J. Nat. Prod. **2001,** *64,* 1169−1173

Haterumaimides F−I, Four New Cytotoxic Diterpene Alkaloids from an Ascidian *Lissoclinum* Species

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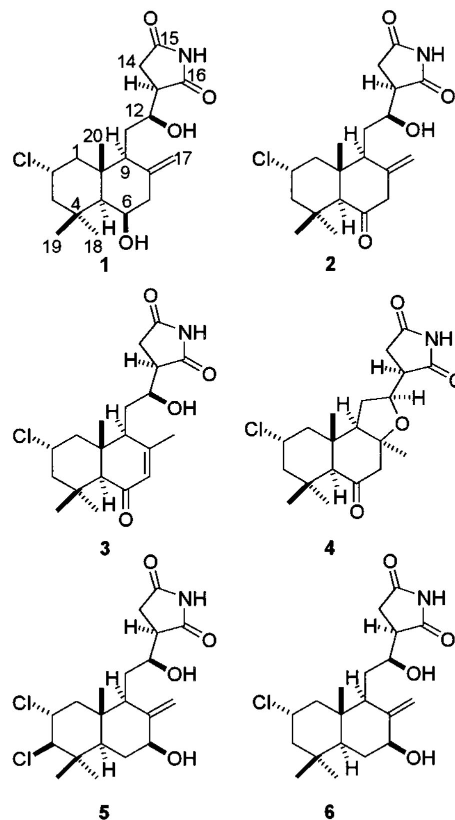
Received February 9, 2001

Four new monochlorinated diterpene alkaloids, haterumaimides F−I (**1**−**4**), and two known ones, dichlorolissoclimide and chlorolissoclimide, were isolated from an ascidian *Lissoclinum* sp. Their structures with absolute stereochemistries were elucidated by chemical and spectral analyses. Haterumaimides F−I (**1**−**4**) inhibited the first cleavage of fertilized sea urchin eggs and exhibited potent to weak cytotoxicities against P388 cells.

Among marine organisms, ascidians have been proven to be a prolific source of unprecedented bioactive secondary metabolites that include a diverse array of amino acid-derived alkaloids, cyclic peptides, and a small, but significant number of acetogenins.[1,2] As part of our ongoing screening for bioactive metabolites from Okinawan marine organisms,[3−5] we investigated an ascidian collected off the coast of Hateruma Island and identified as a *Lissoclinum* sp. Bioassay-guided fractionation led to the isolation of four new cytotoxic diterpene alkaloids, haterumaimides F−I (**1**−**4**), together with two known compounds, dichlorolissoclimide (**5**) and chlorolissoclimide (**6**), which contain a very rare succinimide moiety. This class of diterpene alkaloid, dichlorolissoclimide (**5**), was first isolated from the New Caledonia ascidian *Lissoclinum voeltzkowi* Michaelson by Verbist and co-workers in 1991,[6] and its absolute stereochemistry was determined by X-ray crystallography.[7] Haterumaimides A−E were recently reported from our laboratory.[8] These alkaloids are extremely important because of their potential use as antitumor drugs and physiological tools.[9] In this report, we describe the isolation, structure elucidation, absolute stereostructures, and cytotoxicities of haterumaimides F−I (**1**−**4**).



Results and Discussion

The ascidian *Lissoclinum* sp. was collected off the coast of Hateruma Island, Okinawa, Japan, in June 1996, and kept frozen until use. The animal specimens (1.0 kg, wet wt) were first extracted with acetone. The acetone extract was partitioned between H_2O and EtOAc. The EtOAc extract completely inhibited the cell division of fertilized sea urchin eggs at 10 ppm. The EtOAc extract (3.4 g) was suspended in aqueous MeOH (1:1) and then successively partitioned between the aqueous MeOH and hexane, $CHCl_3$, and 1-BuOH. Cytotoxic activity was found in the $CHCl_3$ extract, while the hexane and 1-BuOH extracts were inactive. Bioassay-guided fractionation of the $CHCl_3$ extract (2.5 g) by a series of chromatographic processes, including a silica gel column, ODS column, HPLC on Si60, and reversed-phase HPLC on ODS, led to the isolation of four new diterpene alkaloids, haterumaimide F (**1**, 0.00049% of wet ascidian), haterumaimide G (**2**, 0.00105%), haterumaimide H (**3**, 0.00111%), and haterumaimide I (**4**, 0.00235%), together with known[6,10] dichlorolissoclimide (**5**, 0.01%) and chlorolissoclimide (**6**, 0.01%).

