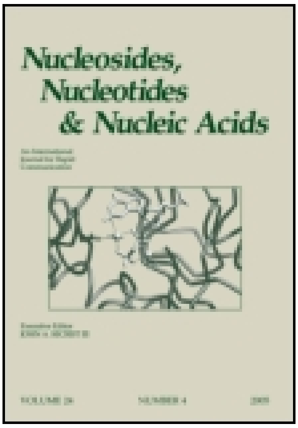

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis and Antiviral Activity of Purine 2′,3′-Dideoxy-2′,3′-Difluoro-D-Arabinofuranosyl Nucleosides

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Published online: 29 Jul 2009.

Nucleosides, Nucleotides and Nucleic Acids, 28:519–536, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903053979

SYNTHESIS AND ANTIVIRAL ACTIVITY OF PURINE 2′,3′-DIDEOXY-2′,3′-DIFLUORO-D-ARABINOFURANOSYL NUCLEOSIDES

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□ *9-(2′,3′-Dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)adenine (**20**), 2-chloro-9-(2′,3′-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)adenine (**22**), as well as their respective α-anomers **21** and **23**, were synthesized by the nucleobase anion glycosylation of intermediate 5-O-benzoyl-2,3-dideoxy-2,3-difluoro-α-D-arabinofuranosyl bromide (**13**) starting from methyl 5-O-benzyl-3-deoxy-3-fluoro-α-D-ribofuranoside (**3**) and methyl 5-O-benzoyl-α-D-xylofuranoside (**10**). These compounds were evaluated as potential inhibitors of HIV-1 and hepatitis C virus in human PBM and Huh-7 Replicon cells, respectively. The adenosine analog **20** demonstrated potent activity against HIV-1 in primary human lymphocytes with no apparent cytotoxicity. Conformation of pentofuranose ring of nucleoside **20** in solution was studied by PSEUROT calculations.*

Keywords Fluoro-nucleosides; purines; antiviral activity; conformational analysis

INTRODUCTION

Fluorine substitution of a hydrogen atom and/or a hydroxyl group in a nucleoside analog can impart a significant influence on its biological

Received 6 April 2009; accepted 13 May 2009.

This paper is dedicated to Dr. Morris Robbins, our colleague and friend, and to honor all his contributions to the field of nucleoside chemistry and biology.

This work was supported in part by NIH grant 2P30-AI-50409 (RFS), 5R37-AI-041980 (RFS) and Department of Veterans Affairs (RFS) and Belarus State Program of FOI "Physiological Active Compounds" (Grant 2.04). R.F.S. is the Founder and Director of RFS Pharma, LLC, and received no compensation for performing this work.

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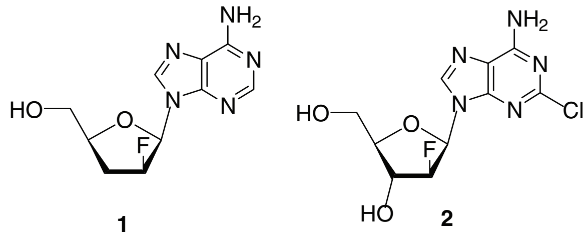


FIGURE 1 Biologically active purine 2′-β-fluoro nucleosides.

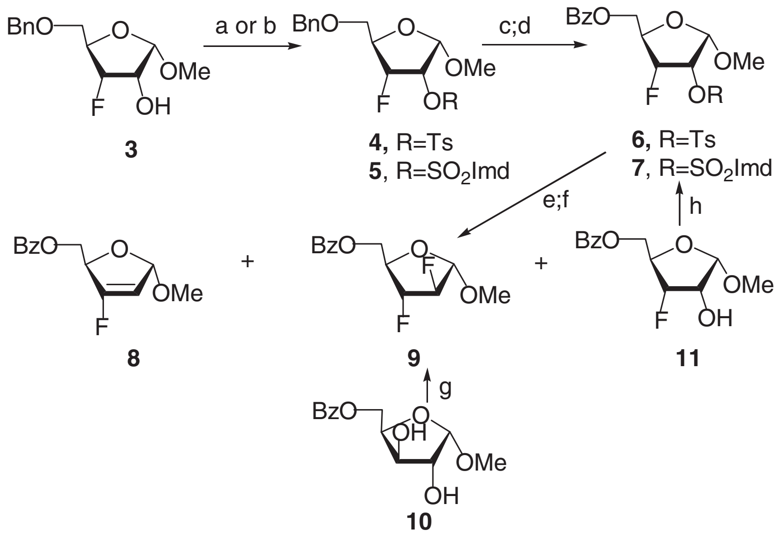
properties. Addition of a fluorine atom to the sugar moiety of nucleosides can improve their antiviral properties by enhancing potency and selectivity of modified nucleosides against HIV or other viruses.[1–4] The structure-activity relationships for pyrimidine and purine monofluoro 2′,3′-dideoxy nucleosides as potential anti-HIV agents has been previously reported.[1,5,6] Of the different fluoro-substituted nucleosides, purine 2′-β-fluoro nucleosides attract particular attention in view of their interesting biological activity, primarily due to the stabilization of the glycosidic bond. For example, lodenosine **1** is anti-HIV agent which possesses increased chemical and metabolic stability (Figure 1).[7]

Among dideoxydifluoro nucleosides with β-D-*arabino*-configuration, a set of pyrimidine nucleosides have been synthesized and evaluated against HIV-1.[5] However, synthesis and study of the antiviral properties of purine nucleosides is of interest for a full biological evaluation of the antiviral potential for this family of nucleosides. Thus, we have synthesized 9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)adenine (**20**) and its 2-chloro derivative **22** which can also be considered as new, closely related nucleoside analogues of purine 2′-β-fluoro nucleosides, lodenosine **1** and clofarabine **2**,[8] respectively, the latter being used as anticancer agent for treatment of pediatric acute leukemia (Figure 1).

We describe herein the synthesis of purine 2′,3′-dideoxy-2′,3′-difluoro nucleosides with β-D-*arabino*-configuration **20** and **22**, their α-anomers **21** and **23**, along with the in vitro anti-HIV and anti-HCV evaluation.

RESULTS AND DISCUSSION

The synthetic route to adenine nucleoside **20** and its α-anomer **21** was briefly reported earlier from methyl 5-*O*-benzyl-2-deoxy-2-fluoro-α-D-arabinofuranoside.[9] Herein, we studied alternative pathways for the preparation of difluoride **9** from the benzyl derivative of methyl 3-fluoro-3-deoxy-D-ribofuranoside (**3**) and methyl 5-O-benzoyl-α-D-xylofuranoside (**10**) (Scheme 1). Riboside **3**, prepared from methyl 5-*O*-benzyl-3-deoxy-3-fluoro-α-D-arabinofuranoside,[10] gave the 2-O-Ts derivative **4** under standard tosylation conditions. The 2-O-SO_2Imd derivative **5** was synthesized



