Chemical Study and Absolute Configuration of a New Marine Secospatane from the Brown Alga *Dilophus okamurae*

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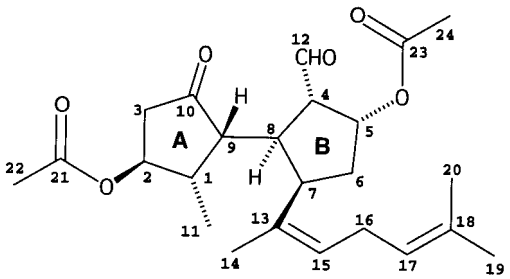
A new diterpene, dilkamural (**1**), of the secospatane class has been isolated from the brown alga *Dilophus okamurae* Dawson, and its structure, including absolute stereochemistry, has been determined by NMR spectroscopic and chemical means.

Marine organisms produce unique secondary metabolites, some of which have important biological and pharmaceutical activities.[1] In the course of our screening for bioactive components of the marine algae growing around Shikoku Island of Japan, we isolated from the brown alga *Dilophus okamurae* Dawson (Dictyotaceae), a secospatane-type diterpenoid **1**, designated dilkamural, having moderate antimicrobial activity against a Gram-positive microorganism. We herein describe the structure and absolute configuration of the new compound, elucidated by the chemical and spectroscopic analyses of **1** and its derivatives.



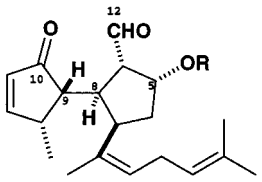
1

D. okamurae Dawson[2] was collected in 1994 at Ehime, Japan, and immediately freeze-dried. The dried algal mass yielded a new secospatane diterpenoid **1** as a colorless oil in 1.9% yield. The molecular formula of $C_{24}H_{34}O_6$ was established by high-resolution mass spectrometry and analysis of the ^{13}C NMR data. The presence of two acetoxyl groups attached to secondary carbons was indicated by the ^1H NMR (C_6D_6) signals at δ 1.60 (s, 3H), 1.61 (s, 3H), 4.80 (dd, 1H), and 5.59 (dd, 1H) and the ^{13}C NMR signals at δ 169.3 (s), 169.8 (s), 75.3 (d), 76.2 (d), 20.6 (q), and 20.7 (q). The NMR showed the signals due to a secondary methyl group (δ 0.69, d, 3H), an aldehyde group [δ 9.86 (1H, d); δ 197.8 (d)], three olefinic methyl groups [δ 1.41, 1.53, 1.66 (each bs, 3H)], and two olefinic protons [δ 5.09, 5.16 (each bt, 1H)]. The ^{13}C NMR showed a ketone carbonyl signal at δ 214.9 (s). The existence of a cyclopentanone and acetoxy groups was supported by the IR bands at 1739 and 1240 cm^{-1}. Extensive NMR work (H−H COSY, C−H COSY, NOESY, HMBC) on this compound led to the structure (**1**) with the secospatane framework.

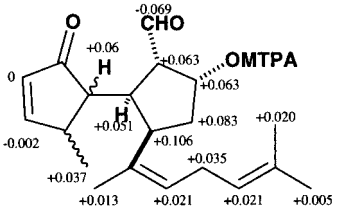
The relative stereochemistry of the substituents on each of the A and B rings was determined by NOEs and coupling constants of the ring protons. The stereochemistry of the A ring relative to the B ring, however, could not be established, because the rings apparently rotate rapidly around the single bond connecting them on the NMR time scale.

When **1** was allowed to stand with silica gel and CH_2Cl_2,[3] (5*R*,13*Z*)-5-acetoxy-10-oxo-4,10-secospata-2,13-(15),17-trien-12-al (**2**) was formed. This compound has been isolated from *Dilophus marginata* Okamura by an Australian group,[4] and its NMR data and stereochemical features were identical with those of the present deacetoxyl compound. However, neither the relative stereochemistry of the A versus B rings nor its absolute configuration had been established for **2**. Therefore, we attempted to elucidate the absolute configuration of the respective rings.[5]

Treatment of **2** with carboxylesterase in 100 mM phosphate buffer (pH 7.0) yielded deacetylated product (**3**). To establish the absolute configuration of the 5-OH group, the (*S*)- and (*R*)-MTPA esters (**4**) were prepared.[6]



2; R=Ac 3 ; R=H 4 ; R=MTPA



4'

The $\Delta\delta$ ($= \delta_S - \delta_R$; ppm) values were calculated for the respective protons, and they are summarized in the formula **4′**. No systematic arrangement of the $\Delta\delta$ values was observed in **4′**; the modified Mosher's method failed

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⊥ Tokushima University.

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 259 and references therein.

(2) The voucher specimen is preserved in this laboratory.

(3) Ishitsuka, O. M.; Kusumi, T.; Ichikawa, A.; Kakisawa, H. *Phytochemistry* **1990**, *29*, 2605.

(4) Ravi, B. N.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 129.

(5) Kusumi, T.; Hamada, T.; Ishitsuka, M. O.; Ohtani, I.; Kakisawa, H. *J. Org. Chem.* **1992**, *57*, 1033.

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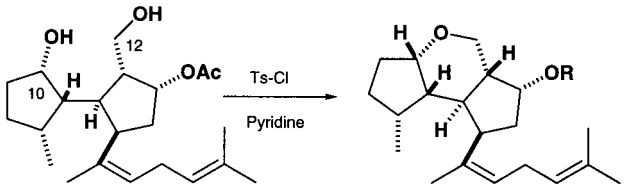
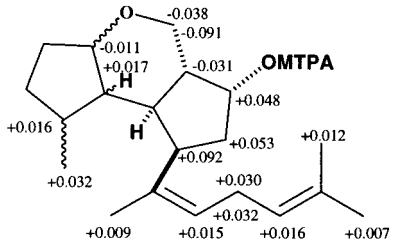
in this case. This anomaly seemed to be caused by the flexibility of the molecule due to the single-bond linkage (C8−C9) between the two rings, which might result in the different conformations of the (*R*)- and (*S*)-MTPA diastereomers (**4**).

