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Jomthonic Acid A, a Modified Amino Acid from a Soil-Derived *Streptomyces*

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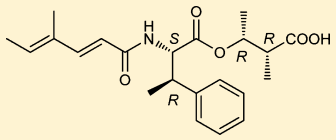
Supporting Information

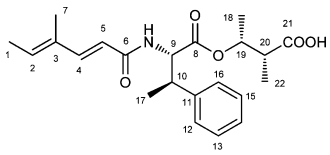
ABSTRACT: Jomthonic acid A (**1**), a new modified amino acid, was isolated from the culture broth of a soil-derived actinomycete of the genus *Streptomyces*. The structure and absolute configuration of **1** were determined by spectroscopic analyses and chemical conversion. Jomthonic acid A (**1**) induced differentiation of preadipocytes into mature adipocytes at 2−50 μM.



Actinomycetes are Gram-positive bacteria that have served as an important source of drug discovery owing to their unparalleled ability to produce structurally diverse secondary metabolites. Despite the recent decline in drug discovery efforts from microbial metabolites, *Streptomyces* is still believed to be a rich source of new and useful compounds, and up to 40% of known microbial metabolites are derived from this group.[1,2] Even after the intensive isolation and screening of this group over several decades, it is estimated that less than 10% of its potential in secondary metabolite biosynthesis has been realized.[3] Genome analyses have shown that *Streptomyces coelicolor* contains 21 biosynthetic gene clusters, and *Streptomyces avermitilis* contains 25 biosynthetic gene clusters for secondary metabolites. The predicted number of gene clusters in these two strains, however, exceeds the number of reported compounds, suggesting that additional rare metabolites await to be discovered.[4,5]

In our continuing search for structurally novel secondary metabolites from *Streptomyces*,[6−8] strain BB47 isolated from a soil sample collected in Bangkok, Thailand, was chosen for chemical investigation because of its potent antagonism against *Colletotrichum*, a causative agent of anthracnose disease in crops.[9] The strain was cultured in A-3 M liquid medium, and the whole culture broth was extracted with 1-butanol. HPLC/UV analysis of the extract and subsequent comparison to our in-house metabolite database suggested that the antifungal principles were clethramycin-type hexaenes[10,11] and piericidins.[12] In addition to these metabolites, an unknown metabolite showing an UV absorption band at 264 nm was detected although it lacked antifungal activity. HPLC/UV-guided purification from the extract led to the isolation of a new modified amino acid, jomthonic acid A (**1**). During diverse biological evaluation, **1** was found to promote the differentiation of preadipocytes into adipocytes. Herein, we report the isolation, structure determination, and biological properties of **1**.

Jomthonic acid A (**1**) was obtained as a pale yellow oil that analyzed for a molecular formula of $C_{22}H_{29}NO_5$ (9 degrees of unsaturation) by interpretation of HR-ESITOFMS. This molecular formula was corroborated by ^{1}H and ^{13}C NMR spectral data (Table 1). Analysis of the combined 1D and 2D NMR data established that **1** possessed eight olefinic methines, two sp^2 quaternary and three carbonyl carbons, four sp^3 methines, and five methyls, in addition to two exchangeable protons. The IR absorptions at 1717 and 1656 cm^{-1} demonstrated the presence of carbonyl functionalities, which was confirmed by the presence of resonances at δ 166.9, 171.3, and 177.5 in the ^{13}C NMR spectrum. The nine degrees of unsaturation inherent in the molecular formula of **1**, coupled with the data showing the presence of three carbonyl and 10 olefinic carbons, indicated that **1** must possess one ring.



jomthonic acid A (**1**)

Further analysis of ^{1}H−^{1}H COSY and HMBC spectra provided three substructures. The first was an unsaturated fatty acid containing two small COSY-defined fragments. The first fragment was a two-carbon unit consisting of a terminal methyl

