

Synthesis and Antiviral and Cytostatic Properties of 3'-Deoxy-3'-fluoro- and 2'-Azido-3'-fluoro-2',3'-dideoxy-D-ribofuranosides of Natural Heterocyclic Bases

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A series of 3'-deoxy-3'-fluoro- and 2'-azido-2',3'-dideoxy-3'-fluoro-D-ribofuranosides of natural heterocyclic bases have been synthesized with the use of universal carbohydrate precursors, viz., 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose and methyl 2-azido-5-*O*-benzoyl-2,3-dideoxy-3-fluoro-β-D-ribofuranoside, respectively. The cytostatic and antiviral activity of the compounds was evaluated against a variety of tumor cell lines and DNA/RNA viruses, respectively. As the most active compound, from both a cytostatic and antiviral activity viewpoint, emerged 3'-deoxy-3'-fluoro-adenosine. It inhibited the proliferation of some tumor cell lines (i.e. murine leukemia L1210 and human T-lymphocyte MT-4) at a concentration of 0.2–2 μg/mL, and proved inhibitory to the replication of positive-stranded RNA viruses (i.e. polio, Coxsackie, Sindbis, Semliki forest), double-stranded RNA viruses (i.e. reo), and some DNA viruses (i.e. vaccinia) at a concentration of 1–4 μg/mL, which is well below the cytotoxicity threshold (40 μg/mL).

Introduction

The analogues of natural 2'-deoxyribonucleosides in which the hydroxy group at C3' is substituted by a fluorine atom in the ribo configuration exhibit a wide range of biological properties^{1–9} following their intracellular conversion to 5'-triphosphates.^{10–13} The latter act as competitive inhibitors^{10–13} and/or alternate substrates^{10,14–17} of some DNA polymerases, depending on the experimental conditions used.^{11,14,15} When being substrates, the analogues of natural compounds are incorporated into a growing DNA chain and terminate it at a site that is strictly complementary to the corresponding template base. Hence, they can be used as an effective tool for DNA sequencing by the method of Sanger et al.^{10–17}

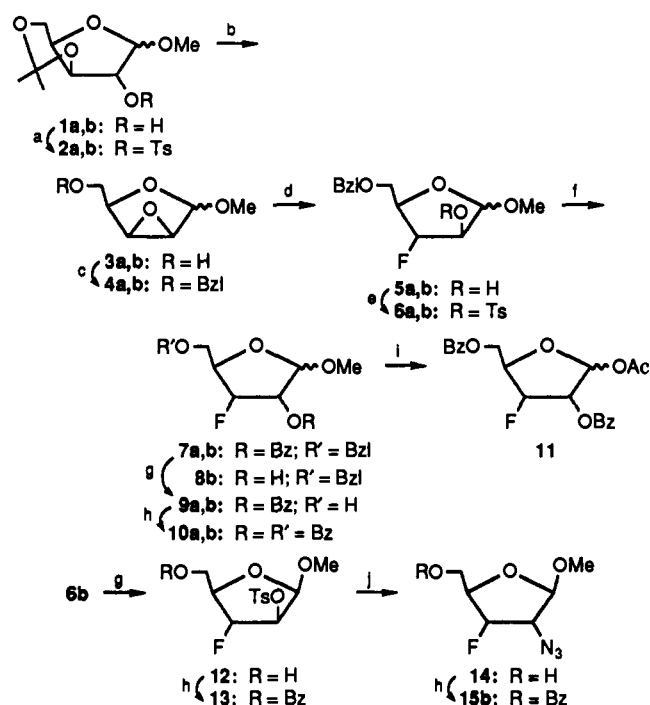
The synthesis and investigation of the biological activity of 3'-deoxy-3'-fluoro analogues of ribonucleosides have received relatively little attention.¹⁸ Thus, Langen et al. briefly described two approaches to the synthesis of 3'-deoxy-3'-fluorouridine and did not find an inhibitory effect of the compound on RNA synthesis *in vitro*.²⁵ Later, another group of investigators gave a more detailed account on the above approach (without reference to the original paper of Langen et al.²⁵). From the physicochemical data²⁶ provided for 3'-deoxy-3'-fluorouridine it appears that the ¹H NMR results and, in particular *J*_{2,F}, differ significantly from those reported by Langen et al.²⁵ Also, Wiebe and his co-workers²⁶ failed to explain the mechanism by which 3'-deoxy-3'-fluorouridine was formed. Recently, two other groups reported the synthesis of 3'-fluoro-3'-deoxyadenosine.^{20,27} The compound proved active against a broad range of viruses encompassing vaccinia, polio, Coxsackie B, Sindbis, Semliki forest, and reovirus.²⁷

The present study was undertaken to develop an universal method for the synthesis of 3'-fluoro-3'-deoxyribonucleosides and various 2'-derivatives thereof, and to provide a detailed account of their biological properties.

Chemistry

1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose (11) was used as the key glycosylating agent for the synthesis of the 3'-fluoro-3'-deoxy analogues of natural ribonucleosides (Scheme I). Initially, starting from D-xylose, individual α- and β-methyl glycosides (1a and 1b) of 3,5-*O*-isopropylidene-D-xylofuranose were prepared by

Scheme I^a



^a (a) TsCl, pyr, room temperature, 10 h (2a, 87.5%; 2b, 86%). (b) (1) AcOH/H₂O, 50 °C, 2 h; (2) MeONa/MeOH, 0–4 °C, 18–24 h (3a, 89%; 3b, 80%). (c) NaH, dioxane, room temperature, 0.5 h; BzIBr, 0 °C, 1 h, room temperature, 1 h (4a, 89%; 4b, 80%). (d) KHF₂/NaF, 1,2-ethylene glycol, reflux, 1.5–4 h (5a, 45%; 5b, 31%). (e) TsCl, pyr, room temperature, 28 h (6a, 93%; 6b, 89%). (f) BzONa, DMSO, dibenzo-18-crown-6, reflux, 4–7 h (7a, 33.6%; 7b, 39.8%, + 8b, 38.5%). (g) H₂, 10% Pd/C, EtOH, room temperature, 72 h (9a, 97%; 9b, 98%). (h) BzCl, pyr, room temperature, 12 h (10a, 94%; 10b, 81%). (i) AcOH/Ac₂O/H₂SO₄, room temperature, 20 h (89–94%). (j) NaN₃, DMSO, 180 °C, 1 h (15b, 61%).

using a modification²⁸ of the method described by Baker et al.²⁹ Tosylation of 1a and 1b in pyridine gave the

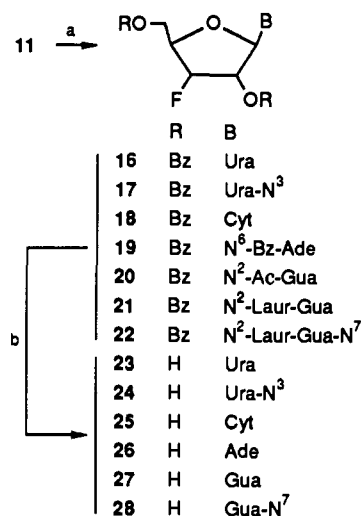
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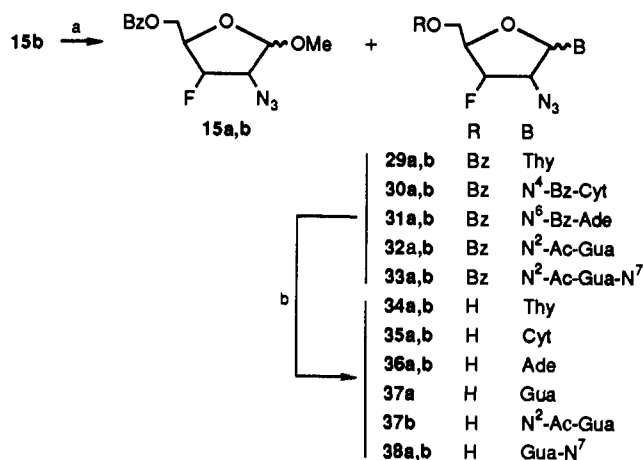
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crystalline tosylates **2a**³⁰ and **2b**, respectively; these compounds were subsequently converted to the corresponding crystalline epoxides **3a** and **3b** in a two-step procedure.²⁹ Epoxides **3a** and **3b** were benzylated with benzyl bromide in dioxane in the presence of NaH³¹ to give upon workup compounds **4a** and **4b** as oils in high yields. Treatment of the α -anomer **4a** with KHF₂ and NaF in 1,2-ethylene glycol,^{32,33} followed by silica gel column chromatography,

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Scheme II^a

^a (a) Persilylated base, (1) SnCl₄, 1,2-dichloroethane, room temperature, 18-24 h; (2) TMS-TfI, 1,2-dichloroethane or CH₃CN, reflux, 1-3 h. (b) NH₃/MeOH, room temperature, 48 h.