The molecular formula of haterumaimide F (**1**) was deduced to be $C_{20}H_{30}ClNO_4$ based on high-resolution FABMS [*m/z* 384.1940 $(M + H)^+$, Δ−0.2 mmu and *m/z* 386.1920 $(M + H)^+ + 2$; intensity ratio (3:1)]. The ^1H and ^{13}C NMR data of haterumaimide F (**1**) are presented in Tables 1 and 2. The deshielded carbon signals resonating at δ 181.0 (s) and 178.7 (s) indicated the presence of two ester, amide, and/or imide functional groups. IR (film) absorption bands at ν_{max} 1710 and 1705 cm^{-1} further supported the presence of these two carbonyls in **1**. Two more deshielded carbon signals at δ 144.3 (s) and 109.5 (t) together with proton signals at δ 4.86 (brs) and 4.82 (brs) clearly demonstrated the presence of an exomethylene moiety in the molecule. Haterumaimide F, therefore, must

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10.1021/np010066n CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 08/31/2001

Table 1. ^{1}H NMR[a] (DMSO-d_6) Data for Haterumaimides F−I (**1**−**4**)

position	**1** (mult, *J*/Hz)	**2** (mult, *J*/Hz)	**3** (mult, *J*/Hz)	**4** (mult, *J*/Hz)
1β	2.06 (ddd, 12.0, 4.0, 1.5)	2.20 (ddd, 12.0, 4.0, 1.5)	2.21 (ddd, 12.0, 4.0, 1.5)	2.01 (ddd, 12.0, 3.5, 1.5)
1α	1.31 (t, 12.0)	1.50 (t, 12.0)	1.59 (t, 12.0)	1.52 (t, 12.0)
2	4.33 (tt, 12.0, 4.0)	4.34 (tt, 12.0, 4.0)	4.33 (tt, 12.0, 4.0)	4.27 (tt, 12.0, 3.5)
3β	1.81 (ddd, 12.0, 4.0, 1.5)	1.78 (ddd, 12.0, 4.0, 1.5)	1.80 (ddd, 12.0, 4.0, 1.5)	1.79 (ddd, 12.0, 3.5, 1.5)
3α	1.45 (t, 12.0)	1.43 (t, 12.0)	1.48 (dd, 12.5, 12.0)	1.52 (t, 12.0)
5	1.11 (brd, 4.0)	2.51 (s)	2.26 (s)	2.10 (s)
6	4.23 (m)			
7β	2.26 (dd, 13.0, 2.5)	2.84 (d, 13.5)	5.73 (q, 1.5)	2.43 (d, 18.5)
7α	2.15 (brd, 13.0)	3.35 (d, 13.5)		2.61 (d, 18.5)
9	1.63 (m)	2.29 (brdd, 8.5, 7.5)	2.47 (ddd, 7.5, 5.4, 1.5)	2.17 (dd, 9.0, 7.5)
11a	1.63 (m)	1.62 (ddd, 12.5, 8.5, 2.5)	1.56 (ddd, 12.0, 9.5, 5.4)	2.10 (dt, 12.0, 9.0)
11b	1.36 (ddd, 11.5, 10.5, 6.0)	1.48 (m)	1.46 (m)	1.54 (ddd, 12.0, 9.0, 5.5)
12	4.00 (dddd, 10.5, 5.5, 5.0, 2.5)	4.05 (m)	4.03 (dddd, 9.5, 5.5, 4.5, 2.5)	4.24 (ddd, 9.0, 5.5, 3.0)
13	2.86 (ddd, 9.5, 4.5, 2.5)	2.98 (ddd, 9.0, 4.8, 2.5)	3.08 (ddd, 9.5, 4.5, 2.5)	3.05 (ddd, 9.0, 5.0, 3.0)
14a	2.54 (dd, 17.5, 5.0)	2.59 (dd, 17.0, 5.0)	2.63 (dd, 17.5, 4.5)	2.66 (dd, 17.5, 9.0)
14b	2.45 (dd, 17.5, 9.5)	2.54 (dd, 17.0, 9.0)	2.51 (dd, 17.5, 9.5)	2.50 (dd, 17.5, 5.0)
17a	4.86 (brs)	4.92 (brs)	1.94 (brd, 1.5)	1.20 (s)
17b	4.82 (brs)	4.85 (brs)		
18	0.97 (s)	0.95 (s)	1.11 (s)	1.15 (s)
19	1.17 (s)	1.15 (s)	1.12 (s)	1.11 (s)
20	0.94 (s)	0.58 (s)	0.80 (s)	0.94 (s)
NH	11.01 (s)	11.05 (s)	11.11 (s)	11.10 (s)
OH-12	4.91 (d, 5.0)	5.04 (d, 4.5)	5.29 (d, 4.5)	
OH-6	4.15 (d, 3.5)			

[a] Recorded at 500 MHz (δ_{DMSO} 2.49).

