*cyclo*Sal-Pronucleotides of 2′-Fluoro-*ara*- and 2′-Fluoro-*ribo*-2′,3′-dideoxyadenosine as a Strategy to Bypass a Metabolic Blockade

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Novel, lipophilic *cyclo*Sal triesters **4a**−**c** and **5a**−**c** were synthesized, respectively, from the *ara*- and *ribo*-configurated 2′-fluorinated-2′,3′-dideoxyadenosines **2** and **3**. The *cyclo*Sal phosphotriesters were used as tools to study the effects of the two different sugar pucker conformations induced by two opposite configurations of the fluorine substituent at C2′ of the dideoxyribose moiety. F-*ara*-ddA (**2**) is known to be an active anti-HIV agent, whereas the *ribo*-analogue **3** is inactive. Hydrolysis studies with the triester precursors **4a**−**c** and **5a**−**c** showed selective formation of the monophosphates of **2** and **3**. The lipophilicity of the triester prodrugs was considerably increased by the *cyclo*Sal mask with respect to ddA (**1**), F-*ara*-ddA (**2**), and F-*ribo*-ddA (**3**). Phosphotriesters **4** and **5** proved to be completely resistant to ADA and AMPDA deamination. In parallel experiments, *ribo*-nucleoside **3** showed a 50-fold faster deamination rate relative to the *ara*-analogue **2**. Against HIV in CEM cells, the phosphotriesters **4** proved to be 10-fold more potent than the parent nucleoside **2**. Furthermore, the prodrugs **4** were active against MSV-induced transformation of C3H/3T3 fibroblasts, while **2** was inactive. More interestingly, the *ribo*-configurated phosphotriesters **5**, prepared from the inactive F-*ribo*-ddA (**3**), showed a level of anti-HIV activity that was even higher than that of F-*ara*-ddA (**2**). Our findings clearly prove that the application of the *cyclo*Sal-pronucleotide concept to F-*ribo*-ddA (**3**) overcomes a metabolic blockade in the formation of the corresponding monophosphate.

Introduction

In addition to the pyrimidine nucleoside analogues (AZT, d4T, ddC, and 3TC) that are used clinically for the treatment of AIDS,[1,2] the only purine nucleoside analogue clinically approved to date is 2′,3′-dideoxyinosine (ddI; Didanosine, Videx), the deamination product of 2′,3′-dideoxyadenosine (ddA, **1**).[3,4] For these nucleoside analogues to show effectiveness against human immunodeficiency virus (HIV), they must be converted intracellularly by cellular enzymes to their corresponding 5′-triphosphates before they can act as inhibitors of the target enzyme, reverse transcriptase (RT).[2a,5] In marked contrast with the pyrimidine 2′,3′-dideoxynucleosides, purine nucleoside analogues like ddA (**1**), or ddI, are extensively catabolized.[3,4,6−12] The main catabolic pathway involves deamination by the ubiquitous enzyme adenosine deaminase (ADA) to yield the corresponding inosine derivatives.[13,14] A further drawback of the purine 2′,3′-dideoxynucleoside analogues is their extreme acid sensitivity which results in the cleavage of the glycosyl bond [ddA (**1**) has a half-life of 35 s at pH 1.0, 1/40.000 of the half-life of adenosine].[15] Since the introduction of fluorine at the 2′-position of the furanose ring in nucleosides decreases chemically catalyzed hydrolysis,[16] particularly for purine nucleosides,[13] the 2′-fluoro analogues of ddA (**1**) were designed to overcome the chemical instability of the parent dideoxynucleoside. These fluorinated nucleosides indeed proved to be completely stable for 20 days at pH 1.0. One of the fluoro analogues, 2′-F-*ara*-2′,3′-dideoxyadenosine (**2**, F-*ara*-ddA; Lodenosine),[17,18] demonstrated anti-HIV-1 and anti-HIV-2 activities comparable to those achieved with ddI in ATH8 cells (EC_{50} = 10 μM).[19,20] Comparable results in the MT-4 test system were also reported by Herdewijn et al.[21] In vivo, F-*ara*-ddA (**2**) was also active against HIV-1 when administered orally in the hu PBL SCID mouse test system.[22,23] Like ddA (**1**), F-*ara*-ddA (**2**) is converted by ADA to F-*ara*-ddI (**6**), but at a 10-fold slower rate.[17] As a consequence, and in contrast to ddA (**1**), the 2′-fluorinated ddA derivative **2** follows a more direct anabolic route toward nucleotide formation initiated by 2′-deoxycytidine kinase (dCK) and/or adenosine kinase (AK).[17] The deamination product F-*ara*-ddI (**6**), unlike its nonfluorinated analogue, does not undergo cleavage by purine nucleoside phosphorylase (PNP) to yield hypoxanthine, which in the case of ddI enters the hypoxanthine salvage pathway leading to inactive metabolites (Scheme 1).[17,24] Moreover, F-*ara*-ddA (**2**) has been found to be a very weak inhibitor of mitochondrial DNA synthesis, even at high doses.[25,26] From resistance studies, it appears that **2** lacks cross-resistance to AZT, ddI, and the nonnucleoside RT inhibitors.[27] In cellular uptake studies, it has been shown that **2** penetrates the cell membrane by passive diffusion and does not require

