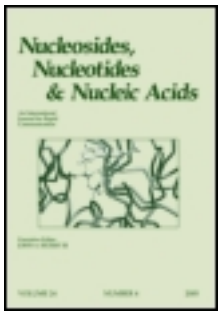

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Derivatives of 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-phenyluracil and 5-Benzyluracil. Synthesis and Biological Properties

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DERIVATIVES OF 1-(2-DEOXY-2-FLUORO-β-D-ARABINOFURANOSYL)-5-PHENYLURACIL AND 5-BENZYLURACIL. SYNTHESIS AND BIOLOGICAL PROPERTIES.[1]

Krzysztof Dziewiszek,[a] Raymond F. Schinazi,[b] Ting-Chao Chou,[a] Tsann-Long Su,[a] Jolanta M. Dzik,[c] Wojciech Rode,[c] and Kyoichi A. Watanabe[a]

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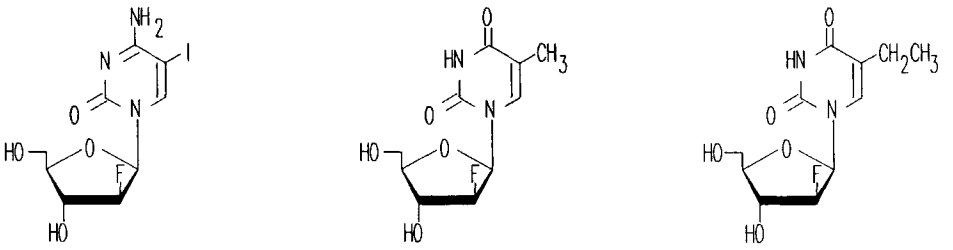
Abstract: A number of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil and -cytosine nucleosides substituted at the 5 position with a nitrophenyl or nitrobenzyl group were synthesized from 5-phenyl- and 5-benzyluracil *via* condensation of the fluorinated sugar, followed by nitration. The corresponding amino analogues were also prepared by reduction of the nitro nucleosides. The uracil nucleosides were converted into the corresponding cytosine nucleosides by way of the triazole intermediates. None of these nucleosides exhibited significant activity against herpes simplex virus type 1 in Vero cells. However, cytosine nucleosides containing the *o*-nitrophenyl, *p*-nitrophenyl, *p*-nitrobenzyl or *p*-aminobenzyl substituent were found to be toxic (even at 1 μM) to uninfected Vero cells, although they were essentially nontoxic in HL-60 cells. The 5'-monophosphates of the uracil nucleosides were inhibitors of the reaction catalyzed by purified Ehrlich ascites carcinoma thymidylate synthase, the 5-phenyluracil nucleotides causing a strong inhibition, competitive *vs* dUMP, described by the K_i value of 0.01 μM.

INTRODUCTION

The 2'-β("up")-fluoro-2'-deoxy-β-D-arabinofuranosyl nucleosides are structurally closest analogues of 2'-deoxynucleosides, since the size of the fluorine atom (Van der Waal's

[1] This paper is dedicated to the memory of Professor Roland K. Robins.



FIAC FMAU FEAU

Figure 1

radius 1.35 Å) is very close to that of hydrogen (1.20 Å), and the 2'-α("down") position is unsubstituted exactly like 2'-deoxynucleosides. Due to the presence of the electronegative fluorine substituent adjacent to the anomeric position, such nucleosides are highly resistant to chemical and enzymic hydrolysis.[2,3]

A number of 5-substituted pyrimidine nucleosides containing the 2-deoxy-2-fluoro-β-D- arabinofuranosyl moiety exhibit anticancer and/or antiviral activities.[4-10] Among these are the 5-iodocytosine and thymine nucleosides, FIAC and FMAU (Figure 1). FIAC has shown clinical efficacy in treatment of herpesvirus infection in phase I[11] and phase II[12] studies with immunosuppressed cancer patients. FMAU has exhibited more potent *in vivo* activity in mice infected with herpes simplex virus.[13] This nucleoside also showed activity against leukemic cells.[14] More recently, the 5-ethyl analogue, FEAU, was shown to be active against woodchuck hepatitis virus *in vivo*.[15] FIAU was recently shown to have activity in humans infected with hepatitis B virus.[16] These antiviral nucleosides are converted *in vivo* into their 5'-triphosphates which inhibit the viral DNA polymerases.[17,18]

We also searched for 2'-fluorinated carbohydrate-containing nucleosides which could have a chemotherapeutic target enzyme in addition to DNA polymerases. Mertes *et al.*[19] synthesized 5-*p*-benzoquinonyl-2'-deoxyuridine 5'-phosphate (**A**) as a potential thymidylate synthase (TS) inhibitor, since such compound may form a stable conjugate (**C**) *via* an intermediate **B** (Figure 2). It was later found that the quinone acted as the Michael acceptor forming two products when treated with methyl mercaptoacetate as model for the active-site nucleophile.[20] More recently, it was reported that the 5-quinone derivatives of 2'-deoxyuridylate in which the quinone cannot act as the Michael acceptor could show some

