Halishigamides A−D, New Cytotoxic Oxazole-Containing Metabolites from Okinawan Sponge *Halichondria* sp.

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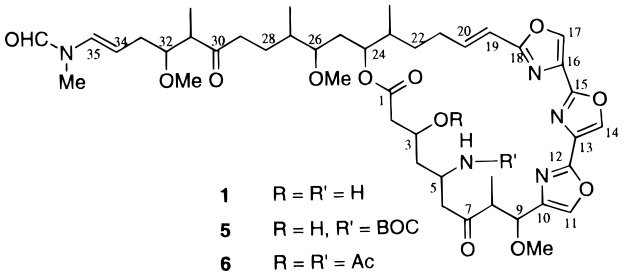
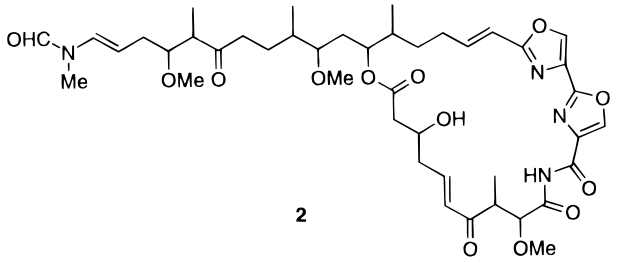
Four new oxazole-containing compounds, halishigamides A−D (**1**−**4**), have been isolated from an Okinawan marine sponge *Halichondria* sp. and the structures elucidated on the basis of 2D NMR data. Halishigamide A (**1**) is the first trisoxazole-containing macrolide possessing a primary amino group in the macrolactone ring.

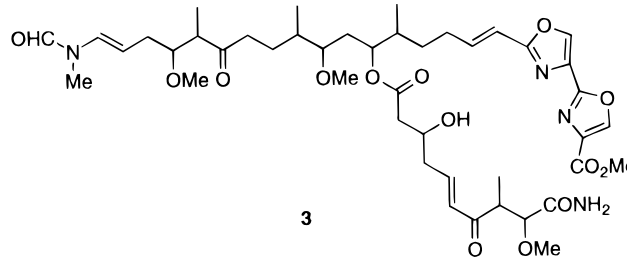
Several cytotoxic and antifugal macrolides containing two or three oxazole rings have been isolated from marine living organisms such as nudibranch egg masses,[1−3] sponges,[4−7] and stony corals.[8] In our studies on bioactive substances from marine organisms,[9−11] we isolated four new oxazole-containing compounds, halishigamides A−D (**1**−**4**), from an Okinawan marine sponge *Halichondria* sp. Here we describe the isolation and structure elucidation of **1**−**4**.

The MeOH extract of the sponge *Halichondria* sp. (order Halichondrida, family Spongiidae) collected off Ishigaki Island, Okinawa, was partitioned between EtOAc and H_2O. The EtOAc-soluble material was subjected to a silica gel column ($CHCl_3$/MeOH, 95:5−85:15). The fraction eluted with 85% $CHCl_3$/MeOH was purified by using centrifugal countercurrent chromatography (EtOAc/MeOH/H_2O) and an HP-Cellulofine column ($CHCl_3$/MeOH) to afford halishigamide A (**1**, 0.0037%, wet weight). The fraction eluted with 95% $CHCl_3$/MeOH in the silica gel column was subjected to a C_{18} column (MeOH/H_2O) followed by C_{18} HPLC (MeOH/H_2O) to afford halishigamides B (**2**, 0.0009%), C (**3**, 0.0004%), and D (**4**, 0.0006%) together with the known compounds halichondramide (**7**)[4] and halichondramide ester (**8**).[5]

Halishigamide A (**1**) was shown to have the molecular formula $C_{44}H_{63}N_5O_{12}$ by HRFABMS. Its IR spectrum indicated the presence of OH and/or NH (3350 cm^{-1}), ester and/or ketone (1700 cm^{-1}), and amide (1670 cm^{-1}) groups. The presence of a primary amino group was suggested by positive coloration to the ninhydrin test as well as treatment of **1** with di-*tert*-butyl carbonate [$(BOC)_2O$] to afford *N*-BOC derivative **5**. The ^1H and ^{13}C NMR data (Table 1) of **1** were similar to those of halichondramide (**7**).[4] The trisoxazole moiety (C-10−C-18) was characterized by three aromatic proton signals at δ 7.96 (s, H-11), 8.58 (s, H-14), and 8.51 (s, H-17). In the ^1H NMR spectrum doubled signals, due to restricted rotation around the C−N bond of the *N*-methyl formamide group, were observed in a ratio of 2:1 for H-34 (δ 5.18 and 5.26), H-35 (δ 6.71 and 7.12), and the *N*-methylformamide group (*N*-Me, δ 3.01 and 3.10; *N*-CHO, δ 8.30 and 8.07). Detailed analyses of the NMR data including $^1H-^1H$ COSY, HOHAHA, HMQC, and HMBC spectra suggested the presence of four structure units, C-7−C-9, C-10−C-18 (trisoxazole), C-19−C-35, and *N*-methylformamide, which were also found in **7**. Halichondramide (**7**) had a double bond at C-5 and C-6, while **1** possessed a methine (C-5, δ_H 4.26; δ_C







