

Carijodienone from the Octocoral *Carijoa multiflora*. A Spiropregnane-Based Steroid

Ana R. Díaz-Marrero,^{*,†} Gina Porras,[†] Zulma Aragón,[†] José M. de la Rosa,[†] Enrique Dorta,[†] Mercedes Cueto,[†] Luis D'Croz,[‡] Juan Maté,[§] and José Darías[†]

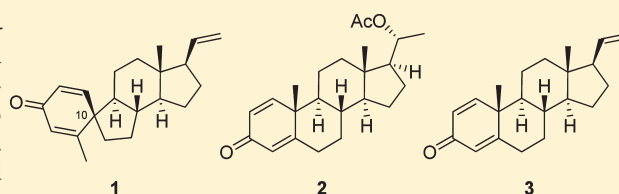
[†]Instituto de Productos Naturales y Agrobiología del CSIC, Avenida Astrofísico F. Sánchez, 3, 38206 La Laguna, Tenerife, Spain

[‡]Departamento de Biología Marina y Limnología, Universidad de Panamá, Panamá

[§]Smithsonian Tropical Research Institute, STRI, Box 0843-03092, Balboa, Panama

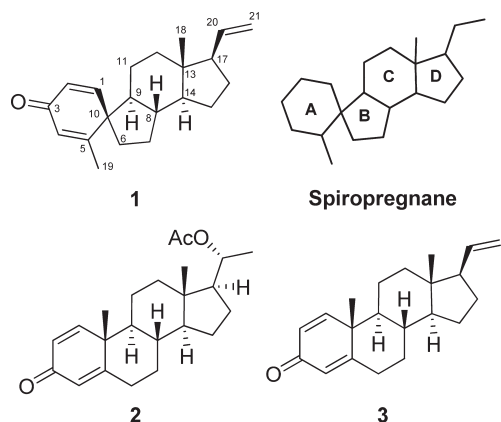
S Supporting Information

ABSTRACT: Two new steroids (**1** and **2**) and the known pregna-1,4,20-trien-3-one (**3**) have been isolated from the Pacific octocoral *Carijoa multiflora*. Compound **1** possesses a novel spiropregnane-based steroidal skeleton. The photochemical transformation of **3** into **1** allowed the assignment of the absolute configuration at C-10 of **1**. The antibacterial activities of compounds **1** and **3** were evaluated against a panel of bacterial strains.



Sterols found in marine invertebrates can arise from the food chain, from biochemical modification of dietary sterols, or from symbiotic relationships between organisms, making the biosynthetic origin and biological function of the frequently complex mixture of this structural class enigmatic.¹ Despite the great abundance of marine sterols, pregnane steroids are rare in the marine environment, and octocorals appear to be their most prolific source,² with the 5 α -pregnane nucleus bearing a vinyl side chain the most common example.³

The search for marine natural products in benthic species from both sides of the Isthmus of Panama⁴ prompted us to study the eastern Pacific octocoral *Carijoa multiflora*. Our previous studies on the chemical constituents of this species have led to the isolation of two chlorinated pregnanes³ and a novel class of chlorinated prostanoid.^{4a} In this new work we report the isolation and structure elucidation of carijodienone (**1**). This new compound possesses a novel carbon skeleton that we have named spiropregnane. This skeleton contains a spiro[4,5]decane core derived from the A–B ring rearrangement of a steroidal nucleus.



An acetone extract of *C. multiflora* (= *Telesto multiflora*) collected at Archipelago Las Perlas, Panama, yielded compounds **1** and **2**, after flash chromatography, gel filtration, and HPLC, along with the known⁵ compound **3**, previously found in *Carijoa* species.^{4a,6} Octocorals of the genus *Carijoa* (Cnidaria: Anthozoa: Octocorallia: Alcyonacea: Clavulariidae)⁷ have proven to be a source of cytotoxic amides,⁸ cytotoxic sterol glycosides,⁹ and the enzyme inhibitory punaglandins,^{10,11} in addition to pregnane steroids.^{2b,6} *C. multiflora* is a common nonzooxanthellate octocoral from the Pacific coast of Panama usually found with polyps expanded during the day in sheltered and shaded crevices or in shallow caverns covering basaltic and coralline substrata. Microscopic examination confirmed the absence of endosymbiotic algae in tissues from the collected specimens.

