# Plakortethers A−G: A New Class of Cytotoxic Plakortin−Derived Metabolites[‡]

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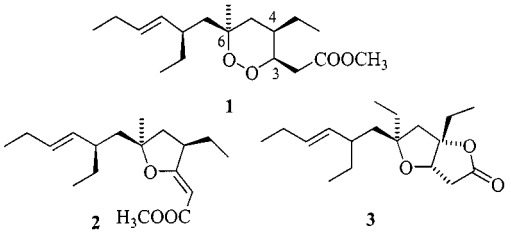
Plakortethers A–G (**4**–**10**) represent a new class of plakortin-related metabolites containing a trisubstituted tetrahydrofuran ring. They have been isolated from the Caribbean sponge *Plakortis simplex* and their stereostructures fully characterized by a combination of spectroscopic data and chemical evidence, based on a three-step conversion of the cycloperoxide plakortin into plakortether B (**5**). Plakortethers A (**4**), B (**5**), D (**7**), and E (**8**) exhibited selective cytotoxicity against the RAW 264-7 cell line (murine macrophage).

## Introduction

Marine sponges of the genus *Plakortis* produce a wide variety of biologically active (antifungal, antibacterial, and antitumor) cycloperoxide metabolites, differing in the ring size (five- or six-membered), in the length and the functionalization of the carbon backbone, and in the stereochemistry of the asymmetric centers. As well as a plethora of cycloperoxides closely resembling the parent compound and antibiotic plakortin (**1**),[2] *Plakortis* sponges have also furnished some structurally related metabolites in the forms of furano esters and plakortones,[3] which lack the peroxide function but still possess the same parent cycloperoxide carbon framework. In the furano esters, such as compound **2**,[4] a single oxygen atom connects C(3) and C(6); in the plakortones [such as plakortone E (**3**)][5] a γ-lactone bond connecting C(1) and C(4) is also present. These derivatives have interesting biological potential, and furano esters in particular have shown cytotoxic and antileishmanial properties, while plakortones are able to enhance $Ca^{2+}$ uptake by the cardiac sarcoplasmic reticulum.[3]




The richness and the variety of pharmacologically active secondary metabolites found in these sponges have prompted us to intensify our chemical investigations into the Caribbean specimen *Plakortis simplex*. As we have already reported, this organism produces plakortin (in remarkable amounts),[4] other known (e.g., plakortide H) and novel (e.g., dihydroplakortin and plakortide J)[1,4] cyclic peroxides, and related bioactive compounds belonging to the classes of furanoesters and of plakortones.[4,5] In this paper we now report the isolation and the stereostructural determination of plakortethers A−G (**4**−**10**), which represent a new class of non-peroxide plakortin derivatives. Their unprecedented structures, characterized by a tetrahydrofuran ring connecting C(6) and C(9), share their carbon backbones and the absolute stereochemistry of their corresponding asymmetric centers with plakortin, as we have demonstrated by a three-step chemical conversion of plakortin into plakortether B. In addition, one of these derivatives, plakortether C (**6**), represents the first chlorinated compound found in a *Plakortis* sponge. Finally, some of the plakortethers exhibited a significant and selective cytotoxic activity against the RAW 264-7 cell line.

## Results and Discussion

A specimen of *P. simplex* (order Homosclerophorida, family Plakinidae) was exhaustively extracted first with methanol and then with chloroform. The methanol extract was partitioned between *n*BuOH and water, and the organic

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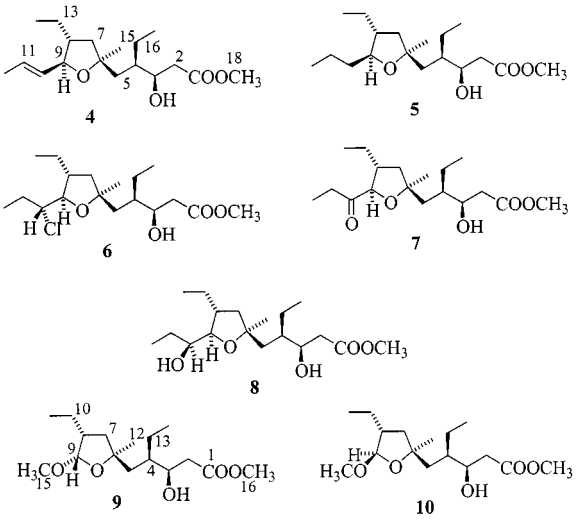
phase, recombined with the $CHCl_3$ extract, was then fractionated by column chromatography on reversed-phase silica gel (RP18), eluting with a system of solvents of decreasing polarity form $H_2O$ to $H_2O$/MeOH (1:9). The less polar fractions were purified by MPLC on silica gel, and fractions eluted with *n*-hexane/EtOAc (1:1 and 4:6) were further chromatographed by HPLC to afford plakortethers A, B, C, F, and G and plakortethers D and E, respectively, in the pure state.




Plakortether A (**4**) was isolated as a colorless oil, $[\alpha]_D$ = +2 ($c$ = 0.18). The electron impact (EI) MS of **4** showed only fragmentation peaks (see Exp. Sect.), although the molecular ion peak was clearly visible in the electrospray (ES) MS at $m/z$ = 313 $[M + H]^+$. High-resolution measurements resulted in the attribution of the molecular formula $C_{18}H_{32}O_4$, implying three degrees of unsaturation, to **4**. The IR (KBr) spectrum of **4** indicated the presence of an ester functionality ($\tilde{\nu}$ = 1742 $cm^{-1}$) and of a hydroxy group ($\tilde{\nu}$ = 3596 $cm^{-1}$).

Detailed analysis of the $^1H$ and $^{13}C$ NMR spectra (Table 1) of plakortether A (**4**) was achieved with the aid of 2D NMR spectroscopic data. In particular, inspection of the $^1H$-$^1H$ COSY spectrum revealed the presence of only two spin systems in the molecule, while the 2D HMQC spectrum associated all these protons with the resonances of the relevant carbon atoms. The first fragment, extending from $H_2$-2 to $H_2$-5, possessed a hydroxy group at C(3) ($\delta_C$ = 71.2; $\delta_H$ = 4.16), coupled with the OH proton (resonating at $\delta$ = 4.40) and a branching ethyl group at C(4). The second fragment spanned $H_2$-7 to $H_3$-12, with an ethyl group linked at C(8), an oxygen atom at C(9) ($\delta_C$ = 86.5; $\delta_H$ = 3.84), and a *trans* double bond between C(10) and C(11) ($\delta_H$ = 5.42 and 5.67, respectively, $J$ = 14.7 Hz). In addition, two uncoupled methyl groups ($\delta_H$ = 1.28, $\delta_C$ = 26.1; $\delta_H$ = 3.70, $\delta_C$ = 52.0) and two unprotonated carbon atoms ($\delta_C$ = 174.1 and 81.2) were part of structure **4**. The first of these latter carbon atoms, attributable to the ester carbonyl group, was confidently placed at C(1), as indicated by the HMBC $H_2$-2/C(1) cross-peak; C(1) also showed a cross-peak with the methyl protons resonating at $\delta$ = 3.70, indic-

