Determination of the Absolute Stereostructure of *seco*-Macrosphelide E Produced by a Fungal Strain from a Sea Hare

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***seco*-Macrosphelide E has been isolated from a strain of *Periconia byssoides* originally separated from the sea hare *Aplysia kurodai*. Its absolute stereostructure, with the same configuration as that of macrosphelide E, have been elucidated on the basis of spectroscopic analyses and unambiguous synthesis.**

Key words *seco*-macrosphelide E; structure elucidation; total synthesis; Keck condensation

As part of our continuing search for antitumor metabolites from marine microorganisms, we have previously isolated pericosine A as an anticancer material and five 16-membered macrolides, macrosphelides E (**1**)-I, as cell adhesion inhibitors from a strain of *Periconia byssoides* OUPS-N133 originally separated from the sea hare *Aplysia kurodai*.[1] Macrosphelide E (**1**) has been found to be the C-3 stereoisomer of macrosphelide A (**3**) which had been isolated as a cell adhesion inhibitor from a strain of *Microsphaeropsis* sp. by Omura and co-workers.[2] In addition, it inhibited the adhesion of human-leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC) potently as macrosphelide A (**3**) did. Total syntheses of these metabolites **1** and **3** were achieved by three research groups involving our group.[3] Further investigation of the secondary metabolites of this fungal strain led to the isolation of the straight-chain triester **2** designated as *seco*-macrosphelide E. Now we report the determination of the absolute stereostructure for *seco*-macrosphelide E (**2**) based on spectroscopic analysis and unambiguous synthesis.

seco-Macrosphelide (**2**) had the molecular formula $C_{16}H_{23}O_8$ established by the $[M]^+$ peak of **2** in high resolution (HR)-EI-MS. Its IR spectrum exhibited bands at 3429, 1718, 1665 and 1647 cm^{-1}, characteristic of hydroxy and carbonyl groups, and a double bond. A close inspection of the ^1H- and ^{13}C-NMR spectra of **2** by distortionless enhancement by polarization transfer (DEPT) and ^1H–^{13}C correlation spectroscopy (COSY) experiments revealed the presence of three secondary methyls, one sp^3-hybridized methylene, five oxygen-bearing sp^3-methines including three hydroxymethines, two 1, 2-disubstituted double bonds, one methoxyl group and three ester carbonyl groups. The ^1H–^1H COSY analysis of **2** led to three partial structural units as shown by bold-faced lines in Fig. 1. The *E*-geometry of both the Δ^6- and Δ^{12}-double bonds was deduced from the coupling constants ($J_{6,7}$=15.7 Hz, $J_{12,13}$=15.6 Hz) of the olefinic protons. The connection of these three units and the remaining ester moieties was determined on the basis of the key heteronuclear multiple bond connectivity (HMBC) correlation summarized in Fig. 1, and the planar structure of **2** was elucidated.

The absolute stereostructure for *seco*-macrosphelide E is assumed to be either 3*R*-isomer **2** or 3*S*-isomer **4**, since **2** is considered to be a precursor of macrosphelides E (**1**) or A (**3**). We have therefore achieved synthesis of both these diastereisomers **2** and **4** in order to resolve the stereochemistry for the natural product.

We reported that the enantioselective hydrolysis of (±)-(4,5)-*anti*-5-acetoxy-4-benzyloxy-2(*E*)-hexenoate **5** using the lipase "Amano P" from *Pseudomonas* sp. in phosphate buffer solution gave the (4*R*,5*S*)-5-acetoxy ester **5** (>99% ee, 48% yield) and the (4*S*,5*R*)-5-hydroxy ester **6** (>99% ee, 44% yield), and methanolysis of (4*R*,5*S*)-**5** provided the (4*R*,5*S*)-**6** in 84% yield.[4] The key compound in the synthesis of (+)-**1** and (+)-**3** is (4*R*,5*S*)-4-benzyloxy-5-silyloxy-2(*E*)-hexenoic acid **7** and this compound was also used for the synthesis of **2** or **4** as a starting material. Condensation of carboxylic acid (4*R*,5*S*)-**7** and commercially available (3*R*)-hydroxy butanoate **8** *via* the Keck procedure[5] (dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), camphorsulfonic acid (CSA)) provided diester (3*R*,8*R*,9*S*)-**9** ($[\alpha]_D$ −23.1° (*c*=0.8, $CHCl_3$)) in 64% yield, which was desi-

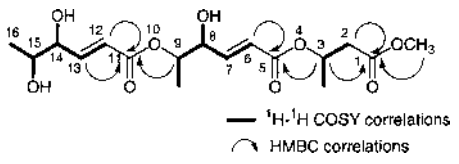
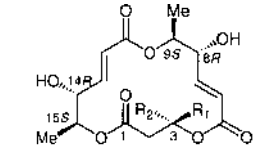
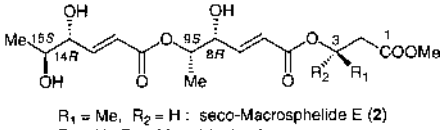


