Antitrypanosomal Cyclic Polyketide Peroxides from the Australian Marine Sponge *Plakortis* sp.

Yunjiang Feng, Rohan A. Davis, Melissa Sykes, Vicky M. Avery, David Camp, and Ronald J. Quinn*

Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia

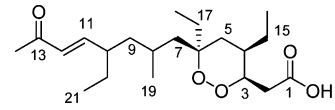
Received September 2, 2009

Bioassay-guided fractionation of the crude extract from the Australian marine sponge *Plakortis* sp. led to the isolation of two new cyclic polyketide peroxides, 11,12-didehydro-13-oxo-plakortide Q (**1**) and 10-carboxy-11,12,13,14-tetranor-plakortide Q (**2**). Antitrypanosomal studies showed that compound **1** had an IC_{50} value of 49 nM against *Trypanosoma brucei brucei*, and compound **2**, where a carboxylic acid is present in the side chain, had a 20-fold reduction of activity. 11,12-Didehydro-13-oxo-plakortide Q (**1**) is the most active peroxide isolated so far against *T. b. brucei*, and it indicates the potential therapeutic value of this class of compounds.

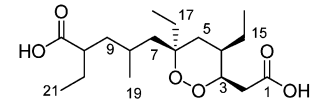
Human African trypanosomiasis (HAT), also known as African sleeping sickness, is a fatal disease caused by two subspecies of a protozoan parasite, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. According to the latest figure from the World Health Organization (WHO), African sleeping sickness threatens more than 60 million people in resource-poor regions of Africa and is the world's third most devastating parasitic disease.[1] Since the disease predominantly afflicts the very poor, it is designated as a neglected tropical disease. The four main drugs (suramin, pentamidine, melarsoprol, and eflorithine) currently used to combat this disease all have undesirable side-effects, and new strains of *T. brucei* are showing resistance to these agents.[2,3] There is an urgent need for the development of new, safer, and more effective drugs to fight African sleeping sickness.

The search for antitrypanosomal agents has predominantly focused on synthetic efforts. Using combinatorial chemistry approaches, a series of purine nitriles have shown potent trypanocidal activity and a high degree of selectivity,[4] and a small library of thiosemicarbazones was reported as trypanocidal by inhibiting the cysteine proteases of *T. brucei*.[5] Most recently, Sanofi-aventis and Drugs for Neglected Diseases initiative (DNDi) announced an agreement for the development, manufacture, and distribution of fexinidazole, a promising new drug for the treatment of African sleeping sickness.[6] Though natural product research has not played a central role in the search for antitrypanosomal therapeutics, there are emerging numbers of compounds from plants and marine organisms with promising activity against trypanosomiasis.[7–9]

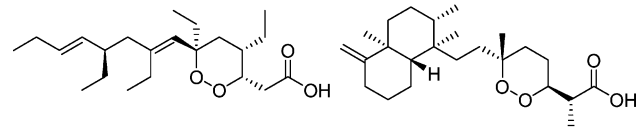
Given the tremendous chemical diversity present in natural products and the promising activity that has already been demonstrated,[7–9] a drug discovery program was established. A 384-well fluorescence-based trypanosomal high-throughput screening (HTS) assay was developed against *T. brucei brucei* and used to screen a prefractionated natural product library consisting of 202 983 fractions. *T. b. brucei* is routinely used in screening for identification of antitrypanosomal compounds that could be potentially developed into HAT drugs.[10] The library was constructed by fractionation of over 18 000 marine and terrestrial biota samples, with collection of 11 fractions each. From the 11 fractions derived from the Australian sponge *Plakortis* sp. a single active fraction (11 of 11) was identified. This fraction showed potent activity against *T. b. brucei* and desirable selectivity against a human embryonic kidney cell line, HEK293 (>10 fold). Bioassay-guided fractionation and purification of the extract afforded two new cyclic polyketide peroxides, 11,12-didehydro-13-oxo-plakortide Q (**1**) and 10-carboxy-11,12,13,14-tetranor-plakortide Q (**2**). In this paper we report the isolation and structural elucidation of the active principles **1** and **2**, along with their biological activity toward *T. b. brucei* and HEK293.



