Raymond F. Schinazi*, Grigorii G. Sivets, Mervi A. Detorio, Tami R. McBrayer, Tony Whitaker, Steven J. Coats and Franck Amblard

Synthesis and antiviral evaluation of 2′,3′-dideoxy-2′,3′-difluoro-D-arabinofuranosyl 2,6-disubstituted purine nucleosides

DOI 10.1515/hc-2015-0174
Received August 14, 2015; accepted September 11, 2015

Abstract: The synthesis of new 2,6-disubstituted purine 2′,3′-dideoxy-2′,3′-difluoro-D-arabino nucleosides is reported. Their ability to block HIV and HCV replication along with their cytotoxicity toward Huh-7 cells, human lymphocyte, CEM and Vero cells was also assessed. Among them, β-2,6-diaminopurine nucleoside **25** and guanosine derivative **27** demonstrate potent anti-HIV-1 activity (EC_{50} = 0.56 and 0.65 μM; EC_{90} = 4.2 and 3.1 μM) while displaying only moderate cytotoxicity in primary human lymphocytes.

Keywords: fluorine; HCV; HIV; nucleoside; purine.

Dedication: This work is dedicated to our friend and colleague Dr. Kyoichi (Kyo) Watanabe who passed away on April 7, 2015. We will miss his wisdom, encyclopedic memory on nucleosides, and adorable smiling face.

Introduction

The chemical synthesis and biochemical properties of fluorine-containing nucleosides have been the focus of numerous studies over the years [1]. Recent advances in medicinal chemistry indicate conclusively that synthetic fluorinated nucleoside analogs represent a valuable class of drugs for the treatment of various diseases [2–5].

***Corresponding author: Raymond F. Schinazi,** Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA; and Veterans Affairs Medical Center, Decatur, GA 30033, USA, e-mail: rschina@emory.edu
Grigorii G. Sivets: Institute of Bioorganic Chemistry, National Academy of Sciences, Acad. Kuprevicha 5, 220141 Minsk, Belarus
Mervi A. Detorio and Franck Amblard: Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA; and Veterans Affairs Medical Center, Decatur, GA 30033, USA
Tami R. McBrayer, Tony Whitaker and Steven J. Coats: Cocrystal Pharma, Inc., Tucker, GA 30084, USA

Introduction of a fluorine atom into nucleoside analogs can lead to a change in biological activity, lipophilicity, or bioavailability. The small size and strong electronegativity of the fluorine atom, which can mimic either a hydrogen or a hydroxyl group, may critically influence the pharmacokinetic properties and/or toxicity of a drug [1]. It has been established that fluorination of either the sugar moiety or the heterocyclic base of a nucleoside may alter its affinity with various metabolic enzymes and can radically affect the conformation of the pentofuranose ring of the nucleoside in solution [6, 7]. Nucleosides containing fluorine at C2′ exhibit potent biological activities [8, 9]. Among them, C2′-β-fluoro purine nucleosides are of special interest because the location of the fluorine atom in the β-orientation can affect their phoshorylation, the metabolic stability of the glycosidic bond and potentially their antiviral and anticancer activities. For instance, fluorinated nucleosides such as sofosbuvir and gemcitabine (Figure 1) have been approved for the treatment of hepatitis C virus (HCV) and various cancers, respectively. On the other hand, lodenosine (2′-β-fluoro-2′,3′-dideoxyadenosine, **1**) displays *in vitro* activity against HIV-1 and appears chemically and metabolically stable (Figure 1) [10].

A number of other purine nucleosides with the 2′-fluoro-β-D-arabinofuranosyl moiety have been synthesized and tested for their antitumor activity, including 9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)guanine (**3**), which displays activity against human leukemic T-cell lines [11, 12]. In addition, 3′-α-fluoro-2′,3′-dideoxyguanosine (**4**) was studied clinically for the treatment of HIV and HBV infected patients (Figure 1) [13, 14]. The HBV studies were subsequently abandoned due to a lack of advantage versus standard of care (http://www.medivir.se/v5/en/uptodate/pressrelease.cfm). In earlier investigations, we disclosed 9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)adenine (**2**), a unique difluorinated nucleoside that was more potent *in vitro* against HIV-1 than lodenosine **1** [15]. Based on this work, we have decided to further evaluate this new class of compounds and wish to describe herein the synthesis of new D-*arabino*-2′,3′-dideoxy-2′,3′-difluoronucleosides along with their biological evaluation for *in vitro* anti-HIV-1 and anti-HCV activities.

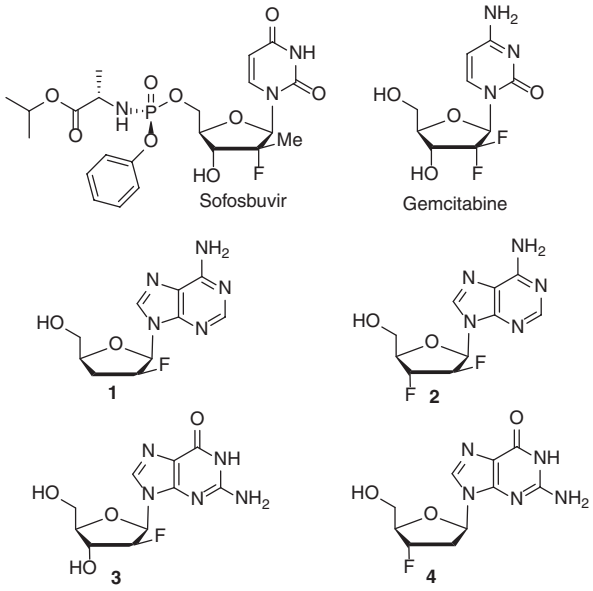
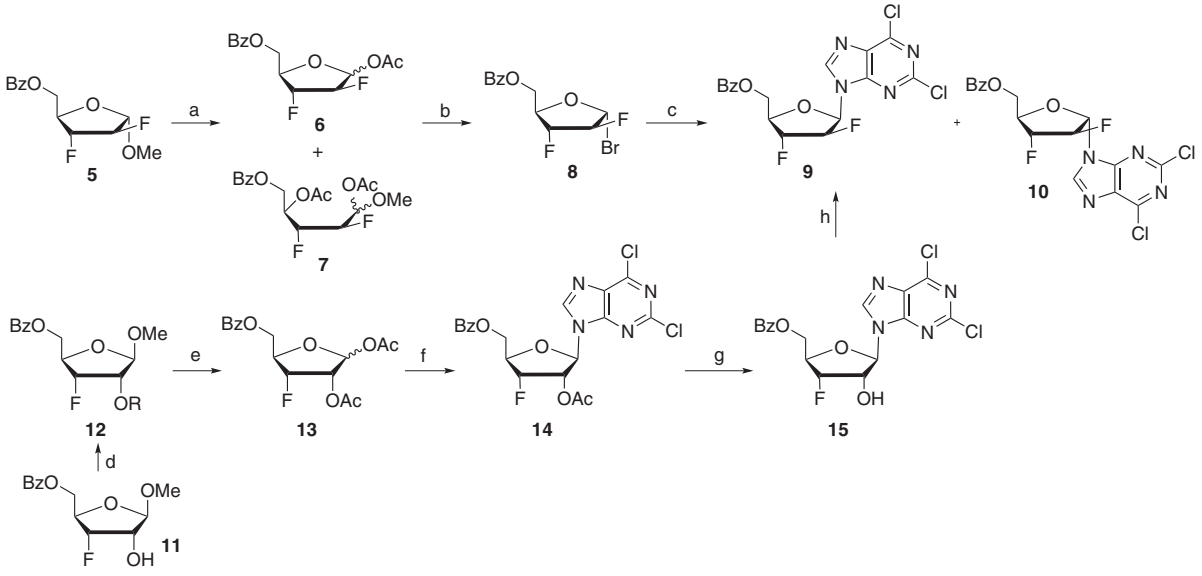


Figure 1 FDA approved 2′-fluoro nucleosides and biologically active fluorine containing purine nucleosides **1–4**.

Results and discussion

In order to prepare our library of purine nucleosides, the synthesis of key 2,6-dichloropurine nucleoside **9** was optimized (Scheme 1).

Thus, treatment of **5** in a mixture of acetic acid/acetic anhydride/H_2SO_4 at room temperature (instead of 4°C) [15], allowed for the formation of 1-*O*-acetyl derivative **6** in 87% yield (α/β ratio ca. 3:1) with no traces of acyclic compound **7** formed. Treatment of **6** with TMSBr in anhydrous CH_2Cl_2 in the presence of the inexpensive and easily available catalyst $ZnBr_2$ lead to almost quantitative formation of 1′-α-bromide **8.** The α-anomeric configuration of bromo sugar **8** was confirmed by the $^3J_{H\text{-}1,F\text{-}2}$ and $^3J_{H\text{-}1,H\text{-}2}$ values (12.6 Hz and < 1.0 Hz, respectively) observed by ^{1}H NMR (Figure 2) and by comparison with known 1′-α-bromo anomer of 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose [16]. Next, reaction of 5-*O*-benzoyl-2,3-dideoxy-2,3-difluoro-α-D-ara-binofuranosyl bromide (**8**) with the sodium salt of 2,6-dichloropurine [17] gave a 4:1 mixture of protected nucleosides **9** and **10,** which were separated by column chromatography on silica gel (Scheme 1). In an effort to improve the β/α ratio during the glycosylation reaction [18–20], the use of the potassium salt of 2,6-dichloropurine was evaluated. Interestingly using this salt in anhydrous acetonitrile led to the formation of N^9-β-D-nucleoside **9** and –α-D-protected nucleoside **10** in 73% and 8% yield, respectively (9/1 ratio). An alternative linear approach to the key intermediate **9** was also investigated from known fluorodeoxysugar **11** (Scheme 1) [21]. Thus, compound **11** was first acetylated in 86% and then allowed to react in a mixture of Ac_2O/AcOH/H_2SO_4 to give acetyl derivatives **13** in 97% yield (α/β ratio ≈ 1:1). The glycosylation of **13** with a silylated 2,6-dichloropurine under Vorbrüggen conditions was investigated. Thus, the coupling reaction



Scheme 1 Reagents and conditions: (a) AcOH/Ac_2O/cc H_2SO_4 0°C to 4°C (**6**, 77%; **7**, 10–12%); or 0°C to rt, (**6**, 87%); (b) TMSBr/CH_2Cl_2/$ZnBr_2$, 0°C to rt; 95%; (c) i) Na salt of 2,6-dichloropurine/CH_3CN, rt (**9**, 55%, **10**, 13%); ii) K salt of 2,6-dichloro purine/CH_3CN, rt, (**9**, 73%, **10**, 8%); (d) Ac_2O/Py, rt, 86%; (e) AcOH/Ac_2O/conc. H_2SO_4, rt, 97%; (f) TMSOTf, silylated 2,6-dichloropurine/CH_3CN/DCE, rt, 89%; (g) $NaHCO_3$, MeOH, rt, 63%; (h) DAST/CH_2Cl_2/Py, rt, 35%.

