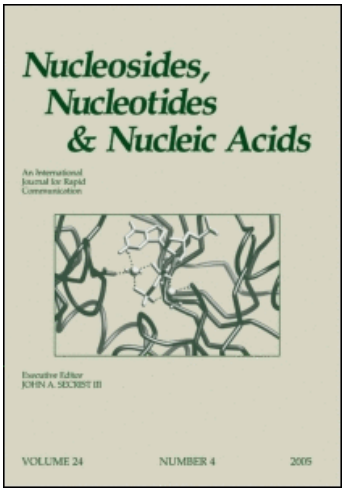

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Nucleosides. Part 3.[1] Synthesis of 2-N-Substituted 1-β-D-Arabinofuranosylisocytosine Derivatives by The Reaction of 2',5'-Dichloro-2',5'-Dideoxyuridine with Amines

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NUCLEOSIDES. PART 3.[1] SYNTHESIS OF 2-N-SUBSTITUTED 1-β-D-ARABINOFURANOSYLISOCYTOSINE DERIVATIVES BY THE REACTION OF 2',5'-DICHLORO-2',5'-DIDEOXYURIDINE WITH AMINES

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Abstract: Reaction of 2',5'-dichloro-2',5'-dideoxyuridine (1) with ammonia and benzylamine afforded the corresponding 2-N-substituted 1-(5-chloro-5-deoxy-β-D-arabinofuranosyl)-isocytosine derivatives (**2** and **10**). Reaction of **1** with ammonia, methylamine, cyclohexylamine, and benzylamine followed by treatment with methanolic sodium methoxide gave the corresponding 2-N-substituted 1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine derivatives (**6**, **11**, and **12**).

Our recent work has demonstrated that the reaction of uridine derivatives with the Vilsmeier-Haack reagent (POX_3/DMF) results in the facile formation of 5-substituted 2',5'-dideoxy-2',5'-dihalogenouridines.[2,3] These 2',5'-dihalogenouridines are versatile intermediates for the preparation of biologically interesting 2'- and/or 5'-deoxyuridines.[4] Previously, we reported the synthesis of 5-substituted 1-(2,5-anhydro-β-D-arabinofuranosyl)uracil derivatives (II) by the reaction of 5-substituted 2',5'-dichloro-2',5'-dideoxyuridines (I) with hydroxide anion.[5,6]

Our continuing investigation of the reactivities of the 2',5'-dichlorouridines toward various nucleophiles led to the discovery of a new method for chemical modification of pyrimidine nucleosides which produced the corresponding 1-(5-chloro-5-deoxy-β-D-arabinofuranosyl)isocytosine and 1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine derivatives by the use of amines as nucleophiles. Thus far a few isocytosines have been synthesized by the reaction of 2,5'-

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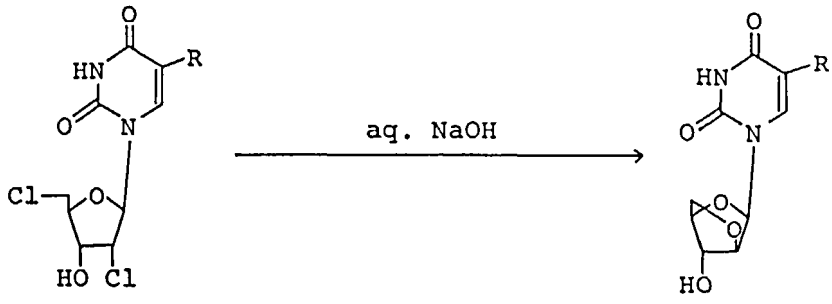


