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Bahamaolides A and B, Antifungal Polyene Polyol Macrolides from the Marine Actinomycete *Streptomyces* sp.

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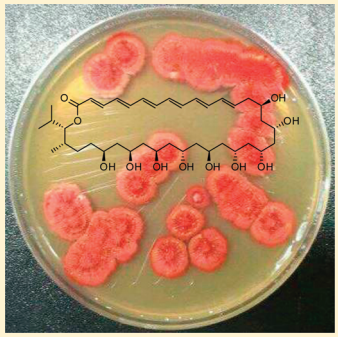
Supporting Information

ABSTRACT: Bahamaolides A and B (**1** and **2**), two new 36-membered macrocyclic lactones, were isolated from the culture of the marine actinomycete *Streptomyces* sp. derived from a sediment sample collected at North Cat Cay in the Bahamas. The planar structures of **1** and **2**, bearing a hexaenone and nine consecutive skipped hydroxy groups, were determined by 1D and 2D NMR, mass, IR, and UV spectra. The absolute configurations of the bahamaolides were established by combined multistep chemical reactions and spectroscopic analysis. Bahamaolide A displayed significant inhibitory activity against *Candida albicans* isocitrate lyase and antifungal activity against various pathogenic fungi.



Actinobacteria are an important source of antibiotics that led the golden era of antibiotic discovery (1940−1960), affording clinically useful antibiotic scaffolds such as aminoglycosides, tetracyclines, erythromycin, vancomycin, and amphotericin.[1] However, the discovery of new bioactive natural products from this promising bacterial group has declined due to redundant studies on heavily investigated terrestrial actinomycetes. Marine environments are now known to harbor unique actinobacterial populations that may produce new secondary metabolites with useful pharmaceutical properties.[2] Despite their biomedical potential, systematic studies on the secondary metabolites of marine actinomycetes have only recently begun.[3] Owing to the focus of efforts on marine microbial natural products over the last 15 years, such as actinomycetes, eubacteria, cyanobacteria, and fungi, the number of novel bioactive chemicals from marine microorganisms has dramatically increased since 1997,[4] resulting in the discovery of drug lead compounds that are currently in clinical trials.[5]

Novel antibacterial compounds such as marinomycin,[6] abyssomicin,[7] marinopyrrole,[8] and lipoxazolidinone[9] have been discovered in marine actinomycetes. Although marine actinomycete-derived antifungal natural products are far less common than their antibacterial analogues, new polyketide spiroketals, the reveromycins,[10] and a new bafilomycin derivative, all with antifungal properties, were recently isolated from marine-derived *Streptomyces* strains.[11] This finding indicates the potential for discovering antifungal lead compounds in marine actinomycetes.

Over the course of our search for new antibiotic lead compounds in marine microbes, we screened various marine-derived actinomycete strains and found that a strain isolated from a seafloor sediment sample collected off North Cat Cay in the Bahamas produces unusual hexaenone-bearing compounds as its major chemical constituents. Further investigation of these secondary metabolites led to the discovery of two new polyene polyol macrocyclic lactones with antifungal activity, bahamaolides A and B (**1** and **2**). Here we report the isolation, structural determination (including absolute configurations), and biological activity of these rare hexaene macrolides.

RESULTS AND DISCUSSION

A bacterial strain CNQ343, *Streptomyces* sp. based on 16S rDNA analysis, was isolated from a sediment sample collected at the depth of 27 m off North Cat Cay in the Bahamas. The actinomycete was cultivated in a seawater-based medium. Time-course chemical analysis of its EtOAc extract by LC/MS revealed the production of polyene compounds with characteristic UV spectra (λ_{max} at 384 nm) and molecular ions $[M + H]^+$ at m/z 709 as major chemical constituents. We then cultivated the bacterium on a large scale (40 L) and purified the polyene compounds, bahamaolides A and B, by the combination of normal-phase and reversed-phase chromatographic techniques.

Bahamaolide A (**1**) was purified as a yellow powder and was determined to have the molecular formula $C_{39}H_{64}O_{11}$ by

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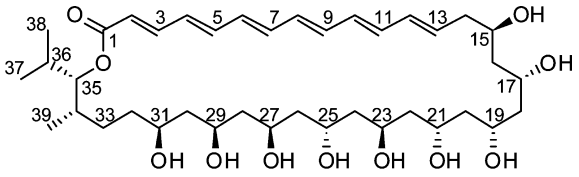
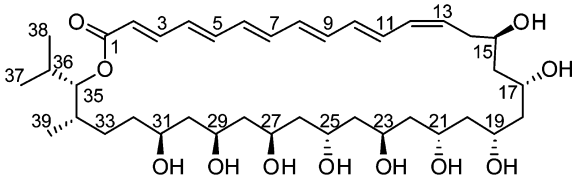
HRFABMS and ^{1}H and ^{13}C NMR data (Table 1). The ^{1}H NMR spectrum of **1** clearly displayed polyunsaturated and



1



2

polyhydroxylated features, with 12 olefinic protons between δ_H 7.63 and 6.12 and 10 protons attached to oxygenated carbons between δ_H 5.08 and 4.03. Further analysis of the ^{1}H NMR spectrum of **1** identified 24 aliphatic protons in the region δ_H 2.75−1.26 and three methyl groups at δ_H 0.96, 0.96, and 0.78. The ^{13}C NMR and DEPT spectra divided the carbon signals into one quaternary carbonyl at δ_C 168.0, 12 olefinic methines from δ_C 146.0 to 121.2, 10 oxygenated methines from δ_C 79.3 to 65.0, 12 aliphatic methylenes from δ_C 48.3 to 30.9, one aliphatic methine at δ_C 30.2, and three methyls at δ_C 20.6, 19.0, and 14.9. Combined analyses of ^{13}C, DEPT, and gHMQC NMR spectra confirmed 55 carbon-bound protons in total; the nine protons unaccounted for in the molecular formula were assigned as exchangeable protons. The IR absorption at 1747 cm^{-1}, coupled with the carbon signal at δ_C 168.0, revealed the existence of an ester functionality. The observation of a UV absorption maximum at 384 nm, 12 olefinic carbons in the ^{13}C NMR spectrum, and corresponding protons in the ^{1}H NMR spectrum indicated that the ester is conjugated to six double bonds. The connectivity from C-1 to C-13 was also confirmed by the array of COSY couplings in both pyridine-d_5 and CD_3OD between the 12 olefinic protons. The HMBC showed a correlation from H-2 (δ_H 6.12) to carbonyl carbon C-1 (δ_C 168.0). Similarly, a combination of gCOSY, gHMQC, and gHMBC experimental data elucidated a partial structure bearing nine hydroxy groups in a repeating 1,3-pattern from C-15 to C-31. All of the ^{1}H and ^{13}C NMR chemical shifts were adequately assigned by detailed analysis of gCOSY, gHMQC, and gHMBC spectroscopic data.

