Isolation of 13 New Ritterazines from the Tunicate *Ritterella tokioka* and Chemical Transformation of Ritterazine B[1]

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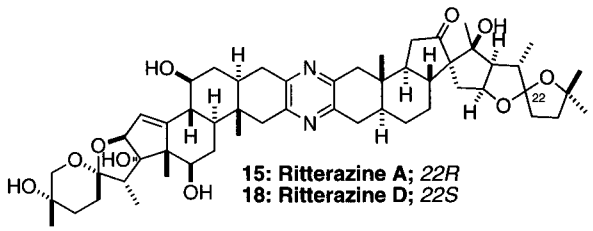
Received January 16, 1997[⊗]

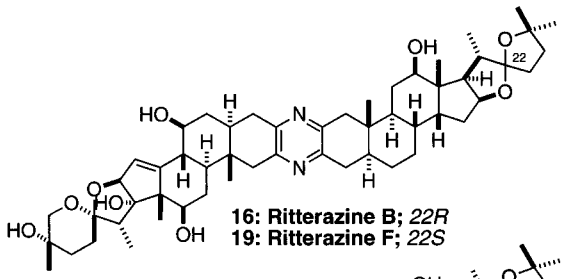
Thirteen new ritterazines, ritterazines N−Z (**2**−**14**), were isolated from the tunicate *Ritterella tokioka*. Chemical transformation of ritterazine B, the most active among the ritterazines, by reduction, oxidation, methanolysis, and acetylation furnished compounds **20**, **21**, **23**, and **25**−**29**. Cytotoxicity of 26 natural products and chemically modified ritterazine B disclosed important structural features of the ritterazines for cytotoxic activity.

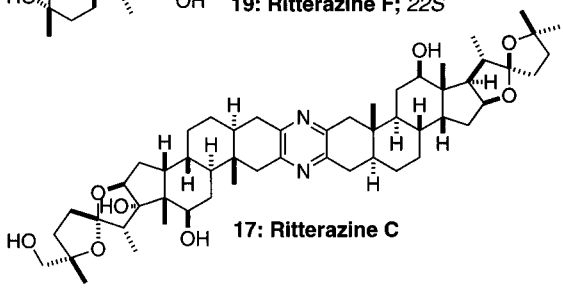
Secondary metabolites of marine invertebrates continue to attract the attention of organic chemists, biochemists, and pharmacologists due to their novel structures and potent biological activities. One such example is cephalostatin 1 (**1**)[2] isolated from the Indian Ocean hemichordate *Cephalodiscus gilchristi*, which exhibited remarkable cytotoxic activity against P388 murine leukemia cells with IC_{50} values of 10^{-4}−10^{-6} ng/mL. In the course of our search for cytotoxic substances from Japanese marine invertebrates, we found potent activity against P388 cells in the lipophilic extract of the tunicate *Ritterella tokioka*[3] collected off the Izu Peninsula. Bioassay-guided isolation afforded ritterazines A−M,[4−6] which are dimeric steroidal alkaloids closely related to cephalostatin 1. Since the ritterazines were not only highly cytotoxic but also structurally unusual, we tried to accumulate knowledge of their structure−activity relationships by examination of the cytotoxic activity of further derivatives of the ritterazines. In this paper, we report the isolation and structure elucidation of ritterazines N−Z (**2**−**14**, Chart 1) and chemical modification of ritterazine B at the terminal 5/6 spiroketal and secondary hydroxyl groups.

Results and Discussion

Isolation. Colonies of the tunicate (9 kg) collected at depths of 3−5 m off the Izu Peninsula were extracted with EtOH. The combined extracts were concentrated and partitioned between water and ethyl acetate. The organic phase was fractionated by the Kupchan partitioning procedure.[7] The CH_2Cl_2 fraction was repeatedly purified by ODS, SiO_2, and Sephadex LH-20 chromatographies. Ritterazines N (**2**), O (**3**), P (**4**), Q (**5**), R (**6**), and S (**7**) (yields: 1.5, 3.8, 0.8, 0.6, 0.3, and 0.5 mg, respectively) were obtained from the MeOH fraction of the initial ODS flash chromatography, whereas ritterazines T (**8**), U (**9**), V (**10**), W (**11**), X (**12**), Y (**13**), and Z (**14**) (yields: 2.3, 3.0, 0.9, 0.7, 0.7, 3.5, and 1.7 mg, respectively) were obtained from the MeCN/H_2O (7:3) fraction together with ritterazines A−C (**15**−**17**).[4−6]







Structure Elucidation of Ritterazines N−Z. Structure elucidation of the new ritterazines was carried out

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⊗ Abstract published in *Advance ACS Abstracts,* June 1, 1997.

(1) Bioactive Marine Metabolites Series. 79. Part 78: Matsunaga, S.; Wakimoto, T.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 2640−2642.

(2) Pettit, G. R.; Inoue M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006−2007.

(3) Colonies of *R. tokioka* were collected by hand using scuba at depths of 2−10 m off the Izu Peninsula, 100 km southwest of Tokyo. They were identified as *R. tokioka* Kott, 1992 (family Polyclinidae, order Enterogona) by Dr. T. Nishikawa (Nagoya University). A voucher specimen (T94-001) is deposited at the Laboratory of Aquatic Natural Products Chemistry, The University of Tokyo.

(4) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1994**, *59*, 6164−6166.

(5) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 608−614.

(6) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6707−6716.

(7) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

(8) The gross structures and relative stereochemistry of the new compounds were assigned on the basis of DQF-COSY, HMQC, HMBC, NOESY, and ROESY data acquired for each compound.

(9) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.

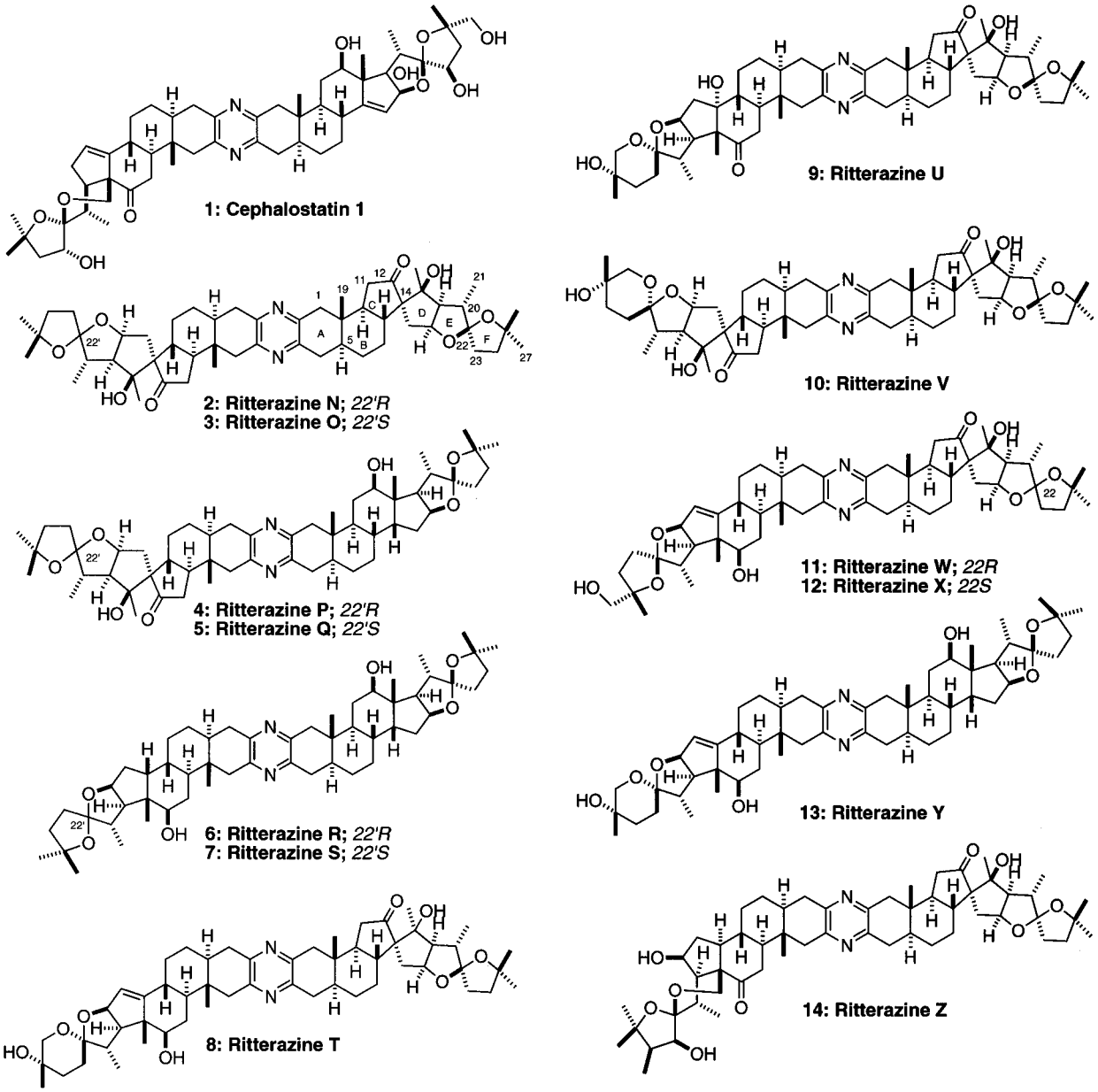
(10) Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

(11) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811−813.

(12) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207−213.

S0022-3263(97)00091-1 CCC: $14.00

Chart 1



by interpretation of spectral data. With the NMR parameters of 13 previously isolated ritterazines in hand,[4−6] interpretation of 2D NMR data for the new compounds was unexceptional (Tables 1 and 2 and tables in the Supporting Information). Ritterazines N−S have a combination of previously encountered steroidal units, whereas one or both of the steroidal halves of ritterazines T−Z are new.[8−12]

Ritterazine N (**2**) had a molecular formula of $C_{54}H_{76}N_2O_8$ as established by HR-FABMS. ^1H and ^{13}C NMR spectra revealed that **2** was symmetric and had a five-membered ketone signal (δ 220.8). Interpretation of 2D NMR data showed that ritterazine N has two units of the eastern hemisphere of ritterazine A.

