Absolute Structural Determination of Stevastelin B

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Stevastelin B, obtained from a culture of a *Penicillium* sp. NK374186, is a novel depsipeptide containing three amino acids and 3,5-dihydroxy-2,4-dimethylstearic acid. The stereochemistry of the three amino acids was determined by HPLC analysis, and the relative configuration of the 3,5-dihydroxy-2,4-dimethylstearic acid was elucidated by chemical conversion and NMR analysis. The absolute stereochemistry of stevastelin B was determined by synthetic methods.

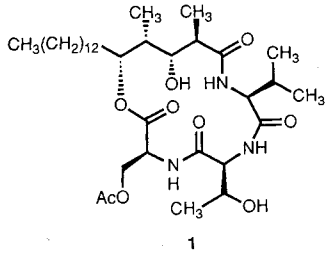
Stevastelin B (**1**)[1] (Fig. 1) is a novel depsipeptide, obtained from a culture of a *Penicillium* sp. NK374186 as the most abundant compound among stevastelins. Stevastelin B contains four components, namely valine, threonine, *O*-acetylserine, and 3,5-dihydroxy-2,4-dimethylstearic acid.

The planar structure of stevastelin B was reported in the previous paper[2]. In this report, we describe the determination of absolute stereochemistry of the amino acid components by HPLC analysis, and the 3,5-dihydroxy-2,4-dimethylstearic acid moiety by NMR analysis and synthetic methods.

Absolute Stereochemistry of Three Amino Acids

The amino acids obtained by the acid hydrolysis of stevastelin B were subjected to HPLC analysis in the usual way, by use of a MIC GEL CRS 10W column to determine the stereochemistry. The results showed that all three amino acids were L-form. That is, the three amino acids which constitute stevastelin B are L-valine, L-threonine, and L-*O*-acetylserine.

Fig. 1. The structure of stevastelin B (**1**).



Elucidation of the Relative Configuration of the Fatty Acid Moiety

Complete reduction of stevastelin B (**1**) with $LiBH_4$ yielded triol **2**. The optical rotation of **2** was $+14.2°$. To elucidate the relative stereochemistry of the fatty acid moiety, triol **2** was converted into two derivatives **3** and **4** (Scheme 1). At first, the triol (**2**) was treated with benzoyl chloride in pyridine, then the resulting monobenzoate was treated with 2,2-dimethoxypropane to give **3**. On the other hand, treatment of the triol **2** with 2,2-dimethoxypropane, followed by benzoyl chloride in pyridine gave **4**.

NMR analysis was performed on **3** and **4** (Fig. 2). The signals of the three acetonide carbons of **3** appeared at δ 19.5, 30.0 and 98.9 ppm in the ^{13}C NMR spectrum. The chemical shifts suggested that H-3 and H-5 had a *syn* relationship according to EVANS' report[3]. Then, *cis* relationships between H-3 and H-4, H-4 and H-5 were elucidated from the observed NOE between H-3 and H-4,

Scheme 1.

