CATALYTIC HYDROGENATION OF 8-ACYLOXY-1-CYANOISOQUINOLINE AND SYNTHESIS OF 9-METHOXY-9-DEETHOXYCRIBROSTATIN 6

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Abstract - Catalytic hydrogenation of 8-acyloxy-1-cyanoisoquinolines (6) – (9) in the presence of 10% Pd-C and synthesis of 9-methoxy isomer (10) of cribrostatin 6 utilizing the compound (26) obtained by this reaction are described.

During the past twenty years, a series of structurally fascinating and biologically active 5,8-isoquinolinedione alkaloids have been isolated from marine sources. Recently Pettit and colleagues have reported isolation and structure determination of cribrostatin 6(1), a dark blue cancer cell growth inhibitor from the marine sponge Cribrochalinasp. on the basis of several spectral data and X-Ray crystal structure analyses. Compound (1) possesses an isoquinolinedione skeleton like cribrostatin 1(2) and 2(3), and inhibits the growth of a number of pathogenic bacteria and fungi. Therefore, the synthesis and bioactivity of compound (1) and its isomers are of interest.

Previously we have reported the catalytic hydrogenation of 1-cyano-7-methoxy-6-methyl-
8-nitroisoquinoline (4) in the presence of 10% Pd-C, which leads to formation of the interesting heterocyclic lactam, 8-methoxy-7-methylpyrolo[2,3,4-ij]isoquinoline-2(1H)-one (5), and the first synthesis of 2 and 3. In furtherance of our study we now report catalytic hydrogenation of 8-acyloxy-1-cyanoisoquinolines (6) over 10% Pd-C, which leads to formation of the interesting heterocyclic lactam, 8-methoxy-7-methylpyrolo[2,3,4-ij]isoquinoline-2(1H)-one (5), and the first synthesis of 2 and 3.

8-Benzylloxy-1-cyanoisoquinoline (16) was prepared from phenol (11) in five steps via isoquinoline (15), according to the modified Pomeranz-Fritsch isoquinoline synthesis. Benzylolation of 11 with benzyl bromide and K₂CO₃ in DMF afforded the benzyloxyaldehyde (12) in 85% yield. Reaction of 12 and aminoacetaldehyde dimethylacetal in benzene for 3 h followed by reduction with NaBH₄ in MeOH for 1 h afforded the amino compound (13) in 88% yield. Treatment of 13 with tosyl chloride in pyridine for 16 h gave the N-tosyl compound (14) in 70% yield. Cyclization of 14 with 6M HCl in dioxane for 3 h followed by desylation with potassium tert-butoxide in tert-butyl alcohol for 1 h under reflux afforded the isoquinoline (15) in 50% yield. Treatment of 15 with m-chloroperoxybenzoic acid in CH₂Cl₂ for 12 h followed by trimethylsilyl cyanide in 1-methyl-2-pyrrolidinone for 2.5 h gave the 1-cyanoisoquinoline (16) in 53% yield (Scheme 1).
MeOH afforded the 8-hydroxy-1-cyanoisoquinolines (17) and (19) in 94% and 78% yields, respectively. Acylation of 17 and 19 with acetyl chloride in pyridine gave 6a and 7a in 91% and 87% yield and with benzoyl chloride afforded 6b and 7b in 83% and 96% yields, respectively. Catalytic hydrogenation of 6a,b and 7a,b over 10% Pd-C in MeOH containing HCl resulted in the intramolecular transfer of the acyl group from the oxygen atom to the nitrogen atom, furnishing the corresponding amides (20a,b and 21a,b) in 90%, 75% and 45%, 45% yields, respectively (Scheme 2).

In case of 1-cyano-5,8-diacetoxyisoquinoline (8), similar conditions afforded compound (22) in 80% yield. The structure of 22 was decided as follows. Benzylation of 22 afforded 23 in 87% yield. Hydrolysis of 23 with HCl in MeOH for 20 h gave 24 in 50% yield. Treatment of 24 with diazomethane in ether for 24 h afforded 25 in 73% yield. The compound obtained by catalytic hydrogenation of 25 was identical to 20a.

Hydrolysis of 8 with HCl in MeOH gave the 8-acetoxy-5-hydroxyisoquinoline (9) in 55% yield. Structure of 9 was confirmed by O-methylation with diazomethane in ether giving 6a. Catalytic hydrogenation of 9 afforded the 1-acetylaminomethylisoquinoline-5,8-dione (26) in 67% yield. Treatment of 26 with POCl₃ in toluene at 110°C for 15 min afforded 10 in 26% yield (Scheme 3).

