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Melleumin A, a novel peptide lactone isolated from the cultured myxomycete *Physarum melleum*

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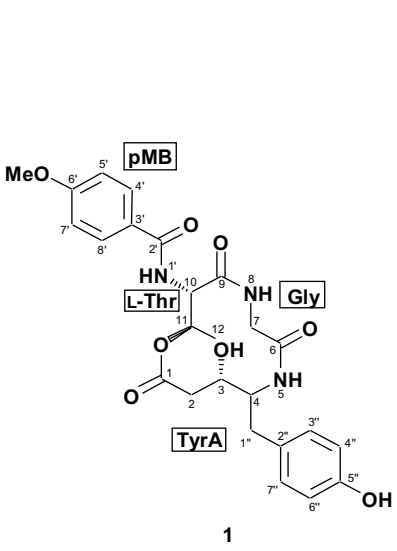
Abstract—Melleumin A (**1**), a novel peptide lactone, has been isolated from the laboratory-cultured plasmodium of myxomycete *Physarum melleum*, and its structure was elucidated by spectral data. Melleumin A (**1**) consisted of four residues (*p*-methoxybenzoic acid, L-threonine, glycine, and an unusual amino acid, a tyrosine-attached acetic acid).

1. Introduction

Myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes.[1] Studies on the constituents of cultured myxomycetes have been very limited except for only one species, *Physarum polycephalum*, from which isolation of several pigments[2] or bioactive lysophosphatidic acid[3] had been described. During our studies in a search for natural products from myxomycetes,[4] we recently studied spore germination experiments of hundreds of field-collected myxomycetes collected in Japan, and succeeded in laboratory culture of plasmodia of several myxomycetes in a practical scale for natural products chemistry studies.[5] As a result, we recently reported isolation of pyrroloiminoquinones and polyene yellow pigments from the laboratory-cultured myxomycetes *Didymium bahiense*,[6] *Didymium iridis*,[7] and *Physarum rigidum*,[8] respectively. Here we describe the isolation and structure elucidation of a novel peptide lactone, melleumin A (**1**), and its *seco* acid methyl ester, melleumin B (**2**), from the cultured plasmodium of the myxomycete *Physarum melleum*.



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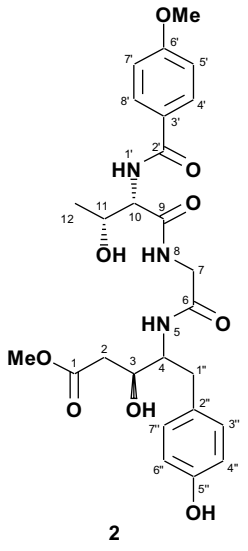

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2. Results and discussions

The fruit bodies of the myxomycetes *P. melleum*[9] were collected at Tokorozawa, Saitama Prefecture, Japan, in June, 2001. The plasmodium of this myxomycete obtained in a plate culture was mass-cultured in the laboratory on agar plates in the presence of *Escherichia*

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coli according to the methods described previously.[5,6] The harvested plasmodial cells (19.5 g from 2112 plates (9 cmϕ)) were extracted with 90% MeOH and 90% acetone, and the combined extract (2.3 g) was partitioned between ethyl acetate and water. The ethyl acetate-soluble layer was subjected to silica gel column chromatography, and the fraction eluting with 5–9% MeOH in chloroform, containing UV(254 nm)-positive spots on TLC, was combined with the corresponding fraction obtained by the same procedures starting from the cultured plasmodial cells (7.5 g from 998 plates (9 cmϕ)). The combined fraction was further separated by HPLC on ODS eluted with 50% MeOH to give two UV(254 nm)-positive compounds, named melleumins A (**1**) and B (**2**), in 0.007% and 0.02% yield, respectively.[10]

