New Macrolides from the Sponge *Chondrosia corticata*

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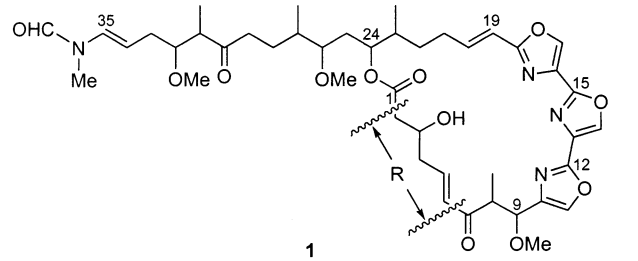
Three new oxazole-containing metabolites, neohalichondramide (**5**), (19*Z*)-halichondramide (**6**), and secohalichondramide (**7**), along with four previously reported compounds of the same structural class were isolated from the marine sponge *Chondrosia corticata* collected from Guam. The structures of novel compounds were determined on the basis of combined spectroscopic analyses. These compounds exhibited significant cytotoxicity and antifungal activity toward the human leukemia cell-line K562 and *Candida albicans*, respectively.

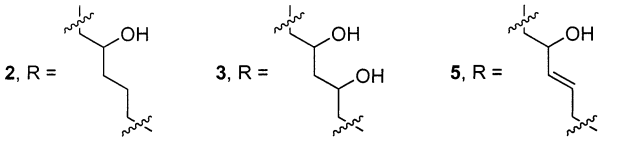
Trisoxazole-containing macrolides and structurally related marine metabolites are widely recognized to exhibit potent and diverse bioactivities such as antifungal activity, cytototoxicity, and icthyotoxicity as well as inhibition of cell division in fertilized sea urchin eggs.[1] Since the concurrent reports of ulapualides and kabiramide C from nudibranch egg masses in the late 1980s,[2,3] compounds of this structural class have been isolated from the nudibranch *Hexabranchus sanguineus*[4,5] and its potential prey, sponges of the genera *Halichondria*,[4,6] *Jaspis*,[7] and *Mycale*,[8−10] and even a taxonomically unrelated stony coral of the genus *Tubastrea*.[11] The structural uniqueness and potent bioactivity of these compounds have attracted considerable biomedical and synthetic interest.[12−16]

In the course of our continuing search for novel secondary metabolites of biomedical and ecological importance from tropical marine animals,[17] we collected the sponge *Chondrosia corticata* Thiel (order Chondrosida, family Chondrillidae), from reef slopes on the south side of Cocos Lagoon, Guam. The crude extract of these specimens exhibited significant cytotoxicity (LC_{50} 4.9 μg/mL) against the human leukemia cell-line K562 as well as antifungal activity (diameter of clear zone 12.5 mm at 25 μg/disk in paper disk method) against *Candida albicans*. Directed by the results of bioactivity tests and ^{1}H NMR analysis, the crude extracts were separated employing solvent-partitioning followed by C_{18} vacuum flash chromatography and C_{18} HPLC to afford several secondary metabolites. Herein we describe the structure elucidation of three new oxazole metabolites (**5**−**7**) along with four known compounds (**1**−**4**) of the same structural class. Those isolated previously were halichondramide (**1**), the predominantly major metabolite (>0.18% of dry weight) of this specimen, and dihydrohalichondramide (**2**) from the sponge *Halichondria* sp.[4] and the nudibranch *Hexabranchus sanguineus*,[4] jaspisamide A (**3**) from the sponge *Jaspis* sp.,[6] and halishigamide D (**4**) from the sponge *Halichondria* sp.[7] The spectral data for these compounds were in good agreement with those reported previously.



1

6 (19*Z*)



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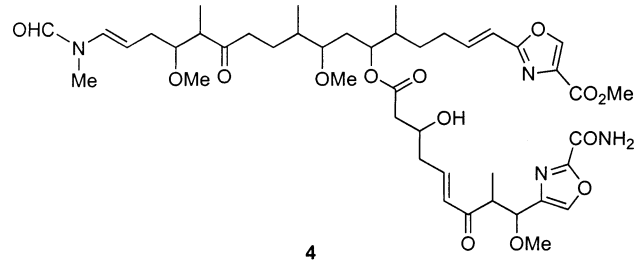