Table 2. ^{13}C NMR[a] (DMSO-d_6) Data of Haterumaimides F−I (**1**−**4**)

C no.	**1** (mult)	**2** (mult)	**3** (mult)	**4** (mult)
1	50.7 (t)	47.6 (t)	47.6 (t)	51.4 (t)
2	56.9 (t)	55.7 (d)	55.2 (d)	54.5 (d)
3	53.6 (t)	51.9 (t)	52.4 (t)	52.3 (t)
4	36.5 (s)	34.7 (s)	34.9 (s)	35.2 (s)
5	54.9 (d)	62.8 (d)	60.7 (d)	59.8 (d)
6	66.6 (d)	207.1 (s)	198.1 (s)	207.9 (s)
7	46.9 (t)	54.7 (t)	127.6 (d)	51.3 (t)
8	144.3 (s)	143.4 (s)	160.1 (s)	79.3 (s)
9	51.7 (d)	50.5 (d)	50.6 (d)	58.0 (d)
10	42.4 (s)	42.8 (s)	44.8 (s)	overlapping
11	29.9 (t)	30.0 (t)	32.0 (t)	30.5 (t)
12	66.9 (d)	66.9 (d)	68.9 (d)	73.7 (d)
13	45.3 (d)	45.4 (d)	45.9 (d)	44.3 (d)
14	28.9 (t)	29.0 (t)	29.6 (t)	30.4 (t)
15	181.0[b] (s)	180.3[b] (s)	180.7[b] (s)	179.5[b] (s)
16	178.7[b] (s)	178.7[b] (s)	178.7[b] (s)	178.2[b] (s)
17	109.5 (t)	110.3 (t)	22.1 (q)	30.2 (q)
18	32.9 (q)	31.9 (q)	32.6 (q)	32.7 (q)
19	23.8 (q)	21.8 (q)	21.6 (q)	21.9 (q)
20	16.9 (q)	16.0 (q)	14.7 (q)	18.1 (q)

[a] Recorded at 125 MHz (δ_{DMSO} 39.5). [b] Exchangeable.

be tricyclic, to account for the six sites of unsaturation required by the molecular formula. The HMBC correlations from the NH proton signal at δ_H 11.1 (s) to δ_C 181.0 (s), 178.7 (s), 28.9 (t), and 45.3 (d) clearly indicated the existence of a rare succinimide moiety in haterumaimide F. The ^{1}H and ^{13}C NMR data thus suggested that the remaining two rings are carbocyclic. A detailed analysis of the DQF-COSY and HOHAHA spectra of haterumaimide F led to the elucidation of three partial structures, C1−C3, C5−C7, which has a secondary hydroxyl group [δ_H 4.15 (d, J = 3.5 Hz)] attached at C-6 (δ_C 66.6), and C9−C-14, which also contains a secondary hydroxyl group [δ_H 4.91 (d, J = 5.0 Hz) attached at C-12 (δ_C 66.9). The connectivity in the foregoing partial structures was established by the HMBC correlations of H_2-3/C-4, H_3-18/C-4, H_3-19/C-4, H-5/C-4, H_3-18/C-3, H_3-19/C-3, H_3-18/C-5, H_3-19/C-5, H_2-7/C-8, H-9/C-8, H_2-17/C-8, H_2-17/C-7, H_2-17/C-9, H_2-1/C-10, H-9/C-10, H-5/C-10, H_3-20/C-10, H_3-20/C-1, H_3-20/C-9, H_3-20/C-5, OH-6/C-6, OH-12/C-12, H-13/C-16, NH/C-16, H_2-14/C-15, NH/C-15, NH/C-13, and NH/C-14, to give the entire carbon framework of haterumaimide F (**1**), leaving the chlorine atom to reside at C-2, as shown in Figure 1. Therefore, the planar structure of haterumaimide F was elucidated as a rare class of monochlorinated diterpene alkaloid, as shown in **1**.

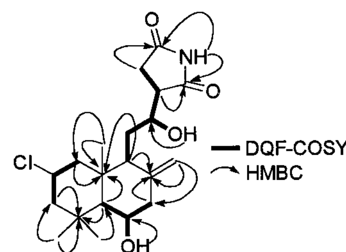

Figure 1. Partial structures of haterumaimide F (**1**) based on HOHAHA (bold line) and some important HMBC correlations (arrows).

Haterumaimide G (**2**) had a molecular formula of $C_{20}H_{28}ClNO_4$, as determined by high-resolution FABMS [m/z 382.1786 (M + H)$^+$, Δ+0.1 mmu]. The molecular formula suggested an additional degree of unsaturation compared to **1**. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) are similar to those of **1** except for the carbon signals at δ 62.8 (d), 207.1 (s), and 54.7 (t) for C-5, C-6, and C-7, respectively, and the proton signals at δ 2.51 (s) for H-5, 3.35 (d, J = 13.5 Hz), and 2.84 (d, J = 13.5 Hz) for H-7α and H-7β, respectively. Extensive analysis of 1D and 2D NMR data led to a planar structure for haterumaimide G (**2**), which puts it in the same class of diterpene alkaloid as **1**, but with a keto group at C-6 instead of a hydroxyl group. In addition to H-5 and H-7, the H-9 signal also appeared downfield compared to that in **1**.

