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Bastimolide B, an Antimalarial 24-Membered Marine Macrolide Possessing a *tert*-Butyl Group

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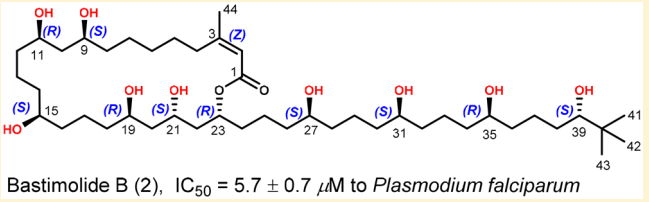
Supporting Information

ABSTRACT: We reported previously the discovery of the potent antimalarial 40-membered macrolide bastimolide A (**1**) from the tropical marine cyanobacterium *Okeania hirsute*. Continued investigation has led to the discovery of a new analogue, bastimolide B (**2**), a 24-membered polyhydroxy macrolide with a long aliphatic chain and unique terminal *tert*-butyl group. Its complete structure was determined by a combination of extensive spectroscopic methods and comparative analysis of its methanolysis products with those of bastimolide A. A methanolysis mechanism for bastimolide A is proposed, and one unexpected isomerization product of the C2−C3 double bond, 2-(*E*)-bastimolide A (**3**), was obtained. Bastimolide B (**2**) showed strong antimalarial activity against chloroquine-sensitive *Plasmodium falciparum* strain HB3. A preliminary investigation of the structure−activity relationship based on six analogues revealed the importance of the double bond as well as the 1,3-diol and 1,3,5-triol functionalities.



Marine cyanobacteria are exceptionally prolific producers of structurally diverse and biologically active secondary metabolites.[1,2] There is one approved anticancer agent that owes its origin to a marine cyanobacterial metabolite and a number of related agents in clinical trials,[3] and while peptides including lipopeptides are the dominant class of metabolites from these organisms, there are also several unique polyhydroxy macrolides with 36-membered rings (caylobolides A[4] and B[5]) or 40-membered rings (amantelides A and B[6] and nuiapolide[7]). Most of these latter agents have shown potent cytotoxic or antichemotactic activities. Recently, we also discovered an intriguing 40-membered-ring polyhydroxy macrolide, named bastimolide A (**1**), and demonstrated its potent and selective antimalarial activity against four multidrug-resistant strains of *Plasmodium falciparum*.[8] Due to the considerable challenge of firm identification of the configurations for 1,*n*-diol ($n \geq 5$) moieties,[9] bastimolide A is the only example for which the planar structure and absolute configuration has been completely identified.[8]

As part of the Panama International Cooperative Biodiversity Group (ICBG) to discover antiparasitic lead compounds from marine microorganisms, further chemical investigation of the new genus/species of tropical marine cyanobacterium *Okeania hirsuta* (PAB-19MAY11-4) was undertaken for its antimalarial constituents. This investigation has now resulted in the isolation of bastimolide B (**2**), a 24-membered macrolide. This new polyketide-derived compound is the first 24-membered macrolide isolated from a marine cyanobacterium and possesses a highly oxidized side chain and unique *tert*-butyl group. Herein, we report its discovery, structure determination, antimalarial activity, and the preliminary structure−activity relationship.

A freeze-dried sample of the cyanobacterium *O. hirsuta* collected from near the Isla Bastimentos Park, Panama, in 2011, was repeatedly extracted with CH_2Cl_2/MeOH (2:1), and the resulting extract was fractionated by silica gel vacuum liquid chromatography (VLC) to produce nine subfractions (A−I). Fraction I was further separated using RP SPE [starting with 20% MeOH in H_2O to 100% MeOH, to produce four fractions]. Fraction 2 (40% MeOH in H_2O) was further separated by RP HPLC to obtain compound **2** (1.8 mg).