To resolve this difficulty, we fixed the conformation by building a new linkage utilizing the aldehyde at C-12 and the carbonyl oxygen at C-10. Reduction of **2** with $NaBH_4$ in methanol gave diol **5** (the stereochemistry of **5** is commented on at the end of the text), in which the conjugated double bond was concomitantly reduced as a single isomer. The intramolecular cyclization of **5** was carried out with TsCl in pyridine to give **6**, which was saponified (NaOMe/MeOH) into **7**.



5 **6: R=Ac 7: R=H 8: R=MTPA**

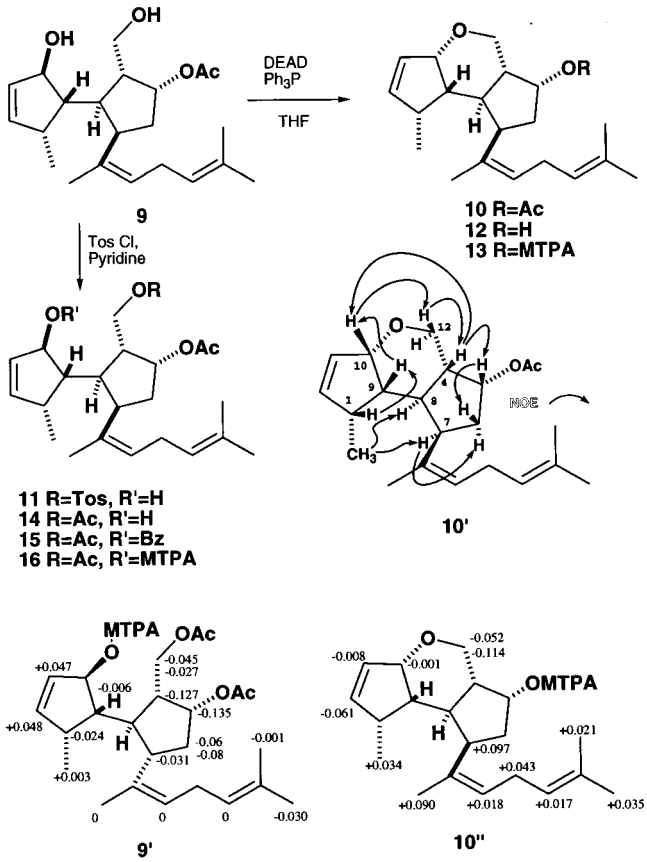
The modified Mosher's method was applied to this compound (see **8′**). Although the *R* configuration of 5-OH was easily deduced by these results, another problem arose: The ^1H NMR spectra (600 MHz, $CDCl_3$) of the MTPA esters (**8**) exhibited heavily overlapped signals in the aliphatic region 2.2−1.2 ppm, which made the assignment of the proton signals and, therefore, the stereochemistry of the substituents extremely difficult.



8'

To overcome this problem, we turned to cyclization of allylic alcohol **9** (the stereochemistry of 10-OH was not determined at this stage), which was successfully obtained by reduction with $NaBH_4$ in the presence of cerium chloride[7] in MeOH. The attempted intramolecular cyclization of diol **9** with TsCl, however, was unsuccessful and only gave C-12 tosylate (**11**). Apparently, the C-10 hydroxyl group was not positioned for a backside attack at the C-12 tosylate carbon (vide infra). We were delighted to find that the Mitsunobu reaction[8] carried out on **9** resulted in the formation of cyclization product (**10**) in a good yield.

The ^1H NMR spectrum of the cyclized product exhibited well-separated signals of the protons, and the relative stereochemistry of this compound was firmly established by the NOEs as shown in **10′**. The cyclized compound **10** was then saponified, giving **12**, and the alcohol was further converted to the (*S*)- and (*R*)-MTPA

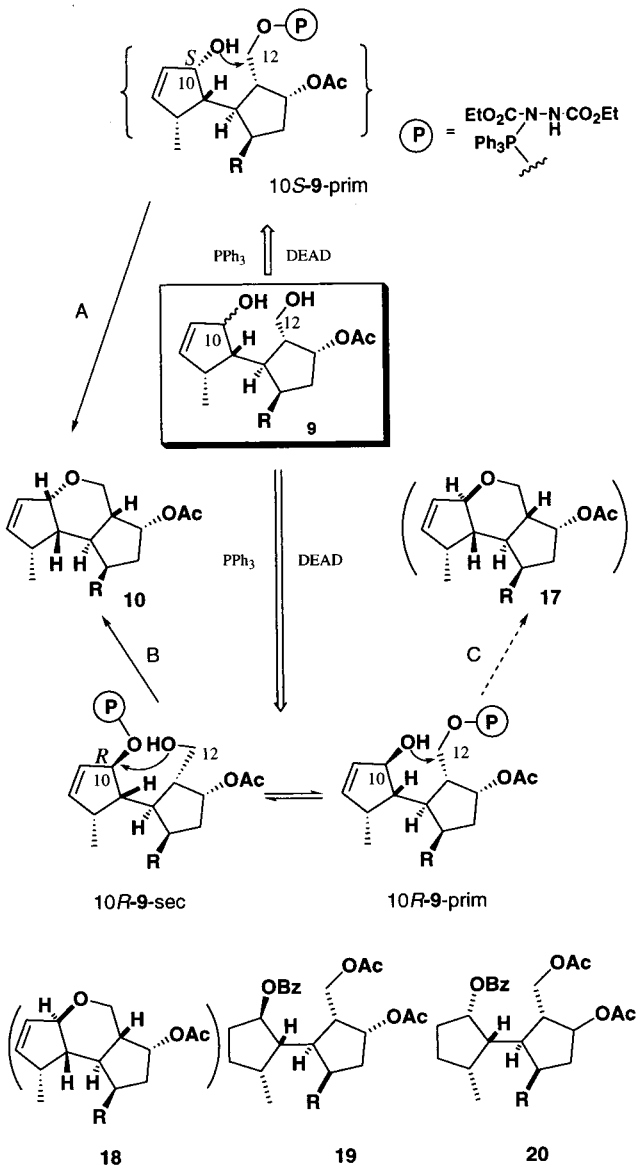


esters (**13**). The $\Delta\delta$ values of the protons summarized in the formula (**10″**) are systematically arranged on the right- and left-hand sides of the MTPA plane, confirming the *R* configuration of the hydroxyl group at C-5. This finding automatically established the absolute stereochemistry of dilkamural (**1**) as shown.