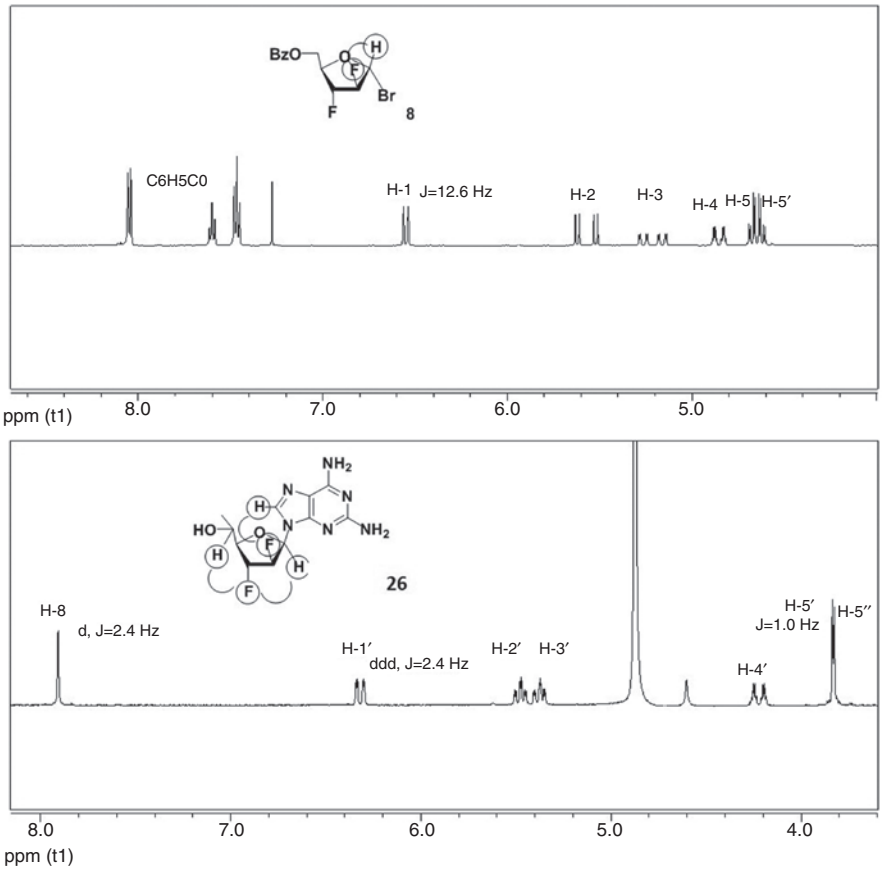
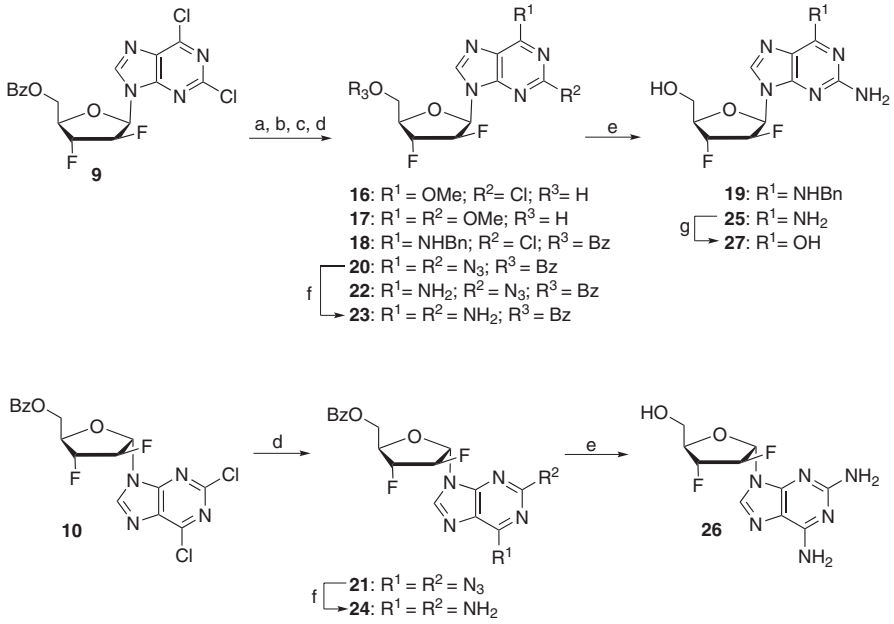


Figure 2 ^{1}H NMR spectra of 1-α-bromide **8** and β-2′,3′-difluoroarabinonucleoside of 2,6-diaminopurine **26**.

in the presence of TMSOTf under reflux in acetonitrile gave a complicated mixture of products from which β-2,6-dichloropurine nucleoside **14** (26%) was isolated. On the other hand, coupling of acetate derivative **13** with silylated 2,6-dichloropurine in a mixture of acetonitile-1,2-dichloroethane, and in the presence of TMSOTf gave desired 2,6-dichloropurine **14** in 89% yield. Selective deprotection of nucleoside **14** with $NaHCO_3$ in methanol produced **15** in 63% yield and final fluorination with diethylaminosulfur trifluoride (DAST) in the presence of pyridine in dichloromethane at room temperature gave β-2′,3′-difluoro arabinonucleoside **9** in 35% yield.

Treatment of protected nucleoside **9** with 1.1 equivalents of sodium methoxide in methanol at room temperature produced 2-chloro-6-methoxypurine arabinoside **16**, in 84% yield. 2,6-Dimethoxypurine arabinoside **17** was prepared in 52% yield by reaction of **9** with 2.7 equivalents of sodium methoxide in methanol (Scheme 2) [22]. The treatment of nucleoside **9** with 5.0 equivalents of benzylamine in methanol at 55°C afforded 2-chloro-6-benzylaminopurine arabinoside **18** in 78% yield. Removal of the acyl group in **18** with saturated methanolic ammonia gave 2-chloro-6-benzylaminopurine analog **19** in 82% yield. Reaction of each β- and α-protected nucleosides of 2,6-dichloropurine **9** and **10** with LiN_3 in EtOH under reflux afforded 2,6-diazido derivatives **20** and **21** in 97% yield. The reduction of both azido groups with $SnCl_2$ in a mixture of dichloromethane-methanol [23] resulted in the formation of 5′-*O*-benzoyl derivatives of N^9-β- and N^9-α-arabinosides **23** (87%) and **24** (91%). It is noteworthy that 2-azido-6-amino derivative **22** was also isolated in 4% yield after reduction of β-protected nucleoside **20** with stannous chloride (Scheme 2). Subsequent debenzoylation of intermediates **23** and **24** with saturated methanolic ammonia, gave pure 2′,3′-dideoxy-2′,3′-difluoronucleosides 2,6-diaminopurine **25** and **26** in 72% and 79% yield, respectively. Guanine nucleoside **28** was prepared by enzymatic deamination of N^9-β-nucleoside **25** in water with calf intestine adenosine deaminase in 85% yield (Scheme 2).

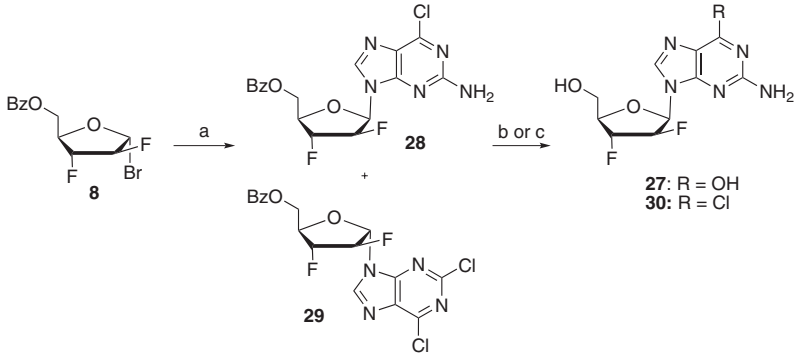
An alternative approach to prepare guanine analog **28** from bromide **8** was also investigated (Scheme 3). Treatment of 2-amino-6-chloropurine with potassium



Scheme 2 Reagents and conditions: (a) MeONa/MeOH, rt, **16,** 84%; (b) MeONa/MeOH, rt and then reflux, **17**, 52%; (c) $BnNH_2$/MeOH, 55°C, **18**, 78%; (d) LiN_3/EtOH, reflux, (**20**, 97%, **21**, 97%); (e) saturated NH_3/MeOH, rt, (**19**, 82%, **25**, 72%, **26**, 79%); (f) $SnCl_2/CH_2Cl_2$/MeOH, rt, (**22**, 4%, **23**, 87%; **24**, 91%); (g) Adenosine deaminase/H_2O, rt, **27**, 85%.

t-butoxide in 1,2-dimethoxyethane followed by coupling of the resulting salt with bromo sugar **8** in acetonitrile at room temperature gave a mixture of N^9-β- and N^9-α-nucleosides **28** and **29** which were separated by column chromatography in 50% and 4% yields, respectively. The benzoyl-protected 2-amino-6-chloropurine analog **28** was converted to guanine derivative **27** (71%) by treatment with 2-mercaptoethanol and sodium methoxide in refluxing methanol. Finally, in order to extend our series of novel purine nucleosides, debenzoylation of protected β-nucleoside **28** with saturated methanolic ammonia at room temperature afforded 2-amino-6-chloropurine analog **30** in 77% yield.

Structures of nucleosides **9, 10**, **14**, **16–30** were confirmed by ^1H, ^{19}F and ^{13}C NMR, UV and mass spectroscopy. Assignment of the configurations of synthesized nucleosides at the anomeric centers are based upon ^1H NMR analysis (long-range couplings between the H-8 proton of the purine and the 2′-β fluorine atom for the β-anomers) and ^{13}C NMR data (characteristic $J_{C\text{-}1',F\text{-}2'}$ coupling constants for β- and α-anomers of purine 2′,3′-difluoro-D-arabinofuranosyl nucleosides) (Tables 1–4). The resonance signal of the purine H-8 proton for 2,6-diaminopurine nucleoside **25** is displayed as a doublet in its ^1H NMR spectrum and the magnitude of the $^5J_{H,F}$ coupling is 2.4 Hz (Figure 2). It should be noted that two $^4J_{H,F}$ the long-range



Scheme 3 Reagents and conditions: (a) K salt of 2-amino-6-chloropurine/CH_3CN, rt, (**28**, 50%, **29**, 4%); (b) saturated NH_3/MeOH, rt, 77%; (c) $HSCH_2CH_2OH$/MeONa/MeOH, reflux, 71%.

Table 1 ^{1}H NMR Chemical shifts of 2′,3′-dideoxy-2′,3′-difluoro nucleosides **9**, **10**, **16**, **17**, **25–27** with D-*arabino*-configurations.

Compound	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	Others
9	6.60 dt	5.46 br.dd	5.35 ddd	4.74 dm	4.65–4.71 m		8.32 (d, 1H, *J*=2.63, H-8), 7.46-8.07 (m, 5H, Bz)
10	6.55 d	5.84 dd	5.47 ddt	5.09 ddt	4.64 dd	4.57 dd	8.24 (s, 1H, H-8), 7.47–8.08 (m, 5H, Bz)
16	6.55 dd	5.52 dddd	5.49 dddd	4.29 dm	3.87 dd	3.84 dd	8.46 (d, 1H, *J*=2.10, H-8), 4.17 (s, 3H, OCH_3)
17	6.49 ddd	5.51 dddd	5.45 dddd	4.26 dm	3.85 dd	3.83 dd	8.24 (d, 1H, *J*=2.13, H-8), 4.13 and 4.02 (2s, 6H, 2×OCH_3)
25	6.32 ddd	5.44 dddd	5.40 dddd	4.22 dm	3.84 ddd	3.82 dd	7.91 (d, 1H, *J*=2.42, H-8)
26	6.22 dd	5.99 ddt	5.36 dddd	4.69 ddt	3.75 dd	3.72 dd	7.84 (s, 1H, H-8)
27	6.15 dd	5.60 dddd	5.56 dddd	4.10 dm	3.65 br.m	3.61 br.m	10.66 (brs, 1H, NH), 7.74 (d, 1H, *J*=2.9, H-8), 6.51 (brs, 2H, NH_2), 5.17 (t, 1H, *J* = 5.64, 5′-OH)

Spectra were obtained in $CDCl_3$ for nucleosides **9**, **10**; in CD_3OD for **16**, **17**, **25**, **26**, and DMSO-d_6 for nucleoside **27**. δ in ppm, *J* in Hz.