Pure bastimolide B (**2**) was thus obtained as an optically active white powder and gave by HRESITOFMS an $[M + Na]^+$ peak at m/z 811.5907 (calcd for $C_{44}H_{84}O_{11}Na$, 811.5906), requiring three degrees of unsaturation. A strong IR absorption

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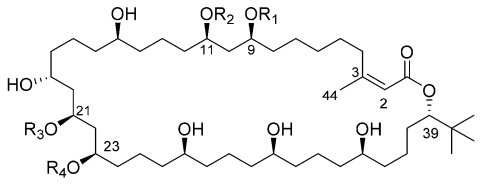
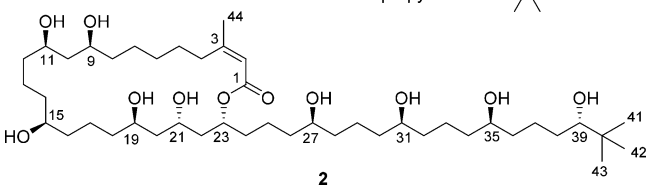


1 $R_1 = R_2 = R_3 = R_4 =$ H, 2,3-*Z*
1a $R_1 = R_2 = R_3 = R_4 =$ H, $\Delta^{3,44}$ isomer of bastimolide A
1i $R_1 = R_2 =$ H, $R_3 + R_4 =$ isopropylidene, 2,3-*Z*
1j $R_1 + R_2 =$ isopropylidene, $R_3 = R_4 =$ H, 2,3-*Z*
1k $R_1 + R_2 =$ isopropylidene, $R_3 + R_4 =$ isopropylidene, 2,3-*Z*
3 $R_1 = R_2 = R_3 = R_4 =$ H, 2,3-*E*

isopropylidene =



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band at 1700 cm^{-1} suggested an ester group, and this was supported by the presence of a carbonyl signal at δ_C 166.7 in the ^{13}C NMR spectrum (Table 1). The relatively shielded shift of this ester carbonyl, together with the UV absorption maximum at 214 nm, suggested α,β-unsaturation; this was supported by HMBC and other ^1H NMR analysis (Supporting Information, Table S1). Furthermore, a methyl group (δ_H 1.77, δ_C 25.4) was located by HMBC at the β-position to define a trisubstituted α,β-unsaturated ester motif. Considering the molecular formula and remainder of the NMR data, the structure of **2** was thus suggested to contain a single ring.

The ^1H NMR spectrum of bastimolide B (pyridine-d_5) showed a single olefinic proton (δ_H 5.83), nine protons on oxygenated carbons at δ_H 3.47−4.59 along with one more deshielded resonance at δ_H 5.63, and nine exchangeable protons at δ_H 5.71−6.12. Additionally, there was an intense 9H singlet at δ_H 1.08 due to three isochronous methyl groups comprising a *tert*-butyl moiety; one singlet methyl group (δ_H 1.77); and 52 methylene protons including 49 that were significantly overlapped at δ_H 1.41−2.19. Between the ^{13}C NMR (Table 1) and HSQC spectra of **2**, all 44 carbon resonances were observable and included one carbonyl, two quaternary carbons (including one olefinic carbon), 10 oxygenated methines (δ_C 66.3−79.3), 26 methylenes, and four methyl groups (including one *tert*-butyl). These data taken together with the co-occurrence of bastimolide A (**1**)[8] suggested that compound **2** was a related polyhydroxy macrolide.

The most obvious difference in the ^1H NMR spectra between bastimolides A and B was the relatively deshielded chemical shift of H-23 (δ_H 5.63) in **2**, instead of the relatively shielded chemical shift of H-23 (δ_H 4.28) in **1**. Correspondingly, H-39 was relatively shielded in bastimolide B (δ_H 3.47 in **2** vs 5.09 in **1**). Based on the above data, together with key HMBC correlations between H-23 and the C-1 ester carbonyl group, the location of lactone formation was established as between C-1 and C-23 in **2** (versus C-1 and C-39 in **1**). Furthermore, detailed interpretation of 1D and 2D NMR spectra (COSY, TOCSY, HSQC, and HMBC) allowed location of one 1,3-diol system (C-9 and C-11), one 1,3,5-triol system (C-19, C-21, and C-23), and six repeating 1,5-diol moieties (Figure 1). The relative configurations of the 1,3-diol group (C-9/C-11) and 1,3,5-triol system (C-19/C-21/C-23) were identified as *syn* and *anti*/*syn* by Kishi's Universal NMR database[10] and the equivalence or nonequivalence of the intervening methylene protons.[8,11] Finally, the *Z*-configured double bond (C-2/C-3) was defined by a selective 1D NOE experiment and the deshielded chemical shift of the C-44 methyl carbon at δ_C 25.4.[8]

