SYNTHESIS AND ANTITUMOR EVALUATION OF OCTAHYDRO-5-HYDROXY-1,5-IMINO-3-BENZAZOCIN-4,7,10-TRIONES

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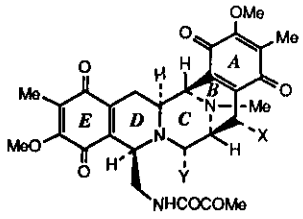
Abstract—— 1,2,3,4,5,6,7,10-Octahydro-5-hydroxy-1,5-imino-3-benzazocin-4,7,10-trione (**3a**) was synthesized from the hexahydro-1,5-imino-9-methoxy-3,8,11-trimethyl-4-oxo-3-benzazocine (**4a**) in six steps and evaluated for its antitumor activity against L1210 *in vitro*.

This paper is dedicated to the memory of Dr. Shun-ichi Yamada, Professor Emeritus, Tokyo University.

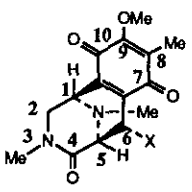
Saframycins A-C (**1a-c**) are among the most representative examples of the antitumor isoquinolinequinone antibiotics.[1] These microbial metabolites exhibit wide-spectrum antimicrobial activity, but **1a** is attracting particular interest because it exhibits strong cytotoxicity toward cultured cells and shows antitumor activity against several experimental tumors including leukemias L1210 and P388, and Ehrlich carcinoma both in ascitic and solid forms.

To design anticancer compounds for practical use and simplify their synthesis, it was decided to eliminate the lefthand half portion from the saframycin core. In a previous paper, a practical synthesis of the ABC ring model (**2a**) and the introduction of a hydroxyl group into C-6 position of **2a** to give **2b** using selenium dioxide

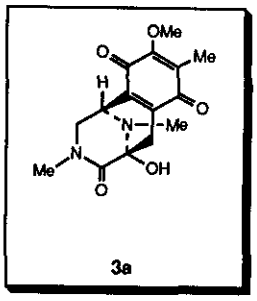
were described.[2] Unfortunately, these compounds (**2a**) (ID_{50}: 0.22 μg/ml) and (**2b**) (ID_{50}: 8.8 μg/ml) were shown to possess low cytotoxic potency relative to **1a** (ID_{50}: 0.0012 μg/ml) against L1210 leukemia *in vitro*. To further evaluate the relationship of the ABC ring with biological activity, we report here the synthesis of an additional tricyclic lactam (**3a**) which has a hydroxyl group at C-5 position.



saframycins
A (1a): X = H, Y = CN
B (1b): X = Y = H
C (1c): X = OMe, Y = H



2a: X = H
2b: X = OH



3a

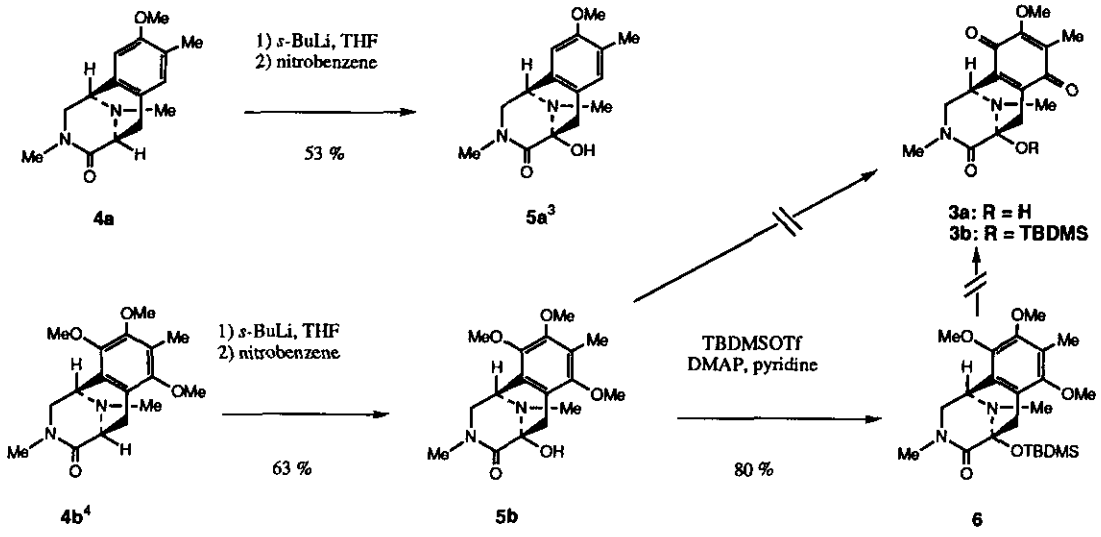
Figure 1

The challenge addressed was the introduction of a hydroxyl group into the C-5 position of the 1,5-imino-3-benzazocine skeleton. Recently, we reported that the regioselective hydroxylation of **4a** by metal-hydrogen interchange and subsequent reaction of the organometallic intermediate with nitrobenzene afforded **5a** in 53% yield.[3] We present here the second example of the introduction of a hydroxyl group at the bridgehead position as follows: the treatment of **4b**[4] with *s*-BuLi in THF at -78 °C followed by oxidation with nitrobenzene afforded **5b** in 63% yield. (Scheme 1). The structure of **5b** was supported by the ^{1}H NMR spectrum, which showed two doublet signals at δ 2.86 and 3.01 assigned to the H-6 protons. With the 5-hydroxyl compound (**5b**) in hand, we then turned our attention to the conversion of the arene (**5b**) into the *p*-quinone (**3a**).