Compound **1** was isolated as a colorless oil with a molecular formula of C₂₁H₂₈O, as determined by the [M]⁺ peak at 296.2162 in the HREIMS spectrum, indicating eight degrees of unsaturation. Its NMR spectra acquired in C₆D₆ provided better dispersion than in CDCl₃, avoiding overlapped key signals. The ¹³C NMR spectrum of **1** (Table 1), together with the information from a DEPT spectrum, showed the presence of 21 carbon signals assigned to two methyls (one olefinic), seven methylenes (one olefinic), eight methines (four olefinic), and four nonprotonated carbons (one carbonyl and one olefinic). The IR absorption at 1660 cm⁻¹ is consistent with an unsaturated carbonyl group, which combined with the aforesaid information suggested a tetracyclic molecule.

The ¹H NMR spectrum of **1** exhibited two singlet methyl signals at δ 0.44 and 1.46 ppm and six olefinic protons. Although the NMR data suggested a C₂₁-pregnane with a vinyl side chain, the presence of an olefinic methyl group in this skeleton raised some doubts

Received: October 20, 2010

Published: January 14, 2011

Table 1. ^1H NMR [500 MHz] and ^{13}C NMR [125 MHz] Data for Compounds **1** and **2**

position	carijodienone (1) ^a		2 ^b	
	δ_{C} , mult.	δ_{H} (J in Hz)	δ_{C} , mult.	δ_{H} (J in Hz)
1	153.0, CH	6.17, d (10.4)	155.9, CH	7.04, d (10.0)
2	127.3, CH	6.27, dd (1.9, 10.4)	127.5, CH	6.22, dd (1.9, 10.5)
3	185.2, C		186.4, C	
4	129.5, CH	6.28, brs	123.8, CH	6.06, s
5	160.6, C		169.3, C	
6	35.0, CH ₂	a: 1.57, m b: 1.32, m	32.9, CH ₂	a: 2.45, ddd (6.2, 13.8, 13.8) b: 2.35, ddd (2.4, 3.8, 13.5)
7	30.2, CH ₂	a: 1.54, m b: 0.89, m	33.7, CH ₂	a: 1.96, m b: 1.04, m
8	42.3, CH	1.31, m	35.4, CH	1.65, m
9	56.3, CH	1.11, ddd (4.8, 11.8, 11.8)	52.5, CH	1.06, m
10	51.9, C		43.6, C	
11	21.7, CH ₂	α : 0.96, m β : 0.92, m	22.8, CH ₂	a: 1.74, m b: 1.57, m
12	37.3, CH ₂	a: 1.50, ddd (3.5, 3.5, 12.6) b: 0.64, ddd (5.4, 12.6, 12.6)	38.9, CH ₂	a: 1.85, ddd (3.3, 3.3, 12.9) b: 1.25, m
13	44.8, C		42.5, C	
14	55.5, CH	0.71, ddd (7.6, 10.8, 12.8)	54.8, CH	1.00, m
15	25.2, CH ₂	a: 1.50, m b: 1.15, dddd (5.8, 5.9, 12.2, 18.1)	25.3, CH ₂	a: 1.77, m b: 1.55, m
16	27.7, CH ₂	a: 1.78, m b: 1.50, m	24.4, CH ₂	a: 1.70, m b: 1.57, m
17	54.9, CH	1.85, dd (8.8, 16.7)	54.8, CH	1.57, m
18	13.2, CH ₃	0.44, s	12.5, CH ₃	0.70, s
19	18.9, CH ₃	1.46, s	18.7, CH ₃	1.21, s
20	139.7, CH	5.75, ddd (7.3, 10.4, 17.0)	72.7, CH	4.83, dq (12.4, 6.2)
21	115.2, CH ₂	5.07, m	19.9, CH ₃	1.14, d (6.2)
	COCH_3		170.4, C	
	COCH_3		21.5, CH ₃	2.01, s

^a NMR data recorded in C₆D₆. ^b NMR data recorded in CDCl₃.

about whether the molecule was indeed a pregnane steroid. Thus, a careful 2D NMR spectroscopic analysis was undertaken.

