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New antimalarial polyketide endoperoxides from the marine sponge *Plakinastrella mamillaris* collected at Fiji Islands

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ABSTRACT

Plakortides R–U, four new polyketide endoperoxides, have been isolated from the marine sponge *Plakinastrella mamillaris*. Their structures were elucidated on the basis of extensive NMR spectroscopic (^{1}H and ^{13}C, COSY, HSQC, HMBC, and ROESY) and MS analyses and by chemical methods. In addition, a new method for the unambiguous stereochemical elucidation of 3,6-disubstituted 1,2-dioxines, frequently isolated from Plakinidae sponges, is reported. Pharmacological analysis demonstrated that plakortide U is endowed with in vitro antiplasmodial activity against a chloroquine-resistant strain.

1. Introduction

Six-membered cyclic peroxides are a large family of oxygenated polyketides characteristic of marine sponges of the family Plakinidae. After the discovery in 1978 of plakortin from *Plakortis halichondroides*,[1] several new derivatives, differing in the length of the side chain attached at C-6, by the presence of methyl- and/or ethyl branching, and in the number and position of double bonds have been isolated and found to exhibit a wide spectrum of biological activities, including antiparasitic and cytotoxic properties.[2–12]

In the frame of a project aimed at the investigation of marine invertebrates of the South Pacific Ocean, we had the opportunity to study the sponge *Plakinastrella mamillaris* collected at Fiji Islands. Previous investigation of the same organism by our research group resulted in the identification of several mono- and polycyclic oxygenated polyketides,[13,14] some of which displayed PPARγ agonistic activity.[13] We now report the results of investigations of the *n*-hexane extract, obtained from a solvent partitioning[15] of the crude methanol extract, which led to the isolation of four new cyclic polyketide peroxides (Fig. 1), which we named plakortides R–U (**1**–**4**).

In particular, plakortide R (**1**) was shown to be the methyl ester of a diastereomer of haterumadioxin A (**5**), previously isolated from the Okinawan sponge *Plakortis lita*.[8] In this paper we report the isolation and the structural elucidation of all new compounds, including a detailed stereochemical analysis of plakortide R (**1**) and the evaluation of their antimalarial activity.

2. Results and discussion

Plakortide R (**1**) has the molecular formula $C_{19}H_{32}O_4$ as determined by HRESIMS (*m*/*z* 347.2188 $[M+Na]^+$, calcd 347.2198), which requires 4° of unsaturation.

A carbomethoxy group was indicated by one methoxy signal (δ_H 3.71, δ_C 52.2) and one acyl carbon at δ_C 172.2. The ^{13}C NMR spectrum also contained 4 olefinic carbon signals attributable to a trisubstituted and a disubstituted double bond [δ_C 138.2 (s) and 124.8 (d), 134.6 (d) and 131.8 (d)], 2 signals at δ_C 83.3 (s) and 77.0 (d) assigned to oxygen-bearing carbon atoms, and 11 aliphatic carbon signals ($4CH_3$, $6CH_2$, and 1CH). Analysis of the 2D NMR spectra, including DQF-COSY, HSQC and HMBC spectra allowed the assignment of the structure of plakortide R (**1**). This was identified as the methyl ester of a compound sharing the entire molecular framework with the known haterumadioxin A (**5**) (Fig. 1), isolated by Uemura and co-workers from an Okinawan *P. lita*.[8] The availability in our laboratories of an original sample of haterumadioxin A,

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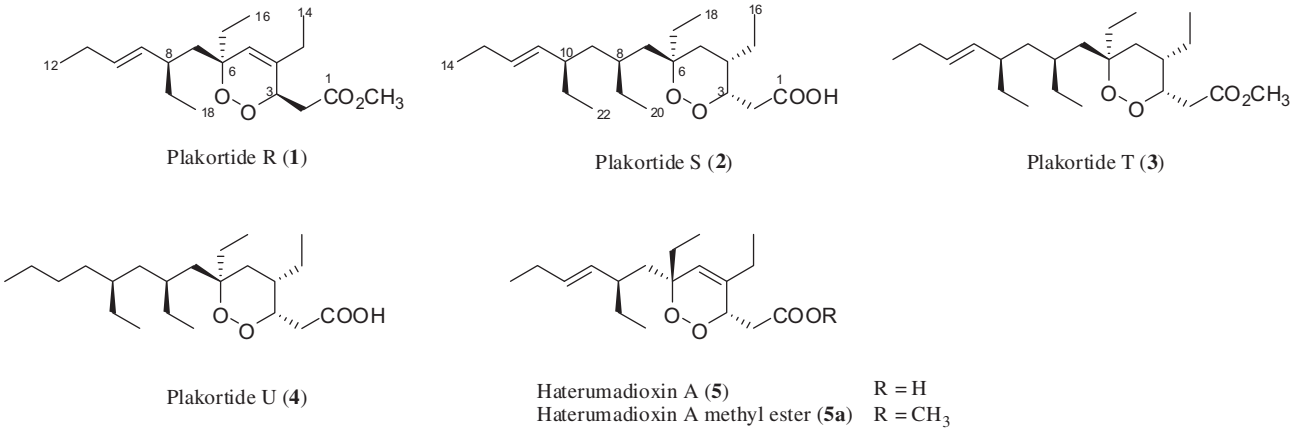

Fig. 1. New cyclic polyketide endoperoxides (**1**–**4**) from *Plakinastrella mamillaris*, the previously reported haterumadioxin A (**5**) and its methyl ester derivative (**5a**).

obtained from our previous investigations on Plakinidae sponges, allowed the preparation of its methyl ester derivative (**5a**) (Fig. 1), whose ^{1}H NMR spectroscopic chemical shifts were carefully compared to those of plakortide R (Table 1 and Experimental section). This comparison evidenced several small but significant differences between the two sets of signals, especially in the resonances of the nuclei attached to the peroxide ring and of the diastereotopic CH_2-7 protons. For example, these latter signals resonated as well-separated double doublets at δ_H 1.59 and 1.86 in plakortide R and as closer signals at δ_H 1.49 and 1.60 in **5a**. Thus, we concluded that plakortide R is a diastereomer of haterumadioxin A methyl ester (**5a**). Having assigned the trans configuration at $\Delta^{9,10}$ of **1** on the basis of the large value of $J_{H\text{-}9/H\text{-}10}$ (15.3 Hz), we concluded that **1** and **5a** should differ in the configuration at one (or two) of the stereogenic centres at C-3, C-6 and C-8.

