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New Polyketide Peroxides from Okinawan Marine Sponge *Plakortis* sp.

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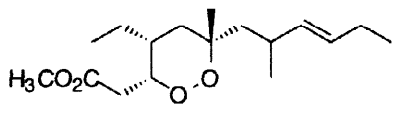
Abstract: New polyketide peroxides **2-4**, isolated from an Okinawan sponge *Plakortis* sp., have been fully characterized by spectroscopic and chemical methods. The absolute stereochemistries have been determined by analysis of MTPA esters of the acyclic derivatives. Compound **2** was cytotoxic against human epidermoid carcinoma KB and murine lymphoma L1210 cells.

Cyclic endoperoxides have been frequently isolated from natural sources.[2,3] Most of them are derived from sterols and fatty acids and are supposed to be formed by oxygen addition to 1,3-dienes. Organic extracts from sponges of the genus *Plakortis* also contain polyketide endoperoxides exemplified by plakortin (**1**).[4] Such compounds are featured by a 1,2-dioxane ring with an acetic group at C-3 and alkyl substituens at C-4 and C-6. Here we report the full spectroscopic characterization of the new peroxides **2-4** isolated from an Okinawan sponge *Plakortis* sp., from which we previously isolated manzamenones and related fatty acid derivatives.[5,6] The relative and absolute stereochemistries have been elucidated by NOEs analysis of the natural specimen and by MTPA method applied to the acyclic derivatives **5-8**.

The organic extract of the sponge was obtained and fractionated as described in the Experimental Section. Beside plakorin[7] and chondrillin,[8,9] which constituted more than 13.0% of the EtOAc soluble fraction of the MeOH extract, the sponge contained a complex mixture of cyclic peroxides (about 2.3% of the EtOAc soluble fraction). Repeated chromatography of this fraction gave **2** together with a mixture containing **3** and **4** as main components. Methylation with CH_2N_2 of this mixture followed by HPLC purification afforded methyl esters **3a** and **4a**.



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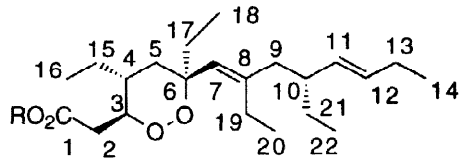

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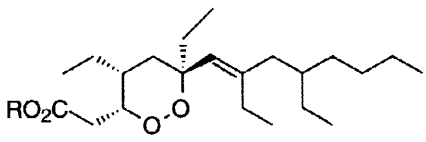
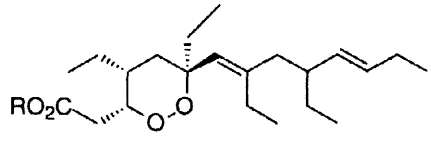
2a R=CH_3;



3 R=H;
3a R=CH_3;



4 R=H;
4a R=CH_3;

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PII: S0040-4020(97)10414-8

Table 1. NMR Data for Compounds **2**, **3a**, and **4a** in $CDCl_3$.

	2		**3a**		**4a**
	^1H δ (m, *J* in Hz)	^{13}C δ(m)	^1H δ (m, *J* in Hz)	^{13}C δ(m)	^1H δ (m, *J* in Hz)
1	-	176.1 (s)	-	n.d.	-
2	2.66 (dd, 15.8 and 3.0) 2.29 (bdd, 15.8 and 9.6)	36.1 (t)	3.03 (dd, 15.6 and 9.3) 2.39 (dd, 15.6 and 3.7)	31.4 (t)	3.02 (dd, 15.7 and 9.3) 2.38 (dd, 15.7 and 3.4)
3	4.18 (ddd, 9.6, 9.6 and 3.0)	81.7 (d)	4.47 (m)	78.8 (d)	4.46 (m)
4	1.57 (m)	37.2 (d)	2.10 (m)	35.6 (d)	2.12 (m)
5	1.98 (m) 1.28 (dd, 12.8, 12.8)	39.3 (t)	1.70 (dd,13.1 and 4.2) 1.25 (m)	35.8 (t)	1.71 (m) 1.28 (dd, 12.9, 12.9)
6	-	85.1 (s)	-	84.2 (s)	-
7	5.17 (s)	127.2 (d)	5.17 (s)	127.0 (d)	5.19 (s)
8	-	141.6 (s)	-	142.8 (s)	-
9	2.10 (m) 1.94 (m)	42.4 (t)	2.02 (dd, 13.4 and 7.9) 1.92 (dd, 13.4 and 8.1)	41.1 (t)	2.12 (m) 1.99 (m)
10	1.98 (m)	42.7 (d)	1.42 (bm)	36.8 (d)	1.99 (m)
11	5.12 (ddd, 15.2, 8.1 and 0.7)	133.3 (d)	1.25 (m) 1.20 (m)	25.8 (t)[a]	5.14 (m)
12	5.37 (ddd, 15.2, 6.5 and 6.5)	131.7 (d)	1.25 (m)	23.3 (t)[a]	5.38 (ddd, 15.2, 6.3 and 6.3)
13	1.96 (m)	25.6 (t)	1.25 (m)	28.7 (t)[a]	1.99 (m)
14	0.95 (t, 7.5)	14.0 (q)	0.90 (m)	14.1 (q)[b]	0.95 (t, 7.5)
15	1.42 (m) 1.09 (m)	23.9 (t)	1.25 (m) 1.18 (m)	32.4 (t)	1.41 (m) 1.16 (m)
16	0.89 (dd, 7.5, 7.5)	10.5 (q)	0.90 (m)	10.8 (q)	0.90 (t, 7.5)
17	1.59 (q, 7.5)	32.8 (t)	1.62 (m)	32.9 (t)	1.58 (m)
18	0.86 (t, 7.5)	7.8 (q)	0.86 (m)	7.7 (q)	0.84 (t, 7.5)
19	2.12 (m)	23.1 (t)	2.23 (m) 2.14 (m)	22.4 (t)	2.23 (m) 2.12 (m)
20	0.97 (t, 7.5)	11.6 (q)	0.97 (t, 7.4)	12.3 (q)	0.97 (t, 7.5 Hz)
21	1.40 (m) 1.16 (m)	27.8 (t)	1.26 (m) 1.12 (m)	25.1 (t)[a]	1.41 (m) 1.16 (m)
22	0.84 (t, 6.4)	12.2 (q)	0.86 (m)	11.1 (q)[b]	0.84 (t, 7.5)
Me			3.71 (s)	51.8 (q)	3.71 (s)