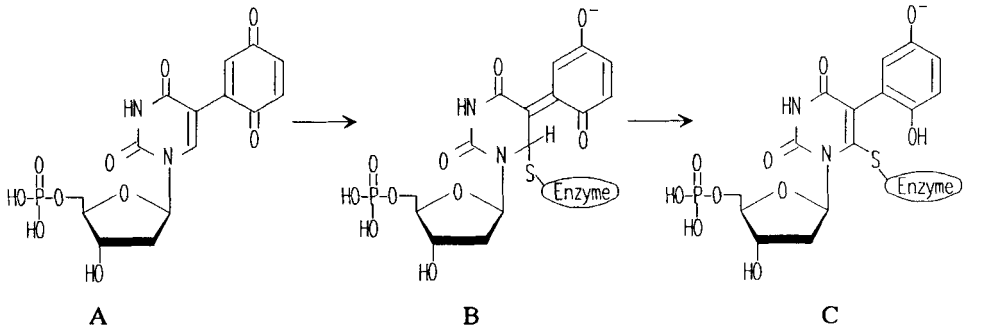


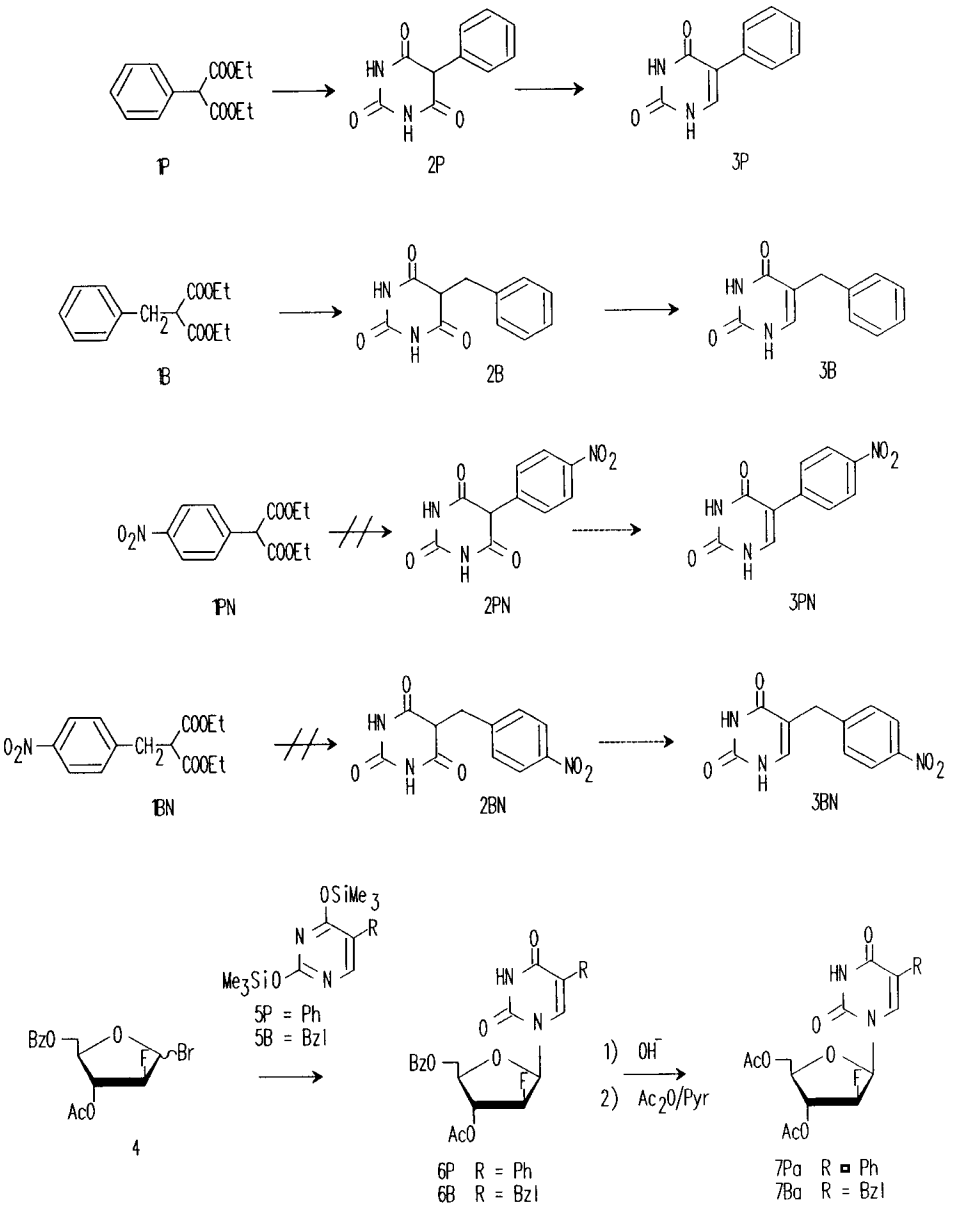
Figure 2

inhibitory activity against TS from various sources (albeit weak activity) and potent anti-herpes simplex virus type 1 (HSV-1) activity.[21]

5-(*p*-Nitrophenyl)uracil nucleosides containing the fluorinated sugar moiety might inhibit TS, because these nucleosides due to the activation at the C6 position or phenyl ring, may bind at the 5'-monophosphate level, to the SH enzyme, thymidylate synthase (TS), forming stable enzyme-substrate conjugates (Figure 2). In this report, we describe the synthesis of 5-(nitrophenyl)-, 5-(aminophenyl)-, 5-(nitrobenzyl)- and 5-(aminobenzyl)uracil and -cytosine nucleosides and their activity against HSV-1 and HL-60 human leukemic cells. We also report the synthesis of the 5'-monophosphates of these nucleosides and their activity against mammalian TS.

CHEMISTRY

The synthesis of the targeted nucleosides was found to be a rather elaborate endeavor. We chose to prepare 5-(*o*- or *p*-nitrophenyl)- and 5-(*o*- or *p*-nitrobenzyl)-uracil (**3P** and **3B**, respectively) and then condense with 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide[4,22] (**4**), since direct nitrophenylation of the 5 position of the uracil nucleoside would be uneconomical due to low yield. Unfortunately, we found that, although diethyl 2-phenylmalonate (**1P**) or 2-benzylmalonate (**1B**) condensed with urea to yield 5-phenylbarbituric acid (**2P**) or 5-benzylbarbituric acid (**2B**), from which 5-phenyluracil (**3P**) or 5-benzyluracil (**3B**) was obtained (Scheme **1**), diethyl 2-(*p*-nitrophenyl)malonate failed to react with urea or thiourea or S-methylisothiourea.



Scheme 1

Nitration of **3P** and **3B** under various conditions always gave inseparable mixtures of their corresponding *o*- and *p*-nitro derivatives (approximately 1:1). We, therefore, switched our strategy, and prepared protected nucleosides (**6P** and **6B**) by condensation of the bis(trimethylsilyl) derivatives (**5P** and **5B**) of 5-phenyl- and 5-benzyl-uracil (**3P** and **3B**) with the halogenated sugar **4**, and then nitrated the products, in the hope that the separation of the nitrated products might be easier at the nucleoside level. Condensation reaction by the Vorbrüggen's procedure[23] afforded the desired β-nucleosides (**6P** and **6B**) as the major products (about 50% yield) along with a small amount of the α-anomers (15%). In order to avoid complications during nitration reaction, the benzoyl group in **6** was replaced by the acetyl group in two steps. The 3',5'-di-O-acetyl nucleosides **7** were treated with ethyl nitrate in a 1:1 mixture of methylene chloride and nitromethane. An approximately 1:1 mixture of *o*- and *p*-nitro derivatives **8** and **9**, respectively, resulted (Scheme **2**). No *m*-nitro product **10** was detected in the reaction mixture. Compounds **8** and **9** were separated on a preparative HPLC (silica gel column with the hexane-ether-methanol system).

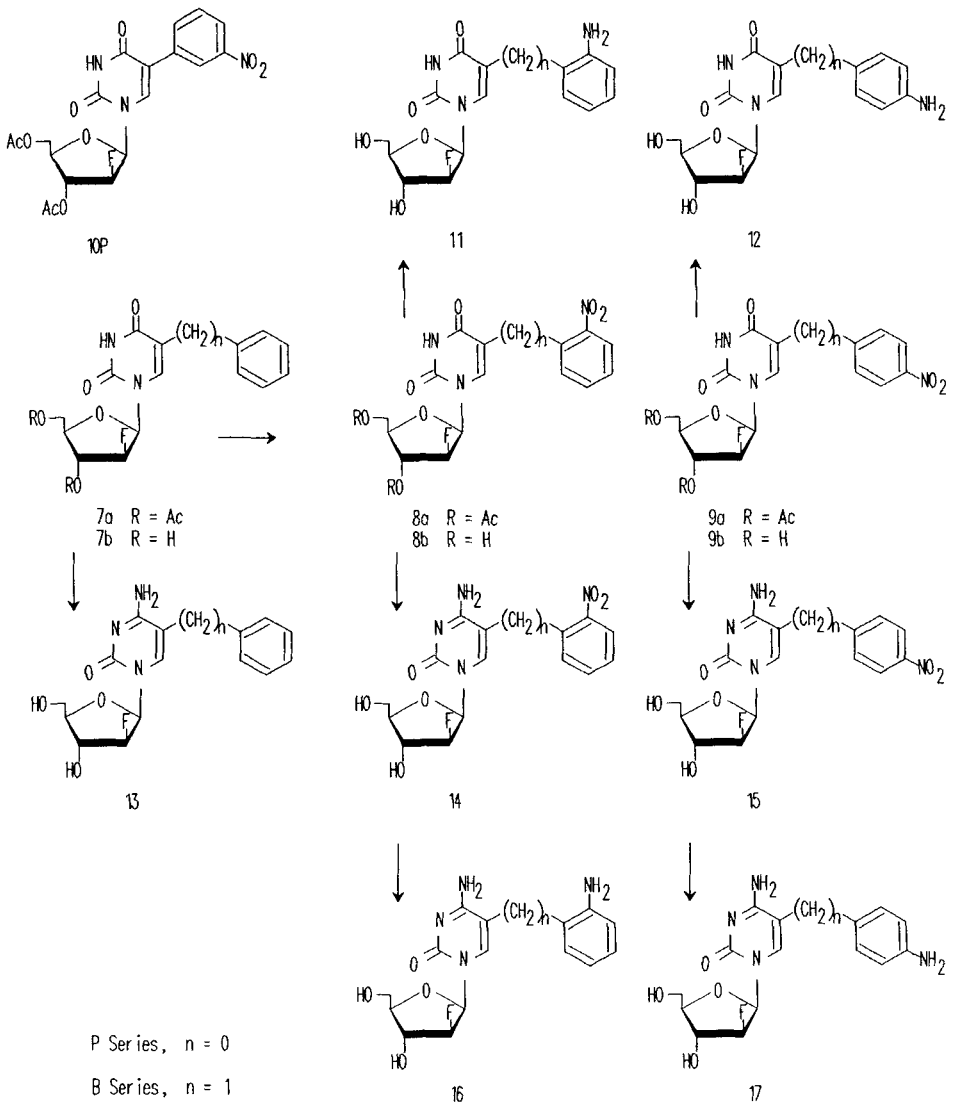
Each nitrated 3',5'-di-O-acetyl nucleoside (**8** or **9**) was converted into the corresponding amino nucleoside (**11** and **12**) by catalytic hydrogenation. They were further converted into the corresponding cytosine nucleoside (**14** and **15**) by way of the triazole intermediate procedure.[24] Catalytic hydrogenation of these cytosine nucleosides afforded their corresponding amino derivatives (**16** and **17**).