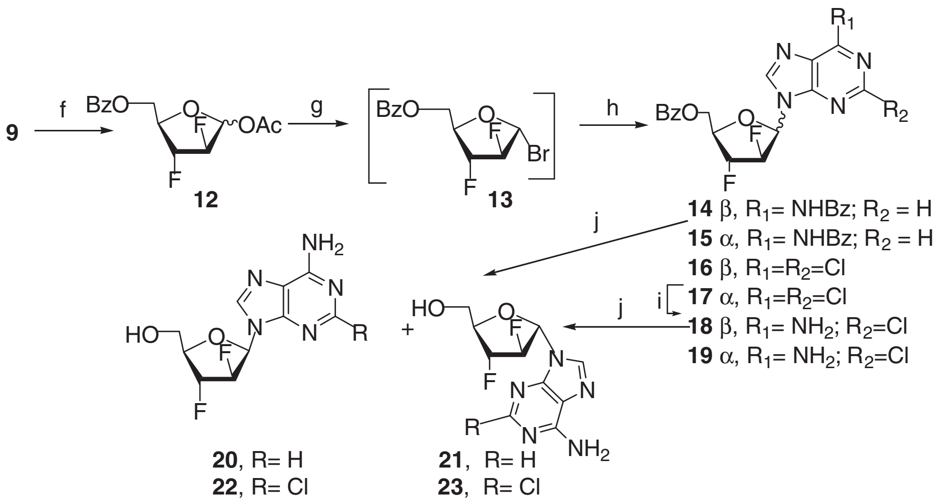
SCHEME 1 Reagents and conditions: a) TsCl/Py, room temperature 18 hours, (**4**, 84%); b) SO_2Cl_2/CH_2Cl_2, −40°C to room temperature, Imd, 0°C to room temperature (**5**, 64%); c) 20% $Pd(OH)_2$/C/cycloxehene, EtOH, reflux, 2–3 hours; d) BzCl/Py, room temperature (c + d, **6** Σ70%; **7** Σ64%); e) KHF_2/45%HF/2,3-butanediol, 160°C, 45 minutes (**9** from **7**, 49%); f) CsF/anh.DMSO/anh.HMPA (11:1,v/v), 190°C, 1 hour (**9**, 10%; **8**, 26%); g) DAST, CH_2Cl_2, room temperature and 27–29°C, **9**, 34%; **11**, 19%; h) SO_2Cl_2/CH_2Cl_2/DMF, −40°C to room temperature, Imd, 0°C to room temperature (**7**, 92%).

from **3** using the method proposed by Hanessian for the preparation of imidazolylsulfonate derivatives of sugars.[11] Debenzylation of **4** and **5** by 20% $Pd(OH)_2$/C in ethanol in the presence of cyclohexene followed by benzoylation resulted in benzoates **6** and **7**. Further, two approaches involving nucleophilic displacement of the 2-OTs group of the riboside **6** by CsF in DMSO/HMPA[12,13] or 2-O-SO_2Imd group of the riboside **7** under treatment with KHF_2/HF/2,3-butanediol[14] were tested for the introduction of a fluorine atom at C-2 position. Difluoride **9** was synthesized under these conditions from the tosylate **6** and the imidazolylsulfonate **7** in 10 and 49% yields (Scheme 1), respectively, after chromatographic isolation.

Concomitant with the nucleophilic displacement of the 2-O-tosyl group in **6** by the fluoride anion, elimination of the tosyl group generated the the 3-fluorovinyl compound **8** as a by-product isolated in 26% yield after chromatography. Thus, the investigated four-step route to **9** from riboside **3** via imidazolylsulfonate **7** resulted in the target difluoride in a moderate 20% combined yield.[9]

The most efficient synthetic route to difluoride **9** was from derivative of methyl α-D-xylofuranoside **10**, which is readily available from D-xylose.[15] The fluorination of **10** was accomplished with excess of DAST in methylene chloride under 27–29°C for 20 hours resulted in the target sugar **9** and methyl 5-*O*-benzoyl-3-deoxy-3-fluoro-α-D-ribofuranoside (**11**) with 34% and 19% yields, respectively. The synthesis of difluoride **9** from **10** involves fluorination of **10** at C-3 resulting in the formation of intermediate 3-fluorodeoxy sugar **11** followed by introduction of a fluorine atom with DAST at C-2 of **11** under mild heating. It should be noted that the preparation of **9** under these conditions is the first example of relatively effective synthesis of vicinal substituted 2,3-dideoxy-2,3-difluoro D-pentofuranose via a double fluorination of a 5-O-acyl derivative of D-pentofuranose with double inversion at the C-2 and C-3 atoms resulting from of a single treatment of the starting sugar by a fluorinating agent. The riboside **11** prepared from xyloside **10** can also be converted into difluoride **9** via imidazolylsulfonate **7.** Synthesis of sulfonate **7** was performed by treatment of **11** with SO_2Cl_2 and imidazole in CH_2Cl_2-DMF in high yield (92%) after column chromatography on silica gel.

The condensation of difluoride **9** with persilylated N^6-benzoyladenine in the presence of $SnCl_4$ in acetonitrile afforded a mixture of β- and α-nucleosides **14** and **15** which was separated into individual anomers by column chromatography on silica gel.[9] The formation of desired β-nucleoside **14** was observed in low yield as a result of the glycosylation of N^6-benzoyladenine by α-methyl glycoside **9**. Therefore, another and more effective approach to β-nucleoside **14** was studied via bromide **13** and experimental details of it are reported in this communication (Scheme 2). Conventional acetolysis of **9** gave a mixture of acetates **12** after chromatography



SCHEME 2 Reagents and conditions: f) $AcOH/Ac_2O$/concn. H_2SO_4 (77%); g) TMS-Br/$CDCl_3$, 2 weeks, room temperature; (**13**, ≈50%); h) **13**/Na-salt of N^{6Bz}Ade/THF, reflux, 5 hours (**14**, 23%; **15**, 9%); h) **11**/Na-salt of 2,6-di-Cl purine/CH_3CN, room temperature (1:1.04), 18 hours (**16**+**17**, 48%); i) saturated NH_3/1,2-DME, room temperature (**18**, 67%; **19**, 25%); j) saturated NH_3/MeOH, room temperature (**20**, 88%, **21**, 81%, **22**, 75%, **23**, 80%).

on silica gel, bromination (TMSiBr/$CDCl_3$)[16] of the latter generated intermediate glycosyl bromide **13** that was reacted, without isolation, with the sodium salt of N^6-benzoyladenine under reflux in tetrahydrofuran to give a mixture of β- and α-nucleosides **14** and **15**. The target β-nucleoside **14** and its α-anomer **15** were isolated by column chromatography on silica gel in 23% and 9% yield, respectively. An analogous route via coupling of 1-α-bromo sugar with the nucleobase was used for the preparation of 2-chloro derivative of **18**.

The condensation of bromide **13** with the sodium salt of 2,6-dichloropurine in acetonitrile[17] when compared to the one of N^6-benzoyladenine gave more complex mixture of products from which a mixture of protected N^9-β/α-nucleosides **16** and **17** (β/α-ratio $\approx$ 2.9:1 according to ^{1}H NMR data) was isolated in 48% overall yield after column chromatography on silica gel (Scheme 2). Treatment of the **16/17** mixture with a saturated solution of ammonia in 1,2-dimethoxyethane[18] at room temperature for 24 hours afforded protected β- and α-nucleosides of 2-chloroadenine **18** and **19** which were successively isolated by chromatography on silica gel in 67 and 25% yield, respectively.

Standard deprotection of individual blocked nucleosides **14**, **15**, **18**, and **19** with methanolic ammonia and subsequent chromatographic purification gave pure 9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)adenine (**20**) and its α-anomer **21**, 2-chloro-9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)adenine (**22**) and its α-anomer **23**, respectively. The structure of nucleosides **20** and **21**, **22**, and **23** was verified by ^{1}H, ^{19}F, ^{13}C NMR and by mass spectroscopy, UV, CD.