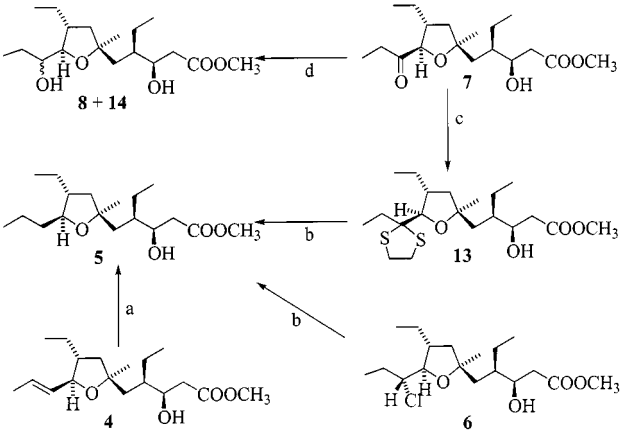
Table 1. $^{13}C$ (125 MHz) and $^1H$ (500 MHz) NMR spectroscopic data of plakortethers A (**4**) and B (**5**) in $CDCl_3$

| Pos. | **4** | | **5** | |
|---|---|---|---|---|
| | $\delta_C$, mult. | $\delta_H$, mult., $J$ in Hz | $\delta_C$, mult. | $\delta_H$, mult., $J$ in Hz |
| 1 | 174.1, C | | 174.2, C | |
| 2a | 37.5, $CH_2$ | 2.48, dd, 15.5, 10.0 | 37.7, $CH_2$ | 2.50, dd, 15.4, 10.3 |
| 2b | | 2.34, dd, 15.5, 2.1 | | 2.32, dd, 15.4, 2.2 |
| 3 | 71.2, CH | 4.16, m | 71.0, CH | 4.13, m |
| 3-OH | | 4.40, d, 7.0 | | 4.80, d, 7.0 |
| 4 | 42.1, CH | 1.84, m | 41.8, CH | 1.87, m |
| 5a | 42.5, $CH_2$ | 1.68[a] | 42.7, $CH_2$ | 1.62[a] |
| 5b | | 1.37, dd, 15.5, 1.8 | | 1.42, dd, 15.5, 1.8 |
| 6 | 81.2, C | | 81.3, C | |
| 7a | 47.0, $CH_2$ | 2.02, dd, 11.5, 9.5 | 47.4, $CH_2$ | 2.00, dd, 12.5, 8.8 |
| 7b | | 1.48, dd, 11.5, 10.9 | | 1.57[a] |
| 8 | 47.2, CH | 1.80, m | 47.2, CH | 1.71, m |
| 9 | 86.5, CH | 3.84, t, 10.8 | 83.7, CH | 3.53, dt, 8.8, 2.9 |
| 10 | 131.2, CH | 5.42, dd, 14.7, 10.8 | 36.4, $CH_2$ | 1.55[a] |
| 11a | 130.0, CH | 5.67, dq, 14.7, 6.2 | 29.5, $CH_2$ | 1.30[a] |
| 11b | | | | 1.27[a] |
| 12 | 17.8, $CH_3$ | 1.69, d, 6.2 | 13.2, $CH_3$ | 0.91, t, 7.3 |
| 13a | 24.1, $CH_2$ | 1.44, m | 25.8, $CH_2$ | 1.57[a] |
| 13b | | 1.13, m | | 1.18, m |
| 14 | 17.8, $CH_3$ | 0.88, t, 7.3 | 12.4, $CH_3$ | 0.93, t, 7.3 |
| 15 | 26.1, $CH_3$ | 1.28, s | 26.1, $CH_3$ | 1.27, s |
| 16a | 25.5, $CH_2$ | 1.31, m | 25.3, $CH_2$ | 1.32[a] |
| 16b | | 1.19, m | | 1.12, m |
| 17 | 12.3, $CH_3$ | 0.92, t, 7.3 | 12.5, $CH_3$ | 0.93, t, 7.3 |
| 18 | 52.0, $CH_3$ | 3.70, s | 51.8, $CH_3$ | 3.70, s |

[a] Overlapped with other signals.

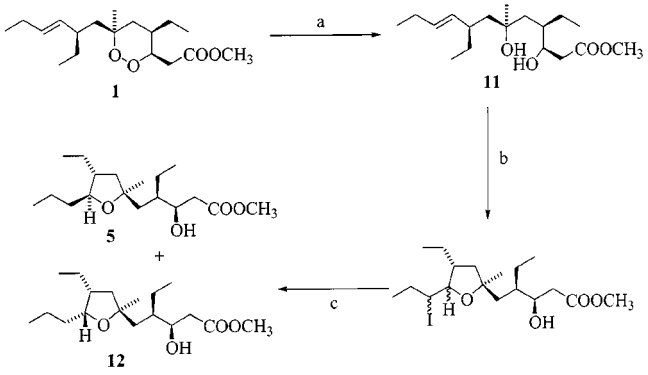
ating the methoxycarbonyl nature of the ester function. The oxygenated carbon atom signal at δ = 81.2 [C(6)] displayed HMBC correlations with $H_3$-15 (δ = 1.28) and with both $H_2$-7 and $H_2$-5, and should therefore constitute the connection point between these fragments. Furthermore, the cross-peak of C(6) with H(9) suggested, in accordance with the molecular formula, that C(6) and C(9) were linked to the same oxygen atom, making up a tetrahydrofuran ring. These data unambiguously indicated the gross structure of plakortether A, which was in accordance with the whole series of HMBC cross-peaks reported in Exp. Sect.

Plakortether B (**5**), a colorless oil with $[\alpha]_D = -13$ ($c$ = 0.12), was identified as the 10,11-dihydro derivative of plakortether A (**4**) by interpretation of the following spectral and chemical evidence. ESMS of **5** showed a pseudomolecular ion peak at $m/z$ = 315 $[M + H]^+$ and the high-resolution measurement determined the molecular formula $C_{18}H_{34}O_4$, which differs from that of compound **4** by two more hydrogen atoms. Both the $^1H$ and $^{13}C$ NMR spectra of **5** (Table 1), assigned through inspection of 2D NMR experiments (COSY, HMQC, and HMBC), closely resembled those of **4** but the olefinic signals of H(10) [and C(10)] and H(11) [and C(11)] were missing, being substituted by signals located in the $sp^3$ region. The relationship between plakortethers A (**4**) and B (**5**) was unambiguously confirmed by chemical interconversion (Scheme 1): Catalytic hydrogenation (10% Pd on charcoal) of **4** yielded a compound identical to **5**, as indicated by $[\alpha]_D$ and NMR spectroscopic data.




Scheme 1. Interconversion between plakortethers: a: $H_2$/Pd; b: $Ph_3SnH/(=NCMe_2CN)_2$ in benzene; c: $HS(CH_2)_2SH/BF_3{\cdot}Et_2O$; d: $NaBH_4$ in MeOH

Determination of the absolute stereochemistry at the five stereogenic carbon atoms in plakortethers A and B appeared particularly challenging. Because of the limited amount of available material, the best way to achieve this goal would have been derivatization with enantiomers of an appropriate auxiliary reagent, followed by comparison of the $^1H$ NMR spectra of the resulting diastereomers. Unfortunately, four of the chiral carbon atoms of **4** and **5** did not support any of the functional groups generally required for this type of analysis. However, in view of the fact that the novel structures possessed the same carbon skeleton as the cyclic peroxide plakortin (**1**), it appeared crucial to verify whether the absolute configuration of plakortethers could somehow be correlated with that of plakortin, recently determined by our group.[4] To this end, we elaborated a three-step semisynthesis of the plakortether B framework from plakortin (Scheme 2).




Scheme 2. Synthesis of plakortether B (**5**) from plakortin (**1**): a: Zn/AcOH; b: *N*-iodosuccinimide in $CH_3CN$; c: $Ph_3SnH/(=NCMe_2CN)_2$ in benzene

Reduction of plakortin with Zn/AcOH afforded the diol **11** in good yield. This was treated with *N*-iodosuccinimide to achieve the electrophilic cyclization of the γ,δ-unsaturated alcohol to the tetrahydrofuran ring.[6] This reaction resulted in the formation of two additional chiral centers, thus affording four different epimers, which were not separated. This mixture of diastereomers was subjected to reduction with triphenyltin hydride/2,2′-azobis(isobutyronitrile), which selectively substituted the iodine with a hydrogen atom, with elimination of one of the chiral centers: The two C(9) epimers thus obtained were purified by HPLC. Since one of these was identical (by $[\alpha]_D$ and NMR spectroscopic data) to plakortether B (**5**), this unambiguously implied that the absolute configurations at C(3), C(4), C(6), and C(8) in **5** could be assigned as the same as those of the corresponding carbon atoms in plakortin (**1**). The (*S*) configuration of the remaining chiral center at C(9) in **5** was inferred from the dipolar H-9/H-15 coupling evidenced in the 2D ROESY spectrum. Finally, the conversion of plakortether A (**4**) into plakortether B (**5**) by catalytic hydrogenation reported above confirmed that compound **4** possessed the same absolute stereochemistry as **5**.

