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HATERUMAIMIDES A–E, FIVE NEW DICHLOROLISSOCLIMIDE-TYPE DITERPENOIDS FROM AN ASCIDIAN, *LISSOCLINUM* SP.[#]

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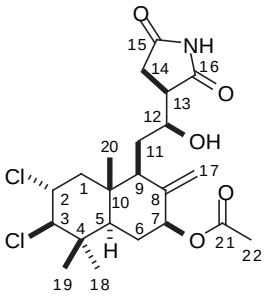
Abstract – Five new dichlorolissoclimide-type diterpenoids, haterumaimides A–E, were isolated from an ascidian, *Lissoclinum* sp., and the absolute stereostructures of haterumaimides A–D and the relative stereostructure of haterumaimide E were determined by spectroscopic and chemical analyses. Haterumaimides A–E inhibited the first cleavage of fertilized sea urchin eggs and exhibited cytotoxicity against P388 cells.

INTRODUCTION

As described previously, in our screening for inhibitors of the cell division of fertilized sea urchin eggs, haterumalide B was isolated from an Okinawan ascidian *Lissoclinum* sp.,[1] and in our screening for cytotoxicity against P388 cells, haterumalides NA-NE were isolated from an Okinawan sponge *Ircinia* sp.[2] Recently, five new dichlorolissoclimide-type diterpenoids, haterumaimides A–E (**1**–**5**), were isolated from an ascidian, *Lissoclinum* sp., collected off the coast of Hateruma Island, Okinawa, Japan, and these inhibited both the first cleavage of fertilized sea urchin eggs and the growth of P388 cells. Although dichlorolissoclimide was isolated from the New Caledonian ascidian *Lissoclinum voeltzkowi* Michaelson by Verbist and co-workers in 1988,[3] and its absolute stereochemistry was determined by X-Ray analysis,[4] these types of compounds are extremely important due to their biological activities, and their potential use as antitumor drugs and physiological tools. Therefore, the absolute stereostructures of the Okinawan haterumaimides A–D (**1**–**4**) and the relative stereostructure of haterumaimide E (**5**) were determined by

[#] Dedicated to Professor Shô Itô in celebration of his 77th birthday.

spectroscopic and chemical analyses. We report here the detailed isolation and structure determination of **1**–**5**.



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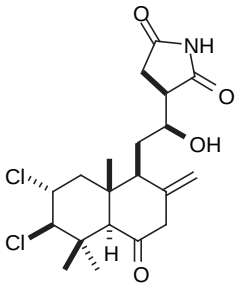

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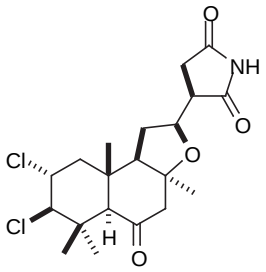

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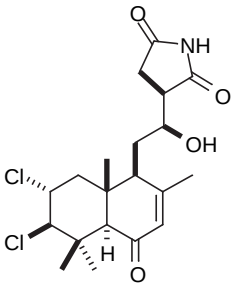

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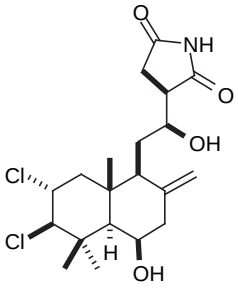

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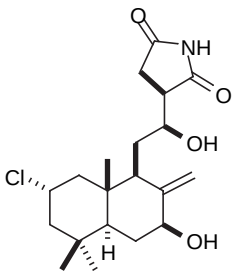

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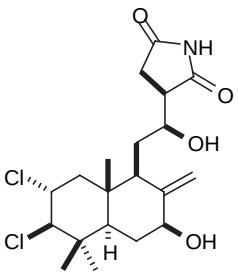