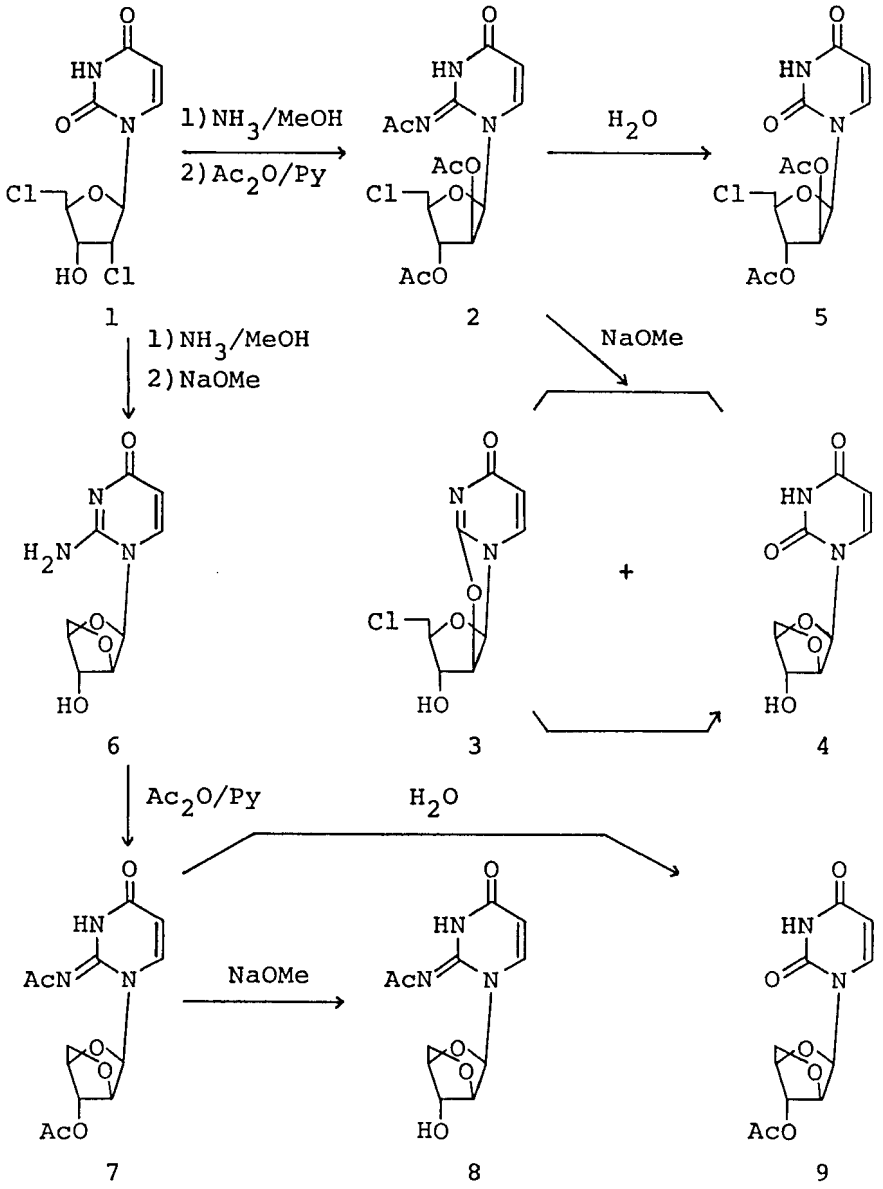
I Scheme 1 II

anhydro-5-fluoro-2',3'-O-isopropylideneuridine[7] or 2,2'-anhydro-1-β-D-arabinofuranosyluracil[8] with ammonia. A recent paper reported that 1-(2,5-anhydro-β-D-arabinofuranosyl)cytosine exhibited highly potent inhibitor of DNA synthesis in cultured L1210 cells.[9] 1-(2,5-Anhydro-β-D-arabinofuranosyl)isocytosine derivatives described in this paper are of interest in view of their structural similarity to biologically active 1-(2,5-anhydro-β-D-arabinofuranosyl)cytosine.