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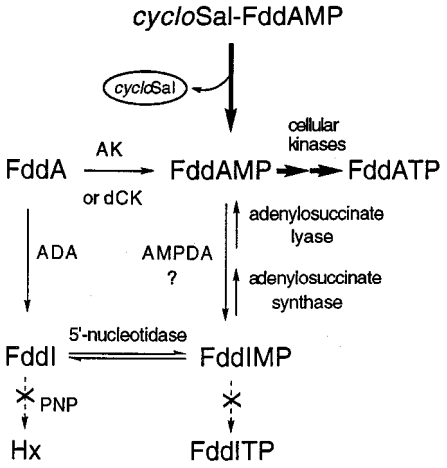
Scheme 1. Metabolism of 2′-Fluorinated Purine Nucleosides (FddAs)

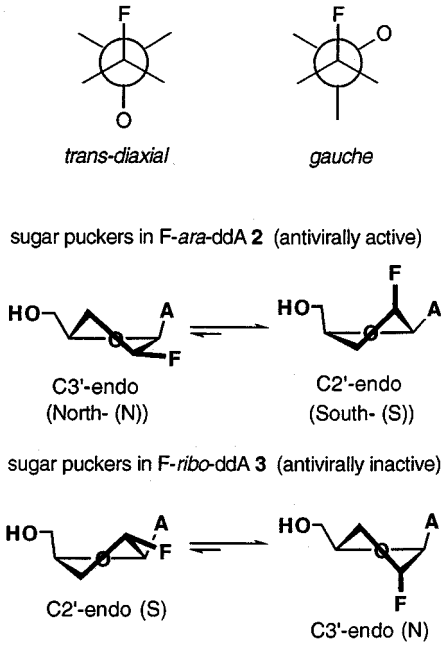


the purine nucleoside transport carrier system to enter the cell.[17] All things considered, F-*ara*-ddA (**2**) offers interesting perspectives as a candidate for anti-HIV therapy. The drug has already completed phase I clinical trials as a single agent, and it has now entered extended trials as a component of a triple-drug combination.[28]

With respect to the configuration at C2′, only the nucleoside analogue with the fluorine atom with the *ara*-configuration (**2**) proved to be antivirally active, while the *ribo*-configurated derivative, 2′-*ribo*-F-2′,3′-dideoxyadenosine (**3**, F-*ribo*-ddA), was devoid of activity.[20,21] We have argued that the different stereochemistry of the fluorine substituent is responsible for such striking biological differences between the two compounds because the configuration at C2′ is associated with a different conformational effect on the glycon.[29] In solution, nucleoside analogues generally show a rapid equilibrium between two equally populated puckering conformations of the glycon: a 3′-*endo* (north, N) conformation and the 2′-*endo* (south, S) conformation. In contrast, a fluorine atom in a 2′,3′-dideoxyribose residue leads to a higher rigidity of the five-membered ring and forces the equilibrium to shift to either extreme (N- or S-form) as a function of its stereochemistry. The origin of the fluorine-induced "stiffness" on the sugar pucker arises from the so-called *gauche* effect resulting from the interaction between the ribose oxygen and the very electronegative fluorine atom. This strong *gauche* effect has a profound stereoelectronic effect on the stereochemical orientation of the neighboring groups. Indeed, the fluorine substituent governs the overall conformation of the sugar ring forcing vicinal electronegative atoms to adopt a *gauche* rotational arrangement instead of the expected *trans*-diaxial orientation (Scheme 2). Due to this *gauche* effect, the axially oriented fluorine atom changes the sugar pucker into a preferred 3′-*endo*-conformation for the F-*ribo*-configuration and a preponderant 2′-*endo*-conformation in the case of the F-*ara*-configuration (Scheme 2).[29] As a result, the fluorine is able to shift the sugar pucker equilibrium to a high percentage (ca. 85%) of only one (N- or S-form) conformation of the tetrahydrofuran ring.[30]

