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Stereochemistry of Pladienolide B

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Abstract Pladienolide B is a 12-membered macrolide isolated from *Streptomyces platensis* Mer-11107. It showed potent *in vitro* and *in vivo* antitumor activities and is a potential lead for novel antitumor agents. The absolute configurations at ten chiral centers were determined on the basis of spectral data of pladienolide B and its chemical transformation products.

Keywords absolute stereochemistry, 12-membered macrolide, VEGF, antitumor activity

Introduction

Pladienolides [1~3], isolated from a culture broth of *Streptomyces platensis* Mer-11107, are structurally unique 12-membered macrolide compounds having a diene and an epoxide on its side-chain. One of the most potent compounds, pladienolide B (**1**), showed potent *in vitro* antiproliferative activities as well as *in vivo* antitumor activities [3]. By the COMPARE analysis, **1** was suggested to possess a novel mechanism of action that attracts great interest for its potential as a novel antitumor agent.

In a previous report [2], the planar structure of **1** was elucidated by extensive spectroscopic analyses, and the physico-chemical properties were described in detail. However, stereochemical configuration for its ten chiral centers has been hitherto unknown. In this report, we describe the elucidation of absolute configurations at all chiral centers of **1**.

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Results and Discussion

Relative Stereochemistry of Macrolide Moiety

Among the ten chiral carbons in **1** (Fig. 1), five of them exist in the macrolide moiety (C-3, C-6, C-7, C-10 and C-11) and another five are in the side chain moiety (C-16, C-18, C-19, C-20 and C-21). The relative stereochemistry of the macrolide moiety was determined on the basis of *J*-coupling and NOESY data of **1**. The ^{1}H- and ^{13}C-NMR spectral data of **1** were measured in pyridine-d_5 and assignments are shown in Table 1. The 1D selective TOCSY [4, 5] and homo-decoupling experiments were performed to determine coupling constants of overlapping proton signals.

Observed proton–proton vicinal coupling constants for C-2 through C-5 were typical value for the staggered conformation (10.0~13.2 Hz for anti-periplanar, 3.2~4.2 Hz for syn-gauche). NOESY spectral analysis, in conjunction with measured *J* values, led to unambiguous stereochemical assignments for all protons in this region, including diastereotopic methylenes H2$\alpha\beta$, H4$\alpha\beta$, and H5$\alpha\beta$ (Fig. 2). On the other hand, protons attached to C-7 through C-11 were all oriented anti-periplanar, as indicated

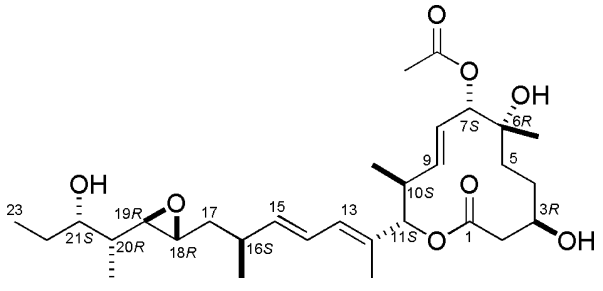


Fig. 1 Structure of pladienolide B (**1**).

Table 1 ^{1}H- and ^{13}C-NMR data for pladienolide B in pyridine-d_5[a]

