JOC Article

Spongolactams, Farnesyl Transferase Inhibitors from a Marine Sponge: Isolation through an LC/MS-Guided Assay, Structures, and Semisyntheses

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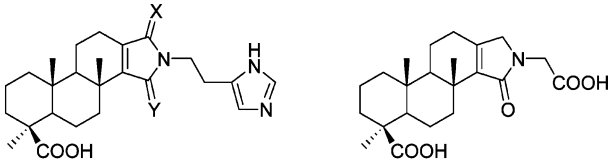

X = H_2, Y = O : spongolactam A
X = O, Y = H_2 : spongolactam B

spongolactam C

Novel nitrogenous diterpenoids, spongolactams A−C (**1**−**3**), were isolated as trace components of an Okinawan marine sponge, *Spongia* sp., by an LC/MS-guided assay for farnesyl transferase (FTase) inhibitors. Their structures were elucidated by spectroscopic analyses. To evaluate their structures and biological activity, the metabolites were semisynthesized from the known furanoditerpene **5**, obtained from the same sponge. Three related compounds **4**, **13**, and **16** were also semisynthesized. The IC_{50} values against FTase for **1**−**3** were 23, 130, and >260 μM, respectively, while the IC_{50} values against a human tumor cell line were 2.0, 3.5, and 20 μM, respectively. The structure−activity relationships within the six compounds suggest some positive correlation between FTase inhibitory and cytotoxic activities.

Introduction

Farnesyl transferase (FTase) is a ubiquitous enzyme that activates Ras protein by farnesylating to anchor the protein to the cell membrane. The farnesylation of Ras specifically occurs at the thiol of the cysteine of the C-terminal CAAX motif (C: cysteine; A: aliphatic amino acid; X: methionine, serine, or glutamine) and is followed by a series of posttranslational modifications.[1] Ras attached to the cell membrane triggers a signal transduction to start cellular proliferation. Mutation of Ras is regarded as a cause of the uncontrolled cell growth of certain cancers because this mutation is frequently found in human cancers, e.g., there is a 90% incidence of Ras mutation in pancreatic cancers.[2] Since the lipid side chain is critical for the Ras function, it has been believed that FTase inhibitors could be candidates for novel cancer chemotherapeutic drugs.[3] The initial reports on FTase inhibitors involved peptidomimetics that mimic the CAAX motif, such as B581, which is a commercially available peptide analogue of Cys-Val-Phe-Met.[4] In addition, FTase inhibitors from natural sources have also been sought and active metabolites have been found. In these studies, FTase inhibitory activity was evaluated by using radioisotope-labeled[5] or fluorescent-labeled substrates,[6] which require careful handling

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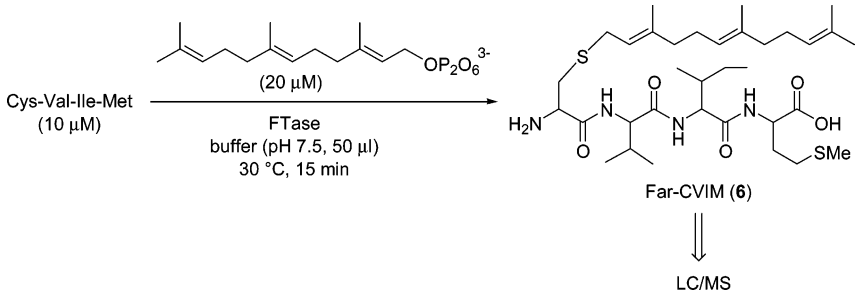
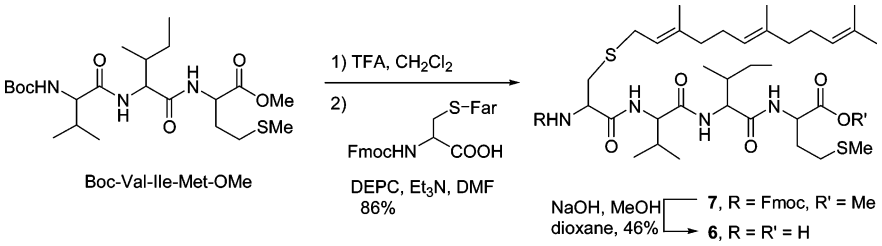
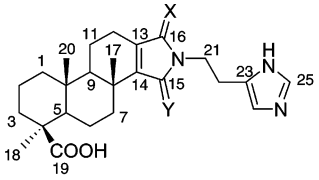
SCHEME 1. FTase-catalyzed Farnesylation of CVIM Followed by LC/MS Analysis



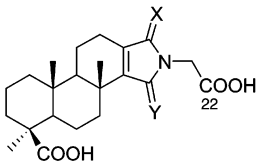
SCHEME 2. Synthesis of Far-CVIM (6)

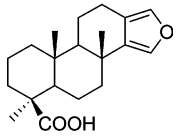


or reduce the affinity to the enzyme, respectively. In the present study, we first devised a convenient method for evaluating the FTase activity by direct LC/MS detection of the farnesylated product from an unlabeled tetrapeptide substrate. Herein, we describe the screening method for FTase inhibitors, isolation of the novel inhibitors named spongolactams A−C (**1**−**3**) from a marine sponge of the genus *Spongia*, and semisyntheses of these metabolites and related substances from a known furanoditerpene, **5**, obtained from the same sponge.



X = H_2, Y = O : spongolactam A (**1**)
X = O, Y = H_2 : spongolactam B (**2**)





X = H_2, Y = O : spongolactam C (**3**)
X = O, Y = H_2 : **4**

5

Results and Discussion

Assay Method for FTase Inhibitors. This simple and unique assay system is composed of (i) FTase reaction with the unlabeled tetrapeptide Cys-Val-Ile-Met (CVIM) and farnesyl pyrophosphate (FPP) and (ii) subsequent quantitative LC/MS analysis of the reaction product *S*-farnesyl-Cys-Val-Ile-Met (Far-CVIM, **6**) without any purification and labeling (Scheme 1). The enzyme was obtained from an FTase overexpressing transformant of *Saccharomyces cerevisiae* as the 30−60% ammonium sulfate fraction.[7] The substrate CVIM was prepared by solid-phase synthesis, using Fmoc chemistry. A standard sample of Far-CVIM (**6**)[8] for quantitative analysis was synthesized from Boc-Val-Ile-Met-OMe[9] and Fmoc-Cys(Far)-OH[10] (Scheme 2). The sensitivity of the product **6** in our LC/MS analysis was first evaluated to demonstrate that **6** was detectable to a minimum of 0.037 pmol/injection (Figure 1) and that an analytical curve showed favorable linearity between 0.1 and 5 pmol/injection (corresponding to 0.1−5 μM in the reaction mixture). In the optimized conditions for the enzyme reaction, 10 μM CVIM and 20 μM FPP were incubated in the presence of the crude enzyme at 30 °C for 15 min. The reaction mixture was then directly analyzed by LC/MS to quantify **6**. The production of **6** was approximately 2−2.5 μM (20−25% yield) in the absence of inhibitors, although this depended on the quality of the enzyme extracted. A synthetic inhibitor, B581,[4] showed an IC_{50} value of 0.77 μM in this assay.

Isolation of Spongolactams A−C from *Spongia* sp. Forty-one specimens of Okinawan marine animals were extracted and partitioned into hexane-, EtOAc-, and water-soluble fractions. The FTase inhibition assay described above was applied to these fractions, indicating the presence of several EtOAc-soluble fractions that showed inhibitory activity of more than 50% at a concentration of 100 μg mL^{-1}. Of these, the EtOAc-soluble fraction obtained from a sponge of the genus *Spongia* indicated 70% inhibition at 20 μg mL^{-1} and was subjected to assay-guided fractionation. The EtOAc-soluble fraction obtained from the sponge (343 g, wet wt) was chromatographed on silica gel to give two active fractions, one of which contained a known compound, spongia-13[16],14-dien-19-oic acid (**5**).[11] Another active fraction was chromatographed on silica gel, ODS, and then by reversed-phase HPLC to give spongolactams A (**1**), B **2**, and C **3** in 2 × 10^{-4}%, 1 × 10^{-4}%, and 6 × 10^{-5}% yields, respectively.