Plakortether C (**6**) was isolated as a colorless oil, $[\alpha]_D = -3$ ($c$ = 0.14). The presence of a chlorine atom in the structure of **6** was initially indicated by the isotopic patterns in both the ES [pseudomolecular ion peak at $m/z$ = 349 and 351 (relative intensity approx. 3:1)] and the EI (the molecular ion was absent and only chlorine-containing fragmentation peaks were visible) mass spectra. The molecular formula of $C_{18}H_{33}ClO_4$ was established for **6** from high-resolution measurements and corroborated by NMR spectroscopic data (Table 2). Inspection of 2D NMR spectra (COSY, HMQC, HMBC) indicated the same gross structure for plakortether C (**6**) as for plakortether B (**5**), except for

Table 2. $^{13}C$ and $^{1}H$ NMR spectroscopic data of plakortethers C (**6**) and D (**7**) in $CDCl_3$

| Pos. | **6** | | **7** | |
|---|---|---|---|---|
| | $\delta_C$, mult. | $\delta_H$, mult., *J* in Hz | $\delta_C$, mult. | δH, mult., *J* in Hz |
| 1 | 171.2, C | | 174.0, C | |
| 2a | 35.8, $CH_2$ | 2.65, dd, 15.4, 10.3 | 38.1, $CH_2$ | 2.57[a] |
| 2b | | 2.34, dd, 15.4, 3.7 | | 2.41, dd, 15.5, 3.0 |
| 3 | 67.8, CH | 4.28, m | 69.2, CH | 4.36, m |
| 4 | 39.2, CH | 1.77, m | 41.6, CH | 1.75[a] |
| 5a | 39.6, $CH_2$ | 1.68, dd, 15.5, 10.3 | 41.3, $CH_2$ | 1.78, dd, 15.0, 12.5 |
| 5b | | 1.50, dd, 15.5, 3.0 | | 1.49, dd, 15.5, 2.0 |
| 6 | 80.0, C | | 85.1, C | |
| 7a | 44.1, $CH_2$ | 2.05, dd, 11.8, 9.6 | 46.2, $CH_2$ | 2.07[a] |
| 7b | | 1.54[a] | | 1.56, dd, 12.0, 9.5 |
| 8 | 40.3, CH | 2.23, m | 45.2, CH | 2.09[a] |
| 9 | 82.8, CH | 3.68, dd, 8.1, 1.5 | 88.3, CH | 4.09, d, 9.5 |
| 10 | 62.5, CH | 3.84, ddd, 9.6, 4.0, 1.5 | 211.3, C | |
| 11a | 27.0, $CH_2$ | 1.88[a] | 32.3, $CH_2$ | 2.56[a] |
| 11b | | 1.83[a] | | 2.56[a] |
| 12 | 9.8, $CH_3$ | 1.05, t, 6.6 | 7.0, $CH_3$ | 1.06, t, 6.6 |
| 13a | 22.5, $CH_2$ | 1.52[a] | 26.3, $CH_2$ | 1.74[a] |
| 13b | | 1.27[a] | | 1.37, m |
| 14 | 17.2, $CH_3$ | 0.92, t, 7.3 | 12.2, $CH_3$ | 0.93, t, 7.3 |
| 15 | 23.2, $CH_3$ | 1.29, s | 26.1, $CH_3$ | 1.32, s |
| 16a | 22.1, $CH_2$ | 1.36, m | 23.5, $CH_2$ | 1.42, m |
| 16b | | 1.18, m | | 1.28, m |
| 17 | 10.0, $CH_3$ | 0.90, t, 7.3 | 12.4, $CH_3$ | 0.91, t, 7.3 |
| 18 | 49.5, $CH_3$ | 3.68, s | 51.3, $CH_3$ | 3.69, s |

[a] Overlapped with other signals.

the chlorine atom. This, according to the proton connectivity established by COSY experiments, must be positioned at C(10). This is in perfect agreement with the chemical shift value of C(10), at δ = 62.5, and with the upfield shifted $^{13}C$ NMR resonances of C(12) (δ = 9.8 in **6**; δ = 13.2 in **5**) and C(8) (δ = 40.3 in **6**; δ = 47.2 in **5**). To the best of our knowledge, this is the first report of a halogen-containing polyketide from a *Plakortis* sponge.

Plakortether C was converted (Scheme 1) into plakortether B (**5**) by reductive dehalogenation carried out with triphenyltin hydride (sodium borohydride proved completely ineffective in this case), thus indicating that the absolute configurations at C(3), C(4), C(6), C(8), and C(9) in plakortether C must be assigned as (3*R*), (4*R*), (6*R*), (8*R*), and (9*R*), identical to those of the corresponding carbons in plakortether B. These data having been gained, it remained only to define the absolute configuration at the chlorine-binding C(10) in **6**. In view of the fact that C(10) was adjacent to the chiral carbon atom C(9) and that the small value of $^{3}J_{H\text{-}9/H\text{-}10}$ (1.5 Hz) indicated that a dominant rotamer (with the two protons in a *gauche* orientation) existed around the C(9)/C(10) axis, our molecule appeared to possess all the requirements essential for application of the *J-based configuration analysis* recently proposed by Murata et al.[7] Murata's method allows relative stereochemistry in acyclic systems to be deduced on the basis of combined analysis of the coupling constants $^{3}J_{H,H}$, $^{3}J_{C,H}$ (both related to conformational parameters through the Karplus rule) and $^{2}J_{C,H}$, the value of which in $^{1}H-C-^{13}C-X$ systems (where X is an oxygen or halogen atom) is related to the dihedral angle between the proton and the heteroatom.[8] We qualitatively determined the four required heteronuclear coupling constants (Figure 1) through an analysis of the phase-sensitive PS-HMBC spectrum; the *J* data obtained indicated a *threo* stereochemical relationship between C(9) and C(10) in plakortether C, as shown in Figure 1. Thus, on the basis of the previously determined (*R*) configuration

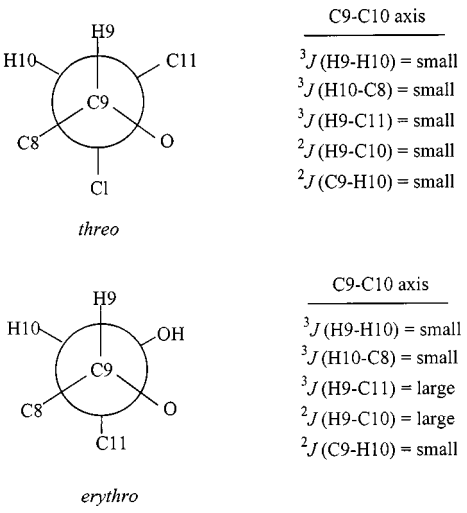



Figure 1. Application of Murata's method along the C(9)/C(10) axes of plakortethers C (top) and E (bottom)

for C(9), we assigned the (*R*) configuration to C(10), unambiguously defining the stereostructure of plakortether C (**6**).

Plakortether D (**7**), a colorless oil with $[\alpha]_D = +7$ ($c = 0.09$), showed a pseudomolecular ion peak at $m/z = 329$ $[M + H]^+$ in the ES mass spectrum. High-resolution measurements on **7** established a molecular formula of $C_{18}H_{32}O_5$, implying three degrees of unsaturation. The IR spectrum of **7** showed absorption bands centered at $\tilde{\nu} = 1745$ and 1719 $cm^{-1}$, attributable to ester and ketone carbonyl groups, respectively. The presence of a ketone group was further supported by the $^{13}C$ NMR resonance at $\delta = 211.3$ (Table 2). Combined analysis of COSY, HMQC, and HMBC NMR spectra indicated the gross structure of plakortether D to be 10-oxoplakortether B [HMBC cross peaks H-8/C(10) and $H_3$-12/C(10)]. It is known that ketone groups induce downfield shifts of the $^{13}C$ NMR resonances of α-carbonatoms and upfield shifts in the resonances of β-carbon atoms. Accordingly, the resonances of C(11) and C(9) in **7** had higher values than those in plakortether B ($\delta = 32.3$ instead of 29.5, and $\delta = 88.3$ instead of 83.7, respectively), while the resonances of C(12) and C(8) in **7** had lower values than those in plakortether B ($\delta = 7.0$ instead of 13.2, and $\delta = 45.2$ instead of 47.2, respectively).