The repeating 1,3-hydroxy group moiety was determined to be connected to the hexaenone through a methylene linker (C-14, H_2-14; δ_C 42.4; δ_H 2.75, 2.60) according to COSY correlations of H-13 (δ_H 6.18)−H_2-14 and of H_2-14−H-15 (δ_H 4.52) and HMBC correlations from H-12 (δ_H 6.32) and H-13 to C-14 and from H_2-14 to C-13 (δ_C 133.9) and C-15 (δ_C 69.1). The other end of this fragment is connected to the adjacent aliphatic methylenes at C-32 and C-33 according to the HMBC correlation from H_2-32 (δ_H 1.97, 1.76) and H_2-33 (δ_H 1.31, 1.26) to C-31 (δ_C 73.3) and the COSY correlation between H_2-32 and H-31. The HMBC correlation from H_2-33 to C-34 (δ_C 34.9) and strong HMBC correlations from the

Table 1. NMR Spectroscopic Data for Bahamaolides A (1) and B (2) in Pyridine-d_5 (800 MHz)

	bahamaolide A (**1**)		bahamaolide B (**2**)	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	168.0, C		167.7, C	
2	121.2, CH	6.12, d (15.0)	121.1, CH	6.14, d (15.0)
3	146.0, CH	7.63, dd (15.0, 11.5)	146.1, CH	7.68, dd (15.0, 11.5)
4	130.3, CH	6.46, m	130.5, CH	6.47, dd (14.5, 11.5)
5	142.2, CH	6.68, dd (14.5, 11.5)	142.3, CH	6.73, dd (14.5, 11.5)
6	132.3, CH	6.38, m	132.7, CH	6.39, m
7	138.8, CH	6.54, dd (14.5, 11.5)	138.7, CH	6.65, dd (14.5, 11.5)
8	132.9, CH	6.37, m	133.5, CH	6.39, m
9	137.1, CH	6.47, m	137.0, CH	6.52, dd (14.5, 11,0)
10	131.9, CH	6.31, m	134.0, CH	6.42, m
11	135.8, CH	6.32, m	130.8, CH	6.89, dd (14.5, 11.0)
12	133.6, CH	6.32, m	130.8, CH	6.29, dd (11.0, 11.0)
13	133.9, CH	6.18, m	131.5, CH	5.76, m
14	42.4, CH_2	2.75, m	37.1, CH_2	3.14, m
		2.60, m		2.68, ddd (13.5, 11.0, 8.5)
15	69.1, CH	4.52, m	69.9, CH	4.44, m
16	44.4, CH_2	2.13, m	43.6, CH_2	2.15, m
		2.10, m		2.01, m
17	67.2, CH	4.70, m	67.3, CH	4.68, m
18	47.6, CH_2	2.25, m	47.3, CH_2	2.20, m
		2.01, m		1.95, m
19	70.7, CH	4.52, m	70.9, CH	4.68, m
20	45.8, CH_2	1.93, m	45.8, CH_2	1.99, m
				1.89, m
21	69.5, CH	4.79, m	69.1, CH	4.83, m
22	47.7, CH_2	1.88, m	47.5, CH_2	1.89, m
		1.82, m		
23	65.0, CH	5.07, m	65.4, CH	5.02, m
24	48.3, CH_2	1.84, m	47.9, CH_2	1.96, m
		1.76, m		1.84, m
25	65.0, CH	5.04, m	65.6, CH	5.01, m
26	48.0, CH_2	1.80, m	47.1, CH_2	2.01, m
				1.94, m
27	69.7, CH	4.74, m	69.5, CH	4.72, m
28	46.1, CH_2	1.94, br d (14.0)	45.9, CH_2	2.07, m
		1.59, m		1.81, m
29	74.3, CH	4.41, m	72.9, CH	4.52, m
30	44.2, CH_2	1.86, m	45.1, CH_2	2.00, m
		1.83, m		1.92, m
31	73.3, CH	4.03, m	72.6, CH	4.06, m
32	36.4, CH_2	1.97, m	36.9, CH_2	2.04, m
		1.76, m		1.79, m
33	30.9, CH_2	1.31, m	31.2, CH_2	1.47, m
		1.26, m		1.39, m
34	34.9, CH	1.75, m	35.0, CH	1.78, m
35	79.3, CH	5.08, br d (9.5)	79.6, CH	5.10, m
36	30.2, CH	1.88, m	30.3, CH	1.92, m
37	20.6, CH_3	0.96, d (6.5)	20.4, CH_3	0.96, d (6.5)
38	19.0, CH_3	0.78, d (6.5)	19.1, CH_3	0.82, d (6.5)
39	14.9, CH_3	0.96, d (6.5)	14.7, CH_3	0.98, d (7.0)

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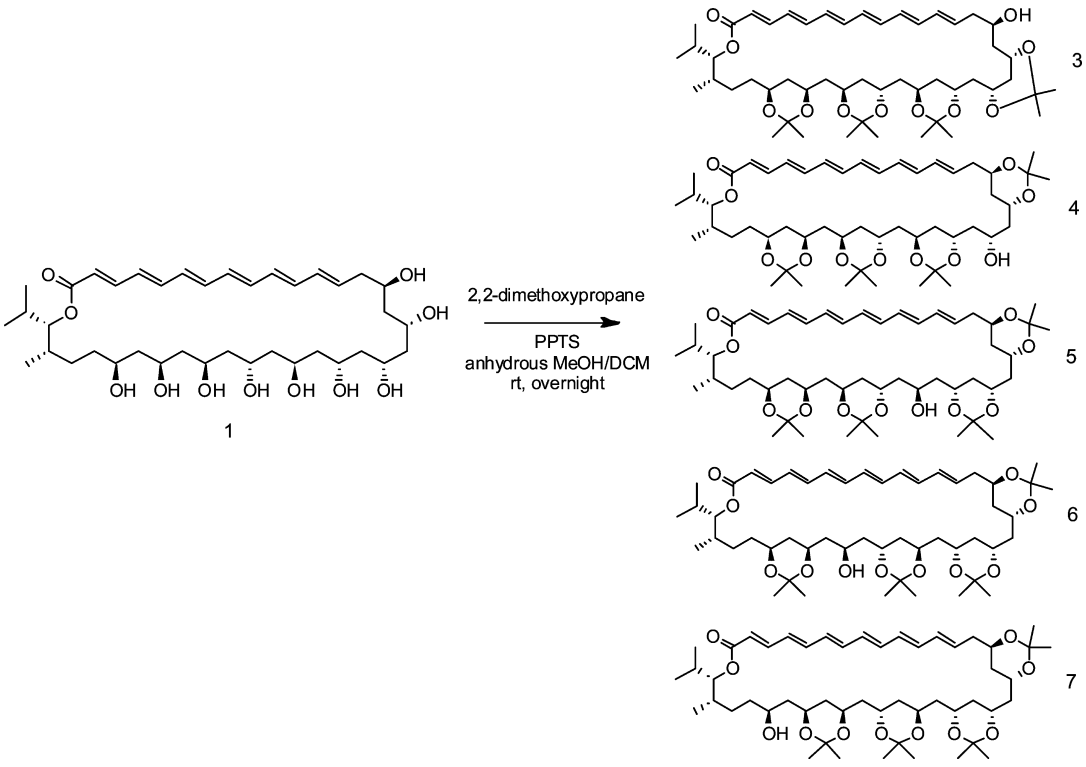