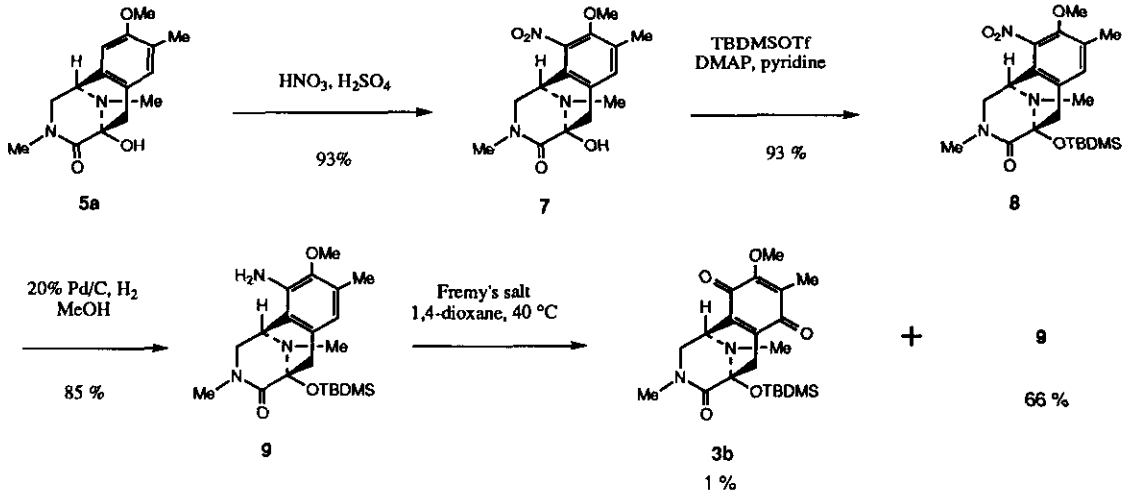
Numerous efforts for the direct conversion of **5b** to the corresponding *p*-quinone (**3a**) were totally unsuccessful and afforded only a polar polymeric material. The alcohol (**5b**) was then protected with a silyl group to give **6** in 80% yield. Oxidative demethylation of **6** to produce **3b** employing the usual agents (*e.g.*, CAN, AgO, HNO_3) was also fruitless.[5]

This prompted us to examine an alternative synthesis of **3a** which featured a different approach (Scheme 2). Nitration of **5a** with HNO_3-H_2SO_4 at 0 °C for 1 h afforded **7** in 93% yield.[6] The nitro group of **7** was assigned to C-10 based upon the observation of extensive nuclear Overhauser effect (NOE) enhancement of the aromatic proton (δ 7.06) when the 8-CH_3 protons (δ 2.33) were irradiated. The alcohol (**7**) was protected with a silyl group to afford **8** in 93% yield. Hydrogenation of **8** with 20% palladium on carbon in ethanol afforded amine (**9**) in 85% yield. Oxidation of the amine (**9**) with potassium nitrosodisulfonate (Fremy's salt)

in methanol at 25 °C failed and only the starting material was recovered. One possible reason for the failure of this oxidation was the poor solubility of the amine (**9**) in most organic solvents. Even in the best solvent, 1,4-dioxane at 40 °C for 16 h, **9** was converted to the *p*-quinone (**3b**) in only 1.4% yield along with restored **9** (66%).

