure 1), was isolated from the culture extract of *Actinomadura* sp. TP-A0878. In this paper, we describe the isolation, structural determination and biological properties of **1**.

RESULTS AND DISCUSSION

The producing strain TP-A0878 was isolated from a compost sample collected at Nomi, Ishikawa, Japan, and identified as a member of *Actinomadura* on the basis of 16S rRNA gene sequence. This strain was cultured in A-3M 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 C_{18} column, to yield nomimicin (**1**) as an optically active, amorphous solid ($[\alpha]_D$ –94, $CHCl_3$). Compound **1** gave a pseudomolecular ion at *m/z* 519.2726 $[M+Na]^+$ in a HR-ESI time-of-flight (TOF) MS measurement, suggesting a molecular formula of $C_{30}H_{40}O_6$, which was subsequently corroborated by the NMR data. The IR spectrum indicated the presence of hydroxyl (3397 cm^{-1}) and carbonyl (1749 cm^{-1}) functionalities. Analysis of ^1H and ^{13}C NMR, and HSQC data established three oxygenated quaternary sp^2 carbons, seven sp^2 carbons (five are proton-bearing), three quaternary sp^3 carbons (one is oxygen-bearing), seven sp^3 methines (two are oxygen-bearing), five sp^3 methylenes and five methyl groups (Table 1). The UV spectrum of **1** was closely similar to that of maklamicin[7] (Figure 1), a spirotetronate polyketide that we recently discovered from a *Micromonospora* strain, suggesting the presence of a tetronic acid functionality in this molecule. This was supported by the ^{13}C chemical shifts of the carbons C-1 (δ_C 167.0), C-2 (δ_C 107.2), C-3 (δ_C 200.3) and C-24 (δ_C 204.5) that closely matched those reported for this structure in maklamicin. As three double bonds and a tetronic acid unit that contains three double bonds and one ring accounted 7 of the 11 double-bond equivalents, **1** must possess four more rings to satisfy the molecular formula.

Interpretation of the COSY and HSQC spectra provided six fragments (indicated by bold lines, Figure 2), and the linkage among these fragments was established by analysis of HMBC correlations. A secondary methyl fragment C-8/C-26 and two large fragments C-7/C-6/C-5/C-10/C-9 and C-11–C-17 were joined to form an oxygenated cyclohexane substructure, based on HMBC correlations from H_3-26 to C-7 and C-9, H-11 to C-5 and C-9, and H-9 to C-11. Furthermore, a series of long-range correlations from a singlet methyl proton resonance H_3-25 to C-3, C-4, C-5 and C-13 fused an additional ring to the cyclohexane ring. Accordingly, a Δ^1-octalin framework with an olefinic extension (C-14 to C-17) and a carbonyl functionality from the tetronic acid unit (C-3) were established to be present in the bottom half of the molecule. The *E*-geometry of the olefinic extension was deduced from a large vicinal coupling constant between H-15 and

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H-16 (15.3 Hz). The remaining fragments C-21/C-22, C-29/C-30 and C-19/C-20/C-28 were established by COSY correlations, and were assembled by HMBC correlations from H_3-28 to C-19, C-20 and C-21, H-30 to C-21, and H-29 to C-20 and C-22 into the upper half of the cyclohexene unit in the top half of the molecule. Finally, a set of HMBC correlations from H_3-27 to C-17, C-18, C-19 and C-23 assigned an oxygenated spirocarbon (C-23) and a quaternary carbon C-18 to be placed in the lower half of the cyclohexene ring, and at the same time, as a juncture to the tetronic acid moiety and to the terminus of the octalin extension, respectively. The spiro connection between the cyclohexene and tetronic acid units was also supported by an HMBC correlation from H_2-22 to C-24, and thus a characteristic pentacycle-condensed spirotetronate structure was assigned for **1** (Figure 2).