In summary, the catalytic hydrogenation of 8-acyloxy-1-cyanoisoquinolines over 10% Pd-C in MeOH containing HCl resulted in the intramolecular transfer of the acyl group from the oxygen atom to the nitrogen atom, furnishing the corresponding amides and 9-methoxy isomer (10) of criblestatin 6 (1) was synthesized by this method.
EXPERIMENTAL

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. $^1$H-NMR spectra at 100 MHz and 270 MHz were measured in CDCl$_3$ or CD$_3$OD+CDCl$_3$ with tetramethylsilane as an internal standard. Anhydrous sodium sulfate was used for drying organic solvent extracts, and the solvent was removed with a rotary evaporator and finally under high vacuum. Column chromatography (flash chromatography) was performed with silica gel 60 (Merck, 230-400 mesh).

2-Benzyloxy-3,5-dimethoxy-4-methylbenzaldehyde (12)

Benzyl bromide (178 mL, 1.04 mmol) and K$_2$CO$_3$ (207 mg, 1.5 mmol) were added to a solution of 2-hydroxy-3,5-dimethoxy-4-methylbenzaldehyde (11) (196 mg, 1 mmol) in DMF (7 mL). The whole was stirred at rt for 20 h, poured into water (40 mL) and extracted with CHCl$_3$ (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 5 : 1) to afford benzyloxyaldehyde (12) (243 mg, 85%, colorless oil). HRMS Calcd for C$_{17}$H$_{18}$O$_4$: 286.1205, Found: 286.1206. Ms m/z (%): 286(M$^+$, 30), 258(22), 195(100), 91(80). $^1$H-NMR( CDCl$_3$ ) δ: 2.21(3H, s), 3.83(3H, s), 3.90(3H, s), 5.12(2H, s), 7.01(1H, s), 7.36(5H, s), 10.18(1H, s).

N-(2-Benzyloxy-3,5-dimethoxy-4-methylbenzyl)-2,2-dimethoxyethylamine (13)

Aminoacetoaldehyde dimethylacetal (266 mg, 2.53 mmol) was added to a solution of benzyloxyaldehyde (12) (660 mg, 2.3 mmol) in benzene (10 mL). The mixture was refluxed in a Dean-Stark apparatus until no further water appeared and the solvent removed under vacuum. The residue was dissolved in MeOH (10 mL) and NaBH$_4$ (87 mg, 2.3 mmol) was added in portions with stirring. The mixture was stirred for an additional 1 h at rt, then diluted with water (40 mL) and extracted with CHCl$_3$ (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with ethyl acetate) to afford amino compound (13) (759 mg, 88%, colorless oil). HRMS Calcd for C$_{21}$H$_{29}$NO$_5$: 375.2046, Found: 375.2043. Ms m/z (%): 375(M$^+$, 25), 300(47), 271(100), 181(29), 91(44). $^1$H-NMR( CDCl$_3$ ) δ: 1.62(1H, s), 2.13(3H, s), 2.67(2H, d, J=6 Hz), 3.31(6H, s), 3.70(2H, s), 3.79(3H, s), 3.80(3H, s), 3.81(3H, s), 4.42(1H, t, J=6 Hz), 4.96(2H, s), 6.56(1H, s), 7.2 - 7.6(5H, m).

N-(2-Benzyloxy-3,5-dimethoxy-4-methylbenzyl)-2,2-dimethoxy-N-tosylethylamine (14)