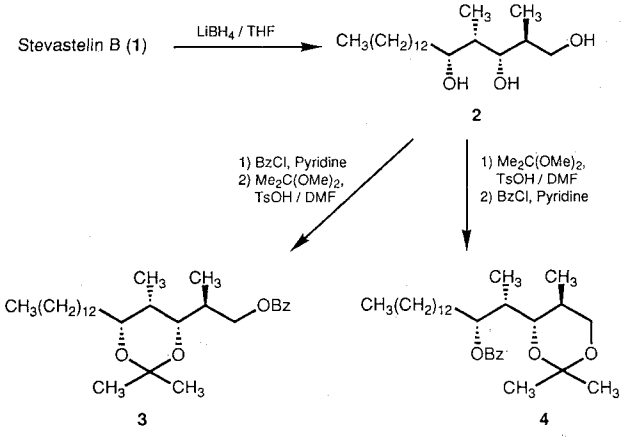
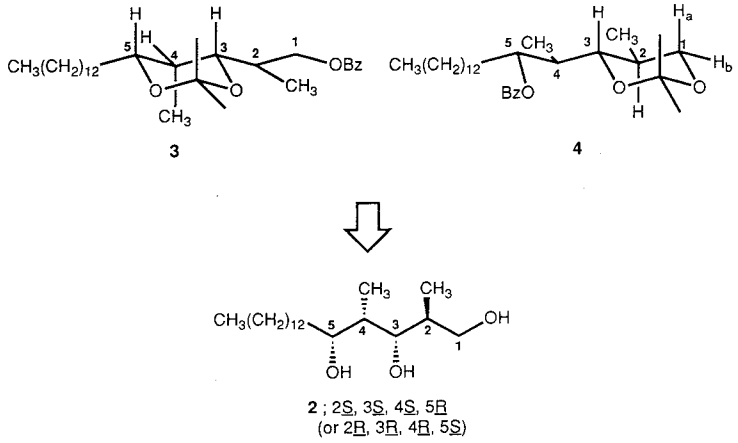
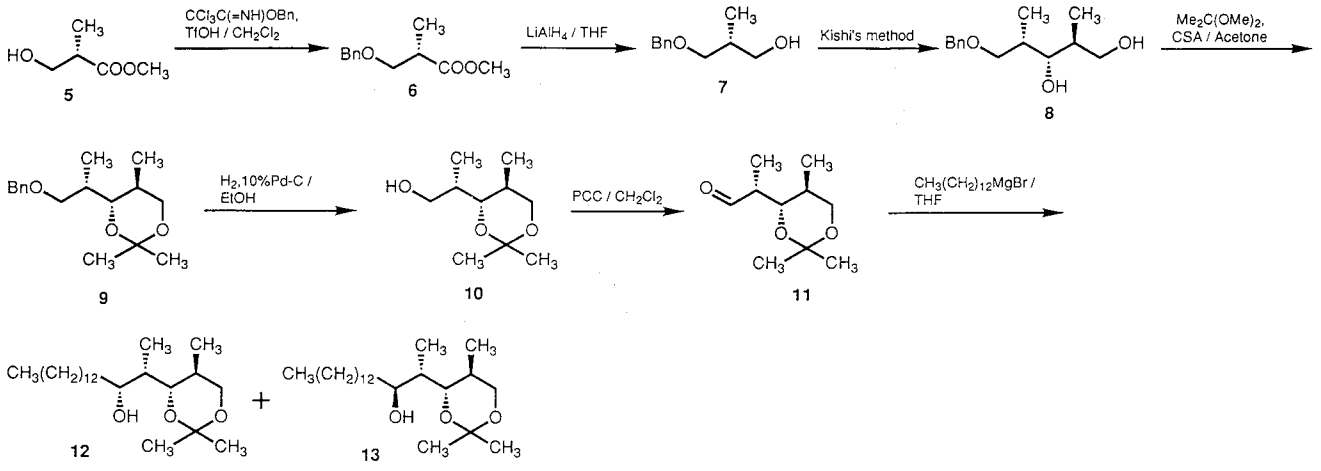


Fig. 2. Relative configurations of fatty acid moiety of stevastelin B.



Scheme 2.



H-4 and H-5, and small coupling constants between H-3 and H-4 (2.0 Hz), H-4 and H-5 (3.0 Hz).

The coupling constant between H-2 and H-3 of **4** was shown to be 10.0 Hz, indicating a *trans*-diaxial relationship of these two protons.

The results showed that two possible configurations of the fatty acid moiety are represented as (2*S*, 3*S*, 4*S*, 5*R*) (isomer **2**) or (2*R*, 3*R*, 4*R*, 5*S*) (Fig. 2).

Synthesis of Triol **2**

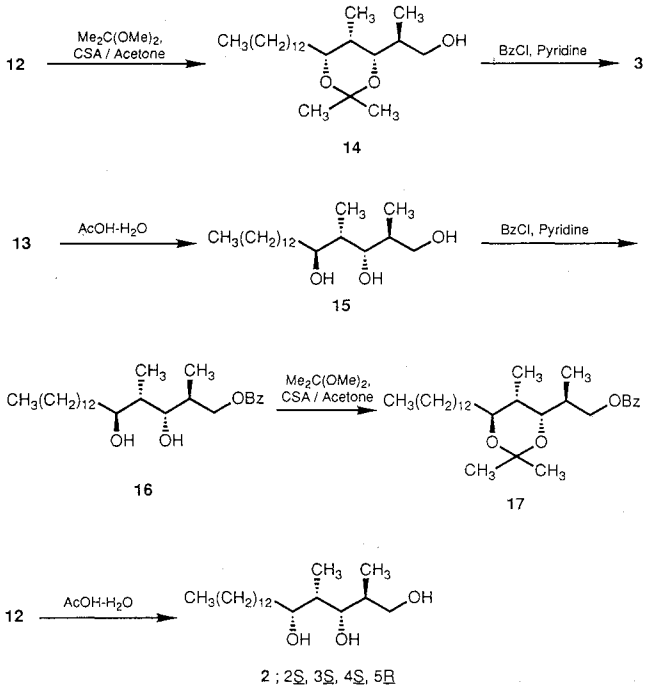
In order to determine the absolute structure of stevastelin B, the (2*S*, 3*S*, 4*S*, 5*R*) isomer (**2**) was synthesized (Scheme 2).

