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CATALYTIC HYDROGENATION OF 8-ACYLOXY-1-CYANOISO-QUINOLINE AND SYNTHESIS OF 9-METHOXY-9-DEETHOXY-CRIBROSTATIN 6

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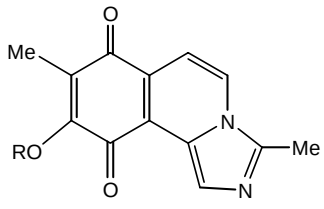
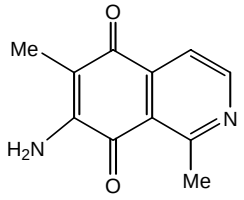
Abstract - Catalytic hydrogenation of 8-acyloxy-1-cyanoisoquinolines (**6**) ~(**9**) in the presence of 10% Pd-C and synthesis of 9-methoxy isomer (**10**) of cribrostatin 6 utilizing the compound (**26**) obtained by this reaction are described.

During the past twenty years, a series of structurally fascinating and biologically active 5,8-isoquinolinedione alkaloids have been isolated from marine sources.[1] 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 *Cribrochalina* sp. on the basis of several spectral data and X-Ray crystal structure analyses.[2] 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.

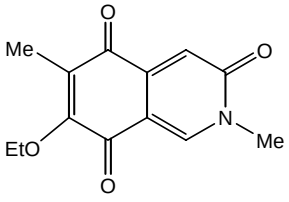
Figure 1



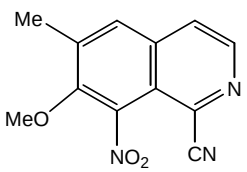
1 R = Et Cribrostatin 6
10 R = Me 9-Methoxy-9-deethoxy-cribrostatin 6



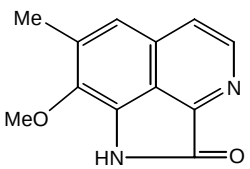
2 Cribrostatin 1



3 Cribrostatin 2



4



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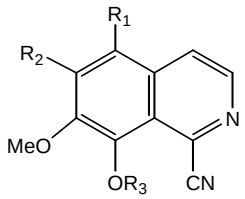
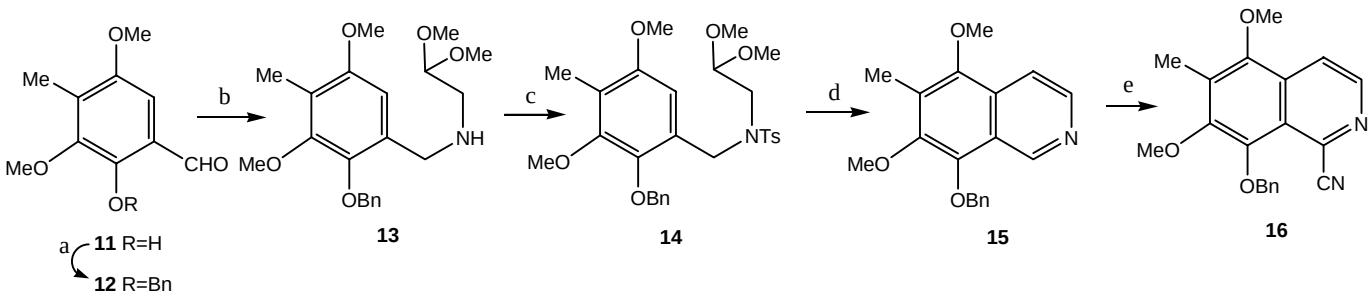


6a R_1 = OMe, R_2 = Me, R_3 = Ac
6b R_1 = OMe, R_2 = Me, R_3 = Bz
7a R_1 = R_2 = H, R_3 = Ac
7b R_1 = R_2 = H, R_3 = Bz
8 R_1 = OAc, R_2 = Me, R_3 = Ac
9 R_1 = OH, R_2 = Me, R_3 = Ac

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(1*H*)-one (**5**),[3] and the first synthesis of **2** and **3**.[4] In furtherance of our study we now report catalytic hydrogenation of 8-acyloxy-1-cyanoisoquinolines (**6**)～(**9**) and synthesis of 9-methoxy isomer (**10**) utilizing this reaction.

