

SYNTHESIS OF CRIBROSTATIN 6

Shinsuke Nakahara* and Akinori Kubo

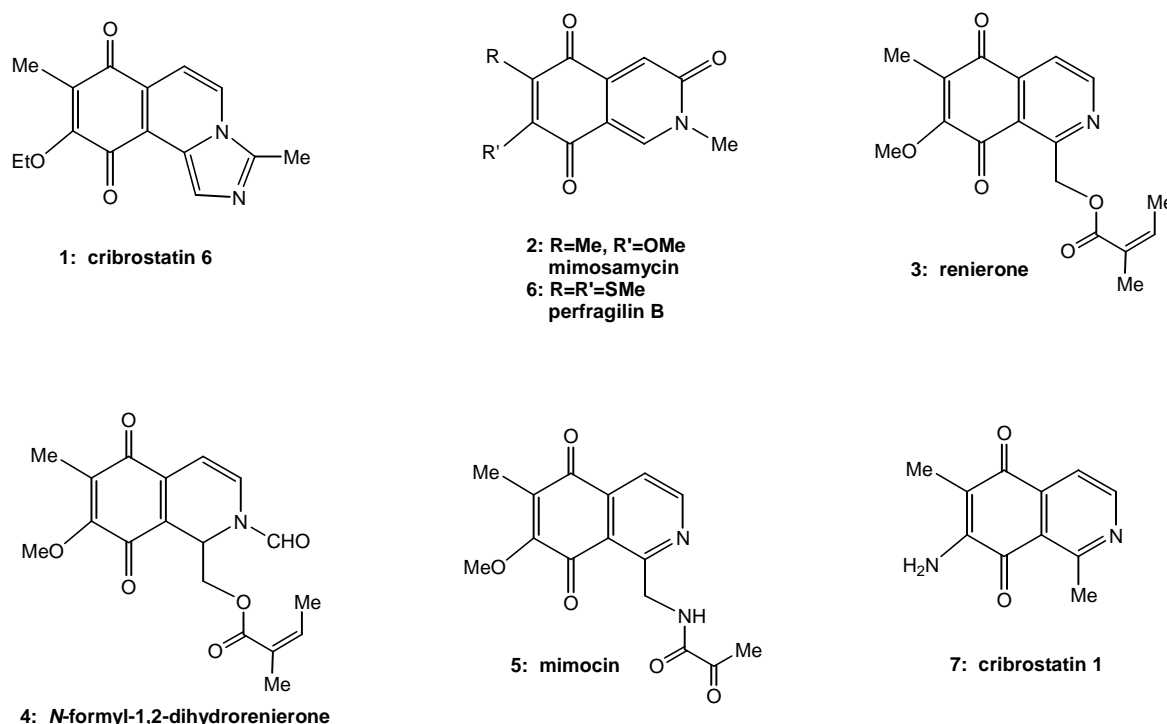
Meiji Pharmaceutical University

2-552-1, Noshio, Kiyose, Tokyo 204-8588, Japan

Abstract – The first synthesis of cribrostatin 6 (**1**), a dark blue cancer cell growth inhibitor was achieved in ten-steps from 2,4-diethoxy-3-methylphenol (**8**), utilizing a catalytic hydrogenation which induces an intramolecular transfer reaction.

Over the past twenty years, a series of structurally interesting and biologically active 5,8-isoquinolinedione alkaloids have been isolated from marine sources and *Actinomyces*.¹ Mimosamycin (**2**) was isolated from the culture filtrate of *Streptomyces lavendulae*^{1a} and showed antimicrobial activity, particularly against mycobacteria. Renierone (**3**)^{1b} and *N*-formyl-1,2-dihydrorenierone (**4**)^{1d} were isolated as major metabolites of *Reniera* sp. Mimocin (**5**), isolated from a *Streptomyces lavendulae* metabolite,^{1c} contains a pyruvamide side chain in place of the angelate ester side chain of **3**. Perfragilin B (**6**), isolated from the Bryozoan *Biflustra perfragilis*,^{1e} contains a methylthio ether group, while cribrostatin 1 (**7**), isolated from the marine sponge *Cribochalina* sp.^{1f} contains an amino group.