The hydroxyl group of methyl (2*S*)-3-hydroxy-2-methylpropionate (**5**) was protected as a benzyl ether (**6**) in 97% yield by use of benzyl trichloroacetimidate. $LiAlH_4$ reduction of **6** gave alcohol **7** in 62% yield. Then **7** was converted into monoprotected triol **8** as an optically pure form by KISHI's method[4].

The diol moiety of **8** was protected as an acetonide (**9**) in 97% yield. Catalytic deprotection of **9** in the presence of Pd-C under atmospheric hydrogen gave alcohol **10** in 91% yield. Pyridinium chlorochromate (PCC) oxidation of **10** provided aldehyde **11** in 94% yield. The Grignard reaction of the aldehyde **11** with $CH_3(CH_2)_{12}MgBr$ in THF was executed and compounds **12** and **13** were isolated in 49% and 18% yields, respectively, after separation on silica gel.

The configurations of the newly introduced asymmetric center in the two products (**12** and **13**) were established as follows (Scheme 3). The major product (**12**) was treated with 2,2-dimethoxypropane and CSA in acetone to give isopropylidene-migrated product **14** in 53% yield along with the recovered starting material **12** (25%). The hydroxyl group of **14** was protected as a benzoyl ester in 99% yield. From comparison between

Scheme 3.



the NMR data, the configuration of the benzoyl ester was identical with **3**.

On the other hand, the minor product (**13**) was treated with 70% aq. AcOH to give triol **15** in 82% yield. The primary hydroxyl group in **15** was selectively protected as a benzoyl ester **16** in 53% yield with the recovered starting material **15** (32%).

Compound **16** was treated with 2,2-dimethoxypropane and CSA in acetone to give **17** in 99% yield. The signals of three acetonide carbons of **17** appeared at δ 23.6, 25.0 and 100.8 ppm in the ^{13}C NMR spectrum. The fact suggested that the six-membered ring of **17** made a twist boat form because of comparison with the ^{13}C NMR data of EVANS' report[3].

Absolute Structure of Stevastelin B

Compound **12** was treated with 70% aq. AcOH to give triol in 91% yield. From comparison between the NMR data, the triol was identical with **2**. Optical rotation of **2** was $+14.1°$ in this case.

Based upon these experiments, the absolute stereochemistry of 3,5-dihydroxy-2,4-dimethylstearic acid moiety was decided to be **2** (2*S*, 3*S*, 4*S*, 5*R*) isomer, and the absolute stereochemistry of stevastelin B was determined (Fig. 1).

Table 1. Retention times and flow rate of amino acids.

Mobile phase	Flow rate (ml / min)	Amino acid	Retention time (min)
I	0.5	L-Serine	10.3
		D-Serine	8.2
I	0.5	L-Threonine	11.5
		D-Threonine	9.1
II	1.0	L-Valine	12.1
		D-Valine	6.4

Experimental

General Procedure

TLC was carried out with Silica gel 60 plates (Merck). Silica gel column chromatography was performed with Silica gel BW-200 (Fuji Silysia Kagaku). ^1H and ^{13}C NMR spectra were recorded with a JEOL JNM-GX400 NMR spectrometer and a Varian GEMINI-200 NMR spectrometer. Mass spectra were recorded with a VG MM-ZAB HF mass spectrometer. Optical rotation was determined with a Perkin-Elmer 241 polarimeter.