Melleumin A (**1**),[11] colorless amorphous solid, $[\alpha]_D^{26}$ +27 (*c* 0.15, MeOH), showed a quasi-molecular ion peak at *m*/*z* 500 $(M+H)^+$ and 522 $(M+Na)^+$ in its positive FAB mass spectrum. The molecular formula of **1** was revealed as $C_{25}H_{30}O_8N_3$ by the HRFABMS data [*m*/*z* 500.1995, $(M+H)^+$, Δ −3.9 mmu]. The UV spectrum of **1** showed absorption maxima at 254 and 225 nm, indicating the presence of conjugated or aromatic system(s). The ^{1}H NMR spectrum of **1** in DMSO-d_6 (Table 1) showed five signals of low-field resonances at δ_H 9.15 (1H, br s), 8.54 (1H, br t), 8.07 (1H, d, J = 8.9 Hz), 6.21 (1H, d, J = 10.4 Hz), and 5.43 (1H, d, J = 3.9 Hz) with no HMQC correlation with any carbons, which were assignable to hydroxyl or amide protons. The ^{1}H NMR also showed signals due to two *p*-substituted benzene rings [δ_H 7.91 and 7.01 (each 2H, d, J = 8.9 Hz); δ_H 6.95 and 6.63 (each 2H, d, J = 8.5 Hz)], one methoxy protons [δ_H 3.81 (3H, s)], four oxygen- or nitrogen-bearing methine protons [δ_H 5.63 (1H, qd, J = 6.4 and 3.4 Hz), 5.00 (1H, dd, J = 8.9 and 3.4 Hz), 4.10 (1H, m), and 3.75 (1H, m)], three sp^3 methylenes [δ_H 3.52 (1H, m) and 3.49 (1H, m); δ_H 2.86 (1H, d, J = 14.7 and 2.4 Hz) and 2.57 (1H, m); δ_H 2.55 (1H, m) and 2.36 (1H, m)], and one secondary methyl group [δ_H 1.20 (3H, d, J = 6.4 Hz)]. These observations were also corroborated by its ^{13}C NMR spectrum (Table 1), aided by DEPT experiments, which gave signals assignable to four carbonyls, two *p*-substituted benzene rings, four sp^3 methines, three sp^3 methylenes, and two methyls including one methoxy group. Since 12 out of 13 unsaturation equivalents were accounted for from the ^{13}C NMR data, compound **1** was inferred to have one ring besides the two benzene rings. Chemical shifts of four carbonyl groups (δ_C 170.7, 169.2, 169.1, and 166.6) were likely

Table 1. ^{1}H and ^{13}C NMR data of melleumins A (**1**) and B (**2**) in DMSO-d_6[a]

Position	**1**			**2**		
	δ_H	J in Hz	δ_C	δ_H	J in Hz	δ_C
1			170.7			171.8
2	2.36	m	38.7	2.29	dd 15.6, 9.5	38.3
	2.55	m		2.38	dd 15.6, 3.7	
3	3.75	m	69.6	3.91	m	67.5
4	4.10	m	54.9[b]	3.84	m	54.7
5	6.21	d 10.4		7.53	d 9.2	
6			169.2[c]			168.5
7	3.49	m	44.4	3.57	dd 5.2, 16.7	42.2
	3.52	m		3.74	dd 6.3, 16.7	
8	8.54	br t 5.6		8.31	br t 5.8	
9			169.1[c]			170.8
10	5.00	dd 8.9, 3.4	54.8[b]	4.30	dd 7.6, 4.9	60.1
11	5.63	qd 6.4, 3.4	71.7	4.08	m	66.7
12	1.20 (3H)	d 6.4	16.1	1.11 (3H)	d 6.4	20.0
1′	8.07	d 8.9		8.02	d 7.6	
2′			166.6			166.3
3′			126.0			126.1
4′,8′	7.91 (2H)	d 8.9	129.4[d]	7.89 (2H)	d 8.9	129.4
5′,7′	7.01 (2H)	d 8.9	113.4	7.01 (2H)	d 8.9	113.5
6′			161.8			161.8
1″	2.86	dd 14.7, 2.4	30.8	2.73	dd 13.7, 5.8	35.0
	2.57	m		2.47	dd 13.7, 8.6	
2″			129.3			129.1
3″,7″	6.95	d 8.5	129.6[d]	6.93	d 8.6	129.9
4″,6″	6.63	d 8.5	115.0	6.59	d 8.6	114.9
5″			155.5			155.4
1-OMe				3.55 (3H)	s	51.2
6′-OMe	3.81 (3H)	s	55.4	3.81 (3H)	s	55.4
3-OH	5.43	d 3.9		5.13	d 5.5	
11-OH				5.09	d 6.1	
5″-OH	9.15	br s		9.12	br s	

