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Dolastanes from the brown alga *Dilophus spiralis*: absolute stereochemistry and evaluation of cytotoxicity

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Abstract

Five new dolastanes (**1**–**5**) were isolated from the brown alga *Dilophus spiralis*. The structural elucidation of the isolated compounds and the assignment of relative stereochemistry were based on analyses of their spectroscopic data. The structure proposed for **1** was confirmed by single crystal X-ray diffraction analysis, whereas its absolute stereochemistry was determined using the modified Mosher's method. The cytotoxicity of **1**–**4** was evaluated against seven cell lines.

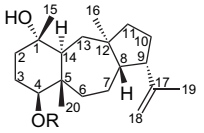
Keywords: Dilophus spiralis; Dolastanes; Cytotoxicity

1. Introduction

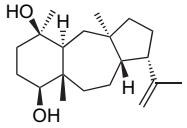
Brown algae of the family Dictyotaceae are found mainly in the tropical and subtropical waters of the Atlantic, Indian and Pacific Oceans, the Sea of Japan, and the Caribbean and Mediterranean Seas. These algae are known to produce a wide spectrum of diverse terpenoid metabolites. Sesquiterpenes and diterpenes of normal biosynthesis have been isolated mainly from species of *Dictyota* and *Dilophus*, whereas meroterpenoids have been reported mostly from *Stypopodium* and *Taonia* specimens.[1,2] Many of these natural products have been evaluated for and proven to possess antibacterial, algicidal, cytotoxic, ichthyotoxic, and antifeedant activities.[1,2]

In continuation of our research program for the isolation of bioactive natural products from marine organisms of the Greek Seas,[3–5] we carried out a preliminary cytotoxicity screening of a number of algal extracts that ranked the dichloromethane extract of *Dilophus spiralis* (syn. *ligulatus*) among the most active ones. Moreover, its interesting chemical profile, in combination with the fact that this species has been the subject of only a few chemical studies led us to the decision of investigating the chemical composition of this brown alga.[6–10]

Herein, we report the isolation, structural elucidation, absolute stereochemistry, and evaluation of the in vitro cytotoxic activity of five new dolastane derivatives (**1**–**5**).



1 R=H
1a R=(*S*)-MTPA
1b R=(*R*)-MTPA



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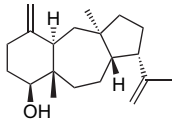

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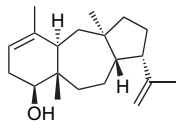

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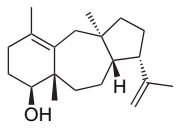

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2. Results and discussion

D. spiralis was collected from Elafonissos island, south of Peloponnese in April of 2004. The freeze-dried alga was

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extracted with CH_2Cl_2 and the dark green oily residue was subjected to a series of chromatographic separations to allow the isolation of metabolites **1**–**5**.