HPLC Examination Using Chiral Column

A chiral column MIC GEL CRS 10W (4.6 × 50 mm, Mitsubishi Chemical Industries Limited) was used. The mobile phase used was: I: 0.1 mM $CuSO_4$, II: 0.5 mM $CuSO_4$. HPLC was carried out under a flow rate of 0.5 ml/minute or 1.0 ml/minute, and monitored by optical density 254 nm.

The retention times and flow rate are listed in Table 1.

The configuration of these amino acids was determined to be L-threonine, L-serine and L-valine.

Triol 2 by Reduction of Stevastelin B (1)

To a solution of **1** (202 mg, 308 μmol) in THF (6 ml) was added $LiBH_4$ (35 mg) and the mixture was stirred under reflux for 2 hours. The reaction mixture was treated with a small amount of water to remove excess reagent. After evaporation of the mixture, the residue was chromatographed on a silica gel column ($CHCl_3$-MeOH) to give **2** (37.0 mg, 112 μmol, 36%). FAB-MS *m/z* 331 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.75 (3H, d, $J=6.9$ Hz), 0.88 (3H, t, $J=6.6$ Hz), 0.92 (3H, d, $J=7.1$ Hz), 1.16~1.68 (25H, m), 1.82~1.98 (1H, m), 2.77 (1H, br-d), 3.32 (1H, dd, $J=3.7$ and 5.9 Hz), 3.59~3.92 (4H, m), 4.36 (1H, s). $[\alpha]_D^{20}$ $+14.2°$ (*c* 1.00, $CHCl_3$).

Triol Acetonide Benzoyl Ester 3 from Triol 2

To a solution of triol **2** (5.1 mg, 15 μmol) in pyridine (1 ml) was added benzoyl chloride (4.2 μl) and the mixture was stirred at 60°C for 5 hours. The reaction mixture was treated with a small amount of water to remove excess reagent. After evaporation of the mixture, the

residue was chromatographed on a silica gel column (hexane-acetone) to give monobenzoate (4.9 mg, 11 μmol). To a solution of this monobenzoate (4.9 mg, 11 μmol) in DMF (1 ml) was added 2,2-dimethoxypropane (20 μl) and *p*-toluenesulfonic acid monohydrate (0.3 mg), and the mixture was stirred at room temperature for 3 days. After quenching with saturated aq. $NaHCO_3$ at 0°C, H_2O was added and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **3** (4.2 mg, 8.8 μmol, 59% from **2**). FAB-MS *m/z* 475 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.87 (3H, d, $J=7.3$ Hz), 0.88 (3H, t, $J=7.0$ Hz), 0.98 (3H, d, $J=7.0$ Hz), 1.17~1.60 (28H, m), 1.37 (3H, s), 2.02~2.12 (1H, m), 3.73 (1H, dd, $J=2.0$ and 10.3 Hz), 3.80~3.87 (1H, m), 4.33 (1H, dd, $J=5.9$ and 10.6 Hz), 4.42 (1H, dd, $J=2.9$ and 10.6 Hz), 7.41~7.59 (3H, m), 8.03~8.19 (2H, m). ^{13}C NMR ($CDCl_3$) δ 4.4, 12.5, 14.1, 19.5, 22.7, 25.4, 29.4, 29.5, 29.7, 30.0, 31.9, 32.4, 32.9, 34.5, 67.0, 73.6, 74.1, 98.9, 128.4, 129.5, 130.6, 132.8, 166.7.

Triol Acetonide Benzoyl Ester **4** from Triol **2**

To a solution of triol **2** (4.5 mg, 14 μmol) in DMF (1 ml) was added 2,2-dimethoxypropane (40 μl) and *p*-toluenesulfonic acid monohydrate (0.5 mg), and the mixture was stirred at room temperature for 15 minutes. After quenching with saturated aq. $NaHCO_3$ at 0°C, H_2O was added and extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give the acetonide. To a solution of this acetonide in pyridine (1 ml) was added benzoyl chloride (10 μl) and the mixture was stirred at 60°C for 4 hours. The reaction mixture was treated with water to remove excess reagent. After evaporation of the mixture, the residue was chromatographed on a silica gel column (hexane-acetone) to give **4** (3.7 mg, 7.8 μmol, 56% from **2**). FAB-MS *m/z* 475 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.70 (3H, d, $J=6.7$ Hz), 0.88 (3H, t, $J=7.0$ Hz), 1.02 (3H, d, $J=7.0$ Hz), 1.12 (3H, s), 1.20~1.45 (24H, m), 1.36 (3H, s), 1.82~1.92 (1H, m), 1.94~2.02 (1H, m), 3.50 (1H, t, $J=11.2$ Hz), 3.66 (1H, dd, $J=11.2$ and 3.1 Hz), 3.68 (1H, dd, $J=10.2$ and 2.0 Hz), 5.26 (1H, q, $J=5.8$ Hz), 7.37~7.56 (3H, m), 8.03~8.09 (2H, m).