Table 1
^{1}H and ^{13}C NMR data (500 and 125 MHz, $CDCl_3$) of plakortide R (**1**)

Position	δ_H[a]	δ_C
1	—	172.2
2	2.57 dd (3.0, 15.1), 2.85 dd (8.8, 15.1)	37.2
3	4.62 br d (8.8)	77.0
4	—	138.2
5	5.48 s	124.8
6	—	83.3
7	1.59 ovl, 1.86 dd (3.5, 14.2)	43.1
8	1.98 ovl	40.9
9	5.13 dd (9.2, 15.3)	134.6
10	5.35 dt (6.5, 15.3)	131.8
11	2.01 ovl	25.6
12	0.97 t (7.5)	14.2
13	1.99 ovl	25.6
14	1.08 t (7.3)	11.9
15	1.58 ovl	30.2
16	0.79 t (7.5)	8.2
17	1.13–1.21 m, 1.32–1.40 m	30.1
18	0.80 t (7.3)	11.8
OMe	3.71 s	52.2

[a] Coupling constants are in parentheses and given in hertz. ^{1}H and ^{13}C assignments aided by COSY, HSQC and HMBC experiments. ovl: overlapped with other signals.

To determine the absolute configuration at C-3, plakortide R (**1**) was reduced by treatment with acetic acid and Zn dust in dry ether to give the diol **1a**, which was in turn esterified at C-3 with *R*-(−)- or *S*-(+)-MTPA chloride in dry CH_2Cl_2 (Fig. 2). Analysis of the obtained MTPA derivatives, **1b**, *S*-(−)- and **1c** *R*-(+)-, respectively, according to the modified Mosher method,[16] established the 3*R* configuration, opposite to that reported for haterumadioxin A (**5**).

The absolute configuration at C-8 was determined using a procedure based on the ^{1}H NMR spectroscopic chemical shift difference of the hydroxymethylene protons in diastereomeric *R*-(+)- and *S*-(−)-MTPA esters,[17] a method frequently used to assign the absolute configuration at C-2 of primary β-alkyl-substituted alcohols.[9,18] Accordingly, plakortide R (**1**) was converted into the alcohol derivative **1d** by mild ozonolysis[2] followed by reduction with $NaBH_4$ as shown in Fig. 3. Compound **1d** was treated with *R*-(−)- and *S*-(+)-MTPA chloride to give the *S*-(−)-MTPA (**1e**) and *R*-(+)-MTPA (**1f**) esters, respectively (Fig. 3). In the ^{1}H NMR spectra, the protons at C-9 of the *R*-(+)-MTPA derivative (**1f**) appeared as two well-separated double doublets at δ_H 4.52 and 4.21, whereas those of the *S*-(−)-MTPA ester (**1e**) were closer at δ_H 4.40 and 4.30. These data suggested the *R* configuration at C-8 of **1**, the same as in haterumadioxin A (**5**).

The analysis of spatial couplings did not allow an unambiguous definition of the relative stereochemistry around the unsaturated six-membered ring. In haterumadioxin A,[8] a strong ROE effect observed between the remote H-2a and H-9, and justified by the folding of the side chain due to π−π stacking between C-4 and C-9 double bonds, has been used to suggest a *cis* relationship between the carbomethoxy methyl substituent at C-3 and the side chain at C-6. Although we observed this ROE contact also for **1**, we reasoned that, given the remote nature of the involved protons, the same contact could also be feasible in the case of trans oriented carbomethoxy and C-6 side chains. Thus, for the sake of an unambiguous assignment of the relative configuration at C-6, we envisaged that saturation of the C-4 olefin could offer a better opportunity to analyze the relative spatial arrangement of the substituents around the 1,2-dioxane ring. Since the chemoselective hydrogenation of double bond in monocyclic 1,2-dioxines has been demonstrated to be practically unfeasible under different experimental conditions,[19] we subjected the side chain truncated monounsaturated derivative **1d** to epoxidation with *m*-CPBA in $CHCl_3$ (Fig. 4). Epoxidation was found to proceed stereoselectively, affording a 3:1 mixture of two diastereomeric epoxide derivatives **1g** and **1h**, which were separated through HPLC and characterized by 1D and 2D NMR spectroscopy.

Analysis of the ROESY spectrum of the major epoxide derivative **1g** clearly evidenced the β-configuration of the epoxide ring and gave definitive information on the stereochemistry of the six-membered ring. In particular ROESY correlations (Fig. 4) H-3/H-5, H-10 and H-5/H-12, H-13 indicated that H-3 and the two ethyl groups on C-4 and C-6 were on the same face of the molecule and, consequently, the carbomethoxy methyl substituent at C-3 and the side chain at C-6 should be cis-oriented.

On the basis of the overall stereochemical analysis, the absolute stereochemistry of plakortide R (**1**) was determined to be 3*R*,6*S*,8*R*.

The same sequence of derivatization reactions was applied to haterumadioxin A methyl ester (**5a**) (Fig. 4). The stereoselective

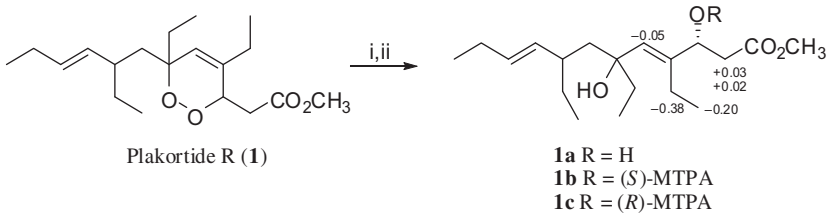

Fig. 2. Conversion of plakortide R (**1**) to diol **1a** and $\Delta\delta$ ($S-R$) values (in ppm) for MTPA ester derivatives **1b** and **1c**. Reagents and conditions: (i) Zn, AcOH, ether; (ii) (*R*)- or (*S*)-MTPACl, DMAP/CH_2Cl_2, rt, 3 h.

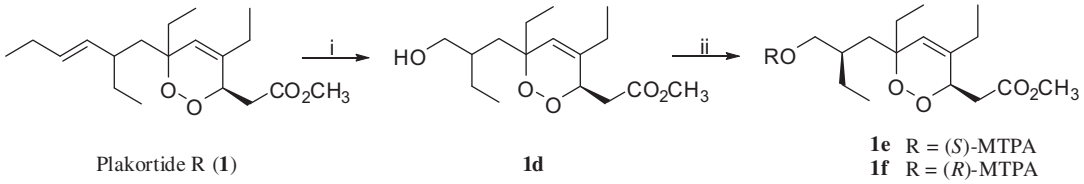

Fig. 3. Conversion of plakortide R (**1**) into alcohol **1d** and its MTPA esters **1e** and **1f**. Reagents and conditions: (i) O_3, CH_2Cl_2, −78 °C, 1 min; then $NaBH_4$, MeOH, 2 h; (ii) (*R*)- or (*S*)-MTPACl, DMAP/CH_2Cl_2, rt, 3 h.