In addition, these nucleosides were selectively phosphorylated at the 5' position by the Yoshikawa procedure,[25] purified by HPLC, and tested for their inhibitory activity against thymidylate synthase from Ehrlich carcinoma cells.

BIOLOGICAL ACTIVITIES

Inhibition of the growth of HL-60 human promyelocytic leukemic cells by these compounds was tested as described previously.[26] The IC_{50} values for these nucleosides were all greater than 0.3 mM, except that for 5-(*p*-aminobenzyl)cytosine nucleoside the IC_{50} was 0.26 μM.

None of the new nucleosides synthesized herein showed activity against HSV-1 except 2'-fluoro-5-phenyl-ara-C (**7Pb**) with the EC_{50} value of 6.0 μM. In contrast, acyclovir and FMAU used as positive controls had EC_{50} values of 0.02 and 0.006 μM, respectively (data not shown). Many nucleosides, however, showed significant cytotoxicity to uninfected cells. In particular, the 2'-fluoro-arabinosyl cytosine nucleosides substituted at C5 with an *o*-nitrophenyl, *p*-nitrobenzyl, *p*-aminophenyl and *p*-aminobenzyl group were highly toxic to



Scheme 2

Vero cells ($IC_{50} \leq 1$ μM). The difference in toxicity between HL-60 and Vero cells may be related to transport or metabolism which may be cell dependent. Furthermore, the difference seen with the 5-(*p*-aminobenzyl)cytosine derivative between HL-60 and Vero cells may be due to the deamination of this compound in HL-60, but not in Vero cells.

The effect of 5-substituted 2'-fluoroarabinosyl pyrimidines on replication of human immunodeficiency virus type 1 (HIV-1) was tested in HIV-1_{IIIB} infected MT4 cells. Again, none of them showed appreciable activity when tested up to 0.1 mM.

Table 1. Inhibition of Ehrlich carcinoma thymidylate synthase by 5-substituted 2'-F-ara-U and 2'-F-ara-C

2'-F-ara-U		2'-F-ara-C	
5-Substituent	IC_{50} (10 μM)	5-Substituent	IC_{50} (10 μM)
o-nitropheny 5'-MP	2.0	*p*-nitrophenyl 5'-MP	*a
p-nitrophenyl 5'-MP	24	*p*-nitrobenzyl 5'-MP	6000
p-nitrobenzyl 5'-MP	45	*o*-nitrophenyl 5'-MP	2000
p-aminophenyl 5'-MP	5.3	*o*-nitrophenyl 3',5'-DP	1500
p-aminophenyl 3,'5'-DP	1.2	Phenyl 5'-MP	950
o-aminophenyl 5'-MP	13	Phenyl 3',5'-DP	330
o-aminophenyl 3',5'-DP	1080	*p*-aminophenyl 5'-MP	1300
p-aminobenzyl 3',5'-DP	41	*o*-aminophenyl 5'-MP	780
Phenyl 5'-MP	0.2	*o*-aminophenyl 3',5'-DP	4700
Benzyl 5'-MP	35	*p*-aminobenzyl 5'-MP	880
		p-aminobenzyl 3',5'-DP	580
		Benzyl 5'-MP	5900
		Benzyl 3',5'-DP	*b

*a: 3.4 mM caused a 17% inhibition; *b: 1.7 mM caused a 23% inhibition.

The 5'-phosphates of 5-substituted 2'-F-ara-U derivatives were thymidylate synthase inhibitors, the strongest being the 5-phenyl derivative with the IC_{50} value of 0.2 μM. Substituents at C5 may be ordered according to their influence on inhibitory activity as follows: phenyl > *o*-nitrophenyl = *p*-aminophenyl > *o*-aminophenyl > *p*-nitrophenyl = benzyl = *p*-nitrobenzyl = *p*-aminobenzyl. Substituting the 3' position with an additional phosphate group ambiguously affected the inhibitory activity (Table I). In agreement with thymidylate synthase pyrimidine specificity,[27,28] the 5'-phosphates of 2'-F-ara-C analogues showed by two to three orders of magnitude lower affinity for the enzyme than their 2'-F-ara-U congeners (Table I). No time-dependent inactivation was observed when the enzyme was preincubated with either 2'-F-ara-5-phenyl-U 5'-MP or 2'-F-ara-5-*o*-nitrophenyl-ara-U 5'-MP, in the presence of methylenetetrahydrofolate, before addition of [5-^{3}H]dUMP under conditions previously described.[29]

Thymidylate synthase inhibition by 2'-F-ara-5-phenyl-U 5'-MP, examined by varying the dUMP concentration with different concentrations of inhibitor, added simultaneously to

the reaction mixture, was found to be competitive, as reflected by intersection at the ordinate of Lineweaver-Burk plots (not shown). Since with the Ehrlich carcinoma enzyme K_m for dUMP is 1.3 μM,[30] the IC_{50} value of 0.2 μM, determined in the presence of 25 μM dUMP, allows to estimate K_i value describing 2'-F-ara-U 5'-MP inhibition as 0.01 μM.[31] Thus, 2'-F-ara-5-phenyl-U 5'-MP is a strong thymidylate synthase inhibitor. It is 10^3-fold stronger than the reaction product, dTMP (K_i = 9.6 μM),[30] and successfully competed with dUMP (K_i/K_m = 0.008).

EXPERIMENTAL SECTION

General Methods:—— Melting points were determined on a Thomas-Hoover capillary apparatus and were uncorrected. Elemental analyses were performed by M.H.W. Laboratories, Phoenix, AZ, and were within 0.4% range. Column chromatography was performed on a silica gel 60 (230-400 mesh, ASTM, Merck). TLC was performed on Merck 60 F_{254} plates with short wave UV-light for visualization. ^{1}H-NMR spectra were recorded on a JEOL-FX 90 Q spectrometer with Me_4Si as the internal standard. UV spectra were recorded on Gilford-Response apparatus. Preparative HPLC was performed on a Rainin Rabbit system with a 21 mm diameter silica gel Dynamax column.

1-(3-O-Acetyl-5-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-phenyluracil (4P). — A solution of 1,3-di-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose[4,22] (3.40 g, 10 mmoles) in CH_2Cl_2 (30 mL) was chilled in an ice-bath, and HBr was bubbled in for 1 h to give the bromosugar **4**. The mixture was kept at 4°C overnight, and then the solvent was removed in vacuo below 35 °C. Traces of AcOH were removed by several co-evaporations with toluene (3 x 20 mL), and the residue **4** was dissolved in CH_2Cl_2 (20 mL).