The reaction course of the Mitsunobu reaction of **9** should be considered here. The reagent (DEAD-PPh_3) would activate the primary alcohol (12-OH) preferentially rather than the secondary alcohol (10-OH). In this case, since the free secondary alcohol would attack at C-12 (10*S* -**9**-prim: route A), the secondary hydroxyl group (10-OH) should have the *S* configuration considering the stereochemistry of **10**. It turned out, however, that the benzoate (**15**) of **9** showed a negative Cotton effect at 230 nm (EtOH), indicating the *R* configuration of 10-OH. The modified Mosher's method carried out on **9** (see **9′**) also supported the *R* configuration of the 10-OH. These results imply that in the Mitsunobu reaction the activated secondary alcohol (C-10) of 10*R*-**9**-sec was attacked by the primary alcohol (12-OH), which resulted in inversion of the configuration at C-10 (route B).

On the basis of these facts, we propose the following reaction course: The phosphonium activator will attach to either the secondary (10*R*-**9**-sec) or primary (10*R*-**9**-prim) alcohols, the latter being sterically preferable. These two species would be in equilibrium.[9] In 10*R*-**9**-prim, attack of the secondary alcohol to the primary carbon (route C) would give sterically strained trans-anti-trans product **17**, while the cyclized trans-anti-cis product **10** from 10*R*-**9**-sec is sterically less compressed (route A).[10] Here, one should recall that the tosylation of **5**

(6) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
(7) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *29*, 2226.
(8) Mitsunobu, O.; Eguchi, M. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 3472.
(9) Robinson, P. L.; Barry, C. N.; Bass, S. W.; Jarvis, S. E.; Evans, S. A. *J. Org. Chem.* **1983**, *48*, 5396.



afforded the cyclized product (**6**: trans-anti-cis), whereas the reaction of **9** with TsCl afforded only the tosylate **11** instead of the expected cyclized trans-anti-trans product **18**. This fact is also compatible with the retarded cyclization of 10*S*-**9**-prim and the instability of the trans-anti-trans system.

Hydrogenation of the benzoate **15** by using a homogeneous catalyst afforded **19**, the ^{1}H NMR data of which were different from those of **20**, which was obtained by monoacetylation of **5** followed by benzoylation. This finding unambiguously establishes the stereochemistry of **5**.

Diterpenoids **1** and **2** exhibited an antimicrobial activity against *Bacillus subtilis*, a Gram-positive microorganism: **1** and **2** showed 12 and 9 mm of clear zone diameter at 10 μg/ disk, respectively, in the agar-disk diffusion method. Compound **1** also showed weak inhibitory activity against a species of plant pathogenic mold (*Colletotrichum lagenarium*). Some secospatanes have been reported as feeding-deterrent substances against the young abalone.[11] Diterpenoid **1**, present in a large amount (1.9% in freeze-dried alga), might therefore serve as a chemical defense substance against mollusks and fish.

(10) Substituted *cis*-hydroindans are known to be more thermodynamically stable than *trans*-hydroindans. The presence of two trans-fused cyclopentane units in **17** would make the tricyclic system less favorable than **10** (or **6**). Validation of this assumption by MM calculation is in progress.

Experimental Section

^{1}H NMR (^{13}C NMR) spectra were recorded at 270 (67.5) and 600 (150) MHz. Chemical shifts are reported relative to TMS (δ 0), and coupling constants are given in hertz.

Algal Collection and Isolation of Dilkamural 1. *D. okamurae* Dawson was collected in May 1994 at the intertidal zones around the Honai Beach, Ehime Prefecture, Japan. The collection was immediately washed with seawater and freeze-dried. Dried alga (100 g) was powdered and soaked in 1 L of EtOAc for 1 day at room temperature with shaking. The EtOAc was evaporated in vacuo to give a dark-green residue (3.0 g) that was applied to a column (2.5 × 20 cm) of COSMOSIL PREP C_{18} (Nakarai Tesque), and the column was eluted with 500 mL of acetonitrile to remove the pigments. The eluate was concentrated under reduced pressure and applied to a column (4 × 45 cm) of Kieselgel 60 (Merck), and the column was eluted with a stepwise gradient solvent system of EtOAc−hexane. The eluate from 20% EtOAc/hexane was charged on a normal-phase HPLC column (2 × 25 cm) of SIL-06 (YMC), and it was eluted with 5% EtOH/hexane to yield pure dilkamural (**1**) (1.9 g): $[\alpha]^{27}_D$ +32° (*c* = 0.30, $CHCl_3$); IR (film) 1738, 1374, 1238, 1174, 1024 cm^{-1}; HRFABMS *m/z* 359.2222 (MH^+ − AcOH, 0 mamu deviation, $C_{22}H_{31}O_4$); low-resolution FABMS obsd *m/z* 359 (MH^+ − AcOH, 20), 299 (MH^+ − 2AcOH, 100), 281 (21); ^{1}H NMR (270 MHz, C_6D_6) δ 9.86 (1H, d, *J* = 1.7 Hz, H-12), 5.59 (1H, ddd, *J* = 7.6, 6.2, 4.3 Hz, H-5), 5.16 (1H, bt, *J* = 6.2 Hz, H-15), 5.09 (1H, bt, *J* = 7.0 Hz, H-17), 4.80 (1H, dd, *J* = 5.4, 1.4 Hz, H-2), 3.48 (1H, ddd, *J* = 7.6, 6.5, 1.7 Hz, H-4), 3.38 (1H, dt, *J* = 8.4, 8.1, 5.9 Hz, H-7), 3.06 (1H, m, H-8), 2.67 (2H, m, H-16), 2.48 (1H, m, H-1), 2.31 (1H, dd, *J* = 9.7, 7.3 Hz, H-9), 2.11 (1H, dd, *J* = 5.4, 19.4 Hz, H-3α), 1.99 (1H, dd, *J* = 19.4, 1.4 Hz, H-3β), 1.90 (1H, ddd, *J* = 14.6, 6.2, 5.9, H-6α), 1.77 (1H, ddd, *J* = 14.6, 8.1, 4.3 Hz, H-6β), 1.66 (3H, s, H-19), 1.61 (3H, s, H-22), 1.60 (3H, s, H-24), 1.53 (3H, s, H-20), 1.41 (3H, s, H-14), 0.69 (3H, d, *J* = 7.3 Hz, H-11); ^{13}C NMR (67.5 MHz, C_6D_6) δ 214.9 (s, C-10), 197.8 (d, C-12), 169.8 (s, C-23), 169.3 (s, C-21), 134.5 (s, C-13), 132.0 (s, C-18), 129.0 (d, C-15), 123.0 (d, C-17), 76.2 (d, C-5), 75.3 (d, C-2), 57.9 (d, C-4), 50.6 (d, C-9), 41.6 (t, C-3), 40.5 (d, C-7), 40.0 (d, C-1), 37.5 (d, C-8), 37.1 (t, C-6), 27.5 (t, C-16), 25.8 (q, C-14), 22.2 (q, C-19), 20.7 (q, C-24), 20.6 (q, C-22), 17.7 (q, C-20), 13.6 (q, C-11).