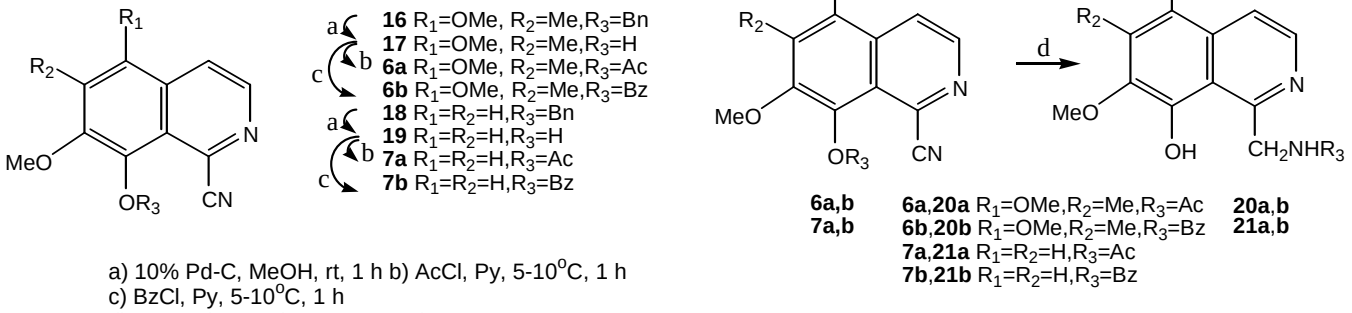
Scheme 1



a) BnBr, K_2CO_3, DMF, rt, 20 h b) i $(MeO)_2CHCH_2NH_2$, C_6H_6, reflux, 3 h ii $NaBH_4$, MeOH, rt, 1 h c) TsCl, Py, rt, 16 h d) i HCl, dioxane, reflux, 3 h ii *tert*-BuOK, *tert*-BuOH, reflux, 1 h e) i *m*-CPBA, CH_2Cl_2, rt, 12 h ii $(Me)_3SiCN$, 1-methylpyrrolidinone,50-60^oC, 2.5 h

8-Benzyloxy-1-cyanoisoquinoline (**16**) was prepared from phenol (**11**) in five steps *via* isoquinoline (**15**), according to the modified Pomeranz-Fritsch iosquinoline synthesis.[5] Benzylation of **11**[6] with benzyl bromide and K_2CO_3 in DMF afforded the benzyloxyaldehyde (**12**) in 85% yield. Reaction of **12** and aminoacetaldehyde dimethylacetal in benzene for 3 h followed by reduction with $NaBH_4$ 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 detosylation 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 CH_2Cl_2 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).

Scheme 2

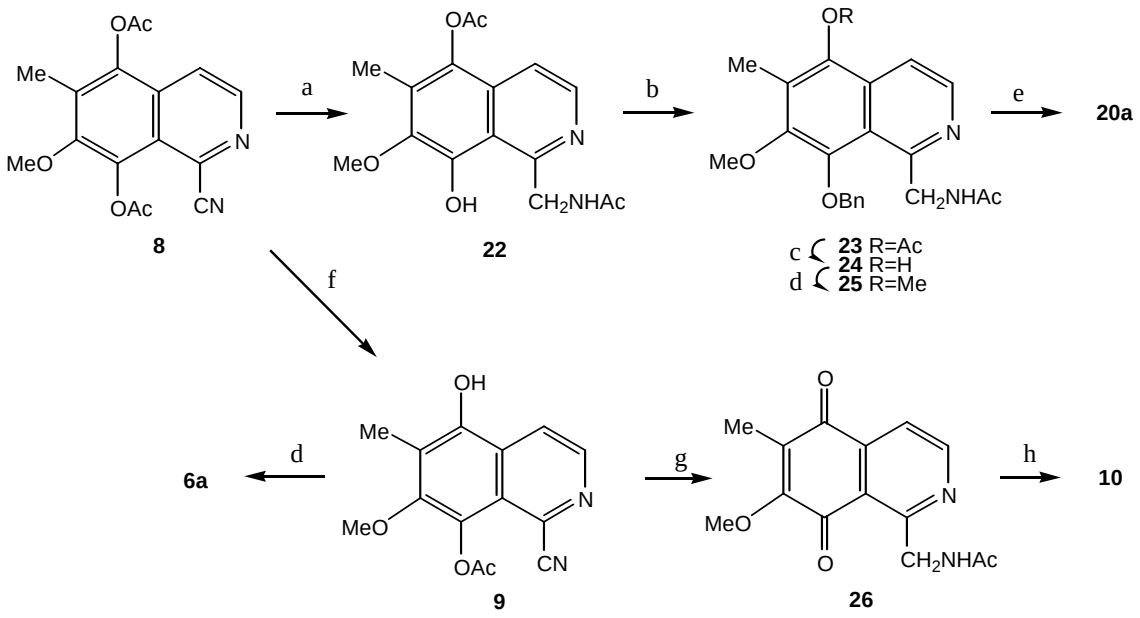


a) 10% Pd-C, MeOH, rt, 1 h b) AcCl, Py, 5-10^oC, 1 h
c) BzCl, Py, 5-10^oC, 1 h
d) 10% Pd-C, HCl, MeOH, rt, 3 h

Catalytic hydrogenation of 8-benzyloxy-1-cyanoisoquinolines (**16**) and (**18**)[7] over 10% Pd-C in

MeOH afforded the 8-hydroxy-1-cyanoiosquinolines (**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[8] (**20a**,**b** and **21a**,**b**) in 90%, 75% and 45%, 45% yields, respectively (Scheme 2).

