Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Notoamides A-D: new indole alkaloids isolated from a marine-derived fungus, *Aspergillus* sp.

Hikaru Kato, Takushi Yoshida, Takanori Tokue, Yuka Nojiri, Hiroshi Hirota, Tomihisa Ohta, Robert M. Williams, and Sachiko Tsukamoto*

List of Supporting Information

1. Fungal strain

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

2. Culture conditions

The fungus was grown on 200 agar plates composed of 50% seawater with 2.0% malt extract, 0.5% peptone, and 1.5% agar at 25 ˚C for 14 days.

3. Extraction and isolation

The cultured plates were extracted with EtOH. The extract was concentrated under reduced pressure and extracted with EtOAc, and then *n*-BuOH. The EtOAc layer was partitioned between hexane and 90% MeOH-H_2O. The aq 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 60% MeOH-H_2O to afford notoamides A (**1**, 3.4 mg), B (**2**, 2.1 mg), C (**3**, 7.9 mg), and D (**4**, 8.9 mg) along with sclerotiamide (**5**, 2.9 mg), stephacidin A (**6**, 6.1 mg), and desoxybrevianamide E (**7**, 0.57 mg).

4. Notoamide A (1)

$[\alpha]_D^{27}$ -112˚ (*c* 0.0836, MeOH); UV (MeOH) λ_{max} (log ε) 247.5 (4.3), 283.0 (3.9, sh), 294.0 (3.7, sh), 330 nm (3.2, sh); IR (film) ν_{max} 3500, 1700 cm^{-1}. ^1H and ^{13}C NMR (acetone-d_6) see Tables 1 and 2. HMBC cross peaks: H-4/C-6, C-8; H-5/C-6, C-7, C-9; H-10 (δ 2.21)/C-2, C-3, C-11, C-12, C-22; H-10 (δ 3.06)/C-2, C-9, C-11, C-12, C-21; H-15/C-17; H-16 (δ 2.65)/C-14, C-15, C-18; H-20 (δ 1.84)/C-18, C-21, C-22; H-21/C-11, C-12, C-22, C-23, C-24; H_3-23/C-3, C-21, C-22, C-24; H_3-24/C-3, C-21, C-22, C-23; H-25/C-6, C-27; H-26/C-7, C-27; H_3-28/C-26, C-27, C-29; H_3-29/C-26, C-27, C-28.

FABMS (positive) *m/z* 464 $[M + H]^+$, 448 $[M + H - 16]^+$; HRFABMS *m/z* 464.21710 ($C_{26}H_{30}N_3O_5$, Δ -1.4 mmu).

5. Notoamide B (2)

$[\alpha]_D^{27}$ -118˚ (*c* 0.0640, MeOH); UV (MeOH) λ_{max} (log ε) 246.0 (4.3), 283.0 (3.8, sh), 294.0 (3.6, sh), 330.0 nm (3.1, sh); IR (film) ν_{max} 3500, 1700 cm^{-1}. ^1H and ^{13}C NMR (acetone-d_6) see Tables 1 and 2. HMBC cross peaks: H-4/C-6, C-8; H-5/C-4, C-7, C-9; H-10 (δ 2.21)/C-2, C-11, C-12; H-10 (δ 3.04)/C-2, C-11, C-12; H-15/C-14; H-16 (δ 1.83)/C-17, C-18; H-20 (δ 1.83)/C-17, C-18; H-21/C-11, C-12, C-22, C-23, C-24; H_3-23/C-3, C-21, C-22, C-24; H_3-24/C-3, C-21, C-22, C-23; H-25/C-6, C-27; H-26/C-7, C-27; H_3-28/C-26, C-27, C-29; H_3-29/C-26, C-27, C-28. FABMS (positive) *m/z* 448 [M + H]; HRFABMS *m/z* 448.22429 ($C_{26}H_{30}N_3O_4$, Δ +0.7 mmu).

