Cytotoxic Pregnane Steroids from the Formosan Soft Coral *Stereonephthya crystalliana*

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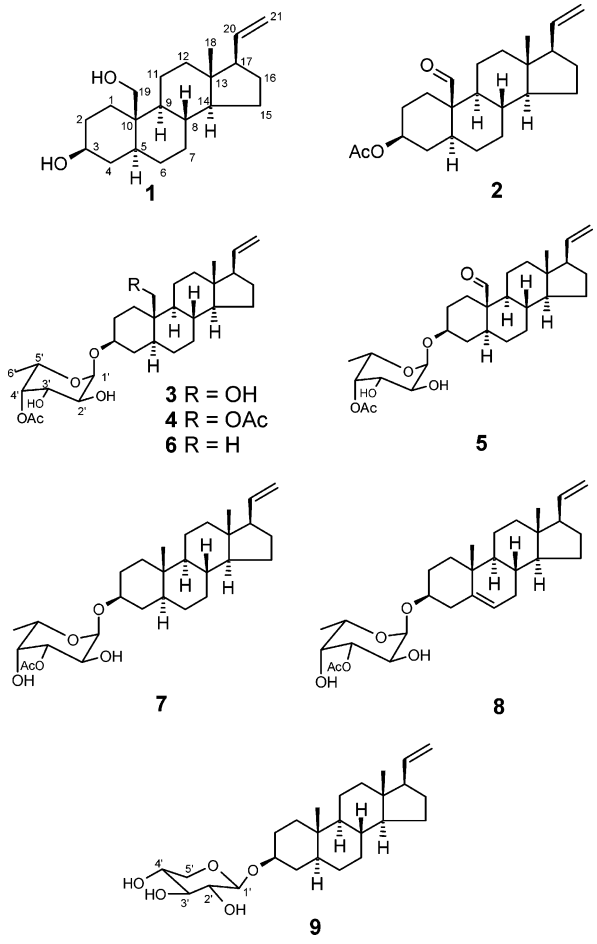
Nine new steroids, stereonsteroids A−I (**1**−**9**), were isolated from the methylene chloride solubles of the Formosan soft coral *Stereonephthya crystalliana* Kükenthal. The structures were elucidated by extensive spectroscopic analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

The family Nephtheidae has afforded bioactive terpenes and steroids.[1] As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Stereonephthya crystalliana* Kükenthal (family Nephtheidae) was studied because the CH_2Cl_2 extract showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[2,3] Bioassay-guided fractionations resulted in the isolation of nine new steroids, stereonsteroids A−I (**1**−**9**).



Results and Discussion

Compound **1** was assigned a molecular formula of $C_{21}H_{34}O_2$ as shown by HREIMS, indicating 5 degrees of unsaturation. ^{13}C NMR and DEPT spectra of **1** exhibited the presence of one methyl, 10 methylene sp^3 C atoms, six methine sp^3 C atoms, one methine sp^2 C atom, two sp^3 quaternary carbons, and one methylene sp^2 C atom, indicating **1** was tetracyclic. The ^1H and ^{13}C (including DEPT and HSQC) NMR spectra (Tables 1 and 2) implied the presence of a tertiary methyl (δ_H 0.64 s; δ_C 13.3 q), a terminal vinyl group (δ_H 4.97 d, J = 16.2 Hz, 4.98 d, J = 10.5 Hz; δ_C 139.9 d, 114.5 t), an oxygenated methine (δ_H 3.65 m; δ_C 71.1 d), and an oxygenated methylene (δ_H 3.81 d, J = 11.4 Hz, 3.95 d, J = 11.4 Hz; δ_C 61.0 t). The foregoing spectral data and a literature survey provided evidence that **1** has a 3-ol pregnane skeleton,[4] with an oxygenated methylene group. This methylene group was assigned to C-19, on the basis of the absence of a methyl singlet (δ 0.80) assignable to the C-19 angular methyl and the presence of an AB doublet at δ 3.81 (J = 11.4 Hz) and 3.95 (J = 11.4 Hz). HMBC correlations between H_2-19 and C-10, C-9, C-1, and C-5 confirmed this assignment. The relative stereochemistry of **1** was established by NOESY experiment. The NOESY correlations observed from H-20 to H_3-18, from H-14 to H-17/H-9, from H_2-19 to H-8/H-2β, and from H-5 to H-3/H-9/H-1α indicated the relative configurations for each ring junction and chiral center. On the basis of these findings, the structure of **1** was established as pregna-20-diene-3β,19-diol.[4]

