

**CHEMISTRY OF RENIERAMYCINS. PART 2.¹ PARTIAL
REDUCTION AND NUCLEOPHILIC SUBSTITUTION OF
HEXAHYDRO-1,5-IMINO-4-OXO-3-BENZAZOCINE-7,10-DIONE:
PROMISING METHOD TO CONSTRUCT RENIERAMYCIN J FROM
RENIERAMYCIN G VIA RENIERAMYCIN E**

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Abstract- The conversion of 1,2,3,4,5,6,7,10-octahydro-9-methoxy-3,8,11-trimethyl-1,5-imino-3-benzazocine-4,7,10-trione (**8**) to the corresponding alkylated compound at C-21 position (**11**) as an ABC ring model of renieramycin J (**1j**) is described. This is a promising method for converting renieramycin G (**1g**) into **1j** via renieramycin E (**1e**).

As part of our search for new metabolites *via* the isolation and characterization of biologically active compounds from Thai marine animals, we recently reported the isolation and structural elucidation of renieramycins J-L (**1j-l**) from a Thai sponge, *Xestospongia* sp., in the vicinity of Sichang Island.² It is interesting to note that these natural products have an acetone residue at C-21. One possible pathway to

generate these compounds is shown in Scheme I which involves partial reduction of type I compound (such as renieramycin G (**1g**)³) to give type II compound (such as renieramycin E (**1e**)⁴), into which an acetone nucleophile can be substituted at the C-21 position⁵ stereoselectively to give type IV compound (such as renieramycin J (**1j**)) *via* type III compound. We report here the preparation of the ABC ring model (**11**) from readily available lactam (**8**) *via* α -amino nitrile (**10a**).

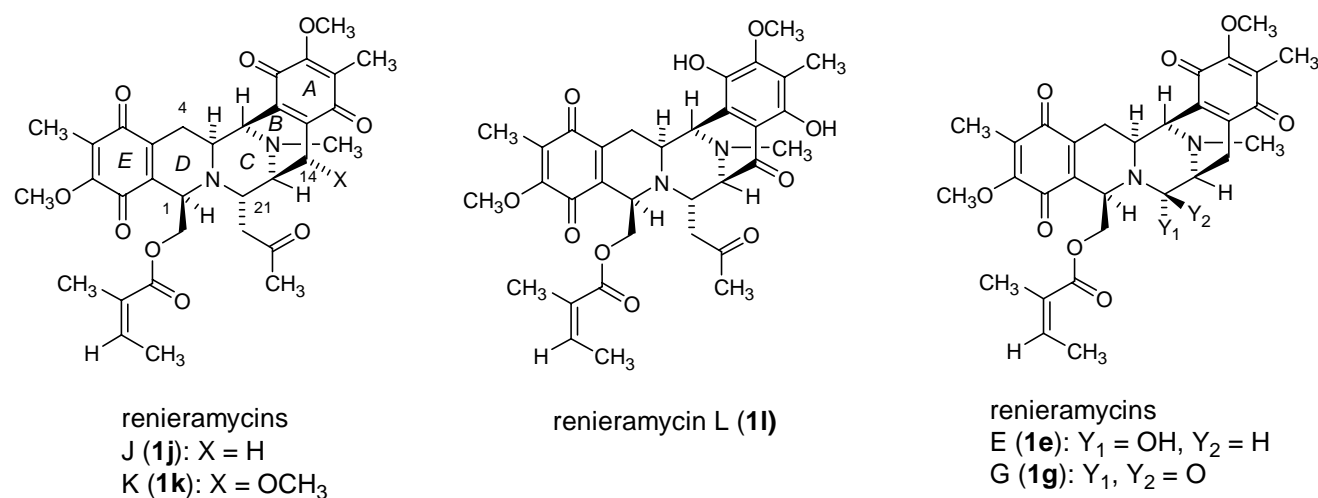
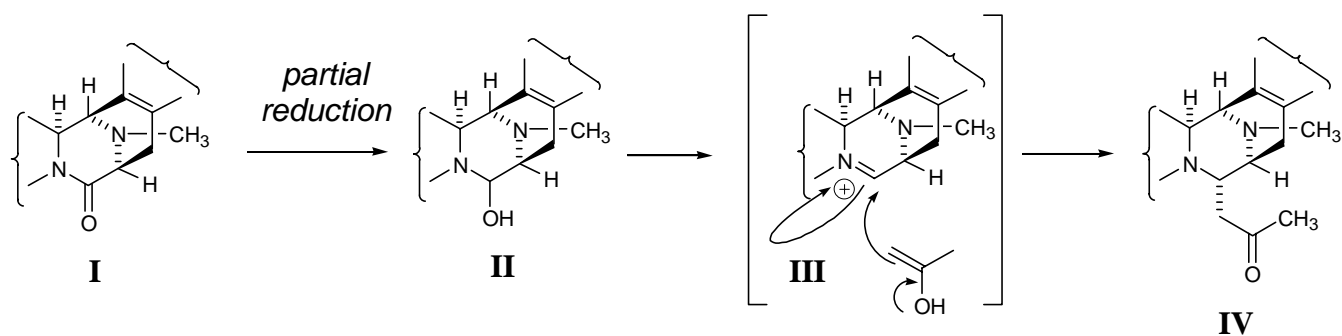