Table 2 Coupling constants (in Hz) for ^{1}H NMR data of 2′,3′-dideoxy-2′,3′-difluoro nucleosides **9**, **10**, **16**, **17**, **25–27** with D-*arabino*-configurations.

	^{3}J(H,H)				^{3}J(H,F)				Others
	1′,2′	2′,3′	3′,4′	4′,5′/4′,5″	H1′,F2	H3′,F2	H2′,F3	H4′,F3	
9	2.56	<1.0	2.38	4.37/n.d.	21.8	9.2	11.94	n.d	$^{5}J_{F2',H8}$ = 2.63 $^{4}J_{1',F3'}$ = 2.56 $^{gem}J_{2',F2'}$ = 50.03 $^{gem}J_{3',F3'}$ = 49.51 $J_{H5',H5''}$ = 11.2
10	<1.0	1.8	1.7	5.48/5.5	15.02	12.6	11.22	23.34	$^{gem}J_{2',F2'}$ = 48.14 $^{gem}J_{3',F3'}$ = 50.12 $J_{H5',H5''}$ =12.14
16	4.16	2.28	3.90	3.31/2.53	16.93	15.45	12.87	24.10	$^{5}J_{F2',H8}$ = 2.1 $^{4}J_{1',F3'}$ = 1.64 $^{gem}J_{2',F2'}$ = 50.65 $^{gem}J_{3',F3'}$ = 51.29 $^{gem}J_{H5',H5''}$= 12.1
17	3.89	2.19	3.80	3.85/5.12	17.29	15.70	12.50	24.36	$^{5}J_{F2',H8}$ = 2.13 $^{4}J_{1',F3'}$ = 1.8 $^{gem}J_{2',F2'}$ = 50.33 $^{gem}J_{3',F3'}$ = 49.51 $J_{H5',H5''}$ = 11.2
25	3.87	2.0	3.85	4.62/5.33	18.37	12.18	14.69	25.0	$^{5}J_{F2',H8}$ = 2.42 $^{4}J_{1',F3'}$ = 2.0 $^{gem}J_{2',F2'}$ = 51.29 $^{gem}J_{3',F3'}$ = 50.65 $^{gem}J_{H5',H5''}$= 12.8 $^{4}J_{5',F3'}$ = 1.0 $^{4}J_{5',F3'}$ = <1.0
26	2.56	2.88	4.17	4.40/3.7	15.37	16.35	14.42	20.52	$^{gem}J_{H5',H5''}$= 11.5 $^{gem}J_{3',F3'}$ = 52.25 $^{gem}J_{2',F2'}$ = 50.33
27	4.16	3.2	3.21	5.48/5.42	16.34	16.03	14.42	22.91	$^{5}J_{F2',H8}$ = 2.9 $^{gem}J_{2',F2'}$ = 50.64 $^{gem}J_{3',F3'}$ = 51.61 $^{gem}J_{H5',H5''}$= 12.8

Table 3 ^{13}C NMR Data of 2′,3′-difluoro nucleosides **9**, **10**, **16**, **17**, **25–27**, **30**.

Compound	Chemical shifts, δ_{TMS}, ppm [J(C,F) in Hz]					Others
	C-1′	C-2′	C-3′	C-4′	C-5′	
9	83.73 d	93.64 dd	91.57 dd	81.31 d	62.46 d	166.17 (s, Ph-***C***=O), 128.81–133.85 (*Ph*-C=O and C-5), 153.57, 152.56, 152.35 (C-6, 2, 4), 144.88 (d, $^4J_{C8,F2'}$ = 5.6, C-8)
10	88.92 d	96.09 dd	94.64 dd	84.23 d	62.40 d	166.08 (s, Ph-***C***=O), 128.13–133.78 (*Ph*-C=O and C-5), 153.71, 152.58, 152.29 (C-6, 2, 4), 143.35 (d, $^4J_{C8,F2'}$ = 3.8, C-8)
16	83.07 dd	93.42 dd	92.69 dd	82.17 dd	60.16 d	161.35 (C-6) 153.34 (C-2) 152.63 (C-4) 142.79 (d, $^4J_{C8,F2'}$ = 4.23, C-8) 119.48 (C-5) 54.44 (OCH_3)
17	82.79 dd	93.51 dd	92.76 dd	81.96 dd	60.19 d	162.26 (C-6 or C-2) 162.02 (C-2 or C-6) 153.07 (C-4) 140.73 (d, $^4J_{C8,F2'}$ = 4.14, C-8) 115.98 (C-5) 54.50 and 53.69 (2×OCH_3)
25	82.63 dd	93.65 dd	92.60 dd	81.82 dd	60.32 d	160.74 (C-6) 156.33 (C-4) 151.32 (C-2) 137.19 (d, $^5J_{C8,F2'}$ = 4.38, C-8) 112.36 (C-5)
26	87.12 dd	96.58 dd	93.99 dd	84.35 dd	60.37 d	160.77 (C-6) 156.36 (C-4) 151.35 (C-2) 136.23 (d, $^5J_{C8,F2'}$ = <2.0, C-8) 113.23 (C-5)
27	81.40 dd	94.10 dd	92.62 dd	81.16 dd	60.55 d	157.24 (C-6) 154.48 (C-4) 151.46 (C-2) 136.60 (d, $^5J_{C8,F2'}$ = 4.36, C-8) 116.59 (C-5)
30	82.62 dd	93.65 dd	92.68 dd	82.1 dd	60.25 d	160.51 (C-2) 153.46 (C-6) 150.52 (C-4) 141.79 (d, $^5J_{C8,F2'}$ = 4.37, C-8) 123.16 (C-5)

Table 4 The ^{13}C NMR Data of 2′,3′-difluoro nucleosides **9**, **10**, **16**, **17**, **25–27**, **30** (Coupling constants given in Hz).

Compound	F-2′				F-3′			
		^2J(C,F)	^3J(C,F)	^4J(C,F)	^3J(C,F)		^2J(C,F)	^3J(C,F)
	C1′,F2′	C3′,F2′	C4′,F2′	C5′,F2′	C1′,F3′	C2′,F3′	C4′,F3′	C5′,F3′
9	16.71	30.20	<1.0	<1.0	<1.0	30.70	27.27	8.71
10	36.25	29.92	<1.0	<1.0	<1.0	30.92	26.00	7.67
16	17.21	28.81	3.08	<1.0	3.76	28.28	25.45	6.26
17	17.88	28.15	2.12	<1.0	3.35	28.65	25.43	6.10
25	17.08	28.67	1.48	<1.0	2.35	28.74	24.93	6.51
26	35.54	28.12	1.50	<1.0	4.99	28.16	24.92	5.14
27	16.85	25.08	3.99	<1.0	4.99	26.85	24.83	5.22
30	16.90	28.80	2.99	<1.0	2.90	28.90	24.93	5.31

Table 5 *In vitro* antiviral activity and cytotoxicity of compounds **9**, **10**, **16**, **17**, **19**, **20**, **22**, **25–28** and **30**.

Compound	Anti-HIV-1 activity (μM)		Anti-HCV activity (μM)		Cytotoxicity, CC_{50} (μM)			
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	PBM	CEM	Vero	Huh-7
9	18	46	<10[a]	<10[a]	23	4.9	33	<10[a]
10	13	27	<10[a]	<10[a]	54	11	23	<10[a]
16	17	60	>10	>10	>100	>100	>100	>10
17	>100	>100	>10	>10	>100	>100	>100	>10
19	32	>100	2.9	9.9	53	14	56	26
20	21	>100	>10	>10	95	24	>100	>10
22	41	>100	>10	>10	40	52	>100	>10
25	0.56	4.2	>10	>10	58	91	>100	>10
26	2.4	14	>10	>10	17	4.9	>100	>10
27	0.66	3.1	>10	>10	30	62	90	>10
28	2.4	18	4.7	10	7.6	ND	>100	9.1
30	0.65	2.4	>10	>10	14	ND	>100	>10
AZT	0.0037	0.047	> 10	> 10	>100	14	56	ND[a]
2′<-*C*-MeC[b]	48	>100	1.8	5.8	>100	>100	>100	>10

[a]Cytotoxicity in Huh-7 cells did not allow for anti-HCV activity determination.

[b]AZT and 2′-*C*-MeC (2′-C-methylcytidine) were used as positive controls for HIV and HCV assays, respectively.

coupling constants of 2.0 Hz and 1.0 Hz between sugar H-1′ and H-5′ protons, and F-3′ substituent, respectively, are exhibited also in the spectrum of difluoride **25** due to the W-arrangements between these protons and fluorine atom at C-3′.

To determine the spectrum of activity of the synthesized purine nucleosides, anti-HIV-1 activity was evaluated versus $HIV\text{-}1_{LAI}$ in primary human peripheral blood mononuclear (PBM) cells and 3′-azido-3′-deoxythymidine (AZT) was used as a positive control. Cytotoxicity was determined in human PBM, human T-lymphoblastoid (CEM), and African Green monkey (Vero) cells [24, 25]. All of the modified purine nucleosides were also evaluated for inhibition of HCV RNA replication at 10 μM in human hepatoma cells (Huh-7) using a subgenomic HCV replicon system and 2′-*C*-methyl-cytidine (2′-C-MeC) as a positive control [26]. Cytotoxicity in Huh-7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and ribosomal RNA (rRNA) [27]. The antiviral and cytotoxicity results are summarized in Table 5. In general, all of the fluoro-containing nucleoside analogs inhibit HIV replication at micromolar concentrations, but show cytotoxicity in the same range in most of the cell systems tested. It is noteworthy though, that 2,6-diaminopurine analog **25** displays submicromolar activity against HIV (EC_{50} = 0.56 μM) while showing only modest cytotoxicity in PBM and CEM (CC_{50} = 58 and 91 μM, respectively) and no toxicity in Vero at concentration up to 100 μM.

Conclusions

A new and efficient procedure for the preparation of key 1′-α-bromosugar **8** by bromination of known acetate **6** under mild catalytic conditions was described. In addition, new and selective synthetic approaches to the key intermediates **9** and **28** were developed. With these compounds in hand, twelve 2′,3′-difluoro-D-arabinofuranosyl 2,6-disubstituted purine nucleosides were prepared which were evaluated as potential anti-HIV-1 and anti-HCV agents. The SAR results indicate that modifications at 2 and 6-positions have effects on antiviral activity and host cell toxicity. The β-2,6-diaminopurine nucleoside **25** demonstrates selective *in vitro* anti-HIV-1 activity (EC_{50} = 0.56 μM) while displaying only moderate cytotoxicity in human lymphocytes. Based on these encouraging results, further structural modifications of the base should allow us to improve the antiviral potency and selectivity of these compounds.

Experimental

Column chromatography was performed on silica gel 60 H (70–230 mesh; Merck, Darmstadt, Germany). All anhydrous solvents were distilled over CaH_2, P_2O_5 or magnesium prior to the use. The UV spectra were recorded on Specord M-400 (Carl Zeiss, Germany). The ^1H, ^{13}C and ^{19}F NMR spectra were recorded in $CDCl_3$, CD_3OD and DMSO-d_6 with Bruker Avance-500-DRX spectrometer at 500.13, 126.76 and 470.59 MHz, respectively. Chemical shifts δ are reported in ppm

downfield from internal $SiMe_4$ (^1H,^{13}C) or external $CFCl_3$ (^{19}F). J values are reported in Hz. NMR assignments were confirmed by 2D (^1H,^1H and ^1H,^{13}C) correlation spectroscopy. Melting points were determined on a Boetius apparatus and were uncorrected. High resolution mass spectra were measured on a mass spectrometer Agilent Q-TOF 6550 (USA) using electrospray ionization.