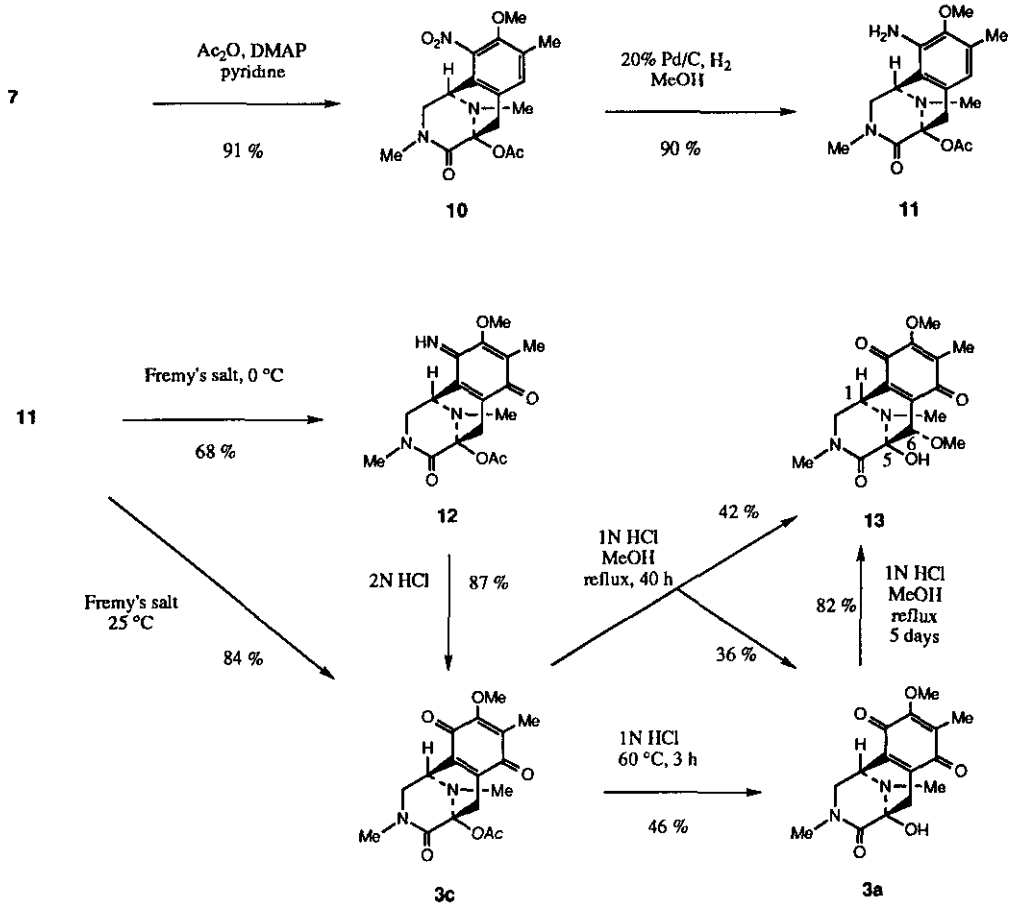


Scheme 1



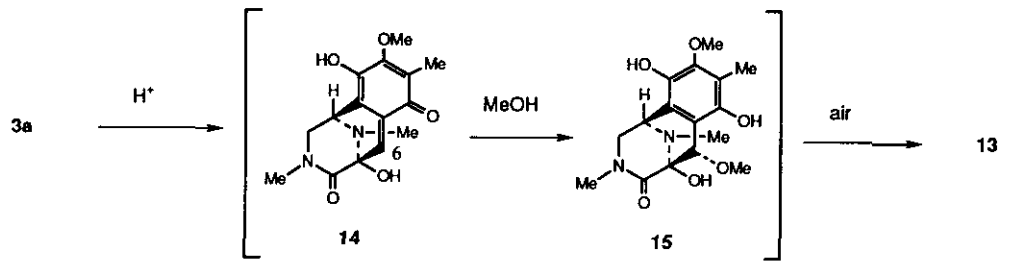
Scheme 2

Since attempts to convert **9** into the *p*-quinone (**3b**) were unsuccessful, we sought to induce this transformation by using the acetyl group as a hydroxyl protecting group (Scheme 3). Protection of the alcohol (**7**) with acetic anhydride and pyridine gave **10** in 91% yield. Hydrogenation of **10** with 20% palladium on carbon in methanol gave **11** in 90% yield. The reaction of **11** with Fremy's salt at 0 °C for 1 h afforded **3c** in 4.5% yield. However, the major product was the iminoquinone (**12**) in 68% yield along with the starting material (**11**) (10.5%). Treatment of **12** with aqueous HCl gave the desired *p*-quinone (**3c**) in 87% yield, while oxidation of **11** with Fremy's salt at 25 °C for 1 h directly gave **3c** in 84% yield.



Scheme 3

We then studied removal of the hydroxyl protecting group of **3c** to obtain the alcohol (**3a**). Hydrolysis of **3c** 1N HCl at 60 °C for 3 h gave **3a** in 46% yield. In contrast, treatment of **3c** with 1N HCl in methanol under reflux gave **3a** and **13** in 36% and 42% yields, respectively. The structure of **13** was supported by the ^{13}C NMR spectrum, which shows two aliphatic methine carbons at δ 52.9 and 73.1 assigned to C-1 and C-6 carbon peaks and an aliphatic quaternary carbon at δ 85.1 (C-5).[7] Interestingly, heating **3a** with 1N HCl in methanol for 5 days afforded **13** in 82% yield. The probable mechanistic pathway for the introduction of methoxyl group at C-6 position of **3a** is shown in Scheme 4. Thus the isomerization of **3a** under acidic conditions generates the enol (**14**). A methoxyl group attacks at C-6 position of **14** from the less-hindered α face to give the hydroquinone (**15**), and this is quickly oxidized by air to afford **13**.



Scheme 4

Finally, the compounds reported here were evaluated as growth inhibitors of murine leukemic cells (L1210). These data are tabulated in Table I. The data suggest that the introduction of a hydroxyl group at C-5 position does not dramatically change the *in vitro* activity.

Table 1

In vitro Activity of Tricyclic Lactam Derivatives Having a Hydroxyl Group at C-5 Position Against L1210 Leukemia.

Compound	ID_{50} (μg/mL)
3a	0.9
3c	5.0
12	2.23
13	1.58
saframycin A (**1a**)	0.0012

Further studies on the synthesis of the righthand half of saframycins are in progress in our laboratories.

ACKNOWLEDGMENTS

We thank Misses S. Yoshioka and T. Koseki in the Analytical Center of Meiji College of Pharmacy for the MS data measurement and microanalytical data.

EXPERIMENTAL SECTION

All melting points were determined with a Yanagimoto micromelting points apparatus and are uncorrected. IR spectra were obtained with a Hitachi 260-10 Infra Red Fourier transform spectrophotometer. ^{1}H-NMR spectra were recorded at 270 MHz by a JEOL JNM-EX 270 spectrometer. Peak multiplicities were denoted by s (single), br s (broad single), d (doublet), t (triplet), q (quartet), m (multiplet) or by a combination of these, *e.g.* dd (double doublet) with coupling constants (*J*) given in Hz. ^{13}C-NMR spectra were recorded at 67.5 MHz (multiplicity determined from off-resonance decoupled or distortionless enhancement by polarization transfer (DEPT) spectra). NMR spectra were measured in $CDCl_3$ and chemical shifts were usually recorded in δ_H values relative to internal tetramethylsilane as a standard. MS spectra were recorded on a JMS-DX 302 instrument with a direct inlet system operating at 70eV. Elemental analyses were carried out using a Perkin-Elmer Model 240B elemental analyzer. All reactions were conducted under an argon atmosphere. Dry solvents and reagents were obtained using standard procedures. 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 E. Merck silica gel 60 (70-230 mesh).

1,2,3,4,5,6-Hexahydro-5-hydroxy-7,9,10-trimethoxy-3,8,11-trimethyl-1,5-imino-3-benzazocin-4-one (**5b**)