Benzyl Ether **6** from Propionate **5**

To a solution of propionate **5** (7.02 g, 59.5 mmol) and benzyl trichloroacetimidate (22.5 g) in CH_2Cl_2 was added TfOH (0.260 ml) at 0°C. After being stirred at room temperature for 2 hours, the reaction mixture was quenched with saturated aq. $NaHCO_3$. The mixture was extracted with EtOAc, and the organic layer was washed with H_2O, then brine, dried over Na_2SO_4, and evaporated. To the residue, hexane and $CHCl_3$ was added and the precipitated white crystal was removed by filtration. After evaporation of the filtrate, the residue was chromatographed on a silica gel column (hexane-acetone) to give **6** (12.0 g, 57.7 mmol, 97%). FAB-MS *m/z* 209 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 1.18 (3H, d, $J=7.1$ Hz), 2.71~2.88 (1H, m), 3.49 (1H, dd, $J=5.9$ and 9.1 Hz), 3.66 (1H, dd, $J=7.3$ and 9.1 Hz), 3.69 (3H, s), 4.52 (3H, s), 7.32 (5H, s).

Diol Monobenzyl Ether **7** from Benzyl Ether **6**

To a stirred suspension of $LiAlH_4$ (3.46 g) in THF (120 ml) was added a solution of **6** (12.0 g, 57.7 mmol) in THF (70 ml) dropwise at 0°C. After being stirred at room temperature for 1 hour, the reaction mixture was cooled to 0°C and quenched with H_2O (3.5 ml), 15% aq. NaOH (3.5 ml), then H_2O (11.5 ml). The resulting gels were removed by filtration and washed with THF. The combined filtrate and washings were dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **7** (6.46 g, 35.8 mmol, 62%). FAB-MS *m/z* 181 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.88 (3H, d, $J=7.0$ Hz), 1.97~2.19 (1H, m), 2.58 (1H, br), 3.42 (1H, dd, $J=8.0$ and 9.1 Hz), 3.55~3.66 (2H, br), 3.56 (1H, dd, $J=4.7$ and 9.1 Hz), 4.52 (2H, s), 7.33 (5H, s). $[\alpha]_D^{20}$ +16.6° (*c* 0.61, $CHCl_3$).

Triol Acetonide Benzyl Ether **9** from Triol Monobenzyl Ether **8**

To a solution of **8**[4] (60.6 mg, 0.255 mmol) in acetone (3 ml) was added 2,2-dimethoxypropane (313 μl) and CSA (5.9 mg), and the mixture was stirred at room temperature for 3 hours. After quenching with saturated aq. $NaHCO_3$ at 0°C, H_2O was added and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **9** (68.6 mg, 0.247 mmol, 97%). FAB-MS *m/z* 279 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.72 (3H, d, $J=7.0$ Hz), 0.88 (3H, d, $J=7.0$ Hz), 1.34 (3H, s), 1.40 (3H, s), 1.76~1.96 (1H, m), 1.97~2.14 (1H, m), 3.30 (1H, dd, $J=6.2$ and 8.9 Hz), 3.44~3.57 (2H, m), 3.66~3.75 (2H, m), 4.50 (2H, s), 7.33 (5H, s).

Triol Acetonide **10** from Triol Acetonide Benzyl Ether **9**

A solution of **9** (68.6 mg, 0.247 mmol) in EtOH (5 ml) was hydrogenated in the presence of 10% Pd-C catalyst under an atmosphere of hydrogen for 18 hours. The catalyst was removed through a Celite pad, and washed with EtOH. The combined filtrate and washings were evaporated and the residue was chromatographed on a silica gel column (hexane-acetone) to give **10** (42.4 mg, 0.226 mmol, 91%). FAB-MS *m/z* 189 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.72 (3H, d $J=6.7$ Hz), 0.99 (3H, d, $J=7.1$ Hz), 1.37 (3H, s), 1.46 (3H, s), 1.77~2.02 (2H, m), 2.38 (1H, br), 3.54 (1H, t, $J=11.2$ Hz), 3.56~3.82 (4H, m).