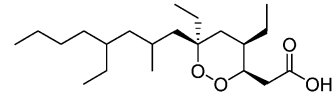
11,12-Didehydro-13-oxo-plakortide Q (**1**)



10-Carboxy-11,12,13,14-tetranor-plakortide Q (**2**)



Plakortide P (**3**) (-)-Sigmosceptrellin B (**4**)



Plakortide Q (**5**)

The freeze-dried and ground *Plakortis* sp. (5 g) was sequentially extracted with *n*-hexane, CH_2Cl_2, and MeOH. The CH_2Cl_2/MeOH extracts were combined and chromatographed using C_{18} bonded silica HPLC (MeOH/H_2O/0.1% TFA). NMR analysis of the active fractions led to two new cyclic polyketide peroxides, 11,12-didehydro-13-oxo-plakortide Q (**1**, 14.5 mg, 0.29% dry weight) and 10-carboxy-11,12,13,14-tetranor-plakortide Q (**2**, 7.0 mg, 0.15% dry weight).

Compound **1** was isolated as an optically active glassy solid. (−)-LRESIMS of **1** showed a strong $[M - H]^-$ ion at *m*/*z* 367. HRESIMS measurement on the $[M + Na]^+$ ion (*m*/*z* 391.2452), in combination with ^1H and ^{13}C NMR spectroscopic data (Table 1), supported the molecular formula of $C_{21}H_{36}O_5$ with four double-bond equivalents. The ^1H NMR spectrum of **1** showed signals due to two olefinic protons (δ_H 6.57 and 6.01), five methyl protons (δ_H 2.17, 0.83, 0.81, 0.78, and 0.74), and several methine and methylene groups. Twenty-one carbons were observed in the ^{13}C NMR experiment. In addition to the two olefinic carbons (δ_C 131.1 and 152.0), two carbonyl carbons (δ_C 172.6 and 197.7) were also

* To whom correspondence should be addressed. Tel: +61 7 37356000. Fax: +61 7 37356001. E-mail: r.quinn@griffith.edu.au.