Ritterazine O (**3**) was isomeric to ritterazine N. Although the gross structure of ritterazine O assigned on the basis of 2D NMR data was identical with that of ritterazine N, ritterazine O was unsymmetrical. Comparison of the NMR data revealed that one half of **3** was identical with the eastern hemisphere of ritterazine A, while the other half was identical with the eastern hemisphere of ritterazine D (**18**).

Ritterazine P (**4**) had a molecular formula of $C_{54}H_{78}N_2O_7$ as determined by HR-FABMS. The eastern hemisphere of ritterazine P including stereochemistry was identical with that of ritterazine B (**16**), while its western hemisphere was the same as the eastern hemisphere of ritterazine A (**15**).

Ritterazine Q (**5**) had the same molecular formula as ritterazine P. NMR data suggested that the western hemisphere of **5** was identical with that of ritterazine O, while its eastern hemisphere was identical with that of ritterazine B. Therefore, **5** is 22′-*epi*-ritterazine P.

Ritterazine R (**6**) had a molecular formula of $C_{54}H_{80}N_2O_6$ as determined by HR-FABMS. NMR data suggested its symmetrical nature; both hemispheres were identical with the eastern hemisphere of ritterazine B.

Ritterazine S (**7**) had the same molecular formula as ritterazine R. NMR data showed that the western hemisphere of ritterazine S was identical with the eastern hemisphere of ritterazine F (**19**), whereas its eastern hemisphere was identical with the eastern hemisphere of ritterazine B: ritterazine S is 22′-*epi*-ritterazine R.

NMR data indicated the similarity of ritterazine T (**8**) to ritterazine A. HRFABMS showed that **8** was smaller

Table 1. ^{13}C NMR Data of Ritterazines N−S (Pyridine-d_5)

no.	2	3	4	5	6	7
1	46.7 t	46.8 t	46.7 t	46.7 t	46.5 t	46.5 t
2	149.0 s	149.0 s	149.0 s	149.0 s	149.1 s	149.0 s
3	149.0 s	149.0 s	149.0 s	149.0 s	149.1 s	149.2 s
4	35.8 t	35.9 t	35.9 t	35.9 t	36.0 t	36.1 t
5	42.0 d	42.0 d	41.5 d	41.5 d	41.5 d	41.5 d
6	29.2 t	29.0 t	28.7 t	29.0 t	28.8 t	29.0 t
7	30.6 t	30.8 t	31.7 t	31.7 t	31.7 t	31.5 t
8	40.5 d	40.5 d	32.7 d	32.5 d	32.6 t	32.5 d
9	50.3 d	50.3 d	45.3 d	45.1 d	45.2 d	44.9 d
10	35.4 s	35.3 s	35.5 s	35.6 s	35.7 s	35.5 s
11	40.8 t	40.9 t	30.6 t	30.4 t	30.5 t	30.5 t
12	220.2 s	220.2 s	72.3 d	72.2 d	72.2 d	72.1 d
13	81.0 s	82.0 s	48.0 s	48.0 s	48.0 s	48.0 s
14	69.1 s	69.2 s	47.9 d	48.0 d	47.9 d	48.0 d
15	36.0 t	36.0 t	32.7 t	32.7 t	32.6 t	32.6 t
16	83.3 d	83.3 d	79.9 d	79.7 d	79.7 d	79.8 d
17	61.9 d	61.8 d	57.5 d	57.4 d	57.5 d	57.5 d
18	23.5 q	23.8 q	13.2 q	13.2 q	13.2 q	13.2 q
19	10.8 q	11.1 q	11.9 q	11.8 q	11.8 q	11.8 q
20	41.0 d	41.0 d	42.0 d	42.0 d	42.4 d	42.3 d
21	19.1 q	19.4 q	14.6 q	14.6 q	14.7 q	14.6 q
22	119.0 s	119.6 s	116.7 s	116.7 s	116.9 s	116.8 s
23	33.0 t	33.1 t	32.9 t	33.3 t	33.8 t	33.3 t
24	37.4 t	37.7 t	38.0 t	38.0 t	37.7 t	38.0 t
25	82.4 s	82.4 s	81.6 s	81.6 s	81.7 s	81.5 s
26	28.7 q	28.7 q	28.8 q	28.8 q	28.8 q	28.8 q
27	30.4 q	30.4 q	30.4 q	30.3 q	30.7 q	30.4 q
1′		46.6 t	46.7 t	46.6 t		46.6 t
2′		148.8 s	148.9 s	148.8 s		149.0 s
3′		148.8 s	148.9 s	148.8 s		149.2 s
4′		35.9 t	35.9 t	35.9 t		36.1 t
5′		41.6 d	41.7 d	41.6 d		41.5 d
6′		29.0 t	29.0 t	29.0 t		29.0 t
7′		30.8 t	30.5 t	30.4 t		31.5 t
8′		40.4 d	40.5 d	40.6 d		32.6 d
9′		49.6 d	50.3 d	50.0 d		44.9 d
10′		35.3 s	35.5 s	35.3 s		35.9 s
11′		40.6 t	40.8 t	40.3 t		30.5 t
12′		218.6 s	220.0 s	218.3 s		72.8 d
13′		82.4 s	81.0 s	82.0 s		48.0 s
14′		69.6 s	69.2 s	69.4 s		48.0 d
15′		36.0 t	33.3 t	33.1 t		33.7 t
16′		80.9 d	83.4 d	80.3 d		79.2 d
17′		60.4 d	61.9 d	60.5 d		57.8 d
18′		21.9 q	23.5 q	21.6 q		13.8 q
19′		11.1 q	10.8 q	10.8 q		11.8 q
20′		38.5 d	41.0 d	38.0 d		41.4 d
21′		15.1 q	19.2 q	15.1 q		17.1 q
22′		119.6 s	119.5 s	119.5 s		117.5 s
23′		33.1 t	33.4 t	34.3 t		33.3 t
24′		37.7 t	37.5 t	37.6 t		37.5 t
25′		82.1 s	82.4 s	82.0 s		81.1 s
26′		28.8 q	28.7 q	28.7 q		28.8 q
27′		30.5 q	30.4 q	30.4 q		30.1 q

Table 2. ^{13}C NMR Data of Ritterazines T−Z (Pyridine-d_5)

no.	8	9	10	11	12	13	14
1	46.8 t	46.6 t	46.6 t	46.7 t	46.4 t	46.2 t	46.4 t
2	149.0 s	148.8 s	148.7 s	148.7 s	148.7 s	148.6 s	148.0 s
3	149.0 s	148.8 s	148.7 s	148.7 s	148.7 s	149.1 s	148.5 s
4	35.5 t	35.9 t	35.5 t	35.7 t	35.7 t	35.8 t	35.1 t
5	41.7 d	41.9 d	42.0 d	41.8 d	41.6 d	41.7 d	41.5 d
6	29.2 t	29.1 t	29.1 t	29.2 t	28.9 t	29.0 t	28.7 t
7	30.7 t	30.6 t	30.7 t	30.6 t	30.6 t	31.8 t	30.2 t
8	40.5 d	40.5 d	40.7 d	40.5 d	41.0 d	32.6 d	40.2 d
9	50.1 d	50.2 d	50.1 d	50.0 d	50.0 d	45.5 d	49.7 d
10	35.6 s	35.5 s	35.6 s	35.8 s	35.4 s	35.9 s	35.6 s
11	40.9 t	40.9 t	41.0 t	41.0 t	41.0 t	30.7 t	40.5 t
12	221.6 s	221.2 s	221.2 s	221.2 s	220.5 s	71.7 d	220.5 s
13	80.9 s	80.9 s	80.4 s	80.9 s	79.8 s	48.5 s	80.6 s
14	69.3 s	69.3 s	69.3 s	69.7 s	70.8 s	47.7 d	68.9 s
15	35.9 t	35.8 t	35.7 t	35.9 t	34.0 t	32.9 t	35.5 t
16	82.8 d	82.8 d	82.8 d	81.9 d	80.4 d	80.0 d	82.5 d
17	61.7 d	61.7 d	61.6 d	61.7 d	60.7 d	57.4 d	61.4 d
18	23.5 q	23.5 q	23.5 q	23.4 q	23.8 q	13.7 q	23.1 q
19	10.9 q	10.9 q	11.0 q	10.9 q	10.9 q	11.9 q	10.5 q
20	40.8 d	40.7 d	40.8 d	40.7 d	38.4 d	42.0 d	40.4 d
21	18.9 q	19.0 q	19.0 q	19.0 q	14.7 q	14.7 q	18.6 q
22	119.9 s	119.8 s	119.9 s	119.6 s	120.6 s	119.0 s	119.2 s
23	32.7 t	32.7 t	32.7 t	32.8 t	33.9 t	33.3 t	32.4 t
24	37.4 t	37.4 t	37.3 t	37.4 t	37.8 t	37.8 t	37.0 t
25	82.3 s	82.5 s	81.7 s	81.6 s	81.6 s	81.4 s	82.3 s
26	28.7 q	28.5 q	28.6 q	28.6 q	28.8 q	28.7 q	28.2 q
27	30.5 q	30.4 q	30.4 q	30.4 q	30.3 q	30.3 q	30.1 q
1′	46.3 t	46.0 t	46.5 t	46.2 t	46.1 t	46.2 t	45.4 t
2′	149.0 s	148.8 s	148.7 s	148.7 s	148.7 s	148.8 s	148.5 s
3′	149.0 s	148.8 s	148.7 s	148.7 s	148.7 s	148.7 s	148.0 s
4′	35.8 t	35.6 t	35.5 t	35.8 t	35.7 t	36.0 t	35.4 t
5′	42.0 d	42.1 d	41.7 d	41.9 d	42.0 d	41.6 d	41.3 d
6′	28.3 t	28.6 t	29.0 t	28.3 t	28.3 t	28.3 t	27.9 t
7′	29.9 t	27.2 t	30.7 t	29.9 t	29.8 t	29.7 t	30.6 t
8′	35.1 d	38.6 d	40.7 d	34.7 d	34.7 d	33.1 d	33.9 d
9′	49.5 d	48.4 d	49.8 d	49.6 d	49.1 d	52.7 d	54.3 d
10′	36.2 s	36.4 s	35.4 s	36.1 s	36.0 s	36.2 s	35.9 s
11′	29.8 t	37.5 t	41.0 t	29.8 t	29.7 t	30.9 t	39.4 t
12′	76.1 d	212.6 s	220.6 s	76.1 d	76.1 d	78.7 d	208.3 s
13′	52.5 s	60.3 s	80.0 s	52.6 s	52.6 s	52.6 s	60.0 s
14′	151.9 s	90.5 s	70.9 s	153.8 s	153.5 s	157.7 s	53.9 d
15′	120.9 d	39.5 t	34.0 t	121.5 d	121.5 d	120.5 d	36.2 t
16′	86.4 d	81.0 d	81.0 d	86.3 d	86.3 d	85.4 d	79.4 d
17′	54.6 d	53.1 d	60.8 d	54.6 d	54.5 d	56.4 d	49.6 d
18′	18.9 q	20.0 q	23.7 q	19.0 q	19.0 q	14.0 q	59.5 t
19′	11.9 q	11.6 q	11.0 q	11.8 q	11.8 q	11.8 q	11.3 q
20′	45.1 d	43.0 d	41.5 d	41.8 d	41.8 d	45.2 d	37.3 d
21′	14.8 q	14.4 q	14.7 q	14.6 q	14.7 q	14.5 q	14.0 q
22′	107.1 s	110.0 s	110.3 s	118.0 s	118.0 s	107.1 s	111.2 s
23′	27.8 t	28.1 t	27.7 t	33.3 t	33.3 t	27.7 t	70.4 d
24′	33.8 t	33.8 t	33.9 t	32.8 t	32.7 t	33.8 t	44.8 d
25′	66.0 s	65.9 s	66.0 s	85.4 s	85.5 s	66.0 s	72.6 s
26′	70.3 t	69.8 t	70.1 t	69.8 t	69.7 t	70.2 t	26.5 q
27′	26.9 q	27.0 q	27.0 q	24.2 q	24.2 q	27.0 q	29.7 q
28′							9.3 q