On the basis of the co-occurrence and shared biogenesis with **1**, along with a careful comparison of ^1H and ^{13}C NMR data of **2** (Table 1) with those of **1**, we propose the absolute configuration of the two metabolites to be the same. To confirm this assignment, we attempted to compare the hydrolysis products of **1** and **2** and show their co-identities. Unfortunately, bastimolide A (**1**) was unreactive to relatively mild acidic (1, 3, and 4 M HCl) or basic conditions (saturated solutions of LiOH, NaOH, or KOH) at different temperatures (30−90 °C). As we have seen in other esters with adjacent sterically bulky and hydrophobic *tert*-butyl groups, such as palmyrolide A,[12] they are highly resistant to hydrolytic conditions. However, treatment of **1** with NaOMe in MeOH and H_2O quickly (<1 h) resulted in production of new products by LCMS. Over a 10-day period of subjecting bastimolide A (**1**) to these conditions, we monitored the consecutive production of several products (Supporting Information). Initially, two compounds (**3** and **1a**) with the same molecular weights (*m*/*z* 788) as bastimolides A and B were observed. Subsequently, three corresponding bastimolide methyl esters (Supporting Information structures **1b**, **1c**, and **1d**, *m*/*z* 820) were produced. Finally, three polar derivatives, the seco acids of **1**, **3**, and **1a** (Supporting Information structures **1e**, **1f**, and **1g**), dominated as the major products with *m*/*z* values of 806. When any of the above bases were used in the presence of MeOH, the same product profiles were obtained in each case, and thus MeOH appears to play an important role in this reaction (Scheme S2). Importantly, in all cases, the C2−C3 double-bond isomerization product, 2-(*E*)-bastimolide A (**3**) (Supporting Information, Scheme S1), was produced from bastimolide A (**1**), and its structure was determined by spectroscopic methods (Supporting Information).

Fortunately, the more vigorous basic hydrolysis conditions (50.0 μg of bastimolide B (**2**), 0.1 mL of NaOMe, 0.4 mL of MeOH, 40 °C, 8 h) were able to affect ester hydrolysis. A comparison of LCMS retention times and fragmentations for the three main hydrolysis products (Supporting Information) from bastimolide A (**1**) and bastimolide B (**2**) showed they were identical. Hence, based on spectroscopic, chemical, and biosynthetic considerations, we assign bastimolide B (**2**) to possess the same absolute configuration as bastimolide A (**1**).

Although the absolute configuration of **1** was established by X-ray crystallographic analysis, electronic circular dichroism (ECD) chiroptical methods were also applied to compounds **1**−**3**.[13] First, the absolute configuration of the known 40-membered-ring polyhydroxy macrolide bastimolide A (**1**) was verified by comparing its computed ECD spectrum with the experimental result. A total of 729 lowest energy conformers of **1**, with relative energies extending from 0 to 10.0 kcal/mol, were searched using the MMFF94S force field and used for structural optimizations and ECD computations. Boltzmann statistics were performed for ECD simulations with a standard deviation of σ 0.4 eV. The predicted ECD curve matched well with the experimental result of bastimolide A (**1**) (Figure S74), suggesting the accuracy and applicability of the ECD method to these macrolides. In the course of these preliminary studies, it

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Table 1. NMR Spectroscopic Data (^{1}H 800 MHz, ^{13}C 200 MHz, Pyridine-d_5) for Bastimolide B (2)