Scheme III^a

^a (a) Persilylated base, (1) SnCl₄, CH₃CN, reflux, 5 h; (2) TMS-TfI, CH₃CN, reflux, 3 h. (b) NH₃/MeOH, room temperature, 48 h.

afforded the fluoride **5a** in 45% yield. Contrary to published data,³³ under similar conditions with β -anomer **4b** as the starting material, fluoride **5b** was obtained in 31% yield, but no 2-fluoro-2-deoxy xylo isomer was isolated. The treatment of tosylate **6b** with potassium benzoate in boiling DMSO in the presence of dibenzo-18-crown-6, followed by silica gel column chromatography, afforded the compounds **7b** and **8b** in 40% and 37% yield, respectively. Benzoylation of the latter compound gave **7b**. As could be expected, a combination of steric and electronic effects of α -OMe group of **6a** proved unfavorable to nucleophilic substitution of the 2-tosyloxy group. As a result, conversion of **6a** to **7a** required longer heating of reagents, and benzoate **7a** was isolated by chromatography in 35% yield. Catalytic hydrogenolysis of **7a** and **7b** in the presence of 10% Pd/C in EtOH gave rise to crystalline **9a** and oily **9b**, respectively; these compounds were subsequently converted to **10a** and **10b** by benzoylation. The total yield of **10a** and **10b** (from **7a** and **7b**, respectively) was greater than 90%. Acetolysis³⁴ of both **10a** and **10b** afforded a

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mixture of the anomeric acetates 11 (α/β ratio \approx 1:1) in a high yield.

As the first step in the synthesis of the 2'-azido-2',3'-dideoxy-3'-fluoro derivatives, nucleophilic displacement of the tosyloxy group in 6a and 6b by an azido group was studied. The reaction could not be accomplished with α -anomer 6a under different conditions due to the reasons mentioned above. On the contrary, when 6b was treated with NaN_3 in DMSO at 200 °C, nucleophilic displacement with inversion of the configuration occurred. However, the yield of the desired compound did not exceed 34%. The following reaction sequence was found to be more effective. Catalytic hydrogenolysis of fluoride 6b gave 12, which was converted to the benzoate 13 by benzylation in 85% yield. Nucleophilic displacement of the tosyloxy group in 13 by treatment with NaN_3 in DMSO led to partial debenylation yielding compound 14. Therefore, the reaction products were treated with benzoyl chloride in pyridine, and after column chromatography methyl 2-azido-5-O-benzoyl-2,3-dideoxy-3-fluoro- β -D-ribofuranoside (15b) (61% yield) and the starting tosylate 13 were isolated. The methyl glycoside 15b was as such used for the synthesis of the nucleosides.

The condensation of the silylated bases with sugars 11 and 15b in the presence of Friedel-Crafts catalysts was used for the synthesis of nucleosides³⁶ (Schemes II and III). Thus, the reaction of persilylated uracil with compound 11 in the presence of excess SnCl_4 [ratio of the reagents (mol) 1.7:1.0:4.16] in 1,2-dichloroethane for 22 h at room temperature followed by column chromatography gave 1-(2,5-di-O-benzoyl-3-deoxy-3-fluoro- β -D-ribofuranosyl)-uracil (16) and the N^3 -isomer 17 in 70% and 8% yield, respectively. It should be emphasized that a decrease of SnCl_4 amount resulted in a higher yield of N^3 -isomer and lower total yield of nucleoside products. The condensation of persilylated cytosine with acetate 11 in the presence of trimethylsilyl triflate (TMS-TfI) (1:1:1, mol) in refluxing 1,2-dichloroethane for 1 h gave the nucleoside 18 in 75% yield.

For the synthesis of the adenine and guanine nucleosides, the results obtained earlier for related compounds were taken into consideration.³⁶⁻³⁸ Thus, to minimize the formation of N^7 -adenine nucleoside, persilylated N^6 -benzoyladenine was glycosylated with compound 11 in the presence of excess SnCl_4 (1:1:2, mol) in 1,2-dichloroethane at room temperature.^{36,37} After column chromatography on silica gel, N^9 -glycoside 19 was obtained in 82% yield. As demonstrated earlier,^{37,38} the use of TMS-TfI instead of SnCl_4 as a condensing agent led to the formation of the guanine N^9 -glycosides as principal product along with formation of the N^7 -isomers. Indeed, the reaction of persilylated N^2 -acetylguanine with 11 and TMS-TfI (1.2:1.0:1.16, mol) followed by chromatography gave the N^9 -glycoside 20 in 62% isolated yield. The TLC data indicated that after deprotection minor amounts of the N^7 -isomer were present in the reaction products. However, when silylated N^2 -acetylguanine was replaced by silylated

N^2 -lauroylguanine in the above condensation, the N^9 -glycoside 21 was obtained as the main product of the reaction along with the N^7 -glycoside 22 (the ratio of N^9/N^7 was 2:1) in 53% yield (combined).

Treatment of the protected nucleosides 16-22 with methanolic ammonia afforded in good to excellent yield the free nucleosides 23-28, respectively.

As glycosylating agents, methyl glycosides have, in particular cases, certain advantages over peracyl derivatives of sugars and peracyl glycosyl halides, although their use requires more vigorous reaction conditions (cf. the data^{39,40}). Thus, the reaction of persilylated thymine with methyl glycoside 15b and TMS-TfI (2.0:1.0:3.0, mol) in refluxing acetonitrile for 5 h followed by chromatography afforded a mixture of the α - and β -anomers 29a,b (the ratio of α/β was 1:4 according to ^1H NMR data) in 58% yield and a mixture of the methyl glycosides 15a,b in 10% yield. Treatment of 29a,b with methanolic ammonia and subsequent chromatography on silica gel afforded 1-(2-azido-2,3-dideoxy-3-fluoro- β -D-ribofuranosyl)thymine (34b) and its α -anomer 34a in 68% and 22% yield, respectively. Along the same line, the synthesis of cytosine nucleosides after standard workup and subsequent chromatography gave a mixture of the methyl glycosides 15a,b (18%), the β -anomer 30b (30%), and the α -anomer 30a (8%). When carried out in the presence of SnCl_4 instead of TMS-TfI, the reaction led to the desired β -nucleoside 30b and its α -anomer 30a in yields of 40% and 10%, along with the mixture of 15a,b (16%). In a related way, the condensation of silylated N^6 -benzoyladenine with methyl glycoside 15b in the presence of excess SnCl_4 (2:1:5, mol) in refluxing 1,2-dichloroethane/acetonitrile (1:2, v/v) mixture for 5 h proceeded analogously, and the products 15a,b, 31b, and 31a were isolated in yields of 13%, 57%, and 19%, respectively. Finally, the reaction of silylated N^2 -acetylguanine with methyl glycoside 15b and TMS-TfI (2.0:1.0:5.0, mol) in refluxing acetonitrile for 3 h afforded a complex mixture of reaction products, the chromatography of which gave the N^9 - α -nucleoside 32a (22%) and the mixture of 32b and 33a,b in 70% yield (combined). Removal of the acyl protecting group from the mixture of nucleosides and subsequent chromatographical separation gave 7-(2-azido-2,3-dideoxy-3-fluoro- β -D-ribofuranosyl)guanine (38b), its α -anomer 38a, and 9-(2-azido-2,3-dideoxy-3-fluoro- β -D-ribofuranosyl)- N^2 -acetylguanine (37b). Long-time treatment of the latter nucleoside with saturated ammonia in methanol at room temperature did not lead to deacylation. On the contrary, treatment of protected nucleosides 30a,b, 31a,b, and 32a with methanolic ammonia afforded in high yield the free nucleosides 35a,b, 36a,b, and 37a, respectively.