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

position	δ_C[a]	δ_H mult (J in Hz)[b]	HMBC[b,c]
1	14.5, CH_3	1.79, d (7.0)	2, 3
2	135.6, CH	5.93, q (7.0)	1, 7
3	133.4, qC		
4	146.8, CH	7.23, d (15.4)	2, 5, 6, 7
5	116.9, CH	5.79, d (15.4)	3, 6
6	166.9, qC		
7	11.9, CH_3	1.74, s	2, 3, 4
8	171.3, qC		
9	57.8, CH	4.88, dd (8.9, 8.0)	6, 8, 10, 11, 17
10	43.3, CH	3.16, dq (8.0, 7.1)	8, 9, 11, 12, 17
11	141.4, qC		
12	127.9, CH	7.22[d]	10, 14
13	128.4, CH	7.28, t (7.8)	11, 15
14	127.2, CH	7.22[d]	12, 16
15	128.4, CH	7.28, t (7.8)	11, 13
16	127.9, CH	7.22[d]	10, 14
17	17.8, CH_3	1.39, d (7.1)	9, 10, 11
18	16.1, CH_3	0.88, d (6.4)	19, 20
19	72.5, CH	4.98, dq (7.1, 6.4)	8, 20, 21
20	44.3, CH	2.59, dq (7.1, 7.2)	18, 19, 21, 22
21	177.5, qC		
22	12.3, CH_3	1.08, d (7.2)	19, 20, 21
9-NH		6.35, d (8.9)	6

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

group (H_3-1) attached to a vinyl methine (H-2). The second fragment was also a two-carbon unit comprising two vinyl protons (H-4 and H-5) mutually coupled with a large coupling constant (15.5 Hz), indicating the *E* geometry at C-4 and C-5. These two fragments were connected to the quaternary carbon C-3 on the basis of HMBC correlations from the methyl proton at δ_H 1.75 (H_3-7) to C-2, C-3, and C-4. This unit was then expanded to include the carbonyl carbon C-6 based on HMBC correlations from H-4 and H-5 to this carbon, providing a 4-methyl-2,4-hexadienoate substructure. This was consistent with the UV spectrum, which displayed an absorption band at λ_{max} 264 nm.[13] The *E* geometry for the C-2−C-3 double bond was assigned by the observation of NOEs between H-2 and H-4 and between H_3-7 and H-5. The second part was elucidated starting from an NH proton at δ_H 6.35 that showed a series of COSY correlations to the methyl proton H_3-17. A monosubstituted benzene ring was established by analysis of the ^{1}H, ^{13}C, and HSQC spectra, showing relatively intense signals at δ_C 127.9 and 128.4, each accounting for two proton-bearing carbons (C-12/C-16 and C-13/C-15, respectively), and resonances at δ_H 7.22 and 7.28 accounting for five protons. The aromatic part was connected to C-10 on the basis of HMBC correlations from H_3-17 and H-9 to C-11 and from H-10 to C-12 and C-16. HMBC correlations from H-9 and H-10 allowed the connection of carbonyl carbon C-8 to C-9, establishing this substructure as a phenylalanine bearing a methyl substitution at the β-position. The third substructure was a 3-hydroxy-2-methylbutanoic acid, which was deduced from a series of COSY correlations from H_3-18 to H_3-22 and HMBC correlations from H-19 and H_3-22 to carbonyl carbon C-21. These three substructures were combined on the basis of HMBC correlations from 9-NH and H-9 to C-6 and from H-19 to C-8, to complete the planar structure of **1** as depicted in Figure 1.

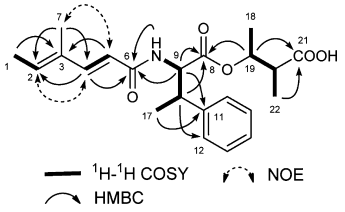

Figure 1. ^{1}H−^{1}H COSY, key HMBC, and key NOESY correlations for **1**.

The absolute stereochemistry of **1** was assigned by application of Marfey's analysis[14] and the chiral anisotropy method.[15] First, the relative configuration of the β-methylphenylalanine (βMePhe) residue was determined by HPLC analysis. A commercially available diastereomeric mixture of βMe-DL-Phe was separated into the (2*RS*,3*SR*)-isomer (*syn*-form) and the (2*RS*,3*RS*)-isomer (*anti*-form) by preparative HPLC, and their relative configurations were assigned by comparing the ^{1}H NMR data with reported values.[16] The acid hydrolysate of **1** was compared with these standards, and the βMePhe residue was determined to have a (2*RS*,3*SR*)-configuration. In a second step, the hydrolysate was derivatized using 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA) and compared with the standard (2*RS*,3*SR*)-βMePhe that was similarly derivatized with L-FDLA. The βMePhe residue was assigned as a (2*S*, 3*R*)-isomer. The remaining stereocenters at C-19 and C-20 of **1** were determined using MTPA and PGME methods[17,18] (Figure 2). Since the recovery of the 3-hydroxy-2-methylbutanoate residue was hampered by difficulties with detection, **1** was tagged with a fluorescent chromophore, 1-pyrenemethylamine, to give the corresponding amide **2**. Alkaline hydrolysis of **2** gave **3**, which was easily detected by TLC. Esterification of **3** with (*R*)- and (*S*)-MTPA acid in the presence of DCC and DMAP gave the (*R*)- and (*S*)-MTPA esters **4** and **5**, respectively. The $\Delta\delta$ value distribution pattern suggested the *R* configuration at C-19. The C-20 stereochemistry was assigned as *R* on the basis of the $\Delta\delta$ values of (*R*)- and (*S*)-PGME amides (**6** and **7**), which showed negative values for H-9, H-10, H-18, and H-19.

