Chemistry of Renieramycins. Part 5.[1] Structure Elucidation of Renieramycin-Type Derivatives O, Q, R, and S from Thai Marine Sponge *Xestospongia* Species Pretreated with Potassium Cyanide[†]

Surattana Amnuoypol,[‡] Khanit Suwanborirux,*,[‡] Sunibhond Pummangura,[‡] Akinori Kubo,[§] Chieko Tanaka,[§] and Naoki Saito*,[§]

Bioactive Marine Natural Products Chemistry Research Unit (BMNCU), Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand, and Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

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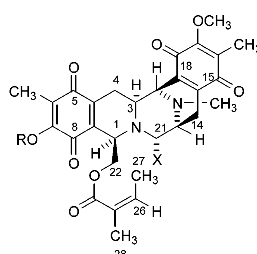
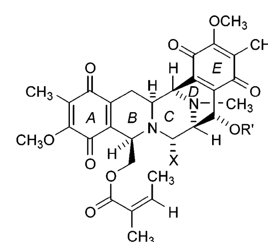
Four minor renieramycin-type derivatives, including renieramycins O (**1o**) and Q−S (**1q**−**s**), were isolated from the sponge *Xestospongia* sp. pretreated with potassium cyanide. Their structures were elucidated by comparison of spectral data with those of recently reported renieramycins M (**1m**) and N (**1n**). The results of transformation and cytotoxicity measurements are also described.

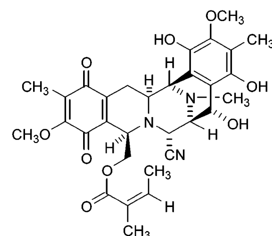
The isoquinolinequinones and their reduced forms, in particular the ecteinascidins, constitute an important class of metabolites that exhibit significant cytotoxicity.[2] These metabolites have been isolated predominantly from marine organisms and Actinomycetes; however, the isolated marine natural products are available only in very minute quantities. We have recently reported the isolation and structure elucidation of renieramycins M (**1m**) and N (**1n**) from a Thai sponge, *Xestospongia* sp., which was pretreated with potassium cyanide.[1a] We have shown that this strategy enables a gram-scale supply of novel more stable natural product derivatives from marine sources. In our continuing studies of the bioactive substances from Thai marine organisms, we further investigated the extracts of *Xestospongia* sp. pretreated with potassium cyanide and were able to obtain four new derivatives of minor compounds named renieramycins O (**1o**), Q (**1q**), R (**1r**), and S (**1s**) in 0.27%, 0.11%, 0.62%, and 0.09% yield, respectively, based on the weight of the ethyl acetate extract. In this paper, we describe their structure elucidation along with some of the transformation procedures for the preparation of their derivatives.



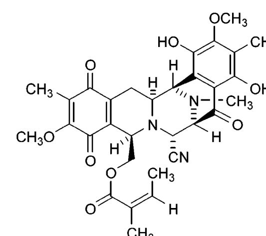
renieramycins
E (**1e**): R = CH_3, X = OH
M (**1m**): R = CH_3, X = CN
S (**1s**): R = H, X = CN



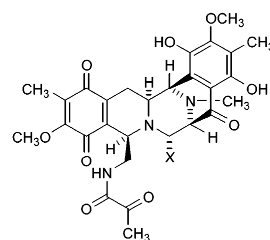
renieramycins
A (**1a**): R' = X = H
F (**1f**): R' = CH_3, X = OH
O (**1o**): R' = H, X = CN
R (**1r**): R' = CH_3, X = CN



renieramycin N (**1n**)



renieramycin Q (**1q**)



saframycins
D (**2d**): X = H
F (**2f**): X = CN

Results and Discussion

Renieramycin O (**1o**) was obtained as a dark yellow amorphous solid. Its structure was elucidated predominantly by interpretation of NMR and MS data and comparison with the spectral data of **1m**. High-resolution MS of **1o** revealed the molecular formula of $C_{31}H_{33}N_3O_9$. Therefore, **1o** might be an oxygenated analogue of **1m**. All protons and carbons were assigned by extensive NMR measurements (including COSY, NOESY, and HMBC techniques). The methylene proton signals of C-14 present in the ^{1}H NMR spectrum of **1m** were not present in that of **1o**. Instead, a new methine proton signal at δ 4.37 ppm appeared as a singlet along with one D_2O exchangeable proton at δ 3.45 ppm. In addition, the methylene carbon signal at δ 21.3 ppm of C-14 in the ^{13}C NMR spectrum of **1m** was not present in that of **1o**. A new methine carbon signal at δ 62.0 ppm was present. Treatment of **1o** with acetic anhydride in pyridine afforded the acetate (**3**) in 52% yield, the ^{1}H NMR spectrum of which showed a downfield shift of the signal for the H-14 proton to δ 5.43 ppm. This compound was also obtained as a minor product (6%) by treating **1n** with acetic anhydride in pyridine, along with the triacetate (**4**; 59%) (Scheme 1). Thus, the structure of **1o** was considered to be 14-hydroxyrenieramycin M.[1,3]

† Dedicated to the late Dr. D. John Faulkner (Scripps) for his pioneering work in the field of renieramycin chemistry.

* To whom correspondence should be addressed. (K.S.) Fax: +662-254-5195. E-mail: skhanit@chula.ac.th. (N.S.) Fax: +81-424-95-8792. E-mail: naoki@my-pharm.ac.jp.
‡ Chulalongkorn University.
§ Meiji Pharmaceutical University.

