Notoamides F−K, Prenylated Indole Alkaloids Isolated from a Marine-Derived *Aspergillus* sp.

Sachiko Tsukamoto,*[,†,‡] Hikaru Kato,[‡] Masayuki Samizo,[‡] Yuka Nojiri,[‡] Hiroyuki Onuki,[§] Hiroshi Hirota,[§] and Tomihisa Ohta[‡]

Graduate School of Science, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, and RIKEN Genomic Sciences Center (GSC), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan

Received July 29, 2008

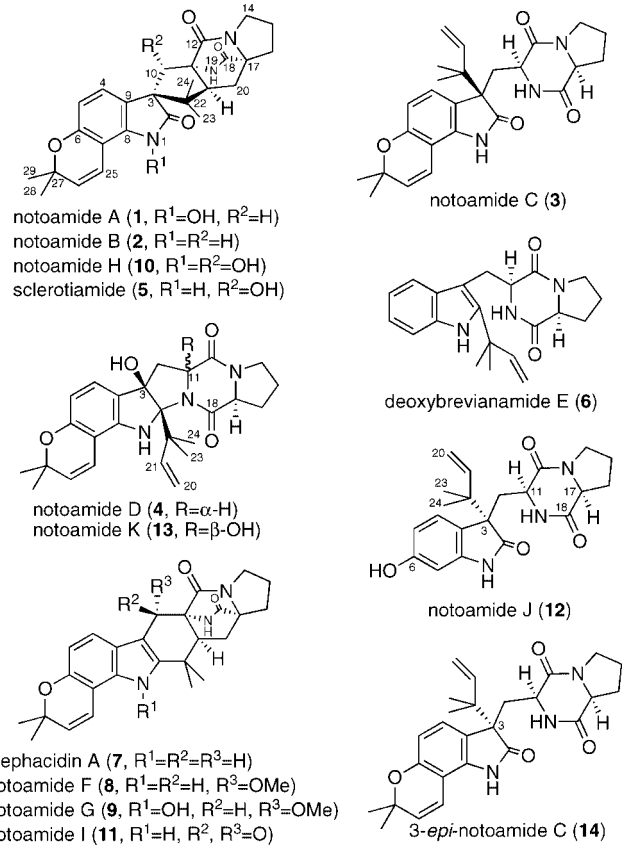
Six new prenylated indole alkaloids, named notoamides F−K (**8**−**13**), were isolated from a marine-derived *Aspergillus* sp. Their structures, including absolute configurations, were elucidated by spectroscopic methods. Notoamide I (**11**) showed weak cytotoxicity against HeLa cells with an IC_{50} value of 21 μg/mL.

A family of prenylated indole alkaloids, including the brevianamides,[1] paraherquamides,[2] stephacidin,[3] asperparalines,[4] marcfortines,[5] notoamides,[6] malbrancheamides,[7] avrainvillamide,[8] and sclerotiamide,[9] are secondary metabolites produced by various fungi of the genera *Aspergillus* and *Penicillium*. These alkaloids contain a diketopiperazine or a bicyclo[2.2.2]diazaoctane ring, which is derived from tryptophan, a cyclic amino acid residue consisting of either proline or pipecolic acid, and one or two isoprene units. Due to their complex ring systems, this family has become the target of synthetic studies. In addition, a wide range of biological activities, including insecticidal, antitumor, anthelmintic, calmodulin inhibitory, and antibacterial, has been reported within this family. Recently, we isolated notoamides A−D (**1**−**4**)[6] (Chart 1) from a marine-derived *Aspergillus* sp. along with the known alkaloids sclerotiamide (**5**),[9] deoxybrevianamide E (**6**),[1c] and stephacidin A (**7**).[3] This *Aspergillus* sp. exhibits an extensive array of co-metabolites within the structurally diverse ring systems of prenylated indole alkaloids. As part of our continuing efforts to discover new alkaloids in fungi, we further examined cultures of this *Asperigillus* sp. for new metabolites. Herein we describe the isolation, structure, and biological activity of six new alkaloids, notoamides F−K (**8**−**13**).[10]

The *Aspergillus* sp.[6] was cultured on agar plates and subjected to extraction with EtOH. After evaporation, the aqueous residue was extracted with EtOAc and then *n*-BuOH. The EtOAc fraction was partitioned between *n*-hexane and 90% MeOH−H_2O. The *n*-BuOH and aqueous MeOH fractions, which contained notoamides, were combined and purified by column chromatography and HPLC to afford notoamides F−K (**8**−**13**).