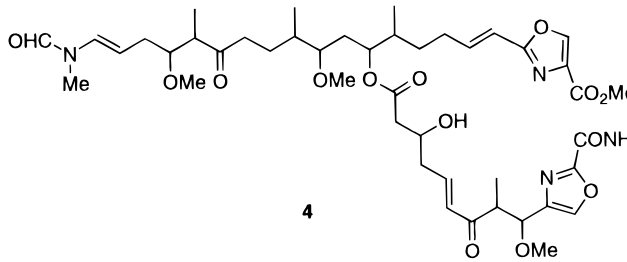


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Table 1. ^{1}H and ^{13}C NMR Data of Halishigamide A (**1**) in CD_3OD

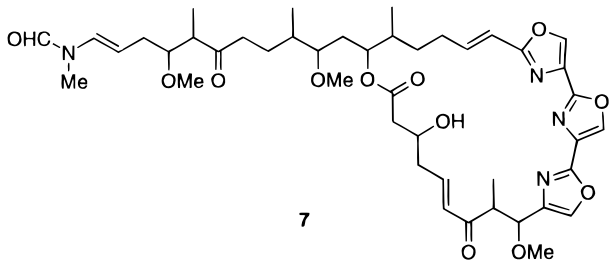
position	δ_H [m, *J* (Hz)]		δ_C (m)	HMBC
1			173.2 (s)	H_2-2, H-24
2	2.60 (d, 15.3)	2.54 (dd, 9.7, 15.3)	43.6 (t)	
3	4.44 (br t, 10.0)		69.5 (d)	H_2-2, H-4
4	2.05 (dt, 13.9, 10.5)	1.84 (d, 13.9)	39.2 (t)	H-5
5	4.25 (br t, 9.9)		49.4 (d)	H-4
6	3.58 (dd, 10.0, 19.0)	2.95 (m)	45.3 (t)	H-5
7			211.3 (s)	H-5, H-9, 8-Me
8	2.93 (m)		52.7 (d)	H-9, 8-Me
9	4.69 (d, 3.6)		78.4 (d)	8-Me, 9-OMe
10			140.9 (s)	H-9, H-11
11	7.96 (s)		139.3 (d)	
12			156.8 (s)	H-11
13			131.6 (s)	H-14
14	8.58 (s)		140.1 (d)	
15			158.3 (s)	H-14
16			130.9 (s)	H-17
17	8.51 (s)		141.0 (d)	
18			164.6 (s)	H-17, H-19, H-20
19	6.64 (d, 16.3)		116.8 (d)	
20	7.03 (dt, 16.3, 6.1)		144.1 (d)	H-19, H_2-21
21	2.42 (m)	2.18 (m)	29.7 (t)	
22	1.83 (m)	1.33 (m)	31.5 (t)	
23	1.66 (m)		38.6 (d)	23-Me
24	5.08 (t, 9.3)		76.3 (d)	H-25, 23-Me
25	1.72 (m)	1.52 (dd, 10.7, 13.8)	34.2 (t)	
26	3.02 (m)		83.2 (t)	H-24, H-28, 27-Me, 26-OMe
27	1.75 (m)		35.5 (d)	H-28, H_2-29, 27-Me
28	1.78 (m)	1.24 (m)	25.9 (t)	H_2-29, 27-Me
29	2.58 (m)[a]		41.8 (t)	
30			215.9 (s)	H_2-29, H-31, 31-Me
31	2.82 (m)		50.2 (d)	31-Me
32	3.46 (m)		83.2 (d)	H-31, H-33, 31-Me, 32-OMe
33	2.51 (m)	2.20 (m)	30.9 (t)	H-31, H-35, 31-Me
34	5.18 (dt, 14.5, 7.8)		107.1 (d)	H-33
	5.26 (dt, 14.5, 7.6)[b]		109.3 (d)[b]	H-33
35	6.71 (d, 14.5)		131.9 (d)	H_2-33, H-34, N-Me, N-CHO
	7.12 (d, 14.5)[b]		127.2 (d)[b]	H-34, N-Me, N-CHO
8-Me	1.28 (d, 7.1)		11.5 (q)	H-9
9-OMe	3.38 (s)		57.5 (q)	H-9
23-Me	0.96 (d, 6.5)		16.0 (q)	H-24
26-OMe	3.33 (s)		58.2 (q)	
27-Me	0.85 (d, 6.5)		15.8 (q)	
31-Me	0.99 (d, 6.5)		12.8 (q)	H-31
32-OMe	3.31 (s)		57.5 (q)	
NMe	3.01 (s)		27.0 (q)	H-35, N-CHO
	3.10 (s)[b]		32.8 (q)[b]	H-35
NCHO	8.30 (s)		165.4 (d)	H-35, N-Me
	8.07 (s)[b]		163.7 (d)[b]	N-Me