1-*O*-Acetyl-5-*O*-benzoyl-2,3-dideoxy-2,3-difluoro-α/β-D-arabinofuranoside (6)

Method A Acetolysis of **5** (0.337 g, 1.24 mmol) for 18 h in a mixture of acetic acid/acetic anhydride/H_2SO_4 was accomplished as described earlier [9]. The product was chromatographed on silica gel, eluting with EtOAc/hexane (ratio 1:5, 1:3 and 1:1) to give **6** (0.286 g, 77%) as a syrup and a diastereomeric mixture of **7** (0.046 g, 10%, oil). ^1H NMR ($CDCl_3$): (ratio of diastereomers α and β ca. 1.0:0.43), δ 7.45–8.05 (m, ArH), 6.06 (dd, 1H, H-1a, $J_{1,F\text{-}2}$ = 5.2, $J_{1,2}$ = 7.37), 5.99 (t, 0.43H, H-1b, $J_{1,F\text{-}2}$ = 6.4, $J_{1,2}$ = 6.4), 5.48 (m, H-4a and H-4b), 5.21 (dm, H-2b), 4.97 (ddd, H-3a), 4.80–4.86 (m, H-5a and H-5b), 4.65 (dm, H-3b), 4.50 (ddd, H-2a), 4.44–4.48 (m, H-5′a and H-5′b), 3.58 (s, OCH_3 b), 3.54 (s, OCH_3 a), 2.17 (s, OAc a), 2.15 (s, OAc b), 2.11 (s, OAc b), 2.10 (s, OAc a); ^{13}C NMR ($CDCl_3$): δ 170.7, 169.9, 169.4, 166.2 (4s, 2C=O, Ac and 2C=O, Bz), 133.4, 129.9, 129.8, 128.8, 129.7, 128.6 (s, 2C_6H_5CO-), 94.4 (dd, $J_{C\text{-}1,F\text{-}2}$ = 30.1, $J_{C\text{-}1,F\text{-}3}$ = 6.3, C-1b), 94.3 (dd, $J_{C\text{-}1,F\text{-}2}$ = 29.2, $J_{C\text{-}1,F\text{-}3}$ = 6.9, C-1a), 88.74 (dd, $J_{C\text{-}2,F\text{-}2}$ = 185.5, $J_{C\text{-}2,F\text{-}3}$ = 16.9, C-2b), 88.33 (dd, $J_{C\text{-}2,F\text{-}2}$ = 183.5, $J_{C\text{-}2,F\text{-}3}$ = 16.0, C-2a), 87.5 (dd, $J_{C\text{-}3,F\text{-}3}$ = 181.5, $J_{C\text{-}3,F\text{-}2}$ = 17.9, C-3a and C-3b), 68.1 (dd, C-4b), 67.9 (dd, C-4a), 62.0 (s, C-5a and C-5b), 58.63 (s, OCH_3 b), 58.0 (s, OCH_3 a), 21.06 (s, CH_3CO), 21.02 (s, CH_3CO), 20.9 (s, CH_3CO); ^{19}F NMR ($CDCl_3$): δ -214.07 (F-2, dddd), -214.4 (F-3, m, $J_{F\text{-}2,F\text{-}3}$ = 9.3) (compound α), -212.74 (F-2, dddd), -213.29 (F-3, m, $J_{F\text{-}2,F\text{-}3}$ = 6.4) (compound β). HRMS (EI). Calcd for $C_{17}H_{20}O_7F_2Na$ $[M+Na]^+$: *m/z* 397.1100. Found: *m/z* 397.1106.

Method B Concentrated H_2SO_4 (0.03 mL) was added to a solution of α-methyl glycoside **1** (0.1 g, 0.37 mmol) in acetic acid (0.78 mL) and acetic anhydride (0.19 mL) at 0°C. The reaction mixture was stirred at this temperature for 20 min and then 2 h at room temperature. The solution was poured into ice. After the ice melted, the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica gel to afford **6** (0.096 g, 87%) as a syrup.

5-*O*-Benzoyl-2,3-dideoxy-2,3-difluoro-α-D-arabinofuranosyl bromide (8)

To a suspension of **6** (0.36 g, 1.2 mmol) and anhydrous $ZnBr_2$ (0.065 g, 0.29 mmol) in anhydrous CH_2Cl_2 (10 mL) TMSBr (0.38 mL, 2.9 mmol) was added at 0°C. The resulting mixture was stirred at 0°C for 1 h, then 18 h at room temperature. The reaction mixture was poured into a cooled saturated solution of $NaHCO_3$, extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, evaporated to dryness, and co-evaporated with anhydrous toluene to give **8** (0.365 g, 95%) as a yellowish oil which was used in the next step without purification. ^1H NMR ($CDCl_3$): δ 7.46–8.04 (m, 5H, Bz), 6.55 (d, 1H, $J_{1,2}$ < 1.0, $J_{1,F\text{-}2}$ = 12.64, H-1), 5.57 (dd, 1H, $J_{2,3}$ < 1.0, $J_{2,F\text{-}2}$ = 49.65, $J_{2,F\text{-}3}$ = 10.68, H-2), 5.21 (ddd, 1H, $J_{3,4}$ = 3.7, $J_{3,F\text{-}2}$ = 19.1, $J_{3,F}$ = 51.4, H-3), 4.85 (ddt, 1H, $J_{4,F}$ = 20.2, H-4), 4.67 (dd, 1H, $J_{5,4}$ = 3.8, $J_{5,5'}$ = 12.8, H-5), 4.62 (dd, 1H, $J_{5,4}$ = 4.4, H-5′); ^{13}C NMR ($CDCl_3$): δ 166.1 (s, C=O, Bz), 133.6, 129.93, 129.28, 128.65 (4s, C_6H_5CO-), 100.1 (dd, $J_{C\text{-}2,F\text{-}2}$ = 190.5, $J_{C\text{-}2,F\text{-}3}$ = 26.37, C-2), 93.89 (dd, $J_{C\text{-}3,F\text{-}2}$ = 32.01, $J_{C\text{-}3,F\text{-}3}$ = 188.6, C-3), 86.57 (dd, $J_{C\text{-}1,F\text{-}3}$ = 14.8, $J_{C\text{-}1,F\text{-}2}$ = 31.95, C-1), 83.65 (d, $J_{C\text{-}4,\ F\text{-}3}$ = 28.8, C-4), 61.9 (d, $J_{C\text{-}5,F\text{-}3}$ = 5.65, C-5); ^{19}F NMR ($CDCl_3$): δ -170.98 (dm, F-2, $J_{F\text{-}2,F\text{-}3}$ = 8.5), -188.31 (dt, F-3).

2,6-Dichloro-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (9) and 2,6-dichloro-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-α-D-arabinofuranosyl)purine (10)

Method A A solution of 1-α-bromide **8** (0.073 g, 0.227 mmol) in anhydrous MeCN (4 mL) was added to a suspension of the sodium salt of 2,6-dichloropurine, prepared from 2,6-dichloropurine (0.045 g, 0.238 mmol) and NaH (7.7 mg of 80% in oil, 0.024 mmol) in anhydrous MeCN (4 mL) under argon. The reaction mixture was stirred at room temperature overnight. Insoluble materials were removed by filtration and washed with MeCN (5 mL). The combined filtrate and washings were concentrated and the residue was chromatographed on silica gel (140 mL), eluting with EtOAc/toluene (ratio 1:8 and 1:4) to afford β- nucleoside **9** (0.054 g, 55%) as a colorless oil which crystallized during storage and α-nucleoside **10** (0.013 g, 13%) as a syrup.

Nucleoside 9

Mp 143–144°C; UV (EtOH), λ_{max}, nm, (ε): 274 (5660), 231 (7300); ^{19}F NMR ($CDCl_3$): δ -188.77 (dm, F-2′or F-3′), -203.62 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{17}H_{13}N_4O_3F_2Cl_2$ $[M+H]^+$: *m/z* 429.0411. Found: *m/z* 429.0418.

Nucleoside 10

UV (EtOH), λ_{max}, nm, (ε): 274 (5660), 231 (7300); ^{19}F NMR ($CDCl_3$): δ -190.48 (m, F-3′), -191.43 (m, F-2′). HRMS (EI). Calcd for $C_{17}H_{13}N_4O_3F_2Cl_2$ $[M+H]^+$: *m/z* 429.0411. Found: *m/z* 429.0416.

Method B To a solution of 2,6-dichloropurine (0.14 g, 0.74 mmol) in anhydrous 1,2-dimethoxyethane (11.5 mL) under argon at 0°C was added potassium *t*-butoxide (0.085 g, 0.75 mmol) and then the resulting solution was stirred for 12 min at room temperature before concentration. A solution of bromide **8** (0.2 g, 0.62 mmol) in anhydrous MeCN (17 mL) was added, under argon, to a suspension of the prepared potassium salt of purine in anhydrous MeCN (20 mL). The mixture was stirred under argon at room temperature for 18 h. Insoluble materials were removed by filtration and the solids were washed with MeCN (20 mL). The combined filtrate and washings were concentrated. The residue was dissolved in anhydrous CH_2Cl_2 (25 mL), and insoluble materials were filtered off and washed with CH_2Cl_2. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with EtOAc/toluene (ratio 1:8 and 1:4) to afford nucleoside **9** (0.195 g, 73%) and nucleoside **10** (0.02 g, 8%).

Methyl 5-*O*-benzoyl-2-*O*-acetyl-3-deoxy-3-fluoro-β-D-ribofuranoside (12)

Acetic anhydride (0.24 mL) was added to a solution of β-methyl riboside **11** (0.171 g, 0.63 mmol) in pyridine (3.5 mL) at room temperature. The mixture was stirred at this temperature for 48 h and then poured onto ice. After the ice melted, the aqueous phase was

extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated. The product was chromatographed on silica gel, eluting with EtOAc/hexane (ratio 1:6 and 1:4) to afford the acetate **12** (0.170 g, 86%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 7.26–8.08 (m, 5H, Ar-H), 5.31 (dt, 1H, $J_{3,F}$ = 52.6, H-3), 5.19 (m, 1H, H-2), 4.99 (t, 1H, $J_{1,2}$ = $J_{1,F\text{-}3}$ =1.6, H-1), 4.52–4.91 (dm, 2H, 2H-5), 4.42 (dm, 1H, H-4), 3.36 (s, 3H, OCH_3), 2.16 (s, 3H, CO$\underline{CH_3}$); ^{13}C NMR ($CDCl_3$): δ 169.8 (s, C=O, Bz), 166.1 (s, C=O, OAc), 133.3, 129.75, 129.62, 129.40 (4s, C_6H_5CO-), 106.05 (s, C-1), 90.0 (d, $J_{C\text{-}3,F\text{-}3}$ = 193.7, C-3), 79.25 (d, $J_{C\text{-}2,F\text{-}2}$= 25.2, C-2), 75.0 (d, $J_{C\text{-}4,F\text{-}3}$=13.97, C-4), 63.92 (d, $J_{C\text{-}5,F\text{-}3}$ = 4.68, C-5), 55.75 (s, OCH_3), 20.61 (s, CO$\underline{CH_3}$); ^{19}F NMR ($CDCl_3$): δ -210.63 (ddd, F-3). HRMS (EI). Calcd for $C_{15}H_{17}O_6FNa$ [M+Na]$^+$: *m/z* 335.0997. Found: *m/z* 335.1005.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-fluoro-α/β-D-ribofuranose (13)