Fig. 1. Typical 2D NMR Correlations in *seco*-Macrosphelide (**2**)



R_1 = Me, R_2 = H : (+)-Macrosphelide E (**1**)
R_1 = H, R_2 = Me : (+)-Macrosphelide A (**3**)



R_1 = Me, R_2 = H : seco-Macrosphelide E (**2**)
R_1 = H, R_2 = Me : triester **4**

Chart 1

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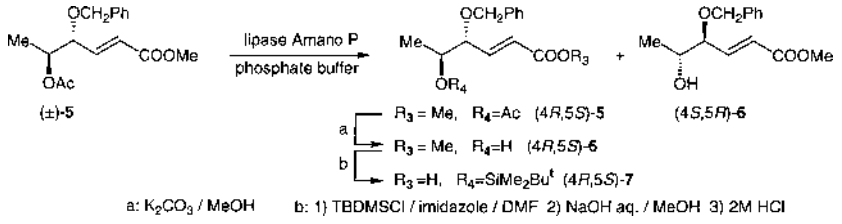

Chart 2

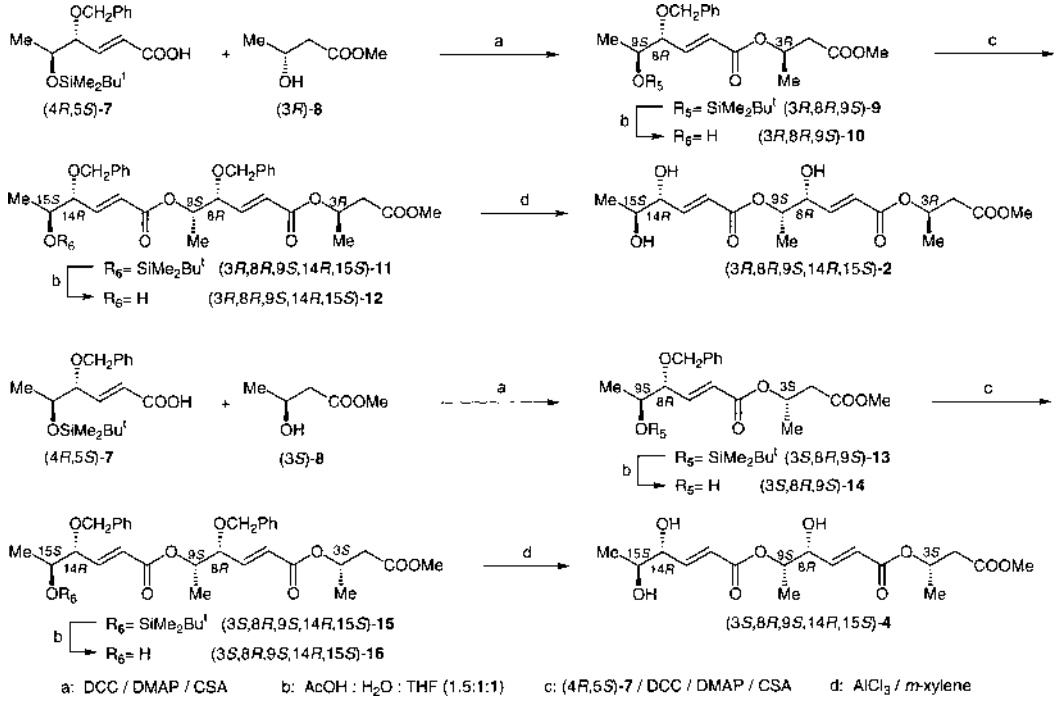

Chart 3

lylated to yield a hydroxy-diester (3*R*,8*R*,9*S*)-**10** ($[\alpha]_D$ −26.5° (*c*=0.71, $CHCl_3$)) in 62% yield. Second condensation of carboxylic acid (4*R*,5*S*)-**7** and (3*R*,8*R*,9*S*)-**10** *via* the Keck procedure[4] afforded triester (3*R*,8*R*,9*S*,14*R*,15*S*)-**11** ($[\alpha]_D$ −35.6° (*c*=0.74, $CHCl_3$)) in 57% yield, which was desilylated to yield a hydroxy-triester (3*R*,8*R*,9*S*,14*R*,15*S*)-**12** ($[\alpha]_D$ −64.3° (*c*=0.43, $CHCl_3$)) in 51% yield. Deprotection of benzyl group in (−)-**12** using $AlCl_3$ in the presence of *m*-xylene[4] gave trihydroxy-triester (3*R*,8*R*,9*S*,14*R*,15*S*)-**2** ($[\alpha]_D$ +40.0° (*c*=0.18, EtOH)) in 46% yield. The synthesis of (3*S*,8*R*,9*S*,14*R*,15*S*)-**4** was carried out by the way as for the preparatiom of (3*R*,8*R*,9*S*,14*R*,15*S*)-**2**. Condensation of carboxylic acid (4*R*,5*S*)-**7** and commercially available (3*S*)-hydroxy butanoate **8** *via* the Keck procedure gave diester (3*S*,8*R*,9*S*)-**13** ($[\alpha]_D$ −9.1° (*c*=0.7, $CHCl_3$)) in 75% yield, which was desilylated to yield a hydroxy-diester (3*S*,8*R*,9*S*)-**14** ($[\alpha]_D$ −42.5° (*c*=0.54, $CHCl_3$)) in 86% yield. Second condensation of carboxylic acid (4*R*,5*S*)-**7** and (3*S*,8*R*,9*S*)-**14** *via* the Keck procedure afforded triester (3*S*,8*R*,9*S*,14*R*,15*S*)-**15** ($[\alpha]_D$ −22.9° (*c*=0.5, $CHCl_3$)) in 69% yield, which was desilylated to yield a hydroxy-triester (3*S*,8*R*,9*S*,14*R*,15*S*)-**16** ($[\alpha]_D$ −44.9° (*c*=0.54, $CHCl_3$)) in 76% yield. Deprotection of benzyl group in (−)-**16** using $AlCl_3$ in the presence of *m*-xylene gave trihydroxy-triester (3*S*,8*R*,9*S*,14*R*,15*S*)-**4** ($[\alpha]_D$ +57.2° (*c*=0.17, EtOH)) in 