FABMS of notoamide F (**8**) showed a quasi-molecular ion peak at *m/z* 462 $[M + H]^+$, and the molecular formula was determined as $C_{27}H_{31}N_3O_4$ on the basis of HRFABMS; thus **8** must have 14 degrees of unsaturation. The ^{1}H NMR spectrum of **8** (Table 1) showed four singlet methyl signals at δ 1.10, 1.387, 1.391, and 1.40, four doublet olefinic signals at δ 5.68 (d, $J = 9.5$ Hz), 6.58 (d, $J = 8.0$ Hz), 6.83 (d, $J = 9.5$ Hz), and 7.45 (d, $J = 8.0$ Hz), a methoxy signal at δ 3.49, an oxygen-bearing signal at δ 5.01 (s), and two D_2O exchangeable signals at δ 7.09 and 10.03. Thus, the spectrum was similar to that of stephacidin A (**7**) except for the presence of a methoxy group. Analysis of 2D NMR data suggested that the methoxy group was attached to C-10, which was indicated by HMBC correlations between 10-OMe (δ 3.49) and C-10 (δ 69.9) and between H-10 (δ 5.01) and C-2 (δ 143.0), C-3 (δ 107.5), C-11 (δ 63.5), C-21 (δ 46.5), and 10-OMe (δ 57.4) (Figure 1). The relative configuration of **8** was established from NOE correlations between H-21 (δ 2.80) and H-19 (δ 7.09) and H_3-23 (δ 1.10) and between H_3-24 (δ 1.387) and H-10 (δ 5.01) (Figure 1). The Cotton effect at 200−250 nm arises from an n−π* transition of the diketopiperazine amide moiety, which is diagnostic for the bicyclo[2.2.2]diazaoctane diketopiperazine core.[11] The CD spectrum of **8** (Figure S1) showed a positive Cotton effect around 225 nm. Thus, the structure of **8**, including the 10*S*,11*R*,17*S*,21*S* configuration, was determined.

Chart 1



The molecular formula of notoamide G (**9**), $C_{27}H_{31}N_3O_5$, was established by high-resolution FABMS and was found to contain an additional oxygen atom when compared to that of **8**. The NMR data of **9** (Table 2) were very similar to those of **8**, except for the absence of the exchangeable NH signal at δ 10.03 in **8**. In addition, a difference was observed in the chemical shift for H-25 (δ 6.83 in **8** and δ 7.87 in **9**). This feature was also found in notoamides A (**1**) and B (**2**),[6] and accordingly **9** was concluded to be the 1-hydroxy derivative of **8**. The CD spectrum of **9** (Figure S2) showed a positive Cotton effect around 225 nm, and the NOE spectrum of **9** suggested that the absolute configuration of **9** was the same as that of **8**.

The HRFABMS of notoamide H (**10**) showed the molecular formula $C_{26}H_{29}N_3O_6$. The ^{1}H and ^{13}C NMR spectra of **10** suggested

* To whom correspondence should be addressed. Tel: +81-43-290-3693. E-mail: sachiko@faculty.chiba-u.jp.
† Chiba University.
‡ Kanazawa University.
§ RIKEN.

10.1021/np800471y CCC: $40.75
Published on Web 11/18/2008

Table 1. ^{1}H and ^{13}C NMR Data for **8**[a]