Plakortether D (**7**) was converted into plakortether B (**5**) through the two-step reduction illustrated in Scheme 1. The dithioketal **13** was prepared by treatment of **7** with ethanedithiol in the presence of boron trifluoride−diethyl ether, and the desulfurization of **13** to the corresponding hydrocarbon, plakortether B (**5**), was then accomplished by the use of 4 equiv. of triphenyltin hydride/2,2′-azobis(isobutyronitrile).[9] This transformation unambiguously indicated that the five chiral carbon atoms in plakortether D [C(3), C(4), C(6), C(8), and C(9)] had to possess the same absolute configurations as the corresponding carbons in plakortether B.

Plakortether E (**8**), a colorless oil with $[\alpha]_D = -2$ ($c = 0.03$), showed HRMS data compatible with the molecular formula $C_{18}H_{34}O_5$, with just one oxygen atom more than plakortether B (**5**). This was confirmed by combined inspection of 2D NMR (COSY, HMQC, HMBC) spectra, allowing the complete assignment of $^1H$ and $^{13}C$ NMR spectroscopic data (Table 3), which indicated that compound **8** may be viewed as the 10-hydroxy derivative of plakortether B.

The absolute stereochemistry of plakortether E (**8**) was correlated with that of the previously described plakortethers by reduction of plakortether D (**7**) with $NaBH_4$ in MeOH, which yielded compound **8** (characterized by $[\alpha]_D$ and NMR), together with its C(10) epimer **14** (Scheme 1). This transformation indicated that plakortether E actually possesses the same absolute configurations as in plakortethers A−D at C(3), C(4), C(6), C(8), and C(9). In order to ascertain the absolute configuration at the remaining chiral center in **8** [C(10)], we applied Murata's *J-based configuration analysis*,[7] as for plakortether C. Evaluation of the required *J* values, accomplished by analysis of $^1H$ NMR coupling constants and of the phase-sensitive PS-HMBC spectrum, was in accordance with the C(9)/C(10) *erythro*

Table 3. $^{13}C$ and $^1H$ NMR spectroscopic data of plakortethers E (**8**) and F (**9**) in $CDCl_3$

| Pos. | **8** | | **9** | |
|---|---|---|---|---|
| | $\delta_C$, mult. | $\delta_H$, mult., *J* in Hz | $\delta_C$, mult. | $\delta_H$, mult., *J* in Hz |
| 1 | 173.2, C | | 173.8, C | |
| 2a | 37.5, $CH_2$ | 2.56, dd, 15.5, 10.5 | 37.6, $CH_2$ | 2.36[a] |
| 2b | | 2.35, dd, 15.5, 1.8 | | 2.34[a] |
| 3 | 69.5, CH | 4.22, m | 71.2, CH | 4.17, m |
| 3-OH | | | | 4.46, d, 7.0 |
| 4 | 41.1, CH | 1.85, m | 42.1, CH | 1.89[a] |
| 5a | 40.6, $CH_2$ | 1.79, dd, 15.0, 12.0 | 42.3, $CH_2$ | 1.70, dd, 15.0, 12.0 |
| 5b | | 1.29[a] | | 1.32[a] |
| 6 | 82.1, C | | 83.3, C | |
| 7a | 47.3, $CH_2$ | 2.06, dd, 12.1, 9.2 | 43.2, $CH_2$ | 1.88[a] |
| 7b | | 1.56, dd, 12.1, 2.0 | | 1.66, dd, 12.5, 11.6 |
| 8 | 39.5, CH | 2.35[a] | 47.0, CH | 2.10, m |
| 9 | 87.4, CH | 3.64, dd, 8.2, 1.5 | 105.8, CH | 4.78, d, 4.5 |
| 10 | 73.1, CH | 3.71, ddd, 9.8, 4.5, 1.5 | 21.7, $CH_2$ | 1.52, m |
| | | | | 1.39, m |
| 11 | 26.0, $CH_2$ | 1.43[a] | 12.7, $CH_3$ | 0.91, t, 6.9 |
| | | 1.45[a] | | |
| 12 | 11.4, $CH_3$ | 1.00, t, 6.6 | 27.6, $CH_3$ | 1.37, s |
| 13a | 27.2, $CH_2$ | 1.68, m | 27.1, $CH_2$ | 1.31[a] |
| 13b | | 1.22[a] | | 1.17, m |
| 14 | 13.1, $CH_3$ | 0.91, t, 7.3 | 12.3, $CH_3$ | 0.93, t, 7.0 |
| 15 | 26.1, $CH_3$ | 1.28, s | 54.5, $CH_3$ | 3.29, s |
| 16 | 25.9, $CH_2$ | 1.22, m | 52.0, $CH_3$ | 3.70, s |
| | | 1.19, m | | |
| 17 | 12.5, $CH_3$ | 0.93, t, 7.3 | | |
| 18 | 51.0, $CH_3$ | 3.73, s | | |

[a] Overlapped with other signals.

stereochemical relationship shown in Figure 1, which implied the (*S*) configuration at C(10). Further evidence for this assignment came from application of the modified Mosher method for secondary alcohols.[10] Treatment of 2 equiv. of **8** with (−)- and (+)-MTPA chloride in dry pyridine provided the (*S*)- and (*R*)-MTPA diesters **15** and **16**, respectively, and the distribution of the $\Delta\delta$ ($\delta_S - \delta_R$) values (Figure 2), reflecting the anisotropic effect of MTPA in accordance with the Mosher model, indicated the (10*S*) configuration. Finally, the same procedure also allowed us to confirm the (*R*) configuration at C(3) (Figure 2).

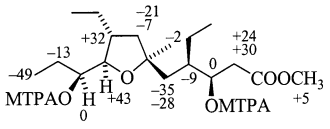


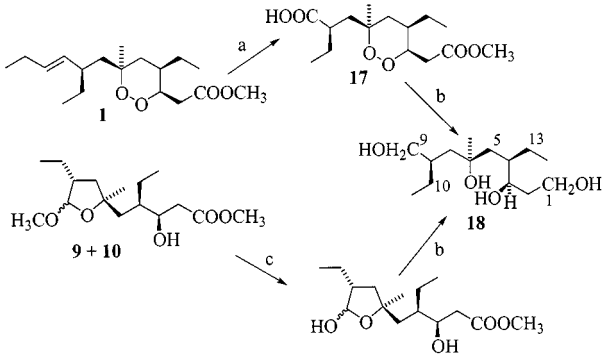

Figure 2. Application of the modified Mosher method for secondary alcohols to plakortether E; $\Delta\delta$ ($\delta_S - \delta_R$) are given in Hz

Plakortethers F (**9**) and G (**10**) can be viewed as two further analogs of this class, although they differ fundamentally from the tetrahydrofuran derivatives described above since, in these cases, C(9) is an acetal carbon atom directly binding a methoxy group rather than the three-carbon chain. Compounds **9** and **10** are the two C(9) epimers.

Plakortether F (**9**), a colorless oil with $[\alpha]_D = +40$ ($c = 0.01$), showed mass spectroscopic data indicating the molecular formula $C_{16}H_{30}O_5$, which requires two degrees of unsaturation. The $^1$H NMR spectrum of **9** (Table 3), exhibited a series of signals very close to those of other plakortethers but revealed the presence of an additional methoxy group resonating at $\delta = 3.29$. The $^1$H NMR signals were arranged into two spin systems with the aid of the 2D COSY spectrum. The first fragment, involving the $H_2$-2/$H_2$-5 part of the molecule, ethyl-branched at C(4), was identical to the corresponding moiety in other plakortethers, whereas the second spin system simply connected $H_2$-7 with H-9, including an ethyl group linked at C(8). The 2D HMQC spectrum associated all the proton resonances with those of their directly linked carbon atoms and, in particular, attributed the value of $\delta = 105.8$, typical of acetal carbon atoms, to C(9). The 2D HMBC spectrum, showing the key long range correlations $H_3$-15/C(9), H-9/C(6), H-8/C(6), and H-4/C(6), allowed us to define the gross structure of plakortether F completely. Finally, the relative stereochemistry around the five-membered ring was assigned by interpretation of the 2D ROESY spectrum. In particular, diagnostic correlations were observed between H-9 and $H_2$-5, H-10a and $H_3$-12, and H-8 and H-5a, implying a *cis* relationship between the protons of each couple.