Haterumaimide H (**3**) had the same molecular formula as **2**, $C_{20}H_{28}ClNO_4$, as determined by high-resolution FABMS [m/z 382.1801 (M + H)$^+$, Δ+1.6 mmu]. Although the molecular formulas are the same, the ^{1}H and ^{13}C NMR data (Tables 1 and 2) are quite different. Disappearance of the exomethylene proton signals and the appearance of the olefinic proton signal at δ 5.73 (q, J = 1.5 Hz) and a methyl signal at δ 1.94 (brd, J = 1.5 Hz) together with

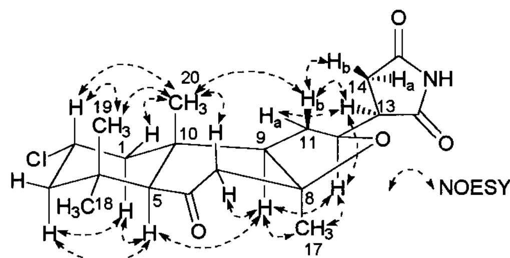

Figure 2. Selected NOESY correlations of haterumaimide I (**4**).

carbon chemical shifts at δ 160.1 (s), 127.6 (d), and 22.1 (q) clearly demonstrated that **3** might be a positional isomer of **2**. The HMBC correlations of the methyl protons at δ 1.94 (brd, $J = 1.5$ Hz) to δ_C 127.6 (d) and 160.1 (s), IR (film) absorptions at ν_{max} 1710 and 1640 cm^{-1}, and UV (MeOH) λ_{max} 238 nm (ϵ 7910) indicated the presence of an α,β-unsaturated keto group in **3**. Detailed analysis of 1D and 2D NMR data finally composed the planar structure of haterumaimide H (**3**) as a positional isomer of **2**. Acid-catalyzed rearrangement of **2** by pyridinium *p*-toluenesulfonate (PPTS) in MeOH gave **3**. The ^{1}H and ^{13}C NMR data, $[\alpha]_D$, and HPLC retention time of the derived **3** were identical to those of haterumaimide H (**3**), thus confirming the structure of **3**.

Haterumaimide I (**4**) had the same molecular formula as those of **2** and **3**, $C_{20}H_{28}ClNO_4$, as deduced from high-resolution FABMS [m/z 382.1786 (M + H)$^+$, Δ+0.1 mmu]. The ^{1}H and ^{13}C NMR data are presented in Tables 1 and 2. The carbonyl carbon signals at δ 207.9 (s), 179.5 (s), and 178.2 (s) suggested that **4** must be tetracyclic to account for the unsaturation required by the molecular formula. The loss of a signal for an olefinic proton at δ 5.73 (q, $J = 1.5$ Hz) and the loss of carbon signals at δ 160.1 (s) and 127.6 (d) together with the loss of a hydroxyl proton at δ 5.29 (d, $J = 4.5$ Hz) in **3** and the appearance of a carbon signal at δ 79.3 (s) and a methyl signal (δ_C 30.2 and δ_H 1.20) clearly indicated the presence of a tetrahydrofuran ring, which was formed through cyclization between the hydroxyl group at C-12 and the β-carbon of the α,β-unsaturated keto group in **3**. Extensive analysis of 1D and 2D NMR data led to the planar structure for haterumaimide I as shown in **4**. Acid treatment [camphorsulfonic acid, 50 °C, 48 h] of **3** gave **4** with identical ^{1}H and ^{13}C NMR data, $[\alpha]_D$, and HPLC retention time, thus confirming the structure of **4**.

The relative stereochemistry of haterumaimide I (**4**) was deduced from the NOESY analysis and the examination of vicinal coupling constants. NOESY correlations (Figure 2) were observed as follows: H-2/H$_3$-20, H-2/H$_3$-19, H$_3$-19/H$_3$-20, H-1β/H$_3$-20, H-3α/H-1α, H-3α/H-5, H-1α/H-5, H-3α/H$_3$-18, H-5/H-9, H-5/H$_3$-17, H-9/H$_3$-17, H-9/H-12, H-7β/H$_3$-20, H-7α/H-9, H-12/H$_3$-17, H-12/H-13, H-11a/H-13, H-11b/H-13, H-11b/H$_3$-20, H-11b/H-14b, and H-13/H-14a.

The NOESY correlations were further confirmed by a series of NOEDF spectral analyses. These NOESY observations together with vicinal coupling constants ($J_{1\alpha-2} = 12.0$, $J_{1\beta-2} = 3.5$, $J_{2-3\alpha} = 12.0$ and $J_{2-3\beta} = 3.5$ Hz) clearly indicated the presence of a *trans*-decalin *cis*-fused to a tetrahydrofuran ring with a C-2 chlorine atom in an α-orientation. Therefore, the relative stereochemistry of **4** was deduced to be 2*S**, 5*S**, 8*S**, 9*R**, 10*R**, and 12*S**. In the succinimide ring, the magnitude of $J_{12-13} = 3.0$ Hz and NOESY correlations of H-11a/H-13, H-11b/H$_3$-20, H-12/H-13, and H-11b/H-14b suggested the plausible conformation shown in Figure 2, and therefore, the relative stereochemistry at C-13 was determined to be 13*R**. The relative

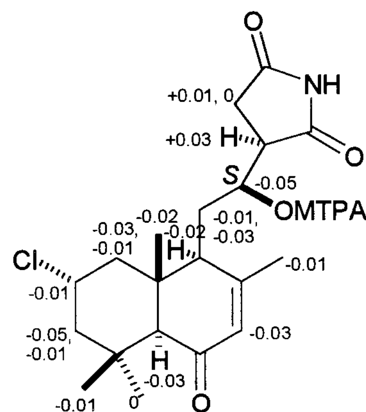

Figure 3. $\Delta\delta$ values ($\delta_S - \delta_R$, ppm) for the MTPA esters of haterumaimide H (**3**), **7a**, and **7b** (500 MHz).

stereochemistries of **1**–**3** were determined in the same way as described above for **4**, except for the C-12 and C-13 centers. The relative stereochemistry of C-6 in **1** was determined to be 6*S** from the NOESY correlations of OH-6/H$_3$-20 and OH-6/H$_3$-19 together with the magnitude of $J_{5,6} = 4.0$ Hz.