A solution of **4b**[4] (192.0 mg, 0.6 mmol) in dry THF (12 mL) was added to a solution of *s*-BuLi (1.05 M, 1.43 mL, 1.5 mmol) in cyclohexane at -78 °C and the mixture was stirred for 1 h at the same temperature. Nitrobenzene (618 μL, 6.0 mmol) was added quickly to this mixture and the stirring was continued for 4 h. After the solution was brought to room temperature over 1 h, the reaction mixture was diluted with ether (40 mL) and extracted with 1N HCl (50 mL x 3). The combined acidic aqueous extracts were made alkaline with diluted 3% NH_4OH and extracted with chloroform (50 mL x 3). The combined extracts were washed with water (50 mL), dried, and concentrated *in vacuo* to give the residue. This material was subjected to chromatography (silica gel, 10 g; elution with 50:1 dichloromethane-methanol) to give a solid, recrystallization of which from ethyl acetate gave **5b** (127.0 mg, 63.0%) as colorless needles, mp 188-188.5 °C (decomp); ν_{max} (KBr) 3250, 1640 cm^{-1}; δ_H 2.19 (3H, s, 8-CH_3), 2.43 (3H, s, NCH_3), 2.84 (3H, s, NCH_3), 2.86, 3.01 (each 1H, d, J = 17.6 Hz, 6-H), 3.09 (1H, dd, J = 11.6, 1.3 Hz, 2-Hβ), 3.68, 3.81, 3.88 (each 3H, s, OCH_3), 3.96 (1H, dd, J = 11.6, 4.3 Hz, 2-Hα), 4.32 (1H, dd, J = 4.3, 1.3 Hz, 1-H), 4.79 (1H, br s, OH); *m/z* (%) 336 (M^+, 12), 265 (29), 264 (100), 234 (18). Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.44; H, 7.22; N, 8.25.

5-*t*-Butyldimethlsilyloxy-1,2,3,4,5,6-hexahydro-7,9,10-trimethoxy-3,8,11-trimethyl-1,5-imino-3-benzazocin-4-one (**6**) A solution of **5b** (33.6 mg, 0.1 mmol) and DMAP (1.3 mg, 0.015 mmol) in dry pyridine (12 mL) was cooled with ice-water, and TBDMSOTf (68.9 μL, 0.3 mmol) was added dropwise over 5 min. The solution was stirred at 25 °C for 1 h. The reaction mixture was poured into water (10 mL) and

extracted with chloroform (10 mL x 3). The combined extracts were washed with water (10 mL), dried, and concentrated *in vacuo* to give a residue. This material was subjected to chromatography (silica gel, 5 g; elution with 5:1 hexane-ethyl acetate) to give a solid, recrystallization of which from ether gave **6** (36.1 mg, 80.2 %) as colorless prisms, mp 132-135 °C; ν_{max} (KBr) 1665 cm^{-1}; δ_H (relative to chloroform at 7.26 as a standard in this case) 0.15, 0.28 (each 3H, s, $SiCH_3$), 0.93 (9H, s, $C(CH_3)_3$), 2.16 (3H, s, 8-CH_3), 2.35 (3H, s, NCH_3), 2.68 (1H, d, J = 17.5 Hz, 6-H), 2.77 (3H, s, NCH_3), 3.03 (1H, dd, J = 11.2, 1.3 Hz, H-2β), 3.05 (1H, d, J = 17.5 Hz, 6-H), 3.66, 3.78, 3.85 (each 3H, s, OCH_3), 3.90 (1H, dd, J = 11.2, 4.6 Hz, 2-Hα), 4.22 (1H, dd, J = 4.6, 1.3 Hz, 1-H); *m/z* (%) 450 (M^+, 4), 394 (13), 393 (46), 380 (12), 379 (45), 378 (100). Anal. Calcd for $C_{23}H_{38}N_2O_5Si$: C, 61.30; H, 8.50; N, 6.22. Found: C, 61.15; H, 8.45; N, 6.11.

1,2,3,4,5,6-Hexahydro-5-hydroxy-9-methoxy-3,8,11-trimethyl-10-nitro-1,5-imino-3-benzazocin-4-one (**7**)

The alcohol (**5a**)[3] (414.0 mg, 1.5 mmol) was added to a mixture of H_2SO_4-63% HNO_3 (1:1, 3.0 mL) and the whole was stirred for 1 h under ice-cooling. The reaction mixture was diluted with water (20 mL), made alkaline with diluted aqueous 3% NH_4OH, and extracted with chloroform (20 mL x 3). The combined extracts were washed with water (20 mL), dried, and concentrated *in vacuo* to give a solid, recrystallization of which from ethyl acetate gave **7** (449.7 mg, 93.4%) as pale yellow prisms, mp 191-192.5 °C (decomp); ν_{max} (KBr) 3260, 1640, 1530 cm^{-1}; δ_H 2.33 (3H, s, 8-CH_3), 2.42 (3H, s, NCH_3), 2.85 (3H, s, NCH_3), 2.93, 3.03 (each 1H, d, J = 17.8 Hz, 6-H), 3.23 (1H, dd, J = 11.9, 1.3 Hz, 2-Hβ), 3.84 (3H, s, OCH_3), 3.89 (1H, dd, J = 11.9, 4.6 Hz, 2-Hα), 4.10 (1H, dd, J = 4.6, 1.3 Hz, 1-H), 4.42 (1H, br s, OH), 7.06 (1H, s, 7-H); *m/z* (%) 321 (M^+, 9), 250 (22), 249 (100), 203 (10), 202 (12). Anal. Calcd for $C_{15}H_{19}N_3O_5$: C, 56.06; H, 5.96; N, 13.08. Found: C, 55.85; H, 6.04; N, 12.97.