10.1021/np900535z
Published on Web 03/17/2010

Table 1. NMR Data for Compounds **1** and **2**

	1					**2**		
	δ_C[a]		δ_H mult. (J in Hz)[a]			δ_C[a]	δ_H mult. (J in Hz)[a]	
position	d_6-DMSO	$CDCl_3$	d_6-DMSO	$CDCl_3$	HMBC[a]	d_6-DMSO	d_6-DMSO	HMBC[a]
1	172.6	175.1				172.7		
2	31.1	31.2	2.68, dd (15.6, 9.6) 2.27, dd (15.6, 4.2)	2.99, dd (16.0, 9.0) 2.40, dd (16.0, 3.5)	1, 3, 4	31.2	2.73, dd (16.2, 9.6) 2.34, dd (16.2, 3.6)	1, 3, 4
3	77.9	78.2	4.30, ddd (9.6, 4.5, 4.2)	4.46, ddd (9.0, 4.5, 3.5)	1, 2, 4, 5, 6	78.0	4.32, m	1, 4
4	33.8	34.4	2.04, m	2.14, m[b]	2, 3, 15, 16	33.8	2.07, m	3, 5, 15, 16
5	31.9	32.7	1.44, dd (13.8, 4.8), 1.22, m[b]	1.53, m[b], 1.25, m[b]	3, 4, 6, 15	31.4	1.46, m[b], 1.28, m[b]	3, 4, 6,
6	82.6	83.6				82.5		
7	44.2	45.1	1.27, m[b], 1.21, m[b]	1.32[b]	5, 6, 8, 17, 19	43.8	1.28, m[b], 1.21, m[b]	5, 6, 8, 9, 19
8	25.2	25.8	1.48, m	1.55, m[b]	6, 7, 19	25.8	2.20, m[b]	7
9	42.8	43.5	1.34, m[b], 1.18, m[b]	1.43, m[b], 1.22, m[b]	8, 10, 11, 19, 20	40.7	1.56, m[b], 1.10, m[b]	8, 11, 19
10	41.8	42.7	2.11, ddd (9.6, 4.8, 4.2)	2.13, m[b]	9, 11, 12, 20, 21	44.7	2.20, m[b]	8, 9, 21
11	152.0	152.5	6.57, dd (15.6, 9.6)	6.53, dd (15.6, 9.5)	9, 13, 20			
12	131.1	131.4	6.01, d (15.6)	6.07, d (15.6)	10, 13, 14			
13	197.7	198.7						
14	26.7	27.0	2.17, s	2.26, s	11, 12, 13			
15	24.3	25.1	1.14, m[b], 1.07, m	1.22, m[b], 1.16, m[b]	3, 4, 5, 16	24.3	1.18, m[b], 1.11, m[b]	3, 4, 5, 16
16	10.8	11.0	0.81, t (7.2)	0.93, t (7.0)	4, 15	10.8	0.85, t (7.2)	4, 15
17	25.2	25.5	1.81, dq (14.4, 7.2) 1.57, dq (14.4, 7.2)	1.91, dq (14.0, 7.0) 1.70, dq (14.0, 7.0)	5, 6, 7, 18	25.0	1.88, q (7.2), 1.58 m[b]	5, 6, 7, 18
18	7.7	8.0	0.74, t (7.8)	0.83, t (7.0)	6, 17	7.6	0.77, t (7.2)	6, 17
19	21.2	21.3	0.83, d (6.6)	0.89, d (7.0)	7, 8, 9	21.1	0.89, d (7.2)	7, 8, 9
20	27.4	28.1	1.39, m, 1.29, m[b]	1.45, m[b], 1.33, m[b]	9, 11, 21	31.2	1.47, m[b], 1.41, m[b]	9, 10, 11, 21
21	11.6	11.8	0.78, t (7.2)	0.87, t (7.5)	10, 20	11.7	0.83, t (7.2)	10, 20
10-COOH						176.9		

[a] Spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C using d_6-DMSO or $CDCl_3$ as solvents at 30 °C. [b] Signals were overlapping.

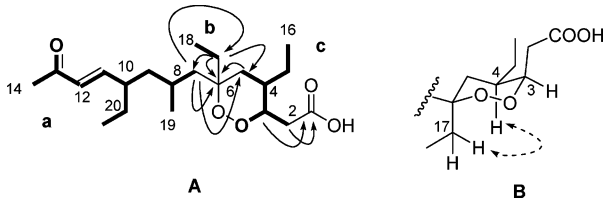


Figure 1. (A) Partial structures **a**, **b**, and **c** and key HMBC correlations and (B) key ROESY correlations for **1**.

observed. The presence of a conjugated methyl enone moiety in **1** was evident on the basis of the UV absorption at λ_{max} 225 nm and upon the observation of the carbon signals at δ_C 26.7, 197.7, 131.1, and 152.0.

Analysis of the ^{1}H and ^{13}C NMR spectroscopic data with the aid of gCOSY, HSQC, and gHMBC data (Table 1) confirmed the conjugated methyl enone moiety and established its connection to a 4-(2-methylhexane) group to form substructure **a** (Figure 1A in bold). The *trans*-configuration of the double bond was established by the large coupling constant of 15.6 Hz between the two olefinic protons. Further analysis of the COSY spectrum in combination with the HSQC spectroscopic data suggested the presence of an ethyl spin system **b** and a (2-oxo-3-ethyl)butyl spin system **c** (Figure 1A in bold).