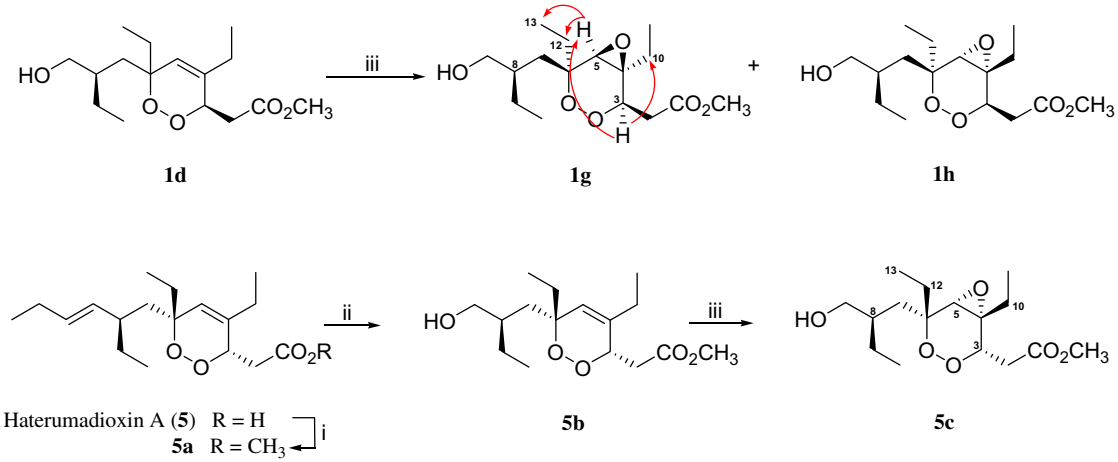

Fig. 4. Conversion of compound **1d** and haterumadioxin A methyl ester (**5a**) to epoxides **1g**, **1h** and **5c**, respectively. Red arrows for selected ROESY correlations of epoxide **1g**. Reagents and conditions: (i) diazomethane; (ii) O_3, CH_2Cl_2, −78 °C, 1 min; then $NaBH_4$, MeOH, 2 h; (iii) *m*-CPBA, $CHCl_3$, rt, overnight.

formation of epoxide **5c**, showing proton resonances of nuclei belonging to the six-membered ring almost superimposable with those of epoxide **1g**, unambiguously demonstrated their enantiomeric relationship in the dioxane ring, thus confirming the stereochemical assignment previously reported.[8]

Plakortide S (**2**) was obtained as a light yellow oil, $[\alpha]_D^{25}$ −149.8 (*c* 3.45, $CHCl_3$). Its molecular formula was determined as $C_{22}H_{40}O_4$ by HRESIMS (*m*/*z* 391.2815 $[M+Na]^+$), which required 3° of unsaturation. The presence of a carboxylic acid functionality was easily inferred by ^{13}C NMR spectroscopic data [δ_C 177.3 (s)] and IR absorptions at 1708 cm^{-1} together with the large band between 3400 and 3000 cm^{-1}. The ^{13}C NMR spectrum of **2** showed 22 carbon resonances including signals for 2 oxygen-bearing carbons at δ_C 83.3 (s) and 78.5 (d), 1 disubstituted double bond [δ_C 133.5 (d) and 132.2 (d)], and 17 other aliphatic carbon signals ($5CH_3$, $9CH_2$, and 3CH) (Table 2). As shown in Fig. 5, the COSY spectrum indicated the presence of two extended spin systems and one isolated ethyl substituent. The assignments of the 1,2-dioxane ring substructure, mostly based on the COSY data, showed a close similarity with other members of the plakortide family.[3]

The attachment of carboxyl group (C-l) to C-2 was based on the HMBC correlations between C-l (δ_C 177.3) and H_2-2 (δ_H 2.37 and 3.01). The branched monounsaturated alkyl chain was unambiguously ascertained by 2D NMR experiments that allowed elucidation of the fragment going from C-7 to C-14. The HMBC correlations H_2-7 (δ_H 1.39 ovl and 1.96 ovl) to C-6 (δ_C 83.3) connected the side chain to C-6, in addition, the isolated ethyl system was linked to C-6 on the basis of the long range heteronuclear coupling H_3-18 (δ_H 0.86)/C-6 (Fig. 5). Finally, the stereochemistry of the C-11 olefin was assigned as *E* on the basis of the coupling constant between H-11 and H-12 (J=15.1 Hz). Therefore the planar structure of plakortide S (**2**) was determined as shown in Fig. 5.

The relative stereochemistry about the peroxide ring was established by ROESY correlations, chemical shifts and coupling constant analysis (Fig. 5).

The pattern of coupling constants, H-5_{ax}/H-4 (J=12.8 Hz) and H-4/H-3 (J=4.4 Hz), was typical of a six-membered ring with an equatorial H-3 and an axial H-4. The ROESY spectrum of **2** showed cross-peaks between H_2-2 and H_2-15 confirming a 3,4-*cis* disubstituted 1,2-dioxane ring, while the correlation of H-4 with H-7a (δ_H 1.96) indicated the 6α (equatorial)-ethyl orientation (Fig. 5). The equatorial orientation of the ethyl group at C-6 was also confirmed by the ^{13}C chemical shift of C-17 at δ_C 29.8.[6] The absolute

Table 2
NMR data ($CDCl_3$) of plakortides S (**2**, 400 MHz) and U (**4**, 500 MHz)

Position	2		4	
	δ_H[a]	δ_C	δ_H[a]	δ_C
1	—	177.3	—	177.1
2	2.37 dd (2.4, 15.7) 3.01 dd (9.3, 15.7)	31.5	2.38 dd (2.5, 15.1) 2.98 dd (9.6, 15.1)	31.6
3	4.48 ddd (2.4, 4.4, 9.3)	78.5	4.48 ddd (2.5, 4.5, 9.6)	78.9
4	2.13–2.23 m	34.6	2.12–2.22 m	34.8
5	1.24 t (12.8) 1.50 ovl	33.0	1.30 ovl 1.49 ovl	33.5
6	—	83.3	—	83.3
7	1.39 ovl 1.96 ovl	35.9	1.33 ovl 1.99 dd (7.2, 14.6)	35.9
8	1.50 ovl	30.9	1.50 ovl	31.6
9	1.01–1.07 m 1.35 ovl	40.7	0.94–1.02 m 1.27 ovl	39.3
10	1.78–1.88 m	42.2	1.25 ovl	36.3
11	5.04 dd (9.0, 15.1)	133.5	1.23 ovl	33.0
12	5.40 dt (6.3, 15.1)	132.2	1.23 ovl	28.9
13	2.01 ovl	25.4	1.29 ovl	23.3
14	0.97 t (7.4)	14.2	0.90 t (7.0)	14.3
15	1.15 ovl 1.21 ovl	25.1	1.09–1.17 m 1.24 ovl	25.2
16	0.92 t (7.4)	11.1	0.93 t (7.3)	11.2
17	1.39 ovl 1.51 ovl	29.8	1.36–1.44 m 1.55 ovl	29.9
18	0.86 t (7.5)	7.1	0.89 t (7.1)	7.4
19	1.41 ovl	25.6	1.28 ovl 1.54 ovl	26.1
20	0.83 t (7.1)	9.4	0.85 t (7.3)	9.9
21	1.15 ovl 1.33 ovl	28.8	1.32 ovl 1.54 ovl	26.5
22	0.82 t (7.3)	11.7	0.84 t (7.3)	10.8

[a] Coupling constants are in parentheses and given in hertz. ^1H and ^{13}C assignments aided by COSY, HSQC and HMBC experiments. ovl: overlapped with other signals.

