JOC The Journal of Organic Chemistry

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Bastimolide A, a Potent Antimalarial Polyhydroxy Macrolide from the Marine Cyanobacterium *Okeania hirsuta*

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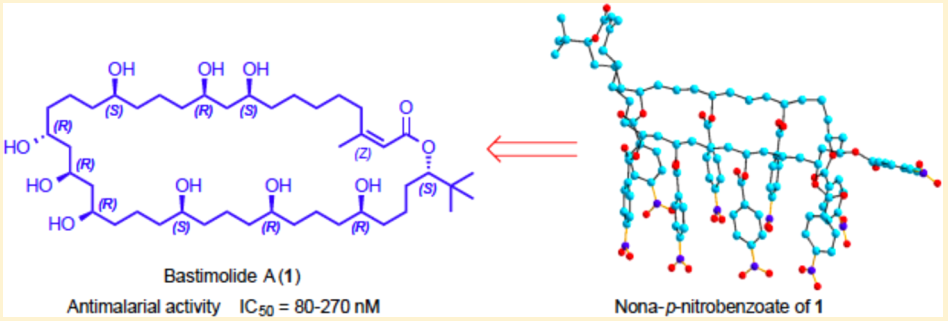
Supporting Information



ABSTRACT: Bastimolide A (**1**), a polyhydroxy macrolide with a 40-membered ring, was isolated from a new genus of the tropical marine cyanobacterium *Okeania hirsuta*. This novel macrolide was defined by spectroscopy and chemical reactions to possess one 1,3-diol, one 1,3,5-triol, six 1,5-diols, and one *tert*-butyl group; however, the relationships of these moieties to one another were obscured by a highly degenerate ^{1}H NMR spectrum. Its complete structure and absolute configuration were therefore unambiguously determined by X-ray diffraction analysis of the nona-*p*-nitrobenzoate derivative (**1d**). Pure bastimolide A (**1**) showed potent antimalarial activity against four resistant strains of *Plasmodium falciparum* with IC_{50} values between 80 and 270 nM, although with some toxicity to the control Vero cells (IC_{50} = 2.1 μM), and thus represents a potentially promising lead for antimalarial drug discovery. Moreover, rigorous establishment of its molecular arrangement gives fresh insight into the structures and biosynthesis of cyanobacterial polyhydroxymacrolides.

INTRODUCTION

Globally, an estimated 3.2 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk, mostly children under five years of age.[1] Fifty years ago, malaria had been eliminated from many areas of the world through effective antimalarial drug treatments, vector control interventions, and disease prevention.[2] However, by the 1980s, the global spread of drug resistance resulted in a substantial increase in disease incidence and mortality.[3,4] Over the last three decades, *Plasmodium falciparum* has developed resistance to all classes of antimalarial drugs and is responsible for a recent increase in malaria-related mortality.[4] Recent reports of increased tolerance to artemisinin derivatives, the last widely

Received: June 4, 2015
Published: July 29, 2015

DOI: 10.1021/acs.joc.5b01264
J. Org. Chem. 2015, 80, 7849−7855

effective class of antimalarials, bolster the medical need for new treatments.[5] Thus, a pressing need exists for the development of new and more efficacious drugs to treat malaria, and it is with this aim that the Panama International Cooperative Biodiversity Group (ICBG) investigated Panamanian microorganisms for antimalarial lead compounds.[6]

In the current research, we report the isolation, structure elucidation, and antimalarial activity of the new macrolide, bastimolide A (**1**, Figure 1), isolated from the cyanobacterium

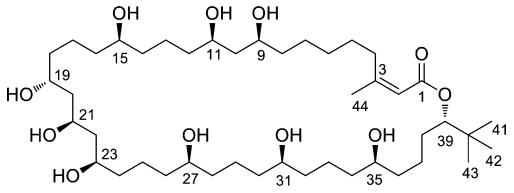


Figure 1. Structure of bastimolide A (**1**).

Okeania hirsuta collected from the Caribbean coast of Panama.[7] Bastimolide A (**1**) showed potent nanomolar level antimalarial activity against four resistant strains of *P. falciparum*.

RESULTS AND DISCUSSION

A freeze-dried sample of cyanobacterium *O. hirsuta* collected from near the Isla Bastimentos Park, Panama, in 2011 was repeatedly extracted with CH_2Cl_2/MeOH (2:1), and a portion of the resulting crude extract (12.0 g) was fractionated by silica gel vacuum liquid chromatography (VLC) to produce nine subfractions (A–I). Fraction I (5.05 g) displayed intriguing ^{1}H NMR resonances and was thus subjected to ^{1}H NMR guided fractionation involving gradient silica gel column chromatography, reversed-phase C18 column, and reversed-phase HPLC. The new compound, bastimolide A (**1**), was isolated from this process in 0.64% yield (76.8 mg).

Bastimolide A (**1**) was obtained as an optically active white powder. Its molecular formula of $C_{44}H_{84}O_{11}$ (three degrees of unsaturation) was determined by HRESITOFMS and NMR data (Table 1). The IR absorption band at 1747 cm^{-1} suggested the presence of an ester group, which was supported by the presence of one carbonyl signal at δ_C 166.9 in the ^{13}C NMR spectrum. The upfield shift of this ester carbonyl, combined with UV absorption at 216 nm, suggested α,β-unsaturation, and by HMBC along with ^{1}H NMR analysis, a methyl group (δ_H 1.82, δ_C 25.4) was located at the β-position to define a trisubstituted olefin. Considering the molecular formula and remainder of the NMR data, the structure of **1** was thus suggested to contain a single ring.