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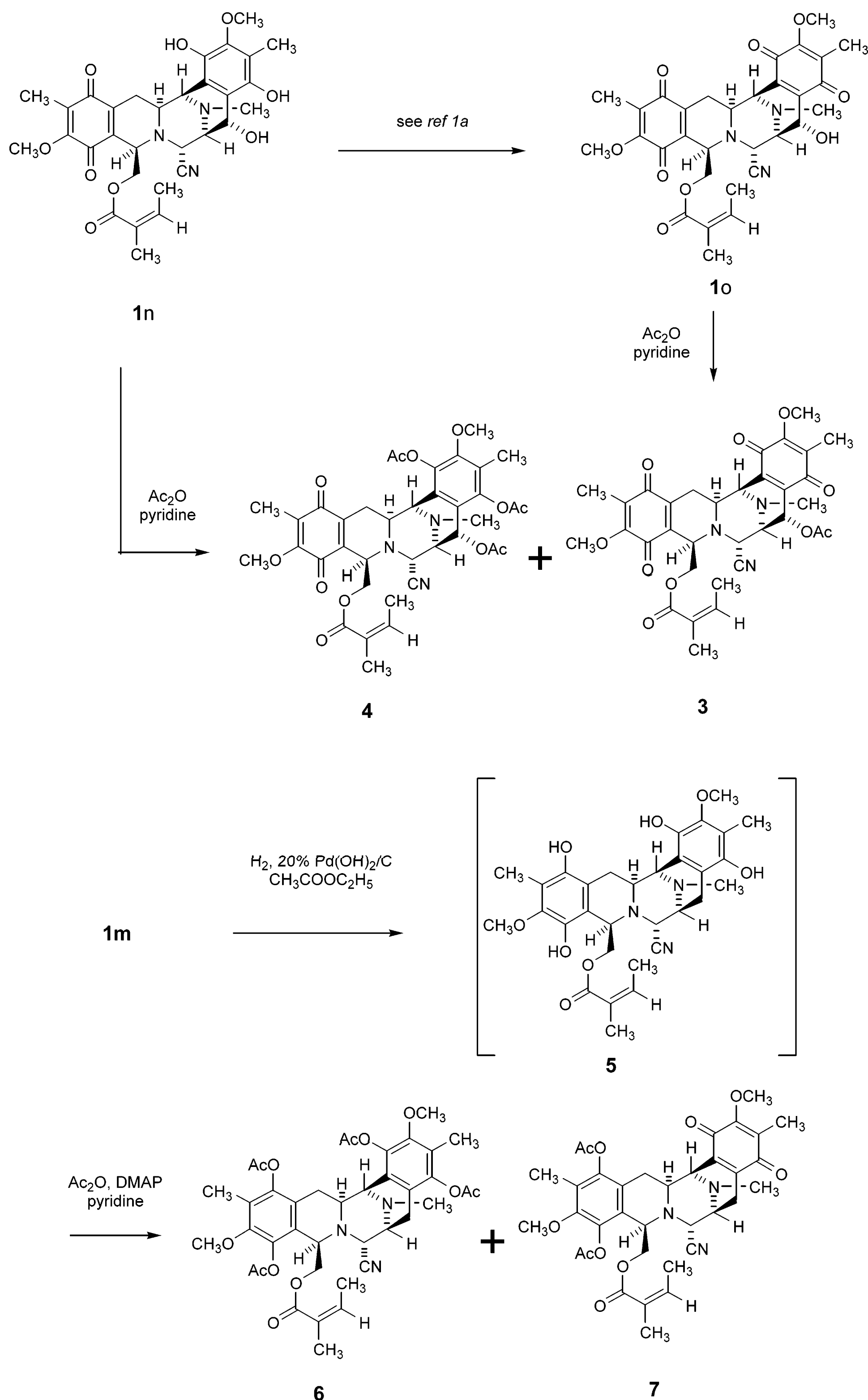
Scheme 1



Renieramycin Q (**1q**) was obtained as a pale yellow amorphous solid. The molecular formula of **1q** was determined to be $C_{31}H_{33}N_3O_9$ on the basis of high-resolution MS. The extinction coefficient at maximum UV absorption of **1q** was half that of bisquinone-type renieramycins. The characteristic D_2O exchangeable protons at δ 11.40 and 5.51 ppm, two carbonyl resonances (δ 185.7 and 180.7 ppm) indicative of a quinone ring, and an unsaturated ketone carbonyl resonance (δ 198.4 ppm) were observed. These data revealed that one of the quinone rings might have been reduced to form a hydroquinone with the peri-carbonyl function, similar to saframycins D (**2d**) and F (**2f**).[4] In addition, the ester carbon signal at δ 166.6 ppm indicated that renieramycin Q (**1q**) had an angelic acid ester side chain in place of the pyruvamide side chain of saframycin F (**2f**).

Renieramycin R (**1r**) was obtained as a pale yellow amorphous solid. Its structure was deduced by comparison of spectral data with those of renieramycins M (**1m**) and O (**1o**). High-resolution MS of **1r** revealed the molecular

Table 1. ^{1}H and ^{13}C NMR Assignment for Renieramycin S (**1s**) in $CDCl_3$

atom no.	^{13}C NMR δ			^{1}H NMR δ	(multi., integral, J in Hz)	HMBC correlations from C no.	NOE correlations
1	56.0	CH		4.03	(ddd, 1H, 3.1, 2.9)	21-H, 7-H	22-H, 22-H
3	54.2	CH		3.12	(d, 1H, 11.3, 3.1, 2.2)	7-H, 9-H, 15-H	4-Hα. 11-H
4	26.0	CH_2	α	2.92	(dd, 1H, 17.7, 2.2)		4-Hβ, 3-H, 11-H
			β	1.40	(ddd, 1H, 17.7, 11.3, 2.8)		4-Hα
5	184.8	C				4-Hα, 6-CH_3	
6	117.2	C				6-CH_3	
7	151.1	C				6-CH_3	
8	180.9	C					
9	133.5	C				22-H, 4-H_2	
10	144.1	C				4-Hα, 4-Hβ	
11	54.2	CH		3.99	(dd, 1H, 3.1, 1.0)	13-H, 4-Hβ, N-CH_3	4-Hα, 3-H, N-CH_3
13	54.5	CH		3.38	(ddd, 1H, 7.7, 2.5, 1.0)	11-H, 21-H, 14-H_2, N-CH_3	14-Hα, 14-Hβ, 21-H
14	21.0	CH_2	α	2.74	(dd, 1H, 21.1, 7.7)		13-H, 14-Hβ
			β	2.31	(d, 1H, 21.1)		14-Hα, 21-H
15	185.8	C				14-H_2, 16-CH_3	
16	128.6	C				16-CH_3	
17	155.2	C				16-CH_3, 17-OCH_3	
18	182.5	C				11-H	
19	135.0	C				11-H, 3-H, 14-H_2	
20	142.1	C				11-H, 13-H, 14-H_2	
21	58.3	CH		4.09	(d, 1H, 2.5)	1-H, 14-H_2	13-H, 14-Hβ
22	61.4	CH_2		4.04	(dd, 1H, 11.9, 2.9)	1-H	22-H, 1-H
				4.65	(dd, 1H, 11.9, 3.1)	9-H	22-H, 1-H
24	166.5	C				22-H, 25-CH_3	
25	126.2	C				25-CH_3, 26-CH_3	
26	140.8	CH		5.96	(qq, 1H, 7.4, 1.5)	25-CH_3, 26-CH_3	25-CH_3, 26-CH_3
27	15.8	CH_3		1.82	(dq, 3H, 7.4, 1.4)		26-H, 25-CH_3
28	20.4	CH_3		1.57	(dq, 3H, 1.5, 1.4)		26-H, 26-CH_3
6-CH_3	8.1	CH_3		1.94	(s, 3H)		
16-CH_3	8.5	CH_3		1.90	(s, 3H)		
17OCH_3	60.9	CH_3		3.98	(s, 3H)		
NCH_3	41.5	CH_3		2.27	(s, 3H)	11-H, 13-H	11-H, 13-H, 5-Hα
CN	116.8	C			7-H		