5-*t*-Butyldimethlsilyloxy-1,2,3,4,5,6-hexahydro-9-methoxy-3,8,11-trimethyl-10-nitro-1,5-imino-3-benzazocin-4-one (**8**) A solution of **7** (321.0 mg, 1.0 mmol) and DMAP (12.2 mg, 0.1 mmol) in dry pyridine (10 mL) was cooled in an ice-water, and TBDMSOTf (689 μL, 3.0 mmol) was added dropwise over 5 min. The solution was stirred at 25 °C for 1 h. The reaction mixture was poured into water (10 mL) and extracted with chloroform (10 mL x 3). The combined extracts were washed with water (10 mL), dried, and concentrated *in vacuo* to give a residue (688 mg). This material was subjected to chromatography (silica gel, 12 g; elution with 10:1 hexane-ethyl acetate) to give a solid, recrystallization of which from ether gave **8** (404.6 mg, 92.9 %) as colorless prisms, mp 159.5-161 °C; ν_{max} (KBr) 1665, 1535 cm^{-1}; δ_H (relative to chloroform at 7.26 as a standard in this case) 0.14, 0.25 (each 3H, s, $SiCH_3$), 0.94 (9H, s, $C(CH_3)_3$), 2.31 (3H, s, 8-CH_3), 2.35 (3H, s, NCH_3), 2.80 (3H, s, NCH_3), 2.85, 3.04 (each 1H, d, J = 17.5 Hz, 6-H), 3.14 (1H, dd, J = 11.6, 1.3 Hz, H-2β), 3.83 (3H, s, OCH_3), 3.88 (1H, dd, J = 11.6, 4.6 Hz, 2-Hα), 4.07 (1H, dd, J = 4.6, 1.3 Hz, 1-H), 7.03 (1H, s, 7-H); *m/z* (%) 379 (M^+ - 56, 17), 378 (66), 364 (33), 363 (100). Anal. Calcd for $C_{21}H_{33}N_3O_5Si$: C, 57.90; H, 7.64; N, 9.65. Found: C, 57.73; H, 7.64; N, 9.59.

10-Amino-5-*t*-butyldimethlsilyloxy-1,2,3,4,5,6-hexahydro-9-methoxy-3,8,11-trimethyl-1,5-imino-3-benzazocin-4-one (**9**) A solution of **8** (261.4 mg, 0.6 mmoL) in ethanol (15 ml) was shaken for 3 h at 25 °C under 1 atm of hydrogen in the presence of 20% palladium on carbon (75 mg). The catalyst was removed by filtration and washed with ethanol (100 mL). The combined filtrates were evaporated and the residue was diluted with 5% aqueous 5% $NaHCO_3$ solution (40 mL), and extracted with chloroform (50 mL x 3). The combined extracts were washed with water (50 mL), dried, and concentrated *in vacuo* to give a solid,

recrystallization of which from acetone gave **9** (206.0 mg, 84.6%) as colorless needles, mp 211-214 °C (decomp); ν_{max} (KBr) 3460, 3360, 1655, 1635 cm^{-1}; δ_H (relative to chloroform at 7.26 as a standard in this case) 0.16, 0.28 (each 3H, s, $SiCH_3$), 0.94 (9H, s, $C(CH_3)_3$), 2.22 (3H, s, 8-CH_3), 2.39 (3H, s, NCH_3), 2.79 (3H, s, NCH_3), 2.83, 2.94 (each 1H, d, J = 17.2 Hz, 6-H), 3.14 (1H, dd, J = 10.9, 1.0 Hz, H-2β), 3.66-3.74 (2H, br s, NH_2), 3.73 (3H, s, OCH_3), 3.89 (1H, dd, J = 10.9, 4.3 Hz, 2-Hα), 3.99 (1H, dd, J = 4.3, 1.0 Hz, 1-H), 6.34 (1H, s, 7-H); *m/z* (%) 405 (M^+, 12), 349 (13),348 (49), 335 (13), 334 (48), 333 (100). Anal. Calcd for $C_{21}H_{35}N_3O_3Si \cdot 1/10H_2O$: C, 61.91; H, 8.71; N, 10.31. Found: C, 61.72; H, 8.68; N, 10.08.

Oxidation of **9** with Potassium Nitrosodisulfonate (Fremy's salt) A solution of the amine (**9**) (40.6 mg, 0.1 mmol) in 1,4-dioxane (4 mL) was added quickly to a stirred solution of Fremy's salt (223.6 mg, 0.5 mmol) in aqueous potassium dihydrogen phosphate (0.02 M, 7.5 mL). The reaction mixture was stirred at 25 °C for 1 h, and then at 40 °C for 16 h, poured into water (10 mL), and extracted with chloroform (10 mL x 3). The combined extracts were washed with water, dried, and concentrated *in vacuo* to give a residue (32.6 mg). Chromatography of this on a silica gel (10 g) column with hexane-ethyl acetate (2:1) as an eluent gave **3b** (0.6 mg, 1.4%) as pale yellow powder. Further elution with hexane-ethyl acetate (1:1) as an eluent gave **9** (26.8 mg, 66.0% recovery).

5-*t*-Butyldimethylsilyoxy-9-methoxy-3,8,11-trimethyl-1,2,3,4,5,6,7,10-octahydro-1,5-imino-3-benzazocin-4-one (**3b**) δ_H 0.13, 0.26 (each 3H, s, $SiCH_3$), 0.92 (9H, s, $C(CH_3)_3$), 1.95 (3H, s, 8-CH_3), 2.33 (3H, s, NCH_3), 2.48 (1H, d, J = 20.1 Hz, 6-H), 2.84 (3H, s, NCH_3), 2.91 (1H, d, J = 20.1 Hz, 6-H), 3.01 (1H, dd, J = 11.6, 1.7 Hz, H-2β), 3.88 (1H, dd, J = 11.6, 5.0 Hz, H-2α), 4.00 (3H, s, OCH_3), 4.10 (1H, dd, J = 5.0, 1.7 Hz, 1-H); *m/z* (%) 420 (M^+, 5), 349 (13),348 (100). High-resolution MS Calcd for $C_{21}H_{32}N_2O_5Si$: 420.2081. Found: 420.2078.