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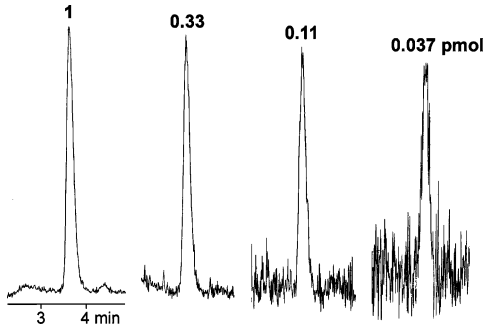

FIGURE 1. Mass chromatograph of Far-CVIM (**6**) at various injection amounts in LC/MS.

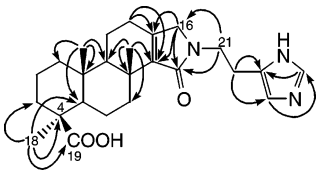

FIGURE 2. Important HMBC correlations of **1**.

Structural Elucidation. Spongolactams A (**1**) and B (**2**) possessed the same molecular formula of $C_{25}H_{35}N_3O_3$, as determined by high-resolution MS. The ^1H and ^{13}C NMR spectra of these compounds were similar, suggesting that these metabolites were isomers. Although several ^{13}C signals were unclear due to the very small amount of sample, the $^1H-^{13}C$ correlation spectra of HMQC and HMBC revealed their presence. The exact chemical shifts were finally determined by using synthetic samples as described hereinafter. The $^1H-^1H$ connectivities were determined by DQF-COSY as follows: $-CH_2-CH_2-CH_2-$, $>CH-CH_2-CH_2-$ ($\times 2$), and $-CH_2-CH_2-$. The isolated protons, CH_3- ($\times 3$), $-CH_2-$, and $-CH=$, were revealed by the HMQC spectrum. These partial structures and the remaining quaternary carbons, $>C<$ ($\times 3$), $>C=$ ($\times 2$), and $-C(O)X$ ($\times 2$), were inferred from the HMBC spectrum. Figure 2 summarizes the important HMBC correlations of spongolactam A (**1**). The signal at δ_C 181.9 in **1** was assigned to a carboxyl group based on its chemical shift and the IR absorption bands at 3600−2400 (br) and 1717 cm^{-1}. The connectivity of the carboxyl group (C-19) to the C-4 quaternary carbon was elucidated by the HMBC correlations of H-18/C-19 and H-18/C-4. The signal at δ_C 172.9 in **1** was assigned to a tertiary amide carbonyl group based on the HMBC correlations from two nitrogen-bearing methylene groups, H-16 and H-21. The presence of a 5-imidazolyl group connecting to the C-22 methylene group was revealed based on typical chemical shifts and the HMBC correlations shown in Figure 2. These findings led us to a determination of the gross structure of **1**. Complete NMR data are summarized in Tables 1 and 2. The relative stereochemistry of **1** was determined by NOESY correlation as shown in Figure 3. One of the most important correlations is H12/H16, which specifies the direction of the lactam carbonyl group (C-15). The structure including the absolute stereochemistry of **1** was finally determined by semisynthesis from the known compound **5**.[11]

The NMR data of isomer **2**, which were assigned by 2D NMR in the same way as for **1**, were similar to those for **1**, except for the signals due to positions 7 and 12−16. Of these, a reversal of chemical shifts between C-15 and C-16 is especially notable (Table 2). These chemical shift differences and a NOESY correlation of H-7/H-15 suggest that **2** possesses a lactam group with the opposite orientation to that of **1**.

The NMR data of the third compound, **3**, are also similar to those for **1**, suggesting that this is a derivative of **1**. Some significant differences in the NMR data are the lack of signals corresponding to the imidazole moiety in **1** and the replacement of the C-22 methylene (δ_C 27.2 and δ_H 2.83) in **1** by a carbonyl group (δ_C174.6) in **3**. The structure of the remainder of the molecule must be the same as that of the corresponding part of **1**, because the NMR data are superimposable. On the basis of the molecular formula $C_{22}H_{31}NO_5$ determined by high-resolution MS, position C-22 proved to be a carboxyl group. The structure of **3** thus determined was confirmed later by semisynthesis. The structures of spongolactams are quite novel and only one related metabolite, haumanamide, has been reported.[12]

Semisyntheses of Spongolactams A−C (1−3). To confirm the structures including absolute configuration, spongolactams A−C (**1**−**3**) were semisynthesized from the known related diterpene **5** (Scheme 3). The absolute structure of **5** was clarified by comparison of the NMR data and specific rotation with those reported.[11] Oxidation of **5** with *m*-chloroperbenzoic acid (MCPBA) gave a 93:7 mixture of hemiacetal **8a** and its 16-hemiacetal isomer **8b**, which are the previously isolated natural products, spongiabutenolides A and B, respectively.[13] The hemiacetal group at C-15 in **8a** was elucidated from a NOESY correlation of H-15 (δ 6.05)/H-7 (δ 1.44 and 2.05) and comparison of the NMR data with those reported. Treatment of a mixture of **8a** and **8b** with histamine gave a 93:7 mixture of hemiaminal **9a** and 16-hemiaminal isomer **9b** in a high yield. The compounds **8a**,**b** and **9a**,**b** are single isomers as to the position of C-15 or C-16, though the configuration is unclear. The aminal moiety of this mixture was then reduced with sodium cyanoborohydride to afford spongolactam B (**2**) only. The minor isomer **9b** might be reduced after isomerization to stable **9a** under acidic conditions. The synthetic **2** was identical with the natural material in all respects.

To synthesize spongolactam A (**1**), hemiacetal **8b** is needed. A mixture of **8a** and **8b** (93:7) was subjected to PCC oxidation to obtain anhydride **10**. Regioselective reduction of anhydride **10** to **8b** was first performed with sodium borohydride, which afforded a 1:3 mixture of **8a** and **8b** in 51% yield. In contrast, a second trial with L-selectride gave **8b** exclusively in 52% yield (Scheme 3). Hemiaminal formation from **8b** followed by hydride reduction at a low temperature provided spongolactam A (**1**) without isomerization to **2**. Comparison of the spectral data between the synthetic and natural specimens verified the absolute stereostructure of **1**.

In the structure of **3**, the histamine moiety of **1** is replaced by glycine. Therefore, **8b** was treated with methyl glycinate, and the resulting hemiaminal was reduced with sodium cyanoborohydride to give methyl ester **11b** (Scheme 3). Hydrolysis of **11b** gave spongolactam C (**3**), which was identical with the natural material in all respects.

Semisyntheses of Other Related Compounds. Compound **4**, the isomer possessing the same lactam moiety as **3** but with opposite orientation, was synthesized from a 93:7 mixture of **8a** and **8b** in the same manner as that for **3** (Scheme 4).

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TABLE 1. ^{1}H NMR Data for Spongolactams A−C (1−3) and 4 (600 MHz, CD_3OD)