46% yield. Both physical data (^{1}H-NMR, ^{13}C-NMR, $[\delta]_D$ and MS) of (3*R*,8*R*,9*S*,14*R*,15*S*))-**2** and (3*S*,8*R*,9*S*,14*R*,15*S*)-**4** quite resembled those of the natural product **2**, respectively, while both retention times of (3*R*,8*R*,9*S*,14*R*,15*S*)-**2** (t_R=19.3 min) and (3*S*,8*R*,9*S*,14*R*,15*S*)-**4** (t_R=25.8 min) by mean of HPLC (column; CHIRALCEL OD (0.46×25 cm), solvent; *n*-hexane–EtOH (10 : 1); detection; 254 nm) were quite different. The retention time of (3*R*,8*R*,9*S*,14*R*,15*S*)-**2** was found to be consistent with that (t_R=19.3 min) of the natural product **2**.

In conclusion, absolute structure of the natural product **2** was found to be methyl (3*R*,8*R*,9*S*,14*R*,15*S*)-8,14,15-trihydroxy-3,9-dimethyl-5,11-dioxo-4,10-dioxahexadeca-(6*E*,12*E*)-dienoate in comparison with unambiguouly synthesized (3*R*,8*R*,9*S*,14*R*,15*S*)-**2**. The absolute configurations due to five chiral centers in **2** were the same as those of macrosphelide E (**1**).

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on JEOL AL 400 or Varian UNITY INOVA-500 spectrometers in $CDCl_3$. Carbon substitution degrees were established by DEPT pulse sequence. The fast atom bombardment mass spectra (FAB-MS) were obtained with JEOL JMS-DX 303 and electron (impact) ionization mass spectra (EI-MS) were recorded a Hitachi M-4000H spectrometer. IR spectra were recorded a JASCO FT/IR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

Culturing and Isolation A strain of *Periconia byssoides* OUPS-N133, separated from the sea hare *Aplysia kurodai*, was cultured at 27 °C for four weeks in a liquid medium (90 l) containing malt extract 1%, glucose 1% and peptone 0.05% in artificial seawater adjusted to pH 7.5. As reported previously,[1b] the AcOEt extract (5.7 g) of the culture filtrate was successively chromatographed on Sephadex LH-20 (CH_2Cl_2–MeOH, 1 : 1) and silica gel (CH_2Cl_2/MeOH). The MeOH–CH_2Cl_2 (1 : 19) eluate (315.7 mg) from silica gel column chromatography was purified by HPLC using MeOH–H_2O (1 : 1) as the eluent to afford **2** (1.2 mg) as a colorless oil.