5-Acetoxy-1,2,3,4,5,6-hexahydro-9-methoxy-3,8,11-trimethyl-10-nitro-1,5-imino-3-benzazocin-4-one (**10**) A solution of **7** (192.6 mg, 0.6 mmol) and DMAP (29.3 mg, 0.24 mmol) in dry pyridine (3 mL) was cooled in an ice-water, and acetic anhydride (1.2 mL) was added dropwise over 5 min. The solution was stirred at 25 °C for 1 h, then removal of the solvent *in vacuo* afforded a residue, which was partitioned between chloroform (30 mL) and water (30 mL). The organic phase was washed with water (10 mL), dried, and concentrated *in vacuo* to give a solid (223 mg), recrystallization of which from ethyl acetate gave **10** (197.4 mg, 90.6%) as colorless needles, mp 198-200.5 °C (decomp); ν_{max} (KBr) 1775, 1670,1535 cm^{-1}; δ_H 2.14 (3H, s, $COCH_3$), 2.32 (3H, s, NCH_3), 2.35 (3H, s, 8-CH_3), 2.86 (3H, s, NCH_3), 3.09 (2H, s, 6-H_2), 3.19 (1H, dd, J = 11.6, 1.7 Hz, H-2β), 3.84 (3H, s, OCH_3), 3.98 (1H, dd, J = 11.6, 4.3 Hz, 2-Hα), 4.09 (1H, dd, J = 4.3, 1.7 Hz, 1-H), 7.09 (1H, s, 7-H); δ_C 15.9 (q, 8-CH_3), 21.2 (q, $COCH_3$), 29.7 (t, C-6), 34.5 (q, NCH_3), 35.2 (q, NCH_3), 54.0 (t, C-2), 56.1 (d, C-1), 62.4 (q, OCH_3), 87.5 (s, C-5), 122.8 (s), 129.4 (s), 132.9 (s), 133.5 (d, C-7), 145.7 (s), 148.9 (s), 166.2 (s, CO), 168.4 (s, CO); *m/z* (%) 363 (M^+, 8), 292 (8), 291 (42), 250 (27), 249 (100), 233 (19). Anal. Calcd for $C_{17}H_{21}N_3O_6$: C, 56.19; H, 5.83; N, 11.57. Found: C, 56.05; H, 5.77; N, 11.44.

5-Acetoxy-10-amino-1,2,3,4,5,6-hexahydro-9-methoxy-3,8,11-trimethyl-1,5-imino-3-benzazocin-4-one (**11**) A solution of **10** (399.3 mg, 1.1 mmol) in methanol (20 mL) was shaken for 2 h at 25 °C under 1 atm of hydrogen in the presence of 20% palladium on carbon (130 mg). The catalyst was removed by filtration and

washed with methanol (100 mL). The combined filtrates were evaporated and the residue was diluted with 5% aqueous $NaHCO_3$ solution (40 mL), and extracted with chloroform (50 mL x 3). The combined extracts were washed with water (50 mL), dried, and concentrated *in vacuo* to give a solid, recrystallization of which from chloroform-ethyl acetate gave **11** (330.4 mg, 90.2%) as colorless needles, mp 234-235 °C (decomp); ν_{max} (KBr) 3480, 3360, 1755, 1645, 1620 cm^{-1}; δ_H 2.13 (3H, s, 8-CH_3), 2.23 (3H, s, $COCH_3$), 2.34 (3H, s, NCH_3), 2.85 (3H, s, NCH_3), 2.96, 3.09 (each 1H, d, J = 16.8 Hz, 6-H), 3.20 (1H, dd, J = 10.8, 1.0 Hz, H-2β), 3.52-3.79 (2H, br s, NH_2), 3.73 (3H, s, OCH_3), 3.97 (1H, dd, J = 10.8, 4.0 Hz, 2-Hα), 4.03 (1H, dd, J = 4.0, 1.0 Hz, 1-H), 6.38 (1H, s, 7-H); *m/z* (%) 333 (M^+, 32), 261 (30), 220 (28), 219 (100), 204 (11). Anal. Calcd for $C_{17}H_{23}N_3O_4 \cdot 1/10H_2O$: C, 60.92; H, 6.98; N, 12.54. Found: C, 60.87; H, 6.94; N, 12.49.

Oxidation of **11** with Potassium Nitrosodisulfonate (Fremy's salt)

Oxidation at 0° C: A solution of the amine (**11**) (99.9 mg, 0.3 mmol) in methanol (4 mL) was added quickly to a stirred solution of Fremy's salt (402.5 mg, 0.9 mmol) in aqueous potassium dihydrogen phosphate (0.02 M, 10.0 mL). The reaction mixture was stirred at 0 °C for 2 h, then poured into water (10 mL), and extracted with chloroform (20 mL x 3). The combined extracts were washed with water, dried, and concentrated *in vacuo* to give a residue (150.8 mg). Chromatography of this on a silica gel (12 g) column with dichloromethane-methanol (500:1) as an eluent gave **3c** (4.7 mg, 4.5%), and elution with dichloromethane-methanol (200:1) as an eluent gave a solid, recrystallization of which from ethyl acetate-ether gave **12** (70.7 mg, 67.9%) as pale yellow needles, mp 191.192.5 °C. Further elution with dichloromethane-methanol (100:1) as an eluent gave **11** (10.5 mg, 10.5% recovery).

Compound (**12**) ν_{max} (KBr) 3650-3350, 3260, 1765, 1675, 1635, 1610 cm^{-1}; δ_H 1.93 (3H, s, 8-CH_3), 2.07 (3H, s, $COCH_3$), 2.25 (3H, s, NCH_3), 2.65 (1H, d, J = 19.5 Hz, 6-H), 2.83 (3H, s, NCH_3), 2.93 (1H, d, J = 19.5 Hz, 6-H), 3.21 (1H, d, J = 11.9 Hz, H-2β), 3.79 (3H, s, OCH_3), 3.94 (1H, dd, J = 11.9, 4.6 Hz, 2-Hα), 4.41 (1H, d, J = 4.6 Hz, 1-H), 11.07 (1H, br s, NH); δ_C 9.5 (q, 8-CH_3), 21.1 (q, *COCH*$_3$), 25.7 (t, C-6), 34.4 (q, NCH_3), 35.0 (q, NCH_3), 52.7 (t, C-2), 55.4 (d, C-1), 61.6 (q, OCH_3), 88.1 (s, C-5), 125.0 (s), 136.4 (s), 137.8 (s), 154.0 (s), 161.0 (s, C-10), 166.3 (s, CO), 168.5 (s, CO), 186.1 (s, C-7); *m/z* (%) 347 (M^+, 5), 306 (19), 303 (100), 290 (20), 274 (18), 262 (31), 261 (30), 247 (20), 234 (39), 233 (37), 219 (50), 205 (10), 191 (11), 44 (22). Anal. Calcd for $C_{17}H_{21}N_3O_5$: C, 58.78; H, 6.09; N, 12.10. Found: C, 58.51; H, 6.14; N, 11.91.