We have further argued that differences in the predominant conformation in solution could account for the differential metabolic profile of the 2′-fluorinated ddA derivatives **2** and **3**. If this difference in metabolism

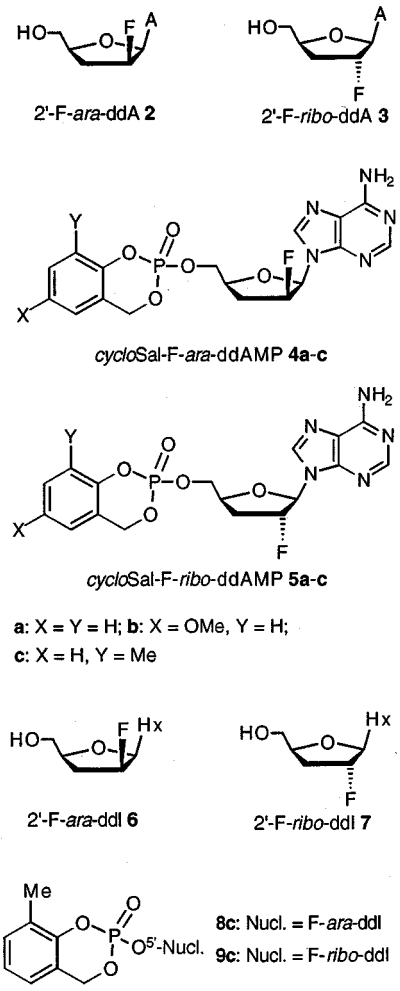
Scheme 2. Equilibrium of the Sugar Pucker Conformation of F-*ara*-ddA (**2**) and F-*ribo*-ddA (**3**)



is limited to the formation of the corresponding monophosphates, this assumption can be studied by the use of masked nucleotide precursors that are able to deliver the nucleotides intracellularly after cell membrane penetration.[31] We have previously shown that our *cyclo*Sal-pronucleotide concept[32] is, in principle, able to fulfill this premise. It has been unequivocally shown for different *cyclo*Sal-d4TMP derivatives that these compounds selectively deliver d4TMP from the lipophilic prodrugs, since the anti-HIV activity of these compounds was found to be independent of thymidine kinase, the enzyme that converts d4T into d4TMP.[33] Moreover, with different *cyclo*Sal-ddAMP and *cyclo*Sal-d4AMP derivatives,[34] a marked increase in biological activity against HIV-1, HIV-2, and murine sarcoma virus (MSV) has been observed.[35] This has been correlated to a successful and efficient intracellular delivery of the corresponding monophosphates through the adenosine deaminase (ADA) bypass.[34,35] Further, it was demonstrated that the latter *cyclo*Sal derivatives were completely resistant to deamination by ADA as well as by adenosine monophosphate deaminase (AMPDA). Therefore, the *cyclo*Sal concept ought to be a good tool to differentiate the effect of fluorine substitution beyond the monophosphate level in both F-ddA nucleoside analogues **2** and **3**.

In this work we report on the synthesis, lipophilic properties, and susceptibility to hydrolysis as well as the biological activity of the *cyclo*Sal-2′-*ara*-fluoro-2′,3′-dideoxyadenosine monophosphates **4a**−**c** (*cyclo*Sal-F-*ara*-ddAMP) and *cyclo*Sal-2′-*ribo*-fluoro-2′,3′-dideoxyadenosine monophosphates **5a**−**c** (*cyclo*Sal-F-*ribo*-d4AMP) as potential nucleotide delivery systems of F-*ara*-ddAMP and F-*ribo*-ddAMP (Chart 1). Furthermore, the effects of the *cyclo*Sal modification on the enzymatic stability against deamination by ADA and AMPDA were evaluated. The properties observed for **4** and **5** were compared to those of the corresponding F-*ara*-ddIMP (**8c**) and F-*ribo*-ddIMP (**9c**) triesters.