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RESULTS AND DISCUSSION

An ascidian, *Lissoclinum* sp., was collected off the coast of Hateruma Island, Okinawa, Japan in June 1996. The animal specimen (1.0 kg, wet weight) was extracted with acetone. The acetone extracts were partitioned between H_2O and EtOAc. The EtOAc extracts completely inhibited the first cleavage of fertilized sea urchin eggs at a concentration of 10 ppm. The EtOAc extracts (3.4 g) was suspended in MeOH–H_2O (1:1) and then extracted with hexane and $CHCl_3$. The activity was found only in the $CHCl_3$ extracts. The $CHCl_3$ extracts (2.5 g) were purified through a series of processes: silica gel column chromatography, ODS column chromatography, HPLC and reversed-phase HPLC by monitoring the activity for sea urchin eggs test. Five new dichlorolissoclimide-type diterpenoids, haterumaimides A–E [(**1**, 0.002% of wet ascidian), (**2**, 0.003%), (**3**, 0.002%), (**4**, 0.003%) and (**5**, 0.0002%)], and known chlorolissoclimide (**6**, 0.01%) and dichlorolissoclimide (**7**, 0.01%) were isolated. The molecular formula of **1** was determined to be $C_{22}H_{31}NO_5Cl_2$ by HRFABMS [*m/z* 460.1657 $(M+H)^+$, calcd for $C_{22}H_{32}NO_5Cl_2$, 460.1658]. The IR spectrum indicated the presence of an imide (1710 cm^{-1}) and exocyclic olefin (1605 cm^{-1}).

The NMR data for **1**–**5** are summarized in Tables 1 and 2. The ^1H NMR spectrum of **1** showed the presence of one imide NH group (δ 11.03), one hydroxyl group (δ 3.34) and four methyl groups as a

singlet (δ 2.10, 1.05, 0.85 and 0.70). In the ^{13}C NMR spectrum, 22 carbon signals were observed, including three carbonyl carbons (δ 180.7, 178.6 and 169.4), two olefin carbons (δ 144.5 and 105.1), two chlorine-bearing carbons (δ 77.0, and 61.5), two oxymethine carbon (δ 73.4 and 66.6) and four methyl carbons (δ 29.2, 20.9, 17.3 and 14.0). The remaining carbon signals were assigned to four methylenes, three methines and two quaternary carbons by HMQC experiment.

Table 1 ^{1}H NMR Data for haterumaimides A–E (**1**–**5**)

position	**1**	**2**	**3**	**4**	**5**
1α	[a] 1.62 dd (13.0, 12.0)	[a] 1.84 dd (13.0, 12.5)	[b] 1.83 dd (13.3, 12.3)	[b] 1.90 dd (12.8, 12.1)	[a] 1.59 t (12.5)
1β	2.24 dd (13.0, 4.2)	2.34 dd (13.0, 4.5)	2.29 dd (13.3, 4.2)	2.47 dd (12.8, 4.2)	2.20 dd (12.5, 4.5)
2	4.45 td (12.0, 4.2)	4.48 td (12.5, 4.5)	4.22 ddd (12.3, 11.0, 4.2)	4.28 ddd (12.1, 11.0, 4.2)	4.40 td (12.5, 4.5)
3	3.90 d (11.0)	3.86 d (11.0)	3.79 d (11.0)	3.78 d (11.0)	3.84 d (11.0)
5	1.63 dd (12.0, 2.0)	2.84 s	2.34 s	2.55 s	1.34 br d (3.0)
6α	1.65 m				4.23 m
6β	1.40 q (12.0)				
7α	5.09 m	2.89 d (13.5)	2.56 d (18.7)	5.81 dd (2.8, 1.3)	2.18 dd (13.5, 4.5)
7β		3.32 d (13.5)	2.72 d (18.7)		2.27 dd (13.5, 2.5)
9	1.70 dd (10.9, 4.0)	2.27 br t (9.5)	2.26 dd (9.0, 7.7)	2.50 dddd (7.0, 2.8, 2.0, 1.3)	1.63 dd (8.5, 5.5)
11α	1.98 ddd (13.0, 7.0, 4.0)	1.64 ddd (14.0, 9.5, 4.0)	2.20 ddd (12.9, 9.0, 5.7)	1.72 dt (15.2, 7.0)	1.64 m
11β	1.40 ddd (13.0, 10.9, 7.0)	1.49 dt (14.0, 9.5)	1.73 ddd (12.9, 10.1, 7.7)	1.61 ddd (15.2, 7.5, 2.0)	1.37 m
12	3.95 td (7.0, 2.1)	4.01 m	4.33 ddd (10.1, 5.7, 4.3)	4.25 ddd (7.5, 7.0, 2.4)	4.00 dddd (10.5, 5.5, 4.5, 1.5)
13	2.85 ddd (10.8, 4.5, 2.1)	2.96 ddd (9.0, 5.0, 2.0)	3.12 ddd (9.1, 5.1, 4.3)	3.13 ddd (9.2, 4.7, 2.4)	2.84 ddd (9.5, 5.0, 1.5)
14α	2.49 dd (17.6, 10.8)	2.53 dd (18.0, 9.0)	2.75 dd (18.0, 9.1)	2.66 dd (17.8, 9.2)	2.44 dd (17.5, 9.5)
14β	2.55 dd (17.6, 4.5)	2.62 dd (18.0, 5.0)	2.64 dd (18.0, 5.1)	2.79 dd (17.8, 4.7)	2.55 dd (17.5, 5.0)
17	5.09 br s, 4.86 br s	4.96 br s, 4.88 br s	1.29 s	2.04 t (1.3)	4.88 br s, 4.84 br s
18	1.05 s	1.14 s	1.41 s	1.39 s	1.14 s
19	0.85 s	1.21 s	1.23 s	1.25 s	1.24 s
20	0.70 s	0.63 s	1.08 s	0.95 s	0.95 s
22	2.10 s				
NH	11.03 s	11.10 s			11.03 s
OH	3.34 br s	5.05 d (5.0)			4.94 d (4.5), 4.36 d (4.0)