Compound **2** had a molecular formula of $C_{23}H_{34}O_3$ as determined by HREIMS, indicating 6 degrees of unsaturation. The ^1H and ^{13}C NMR (including DEPT) spectra suggested the presence of a tertiary methyl (δ_H 0.53 s; δ_C 12.8 q), a terminal vinyl group (δ_H 4.96 d, J = 17.1 Hz, 4.97 d, J = 10.5 Hz; δ_C 139.5 d, 114.8 t), a secondary acetoxyl (δ_H 4.72 m, 2.00 s; δ_C 72.8 d, 170.8 s), and an aldehyde (δ_H 10.03 s; δ_C 208.3 s). Comparison of ^1H and ^{13}C NMR spectra data with those of **1** and a literature survey suggested that **2** has a 3-*O*-acetoxyl pregnane skeleton, with an aldehyde group. This aldehyde was assigned to C-19, on the basis of the absence of a methyl singlet (δ 0.80) assignable to the C-19 angular methyl. HMBC correlations between H-19 and C-10, C-9, C-1, and C-5 helped ascertain this assignment. The relative stereochemistry of **2** was deduced from a NOESY experiment. Therefore, the structure of **1** can be formulated as pregna-20-dien-3β-acetoxyl-19-al.[4]

The molecular formula of **3** proved to be $C_{29}H_{46}O_7$ from HRFABMS, DEPT, and ^{13}C NMR data. The seven degrees of unsaturation inherent in the molecular formula of **3** could be accounted for by only one carbon−carbon double bond and one ester carbonyl group. Hence, **3** possessed five rings. The ^1H NMR spectral data of **3** in $CDCl_3$ were similar to those of **1**, except that there were additional signals at δ 4.0−5.8 and at δ 2.15, suggesting the presence of an acetylated sugar moiety in the molecule. The

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Table 1. ^{1}H NMR Data of **1**−**5** (300 MHz, in $CDCl_3$)[a]

H	1	2	3	4	5
1	0.85 m	0.99 m	0.86 m	0.98 m	0.96 m
	2.28 dt (13.5, 3.3)[b]	2.43 dt (13.2, 3.3)	2.27 dt (13.2, 3.2)	2.23 m	2.42 m
2	1.40 m	1.36 m	1.39 m	1.41 m	1.40 m
	1.90 m	1.93 m	1.45 m	1.88 m	1.85 m
3	3.65 m	4.72 m	3.60 m	3.60 m	3.59 m
4	1.01 m	1.50 m	0.99 m	1.00 m	1.44 m
	1.74 m	1.80 m	1.72 m	1.73 m	1.88 m
5	1.24 m	1.45 m	1.22 m	1.32 m	1.37 m
6	1.17 m	1.54 m	1.55 m	1.33 m	1.38 m
	1.28 m	1.78 m	1.70 m	1.89 m	1.97 m
7	1.18 m	1.11 m	0.90 m	0.93 m	1.12 m
	1.26 m	1.81 m	1.76 m	2.16 m	1.93 m
8	1.53 m	1.45 m	1.54 m	1.53 m	1.70 m
9	0.74 m	0.97 m	0.72 m	0.77 m	0.98 m
11	1.57 m	1.24 m	1.56 m	1.38 m	1.31 m
	1.72 m	1.72 m	1.68 m	1.67 m	1.75 m
12	1.39 m	1.02 m	1.39 m	1.34 m	1.02 m
	1.70 m	1.68 m	1.75 m	1.76 m	2.37 m
14	1.04 m	0.98 m	1.15 m	1.12 m	0.97 m
15	1.73 m	1.20 m	1.21 m	1.21 m	1.23 m
		1.72 m	1.62 m	1.70 m	1.75 m
16	1.58 m	1.59 m	1.56 m	1.57 m	1.57 m
	1.80 m	1.82 m	1.81 m	1.84 m	1.80 m
17	1.97 m	1.97 m	1.96 m	1.97 m	1.98 m
18	0.64 s	0.53 s	0.61 s	0.58 s	0.53 s
19	3.81 d (11.4)	10.03 s	3.77 d (12.0)	4.22 d (12.3)	10.03 s
	3.95 d (11.4)		3.90 d (12.0)	4.35 d (12.3)	
20	5.78 ddd	5.73 ddd	5.74 ddd	5.74 ddd	5.74 ddd
	(16.2, 10.5, 7.8)	(17.1, 10.5, 7.8)	(16.5, 10.8, 7.8)	(17.1, 10.2, 7.6)	(17.1, 10.3, 7.8)
21	4.97 d (16.2)	4.96 d (17.1)	4.94 d (16.5)	4.96 d (17.1)	4.96 d (17.1)
	4.98 d (10.5)	4.97 d (10.5)	4.95 d (10.8)	4.97 d (10.2)	4.97 d (10.3)
1′			5.02 d (3.6)	5.04 d (3.6)	5.01 m
2′			3.74 dd (9.6, 3.6)	3.73 dd (9.6, 3.6)	3.72 dd (9.6, 3.6)
3′			3.92 dd (9.6, 3.0)	3.91 dd (9.6, 3.0)	3.88 dd (9.6, 3.0)
4′			5.16 br d (3.0)	5.21 br d (3.0)	5.20 br d (3.0)
5′			4.09 br q (6.3)	4.11 br q (6.3)	4.08 br q (6.3)
6′			1.10 d (6.3)	1.14 d (6.3)	1.13 d (6.3)
OAc		2.00 s	2.15 s	2.18 s, 2.07 s	2.17 s

