Determination of Absolute Configuration and Biological Activity of New Immunosuppressants, Mycestericins D, E, F and G†

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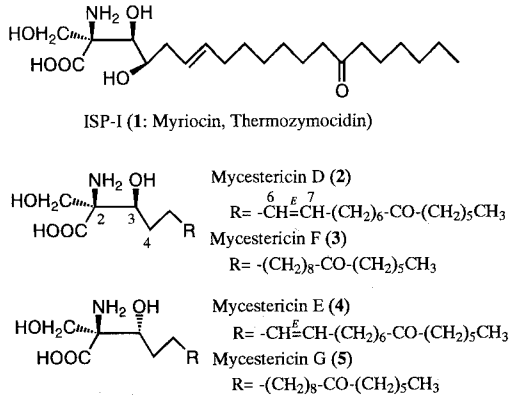
Mycestericins D, E, F and G were isolated from the culture broth of *Mycelia sterilia* ATCC 20349 as potent immunosuppressants. Mycestericins F and G were identical with dihydromycestericins D and E, respectively. Their absolute configurations were determined by use of the modified MOSHER's method and by comparison of the CD spectra of their benzoate derivatives with those of synthetic analogs. Mycestericins D, E, F and G suppressed the proliferation of lymphocytes in the mouse allogeneic mixed lymphocyte reaction.

We have isolated a potent immunosuppressant, ISP-I[2] (**1**: myriocin[3,4], thermozymocidin[5]), from the culture broth of *Isaria sinclairii* (ATCC 24400) and reported that it was 10 to 100 times more potent than cyclosporin A in terms of suppressing lymphocyte proliferation in mouse allogeneic mixed lymphocyte reaction (MLR) *in vitro*, and allo-reactive cytotoxic T lymphocytes generation and T cell-dependent antibody production *in vivo*. A search for new compounds having more potent activity than **1** led to the isolation of mycestericins A, B, C, D (**2**) and E (**4**) as minor analogs from an ISP-I-producing strain, *Mycelia sterilia* (ATCC 20349)[6]. The plane structures of **2** and **4** were clarified and it was established that they are in a diastereo isomeric relationship to each other[6]. However, their absolute configurations were not elucidated. Further study led to the isolation of two more new active compounds, mycestericins F (**3**) and G (**5**), from the same strain.

The present paper describes in detail the elucidation of the structures and absolute configurations[7], as well as the biological activities, of **2**, **3**, **4** and **5**.

Fig. 1. The structures of ISP-I and mycestericins D, E, F, G.



Results and Discussion

Isolation of Mycestericins D, E, F and G

Mycestericins D (**2**) and E (**4**) were isolated from the culture broth of *M. sterilia* as described in a previous paper[6]. Further examination of the crude fraction containing **2** and **4** by means of preparative HPLC gave **3** and **5**.

Determination of Plane Structures of Mycestericins F and G

Mycestericin F

Mycestericin F (**3**) was obtained as a white powder.

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The molecular formula was determined as $C_{21}H_{41}NO_5$ by HRFAB-MS (Calcd for $C_{21}H_{42}NO_5$: 388.3065, Found: 388.3049). The formula has two hydrogen atoms more than that of **2**. The IR and ^1H NMR spectra of **3** were similar to those of **2**. However, the C-6, 7 olefin signals of **2** (IR: 970 cm^{-1}, ^1H NMR: δ 5.4~5.5, $-CH=CH-$ and δ 2.0, $-CH_2-CH=CH-CH_2-$) were absent in the spectra of **3**. Instead, a signal due to $-CH_2-\times 4$ at 1.29 ppm was observed in the ^1H NMR spectrum of **3**. These data suggested that **3** is the 6,7-dihydro derivative of **2**. It was confirmed by the catalytic hydrogenation of **2**.

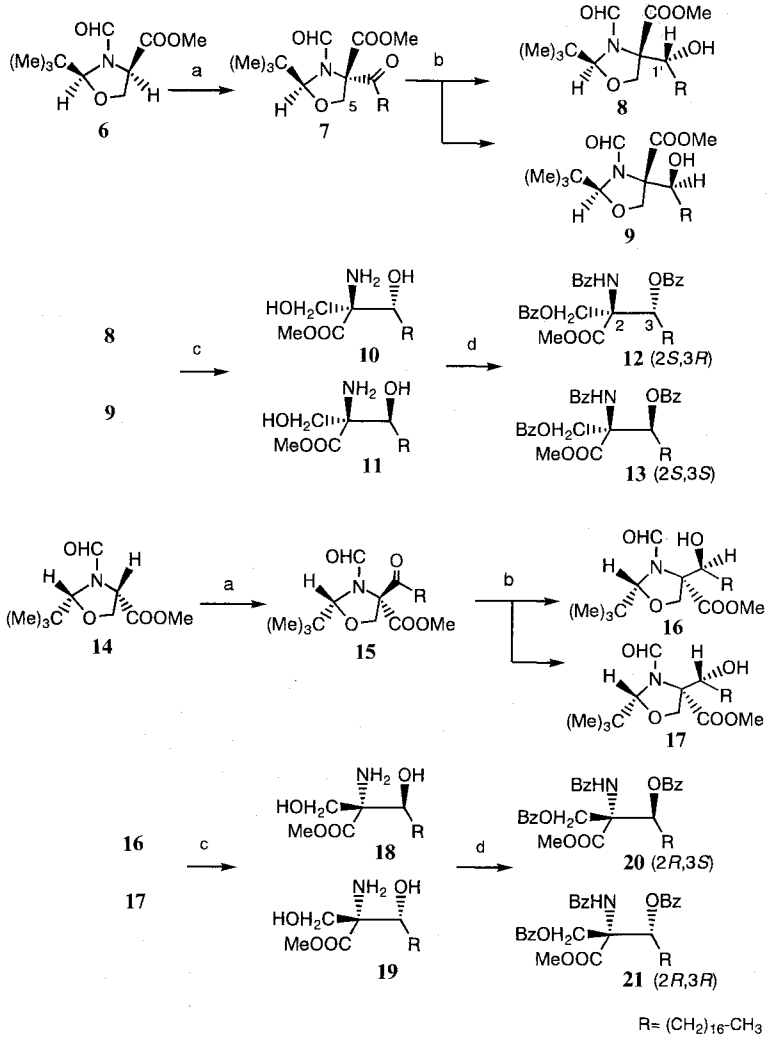
Mycestericin G

Mycestericin G (**5**), $C_{21}H_{41}NO_5$ (HRFAB-MS Calcd for $C_{21}H_{42}NO_5$: 388.3065, Found: 388.3032), was obtained as a white powder. Although the ^1H NMR spectrum of **5** was very similar to that of **3**, the signal of methylene protons (δ: 1.53, 2H) at C-4 of **3** was replaced by signals at δ 1.62 and δ 1.36 in the spectrum of **5**. The spectra (IR, ^1H NMR, MS) of **5** were identical with those of dihydromycestericin E.