position	δ_C, type[a]	δ_H (J in Hz)	HMBC[a]	TOCSY
1	166.7, C			
2	117.5, CH	5.83, s	1, 3, 4, 44	44
3	160.7, C			
4	33.7, CH_2	3.14, dd (8, 4); 2.41, m	2, 3, 5, 6, 44	5
5	28.9, CH_2	1.52, m	3, 4, 6, 7	4, 6
6	30.2, CH_2	1.46, m	4, 5, 7	4, 5, 7, 9
		1.42, m		
7	26.1, CH_2	1.65, m[b]		
		1.60, m[b]		
8	39.3, CH_2	1.65−1.98, m[b]		
9	71.7, CH	4.23, br s		5, 6, 7, 10, 9-OH
10	44.5, CH_2	2.04, m[b]		9, 11
		1.96, m[b]		
11	71.7, CH	4.32, brs		9, 10, 11-OH
12	39.2, CH_2	1.65−1.98, m[b]		
13	22.4, CH_2	1.77, m[b]		
14	39.1, CH_2	1.65−1.98, m[b]		
15	71.4, CH	3.96, m[b]		15-OH
16	39.1, CH_2	1.65−1.98, m[b]		
17	21.5, CH_2	1.77, m[b]		
18	39.1, CH_2	1.65−1.98, m[b]		
19	68.3, CH	4.51, brs		20, 21, 22, 19-OH
20	44.9, CH_2	2.01, m	19, 21, 22	19, 21, 22, 23, 19-OH, 21-OH
21	66.3, CH	4.59, br s		19, 20, 22, 23, 21-OH
22	44.0, CH_2	2.34, ddd (8, 4, 4); 2.05, m[b]	21, 23, 24	19, 20, 21, 23, 24, 21-OH
23	71.6, CH	5.63, m	1, 21, 24, 25	21, 22, 24
24	35.9, CH_2	1.87, m[b]		
25	22.7, CH_2	1.70, m[b]		
26	37.4, CH_2	1.65−1.98, m[b]		
27	70.7, CH	4.07, m[b]		27-OH
28	38.3, CH_2	1.65−1.98, m[b]		
29	23.4, CH_2	1.77, m[b]		
30	38.8, CH_2	1.65−1.98, m[b]		
31	71.6, CH	3.94, m[b]		31-OH
32	38.9, CH_2	1.65−1.98, m[b]		
33	23.4, CH_2	1.77, m[b]		
34	39.0, CH_2	1.65−1.98, m[b]		
35	71.2, CH	3.88, brs		37, 35-OH
36	37.6, CH_2	1.65−1.98, m[b]		
37	24.7, CH_2	2.16, m[b]		
		1.94, m[b]		
38	32.7, CH_2	1.62, m		
39	79.3, CH	3.47, m	38, 40, 41−43	37, 38, 39-OH
40	35.8, C			
41−43	26.8, CH_3	1.08, s	39, 40	
44	25.4, CH_3	1.77, s	2, 3, 4	
9-OH		6.04, br d (2.4)[b]		8, 9, 11
11-OH		6.12, br d (3.2)		10, 11
15-OH		5.76, br d (4.8)		15
19-OH		5.87, d (4.0)		19, 20
21-OH		6.06, d (4.8)		21, 22
27-OH		5.74[b]		27
31-OH		5.72		31
35-OH		5.74[b]		35
39-OH		5.71, d (5.6)		39

[a]HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. [b]Signal overlapped with other signals.

was observed that the preferential conformations of **1** contained several hydrogen bonds, resulting in a relatively rigid structure for **1** (Figure S76).

For **3**, the experimental ECD spectrum was almost opposite to that of the calculated spectrum and implied that the 39*R*-epimer of **1** was also a structural possibility. Thus, conforma-

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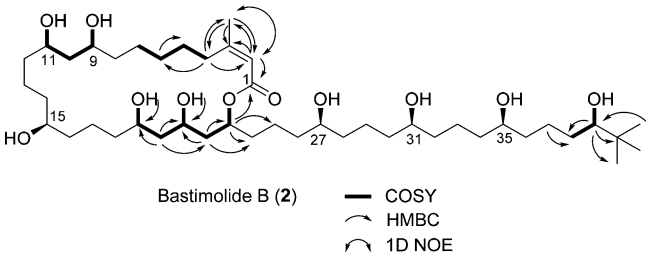

Figure 1. Key NMR-based connections for bastimolide B (**2**).