The assignments of the configurations of nucleosides **20–23** at the anomeric centers were based upon NMR data. The diagnostic for the β-anomeric configurations of **20** and **22** is the $^5J_{H,F}$ long-range coupling of H-C(8) to a 2′-β-fluorine atom of 2.3 and 2.27 Hz, respectively, exhibited in their ^{1}H NMR spectra (Tables 1 and 2). Similarly, the presence of the similar five-bond coupling of 2.9 and 3.0 Hz (see Experimental) for intermediate protected nucleosides **14** and **18** supported their β-structural assignment. This coupling is generally indicative of a spatial proximity of the nuclei involved and is not observed in the α-anomers **15** and **19**, **21**, and **23**. The most informative feature of the ^{1}H NMR spectra for the ones in comparison with the corresponding β-nucleosides **14** and **19**, **20**, and **22** is the shift of H-2′ and H-4′ resonances in a lower field for the α-anomers.[19,20]

Of interest are some other observations from the NMR data (Table 2). Long-range coupling of H-1′ to 3′-fluorine atom of 1.86 and 0.9 Hz in ^{1}H NMR spectra of nucleosides **20** and **22** argues for W-shape configuration of H-1′ and F-3′, and the β-anomeric configuration of adenine and 2-chloroadenine nucleosides. A four-bond coupling (0.91 Hz) between H-1 and H-3 atoms of difluoride **9** is exhibited in its ^{1}H NMR spectrum due to W-arrangement[21] between these protons in the case of α-methyl glycoside

TABLE 1 ^{1}H NMR chemical shifts of 2,3-dideoxy-2,3-difluoro sugars **9**, **12** and nucleosides **20–23** with D-*arabino*-configurations. δ in ppm; J in Hz

Cmpd.	H-1 or H-1′	H-2 or H-2′	H-3 or H-3′	H-4 or H-4′	H-5a or H-5′a	H-5b or H-5′b	Others
9	5.13 br.d	5.08 ddd	5.10 dddt	4.49–4.61 m			3.46 (s, OMe), 8.08 (m, 1H), 7.60 (m, 2H), 7.48 (m, 2H, Bz)
12	6.42 br.d	5.21 dd	5.20 ddd	4.70 ddt	4.57 dd	4.55 dd	2.12 (s, 3H, CH_3CO), 8.04 (d, 1H), 7.57 (t, 2H), 7.44 (t, 2H, Bz)
20	6.53 ddd	5.50 dddd	5.46 ddd	4.30 dm	3.89 ddd	3.85 dd	8.30 (d, 1H, $J = 2.3$, H-8), 8.21 (s, 1H, H-2)
22	6.42 ddd	5.74 dddd	5.57 dddd	4.20 dm	3.72 br.m	3.69 br.m	8.27 (d, 1H, $J = 2.27$, H-8), 7.94 (br.s, 2H, NH_2),5.27 (br.s, 1H, 5′-OH),$^{gem}J_{5'a,\,5'b} \sim 13$
21	6.53 dd	6.04 ddt	5.47 dddd	4.81 ddt	3.83 dd	3.79 ddd	8.40 (s, 1H, H-8) 8.39 (s, 1H, H-2) $^4J_{5'b,\,F3'} = 1.15$
23	6.35 dd	6.06 ddt	5.45 ddt	4.63 dm	3.61 dd	3.58 dd	8.31 (s, 1H, H-8), 5.26 (t, 1H, $J = 5.66$, 5′-OH), 7.90 (br.s, 2H, NH_2)

Spectra were obtained in $CDCl_3$ for sugars **9** and **12**, in CD_3OD and DMSO-d_6 for nucleosides **20**, **21** and **22**, **23**, respectively. Spectral data for pure α-anomer of **12** are presented.

TABLE 2 Coupling constants (in Hz) for ^{1}H NMR data of 2,3-dideoxy-2,3-difluoro sugars **9**, **12** and nucleosides **20–23** with D-*arabino*-configurations

	^3J(H,H)				^3J(H,F)				
	1,2 or 1′,2′	2,3 or 2′,3′	3,4 or 3′,4′	4,5a/4,5b or 4′,5′a/ 4′,5′b	H1,F2 or H1′,F2	H3,F2 or H3′,F2	H2,F3 or H2′,F3	H4,F3 or H4′,F3	Others
9	<0.8	0.91	4.11	n.d.	10.03	19.76	13.44	22.60	$^4J_{3,1} = 0.91$
12	<1.0	<1.0	3.24	4.5/4.69	10.57	17.49	12.21	24.14	$^{gem}J_{2,F2} = 48.17$ $^{gem}J_{3,F3} = 51.0$ $^{gem}J_{H5a,H5b} = 12.71$
20	3.97	2.17	3.76	4.27/4.85	17.67	15.29	12.82	24.80	$^5J_{F2',H8} = 2.3$ $^4J_{1',F3'} = 1.86$ $^{gem}J_{2',F2'} = 50.52$ $^{gem}J_{3',F3'} = 51.24$ $J_{H5'a,H5'b} = 12.8$
22	4.76	3.35	4.73	4.78	14.54	16.58	14.77	22.31	$^5J_{F2',H8} = 2.27$ $^4J_{1',F3'} = 0.9$ $^{gem}J_{2',F2'} = 50.86$ $^{gem}J_{3',F3'} = 52.16$
21	2.42	2.74	3.90	5.04/4.98	15.04	15.90	14.0	21.16	$^{gem}J_{2',F2'} = 49.70$ $^{gem}J_{3',F3'} = 51.70$ $^{gem}J_{H5'a,H5'b} = 12.8$
23	3.24	3.37	4.32	6.30/5.26	15.11	15.39	17.06	20.44	$^{gem}J_{H5'a,H5'b} = 2.29$ $^{gem}J_{3',F3'} = 52.35$ $^{gem}J_{2',F2'} = 50.28$

TABLE 3 The ^{13}C NMR data (chemical shifts in ppm)

	Chemical shifts, δ_{TMS}, ppm [J(C,F)in Hz]					
Compd.	C-1 or C-1′	C-2 or C-2′	C-3 or C-3′	C-4 or C-4′	C-5 or C-5′	Others
9	106.03 dd	96.81 dd	95.04 dd	80.19 d	62.95 d	166.14 (s, Ph-***C***=O, arom), 133.30, 129.78, 129.55, 128.47 (*Ph*-C=O), 54.98 (s, OCH_3)
12	98.84 dd	96.86 dd	94.38 dd	82.76 d	62.81 d	169.34 (s, CH_3-***C***=O), 166.21 (s, Ph-***C***=O), 133.54, 129.91, 129.8, 128.59 (*Ph*-C=O), 21.05 (s, ***C***H_3-C=O)
20	83.07 dd	93.57 dd	92.69 dd	82.15 dd	60.26 d	156.09 (C-6) 152.83 (C-2) 149.13 (C-4) 140.26 (d, $^5J_{C8,F2'} = 4.6$, C-8) 118.49 (C-5)
22	81.75 dd	93.93 dd	92.99 dd	81.11 dd	60.40 d	157.38(C-6) 153.95(C-2) 150.70(C-4) 140.69 (d, $^5J_{C8,F2'} = 3.75$, C-8) 117.89 (C-5)
21	87.95 dd	96.90 dd	93.93 dd	85.13 dd	60.30 d	152.93(C-6) 148.68(C-2) 147.94(C-4) 141.02 (s, $^5J_{C8,F2'} <1.0$, C-8) 119.37 (C-5)
23	86.58 dd	97.03 dd	94.46 dd	84.04 dd	60.66 d	157.42(C-6) 153.87(C-2) 150.56(C-4) 140.06 (s, C-8) 118.69 (d, $J = 5.51$, C-5)

9, but it is not found for α-1-O-acetate **12**. Interesting stereochemical peculiarities of compounds **20**, **22,** and **9** is well represented by ^{1}H NMR data described above.