[a] 500 MHz for ^{1}H, 125 MHz for ^{13}C. Assignments were made by HMQC and HMBC analyses.
[b] Signals may be reversed.
[c] Signals may be reversed.
[d] Signals may be reversed.

to be assigned to amides (or esters), and the nature of all these ^{1}H and ^{13}C NMR data was strongly suggestive that **1** was a peptide-related compound. The ^{1}H–^{1}H COSY spectrum of **1** showed connectivities for two amino acid residues: threonine (Thr) [H_3-12/H-11/H-10/NH-1′] and glycine (Gly) [H_2-7/NH-8], along with another proton-network included in an unusual amino acid residue [H_2-2/H-3/H-4/H_2-1″(NH-5)], as shown in Figure 1. Interpretation of the HMBC spectral data of **1** (Fig. 1) revealed the presence of a *p*-methoxybenzoic acid group (*p*MB) [C-2′ to C-8′ position; HMBC correlations: MeO/C-6′, H-5′/C-3′ (H-7′/C-3′), H-5′/C-7′ (H-7′/C-5′), H-4′/C-6′ (H-8′/C-6′), H-4′/C-8′ (H-8′/C-4′), and H-4′/C-2′ (H-8′/C-2′)] and a *p*-hydroxyphenyl group [C-2″ to C-7″ position; HMBC correlations: H-3″/C-5″ (H-7″/C-5″), H-3″/C-7″ (H-7″/C-3″), H-4″/C-2″ (H-6″/C-2″), H-4″/C-5″ (H-6″/C-5″), and H-4″/C-6″ (H-6″/C-4″)], and this *p*-hydroxyphenyl group was revealed to be connected to C-1″ [HMBC correlations: H_2-1″/C-2″, H_2-1″/C-3″(C-7″), and H-3″(H-7″)/C-1″], constructing an unusual amino acid residue (C-1 to C-4 and C-1″ to C-7″ position), which corresponded to a tyrosine-attached acetic acid (TyrA).[12] The HMBC correlation data clearly revealed the connectivity of these partial structural residues from TyrA to Gly (NH-5/C-6), from Gly to Thr (NH-8/C-9), and from Thr to *p*MB (NH-1′/C-2′), thus giving rise to the sequence of four residues as *p*MB-Thr-Gly-TyrA. This sequence was also corroborated by the observation of the ROESY correlations for 5-NH/H-7, 8-NH/H-10, and 1′-NH/H-4′(H-8′). The oxymethine proton on C-11 having low-field resonance (δ_H 5.63) showed HMBC cross-peak with C-1 carbonyl (δ_C 170.7), which suggested the presence of a linkage between the hydroxyl group of Thr (C-11) and the carbonyl group of TyrA (C-1) through an ester bond to construct the whole molecule of melleumin A as **1**.