The biological activity of jomthonic acid A (**1**) is still under investigation. Initial screening showed that **1** was active in an assay designed to detect antidiabetic and antiatherogenic activities using mouse ST-13 preadiopocytes.[19] At 2 to 50 μM **1** displayed inducing activity of preadipocyte differentiation into mature adipocytes. At the highest concentration about 80% of preadipocytes were differentiated into adipocytes. Preadipocyte differentiation is associated with the production of adipocytokines, proteinogenic hormones secreted by mature adipocytes and beneficial to relieve lifestyle deseases.[20] **1** was inactive in a cancer-cell cytotoxicity assay (IC_{50} >100 μM against MCF7 human breast cancer cells) and in an antimicrobial assay (MIC >50 μg/mL against *Escherichia coli*, *Micrococcus luteus*, and *Candida albicans*).

Jomthonic acid A (**1**) is an interesting modified amino acid that contains rare structural features, and no related metabolites have been reported. The unsaturated fatty acid moiety 4-methyl-2*E*,4*E*-hexadienoate present in **1** has been found only in salinamide C[21] from *Streptomyces* and daldinin F[13] from *Hypoxylon*. β-Methylphenylalanine is a rare amino acid reported

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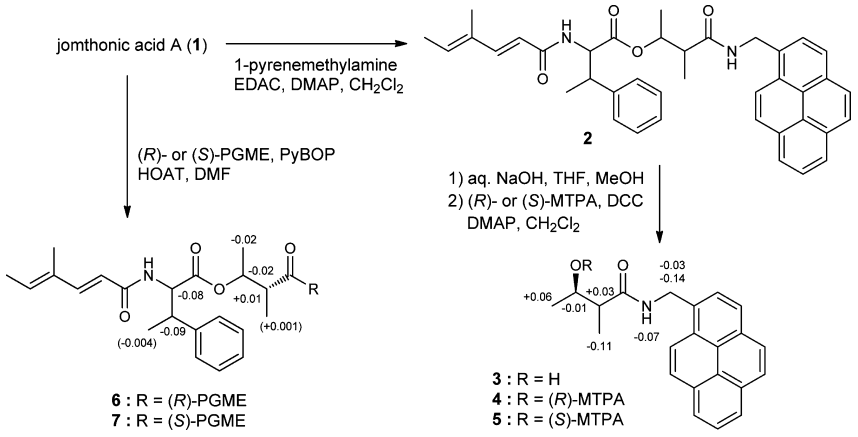

Figure 2. Preparation of **3** and ^{1}H NMR $\Delta\delta_{S-R}$ values for MTPA esters (**4** and **5**) of **3** and PGME amides (**6** and **7**) of **1**.

from two classes of microbial metabolites, bottromycins[22] from *Streptomyces* and AK toxins[23] from *Alternaria*. While these compounds contain the (2*S*,3*S*)-isomer of this unusual amino acid, **1** is the first natural product that contains the (2*S*,3*R*)-isomer. The discovery of jomthonic acid A (**1**) provides additional evidence that *Streptomyces* still has great potential as a source of novel secondary metabolites. Further structural analysis of minor analogues of **1** present in the culture extract is currently in progress.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was measured using a JASCO DIP-3000 polarimeter. The UV spectrum was recorded on a Hitachi U-3210 spectrophotometer. The IR spectrum was 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 7.27, δ_C 77.0). HR-ESITOFMS were recorded on a Bruker microTOF Focus. Silica gel 60-C18 (Nacalai Tesque, 250−350 mesh) was used for ODS column chromatography.