Position	^{13}C (ppm)	^{1}H (δ=ppm, J=Hz)	Position	^{13}C (ppm)	^{1}H (δ=ppm, J=Hz)
1	170.2	—	18	56.2	2.86 (ddd, 6.0, 5.8, 1.8)
2	39.5	2.75 (dd, 14.0, 4.2, Hβ)	19	61.3	2.99 (dd, 8.1, 1.8)
		2.68 (dd, 14.0, 3.2, Hα)			
3	69.1	4.07 (ddddd, 10.0, 7.1, 4,2, 3.5, 3.2)	20	41.5	1.42 (ddq, 8.1, 4.4, 6.8)
4	30.1	1.93 (dddd, 13.2, 13.2, 3.5, 3.5, Hα)	21	72.9	3.95 (ddd, 8.3, 4.4, 4.0)
		1.77 (dddd, 13.2, 13.2, 10.0, 3.5, Hβ)			
5	37.0	2.08 (ddd, 13.2, 13.2, 3.5, Hβ)	22	27.9	1.73 (ddq, 13.7, 8.1, 7.3)
		1.67 (ddd, 13.2, 13.2, 3.5, Hα)			1.65 (ddq, 13.7, 4.0, 7.3)
6	72.3	—	23	10.3	1.05 (t, 7.3)
7	79.1	5.51 (d, 9.8)	6-Me	24.1	1.43 (3H, s)
8	126.7	6.24 (dd, 15.2, 9.8)	10-Me	16.0	0.84 (3H, d, 6.7)
9	139.4	5.78 (dd, 15.2, 10.0)	12-Me	11.3	1.77 (3H, br s)
10	40.2	2.64 (ddq, 10.7, 10.0, 6.7)	16-Me	20.6	1.06 (3H, d, 6.8)
11	82.3	5.30 (d, 10.7)	20-Me	10.1	1.12 (3H, d, 6.8)
12	131.3	—	Ac(CO)	169.5	—
13	130.6	6.31 (br d, 10.9)	Ac(Me)	20.4	1.93 (s)
14	124.2	6.43 (dd, 15.0, 10.9)	3-OH	—	5.54 (br d, 7.1)
15	141.1	5.70 (dd, 15.0, 8.2)	6-OH	—	6.09 (br s)
16	35.1	2.51 (dddq, 8.4, 8.2, 5.8, 6.8)	21-OH	—	5.93 (br d, 5.7)
17	39.4	1.67 (ddd, 14.0, 5.8, 5.8)			
		1.53 (ddd, 14.0, 8.4, 6.0)			

[a] Data were collected using approximately 14 mg of pladienolide B at 30°C. Chemical shifts were referenced to internal solvent peaks δ_H 8.71 (H-2) and δ_C 149.2 (C-2).

by their large coupling constants (9.8～15.2 Hz) and NOESY correlations (Fig. 2). On the basis of these torsionally fixed subfragments (C2～C5 and C7～C11), NOESY analysis of the macrolide ring was carried out. NOESY correlations were observed between H4β/H9, H4β/H7, H5β/H8, 6-CH_3/H4α, 6-CH_3/H5α, and 6-CH_3/H7, which established the macrolide ring configuration as well as the conformation as shown in Fig. 2. Thus, the stereochemistry of macrolide moiety was determined to be 3*R**, 6*R**, 7*S**, 10*S**, 11*S**.

Relative Stereochemistry of Side Chain Moiety

The epoxide ring in the side chain moiety of **1** has the *trans*-configuration 18*R**, 19*R** as readily discerned by the $J_{H18/H19}$ value of 1.8 Hz [6]. To confirm other chiral centers in the side chain, chemical degradation and derivatization were carried out as shown in Scheme 1. First, secondary alcohols of **1** were protected as *tert*-butyldimethylsilyl (TBS) ethers, and C-14/C-15 double bond was oxidatively cleaved by treatments with OsO_4 and $NaIO_4$. The resulting aldehyde **2** derived from the side chain was reduced with $NaBH_4$ to provide alcohol **3**, which was transformed to