It is noteworthy that the formation of the β -anomers of nucleosides as principal products in the reaction of methyl glycoside 15b with the silylated bases may be ascribed to steric hindrances and/or electronic effects by the 2-azido group in the formation of the α -anomers. The anomeric mixture of the methyl glycosides 15a,b could be used for the synthesis of the corresponding nucleosides, the α/β ratio of the latter being unchanged.

The structure of all the compounds that were synthesized was proven by ^1H NMR data (Tables I-IV) and UV and CD spectroscopy, taking into account previous remarks.^{15,36-38} The ^1H NMR data recorded for 3'-fluoro-3'-deoxyuridine (23) are in agreement with those reported

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Table I. ¹H NMR Spectral Data of Sugars

no.	chemical shifts						others
	H-1	H-2	H-3	H-4	H-5	H-5'	
5a	4.87 s	4.04 d	4.77 dd	4.33 ddt	3.66 dd	3.61 dd	7.35–7.26 (m, PhCH ₂), 4.65 (br s, 2-OH), 4.59 (dd, PhCH ₂), 3.36 (s, OCH ₃)
5b	~4.92 d	~4.31 m	4.86 dm	4.24 ddt		3.58 m	7.39–7.26 (m, PhCH ₂), 4.49 (dd, PhCH ₂), 3.43 (s, OCH ₃)
6a	4.96 s	4.83 dm	4.86 ddd	4.27 ddt	3.63 dd	3.59 dd	7.80–7.28 (m, PhCH ₂ + CH ₃ PhSO ₂), 4.57 (dd, PhCH ₂), 3.30 (s, OCH ₃), 2.45 (s, CH ₃ PhSO ₂)
6b	~4.86 m	~4.95 ddd	~5.06 ddd	4.19 ddt	3.58 dd	3.54 dd	7.85–7.25 (m, PhCH ₂ + CH ₃ PhSO ₂), 4.55 (s, PhCH ₂), 3.25 (s, OCH ₃), 2.45 (s, CH ₃ PhSO ₂)
7a	5.30 d	5.08 dm	5.23 ddd	4.50 ddt	3.70 dd	3.63 dd	8.15–7.30 (m, PhCH ₂ + Bz), 4.61 (dd, PhCH ₂), 3.47 (s, OCH ₃)
7b	5.15 t	5.35 m	5.28 dt	4.49 ddt		3.66 m	7.61–7.27 (m, PhCH ₂ + Bz), 4.63 (dd, PhCH ₂), 3.43 (s, OCH ₃)
8b	4.87 t	4.17 m	5.02 dt	4.37 ddt	3.61 dd	3.57 dd	7.38–7.27 (m, PhCH ₂), 4.59 (dd, PhCH ₂), 3.39 (s, OCH ₃)
9a	5.33 d	5.04 ddd	5.19 ddd	4.50 ddt	3.91 dd	3.83 dd	8.14–7.44 (m, Bz), 3.50 (s, OCH ₃)
9b	5.19 t	5.35 m	5.36 ddd	4.49 ddt	3.87 dd	3.74 dd	8.12–7.42 (m, Bz), 3.50 (s, OCH ₃)
10a	5.35 d	5.12 ddd	5.30 ddd	4.68 ddt	4.60 dd	4.53 dd	8.15–7.44 (m, 2Bz), 3.50 (s, OCH ₃)
10b	5.13 t	5.45 m	5.44 m	4.71		4.46 m	8.12–7.39 (m, 2Bz), 3.40 (s, OCH ₃)
11b	6.41 t	5.58 ddd	5.46 dt	4.74 ddd	4.68 dd	4.48 dd	1.98 (s, OAc) 8.11–7.45 (m, Bz)
11a	6.65 d	5.35 dt	5.37 ddd	4.85 ddt	4.58 dd	4.51 dd	2.17 (s, OAc)
12	~4.90	~4.93 ddd	5.21 ddd	4.17 ddt	3.73 dd	3.66 dd	7.83–7.38 (m, CH ₃ PhSO ₂), 3.40 (s, OCH ₃), 2.47 (s, CH ₃ PhSO ₂)
13	~4.98	4.93 ddd	5.25 ddd	4.35 ddt		4.42 m	8.06–7.34 (m, Bz + CH ₃ PhSO ₂), 3.30 (s, OCH ₃), 2.45 (s, CH ₃ PhSO ₂)
15a	5.15 d	3.30 ddd	5.16 ddd	4.68 ddt	4.53 dd	4.47 dd	8.00–7.48 (m, Bz), 3.60 (s, OCH ₃)
15b	4.88 t	4.06 m	5.36 dt	4.61		4.41	8.10–7.43 (m, Bz), 3.35 (s, OCH ₃)

m

Table II. Coupling Constants

no.	1,2	2,3	3,4	1,F	2,F	3,F	4,F
5a	<1.0	<1.0	1.8		12.9	51.6	26.2
5b		4.8	4.2		~21.0	55.8	22.3
6a	1.2	1.2	4.8		15.6	51.6	20.4
6b	4.2	6.0	4.8		~22.0	54.0	~21.9
7a	4.8	5.4	1.2		23.4	55.2	26.4
7b	1.8	3.6	3.6	1.8		54.0	20.4
8b	1.8	3.6	3.6	1.2	9.2	54.6	21.6
9a	4.8	6.0	2.4		22.8	56.4	26.4
9b ^a	1.8	4.8	3.6	1.8	9.0	53.4	21.0
10a	4.8	6.0	2.4		22.3	55.2	25.2
11a	4.8	4.8	1.2		26.4	55.2	25.8
11b	1.8	4.8	4.8	1.8	8.4	51.6	18.6
12	4.6	6.0	4.8		23.4	56.4	21.6
13	4.8	6.6	4.8		21.0	56.1	
15a	4.2	5.4	1.8		27.0	56.4	26.4
15b	1.8	4.8	4.8	1.8	7.2	53.4	

^a ⁴J_{5,F} = 1.2.

by Kowollik²⁵ and are actually opposite to the data of Misra et al.²⁶ Analysis of the *J* and δ values suggests that the compound, to which was assigned the structure of 3'-fluoro-3'-deoxyuridine,²⁶ probably corresponds to 1-(3-fluoro-3-deoxy- β -D-arabinopyranosyl)uracil. This suggestion is based on the following features of ¹H NMR spectrum: (a) the supposed pyranoside is in conformational equilibrium (${}^4C_1 \rightleftharpoons {}^4C_1$) in solution, as indicated by the values of the coupling constants: viz. H4':H5', F3':H2', and F3':H4' as well as H1':H2', H2':H3', and H3':H4' (see, e.g., refs 41, 42); (b) the values of ⁴J_{F,1'} and ⁴J_{F,5'} agree well with a near-planar W arrangement of the corresponding nuclei in the 4C_1 conformation of the pyranose ring (see, e.g., refs 42, 43).