position	δ_H, J in Hz	δ_C, mult.	HMBC
1	10.03 br s		9
2		143.0 qC	
3		107.5 qC	
4	7.45 d 8.0	119.7 CH	6, 8
5	6.58 d 8.0	110.7 CH	6, 7, 9
6		149.4 qC	
7		106.0 qC	
8		134.2 qC	
9		123.4 qC	
10	5.01 s	69.9 CH	2, 3, 11, 21, 10-OMe
11		63.5 qC	
12		168.1 qC	
14	3.25 m	44.3 CH_2	16
	3.39 m		
15	1.89 m	25.0 CH_2	
	1.92 m		
16	1.89 m	29.8 CH_2	
	2.65 m		
17		67.5 qC	
18		173.1 qC	
19	7.09br s		17
20	2.12 (2H) m	31.2 CH_2	17, 18
21	3.30 m	46.5 CH	
22		36.0 qC	
23	1.10 (3H) s	23.0 CH_3	2, 24
24	1.387 (3H) s	28.8 CH_3	2, 23
25	6.83 d 9.5	118.6 CH	6, 27
26	5.68 d 9.5	130.0 CH	7, 27
27		76.0 qC	
28	1.391 (3H) s	27.6 CH_3	26, 27, 29
29	1.40 (3H) s	27.7 CH_3	26, 27, 28
10-OMe	3.49 (3H) s	57.4 CH_3	10

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

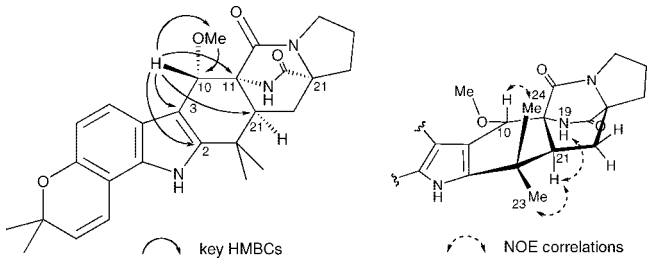


Figure 1. Key HMBCs and NOE correlations for **8**.

that it possessed a different nucleus than **8** and **9** and were almost superimposable on those of sclerotiamide (**5**),[9] except for differences for H-25 (δ 7.22 in **10** and δ 6.66 in **5**) and C-2 (δ 169.6 in **10** and δ 179.4 in **5**), which indicated that **10** was the 1-hydroxy derivative of **5**. The absolute configuration of **10** was established as 3*R*,11*R*,17*S*,21*S* by its CD spectrum (Figure S3).

Notoamide I (**11**) showed a molecular ion peak at *m*/*z* 446 [M + H]$^+$ in the FABMS, and the molecular formula was determined as $C_{26}H_{27}N_3O_4$ by HRFABMS. The ^{1}H and ^{13}C NMR spectra (Table 4) were similar to those of stephacidin A (**7**), except for the presence of an additional carbonyl carbon at δ 184.0 and the absence of an oxymethine signal (H-10 in **7**). On the basis of 2D NMR data including HMBC correlations (Figure 2) and the molecular formula, **11** was established to be 10-oxostephacidin A. Thus, the carbon at δ 184.0 corresponds to an α,β-unsaturated carbonyl carbon (C-10). The CD spectrum of **11** (Figure S4) showed that its absolute configuration was the same as that of other notoamides.

Notoamide J (**12**) had the molecular formula $C_{21}H_{25}N_3O_4$ as established by HRFABMS. The ^{1}H and ^{13}C NMR spectra (Table 5) showed that the structure of **12** was partially the same as that of 3-*epi*-notoamide C (**14**).[6b] In the ^{1}H NMR spectrum, differences were observed in the presence of signals for a 1,2,4-trisubstitued phenyl group [δ 6.46 (d, J = 1.0 Hz, H-7), 6.48 (dd, J = 8.0, 1.0 Hz, H-5),

Table 2. ^{1}H and ^{13}C NMR Data for **9**[a]

position	δ_H, J in Hz	δ_C, mult.	HMBC
1			
2		151.8 qC	
3		113.6 qC	
4	7.45 d 8.0	124.4 CH	6, 8
5	6.82 d 8.0	117.3 CH	7, 9
6		155.5 qC	
7		112.8 qC	
8		141.4 qC	
9		129.1 qC	
10	4.33 s	76.7 CH	11, 12, 10-OMe
11		62.8 qC	
12		169.0 qC	
14	3.48 (2H) t 7.0	44.8 CH_2	15, 16
15	1.91 m	25.1 CH_2	
	2.10 m		
16	1.96 m	31.6 CH_2	17, 18
	2.16 m		
17		67.6 qC	
18		172.3 qC	
19	7.34 br s		
20	1.91 (2H) m	29.6 CH_2	
21	3.63 dd 10.0, 8.5	51.2 CH	
22		37.3 qC	
23	1.40 (3H) s	22.8 CH_3	2, 21, 22, 24
24	1.27 (3H) s	30.3 CH_3	2, 21, 22, 23
25	7.87 d 10.0	116.7 CH	6
26	5.87 d 10.0	133.4 CH	7
27		77.1 qC	
28	1.429 (3H) s	28.0 CH_3	26, 27, 29
29	1.433 (3H) s	28.0 CH_3	26, 27, 28
10-OMe	3.46 (3H) s	60.5 CH_3	10