Chart 1. Structures of F-*ara*-ddA (**2**), F-*ribo*-ddA (**3**), F-*ara*-ddI (**6**), F-*ribo*-ddI (**7**), and Title *cyclo*Sal Phosphotriesters **4**, **5**, **8c**, and **9c**



Results and Discussion

Chemistry. F-*ara*-ddA (**2**) and F-*ribo*-ddA (**3**) were synthesized as described previously.[21] In order to compare the physical and biological properties of the adenine and hypoxanthine analogues, we prepared F-*ara*-ddI (**6**) and F-*ribo*-ddI (**7**), respectively, from F-*ara*-ddA (**2**) and F-*ribo*-ddA (**3**), by enzymatic deamination with ADA in nearly quantitative yields.[13] All nucleoside derivatives **2**, **3**, and **6** showed spectroscopic properties identical to those described before.[20,21] The synthesis of the *cyclo*Sal-FddAMP triesters **4** and **5**, as well as the *cyclo*Sal-FddIMP triesters **8c** and **9c**, was performed in a similar fashion as for the nonfluorinated nucleoside analogues without protecting the exocyclic amino group of nucleosides **2** and **3**.[33] The *cyclo*Sal residue was introduced regioselectively by the appropriate chlorophosphanes[36] which favored the 5′-*O*-modification, relative to the 5′-*O*,*N*-di-*cyclo*Sal-modified phosphotriesters, by a 6:1 ratio. Both the *cyclo*Sal-F-ddAMP (**4a**−**c** and **5a**−**c**) and *cyclo*Sal-F-ddIMP (**8c** and **9c**) triesters were obtained in good yield as 1:1.6 diastereomeric mixtures with respect to the configuration at the phosphorus center.

The mixtures of diastereomers were characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, as well as by electrospray mass spectrometry (ESI, positive mode) and UV spectroscopy. The purity of the products was verified by RP-HPLC using different gradient systems (water/acetonitrile gradients). Prior to antiviral activity evaluation, small amounts of *cyclo*Sal phosphotriesters **4a**−**c** and **5a**−**c** were additionally purified by semipreparative HPLC using acetonitrile/water eluants to ensure that traces of nucleoside impurities had been eliminated.

Table 1. Hydrolysis Half-Lives ($t_{1/2}$) in Phosphate Buffer and log *P* Values of Nucleoside Analogues **2**, **3**, **6**, and **7** and *cyclo*Sal-FddA(I)MPs **4**, **5**, **8c**, and **9c**

compd	subst X	hydrolysis ($t_{1/2}$) at pH 7.3[a] (h)[b]	log *P* [c]
4a	H	8.9	0.95
4b	5-OMe	13.0	1.04
4c	3-Me	21.6	1.29
2		na[d]	−0.16[14]
8c	3-Me	22.2	0.51
6		na[d]	−1.19
5a	H	8.4	0.72
5b	5-OMe	12.3	0.71
5c	3-Me	23.4	0.93
3		na[d]	−0.20
9c	3-Me	22.7	0.24
7		na[d]	−0.96
AZT		na[d]	0.025[14]

[a] pH 7.3: 25 mM phosphate buffer (Sörensen) at 37 °C. [b] Half-lives were determined from the decreasing peak of the starting phosphotriester and are the mean of duplicate experiments; values are given in hours. [c] log *P*: log of the partition coefficient in 1-octanol/water mixtures. [d] na, not available.

Determination of the Partition Coefficients (log *P* Values). The partition coefficients (log *P* values) of the *cyclo*Sal-FddA(I)MPs **4**,**5** and **8**,**9**, as well as of those of the parent nucleoside analogues **2**,**3** and **6**,**7**, were determined as previously described for the nonfluorinated analogues in 1-octanol/water.[34] The calculated log *P* values are summarized in Table 1. The *P* values of the phosphotriesters studied here were found to be between 14 and 30, whereas the *P* value for F-*ara*-ddA (**2**) was 0.66.[14] That means that the lipophilicity increased by at least 22-fold. The log *P* of the parent nucleosides **2** and **3** did not differ greatly, whereas the *P* values for the *cyclo*Sal-F-*ara*-ddAMP and *cyclo*Sal-F-*ribo*-ddAMP triesters differed by ca. 2-fold. This unexpected lipophilicity difference of the triesters cannot be explained, but further molecular modeling and NMR studies are in progress to elucidate possible structural differences. As expected, all hypoxanthine-bearing derivatives showed a marked decrease in lipophilicity relative to the corresponding adenine-bearing compounds. Compared to AZT, which has a log *P* of 0.025[36,37] and enters mammalian cells only by passive, nonfacilitated diffusion,[38] the *cyclo*Sal-FddAMP triesters **4** and **5** described here may show improved cellular uptake by passive diffusion as would be expected from their log *P* values. However, high lipophilicity per se may not be the only factor that determines intracellular accumulation, and a direct correlation of lipophilicity and ease of membrane penetration is not always possible. Furthermore, very high lipophilicity would cause reduced water solubility and possible dosing problems. Judging by the log *P* value alone, F-*ara*-ddA (**2**) is more lipophilic (−0.18)[14] than ddA (**1**, −0.29).[14] However, Masood et al. have reported that the rate of entry for ddA (**1**) was twice that of F-*ara*-ddA (**2**).[17] The same was true for the corresponding hypoxanthine derivatives.