tional searches were performed using the MMFF94S force field for (2*E*)-bastimolide A (**3a**) as well as (39*R*)-bastimolide A (**3b**). All geometries (746 lowest energy conformers for **3a** and 1239 for **3b**, respectively) with relative energies from 0 to 10.0 kcal/mol were used in optimizations at the B3LYP/6-31G(d) level using the Gaussian09 package. The B3LYP/6-31G(d)-optimized conformers (124 lowest energy conformers for **3a** and 167 for **3b**) with relative energy from 0 to 2.0 kcal/mol were then reoptimized at the B3LYP/6-311+G(d) level. Boltzmann statistics were performed for ECD simulations with a standard deviation (σ) of 0.2 eV. The predicted ECD for **3a** matched better with the experimental result of **3** (Figure S77), suggesting that **3** was indeed the 2*E*-isomer of bastimolide A (**1**). Interestingly, the ECD curve of **3** was principally affected by the double-bond configuration between C-2 and C-3 rather than the C-39 stereogenic carbon, suggesting a new application of ECD calculations to small-molecule molecular structures. Further, our calculations suggested that compound **3a** possesses a rigid structure due to the presence of hydrogen bonds, whereas in **3b** the change in direction of the C-1 carbonyl was predicted to disrupt this hydrogen bond network and increase structural flexibility (Figure S76). Finally, attempts to confirm the absolute configuration of bastimolide B (**2**) by the ECD method were unsuccessful. At the present time, the presence of multiple different hydrogen bonds among the side chain hydroxy groups in **2** prevented this type of computational approach.

Bastimolide A (**1**), bastimolide B (**2**), and 2-(*E*)-bastimolide A (**3**), together with three previously prepared acetonide derivatives of bastimolide A (**1i**–**1k**),[8] were evaluated for their antimalarial activities against chloroquine-sensitive *Plasmodium falciparum* strain HB3. The natural products (**1** and **2**) and their derivatives had widely varying antimalarial effects (Table 2). While the natural products **1** and **2** showed pronounced antimalarial activity with EC_{50} values of 2.6 ± 0.2 and 5.7 ± 0.7 μM, respectively, it was 2-(*E*)-bastimolide A (**3**) that showed the greatest potency, with an EC_{50} value of 1.4 ± 0.5 μM. The acetonide derivative of the C-21/C-23 hydroxy groups (**1i**) had an 8-fold decrease in potency, whereas the C-9/C-11 acetonide (**1j**) had nearly the same potency as bastimolide A (**1**), indicating that the 9-OH/11-OH groups have little effect on the antimalarial activity.

Table 2. Antimalarial Activity of Compounds 1–3 and 1i–1k against Chloroquine-Sensitive *P. falciparum* Strain HB3

compound	IC_{50} (μM)
bastimolide A (**1**)	2.6 ± 0.2
bastimolide B (**2**)	5.7 ± 0.7
2-(*E*)-bastimolide A (**3**)	1.4 ± 0.5
acetonide A (**1i**)	20 ± 3
acetonide B (**1j**)	2.3 ± 0.2
acetonide C (**1k**)	9.7 ± 1.7
chloroquine	0.063 ± 0.025

In summary, we report here the isolation and structure elucidation of bastimolide B (**2**), a new 24-membered polyhydroxy macrolide with a unique terminal *tert*-butyl group. The bastimolides show considerable antimalarial activity against chloroquine-sensitive *P. falciparum* strain HB3 and represent an intriguing new class of antimalarial agent. The discovery of this new polyhydroxy macrolide further suggests that the cyanobacterial genus *Okeania* should be a significant resource of structurally interesting molecules in the future.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2000 polarimeter. UV/visual-light spectra were recorded on a Beckman Coulter DU 880 spectrophotometer. ECD spectra were recorded in MeOH using a JASCO J-810 spectropolarimeter. IR spectra were recorded using a Thermo Electron Corporation Nicolet IR 100 FT-IR and KBr plates. NMR spectra were recorded on Varian 500 MHz or vs800 MHz spectrometers in methanol-d_4 or pyridine-d_5. Chemical shifts δ are reported using tetramethylsilane as an internal standard. High-resolution mass spectra were carried out on an Agilent 6230 TOF-MS under positive ion ESI-TOF-MS conditions in the University of California, San Diego (UCSD), Small Molecule MS Facility. All solvents were purchased as HPLC grade.