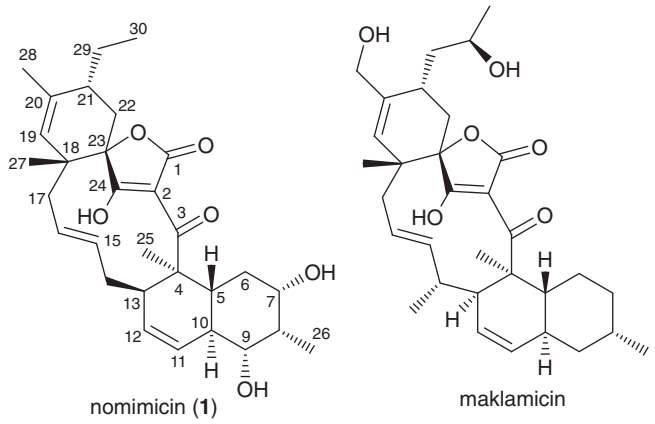

Figure 1 Structures of nomimicin (**1**) and maklamicin.

The relative configuration was elucidated by NOESY experiments and *J*-based configuration analysis (Figures 3 and 4). NOEs between H-5, H-7 and H-9, and large scalar couplings ($^3J_{HH} > 10$ Hz) shown by H-5/H-6α, H-6α/H-7 and H-5/H-10 provided the 1,3-diaxial relationships between H-5, H-7 and H-9, and a *trans* ring fusion in the octalin unit. The axial orientation of the methyl group at C-8 was confirmed by an NOE between H_3-26 and H-10. NOEs between H-10 and H_3-25, and between H_3-25 and H-13 placed the H_3-25 methyl and H-13 on the same side of the octalin ring, with respect to the

Table 1 ^{1}H and ^{13}C NMR data for nomimicin (1) in $CDCl_3$

Position	δ_C[a]	δ_H *mult (J in Hz)*[b]	*HMBC*[b,c]	*COSY*[b]	*NOESY*[b]
1	167.0, qC				
2	107.2, qC				
3	200.3, qC				
4	49.4, qC				
5	34.9, CH	1.55, br.dd (11.3, 11.0)	4, 6, 7, 9, 25	6α, 10	7, 9
6α	30.7, CH_2	1.32, ddd (11.7, 11.6, 11.3)	4, 5, 7, 10	5, 6β, 7	6β
6β		2.18, br.d (11.7)	4, 5, 7, 8, 10	6α, 7	6α, 7
7	71.1, CH	3.96, ddd (11.6, 4.6, 4.6)	5, 6, 8, 9, 26	6α, 6β	5, 6β, 8, 9
8	41.5, CH	2.45, m	6, 7, 9, 10, 26	26	7, 9, 26
9	74.0, CH	3.45, dd (10.7, 4.8)	5, 8, 10, 11, 26	10	5, 7, 8
10	36.9, CH	1.97, m	9	5, 9	11, 25, 26
11	122.7, CH	5.81, d (10.0)	5, 9, 13	12	10
12	131.2, CH	5.60, ddd (10.0, 5.1, 2.5)	4, 10, 13	11, 13	13, 14a
13	37.7, CH	2.82, br.dd (5.1, 5.1)	4, 11, 12, 14, 15, 25	12, 14b	12, 15, 25
14a	36.4, CH_2	1.99, m	15	14b	12, 15
14b		1.74, m	4, 13, 15, 16	13, 14a, 15	16
15	136.8, CH	5.54, dddd (15.3, 10.7, 2.4, 2.4),	14, 17	14b, 16	13, 14a, 17b
16	123.1, CH	5.04, dddd (15.3, 11.5, 2.4, 2.4)	14, 17	15, 17b	14b, 17a, 27
17a	42.6, CH_2	1.93, m	15, 16, 18, 19, 27	17b	16, 17b, 19
17b		2.40, dd (13.8, 11.5)	15, 16, 23	16, 17a	15, 17a
18	39.3, C				
19	129.1, CH	4.97	17, 18, 23, 28		17a, 27, 28
20	133.7, C				
21	39.0, CH	1.96, m	30	22β	22β
22α	29.4, CH_2	1.73, m	21, 23, 24, 29	22β	22β
22β		2.26, dd (14.8, 7.7)	18, 21, 23, 24, 29	21, 22α	21, 22α, 27
23	86.2, qC				
24	204.5, qC				
25	16.1, CH_3	1.61, s	3, 4, 5, 13		10, 13
26	5.5, CH_3	0.99, d (7.0)	7, 8, 9		8, 10
27	23.8, CH_3	1.22, s	17, 18, 19, 23		16, 19, 22β
28	22.1, CH_3	1.73, s	19, 20, 21		19
29	25.1, CH_2	1.60, m	22	30	
		1.72, m	20, 22, 30	30	
30	12.9, CH_3	0.91, t (7.4)	21, 29	29a, 29b	