Figure 1. Derivatization of **1** to yield tetraacetonides **3**–**7**.

doublet methyl group (H_3-39, δ_H 0.96) to C-34 and C-33 led to the assigned C-33–C-34–C-39 connectivity. The H-34 methine proton (δ_H 1.75) showed a COSY correlation with H-35 (δ_H 5.08), which connected the C-34 end of the fragment to C-35. This was supported by the HMBC correlation from H_3-39 to C-35 (δ_C 79.3). The final two methyl groups (δ_C 20.6, δ_H 0.96; δ_C 19.0, δ_H 0.78) were assigned as a dimethyl moiety by their common COSY correlation with H-36 (δ_H 1.88) and HMBC coupling to C-36 (δ_C 30.2). This isopropyl group was connected to C-35 according to the HMBC correlation from H_3-37 and H_3-38 to C-35. The hexaenone moiety accounts for seven out of the eight degrees of unsaturation inherent in the molecular formula, which suggests that bahamaolide A (**1**) must possess a single ring. The connectivity of the ring was assigned by long-range coupling from H-35 to C-1 and completed the planar macrolide structure of bahamaolide A (**1**).

The carbinol proton signals are sufficiently separated to completely assign their corresponding chemical shifts in the ^{1}H NMR spectrum in pyridine-d_5, but the olefinic protons overlap significantly in this solvent, which makes the assignment of the alkene geometries challenging. This problem was overcome by changing the NMR solvent to CD_3OD. Although the ^{1}H NMR spectrum at 900 MHz in CD_3OD displayed highly congested signals for the carbinol protons, the alkene protons were well separated without a second-order peak (see Figure S3 and Table S1). On the basis of the large vicinal ^{1}H–^{1}H coupling constants (J = 14.5–15.5 Hz), the alkene configurations were assigned as 2*E*, 4*E*, 6*E*, 8*E*, 10*E*, and 12*E*.

The dimethyl carbon chemical shifts in the acetonide derivatives of a 1,3-diol indicate their relative configurations.[12] When a 1,3-diol is *syn*, its acetonide dimethyl carbons show two distinctive chemical shifts around δ_C 19 and 30, but when it is *anti*, the carbons display nearly identical signals near δ_C 25. To apply this rule to the relative configurations of the repeating 1,3-diol chain moiety, bahamaolide A (**1**) was derivatized using 2,2-dimethoxypropane to yield five tetraacetonide products (**3**–**7**) (Figure 1).

On the basis of the ^{1}H and ^{13}C, COSY, HMQC, and HMBC NMR spectra of the acetonide products (**3**–**7**), compounds **3** and **7** were used to determine the relative polyol configuration. The NMR spectra, including the ROESY spectra of **3** and **7**, were further analyzed to assign the ^{1}H and ^{13}C chemical shifts, particularly of the nine acetonide-protected hydroxy groups.

The ^{1}H, ^{13}C, DEPT, and HMQC NMR spectra of tetraacetonide **3**, which has a free alcohol (δ_H 2.77, d, J = 3.5 Hz) at C-15, showed that eight ^{1}H singlet methyl groups correlated with several methyl carbons (δ_C 30.5–δ_H 1.51; δ_C 30.1–δ_H 1.39; δ_C 25.1–δ_H 1.35; δ_C 24.7–δ_H 1.29; δ_C 24.5–δ_H 1.34; δ_C 24.4–δ_H 1.32; δ_C 19.5–δ_H 1.35; δ_C 19.4–δ_H 1.28), indicating the presence of two *syn* diols and two *anti* diols (Table 2). As shown in Figure 2a, H-17 and H-19 displayed a common ROESY correlation with the H_3-41 methyl group (δ_H 1.28), which is bound to C-41 (δ_C 19.4). This methyl group and another methyl group (C-42: δ_H 1.39–δ_C 30.1) are coupled to the acetal carbon at δ_C 98.8 in the HMBC spectrum, revealing that this acetal structure is in a chair conformation and that the hydroxy groups at C-17 and C-19 are in the *syn* orientation. Because H-21 and H-23 correlate with H_3-44 (δ_H 1.29, δ_C 24.7) and H_3-45 (δ_H 1.35, δ_C 25.1), respectively, which show HMBC correlations to the same acetal carbon (δ_C 100.3), the relative orientation of these two hydroxy groups must be *anti*. The relative configuration of the two alcohols at C-25 and C-27 was established as *anti* on the basis of the chemical shifts of dimethyl carbons C-47 and C-48 (δ_C 24.5 and 24.4, respectively). Similarly, the diol moiety at C-29 and C-31 was found to be *syn* (Figure 2a).

In contrast, the other tetraacetonide (**7**) was revealed to have a free hydroxy group (δ_H 3.52, br s) at C-31 on the basis of

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Table 2. NMR Spectroscopic Data for Tetraacetonides 3 and 7 in Toluene-d_8 (800 MHz)

