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

Shang-Kwei Wang,[†] Chang-Feng Dai,[§] and Chang-Yih Duh*[,⊥]

*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan, Republic of China, Department of Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China, and Institute of Oceanography, National Taiwan University, Taipei, Taiwan, Republic of China*

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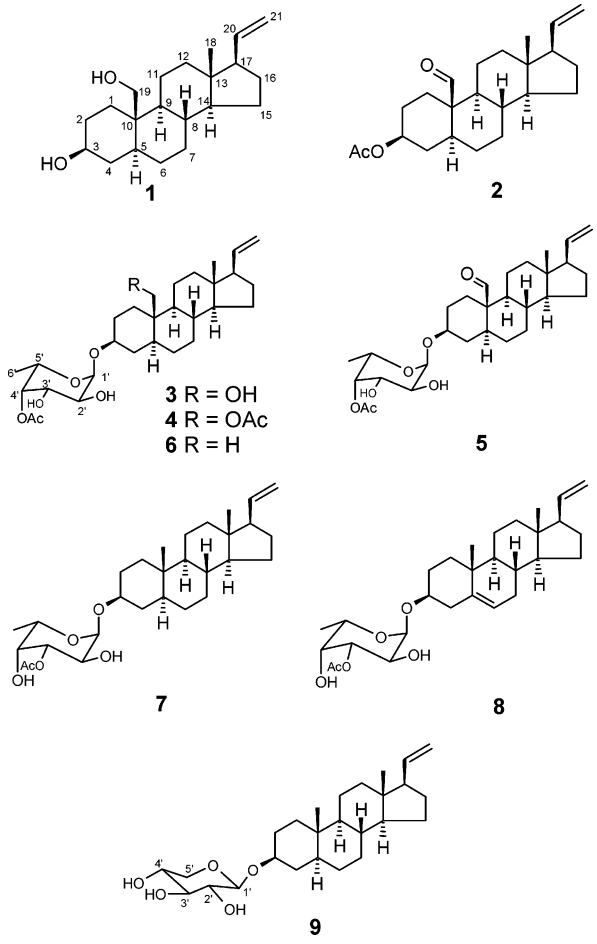
Nine new steroids, stereonsteroids A−I (**1**−**9**), were isolated from the methylene chloride solubles of the Formosan soft coral *Stereonephthya crystalliana* Kükenthal. The structures were elucidated by extensive spectroscopic analysis, and their cytotoxicity against selected cancer cells was measured 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 ($\delta_H$ 0.64 s; $\delta_C$ 13.3 q), a terminal vinyl group ($\delta_H$ 4.97 d, $J$ = 16.2 Hz, 4.98 d, $J$ = 10.5 Hz; $\delta_C$ 139.9 d, 114.5 t), an oxygenated methine ($\delta_H$ 3.65 m; $\delta_C$ 71.1 d), and an oxygenated methylene ($\delta_H$ 3.81 d, $J$ = 11.4 Hz, 3.95 d, $J$ = 11.4 Hz; $\delta_C$ 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 ($\delta$ 0.80) assignable to the C-19 angular methyl and the presence of an AB doublet at $\delta$ 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-2$\beta$, and from H-5 to H-3/H-9/H-1$\alpha$ 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-3$\beta$,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 ($\delta_H$ 0.53 s; $\delta_C$ 12.8 q), a terminal vinyl group ($\delta_H$ 4.96 d, $J$ = 17.1 Hz, 4.97 d, $J$ = 10.5 Hz; $\delta_C$ 139.5 d, 114.8 t), a secondary acetoxyl ($\delta_H$ 4.72 m, 2.00 s; $\delta_C$ 72.8 d, 170.8 s), and an aldehyde ($\delta_H$ 10.03 s; $\delta_C$ 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 ($\delta$ 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-3$\beta$-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 $\delta$ 4.0−5.8 and at $\delta$ 2.15, suggesting the presence of an acetylated sugar moiety in the molecule. The

* To whom correspondence should be addressed. Tel: 886-7-525-2000, ext. 5036. Fax: 886-7-525-5020. E-mail: yihduh@mail.nsysu.edu.tw.
[†] Kaohsiung Medical University.
[§] National Taiwan University.
[⊥] National Sun Yat-sen University.

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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 $\delta$ 65 and 100 and a carbonyl group at $\delta$ 171.7. A sharp signal at $\delta_H$ 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 ($\delta$ 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′ ($\delta$ 5.16) and $CH_3COO$ indicated that the acetate ester was at the sugar C-4′ position. The coupling constants of the anomeric proton ($\delta$ 5.02, $J_{H-1',\ H-2'} = 3.6$ Hz) and H-2′ ($\delta$ 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 ($\delta_H$ 4.22, 4.35) to C-10 ($\delta_C$ 37.9), C-9 ($\delta_C$ 54.6), C-1 ($\delta_C$ 32.1), C-5 ($\delta_C$ 45.0), and an acetyl group ($\delta_C$ 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 ($\delta_H$ 10.03) and C-10 ($\delta_C$ 51.8), C-9 ($\delta_C$ 52.8), C-1 ($\delta_C$ 31.0), and C-5 ($\delta_C$ 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 ($\delta_H$ 0.82) and C-10 ($\delta_C$ 35.8), C-9 ($\delta_C$ 54.7), C-1 ($\delta_C$ 37.6), and C-5 ($\delta_C$ 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 ($\delta$) 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).

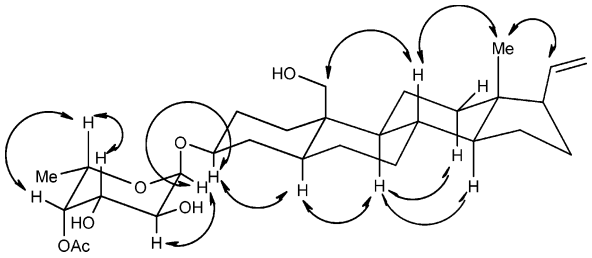


**Figure 1.** Selective NOESY correlations of **3**.

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′ ($\delta_H$ 5.03) and C-3 ($\delta_C$ 77.6) secured the final structure of **7** as 3$\beta$-(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 ($\delta_H$ 1.03) to C-10 ($\delta_C$ 36.9), C-9 ($\delta_C$ 50.5), C-1 ($\delta_C$ 37.4), and C-5 ($\delta_C$ 38.8) and from H-6 ($\delta_H$ 5.37) to C-10, C-7 ($\delta_C$ 32.1), C-5, and C-4 ($\delta_C$ 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 $\beta$-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 $\beta$-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′ ($\delta_H$ 4.90) and C-3 ($\delta_C$ 77.3) secured the final structure of **9** as 3$\beta$-($\beta$-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.

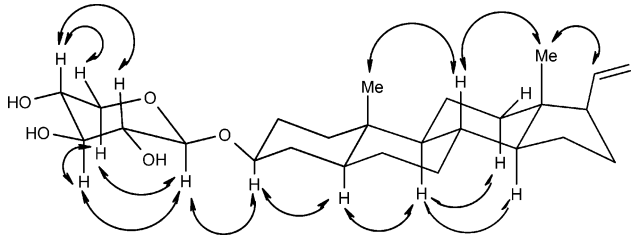


**Figure 2.** Selective NOESY correlations of **9**.

**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 $ED_{50}$ ($\mu$g/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 $\mu$g/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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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) $\nu_{max}$ 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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