The above solution was added to **5P** [freshly prepared by refluxing **3P** (2.82 g, 15 mmoles) in $(Me_3Si)_2NH$ (35 mL) and $(NH_4)_2SO_4$ (50 mg) until a clear solution was obtained (2 h), and then excess $(Me_3Si)_2NH$ was removed by evaporation in vacuo], and the mixture was stirred at room temperature. The reaction was monitored by TLC (hexane/EtOAc 3:2), and after 5 d, MeOH (5 mL) was added dropwise and the mixture was filtered through a Celite pad. The Celite was thoroughly washed with CH_2Cl_2. The combined filtrate and washings were extracted with H_2O (3 x 30 mL), dried over Na_2SO_4, concentrated to an oily residue which was flash chromatographed using hexane/EtOAc (75:25 - 70:30). The α-anomer (0.345 g, 7.6%, isolated as a foam) was eluted from the column first, followed by the desired β-nucleoside (**6P**, 1.870 g, 40%) as crystals; m.p. 167-168 °C; ^{1}H NMR ($CDCl_3$) for the α-anomer δ 2.03 (s, 3H, OAc), 4.57 (d, 2H, H-5$_a$',5$_b$'), 4.79 (m, 1H, H-4'), 5.43 (dt, 1H,

H-2', $J_{2'F}$ = 49.1 Hz), 5.48 (dt, 1H, H-3',$J_{3'F}$ = 9.9 Hz), 6.20 (dd, 1H, H-1', $J_{1'F}$ = 14.3, $J_{1'2'}$ ≈ 1.4Hz), 7.2-8.1 (m, 11H, Bz, Ph, H-6), 9.0 (s, 1H, NH, exchangeable), ^{1}H NMR ($CDCl_3$) for **6P** δ 2.17 (s, 3H, OAc), 4.38 (m, 1H, H-4'), 4.71 (d, 2H, H-5_a',5_b'), 5.22 (dd, 1H, H-2', $J_{2'1'}$ = 2.7, $J_{2'F}$ = 51.2 Hz), 5.41 (dd, 1H, H-3', $J_{3'F}$ = 14.1 Hz), 6.14 (dd, 1H, H-1', $J_{1'2'}$ = 2.7, $J_{1'F}$ = 21.9 Hz), 7.2-8.0 (m, 11H, Ph, Bz, H-6), 9.04 (s, 1H, NH, exchangeable). *Anal.* Calcd. for $C_{24}H_{21}FN_2O_7$: C, 61.54; H, 4.5; N, 5.98. Found: C, 61.37; H, 4.74; N, 5.64.

In a similar manner, **1-(3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-benzyluracil (6B)** (55% yield) was obtained along with its α-anomer (15%). ^{1}H NMR ($CDCl_3$) data for **6B** δ 2.16 (s, 3H, OAc), 3.51 (s, 2H, CH_2Bn), 4.33 (m, 1H, H-4'), 4.57 (d, 2H, H-$5'_a$,$5'_b$), 5.14 (dd, 1H, H-2', $J_{2'F}$ = 49.9 Hz), 5.36 (dd, 1H, H-3',$J_{3'F}$ = 16.5 Hz), 6.25 (dd, 1H, H-1', $J_{1'2'}$ = 2.5, $J_{1'F}$ = 22.2 Hz), 7.1-8.1 (m, 11H, H-6, Ph, Bn), 9.03 (s, 1H, NH, exchangeable); ^{1}H NMR ($CDCl_3$) for α anomer δ 1.93 (s, 3H, OAc), 3.66 (AB, 2H, CH_2Ph), 4.52 (s, 2H, H-$5'_a$,$5'_b$), 5.37 (dd, 1H, H-2', $J_{2'F}$ = 49.7 Hz), 5.41 (bd, 1H, H-3', $J_{3'f}$ = 17.3 Hz), 5.98 (dd, 1H, H-1', $J_{1'2'}$ ≈ 1.5, $J_{1'F}$ = 15.4 Hz), 6.91 (s, 1H, H-6), 7.2-8.2 (m, 10H, Bz, Bn), 9.35 (s, 1H, NH, exchangeable). *Anal.* Calcd. for $C_{25}H_{23}FN_2O_7$: C, 62.24; H, 4.81; N, 5.81. Found: C, 62.51; H, 4.78; N, 5.90.

1-(3,5-Di-O-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-phenyluracil (7Pa). Compound **6P** (0.425 g, 0.91 mmol) was dissolved in saturated NH_3/MeOH. After 2 d at room temperature, the solution was concentrated in vacuo. The residue was co-evaporated with toluene (2x10 mL) and then acetylated using pyridine and acetic anhydride. Compound **7Pa** was obtained as a yellow oil (0.412 g) which was used directly in the next step without further purification. ^{1}H NMR ($CDCl_3$) for **7Pa** δ 2.01, 2.15 (2s, 6H, 2 x OAc), 4.25 (m, 1H, H-4'), 4.43 (d, 2H, H-$5'_a$,$5'_b$), 5.16 (dd, 1H, H-2', $J_{2'F}$ = 49.5 Hz), 5.25 (dd, 1H, H-3' , $J_{3'F}$ = 17.1 Hz), 6.31 (dd, 1H, H-1', $J_{1'2'}$ = 2.9, $J_{1'F}$ = 21.5Hz), 7.2-8.0 (m, 6H, H-6, Bn), 9.58 (s, 1H, NH, exchangeable). *Anal.* Calcd for $C_{19}H_{19}FN_2O_7$: C, 56.16; H, 4.75; N, 6.89. Found: C, 56.29; H, 4.75; N 6.76.

1-(3,5-Di-O-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-benzyluracil (7Ba) was obtained by using a similar procedure. ^{1}H NMR ($CDCl_3$) for **7Ba** δ 2.05, 2.14 (2s, 6H, 2 x OAc), 3.66 (s, 2H, CH_2Ph), 4.24 (m, 3H, H-4', H-$5'_a$,$5'_b$), 5.07 (dd, 1H, H-2', $J_{2'1'}$ ≈ 2.5, $J_{2'F}$ = 50.1Hz), 5.18 (bd, 1H, H-3', $J_{3'2'}$ ≈ 0, $J_{3'F}$ = 16.2Hz), 6.18 (dd, 1H, H-1', $J_{1'2'}$ = 2.5, $J_{1'F}$ = 22.2Hz), 7.25 (s, 5H, Bn), 7.50 (s, 1H, H-6), 9.40 (s, 1H, NH, exchangeable). *Anal.* Calcd for $C_{20}H_{21}FN_2O_7$: C, 57.14; H, 5.04; N, 6.66. Found: C, 57.32; H, 5.23; N, 6.51.

1-(3,5-Di-O-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*o*-and *p*-nitrophenyl)uracil (8Pa and 9Pa) Compound **7Pa** (0.522 g, 1.3 mmole) was dissolved in a

1:1 mixture of CH_2Cl_2 and $MeNO_2$ (5 mL) at -10°C. To this solution was added successively $EtONO_2$ (0.1 mL) and conc. H_2SO_4 (0.36 mL). After 1.5 h, all starting material was consumed (TLC: hexane/Et_2O/MeOH, 30:70:5, plate was developed three times). The reaction was neutralized with $NaHCO_3$, and the mixture was extracted with EtOAc (3 x 25 mL). The combined extracts were washed (H_2O, 2 x 5 mL), dried (Na_2SO_4), and concentrated to an oily residue (0.566 g) which was purified by HPLC (hexane/Et_2O/MeOH, 30:70:1.5) to give **9Pa** (0.238 g, 41%, foam) and **8Pa** (0.209 g, 36%, m.p.79-81°C). ^{1}H NMR for **9Pa** (acetone-d_6) δ 4.38 (m, 3H, H-4', H-5'$_a$,5'$_b$), 5.30 (ddd, 1H, H-2', $J_{2'1'}$ = 3.3, $J_{2'3'}$ = 2.6, $J_{2'F}$ = 50.2 Hz), 5.33 (m, 1H, H-3', $J_{3'F}$ = 18.7Hz), 6.24 (dd, 1H, H-1', $J_{1'2'}$ = 3.4, $J_{1'F}$ = 20.4 Hz), 7.92 (d, 1H, H-6, J = 1.9 Hz), 7.99 (m, 4H, Ph, $J_{a,b}$ = 9.3Hz). This intermediate was not purified further but directly used in the next step. ^{1}H NMR for **8Pa** (acetone-d_6) δ 4.42 (m, 3H, H-4', H-5'$_a$,5'$_b$), 5.37 (m, 1H, H-3', $J_{3'F}$ = 18.7 Hz), 5.41 (ddd, 1H, H-2',$J_{2'1'}$ = 3.3, $J_{2'3'}$ = 1.5, $J_{2'F}$ = 50.6Hz), 6.34 (dd, 1H, H-1', $J_{1'2'}$ = 3.3, $J_{1'F}$ = 20.3 Hz), 7.4-8.1 (m, 5H, H-6, Ph). *Anal.* Calcd. for $C_{19}H_{18}FN_3O_9$: C, 50.56; H, 4.02; N, 9.31. Found: C, 50.21; H, 4.29; N 9.00%