	tetraacetonide 3		tetraacetonide 7	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	166.5, C		167.2, C	
2	120.7, CH	5.93, d (15.0)	121.1, CH	5.83, d (15.0)
3	145.0, CH	7.51, dd (15.0, 11.5)	146.4, CH	7.40, dd (15.0, 10.5)
4	129.8, CH	6.00, m	130.2, CH	5.96, m
5	140.9, CH	6.13, dd (14.5, 11.0)	141.8, CH	5.97, m
6	135.6, CH	6.09, m	132.5, CH	5.87, m
7	137.3, CH	6.05, dd (14.0, 11.0)	132.4, CH	6.13, m
8	131.3, CH	6.00, m	135.5, CH	6.15, m
9	133.4, CH	6.02, m	136.3, CH	6.10, m
10	131.8, CH	5.96, dd (14.5, 11.0)	135.3, CH	6.15, m
11	134.6, CH	6.11, m	138.1, CH	6.05, dd (14.5, 11.0)
12	132.4, CH	6.01, m	133.1, CH	5.89, m
13	132.2, CH	5.77, m	131.9, CH	6.09, m
14	40.2, CH_2	2.34, ddd (13.5, 3.5, 3.5)	40.5, CH_2	2.69, ddd (13.5, 3.5, 3.5)
		2.33, ddd (13.5, 11.0, 2.5)		1.92, ddd (13.5, 11.0, 2.5)
15	68.5, CH	4.04, m	66.0, CH	4.02, m
16	41.3, CH_2	1.61, ddd (14.0, 5.0, 4.0)	36.5, CH_2	2.24, ddd (14.0, 5.0, 4.0)
		1.57, m		1.63, m
17	67.2, CH	4.15, m	64.9, CH	4.03, m
18	35.9, CH_2	1.24, m	40.9, CH_2	1.84, m
		1.18, m		1.80, m
19	62.9, CH	4.00, m	67.5, CH	3.75, m
20	43.1, CH_2	1.97, m	35.4, CH_2	1.17, m
		1.38, m		0.90, m
21	62.5, CH	3.84, m	65.1, CH	3.91, m
22	39.3, CH_2	1.37, m	40.8, CH_2	1.37, m
		1.24, m		
23	62.4, CH	4.02, m	62.5, CH	4.13, m
24	42.5, CH_2	1.35, m	43.5, CH_2	1.38, m
		1.31, m		1.36, m
25	62.3, CH	4.07, m	62.2, CH	4.23, m
26	38.9, CH_2	1.36, m	43.9, CH_2	1.30, m
		1.25, m		1.20, m
27	65.8, CH	3.99, m	64.7, CH	3.99, m
28	42.6, CH_2	1.98, m	39.3, CH_2	0.97, m
		1.39, m		0.78, m
29	65.7, CH	3.93, m	71.7, CH	3.73, m
30	36.5, CH_2	1.44, m	43.8, CH_2	1.62, m
		1.23, m		1.61, m
31	70.0, CH	3.70, m	73.5, CH	3.70, m
32	34.0, CH_2	1.74, m	34.8, CH_2	1.84, m
		1.58, m		1.71, m
33	29.6, CH_2	1.25, m	31.5, CH_2	1.41, m
				1.09, m
34	34.6, CH	1.64, m	34.3, CH	1.67, m
35	79.3, CH	5.02, dd (10.0, 2.5)	78.3, CH	5.13, dd (10.0, 2.5)
36	29.9, CH	1.87, m	30.6, CH	1.87, m
37	19.9, CH_3	1.02, d (6.5)	20.7, CH_3	1.02. d (6.5)
38	18.5, CH_3	0.76, d (6.5)	18.8, CH_3	0.75, d (6.5)
39	13.7, CH_3	0.98, d (7.0)	15.3, CH_3	0.95, d (7.0)
40	98.8, C		100.2, C	
41	19.4, CH_3	1.28, s	27.7, CH_3	1.46, s
42	30.1, CH_3	1.39, s	25.3, CH_3	1.40, s
43	100.3, C		98.6, C	
44	24.7, CH_3	1.29, s	19.8, CH_3	1.26, s
45	25.1, CH_3	1.35, s	31.0, CH_3	1.40, s
46	100.2, C		100.9, C	
47	24.5, CH_3	1.34, s	25.0, CH_3	1.45, s
48	24.4, CH_3	1.32, s	25.1, CH_3	1.41, s
49	98.4, C		99.1, C	
50	30.5, CH_3	1.51, s	30.7, CH_3	1.39, s
51	19.5, CH_3	1.35, s	20.3, CH_3	1.34, s

^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR spectra. As observed with **3**, the analysis of ^{1}H, ^{13}C, DEPT, and HMQC spectra connected all of the singlet methyl groups to methyl carbons (δ_C 31.0–δ_H 1.39; δ_C 30.7–δ_H 1.39; δ_C 27.7–δ_H 1.46; δ_C 25.3–δ_H 1.40; δ_C 25.1–δ_H 1.41; δ_C 25.0–δ_H 1.45; δ_C 20.3–δ_H 1.34; δ_C 19.8–δ_H 1.26), suggesting the presence of two *syn* and two *anti* diol relationships (Table 2). Specifically, the C-41 and C-42 dimethyl protons (δ_C 27.7–δ_H 1.46; 25.3–δ_H 1.40), which displayed HMBC correlations with the C-40 acetal carbon at δ_C 100.2, coupled through space with the carbinol protons at δ_C 4.03 (H-17) and 4.02 (H-15), respectively. This finding unambiguously established an *anti* relationship (Figure 2b). The *syn* relationship between the two hydroxy groups at C-19 and C-21 was inferred from the chemical shifts of dimethyl carbons C-44 (δ_C 31.0) and C-45 (δ_C 19.8), which were assigned to this acetal ring on the basis of ROESY and HMBC spectra. In a process similar to that used to determine the *anti* diol relationship at C-15 and C-17, the relative configuration of the diol moiety at C-23 and C-25 was established as *anti*, in agreement with the chemical shifts of dimethyl carbons C-47 and C-48 (δ_C 25.0 and 25.1). The two hydroxy groups at C-27 and C-29 were determined to be *syn* on the basis of two distinct carbon peaks corresponding to the C-50 and C-51 methyl groups (δ_C 30.7 and 20.3). As a result of the spectroscopic analysis of tetraacetonides **3** and **7**, the relative configuration of the polyol moiety was assigned as 15*R**, 17*S**, 19*S**, 21*S**, 23*S**, 25*R**, 27*S**, 29*S**, and 31*S**.

To determine the relative configuration of the stereogenic centers at C-34 and C-35, hetero half-filtered TOCSY (HETLOC) and ROESY experiments were performed in CD_3OD, which avoids the overlap of the methyl groups. The HETLOC NMR spectroscopic data showed that the H-35–C-39 $^3J_{CH}$ value was 6.0 Hz, which is large enough to assign an *anti* relationship in oxygenated systems.[13] In the case where H-35 is *anti* to C-39, only one rotamer can satisfy the observed ROESY correlations around the stereogenic centers at C-34 and C-35 (Figure 3). Therefore, the relative configurations of C-34 and C-35 were assigned as 34*S** and 35*S**.

The absolute configuration of the repeating 1,3-diol moiety in bahamaolide A (**1**) was determined by the application of the modified Mosher's method.[14] The tetraacetonide product (**7**) was treated with either (*R*)- or (*S*)-MTPA-Cl to yield (*S*)- and (*R*)-MTPA esters **8** and **9**, respectively. The ^{1}H chemical shifts around the esterified position (C-31) of **8** and **9** were adequately assigned using ^{1}H, COSY, HMQC, and HMBC NMR experiments. The analysis of $\Delta\delta_{S-R}$ values allowed the

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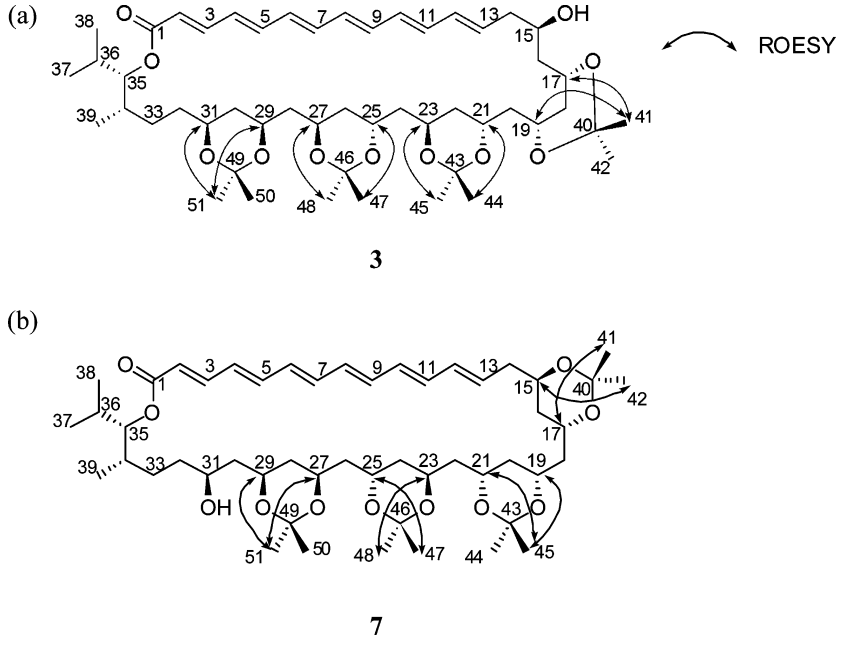

Figure 2. Key ROESY correlations of tetraacetonide (a) **3** and (b) **7** for assigning the relative configurations of the repeating 1,3-diol moiety of **1**.