Absolute Structures of Mycestericins D, E, F and G

The absolute configurations of **2**, **3**, **4** and **5** were determined by comparison of the CD spectra of their *N,O,O'*-tribenzoyl methyl esters (**22**~**25**) with those of synthetic compounds **12**, **13**, **20** and **21**. The syntheses are outlined in Scheme 1. Stereoselective acylation of the protected D-serine **6**[8,9] with stearoyl chloride afforded the β-keto ester **7**. The ketonic carbonyl oxygen and the ester carbonyl carbon were confirmed to be oriented to the same side in the same plane, based on NOE experiments (Fig. 2). Therefore, it is considered that the stereo-

Scheme 1. Syntheses of **12**, **13**, **20** and **21**.



Reagents: a) $CH_3(CH_2)_{16}COCl$ (1.2 eq.), LDA (1.2 eq.), THF, -100°C; b) $NaBH_4$ (1.2 eq.), MeOH; c) 6 N HCl, MeOH, 80°C, 1 hour; d) Bz_2O, Et_3N, 40°C, 2 hours.

Fig. 2. ^1H-^1H correlations of **7** obtained in an NOE experiment.

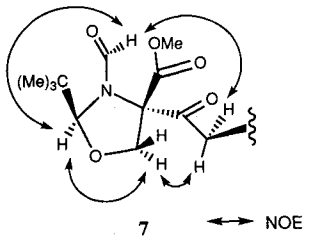
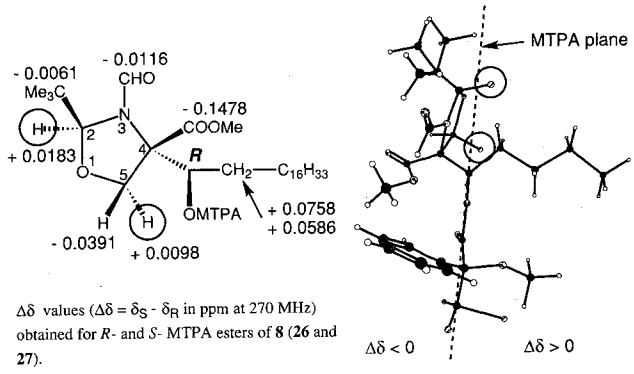


Fig. 3. Application of the modified MOSHER's method to **8**.



Δδ values (Δδ = δ_S - δ_R in ppm at 270 MHz) obtained for *R*- and *S*- MTPA esters of **8** (**26** and **27**).

Fig. 4. Synthesis of benzoate-Me ester derivatives of **2**~**5** (**22**~**25**).

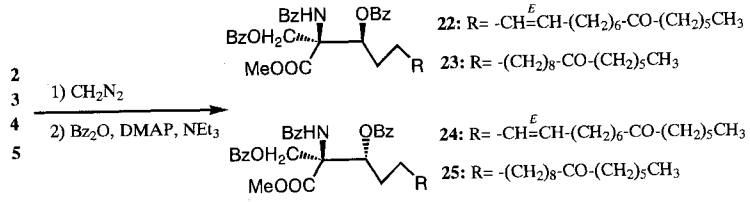


Fig. 5. CD spectra of **13**, **21**, **22** and **23** (in MeOH).

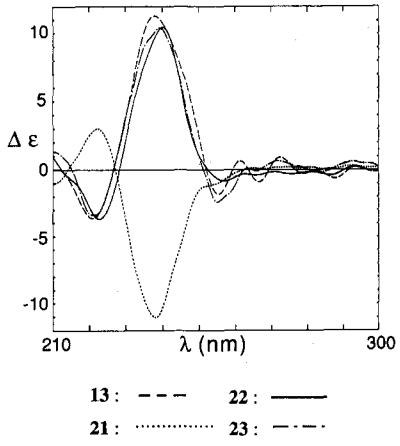
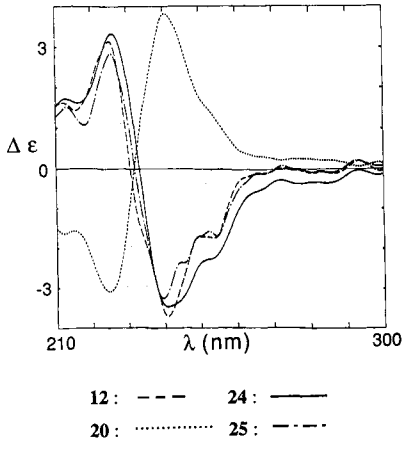
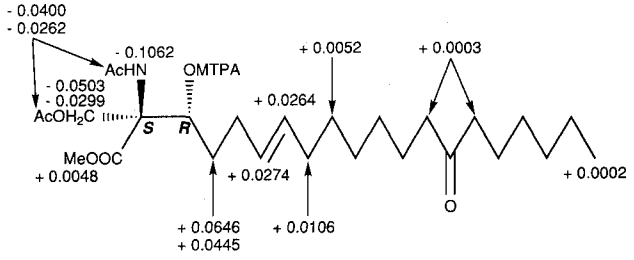


Fig. 6. CD spectra of **12**, **20**, **24** and **25** (in MeOH).