The ¹H NMR spectra of 3'-fluoro-3'-deoxyadenosine (26) and -guanosine (27) are in accord with those obtained for the same compounds obtained by alternative procedures.^{20,21,23,24} However, the ¹H NMR data of 3'-fluoro-3'-deoxyadenosine differ from the ¹H NMR data of the compound²² obtained by transglycosylation of 3'-fluoro-

3'-deoxyuridine,^{25,26} with adenine.

The site of glycosylation of uracil and guanine was determined by comparison of the ¹H NMR, UV, and CD spectra of the corresponding compounds with those of the pairs of related N¹- and N³-, and N⁹- and N⁷-isomers,^{15,37,44,45} respectively. The most informative feature of the ¹H NMR spectra of the α -anomers is the 0.38–0.41 ppm shift of H4' resonance signal to a lower field when going from β - to α -anomers (see, e.g., refs 15, 45).

Cytostatic and Antiviral Properties of the Test Compounds in Vitro

The 3'-fluoro-3'-deoxynucleosides were evaluated for their inhibitory effect on the proliferation of different tumor cell lines in vitro. 3'-Fluoro-3'-deoxyadenosine (26) proved invariably more cytostatic than its uridine (23), cytidine (25), and guanosine (27) counterparts. However, the cytostatic effects of the compounds varied markedly from one tumor cell line to another (Table V). Compound 26 was 7–17-fold more cytostatic to MT-4 than L1210 or Raji cells and at least 100-fold more cytostatic to MT-4 than Molt/4F cells. In contrast, the guanine derivative proved at least 20-fold more effective against L1210 than MT-4 cells (Table V).

The compounds listed in Table VI were also evaluated for their inhibitory effects on a number of viruses including the DNA viruses herpes simplex virus type 1, herpes simplex virus type 2, and vaccinia virus and the RNA viruses vesicular stomatitis virus, Sindbis virus, Coxsackie virus type B 4, polio virus type 1, reovirus type 1, Semliki forest virus, parainfluenza virus type 3, and human immunodeficiency virus type 1. None of the 2'-azido-3'-fluoro-substituted 2',3'-dideoxynucleoside analogues (i.e. 34a, 34b, 35b, and 36b) showed significant antiviral activity at subtoxic concentrations. However, among the 3'-fluoro-substituted 3'-deoxynucleoside analogues, 3'-fluoro-3'-deoxyadenosine (26) proved to be markedly inhibitory to the replication of a number of viruses including vaccinia virus, polio virus-1, Coxsackie virus B4, Sindbis virus,

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Table III. ¹H NMR Spectral Data of Nucleosides

no.	chemical shifts								others
	H-8 (H-6)	H-2 (H-5)	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	
23	7.83 d	5.71 d	5.90 d	4.26 ddd	4.96 dd	4.18 dt	3.63 dd	3.57 dd	
24	7.58 d	5.56 d	6.17 d	4.85 m	5.05 ddd	4.03 ddt	3.72 dd		
25	7.73 d	5.78 d	5.91 d	4.26 ddd	4.92 dd	4.13 dt	3.63 dd	3.50 dd	7.24 (br s, NH), 7.18 (br s, NH), 5.70 (d, 2'-OH), 5.21 (t, 5'-OH)
26	8.37 s	8.15 s	5.96 d	4.95 m	5.10 dd	4.31 dt		3.68 m	7.40 (br s, NH ₂), 5.92 (d, 2'-OH), 5.78 dd, 5'-OH)
27	7.92 s		5.75 d	4.76 ddd	5.04 dd	4.21 dt		3.59 d	6.54 (br s, NH ₂), 5.88 (d, 2'-OH), 5.42 (t, 5'-OH)
28	8.30 s		6.01 d	4.74 ddd	4.99 dd	4.18 dt		3.62 dd	3.56 dd
34a	7.27 d		6.29 d	4.78 ddd	5.36 ddd	4.69 ddt		3.52 m	6.25 (br s, NH ₂), 5.86 (d, 2'-OH), 5.20 (t, 5'-OH)
34b	7.68 d		6.06 d	4.37 ddd	5.35 dd	4.28 ddt		3.65 m	11.40 (s, NH), 5.37 (t, 5'-OH), 1.80 (d, 5-CH ₃)
35a	7.44 d	5.75 d	6.31 d	4.69 ddd	5.34 ddd	4.57 ddt		3.53 m	8.27 (s, NH), 5.19 (t, 5'-OH)
35b	7.75 d	5.80 d	6.15 d	4.22 ddd	5.32 dd	4.25 m		3.65 m	7.33 (s, NH), 7.31 (s, NH), 5.28 (t, 5'-OH)
36a	8.15 s	8.03 s	6.55 d	4.90 ddd	5.46 ddd	4.77 ddt		3.56 m	7.27 (s, NH ₂), 5.19 (t, 5'-OH)
36b	8.41 s	8.17 s	6.16 d	5.11 ddd	5.53 dd	4.39 dt		3.68 m	7.42 (s, NH ₂), 5.64 (t, 5'-OH)
37a	7.62 s		6.28 d	4.69 ddd	5.46 dd	4.60 dt		3.55 m	10.65 (br s, NH), 6.49 (br s, NH ₂)
37b	8.00 s		5.89 d	4.93 ddd	5.47 dd	4.30 dt		3.62 m	5.67 (s, NH), 5.35 (t, 5'-OH), 1.76 (s, Ac)
38a	7.93 s		6.68 d	4.78 m	5.40 dd	4.78 m		3.56 m	6.20 (br s, NH ₂), 5.25 (t, 5'-OH)
38b	8.40 s		6.26 d	4.92 ddd	5.44 dd	4.29 dt		3.64 m	11.02 (s, NH), 6.30 (br s, NH ₂), 5.27 (t, 5'-OH)

Table IV. Coupling Constants

no.	coupling constants (Hz)									others
	1',2'	2',3'	3',4'	4',5'	4',5''	2',F	3',F	4',F		
23	7.8	4.2	<1.0	3.0	3.6	25.2	54.0	28.2	7.8 (5,6), 12.0 (5',5'')	
24	5.4	4.8	3.6	6.0	6.0	31.2	54.0	23.4	7.8 (5,6), 10.8 (5',5'')	
25	7.8	4.8	<1.0	3.6	3.6	24.0	54.6	27.0	7.2 (5,6), 6.0 (2',OH-2'), 12.0 (5',5'')	
26	7.8	4.2	<1.0	3.0	3.0	26.4	54.6	27.6		
27	8.4	4.8	<1.0	3.7	3.7	25.2	54.0	27.6		
28	8.6	5.8	<1.0	4.3	4.3	25.2	55.7	27.8	12.5 (5',5'')	
34a	6.9	4.8	1.2	4.2	4.2	25.2	54.0	23.4	1.2 (6, CH ₃ -5), 5.4 (5'-OH-5', 5'',OH-5')	
34b	8.4	4.8	<1.0	3.0	3.0	24.0	53.4	27.6	1.2 (6, CH ₃ -5), 4.8 (5',OH-5', 5'',OH-5')	
35a	6.0	4.8	1.8	3.6	3.6	24.0	54.0	22.8	7.8 (5,6), 5.4 (5',OH-5', 5'',OH-5')	
35b	7.8	3.6	<1.0			24.6	54.0	26.4	7.8 (5,6), 5.4 (5',OH-5', 5'',OH-5')	
36a	6.6	4.8	1.2	3.9	3.9	26.1	54.3	24.6	12.0 (5,5''), 5.4 (5',OH-5', 5'',OH-5')	
36b	8.4	4.8	<1.0	3.6	3.6	25.8	53.4	27.6	12.0 (5',5''), 6.0 (5',OH-5', 5'',OH-5')	
37a	6.3	4.8	<1.0	3.6	3.6	26.4	55.2	24.0		
37b	8.4	4.2	<1.0	4.2	4.2	24.6	54.0	27.6	5.4 (5',OH-5', 5'',OH-5')	
38a	6.6	4.8	<1.0				54.0			
38b	8.4	4.2	<1.0	4.2	4.2	25.2	54.0	27.6	6.0 (5',OH-5', 5'',OH-5')	

Table V. Cytostatic Activity of Test Compounds against Different Tumor Cell Lines

no.	50% inhibitory concentration (IC ₅₀), ^a μg/mL			
	murine leukemia	human B-lymphoblast	human T-lymphoblast	human T-lymphoblast
	L1210	Raji	Molt/4F	MT-4
23	>100	>100	>100	6.9
25	39	98	65	7.9
26	1.6	3.9	28	0.22
27	5.0	29	75	>100

^a Required to reduce the viability of the tumor cells by 50%.

reovirus-1, and Semliki forest virus. Its minimum inhibitory concentration (MIC) for these viruses ranged from 1 to 7 μg/mL, a concentration that is well below the cytotoxicity threshold of the compound (MCC ≥ 40 μg/mL) (Table VI). 3'-Fluoro-3'-deoxyguanosine (27) was inhibitory to vaccinia virus, Coxsackie virus B4, Semliki forest virus, and Sindbis virus at a 10-fold higher concentration than 26. Compounds 23 and 25 were only marginally active against those viruses that were sensitive to 26.