[a] Assigned by COSY, HSQC, and HMBC experiments. [b] *J* values (in Hz) in parentheses.

^{13}C NMR spectral data of **3** were also similar to those of **1**, except for five additional oxymethine carbons between δ 65 and 100 and a carbonyl group at δ 171.7. A sharp signal at δ_H 2.15 (3H, s) showed that the carbonyl group was probably derived from an acetyl residue. Further, a methyl signal at 1.10 ppm (3H, d, $J = 6.3$ Hz) together with the presence of a ^{13}C NMR acetal resonance (δ 96.7, d) suggested the presence of a cyclized, acetylated 6′-deoxyhexose unit. Comparison of the ^{13}C NMR data with those of 6′-deoxyhexose acetate models showed that **3** contained an acetylated 6′-deoxyhexose ring in the pyranose form.[5,6] The ^{13}C and ^{1}H NMR spectra of **3** immediately suggested that the aglycon was **1**. By subtraction of the molecular formula of **1** from the overall formula of **3** the sugar component was shown to possess the composition $C_8H_{12}O_5$. Elimination of one acetyl residue from the formula left $C_6H_{10}O_4$, which is the formula of a typical deoxy-hexose.

The ^{1}H−^{1}H COSY of **3** revealed contiguous coupling between H-1′ and H-2′, H-2′ and H-3′, H-3′ and H-4′, and H-5′ and 5′-CH_3. A HMBC cross-peak between H-4′ (δ 5.16) and CH_3COO indicated that the acetate ester was at the sugar C-4′ position. The coupling constants of the anomeric proton (δ 5.02, $J_{H-1',\ H-2'} = 3.6$ Hz) and H-2′ (δ 3.74, $J_{H-2',\ H-3'} = 9.6$ Hz) of **3** suggested an equatorial orientation for the anomeric proton, thus confirming the α or axial hemiacetal linkage to the algycon and an axial orientation for both H-2′ (dd, 3.6, 9.6 Hz) and H-3′ (dd, 3.0, 9.6 Hz). Furthermore, H-4′ (a broad doublet with $J = 3.0$ Hz) had to be equatorial, *cis* to both H-3′ and H-5′. A NOESY correlation from H-3′ to H-5′ confirmed the 1,3-diaxial relationship of the latter protons (Figure 1). Because the anomeric oxygen is *trans* to CH_3-6′, the monosaccharide belongs to the α-series. Thus, the sugar component in the marine-derived saponin **3** was concluded to be 4′-*O*-acetyl-α-fucopyranose.[5,6] A HMBC correlation between the anomeric proton at 5.02 ppm (C-1′) and a carbon at 76.6 ppm (C-3) connected the monosaccharide to the A ring of **1** and yielded the final structure, **3**. However, the absolute stereochemistry of the fucose in **3** could not be conclusively assigned due to the limited amount of sample available for further studies.

