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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Received September 10, 1990

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′-fluoroadenosine. 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]

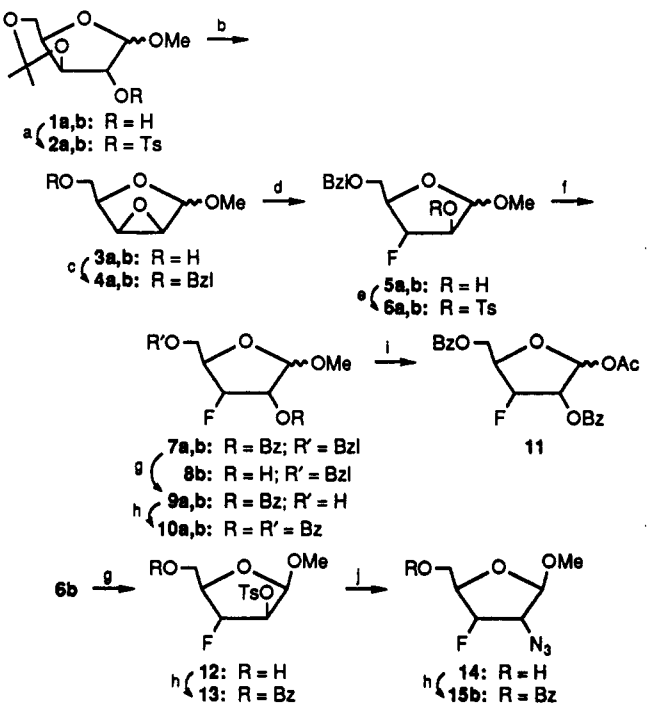
The synthesis and investigation of the biological activity of 3′-deoxy-3′-fluoro analogues of ribonucleosides have received relatively little attention.[18] Thus, Langen et al. briefly described two approaches to the synthesis of 3′-deoxy-3′-fluorouridine and did not find an inhibitory effect of the 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 data[26] provided for 3′-deoxy-3′-fluorouridine it appears that the ^{1}H NMR results and, in particular $J_{2',F}$, differ significantly from those reported by Langen et al.[25] Also, Wiebe and his co-workers[26] 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 using a modification[28] of the method described by Baker et al.[29] Tosylation of **1a** and **1b** in pyridine gave the

Scheme I[a]



[a] (a) TsCl, pyr, room temperature, 10 h (**2a**, 87.5%; **2b**, 86%). (b) (1) $AcOH/H_2O$, 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; BzlBr, 0 °C, 1 h, room temperature, 1 h (**4a**, 89%; **4b**, 80%). (d) KHF_2/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) H_2, 10% Pd/C, EtOH, room temperature, 72 h (**9a**, 97%; **9b**, 98%). (h) BzCl, pyr, room temperature, 12 h (**10a**, 94%; **10b**, 81%). (i) $AcOH/Ac_2O/H_2SO_4$, room temperature, 20 h (89–94%). (j) NaN_3, DMSO, 180 °C, 1 h (**15b**, 61%).

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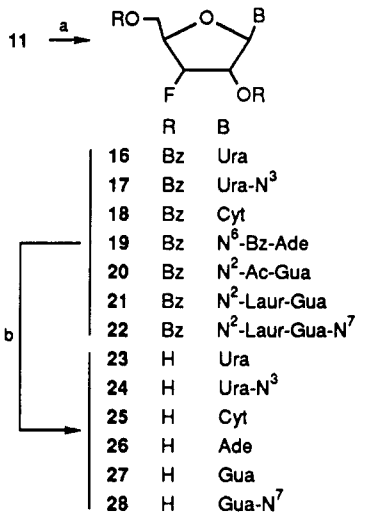
crystalline tosylates **2a**[30] and **2b**, respectively; these compounds were subsequently converted to the corresponding crystalline epoxides **3a** and **3b** in a two-step procedure.[29] Epoxides **3a** and **3b** were benzylated with benzyl bromide in dioxane in the presence of NaH[31] to give upon workup compounds **4a** and **4b** as oils in high yields. Treatment of the α-anomer **4a** with KHF_2 and NaF in 1,2-ethylene glycol,[32,33] followed by silica gel column chromatography,