p-Toluenesulfonyl chloride(492 mg, 2.59 mmol) was added to a solution of 13 (810 mg, 2.16 mmol) in pyridine (3 mL) in portions with stirring. The mixture was stirred for an additional 16 h at rt, then diluted with water (50 mL) and extracted with Et$_2$O (3 x 20 mL). The extract was washed with 1% HCl, brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford N-tosyl compound (14) (800 mg, 70%, colorless oil). HRMS Calcd for C$_{28}$H$_{35}$NO$_7$: 529.2134, Found: 529.2133. Ms m/z (%): 529(M$^+$, 19), 438(100), 406(73), 251(46), 91(47). $^1$H-NMR( CDCl$_3$ ) δ: 2.11(3H, s), 2.40(3H, s), 3.18(6H, s), 3.20(2H, d, J=6 Hz), 3.68(3H, s), 3.78 (3H, s), 4.38 (2H, s), 4.90(2H, s), 4.95(1H, t, J=6 Hz), 6.51(1H, s), 7.23(1H, d, J=8 Hz), 7.34(5H, s), 7.66(1H, d, J=8 Hz).
8-Benzyl-30xy-5,7-dimethoxy-6-methylisoquinoline (15)
The N-tosyl compound (14) (265 mg, 0.5 mmol) in dioxane (6.5 mL) was treated with 6 N HCl (0.5 mL). The solution was boiled under reflux for 3 h, then poured into water (50 mL), basified with 5% aqueous NaHCO₃ solution and extracted with Et₂O (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was dissolved in tert-butyl alcohol (1.1 mL) and potassium tert-butoxide (163 mg, 1.46 mmol) was added. The mixture was refluxed for 1 h and poured into water (50 mL), and extracted with CHCl₃ (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford isoquinoline (15) (78 mg, 50%, colorless oil). HRMS Calcd for C₁₉H₁₉NO₃: 309.1365, Found: 309.1388. Ms m/z (%): 309(M⁺, 17), 218(100), 91(42).

1-H-NMR( CDCl₃) ́: 2.40(3H, s), 3.82(3H, s), 3.94(3H, s), 5.12 (2H, s), 7.2 - 7.6(5H, m), 7.69(1H, d, J=6 Hz), 8.89(1H, d, J=6 Hz), 9.42(1H, s).

8-Benzyl-1-cyano-5,7-dimethoxy-6-methylisoquinoline (16)
A solution of 80% m-chloroperoxybenzoic acid (1.62 g, 7.5 mmol) in CH₂Cl₂ (40 mL) was added drop wise to 15 (1.55 g, 5 mmol) in CH₂Cl₂ (20 mL) with stirring. The mixture was stirred at rt for 12 h, washed with 2% aqueous NaHCO₃ solution and brine, then dried and concentrated. The residue was dissolved in 1-methyl-2-pyrrolidinone (13 mL) and trimethylsilyl cyanide (5.5 mL) was added. The mixture was stirred at 50 ~ 60 °C for 2.5 h and poured into water (50 mL), and extracted with CHCl₃ (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 2 : 1) to afford 1-cyanoisoquinoline (16) (0.89 g, 53%). mp 113-114.5 °C (colorless prisms from CHCl₃-hexane). Anal. Calcd for C₂₀H₁₈N₂O₃: C, 71.84 %, H, 5.43 %, N, 8.38. Found: C, 72.08 ; H, 5.25 ; N, 8.16. IR(KBr) cm⁻¹: 2230. ¹H-MNR( CDCl₃) ́: 2.41(3H, s), 3.84(3H, s), 3.89(3H, s), 5.23(2H, s), 7.2 - 7.4(3H, m), 7.5 - 7.7(2H, m), 7.90(1H, d, J=6 Hz), 8.45(1H, d, J=6 Hz). Ms m/z (%): 334(M⁺, 45), 243(64), 215(51), 91(100).

1-Cyano-8-hydroxy-5,7-dimethoxy-6-methylisoquinoline (17)
The 8-benzyloxy-1-cyanoisoquinoline (16) (600 mg, 1.79 mmol) in MeOH (30 mL) was hydrogenated at 1 atm for 1 h using 10% Pd-C (200 mg) as a catalyst. The catalyst was filtered off and the filtrate was poured into water (100 mL), and extracted with CHCl₃ (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was recrystallized from benzene to give 1-cyano-8-hydroxyisoquinoline (17) (410 mg, 94%) as a yellow needles. mp 155-156.5 °C. Anal. Calcd for C₁₃H₁₂N₂O₂: C, 63.92 ; H, 4.95 ; N, 11.74. Found: C, 63.99 ; H, 4.85 ; N, 11.25. IR(KBr) cm⁻¹: 3200, 2220. ¹H-MNR( CDCl₃) ́: 2.43(3H, s), 3.84(3H, s), 3.89(3H, s), 4.6 - 5.3(1H, br), 7.98 (1H, d, J=6 Hz), 8.56(1H, d, J=6 Hz). Ms m/z (%): 244(M⁺, 100), 229(93), 201(20).