[a] 2H. [b] These resonances are derived from a minor rotational isomer.

49.4) and a methylene group (C-6, δ_H 3.58 and 2.95; δ_C 45.3). Connection from C-2 to C-6 was assigned by ^{1}H−^{1}H COSY cross-peaks and HOHAHA correlations. HMBC correlations for H_2-2/C-1 and H-24/C-1 indicated that C-2 was attached to C-24 via an ester carbonyl group at C-1. On the other hand, HMBC correlations for H-5/C-7 and H-9/C-7 established the bond of C-6 to C-7. The ^{13}C chemical shifts of C-3 (δ 69.5) and C-5 (δ 49.4) implied that a hydroxy and an amino group were attached to C-3 and C-5, respectively. This assignment was established by analyses of spectroscopic data of the corresponding diacetyl derivative **6**, which was obtained by acetylation of **1**. IR absorptions at 1740 and 1680 cm^{-1} were attributed to ester and amide carbonyl groups of **6**, respectively. The ^{1}H NMR and HMQC data of **6** revealed the presence of two acetyl methyl signals (δ_H 1.97, 6H; δ_C 20.5 and 23.4).[12] In the ^{1}H−^{1}H COSY spectrum H-5 (δ 4.56) was coupled to an amide NH signal (δ 7.54). Thus, the structure of halishigamide A (**1**) was deduced to be the 5,6-dihydro-5-amino form of halicondramide (**7**).

HRFABMS data of halishigamide B (**2**) established the molecular formula $C_{43}H_{60}N_4O_{13}$, which was the same as that of halichondramide imide (**9**).[5] Although the spectral data of **2** were similar to those of **9**, the ^{1}H NMR spectrum of **2** showed that chemical shifts of an imide proton [δ 10.46 (brs)] and one of two oxazole protons [δ 8.12 (s)] of **2** differed from those [δ 9.86 (brs) and 7.75 (s), respectively] of **9**. The imide moiety of halishigamide B (**2**) was assigned on the basis of analyses of HMBC and NOESY spectra. In the HMBC spectrum the imide proton (NH-10) was coupled to two imide carbonyl carbons at δ 158.2 (C-12) and 172.1 (C-10), the latter of which showed a long-range correlation to H-9. The NOESY spectrum revealed a cross-peak for 9-OMe/NH-10, indicating that the imide portion was attached to C-9. The chemical shift of C-12 corresponded well to that of an imide carbon connected to an oxazole ring.[8] Thus, the structure of halishigamide B was concluded to be **2**.

HRFABMS data of halishigamide C (**3**) showed it to have the same molecular formula, $C_{44}H_{64}N_4O_{14}$, as that



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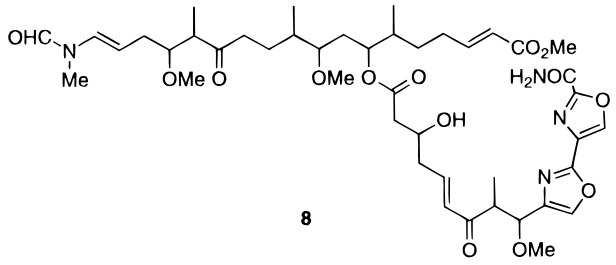

