Polyhalogenated Monoterpenes from *Plocamium cartilagineum* from the Portuguese Coast

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Examination of the red alga *Plocamium cartilagineum* (L.) Dixon collected at two locations along the Portuguese coast yielded two new acyclic polyhalogenated monoterpenes, (3*Z*,7*E*)-5,8-dibromo-2,6-dichloro-2,6-dimethylocta-3,7-dien-1-al (**2**) and (*E*)-3,8-dibromo-2,7-dichloro-2,6-dimethyloct-5-ene (**3**), and the known compounds **1**, **4**−**6**, and **8**. The structures were established by spectral methods.

Red algae of the genus *Plocamium* have been shown to be a rich source of acyclic and cyclic halogenated monoterpenes that vary for a given species depending on collection location and season.[1] The largest variety of halogenated monoterpenes has been isolated from *Plocamium cartilagineum* with collections ranging from the Antarctic Peninsula,[2,3] the Pacific coasts of North America,[4−8] Australia,[9,10] New Zealand,[11] Chile,[12] the Spanish Atlantic coast,[13−15] and the British coast.[16]

In this paper, we wish to describe the structure elucidation of two new and five known polyhalogenated monoterpenes from *P. cartilagineum* (L.) Dixon (Plocamiaceae) collected at two locations along the west coast of Portugal.

A fresh sample of *P. cartilagineum* collected at Sesimbra was successively extracted with hexane and $CHCl_3$. Fractionation of the hexane extract by repeated Si gel chromatography afforded three main fractions. The least polar fraction was found to consist predominantly of compound **1** (37% of the extract); this compound was identified from its physical and spectroscopic properties, which were identical to those reported in literature.[13] The relative stereochemistry of the four chiral centers of **1** was confirmed by NOESY experiments.

The second group of fractions was purified following a sequence of Si gel, preparative HPTLC, and reversed-phase HPLC to yield (3*Z*,7*E*)-5,8-dibromo-2,6-dichloro-2,6-dimethylocta-3,7-dien-1-al (**2**), an oil whose HREIMS corresponds to a molecular formula of $C_{10}H_{12}OBr_2Cl_2$. The presence of fragment ion clusters corresponding to $M^+ - Cl$ (*m/z* 341, 343, 345, 347), $M^+ - Cl - HBr$ (*m/z* 261, 263, 265), $M^+ - Br - Cl_2$ (*m/z* 227, 229), and $M^+ - HCl - Br_2$ (*m/z* 182, 184) illustrates the halogen content. The base peak at *m/z* 167, 169, 171 was assigned to the ion $[CH_3{-}CCl{-}CH{=}CH{-}Br]^+$ by comparison with related monoterpenes.[4] The ^{1}H-NMR spectrum of **2** (Table 1) displays two methyl signals at δ 1.80 and δ 1.84, and one singlet at δ 9.52, which indicates the presence of an α-trisubstituted aldehyde. DQF−COSY measurements allowed the following spin systems to be discerned: an AB quartet $-CH_B{=}CH_AX$ at δ 6.27 and δ 6.50 and a three-proton AMX system $(R)_3C{-}CH_E{=}CH_D{-}CH_CX-$ at δ 4.55 (H_C), δ 5.77 (H_D), and δ 6.76 (H_E). The double bonds Δ^3 and Δ^7 were assigned as *cis* and *trans*, respectively, on the basis of the interproton coupling constants J_{DE} = 10.5 Hz and J_{AB} = 13.2 Hz. The $^3J_{HH(trans)}$ coupling constants of Δ^3 olefinic protons in (3*E*,7*E*)-2,6-dimethyloctadiene polyhalogenated monoterpenes lie in the range of 15 to 17 Hz. In the ^{13}C-NMR spectrum of **2** (Table 1), the chemical shift of C-8 (δ 111.2 ppm) indicated a vinyl bromide functionality. The remaining three sp^2 olefinic carbons absorb at δ 128.5 (C-7), δ 134.7 (C-3), and δ 144.4 (C-4) ppm. The halogen substitution at C-5 and C-6 was established by comparison of the ^{1}H NMR, ^{13}C NMR, and MS of **2** with those of known compounds.[1] The proton and carbon chemical shifts of the C-10 methyl group (δ 1.80 and δ 29.7 ppm) suggest a (5*R**,6*R**) stereochemistry, in accordance with the empirical rules of Mynderse and Faulkner[4] and Crews.[17] The C-9 methyl group (δ 1.80 and δ 30.8 ppm) is bonded to the quaternary allylic chlorine-bearing C-2 (δ 73.8 ppm). The observed $^3J_{HH}$ value of 2.1 Hz of the vicinal H_C-H_D coupling suggests a rigid carbon framework with a dihedral angle $H_C-C-C-H_D$ of about 90°. This conformation could result from a steric interaction between the two *cis* substituent groups of the Δ^3 double bond. The anomalous upfield shift of C-5 (δ 44.4 ppm) could also be explained on the basis of a C-3 substituent γ-effect. An unusual and rigid architecture for cartilagineal, the first monoterpene aldeyde isolated from *P. cartilagineum*, has been observed previously by Crews and Kho.[5] To our knowledge, compound **2** represents the first natural halogenated 2,6-dimethyloctadiene with a *cis* Δ^3 double bond. Rapid decomposition of this sample at room temperature precluded any further spectroscopic studies. An autocatalytic decomposition of cartilagineal has also been reported by Faulkner *et al.*[18]