The NMR spectra of plakortide T (**3**), $[\alpha]_D^{25}$ −144.6 (*c* 0.13, $CHCl_3$), were superimposable to those registered for compound **2a**, the methyl ester of plakortide S obtained by methylation of **2** with diazomethane. The matching of optical rotation data confirmed the common absolute stereochemistry.[21]

The ^1H NMR spectrum of plakortide U (**4**) resembles that of **2**, but the absence of the deshielded signals for H-11 and H-12 vinyl protons and the molecular formula of **4**, $C_{22}H_{42}O_4$, established by HRESIMS (*m*/*z* 393.2998 $[M+Na]^+$, calcd for $C_{22}H_{42}NaO_4$), suggested that plakortide U is the dihydro derivative of **2**. 2D NMR spectroscopic analysis allowed the assignment of all the resonances (Table 2). The complete matching of the ^1H and ^{13}C NMR signals of the 1,2-dioxane ring system in **4**, compared with those of **2** and **3**, indicated that the stereochemistry of the peroxy subunit was the same as that assigned for plakortides S and T. Finally, careful hydrogenation of the side chain double bond in plakortide S (**2**) with Pt/C at room temperature under H_2 (1 atm) for 5 min afforded a semisynthetic plakortide U whose NMR spectroscopic and optical rotation data[22] were identical to those of a natural sample of **4**, thus securing the assignment made.

Plakortides R–U (**1**–**4**) were tested for their antiplasmodial activity in vitro against chloroquine-resistant FcM29 strain. Plakortide U (IC_{50} 0.80 μM) proved to be the most active member of this series, while the remaining compounds showed a moderate antiplasmodial activity (IC_{50} range: 5–50 μM). None of the above compounds showed significant cytotoxicity on Vero cells (DT50 in the range 90–120 μM).

In conclusion, in this paper we have reported the structural elucidation of four new plakortide derivatives from a *Plakinastrella* sponge. Structural analysis of plakortide R provided an unambiguous method for the stereochemical elucidation of 3,6-disubstituted 1,2-dioxines, frequently isolated from Plakinidae sponges.

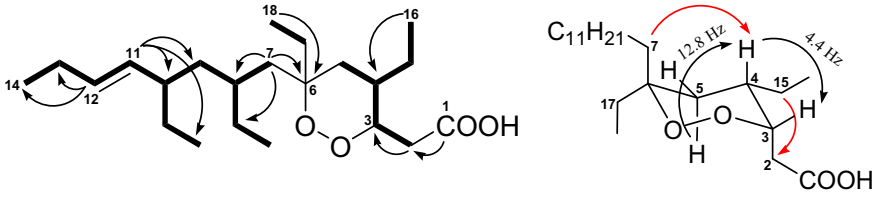

Fig. 5. COSY connectivities (bold bonds), HMBC (black arrows), ROESY (red arrows) correlations and key *J* couplings for plakortide S (**2**).

stereochemistry at C-3 was established following the same protocol already described for plakortide R (**1**). Reductive peroxide ring opening on the methyl ester derivative **2a** followed by derivatization at C-3 with *R*-(−)- or *S*-(+)-MTPA chloride (Fig. 6) afforded the MTPA esters, **2c** and **2d**, respectively. Analysis of $\Delta\delta$ pattern[16] (Fig. 6) enabled us to assign the *S* configuration at C-3. Consequently, the absolute configurations at C-4 (*R*) and C-6 (*S*) were established on the basis of the above deduced relative geometry.

It is interesting to note that, although plakortides R (**1**) and S (**2**) are closely related polyketides, differing only in the presence of an additional ketide unit in **2**, they have the enantiomeric configuration at the C-3 peroxidic carbon. The occurrence in the same sponge of epimeric endoperoxides has already been reported,[20] although the biosynthetic reason for this 'loss of stereospecificity' is still unknown. The *R* configuration at C-10 was established by the analysis of the pattern of the resonances of H_2-11 in the *S*- and *R*-MTPA esters (**2f** and **2g**) obtained following the procedure reported in Fig. 7.

Although the configuration at C-8 could not be determined, we tentatively proposed its assignment as *R* on the basis of the close biogenetic relationship between plakortides R (**1**) and S (**2**).

3. Experimental section

3.1. General procedures

Specific rotations were measured on a Perkin–Elmer 243 B polarimeter. High-resolution ESIMS spectra were performed with a Micromass QTOF Micromass spectrometer. ESIMS experiments were performed on an Applied Biosystem API 2000 triple-quadrupole mass spectrometer. IR spectra were recorded on a PerkinElmer FT-IR 100 spectrometer. NMR spectra were obtained on Varian Inova 400 MHz spectrometer (^1H at 400 MHz, ^{13}C at 100 MHz), Varian Inova 500 NMR spectrometer (^1H at 500 MHz, ^{13}C at 125 MHz, respectively) and Varian Inova 700 MHz spectrometer (^1H at 700 MHz, ^{13}C at 175 MHz, respectively) equipped with Sun hardware, δ (ppm), *J* in hertz, spectra referred to $CDCl_3$ (δ_H 7.27, δ_C 77.0) as an internal standard. Through-space ^1H connectivities were evidenced using a ROESY experiment with mixing times of 200 ms. HPLC was performed using a Waters Model 510 pump equipped with Waters Rheodine injector and a differential refractometer, model 401. Silica gel (200–400 mesh) was used for flash chromatography. The purities of compounds were determined to be greater than 95% by HPLC.

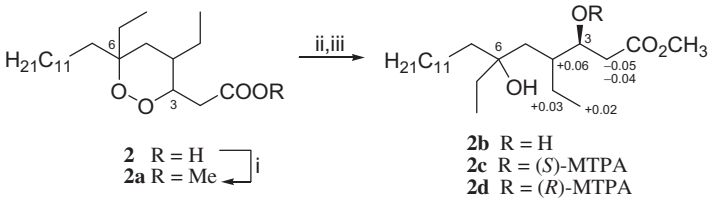

Fig. 6. Conversion of plakortide S (**2**) into methyl ester **2a**, then to diol **2b** and $\Delta\delta$ ($S-R$) values (in ppm) for MTPA ester derivatives **2c** and **2d**. Reagents and conditions: (i) diazomethane; (ii) Zn, AcOH, ether; (iii) (*R*)- or (*S*)-MTPACl, DMAP/CH_2Cl_2, rt, 3 h.

Plakortide S (**2**)

2e R = H
2f R = (*S*)-MTPA
2g R = (*R*)-MTPA

Fig. 7. Conversion of plakortide S (**2**) into methyl ester and then to alcohol **2e** and its MTPA derivatives **2f** and **2g**. Reagents and conditions: (i) diazomethane; (ii) O_3 in CH_2Cl_2, −78 °C, 1 min; then $NaBH_4$, MeOH, 2 h; (iii) (*R*)- or (*S*)-MTPACl, DMAP/CH_2Cl_2, rt, 3 h.