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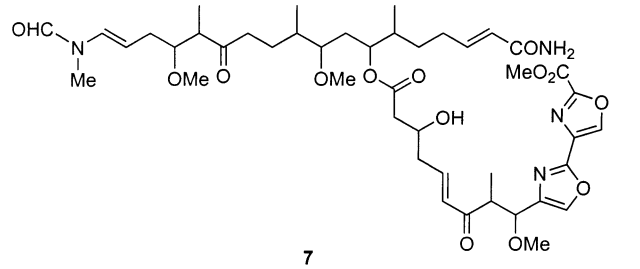

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Neohalichondramide (**5**) was isolated as an amorphous solid that analyzed for $C_{44}H_{60}N_4O_{12}$ on the basis of combined HRFABMS and ^{13}C NMR analyses. The spectral data for this compound were very similar to those obtained for halichondramide (**1**) and isohalichondramide (**2**).[4] Detailed examination of the 1D and 2D NMR data revealed the presence of the same trisoxazole moiety, lactone structure, and side chain including characteristic isomeric mixtures of the foramido group in **5** as found in the other compounds.

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Table 1. ^{1}H and ^{13}C NMR Assignments for Compound **5**[a]

position	δ_H	δ_C	HMBC	position	δ_H	δ_C	HMBC
1		172.3 C		25	1.66, ddd (14.7, 9.8, 2.0)	33.6 CH_2	C-24, C-26
2	2.60, ddd (14.2, 2.4, 1.0)	43.8 CH_2	C-1, C-3, C-4		1.49, ddd (14.7, 10.7, 2.5)		
	2.49, dd (14.2, 10.7)			26	3.08, m	81.9 CH	26-OMe
3	4.73, m	70.3 CH		27	1.73, m	35.3 CH	
4	5.86, dd (15.6, 6.4)	138.3 CH	C-2, C-3, C-5, C-6	28	1.76, m; 1.24, m	25.7 CH_2	C-26, C-27, C-29, C-30
5	6.03, dddd (15.6, 7.8, 6.4, 1.0)	124.3 CH	C-3, C-4, C-6				27-Me
6	3.47, dd (16.7, 7.8); 3.33, m	46.5 CH_2	C-4, C-5, C-7	29	2.52 (2H), m	41.3 CH_2	C-27, C-28, C-30
7		209.6 C		30		213.1 C	
8	3.45, m	48.0 CH	C-7, C-9, C-10, 8-Me	31	2.79, dq (8.8, 7.3)	49.5 CH	C-30, C-32, C-33, 31-Me
9	4.68, d (6.8)	78.6 CH	C-7, C-8, C-10, C-11	32	3.43, m	83.3 CH	C-31, C-34, 32-OMe
			8-Me, 9-OMe	33	2.45, m	30.9 CH_2	C-31, C-32, C-34, C-35
10		140.3 C			2.15, dddd (14.7, 8.3, 5.4, 1.0)		
11	7.98, s	138.4 CH	C-10, C-12	34[b]	5.10, ddd (14.2, 8.3, 6.4)	105.3 CH	C-32, C-33, C-35
12		156.1 C		34[c]	5.13, m	107.4 CH	
13		131.9 C		35[b]	6.77, d (14.2)	131.6 CH	C-33, C-34, NMe, CHO
14	8.60, s	139.4 CH	C-13, C-15	35[c]	7.11, d (14.7)	127.0 CH	
15		157.1 C		3-OH	4.87, d (4.9)		C-2, C-3
16		130.9 C		8-Me	0.88, d (6.8)	11.1 CH_3	C-7, C-8, C-9
17	8.55, s	139.5 CH	C-16, C-18	9-OMe	3.30, s	56.9 CH_3	C-9
18		163.7 C		23-Me	0.939, d (6.8)	15.7 CH_3	C-22, C-23, C-24
19	6.45, d (16.1)	115.8 CH	C-18, C-20, C-21	26-OMe	3.28, s	58.0 CH_3	C-26
20	7.07, ddd (16.1, 7.8, 6.8)	144.8 CH	C-18, C-21, C-22	27-Me	0.82, d (6.8)	15.7 CH_3	C-26, C-27, C-28
21	2.45, m; 2.29, m	28.9 CH_2	C-19, C-20, C-22, C-23	31-Me	0.943, d (7.3)	12.7 CH_3	C-30, C-31, C-32
22	1.80, m; 1.35, m	31.9 CH_2	C-20, C-21, C-23, C-24	32-OMe	3.25, s	57.5 CH_3	C-32
			23-Me	NMe[b]	2.96, s	27.2 CH_3	C-35, CHO
23	1.77, m	37.3 CH		NMe[c]	3.08, s	33.0 CH_3	
24	5.06, m	75.0 CH	C-1, C-26	NCHO[b]	8.33, br s	162.8 CH	C-35, NMe
				NCHO[c]	8.08, br s	161.6 CH	

[a] NMR data were obtained in acetone-d_6 solutions. Assignments were aided by a combination of ^{1}H COSY, TOCSY, *g*HSQC, and *g*HMBC experiments. [b,c] Denote chemical shifts for the major and minor rotational isomer, respectively.