[a] Recorded at 500 MHz (δ_{DMSO} 2.49), [b] Recorded at 600 MHz (δ_{CD3OD} 3.30)

Table 2 ^{13}C NMR Data for haterumaimides A–E (**1**–**5**)

position	**1**	**2**	**3**	**4**	**5**
1	[a] 47.7	[a] 47.8	[b] 53.2	[b] 50.3	[a] 50.1
2	61.5	60.8	60.5	61.1	62.0
3	77.0	76.7	78.7	78.7	78.1
4	41.4	40.0	42.6	42.3	42.1
5	50.4	62.9	63.2	63.9	54.8
6	29.5	205.8	209.1	200.2	67.4
7	73.4	54.9	53.2	129.6	46.8
8	144.5	142.9	81.6	162.7	143.6
9	48.7	50.3	60.4	53.2	51.3
10	39.9	41.9	40.5	45.9	41.4
11	30.2	29.9	32.5	34.1	29.9
12	66.6	66.9	76.1	71.2	66.9
13	45.4	45.4	46.7	48.2	45.3
14	28.9	29.0	32.6	31.3	28.9
15	178.6*	178.6*	180.8	181.2	178.7*
16	180.7*	180.8*	181.9	183.1	180.9*
17	105.1	110.6	31.0	23.2	109.6
18	29.2	28.5	30.7	30.4	29.1
19	17.3	16.8	18.2	17.9	18.5
20	14.0	15.8	19.3	15.8	16.5
21	169.4				
22	20.9				

[a] Recorded at 125 MHz (δ_{DMSO} 39.5), [b] Recorded at 150 MHz (δ_{CD3OD} 49.5), * exchangeable

A detailed analysis of the DQF-COSY spectrum of **1** gave three partial structures, C1–C3, C5–C7 and C9–C14 (Figure 1). In addition, the presence of a five-membered imide ring in **1** was revealed by correlations in the HMBC spectrum, H-13/C15, H-13/C16, H-14/C15 and H-14/C16. The connections in the decalin structure were clarified by the HMBC correlations (H-1/C10, H-3/C4, H-5/C10, H-9/C7, H-9/C8 and H-9/C10). HMBC correlations suggested the presence of an exocyclic olefin structure (H-18/C7, H-18/C8 and H-18/C9) and an acetoxy group (H-7/C21 and H-22/C21). Thus, the gross structure of haterumaimide A was clarified as shown in formula (**1**). The gross structures of **2**–**5** were determined in the same manner as described above for **1**.

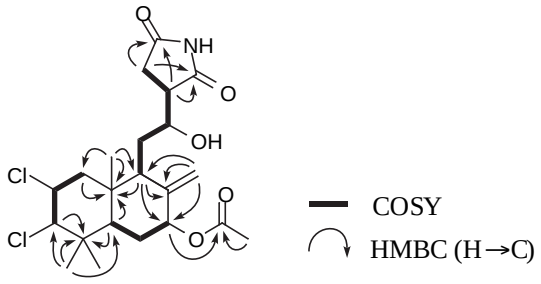

Figure 1. Partial structures of haterumaimide A (**1**) based on DQF-COSY spectrum and selected HMBC correlations.