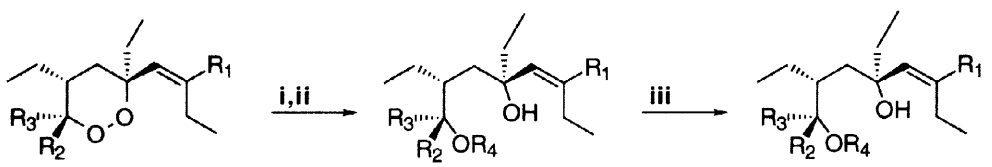
[a,b] Assignments with the same superscripted letter are interchangeable.

Compound **2**, $[\alpha]_D^{23}$ + 76.2° (*c* 1.6, $CHCl_3$), had the molecular formula $C_{22}H_{38}O_4$ with the highest peak in its EIMS occurring at *m/z* 337 ($C_{20}H_{33}O_4$, HREIMS *m/z* 337.2366) for the loss of an ethyl group from the parent molecule. The IR absorption at 1717 cm^{-1} together with the large band between 3480 and 2550 cm^{-1} supported the presence of carboxylic acid (δ 176.1 in the ^{13}C-NMR spectrum), as well as a small band at 1192 cm^{-1} was significant for the peroxidic linkage. The ^{13}C-NMR spectrum of **2** showed 22 carbon resonances including signals for two carbons bearing oxygen at δ 85.1 (s) and 81.7 (d), one trisubstituted (δ 141.6 and 127.2) and one disubstituted (δ 133.3 and 131.7) double bond. The down-field shifted H-3 (δ 4.18) correlated with the C-2 protons (δ 2.66 and 2.29) and with the methine signal at δ 1.57 (H-4). This latest hydrogen also showed cross-peaks with the mutually coupled signals at δ 1.98 (H-5a) and 1.28 (H-5b) as well as with the methylene protons at δ 1.42 and 1.09 (H_2-15), both coupled to the methyl group at δ 0.89 (CH_3-16). The branched alkyl chain was unambiguously ascertained by 2D NMR experiments which allowed elucidation of

C9-C11 and C12-C14 fragments (Table 1). Upon analysis of the COSY spectrum, H-10 further correlated to the methylene hydrogens at δ 1.40 (H–21a) and 1.16 (H-21b), both in turn coupled to the methyl triplet at δ 0.84 (H_3-22). The remaining proton signals were attributed to two isolated ethyl groups resonanting at δ 1.59 (H_2-17), δ 0.86 (H_3-18), δ 2.12 (H_2-19) and δ 0.97 (H_3-20). The HMBC data allowed the partial structures to be connected and completed the assignment. The NOESY spectrum of **2** showed cross-peaks between one C-2 proton (δ 2.29) and H-4 and between H-3 and H-5b. Consistent with those observations, H-5b/H-4 and H-4/H-3 exhibited large coupling constants (Table 1) expected for interactions between axial protons. This agreed with a 3,4-*trans* disubstituted 1,2-dioxane ring adopting a chair conformation. In addition, NOEs between H-7 and H-4, and between H-5b and H_2-17 allowed establishment of the axial orientation of the major alkyl chain. Finally, the *E* geometry of C11-C12 was assigned on the basis of a large coupling constant (J = 15.2 Hz) between H-11 (δ 5.12) and H-12 (δ 5.37), whereas the trisubstituted double bond C7-C8 was determined as *E* because of intense NOEs between H-7 (δ 5.17) and both C-9 protons (δ 2.10 and 1.94), as well as between H_2-17 (δ 1.59) and H_2-19 (δ 2.12).