1-Cyano-8-hydroxy-7-methoxy-5,7-dimethoxy-6-methylisoquinoline (19)
Catalytic hydrogenation of 8-benzyloxy-1-cyanoisoquinoline (18) was carried out by the same procedure as used for 16. 78% yield. mp 176-178 °C (yellow needles from CHCl₃). Anal. Calcd for C₁₅H₁₈N₂O₂: C, 65.99 ; H, 4.03 ; N, 13.99. Found: C, 65.93 ; H, 3.83 ; N, 13.82.
IR (KBr) cm\(^{-1}\): 3240, 2230. \(^1\)H-NMR (CD\(_3\)OD+CDCl\(_3\)) \(\delta\): 4.06(3H, s), 7.37(1H, d, \(J = 8\) Hz), 7.57(1H, d, \(J = 8\) Hz), 7.73(1H, d, \(J = 6\) Hz), 8.40(1H, d, \(J = 6\) Hz). Ms \(m/\ell\) (%): 200(M\(^+\), 100), 185(83), 157(40).

8-Acetyloxy-1-cyano-5,7-dimethoxy-6-methylisoquinoline (6a)
Acetyl chloride (474 mg, 3 mmol) was added to a solution of 8-hydroxyisoquinoline (17) (488 mg, 2 mmol) in pyridine (5 mL) with stirring at 0 °C. The whole was stirred at 5-10 °C for 1 h, poured into cold water (50 mL). The precipitated crystals were collected and recrystallized from acetone-hexane to give 6a (521 mg, 91%) as a colorless prisms. mp 151-152 °C. Anal. Calcd for C\(_{15}\)H\(_{14}\)N\(_2\)O\(_4\): C, 62.93; H, 4.93; N, 9.79. Found: C, 62.85; H, 4.76; N, 9.70. IR (KBr) cm\(^{-1}\): 2310, 1775. \(\text{\(^1\)H-MNR (CDCl}_3\)} \(\delta\): 2.43(3H, s), 7.37(1H, d, \(J = 8\) Hz), 7.57(1H, d, \(J = 8\) Hz), 7.73(1H, d, \(J = 6\) Hz), 8.40(1H, d, \(J = 6\) Hz). Ms \(m/\ell\) (%): 200(M\(^+\), 100), 185(83), 157(40).

8-Benzoyloxy-1-cyano-5,7-dimethoxy-6-methylisoquinoline (6b)
Acylation of 6b, 7a, 7b were carried out by the same procedure as used for 6a.

8-Benzoyloxy-1-cyano-5,7-dimethoxy-6-methylisoquinoline (6b)
83% yield. mp 152-153.5 °C (colorless prisms from acetone). Anal. Calcd for C\(_{20}\)H\(_{16}\)N\(_2\)O\(_4\): C, 68.96; H, 4.63; N, 8.04. Found: C, 69.12; H, 4.48; N, 7.95. IR (KBr) cm\(^{-1}\): 2220, 1745. \(\text{\(^1\)H-MNR (CDCl}_3\)} \(\delta\): 2.48(3H, s), 3.90(3H, s), 7.5 - 7.8(3H, m), 8.08(1H, d, \(J = 6\) Hz), 8.3 - 8.5(2H, m), 8.61(1H, d, \(J = 6\) Hz). Ms \(m/\ell\) (%): 348(M\(^+\), 6), 105(100), 77(18).

8-Acetyloxy-1-cyano-7-methoxyisoquinoline (7a)
87% yield. mp 141.5-143.5 °C (colorless needles from acetone-hexane). Anal. Calcd for C\(_{13}\)H\(_{10}\)N\(_2\)O\(_3\): C, 64.46; H, 4.16; N, 11.57. Found: C, 64.33; H, 4.02; N, 11.41. IR (KBr) cm\(^{-1}\): 2220, 1745. \(\text{\(^1\)H-MNR (CDCl}_3\)} \(\delta\): 2.48(3H, s), 3.94(3H, s), 7.53(1H, d, \(J = 8\) Hz), 7.73(1H, d, \(J = 8\) Hz), 8.43(1H, d, \(J = 6\) Hz). Ms \(m/\ell\) (%): 242(M\(^+\), 3), 200(100), 185(31).