The relative stereochemistry of **3** was determined as follows. In the decalin ring and tetrahydrofuran ring, based on the magnitude of $J_{1\alpha,2}$=12.3 Hz, $J_{1\beta,2}$=4.2 Hz, $J_{2,3}$=11.0 Hz, $J_{9,11\alpha}$=9.0 Hz, $J_{9,11\beta}$=7.7 Hz, $J_{11\alpha,12}$=5.7 Hz and $J_{11\beta,12}$=10.1 Hz and NOESY correlations of H-1α/H-3, H-1α/H-5, H-1α/H-9, H-1β/H-2, H-1β/H-20, H-2/H-19, H-3/H-5, H-3/H-18, H-5/H-17, H-7α/H-17, H-7β/H-20, H-9/H-12, H-9/H-17, H-11β/H-20, H-12/H-17 and H-19/H-20, the relative stereochemistry of **3** was determined to be 2*R**, 3*R**, 5*R**, 8*S**, 9*R**, 10*R** and 12*S**. In the imide ring, the magnitude of $J_{12,13}$=4.3 Hz, the NOESY correlations of H-11α/H-13, H-11β/H-13, H-11β/H-14α, H-11β/H-14β and H-12/H-13, and the weak NOESY correlation of H-12/H-14β suggested the restricted rotation of the bond between C12 and C13. Because **3** adopt the plausible conformation shown in Figure 2, the relative stereochemistry of C13 was determined to be13*R**.

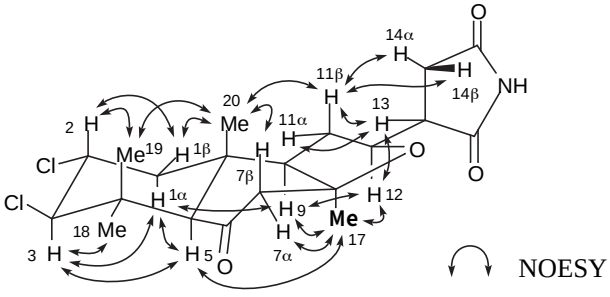

Figure 2. Selected NOESY correlations of haterumaimide C (**3**).

Thus, the relative stereochemistry of haterumaimide D was clarified as shown in formula (**3**). The relative stereostructures of **1**–**2** and **4**–**5** were determined in the same manner as described above for **3**.

The absolute stereochemistries of **1**–**4** were determined as follows. Methanolysis of **1** gave the known **7**. The ^{1}H and ^{13}C NMR spectra, $[\alpha]_D$ and HPLC retention time of the derived **7** were identical to those of the natural product (**7**). Thus, the absolute stereochemistry of haterumaimide A was determined as depicted in formula (**1**). The absolute stereochemistry of C12 in **4** was determined using a modified Mosher's method.[5] Esterification of **4** gave the (*S*)- and (*R*)-MTPA esters (**8a** and **8b**), the ^{1}H NMR signals of which were assigned based on the 2D NMR spectra, and the $\Delta\delta$ values (δ_S–δ_R, ppm) were then calculated. The results established that the absolute stereochemistry of C12 in **4** was *S* (Figure 3). Thus, the absolute stereochemistry of haterumaimide D was determined to be 2*R*, 3*R*, 5*R*, 8*S*, 9*R*, 10*R*, 12*S* and 13*R* as depicted in formula (**4**). Acid treatment of **4** [1M HCl–THF (1:3), 50°C, 18 h] afforded **3**. The ^{1}H NMR spectrum and $[\alpha]_D$ of the derived **3** were identical to those of the natural product (**3**). Thus, the absolute stereochemistry of haterumaimide C was determined as depicted in formula (**3**). Isomerization of the double bond of **2** by PPTS in methanol gave the conjugated ketone (**4**). The ^{1}H and ^{13}C NMR spectra, $[\alpha]_D$ and HPLC retention time of the derived **4** were identical to those of the natural product (**4**). Thus, the absolute stereochemistry of haterumaimide D was determined as depicted in formula (**4**). Although haterumaimides C (**3**) and D (**4**) possess the possibility as artifacts of haterumaimide B (**2**), the presence of **3** and **4** was observed in the flesh extract of the animal specimen. However the absolute stereochemistry of haterumaimide E was not determined, its absolute stereochemistry was presumed as depicted in formula (**5**).