In a similar manner, **1-(3,5-di-O-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*o*-nitrobenzyl)uracil (8Ba)** (130 mg, 12%, yellow oil) and **1-(3,5-di-O-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-nitrobenzyl)uracil (9Ba)** (0.36 g, 41%, foam) were prepared from **7Ba** (0.764g, 1.8mmol). ^{1}H NMR (acetone-d_6) for **8Ba** δ 3.95 (s, 2H, CH_2Ph), 4.31 (m, 3H, H-4', H-5'$_a$,5'$_b$), 5.24 (ddd, 1H, H-2', $J_{2'1'}$ = 3.1, $J_{2'3'}$ = 1.4, $J_{2'F}$ = 50.6 Hz), 5.27 (m, 1H, H-3', $J_{3'F}$ = 18.1 Hz), 6.20 (dd, 1H, H-1', $J_{1'2'}$ = 3.1, $J_{1'F}$ = 21.0Hz), 7.4-8.0 (m, 5H, H-6, Bn) and **9Ba** δ 3.80 (s, 2H, CH_2Ph), 4.37 (m, 3H, H-4', H-5'$_a$,5'$_b$), 5.26 (ddd, 1H, H-2', $J_{2'1'}$ = 3.3, $J_{2'3'}$ = 1.4, $J_{2'F}$ = 50.5 Hz), 5.31 (m, 1H, H-3', $J_{3'F}$ = 18.7Hz), 6.24 (dd, 1H, H-1', $J_{1'2'}$ = 3.3, $J_{1'F}$ = 21.0 Hz), 7.4-8.2 (m, 5H, H-6, Bn, J_{ab} = 8.8Hz). Compound **8Ba**, however, was not stable enough to prepare sufficient amount for further syntheses. *Anal.* Calcd. for $C_{20}H_{20}FN_3O_9$: C, 51.61; H, 4.33; N, 9.03. Found: C, 51.68; H, 4.46; N, 8.71.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-nitrophenyl)uracil (9Pb). Compound **9Pa** (0.050 g, 0.11 mmole) was dissolved in saturated NH_3/MeOH (10 mL) at room temperature. After 1 d (TLC hexane/Et_2O/MeOH 30:70:5, the plate was developed twice), the mixture was concentrated, and the residue chomatographed on a silica gel column using $CHCl_3$/MeOH (98:2 v/v), giving **9Pb** as yellow crystals (0.033 g, 81%); m.p. 255-257°C decomp. (from chloroform-methanol): ^{1}H NMR (DMSO-d_6) δ 3.74 (m, 3H, H-4', H-5'$_a$,5'$_b$), 4.31 (m, 1H, H-3', $J_{3'F} \approx$ 20 Hz), 5.22 (dt, 1H, H-2', $\Sigma J_{2'3'}$, $J_{2'1'}$ = 9.3, $J_{2'F}$ = 53.2 Hz), 5.36 (t, 1H, 5'-OH, exchangeable), 5.95 (d, 1H, 3'-OH, exchangeable), 6.24 (dd, 1H, H-1', $J_{1'2'}$ =

4.6, $J_{1'F}$ = 11.4 Hz), 8.02 (dd, 4H, Ph, J_{ab} = 9.0 Hz), 8.36 (s, 1H, H-6), 11.82 (s, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 313.5 nm (ε16107), λmin 248.0nm (ε6479), (0.01 N HCl in methanol/water 1:1) λmax 312.5 nm (ε 16150), λmin 247.5 nm (ε 6650), (0.01N NaOH in methanol/water 1:1) λmax 336.0 nm (ε 13200), λmin 292.0 nm (ε 8900). *Anal.* Calcd. for $C_{15}H_{14}FN_3O_7$: C, 49.05; H, 3.84; N, 11.44. Found: C, 48.96; H, 4.02; N, 11.16.

In a similar manner, **5-benzyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (7Bb)** (0.078 g, 93%, m.p.123-124.5°C from methanol), **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-phenyluracil (7Pb)** (0.053g, 96% m.p.102-4°C from methanol), **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*o*-nitrophenyl)uracil (8Pb)** (0.047 g, 93%, m.p.118-120°C from chloroform-methanol) and **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-nitrobenzyl)uracil (9Bb)** (0.093 g, 94%, m.p.179-180°C from chloroform-methanol) were prepared from **7Ba** (0.120 g, 0.25 mmole), **7Pa** (0.080 g, 0.17 mmole), **8Pa** (0.062 g, 0.14 mmole) and **9Ba** (0.120 g, 0.26 mmole), respectively. ^{1}H NMR for **7Bb** (DMSO-d_6) δ 3.2, 3.3 (d, s, 2H, H-5'$_a$,5'$_b$), 3.5 (s, 2H, CH_2Ph), 3.7 (m, 1H, H-4'), 4.2 (m, 1H, H-3', $J_{3'F}$ = 19.2 Hz), 5.0 (m, 1H, H-2', $\Sigma J_{2'1',2'3'}$ = 8.0, $J_{2'F}$ = 52.4 Hz), 5.1 (t, 1H, 5'OH, exchangeable) 5.9 (d, 1H, 3'OH, exchangeable), 6.1 (dd, 1H, H-1', $J_{1'2'}$ = 4.4, $J_{1'F}$ = 15.4 Hz), 7.2 (m, 5H, Ph), 7.6 (d, 1H, H-6, J = 1.4 Hz), 11.5 (s, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 266.0 nm (ε 9900), λmin 236.5 nm (ε 3200), (0.01 N HCl in methanol/water 1:1) λmax 266.0 nm (ε 9850), λmin 237.0 nm (ε 3200), (0.01 N NaOH in methanol/water 1:1) λmax 265.5 nm (ε 7400), λmin 246.0 nm (ε 5450). *Anal.* Calcd. for **7Bb**, $C_{16}H_{17}FN_2O_5$: C, 57.14; H, 5.10; N, 8.33. Found: C, 57.39; H, 5.32; N, 8.45.

^{1}H NMR for **7Pb** (DMSO-d_6) δ 3.7 (m, 3H, H-4', H-5'$_a$,5'$_b$), 4.27 (m, 1H, H-3', $J_{3'F}$ = 19.2 Hz), 5.15(m, 1H, H-2', $\Sigma J_{2'1',2'3'}$ = 8.8, $J_{2'F}$ = 53.2 Hz), 5.23 (t, 1H, 5'-OH, exchangeable), 5.92 (d, 1H, 3'OH, exchangeable), 6.22 (dd, 1H, H-1', $J_{1'2'}$ = 4.7, $J_{1'F}$ = 12.9 Hz), 7.4 (m, 5H, Ph), 8.02 (s, 1H, H-6), 11.66 (s, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 235.0 nm (ε 12000), λmax 279.5 nm (ε 9650), λmin 217.5 nm (ε 8900), λmin 258.5 nm (ε 6850), (0.01N HCl in methanol/water 1:1) λmax 236.0 nm (ε 11950), λmax 279.5 nm (ε 9600), λmin 218.5 nm (ε 8950), λmin 259.0 nm (ε 6850), (0.01 N NaOH in methanol/water 1:1), λmax 240.0 nm (ε 12500), λmax 273.5 nm (ε 8700), λmin 233.5 nm (ε 12350), λmin 267.0 nm (ε 8600). *Anal.* Calcd. for **7Pb** ($C_{15}H_{15}FN_2O_5$): C, 55.90; H, 4.69; N, 8.69. Found: C, 55.74; H, 4.87; N, 8.40.