Compound **1**, obtained as a colorless oil, had a molecular formula of $C_{20}H_{34}O_2$ (*m/z* 306.2532 $[M]^+$), as deduced from the HR-EIMS and ^{13}C NMR spectra. The fragment ion at *m/z* 288 $[M-H_2O]^+$ in the mass spectrum, as well as the absorption band at 3330 cm^{-1} in the IR spectrum indicated the presence of at least one hydroxyl group. The ^{13}C NMR spectrum revealed 19 carbon signals, which as determined from DEPT experiments corresponded to four quaternary carbon atoms, four methines, eight methylenes, and four methyls. The structural elements displayed in the ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2) included four tertiary methyl groups ($\delta_{H/C}$ 0.89/11.5, 0.93/18.8, 1.10/22.8, 1.70/24.3), one 1,1-disubstituted double bond ($\delta_{H/C}$ 4.76, 4.85/111.9, δ_C 147.7), one oxygenated methine ($\delta_{H/C}$ 3.19/78.3), and one oxygenated quaternary carbon (δ_C 72.5). Since the carbon–carbon double bond accounted for one of the four degrees of unsaturation, the molecular structure of **1** was determined as tricyclic. The long-range coupling between H_2-18 and H_3-19 observed in the COSY spectrum indicated the presence of an isopropenyl group, whereas the correlations of C-17 with H-9, H_2-18, and H_3-19 in the HMBC spectrum fixed its position. The methine proton H-9 displayed additional HMBC correlations with C-8, C-10, C-11, and C-12, while H_3-16 was correlated with C-8 and C-12. The COSY correlations of H-8/H-9, H-9/H_2-10, and H_2-10/H_2-11 confirmed the assignment of the five-membered ring. The cross peaks of H_2-6/H_2-7, H_2-7/H-8, and H_2-13/H-14, as well as the HMBC correlations of C-5 with H_2-6 and H_3-20, C-8 with H_2-7, C-12 with H_2-13, and C-14 with H_2-13 and H_3-20 concluded the assignment of the seven-membered ring. Finally, the correlations of C-1 with H_2-2, H-14, and H_3-15, C-4 with H_2-3 and H_3-20, and C-15 with H_2-2 and H-14 observed in the HMBC spectrum, in conjunction with the cross peaks of H_2-2/H_2-3 and H_2-3/H-4 in the COSY spectrum identified the six-membered ring.

The relative configuration of **1** was established by analysis of the key correlations displayed in the NOESY spectrum. Lack of NOE enhancement between H-14 and H_3-20

Table 2
^{13}C NMR data of compounds **1**–**5**

No.	**1**		**2**		**3**		**4**		**5**	
1	72.5	s	72.9	s	150.3	s	136.2	s	128.0	s
2	41.3	t	39.9	t	35.6	t	120.0	d	29.9	t
3	28.5	t	26.6	t	32.2	t	32.0	t	26.7	t
4	78.3	d	78.5	d	78.0	d	75.0	d	72.3	d
5	42.9	s	42.9	s	44.6	s	41.5	s	43.2	s
6	37.9	t	36.5	t	34.9	t	33.0	t	39.1	t
7	22.9	t	22.9	t	23.0	t	23.0	t	21.7	t
8	48.7	d	48.1	d	48.2	d	47.8	d	57.9	d
9	49.4	d	49.4	d	49.5	d	49.5	d	48.4	d
10	28.5	t	28.5	t	28.6	t	28.5	t	29.2	t
11	44.3	t	44.6	t	44.7	t	44.3	t	41.6	t
12	44.5	s	44.5	s	44.7	s	44.9	s	45.3	s
13	40.4	t	40.3	t	44.4	t	43.0	t	40.2	t
14	52.9	d	50.7	d	46.9	d	47.1	d	134.0	s
15	22.8	q	31.4	q	107.7	t	23.0	q	21.2	q
16	18.8	q	19.0	q	19.1	q	18.9	q	18.6	q
17	147.7	s	147.8	s	147.7	s	147.8	s	148.7	s
18	111.9	t	111.8	t	111.9	t	112.0	t	111.1	t
19	24.3	q	24.4	q	24.4	q	24.3	q	24.3	q
20	11.5	q	11.5	q	9.4	q	9.5	q	23.5	q

All spectra were recorded in $CDCl_3$ at 50.3 MHz. Chemical shifts are expressed in parts per million.