seco-Macrosphelide (**2**): A colorless oil, $[\delta]_D$ +56.0° (*c* 0.10, EtOH); UV λ_{max} (EtOH) nm (log ε): 215 (4.21); IR ν_{max} (neat) cm^{-1}: 3429 (OH), 1718 (ester), 1665, 1647 (C=C); EI-MS *m/z*: 374 (M^+, 0.2%), 212 (28), 111 (99), 84 (100), 69 (5); HR-EI-MS *m/z*: 374.1564 (M^+) (Calcd for $C_{16}H_{23}O_8$: 374.1575); ^{1}H-NMR δ ppm ($CDCl_3$, 500 MHz): 1.19 (3H, d, *J*=6.4 Hz, H-16), 1.29 (3H, d, *J*=6.6 Hz, 9-CH_3), 1.34 (3H, d, *J*=6.2 Hz, 3-CH_3), 2.02 (1H, br s, 15-OH), 2.47 (1H, br s, 14-OH), 2.52 (1H, br s, 8-OH), 2.55 (1H, dd, *J*=15.5, 5.2 Hz, H-2A), 2.69 (1H, dd, *J*=15.5, 6.2 Hz, H-2B), 3.69 (3H, s, 1-OCH_3), 3.99 (1H, br s, H-15), 4.33 (1H, br s, H-14), 4.45 (1H, br s, H-8), 5.10 (1H, qd, *J*=6.6, 3.2 Hz, H-9), 5.34 (1H, quintet d, *J*=6.2, 5.2 Hz, H-3), 6.12 (1H, dd, *J*=15.7, 1.8 Hz, H-6), 6.15 (1H, dd, *J*=15.6, 1.8 Hz, H-12), 6.91 (1H, dd, *J*=15.7, 4.2 Hz, H-7), 6.99 (1H, dd, *J*=15.6, 4.2 Hz, H-13); ^{13}C-NMR δ ppm ($CDCl_3$, 125 MHz): 14.95 (C-16), 17.71 (9-CH_3), 19.91 (3-CH_3), 40.64 (C-2), 51.87 (1-OCH_3), 67.56 (C-3), 69.85 (C-15), 73.36 (C-8), 73.50 (C-9), 74.51 (C-14), 122.05 (C-12), 122.75 (C-6), 144.64 (C-7), 146.56 (C-13), 165.15 (C-5), 165.91 (C-11), 170.81 (C-1); CD λ (*c* 2.68×10^{-4} M in EtOH)/nm 296 ($\Delta\varepsilon$ 0), 248 (−0.34), 239 (0) and 214 (+9.72).

Methyl (3*R*,8*R*,9*S*)-8-Benzyloxy-9-*tert*-butyldimethylsiloxy-3-methyl-5-oxo-4-oxadeca (6*E*)-Enoate (9) To a mixture of DCC (2.260 g, 11 mmol), DMAP (1.780 g, 14.6 mmol) and (+)-CSA (1.700 g, 14.6 mmol) in CH_2Cl_2 (45 ml) was added a solution of (4*R*,5*S*)-**7** (2.546 g, 7.3 mmol) and (3*R*)-**8** (1.720 g, 14.6 mmol) in CH_2Cl_2 (20 ml) and the reaction mixture was stirred for 3 d at room temperature. After the generated precipitate was filtered off and the filtrate was washed with 2 M aqueous HCl and 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (110 g, *n*-hexane : AcOEt=10 : 1) to give (3*R*,8*R*,9*S*)-**9** (2.106 g, 64%) as a homogenous oil. (3*R*,8*R*,9*S*)-**9**; IR (neat): 1743 cm^{-1}; $[\alpha]_D^{25}$ −23.1° (*c*=0.8, $CHCl_3$); ^{1}H-NMR: δ: 0.00 (3H, s), 0.03 (3H, s), 0.85 (9H, s), 1.19 (3H, d, *J*=6 Hz), 1.32 (3H, d, *J*=6 Hz), 2.52, 2.69 (each 1H, dd, *J*=6, 16 Hz), 3.66 (3H, s), 3.75 (1H, dt, *J*=2, 6 Hz), 3.80 (1H, qd, *J*=6 Hz), 4.42, 4.59 (each 1H, d, *J*=12 Hz), 5.33 (1H, dq, *J*=6 Hz), 5.99 (1H, dd, *J*=2, 16 Hz), 6.90 (1H, dd, *J*=6, 16 Hz), 7.23—7.36 (5H, m). FAB-MS *m/z*: 451 (M^++1); *Anal.* Found: C, 63.67; H, 8.45. Calcd for $C_{24}H_{38}O_6Si$: C, 63.97; H, 8.50%.