Initial ^{1}H NMR analysis (Supporting Information) of bastimolide A (**1**) in DMSO-d_6 identified the most intriguing resonance as an intense singlet at δ_H 0.96 (nine protons) and was attributed to three isochronous methyl groups comprising a *tert*-butyl moiety. Nine exchangeable protons at δ_H 4.20–4.48 were assigned as hydroxy protons due to lack of correlation with carbon atoms by HSQC. Correspondingly, 10 carbon signals were observed at δ_C 66.0–77.8, and these correlated with nine protons on oxygenated carbons at δ_H 3.34–3.77 along with one more downfield resonance δ_H 4.65 and thus began to define bastimolide A (**1**) as a polyhydroxy macrolide. In addition to the above 10 methines and four methyl groups, the NMR spectra of **1** contained 26 methylenes and one quaternary carbon and thus accounted for all of the atoms defined in the $C_{44}H_{84}O_{11}$ molecular formula. However, 50 methylene protons were significantly overlapped between δ1.15–1.55, and this greatly impeded assembly of the planar structure of compound **1**.

Nevertheless, changing the solvent to pyridine-d_5 and employing 800 MHz NMR improved the dispersion between the various NMR signals (Table 1). Analysis of 1D and 2D NMR spectra (COSY, TOCSY, ROESY, HSQC, and HMBC) under these conditions allowed deduction of six partial structures (I–VI, Figure 2) and again accounted for all of the atoms in the molecular formula. As noted above, HMBC correlations of the lone olefinic proton H-2 to C-1/C-3/C-4/C-44 and H-44 to C-2/C-3/C-4 allowed formation of the trisubstituted α,β-unsaturated lactone moiety. Moreover, HMBC correlations from H-4 to C-2/C-3/C-44 and from H-2 and H-44 to C-4 allowed extension of substructure I to include the TOCSY- and COSY-derived sequence C-4–C-11. Similarly, substructure II, which included the *tert*-butyl group, lactone methine, adjacent three methylenes, and a distal secondary alcohol, was identified by a combination of TOCSY and HMBC correlations as shown in Figure 2. Partial structures I and II could be connected through key HMBC correlations between H-39 at δ_C 5.09 and the ester carbonyl carbon at δ_C 166.9 as well as the olefin carbon C-2 at δ_C 117.3. The downfield shifts of H/C-39 (Table 1) were consistent with this assembly of atoms.

One 1,3,5-triol group could be assembled by successive TOCSY correlations between H19/H20/H21/H22/H23 (partial structure III). Five 1,5-diol relationships were indicated by a cluster of almost identical chemical shifts for 10 methylene carbon signals at δ 38.6 plus five at δ 23.3 as well as seven oxygen-bearing methine signals at δ 68.8–72.0, three of which resonated at δ 71.2. Due to the significantly overlapped proton and carbon spectra, plus the numerous remote stereocenters in bastimolide A, NMR methods were considered insufficient to unequivocally establish its complete structure. Additionally, neither ESI-MS^3 (positive mode) nor ESI-MS^4 (negative mode) fragmentation data gave structurally useful information for bastimolide A (**1**) (Supporting Information).

Nevertheless, we explored the relative configurations of the 1,3-diol and 1,3,5-triol groups using a combination of methods, including the NMR features of the corresponding acetonide derivatives, Kishi's Universal NMR Database, and the equivalence or nonequivalence of methylene proton chemical shifts in these diol and triol systems. The acetonide methyl group ^{13}C NMR chemical shifts for 1,3-diols are highly informative of relative configuration.[8] For the *syn*-acetonide (chair form with a lower strain energy), these appear around 20 or 30 ppm depending on their axial or equatorial orientation, whereas for those of an *anti*-acetonide (the skew form yields identical methyl groups of higher strain energy) both are near 25 ppm. Treatment of **1** with 2,2-dimethoxypropane and *p*-toluenesulfonic acid afforded two monoacetonides (**1a** and **1b**) and one bis-acetonide (**1c**) (Scheme 1). Because the observed acetonide methyl groups were all located at either 20.3 or 30.6 ppm in bis-acetonide **1c**, the diols at C-9/C11 and C21/C23 were both assigned the *syn* configuration. The fact that acetonide derivatization did not occur at C19/C21, presumably due to greater steric hindrance at this position, led to the deduction of *anti* configuration for the C19/C21 diol.

The relative configurations of the 1,3-diol and 1,3,5-triol moieties **1** were also independently assigned using Kishi's Universal NMR Database (Supporting Information). The ^{13}C NMR chemical shifts of C-9/C-11 were in good agreement