In the ^{13}C NMR data, signals of an α,β-unsaturated carbonyl group were noticeably shifted from δ 202.3 (C, C-7), 146.7 (CH, C-5), and 133.8 (CH, C-6) in **1** to δ 209.6 (C), 138.3 (CH), and 124.3 (CH) in **5** (Table 1). Corresponding differences were observed in the ^{1}H NMR data, in which signals of the olefinic protons were shifted upfield from δ 7.26 (1H, ddd, J = 16.1, 7.3, 6.3 Hz, H-5) and 6.16 (1H, d, J = 16.1 Hz, H-6) in **1** to δ 6.03 (1H, dddd, J = 15.6, 7.8, 6.4, 1.0 Hz) and 5.86 (1H, dd, J = 15.6, 6.4 Hz) in **5**. The H-3 oxymethine proton was also shifted from δ 4.35 (1H, m) to 4.73 (1H, m), and a proton COSY experiment showed direct spin-coupling (J = 6.4 Hz) between the oxymethine and olefinic proton at δ 5.86. These spectral changes were consistent with a migration of the double bond from C-5 to C-4, which was confirmed by a *g*HMBC (gradient-HMBC) experiment in which several correlations were observed between the olefinic (H-4, H-5), allylic (H-6), and hydroxyl (3-OH) protons and neighboring carbons including the carbonyl carbons at C-1 and C-7 (Table 1). Thus, the structure of neohalichondramide (**5**) was determined to be an analogue of halichondramide (**1**) possessing the C-4 double bond.

The molecular formula of compound **6** was deduced as $C_{44}H_{60}N_4O_{12}$, identical to those of **1** and **5**, by combined HRFABMS and ^{13}C NMR spectrometry. The spectral data of this compound were very similar to those obtained for **1** and **5**. However, the ^{13}C NMR data showed noticeable differences (0.5−2 ppm) in the chemical shifts of carbons at C-18−C-25 and substituents at this portion. In the ^{1}H NMR data, the C-19 olefinic protons at δ 7.15 (1H, dt, J = 16.1, 7.3 Hz, H-20) and 6.42 (1H, d, J = 16.1 Hz, H-19) in **1** were replaced by those at δ 6.35 (1H, s) and 6.35 (1H, t, J = 6.8 Hz) in **6**. Despite the spectral differences, however, combined 2D NMR experiments showed that **6** had the same proton−proton and proton−carbon correlations throughout the entire molecule as other halichondramides, in particular, **1** and **5**. The newly appearing olefinic protons were placed at H-19 and H-20, on the basis of *g*HMBC correlations between these protons and the C-18 and C-21 carbons at δ 162.5 (C) and 28.6 (CH_2), respectively. Accordingly, compound **6** possessed the 19*Z* double bond instead of the 19*E* geometry found in the other compounds. The significant upfield shift of H-20 between these compounds was attributable to the diamagnetic effect of the oxazole ring and inversely correlated with the downfield shift of the allylic H-21 methylene protons from δ 2.49 (1H, m) and 2.30 (1H, dt, J = 14.5, 7.3 Hz) in **1** to δ 3.11 (1H, m) and 2.89 (1H, m) in **6**. A three-dimensional model study using structurally related compounds revealed that the 19*E* double bond placed the H-20 olefinic proton spatially proximal to the oxazole ring, while the 19*Z* geometry reversed the orientation and located the H-21 allylic protons near to the oxazole ring.[10] Thus the structure of compound **6** was defined to be a 19*Z* derivative of halichondramide.