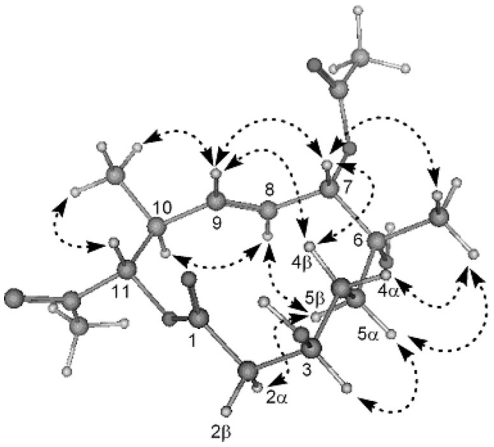
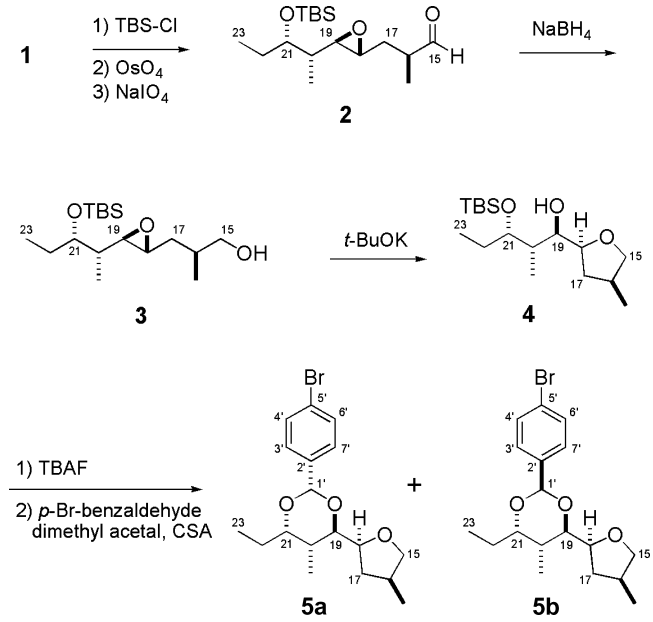


Fig. 2 Key NOESY correlations and relative stereochemistry for the macrolide moiety (C-1～C11) in **1**.

Vicinal *J*-value in Hz, H2α/H3: 3.2, H2β/H3: 4.2, H3/H4α: 3.5, H3/H4β: 10.0, H4α/H5α: 3.5, H4α/H5β: 13.2, H4β/H5α: 13.2, H4β/H5β: 3.5, H7/H8: 9.8, H8/H9: 15.2, H9/H10: 10.0, H10/H11: 10.7.



Scheme 1 Oxidative degradation and cyclization of **1**.

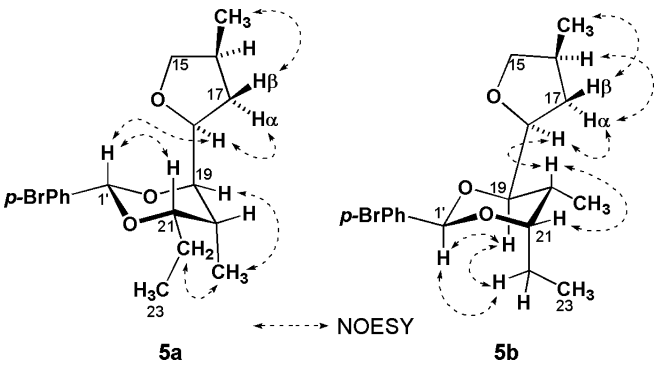


Fig. 3 NOESY correlations and relative stereochemistry for **5a** and **5b**.

tetrahydrofuran compound **4** by treatment with potassium *tert*-butoxide. In the tetrahydrofuran compound **4**, the C-18 chiral center was inverted. After removal of the TBS group, the resulting 1,3-diol compound was treated with 4-bromobenzaldehyde dimethyl acetal to provide the epimeric benzylidene acetals **5a** and **5b**. Stereochemistries of **5a** and **5b** were then evaluated by *J*-coupling and NOESY data. As shown in Fig 3, NOESY data clearly indicated the relative stereochemistry of (16*S**, 18*S**) for tetrahydrofuran ring and (19*R**, 20*R**, 21*S**) for 1,3-dioxolane ring. Since the relative configuration of C18/C19 was originally identified in **1**, relative stereochemistry of all five chiral carbons in the side-chain moiety was determined to be 16*S**, 18*R**, 19*R**, 20*R**, 21*S**.

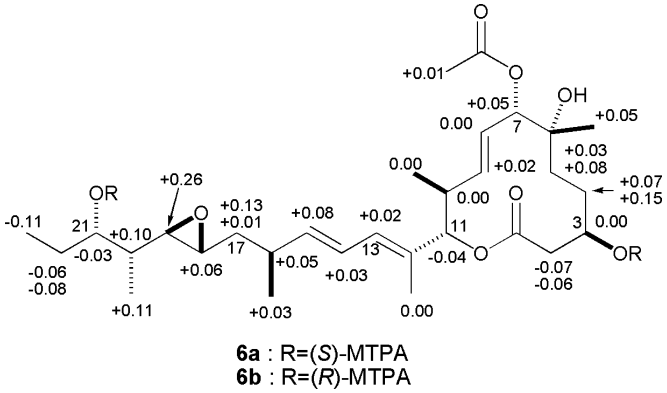


Fig. 4 $\Delta\delta$ values [δH (ppm)$=\delta_S-\delta_R$] obtained for the 3,21-bis-MTPA esters **6a** and **6b**.