None of the compounds was inhibitory to HIV-1 replication in MT-4 cells at subtoxic concentrations (data not

Table VI. Activity of Test Compounds against Different Viruses in Different Cell Systems

no.	minimum inhibitory concentration (MIC), ^a μg/mL														
	minimum cytotoxic concentration ^b (MCC), μg/mL			HeLa cells											
				primary rabbit kidney (PRK) cells					African green monkey kidney (Vero B) cells						
	PRK cells	HeLa cells	Vero B cells	herpes simplex virus-1 (KOS)	herpes simplex virus-2 (G)	vaccinia virus	vesicular stomatitis virus	polio virus-1	Coxsackie virus-B4	Sindbis virus	para-influenza virus-3	reovirus-1	Semliki forest virus		
23	>400	>400	>400	>400	>400	>400	>400	>400	150	300	300	300	>400	150	>400
25	>400	>400	>400	>400	>400	>400	>400	(300)	150	150	300	300	>400	150	>400
26	>40	40	40	>10	>10 (20)	2	>40	>10	4	2	7	4	>4	1	4
27	200	>400	400	>100	>100	10	>200	>200	150	150	40	40	>100	>200	40
34a	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
34b	>400	>400	>400	>400	>400	>400	<400	>400	>400	>400	>400	>400	>400	>400	>400
35b	>400	>400	>400	>400	>400	>400	>300	>400	>400	>400	>400	>400	>400	>400	>400
36b	>400	>400	>400	>400	>200	300	300	>200	>200	300	>200	>200	>200	>200	>200

^a Required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The multiplicity of infection (MOI) was invariably 100 × CCID₅₀, that is 100 times the virus dose needed to infect 50% of the cell cultures. ^b Required to cause a microscopically detectable alteration of normal cell morphology.

shown). The mechanism of antiviral action of **26** and **27** remains to be resolved. It should be worth pursuing the synthesis of closely related derivatives of **26** in attempts to decrease its toxicity and increase its antiviral potency.

The antiviral activity spectrum of **26** and **27** includes some (\pm)DNA viruses such as pox (vaccinia), (+)RNA viruses such as picorna (polio, Coxsackie), toga (Sindbis, Semliki forest), and (\pm)RNA viruses such as reo. This spectrum is clearly different from the activity spectrum of the acyclic and carbocyclic adenosine analogues [i.e. (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA), carbocyclic 3-deazaadenosine (C-c³Ado), neplanocin A, 3-deazaneplanocin A] which encompasses (-)RNA viruses [i.e. paramyxo (parainfluenza), arena (Junin, Tacaribe), rhabdo (vesicular stomatitis)] instead of (+)RNA viruses.⁴⁶ The antiviral activity of these adenosine analogues has been attributed to an inhibition of *S*-adenosylhomocysteine hydrolase,⁴⁷ and, consequently, inhibition of transmethylation reactions starting from *S*-adenosylmethionine as the methyl donor. From the antiviral activity spectrum shown by compound **26**, it is unlikely that this adenosine analogue would achieve its antiviral action through inhibition of *S*-adenosylhomocysteine hydrolase.

The synthesis and antiviral activity of compound **26** have been the subject of a previous report.²⁷ The results that were independently obtained with the present preparation confirm those obtained previously.

Experimental Section

Melting points were determined with a Boethius (GDR) apparatus and are uncorrected. IR spectra were recorded with a UR-20 (GDR) spectrophotometer, UV spectra were recorded with a Specord UV-vis (GDR) spectrophotometer, and ¹H NMR spectra were recorded with a Bruker WM-360 (FRG) spectrometer with tetramethylsilane as an internal standard (s = singlet; d = doublet, t = triplet, m = multiplet). Standard Silufol UV₂₅₄ (Czechoslovakia), Kieselgel 60F (Merck, FRG) plates were used for TLC of sugars and nucleosides, respectively. The following solvent systems were used: hexane-ethyl acetate, 4:1, v/v (A), CHCl₃-MeOH, 19:1 (B), CHCl₃-MeOH, 4:1 (C), CHCl₃-MeOH, 9:1 (D), *i*-PrOH-H₂O-20% NH₄OH, 7:2:1 (E). Column chromatography was performed on silica gel L 40 × 100 (Chemapol, Czechoslovakia). Anhydrous solvents were obtained as follows: pyridine was successively refluxed and distilled over potassium hydroxide and calcium hydride; dimethyl sulfoxide was heated at 100 °C over KOH for 8 h and distilled in vacuo; 1,2-dichloroethane was kept over phosphorous anhydride for 12 h and distilled; acetonitrile was distilled twice over phosphorous anhydride, kept over calcium hydride, and distilled. In all conversion reactions, freshly distilled SnCl₄ and trimethylsilyl trifluoromethanesulfonate (Fluka, Switzerland) were used. The solutions of compounds in organic solvents were dried with anhydrous sodium sulfate for 4 h. The reactions were performed at 20 °C, unless stated otherwise. Elemental analyses were carried out in Microanalytical Laboratorium at the Institute of Organic Chemistry, Ukrainian Academy of Sciences (Kiev, USSR).

Methyl 5-O-benzyl-3-fluoro-3-deoxy- α -D-arabinofuranoside (5a) and its β -anomer (5b) were obtained^{32,33} in 40–45% and 30% yield, respectively.

Methyl 5-O-Benzyl-2-O-tosyl-3-fluoro-3-deoxy- α -D-arabinofuranoside (6a) and Its β -Anomer (6b). A mixture of 2.9 g (11.31 mmol) of **5a** and 5.0 g (26.22 mmol) of tosyl chloride in 50 mL of anhydrous pyridine was stirred for 28 h. The solvent was evaporated, and the residue was dissolved in CHCl₃ (300 mL) and washed with saturated NaHCO₃ solution (2 × 100 mL). The organic layer was separated, evaporated, coevaporated with toluene, and purified by column chromatography, using a linear EtOAc gradient (0–50%, v/v; 2 L) in hexane to yield 4.32 g (93%) of **6a** as a syrup: *R*_f 0.44 (A).

In a similar way, starting from 3.9 g (15.21 mmol) of **5b** and 6.0 g (31.47 mmol) of tosyl chloride, 5.54 g (89%) of **6b** was obtained: mp 73–74 °C (EtOH); *R*_f 0.32 (A). Anal. (C₂₀H₂₃FO₆S) C, H, F, S.

Methyl 5-O-Benzyl-2-O-benzoyl-3-fluoro-3-deoxy- α -D-ribofuranoside (7a). A mixture of 0.44 g (1.07 mmol) of **6a**, 1.83 g (12.67 mmol) of potassium benzoate, and 4.6 g (12.67 mmol) of dibenzo-18-crown-6 in 10 mL of anhydrous DMSO was heated at 205–210 °C for 7 h. After cooling, the reaction mixture was diluted with 300 mL of CHCl₃ and washed with H₂O (3 × 100 mL). The organic layer was separated, dried, and evaporated. The residue was chromatographed with a linear EtOAc gradient (0–50%, v/v, 1 L) in hexane to yield 0.13 g (33.6%) of **7a** as a syrup: *R*_f 0.42 (A).