Kinetic Studies. As has been shown before, the *cyclo*Sal phosphotriesters release the nucleotides and the masking group selectively by a controlled, chemically induced tandem reaction. The degradation pathway has been previously demonstrated.[33] The success of the concept has been demonstrated with the intracellular delivery of d4TMP,[33] ACVMP,[39] ddAMP,[23,35] and d4AMP[35] from the corresponding masked precursors. These compounds can thus be considered as efficient thymidine kinase or adenosine deaminase bypass agents. Here, the degradation of the *cyclo*Sal-F-*ara*-ddAMPs **4** and *cyclo*Sal-F-*ribo*-ddAMPs **5** by chemical hydrolysis was followed at pH 7.3 in phosphate buffer as described before.[33a]

As seen in Table 1, chemical hydrolysis in 25 mM phosphate buffer, pH 7.3, gave half-lives for the triesters **4** and **5** between 9 and 23 h. Again, only the monophosphates and the salicyl alcohol were detected chromatographically by HPLC. Interestingly, the position of the fluorine at C2′ of the glycon had no influence on the hydrolytic stability of the triesters. Furthermore, there was no effect related to the different nature of the heterocycle base attached to the fluorinated glycon (compare **4c** and **8c**, as well as **5c** and **9c**). Compared to the corresponding nonfluorinated *cyclo*Sal-ddAMP derivative,[35] the half-lives at pH 7.3 were 2-fold lower. On the other hand, they were nearly identical to the $t_{1/2}$ values found for the *cyclo*Sal-d4AMP triesters.[35] Thus, although the fluorine is separated by six bonds from the site of hydrolysis, it clearly has an activating effect which can be compared to that caused by the olefinic bond in d4A. In 10% FCS (fetal calf serum)-containing RPMI culture medium, the half-lives of the degradation of the triesters **4a**−**c** were found to be slightly shorter (7.2, 9.5, and 13.6 h, respectively; the values represent only the disappearance of their triesters, data not shown), most probably due to the more basic pH of this medium relative to the phosphate buffer. Additional experiments in acidic media have not been done systematically because others have shown before that due to the introduction of the electronegative fluorine, the FddAs **2** and **3** do not undergo cleavage of the glycosyl bond as do ddA and d4A.[13,20] Thus, only the *cyclo*Sal-F-*ara*-ddAMP (**4c**) was incubated at pH 3.0 and 1.5 in a 25 mM glycine/HCl buffer. As expected, no deglycosylation occurred, but a pH-dependent hydrolysis of the phosphotriester was detected: **4c** showed a half-life of 284 h at pH 3.0 and 19 h at pH 1.5. The considerable decrease of $t_{1/2}$ at pH 1.5 is due to acid-catalyzed phosphate ester hydrolysis. Additional hydrolysis studies in culture medium, plasma, and crude cell extracts are in progress in our laboratory.

Adenosine Deaminase Studies. As mentioned in the Chemistry section, both FddAs **2** and **3** were deaminated by ADA (EC 3.5.4.4; from calf intestinal mucosa) to yield the corresponding FddI derivatives **6** and **7**. It can be assumed that loss in biological potency with these nucleosides is related to this enzymatic catabolism, although the FddIs generated are not subject to phosphorolysis by PNP. We have already shown that *cyclo*Sal derivatives of ddA and d4A are not substrates for either ADA or AMPDA.[35] Here, we confirmed again the stability[40] of the *cyclo*Sal-FddA derivatives **4** and **5** against deamination by both ADA (0.5 U of ADA in a 10 mM phosphate buffer, pH 6.8, 37 °C) and AMPDA (EC 3.5.4.6; from rabbit muscle; 2 U of AMPDA in 10 mM phosphate buffer, pH 6.8, 37 °C). The chosen derivatives for this study were the 3-methyl-*cyclo*Sal compounds **4c** and **5c**. The lack of deamination of the *cyclo*Sal derivatives guarantees the intracellular delivery of the fluorinated adenine nucleotides and prevents these compounds from functioning as FddIMP pronucleotides.

