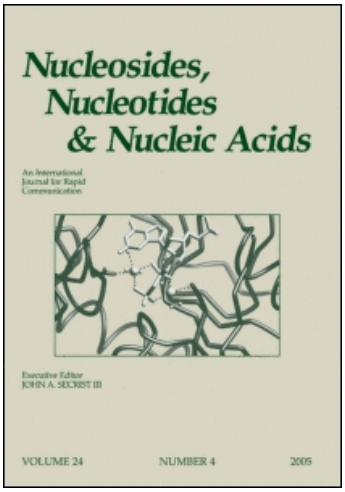


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biological Properties of *2*-Amino-3-fluoro-2,3-Dideoxy-D-Pentofuranosides of Natural Heterocyclic Bases

Igor A. Mikhailopulo[a]; Grigorii G. Sivets[a]; Tamara I. Pricota[a]; Nicolai E. Poopeiko[a]; Jan Balzarini[b]; Erik De Clercq[b]
[a] Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk, USSR [b] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

To cite this Article Mikhailopulo, Igor A. , Sivets, Grigorii G. , Pricota, Tamara I. , Poopeiko, Nicolai E. , Balzarini, Jan and De Clercq, Erik(1991) 'Synthesis and Biological Properties of *2*-Amino-3-fluoro-2,3-Dideoxy-D-Pentofuranosides of Natural Heterocyclic Bases', Nucleosides, Nucleotides and Nucleic Acids, 10: 8, 1743 — 1757
To link to this Article: DOI: 10.1080/15257779108043059
URL: http://dx.doi.org/10.1080/15257779108043059

NUCLEOSIDES & NUCLEOTIDES, 10(8), 1743-1757 (1991)

SYNTHESIS AND BIOLOGICAL PROPERTIES OF 2-AMINO-3-FLUORO-2,3-DIDEOXY-D-PENTOFURANOSIDES OF NATURAL HETEROCYCLIC BASES

Igor A. Mikhailopulo[1], Grigorii G. Sivets[1], Tamara I. Pricota[1], Nicolai E. Poopeiko[1], Jan Balzarini[2] and Erik De Clercq[2]

[1]*Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Zhodinskaya 5/2, 220600 Minsk, USSR and* [2]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Abstract. The oxidation of methyl 5-O-benzyl-3-deoxy-3-fluoro-α-D-arabinofuranoside (**1**) with $DMSO/Ac_2O$ afforded a ~ 2:1 mixture of 2-keto derivatives with *erythro* and *threo* configuration resulting from isomerization at C3. Successive treatment of the above mixture with $MeONH_2$, $LiAlH_4$, and S-ethyl trifluoroacetate followed by silica gel chromatography afforded methyl 5-O-benzyl-2,3-dideoxy-3-fluoro-2-(trifluoroacetamido)-α-D-ribofuranoside (**6b**) and its *lyxo* isomer **7b** in a total yield of 25% and 5%, respectively. The *arabino* analogue **25** was prepared from **6b**. Compounds **6b**, **7b** and **25** were converted to the corresponding 5-O-benzoyl derivatives **8a**, **9** and **26**. A series of 2'-amino-2',3'-dideoxy-3'-fluoro-β-D-ribo- and -α-D-lyxofuranosides of natural heterocyclic bases have been synthesized starting from **8a** and **9**. None of the test compounds had any antiviral activity. 3'-Fluoro-2'-amino-2',3'-dideoxycytidine (**16**) was the only compound showing inhibition of murine L1210 and human Molt/4F cell proliferation (50% effective concentration: 39-42 μg/ml).

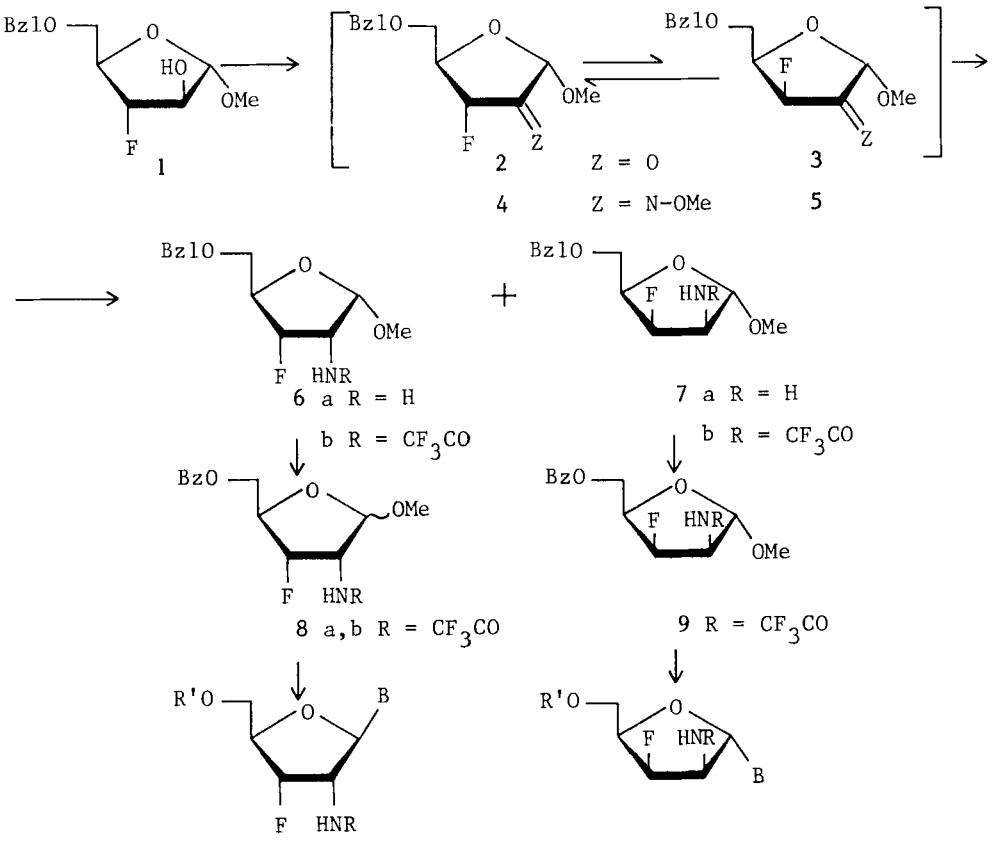
Among the known 2'-amino-2'-deoxy-β-D-ribofuranosides of natural heterocyclic bases (for the main synthetic methods see survey by Moffatt[1]), 2'-amino-2'-deoxyadenosine and -guanosine (both nucleoside antibiotics[2]) exhibit antiviral and cytostatic activities.[3-5] The biological activity of the latter compounds may depend, to some extent, on the basicity of the 2'-amino group. Substitution of a hydrogen atom for the hydroxyl group at C-3' should increase the basicity of the 2'-amino group. The synthesis of such analogues has recently been reported.[5,6] On the contrary, replacement of the 3'-hydroxyl group by the electronegative fluorine atom should give rise to the opposite effect, and thus reduce the basicity of the 2'-amino group. Both types of C-3' modification increase the stability toward phosphorylases.[7]