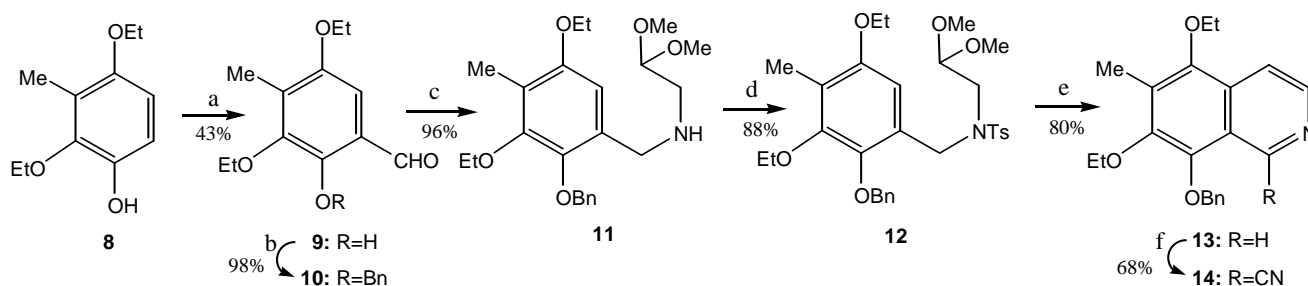
Figure 1



Recently Pettit and colleagues reported the isolation of cribrostatin **6** (**1**) from the marine sponge *Cribrochalina* sp. The structure determination of **1**, based on spectral data and X-Ray crystal structure analyses was also reported,² and concluded that **1** possesses an isoquinolinedione skeleton, similar to mimosamycin (**2**), but contains a fused imidazole ring, in this class of compounds. Cribrostatin **6** shows good biological activity, as a growth inhibitor of cancer cells and a number of pathogenic bacteria and fungi. Therefore, the synthesis and bioactivity of compound (**1**) are of interest.

We have previously reported the catalytic hydrogenation of 8-acyloxy-1-cyanoisoquinoline resulting in the intramolecular transfer of the acyl group from the oxygen atom to the nitrogen atom, to furnish the corresponding amide.³ We describe herein the synthesis of cribrostatin **6** (**1**) utilizing this intramolecular transfer reaction.

Scheme 1



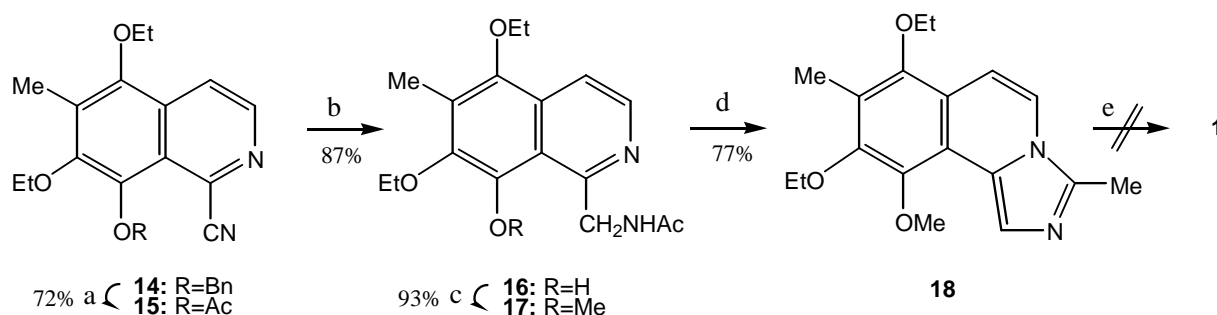
a) hexamine, AcOH, 135-140°C, 2 h b) BnBr, K₂CO₃, DMF, rt, 48 h c) i) (MeO)₂CHCH₂NH₂, C₆H₆, reflux, 3 h
ii) NaBH₄, MeOH, rt, 1 h d) TsCl, Py, rt, 20 h e) i) HCl, dioxane, reflux, 3.5 h ii) *tert*-BuOK, *tert*-BuOH, reflux, 1 h
f) i) *m*-CPBA, CH₂Cl₂, rt, 16 h ii) (Me)₃SiCN, 1-methylpyrrolidinone, 55-65°C, 50 h

8-Benzyloxy-1-cyanoisoquinoline (**14**), a key intermediate in the synthesis of cribrostatin **6** (**1**), was prepared from phenol (**8**)⁴ in six steps *via* isoquinoline (**13**), according to the modified Pomeranz-Fritsch isoquinoline synthesis (Scheme 1).⁵ Duff reaction⁶ of phenol (**8**) with hexamine in AcOH afforded the *o*-hydroxy aldehyde (**9**) in 43% yield. Benzoylation of **9** with benzyl bromide and K₂CO₃ in DMF afforded benzyloxy aldehyde (**10**) in 98% yield. Reaction of **10** with aminoacetaldehyde dimethylacetal in benzene for 3 h followed by reduction with NaBH₄ in MeOH for 1 h gave amino compound (**11**) in 96% yield. Treatment of **11** with tosyl chloride in pyridine for 20 h gave the *N*-tosyl compound (**12**) in 88% yield. Cyclization of **12** with 6M HCl in dioxane for 3.5 h followed by detosylation with potassium *tert*-butoxide in *tert*-butyl alcohol for 1 h under reflux, afforded the isoquinoline (**13**) in 80% yield. Treatment of **13** with *m*-chloroperoxybenzoic acid in CH₂Cl₂ for 16 h followed by trimethylsilyl cyanide in 1-methyl-2-pyrrolidinone for 50 h gave the 8-benzyloxy-1-cyanoisoquinoline (**14**) in 68% yield, (using potassium cyanide and benzoyl chloride in water gave a 57% yield).

Debenzylation of **14** with 10% Pd-C in MeOH for 2 h followed by acetylation with acetyl chloride in pyridine at room temperature for 1 h afforded the 8-acetoxy-1-cyanoisoquinoline (**15**) in 72% yield. Catalytic hydrogenation of **15** over 10% Pd-C in MeOH containing HCl resulted in the intramolecular transfer of the acetyl group from the oxygen to the nitrogen atom, furnishing the amide (**16**) in 87% yield. *O*-Methylation of **16** with diazomethane in ether gave **17** in 93% yield. Treatment of **17** with POCl₃⁷ in

toluene at 110-115 °C for 15 min afforded imidazo[5,1-*a*]isoquinoline (**18**) in 77% yield. Unfortunately, final conversion of **18** into target molecule (**1**) under a variety of oxidative conditions using ceric ammonium nitrate (CAN)⁸ was unsuccessful (Scheme 2).

