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FOUR NEW KABIRAMIDES FROM THE THAI SPONGE, *PACHASTRISSA NUX*

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Abstract – Four new cytotoxic kabiramides (**1-4**) together with the known kabiramides B, C, and D have been isolated from the sponge *Pachastrissa nux* collected in the Gulf of Thailand. The structures of the new entities were elucidated by spectroscopic methods. Kabiramide C derivatives (**8-11**) were prepared and evaluated for cytotoxicity together with the natural congeners.

INTRODUCTION

Trisoxazole-class macrolides have solely been found from marine sources. Their structures are characterized by the presence of three consecutive oxazole rings within a 25-membered macrolide. The first discovered members of this class were kabiramide C and ulapualides, which were reported in 1986 as cytotoxic or antifungal metabolites.[1] Although initially isolated from nudibranch eggmasses, a later discovery of a number of related compounds from marine sponges suggested dietary origins.[2] Additional examples of sponge-derived trisoxazole macrolides include halichondramides, mycalolides, and thiomycalolides[3] and together there are more than 40 different members.[4] In almost all cases these drugs are highly cytotoxic. The mechanism of cytotoxicity has been established for several members

and involves their ability to depolymerize F-actin and sequester G-actin thereby inhibiting essential cell functions that include motility, cytokinesis and intracellular trafficking.[5] A complementary high resolution structural analysis of kabiramide C bound to G-actin confirms the finding that kabiramide C and related drugs can be viewed as biomolecular mimics of gelsolin and CapG, two well studied proteins that bind to the barbed end of the actin filament.[5,6] This and other crystallographic studies have also led to the determination of the absolute stereochemistry of kabiramide C, halichondramide, and ulapualide A.[6] Functional fluorescent and non-fluorescent derivatives of kabiramide C were prepared that serve as unique probes for imaging changes in the distribution of barbed ends within cells and for quantitative analysis of actin in vitro.[7] A project aimed at isolating kabiramide C from the Thai sponge *Pachastrissa nux* led to the discovery of four new congeners (**1**-**4**). Derivatives (**8**-**11**) of kabiramide C were prepared to study the effect of structural modifications on the macrolide ring to cytotoxicity. In this work we describe the isolation and structure elucidation of the new kabiramides and the cytotoxicity of compounds (**1**-**10**).