The similarity of the resonances of the C/D ring carbon atoms to those of the pregnane congener **3**,⁵ also isolated in this work, the COSY spin system observed from H-17 through H-8, and the HMBC correlations of H-17/C-18, C-20 confirmed the C/D rings with a vinyl side chain attached at C-17. Mutual ^1H NMR J -coupling and a COSY correlation located two vicinal sp^2 methines at δ_{C} 153.0 (C-1) and 127.3 (C-2) ppm. The presence of a cross-conjugated ketone (δ 185.2, C-3) and a quaternary carbon characteristic of a spirocarbon at δ 51.9 (C-10) in the A-ring were established by the following HMBC correlations: H-1/C-3, C-5, C-6, C-10; H-2/C-4, C-10; and H-4/C-19, C-10. Finally, the HMBC cross-peaks of H-6/C-9, C-10 allowed the connection of ring A to the C/D ring system, and a COSY correlation of H₂-6 and H₂-7 together with the HMBC correlation of H₂-7 with C-8 established a spiro[4,5]decane moiety in a pregnane scaffold, allowing us to disclose the planar structure as depicted in **1**.

A 2D NOESY experiment established the relative configuration of the spirocarbon as well as the remaining stereogenic centers of the steroidal nucleus as shown in Figure 1. The NMR chemical shifts of C-18 (δ_{H} 0.44, δ_{C} 13.2) suggested that Me-18 was β and that rings C/D were *trans* fused, which was corroborated by NOE.

A NOESY experiment of **1** acquired in C₆D₆ showed spatial correlation of H-1 with both a multiplet constituted by H₂-11 and H-7b and a multiplet constituted by H-6b and H-8. The energy-minimized conformation¹² of **1** indicates that only H-11 β from the first multiplet is at a suitable interatomic distance (2.238 Å) to give a NOE with H-1. Hence, the correlation between H-1 and H-8 of the second multiplet justifies the strong NOE observed. This rationale was reinforced by the NOE observed between H₃-19 and H-9, allowing us to assign a relative R^* configuration for C-10 as represented in Figure 1.

Spirodienones have been obtained by irradiation of various bicyclohexadienones.¹³ Earlier work published in 1966 provided the configurational assignment of the spirocarbons in the dienones **5** (10R) and **7** (10S), which are photochemically¹⁴ derived from the epimeric dehydrotestosterone acetates **4** and **6**, respectively (Figure 2). On the basis of this work, we could confirm the configuration of compound **1** by phototransformation of compound **3**, using a mercury lamp, to obtain a spiro[4,5]dienone that was identical to compound **1** in all respects.¹⁵ Since the absolute configuration of **3** has been determined,⁵ we could, therefore, establish the absolute configuration of **1** as 8S, 9S, 10R, 13R, 14S, 17R. Compound **3** is highly stable, and spontaneous phototransformation to yield **1** has never been observed under laboratory