Deacetoxylation of 1. A solution of dilkamural (**1**; 0.5 g, 1.20 mmol) in CH_2Cl_2 (10 mL) was allowed to stand with 2 g of Kieselgel 60 (Merck) overnight. The silica gel was washed with CH_2Cl_2, and the washing was concentrated to give **2** quantitatively. Compound **2**: $[\alpha]^{27}_D$ +33° (*c* = 0.33, $CHCl_3$); IR (film) 1738, 1703, 1460, 1374, 1342, 1237, 1067, 1024 cm^{-1}; HRFABMS *m/z* 359.2223 (MH^+, 0.1 mamu deviation, $C_{22}H_{30}O_4$); low-resolution FABMS obsd *m/z* 359 (MH^+, 8), 299 (MH^+ − AcOH, 13); ^{1}H NMR (270 MHz, C_6D_6) δ 9.82 (1H, d, *J* = 1.5 Hz, H-12), 6.77 (1H, dd, *J* = 5.9, 3.0 Hz, H-2), 5.77 (1H, dd, *J* = 5.9, 1.9 Hz, H-3), 5.64 (1H, ddd, *J* = 7.3, 6.8, 3.5, H-5), 5.18 (1H, bt, *J* = 7.3 Hz, H-15), 5.08 (1H, bt, *J* = 6.3 Hz, H-17), 3.73 (1H, ddd, *J* = 10.0, 7.3, 1.5 Hz, H-4), 3.46 (1H, m, H-7), 3.17 (1H, m, H-8), 2.63 (3H, m, H-1, 16), 2.14 (2H, m, H-6α, 9), 1.82 (1H, m, H-6β), 1.65 (3H, s, H-14), 1.59 (3H, s, H-24), 1.54 (3H, s, H-20), 1.45 (3H, s, H-19), 0.88 (3H, d, *J* = 7.3 Hz, H-11).

Hydrolysis of 2. A solution of **2** (0.107 g, 0.30 mmol) in MeOH (1 mL) was added into 200 mM phosphate buffer (pH 7.0; 50 mL) containing 12 mg of carboxyl esterase (EC 3.1.1.1, crude product from *Streptomyces rochei*) (Wako Pure Chemical), and the mixture was incubated at 30 °C for 24 h. The mixture was extracted with EtOAc (3 × 50 mL), and the combined EtOAc extract was washed with water (3 × 100 mL)

(11) Taniguchi, K.; Kurata, K.; Suzuki, M. *Kagaku to Seibutsu* **1994**, *32*, 434.

and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded an oil, which was purified by HPLC [YMC-pack SIL-06 (2 × 25 cm) (YMC)]. Elution with 20% EtOH/hexane yielded the hydrolyzed product **3** (0.061 g, 0.19 mmol, 63%). Compound **3**: IR (film) 3423, 1704, 1381, 1344, 1077 cm^{-1}; HRFABMS *m/z* 317.2113 (MH^+, 0.3 mamu deviation, $C_{20}H_{29}O_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 9.88 (1H, d, J = 1.9 Hz, H-12), 7.59 (1H, dd, J = 5.7, 3.0 Hz, H-2), 6.02 (1H, dd, J = 5.7, 1.6 Hz, H-3), 5.26 (1H, bt, J = 7.4 Hz, H-15), 5.06 (1H, bt, J = 6.2 Hz, H-17), 4.80 (1H, dt, J = 5.9, 3.0 Hz, H-5), 3.67 (1H, ddd, J = 8.6, 8.4, 6.2 Hz, H-7), 3.55 (1H, ddd, J = 11.1, 5.9, 1.9 Hz, H-4), 3.00 (2H, m, H-1,8), 2.76 (2H, m, H-16), 2.33 (1H, dd, J = 9.2, 5.9 Hz, H-9), 2.13 (1H, m, H-6α), 1.88 (1H, ddd, J = 14.2, 8.4, 3.0 Hz, H-6*β*), 1.71 (3H, s, H-14), 1.66 (3H, s, H-20), 1.65 (3H, s, H-19), 1.17 (3H, d, J = 7.3 Hz, H-11).