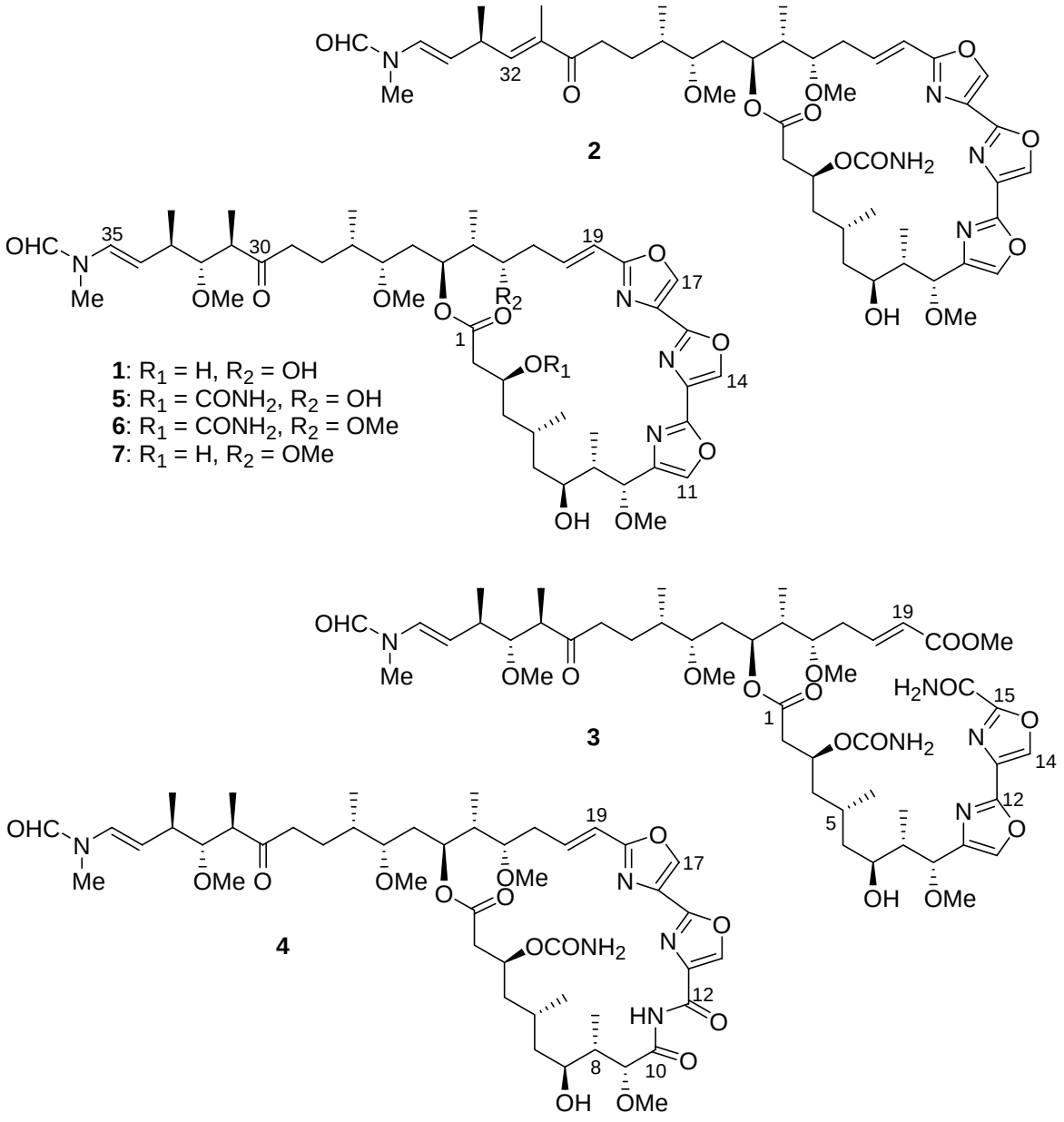
RESULTS AND DISCUSSION

Lipophilic extracts of the sponge *P. nux* were separated by repeated chromatography to give four new compounds, kabiramides F (**1**), G (**2**), H (**3**), and I (**4**) in addition to the known kabiramides B (**5**), C (**6**), and D (**7**).[1,3] Kabiramide C was the major constituent of this sponge.

The molecular formula $C_{46}H_{68}N_4O_{13}$ of kabiramide F (**1**) was deduced from HRESIMS. Both the ^1H and ^{13}C NMR data (Table 1) of **1** resembled to those of kabiramide B (**5**).[3] However, the proton signal (δ 4.32) assigned for H-3 in **1** appeared at higher field than that (δ 5.16) of **5**. Together with the difference of the molecular formulae of **1** and **5** ($C_{47}H_{69}N_5O_{14}$), it was suggested that **1** has a hydroxyl group instead of a carbamoyl group at C-3 in **5.** The remaining portions were elucidated to be the same as **5**.

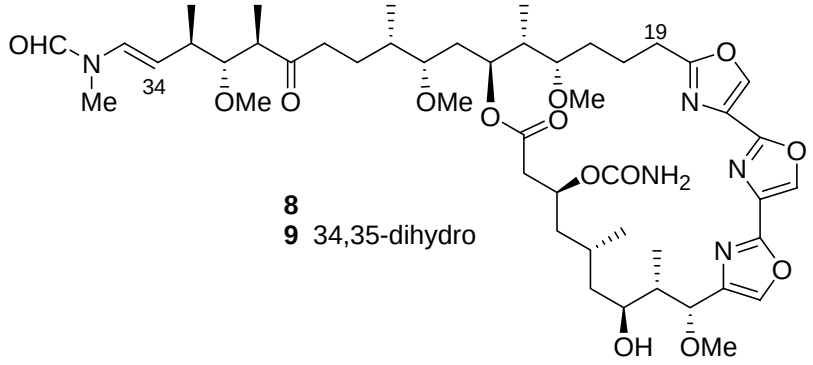
The molecular formula of compound (**2**), $C_{47}H_{67}N_5O_{13}$, indicated that it was short of a MeOH unit compared to that of kabiramide C (**6**), as shown by the presence of only three methoxyl signals (δ 3.31, 3.43 x 2; δ 57.5, 57.6, 57.7, Table 1) instead of four in **6**. The presence of an α-substituted enone moiety (δ 6.39; δ 135.8 s, 144.2 d, 202.3 s) was revealed by HMBC correlations from δ 1.82 (31-Me) to the enone carbons. COSY correlations starting from the olefinic proton signal at δ 7.17 (H-35) to the β-enone proton at δ 6.39 (H-32) and also HMBC correlation from H-29 (δ 2.68) to the ketone carbonyl at δ 202.3 (C-30) allowed us to fix the position of the enone moiety. The double bond geometry was elucidated as *E* by comparing chemical shifts of 31-Me (δ 11.6) with those of tiglates (ca. δ 12), angelates (ca. δ 20), and similar enone moieties. As the remaining portion of **2** showed almost the same NMR spectral data to those of **6**, the structure of **2** was elucidated as shown.