HREIMS, DEPT, and ^{13}C NMR spectra revealed compound **4** to have a molecular formula of $C_{31}H_{48}O_8$. The ^{1}H and ^{13}C NMR spectral data of **4** resembled those of **3**, except that the primary hydroxyl at C-19 in **3** was replaced by a primary acetoxyl in **4**. HMBC correlations from H_2-19 (δ_H 4.22, 4.35) to C-10 (δ_C 37.9), C-9 (δ_C 54.6), C-1 (δ_C 32.1), C-5 (δ_C 45.0), and an acetyl group (δ_C 171.3) clearly positioned the acetoxyl at C-19. However, the absolute stereochemistry of the fucose in **4** could not be conclusively assigned due to the limited amount of sample.

Compound **5** was shown to have the molecular formula $C_{29}H_{44}O_7$ by mass spectrometry and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectral data of **5** were analogous to those of **3**, except for the replacement of the C-19 hydroxy by an aldehyde in **5**. HMBC correlations between H-19 (δ_H 10.03) and C-10 (δ_C 51.8), C-9 (δ_C 52.8), C-1 (δ_C 31.0), and C-5 (δ_C 43.4) helped position the aldehyde at C-19. The absolute configuration of the fucose sugar in **5** could not be established because of the limited amount of material.

Compound **6** analyzed for $C_{29}H_{46}O_6$ by mass spectrometry in combination with interpretation of ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectral data (Tables 3 and 2) of **6** in $CDCl_3$ were similar to those of **3** except for the absence of hydroxyl at C-19. HMBC correlations between H_3-19 (δ_H 0.82) and C-10 (δ_C 35.8), C-9 (δ_C 54.7), C-1 (δ_C 37.6), and C-5 (δ_C 34.5) confirmed this assignment. However, the absolute configuration of the fucose sugar in **6** could not be established because of the limited amount of material.

Table 2. ^{13}C NMR Spectral Data (δ) of **1**−**9**

C	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[b]
1	31.3	30.8	31.6	32.1	31.0	37.6	37.6	37.4	37.6
2	32.2	28.5	28.4	29.5	30.4	29.5	29.5	29.7	30.0
3	71.1	72.8	76.6	77.3	76.9	77.7	77.6	78.2	77.3
4	38.1	35.6	34.6	34.8	36.1	34.5	34.5	38.8	34.7
5	45.1	43.4	44.9	45.0	43.4	44.8	44.9	140.2	44.7
6	28.3	28.3	29.8	28.3	28.4	28.8	28.8	122.2	28.9
7	32.1	32.0	32.1	31.9	32.0	32.2	32.2	32.1	32.3
8	36.2	37.1	36.1	36.0	37.1	35.7	35.7	32.1	35.8
9	55.0	52.8	55.0	54.6	52.8	54.7	54.7	50.5	54.5
10	39.4	51.7	39.4	37.9	51.8	35.8	35.8	36.9	35.5
11	22.7	21.4	22.7	21.9	21.5	20.9	20.9	20.8	20.9
12	38.6	37.4	38.1	38.2	37.4	37.1	37.1	37.4	37.2
13	43.8	43.4	43.8	43.7	43.4	43.7	43.7	43.5	43.7
14	56.0	55.8	56.0	55.4	55.8	55.7	55.7	56.0	55.5
15	24.8	24.7	24.8	24.8	24.7	24.9	24.8	24.9	24.8
16	27.2	27.1	27.2	27.2	27.2	27.3	27.3	27.3	27.3
17	55.5	55.3	55.4	56.0	55.4	55.5	55.5	55.4	55.5
18	13.3	12.8	13.2	13.0	12.8	13.0	12.4	12.8	12.9
19	61.0	208.3	60.7	62.8	208.4	12.4	13.0	19.5	12.2
20	139.9	139.5	139.9	139.8	139.6	140.0	140.0	139.9	140.0
21	114.5	114.8	114.6	114.6	114.8	114.5	114.5	114.6	114.6
1′			96.7	97.2	97.3	97.0	97.2	97.5	102.9
2′			69.3	69.5	70.1	69.5	66.9	66.9	75.0
3′			69.5	70.1	69.5	70.1	74.1	74.1	78.4
4′			73.7	73.0	73.0	73.1	71.0	71.0	71.1
5′			65.2	65.3	65.4	65.2	65.8	65.9	67.0
6′			16.3	16.3	16.3	16.3	16.1	16.1	
OAc		170.8	171.7	171.3	171.3	171.4	171.1	171.1	
		21.3	20.9	171.3	20.9	20.9	21.3	21.3	
				20.9					
				21.3					

[a] Recorded in $CDCl_3$ at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments). [b] Recorded in d_5-pyridine at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments).