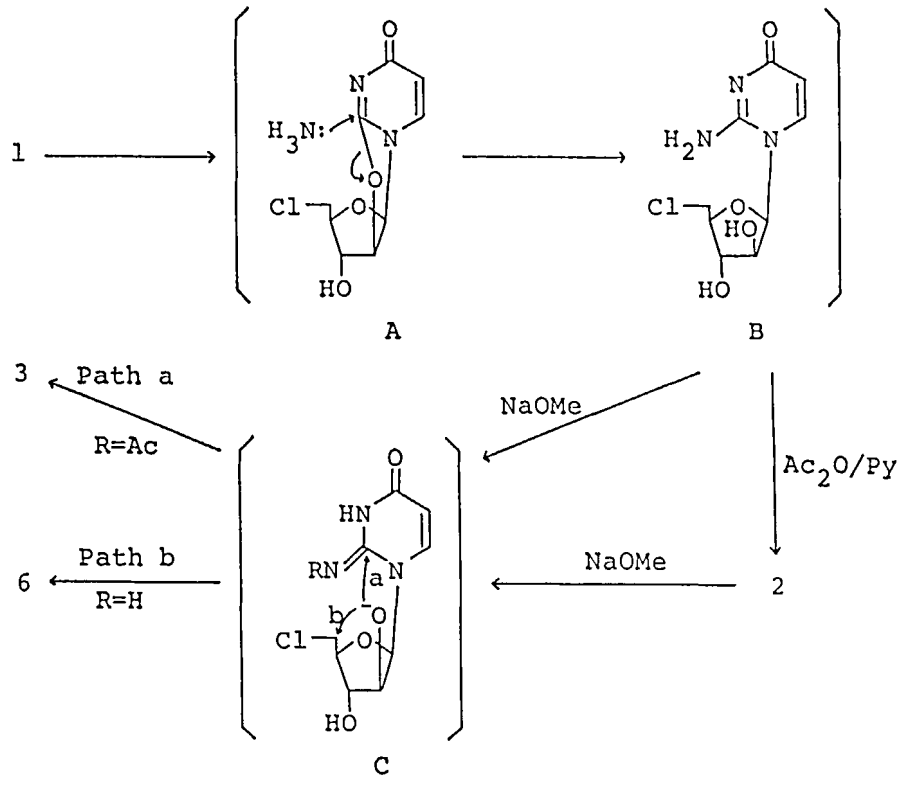
Results and Discussion

2',5'-Dichloro-2',5'-dideoxyuridine (1) was treated with methanolic ammonia in a sealed tube at room temperature for 3 days. The crude product thus obtained was acetylated by Ac_2O-pyridine to afford 2-N-acetyl-1-(2,3-di-O-acetyl-5-chloro-5-deoxy-β-D-arabinofuranosyl)isocytosine (2) in 97% yield. The structure of 2[10] was supported by microanalysis and spectral data. The ultimate proof of the structure of **2** rests upon its comparison with the authentic sample prepared by the ammonolysis and subsequent acetylation of 5'-chloro-5'-deoxy-2,2'-anhydrouridine (**3**).

A plausible mechanism for the formation of **2** from **1** is outlined in Scheme 3. The base-catalyzed cyclization of **1** could give a 2,2'-anhydro intermediate **A**, which undergoes ammonolysis of the 2,2'-anhydrobond by an attack of ammonia on the 2-position to give an arabinofuranosyl intermediate **B**. Acetylation of the intermediate **B** produces **2**.

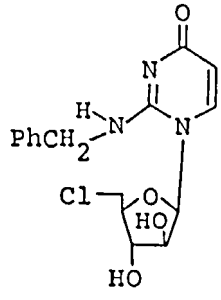


Scheme 2



Scheme 3

When methylamine and cyclohexylamine were employed instead of ammonia in the reaction with **1**, the corresponding 2-N-substituted arabinofuranosylisocytosine derivatives could not be isolated in pure form even after acetylation. The reaction of **1** with benzylamine without subsequent acetylation, however, afforded 2-N-benzyl-1-(5-chloro-5-deoxy-β-D-arabinofuranosyl)isocytosine (**10**) in 54% yield.



10

Treatment of **2** with sodium methoxide under reflux for 12 min gave **3** and the known 1-(2,5-anhydro-β-D-arabinofuranosyl)uracil (**4**)[5,6,11] in 66% and 5% yield, respectively. This result indicates that the arabinofuranosylisocytosine (**2**) which possesses an acetyl group at the 2-N-position undergoes cyclization predominantly between the 2- and 2'-positions (path a in Scheme 3) rather than between the 2'-

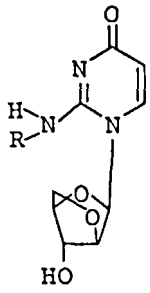
and 5'-positions (path b in Scheme 3). When the reaction was carried out under reflux for 5 hr, **4** was obtained in 83% yield as a sole product. Compound **3** is easily converted into **4** by treatment with aqueous sodium hydroxide.[10] On the other hand, treatment of **2** with boiling water afforded the hydrolysis product, 1-(2,3-di-O-acetyl-5-chloro-5-deoxy-β-D-arabinofuranosyl)uracil (**5**), in 80% yield.