Compound (**3**) had a formula $C_{48}H_{75}N_5O_{16}$ as determined by HRFABMS. In the ^{1}H NMR spectrum of **3**, one of the oxazole proton signals disappeared, while a signal (δ 4.04 s) for a methyl ester was observed instead. This finding suggests that **3** is a secomacrolide related to those reported previously, containing a methyl ester and a primary amide group in the place of one of the oxazole rings.[3,4] The presence of two oxazoles and ester/amide functionality was supported by the HMBC correlations of H-11/C-10,12; H-14/C-13,15; 18-OMe/C-18; and H-19,20/C-18. The remaining portion of the structure of **3** was elucidated to be the same as kabiramide C (**6**).

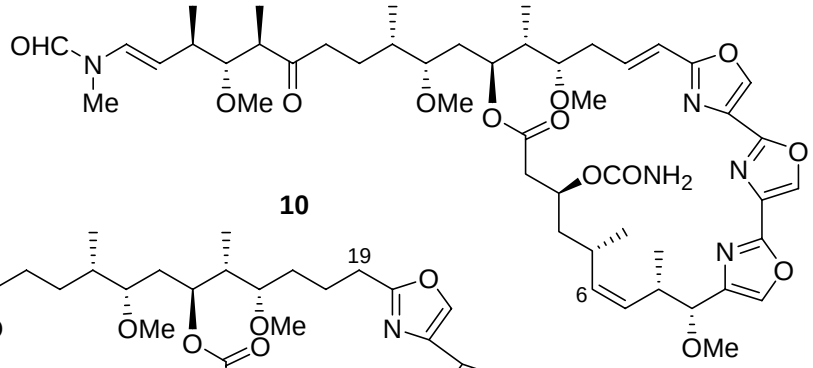


Compound (**4**), $C_{47}H_{71}N_5O_{15}$, also lacked NMR signals for one of the oxazole rings, whereas no additional proton signal was observed. In the ^{13}C NMR spectra, two carbonyl signals appeared at δ 174.8 and 162.2, which were reminiscent of those (δ 172.1, 158.0) observed for an imide functionality of halishigamide B.[4c] The location of the imide was determined by observing HMBC correlations:

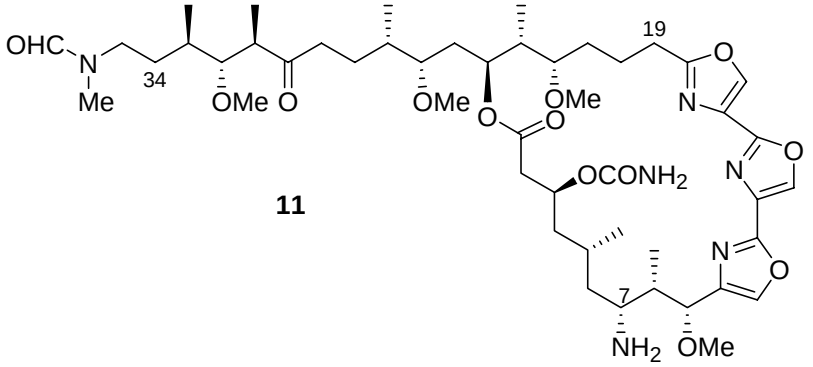
8-Me,9-OMe/C-9, H-9/C-10 (δ 174.8), H-14/C-13, H-17/C-16,18, and H-19,20/C-18. The rest of the structure of **4** was the same as of kabiramide C (**6**) as shown by the NMR data (Table 1).



8
9 34,35-dihydro



10



11

During the course of our development of kabiramide C (**6**) as staining reagents for live cells,[7b] we were intrigued about the structural requirements within the macrolide that were required for cytotoxicity. A comparison of the crystallographic results for trisoxazole macrolides and reidispongiolide A suggests that structural modification for the portions of C-3 to C-9 and also of C-19 to C-22 do not affect the affinity of the drug for actin.[6a,6c,8] With a sufficient amount of **6** in hand, we prepared several new kabiramide C derivatives (**8**-**11**).

