Efficient Synthesis of Clitocine via 1,3-*N* (*endo*) to *N* (*exo*) Migration: A Revision to Kini's Work

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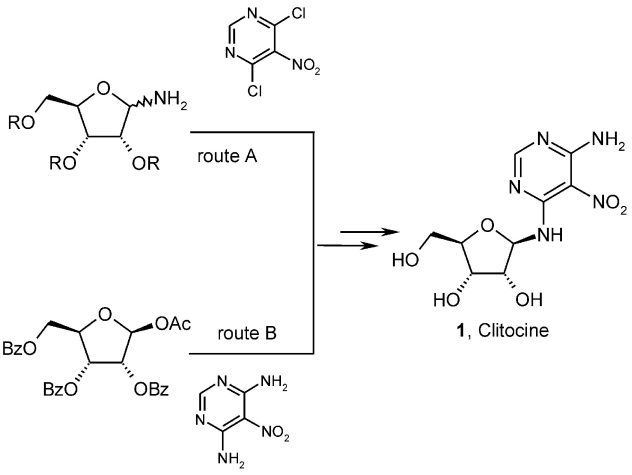
This paper is dedicated to Prof. James D. White.

Abstract: Efficient synthesis of clitocine has been accomplished via unprecedented 1,3-*N* (*endo*) to *N* (*exo*) migration as a key transformation. Incorporation of *p*-chlorobenzoyl (PCB) group as a protecting group led to the easy solidification of the intermediate of **7**, thus making the isolation very facile and to the minimization of the epimerization of the anomeric center at the final deprotection stage.

Key words: 1,3-*N* (*endo*) to *N* (*exo*) migration, isomerizations, clitocine

Clitocine [6-amino-5-nitro-4-(β-D-ribofuranosylamino)pyrimidine], isolated from the mushroom *Clitocybe inversa* by Kubo et al.,[1] has potent insecticidal activity against *Pectinophora gossypiella*. Later it was found that clitocine has also strong cytostatic effect towards several leukemia cell lines and is an inhibitor of adenosine kinase.[2] The interesting biological activity together with biogenetically close relationship with adenosine triggered intensive synthetic studies to lead to the completion of two independent total syntheses of **1**. Kamikawa's group[3] used the condensation reaction of ribofuranosylamine derivatives with 4,6-dichloro-5-nitropyrimidine as a key step (Scheme 1, route A), while Kini's group[2] employed a direct coupling of silylated 4, 6-diamino-5-nitropyrimidine (**3**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) as a critical step (Scheme 1, route B). Compared to the former synthesis, the latter approach is more efficient in terms of chemical yield and stereocontrol, particularly at the anomeric center.

As a part of our interest towards further biological evaluation of **1**, we repeated Kini's synthesis. In the repetition of the coupling reaction of **3**[4] and **4**, we were surprised by the observation that no desired product was formed on the basis of the ^{1}H NMR spectrum of the crude mixture: neither the doublet NH proton at δ = 9.63 ppm nor the broad NH_2 protons at δ = 8.62 ppm of the expected product **8** were observed.[5] However, column chromatography of the crude product provided pure **8** in 28% yield and a 3:1 mixture of the desired **8** and the side product in 23% yield that was later identified as the *endo*-isomer **6**. Hinted by the



Scheme 1

isomerization of the *endo*-product **6** to the *exo*-product **8** during silica gel chromatography, we further treated the 3:1 mixture with silica gel (100% wt/wt to the mixture) for 24 hours in dichloromethane to reach the equilibrated ratio of 93:7. Thus, contrary to the reported result, the *endo*-product **6** was formed exclusively as a kinetic product, which was isomerized to the thermodynamically controlled *exo*-product **8** through 1,3-*N* (*endo*) to *N* (*exo*) migration under acid catalysis. Another drawback of Kini's synthesis is the partial epimerization of the anomeric center at the final deprotection stage: 98:2 of β:α anomer ratio of **8** was deteriorated to 92:8 of β:α anomer ratio of **1** under the deprotection conditions (Scheme 2).