Reaction of **1** with methanolic ammonia at room temperature for 3 days followed by treatment with methanolic sodium methoxide under reflux for 3 h resulted in the formation of 1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine (**6**) in 56% yield. A reasonable mechanism for the formation of the 2',5'-anhydroisocytidine (**6**) is as follows: the intermediate **B**, which is produced via the same reaction sequence for the formation of **2** from **1**, is converted into **6** via the intermediate **C** (R=H) by a base-catalyzed cyclization between the 2'- and 5'-positions (path b in Scheme 3).

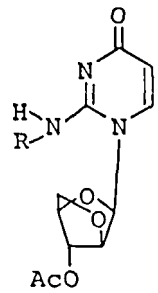
Acetylation of **6** afforded 2-N-acetyl-1-(3-O-acetyl-2,5-anhydro-β-D-arabinofuranosyl)isocytosine (**7**)[10] in 73% yield. Treatment of **7** with methanolic sodium methoxide under reflux for 30 min caused deacetylation of only the sugar moiety to give 2-N-acetyl-1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine (**8**)[10] in 43% yield. Heating of **7** in water for 6 hr, however, afforded 1-(3-O-acetyl-2,5-anhydro-β-D-arabinofuranosyl)uracil (**9**) in 52% yield. The hydrolysis of the 2-N-acetyl derivatives (**2** and **7**) proceeded smoothly upon treatment with boiling water to yield the corresponding 2-oxo derivatives (**5** and **9**). The conversion of the N-acetylimino group into the oxo group at the 2-position of the base moiety preceded the hydrolysis of the O-acetyl group of the sugar moiety under these conditions.

Analogously, the reaction of **1** with cyclohexylamine or benzylamine in methanol at room temperature followed by treatment with methanolic sodium methoxide under reflux afforded the corresponding 2-N-substituted 2',5'-anhydroisocytidine derivatives (**11a**) and (**11b**) in 91% and 86% yield, respectively. When methylamine was employed as an amine, the resulting 2-N-methylisocytidine derivative was subsequently acetylated without purification to give the corresponding 3'-O-acetylisocytidine (**12a**) in 79% yield. Similarly, treatment of **1** with cyclohexylamine and benzylamine afforded the acetylated 2',5'-anhydroisocytidines (**12b**) and (**12c**).

Biological evaluation of the 2-N-substituted 1-β-D-arabinofuranosylisocytosine derivatives is now in progress.



11a; R=⟨H⟩
11b; R=CH_2Ph



12a; R=CH_3
12b; R=⟨H⟩
12c; R=CH_2Ph

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Elemental analyses were carried out at the Microanalytical Laboratory of our university. Proton magnetic resonance spectra (60 MHz) were recorded on a Hitachi; R-20B nuclear magnetic resonance spectrometer with tetramethylsilane ($CDCl_3$) and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DMSO-d_6) as internal standards. Chemical shifts are reported in parts per million (δ), the J values are given in hertz, and signals are quoted as a s (singlet), d (doublet), or br (broad); J values are first order. Mass spectra (Ms) were taken on a JEOL JMS-D300 machine operating at 70 eV. Ultraviolet spectra were obtained from ethanol on a Shimazu 323 spectraphotometer. Column chromatography was carried out on silica gel (Wakogel C-300).

2-N-Acetyl-1-(2,3-di-O-acetyl-5-chloro-β-D-arabinofuranosyl)isocytosine (2). a) A solution of 1 (500 mg, 1.8 mmol) in methanolic ammonia (50 ml) was stirred in a sealed tube for 3 days at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml). The mixture was stirred for 1 day at room temperature and poured into ice-water and the solution was extracted with chloroform. The extract was washed with a saturated $NaHCO_3$ solution, a saturated $NaHSO_4$ solution and then water. The solvent was removed under reduced pressure and the residue was

crystallized from water. Recrystallization from water gave analytically pure 2 (669 mg, 97%), mp. 132.5-134°C. UV λmax: 213 (ε 11100), 255 (ε 20100) and 265 nm (sh) (ε 16700). Ms (m/z): 388 (M^++1). ^{1}H NMR ($CDCl_3$):δ 12.90 (1H, br, HN^3), 7.78 (1H, d, J=8.3 Hz, H-6), 6.58 (1H, d, J=4.1 Hz, H-1'), 5.88 (1H, dd, J=8.3 and 2.1 Hz, H-5), 5.55 (1H, dd, J=4.1 and 1.7 Hz, H-2'), 5.17 (1H, dd, J=4.0 and 1.7 Hz, H-3'), 4.26 (1H, m, H-4'), 3.91 (2H, d, J=3.8 Hz, H-5'), 2.17 (6H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$).