The molecular formula of melleumin B (**2**)[13] was revealed as $C_{26}H_{33}O_9N_3$ by the HRFABMS data [*m*/*z* 532.2338, $(M+H)^+$, Δ +4.3 mmu], having one methanol molecule more than melleumin A (**1**). Although the optical rotation ($[\alpha]_D$ value) of **2** was almost zero, **2** was optically active since it showed a characteristic CD curve [λ_{ext} 212 ($\Delta\varepsilon$ −6.1), 224 (−1.9), and 252 nm (+2.7)]. The ^{1}H and ^{13}C NMR spectral data of **2** (Table 1) as well as its UV absorption were almost parallel to those of compound **1**, though the oxygen- and nitrogen-bearing methine protons on C-11 and C-10, respectively, resonated in relatively higher field for **2** [δ_H 4.08 (H-11) and 4.30 (H-10)] than those of **1** [δ_H 5.63 (H-11) and 5.00 (H-10)]. The HMBC spectrum of **2** did not show correlation from H-11 to C-1 carbonyl carbon, while another hydroxyl proton [δ_H 5.09 (1H, d, J = 6.1 Hz)] was observed in the ^{1}H NMR spectrum of **2** and this signal was assignable to the OH group on C-11 (11-OH) based on the ^{1}H–^{1}H COSY correlation with H-11 (δ_H 4.08). On the other hand, another methoxy signal [δ_H 3.55 (3H, s)] was observed in the ^{1}H NMR spectrum of **2**, and this methoxy proton showed HMBC correlation with the C-1 carbonyl group (δ_C 171.8). From these results, melleumin B (**2**) was deduced to be a *seco* acid methyl ester derivative of compound **1**, which was supported by the ^{1}H–^{1}H COSY and HMBC spectral data of **2**. Treatment of **1** with 28% MeONa afforded **2**, which was detected on the basis of TLC examination.[14] Since methanol was used during isolation processes, **2** was likely to be an artifact of isolation produced from **1**.

The stereochemistry of the chiral centers at C-3, C-10, and C-11 positions was examined by using compound **2**. After acid hydrolysis of **2** (6 N HCl, 110 °C, 12 h), the resulting hydrolyzate was subjected to chiral TLC analysis using L-Thr and D-Thr reference samples to reveal that the threonine residue was L (10*S*,11*R*).[15] In addition, compound **2** was converted into its (*R*)- and (*S*)-MTPA esters (**3** and **4**, respectively),[16] and on the basis of the modified Mosher's method,[17] the absolute configurations of the carbons bearing secondary hydroxyl group (C-3 and C-11) were revealed as 3*S* and 11*R* (Fig. 2). The 11*R*-configuration was also suggested by the chiral TLC analysis (vide supra). The absolute stereochemistry of the C-4 position, however, remained undefined.

The crude extract of the cultured plasmodium of *P. melleum* exhibited antimicrobial activity against *Bacillus subtillis*, but the fraction containing melleumins A (**1**) and B (**2**) was inactive. The antimicrobial activity was likely to be ascribable to unstable yellow pigments,[8]

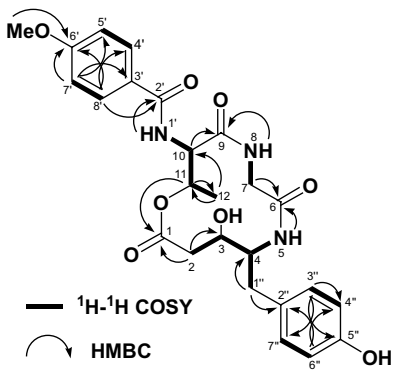

Figure 1. Key ^{1}H–^{1}H COSY and HMBC data of **1**.

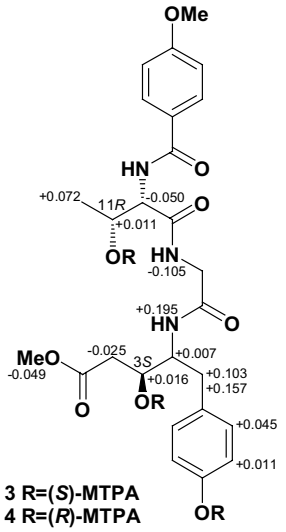

Figure 2. $\Delta\delta$ values of (*R*)- and (*S*)-MTPA esters (**3** and **4**).

purification of which is not currently successful and is still under investigation in our laboratory. Melleumins A (**1**) and B (**2**) did not show cytotoxicity against VCR-resistant KB cell lines and TRAIL (TNF-related apoptosis inducing ligand)-resistant KOB (adult T cell leukemia) cell lines.[4a,18]