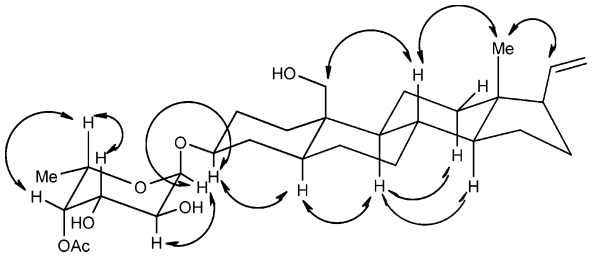

Figure 1. Selective NOESY correlations of **3**.

Compound **7** gave a molecular formula of $C_{29}H_{46}O_6$, as indicated by HREIMS and ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data of **7** in $CDCl_3$ resembled those of **6** except for some ^1H and ^{13}C NMR shift differences in the sugar portion. The sugar moiety was readily assigned to be a 3-*O*-acetyl-α-fucose by interpretation of $^1H-^1H$ COSY data together with HMBC cross-peak H-3′/CH_3COO, coupling constants of H-2′ (J = 4.2, 11.4 Hz), and NOESY correlations H-3′/H-4′, H-5′ and H-4′/H-5′. The HMBC correlation between H-1′ (δ_H 5.03) and C-3 (δ_C 77.6) secured the final structure of **7** as 3β-(3′-*O*-acetyl-α-fucopyranosyloxy)pregna-20-diene.

Compound **8** had a molecular formula of $C_{29}H_{44}O_6$ as determined by HREIMS as well as ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data of **8** in $CDCl_3$ were analogous to those of **7** except for the presence of a double bond between C-5 and C-6 in **8**. HMBC correlations from H_3-19 (δ_H 1.03) to C-10 (δ_C 36.9), C-9 (δ_C 50.5), C-1 (δ_C 37.4), and C-5 (δ_C 38.8) and from H-6 (δ_H 5.37) to C-10, C-7 (δ_C 32.1), C-5, and C-4 (δ_C 38.8) helped ascertain the positioning of the double bond. The absolute configuration of the fucose sugar in **8** could not be established because of the limited amount of material.

HREIMS, DEPT, and ^{13}C NMR spectra revealed compound **9** to have a molecular formula of $C_{26}H_{42}O_5$. The ^1H and ^{13}C NMR spectral data of **9** in $CDCl_3$ were similar to those of **7** except for the sugar portion. The sugar moiety was readily assigned to be a β-xylose[7] by interpretation of $^1H-^1H$ COSY data, coupling constants of H-2′ (t, J = 8.7 Hz), H-3′ (t, J = 8.7 Hz), H-4′ (ddd, J = 11.1, 8.7, 4.8 Hz), and H-5′ (dd, J = 11.1, 4.8 Hz), and NOESY correlations (Figure 2) H-1′/H-3′ and H-5_{ax}′ and H-4′/ H-2′ and H-5_{eq}′. The presence of a β-xylopyranose moiety in **9** was confirmed by comparing the ^{13}C NMR chemical shifts of the sugar unit in **9** with those of known sugars.[7] The HMBC correlation between H-1′ (δ_H 4.90) and C-3 (δ_C 77.3) secured the final structure of **9** as 3β-(β-xylopyranosyloxy)-5α-pregna-20-ene.

The cytotoxicity of compounds **1**−**9** is shown in Table 4. Compounds **1**−**3** and **5** showed cytotoxicity against the P-388 cell line. Compounds **2** and **3** showed cytotoxicity against the HT-29 cell line. Oxygenation at C-19 may be important for the cytotoxicity.

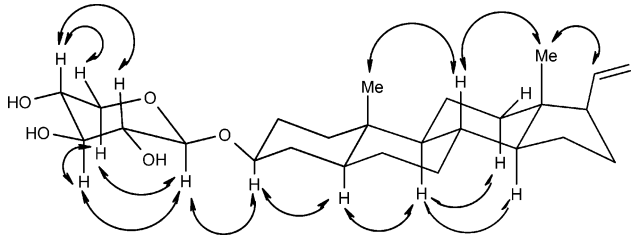

Figure 2. Selective NOESY correlations of **9**.