Oxidation at 25 °C: A solution of the amine (**11**) (299.7 mg, 0.9 mmol) in methanol (12 mL) was added quickly to a stirred solution of Fremy's salt (1.21g, 2.7 mmol) in aqueous potassium dihydrogen phosphate (0.02 M, 30.0 mL). The reaction mixture was stirred at 25 °C for 1 h, poured into water (20 mL), and extracted with chloroform (20 mL x 3). The combined extracts were washed with water, dried, and concentrated *in vacuo* to give a residue (313.2 mg). Chromatography of this on a silica gel (15 g) column with dichloromethane-methanol (500:1) as an eluent gave a solid, recrystallization of which from ethyl acetate-ether gave **3c** (261.6 mg, 83.5%) as pale yellow needles, mp 181.5-184 °C (decomp). Further elution with dichloromethane-methanol (100:1) as an eluent gave **11** (43.1 mg, 14.4% recovery).

5-Acetoxy-9-methoxy-3,8,11-trimethyl-1,2,3,4,5,6,7,10-octahydro-1,5-imino-3-benzazocin-4-one (**3c**) ν_{max} (KBr) 1770, 1670, 1645, 1615 cm^{-1}; δ_H 1.96 (3H, s, 8-CH_3), 2.13 (3H, s, $COCH_3$), 2.29 (3H, s, NCH_3),

2.72 (1H, d, J = 20.1 Hz, 6-H), 2.90 (3H, s, NCH_3), 2.98 (1H, d, J = 20.1 Hz, 6-H), 3.08 (1H, dd, J = 11.9, 1.3 Hz, H-2β), 3.98 (1H, dd, J = 11.9, 4.3 Hz, 2-Hα), 4.02 (3H, s, OCH_3), 4.13 (1H, dd, J = 4.3, 1.3 Hz, 1-H); δ_C 8.9 (q, 8-CH_3), 21.1 (q, $COCH_3$), 25.6 (t, C-6), 34.4 (q, NCH_3), 35.0 (q, NCH_3), 52.1 (t, C-2), 54.5 (d, C-1), 61.0 (q, OCH_3), 87.8 (s, C-5), 129.7 (s), 135.7 (s), 140.5 (s), 155.4 (s), 166.4 (s, CO), 168.4 (s, CO), 182.4 (s, C-10), 185.8 (s, C-7); *m/z* (%) 348 (M^+, 14), 307 (14), 306 (74), 291 (27), 278 (21), 263 (11), 262 (14), 261 (16), 249 (33), 248 (25), 247 (100), 232 (13), 231 (20), 221 (11), 220 (55), 218 (55), 206 (29), 205 (13), 204 (16), 192 (18). Anal. Calcd for $C_{17}H_{20}N_2O_6$: C, 58.61; H, 5.79; N, 8.04. Found: C, 58.567; H, 5.77; N, 7.83.

Transformation of **12** to the quinone (**3c**) A solution of the iminoquinone (**12**) (52.1 mg, 0.15 mmol) in 2N HCl (4 mL) was stirred at 25 °C for 30 min. The reaction mixture was diluted with water (10 mL), made alkaline with 5% aqueous $NaHCO_3$ solution, and extracted with chloroform (10 mL x 3). The combined extracts were washed with water (10 mL), dried, and concentrated *in vacuo* to give a solid (51.9 mg), recrystallization of which from ethyl acetate gave **3c** (46.3 mg, 86.7%) as pale yellow needles, whose spectra were identical with those of the authentic sample described above.

5-Hydroxy-9-methoxy-3,8,11-trimethyl-1,2,3,4,5,6,7,10-octahydro-1,5-imino-3-benzazocin-4-one (**3a**)
A solution of THE acetate (**3c**) (10.44 mmol) in 1N HCl (2 mL) was stirred at 25 °C for 3 h, and then at 60 °C for 3 h. The reaction mixture was diluted with water (10 mL), made alkaline with 5% aqueous $NaHCO_3$ solution, and extracted with chloroform (10 mL x 3). The combined extracts were washed with water (10 mL), dried, and concentrated *in vacuo* to give a solid (7.2 mg), recrystallization of which from ethyl acetate gave **3a** (4.2 mg, 45.8%) as pale yellow needles, mp 194-200 °C (decomp) ν_{max} (KBr) 3400-3050, 1650, 1615 cm^{-1}; δ_H 1.97 (3H, s, 8-CH_3), 2.40 (3H, s, NCH_3), 2.64, 2.81 (each 1H, d, J = 20.1 Hz, 6-H), 2.88 (3H, s, NCH_3), 3.05 (1H, dd, J = 11.9, 1.3 Hz, H-2β), 3.88 (1H, dd, J = 11.9, 4.9 Hz, 2-Hα), 4.03 (3H, s, OCH_3), 4.17 (1H, dd, J = 4.9, 1.3 Hz, 1-H), 4.27 (1H, br s, OH); *m/z* (%) 306 (M^+, 58), 291 (21), 278 (21), 262 (13), 262 (19), 261 (16), 249 (18), 235 (33), 234 (63), 232 (22), 231 (40), 221 (18), 220 (100), 219 (13), 218 (21), 217 (14), 206 (46), 205 (48), 204 (22), 192 (38), 191 (11), 190 (22), 147 (12), 83 (10), 44 (14), 42 (15). Anal. Calcd for $C_{15}H_{18}N_2O_5 \cdot 1/10H_2O$: C, 58.47; H, 5.95; N, 9.09. Found: C, 58.36; H, 5.81; N, 8.97.