Microorganism. Strain BB47 was isolated from a soil sample collected in Bangkok, Thailand. The strain was identified as a member of the genus *Streptomyces* on the basis of 98.7% similarity in the 16S rRNA gene sequence (1425 nucleotides; GenBank accession number HM051280) to *Streptomyces catenulae* ISP 5258 (accession number AY999778).

Fermentation. Strain BB47 cultured on a Bn-2 slant [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 Chemicals USA, Inc.) 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 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 flasks were placed 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-3 M production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Traders Protein) 1.5%, yeast extract 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 1 h. The mixture was centrifuged at 5000 rpm for 10 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent gave approximately 12.5 g of extract from 2 L of culture. The crude extract (12.5 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). Fraction 4 was concentrated to provide 1.4 g of brown oil, which was further purified by reversed-phase ODS column chromatography with a gradient of MeCN/0.15% KH_2PO_4 (pH 3.5) (2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2 v/v). Fraction 6 was evaporated, the remaining aqueous solution was extracted with EtOAc, and the organic layer was concentrated to give a brown solid (114 mg). Final purification was achieved by repeated C_{18} RP HPLC using a Cosmosil 5C18-AR-II column (Nacalai Tesque Inc., 20 × 250 mm) with MeCN/0.15% KH_2PO_4 (pH 3.5) (50:50), followed by evaporation and extraction with EtOAc, yielding jomthonic acid A (**1**, 64 mg).

*Jomthonic acid A (**1**):* pale yellow oil; $[\alpha]^{24}_D$ −3.0 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ε) 264 (4.72) nm; IR (ATR) ν_{max} 3321, 2981, 1717, 1656 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HR-ESITOFMS $[M - H]^-$ 386.1973 (calcd for $C_{22}H_{28}NO_5$ 386.1973).

Stereochemical Determination of β-Methylphenylalanine Residue. Jomthonic acid A (**1**, 1 mg) was hydrolyzed at 140 °C with 6 N HCl for 20 h. The dried hydrolysate and standards of *syn*- and *anti*-forms of racemic β-methylphenylalanine (β-MePhe)[16] were analyzed by HPLC using an XTerra RP_{18} column (Waters, 5 μm, 4.6 × 250 mm), starting with 15% MeOH/85% 10 mM NH_4HCO_3 followed by a gradient elution profile to 25% MeOH/75% 10 mM NH_4HCO_3 over 12 min at a flow rate of 1.0 mL/min, with monitoring at 254 nm. Retention times for the standards were 6.46 min for (2*RS*,3*SR*)-β-MePhe (*syn*-form) and 6.87 min for (2*RS*,3*RS*)-β-MePhe (*anti*-form), while the hydrolysate gave a peak at 6.44 min.

To the remaining hydrolysate was added a 0.1 M $NaHCO_3$ solution (20 μL) and a solution of 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA) in acetone (1%, 25 μL). The reaction vial was sealed and incubated at 35 °C for 1.5 h. To quench the reaction, 20 μL of 1 N HCl was added and then diluted with 800 μL of MeCN. L-FDLA derivatives of (2*RS*,3*SR*)-β-MePhe were prepared in the same manner as described above. The Marfey's derivatives of the hydrolysate and standards were analyzed by HPLC using an XTerra RP_{18} column (Waters, 5 μm, 4.6 × 250 mm). The reaction mixtures were analyzed starting with 40% MeCN/60% 0.15% KH_2PO_4 (pH 3.5) followed by a gradient elution profile to 80% MeCN/20% 0.15% KH_2PO_4 over 30 min at a flow rate of 1.0 mL/min, monitoring at 340 nm. Retention times for the amino acid standards were 14.5 min for (2*S*,3*R*)-β-L-MePhe and 15.4 min for (2*R*,3*S*)-β-D-MePhe, while the hydrolysate gave a peak at 14.5 min.