^{1}H NMR for **8Pb** (DMSO-d_6) δ 3.68 (m, 3H, H-4', H-5'$_a$,5'$_b$), 4.29 (m, 1H, H-3', $J_{3'F}$ ≈ 20 Hz), 5.21 (t, 1H, 5'OH, exchangeable), 5.26 (dt, 1H, H-2',$\Sigma J_{2'1',2'3'}$ ≈ 8.5, $J_{2'F}$ = 53.2 Hz),

5.92 (d, 1H, 3'OH, exchangeable), 6.22 (dd, 1H, H-1', $J_{1'2'}$ = 4.7, $J_{1'F}$ = 13.2 Hz), 7.2-8.0 (m, 5H, H-6, Ph), 11,77 (bs, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 223.0 nm (ε 16150), λmax 271. 0nm (ε 13650), λmin 216.0 nm (ε 14500), λmin 247.5 nm (ε 10100), (0.01 N HCl in methanol/water 1:1) λmax 223.5 nm (ε 16100), λmax 272.0 nm (ε 13550), λmin 217.0 nm (ε 16000), λmin 249.0 nm (ε 9950), (0.01 N NaOH in methanol/water 1:1), λmax 238.0 nm (ε 16600), λmin 230.5 nm (ε 16150). *Anal.* Calcd. for **8Pb** ($C_{15}H_{14}FN_3O_7$ x $0.5H_2O$): C, 47.88; H, 4.02; N, 11.17. Found: C, 48.23; H, 4.22; N, 10.66.

1H NMR for **9Bb** (DMSO-d_6) δ 3.66 (m, 5H, H-4', H-5'$_a$,5'$_b$, CH_2Ph), 4.27 (m, 1H, H-3', $J_{3'F} \approx$ 20.5 Hz), 5.06 (dt, 1H, H-2', $\Sigma J_{2'1',2'3'}$ = 7.7, $J_{2'F}$ = 52.7 Hz), 5.07 (t, 1H, 5'OH, exchangeable), 5.90 (d, 1H, 3'OH, exchangeable), 6.15 (dd, 1H, H-1', $J_{1'2'}$ = 4.4, $J_{1'F}$ = 15.4 Hz), 7.75 (d, 1H, H-6, J ≈ 1.4 Hz), 7.84 (m, 4H, Ph, J_{ab} = 8.8 Hz), 11.54 (s, 1H, NH, exchangeable). UV methanol/water 1:1) λmax 272.0nm (ε 19450) λmin 235.0 nm (ε 6200), (0.01 N HCl in methanol/water 1:1) λmax 272.5 nm(ε 19400), λmin 235.5 nm (ε 6200), (0.01N NaOH in methanol/water 1:1) λmax 271.0 nm (ε 16300), λmin 242.5nm (ε 9800). *Anal.* Calcd. for **9Bb** ($C_{16}H_{16}FN_3O_7$): C, 50.39; H, 4.23; N, 11.02. Found: C, 50.29; H, 4.30; N, 11.02.

1-(2-Deoxy-2-fluoro-ß-D-arabinofuranosyl)-5-(p-nitrophenyl)cytosine (15P). To a suspension of 1,2,4-triazole (0.286 g, 4.1 mmol) in MeCN (2.4 mL) was added $POCl_3$ (0.13 g, 0.082 mL, 0.85 mmol) under argon. The mixture was cooled to 0°C and then Et_3N (0.40 g, 0.554 mL, 4.0 mmol) was added. After stirring for 15 min, a solution of **9Pa** (0.103 g, 0.23 mmol) in MeCN (1.4 mL) was added. After being kept at room temperature for 1.5 h, the solution was stirred with 10% $NaHCO_3$ aqueous solution (10 mL) and the extracted $CHCl_3$ (10 mL x 3). The combined organic extracts were concentrated in vacuo, the residue was dissolved in 3 mL of NH_4OH-dioxane (1:3) and stirred at room temperature for 8 h. The mixture was evaporated in vacuo to dryness, co-evaporated with toluene (5 mL x 4) and the residue was then treated with 10 mL of saturated methanolic ammonia in a sealed vessel overnight. The mixture was concentrated in vacuo to a small volume and chromatographed on a silica gel column (2 x 20 cm) using $CHCl_3$/MeOH)10:1 v/v) as the eluent to give **15P** (66 mg, 79%), m.p. 290-294°C decomp. (from methanol). ^{1}H NMR (DMSO-d_6) δ 3.58 (m, 2H, H-5'$_a$,5'$_b$), 3.79 (m, 1H, H-4'), 4.20 (m, 1H, H-3', $J_{3'F} \approx$ 20 Hz), 5.06 (dt, 1H, H-2', $\Sigma J_{2'1',2'3'}$ = 7.4, $J_{2'F}$ = 53.5 Hz), 5.07 (t, 1H, 5'OH, exchangeable), 5.89 (d, 1H, 3'OH, exchangeable), 6.19 (dd, 1H, H-1', $J_{1'2'}$ = 4.3, $J_{1'F}$ = 16.1 Hz), 6.8 (b, 2H, NH_2, exchangeable), 7.83 (s, 1H, H-6), 7.92 (m, 4H, Ph, J_{ab} = 8.8 Hz). UV (methanol/water 1:1) λmax 226.5 nm (ε 21100), λmax 286.0 nm (ε 12850), λmin 213.0 nm (ε 20350), λmin 260.5 nm (ε 9900), (0.01

N HCl in methanol/water 1:1) λmax 292.5 nm (ε 17800), λmin 247.5 nm (ε 7200), (0.01 N NaOH in methanol/water 1:1) λmax 287.0 nm (ε 12650), λmin 260.5 nm (ε 9750). *Anal.* Calcd. for HCl salt ($C_{15}H_{15}FN_4O_6$ x HCl x $2H_2O$): C, 40.96; H, 4.81; N, 12.74. Found: C, 40.61; H, 4.84; N, 13.03.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-phenylcytosine (7Pb) (0.125 g, 64%), **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-benzylcytosine (7Bb)** (0.215 g, 65%), **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*o*-nitrophenyl)cytosine (14P)** (0.080 g, 95%) and **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-nitrobenzyl)cytosine (15B)** (0.104 g, 97%) were obtained by following the same procedure but using the corresponding uracil nucleosides, **7Pa** (0.281 g, 0.60 mmole), **7Ba** (0.476 g 0.99 mmole), **8Pa** (0.104 g, 0.23 mmole) and **9Ba** (0.131 g, 0.28 mmole), respectively. Compound **7Pb** had the following characteristics: mp 254-254.5°C (from methanol). ^{1}H NMR (DMSO-d_6) δ 3.5 (m, 2H, H-5'$_a$,5'$_b$), 3.8 (m, 1H, H-4'), 4.2 (m, 1H, H-3',$J_{3'F}$ = 19.8 Hz), 5.01 (m, 1H, H-2', $J_{2'F}$ = 51.9 Hz), 5.04 (t, 1H, 5'-OH, exchangeable), 5.8 (d, 1H, 3'-OH, exchangeable), 6.2 (dd, 1H, H-1', $J_{1'2'}$ = 4.1, $J_{1'F}$ = 17.3 Hz), 6.4 (b, 2H, NH_2, exchangeable) 7.4 (m, 5H, Ph), 7.6 (d, 1H, H-6, J = 1.4 Hz). UV (methanol/water 1:1) λmax 234.5 nm (ε 14550), λmax 282.5 nm (ε 7200), λmin 224.5 nm (ε 13750), λmin 270.0 nm (ε 6300), (0.01 N HCl in methanol/water 1:1) λmax 228.0 nm (ε 12800), λmax 292.5 nm (ε 9100), λmin 220.5 nm (ε 12300), λmin 263.0 nm (ε 4550), (0.01 N NaOH in methanol/water 1:1) λmax 234.0 nm (ε 15100), λmax 282.5 nm (ε 7500), λmin 229.0 nm (ε 15000), λmin 270.0 nm (ε 6600). *Anal.* Calcd. for $C_{15}H_{16}FN_3O_4$ x $1/2H_2O$: C, 54.54; H, 5.19; N, 12.72. Found: C, 54.74; H, 5.21; N, 12.74.