than **15** by two oxygen atoms. Comparison of NMR data disclosed that its eastern hemisphere was identical with that of ritterazine A. Therefore, the western hemisphere has two less oxygen atoms than the western hemisphere of ritterazine A. Interpretation of 2D NMR data implied that the hydroxyl groups on C7′ and C17′ in the western hemisphere of **15** were replaced by hydrogen atoms. Therefore, ritterazine T was assigned as 7′,17′-didehydroxyritterazine A.

Ritterazine U (**9**) had one more oxygen atom than ritterazine T (**8**) as evidenced by HR-FABMS data. NMR spectra indicated that the eastern hemisphere of **9** was the same as that of ritterazine T. HMBC data implied the presence of an additional ketone (δ 212.6) and an oxygenated nonprotonated carbon (δ 90.5) in the eastern hemisphere: no sp^2 carbon signals other than those of the pyrazine was observed. ^{1}H−^{13}C long-range couplings observed in the HMBC spectrum allowed the placement of the ketone at C12′ and the hydroxyl group at C14′. Axial-orientation of 14′-OH was deduced on the basis of ROESY cross peaks: H9′/14′OH, H16′/14′OH, and H17′/14′OH (Figure 1). Therefore, ritterazine U has a C/D *trans* junction. Relative stereochemistry of the rest of the western hemisphere was the same as that of **8**.

The ^{13}C NMR spectrum implied the presence of two strained ketones (δ 221.2 and 220.6) in ritterazine V (**10**), which has one more oxygen atom than **2**. The eastern hemisphere of **10** was identical with that of **2**, which was readily inferred from NMR data. HMBC cross peaks indicated that the other half had the rearranged skeleton terminating in a 5/6 spiroketal. Stereochemistries of C13′, C14′, C17′, and C20′ were the same as those of other ritterazines. An NOE between H16′ and H26′β suggested 22′*R* stereochemistry.

Ritterazine W (**11**) was an isomer of **8** with respect to the terminal spiroketal; a 5/6 spiroketal in **8** was isomerized to a 5/5 spiroketal.

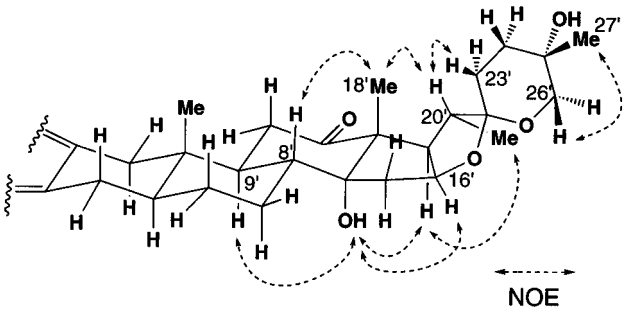


Figure 1. Stereochemistry of ritterazine U (**7**).

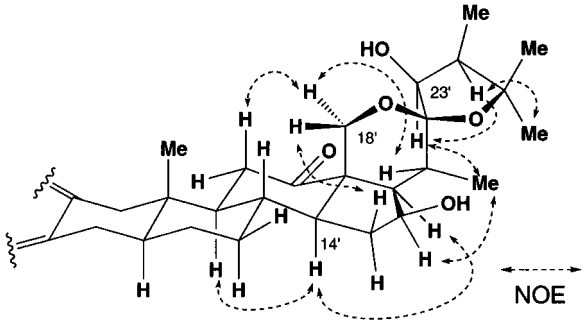


Figure 2. Stereochemistry of ritterazine Z (**14**).

Ritterazine X (**12**) was assigned as 22-*epi*-ritterazine W on the basis of 2D NMR data.

Ritterazine Y (**13**) was a hybrid of ritterazine B and ritterazine T. The eastern hemisphere of **13** was identical to that of ritterazine B, whereas the western hemisphere identical to that of ritterazine T. Therefore, **13** was 7′,17′-didehydroxyritterazine B.

Ritterazine Z (**14**) had a molecular formula of $C_{55}H_{78}N_2O_9$ as established by HR-FABMS. The eastern hemisphere of **14** including the stereochemistry was the same as that of **8**. In the western hemisphere, a ketone (δ 208.3) could be placed at C12′ on the basis of HMBC cross peaks: H11′α/C12′, H11′β/C12′, and H17′/C12′. In addition, oxygenated methylene protons (δ 3.52, 4.08) were long-range coupled to C14′; one of them (δ 4.08) was further coupled to C12′, C13′, C17′, and C22′, revealing a connection between C18′ and C22′ through an oxygen bridge, which is reminiscent of cephalostatin 1 (**1**). The relative stereochemistry of each steroidal unit was deduced from NOESY data measured in pyridine-d_5 at 263 K. H14′ was assigned as axial on the basis of NOESY cross peaks: H18′α/H15′β, H14′/H9′, and H14′/H17′. Therefore, the western hemisphere of ritterazine Z has a C/D trans junction. Further NOESY cross peaks (Me-21′/H23′ and H23′/H24′) suggested both OH-23′ and Me-28′ were β-oriented (Figure 2).

Because of the paucity of material, neither absolute stereochemistry nor the orientation of the steroidal units with respect to the pyrazine ring was determined for compounds **2**−**14**. However, it is likely that they share common structural features with ritterazines B and C, whose structures were unambiguously determined.[4,13]

Chemical Modification of Ritterazine B. In order to obtain further information of the structure−activity relationships of ritterazines, chemical transformations of ritterazine B (**16**) were carried out. Ritterazine B was chosen because (1) it is the most abundant and most potent cytotoxin among natural ritterazines, (2) ritterazine B is stable under esterification conditions, which decompose ritterazine A, and (3) the terminal 5/6 spiroketal is more labile than the 5/5 spiroketal at the other end and can be selectively modified.