Elucidation of Absolute Stereochemistry

The absolute configuration of **1** was determined by applying the modified Mosher's method [7]. The secondary alcohol groups at C-3 and C-21 of **1** were esterified with (*R*)- and (*S*)-MTPA chloride to obtain 3,21-bis-(*S*)-MTPA-ester and 3,21-bis-(*R*)-MTPA-ester, respectively. The ^{1}H-NMR chemical shift differences ($\Delta\delta$) for these diastereomeric esters are shown in Fig. 4. The observed $\Delta\delta$ values indicated the absolute stereochemistry of 3*R* and 21*S*, which led to the overall stereochemical assignments of 3*R*, 6*R*, 7*S*, 10*S*, 11*S*, 16*S*, 18*R*, 19*R*, 20*R*, 21*S*. It should be noted that two MTPA-ester groups attached at C-3 and C-21 are spatially far enough and their mutual interference is considered to be negligible. This is evidenced by very small $\Delta\delta$ values observed for the middle part of the molecule, *e.g.* H-10, H-13, 12-CH_3, 10-CH_3.

It is noteworthy that very recently Kanada *et al.* reported the total synthesis of pladienolides [8] which fully corroborated the stereochemistry proposed in this report.

Experimental

General

Electrospray ionization (ESI) mass spectra were obtained on a ThermoElectron SSQ7000 mass spectrometer. Accurate mass measurements were conducted with a Micromass Q-Tof Ultima Global spectrometer equipped with ESI ionization source. NMR measurements were performed using either a Bruker AVANCE 600, a Varian Unity INOVA 500, or a Varian Mercury 400 spectrometer. Chemical shifts were referenced to internal solvent peaks or tetramethylsilane (TMS). The ^{1}H- and ^{13}C-NMR spectral assignments were made by analyzing two dimensional NMR experiments: COSY, NOESY, TOCSY, HSQC, and

HMBC. The 1D TOCSY experiments were performed with shaped pulse-selective excitation and a spin lock mixing time of 140 ms. The NOESY spectra were obtained with mixing time of 800 ms. Purification of the reaction products was performed either on a silica gel (Silica Gel 60N, 100～210 μm, KANTO) or an NH-silica gel (Chromatorex-NH, 200～350 mesh, Fujisilysia) column chromatography. Each fraction eluted from the column was monitored by TLC (silica gel 60 F254, Merck) using Vaughn's reagent as the visualizing agent and appropriate fractions were collected.

Materials

1 was isolated from the culture broth of *Streptomyces platensis* Mer-11107 as previously described [1].

Oxidative Degradation and Cyclization of 1

To a stirred solution of **1** (crude product from fermentation broth, *ca.* 60% content in *tert*-BuOH, 2.18 g) in DMF (21.8 ml), imidazole (1.66 g, 24.4 mmol) and *tert*-butylchlorodimethylsilane (3.68 g, 24.4 mmol) were added and stirred at room temperature for 2 hours. Then the reaction mixture was partitioned between EtOAc and water. The organic layer was washed with an additional portion of water and brine, dried over anhydrous $MgSO_4$, and evaporated. The resulting residue was purified by silica gel column chromatography (heptane : EtOAc=4 : 1) to obtain 3, 21-bis(*tert*-butyldimethylsilyl) pladienolide B (1.68 g).