MTPA Esters of 3. The hydrolyzed compound **3** (30 mg, 0.09 mmol) was dissolved in 1 mL of CH_2Cl_2, into the solution were added DMAP (0.1 mmol), DCC (0.1 mmol) and (*S*)- or (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) (0.2 mmol), and the reaction mixture was allowed to stand at room temperature for 18 h. The solvent was evaporated in vacuo, and the crude product was purified by HPLC (see above). Elution with 5% EtOH/hexane yielded the MTPA esters **4** [(*S*)-MTPA ester, 12 mg, 0.023 mmol, 25%; (*R*)-MTPA ester, 9 mg, 0.017 mmol, 19%). **4** [(*R*)-MTPA ester]: IR (film) 1749, 1700, 1452, 1273, 1171, 1123, 1021 cm^{-1}; HRFABMS *m/z* 532.2441 (M^+, 0.5 mamu deviation, $C_{30}H_{35}F_3O_5$); ^1H NMR (270 MHz, $CDCl_3$) δ 9.58 (1H, d, J = 2.2 Hz, H-12), 7.58 (1H, dd, J = 5.9, 3.0 Hz, H-2), 7.42 (5H, m, MTPA), 6.01 (1H, dd, J = 5.9, 1.9 Hz, H-3), 5.86 (1H, dt, J = 6.5, 2.7 Hz, H-5), 5.29 (1H, bt, J = 6.6 Hz, H-15), 5.02 (1H, bt, J = 7.3 Hz, H-17), 3.71 (1H, ddd, J = 10.7, 6.5, 2.2 Hz, H-4), 3.49 (3H, s, MTPA), 3.44 (1H, m, H-7), 2.96 (2H, m, H-1, 8), 2.68 (2H, m, H-16), 2.30 (2H, m, H-6α, 9), 1.92 (1H, ddd, J = 14.8, 8.4, 2.7 Hz, H-6*β*), 1.71 (3H, s, H-14), 1.65 (3H, s, H-20), 1.63 (3H, s, H-19), 1.11 (3H, d, J = 7.3 Hz, H-11). **4** [(*S*)-MTPA ester]: δ 9.50 (1H, d, J = 1.6 Hz, H-12), 7.57 (1H, dd, J = 5.9, 2.0 Hz, H-2), 7.44 (5H, m, MTPA), 6.01 (1H, dd, J = 5.9, 1.6 Hz, H-3), 5.92 (1H, dt, J = 6.3, 3.5 Hz, H-5), 5.31 (1H, bt, J = 7.6 Hz, H-15), 5.03 (1H, bt, J = 7.3 Hz, H-17), 3.77 (1H, m, H-4), 3.54 (1H, m, H-7), 3.48 (3H, s, MTPA), 3.01 (2H, m, H-1, 8), 2.71 (2H, m, H-16), 2.36 (2H, m, H-9, 6α), 2.00 (1H, ddd, J = 14.3, 8.1, 3.5 Hz, H-6*β*), 1.70 (3H, s, H-14), 1.67 (3H, s, H-20), 1.64 (3H, s, H-19), 1.14 (3H, d, J = 7.6 Hz, H-11).

Sodium Borohydride Reduction of 2. A solution of **2** (0.212 g, 0.59 mmol) in MeOH (5 mL) was treated with a MeOH solution (1 mL) of $NaBH_4$ (0.045 g, 1.18 mmol) with stirring at 0 °C. The mixture was stirred for 10 min, and water (50 mL) was added. The product was extracted with EtOAc (3 × 50 mL). The EtOAc extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification was performed by HPLC (see above) eluted with 20% EtOH/hexane, yielding the 1,4-reduced diol **5** (95 mg, 0.26 mmol, 44%). **5**: IR (film) 3370, 1738, 1450, 1376, 1244, 1025 cm^{-1}; HREIMS *m/z* 364.2599 (M^+, 1.4 mamu deviation, $C_{22}H_{36}O_4$); ^1H NMR (270 MHz, $CDCl_3$) δ 5.45 (1H, dt, J = 6.8, 3.5 Hz, H-5), 5.12 (1H, bt, J = 7.8 Hz, H-15), 5.07 (1H, bt, J = 7.3 Hz, H-17), 4.34 (1H, t, J = 4.3 Hz, H-10), 3.92 (1H, dd, J = 12.2, 4.1 Hz, H-12), 3.88 (1H, dd, J = 12.2, 5.1 Hz), 3.53 (1H, dt, J = 8.1, 7.8, 4.9 Hz, H-7), 2.74 (3H, m, H-8, 16), 2.36 (2H, m, H-4, 9), 2.23 (1H, m, H-1), 2.09 (3H, s, H-24), 2.04 (1H, m, H-6α), 1.83 (3H, m, H-2α, 3α, 6*β*), 1.69 (3H, s, H-14), 1.66 (3H, s, H-20), 1.63 (3H, s, H-19), 1.52 (2H, m, H-2*β*, 3*β*), 1.11 (3H, d, J = 7.3 Hz, H-11).

Cyclization of 5 with Tosyl Chloride. The diol **5** (0.095 g, 0.26 mmol) was dissolved in pyridine (6 mL), and the solution was cooled in an ice bath. Tosyl chloride (0.05 g, 0.27 mmol) was added to this solution in two portions at 1 h intervals with stirring. The reaction mixture was allowed to warm to room temperature, and after 24 h, an aqueous $NaHCO_3$ solution (100 mL) was added. The mixture was extracted with EtOAc (3 × 50 mL). The EtOAc extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification was performed by HPLC (see above), eluted with 5% EtOH/hexane to yield the cyclic ether **6** (26 mg, 0.075 mmol, 29%). **6**: IR (film) 1738, 1375, 1358, 1238, 1100, 1078 cm^{-1}; HREIMS *m/z* 346.2509 (M^+, 0.1 mamu deviation, $C_{22}H_{34}O_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 5.27 (1H, dt, J = 5.7, 1.9 Hz, H-5), 5.15 (1H, bt, J = 7.6 Hz, H-15), 5.06 (1H, bt, J = 7.3 Hz, H-17), 3.92 (1H, m, H-10), 3.80 (1H, dd, J = 10.5, 4.6 Hz, H-12*β*), 3.60 (1H, dd, J = 10.5, 9.2 Hz, H-12α), 3.49 (1H, dt, J = 8.6, 5.1 Hz, H-7), 2.73 (2H, m, H-16), 2.07 (4H, m, H-1, 6, 8), 2.10 (3H, s, H-24), 1.89 (2H, m, H-3α, 9), 1.69 (3H, s, H-14), 1.66 (3H, s, H-20), 1.63 (2H, m, 2α, 3*β*), 1.62 (3H, s, H-19), 1.48 (1H, m, H-2*β*), 0.99 (3H, d, J = 7.0 Hz, H-11).

Hydrolysis of 6. The cyclized product **6** (30 mg, 0.087 mmol) was dissolved in MeOH (150 μL), and the solution was treated with a 1 M solution of sodium methoxide in methanol (15 μL). The mixture was allowed to stand for 4 h at room temperature and poured onto a 200 mM phosphate buffer solution (pH 7.5; 5 mL). The product was extracted with EtOAc (3 × 50 mL). The organic extract was washed with water and dried over anhydrous $MgSO_4$, and the solution was concentrated in vacuo. Purification by HPLC (see above) eluted with 20% EtOH/hexane afforded the deacetylated product **7** (17 mg, 0.056 mmol, 64%). This product was directly used for the next reaction.