Anal. Calcd for $C_{15}H_{18}ClN_3O_7$: C, 46.46; H, 4.68; N, 10.84. Found: C, 46.22; H, 4.67; N, 10.68.

b) A solution of **3** (100 mg, 0.41mmol) in methanolic ammonia (15 ml) was stirred in a sealed tube for 2 days at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in a mixture of pyridine (1 ml) and acetic anhydride (1 ml) and the reaction mixture was stirred for 1 day at room temperature and poured into ice-water. The solution was extracted with chloroform and the extract was washed with a saturated $NaHCO_3$ solution, a saturated $NaHSO_4$ solution and then water. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column eluting with benzene-ethyl acetate (2:1). The solvent was removed under reduced pressure and the residue was crystallized from water. Recrystallization from water gave analytically pure 2 (94 mg, 59%), which was identical with the compound prepared above.

Reaction of 2 with Sodium Methoxide. a) A solution of **2** (388 mg, 1 mmol) in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (50 ml)] was refluxed for 12 min. The solvent was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure and the residue was chromatographed on a silica gel column eluting with chloroform-methanol (20:1). The elution of the faster fraction gave **4** (11 mg, 5 %), which was identical with an authentic sample.[11] The elution of the slower fraction gave **3** (162 mg, 66 %), which was identical in every respect with an authentic sample.[12]

b) A solution of **2** (388 mg, 1 mmol) in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (50 ml)] was refluxed for 5 hr. The solvent was

neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The resulting precipitate was recrystallized from methanol to give **4** (187 mg, 83 %), which was identical with the compound prepared above.

1-(2,3-Di-O-acetyl-5-chloro-5-deoxy-β-D-arabinofuranosyl)-uracil (5). A suspension of **2** (150 mg, 0.39 mmol) in water (15 ml) was refluxed for 1.5 hr. The solution was extracted with chloroform. The solvent was removed under reduced pressure and the residue was crystallized from ether. Recrystallization from ethanol gave analytically pure **5** (107 mg, 80 %), mp. 149-150°C. UV λmax: 206 (ε 7760) and 257 nm(ε 10100). Ms (m/z): 347 (M^++1). ^{1}H NMR ($CDCl_3$):δ 9.83 (1H, br, HN^3), 7.59 (1H, d, J=8.3 Hz, H-6), 6.33 (1H, d, J=4.4 Hz, H-1'), 5.78 (1H, d, J=8.3 Hz, H-5), 5.43 (1H, dd, J=4.4 and 1.5 Hz, H-2'), 5.21 (1H, dd, J=3.8 and 1.5 Hz, H-3'), 4.23 (1H, m, H-4'), 3.91 (2H, br d, J=4.4 Hz, H-5'), 2.15 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$).

Anal. Calcd for $C_{13}H_{15}ClN_2O_7$: C, 45.03; H, 4.36; N, 8.08. Found: C, 45.18; H, 4.37; N, 8.26.

1-(2,5-Anhydro-β-D-arabinofuranosyl)isocytosine (6). A solution of **1** (1.5 g, 5.3 mmol) in methanolic ammonia (100 ml) was stirred in a sealed tube for 3 days at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in methanolic sodium methoxide [prepared from Na (1.2 g, 53 mmol) in absolute methanol (100 ml)]. The mixture was refluxed for 3 hr and neutralized with Amberlite CG-50 (H^+). The exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The resulting precipitate was recrystallized from 95 % ethanol solution to afford **6** (667 mg, 56 %), mp. 268°C. UV λmax: 207 (ε 25500), 227 (sh) (ε 11800) and 251 nm (sh) (ε 6100). Ms (m/z): 225 (M^+). ^{1}H NMR (DMSO-d_6):δ 7.82 (1H, d, J=8.0 Hz, H-6), 6.10 (1H, s, H-1'), 5.73 (1H, d, J=8.0 Hz, H-5), 5.93-4.79 (3H, br, NH_2 and OH), 4.54 (3H, br, H-2', H-3' and H-4'), 4.01 (2H, br s, H-5').