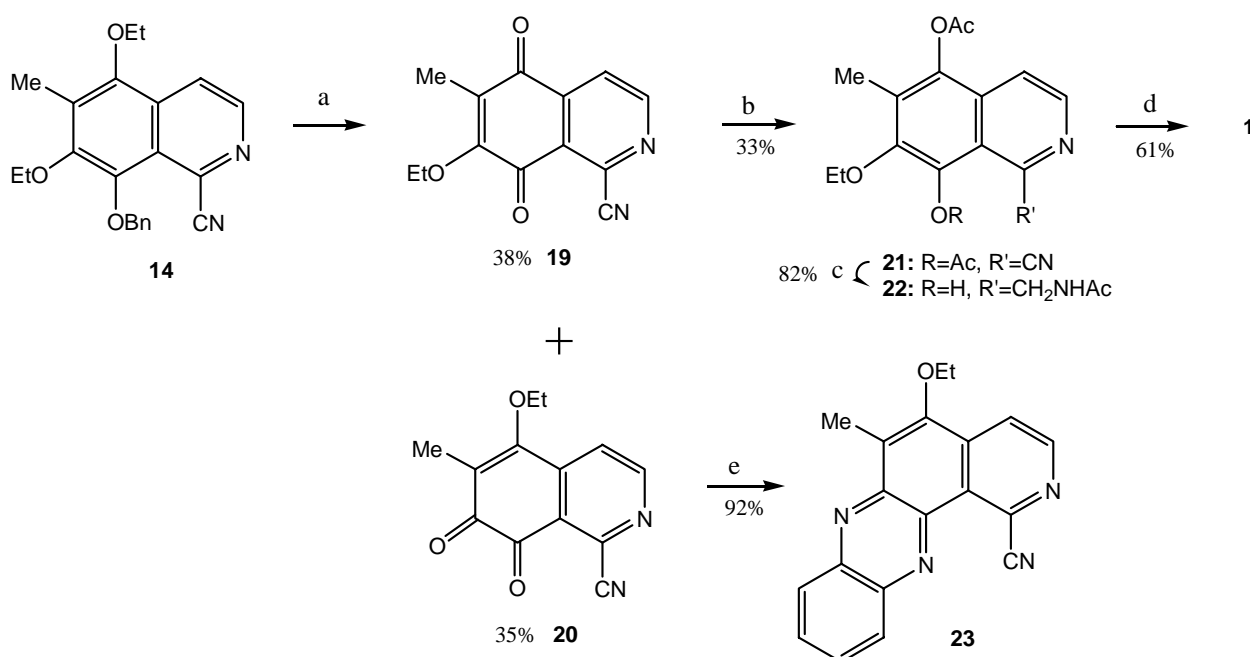
Scheme 2



a) i) 10% Pd-C, MeOH, H₂, rt, 2 h ii) AcCl, Py, rt, 1 h b) 10% Pd-C, HCl, MeOH, H₂, rt, 3 h c) CH₂N₂, Et₂O, rt, 3 h d) POCl₃, toluene, 110-115 °C, 15 min e) CAN

Hence, we studied an alternate synthetic route utilizing air oxidation of imidazo[5,1-*a*]isoquinoline for the final step (Scheme 3).

Scheme 3



a) CAN, MeCN, H₂O, 0-5 °C, 0.5 h b) Zn, (AcO)₂O, rt, 1 h c) 10% Pd-C, HCl, MeOH, H₂, rt, 3 h d) i) POCl₃, toluene, 115 °C, 15 min ii) HCl, MeOH, rt, 20 h iii) O₂ e) *o*-C₆H₄(NH₂)₂, EtOH, rt, 1 h

Oxidation of **14** with CAN in aqueous acetonitrile at 0-5 °C for 0.5 h furnished the desired *p*-quinone (**19**)

and the corresponding *o*-quinone isomer (**20**) in 38% and 35% yields, respectively. To verify the *o*-quinone structure of **20**, it was condensed with *o*-phenylenediamine to afford the corresponding pyrido[3,4-*a*]phenazine (**23**) in 92% yield. Treatment of *p*-quinone (**19**) with zinc powder in acetic anhydride at room temperature for 1 h, afforded diacetate (**21**) in 33% yield. Catalytic hydrogenation of **21** over 10% Pd-C in MeOH containing HCl afforded the amide (**22**) in 82% yield. Finally, treatment of **22** with POCl₃ in toluene at 115 °C for 15 min followed by hydrolysis with HCl in MeOH and air oxidation gave the desired cribrostatin 6 (**1**) in 61% yield. The spectroscopic data of synthetic **1** matched with those of the authentic sample in all respects.

In summary, cribrostatin 6 (**1**) was synthesized in ten-steps from known compounds. Catalytic hydrogenation induced intramolecular transfer was utilized to transfer an acetyl group from oxygen to nitrogen and the final step employed air oxidation to form the imidazo[5,1-*a*]isoquinolinedione.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ¹H-NMR spectra at 270 MHz were measured in CDCl₃ 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).