composition of $C_{32}H_{35}N_3O_9$. The ^{1}H NMR spectrum contained a methoxyl signal at δ 3.54 ppm, and the absence of coupling between the signals at δ 3.84 ppm and a singlet at δ 3.44 ppm required a dihedral angle of 80−90°. These data, in addition to the almost identical chemical shifts and coupling constants of the remaining signals as well as characteristic carbon signals including C-14 (δ 70.0 ppm, s) and OCH_3 (δ 59.5 ppm, q), enabled the structure of **1r** to be determined as 14α-methoxyrenieramycin M.[5] According to our procedure for the introduction of a methoxyl group at C-14 using selenium oxide-assisted oxidation,[6] treatment of **1m** with selenium oxide (10 equiv) in methanol at 80 °C provided **1r** in 35.1% yield, which was identical to the isolated compound in all respects.[7]

Renieramycin S (**1s**) was obtained as pale yellow needles (mp 179−180 °C). The molecular formula of **1s** was determined to be $C_{30}H_{31}N_3O_8$ by EIMS and elemental analysis, and it was 14 mass units less than that of **1m**. Comparison of spectral data with those of **1m** and the fact that **1m** was obtained after treating **1s** with CH_2N_2 led to the conclusion that **1s** was lacking one of the methyl groups of the vinylogous esters of renieramycin M. However, there are two possible orientations of the methyl ester substituents at C-7 and C-17. Thus, all protons and carbons of **1s** were assigned after extensive NMR measurements using COSY, NOESY, HMQC, and HMBC techniques (Table 1). Selected HMBC correlation data are shown in Figure 1. Thus, the structure of **1s** was confirmed to be 7-demethylrenieramycin M.

Finally, we were very interested in the preparation of the hydroquinone tetraacetate (**6**) from **1m** (Scheme 1) because Martinez and Corey have recently reported that a structural analogue of ecteinascidin 743, named phthalascidin, exhibits antitumor activity.[8] In addition,

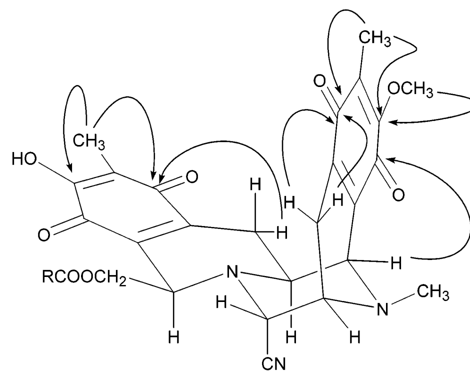


Figure 1.

Myers and co-workers have reported that a designed analogue, QAD, which has been modified by replacement of the pyruvamide side chain of saframycin A with a quinaldic acid amide and by transformation of its two quinone rings into hydroquinone methyl ethers, becomes a potent antitumor compound.[9] Hydrogenation of **1m** with 20% $Pd(OH)_2/C$ in ethyl acetate for 1 h gave the leuco compound (**5**) in quantitative yield. Acetylation of **5** with acetic anhydride and DMAP in dry pyridine gave the tetraacetyl derivative (**6**) and the diacetyl derivative (**7**)[10] in 74% and 11% yields, respectively.[11]

Cytotoxicity against two cell lines of the new renieramycins and their derivatives is summarized in Table 2. The newly isolated compounds showed similar activity to **1m** and **1n**. The presence of the oxygen-containing functionalities (OH, OCH_3, and OAc) at position 14 was much less active; however, it is quite interesting that the acetylated bishydroquinone and monohydroquinone derivatives are more active than the newly isolated renieramycins.

Table 2. Cytotoxicity of Renieramycins and Related Acetates against Several Cancer Cell Lines

	cell line[a]	
	HCT116	QG56
renieramycin O (**1o**)	0.028	0.040
renieramycin Q (**1q**)	0.059	0.071
renieramycin R (**1r**)	0.023	0.029
renieramycin S (**1s**)	0.015	0.026
compound **3**	0.12	0.30
compound **4**	0.63	1.60
compound **6**	0.003	0.0079
compound **7**	0.0019	0.0039
renieramycin M (**1m**)	0.0079	0.019
renieramycin N (**1n**)	0.0056	0.011

[a] HCT116 = human colon carcinoma; QG56 = human lung carcinoma; values reported are IC_{50} in μM.

In conclusion, we have succeeded in isolating four minor renieramycin derivatives from the Thai sponge *Xestospongia* sp. which was pretreated with potassium cyanide. Detailed studies of the biological activities of renieramycin analogues and the preparation of additional derivatives, which are necessary for the clarification of the mechanism of action for the evaluation of antitumor activity, are under way.

Experimental Section

General Experimental Procedure. All melting points were determined using a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were measured on a Horiba-SEPA. CD was obtained on a JASCO J-720WI. IR spectra were obtained on a Hitachi 260-10 spectrophotometer. UV spectra were determined with a Hitachi 340 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.65 MHz, respectively, on a JEOL-JNM-LA 500 FT-NMR spectrometer and at 270 and 67.5 MHz, respectively, on a JEOL-JNM-EX 270 spectrometer (ppm, J in Hz with TMS as internal standard). Mass spectra were recorded on JMS-DX 302 and JMS-700 instruments with a direct inlet system operating at 70 eV. Elemental analyses were conducted on Perkin-Elmer model 240B and Yanaco MT-6 CHN CORDER elemental analyzers.