19,20-Dihydrokabiramide C (**8**) and 19,20,34,35-tetrahydrokabiramide C (**9**) were prepared by catalytic hydrogenation of **6**. The structure of **8** was confirmed by the absence of two olefinic proton signals at δ 6.28 (H-19) and 7.45 (H-20) in the ^{1}H NMR spectrum of **6**. The absence of additional two olefinic proton signals at δ 5.07 (H-34) and 6.45 (H-35) in the ^{1}H NMR spectrum of **6** also supported the structure of **9**. The Δ^6- kabiramide C (**10**) was obtained as a by-product in the preparation of 7-azidokabiramide C via the Mitsunobu reaction by using hydrazoic acid as a nucleophile in the presence of triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate.[7a] 7-Azidokabiramide C was then converted to 7-amino-19,20,34,35-tetrahydrokabiramide C (**11**) by catalytic hydrogenation. The ^{1}H

Table 1. NMR Data for Compounds (**1-4)** in $CDCl_3$ (*J* in Hz)

C#	**1**		**2**		**3**		**4**	
1	174.0		171.6		170.1		168.8	
2	44.4	2.40 m, 2.53 m	43.0	2.14 m, 2.59 m	40.5	2.60 m, 2.65 m	40.4	2.56 m, 2.65 m
3	67.1	4.32 m	69.3	5.16 m	69.6	5.18 m	69.2	5.21 m
3-CO			157.2		nd		nd	
4	44.9	1.10 m, 1.86 m	45.0	1.33 m, 1.84 m	41.3	1.31 m, 1.85 m	40.9	1.35 m, 1.80 m
5	25.6	2.17 m	25.1	1.93 m	25.7	1.86 m	26.4	1.78 m
5-Me	19.9	0.98 d (7)	18.2	0.97 d (7)	20.5	0.99 d (7)	20.2	1.02 d (2)
6	44.0	1.57 m	43.6	1.68 m	43.6	1.49 m	41.3	1.55 m, 1.65 m
7	72.1	3.88 m	73.4	3.84 m	71.1	3.72 m	80.5	4.00 dt (3, 9)
8	41.2	2.16 m	37.3	2.15 m	41.7	1.98 m	43.1	2.10 m
8-Me	12.9	0.90 d (7)	10.7	0.99 d (7)	11.2	0.90 d (7)	14.5	1.18 d (6)
9	79.3	4.49 d (7)	78.3	4.81 brs	78.6	4.69 d (2)	82.3	3.65 d (11)
9-OMe	57.4	3.36 s	57.6	3.43 s	57.8	3.41 s	58.9	3.65 s
10	140.2		141.5		141.6		174.8	
11	136.7	7.63 s	135.6	7.58 s	136.5	7.67 s		
12	155.5		155.4		154.1		162.2	
13	130.9		131.2		132.3		136.8	
14	137.0	8.10 s	136.8	8.09 s	140.8	8.41 s	141.6	8.26 s
15	156.6		156.4		152.8		156.1	
16	129.8		129.9		156.6		130.4	
17	137.2	8.05 s	137.1	8.03 s			138.4	8.14 s
18	163.2		163.2		168.2		162.3	
18-OMe					53.6	4.04 s		
19	115.5	6.28 d (15)	115.5	6.29 d (16)	125.7	5.98 d (15)	117.8	6.45 d (16)
20	143.0	7.10 dt (15, 7)	142.0	7.45 ddd (16, 10, 6)	141.1	6.81 dt (15, 7)	138.7	6.91 dt (16, 7)
21	37.8	2.35 m, 2.58 m	33.9	2.41 m, 2.82 m	33.1	2.45 m, 2.50 m	34.6	2.50 m, 2.62 m
22	68.5	4.10 m	79.2	3.68 m	80.5	3.22 m	80.7	3.26 dt (11, 6)
22-OMe			57.5	3.43 s	57.6	3.32 s	57.8	3.36 s
23	42.0	1.57 m	40.5	1.85 m	40.0	1.82 m	40.2	1.85 m
23-Me	9.0	0.93 d (7)	8.4	0.87 d (7)	9.4	0.91 d (7)	9.5	0.94 d (7)
24	73.4	5.08 m	74.0	5.32 m	73.3	5.18 m	72.3	5.20 m
25	33.4	1.49 m	33.1	1.46 m, 1.67 m	31.8	1.52 m	32.0	1.35 m, 1.57 m
26	81.9	3.00 m	82.0	3.02 m	81.8	2.92 m	81.8	2.96 m
26-OMe	58.1	3.31 s	57.7	3.31 s	58.1	3.32 s	58.2	3.33 s
27	34.6	1.73 m	34.6	1.77 m	34.6	1.70 m	34.6	1.71 m
27-Me	15.5	0.83 d (7)	15.7	0.84 d (7)	15.3	0.84 d (7)	15.2	0.84 d (7)
28	24.9	1.28 m	26.5	1.34 m, 1.84 m	24.9	1.27 m, 1.80 m	24.9	1.25 m, 1.78 m
29	42.3	2.52 m	35.6	2.68 m	42.4	2.50 m	42.3	2.53 m
30	214.1		202.3		214.3		214.3	
31	49.1	2.68 m	135.8		49.1	2.70 m	49.2	2.65 m
31-Me	13.6	0.92 d (7)	11.6	1.82 d (1)	13.6	0.92 d (7)	13.7	0.92 d (7)
32	87.3	3.28 m	144.2	6.39 dd (1,10)	87.3	3.30 m	87.3	3.32 m
32-OMe	61.9	3.34 s			61.4	3.34 s	61.4	3.35 s
33	37.5	2.40 m	34.3	3.32 m	37.4	2.38 m	37.4	2.40 m
33-Me	19.4	1.15 d (7)	20.8	1.21 d (7)	19.4	1.16 d (7)	19.4	1.16 d (7)
34	111.4	5.11 m	113.2	5.03 dd (7, 15)	113.1	5.10 dd (9, 14)	113.1	5.10 dd (9, 14)
35	128.8	7.13 d (14) (6.45 d, 14)	128.3	7.17 d (15) (6.47 d, 15)	128.8	7.13 d (14) (6.46 d, 14)	128.8	7.13 d (14) (6.46 d, 14)
NMe	27.6	3.03 s (3.07 s)	27.5	3.01 s (3.05 s)	27.6	3.04 s (3.08 s)	27.6	3.04 s (3.08 s)
NCHO	160.9	8.07 s (8.28 s)	160.9	8.07 s (8.27 s)	160.0	8.07 s (8.29 s)	160.9	8.08 s (8.29 s)