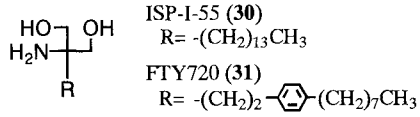
selective reduction of the β-keto ester **7** with $NaBH_4$ gave the (1′*R*)-hydroxy compound **8** as the major product and the (1′*S*)-hydroxy compound **9** as the minor product by hydride attack from the less hindered side because of the presence of the formyl group at the *re*-face of the ketone. The absolute configuration of the (1′*R*)-hydroxy group of the major product **8** was confirmed by means of the modified MOSHER's method[10] (Fig. 3). Consequently, the 1′-hydroxy group of the minor product **9** should have *S*-configuration. Treatment of **8** and **9** with 6N HCl afforded the esters **10** and **11**, which were benzoylated to give **12** and **13**, respectively. By a similar procedure, **20** (2*R*,3*S*) and **21** (2*R*,3*R*) as major and minor products, respectively, were prepared from protected L-serine **14**[8]. On the other hand, the natural products **2**~**5** were treated with CH_2N_2, followed by benzoylation with benzoic anhydride (Bz_2O) and *N*,*N*-dimethylaminopyridine (DMAP) in Et_3N to afford the *N*,*O*,*O*′-tribenzoyl methyl esters (**22**~**25**, Fig. 4). As shown in Fig. 5, the CD curves of **22** and **23** showed good coincidence with that of **13** (2*S*,3*S*). Further, the CD curves of **24** and **25** showed good coincidence with that of **12** (2*S*,3*R*) (Fig. 6). The 3-hydroxy group of **4** was also determined to have *R*-configuration by means

Fig. 7. Application of MOSHER's method to mycestericin E.



$\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ in ppm at 600 MHz) obtained for *R*- and *S*- MTPA esters of diacetylmycestericin E methyl ester (**28** and **29**).

Fig. 8. The structures of ISP-I-55 and FTY720.



of the modified MOSHER's method (Fig. 7).

Consequently, the absolute configurations of mycestericins D, E, F and G were determined to be **2** (2*S*,3*S*), **3** (2*S*,3*S*), **4** (2*S*,3*R*) and **5** (2*S*,3*R*), as shown in Fig. 1.

The structures of **4** and **5** were unambiguously confirmed by total synthesis[7].

Immunosuppressive Activity

The effect of **2**, **3**, **4** and **5** on the mouse allogeneic mixed lymphocyte reaction (MLR)[2] was examined in comparison with that of **1**. Table 1 shows the IC_{50} values of **1**~**5**. From these results, the following structure-activity relationships were apparent. 1) The hydroxy group at C-4 has no effect on the activity (**1**→**2**). 2) The configuration of the 3-hydroxyl group is unimportant (**2**→**4**, **3**→**5**). 3) The C-6 olefin group increases the activity (**2**→**3**, **4**→**5**). These structure-activity relationships provided clues that led to the development of 2-substituted 2-amino-1,3-propanediols, ISP-I-55 (**30**)[11] and FTY720 (**31**)[12] (Fig. 8).

Experimental

General Methods

Melting points were determined on a Yanagimoto micro melting point apparatus without correction. Optical rotations were measured with a Jasco DIP-181 or Jasco DIP-140 digital polarimeter. UV and IR spectra were taken on a Shimadzu UV-2200 UV-VIS recording spectrophotometer and a Shimadzu IR-435 infrared spectrophotometer, respectively. CD spectra were measured on a Jasco J-720 spectrophotometer. The concentration of benzoate derivatives was determined from the UV spectra by using a calibration curve for propyl benzoate. ^1H NMR and ^{13}C NMR spectra were taken on a JEOL FX-200, JEOL EX-270, Bruker AC-300 or Bruker AM-600 spectrometer with TMS as an internal standard. Mass spectra (EI-MS and FAB-MS) were taken on a JEOL JMS-01SG, JEOL JMS-HX100 or JMS-HX110 spectrometer. HPLC was performed on a Shimadzu LC-8A system [eluent MeOH-H_2O (65:35 v/v); column temperature 40°C; UV detector (210 nm)]. Analytical HPLC was carried out with a YMC-ODS AM 313 column (i.d. 6 mm × 250 mm) (flow rate 1.0 ml/minute). Preparative HPLC was carried out with a YMC-ODS SH-343-5 column (i.d. 20 mm × 250 mm) (flow rate 7.0 ml/minute). Analytical TLC and preparative TLC were performed on Kieselgel 60 F254 (Merck) and Kieselgel 60 PF254 (Merck), respectively. Organic extracts of reaction mixtures were washed successively with 1 N HCl, 5% $NaHCO_3$ and saturated aqueous NaCl and dried over anhydrous magnesium sulfate, unless otherwise specified.

Table 1. Effect of ISP-I and mycestericins on mouse allogeneic MLR.

Compound	IC_{50} (nM)
ISP-I (**1**)	8.0
Mycestericin D (**2**)	16
E (**4**)	13
F (**3**)	120
G (**5**)	370

Isolation and Purification

Mycestericins D (**2**) and E (**4**) were isolated from the culture broth of *M. sterilia* as described in the previous paper[6]. A crude fraction of **2** and **4** (650 mg) containing **3** and **5** was subjected to preparative HPLC to give **3** (5.8 mg; retention time, 109~112 minutes) and **5** (9.0 mg; retention time, 115~118 minutes).

3: MP 190~191.5°C. $[\alpha]_D^{25}$ −7.94° (*c* 0.106, MeOH). IR ν_{max} (KBr) cm^{-1}: 3375, 3100, 2910, 2830, 1705, 1665, 1580, 1045. ^1H NMR (CD_3OD, 300 MHz) δ: 3.98 (1H, d, J=11.1 Hz, 21H), 3.82 (1H, d, J=11.1 Hz, 21-H), 3.81 (1H, m, 3-H), 2.43 (4H, t, J=7.3 Hz, 13-, 15-H_2), 1.53 (6H, m, 4-, 12-, 16-H_2), 1.29 (20H, m, 5-~11-, 17-~19-H_2), 0.90 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS *m/z* 388 $[(M+H)^+]$, 282, 246.

5: MP 187~189°C. $[\alpha]_D^{25}$ −6.81° (*c* 0.106, MeOH). IR ν_{max} (KBr) cm^{-1}: 3400, 3100, 2910, 2830, 1710, 1660, 1580, 1045. ^1H NMR (CD_3OD, 300 MHz) δ: 3.93 (1H, d, J=11.1 Hz, 21H), 3.83 (1H, m, 3-H), 3.81 (1H, d, J=11.1 Hz, 21-H), 2.44 (4H, t, J=7.13 Hz, 13-, 15-H_2), 1.62 (1H, m, 4-H), 1.54 (4H, qui, J=6.5 Hz, 12-, 16-H_2), 1.36 (1H, m, 4-H), 1.30 (20H, m, 5-~11-, 17-~19-H_2), 0.90 (3H, t, J=6.6 Hz, 20-H_3). FAB-MS *m/z* 388 $[(M+H)^+]$, 282, 246.

Mouse Allogeneic Mixed Lymphocyte Reaction (MLR)

The effect of mycestericins on mouse allogeneic MLR was examined by the method described in our previous paper[2].