The absolute stereochemistries of **1**–**4** were determined as follows. The absolute stereochemistry at C-12 of **3** was determined using the modified Mosher's method.[11] Esterification of **3** gave (*S*)- and (*R*)-MTPA esters, **7a** and **7b**. The ^{1}H NMR signals of (*S*)- and (*R*)-MTPA esters were assigned on the basis of 2D NMR data, and the $\Delta\delta$ ($\delta_S - \delta_R$, ppm) values were then calculated as shown in Figure 3. The positive and negative $\Delta\delta$ values were systematically arranged on the right and left sides of the chiral center, and the results finally disclosed a 12*S* configuration in **3** (Figure 3). Since the acid treatment of **3** [camphorsulfonic acid, 50 °C, 48 h] afforded **4** with ^{1}H and ^{13}C NMR data, $[\alpha]_D$, and HPLC retention time identical to those of the natural product **4**, the absolute stereochemistry of **4** was determined to be 2*S*, 5*S*, 8*S*, 9*R*, 10*R*, 12*S*, and 13*R*, and that of **3** was 2*S*, 5*S*, 8*S*, 9*R*, 10*R*, and 13*R*, as shown in **4** and **3**, respectively. The absolute stereochemistry of haterumaimide G (**2**) was deduced to be as depicted in **2**, since acid-catalyzed rearrangement [PPTS, rt, 32 h] of **2** furnished **3** with identical ^{1}H and ^{13}C NMR data, $[\alpha]_D$, and HPLC retention time. The absolute stereochemistry of haterumaimide F (**1**) was tentatively deduced to be as depicted in **1** based on assumptions regarding the biosynthetic relationship between the imides (**1**–**4**).

Dichlorolissoclimide (**5**)[6] and chlorolissoclimide (**6**)[10] were identified from a comparison of ^{1}H and ^{13}C NMR data to reported values. Although haterumaimides H and I seemed to be artifacts of haterumaimide G, the lack of a change in haterumaimide G upon heating and the lack of a change in haterumaimide H upon treatment with PPTS for overnight confirmed that these are natural products. Also, acidic conditions were not used during isolation.

Biological Activities. Haterumaimides F (**1**), G (**2**), and H (**3**) completely inhibited the first cleavage of fertilized sea urchin eggs at 3 ppm, while haterumaimide I (**4**) inhibited the cell division of fertilized sea urchin eggs by 80% at 5 ppm. Haterumaimides F–I (**1**–**4**) exhibited potent to weak cytotoxicities against mouse lymphocytic leukemia cells (P388), with IC_{50} values of 0.0055, >10, 2.7, and >10 μg/mL, respectively. Haterumaimide G (**2**) and haterumaimide I (**4**) showed weak cytotoxicities compared to haterumaimide F (**1**) and haterumaimide H (**3**). Accordingly, the secondary hydroxyl groups at C-6 and C-12 seem to be very important for in vitro cytotoxicity.

Conclusion

Chlorinated labdane alkaloids having a succinimide moiety are extremely rare in nature. So far, only seven such compounds have been reported, including those in our recent report.[6,8,10] In this report, we have described the structures, absolute stereostructures, and structure/activity relationship of haterumaimides F−I. These types of alkaloids may be extremely important as pharmacological "lead" compounds. Further chemical and biological studies are underway in our laboratory.

Experimental Section

General Experimental Procedures. The ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a JEOL α-500 spectrometer, and the ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks [δ_H 2.49 and δ_C 39.5 in DMSO-d_6 and δ_H 7.26 in $CDCl_3$]. IR spectra were measured using a JASCO FT/IR-300 spectrometer, and optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were obtained in methanol using a JASCO UVDEC 610 spectrophotometer. High-resolution mass spectra (HRFABMS) were determined on a JEOL JMS-LG 2000 mass spectrometer. Column chromatography was performed on Kieselgel 60 (70−230 mesh, Merck) and Cosmosil 75C18-OPN (Nacalai tesque). High-performance liquid chromatography (HPLC) was performed using a COSMOSIL Si60 HPLC column (5SL, 10 × 250 mm) and a COSMOSIL packed ODS HPLC column (5C18, 10 × 250 mm). Preparative TLC was performed using Kieselgel 60 F_{254} DC-fertigplatten (Merck). All solvents used were reagent grade. Pyridine was distilled from calcium hydride.