DOI: 10.1021/acs.joc.5b01264
J. Org. Chem. 2015, 80, 7849–7855

Table 1. NMR Data for Bastimolide A (1) (δ) (ppm) (Multiplicity, *J*, Hz)

position	^{13}C[a]	^{1}H[a]	HMBC	TOCSY
1	166.9			
2	117.3	5.89, s	1, 3, 4, 44	44
3	160.9			
4	33.9	2.62, ddd, 12.0, 8.0, 8.0	1, 2, 3, 6, 44	5, 6, 7
		2.96, ddd, 12.0, 8.0, 8.0	1, 2, 3, 6, 44	5, 6, 7
5	29.0	1.54, quintet, 8.0	3, 4, 6, 7	4, 6, 7, 8
6	30.5	1.41, m	4, 5, 7, 8	4, 5, 7, 8, 9
		1.46, m	4, 5, 7, 8	4, 5, 7, 8, 9
7	26.3	1.69[b]		4, 5, 6, 9
		1.57[b]		
8	39.2	1.70[b]		5, 6, 9
		1.65[b]		
9	72.0	4.17, brs		5, 6, 7, 8, 10, 9-OH
10	45.1	1.91[b]		9, 11, 9-OH, 11-OH
		1.84[b]		
11	72.2	4.23, brs		10, 12, 13, 9-OH, 11-OH
12	39.4	1.81[b]		11
13	23.6	2.10[b]		11, 15
14	39.2	1.78[b]		
15	71.6	3.93[b]		13
16	39.1	1.78[b]		
17	22.7	1.78[b]		
18	39.0	1.93[b]		
		1.82[b]		
19	68.8	4.45, brs		18, 20, 21, 22, 19-OH
20	45.1	2.07, t, 2.6		18, 19, 21, 23, 19-OH, 21-OH
21	69.4	4.76, brs		19, 20, 22, 23, 24, 21-OH
22	45.4	2.03, ddd, 13.6, 6.0, 4.0		19, 20, 21, 23, 24, 21-OH, 23-OH
		2.13[b]		
23	71.2	4.28, brs		19, 20, 21, 22, 23-OH
24	39.0	1.90[b]		21, 22
		1.79[b]		
25	22.5	1.78[b]		
26	39.0	1.78[b]		
27	71.2	3.96[b]		
28	38.9	1.78[b]		
29	23.3	1.78[b]		
30	38.8	1.78[b]		
31	71.2	3.96[b]		
32	38.6	1.78[b]		
33	23.3	2.13,[b] 1.97[b]		35
34	38.6	1.76[b]		
35	71.1	3.90, brd		33, 39
36	38.6	1.69[b]		
		1.76[b]		
37	22.9	1.76,[b] 1.80[b]		
38	30.6	1.67−1.69		
39	79.9	5.09, dd, 10.4, 2.4 Hz	1, 2, 40, 41−43	35
40	35.1			
41−43	26.5	0.96, s	39, 40	
44	25.4	1.82, s	2, 3, 4	
9-OH		6.14[b]		9
11-OH		6.19, d, 3.2		11
19-OH		5.99, d, 3.2		18, 19, 20, 22
21-OH		6.25, d, 2.4		19, 21, 20, 23, 19-OH
23-OH		6.14[b]		21, 23
15, 27, 31, 35-OH		5.71−5.76[b]		

[a]800 MHz for ^{1}H NMR and 200 MHz for ^{13}C NMR in pyridine-d_5. [b]Signal overlapped with other signals.

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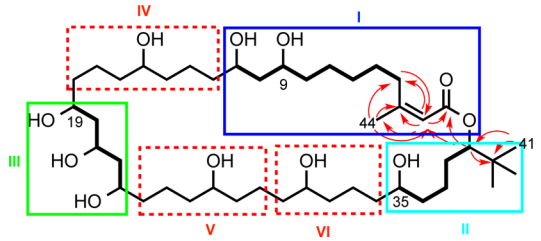


Figure 2. Key correlations deduced from COSY (bold bonds) and HMBC data (red curves) leading to partial structures (I–VI) of bastimolide A (**1**).

with *syn* arrangement of the 1,3-diol model system. The 1,3,5-triol system was assigned as either *anti*/*syn* or *syn*/*anti* between C-19/C-21/C-23 on the basis of comparison of the ^{13}C NMR shift at C-21 with the characteristic central carbon of model systems.[9]

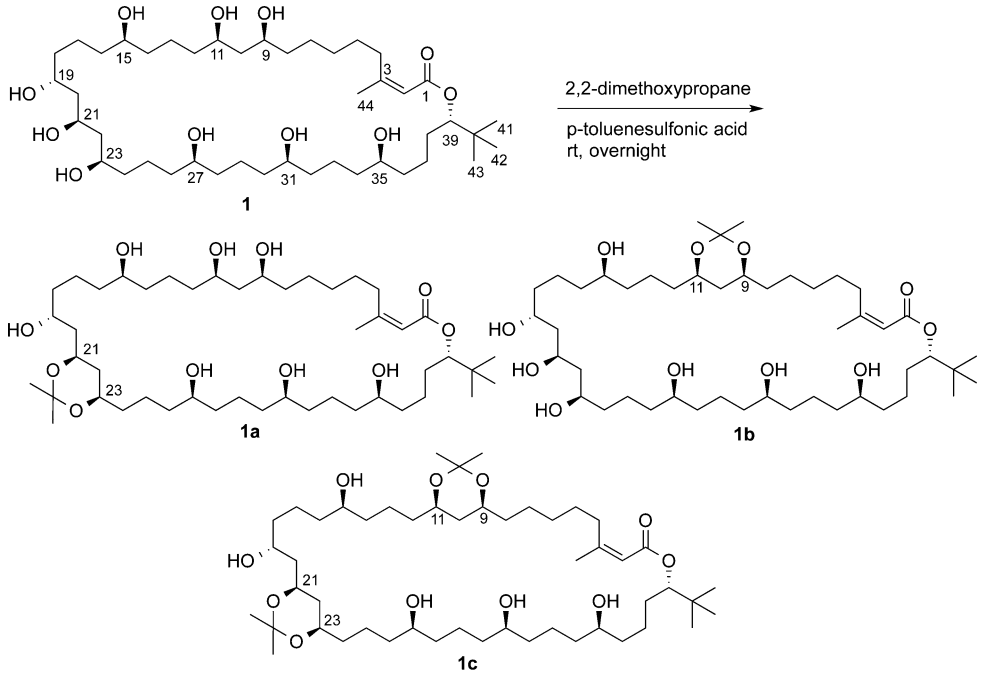
Additionally, the chemical shifts of the two protons at H_2-10 as well as those at H_2-22 were separated by 0.07 and 0.10 ppm, respectively. This contrasted with those at H_2-20, which were isochronous (Table 1). The nonequivalent methylene protons result from the deshielding effects from two proximate *syn* configured oxygen atoms, whereas the magnetically equivalent methylene protons (H_2-20) are equally situated relative to the two antioriented oxygen atoms.[10] As a result of these three analyses, the relative configurations of the 1,3-diol (C-9/C-11) and 1,3,5-triol (C-19/C-21/C-23) were assigned *syn* and *anti*/*syn*, respectively.