Anal. Calcd for $C_9H_{11}N_3O_4 \cdot 1/2H_2O$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.23; H, 5.06; N, 17.67.

2-N-Acetyl-1-(3-O-acetyl-2,5-anhydro-β-D-arabinofuranosyl)-isocytosine (7). A mixture of **6** (500 mg, 2.22 mmol), pyridine (6 ml), and acetic anhydride (4 ml) was stirred at

room temperature for 1 hr and poured into ice-water. The solution was extracted with chloroform and the extract was washed with a saturated $NaHCO_3$ solution, a saturated $NaHSO_4$ solution and then water. The solvent was removed under reduced pressure. The resulting precipitate was recrystallized from water to afford **7** (498 mg, 73 %), mp. 207°C. UV λ max: 214 (ε 10200), 255 (ε 19000) and 272 nm (ε 14200). Ms (m/z): 310 (M^++1). ^{1}H NMR ($CDCl_3$):δ 12.99 (1H, br s, HN^3), 7.93 (1H, d, J=8.3 Hz, H-6), 6.27 (1H, s, H-1'), 5.91 (1H, br d, J=8.3 Hz, H-5), 5.29 (1H, br d, J=2.7 Hz, H-3'), 4.86 (2H, m, H-2' and H-4'), 4.11 (2H, br s, H-5'), 2.23 (3H, s, $COCH_3$), 2.18 (3H, s, $COCH_3$).

Anal. Calcd for $C_{13}H_{15}N_3O_6$: C, 50.48; H, 4.89; N, 13.59. Found: C, 50.20; H, 4.94; N, 13.41.

2-N-Acetyl-1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine (8). A solution of **7** (250 mg, 0.81 mmol) in methanolic sodium methoxide [prepared from Na (186 mg, 8.1 mmol) in absolute methanol (30 ml)] was refluxed for 30 min. The mixture was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The residue was dissolved in water and the solution was extracted with chloroform. The extract was removed under reduced pressure. The resulting precipitate was recrystallized from ethanol to afford **8** (94 mg, 43%), mp. 215-216°C. UV λmax: 214 (ε 10000), 256 (ε 19500) and 274 nm (ε 14100). Ms (m/z): 268 (M^++1). ^{1}H NMR (DMSO-d_6):δ 12.93 (1H, br, HN^3), 8.15 (1H, d, J=8.3 Hz, H-6), 6.34 (1H, s, H-1'), 6.10 (1H, d, J=3.0 Hz, OH), 5.95 (1H, d, J=8.3 Hz, H-5), 4.57 (2H, br s, H-2' and H-3'), 4.45 (1H, t, J=2.6 Hz, H-4'), 4.04 (2H, br s, H-5'), 2.12 (3H, s, $COCH_3$).

Anal. Calcd for $C_{11}H_{13}N_3O_5$: C, 49.43; H, 4.90; N, 15.73. Found: C, 49.39; H, 5.03; N, 15.66.

1-(3-O-Acetyl-2,5-anhydro-β-D-arabinofuranosyl)uracil (9). A suspension of **7** (150 mg, 0.49 mmol) in water (15 ml) was refluxed for 2 hr. The solution was extracted with chloroform and the extract was dried with sodium sulfate. The solvent was removed under reduced pressure. The resulting precipitate was recrystallized from ethanol to afford **9** (68 mg, 52 %), mp. 232-233°C. UV λmax: 204 (ε 8850) and 262 nm (ε 10500). Ms (m/z): 269 (M^++1). ^{1}H NMR (DMSO-d_6):δ 11.41 (1H, br, HN^3), 7.89 (1H, d, J=8.1 Hz, H-6), 6.01 (1H, s, H-

1'), 5.67 (1H, dd, J=8.1 and 1.5 Hz, H-5), 5.30 (1H, d, J=2.4 Hz, H-3'), 4.83 (1H, s, H-2'), 4.57 (1H, dd, J=2.4 and 1.1 Hz, H-4'), 4.12 (2H, br s, H-5'), 2.17 (3H, s, $COCH_3$).

Anal. Calcd for $C_{11}H_{12}N_2O_6$: C, 49.25; H, 4.51; N, 10.45. Found: C, 49.20; H, 4.55; N, 10.37.