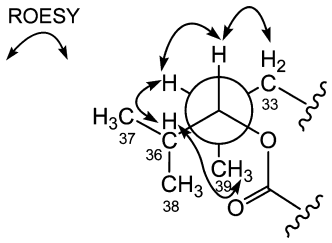

Figure 3. ROESY correlations determining the relative configurations of C-34 and C-35 (in CD_3OD).

absolute configuration at C-31 to be assigned as *S* (Figure 4). The relative configurations assigned above were used to assign the absolute configuration of the polyol structure as 15*R*, 17*S*, 19*S*, 21*S*, 23*S*, 25*R*, 27*S*, 29*S*, and 31*S*.

The absolute configuration of the asymmetric center at C-35 was also determined through multistep chemical derivatizations, including an MTPA esterification. We attempted a methanolysis of **1** to obtain a free hydroxy at C-35. However, possibly due to steric hindrance, the yield of the reaction was very low, and prolonging the reaction resulted in the formation of the saponification product. Consequently, bahamaolide A was treated instead with potassium hydroxide to produce linear saponification product **10**, followed by methylation with TMS-diazomethane to afford linear methyl ester **11**. In the final step toward determining the absolute configuration at C-35, methyl ester **11** was derivatized using either (*R*)- or (*S*)-MTPA-Cl to yield bis-(*S*)- and (*R*)-MTPA esters **12** and **13**, respectively (Figure 5). The ^{1}H chemical shifts around C-31 and C-35 were assigned by ^{1}H and COSY NMR spectroscopic analysis. Because the 31*S* configuration had already been assigned, we could utilize the distribution of $\Delta\delta_{S-R}$ values for a 1,*n*-diol (*n* odd) to establish the absolute configuration at C-35.[15] The relative configurations of 34*S** and 35*S** were used to assign the absolute configuration at C-34 as *S*, thereby completing the stereochemical assignment of bahamaolide A (Figure 6).

Bahamaolide B (**2**) was obtained as a yellow powder, and the molecular formula was determined to be $C_{39}H_{64}O_{11}$ by HRFABMS and ^{1}H and ^{13}C NMR spectroscopic data (Table 1). The general features of the NMR, UV, and mass spectra of **2** were very similar to those of **1**, indicating a structurally related isomer of **1**. Detailed analysis of ^{1}H and ^{13}C, COSY, HSQC, HMBC, and ROESY NMR spectra revealed that the only difference between **1** and **2** was in the alkene geometry at C-12. The H-12−H-13 coupling constant was 11.0 Hz, characteristic of the 12*Z* configuration supported by ROESY analysis.

To determine the absolute configuration of **2**, the alkenes in **1** and **2** were first reduced by hydrogenation. The two hydrogenation products (**12** and **13**) derived from **1** and **2** displayed virtually identical ^{1}H NMR spectra (Figures S27 and S28) and CD spectra (Figure S29), which enabled us to assume an absolute configuration for the stereogenic centers in **2** identical with those assigned in **1**.

Because polyene polyol macrolides, such as the clinically used amphotericin B, have been reported to possess antifungal

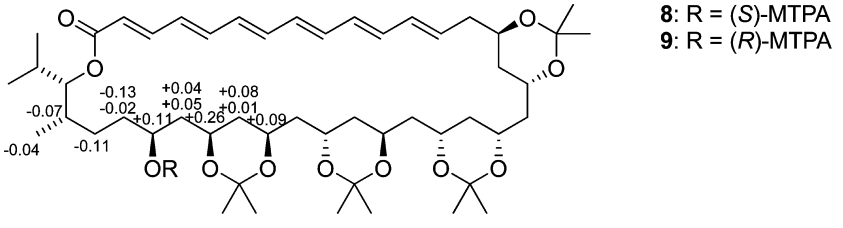

Figure 4. $\Delta\delta_{S-R}$ values of **8** and **9** in toluene-d_8.

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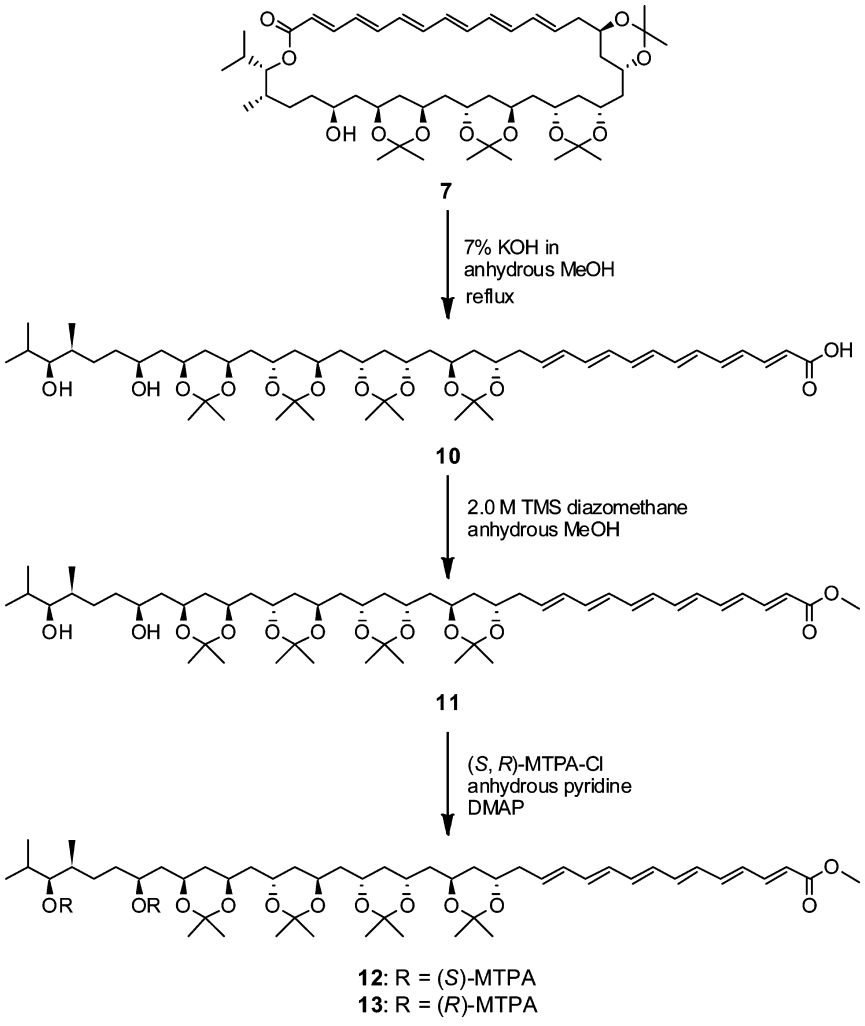

Figure 5. Multistep chemical modification of **7** to afford bis-(*S*)- and (*R*)-MTPA esters **12** and **13**.