Acknowledgements

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References and notes

1. Steglich, W. *Pure Appl. Chem.* **1989**, *61*, 281–288.
2. (a) Steffan, B.; Praemassing, M.; Steglich, W. *Tetrahedron Lett.* **1987**, *28*, 3667–3670; (b) Nowak, A.; Steffan, B. *Liebigs Ann.* **1997**, 1817–1821; (c) Nowak, A.; Steffan, B. *Angew. Chem., Int. Ed.* **1998**, *37*, 3139–3141; (d) Blumenthal, F.; Polborn, K.; Steffan, B. *Tetrahedron* **2002**, *58*, 8433–8437; (e) Eisenbarth, S.; Steffan, B. *Tetrahedron* **2000**, *56*, 363–365.
3. Murakami-Murofushi, K.; Shinoda, M.; Kaji, K.; Yoshida, S.; Murofushi, H. *J. Biol. Chem.* **1992**, *267*, 21512–21517.
4. For studies on field-collected wild myxomycetes, see: (a) Kamata, K.; Onuki, H.; Hirota, H.; Yamamoto, Y.; Hayashi, M.; Komiyama, K.; Sato, M.; Ishibashi, M. *Tetrahedron* **2004**, *60*, 9835–9839; (b) Nakatani, S.; Yamamoto, Y.; Hayashi, M.; Komiyama, K.; Ishibashi, M. *Chem. Pharm. Bull.* **2004**, *52*, 368–370; (c) Iwata, D.; Ishibashi, M.; Yamamoto, Y. *J. Nat. Prod.* **2003**, *66*, 1611–1612; (d) Nakatani, S.; Naoe, A.; Yamamoto, Y.; Yamauchi, T.; Yamaguchi, N.; Ishibashi, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2879–2881; (e) Misono, Y.; Ishikawa, Y.; Yamamoto, Y.; Hayashi, M.; Komiyama, K.; Ishibashi, M. *J. Nat. Prod.* **2003**, *66*, 999–1001; (f) Naoe, A.; Ishibashi, M.; Yamamoto, Y. *Tetrahedron* **2003**, *59*, 3433–3435; (g) Ishikawa, Y.; Ishibashi, M.; Yamamoto, Y.; Hayashi, M.; Komiyama, K. *Chem. Pharm. Bull.* **2002**, *50*, 1126–1127.
5. Ishibashi, M. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 2003; Vol. 29, pp 223–262.
6. (a) Ishibashi, M.; Iwasaki, T.; Imai, S.; Sakamoto, S.; Yamaguchi, K.; Ito, A. *J. Nat. Prod.* **2001**, *64*, 108–110; (b) Misono, Y.; Ishibashi, M.; Ito, A. *Chem. Pharm. Bull.* **2003**, *51*, 612–613.
7. Nakatani, S.; Kiyota, M.; Matsumoto, J.; Ishibashi, M. *Biochem. Sys. Ecol.*, in press.
8. Misono, Y.; Ito, A.; Matsumoto, J.; Sakamoto, S.; Yamaguchi, K.; Ishibashi, M. *Tetrahedron Lett.* **2003**, *44*, 4479–4481.
9. The fruit bodies of *Physarum melleum* were collected at Tokorozawa, in Saitama Prefecture, Japan, in June 2001. A voucher specimen (01-19) is maintained at Graduate School of Pharmaceutical Sciences, Chiba University and Fukui Botanical Garden.
10. The plasmodium of *Physarum melleum* was mass-cultured in the laboratory on agar plates in the presence of *Escherichia coli* according to the methods described previously.[5,6] The harvested plasmodial cells (19.5 g from 2112 plates (9 cmϕ)) were extracted with 90% MeOH (800 mL × 3) and 90% acetone (800 mL × 1). The combined MeOH and acetone extracts (2.26 g) were partitioned between ethyl acetate (200 mL × 7) and water (250 mL). The ethyl acetate-soluble layer (204 mg) was subjected to silica gel column chromatography (15 × 270 mm) eluted with 2–100% MeOH in chloroform, and the fraction (9.3 mg) eluting with 5–9% MeOH in chloroform, containing UV(254 nm)-positive spots on TLC, was combined with the corresponding fraction (9.3 mg) obtained by the same procedures starting from the cultured plasmodial cells (7.5 g from 998 plates (9 cmϕ)). These combined fractions were separated by HPLC on ODS (Develosil ODS HG-5; 10 × 250 mm; eluent, 50% MeOH; flow rate, 1.8 mL/min; detection, UV at 254 nm) to give melleumin A (**1**, 1.9 mg, t_R = 19.9 min) and melleumin B (**2**, 6.5 mg, t_R = 14.8 min).