Hydrogenation of **2**

A solution of **2** (10.0 mg) in MeOH (30 ml) was subjected to hydrogenation over 5% palladium carbon (20 mg). The catalyst was filtered off and the solvent was evaporated. The residue was purified by preparative HPLC [eluent MeOH-H_2O (70:30 v/v); flow rate 8.0 ml/minute] to give dihydromycestericin D as a white powder (9.39 mg, 93.4%). This compound was identical with **3**.

Hydrogenation of **4**

By a procedure similar to that used for the preparation of **3** from **2**, **5** (9.65 mg) was obtained as a white powder in 96.0% yield from **4** (10.0 mg).

Methyl (2*S*,4*S*)-2-*t*-Butyl-3-formyl-4-octadecanoyloxazolidine-4-carboxylate (**7**)

Compound **7** was synthesized from methyl (2*S*,4*R*)-2-*t*-butyl-3-formyloxazolidine-4-carboxylate (**6**, 1.27 g) and stearoyl chloride (2.14 g) in the reported manner.[9] The product was purified to give **7** as an oil (1.302 g, 45.8%) by chromatography on a silica gel column using *n*-hexane-EtOAc (9:1). $[\alpha]_D^{25}$ +3.01° (*c* 1.22, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3000, 2915, 2850, 1750, 1725, 1680, 1280, 1230. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.32, 8.25 (1H, 2s, CHO), 5.48, 5.08 (1H, 2s, 2-H), 4.71, 4.61 (1H, 2d, J=10.2 and 9.0 Hz, 5-H), 4.16, 4.08 (1H, 2d, J=10.2 and 9.0 Hz, 5-H), 3.88, 3.85 (3H, 2s, OCH_3), 2.68 (1H, dt, J=17.8 and 7.6 Hz, 2'-H), 2.49 (1H, m, 2'-H), 1.60 (2H, m, 3'-H_2), 1.25 (28H, m, 4'-~17'-H_2), 0.98, 0.94 (9H, 2s, *t*-Bu), 0.88 (3H, t, J=6.5 Hz, 18'-H_3). *Anal.* Calcd for $C_{28}H_{51}NO_5$: C 69.82, H 10.67, N 2.91. Found: C 69.74, H 10.91, N 2.81.

Methyl (2*R*,4*R*)-2-*t*-Butyl-3-formyl-4-octadecanoyloxazolidine-4-carboxylate (**15**)

By a procedure similar to that used for the preparation of **7**, **15** (1.515 g) was obtained as an oil in 53.3% yield from methyl (2*R*,4*S*)-2-*t*-butyl-3-formyloxazolidine-4-carboxylate (**14**, 1.27 g) and stearoyl chloride (2.14 g). $[\alpha]_D^{25}$ −2.20° (*c* 2.11, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3000, 2915, 2850, 1750, 1725, 1680, 1280, 1230. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.32, 8.25 (1H, 2s, CHO), 5.48, 5.08 (1H, 2s, 2-H), 4.72, 4.61 (1H, 2d, J=10.2 and 9.0 Hz, 5-H), 4.16, 4.08 (1H, 2d, J=10.2 and 9.0 Hz, 5-H), 3.88, 3.85 (3H, 2s, OCH_3), 2.68 (1H, dt, J=17.8 and 7.6 Hz 2'-H), 2.49 (1H, m, 2'-H), 1.61 (2H, m, 3'-H_2), 1.25 (28H, m, 4'-~17'-H_2), 0.98, 0.94 (9H, 2s, *t*-Bu), 0.88 (3H, t, J=6.6 Hz, 18'-H_3). *Anal.* Calcd for $C_{28}H_{51}NO_5$: C 69.82, H 10.67, N 2.91. Found: C 69.94, H 10.93, N 3.01.

Methyl (2*S*,4*S*)-2-*t*-Butyl-3-formyl-4-(1(*R*)-hydroxyoctadecyl)oxazolidine-4-carboxylate (**8**) and Methyl (2*S*,4*S*)-2-*t*-Butyl-3-formyl-4-(1(*S*)-hydroxyoctadecyl)-oxazolidine-4-carboxylate (**9**)

Sodium borohydride (10 mg) was added to a solution of **7** (100 mg) in MeOH (15 ml) at 0°C. After 15 minutes, H_2O (30 ml) was added to the mixture, then MeOH was evaporated off. The H_2O solution was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with saturated aqueous NaCl, dried and concentrated to give an oily residue. The residue was purified by preparative HPLC [column: YMC-ODS SH-343-5 (i.d. 20 mm × 250 mm), mobile phase: MeOH-H_2O (93:7), flow rate: 8.0 ml/minute, temperature: 40°C, detection: UV at 210 nm] to give **8** (69.8 mg, 69.5%) and **9** (14.3 mg, 14.2%).

8: $[\alpha]_D^{25}$ +28.45° (*c* 0.113, MeOH). IR ν_{max} ($CHCl_3$) cm^{-1}: 3350, 3000, 2905, 2840, 1755, 1730, 1665, 1215. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.70, 8.46 (1H, 2s, CHO), 5.39, 4.99 (1H, 2s, 2-H), 5.03 (1H, d, J=11.2 Hz, OH), 4.50, 4.40 (1H, 2d, J=9.4 Hz, 5-H), 3.95 (1H, br t-like, J=10.7 Hz, 1'-H), 3.90 (1H, d, J=9.4 Hz, 5-H), 3.83, 3.80 (3H, 2s, OMe), 1.64 (1H, m, 2'-H), 1.46 (1H, m, 2'-H), 1.25 (30H, br s, 3'-~17'-H_2), 1.00, 0.94 (9H, 2s,-Bu), 0.88 (3H, t, J=6.3 Hz, 18'-H_3). *Anal.* Calcd for $C_{28}H_{53}NO_5$: C 69.52, H 11.04, N 2.90. Found: C 69.72, H 11.25, N 2.94.