ADA-catalyzed deamination of the parent nucleoside analogue F-*ara*-ddA (**2**) was achieved after 30 min, whereas deamination of F-*ribo*-ddA (**3**) was very rapid and complete to F-*ribo*-ddI within 0.6 min. These enzymatic reactions were performed with only 0.25 U of enzyme and monitored by UV spectroscopy (260 nm). In our hands, F-*ribo*-ddA (**3**) deaminated even faster than ddA (2 min) and d4A (10 min).[35] The probable reason for the 50-fold difference in the rate of deamination of F-*ribo*-ddA (**3**) over ddA may be associated with the preponderance of the C3′-*endo* (N) conformer. Conversely, F-*ara*-ddA (**2**), which is predominately C2′-*endo* (S), undergoes a more sluggish reaction.

Antiviral Evaluation. The efficient adenosine deaminase bypass of *cyclo*Sal-ddAMP and *cyclo*Sal-d4AMP phosphotriesters[35] and other pronucleotide approaches[41] has been reported before. These results demonstrated (1) the successful membrane penetration of the *cyclo*Sal triesters and (2) the intracellular delivery of the corresponding nucleotides ddAMP or d4AMP. Consequently, a 100−600-fold increase in biological potency against HIV-1 and HIV-2 in CEM/O cells was observed. This marked improvement in bioactivity has been attributed to the ability of these compounds to bypass up to four metabolic steps that may otherwise be needed for the conversion of ddA/d4A into ddAMP/d4AMP.

The *cyclo*Sal compounds studied here were also expected to deliver the corresponding FddA nucleotides because of their common characteristics with the *cyclo*Sal-ddAMP and *cyclo*Sal-d4AMP triesters. Thus, a commensurate increase in the antiviral potency of the F-*ara*-ddAMP prodrugs was anticipated. Additionally, it was of interest to see whether the *cyclo*Sal modification would have an influence on the biological activity of F-*ribo*-ddA (**3**), a compound that was completely inactive against HIV in several cell lines (MT-4, ATH8).[20,21] A tentative hypothesis was that the different sugar conformation in F-*ribo*-ddA (**3**) may have accounted for the blockade of its metabolic conversion to the monophosphate level and that the pronucleotide approach would circumvent such a blockade and convert it into an active compound. The parent nucleosides F-*ara*-ddA (**2**) and F-*ribo*-ddA (**3**) as well as the corresponding *cyclo*Sal compounds **4** and **5** were evaluated for their ability to inhibit the replication of HIV-1 and HIV-2 in rapidly dividing human T-lymphocytic CEM cells. Additionally, the compounds were evaluated against Moloney murine sarcoma virus (MSV)-induced transformation of murine C3H/3T3 embryo fibroblasts (Table 2). The two *cyclo*Sal-FddIMP triesters **8c** and **9c** and their parent nucleosides **6** and **7** were also included in the antiviral tests, and the antiviral activities of the nucleoside analogues ddA and d4T appear in Table 2 for comparison. All test compounds **4a**−**c** and **5a**−**c**

Table 2. Inhibitory Effects of the *cyclo*Sal Phosphotriesters **4a**−**c**, **5a**−**c**, **8c**, and **9c** as Well as Nucleosides **1**−**3**, **6**, and **7** on the Replication of HIV-1 and HIV-2 in Wild-Type CEM Cells and MSV in C3H/3T3 Cells

		antiviral activity EC_{50} (μM)[a]			cytotoxicity		
compd	subst X	CEM HIV-1	CEM HIV-2	C3H/3T3 MSV	CEM CC_{50} (μM)[b]	C3H/3T3 MCC (μM)[c]	SI[e] (CEM)
4a	H	4.53	1.85	1.7	137	>50	74
4b	5-OMe	1.5	5.67	nd[d]	121	nd[d]	80
4c	3-Me	3.67	3.3	nd[d]	146	nd[d]	44
2		36.7	40.0	>50	>250	>50	>6.8
8c	3-Me	3.57	3.57	>50	91.9	>50	26
6		41.7	30.0	>50	>250	>50	>8
5a	H	19.0	13.0	4.7	>250	>50	>19
5b	5-OMe	20.0	25.0	nd[d]	>250	nd[d]	>12
5c	3-Me	11.7	12.5	2.83	118	>50	10
3		>250	>250	>50	>250	>50	na[f]
9c	3-Me	75.0	31.7	>50	>250	>50	>8
7		>250	>250	>50	>250	>50	na[f]
ddA (**1**)		4.33	4.55	39.1	>250	>50	>57
d4T		0.18	0.26	nd[d]	56	nd[d]	311