8-Benzoyloxy-1-cyano-7-methoxyisoquinoline (7b)
96% yield. mp 173-174 °C (colorless prisms from acetone-hexane). Anal. Calcd for C\(_{18}\)H\(_{12}\)N\(_2\)O\(_3\): C, 71.04; H, 3.98; N, 9.21. Found: C, 71.02; H, 3.71; N, 9.28. IR (KBr) cm\(^{-1}\): 2220, 1745. \(\text{\(^1\)H-MNR (CDCl}_3\)} \(\delta\): 3.92(3H, s), 7.4 - 7.7(3H, m), 7.87(1H, d, \(J = 8\) Hz), 8.2 - 8.4(2H, m). Ms \(m/\ell\) (%): 304(M\(^+\), 4), 105(100), 77(26).

1-Acetylaminomethyl-8-hydroxy-5,7-dimethoxy-6-methylisoquinoline (20a)
The 8-acetyloxy-1-cyanoisoquinoline (6a) (87 mg, 0.3 mmol) in MeOH (15 mL) containing concentrated HCl (0.15 mL) was hydrogenated at 1 atm for 3 h using 10% Pd-C (87 mg) as a catalyst. The catalyst was filtered off, the filtrate was poured into water (60 mL), adjusted to pH 7 with saturated aqueous NaHCO\(_3\) and extracted with CHCl\(_3\) (3 x 20 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1 : 1) to afford 1-acetylaminomethylisoquinoline (20a) (78 mg, 90%). mp 149-151 °C (colorless prisms from Et\(_2\)O-hexane). Anal. Calcd for C\(_{15}\)H\(_{18}\)N\(_2\)O\(_4\): C, 62.05; H, 6.25; N, 9.65. Found: C, 62.35; H, 6.20; N, 9.65. IR (KBr) cm\(^{-1}\): 3380, 3680 - 2880, 1640. \(\text{\(^1\)H-MNR (CDCl}_3\)} \(\delta\): 2.18(3H, s), 5.34(2H, d, \(J = 5\) Hz), 5.9 - 6.6(1H, br), 7.66(1H, d, \(J = 6\) Hz), 7.9 - 8.1(1H, br s), 8.28(1H, d, \(J = 6\) Hz). Ms \(m/\ell\) (%): 290(M\(^+\), 65), 275(50), 216(100).
Catalytic hydrogenation of 20b, 21a, 21b, 22 were carried out by the same procedure as used for 20a.

1-Benzoylaminomethyl-8-hydroxy-5,7-dimethoxy-6-methylisoquinoline (20b)  
75% yield. mp 196-197 °C (colorless prisms from CHCl₃-benzene). Anal. Calcd for C₂₀H₂₀N₂O₄: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.06; H, 5.81; N, 7.97. IR(KBr) cm⁻¹: 3340, 3290, 1655. ¹H-MNR (CDCl₃): 2.43(3H, s), 3.84(6H, s), 5.52(2H, d, J=4 Hz), 7.4 - 7.6(3H, m), 7.71(1H, d, J=6 Hz), 8.01(1H, dd, J=8, 1.5 Hz), 8.34(1H, d, J=6 Hz), 8.81(1H, br s). Ms m/z (%): 352(m⁺, 53), 337(31), 247(26), 105(100), 77(32).

1-Acetylaminomethyl-8-hydroxy-7-methoxyisoquinoline (21a)  
45% yield. mp 61.5-63.5 °C (colorless needles from MeOH). Anal. Calcd for C₁₃H₁₄N₂O₃·1/₅H₂O: C, 62.43; H, 5.81; N, 11.21. Found: C, 62.42; H, 5.59; N, 11.11. IR(KBr) cm⁻¹: 3400, 3250, 1640. ¹H-NMR (CD₃OD+CDCl₃): 2.15(3H, s), 4.02(3H, s), 5.26(2H, s), 7.35(1H, d, J=8.6 Hz), 7.44(1H, d, J=5.6 Hz), 7.46(1H, d, J=8.6 Hz), 8.17(1H, d, J=5.6 Hz). Ms m/z (%): 246(M⁺, 94), 203(100), 186(89), 172(59).

1-Benzoylaminomethyl-8-hydroxy-7-methoxyisoquinoline (21b)  
45% yield. mp 195-196 °C (colorless prisms from CHCl₃-MeOH). Anal. Calcd for C₁₈H₁₆N₂O₃: C, 70.11; H, 5.23; N, 9.09. Found: C, 70.07; H, 5.14; N, 9.15. IR(KBr) cm⁻¹: 3600 - 2900, 3230, 1640. ¹H-NMR (CD₃OD+CDCl₃): 3.95(3H, s), 5.42(2H, s), 7.3 - 7.6(6H, m), 7.8 - 8.0(2H, m), 8.18(1H, d, J=6 Hz). Ms m/z (%): 308(M⁺, 60), 203(100), 186(47), 105(58), 77(44).