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

Table 3. ^{1}H and ^{13}C NMR Data for **10**[a]

position	δ_H, J in Hz	δ_C, mult.	HMBC
1			
2		169.6 qC	
3		67.9 qC	
4	7.03 d 8.0	126.0 CH	3, 6, 8
5	6.47 d 8.0	109.9 CH	6, 7, 9
6		154.0 qC	
7		106.2 qC	
8		138.1 qC	
9		118.1 qC	
10	5.40 d 8.5	73.3 CH	2
11		66.5 qC	
12		169.6 qC	
14	3.48 (2H) m	44.0 CH_2	15, 16
15	1.91 m	25.1 CH_2	14, 16
	2.06 m		16
16	2.66 (2H) m	29.8 CH_2	14, 15, 17, 18, 20
17		69.9 qC	
18		173.4 qC	
19	7.08br s		
20	1.82 m	31.2 CH_2	17, 18, 21, 22
	2.02 m		17, 21
21	3.80 t 9.5	56.2 CH	11, 12, 20, 22, 23, 24
22		44.7 qC	
23	0.74 (3H) s	23.2 CH_3	3, 21, 22, 24
24	0.86 (3H) s	19.7 CH_3	3, 21, 22, 23
25	7.22 d 10.0	117.4 CH	6, 7, 8, 27
26	5.75 d 10.0	130.4 CH	7, 27, 28, 29
27		76.5 qC	
28	1.41 (3H) s	27.9 CH_3	26, 27, 29
29	1.42 (3H) s	28.2 CH_3	26, 27, 28
10-OH	4.67 d 8.5		

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

and 7.01 (d, J = 8.0 Hz, H-4)], which replaced the signals derived from a second isoprene unit [H-25, H-26, H_3-28, and H_3-29]. The position of the hydroxy group at C-6 was confirmed by the HMBC correlation between H-5 (δ 6.48) and C-9 (δ 120.9). Consequently, **12** was identified as an 2-oxo-6-hydroxyindole derivative, which was

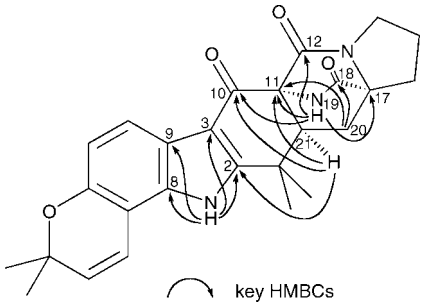


Figure 2. Key HMBCs for **11**.

Table 4. ^{1}H and ^{13}C NMR Data for **11**[a]

position	δ_H, *J* in Hz	δ_C, mult.	HMBC
1	11.75 br s		2, 3, 8, 9
2		158.1 qC	
3		109.9 qC	
4	7.75 d 8.4	120.6 CH	3, 6, 8
5	6.68 d 8.4	112.1 CH	6, 7, 9
6		149.0 qC	
7		105.7 qC	
8		133.1 qC	
9		118.7 qC	
10		184.0 qC	
11		67.1 qC	
12		166.8 qC	
14	3.31 m	43.7 CH_2	12, 15, 16, 17
	3.37 m		12, 15, 16, 17
15	1.85 m	24.1 CH_2	14, 16, 17
	2.02 m		14, 16, 17
16	1.86 m	28.5 CH_2	14, 15, 17, 18, 20
	2.66 m		14, 15, 17, 18, 20
17		67.0 qC	
18		171.9 qC	
19	8.69 br s		10, 11, 12, 17
20	2.08 m	30.7 CH_2	11, 17, 18, 21, 22
	2.11 m		11, 17, 18, 21, 22
21	2.82 m	51.0 CH	2, 10, 11, 12, 20, 22, 23, 24
22		35.6 qC	
23	1.23 (3H) s	21.1 CH_3	2, 21, 22, 24
24	1.42 (3H) s	26.8 CH_3	2, 21, 22, 23
25	7.04 d 9.9	117.3 CH	6, 7, 8, 27
26	5.82 d 9.9	130.4 CH	7, 27, 28, 29
27		75.6 qC	
28	1.38 (3H) s	27.2 CH_3	26, 27, 29
29	1.39 (3H) s	27.2 CH_3	26, 27, 28