Compounds **3** was characterized as the methyl ester derivative **3a**. The spectral properties of **3a**, $[\alpha]_D^{23}$ +12.4 ° (*c* 0.5, $CHCl_3$), were very similar to those of the methyl ester of **2**, except for the absence of the C11-C12 double bond and for the relative stereochemistry at C-3. Accordingly, the EIMS spectrum showed a highest peak at *m/z* 353, corresponding to the fragment having the molecular formula $C_{21}H_{37}O_4$ (HREIMS *m/z* 353.2683), and the peroxide ring was featured by signals at δ 3.03 and 2.39 (H_2-2), 4.47 (H-3), 2.10 (H-4), 1.70 and 1.25 (H_2-5). Comparison of the spectral data of **3a** with those reported for **1**[4] suggested a *cis* relation for the ring substituents at C-3 and C-4, which was confirmed by the NOEs from H-4 to the equatorial protons H-3 and H-5a. Moreover, the NOESY spectrum also showed cross-peaks between H-4 and H-7 (δ 5.17), as well as between H-7 and the methylene protons at C-9 (δ 2.02 and 1.92), thus suggesting for C-6 and for the double bond C-7/C-8 the same stereochemistry of **2**.

Treatment of **2a** (the methyl ester of **2**) with H_2 / (10%) Pd on charcoal afforded the diol **5**, which was esterified at C-3 by (*R*)- or (*S*)-MTPA chloride in dry pyridine (Scheme 1). Upon analysis of the MTPA derivatives **5a** and **5b**,[10,11] the absolute configuration of **5** at C-3 was *S*. Whereby coupled with the relative

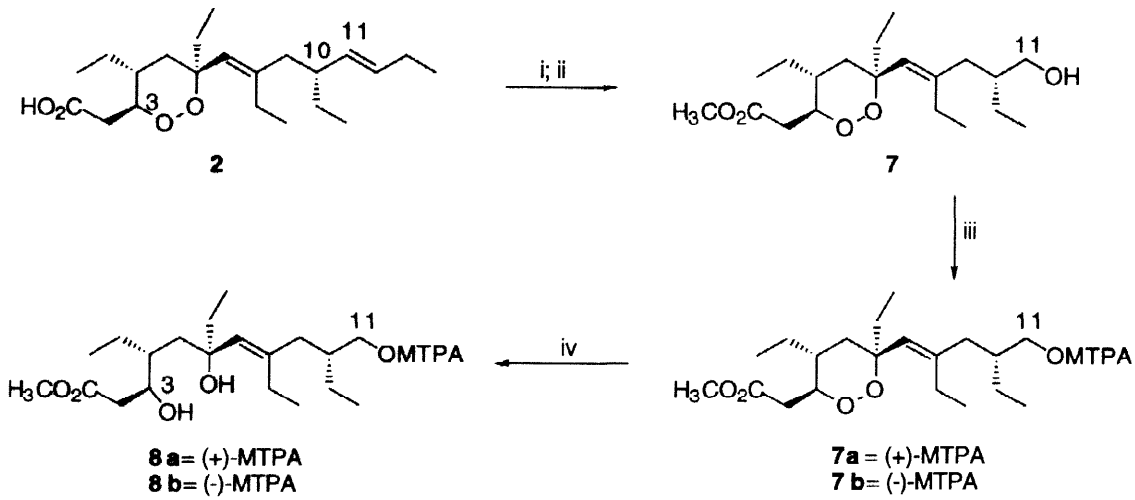


2a **3a** **5** **6** **5a** and **5b** **6a** and **6b**

	R_1	R_2	R_3	R_4
2a	C_8H_{15}	CH_2CO_2Me	H	-
3a	C_8H_{17}	H	CH_2CO_2Me	-
5	C_8H_{17}	CH_2CO_2Me	H	H
6	C_8H_{17}	H	CH_2CO_2Me	H
5a	C_8H_{17}	CH_2CO_2Me	H	(*S*)-MTPA
5b	C_8H_{17}	CH_2CO_2Me	H	(*R*)-MTPA
6a	C_8H_{17}	H	CH_2CO_2Me	(*S*)-MTPA
6b	C_8H_{17}	H	CH_2CO_2Me	(*R*)-MTPA

i. CH_2N_2 in THF, r.t., 1h (100%); **ii.** H_2-(10%) Pd/C, r.t., overnight (90%); **iii.** (*R*)- or (*S*)-MTPA Cl in pyridine, r.t., 2 h.