3,5-Diethoxy-2-hydroxy-4-methylbenzaldehyde (9). Hexamethylenetetramine (28 g, 0.2 mol) was added to a solution of 2,4-diethoxy-3-methylphenol (**8**)(13.0 g, 66.2 mmol) in acetic acid (200 mL). The mixture was stirred at 135-145 °C for 2 h and poured into water (500 mL). The precipitated crystals were collected by filtration and recrystallized from CHCl₃-hexane to give **9**(6.43 g, 43%) as light yellow needles. mp 54-55 °C. *Anal.* Calcd for C₁₂H₁₆O₄: C, 64.27; H, 7.19. Found: C, 64.18; H, 7.02. IR(KBr) cm⁻¹:1652.

¹H-NMR (CDCl₃) δ: 1.39(3H, t, *J*=6.9 Hz), 1.44(3H, t, *J*=6.9 Hz), 2.23(3H, s), 4.01(2H, q, *J*=6.9 Hz), 4.11(2H, q, *J*=6.9 Hz), 6.68(1H, s), 9.80(1H, s), 10.81(1H, br s). Ms *m/z* (%): 224(M⁺, 100), 196(18), 168(88), 139(20).

2-Benzyloxy-3,5-diethoxy-4-methylbenzaldehyde (10). Benzyl bromide (4.62 g, 27.04 mmol) and K₂CO₃ (5.39 g, 39 mmol) were added to a solution of 3,5-diethoxy-2-hydroxy-4-methylbenzaldehyde (**9**)(5.8 g, 26 mmol) in DMF (100 mL). The whole was stirred at rt for 48 h, poured into water (200 mL). The precipitated crystals were collected and recrystallized from hexane to give **10**(7.98 g, 98%) as colorless prisms. mp 53-54 °C. HRMS Calcd for C₁₉H₂₂O₄: 314.1518, Found: 314.1515. IR(KBr) cm⁻¹: 1680. Ms *m/z* (%): 314(M⁺, 37), 223(100), 167(64), 91(51). ¹H-NMR (CDCl₃) δ: 1.41(3H, t, *J*=6.9 Hz), 1.42(3H, t, *J*=6.9 Hz), 2.23(3H, s), 4.04(2H, q, *J*=6.9 Hz), 4.10(2H, q, *J*=6.9 Hz), 5.11(2H, s), 6.97(1H, s), 7.36(5H, br s), 10.13(1H, s).

***N*-(2-Benzyloxy-3,5-diethoxy-4-methylbenzyl)-2,2-dimethoxyethylamine (11).** Aminoacetaldehyde dimethyl acetal (2.92 g, 27.5 mmol) was added to a solution of benzyloxy aldehyde (**10**)(7.86 g, 25 mmol) in benzene (120 mL). The mixture was refluxed in a Dean-Stark apparatus until no further water appeared (3 h) and the solvent removed under vacuum. The residue was dissolved in MeOH (120 mL) and NaBH₄ (0.95 g, 25 mmol) was added in portions with stirring at 0-5 °C. The mixture was stirred for an additional 1 h at rt, then diluted with water (200 mL) and extracted with CHCl₃ (3 x 50 mL). The extract was washed

with brine, dried and concentrated. The residue was chromatographed (eluting with ethyl acetate) to afford amino compound (**11**)(9.69 g, 96%, colorless oil). HRMS Calcd for C₂₃H₃₃NO₅: 403.2359, Found: 403.2353. Ms *m/z* (%): 403(M⁺, 27), 328(34), 299(100), 209(47), 91(38). ¹H-NMR(CDCl₃) : 1.36(3H, t, *J*=6.9 Hz), 1.40(3H, t, *J*=6.9 Hz), 2.16(3H, s), 2.68(2H, q, *J*=5.6 Hz), 3.31(6H, s), 3.69(2H, s), 4.00(2H, q, *J*=6.9 Hz), 4.04(2H, q, *J*=6.9 Hz), 4.44(1H, q, *J*=5.6 Hz), 4.99(2H, s), 6.55(1H, s), 7.34-7.45(5H, m).

***N*-(2-Benzyloxy-3,5-diethoxy-4-methylbenzyl)-2,2-dimethoxy-*N*-tosylethylamine (12).** *p*-Toluene-sulfonyl chloride(5.24 g, 27.5 mmol) was added to a solution of ethylamine (**11**)(10.1 g, 25 mmol) in pyridine (55 mL) in portions with stirring at 0-5 °C. The mixture was stirred for an additional 20 h at rt, then diluted with water (200 mL) and extracted with Et₂O (3 x 80 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 (**12**)(12.27 g, 88%, colorless oil). HRMS Calcd for C₃₀H₃₉NO₇S: 557.2447, Found: 557.2442. Ms *m/z* (%): 557(M⁺, 12), 466(100), 434(42), 311(28), 279(20), 209(16).