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

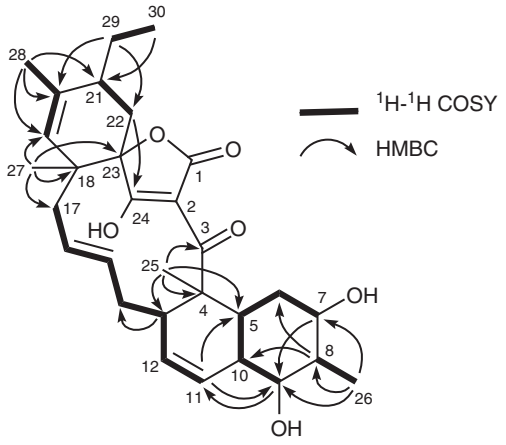

Figure 2 ^{1}H-^{1}H COSY and selected HMBC correlations for **1**.

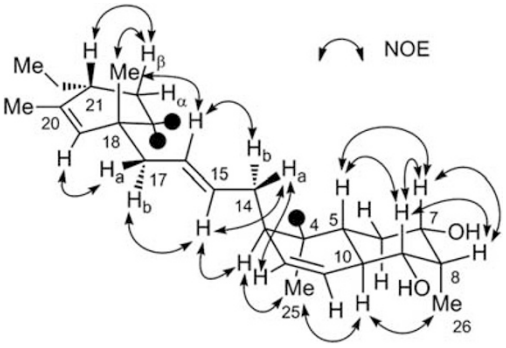

Figure 3 Conformation and configuration of Δ^1-octalin and cyclohexene moieties of **1** determined by NOESY data and *J*-based analysis.

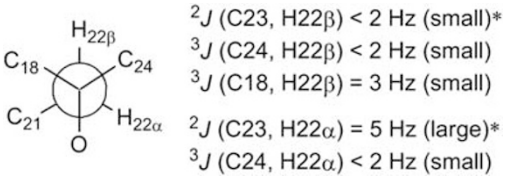

Figure 4 Configuration at C-23 of **1** determined on the basis of $^2J_{CH}$ and $^3J_{CH}$ values. *Absolute values.

axial H-10 proton. A series of NOESY correlations for H-13/H-15, H-15/H-17b, H-17a/H-19, H-14b/H-16 and H-16/H_3-27, established a zigzag conformation of the C-13 to C-18 chain, and the configuration at C-18 relative to C-13. Furthermore, NOEs between H-21 and H-22β, and between H-22β and H_3-27 established that these protons were located on the same side of the cyclohexene ring (Figure 3). Finally, the configuration at the spirocarbon C-23 was determined using the *J*-based configuration analysis.[8] Heteronuclear long-range coupling constants $^2J_{CH}$ and $^3J_{CH}$ were determined by *J*-resolved HMBC experiments.[9,10] *J*-Resolved HMBC spectra were measured on a Varian INOVA-500 spectrometer at 20 °C using a microtube (Shigemi Inc., Tokyo, Japan) in $CDCl_3$ (10 mg of **1** in 0.25 ml). In the *J*-resolved HMBC spectra, $^2J_{CH}$ and $^3J_{CH}$ values are obtained as absolute values. The small coupling constant between H-22β and C-23 ($^2J_{CH} < 2$ Hz), and the large coupling constant between H-22α and C-23 ($^2J_{CH} = 5$ Hz) suggested the anti-relationship of H-22β and the oxygen atom at C-23 (Figure 4).

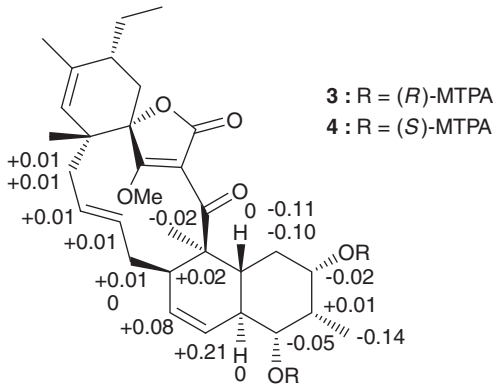

Figure 5 $\Delta\delta_{S-R}$ values for bisMTPA esters (**3** and **4**) of **2**.