Animal Material. The ascidian *Lissoclinum* sp. was collected by hand from the coast of Hateruma Island, Okinawa, in June 1996, and identified as a *Lissoclinum* sp. by Dr. Yuichi Hirose of the University of the Ryukyus. The identified ascidian was kept frozen until use. A voucher specimen was deposited at the University of the Ryukyus (Specimen no. URKU-31), and color photographs have been deposited as Supporting Information.

Extraction and Isolation. The ascidian *Lissoclinum* sp. (1.0 kg, wet wt) was extracted with acetone and then concentrated in vacuo to give an acetone extract. The acetone extract was partitioned between H_2O and EtOAc. The EtOAc extract completely inhibited the first cleavage of fertilized sea urchin eggs at 10 ppm. The EtOAc extract (3.4 g) was suspended in aqueous MeOH (1:1) and then successively partitioned between the aqueous MeOH and hexane, $CHCl_3$, and 1-BuOH. All three extracts were tested for cytotoxicity against fertilized sea urchin eggs. Only the $CHCl_3$ extract completely inhibited the cell division of fertilized sea urchin eggs at 5 ppm. The active $CHCl_3$ extract (2.5 g) was first chromatographed on Si gel (300 g) using hexane with increasing proportions of EtOAc and EtOAc with increasing proportions of MeOH, and the column was finally washed with MeOH to give nine fractions. All nine fractions were tested for cytotoxicity against fertilized sea urchin eggs at 5 ppm. Only fraction 5 and fraction 6 completely inhibited the cell division of the fertilized sea urchin eggs at 5 ppm. The active fifth fraction (0.4 g) was further chromatographed on ODS (150 g) using 35% H_2O in MeOH and MeOH to give two fractions. The active polar fraction (0.3 g) was subjected to further separation by HPLC on Si60 using hexane−CH_2Cl_2−EtOAc−MeOH (12:4:3:1) to give 12 fractions. The 11th fraction was purified by reversed-phase HPLC on ODS using MeOH−H_2O−CH_3CN (5.5:3.5:1) to afford haterumaimide F (**1**, 4.9 mg). The fifth fraction was subjected to reversed-phase HPLC on ODS using MeOH−H_2O−CH_3CN (6.5:3:0.5) to give haterumaimide G (**2**, 10.5 mg). The sixth fraction was subjected to reversed-phase HPLC on ODS using MeOH−H_2O−CH_3CN (6.5:2.7:0.8) to give a fraction that was finally purified by normal-phase HPLC on Si60 using hexanes−EtOAc−MeOH (7.2:2.3:0.5) to afford haterumaimide H (**3**, 11.1 mg) and haterumaimide I (**4**, 23.5 mg). The active sixth fraction (0.4 g) from the first column was further chromatographed on ODS (150 g) using 30% H_2O in MeOH and MeOH to give two fractions. The active polar fraction (0.3 g) was subjected to further purification by HPLC on Si60 using hexane−CH_2Cl_2−EtOAc−MeOH (10:3:6:1) to afford dichlorolissoclimide (**5**, 100.1 mg) and chlorolissoclimide (**6**, 98.3 mg).

Haterumaimide F (1): colorless oil; $[\alpha]^{29}_D$ +53.7° (*c* 0.35, MeOH), FT/IR ν_{max} 3410, 2920, 1710, 1705, 1405, and 1180 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 nm (3.57); ^{1}H and ^{13}C NMR (DMSO-d_6) data are listed in Tables 1 and 2; HRFABMS *m*/*z* $(M + H)^+$ 384.1940 (calcd for $C_{20}H_{31}ClNO_4$, 384.1942, Δ−0.2 mmu).

Haterumaimide G (2): colorless oil; $[\alpha]^{29}_D$ +63.5° (*c* 0.58, MeOH), FT/IR ν_{max} 3410, 2905, 1710, 1705, 1400, and 1180 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 207 nm (3.56); ^{1}H and ^{13}C NMR (DMSO-d_6) data are listed in Tables 1 and 2; HRFABMS *m*/*z* $(M + H)^+$ 382.1786 (calcd for $C_{20}H_{29}ClNO_4$, 382.1785, Δ+0.1 mmu).

Haterumaimide H (3): colorless oil; $[\alpha]^{32}_D$ +47.6° (*c* 0.31, MeOH), FT/IR ν_{max} 3400, 2850, 1710, 1640, 1390, 1200, and 1050 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 238 (3.90) and 205 nm (3.70); ^{1}H and ^{13}C NMR (DMSO-d_6) data are listed in Tables 1 and 2; HRFABMS *m*/*z* $(M + H)^+$ 382.1801 (calcd for $C_{20}H_{29}ClNO_4$, 382.1785, Δ+1.6 mmu).

Haterumaimide I (4): colorless oil; $[\alpha]^{32}_D$ +62.0° (*c* 0.77, MeOH), FT/IR ν_{max} 3405, 2900, 1715, 1700, 1380, 1200, and 1080 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 nm (3.56); ^{1}H and ^{13}C NMR (DMSO-d_6) data are listed in Tables 1 and 2; HRFABMS *m*/*z* $(M + H)^+$ 382.1786 (calcd for $C_{20}H_{31}ClNO_4$, 382.1785, Δ+0.1 mmu).