Methyl 5-O-Benzyl-2-O-benzoyl-3-fluoro-3-deoxy- β -D-ribofuranoside (7b) and Methyl 5-O-Benzyl-3-fluoro-3-deoxy- β -D-ribofuranoside (8b). A mixture of 1.23 g (2.99 mmol) of **6b**, 4.5 g (31.2 mmol) of potassium benzoate, and 4.5 g (12.4 mmol) of dibenzo-18-crown-6 in 45 mL of anhydrous DMSO was heated at 205–210 °C for 4 h. The workup and isolation, performed as described above, yielded 0.43 g (39.8%) of **7b** as a syrup (*R*_f 0.35 (A)) and 0.28 g (38.5%) of **8b** as a syrup (*R*_f 0.13 (A)).

Methyl 2-O-Benzoyl-3-fluoro-3-deoxy- α -D-ribofuranoside (9a) and Its β -Anomer (9b). A mixture of 1.7 g of 10% Pd/C and 1.7 g (4.71 mmol) of **7a** in 150 mL of EtOH was stirred in H₂ atmosphere for 72 h. The catalyst was filtered off and washed with EtOH (2 × 50 mL). The filtrates were evaporated, and the residue was crystallized from Et₂O to give 1.24 g (97%) of **9a**: mp 104–105 °C, *R*_f 0.12 (A). Anal. (C₁₃H₁₅FO₅) C, H, F.

Similarly, starting from 1.36 g (3.77 mmol) of **7b** and 1.4 g of 10% Pd/C in 130 mL of EtOH, 1.0 g (98%) of **9b** as a syrup was produced: *R*_f 0.1 (A).

Methyl 2,5-Di-O-benzoyl-3-fluoro-3-deoxy- α -D-ribofuranoside (10a) and Its β -Anomer (10b). A mixture of 1.3 mL (1.58 g; 11.23 mmol) of benzoyl chloride and 1.3 g (4.81 mmol) of **9a** in 12 mL of anhydrous pyridine was stirred for 12 h and then poured into 50 mL of H₂O, containing 10 g of ice, and extracted by CHCl₃ (3 × 100 mL). The organic extracts were combined, washed, dried, evaporated, and coevaporated with toluene (2 × 50 mL). The residue was purified by column chromatography, using a linear EtOAc gradient (0–50%, v/v, 1 L) in hexane to yield 1.7 g (94%) of **10a** as a syrup: *R*_f 0.44 (A).

Similarly, starting from 1.0 g (3.7 mmol) of **9b** and 0.54 mL (0.65 g, 4.6 mmol) of benzyl chloride, 1.12 g (81%) of **10b** was obtained as a syrup: *R*_f 0.42.

1-O-Acetyl-2,5-di-O-benzoyl-3-fluoro-3-deoxy- α,β -D-ribofuranose (11). Concentrated H₂SO₄ (0.7 mL) was added to the solution of 1.3 g (3.34 mmol) of **10a** in 10 mL of AcOH and 1.2 mL of Ac₂O, the mixture was stirred for 20 h and poured into 5% aqueous solution of NaHCO₃ (100 mL), containing 50 g of ice, and after the ice melted, it was extracted with CHCl₃ (2 × 100 mL). The organic extracts were combined, evaporated, and coevaporated with toluene (2 × 50 mL). The residue was purified with column chromatography, using a linear EtOAc gradient (0–50%, v/v; 1 L) in hexane to yield 1.31 g (94%) of **11** as a syrup: *R*_f 0.37 (A).

In a similar way, starting from 0.5 g (1.33 mmol) of **10b**, 0.48 g (89%) of **11** was obtained.

3'-Fluoro-3'-deoxyuridine (23) and Its 3-N-Isomer (24). A solution of SnCl₄ (2.35 mL, 5.22 g, 20.03 mmol), 1.94 g (4.82 mmol) of **11**, and 1.71 g (2.1 mL, 5.48 mmol) of bis(trimethylsilyl) derivative of uracil in anhydrous 1,2-dichloroethane (90 mL) was stirred for 22 h. The reaction mixture was diluted with CHCl₃ (60 mL) and washed with 5% aqueous NaHCO₃ (150 mL). The water layer was washed with CHCl₃ (2 × 50 mL). The organic extracts were combined, dried, and evaporated. The residue was chromatographed, using a linear EtOAc gradient (20–80%, v/v; 2 L) in hexane to yield 1.54 g (70%) of **16** as foam (*R*_f 0.70 (B)) and 0.18 g (8%) of its isomer **17** (mp 170–171 °C (EtOH); *R*_f 0.55 (B)).

The solution of 0.4 mL (0.88 mmol) of **16** in methanol (20 mL), saturated with ammonia at 0 °C, was kept for 48 h and evaporated. The residue was treated with Et₂O (50 mL): the precipitate was filtered off and crystallized from EtOH to yield 0.16 g (74%) of **23**: mp 197–198 °C (lit.²⁷ mp 198–199 °C); *R*_f 0.49 (C); UV (EtOH) λ_{\max} 263 nm (ϵ 9800). Anal. (C₉H₁₁FN₂O₅) C, H, F, N.

(46) De Clercq, E. *Biochem. Pharmacol.* 1987, 36, 2567.

(47) Cools, M.; De Clercq, E. *Biochem. Pharmacol.* 1989, 38, 1061.

Similarly, starting from 60 mg (0.13 mmol) of 17, 27 mg (87%) of 24 was obtained: mp 165 °C (EtOH); R_f 0.24 (D); UV (EtOH) λ_{\max} 262 nm (ϵ 6200); UV (0.1 N NaOH) λ_{\max} 298 nm.

3'-Fluoro-3'-deoxycytidine (25). A solution of TMS-TfI (5 mL, 0.61 g, 2.7 mmol), 1.1 g (2.73 mmol) of 11, and 0.67 g (2.7 mmol) of bis(trimethylsilyl) derivative of cytosine in anhydrous 1,2-dichloroethane (25 mL) was refluxed for 1 h. After standard treatment, the residue was purified by column chromatography, using a linear MeOH gradient (2–10%, v/v, 2 L) in CHCl_3 . The fractions, containing 18, were collected, evaporated, and crystallized from EtOH to yield 0.92 g (75%) of 18: mp 203–205 °C; R_f 0.25 (B).

Standard debenzoylation of 0.41 g (0.9 mmol) of 18 followed by crystallization from EtOH yielded 0.18 g (81%) of 25: mp 221–223 °C; (lit.²⁷ mp 217–218 °C); R_f 0.16 (C); UV (EtOH) λ_{\max} 270 nm (ϵ 8400). Anal. ($\text{C}_9\text{H}_{12}\text{FN}_3\text{O}_4$) C, H, F, N.

3'-Fluoro-3'-deoxyadenosine (26). A solution of SnCl_4 (0.97 mL, 2.16 g, 8.31 mmol), 1.7 g (4.22 mmol) of 11 and 1.6 g (4.17 mmol) of bis(trimethylsilyl) derivative of N^6 -benzoyladenine in anhydrous 1,2-dichloroethane (30 mL) was stirred for 18 h. After standard workup, the residue was purified with column chromatography, using a linear MeOH gradient (2–10%, v/v; 2 L) in CHCl_3 . The fractions, containing 19, were collected and evaporated to yield 1.99 g (82%) of 19 as a foam: R_f 0.85 (B); UV (EtOH) λ_{\max} 280 nm (ϵ 20000).