Figure 1



Scheme 1

The challenge we faced was to partially reduce the lactam carbonyl into the corresponding cyclic iminal (Scheme 1). A preliminary experiment was carried out using readily available lactam (**2a**)^{6a} and DIBAL-H.⁷ After numerous attempts to prepare of **3a** under various conditions, we found that 4 equivalents of reagent in THF at -65°C was the best in terms product yield (81%) along with amine (**4**) in 18% yields. The ¹³C-NMR spectrum of **3a** showed C-21 as a doublet at δ 86.5. However,

decomposition occurred during workup because **3a** has a relatively unstable cyclic aminal functionality, and on separation by column chromatography and in large-scale conditions, the yield of the product dramatically decreased. This problem was solved using the recent procedure of Martinez and Corey.⁸ The lactam carbonyl of **2a** was reduced cleanly by treatment with 4 equivalents of $\text{LiAlH}_2(\text{OC}_2\text{H}_5)_2$ ⁹ or sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al^R) in toluene at 0°C to the corresponding cyclic aminal (**3a**), which, upon exposure to KCN, provided amino nitrile (**5a**) in 86% and 81% overall yields, respectively. An NOE between H-14 β and H-21 revealed the relative stereochemistry at C-21 of **5a**. A similar partial reduction using Red-Al^R and substitution sequence of **2b**^{6b} gave amino nitrile (**5b**) *via* **3b** in 85% yield, the stereochemical structure of **5b** was confirmed by X-Ray crystallographic analysis (Figure 3).

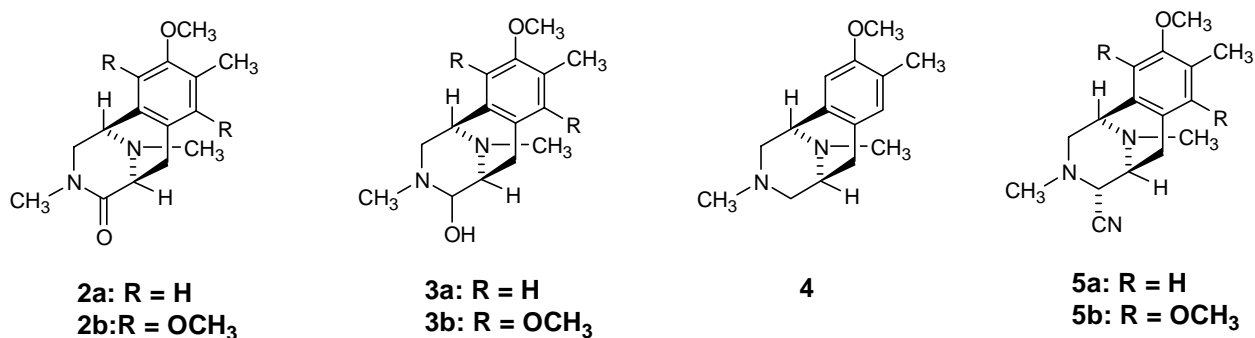


Figure 2

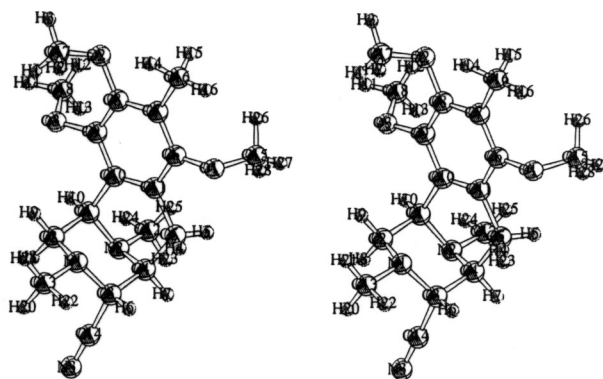
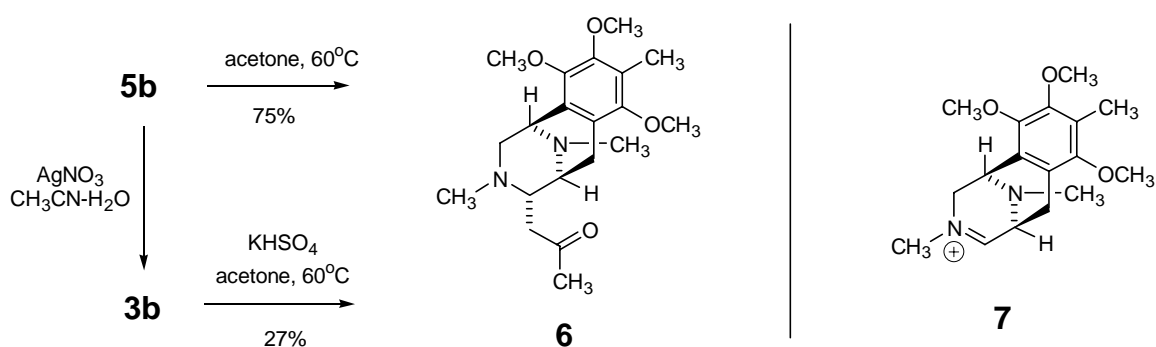