MTPA Esters of 7. The (*S*)- and (*R*)-MTPA esters of **7** (each 8 mg, 0.026 mmol) were prepared according to the aforementioned method, yielding the MTPA esters **8** [(*S*)-MTPA ester, 12 mg, 0.023 mmol, 89%; (*R*)-MTPA ester, 11 mg, 0.021 mmol, 81%]. **8** [(*S*)-MTPA ester]: IR (film) 1746, 1275, 1240, 1170, 1123, 1022 cm^{-1}; HRFABMS *m/z* 520.2805 (M^+, 0.5 mamu deviation, $C_{30}H_{39}F_3O_4$); ^1H NMR (600 MHz, $CDCl_3$; several signals are overlapping) δ 7.55 (2H, m, MTPA), 7.42 (3H, m, MTPA), 5.16 (1H, m, H-5), 5.46 (1H, bt, J = 7.5 Hz, H-15), 5.05 (1H, bt, J = 7.2 Hz, H-17), 3.97 (1H, m, H-10), 3.76 (1H, dd, J = 10.5, 4.9 Hz, H-12*β*), 3.56 (3H, s, MTPA), 3.45 (2H, m, H-7, 12α), 2.77 (1H, m, H-16a), 2.63 (1H, m, H-16b), 2.06 (3H, m, H-4, 6, 8), 1.99 (1H, m, H-1), 1.90 (1H, m, H-9), 1.82 (1H, m, H-3α), 1.69 (3H, s, H-14), 1.67 (3H, s, H-20), 1.62 (3H, s, H-19), 1.58 (2H, m, H-2α, 3*β*), 1.36 (1H, m, H-2*β*), 0.93 (3H, d, J = 7.2 Hz, H-11). **8** [(*R*)-MTPA ester]: ^1H NMR (600 MHz, $CDCl_3$; several signals are overlapping) δ 7.52 (2H, m, MTPA), 7.42 (3H, m, MTPA), 5.41 (1H, dt, J = 4.2, 1.7 Hz, H-5), 5.15 (1H, bt, J = 7.2 Hz, H-15), 5.03 (1H, bt, J = 6.3 Hz, H-17), 3.98 (1H, m, H-10), 3.80 (1H, dd, J = 10.8, 7.2 Hz, H-12α), 3.55 (1H, dd, J = 10.8, 3.0 Hz, H-12*β*), 3.54 (3H, s, MTPA), 3.35 (1H, dt, J = 9.0, 6.0 Hz, H-7), 2.74 (1H, m, H-16a), 2.59 (1H, m, H-16b), 2.09 (1H, m, H-4), 1.97 (1H, m, H-1), 1.88 (1H, m, H-9), 1.82 (1H, m, H-3α), 1.68 (3H, s, H-14), 1.66 (3H, s, H-20), 1.61 (3H, s, H-19), 1.58 (2H, m, H-3*β*, 2α), 1.36 (1H, m, H-2*β*), 0.89 (3H, d, J = 7.6 Hz, H-11).

Sodium Borohydride Reduction of 2 in the Presence of Cerium Chloride. To a methanolic solution (15 mL) of **2** (0.462 g, 1.29 mmol) and $CeCl_3 \cdot 7H_2O$ (0.961 g, 2.58 mmol) was added a methanol solution (2 mL) of $NaBH_4$ (0.097 g, 2.58 mmol) with stirring at 0 °C. The reaction mixture was allowed to stand for 10 min, and 100 mL of water was added. The mixture was extracted with EtOAc (3 × 100 mL), and the extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. After purification by HPLC (see above) eluted with 20% EtOH/hexane, diol **9** (0.145 g, 0.40 mmol, 31%) was obtained. **9**: IR (film) 3370, 1738, 1440, 1376, 1245, 1107, 1042 cm^{-1}; HRFABMS *m/z* 345.2423 ($MH^+ - H_2O$, 0.6 mamu deviation, $C_{22}H_{33}O_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 5.90 (1H, m, H-2), 5.64 (1H, bd, J = 5.7 Hz, H-3), 5.52 (1H, dt, J = 6.2, 4.1 Hz, H-5), 5.15 (1H, bt, J = 7.3 Hz, H-15), 5.06 (1H, bt, J = 7.0 Hz, H-17), 4.67 (1H, bd, J = 7.8 Hz, H-10), 3.87 (1H, dd, J = 11.9, 4.1 Hz, H-12a), 3.70 (1H, dd, J = 11.9, 6.5 Hz, H-12b), 3.50 (1H, ddd, J = 11.3, 9.7, 6.5 Hz, H-7), 2.71 (5H, m, H-1, 4, 9, 16), 2.34 (1H, m, H-8), 2.08 (3H, s, H-24), 2.04 (1H, m, H-6α), 1.67 (1H, m, H-6*β*), 1.71 (3H, s, H-14), 1.70 (3H, s, H-20), 1.62 (3H, s, H-19), 0.91 (3H, d, J = 7.3 Hz, H-11).

Cyclization of 9 by the Mitsunobu Reaction. A solution of **9** (0.110 g, 0.30 mmol), diethyl azodicarbonate (DEAD as a 40% toluene solution, 150 μL, 0.34 mmol), and triphenylphosphine (90 mg, 0.34 mmol) in THF (2 mL) was allowed to stand at room temperature overnight. The reaction mixture was

concentrated, and the product was purified by HPLC eluted with 5% EtOH/hexane to yield the cyclic ether **10** (56 mg, 0.163 mmol, 54%). **10**: IR (film) 1738, 1372, 1237, 1073, 932 cm^{-1}; HREIMS *m/z* 344.2342 (M^+, 0.9 mamu deviation, $C_{22}H_{32}O_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 5.95 (1H, m, H-2), 5.73 (1H, m, H-3), 5.26 (1H, bt, J = 4.3 Hz, H-5), 5.19 (1H, bt, J = 7.0 Hz, H-15), 5.06 (1H, bt, J = 7.0 Hz, H-17), 4.33 (1H, bd, J = 3.5 Hz, H-10), 3.88 (1H, dd, J = 10.3, 6.8 Hz, H-12α), 3.54 (2H, m, H-7, 12β), 2.76 (3H, m, H-1, 16), 2.11 (5H, m, H-4, 6, 8, 9), 2.05 (3H, s, H-24), 1.69 (3H, s, H-14), 1.67 (3H, s, H-20), 1.62 (3H, s, H-19), 1.07 (3H, d, J = 7.3 Hz, H-11).