In continuation of studies[8-19] on the synthesis and investigation of the biological properties of fluorodeoxy analogues of natural nucleosides, we have developed a versatile method for the preparation of various 2-substituted 3-fluoro-3-deoxypentofuranosides.[11,12] The present report deals with the preparation and biological evaluation of some 2-amino-2,3-dideoxy-3-fluoro-D-pentofuranosides of natural heterocyclic bases. A preliminary account of part of this work has appeared.[12]

CHEMISTRY

As the starting compound we chose methyl 5-O-benzyl-3-deoxy-3-fluoro-α-D-arabinofuranoside (**1**)[10] that has also been used in an alternative synthesis of aminofluorodideoxynucleosides[12] (Scheme 1). Oxidation of **1** with $DMSO/Ac_2O$[13] gave the mixture of isomeric ketones **2** and **3** as hydrates in the ratio of ~ 2:1 according to ^1H NMR data. The treatment of the above mixture of ketones with $MeONH_2$ afforded the corresponding oximes **4** and **5**, which, without additional purification, were converted by the action of $LiAlH_4$ in THF[14] to the amines **6a** and **7a**, respectively. The reaction of the mixture of **6a** or **7a** with S-ethyl trifluoroacetate[15], followed by silica gel column chromatography, afforded the individual methyl 5-O-benzyl-2,3-dideoxy-3-fluoro-2-(trifluoroacetamido)-α-D-ribofuranoside (**6b**) and its *lyxo* isomer **7b** in a total yield of 25% and 5%, respectively. Debenzylation of **6b** and **7b** by 20% $Pd(OH_2)/C$ in ethanol in the presence of cyclohexene[16] followed by benzoylation resulted in high yields of benzoates **8a** and **9**, respectively.

The condensation of α-methyl glycoside **8a** with persilylated N^6-benzoyladenine in the presence of $SnCl_4$ (five-fold molar excess relative to the glycosylating agent **8a**; cf. the data[10,12,17]) in refluxing acetonitrile followed by standard work-up and silica gel column chromatography afforded N^9-β-nucleoside **10** in a yield of 53%. When silylated N^6-benzoyladenine was replaced by silylated N^4-benzoylcytosine or thymine in the above condensation, the N^1-β-nucleosides **11** and **12** were obtained in 82% and 63% yield, respectively. Surprisingly, the condensation of persilylated cytosine with benzoate **8a** in acetonitrile in the presence of trimethylsilyl triflate (TMS-Tfl) gave a complex mixture of products from which we failed to isolate any nucleoside compounds.[10] The only individual product, isolated in 36% yield by silica gel column chromatography, proved to be β-methyl glycoside **8b**. Further, the condensation of sily-



	R'	B	R
10	Bz	N^6-Bz-Ade	CF_3CO
11	Bz	N^4-Bz-Cyt	CF_3CO
12	Bz	Thy	CF_3CO
13	Bz	N^2-Ac-Gua	CF_3CO
14	Bz	N^2-Ac-Gua-N^7	CF_3CO
15	H	Ade	H
16	H	Cyt	H
17	H	Thy	H
18	H	Gua	H
19	H	Gua-N^7	H

	R'	B	R
20	Bz	N^6-Bz-Ade	CF_3CO
21	H	Ade	H

Scheme 1

lated N^2-acetylguanine with methyl glycoside **8a** in the presence of TMS-Tfl in acetonitrile gave N^9- and N^7-β-nucleosides **13** and **14** in 40% and 20% yield, respectively (cf.[10]). In contrast to the transglycosylation reaction of N^6-octanoyladenine with 2'-deoxy-2'-trifluoroacetamidouridine, which affords a mixture of α- and β-guanine nucleosides[18], only the formation of β-anomers was observed in all the cases studied. We have also found that the use of β-methyl glycoside **8b** in the above condensation with persilylated N^6-benzoyladenine afforded nucleoside **10** in 48% yield. Treatment of 10-14 with methanolic ammonia gave the respective free nucleosides **15-19**.

With methyl glycoside **9** as a glycosylating agent the synthesis similar to that described above for ribonucleoside **10** gave *lyxo* nucleoside **20** (40%); deprotection yielded 9-(2-amino-2,3-dideoxy-3-fluoro-α-D-lyxofuranosyl)adenine (**21**).