Table 1
^1H NMR data of compounds **1**–**5**

No.		**1**		**2**		**3**		**4**		**5**
2	a	1.75 m	a	1.67 m	α	2.05 m		5.30 br s		2.11 m
	b	1.48 m	b	1.53 m	β	2.27 m				
3	a	1.73 m	a	1.83 m	α	1.83 m	α	2.21 m	α	1.85 m
	b	1.50 m	b	1.58 m	β	1.45 m	β	1.94 m	β	1.73 m
4		3.19 dd (11.2, 4.1)		3.13 dd (11.5, 3.9)		3.29 dd (11.7, 4.4)		3.40 dd (10.3, 6.1)		3.59 dd (10.2, 4.1)
6	α	1.32 m	α	1.27 m	α	1.47 m	α	1.28 m	α	1.19 m
	β	2.08 m	β	2.08 m	β	2.13 m	β	2.14 m	β	2.08 m
7	a	1.61 m	a	1.62 m	a	1.64 m	a	1.67 m	a	1.53 m
	b	1.37 m	b	1.36 m	b	1.56 m	b	1.46 m	b	1.23 m
8		2.05 m		2.14 m		2.10 m		2.18 m		1.77 m
9		2.84 td (11.2, 9.3)		2.84 td (11.0, 9.3)		2.84 td (11.4, 9.3)		2.85 td (11.4, 9.3)		2.78 td (11.2, 9.3)
10		1.78 m		1.79 m		1.78 m		1.77 m		1.89 m
11		1.44 m		1.42 m		1.42 m		1.39 m		1.39 m
13	α	2.00 m	α	1.87 m	α	1.76 m	α	1.92 dd (13.7, 3.3)	α	2.58 d (13.3)
	β	1.22 m	β	1.39 m	β	1.39 m	β	1.10 t (13.7)	β	1.70 m
14		1.34 m		1.19 m		1.90 m		2.08 m		
15		1.10 s		1.16 s	a	4.76 br s		1.60 s		1.59 s
					b	4.59 br s				
16		0.93 s		0.92 s		0.97 s		0.95 s		0.74 s
18	a	4.85 br s	a	4.86 br s	a	4.86 br s	a	4.86 br s	a	4.72 br s
	b	4.76 br s	b	4.76 br s	b	4.76 br s	b	4.77 br s	b	4.66 br s
19		1.70 s		1.70 s		1.71 s		1.71 s		1.66 s
20		0.89 s		1.03 s		0.72 s		0.82 s		0.95 s

All spectra were recorded in $CDCl_3$ at 400 MHz. Chemical shifts are expressed in parts per million and *J* values in parentheses are reported Hz.

suggested the trans fusion of the six- and seven-membered rings, whereas lack of NOE enhancement between H-8 and H_3-16 indicated the trans fusion of the five- and seven-membered rings. In addition, the strong NOE interactions observed between H-14 and H_3-16, as well as H-8 and both H-9 and H_3-20 provided evidence that H-14 and H_3-16 were cofacial on one side of the molecule, while H-8, H-9, and H_3-20 were on the opposite side. The cross peaks of H-4/H-14 and H_3-15/H_3-20, in combination with the lack of NOE enhancements for H-4/H_3-20, H-14/H_3-15 or H_3-15/H_3-16 determined the relative stereochemistry at C-1 and C-4. The proposed structure of **1** was confirmed by single crystal X-ray diffraction analysis (Fig. 1).[11]

The absolute stereochemistry of **1** was determined by application of the modified Mosher's method.[12] When **1** was treated with (*R*)- and (*S*)-MTPA chloride, the secondary hydroxyl group at C-4 reacted to give the (*S*)- and (*R*)-MTPA derivatives (**1a** and **1b**), respectively. The ^{1}H NMR chemical shifts of **1a** and **1b** were assigned by analysis of ^{1}H, HSQC, and COSY NMR spectra. The calculation of the $\Delta\delta_{S-R}$ values, shown in Figure 2, clearly defined the absolute configuration of C-4 as *S* and subsequently, on the basis of its relative stereochemistry, established the absolute configuration of **1** as depicted.