no.	**1** δ mult (*J*, Hz)	**2** δ mult (*J*, Hz)	**3** δ mult (*J*, Hz)	**4** δ mult (*J*, Hz)
1	0.93 dt (4.2, 13.4)	0.94 dt (4.4, 13.4)	0.96 dt (4.2, 13.2)	0.97 dt (4.2, 13.2)
	1.82 m	1.84 br d (13.4)	1.84 br d (13.2)	1.86 m
2	1.44 br d (13.8)	1.44 br d (14.0)	1.45 br d (13.4)	1.45 br d (14.4)
	1.96 tq (3.8, 13.8)	1.95 m	1.95 tq (3.0, 13.4)	1.95 tq (3.0, 13.8)
3	1.02 dt (4.2, 13.2)	1.04 dt (4.4, 12.6)	1.04 dt (4.2, 13.8)	1.05 dt (4.2, 13.0)
	2.11 br d (13.2)	2.12 br d (12.6)	2.11 br d (13.2)	2.12 br d (13.2)
5	1.13 dd (12.6, 3.0)	1.15 m	1.16 m	1.18 m
6	1.84 m	1.89 m	1.85 br d (13.4)	1.90 m
	2.06 dq (3.2, 13.4)	2.10 m	2.06 dq (3.0, 13.4)	2.12 m
7	1.14 m	1.31 dt (3.4, 13.4)	1.21 dt (3.0, 13.2)	1.39 dt (3.0, 13.2)
	2.74 dt (13.0, 3.2)	1.79 dt (13.4, 3.4)	2.73 dt (13.2, 3.0)	1.86 dt (13.6, 3.0)
9	1.12 br d (12.6)	1.14 m	1.16 m	1.18 m
11	1.49 ddt (12.2, 5.8, 12.2)	1.49 ddt (13.0, 6.6, 13.0)	1.52 dq (5.4, 12.0)	1.54 dq (5.4, 12.0)
	1.85 m	1.87 m	1.87 m	1.90 m
12	2.23 ddd (18.6, 11.2, 6.4)	2.02 m	2.28 ddd (18.2, 11.4, 6.8)	2.06 m
	2.34 dd (18.6, 5.6)	2.29 dd (17.6, 4.6)	2.40 dd (18.2, 5.6)	2.33 dd (17.4, 5.4)
15		3.73 dd (19.2, 3.7)		3.97 dd (18.6, 1.6)
		3.82 d (19.2)		4.06 d (18.6)
16	3.64 d (19.2)		3.83 d (18.8)	
	3.71 d (19.2)		3.91 d (18.8)	
17	1.14 s	1.13 s	1.17 s	1.19 s
18	1.18 s	1.19 s	1.19 s	1.20 s
20	0.85 s	0.86 s	0.86 s	0.88 s
21	3.62 m	3.67 m	3.98 d (17.0)	4.17 s
			4.02 d (17.0)	
22	2.83 t (7.0)	2.85 t (7.0)		
24	6.83 br s	6.82 br s		
25	7.65 br s	7.62 br s		

TABLE 2. ^{13}C NMR Data for Spongolactams A−C (1−3) and 4 (150 MHz, CD_3OD)

no.	**1** δ mult	**2**[a] δ mult	**3**[a] δ mult	**4**[a] δ mult
1	41.3 t	41.2 t	41.3 t	41.2 t
2	20.3 t	20.2 t	20.3 t	20.2 t
3	39.4 t	39.3 t	39.3 t	39.2 t
4	44.9 s	44.9 s	44.8 s	44.8 s
5	58.5 d	58.3 d	58.5 d	58.3 d
6	20.9 t	21.1 t	20.9 t	21.1 t
7	37.8 t	39.3 t	37.7 t	39.2 t
8	37.1 s	37.7 s	37.1 s	37.8 s
9	57.9 d	56.9 d	57.8 d	56.8 d
10	39.2 s	39.2 s	39.2 s	39.2 s
11	18.7 t	18.4 t	18.7 t	18.5 t
12	27.0 t	23.1 t	27.3 t	23.2 t
13	151.1 s	129.4	151.7 s	129.1 s
14	141.6 s	163.7 s	141.2 s	164.0 s
15	172.9 s	50.5 t	173.1 s	50.9 t
16	53.6 t	174.1 s	54.1 t	174.3 s
17	21.3 q	22.4 q	21.3 q	22.5 q
18	29.3 q	29.4 q	29.3 q	29.3 q
19	181.9 s[a]	181.6 s	181.5 s	181.4 s
20	14.7 q	14.7 q	14.7 q	14.7 q
21	42.9 t	43.3 t	45.3 br t	46.2 br t
22	27.2 t	27.1 t	174.6 br s	175.4 br s
23	135.8 br s[a]	135.9 br s		
24	118.1 br d[a]	118.0 br d		
25	136.1 br d[a]	136.2 br d		

[a] Data obtained by using the corresponding synthetic samples.

Reductive amination of **8a**,**b** with methyl glycinate yielded methyl ester **11a**, which was then hydrolyzed to **4**.

A tryptamine derivative, **13**, was next prepared in a similar way to the above method with **8a**,**b** and tryptamine. The intermediate aminal **12** (9:1 mixture of **12** and its 16-hemiaminal isomer) was then converted to **13** by reduction.

To evaluate the importance of the histamine moiety in spongolactams, a simpler derivative **16** was also synthesized from phthalimide **14**, which was prepared from phthalic anhydride and histamine.[14] Hydride reduction of **14** gave aminal **15**, which was then reduced with triethylsilane under acidic conditions to afford **16**.

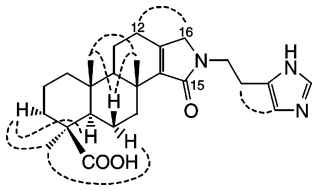

FIGURE 3. Partial NOESY correlations of **1**.

Biological Activities. Inhibitory activities of the natural and synthetic substances against FTase were evaluated by our LC/MS-based assay. The IC_{50} values are summarized in Table 3. The synthetic sample of spongolactam A (**1**) showed an IC_{50} value of 23 μM, which was similar to the value (22 μM) of the natural material and was the highest activity among the compounds tested. The activity of spongolactams B (**2**) and C (**3**) was determined only with the synthetic samples due to inadequate amounts of natural samples. Spongolactam B (**2**) was 6 times less active than **1**, despite the two compounds differing in only the direction of the lactam group. Spongolactam C (**3**) and its isomer **4** were inactive, suggesting that the histamine moiety, as well as the lactam orientation, is important for activity. However, the histamine moiety seems not to be essential because the small derivative **16** is inactive. Replacement of the histamine group of **2** with an indole group as in the derivative **13** did not change the activity.

Cytotoxicity of these compounds was also evaluated against a human vulval-derived epidermoid carcinoma cell line, A431 (Table 3). Roughly speaking, some correlation between FTase

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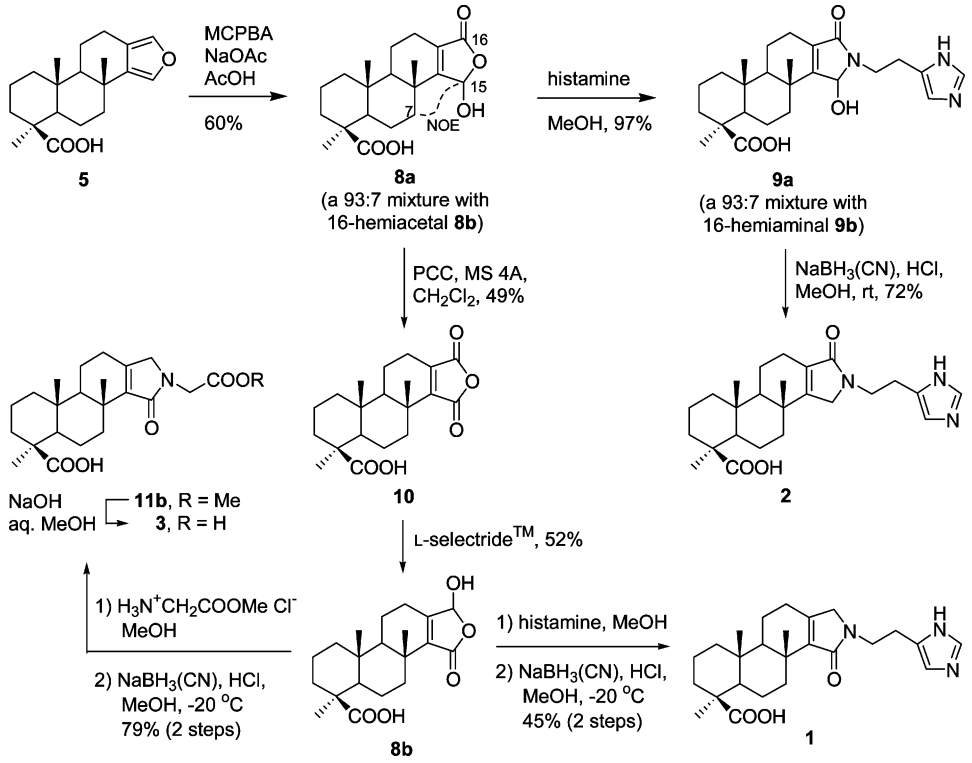
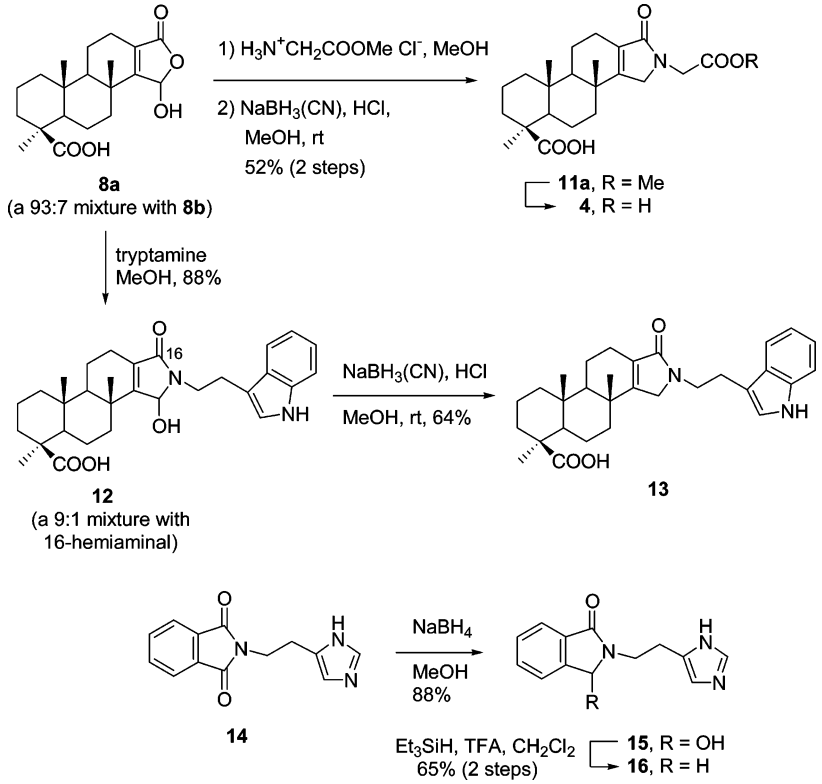
SCHEME 3. Synthesis of Spongolactams A (1), B (2), and C (3)