Figure 3

We then investigated the conversion of **5b** into compound (**6**) *via* **3b**. Treatment of **5b** with silver nitrate¹⁰ in acetonitrile and water at 25°C regenerated **3b**,¹¹ which, on treatment at 60°C for several hours, gave an inseparable mixture of the decomposition products. Another approach was based on the treatment of **5b** with silver nitrate in acetonitrile and water, followed by substitution with acetone in the presence of KHSO₄ to afford the desired compound (**6**) with a maximum yield of only 27%. An NOE between H-14 β (δ 2.46, d, J = 18.0 Hz) and H-21 (δ 3.24, dd, J = 8.3, 3.3Hz) revealed the relative stereochemistry at C-21 of **6**. Actually, the direct transformation of **5b** with silver nitrate in acetone at 60°C gave the compound (**6**) in 75% yield *via* **7**.

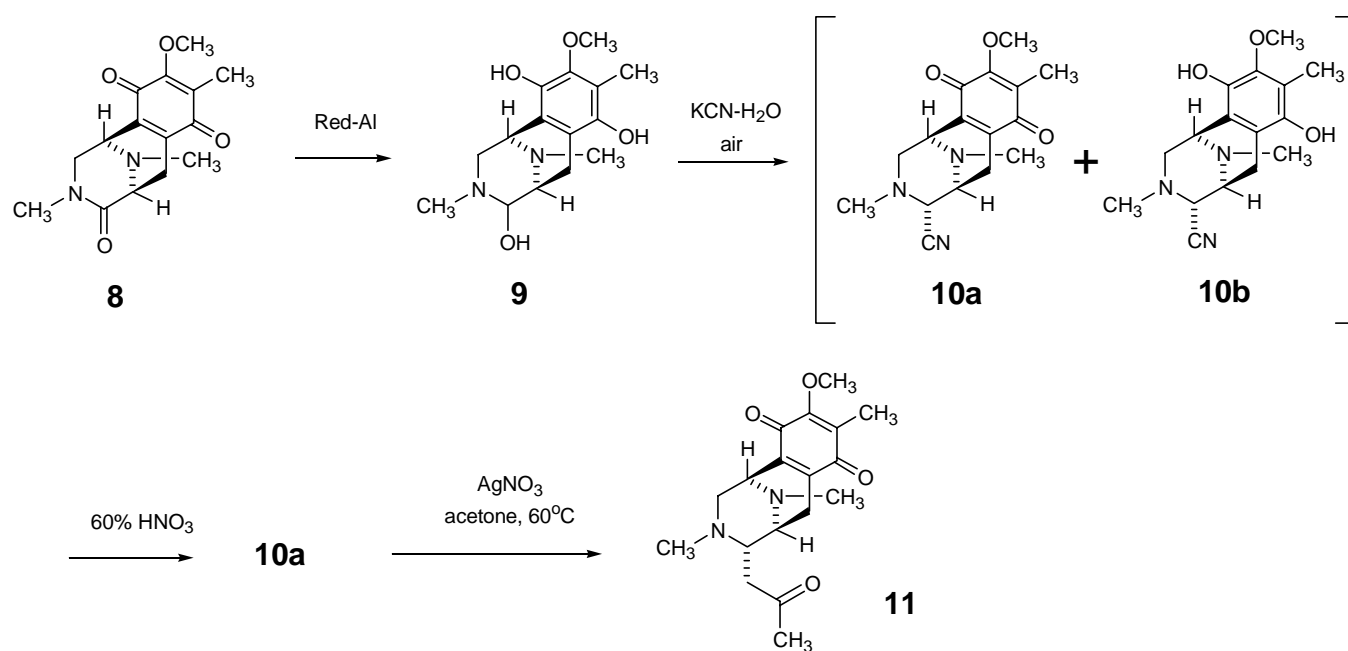


Scheme 2

Encouraged by the results of the above model conversion, we carried out the transformation of quinone (**8**)¹² into the corresponding adduct (**11**). Reduction of **8** with Red-Al^R at 0°C for 1 h gave the leuco compound (**9**), which was treated with KCN in water to give a mixture of **10a** and **10b**, which, in turn, was subjected to oxidation with 60% HNO₃ to afford **10a** in 74% overall yield from **8** (Scheme 3). Treatment of **10a** with silver nitrate in acetone at 60°C gave final product (**11**) in 31% yield. Thus, we prepared **11**, which has all the skeletal features of the right half of renieramycin J.