(1) Acid Methanolysis. In expectation of obtaining the C22′-dimethyl acetal, ritterazine B was treated with 10% HCl/MeOH. Two major products, **20** and **21**, both of which had a molecular formula of $C_{55}H_{76}N_2O_8$, were obtained; one water molecule was lost rather than formation of a methanol adduct. The ^{13}C NMR spectrum indicated the presence of one each of 5/5 and 5/6 spiroketal in **20**, whereas **21** had two 5/5 spiroketals; thus, the isomerization of the terminal 5/6 spiroketal had taken place. We experienced a similar isomerization of ritterazine B to ritterazine C by mild acid treatment.[5] Two oxygenated carbon signals assigned to C16′ and C17′ in ritterazine B were missing in **20**, which exhibited two new signals at δ 210.4 and 54.0 in the ^{13}C NMR spectrum. The C14′ sp^2 carbon experienced a downfield shift of 34 ppm. Interpretation of 2D NMR data including the HMBC spectrum implied that C15′ was oxygenated to a ketone and the C16′ hydroxyl group was replaced by a hydrogen atom. This is consistent with dehydration of the C17′ alcohol to form an unsaturated spiroketal that was isomerized to a ketone after acid hydrolysis of the spiroketal group. In order to satisfy the molecular formula, the resulting C21′-hemiacetal must further cyclize with the C12′ hydroxyl group. A large coupling constant as observed in the COSY spectrum and the absence of NOE between H17′ and H20′ as well as a NOESY cross peak between H17′ and H21′ revealed that the stereochemistry at C17′ was inverted. An intense NOESY cross peak between H12′ and H20′ indicated that the newly formed tetrahydropyran ring was in the boat form. The stereochemistry at C22′ was assigned on the basis of a NOESY cross peak between H21′ and the axial proton on C23′. The structure of **21** was assigned in the same way. The ring system of **21** is similar to that of cephalostatin 6 (**22**).[14]

(2) Reduction with $LiAlH_4/AlCl_3$. Though the spiroketals appear to resist hydride reduction, there are precedents for the reduction of the steroidal 6/6 spiroketal systems with $LiAlH_4$ in the presence of acid.[15,16] The reaction is suggested to proceed first by protonation of the oxygen on C26′, rupture of the spiroketal system with concomitant formation of the oxonium intermediate, and then migration of a hydrogen on C26′ to C22′, resulting in the formation of a C26′ aldehyde, which is reduced to a primary alcohol.[15] It should be noted that there must be a hydrogen on the carbon bearing the spiroketal oxygen (C26′ in case of **16**) for the acid-catalyzed rearrangement to take place. The 5/5 spiroketal system in the eastern hemisphere of **16**, which lacks the pertinent hydrogen to migrate, is not expected to be reduced. Treatment of ritterazine B with $LiAlH_4/AlCl_3$ afforded the hexol **23** together with ritterazine C. Only one isomer of the hexol was formed, though the stereochemistry at C22 has not been determined. Ritterazine C was formed by acid-catalyzed isomerization and resisted reduction because of the absence of a hydrogen atom at C25.

(3) Oxidation. Oxidation of ritterazine B (**16**) with 1 equiv of PCC/Al_2O_3 complex[17] resulted in selective oxida-

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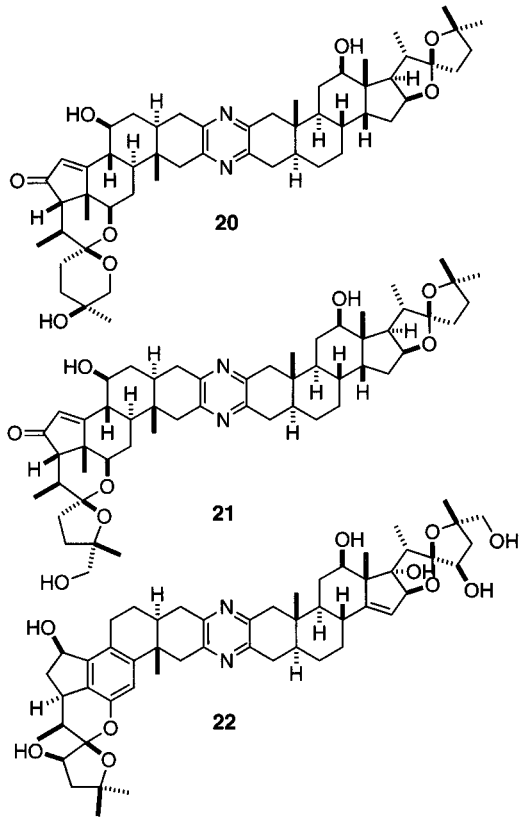
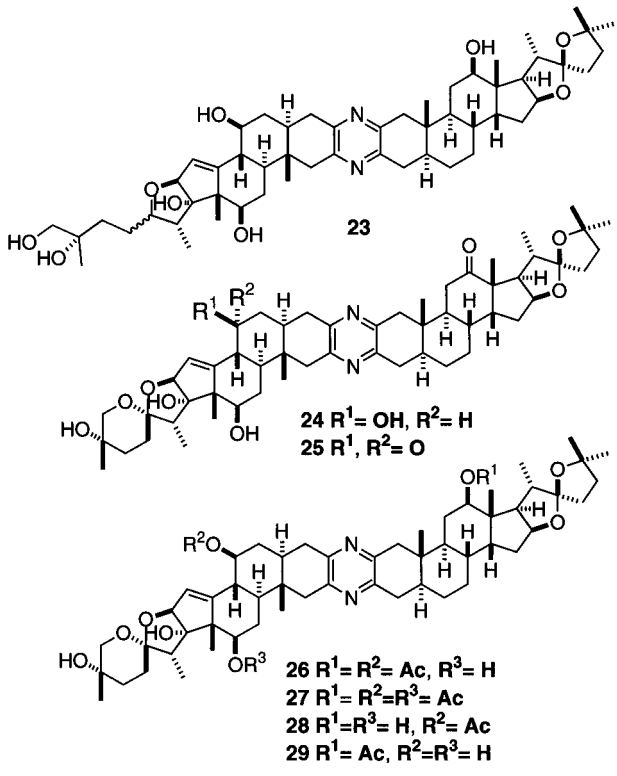


tion of C12 alcohol to afford ritterazine H (**24**), whereas excess reagent promoted further oxidation to give the



diketone **25**. HRFABMS indicated that **25** had two less hydrogen atoms than **23**. Interpretation of 2D NMR data revealed that the C6′ methylene and C8′ methylene protons were shifted downfield; other ^{1}H NMR signals of **25** were comparable to those of **24**. Therefore, **25** is 7′-ketoritterazine H.

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Table 3. Cytotoxic Activity of Ritterazines A−Z and Compounds 20, 21, and 23−29 (IC_{50}, μg/mL)[a]

ritterazine A (**15**)	0.0035	ritterazine R (**6**)	2.1
ritterazine B (**16**)	0.000 15	ritterazine S (**7**)	0.46
ritterazine C (**17**)	0.092	ritterazine T (**8**)	0.46
ritterazine D (**18**)	0.016	ritterazine U (**9**)	2.1
ritterazine E	0.0035	ritterazine V (**10**)	2.1
ritterazine F (**19**)	0.000 73	ritterazine W (**11**)	3.2
ritterazine G	0.000 73	ritterazine X (**12**)	3.0
ritterazine H (**24**)	0.016	ritterazine Y (**13**)	0.0035
ritterazine I	0.014	ritterazine Z (**14**)	2.0
ritterazine J	0.013	**20**	2.1
ritterazine K	0.0095	**21**	2.5
ritterazine L	0.010	**23**	0.24
ritterazine M	0.015	**25**	0.018
ritterazine N (**2**)	0.46	**26**	0.8
ritterazine O (**3**)	2.1	**27**	7.6
ritterazine P (**4**)	0.71	**28**	0.092
ritterazine Q (**5**)	0.57	**29**	0.0035

[a] The IC_{50} values for ritterazines A−M were redetermined by carrying out the cytotoxicity test together with the new compounds.

(4) Acetylation. Acetylation of ritterazine B (**16**) with Ac_2O/pyridine at rt for 40 min afforded the diacetate **26** and the triacetate **27**. ^{1}H NMR data of **26** and **27** were consistent with 7′,12-diacetylritterazine B and 7′,12′,12-triacetylritterazine B, respectively. In order to obtain monoacetylated derivatives, the reaction was carried out by dilution with toluene. After separation of the products by HPLC, two monoacetates **28** and **29** were obtained together with **26**. ^{1}H NMR data indicated that **28** was 7′-acetylritterazine B, while **29** was 12-acetylritterazine B.

Cytotoxic Activity. Cytotoxic activity against P388 murine leukemia cells of ritterazines A−Z and compounds **20**, **21**, **23**, and **25**−**29** are shown in Table 3. Ritterazines N−S (**2**−**7**) having two nonpolar steroidal units[18] were much less active than ritterazine B. Ritterazines T−Y (**8**−**13**) are related to ritterazine A and B, composed of polar and nonpolar steroidal units, lacking the C7′ and C17′ hydroxyl groups. Ritterazine Y (**13**) differs from ritterazine B in the absence of the two hydroxyl groups; ritterazines T−X can be considered as derivatives of **13** with further modifications. Ritterazine T has a rearranged nonpolar steroidal unit, while ritterazine U is an oxidized analog of ritterazine T. In ritterazine V, both steroidal units are rearranged. This is the only example in the ritterazines in which a polar steroidal unit is rearranged. Ritterazines W and X have the 5/5 spiroketal terminus in the polar steroidal unit instead of the 5/6 spiroketal terminus in ritterazine T. Although ritterazines T−X (**8**−**12**) are marginally active, ritterazine Y (**13**) is a potent cytotoxin. However, it is 30 times less potent than ritterazine B. The modifications of ritterazine Y, i.e., rearrangement of steroid skeleton(s) and isomerization of the 5/6 spiroketal to the 5/5 spiroketal, significantly diminished the cytotoxic activity. Similar modifications of ritterazine B to yield ritterazine A and ritterazines C−M also caused significant decrease in the cytotoxic activity.[4−6] Ritterazine Z (**14**), which is apparently related to cephalostatin 1 (**1**), is the only isomer that forms an oxygen bridge between C18 and C22. Weak cytotoxic activity of this compound

(18) The steroidal units of ritterazines can be classified into two groups: those possessing a hydroxyl group or its equivalent at C26 are called polar steroidal units because they have more than two hydroxyl groups; those having no substituent on C26 are called nonpolar steroidal units because they have one hydroxyl group.

is probably due to the presence of the rearranged nonpolar unit in the eastern hemisphere.[19]

Significant contribution of the terminal 5/6 spiroketal to cytotoxicity is evident by comparing the activity of ritterazines B and C, which is further supported by the cytotoxic activity of compounds **20**, **21**, and **23**. Compounds **20** and **21**, in which the terminal spiroketal in the polar steroid units is translocated, have considerably diminished activity compared with the parent compounds **15** and **16**. Therefore, it was suggested that the spatial arrangement of the 5/6 spiroketal with respect to the rest of the skeleton is of importance for the potent cytotoxic activity. Compound **23**, which retains the ring E′, was weakly active but 10 times more potent than **20** and **21**. It must be noted that the presence of the 5/6 spiroketal at the right position in the polar steroid unit is necessary but not sufficient for the potent cytotoxic activity: symmetric or nearly symmetric ritterazines (ritterazines J, K, L, and M) having two polar steroidal units with 5/6 spiroketal are 100 times less active than ritterazine B.[6]

The importance of the hydroxyl groups in ritterazine B was verified by the weak activity of compounds **25**−**29**. We previously showed that oxidation of the C12 alcohol to a ketone (ritterazine B to H) diminished the activity;[6] similarly, oxidation of C7′ further decreased the activity. Introduction of one or more acetyl groups affected the cytotoxicity, which indicates significant contribution of all three secondary hydroxyl groups to the potent cytotoxicity. The higher the number of the acetyl groups the weaker the activity. Interestingly, 7′-acetate **28** was 30 times less potent than the 12-acetate **29**. It is likely that the introduction of a bulky functionality at C7′ hindered the binding to a target molecule.