The connectivities between substructures **a**, **b**, and **c** through an oxygenated quaternary carbon were established by detailed analysis of the HMBC correlations (Figure 1A). The HMBC correlations from the H-7 methylene protons (δ_H 1.21 and 1.27) in **a** to the carbons at δ_C 82.6 (C-6), 31.9 (C-5), and 25.2 (C-17) established the connectivities of substructures **a**, **b**, and **c** through the C-6 quaternary carbon. This connectivity was confirmed by the correlations from the H-17 methylene protons (δ_H 1.57 and 1.81) in **b** to the carbons at δ_C 82.6 (C-6), 44.2 (C-7), and 31.9 (C-5). It was also supported by the correlations from the H-5 methylene protons (δ_H 1.22 and 1.44) in **c** to the carbon at δ_C 82.6 (C-6). Additional HMBC correlations were observed from the H-3 methine proton (δ_H 4.30) and H-2 methylene protons (δ_H 2.68 and 2.27) in **c** to a carbonyl carbon at δ_C 172.6, indicating the attachment of a carboxylic acid group to substructure **c**. Finally, the formation of the peroxide ring was established on the basis of the molecular formula, double-bond equivalents, and the carbon chemical shifts at C-3 and C-6 (δ_C 78.1 and 82.6). Therefore the planar structure was established as **1**.

The relative configuration of the peroxide ring in plakortide Q (**5**) and analogues can be established on the basis of the carbon chemical shifts of the methylene carbons at C-2, C-15, and C-17.[11,12] The acetic acid substituent at C-3 occupies an equatorial or an axial position when C-2 resonates above δ_C 36 or below δ_C 32, respectively. In a similar manner, the ethyl substituent at the C-6 position has an equatorial or an axial position when C-17 resonates above δ_C 30 or below δ_C 27, respectively. The ethyl substituent at C-4 occupies an equatorial position with methylene C-15 resonating near δ_C 25, and no axial position for this substituent has been reported in the literature. Comparison of the ^{13}C NMR data of compound **1** (in $CDCl_3$, Table 1) with the literature values determined that **1** had the same relative configuration as that of plakortide Q with an axial acetic acid substituent at C-3 (C-2, δ_C 31.2), an axial ethyl substituent at C-6 (C-17, δ_C 25.5), and an equatorial ethyl substituent at C-4 (C-15, δ_C 25.1). Further support for the relative configuration of **1** was obtained from a ^{1}H NMR selective decoupling experiment in $CDCl_3$ and ROESY data (Figure 1B). Irradiation of H-2 at δ_H 2.40 simplified the signal of H-3 at δ_H 4.46 to a doublet of doublets (J = 4.5 and 9.0 Hz). The $^3J_{H\text{-}3/H\text{-}4}$ coupling constant of 4.5 Hz was in agreement with a $^3J_{H\text{-}3/H\text{-}4}$ equatorial/axial coupling. A ROESY correlation was also observed from H-4 to the H-17 methylene proton (δ_H 1.81), which confirmed the axial orientation of H-4 and ethyl substituent at the C-6 position. With the relative configuration about the peroxide ring determined, compound **1** was assigned as 11,12-didehydro-13-oxo-plakortide Q.

The minor metabolite **2** was also obtained as an optically active glassy solid. Comparison of the ^{1}H and ^{13}C NMR data (Table 1)

suggested that **2** had a similar structure to **1**. The only difference was that the conjugated methyl enone moiety in **1** was replaced by a carboxylic acid in **2**. This was supported by the HMBC correlation from the H-20 methylene protons (δ_H 1.47 and 1.41) to the carboxylic acid carbon at δ_C 176.9. HRESIMS measurement supported the planar structure **2**. Comparison of the ^{13}C NMR data of **1** and **2** suggested that compound **2** had the same relative configuration around the cyclic peroxide as that of **1**. Hence, compound **2** was assigned as 10-carboxy-11,12,13,14-tetranor-plakortide Q.

The antitrypanosomal activity of **1** and **2** was evaluated *in vitro* against *T. b. brucei*. Compound **1** exhibited potent activity against *T. b. brucei* with an IC_{50} value of 49 nM. Replacement of the enone with a carboxylic acid group in the side chain resulted in the reduction of the activity by almost 20-fold, with **2** showing an IC_{50} of 940 nM. A preliminary cytotoxicity study indicated that **1** had an IC_{50} value of 5.1 μM against the human embryonic kidney cell line HEK293, showing a 105-fold selectivity for antitrypanosomal activity, while **2** showed 100% inhibition of HEK293 at 83 μM.