The molecular formula of compound **7** was established as $C_{44}H_{64}N_4O_{14}$ by HRFABMS and ^{13}C NMR data. The spectral data of this compound were highly comparable with those of other halichondramides. However, the ^{1}H NMR data revealed the disappearance of a signal of an oxazole proton from the region of δ 7.8−8.7 that was coupled with the appearance of an oxymethyl proton at δ 3.99 (3H, s). The ^{13}C NMR spectra also indicated the cleavage of an oxazole ring and appearance of a methyl

Table 2. Results of Bioactivity Tests for Compounds **1**−**7**[a]

compound	K562 LC_{50} (μg/mL)	*C. albicans* $D_{i.z.}$ (mm)[b]	*A. niger* $D_{i.z.}$ (mm)[b]
1	0.19	18	21
2	0.32	19	11
3	0.31	18	10
4	92.0		
5	0.38	20	15
6	0.90	10	10
7	>500	15	

[a] Cytotoxicity and antifungal activity were measured by MTT method and paper disk method, respectively. [b] Numbers in the column are diameter of clear zone at the concentration of 2 μg per disk.

ester at δ 156.2 (C) and 53.5 (CH_3). Detailed interpretation of 2D NMR experiments, combined with comparison of the spectral data with other compounds, showed that the C-16−C-18 oxazole ring was cleaved to a methyl ester and primary amide, as observed for halichondramide ester and halishigamides C and D (**4**).[4,6]

All of the previously reported secohalichondramides possessed primary amide and ester carbonyl carbons at the enamine (C-10, C-13, and C-16) and oxyimine (C-12, C-15, and C-18) carbons of the corresponding oxazole ring, respectively. Comparison of the ^{1}H and ^{13}C NMR data showed striking similarity between those from **7** and halichondramide ester containing the primary amide and methyl ester group at C-16 and C-18, respectively.[4] However, the *g*HMBC data showed long-range correlation between the C-16 carbonyl carbon at δ 156.2 and methyl proton at δ 3.99, while the C-18 carbonyl carbon at δ 167.7 was correlated to the H-20 olefinic proton at δ 6.73. These data secured the placement of the methyl ester and primary amide group at C-16 and C-18, respectively, which is a new pattern of oxazole ring cleavage in these compounds. Thus, secohalichondramide (**7**) was structurally defined to be a seco-oxazole derivative of halichondramide.

Oxazole-containing macrolides exhibit potent cytotoxicity and antifungal activity that is attributable to the actin-depolymerizing ability of these metabolites.[12] In our measurement, trisoxazole-containing macrolides **1**−**3**, **5**, and **6** exhibited significant cytotoxicity toward the human leukemia cell line K562, while the secomacrolides **4** and **7** showed much weaker activity. A similar trend was also observed in the antifungal activity test against the fungi *Candida albicans* and *Aspergillus niger*, in which several compounds exhibited significant activity against test organisms but compounds **4** and **7** were generally less active (Table 2).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. IR spectra were recorded on a Mattson Galaxy spectrophotometer. NMR spectra were recorded in acetone-d_6 solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectra were provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The specimens of *Chondrosia corticata* (order Chondrosida, family Chondrillidae) was collected by hand using scuba at 15−30 m depth on the outer reef slope on the south side of Cocos Lagoon, Guam, on July 28, 1998. The growth form of *C. corticata* varies with its environment. Exposed on fore reefs where V.J.P. made collections it tends to form small (2−3 × 5−10 cm) stolons or cushions, but in caverns it forms large, 1−3 cm thick mats. Exposed animals are near black, but in caverns and crevices they become progressively lighter brownish-gray with increasing darkness. This sponge has a smooth dark surface, is soft and compressible, has a slippery but not slimy feel, and lacks an obvious odor. The sponge was identified by John Hooper as previously reported in a taxonomic inventory of the sponges of the Marianas.[18] A photograph of the sponge can be found on the Florida Museum of Natural History website (http://www.flmnh.ufl.edu/reefs/guam/guamimg/porifera/Pages/Image19.html).

Extraction and Isolation. The fresh collection was dried under shade, transported to the laboratory, and kept at −25 °C until investigated chemically. The specimens were lyophilized (dry wt 1.08 kg), macerated, and repeatedly extracted with MeOH (6 L × 3) and CH_2Cl_2 (6 L × 2). The combined crude extract (ca. 128 g) was partitioned between *n*-BuOH and H_2O, and then the *n*-BuOH layer (28.32 g) re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (13.96 g) was subjected to C_{18} reversed-phase vacuum flash chromatography using gradient mixtures of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH) and finally acetone. The fraction (1.94 g) eluted with 20% aqueous MeOH was dried and separated by reversed-phase HPLC (YMC ODS-A column, 1 cm × 25 cm, 25% aqueous MeOH) to yield in the order of elution **7**, **4**, **3**, **1**, **6**, and **2**. Purification of the oxazole-containing metabolites was accomplished by reversed-phase HPLC (30% aqueous MeOH for **7** and **4**, 40% aqueous MeCN for **3**, 35% aqueous MeCN for **1**, **6**, and **2**, respectively) to afford these compounds as amorphous solids.