3.2. Sponge material and separation of individual compounds

P. mamillaris Kirkpatrick, 1900 (order Homosclerophorida, family Plakinidae) was collected at −22 m depth at Fiji Islands, in May 2007 (Coral Reef Initiative in the South Pacific project granted by the Agence Française de Développement). The sample was frozen immediately after collection and lyophilized to yield 171 g of dry mass. The sponge was identified by Dr. John Hooper, Queensland Museum, Brisbane, Australia, where a voucher specimen is deposited under the accessing number G324613.

The lyophilized material (171 g) was extracted with methanol (3×1.5 L) at room temperature and the crude methanolic extract (40 g) was subjected to a modified Kupchan's partitioning procedure[15] as follows. The methanol extract was dissolved in a mixture of MeOH/H_2O containing 10% H_2O and partitioned against *n*-hexane to give 17.3 g of the crude extract. The water content (% v/v) of the MeOH extract was adjusted to 30% and partitioned against $CHCl_3$ to give 16.6 g of the crude extract The aqueous phase was concentrated to remove MeOH and then extracted with *n*-BuOH (2.4 g of crude extract).

The *n*-hexane extract (8 g) was fractionated by silica gel MPLC using a solvent gradient system from *n*-hexane to EtOAc.

The fraction eluted with hexane/EtOAc 98:2 (995 mg) was further purified by HPLC on a Nucleodur 100-5 C18 (5 μm; 10 mm i.d.×250 mm) with 90% MeOH/H_2O as eluent (flow rate 5 mL/min) to give 24.8 mg of plakortide R (**1**) (t_R=16.2 min) and 14.3 mg of plakortide T (**3**) (t_R=39.3 min).

The fraction eluted with hexane/EtOAc 94:6 (2.10 g) was purified on a Nucleodur 100-5 C18 (5 μm; 10 mm i.d.×250 mm) with 90% MeOH/H_2O as eluent (flow rate 3.5 mL/min) to give 159 mg of plakortide S (**2**) (t_R=23.1 min) and 10.3 mg of plakortide U (**4**) (t_R=38.1 min).

3.3. Characteristic data for each compounds

*3.3.1. Plakortide R (**1**).* Light yellow oil; $[\alpha]_D^{25}$ −29.8 (*c* 0.26, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3023–2965, 1735, 1624, 1461 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data in $CDCl_3$ given in Table 1; ESIMS: *m*/*z* 347.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{19}H_{32}NaO_4$: 347.2198; found 347.2188 $[M+Na]^+$.

*3.3.2. Plakortide S (**2**).* Light yellow oil; $[\alpha]_D^{25}$ −149.8 (*c* 3.45, $CHCl_3$); IR ν_{max} (liquid film) 3400–3000 (br), 1708 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data in $CDCl_3$ given in Table 2; ESIMS: *m*/*z* 391.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{22}H_{40}NaO_4$: 391.2824; found 391.2815 $[M+Na]^+$.

*3.3.3. Plakortide T (**3**).* Light yellow oil; $[\alpha]_D^{25}$ −144.6 (*c* 0.13, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3017–2966, 1734, 1603, 1458 cm^{-1}; ESIMS: *m*/*z* 405.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{23}H_{42}NaO_4$: 405.2981; found 405.2972 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.41 (1H, dt, *J*=6.2, 15.2 Hz, H-12), 5.05 (1H, dd, *J*=9.1, 15.2 Hz, H-11), 4.50 (1H, ddd, *J*=2.4, 4.1, 8.7 Hz, H-3), 3.71 (3H, s, O–CH_3), 3.00 (1H, dd, *J*=9.2, 15.6 Hz, H-2a), 2.38 (1H, dd, *J*=3.4, 15.6 Hz, H-2b), 2.23–2.11 (1H, m, H-4), 2.01 (2H, ovl, H_2-13), 1.97 (1H, ovl, H-7), 1.88–1.78 (1H, m, H-10), 1.52 (1H, ovl, H-5a), 1.50 (1H, ovl, H-8), 1.49 (1H, ovl, H-17a), 1.41 (1H, ovl, H-19), 1.40 (1H, ovl, H-17b), 1.38 (1H, ovl, H-7b), 1.35 (1H, ovl, H-9a), 1.33 (1H, ovl, H-21a), 1.24 (1H, t, *J*=13.1 Hz, H-5b), 1.22 (1H, ovl, H-15a), 1.16 (1H, ovl, H-15b), 1.15 (1H, ovl, H-21b), 1.08–1.00 (1H, m, H-9b), 0.97 (3H, t, *J*=7.6 Hz, H_3-14), 0.93 (3H, t, *J*=7.2 Hz, H_3-16), 0.87 (3H, t, *J*=7.6 Hz, H_3-18), 0.85 (3H, t, *J*=7.2 Hz, H_3-20), 0.83 (3H, t, *J*=7.4 Hz, H_3-14). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 172.3 (C-1), 133.5 (C-11), 132.2 (C-12), 83.1 (C-6), 78.6 (C-3), 51.9 (OCH_3), 42.2 (C-10), 40.6 (C-9), 35.9 (C-7), 34.6 (C-4), 33.0 (C-5), 31.3 (C-2), 30.9 (C-8), 29.8 (C-17), 28.8 (C-21), 25.6 (C-19), 25.4 (C-13), 25.2 (C-15), 14.2 (C-14), 11.8 (C-22), 11.1 (C-16), 9.4 (C-20), 7.2 (C-18).

*3.3.4. Plakortide U (**4**).* Light yellow oil; $[\alpha]_D^{25}$ −109.0 (*c* 0.09, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3300–3000 (br), 1715, 1462 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data in $CDCl_3$ given in Table 2; ESIMS: *m*/*z* 393.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{22}H_{42}NaO_4$: 393.2981; found 393.2998 $[M+Na]^+$.

3.4. Esterification with diazomethane of plakortide S (2) and haterumadioxin A (5)

Plakortide S (**2**) and haterumadioxin A (**5**) were dissolved in ether and the resulting solutions were added dropwise to an ethereal solution of CH_2N_2 (ca. 20 equiv) at 0 °C. The cloudy yellow mixtures were stirred for 10 min and then concentrated under reduce pressure to give methyl ester of plakortide S (**2a**) and haterumadioxin A methyl ester (**5a**).