Table 3. ^1H NMR Data of **6**−**9** (300 MHz)

H	**6**[a]	**7**[a]	**8**[a]	**9**[b]
1	1.08 m	1.03 m	1.05 m	0.92 m
	1.74 m	1.68 m	1.72 m	1.65 m
2	1.55 m	1.52 m	1.62 m	1.68 m
	1.86 m	1.84 m	1.94 m	2.08 m
3	3.56 m	3.58 m	3.52 m	3.90 m
4	1.32 m	1.29 m	1.72 m	1.37 m
	1.68 m	1.63 m	1.89 m	1.81 m
5	1.12 m	1.14 m		0.99 m
6	1.35 m	1.30 m	5.37 d (5.1)	1.16 m
	1.84 m			
7	0.96 m	0.92 m	2.02 m	0.82 m
	1.77 m	1.73 m		1.55 m
8	1.42 m	1.36 m	1.56 m	1.21 m
9	0.68 m	0.68 m	0.96 m	0.53 m
11	1.36 m	1.28 m	1.50 m	1.15 m
	1.60 m	1.55 m	1.58 m	1.46 m
12	1.04 m	1.02 m	1.13 m	0.92 m
	1.72 m	1.76 m	1.92 m	1.61 m
14	1.04 m	1.01 m	1.03 m	0.87 m
15	1.24 m	1.06 m	1.23 m	1.18 m
	1.72 m	1.66 m	1.75 m	1.60 m
16	1.62 m	1.56 m	1.60 m	1.53 m
	1.86 m	1.78 m	1.78 m	1.64 m
17	1.95 m	1.96 m	1.96 m	1.93 m
18	0.59 s	0.59 s	0.62 s	0.45 s
19	0.82 s	0.83 s	1.03 s	0.66 s
20	5.76 ddd (16.2, 11.1, 7.8)[c]	5.76 ddd (16.8, 10.2, 8.1)	5.78 ddd (15.9, 11.4, 7.8)	5.74 ddd (15.6, 11.4, 7.8)
21	4.96 d (16.2)	4.96 d (16.8)	4.98 d (15.9)	4.98 m
	4.97 d (11.1)	4.97 d (10.2)	4.97 d (11.4)	
1′	5.04 d (3.6)	5.03 d (4.2)	5.05 d (4.2)	4.90 d (8.7)
2′	3.74 dd (10.8, 3.6)	3.91 dt (4.2, 11.4)	3.93 dt (4.2, 11.4)	4.00 t (8.7)
3′	3.92 dd (10.8, 3.0)	5.05 dd (10.5, 3.0)	5.07 dd (10.5, 3.0)	4.19 t (8.7)
4′	5.21 br d (3.0)	3.84 br s	3.85 br s	4.25 ddd (11.1, 8.7, 4.8)
5′	4.12 br q (6.3)	4.11 q (6.3)	4.12 q (6.6)	3.76 t (11.1)
				4.39 dd (11.1, 4.8)
6′	1.14 d (6.3)	1.26 d (6.3)	1.26 d (6.6)	
OAc	2.18 s	2.18 s	2.19 s	

[a] Recorded in $CDCl_3$ (assigned by COSY, HSQC, and HMBC experiments). [b] Recorded in d_5-pyridine (assigned by COSY, HSQC, and HMBC experiments). [c] J values (in Hz) in parentheses.

Table 4. Cytotoxicity[a] of **1**−**9**

compound	cell lines ED_{50} (μg/mL)	
	HT-29	P-388
1	7.5	3.5
2	1.7	1.6
3	2.7	3.1
4	5.2	4.9
5	9.3	3.8
6	10.8	9.4
7	6.2	13.3
8	5.3	5.4
9	7.7	5.9