SCHEME 4. Synthesis of Spongolactam-Related Compounds



inhibition and cytotoxicity is observed. FTase could be a molecular target in the expression of spongolactam cytotoxicity.

Experimental Section

Production of Yeast FTase. The *Saccharomyces cerevisiae* SP1 strain that was transformed with two plasmids, YEpDPR1 and pBH28, was developed by F. Tamanoi et al. as a clone that overexpressed FTase.[7] The yeast was precultured in 2 mL of synthetic complete and drop-out media (-Leu, -Ura, -bacto agar) at 27 °C, 140 rpm for 24 h. The precultured broth was added into 100 mL of the same medium and cultured at 27 °C, 120 rpm for 27 h. The cells were collected from 500 mL of the broth by centrifugation at 500 *g* and washed with water to give 3 g of wet

TABLE 3. FTase Inhibitory Activity and Cytotoxicity of Spongolactams and Its Derivatives

	IC_{50}, μM	
compds	FTase inhibition	cytotoxicity[a]
1	23 (22)[b]	2.0
2	130	3.5
3	>260	20
4	>260	14
13	130	1.8
16	>440	22
B581	0.9	n.d.

[a] Cytotoxicity against a human tumor cell line, A431 (American vulval-derived epidermoid carcinoma). [b] A value for the natural sample of **1** is in parentheses.

cells. The following procedure was carried out at 4 °C. The cells were broken with 10 mL of quartz sand and 1 mL of buffer A (containing 0.1 M MES-NaOH pH 6.5, 0.1 mM $MgCl_2$, 35 μg mL^{-1} N^{α}-tosyl-L-lysine chloromethyl ketone hydrochloride, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF, 3.1 μg mL^{-1} bestatine, and 6.7 μg mL^{-1} pepstatine). The mixture was washed with 13 mL of buffer A and the washing was centrifuged at 100 000 *g* for 1 h. The supernatant was treated with ammonium sulfate and 30−60% ammonium sulfate fraction was dialyzed against 500 mL of buffer B (containing 25 mM Tris-HCl pH 7.5, 1 mM DTT, 0.1 mM $MgCl_2$, and 0.1 mM EGTA). The dialyzed solution (0.9 mL) was treated with octyl β-D-glucopyranoside (final 0.2%) and stored at −85 °C until used as the crude FTase solution.

Enzyme Reaction and LC-MS Analysis. To a mixture (total 45 μL) of 25 μL of water, 5 μL of reaction buffer (10×, 500 mM HEPES-NaOH pH 7.5, 50 mM $MgCl_2$, 100 μM $ZnCl_2$, 2% octyl β-D-glucopyranoside, and 50 mM DTT), 5 μL of FPP[15] (10×, 200 μM), 5 μL of CVIM (10×, 100 μM), and 5 μL of sample solution (10% DMSO) in a 1.5-mL tube was added 5 μL of a crude FTase solution at 4 °C. The mixture was incubated at 30 °C for 15 min. The reaction was quenched by adding 200 μL of the HPLC solution (70% MeCN−0.1% HCOOH), and a portion (5 μL) was injected for the LC/MS analysis under the following conditions: Develosil ODS-UG-5 (4.6 × 150 mm), 70% MeCN−0.1% HCOOH, 1 mL/min, ESI(+) mode, ionization chamber 70 °C, cone voltage 30 V, detected by single-ion recording at *m/z* 669.2 $(M + H)^+$ of Far-CVIM (**6**). Quantitative analysis was done by injecting synthetic **6** (Figure 1).

Fmoc-Cys(*S*-farnesyl)-Val-Ile-Met-OMe (7). To a solution of Boc-Val-Ile-Met-OMe[9] (22.7 mg, 0.048 mmol) in dry CH_2Cl_2 (1 mL) was added TFA (1 mL), and the mixture was stirred at 0 °C for 1.5 h. The mixture was repeatedly concentrated with benzene to afford crude Val-Ile-Met-OMe TFA salt (24.7 mg).

The TFA salt and Fmoc-Cys(*S*-farnesyl)-OH[10] (42.4 mg, 0.078 mmol) were dissolved in dry DMF (0.5 mL). To this solution were added dry Et_3N (17 μL, 0.12 mmol) and diethyl cyanophosphonate (10 μL, 0.058 mmol), and the mixture was stirred at 0 °C for 4 h. The resulting mixture was diluted with 30 mL of hexane−EtOAc (1:2) and washed with 10% citric acid (3 mL), saturated sodium bicarbonate (3 mL), water (3 mL), and brine (3 mL) sequentially, and then dried. Some precipitate formed was dissolved in $CHCl_3$ and combined with the previous organic solution. The combined organic solution was concentrated and the residue (52 mg) was purified twice by column chromatography [(i) 5 g of silica gel, $CHCl_3$−EtOAc (9:1); (ii) 5 g of alumina, $CHCl_3$−EtOAc (9:1)] to give Fmoc-Cys(*S*-farnesyl)-Val-Ile-Met-OMe (**7**) (37.4 mg, 86%): colorless powder, R_f 0.67 [(silica gel TLC, $CHCl_3$−EtOAc (1:1)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.9−1.1 (12H, m), 1.15 (1H, m), 1.45 (1H, m), 1.59 (6H, s), 1.67 (6H, s), 1.9−2.1 (11H, m), 2.07 (3H, s), 2.25 (1H, m), 2.50 (2H, t, J = 7.1 Hz), 2.87 (2H, d, J = 6.0 Hz), 3.22 (2H, d, J = 7.1 Hz), 3.73 (3H, s), 4.23 (2H, m), 4.25−4.45 (4H, m), 4.68 (1H, m), 5.07 (2H, m), 5.26 (1H, t, J = 7.0 Hz), 5.72 (1H, br), 6.60 (1H, br d, J = 7.8 Hz), 6.74 (1H, m), 7.31 (2H, t, J = 7.6 Hz), 7.41 (2H, t, J = 7.4 Hz), 7.58 (2H, d, J = 7.4 Hz), 7.77 (2H, d, J = 7.4 Hz); FAB-MS (NBA) *m/z* (rel. intensity) 905 $[M + H]^+$ (5), 857 (4), 341 (15), 305 (17), 277 (17), 219 (46), 179 (87), 154 (100), 136 (83).