¹H-NMR(CDCl₃) : 1.34(3H, t, *J*=6.9 Hz), 1.37(3H, t, *J*=6.9 Hz), 2.13(3H, s), 2.42(3H, s), 3.18(6H, s), 3.20(2H, q, *J*=5.6 Hz), 3.82(2H, q, *J*=6.9 Hz), 3.99(2H, q, *J*=6.9 Hz), 4.37(2H, s), 4.37(1H, t, *J*=5.6 Hz), 4.92(2H, s), 6.46(1H, s), 7.26(2H, dd, *J*=8.3, 1.7 Hz), 7.32-7.38(5H, m), 7.66(2H, dd, *J*=8.3, 1.7 Hz).

8-Benzyloxy-5,7-diethoxy-6-methylisoquinoline (13). A mixture of *N*-tosyl compound (**12**)(12.27 g, 22 mmol) and 6 N HCl (22 mL) in dioxane (250 mL) was boiled under reflux for 3.5 h, then poured into water (250 mL), basified with 5% aqueous NaHCO₃ solution and extracted with Et₂O (3 x 150 mL). The extract was washed with brine, dried and concentrated. The residue was dissolved in *tert*-butyl alcohol (50 mL) and potassium *tert*-butoxide (7.4 g, 66 mmol) was added. The mixture was refluxed for 1 h and poured into water (250 mL). The precipitated crystals were collected by filtration and chromatographed (eluting with hexane-ethyl acetate 5 : 1) to afford **13**(5.94 g, 80%. mp 122-123 °C, colorless prisms from CHCl₃-hexane). *Anal.* Calcd for C₂₁H₂₃NO₃ : C, 74.75 ; H, 6.87 ; N, 4.15. Found: C, 74.89 ; H, 7.07 ; N, 4.02. Ms *m/z* (%) : 337(M⁺, 14), 246(76), 218(18), 190(100), 162(25), 91(20). ¹H-NMR (CDCl₃) : 1.43(3H, t, *J*=6.9 Hz), 1.52(3H, t, *J*=6.9 Hz), 2.42(3H, s), 4.02(2H, q, *J*=6.9 Hz), 4.22(2H, q, *J*=6.9 Hz), 5.17(2H, s), 7.32-7.44(3H, m), 7.53(2H, dd, *J*=7.6, 1.7 Hz), 7.74(1H, d, *J*=5.9 Hz), 8.44(1H, d, *J*=5.9 Hz), 9.45(1H, s).

8-Benzyloxy-1-cyano-5,7-diethoxy-6-methylisoquinoline (14). 75% *m*-Chloroperoxybenzoic acid (3.11 g, 13.5 mmol) was added to isoquinoline (**13**)(3.04 g, 9 mmol) in CH₂Cl₂ (100 mL) with stirring at 0-5 °C. The mixture was stirred at rt for 16 h, washed with 2% aqueous NaHCO₃ solution and brine, then dried and concentrated. The residue was dissolved in 1-methyl-2-pyrrolidinone (23 mL), and trimethylsilyl cyanide (7.2 g, 73 mmol) was added. The mixture was stirred at 55-65 °C for 50 h, and the solvent was removed under reduced pressure. The residue was recrystallized from CHCl₃-hexane to give 1-cyanoisoquinoline (**14**)(2.23 g, 68%) as colorless prisms. mp 163-164 °C. *Anal.* Calcd for C₂₂H₂₂N₂O₃ : C, 72.91 ; H, 6.12 ; N, 7.73. Found: C, 73.05 ; H, 6.26 ; N, 7.59. IR(KBr) cm⁻¹: 2232. Ms *m/z* (%): 362(M⁺, 57), 271(20), 243(26), 215(100), 187(25), 91(54). ¹H-NMR(CDCl₃) : 1.36(3H, t, *J*=6.9 Hz), 1.52(3H, t, *J*=6.9 Hz), 2.43(3H, s), 4.00(2H, q, *J*=6.9 Hz), 4.17(2H, q, *J*=6.9 Hz), 5.29(2H, s), 7.30-7.39(3H, m), 7.63(2H, dd, *J*=7.6, 1.7 Hz), 7.98(1H, d, *J*=5.6 Hz), 8.54(1H, d, *J*=5.6 Hz).

8-Acetoxy-1-cyano-5,7-diethoxy-6-methylisoquinoline (15). The 8-benzyloxy-1-cyanoisoquinoline (**14**)(1.71 g, 4.7 mmol) in MeOH (120 mL) was hydrogenated for 2 h using 10% Pd-C (420 mg) as a

catalyst under H₂ atmosphere. The catalyst was filtered off and the solvent was removed. Acetyl chloride (3.4 mL, 23.8 mmol) was added in portions to a solution of the residue in pyridine (10 mL) with stirring at 0 °C. The whole was stirred at rt for 1 h and poured into cold water (250 mL). The precipitated crystals were collected and recrystallized from CHCl₃-hexane to give **15** (1.07 g, 72%) as colorless prisms. mp 145-146 °C. *Anal.* Calcd for C₁₇H₁₈N₂O₄: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.07; H, 5.94; N, 8.77. IR(KBr) cm⁻¹: 2232, 1772. Ms *m/z* (%): 314(M⁺, 4), 272(100), 243(58), 215(64), 187(17). ¹H-NMR (CDCl₃) δ: 1.45(3H, t, *J*=6.9 Hz), 1.53(3H, t, *J*=6.9 Hz), 2.44(3H, s), 2.57(3H, s), 4.03(2H, q, *J*=6.9 Hz), 4.12(2H, q, *J*=6.9 Hz), 8.05(1H, d, *J*=5.6 Hz), 8.59(1H, d, *J*=5.6 Hz).