Scheme 1

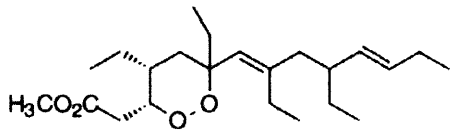


i. CH_2N_2 in THF, r.t., 1h (95%); **ii.** O_3 in MeOH, and then $NaBH_4$ -78° C, 20 min. (90%); **iii.** (+)- or (-)-MTPA Cl in pyridine, r.t., 4h (100%); **iv.** H_2-(10%) Pd/C, r.t., 2h (90%).

Scheme 2.

geometry above described, this established the complete absolute stereochemistry of the peroxide ring in **2** as *3S,4R, 6S*. In the same way, reductive cleavage of the peroxide ring in **3a** gave the diol **6**. Reaction with MTPA chlorides and analysis of the Mosher's derivatives **6a** and **6b** allowed assignment of *R* configuration at C3 in **6**, thus confirming that the absolute stereochemistry of **3a** was *3R,4R, 6S*. Recently a MTPA method has been proposed to assign the absolute stereochemistry at C-2 of primary β-methyl alcohol.[12,13] This procedure is based on the ^{1}H-NMR chemical shift difference of the hydroxymethylene protons in diasteromeric (+)- and (-)-MTPA esters. When the carbon β has *R* configuration, the two hydroxymethylene protons appear more apart in the (+)-MTPA derivative than in the (-)-MTPA. The contrary is true for the (*S*)-configuration.[12] Accordingly, compound **2a** was transformed into the alcohol derivative **7** by mild ozonolysis[14] followed by reduction with $NaBH_4$ (Scheme 2). Treatment of **7** with (+)- and (-)-MTPA chlorides gave the 11-(+) -MTPA (**7a**) and 11-(-)-MTPA (**7b**) esters, respectively. The 11-methylene protons resonated as two double doublets at δ 4.33 and 4.21 in the ^{1}H-NMR spectrum of **7a** and at δ 4.28 and 4.24 in that of **7b**. As the signal of H-3 (δ 4.23) partially overlapped one of the hydroxymethylene protons, the diols **8a** and **8b** were prepared by hydrogenolysis. In the ^{1}H-NMR spectra, the methylene signals of the (+)-MTPA derivative (**8a**) appeared well separated at δ 4.32 and 4.14, whereas those of the (-)-MTPA ester (**8b**) were very close at δ 4.24 and 4.22. These data were in well agreement with those previously reported in models[12] and suggested *R* configuration at C-10 of **2.**

The methyl ester **4a** was epimeric at C-3 with **2a** and shows the same substitution pattern of the endoperoxide ring in **3a**. Except for the optical rotation value, $[\alpha]_D^{23}$ + 49.8° (*c* 0.5, $CHCl_3$), spectral properties of **4a** were similar to those of **9**, previously reported with limited data by a collection of *Plakortis halichondrioides*.[15] The literature data allow no more accurate structural comparison between **4** and **9**, even if the different polarimetric properties suggest that they could be diasteromeric. The structure and the stereochemistry of **4a** were definitively proved by reduction with H_2/Pd which gave the diol **6** as main product, thus demonstrating that the natural acids **3** and **4** share the same absolute stereochemistry of the dioxane ring.



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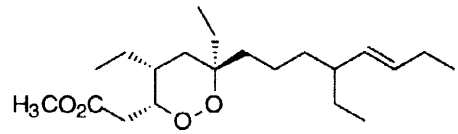

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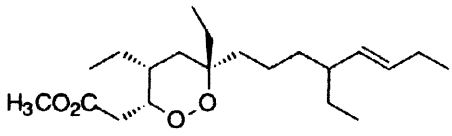

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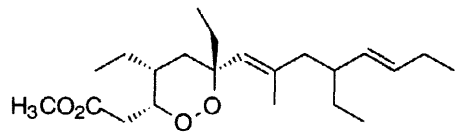

12

The spectroscopic analysis of **2-4** proves that those compounds occur in a chair conformation in solution. Although the elucidation of the elusive stereochemistry at C-6 has been accomplished by NOEs analysis, a simple comparison of ^{1}H-NMR data of **2a-3a** with those reported in literature for plakortide F-H (**10-12**)[16] reveals significant differences whereby the major alkyl chain is α (**11** and **12**) or β (**2a,3a** and **10**). In particular, one methylene proton of the C6 tertiary ethyl group in **11** and **12** (δ 2.05) is significantly further downfield than the corresponding hydrogen in **2a** (δ 1.60), **3a** (δ 1.62) and **10** (δ 1.48). This shift is affected neither by the type of alkyl chain nor by the ring substituents, and hence can be of general application to assign the relative stereochemistry of the quaternary stereocenter.

Despite the common occurrence of polyketide peroxides in marine sponges, their relative and absolute stereochemistry is not always reported. At the best of our knowledge, this is the first complete assignment of the absolute stereochemistry in plakortin-like endoperoxides. Compound **2** has cytotoxic activity against human epidermoid carcinoma KB (IC_{50}, 0.4 μg/mL) and murine lymphoma L1210 (IC_{50}, 1.1 μg/mL) cells. On the contrary, the methyl ester derivatives **2a-4a** were completely inactive.