The fractions (1.88 g) eluted with 10% aqueous MeOH from vacuum flash chromatography was dried and separated by reversed-phase HPLC (35% aqueous MeCN) to yield, in the order of elution, **1**, **6**, **2**, and **5**. Final purification of these compounds was accomplished by reversed-phase HPLC (35% aqueous MeCN for **1**, **6**, **2** and 30% aqueous MeCN for **5**, respectively). The overall amount of purified compound was 2258.2, 29.9, 8.7, 13.8, 12.1, 15.2, and 32.1 mg for **1**−**7**, respectively.

Halichondramide (1): colorless amorphous solid, mp 71−73 °C [lit. 66−68 °C];[4] $[\alpha]^{25}_D$ −128.1° (*c* 0.19, MeOH) {lit. $[\alpha]^{25}_D$ −100.7° (*c* 0.42, MeOH)};[4] UV (MeOH) λ_{max} (log ϵ) 230 (4.53) nm [lit. 231 (4.37) nm];[4] IR (KBr) ν_{max} 3500−3300 (br), 2935, 1710, 1700, 1690, 1660, 1460 cm^{-1}; HRFABMS *m/z* 859.4069 $[M + Na]^+$ (calcd for $C_{44}H_{60}N_4O_{12}Na$, 859.4105).

Dihydrohalichondramide (2): colorless amorphous solid; $[\alpha]^{25}_D$ −60.9° (*c* 0.61, MeOH) {lit. $[\alpha]^{25}_D$ −69.7° (*c* 1.68, MeOH)};[4] UV (MeOH) λ_{max} (log ϵ) 241 (4.64) nm [lit. 247 (4.51) nm];[4] IR (KBr) ν_{max} 3500−3300 (br), 2935, 1700, 1690, 1660, 1460 cm^{-1}; HRFABMS *m/z* 861.4237 $[M + Na]^+$ (calcd for $C_{44}H_{62}N_4O_{12}Na$, 861.4261).

Jaspisamide A (3): colorless amorphous solid; $[\alpha]^{25}_D$ −65.2° (*c* 0.52, MeOH) {lit. $[\alpha]^{25}_D$ −51° (*c* 0.13, MeOH)};[7] UV (MeOH) λ_{max} (log ϵ) 244 (4.63) nm [lit. 244 (4.38) nm];[7] IR (KBr) ν_{max} 3500−3300 (br), 2935, 1705, 1655, 1465, 1380 cm^{-1}; HRFABMS *m/z* 877.4256 $[M + Na]^+$ (calcd for $C_{44}H_{62}N_4O_{13}Na$, 877.4211).

Halishigamide D (4): colorless amorphous solid; $[\alpha]^{25}_D$ −46.1° (*c* 0.06, MeOH) {lit. $[\alpha]^{25}_D$ −88° (*c* 0.03, MeOH)};[6] UV (MeOH) λ_{max} (log ϵ) 241 (sh, 4.18) nm [lit. 235 (4.18) nm];[6] IR (KBr) ν_{max} 3500−3300 (br), 2935, 1730, 1720, 1675, 1455, 1380 cm^{-1}; HRFABMS *m/z* 895.4294 $[M + Na]^+$ (calcd for $C_{44}H_{64}N_4O_{14}Na$, 895.4317).

Neohalichondramide (5): colorless amorphous solid; $[\alpha]^{25}_D$ −67.0° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 241 (sh, 4.29), 225 (4.32) nm; IR (KBr) ν_{max} 3500−3300 (br), 2935, 1725, 1715, 1660, 1460 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 837.4283 $[M + H]^+$ (calcd for $C_{44}H_{61}N_4O_{12}$, 837.4286).