6. Notoamide C (3)

$[\alpha]_D^{27}$ +23˚ (*c* 0.255, MeOH); UV (MeOH) λ_{max} (log ε) 247.5 (4.3), 283.0 (3.9, sh), 294.0 (3.7, sh), 319.5 nm (3.4, sh); IR (film) ν_{max} 3500, 1700, 1650 cm^{-1}. ^1H and ^{13}C NMR (acetone-d_6) see Tables 1 and 2. HMBC cross peaks: H-1/C-3, C-9; H-4/C-3, C-6, C-8; H-5/C-6, C-7, C-9; H-10 (δ 2.65)/C-2, C-3, C-11; H-10 (δ 2.76)/C-2, C-3, C-9, C-11, C-12; H-11/C-3, C-12; H-14 (δ 3.19)/C-15, C-16; H-14 (δ 3.40)/C-15, C-16; H-16 (δ 1.30)/C-17; H-17/C-16, C-18; H-20 (δ 4.98)/C-21, C-22; H-20 (δ 5.04)/C-22; H-21/C-24; H_3-23/C-3, C-21, C-22, C-24; H_3-24/C-3, C-21, C-22, C-23; H-25/C-6, C-7, C-8, C-27; H-26/C-7, C-27, C-28, C-29; H_3-28/C-26, C-27, C-29; H_3-29/C-26, C-27, C-28. FABMS (positive) *m/z* 450 [M + H]; HRFABMS *m/z* 450.24152 ($C_{26}H_{32}N_3O_4$, Δ +2.2 mmu).

7. Notoamide D (4)

$[\alpha]_D^{27}$ -163˚ (*c* 0.321, MeOH); UV (MeOH) λ_{max} (log ε) 237.5 (4.5), 286.5 (4.0), 331.0 nm (3.8); IR (film) ν_{max} 3500, 1700 cm^{-1}. ^1H and ^{13}C NMR (acetone-d_6) see Tables 1 and 2. HMBC cross peaks: H-1/C-3, C-9; H-4/C-3, C-6, C-8; H-5/C-7, C-9; H-10 (δ

2.47)/C-2, C-3, C-9; H-10 (δ 2.75)/C-3, C-9, C-11, C-12; H-11/C-10, C-12, C-18; H-14 (δ 3.35)/C-16; H-14 (δ 3.47)/C-15, C-16; H-15/C-17; H-16 (δ 2.05)/C-18; H-16 (δ 2.18)/C-14; H-17/C-16, C-18; H-20 (δ 4.88)/C-22; H-20 (δ 4.93)/C-21, C-22; H-21/C-22, C-23, C-24; H_3-23/C-2, C-21, C-22, C-24; H_3-24/C-2, C-21, C-22, C-23; H-25/C-6, C-7, C-8, C-27; H-26/C-7, C-27; H_3-28/C-26, C-27, C-29; H_3-29/C-26, C-27, C-28; 3-OH/C-10. NOE correlations: H-10/H-21; 3-OH/H-4, H-21, H_3-23. FABMS (positive) *m/z* 450 [M + H]; HRFABMS *m/z* 450.23999 ($C_{26}H_{32}N_3O_4$, Δ -0.7 mmu).

8. Stereochemical analysis of notoamides C (3) and D (4)

A solution of **3** or **4** (50 μg) in 6 M HCl (100 μL) was heated at 110 ˚C for 12 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 of 5:3:5.

9. Cytotoxicity assay

Cytotoxicity test was carried out with HeLa, KB, and L1210 cells. 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. For cytotoxicity assay using HeLa or KB cells, the cells were seeded into 96-well microplates (3×10^3 cells/well) and pre-cultured for a 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 addition of 200 μL of DMSO, the optical density at 570 nm was measured with a microplate reader. For cytotoxicity assay using L1210 cells, samples were added to the culture medium containing cells (5×10^4 cells/mL) as DMSO solutions, and the concentration of DMSO was 0.1% in the medium. The cells (1×10^4 cells) in 200 μL of the medium were seeded into 96-well microplates. After incubation at 37 ˚C for 3 days, 25 μL of MTT solution (2 mg/mL in PBS) were added to the cell culture, and the mixture was further incubated under the same conditions for 4 h. After removal of the

solution, 200 μL of DMSO was added to the plate, and the optical density at 570 nm was measured with a microplate reader.