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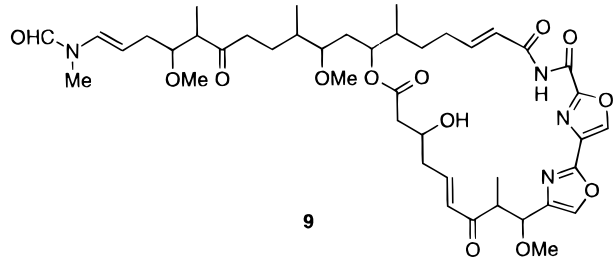

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of halichondramide ester (**8**).[5] The ^{1}H NMR spectrum of **3** contained proton signals due to two oxazoles (δ_H 8.26 and 8.29), a methyl ester (δ_H 3.95), and a primary amide (δ_H 5.62 and 6.50), which were also observed for **8**. The ^{13}C NMR data revealed the presence of carbon signals due to two oxazole rings and ester (δ_C 161.4) and amide (δ_C 173.6) carbonyls. In the decoupled HMBC (D-HMBC)[13] spectrum of **3**, H-8 was coupled to the amide carbonyl carbon, while H-14 (δ_H 8.29) and 12-OMe (δ_H 3.95) were coupled to ester carbonyl carbon (δ_C 161.4). Thus, the structure of halishigamide C was assigned as **3**.

The ^{1}H and ^{13}C NMR spectra of halishigamide D (**4**) resembled those of halishigamide C (**3**), and its molecular formula of **3** was established as $C_{44}H_{64}N_4O_{14}$ by HRFABMS. Its ^{1}H and ^{13}C NMR data suggested the presence of two oxazole rings and ester and amide carbonyl groups. Assignment of the ester and amide carbonyl groups was based on the D-HMBC technique adjusted to small long-range coupling (2 Hz), which showed the four-bond couplings from the ester methyl protons (15-OMe) to C-16 and from H-11 to the amide carbonyl carbon (C-13). Thus, the structure of halishigamide D was elucidated as **4**.

Halishigamides A−D (**1**−**4**) are new congeners of halichondramide (**7**). Halishigamide A (**1**) is the first halichondramide-related macrolide possessing a primary amino group in the macrolactone ring. Compound **1** exhibited potent cytotoxic activity against murine lymphoma L1210 and human epidermoid carcinoma KB cells (IC_{50} 0.0036 and 0.012 μg/mL, respectively) and antifungal activity against *Trichophyton mentagrophytes* (MIC, 0.1 μg/mL). On the other hand, halishigamides B−D (**2**−**4**) had weak cytotoxicity against L1210 (IC_{50} 4.4, 5.2, and 1.1 μg/mL, respectively) and KB cells (IC_{50} 7.5, 6.5, and 1.8 μg/mL, respectively) and modest antifungal activity against *T. mentagrophytes* (MIC 25, 25, and 6.5 μg/mL, respectively).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-360 polarimeter. The IR and UV spectra were taken on a JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 and ARX-500 spectrometer, respectively. FAB mass spectra were obtained on a JEOL HX-110 spectrometer using glycerol as a matrix. The pulse sequence of D-HMBC was $90°_x(^1H)$-Δ-$90°_{\phi1}(^{13}C)$-t_1/2-$180°_y(^1H)$-t_1/2-$90_{\phi2}(^{13}C)$-Δ-$AQ_{\phi3}(^1H,^{13}C$-decoupling); $\phi1 = x, -x$; $\phi2 = x, x, -x, -x$; $\phi3 = x, -x, -x, x$. The experimental conditions were as follows: $t_1 \times t_2 = 256 \times 1$ K, times = 256, pulse delay = 1.0 s, Δ = 50 or 250 ms. F1 width (115 ppm) was reduced to one half of the conventional width to enhance the degital resolution, giving a spectrum with folded signals.

Sponge Material. The sponge *Halichondria* sp. (order Halichondrida, family Spongiidae) was collected off Ishigaki Island, Okinawa, and kept frozen until used. Preserved sponge has a dark brown exterior and deep fawn interior. The specimen is firm. The surface of the sponge is covered a brown pigment layer with many pigment cells. The mesohyl is quite dense. Spicules are aligned in loose tracts or without orientation. Spicules are oxeas 427 × 10 μm with some thinner forms 335 × 4 μm. Oxeas have long pointed ends, and some stylote or strongylote modifications occur. The voucher specimen (SS-369) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University, and James Cook University of North Queensland.