Experimental Section

General Methods. Optical rotations were determined on a JASCO DIP-370 polarimeter. 1D- and 2D-NMR spectra were recorded on Bruker ARX-500 and Bruker AMX-600 spectrometers. The $CHCl_3$ resonances at δ 7.26 and δ 77.0 were used as internal references. EIMS spectra were obtained on a JEOL DX-303 spectrometer operating at 70 eV. Infrared data were recorded by JASCO FT/IR-230 spectrometer.

Collection, Extraction and Purification. The sponge *Plakortis* sp. (SS11) collected off Manzamo Okinawa, was the same as that previously reported.[5] The frozen material (1.7 kg, wet weight) was extracted with MeOH (2 x 1.5 L). After removing the volatile solvent, the aqueous residue was diluted by fresh water (350 mL) and extracted first with EtOAc (3x 350 mL) and later with *n*-BuOH (3 x 250 mL) to give 1.49 g of EtOAc extract and 4.98 g of BuOH extract. The crude EtOAc soluble material (1.49 g) was separated by silica gel column (130 g SiO_2) with solvent mixtures of increasing polarity from *n*-hexane/EtOAc 9:1 through EtOAc and $CHCl_3$ to MeOH. The earlier fractions (60 mg) were further chromatographied by Sephadex LH-20 eluting with $CHCl_3$/MeOH (1:1). The fractions positive in green to an acidic solution of anisaldehyde were joined and purified by reverse phase HPLC (Shiseido Capcell Pack C-18 column, 10x250 mm, MeOH/H_2O/TFA 85:15:0.125, 2.5 mL/min, detector UV 205 nm) to yield **2** (8.3 mg, t_R 31.0 min) and other four peaks containing peroxide

mixtures. Methylation of these fractions with CH_2N_2 in Et_2O (2 ml) at room temperature for 10 min, followed by HPLC first on reverse phase (Shiseido Capcell Pack C-18 column, 10x250 mm, CH_3CN/H_2O 92:8, 1.0 mL/min, detector UV 205 nm) and then on normal phase (YMC-Pack Sil-06 column, $CHCl_3$ 100%, 0.8 mL/min) afforded the isolation of **3a** (1.5 mg, t_R 8.5 min) and **4a** (1.3 mg, t_R 11.5 min)

Compound 2. Pale yellow oil, $[\alpha]_D^{23}$ + 76.2° (c 1.6, $CHCl_3$); IR (film) 3480-2550 (COOH), 2963, 2929, 2875, 1717 (CO), 1192 (peroxide) cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$, 600 MHz) see Table 1; EIMS *m/z*: 337 (5, M-29), 321 (10, M-45), 279 (5, M-29-58), 223 (5), 185 (25), 97 (40), 55 (100, $C_4H_7^+$); HREIMS *m/z* 337.2366 [$(M-29)^+$, C_{20} H_{33} O_4 Δ −3.7 mmu].

Compound 2a. Colorless oil, $[\alpha]_D^{23}$ +83.1° (c 0.9, $CHCl_3$); IR (film) 3480-2550 (COOH), 2963, 2931, 2875, 1745 (CO), 1457, 1437, 1191, 1178 (peroxide) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.38 (1H, ddd, *J* = 15.2, 6.3 and 6.3 Hz, H-12), 5.19 (1H, s, H-7), 5.12 (1H, bd, *J* = 15.3 and 8.1 Hz, H-11), 4.19 (1H, ddd, *J* = 12.7, 9.5 and 3.2 Hz, H-3), 3.69 (3H, s, CH_3O-), 2.61 (1H, dd, *J* = 15.7 and 2.5 Hz, H-2a), 2.28 (1H, bdd, *J* = 15.7 and 9.1 Hz, H-2b), 2.11 (3H, m, H-9a and Hs-19), 1.98 (5H, m, H-5a, H-9b, H-10 and Hs-13), 1.60 (2H, q, *J* =7.5 Hz, Hs-17), 1.57 (1H, m, H-4), 1.42 (2H, m, H-21a and H-15a), 1.28 (1H, t, *J* = 12.5 Hz, H-5b), 1.17 (1H, m, H-21b), 1.10 (1H, m, H-15b), 0.96 (3H, t, *J* = 7.5 Hz, CH_3-20), 0.95 (3H, t, *J* = 7.5 Hz, CH_3-14), 0.89 (3H, t, *J* = 7.5 Hz, CH_3-16), 0.84 (6H, t, *J* = 7.5 Hz, CH_3-18 and CH_3-22); EIMS *m/z*: 351 (20, M-29), 199 (90), 97 (80), 55 (100, $C_4H_7^+$).