Plakortether G (**10**), $[\alpha]_D = -14$ ($c = 0.02$), possessing the same molecular formula ($C_{16}H_{30}O_5$ by HRMS) as plakortether F (**9**) was deduced to be that compound's C(9) epimer from the following spectral evidence. The NMR spectroscopic data of these compounds were very similar, although not identical, and analysis of 2D NMR spectra of **10**, including COSY, HMQC and HMBC experiments, indicated the same gross structure as in compound **9**. On the other hand, differences between the two compounds were found in the dipolar couplings inferred from the 2D ROESY spectrum, in which plakortether G displayed the cross-peaks H-9/$H_3$-12 and H-8/H-5a, in agreement with a change of stereochemistry at C(9).

As in the cases of plakortethers A−D, we determined the absolute stereochemistries of plakortethers F and G by correlating their structures with that of plakortin (**1**). In particular, the general strategy chosen in this case consisted of the two-step conversion of both plakortin and plakortether F (or G) into the same molecule, namely the tetraol **18** (Scheme 3). Oxidative cleavage ($KMnO_4$/$NaIO_4$ in *t*BuOH) of the $\Delta^{9,10}$ double bond of plakortin afforded compound **17** in high yield.[11] Treatment of **17** with an excess of $LiAlH_4$ in dry ether reduced the ester carbonyl group, the carboxylic group, and the endoperoxide function, yielding compound **18**. The same tetraol **18** was obtained from a mixture of plakortether F and G after acidic hydrolysis, followed by reduction with $LiAlH_4$ in dry ether. This result unambiguously indicated that the absolute configurations at C(3), C(4), C(6), and C(8) in both plakortethers F (**9**) and G (**10**) can be assigned as the same as those at the corresponding carbon atoms in plakortin (**1**). Finally, the absolute configurations at C(9) in both **9** (*S*) and **10** (*R*) were easily assigned on the basis of the relative stereochemistry deduced above.




Scheme 3. Conversion of plakortin and of plakortether F−G to tetraol **18**: a: $NaIO_4$/$KMnO_4$ in *t*BuOH; b: $LiAlH_4$ in dry diethyl ether; c: $H^+$/$H_2O$

All the isolated compounds were evaluated for cytotoxic activity against two different cell lines: WEHI 164 (murine fibrosarcoma) and RAW 264-7 (murine macrophage). The results obtained are shown in Table 4. All the tested compounds were inactive against the first cell line, while, interestingly, plakortethers A (**4**), B (**5**), D (**7**), and E (**8**) proved to be selectively active against the second cell line. It is worthy of note that, among the class of $C_{18}$ plakortethers, only the chlorine derivative, plakortether C (**6**), was shown to be inactive.

Table 4. Cytotoxic activities of plakortethers A−G (**4**−**10**); $IC_{50}$ is expressed in μg/mL; all the measurements were repeated on triplicate samples; the data reported are the mean of them

| | WEHI 164[a] | RAW 264−7[b] |
|---|---|---|
| **4** | > 20 | 7.9 |
| **5** | > 20 | 10.0 |
| **6** | > 20 | > 20 |
| **7** | 19 | 8.4 |
| **8** | > 20 | 11.6 |
| **9** | > 20 | > 20 |
| **10** | > 20 | > 20 |

[a] Murine fibrosarcoma. [b] Murine macrophages.

## Experimental Section

**General Remarks:** Optical rotations ($CHCl_3$): Perkin−Elmer 192 polarimeter equipped with a sodium lamp (λ = 589 nm) and a 10-cm microcell. IR (KBr): Bruker model IFS-48 spectrophotometer. Low- and high-resolution EI (70 eV) and FAB mass spectra (CsI ions, glycerol matrix): VG Prospec (FISONS) mass spectrometer. ES mass spectra: LCQ FINNIGAN MAT. $^1H$ (500 MHz) and $^{13}C$ (125 MHz) NMR spectra: Bruker AMX 500 spectrometer; chemical shifts are referenced to the residual solvent signal ($CDCl_3$: $\delta_H$ = 7.26; $\delta_C$ = 77.0). For more accurate measurement of the coupling constants, the one-dimensional $^1H$ NMR spectra were transformed at 64 K points, achieving a digital resolution of 0.09 Hz. The multiplicities of $^{13}C$ resonances were determined by DEPT experiments. Homonuclear $^1H$ connectivities were determined by COSY experiments. One-bond heteronuclear $^1H$-$^{13}C$ connectivities were determined with the 2D HMQC pulse sequence, using a BIRD pulse 0.50 s before each scan to suppress the signals originating from protons not directly bonded to $^{13}C$ (interpulse delay set for $^1J_{CH}$ = 125 Hz). During the acquisition time, $^{13}C$ broadband decoupling was performed by the use of the GARP sequence. Two- and three-bond $^1H$-$^{13}C$ connectivities were determined by 2D HMBC experiments. The phase-sensitive (PS)-HMBC spectra were recorded with the delay set at 40 ms and a data size of 2 K (*F2*) × 128 (*F1*) points. Medium-pressure liquid chromatography (MPLC): Büchi 861 apparatus and Merck SI60 (230−400 mesh) and RP18 stationary phase. High-performance liquid chromatography (HPLC) separations in isocratic mode: Beckman apparatus equipped with refractive index detector and with LUNA SI60 (250 × 4 mm) or RP18 columns.

**Collection, Extraction, and Isolation:** A specimen of *Plakortis simplex* was collected in Summer 1998 on the coast of Berry Island (Bahamas), and identified by Prof. M. Pansini (Università di Genova). A voucher specimen has been deposited at the Istituto di Zoologia, Università di Genova, Italy with the ref. No. 2006. The organism was immediately frozen after collection and kept frozen until extraction, when the sponge (57 g, dry weight after extraction) was homogenized and extracted, in sequence, with methanol (4 × 500 mL) and with chloroform (4 × 500 mL). The methanol extract was initially partitioned between $H_2O$ and *n*BuOH and the organic phases were then combined and concentrated in vacuo to afford 29.3 g of a brown-colored viscous oil. This was subjected to chromatography on a column packed with RP18 silica gel and eluted with a system of solvents of decreasing polarity from $H_2O$ to MeOH/$H_2O$ (1:9). Fractions eluted with MeOH/$H_2O$ (8:2 and 9:1) were combined (13.3 g) and further chromatographed by MPLC ($SiO_2$, 230−400 mesh; solvent gradient system of increasing polarity from *n*-hexane to MeOH). Fractions eluted with EtOAc/*n*-hexane (1:1) were purified by HPLC (eluent *n*-hexane/EtOAc, 88:12; flow 0.8 mL/min) to afford, in order of elution, plakortether B (**5**, 13 mg), plakortether A (**4**, 22 mg), plakortether C (**6**, 15 mg), plakortether F (**9**, 11 mg), and plakortether G (**10**, 4 mg) in pure states. Fractions eluted with EtOAc/*n*-hexane (6:4) were purified by HPLC (eluent *n*-hexane/EtOAc, 75:25; flow 0.7 mL/min) to afford, in order of elution, plakortether D (**7**, 18 mg) and plakortether E (**8**, 15 mg), in pure states.