Desilylation of (3*R*,8*R*,9*S*)-9 A mixture of (−)-**9** (1.758 g, 3.9 mmol) in the mixed solvent (AcOH (7.5 ml), H_2O (5 ml) and THF (5 ml)) was stirred for 12 h at 80 °C. The reaction mixture was evaporated and the residue was diluted with H_2O, extracted with Et_2O. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (25 g, *n*-hexane : AcOEt=5 : 1) to give (3*R*,8*R*,9*S*)-**10** (0.822 g, 62%) as a homogeneous oil. (3*R*,8*R*,9*S*)-**10**; IR (neat): 3484, 1722 cm^{-1}; $[\alpha]_D^{26}$ −26.5° (*c*=0.71, $CHCl_3$); ^{1}H-NMR: δ: 1.12 (3H, d, *J*=6 Hz), 1.32 (3H, d, *J*=6 Hz), 2.51—2.63 (1H, br s), 2.52, 2.68 (each 1H, dd, *J*=6, 16 Hz), 3.65 (3H, s), 3.89 (1H, ddd, *J*=2, 4, 6 Hz), 3.92 (1H, qd, *J*=4, 6 Hz), 4.39, 4.61 (each 1H, d, *J*=12 Hz), 5.32 (1H, dq, *J*=6 Hz), 6.00 (1H, dd, *J*=2, 16 Hz), 6.86 (1H, dd, *J*=6, 16 Hz), 7.23—7.38 (5H, m). FAB-MS *m/z*: 337 (M^++1); *Anal.* Found: C, 63.85; H, 7.32. Calcd for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19%.

Methyl (3*R*,8*R*,9*S*,14*R*,15*S*))-8,14-Dibenzyloxy-15-*tert*-butyldimethylsiloxy-3,9-dimethyl-5,11-dioxo-4,10-dioxadeca (6*E*,12*E*)-Dienoate (11) To a mixture of DCC (0.760 g, 3.7 mmol), DMAP (0.600 g, 4.9 mmol) and (+)-CSA (0.570 g, 2.5 mmol) in CH_2Cl_2 (30 ml) was added a solution of (3*R*,8*R*,9*S*)-**10** (0.822 g, 2.5 mmol) and (4*R*,5*S*)-**7** (1.620 g, 4.6 mmol) in CH_2Cl_2 (10 ml) and the reaction mixture was stirred for 12 h at room temperature. After the generated precipitate was filtered off and the filtrate was washed with 2 M aqueous HCl and 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (40 g, *n*-hexane : AcOEt=10 : 1) to give (3*R*,8*R*,9*S*,14*R*,15*S*)-**11** (0.889 g, 57%) as a homogenous oil. (3*R*,8*R*,9*S*,14*R*,15*S*)-**11**; IR (neat): 1732 cm^{-1}; $[\alpha]_D^{23}$ −35.6° (*c*=0.74, $CHCl_3$); ^{1}H-NMR: δ: 0.01 (3H, s), 0.03 (3H, s), 0.85 (9H, s), 1.19 (3H, d, *J*=6 Hz), 1.27 (3H, d, *J*=6 Hz), 1.33 (3H, d, *J*=6 Hz), 2.52, 2.69 (each 1H, dd, *J*=6, 16 Hz), 3.66 (3H, s), 3.77 (1H, ddd, *J*=2, 6, 6 Hz), 3.84 (1H, dq, *J*=6 Hz), 4.11 (1H, ddd, *J*=2, 4, 6 Hz), 4.44, 4.49, 4.60, 4.63 (each 1H, d, *J*=12 Hz), 5.10 (1H, dq, *J*=4, 6 Hz), 6.03 (1H, dd, *J*=2, 16 Hz), 6.07 (1H, dd, *J*=2, 16 Hz), 6.85 (1H, dd, *J*=6, 16 Hz), 6.90 (1H, dd, *J*=2, 16 Hz), 7.22—7.36 (10H, m). *Anal.* Found: C, 66.73; H, 8.08. Calcd for $C_{37}H_{52}O_9Si$: C, 66.44; H, 7.84%.