Finally, the compounds synthesized here were evaluated in terms their inhibitory activity against several cancer cell lines (Table). The data revealed that the introduction of a cyano group at C-21 slightly changes the *in vitro* activity of the compounds.

In summary, we succeeded in the preparation of the ABC ring model of renieramycin J.



Scheme 3

Table. *In vitro* Cytotoxicity of ABC Ring Model Compounds Against Several Cancer Cell Lines

Compound	HCT116	QC56	NCI-H460	DLD1
2a	> 50	> 50	> 50	> 50
2b	> 50	> 50	> 50	> 50
5a	11	26	6.4	28
5b	33	> 50	15	> 50
6	> 50	> 50	NT	NT
8	2.4	1.9	29	9.3
10a	0.084	0.24	NT	NT
11	3.3	1.5	NT	NT

HCT116 (colorectal cancer); QC56 (lung cancer); NCI-H460 (lung cancer); DLD1 (colorectal cancer); values recorded are IC₅₀ in μM. NT: not tested..

EXPERIMENTAL

All melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were obtained with a SHIMAZU FT-IR 8300 spectrophotometer. ¹H-NMR spectra were recorded at 300 MHz by a JEOL AIL300 spectrometer. ¹³C-NMR spectra were recorded at 75 MHz (multiplicity determined from off-resonance decoupled or distortionless enhancement by polarization transfer (DEPT) spectra). Chemical shifts were usually recorded in δ_H values relative to tetramethylsilane

as the internal standard. MS spectra were recorded on a JMS-DX 302 instrument with a direct inlet system operating at 70 eV. Elemental analyses were carried out using a Perkin Elmer Model 240B elemental analyzer. Anhydrous sodium sulfate was used for drying organic solvent extracts. Removal of the solvent was done with a rotary evaporator and, finally, under high vacuum. Column chromatography was performed with Kieselgel 60 (Merck, 70-230).

4-Cyano-1,2,3,5,6-hexahydro-9-methoxy-3,8,11-trimethyl-1,5-imino-3-benzazocine (5a)

Method A: 1.0 M LiAlH₄ in dry ether (0.8 mL, 0.8 mmol) was cooled with ice-cold water, after which ethyl acetate (70.4 mg, 0.8 mmol) was added dropwise over 5 min, and the mixture was stirred at 0°C for 2 h. A solution of **2a**^{6a} (52.0 mg, 0.2 mmol) in dry THF (5 mL) was added to the above mixture, and the whole was stirred for 1 h at the same temperature. After the reaction mixture was quenched with acetic acid (262 mg, 4.36 mmol), a solution of potassium cyanide (78 mg, 1.2 mmol) in water (0.6 mL) was added dropwise over 10 min, and this mixture was stirred subsequently at 25°C for 1 h. The reaction mixture was poured into water (30 mL) and extracted with ethyl acetate (20 mL x 3). The combined extracts were washed with 5% NaHCO₃ (20 mL), dried, and concentrated *in vacuo* to give a solid, the recrystallization of which from ethyl acetate-hexane gave **5a** (45.9 mg, 85.5%) as colorless prisms.

Method B: A solution of **2a** (390 mg, 1.5 mmol) in dry THF (20 mL) was cooled with ice-cold water, after which 65 wt% sodium bis(2-methoxyethoxy)aluminum hydride in toluene (Red-Al^R: 1.87 g, 6.0 mmol) was added dropwise over 10 min, and the mixture was stirred at 0°C for 1 h. After the reaction mixture was quenched with acetic acid (1.96 g, 33 mmol), a solution of potassium cyanide (586 mg, 9 mmol) in water (4.5 mmol) was added dropwise for 10 min, and this mixture was stirred subsequently at 25°C for 1 h. The reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (30 mL x 3). The combined extracts were washed with 5% NaHCO₃ (30 mL), dried, and concentrated *in vacuo* to give a solid, the recrystallization of which from ethyl acetate-hexane gave **5a** (329 mg, 80.8%) as colorless prisms.