Extraction and Isolation. The sponge (1.0 kg, wet weight) was extracted with MeOH (1 L × 2) and then evaporated *in vacuo* to give a residue (42.3 g). The EtOAc-soluble material (5.16 g) of the residue was subjected to a silica gel column ($CHCl_3$/MeOH 85:15) followed by centrifugal countercurrent chromatography (Model LLB-M, Sanki Laboratories, Inc.; EtOAc/MeOH/H_2O, 2:1:2, descending mode; flow rate 2.0 mL/min; rotor speed 1800 rpm; detection at 262 nm) and an HP-Cellulofine column ($CHCl_3$/MeOH, 1:1) to afford halishigamide A (**1**, 0.0037%, wet weight). The fraction eluted with $CHCl_3$/MeOH (95:5) of the silica gel column was subjected to a C_{18} column (Develosil LOP ODS, 24 × 360 mm, MeOH/H_2O, 80:20) followed by C_{18} HPLC (Develosil ODS-HG-5, 10 × 250 mm; MeOH/H_2O, 75:25; flow rate, 2.0 mL/min; UV detection at 240 nm) to give halishigamides B (**2**, 0.0009%, t_R 21.6 min), C (**3**, 0.0004%, t_R 13.8 min), and D (**4**, 0.0006%, t_R 12.8 min).

Halishigamide A (1): colorless amorphous solid; $[\alpha]^{25}_D$ +38° (*c* 0.51, MeOH); UV (MeOH) λ_{max} 262.5 nm (ϵ 15 000); IR (film) ν_{max} 3600−3200 (br), 1700, 1670 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); FABMS (pos) *m*/*z* 855 $(M + H)^+$; HRFABMS *m*/*z* 854.4548 $(M + H)^+$, calcd for $C_{44}H_{64}N_5O_{12}$, 854.4552.

Halishigamide B (2): colorless amorphous solid; $[\alpha]^{25}_D$ −72° (*c* 0.06, MeOH); UV (MeOH) λ_{max} 260 (sh), 231 nm (ϵ 66 000); IR (film) ν_{max} 3600−3200 (br), 1750, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, d, J = 6.7 Hz, 27-Me), 0.94 (3H, d, J = 6.7 Hz, 23-Me), 0.99 (3H, d, J

= 6.9 Hz, 31-Me), 1.15 (3H, d, J = 7.1 Hz, 8-Me), 1.29 (1H, m, H-28), 1.35 (1H, m, H-22), 1.53 (1H, m, H-25), 1.63 (1H, m, H-25), 1.67 (1H, m, H-22), 1.76 (1H, m, H-27), 1.78 (1H, m, H-28), 1.79 (1H, m, H-23), 2.15 (1H, m, H-33), 2.22 (1H, m, H-21), 2.42 (1H, m, H-21), 2.45 (1H, m, H-2), 2.46 (1H, m, H-33), 2.48 (1H, m, H-4), 2.52 (2H, m, H_2-29), 2.54 (1H, m, H-2), 2.58 (1H, m, H-4), 2.72 (1H, m, H-31), 2.99 (1H, m, H-26), 3.04 (0.7H, s, NMe), 3.07 (0.3H, s, NMe), 3.29 (3H, s, 26-OMe), 3.32 (3H, s, 32-OMe), 3.38 (3H, s, 9-OMe), 3.39 (1H, m, H-8), 3.45 (1H, m, H-32), 4.03 (1H, d, J = 9.6 Hz, H-9), 4.36 (1H, m, H-3), 5.04 (1H, m, H-24), 5.10 (0.7H, m, H-34), 5.12 (0.3H, m, H-34), 6.27 (1H, d, J = 16.2 Hz, H-6), 6.34 (1H, d, J = 16.0 Hz, H-19), 6.52 (0.7H, d, J = 14.1 Hz, H-35), 7.01 (1H, m, H-5), 7.05 (1H, m, H-20), 7.19 (0.3H, d, J = 14.7 Hz, H-35), 8.07 (0.3H, s, NCHO), 8.12 (1H, s, H-17), 8.29 (0.7H, s, NCHO), 8.38 (1H, s, H-14), 10.46 (1H, br s, NH-10); ^{13}C NMR ($CDCl_3$) δ 13.6 (q, 31-Me), 14.9 (q, 8-Me), 15.3 (q, 27-Me), 15.4 (q, 23-Me), 24.9 (t, C-28), 27.5 (NMe), 29.7 (t, C-21), 30.5 (t, C-33), 31.1 (t, C-22), 32.4 (t, C-25), 33.0 (q, NMe), 34.0 (d, C-27), 35.6 (d, C-23), 40.5 (t, C-4), 41.4 (t, C-29), 42.3 (t, C-2), 44.6 (d, C-8), 48.9 (d, C-31), 57.6 (q, 26-OMe), 57.8 (q, 32-OMe), 59.5 (q, 9-OMe), 66.9 (d, C-3), 75.2 (d, C-24), 81.8 (d, C-26), 82.5 (d, C-32), 85.3 (d, C-9), 105.3 (d, C-34), 106.0 (d, C-34), 114.5 (d, C-19), 126.5 (d, C-35), 129.7 (s, C-16), 130.4 (d, C-35), 133.2 (d, C-6), 137.2 (s, C-13), 138.4 (d, C-17), 143.2 (d, C-14), 144.0 (d, C-20), 144.5 (d, C-5), 155.4 (s, C-15), 158.0 (s, C-12), 162.1 (s, C-18), 162.1 (d, N-CHO), 163.3 (d, N-CHO), 171.5 (s, C-1), 172.1 (s, C-10), 201.2 (s, C-7), 213.8 (s, C-30); FABMS (pos) m/z 842 $(M + H)^+$; HRFABMS m/z 841.9798 $(M + H)^+$, calcd for $C_{43}H_{60}N_4O_{13}$ 841.9804.