The geometry of the C-2–C-3 double bond was defined using a selective 1D NOE experiment (Supporting Information). Irradiation of H-2 resulted in a strong enhancement of H_3-44, indicating a *Z* configured olefin. The *Z*-geometry was additionally supported from the downfield chemical shift of the C-44 methyl carbon at δ_C 25.4.[11]

Whereas a number of conceptual approaches were examined for attempting to relate the chirality at the seven remote sites of stereochemistry, none were successful, and thus, we attempted to crystallize bastimolide A as well as several derivatives for a single-crystal X-ray diffraction study. Due to the viscous nature of concentrated solutions of bastimolide A (**1**) in various solvents, it was not possible to form crystals of the underivatized compound. Thus, a number of different per-esters were explored for their propensity to form crystals, and ultimately, we discovered that the nona-*p*-nitrobenzoate derivative (**1d**) formed well-ordered crystals (Scheme 2). Crystals were prepared by slowly diffusing hexane into an acetone solution of the nona-*p*-nitrobenzoate derivative. Over a 3-week period, tiny single crystals (0.28 × 0.22 × 0.08 mm, ca. 6.63 μg) of **1d** formed that were suitable for X-ray diffraction analysis. As a result, the planar and stereostructure of **1d** were firmly established as shown in Figure 3 with the absolute configuration of 9*S*,11*R*,15*S*,19*R*,21*R*,23*R*,27*S*,31*R*,35*R*,39*S* through refinement of Flack's parameter [x = 0.1(4)].[12]

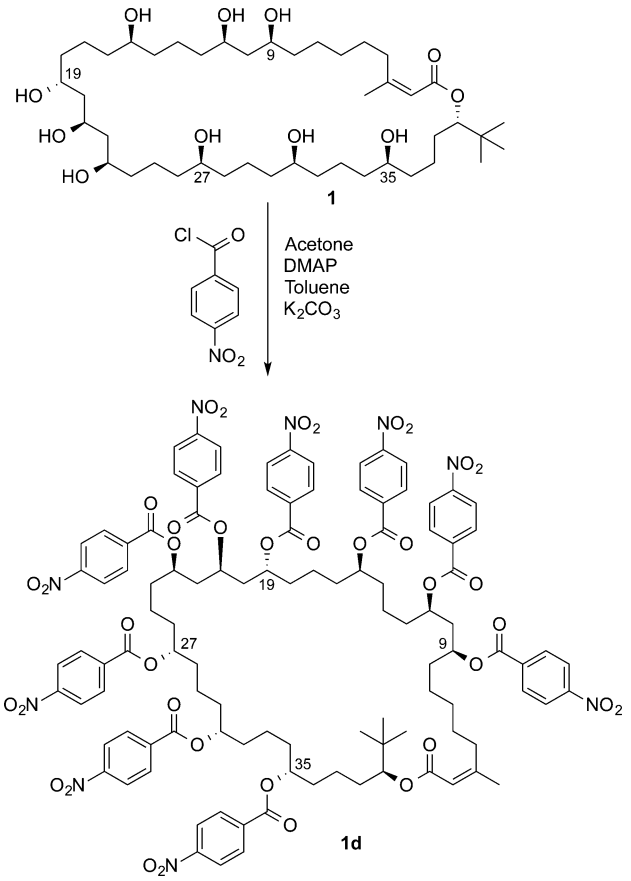
Bastimolide A is the first new secondary metabolite to be reported from the new genus of cyanobacterium described as *Okeania*.[7] Its structure is unique for several reasons, including the large 40-membered macrolactone ring and the repeating occurrence of six 1,5-diol groups. This type of structural architecture has only been previously reported for two other cyanobacterial natural products, caylobolides A and B, each possessing a 36-membered ring, which were reported from the cyanobacterium *Lyngbya majuscula*[13] and a mixture of two cyanobacteria, *L. majuscula* and *Phormidium* spp.,[14] respectively. In this latter case, however, the location of functional groups about the macro-ring were located relative to one another by NMR methods and are quite different from those found for bastimolide A by crystallographic data. Further, most of the configurational aspects of the caylobolides were unresolved. Another notable feature of bastimolide A is the occurrence of a *tert*-butyl group moiety, which is quite rare among natural products, although it is seen in other marine cyanobacterial metabolites such as the apratoxins.[15] The biosynthesis of *tert*-butyl groups remains unstudied at a biochemical level, although