mp 149-150°C; ν_{\max} 2945, 2793, 2220, 1506, 1232, 1105 cm⁻¹; δ_{H} 2.17 (3H, s, 8-CH₃), 2.27 and 2.35 (each 3H, s, *N*-CH₃), 2.48 (1H, d, *J* = 17.6 Hz, 6-H β), 2.61 (1H, d, *J* = 10.8 Hz, 2-H β), 2.86 (1H, dd, *J* = 10.8, 3.1 Hz, 2-H α), 3.09 (1H, dd, *J* = 17.6, 8.0 Hz, 6-H α), 3.32 (1H, dd, *J* = 8.0, 2.0 Hz, 5-H), 3.62 (1H, d, *J* = 3.1 Hz, 1-H), 3.74 (1H, d, *J* = 2.0 Hz, 4-H), 3.80 (3H, s, OCH₃), 6.47 and 6.82 (each 1H, s, Ar-H); δ_{C} 16.1 (q, 8-CH₃), 25.0 (t, C-6), 41.6 (q, NCH₃), 43.7 (q, NCH₃), 55.3 (q, OCH₃), 55.5 (d, C-5), 56.8 (t, C-2), 58.5 (d, C-1), 61.7 (d, C-4), 108.5 (d), 116.3 (s, CN), 125.2 (s), 125.2 (s), 129.5 (d), 133.1 (s), 155.9 (s); *m/z* (%) 271 (M⁺, 2), 189 (20), 188 (100); Anal. Calcd for C₁₆H₂₁N₃O · 1/5H₂O: C, 69.94; H, 7.78; N, 15.29. Found: C, 70.26; H, 7.72; N, 14.98.

4-Cyano-1,2,3,5,6-hexahydro-7,9,10-trimethoxy-3,8,11-trimethyl-1,5-imino-3-benzazocine (5b)

Method A: 1.0 M LiAlH₄ in dry ether (0.4 mL, 0.4 mmol) was cooled with ice-cold water, after which ethyl acetate (35.2 mg, 0.4 mmol) was added dropwise for 5 min, and the mixture was stirred at 0°C for 1 h. A solution of **2b**^{6b} (32.0 mg, 0.1 mmol) in dry THF (5 mL) was added to the above mixture and the whole was stirred for 35 min at the same temperature. After the reaction was quenched with acetic acid (131 mg, 2.2 mmol), a solution of potassium cyanide (39.7 mg, 1.6 mmol) in water (0.3 mmol) was added dropwise over 10 min, and this mixture was stirred subsequently at 25°C for 2 h. The reaction mixture was poured into water (10 mL) and extracted with ethyl acetate (10 mL x 3). The combined

extracts were washed with 5% NaHCO₃ (20 mL), dried, and concentrated *in vacuo* to give a solid, the recrystallization of which from ethyl acetate-hexane gave **5b** (26.0 mg, 78.5%) as colorless prisms.

Method B: A solution of **2b** (320 mg, 1.0 mmol) in dry THF (13 mL) was cooled with icewater, after which 65 wt% sodium bis(2-methoxyethoxy)aluminum hydride in toluene (Red-Al[®]: 1.244 g, 4.0 mmol) was added dropwise over 10 min, and the mixture was stirred at 0°C for 1 h. After the reaction mixture was quenched with acetic acid (1.31 g, 21.8 mmol), a solution of potassium cyanide (391 mg, 6 mmol) in water (3.0 mL) was added dropwise for 10 min, and this mixture was stirred subsequently at 25°C for 4 h. The reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (30 mL x 3). The combined extracts were washed with 5% NaHCO₃ (30 mL), dried, and concentrated *in vacuo* to give a solid, the recrystallization of which from ethyl acetate-hexane gave **5b** (281 mg, 85%) as colorless prisms.

mp 150-151°C; ν_{\max} 2939, 2222, 1464, 1406, 1115, 1074 cm⁻¹; δ_{H} 2.19 (3H, s, 8-CH₃), 2.26 and 2.31 (each 3H, s, *N*-CH₃), 2.44 (1H, d, *J* = 18.2 Hz, 6-H β), 2.57 (1H, d, *J* = 11.0 Hz, 2-H β), 2.84 (1H, dd, *J* = 11.0, 3.1 Hz, 2-H α), 3.03 (1H, dd, *J* = 18.2, 8.1 Hz, 6-H α), 3.35 (1H, dd, *J* = 8.1, 1.0 Hz, 5-H), 3.39 (1H, d, *J* = 3.1 Hz, 1-H), 3.70 (1H, d, *J* = 1.0 Hz, 4-H), 3.70, 3.81, and 3.83 (each 3H, s, OCH₃); δ_{C} 9.3 (q, 8-CH₃), 20.8 (t, C-6), 41.5 (q, NCH₃), 43.7 (q, NCH₃), 52.4 (d, C-1), 54.8 (d, C-5), 55.7 (t, C-2), 59.6, 60.0, 60.5 (each q, OCH₃), 61.7 (d, C-4), 116.3 (s, CN), 123.0 (s), 123.4 (s), 126.2 (s), 146.4 (s), 149.4 (s), 151.0 (s); *m/z* (%) 331 (M⁺, 2), 248 (100); Anal. Calcd for C₁₈H₂₅N₃O₃: C, 65.23; H, 7.60; N, 12.68; Found: C, 65.25; H, 7.56; N, 12.69.