NMR spectrum of **10** confirmed the presence of two additional olefinic proton signals at δ 5.29 (H-6) and at δ 5.49 (H-7). The structure of **11** was confirmed by the absence of olefinic proton signals (H-19, H-20, H-34, and H-35). Compound (**11**) also showed the green fluorescence under ultraviolet light at wavelength of 365 nm after spraying with fluorescamine solution, indicating the presence of primary amino group in this compound.

Compounds (**1**, **2**) and (**8**-**10**) showed nearly the same range of cytotoxicity as those of kabiramides B (**5**), C (**6**), and D (**7**) and more potent activity than those of **3** and **4** (Table 2), suggesting that an intact macrocyclic ring with three consecutive oxazole rings is important for the cytotoxic properties. Due to limited quantity, compound (**11**) was not tested. As shown by the previous crystallographic studies on kabiramide C with actin,[6] the macrocyclic ring located in a proximate region of a hydrophobic patch consisting of Ile341, Ile345, Ser348, and Leu349. Therefore, the polar functional groups, a methyl ester and a primary amide group in **3** and an imide in **4** may contribute to the less binding affinity to G-actin, resulting in weaker cytotoxicity. Moreover, double bonds at C-19 and C-34 and a hydroxyl group at C-7 did not affect the activity.

Table 2. Cytotoxicity of Compounds **1**-**10** (IC_{50}, μg/mL)

Compound	A-549	HT-29	KB	BC	NCI-H187
1	0.05	0.05	0.06	0.06	0.17
2	0.05	0.1	N/A	N/A	N/A
3	20	>50	N/A	N/A	N/A
4	2	0.4	N/A	N/A	N/A
5	0.05	0.05	N/A	N/A	N/A
6	0.05	0.1	0.04	0.03	0.18
7	N/A	N/A	0.04	0.03	0.52
8	N/A	N/A	0.03	0.03	0.2
9	N/A	N/A	0.04	0.04	1.2
10	N/A	N/A	0.06	0.04	0.18

N/A: no data.

A-549: Human lung carcinoma, HT-29: human colon adenocarcinoma, KB: oral human epidermal carcinoma, BC: breast cancer, and NCI-H187: human small cell lung cancer.