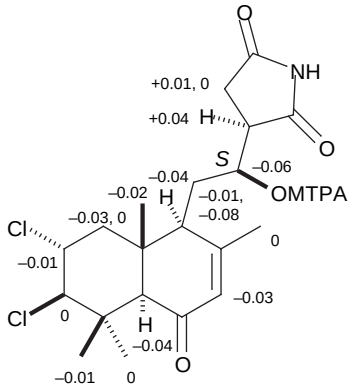

Figure 3. $\Delta\delta$ values (δ_S–δ_R, ppm) for the MTPA esters (**8a**) and (**8b**) (400 MHz).

BIOLOGICAL ACTIVITIES

Haterumaimides A, B and D (**1**, **2** and **4**) inhibited the cleavage of fertilized sea urchin eggs, by 10%, 100% and 100% at 3 ppm, respectively. Haterumaimides A–E (**1**–**5**) exhibited potent cytotoxicity against

mouse lymphocytic leukemia cells (P388), with IC_{50} values of 3.5×10^{-3}, 2.1, >10 (12%), 0.9 and 4.1×10^{-3} μg/mL, respectively.

CONCLUSION

Five new dichlorolissoclimide-type diterpenoids, haterumaimides A–E, were isolated from an ascidian, *Lissoclinum* sp., and the absolute stereostructures of haterumaimides A–D and the relative stereostructure of haterumaimide E were determined by spectroscopic and chemical analyses. These haterumaimides A–E inhibited the first cleavage of fertilized sea urchin eggs and were cytotoxic against P388 cells. These types of compounds are important as pharmacological lead compounds. Research in this area is now in progress in our laboratory.

EXPERIMENTAL

GENERAL ASPECTS

Optical rotations were measured with a JASCO DIP-1000 polarimeter. The ^{1}H, ^{13}C and 2D NMR spectra were recorded on a JEOL JNM-A400 [400 MHz (^{1}H) and 100 MHz (^{13}C)], a JEOL JNM-A500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] or a JEOL JNM-A600 [600 MHz (^{1}H) and 150 MHz (^{13}C)] spectrometer. The ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks [(δ_H=3.30 and δ_C=49.5 in methanol-d_4), (δ_H=2.49 and δ_C=39.5 in DMSO-d_6) or (δ_H=7.26 in $CDCl_3$)]. IR spectra were measured using a JASCO FT/IR-300 or a JASCO FT/IR-230 spectrophotometer. LREIMS measurements were taken on a HITACHI M-2500 mass spectrometer. High-resolution mass spectra (HRFABMS) and LRFABMS were obtained on a JEOL JMS-LG2000 mass spectrometer. Column chromatography was performed on Kieselgel 60 (Merck), silica gel (Fuji Silysia gel FL60D) or Cosmosil 75C18-OPN (Nacalai tesque). High performance liquid chromatography (HPLC) was performed using a COSMOSIL Si60 HPLC column (5SL, 10x250 mm) or a COSMOSIL-packed ODS HPLC column (5C18, 10×250 mm). Preparative TLC was carried out using Kieselgel 60 F_{254} DC-Fertigplatten (Merck). All solvents used were reagent grade. Pyridine was distilled from calcium hydride.

ISOLATION

An ascidian, *Lissoclinum* sp., was collected off the coast of Hateruma Island, Okinawa, Japan in June 1996. The sample (1.0 kg, wet weight) was extracted with acetone (1.5 L) for 7 d at rt. After filtration, the residue was extracted with acetone (1.0 L) for 1 d at rt. The combined extracts were evaporated. The extracts containing H_2O were extracted with EtOAc (0.5 L×3). The EtOAc extracts completely inhibited the first cleavage of fertilized sea urchin eggs at a concentration of 10 ppm. The EtOAc extracts (3.4 g)