11. Melleumin A (**1**): Colorless amorphous solid; $[\alpha]_D^{26}$ +27 (*c* 0.15, MeOH); CD (MeOH) λ_{ext} 208 ($\Delta\varepsilon$ −3.3) and 244 nm (+4.7); UV (MeOH) λ_{max} 254 (ε 15,000) and 225 nm (13,000); ν_{max} 3420, 2930, 1720, 1640, 1610, 1510, and 1250 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 500 $(M+H)^+$ and 522 $(M+Na)^+$; HRFABMS calcd for $C_{25}H_{30}O_8N_3$ $(M+H)^+$ 500.2034, found *m/z* 500.1995.
12. McConnel, R. M.; Frizzell, D.; Camp, A.; Evans, A.; Jones, W.; Cagle, C. *J. Med. Chem.* **1991**, *34*, 2298–2300.
13. Melleumin B (**2**): Colorless amorphous solid; $[\alpha]_D^{26}$ ca. 0 (*c* 0.32, MeOH); CD (MeOH) λ_{ext} 212 ($\Delta\varepsilon$ −6.1), 224 (−1.9), and 252 nm (+2.7); UV (MeOH) λ_{max} 254 (ε 13,000) and 226 nm (10,000); IR (film) ν_{max} 3410, 2925, 1730, 1660, 1640, 1630, 1610, 1510, 1500, 1440, and 1260 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 532 $(M+H)^+$ and 554 $(M+Na)^+$; HRFABMS calcd for $C_{26}H_{34}O_9N_3$ $(M+H)^+$ 532.2295, found *m/z* 532.2338.
14. Treatment of **1** (0.1 mg) with 28% MeONa (10 μL) and MeOH (500 μL) at room temperature for 5 min afforded **2**, which was detected on the basis of TLC examination (silica gel; $CHCl_3$/MeOH, 9:1).
15. Compound **2** (0.9 mg) was treated with 6 N HCl (1.5 mL) and heated at 110 °C for 12 h. The reaction mixture was cooled to room temperature and evaporated to dryness, and the resulting hydrolyzate was subjected to the chiral TLC analysis [Merck HPTLC plate CHIR Art. 14101; MeOH/H_2O/CH_3CN = 1:1:4; visualization, ninhydrin; R_f-values: L-Thr and L-allo-Thr (both ca. 0.6), D-Thr and D-*allo*-Thr (both ca. 0.55), and the hydrolyzate (ca. 0.6)]. The acidic hydrolyzate was examined by TLC using L-Thr and L-*allo*-Thr reference samples to reveal that the hydrolyzate contained not L-*allo*-Thr but L-Thr [TLC (RP-18 F_{254S}, Merck 1.15423; MeOH 100%), R_f-values: L-Thr (0.56), L-allo-Thr (0.61), and the hydrolyzate (0.54)].
16. A solution of **2** (0.9 mg) in dry pyridine (20 μL) was treated with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA-Cl] (5.6 μL) at rt for 3 h. After addition of methanol, the reaction mixture was evaporated and purified by a silica gel HPLC (YMC-pack SIL-06; 6 × 250 mm; flow rate, 2.0 mL/min; eluent, hexane/isopropanol = 85:15; detection, UV at 254 nm) to give the (*S*)-MTPA ester (**3**, 1.0 mg): ^1H NMR ($CDCl_3$) δ_H 2.638 (1H, d, *J* = 6.4 Hz, H-2), 5.529 (1H, td, *J* = 6.4 and 2.2 Hz, H-3), 4.501 (1H, m, H-4), 6.339 (1H, d, *J* = 9.1 Hz, H-5), 3.649 (1H, d, *J* = 5.8 Hz, H-7), 6.636 (1H, br t, *J* = 5.8 Hz, H-8), 4.647 (1H, dd, *J* = 8.0 and 4.5 Hz, H-10), 5.732 (1H, qd, *J* = 6.2 and 4.5 Hz, H-11), 1.390 (1H, d, *J* = 6.2 Hz, H-12), 6.812 (1H, d, *J* = 8.0 Hz, H-1′), 7.658 (2H, d, *J* = 8.8 Hz, H-4′ and H-8′), 6.897 (2H, d, *J* = 8.8 Hz, H-5′ and H-7′), 2.795 (1H, dd, *J* = 14.0 and 6.3 Hz, H-1″), 2.648 (1H, m, H-1″), 7.094 (2H, d, *J* = 8.4 Hz, H-3″ and H-7″), 6.961 (2H, d, *J* = 8.4 Hz, H-4″ and H-6″), 3.573 (3H, s,