Successive Si gel and Florisil chromatography of the third group of fractions followed by reversed-phase LPLC and HPLC yielded (*E*)-3,8-dibromo-2,7-dichloro-2,6-dimethyloct-5-ene (**3**), whose HRMS corresponds to a molecular formula of $C_{10}H_{16}Br_2Cl_2$. The ^{1}H NMR (Table 1) and ^{1}H−^{1}H COSY spectra indicated several isolated proton clusters as follows: a methine proton resonating at δ 4.61 (dd, J = 9.9, 5.7 Hz) coupled to each proton of an adjacent methylene group with signals at δ 3.62 (dd, J = 12.3, 9.9 Hz) and δ 3.68 (dd, J = 12.3, 5.7 Hz); two methylene protons at δ 2.61 and δ 3.18 both coupled to a vinyl proton at δ 5.71 (br t, J = 6.3, 6.6 Hz) and also to a methine proton at δ 4.07 (dd, J = 10.8, 2.1 Hz); three methyl singlets at δ 1.69, 1.73, and 1.80.

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Table 1. ^{1}H- and ^{13}C-NMR Assignments for Compounds **2**, **3**, and **8** in $CDCl_3$

	compound					
	2		3		8	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	9.52 (s)	a	1.73 (s)[b]	26.9	6.39 (s)	132.3
2		73.8		71.6		136.5
3	6.76 (d, 10.5)	134.7	4.07 (dd, 10.8, 2.1)	63.9	6.63 (d, 16.3)	124.2
4	5.77 (dd, 10.5, 1.8)	144.4	2.61 (ddd, 15.9, 10.8, 6.3) 3.18 (ddd, 15.9, 6.6, 2.1)	33.0[d]	6.47 (dd, 16.3, 8.1)	124.3
5	4.55 (d, 1.8)	44.4	5.71 (br t, 6.6, 6.3)	129.4	4.58 (d, 8.1)	69.6
6		73.0		134.0		71.8
7	6.27 (d, 13.2)	128.5	4.61 (dd, 9.9, 5.7))	66.0	6.09 (dd, 17.0, 10.6)	139.8
8	6.50 (d, 13.2)	111.2	3.62 (dd, 12.3, 9.9) 3.68 (dd, 12.3, 5.7)	32.7[e]	5.29 (d, 10.5) 5.41 (d, 17.0)	116.5
9	1.80 (s)	30.8	1.80 (s)[c]	33.2[f]	6.76 (s)	69.3
10	1.84 (s)	29.7	1.69 (s)	10.7	1.78 (s)	25.4

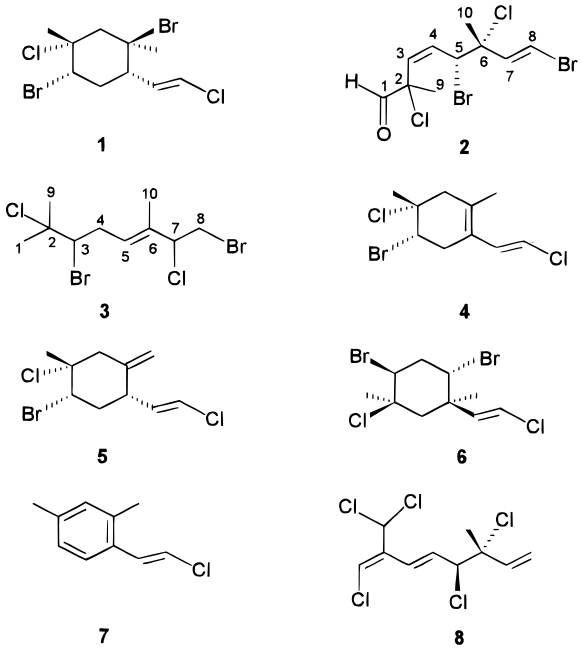
[a] The carbonyl resonance could not be assigned due to sample decomposition after 20 000 accumulations and high background noise level of the ^{13}C spectrum. [b–c, d–f] These assignments may be interchangeable.

