Novel Exocyclic Nucleoside Related to Clitocine: A Convergent Synthesis of 3′-Azido-2′,3′-dideoxy Clitocine

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Abstract: The synthesis of a new clitocine derivative was achieved through a convergent strategy. A protected 4,6-diamino-5-nitropyrimidine was condensed with *p*-chlorobenzoyl (PCB)-protected methyl 3′-azido-2′,3′-dideoxyribofuranoside, followed by subsequently deprotection to give the desired product.

Key words: nucleoside analogues, exocyclic nucleosides, convergent synthesis, clitocine, antiviral drug

Clitocine (**1**, Figure 1), 6-amino-5-nitro-4-(β-D-ribofuranosylamino) pyrimidine, is a naturally occurring amino exocyclic nucleoside isolated from the mushroom, *Clitocybe inversa*,[1] whose syntheses have been reported.[2] Clitocine has already been demonstrated to exhibit strong insecticidal activity against the pink bollworm *Pectinophora gossypiella*[1] and cytotoxic activities against some cancer cell lines [L1210, WI-L2, 3LL, DU145, K-562, MCF7, U251, human cervical cancer cells (HeLa) and CCRF-CEM].[2a,3] It is structurally similar to adenosine and this makes it to be a potential inhibitor for adenosine kinase.[4] The successful applications of clitocine as a therapeutic composite to treat diseases associated with HepG2 liver cancer cells,[5] P-glycoprotein tumor cells,[6] and nonsense mutations[7] have been disclosed. Because of these interesting pharmacological activities, several research groups have synthesized various acyclic,[8] carbocyclic,[9] 4-substituted amino,[10] 5′-deoxy,[4a,b] and 2′-deoxy[11] analogues of clitocine.

Following many successful nucleoside drugs related to acyclovir (**2**), such as valaciclovir (**3**), and penciclovir (**4**), which can be seen as acyclic nucleosides; we believe that it is worthwhile to explore some exocyclic structures. In continuation of our interest in the synthesis of novel nucleosides, we wished to investigate the synthesis and biological activities of compounds containing the clitocine aglycon, which might be regarded as an exocyclic nucleoside structure.

Zidovudine (AZT, **5**) is the first approved drug for treatment of AIDS, and is characterized by a 3′-azido substituent. 3′-Azido functionality can also be easily reduced to 3′-amino, a characteristic group of oligonucleotide N3′–P5′ phosphoramidates that had been developed as antisense drugs.[12] Based on above considerations, 3′-azido-substituted clitocine could be considered as a potential antitumor and antiviral target. We report herein the synthesis of the new 3′-azido-2′,3′-dideoxy clitocine [**6**, 6-amino-5-nitro-4-(3′-azido-2′,3′-dideoxy-β-D-ribofuranosylamino)-pyrimidine].

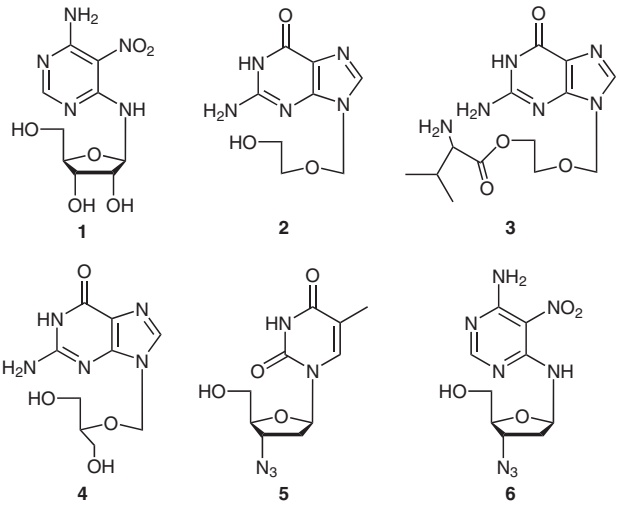

Figure 1 Some nucleoside structures

Two synthetic building blocks were required, a protected base and a sugar. The base 4,6-diamino-5-nitro-pyrimidine (**7**) was prepared from 4,6-dihydroxy-pyrimidine employing a three-step method,[13] and then silylated to give TMS protected **8**.[2a]

We initially used TBDPS-protected methyl 3-azido-2,3-dideoxy-D-erythro-pentofuranoside as a sugar source, which was easily prepared from 2-deoxy-D-ribose (**9**).[14] But further coupling failed to meet our expectation. The protected base **8** was glycosylated with silylated sugar in 1,2-dichloroethane with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the Lewis acid catalyst at room temperature for 72 hours, but no detectable product was observed. The lability of silylated sugar under acidic condition might be responsible for this failure.