1-OMe), 3.838 (3H, s, 6′-OMe), 3.459 (3H, s, MTPA-OCH_3), 3.501 (3H, s, MTPA-OCH_3), 3.635 (3H, s, MTPA-OCH_3), and 7.34–7.59 (15H, m, MTPA-Ph); FABMS *m*/*z* 1180 $(M+H)^+$. The (*R*)-MTPA ester (**4**) was also prepared from **2** by the same procedures using (*S*)-MTPA chloride. **4**: ^1H NMR ($CDCl_3$) δ_H 2.663 (1H, dd, $J = 6.1$ and 2.6 Hz, H-2), 5.513 (1H, td, $J = 6.1$ and 1.9 Hz, H-3), 4.494 (1H, m, H-4), 6.144 (1H, d, $J = 9.4$ Hz, H-5), 3.749 (1H, dd, $J = 16.8$ and 5.7 Hz, H-7), 3.603 (1H, dd, $J = 16.8$ and 5.7 Hz, H-7), 6.741 (1H, t, $J = 5.7$ Hz, H-8), 4.697 (1H, dd, $J = 7.9$ and 3.5 Hz, H-10), 5.721 (1H, qd, $J = 6.6$ and 3.5 Hz, H-11), 1.318 (1H, d, $J = 6.6$ Hz, H-12), 6.795 (1H, d, $J = 7.9$ Hz, H-1′), 7.603 (2H, d, $J = 9.0$ Hz, H-4′ and H-8′), 6.882 (2H, d, $J = 9.0$ Hz, H-5′ and H-7′), 2.692 (1H, dd, $J = 14.2$ and 6.3 Hz, H-1″), 2.491 (1H, dd, $J = 14.2$ and 9.1, H-1″), 7.049 (2H, d, $J = 8.7$ Hz, H-3″ and H-7″), 6.950 (2H, d, $J = 8.7$ Hz, H-4″ and H-6″), 3.622 (3H, s, 1-OMe), 3.831 (3H, s, 6′-OMe), 3.456 (3H, s, MTPA-OCH_3), 3.559 (3H, s, MTPA-OCH_3), 3.624 (3H, s, MTPA-OCH_3), and 7.33–7.60 (15H, m, MTPA-Ph); FABMS *m*/*z* 1180 $(M+H)^+$.

17. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4902–4906.
18. Rho, M.-C.; Hayashi, M.; Fukami, A.; Obata, R.; Sunazuka, T.; Tomoda, H.; Komiyama, K.; Ōmura, S. *J. Antibiot.* **2000**, *53*, 1201–1206.