Arabinosides **25** and **26** were synthesized to confirm the structures of compounds **6-9** (Scheme 2). Methyl riboside **22**[10] was debenzoylated and then tosylated by standard procedures to give tosylate **23** in a total yield of 84%. The reaction of **23** with NaN_3 in DMSO at 190°C gave azide **24**, which was converted into arabinoside **25** by successive treatment with triphenylphosphine in pyridine[19], aqueous ammonia and S-ethyl trifluoroacetate. The total yield for the conversion of **23** to **25** was 46%. Benzoate **26** was synthesized as described above for **8** and **9**.

^1H NMR spectroscopy of **8a,b**, **9** and **26** did not provide much information because of overlapping of the resonances H2, H4 and H5. On the contrary, in the ^1H NMR spectra of **6a,b**, **7b** and **25** all the resonances were assigned using the double resonance technique (Tables 1 and 2). It should be emphasized that isomerization at C-3 occurs during oxidation of **1**. In the ^1H NMR spectrum of the products of the oxidation of **1**, the resonances corresponding to two isomers **2** and **3** are present. The comparison of ^1H NMR spectra of riboside **6b** and lyxoside **7b** with that of arabinoside **25** can be useful to confirm the structures of the former. In the case of 1,2-*trans* relationship of H1 and H2 protons (compounds **7b** and **25**), the anomeric proton resonances in the ^1H NMR spectra were singlets ($J_{1,2}$ < 1.0 Hz), whereas a doublet ($J_{1,2}$ = 5.1 Hz) was noted for the H1 signal of **6b** with 1,2-*cis* location of the same protons. A similar trend was observed for the other vicinal proton coupling constants of the furanose rings. It should be also pointed out that the H1 resonances in the ^1H NMR spectra of **7b** and **25** appeared upfield *vs.* the same signal of **6b**.

Table 1. ^{1}H NMR Spectral Data of Sugars and Nucleosides. Chemical Shifts*.

Compd :	H-1:	H-2:	H-3:	H-4:	H-5:	H-5':	H-8: (H-6)	H-2: (H-5)	Others
	4.66		4.50	4.40		3.72			7.32-7.38 (m, Ph); 4.63 (d, Ph$\underline{CH_2}$);
	s		dd	dm		m			4.57 (d, Ph$\underline{CH_2}$); 3.48 (s, OCH_3)
2/3	4.90		5.10	4.42	3.92	3.80			7.32-7.38 (m, Ph); 4.67 (d, Ph$\underline{CH_2}$);
	s		dd	dm	dd	dd			4.61 (d, Ph$\underline{CH_2}$); 3.51 (s, OCH_3)
6b	5.06	4.60	5.00	4.46	3.63	3.58			7.26-7.38 (m, Ph); 6.78 (d, N$\underline{H}$);
	d	ddd	dq	dm	dd	dd			4.75 (d,Ph$\underline{CH_2}$); 4.63 (d, Ph$\underline{CH_2}$);
									3.45 (s, OCH_3)
7b	4.85	4.61	5.43	4.38	3.71	3.61			7.31-7.38 (m, Ph); 7.95 (d, N$\underline{H}$);
									4.66 (d, Ph$\underline{CH_2}$); 4.59 (d, Ph$\underline{CH_2}$);
	br.s	ddd	dt	dm	dm	dm			3.37 (s,OCH_3)
25	4.91	4.63	4.92	4.48	3.72	3.67			7.27-7.41 (m, Ph); 7.76 (d, N$\underline{H}$);
									4.66 (d, Ph$\underline{CH_2}$); 4.53 (d, Ph$\underline{CH_2}$);
	s	dd	bd	dm	dd	dd			3.41 (s, OCH_3)
15	5.81	4.14	5.05	4.33	3.70		8.31	8.16	7.38 (br.s, $6NH_2$); 1.86 (br.s,
	d	ddd	dd	dt	d				$2'NH_2$); 5.80 (t, 5'-OH)
16	5.83	3.49	4.93	4.19	3.65	3.57	7.76	5.88	
	d	ddd	dd	dt	dd	dd	d	d	
17†	5.94	3.61	4.98	4.28	3.77		7.76		1.89 (d, CH_3)
	d	ddd	dd	dt	d		d		
18	5.57	4.00	4.96	4.20	3.63		7.92		6.30 (s, $6NH_2$); 1.83 (br.s, $2'NH_2$)
	d	ddd	dd	dt	br.s		s		5.40 (t, 5'-OH)
19	5.83	3.97	4.96	4.19	3.65	3.57	8.26		6.27 (s, $6NH_2$); 1.77 (br.s, $2'NH_2$)
	d	ddd	dd	dt	dd	dd	s		5.40 (t, 5'-OH)
21	5.82	4.45	5.08	4.63	3.68	3.55	8.32	8.15	7.15 (br.s, $6NH_2$); 1.75 (br.s,
	d	ddd	ddd	ddt	dd	dd	s	s	$2'NH_2$); 4.68 (t, 5'-OH)

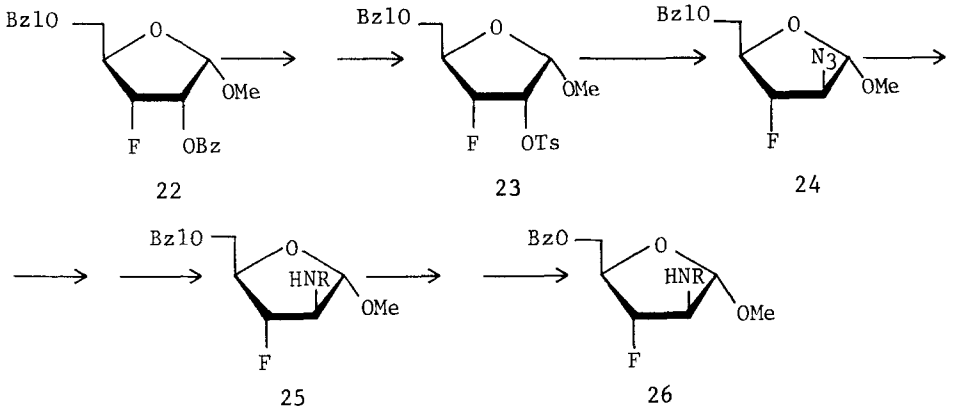
*The spectra of sugars and nucleosides were taken in $CDCl_3$ and DMSO-d_6 (without and with addition of D_2O)
†In CD_3OH.