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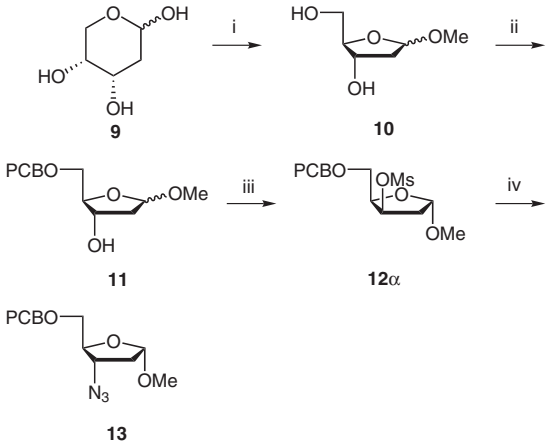
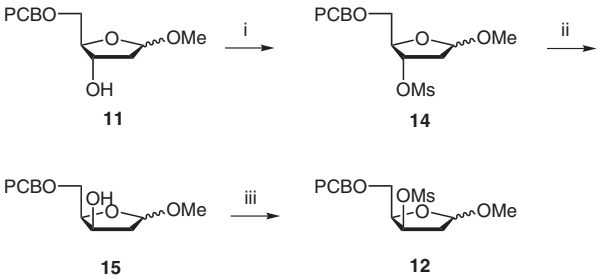


Scheme 1 Synthesis of PCB protected sugar. *Reagents and conditions*: (i) HCl, MeOH, r.t.; (ii) *p*-chlorobenzoyl chloride, Et_3N, CH_2Cl_2, ice bath (90% for two steps); (iii) Ph_3P, DEAD, MsOH, toluene, 60–70 °C (86%); (iv) NaN_3, DMF, 110 °C (70%).

To address the above-mentioned protecting problem, we changed the *tert*-butyldiphenylsilyl (TBDPS) protecting group to *p*-chlorobenzoyl (PCB) group. Two routes were used to provide PCB protected sugar starting from 2-deoxy-D-ribose (**9**). The first route (Scheme 1) employed a Mitsunobu reaction as a key step to achieve inversion of the configuration at 3-OH. The starting **9** was first quantitatively methylated at position 1 with hydrogen chloride in methanol.[15] The obtained methyl glycoside **10** was selectively 5-O-benzoylated using *p*-chlorobenzoyl chloride in dichloromethane and triethylamine, afforded **11** as an α/β mixture in a yield of 90%. The mixture of anomers could be used in the next step without separation, which was treated with triphenylphosphine, diethyl azodicarboxylate (DEAD), and methanesulfonic acid in toluene at 60–70 °C to give **12α** in 86% yield.[16] It is of interest to note that 1α-anomer of **12** was found as the only main product after Mitsunobu reaction, whose structure was unambiguously determined by X-ray analysis of a single crystal (Figure 2)[17]. The **12α** were then heated in DMF with an excess of sodium azide at 110 °C to give the corresponding azido compounds **13** in 70% yield. Pure compound **12α** is very stable after recrystallization, but slow racemization at C-1 of **13** was observed after prolonged storage at room temperature.



Scheme 2 Synthesis of 3β-OH sugar. *Reagents and conditions*: (i) MsCl, Et_3N, CH_2Cl_2, ice bath (90%); (ii) $NaNO_2$, DMF, 120 °C (62%); (iii) MsCl, Et_3N, CH_2Cl_2, ice bath (92%).