Extraction and Initial Separation of *Xestospongia* sp. Pretreated with KCN. For details of the 2001 recollection (8.4 kg, wet weight) of the Thai blue marine sponge *Xestospongia* sp., see ref 1a. Fractions from the original (16.2 g) ethyl acetate-soluble fraction prepared from this re-collection were further investigated.

Isolation of Renieramycins O (1o) and Q−S (1q−s). After removing crystals of renieramycins M and N from the fractions, the combined mother liquor was concentrated in vacuo to give a residue (1.124 g), which was subjected to flash silica gel column chromatography to give four renieramycin-type compounds. These products were finally purified by preparative silica gel TLC using the appropriate solvent systems.

Renieramycin O (1o): 43.5 mg, dark yellow amorphous solid; $[\alpha]^{27}_D$ −134.4 (c 0.7, $CHCl_3$). All the spectroscopic data were identical with those of an authentic sample that was easily prepared from **1n** by air oxidation.[1a]

Renieramycin Q (1q): 18.5 mg, pale yellow amorphous solid, $[\alpha]^{18}_D$ −69.8 (c 0.1, $CHCl_3$); CD $\Delta\epsilon$ nm (c 92 μM, methanol, 22 °C) +3.3 (378), −4.0 (291), +2.5 (261), −1.6 (244), +8.2 (220); IR ($CHCl_3$) 3510, 2940, 2220w, 1710, 1680, 1660, 1640, 1620 cm^{-1}; UV λ_{max} (log ϵ) 224 (4.02), 276 (4.07), 374 (3.61) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.40 (1H, br s, 15-OH), 5.81 (1H, qq, J = 7.3, 1.7 Hz, 26-H), 5.51 (1H, br s, 18-OH), 4.31 (1H, d, J = 1.0 Hz, 21-H), 4.28 (1H, dd, J = 3.1, 0.5 Hz, 11-H), 4.09 (1H, dd, J = 11.6, 3.4 Hz, 22-H), 4.02 (1H, dd, J = 11.6, 2.5 Hz, 22-H), 3.95 (1H, dt, J = 3.4, 2.5 Hz, 1-H), 3.95 (3H, s, 7-OCH_3), 3.79 (3H, s, OCH_3), 3.37 (1H, dd, J = 1.0, 0.5 Hz, 13-H), 3.23 (1H, ddd, J = 11.0, 3.1, 2.5 Hz, 3-H), 3.03 (1H, dd, J = 17.7, 2.5 Hz, 4-Hα), 2.38 (3H, s, NCH_3), 2.09 (3H, s, 16-CH_3), 1.85 (3H, s, 6-CH_3), 1.68 (3H, dq, J = 7.3, 1.6 Hz, 27-H), 1.54 (3H, dq, J = 1.6, 1.3 Hz, 28-H), 1.54 (1H, ddd, J = 17.7, 11.0, 2.5 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.4 (C-14), 185.7 (C-5), 180.7 (C-8), 166.6 (C-24), 156.0 (C-7), 155.0 (C-15), 153.0 (C-17), 141.4 (C-10), 139.6 (C-26), 139.3 (C-18), 135.6 (C-9), 128.0 (C-6), 126.5 (C-25), 118.4 (C-16), 117.7 (C-19), 115.9 (21-CN), 112.2 (C-20), 66.2 (C-13), 62.0 (C-22), 61.1 (7-OCH_3), 61.0 (17-OCH_3), 56.5 (C-11), 56.0 (C-21), 54.8 (C-1), 53.8 (C-3), 42.4 (NCH_3), 24.1 (C-4), 19.8 (C-28), 15.4 (C-27), 8.8 (16-CH_3), 8.6 (6-CH_3); EIMS m/z (%) 591 (M^+, 1), 480 (4), 478 (7), 476 (6), 451 (8), 359 (13), 315 (25), 236 (100), 235 (74), 220 (19), 217 (15), 204 (27), 83 (25), 55 (19); HR-EIMS m/z 591.2222 [M^+] (calcd for $C_{31}H_{33}N_3O_9$, 591.2217).

Renieramycin R (1r): 101.2 mg, pale yellow amorphous solid; $[\alpha]^{18}_D$ −17.6 (c 0.1, $CHCl_3$); CD $\Delta\epsilon$ nm (c 88 μM, methanol, 22 °C) −0.7 (346), −0.2 (315), −2.7 (275), +10.7 (214); IR ($CHCl_3$) 2930, 2830, 2220w, 1715, 1660, 1650, 1620 cm^{-1}; UV λ_{max} (log ϵ) 269 (4.62), 370 (3.12) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.97 (1H, qq, J = 7.3, 1.5 Hz, 26-H), 4.34 (1H, dd, J = 11.6, 3.4 Hz, 22-H), 4.17 (1H, d, J = 2.4 Hz, 21-H), 4.13 (1H, dd, J = 11.6, 3.4 Hz, 22-H), 4.08 (1H, dd, J = 3.1, 1.0 Hz, 11-H), 4.01 (3H, s, 7-OCH_3), 4.01 (1H, br s, 1-H), 3.97 (3H, s, 17-OCH_3), 3.84 (1H, s, 14-Hβ), 3.54 (3H, s, 14-OCH_3), 3.44 (1H, br d, 13-H), 3.02 (1H, ddd, J = 11.6, 3.1, 2.8 Hz, 3-H), 2.86 (1H, dd, J = 17.1, 2.5 Hz, 4-Hα), 2.48 (3H, s, NCH_3), 1.92 (3H, s, 16-CH_3), 1.92 (3H, s, 6-CH_3), 1.83 (3H, dq, J = 7.3, 1.6 Hz, 27-H), 1.59 (3H, dq, J = 1.6, 1.5 Hz, 28-H), 1.26 (1H, ddd, J = 17.1, 11.6, 2.5 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.3 (C-5), 185.1 (C-15), 182.9 (C-18), 180.8 (C-8), 166.7 (C-24), 155.8 (C-7), 154.9 (C-17), 141.2 (C-10), 140.8 (C-20), 140.3 (C-26), 135.6 (C-9), 135.2 (C-19), 129.8 (C-6), 128.3 (C-16), 126.3 (C-25), 116.5 (CN), 70.0 (C-14), 62.8 (C-22), 61.0 (7-OCH_3), 60.9 (17-OCH_3), 60.0 (C-13), 59.5 (14-OCH_3), 56.6 (C-21), 56.3 (C-1), 54.5 (C-11), 53.4 (C-3), 42.7 (NCH_3), 25.1 (C-4), 20.3 (C-28), 15.7 (C-27), 8.7 (16-CH_3), 8.7 (6-CH_3); EIMS m/z (%) 605 (M^+, 3), 577 (5), 462 (6), 460 (7), 290 (8), 275 (8), 260 (8), 243 (30), 221 (19), 220 (100), 219 (23), 218 (63), 205 (10), 204 (12), 83 (9), 55 (7); HR-EIMS m/z 605.2375 [M^+] (calcd for $C_{32}H_{35}N_3O_9$, 605.2373).