**Plakortether A (4):** Colorless oil. $[\alpha]_D^{25}$ = +2 (*c* = 0.18 in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3596, 1742, 1069, 915 $cm^{-1}$. $^1H$ and $^{13}C$ NMR ($CDCl_3$): see Table 1. HMBC ($CDCl_3$): H-3/C-1, $H_2$-2/C-1, $H_2$-2/C-4, $H_2$-5/C-7, $H_2$-5/C-6, $H_2$-5/C-15, $H_2$-7/C-15, $H_2$-7/C-6, H-8/C-6, $H_3$-15/C-6, H-9/C-6, $H_3$-18/C-1. ROESY ($CDCl_3$): H-9/$H_3$-15, H-8/$H_2$-5. EIMS (70 eV): *m*/*z* = 297 [M − Me], 279 [M − Me − OH], 153 (base peak, M − side chain: $C_{10}H_{17}O$); ESMS (positive ions): *m*/*z* = 313 $[M + H]^+$. HRESMS: *m*/*z* = 313.2396, calcd. for $C_{18}H_{33}O_4$ *m*/*z* = 313.2381.

**Plakortether B (5):** Colorless oil. $[\alpha]_D^{25}$ = −13 (*c* = 0.12 in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3599, 1742, 1072, 915 $cm^{-1}$. $^1H$ and $^{13}C$ NMR ($CDCl_3$): see Table 1. EIMS (70 eV): *m*/*z* = 299 [M − Me], 281 [M − Me − OH], 155 (base peak, M − side chain: $C_{10}H_{19}O$); ESMS (positive ions): *m*/*z* = 315 $[M + H]^+$. HRESMS: *m*/*z* = 315.2544, calcd. for $C_{18}H_{33}O_4$ *m*/*z* = 315.2537.

**Catalytic Hydrogenation of Plakortether A:** Palladium on charcoal catalyst (10%; 3 mg) was added to plakortether A (**4**; 15 mg) in dry EtOH. The solution was stirred at room temperature under hydrogen for 5 h. The catalyst was then removed by filtration and the solvent was evaporated to obtain a mixture, which, purified by HPLC on SI60 column (eluent *n*-hexane/EtOAc, 95:5), afforded plakortether B (**5**) in a pure state (13.5 mg, 90% yield).

**Reduction of Plakortin:** Plakortin (**1**; 50 mg) in dry ether (200 μL) was treated with acetic acid (250 μL) and an excess (100 mg) of Zn dust and then stirred vigorously for 24 h at room temperature. After confirmation of disappearance of the starting material by TLC, the solution was neutralized with $Na_2CO_3$ and the solid was removed by filtration. The solvent was then evaporated and the obtained product was partitioned between $H_2O$ and $CHCl_3$. The organic phase contained compound **11** (43 mg), in a pure state.

**Compound 11:** Colorless oil. $[\alpha]_D^{25}$ = −5 (*c* = 0.16 in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3340, 2969 $cm^{-1}$. ESMS: *m*/*z* = 315 $[M + H]^+$, 337 $[M + Na]^+$. EIMS: *m*/*z* = 296 [M − 18], 281 [M − 33]. $^1H$ NMR ($CDCl_3$): δ = 5.53 (dt, H-10, *J* = 15.2, 6.2 Hz), 5.21 (dd, H-9, *J* = 15.2, 9.0 Hz), 4.16 (dt, H-3, *J* = 10.4, 2.1 Hz), 3.70 (s, $H_3$-18), 2.46 (dd, H-2a, *J* = 15.9, 10.4 Hz), 2.34 (dd, H-2b, *J* = 15.9, 2.1 Hz), 2.13 (m, H-8), 2.03 (q, $H_2$-11, *J* = 6.2 Hz), 1.91 (m, H-4), 1.67 (dd, H-5a, *J* = 13.5, 9.8 Hz), 1.55 (H-7a, overlapped), 1.53 (H-7b, overlapped), 1.38 (m, H-13a), 1.35 (H-5b, overlapped), 1.27 (m, H-16a), 1.21 (m, H-13b), 1.17 (s, $H_3$-15), 1.13 (m, H-16b), 0.98 (t, $H_3$-12, *J* = 6.2 Hz), 0.92 (t, $H_3$-17, *J* = 7.3 Hz), 0.84 (t, $H_3$-14, *J* = 6.3 Hz).

**Synthesis of Plakortether B from Compound 11:** Compound **11** (32 mg, 0.102 mmol) was dissolved in dry $CH_3CN$ (4 mL) under an argon flow at 0 °C and *N*-iodosuccinimide (34 mg, 0.145 mmol) was then added to the solution. The reaction was then stirred for 3 h at room temperature. The obtained mixture was dried and then purified on a silica gel column by elution with a solvent gradient from *n*-hexane to *n*-hexane/EtOAc (1:1). The mixture of four epi-

mers (30 mg) was dissolved in dry benzene (3 mL) and an excess (5:1) of triphenyltin hydride was then added dropwise to the solution, followed by a spatula tip of 2,2′-azobis(isobutyronitrile) (AIBN). The solution was stirred at 95 °C under reflux for 2 h. After this time, the solution was cooled at room temperature, filtered through Celite, and concentrated in vacuo, and the obtained mixture was purified by HPLC (LUNA SI60 250 × 4; eluent *n*-hexane/EtOAc, 95:5). Only two main products were obtained. The first one (18 mg) was identical (by $[\alpha]_D$ and NMR spectroscopic data) to plakortether B (**5**), the second, **12** (8 mg), was identified as its C(9) epimer.

**Compound 12:** Colorless oil. $[\alpha]_D^{25} = -2$ ($c = 0.05$ in $CHCl_3$). ESMS (positive ions): $m/z$ = 315 $[M + H]^+$, 337 $[M + Na]^+$. $^1H$ NMR ($CDCl_3$): δ = 4.09 (m, H-3), 3.70 (s, $H_3$-18), 3.48 (m, H-9), 2.49 (dd, H-2a, $J$ = 15.4, 10.3 Hz), 2.33 (dd, H-2b, $J$ = 15.4, 2.2 Hz), 2.00 (dd, H-7a, $J$ = 12.5, 8.8 Hz), 1.88 (m, H-4), 1.69 (m, H-8,), 1.62 (H-5a, overlapped), 1.57 (H-7b, overlapped), 1.53 (H-13a, overlapped), 1.52 (H-10a, overlapped), 1.49 (H-10b, overlapped), 1.45 (dd, H-5b, $J$ = 15.5, 1.9 Hz), 1.32 (H-16a, overlapped), 1.28 ($H_2$-11, overlapped), 1.27 (s, $H_3$-15), 1.18 (m, H-13b), 1.12 (m, H-16b), 0.94 (t, $H_3$-17, $J$ = 7.3 Hz), 0.93 (t, $H_3$-14, $J$ = 7.3 Hz), 0.90 (t, $H_3$-12, $J$ = 7.3 Hz).

**Plakortether C (6):** Colorless oil. $[\alpha]_D^{25} = -3$ ($c = 0.14$ in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3585, 1742, 1072, 915 $cm^{-1}$. $^1H$ and $^{13}C$ NMR ($CDCl_3$): see Table 2. EIMS (70 eV): $m/z$ = 333, 335 (ratio 3:1, M − Me), 315, 317 (3:1, M − Me − OH), 277 [M − Me − OH − HCl], 189, 191 (3:1, base peak, M − side chain: $C_{10}H_{18}ClO$); ESMS (positive ions): $m/z$ = 349, 351 (ratio 3:1) $[M + H]^+$. HRESMS: $m/z$ = 349.2135, calcd. for $C_{18}H_{34}{}^{35}ClO_4$ $m/z$ = 349.2147.

**Dehalogenation of Plakortether C (6):** Plakortether C (6 mg, 0.017 mmol) was dissolved in dry benzene (2 mL) and an excess (5:1) of triphenyltin hydride and a spatula tip of 2,2′-azobis(isobutyronitrile) (AIBN) were then added to the solution, which was then stirred at 95 °C under reflux for 3 h. After this time, the solution was allowed to cool to room temperature, filtered through Celite, concentrated in vacuo, and purified by HPLC (LUNA SI60; eluent *n*-hexane/EtOAc, 95:5), yielding plakortether B (**5**, 4.5 mg).