Esterification of 3. To a solution of haterumaimide H (**3**, 0.6 mg, 1.6 μmol) in pyridine (0.2 mL) was added (−)-MTPA chloride (15.6 mg, 61.8 μmol). After stirring at room temperature for 4 h, methanol (0.1 mL) was added, and the mixture was stirred for 1.5 h; H_2O (0.1 mL) was then added and stirred for 1 h. The mixture was diluted with H_2O (0.3 mL) and then extracted with EtOAc (0.4 mL × 3). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residual oil was purified by preparative TLC, R_f = 0.50 [hexanes−EtOAc−MeOH (6.5:3.0:0.5)] to give (*S*)-MTPA ester (**7a**, 0.4 mg, 45%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42−7.38 (5H, m, MPTA phenyl protons), 5.81 (1H, q, *J* = 1.5 Hz, H-7), 5.63 (1H, ddd, *J* = 9.5, 5.5, 2.5 Hz, H-12), 4.09 (1H, tt, *J* = 12.5, 3.5 Hz, H-2), 3.40 (3H, s, MTPA OC*H*$_3$), 3.24 (1H, ddd, *J* = 9.5, 5.5, 2.0 Hz, H-13), 2.79 (1H, dd, *J* = 18.0, 9.5 Hz, H-14a), 2.71 (1H, dd, *J* = 18.0, 5.5 Hz, H-14b), 2.20 (1H, ddd, *J* = 12.0, 3.5, 2.0 Hz, H-1β), 2.10 (1H, ddd, *J* = 12.0, 3.5, 2.0 Hz, H-3β), 2.07 (3H, brs, C*H*$_3$-17), 2.01 (1H, s, H-5), 1.95 (1H, ddd, *J* = 9.5, 5.5, 1.5 Hz, H-9), 1.92 (1H, ddd, *J* = 13.0, 9.5, 5.4 Hz, H-11a), 1.58 (1H, ddd, *J* = 13.0, 7.5, 5.5 Hz, H-11b), 1.43 (1H, t, *J* = 12.5 Hz, H-1α), 1.38 (1H, t, *J* = 12.0 Hz, H-3α), 1.19 (6H, s, C*H*$_3$-18, C*H*$_3$-19), 0.87 (3H, s, C*H*$_3$-20); HRFABMS *m*/*z* $(M + H)^+$ 598.2171 (calcd for $C_{30}H_{36}ClF_3NO_6$, 598.2183, Δ−1.2 mmu). Using the same procedure as described above, (*R*)-MTPA ester (**7b**, 0.4 mg, 45%) was obtained from the reaction between (+)-MTPA chloride (13.5 mg, 53.4 μmol) and **3** (0.6 mg, 1.6 μmol) in pyridine (1.5 mL): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44−7.40 (5H, m, MTPA phenyl protons), 5.84 (1H, q, *J* = 1.5, H-7), 5.68 (1H, ddd, *J* = 10.0, 5.0, 2.0 Hz, H-12), 4.10 (1H, tt, *J* = 12.0, 4.0 Hz, H-2), 3.45 (3H, s, MTPA OC*H*$_3$), 3.21 (1H, ddd, *J* = 9.5, 5.5, 2.5 Hz, H-13), 2.78 (1H, dd, *J* = 18.0, 9.0 Hz, H-14a), 2.71 (1H, dd, *J* = 18.0, 5.5 Hz, H-14b), 2.23 (1H, ddd, *J* = 12.0, 3.5, 2.0 Hz, H-1β), 2.15 (1H, ddd, *J* = 12.0, 3.5, 2.0 Hz, H-3β), 2.08 (3H, brs, C*H*$_3$-17), 2.04 (1H, s, H-5), 1.97 (1H, ddd, *J* = 9.5, 5.5, 1.5 Hz, H-9), 1.93 (1H, ddd, *J* = 12.9, 9.4, 5.5 Hz, H-11a), 1.61 (1H, ddd, *J* = 13.0, 7.5, 5.5 Hz, H-11b), 1.44 (1H, t, *J* = 12.0 Hz, H-1α), 1.39 (1H, t, *J* = 12.0 Hz, H-3α), 1.20 (3H, s, C*H*$_3$-19), 1.19 (3H, s, C*H*$_3$-18), 0.89 (3H, s, C*H*$_3$-20); HRFABMS *m*/*z* $(M + H)^+$ 598.2213 (calcd for $C_{30}H_{36}ClF_3NO_6$, 598.2183, Δ+3.0 mmu).