10. Flow cytometric analysis

Cells were harvested by trypsin digestion, washed with cold phosphate-buffered saline (PBS). The cells were collected by brief centrifugation and treated with PBS containing 500 μg/mL RNase A at 37 ^{o}C for 20 min and then with 10 μg/mL propidium iodide at room temperature for 15 min. Samples were then subjected to flow cytometric analysis.

Table S1. ^{1}H NMR data for compounds **1-4** in acetone-d_6.

No.	**1**	**2**	**3**	**4**
1		9.47 br s	10.30 br s	6.60 br s
4	7.09 d 8.5	7.08 d 8.5	7.00 d 8.3	7.01 d 8.0
5	6.45 d 8.5	6.41 d 8.5	6.34 d 8.3	6.15 d 8.0
10	2.21 d 15.0	2.21 d 14.5	2.65 dd 14.7, 4.9	2.47 dd 13.0, 8.0
	3.06 d 15.0	3.04 d 14.5	2.76 dd 14.7, 4.9	2.75 dd 13.0, 11.0
11			3.99 br t 4.9	3.82 dd 11.5, 8.0
14	3.45 dt 11.5, 6.5	3.45 dt 11.5, 6.5	3.19 dt 11.7, 5.9	3.35 ddd 11.0, 8.0, 5.0
	3.49 ddd 11.5, 7.5, 5.0	3.49 ddd 11.5, 7.5, 5.0	3.40 dt 11.7, 8.3	3.47 dt 11.0, 8.0
15	1.82 m	1.83 m	1.71 m	1.88 m
	1.90 m	1.90 m	1.71 m	1.88 m
16	1.82 m	1.83 m	1.30 m	2.05 m
	2.65 ddd 13.0, 7.0, 4.5	2.65 ddd 13.0, 7.0, 4.5	2.00 m	2.18 ddd 18.0, 7.0, 6.0
17			3.89 dd 10.3, 6.4	4.07 t 8.0
19	8.17 br s	7.99 br s	6.27 br s	
20	1.84 m	1.83 m	4.98 dd 17.6, 1.0	4.88 dd 11.0, 1.5
	2.00 m	2.02 m	5.04 dd 10.3, 1.0	4.93 dd 17.5, 1.5
21	3.32 dd 10.5, 8.5	3.36 dd 10.5, 8.5	6.13 dd 17.6, 10.3	6.34 dd 17.5, 11.0
23	0.75 s	0.80 s	1.00 s	1.25 s
24	0.81 s	0.83 s	1.07 s	1.28 s
25	7.72 d 10.5	6.64 d 10.0	6.59 d 10.7	6.40 d 10.0
26	5.72 d 10.5	5.74 d 10.0	5.73 d 10.7	5.63 d 10.0
28	1.38 s	1.39 s	1.39 s	1.36 s
29	1.41 s	1.40 s	1.42 s	1.38 s
3-OH				4.33 br s

Table S2. ^{13}C NMR data for compounds **1**-**4** in acetone-d_6.

No.	**1**	**2**	**3**	**4**
2	178.8 C	184.0 C	182.3 C	92.7 C
3	60.2 C	62.6 C	56.6 C	89.4 C
4	126.9 CH	127.4 CH	128.8 CH	124.7 CH
5	110.3 CH	109.6 CH	108.8 CH	108.3 CH
6	154.3 C	153.7 C	153.8 C	155.3 C
7	106.4 C	105.8 C	105.7 C	106.5 C
8	138.5 C	139.2 C	139.9 C	146.8 C
9	120.2 C	123.5 C	121.3 C	124.9 C
10	34.7 CH_2	34.8 CH_2	32.9 CH_2	36.1 CH_2
11	66.9 C	67.1 C	54.7 CH	60.0 CH
12	170.3 C	170.0 C	164.8 C	166.5 C
14	44.3 CH_2	44.3 CH_2	45.5 CH_2	45.7 CH_2
15	25.4 CH_2	25.4 CH_2	22.1 CH_2	24.3 CH_2
16	30.4 CH_2	30.4 CH_2	29.1 CH_2	29.0 CH_2
17	69.3 C	69.3 C	59.0 CH	62.2 CH
18	174.1 C	174.1 C	169.0 C	173.2 C
20	31.0 CH_2	31.0 CH_2	113.7 CH_2	111.7 CH_2
21	57.1 C	56.8 CH	144.4 CH	146.4 CH
22	46.6 C	46.4 C	43.3 C	45.3 C
23	23.9 CH_3	23.8 CH_3	21.8 CH_3	24.8 CH_3
24	20.4 CH_3	20.3 CH_3	22.8 CH_3	25.2 CH_3
25	118.1 CH	117.6 CH	117.6 CH	117.9 CH
26	130.5 CH	131.2 CH	131.0 CH	129.5 CH
27	76.3 C	76.6 C	76.7 C	76.5 C
28	27.8 CH_3	28.03 CH_3	28.0 CH_3	27.9 CH_3
29	28.1 CH_3	28.02 CH_3	28.4 CH_3	28.4 CH_3