Scheme 1. Preparation of Acetonide Derivatives 1a–c from Bastimolide A (1)



DOI: 10.1021/acs.joc.5b01264
J. Org. Chem. 2015, 80, 7849–7855

Scheme 2. Preparation of the Nona-*p*-nitrobenzoate Derivative 1d of Bastimolide A (1)



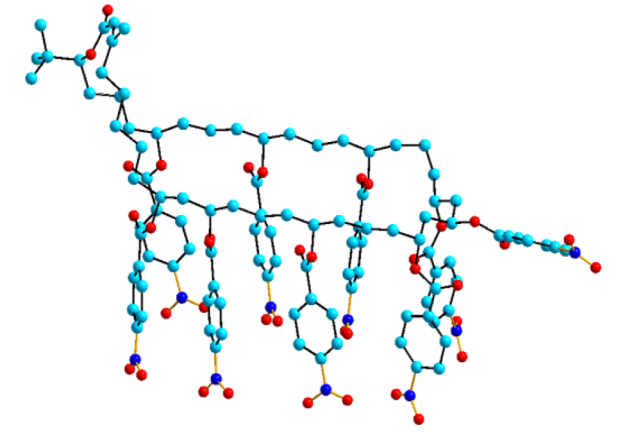


Figure 3. Perspective drawing of the nona-*p*-nitrobenzoate derivative **1d** of bastimolide A (**1**). Hydrogen atoms are omitted for clarity.

bioinformatic analysis of the apratoxin cluster suggests that the three methyl groups may be added from *S*-adenosyl methionine (SAM) to a suitably activated and tethered acetate unit.[16] The occurrence of a sterically bulky and hydrophobic *tert*-butyl group adjacent to the lactone ester in bastimolide may protect it from hydrolysis, as was found for palmyrolide A.[17]

A major focus of the Panama ICBG program has been the discovery of new classes of agents for the treatment of neglected tropical diseases such as malaria, leishmaniasis, and Chagas disease.[18,19] Thus, bastimolide A (**1**) was evaluated against a panel of these various human parasites and, remarkably, showed highly potent and selective antimalarial activity against four resistant strains of *Plasmodium falciparum* [TM90-C2A (chloroquine, mefloquine, and pyrimethamine resistant), TM90-C2B (chloroquine, mefloquine, pyrimethamine, and atovaquone resistant), W2 (chloroquine and pyrimethamine resistant), and TM91-C235 (chloroquine, mefloquine, and pyrimethamine resistant)] with IC_{50} values of 80, 90, 140, and 270 nM, respectively. By contrast, the activity toward the other parasites as well as to control mammalian cell lines was quite modest [*Trypanosoma cruzi* (Chagas' disease) 6.5 μM; *Leishmania donovani* (leishmaniasis) 3.0 μM; mammalian cell cytotoxicity (MCF-7) 3.1 μM, (Vero epithelial cells) 2.1 μM)].

In summary, we report here the isolation and structure elucidation of bastimolide A (**1**), an intriguing polyhydroxy macrolide possessing a 40-membered ring, 10 stereocenters segregated into seven distinct sites in the molecule, and a *tert*-butyl terminus which appears to stabilize the lactone ring from hydrolysis. Compound **1** possesses potent and selective antimalarial activity against four multidrug resistant strains of *P. falciparum* and represents a promising new class of antimalarial agent. The discovery of this new natural product also suggests that the cyanobacterial genus *Okeania* will be a significant resource of structurally interesting molecules in the future. Ongoing studies are focused on assessing its in vivo activity, molecular and cellular mechanisms of action, and structure−activity relationships as provided by naturally occurring analogues and semisynthetic derivatives.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a polarimeter, while CD spectra were recorded in MeOH using a spectropolarimeter. IR spectra were recorded using KBr plates. NMR spectra were recorded on 500, 600, or 800 MHz spectrometers in methanol-d_4, DMSO-d_6, or pyridine-d_5. Chemical shifts (δ) are reported using TMS as an internal standard. For the HETLOC experiments, 128 scans (4 K) and 512 experiments were obtained with zero-filling at F1 to 4 K. A mixing time of 60−80 ms was used. X-ray diffraction data were obtained with Cu Kα radiation (λ = 1.54718 Å). All solvents were purchased as HPLC grade.

Sampling and Taxonomic Characterization. The clump-forming cyanobacterium was collected in May 2011 by hand using snorkeling methods in a bay near the Isla Bastimentos Park (9° 17.411 N, 82° 11.387 W) in the Republic of Panama. A 1 L sample was preserved in a 1:1 EtOH−seawater solution and stored at −20 °C until it was extracted. A small sample of cyanobacterial biomass was also preserved in RNA stabilization reagent (RNAlater, Qiagen) for subsequent 16S rRNA sequencing and analysis. Voucher specimen number PAB-19MAY11-4 (recollection of the *Okeania hirsuta* holotype PAB-10-FEB-10-1^T; GenBank account no. KC986936; U.S. herbarium of the National Museum of Natural History, deposition no. US 217947) is deposited at the Gerwick Laboratory sample repository, Scripps Institution of Oceanography, University of California, San Diego, California.