Renieramycin S (1s): 15.0 mg, pale yellow needles from ethyl acetate ether, mp 179−180 °C; $[\alpha]^{20}_D$ −38.8 (c 0.1, $CHCl_3$); CD $\Delta\epsilon$ nm (c 92 μM, methanol, 22 °C) −2.8 (359), −1.8 (309), −7.8 (282), +7.6 (261), −3.2 (230), +1.6 (212), −2.0 (202); IR (KBr) 3280, 2950, 2220w, 1720, 1660, 1645, 1630 cm^{-1}; UV λ_{max} (log ϵ) 269 (4.62), 370 (3.12) nm; ^{1}H NMR and ^{13}C NMR spectral data: see Table 1; EIMS m/z (%) 561 (M^+, 5), 449 (20), 448 (74), 446 (13), 423 (10), 260 (29), 230 (16), 229 (100), 221 (28), 220 (71), 219 (11), 218 (21), 205 (14), 204 (11), 203 (21), 100 (77), 85 (19), 83 (25), 55 (41), 54 (10), 53 (10); HR-EIMS m/z 561.2112 [M^+] (calcd for $C_{30}H_{31}N_3O_8$, 561.2111).

Transformation of Renieramycin M (1m) into Renieramycin R (1r). A solution of renieramycin M (**1m**, 58.7 mg, 0.10 mmol) and selenium oxide (116.3 mg, 1.05 mmol) in methanol (10 mL) was heated at 80 °C for 160 h. The reaction mixture was diluted with water (20 mL), made alkaline with 5% $NaHCO_3$, and extracted with chloroform (20 mL × 3). The combined extracts were washed with water (20 mL), dried, and concentrated in vacuo to give a residue (65.0 mg), the purification of which by silica gel flash column chromatography (hexane/ethyl acetate, 2:1) afforded renieramycin R (**1r**, 21.7 mg, 35.1%) as a yellow solid, which gave spectral data (^{1}H NMR and ^{13}C NMR, IR) that were in complete agreement with those of the isolated derivative described above.

Methylation of Renieramycin S (1s). Ethereal diazomethane solution (0.4 mL) was added dropwise to a cooled solution of renieramycin S (**1s**, 2.8 mg, 0.005 mmol) in THF (0.5 mL), and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was diluted with water (10 mL) and extracted with chloroform (10 mL × 3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give a residue (3.0 mg), chromatography of which on preparative layer silica gel plates (Merck 5715, hexane/ethyl

acetate, 3:2) afforded renieramycin M (**1m**, 0.9 mg, 31.3%) and **1s** (0.3 mg, 10.7% recovery).

Acetylation of Renieramycin O (1o). Acetic anhydride (0.2 mL) was added to a solution of renieramycin O (**1o**, 13.6 mg, 0.023 mmol) in pyridine (0.5 mL), and the reaction mixture was allowed to stand for 1 h at room temperature. The reaction mixture was diluted with water (10 mL) and extracted with chloroform (10 mL × 3). The combined extracts were washed with water (10 mL), dried, and concentrated in vacuo to give a residue (16.2 mg), the purification of which by silica gel column chromatography (hexane/ethyl acetate, 3:1) afforded the acetate (**3**, 7.7 mg, 51.7%) as a pale yellow amorphous solid; $[\alpha]^{18}_{D}$ −63.4 (*c* 0.22, $CHCl_3$); IR ($CHCl_3$) 1725, 1718, 1700, 1660, 1650, 1640 cm^{-1}; UV λ_{max} (log ϵ) 269 (4.62), 370 (3.12) nm; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 5.96 (1H, qq, J = 7.3, 1.5 Hz, 26-H), 5.43 (1H, s, 14-Hβ), 4.37 (1H, d, J = 2.6 Hz, 21-H), 4.30 (1H, dd, J = 11.6, 2.6 Hz, 22-H), 4.21 (each 1H, dd, J = 11.6, 3.3 Hz, 22-H), 4.16 (1H, d, J = 3.0 Hz, 11-H), 4.03 (1H, br s, 1-H), 4.03, 4.01 (6H, s, 7-OCH_3 and 17-OCH_3), 3.31 (1H, br d, 13-H), 3.08 (1H, ddd, J = 11.6, 3.0, 2.6 Hz, 3-H), 2.89 (1H, dd, J = 16.8, 2.6 Hz, 4-Hα), 2.46 (3H, s, NCH_3), 1.93, 1.92 (6H, s, 6-CH_3 and 16-CH_3), 1.80 (3H, dq, J = 7.3, 1.7 Hz, 27-H), 1.60 (3H, dq, J = 1.7, 1.5 Hz, 28-H), 1.26 (1H, ddd, J = 16.8, 11.6, 2.6 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 185.2 (C-5), 184.1 (C-15), 182.4 (C-18), 180.6 (C-8), 169.7 (O*C*OCH_3), 166.7 (C-24), 155.9 (C-7), 155.1 (C-17), 140.8 (C-10), 140.2 (C-20), 138.6 (C-26), 137.3 (C-9), 135.7 (C-19), 129.6 (C-6), 128.1 (C-16), 126.3 (C-25), 116.1 (CN), 63.2 (C-22), 61.7 (C-13), 61.4 (C-14), 61.0 (OCH_3), 61.0 (OCH_3), 56.3 (C-1), 56.0 (C-21), 54.6 (C-11), 53.7 (C-3), 42.4 (NCH_3), 25.0 (C-4), 20.7 (OCO*C*H_3), 20.3 (C-28), 15.6 (C-27), 8.7 (16-CH_3), 8.7 (6-CH_3); EIMS *m*/*z* (%) 634 (M$^+$ + 1, 13), 633 (M$^+$, 1), 607 (7), 309 (16), 220 (44), 219 (32), 218 (76), 155 (47), 154 (13), 153 (12), 149 (10), 135 (37), 121 (15), 119 (100), 103 (54), 101 (24), 89 (18), 87 (16), 85 (84), 55 (14); HRFABMS *m*/*z* 634.2399 [M$^+$ + 1] (calcd for $C_{33}H_{36}N_3O_{10}$, 634.2401).