Table S3. Cytotoxicity of **1-4** against HeLa and L1210 cell lines.

Compound	IC_{50} (μg/mL)	
	HeLa	L1210
1	27	29
2	52	36
3	50	22
4	*a*	*a*

a IC_{50} value was more than 100 μg/mL.

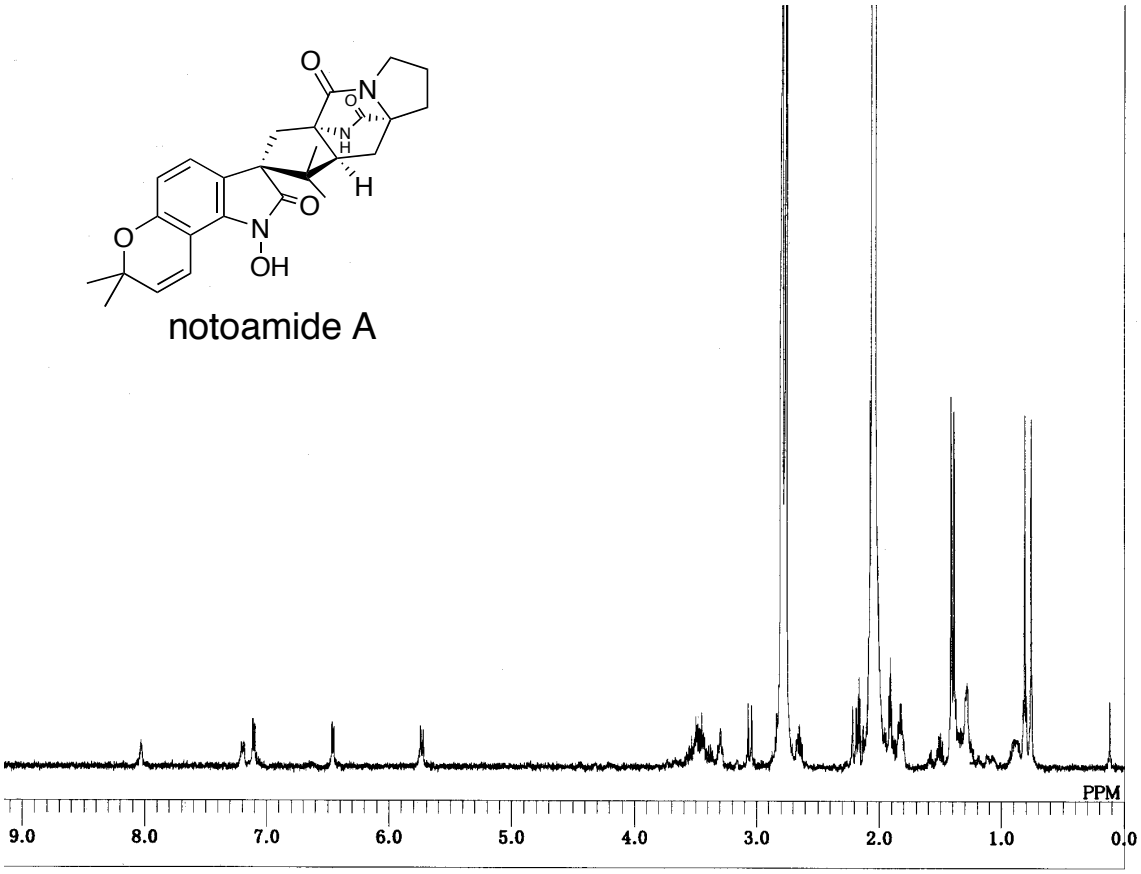

Figure S1. ^{1}H NMR spectrum of notoamide A (**1**) in acetone-d_6.