1N HCl (1 mL) was added to a stirred solution of **3a** (104.4 mg, 0.3 mmol) in methanol (9 mL), and the resulting solution was heated at reflux for 40 h. The reaction mixture was diluted with water (50 mL), made alkaline with 5% aqueous $NaHCO_3$ solution, and extracted with ethyl acetate (10 mL x 3). The combined extracts were washed with water (10 mL), dried, and concentrated *in vacuo* to give a residue (73.7 mg). Chromatography of this on a silica gel (15 g) column with dichloromethane-methanol (200:1) as an eluent gave **20** (7.0 mg, 6.7% recovery), and elution with dichloromethane-methanol (100:1) as an eluent gave a solid, recrystallization of which from ethyl acetate-ether gave **13** (42.3 mg, 42.0%) as pale yellow needles, mp 158-159 °C. Further elution with dichloromethane-methanol (80:1) as an eluent gave **3a** (32.7 mg, 35.6%).

5,9-Dimethoxy-3,8,11-trimethyl-1,2,3,4,5,6,7,10-octahydro-1,5-imino-3-benzazocin-4-one (**13**)
ν_{max} (KBr) 3610, 3560, 3500-3200, 1650, 1615 cm^{-1}; δ_H 2.00 (3H, s, 8-CH_3), 2.59 (3H, s, NCH_3), 2.88 (3H, s, NCH_3), 2.94 (1H, dd, J = 12.5, 1.0 Hz, H-2β), 3.74 (3H, s, 6-OCH_3), 3.96 (1H, dd, J = 12.5, 5.3

Hz, 2-Hα), 4.00 (3H, s, OCH_3), 4.03 (1H, s, 6-H), 4.23 (1H, dd, J = 5.3, 1.0 Hz, 1-H); δ_C 8.9 (q, 8-CH_3), 34.8 (q, NCH_3), 36.1 (q, NCH_3), 49.9 (t, C-2), 52.9 (d, C-1), 61.0 (q, OCH_3), 62.4 (q, OCH_3), 73.1 (d, C-6), 85.6 (s, C-5), 130.3 (s), 137.3 (s), 139.3 (s), 155.4 (s), 169.3 (s, CO), 182.5 (s, C-10), 186.1 (s, C-7);*m/z* (%) 336 (M^+, 100), 321 (37), 250 (16), 234 (15), 221 (23), 220 (38), 219 (99), 218 (32), 206 (66), 205 (37), 204 (38), 191 (17), 190 (22), 42 (16). Anal. Calcd for $C_{16}H_{20}N_2O_6 \cdot 1/2H_2O$: C, 55.65; H,6.13; N, 8.11. Found: C, 55.77; H, 6.21; N, 8.33.

Reaction of **3a** with 1N HCl-methanol

1N HCl (1 mL) was added to a stirred solution of the quinone (**3a**) (9.2 mg, 0.03 mmol) in methanol (9 mL), and the resulting solution was heated under reflux for 5 days. The reaction mixture was diluted with water (50 mL), made alkaline with 5% aqueous $NaHCO_3$ solution, and extracted with ethyl acetate (10 mL x 3). The combined extracts were washed with water (10 mL), dried, and concentrated *in vacuo* to give a solid (8.9 mg), recrystallization of which from ethyl acetate-ether gave **13** (8.3 mg, 82.2%), whose spectra were identical with those of the authentic sample described above.

In vitro Cytotoxicity Assays

In vitro antitumor activity was determined using a cultured cell line of L1210 mouse leukemia. Cells were maintained in suspension culture using Eagle's minimum essential medium with Hank's salt (Gibco Laboratories, New York) supplemented with 10% fetal calf serum (Gibco Laboratories). Cells were suspended at a concentration of 3 x 10^4 cells/mL and incubated at 37 °C for 24 h to initiate exponential growth. These cultures were exposed to a series of 10-fold dilutions of samples. After incubation for 3 days, the cell concentrations were determined by electronic counting with a Toa Micro Counter (Toa Electronics, Ltd., Tokyo). The percent growth-inhibition (I) at each dose level was calculated according to the formula.

$I = (C_3 - T_3)/(C_3 - C_0) \times 100$: T_3 (the cell concentration in the culture tube with each dilution of drugs after 3 days); C_3 (the cell concentration in the corresponding control); C_0 (the cell concentration in the culture tube before 3 days of incubation). C_0 was within the range of 0.8 x 10^5 ~ 1.04 x 10^5 cells/mL. C_3 was within the range 1.04 x 10^6 ~ 1.18 x 10^6 cells/mL. ID_{50} (50% inhibition dose) was determined by interpolation (ip).

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5 In our total synthesis of (±)-saframycin B, we found it best in terms of the product yield to employ partial demethylation with boron tribromide followed by oxidative demethylation procedure to convert the polymethoxyarene to a *p*-quinone system; A. Kubo, N. Saito, H. Yamato, K. Masubuchi, and M. Nakamura, *J. Org. Chem.*, 1988, **53**, 4295. However, treating **6** with boron tribromide in dichloromethane allowed only an unstable polymeric material to be obtained.

6 Nitration of **4a** with HNO_3-H_2SO_4 at 0 °C for 2 h afforded the corresponding 10-nitro derivative in 80% yield; N. Saito, Y. Obara, M. Azumaya, and A. Kubo, *Chem. Pharm. Bull.*, 1992, **40**, 2620. Treatment of this compound with 10 equiv of nitrobenzene in THF at -78 °C in the presence of 5 equiv of *s*-BuLi provided the alcohol (**7**) in 11% yield.

7 Stereochemistry of the methoxyl group in **13** was assigned based upon the clear observation of nuclear Overhauser effect (NOE) between 6-OMe (δ 3.74) and 11-Me (δ 2.59).

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