*3.4.1. Haterumadioxin A methyl ester (**5a**).* $[\alpha]_D^{25}$ −96 (*c* 0.81, MeOH); ESIMS: *m*/*z* 347.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{19}H_{32}NaO_4$:

347.2198; found 347.2203 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.49 (1H, s, H-5), 5.27 (1H, dt, J=6.5, 15.3 Hz, H-10a), 5.08 (1H, dd, J=9.1, 15.3 Hz, H-10b), 4.61 (1H, br d, J=8.7 Hz, H-3), 3.73 (3H, s, -OCH_3), 2.90 (1H, dd, J=8.7, 15.3 Hz, H-2a), 2.56 (1H, dd, J=2.9, 15.3 Hz, H-2b), 2.00 (2H, ovl, H_2-11), 1.98 (2H, ovl, H_2-13), 1.92 (1H, ovl, H-8), 1.82–1.66 (1H, m, H-15a), 1.63 (1H, ovl, H-15b), 1.60 (1H, ovl, H-7a), 1.54–1.44 (1H, m, H-7b), 1.43–1.34 (1H, m, H-17a), 1.20–1.12 (1H, m, H-17b), 1.06 (3H, t, J=7.3 Hz, H_3-14), 0.96 (3H, t, J=7.6 Hz, H_3-12), 0.87 (3H, t, J=7.6 Hz, H_3-16), 0.79 (3H, t, J=7.6 Hz, H_3-18).

*3.4.2. Plakortide S methyl ester (**2a**).* $[\alpha]_D^{25}$ −142.8 (c 0.55, $CHCl_3$); ESIMS: m/z 405.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{23}H_{42}NaO_4$: 405.2981; found 405.2979 $[M+Na]^+$. ^1H and ^{13}C NMR spectroscopic data superimposable to those reported for plakortide T (**3**) see Section 3.3.

3.5. Reduction of plakortide R (1) and plakortide S methyl ester (2a)

Plakortide R (**1**) (2.5 mg, 7.7×10^{-3} mmol) in dry ether (100 μL) was treated with acetic acid (40 μL) and excess Zn dust (10 mg, 0.33 mmol). The mixture was stirred vigorously until the starting material disappeared as evident by TLC analysis (24 h). The solution was neutralized with Na_2CO_3 and the solid removed by filtration. The solution was concentrated and the residue mixture was partitioned between H_2O and $CHCl_3$. The organic phase was evaporated to give **1a** (1.7 mg, 67.7% yield).

*3.5.1. Compound **1a**.* Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.51 (1H, dt, J=6.4, 15.3 Hz, H-10), 5.24 (1H, dd, J=9.5, 15.3 Hz, H-9), 5.02 (1H, s, H-5), 4.75 (1H, dd, J=3.4, 9.9 Hz, H-3), 3.71 (3H, s, OCH_3), 2.84 (1H, dd, J=9.9, 15.7 Hz, H-2a), 2.55 (1H, dd, J=3.4, 15.7 Hz, H-2b), 1.05 (3H, t, J=7.4 Hz, H_3-14), 0.99 (3H, t, J=7.4 Hz, H_3-12), 0.90 (3H, t, J=7.4 Hz, H_3-16), 0.82 (3H, t, J=7.3 Hz, H_3-18).

The same procedure was applied to plakortide S methyl ester (**2a**) (2.6 mg, 6.8×10^{-3} mmol) to afford diol **2b** (2.0 mg, 76.5% yield).

*3.5.2. Compound **2b**.* Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.41 (1H, dt, J=6.5, 15.2 Hz, H-12), 5.05 (1H, dd, J=9.2, 15.2 Hz, H-11), 4.19 (1H, dt, J=2.5, 10.9 Hz, H-3), 3.72 (3H, s, OCH_3), 2.60 (1H, dd, J=10.9, 16.1 Hz, H-2a), 2.38 (1H, dd, J=2.5, 16.1 Hz, H-2b), 2.05–1.97 (1H, m, H-4), 0.97 (3H, t, J=7.6 Hz, H_3-14), 0.96 (3H, t, J=7.6 Hz, H_3-16), 0.89 (3H, t, J=7.5 Hz, H_3-18), 0.84 (3H, t, J=7.3 Hz, H_3-20), 0.82 (3H, t, J=7.3 Hz, H_3-22).

3.6. General procedure for the preparation of MTPA esters

Samples of 1.0–1.2 mg were dissolved in freshly distilled CH_2Cl_2 (500 μL) and treated with a catalytic amount of triethylamine (2 μL), (−)- or (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) (10 μL, 0.054×10^{-3} mmol) and a catalytic amount of 4-(dimethylamino)pyridine. The mixture was left to stand at room temperature for 3 h. After removal of the solvent, the reaction mixtures were purified by HPLC on a LUNA 5μ Silica(2) (5μ; 4.60 mm i.d.×250 mm) column with 90% n-hexane/EtOAc as eluent (flow rate 1 mL/min) to afford pure MTPA esters **1b**, **1c**; **2c**, **2d**; **1e**, **1f** and **2f**, **2g**.

*3.6.1. [(S)-MTPA ester **1b**].* ESIMS: m/z 565.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{29}H_{41}F_3NaO_6$: 565.2753; found 565.2760 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.21 (1H, dd, J=3.3, 10.1 Hz, H-3), 5.38 (1H, dt, J=6.4, 15.3 Hz, H-10), 5.18 (1H, dd, J=8.9, 15.3 Hz, H-9), 5.17 (1H, s, H-5), 2.89 (1H, dd, J=10.1, 16.2 Hz, H-2a), 2.66 (1H, dd, J=3.3, 16.2 Hz, H-2b), 1.85 (2H, ovl, H_2-13), 0.87 (3H, t, J=7.4 Hz, H_3-14).

*3.6.2. [(R)-MTPA ester **1c**].* ESIMS: m/z 565.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{29}H_{41}F_3NaO_6$: 565.2753; found 565.2775 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.28 (1H, ovl, H-3), 5.38 (1H, dd, J=6.5, 15.2 Hz, H-10), 5.18 (1H, dd, J=8.8, 15.2 Hz, H-9), 5.22 (1H, s, H-5), 2.86 (1H, dd, J=10.0, 15.9 Hz, H-2a), 2.64 (1H, dd, J=3.8, 15.9 Hz, H-2b), 2.27–2.19 (2H, m, H_2-13), 1.07 (3H, t, J=7.4 Hz, H_3-14).

*3.6.3. [(S)-MTPA ester **2c**].* ESIMS: m/z 623.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{33}H_{51}F_3NaO_6$: 623.3535; found 623.3558 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.85–5.79 (1H, m, H-3), 5.41 (dt, J=6.3, 15.3 Hz, H-12), 5.04 (dd, J=9.2, 15.3 Hz, H-11), 2.71 (1H, dd, J=8.3, 16.0 Hz, H-2b), 2.59 (1H, dd, J=6.0, 16.0 Hz, H-2a), 2.05–1.97 (2H, m, H_2-13), 1.93–1.87 (1H, m, H-4), 1.87–1.81 (1H, m, H-10), 1.40 (2H, ovl, H_2-15), 0.97 (3H, t, J=7.5 Hz, H_3-14), 0.95 (3H, t, J=7.5 Hz, H_3-16), 0.83 (3H, ovl, H_3-18), 0.83 (3H, ovl, H_3-20), 0.83 (3H, ovl, H_3-22).