The absolute stereochemistry was determined by applying the modified Mosher method[11] to the secondary hydroxyl groups at C-7 and C-9, both of which were in equatorial orientation. After methylation of the enolic hydroxyl group at C-24 with $TMSCHN_2$ in $CHCl_3$/MeOH, the methylated product **2** was reacted with (*S*)- and (*R*)-MTPA (α-methoxy-α-trifluoromethylphenylacetyl) chloride, yielding bis-(*R*)- and (*S*)-MTPA esters (**3** and **4**), respectively. Calculation of the $\Delta\delta_H(S\text{-}R)$ values identified H-11 to H_2-17 to have positive signs, whereas H_2-6 and H_3-25 negative signs (Figure 5). These data allowed assignment of both the absolute configurations at C-7 and C-9 as *R*. Although signs were mixed for the protons flanked by the MTPA ester groups, the sign distribution pattern is in good accordance with that reported for bisMTPA derivatives of cyclohexane-1,3-diols.[12] The deduced absolute configuration was identical with that known for this antibiotic family.[13,14]

Spirotetronates represented by kijanimicin[15] feature a *trans*-Δ^1-octalin unit and a tetronic acid moiety spiro-linked with a cyclohexene ring. To date, over 50 related metabolites have been discovered from actinomycetes. Their structural variations are largely derived from the glycosylation pattern and the length of the carbon chain connecting the octalin and the cyclohexene units. Maklamicin is the first example to have the shortest four-carbon linker, and nomimicin (**1**) is a new member of this class.

Nomimicin (**1**) showed antimicrobial activities against *Micrococcus luteus*, *Candida albicans* and *Kluyveromyces fragilis* with MIC values of 6.3, 12.5 and 12.5 μg ml^{-1}, respectively, whereas it was inactive against *Escherichia coli*. In contrast, maklamicin is not active against these yeasts, suggesting the effect of the hydroxylation pattern on antifungal activities. Compound **1** displayed weak cytotoxicity against human cervical cancer cells HeLa and human breast cancer cells MCF7, with IC_{50} values of 74 and 59 μM, respectively.

EXPERIMENTAL PROCEDURES

General experimental procedures

Optical rotations were measured using a JASCO DIP-3000 polarimeter (JASCO Corporation, Tokyo, Japan). UV spectrum was recorded on a Hitachi U-3210 spectrophotometer (Hitachi, Tokyo, Japan). IR spectrum was measured on a Perkin Elmer Spectrum 100 (Perkin-Elmer, Fremont, CA, USA). ^{13}C NMR spectra were measured on a Bruker AVANCE 400 spectrometer, and ^{1}H and 2D NMR spectra were obtained on a Bruker AVANCE 500 spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts were referenced to residual solvent signals (δ_H 7.26, δ_C 77.0). *J*-Resolved HMBC experiments were performed on a Varian INOVA-500 spectrometer (Varian Inc., Palo Alto, CA, USA). HR-ESITOFMS were recorded on a Bruker microTOF focus. Silica Gel 60 (Kanto

Chemical Co., Inc., Tokyo, Japan; 63-210 mesh) and Silica Gel 60-C18 (Nacalai Tesque, Kyoto, Japan; 250-350 mesh) were used for silica gel and octa decyl silica (ODS) column chromatographies, respectively. HPLC separation was performed using an XTerra RP_{18} (Waters Corporation, Milford, MA, USA; 7 μm, 19 × 300 mm) with a photodiode array detector.

Producing microorganism

Strain TP-A0878 was isolated from a compost sample collected at Nomi, Ishikawa, Japan, in 2007. The strain was identified as a member of the genus *Actinomadura* on the basis of 100% identity of 16S rRNA gene sequence (1462 nucleotides; DDBJ accession number AB488798) with *Actinomadura* sp. TFS 455 (accession number EF2120220).