2-N-Benzyl-1-(5-chloro-5-deoxy-β-D-arabinofuranosyl)isocytosine (10). A mixture of **1** (281 mg, 1 mmol), benzylamine (10 ml), and methanol (10 ml) was stirred at room temperature for 24 hr. The solvent was removed under reduced pressure and the residue was dissolved in water (200 ml). The solution was extracted with chloroform. The aqueous solution was evaporated in vacuo and the resulting precipitate was collected by filtration. Recrystallization from water gave analytically pure **10** (189 mg, 54%), mp 169-171°C. UV λmax: 218 (ε 27900) and 257 nm (sh) (ε 6760). Ms (m/z): 351 (M^+). ^1H NMR (DMSO-d_6):δ 7.84-7.12 (7H, br, Ph, HN^2 and H-6), 6.24-5.73 (3H, br, H-1' and OH), 5.62 (1H, d, J=8 Hz, H-5), 4.56 (2H, br d, J=4.5 Hz, $PhCH_2$), 4.43-3.77 (5H, br, H-2', H-3', H-4', and H-5').

Anal. Calcd for $C_{16}H_{18}ClN_3O_4 \cdot 1/3H_2O$: C, 53.71; H, 5.26; N, 11.74. Found: C, 53.66; H, 5.34; N, 11.66.

2-N-Cyclohexyl-1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine (11a). A mixture of **1** (281 mg, 1 mmol), cyclohexylamine (10 ml), and methanol (10 ml) was stirred for 2 days at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (30 ml)]. The mixture was refluxed for 1.5 hr. The mixture was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The resulting precipitate was collected by filtration. Recrystallization from water gave analytically pure **11a** (279 mg, 91 %), mp. 256-256.5°C. UV λ max: 215 (ε 32700), 258 nm (sh) (ε 7140). Ms (m/z): 307 (M^+). ^1H NMR(DMSO-d_6):δ 7.21 (1H, d, J=7.7 Hz, H-6), 6.45 (1H, br d, J=7.5 Hz, HN^2), 6.15 (2H, br s, H-1' and OH), 5.60 (1H, d, J=7.7 Hz, H-5), 4.53 (3H, br s, H=2', H-3' and H-4'), 3.97 (2H, br s, H-5'), 2.10-0.97 (11H, m $N\text{-}C_6H_{11}$).

Anal. Calcd for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67. Found: C, 58.35; H, 6.98; N, 13.70.

2-N-Benzyl-1-(2,5-anhydro-β-D-arabinofuranosyl)isocytosine (11b). A mixture of **1** (281 mg, 1 mmol), benzylamine (10 ml), and methanol (10 ml) was stirred for 1 day at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (30 ml)]. The mixture was refluxed for 30 min. The solvent was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The residue was dissolved in chloroform (200 ml) and the solvent was extracted with water (200 ml). The aqueous solution was removed under reduced pressure and the residue was crystallized from methanol. Recrystallization from methanol gave an analytically pure **11b** (271 mg, 86 %), mp. 276-278°C. UV λmax: 217 (ε 25000) and 256 nm (sh) (ε 5720). Ms (m/z): 315 (M^+). ^1H NMR (DMSO-d_6):δ 7.78 (1H, d, J=8.1 Hz, H-6), 7.33 (6H, br s, C_6H_5 and HN^2), 6.15 (2H, br s, H-1' and OH), 5.59 (1H, d, J=8.1 Hz, H-5), 4.54 (5H, br s, $C_6H_5CH_2$, H-2', H-3' and H-4'), 4.01 (2H, br s, H-5').

Anal. Calcd for $C_{15}H_{15}N_3O_4$: C, 60.94; H, 5.43; N, 13.33. Found: C, 60.76; H, 5.48; N, 13.27.

2-N-Methyl-1-(3-O-acetyl-2,5-anhydro-β-D-arabinofuranosyl)-isocytosine (12a). A mixture of **1** (281 mg, 1 mmol) in methanolic methylamine (30 ml) was stirred for 2 days at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (30 ml)] and the mixture was refluxed for 1.5 hr. The mixture was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The residue was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (2 ml) and the mixture was stirred for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column eluting with chloroform-methanol (15:1) to afford **12a**. Recrystallization from ethanol gave analytically pure **12a** (195 mg, 69%), mp. 210-212°C. UV λmax: 212 (ε 25400) and 254 nm (sh) (ε 6440). Ms (m/z): 282 (M^++1). ^1H NMR (DMSO-d_6):δ 7.79 (1H, d, J=7.7 Hz, H-6), 7.22 (1H, br d, J=4.2 Hz, HN^2), 6.03 (1H, s, H-1'), 5.65 (1H, d, J=7.7 Hz, H-5), 5.38 (1H, d, J=2.4 Hz, H-3'), 4.85 (1H, s, H-2'), 4.73 (1H, d, J=2.4 Hz, H-4'), 4.13 (2H, br s, H-5'), 2.78 (3H, d, J= 3.8 Hz, NCH_3), 2.15 (3H, s, $COCH_3$).