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in DMSO-d_6.

supported by the ^{13}C NMR chemical shift of C-6 (δ 158.9). Acid hydrolysis of **12**, followed by analysis by TLC on a chiral stationary phase, showed the presence of L-proline.[12] NOE experiments of **12** revealed a correlation between H-11 and H-17, which indicates that the diketopiperazine ring is of *cis* configuration. Therefore, the stereogenic centers of **12** were determined as being 11*S*,17*S*. The 3*R* configuration of **12** was indicated by its CD spectrum (Figure S5), which was identical to that of **14**.

The ^{1}H and ^{13}C NMR spectra of notoamide K (**13**) (Table 6) were similar to those of notoamide D (**4**).[6a] The molecular formula of **13**, $C_{26}H_{31}N_3O_5$, was established by HRFABMS and was found to contain an additional oxygen atom compared with **4**. The NMR spectra of **13** showed the presence of a hydroxy signal at δ 5.57 (11-OH) and an additional oxygen-bearing carbon at δ 106.63 (C-11) in **13** instead of a methine hydrogen at δ 3.82 (H-11) and a methine carbon at δ 60.0 (C-11) in **4**. This clearly suggested that **13** was 11-hydroxynotoamide D, which was supported by the analysis of 2D NMR data. The β-configuration of the 11-hydroxy group was identified by NOE correlations between H-21 and 3-OH and 11-OH (Figure 3). Analysis of the acid hydrolysate of **13** by TLC on a chiral stationary phase showed again the presence of

Table 5. ^{1}H and ^{13}C NMR Data for **12**[a]

position	δ_H, *J* in Hz	δ_C, mult.	HMBC
1	9.61 br s		
2		182.7 qC	
3		57.9 qC	
4	7.01 d 8.0	127.4 CH	6, 8
5	6.48 dd 8.0, 1.0	109.1 CH	6, 7, 9
6		158.9 qC	
7	6.46 d 1.0	98.6 CH	5, 6, 9
8		144.8 qC	
9		120.9 qC	
10	2.07 d 14.5	32.1 CH_2	9, 11
	3.10 d 14.5		2, 3, 9, 11, 12
11	3.26 m	53.2 CH	3, 10, 12
12		165.8 qC	
14	3.32 m	46.0 CH_2	
	3.43 m		
15	1.77 m	23.2 CH_2	
	1.89 m		
16	1.94 m	28.7 CH_2	18
	2.11 m		
17	3.98 t 8.0	59.3 CH	16, 18
18		170.2 qC	
19	6.28 br s		
20	5.01 dd 17.5, 1.0	113.8 CH_2	21, 22
	5.08 dd 10.5, 1.0		22
21	6.11 dd 17.5, 10.5	144.3 CH	23, 24
22		42.8 qC	
23	1.05 (3H) s	22.0 CH_3	3, 21, 22, 24
24	1.10 (3H) s	22.8 CH_3	3, 21, 22, 23
6-OH	8.50 br s		

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

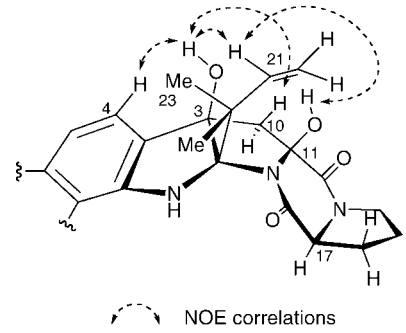


Figure 3. NOE correlations for **13**.

L-proline in **13**. Thus, the absolute configuration of **13** was established as 2*S*,3*R*,11*S*,17*S*.