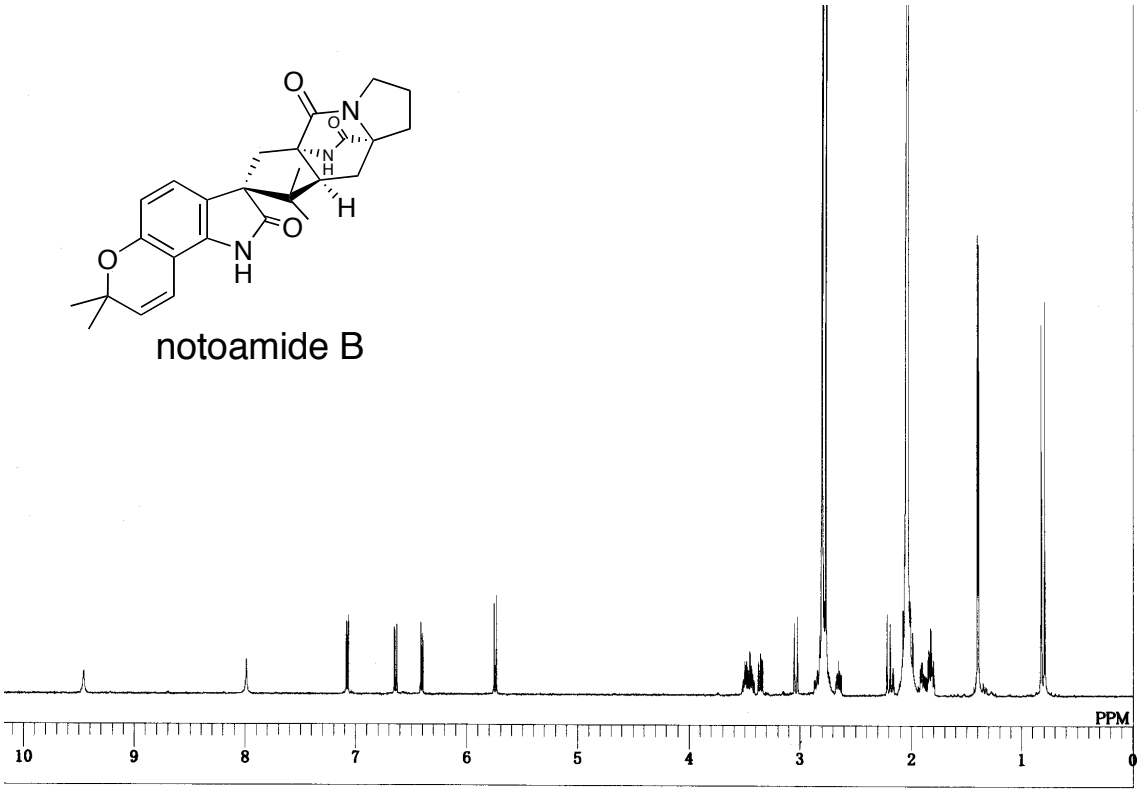

Figure S2. ^{1}H NMR spectrum of notoamide B (**2**) in acetone-d_6.