Anal. Calcd for $C_{12}H_{15}N_3O_5 \cdot 1/3H_2O$: C, 50.17; H, 5.50; N, 14.63. Found: C, 50.00; H, 5.61; N, 14.52.

2-N-Cyclohexyl-1-(3-O-acetyl-2,5-anhydro-β-D-arabinofuranosyl)isocytosine (12b). A mixture of **1** (281 mg, 1 mmol), cyclohexylamine (10 ml), and methanol (10 ml) was stirred for 2 days at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (30 ml)]. The mixture was refluxed for 1.5 hr. The mixture was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The residue was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (2 ml) and the mixture was stirred for 1 hr at room temperature. The solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column eluting with chloroform-methanol (30:1). The solvent was removed under reduced pressure and the residue was crystallized from ethanol. Recrystallization from ethanol gave analytically pure **12b** (343 mg, 98 %), mp. 275-277°C. UV λmax: 215 (ε 25400) and 256 nm (sh)(ε 5720). Ms (m/z): 349 (M^+). ^1H NMR (DMSO-d_6):δ 7.75 (1H, d, J=8.1 Hz, H-6), 6.56 (1H, br d, J=7.4 Hz, HN^2), 6.10 (1H, br s, H-1'), 5.63 (1H, d, J=8.1 Hz, H-5), 5.34 (1H, dd, J=2.4 and 0.8 Hz, H-3'), 4.82 (2H, br, H-2' and H-4'), 4.07 (2H, br s, H-5'), 2.05-1.05 (11H, m, NC_6H_{11}).

Anal. Calcd for $C_{17}H_{23}N_3O_5$: C, 58.44; H, 6.64; N, 12.03. Found: C, 58.32; H, 6.71; N, 12.04.

2-N-Benzyl-1-(3-O-acetyl-2,5-anhydro-β-D-arabinofuranosyl)-isocytosine (12c). A mixture of **1** (281 mg, 1 mmol), benzylamine (10 ml), and methanol (10 ml) was stirred for 1 day at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in methanolic sodium methoxide [prepared from Na (230 mg, 10 mmol) in absolute methanol (30 ml)]. The mixture was refluxed for 30 min. The solvent was neutralized with Amberlite CG-50 (H^+) and the exchanger was washed with methanol. The combined solvents were removed under reduced pressure. The residue was dissolved in chloroform (200 ml) and the solvent was extracted with water (200 ml). The aqueous solution was removed under reduced pressure and the residue was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (2 ml). The mixture

was stirred for 20 min at room temperature and the solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column eluting with chloroform-methanol (30:1). The solvent was removed under reduced pressure and the residue was crystallized from ether. Recrystallization from water gave analytically pure **12c** (267 mg, 75 %), mp. 237-238°C. UV λmax: 216 (ε 28300) and 255 nm (sh) (ε 6720). Ms (*m/z*): 358 (M^++1). ^{1}H NMR (DMSO-d_6):δ 7.83 (1H, d, J=8.1 Hz, H-6), 7.67 (1H, br, HN^2), 7.30 (5H, br s, $C_6H_5CH_2$), 6.10 (1H, br s, H-1'), 5.67 (1H, d, J=8.1 Hz, H-5), 5.38 (1H, br d, J= 2.7 Hz, H-3'), 4.92-4.68 (2H, br, H-2' and H-4'), 4.58 (2H, br d, J=5.1 Hz, $C_6H_5CH_2$), 4.12 (2H, br s, H-5'), 2.15 (3H, s, $COCH_3$).

Anal. Calcd for $C_{18}H_{19}N_3O_5$: C, 60.49; H, 5.36; N, 11.76. Found: C, 60.68; H, 5.34; N, 11.76.

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