were suspended in MeOH–H_2O (1:1) and then extracted with hexane and $CHCl_3$. The active $CHCl_3$ extracts (2.5 g) were first chromatographed on silica gel using hexane with increasing proportions of EtOAc and MeOH to give nine fractions. The active fifth fraction (438 mg) was further chromatographed on ODS using 35% H_2O in MeOH and MeOH to give two fractions. The active polar fraction (300 mg) was subjected to further separation by HPLC using hexane–CH_2Cl_2–EtOAc–MeOH (12:4:3:1) to give twelve fractions. The fifth fraction was purified by HPLC using MeOH–H_2O–MeCN (13:6:1) to afford haterumaimide A (**1**, 23 mg). The eighth fraction was subjected to HPLC using MeOH–H_2O–MeCN (11:7:2) to give haterumaimide B (**2**, 31 mg), haterumaimide C (**3**, 17 mg) and a fraction (8 mg) that contained **4**. The fraction containing **4** was finally purified by HPLC using hexane–EtOAc–MeOH (23:15:2) to afford haterumaimide D (**4**, 7 mg). The ninth fraction was purified by HPLC using MeOH–H_2O–MeCN (11:7:2) to furnish two compounds of **4** (24 mg) and **2** (4 mg). The twelfth fraction was purified by HPLC using MeOH–H_2O–MeCN (11:7:2) to afford haterumaimide E (**5**, 1.9 mg). The active sixth fraction (415 mg) of the first column was chromatographed on ODS using 30% H_2O in MeOH and MeOH. The active polar fraction (315 mg) was purified by HPLC using hexane–CH_2Cl_2–EtOAc–MeOH (10:3:6:1) to afford a fraction (9 mg), which contained **3** and **4**, and known chlorolissoclimide (**6**, 100 mg) and dichlorolissoclimide (**7**, 98 mg). The fraction containing **3** and **4** was finally purified by HPLC using hexane–CH_2Cl_2–EtOAc–MeOH (12:4:3:1) to afford **3** (2 mg) and **4** (3 mg).

Haterumaimide A–E (1–5). **1**: $[\alpha]_D^{29}$=+31.3° (*c* 0.133, MeOH); IR (film) ν_{max} 3430, 2950, 1710, 1605, 1380, 1230, 1150, 1050 cm^{-1}; HRFABMS: calcd for $C_{22}H_{32}NO_5Cl_2$, 460.1658: found *m/z* 460.1657 $(M+H)^+$. **2**: $[\alpha]_D^{33}$=+32.6° (*c* 0.484, MeOH); IR (film) ν_{max} 3450, 2920, 1715, 1710, 1625, 1350, 1180 cm^{-1}; HRFABMS: calcd for $C_{20}H_{28}NO_4Cl_2$, 416.1395: found *m/z* 416.1371 $(M+H)^+$. **3**: $[\alpha]_D^{28}$=+66.6° (*c* 0.062, MeOH); IR ($CHCl_3$) ν_{max} 3400, 1780, 1720, 1350, 1280, 1180, 1170 cm^{-1}; HRFABMS: calcd for $C_{20}H_{28}NO_4Cl_2$, 416.1396: found *m/z* 416.1425 $(M+H)^+$. **4**: $[\alpha]_D^{29}$=–27.7° (*c* 0.165, MeOH); IR ($CHCl_3$) ν_{max} 3400, 1780, 1720, 1670, 1350, 1180, 1100 cm^{-1}; HRFABMS: calcd for $C_{20}H_{28}NO_4Cl_2$, 416.1395: found *m/z* 416.1423 $(M+H)^+$. **5**: $[\alpha]_D^{29}$=+29.6° (*c* 0.158, MeOH); IR (film) ν_{max} 3450, 2910, 1715, 1705, 1610, 1170 cm^{-1}; HRFABMS: calcd for $C_{20}H_{30}NO_4Cl_2$, 418.1552: found *m/z* 418.1529 $(M+H)^+$.

Methanolysis of 1. To a solution of haterumaimide A (**1**, 2.1 mg, 4.6 μmol) in MeOH (0.5 mL) was added sodium methoxide (2.0 mg, 37.0 μmol) and the mixture was stirred at rt for 4 h and then diluted with water (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 evaporated. The residue was

purified by HPLC using Hexane–EtOAc–MeOH (25:13:2) to give alcohol (**7**) (t_R=23.8 min, 1.6 mg, 83%): $[\alpha]_D^{29}$=+31.3° (*c* 0.113, MeOH).