The physical properties of **7Bb** are as follows: m.p.191-193°C decomp. (from methanol). ^{1}H NMR (DMSO-d_6) δ 3.1-3.8 (m, 5H, H-4', H-5'$_a$,5'$_b$, CH_2Ph), 4.1 (m, 1H, H-3', $J_{3'F}$ = 18.9 Hz), 4.9 (m, 1H, H-2', $J_{2'F}$ = 52.6 Hz), 5.0 (t, 1H, 5'OH, exchangeable), 5.8 (d, 1H, 3'-OH, exchangeable), 6.1(dd, 1H, H-1', $J_{1'2'}$ = 3.7 Hz, $J_{1'F}$ = 18.6 Hz), 6.8 (b, 2H, NH_2, exchangeable), 7.3 (m, 6H, Ph, H-6). UV (methanol/water 1:1) λmax 277.5 nm (ε 7700), λmin 257.5 nm (ε 5700), 0.01N HCl in methanol water 1:1 λmax 288.5 nm (ε 10500), λmin 247.0 nm (ε 2650), (0.01 N NaOH in methanol/water 1:1) λmax 278.5 nm (ε 7650), λmin 256.5 nm (ε 5600). *Anal.* Calcd. for $C_{16}H_{18}FN_3O_4$: C, 57.31; H, 5.41; N, 12.53. Found: C, 56.98; H, 5.60; N, 12.13.

Compound **14P** had the following characteristics: m.p. 265-8°C decomp. (from acetonitrile-water), ^{1}H NMR (DMSO-d_6) δ 3.5 (m, 2H, H-5'$_a$,5'$_b$), 3.7(m, 1H, H-4'), 4.1 (m, 1H, H-3', $J_{3'F} \approx$ 19 Hz), 4.9 (m, 1H, H-2', $J_{2'F}$ = 52.1Hz), 4.91 (t, 1H, 5'-OH, exchangeable), 5.79 (d, 1H, 3'-OH, exchangeable), 6.03 (dd, 1H, H-1', $J_{1'2'}$ = 3.8Hz, $J_{1'F}$ = 17.1 Hz), 7.2-8.2

(m, 5H, Ph, H-6), UV (methanol/water 1:1) λmax 272.0 nm, λmin 265.5 nm, (0.01 N HCl in methanol/water 1:1) λmax 282.0 nm (ε 9200), λmin 250.5 nm (ε 6200), (0.01 N NaOH in methanol/water 1:1) λmax 272.0 nm, λmin 267.5 nm. *Anal.* Calcd. for $C_{15}H_{15}FN_4O_6$ x $1/2H_2O$: C, 48.00; H, 4.30; N, 14.93. Found: C, 47.98; H, 4.44; N, 14.99.

The physical properties of **15B** are as follows: m.p. 256-259°C decomp. (from methanol), ^{1}H NMR (DMSO-d_6) δ 3.51 (AB, 2H, CH_2Ph), 3.83 (m, 3H, H-4', H-5'$_a$,5'$_b$), (m, 1H, H-3', $J_{3'F} \approx$ 16Hz), 5.01 (m, 1H, H-2', $J_{2'F}$ = 53.2 Hz), 5.9 (b, 2H, NH_2, exchangeable), 6.12 (dd, 1H, H-1', $J_{1'2'}$ = 4.0, $J_{1'F}$ = 16.9 Hz), 7.68 (s, 1H, H-6), 7.84 (m, 4H, Ph, J_{ab} = 8.8 Hz). UV (methanol/water 1:1) λmax 274.0 nm (ε 12300), λmin 238.5 nm (ε 9250), (0.01 N HCl in methanol water 1:1) λmax 284.0nm (ε 13250), λmin 241.0 nm (ε 6650), (0.01N NaOH in methanol water 1:1) λmax 274.5nm (ε 12100), λmin 240.0 nm (ε 9350). *Anal.* Calcd. for $C_{16}H_{17}FN_4O_6$: C, 50.52; H, 4.51; N 14.73. Found: C, 50.64; H, 4.78; N, 14.47.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-aminophenyl)uracil (12P). Compound **9P** (72 mg, 0.20 mmol) was hydrogenated in MeOH (15 mL) at normal pressure over 5% moist Pd/C (0.015g). The reduction was completed within 2 h (TLC $CHCl_3$/MeOH, 8:2 v/v). After removal of the catalyst by filtration, the filtrate was concentrated, and the residue chromatographed on a silica gel column (CH_2Cl_2/MeOH, 95:5 v/v) to give **12P** (60 mg, 91%), m.p. 241-5°C decomp.(from methanol). ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 2H, H-5'$_a$,5'$_b$), 3.8 (m, 1H, H-4') 4.26 (m, 1H, H-3', $J_{3'F}$ = 20.8 Hz), 5.14 (m, 1H, H-2', $J_{2'F} \approx$ 53, $\Sigma J_{2'1',2'3'} \approx$ 9 Hz), 5.17 (m, 3H, 5'OH, NH_2, exchangeable), 5.91 (d, 1H, 3'OH, exchangeable), 6.19 (dd, 1H, H-1', $J_{1'2'}$ = 4.2, $J_{1'F}$ = 13.0 Hz), 6.89 (m, 4H, Ph, J_{ab} = 8.5Hz), 7.8 (d, 1H, H-6 J = 1.4 Hz), 11.5 (s, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 251.5 nm (ε 23600), λmax 294.0 nm (ε 12300), λmin 224.0 nm (ε 11000), λmin 281.5 nm (ε 11500), (0.01 N HCl in methanol/water 1:1) λmax 241.0nm (ε 15500), λmax 278.0nm (ε 15700) λmin 224.0 nm (ε 12100), λmin 259.0 nm (ε 11700), (0.01 N NaOH in methanol/water 1:1) λmax 252.5 nm (ε 22300), λmin 231.5 nm (ε 17500). *Anal.* Calcd. for $C_{15}H_{16}FN_3O_5$: C, 53.41; H 4.78; N, 12.46. Found: C, 53.28; H, 4.94; N, 12.65.

In a similar manner, **1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*o*-aminophenyl)uracil (11P)** mp of HCl salt 223-6°C decomp. (from ethanol). ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 2H, H-5'$_a$,5'$_b$), 3.7 (m, 1H, H-4'), 4.24 (m, 1H, H-3', $J_{3'F}$ = 19.3 Hz), 5.13 (m, 1H, H-2', $J_{2'F}$ = 52.8Hz), 6.19 (dd, 1H, H-1', $J_{1'2'}$ = 4.4, $J_{1'F}$ = 14.2 Hz), 7.25 (m, 4H, Ph), 7.9 (d, 1H, H-6 J = 0.8 Hz), 11.8 (s, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 266.0 nm (ε 9900), λmin 252.5 nm (ε 8500), (0.01 N HCl in methanol/water 1:1) λmax 230.0 nm (ε 11700), λmax 278.5 nm (ε 10800) λmin 219.5 nm (ε 11000),λmin 252.0 nm (ε