Acetylation of Renieramycin N (1n). Acetic anhydride (0.4 mL) was added to a solution of renieramycin N (**1n**, 23.8 mg, 0.04 mmol) in pyridine (1.0 mL), and the reaction mixture was allowed to stand for 3 h at room temperature. The reaction mixture was diluted with water (10 mL) and extracted with chloroform (10 mL × 3). The combined extracts were washed with water (10 mL), dried, and concentrated in vacuo to give a residue (32.6 mg), the purification of which by silica gel column chromatography (hexane/ethyl acetate, 2:1) afforded the triacetate (**4**, 16.9 mg, 58.9%) as an amorphous solid and the acetate (**3**, 1.6 mg, 6.2%).

Compound 4: $[\alpha]^{25}_{D}$ −42.7 (*c* 0.56, $CHCl_3$); IR ($CHCl_3$) 1760, 1750, 1720, 1710, 1660 cm^{-1}; UV λ_{max} (log ϵ) 266 (3.94), 280sh (3.85), 370 (2.70) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.94 (1H, qq, J = 6.4, 1.5 Hz, 26-H), 5.53 (1H, s, 14-Hβ), 4.39 (1H, d, J = 2.4 Hz, 21-H), 4.09 (1H, dd, J = 11.0, 4.3 Hz, 22-H), 4.04−4.02 (2H, m, 1-H and 22-H), 4.01 (3H, s, 7-OCH_3), 3.84 (1H, d, J = 2.1 Hz, 11-H), 3.75 (3H, s, 17-OCH_3), 3.30 (1H, br d, 13-H), 3.10 (1H, ddd, J = 11.3, 3.0, 2.7 Hz, 3-H), 2.94 (1H, dd, J = 17.4, 2.6 Hz, 4-Hα), 2.45 (3H, s, NCH_3), 2.44, 2.17, 2.08 (9H, s, $OCOCH_3$), 2.04 (3H, s, 16-CH_3), 1.92 (3H, s, 6-CH_3), 1.83 (3H, dq, J = 7.3, 1.7 Hz, 27-H), 1.53 (1H, ddd, J = 17.4, 11.3, 2.1 Hz, 4-Hβ), 1.51 (3H, dq, J = 1.7, 1.5 Hz, 28-H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.5 (C-5), 180.5 (C-8), 170.3 (O*C*OCH_3), 168.4 (O*C*OCH_3), 167.6 (C-24), 166.9 (O*C*OCH_3), 155.9 (C-7), 150.7 (C-17), 145.9 (C-15), 141.1 (C-10), 139.8 (C-18), 139.4 (C-26), 135.6 (C-9), 127.9 (C-6), 126.9 (C-25), 125.6 (C-16), 123.1 (C-19), 122.3 (C-20), 116.4 (CN), 63.1 (C-14), 61.7 (C-13), 61.0 (C-22), 60.9 (7-OCH_3), 60.9 (17-OCH_3), 57.0 (C-11), 57.0 (C-21), 55.8 (C-1), 54.9 (C-3), 42.0 (NCH_3), 24.1 (C-4), 20.8 (OCO*C*H_3), 20.7 (OCO*C*H_3), 20.3 (OCO*C*H_3), 20.1 (C-28), 15.5 (C-27), 10.1 (16-CH_3), 8.6 (6-CH_3); EIMS *m*/*z* (%) 719 (M$^+$, 6), 609 (7), 608 (19), 579 (9), 403 (11), 402 (49), 359 (13), 317 (9), 305 (18), 304 (100), 262 (25), 220 (13), 218 (18), 100 (11), 35 (11); HR-EIMS *m*/*z* 719.2683 [M$^+$] (calcd for $C_{37}H_{41}N_3O_{12}$, 719.2690).

Reductive Acetylation of Renieramycin M (1m). A solution of renieramycin M (**1m**, 46.0 mg, 0.08 mmol) in ethyl acetate (8 mL) was hydrogenated over 20% $Pd(OH)_2$/C (23.0 mg) at 1 atm for 3 h. The catalyst was removed by filtration and washed with ethyl acetate (100 mL). The combined filtrates were concentrated in vacuo to give the leuco compound (**5**, 58.6 mg) as a colorless amorphous solid.