Desilylation of (3*R*,8*R*,9*S*,14*R*,15*S*)-11 A mixture of (3*R*,8*R*,9*S*,14*R*,15*S*)-**11** (0.809 g, 1.3 mmol) in the mixed solvent (AcOH (4.5 ml), H_2O (3 ml) and THF (3 ml)) was stirred for 12 h at 80 °C. The reaction mixture was evaporated and the residue was diluted with H_2O, extracted with Et_2O. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane : AcOEt=5 : 1) to give (3*R*,8*R*,9*S*,14*R*,15*S*)-**12** (0.358 g, 51%) as a homogeneous oil. (3*R*,8*R*,9*S*,14*R*,15*S*)-**12**; IR (neat): 3505, 1720 cm^{-1}; $[\alpha]_D^{23}$ −64.3° (*c*=0.43, $CHCl_3$); ^{1}H-NMR: δ: 1.14 (3H, d, *J*=6 Hz), 1.28 (3H, d, *J*=6 Hz), 1.32 (3H, d, *J*=6 Hz), 2.00 (1H, br s), 2.52, 2.68 (each 1H, dd, *J*=6, 16 Hz), 3.66 (3H, s), 3.89 (1H, ddd, *J*=2, 6, 6 Hz), 3.90 (1H, dq, *J*=4, 6 Hz), 4.07 (1H, ddd, *J*=2, 4, 6 Hz), 4.40, 4.48, 4.62, 4.63 (each 1H, d, *J*=12 Hz), 5.09 (1H, dq, *J*=4, 6 Hz), 5.33 (1H, qt, *J*=6, 6 Hz), 6.03 (1H, dd, *J*=2, 16 Hz), 6.06 (1H, dd, *J*=2, 16 Hz), 6.84 (1H, dd, *J*=6, 16 Hz), 6.90 (1H, dd, *J*=6, 16 Hz), 7.24—7.36 (10H, m). ^{13}C-NMR: δ 15.2 (q), 18.1 (q), 20.0 (q), 40.6 (t), 51.8 (q), 67.7 (d), 69.2 (d), 71.4 (t), 71.6 (t), 71.7 (d), 79.5 (d), 82.0 (d), 124.0 (d), 124.2 (d), 127.4 (d), 127.6 (d), 127.6 (d), 127.7 (d), 128.2 (d), 128.3 (d), 137.4 (s), 137.4 (s), 144.0 (d), 144.5 (d), 164.6 (s), 164.7 (s), 170.3 (s). FAB-MS *m/z*: 555 (M^++1); *Anal.* Found: C, 66.92; H, 7.16. Calcd for $C_{31}H_{38}O_9$: C, 67.13; H, 6.91%.

Methyl (3*R*,8*R*,9*S*,14*R*,15*S*))-8,14,15-Hydroxy-3,9-dimethyl-5,11-dioxo-4,10-dioxadeca (6*E*,12*E*)-Dienoate (2) To a mixture of $AlCl_3$ (0.146 g, 1.1 mmol) in CH_2Cl_2 (2 ml) was added dropwise a solution of (3*R*,8*R*,9*S*,14*R*,15*S*)-**12** (0.060 g, 0.11 mmol) and *m*-xylene (1 ml) at −20 °C, and the reaction mixture was stirred for 1 h at 0 °C. The reaction mixture was diluted with saturated brine and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was subjected to preparative SiO_2 thin layer chromatography (*n*-hexane : AcOEt=4 : 1) to give (3*R*,8*R*,9*S*,14*R*,15*S*)-**2** (0.017 g, 46%) as colorless oil. (3*R*,8*R*,9*S*,14*R*,15*S*)-**2**; IR (KBr): 3438, 1708 cm^{-1}; $[\alpha]_D^{23}$ +40.0° (*c*=0.18, EtOH); HR-MS (FAB-MS, matrix: *m*-nitrobenzyl alcohol (NBA)): Calcd for $C_{17}H_{27}O_9$ (M^++1) 375.1655; Found 375.1649. ^{1}H-NMR and ^{13}C-NMR data of (3*R*,8*R*,9*S*,14*R*,15*S*)-**2** were identical with those of the natural product **2**.

Methyl (3*S*,8*R*,9*S*)-8-Benzyloxy-9-*tert*-butyldimethylsiloxy-3-methyl-5-oxo-4-oxadeca (6*E*)-Enoate (13) To a mixture of DCC (3.030 g, 14.7 mmol), DMAP (2.390 g, 19.6 mmol) and (+)-CSA (2.280 g, 9.8 mmol) in CH_2Cl_2 (50 ml) was added a solution of (4*R*,5*S*)-**7** (3.425 g, 9.8 mmol) and (3*S*)-**8** (2.310 g, 19.6 mmol) in CH_2Cl_2 (20 ml) and the reaction mixture was stirred for 2 d at room temperature. After the generated precipitate was filtered off and the filtrate was washed with 2 M aqueous HCl and 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (200 g, *n*-hexane : AcOEt=20 : 1) to give (3*S*,8*R*,9*S*)-**13** (3.316 g, 75%) as a homogenous oil. (3*S*,8*R*,9*S*)-**13**; IR (neat): 2953, 1743, 1722 cm^{-1}; $[\alpha]_D^{22}$ −9.1° (*c*=0.7, $CHCl_3$); ^{1}H-NMR: δ: 0.00 (3H, s), 0.02 (3H, s), 0.84 (9H, s), 1.18 (3H, d, *J*=6 Hz), 1.32 (3H, d, *J*=6 Hz), 2.56, 2.69 (each 1H, dd, *J*=6, 16 Hz), 3.65 (3H, s), 3.73 (1H, ddd, *J*=2, 6 Hz), 3.80 (1H, dq, *J*=6, 6 Hz), 4.42, 4.58 (each 1H, d, *J*=12 Hz), 5.33 (1H, sixtet, *J*=6 Hz), 5.98 (1H, dd, *J*=2, 16 Hz), 6.88 (1H, dd, *J*=6, 16 Hz), 7.23—7.35 (5H, m). FAB-MS *m/z*: 451 (M^++1); *Anal.* Found: C, 64.02; H, 8.69. Calcd for $C_{24}H_{38}O_6Si$: C, 63.97; H, 8.50%.