Halishigamide C (3): colorless amorphous solid; $[\alpha]^{27}_D$ −70° (c 0.12, $CHCl_3$); UV (MeOH) λ_{max} 231 nm (ϵ 56 000); IR (film) ν_{max} 3600 ∼ 3200 (br), 2920, 1710, 1690, 1650, 1150, 1090 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, d, J = 6.7 Hz, 27-Me), 0.93 (3H, d, J = 6.8 Hz, 23-Me), 0.99 (3H, d, J = 6.8 Hz, 31-Me), 1.19 (3H, d, J = 7.1 Hz, 8-Me), 1.27 (1H, m, H-28), 1.30 (1H, m, H-22), 1.57 (1H, m, H-22), 1.59 (1H, m, H-25), 1.72 (1H, m, H-27), 1.77 (2H, m, H-23 and H-28), 2.14 (1H, m, H-33), 2.36 (1H, m, H-21), 2.40 (1H, m, H-21), 2.45−2.55 (7H, m, H_2-2, H_2-4, H_2-29, and H-33), 2.73 (1H, m, H-31), 2.99 (1H, , H-26), 3.03 (2.1H, s, N-Me), 3.07 (0.9H, s, N-Me), 3.22 (1H, m, H-8), 3.29, (3H, s, 32-OMe), 3.33 (3H, s, 26-OMe), 3.42 (3H, s, 9-OMe), 3.46 (1H, m, H-32), 3.85 (1H, d, J = 6.7 Hz, H-9), 3.95 (3H, s, 12-OMe), 4.21 (1H, m, H-3), 5.07 (1H, m, H-34), 5.13 (1H, m, H-24), 5.62 (1H, br s, 10-NH), 6.24 (1H, d, J = 15, 8 Hz, H-6), 6.37 (1H, d, J = 16.1 Hz, H-19), 6.50 (1H, br s, 10-NH), 6.52 (0.7H, d, J = 14.2 Hz, H-35), 6.84 (1H, m, H-20), 6.89 (1H, m, H-5), 7.18 (0.3H, d, J = 14.5 Hz, H-35), 8.06 (0.3H, s, NCHO), 8.26 (1H, s, H-17), 8.28 (0.7H, s, NCHO), 8.29 (1H, s, H-14); ^{13}C NMR ($CDCl_3$) δ 12.8 (q, 31-Me), 13.1 (q, 8-Me), 14.9 (q, 23-Me), 15.4 (q, 27-Me), 24.9 (t, C-28), 27.6 (N-Me), 30.2 (t, C-21), 30.5 (t, C-33), 30.9 (t, C-22), 31.3 (t, C-25), 34.5 (q, NMe), 34.6 (d, C-27), 36.3 (d, C-23), 39.6 (t, C-4), 41.4 (t, C-29), 41.4 (t, C-2), 46.2 (d, C-8), 48.9 (d, C-31), 52.3 (q, 10-OMe), 57.8 (q, 32-OMe), 58.0 (q, 26-OMe), 59.5 (q, 9-OMe), 66.9 (d, C-3), 75.4 (d, C-24), 81.8 (d, C-26), 82.4 (d, C-32), 83.9 (d, C-9), 105.2 (d, C-34), 106.2 (d, C-34), 116.0 (d, C-19), 126.4 (d, C-35), 130.3 (s, C-16), 130.4 (d, C-35), 134.3 (d, C-6), 138.7 (s, C-13), 138.7 (d, C-17), 142.2 (d, C-14), 143.2 (d, C-20), 143.7 (d, C-5), 155.3 (s, C-15), 160.8 (d, N-CHO), 161.4 (s, C-12), 162.1 (s, C-18), 162.2 (d, N-CHO), 171.9 (s, C-1), 173.6 (s, C-10), 198.4 (s, C-7), 213.5 (s, C-30); FABMS (pos) m/z 873 $(M + H)^+$; HRFABMS m/z 873.4522 $(M + H)^+$, calcd for $C_{44}H_{65}N_4O_{14}$ 873.4500.