1-Acetylaminoethyl-5,7-diethoxy-8-hydroxy-6-methylisoquinoline (16). The 8-acetoxy-1-cyanoisoquinoline (**15**) (314 mg, 1 mmol) in MeOH (50 mL) containing concentrated HCl (0.5 mL) was hydrogenated for 3 h using 10% Pd-C (314 mg) as a catalyst under H₂ atmosphere. The catalyst was filtered off, the filtrate was poured into water (250 mL), adjusted to pH 7 with saturated aqueous NaHCO₃ solution and extracted with CHCl₃ (3 x 40 mL). The extract was washed with brine, dried and concentrated. The residue was recrystallized from CHCl₃-hexane to give 1-acetylaminoethylisoquinoline (**16**) (277 mg, 87%) as light yellow prisms. mp 156-157 °C. *Anal.* Calcd for C₁₇H₂₂N₂O₄: C, 64.13; H, 6.97; N, 8.80. Found: C, 64.33; H, 6.96; N, 8.60. IR(KBr) cm⁻¹: 3380, 3080, 1658. Ms *m/z* (%): 318(M⁺, 70), 289(100), 247(45), 230(75), 202(24). ¹H-NMR(CDCl₃) δ: 1.41(3H, t, *J*=7.3 Hz), 1.46(3H, t, *J*=7.3 Hz), 2.12(3H, s), 2.36(3H, s), 3.90(2H, q, *J*=7.3 Hz), 3.96(2H, q, *J*=7.3 Hz), 5.25(2H, d, *J*=4.3 Hz), 6.27(1H, br), 7.63(1H, d, *J*=5.6 Hz), 7.90(1H, br s), 8.23(1H, d, *J*=5.6 Hz).

1-Acetylaminoethyl-5,7-diethoxy-8-methoxy-6-methylisoquinoline (17). 8-Hydroxyisoquinoline (**16**) (478 mg, 1.5 mmol) was added to an ether solution containing excess of CH₂N₂ and the mixture was stirred at rt for 3 h. The solvent was evaporated and the residue was chromatographed (eluting with ethyl acetate) to afford **17** (462 mg, 93%) mp 100-101 °C (colorless prisms from CHCl₃-hexane). HRMS Calcd for C₁₈H₂₄N₂O₄: 332.1736, Found: 332.1739. IR(KBr) cm⁻¹: 3292, 1644. Ms *m/z* (%): 332(M⁺, 89), 289(100), 245(28), 229(21), 217(15). ¹H-NMR(CDCl₃) δ: 1.38(3H, t, *J*=7.3 Hz), 1.45(3H, t, *J*=7.3 Hz), 2.09(3H, s), 2.33(3H, s), 3.91(2H, q, *J*=7.3 Hz), 3.95(3H, s), 4.07(2H, q, *J*=7.3 Hz), 5.10(2H, d, *J*=4.0 Hz), 7.66(1H, d, *J*=5.6 Hz), 7.88(1H, br s), 8.24(1H, d, *J*=5.6 Hz).

7,9-Diethoxy-10-methoxy-3,8-dimethylimidazo[5,1-*a*]isoquinoline (18). 8-Methoxyisoquinoline (**17**) (332 mg, 1 mmol) in toluene (5 mL) was treated with POCl₃ (767 mg, 5 mmol) under stirring at 110-115 °C for 15 min, then poured into cold water (80 mL), adjusted to pH 7 with saturated aqueous NaHCO₃ solution 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 **18** (242 mg, 77%, light yellow oil). HRMS Calcd for C₁₈H₂₂N₂O₃: 314.1631, Found: 314.1632. Ms *m/z* (%): 314(M⁺, 100), 285(18), 257(17), 214(13). ¹H-NMR(CDCl₃) δ: 1.44(3H, t, *J*=6.9 Hz), 1.48(3H, t, *J*=6.9 Hz), 2.31(3H, s), 2.66(3H, s), 3.94(2H, q, *J*=6.9 Hz), 3.98(3H, s), 4.17(2H, q, *J*=6.9 Hz), 7.02(1H, d, *J*=7.6 Hz), 7.48(1H, d, *J*=7.6 Hz), 7.90(1H, s).