9: $[\alpha]_D^{25}$ +1.13° (*c* 0.053, MeOH). IR ν_{max} ($CHCl_3$) cm^{-1}: 3370, 3000, 2905, 2840, 1750 (sh), 1725, 1660, 1215. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.39, 8.19 (1H, 2s, CHO), 5.33, 5.16 (1H, m and br s, OH), 4.85 (1H, s, 2-H), 4.36, 4.01 (1H, 2d, J=8.9 and 9.2 Hz, 5-H), 4.17 (1H, m, 1'-H), 3.92, 3.66 (1H, 2d, J=8.9 and 9.2 Hz, 5-H), 3.79, 3.78 (3H, 2s, OMe), 1.79 (1H, m, 2'-H), 1.59 (1H, m, 2'-H), 1.25 (30H, br s, 3'-~17'-H_2), 1.01, 0.97 (9H, 2s, *t*-Bu), 0.88 (3H, t, J=6.6 Hz, 18'-H_3). *Anal.* Calcd for $C_{28}H_{53}NO_5$: N 2.90. Found: N 3.02.

Methyl (2*R*,4*R*)-2-*t*-Butyl-3-formyl-4-(1(*S*)-hydroxyoctadecyl)oxazolidine-4-carboxylate (**16**) and Methyl (2*R*,4*R*)-2-*t*-Butyl-3-formyl-4-(1(*R*)-hydroxyoctadecyl)-oxazolidine-4-carboxylate (**17**)

By a procedure similar to that used for the preparation of **8** and **9**, **16** (67.2 mg) and **17** (12.1 mg) were obtained as oils in yields of 66.9 and 12.0% from **15** (100 mg), respectively.

16: $[\alpha]_D^{25}$ −32.2° (*c* 0.116, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3350, 3000, 2905, 2840, 1755, 1735, 1665, 1215. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.70, 8.46 (1H, 2s, CHO), 5.39, 4.99 (1H, 2s, 2-H), 5.03 (1H, d, J=11.2 Hz, OH), 4.50, 4.40 (1H, 2d, J=9.4 Hz, 5-H), 3.95 (1H, br t-like, J=10.7 Hz, 1'-H), 3.90 (1H, d, J=9.4 Hz, 5-H), 3.83, 3.80 (3H, 2s, OMe), 1.64 (1H, m, 2'-H), 1.47 (1H, m, 2'-H), 1.25 (30H, br s, 3'-~17'-H_2), 0.99, 0.94 (9H, 2s, *t*-Bu), 0.88 (3H, t, J=6.3 Hz, 18'-H_3).

17: $[\alpha]_D^{25}$ −0.20° (*c* 0.044, MeOH). IR ν_{max} ($CHCl_3$) cm^{-1}: 3350, 3000, 2905, 2840, 1750 (sh), 1730, 1660, 1215. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.39, 8.19 (1H, 2s, CHO), 5.33, 5.16 (1H, m and br s, OH), 4.85 (1H, s,

2-H), 4.36, 4.01 (1H, 2d, J=8.9 and 9.2 Hz, 5-H), 4.18 (1H, m, 1'-H), 3.92, 3.66 (1H, 2d, J=8.9 and 9.2 Hz, 5-H), 3.79, 3.78 (3H, 2s, OMe), 1.79 (1H, m, 2'-H), 1.58 (1H, m, 2'-H), 1.25 (30H, br s, 3'-~17'-H_2), 1.01, 0.97 (9H, 2s, t-Bu), 0.88 (3H, t, J=6.6 Hz, 18'-H_3).

Methyl 2-Amino-3-hydroxy-2-hydroxymethyleicosanoates (**10** (2*S*,3*R*), **11** (2*S*,3*S*), **18** (2*R*,3*S*) and **19** (2*R*,3*R*))

Compound **8** (10 mg), **9** (10 mg), **16** (10 mg) or **17** (10 mg) was treated with 6 N methanolic HCl at 80°C for 1 hour. The mixture was applied to an IRA-93zu (eluent, MeOH) column and the eluate was concentrated to give a residue. The residue was purified by preparative TLC [solvent, $CHCl_3$-MeOH-H_2O (65:35:2)] to give **10** (4.9 mg, 61.2%), **11** (4.8 mg, 59.9%), **18** (5.8 mg, 72.4%) or **19** (5.1 mg, 64.6%), respectively.

10: MP 60~61°C. $[\alpha]_D^{25}$ +25.31° (c 1.05, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3570 (sh), 3450 (sh), 3390, 3000, 2915, 2840, 1730, 1225. ^1H NMR (CD_3OD, 270 MHz) δ: 3.85 (1H, d, J=10.6 Hz, 21-H), 3.74 (4H, m, 3-H and OMe), 3.51 (1H, d, J=10.6 Hz, 21-H), 1.54 (1H, m, 4-H), 1.28 (31H, m, 4-H, 5-~19-H_2), 0.90 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS m/z: 388 [$(M+H)^+$], 370, 328, 118, 55, 43.

11: MP 65~69.5°C. $[\alpha]_D^{25}$ +3.40° (c 0.92, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3600 (sh), 3500 (sh), 3390, 3000, 2910, 2840, 1730, 1225. ^1H NMR (CD_3OD, 270 MHz) δ: 3.88 (1H, d, J=10.9 Hz, 21-H), 3.73 (3H, s, OMe), 3.67 (1H, dd, J=10.2 and 2.0 Hz, 3-H), 3.64 (1H, d, J=10.9 Hz, 21-H), 1.51 (2H, m, 4-H_2), 1.28 (30H, m, 5-~19-H_2), 0.90 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS m/z 388 [$(M+H)^+$], 370, 328, 310, 118, 57, 43.

18: MP 60~60.5°C. $[\alpha]_D^{25}$ −23.21° (c 1.23, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3570 (sh), 3450 (sh), 3390, 3000, 2915, 2835, 1730, 1225. ^1H NMR (CD_3OD, 270 MHz) δ: 3.85 (1H, d, J=10.6 Hz, 21-H), 3.74 (4H, m, 3-H and OMe), 3.51 (1H, d, J=10.6 Hz, 21-H), 1.56 (1H, m, 4-H), 1.29 (31H, m, 4-H, 5-~19-H_2), 0.90 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS m/z: 388 [$(M+H)^+$], 370, 328, 118, 55, 43.

19: MP 69~71°C. $[\alpha]_D^{25}$ −5.01° (c 0.621, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3605 (sh), 3500 (sh), 3390, 3000, 2910, 2840, 1735, 1225. ^1H NMR (CD_3OD, 270 MHz) δ: 3.88 (1H, d, J=10.9 Hz, 21-H), 3.73 (3H, s, OMe), 3.67 (1H, dd, J=10.2 and 2.0 Hz, 3-H), 3.64 (1H, d, J=10.9 Hz, 21-H), 1.51 (2H, m, 4-H_2), 1.28 (30H, m, 5-~19-H_2), 0.90 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS m/z 388 [$(M+H)^+$], 370, 328, 310, 118, 57, 43.