EXPERIMENTAL

General Method

Optical rotation was measured on a Jasco DIP-1000 polarimeter. IR spectra were recorded on a Jasco FT/IR-300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol A500 NMR instrument and a Bruker AVANCE DPX-300 NMR spectrometer. MS were measured on a Jeol JMS-700 instrument and a Micromass LCT mass spectrometer.

Isolation

The first specimen (12 kg, wet) of the black sponge *Pachastrissa nux* was collected off Sichang Island in the Gulf of Thailand in May, 1997. The sponge was repeatedly extracted with 15 L of acetone, and its lipophilic portion was taken to give 69 g of an oil. This extract was successively separated on vacuum flash column (silica gel), silica gel column, and repeatedly by HPLC (RP18, MeOH-H_2O) to give four new kabiramides (**1**-**4**) in the amount of 4.8, 5.5, 3.6, and 2.4 mg together with kabiramides B (**5**, 21.8 mg) and C (**6**, 79.3 mg).

The second sponge specimen (15 kg, wet) was collected in February, 2003 at a nearby location of the first collection and exhaustively extracted with MeOH (11 L). The EtOAc layer (24 g) prepared from liquid-liquid partition of the MeOH extract was separated successively by silica gel, Sephadex LH-20, silica gel (flash column), and by RP18 reversed phase HPLC column to yield kabiramide F (**1**, 14.0 mg) along with kabiramides C (**6**, 1.61 g) and D (**7**, 38.0 mg).

Compound (1): $[\alpha]_D$ -26 (*c* 0.46, $CHCl_3$); IR (neat) 3420, 2930, 1720, 1660, 1100 cm^{-1}; ESIMS *m/z* $[M+Na]^+$ 907.4679 ($C_{46}H_{68}N_4O_{13}Na$, Δ -2.2 ppm).

Compound (2): $[\alpha]_D$ +38 (*c* 0.40, $CHCl_3$); IR (neat) 3480, 3360, 2930, 1730, 1655, 1085 cm^{-1}; FABMS *m/z* $[M+H]^+$ 910.4854 ($C_{47}H_{68}N_5O_{13}$, Δ +4.4 ppm).

Compound (3): $[\alpha]_D$ -35 (*c* 0.30, $CHCl_3$); IR (neat) 3420, 2930, 1720, 1680, 1650, 1090 cm^{-1}; FABMS *m/z* $[M+H]^+$ 978.5289 ($C_{48}H_{76}N_5O_{16}$, Δ +0.2 ppm).

Compound (4): IR (neat) 3350, 2920, 1655, 1090 cm^{-1}; FABMS *m/z* $[M+H]^+$ 946.5001 ($C_{47}H_{72}N_5O_{15}$, Δ -2.5 ppm).

19,20-Dihydrokabiramide C (8) and 19,20,34,35-tetrahydrokabiramide C (9). A solution of **6** (14.3 mg, 15.2 μmol) in MeOH (500 μL) was cooled in an ice bath, and 10% Pd/C (1.5 mg) was added under nitrogen stream. Then the suspension was stirred under hydrogen atmosphere for 3 h, filtered, and the solvent was evaporated to give a residue that was further purified by preparative silica gel TLC (CH_2Cl_2-MeOH = 20:1) to give **8** (6.1 mg, 42 %) and **9** (5.9 mg, 41 %) as white amorphous solid.

19,20-Dihydrokabiramide C (8): ^1H NMR ($CDCl_3$, 300 MHz) δ 8.26 (0.7H, s, 35-NCHO), 8.07 (1H, s, H-14), 8.05 (1H, s, H-17 and 0.3H, s, 35-NCHO), 7.54 (1H, s, H-11), 7.10 (0.3H, d, *J* = 14.4 Hz, H-35), 6.43 (0.7H, d, *J* = 14.4 Hz, H-35), 6.32 (2H, br s, 3-$OCONH_2$), 5.28 (1H, dd, *J* = 10.1, 5.6 Hz, H-24), 5.22 (1H, t, *J* = 11.0 Hz, H-3), 5.08 (1H, m, H-34), 4.75 (1H, br s, H-9), 3.80 (1H, H-7), 3.44-3.39 (1H, H-22), 3.44 (3H, s, 9-OMe), 3.39 (3H, s, 22-OMe), 3.31 (6H, s, 26-OMe and 32-OMe), 3.27 (1H, H-32), 3.05 (1H, s, 35-NMe), 3.01 (2H, s, 35-NMe), 3.07-1.20 (26H), 1.13 (3H, d, *J* = 6.9 Hz, 33-Me), 1.05 (3H, d, *J* = 7.5 Hz, 8-Me), 0.97-0.78 (12H, 4 × Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 213.8 (s), 170.7 (s), 166.6 (s), 161.9 (d), 160.7 (d), 156.7 (s), 156.2 (s), 155.2 (s), 141.3 (s), 137.9 (d), 137.0 (d), 135.2 (d), 131.0 (s),