Oxidation of 8-benzyloxy-1-cyano-5,7-diethoxy-6-methylisoquinoline (14) with CAN. A solution of CAN (5.48 g, 10 mmol) in water (6 mL) was added drop wise to **14** (810 mg, 2.24 mmol) suspended in acetonitrile-water (5 : 2, 140 mL) with stirring at 0-5 °C. The mixture was stirred for an additional 0.5 h, diluted with water (200 mL), adjusted to pH 7 with saturated aqueous NaHCO₃ solution and extracted

with CHCl_3 (3 x 50 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed on a silica gel column. Elution with ethyl acetate-hexane (1 : 10) gave a less polar *p*-quinone (**19**)(205 mg, 38%) and further elution with ethyl acetate-hexane (1 : 3) gave a more polar *o*-quinone (**20**)(192 mg, 35%). *p*-quinone (**19**) : mp 112-113.5 (yellow needles from CHCl_3 -hexane). HRMS Calcd for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_3$: 242.0691, Found: 242.0692. Ms m/z (%): 242(M^+ , 100), 214(21), 198(48), 186(36), 170(17), 158(24), 142(22), 130(34), 103(15). IR(KBr) cm^{-1} : 2240, 1676, 1662. $^1\text{H-NMR}$ (CDCl_3) : 1.44(3H, t, $J=6.9$ Hz), 2.14(3H, s), 4.59(2H, q, $J=6.9$ Hz), 8.13(1H, d, $J=5.0$ Hz), 9.08(1H, d, $J=5.0$ Hz). *o*-quinone (**20**) : mp 210-211 (red prisms from CHCl_3). Anal. Calcd for $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_3$: C, 64.46 ; H, 4.16 ; N, 11.56. Found: C, 64.26 ; H, 4.22 ; N, 11.85. IR(KBr) cm^{-1} : 1708, 1652. Ms m/z (%): 242(M^++2 , 6), 214(100), 186(97), 158(26), 130(33), 103(15). $^1\text{H-NMR}$ (CDCl_3) : 1.54(3H, t, $J=6.9$ Hz), 2.16(3H, s), 4.29(2H, q, $J=6.9$ Hz), 7.85(1H, d, $J=5.0$ Hz), 8.98(1H, d, $J=5.0$ Hz). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) : 10.48(CH_3), 15.80(CH_3CH_2), 70.87(OCH_2), 115.40(CN), 121.17(C_4), 125.59(C_9), 128.40(C_6), 132.58(C_1), 142.42(C_{10}), 156.19(C_3), 160.93(C_5), 175.63(C_8), 178.74(C_7).

1-Cyano-5-ethoxy-6-methylpyrido[3,4-*a*]phenazine (23). A mixture of *o*-quinone (**20**)(48 mg, 0.2 mmol) and *o*-phenylenediamine (22 mg, 0.2 mmol) in EtOH (8 mL) was stirred at rt for 1 h. The precipitated crystals were collected by filtration and recrystallized from CHCl_3 -hexane to give **23**(58 mg, 92%) as yellow prisms. mp 237-238. HRMS Calcd for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}$: 314.1168, Found: 314.1171. Ms m/z (%): 314(M^+ , 75), 285(100), 270(42), 257(50), 230(33). IR(KBr) cm^{-1} : 2232. $^1\text{H-NMR}$ (CDCl_3) : 1.61(3H, t, $J=6.9$ Hz), 2.85(3H, s), 4.19 (2H, q, $J=6.9$ Hz), 7.87-7.96(2H, m), 8.19(1H, d, $J=5.3$ Hz) 8.24-8.30(1H, m), 8.45-8.50(1H, m), 8.99(1H, d, $J=5.3$ Hz).

5,8-Diacetoxy-1-cyano-7-ethoxy-6-methylisoquinoline (21). Zinc powder (177 mg, 2.7mmol) was added in portions to a solution of *p*-quinone (**19**)(218 mg, 0.9 mmol) in acetic anhydride (3 mL) with stirring at rt. The whole was stirred at rt for an additional 1 h. Then the insoluble materials were filtered off, and the filtrate was evaporated under reduced pressure, diluted with water (50 mL), adjusted to pH 7 with saturated aqueous NaHCO_3 solution and extracted with ethyl acetate (3 x 10 mL). The extract was washed with brine, dried and concentrated. The residue was chromatographed (eluting with hexane-ethyl acetate 10 : 1) to afford isoquinoline (**21**)(97.5 mg, 33%,). mp 196-197 (colorless prisms from CHCl_3 -hexane). Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_5$: C, 62.19 ; H, 4.91 ; N, 8.53. Found: C, 62.39 ; H, 5.03 ; N, 8.42. IR(KBr) cm^{-1} : 2236, 1772. Ms m/z (%): 328(M^+ , 2), 286(69), 244(100), 216(50), 187(12). $^1\text{H-NMR}$ (CDCl_3) : 1.46(3H, t, $J=6.9$ Hz), 2.31(3H, s), 2.51(3H, s), 2.58(3H, s), 4.14(2H, q, $J=6.9$ Hz), 7.72(1H, d, $J=5.9$ Hz), 8.60(1H, d, $J=5.9$ Hz).

1-Acetylaminoethyl-5-acetoxy-7-ethoxy-8-hydroxy-6-methylisoquinoline (22). 5,8-Diacetoxy-isoquinoline (**21**)(131 mg, 0.4 mmol) in MeOH (20 mL) containing concentrated HCl (0.2 mL) was hydrogenated for 3 h using 10% Pd-C (131 mg) as a catalyst under H_2 atmosphere. The catalyst was filtered off, the filtrate was poured into water (60 mL), adjusted to pH 7 with saturated aqueous NaHCO_3 solution and extracted with CHCl_3 (3 x 30 mL). The extract was washed with brine, dried and concentrated. The residue was recrystallized from CHCl_3 -hexane to give **22**(109 mg, 82%) as light yellow prisms. mp 180-181.5. HRMS Calcd for $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5$: 332.1372, Found: 332.1363. Ms m/z (%): 332(M^+ , 100), 289(71), 261(61), 247(31), 230(61), 219(45), 202(61). IR(KBr) cm^{-1} : 3360, 3160, 1764, 1658. $^1\text{H-NMR}$ (CDCl_3) : 1.45(3H, t, $J=6.9$ Hz), 2.15(3H, s), 2.27(3H, s), 2.47(3H, s), 4.00(2H, q, $J=6.9$ Hz),

5.23(2H, d, $J=5.2$ Hz), 7.32(1H, d, $J=5.9$ Hz), 7.84(1H, br s), 8.28(1H, d, $J=5.9$ Hz).