As yet, structure−activity relationships of cephalostatins have not been reported. However, examination of their analogs prepared by synthesis disclosed the importance of the $\Delta^{14,15}$ double bond and the nonsymmetric structure.[20,21] Recently synthesized dihydrocephalostatin 1, which has a C/D-trans junction in one unit steroid, was reported to be as potent as cephalostatin 1.[22]

Experimental Section[23]

Extraction and Isolation. Specimens of *R. tokioka* were collected off the Izu Peninsula in August 1994 and kept frozen until processed. The thawed samples were freed from macroepibionts, sand, and other debris before extraction. The cleaned animals (9 kg) were homogenized in a Waring blender and extracted with ethanol (4 × 10 L). The combined extracts were concentrated and partitioned between water (2 L) and ethyl acetate (4 × 1.5 L). The ethyl acetate-soluble portion (44.5 g) was partitioned between H_2O/MeOH (1:9) and *n*-hexane. Water was added to the aqueous MeOH phase to adjust the MeOH concentration to 60%, and the mixture was extracted with CH_2Cl_2. The active CH_2Cl_2 layer (12.2 g) was subjected to flash chromatography on ODS (5 × 15 cm) with MeCN/H_2O (5:5), MeCN/H_2O (7:3), MeCN/H_2O (9:1), MeOH, and MeOH/$CHCl_3$/H_2O (7:3:0.5). The fraction eluted with MeCN/H_2O (7:3) (2.45 g) was gel-filtered on Sephadex LH-20 (6 × 90 cm) with C_6H_{14}/CH_2Cl_2/MeOH (4:5:1). The active fractions were combined and purified by ODS-HPLC (2 × 25 cm) with MeCN/H_2O (6:4) to yield ritterazines A (**15**), B (**16**), T (**8**), U (**9**), V (**10**), W (**11**), X (**12**), Y (**13**), and Z (**14**) (yields, 7.7, 34.5, 2.3, 3.0, 0.9, 0.7, 0.7, 3.5, and 1.7 mg, respectively) as colorless glassy solids. The fraction eluted with MeOH in ODS flash chromatography (1.0 g) was chromatographed on SiO_2 (4.0 × 20 cm, $CHCl_3$ → 50% MeOH/$CHCl_3$ and 50% MeOH/EtOAc) to afford 23 fractions. Fractions 4−6 were combined and separated by ODS-HPLC (95% MeOH, 1 × 25 cm, UV detection at 210 nm), followed by purification on a SiO_2 short column (1.0 × 5 cm, $CHCl_3$ → 1% MeOH/$CHCl_3$) to furnish ritterazine N (**2**, yield 1.5 mg). Fraction 9 was separated by ODS-HPLC (95% MeOH, 1 × 25 cm, UV detection at 210 nm) to yield ritterazine O (**3**, yield 3.8 mg). Fractions 10−16 were combined and separated by ODS-HPLC (1 × 25 cm, UV detection at 210 nm), first with 95% MeOH and then with MeCN, to afford ritterazines P (**4**), Q (**5**), R (**6**), and S (**7**) (yield, 0.8, 0.6, 0.5, and 0.5 mg, respectively).

Ritterazine N (2): $[\alpha]_D$ +121.7 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 289 (ϵ 9388), 309 sh nm; HR-FABMS (positive; 3-nitrobenzyl alcohol matrix) *m*/*z* 881.5629 ($C_{54}H_{77}N_2O_8$, Δ −5.1 mmu); ^{13}C NMR data in benzene-d_6 at 300 K, see Table 1; ^{1}H NMR data in benzene-d_6 at 300 K, see Table 1 (Supporting Information).

Ritterazine O (3): $[\alpha]_D$ +108.6 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 289 (ϵ 9379), 308 sh nm; HR-FABMS (positive; 3-nitrobenzyl alcohol matrix) *m*/*z* 881.5719 ($C_{54}H_{77}N_2O_8$, Δ +3.9 mmu); ^{13}C NMR data in benzene-d_6 at 300 K, see Table 1; ^{1}H NMR data in benzene-d_6 at 300 K, see Tables 1 and 2 (Supporting Information).

Ritterazine P (4): $[\alpha]_D$ +42.5 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 289 (ϵ 10618), 309 sh nm; HR-FABMS (positive; 3-nitrobenzyl alcohol matrix) *m*/*z* 867.5815 ($C_{54}H_{79}N_2O_7$, Δ −7.2 mmu); ^{13}C NMR data in benzene-d_6 at 300 K, see Table 1; ^{1}H NMR data in p benzene-d_6 at 300 K, see Tables 1 and 2 (Supporting Information).

Ritterazine Q (5): $[\alpha]_D$ +57.8 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 289 (ϵ 11225), 309 sh nm; HR-FABMS (positive; 3-nitrobenzyl alcohol matrix) *m*/*z* 867.5938 ($C_{54}H_{79}N_2O_7$, Δ +5.0 mmu); ^{13}C NMR data in benzene-d_6 at 300 K, see Table 1; ^{1}H NMR data in benzene-d_6 at 300 K, see Tables 1 and 2 (Supporting Information).

Ritterazine R (6): $[\alpha]_D$ +26.3 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 288 (ϵ 9557), 309 sh nm; HR-FABMS (positive; 3-nitrobenzyl alcohol matrix) *m*/*z* 853.6071 ($C_{54}H_{81}N_2O_6$, Δ −2.4 mmu); ^{13}C NMR data in benzene-d_6 at 300 K, see Table 1; ^{1}H NMR data in benzene-d_6 at 300 K, see Table 1 (Supporting Information).

Ritterazine S (7): $[\alpha]_D$ +43.3 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 290 (ϵ 10480), and 308 sh nm; HR-FABMS (positive; 3-nitrobenzyl alcohol matrix) *m*/*z* 853.6045 ($C_{54}H_{81}N_2O_6$, Δ −5.0 mmu); ^{13}C NMR data in benzene-d_6 at 300 K, see Table 1; ^{1}H NMR data in benzene-d_6 at 300 K, see Tables 1 and 2 (Supporting Information).

Ritterazine T (8): $[\alpha]_D$ +106.6 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 290 (ϵ 9874), 308 sh nm; HR-FABMS (positive; glycerol matrix) *m*/*z* 881.5719 ($C_{54}H_{77}N_2O_8$, Δ +3.9 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^{1}H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Ritterazine U (9): $[\alpha]_D$ +89.0 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 290 (ϵ 9424), 308 sh nm; HR-FABMS (positive; glycerol matrix) *m*/*z* 897.5646 ($C_{54}H_{77}N_2O_9$, Δ +1.7 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^{1}H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Ritterazine V (10): $[\alpha]_D$ +109.2 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 290 (ϵ 9209), 308 sh nm; HR-FABMS (positive; glycerol matrix) *m*/*z* 897.5627 ($C_{54}H_{77}N_2O_9$, Δ −0.2 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^{1}H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Ritterazine W (11): $[\alpha]_D$ +120.4 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 290 (ϵ 8155), 308 sh nm; HR-FABMS (positive; glycerol matrix) *m*/*z* 881.5691 ($C_{54}H_{77}N_2O_8$, Δ +1.1 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^{1}H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Ritterazine X (12): $[\alpha]_D$ +108.0 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} 290 (ϵ 8816), 308 sh nm; HR-FABMS (positive; glycerol

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(23) For the general procedure, see ref 5.

matrix) *m*/*z* 881.5632 ($C_{54}H_{77}N_2O_8$, Δ −4.8 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^1H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Ritterazine Y (13): $[\alpha]_D$ +57.4 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 289 (ε 9156), 308 sh nm; HR-FABMS (positive; glycerol matrix) *m*/*z* 867.5938 ($C_{54}H_{79}N_2O_7$, Δ +5.0 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^1H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Ritterazine Z (14): $[\alpha]_D$ +105.8 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 289 (ε 8958), 308 sh nm; HR-FABMS (positive; glycerol matrix) *m*/*z* 911.5773 ($C_{55}H_{79}N_2O_9$, Δ −1.2 mmu); ^{13}C NMR data in pyridine-d_5 at 300 K, see Table 2; ^1H NMR data in pyridine-d_5 at 300 K, see Tables 3 and 4 (Supporting Information).

Acid Methanolysis of Ritterazine B. Ritterazine B (3.2 mg) was dissolved in 10% dry HCl/MeOH (0.5 mL), and the mixture was stirred at room temperature for 3 days. The reaction mixture was freed from solvent and separated by ODS-HPLC (UV detection at 286 nm, 60% MeCN) to furnish two products; **20** (yield 0.4 mg 13%) and **21** (yield 0.8 mg 26%).