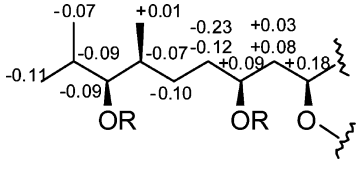

Figure 6. $\Delta\delta_{S-R}$ values of **12** and **13** in pyridine-d_5.

properties, the biological activities of **1** and **2** were initially evaluated by antifungal assays with various pathogenic fungal strains, including *Aspergillus fumigatus* HIC 6094, *Trichophyton rubrum* IFO 9185, *T. mentagrophytes* IFO4 0996, and *Candida albicans* ATCC 10231. Bahamaolide A (**1**) showed moderate antifungal activity against all tested fungal strains with an MIC value of 12.5 μg/mL, but bahamaolide B (**2**) did not display significant inhibitory activity against any tested strains. Because bahamaolide A exhibited cell-based antifungal activity, we then tested the inhibitory activities of the bahamaolides against *C. albicans* isocitrate lyase (ICL), which is a recently highlighted antifungal target enzyme utilizing multiple metabolic networks and carbon sources and thus plays an important role in fungal pathogenesis.[16] Interestingly, bahamaolide A displayed significant ICL inhibitory activity, with an IC_{50} of 7.65 μg/mL (10.8 μM), whereas amphotericin B did not inhibit ICL, even at high concentrations (100 μg/mL). Bahamaolide B showed weak ICL inhibitory activity, with an IC_{50} of 90 μg/mL (127 μM). In molar concentration, bahamaolide A's IC_{50} (10.8 μM) is lower than that of the positive control compound, 3-nitropropionate (IC_{50} 20.1 μM).

Bahamaolides A and B (**1** and **2**) were also tested in antibacterial assays against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633, and *Micrococcus luteus* IFO 12708) and Gram-negative bacteria (*Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 3851, and *Escherichia coli* ATCC 35270). However, these compounds did not show significant activity in these assays.

The cytotoxicities of **1** and **2** were measured against several human cancer cell lines, including A549 (lung cancer), HCT116 (colon cancer), MDA-MB-231 (breast cancer), and SK-HEP-1 (liver cancer). Bahamaolides A and B did not display significant inhibitory activity even at high concentrations (100 μg/mL).

Bahamaolides A and B (**1** and **2**), both bearing a hexaenone and contiguous 1,3-diol groups, are structurally new 36-membered macrocyclic lactones belonging to the hexaene macrolide class, apparently derived from the polyketide synthase type I pathway. Hexaene macrolides are quite rare in nature, even though pentaene and heptaene macrolides are common.[17,18] To the best of our knowledge, the only other well-characterized hexaene-class macrolides are dermostatins A and B, isolated from *Streptomyces viridogriseus*.[12,19] Compared with dermostatin A (Figure 7), the bahamaolides have one less alkene at C-32 and

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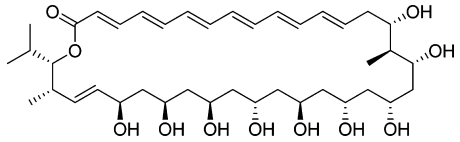

Figure 7. Structure of dermostatin A.

one less methyl group at C-16. Furthermore, the absolute configuration of the C-15 stereogenic center in bahamaolides A and B is opposite that of dermostatin A. This inconsistency could be due to modular variations in the biosynthetic pathways of both dermostatins and bahamaolides, which may involve different ketoreductases that possess opposing stereochemical specificities.[20]

The structural determination of polyene polyol macrolides is still challenging due to overlapping signals in the polyene and polyol NMR regions; the complete NMR assignment of the dermostatins has not yet been achieved.[12,19] We overcame this difficulty using two different NMR solvents (CD_3OD, pyridine-d_5) and high-field (800 and 900 MHz) NMR spectrometers. In addition, the absolute configuration of every stereogenic center in bahamaolide A was unambiguously determined by multistep chemical derivatization and subsequent spectroscopic analyses.

In biological studies, several classes of ICL inhibitors have been reported previously, including terpenoids, indole alkaloids, bromophenols, and hydroquinones.[21] However, no compounds belonging to the polyene polyol macrolide class, one of the most popular antifungal agent groups, had been identified as ICL inhibitors. Bahamaolide A is the first polyene polyol macrolide ICL inhibitor. The discovery of bahamaolides A and B from a marine actinomycete and the biological significance of bahamaolide A as a *C. albicans* isocitrate lyase inhibitor provide additional evidence to support the claim that marine environments are a prolific source of new bioactive chemical entities.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a Jasco P-1020 polarimeter with a 5 cm cell. UV spectra were measured using a Perkin-Elmer Lambda 35 UV/vis spectrometer. CD spectra were collected with a JACSO model J-715 CD spectrometer with a 0.5 cm cell. IR spectra were acquired using a Thermo Nicolet iS10 IR spectrometer. Low-resolution LC/MS data were obtained with an Agilent Technologies 6130 quadrupole mass spectrometer coupled with an Agilent Technologies 1200 series HPLC using a reversed-phase C_{18} column (Phenomenex Luna, 100 mm × 4.60 mm). High-resolution fast-atom bombardment (HRFAB) mass spectra were acquired with a Jeol JMS700 high-resolution mass spectrometer at the Korea Basic Science Institute in Daegu. NMR spectra were obtained on Bruker 800 and 900 MHz NMR spectrometers at the Korea Basic Science Institute in Ochang and Bruker 500 MHz and Varian Inova 300 MHz NMR spectrometers at the College of Pharmacy, Seoul National University.

Bacterial Identification. The bacterium (strain CNQ343) was isolated from a sediment sample collected at the depth of 27 m from North Cat Cay in the Bahamas in 2000. The chromosomal DNA from *Streptomyces* sp. CNQ343 was extracted using a G-spin genomic DNA extraction kit, and the isolated DNA was amplified by PCR using universal primers 27F and 1492R, corresponding to position 27 in the forward direction and 1492 in the reverse direction of the *E. coli* 16S rDNA sequence. For PCR, the reaction elements included 5 μL of the template DNA, 5 μL of 10× Taq buffer, 1 μL of 10 mM mixed dATP, dTTP, dGTP, and dCTP, 1 μL of sense-27F primer (10 pmol/μL), 1 μL of antisense-1492R primer (10 pmol/μL), and 5 U of *Taq* DNA polymerase, which were prepared in a final volume of 50 μL. The PCR thermal conditions were set as follows: preheating for denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min 30 s, and additional extension at 72 °C for 10 min. The sequencing analysis data obtained from COSMO Co., Ltd. showed that CNQ343 was most closely related to the partial sequence of *Streptomyces chartreusis* (98% identity), identifying this strain as *Streptomyces* sp. The strain was deposited in The National Center for Biotechnology Information, United States, with an accession number of AGDE01000038.