Scheme 3



a) 10% Pd-C, HCl, MeOH, rt, 3 h b) BnBr, K_2CO_3, DMF, 0°C, 1 h then rt, 15 h c) HCl, MeOH, rt, 20 h d) CH_2N_2, Et_2O, rt, 24 h e) 10% Pd-C, MeOH, rt, 1 h f) sat. HCl, MeOH, rt, 5 d g) i 10% Pd-C, HCl, MeOH, rt, 2 h ii O_2 h) $POCl_3$, toluene, 110°C, 15 min

In case of 1-cyano-5,8-diacetoxyisoquinoline (**8**),[9] 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 $POCl_3$[10] in toluene at 110℃ 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 cribrostatin 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_3OD+$CDCl_3$ 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 μL, 1.04 mmol) and K_2CO_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_2O (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 $C_{28}H_{35}NO_7S$: 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-Benzyloxy-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 $NaHCO_3$ solution and extracted with Et_2O (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 $CHCl_3$ (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 isoquinoline (**15**) (78 mg, 50%, colorless oil). HRMS Calcd for $C_{19}H_{19}NO_3$: 309.1365, Found: 309.1388. Ms *m/z* (%): 309(M^+, 17), 218(100), 91(42). ^{1}H-NMR ($CDCl_3$) δ：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-Benzyloxy-1-cyano-5,7-dimethoxy-6-methylisoquinoline (**16**)

A solution of 80% *m*-chloroperoxybenzoic acid (1.62 g, 7.5 mmol) in CH_2Cl_2 (40 mL) was added drop wise to **15** (1.55 g, 5 mmol) in CH_2Cl_2 (20 mL) with stirring. The mixture was stirred at rt for 12 h, washed with 2% aqueous $NaHCO_3$ 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 ℃ for 2.5 h and poured into water (50 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 2 : 1) to afford 1-cyanoisoquinoline (**16**) (0.89 g, 53%). mp 113-114.5 ℃ (colorless prisms from $CHCl_3$-hexane). *Anal.* Calcd for $C_{20}H_{18}N_2O_3$：C, 71.84；H, 5.43；N, 8.38. Found: C, 72.08；H, 5.25；N, 8.16. IR(KBr) cm-^{1}：2230. ^{1}H-MNR ($CDCl_3$) δ：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 $CHCl_3$ containing MeOH (3 x 30 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 ℃. *Anal.* Calcd for $C_{13}H_{12}N_2O_3$：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. ^{1}H-MNR ($CDCl_3$) δ：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-methoxyisoquinoline (**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 ℃ (yellow needles from $CHCl_3$). *Anal.* Calcd for $C_{11}H_8N_2O_2$：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 ($CD_3OD+CDCl_3$) δ : 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/z* (%) : 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 ℃. The whole was stirred at 5-10 ℃ 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 ℃. *Anal.* Calcd for $C_{15}H_{14}N_2O_4$: 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. ^{1}H-MNR ($CDCl_3$) δ : 2.43(3H, s), 2.57(3H, s), 3.88(3H, s), 3.91(3H, s), 8.00(1H, d, *J*=6 Hz), 8.54(1H, d, *J*=6 Hz). Ms *m/z* (%) : 286(M^+, 2), 244(100), 229(83).

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 ℃ (colorless prisms from acetone). *Anal.* Calcd for $C_{20}H_{16}N_2O_4$: 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. ^{1}H-MNR ($CDCl_3$) δ : 2.48(3H, s), 3.90(3H, s), 3.94(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/z* (%) : 348(M^+,6), 105(100), 77(18).

8-Acetyloxy-1-cyano-7-methoxyisoquinoline (7a)

87% yield. mp 141.5-143.5 ℃ (colorless needles from acetone-hexane). *Anal.* Calcd for $C_{13}H_{10}N_2O_3$: C, 64.46 ; H, 4.16 ; N, 11.57. Found: C, 64.33 ; H, 4.02 ; N, 11.41. IR(KBr) cm-1: 2220, 1775. ^{1}H-NMR ($CDCl_3$) δ : 2.48(3H, s), 3.94(3H, s), 7.53(1H, d, *J*=8 Hz), 7.73(1H, d, *J*=6 Hz), 7.76(1H, d, *J*=8 Hz), 8.43(1H, d, *J*=6 Hz). Ms *m/z* (%) : 242(M^+, 3), 200(100), 185(31).