The ^{13}C-NMR data (Table 1) revealed the presence of a double bond (δ 129.4 and 134.0 ppm), three halogen-bearing carbons (δ 63.9, 66.0, and 71.6 ppm), and two methyl groups (δ 10.7 and δ 26.9 ppm), respectively bonded to a sp^2 and quaternary carbon. Three close resonances (δ 32.7, 33.0, and 33.2 ppm) were attributed to the primary carbons $-CH_2-$ and CH_2X- and the remaining methyl group. The above spectral data was consistent with the framework $XC(CH_3)_2-CHX-CH_2-CH{=}C(CH_3)-CHX-CH_2X$. The halogen substitution pattern was assigned by comparison of the ^{1}H-NMR and ^{13}C-NMR data with those of known compounds containing the structural fragments $-CH_2-CH{=}C(CH_3)-CHCl-CH_2Br$,[11] $-CH{=}C(CH_3)-CHBr-CH_2Cl$,[19] $ClC(CH_3)_2-CHBr-CH_2-$,[20] and $BrC(CH_3)_2-CHCl-CH_2-$.[21] The *E*-stereochemistry of the double bond Δ^5 was deduced from the ^{13}C upfield chemical shift for C-10 (δ 10.7 ppm) due to a γ-effect from C-4 in a *cis*-relationship.[11] No information on the configuration of the halogen substituents at C-3 and C-7 could be obtained.

Successive flash chromatography of the $CHCl_3$ extract followed by repeated HPLC gave the conjugated diene **4** ($C_{10}H_{13}BrCl_2$), its exocyclic methylene isomer **5**, and compound **6** ($C_{10}H_{14}Br_2Cl_2$), whose spectral data corresponds to those for known compounds previously isolated from *P. cartilagineum* from the British coast.[16] The structures of **1**, **4**, and **5** were confirmed by a series of chemical interconversions.

Reaction of **1** with silver acetate in glacial HOAc gave the diene **4,** which, upon treatment with DBU, afforded the aromatic compound **7**. The (*E*)-vinyl chloride **7** was also obtained from 2,4-dimethylbenzaldehyde by means of the $CHCl_3-CrCl_2$ system of Takai.[22] Treatment of **1** with DMF at 140 °C gave a mixture of **4**, **5**, and **7**.

From the hexane extract of a *P. cartilagineum* specimen collected at Figueira da Foz metabolites **1**, **2**, and **8** were isolated. The ^{1}H NMR and MS of the pentachlorinated monoterpene **8** ($C_{10}H_{11}Cl_5$) corresponds to those of a known compound previously isolated from *P. cartilagineum* at La Jolla.[4] The negative optical rotation of **8** and its ^{13}C-NMR data, here reported for the first time in literature (Table 1), confirms the (*E*)-geometry of the Δ^1 double bond[4] and the (5*S**,6*R**) stereochemistry. The olefinic carbons C-3 and C-4 have similar chemical shifts (δ 124.2 and δ 124.3 ppm), whereas these resonances appear at δ 126.6 and δ 119.0 ppm in the corresponding (*Z*) isomer.[1] The C-6 methyl shift (δ 25.4 ppm) is characteristic of an erythro C-5,C-6 configuration.



The crude hexane extract of *P. cartilagineum* exhibited strong ichthyotoxic activity for the fish *Lebistes reticulatus* (< 50 mg/L).