Methyl 2-Benzamido-3-benzoyloxy-2-benzoyloxymethyleicosanoates (**12** (2*S*,3*R*), **13** (2*S*,3*S*), **20** (2*R*,3*S*) and **21** (2*R*,3*R*))

Benzoic anhydride (90 mg) was added to a solution of **10** (12 mg), **11** (3.5 mg), **18** (5.0 mg) or **19** (5.0 mg) and *N*,*N*-dimethylaminopyridine (11 mg) in Et_3N (1.0 ml) and the mixture was stirred at 40°C for 2 hours. Ice-water was added and the mixture was extracted with $CHCl_3$. The organic layer was washed and concentrated to give an oily residue. The residue was purified by preparative TLC [solvent, *n*-hexane-EtOAc (6:4)] to give **12** (16.8 mg, 77.5%), **13** (3.0 mg, 47.5%), **20** (6.3 mg, 69.8%) or **21** (5.7 mg, 63.1%), respectively, as an oil.

12: $[\alpha]_D^{25}$ +4.83° (c 0.116, $CHCl_3$). CD λ_{ext} (EtOH) nm: 241 ($\Delta\varepsilon$ = −3.70), 225 (3.13). IR ν_{max} ($CHCl_3$) cm^{-1}: 3395, 3000, 2910, 2840, 1720, 1670, 1265, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 7.99~7.19 (16H, m, NH and aromatic H), 5.84 (1H, dd, J=10.6 and 2.3 Hz, 3-H), 5.39 (1H, d, J=11.7 Hz, 21-H), 5.01 (1H, d, J=11.7 Hz, 21-H), 3.86 (3H, s, OMe), 1.91 (1H, m, 4-H), 1.75 (1H, m, 4-H), 1.25 (30H, m, 5-~19-H_2), 0.88 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS m/z: 700 [$(M+H)^+$], 578, 105. HRFAB-MS Calcd for $C_{43}H_{58}NO_7$: 700.4215 [$(M+H)^+$]. Found: m/z 700.4219.

13: $[\alpha]_D^{25}$ +44.04° (c 0.045, $CHCl_3$). CD λ_{ext} (EtOH) nm: 238 ($\Delta\varepsilon$ = 11.3), 221 (−3.65). IR ν_{max} ($CHCl_3$) cm^{-1}: 3395, 3000, 2910, 2840, 1725, 1665, 1270, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.08~7.39 (16H, m, NH and aromatic H), 5.93 (1H, dd, J=9.2 and 4.6 Hz, 3-H), 5.17 (2H, s, 21-H_2), 3.85 (3H, s, OMe), 1.89 (2H, m, 4-H_2), 1.25 (30H, m, 5-~19-H_2), 0.88 (3H, t, J=6.3 Hz, 20-H_3), FAB-MS m/z: 700 [$(M+H)^+$], 578, 105. HRFAB-MS Calcd for $C_{43}H_{58}NO_7$: 700.4215 [$(M+H)^+$]. Found: m/z 700.4224.

20: $[\alpha]_D^{25}$ −3.80° (c 0.111, $CHCl_3$). CD λ_{ext} (EtOH) nm: 241 ($\Delta\varepsilon$ = 3.83), 225 (−3.07). IR ν_{max} ($CHCl_3$) cm^{-1}: 3395, 3000, 2910, 2840, 1720, 1670, 1265, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.01~7.21 (16H, m, NH and aromatic H), 5.84 (1H, dd, J=10.6 and 2.3 Hz, 3-H), 5.39 (1H, d, J=11.7 Hz, 21-H), 5.01 (1H, d, J=11.7 Hz, 21-H), 3.86 (3H, s, OMe), 1.91 (1H, m, 4-H), 1.75 (1H, m, 4-H), 1.25 (30H, m, 5-~19-H_2), 0.88 (3H, t, J=6.3 Hz, 20-H_3). FAB-MS m/z: 700 [$(M+H)^+$], 578, 105. HRFAB-MS Calcd for $C_{43}H_{58}NO_7$: 700.4215 [$(M+H)^+$]. Found: m/z 700.4203.

21: $[\alpha]_D^{25}$ −34.36° (c 0.103, $CHCl_3$). CD λ_{ext} (EtOH) nm: 238 ($\Delta\varepsilon$ = −11.0), 222 (−3.01). IR ν_{max} ($CHCl_3$) cm^{-1}: 3395, 3000, 2910, 2840, 1725, 1665, 1265, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.08~7.38 (16H, m, NH and aromatic H), 5.93 (1H, dd, J=9.2 and 4.6 Hz, 3-H), 5.17 (2H, s, 21-H_2), 3.85 (3H, s, OMe), 1.88 (2H, m, 4-H_2), 1.25 (30H, m, 5-~19-H_2), 0.88 (3H, t, J=6.3 Hz, 20-H_3), FAB-MS m/z: 700 [$(M+H)^+$], 578, 105. HRFAB-MS Calcd for $C_{43}H_{58}NO_7$: 700.4215 [$(M+H)^+$]. Found: m/z 700.4216.

N,*O*,*O*'-Tribenzoyl Methyl Esters of **2**, **3**, **4** and **5** (**22**, **23**, **24** and **25**)

Excess ethereal diazomethane was added to a solution of **2** (29.4 mg), **3** (10 mg), **4** (22.4 mg) or **5** (10 mg) in MeOH (50 ml) at 0°C and the mixture was kept standing at room temperature for 15 minutes. The solvent was evaporated off and the residue was purified by preparative TLC [solvent, $CHCl_3$-MeOH (9:1)] to give the corresponding methyl ester (27.4 mg, 3.8 mg, 19.1 mg or 4.6 mg) of **2**, **3**, **4** or **5**, respectively, as an oil.

By a procedure similar to that used for the preparation of **12**, **13**, **20** and **21**, **22** (14.4 mg), **23** (9.4 mg), **24** (3.2 mg)

and **25** (3.7 mg) were obtained as oils in 72.7%, 69.8%, 43.4% and 63.1% yields from the above methyl esters (10 mg, 3.8 mg, 10 mg and 4.6 mg) of **2**, **3**, **4** and **5**, respectively.