β-Methyl riboside **12** (0.17 g, 0.54 mmol) was dissolved in a mixture of acetic acid (1.16 mL), acetic anhydride (0.28 mL) and concentrated H_2SO_4 (0.05 mL) at 0–5°C. The mixture was stirred at this temperature for 5 min and then 150 min at room temperature. The solution was poured onto ice. After the ice melted, the aqueous phase was extracted with $CHCl_3$ (3 × 20 mL), and then after adding cold aqueous 5% $NaHCO_3$ to the aqueous phase, it was again extracted with $CHCl_3$ (2 × 20 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated to dryness to afford **13** (0.179 g, 97%) as a syrup. ^{1}H NMR ($CDCl_3$): (ratio of α and β anomers ca. 1.0:1.0), δ 8.09 (d, 2H, Bz), 8.04 (d, 2H, Bz), 7.60 (t, 2H, Bz), 7.49 (m, 4H, Bz), 6.53 (d,1H, $J_{1,2}$ = 4.6, H-1a), 6.26 (t, 1H, $J_{1,2}$ = $J_{1,F\text{-}3}$ =1.8, H-1b), 5.37 (dt, 1H, H-3a), 5.26 (dm, 1H, H-3b), 4.81 (dm, 1H, H-4a), 4.63–4.71 (2m, 2H, 2H-5b), 4.55 (dd, 1H, H-5a), 4.49 (dd, 1H, H-5′a), 4.46 (dm, 1H, H-4b), 2.20, 2.19, 2.17 and 1.97 (4s, 12H, 4 CO$\underline{CH_3}$); ^{13}C NMR ($CDCl_3$): δ 169.91, 169.78, 169.59, 169.10, 165.89, 165.85 (6s, C=O, 2Bz and 4Ac), 133.53, 133.45, 129.74, 129.45, 129.18, 128.65, 128.54 (8s, $2C_6H_5CO$-), 98.2 and 93.7 (2s, C-1a, C-1b), 89.1 and 88.4 (2d, $J_{C\text{-}3,F\text{-}3}$ = 194.4, $J_{C\text{-}3,F\text{-}3}$ = 191.56, C-3a, C-3b), 82.25 and 80.7 (2d, $J_{C\text{-}2,F\text{-}2}$ = 25.2, $J_{C\text{-}2,F\text{-}2}$ = 25.0, C-2a, C-2b), 74.7 and 71.2 (2d, $J_{C\text{-}4,F\text{-}3}$ = 14.4, $J_{C\text{-}4,F\text{-}3}$ = 15.1, C-4a, C-4b), 63.36 and 63.14 (2d, $J_{C\text{-}5a,F\text{-}3a}$ = 9.16, $J_{C\text{-}5b,F\text{-}3b}$ = 5.21, C-5a, C-5b), 21.1, 20.1, 20.47, 20.37 (4s, $COCH_3$); ^{19}F NMR ($CDCl_3$): δ 197.91 (dd, F-3a), -184.78 (dt, F-3b). HRMS (EI). Calcd for $C_{16}H_{17}O_7FNa$ [M+Na]$^+$: *m/z* 363.0947. Found: *m/z* 363.0952.

2,6-Dichloro-9-(5′-*O*-benzoyl-2′-*O*-acetyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)purine (14)

A mixture of 2,6-dichloropurine (0.148 g, 0.33 mmol) and a catalytic amount of $(NH_4)_2SO_4$, HMDS (3 mL) and anhydrous toluene (12 mL) was heated under reflux for 2 h under argon. After cooling the clear solution was concentrated under reduced pressure to give a residue. To a solution of this trimethylsilyl derivative and the diacetate **13** (0.217 g, 0.64 mmol) in a mixture of anhydrous MeCN (10.8 mL) and 1,2-dichloroethane (2.7 mL) was added TMSOTf (0.18 mL, 0.96 mmol) and the reaction mixture was stirred at room temperature for 90 min. After the standard work-up, the residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (ratio 1:2 and 1:1) to yield nucleoside **14** (0.266 g, 89%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 8.21 (s, 1H, H-8), 7.44-8.03 (m, 5H, Bz), 6.31 (d, 1H, $J_{1′,2′}$ = 7.1, H-1′), 5.92 (ddd, 1H, $J_{2′,3′}$ = 4.7, $J_{2′,F\text{-}3′}$ = 18.65, H-2′), 5.65 (ddd, 1H, $J_{3′,4′}$ = 1.82, $J_{3′,F\text{-}3′}$ = 52.7, H-3′), 4.79–4.82 (m, 2H, H-4′ and H-5′), 4.61 (dm, 1H, H-5″), 2.19 (s, 3H, CO$\underline{CH_3}$); ^{13}C NMR ($CDCl_3$): δ 169.7 (s, C=O, Bz), 165.8 (s, C=O, OAc), 153.4, 152.60, 152.40 (3s, C-2, C-4, C-6), 144.0 (s, C-8), 133.8, 131.4, 129.67, 129.58, 128.8 (5s, C_6H_5CO-, C-5), 89.3 (d, $J_{C\text{-}3′,F\text{-}3′}$ = 190.98, C-3′), 85.9 (s, C-1′), 81.8 (d, $J_{C\text{-}2′,F\text{-}3′}$ = 24.5, C-2′), 73.45 (dd, $J_{C\text{-}4′,F\text{-}3′}$ = 15.58, C-4′), 62.95 (d, $J_{C\text{-}5,F\text{-}3}$ = 8.93, C-5′), 20.6 (s, $COCH_3$); ^{19}F NMR ($CDCl_3$): δ -198.59 (dt, F-3′); UV (EtOH), λ_{max}, nm, (ε): 274 (6500), 230 (11300). HRMS (EI). Calcd for $C_{14}H_{14}O_5F$ [M-base]$^+$: *m/z* 281.0925. Found: *m/z* 281.0928.

2,6-Dichloro-9-(5′-*O*-benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)purine (15)

A cooled solution of nucleoside **14** (0.175 g, 0.37 mmol) in anhydrous MeOH (14.0 mL) was treated with solid anhydrous $NaHCO_3$ (0.117 g, 1.4 mmol). The reaction mixture was stirred at room temperature for 1 h, then neutralized with glacial acetic acid, concentrated, and co-evaporated with ethanol to dryness. The residue was chromatographed on silica gel, eluting with EtOAc/hexane (ratio 1:2 and 1:1) to afford nucleoside **15** (0.1 g, 63%) as an amorphous powder. ^{1}H NMR ($CDCl_3$ +CD_3OD): δ 8.35 (s, 1H, H-8), 7.43–8.01 (m, 5H, Bz), 6.11 (d, 1H, $J_{1′,2′}$ = 7.0, H-1′), 5.30 (ddd, 1H, $J_{3′,2′}$ = 4.4, $J_{3′,4′}$ = 1.85, $J_{3′,F\text{-}3′}$ = 53.5, H-3′), 5.04 (ddd, 1H, $J_{2′,F\text{-}3′}$ = 20.49, H-2′), 4.64–4.79 (m, 2H, H-4′ and H-5′), 4.58 (dd, 1H, H-5″); ^{13}C NMR ($CDCl_3$): δ 166.1 (s, C=O, Bz), 153.0, 152.6, 151.8 (3s, C-2, C-4, C-6), 145.1 (s, C-8), 133.5, 131.1, 128.80, 128.50 (4s, C_6H_5CO-), 129.4 (C-5), 91.1 (d, $J_{C\text{-}3′,F\text{-}3′}$ = 190.98, C-3′), 88.0 (s, C-1′), 80.9 (d, $J_{C\text{-}4′,F\text{-}3′}$ = 24.5, C-4′), 72.8 (dd, $J_{C\text{-}2′,F\text{-}3′}$ = 15.58, C-2′), 63.0 (d, $J_{C\text{-}5′,F\text{-}3′}$ = 8.93, C-5′); ^{19}F NMR ($CDCl_3$): -200.58 (dt, F-3′); UV (EtOH) λ_{max}, nm, (ε): 274 (5580), 231 (9500). HRMS (EI). Calcd for $C_{17}H_{14}N_4O_4FCl_2$ [M+H]$^+$: *m/z* 427.0368. Found: *m/z* 427.0363.

2,6-Dichloro-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (9) from nucleoside 15

To a suspension of nucleoside **15** (0.1 g, 0.23 mmol) in anhydrous dichloromethane (4.5 mL) was added pyridine (0.058 mL, 0.72 mmol) and diethylaminosulfur trifluoride (0.086 mL, 0.64 mmol) at 0°C. The reaction mixture was stirred at room temperature (25°C) for 14 h, and then poured onto cold aqueous 5% solution of $NaHCO_3$. The aqueous phase was extracted with $CHCl_3$ (3 × 30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was chromatographed on silica gel, using a linear gradient AcOEt in hexane (0→33%) to afford nucleoside **9** (0.035 g, 35%) as a colorless oil.

2-Chloro-6-methoxy-9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (16)

To a solution of nucleoside **9** (0.008 g, 0.019 mmol) in anhydrous MeOH (1.7 mL), 0.1 mL of a 0.22 N solution of sodium methoxide in methanol was added. The reaction mixture was stirred at room temperature for 18 h, then neutralized with acetic acid, concentrated, and co-evaporated with a mixture of toluene/ethanol (1:1, 20 mL) to dryness. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH (ratio 20:1 and 10:1) to afford nucleoside **16** (0.005 g,

84%); mp 174–176°C (EtOH); UV (EtOH) λ_{max}, nm, (ε): 207 (14250), 258 (10100); ^{19}F NMR (CD_3OD): δ -196.61 (m, F-2′ or F-3′), -204.7 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{11}H_{12}N_4O_3F_2Cl$ [M+H]$^+$: *m/z* 321.0566. Found: *m/z* 321.0562.

2,6-Dimethoxy-9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (17)

To a solution of nucleoside **9** (0.031 g, 0.072 mmol) in anhydrous MeOH (2.5 mL), 0.22 mL of a 1 *N* solution of sodium methoxide in methanol was added. The reaction mixture was stirred at room temperature for 18 h, heated under reflux for 90 min, neutralized with acetic acid, and concentrated and co-evaporated with a mixture of toluene/ethanol (1:1, 50 mL) to dryness. The residue was chromatographed on silica gel, eluting with $CHCl_3$, $CHCl_3$/hexane/MeOH (10:5:1) to afford nucleoside **17** (0.012 g, 52%) as a syrup. UV (EtOH) λ_{max}, nm, (ε): 212 (6500), 240 (9500), 262 (10300); ^{19}F NMR (CD_3OD): δ -196.28 (m, F-2′ or F-3′), -204.6 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{12}H_{15}N_4O_4F_2$ [M+H]$^+$: *m/z* 317.1061. Found: *m/z* 317.1066. Calcd for $C_{12}H_{14}N_4O_4F_2Na$ [M+Na]$^+$: *m/z* 339.0081. Found: *m/z* 339.0081.

2-Chloro-6-benzylamino-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (18)

To a solution of nucleoside **9** (0.011 g, 0.025 mmol) in anhydrous MeOH (2.3 mL) was added benzylamine (0.014 mL, 0.128 mmol). The reaction mixture was stirred at 55°C for 4 h, and then concentrated. The residue was chromatographed on silica gel, eluting with EtOAc/hexane (ratio 2:3 and 3:2) to afford nucleoside **18** (0.01 g, 78%); mp 164–166°C (MeOH); ^{1}H NMR ($CDCl_3$): δ 7.3–8.07 (5m, 10H, Bz and $C_6H_5CH_2$-), 7.92 (br.s, 1H, H-8), 6.53 (dt, 1H, $J_{1',F-2'}$ = 22.6, H-1′), 6.29 (br.s, 1H, NH), 5.25-5.48 (m, 2H, H-2′ and H-3′), 4.82 (br.s, 2H, -$CH_2C_6H_5$), 4.54-4.7 (m, 3H, H-5′, H-5″ and H-4′); ^{13}C NMR ($CDCl_3$): δ 166.2 (s, C=O, Bz), 156.1, 149.8, 137.9 (C-6, C-2, C-4), 139.45 (d, $J_{C-8,F-2'}$ = 4.6, C-8), 137.9, 133.7, 129.94, 129.86, 129.25, 128.93, 128.75, 128.19, 127.85 (C_6H_5CO- and $C_6H_5CH_2$-), 118.2 (C-5), 93.9 (dd, $J_{C-2',F-2'}$ = 183.5, $J_{C-2',F-3'}$ = 30.3, C-2′), 92.0 (dd, $J_{C-3',F-3'}$ = 192.49, $J_{C-3',F-2'}$ = 30.1, C-3′), 83.2 (d, $J_{C-1',F-2'}$ = 16.8, C-1′), 80.6 (d, $J_{C-4',F-3'}$ = 27.2, C-4′), 62.7 (d, $J_{C-5',F-3'}$ = 9.3, C-5′), 45.0 (s, $C_6H_5CH_2$-); ^{19}F NMR ($CDCl_3$): δ -188.8 (m, F-2′ or F-3′), -203.78 (m, F-3′ or F-2); UV (MeOH) λ_{max}, nm, (ε): 216 (17300) 232 sh, 272 (12100). HRMS (EI). Calcd for $C_{24}H_{20}N_5O_3F_2ClNa$ [M+Na]$^+$: *m/z* 522.1120. Found: *m/z* 522.1126.