3,21-Bis(*tert*-butyldimethylsilyl)pladienolide B

ESI-MS *m/z* 765 $(M+H)^+$; ^{1}H-NMR (400 MHz, CD_3OD, TMS=0 ppm) δ 6.36 (1H, dd, *J*=15.0, 10.8 Hz), 6.14 (1H, d, *J*=10.8 Hz), 5.75 (1H, dd, *J*=15.0, 9.6 Hz), 5.68 (1H, dd, *J*=15.0, 8.4 Hz), 5.61 (1H, dd, *J*=15.0, 9.6 Hz), 5.07 (1H, d, *J*=9.6 Hz), 4.94 (1H, d, *J*=10.8 Hz), 3.99～3.93 (1H, m), 3.80～3.75 (1H, m), 2.77 (1H, dt, *J*=2.4, 6.0 Hz), 2.66 (1H, dd, *J*=8.4, 3.0 Hz), 2.64～2.48 (3H, m), 2.42 (1H, dd, *J*=15.0, 4.8 Hz), 2.11 (3H, s), 1.77 (3H, s), 1.75～1.67 (2H, m), 1.63～1.36 (6H, m), 1.33～1.26 (1H, m), 1.22 (3H, d, *J*=6.6 Hz), 0.96 (9H, s), 0.95 (9H, s), 0.93 (3H, d, *J*=6.8 Hz), 0.90 (3H, d, *J*=6.8 Hz), 0.38 (3H, t, *J*=7.2 Hz), 0.14 (3H, s), 0.13 (3H, s), 0.12 (6H, s).

To a stirred solution of 3,21-bis(*tert*-butyldimethylsilyl)pladienolide B (60 mg, 0.078 mmol) in THF - water (50% v/v, 2 ml), 2% solution of OsO_4 in water (0.199 ml, 0.016 mmol) and 50% aqueous solution of 4-methylmorpholine-4-oxide (0.018 ml, 0.078 mmol) were added and stirred at room temperature for 24 hours. Then sodium sulfite (19.8 mg, 0.156 mmol) was added to the solution and the mixture was stirred for additional 1 hour. The reaction mixture was diluted with EtOAc, and washed with portions of water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (heptane : EtOAc=1 : 1) to obtain the diol compound (67.6 mg). The diol compound (67.6 mg, 0.085 mmol) and sodium periodate (181 mg, 0.085 mmol) were dissolved in THF–water (50% v/v, 2 ml) and stirred at room temperature for 1 hour. The mixture was diluted with EtOAc, and washed with portions of water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (heptane : EtOAc= 20 : 1～2 : 1) to obtain macrolide derived aldehyde (36 mg, y. 88%) and side-chain derived aldehyde **2** (19.3 mg, y. 72%).

Side Chain Derived Aldehyde (**2**)

ESI(+)MS *m/z* 315 $(M+H)^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, TMS=0 ppm) δ 9.67 (1H, d, *J*=0.8 Hz), 3.76～3.67 (1H, m), 2.80～2.72 (1H, m), 2.67 (1H, dd, *J*=8.0, 2.4 Hz), 2.63～2.52 (1H, m), 2.05 (1H, ddd, *J*=13.6, 8.0, 4.4 Hz), 1.56～1.42 (3H, m), 1.39～1.28 (1H, m), 1.20 (1H, dd, *J*=7.6, 0.8 Hz), 0.89 (9H, s) 0.87 (3H, t, *J*=6.8 Hz), 0.81 (3H, t, *J*=7.6 Hz), 0.06 (6H, s).

Aldehyde **2** (0.29 g, 0.92 mmol) was dissolved in THF (3.0 ml) and the solution was stirred at room temperature. $NaBH_4$ (52 mg, 1.38 mmol) was added to the solution and the mixture was stirred for 3 hours. Aqueous NH_4Cl was added, and the mixture was stirred vigorously. Then the mixture was extracted with 2 portions of EtOAc. The combined organic layer was washed with saturated NH_4OH, dried over anhydrous $MgSO_4$, filtered, and evaporated to provide the crude alcohol **3** (0.29 g, y. quant.).