X-Ray structure determination of **5b**

The crystal of **5b** (C₁₈H₂₅N₃O₃) belongs to the monoclinic space group P2₁/c (#14) with cell constants *a* = 9.218 (1) Å, *b* = 26.977 (5) Å, *c* = 9.179 (1) Å, β = 53.186 (8)°, *V* = 1827.4 (5) Å³, *Z* = 4, and *D_c* = 1.205 g/cm³. All measurements were conducted on a Rigaku AFC7S diffractometer with filtered Cu-K α radiation. The data were collected at a temperature of 25 ± 1°C to a maximum 2θ value of 136.0°. A total of 3306 reflections were collected. The linear absorption coefficient, μ , for Cu-K α radiation was 6.72 cm⁻¹. The structure was solved by direct methods^{13a} and expanded using the Fourier technique.^{13b} The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included, but their positions were not refined: isotropic B values were refined. The final cycle of full-matrix leastsquares refinement was based on 2862 observed reflections [*I* > 3.00 (*I*)] and 218 variable parameters and converged (largest parameter shift was 0.01 times its esd) with unweighted and weighted agreement factors of *R* = 0.044 and *R_w* = 0.080. Neutral atom scattering factors were taken from Cromer and Waber.^{13c} Anomalous dispersion effects were included in *F*_{calc},^{13d} and the values for *f*' and *f*'' were those of Creagh and McAuley.^{13e} The values for mass attenuation coefficients were those of Creagh and Hubble.^{13f} All calculations were performed using the teXan^{12g} crystallographic software package of Molecular Structure Corporation. The drawing of the molecule was made by ORTEP.

1,2,3,5,6-Hexahydro-4-hydroxy-9-methoxy-3,8,11-trimethyl-1,5-imino-3-benzazocine (**3a**)

Silver nitrate (60.8 mg, 0.358 mmol) was added to a solution of **5a** (23.3 mg, 0.08 mmol) in acetonitrile (3 mL) and water (2 mL) at 0°C, and the suspension was stirred at 25°C for 4 h. The reaction mixture was partitioned between chloroform (10 mL x 3) and 5% NaHCO₃ (20 mL), and the combined organic layer was washed with water (10 mL), dried, and concentrated *in vacuo* to give a residue (**3a**: 23.0 mg) as pale

yellow oil; δ_{H} 2.17 (3H, s, 8-CH₃), 2.26 and 2.30 (each 3H, s, *N*-CH₃), 2.39 (1H, d, $J = 10.0$ Hz, 2-H β), 2.40 (1H, d, $J = 18.0$ Hz, 6-H β), 2.96 (1H, dd, $J = 18.0, 7.9$ Hz, 6-H α), 3.01 (1H, dd, $J = 10.0, 3.6$ Hz, 2-H α), 3.09 (1H, dd, $J = 7.9, 1.0$ Hz, 5-H), 3.50 (1H, d, $J = 3.6$ Hz, 1-H), 3.79 (3H, s, OCH₃), 4.19 (1H, d, $J = 1.0$ Hz, 4-H), 6.46 and 6.85 (each 1 H, s, Ar-H); δ_{C} 16.1 (q, 8-CH₃), 24.0 (t, C-6), 41.4 (q, 2 x NCH₃), 53.6 (t, C-2), 55.3 (q, OCH₃), 58.6 (d, C-1), 58.8 (d, C-5), 86.5 (d, C-4), 108.6 (d), 125.0 (s), 125.1 (s), 129.9 (d), 133.3 (s), 155.8 (s).

Preparation of **6**

Method A (two-step procedure): Silver nitrate (49.0 mg, 0.288 mmol) was added to a solution of **5b** (23.8 mg, 0.072 mmol) in acetonitrile (3 mL) and water (2 mL) at 0°C, and the suspension was stirred at 25°C for 4 h. The reaction mixture was partitioned between chloroform (10 mL x 3) and 5% NaHCO₃ (20 mL), and the combined organic layer was washed with water (10 mL), dried, and concentrated *in vacuo* to give a residue (**3b**: 25.7 mg) as pale yellow oil, which was used in the next step without further purification; δ_{H} 2.19 (3H, s, 8-CH₃), 2.27 (6H, s, 2 x *N*-CH₃), 2.33 (1H, d, $J = 11.0$ Hz, 2-H β), 2.36 (1H, d, $J = 18.2$ Hz, 6-H β), 2.92 (1H, dd, $J = 18.2, 8.1$ Hz, 6-H α), 3.00 (1H, dd, $J = 11.0, 3.6$ Hz, 2-H α), 3.12 (1H, dd, $J = 8.1, 1.0$ Hz, 5-H), 3.71, 3.81, and 3.82 (each 3H, s, OCH₃), 3.90 (1H, d, $J = 3.1$ Hz, 1-H), 4.17 (1H, d, $J = 1.0$ Hz, 4-H); δ_{C} 9.3 (q, 8-CH₃), 19.8 (t, C-6), 41.4 (q, 2 x NCH₃), 52.5 (d, C-1), 52.5 (t, C-2), 57.9 (d, C-5), 59.5, 60.0, 60.5 (each q, OCH₃), 86.5 (d, C-4), 123.5 (s), 123.7 (s), 130.1 (s), 144.7 (s), 149.2 (s), 151.6 (s).