Compound 5: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 5.85 (1H, qq, J = 7.3, 1.7 Hz, 26-H), 5.40 (2H, br s, OH × 2), 4.56 (1H, dd, J = 10.9, 3.0 Hz, 22-H), 4.33 (1H, br s, OH), 4.27 (1H, dd, J = 4.0, 3.0 Hz, 1-H), 4.14 (1H, d, J = 2.1 Hz, 11-H), 4.14 (1H, d, J = 2.6 Hz, 21-H), 3.98 (1H, br s, OH), 3.96 (1H, dd, J = 10.9, 4.0 Hz, 22-H), 3.74, 3.73 (6H, s, OCH_3), 3.39 (1H, br d, 13-H), 3.27 (1H, ddd, J = 11.9, 2.6, 2.5 Hz, 3-H), 3.04 (1H, dd, J = 15.5, 2.6 Hz, 14-Hα), 2.83 (1H, dd, J = 17.8, 7.9 Hz, 4-Hα), 2.42 (1H, d, J = 17.8 Hz, 14-Hβ), 2.30 (3H, s, NCH_3), 2.16, 2.14 (6H, s, 6-CH_3, 16-CH_3), 1.89 (1H, dd, J = 15.5, 11.9 Hz, 4-Hβ), 1.80 (3H, dq, J = 7.3, 1.3 Hz, 27-H), 1.43 (3H, dq, J = 1.7, 1.3 Hz, 28-H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 167.8 (C-24), 143.9, 143.6, 143.6, 143.0, 140.9, 139.1, 137.7 (C-26), 127.6, 118.8, 118.4, 118.0, 117.6, 116.6, 116.4 (CN), 115.7, 64.3 (C-22), 61.0 (OCH_3), 60.9 (OCH_3), 60.7 (C-21), 56.9 (C-1), 56.7 (C-3), 56.4 (C-11), 55.3 (C-13), 41.7 (NCH_3), 25.3 (C-4), 21.1 (C-14), 20.1 (C-28), 15.4 (C-27), 9.3 (Ar−CH_3), 9.2 (Ar−CH_3). This material was used in the next step without further purification. Acetic anhydride (1.2 mL) was added to a solution of crude **5** (58.6 mg) and 4-(dimethylamino)pyridine (2.0 mg) in pyridine (3.0 mL), and the reaction mixture was allowed to stand for 3 h at room temperature. The reaction mixture was diluted with water (20 mL) and extracted with chloroform (20 mL × 3). The combined extracts were washed with water (20 mL), dried, and concentrated in vacuo to give a residue (340 mg), the purification of which by silica gel column chromatography (hexane/ethyl acetate, 2:1) afforded the diacetate **7** (5.5 mg, 11.0%) as a pale yellow amorphous solid. Further elution with hexane/ethyl acetate (1:1) afforded tetraacetate **6** as a solid, recrystallization of which from ethyl acetate/ether afforded pure **6** (44.5 mg, 74.4%) as colorless needles.

Compound 6: mp 230−231 °C; $[\alpha]^{20}_{D}$ +3.7 (*c* 1.0, $CHCl_3$); CD $\Delta\epsilon$ nm (*c* 67 μM, methanol, 24 °C) +0.8 (274), −1.8 (238), −2.3 (282), +75.6 (203), −3.2 (230); IR (KBr) 1760, 1720, 1380, 1190 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 6.05 (1H, qq, J = 7.3, 1.7 Hz, 26-H), 4.10 (2H, m, 1-H and 22-H), 4.00 (1H, d, J = 2.6 Hz, 21-H), 3.74, 3.71 (6H, s, OCH_3), 3.68 (1H, m, 22-H), 3.68 (1H, d, J = 2.3 Hz, 11-H), 3.33 (1H, ddd, J = 7.9, 2.6, 1.3 Hz, 13-H), 3.22 (1H, ddd, J = 11.6, 2.6, 2.3 Hz, 3-H), 2.82 (1H, dd, J = 17.8, 7.9 Hz, 14-Hα), 2.71 (1H, dd, J = 15.5, 2.6 Hz, 4-Hα), 2.38, 2.37, 2.33, 2.28 (12H, s, $OCOCH_3$), 2.27 (1H, d, J = 17.8 Hz, 14-Hβ), 2.23 (3H, s, NCH_3), 2.06, 2.04 (6H, s, ArCH_3), 1.96 (3H, dq, J = 7.3, 1.7 Hz, 27-H), 1.89 (1H, dd, J = 15.5, 11.6 Hz, 4-Hβ), 1.70 (3H, dq, J = 1.7, 1.5 Hz, 28-H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 168.3 (O*C*OCH_3), 168.0 (O*C*OCH_3), 167.8 (O*C*OCH_3), 167.8 (O*C*OCH_3), 167.1 (C-24), 148.1 (C-17), 148.1 (C-7), 144.9 (C-15), 144.1 (C-5), 140.2 (C-18), 139.5 (C-26), 139.2 (C-8), 127.1 (C-25), 124.5 (C-16), 124.4 (C-6), 124.3 (C-19), 124.1 (C-9), 123.6 (C-20), 123.3 (C-10), 117.4 (CN), 67.9 (C-22), 61.0 (C-21), 60.8 (OCH_3), 60.8 (OCH_3), 57.2 (C-11), 56.2 (C-1), 56.0 (C-3), 54.7 (C-13), 41.5 (NCH_3), 26.4 (C-4), 20.9 (C-14), 20.7 (OCO*C*H_3), 20.5 (OCO*C*H_3), 20.4 (C-28), 20.3 (OCO*C*H_3), 20.3 (OCO*C*H_3), 15.7 (C-27), 10.0 (ArCH_3), 9.8 (CH_3); EIMS *m*/*z* (%) 747 (M$^+$, 1), 635 (6), 634 (10), 344 (34), 305 (22), 304 (100), 262 (20), 220 (10); *anal.* C 61.90%, H 5.95%, N 5.56%, calcd $C_{39}H_{45}N_3O_{12}\cdot 1/2H_2O$, C 61.90%, H 6.07%, N 5.46%.

Compound 7: $[\alpha]^{21}_{D}$ +33.1 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) 2950, 1760, 1720, 1650, 1370, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 6.07 (1H, qq, J = 7.3, 1.7 Hz, 26-H), 4.34 (1H, dd, J = 11.2, 3.0 Hz, 22-H), 4.13 (1H, dd, J = 3.3, 3.0 Hz, 1-H), 4.01 (1H, d, J = 2.6 Hz, 21-H), 3.99 (3H, s, OCH_3), 3.97 (1H, d, J = 2.3 Hz, 11-H), 3.73 (3H, s, OCH_3), 3.73 (1H, dd, J = 11.2, 3.0 Hz, 22-H), 3.34 (1H, ddd, J = 7.9, 2.6, 1.3 Hz, 13-H), 3.20 (1H, ddd, J = 12.3, 2.6, 2.3 Hz, 3-H), 2.76 (1H, dd, J = 20.8, 7.6 Hz, 14-Hα), 2.57 (1H, dd, J = 15.5, 2.6 Hz, 4-Hα), 2.36, 2.35 (6H, s, $OCOCH_3$), 2.27 (3H, s, NCH_3), 2.27 (1H, d, J = 20.8 Hz, 14-Hβ), 2.10 (3H, s, 6-CH_3), 1.94 (3H, s, 16-CH_3), 1.92 (3H, dq, J = 7.3, 1.3 Hz, 27-H), 1.76 (3H, dq, J = 1.7, 1.3 Hz, 28-H), 1.66 (1H, dd, J = 15.5, 12.3 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 186.1 (C-15), 182.8 (C-18), 168.3 (O*C*OCH_3), 168.2