Compound **2**, obtained as a colorless oil, had a molecular formula of $C_{20}H_{34}O_2$ (*m/z* 306.2552 $[M]^+$), as deduced from the HR-EIMS and ^{13}C NMR spectra. Both ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) closely resembled those of compound **1**. Analysis of the 2D NMR spectra (HSQC, HMBC and COSY) suggested the two to have identical planar structures. Therefore, the difference between them should have been stereochemical. Inspection of the NOESY spectrum revealed

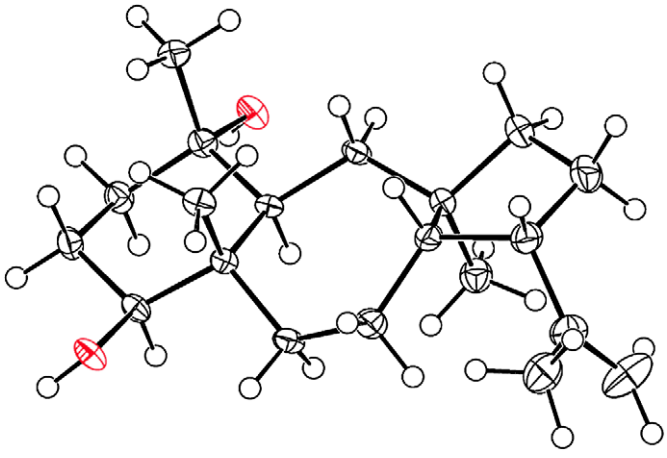

Figure 1. ORTEP drawing of **1**.

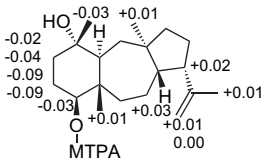

Figure 2. $\Delta\delta_{S-R}$ values (ppm) for the C-4 MTPA derivatives of **1** in $CDCl_3$.

cross peaks of H-8/H-9, H-8/H_3-20, H-14/H_3-16, H-4/H-14, as well as lack of NOE enhancements between H-14 and H_3-20 and H-8 and H_3-16, as in the case of **1**, thus determining the same relative stereochemistry for C-4, C-5, C-8, C-9, C-12, and C-14. However, the absence of NOE interaction between H_3-15 and H_3-20 indicated that the difference of **1** and **2** was the relative configuration of C-1.

Compound **3**, obtained as a colorless oil, had a molecular formula of $C_{20}H_{32}O$ (*m/z* 288.2472 $[M]^+$), as deduced from the HR-EIMS and ^{13}C NMR spectra. Analysis of the spectroscopic data of **3** (Tables 1 and 2) showed a high degree of similarity with metabolites **1** and **2**. In agreement with the molecular formula, it was clear that the difference was the absence of one hydroxyl group and the formation of a second carbon–carbon double bond. This was verified from the ^{13}C NMR spectrum, where it was evident through DEPT experiments that instead of the two oxygenated carbon signals, only the secondary one could be observed, in conjunction with the absence of a methyl and the presence of two more olefinic carbon signals, assigned to a second 1,1-disubstituted double bond. The replacement of H_3-15 by a second exomethylene (δ_H 4.59, 4.76) was also obvious in the ^{1}H NMR spectrum and further confirmed from the observed HMBC correlations of C-1 with H_2-2 and H-14. The relative stereochemistry of **3** was established by analysis of the key correlations displayed in the NOESY spectrum, in accordance with that of **1** and **2**.

Compound **4** was obtained as a colorless oil. Combination of ^{13}C NMR and HR-EIMS data suggested the same molecular formula as in **3** (*m/z* 288.2447 $[M]^+$) and interpretation of its spectroscopic data (Tables 1 and 2) indicated that it was an isomer of the latter. Comparison of the ^{1}H and ^{13}C NMR data for the two metabolites revealed that the difference between them was the replacement of the second 1,1-disubstituted double bond by a trisubstituted double bond. This was clear from the ^{1}H NMR spectrum, where the second exomethylene had given its place to a second vinylic methyl (δ_H 1.60) and an olefinic proton (δ_H 5.30). More specifically, after analysis of the 2D NMR spectra, it was evident that the double bond between C-1 and C-15 was shifted between C-1 and C-2, which was further verified by the HMBC correlations of both C-3 and C-15 with H-2. The relative stereochemistry of **4**, as established by analysis of the NOESY spectrum, was found in accordance with that of **1**–**3**.