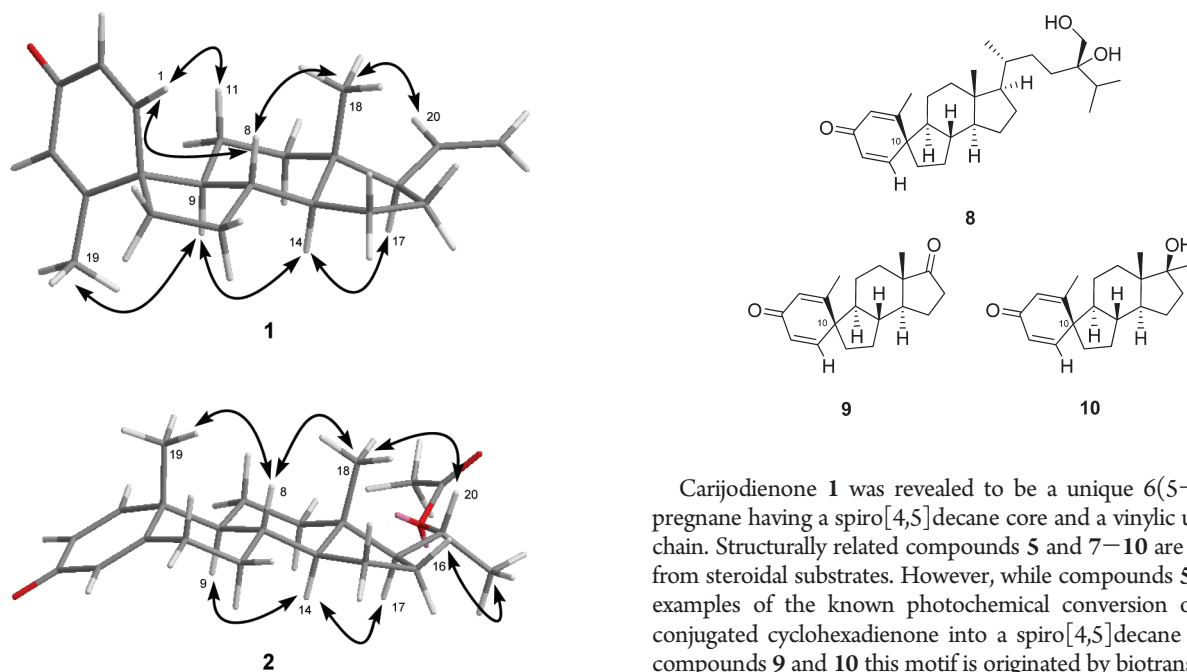


Figure 1. Selected NOEs for compounds 1 and 2.

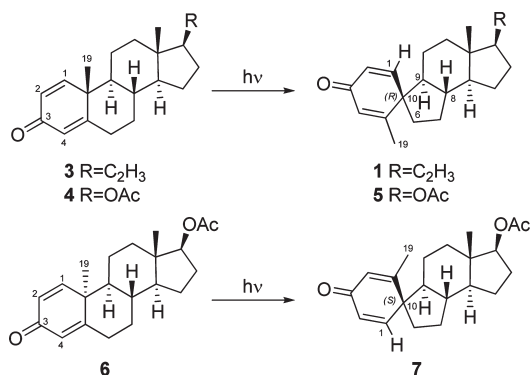


Figure 2. Photochemical transformation of 3, 4, and 6 into the 1, 5, and 7 6(5–10)-abeosteroids, respectively.

conditions (room temperature and long exposure to sunlight). Therefore, cariodienone (**1**) is suggested to be a *de novo* compound produced by *C. multiflora*.

Compound **2** was isolated as a colorless oil with a m/z 356.2328 corresponding to a molecular formula of $C_{23}H_{32}O_3$, indicative of eight degrees of unsaturation. The NMR data of **2** (Table 1) resemble those of the known compound **3** (Table S1, Supporting Information), with the primary difference being a saturated side chain bearing a secondary acetate group at C-20 (δ_H 4.83 dq, $J = 12.4, 6.2$ Hz; δ_C 72.7 ppm) instead of the vinylic side chain of compound **3**. The planar structure of compound **2** was confirmed by COSY, HSQC, and HMBC experiments.

The relative configuration of **2** was assigned on the basis of a 2D NOESY experiment. The C-5/C-10, C-8/C-9, and C-13/C-14 *trans*-fused ring junctions were established by NOE correlations of H-8 with H₃-18 and H₃-19. The NOEs observed between H₃-18 and H-20 indicated a β -disposition for the side chain, and the NOE observed between H₃-21 and H-16 β indicated a 20R* relative configuration (Figure 1).

Cariodienone **1** was revealed to be a unique 6(5–10)-abeopregnane having a spiro[4,5]decane core and a vinylic unit as side chain. Structurally related compounds **5** and **7**–**10** are all derived from steroidal substrates. However, while compounds **5** and **7** are examples of the known photochemical conversion of a cross-conjugated cyclohexadienone into a spiro[4,5]decane system, in compounds **9** and **10** this motif is originated by biotransformation of an exogenous steroidal substrate using microalgae.^{16,17} Therefore, although the well-known photochemical mechanisms¹⁸ that involve the cyclohexadienone \rightarrow spiro[4,5]decane rearrangement could explain the genesis of **1** from the naturally occurring **3** in *C. multiflora*, enzymatically induced mechanisms should not be discounted. The formation of the conjugated 6(5–10)-abeodienone system of **1** from a parent sterol skeleton may occur prior to steroidogenesis because **8**, a compound isolated from the soft coral *Nephthea chabrolii*,¹⁹ possesses a steroidal network with a full side chain and embodies the same spiro[4,5]decane subunit as **1**. Compound **1** is a unique spiropregnane that has been found in a living organism.