20: HR-FABMS (positive; glycerol matrix) *m*/*z* 881.5677 [$C_{54}H_{77}N_2O_8$ $(M + H)^+$, Δ −0.3 mmu]; ^1H NMR (pyridine-d_5 at 300 K) δ 0.75 (3H, s, H19), 0.92 (3H, s, H19′), 1.12 (1H, m, H7α), 1.18 (3H, d, *J* = 6.6 Hz, H21), 1.19 (3H, s, H26), 1.27 (1H, m, H6β), 1.27 (3H, s, H18), 1.31 (1H, m, H9′), 1.32 (3H, s, H27′), 1.34 (3H, s, H18′), 1.36 (1H, m, H9), 1.43 (3H, s, H27), 1.44 (1H, m, H23′α), 1.49 (1H, m, H6α), 1.49 (1H, m, H7β), 1.59 (1H, m, H5), 1.68 (1H, m, H8), 1.68 (1H, m, H24α), 1.69 (1H, m, H11β), 1.72 (3H, d, *J* = 5.4 Hz, H21′), 1.76 (1H, m, H11′β), 1.78 (1H, m, H15α), 1.79 (1H, m, H6′β), 1.86 (1H, m, H15β), 1.88 (1H, m, H5′), 1.91 (1H, m, H24′β), 2.02 (1H, m, H20), 2.02 (1H, m, H23α), 2.04 (1H, m, H24β), 2.05 (1H, m, H11α), 2.08 (1H, m, H14), 2.09 (1H, m, H11′α), 2.12 (1H, m, H23β), 2.20 (1H, m, H17′), 2.20 (1H, m, H20′), 2.23 (1H, m, H6′α), 2.42 (1H, m, H24′α), 2.66 (1H, m, H23′β), 2.69 (1H, m, H4β), 2.71 (1H, d, *J* = 16.9 Hz, H1′α), 2.72 (1H, d, *J* = 16.9 Hz, H1α), 2.78 (1H, m, H4′β), 2.82 (1H, m, H8′), 2.96 (1H, dd, *J* = 18.8, 6.2 Hz, H4α), 2.97 (1H, dd, *J* = 18.5, 6.2 Hz, H4′α), 3.16 (1H, m, H17), 3.17 (1H, d, *J* = 16.9 Hz, H1′β), 3.18 (1H, d, *J* = 16.9 Hz, H1β), 3.45 (1H, br d, *J* = 11.0 Hz, H12′), 3.64 (1H, m, H12), 3.69 (1H, br d, *J* = 11.4 Hz, H26′β), 4.00 (1H, m, H7′), 4.21 (1H, d, *J* = 11.0 Hz, H26′α), 4.79 (1H, dd, *J* = 6.9, 6.9 Hz, H16), 5.83 (1H, d, *J* = 4.6 Hz, 12OH), 6.37 (1H, d, *J* = 5.4 Hz, 7′OH), 6.67 (1H, s, H15′); ^{13}C NMR (pyridine-d_5 at 300 K) δ 11.8 (q, C19), 11.9 (q, C19′), 12.7 (q, C18), 14.7 (q, C21), 15.2 (q, C21′), 17.7 (q, C18′), 26.1 (t, C11′), 27.2 (q, C27′), 28.8 (q, C26), 28.9 (t, C23′), 29.0 (t, C6), 30.2 (q, C27), 30.7 (t, C11), 32.0 (t, C7), 32.3 (t, C24′), 32.6 (d, C8), 32.9 (t, C15), 33.2 (t, C23), 35.6 (t, C4′), 35.8 (s, C10), 36.0 (t, C4), 36.1 (s, C10′), 37.8 (d, C20′), 37.8 (t, C24), 38.7 (t, C6′), 39.7 (d, C5′), 41.7 (d, C5), 42.0 (d, C20), 44.0 (d, C8′), 45.6 (d, C9), 46.2 (t, C1′), 46.2 (t, C1), 47.8 (d, C14), 48.5 (s, C13), 48.8 (s, C13′), 50.9 (d, C9′), 54.0 (d, C17′), 57.6 (d, C17), 65.7 (s, C25′), 69.7 (t, C26′), 70.0 (d, C7′), 71.7 (d, C12), 77.4 (d, C12′), 80.0 (d, C16), 81.3 (s, C25), 100.1 (s, C22′), 117.1 (s, C22), 128.4 (d, C15′), 148.5 (s, C2), 148.5 (s, C3′), 149.5 (C2′), 149.5 (s, C3), 185.4 (s, C14′), 210.4 (s, C16′).

21: HR-FABMS (positive; glycerol matrix) *m*/*z* 881.5641 ($C_{54}H_{77}N_2O_8$ $(M + H)^+$, Δ −3.9 mmu); ^1H NMR (pyridine-d_5 at 300 K) δ 0.75 (3H, s, H19), 0.81 (3H, s, H19′), 1.11 (1H, m, H7α), 1.18 (3H, d, *J* = 6.9 Hz, H21), 1.19 (3H, s, H26), 1.26 (1H, m, H9′), 1.27 (1H, m, H6β), 1.27 (3H, s, H18), 1.32 (3H, s, H27′), 1.36 (1H, m, H9), 1.40 (3H, s, H18′), 1.43 (3H, s, H27), 1.49 (1H, m, H6α), 1.49 (1H, m, H7β), 1.57 (3H, d, *J* = 6.2 Hz, H21′), 1.58 (1H, m, H5), 1.67 (1H, m, H24α), 1.67 (1H, m, H24′b), 1.68 (1H, m, H8), 1.68 (1H, m, H11β), 1.73 (1H, m, H6′β), 1.73 (1H, m, H15α), 1.74 (1H, m, H11′β), 1.82 (1H, m, H5′), 1.82 (1H, m, H15β), 1.95 (1H, m, H23′α), 1.99 (1H, m, H11′α), 2.02 (1H, m, H20), 2.02 (1H, m, H23α), 2.02 (1H, m, H24β), 2.04 (1H, m, H11α), 2.09 (1H, m, H14), 2.09 (1H, m, H17′), 2.12 (1H, m, H23β), 2.20 (1H, m, H6′α), 2.20 (1H, m, H23′β), 2.31 (1H, m, H20′), 2.56 (1H, m, H24′β), 2.66 (1H, d, *J* = 16.9 Hz, H1′α), 2.67 (1H, m, H4β), 2.71 (1H, d, *J* = 16.9 Hz, H1α), 2.75 (1H, m, H4′β), 2.76 (1H, m, H8′), 2.94 (1H, m, H4α), 2.98 (1H, m, H4′α), 3.11 (1H, d, *J* = 16.9 Hz, H1′β), 3.15 (1H, m, H17), 3.18 (1H, d, *J* = 16.9 Hz, H1β), 3.37 (1H, m, H12′), 3.66 (1H, m, H12), 3.83 (1H, dd, *J* = 10.0, 8.5 Hz, H26′b), 3.95 (1H, d, *J* = 11.0 Hz, H26′a), 3.97 (1H, m, H7′), 4.79 (1H, dd, *J* = 6.9, 6.9 Hz, H16), 5.23 (1H, d, *J* = 8.5 Hz, 26′OH), 5.82 (1H, d, *J* = 4.6 Hz, 12OH), 6.34 (1H, d, *J* = 5.4 Hz, 7′OH), 6.65 (1H, s, H15′); ^{13}C NMR (pyridine-d_5 at 300 K) δ 11.8 (q, C19′), 11.9 (q, C19), 12.9 (q, C18), 14.3 (q, C21), 14.7 (q, C21′), 17.7 (q, C18′), 23.6 (q, C27′), 26.2 (t, C11′), 28.8 (q, C26), 29.0 (t, C6), 30.3 (q, C27), 30.8 (t, C11), 31.8 (t, C7), 32.2 (t, C24′), 32.4 (d, C8), 32.7 (t, C15), 33.4 (t, C23), 34.7 (d, C20′), 35.6 (t, C4′), 36.0 (t, C4), 36.0 (s, C10), 36.0 (t, C23′), 36.2 (s, C10′), 37.8 (t, C24), 38.7 (t, C6′), 39.9 (d, C5′), 41.6 (d, C5), 42.0 (d, C20), 44.1 (d, C8′), 45.5 (d, C9), 46.0 (t, C1′), 46.3 (t, C1), 48.7 (s, C13), 49.4 (s, C13′), 49.4 (d, C14), 50.8 (d, C9′), 54.3 (d, C17′), 57.6 (d, C17), 69.5 (d, C7′), 69.9 (t, C26′), 71.8 (d, C12), 77.5 (d, C12′), 80.0 (d, C16), 81.4 (s, C25), 86.4 (s, C25′), 111.3 (s, C22′), 117.1 (s, C22), 128.3 (d, C15′), 148.0 (s, C2), 148.0 (s, C3′), 149.0 (C2′), 149.0 (s, C3), 185.4 (s, C14′), 209.7 (s, C16′).

$LiAlH_4$/$AlCl_3$ Reduction of Ritterazine B. To a solution of ritterazine B (1.6 mg) in dry THF (50 mL) were added $LiAlH_4$ (excess) and $AlCl_3$ (excess), and the mixture was stirred for 24 h at room temperature. The reaction was stopped by addition of 1 N HCl (1 mL), and the mixture was extracted with EtOAc (1 mL × 3). The organic layer was evaporated and separated by ODS-HPLC (UV detection at 286 nm, 60% MeCN) to furnish not only **23** (yield 0.5 mg, 31%) but also ritterazine C (yield 0.6 mg, 38%).