Notoamide I (**11**) showed weak cytotoxicity against HeLa cells with an IC_{50} value of 21 μg/mL, whereas for notoamides F (**8**), J (**12**), and K (**13**) the IC_{50} values were more than 50 μg/mL.[13]

Experimental Section

General Experimental Procedures. Optical rotations were determined with a HORIBA SEPA-300 high sensitive polarimeter. CD spectra were measured on a JASCO J-725 spectropolarimeter in MeOH. UV spectra were measured on a Shimadzu UV-1600 UV−visible spectrophotometer. NMR spectra were recorded on a JEOL GSX500 and a Bruker Avance 500 NMR spectrometer in acetone-d_6 or DMSO-d_6. Chemical shifts were referenced to the residual solvent peaks (δ_H 2.04 and δ_C 29.8 for acetone-d_6; δ_H 2.49 and δ_C 39.5 for DMSO-d_6). Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Fungal Strain. The strain of fungus, *Aspergillus* sp., was isolated from the mussel *Mytilus edulis galloprovincialis* collected off Noto Peninsula in the Japan Sea and was identified on the basis of morphological evaluation by TechnoSuruga Co., Ltd. (Shizuoka, Japan). A voucher specimen is deposited at Chiba University with the code MF297-2.

Culture Conditions. The fungus was grown on 1800 agar plates composed of 50% seawater with 2.0% malt extract, 0.5% peptone, and 1.5% agar at 25 °C for 14 days.

Extraction and Isolation. The cultured plates (1800 plates) were extracted with EtOH. The extract was concentrated under reduced pressure

Table 6. ^{1}H and ^{13}C NMR Data for **13**[a]

position	δ_H, J in Hz	δ_C, mult.	HMBC
1	7.65 br s		
2		110.9 qC	
3		90.6 qC	
4	6.94 d 8.0	123.5 CH	6, 8
5	6.07 d 8.0	106.60 CH	6, 7, 9
6		154.7 qC	
7		104.1 qC	
8		144.2 qC	
9		125.7 qC	
10	2.45 d 14.0	48.8 CH_2	2, 3, 9, 12, 17
	3.31 d 14.0		2, 3, 9, 12, 17
11		106.63 qC	
12		163.6 qC	
14	3.30 m	46.1 CH_2	15
	3.44 m		15
15	1.86 m	23.9 CH_2	
	1.93 m		
16	1.96 m	29.4 CH_2	18, 15, 17
	2.25 m		18
17	4.33 t 7.5	59.7 CH	16, 18
18		172.3 qC	
20	4.98 dd 10.5, 1.0	113.3 CH_2	21, 22
	5.06 dd 17.5, 1.0		21, 22
21	6.25 dd 17.5, 10.5	145.6 CH	
22		45.6 qC	
23	1.22 (3H) s	22.1 CH_3	2, 21, 22, 24
24	1.26 (3H) s	23.3 CH_3	2, 21, 22, 23
25	6.45 d 9.5	118.3 CH	6, 7, 8, 27
26	5.55 d 9.5	128.8 CH	7, 27, 28, 29
27		75.9 qC	
28	1.34 (3H) s	27.9 CH_3	26, 27, 29
29	1.35 (3H) s	29.0 CH_3	26, 27, 28
3-OH	4.65 br s		
11-OH	5.57 br s		

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

and extracted with EtOAc and then *n*-BuOH. The EtOAc layer was partitioned between *n*-hexane and 90% MeOH−H_2O. The aqueous MeOH fraction (2.5 g) and *n*-BuOH fraction (1.4 g) were combined and subjected to ODS chromatography with MeOH−H_2O. The fraction eluted with 80% MeOH−H_2O was purified by reversed-phase HPLC with MeOH−H_2O to afford notoamides F (**8**, 5.3 mg), G (**9**, 0.47 mg), H (**10**, 0.28 mg), I (**11**, 3.0 mg), J (**12**, 2.3 mg), and K (**13**, 4.2 mg) along with cyclopenol[14] (24.2 mg) and viridicatol[15] (70.0 mg).

Notoamide F (8): $[\alpha]_D^{21}$ +1.9 (*c* 0.27, MeOH); CD (MeOH), see Figure S1; UV (MeOH) λ_{max} (log ε) 239 nm (4.1), 295 (sh, 3.5), 307 (3.6), 338 (sh, 3.2), 352 (sh, 3.0); NMR data (acetone-d_6), see Table 2; NOESY cross-peaks H-10/H-4, H_3-24, 10-OMe; H-21/H-19, H_3-23; FABMS (positive) *m/z* 462 $[M + H]^+$, 430 $[M + H - MeOH]^+$; HRFABMS $[M + H]^+$ *m/z* 462.2372 (calcd for $C_{27}H_{32}N_3O_4$, 462.2393).