Cycloperoxides have been frequently isolated from marine sponges of the genus *Plakortis* sp.[13] This class of compounds structurally differs in the ring size (five- or six-membered), in the length and the functionalization of the carbon backbone, and in the configurations of the chiral centers.[13] Other marine sponges such as *Diacarnus* sp.[9,14] and *Latrunculia* sp.[15,16] have also yielded cycloperoxides with mono-, bi-, or tricyclic substituents in the carbon backbone. A variety of biological activities have been reported for cycloperoxides including antimicrobial,[17] antimalarial,[18] and anticancer.[11,19,20] More recently a number of cycloperoxides including plakortide P (**3**)[8] and (−)-sigmosceptrellin B (**4**)[9] have been reported to possess antitrypanosomal activity. Plakortide P (**3**) was first isolated from the Caribbean marine sponge *Plakortis halichondrioides*[21] and has subsequently been isolated from the Brazilian marine sponge *Plakortis angulospiculatus* and shown to have potent antiparasitic activity against *T. cruzi* and *Leishmania chagasi*.[8] (−)-Sigmosceptrellin B (**4**) along with a number of other cycloperoxides were identified from the Papua New Guinean marine sponge *Diacarnus bismarckensis*.[9] An activity study suggested that compound **4** had an IC_{50} value of 0.2 μg/mL (510 nM) against *T. brucei*.[9] 11,12-Didehydro-13-oxo-plakortide Q (**1**) isolated in this study had an IC_{50} of 49 nM against *T. b. brucei* and is the most active cyclic polyketide peroxide against *T. b. brucei*. This result, along with other literature reports, identifies the cyclic peroxide structure class as potential leads for development of antitrypanosomal drugs.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco P-1020 polarimeter. IR and UV spectra were recorded on a Bruker Tensor 27 spectrometer and an Agilent 8453 UV/vis spectrophotometer, respectively. NMR spectra were recorded at 30 °C on either a Varian 500 or 600 MHz Unity INOVA spectrometer. The latter spectrometer was equipped with a triple-resonance cold probe. The ^1H and ^{13}C chemical shifts were referenced to the solvent peak for DMSO-d_6 at δ_H 2.49 and δ_C 39.5, and $CDCl_3$ at δ_H 7.26 and δ_C 77.0. LRESIMS were recorded on a Waters ZQ mass spectrometer. HRESIMS were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. A Bioline orbital shaker was used for large-scale sponge extractions. Alltech Davisil 40−60 μm 60 Å C_{18} bonded silica was used for preadsorption work. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. A ThermoElectron C_{18} Betasil 5 μm 143 Å column (21.2 mm × 150 mm) was used for semipreparative HPLC separations. All solvents used for chromatography, UV, $[\alpha]_D$, and MS were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered. *T. b. brucei* BS427 cells were kindly supplied by Dr. Achim Schnaufer (University of Edinburgh), while at the Seattle Biomedical Research Institute. HEK293 was purchased from the American Type Culture Collection (ATCC).

Animal Material. The sponge *Plakortis* sp. was collected at Ribbon Reef, Queensland, Australia, by scuba diving (−24 m) during November 2001. It was kept frozen prior to freeze-drying and extraction. A voucher sample (G319109) has been lodged at the Queensland Museum, Brisbane, Australia. The sponge has a massive and bulbous growth form. It is mottled gray-brown alive and stains a beige exterior and interior in EtOH. It contains several oscules (5 mm) with sieve plates scattered over the surface. The sponge has a rubbery, but compressible texture. The surface ornamentation is smooth. The ectosomal skeleton is not distinctive from choanosome. The choanosomal skeleton contains a densely packed criss-cross of thin, centroangulate oxeas. Megasclere details include oxeas, 100−120 μm. Microsclere details include diods, 10 μm.