6100). *Anal.* Calcd. for $C_{15}H_{16}FN_3O_5$ x HCl x 1/2 H_2O: C, 47.07; H, 4.74; N 10.98. Found: C, 46.98; H, 4.79; N, 10.68.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-aminobenzyl)uracil (12B), m.p. of HCl salt 210-6°C decomp. (from ethanol), ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 8H, C$\underline{H_2}$Ph, H-5'$_a$,5'$_b$, 2 x OH, NH_2), 3.7 (m, 1H, H-4'), 4.0 (m, 1H, H-3', $J_{3'F}$ = 19.2 Hz), 5.03 (m, 1H, H-2', $J_{2'F}$ = 52.9 Hz), 6.12 (dd, 1H, H-1', $J_{1'2'}$ = 4.4, $J_{1'F}$ = 12.9 Hz), 7.2 (m, 4H, Ph), 7.7 (s, 1H, H-6), 11.5 (s, 1H, NH, exchangeable). UV (methanol/water 1:1) λmax 265.0 nm (ε 9000), λmin 251.5 nm (ε 7900), (0.01 N HCl in methanol/water 1:1) λmax 266.0 nm (ε 9000) λmin 236.5 nm (ε 3100), (0.01 N NaOH in methanol/water 1:1) λmax 263.5 nm (ε 7200). *Anal.* Calcd. for $C_{15}H_{16}FN_3O_5$ x HCl x H_2O: C, 47.36; H, 5.22; N, 10.36. Found: C, 47.60; H, 5.18; N 9.93.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*o*-aminophenyl)cytosine (16P).m.p.of HCl salt 192-200°C decomp. (from methanol). ^{1}H NMR (DMSO-d_6) δ 3.5 (m, 2H, H-5'$_a$,5'$_b$), 3.8 (m, 1H, H-4'), 4.20 (m, 1H, H-3', $J_{3'F}$ = 19.2 Hz), 4.8 (b, 2xOH, NH_2) 5.1 (m, 1H, H-2', $J_{2'F}$ = 52.8 Hz), 6.2 (dd, 1H, H-1', $J_{1'2'} \approx 4$, $J_{1'F}$ = 14.4 Hz), 7.68 (s, 1H, H-6), 6.6-7.3 (m, 4H, Ph), 7.9 (s, 1H, H-6), 8.9 (s, 3H, NH_2HCl). UV (methanol/water 1:1) λmax 278.0 nm (ε 8350), λmin 260.0 nm (ε 6900), (0.01 N HCl in methanol/water 1:1) λmax 286.5 nm (ε 10650), λmin 255.5 nm (ε 4850), (0.01 N NaOH in methanol/water 1:1) λmax 278.0 nm (ε 8600), λmin 260.0 nm (ε 7200). *Anal.* Calcd. for $C_{15}H_{17}FN_4O_4$: C, 53.57; H, 5.10; N, 16.66. Found: C, 53.37; H, 5.21; N, 16.54.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-aminophenyl)cytosine (17P) m.p. 272-6°C decomp. (from ethanol). ^{1}H NMR (DMSO-d_6) δ 3.5 (m, 2H, H-5'$_a$,5'$_b$), 3.75 (m, 1H, H-4'), 4.15 (m, 1H, H-3', $J_{3'F}$ = 19.8 Hz), 5.03 (m, 1H, H-2', $J_{2'F}$ = 52.8 Hz), 5.05 (t, 1H, 5'OH, exchangeable), 5.24 (s, 2H, NH_2,exchangeable), 5.85 (d, 1H, 3'OH, exchangeable), 6.12 (dd, 1H, H-1', $J_{1'2'}$ = 4.1, $J_{1'F}$ = 17.0 Hz), 6.8 (m, 4H, Ph, J_{ab} = 8.5 Hz), 7.4 (d, 1H, H-6, J = 1.4 Hz). UV (methanol/water 1:1) λmax 248.5 nm (ε 16800), λmin 224.5 nm (ε 10700), (0.01 N HCl in methanol/water 1:1) λmax 288.5 nm (ε 10800), λmin 259.0 nm (ε 7500), (0.01 N NaOH in methanol/water 1:1) λmax 248.0 nm (ε 17300), λmin 225.5 nm (ε 11800). *Anal.* Calcd. for $C_{15}H_{17}FN_4O_4$: C, 53.57; H, 5.10; N 16.66. Found: C, 53.61; H, 5.14; N, 16.64%

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(*p*-aminobenzyl)cytosine (17B),m.p.of HCl salt 230-40°C decomp. (from ethanol), ^{1}H NMR (DMSO-d_6) δ 3.5 (m, 2H, H-5'$_a$,5'$_b$), 3.8 (m, CH_2, NH_2, 2xOH), 4.1 (m, 1H, H-4'), 4.2 (m, 1H, H-3', $J_{3'F} \approx$ 16Hz), 5.12 (m, 1H, H-2', $J_{2'F}$ = 53.0 Hz), 6.13 (dd, 1H, H-1', $J_{1'2'}$ = 4.0, $J_{1'F}$ = 15.1Hz), 7.2 (m, 4H, Ph, J_{ab} = 8.3 Hz) 7.9 (s, 1H, H-6), 9.2 (s, NH_2HCl exchangeable). UV (methanol/water 1:1) λmax

234.5 nm (ϵ 21200), λmax 277.0 nm (ϵ 11600), λmin 266.0 nm (ϵ 10850), (0.01 N HCl in methanol/water 1:1) λmax 285.0 nm (ϵ 12900), λmin 245.5 nm (ϵ 6500), (0.01N NaOH in methanol/water 1:1) λmax 234.0 nm (ϵ 25700), λmax 275.5 nm (ϵ 11650), λmin 226.5 nm (ϵ 24750), λmin 266.0 nm (ϵ 11200). *Anal.* Calcd. for $C_{16}H_{19}FN_4O_4$ x 2 HCl: C, 45.40; H, 5.00; N, 13.24. Found: C, 45.18; H, 5.06; N, 13.22.

Preparation of nucleoside 5'-monophosphates. To a solution of nucleoside (14.0 µmole) in triethylphosphate (0.5 mL) was added 35 µL of 1.2 *M* solution of $POCl_3$ in triethylphosphate [freshly prepared from $POCl_3$ (0.11 mL) in triethylphosphate (1 mL)], and the mixture was stirred for 8 h at room temperature. Water (0.3 mL) was added, and the stirring was continued for another h, and then neutralized with Et_3N to pH 7. The mixture was concentrated to dryness in SpeedVac concentrator. The product monophosphate was isolated and purified by HPLC using the stational phase: Microsorb 5 µm C18, 25 cm bed, and the mobile phase: A: 0.1 *N* triethylamine carbonate; B: 30% H_2O in MeCN. Gradient: 10 min with mobile phase A, followed by 10 - 70 min from 0 to 100% B in A. Flow rate was 1 mL/min. The yield was determined spectrophotometrically using the ϵ value for the corresponding nucleoside reported above.

Cell Growth Inhibition: HL-60 cells at 4 x 10^5 cells/mL were incubated with various drug concentrations at 37 °C for 5 d. Cells were counted with a hemocytometer. Viability was determined by trypan blue exclusion on d 1, 3 and 5. The relationship between dose and number of viable cells for a particular compound was analyzed using a computer program based on the median-effect equation derived by Chou and Talalay.[26,32]

Antiviral and Cytotoxic Assays: The compounds examined in this study were screened for antiviral activity against HSV-1 by using a plaque reduction assay in Vero cells using the method described previously.[33-35] The cytotoxic activities of the drugs were measured for 3 d in rapidly dividing Vero cells, as described previously.[35] The trypsinized cells were counted with a hemacytometer in the presence of 3% trypan blue.

The effect of new nucleosides synthesized herein on the replication of HIV-1 was tested in acutely infected MT4 cells grown for four days in RPMI-1640 medium supplemented with 10% fetal calf serum at 37 °C and 5% CO_2, as described previously.[28] Inhibition of cytopathic effect (CPE) and the reverse transcriptase (RT) in the supernatant of cell culture were measured using AZT as a positive control. No inhibition of CPE or RT was observed for all of these compounds at 0.1 mM.

Thymidylate Synthase Inhibition Studies: Electrophoretically homogeneous preparations of thymidylate synthase from Ehrlich ascites carcinoma cells were obtained as

previously described.[36] [5-^{3}H]dUMP tritium release was determined as previously described,[29] all measurements being done in triplicate. The studied nucleotides were added to the reaction mixture as neutral aqueous solutions.

The IC_{50} values, defined as a nucleotide concentration causing 50% inhibition of enzyme reaction, were determined in the presence of 25 μM [5-^{3}H]dUMP in the reaction mixture. To identify the type of inhibition involved, the effect of a nucleotide on the dependence of reaction rate on dUMP concentration, in the form of Lineweaver-Burk plots, was analyzed as previously reported.[29]

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