MTPA Esters of 12. The cyclized compound **10** (14 mg, 0.041 mmol) was saponified (NaOH/MeOH) to give the deacetylated product **12** (12 mg, 0.039 mmol, 95%). Halves of **12** (6 mg, 0.017 mmol) were converted to (*S*)- and (*R*)-MTPA esters by the aforementioned method to yield the diastereomeric MTPA esters **13** [(*S*)-MTPA ester, 8 mg, 0.015 mmol, 88%; (*R*)-MTPA ester, 7 mg, 0.013 mmol, 76%]. **13** [(*S*)-MTPA ester]: IR (film) 1748, 1452, 1272, 1180, 1125, 1019 cm^{-1}; HRFABMS *m/z* 518.2650 (M^+, 0.6 mamu deviation, $C_{30}H_{37}F_3O_4$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.51 (2H, m, MTPA), 7.40 (3H, m, MTPA), 5.90 (1H, m, H-2), 5.68 (1H, m, H-3), 5.44 (1H, bs, H-5), 5.21 (1H, bt, J = 7.3 Hz, H-15), 5.05 (1H, bt, J = 7.3 Hz, H-17), 4.36 (1H, bd, J = 3.8 Hz, H-10), 3.84 (1H, m, H-12α), 3.49 (1H, m, H-12β), 3.54 (3H, s, MTPA), 3.44 (2H, m, H-7), 2.80 (1H, m, H-16α), 2.65 (2H, m, H-1, 16β), 2.13−2.17 (5H, m, H-4, 6, 8, 9), 1.70 (3H, s, H-14), 1.69 (3H, s, H-20), 1.65 (3H, s, H-19), 1.00 (3H, d, J = 7.3 Hz, H-11). **13** [(*R*)-MTPA ester]: ^1H NMR (270 MHz, $CDCl_3$) δ 7.49 (2H, m, MTPA), 7.40 (3H, m, MTPA), 5.96 (1H, m, H-2), 5.69 (1H, m, H-3), 5.40 (1H, dt, J = 3.5 Hz, H-5), 5.19 (1H, bt, J = 7.3 Hz, H-15), 5.04 (1H, bt, J = 7.3 Hz, H-17), 4.36 (1H, bd, J = 5.7 Hz, H-10), 3.89 (1H, m, H-12α), 3.60 (1H, m, H-12β), 3.53 (3H, s, MTPA), 3.34 (1H, m, H-7), 2.76 (1H, m, H-16α), 2.61 (2H, m, H-1, 16β), 2.12−2.18 (5H, m, H-4, 6, 8, 9), 1.69 (3H, s, H-14), 1.67 (3H, s, H-20), 1.61 (3H, s, H-19), 0.97 (3H, d, J = 7.3 Hz, H-11).

Attempted Cyclization of 9. To a solution of **9** (25 mg, 0.07 mmol) in pyridine (1 mL), cooled in an ice bath, was added TsCl (26 mg, 0.14 mmol) in two portions at 1 h intervals with stirring. The reaction mixture was allowed to warm to room temperature, and after 24 h, 100 mL of aqueous $NaHCO_3$ was added. The mixture was extracted with EtOAc (3 × 50 mL), and the EtOAc extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated. HPLC purification by elution with 20% EtOH/hexane gave C12-tosylate **11** (15 mg, 0.029 mmol, 41%). **11**: IR (film) 3517, 1738, 1452, 1362, 1242, 1177, 1108, 948 cm^{-1}; HRFABMS *m/z* 439.2312 ($MH^+ - H_2O - CH_3COOH$, 0.6 mamu deviation, $C_{27}H_{35}O_3S$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.80 (2H, d, J = 8.6 Hz, 12-OTs), 7.34 (2H, d, J = 7.8 Hz, 12-OTs), 5.88 (1H, m, H-2), 5.58 (1H, bd, J = 5.7 Hz, H-3), 5.40 (1H, dt, J = 6.2, 4.1 Hz, H-5), 5.13 (1H, bt, J = 7.0 Hz, H-15), 5.03 (1H, bt, J = 7.0 Hz, H-17), 4.47 (1H, dd, J = 9.2, 4.3 Hz, H-12a), 4.40 (1H, bt, J = 7.3 Hz, H-10), 4.23 (1H, t, J = 9.2 Hz, H-12b), 3.44 (1H, m, H-7), 2.70 (5H, m, H-1, 4, 9, 16), 2.44 (3H, s, ArMe), 2.11 (1H, m, H-8), 1.97 (1H, m, H-6α), 1.97 (3H, s, H-24), 1.80 (1H, m, H-6β), 1.69 (3H, s, H-14), 1.64 (3H, s, H-20), 1.61 (3H, s, H-19), 1.43 (1H, bd, J = 7.3 Hz, 10-OH), 0.86 (3H, d, J = 7.0 Hz, H-11).