Table 2. Coupling Constants.

Compd:	1',2':	2',3':	3',4':	4',5':	4',5":	2',F:	3',F:	4',F:	Others
2			1.8				54.0	26.4	
3			8.4	2.4	3.6		52.8	18.0	11.4 (5',5")
6 b	5.1	5.4	1.2	3.3	3.6	25.2	56.4	26.0	10.2 (5'5"); 8.4 (NH,2')
7 b	< 1.0	6.0	6.0	3.6	2.1	14.4	53.4	15.6	1.2 (5',F); 2.1 (5",F); 10.2 (5',5")
25	< 1.0	< 1.0	< 1.0	1.2	1.8	16.2	52.2	28.0	10.2 (5',5"); 9.3 (NH,2')
15	9.0	4.2	< 1.0	3.3	3.3	28.8	54.0	27.6	
16	9.0	4.2	< 1.0	3.0	3.6	28.2	54.0	27.6	7.5 (5,6); 12.0 (5',5")
17	9.0	4.2	< 1.0	2.4	2.4	27.6	54.0	28.2	2.4 (6,CH_3-5)
18	9.0	4.2	< 1.0	4.2	4.2	28.8	54.0	27.6	5.4 (5',OH-5'; 5",OH-5")
19	9.0	4.2	< 1.0	3.0	3.6	28.8	54.0	27.6	12 (5',5"); 5.4 (5', OH-5', 5", OH-5')
21	7.8	3.4	1.8	6.3	6.3	28.8	54.0	30.6	1.2 (5",F); 9.6 (5',5")

Table 3. U.V. and C.D. Spectral Data of 2'-amino-3'-fluoro-2',3'-dideoxynucleosides

Compd	U.V. H_2O		C.D. H_2O		
	λ_{max} (nm):	$\varepsilon .10^{-3}$:	λ (nm):	$[\theta .10^{-3}]$:	λ(nm), $[\theta = 0]$
15	261	15.5	205	- 4.5	209
			230	+ 2.7	245
			267	- 3.6	288
16	236.5	8.5	220	- 15.8	243
	270.5	9.0	270	+ 7.9	300
17	265	8.1	200	+ 14.2	214
			230	- 4.0	255
			275	+ 4.0	310
18 pH 1	256	12.7			
	275.8 (sh)	9.0			
pH 7	254	11.3	203	- 23.4	236
	270 (sh)	8.1	215	+ 10.8	262
			248	- 2.2	315
pH 14	258-270	10.8			
19 pH 1	250	5.6			
	270	5.1			
pH 7	216	17.4	200	+ 10.4	246
	239	5.4	218	- 14.7	269
	288	5.9	293	+ 1.8	320
pH 14	215	17.1			
	236 (sh)	6.1			
	285	4.9			
21	261	13.4	202	- 6.9	233
			260	+ 4.9	304



Scheme 2

The structure of nucleosides **15-19** and **21** was proved by ^{1}H NMR (Table 1 and 2) and CD (Table 3) spectroscopy. Moreover, 2'-azido-2',3'-dideoxy-3'-fluoro-β-D-ribofuranosides of adenine, cytosine and thymine[10] were converted[12] to the appropriate 2'-amino derivatives. The nucleosides obtained by this way were found to be identical with the compounds **15**, **16** and **17**, respectively.

BIOLOGICAL PROPERTIES

The test compounds **15-18** were evaluated for their inhibitory effects on the replication of HSV-1, HSV-2, VV, VSV, polio virus-1, Coxsackie virus B4, Sindbis virus, parainfluenza virus-3, reovirus, Semliki forest virus and HIV-1, as well as for their inhibitory effects on L1210, Molt/4F and MT-4 tumor cell proliferation. None of the compounds proved inhibitory to virus replication or MT-4 cell proliferation at concentrations up to 100-400 μg/ml. However, 3'-fluoro-2'-amino-2',3'-dideoxycytidine (**16**) was inhibitory to L1210 and Molt/4F cell growth at a 50% effective concentration (EC_{50}) of 39 and 42 μg/ml, respectively. It would now be imperative to investigate the cytostatic activity and intracellular metabolism of compound **16** in various tumor cell lines.

EXPERIMENTAL SECTION

Melting points were determined with a Boethius (GDR) apparatus and are uncorrected. IR spectra were recorded with UR-20 (GDR) spectrophotometer, UV spectra were recorded with a Specord UV-VIS (GDR) spectrophotometer, CD spectra were recorded with a J-20 (JASCO, Japan) spectropolarimeter, the ^{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; br.s = broad signal). Standard Silufol UV_{254} (Czechoslovakia) and Kieselgel 60 F_{254} (Merck, FRG) plates were used for TLC of sugars and nucleosides, respectively. As solvent systems were used (v/v): hexane-ethylacetate, 3:1 (A), $CHCl_3$-MeOH, 15:1 (B), CHCl-3-MeOH, 4:1 (C), i-PrOH-H_2O-20% NH_4OH, 7:2:1 (D). Column chromatography was performed on silica gel L 40/100 μ (Czechoslovakia), silica gel Woelm containing 20% of water (Woelm, FRG) and aluminium oxide neutral (grade II) according to Brockman (Reanal, Hungary). Anhydrous solvents were obtained as described.[10] In all condensation reactions, freshly distilled $SnCl_4$ and trimethylsilyl trifluoromethanesulfonate (Fluka, Switzerland) were used. The solutions of compounds in organic solvents were dried with anhydrous sodium sulphate for 4 h. The reactions were performed at 20°C, unless stated otherwise.