Extraction and Isolation. The freeze-dried and ground sponge (5 g) was poured into a conical flask (1 L), *n*-hexane (250 mL) was added, and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity then discarded. CH_2Cl_2/MeOH (4:1, 250 mL) was added to the defatted sponge material in the conical flask and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity and set aside. MeOH (250 mL) was added, and the MeOH/sponge mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the biota was extracted with another volume of MeOH (250 mL) while being shaken at 200 rpm for 16 h. All CH_2Cl_2/MeOH extracts were combined and dried under reduced pressure to yield a dark brown solid (1.19 g). A portion of this material (1.0 g) was preadsorbed to C_{18}-bonded silica (1 g), then packed into a stainless steel cartridge (10 × 30 mm) that was subsequently attached to a C_{18} semipreparative HPLC column. Isocratic HPLC conditions of 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for the first 10 min; then a linear gradient to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from the start of the HPLC run, and fractions 55 and 52 afforded 11,12-didehydro-13-oxo-plakortide Q (**1**, 14.5 mg, 0.29% dry weight) and 10-carboxy-11,12,13,14-tetranor-plakortide Q (**2**, 7.0 mg, 0.15% dry weight), respectively.

11,12-Didehydro-13-oxo-plakortide Q (1): colorless, glassy solid; $[\alpha]^{28}_D$ −83 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.69) and 225 (3.39) nm; IR ν_{max} (KBr) 3434 (br), 2963, 2932, 1639, 1462, 1380, 1255, 1202, 985, 755, 665 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* 391.2452 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_5Na$, 391.2455).

10-Carboxy-11,12,13,14-tetranor-plakortide Q (2): colorless, glassy solid; $[\alpha]^{28}_D$ −51 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.32) nm; IR ν_{max} (KBr) 3428, 2964, 2935, 2878, 1712, 1462, 1381, 1202, 756, 665, 596 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* 367.2104 $[M + Na]^+$ (calcd for $C_{18}H_{32}O_6Na$, 367.2091).

Trypanosome Assay. *T. b. brucei* BS427 cells were maintained in log phase growth in 25 cm^2 tissue culture flasks (Corning) by subculturing at either 24 or 48 h intervals. Cells were grown in complete HMI-9 medium,[22] supplemented with 10% FCS and 100 IU/mL penicillin/streptomycin with incubation at 5% CO_2 at 37 °C in humidified conditions. Cells were diluted to 2000 cells/mL in HMI-9 media, and 55 μL/well was added to a black/clear-bottomed 384-well lidded plate with a Multidrop liquid handler under sterile conditions. Plates were incubated for 24 h at 37 °C and 5% CO_2 prior to addition of 5 μL of prediluted compounds/DMSO for control wells. Compounds in 100% DMSO or 100% DMSO for controls were prediluted 1:20 in high-glucose DMEM medium with L-glutamine supplemented with 1 × nonessential amino acids (NEAA) and 1 mM sodium pyruvate without FCS, by a Minitrack robotic liquid handler. The final DMSO concentration in the assay was 0.417%. Plates were incubated for a further 48 h at 37 °C in 5% CO_2 before the addition of 10 μL of Alamar Blue to each well (diluted in the HMI-9 medium supplemented with 10% FCS) to a final concentration of 10% in the assay. The plate was then incubated for 2 h under the same conditions before incubation for a further 22 h, light protected, at room temperature. Wells were read at excitation 535 nm, emission 590 nm on a Victor II Wallac plate reader. Reference drugs used in the assay were pentamidine, diminazene aceturate, and puromycin.

HEK293 Assay. HEK293 cells were maintained in a growth media comprised of high-glucose DMEM with L-glutamine, supplemented with 1 × NEAA and 1 mM sodium pyruvate. Growth conditions were in 5% CO_2 at 37 °C, under humidified conditions. Cells at 80% confluence were harvested and diluted in the growth media to 7.27 × 10^4 cells/

mL. A 55 μL amount per well of diluted cells was added to a black/clear-bottomed 384-well lidded plate with a multidrop liquid handler under sterile conditions. Incubation times, compound additions, and plate read were as per the trypanosome viability assay, except that the Alamar Blue was diluted in HEK293 growth media before addition. The reference drug used was puromycin.