Compound 3a. Colorless oil, $[\alpha]_D^{23}$ 12.4° (c 0.4, $CHCl_3$); IR (film) 2965, 2953, 1746 (CO), 1186 (peroxide) cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$, 600 MHz) see Table 1; EIMS *m/z*: 353 (5, M-29), 279 (20, M-29-74), 195 (40), 57 (100, $C_4H_9^+$); HREIMS *m/z* 353.2683 [$(M-29)^+$, C_{21} H_{37} O_4 Δ −2.6 mmu]..

Compound 4a. Colorless oil, $[\alpha]_D^{23}$ + 49.8° (c = 0.2, $CHCl_3$); IR (film) 2963, 2943, 1745 (CO), 1180 (peroxide) cm^{-1}; ^1H-NMR ($CDCl_3$, 600 MHz) see Table 1; EIMS *m/z*: 351 (20, M-29), 199 (90), 97 (80), 55 (100, $C_4H_7^+$); HREIMS *m/z* 351.2539 [$(M-29)^+$, C_{21} H_{35} O_4 Δ +1.0 mmu]..

Absolute stereochemistry of 2 at C-3. A solution of the acid **2** (2.4 mg, 7.1 μmol) in THF (2mL) was reacted with CH_2N_2 for 1h. Evaporation of the solvent gave the pure ester **2a** (3.5 mg), which was reduced in EtOH (2 mL) by H_2 and 10% Pd on charcoal. The reaction was stirred at r.t. overnight, and then filtered on paper (Advantec n.2). The filtered solution was dried under vacuum and the resulting residue was chromatographied by HPLC (Shiseido Capcell Pack C-18 column, 4.6x250 mm; $MeOH/H_2O$ 88:12; 1 mL/min.) to give pure **5**. Compound **5** was dissolved in 1 mL of pyridine and divided into two aliquots. One of this was treated under argon with 25 μL of (*R*)-MTPA chloride, and the other one with the same amount of (*S*)-MTPA chloride. The reaction mixture was stirred under argon at room temperature for 4h, then 1 mL of MeOH was added to each solution. Removing of the organic solvent under vacuum gave dark yellow oily fractions which were first chromatographied on silica gel ($CHCl_3$/MeOH 95:5) and then purified by HPLC (Shiseido Capcell Pack C-18, 4.6x250 mm; $MeOH/H_2O$ 92:8; 1 mL/min.) in order to afford the (*S*)-MTPA ester **5a** (0.8 mg) and the (*R*)-MTPA ester **5b** (0.6 mg), respectively from the reactions with (*R*)-MTPA -and (*S*)-MTPA chlorides. **Compound 5**. ^1H-NMR ($CDCl_3$) δ: 4.89 (1H, s), 3.85 (1H, ddd, *J* = 7.5, 3.1, 1.5 Hz), 3.72 (3H, s), 2.62 (1H, dd, *J* = 16.2 and 2.5 Hz), 2.38 (1H, dd, *J* = 16.2 and 10.0 Hz), 2.36 (1H, m), 2.24 (1H, m), 1.94 (1H, dd, *J* = 13.7 and 6.8 Hz), 1.88 (1H, dd, *J* = 13.7 and 6.8 Hz), 1.67 (1H, m), 1.40 (1H, m), 1.26 (m), 0.99 (3H, t, *J* = 7.5 Hz), 0.91 (3H, t, *J* = 7.5 Hz), 0.89 (3H, t, *J* = 6.6 Hz), 0.87 (3H, t, *J* = 7.5 Hz), 0.84 (3H, t, *J* = 7.5 Hz); EIMS (*m/z*): 366 (5, M-18), 355 (10, M-29), 337 (100, M-29-18), 225 (80), 113 (90). **Compound 5a**. [(*S*)-MTPA ester derivative of **5**]; ^1H-NMR ($CDCl_3$) δ: 7.49 (2H, m),7.39 (bs, 3H), 5.71 (1H, m, H-3),