(19*Z*)-Halichondramide (6): colorless amorphous solid; $[\alpha]^{25}_D$ −87.2° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (4.38) nm; IR (KBr) ν_{max} 3500−3250 (br), 2935, 1700, 1660, 1385 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 8.62 (1H, s, H-14), 8.57 (1H, s,

H-17), 8.34 (s, N-CHO major), 8.09 (s, N-CHO minor), 8.08 (1H, s, H-11), 7.10 (d, J = 14.7 Hz, H-35 minor), 7.08 (1H, dt, J = 16.1, 7.8 Hz, H-5), 6.77 (d, J = 14.2 Hz, H-35 major), 6.35 (1H, s, H-19), 6.35 (1H, t, J = 6.8 Hz, H-20), 6.14 (1H, d, J = 16.1 Hz, H-6), 5.20 (1H, ddd, J = 10.3, 3.4, 2.0 Hz, H-24), 5.12 (1H, m, H-34 minor), 5.10 (ddd, J = 14.2, 8.1, 6.8 Hz, H-34 major), 4.35 (1H, d, J = 9.3 Hz, H-9), 4.28 (1H, m, H-3), 3.98 (1H, dq, J = 9.3, 6.8 Hz, H-8), 3.43 (1H, m, H-32), 3.31 (3H, s, 26-OMe), 3.25 (3H, s, 32-OMe), 3.12 (1H, br d, J = 10.7 Hz, H-26), 3.11 (1H, m, H-21), 3.10 (3H, s, 9-OMe), 3.08 (s, N-Me minor), 2.96 (s, N-Me major), 2.89 (1H, m, H-21), 2.79 (1H, m, H-31), 2.62 (1H, m, H-4), 2.59 (1H, dd, J = 14.6, 5.4 Hz, H-2), 2.55 (1H, m, H-2), 2.53 (2H, m, H-29), 2.51 (1H, m, H-4), 2.46 (1H, m, H-33), 2.15 (ddd, J = 14.7, 8.1, 5.4 Hz, H-33), 1.96 (1H, m, H-23), 1.76 (1H, m, H-28), 1.74 (1H, m, H-27), 1.67 (2H, m, H-22, H-25), 1.54 (1H, br dd, J = 14.7, 9.8 Hz, H-25), 1.45 (1H, dddd, J = 13.2, 10.3, 9.8, 4.9 Hz, H-22), 1.25 (1H, m, H-28), 1.09 (3H, d, J = 6.8 Hz, 23-Me), 0.94 (3H, d, J = 7.3 Hz, 31-Me), 0.84 (3H, d, J = 6.8 Hz, 8-Me), 0.82 (3H, d, J = 6.8 Hz, 27-Me); ^{13}C NMR (acetone-d_6) δ 213.1 (C, C-30), 202.4 (C, C-7), 173.7 (C, C-1), 162.8 (CH, N-CHO major), 162.5 (C, C-18), 161.6 (CH, N-CHO minor), 157.2 (C, C-15), 156.5 (C, C-12), 144.9 (CH, C-20), 144.8 (CH, C-5), 139.8 (C, C-10), 139.7 (CH, C-17), 139.3 (CH, C-14), 139.0 (CH, C-11), 134.6 (CH, C-6), 132.2 (C, C-16), 131.7 (CH, C-35 major), 131.5 (C, C-13), 127.0 (CH, C-35 minor), 114.2 (CH, C-19), 107.3 (CH, C-34 minor), 105.3 (CH, C-34 major), 83.3 (CH, C-32), 82.1 (CH, C-26), 78.1 (CH, C-9), 75.7 (CH, C-24), 68.3 (CH, C-3), 58.2 (CH_3, 26-OMe), 57.5 (CH_3, 32-OMe), 56.5 (CH_3, 9-OMe), 49.4 (CH, C-31), 44.4 (CH, C-8), 42.9 (CH_2, C-2), 41.3 (CH_2, C-29), 40.4 (CH_2, C-4), 37.8 (CH, C-23), 35.5 (CH, C-27), 33.0 (CH_3, N-Me minor), 32.3 (CH_2, C-22), 31.4 (CH_2, C-25), 30.9 (CH_2, C-33), 28.6 (CH_2, C-21), 27.2 (CH_3, N-Me major), 25.9 (CH_2, C-28), 15.6 (CH_3, 27-Me), 14.6 (CH_3, 23-Me), 14.4 (CH_3, 8-Me), 12.7 (CH_3, 31-Me); HRFABMS m/z 859.4082 $[M + Na]^+$ (calcd for $C_{44}H_{60}N_4O_{12}Na$, 859.4105).