*3.6.4. [(R)-MTPA ester **2d**].* ESIMS: m/z 623.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{33}H_{51}F_3NaO_6$: 623.3535; found 623.3543 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.81–5.77 (1H, m, H-3), 5.41 (1H, dt, J=6.3, 15.3 Hz, H-12), 5.04 (1H, dd, J=9.2, 15.3 Hz, H-11), 2.75 (1H, dd, J=8.3, 15.8 Hz, H-2b), 2.64 (1H, dd, J=4.6, 15.8 Hz, H-2a), 2.03–1.97 (2H, m, H_2-13), 1.84 (1H, ovl, H-4), 1.82 (1H, ovl, H-10), 1.37 (2H, ovl, H_2-15), 0.95 (3H, t, J=7.5 Hz, H_3-14), 0.93 (3H, t, J=7.4 Hz, H_3-16), 0.84 (3H, t, J=7.7 Hz, H_3-22), 0.82 (3H, t, J=7.7 Hz, H_3-20), 0.80 (3H, t, J=7.4 Hz, H_3-18).

*3.6.5. [(S)-MTPA ester **1e**].* ESIMS: m/z 539.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{26}H_{35}F_3NaO_7$: 539.2233; found 539.2245 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.56 (1H, dd, J=2.9, 15.9 Hz, H-2a), 2.86 (1H, dd, J=8.8, 15.9 Hz, H-2b), 4.30 (1H, dd, J=6.4, 10.9 Hz, H-9a), 4.40 (1H, dd, J=3.9, 10.9 Hz, H-9b), 5.43 (1H, s, H-5).

*3.6.6. [(R)-MTPA ester **1f**].* ESIMS: m/z 539.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{26}H_{35}F_3NaO_7$: 539.2233; found 539.2252 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.57 (1H, dd, J=2.7, 16.0 Hz, H-2a), 2.87 (1H, dd, J=9.0, 16.0 Hz, H-2b), 4.21 (1H, dd, J=6.0, 11.1 Hz, H-9a), 4.52 (1H, dd, J=4.1, 11.1 Hz, H-9b), 5.43 (1H, s, H-5).

*3.6.7. [(S)-MTPA ester **2f**].* ESIMS: m/z 597.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{30}H_{45}F_3NaO_7$: 597.3015; found 597.3030 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.37 (1H, dd, J=3.6, 15.9 Hz, H-2a), 2.99 (1H, dd, J=9.3, 15.9 Hz, H-2b), 4.28–4.22 (2H, m, H_2-11), 4.54–4.46 (1H, m, H-3).

*3.6.8. [(R)-MTPA ester **2g**].* ESIMS: m/z 597.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{30}H_{45}F_3NaO_7$: 597.3015; found 597.3027 $[M+Na]^+$. Selected ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.37 (1H, dd, J=3.7, 15.8 Hz, H-2a), 3.00, (1H, dd, J=9.0, 15.8 Hz, H-2b), 4.16 (1H, dd, J=5.5, 11.0 Hz, H-11a), 4.36 (1H, dd, J=5.1, 11.0 Hz, H-11b), 4.54–4.48 (1H, m, H-3).

3.7. Ozonolysis of plakortide R (1), plakortide S methyl ester (2a) and haterumadioxin A methyl ester (5a)

A stream of O_3 was bubbled into a solution of **1** (10 mg, 30.9×10^{-3} mmol) in CH_2Cl_2 (2 mL) kept at −78 °C until a blue-colour solution resulted. After stirring for 1 min, excess of O_3 was removed upon bubbling N_2 and to the colourless solution was added dry MeOH (1 mL) followed by an excess of $NaBH_4$ (5.8 mg, 154.5×10^{-3} mmol). After stirring at −78 °C for 2 h, the reaction mixture was left to warm to room temperature and treated with dry MeOH (1 mL). The solution was concentrated and the resulting mixture was partitioned between $CHCl_3$ and H_2O (3×4 mL). The organic phase was evaporated to give alcohol **1d** (7.0 mg, 75.5% yield).

Compound ***1d***: Selected ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.50 (1H, s, H-5), 4.67 (1H, br d, J=9.3 Hz, H-3), 3.73 (3H, s, OCH_3), 3.72 (1H, dd, J=4.1, 11.4 Hz, H-9a), 3.54 (1H, dd, J=5.1, 11.4 Hz, H-9b), 2.91 (1H, dd, J=9.3, 16.4 Hz, H-2a), 2.57 (1H, dd, J=2.9, 16.4 Hz, H-2b), 1.09 (3H, t, J=7.4 Hz, H_3-11).

The same procedure was applied to compounds **2a** (2.2 mg, 5.8×10^{-3} mmol) affording alcohol **2e** (1.6 mg, 77.0% yield) and **5a** (3.5 mg, 11.3×10^{-3} mmol) affording alcohol **5b** (2.0 mg, 59% yield).

Compound ***2e***: Selected ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.55–4.47 (1H, m, H-3), 2.87–2.81 (1H, m, H-11a), 3.71 (3H, s, OCH_3), 3.68–3.63 (1H, m, H-11b), 3.00 (1H, dd, J=9.3, 15.8 Hz, H-2a), 2.38 (1H, dd, J=3.5, 15.8 Hz, H-2b).

Compound ***5b***: Selected ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.56 (1H, s, H-5), 4.71 (1H, br d, J=9.0 Hz, H-3), 3.73 (3H, s, OCH_3), 3.62 (1H, dd, J=5.0, 11.2 Hz, H-9a), 3.48 (1H, dd, J=4.1, 11.2 Hz, H-9b), 2.79 (1H, dd, J=9.2, 15.8 Hz, H-2a), 2.61 (1H, dd, J=3.1, 15.8 Hz, H-2b), 1.09 (3H, t, J=7.4 Hz, H_3-11).

3.8. Mild hydrogenation of plakortide S (2)

An oven-dried 10 mL flask was charged with 10% platinum on carbon (2 mg) and plakortide S (**2**) (5 mg, 13.6×10^{-3} mmol). Absolute methanol (1 mL) and dry THF (1 mL) were added, and the flask was evacuated, flushed with argon and then with hydrogen. The reaction mixture was stirred at room temperature under H_2 (1 atm) for 5 min. The mixture was filtered through Celite and the recovered filtrate was concentrated. The residue was purified by HPLC on a Nucleodur 100-5 C18 (5 μm; 4.6 mm i.d.×250 mm) with 90% MeOH/H_2O as eluent (flow rate 1 mL/min) to give semisynthetic plakortide U (0.7 mg, 13.9% yield).

3.8.1. Semisynthetic plakortide U. Light yellow oil; $[\alpha]_D^{22}$ −105.0 (*c* 0.05, $CHCl_3$); ^{1}H and ^{13}C NMR spectroscopic data superimposable to those reported for plakortide U (**4**) see Section 3.3. ESIMS: *m*/*z* 393.3 $[M+Na]^+$. HRMS (ESI): calcd for $C_{22}H_{42}NaO_4$ 393.2981 found 393.2995 $[M+Na]^+$.