23: HR-FABMS (positive; glycerol matrix) *m*/*z* 901.5888 ($C_{54}H_{81}N_2O_9$, Δ −5.4 mmu); ^1H NMR (pyridine-d_5 at 300 K) δ 0.75 (3H, s, H19), 0.86 (1H, m, H7α), 0.86 (3H, s, H19′), 1.10 (1H, m, H6β), 1.17 (1H, m, H9′), 1.18 (3H, d, *J* = 7.0 Hz, H21), 1.18 (3H, s, H26), 1.24 (1H, m, H7β), 1.24 (3H, d, *J* = 67.5 Hz, H21′), 1.26 (3H, s, H18), 1.34 (1H, m, H9), 1.40 (3H, s, H27′), 1.43 (3H, s, H27), 1.48 (1H, m, H6α), 1.50 (3H, s, H18′), 1.55 (1H, m, H5), 1.67 (1H, m, H11β), 1.67 (1H, m, H23a), 1.67 (1H, m, H24α), 1.70 (1H, m, H8), 1.73 (1H, m, H6′β), 1.79 (1H, m, H15α), 1.83 (1H, m, H5′), 1.94 (2H, m, H23′), 1.96 (1H, m, H11′β), 2.01 (1H, m, H20), 2.04 (1H, m, H11α), 2.06 (1H, m, H15β), 2.08 (1H, m, H14), 2.08 (1H, m, H24β), 2.12 (1H, m, H23β), 2.19 (1H, m, H6′α), 2.19 (1H, m, H11′α), 2.32 (2H, m, H24′), 2.43 (1H, m, H8′), 2.62 (1H, m, H20′), 2.66 (1H, d, *J* = 17.0 Hz, H1′α), 2.66 (1H, m, H4β), 2.70 (1H, d, *J* = 18.0 Hz, H1α), 2.77 (1H, dd, *J* = 17.5, 11.5 Hz, H4′β), 2.92 (1H, dd, *J* = 17.5, 4.5 Hz, H4a), 2.98 (1H, dd, *J* = 17.5, 4.8 Hz, H4′α), 3.12 (1H, d, *J* = 17.0 Hz, H1′β), 3.15 (1H, m, H17), 3.17 (1H, d, *J* = 18.0 Hz, H1β), 3.64 (1H, m, H12), 3.87 (1H, d, *J* = 9.5 Hz, H26′a), 3.89 (1H, d, *J* = 9.5 Hz, H26′b), 4.04 (1H, m, H7′), 4.24 (1H, m, H22′), 4.29 (1H, m, H12′), 4.78 (1H, dd, *J* = 7.0, 6.5 Hz, H16), 5.34 (1H, s, H16′), 5.80 (1H, br s, 12OH), 6.06 (1H, s, H15′), 6.19 (1H, br s, 12′OH).

Oxidation of Ritterazine B. 1. To a solution of ritterazine B (1.0 mg) in dry CH_2Cl_2 (0.1 mL), was added PCC/Al_2O_3 complex (1.1 mg). The mixture was stirred at room temperature for 20 h. The reaction mixture was filtered and separated by ODS-HPLC (UV detection at 286 nm, MeCN) to furnish ritterazine H (yield 0.5 mg 51%) and ritterazine C (yield 0.3 mg 27%).

2. To the solution of ritterazine B (1.2 mg) in dry CH_2Cl_2 (0.1 mL) was added PCC/Al_2O_3 complex (20 mg). The mixture was stirred at room temperature for 4 h until no starting material was observed by TLC. The reaction mixture was filtered and separated by ODS-HPLC (UV detection at 286 nm, MeCN) to furnish four products. The major product was 7′-ketoritterazine H (**25**, yield 0.4 mg 34%). The other three products could not be characterized due to limited amounts of samples (<0.1 mg).

7′-Ketoritterazine H (25): HR-FABMS (positive; glycerol matrix) *m*/*z* 895.5461 ($C_{54}H_{75}N_2O_9$, Δ −1.2 mmu); ^1H NMR (pyridine-d_5 at 300 K) δ 0.78 (3H, s, H19), 1.03 (3H, s, H19′), 1.07 (3H, d, *J* = 6.5 Hz, H21), 1.11 (1H, m, H7α), 1.19 (3H, s, H26), 1.21 (3H, s, H18), 1.26 (3H, s, H27′), 1.27 (3H, d, *J* = 8.5 Hz, H21′), 1.30 (1H, m, H6β), 1.30 (3H, s, H18′), 1.34 (1H, m, H15α), 1.40 (3H, s, H27), 1.50 (1H, m, H6α), 1.51 (1H, m, H23′β), 1.55 (1H, m, H5), 1.56 (1H, m, H7β), 1.65 (1H, m, H23α), 1.67 (1H, m, H9), 1.67 (1H, m, H24α), 1.70 (1H, m, H9′), 1.73 (1H, m, H15β), 1.86 (1H, dq, *J* = 9.0, 6.5 Hz, H20),

1.86 (1H, m, H24′α), 1.99 (1H, m, H11′*β*), 1.99 (1H, m, H23*β*), 2.03 (1H, m, H8), 2.04 (1H, m, H24*β*), 2.16 (1H, m, H24′*β*), 2.19 (1H, q, *J* = 8.5 Hz, H20′), 2.21 (1H, m, H14), 2.22 (1H, m, H5′), 2.22 (1H, m, H6′α), 2.24 (1H, m, H11′α), 2.47 (1H, m, H11α), 2.49 (1H, m, H6′*β*), 2.51 (1H, m, H23′α), 2.60 (1H, dd, *J* = 13.0, 12.4 Hz, H11*β*), 2.63 (1H, d, *J* = 16.0 Hz, H1α), 2.69 (1H, m, H4*β*), 2.76 (1H, d, *J* = 18.0 Hz, H1′α), 2.77 (1H, m, H4′*β*), 2.96 (1H, m, H4α), 2.98 (1H, m, H4′α), 3.01 (1H, d, *J* = 16.0 Hz, H1*β*), 3.22 (1H, d, *J* = 18.0 Hz, H1′*β*), 3.23 (1H, d, *J* = 11.5 Hz, H8′), 3.50 (1H, dd, *J* = 9.0, 7.5 Hz, H17), 3.59 (1H, dd, *J* = 11.0, 2.0 Hz, H26′α), 4.00 (1H, d, *J* = 11.0 Hz, H26′*β*), 4.14 (1H, dd, *J* = 12.0, 6.0 Hz, H12′), 4.66 (1H, dd, *J* = 7.5, 6.5 Hz, H16), 5.10 (1H, s, 17′OH), 5.22 (1H, s, 16′), 6.76 (1H, s, H15′).

Acetylation of Ritterazine B. 1. To a stirred solution of Ac_2O (0.1 mL) was added ritterazine B (3.2 mg) in pyridine (0.2 mL). After 40 min, the reaction mixture was diluted with MeOH (0.1 mL) and separated by ODS-HPLC (UV detection at 286 nm, MeOH) to furnish 7′,12-diacetylritterazine B (**26**, yield 0.5 mg 14%) and 7′,12,12′-triacetylritterazine B (**27**, yield 1.9 mg 53%).

2. Ritterazine B (2.8 mg) was dissolved in dry toluene (0.1 mL) and pyridine (0.2 mL), and to this mixture was added Ac_2O (0.1 mL). The mixture was stirred at room temperature for 40 min. The reaction mixture was diluted with MeOH (0.1 mL) and separated by ODS-HPLC (UV detection at 286 nm, MeCN) to furnish 7′-acetylritterazine B (**28**, yield 0.5 mg 16%), 12-acetylritterazine B (**29**, yield 0.5 mg 16%), and 7′,12-diacetylritterazine B (**26**, yield 1.7 mg 55%).

7′,12-Diacetylritterazine B (26): HR-FABMS (positive; glycerol matrix) *m*/*z* 983.5978 ($C_{58}H_{83}N_2O_{11}$, Δ −1.9 mmu); ^{1}H NMR (pyridine-d_5 at 300 K) *δ* 0.72 (3H, s, H19), 0.81 (3H, s, H19′), 1.06 (1H, m, H7α), 1.12 (3H, d, *J* = 6.5 Hz, H21), 1.17 (3H, s, H18), 1.17 (3H, s, H26), 1.18 (1H, m, H9′), 1.22 (1H, m, H6*β*), 1.22 (3H, s, H27′), 1.25 (3H, d, *J* = 6.5 Hz, H21′), 1.33 (3H, s, H18′), 1.39 (3H, s, H27), 1.40 (1H, m, H9), 1.43 (1H, m, H6′*β*), 1.45 (1H, m, H6α), 1.45 (1H, m, H7*β*), 1.50 (1H, m, H11*β*), 1.51 (1H, m, H23′*β*), 1.56 (1H, m, H5), 1.63 (1H, m, H8), 1.65 (1H, m, H24α), 1.78 (1H, m, H15α), 1.81 (1H, m, H23α), 1.81 (1H, m, H24′α), 1.82 (1H, m, H5′), 1.83 (1H, m, H15*β*), 1.86 (1H, m, H11′*β*), 1.97 (1H, m, H20), 2.00 (1H, m, H11α), 2.00 (1H, m, H24*β*), 2.00 (3H, s, 7′Ac), 2.04 (1H, m, H14), 2.07 (3H, s, 12Ac), 2.08 (1H, m, H23*β*), 2.09 (1H, m, H6′α), 2.09 (1H, m, H24′*β*), 2.17 (1H, m, H11′α), 2.19 (1H, q, *J* = 6.5 Hz, H20′), 2.49 (1H, m, H17), 2.50 (1H, m, H23′α), 2.53 (1H, m, H8′), 2.65 (1H, m, H4*β*), 2.66 (1H, d, *J* = 16.5 Hz, H1′α), 2.67 (1H, d, *J* = 16.5 Hz, H1α), 2.70 (1H, m, H4′*β*), 2.92 (1H, m, H4α), 2.94 (1H, m, H4′α), 3.08 (1H, d, *J* = 16.5 Hz, H1*β*), 3.12 (1H, d, *J* = 16.5 Hz, H1′*β*), 3.60 (1H, br d, *J* = 12.0 Hz, H26′α), 3.99 (1H, d, *J* = 12.0 Hz, H26′*β*), 4.15 (1H, dd, *J* = 11.5, 4.5 Hz, H12′), 4.65 (1H, s, 12′OH), 4.77 (1H, dd, *J* = 6.5, 6.5 Hz, H16), 4.90 (1H, m, H12), 5.06 (1H, s, 17′OH), 5.12 (1H, s, H16′), 5.25 (1H, m, H7′), 5.70 (1H, s, H15′).