To minimize the partial epimerization of the anomeric center, we changed the benzoyl protecting group to *p*-chlorobenzoyl (PCB) group on the assumption that more electron deficient aromatic ring would facilitate the methanolysis reaction. In the same way as with the benzoate derivatives, 1-acetoxy-PCB-protected ribofuranose **5** was prepared without any incidence. Coupling of **3** and **5** under the conditions used by Kini et al., provided exclusively the *endo*-product **7**. Serendipitously, the kinetic *endo*-product **7** was easily solidified in high purity simply by triturating the crude reaction mixture with ethyl acetate–diethyl ether co-solvent to make the isolation of the prod-

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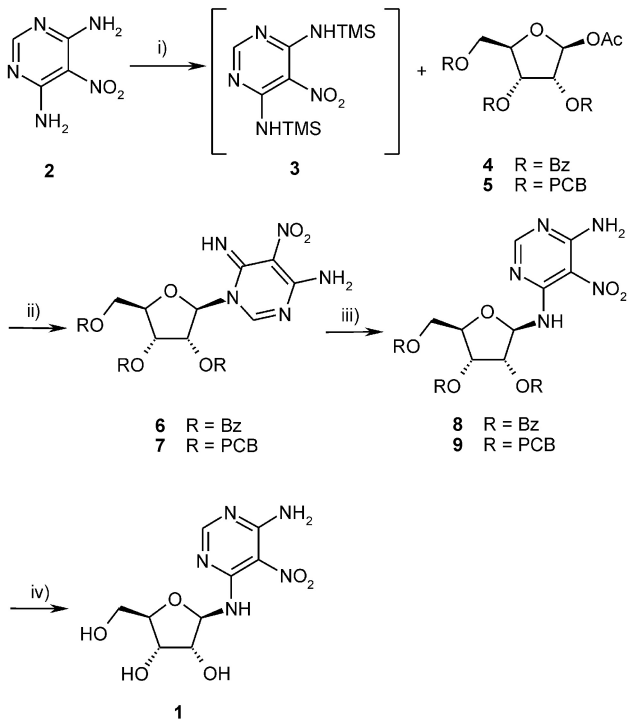

Scheme 2 *Reagents and conditions*: i) HMDS, pyridine, H_2SO_4, $(NH_4)_2SO_4$; ii) TMSOTf; iii) silica gel or HOAc; iv) NaOMe, MeOH–dioxane.

uct very facile (for the ^{1}H NMR spectra of **7** and **9**, see Figure 1). Furthermore, X-ray analysis of a single crystal of **7** unambiguously determined the structure of the *endo*-product (Figure 2).[6] Subsequent isomerization of the *endo*-product **7** by silica gel for 24 hours gave exclusively the *exo*-product **9** in high yield and purity.[7] Besides silica gel, Brønsted acid such as acetic acid also caused isomerization to reach the *exo*:*endo* ratio of 96:4 after 24 hours but Lewis acids like lithium chloride or magnesium chloride did not trigger isomerization.

As expected, the deprotection of the PCB group proceeded much faster (in 2 h at 0 °C) than the benzoyl group (in 17 h at 0–8 °C) to result in no epimerization of the

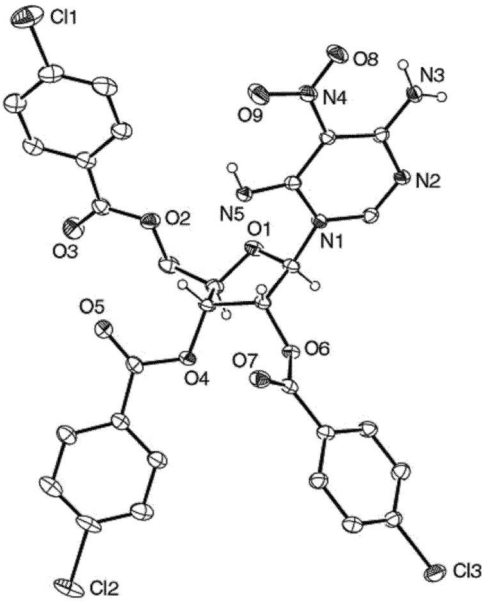

Figure 2 ORTEP plot of **7**.

anomeric center. Simple recrystallization of the crude reaction mixture in methanol provided **1** having 98:2 ratio of β/α anomer in 51% yield. One more recrystallization cycle of the filtrate provided additional 32% of **1** having the same purity of the first crop.[8]

In conclusion, we have discovered that the *endo*-product **6** or **7** was kinetically formed in the glycosylation stage, which was isomerized to the thermodynamic *exo*-product **8** or **9**, respectively, under acid catalysis. Fortunately, the incorporation of the PCB group facilitated the solidification of the *endo*-product **7**, thus making the isolation and purification very facile. Isomerization and subsequent deprotection underwent without any sign of the deterioration of the anomeric purity. The established synthesis provides highly pure clitocine (**1**) without any column separation and delineates the new reaction pathway, 1,3-*N* (*endo*) to *N* (*exo*) migration.