Far-CVIM (6). To a solution of **7** (4.5 mg, 0.005 mmol) in a mixture of MeOH (0.75 mL) and dioxane (0.25 mL) was added a 0.5 M NaOH solution (25 μL), then the mixture was stirred at room temperature for 30 h. The resulting mixture was adsorbed on silica gel (1 g) and applied on a silica gel column (4 g), which was then eluted with $CHCl_3$−MeOH−water (90:9:1). The fraction containing the desired product was subjected to HPLC [Develosil ODS-10 (20 × 250 mm), 80% MeOH-20 mM NH_4OAc, 7.0 mL/min, detected at 215 nm] to afford Far-CVIM (**6**) (1.5 mg, 46%): colorless powder, R_f 0.15 [(silica gel TLC, $CHCl_3$−MeOH 9:1], t_R = 19.0 min [Develosil ODS-HG-5 (4.6 × 250 mm), 80% MeOH−20 mM NH_4OAc, 1.0 mL/min]; ^{1}H NMR [600 MHz, $CDCl_3$−CD_3OD (4:1)] δ 0.84 (3H, m), 0.90 (6H, m), 0.95 (1.5H, d, J = 7.0 Hz), 0.97 (1.5H, d, J = 7.0 Hz), 1.10 (1H, m), 1.48 (1H, m), 1.57 (6H, s), 1.64 (4.5H, s), 1.67 (1.5H, s), 1.83 (1H, m), 1.94 (2H, m), 2.0−2.1 (8H, m), 2.07 (3H, s), 2.13 (1H, m), 2.49 (2H, m), 2.76 (0.5H, dd, J = 14.0, 6.6 Hz), 2.81 (0.5H, dd, J = 14.0, 6.6 Hz), 2.93 (0.5H, dd, J = 14.0, 6.6 Hz), 3.00 (0.5H, dd, J = 14.0, 6.6 Hz), 3.17 (1H, m), 3.26 (1H, d, J = 7.8 Hz), 4.17 (2H, m), 4.55 (1H, m), 5.06 (2H, m), 5.21 (1H, m); FAB-MS (NBA) *m/z* (rel. intensity) 669 $[M + H]^+$ (22), 407 (10), 316 (8), 263 (39), 219 (24), 175 (36), 133 (88), 109 (100).

Collection of Marine Animals and Identification of the Sponge. Forty-one marine organisms were collected off Akajima Island in Okinawa, Japan. The sponge used in this study was collected at a depth of 20 m and identified as *Spongia* to the genus level by Dr. John N. A. Hooper of Queensland Museum and registered in the Queensland Museum collections (voucher specimen No. QM G315989).

Isolation of Spongolactams. Conventional solvent partition of the collected marine organisms gave hexane-, EtOAc-, and water-soluble materials. Among them the EtOAc-soluble fraction obtained from the sponge of the genus *Spongia* indicated FTase inhibitory activity of IC_{50} < 20 μg mL^{-1}. The collected sponge (343 g, wet wt) was immediately frozen and stored at −30 °C until used. The freeze-dried sponge was homogenized in 1 L of EtOH−MeOH (4:1) and stood at room temperature for 3 days. The mixture was filtered, and the filtrate was concentrated. The resulting solid residue (16.2 g) was dissolved in 90% aqueous MeOH (200 mL) and washed twice with hexane (200 mL). The aqueous MeOH layer was concentrated, dissolved in water (100 mL), and extracted twice with EtOAc (100 mL). The combined EtOAc layers were concentrated to give an EtOAc fraction (3.14 g) as an oil. The fraction was chromatographed on silica gel (60 g) with benzene−EtOAc (4:1 and 1:1), EtOAc, and then MeOH to give 5 fractions. A portion (0.6 g) of the first fraction (1.6 g), which was eluted with benzene−EtOAc (4:1), was recrystallized from MeOH to give furanoditerpene **5** (297 mg) as a colorless powder: $[\alpha]^{24}_D$ +10 (*c* 0.50, $CHCl_3$) (lit.[11] +15°).

A portion (420 mg) of the fifth fraction (464 mg), which was eluted with MeOH, was chromatographed on silica gel (40 g) with $CHCl_3$−MeOH (98:2, 95:5, 90:10, 85:15, 80:20, and 50:50), and then MeOH to give 6 fractions. Fractions (total 55.5 mg) eluted with $CHCl_3$−MeOH (85:15 and 80:20) were combined and chromatographed on silica gel (25 g) with $CHCl_3$−90% aq MeOH (85:15, 80:20, and 75:25) to give four fractions. Fractions (total 16.2 mg) eluted with $CHCl_3$−90% aq MeOH (85:15 and 80:20) were combined and chromatographed on ODS (2 g) with 70%, 80%, 90% aq MeOH, and then MeOH to give 5 fractions. Fractions eluted with 70% and 80% aq MeOH were combined and the material (8.4 mg) was subjected to reverse-phase HPLC [Develosil ODS HG-5 (ϕ 10 × 250 mm), Nomura Chemical Co., Ltd., 60% MeOH in 20

(15) Davisson, V. J.; Woodside, A. B.; Poulter, C. D. *Methods Enzymol.* **1985**, *110*, 130.

mM NH_4OAc, 3.0 mL/min, UV 225 nm]. Three peaks at retention times of 32.3, 21.7, and 14.0 min were collected and concentrated to give spongolactams A (**1**, 0.8 mg), B (**2**, 0.5 mg), and C (**3**, 0.2 mg), respectively.

Spongolactam A (**1**): colorless powder, $[\alpha]^{25}_D$ −37 (*c* 0.043, MeOH); UV (MeOH) λ_{max} 208 (ϵ 16000), 238 (sh, 3700) nm; HR ESI-TOF-MS (positive) found *m*/*z* 426.2743 $[M + H]^+$, calcd. for $C_{25}H_{36}N_3O_3$ 426.2751.

Spongolactam B (**2**): colorless powder, $[\alpha]^{25}_D$ −12 (*c* 0.03, MeOH); UV (MeOH) λ_{max} 208 (ϵ 13000), 238 (sh, 3400) nm; HR ESI-TOF-MS (positive) found *m*/*z* 426.2740 $[M + H]^+$, calcd for $C_{25}H_{36}N_3O_3$ 426.2751.

Spongolactam C (**3**): colorless powder, $[\alpha]^{27}_D$ −30 (*c* 0.016, MeOH); UV (MeOH) λ_{max} 215 (ϵ 13000), 243 (sh, 3300) nm; HR ESI-TOF-MS (positive) found *m*/*z* 390.2260 $[M + H]^+$, calcd for $C_{22}H_{32}NO_5$ 390.2275.

Mixture of Hemiacetals 8a and 8b (93:7). A solution of **5** (224 mg, 0.709 mmol), anhydrous sodium acetate (241 mg, 2.93 mmol), *m*-chloroperbenzoic acid (307 mg, 1.78 mmol), and acetic acid (0.25 mL) in $CHCl_3$ (10 mL) was stirred at room temperature for 40 min. The reaction mixture was diluted with saturated $NaHCO_3$ (5 mL), adjusted to pH 3 with 6 N HCl, and then extracted with ether three times. The combined organic layers were concentrated and the residue was chromatographed on silica gel with $CHCl_3$−MeOH (30:1) to give an isomeric mixture of hemiacetals **8a** and **8b** in the ratio of 93:7 (149 mg, 60%): colorless powder, IR (KBr) 3600−2400 (br), 1734, 1710, 1696, 1178, 1088, 919, 912, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$:CD_3OD 3:10, data for **8a**) δ 0.87 (3H, s), 0.93 (1H, m), 1.02 (1H, m), 1.07 (1H, d, J = 11.0 Hz), 1.14 (1H, dd, J = 12.5, 2.5 Hz), 1.19 (3H, s), 1.27 (3H, s), 1.44 (2H, m), 1.56 (1H, m), 1.78−1.98 (4H, m), 1.98−2.17 (4H, m), 2.33 (1H, dd, J = 16.0, 4.0 Hz), 6.05 (1H, d, J = 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD 3:5, data for **8a**) δ 14.7 (q), 17.9 (t), 19.9 (t), 20.4 (t), 20.5 (q), 22.1 (t), 29.3 (q), 37.6 (t), 38.0 (s), 39.0 (t), 39.1 (s), 41.0 (t), 44.5 (s), 56.4 (d), 58.0 (d), 98.9 (d), 127.7 (s), 170.2 (s), 173.7 (s), 181.0 (s); FABMS (glycerol) *m*/*z* 347 $[M - H]^-$.