Sampling and Taxonomic Characterization. The cyanobacterium was identified as *Okeania hirsuta*.[8]

Extraction and Isolation. The sample was extracted with 2:1 CH_2Cl_2/MeOH and fractionated to obtain 76.8 mg of bastimolide A as previously described.[8] Fraction 2 (40% MeOH in H_2O) from the RP SPE fractionation step was further separated by RP HPLC (Synergi 10 μm C18, 80 Å, 250 × 10 mm, 70% MeOH/H_2O at 3 mL/min, detection at 210, 230, and 250 nm) to obtain pure compound **2** (1.8 mg) at t_R 34.0 min.

*Bastimolide B (**2**):* white powder; $[\alpha]^{25}_D$ −11.3 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.24) nm; ECD (0.63 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 222 (−5.12); IR (KBr) ν_{max} 3399, 2931, 1700, 1383, 1114 cm^{-1}; ^1H NMR (pyridine-d_5, 800 MHz) and ^{13}C NMR (pyridine-d_5, 200 MHz), see Table 1; ^1H NMR (CD_3OD, 500 MHz) 5.70 (H-2, 1H, s), 5.06 (H-23, 1H, m), 3.90 (H-21, 1H, m), 3.85 (H-19, 1H, m), 3.78 (1H, m), 3.73 (1H, m), 3.62 (1H, m), 3.54 (3H, overlapped), 3.12 (H-39, 1H, d, 10.5 Hz), 2.88 (H-4, 1H, m), 2.40 (H-4, 1H, m), 1.91 (H-44, 3H, s, $-CH_3$), 1.83 (H-22, 1H, m), 1.72 (H-22, 1H, m), 1.65 (H-10, 1H, m), 1.56 (H-20, 2H, m), 1.51 (H-10, 1H, m), 1.29–1.73 (44H, overlapped), 0.89 (H-41, 42, 43, 9H, s, $-3CH_3$); ^{13}C NMR (CD_3OD, 125 MHz) 167.8 (C-1), 117.1 (C-2), 162.6 (C-3), 80.5 (C-39), 71.8 (C-23), 68.8 (C-19), 66.7 (C-21), 71.5 (C-9), 71.1 (C-11), 70.8 (C-27), [72.4, 72.4, 72.2, (C-15, 31, 35)], 44.3 (C-10), 44.1 (C-20), 43.5 (C-22), [38.5, 38.4, 38.4, 38.4, 38.4, 38.3, 38.2, 38.1, 37.9, 37.0, 36.9, (C-8, 12, 14, 16, 18, 26, 28, 30, 32, 34, 36)], 35.9 (C-24), 35.8 (C-40), 34.2 (C-4), 32.3 (C-38), 30.6 (C-6), 29.3 (C-5), 26.4 (C-41, 42, 43), 26.0 (C-7), 25.4 (C-44), [24.4, 23.0, 23.0, 22.5, 21.9, 21.4, (C-13, 17, 25, 29, 33, 37)]; ESITOFMS *m/z* 811.62 $[M + Na]^+$; ESITOFMS *m/z* 787.54 $[M - H]^-$; HRESITOFMS *m/z* 811.5907 $[M + Na]^+$ (calcd for $C_{44}H_{84}O_{11}Na$, 811.5906).

Preparation of the Isomerization Product 2-(*E*)-Bastimolide A (3). To a stirred solution of **1** (5.0 mg) in MeOH (1.0 mL) was added 0.5 mL of NaOMe in MeOH. The reaction mixture was stirred at room temparature for 8 h and then quenched with distilled H_2O. The mixture was extracted with EtOAc, and the organic extract was evaporated to dryness. The resulting residue was purified by RP HPLC (Synergic 10 μm C18, 80 Å, 250 × 10 mm, 70% MeOH/H_2O at 3 mL/min, detection at 210, 230, and 250 nm) to obtain pure compound **3** (2.4 mg).