Cultivation and Extraction. To maximize the yield of bahamaolides A and B (**1** and **2**), serial cultivation procedures were employed. The bacterium on a YPM agar plate was used to inoculate 2 mL of seawater-based YPM medium (4 g of mannitol, 2 g of yeast extract, 2 g of peptone in 1 L of artificial seawater) in a 50 mL flask. After a three-day cultivation at 30 °C with shaking at 200 rpm, 10 mL of the culture was used to inoculate 200 mL of YPM medium in a 500 mL flask with further incubation at 30 °C with shaking at 200 rpm for three days. Finally, 30 mL of the culture in a 500 mL flask was used to inoculate 2.8 L Fernbach flasks containing 1 L of YPM medium. In total, 40 L of bacterial culture was incubated at 30 °C with shaking at 200 rpm. After seven days of cultivation, sterilized XAD-7 (20 g/L) resin was added to obtain organic products, and the culture, with resin, was shaken at 200 rpm for 2 h. The resin was filtered through cheesecloth, washed with distilled water, and eluted with acetone. The acetone extract was dried *in vacuo* to yield 2 g of extract.

Isolation of Bahamaolides A and B (1 and 2). The extract was fractionated by flash column chromatography on silica gel (100 g) using a step elution with combinations of hexane, EtOAc, and MeOH (hexane/EtOAc, 1:1; 100% EtOAc; EtOAc/MeOH, 10:1, 5:1, 2:1; and 100% methanol). Bahamaolides A and B eluted in the ethyl acetate/methanol (5:1) fraction. This fraction was purified further by semipreparative reversed-phase HPLC (Phenomenex Luna 5 μm C_8 (2) 250 × 10.0 mm column, flow rate 2 mL/min, UV 360 nm detection) using isocratic 40% aqueous CH_3CN to afford bahamaolide A (36 mg) and bahamaolide B (6 mg) as yellow powders. Purified bahamaolides A and B (**1** and **2**) eluted at 39 and 41 min in the HPLC run, respectively.

*Bahamaolide A (**1**):* yellow powder, $[\alpha]^{25}_{D}$ −190 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 384 (5.48) nm; IR (neat) ν_{max} 3370, 2925, 1698, 1559 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) see Table 1; HRFABMS *m/z* 731.4341 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{11}Na$, 731.4346).

*Bahamaolide B (**2**):* yellow powder, $[\alpha]^{25}_{D}$ −130 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 384 (5.45) nm; IR (neat) ν_{max} 3354, 2927, 1695, 1564 cm^{-1}; For ^1H and ^{13}C NMR (pyridine-d_5) see Table 1; HRFABMS *m/z* 731.4344 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{11}Na$, 731.4346).

Preparation of Tetraacetonide Derivatives of 1 (3−7). Pyridinium *p*-toluenesulfonate (5 mg) and distilled 2,2-dimethoxypropane (4 mL) were added to a solution of bahamaolide A (20 mg) in anhydrous MeOH (200 μL) and anhydrous CH_2Cl_2 (2 mL) at room temperature (rt). The mixture was stirred in the dark under argon for 48 h, and then the reaction was quenched with saturated $NaHCO_3$ solution. The mixture was fractionated using a step gradient elution system with H_2O/MeOH (20%, 40%, 60%, 80%, and 100% MeOH in H_2O). The 100% MeOH fraction contained the five desired tetraacetonide derivatives and was purified on a reversed-phase C_{18} column (Phenomenex Luna 5 μm C_{18} (2) 250 × 10.0 mm column, flow rate 2 mL/min, UV 360 nm detection) using a solvent gradient (50% to 100% aqueous MeOH) for 20 min and 100% MeOH after 20 min. The following five derivatives (**3**−**7**) were obtained: tetraacetonide **3** (3.0 mg) at t_R 32.0 min, tetraacetonide **4** (2.5 mg) at t_R 32.5 min, tetraacetonide **5** (0.5 mg) at t_R 36.0 min, tetraacetonide **6** (5 mg) at t_R 38.0 min, and tetraacetonide **7** (4 mg) at t_R 41.0 min. The molecular formulas of the five derivatives were each confirmed as being $C_{51}H_{80}O_{11}$ by ESIMS analysis ($[M + Na]^+$ *m/z* at 891.5). For ^1H and ^{13}C NMR data of **3** and **7**, see Table 2.

MTPA Esterification of Tetraacetonide 7. Tetraacetonide **7** (4.0 mg) was separated into two equal portions and dried completely under high vacuum. Freshly distilled anhydrous pyridine (1 mL) and a catalytic amount of dimethylaminopyridine were added to each

portion. The reaction mixtures were stirred at room temperature for 10 min. Either (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA) (30 μL) was added to each vial. The reactions were quenched with MeOH after 1 h. The products were purified on a reversed-phase C_{18} column (Phenomenex Luna 5 μm C_{18} (2) 250 × 10.0 mm column, flow rate 2 mL/min, UV 360 nm detection) using a gradient system (40% to 100% aqueous MeOH) for 35 min and 100% MeOH after 35 min. (*S*)-MTPA ester **8** (1.2 mg) and (*R*)-MTPA ester **9** (1.2 mg) eluted at 64.5 and 64.2 min, respectively. The molecular formulas of the two derivatives (**8** and **9**) were each confirmed as being $C_{61}H_{87}F_3O_{13}$ by ESIMS analysis ($[M + Na]^+$ *m*/*z* at 1107.5). The $\Delta\delta_{S-R}$ values around the stereogenic centers of MTPA esters **8** and **9** were assigned by ^{1}H NMR, COSY, HMQC, and HMBC NMR experiments.

Saponification of Tetraacetonide 7. KOH (7%) was added to a room-temperature solution of tetraacetonide **7** (7.0 mg) in anhydrous MeOH (2 mL).[22] The solution was stirred at reflux for 24 h and quenched with saturated NH_4Cl solution. The reaction mixture was fractionated using a step gradient elution system with H_2O/MeOH (20%, 40%, 60%, 80%, and 100% MeOH in H_2O). The 100 MeOH fraction contained the desired product and was dried *in vacuo*. It was purified on a reversed-phase C_{18} column (Phenomenex Luna 5 μm C_{18} (2) 250 × 10.0 mm column, flow rate 2 mL/min, UV 360 nm detection) using a gradient system (50% to 100% aqueous MeOH) for 20 min and 100% MeOH after 20 min. Saponification product **10** (5.0 mg) eluted at 30.0 min. The molecular formula of **10** was confirmed as being $C_{51}H_{82}O_{12}$ by ESIMS analysis ($[M + Na]^+$ *m*/*z* at 909.5).

Methylation of Saponification Product 10. The purified saponification product (**10**, 5.0 mg) was dried under high vacuum and dissolved in anhydrous MeOH (2 mL). A solution of 2.0 M TMS-diazomethane in tetrahydrofuran (3 mL) was added to the solution. The solution was stirred for 2 h, and the reaction was quenched by adding acetic acid. The mixture was fractionated using a step gradient elution system with H_2O/MeOH (20%, 40%, 60%, 80%, and 100% MeOH in H_2O). The 100% MeOH fraction contained methylated product **11** and was purified on a reversed-phase C_{18} column (Phenomenex Luna 5 μm C_{18} (2) 250 × 10.0 mm column, flow rate 2 mL/min, UV 360 nm detection) using a gradient system (50% to 100% aqueous MeOH) for 20 min and 100% MeOH after 20 min. Methyl ester **11** (3.0 mg) eluted at 31.5 min in this HPLC run. The molecular formula of **11** was confirmed as being $C_{52}H_{84}O_{12}$ by ESIMS analysis ($[M + Na]^+$ *m*/*z* at 923.5).