Side Chain Derived Alcohol (**3**)

ESI(+)MS *m/z* 339 $(M+Na)^+$; ^{1}H-NMR (400 MHz, CD_3OD, TMS=0 ppm), δ 3.83～3.76 (1H, m), 3.52～3.42 (2H, m), 3.37～3.33 (1H, m), 2.90～2.84 (1H, m), 2.68 (1H, dd, *J*=8.0, 2.4 Hz), 1.90～1.79 (1H, m), 1.71 (1H, td, *J*=13.6, 5.6 Hz), 1.66～1.52 (2H, m), 1.45～1.22 (2H, m), 1.04 (3H, d, *J*=6.8 Hz), 0.97 (3H, d, *J*=7.6 Hz), 0.96 (9H, s), 0.89 (3H, t, *J*=7.6 Hz), 0.13 (6H, s).

Alcohol **3** (0.29 g, 0.92 mmol) was dissolved in MeOH (6.0 ml) and the solution was stirred at room temperature. Potassium *tert*-butoxide (0.10 g, 9.2 mmol) was added to the solution, and the mixture was stirred for 48 hours. The reaction mixture was partitioned between EtOAc and water. The organic layer was washed with 2 portions of brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column

chromatography (hexane : EtOAc=6 : 1～4 : 1) to obtain cyclized **4** (0.25 g, y. 86%).

Tetrahydrofuran Compound (**4**)

ESI(+)MS *m/z* 339 $(M+Na)^+$, ^{1}H-NMR (400 MHz, $CDCl_3$, TMS=0 ppm) δ 4.00 (1H, ddd, *J*=10.0, 6.0, 4.0 Hz), 3.96～3.86 (2H, m), 3.77 (1H, ddd, *J*=9.2, 4.0, 2.0 Hz), 3.37 (1H, t, *J*=8.0 Hz), 3.25～3.20 (1H, m), 2.40～2.28 (1H, m), 2.0～1.90 (1H ,m), 1.64～1.46 (4H, m), 1.06 (3H, d, *J*=7.2 Hz), 0.89 (9H, s), 0.87 (3H, t, *J*=7.6 Hz), 0.81 (3H ,d, *J*=6.8 Hz), 0.08 (3H, s), 0.00 (3H, s).

Tetrahydrofuran compound **4** (0.24 g, 0.76 mmol) was dissolved in THF (5.0 ml) and the solution was stirred at room temperature. A 1.0 M solution of tetrabutylammonium fluoride in THF (0.84 ml, 0.84 mmol) was added dropwise and the mixture was stirred for 2 hours. The reaction mixture was evaporated and the resulting residue was partitioned between EtOAc and water. The organic layer was washed with 2 portions of brine, dried over anhydrous $MgSO_4$, filtered, and evaporated to provide crude product of 1,3-diol (0.16 g, y. quant.). This was dissolved in CH_2Cl_2 (15 ml); 10-camphorsulfonic acid (35 mg, 0.15 mmol) and 4-bromobenzaldehyde dimethyl acetal (0.59 g, 1.52 mmol) were added to the solution. The mixture was stirred at reflux for 24 hours and then evaporated. The residue was partitioned between EtOAc and water. The organic layer was washed with 2 portions of brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. To the residue was added aliquot of *n*-hexane and the resulting precipitate was removed by filtration. The filtrate was concentrated by evaporation and purified by silica gel column chromatography (*n*-hexane : EtOAc= 10 : 1～4 : 1) to obtain two epimeric 4-bromobenzylidene acetals **5a** (0.10 g, y. 36%) and **5b** (0.06 g, y. 21%).

4-Bromobenzylidene Acetal (**5a**)