2-Chloro-6-benzylamino-9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (19)

A solution of nucleoside **18** (0.01 g, 0.02 mmol) in MeOH (6 mL) saturated at 0°C with ammonia was kept for 24 h at room temperature and then evaporated. The residue was chromatographed on silica gel, eluting with CH_2Cl_2, then CH_2Cl_2/MeOH (ratio 30:1 and 6:1) to afford nucleoside **19** (0.0065 g, 82%) as a syrup. ^{1}H NMR (CD_3OD): δ 8.19 (br.s, 1H, H-8), 7.21–7.39 (d and 2t, 5H, $C_6H_5CH_2$-), 6.53 (ddd, 1H, H-1′), 5.36–5.45 (m, 2H, H-2′ and H-3′), 4.74 (br.s, 2H, -$CH_2C_6H_5$), 4.26 (ddt, 1H, H-4′), 3.85 (dd, 1H, H-5′), 3.82 (dd, 1H, H-5″); ^{13}C NMR (CD_3OD): δ 154.6, 149.6, 131.6 (C-6, C-2, C-4), 140.0 (br.s, C-8), 129.4, 128.25, 127.5, 128.3, 127.0 ($C_6H_5CH_2$-), 117.7 (C-5), 93.6 (dd, $J_{C-2',F-2'}$ = 182.1, $J_{C-2',F-3'}$ = 28.3, C-2′), 92.7 (dd, $J_{C-3',F-2'}$ = 28.6, $J_{C-3',F-3'}$ = 192.0, C-3′), 82.9 (d, $J_{C-1',F-2'}$ = 17.7, C-1′), 82.0 (d, $J_{C-4',F-3'}$ = 26.0, C-4′), 60.2 (d, $J_{C-5',F-3'}$ = 4.6, C-5′), 43.9 (s, $C_6H_5CH_2$-); ^{19}F NMR (CD_3OD): δ -196.1 (m, F-2′ or F-3′), -204.58 (m, F-3′ or F-2′); UV (MeOH) λ_{max}, nm, (ε): 211 (12600), 271 (8900). HRMS (EI). Calcd for $C_{17}H_{17}N_5O_2F_2Cl$ [M+H]$^+$: *m/z* 396.1039. Found: *m/z* 396.1034.

2,6-Diazido-9-(5′-O-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (20)

Nucleoside **9** (0.075 g, 0.175 mmol) was treated with LiN_3 (0.045 g, 0.92 mmol) in EtOH (10 mL) under reflux for 110 min. The reaction mixture was concentrated and the residue was dissolved in chloroform (5 mL). After filtration, the filtrate was concentrated and the residue was chromatographed on silica gel, eluting with EtOAc/petroleum ether (ratio 1:5, 1:4 and 1:2) to afford nucleoside **20** (0.075 g, 97%) as an amorphous powder. ^{1}H NMR ($CDCl_3$): δ 8.10 (d, 1H, $J_{H-8,F-2'}$ = 1.93, H-8), 7.44–8.06 (3m, 5H, Bz), 6.53 (dt, 1H, $J_{1',2'}$ = 2.56, $J_{1',F-2'}$ = 22.1, $J_{1',F-3'}$ = 2.56, H-1′), 5.44 (dd, 1H, $J_{3',4'}$ < 1.0, $J_{3',F-2'}$ = 12.42, $J_{3',F'}$ = 50.44, H-3′), 5.31 (ddd, 1H, $J_{2',3'}$ < 1.0, $J_{2',F-2'}$ = 49.51, $J_{2',F-3'}$ = 9.46, H-2′), 4.62 (dm, 1H, H-4′), 4.69 (dd, 1H, H-5′), 4.65 (dd, 1H, H-5″); ^{13}C NMR ($CDCl_3$): δ 166.1 (s, C=O, Bz), 133.7, 129.85, 129.19, 128.8 (C_6H_5CO-), 156.6, 154.2, 153.5 (C-2, C-4, C-6), 142.5 (d, $J_{C-8,F-2'}$ = 6.0, C-8), 121.0 (C-5), 93.04 (dd, $J_{C-2',F-2'}$ = 184.7, $J_{C-2',F-3'}$ = 30.3, C-2′), 93.89 (dd, $J_{C-3',F-2'}$ = 30.0, $J_{C-3',F-3'}$ = 192.1, C-3′), 83.4 (d, $J_{C-1',F-2'}$ = 16.8, C-1′), 80.8 (d, $J_{C-4',F-3'}$ = 27.1, C-1′), 62.55 (d, $J_{C-5',F-3'}$ = 8.9, C-5′); ^{19}F NMR ($CDCl_3$): δ -188.9 (m, F-2′), -203.74 (m, F-3′); UV (EtOH) λ_{max}, nm, (ε): 232 (7300), 270 (2240), 297 (1120). HRMS (EI). Calcd for $C_{17}H_{13}N_{10}O_3F_2$ [M+H]$^+$: *m/z* 443.1140. Found: *m/z* 443.1140.

2,6-Diazido-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-α-D-arabinofuranosyl)purine (21)

Starting from α-nucleoside **10** (0.014 g, 0.033 mmol) and using the procedure described above for the preparation of **20**, nucleoside **21** (0.014 g, 97%) was obtained as a syrup. ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1H, H-8), 7.46–8.08 (m, 5H, Bz), 6.44 (dd, 1H, $J_{1',2'}$ = 1.0, $J_{1',F-2'}$ = 15.6, H-1′), 5.88 (ddt, 1H, $J_{2',F-2'}$ = 48.82, $J_{2',F-3'}$ = 12.3, H-2′), 5.44 (dddd, 1H, $J_{3',4'}$ = 2.5, $J_{3',F-2'}$ = 13.4, $J_{3',F-3'}$ = 50.0, H-3′), 5.05 (dm, 1H, H-4′), 4.62 (dd, 1H, H-5′), 4.57 (dd, 1H, H-5″); ^{13}C NMR ($CDCl_3$): δ 166.0 (C=O, Bz), 133.6, 129.83, 129.10, 128.6 (C_6H_5CO-), 156.6, 154.4, 153.1 (C-2, C-4, C-6), 141.2 (d, $J_{C-8,F-2'}$ = 3.6, C-8), 121.7 (C-5), 96.3 (dd, $J_{C-2',F-2'}$ = 188.0, $J_{C-2',F-3'}$ = 28.9, C-2′), 94.2 (dd, $J_{C-3',F-2'}$ = 29.1, $J_{C-3',F-3'}$ = 184.87, C-3′), 88.5 (dd, $J_{C-1',F-2'}$ = 36.9, $J_{C-1',F-3'}$ = 2.18, C-1′), 83.45 (d, $J_{C-4',F-3'}$ = 25.88, C-4′), 62.4 (d, $J_{C-5',F-3'}$ = 7.3, C-5′); ^{19}F NMR ($CDCl_3$): δ -191.47 (dm, F-2′), -191.85 (m, F-3′); UV (EtOH) λ_{max}, nm (ε): 228 (7300), 271 (2240), 298 (1120). HRMS (EI). Calcd for $C_{17}H_{13}N_{10}O_3F_2$ [M+H]$^+$: *m/z* 443.1140. Found: *m/z* 443.1142.

2,6-Diamino-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (23)

Anhydrous $SnCl_2$ (0.081 g, 0.427 mmol) was added at room temperature, under argon, to a solution of nucleoside **20** (0.075 g, 0.169 mmol) in a mixture of anhydrous CH_2Cl_2 (10 mL) and MeOH (1.2 mL). The reaction mixture was stirred for 2 h, and then poured onto a cooled saturated aqueous solution of $NaHCO_3$. After stirring, the prepared suspension was filtered and the precipitate was washed with $CHCl_3$

(30 mL). After separation of the organic phase, the aqueous layer was extracted again with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel, eluting with EtOAc/hexane (2:1) and EtOAc/hexane/MeOH (ratio 20:10:2) to afford nucleoside **22** (0.003 g, 4%) as a syrup and nucleoside **23** (0.058 g, 87%).

Nucleoside 22 ^{1}H NMR ($CDCl_3$): δ 7.92 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 3.1, H-8), 7.46–8.08 (m, 5H, Bz), 6.48 (dt, 1H, $J_{1',2'} = J_{1',F\text{-}3'}$ = 2.56, $J_{1',F\text{-}2'}$ = 22.7, H-1′), 5.73 (br.s, 2H, NH_2), 5.44 (dd, 1H, $J_{3',4'}$ ~ 1.6, $J_{3',F\text{-}2'}$ = 12.6, $J_{3',F'}$ = 49.7, H-3′), 5.26 (ddd, 1H, $J_{2',3'}$ < 1.0, $J_{2',F\text{-}2'}$ = 49.4, $J_{2',F\text{-}3'}$ = 9.3, H-2′), 4.69 (dd, 1H, H-5′), 4.56–4.66 (m, 2H, H-5″ and H-4′); ^{13}C NMR ($CDCl_3$): δ 166.15 (s, C=O, Bz), 157.2, 156.0, 151.2 (C-6, C-4, C-2), 139.4 (d, $J_{C\text{-}8,F\text{-}2'}$ = 6.0, C-8), 133.7, 129.86, 129.28, 128.7 (C_6H_5CO-), 116.6 (C-5), 93.9 (dd, $J_{C\text{-}2',F\text{-}2'}$ = 184.4, $J_{C\text{-}2',F\text{-}3'}$ = 30.16, C-2′), 91.7 (dd, $J_{C\text{-}3',F\text{-}2'}$ = 29.8, $J_{C\text{-}3',F\text{-}3'}$ = 191.29, C-3′), 83.2 (d, $J_{C\text{-}1',F\text{-}2'}$ = 16.9, C-1′), 80.5 (d, $J_{C\text{-}4',F\text{-}3'}$ = 27.9, C-4′), 62.7 (d, $J_{C\text{-}5',F\text{-}3'}$ = 8.9, C-5′); ^{19}F NMR ($CDCl_3$): δ -188.88 (m, F-2′ or F-3′), -203.85 (m, F-3′ or F-2′); IR (film): 2120 cm^{-1} (N_3); UV (EtOH) λ_{max}, nm, (ε): 232 (12400), 270 (8100). HRMS (EI). Calcd for $C_{17}H_{15}N_8O_3F_2$ $[M+H]^+$: *m/z* 417.1235. Found: *m/z* 417.1239.