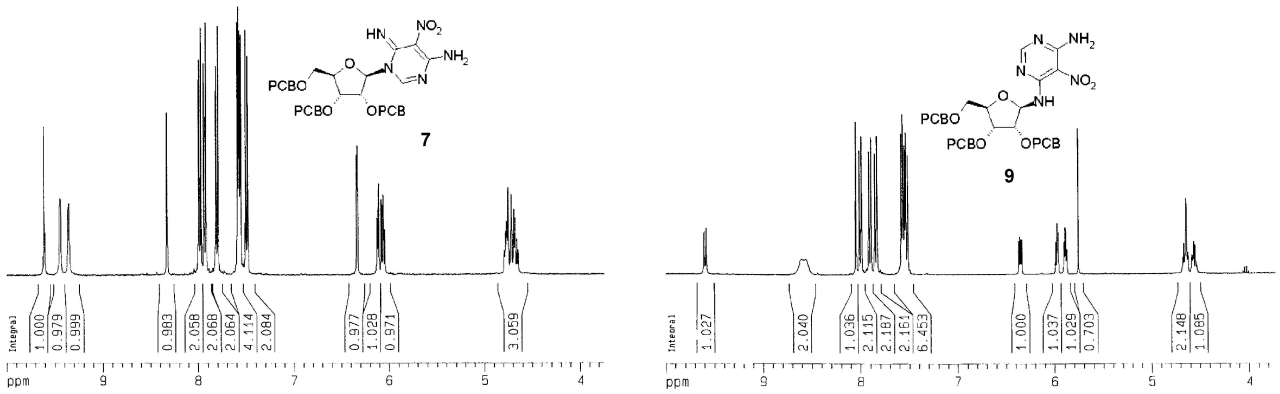

Figure 1 ^{1}H NMR spectra of **7** and **9**.