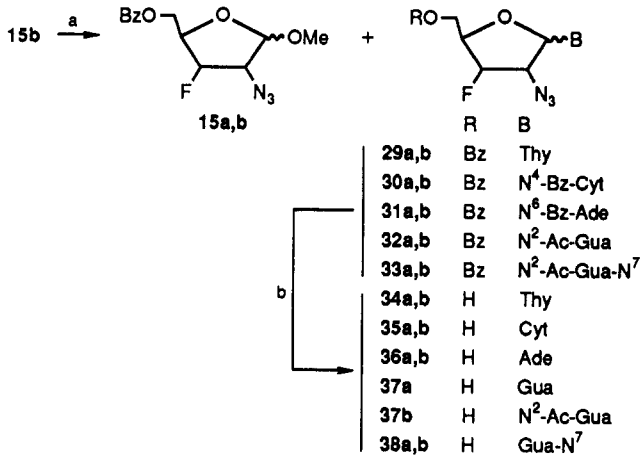
Scheme II[a]



	R	B
16	Bz	Ura
17	Bz	Ura-N^3
18	Bz	Cyt
19	Bz	N^6-Bz-Ade
20	Bz	N^2-Ac-Gua
21	Bz	N^2-Laur-Gua
22	Bz	N^2-Laur-Gua-N^7
23	H	Ura
24	H	Ura-N^3
25	H	Cyt
26	H	Ade
27	H	Gua
28	H	Gua-N^7

[a] (a) Persilylated base, (1) $SnCl_4$, 1,2-dichloroethane, room temperature, 18–24 h; (2) TMS-Tfl, 1,2-dichloroethane or CH_3CN, reflux, 1–3 h. (b) NH_3/MeOH, room temperature, 48 h.

Scheme III[a]



	R	B
29a,b	Bz	Thy
30a,b	Bz	N^4-Bz-Cyt
31a,b	Bz	N^6-Bz-Ade
32a,b	Bz	N^2-Ac-Gua
33a,b	Bz	N^2-Ac-Gua-N^7
34a,b	H	Thy
35a,b	H	Cyt
36a,b	H	Ade
37a	H	Gua
37b	H	N^2-Ac-Gua
38a,b	H	Gua-N^7

[a] (a) Persilylated base, (1) $SnCl_4$, CH_3CN, reflux, 5 h; (2) TMS-Tfl, CH_3CN, reflux, 3 h. (b) NH_3/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%. Acetolysis[34] 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 benzoylation in 85% yield. Nucleophilic displacement of the tosyloxy group in **13** by treatment with NaN_3 in DMSO led to partial debenzoylation 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 nucleosides[35] (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-Tfl) (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-Tfl 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-Tfl (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-Tfl (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-Tfl, 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-Tfl (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. ^{1}H NMR Spectral Data of Sugars

no.	chemical shifts H-1	H-2	H-3	H-4	H-5	H-5′	others
5a	4.87 s	4.04 d	4.77 dd	4.33 ddt	3.66 dd	3.61 dd	7.35–7.26 (m, *Ph*CH$_2$), 4.65 (br s, 2-OH), 4.59 (dd, PhC*H*$_2$), 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, *Ph*CH$_2$), 4.49 (dd, PhC*H*$_2$), 3.43 (s, OCH$_3$)
6a	496 s	4.83 dm	4.86 ddd	4.27 ddt	3.63 dd	3.59 dd	7.80–7.28 (m, *Ph*CH$_2$ + CH$_3$*Ph*SO$_2$), 4.57 (dd, *Ph*CH$_2$), 3.30 (s, OCH$_3$), 2.45 (s, C*H*$_3$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, *Ph*CH$_2$ + CH$_3$*Ph*SO$_2$), 4.55 (s, PhC*H*$_2$), 3.25 (s, OCH$_3$), 2.45 (s, C*H*$_3$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, *Ph*CH$_2$ + Bz), 4.61 (dd, PhC*H*$_2$), 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, *Ph*CH$_2$ + Bz), 4.63 (dd, PhC*H*$_2$), 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, *Ph*CH$_2$), 4.59 (dd, PhC*H*$_2$), 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$_3$*Ph*SO$_2$), 3.40 (s, OCH$_3$), 2.47 (s, C*H*$_3$PhSO$_2$)
13	~4.98	4.93 ddd	5.25 ddd	4.35 ddt	4.42 m		8.06–7.34 (m, Bz + CH$_3$*Ph*SO$_2$), 3.30 (s, OCH$_3$), 2.45 (s, C*H*$_3$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	m	4.41	8.10–7.43 (m, Bz), 3.35 (s, OCH$_3$)