Experimental Section

General Experimental Procedures. Mps were determined on a Reichert microscope and are uncorrected. Optical rotations were recorded with Perkin-Elmer 241 MC and Optical Activity Ltd. polarimeters using $CHCl_3$ as solvent. IR spectra were measured on Perkin-Elmer 157G and Bruker IFS25 FT-IR infrared spectrometers. UV spectra were recorded in hexane on Philips PU 870 and Milton Roy Spectronic 1201 spectrophotometers. ^{1}H-NMR spectra were measured at 250 MHz on a Bruker WM 250, at 300 MHz on a GE NMR 300 and a Varian Unity-300, at 400 MHz on a Varian XL-400, and at 600 MHz on a Varian Unity-600. 2D correlated spectroscopy was undertaken on a GE NMR 300 for compounds **1**, **3**, **4**, and **6**; on a Varian XL-400 for compounds **1**, **4**, and **6**; and on a Varian Unity-600 for compound **2**. ^{13}C-NMR spectra were measured at 75.5 MHz on a Bruker WM 250 and a GE NMR 300,

and at 100.6 MHz on a Varian XL-400. $CDCl_3$ was used as solvent with TMS as the internal standard. EIMS were recorded on a JEOL JMSDX 300 and a Micromass 7070F operating at 70 eV. HREIMS were recorded on a JEOL JMSDX 300. Reversed-phase HPLC of compound **2** was carried out with a Waters 6000A connected to a UV detector using a μ-Bondapak C-18 column (250 mm × 4.6 mm, 5 mm). A Spectra Physics 100 was used for HPLC separations of compounds **1**, **3**, and **4**−**8** using a Hypersil H50 ODS column (250 mm × 46 mm, 5 mm). Michel-Miller columns filled with Si 60 and Lichroprep RP-18 were used in normal and reversed-phase LPLC. The LPLC system was equipped with an ISCO UA-6 detector set at 254 nm and a 5-mm preparative flow cell. The mobile phase was delivered by a Fluid Metering QSY pump. Merck Si gel of 70−230 mesh and 230−400 mesh were used for normal pressure and flash column chromatography, while aluminium-backed sheets coated with silica $60F_{254}$, 0.20-mm thick, were used for TLC. Merck Si gel plates 0.25-mm and 2-mm thick were used for preparative HPTLC.

Plant Material. The algal material was collected in Sesimbra and Figueira da Foz in July 1990, and air dried. A voucher specimen is deposited at the herbarium of IPIMAR, Lisbon.

Extraction and Isolation. Air dried alga (4 kg) collected at Sesimbra was successively extracted with hexane and $CHCl_3$. Si gel chromatography of a portion of the hexane extract (12 g from 16 g dry wt), using hexane with increasing proportions of EtOAc as eluent, afforded three groups of fractions that were combined according to their composition. From the least polar group of fractions compound **1** (6 g) was isolated, mp 115−116° (MeOH), $[\alpha]_D$ −84° (*c* 4.3, $CHCl_3$), {lit.[13] mp 121−122°, $[\alpha]_D$ −88°}. ^{1}H-NMR, ^{13}C-NMR, and MS data of **1** were in agreement with published values.[13] The second group of fractions (0.43 g) was eluted with light petroleum on a Si gel column followed by a step gradient of EtOAc (5−50%) in hexane. Further purification of one fraction (0.14 g) by successive HPTLC (hexane/EtOAc, 95:5) and HPLC (MeOH/H_2O, 85:15) yielded 0.01 g of **2**, isolated as an oil. The most polar group of fractions was submitted to successive Si gel (gradient elution from 0−100% EtOAc in hexane), Florisil (hexane elution), and reversed-phase LPLC (MeOH/H_2O, 75:25 elution). Final purification by reversed phase HPLC using MeOH/H_2O (82:18) as eluent yielded 0.028 g of **3**. Successive flash chromatography of the $CHCl_3$ extract (37 g) using gradient elution of hexane/EtOAc, followed by reversed-phase LPLC (MeOH/H_2O, 85:15) afforded two main fractions. One fraction was purified by repeated reversed-phase LPLC (MeOH/H_2O, 85:15) and HPLC (CH_3CN/MeOH/H_2O, 50:15:35) yielding compounds **4** (0.018 g), mp 103−104° (MeOH), $[\alpha]_D$ −27° (*c* 0.73, $CHCl_3$) {lit. 104−105°, $[\alpha]_D$ −13°[16]} and **6** (0.007 g) mp 49−50° (MeOH), $[\alpha]_D$ +42° (*c* 0.68, $CHCl_3$) {lit. oil, $[\alpha]_D$ +32°[16]}. The second fraction was submitted to successive normal- and reversed-phase LPLC, eluted with hexane and MeOH/H_2O, 85:15 respectively, and finally purified by reversed-phase HPLC to yield compound **5** (0.013 g), mp 49−50° (MeOH), $[\alpha]_D$ −85° (*c* 1.3, $CHCl_3$) {lit. oil, $[\alpha]_D$ −71°[16]}. ^{1}H-NMR, ^{13}C-NMR and MS data of **4**−**6** were in agreement with published values.[16] A specimen of *P. cartilagineum* (1 kg) collected at Figueira da Foz was extracted with hexane yielding 0.5 g of **1** by crystallization. Si gel flash chromatography of the extract (4 g) using a step gradient of EtOAc (0−50%) in hexane followed by reversed-phase LPLC (MeOH/H_2O, 85:15) afforded two main fractions. Reversed-phase HPLC (MeOH/H_2O, 85:15) of the less polar fraction yielded compound **8** (0.003 g), oil, $[\alpha]_D$ −17° (*c* 0.14, $CHCl_3$) {lit. $[\alpha]_D$ −39°[16]}. ^{1}H- and ^{13}C-NMR data, see Table 1.