3.9. Epoxidation of compounds 1d and 5b

To a solution of **1d** (5.6 mg, 18.7×10^{-3} mmol) in $CHCl_3$ (2 mL) was added slowly *m*-chloroperbenzoic acid (4.7 mg, 27.2×10^{-3} mmol). The mixture was stirred overnight at room temperature, and then CH_2Cl_2 (3×20 mL) and 5% aq Na_2SO_3 solution (30 mL) were added. The combined CH_2Cl_2 extracts were washed successively with a saturated aq solution of $NaHCO_3$ (30 mL) and then water (30 mL), dried over anhydrous $NaSO_4$ and evaporated to dryness to give 5 mg of crude epoxide, which was purified by HPLC on a Nucleodur 100-5 C18 (5 μm; 4.6 mm i.d.×250 mm) with 70% MeOH/H_2O as eluent (flow rate 1 mL/min) to give epoxide **1g** (2.1 mg, 35.5% yield) (t_R=7.5 min) and epoxide **1h** (0.6 mg, 10% yield) (t_R=9.0 min).

The same procedure was applied to **5b** (2.0 mg, 6.7×10^{-3} mmol) to afford the major epoxide **5c** (1.4 mg, 66% yield).

3.9.1. Epoxide ***1g***. ESIMS: *m*/*z* 339.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{16}H_{28}NaO_6$: 339.1784; found 339.1793 $[M+Na]^+$. ^{1}H NMR (700 MHz, $CDCl_3$) δ (ppm): 4.57 (1H, dd, J=3.6, 8.8 Hz, H-3), 3.74 (3H, s, -OCH_3), 3.66 (1H, dd, J=4.3, 11.3 Hz, H-9a), 3.54 (1H, dd, J=5.3, 11.3 Hz, H-9b), 3.01 (1H, dd, J=9.0, 15.9 Hz, H-2a), 3.13 (1H, s, H-5), 2.68 (1H, dd, J=3.9, 15.9 Hz, H-2b), 2.19 (2H, dd, J=9.9, 15.6 Hz, H_2-7), 1.94–1.86 (1H, m, H-10a), 1.79–1.71 (1H, m, H-12a), 1.56 (1H, ovl, H-12b), 1.55 (1H, ovl, H-10b), 1.50 (1H, ovl, H-8), 1.50–1.44 (1H, m, H-14a), 1.36–1.28 (1H, m, H-14b), 0.98 (3H, t, J=7.4 Hz, H_3-11), 0.97 (3H, t, J=7.5 Hz, H_3-13), 0.94 (3H, t, J=7.4, H_3-15). ^{13}C NMR (175 MHz, $CDCl_3$): 170.6 (C-1), 80.9 (C-6), 76.4 (C-3), 65.8 (C-9), 59.7 (C-4), 61.4 (C-5), 51.9 (-OCH_3), 37.2 (C-8), 33.0 (C-7), 26.8 (C-12), 25.9 (C-14), 23.9 (C-10), 11.3 (C-15), 7.5 (C-13), 7.2 (C-11).

3.9.2. Epoxide ***1h***. ESIMS: *m*/*z* 339.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{16}H_{28}NaO_6$: 339.1784; found 339.1798 $[M+Na]^+$. ^{1}H NMR (700 MHz, $CDCl_3$) δ (ppm): 4.65 (1H, dd, J=3.5, 8.9 Hz, H-3), 3.74 (1H, dd, J=3.7, 11.3 Hz, H-9a), 3.73 (3H, s, −OCH_3), 3.54 (1H, dd, J=4.8, 11.3 Hz, H-9b), 3.06–2.96 (1H, m, H-2a), 2.94 (1H, s, H-5), 2.64 (1H, dd, J=3.5, 16.7 Hz, H-2b), 1.89 (2H, dd, J=9.5, 15.0 Hz, H_2-7), 1.85–1.79 (1H, m, H-10a), 1.59 (2H, ovl, H_2-12), 1.57 (1H, ovl, H-10b), 1.49 (1H, ovl, H-8), 1.47 (1H, ovl, H-14a), 1.38–1.28 (1H, m, H-14b), 0.99 (3H, t, J=7.4 Hz, H_3-11), 0.98 (3H, t, J=7.5 Hz, H_3-13), 0.94 (3H, t, J=7.4, H_3-15). ^{13}C NMR (175 MHz, $CDCl_3$): 171.3 (C-1), 82.5 (C-6), 76.2 (C-3), 65.1 (C-9), 61.9 (C-4), 58.9 (C-5), 52.2 (−OCH_3), 37.6 (C-8), 32.3 (C-7), 27.1 (C-10), 26.8 (C-14), 24.5 (C-12), 11.8 (C-15), 8.9 (C-11), 8.3 (C-13).

3.9.3. Epoxide ***5c***. ESIMS: *m*/*z* 339.2 $[M+Na]^+$. HRMS (ESI): calcd for $C_{16}H_{28}NaO_6$: 339.1784; found 339.1789 $[M+Na]^+$. ^{1}H NMR (700 MHz, $CDCl_3$) δ (ppm): 4.54 (1H, dd, J=3.7, 9.3 Hz, H-3), 3.74 (3H, s, −OCH_3), 3.70 (1H, dd, J=3.7, 11.3 Hz, H-9a), 3.54 (1H, dd, J=4.8, 11.3 Hz, H-9b), 3.12 (1H, s, H-5), 3.01 (1H, dd, J=9.4, 15.6 H-2a), 2.64 (1H, dd, J=3.7, 15.6 Hz, H-2b), 2.11–2.05 (1H, m, H-7a), 1.99–1.93 (1H, m, H-12a), 1.93–1.87 (1H, m, H-10a), 1.81–1.75 (1H, m, H-12b), 1.72–1.64 (1H, m, H-7b), 1.56–1.50 (1H, m, H-10b), 1.44 (1H, ovl, H-8), 1.43 (1H, ovl, H-14a), 1.33–1.29 (1H, m, H-14b), 0.98 (3H, t, J=7.4 Hz, H_3-11), 0.96 (3H, t, J=7.5 Hz, H_3-13), 0.92 (3H, t, J=7.4, H_3-15).

3.10. In vitro antiplasmodial activity

The *Plasmodium falciparum* FcM29 chloroquine-resistant strain was cultured according to Trager and Jensen,[23] with modifications.[24] Antiplasmodial activity was determined following the [^{3}H]-hypoxanthine (Amersham-France) incorporation method.[25]

3.11. In vitro cytotoxicity

Toxicity was estimated using Vero cells (normal monkey kidney cells) cultured in the same conditions as *P. falciparum*, except for the replacement of 5% human serum with 10% foetal calf serum. After the addition of drugs to be tested at increasing concentrations, cell growth was estimated by [^{3}H]-hypoxanthine incorporation following a 48-h incubation and was compared with a control sample.[26]

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