7′,12,12′-Triacetylritterazine B (27): HR-FABMS (positive; glycerol matrix) *m*/*z* 1025.6115 ($C_{60}H_{85}N_2O_{12}$, Δ +1.2 mmu); ^{1}H NMR (pyridine-d_5 at 300 K) *δ* 0.72 (3H, s, H19), 0.80 (3H, s, H19′), 1.07 (1H, m, H7α), 1.12 (3H, d, *J* = 6.5 Hz, H21), 1.13 (1H, m, H9′), 1.17 (3H, s, H18), 1.17 (3H, s, H26), 1.24 (1H, m, H6*β*), 1.24 (3H, s, H27′), 1.26 (3H, d, *J* = 6.5 Hz, H21′), 1.38 (3H, s, H18′), 1.39 (3H, s, H27), 1.40 (1H, m, H9), 1.41 (1H, m, H6′*β*), 1.45 (1H, m, H7*β*), 1.46 (1H, m, H6α), 1.50 (1H, m, H11*β*), 1.51 (1H, m, H23′*β*), 1.55 (1H, m, H5), 1.63 (1H, m, H8), 1.65 (1H, m, H24α), 1.76 (1H, m, H11′*β*), 1.76 (1H, m, H15α), 1.79 (1H, m, H5′), 1.81 (1H, m, H24′α), 1.83 (1H, m, H15*β*), 1.83 (1H, m, H23α), 1.96 (3H, s, 7′Ac), 1.97 (1H, m, H20), 2.00 (1H, m, H11α), 2.01 (1H, m, H24*β*), 2.04 (1H, m, H14), 2.06 (1H, m, H23*β*), 2.07 (3H, s, 12Ac), 2.08 (1H, m, H6′α), 2.09 (1H, m, H24′*β*), 2.12 (3H, s, 12′Ac), 2.22 (1H, q, *J* = 6.5 Hz, H20′), 2.26 (1H, m, H11′α), 2.49 (1H, m, H17), 2.50 (1H, m, H23′α), 2.54 (1H, m, H8′), 2.62 (1H, d, *J* = 17.0 Hz, H1′α), 2.67 (1H, d, *J* = 17.0 Hz, H1α), 2.67 (1H, m, H4*β*), 2.69 (1H, m, H4′*β*), 2.93 (1H, m, H4′α), 2.95 (1H, m, H4α), 3.07 (1H, d, *J* = 17.0 Hz, H1*β*), 3.09 (1H, d, *J* = 17.0 Hz, H1′*β*), 3.65 (1H, br d, *J* = 11.0 Hz, H26′α), 4.00 (1H, d, *J* = 11.0 Hz, H26′*β*), 4.45 (1H, s, 17′OH), 4.78 (1H, dd, *J* = 6.5, 6.5 Hz, H16), 4.90 (1H, m, H12), 4.98 (1H, s, H16′), 5.20 (1H, m, H7′), 5.55 (1H, dd, *J* = 11.5, 4.5 Hz, H12′), 5.72 (1H, s, H15′).

7′-Acetylritterazine B (28): HR-FABMS (positive; glycerol matrix) *m*/*z* 941.5954 ($C_{56}H_{81}N_2O_{10}$, Δ +6.3 mmu); ^{1}H NMR (pyridine-d_5 at 300 K) *δ* 0.76 (3H, s, H19), 0.82 (3H, s, H19′), 1.12 (1H, m, H7α), 1.17 (1H, m, H9′), 1.18 (3H, d, *J* = 6.5 Hz, H21), 1.19 (3H, s, H26), 1.22 (3H, s, H18), 1.25 (1H, m, H6*β*), 1.25 (3H, d, *J* = 7.0 Hz, H21′), 1.27 (3H, s, H27′), 1.33 (3H, s, H18′), 1.36 (1H, m, H9), 1.43 (1H, m, H6′*β*), 1.43 (3H, s, H27), 1.49 (1H, m, H6α), 1.49 (1H, m, H7*β*), 1.51 (1H, m, H23′*β*), 1.56 (1H, m, H5), 1.66 (1H, m, H23α), 1.68 (1H, m, H8), 1.69 (1H, m, H11*β*), 1.69 (1H, m, H24α), 1.79 (1H, m, H15α), 1.82 (1H, m, H5′), 1.82 (1H, m, H24′α), 1.86 (1H, m, H11′*β*), 1.86 (1H, m, H15*β*), 2.00 (3H, s, 7′Ac), 2.02 (1H, m, H24*β*), 2.03 (1H, m, H20), 2.04 (1H, m, H11α), 2.06 (1H, m, H6′α), 2.08 (1H, m, H24′*β*), 2.09 (1H, m, H14), 2.11 (1H, m, H23*β*), 2.17 (1H, m, H11′α), 2.18 (1H, q, *J* = 7.0 Hz, H20′), 2.51 (1H, m, H23′α), 2.53 (1H, m, H8′), 2.67 (1H, d, *J* = 17.0 Hz, H1′α), 2.68 (1H, m, H4*β*), 2.69 (1H, m, H4′*β*), 2.70 (1H, d, *J* = 17.0 Hz, H1α), 2.90 (1H, m, H4′α), 2.93 (1H, m, H4α), 3.12 (1H, d, *J* = 17.0 Hz, H1′*β*), 3.15 (1H, m, H17), 3.18 (1H, d, *J* = 17.0 Hz, H1*β*), 3.59 (1H, br d, *J* = 11.0 Hz, H26′α), 3.65 (1H, m, H12), 3.99 (1H, d, *J* = 11.0 Hz, H26′*β*), 4.15 (1H, dd, *J* = 11.0, 4.5 Hz, H12′), 4.66 (1H, s, 12′OH), 4.79 (1H, dd, *J* = 7.0, 6.5 Hz, H16), 5.06 (1H, s, 17′OH), 5.12 (1H, s, H16′), 5.24 (1H, ddd, *J* = 10.5, 10.0, 4.0 Hz, H7′), 5.70 (1H, s, H15′) 5.82 (1H, d, *J* = 5.0 Hz, 12OH).

12-Acetylritterazine B (29): HR-FABMS (positive; glycerol matrix) *m*/*z* 941.5952 ($C_{56}H_{81}N_2O_{10}$, Δ −1.2 mmu); ^{1}H NMR (pyridine-d_5 at 300 K) *δ* 0.72 (3H, s, H19), 0.84 (3H, s, H19′), 1.05 (1H, m, H7α), 1.12 (3H, d, *J* = 6.0 Hz, H21), 1.17 (3H, s, H18), 1.17 (3H, s, H26), 1.19 (1H, m, H9′), 1.22 (1H, m, H6*β*), 1.22 (3H, s, H27′), 1.26 (3H, d, *J* = 7.0 Hz, H21′), 1.33 (3H, s, H18′), 1.39 (1H, m, H9), 1.39 (3H, s, H27), 1.45 (1H, m, H6α), 1.45 (1H, m, H7*β*), 1.45 (1H, m, H23′*β*), 1.49 (1H, m, H11*β*), 1.56 (1H, m, H5), 1.63 (1H, m, H8), 1.65 (1H, m, H24α), 1.66 (1H, m, H23α), 1.74 (1H, m, H6′*β*), 1.76 (1H, m, H15α), 1.81 (1H, m, H15*β*), 1.86 (1H, m, H24′α), 1.87 (1H, m, H11′*β*), 1.88 (1H, m, H5′), 1.96 (1H, m, H20), 1.99 (1H, m, H11α), 2.00 (1H, m, H24*β*), 2.04 (1H, m, H14), 2.07 (3H, s, 12Ac), 2.09 (1H, m, H23*β*), 2.15 (1H, m, H24′*β*), 2.18 (1H, m, H11′α), 2.21 (1H, m, H6′α), 2.21 (1H, q, *J* = 7.0 Hz, H20′), 2.41 (1H, dd, *J* = 11.0, 10.0 Hz, H8′), 2.48 (1H, m, H17), 2.50 (1H, m, H23′α), 2.65 (1H, m, H4*β*), 2.67 (1H, d, *J* = 16.5 Hz, H1′α), 2.68 (1H, d, *J* = 15.5 Hz, H1α), 2.80 (1H, m, H4′*β*), 2.93 (1H, dd, *J* = 18.0, 4.9 Hz, H4α), 3.02 (1H, dd, *J* = 17.7, 5.2 Hz, H4′α), 3.09 (1H, d, *J* = 15.5 Hz, H1*β*), 3.15 (1H, d, *J* = 16.5 Hz, H1′*β*), 3.60 (1H, br d, *J* = 11.0 Hz, H26′α), 4.01 (1H, d, *J* = 11.0 Hz, H26′*β*), 4.07 (1H, m, H7′), 4.21 (1H, dd, *J* = 11.0, 4.5 Hz, H12′), 4.66 (1H, s, 12′OH), 4.77 (1H, dd, *J* = 7.0, 6.5 Hz, H16), 4.90 (1H, m, H12), 5.00 (1H, s, 17′OH), 5.25 (1H, s, H16′), 6.13 (1H, s, H15′).

Acknowledgment. We are grateful to Professor P. J. Scheuer of University of Hawaii for reading the manuscript. Thanks are also due to Dr. T. Nishikawa of Nagoya University for identification of the tunicate and to Dr. K. Yamada of Sagami Chemical Research Center for her help in cytotoxicity testing. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture, and Sports of Japan and by the Ciba-Geigy Foundation. A JSPS Research Fellowship for Young Scientists to S.F. is also acknowledged.

Supporting Information Available: Copies of ^{1}H NMR spectra of the new compounds, COSY, HMQC, ROESY, and HMBC spectra of **14**, and tables of ^{1}H NMR data for **2**−**14** (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO970091R