(3*Z*,7*E*)-5,8-Dibromo-2,6-dichloro-2,6-dimethylocta-3,7-dien-1-al (2): oil; HREIMS *m/z* 375.8640 (calcd for $C_{10}H_{12}O^{79}Br_2{}^{35}Cl_2$, 375.8633); EIMS *m/z* 376, 378, 378, 380, 382, 384 $[M]^+$, 341, 343, 345, 347 $[M - Cl]^+$, 297, 299, 301, 303 $[M - Br]^+$, 261, 263, 265 $[M - Cl - HBr]^+$, 227, 229 $[M - Br - Cl_2]^+$, 183, 185, $[M - Br_2 - Cl]^+$, 182, 184, $[M - HCl - Br_2]^+$, 167, 169, 171 (base peak) $[C_4H_5BrCl]^+$; ^{1}H- and ^{13}C-NMR data, see Table 1.

(*E*)-3,8-Dibromo-2,7-dichloro-2,6-dimethyloct-5-ene (3): oil, $[\alpha]_D$ −53° (*c* 0.67, $CHCl_3$); HREIMS *m/z* 363.8996 (calcd for $C_{10}H_{16}{}^{79}Br_2{}^{35}Cl_2$, 363.8997); EIMS *m/z* 376, 364, 366, 368, 370 $[M]^+$, 329, 331, 333, 335 $[M - Cl]^+$, 284, 286, 288, 290 $[M - HBr]^+$, 250, 252, 254 $[M - Br - Cl]^+$, 249, 251, 253 $[M - HBr - Cl]^+$, 135 $[M - HBr - Br - Cl_2]^+$, 81 (base peak) $[C_6H_9]^+$. ^{1}H- and ^{13}C-NMR data, see Table 1.

Conversion of 1 to 4, 5, and 7. A solution of **1** (100 mg, 0.27 mmol) and AgOAc (47.5 mg, 0.28 mmol) in glacial AcOH was treated according to the procedure described[16] to obtain **4** (66 mg, 0.23 mmol), identical in all aspects to an authentic sample. Compound **4** (12 mg, 0.04 mmol) in a N_2 atmosphere and dry THF (10 mL) was reacted with a slight excess of DBU at 30 °C for 24 h, to yield the aromatic compound **7** (4 mg, 0.02 mmol). Its ^{1}H-NMR and MS data were identical to those reported in literature.[9] A solution of **1** (60 mg, 0.16 mmol) in DMF (5 mL) was treated according to the procedure described[16] to obtain a mixture of **4** (10 mg, 0.19 mmol), **5** (17 mg, 0.06 mmol), and **7** (20 mg, 0.1 mmol) that was separated by Si gel chromatography (hexane elution) followed by reversed-phase HPLC (MeOH/H_2O, 85:15).