Standard debenzoylation of 1.1 g (1.89 mmol) of 19 followed by crystallization from EtOH yielded 0.38 g (75%) of 26: mp 211–212 °C; (lit.²⁷ mp 205 °C); R_f 0.52 (C); UV (EtOH) λ_{\max} 260 nm (ϵ 14600). Anal. ($\text{C}_{10}\text{H}_{12}\text{FN}_5\text{O}_3$) C, H, F, N.

3'-Fluoro-3'-deoxyguanosine (27) and 7-N-Isomer (28). (a) A solution of TMS-TfI (0.91 mL, 1.11 g, 5.0 mmol), 1.74 g (4.32 mmol) of 11, and 2.22 g (5.10 mmol) of silyl derivative of N^2 -acetylguanine in anhydrous 1,2-dichloroethane (50 mL) was refluxed for 1.5 h. After standard workup, the residue was chromatographed, using a linear MeOH gradient (1–6%, v/v; 2 L) in CHCl_3 . The fractions, containing nucleoside 20, were collected and evaporated to yield 1.44 g (62%) of 20 as a foam: R_f 0.42 (B).

Standard deprotection of 1.2 g (2.24 mmol) of 20 followed by crystallization from H_2O yielded 0.45 g (70%) of 27: mp 289–291 °C dec (lit.²⁴ mp 275–277 °C); R_f 0.73 (E); UV (H_2O) λ_{\max} 253 nm (ϵ 14100), 269 nm shoulder (ϵ 10100); UV (0.1 N NaOH) λ_{\max} 257–270 nm (ϵ 8600); UV (0.1 N HCl) λ_{\max} 259 nm (ϵ 15400), 275 nm shoulder (ϵ 9200). Anal. ($\text{C}_{10}\text{H}_{12}\text{FN}_5\text{O}_4$) C, H, F, N.

(b) A solution of TMS-TfI (0.225 mL, 0.8 g, 1.35 mmol), 1.12 g (2.73 mmol) of 11, and 1.78 g (3.24 mmol) of bis(trimethylsilyl) derivative of N^2 -lauroylguanine in anhydrous acetonitrile (30 mL) was refluxed for 3 h. The reaction mixture was evaporated, and the residue was dissolved in CHCl_3 (150 mL). After standard workup the residue was chromatographed, using a linear EtOAc gradient (0–60%, v/v; 3 L) in benzene. The fractions, containing the individual products, were collected and evaporated to yield 0.33 g (18%) of 22 as a foam and 0.64 g (35%) of 21 as a foam.

Standard deprotection of 0.58 g (0.86 mmol) of 21 followed by crystallization from H_2O yielded 0.125 g (51%) of a product that was identical with 27 in all respects.

In a similar way, starting from 0.28 g (0.41 mmol) of 22, 55 mg (46.6%) of 28 was produced: mp 284–286 °C dec (H_2O); R_f 0.71 (E); UV (H_2O) λ_{\max} 239 nm (ϵ 7600), 289 nm (ϵ 8800); UV (0.1 N NaOH) λ_{\max} 285 nm (ϵ 8700); UV (0.1 N HCl) λ_{\max} 255 nm (ϵ 17100), 266 nm shoulder (ϵ 15400).

Methyl 2-O-tosyl-3-fluorodeoxy- β -D-arabinofuranoside (12) was obtained, as described above, starting from 2.46 g (5.99 mmol) of 6b and 2.5 g of 10% Pd/C in 250 mL of EtOH. The residue was crystallized from Et_2O with hexane added until slight turbidity: 1.7 g (88%) of 12 was obtained: mp 60 °C; R_f 0.1 (A). Anal. ($\text{C}_{13}\text{H}_{17}\text{FO}_8\text{S}$) C, H, F, S.

Methyl 5-O-benzoyl-2-O-tosyl-3-fluoro-3-deoxy- β -D-arabinofuranoside (13) was obtained, as described above, starting from 0.8 g (2.49 mmol) of 12 and 0.32 mL (0.38 g, 2.73 mmol) of benzoyl chloride in 20 mL of pyridine to yield 0.86 g (81%) of 13: mp 85–86 °C (EtOH); R_f 0.53 (A). Anal. ($\text{C}_{20}\text{H}_{21}\text{FO}_7\text{S}$) C, H, F, S.

Methyl 2-Azido-5-O-benzoyl-3-fluoro-2,3-dideoxy- β -D-ribofuranoside (15b). A mixture of 5.2 g (80 mmol) of sodium azide and 4.1 g (9.65 mmol) of 13 in 70 mL of anhydrous DMSO was stirred for 1 h at 180 °C. After standard workup, the residue

was benzoylated with a standard procedure and chromatographed, using a linear Et_2O gradient (10–50%, v/v; 2 L) in hexane. The fractions, containing individual products, were collected and evaporated to yield 1.75 g (61%) of azide 15b as a syrup: R_f 0.8 (A); IR (film) 2125 cm^{-1} (N_3) and 0.92 g of the starting material 13.

2'-Azido-3'-fluoro-2',3'-dideoxythymidine (34b) and Its α -Anomer (34a). A mixture of 2.87 mL (5.51 g, 15.81 mmol) of TMS-TfI, 1.56 (5.28 mmol) of 15b, and 2.85 g (10.54 mmol) of bis(trimethylsilyl) derivative of thymine in acetonitrile (60 mL) was refluxed for 3 h. After standard workup, the residue was chromatographed with a linear EtOH gradient (10–90%, v/v; 2 L) in hexane to yield 0.16 g (10%) of mixture of starting azide 15b and its α -anomer 15a, 1.2 g (58%) of mixture of nucleosides 29a and 29b, and 0.25 g of a product with undetermined structure. A mixture of 29a,b (1.0 g, 2.09 mmol) was debenzoylated with a standard procedure and chromatographed with a linear MeOH gradient (1–10%, v/v; 1 L) in CHCl_3 to give 0.5 g (68%) of 34b: mp 147 °C (EtOH); R_f 0.59 (D); UV (EtOH) λ_{\max} 266 nm (ϵ 9900); IR (KBr) 2140 cm^{-1} (N_3) [Anal. ($\text{C}_{10}\text{H}_{12}\text{FN}_5\text{O}_4$) C, H, F, N] and 0.165 g (22%) of its α -anomer 34a: mp 130–131 °C (EtOH); R_f 0.48 (D); UV (EtOH) λ_{\max} 269 nm (ϵ 10000); IR (KBr) 2125 cm^{-1} (N_3). Anal. ($\text{C}_{10}\text{H}_{12}\text{FN}_5\text{O}_4$) C, H, F, N.

2'-Azido-3'-fluoro-2',3'-dideoxycytidine (35b) and Its α -Anomer 35a. A mixture of 0.74 mL (1.64 g, 6.3 mmol) of SnCl_4 , 0.62 g (2.09 mmol) of 15b, and 1.5 g (4.2 mmol) of bis(trimethylsilyl) derivative of N^4 -benzoylcytosine in anhydrous acetonitrile (20 mL) was refluxed for 5 h. After standard workup, the residue was chromatographed with a linear EtOAc gradient (10–19%, v/v; 2 L) in hexane to yield 0.1 g (16%) of the mixture of starting sugar 15b and its α -anomer 15a, 0.4 g (40%) of 30b as a foam: R_f 0.70 (B), and 0.1 g (10%) of its α -anomer 30a as a foam: R_f 0.53 (B).

Standard debenzoylation of 0.3 g (0.62 mmol) of 30b and subsequent chromatography with a linear MeOH gradient (0–25%, v/v; 0.5 L) in CHCl_3 , afforded 0.15 g (89%) of 35b: mp 193–194 °C (EtOH); R_f 0.41 (C); UV (EtOH) λ_{\max} 270 nm (ϵ 8200). Anal. ($\text{C}_9\text{H}_{11}\text{FN}_5\text{O}_3$) C, H, F, N.