Benzoylation of 14. A solution of **9** (32 mg, 0.09 mmol) and Ac_2O (300 μL) in pyridine (1 mL) was stirred in an ice bath for 15 min. The usual workup and purification by HPLC yielded the C12-acetate **14** (24 mg, 0.047 mmol, 52%), which was dissolved in 1 mL of pyridine. The solution was cooled in an ice bath and treated with 100 μL of benzoyl chloride, and the mixture was stirred for 30 min. An aqueous $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The EtOAc extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by HPLC eluted with 5% EtOH/hexane yielded the C10-benzoate **15** (24 mg, 0.047 mmol, 52%). **15**: IR (film) 1741, 1717, 1366, 1272, 1248, 1112, 1070, 1027 cm^{-1}; HRFABMS *m/z* 386.2455 ($M^+ - C_7H_6O_2$, 0.2 mamu deviation, $C_{24}H_{34}O_4$); ^1H NMR (270 MHz, $CDCl_3$) δ 8.01 (2H, d, J = 8.1 Hz, 10-OBz), 7.52 (3H, m, 10-OBz), 6.04 (1H, m, H-2), 5.85 (1H, bd, J = 5.9 Hz, H-3), 5.74 (1H, bd, J = 7.8 Hz, H-10), 5.50 (1H, m, H-5), 5.24 (1H, bt, J = 7.6 Hz, H-15), 5.08 (1H, bt, J = 7.0 Hz, H-17), 4.11 (1H, dd, J = 11.1, 8.6 Hz, H-12a), 3.97 (1H, dd, J = 11.1, 4.6 Hz, H-12b), 3.51 (1H, m, H-7), 2.76 (4H, m, H-1, 4, 16), 2.39 (2H, m, H-8, 9), 2.09 (1H, m, H-6α), 2.03 (3H, s, H-24), 1.87 (1H, m, H-6β), 1.80 (3H, s, H-14), 1.70 (3H, s, H-20), 1.69 (3H, s, 12-OAc), 1.64 (3H, s, H-19), 0.98 (3H, d, J = 7.0 Hz, H-11).

Preparation of MTPA Esters of 14. The (*S*)- and (*R*)-MTPA esters of **14** (each 15 mg, 0.038 mmol) were prepared according to the aforementioned method. MTPA **16** [(*S*)-MTPA ester, 14 mg, 0.023 mmol, 61%; (*R*)-MTPA ester, 14 mg, 0.023 mmol, 61%]. **16** [(*S*)-MTPA ester]: IR (film) 1743, 1367, 1246, 1170, 1025 cm^{-1}; HRFABMS *m/z* 386.2451 ($M^+ - C_7H_6O_2$, 0.6 mamu deviation, $C_{24}H_{34}O_4$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.55 (2H, m, MTPA), 7.40 (3H, m, MTPA), 6.08 (1H, m, H-2), 5.81 (1H, m, H-3), 5.74 (1H, bd, J = 5.4 Hz, H-10), 5.18 (2H, m, H-5, 15), 5.05 (1H, bt, J = 7.0 Hz, H-17), 4.06 (1H, dd, J = 11.3, 7.6 Hz, H-12a), 3.94 (1H, dd, J = 11.3, 5.4 Hz, H-12b), 3.58 (3H, s, MTPA), 3.38 (1H, m, H-7), 2.77 (1H, m, H-1), 2.71 (2H, m, H-16), 2.28 (1H, m, H-9), 2.27 (1H, m, H-8), 2.03 (3H, s, H-24), 2.01 (3H, s, 12-OAc), 1.84 (1H, m, H-6α), 1.71 (1H, m, H-6β), 1.69 (3H, s, H-14), 1.62 (3H, s, H-20), 1.59 (3H, s, H-19), 0.92 (3H, d, J = 6.8 Hz, H-11). **16** [(*R*)-MTPA ester]: ^1H NMR (270 MHz, $CDCl_3$) δ 7.52 (2H, m, MTPA), 7.42 (3H, m, MTPA), 6.03 (1H, m, H-2), 5.76 (1H, m, H-3), 5.73 (1H, bd, J = 5.4 Hz, H-10), 5.31 (1H, m, H-5), 5.19 (1H, bt, J = 6.8 Hz, H-15), 5.05 (1H, m, H-17), 4.10 (1H, dd, J = 11.3, 8.6 Hz, H-12a), 3.97 (1H, dd, J = 11.3, 5.4 Hz, H-12b), 3.46 (3H, s, MTPA), 3.41 (1H, m, H-7), 2.79 (1H, m, H-1), 2.72 (2H, m, H-16), 2.40 (1H, m, H-4), 2.28 (1H, m, H-9), 2.28 (1H, m, H-8), 2.02 (3H, s, H-24), 2.01 (3H, s, 12-OAc), 1.92 (1H, m, H-6α), 1.77 (1H, ddd, J = 13.8, 7.6, 4.3 Hz, H-6β), 1.69 (3H, s, H-14), 1.62 (6H, s, H-19, 20), 0.92 (3H, d, J = 7.0 Hz, H-11).

Preparation of 19 with Wilkinson's Catalyst. A solution of **15** (8 mg, 0.015 mmol) in MeOH (5 mL) was stirred with Wilkinson's catalyst [chlorotris(triphenylphosphine)rhodium-(I)] (0.05 g) under hydrogen at room temperature for 1 h. The mixture was applied onto a silica gel column (0.5 × 25 cm) [Kieselgel 60 (Merck)], and the column was eluted with EtOAc/hexane (1:3). Purification was performed by HPLC (see above) eluted with 2% EtOAc/hexane, yielding **19** (4 mg, 0.008 mmol, 53%): ^1H NMR (270 MHz, $CDCl_3$) δ 8.00 (2H, m), 7.56 (1H, m), 7.45 (2H, m), 5.51 (1H, m), 5.22 (1H, bt, J = 7.3 Hz), 5.08 (2H, m), 4.07 (1H, dd, 11.0, 8.9 Hz), 3.89 (1H, dd, J = 11.0, 4.3 Hz), 3.43 (1H, m), 2.02 (3H, s), 1.80 (3H, s), 1.70 (3H, s), 1.64 (6H, s), 0.88 (3H, d, J = 7.0 Hz).

Preparation of 20. A solution of **5** (20 mg, 0.07 mmol) and Ac_2O (300 μL) in pyridine (1 mL) was stirred at 0 °C for 15 min. Workup and HPLC separation gave C12-acetate of **5**. This acetate (13 mg, 0.03 mmol) was treated with benzoyl chloride to yield **20** (3 mg, 0.006 mmol, 20%); ^1H NMR (270 MHz, $CDCl_3$) δ 7.99 (2H, m), 7.56 (1H, m), 7.44 (2H, m), 5.85 (1H, m), 5.25 (1H, bt, J = 6.8 Hz), 5.13 (2H, m), 4.81 (1H, bd, J = 6.2 Hz), 4.49 (1H, bs), 3.43 (1H, m), 2.01 (3H, s), 1.90 (3H, s), 1.83 (3H, s), 1.70 (3H, s), 1.63 (3H, s), 0.92 (3H, d, J = 7.6 Hz).

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