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 Kowollik[25] 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 ^{1}H NMR spectrum: (a) the supposed pyranoside is in conformational equilibrium ($_4C^1 \rightleftarrows {}^4C_1$) 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 ^{1}H 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 ^{1}H NMR data of 3′-fluoro-3′-deoxyadenosine differ from the ^{1}H NMR data of the compound[22] 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 ^{1}H NMR, UV, and CD spectra of the corresponding compounds with those of the pairs of related N^1- and N^3-, and N^9- and N^7-isomers,[15,37,44,45] respectively. The most informative feature of the ^{1}H 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. ^{1}H NMR Spectral Data of Nucleosides

	chemical shifts								
no.	H-8 (H-6)	H-2 (H-5)	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	others
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	6.25 (br s, NH_2), 5.86 (d, 2'-OH), 5.20 (t, 5'-OH)
34a	7.27 d		6.29 d	4.78 ddd	5.36 ddd	4.69 ddt	3.52 m		11.40 (s, NH), 5.18 (t, 5'-OH), 1.80 (d, 5-CH_3)
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.	1',2'	2',3'	3',4'	4',5'	4',5''	2',F	3',F	4',F	others
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

	50% inhibitory concentration (IC_{50}),[a] μg/mL			
no.	murine leukemia L1210	human B-lymphoblast Raji	human T-lymphoblast Molt/4F	human T-lymphoblast 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

	minimum cytotoxic concentration[b] (MCC), μg/mL			minimum inhibitory concentration (MIC),[a] μg/mL											
				primary rabbit kidney (PRK) cells		HeLa cells			African green monkey kidney (Vero B) cells						
no.	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	reo-virus-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 × $CCID_{50}$, 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 (±)DNA viruses such as pox (vaccinia), (+)RNA viruses such as picorna (polio, Coxsackie), toga (Sindbis, Semliki forest), and (±)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-c^{3}Ado), 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 ^{1}H 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 UV_{254} (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), $CHCl_3$–MeOH, 19:1 (B), $CHCl_3$–MeOH, 4:1 (C), $CHCl_3$–MeOH, 9:1 (D), *i*-PrOH–H_2O–20% NH_4OH, 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 $SnCl_4$ 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 $CHCl_3$ (300 mL) and washed with saturated $NaHCO_3$ 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. ($C_{20}H_{23}FO_6S$) 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 $CHCl_3$ and washed with H_2O (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 H_2 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 Et_2O to give 1.24 g (97%) of **9a**: mp 104–105 °C, R_f 0.12 (A). Anal. ($C_{13}H_{15}FO_5$) 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 H_2O, containing 10 g of ice, and extracted by $CHCl_3$ (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 H_2SO_4 (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 Ac_2O, the mixture was stirred for 20 h and poured into 5% aqueous solution of $NaHCO_3$ (100 mL), containing 50 g of ice, and after the ice melted, it was extracted with $CHCl_3$ (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 $SnCl_4$ (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 $CHCl_3$ (60 mL) and washed with 5% aqueous $NaHCO_3$ (150 mL). The water layer was washed with $CHCl_3$ (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 Et_2O (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. ($C_9H_{11}FN_2O_5$) C, H, F, N.

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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-Tfl (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. ($C_9H_{12}FN_3O_4$) 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 20 000).

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 14 600). Anal. ($C_{10}H_{12}FN_5O_3$) C, H, F, N.

3'-Fluoro-3'-deoxyguanosine (27) and 7-N-Isomer (28). (a) A solution of TMS-Tfl (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 14 100), 269 nm shoulder (ε 10 100); UV (0.1 N NaOH) λ_{max} 257–270 nm (ε 8600); UV (0.1 N HCl) λ_{max} 259 nm (ε 15 400), 275 shoulder (ε 9200). Anal. ($C_{10}H_{12}FN_5O_4$) C, H, F, N.