Similarly, starting from 70 g (0.15 mmol) of 30a, 36 mg (91%) of the α -anomer 35a was obtained as a syrup: R_f 0.3 (C); UV (EtOH) λ_{\max} 272 nm (ϵ 8600).

2'-Azido-3'-fluoro-2',3'-dideoxyadenosine (36b) and Its α -Anomer (36a). A mixture of SnCl_4 (1.22 mL, 2.72 g, 10.44 mmol), 0.62 g (2.09 mmol) of 15b, and 1.63 g (4.18 mmol) of bis(trimethylsilyl) derivative of N^6 -benzoyladenine in acetonitrile/1,2-dichloroethane mixture (60 mL, 2:1; v/v) was refluxed for 5 h. After standard workup, the residue was chromatographed with a linear EtOAc gradient (10–90%, v/v; 2 L) in hexane to yield 0.08 g (12%) of the mixture of starting azide 15b and its α -anomer 15a, 0.6 g (57%) of 31b as a foam: R_f 0.55 (B).

From 0.5 g (1 mmol) of 31b, following the above procedure, 0.25 g (85%) of 36b was obtained: mp 198–199 °C (EtOH); R_f 0.54 (D) (EtOH) λ_{\max} 258 nm (ϵ 14900). Anal. ($\text{C}_{10}\text{H}_{11}\text{FN}_5\text{O}_2$) C, H, F, N.

In a similar way, starting from 0.2 g (0.4 mmol) of 31a, 0.1 g (85%) of 36a was obtained: mp 94–95 °C (absolute EtOH + Et_2O); R_f 0.49 (D); UV (EtOH) λ_{\max} 258 nm (ϵ 14600). Anal. ($\text{C}_{10}\text{H}_{11}\text{FN}_5\text{O}_2$) C, H, F, N.

9-(2-Azido-3-fluoro-2,3-dideoxy- β -D-ribofuranosyl)-2-N-acetylguanine (37b), 9-(2-Azido-3-fluoro-2,3-dideoxy- α -D-ribofuranosyl)guanine (37a), 7-(2-Azido-3-fluoro-2,3-dideoxy- β -D-ribofuranosyl)guanine (38b), and Its α -Anomer (38a). A mixture of TMS-TfI (0.94 mL, 1.16 g, 5.1 mmol), 0.31 g (1.05 mmol) of 15b, and 0.7 g (2.09 mmol) of silyl derivative of N^2 -acetylguanine in anhydrous acetonitrile (40 mL) was refluxed for 3 h. After standard workup, the residue was applied to a chromatographic column, which was eluted with a linear MeOH gradient (0–5%, v/v; 1 L) in CHCl_3 . The fraction containing the mixture of 32b, 33a, and 33b [0.315 g (70%)] and the fraction containing 0.1 g (22%) of individual 32a were isolated. Each of the fractions was treated with MeOH/ NH_3 (for 3 days), evaporated, and chromatographed with a linear MeOH gradient (0–25%, v/v; 1 L) in CHCl_3 as eluent. Starting from 0.315 g of a mixture of nucleosides 32b, 33a, and 33b, 0.16 g of the mixture (R_f 0.42 (C)) of 37b and 38b was obtained in the ratio of 1:1.5 (according to ^1H NMR) and 55 mg of 38a. The additional chromatography

of **37b** and **38b** with a linear MeOH gradient (0–25%, v/v; 0.6 L) in CHCl₃ gave the individual nucleosides **37b** and **38b**.

38a: *R_f* 0.29 (C); UV (20% EtOH in H₂O) 241 nm shoulder (ε 7020), λ_{max} 280 nm (ε 7400); UV (0.1 N NaOH) 237 nm shoulder (ε 8600), λ_{max} 274 nm (ε 6200); UV (0.1 N HCl) λ_{max} 258 nm (ε 9200), λ_{max} 269 nm (ε 8600).

37b: UV (20% EtOH in H₂O) λ_{max} 254 nm (ε 15 200), 268 nm shoulder (ε 11 500); UV (0.1 N NaOH) broad max 258–270 nm (ε 13 700); UV (0.1 N HCl) λ_{max} 258 nm (ε 15 000), 274 nm shoulder (ε 10 960).

38b: UV (20% EtOH in H₂O) λ_{max} 287 nm (ε 7400), 238 nm shoulder (ε 6150); UV (0.1 N NaOH) λ_{max} 283 nm (ε 6900) 236 nm shoulder (ε 9100); UV (0.1 N HCl) λ_{max} 259 nm (ε 8600) and 267 nm (ε 8070).

Deprotection of 0.1 g of **32a** afforded 40 mg of **37a**: *R_f* 0.2 (C); UV (20% EtOH in H₂O) λ_{max} 254 nm (ε 14 500), 269 nm shoulder (ε 12 000); UV (0.1 N NaOH), broad max 260–270 nm (ε 9600); UV (0.1 N HCl) λ_{max} 259 nm (ε 12 400), 274 nm shoulder (ε 8900).

Inhibition of L1210, Raji, Molt/4F, and MT-4 Cell Proliferation. All assays were performed in flat-bottomed microtests III Plates (96 wells) as previously described.⁴⁸ Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5 × 10⁴ L1210 or Molt/4F cells/well (200 μL), 6.25 × 10⁴ MT-4 cells/well or 7.5 × 10⁴ Raji cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 h (L1210 cells), 72 h (Molt/4F and Raji cells), or 120 h (MT-4 cells) at 37 °C in a humidified CO₂-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (L1210, Raji, Molt/4F) or a blood cell counting chamber by trypan blue dye exclusion (MT-4). The IC₅₀ was defined as the concentration of compound that reduced the number of viable cells by 50%.

Antiviral Assays. The antiviral assays, other than HIV-1, were based on an inhibition of virus-induced cytopathogenicity in either HeLa cell, Vero cell, or primary rabbit kidney cell cultures, following previously established procedures.⁴⁹ Briefly, confluent cell cultures in microtiter trays were inoculated with 100 CCID₅₀ of virus, 1 CCID₅₀ being the virus dose required to infect 50% of the cell cultures. After a 1-h virus adsorption period, residual virus was removed, and the cell cultures were incubated

in the presence of varying concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Inhibition of HIV-1-Induced Cytopathogenicity in MT-4 Cells. Human 5 × 10⁵ MT-4 cells were infected with 100 CCID₅₀ HIV-1 (strain HLTV-III_B)/mL and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds.⁵⁰ After 5 days of incubation at 37 °C, the number of viable cells was determined in a blood cell counting chamber by trypan blue dye exclusion.

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Design, Synthesis, and Pharmacological Evaluation of Ultrashort- to Long-Acting Opioid Analgetics

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In an effort to discover a potent ultrashort-acting μ opioid analgetic that is capable of metabolizing to an inactive species independent of hepatic function, several classes of 4-anilidopiperidine analgetics were synthesized and evaluated. One series of compounds displayed potent μ opioid agonist activity with a high degree of analgesic efficacy and an ultrashort to long duration of action. These analgetics, 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic acid alkyl esters, were evaluated in vitro in the guinea pig ileum for μ opioid activity, in vivo in the rat tail withdrawal assay for analgesic efficacy and duration of action, and in vitro in human whole blood for their ability to be metabolized in blood. Compounds in this series were all shown to be potent μ agonists in vitro, but depending upon the alkyl ester substitution the potency and duration of action in vivo varied substantially. The discrepancies between the in vitro and in vivo activities and variations in duration of action are probably due to different rates of ester hydrolysis by blood esterase(s). The SAR with respect to analgesic activity and duration of action as a function of the various esters synthesized is discussed. It was also demonstrated that the duration of action for the ultrashort-acting analgetic, **8**, does not change upon prolonged infusion or administration of multiple bolus injections.

Fentanyl, a potent short-acting analgetic, is used clinically during surgical procedures as an adjunct to gaseous

anesthesia.¹ As a result of fentanyl's clinical success and the desire to more clearly define the structural require-