Nucleoside 23 mp 90–92°C; ^{1}H NMR ($CDCl_3$): δ 7.73 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 3.2, H-8), 7.46–8.05 (3m, 5H, Bz), 6.37 (dt, 1H, $J_{1',2'} = J_{1',F\text{-}3'}$ = 3.2, $J_{1',F\text{-}2'}$ = 23.02, H-1′), 5.67 (br.s, 2H, NH_2), 5.41 (ddd, 1H, $J_{3',4'}$ = 1.3, $J_{3',F\text{-}2'}$ = 12.9, $J_{3',F'}$ = 49.95, H-3′), 5.26 (ddd, 1H, $J_{2',3'}$ < 1.0, $J_{2',F\text{-}2'}$ = 49.46, $J_{2',F\text{-}3'}$ = 9.71, H-2′), 4.85 (br.s, 2H, NH_2), 4.66 (dd, 1H, H-5′), 4.62 (dd, 1H, H-5″), 4.56 (ddt, 1H, H-4′); ^{13}C NMR ($CDCl_3$): δ 166.2 (s, C=O, Bz), 160.2, 156.1, 151.9 (C-6, C-4, C-2), 137.1 (d, $J_{C\text{-}8,F\text{-}2'}$ = 6.4, C-8), 133.7, 129.88, 129.32, 128.73, 128.47 (C_6H_5CO-), 113.7 (C-5), 94.1 (dd, $J_{C\text{-}2',F\text{-}2'}$ = 183.7, $J_{C\text{-}2',F\text{-}3'}$ = 30.2, C-2′), 91.8 (dd, $J_{C\text{-}3',F\text{-}2'}$ = 29.8, $J_{C\text{-}3',F\text{-}3'}$ = 191.42, C-3′), 82.8 (d, $J_{C\text{-}1',\ F\text{-}2'}$ = 16.9, C-1′), 80.1 (d, $J_{C\text{-}4',F\text{-}3'}$ = 27.0, C-4′), 62.8 (d, $J_{C\text{-}5',F\text{-}3'}$ = 8.5,C-5′); ^{19}F NMR ($CDCl_3$): δ -188.85 (m, F-2′ or F-3′), -203.75 (m, F-3′ or F-2′); UV (EtOH) λ_{max}, nm, (ε): 235 (7350), 256 (6690), 277 (6180). HRMS (EI). Calcd for $C_{17}H_{17}N_6O_3F_2$ $[M+H]^+$: *m/z* 391.1396. Found: *m/z* 391.1422.

2,6-Diamino-9-(5′-*O*-benzoyl-2′,3′-dideoxy-2′,3′-difluoro-α-D-arabinofuranosyl)purine (24)

Starting from α-nucleoside **21** (0.015 g, 0.034 mmol) and using the procedure described above for the preparation of **20**, nucleoside **24** (0.012 g, 91%) was obtained as a syrup; ^{1}H NMR ($CDCl_3$): δ 7.63 (s, 1H, H-8), 7.42–8.07 (m, 5H, Bz), 6.26 (dd, 1H, $J_{1',2'}$<1.0, $J_{1',F\text{-}2'}$ = 16.5, $J_{1',F\text{-}3'}$ = 1.56, H-1′), 6.01 (ddt, 1H, $J_{2',3'}$ = 1.9, $J_{2',F\text{-}2'}$ = 49.7, $J_{2',F\text{-}3'}$ = 13.1, H-2′), 5.44 (br.s, 2H, NH_2), 5.38 (ddd, 1H, $J_{3',4'}$ = 1.3, $J_{3',F\text{-}2'}$ = 16.0, $J_{3',F'}$ n.d., H-3′), 5.01 (dq, 1H, H-4′), 4.77 (br.s, 2H, NH_2), 4.60 (dd, 1H, H-5′), 4.57 (dd, 1H, H-5″); ^{19}F NMR ($CDCl_3$): δ -191.72 (m, F-2′ or F-3′), -194.23 (m, F-3′ or F-2′); UV (EtOH) λ_{max}, nm, (ε): 235 (7350), 256 (6600), 277 (6150). HRMS (EI). Calcd for $C_{17}H_{17}N_6O_3F_2$ $[M+H]^+$: *m/z* 391.1396. Found: *m/z* 391.1420.

2,6-Diamino-9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (25)

A solution of nucleoside **23** (0.058 g, 0.164 mmol) in MeOH (25 mL) saturated at 0°C with ammonia was kept for 18 h at room temperature and then concentrated. The residue was chromatographed on silica gel, eluting with $CHCl_3$, $CHCl_3$/MeOH (ratio 15:1 and 5:1) to afford nucleoside **25** (0.031 g, 72%); mp 236–238°C (EtOH); UV (EtOH) λ_{max}, nm (ε): 215 (18450), 256 (7020), 277 (7280); ^{19}F NMR (CD_3OD): δ -195.19 (m, F-2′ or F-3′), -204.95 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{10}H_{13}N_6O_2F_2$ $[M+H]^+$: *m/z* 287.1100. Found: *m/z* 287.1103.

2,6-Diamino-9-(2′,3′-dideoxy-2′,3′-difluoro-α-D-arabinofuranosyl)purine (26)

Starting from α-nucleoside **24** (0.012 g, 91%) and using the procedure described above for the preparation **23**, nucleoside **26** (0.007 g, 79%) was prepared as an amorphous powder; UV (EtOH) λ_{max}, nm (ε): 215 (18400), 256 (7000), 277 (7240); ^{19}F NMR ($CDCl_3$): δ -196.56 (m, F-2′ or F-3′), -197.764 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{10}H_{13}N_6O_2F_2$ $[M+H]^+$: *m/z* 287.1100. Found: *m/z* 287.1104.

9-(2′,3′-Dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl) guanine (27) from nucleoside 25

To a solution of nucleoside **25** (0.02 g, 0.07 mmol) in water (3 mL) was added adenosine deaminase (15 μL). The resulting solution was stirred at 23°C for 48 h, and concentrated after addition of methanol. The residue was chromatographed on silica gel, eluting with $CHCl_3$, $CHCl_3$/MeOH (ratio 10:1 and 5:1) to afford nucleoside **25** (0.017 g, 85%); mp > 260°C (MeOH); UV (H_2O) λ_{max}, nm (ε): 251 (14200), 270 (sh); ^{19}F NMR (DMSO-d_6): δ -191.72 (m, F-2′ or F-3′), -194.23 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{10}H_{12}N_5O_3F_2$ $[M+H]^+$: *m/z* 288.0908. Found: *m/z* 288.0904. Calcd for $C_{10}H_{11}N_5O_3F_2Na$ $[M+Na]^+$: *m/z* 310.0728. Found: *m/z* 310.0724.

2-Amino-6-chloro-9-(5′-O-benzoyl-2′,3′-dideoxy-2′, 3′-difluoro-β-D-arabinofuranosyl)purine (28) and 2-amino-6-chloro-9-(5′-O-benzoyl-2′,3′-dideoxy-2′, 3′-difluoro-α-D-arabinofuranosyl)purine (29)

2-Amino-6-chloropurine potassium salt was prepared by adding (0.024 g, 0.151 mmol) potassium *t*-butoxide to a solution of 2-amino-6-chloropurine (0.036 g, 0.21 mmol) in anhydrous 1,2-dimethoxyethane (11 mL) under argon at 0°C and the resulting solution was stirred for 40 min at room temperature and then concentrated. To a suspension of the prepared potassium salt of 2-amino-6-chloropurine in anhydrous MeCN (9 mL) was added, under argon, a solution of bromide **8** (0.060 g, 0.187 mmol) in anhydrous MeCN (5 mL). The reaction mixture was stirred under argon at room temperature for 18 h. Insoluble materials were removed by filtration and the solids were washed with MeCN (5 mL). The combined filtrate and washings were concentrated. The residue was dissolved in anhydrous CH_2Cl_2 (5 mL), filtered off and insoluble materials were washed with CH_2Cl_2. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel, eluting with EtOAc/hexane (1:1) to afford β-nucleoside **28** (0.038 g, 50%) as a white amorphous powder and α-nucleoside **29** (0.003 g, 8%) as a syrup.

β-Nucleoside 28 ^{1}H NMR ($CDCl_3$): δ 7.97 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 2.8, H-8), 7.45–8.05 (m, 5H, Bz), 6.41 (dt, 1H, $J_{1',2'}$ = 2.5, $J_{1',F\text{-}2'}$ = 19.8, H-1′), 5.46

(dd, 1H, $J_{2',3'}$ < 1.0, $J_{2',F-2'}$ = 49.9, $J_{2',F-3'}$ = 12.78, H-2′), 5.29 (ddd, 1H, $J_{3',4'}$ = 2.46, $J_{3',F-2'}$ = 25.0, $J_{3',F'}$ = 49.95, H-3′), 5.29 (br.s, 2H, NH_2), 4.67 (d, 2H, H-5′ and H-5″), 4.58 (ddt, 1H, H-4″); ^{13}C NMR ($CDCl_3$): δ 166.2 (s, C=O, Bz), 133.7, 129.85, 129.19, 128.75 (C_6H_5CO-), 159.2, 153.4, 151.9 (C-2, C-6, C-4), 141.1 (d, $J_{C-8,F-2'}$ = 6.1, C-8), 125.0 (C-5), 94.1 (dd, $J_{C-2',F-2'}$ = 183.9, $J_{C-2',F-3'}$ = 30.0, C-2′), 91.68 (dd, $J_{C-3',F-2'}$ = 30.0, $J_{C-3',F-3'}$ = 192.07, C-3′), 83.15 (d, $J_{C-1',F-2'}$ = 16.7, C-1′), 80.6 (d, $J_{C-4',F-3'}$ = 27.1, C-4′), 62.6 (d, $J_{C-5',F-3'}$ = 8.9, C-5′); ^{19}F NMR ($CDCl_3$): δ -188.82 (m, F-2′ or F-3′), -203.76 (m, F-3′ or F-2′); UV (MeOH) λ_{max}, nm, (ε): 232 (16350), 308 (6600). HRMS (EI). Calcd for $C_{17}H_{15}N_5O_3F_2Cl$ [M+H]$^+$: *m/z* 410.0831. Found: *m/z* 410.0834.

α-Nucleoside 29 ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H, H-8), 7.46–8.08 (3m, 5H, Bz), 6.34 (d, 1H, $J_{1',F-2'}$ = 15.8, H-1′), 5.90 (dd, 1H, $J_{2',3'}$ < 1.0, $J_{2',F-2'}$ = 48.6, $J_{2',F-3'}$ = 12.49, H-2′), 5.42 (ddd, 1H, $J_{3',4'}$ = 2.46, $J_{3',F-2'}$ = 25.0, $J_{3',F'}$ = 49.95, H-3′), 5.16 (br.s, 2H, NH_2), 5.02 (dm, 1H, H-4′). 4.61 (dd, 1-H, H-5′), 4.58 (dd, 1H, H-5″); ^{13}C NMR ($CDCl_3$): δ 166.0 (C=O, Bz), 133.6, 129.83, 129.17, 128.6 (C_6H_5CO-), 159.1, 153.0, 152.0 (C-2, C-6, C-4), 139.8 (d, $J_{C-8,F-2'}$ = 3.2, C-8), 125.7 (C-5), 96.3 (dd, $J_{C-2',F-2'}$ = 187.8, $J_{C-2',F-3'}$ = 29.1, C-2′), 94.3 (dd, $J_{C-3',F-2'}$ = 29.6, $J_{C-3',F-3'}$ = 185.7, C-3′), 88.2 (dd, $J_{C-1',F-2'}$ = 36.4, $J_{C-1',F-3'}$ = 2.9, C-1′), 83.1 (d, $J_{C-4',F-3'}$ = 25.6, C-4′), 62.5 (d, $J_{C-5',F-3'}$ = 6.6, C-5′); ^{19}F NMR ($CDCl_3$): δ -191.63 (m, F-2′ or F-3′), -192.6 (m, F-3′ or F-2′); UV (MeOH) λ_{max}, nm, (ε): 232 (16450), 308 (6700). HRMS (EI). Calcd for $C_{17}H_{15}N_5O_3F_2Cl$ [M+H]$^+$: *m/z* 410.0831. Found: *m/z* 410.0833.