A second route was designed to overcome tedious workup of Mitsunobu reaction and improve productivity of 3β-OH sugar **12** (Scheme 2). Two additional steps were used to realize the inversion of 3-OH. First, 3α-OH was mesylated to give **14** in a yield of 90%. Then a Walden inversion was performed in the next step with substitution of the methanesulfonate group in position 3. Sodium nitrite was then used as O-nucleophile, and the compound **15** with free hydroxy groups was isolated in 62% yield. Subsequent mesylation of **15** gave **12** (1:1 ratio of α/β) in 92% yield.

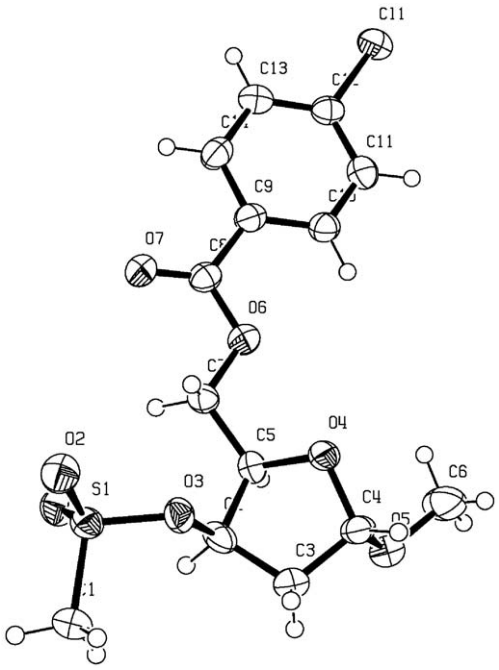
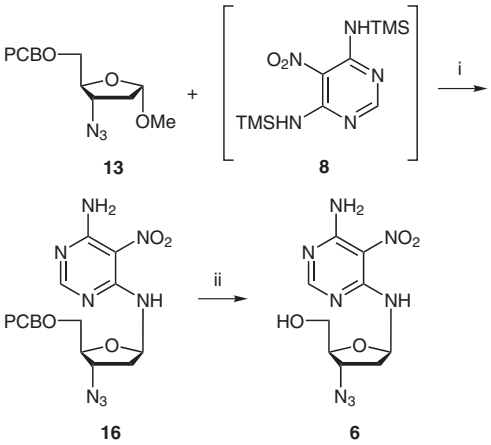


Figure 2 ORTEP plot of compound **12α**

Finally, the silylated base **8** was directly glycosylated with azide substituted sugar **13** in 1,2-dichloroethane with TMSOTf as the Lewis acid catalyst to give 6-amino-5-



Scheme 3 Synthesis of 3′-azido-2′,3′-dideoxy clitocine (**6**). *Reagents and conditions*: (i) TMSOTf, DCE, r.t. (16%); (ii) NaOMe, MeOH, r.t. (65%).

nitro-4-(3′-azido-5′-*O*-*p*-chlorobenzoyl-2′,3′-dideoxy-β-D-ribo-furanosylamino) pyrimidine (**16**) as a white solid in 16% yield after chromatography.[18] No significant amount of α-anomer was isolated. Treatment of **16** with a solution of NaOMe in methanol gave nucleoside **6** in 65% yield[19] (Scheme 3).

In conclusion, we have developed an efficient method to prepare the novel 3′-azido-2′,3′-dideoxy clitocine analogue. The synthesis was accomplished via convergent route, which should be applicable to the synthesis of similarly functionalized analogues of other pyrimidine as well as purine nucleosides.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(17) The CCDC number of compound **12α** is 777653.