Compound **3** was found to be practically inactive (MIC values over 40 $\mu\text{g}/\text{mL}$) against all bacterial strains under study. However, **1** caused a significant growth rate decrease in the ranges 6–8 and 5–10 $\mu\text{g}/\text{mL}$ against *B. cereus* and *K. pneumoniae*, respectively, but this effect seems to diminish as the concentration of the compound increases.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter using a Na lamp at 25 °C. IR spectra were recorded on a Perkin-Elmer 1650/FTIR spectrometer. UV spectra were recorded on a Varian Cary E1 UV–visible spectrophotometer. ¹H NMR and ¹³C NMR, HSQC, HMBC, and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ¹H NMR and at 125 MHz for ¹³C NMR. All ¹³C and ¹H NMR spectra were internally referenced to the residual solvent signal (CDCl₃: δ_C 77.0 ppm, δ_H 7.25 ppm; C₆D₆: δ_C 128.0 ppm, δ_H 7.16 ppm). Two-dimensional NMR spectra were obtained using the standard Bruker software. EIMS and HRMS data were obtained on a Micromass Autospec spectrometer. HPLC separations were performed on an Agilent 1200 Series Quaternary LC system using a Jaigel-Sil-043-10 semipreparative column (10 μm , 20 \times 250 mm) eluted with hexane–EtOAc mixtures and a Jaigel GS-300 column (10 μm , 20 \times 500 mm) eluted with MeOH. The gel filtration column (Sephadex LH-20) used hexane–MeOH–CH₂Cl₂ (3:1:1) as solvent. The spray reagent for TLC was H₂SO₄–H₂O–AcOH (1:4:20).

Biological Material. *Carijoa multiflora* was collected by scuba diving off Archipelago Las Perlas, Panama, at –15 m. A voucher specimen has been deposited at the Smithsonian Research Institute (Panama) under code CJm09.

Extraction and Isolation. A fresh sample of *C. multiflora* (932 g) was extracted in acetone (3 × 500 mL). The acetone extracts were combined and concentrated *in vacuo* to afford 61.5 g of a dark brown gum. The extract was partitioned between H₂O (400 mL) and EtOAc (3 × 200 mL). The EtOAc fraction was evaporated under reduced pressure (18.3 g) and chromatographed on a silica gel column using a gradient from 100% hexane to 100% EtOAc. The fraction eluted with hexane–EtOAc (8:2) contained a mixture of steroidal compounds (1.2 g). Gel filtration chromatography afforded two fractions, which were further purified by HPLC. Pure compound 1 (2.6 mg) along with the known 3⁵ (95.0 mg) were obtained after HPLC of fraction A4 (125.5 mg, Jaigel-Sil-043-10 column, hexane–EtOAc (85:15), 5.0 mL/min), whereas compound 2 (2.6 mg) was purified after HPLC of fraction A9 (50.4 mg, Jaigel GS-300 column, 100% MeOH, 5.0 mL/min).

Carijodienone (1): colorless oil; $[\alpha]_{\text{D}}^{20} -11.5$ (*c* 0.26, CH₂Cl₂); IR (film) ν_{max} 1660, 1620 cm⁻¹; UV (MeOH) λ_{max} (log ϵ) 285 nm (2.77); ¹H and ¹³C NMR recorded in C₆D₆, see Table 1; ¹H and ¹³C NMR in CDCl₃, see Table S1; EIMS *m/z* 296 [M]⁺ (91), 227 (73), 121 (100), 91 (48); HREIMS [M]⁺ *m/z* 296.2162 (calcd for C₂₁H₂₈O, 296.2140).