Extraction and Isolation. The preserved biomass and alcohol solution were filtered through cheesecloth, and the crude cyanobacterial biomass was extracted by soaking for 15 min in 600 mL of 2:1 CH_2Cl_2−MeOH with manual fragmentation of the cyanobacterial clumps. The organic layer was then filtered through cheesecloth, and the cyanobacterial biomass was repetitively extracted six times by submerging in 2:1 CH_2Cl_2−MeOH with mild warming (<40 °C) for 30 min and then filtering through cheesecloth. The aqueous layer was concentrated to a reduced volume (ca. 500 mL) by rotary evaporation (<40 °C) and then extracted with an equal volume of CH_2Cl_2. All of the organic layers were combined and concentrated by rotary evaporation to afford 12.1 g of a black−green paste. A small portion

DOI: 10.1021/acs.joc.5b01264
J. Org. Chem. 2015, 80, 7849−7855

(0.1 g) was preserved for future bioassays, and the remainder was purified by vacuum liquid chromatography (VLC) with 300 mL of silica gel (type H, 10−40 μm, Sigma-Aldrich) in a 10 cm diameter by 9 cm high glass vacuum funnel subtended with a glass frit. Fractions of 500 mL volume were collected using a stepped gradient of hexanes−EtOAc−MeOH (nine fractions, 100% hexanes, 10% EtOAc/90% hexanes, 20% EtOAc/80% hexanes, then 20% increments to 100% EtOAc, 25% MeOH/75% EtOAc, and 100% MeOH). The fraction eluting with 100% MeOH (fraction I, 5.05 g) was further separated using RP SPE [500 mg of SPE, stepwise gradient solvent system of decreasing polarity starting with 20% MeOH in H_2O to 100% MeOH, to produce four fractions]. Fraction 3 (60% MeOH in H_2O) was further separated by RP HPLC (Synergic 10 μm C18, 80 Å, 250 × 10 mm, 70% ACN/H_2O at 3 mL/min, detection at 210, 230, and 250 nm) to obtain pure compound **1** (76.8 mg, 0.64% of extract) at t_R 21.0 min.

Bastimolide A (1): white powder; $[\alpha]^{25}_D$ = −11.7 (*c* 1.75, MeOH); UV (MeOH) λ_{max} (log ε) 216 (*c* 2.3 mg/mL) nm; IR (KBr) ν_{max} 3419, 2931, 1747, 1650, 1458 cm^{-1}; ^1H NMR (pyridine-d_5, 800 MHz) and ^{13}C NMR (pyridine-d_5, 200 MHz), see Table 1; ESITOFMS *m/z* 789.30 $[M + H]^+$; ESITOFMS *m/z* 833.54 $[M + HCOO]^-$; HRESITOFMS *m/z* 811.5905 $[M + Na]^+$ (calcd for $C_{44}H_{84}O_{11}Na$, 811.5906).

Preparation of Acetonide Derivatives of 1 (1a−c). A mixture of **1** (18.0 mg), 2,2-dimethoxypropane (3.0 mL), and *p*-TsOH (1.0 mg) was stirred at room temperature for 4 h and then quenched with saturated $NaHCO_3$ solution. The reaction mixture was extracted with EtOAc, the organic solvents were removed in vacuo, and the crude mixture was purified over a 200 mg C-18 Sep-Pak followed by RP-HPLC (Synergic 10 μm C18, 80 Å, 250 × 10 mm, 80% ACN/H_2O at 3 mL/min) to obtain **1a** (1.3 mg, 6.8%) at t_R 12.5 min, **1b** (1.8 mg, 9.5%) at t_R 16.0 min, and **1c** (9.8 mg, 50.0%) at t_R 28.0 min.

1a: white powder; $[\alpha]^{25}_D$ = −9.1 (*c* 0.33, MeOH); ^1H NMR (MeOH-d_4, 500 MHz) 5.70 (1H, s), 4.76 (1H, d, 9.5 Hz), 4.13 (1H, m), 3.93 (1H, m), 3.76 (3H, overlapped), 3.56 (4H, overlapped), 2.65 (2H, overlapped), 1.92 (−CH_3, s), 1.47 (−CH_3, s), 1.33 (−CH_3, s), 1.28−1.71 (49H, overlapped), 1.14 (1H, ddd, 12.0, 12.0, 12.0 Hz), 0.91 (−3CH_3, s); ^{13}C NMR (MeOH-d_4, 125 MHz), 168.1 (C-1), 162.4 (C-3), 117.1 (C-2), 99.8 (acetonide), 80.9 (C-39), 72.3 (C-11), 72.1 (C-9), [71.9, 71.9, 71.6, 71.5 (C-15, 27, 31, 35)], 70.2 (C-23), 68.3 (C-19), 67.6 (C-21), 44.9 (C-10), 44.7 (C-20), [38.6, 38.6, 38.4, 38.4, 38.3, 38.3, 38.2, 38.0, 38.0, 37.8, 37.8, 37.6, (C-8, 12, 14, 16, 18, 24, 26, 28, 30, 32, 34, 36)], 37.1 (C-22), 35.6 (C-40), 34.3 (C-4), 30.9 (C-6), 30.7 (C-38), 30.7 (acetonide), 29.4 (C-5), 26.5 (C-41, 42, 43), 26.4, (C-7), 25.4 (C-44), [23.5, 22.9, 22.8, 22.6, 22.1, 21.5 (C-13, 17, 25, 29, 33, 37)], 20.2 (acetonide); ESITOFMS *m/z* 851.66 $[M + Na]^+$; ESITOFMS *m/z* 827.66 $[M - H]^-$; HRESITOFMS *m/z* 851.6218 $[M + Na]^+$ (calcd for $C_{47}H_{88}O_{11}Na$, 851.6219).