Methyl ester 11: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.29 (1H, dd, *J* = 15.0, 10.5 Hz), 6.80 (1H, dd, *J* = 15.0, 10.5 Hz), 6.55 (1H, dd, *J* = 15.0, 10.0 Hz), 6.51−6.26 (6H, m), 6.19 (1H, dd, *J* = 15.0, 10.5 Hz), 5.96 (1H, d, *J* = 15.0 Hz), 5.77 (1H, m), 5.75 (1H, m), 5.23 (1H, m), 4.25 (1H, m), 4.07 (1H, m), 4.04−3.77 (8H, m), 3.65 (3H, s), 2.86 (1H, m), 2.26 (1H, m), 1.67−1.37 (8H, m), 1.33 (3H, s), 1.31 (3H, s), 1.30−1.11 (10H, m), 1.26 (3H, s), 1.25 (3H, s), 1.22 (3H, s), 1.22 (3H, s), 1.22 (3H, s), 1.21 (3H, s), 1.02 (1H, m), 0.94 (1H, m), 0.85 (3H, d, *J* = 6.5 Hz), 0.78 (3H, d, *J* = 7.0 Hz), 0.75 (3H, d, *J* = 7.0 Hz), 0.52 (1H, m), 0.43 (1H, m).

MTPA Esterification of Methyl Ester 11. Methyl ester **11** (3.0 mg) was separated into two equal portions and placed into separate vials. The same procedure described for the MTPA esterification of tetraacetonide **7** was performed for the esterification of **11** with either (*R*)- or (*S*)-MTPA chloride and yielded bis-(*S*)- and bis-(*R*)-MTPA esters **12** and **13**, respectively. The reactions were monitored by LC/MS and quenched with MeOH after 3 h. Bis-MTPA esters **12** and **13** were purified on a reversed-phase C_{18} column (Phenomenex Luna 5 μm C_{18} (2) 250 × 10.0 mm column, flow rate 2 mL/min, UV 360 nm detection) using a gradient system (40% to 100% aqueous MeOH (0.1% formic acid)) for 35 min and 100% MeOH (0.1% formic acid) from 35 to 70 min. Bis-(*S*)-MTPA ester **12** (0.2 mg) and bis-(*R*)-MTPA ester **13** (0.5 mg) eluted at 62 and 63 min, respectively. The molecular formulas of **12** and **13** were each confirmed as being $C_{72}H_{98}F_6O_{16}$ by ESIMS analysis ($[M + Na]^+$ *m*/*z* at 1355.6). The $\Delta\delta_{S-R}$ values around the stereogenic centers of the two derivatives were assigned by ^{1}H and COSY NMR experiments.

Hydrogenation of 1 and 2. Bahamaolides A and B (**1** and **2**), each in a separate vial, were suspended with a 10% Pd/C catalyst in absolute EtOH (1.5 mL). The reaction mixtures were hydrogenated under 1 atm of H_2 at rt. Each product was filtered through a polytetrafluoroethylene syringe filter and dried *in vacuo*. The molecular formulas of the resultant products **14** and **15** (derived from bahamaolides A and B, respectively) were each confirmed to be $C_{39}H_{76}O_{11}$ by ESIMS analysis ($[M + H]^+$ *m*/*z* at 721.5).

Hydrogenation product 14: CD (2.1 × 10^4 M, MeOH), λ_{max} (Δε) 314 (+0.10), 362 (+0.40), 397 (+0.28), 430 (+0.70) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.33 (1H, m), 4.68 (1H, dd, *J* = 8.0, 3.5 Hz), 4.07 (2H, m), 4.01 (2H, m), 3.95 (1H, m), 3.89 (1H, m), 3.81 (1H, m), 3.66 (1H, m), 2.34 (2H, m), 2.18 (2H, m), 2.02 (1H, m), 1.91 (1H, m), 1.77 (1H, m), 1.69−1.50 (13H, m), 1.50−1.39 (3H, m), 1.39−1.24 (25H, m), 0.90 (3H, d, *J* = 6.5 Hz), 0.90 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz).

Hydrogenation product 15: CD (2.1 × 10^4 M, MeOH), λ_{max} (Δε) 314 (+0.10), 362 (+0.43), 397 (+0.32), 430 (+0.72) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.33 (1H, m), 4.68 (1H, dd, *J* = 8.0, 3.5 Hz), 4.07 (2H, m), 4.01 (2H, m), 3.95 (1H, m), 3.89 (1H, m), 3.81 (1H, m), 3.66 (1H, m), 2.34 (2H, m), 2.18 (2H, m), 2.02 (1H, m), 1.91 (1H, m), 1.77 (1H, m), 1.69−1.50 (13H, m), 1.50−1.39 (3H, m), 1.39−1.24 (25H, m), 0.90 (3H, d, *J* = 6.5 Hz), 0.90 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz).

Antifungal Activity Assay. YPD medium (1% yeast extract, 2% peptone, 2% dextrose) was used for cultivation of *Candida albicans*. After incubation for 48 h at 28 °C, cells were harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes* were plated on potato dextrose agar and incubated for 2 weeks at 28 °C. Spores were washed three times with sterile distilled water and resuspended in potato dextrose broth (PDB, Difco) to prepare an initial inoculum size of 10^5 spores/mL. We mixed 90 μL of PDB (10^4 cells/mL) with 10 μL of test compound solutions (bahamaolides A and B) in 5% DMSO in each well of a 96-well plate. A culture of DMSO (0.5%) was used as a solvent control, and a culture supplemented with amphotericin B was used as a positive control. Culture plates were incubated at 28 °C for 48−72 h. The MIC values were determined at the lowest concentration where test compounds inhibited fungal growth.

ICL Activity Assay. ICL enzyme activity was determined using Dixon and Kornberg's method.[23] The basic concept of this method is to spectrophotometrically measure the formation of glyoxylate phenylhydrazone at 324 nm in the presence of phenylhydrazine and isocitrate. A 1 mL aliquot of the reaction mixture contained 20 mM sodium phosphate buffer (pH 7.0), 1.27 mM *threo*-DL (+) isocitrate, 3.75 mM $MgCl_2$, 4.1 mM phenylhydrazine, and 2.5 μg/mL purified ICL.[24] The reaction was performed at 37 °C for 30 min with and without a prescribed concentration of the inhibitor dissolved in DMSO (final concentration, 1%). The effect of the inhibitor on ICL was calculated as a percentage, relative to solvent-treated control, and the IC_{50} values were calculated using nonlinear regression analysis (percent inhibition versus concentration). Protein concentration was determined by the method of Bradford[25] using Bio-Rad protein assay kit (Bio-Rad) and bovine serum albumin as a standard. The ICL inhibitor 3-nitropropionate was used as a positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of **1** and **2** and their derivatives are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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