Compound 2: colorless oil; $[\alpha]_{\text{D}}^{20} +39.3$ (*c* 0.28, CH₂Cl₂); IR (film) ν_{max} 1727, 1654 cm⁻¹; ¹H and ¹³C NMR in CDCl₃, see Table 1; EIMS *m/z* 356 [M]⁺ (2), 296 [M – C₂H₄O₂]⁺ (14), 175 (21), 122 (100), 91 (28), 55 (22); HREIMS [M]⁺ *m/z* 356.2328 (calcd for C₂₃H₃₂O₃, 356.2351).

Pregna-1,4,20-trien-3-one (3): amorphous solid; $[\alpha]_{\text{D}}^{20} +35.5$ (*c* 2.0, CH₂Cl₂) (lit.⁵ $[\alpha]_{\text{D}}^{20} +36.9$ (*c* 1.0, CHCl₃), lit.⁶ $[\alpha]_{\text{D}}^{20} +36.0$ (*c* 0.23, CHCl₃)); ¹³C NMR in CDCl₃, see Table S1.

Photochemical Interconversion of Compound 3 into Carijodienone (1) (ref 13). A solution of dienone 3 (80.0 mg, 0.27 mmol) in 3.0 mL of dioxane was irradiated with a Hanovia medium-pressure mercury lamp for 2.5 h. The solvent was removed *in vacuo*, and the residue chromatographed by HPLC (Jaigel-Sil-043-10 column, gradient from 100% hexane to hexane–EtOAc (85:15) for 40 min, then isocratically in hexane–EtOAc (85:15), 5.0 mL/min) to give 8.0 mg (10% yield) of carijodienone (1). Synthetic 1: $[\alpha]_{\text{D}}^{20} -31.3$ (*c* 0.80, CH₂Cl₂). See Supporting Information for comparison of ¹H NMR spectra.

Antibacterial Activity. Antibacterial activity was determined by the broth macrodilution method against the following strains: *Staphylococcus aureus*, *Salmonella* sp., *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

Briefly, bacterial strains were grown aerobically at 28 °C in tryptic soy broth (TSB) for 16–20 h in an orbital shaker. A set of tubes with different concentrations of compounds 1 and 3 prepared in TSB were next inoculated with the microorganisms ((1–5) × 10⁵ colony forming units/mL) and incubated overnight. Broth tubes that appeared turbid were indicative of bacterial growth, while tubes that remained clear indicated no growth. The MIC, defined as the lowest concentration of inhibitory compound at which no growth was observed, was evaluated in triplicate for each compound (within the range 1–40 μg/mL). Cultures prepared under the same conditions but without compounds and cultures with the same proportions of DMSO (<1%) were used as controls. The growth of broth tubes without turbidity was further examined by counting the viable cells on tryptic soy agar (TSA) plates.

■ ASSOCIATED CONTENT

Supporting Information. ¹H and ¹³C NMR spectra of 1–3 and comparison of ¹H NMR of natural and synthetic 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +34 922 252 144. Fax: +34 922 260 135. E-mail: ardiáz@ipna.csic.es.

■ ACKNOWLEDGMENT

This work was supported by the Ministerio de Ciencia e Innovación (BIO2007-61745, SAF2009-08039, SAF2010-16858). A.R.D.-M. acknowledges financial support from Programa JAE-Doc (CSIC). The STRI provided support and facilities for field work. The authors thank J. del Rosario for technical support and Dr. A. J. Herrera for assistance in completion of the photochemical reaction. The Government of Panama granted permission for the collection of the samples.