*1-Pyrenemethylamide of **1** (**2**).* Jomthonic acid A (**1**, 13 mg, 0.034 mmol) was treated with 1-pyrenemethylamine hydrochloride (25 mg, 0.093 mmol) in dry DMF (2.5 mL) containing DMAP (4.0 mg, 0.034 mmol) and EDAC (33 mg, 0.17 mmol) for 18 h at room temperature. The reaction mixture was diluted with ice−water and extracted with EtOAc. The organic layer was concentrated to dryness, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 1:0−1:1) to give **2** (5.6 mg, 27% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (3H, d, *J* = 6.5 Hz, H-18), 1.08 (3H, d, *J* = 7.0 Hz, H-22), 1.09 (3H, d, *J* = 7.1 Hz, H-17), 1.69 (3H, s, H-7), 1.73 (3H, d, *J* = 7.1 Hz,

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H-1), 2.38 (1H, dq, J = 5.4, 7.0 Hz, H-20), 2.88 (1H, dq, J = 9.0, 7.1 Hz, H-10), 4.30 (1H, dd, J = 9.0, 8.5 Hz, H-9), 4.84 (1H, dq, J = 5.4, 6.5 Hz, H-19), 4.95 (1H, dd, J = 14.5, 4.3 Hz, NHCH_2), 5.38 (1H, dd, J = 14.5, 6.9 Hz, NHCH_2), 5.69 (1H, d, J = 15.3 Hz, H-5), 5.82 (1H, d, J = 8.5 Hz, 9-NH), 5.83 (1H, q, J = 7.1 Hz, H-2), 6.56 (1H, dd, J = 6.9, 4.3 Hz, N*H*CH$_2$), 6.76 (2H, d, J = 7.4 Hz, H-12 and H-16), 6.96 (2H, dd, J = 7.4, 7.4 Hz, H-13 and H-15), 7.06 (1H, t, J = 7.4 Hz, H-14), 7.16 (1H, d, J = 15.3 Hz, H-4), 8.01−8.07 (4H, m), 8.16−8.23 (4H, m), 8.36 (1H, d, J = 9.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8 (C-7), 13.8 (C-22), 14.4 (C-1), 17.0 (C-18), 17.9 (C-17), 41.6 (NHCH$_2$), 42.6 (C-10), 46.7 (C-20), 58.3 (C-9), 72.6 (C-19), 116.6 (C-5), 123.4, 124.78, 124.81, 125.0, 125.2, 125.3, 126.0, 127.1 (C-14), 127.40, 127.43, 127.6 (C-13, C-15), 128.0, 128.2 (C-12, C-16), 128.9, 130.9, 131.0, 131.3, 132.0, 133.2 (C-3), 135.8 (C-2), 141.0 (C-11), 146.8 (C-4), 166.5 (C-6), 171.2 (C-8), 172.4 (C-21); HR-ESITOFMS m/z 599.2921 $[M - H]^-$ (calcd for $C_{39}H_{39}N_2O_4$ 599.2915).

*Hydrolysis of **2** to Yield **3**.* Compound **2** (4.5 mg, 7.5 μmol) was hydrolyzed at 0−5 °C with 1 M NaOH (450 μL) in MeOH/THF (1:1, 900 μL) for 16 h at room temperature. To stop the reaction, 0.5 N HCl (2.25 mL) was added. The reaction mixture was then diluted with 2.25 mL of H_2O and lyophilized. The residual solid was subjected to HPLC purification using an XTerra RP$_{18}$ column (Waters, 10 μm, 19 × 300 mm) with an isocratic solvent system of MeCN/0.15% KH_2PO_4 aqueous solution (pH 3.5) (40:60) at a flow rate of 10 mL/min, yielding **3** (2.5 mg, 100% yield): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25 (3H, d, J = 6.3 Hz, H-18), 1.25 (3H, d, J = 7.1 Hz, H-22), 2.21 (1H, dq, J = 7.1, 6.4 Hz, H-20), 3.90 (1H, dq, J = 6.4, 6.3 Hz, H-19), 5.18 (1H, d, J = 15.1 Hz, NHCH_2), 5.20 (1H, d, J = 15.1 Hz, NHCH_2), 6.13 (1H, br s, N*H*CH$_2$), 7.98 (1H, d, J = 7.7 Hz), 8.03−8.10 (3H, m), 8.15−8.23 (4H, m), 8.26 (1H, d, J = 9.2 Hz); HR-ESITOFMS m/z 354.1462 $[M + Na]^+$ (calcd for $C_{22}H_{21}NO_2Na$ 354.1465).

*Preparation of (R)-MTPA Ester and (S)-MTPA Ester of **3** (**4** and **5**).* To a solution of **3** (1.0 mg, 2.6 μmol) in dry CH_2Cl_2 (100 μL) was added (*R*)-MTPA acid (1.0 mg, 4.3 μmol), DCC (1.0 mg, 4.8 μmol), and DMAP (trace amount) at room temperature. After stirring for 18 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 1:0−1:1) to give (*R*)-MTPA ester **4** (0.6 mg). (*S*)-MTPA ester **5** was obtained by the same experimental procedure as described for (*R*)-MTPA ester **4**.