Methyl 5-O-benzyl-2,3-dideoxy-3-fluoro-2-trifluoroacetamido-α-D-ribofuranoside (6b) and methyl 5-O-benzyl-2,3-dideoxy-3-fluoro-2-trifluoroacetamido-α-D-lyxofuranoside (7b). Acetic anhydride (16.5 ml) was added to the solution of 3.4 g (13.26 mmol) of **1** in 25 ml of anhydrous DMSO, the mixture was stirred for 20 h and then poured into a mixture of ice and water (70 ml), and after the ice melted, it was extracted with $CHCl_3$ (3 x 150 ml). The organic extracts were combined, washed with 5% aqueous solution of $NaHCO_3$ (100 ml), water (3 x 100 ml), dried and evaporated *in vacuo* to give 3.4 g of the mixture of ketones **2** and **3** as syrup.

Without purification, this mixture (3.4 g) was dissolved in anhydrous pyridine (30 ml) and treated with $MeONH_2$.HCl (1.4 g, 16.76 mmol). After stirring for 48 h, the mixture was evaporated, water (40 ml) was added to the residue and the mixture was extracted with $CHCl_3$ (3 x 50 ml). The organic extracts were combined, washed with water (2 x 20 ml), dried and evaporated to give 3.7 g of the mixture of oximes **4** and **5** as a syrup. This mixture was dissolved in anhydrous THF, cooled to 0°C, then $LiAlH_4$ (1.32 g, 34.78 mmol) was added portionwise and the mixture was stirred for 2 h at 0°C and then for 10 h at room temperature. The reaction mixture was poured into ethylacetate (30 ml) containing ice (10 g), stirred, and after the ice melted, water (20 ml) was added, the precipitate was filtered off, washed with $CHCl_3$ (150 ml) and MeOH (25 ml). The combined filtrates were evaporated, water (60 ml) was added to the residue and the mixture was extracted with $CHCl_3$ (3 x 150 ml). The organic extracts were combined, dried and evaporated. The residue was purified by islica gel L (90 ml) column chromatography, eluted with hexane-ethylacetate (4:1, v/v; 600 ml), then with $CHCl_3$ (9:1, v/v; 500 ml) to afford 1.57 g of the mixture of amines **6a** and **7a** as a syrup.

The above mixture was co-evaporated with benzene (2 x 50 ml), dissolved in anhydrous MeOH (20 ml), treated with CF_3COSEt (2 ml, 2.5 g, 10.11 mmol) under stirring for 18 h and evaporated. The residue was chromatographed on the silica gel L (110 ml) column eluted with a linear ether gradient (0-50%, v/v; 2 x 500 ml) in hexane. The fractions containing individual products were collected and evaporated to yield 1.18 g (25%) of riboside **6b** as a syrup [Rf 0.61 (A); IR (film) 1730 cm-1, νco of COCF3 group] and 233 mg (5%) of lyxoside **7b** [m.p. 73-74°C (from ether-hexane); R_f 0.49 (A); IR (KBr) 1730 cm^{-1}].

Methyl 5-O-benzoyl-2,3-dideoxy-3-fluoro-2-trifluoroacetamido-α-D-ribofuranoside (8a) and methyl 5-O-benzoyl-2,3-dideoxy-3-fluoro-2-trifluoroacetamido-α-D-lyxofuranoside (9). To a solution of **6b** (0.35 g, 1.0

mmol) in 16 ml anhydrous ethanol, 20% $Pd(OH)_2/C$ (0.7 g) and freshly distilled cyclohexene (16 ml) were added and the mixture was refluxed for 2.5 h. After cooling to room temperature, catalyst was filtered off, washed with ethanol (150 ml), the combined filtrates were evaporated, the residue was dissolved in anhydrous pyridine (6 ml), benzoyl chloride (0.35 ml, 0.42 g, 3.03 mmol) was added and the reaction mixture was stirred for 18 h. The reaction mixture was poured under vigorous stirring into 20 ml of a ice/water mixture, and after the ice melted, it was extracted with $CHCl_3$ (3 x 80 ml). The organic extracts were combined, washed with 5% aqueous $NaHCO_3$ (50 ml), then with water (50 ml), dried and evaporated. The residue was purified by silica gel L (120 ml) column chromatography, using a linear EtOAc gradient (0-33%, v/v; 2 x 500 ml) in hexane to yield 0.33 g (91%) of riboside **8a** as a syrup: R_f 0.55 (A).

In a similar way, starting from 0.39 g (1.11 mmol) of **7b**, 0.33 g (81%) of lyxoside **9** was obtained: m.p. 116-117°C (from ether-hexane), Rf 0.49 (A).

9-(2-Amino-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)adenine (15). A solution of $SnCl_4$ (0.54 ml, 1.20 g, 4.68 mmol), 0.35 g (0.96 mmol) of **8a** and bis-trimethylsilyl derivative of N^6-benzoyladenine [obtained from 0.36 g (1.5 mmol) N^6-benzoyladenine] in anhydrous acetonitrile (12.5 ml) was refluxed for 30 min. After cooling to room temperature, the reaction mixture was poured into 5% aqueous $NaHCO_3$ (200 ml) and the mixture was extracted with $CHCl_3$ (3 x 100 ml). The organic extracts were combined, dried and evaporated. The residue was purified by silica gel L (110 ml) column chromatography, using a linear methanol gradient (0-6%, v/v; 2 x 500 ml) in $CHCl_3$ to yield 0.29 g (53%) of **10**: m.p. 218-219°C (from ethanol); R_f 0.66 (B).