Compound **5** could not be isolated in pure form, but was rather part of an inseparable mixture with **4** (at a ratio of 63:37, respectively, as observed from GC analysis). However, due to the close resemblance of the two metabolites, it was possible after careful inspection of the spectroscopic data (Tables 1 and 2) and the information obtained by the EIMS spectrum (*m/z* 288 $[M]^+$) to determine that **5** was an isomer of **3** and **4**. In particular, the trisubstituted double bond between C-1 and C-2 present in **4** was replaced by a tetrasubstituted double bond, which was placed between C-1 and C-14, since correlations of both C-1 and C-14 with H_2-2, H_2-13, and H_3-15 could be observed in the HMBC spectrum. The relative stereochemistry of **5**, found in accordance with

that of **1**–**4**, was established by analysis of the NOESY spectrum.

The absolute configuration for compounds **2**–**5** was not determined, but on the basis of biogenetic considerations it is expected to be identical to that of **1**.

To the best of our knowledge, only about 25 diterpenes of the dolastane class, featuring a hydrobenzo[*f*]azulene ring system, have been reported, isolated exclusively from marine sources.[13] The trans arrangement of the methyl group at C-12 and the methine at C-9 depicted for **1**–**5** has been found only in one algal metabolite in the past,[14] whereas the isopropenyl moiety has been encountered only in three soft coral metabolites possessing though the opposite relative stereochemistry at C-9.[15] In the present study, the unambiguous determination of the absolute stereochemistry through single crystal X-ray diffraction analysis and application of the modified Mosher's method is combined with detailed spectroscopic analyses and assignment of all NMR resonances supplementing the relevant literature.

The in vitro cytotoxic activity of metabolites **1**–**4** was evaluated against seven different cell lines. The results showed that **2** and **3** did not exhibit any significant activity (>30 μg/mL), whereas **1** was found moderately cytotoxic against L16 (11.2 μg/mL) and A549 (12.6 μg/mL), and **4** against HeLa (17.3 μg/mL), PC3 (17.7 μg/mL), MCF7 (18.5 μg/mL), HT29 (19.6 μg/mL) and A431 (21.4 μg/mL) cell lines. The fact that the minor structural differences observed between **1**–**4** induce intriguingly different levels of activity is currently under investigation through isolation of other related metabolites, semisynthetic preparation of a series of analogues and their in silico studies thereafter.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer model 341 polarimeter with a 1 dm cell. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained on a Paragon 500 Perkin–Elmer spectrometer. NMR spectra were recorded on Bruker AC 200 and Bruker DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). The 2D experiments (HSQC, HMBC, COSY, NOESY) were performed using standard Bruker microprograms. High resolution mass spectra were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana, USA. Low resolution EI mass spectra were measured on a Hewlett Packard 5973 mass spectrometer. Column chromatography separations were performed with Kieselgel 60 (Merck). HPLC separations were conducted using a CECIL 1100 Series liquid chromatography pump equipped with a GBC LC-1240 refractive index detector, using a Spherisorb S10W (Phase Sep, 25 cm×10 mm) column. TLC were performed with Kieselgel 60 F_{254} (Merck aluminum support plates) and spots were detected after spraying with 15% H_2SO_4 in MeOH reagent and heating at 100 °C for 1 min. The lyophilization was carried out in a Freezone 4.5 freeze dry system (Labconco).

3.2. Plant material

D. spiralis was collected by hand in Elafonissos island, south of Peloponnese, Greece, at a depth of 0.1–1 m in April of 2004. The alga was identified by the authors and a voucher specimen (ATPH/MO/159) is kept at the Herbarium of the Department of Pharmacognosy and Chemistry of Natural Products, University of Athens.