*(R)-MTPA Ester (**4**):* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.20 (3H, d, J = 7.2 Hz, H-22), 1.34 (3H, d, J = 6.4 Hz, H-18), 2.50 (1H, dq, J = 7.2, 7.2 Hz, H-20), 4.85 (1H, dd, J = 14.4, 4.4 Hz, NHCH_2), 5.27 (1H, dd, J = 14.4, 6.3 Hz, NHCH_2), 5.33 (1H, dq, J = 7.2, 6.4 Hz, H-19), 5.92 (1H, br t, J = 5.4 Hz, N*H*CH$_2$); HR-ESITOFMS m/z 570.1864 $[M + Na]^+$ (calcd for $C_{32}H_{28}F_3NO_4Na$ 570.1863).

*(S)-MTPA Ester (**5**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.10 (3H, d, J = 7.1 Hz, H-22), 1.40 (3H, d, J = 6.4 Hz, H-18), 2.53 (1H, dq, J = 6.7, 7.1 Hz, H-20), 4.71 (1H, dd, J = 14.4, 4.2 Hz, NHCH_2), 5.24 (1H, dd, J = 14.4, 6.5 Hz, NHCH_2), 5.32 (1H, dq, J = 6.7, 6.4 Hz, H-19), 5.85 (1H, br t, J = 5.4 Hz, N*H*CH$_2$); HR-ESITOFMS m/z 570.1878 $[M + Na]^+$ (calcd for $C_{32}H_{28}F_3NO_4Na$ 570.1863).

*Preparation of (R)-PGME Amide and (S)-PGME Amide of **1** (**6** and **7**).* To a solution of jomthonic acid A (**1**, 5.0 mg, 13 μmol) in dry DMF (0.35 mL) and dry triethylamine (0.35 mL) were added (*R*)-PGME (5.2 mg, 26 μmol), PyBOP (14 mg, 26 μmol), and HOAT (3.5 mg, 26 μmol) at room temperature. After stirring for 1 h, the reaction mixture was passed through an ODS column cartridge with 5% HCO_2H (25 mL), and the column was eluted with MeOH. The eluent was concentrated, and the residue was purified by HPLC (Cosmosil 5C18-AR-II column, Nacalai Tesque Inc., 10 × 250 mm) with MeCN/0.1% HCO_2H (55:45), followed by evaporation and extraction with EtOAc to give (*R*)-PGME amide **6** (2.4 mg, 35% yield). In the same manner as described for **6**, (*S*)-PGME amide **7** was prepared from **1**.

*(R)-PGME amide (**6**):* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.96 (3H, d, J = 6.4 Hz, H-18), 1.09 (3H, d, J = 7.0 Hz, H-22), 1.37 (3H, d, J = 7.1 Hz, H-17), 2.44 (1H, dq, J = 6.7, 7.0 Hz, H-20), 3.23 (1H, dq, J = 7.5, 7.1 Hz, H-10), 4.91 (1H, dd, J = 8.9, 7.5 Hz, H-9), 4.94 (1H, dq, J = 6.7, 6.4 Hz, H-19); HR-ESITOFMS m/z 557.2651 $[M + Na]^+$ (calcd for $C_{31}H_{38}N_2O_6Na$ 557.2622).

*(S)-PGME amide (**7**):* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.93 (3H, d, J = 6.4 Hz, H-18), 1.09 (3H, d, J = 7.1 Hz, H-22), 1.36 (3H, d, J = 7.1 Hz, H-17), 2.45 (1H, dq, J = 6.7, 7.1 Hz, H-20), 3.15 (1H, dq, J = 7.9, 7.1 Hz, H-10), 4.84 (1H, dd, J = 8.5, 7.9 Hz, H-9), 4.91 (1H, dq, J = 6.7, 6.4 Hz, H-19); HR-ESITOFMS m/z 557.2623 $[M + Na]^+$ (calcd for $C_{31}H_{38}N_2O_6Na$ 557.2622).

Biological Assays. Cytotoxicity assay,[24] antimicrobial assay,[24] and preadipocyte differentiation assay[17] were carried out according to the procedures previously reported.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra of jomthonic acid A (**1**) and ^{1}H NMR spectra of **2**−**7**. This material is available 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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