(O*C*OCH$_3$), 167.0 (C-24), 155.4 (C-17), 149.3 (C-7), 144.2 (C-5), 141.8 (C-20), 140.4 (C-26), 139.0 (C-8), 135.4 (C-19), 128.9 (C-16), 126.7 (C-25), 124.6 (C-6), 124.5 (C-9), 124.0 (C-10), 117.2 (CN), 66.7 (C-22), 60.9 (OCH$_3$), 60.7 (OCH$_3$), 60.0 (C-21), 56.5 (C-1), 55.4 (C-3), 54.8 (C-13), 54.7 (C-11), 41.5 (NCH$_3$), 27.7 (C-4), 21.2 (C-14), 20.6 (OCO*C*H$_3$), 20.6 (C-28), 20.3 (OCO*C*H$_3$), 15.9 (C-27), 10.0 (6-CH$_3$), 8.7 (16-CH$_3$); EIMS *m*/*z* (%) 661 (M$^+$, 16), 549 (7), 548 (17), 506 (5), 304 (7), 260 (18), 221 (23), 220 (100), 219 (42), 218 (18), 204 (9), 100 (5), 55 (5); HR-EIMS *m*/*z* 661.2639 [M$^+$] (calcd for $C_{35}H_{39}N_3O_{10}$, 661.2636).

Assay for Cytotoxicity. A single-cell suspension of HCT116 cells (2×10^3 cells/well) was added to the serially diluted test compounds in a microplate. The cells were then cultured for 4 days. The degree of cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50% compared with untreated control.

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Supporting Information Available: Color picture of *Xestospongia* sp., ^{1}H NMR spectra of renieramycins Q−S (**1q**−**s**), and a flowchart for extraction and separation. These materials are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Part 3: Suwanborirux, K.; Amnuoypol, S.; Plubrukarn, A.; Pummangura, S.; Kubo, A.; Tanaka, C.; Saito, N. *J. Nat. Prod.* **2003**, *66*, 1441−1446. (b) Part 4: Saito, N.; Koizumi, Y.; Tanaka, C.; Suwanborirux, K.; Amnuoypol, S.; Kubo, A. *Heterocycles* **2003**, *61*, 79−86.

(2) Arai, T.; Kubo, A. In *The Alkaloids*; Brossi, A., Ed.; Academic: New York, 1983; Vol. 21, pp 55−100. (b) Tomson, R. H. *Naturally Occurring Quinones III*; Chapman and Hall: New York, 1987; pp 633−686. (c) Kubo, A.; Saito, N. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1992; Vol. 10, pp 77−145. (d) Ozturk, T. In *The Alkaloids*; Cordell, G. A., Ed.; Academic: New York, 2000; Vol. 53, pp 119−238. (e) Scott, J. D.; Williams, R. M. *Chem. Rev.* **2002**, *102*, 1669−1730.

(3) This compound was transformed from **1n** by air oxidation in the basic conditions.[1a] Renieramycin O (**1o**) is 21-cyanorenieramycin A, which was isolated from a *Reniera* sp. sponge and constitutes the first example of this type of alkaloid from marine organisms; see: Frincke, J. M.; Faulkner, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 265−269.

(4) Kubo, A.; Saito, N.; Kitahara, Y.; Takahashi, K.; Yazawa, K.; Arai, T. *Chem. Pharm. Bull.* **1986**, *35*, 440−442. (b) Mikami, Y.; Takahashi, K.; Yazawa, K.; Hour-Young, C.; Arat, T.; Saito, N.; Kubo, A. *J. Antibiot.* **1988**, *41*, 734−740.

(5) This compound is modified by replacement of the hydroxyl group at C-21 of renieramycin F (**1f**) with a cyano group; see: He, H.; Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 5822−5824.

(6) Saito, N.; Ohira, Y.; Kubo, A. *Chem. Pharm. Bull.* **1990**, *38*, 821−823. (b) Saito, N.; Ohira, Y.; Wada, N.; Kubo, A. *Tetrahedron* **1990**, *46*, 7711−7728. (c) Saito, N.; Nishida, M.; Kubo, A. *Chem. Pharm. Bull.* **1991**, *39*, 1243−1345. (d) Saito, N.; Harada, S.; Nishida, M.; Inouye, I.; Kubo, A. *Chem. Pharm. Bull.* **1995**, *43*, 777−782.

(7) Treatment of **1m** with selenium oxide in acetic acid at room temperature for 6 days gave **1o** and **3** in 11% and 11% yields, respectively.

(8) Martinez, E. J.; Owa, T.; Schreiber, S. L.; Corey, E. J. *Proc. Natl. Acad. Sci., U.S.A.* **1999**, *96*, 3496−3501. (b) Martinez E. J.; Corey, E. J. *Org. Lett.* **2000**, *2*, 993−996. (c) Martinez, E. J.; Corey, E. J.; Owa, T. *Chem. Biol.* **2001**, *8*, 1151−1160.

(9) Myers A. G.; Lanman, B. A. *J. Am. Chem. Soc.* **2002**, *124*, 12969−12971. (b) Plowright, A. T.; Schaus, S. E.; Myers, A. G. *Chem. Biol.* **2002**, *9*, 607−618.

(10) In the ^{1}H NMR spectrum of **7**, the diagnostic homoallylic coupling (ca. 3 Hz) between 1-H (δ 4.13 ppm) and 4-Hβ (δ 1.66 ppm) through five bonds was negligible, and this phenomenon revealed that this compound might have retained the hydroquinone moiety at ring A. Additional evidence was provided by HMBC experiments. See Experimental Section.

(11) The direct conversion of **1m** into **6** by reductive acetylation using zinc powder and acetic anhydride failed. In contrast, acetylation of **5** with acetic anhydride and acetic acid (4:1 w/w) at 100 °C for 1 h afforded **6** in low yield (15%, 2 steps).

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