3.3. Extraction and isolation

The freeze-dried alga (272 g) was exhaustively extracted with CH_2Cl_2 at room temperature. Evaporation of the solvent in vacuo afforded a dark green oily residue (9.2 g) that was subjected to vacuum column chromatography on silica gel, using *c*-hexane with increasing amounts of EtOAc, followed by EtOAc with increasing amounts of MeOH as the mobile phase, to afford fifteen fractions (A1–A15). Fraction A3 (20% EtOAc, 1.17 g) was further fractionated by gravity column chromatography on silica gel, using *c*-hexane with increasing amounts of EtOAc as the mobile phase, to yield twenty-one fractions (A3a–A3u). Fractions A3i (2% EtOAc, 81.7 mg) and A3j (2% EtOAc, 28.2 mg) were purified separately by normal phase HPLC, using *c*-hexane/EtOAc (95:5) as eluent, to yield compounds **3** (6.4 mg) and **4** (5.8 mg) in pure form and an inseparable mixture of **4** and **5** (1.6 mg). Fractions A3s (50% EtOAc, 13.8 mg) and A3t (50% EtOAc, 20.2 mg) were purified separately by normal phase HPLC, using *c*-hexane/EtOAc (50:50) as eluent, to yield compounds **1** (17.9 mg) and **2** (2.6 mg) in pure form.

3.3.1. (1*S*,4*S*,8*S*,14*S*)-1,4-Dihydroxy-17-dolastene (**1**)

Colorless oil; $[\alpha]_D^{20}$ −8.9 (*c* 0.045, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 246.0 (2.90) nm; IR (thin film) ν_{max} 3330, 2936, 1706, 1454, 1381 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-EIMS *m/z* 306.2532 $[M]^+$ (calcd for $C_{20}H_{34}O_2$ 306.2559); EIMS 70 eV *m/z* (rel int. %): 55 (74), 67 (80), 79 (86), 93 (93), 107 (95), 119 (79), 133 (65), 147 (64), 159 (100), 177 (56), 187 (42), 201 (30), 219 (41), 245 (31), 273 (26), 288 (43), 306 (9).

3.3.2. (1*R*,4*S*,8*S*,14*S*)-1,4-Dihydroxy-17-dolastene (**2**)

Colorless oil; $[\alpha]_D^{20}$ −14.3 (*c* 0.035, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 245.0 (2.62) nm; IR (thin film) ν_{max} 3367, 2927, 1691, 1459, 1372 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-EIMS *m/z* 306.2552 $[M]^+$ (calcd for $C_{20}H_{34}O_2$ 306.2559); EIMS 70 eV *m/z* (rel int. %): 55 (88), 67 (84), 81 (82), 93 (92), 107 (91), 121 (77), 133 (50), 147 (100), 159 (83), 175 (30), 188 (47), 201 (37), 206 (71), 219 (49), 232 (26), 245 (23), 273 (15), 288 (14), 306 (4).

3.3.3. (4*S*,8*S*,14*R*)-4-Hydroxy-1(15),17-dolastadiene (**3**)

Colorless oil; $[\alpha]_D^{20}$ −45.7 (*c* 0.035, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 247.0 (2.30) nm; IR (thin film) ν_{max} 3339,

2927, 1642, 1445, 1376 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-EIMS *m/z* 288.2472 $[M]^+$ (calcd for $C_{20}H_{32}O$ 288.2453); EIMS 70 eV *m/z* (rel int. %): 55 (43), 67 (56), 79 (65), 91 (75), 107 (68), 119 (55), 133 (53), 145 (55), 159 (100), 175 (32), 187 (27), 201 (33), 217 (23), 232 (32), 245 (38), 255 (17), 273 (18), 288 (24).