Conversion of 2,4-Dimethylbenzaldehyde to 7. Anhydrous $CrCl_2$ (0.55 g, 4.3 mmol) was suspended in dry THF (10 mL) under an argon atmosphere. A solution of 2,4-dimethylbenzaldehyde (0.17 g, 1.2 mmol) and $CHCl_3$ (1 mL) in THF (5 mL) was added dropwise at 0 °C, and the resulting suspension was heated at 65 °C for 6 h. The reaction mixture was poured into H_2O, extracted with Et_2OH, and dried over Na_2SO_4. Purification by Si gel chromatography (hexane elution) followed by reversed-phase HPLC (MeOH/H_2O, 85:15) afforded a nearly 85:15 mixture of *trans*- and *cis*-1-(2′-chlorovinyl)-2,4-dimethylbenzene (**7**) (0.03 g, 0.18 mmol). In the ^{1}H-NMR spectrum of **7** the two *cis* vinyl protons appears at δ 6.3 and δ 6.71 (d, J = 7.8 Hz), whereas the *trans* protons are centered at δ 6.99 and δ 6.45 (d, J = 13.2 Hz).

Bioassay. The hexane extract is lethal for the fish *Lesbites reticulatus* in 1 h at concentrations < 50 mg/L.

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References and Notes

(1) Naylor, S.; Hanke, F. J.; Manes, L. V; Crews, P. *Prog. Chem. Org. Nat. Prod.* **1983,** *44*, 189−241, and references cited therein.
(2) Stierle, D. B.; Sims, J. J. *Tetrahedron* **1979**, *35*, 1261−1265.
(3) Rovirosa, J.; Sanchez, I.; Palacios, Y.; Darias, J.; San-Martin, A. *Bol. Soc. Chil. Quím.* **1990**, *35*, 131−135.
(4) Mynderse, J. S.; Faulkner, D. J. *Tetrahedron* **1975**, *31*, 1963−1967.
(5) Crews, P.; Kho, E. *J. Org. Chem.* **1974**, *39*, 3303−3304.
(6) Crews, P.; Kho-Wiseman, E.; Montana, P. *J. Org. Chem.* **1978,** *43*, 116−120.
(7) Mynderse, J. S.; Faulkner, D. J. *Phytochemistry* **1978,** *17*, 237−240.
(8) Crews, P.; Naylor, S.; Hanke, F. J.; Hogue, E. R; Kho, E.; Braslau, R. *J. Org. Chem.* **1984**, *49*, 1371−1377.
(9) Capon, R. J.; Engelhardt, L. M.; Ghisalberti, E. L.; Jefferies, P. R.; Patrick, V. A.; White, A. H. *Aust. J. Chem.* **1984**, *37*, 537−544.
(10) Norton, R. S.; Warren, R. G.; Wells, R. J. *Tetrahedron Lett.* **1977**, 3905−3908.
(11) Blunt, J. W.; Bowman, N. J.; Munro, M. H. G.; Parsons, M. J.; Wright, G. J.; Kon, Y. K. *Aust. J. Chem.* **1985**, *38*, 519−525.
(12) San-Martin, A.; Negrete, R.; Rovirosa, J. *Phytochemistry* **1991**, *30*, 2165−2169.
(13) González, A. G.; Arteaga, J. M.; Martín, J. D.; Rodríguez, M. L.; Fayos, J.; Ripolls, M. M. *Phytochemistry* **1978**, *17*, 947−948.
(14) Castedo, L.; Garcia, M. L.; Quiñoá, E.; Riguera, R. R. *J. Nat. Prod.* **1984**, *47*, 724−726.
(15) Konig, G. M.; Wright, A. D.; Sticher, O. *J. Nat. Prod.* **1990**, *53*, 1615−1618.
(16) Higgs, M. D.; Vanderah, D. J.; Faulkner, D. J. *Tetrahedron* **1977**, *33*, 2775−2780.
(17) Crews, P. *J. Org. Chem.* **1977**, *42*, 2634−2636.
(18) Ireland, C.; Stallard, M. O.; Faulkner, D. J. *J. Org. Chem.* **1976**, *41*, 2461−2465.
(19) Stierle, D. B.; Sims, J. J. *Tetrahedron Lett.* **1984**, 153−156.
(20) Fuller, R. W.; Cardellina, J. H.; Kato, Y.; Brinen, L. S.; Clardy, J.; Snader, K. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 3007−3011.
(21) Fuller, R. W.; Cardellina, J. H.; Jurek, J.; Scheuer, P. J.; Lindner, B. A.; McGuire, M.; Gray, G. N.; Steiner, J. R.; Clardy, J.; Menez, E.; Shoemaker, R. H.; Newman, D. J.; Snader, K. M.; Boyd, M. R. *J. Med. Chem.* **1994**, *37*, 4407−4411.
(22) Takai, K.; Nitta K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408−7411.

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