HRESI(+)MS *m/z* 391.0855 [calcd. for $C_{18}H_{25}O_3BrNa$ $(M+Na)^+$, 391.0885, Δ−3.0 mmu]; ^{1}H-NMR (600 MHz, C_6D_6, solvent peaks δ_H 7.15 and δ_C 128.0 were used as reference), δ 7.34 (2H, m, H-4′,6′), 7.31 (2H, m, H-3′,7′), 5.48 (1H, s, H-1′), 4.38 (1H, ddd, *J*=9.0, 9.0, 6.2, H-18), 3.91 (1H, ddd, *J*=8.0, 5.3, 2.5, H-21), 3.76 (1H, dd, *J*=8.1, 7.2, H-15α), 3.58 (1H, d, *J*=9.0, H-19), 3.18 (1H, dd, *J*=8.1, 8.1, H-15β), 2.04 (1H, m, H-16), 1.97 (1H, dq, *J*=2.5, 7.1, H-20), 1.93 (1H, ddd, *J*=12.4, 7.2, 6.2, H-17α), 1.62 (1H, ddq, *J*=13.9, 8.0, 7.4, H-22a), 1.26 (1H, m, H-17β), 1.26 (1H, m, H-22b), 1.17 (3H, d, *J*=7.1, 20-CH_3), 0.88 (3H, t, *J*=7.4, CH_3-23), 0.81 (3H, d, *J*=6.6, 16-CH_3); ^{13}C-NMR δ 139.3 (C-2′), 131.4 (C-4′,6′), 128.4 (C-3′,7′), 122.7 (C-5′), 96.0 (C-1′), 83.5 (C-19), 76.9 (C-21), 76.8 (C-18), 74.9 (C-15), 38.8 (C-17), 34.6 (C-16), 31.4 (C-20), 25.9 (C-22), 17.4 (16-CH_3), 13.2 (20-CH_3), 10.1 (C-23).

4-Bromobenzylidene Acetal (**5b**)

HRESI(+)MS *m/z* 369.1036 [calcd. for $C_{18}H_{26}O_3Br$ $(M+H)^+$, 369.1065, Δ−2.9 mmu]; ^{1}H-NMR (600 MHz, C_6D_6, solvent peaks δ_H 7.15 and δ_C 128.0 were used as reference) δ 7.35 (2H, m, H-3′,7′), 7.31 (2H, m, H-4′,6′), 5.51 (1H, s, H-1′), 3.96 (1H, ddd, *J*=9.5, 7.1, 4.5, H-18), 3.82 (1H, dd, *J*=8.5, 8.5, H-15α), 3.75 (1H, dd, *J*=10.2, 4.5, H-19), 3.69 (1H, ddd, *J*=12.0, 5.0, 3.9, H-21), 3.35 (1H, dd, *J*=8.5, 8.5, H-15β), 2.01 (1H, m, H-16), 2.01 (1H, m, H-20), 1.82 (1H, ddd, *J*=12.4, 7.1, 7.1, H-17α), 1.64 (1H, ddq, *J*=11.8, 12.0, 7.3, H-22a), 1.57 (1H, ddd, *J*=12.2, 9.5, 9.5, H-17β), 1.10 (1H, ddq, *J*=11.8, 3.9, 7.3, H-22b), 0.87 (3H, t, *J*=7.3, CH_3-23), 0.84 (3H, d, *J*=6.6, 16-CH_3), 0.62 (3H, d, *J*=7.2, 20-CH_3), ^{13}C-NMR δ 139.3 (C-2′), 131.4 (C-4′, 6′), 128.4 (C-3′,7′), 122.6 (C-5′), 93.0 (C-1′), 80.9 (C-18), 79.6 (C-19), 77.7 (C-21), 75.3 (C-15), 36.2 (C-16), 35.4 (C-17), 34.5 (C-20), 18.2 (C-22), 16.9 (16-CH_3), 12.9 (20-CH_3), 10.4 (C-23).

Preparation of (*S*)- and (*R*)-3,21-Bis-MTPA Esters 6a and 6b

To a solution of **1** (2.0 mg) in pyridine (100 μl), 8.2 mg of *N*,*N*-dimethylaminopyridine (as dichloromethane solution, 200 μl) and (*R*)-(−)-MTPA chloride (20 μl) were added and stirred for 1 hour at room temperature. The reaction mixture was then partitioned between dichloromethane (2 ml) and H_2O (2 ml) and the organic layer was dried under a nitrogen gas stream. The residue was purified by NH-silica gel chromatography (*n*-hexane/EtOAc 3 : 2) to afford the 3,21-bis-(*S*)-MTPA ester **5a** (1.2 mg). In the parallel manner, **1** (2.0 mg) was reacted with (*S*)-(+)-MTPA chloride to obtain the 3,21-bis-(*R*)-MTPA ester **5b** (1.1 mg).