Notoamide G (9): $[\alpha]_D^{22}$ +13 (*c* 0.014, MeOH); CD (MeOH), see Figure S2; UV (MeOH) λ_{max} (log ε) 258 nm (sh, 3.7), 267 (sh, 3.6), 305 (sh, 3.3); NMR data (acetone-d_6), see Table 2; NOESY cross-peaks H-10/H_3-24, 10-OMe; H-21/H_3-23; FABMS (positive) *m/z* 478 $[M + H]^+$; HRFABMS $[M + H]^+$ *m/z* 478.2341 (calcd for $C_{27}H_{32}N_3O_5$, 478.2342).

Notoamide H (10): $[\alpha]_D^{27}$ −46.7 (*c* 0.012, MeOH); CD (MeOH), see Figure S3; UV (MeOH) λ_{max} (log ε) 249 nm (4.3), 283 (sh, 3.9), 294 (sh, 4.7), 332 (sh, 3.2); NMR data (acetone-d_6), see Table 3; NOESY cross-peaks H-4/H-10, H_3-24; H-10/H_3-24; H-20β (δ 1.82)/H_3-24, H-16; H-20α (δ 2.02)/H-16; H-21/H_3-23, H-19; FABMS (positive) *m/z* 480 $[M + H]^+$; HRFABMS $[M + H]^+$ *m/z* 480.2134 (calcd for $C_{26}H_{30}N_3O_6$, 480.2134).

Notoamide I (11): $[\alpha]_D^{29}$ +31 (*c* 0.1, MeOH−$CHCl_3$ 1:1); CD (MeOH), see Figure S4; UV (MeOH) λ_{max} (log ε) 237 nm (4.3), 297 (3.7), 338 (3.5); NMR data (DMSO-d_6), see Table 4; NOESY cross-peaks: H-21/H-19, H_3-24, H-20β (δ 2.11); H-20α (2.08)/H_3-23; FABMS (positive) *m/z* 446 $[M + H]^+$; HRFABMS $[M + H]^+$ *m/z* 446.2083 (calcd for $C_{26}H_{28}N_3O_4$, 446.2080).

Notoamide J (12): $[\alpha]_D^{19}$ −156 (*c* 0.067, MeOH); CD (MeOH), see Figure S5; UV (MeOH) λ_{max} (log ε) 236 nm (4.3), 286 (3.8), 337 (3.6); NMR data (acetone-d_6), see Table 5; NOESY cross-peaks H-11/H-17, H-19; FABMS (positive) *m/z* 384 $[M + H]^+$; HRFABMS $[M + H]^+$ *m/z* 384.1924 (calcd for $C_{21}H_{26}N_3O_4$, 384.1923).

Notoamide K (13): $[\alpha]_D^{21}$ −128 (*c* 0.14, MeOH); CD (MeOH), see Figure S6; UV (MeOH) λ_{max} (log ε) 273 nm (3.6), 294 (sh, 3.5), 318 (2.9); NMR data (acetone-d_6), see Table 6; NOESY cross-peaks 3-OH/H-4, H-10, H-21; H-16/H-17; H-11/H-21; FABMS (positive) *m/z* 466 $[M + H]^+$; HRFABMS $[M + H]^+$ *m/z* 466.2340 (calcd for $C_{26}H_{32}N_3O_5$, 466.2342).

Stereochemical Analysis of Notoamides J (12) and K (13). A solution of **12** or **13** (50 μg) in 6 M HCl (100 μL) was heated at 110 °C for 14 h. The freeze-dried solution dissolved in H_2O was analyzed on CHIRALPLATE for enatiomeric resolution by TLC (Macherey-Nagel) with the solvent system MeOH−CH_3CN−H_2O in the ratio 5:3:5.