Acknowledgment. Financial support from DNDi and Deutsche Gesellschaft für Technische Zusammenarbeit GmbH on behalf of the Government of the Federal Republic of Germany is acknowledged. We thank H. T. Vu for acquiring the HRESIMS measurements and C. Lewis and K. Watts for their assistance in the preparation of the screening library. We thank J. N. A. Hooper from the Queensland Museum for sponge collection and identification. We also thank the reviewers of this paper for valuable contributions to the assignment of the relative configuration.

Supporting Information Available: ^{1}H and ^{13}C NMR data for **1** and **2** and a photograph of the sponge *Plakortis* sp. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) World Health Organization, http://www.who.int/mediacentre/factsheets/fs259/en/.
(2) Milord, F.; Pepin, J.; Loko, L.; Ethier, L.; Mpia, B. *Lancet* **1992**, *340*, 652–655.
(3) Mpia, B.; Pepin, J. *Trop. Med. Int. Health* **2002**, *7*, 775–779.
(4) Mallari, J. P.; Shelat, A. A.; Obrien, T.; Caffrey, C. R.; Kosinski, A.; Connelly, M.; Harbut, M.; Greenbaum, D.; McKerrow, J. H.; Guy, R. K. *J. Med. Chem.* **2008**, *51*, 545–552.
(5) Mallari, J. P.; Shelat, A. A.; Kosinski, A.; Caffrey, C. R.; Connelly, M.; Zhu, F.; McKerrow, J. H.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2883–2885.
(6) Drugs for Neglected Disease Initiative, http://www.dndi.org.
(7) Salem, M. M.; Werbovetz, K. A. *Curr. Med. Chem.* **2006**, *13*, 2571–2598.
(8) Kossuga, M. H.; Nascimento, A. M.; Reimao, J. Q.; Tempone, A. G.; Taniwaki, N. N.; Veloso, K.; Ferreira, A. G.; Cavalcanti, B. C.; Pessoa, C.; Moraes, M. O.; Mayer, A. M. S.; Hajdu, E.; Berlinck, R. G. S. *J. Nat. Prod.* **2008**, *71*, 334–339.
(9) Rubio, B. K.; Tenney, K.; Ang, K.; Abdulla, M.; Arkin, M.; McKerrow, J.; Crews, P. *J. Nat. Prod.* **2009**, *72*, 218–222.
(10) Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. *Nat. Rev. Drug Discovery* **2005**, *4*, 727–740.
(11) Berrue, F.; Thomas, O. P.; Funel-Le Bon, C.; Reyes, F.; Amade, P. *Tetrahedron* **2005**, *61*, 11843–11849.
(12) Harrison, B.; Crews, P. *J. Nat. Prod.* **1998**, *61*, 1033.
(13) *Dictionary of Natural Products*; Chapman and Hall/CRC: U.K., 2009; version 17:2.
(14) D'Ambrosio, M.; Guerriero, A.; Deharo, E.; Debitus, C.; Munoz, V.; Pietra, F. *Helv. Chim. Acta* **1998**, *81*, 1285–1292.
(15) He, H.; Faulkner, D. J. *J. Org. Chem.* **1991**, *56*, 2112–2115.
(16) Capon, R. J.; MacLeod, J. K.; Willis, A. C. *J. Org. Chem.* **1987**, *52*, 339–342.
(17) Lim, C. W.; Cha, Y. J.; Kim, J. S. *J. Fish. Sci. Technol.* **2005**, *8*, 6–9.
(18) Fattorusso, E.; Parapini, S.; Campagnuolo, C.; Basilico, N.; Taglialatela-Scafati, O.; Taramelli, D. *J. Antimicrob. Chemother.* **2002**, *50*, 883–888.
(19) Fattorusso, E.; Taglialatela-Scafati, O.; Di Rosa, M.; Ianaro, A. *Tetrahedron* **2000**, *56*, 7959–7967.
(20) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1993**, *56*, 1827–30.
(21) Jimenéz, M. del S.; Garzón, S. P.; Rodríguez, A. D. *J. Nat. Prod* **2003**, *66*, 655–661.
(22) Hirumi, H.; Hirumi, K. *J. Parasitol.* **1989**, *75*, 985–989.

NP900535Z