The solution of 0.26 g (0.45 mmol) of **10** in methanol (35 ml), saturated with ammonia at 0°C, was kept for 168 h and evaporated. The residue was purified by silica gel Woelm (160 ml) column chromatography and eluted with $CHCl_3$-MeOH (15:1, v/v) to yield 0.11 g (90%) of **15**: m.p. 185-186°C (from ethanol); Rf 0.22 (C).

1-(2-Amino-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)cytosine (16). *Method A.* A solution of $SnCl_4$ (0.42 ml, 0.93 g, 3.64 mmol), 0.26 g (0.71 mmol) of **8a** and bis-trimethylsilyl derivative of N^4-benzoylcytosine [obtained from 0.26 g (1.21 mmol) of N^4-benzoylcytosine] in anhydrous acetonitrile (12 ml) was refluxed for 2 h. After standard work-up, the residue was purified by silica gel L (100 ml) column chromatography, using a linear ethyl acetate gradient (20-100%, v/v; 2 x 300 ml) in hexane to afford 0.32 g (82%) of **11** as a syrup.

Deprotection of **11** as described above followed by chromatography on Dowex AG 1x8 (200-400 mesh, OH^-form, 100 ml) using water as an eluent yielded 0.13 g (91%) of **16** as an amorphous powder after precipitation of the ethanolic solution of nucleoside with ether; Rf 0.51 (D).

Method B. A solution of TMS-Tfl (0.8 ml, 0.94 g, 4.3 mmol), 0.33 g (0.9 mmol) of **8a** and trimethylsilyl derivative of cytosine [obtained from 0.2 g (1.8 mmol) of cytosine] in anhydrous acetonitrile (16 ml) was refluxed for 1.5 h. After standard work-up, the residue was chromatographed on silica gel L (80 ml), using a linear methanol gradient (0-6%, v/v; 2 x 500 ml) in $CHCl_3$ to yield 0.12 g (36% relative to the starting compound **8a**) of **8b** as the only identified compound: m.p. 128-131°C (from $CHCl_3$); R_f 0.41 (A).

1-(2-Amino-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)thymine (17). A solution of $SnCl_4$ (0.64 ml, 1.42 g, 5.55 mmol), 0.39 g (1.07 mmol) of **8a** and bis-trimethylsilyl derivative of thymine [obtained from 0.3 g (2.38 mmol) thymine] in anhydrous acetonitrile (16 ml) was refluxed for 45 min. After standard work-up, the residue was purified by silica gel L (100 ml) column chromatography, using a linear methanol gradient (0-6%, v/v; 2 x 500 ml) in $CHCl_3$ to yield 0.3 g (63%) of **12**: m.p. 184-185°C (from $CHCl_3$), R_f 0.61 (B).

Standard deprotection of 0.3 g (0.67 mmol) of **12** followed by chromatography on silica gel L (140 ml), using a linear methanol gradient (0-15%, v/v; 2 x 600 ml), yielded 0.15 g (86%) of **17**: m.p. 63-65°C (from ethanol-ether mixture); R_f 0.3 (C).

9-(2-Amino-2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)guanine (18) and its 7-N-isomer (19). A mixture of TMS-Tfl (0.76 ml, 0.9 g, 4.08 mmol), 0.31 g (0.85 mmol) of **8a** and silyl derivative of N^2-acetyl-guanine [obtained from 0.3 g (1.27 mmol) of N^2,$N^{7(9)}$-diacetyl-guanine[20]] in anhydrous acetonitrile (12.5 ml) was refluxed for 30 min. After standard work-up, the residue was applied onto a linear methanol gradient (0-6%, v/v; 2 x 500 ml) in $CHCl_3$, to yield 90 mg (20%) of **14**: mp 136-137°C (MeOH); R_f 0.48 (B), and 180 mg (40%) of **13**: mp 265-266°C (EtOH); R_f 0.34 (B).

A solution of 180 mg (0.34 mmol) of **13** in methanol (30 ml), saturated with ammonia at 0°C, was kept for 168 h and evaporated. The residue was triturated with methanol (30 ml), the precipitate was filtered off, washed with hexane-ether (1:1, v/v; 30 ml), ether (30 ml) and dried to yield 70 mg (72%) of **18** as an amorphous powder, R_f 0.53 (D).

In a similar way, starting from 90 mg (0.17 mmol) of **14**, 35 mg (72%) of **19** was obtained as an amorphous powder, R_f 0.50 (D).

9-(2-Amino-2,3-dideoxy-α-D-lyxofuranosyl)adenine (21) was obtained, as described above for **15**, starting from 0.33 g (0.9 mmol) of **9** and silyl derivative of N^6-benzoyladenine [obtained from 0.4 g (1.67 mmol) of N^6-benzoyladenine] to yield 0.21 g (41%) of **20**: mp 140-141°C (EtOH); R_f 0.66 (B).

Standard debenzoylation of 0.21 g (0.37 mmol) of **20** and subsequent chromatography on Dowex 1x8 (200-400 mesh, OH^--form, 60 ml using water as an eluent afforded 70 mg (71%) of **21**:mp 187-188°C (EtOH); R_f 0.65 (D).

Methyl 5-O-benzyl-3-deoxy-3-fluoro-2-O-tosyl-α-D-ribofuranose (23). A solution of 2.1 g (5.82 mmol) of **22**[10] in methanol (35 ml), saturated with ammonia at 0°C, was stirred for 48 h and evaporated. The residue was coevaporated with benzene (2 x 30 ml), dissolved in anhydrous pyridine (20 ml), tosyl chloride (2.0 g, 10.5 mmol) was added and the reaction mixture was stirred for 18 h. The reaction mixture was poured under vigorous stirring into 50 ml of an ice/water mixture, and, after the ice melted, it was extracted with $CHCl_3$ (3 x 90 ml). The combined organic extracts were washed with 5% aqueous $NaHCO_3$ (100 ml), then with water (50 ml), dried and evaporated. The residue was purified by Al_2O_3 (100 ml) column chromatography, using a linear EtOAC gradient (0-50%, v/v; 2 x 750 ml) in hexane to yield 2.0 g (84%) of **23** as a syrup; R_f 0.14 (A).