Halishigamide D (4): colorless amorphous solid; $[\alpha]^{25}_D$ −88° (c 0.03, MeOH); UV (MeOH) λ_{max} 235 nm (ϵ 15 000); IR (film) ν_{max} 3600 ∼ 3200 (br), 2920, 1730, 1690, 1645, 1090 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, d, J = 6.7 Hz, 27-Me), 0.89 (3H, d, J = 7.0 Hz, 8-Me), 0.93 (3H, d, J = 6.8 Hz, 23-Me), 0.99 (3H, d, J = 6.8 Hz, 31-Me), 1.27 (1H, m, H-22), 1.33 (1H, m, H-28), 1.56 (2H, m, H_2-25), 1.58 (1H, m, H-22), 1.74 (1H, m, H-27), 1.78 (2H, m, H-23 and H-28), 2.13 (1H, m, H-33), 2.25 (1H, m, H-21), 2.39 (1H, m, H-21), 2.4−2.6 (7H, m, H_2-2, H_2-4, H_2-29, and H-33), 2.74 (1H, m, H-31), 2.97 (1H, m, H-26), 3.03 (2H, s, N-Me), 3.07 (1H, s, N-Me), 3.18 (3H, s, 9-OMe), 3.29 (3H, s, 32-OMe), 3.33 (3H, s, 26-OMe), 3.46 (1H, m, H-32), 3.48 (1H, m, H-8), 4.02 (3H, s, 15-OMe), 4.21 (1H, br s, H-3), 4.47 (1H, d, J = 9.5 Hz, H-9), 5.08 (1H, m, H-34), 5.16 (1H, m, H-24), 5.52 (1H, br s, 13-NH), 6.27 (1H, d, J = 15.7 Hz, H-6), 6.31 (1H, d, J = 15.9 Hz, H-19), 6.52 (0.7H, d, J = 15.9 Hz, H-35), 6.76 (1H, dt, J = 15.9, 7.0 Hz, H-20), 6.84 (1H, br s, 13-NH), 6.96 (1H, dt, J = 15.6, 7.5 Hz, H-5), 7.18 (0.3H, d, J = 14.5 Hz, H-35), 7.81 (1H, s, H-11), 8.06 (0.3H, s, NCHO), 8.10 (1H, s, H-17), 8.29 (0.7H, s, NCHO); ^{13}C NMR ($CDCl_3$) δ 13.0 (q, 31-Me), 14.1 (q, 8-Me), 14.9 (q, 23-Me), 15.5 (q, 27-Me), 24.8 (t, C-28), 27.6 (N-Me), 30.1 (t, C-21), 30.5 (t, C-33), 30.9 (t, C-22), 31.9 (t, C-25), 34.4 (q. N-Me), 34.4 (d, C-27), 36.2 (d, C-23), 39.5 (t, C-4), 41.4 (t, C-29), 41.4 (t, C-2), 47.1 (d, C-8), 49.0 (d, C-31), 53.4 (q, 15-OMe), 57.2 (q, 9-OMe), 57.8 (q, 32-OMe), 58.1 (q, 26-OMe), 66.9 (d, C-3), 75.2 (d, C-24), 77.6 (d, C-9), 81.9 (d, C-26), 82.5 (d, C-32), 105.3 (d, C-34), 106.2 (d, C-34), 116.4 (d, C-19), 126.4 (d, C-35), 130.5 (d, C-35), 132.6 (s, C-16), 132.6 (d, C-6), 140.0 (d, C-17), 140.1 (d, C-11), 140.8 (s, C-10), 141.6 (d, C-20), 143.1 (d, C-5), 148.2 (s, C-12), 160.3 (s, C-15), 160.7 (d, N-CHO), 162.1 (s, C-18), 162.2 (d, N-CHO), 168.1 (s, C-13), 172.0 (s, C-1), 201.4 (s, C-7), 213.6 (s, C-30); FABMS (pos) m/z 873 $(M + H)^+$; HRFABMS m/z 873.4508 $(M + H)^+$, calcd for $C_{44}H_{65}N_4O_{14}$ 873.4500).