8-Benzoyloxy-1-cyano-7-methoxyisoquinoline (7b)

96% yield. mp 173-174 ℃ (colorless prisms from acetone-hexane). *Anal.* Calcd for $C_{18}H_{12}N_2O_3$: 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. ^{1}H-NMR ($CDCl_3$) δ: 3.92(3H, s), 7.4 - 7.7(3H, m), 7.60(1H, d, *J*=8 Hz), 7.80(1H, d, *J*=6 Hz), 7.87(1H, d, *J*=8 Hz), 8.2 - 8.4(2H, m). Ms *m/z* (%) : 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℃ (colorless prisms from Et_2O-hexane). *Anal.* Calcd for $C_{15}H_{18}N_2O_4$: 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. ^{1}H-MNR ($CDCl_3$) δ : 2.18(3H, s), 2.40(3H, s),3.83(6H, 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/z* (%) : 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 ℃ (colorless prisms from $CHCl_3$-benzene). *Anal.* Calcd for $C_{20}H_{20}N_2O_4$: 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. [1]H-MNR ($CDCl_3$) δ: 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(IH, 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 ℃ (colorless needles from MeOH). *Anal.* Calcd for $C_{13}H_{14}N_2O_3 \cdot 1/5H_2O$: 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. [1]H-NMR (CD_3OD+$CDCl_3$) δ : 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 ℃ (colorless prisms from $CHCl_3$-MeOH). *Anal.* Calcd for $C_{18}H_{16}N_2O_3$: 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. [1]H-NMR (CD_3OD+$CDCl_3$) δ : 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 ℃ (decomp) (colorless prisms from $CHCl_3$-MeOH). *Anal.* Calcd for $C_{16}H_{18}N_2O_5$: 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. [1]H-NMR (CD_3OD+$CDCl_3$) δ : 2.12(3H, s), 2.27(3H, s), 2.46(3H, s), 3.70(2H, br), 3.84(3H, s), 5.21(2H, s), 7.31(1H, d, *J*=6 Hz), 8.22(1H, d, *J*=6 Hz). Ms *m/z* (%) : 318(M^+, 62), 300(28), 275(60), 258(31), 233(55), 216(100).

1-Acetylaminomethyl-5-acetyloxy-8-benzyloxy-7-methoxy-6-methylisoquinoline (**23**)

Benzyl bromide (36 μL, 0.3 mmol) and K_2CO_3 (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 ℃, then for additional 15 h at rt, poured into water (10 mL) and extracted with $CHCl_3$ (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 ℃ (colorless powder from $CHCl_3$-hexane). *Anal.* Calcd for $C_{23}H_{24}N_2O_5$: 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. [1]H-NMR ($CDCl_3$) δ : 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 $NaHCO_3$. 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 $CHCl_3$-MeOH). *Anal.* Calcd for $C_{21}H_{22}N_2O_4 \cdot 1/10H_2O$: 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. ^{1}H-NMR (CD_3OD+$CDCl_3$) 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 CH_2N_2 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 $CHCl_3$-hexane). *Anal.* Calcd for $C_{22}H_{24}N_2O_4$: 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. ^{1}H-NMR ($CDCl_3$) δ : 2.10(3H, s), 2.37(3H, s), 3.82(3H, s), 3.85(3H, s), 5.10(2H, s), 5.17(2H, d, *J*=4 Hz), 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, ^{1}H-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 $NaHCO_3$ and extracted with $CHCl_3$ (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-$CHCl_3$-hexane). *Anal.* Calcd for $C_{14}H_{12}N_2O_4$: 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. ^{1}H-MNR (CD_3OD+$CDCl_3$) 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, ^{1}H-NMR) of a compound thus obtained match those of **6a**.

1-Acetylaminomethyl-7-methoxy-6-methyl-5,8-isoquinolinedione (**26**)

8-Acetyloxy-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 $NaHCO_3$ and extracted with $CHCl_3$ (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 149-15 ℃ (light yellow needles from $CHCl_3$-hexane). HRMS Calcd for $C_{14}H_{14}N_2O_4$: 274.0954, Found: 274.0962. IR(KBr) cm-1: 3290, 1665, 1645. ^{1}H-NMR ($CDCl_3$) 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 ℃ 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 ℃< (dark blue powder from acetone). *Anal.* Calcd for $C_{14}H_{12}N_2O_3$：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（$CDCl_3$）δ：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).

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