**Plakortether D (7):** Colorless oil. $[\alpha]_D^{25} = +7$ ($c = 0.09$ in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3600, 1745, 1719 1068, 911 $cm^{-1}$. $^1H$ and $^{13}C$ NMR ($CDCl_3$): see Table 2. EIMS (70 eV): $m/z$ = 271 (base peak, M − shorter side chain: $C_{15}H_{27}O_4$); ESMS (positive ions): $m/z$ = 329 $[M + H]^+$. HRESMS: $m/z$ = 329.2335, calcd. for $C_{18}H_{33}O_5$ $m/z$ = 329.2310.

**Synthesis of Dithioketal 13 and Conversion into Plakortether B:** Plakortether D (6 mg, 0.018 mmol) and 1,2-ethanedithiol (3.8 mg, 0.040 mmol) were mixed under argon and stirred, followed by dropwise addition of boron trifluoride−diethyl ether (300 μL). The solution was allowed to stir for 3 h. The mixture was diluted with EtOAc (5 mL) and washed sequentially with three 5-mL portions of 5% NaOH, water, and brine. The organic layer was then dried with anhydrous $Na_2SO_4$. Removal of the solvent gave 5.1 mg of dithioketal **13**. This was dissolved in dry benzene (500 μL), and triphenyltin hydride (5 equiv.) and catalytic amounts of azobis(isobutyronitrile) were then added. The reaction flask (equipped with a reflux condenser) was heated at 90 °C in an oil bath. The reaction mixture was stirred for 14 h and then cooled at room temperature and concentrated in vacuo. The obtained mixture was purified by HPLC (LUNA, SI60; eluent *n*-hexane/EtOAc, 95:5) to yield pure plakortether B (**5**; 3.0 mg).

**Compound 13:** Amorphous solid. IR (KBr): $\tilde{\nu}$ = 3642, 1748, 985, 974 $cm^{-1}$. FABMS (glycerol matrix, positive ions): $m/z$ = 405 $[M + H]^+$. $^1H$ NMR ($CDCl_3$): δ = 4.36 (m, H-3), 3.91 (d, H-9, $J$ = 7.3 Hz), 3.70 (s, $H_3$-18), 2.59 (m, $H_2$-2′/$H_2$-3′), 2.56 (dd, H-2a, $J$ = 15.7, 8.5 Hz), 2.47 (dd, H-2b, $J$ = 15.7, 4.3 Hz), 2.42 (m, H-8), 2.07 (H-7a, overlapped), 2.07 (H-11a, overlapped), 1.97 (dq, H-11b, $J$ = 15.5, 7.7 Hz), 1.83 (dd, H-5a, $J$ = 15.0, 12.5 Hz), 1.82 (m, H-4), 1.79 (m, H-13a), 1.65 (dd, H-7b, $J$ = 12.0, 9.5 Hz), 1.49 (dd, H-5b, $J$ = 15.0, 2.0 Hz), 1.45 (m, H-16a), 1.42 (H-13b, overlapped), 1.40 (s, $H_3$-15), 1.28 (m, H-16b), 1.09 (t, $H_3$-12, $J$ = 7.7 Hz), 0.93 (t, $H_3$-14, $J$ = 6.4 Hz), 0.92 (t, $H_3$-17, $J$ = 7.3 Hz).

**Plakortether E (8):** Colorless oil. $[\alpha]_D^{25} = -2$ ($c = 0.03$ in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3602, 1743, 1069, 915 $cm^{-1}$. $^1H$ and $^{13}C$ NMR ($CDCl_3$): see Table 3; ESMS (positive ions): $m/z$ = 331 $[M + H]^+$. HRESMS: $m/z$ = 329.2496, calcd. for $C_{18}H_{35}O_5$ $m/z$ = 331.2486.

**Reduction of Plakortether D (7) with $NaBH_4$:** Plakortether D (5 mg, 0.016 mmol) was dissolved in MeOH (5 mL), and $NaBH_4$ powder (1 mg) was added to the solution. The reaction mixture was kept at 50 °C for 2.5 h whilst stirring, and it was then concentrated in vacuo and partitioned between water and EtOAc. The organic phase was concentrated and purified by HPLC on an SI60 column (eluent *n*-hexane/EtOAc, 7:3) to yield plakortether E (**8**; 2.0 mg) (characterized by $[\alpha]_D$ and NMR), together with its C(10) epimer (**14**; 1.3 mg).

**Compound 14:** Amorphous solid. $[\alpha]_D = -10$ ($c = 0.01$ in $CHCl_3$). ESMS (positive ions): $m/z$ = 331 $[M + H]^+$. $^1H$ NMR ($CDCl_3$): δ = 4.22 (m, ,H-3, 3.73 (s, $H_3$-18, 3.49 (dd, H-9, $J$ = 8.2, 2.2 Hz), 3.38 (dt, H-10, $J$ = 6.5, 2.2 Hz), 2.60 (dd, H-2a, $J$ = 15.5, 10.5 Hz), 2.33 (H-8, overlapped), 2.32 (dd, H-2b, $J$ = 15.5, 1.8 Hz), 2.05 (dd, H-7a, $J$ = 12.1, 9.2 Hz), 1.84 (m, H-4, 1.76), (dd, H-5a, $J$ = 15.0, 12.0 Hz), 1.58 (dd, H-7b, $J$ = 12.1, 2.0 Hz), 1.58 (H-13a, overlapped), 1.52 (m, H-11a), 1.50 (m, H-11b), 1.30 (H-5b, overlapped), 1.29 (s, $H_3$-15), 1.22 (m, H-13b), 1.18 (m, $H_2$-16), 1.00 (t, $H_3$-12, $J$ = 6.6 Hz), 0.91 (t, $H_3$-17, $J$ = 7.3 Hz), 0.89 (t, $H_3$-14, $J$ = 7.3 Hz).

**Preparation of MTPA Esters of Plakortether E:** Compound **8** (2 mg) was dissolved in dry pyridine (0.5 mL), treated with (−)-MTPA chloride (15 μL), and 4-(dimethylamino)pyridine (DMAP, a spatula tip), and then maintained at room temperature whilst stirring overnight. After removal of the solvent, the reaction mixture was purified by HPLC on SI60 column (eluent *n*-hexane/EtOAc, 95:5), to afford (*S*)-MTPA ester **15** in a pure state (2.4 mg). With (+)-MTPA chloride, the same procedure afforded (*R*)-MTPA ester **16** in the same yield.

**(*S*)-MTPA Ester 15:** Amorphous solid. IR (KBr): $\tilde{\nu}$ = 2868, 1743, 1688, 1572, 1541, 1389, 1188, 1119 $cm^{-1}$. FABMS (glycerol matrix, positive ions): $m/z$ = 763 $[M + H]^+$. $^1H$ NMR ($CDCl_3$): δ = 7.35 and 7.45 (MTPA phenyl protons) 5.68 (m, H-3), 4.99 (m, H-10), 3.71 (dd, H-9, $J$ = 8.1, 3.7 Hz), 3.68 (s, $H_3$-18), 2.65 (dd, H-2a, $J$ = 16.2, 8.8 Hz), 2.57 (dd, H-2b, $J$ = 16.2, 4.4), 1.81 (dd, H-7a, $J$ = 11.8, 2.0 Hz), 1.79 (m, H-8), 1.74 (m, $H_2$-11), 1.68 (m, $H_1$-4), 1.44 (dd, H-5a, $J$ = 14.7, 5.1 Hz), 1.31 (H-7b, overlapped), 1.30 ($H_2$-13, overlapped), 1.29 (m, $H_2$-16), 1.26 (H-5b, overlapped), 1.10 (s, $H_3$-15), 0.87 (t, $H_3$-17, $J$ = 7.3 Hz), 0.86 (t, $H_3$-14, $J$ = 7.3 Hz), 0.82 (t, $H_3$-12, $J$ = 7.3 Hz).