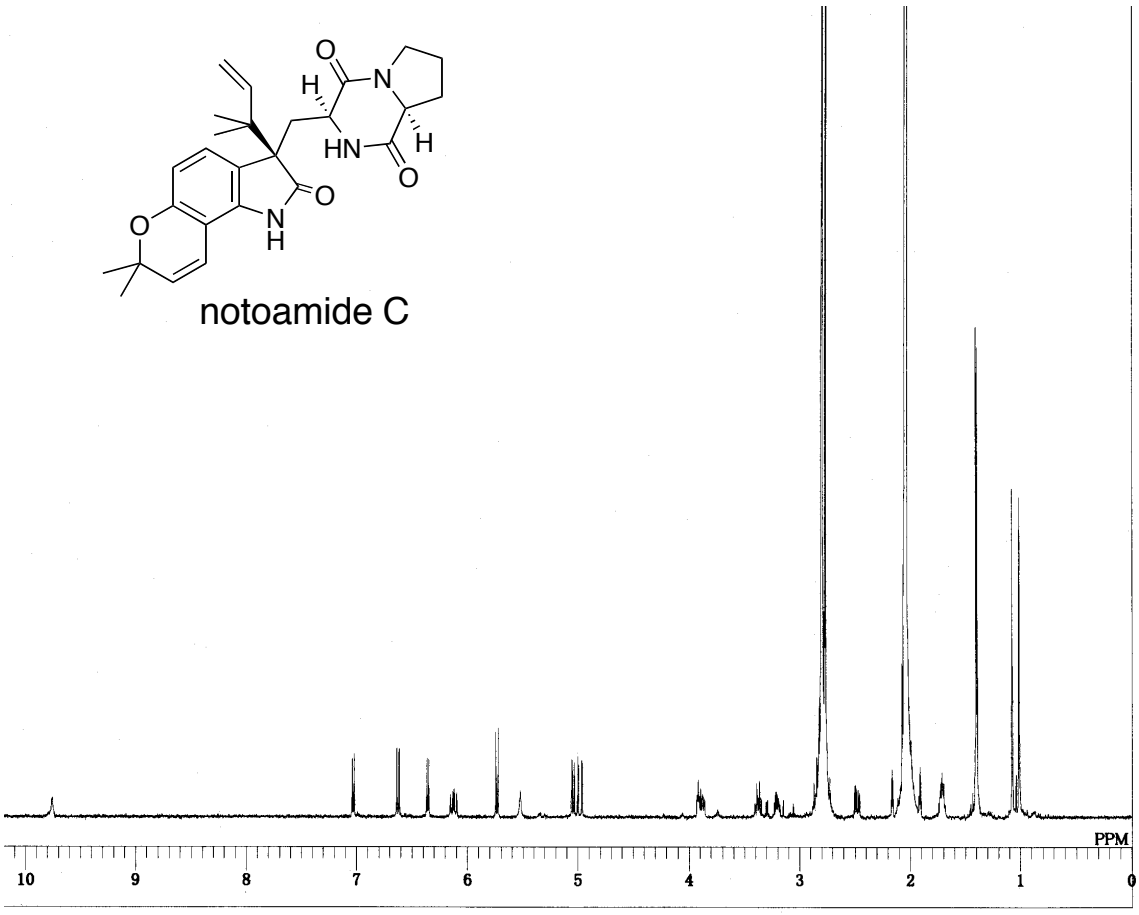

Figure S3. ^{1}H NMR spectrum of notoamide C (**3**) in acetone-d_6.