*2-(E)-Bastimolide A (**3**):* white powder; $[\alpha]^{25}_D$ −10 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.86) nm; ECD (0.63 mM, MeOH)

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λ_{max} ($\Delta\varepsilon$) 225 (5.82); IR (KBr) ν_{max} 3379, 2933, 2861, 2360, 2338, 1650, 1457, 1324, 1223, and 672 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) 6.29 (−OH, br s), 6.24 (−OH, br s), 6.20 (−OH, br s), 6.16 (−OH, br s), 6.03 (−OH, d, 3.5), 5.97 (1H, s), 5.80−5.75 (4OH, overlapped), 5.12 (1H, t, 6.0), 4.75 (1H, br s), 4.46 (1H, br s), 4.26 (2H, overlapped), 4.17 (1H, br s), 3.95 (3H, overlapped), 3.85 (1H, s), 2.29 (3H, s), 2.15−1.58 (47H, overlapped), 1.50 (1H, m), 1.41 (2H, m), 1.30 (2H, m), 0.96 (9H, s); ^{13}C NMR (pyridine-d_5, 125 MHz) 167.3 (C-1), 160.7 (C-3), 116.5 (C-2), 80.1 (C-39), 72.1 (C-11), 71.9 (C-9), [71.5, 71.4, 71.3, 71.3, 71.2 (C-15, 23, 27, 31, 35)], 69.5 (C-21), 68.6 (C-19), [45.5, 45.5, 45.0 (C-10, 20, 22)], 41.3 (C-4), [39.4, 39.3, 39.1, 39.0, 39.0, 39.0, 39.0, 39.0, 38.9, 38.7, 38.7, 38.7 (C-8, 12, 14, 16, 18, 24, 26, 28, 30, 32, 34, 36)], 35.1 (C-40), 30.7 (C-38), 30.0 (C-6), 28.0 (C-5), 26.5 (C-41, 42, 43), 26.1 (C-7), [24.0, 23.3, 23.3, 22.9, 22.7, 22.6 (C-13, 17, 25, 29, 33, 37)], 19.2 (C-44); ^1H NMR (CD_3OD, 500 MHz) 5.70 (1H, s), 4.77 (1H, d, 10.5 Hz), 4.00 (1H, t, 5.5 Hz), 3.83 (1H, br s), 3.76 (3H, overlapped), 3.59−3.47 (4H, overlapped), 2.20 (2H, t, 7.0 Hz), 2.15 (3H, s), 1.64−1.28 (50H, overlapped), 0.91 (9H, s); ^{13}C NMR (CD_3OD, 125 MHz): 168.6 (C-1), 161.8 (C-3), 116.7 (C-2), 81.1 (C-39), 72.3 (C-11), 72.3 (C-9), [72.1, 72.0, 71.5, 71.5, 70.8 (C-15, 23, 27, 31, 35)], 69.0 (C-21), 68.5 (C-19), [45.4, 45.1, 44.8 (C-10, 20, 22)], 41.8 (C-4), [38.7, 38.5, 38.4, 38.4, 38.3, 38.3, 38.3, 38.2, 38.2, 38.0, 38.0, 38.0 (C-8, 12, 14, 16, 18, 24, 26, 28, 30, 32, 34, 36)], 35.5 (C-40), 30.9 (C-38), 30.2 (C-6), 28.5 (C-5), 26.4 (C-41, 42, 43), 26.2 (C-7), [23.7, 23.0, 22.9, 22.5, 22.4, 22.3 (C-13, 17, 25, 29, 33, 37)], 19.0 (C-44); HRESITOFMS m/z 789.6078 $[M + H]^+$ (calcd for $C_{44}H_{85}O_{11}$, 789.6086).

Computational Section. Quantum theory is well developed and used in energy calculations, analytic gradients, and true analytic frequencies study. The ECD spectra of compounds **1** and **3** were calculated by time-dependent density functional theory and compared with experimental spectra. Before ECD calculations, all the conformers were optimized by the Gaussian09 program to ensure that all the conformers were the optimum structures with low energetics.[14] After the calculations of ECD for each conformation, Boltzmann statistics were used to simulate their corresponding values, respectively.

Antimalarial Activity. Antimalarial activities of all compounds were assayed following the procedures previously reported, which used Pico-Green to assess parasite growth inhibition by drugs, and chloroquine was used as positive control.[15]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00917.

^1H and ^{13}C NMR, DEPT, HSQC, HMBC, COSY, NOE, ESI-MS, HR-ESI-MS^2, and HR-ESI-MS spectra of the new compound **2**; ECD and calculated ECD data for compounds **1** and **3** (PDF)

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Notes

The authors declare no competing financial interest.

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