Fermentation

Strain TP-A0878 cultured on a Bn-2 slant (soluble starch 0.5%, glucose 0.5%, meat extract (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) 0.1%, yeast extract (Difco Laboratories, Becton, Dickinson and Company, Sparks, MD, USA) 0.1%, NZ-case (Wako Chemicals USA, Inc., Richmond, VA, USA) 0.2%, NaCl 0.2%, $CaCO_3$ 0.1%, agar 1.5%) was inoculated into 500-ml K-1 flasks, each containing 100 ml of V-22 seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case (Wako Chemicals USA, Inc.) 0.3%, yeast extract (Difco Laboratories) 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 flasks were placed on a rotary shaker (200 r.p.m.) at 30 °C for 4 days. The seed culture (3 ml) was transferred into 500-ml K-1 flasks, each containing 100 ml of A-3M production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Traders Protein, Lubbock, TX, USA) 1.5%, yeast extract 0.3% and Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) 1%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were placed on a rotary shaker (200 r.p.m.) at 30 °C for 6 days.

Extraction and isolation

At the end of the fermentation period, 100 ml of 1-butanol were added to each flask and they were allowed to shake for 1 h. The mixture was centrifuged at 6000 r.p.m. for 10 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent gave 3.0 g of the crude extract from 2-l culture. This was subjected to silica gel column chromatography with a stepwise gradient of $CHCl_3$/MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1 and 0:1 v/v). Fractions 5 (2:1) and 6 (1:1) were concentrated to provide 0.47 g of a brown solid, which was further purified by reversed-phase ODS column chromatography with a gradient of MeCN/0.15% KH_2PO_4 buffer (pH 3.5; 2:8, 3:7, 4:6, 5:5, 6:4, 7:3 and 8:2 v/v). Fraction 6 (7:3) was evaporated to an aqueous solution, which was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give a pale yellow solid (90 mg). Final purification was achieved by repeated preparative HPLC with MeCN/0.1% HCO_2H (MeCN concentration: 50% for 0–8 min; 50–80% for 8–42 min; 14 ml min^{-1}; UV detection at 254 nm), followed by evaporation and extraction with EtOAc, to yield nomimicin (**1**, 19 mg, t_R 22.5 min).

Nomimicin (1)

Colorless amorphous solid; $[\alpha]^{23}_D$ –94 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 250 (3.97), 293 (3.89) nm; IR (attenuated total reflection) ν_{max} 3397, 1749 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESITOFMS $[M+Na]^+$ 519.2726 (calcd for $C_{30}H_{40}O_6Na$, 519.2717).

Methylation of 1 to yield 2

24-*O*-Methyl ether of **1** (**2**): To a solution of **1** (5.0 mg, 0.010 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 chromatography (hexane/EtOAc = 20:1–1:1) to give **2** (3.5 mg) in 60% yield: colorless amorphous solid; $[\alpha]^{25}_D$ –52 (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (3 H, t, *J* = 7.6 Hz, H-30), 0.96 (3 H, d, *J* = 7.0 Hz, H-26), 1.04 (3 H, s, H-27), 1.20 (1 H, ddd, *J* = 11.9, 11.9, 11.7 Hz, H-6α), 1.72 (3 H, s, H-28), 1.59 (1 H, m, H-6β), 1.53 (3 H, s, H-25), 1.60 (1 H, m, H-5), 1.62 (1 H, m, H-29a), 1.66 (1 H, m, H-29b), 1.66 (1 H, m, H-22α), 1.84 (1 H, m, H-17a), 1.85 (2 H, m, H-14), 1.88 (1 H, m, H-21), 1.91 (1 H, m, H-10), 2.18 (1 H, dd, *J* = 14.6, 7.1 Hz, H-22β), 2.38 (1 H, m, H-8), 2.40 (1 H, m, H-17b), 2.87 (1 H, br.dd, *J* = 5.0, 5.0 Hz, H-13), 3.43 (1 H, dd, *J* = 10.7, 4.7 Hz, H-9), 3.95 (1 H, ddd, *J* = 11.7, 4.3, 4.3 Hz, H-7), 4.07 (3 H, s, 24-OCH_3), 4.86 (1 H, s, H-19), 5.02 (1 H, dd, *J* = 15.1, 11.4 Hz, H-16), 5.30 (1 H, dddd, *J* = 15.1, 10.7, 2.1, 2.1 Hz, H-15), 5.57 (1 H, ddd, *J* = 10.0, 5.0, 2.4 Hz, H-12), 5.81 (1 H, d, *J* = 10.0 Hz, H-11); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.4 (C-26), 13.0 (C-30), 15.5 (C-25), 22.2 (C-28), 23.7 (C-27), 24.8 (C-29), 29.6 (C-6), 29.7 (C-22), 35.4 (C-5), 35.9 (C-14), 36.4 (C-10), 37.6 (C-13), 39.2 (C-21), 39.3 (C-18), 41.8 (C-8), 43.6 (C-17), 51.6 (C-4), 65.8 (24-OCH_3), 71.0 (C-7), 74.1 (C-9), 86.0 (C-23), 111.9 (C-2), 123.4 (C-11), 126.9 (C-16), 129.7 (C-19), 131.4 (C-15), 131.7 (C-12), 133.7 (C-20), 168.2 (C-1), 193.7 (C-24), 196.6 (C-3); HR-ESITOFMS $[M+Na]^+$ 533.2865 (calcd for $C_{31}H_{42}O_6Na$, 533.2874).