1-Acetylaminomethyl-5-acetyloxy-8-hydroxy-7-methoxy-6-methylisoquinoline (22)  
80% yield. mp 220-222 °C (decomp) (colorless prisms from CHCl₃-MeOH). Anal. Calcd for C₁₆H₁₈N₂O₅: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.07; H, 5.72; N, 8.64. IR(KBr) cm⁻¹: 3360, 1760, 1660. ¹H-NMR (CD₃OD+CDCl₃): 2.12(3H, s), 2.27(3H, s), 2.46(3H, s), 3.85(3H, s), 5.17(2H, s), 5.21(2H, d, J=5 Hz), 7.2 - 8.1(7H, m), 8.12(1H, d, J=6 Hz). Ms m/z (%): 318(M⁺, 62), 300(28), 275(31), 233(55), 216(100).

1-Acetylaminomethyl-5-acetyloxy-8-benzyloxy-7-methoxy-6-methylisoquinoline (23)  
Benzyl bromide (36 µL, 0.3 mmol) and K₂CO₃ (41 mg, 0.3 mmol) were added to a solution of 22 (64 mg, 0.2 mmol) in DMF (1.5 mL). The whole was stirred for 1 h at 0 °C, then for additional 15 h at rt, poured into water (10 mL) and extracted with CHCl₃ (3 x 3 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1:1) to afford 23 (71 mg, 87%). mp 134-135 °C (colorless powder from CHCl₃-hexane). Anal. Calcd for C₂₃H₂₄N₂O₅: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.82; H, 5.83; N, 6.91. IR(KBr) cm⁻¹: 3400, 3320, 1755, 1640. ¹H-NMR (CDCl₃): 2.10(3H, s), 2.28(3H, s), 2.46(3H, s), 3.85(3H, s), 5.17(2H, s), 5.21(2H, d, J=5 Hz), 7.2 - 7.8(7H, m), 8.24(1H, d, J=6 Hz). Ms m/z (%): 408(M⁺, 36), 317(49), 275(97), 216(92), 91(100).

1-Acetylaminomethyl-8-benzyloxy-5-hydroxy-7-methoxy-6-methylisoquinoline (24)  
A saturated methanol (6.8 ml) with HCl was added to a solution of 23 (88 mg, 0.22 mmol) in MeOH (3.4 mL). The whole was stirred at rt for 20 h, poured into water (40 mL) and
adjusted to pH 7 with saturated aqueous NaHCO₃. The precipitated crystals were collected by filtration and chromatographed (eluting with hexane-ethyl acetate 1:1) to afford 24 (40 mg, 50%). mp 153-155 °(colorless powder from CHCl₃-MeOH). Anal. Calcd for C₂₁H₂₂N₂O₄·1/10H₂O: C, 68.50; H, 6.08; N, 7.61. Found: C, 68.34; H, 5.96; N, 7.49. IR(KBr) cm⁻¹: 3640 - 2840, 3370, 3230, 1650. ¹H-NMR (CDCl₃+CDCl₃) δ: 2.09(3H, s), 2.37(3H, s), 3.98(3H, s), 5.12(2H, s), 5.19(2H, s), 7.3 - 7.8(5H, m), 7.92(1H, d, J = 6 Hz), 8.24(1H, d, J = 6 Hz). Ms m/z (%): 366(M⁺, 18), 334(27), 291(34), 275(59), 233(58), 216(100), 91(80).

1-Acetylaminomethyl-8-benzyloxy-5,7-dimethoxy-6-methylisoquinoline (25)
5-Hydroxyisoquinoline (24) (29 mg, 0.08 mmol) was added to an ether solution containing excess of CH₂N₂ and the mixture was stirred at rt for 24 h. The solvent was evaporated and the residue was chromatographed (eluting with hexane-ethyl acetate 1:1) to afford 25 (22 mg, 73%). mp 95-96.5 °(colorless prisms from CHCl₃-hexane). Anal. Calcd for C₂₂H₂₄N₂O₄·1/2H₂O: C, 69.45; H, 6.36; N, 6.36. Found: C, 69.34; H, 6.37; N, 7.34. IR(KBr) cm⁻¹: 3360, 1670. ¹H-NMR (CDCl₃) δ: 2.10(3H, s), 2.37(3H, s), 3.82(3H, s), 3.85(3H, s), 5.10(2H, s), 5.17(2H, s), 7.3 - 7.5(3H, m), 7.5 - 7.8(4H, m), 8.23(1H, d, J = 6 Hz). Ms m/z (%): 380(M⁺, 25), 289(53), 247(58), 230(100), 91(38).