*3.3.4. (4S,8S,14R)-4-Hydroxy-1,17-dolastadiene (**4**)*

Colorless oil; $[\alpha]_D^{20}$ −20.0 (*c* 0.040, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 246.0 (1.87) nm; IR (thin film) ν_{max} 3413, 2927, 1638, 1440, 1379 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HR-EIMS *m/z* 288.2447 $[M]^+$ (calcd for $C_{20}H_{32}O$ 288.2453); EIMS 70 eV *m/z* (rel int. %): 55 (62), 67 (61), 81 (77), 95 (71), 107 (68), 123 (85), 133 (40), 145 (32), 159 (100), 177 (89), 187 (21), 204 (39), 217 (23), 232 (17), 244 (12), 255 (5), 273 (7), 288 (36).

*3.3.5. (4S,8S)-4-Hydroxy-1(14),17-dolastadiene (**5**)*

^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS 70 eV *m/z* (rel int. %): 55 (49), 67 (47), 79 (60), 93 (82), 107 (82), 121 (72), 133 (100), 145 (49), 159 (76), 173 (19), 187 (80), 205 (17), 218 (13), 227 (12), 255 (18), 270 (12), 273 (11), 288 (55).

*3.4. Preparation of MTPA derivatives of **1***

Compound **1** (2.0 mg) was treated with (*R*)-MTPA chloride (5 μL) in freshly distilled dry pyridine (1 mL) and left under constant stirring at room temperature for 16 h. The reaction was quenched by the addition of 1 mL of H_2O and the mixture was partitioned between the aqueous and the organic layer. After evaporation of the organic layer in vacuo, the residue was purified by normal phase HPLC, using *c*-hexane/EtOAc (50:50) as eluent, to obtain the (*S*)-MTPA derivative (**1a**, 2.1 mg). The (*R*)-MTPA derivative (**1b**, 2.8 mg) was prepared with (*S*)-MTPA chloride and purified in the same manner.

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References and notes

1. Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2007**, *24*, 31–86 and earlier reviews in this series.
2. MarinLit database, Department of Chemistry, University of Canterbury: http://www.chem.canterbury.ac.nz/marinlit/marinlit.shtml.
3. Kladi, M.; Vagias, C.; Papazafiri, P.; Furnari, G.; Serio, D.; Roussis, V. *Tetrahedron* **2007**, *63*, 7606–7611.
4. Gavagnin, M.; Carbone, M.; Amodeo, P.; Mollo, E.; Vitale, R. M.; Roussis, V.; Cimino, G. *J. Org. Chem.* **2007**, *72*, 5625–5630.
5. Kontiza, I.; Abatis, D.; Malakate, K.; Vagias, C.; Roussis, V. *Steroids* **2006**, *71*, 177–181.
6. Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S.; Mayol, L. *J. Chem. Soc., Chem. Commun.* **1976**, 1024–1025.
7. Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1979**, *18*, 1895–1897.
8. Tringali, C.; Oriente, G.; Piattelli, M.; Geraci, C.; Nicolosi, G.; Breitmaier, E. *Can. J. Chem.* **1988**, *66*, 2799–2802.
9. Bouaicha, N.; Pesando, D.; Puel, D.; Tringali, C. *J. Nat. Prod.* **1993**, *56*, 1747–1752.
10. Bouaicha, N.; Tringali, C.; Pesando, D.; Mallea, M.; Roussakis, C.; Verbist, J. F. *Planta Med.* **1993**, *59*, 256–258.
11. Crystallographic data (excluding structure factors) for **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 666585. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
12. Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.
13. Hiersemann, M.; Helmboldt, H. *Top. Curr. Chem.* **2005**, *243*, 73–136.
14. González, A. G.; Martin, J. D.; Norte, M.; Rivera, P.; Perales, A.; Fayos, J. *Tetrahedron* **1983**, *39*, 3355–3357.
15. Braekman, J. C.; Daloze, D.; Schubert, R.; Albericci, M.; Tursch, B.; Karlsson, R. *Tetrahedron* **1978**, *34*, 1551–1556.