Cribrostatin 6 (1). A mixture of 1-acetylaminoethyl-5-acetoxy-8-hydroxyisoquinoline (**22**)(66 mg, 0.2 mmol) and POCl_3 (168 mg, 1.1 mmol) in toluene (1 mL) was stirred at 115 °C for 15 min, then poured into cold water (10 mL), adjusted to pH 7 with saturated aqueous NaHCO_3 solution and extracted with CHCl_3 (3 x 5 mL). The extract was washed with brine, dried and concentrated. The residue was added to a solution of concentrated HCl (0.6 mL) in MeOH (0.6 mL). The solution was stirred at rt for 20 h, then poured into cold water (50 mL), adjusted to pH 7 with saturated aqueous NaHCO_3 solution and extracted with CHCl_3 (3 x 30 mL). The extract was washed with brine, dried and concentrated. The residue was recrystallized from acetone to give **1**(33 mg, 61%) as dark blue needles. mp 171-172 °C. HRMS Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_3$: 270.1004, Found: 270.1002. Ms m/z (%): 270(M^+ , 100), 242(11), 214(33), 185(16), 172(9), 157(7), 145(14), 116(7). IR(KBr) cm^{-1} : 2928, 1664, 1630, 1614, 1528, 1318, 1174. $^1\text{H-NMR}$ (CDCl_3) δ : 1.43(3H, t, $J=6.9$ Hz), 2.07(3H, s), 2.71(3H, s), 4.41(2H, q, $J=6.9$ Hz), 7.21(1H, d, $J=7.3$ Hz), 7.87(1H, d, $J=7.3$ Hz), 8.26(1H, s). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 9.17, 12.58, 16.00, 69.65, 107.75, 123.54, 123.92, 124.73, 125.02, 125.73, 130.01, 137.65, 156.19, 180.63, 184.90.

REFERENCES

- 1 a) T. Arai, K. Yazawa, Y. Mikami, A. Kubo, and K. Takahashi, *J. Antibiot.*, 1976, **29**, 398. b) D. E. McIntyre, D. J. Faulkner, D.V. Engen, and J. Clardy, *Tetrahedron Lett.*, 1979, 4163. c) A. Kubo, S. Nakahara, R. Iwata, K. Takahashi, and T. Arai, *Tetrahedron Lett.*, 1980, **21**, 3207. d) J. M. Frincke and D. J. Faulkner, *J. Am. Chem. Soc.*, 1982, **104**, 265. e) F. J. Schmitz, F. S. DeGuzman, Y.-H. Choi, M. B. Hossain, S. K. Rizvi, and D. van der Helm, *Pure Appl. Chem.*, 1990, **62**, 1393. f) G. R. Pettit, J. C. Collins, D. L. Herald, D. L. Doubek, M. R. Boyd, J. M. Schmidt, J. N. A. Hooper, and L. P. Tackett, *Can. J. Chem.*, 1992, **70**, 1170. g) A. J. Blackman, C. E. Ralph, B. W. Skelton, and A. H. White, *Aust. J. Chem.*, 1993, **46**, 213. h) Y.-H. Choi, A. Park, F. J. Schmitz, and I. van Aitena, *J. Nat. Prod.*, 1993, **56**, 1431. i) A. Park and F. J. Schmitz, *Tetrahedron Lett.*, 1993, **34**, 3983. j) M. Kobayashi, S. R. Rao, R. Chavakula, and N. S. Sarma, *J. Chem. Res. (S)*, **1994**, 282.
- 2 G. R. Pettit, J. C. Collins, J. C. Knight, D. L. Herald, R. A. Nieman, M. D. Williams, and R. K. Pettit, *J. Nat. Prod.*, 2003, **66**, 544.
- 3 S. Nakahara and A. Kubo, *Heterocycles*, 2003, **60**, 2717.
- 4 S. Nakahara, R. Numata, Y. Tanaka, and A. Kubo, *Heterocycles*, 1995, **41**, 651.
- 5 A. J. Birch, A. H. Jackson, and P. V. R. Shannon, *J. Chem. Soc., Perkin Trans. I*, 1974, 2185.
- 6 J. C. Duff, *J. Chem. Soc.*, 1941, 547.
- 7 H. Zimer, D. G. Glasgow, M. McClanhan, and T. Novinson, *Tetrahedron Lett.*, 1968, 2805.
- 8 A. Kubo, Y. Kitahara, S. Nakahara, and R. Numata, *Chem. Pharm. Bull.*, 1983, **31**, 341.