3,21-Bis-(*S*)-MTPA Ester (**6a**)

ESI(+)MS *m/z* 991.4 $(M+Na)^+$; ^{1}H-NMR (500 MHz, CD_3CN, solvent peaks δ_H 1.93 and δ_C 1.3 were used as reference): δ 7.55 (4H, m, Ph), 7.43 (6H, m, Ph), 6.28 (1H, dd, *J*=15.1, 10.7, H-14), 5.99 (1H, br d, *J*=10.7, H-13), 5.66 (1H, dd, *J*=15.4, 9.7, H-8), 5.61 (1H, dd, *J*=15.1, 8.4, H-15), 5.52 (1H, dd, *J*=15.4, 9.7, H-9), 5.13 (1H, m, H-3), 5.08 (1H, m, H-21), 4.98 (1H, d, *J*=9.7, H-7), 4.89 (1H, d, *J*=10.7, H-11), 3.54 (3H, s, CH_3O), 3.49 (3H, s, CH_3O), 2.68 (1H, ddd, *J*=5.9, 5.9, 2.0, H-18), 2.66 (1H, dd, *J*=15.4, 3.7, H-2β), 2.56 (1H, dd, *J*=15.4, 4.4, H-2α), 2.56 (1H, ddq, *J*=10.7, 9.7, 6.7, H-10), 2.43 (1H, m, H-16), 2.40 (1H, dd, *J*=8.4, 2.0, H-19), 2.02 (3H, s, 7-OAc), 1.69 (3H, s, 12-CH_3), 1.66 (1H, m, H-22b), 1.64 (1H, m, H-4β), 1.64

(1H, m, H-5β), 1.61 (1H, m, H-22a), 1.60 (1H, m, H-17β), 1.56 (1H, m, H-4α), 1.52 (1H, m, H-20), 1.43 (1H, m, H-5α), 1.36 (1H, m, H-17α), 1.14 (3H, s, 6-CH_3), 1.03 (3H, d, J=7.1, 16-CH_3), 0.86 (3H, d, J=6.7, 20-CH_3), 0.79 (3H, d, J=6.7, 10-CH_3), 0.77 (3H, t, J=7.4, CH_3-23).

3,21-Bis-(*R*)-MTPA Ester (**6b**)

ESI(+)MS *m/z* 991.4 $(M+Na)^+$; ^{1}H-NMR (500 MHz, CD_3CN, solvent peaks δ_H 1.93 and δ_C 1.3 were used as reference): δ 7.56 (4H, m, Ph), 7.43 (6H, m, Ph), 6.25 (1H, dd, J=15.1, 10.7, H-14), 5.97 (1H, br d, J=10.7, H-13), 5.66 (1H, dd, J=15.4, 9.7, H-8), 5.53 (1H, dd, J=15.1, 8.4, H-15), 5.50 (1H, dd, J=15.4, 9.7, H-9), 5.13 (1H, m, H-3), 5.11 (1H, m, H-21), 4.93 (1H, d, J=9.7, H-7), 4.93 (1H, d, J=10.7, H-11), 3.58 (3H, s, CH_3O), 3.54 (3H, s, CH_3O), 2.72 (1H, m, H-2β), 2.68 (1H, m, H-2α), 2.62 (1H, ddd, J=6.0, 6.0, 2.0, H-18), 2.56 (1H, ddq, J=10.7, 9.7, 6.7, H-10), 2.38 (1H, m, H-16), 2.14 (1H, m, H-19), 2.01 (3H, s, 7-OAc), 1.74 (1H, m, H-22b), 1.69 (3H, s, 12-CH_3), 1.67 (1H, m, H-22a), 1.59 (1H, m, H-17β), 1.56 (1H, m, H-5β), 1.49 (2H, m, H-4$\alpha\beta$), 1.42 (1H, m, H-20), 1.40 (1H, m, H-5α), 1.23 (1H, m, H-17α), 1.09 (3H, s, 6-CH_3), 1.01 (3H, d, J=6.7, 16-CH_3), 0.88 (3H, t, J=7.4, CH_3-23), 0.79 (3H, d, J=6.7, 10-CH_3), 0.75 (3H, d, J=7.1, 20-CH_3).

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