4.99 (1H, s, H-7), 3.58 (3H, s, -OCH_3), 3.49 (3H, s, -OCH_3), 2.62 (1H, d, J = 1.8 Hz, H-2a), 2.61 (1H, d, J = 4.1 Hz, H-2b), 2.43 (1H, m, H-19a), 2.17 (1H, m, H-19b), 1.99 (1H, dd, J = 13.9 and 5.7 Hz, H-9a), 1.86 (1H, m, H-4), 1.83 (1H, dd, J = 13.9 and 8.2 Hz, H-9a), 1.59 (2H, m, Hs-17), 1.48 (1H, m, H-15a), 1.46 (2H, m, Hs-5), 1.40 (1H, m, H-10), 1.26 (8H, m), 1.20 (1H, m, H-15b), 0.98 (3H, t, J = 7.4 Hz, CH_3-20), 0.91 (3H, t, J = 7.4 Hz, CH_3-16), 0.90 (3H, t, J = 7.4 Hz, CH_3-18), 0.88 (3H, t, J = 6.5 Hz, CH_3-14 or CH_3-22), 0.81 (3H, t, J = 7.4 Hz, CH_3-22 or CH_3-14). **Compound 5b**. [(*R*)-MTPA ester derivative of **5**]; ^{1}H-NMR ($CDCl_3$) δ: 7.52 (2H,m),7.38 (bs, 3H), 5.74 (1H, m, H-3), 4.98 (1H, s, H-7), 3.64 (3H, s, -OCH_3), 3.52 (3H, s, -OCH_3), 2.64 (1H, d, J = 1.7 Hz, H-2a), 2.63 (1H, d, J = 4.3 Hz, H-2b), 2.47 (1H, m, H-19a), 2.17 (1H, m, H-19b), 2.03 (1H, dd, J = 13.6 and 5.5 Hz, H-9a), 1.85 (1H, dd, J = 13.6 and 8.3 Hz, H-9a), 1.81 (1H, m, H-4), 1.58 (2H, m, Hs-17), 1.44 (1H, dd, H-5a), 1.38 (2H, dd, H-5b), 1.41 (1H, m, H-10), 1.26 (8H, m), 1.26 (1H, m, H-15a), 1.06 (1H, m, H-15b), 1.02 (3H, t, J = 7.4 Hz, CH_3-20), 0.89 (6H, t, J = 7.4 Hz, CH_3-14 and CH_3-18), 0.83 (3H, t, J = 7.4 Hz, CH_3-22), 0.78 (3H, t, J = 7.4 Hz, CH_3-16). **Chemical shift differences in MTPA esters 5a and 5b** ($\Delta\delta = \delta_{5a} - \delta_{5b}$). ^{1}H-NMR ($CDCl_3$): + 0.01 (H-7),- 0.06 ppm (OCH_3), - 0.02 ppm (H-2a), - 0.02 ppm (H-2b), + 0.05 ppm (H-4), + 0.05 ppm (H-4), + 0.02 ppm (H-5a), + 0.08 ppm (H-5b), + 0.04 ppm (H-19a), + 0.22 ppm (H-15a), + 0.14 ppm (H-15b), + 0.13 ppm (H_3-16).

Absolute stereochemistry of 3 at C-3. The absolute stereochemistry of **3** was determined as above described for **2**. Starting from 1.0 mg (2.7 μmol) of **3a**, (*S*)-MTPA (**6a**, 0.4 mg) and (*R*)-MTPA (**6b**, 0.4 mg) esters were obtained. **Compound 6**. ^{1}H-NMR ($CDCl_3$) δ: 4.91 (1H,s), 4.18 (1H,d, J = 9.3 Hz), 3.71 (3H, s), 2.57 (1H,dd, J = 16.4 and 10.8 Hz), 2.39 (1H, m), 2.35 (1H,d, J = 16.2 Hz), 2.18 (1H, m), 1.96 (1H,dd, J = 13.6 and 6.5 Hz), 1.92 (1H, m), 1.88 (1H,dd, J = 13.6 and 6.5 Hz), 1.40 (1H, bs), 1.27 (m), 0.98 (3H, t, J = 7.5 Hz), 0.92 (9H, bt), 0.84 (3H, t, J = 7.3 Hz); EIMS (*m/z*): 366 (5, M-18), 355 (10, M-29), 337 (100, M-29-18), 225 (80), 113 (90). **Compound 6a.** [(*S*)-MTPA ester derivative of **6**]; ^{1}H-NMR ($CDCl_3$) δ: 7.51 (2H,m),7.39 (bs, 3H), 5.89 (1H, m, H-3), 4.85 (1H, s, H-7), 3.65 (3H, s, -OCH_3), 3.54 (3H, s, -OCH_3), 2.74 (1H, dd, J = 16.0 and 8.5 Hz, H-2a), 2.59 (1H, dd, J = 16.0 and 4.4 Hz, H-2b), 2.30 (2H, q, J = 7.6 Hz, Hs-19), 1.89 (3H,m, H-4 and H_2-9), 1.48 (m), 1.26 (m), 1.20 (m), 0.98 (3H, t, J = 7.6 Hz, CH_3-20), 0.92 (3H), 0.90 (6H), 0.85 (3H). **Compound 6b.** [(*R*)-MTPA ester derivative of **6**]; ^{1}H-NMR ($CDCl_3$) δ: 7.56 (2H,m), 7.40 (bs, 3H), 5.90 (1H, m, H-3), 4.89 (1H, s, H-7), 3.61 (3H, s, -OCH_3), 3.50 (3H, s, -OCH_3), 2.71 (1H, dd, J = 16.0 and 7.9 Hz, H-2a), 2.55 (1H, dd, J = 16.0 and 5.1 Hz, H-2b), 2.32 (2H, m, J = 7.6 Hz, Hs-19), 1.91 (3H,m, H-4 and H_2-9), 1.48 (m), 1.43 (m), 1.26 (m), 1.20 (m), 0.99 (3H, t, J = 7.6 Hz, CH_3-20), 0.92 (3H), 0.91 (3H), 0.90 (3H), 0.83 (3H). **Chemical shift differences in MTPA esters 6a and 6b** ($\Delta\delta = \delta_{6a} - \delta_{6b}$). ^{1}H-NMR ($CDCl_3$): - 0.04 (H-7), + 0.04 ppm (OCH_3), + 0.03 ppm (H-2a), + 0.04 ppm (H-2b), - 0.02 (H_2-19), - 0.02 (H-4), - 0.01 (H_3-20).