Isomerization of 2. To a solution of **2** (0.8 mg, 2.1 μmol) in methanol (0.5 mL) was added PPTS (1.0 mg, 3.9 μmol), and the mixture was stirred at room temperature for 32 h and then quenched with 10% aqueous NH_3 (0.5 mL). The solvent was evaporated, and the residue was extracted with ether (0.5 mL × 3). The combined ether extract was washed with

saturated NaCl solution, dried ($MgSO_4$), and then evaporated. The residue was purified by normal-phase HPLC on Si60 using hexanes−EtOAc−MeOH (7.2:2.3:0.5) as a mobile phase to give **3** (t_R = 14.4 min, 0.5 mg, 62%) as a colorless oil: $[\alpha]^{29}_D$ +47.8° (*c* 0.04, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.10 (1H, s), 5.73 (1H, q, J = 1.5 Hz), 5.20 (1H, brs), 4.32 (1H, tt, J = 12.0, 4.0 Hz), 4.03 (1H, ddd, J = 9.5, 4.5, 2.7 Hz), 3.05 (1H, ddd, J = 9.4, 4.5, 2.8 Hz), 2.63 (1H, dd, J = 17.5, 4.5 Hz), 2.51 (1H, dd, J = 17.5, 9.5 Hz), 2.45 (1H, ddd, J = 7.8, 5.5, 2.0 Hz), 2.26 (1H, s), 2.21 (1H, dd, J = 12.0, 4.5 Hz), 1.95 (3H, brs), 1.80 (1H, dd, J = 12.0, 4.5 Hz), 1.59 (1H, t, J = 12.0 Hz), 1.56 (1H, m), 1.47 (t, J = 12.0 Hz), 1.46 (1H, m), 1.13 (3H, s), 1.11 (3H, s), 0.82 (3H, s); ^{13}C NMR (DMSO, 125 MHz) 47.6 (t, C-1), 55.2 (d, C-2), 52.3 (d, C-3), 34.8 (s, C-4), 60.7 (d, C-5), 198.0 (s, C-6), 127.6 (d, C-7), 160.2 (s, C-8), 50.6 (d, C-9), 44.8 (s, C-10), 32.0 (t, C-11), 68.9 (d, C-12), 46.0 (d, C-13), 29.7 (t, C-14), 181.4 (s, C-15), 177.7 (s, C-16), 22.1 (q, C-17), 32.6 (q, C-18), 21.6 (q, C-19), 14.7 (q, C-20).

Acid Treatment of 3. To a solution of **3** (1.6 mg, 4.1 μmol) in methanol (0.5 mL) was added camphorsulfonic acid (4.0 mg, 16.4 μmol), and the mixture was stirred at 50 °C for 48 h and then quenched with 10% aqueous NH_3 (0.5 mL). The solvent was evaporated, and the residue was extracted with ether (0.5 mL × 3). The combined ether extract was washed with brine, dried ($MgSO_4$), and then evaporated. The residue was purified by normal-phase HPLC on Si60 using hexanes−EtOAc−MeOH (7.2:2.3:0.5) as a mobile phase to give **4** (t_R = 10.9 min, 0.4 mg, 25%) as a colorless oil: $[\alpha]^{29}_D$ +62.1° (*c* 0.03, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.11 (1H, s), 4.26 (1H, tt, J = 12.0, 4.0 Hz), 4.24 (1H, ddd, J = 9.0, 5.5, 2.0 Hz), 3.05 (1H, m), 2.65 (1H, dd, J = 17.5, 9.0 Hz), 2.61 (1H, d, J = 18.5 Hz), 2.50 (1H, dd, J = 17.5, 5.0 Hz), 2.43 (1H, d, J = 18.5 Hz), 2.17 (1H, t, J = 9.0 Hz), 2.10 (1H, s), 2.11 (1H, dt, J = 12.0, 9.0 Hz), 2.00 (1H, dd, J = 12.0, 4.0 Hz), 1.80 (1H, m), 1.55 (1H, m), 1.52 (1H, t, J = 12.0 Hz), 1.50 (1H, t, J = 12.0 Hz), 1.20 (3H, s), 1.15 (3H, s), 1.11 (3H, s), 0.94 (3H, s); ^{13}C NMR (DMSO, 125 MHz) 51.4 (t, C-1), 54.5 (d, C-2), 52.3 (t, C-3), 35.2 (s, C-4), 59.8 (d, C-5), 208.0 (s, C-6), 50.9 (t, C-7), 79.2 (s, C-8), 58.0 (d, C-9), 40.1 (s, C-10), 30.5 (t, C-11), 73.8 (d, C-12), 44.6 (d, C-13), 30.2 (t, C-14), 179.2 (s, C-15), 178.0 (s, C-16), 30.2 (q, C-17), 32.8 (q, C-18), 21.9 (q, C-19), 18.0 (q, C-20).

Acknowledgment. We thank Professor Tatsuo Higa, University of the Ryukyus, for his valuable suggestions throughout this study and Dr. Yuichi Hirose, University of the Ryukyus, for identifying the ascidian. This work was supported in part by Wako Pure Chemical Industries Ltd. and by Grants-in-Aid (Nos. 11558079 and 12045235) from the Ministry of Education, Science, Sports, and Culture of Japan.

Supporting Information Available: Photos of *Lissoclinum* sp. This material is available free of charge via the Internet at http://pub.acs.org.

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NP010066N