Mixture of Hemiaminals 9a and 9b. A solution of **8a**/**8b** (93:7) (30.9 mg, 0.089 mmol) and histamine (40 mg, 0.36 mmol) in MeOH (2 mL) was stirred at room temperature for 6 h. The reaction mixture was concentrated, and the residue was chromatographed on silica gel with $CHCl_3$−MeOH (6:1) to give an isomeric mixture of hemiaminals **9a** and **9b** in the ratio of 93:7 (37.9 mg, 97%): colorless powder; IR (KBr) 3600−2300 (br), 3323, 3144, 1733, 1683 (sh), 1635, 1559, 1238, 1194, 1175, 1097, 626 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, data for **9a**) δ 0.87 (3H, s), 0.93 (1H, td, J = 13.2, 3.2 Hz), 1.03 (1H, td, J = 13.2, 3.2 Hz), 1.07 (1H, br d, J = 12.0 Hz), 1.13 (1H, br d, J = 12.4 Hz), 1.15 (1H, m), 1.19 (3H, s), 1.21 (3H, s), 1.32 (1H, td, J = 13.2, 3.2 Hz), 1.43 (1H, br d, J = 14.0 Hz), 1.53 (1H, qd, J = 12.4, 6.4 Hz), 1.80−1.90 (3H, m), 1.90−2.15 (4H, m), 2.31 (1H, dd, J = 18.0, 6.0 Hz), 2.77 (2H, t, J = 7.2 Hz), 3.04 (1H, m), 3.10 (1H, m), 5.72 (1H, s), 6.86 (1H, s), 7.63 (1H, s); ^{13}C NMR (100 MHz, CD_3OD, data for **9a**) δ 14.9 (q), 18.2 (t), 20.2 (t), 20.8 (t), 20.8 (q), 22.6 (t), 28.7 (t), 29.4 (q), 37.7 (t), 38.9 (s), 39.2 (s), 39.3 (t), 41.3 (t), 44.8 (s), 46.6 (t), 56.9 (d), 58.2 (d), 96.3 (d), 118.3 (d), 128.4 (s), 135.8 (d), 136.3 (s), 169.5 (s), 175.1 (s), 181.5 (s); HR ESI-TOF-MS found *m*/*z* 442.2624 $[M + H]^+$, calcd for $C_{25}H_{36}N_3O_4$ 442.2700.

Spongolactam B (2). To a solution of **9a**/**9b** (93:7) (23.6 mg, 0.054 mmol) in a mixture of MeOH (0.6 mL) and THF (0.3 mL) were added $NaBH_3CN$ (2.5 mg, 0.04 mmol) and then 12 N HCl (0.002 mL), and the mixture was stirred at room temperature for 6 h. The reaction mixture was treated with saturated $NaHCO_3$ (0.1 mL) and concentrated to dryness, then chromatographed on silica gel with $CHCl_3$−MeOH (6:1) to give **2** (16.4 mg, 72%): colorless powder, mp 183−190 °C; $[\alpha]^{28}_D$ −28 (*c* 0.23, MeOH); UV (MeOH) λ_{max} 213 (ϵ 14600), 236 (ϵ 3200, sh) nm; IR (KBr) 3448, 3152, 1669, 1655, 1463, 1215, 1178, 625 cm^{-1}; NMR (Tables 1 and 2).

Acid Anhydride 10. A mixture of **8a**/**8b** (93:7) (58.5 mg, 0.168 mmol) and powdered molecular sieves 4A in dry CH_2Cl_2 (7 mL) was stirred at room temperature for 5 min. To the resulting mixture was added pyridinium chlorochromate (PCC) (794 mg, 3.68 mmol), and the mixture was stirred at room temperature for 2 h. To the reaction mixture was added an additional PCC (206 mg) and the mixture was further stirred for 2 h. The reaction mixture was diluted with ether (3 mL) and then passed through a silica gel pad. The residue on the pad was washed with ether. The filtrate and washings were combined and concentrated, and the residue was chromatographed on silica gel (hexane−EtOAc 2:1) to give **10** (28.2 mg, 49%): colorless powder; mp 230−240 °C; $[\alpha]^{26}_D$ −66 (*c* 0.25, $CHCl_3$); UV (CH_3CN) λ_{max} 203 (ϵ 10600), 260 (ϵ 5100) nm; IR (KBr) 3600−2500 (br), 1843, 1767, 1697, 1274, 894 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, s), 0.93 (1H, td, J = 13.2, 4.0 Hz), 1.05 (1H, td, J = 13.2, 4.0 Hz), 1.13 (1H, br d, J = 10.8 Hz), 1.15 (1H, dd, J = 12.4, 2.8 Hz), 1.26 (3H, s), 1.27 (3H, s), 1.35 (1H, td, J = 13.2, 3.6 Hz), 1.42−1.55 (2H, m), 1.84 (1H, br d, J = 12.8 Hz), 1.90−2.02 (3H, m), 2.09 (1H, qd, J = 13.2, 3.2 Hz), 2.19 (1H, br d, J = 13.2 Hz), 2.29 (1H, ddd, J = 20.0, 11.2, 7.2 Hz), 2.56 (1H, m), 2.60 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1 (q), 16.8 (t), 18.9 (t), 19.3 (t), 21.1 (q), 22.6 (t), 28.7 (q), 36.0 (t), 36.7 (s), 37.8 (t), 38.2 (s), 40.0 (t), 43.7 (s), 55.4 (d), 56.8 (d), 143.1 (s), 153.4 (s), 163.1 (s), 164.7 (s), 182.1 (s); HR ESI-TOF-MS found *m*/*z* 347.1835 $[M + H]^+$, calcd for $C_{20}H_{27}O_5$ 347.1853.

Hemiacetal 8b. To a cooled solution of **10** (25.3 mg, 0.073 mmol) in anhydrous THF (2 mL) at −78 °C was added a 1 M solution of L-selectride in THF (0.075 mL), and the mixture was stirred for1.5 h. Additional L-selectride (0.025 mL) was added and the mixture was stirred for 30 min. The reaction mixture was diluted with 1 N HCl (1 mL) and water (2 mL), and the mixture was extracted with ether three times. The combined organic layers were concentrated and the residue was chromatographed on silica gel with $CHCl_3$−MeOH (30:1) to give **8b** (13.3 mg, 52%): colorless powder; ^1H NMR (400 MHz, $CDCl_3$:CD_3OD 1:1) δ 0.86 (3H, s), 0.93 (1H, td, J = 13.5, 3.6 Hz), 1.02 (1H, td, J = 13.5, 3.0 Hz), 1.12 (1H, br d, J = 12.6 Hz), 1.13 (1H, br d, J = 12.6 Hz), 1.18 (3H, s), 1.22 (3H, s), 1.23 (1H, m), 1.44−1.56 (2H, m), 1.84 (1H, br d, J = 12.8 Hz), 1.85−2.00 (4H, m), 2.07 (1H, m), 2.15 (1H, br d, J = 13.2 Hz), 2.60 (1H, br d, J = 13.6 Hz), 5.82 (1H, s).