[a] For significant activity of pure compounds, an ED_{50} of ≤4.0 μg/mL is required.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26-30 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. MS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *Stereonephthya crystalliana* was collected at Green Island, off Taiwan, in September 2001, at a depth of 4−5 m, and was stored for 1 month in a freezer until extraction. A voucher specimen, NSUGN-049 (identified by one of the authors, C.-F.D.), was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *S. crystalliana* were freeze-dried to give 1.10 kg of a solid, which was extracted with CH_2Cl_2 (3.0 L × 3). After removal of solvent in vacuo, the residue (28 g) was chromatographed over Si gel 60 using *n*-hexane and *n*-hexane−EtOAc mixtures of increasing polarity. Elution with *n*-hexane−EtOAc (9:1) gave fractions containing compound **2**, with *n*-hexane−EtOAc (1:1) gave fractions containing compounds **6**−**8**, with *n*-hexane−EtOAc (4:6) gave fractions containing compounds **1** and **5**, with *n*-hexane−EtOAc (3:7) gave fractions containing compound **4**, with *n*-hexane−EtOAc (1:9) gave fractions containing compound **3**, and with *n*-hexane−EtOAc (2:8) gave fractions containing compound **9**. Compounds **1** (5 mg) and **5** (2 mg) were further purified by Si gel column chromatography, eluting with *n*-hexane−acetone (9:1). Compounds **6** (2 mg), **7** (1 mg), and **8** (2 mg) were further purified by Si gel (immersed with 8% $AgNO_3$) column chromatography by eluting with CH_2Cl_2−acetone (19:1) as solvent system. Compound **2** (4 mg) was further purified by Si gel column chromatography eluting with CH_2Cl_2 as solvent system. Compound **3** (2 mg) was further purified by Si gel column chromatography by eluting with CH_2Cl_2−MeOH (97:3) as solvent system. Compound **4** (2 mg) was further purified by Si gel column chromatography eluting with CH_2Cl_2−MeOH (9:1) as solvent system. Compound **9** (2 mg) was further purified by Si gel (immersed with 8% $AgNO_3$) column chromatography eluting with CH_2Cl_2−MeOH (97:3) as solvent system.

Stereonsteroid A (1): $[\alpha]_D^{25}$ +18.6 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3520 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* 318 $[M]^+$ (2), 300 (5), 288 (14), 270 (9), 232 (10), 201 (12), 173 (18), 145 (28), 131 (42), 105 (36), 67 (100); HREIMS *m*/*z* 318.2553 (calcd for $C_{21}H_{34}O_2$, 318.2550).

Stereonsteroid B (2): $[\alpha]_D^{25}$ +15.4 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 1738, 1730 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* 358 $[M]^+$ (3), 330 (5), 302 (4), 269 (8), 200 (14), 148 (70), 91 (100); HREIMS *m*/*z* 358.2494 (calcd for $C_{23}H_{34}O_3$, 358.2499).

Stereonsteroid C (3): $[\alpha]_D^{25}$ −22.0 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3300, 1746 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 529.3132 (calcd for $C_{29}H_{46}O_7Na$, 529.3136).

Stereonsteroid D (4): $[\alpha]_D^{25}$ −30.3 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3460, 1730 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 571.3246 (calcd for $C_{31}H_{48}O_8Na$, 571.3241).

Stereonsteroid E (5): $[\alpha]_D^{25}$ −21.8 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3510, 1740, 1730 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 527.2983 (calcd for $C_{29}H_{44}O_7Na$, 527.2980).

Stereonsteroid F (6): $[\alpha]_D^{25}$ −30.6 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3420, 1735 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 513.3190 (calcd for $C_{29}H_{46}O_6Na$, 513.3187).

Stereonsteroid G (7): $[\alpha]_D^{25}$ −31.4 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3360, 1730 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 513.3185 (calcd for $C_{29}H_{46}O_6Na$, 513.3187).

Stereonsteroid H (8): $[\alpha]_D^{25}$ −41.5 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3450, 1740 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 511.3034 (calcd for $C_{29}H_{44}O_6Na$, 511.3031).

Stereonsteroid I (9): $[\alpha]_D^{25}$ −52.6 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3480 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 457.2922 (calcd for $C_{26}H_{42}O_5Na$, 457.2926).

Cytotoxicity Testing. P-388 cells were kindly supplied by J. M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[3]

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References and Notes

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2005**, *22*, 15−61, and references therein.
(2) Geran, R I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1−91.
(3) Hou, R.-S.; Duh, C.-Y.; Chiang, M. Y.; Lin, C.-N. *J. Nat. Prod.* **1995**, *58*, 1126−1130.
(4) Schow, S. R.; McMorris, T. C. *Steroids* **1977**, *30*, 389−392.
(5) Corgiat, J. M.; Scheuer, P. J.; Rios Steiner, J. L.; Clardy, J. *Tetrahedron* **1993**, *49*, 1557−1561.
(6) Reuben, J. *J. Am. Chem. Soc.* **1984**, *106*, 6180−6186.
(7) Seo, S.; Tomit, Y.; Tori, K.; Yoshimra, Y. *J. Am. Chem. Soc.* **1978**, *100*, 3331−3339.

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