^{13}C NMR data presented in Tables 3 and 4 provide further support for the assignments of the configurations of nucleosides **20–23** at the anomeric centers which were made in terms of ^{1}H NMR data, long-range coupling constants between C-8 of heterocyclic base and 2′-β-fluorine atom (4.6 and 3.75 Hz) that were observed only for β-nucleosides **20** and **22**, respectively. The carbon resonances of the bases in ^{13}C NMR spectra of nucleosides **20–23** are in good accord with ^{13}C NMR spectral data obtained earlier for closely related purine 2′-β-fluoro nucleosides.[20] ^{19}F NMR data (see Experimental) of 2,3-dideoxy-2,3-difluoro sugars **9**, **12** and nucleosides **20–23** with D-*arabino*-configurations are evidence in favour of the assigned structures of synthesized sugars and nucleosides. F-2′ and F-3′ resonance signals of sugars and nucleosides with two fluorine atoms in *trans*-arrangement are

TABLE 4 The ^{13}C NMR data (coupling constants in Hz)

	F-2				F-3			
	^2J(C,F)		^3J(C,F)	^4J(C,F)	^3J(C,F)	^2J(C,F)		^3J(C,F)
Compd.	C1,F2 or C1′,F2	C3,F2 or C3′,F2	C4,F2 or C4′,F2	C5,F2 or C5′,F2	C1,F3 or C1′,F3	C2,F3 or C2′,F3	C4,F3 or C4′,F3	C5,F3 or C5′,F3
9	35.30	30.45	<1.0	<1.0	4.20	28.10	27.20	5.40
12	36.87	30.85	<1.0	<1.0	1.52	29.43	28.23	6.49
20	17.35	28.9	2.84	<1.0	3.55	28.70	25.51	6.39
22	17.42	27.30	3.35	<1.0	5.89	27.63	24.36	4.63
21	36.40	28.02	2.08	<1.0	4.98	28.71	24.55	5.66
23	35.50	27.36	3.40	<1.0	6.42	27.11	24.30	4.68

revealed as complex multiplets in their ^{19}F NMR spectra, but the magnitude of $^3J_{F\text{-}2,F\text{-}3}$ (8.08–8.48 Hz) did not profoundly differ for sugars **9**, **12**, and target nucleosides.

Compounds **20–23** were evaluated as potential inhibitors of HIV-1 and HCV in primary human peripheral blood mononuclear (PBM) and Huh-7 Replicon cells, respectively. The adenine nucleoside **20** demonstrated potent activity against HIV-1 in primary human lymphocytes with a median effective concentration (EC_{50}) of 0.72 μM with no apparent cytotoxicity in three different cell systems up to 100 μM. The 2-chloro derivative **22** was moderately active with an EC_{50} of 14.1 μM, but antiviral activity could be secondary to its cytotoxicity in human PBM cells (Table 5).

TABLE 5 Anti-HIV and anti-HCV activities of nucleosides **20–23**

	Anti-HIV-1 activity in human PBM cells and cytotoxicity in					Anti-HCV activity and toxicity in replicon assay Huh7 cells		
			CC_{50} (μM)					
Compound	EC_{50} (μM)	EC_{90} (μM)	PBM	CEM	Vero	EC_{50} (μM)	EC_{90} (μM)	CC_{50}[b] (μM)
20	0.72	10.3	>100	>100	>100	>10	>10	>10
21	5.6	>100	>100	>100	>100	>10	>10	>10
22	14.1	46.3	13.6	70.2	>100	>10	>10	>10
23	60.1	>100	9.4	>100	>100	>10	>10	>10
2′-β-FddA[a]	4.4	—	>100	—	—	—	—	—
AZT[c]	0.0026	0.010	>100	14.3	56.0	—	—	—
2′-C-Me-C[c]	—	—	—	—	—	1.3	5.4	>10

[a]The anti-HIV activity of lodenosine (2′-β-FddA) **1** in PBM cells was taken from literature [23] and presented for comparison.

[b]Toxicity in Huch-7 Replicon cells.

[c]AZT and 2′-C-Me C were used as positive controls for the HIV and HCV assays.

It should be stressed that nucleoside **20** as an analogue of lodenosine **1** displays higher antiviral activity than 2′-β-FddA[23] with similar cytotoxic effect in the same cell system (Table 5). None of the compounds were effective against HCV replication in a Huh-7 based replicon system when tested at 10 μM. The methodologies for evaluating the antiviral activity against HIV, HCV and cytotoxicity have been published elsewhere.[24, 25]

Considering that 9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl) adenine (**20**) as new closely related nucleoside analogue of known anti-HIV agent, lodenosine **1**, both exhibit anti-HIV activity in vitro tests, it is interesting to compare the conformational peculiarities of the both 2′-β-fluoro nucleosides possessing antiviral activity, namely, N/S equilibrium of their pentofuranose rings in solution.

Conformational analysis of the pentofuranose ring of the adenine β-nucleoside **20** was performed employing the PSEUROT 6.3 program[26] and the results have been compared with those for closely related deoxyfluoro nucleoside **1**[22] (Table 6). All calculations have been performed aiming at achieving: (i) the minimal rms and $|\Delta J|$ values, and (ii) maximal correspondence of the pseudorotational parameters obtained using only four vicinal [H,F] couplings and simultaneous analysis of seven [H,H] and [H,F] couplings.[27,28] Scale factors for the $^3J_{\mathrm{H,H}}$ and $^3J_{\mathrm{H,F}}$ were 1.0 and 0.2, respectively.

According to the data of PSEUROT analysis for nucleosides **20** and **1** with application of ^{1}H NMR data prepared in the same deuterated solvent it can be concluded that pentofuranose rings of the both purine nucleosides are predominantly in the *S*-type conformations, the degree of populations being very similar (76% and 71%). It is noteworthy that the dominating conformations for N/S equilibrium of the pentofuranose rings **20** and **1** occupy a narrow close segment of pseudorotational wheel for these nucleosides (Table 6).

Further, comparison of $N \leftrightarrow S$ pseudorotational equilibrium of 2′-β-FddA **1** and difluoride **20** possesing anti-HIV activity with the one reported earlier[13,27,29] for isomeric adenine nucleosides with 2′-α-fluoro atom and without antiviral activity, 9-(2′,3′-dideoxy-2′-fluoro-β-D-*erythro*-pentofuranosyl)adenine (2′-α-FddA) and 2′,3′-dideoxy-2′,3′-difluoro–adenosine, permits us to note that the conformation of pentofuranose rings of 2′-β-fluoro anti-HIV nucleosides in solution differ from the stereochemistry of inactive adenine nucleosides by pseudorotational parameters.

In conclusion, the preferred conformation of adenine 2′-β-fluoro nucleosides **20** and **1** appear to play an important role in their anti-HIV activity. The synthesis of new purine dideoxydifluoro nucleosides with β-D-*arabino*-configuration is of interest for the development of novel nucleoside analogues with potential antiviral activity and for the continuing search

TABLE 6 Pseudorotational parameters of adenine nucleosides **20** and **1**

	Pseudorotational parameters of nucleosides[a]										
							ΔG_{eff}[c)]				
Compound	P_N (°)	ψ_N (°)	P_S (°)	ψ_S (°)	rms (Hz)	$\lvert\Delta J\rvert$ (Hz)	1′-2′	2′-3′ (2′F)	2′-3′ (3′F)	3′-4′	%S
20 (CD_3OD)	−15.9 ($_2E$)	*48*	137.7 ($_1T^2$)	*32*	.11	.13 (H,H) .20 (H,F)	−8.370	.372	−3.906	2.700	77
Using 4 vicinal [H,F] coupling constants	−20.4 ($_2E$)	*48*	139.8 ($_1T^2$)	*32*	.02	.03					76
1[b] (CD_3OD)	−13.7 ($_2E$)	29.3	129.3 ($_1E$) 145.9 ($_1T^2$)	43.1 39.9	.06	.12					71

[a]All data resulted from analysis of the [H,H] and [H,F] coupling constants, if is not specified. The ψ values given in italics and underlined are kept fixed during the final minimization.