A mixture of crude **3b** and KHSO₄ (29.8 mg, 0.22 mmol) in acetone (5 mL) was heated at 60°C for 4 h. After the solvent was removed *in vacuo*, the residue was diluted with water (5 mL), and extracted with chloroform (5 mL x 3). The combined extracts were washed with 5% NaHCO₃ (5 mL), dried, and concentrated *in vacuo* to give a residue (28.9 mg). The residue was subjected to chromatography on preparative layer silica gel plates (Merck 5715, solvent system-20:1 chloroform:methanol) to give **6** (7.1 mg, 27.3%) as a solid.

Method B (one-pot procedure): Silver nitrate (48.3 mg, 0.284 mmol) was added to a solution of **5b** (22.4 mg, 0.068 mmol) in acetone (5 mL) at 0°C, and the mixture was stirred at 25°C for 1 h, warmed to 60°C and stirred for 2 h. After the solvent was removed *in vacuo*, the residue was diluted with water (5 mL), and extracted with chloroform (5 mL x 3). The combined extracts were washed with 5% NaHCO₃ (5 mL), dried, and concentrated *in vacuo* to give a solid (35 mg), which was recrystallized from ethyl acetate-hexane to give **6** (18.3 mg, 75%) as colorless prisms.

mp 96-97°C; ν_{max} 2926, 1709, 1458, 1406, 1111, 1076 cm⁻¹; δ_{H} 2.10 (3H, s, NCH₃), 2.18 (3H, s, 8-CH₃), 2.20 (3H, s, COCH₃), 2.22 (3H, s, NCH₃), 2.37 (1H, dd, $J = 11.0, 2.0$ Hz, 2-H β), 2.46 (1H, d, $J = 18.0$ Hz, 6-H β), 2.72 (1H, dd, $J = 16.7, 3.3$ Hz, 4-CHCO), 2.78 (1H, dd, $J = 11.0, 3.3$ Hz, 2-H α), 2.85 (1H, d, $J = 8.0$ Hz, 5-H), 2.99 (1H, dd, $J = 18.0, 8.0$ Hz, 6-H α), 3.03 (1H, dd, $J = 16.7, 8.3$ Hz, 4-CHCO), 3.24 (1H, dd, $J = 8.3, 3.3$ Hz, 4-H), 3.71 (3H, s, OCH₃), 3.81 (6H, s, 2 x OCH₃), 3.88 (1H, dd, $J = 3.3, 2.0$ Hz, 1-H); δ_{C} 9.3 (q, 8-CH₃), 22.5 (t, C-6), 31.1 (q, COCH₃), 36.2 (t, 4-CH₂CO), 41.8 (q, NCH₃), 42.5 (q, NCH₃), 53.2 (d, C-1), 54.1 (t, C-2), 56.0 (d, C-5), 59.5, 60.0, 60.5 (each q, OCH₃), 61.9 (d, C-4), 122.9 (s), 124.7 (s), 127.0 (s), 146.3 (s), 149.0 (s), 150.9 (s), 209.9 (s, CO); m/z (%) 362 (M⁺, 2), 276 (12), 249 (16), 248 (100), 218 (9); High-resolution ms Calcd for C₂₀H₃₀N₂O₄: 362.2206. Found: 362.2208.