Catalytic hydrogenation of 25 was carried out by the same procedure as used for 16 in 60% yield and the spectral data(IR, ¹H-NMR) of a compound thus obtained match those of 20a.

8-Acetyloxy-1-cyano-5-hydroxy-7-methoxy-6-methylisoquinoline (9)
5,8-Diacetyloxyisoquinoline (8) (16 mg, 0.05 mmol) was added to a saturated methanol (4 mL) with HCl. The whole was stirred at rt for 5 day, poured into water (20 mL), adjusted to pH 9 with saturated aqueous NaHCO₃ and extracted with CHCl₃ (3 x 5 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1:2) to afford 26 (6 mg, 44%). mp 215-216 °(light yellow needles from MeOH-CHCl₃-hexane). Anal. Calcd for C₁₄H₁₂N₂O₄: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.89; H, 4.26; N, 10.34. IR(KBr) cm⁻¹: 3390, 2230, 1780. ¹H-NMR (CDCl₃+CDCl₃) δ: 2.37(3H, s), 2.55(3H, s), 3.89(3H, s), 8.22(1H, d, J = 6 Hz), 8.47(1H, d, J = 6 Hz). Ms m/z (%): 272(M⁺, 1), 230(100), 215(44).

O-Methylation of 9 was carried out by the same procedure as used for 24 in 68% yield and the spectral data(IR, ¹H-NMR) of a compound thus obtained match those of 6a.

1-Acetylaminomethyl-7-methoxy-6-methyl-5,8-isoquinolinedione (26)
8-Acetoxy-1-cyanoisoquinoline (9) (6 mg, 0.02 mmol) in MeOH (3 mL) containing concentrated HCl (30 μL) was hydrogenated at 1 atm for 2 h using 10% Pd-C (12 mg) as a catalyst. The catalyst was filtered off, the filtrate was poured into water (15 mL), adjusted to pH 7 with 1 % aqueous NaHCO₃ and extracted with CHCl₃ (3 x 5 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 1:2) to afford 5,8-isoquinolinedione (26) (4 mg, 67%). mp 149-151 °(light yellow needles from CHCl₃-hexane). HRMS Calcd for C₁₄H₁₄N₂O₄: 274.0954, Found: 274.0962. IR(KBr) cm⁻¹: 3290, 1665, 1645. ¹H-NMR (CDCl₃) δ: 2.08(3H, s), 2.12(3H, s),
4.20(3H, s), 5.06(2H, d, $J = 5$ Hz), 7.2 - 7.4(1H, br), 7.87(1H, d, $J = 5$ Hz), 8.84(1H, d, $J = 5$ Hz).

$Ms \, m/z$ (%): 274(M$^+$, 55), 231(100), 43(35).

9-Methoxy-9-deethoxycribrostatin 6 (10)
5,8-Isoquinolinedione (26) (79 mg, 0.29 mmol) in toluene (4 mL) was treated with POCl$_3$ (552 mg, 3.6 mmol). The solution was stirred at 110 $\degree$C for 15 min, then poured into cold water (30 mL), adjusted to pH 7 with saturated aqueous NaHCO$_3$ and extracted with CHCl$_3$ (3 x 10 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 2 : 1) to afford 10 (19 mg, 26%). $mp$ 300 $\degree$C (dark blue powder from acetone). $Anal.$ Calcd for C$_{14}$H$_{12}$N$_2$O$_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.60; H, 4.55; N, 10.95. $IR$(KBr) cm$^{-1}$: 1665, 1635. $^1$H-MNR (CDCl$_3$) $\delta$: 2.06(3H, s), 2.70(3H, s), 4.13(3H, s), 7.10(1H, d, $J = 8$ Hz), 7.80(1H, d, $J = 8$ Hz), 8.18(1H, s).

$Ms \, m/z$ (%): 256(M$^+$, 100), 213(22).

REFERENCES