Desilylation of (−)-13 A mixture of (3*S*,8*R*,9*S*)-**13** (3.139 g, 7 mmol) in the mixed solvent (AcOH (15 ml), H_2O (10 ml) and THF (10 ml)) was stirred for 12 h at 80 °C. The reaction mixture was evaporated and the residue was diluted with H_2O, extracted with Et_2O. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (50 g, *n*-hexane : AcOEt=5 : 1) to give (3*S*,8*R*,9*S*)-**14** (2.031 g, 86%) as a homogeneous oil. (3*S*,8*R*,9*S*)-**14**; IR (neat): 3483, 1721 cm^{-1}; $[\alpha]_D^{22}$ −42.5° (*c*=0.54, $CHCl_3$); ^{1}H-NMR: δ: 1.14 (3H, d, *J*=6 Hz), 1.34 (3H, d, *J*=6 Hz), 2.32 (1H, br s), 2.54, 2.70 (each 1H, dd, *J*=6, 16 Hz), 3.66 (3H, s), 3.91—3.97 (1H, m), 3.88—3.92 (1H, m), 4.41, 4.63 (each 1H, d, *J*=12 Hz), 5.34 (1H, sixtet, *J*=6 Hz), 6.03 (1H, dd, *J*=2, 16 Hz), 6.89 (1H, dd, *J*=6, 16 Hz), 7.25—7.37 (5H, m). FAB-MS *m/z*: 337 (M^++1); *Anal.* Found: C, 63.43; H, 7.17. Calcd for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19%.

Methyl (3*S*,8*R*,9*S*,14*R*,15*S*))-8,14-Dibenzyloxy-15-*tert*-butyldimethylsiloxy-3,9-dimethyl-5,11-dioxo-4,10-dioxadeca (6*E*,12*E*)-Dienoate (15) To a mixture of DCC (1.700g, 8.2 mmol), DMAP (1.340 g, 10.9 mmol) and

(+)-CSA (1.280 g, 5.5 mmol) in CH_2Cl_2 (40 ml) was added a solution of (3*S*,8*R*,9*S*)-**14** (1.900 g, 5.7 mmol) and (4*R*,5*S*)-**7** (1.930 g, 5.5 mmol) in CH_2Cl_2 (10 ml) and the reaction mixture was stirred for 48 h at room temperature. After the generated precipitate was filtered off and the filtrate was washed with 2 M aqueous HCl and 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (50 g, *n*-hexane : AcOEt=10 : 1) to give (3*S*,8*R*,9*S*,14*R*,15*S*)-**15** (2.554 g, 69%) as a homogenous oil. (3*S*,8*R*,9*S*,14*R*,15*S*)-**15**; IR (neat): 2932, 1722 cm^{-1}; $[\alpha]_D^{25}$ −22.9° (*c*=0.5, $CHCl_3$); ^{1}H-NMR: δ: 0.02 (3H, s), 0.04 (3H, s), 0.86 (9H, s), 1.20 (3H, d, *J*=6 Hz), 1.28 (3H, d, *J*=6 Hz), 1.35 (3H, d, *J*=6 Hz), 2.54, 2.71 (each 1H, dd, *J*=6, 16 Hz), 3.68 (3H, s), 3.78 (1H, ddd, *J*=2, 6, 6 Hz), 3.85 (1H, dq, *J*=6, 6 Hz), 4.11—4.15 (1H, m), 4.45, 4.51, 4.61, 4.63 (each 1H, d, *J*=12 Hz), 5.12 (1H, dq, *J*=4, 6 Hz), 5.35 (1H, sixtet, *J*=6, 16 Hz), 6.04 (1H, dd, *J*=2, 16 Hz), 6.08 (1H, dd, *J*=2, 16 Hz), 6.87 (1H, dd, *J*=6, 16 Hz), 6.91 (1H, dd, *J*=6, 16 Hz), 7.24—7.36 (10H, m). *Anal.* Found: C, 66.87; H, 7.89. Calcd for $C_{37}H_{52}O_9Si$: C, 66.44; H, 7.84%.