Methyl 2-azido-5-O-benzyl-2,3-dideoxy-3-fluoro-α-D-arabinofuranoside (24). To a solution of **23** (0.5 g, 1.22 mmol) in anhydrous DMSO (6.5 ml), NaN_3 (0.44 g, 6.77 mmol) was added and the reaction mixture was

stirred at 190°C for 25 min. After cooling to room temperature, water (40 ml) was added and the mixture was extracted with $CHCl_3$ (3 x 50 ml). The combined organic extracts were washed with water (3 x 20 ml), dried and evaporated. The residue was purified by silica gel L (90 ml) column chromatography, using a linear EtOAc gradient (0-25%, v/v; 2 x 350 ml) in hexane to yield 0.26 g (76%) of **24** as a syrup; R_f 0.62 (A); IR (film) 2100 cm^{-1}, N_3. ^1H NMR ($CDCl_3$): δ 7.30-7.36 (m, 5H, Ph), 4.95 (s, 1H, H-1), 4.90 (ddd, 1H, H-3, $J_{3,F}$ = 53.4, $J_{3,2}$ = 2.4, $J_{3,4}$ = 4.8 Hz), 4.64 and 4.59 (2d, 2H, CH_2Ph, J = 12 Hz), 4.36 (dm, 1H, H-4, $J_{4,F}$ = 22.2, $J_{4,5'}$ = 5.4 Hz), 4.10 (dm, 1H, H-2, $J_{2,F}$ = 17.4 Hz), 3.66 (d, 2H, H-5 + H-5'); 3.42 (s, 3H, OCH_3).

Methyl 5-O-benzyl-2,3-dideoxy-3-fluoro-2-trifluoroacetamido-D-arabinofuranoside (25). A solution of **24** (0.3 g, 1.07 mmol) and triphenylphosphine (0.7 g, 2.67 mmol) in anhydrous pyridine (4 ml) was stirred for 10 h. Concentrated ammonium hydroxide was then added and the solution was stirred for an additional 18 h. The mixture was evaporated, the residue was coevaporated with benzene (2 x 25 ml), dissolved in anhydrous methanol and treated with CF_3COSEt (1 ml, 1.25 g, 5.05 mmol) under stirring for 6 h. The mixture was evaporated, the residue was chromatographed on the silica gel L (120 ml) column, and eluted with a linear EtOAc gradient (0-33%, v/v; 2 x 600 ml) in hexane to yield 0.23 g (61%) of **25** as a syrup: R_f 0.49 (A); IR (film) 1730 cm^{-1}.

Methyl 5-benzoyl-2,3-dideoxy-3-fluoro-2-trifluoroacetamido-D-arabinofuranoside (26) was obtained, as described above for **8a** and **9**, starting from 0.15 g (0.43 mmol) of **25** to yield 0.11 g (70%) of **26**: mp 104-105°C (pentane-ether); R_f 0.49 (A). =1H NMR ($CDCl_3$) δ 7.99-7.45 (m, 5H, Ph), 7.07 (d, 1H, NH, $J_{NH,2}$ = 7.2 Hz), 5.02 (ddd, 1H, H-3, $J_{3,F}$ = 52.8, J = 1.2, J = 3.6 Hz), 5.00 (s, 1H, H-1), 4.67-4.52 (m, 4H, H-2, H-4, H-5, and H-5'), 3.43 (s, 3H, OCH_3).

Antiviral activity. HIV-1 (strain $HTLV\text{-}III_B$)-induced cytopathogenicity assays in MT-4 cells were carried out as described earlier[21]. The virus was prepared from the culture supernatant of HIV-1-infected MT-4 cells. The antiviral assays, other than HIV-1, were based on an inhibition of vesicular stomatitis virus (VSV), polio virus-1 and Coxsackie virus B4-induced cytopathogenicity in HeLa cells, Coxsackie virus B4, Sindbis virus, parainfluenza virus-3, reovirus-1 and Semliki forest virus-induced cytopathogenicity in Vero cells, or herpes simplex virus type 1 (HSV-1), HSV-2, vaccinia virus (VV) and vesicular stomatitis virus (VSV)-induced cytopathogenicity in primary rabbit kidney cell cultures, following previously established procedures[22]. 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 hr 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.

Cytostatic activity. The cytostatic assays were performed according to a previously established procedure[23]. All assays were performed in flat-bottomed 96-wells Microtest plates. Briefly, murine leukemia (L1210), human T-lymphoblast Molt/4F or human T-lymphocyte MT-4 cells

Comp.	M.W.					Calc.,%				Found,%			
N°	C	H	F	N	O	C	H	F	N	C	H	F	N
7b	15	17	4	1	4	51.29	4.88	21.62	3.99	51.42	4.98	21.50	3.75
			351.26										
8b	15	15	4	1	5	49.31	4.14	20.79	3.83	49.51	4.28	20.74	3.80
			365.22										
9	15	15	4	1	5	49.31	4.14	20.79	3.83	49.49	4.24	20.65	3.91
			365.22										
15	10	13	1	6	2	44.78	4.88	7.08	31.33	44.90	4.94	6.91	31.30
			268.25										
16	9	13	1	4	3	44.26	5.36	7.78	22.94	44.47	5.41	7.62	23.09
			244.22										
17	10	11	1	3	4	46.33	5.44	7.33	16.21	46.40	5.30	7.40	16.15
			259.24										
18	10	13	1	6	3	42.25	4.61	6.68	29.54	42.47	4.76	6.60	29.44
			284.25										
19	10	13	1	6	3	42.25	4.61	6.68	29.54	42.24	4.71	6.52	29.65
			284.25										
21	10	13	1	6	2	44.78	4.88	7.08	31.33	44.85	4.75	6.94	31.40
			268.25										
26	15	15	1	1	5	49.31	4.14	20.79	3.83	49.20	4.31	20.94	3.96
			365.22										