[b]The pseudorotational parameters of lodenosine **1** have been calculated previously[22] and included in the table for comparison of stereochemistry of adenine nucleosides.

[c]$\Delta G_{eff} = -3.72[(\alpha_{FCC} + \alpha_{HCC})/2 - 110]$ along a given C—C bond.[27]

for a stereochemical rationale[30] for the activity of anti-HIV nucleosides fluorinated on carbohydrate moiety.

EXPERIMENTAL

Column chromatography was performed on silica gel 60 H (70–230 mesh; Merck, Germany). TLC: aluminium-backed silica gel 60 F_{254} sheets (Merck, Germany); eluents: hexane/AcOEt 3:1 (A), hexane/AcOEt 1:2 (B), $CHCl_3$/MeOH 4:1 (C). All the anhydrous solvents were distilled over CaH_2, P_2O_5 or sodium prior to the reaction. The UV and CD spectra were recorded on Specord M-400 (Carl Zeiss, Germany) and a J-20 (JASCO, Japan) spectropolarimeter, respectively.^{1}H, ^{13}C, and ^{19}F NMR Spectra were recorded in $CDCl_3$, CD_3OD and (D_6)DMSO with Bruker Avance-500-DRX spectrometer at 500.13, 126.76, and 470.593 MHz, respectively. NMR (δ values) are in ppm downfield from internal $SiMe_4$ (^{1}H, ^{13}C) or external $CFCl_3$ (^{19}F). J values are reported in Hz. All NMR assignments were confirmed by two-dimensional (^{1}H, ^{1}H and ^{1}H, ^{13}C) correlation spectroscopy. Mass spectra were recorded on a chromatomass spectrometer with HPLC-Accela and LCQ Fleet mass detector (Thermo Electron Corporation, USA).

Methyl 5-O-benzoyl-3-deoxy-3-fluoro-2-O-[(4-methylphenyl)sulfonyl]-α-D-ribofuranoside (6)

Standard tosylation of **3** (1.49 g, 5.83 mmol) with TsCl in pyridine followed by column chromatography on silica gel (80 mL), using for elution mixture EtOAc/hexane 1:3 and 1:1, afforded tosylate **4** (2.0 g, 84%) as a syrup. R_f 0.31 (A). ^{1}H NMR ($CDCl_3$): 7.78 (d, 2H, $CH_3C_6H_4SO_2$-), 7.22–7.35 (m, 7H, $C_6H_5CH_2$-, $CH_3C_6H_4SO_2$-), 4.89 (d, 1H, H-1, $J_{1,2}$ = 4.8, H-1), 4.80 (ddd, 1H, $J_{3,2}$ = 4.8, $J_{3,4}$ = 1.2, $J_{3,F}$ = 60.0, H-3), 4.73 (dm, 1H, $J_{2,F}$ = 19.2, H-2), 4.55 (d,1H, $PhCH_2$), 4.45 (d,1H, $PhCH_2$), 4.38 (dm, 1H, H-4, $J_{4,F}$ = 25.8), 3.58 (dd, 1H, H-5, $J_{5,4}$ = 2.5, $J_{5,5'}$ = 10.8), 3.53 (dd, 1H, H-5′, $J_{5,4}$ = 2.9), 3.33 (s, 3H, OCH_3), 2.43 (s, 3H, $CH_3C_6H_4SO_2$-). ^{13}C ($CDCl_3$): 145.4, 137.5, 129.9, 129.3, 128.7, 128.3, 128.1 and 127.8 (s, Bzl and $CH_3C_6H_4SO_2$-),101.3 (s, C-1), 89.1 (d, $J_{C\text{-}3,F}$ = 192.3, C-3), 81.8 (d, $J_{C\text{-}2,F}$ = 23.6, C-2), 75.5 (d, $J_{C\text{-}4,F}$ = 14.7, C-4), 73.8 (s, -$CH_2C_6H_5$), 69.1 (d, $J_{C\text{-}5,F}$ = 9.5, C-5), 55.9 (s, OCH_3), 21.8 (s, $CH_3C_6H_4SO_2$-). Anal. calc. for $C_{20}H_{23}FO_6S$: C, 58.52; H, 5.65; Found : C, 58.63; H, 5.58. ^{19}F ($CDCl_3$): -193.45 (dt, F-3).

To a solution of **4** (1.8 g, 4.38 mmol) in 85 mL anhydrous ethanol, 20% $Pd(OH)_2$/C (3.26 g) and 85 mL cyclohexene was added. The reaction mixture was refluxed for 150 minutes, catalyst was filtered off and washed with EtOH (100 mL). The filtrate was evaporated and coevaporated with toluene (2 × 30 mL). Standard benzoylation of oil product (1.5 g) followed by silica gel (90 mL) column chromatography, using for elution mixture EtOAc/hexane 1:6, 1:2, and 1:1, afforded benzoyl derivative **6** (1.3 g, 70%) as syrup. R_f 0.32 (A). ^{1}H NMR ($CDCl_3$): 8.04 (d, 2H, Bz), 7.80 (d, 2H, J = 8.1, $CH_3C_6H_4SO_2$-), 7.63 (m, 1H, Bz), 7.48 (m, 2H, Bz), 7.30 (d, 2H, J = 8.1, $CH_3C_6H_4SO_2$-),4.94 (d, 1H, H-1, $J_{1,2}$ = 4.6), 4.91 (ddd, 1H, $J_{3,2}$ = 5.4, $J_{3,4}$ = 1.2, $J_{3,F}$ = 56.4, H-3), 4.76 (ddd, 1H, $J_{2,F}$ = 22.8, H-2), 4.60 (dm, 1H, H-4, $J_{4,F}$ = 25.2), 4.51 (dd, 1H, H-5, $J_{5,4}$ = 3.5, $J_{5,5'}$ = 12.0), 4.43 (dd, 1H, H-5′, $J_{5,4}$ = 3.6), 3.39 (s, 3H, OCH_3), 2.44 (s, 3H, $CH_3C_6H_4SO_2$-). ^{13}C ($CDCl_3$): 165.9 (s, C = O, Bz), 145.6, 133.7, 132.9, 130.0, 129.8, 129.3, 128.8 and 128.2 (s, Bz and $CH_3C_6H_4SO_2$-), 101.2 (s, C-1), 88.4 (d, $J_{C\text{-}3,F}$ = 194.6, C-3), 80.3 (d, $J_{C\text{-}2,F}$ = 25.0, C-2), 75.2 (d, $J_{C\text{-}4,F}$ = 15.0, C-4), 63.5 (d, $J_{C\text{-}5,F}$ = 9.1, C-5), 56.0 (s, OCH_3), 21.8 (s, $CH_3C_6H_4SO_2$-). Anal. calc. for $C_{20}H_{21}FO_7S$: C, 56.59; H, 4.99; Found : C, 56.63; H, 5.08. ^{19}F ($CDCl_3$): -192.97 (ddd, F-3).