Desilylation of (3*S*,8*R*,9*S*,14*R*,15*S*)-15 A mixture of (3*S*,8*R*,9*S*,14*R*,15*S*)-**15** (2.370 g, 3.5 mmol) in the mixed solvent (AcOH (8 ml), H_2O (5 ml) and THF (5 ml)) was stirred for 12 h at 80 °C. The reaction mixture was evaporated and the residue was diluted with H_2O, extracted with Et_2O. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane : AcOEt=5 : 1) to give (3*S*,8*R*,9*S*,14*R*,15*S*)-**16** (1.482 g, 76%) as a homogeneous oil. (3*S*,8*R*,9*S*,14*R*,15*S*)-**16**; IR (neat): 3505, 1720, 1657 cm^{-1}; $[\alpha]_D^{25}$ −44.9° (*c*=0.54, $CHCl_3$); ^{1}H-NMR: δ: 1.15 (3H, d, *J*=6 Hz), 1.30 (3H, d, *J*=6 Hz), 1.34 (3H, d, *J*=6 Hz), 2.28 (1H br s), 2.54, 2.70 (each 1H, dd, *J*=6, 16 Hz), 3.67 (3H, s), 3.93 (1H, ddd, *J*=2, 4, 6 Hz), 3.96 (1H, dq, *J*=4, 6 Hz), 4.11 (1H, ddd, *J*=2, 4, 6 Hz), 4.42, 4.49, 4.65, 4.65 (each 1H, d, *J*=12 Hz), 5.11 (1H, qd, *J*=4, 6 Hz), 5.35 (1H, sixtet, *J*=6 Hz), 6.06 (1H, dd, *J*=2, 16 Hz), 6.08 (1H, dd, *J*=2, 16 Hz), 6.86 (1H, dd, *J*=6, 16 Hz), 6.91 (1H, dd, *J*=6, 16 Hz), 7.24—7.38 (10H, m). ^{13}C-NMR: δ 15.2 (q), 18.2 (q), 20.0 (q), 40.6 (t), 51.8 (q), 67.7 (d), 69.2 (d), 71.4 (t), 71.6 (t), 71.7 (d), 79.4 (d), 81.9 (d), 124.0 (d), 124.2 (d), 127.4 (d), 127.6 (d), 127.6 (d), 127.7 (d), 128.2 (d), 128.3 (d), 137.4 (s), 137.4 (s), 144.0 (d), 144.6 (d), 164.7 (s), 164.7 (s), 170.3 (s). FAB-MS *m*/*z*: 555 (M^++1); *Anal.* Found: C, 66.83; H, 6.90. Calcd for $C_{31}H_{38}O_9$: C, 67.13; H, 6.91%.

Methyl (3*S*,8*R*,9*S*,14*R*,15*S*)-8,14,15-Hydroxy-3,9-dimethyl-5,11-dioxo-4,10-dioxadeca (6*E*,12*E*)-Dienoate (4) To a mixture of $AlCl_3$ (0.130 g, 1.0 mmol) in CH_2Cl_2 (2 ml) was added dropwise a solution of (3*S*,8*R*,9*S*,14*R*,15*S*)-**16** (0.055 g, 0.1 mmol) and *m*-xylene (1 ml) at −20 °C and the reaction mixture was stirred for 1 h at 0 °C. The reaction mixture was diluted with saturated brine and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was subjected to preparative SiO_2 thin layer chromatography (*n*-hexane : AcOEt=4 : 1) to give (3*S*,8*R*,9*S*,14*R*,15*S*)-**4** (0.017 g, 46%) as colorless oil. (3*S*,8*R*,9*S*,14*R*,15*S*)-**4**; IR (KBr): 3438, 1708 cm^{-1}; $[\alpha]_D^{22}$ +57.2° (*c*=0.17, EtOH); ^{1}H-NMR: δ: 1.14 (3H, d, *J*=6 Hz), 1.23 (3H, d, *J*=6 Hz), 1.31 (3H, d, *J*=6 Hz), 2.52, 2.66 (each 1H, dd, *J*=6, 16 Hz), 3.65 (3H, s), 3.92 (1H, dq, *J*=4, 6 Hz), 4.25 (1H, ddd, *J*=2, 4, 6 Hz), 4.41 (1H, ddd, *J*=2, 4, 6 Hz), 5.04 (1H, dq, *J*=4, 6 Hz), 5.30 (1H, qt, *J*=6, 6 Hz), 6.09 (1H, dd, *J*=2, 16 Hz), 6.10 (1H, dd, *J*=2, 16 Hz), 6.88 (1H, dd, *J*=6, 16 Hz), 6.96 (1H, dd, *J*=6, 16 Hz). ^{13}C-NMR: δ 14.8 (q), 17.8 (q), 20.0 (q), 40.6 (t), 52.0 (q), 67.7 (d), 70.0 (d), 72.8 (d), 73.2 (d), 74.6 (d), 121.8 (d), 122.5 (d), 145.0 (d), 146.6 (d), 165.2 (s), 165.7 (s), 170.6 (s). HR-MS (FAB-MS, matrix: *m*-nitrobenzyl alcohol (NBA)): Calcd for $C_{17}H_{27}O_9$ (M^++1) 375.1655; Found 375.1684.

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