Aldehyde **11** from Triol Acetonide **10**

A mixture of **10** (40.6 mg, 0.216 mmol) and PCC (140 mg) in CH_2Cl_2 (2 ml) was stirred at room temperature for 2 hours. After addition of Et_2O (3 ml), the

whole mixture was chromatographed on a silica gel column (Et_2O) to give **11** (37.5 mg, 0.202 mmol, 94%). FAB-MS *m/z* 187 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.77 (3H, d, $J=6.7$ Hz), 1.15 (3H, d, $J=7.1$ Hz), 1.33 (3H, s), 1.43 (3H, s), 1.74~2.03 (1H, m), 2.47 (1H, dq, $J=2.6$ and 7.1 Hz), 3.59 (1H, t, $J=11.2$ Hz), 3.75 (1H, dd, $J=5.2$ and 11.2 Hz), 4.12 (1H, dd, $J=2.6$ and 10.3 Hz), 9.66 (1H, s).

Triol Acetonide **12** and **13** from Aldehyde **11**

A mixture of Mg (16.7 mg) and $CH_3(CH_2)_{12}Br$ (160 mg) in THF (3 ml) was stirred at 60°C under Ar atmosphere. After 2 hours, a suspension of **11** (37.5 mg, 0.202 mmol) and molecular sieves (4A, powder) in THF (2 ml) was added dropwise at room temperature, and the mixture was stirred 45 minutes. After quenching with saturated aq. NH_4Cl at 0°C, the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **12** (36.9 mg, 99.5 μmol, 49%) and **13** (13.3 mg, 35.8 μmol, 18%). **12**: FAB-MS *m/z* 371 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.71 (3H, d, $J=6.6$ Hz), 0.88 (3H, t, $J=6.8$ Hz), 0.93 (3H, d, $J=7.0$ Hz), 1.17~1.77 (25H, m), 1.37 (3H, s), 1.48 (3H, s), 1.78~2.04 (1H, m), 3.41 (1H, s), 3.53 (1H, t, $J=11.3$ Hz), 3.66~3.82 (3H, m). $[\alpha]_D^{20}$ +36.8° (*c* 1.00, $CHCl_3$). **13**: FAB-MS *m/z* 371 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.70 (3H, d, $J=6.7$ Hz), 0.88 (3H, t, $J=6.8$ Hz), 1.05 (3H, d, $J=7.1$ Hz), 1.18~1.60 (24H, m), 1.37 (3H, s), 1.48 (3H, s), 1.66~1.76 (1H, m), 1.82~2.06 (1H, m), 2.82 (1H, d, $J=8.1$ Hz), 3.44~3.57 (1H, m), 3.54 (1H, t, $J=11.5$ Hz), 3.71 (1H, dd, $J=5.1$ and 11.5 Hz), 3.94 (1H, dd, $J=2.4$ and 10.3 Hz). $[\alpha]_D^{20}$ +30.6° (*c* 0.50, $CHCl_3$).

Triol **2** from Triol Acetonide **12**

Compound **12** (15.0 mg, 40.4 μmol) in 70% aq. AcOH (1 ml) was stirred at room temperature for 20 hours. After evaporation of the mixture, the residue was chromatographed on a silica gel column (hexane-acetone) to give **2** (12.1 mg, 36.6 μmol, 91%). $[\alpha]_D^{20}$ +14.1° (*c* 1.23, $CHCl_3$).

Triol Acetonide **14** from Triol Acetonide **12**

To a solution of **12** (8.4 mg, 22.7 μmol) in acetone (1 ml) was added 2,2-dimethoxypropane (27.9 μl) and CSA (0.8 mg), and the mixture was stirred at room temperature for 3 hours. After neutralization with Et_3N, the mixture was evaporated and the residue was chromatographed on a silica gel column (hexane-acetone) to give **14** (5.5 mg, 14.9 μmol, 53%) and **12** (2.1 mg, 5.66 μmol, 25%) as a starting material. **14**: FAB-MS *m/z* 371 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.76 (3H, d, $J=7.0$ Hz), 0.87 (3H, d, $J=6.8$ Hz), 0.88 (3H, t, $J=6.1$ Hz), 1.16~1.60 (25H, m), 1.39 (3H, s), 1.46 (3H, s), 1.82~2.05 (1H, m), 3.17 (1H, dd, $J=2.8$ and 8.5 Hz), 3.48~3.68 (2H, m), 3.74 (1H, dd, $J=2.1$ and 9.7 Hz), 3.80~3.89 (1H, m).