Esterification of 4. To a solution of haterumaimide D (**4**, 0.5 mg, 1.2 μmol) in pyridine (0.1 mL) was added (–)-MTPA chloride (10 mg, 40 μmol). After being stirred at rt for 1.5 h, the mixture was diluted with methanol (0.1 mL) and stirred at rt for 1 h. After dilution with H_2O (0.2 mL), the products were extracted with EtOAc. The EtOAc soln. was dried (Na_2SO_4) and concentrated *in vacuo*. The residual oil was separated by preparative TLC [R_f=0.56 (hexane–EtOAc 1:1)] to give the (*S*)-MTPA ester (**8a**, 0.4 mg, 55%). By the same procedure as described above, the (*R*)-MTPA ester (**8b**) was obtained by making to react **4** with (+)-MTPA chloride. **8a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46–7.40 (m, 5H), 5.86 (dd, *J*=2.6, 1.4 Hz, 1H), 5.64 (ddd, *J*=9.3, 5.5, 2.4 Hz, 1H), 4.14 (dt, *J*=11.2, 4.1 Hz, 1H), 3.59 (d, *J*=11.2 Hz, 1H), 3.42 (s, 3H), 3.24 (ddd, *J*=9.2, 5.7, 2.4 Hz, 1H), 2.79 (dd, *J*=18.0, 9.2 Hz, 1H), 2.72 (dd, *J*=18.0, 5.7 Hz, 1H), 2.38 (dd, *J*=12.7, 4.1 Hz, 1H), 2.20 (s, 1H), 2.13 (ddt, *J*=7.5, 2.6, 1.4 Hz, 1H), 2.09 (t, *J*=1.4 Hz, 3H), 1.98 (ddd, *J*=14.8, 7.5, 5.5 Hz, 1H), 1.67 (dd, *J*=12.7, 11.2 Hz, 1H), 1.59 (ddd, *J*=14.8, 9.3, 2.6 Hz, 1H), 1.42 (s, 3H), 1.27 (s, 3H), 0.93 (s, 3H); HRFABMS: calcd for $C_{30}H_{35}NO_6Cl_2F_3$, 632.1794: found *m/z* 632.1797 $(M+H)^+$. **8b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.47–7.41 (m, 5H), 5.89 (t, *J*=1.3 Hz, 1H), 5.70 (ddd, *J*=10.4, 5.2, 2.3 Hz, 1H), 4.15 (dt, *J*=11.0, 4.3 Hz, 1H), 3.59 (d, *J*=11.0 Hz, 1H), 3.49 (s, 3H), 3.20 (ddd, *J*=8.5, 6.0, 2.3 Hz, 1H), 2.78 (dd, *J*=17.9, 8.5 Hz, 1H), 2.72 (dd, *J*=17.9, 6.0 Hz, 1H), 2.41 (dd, *J*=12.8, 4.3 Hz, 1H), 2.24 (s, 1H), 2.17 (ddt, *J*=8.4, 2.0, 1.3 Hz, 1H), 2.09 (t, *J*=1.3 Hz, 3H), 1.97 (ddd, *J*=14.1, 8.4, 5.2 Hz, 1H), 1.67 (ddd, *J*=14.1, 10.4, 2.0 Hz, 1H), 1.67 (dd, *J*=12.8, 11.0 Hz, 1H), 1.42 (s, 3H), 1.28 (s, 3H), 0.95 (s, 3H); HRFABMS: calcd for $C_{30}H_{35}NO_6Cl_2F_3$, 632.1794: found *m/z* 632.1819 $(M+H)^+$.

Acid treatment of 4. To a solution of haterumaimide D (**4**, 0.4 mg, 1.0 μmol) in tetrahydrofuran (0.15 mL) was added 1M hydrochloric acid (0.05 mL). After being stirred at 50°C for 18 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using hexane–EtOAc (2:1) to give **3** (0.3 mg, 75%) as a colorless solid: $[\alpha]_D^{31}$=+60° (*c* 0.023, MeOH); FABMS *m/z* 416 $(M+H)^+$.

Isomerization of 2. To a solution of haterumaimide B (**2**, 1.1 mg, 2.6 μmol) in methanol (0.5 mL) was added PPTS (1.0 mg, 3.9 μmol) and the mixture was stirred at rt for 2 h and then quenched with aqueous NH_3 (0.5 mL). 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 HPLC using MeOH–H_2O–MeCN (11:7:2) to give **4** (t_R =20.2 min, 0.6 mg, 55%): $[\alpha]_D^{29}$=+57.5° (*c* 0.024, MeOH).

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