Derivatization of Halishigamide A (1) with $(BOC)_2O$. To a solution of halishigamide A (**1**, 1.2 mg) in CH_2Cl_2 (200 μL) were added Et_3N (2.4 μL), DMAP (0.1 mg), and $(BOC)_2O$ (2.3 mg), and the reaction mixture was stirred at room temperature for 10 h. After evaporation of the solvent, the residue was subjected to a silica gel column (hexane/EtOAc 1:9) to give *N*-BOC derivative (**5**, 0.9 mg): colorless oil; IR (neat) ν_{max} 3400 (br), 2900, 1740, 1720, 1680, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, d, J = 6.8 Hz), 0.87 (3H, d, J = 6.8 Hz), 0.99 (6H, d, J = 7.3 Hz), 1.43 (9H, s), 1.4−1.5 (3H, m), 1.74 (2H, m), 2.1−2.3 (2H, m), 2.4 ∼ 2.6 (3H, m), 2.73 (1H, m), 2.95 (1H, m), 3.01 (1H, m), 3.03 (2H, s, NMe), 3.07 (1H, s, NMe), 3.29 (3H, s), 3.31 (3H, s), 3.45 (1H, m), 3.44 (3H, s), 4.18 (1H, m), 4.46 (1H, m), 5.08 (1H, m), 5.11 (1H, m), 5.29 (1H, m), 5.76 (1H, br), 6.41 (1H, d, J = 16.2 Hz), 6.51 (0.7H, d, J = 14.0 Hz, H-35), 6.05 (1H, m), 7.19 (0.3 Hz, d, J = 14.0 Hz), 7.57 (1H, s), 8.05 (1H, s), 8.06 (0.3H, s), 8.08 (1H, s), 8.28 (0.7H, s); FABMS (pos) m/z 954 $(M + H)^+$; HRFABMS m/z 954.5083 $(M + H)^+$, calcd for $C_{49}H_{71}N_5O_{14}$, 954.5075.

Acetylation of Halishigamide A (1). A solution of halishigamide A (**1**, 2.0 mg) in pyridine (0.2 mL) and acetic anhydride (0.2 mL) was stirred at room temperature for 14 h, and the solvent was removed. The residue was partitioned between EtOAc (1 mL × 3) and H_2O (1 mL). The EtOAc-soluble material was subjected to C_{18} HPLC (Develosil ODS-HG-5, Nomura Chemical, 10 × 250 mm; MeOH/H_2O, 83:17; flow rate 2.5 mL/min; detection at 240 nm) to afford **6** (0.7 mg) as a colorless oil. **6**: IR (film) ν_{max} 2900, 1740, 1700, 1680, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (3H, d, J = 6.8 Hz, 27-Me), 0.87 (3H, d, J = 6.8 Hz, 23-Me), 0.97 (3H, d, J = 7.3 Hz, 8-Me), 0.98 (3H, d, J = 7.4 Hz, 31-Me), 1.44 (1H, m), 1.46 (1H, m), 1.50 (1H, m), 1.74 (2H, m), 1.97 (6H, s, AcO), 2.06 (2H, m), 2.14 (2H, m), 2.24 (1H, m), 2.51 (2H, m), 2.73 (1H, m, H-31), 2.97 (1H, m, H-26), 3.01 (1H, m, H-8), 3.03 (2H, s, NMe), 3.07 (1H, s, NMe), 3.29 (3H, s, 32-OMe), 3.32 (3H, s, 26-OMe), 3.45 (1H, m, H-32), 3.46 (3H, s, 9-OMe), 4.56 (1H, m, H-5), 4.84 (1H, d, J = 3.7 Hz, H-9), 5.08 (2H, m, H-24 and H-34), 5.29 (1H, m, H-3), 6.45 (1H, d, J = 16.2 Hz, H-19), 6.52 (0.7H, d, J = 14.0 Hz, H-35), 6.81 (1H, m, H-20), 7.18 (0.3 Hz, d, J = 14.0 Hz, H-35), 7.54 (1H, m, 5-NH), 7.57 (1H, s, H-11), 8.05 (1H, s, H-17), 8.06 (0.3H, s, N-CHO), 8.10 (1H, s, H-14), 8.28 (0.7H, s, NCHO); FABMS (pos) m/z 938 $(M + H)^+$; HRFABMS m/z 938.4754 $(M + H)^+$, calcd for $C_{48}H_{67}N_5O_{14}$, 938.4765.

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