1b: white powder; $[\alpha]^{25}_D$ = −16.1 (*c* 0.60, MeOH); ^1H NMR (MeOH-d_4, 500 MHz) 5.69 (1H, s), 4.76 (1H, dd, 10.5, 1.5 Hz), 4.00 (1H, m), 3.89 (1H, m), 3.83 (1H, m), 3.75 (1H, m), 3.55 (4H, overlapped), 2.72 (1H, m), 2.56 (1H, m), 1.92 (−CH_3, s), 1.45 (−CH_3, s), 1.33 (−CH_3, s), 1.29−1.65 (49H, overlapped), 1.05 (1H, ddd, 13.5, 13.5, 13.5 Hz), 0.91 (−3CH_3, s); ^1H NMR (pyridine-d_5, 500 MHz) 6.28 (1H, d, 3.0 Hz, −OH), 6.18 (1H, d, 3.0 Hz, −OH), 6.04 (1H, d, 4.0 Hz, −OH), 5.91 (1H, s), 5.80 (1H, d, 5.0 Hz, −OH), 5.70−5.77 (5-OH, overlapped), 5.10 (1H, d, 9.5 Hz), 4.77 (1H, br s), 4.47 (1H, br s), 4.29 (1H, br s), 3.95 (6H, overlapped), 2.97 (1H, m), 2.62 (1H, m), 1.84 (3H, −CH_3), 1.56 (3H, −CH_3), 1.53 (3H, −CH_3), 1.07−2.21 (50H, overlapped), 0.96 (9H, −3CH_3); ^{13}C NMR (MeOH-d_4, 125 MHz), 168.1 (C-1), 117.2 (C-2), 162.3 (C-3), 99.8 (acetonide), 80.9 (C-39), [72.4, 72.1, 72.1, 72.0 (C-15, 27, 31, 35)], 70.6 (C-11), 70.4 (C-9), 70.4 (C-23), 69.0 (C-21), 68.3 (C-19), 45.3 (C-22), 44.8 (C-20), [38.6, 38.5, 38.4, 38.3, 38.3, 38.3, 38.2, 38.0, 37.9, 37.9, 37.9, 37.4, (C-8, 12, 14, 16, 18, 24, 26, 28, 30, 32, 34, 36)], 37.4 (C-10), 35.5 (C-40), 34.3 (C-4), 30.8 (C-6), 30.7 (C-38), 30.6 (acetonide), 29.3 (C-5), 25.8, (C-7), 26.4 (C-41, 42, 43), 25.4 (C-44), [23.5, 23.0, 22.9, 22.3, 22.2, 22.1 (C-13, 17, 25, 29, 33, 37)], 20.3 (acetonide); ESITOFMS *m/z* 851.66 $[M + Na]^+$; ESITOFMS *m/z* 827.67 $[M - H]^-$; HRESITOFMS *m/z* 851.6218 $[M + Na]^+$ (calcd for $C_{47}H_{88}O_{11}Na$, 851.6219).

1c: white powder; $[\alpha]^{25}_D$ = −16.8 (*c* 3.0, MeOH); ^1H NMR (MeOH-d_4, 500 MHz) 5.69 (1H, s), 4.76 (1H, dd, 10.5, 2.0 Hz), 4.12 (1H, m), 3.89 (3H, overlapped), 3.77 (1H, m), 3.55 (4H, overlapped), 2.63 (2H, overlapped), 1.92 (−CH_3, s), 1.46 (−CH_3, s), 1.45 (−CH_3, s), 1.33 (−CH_3, s), 1.28−1.66 (48H, overlapped), 1.14 (1H, m), 1.05 (1H, m), 0.91 (−3CH_3, s); ^1H NMR (pyridine-d_5 + 1 drop D_2O, 500 MHz) 5.83 (1H, s), 5.01 (1H, d, 10.0 Hz), 4.41 (1H, m), 4.23 (1H, m), 3.79−4.02 (7H, overlapped), 2.64−2.81 (2H, overlapped), 1.82 (3H, −CH_3), 1.52 (3H, −CH_3), 1.51 (6H, −2CH_3), 1.49 (3H, −CH_3), 1.11−2.12 (50H, overlapped), 0.90 (9H, −3CH_3); ^{13}C NMR (MeOH-d_4, 125 MHz), 168.1 (C-1), 162.3 (C-3), 117.1 (C-2), 99.8 (2C, acetonide), 80.9 (C-39), [72.3, 72.1, 72.0, 71.9 (C-15, 27, 31, 35)], 70.3 (C-11),70.2 (C-9), 70.2 (C-23), 68.3 (C-19), 67.6 (C-21), 44.7 (C-20), [38.4, 38.4, 38.4, 38.3, 38.3, 38.3, 38.0, 37.9, 37.9, 37.7, 37.5, 37.4, (C-8, 12, 14, 16, 18, 24, 26, 28, 30, 32, 34, 36)], 37.4 (C-10), 37.2 (C-22), 35.6 (C-40), 34.3 (C-4), 30.8 (C-6), 30.7 (C-38), 30.7 (acetonide), 30.6 (acetonide), 29.4 (C-5), 26.5 (C-41, 42, 43), 25.8 (C-7), 25.4 (C-44), [23.5, 22.9, 22.9, 22.0, 22.0, 21.6 (C-13, 17, 25, 29, 33, 37)], 20.3 (2C, acetonide]; ^{13}C NMR (pyridine-d_5 + 1 drop D_2O, 125 MHz), 166.8 (C-1), 161.0 (C-3), 116.8 (C-2), 98.8 (acetonide), 98.7 (acetonide), 79.8 (C-39), [71.3, 70.8, 70.8, 70.7 (C-15, 27, 31, 35)], 69.3 (C-23), 69.3 (C-11), 69.3 (C-9), 67.1 (C-19), 67.0 (C-21), 44.7 (C-20), 36.9 (C-10), [38.4, 38.4, 38.3, 38.3, 38.0, 37.9, 37.9, 37.8, 37.7, 37.6, 37.6, 37.0, (C-8, 12, 14, 16, 18, 24, 26, 28, 30, 32, 34, 36)], 36.8 (C-22), 34.9 (C-40), 33.6 (C-4), 30.8 (acetonide), 30.7 (acetonide), 30.2 (C-38), 30.1 (C-6), 28.6 (C-5), 26.2 (C-41, 42, 43), 25.2 (C-7), 25.2 (C-44), [23.2, 22.9, 22.8, 21.9, 21.8, 21.2 (C-13, 17, 25, 29, 33, 37)], 20.3 (2C, acetonide); ESITOFMS *m/z* 891.71 $[M + Na]^+$; ESITOFMS *m/z* 867.61 $[M - H]^-$; HRESITOFMS *m/z* 891.6531 $[M + Na]^+$ (calcd for $C_{50}H_{92}O_{11}Na$, 891.6532).