Triol Acetonide Benzoyl Ester **3** from Triol Acetonide **14**

To a solution of **14** (5.5 mg, 14.8 μmol) in pyridine (1 ml) was added benzoyl chloride (13.2 μl) and the mixture was stirred at room temperature for 15 hours. The reaction mixture was treated with water to remove excess reagent, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **3** (7.0 mg, 14.7 μmol, 99%).

Triol **15** from Triol Acetonide **13**

Compound **13** (16.2 mg, 43.7 μmol) in 70% aq. AcOH (1 ml) was stirred at room temperature for 15 hours. After evaporation of the mixture, the residue was chromatographed on a silica gel column (hexane-acetone) to give **15** (11.9 mg, 36.0 μmol, 82%). FAB-MS *m/z* 331 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.73 (3H, d, $J=6.9$ Hz), 0.88 (3H, t, $J=6.5$ Hz), 1.05 (3H, d, $J=7.1$ Hz), 1.14~1.70 (25H, m), 1.82~1.97 (1H, m), 2.59 (1H, br-d), 3.45 (1H, br), 3.60~3.74 (3H, m), 3.92~4.03 (2H, m).

Triol Monobenzoyl Ester **16** from Triol **15**

To a solution of **15** (11.9 mg, 36.0 μmol) in pyridine (0.5 ml) was added benzoyl chloride (17.8 μl) and the mixture was stirred at room temperature for 24 hours. The reaction mixture was treated with water to remove excess reagent, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **16** (8.3 mg, 19.1 μmol, 53%) and **15** (3.8 mg, 11.5 μmol, 32%) as a starting material. **16**: FAB-MS *m/z* 435 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.5$ Hz), 0.97 (3H, d, $J=6.9$ Hz), 1.04 (3H, d, $J=7.1$ Hz), 1.15~1.74 (27H, m), 1.90~2.15 (1H, m), 3.62~3.70 (1H, m), 3.86 (1H, dd, $J=1.8$ and 10.0 Hz), 4.38 (1H, dd, $J=3.7$ and 11.0 Hz), 4.60 (1H, dd, $J=5.1$ and 11.0 Hz), 7.40~7.62 (3H, m), 8.03~8.12 (2H, m).

Triol Acetonide Benzoyl Ester **17** from Triol Monobenzoyl Ester **16**

To a solution of **16** (7.9 mg, 18.2 μmol) in acetone (1 ml) was added 2,2-dimethoxypropane (22.3 μl) and CSA (0.5 mg), and the mixture was stirred at room temperature for 15 minutes. After quenching with saturated aq. $NaHCO_3$ at 0°C, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4. After evaporation, the residue was chromatographed on a silica gel column (hexane-acetone) to give **17** (8.6 mg, 18.1 μmol, 99%). FAB-MS *m/z* 475 $(M+H)^+$. ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.5$ Hz), 0.90 (3H, d, $J=6.9$ Hz), 1.01 (3H, d, $J=6.7$ Hz), 1.16~1.56 (27H, m), 1.31 (3H, s), 1.66~1.78 (1H, m), 1.95~2.13 (1H, m), 3.17~3.28 (1H, m), 3.77 (1H, dd, $J=4.4$ and 10.9 Hz), 4.36~4.40 (2H, m), 7.39~7.61 (3H, m), 8.03~8.09 (2H, m). ^{13}C NMR ($CDCl_3$) δ 11.6, 13.2,

14.1, 22.7, 23.6, 25.0, 26.1, 29.4, 29.6, 29.7, 31.9, 33.0, 35.0, 38.4, 66.9, 69.6, 75.2, 100.8, 128.3, 129.5, 130.5, 132.8, 166.7. $[\alpha]_D^{20}$ $-15.0°$ (c 0.42, $CHCl_3$).

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