(18) **6-Amino-5-nitro-4-(3′-azido-5′-*O*-*p*-chloro-benzoyl-2′,3′-dideoxy-β-D-ribofuranosylamino) Pyrimidine (16)**
TMS-protected 4,6-diamino-5-nitropyrimidine **8** (0.86 g, 2.9 mmol) was dissolved in DCE (10 mL). To this solution, methyl 3′-azido-5′-*O*-*p*-chlorobenzoyl-2′,3′-dideoxy-β-D-ribofuranoside (**13**, 0.75 g, 2.4 mmol) and TMSOTf (0.72 mL, 4.0 mmol) were added, the mixture was stirred for 48 h at r.t., and 10% $NaHCO_3$ (15 mL) was added. After 20 min stirring, CH_2Cl_2 (20 mL) was added to the resulting suspension; the mixture was filtered through Hyflo Super Cel; the organic layer was separated, washed with H_2O (10 mL), and dried with Na_2SO_4. Nucleoside **16** (0.11 g) and recovered sugar **13** (0.27 g) were isolated by silica gel chromatography (elution with EtOAc–PE = 1:2). The yield was 16% based on recovered starting material. A white solid was afforded after recrystallizaion in EtOAc; mp 178–180 °C. IR (KBr): ν = 3440, 3332, 2109, 1389, 1344, 1290, 1245 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ = 9.57 (1 , d, $J_{NH-H1'}$ = 8.51 Hz, NH), 8.61 (2 H, d, J_{NH2} = 15.10 Hz, NH_2), 8.00 (1 H, s, C2-H), 7.98 (2 H, m, ArH), 7.61 (2 H, m, ArH), 6.32 (1 H, m, C1′-H), 4.56 (1 H, m, C4′-H), 4.36 (3 H, m, C5′-H, C3′-H), 2.62 (1 H, dd, *J* = 5.69, 12.80 Hz, C2′-H), 2.20 (1 H, m, C2′-H) ppm. ^{13}C NMR (500 MHz, DMSO-d_6): δ = 164.66 (C=O), 159.29 (C-2), 158.58 (C-6), 156.02 (C-4), 138.46 (CCl), 131.11 (2 C, Ar), 128.98 (2 C, Ar), 128.17 (CC=O), 112.03 (C-5), 81.11 (C-1′), 80.62 (C-4′), 64.83 (C-5′), 61.68 (C-3′), 36.77 (C-2′) ppm.

(19) **6-Amino-5-nitro-4-(3′-azido-2′,3′-dideoxy-β-D-ribofuranosylamino) Pyrimidine 6**
To a solution of nucleoside **16** (90 mg, 0.2 mmol) in MeOH (10 mL) cooled to 0 °C was added 0.1 M NaOMe in MeOH

(0.64 mL), and the mixture was stirred at r.t. for 18 h in the argon atmosphere. The reaction mixture was neutralized with Dowex 50 (H^+), and the resin was rapidly filtered. After evaporation under vacuum to dry, a light yellow solid product **6** was isolated from the residue by silica gel chromatography, elution with an EtOAc–PE (3:2). The yield was 40 mg (65%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.39 (1 H, d, $J_{NH–H1'}$ = 8.2 Hz, NH), 8.56 (2 H, br s, NH_2), 8.00 (1 H, s, C2-H), 6.22 (1 H, td, J = 6.0 Hz, $J_{NH–H1'}$ = 8.2 Hz, C1′-H), 5.23 (1 H, t, J = 5.0 Hz, OH), 4.33 (1 H, td, J = 4.2 Hz, $J_{H2α'–H3'}$ = 7.0 Hz, C3′-H), 3.88 (1 H, q, J = 3.58, 3.58, 3.60 Hz, C4′-H), 3.51 (2 H, t, J = 4.2 Hz, C5′-H), 2.39 (1 H, ddd, $J_{H2α'–H1'}$ = 6.0 Hz, $J_{H2α'–H3'}$ = 7.0 Hz, $J_{H2β'–H2α'}$ = 13.0 Hz, C2′-$H_α$), 2.27 (1 H, ddd, $J_{H2β'–H3'}$ = 4.2 Hz, $J_{H2β'–H1'}$ = 6.0 Hz, $J_{H2β'–H2α'}$ = 13.0 Hz, C2′-$H_β$) ppm. ^{13}C NMR (500 MHz, DMSO-d_6): δ = 159.98 (C-2), 159.29 (C-6), 156.49 (C-4), 109.99 (C-5), 84.62 (C-1′), 82.21 (C-4′), 62.14 (C-5′), 62.08 (C-3′), 38.40 (C-2′) ppm.