Absolute stereochemistry at C-10 of compound 2. The ester **2a** (2.6 mg, 7 μmol), prepared from methylation of **2** in THF, was stirred for 10 min. with a saturated ozone solution (about 3 mL) at -78° C according to the reference 14. Briefly, a O_3 stream was bubbled into MeOH at -78° C till a blue-colored solution resulted. Then, 3 mL of this solution were added to **2a** in MeOH at - 78° C. After 20 min., the excess ozone was removed by a stream of nitrogen and $NaBH_4$ (in excess) was added and the resultiing suspension was stirred for 20 min at -78° C. The reaction mixture was allowed to warm at room temperature and the excess of $NaBH_4$ was destroyed by 300 μL of CH_3CO_2H. Extraction with ethyl acetate (6 mL), followed by evaporation of the organic layer gave the compound **7** (2.0 mg), which was divided in two fractions and treated in pyridine with (*S*)- and

(*R*)-MTPA chlorides. The reactions were stirred under argon at room temperature for 4h and then extracted with EtOAc and 3% HCl. Chromatography of these fractions on silica gel column (100% $CHCl_3$) gave the (+)-MTPA ester **7a** (0.6 mg) from the (*S*)-(+)-MTPA chloride, and the (-)-MTPA ester **7b** (0.6 mg) from (*R*)-(-)-MTPA chloride. Hydrogenolysis of **7a** and **7b**, followed by purification on silica gel column afforded the corresponding diols **8a** (0.5 mg) and **8b** (0.6 mg). **Compound 7.** ^{1}H-NMR ($CDCl_3$) δ: 5.20 (1H, s), 4.22 (1H, m), 3.68 (3H, s), 3.76 (2H, bs), 2.61 (1H, dd, *J* = 16.1 and 3.1 Hz), 2.53 (1H, dd, *J* = 16.1 and 5.4 Hz), 2.27 (2H, m), 2.10 (1H, m), 1.95 (1H, dd, *J* = 13.1 and 3.9 Hz), 1.86 (1H, dd, *J* = 13.3 and 9.8 Hz), 1.62 (4H, m), 1.25 (m), 1.11 (1H, m), 0.99 (3H, t, *J* =7.6 Hz), 1.91 (9H, m); EIMS (*m/z*): 327 (8, M-29), 309 (20, M-29-18), 199 (70), 127 (90), 57 (100). **Compound 8a.** [(+)-MTPA ester] ^{1}H-NMR ($CDCl_3$) δ: 7.51 (2H, m), 7.40 (3H, m), 4.88 (1H, s), 4.32 (1H, dd, *J* = 10.8 and 5.0 Hz), 4.14 (1H, dd, *J* = 10.8 and 5.7 Hz), 3.84 (1H, bt, *J* = 7.5 Hz), 3.71 (3H, s), 3.54 (3H, s), 2.64 (1H, dd, *J* = 16.9 and 2.8 Hz), 2.37 (2H, m), 2.29 (2H, m), 1.98 (2H, d, *J* = 7.2 Hz), 1.79 (1H, m), 1.73 (1H, dd, *J* = 15.1 and 6.6 Hz), 1.64 (1H, m), 1.32 (4H, m), 1.25 (3H, m), 0.95 (3H, t, *J* =7.6 Hz), 0.90 (3H, t, *J* =7.4 Hz), 0.87 (3H, t, *J* =7.5 Hz), 0.83 (3H, t, *J* =7.6 Hz). **Compound 8b.** [(+)-MTPA ester] ^{1}H-NMR ($CDCl_3$) δ: 7.51 (2H, m), 7.40 (3H, m), 4.87 (1H, s), 4.24 (1H, dd, *J* = 10.7 and 4.3 Hz), 4.22 (1H, dd, *J* = 10.7 and 4.2 Hz), 3.84 (1H, bdd, *J* = 10.5 and 2.6 Hz), 3.71 (3H, s), 3.54 (3H, s), 2.64 (1H, dd, *J* = 16.4 and 2.4 Hz), 2.36 (3H, m), 2.27 (2H, m), 1.97 (2H, d, *J* = 7.4 Hz), 1.80 (1H, m), 1.72 (1H, dd, *J* = 15.1 and 6.6 Hz), 1.64 (1H, m), 1.37 (2H, m), 1.32 (2H, m), 1.25 (3H, m), 0.95 (3H, t, *J* =7.5 Hz), 0.90 (3H, t, *J* =7.7 Hz), 0.88 (3H, t, *J* =7.5 Hz), 0.85 (3H, t, *J* =7.7 Hz).

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References and Notes

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