Methyl 5-O-benzoyl-3-deoxy-3-fluoro-2-O-(imidazolylsulfonyl)-α-D-ribofuranoside (7)

Method A. Sulfuryl chloride (0.8 mL, 9.87 mmol) was added dropwise to a solution of fluoride **3** (1.24 g, 4.84 mmol) in anhydrous CH_2Cl_2 (15 mL) at −40°C. The reaction mixture was stirred at this temperature during 1 hour and then temperature was gradually raised to room temperature for 3 hours. After cooling to 0°C, imidazole (3.35 g, 49.1 mmol) was added to prepared solution and the reaction mixture was stirred at room temperature for 18 hours. The solution was diluted CH_2Cl_2 (20 mL), washed cold water (10 mL), the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over anh. Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica gel (70 mL), using a linear gradient of EtOAc (0→50%, v/v; 500 mL) in hexane, to afford syrupy **5** (1.2 g, 64%). R_f 0.73 (B). ^{1}H NMR ($CDCl_3$): 7.16–7.90 (m, 8H, Ar-H), 4.89 (d, 1H, H-1, $J_{1,2}$ = 4.6), 4.82 (ddd, 1H, $J_{3,2}$ = 5.3, $J_{3,4}$ = 1.0, $J_{3,F}$ = 59.6, H-3), 4.81 (dt, 1H, $J_{2,F}$ = 21.3, H-2), 4.57 (d,1H, $PhCH_2$), 4.48 (d,1H, $PhCH_2$), 4.43 (dm, 1H, H-4, $J_{4,F}$ = 25.4), 3.63 (dd, 1H, H-5, $J_{5,4}$ = 2.4, $J_{5,5'}$ = 10.7), 3.59 (dd, 1H, H-5′, $J_{5,4}$ = 1.8), 3.37 (s, 3H, OCH_3). ^{13}C ($CDCl_3$): 137.5, 137.1, 131.4, 128.8, 128.7, 128.3, 127.9 and 118.4 (s, $C_6H_5CH_2$- and $C_3H_3N_2SO_2$-), 100.4 (s, C-1), 88.5 (d, $J_{C\text{-}3,F}$ = 194.3, C-3), 81.8 (d, $J_{C\text{-}2,F}$ = 23.5, C-2), 78.4 (d, $J_{C\text{-}4,F}$ = 14.5, C-4), 73.9 (s, -$CH_2C_6H_5$), 69.0 (d, $J_{C\text{-}5,F}$ = 9.5, C-5), 55.8 (s, OCH_3). ^{19}F ($CDCl_3$): -193.83 (ddd, F-3). Anal. calc. for $C_{16}H_{19}FN_2O_6S$: C, 49.73; H, 4.96; N, 7.25; Found: C, 49.70; H, 5.02; N, 7.35.

To a solution of imidazolylsulfonate **5** (1.2 g, 3.12 mmol) in 57 mL anhydrous ethanol, 20% $Pd(OH)_2/C$ (2.4 g) and 57 mL cyclohexene was added and the reaction mixture was refluxed for 150 minutes, catalyst was filtered off and washed with EtOH (100 mL). The filtrate was evaporated and coevaporated with toluene (2 × 30 mL). Standard benzoylation of oil product (1.2 g) followed by silica gel (70 mL) column chromatography afforded benzoyl derivative **7** (0.75 g, 64%) as syrup. R_f 0.79 (B). ^{1}H NMR ($CDCl_3$): 7.10–7.98 (m, 8H, Ar-H), 4.97 (ddd, 1H, $J_{3,2} = 5.7, J_{3,4} = 1.8, J_{3,F} = 56.4$, H-3), 4.91 (d, 1H, H-1, $J_{1,2} = 4.6$), 4.78 (dt, 1H, $J_{2,F} = 20.0$, H-2), 4.62 (dm, 1H, H-4, $J_{4,F} = 24.8$), 4.57 (dd, 1H, H-5, $J_{5,4} = 3.4$, $J_{5,5'} = 12.2$), 4.43 (dd, 1H, H-5′, $J_{5,4} = 3.2$), 3.38 (s, 3H, OCH_3). ^{13}C ($CDCl_3$): 165.8 (s, C = O, Bz), 137.1, 133.9, 131.5, 129.6, 129.2, 128.9 and 118.3 (s, C_6H_5CO- and $C_3H_3N_2SO_2$-), 100.2 (s, C-1), 87.8 (d, $J_{C\text{-}3,F} = 196.7$, C-3), 80.1 (d, $J_{C\text{-}2,F} = 24.9$, C-2), 78.0 (d, $J_{C\text{-}4,F} = 14.9$, C-4), 63.1 (d, $J_{C\text{-}5,F} = 8.3$, C-5), 56.0 (s, OCH_3). ^{19}F ($CDCl_3$): -193.55 (dt, F-3). Anal. calc. for $C_{16}H_{17}FN_2O_7S$: C, 48.00; H, 4.28; N, 6.99; Found : C, 48.09; H, 4.32; N, 7.15.

Method B. Sulfuryl chloride (0.15 ml, 1.85 mmol) was added dropwise to a solution of fluorosugar **11** (0.17 g, 0.63 mmol) in anhydrous CH_2Cl_2 (2 mL) and DMF (0.53 ml) at −40°C. The reaction mixture was stirred at this temperature during 50 minutes and then for 90 minutes at room temperature. After cooling to 0°C, 0.55 g (8.08 mmol) imidazole was added to prepared solution and the reaction mixture was stirred at room temperature for 18 hours. After standard work-up and removal of solvent, the residue was chromatographed on a silica gel (25 mL), using for elution mixture EtOAc/hexane 1:3, 1:2, and 2:1, to afford syrupy **7** (0.232 g, 92%) which was identical to imidazolylsulfonate **7** prepared from fluoride **3**.

Methyl 5-O-benzoyl-2,3-dideoxy-2,3-difluoro-α-D-arabinofuranoside (9)

Method A. To a stirred solution of **6** (1.2 g, 2.83 mmol) in anhydrous DMSO (8 ml) and HMPA (0.7 mL) was added freshly dried cesium fluoride (2.3 g, 15.1 mmol). The reaction mixture was stirred at 190°C for 70 minutes. After cooling to room temperature the reaction mixture was poured into water (30 ml), and extracted with EtOAc (4 × 90 mL). The combined organic extracts was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica gel (200 mL), using a linear gradient of EtOAc (0→33%, v/v; 600 mL) in hexane, to afford syrupy difluoride **9** (0.08 g, 10%). R_f 0.74 (A). ^{19}F ($CDCl_3$): −195.78 (F-2, m), −194.28 (F-3, m, $J_{F\text{-}2,F\text{-}3} = 8.08$). HPLC/APCI-MS, m/z 272 M^+, 241 $(M\text{-}OCH_3)^+$. Anal. calc. for $C_{13}H_{14}F_2O_4$: C, 57.35; H, 5.18. Found: C, 56.90; H, 5.02.