■ REFERENCES

- (1) (a) Lafont, R.; Mathieu, M. *Ecotoxicology* **2007**, *16*, 109–130. (b) D'Auria, M. D.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839–1895. (c) Kerr, R. G.; Baker, B. J. *Nat. Prod. Rep.* **1991**, *8*, 465–497.
- (2) (a) Ortega, M. J.; Zubia, E.; Rodríguez, S.; Carballo, J. L.; Salvá, J. *Eur. J. Org. Chem.* **2002**, 3250–3253, and references therein. (b) Lorenzo, M.; Cueto, M.; D'Croz, L.; Maté, J. L.; San-Martín, A.; Darias, J. *Eur. J. Org. Chem.* **2006**, 582–585. (c) Huang, X.; Deng, Z.; Zhu, X.; van Ofwegen, L.; Proksch, P.; Lin, W. *Helv. Chim. Acta* **2006**, *89*, 2020–2026. (d) Poza, J. J.; Fernández, R.; Reyes, F.; Rodríguez, J.; Jiménez, C. *J. Org. Chem.* **2008**, *73*, 7978–7984.
- (3) Dorta, E.; Díaz-Marrero, A. R.; Cueto, M.; D'Croz, L.; Maté, J. L.; San-Martín, A.; Darias, J. *Tetrahedron Lett.* **2004**, *45*, 915–918.
- (4) (a) Dorta, E.; Díaz-Marrero, A. R.; Cueto, M.; D'Croz, L.; Maté, J. L.; Darias, J. *Org. Lett.* **2004**, *6*, 2229–2232. (b) Cueto, M.; D'Croz, L.; Maté, J. L.; San-Martín, A.; Darias, J. *Org. Lett.* **2005**, *7*, 415–418. (c) Lorenzo, M.; Brito, I.; Cueto, M.; D'Croz, L.; Darias, J. *Org. Lett.* **2006**, *8*, 5001–5004. (d) Díaz-Marrero, A. R.; Cueto, M.; D'Croz, L.; Darias, J. *Org. Lett.* **2008**, *10*, 3057–3060.
- (5) Higgs, M. D.; Faulkner, J. *Steroids* **1977**, *30*, 379–388.
- (6) Ciavatta, M. L.; Gresa, M. P. L.; Manzo, E.; Gavagnin, M.; Wahidulla, S.; Cimino, G. *Tetrahedron Lett.* **2004**, *45*, 7745–7748.
- (7) Fabricius, K.; Alderslade, P. *Soft Corals and Sea Fans: A comprehensive guide to the tropical shallow water genera of the central-west Pacific, the Indian Ocean and the Red Sea*. Australian Institute of Marine Science: Townsville, Qld., 2001; pp 1–264.
- (8) Liyanage, G. K.; Schmitz, F. J. *J. Nat. Prod.* **1996**, *59*, 148–151.
- (9) Maia, L. F.; Epifanio, R. D.; Fenical, W. *J. Nat. Prod.* **2000**, *63*, 1427–1430.
- (10) Verbitski, S. M.; Mullally, J. E.; Fitzpatrick, F. A.; Ireland, C. M. *J. Med. Chem.* **2004**, *47*, 2062–2070.
- (11) Baker, B. J.; Okuda, R. K.; Yu, P. T. K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1985**, *107*, 2976–2977.
- (12) *PCModel* (v, 7.0); Serena Software: Bloomington, IN, 1999.
- (13) Caine, D.; Chu, C. Y.; Graham, S. L. *J. Org. Chem.* **1980**, *45*, 3790–3797.
- (14) Frei, J.; Ganter, C.; Kägi, D.; Kocsis, K.; Miljkovic, M.; Siewinski, A.; Wenger, R.; Schaffner, K.; Jeger, O. *Helv. Chim. Acta* **1966**, *49*, 1049–1105.
- (15) A slight difference is observed in the magnitude of the specific rotation of the natural ($[\alpha]_{\text{D}}^{20} -11.5$ (*c* 0.26, CH₂Cl₂)) and synthetic ($[\alpha]_{\text{D}}^{20} -31.3$ (*c* 0.80, CH₂Cl₂)) 1.
- (16) DellaGreca, M.; Fiorentino, A.; Pinto, G.; Pollio, A.; Previtiera, L. *J. Org. Chem.* **1996**, *61*, 7178–7179.
- (17) DellaGreca, M.; Previtiera, L.; Fiorentino, A.; Pinto, G.; Pollio, A. *Tetrahedron* **1996**, *52*, 13981–13990.
- (18) Schaffner, K. In *Advances in Photochemistry*; Noyes, W. A., Jr.; Hammond, G. S.; Pitts, J. N., Jr., Eds; Wiley & Sons, Inc.: New York, 1966; Vol. 4, pp 81–112.
- (19) Su, J.-H.; Lin, F.-Y.; Huang, H.-C.; Dai, C.-F.; Wu, Y.-C.; Hu, W.-P.; Hsu, C.-H.; Sheu, J.-H. *Tetrahedron* **2007**, *63*, 703–707.