**(*R*)-MTPA Ester 16:** Amorphous solid. IR (KBr): $\tilde{\nu}$ = 2868, 1745, 1890, 1572, 1541, 1389 $cm^{-1}$. FABMS (glycerol matrix, positive ions): $m/z$ = 763 $[M + H]^+$. $^1H$ NMR ($CDCl_3$): δ = 7.32 and 7.55 (MTPA phenyl protons), 5.68 (m, H-3), 4.99 (m, H-10), 3.67 (s, $H_3$-18), 3.62 (dd, H-9, $J$ = 8.8, 5.1 Hz), 2.61 (dd, H-2a, $J$ = 16.2, 8.1 Hz), 2.51 (dd, H-2b, $J$ = 16.2, 4.4 Hz), 1.85 (dd, H-7a, $J$ = 11.0, 8.8 Hz), 1.77 (m, $H_2$-11), 1.72 (m, H-8), 1.70 (m, H-4), 1.51 (dd, H-5a, $J$ = 14.7, 5.1 Hz), 1.37 (m, H-13a), 1.33 (H-7a, over-

lapped), 1.32 (H-5b, overlapped), 1.28 (m, $H_2$-16), 1.12 (m, H-13b), 1.11 (s, $H_3$-15), 0.92 (t, $H_3$-12, $J$ = 7.3 Hz), 0.86 (t, $H_3$-17, $J$ = 7.3 Hz), 0.70 (t, $H_3$-14, $J$ = 7.3 Hz).

**Plakortether F (9):** Colorless oil. $[\alpha]_D^{25} = +40$ ($c$ = 0.02 in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3602, 1744, 1075, 918 $cm^{-1}$. $^1H$ and $^{13}C$ NMR ($CDCl_3$): see Table 3. EIMS (70 eV): $m/z$ = 271 (M − OMe), 143 (base peak, M − side chain); ESMS (positive ions): $m/z$ = 303 $[M + H]^+$. HRESMS: $m/z$ = 303.2182, calcd. for $C_{16}H_{31}O_5$ $m/z$ = 303.2173.

**Plakortether G (10):** Colorless oil. $[\alpha]_D^{25} = -14$ ($c$ = 0.02 in $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3602, 1744, 1077, 917 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): δ = 4.65 (d, H-9, $J$ = 3.2 Hz), 4.37 (m, H-3), 3.69 (s, $H_3$-16), 3.42 (s, $H_3$-15), 2.62 (dd, H-2a, $J$ = 15.5, 10.5 Hz), 2.37 (dd, H-2b, $J$ = 15.5, 2.5 Hz), 2.19 (m, H-8), 2.04 (dd, H-7a, $J$ = 11.5, 9.5 Hz), 1.84 (dd, H-5a, $J$ = 15.5, 12.5 Hz), 1.76 (m, H-4), 1.57 (m, H-10a), 1.48 (dd, H-7b, $J$ = 1.5, 2.0 Hz), 1.37 (H-13a, overlapped), 1.36 (H-10b, overlapped), 1.36 (H-5b, overlapped), 1.31 (s, $H_3$-12), 1.18 (m, H-13b), 0.92 (t, $H_3$-11, $J$ = 7.0 Hz), 0.91 (t, $H_3$-14, $J$ = 7.0 Hz). $^{13}C$ NMR($CDCl_3$): δ = 173.9 (C-1), 107.1 (C-9), 83.8 (C-6), 71.4 (C-3), 54.8 (C-15), 52.0 (C-16), 47.7 (C-8), 43.6 (C-7), 42.2 (C-4), 42.1 (C-5), 37.6 (C-2), 27.1 (C-13), 26.9 (C-12), 21.9 (C-10), 12.9 (C-11), 12.3 (C-14). EIMS (70 eV): $m/z$ = 271 (M − OMe), 143 (base peak, M − side chain); ESMS (positive ions): $m/z$ = 303 $[M + H]^+$. HRESMS: $m/z$ = 303.2180, calcd. for $C_{16}H_{31}O_5$ $m/z$ = 303.2173.

**Oxidative Cleavage of Plakortin and Reduction to Tetraol 18:** $Na_2CO_3$ (0.04 M, 28 mL) and an aqueous solution (150 mL) of $KMnO_4$ (0.023 M, and $NaIO_4$ (0.09 M) were added to a solution of plakortin (140 mg) in *t*BuOH (50 mL). The reaction was allowed to proceed at 37 °C for 20 h with stirring. After acidification with 5 N $H_2SO_4$, the solution was decolorized with a saturated solution of oxalic acid and extracted with diethyl ether (200 mL, twice). The combined organic phases were dried with $Na_2SO_4$, filtered, and then concentrated in vacuo. The obtained fraction was purified by HPLC (LUNA, SI60; eluent *n*-hexane/EtOAc, 7:3) to yield compound **17**[11] (100 mg, 80% yield) in a pure state. Compound **17** (90 mg) was dissolved in dry diethyl ether, $LiAlH_4$ (5 equiv.) was added, and the solution was stirred for 3 h at room temperature. After completion of the reaction, $H_2O$ was added and the solution was extracted with chloroform. Concentration of the organic phase in vacuo yielded tetraol **18** (75 mg), in a pure state.

**Compound 18:** Amorphous solid. $[\alpha]_D = -6$ ($c$ = 0.04 in $CHCl_3$). ESMS (positive ions): $m/z$ = 263 $[M + H]^+$. $^1H$ NMR ($CDCl_3$): δ = 5.62 (bs, OH-3, 5.28 (s, OH-6, 4.77 (bs, OH-9, 4.10 (bs, OH-1, 3.95 (H-3, overlapped), 3.92 (H-1a, overlapped), 3.78 (H-1b, overlapped), 3.74 (H-9a, overlapped), 3.32 (bt, H-9b, $J$ = 7.5 Hz), 2.01 (dd, H-5a, $J$ = 15.0, 12.0 Hz), 1.95 (m, H-4), 1.81 (m, H-8), 1.77 (m, H-2a), 1.63 (dd, H-7a, $J$ = 12.1, 9.2 Hz), 1.59 (dd, H-7b, $J$ = 12.1, 2.0 Hz), 1.48 (m, H-2b), 1.31 ($H_2$-10, overlapped), 1.29 (H-13a, overlapped), 1.23 (s, $H_3$-12), 1.18 (m, H-13b), 1.12 (dd, H-5b, $J$ = 15.0, 4.0 Hz), 0.92 (t, $H_3$-11, $J$ = 7.3 Hz), 0.91 (t, $H_3$-14, $J$ = 7.3 Hz).

**Hydrolysis of Plakortethers F−G and Reduction:** A mixture of plakortethers F (**9**; 3 mg) and G (**10**; 3 mg) was treated with HCl (0.2 N; 500 μL) for 1 h at 60 °C. The solution was then cooled at room temperature, neutralized with $NH_4OH$, and extracted with EtOAc. The organic phase, dried with $Na_2SO_4$, was then subjected to reduction with $LiAlH_4$ in dry ether by the same procedure as described above. Tetraol **18** (2 mg) was thus obtained as single product.

**Cytotoxic Activity:** WEHI 164 cells (murine fibrosarcoma cell line) and RAW 264-7 cells (murine macrophage) were maintained in adhesion on Petri dishes with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (heat-deactivated only for WEHI 164 cells) (FBS), HEPES (25 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). WEHI 164 and RAW 264-7 cells ($4 \times 10^3$ cells) were plated on 96-well plates and allowed to adhere at 37 °C in 5% $CO_2$/95% air for 2 h. Thereafter, the medium was replaced with fresh medium (50 μL) and then aliquots (75 μL) of 1:2 (v/v) serial dilution of test compounds **4**−**10** were added and the cells were incubated for 72 h. The cells' viability was assessed through an MTT conversion assay. After 72 h, MTT (5 mg/mL, 25 μL) was added and the cells were incubated for an additional 3 h. After this time, the cells were lysed and the dark blue crystals were solubilized with a solution (100 μL) containing 50% (v/v) *N*,*N*-dimethylformamide, 20% (w/v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620-nm filter. The viability of each cell line in response to treatment with compounds **4**−**10** was calculated as % dead cells = 100 − (OD treated/OD control) × 100. The results of cytotoxic activity are expressed as $IC_{50}$ (μg/mL) in Table 4.

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[11] Compound **17** has been already obtained from plakortin. Its physical data are reported in ref.[4]

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