Transformation of **8** to aminonitrile (**10a**) via **9**

A solution of **8**¹² (51.2 mg, 0.176 mmol) in dry THF (10 mL) was cooled with ice-cold water, after which 65 wt% sodium bis(2-methoxyethoxy)aluminum hydride in toluene (Red-Al[®]: 328 mg, 1.06 mmol) was added dropwise over 10 min, and the mixture was stirred at 0°C for 1 h. After the reaction mixture was quenched with acetic acid (346 mg, 5.76 mmol), a solution of potassium cyanide (71 mg, 1.09 mmol) in water (0.7 mmol) was added dropwise over 10 min, and this mixture was subsequently stirred at 25°C for 1 h. The reaction mixture was poured into water (50 mL) and extracted with chloroform (30 mL x 3). The combined extracts were washed with 5% NaHCO₃ (30 mL), dried, and concentrated *in vacuo*. The residue (60.1 mg), which showed two major spots on TLC, was subsequently treated with 60% HNO₃ (1 mL) at 0°C for 1 h. This was followed by dilution with water (10 mL) and extraction with chloroform (10 mL x 4). The combined extracts were washed with water (20 mL), dried, and concentrated *in vacuo* to give a solid, the recrystallization of which from ether gave **10a** (39.1 mg, 74%) as yellow prisms; mp 126-127°C (lit.,⁷ mp 122-124°C (decomp)); ν_{\max} 2926, 2226, 1653, 1616, 1308, 1231 cm⁻¹; δ_{H} 1.96 (3H, s, 8-CH₃), 2.44 (1H, d, J = 20.8 Hz, 6-H β), 2.22 and 2.24 (each 3H, s, *N*-CH₃), 2.39 (1H, dd, J = 11.6 Hz, 1.4 Hz, 2-H β), 2.66 (1H, dd, J = 20.8, 7.4 Hz, 6-H α), 2.73 (1H, dd, J = 11.6, 3.3 Hz, 2-H α), 3.24 (1H, dd, J = 7.4, 1.8 Hz, 5-H), 3.55 (1H, d, J = 1.8 Hz, 4-H), 3.74 (1H, dd, J = 3.3, 1.4 Hz, 1-H), 4.01 (3H, s, OCH₃); δ_{C} 8.7 (q, 8-CH₃), 20.9 (t, C-6), 41.5 (q, NCH₃), 43.3 (q, NCH₃), 51.1 (d, C-1), 53.0 (t, C-2), 54.5 (d, C-5), 61.0 (q, OCH₃), 61.0 (d, C-4), 115.7 (s, CN), 128.8 (s), 137.2 (s), 141.0 (s), 155.3 (s), 182.4 (s), 187.0 (s); m/z (%) 301 (M⁺, 7), 220 (26), 219 (100), 218 (96), 204 (55), 202 (11), 201 (22), 190 (19), 176 (20); Anal. Calcd for C₁₆H₁₉N₃O₃ · 1/5H₂O: C, 63.02; H, 6.41; N, 13.78; Found: C, 63.33; H, 6.45; N, 13.65.

Preparation of the compound (11).

Silver nitrate (53.7 mg, 0.316 mmol) was added to a solution of **10a** (23.7 mg, 0.079 mmol) in acetone (5 mL) at 0°C, and the mixture was stirred at 25°C for 1 h, and then warmed to 60°C and stirred for 2 h. After the solvent was removed *in vacuo*, the residue was diluted with water (5 mL) and extracted with chloroform (5 mL x 3). The combined extracts were washed with 5% NaHCO₃ (5 mL), dried, and concentrated *in vacuo* to give a solid (20.5 mg), which was recrystallized from ether to give **11** (8.1 mg, 31.2%) as pale yellow prisms; mp 117-118°C; ν_{\max} 2929, 1710, 1649, 1617, 1306, 1233, 1156 cm⁻¹; δ_{H} 1.95 (3H, s, 8-CH₃), 2.13 (3H, s, *N*-CH₃), 2.19 (3H, s, COCH₃), 2.19 (1H, d, J = 11.6 Hz, 2-H β), 2.20 (3H, s, *N*-CH₃), 2.23 (1H, d, J = 21.9 Hz, 6-H β), 2.64 (1H, dd, J = 16.9, 2.9 Hz, 4-CHCO), 2.67 (1H, dd, J = 11.6, 3.3 Hz, 2-H α), 2.69 (1H, dd, J = 21.9, 6.2 Hz, 6-H α), 2.82 (1H, br d, J = 6.2 Hz, 5-H), 3.00 (1H, dd, J = 16.9, 8.5 Hz, 4-CHCO), 3.14 (1H, dd, J = 8.5, 2.9 Hz, 4-H), 3.71 (1H, d, J = 3.3 Hz, 1-H), 3.99 (3H, s, OCH₃); δ_{C} 8.8 (q, 8-CH₃), 22.5 (t, C-6), 31.0 (s, COCH₃), 35.9 (t, 4-CH₂CO), 41.6 (q, NCH₃), 42.0 (q, NCH₃), 51.2 (t, C-2), 51.6 (d, C-1), 55.6 (d, C-5), 60.9 (q, OCH₃), 61.2 (d, C-4), 128.9 (s), 137.0 (s), 142.2 (s), 155.2 (s), 182.9 (s), 187.4 (s), 209.0 (s); m/z (%) 332 (M⁺, 31), 275 (10), 246 (16), 221 (14), 220 (100), 204 (12), 114 (69), 70 (27); High-resolution MS Calcd for C₁₈H₂₄N₂O₄: 332.1736. Found: 332.1742.

Typical cytotoxicity assay

A single-cell suspension of HCT116 cells (2 x 10³ cells/well) was added to the serially diluted test compounds in a micro plate. The cells were then cultured for 4 days. The degree of cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan). IC₅₀ was expressed as the concentration as

which cell growth was inhibited by 50% compared with the control.

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