Vinyl fluoride **8** (0.183 g, 26%) as syrup. R_f 0.65 (A). ^{1}H NMR ($CDCl_3$): 8.04 (d, 2H, Bz), 7.59 (m, 1H, Bz), 7.48 (m, 2H, Bz), 5.79 (m, 1H, H-2, $J_{2,1} = 1.3, J_{2,4} = J_{2,F} = 4.2$), 5.35 (t, 1H, H-1, $J_{1,F} < 1.0, J_{1,4} = 1.3$), 5.09 (m, H-4), 4.65 (dd, 1H, H-5, $J_{5,4} = 2.8$, $J_{5,5'} = 12.1$), 4.41 (dd, 1H, H-5′, $J_{5',4} = 4.0$), 3.44 (s, 3H, OCH_3). ^{13}C($CDCl_3$): 166.2 (s, C=O, Bz), 161.5 (d, $J_{C\text{-}3,F} = 287.0$, C-3), 133.4, 129.9, 129.8, 128.6 (s, C_6H_5CO-), 106.4 (d, $J_{C\text{-}2,F} = 15.1$, C-2), 101.8 (d, $J_{C\text{-}1,F} = 7.2$, C-1), 76.6 (d, $J_{C\text{-}4,F} = 25.4$, C-4), 63.4 (d, $J_{C\text{-}5,F} = 1.9$, C-5), 54.3 (s, OCH_3). ^{19}F ($CDCl_3$): −131.6 (m, F-3). HPLC/APCI-MS, m/z 252 M^+. Anal. calc. for $C_{13}H_{13}FO_4$: C, 61.90; H, 5.19. Found: C, 62.10; H, 5.27.

Method B. To a solution of **7** (0.75 g, 1.87 mmol) in freshly distilled 2,3-butanediol (7.2 mL) was added KHF_2 (0.61 g, 7.8 mmol) and suspension was stirred under argon at 160°C for several minutes, and then HF (0.36 mL, 46% in H_2O) was added to this mixture. The reaction mixture was stirred at 160°C during 50 minutes. After the standard work up and removal of solvent, the residue was chromatographed on a silica gel (100 mL), using for elution mixture EtOAc/hexane 1:8, 1:6 to afford syrup difluoride **9** (0.25 g, 49%) which was identical to the one described above.

Method C. To a solution of xyloside **10** (0.3 g, 1.12 mmol) in anhydrous CH_2Cl_2 (6.5 mL) at room temperature was added under argon (0.82 ml, 6.19 mmol) DAST and the reaction mixture was stirred at this temperature during 150 minutes and then for 18 hours at 27–29°C under argon. After cooling, the reaction mixture was poured into saturated cooled aqueous $NaHCO_3$ (75 mL). When evolution of gas ceased, it extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over anh. Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel (90 mL), using for a linear gradient of hexane/EtOAc/2:1 (v/v; 500 mL) in hexane/EtOAc/8:1, to afford difluoride **9** (0.103 g, 34%) and fluoride **11** (0.058 g, 19%) which was identical to the one prepared earlier.[15] ^{19}F for **11** ($CDCl_3$): -195.58 (dt, F-3).

1-O-Acetyl-5-O-benzoyl-2,3-dideoxy-2,3-difluoro-α/β-D-arabinofuranoside (12)

Concentrated H_2SO_4 (0.1 mL) was added to a solution of fluoride **9** (0.337 g, 1.24 mmol) in acetic acid (2.33 mL) and acetic anhydride (0.59 mL) at 0°C. The reaction mixture was stirred at this temperature during 30 minutes and left at +4°C for 18 hours, then it was poured into mixture ice/water. After ice melted, the aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic extracts was washed aqueous $NaHCO_3$, dried over anh. Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica gel (55 mL), using for elution mixture EtOAc/hexane 1:3 and 1:1 to afford **12** (0.286 g, 77%) as syrup. R_f 0.48 (A). ^{1}H NMR ($CDCl_3$): (α, β ratio ca. 3:1), 7.43–8.08 (m, ArH), 6.42 (d, H-1 α, $J_{1,F\text{-}2} = 10.57$), 6.39 (d, H-1 β, $J_{1,2} = 4.7$), 5.29–5.49 (m, H-2 β and H-3 β), 5.21 (dd, H-2 α), 5.20 (ddd, H-3 α), 4.70 (ddt, H-4 α), 4.40–4.60 (m, H-5 α, β and H-5′ α, β, H-4 β), 2.12 (s, OAc α), 2.00 (s, OAc β). HPLC/APCI-MS, m/z 241 $(M\text{-}CH_3COO)^+$. ^{19}F ($CDCl_3$, for α-anomer): −195.3 (F-2, m), −192.9 (F-3, m, $J_{F\text{-}2,F\text{-}3} = 8.28$). Anal. calc. for $C_{14}H_{14}F_2O_5$: C, 56.00; H, 4.70; Found : C, 56.11; H, 4.65.

9-(2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)adenine (20) and its α-anomer (21)

TMSiBr (0.2 ml, 1.5 mmol) was added to a solution of **12** (0.19 g, 0.64 mmol) in $CDCl_3$ (1.4 mL) at 0°C and the mixture was stirred at this temperature for 30 minutes. After standing at room temperature for 14 days, the reaction mixture was evaporated, and coevaporated with anhydrous toluene (3 × 4 mL) and **13** (content ≈50% according to TLC data and ^{1}H NMR data, 6.55 ppm, d, $J_{1,F}$ = 12.6, H-1 and 4.85 ppm, ddt, H-4 for 1-α-bromo sugar **13**) was used in the next step without purification.

The solution of **13** in anhydrous THF (10 mL) was added to sodium salt of N^6-benzoyladenine, prepared from 0.098 g (0.40 mmol) N^6-benzoyladenine and NaH in oil (14 mg of 80% in oil, 0.46 mmol). The reaction mixture was refluxed for 5 hours, filtered off, and washed with CH_2Cl_2. After evaporation of combined filtrates, the residue was chromatographed on silica gel (120 mL), using a linear gradient of EtOAc (0→66%, v/v; 500 mL) in hexane, to afford syrupy nucleoside **15** (14 mg, 9%). R_f 0.44 (B). ^{1}H NMR ($CDCl_3$): 9.09(s, 1H, NH), 8.84 (s, 1H, H-2), 8.15 (s, 1H, H-8), 7.48–8.08 (5m, 10H, 2Bz), 6.55 (dd, 1H, $J_{1',2'}$ = 1.1, $J_{1',F\text{-}2'}$ = 15.6, H-1′), 6.03 (ddm, 1H, H-2′), 5.46 (ddm, 1H, H-3′), 5.11 (dm, 1H, H-4′), 4.64 (dd, 1H, H-5′), 4.60 (dd, 1H, H-5″). HPLC/APCI-MS, m/z 479 M^+.

14 (36 mg, 23%) as syrup. R_f 0.33 (B) ^{1}H NMR ($CDCl_3$): 9.16 (s, 1H, NH), 8.14 (s, 1H, H-2), 8.25 (d, 1H, $J_{F2',H8}$ = 2.9, H-8), 7.47–8.08 (5m, 10H, 2Bz), 6.68 (dt, 1H, $J_{1',F\text{-}2'}$ = 22.3, $J_{1',2'}$ = $J_{1',F\text{-}3'}$ = 2.67, H-1′), 5.47 (dm, 1H, H-2′), 5.38 (ddd, 1H, H-3′), 4.62–4.75 (m, 3H, H-4′, 2H-5′). HPLC/APCI-MS, m/z 479 M^+.