Spongolactam A (1). A solution of hemiacetal **8b (**3.1 mg, 0.0089 mmol) and histamine (7.6 mg, 0.068 mmol) in MeOH (0.5 mL) was stirred at room temperature for 4 h. To the cooled mixture at −17 to −20 °C were added $NaBH_3CN$ (3.1 mg, 0.049 mmol) and then 0.48 M HCl in MeOH (0.05 mL), and the mixture was stirred at the same temperature for 1.5 h. The reaction mixture was dried and the residue was chromatographed on silica gel with $CHCl_3$−MeOH (1:6) and then HPLC [Develosil ODS-5 (ϕ 10 × 250 mm), 20 mM NH_4OAc in 60% MeOH, 3 mL/min, detected at 212 nm] afford spongolactam A (**1**) (1.7 mg, 45%, t_R = 38−41 min): colorless powder, mp 210−212 °C; $[\alpha]^{27}_D$ −34 (*c* 0.29, MeOH); UV λ_{max} (CH_3OH) 213 (ϵ 13500), 236 (3300, sh) nm; IR (KBr) 3600−2400 (br), 3302, 3131, 1717, 1655, 1630, 1456, 1230, 1186 cm^{-1}; NMR (Tables 1 and 2).

Methyl Ester 11b. To a solution of methyl glycinate hydrochloride (48.4 mg, 0.386 mmol) and triethylamine (0.054 mL, 0.39 mmol) in MeOH (0.2 mL) was added a solution of hemiacetal **10a** (12.1 mg, 0.035 mmol) in MeOH (0.8 mL), and the mixture was stirred at room temperature for 2 h. The mixture was cooled to −20 °C, and $NaBH_3CN$ (10 mg, 0.16 mmol) and 1 N HCl in MeOH (0.16 mL) were added gradually. The mixture was stirred at −17 to −20 °C for 1 h. The reaction mixture was diluted with 1 N HCl (2 mL) and then water (3 mL), and the mixture was extracted three times with EtOAc (10 mL). The combined organic layers were concentrated to give an oil, which was chromatographed on silica gel ($CHCl_3$−MeOH 10:1) to afford methyl ester **11b** (11.1 mg, 79% in 2 steps): colorless powder, mp 270 °C dec; $[\alpha]^{22}_D$ −36 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 214 (ϵ 16000), 237 (3200, sh)

nm; IR (KBr) 1752, 1712, 1637, 1453, 1216, 1190, 899 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3-CD_3OD$ 9:1) δ 0.84 (3H, s), 0.91 (1H, td, J = 13.2, 4.4 Hz), 1.01 (1H, td, J = 13.2, 4.4 Hz), 1.12 (1H, m), 1.13 (1H, d, J = 12.4 Hz), 1.18 (3H, s), 1.21 (3H, s), 1.22 (1H, m), 1.48 (2H, m), 1.82 (1H, m), 1.86 (2H, m), 1.93 (1H, qt, J = 14.0, 3.6 Hz), 2.04 (1H, qd, J = 14.0, 2.8 Hz), 2.14 (1H, br d, J = 12.8 Hz), 2.26 (1H, ddd, J = 18.0, 11.2, 6.4 Hz), 2.37 (1H, dd, J = 18.8 Hz, 6.0 Hz), 2.75 (1H, dt, J = 9.6, 3.6 Hz), 3.74 (3H, s), 3.78 (1H, d, J = 18.4 Hz), 3.87 (1H, d, J = 18.4 Hz), 4.14 (1H, d, J = 17.6 Hz), 4.21 (1H, d, J = 17.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3-CD_3OD$ 9:1) δ 14.1 (q), 17.6 (t), 19.2 (t), 19.8 (t), 20.9 (q), 26.6 (t), 28.9 (q), 35.9 (s), 36.4 (t), 38.1 (t), 38.3 (t), 40.3 (t), 43.3 (t), 43.7 (s), 52.3 (q), 52.9 (t), 56.4 (d), 57.3 (d), 140.5 (s), 149.9 (s), 170.4 (s), 171.7 (s), 180.6 (s); HR ESI-TOF-MS m/z 404.2428 $[M + H]^+$, calcd for $C_{23}H_{34}NO_5$ 404.2432.

Spongolactam C (3). To a solution of methyl ester **11b** (9.0 mg, 0.022 mmol) in MeOH (1 mL) was added 1 N NaOH (2 mL), and the mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with 1 N HCl (3 mL) and then water (3 mL), and the mixture was extracted three times with EtOAc (10 mL). The combined organic layers were concentrated, and the residue was purified by silica gel TLC ($CHCl_3-MeOH-AcOH$ 10:1:0.1) and then by HPLC [Develosil ODS-5 (ϕ 10 × 250 mm), 60% MeOH−20 mM NH_4OAc, 3 mL/min, detected at 216 nm] to give spongolactam C (**3**) (4.0 mg, 47%, t_R = 12.5−17.5 min). Although a ^1H NMR spectrum was not superimposable on that of the natural compound, after concentration followed by lyophilization of a solution in a mixture of 25% NH_4OH (0.006 mL) and MeOH (1 mL), the spectra turned out to be identical with those of the natural sample. **3**: colorless powder; mp 290−300 °C dec; $[\alpha]^{27}_D$ −26° (*c* 0.018, MeOH); UV (MeOH) λ_{max} 216 (ϵ 12200), 238 (3600, sh) nm; IR (KBr) 3600−2400, 1763, 1672, 1655, 1644, 1459, 1244, 1193 1042, 780 cm^{-1}.

Methyl Ester 11a. The same reaction for **11b** was conducted with **8a**/**8b** (93:7) (4.5 mg, 0.013 mmol). The crude product was purified by column chromatography (silica gel, $CHCl_3-MeOH$ 10:1) followed by TLC [silica gel, $CHCl_3-MeOH$ (10:1)] to give **11a** (2.7 mg, 52% in 2 steps): colorless powder; UV (MeOH) λ_{max} 215 (ϵ 10200), 236 (3500, sh) nm; IR (KBr) 3600−2800 (br), 1752, 1715, 1685, 1661, 1458 1212, 1174, 753 cm^{-1}; $[\alpha]^{28}_D$ −32 (*c* 0.15, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD) δ 0.90 (1H, m), 0.91 (3H, s), 0.98 (1H, td, J = 13.2, 4.0 Hz), 1.11 (1H, dd, J = 13.2, 2.4 Hz), 1.16 (3H, s), 1.18 (1H, m), 1.20 (3H, s), 1.32 (1H, m), 1.36 (1H, m), 1.42 (1H, m), 1.53 (1H, qd, J = 12.0, 6.4 Hz), 1.81−1.97 (4H, m), 1.98−2.15 (3H, m), 2.20 (1H, br q, J = 10.5 Hz), 2.32 (1H, dd, J = 16.4, 4.8 Hz), 3.72 (3H, s), 3.96 (1H, dd, J = 19.2, 2.0 Hz), 4.05 (1H, dt, J = 19.2, 2.4 Hz), 4.22 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 14.1 (q), 17.4 (t), 19.1 (t), 19.9 (t), 22.2 (q), 22.2 (t), 28.9 (q), 36.6 (s), 38.1 (t, 2C), 38.2 (s), 40.0 (t), 43.4 (t), 43.8 (s), 52.1 (q), 55.5 (d), 57.1 (d), 128.5 (s), 161.5 (s), 170.1 (s), 172.3 (s), 181.7 (s); HR ESI-TOF-MS m/z 404.2428 $[M + H]^+$, calcd for $C_{23}H_{34}NO_5$ 404.2432.

Isomer 4. Hydrolysis of **11a** (10.5 mg) was conducted by the same procedure as the above one. The crude product was purified by column chromatography (silica gel, $CHCl_3-MeOH-AcOH$ 10:1:0.1) followed by HPLC [Develosil ODS-5 (ϕ 10 × 250 mm), 60% MeOH in 20 mM NH_4OAc, 3 mL/min, detected at 216 nm] to afford **4** (3.1 mg, 33%, t_R = 9.4−12 min): colorless powder; mp 204−207 °C; $[\alpha]^{28}_D$ −28 (*c* 0.29, MeOH); UV (MeOH) λ_{max} 214 (ϵ 11800), 238 (3600, sh) nm; IR (KBr) 3600−2400 (br), 1725, 1664, 1458, 1246, 1226, 1162, 753 cm^{-1}; HR ESI-TOF-MS m/z 390.2252 $[M + H]^+$, calcd for $C_{22}H_{32}NO_5$ 390.2275.