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(5) We observed peaks at δ = 9.61 (s, 1 H), 9.43 (s, 1 H), and 9.35 (s, 1 H) in DMSO-d_6, which are typical peaks of the *endo*-product **6**. Since **6** was not solidified under various conditions, it was difficult to purify the product thoroughly.
(6) X-ray data for the compound **7**: $C_{30}H_{22}Cl_3N_5O_9$, monoclinic, space group $P2_1$ with cell parameters: a = 14.6041 (11) Å, b = 6.8064 (5) Å, c = 16.1106 (12) Å; α = 90°, β = 108.0770 (10)°, γ = 90°; V = 1522.4 (2) Å^3; D_c = 1.533 mg/m^3; Z = 2.
(7) **4-Amino-6-imino-5-nitro-1-*N*-(2,3,5-tri-*O*-*para*-chlorobenzoyl-β-D-ribofuranosyl)pyrimidine (7).**
A suspension of 4, 6-diamino-5-nitropyrimidine (1.0 g, 6.45 mmol) in HMDS (30 mL) was treated with pyridine (4.8 mL), H_2SO_4 (0.2 mL) and ammonium sulfate (60 mg). The mixture was refluxed at 130 °C for 18 h, and HMDS was evaporated in vacuo. The residual solid was dissolved in MeCN (50 mL), cooled to 0 °C, and treated with 1-*O*-acetyl-2,3,5-tri-*O*-*para*-chlorobenzoyl-D-ribofuranose (**5**, 4.5 g, 7.42 mmol) and TMSOTf (2.0 mL, 11.0 mmol). The mixture was stirred at 0 °C for 23 h, and it turned into dark clear solution. Then, MeCN was evaporated in vacuo, and the residue was treated with sat. $NaHCO_3$ (100 mL). The aqueous layer was extracted with CH_2Cl_2 (100 mL × 2), and the combined organic phase was dried over anhyd $MgSO_4$. After concentration, the residue was triturated with EtOAc (4 mL) and enough amount of Et_2O. The resulting solid was filtered and washed with Et_2O to give the *endo*-product **7** (2.44 g, 55%) as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.61 (1 H, s), 9.44 (1 H, s), 9.36 (1 H, s), 8.33 (1 H, s), 7.98 (2 H, dd, J = 6.8, 2.0 Hz), 7.94 (2 H, dd, J = 6.8, 2.0 Hz), 7.81 (2 H, dd, J = 6.8, 2.0 Hz), 7.57 (4 H, 2 dd, J = 6.8, 2.0 Hz), 7.50 (2 H, dd, J = 6.8, 2.0 Hz), 6.33 (1 H, d, J = 2.4 Hz), 6.12 (1 H, dd, J = 6.4, 2.4 Hz), 6.06 (1 H, dd, J = 7.2, 6.4 Hz), 4.60–4.80 (3 H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.8, 164.0, 163.9, 158.5, 152.3, 148.5, 139.0, 138.9, 138.7, 131.4, 131.3, 131.2, 129.2, 129.1, 128.2, 127.7, 127.5, 111.3, 91.2, 78.7, 74.1, 70.4, 63.7. IR: 3424, 3300, 1713, 1632, 1589, 1478, 1396, 1252, 1087, 1009, 752 cm^{-1}. UV/Vis (CH_2Cl_2): λ_{max} = 243, 374 nm.
6-Amino-5-nitro-4-[(2,3,5-tri-*O*-*para*-chlorobenzoyl-β-D-ribofuranosyl)amino]pyrimidine (9).
A solution of **7** (2.166 g, 3.08 mmol) in methylene chloride (60 mL) was stirred with silica gel (2.2 g) for 20 h. Silica gel was filtered off and washed with CH_2Cl_2. The filtrate was concentrated to give **9** (2.06 g, 95%) as an yellow foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.60 (1 H, d, J = 8.0 Hz), 8.58 (2 H, br s), 8.05 (1 H, s), 8.00 (2 H, dd, J = 6.8, 2.0 Hz), 7.90 (2 H, dd, J = 6.8, 2.0 Hz), 7.84 (2 H, dd, J = 6.8, 2.0 Hz), 7.55 (6 H, m), 6.35 (1 H, dd, J = 8.4, 4.8 Hz), 5.97 (1 H, dd, J = 5.6, 4.8 Hz), 5.89 (1 H, dd, J = 6.0, 5.2 Hz), 4.65 (2 H, m), 4.55 (1 H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.5, 164.8, 164.7, 160.0, 159.4, 157.4, 139.8, 139.7, 139.4, 132.0, 131.9, 129.9, 129.8, 129.7, 128.9, 128.3, 128.2, 113.4, 84.8, 78.7, 75.1, 72.1, 64.8. UV/Vis (CH_2Cl_2): λ_{max} = 240, 331 nm.
(8) **Clitocine (1).**
6-Amino-5-nitro-4-[(2,3,5-tri-*O*-*para*-chlorobenzoyl-β-D-ribofuranosyl)amino]pyrimidine (**9**, 1.93 g, 2.75 mmol) was dissolved in 1,4-dioxane (26 mL) and diluted with MeOH (65 mL). After cooling to 0 °C, the solution was treated with MeONa (0.55 mmol, 0.1 mL of 28% MeOH soln was diluted with 0.4 mL of MeOH) and stirred at 0 °C for 2 h. The resulting solution was stirred with Amberlite IR-120 (900 mg, 3.96 mol equiv, acidic resin was washed with distilled H_2O and MeOH before use) for 30 min. The resin was filtered off and the filtrate was concentrated in vacuo. The residue was triturated with Et_2O and washed with Et_2O (10 mL × 3). The solid was recrystallized from hot MeOH (135 mL) to give **1** (≥ 98% of β-anomer, 400 mg, 51%) as a slightly yellow solid. More clitocine (**1**, ≥ 98% of β-anomer, 255 mg, 32%) was obtained from the mother liquor. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.28 (1 H, d, J = 7.6 Hz), 8.56 (2 H, s), 8.00 (1 H, s), 5.79 (1 H, dd, J = 7.6, 3.6 Hz), 5.20 (1 H, d, J = 5.2 Hz), 5.06 (1 H, t, J = 4.8 Hz), 4.92 (1 H, d, J = 6.0 Hz), 4.05 (1 H, m), 3.93 (1 H, m), 3.79 (1 H, m), 3.53 (1 H, m), 3.47 (1 H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 160.2, 159.5, 156.8, 112.8, 87.0, 84.8, 75.9, 70.8, 61.3.