Elemental analyses were carried out in a Microanalytical Laboratorium at the Institute of Organic Chemistry, Ukrainian Academy of Sciences (Kiev, USSR).

were suspended in growth medium and added to the microplate wells at a density of 5 x 10^4 L1210 or Molt/4F cells, or 6.25 x 10^4 MT-4 cells/well (200 µl) in the presence or varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 hr (L1210 cells), 72 hr (Molt/4F cells) or 120 hr (MT-4 cells) at 37°C in a humidified, CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted either in a Coulter counter (Coulter Electronics Ltd., Harpenden, Herts, U.K.) (L1210 and Molt/4F cells) or an hematocytometer following trypan blue dye exclusion (MT-4 cells). The IC_{50} is defined as the compound concentration that reduces the number of viable cells by 50%.

Acknowledgments

This research was supported in part by the AIDS Basic Research Programme of the European Community and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (no. 3.0040.83), the Belgian Geconcerteerde Onderzoeksacties (no. 85/90-79) and the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek (no. 7.0049.90). I.A.M. is deeply grateful to the Alexander von Humboldt-Stiftung (Bonn - Bad-Godesberg, FRG) for partial financial support of this work.

References

1. Moffatt, J.G. In *Nucleoside Analogues. Chemistry, Biology, and Medical Applications*; Walker, R.T.; De Clercq, E.; Eckstein, F.; Eds.; Plenum: New York and London, **1979**; pp 71-164.
2. Isono, K. *J. Antibiot. (Tokyo)* **1988**, *41*, 1711.
3. De Clercq, E.; Balzarini, J.; Descamps, J.; Eckstein, F. *Biochem. Pharmacol.* **1980**, *29*, 1949.
4. De Clercq, E.; Van Aerschot, A.; Herdewijn, P.; Baba, M.; Pauwels, R.; Balzarini, J. *Nucleosides Nucleotides*, **1989**, *8*, 659.
5. Herdewijn, P.; Balzarini, J.; Pauwels, R.; Janssen, G.; Van Aerschot, A.; De Clercq, E. *Nucleosides Nucleotides*, **1989**, *8*, 1231.
6. Wu, J.C.; Pathak, T.; Tong, W.; Vial, J.-M.; Remand, G.; Chattopadhyaya, J. *Tetrahedron*, **1988**, *44*, 6705.
7. Zinchenko, A.I.; Eroshevskaya, L.A.; Barai, V.N.; Mikhailopulo, I.A. *Nucl. Acids Res. Symp. Series* **1987**, *18*, 137.
8. Zaitseva, G.V.; Kvasyuk, E.I.; Poopeiko, N.E.; Kulak, T.I.; Pashinnik, V.E.; Tovstenko, V.I.; Markovaskii, L.N.; Mikhailopulo, I.A. *Bioorgan. Chem. (Moscow)* **1988**, *14*, 1275.
9. Kvasyuk, E.I.; Zaitseva, G.V.; Savochkina, L.P.; Chidgeavadze, Z.G.; Beabealashvilli, R.Sh.; Lange, P.; Mikhailopulo, I.A. *Bioorgan. Chem. (Moscow)* **1989**, *15*, 781.
10. Mikhailopulo, I.A.; Poopeiko, N.E.; Pricota, T.I.; Kvasyuk, E.I. Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1991**, in press.
11. Mikhailopulo, I.A.; Poopeiko, N.E.; Sivets, G.G.; Pricota, T.I. *EUROCARB. V. Fifth European Symposium on Carbohydrates*. Prague, Czechoslovakia, August 21-25, **1989**, Abstracts, A-93.
12. Mikhailopulo, I.A.; Poopeiko, N.E., Pricota, T.I.; Sivets, G.G.; Balzarini, J.; De Clercq, E. *Coll. Czech. Chem. Comm. Special Issue 1*, **1990**, *55*, 97.
13. Sova, H.; Thomas, G.H.S. *Canad. J. Chem.* **1966**, *44* 836.
14. Herscovi, J.; Egron, M.-J.; Antonakis, K. *J. Chem. Soc. Perkin. Trans. I.*, **1988**, 1219.
15. Wolfrom, M.L.; Conigliaro, P.J. *Carbohyd. Res.* **1969**, *11*, 63.
16. Hanessian, S.; Liak, T.J.; Vanesse, B. *Synthesis*, **1981**, no. 5, 367-369.
17. Dyatkina, N.B.; Krayevsky, A.A.; Azhaev, A.V. *Bioorgan. Chem. (Moscow)* **1986**, *12*, 1048.
18. Imazawa, M.; Eckstein, F. *J. Org. Chem.* **1979**, *44*, 2039.
19. Mungall, W.S.; Greene, G.L.; Heavner, G.A.; Letsinger, R. *J. Org. Chem.* **1975**, *40*, 1659.
20. Furukawa, Y.; Honjo, M. *Chem. Pharm. Bull.* **1968**, *16*, 1076.
21. Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D.G.; De Clercq, E. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 332.

22. De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R.T.; Jones, A.S.; Torrence, P.F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563.
23. De Clercq, E.; Balzarini, J.; Torrence, P.F.; Mertes, M.P.; Schmidt, C.L.; Shugar, D.; Barr, P.J.; Jones, A.S.; Verhelst, G.; Walker, R.T. *Mol. Pharmacol.* **1981**, *19*, 321.

Received 2/27/91
Accepted 5/3/91