Aminal 12. A solution of **8a**/**8b** (93:7) (35.2 mg, 0.090 mmol) and tryptamine (34 mg, 0.21 mmol) in MeOH (5 mL) was stirred at room temperature for 2.5 h. The reaction mixture was concentrated and the residue was chromatographed on silica gel with $CHCl_3-MeOH$ (20:1) to give a 9:1 mixture of aminal **12** and its 16-hemiaminal isomer (43.5 mg, 88%): pale yellow powder; IR (KBr) 3600−2400 (br), 1718, 1457, 1173, 1096, 743 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, data for **12**) δ 0.86 (3H, s), 0.87−1.36 (6H, m), 1.18 (3H, s), 1.19 (3H, s), 1.28 (3H, s), 1.43 (1H, m), 1.50 (1H, m), 1.74−2.16 (8H, m), 2.29 (1H, dd, J = 18.4, 6.0 Hz), 2.94 (2H, t, J = 7.0 Hz), 3.06 (1H, m), 3.14 (1H, m), 5.74 (1H, s), 6.96 (1H, dt, J = 1.0, 8.0 Hz), 7.05 (1H, s), 7.06 (1H, dt, J = 1.0, 8.0 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 8.0 Hz); HR ESI-TOF-MS m/z 491.2846 $[M + H]^+$, calcd for 491.2904 $C_{30}H_{39}N_2O_4$.

Lactam 13. To a solution of **12** and its isomer (9:1) (40.9 mg, 0.084 mmol) in a 1:1 mixture of MeOH and THF were added $NaBH_3CN$ (8.2 mg, 0.13 mmol) and 12 N HCl (0.005 mL), and the mixture was stirred at room temperature for 4 h. The reaction mixture was treated with saturated $NaHCO_3$ and concentrated. The residue was chromatographed on silica gel ($CHCl_3-MeOH$ 8:1) to give **13** (25.1 mg, 64%): pale yellow powder; mp 270−280 °C dec; $[\alpha]^{22}_D$ −38 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 220 (ϵ 38900), 281 (ϵ 5700) nm; IR (KBr) 3600−2400 (br), 1695, 1668, 1636, 1459, 1215, 1173, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1) δ 0.83 (3H, s), 0.91 (1H, td, J = 13.2, 3.0 Hz), 1.01 (1H, m), 1.02 (3H, s), 1.08 (2H, br d, J = 11.6 Hz), 1.11 (1H, td, J = 13.2, 3.6 Hz), 1.21 (3H, s), 1.44 (1H, m), 1.47 (1H, m), 1.62 (1H, br d, J = 12.4 Hz), 1.83 (2H, m), 1.85 (1H, m), 1.90 (1H, m), 1.95 (1H, m), 2.02 (1H, m), 2.08 (1H, m), 2.15 (1H, br d, J = 13.2 Hz), 2.36 (1H, dd, J = 17.2, 5.2 Hz), 3.05 (2H, t, J = 6.8 Hz), 3.55 (1H, dd, J = 19.2, 1.6 Hz), 3.65 (1H, dt, J = 19.2, 2.0 Hz), 3.73 (2H, t, J = 6.8 Hz), 7.05 (1H, s), 7.05 (1H, t, J = 7.4 Hz), 7.14 (1H, t, J = 8.0 Hz), 7.38 (1H, d, J = 8.4 Hz), 7.52 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3-CD_3OD$ 1:1) δ 14.4 (q), 17.9 (t), 19.6 (t), 20.3 (t), 22.3 (q), 22.6 (t), 25.0 (t), 29.2 (q), 36.9 (s), 38.5 (t), 38.5 (s), 38.7 (t), 40.6 (t), 44.2 (t), 44.2 (s), 50.5 (t), 56.0 (d), 57.6 (d), 111.9 (d), 112.7 (s), 118.7 (d), 119.3 (d), 122.0 (d), 122.9 (d), 128.0 (s), 129.2 (s), 137.2 (s), 162.6 (s), 173.6 (s), 181.0 (s); HR ESI-TOF-MS found m/z 475.2885 $[M + H]^+$, calcd for $C_{30}H_{39}N_2O_3$ 475.2955.

Aminal 15. Phthalimide **14** was prepared from phthalic anhydride and histamine according to the literature.[7] To a solution of **14** (20.8 mg, 0.086 mmol) in MeOH (1 mL) was added sodium borohydride (5.9 mg, 0.16 mmol) and the mixture was stirred at room temperature for 1 h. The mixture was treated with an additional sodium borohydride (6.3 mg, 0.17 mmol) and stirred for 1 h. The reaction mixture was diluted with $CHCl_3$ (3 mL) and chromatographed on silica gel (5 g) with $CHCl_3-MeOH$ (3:1). The product was further purified by HPLC [Develosil ODS-UG-5 (ϕ 10 × 250 mm), 30% MeOH−20 mM NH_4OAc, 3 mL/min, detected at 210 nm] to give aminal **15** (18.4 mg, 88%, t_R = 12.5−15 min): colorless powder; mp 44−46 °C; UV (MeOH) λ_{max} 250 nm (ϵ 9800); IR (KBr) 3217, 1683, 1471, 1208, 1107, 1063, 803, 747 697, 622 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 3.01 (2H, m), 3.72 (1H, m), 3.94 (1H, m), 5.77 (1H, s), 6.93 (1H, s), 7.51 (1H, m), 7.60 (1H, s), 7.61 (1H, m), 7.69 (1H, d, J = 7.2 Hz), 7.79 (1H, br); ^{13}C NMR (100 MHz, CD_3OD) δ 26.5 (t), 40.4 (t), 83.1 (d), 117.8 (d), 123.7 (d), 124.6 (d), 130.7 (d), 132.7 (s), 133.5 (d), 135.4 (s, br), 135.9 (d), 146.2 (s), 169.3 (s); HR ESI-TOF-MS m/z 226.0962 $[M - OH]^+$, calcd for $C_{13}H_{12}N_3O$ 226.0975.

Lactam 16. To a solution of hemiaminal **15** (4.1 mg, 0.017 mmol) in dry CH_2Cl_2 (2 mL) was added trifluoroacetic acid (2.6 mL), and the mixture was stirred at 0 °C for 15 min. Triethylsilane (3.4 mL) was then added and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with saturated $NaHCO_3$ (1 mL) and concentrated. The residue was chromatographed on silica gel with $CHCl_3-MeOH$ (5:1) and then subjected to HPLC [Develosil ODS-5 (ϕ 10 × 250 mm), 45% MeOH−20 mM NH_4OAc, 1.2 mL/min, detected at 220 nm] to give lactam **16** (2.5 mg, 64.8%, t_R = 22−37 min): colorless powder; mp 204−207 °C; IR (KBr) 3300−2300 (br), 1682, 1000, 885, 740 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 2.96 (2H, t, J = 7.1 Hz), 3.87 (2H, t, J = 7.1 Hz), 4.37 (2H, s), 6.84 (1H, s), 7.45 (1H, t, J = 7.6 Hz), 7.49 (1H, d, J = 7.6 Hz), 7.56 (1H, dt, J = 7.3, 1.2 Hz), 7.58 (1H, d, J = 1.2 Hz), 7.73 (1H, d, J = 7.0 Hz); ^{13}C NMR

(100 MHz, CD_3OD) δ 26.9 (t), 43.6 (t), 51.6 (t), 117.7 (d, br), 124.0 (d), 124.2 (d), 129.1 (d), 132.8 (s), 133.4 (d), 136.0 (s, br), 136.3 (d), 143.3 (s), 170.1 (s); HR ESI-TOF-MS *m*/*z* 228.1131 $[M + H]^+$, calcd for $C_{13}H_{14}N_3O$ 228.1131.

Cytotoxicity Test. Details on the cytotoxicity test with use of a human tumor cell line, A431, are described in our ref 16.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1**−**4** and ^1H NMR spectra of **6**, **7**, **9a/b**, **11a**, **11b**, **12**, **13**, **15**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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