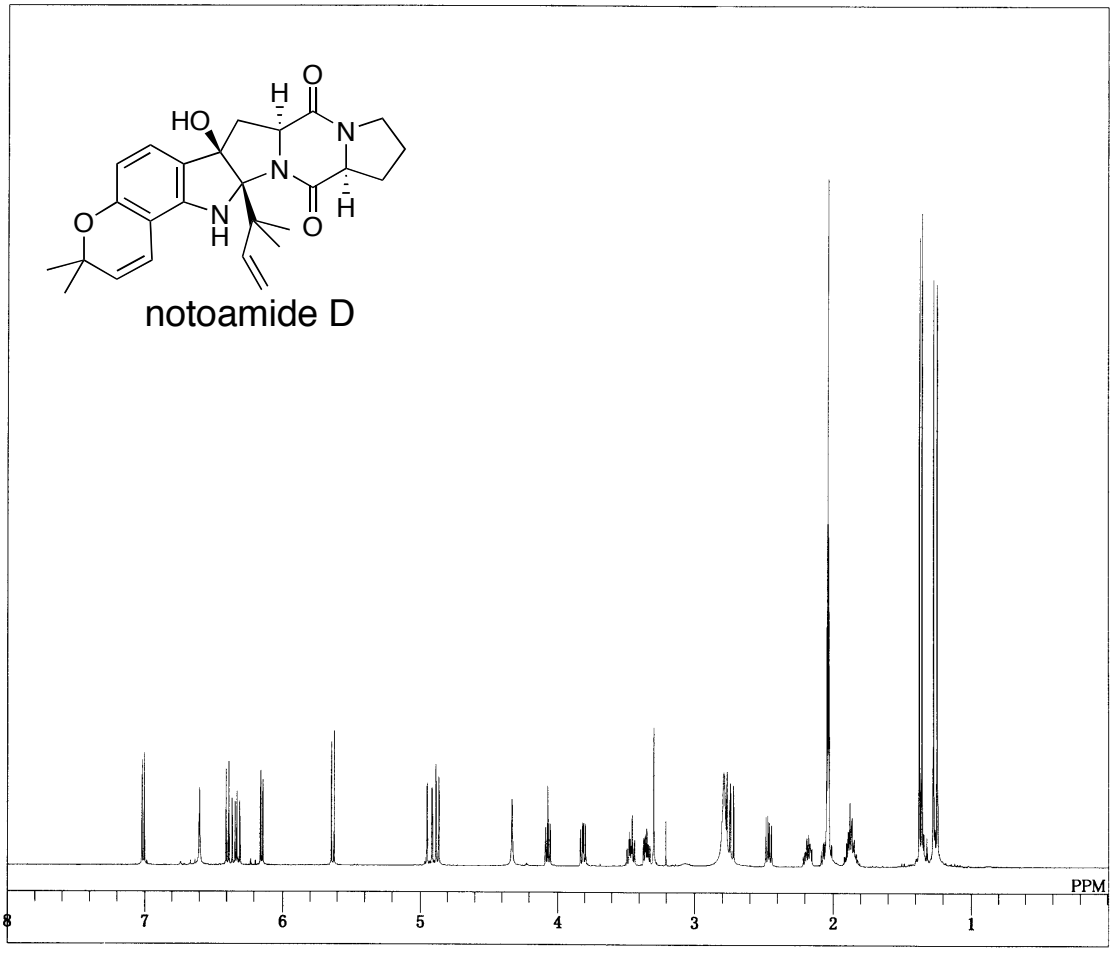

Figure S4. ^{1}H NMR spectrum of notoamide D (**4**) in acetone-d_6.

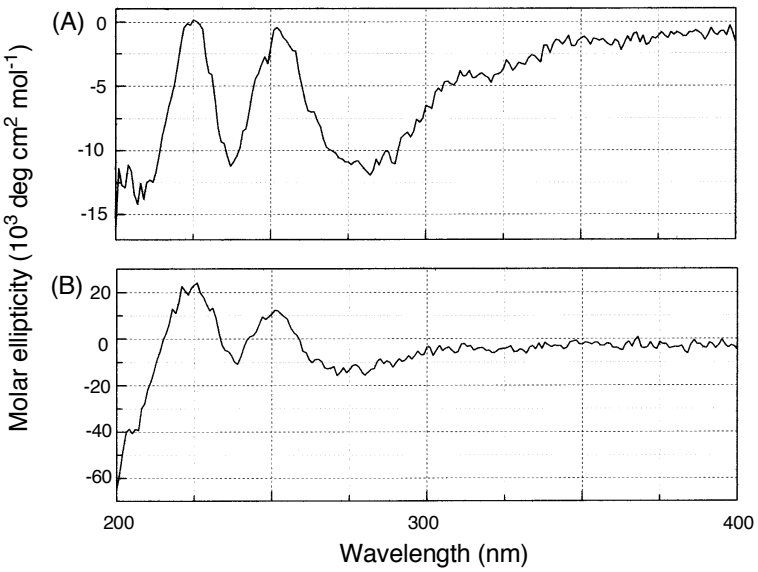

Figure S5. CD spectra of **1** (A) and **2** (B) in MeOH.