22: $[\alpha]_D^{25}$ +38.60° (*c* 0.251, $CHCl_3$). CD λ_{ext} (EtOH) nm: 240 ($\Delta\varepsilon$=10.5), 223 (−3.69). IR ν_{max} ($CHCl_3$) cm^{-1}: 3395, 3000, 2910, 2840, 1725, 1665, 1265, 970, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.09~7.37 (16H, m, NH and aromatic H), 5.91 (1H, dd, *J*=8.9 and 4.0 Hz, 3-H), 5.31 (2H, m, 6- and 7-H) 5.17 (1H, d, *J*=11.7 Hz, 21-H), 5.15 (1H, d, *J*=11.7 Hz, 21-H), 3.85 (3H, s, OMe), 2.37 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 2.36 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 2.12 (1H, m, 5-H), 1.98 (3H, m, 5-H and 8-H_2), 1.81 (2H, m, 4-H_2), 1.53 (4H, m, 12- and 16-H_2), 1.27 (12H, m, 9-~11- and 17-~19-H_2), 0.88 (3H, t, *J*=6.3 Hz, 20-H_3). FAB-MS *m/z*: 712 [$(M+H)^+$], 590, 105. HRFAB-MS Calcd for $C_{43}H_{54}NO_8$: 712.3851 [$(M+H)^+$]. Found: *m/z* 712.3860.

23: $[\alpha]_D^{25}$ +37.61° (*c* 0.031, $CHCl_3$). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$=10.5), 221 (−3.41). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3000, 2910, 2845, 1725, 1665, 1265, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.08~7.36 (16H, m, NH and aromatic H), 5.94 (1H, dd, *J*=9.6 and 4.3 Hz, 3-H), 5.18 (1H, d, *J*=11.9 Hz, 21-H), 5.16 (1H, d, *J*=11.9 Hz, 21-H), 3.85 (3H, s, OMe), 2.37 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 2.36 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 1.88 (2H, m, 4-H_2), 1.53 (4H, m, 12- and 16-H_2), 1.26 (20H, m, 5-~11- and 17-~19-H_2), 0.88 (3H, t, *J*=6.3 Hz, 20-H_3). FAB-MS *m/z*: 714 [$(M+H)^+$]. HRFAB-MS Calcd for $C_{43}H_{56}NO_8$: 714.4008 [$(M+H)^+$]. Found: *m/z* 714.4020.

24: $[\alpha]_D^{25}$ +6.02° (*c* 0.138, $CHCl_3$). CD λ_{ext} (EtOH) nm: 241 ($\Delta\varepsilon$=−3.46), 226 (3.33). IR ν_{max} ($CHCl_3$) cm^{-1}: 3390, 3000, 2910, 2840, 1720, 1670, 1265, 965, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 7.99~7.17 (16H, m, NH and aromatic H), 5.81 (1H, dd, *J*=10.2 and 2.3 Hz, 3-H), 5.38 (1H, d, *J*=11.5 Hz, 21-H), 5.33 (2H, m, 6-, 7-H), 5.01 (1H, d, *J*=11.5 Hz, 21-H), 3.86 (3H, s, OMe), 2.37 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 2.35 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 2.07 (3H, m, 5-H and 8-H_2), 1.87 (3H, m, 4-H_2 and 5-H), 1.53 (4H, m, 12- and 16-H_2), 1.26 (12H, m, 9-~11- and 17-~19-H_2), 0.87 (3H, t, *J*=6.6 Hz, 20-H_3). FAB-MS *m/z*: 712 [$(M+H)^+$], 590, 105. HRFAB-MS Calcd for $C_{43}H_{54}NO_8$: 712.3851 [$(M+H)^+$]. Found: *m/z* 712.3839.

25: $[\alpha]_D^{25}$ +5.78° (*c* 0.054, $CHCl_3$). CD λ_{ext} (EtOH) nm: 239 ($\Delta\varepsilon$=−3.25), 226 (2.84). IR ν_{max} ($CHCl_3$) cm^{-1}: 3390, 3000, 2910, 2840, 1720, 1670, 1265, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 7.99~7.17 (16H, m, NH and aromatic H), 5.85 (1H, dd, *J*=10.9 and 2.6 Hz, 3-H), 5.39 (1H, d, *J*=11.5 Hz, 21-H), 5.01 (1H, d, *J*=11.5 Hz, 21-H), 3.87 (3H, s, OMe), 2.37 (2H, t, *J*=7.6 Hz, 13- or 15-H_2), 2.35 (2H, t, *J*=7.3 Hz, 13- or 15-H_2), 1.90 (1H, m, 4-H), 1.74 (1H, m, 4-H), 1.55 (4H, m, 12- and 16-H_2), 1.20 (12H, m, 5-~11- and 17-~19-H_2), 0.87 (3H, t, *J*=6.6 Hz, 20-H_3). FAB-MS *m/z*: 714 [$(M+H)^+$]. HRFAB-MS Calcd for $C_{43}H_{56}NO_8$: 714.4008 [$(M+H)^+$]. Found: *m/z* 714.4001.

(*R*)-MTPA Ester of **8** (**26**)

N,*N*-Dimethylaminopyridine (7.3 mg), Et_3N (3.1 μl) and (*S*)-(+)-MTPA chloride (5.6 μl) were added to a solution of **8** (7.3 mg) in dry CH_2Cl_2 (0.5 ml), and the mixture was stirred at room temperature for 4 hours. 3-(Dimethylamino)propylamine (3.8 μl) was added to the mixture, then the solvent was evaporated off. The residue was purified by preparative TLC [solvent, *n*-hexane-EtOAc (8:2)] to give **26** as an oil (7.1 mg, 66.0%). IR ν_{max} ($CHCl_3$) cm^{-1}: 2910, 2840, 1755, 1675, 1220, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.51, 8.06 (1H, 2s, CHO), 7.53~7.51 (2H, m, aromatic H), 7.43~7.41 (3H, m, aromatic H), 6.37, 5.67 (1H, m and dd, *J*=10.0 and 2.3 Hz, 1′-H), 5.33, 4.34 (1H, 2s, 2-H), 4.44 (1H, d, *J*=9.9 Hz, 5-H), 4.02 (1H, d, *J*=9.9 Hz, 5-H), 3.75, 3.72 (3H, 2s, COOMe), 3.48, 3.41 (3H, 2q, *J*=1.3 Hz, OMe), 1.59 (1H, m, 2′-H), 1.40 (1H, m, 2′-H), 1.26 (30H, m, 3′-~17′-H_2), 0.93, 0.89 (9H, 2s, *t*-Bu), 0.88 (3H, t, *J*=6.9 Hz, 18′-H_3). FAB-MS *m/z*: 700 [$(M+H)^+$], 670, 614, 380, 189. HRFAB-MS Calcd for $C_{38}H_{61}NO_7F_3$: 700.4402 [$(M+H)^+$]. Found: *m/z* 700.4396.