Preparation of the Nona-p-nitrobenzoate Derivative of Bastimolide A (1d). To a stirred solution of **1** (5.0 mg, 0.0063 mmol) in dry acetone (1.0 mL) and toluene (1.0 mL) were added *p*-nitrobenzoyl chloride (60.0 mg, 0.32 mmol) and DMAP (50.0 mg). The reaction mixture was stirred at 70 °C for 7 d and then quenched with distilled water. The mixture was extracted with CH_2Cl_2, and the organic extract was evaporated to dryness. The resulting residue was purified by column chromatography on Si gel eluting with EtOAc−hexanes (20:80) followed by recrystallization from acetone to yield **1d** (4.8 mg, 35.6%) as a white powder.

1d: ^1H NMR ($CDCl_3$, 600 MHz) 7.89−8.24 (36H, overlapped), 5.59 (1H, s), 5.26 (5H, overlapped), 5.12 (4H, overlapped), 4.68 (1H, d, 10.2), 2.59 (1H, m), 2.40 (1H, m), 2.24 (2H, m), 2.19 (1H, m), 2.12 (1H, m), 2.01 (2H, m), 1.81 (−CH_3, s), 1.23−1.81 (44H, overlapped), 0.84 (−3CH_3, s); HRESITOFMS *m/z* 2152.6905 $[M + Na]^+$ (calcd for $C_{107}H_{111}N_9O_{38}Na$, 2152.6922).

X-ray Crystallographic Analysis of 1d. Colorless crystals of bastimolide A (**1**) were obtained from acetone−hexanes (95:5). The crystal data were recorded at 100(2) K on an Agilent Gemini ultra diffractometer with Cu Kα radiation (λ = 1.54718 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. The crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre with the deposition no. 1058586. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [http://www.ccdc.cam.ac.uk/deposit/]. Crystal data for **1d**: $C_{110}H_{117}N_9O_{39}$, M_r = 2189.13, monoclinic, space group *P*2(1) with *a* = 10.7088(6) Å, *b* = 44.986(2) Å, *c* = 11.2096(6) Å, β = 91.109(3)°, *V* = 5399.1(5) $Å^3$, *Z* = 2, D_x = 1.347 g/cm^3, μ(Cu Kα) = 0.866 mm^{-1}, and *F*(000) = 2298. Crystal dimensions: 0.28 × 0.22 × 0.08 mm^3. Independent reflections: 12031 (R_{int} = 0.0447). The final R_1 values were 0.1129, Flack parameter = 0.1(4), wR2 = 0.2987 ($I > 2\sigma(I)$).

DOI: 10.1021/acs.joc.5b01264
J. Org. Chem. 2015, 80, 7849−7855

Bioassays. All bioassays were evaluated in duplicate, testing at 10, 2, 0.4, 0.08, and 0.016 μg/mL. Malaria bioassays were performed as previously reported by our program; we used Sybr Green to assess parasite growth inhibition by drugs and used chloroquine and atovaquone as positive controls.[20] Chagas' bioassays were tested following the protocol of Buckner et al. and using nifurtimox as a positive control (IC_{50} = 3−5 μg/mL).[21] Leishmaniasis bioassays were performed using a method previously employed in our program, based on parasite DNA fluorescence.[20] In this latter assay, amphotericin B was used as the positive control and had an IC_{50} of 80 ng/mL. Cytotoxicity bioassays were performed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay protocol using Vero cells.[22]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01264.

X-ray crystallographic data for **1d** (CIF)
Full NMR data of **1**, ^{1}H and ^{13}C NMR spectra of **1a**−**1d**, and MS data for all compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. X. Huang (UCSD), Dr. Y. Su (UCSD), and Prof. A. L. Rheingold (UCSD) for assistance with NMR, HRMS, and X-ray data acquisition, respectively. This work was supported by NIH TW006634, NS053398, and CA100851 (W.H.G.), NSFC 41322037 (C.L.S.), and NSFC 41130858 (C.L.S. and C.Y.W.).

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DOI: 10.1021/acs.joc.5b01264
J. Org. Chem. 2015, 80, 7849−7855