(b) A solution of TMS-Tfl (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 17 100), 266 nm shoulder (ε 15 400).

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. ($C_{13}H_{17}FO_6S$) 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. ($C_{20}H_{21}FO_7S$) 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-Tfl, 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. ($C_{10}H_{12}FN_5O_4$) 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 10 000); IR (KBr) 2125 cm^{-1} (N_3). Anal. ($C_{10}H_{12}FN_5O_4$) 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. ($C_9H_{11}FN_6O_3$) 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 14 900). Anal. ($C_{10}H_{11}FN_8O_2$) 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 14 600). Anal. ($C_{10}H_{11}FN_8O_2$) 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-Tfl (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 $CHCl_3$ gave the individual nucleosides **37b** and **38b**.

38a: R_f 0.29 (C); UV (20% EtOH in H_2O) 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 H_2O) 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 H_2O) 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 H_2O) 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 5×10^4 L1210 or Molt/4F cells/well (200 μL), 6.25×10^4 MT-4 cells/well or 7.5×10^4 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 CO_2-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 IC_{50} 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 $CCID_{50}$ of virus, 1 $CCID_{50}$ 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 5×10^5 MT-4 cells were infected with 100 $CCID_{50}$ HIV-1 (strain $HLTV\text{-}III_B$)/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.

Acknowledgment. This work was supported by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Project no. 3.0040.83 and 3.0040.87), the Belgian Geconcerteerde Onderzoeksacties (Conventie no. 85/90-79), and the AIDS Basic Research Programme of the European Communities). We would like to thank Lizette van Berckelaer and Ann Absillis for excellent technical assistance. I.A.M. is deeply grateful to the Alexander von Humboldt-Stiftung (Bonn-Bad-Godesberg, Germany) for partial financial support.

Registry No. **5a**, 14980-09-7; **5b**, 28867-47-2; **6a**, 126716-23-2; **6b**, 126716-24-3; **7a**, 124939-87-3; **7b**, 124939-88-4; **8b**, 133776-28-0; **9a**, 126737-61-9; **9b**, 126716-26-5; **10a**, 126716-27-6; **10b**, 112695-36-0; **11a**, 133814-60-5; **11b**, 122654-34-6; **12**, 133776-08-6; **13**, 133776-09-7; **14**, 133776-10-0; **15a**, 133776-11-1; **15b**, 133776-29-1; **16**, 112668-58-3; **17**, 133776-12-2; **18**, 133776-13-3; **19**, 112668-60-7; **20**, 133776-14-4; **21**, 133776-15-5; **22**, 133776-16-6; **23**, 57994-13-5; **24**, 133776-17-7; **25**, 123402-20-0; **26**, 75059-22-2; **27**, 123402-21-1; **28**, 133794-39-5; **29a**, 133776-18-8; **29b**, 133776-30-4; **30a**, 133776-19-9; **30b**, 133776-31-5; **31a**, 133776-20-2; **31b**, 133776-32-6; **32a**, 133776-21-3; **32b**, 133776-33-7; **33a**, 133776-22-4; **33b**, 133776-34-8; **34a**, 133776-23-5; **34b**, 132776-27-3; **35a**, 133776-24-6; **35b**, 133776-35-9; **36a**, 133776-25-7; **36b**, 123334-78-1; **37a**, 133776-26-8; **37b**, 133776-36-0; **38a**, 133776-27-9; **38b**, 133776-37-1; bis(trimethylsilyl)uracil, 10457-14-4; bis(trimethylsilyl)cytosine, 18037-10-0; bis(trimethylsilyl)-N^6-benzoyladenine, 18055-46-4; bis(trimethylsilyl)-N^2-acetylguanine, 133776-38-2; bis(trimethylsilyl)-N^2-lauroylguanine, 133776-39-3; bis(trimethylsilyl)-N^4-benzoylcytosine, 133776-40-6.

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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-

0022-2623/91/1834-2202$02.50/0