2-Amino-6-chloro-9-(2′,3′-dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl)purine (30)

A solution of nucleoside **28** (0.018 g, 0.043 mmol) in MeOH (5 mL) saturated at 0°C with ammonia was kept for 18 h at room temperature and then concentrated. The residue was chromatographed on silica gel, eluting with $CHCl_3$, then $CHCl_3$/MeOH (ratio 15:1 and 5:1) to afford nucleoside **30** (0.01 g, 77%) as a syrup; UV (EtOH) λ_{max}, nm, (ε): 220 (18900), 247 (9000), 309 (7600); ^{1}H NMR (CD_3OD): δ 8.21 (d, 1H, $J_{H-8,F-2'}$ = 2.4, H-8), 6.43 (ddd, 1H, $J_{1',2'}$ = 3.5, $J_{1',F-2'}$ = 17.3, $J_{1',F-3'}$ = 1.9, H-1′), 5.40–5.45 (dm, 2H, H-2′ and H-3′), 4.25 (dm, 1H, H-4′), 3.85 (dd, 1H, H-5′), 3.83 (dd, 1H, H-5″); ^{19}F NMR (CD_3OD): δ -195.72 (m, F-2′ or F-3′), -204.7 (m, F-3′ or F-2′). HRMS (EI). Calcd for $C_{10}H_{11}N_5O_2F_2Cl$ [M+H]$^+$: *m/z* 306.0569. Found: *m/z* 306.0573.

9-(2′,3′-Dideoxy-2′,3′-difluoro-β-D-arabinofuranosyl) guanine (27) from nucleoside 28

To a solution of nucleoside **28** (0.02 g, 0.049 mmol) in MeOH (2 mL), 2-mercaptoethanol (0.14 mL, 0.2 mmol) and sodium methoxide 0.011 g (0.2 mmol) were added. The reaction mixture was heated under reflux for 3 h. After cooling to room temperature, the resulting solution was neutralized with acetic acid and solvents were removed under reduced pressure. The residue was purified on silica gel, eluting with $CHCl_3$, then $CHCl_3$/MeOH (ratio 10:1 and 5:1) to afford nucleoside **27** (0.01 g, 71%).

Acknowledgments: This work was supported in part by NIH grant 5P30-AI-50409 (CFAR), by the Department of Veterans Affairs and by the Belarus State Program of FOI 'Chempharmsynthesis'(Grants 2.19 and 4.20). Dr. Schinazi is the Chairman and a major shareholder of CoCrystal Pharma, Inc. Emory University received no funding from Cocrystal Pharma, Inc., to perform this work and *vice versa*.

References

[1] Meng, W. D.; Qing, F. L. Fluorinated nucleosides as antiviral and antitumor agents. *Curr. Top Med. Chem.* **2006**, *6*, 1499–1528.

[2] Ray, A. S.; Schinazi, R. F.; Murakami, E.; Basavapathruni, A.; Shi, J.; Zorca, S. M.; Chu, C. K.; Anderson, K. S. Probing the mechanistic consequences of 5-fluorine substitution on cytidine nucleotide analogue incorporation by HIV-1 reverse transcriptase. *Antivir. Chem. Chemother*. **2003**, *14*, 115–125.

[3] Coats, S. J.; Garnier-Amblard, E. C.; Amblard, F.; Etheshami, M.; Amiralaei, S.; Zhang, H.; Zhou, L.; Boucle, S. R. L.; Lu, X.; Bondada, L.; et al. Chutes and ladders in hepatitis C nucleoside drug development. *Antiv. Res.* **2014**, *102*, 119.

[4] Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi R. F.; Watanabe, K. A.; Otto, M. J.; et al. Design, synthesis, and antiviral activity of 2′-deoxy-2′-fluoro-2′-C-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005**, *48*, 5504–5508.

[5] Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discov.* **2013**, *12*, 447–464.

[6] Michailidis, E.; Marchand, B.; Kodama, E. N.; Singh, K.; Matsuoka, M.; Kirby, K. A.; Ryan, E. M.; Sawani, A. M.; Nagy, E.; Ashida, N.; et al. Mechanism of inhibition of HIV-1 reverse transcriptase by 4′-Ethynyl-2-fluoro-2′-deoxyadenosine triphosphate, a translocation-defective reverse transcriptase inhibitor. *J. Biol. Chem.* **2009**, *284*, 35681–35691.

[7] Mikhailopulo, I. A.; Pricota, T. I.; Sivets, G. G.; Altona, C. 2′-chloro-2′,3′-dideoxy-3′-fluoro-d-ribonucleosides: synthesis, stereospecificity, some chemical transformations, and conformational analysis. *J. Org. Chem.* **2003**, *68*, 5897–5908.

[8] Shi, J.; Zhou, L.; Zhang, H.-W.; McBrayer, T. R.; Detorio, M. A.; Johns, M.; Bassit, L.; Powdrill, M. H.; Whitaker, T.; Coats, S. J.; et al. Synthesis and antiviral activity of 2′-deoxy-2′-fluoro-2′-C-methyl-7-deazapurine nucleosides, their phosphoramidate prodrugs and 5′-triphosphates. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7094–7098.

[9] Guo, X.; Li, Y.; Tao, L.; Wang, Q.; Wang, S.; Hu, W.; Pan, Z.; Yang, Q.; Cui, Y.; Ge, Z.; et al. Synthesis and anti-HIV-1 activity of 4-substituted-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine analogues. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6770–6772.

[10] Marquez, V. E.; Tseng, C. K.-H.; Kelly, J. A.; Mitsuya, H.; Broder, S.; Roth, J. S.; Driscoll J. S. 2′,3′-Dideoxy-2′-fluoro-ara-A. An acid-stable purine nucleoside active against human immunodeficiency virus (HIV). *Biochem. Pharmacol.* **1987**, *36*, 2719–2722.

[11] Chu, C. K.; Matulic-Adamic, J.; Huang, J. T.; Chou, T. C.; Burchenal, J. H.; Fox, J. J.; Watanabe K. A. Nucleosides. CXXXV. Synthesis of some 9-(2-deoxy-2-fluoro-beta-D-

arabinofuranosyl)-9H-purines and their biological activities. *Chem. Pharm. Bul.* **1989**, *37*, 336–339.

[12] Mongometry, J. A.; Shortnancy, A. T.; Carson, D. A.; Secrist, J. A. 9-(2-Deoxy-2-fluoro-beta-D-arabinofuranosyl)guanine: a metabolically stable cytotoxic analogue of 2′-deoxyguanosine. *J. Med. Chem.* **1986**, *29*, 2389–2392.

[13] Hafkemeyer, P.; Keppler-Hafkemeyer, A.; al Haya, M. A.; von Janta-Lipinski, M.; Matthes E.; Lehmann C.; Offensperger, W. B.; Offensperger, S.; Gerok, W.; Blum, H. E.; Inhibition of duck hepatitis B virus replication by 2′,3′-dideoxy-3′-fluoroguanosine *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.* **1996**, *40*, 792–794.

[14] Cihlar, T.; Ray, A. S. Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine. *Antivir. Res.* **2010**, *85*, 39–58.

[15] Sivets, G. G.; Kalinichenko, E. N.; Mikhailopulo, I. A.; Detorio, M. A.; McBrayer, T. R.; Whitaker, T.; Schinazi, R. F. Synthesis and antiviral activity of purine 2′,3′-dideoxy-2′, 3′-difluoro-D-arabinofuranosyl nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2009**, *28*, 519–536.

[16] Howell, H. G.; Brodfuehre, P. R.; Bundidge, S. P.; Benigni, D. A.; Sapino, S. P. Antiviral nucleosides. A stereospecific, total synthesis of 2′-fluoro-2′-deoxy-β-D-arabinofuranosyl nucleosides *J. Org. Chem.* **1988**, *53*, 85–88.

[17] Kazimierczuk, Z.; Cottam H. B.; Revankar, G. R.; Robins, R. K. Synthesis of 2′-deoxytubercidin, 2′-deoxyadenosine, and related 2′-deoxynucleosides via a novel direct stereospecific sodium salt glycosylation procedure *J. Am. Chem. Soc.* **1984**, *106*, 6379–6382.

[18] Seela, F.; Xu, K.; Chittepu, P. Fluorinated Pyrrolo[2,3-*d*]pyrimidine Nucleosides: 7- Fluoro-7-deazapurine 2′-Deoxyribofuranosides and 2′-Deoxy-2′-fluoroarabinofuranosyl Derivatives. *Synthesis* **2006**, *12*, 2005–2012.

[19] Bauta, W. E.; Schulmeier, B. E.; Burke, B.; Puente, J. F.; Cantrell, W. R.; Lovett, D.; Goebel, J.; Anderson, B.; lonescu, D.; Guo, R. C. A new process for antineoplastic agent clofarabine. *Org. Process. Res. Dev.* **2004**, *8*, 889–896.

[20] Sivets, G. G.; Boghok, T. S.; Kalinichenko, E. N. In XIX International Roundtable on Nucleosides, Nucleotides and Nucleic Acids (IRT 2010) Lyon. Abstract PA010, August 29 – September 3, 2010, 92.

[21] Mikhailopulo, I. A.; Sivets, G. G. *Helv.* A novel route for the synthesis of deoxy fluoro sugars and nucleosides. *Chim. Acta* **1999**, *82*, 2052–2065.

[22] Zhang, H.-W.; Coats, S. J.; Bondada, L.; Amblard, F.; Detorio, M.; Asif, G.; Fromentin, E.; Solomon, S.; Obikhod, A.; Whitaker, T.; et al. Synthesis and evaluation of 3′-azido-2′, 3′-dideoxypurine nucleosides as inhibitors of human immunodeficiency virus. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 60–64.

[23] Tennilä, T.; Azhayeva, E.; Vepsäläinen, J.; Laatikainen, R.; Azhayev, A.; Mikhailopulo I. A. Oligonucleotides containing 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine and -guanine: synthesis, hybridization and antisense properties. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 1861–1864.

[24] Schinazi, R. F.; Sommadossi, J. P.; Saalmann, V.; Cannon, D. L.; Xie, M.-W.; Hart, G. C.; Smith, G. A.; Hahn, E. F. Activities of 3′-azido-3′-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061–1067.

[25] Stuyver, L. J.; Lostia, S.; Adams, M.; Mathew, J.; Pai, B. S.; Grier, J.; Tharnish, P. M.; Choi, Y.; Chong, Y.; Choo, H.; et al. Antiviral activities and cellular toxicities of modified 2′,3′-dideoxy-2′,3′-didehydrocytidine analogues. *Antimicrob. Agents Chemother.* **2002**, *46*, 3854–3860.

[26] Rondla, R.; Coats, S. J.; McBrayer, T. R.; Crier, J.; Johns, M.; Tharnish, P. M.; Whitaker T.; Zhou L-H.; Schinazi R. F. Anti-hepatitis C virus activity of novel β-d-2′-C-methyl-4′-azido pyrimidine nucleoside phosphoramidate prodrugs. *Antivir. Chem. Chemother.* **2009**, *20*, 99–106.

[27] Stuyver, L. J.; Whitaker, T.; McBrayer, T. R.; Hernandez-Santiago, B. I.; Lostia, S.; Tharnish, P. M.; Ramesh, M.; Chu, C. K.; Jordan, R.; Shi, J.; et al. Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture. *Antimicrob. Agents Chemother.* **2003**, *47*, 244–254.

Supplemental Material: The online version of this article (DOI: 10.1515/hc-2015-0174) offers supplementary material, available to authorized users.