Bis-(*R*)-MTPA ester of 2 (3)

(*S*)-MTPA chloride (5.0 μl, 27 μmol) was added to a solution of **2** (1.7 mg, 3.3 μmol) in dry pyridine (1 ml) at room temperature. After 18 h, the reaction mixture was concentrated to dryness and purified by silica-gel column chromatography (*n*-hexane/EtOAc = 1:0-1:1), to yield **3** (1.6 mg, 42%): ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (3 H, d, *J* = 7.0 Hz, H-26), 0.91 (3 H, t, *J* = 7.4 Hz, H-30), 1.04 (3 H, s, H-27), 1.34 (1 H, ddd, *J* = 12.0, 12.0, 12.0 Hz, H-6α), 1.73 (3 H, s, H-28), 1.79 (1 H, m, H-14), 1.82 (1 H, m, H-5), 1.82 (1 H, m, H-6β), 1.85 (1 H, m, H-14), 1.85 (1 H, m, H-17a), 2.20 (1 H, m, H-10), 2.40 (dd, *J* = 14.3, 11.5 Hz, H-17b), 2.78 (1 H, m, H-8), 2.83 (1 H, dd, *J* = 5.0, 5.0 Hz, H-13), 4.87 (1 H, s, H-19), 4.91 (1 H, dd, *J* = 11.5, 4.7 Hz, H-9), 5.05 (1 H, dd, *J* = 14.8, 11.5 Hz, H-16), 5.10 (1 H, d, *J* = 10.0 Hz, H-11), 5.28 (1 H, m, H-15), 5.30 (1 H, m, H-7), 5.48 (1 H, ddd, *J* = 10.0, 5.0, 2.4 Hz, H-12); HR-ESITOFMS m/z 965.3652 $[M+Na]^+$ (calcd for $C_{51}H_{56}F_6O_{10}Na$, 965.3670).

Bis-(*S*)-MTPA ester of 2 (4)

In the same manner as described for **3**, **4** (1.3 mg) was prepared from 1.7 mg of **2**: ^1H NMR (500 MHz, $CDCl_3$) δ 0.73 (3 H, d, *J* = 7.0 Hz, H-26), 0.91 (3 H, t, *J* = 7.4 Hz, H-30), 1.05 (3 H, s, H-27), 1.25 (1 H, ddd, *J* = 11.7, 11.7, 11.7 Hz, H-6α), 1.72 (1 H, m, H-6β), 1.73 (3 H, s, H-28), 1.80 (1 H, m, H-14), 1.82 (1 H, m, H-5), 1.85 (1 H, m, H-14), 1.86 (1 H, m, H-17a), 2.20 (1 H, m, H-10), 2.41 (dd, *J* = 14.0, 11.7 Hz, H-17b), 2.79 (1 H, m, H-8), 2.85 (1 H, dd, *J* = 5.0, 5.0 Hz, H-13), 4.86 (1 H, dd, *J* = 11.6, 4.7 Hz, H-9), 4.88 (1 H, s, H-19), 5.06 (1 H, dd, *J* = 14.9, 11.7 Hz, H-16), 5.28 (1 H, m, H-7), 5.29 (1 H, m, H-15), 5.32 (1 H, d, *J* = 10.0 Hz, H-11), 5.56 (1 H, ddd, *J* = 10.0, 5.0, 2.4 Hz, H-12); HR-ESITOFMS m/z 965.3679 $[M+Na]^+$ (calcd for $C_{51}H_{56}F_6O_{10}Na$, 965.3670).

Biological assays

Antimicrobial assay[16] and cytotoxic assay[17] were carried out according to the procedures previously described.

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