129.3 (s), 128.6 (d), 124.6 (d), 113.0 (d), 111.3 (d), 87.3 (d), 87.2 (d), 81.9 (d), 81.2 (d), 78.3 (d), 75.1 (d), 73.2 (d), 68.8 (d), 61.4 (q), 58.3 (q), 57.9 (q), 57.7 (q), 49.2 (d), 49.1 (d), 45.2 (t), 44.0 (t), 43.2 (t), 42.5 (t), 40.3 (d), 37.7 (d), 37.5 (d), 37.1 (d), 34.7 (d), 33.2 (q), 32.8 (t), 31.8 (t), 29.8 (t), 28.1 (t), 27.7 (q), 25.2 (t), 25.0 (d), 22.0 (t), 19.5 (q), 17.8 (q), 15.7 (q), 13.7 (q), 10.7 (q), 7.9 (q); ESIMS *m/z* $[M + Na]^+$ 966.5060 ($C_{48}H_{73}N_5O_{14}Na$, Δ +0.8 ppm).

19,20,34,35-Tetrahydrokabiramide C (9): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06 (1H, s, H-14), 8.05 (1H, s, H-17), 8.02 (0.6H, s, 35-NCHO), 7.99 (0.4H, s, 35-NCHO), 7.53 (1H, s, H-11), 6.31 (2H, br s, 3-$OCONH_2$), 5.28 (1H, dd, *J* = 10.2, 5.6 Hz, H-24), 5.21 (1H, t, *J* = 11.2 Hz, H-3), 4.75 (1H, br s, H-9), 3.80 (1H, H-7), 3.44-3.39 (1H, H-22), 3.44 (3H, s, 9-OMe), 3.39 (3H, s, 22-OMe), 3.31 (3H, s, 26-OMe), 3.27 (3H, s, 32-OMe), 3.23 (1H, H-32), 2.90 (1.2H, s, 35-NMe), 2.83 (1.8H, s, 35-NMe), 3.00-1.20 (30H), 1.03-0.77 (18H, 6 × Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 213.6 (s), 213.3 (s), 170.7 (s), 166.6 (s), 162.3 (d), 162.2 (d), 156.7 (s), 156.2 (s), 155.2 (s), 141.3 (s), 137.8 (d), 137.0 (d), 135.2 (d), 131.0 (s), 129.3 (s), 88.0 (d), 87.8 (d), 81.9 (d), 81.2 (d), 78.3 (d), 75.1 (d), 73.1 (d), 68.8 (d), 60.9 (q), 58.3 (q), 57.9 (q), 57.7 (q), 48.3 (d), 47.9 (t), 45.1 (t), 44.0 (t), 43.2 (t), 42.7 (t), 42.2 (t), 40.3 (t), 37.2 (d), 34.8 (d), 34.6 (q), 32.8 (2C, d and t), 32.3 (d), 31.8 (t), 29.8 (t), 29.6 (q), 29.1 (t), 28.0 (t), 27.4 (t), 25.3 (t), 25.0 (d), 22.0 (t), 17.3 (q), 17.2 (q), 15.7 (q), 13.8 (q), 10.7 (q), 7.9 (q); ESIMS *m/z* $[M + Na]^+$ 968.5200 ($C_{48}H_{75}N_5O_{14}Na$, Δ -0.8 ppm).