Cytotoxicity Assay. Cytotoxicity was evaluated in HeLa cells. HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) under a humidified atmosphere of 5% CO_2 at 37 °C. The cells were seeded into 96-well microplates (3×10^3 cells/well) and precultured for one day. The medium was replaced with that containing test compounds at various concentrations, and the cells were further cultured at 37 °C for 3 days. The medium was then replaced with 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.2 mg/mL in medium), and the cells were incubated under the same conditions for 4 h. After the addition of 200 μL of DMSO, the optical density at 570 nm was measured with a microplate reader.

Acknowledgment. This work was supported by Grants-in-Aid for Scientific Research (Nos. 18032033 and 19310140) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and also by a grant from the Keimeikai Foundation.

Supporting Information Available: ^{1}H NMR and CD spectra for notoamides F−K (**8**−**13**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Birch, A. J.; Wright, J. J. *Tetrahedron* **1970**, *26*, 2329–2344. (b) Birch, A. J.; Russel, R. A. *Tetrahedron* **1972**, *28*, 2999–3008. (c) Steyn, P. S. *Tetrahedron* **1973**, *29*, 107–120.
(2) (a) Yamazaki, M.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135–136. (b) Ondeyka, J. G.; Goegelman, R. T.; Schaeffer, J. M.; Kelemen, L.; Zitano, L. *J. Antibiot.* **1990**, *43*, 1375–1379. (c) Liesch, J. M.; Wichmann, C. F. *J. Antibiot.* **1990**, *43*, 1380–1386. (d) Blanchflower, S. E.; Banks, R. M.; Everet, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1991**, *44*, 492–497. (e) Blanchflower, S. E.; Banks, R. M.; Everet, J. R.; Reading, C. *J. Antibiot.* **1993**, *46*, 1355–1363.
(3) Qian-Cutrone, J.; Haung, S.; Shu, Y.-Z.; Vyas, D.; Fairchild, C.; Menendez, Z.; Krampitz, K.; Dalterio, R.; Klohr, S. E.; Gao, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14556–14557.
(4) Hayashi, H.; Nishimoto, Y.; Nozaki, H. *Tetrahedron Lett.* **1997**, *38*, 5655–5658.
(5) (a) Polonsky, J.; Merrien, M.-A.; Prange, T.; Pascard, C. *J. Chem. Soc., Chem. Commun.* **1980**, 601–602. (b) Prange, T.; Billion, M.-A.; Vuilhorgne, M.; Pascard, C.; Polonsky, J. *Tetrahedron Lett.* **1981**, *22*, 1977–1980.
(6) (a) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2254–2256. (b) Grubbs, A. W.; Artman, G. D., III.; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2257–2261. (c) Greshock, T. J.; Grubbs, A. W.; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2262–2265.
(7) Martinez-Luis, S.; Rodriguez, R.; Acevedo, L.; Gonzalez, M. C.; Lira-Rocha, A.; Mata, R. *Tetrahedron* **2006**, *62*, 1817–1822.
(8) (a) Fenical, W.; Jensen, P. R.; Cheng, X. C. Avrainvillamide, a Cytotoxic Marine Natural Product, and the Derivatives Thereof. U.S Patent 6,066,635, 2000. (b) Sugie, Y.; Hirai, H.; Inagaki, T.; Ishiguro, M.; Kim, Y.-J.; Kojima, Y.; Sakakibara, T.; Sakemi, S.; Sugiura, A.; Suzuki, Y.; Brennan, L.; Duignan, J.; Huang, L. H.; Sutcliffe, J.; Kojima, N. *J. Antibiot.* **2001**, *54*, 911–916.
(9) Authrine, C.; Gloer, J. B. *J. Nat. Prod.* **1996**, *59*, 1093–1095.
(10) Notoamide E was discovered as a precursor of other notoamides. The details of this report will appear elsewhere soon. Tsukamoto, S.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M., submitted.
(11) Williams, R. M.; Kwast, E.; Coffman, H.; Glinka, T. *J. Am. Chem. Soc.* **1989**, *111*, 3064–3065.
(12) Günther, K.; Martens, J.; Schickedanz, M. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 506.
(13) The cytotoxicy of notoamides G and H could not be tested because these compounds decomposed before testing.
(14) Mohammed, Y. S.; Luckner, M. *Tetrahedron Lett.* **1963**, 1953–1963.
(15) Luckner, M.; Mohammed, Y. S. *Tetrahedron Lett.* **1964**, 1987–1989.

NP800471Y