Secohalichondramide (7): colorless amorphous solid; $[\alpha]^{25}_D$ −46.3° (c 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 240 (sh, 4.08), 213 (4.19) nm; IR (KBr) ν_{max} 3500−3250 (br), 2925, 1740, 1715, 1675, 1450, 1380 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 8.82 (1H, s, H-14), 8.34 (s, N−CHO major), 8.14 (1H, s, H-11), 8.08 (s, N-CHO minor), 7.11 (d, J = 14.7 Hz, H-35 minor), 7.02 (1H, dt, J = 15.6, 7.3 Hz, H-5), 6.77 (d, J = 14.2 Hz, H-35 major), 6.73 (1H, dt, J = 15.6, 7.3 Hz, H-20), 6.30 (1H, d, J = 15.6 Hz, H-6), 5.97 (1H, d, J = 15.6 Hz, H-19), 5.15 (dt, J = 14.7, 7.8 Hz, H-34 minor), 5.11 (m, H-34 major), 5.09 (1H, m, H-24), 4.44 (1H, d, J = 9.8 Hz, H-9), 4.26 (1H, m, H-3), 3.99 (3H, s, 16-OMe), 3.53 (1H, dq, J = 9.8, 6.8 Hz, H-8), 3.44 (1H, m, H-32), 3.29 (3H, s, 26-OMe), 3.26 (3H, s, 32-OMe), 3.11 (3H, s, 9-OMe), 3.08 (s, N-Me minor), 3.06 (1H, m, H-26), 2.96 (s, N-Me major), 2.79 (1H, dq, J = 8.3, 6.8 Hz, H-31), 2.59 (1H, dd, J = 15.1, 4.4 Hz, H-2), 2.56 (2H, m, H-4), 2.54 (2H, m, H-29), 2.52 (1H, m, H-2), 2.48 (1H, m, H-33), 2.26 (1H, m, H-21), 2.17 (1H, ddd, J = 13.1, 7.3, 6.8 Hz, H-21), 2.15 (1H, m, H-33), 1.76 (1H, m, H-23), 1.74 (2H, m, H-27, H-28), 1.61 (1H, m, H-22), 1.57 (2H, m, H-25), 1.29 (1H, m, H-22), 1.23 (1H, m, H-28), 0.95 (3H, d, J = 6.8 Hz, 31-Me), 0.91 (3H, d, J = 6.8 Hz, 23-Me), 0.832 (3H, d, J = 6.8 Hz, 8-Me), 0.830 (3H, d, J = 6.8 Hz, 27-Me); ^{13}C NMR (acetone-d_6) δ 213.2 (C, C-30), 201.6 (C, C-7), 171.8 (C, C-1), 167.7 (C, C-18), 162.9 (CH, N-CHO major), 161.6 (CH, N-CHO minor), 156.2 (C, C-16), 155.5 (C, C-12), 153.8 (C, C-15), 144.33 (CH, C-20), 144.31 (CH, C-5), 142.8 (CH, C-14), 140.6 (C, C-10), 139.5 (CH, C-11), 133.0 (C, C-13), 133.0 (CH, C-6), 131.7 (CH, C-35 major), 127.0 (CH, C-35 minor), 125.1 (CH, C-19), 107.4 (CH, C-34 minor), 105.3 (CH, C-34 major), 83.4 (CH, C-32), 82.2 (CH, C-26), 78.2 (CH, C-9), 75.2 (CH, C-24), 68.0 (CH, C-3), 58.0 (CH_3, 26-OMe), 57.5 (CH_3, 32-OMe), 56.7 (CH_3, 9-OMe), 53.5 (CH_3, 16-OMe), 49.5 (CH, C-31), 47.2 (CH, C-8), 43.0 (CH_2, C-2), 41.3 (CH_2, C-29), 40.9 (CH_2, C-4), 37.2 (CH, C-23), 35.2 (CH, C-27), 33.0 (CH_3, N-Me minor), 32.1 (CH_2, C-25), 31.7 (CH_2, C-22), 30.9 (CH_2, C-33), 30.0 (CH_2, C-21), 27.2 (CH_3, N-Me major), 25.7 (CH_2, C-28), 15.7 (CH_3, 27-Me), 15.2 (CH_3, 23-Me), 14.4 (CH_3, 8-Me), 12.7 (CH_3, 31-Me); HRFABMS m/z 895.4358 $[M + Na]^+$ (calcd for $C_{44}H_{64}N_4O_{14}Na$, 895.4317).

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