Δ^6- Kabiramide C (10). A paste of sodium azide (260 mg, 4 mmol) in H_2O (260 μL) was stirred on an ice bath, and benzene (1.6 mL) was added. Concentrated sulfuric acid (106 μL, 2 mmol) was carefully added dropwise to the reaction mixture. After stirring for 10 min, the organic layer containing hydrazoic acid was separated and dried over anhydrous $MgSO_4$. To the mixture of **6** (286 mg, 304 μmol) and PPh_3 (158 mg, 602 μmol) in dry THF (3 mL) stirred on an ice bath under a nitrogen atmosphere, the solution of hydrazoic acid in benzene (300 μL) was added. After stirring for 15 min, DIAD (117 μL, 604 μmol) was added dropwise over 1 min to the reaction mixture. The reaction mixture was stirred at rt for 4 h. After concentration the resulting residue was separated using Sephadex LH-20 (hexane-CH_2Cl_2-MeOH = 2:1:1) and preparative silica gel TLC (CH_2Cl_2-MeOH = 20:1) to give **10** as white amorphous solid (115 mg, 41%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (0.7H, s, 35-NCHO), 8.05 (1H, s, H-14 and 0.3H, s, 35-NCHO), 8.00 (1H, s, H-17), 7.53 (1H, s, H-11), 7.50 (1H, m, H-20), 7.13 (0.3H, d, *J* = 14.4 Hz, H-35), 6.42 (0.7H, d, *J* = 14.4 Hz, H-35), 6.26 (1H, d, *J* = 16.0 Hz, H-19), 5.49 (1H, t, *J* = 10.3 Hz, H-6 or H-7), 5.29 (1H, t, *J* = 10.3 Hz, H-6 or H-7), 5.27 (1H, m, H-24), 5.10 (1H, m, H-3), 5.08 (1H, m, H-34), 4.28 (1H, s, H-9), 3.75 (1H, m, H-22), 3.43 (3H, s, OMe), 3.42 (3H, s, OMe), 3.31 (3H, s, OMe), 3.29 (3H, s, OMe), 3.40-3.20 (1H), 3.04 (1H, s, 35-NMe), 3.00 (2H, s, 35-NMe), 3.20-1.30 (19H), 1.12 (3H, d, *J* = 6.8 Hz, 33-Me), 0.98 (3H, d, *J* = 6.4 Hz, 8-Me), 0.90-0.78 (12H, 4 × Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 213.9 (s), 213.8 (s), 171.6 (s), 163.0 (s), 161.9 (d), 160.6 (d), 157.2 (s), 156.1 (s), 155.2 (s), 142.3 (s),

142.2 (d), 136.7 (d), 136.5 (d), 135.3 (d), 135.1 (d), 131.1 (s), 130.3 (d), 129.8 (s), 128.6 (d), 124.6 (d), 115.2 (d), 113.0 (d), 111.3 (d), 87.4 (d), 87.3 (d), 82.7 (d), 82.0 (d), 78.9 (d), 73.9 (d), 69.3 (d), 61.4 (q), 59.2 (q), 57.9 (q), 57.5 (q), 49.2 (d), 49.1 (d), 44.5 (t), 43.2 (t), 42.5 (t), 42.4 (t), 40.6 (d), 37.7 (d), 37.5 (d), 35.7 (d), 34.6 (d), 33.9 (t), 33.2 (2C, t and q), 27.8 (d), 27.7 (q), 25.1 (t), 20.1 (q), 19.5 (q), 15.7 (q), 13.7 (q), 12.9 (q),8.6 (q); ESIMS *m/z* $[M + Na]^+$ 946.4781 ($C_{48}H_{69}N_5O_{13}Na$, Δ -3.3 ppm).

7-Amino-19,20,34,35-tetrahydro kabiramide C (11). 7-Azidokabiramide C[7a] (3.0 mg, 3.2 μmol) was dissolved in MeOH (500 μL) and Pd/C (0.5 mg) was added under nitrogen. The suspension was stirred at rt under hydrogen atmosphere for 2 h. After removing the catalyst by filtration the solution was concentrated to give a residue which was then purified by preparative silica gel TLC (CH_2Cl_2-MeOH = 10:1) to give **11** (1.5 mg, 48%) as white amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06 (1H, s, H-14), 8.04 (1H, s, H-17), 8.03 (0.6H, s, 35-NCHO), 8.00 (0.4H, s, 35-NCHO), 7.52 (1H, s, H-11), 6.52 (2H, br s, 3-$OCONH_2$), 5.28 (1H, m, H-24), 5.18 (1H, m, H-3), 4.54 (1H, br s, H-9), 3.45-3.35 (1H, H-22), 3.52 (3H, s, 9-OMe), 3.39 (3H, s, 22-OMe), 3.31 (3H, s, 26-OMe), 3.28 (3H, s, 32-OMe), 3.35-3.20 (1H, H-32), 2.92 (1.2H, s, 35-NMe), 2.84 (1.8H, s, 35-NMe), 3.00-1.20 (30H), 1.04-0.66 (18H, 6 × Me); ESIMS *m/z* $[M + H]^+$ 945.5542 ($C_{48}H_{77}N_6O_{13}$, Δ -0.6 ppm).

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