Deprotection of **14** and **15** with methanol saturated at 0°C by ammonia and subsequent chromatographic purification on silica gel using for elution mixture $CHCl_3$:MeOH-20:1 and 6:1 afforded adenine nucleosides **20** (18 mg, 88%) and **21** (6.4 mg, 81%), respectively. Compound **20**. R_f 0.64 (C). m.p. 166–169°C (from EtOH/ether); UV (EtOH) λ_{max}, nm (ε): 259 (14000), λ_{min}, nm (ε): 227 (2000). CD (EtOH), λ, nm ($[\theta]\cdot 10^{-3}$): 205 (+6.4), 214(0), 218 (-2.2), 226 (0), 248 (+1.3), 252 (0), 263 (−1.7), 288 (0). HPLC/APCI-MS, m/z 271 M^+. ^{19}F (CD_3OD):−204.6 (m, F-C2′ or F-C3′, $J_{F\text{-}2',F\text{-}3'}$ is not determined), −195.8 (m, F-C2′ or F-C3′). Anal. calc. for $C_{10}H_{11}F_2N_5O_2$: C, 44.28; H, 4.09; Found: C, 44.19; H, 4.00.

Compound **21**. R_f 0.64 (C). m.p. 96–100°C (from EtOH/ether); UV (EtOH) λ_{max}, nm (ε): 259 (14300), λ_{min}, nm (ε): 227 (2100). CD (EtOH)), λ, nm ($[\theta]\cdot 10^{-3}$): 205 (+6.2), 214(0), 217 (-4.7), 220 (0), 230 (+1.9), 255 (+0.8), 260 and 288 (0). HPLC/APCI-MS, m/z 271 M^+. ^{19}F (CD_3OD):−187.29 (m, F-C2′ or F-C3′), −183.08 (m, F-C2′ or F-C3′, $J_{F\text{-}2',F\text{-}3'}$ is not determined). Anal. calc. for $C_{10}H_{11}F_2N_5O_2$: C, 44.28; H, 4.09; Found: C, 44.35; H, 4.17.

2-Chloro-9-(2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl) adenine (22) and its α-anomer (23)

A suspension of 2,6-dichloropurine (0.064 g, 0.332 mmol) in anhydrous acetonitrile (3.0 mL) at room temperature was treated NaH (11.0 mg of 80% in oil, 0.034 mmol), and the mixture was stirred for 30 minutes under argon. To this stirred suspension, a solution bromide **13** prepared from **12** (0.19 g, 0.64 mmol) as described above was added in anhydrous acetonitrile (4 mL) and the reaction mixture was stirred at room temperature overnight. Insoluble material was removed by filtration and washed with acetonitrile (5 mL). The combined filtrate and washings were evaporated and the residue was chromatographed on silica gel (130 mL) using for elution mixture EtOAc/hexane 1:3 and 2:3 to afford mixture nucleosides **16** and **17** (0.066 g, 48%) as syrup. R_f 0.48 (A). ^{1}H NMR ($CDCl_3$): 8.32 (d, H-8β, $J_{F2',H8} = 2.9$, 2.9H), 8.26 (s, H-8α, 1H), 7.44–8.06 (m, 4Bz), 6.59 (dt, 1H, $J_{1',F\text{-}2'} = 21.97$, $J_{1',2'} = J_{1',F\text{-}3'} = 2.6$, H-1′ β), 6.56 (br. d, 1H'α, $J_{1',2'} < 1.0$, $J_{1',F\text{-}2'} = 19.1$, H-1′), 5.75–5.93 (m, H-2′α and H-3′α), 5.46 (dd, H-2′β), 5.36(ddd, H-3′β), 5.09 (dm, H-4′α), 4.52–4.75 (m, 2H-5′α, 2H-5′β and H-4′β). (UV (EtOH) λ_{max}, nm (ε): 274 (5660), 231 (7300).

A solution of mixture nucleosides **16** and **17** (0.066 g, 0.154 mmol) in anhydrous 1,2-dimethoxyethane (10 mL) was saturated by dry ammonia for 4 hours and then left for 18 hours at room temperature. The reaction mixture was filtered off and washed with CH_2Cl_2. The combined filtrate and washings were evaporated and the residue was chromatographed on silica gel (130 mL), using a linear gradient of EtOAc (0→66%, v/v; 500 mL) in hexane, to afford nucleoside **18** as foam (42 mg, 67%). R_f 0.60 (B). ^{1}H NMR ($CDCl_3$): 7.45–8.06 (3m, 5H, Bz), 7.99 (d, 1H, $J_{F2',H8} = 3.0$, H-8), 6.52 (dt, 1H, $J_{1',F2'} = 12.5$, H-1′), 6.32 (br. s, 2H, NH_2), 5.41 (dd, H-2′), 5.31 (ddd, H-3′), 4.54–4.70 (m, 2H-5′and H-4′). HPLC/APCI-MS, m/z, 410 and 412, Cl^{35}/Cl^{37} ratio ≈ 3:1, M^+. Nucleoside **19** (16 mg, 25%) as foam. R_f 0.46 (B). ^{1}H NMR ($CDCl_3$): 7.47–8.10 (3m, 5H, Bz), 7.97 (s, 1H, H-8), 6.48 (d, 1H, $J_{1',F2'} = 14.9$, H-1′), 6.18 (br. s, 2H, NH_2), 5.88 (dm, 1H, H-2′), 5.44 (dm, 1H, H-3′), 5.07 (dm, 1H, H-4′), 4.63 (dd, 1H, H-5′), 4.58 (dd, 1H, H-5″). HPLC/APCI-MS, m/z 410 and 412, Cl^{35}/Cl^{37} ratio ≈ 3:1, M^+.

Solution of nucleoside **18** (36 mg, 0.088 mmol) in 10 mL methanol saturated at 0°C by ammonia was kept during 195 minutes at room temperature and then was evaporated. The residue was chromatographed on silica gel (40 mL) using for elution mixture $CHCl_3$:MeOH-20:1 and 15:1 to afford nucleoside **22** (20 mg, 75%). R_f 0.75 (c). m.p. 171–173°C ($CHCl_3$/EtOH). UV λ_{max}, nm (ε): 263 (14900) at pH 7, 263 (15000) at pH 13, 263 (13900) at pH 1. CD (EtOH), λ, nm ($[\theta]\cdot 10^{-3}$): 207 (+12.9), 212(0), 217 (−4.0), 231 (0), 260 (−1.7), 288 (0). HPLC/APCI-MS, m/z 307 and 309, Cl^{35}/Cl^{37} ratio ≈ 3:1, $(M+H)^+$. ^{19}F (DMSO-d_6): −202.47 (m, F-C2′), −196.40 (m, F-C3′, $J_{F\text{-}2',F\text{-}3'} = 8.48$). Anal. calc. for $C_{10}H_{10}F_2ClN_5O_2$: C, 39.29; H, 3.30; Cl, 11.60; Found : C, 39.40; H, 3.47; Cl, 11.72.

In a similar way, nucleoside **23** (6 mg, 80%) was prepared starting from nucleoside **19** (10 mg, 0.024 mmol). R_f 0.75 (C). m.p. 176–178°C ($CHCl_3$/EtOH). UV λ_{max}, nm (ε): 263 (14500) at pH 7, 263 (14700) at pH 13, 263 (13900) at pH 1. CD (EtOH), λ, nm ($[\theta]\cdot10^{-3}$): 205 (+11.6), 212(0), 217 (−8.5), 228 (0), 255 (+1.1), 269 (0). HPLC/APCI-MS, m/z 307 and 309, Cl^{35}/Cl^{37} ratio ≈ 3:1, $(M+H)^+$. ^{19}F (DMSO-d_6): −195.21 (m, F-C2′, $J_{F\text{-}2',F\text{-}3'} = 8.48$), −195.76 (m, F-C3′). Anal. calc. for $C_{10}H_{10}F_2ClN_5O_2$: C, 39.29; H, 3.30; Cl, 11.60; Found : C, 39.38; H, 3.20; Cl, 11.69.

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