(*S*)-MTPA Ester of **8** (**27**)

By a procedure similar to that used for the preparation of **26**, **27** (6.1 mg) was obtained as an oil in 58.1% yield from **8** (7.3 mg) and (*R*)-(−)-MTPA chloride (5.6 μl). IR ν_{max} ($CHCl_3$) cm^{-1}: 2910, 2840, 1755, 1675, 1230, 710. ^1H NMR ($CDCl_3$, 270 MHz) δ: 8.50, 7.90 (1H, 2s, CHO), 7.53~7.52 (2H, m, aromatic H), 7.43~7.39 (3H, m, aromatic H), 6.37, 5.68 (1H, m and dd, *J*=9.9 and 2.3 Hz, 1′-H), 5.35, 4.26 (1H, 2s, 2-H), 4.42, 4.40 (1H, 2d, *J*=9.9 Hz, 5-H), 4.08, 4.03 (1H, 2d, *J*=9.9 Hz, 5-H), 3.75, 3.58 (3H, 2s, COOMe), 3.62, 3.52 (3H, 2q, *J*=1.3 Hz, OMe), 1.65 (1H, m, 2′-H), 1.47 (1H, m, 2′-H), 1.26 (30H, m, 3′-~17′-H_2), 0.92, 0.84 (9H, 2s, *t*-Bu), 0.88 (3H, t, *J*=6.9 Hz, 18′-H_3). FAB-MS *m/z*: 700 [$(M+H)^+$], 670, 614, 380, 189. HRFAB-MS Calcd for $C_{38}H_{61}NO_7F_3$: 700.4402 [$(M+H)^+$]. Found: *m/z* 700.4415.

(*R*)-MTPA Ester of *N*,21-*O*-Diacetyl Mycestericin E Methyl Ester (**28**)

Pyridine (1.0 ml) and acetic anhydride (1.0 ml) were added to a solution of methyl ester of **4** (20.0 mg) in CH_2Cl_2 (1.5 ml) at 0°C, and the mixture was kept standing at 0°C for 3 hours. Ice-water was added and the whole was extracted with $CHCl_3$. The organic solution was washed and concentrated to give an oily residue. The residue was purified by preparative TLC [solvent, $CHCl_3$-MeOH (97:3)] to give the *N*,21-*O*-diacetyl compound as an oil (9.4 mg, 38.8%).

By a procedure similar to that used for the preparation of **26**, **28** (3.1 mg) was obtained as an oil in 42.8% yield from the diacetyl compound (5.0 mg). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3000, 2910, 2845, 1745, 1715, 1685, 1270 (sh), 1220, 715. ^1H NMR ($CDCl_3$, 270 MHz) δ: 7.58~7.57 (2H, m, aromatic H), 7.44~7.41 (3H, m, aromatic

H), 6.19 (1H, br s, NH), 5.74 (1H, dd, J=9.7 and 2.5 Hz, 3-H), 5.36 (1H, dt, J=15.3 and 6.6 Hz, 7-H), 5.26 (1H, dt, J=15.3 and 6.4 Hz, 6-H), 4.93 (1H, d, J=11.3 Hz, 21-H), 4.48 (1H, d, J=11.3 Hz, 21-H), 3.72 (3H, s, COOMe), 3.51 (3H, s, OMe), 2.37 (4H, t, J=7.4 Hz, 13- and 15-H_2), 1.99 (3H, s, Ac), 1.96 (3H, s, Ac), 1.94 (2H, m, 8-H_2), 1.93 (2H, m, 5-H_2), 1.78 (1H, m, 4-H), 1.65 (1H, m, 4-H), 1.56 (4H, qui, J=7.2 Hz, 12- and 16-H_2), 1.32 (2H, m, 9-H_2), 1.28 (10H, m, 10-, 11- and 17-~19-H_2), 0.88 (3H, t, J=7.0 Hz, 20-H_2). FAB-MS m/z: 700 [$(M+H)^+$], 640, 466, 189, 43. HRFAB-MS Calcd for $C_{36}H_{53}NO_9F_3$: 700.3674 [$(M+H)^+$]. Found: m/z 700.3663.

(*S*)-MTPA Ester of *N*,21-*O*-Diacetyl Mycestericin E Methyl Ester (**29**)

By a procedure similar to that used for the preparation of **28**, **29** (3.9 mg) was obtained as an oil in 58.1% yield from *N*,21-*O*-diacetyl mycestericin E methyl ester (5.0 mg) and (*R*)-(−)-MTPA chloride (5.6 μl). IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3000, 2910, 2850, 1745, 1710, 1685, 1250 (sh), 1220, 720. ^1H NMR ($CDCl_3$, 270 MHz) δ: 7.57~7.56 (2H, m, aromatic H), 7.43~7.41 (3H, m, aromatic H), 6.09 (1H, br s, NH), 5.73 (1H, dd, J=9.4 and 2.6 Hz, 3-H), 5.38 (1H, dt, J=15.3 and 6.5 Hz, 7-H), 5.29 (1H, dt, J=15.3 and 6.6 Hz, 6-H), 4.88 (1H, d, J=11.4 Hz, 21-H), 4.45 (1H, d, J=11.4 Hz, 21-H), 3.72 (3H, s, COOMe), 3.55 (3H, s, OMe), 2.37 (4H, t, J=7.4Hz, 13- and 15-H_2), 1.99 (1H, m, 5-H), 1.96 (3H, s, Ac), 1.95 (2H, m, 8-H_2), 1.94 (1H, m, 5-H), 1.92 (3H, s, Ac), 1.82 (1H, m, 4-H), 1.71 (1H, m, 4-H), 1.56 (4H, qui, J=7.3 Hz, 12- and 16-H_2), 1.33 (2H, m, 9-H_2), 1.28 (10H, m, 10-, 11- and 17-~19-H_2), 0.88 (3H, t, J=6.8 Hz, 20-H_3). FAB-MS m/z: 700 [$(M+H)^+$], 640, 466, 189, 43. HRFAB-MS Calcd for $C_{36}H_{53}NO_9F_3$: 700.3674 [$(M+H)^+$]. Found: m/z 700.3681.

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