[a] 50% effective concentration. [b] 50% cytotoxic concentration. [c] Minimal inhibitory concentration. [d] nd, not determined. [e] Selectivity index. [f] na, not available.

were free from the parent nucleosides **2**,**3** and **6**,**7** as verified by analytical RP-HPLC (>99% purity).

As anticipated, F-*ribo*-ddA (**3**) and F-*ribo*-ddI (**7**) were devoid of any antiretroviral activity (EC_{50} > 250 μM). On the other hand, F-*ara*-ddA (**2**) and the corresponding F-*ara*-ddI (**6**) proved to be equally active against HIV-1- and HIV-2-induced cytopathogenicity in CEM cells (EC_{50} ca. 30−40 μM). This is in excellent agreement with the activity found in MT-4 cells (EC_{50} ca. 35 μM).[21] In the same assay, the nonfluorinated ddA exhibited a 10-fold higher potency despite the expected higher levels of F-*ara*-ddADP and F-*ara*-ddATP metabolites. It is known that in MOLT-4 cells, for example, F-*ara*-ddADP and F-*ara*-ddATP levels are 20- and 5-fold greater, respectively, than the levels of ddADP and ddATP over the same period of time.[17] An interesting finding was that the *cyclo*Sal-F-*ara*-ddAMPs **4** were about 10-fold more potent against HIV-1 and HIV-2 than the parent nucleoside **2**. This increase made the triesters equivalent to ddA (**1**, EC_{50} = 4.4 μM) with respect to antiviral potency and selectivity index (Table 2).[35] The same was true for the anti-HIV activity of 3-methyl-*cyclo*Sal-F-*ara*-ddIMP (**8c**). Inhibition of MSV-induced cell transformation was noted only for compound **4a** (EC_{50} = 1.7 μM), whereas both nucleosides **2** and **3**, and the F-*ara*-ddIMP triester **8c**, were completely inactive. Consequently, due to the *cyclo*Sal modification, F-*ara*-ddA (**2**) must have been converted to a potent anti-MSV active compound without a change in the MIC value (invariably >50 μM). Additional important results came from the antiviral evaluation of the *cyclo*Sal-F-*ribo*-ddAMPs **5**. In contrast to the inactive nucleoside analogue, F-*ribo*-ddA (**3**), all triesters **5** showed antiviral activity in the 12−25 μM range. The most potent compound was the 3-methyl-substituted *cyclo*Sal derivative **5c** (EC_{50} = 12 μM), which translated into a greater than 25-fold increase in potency with respect to **3**. There was no correlation between the antiviral potency and the substitution pattern of the *cyclo*Sal moiety.[33a,35] Moreover, the *cyclo*Sal-F-*ribo*-ddAMPs **5** were 3−4-fold more potent than the nucleoside F-*ara*-ddA (**2**), a compound that has recently completed phase I clinical trials.[28] However, there is still a 4−5-fold difference in potency between the *ara*- and *ribo*-*cyclo*Sal-FddAMPs **4** and **5** in favor of the *ara*-analogues. This difference is essentially the same as that found by Mitsuya and Marquez for the IC_{50} values of the triphosphates F-*ara*-ddATP (12 μM) and F-*ribo*-ddATP (50.1 μM) with recombinant HIV reverse transcriptase.[42] The fact that we observed exactly the same 4−5-fold difference in the antiviral activities of **4** and **5** suggests that continued phosphorylation to the triphosphate level seems not to be influenced by the different C2′ configuration of the fluorine. However, we have no information about the intracellular amounts generated of the respective FddA triphosphates. The results, nevertheless, clearly show that the inactive F-*ribo*-ddA (**3**) was converted into an active compound. This means that the *cyclo*Sal strategy helped bypass the critical step in the activation of F-*ribo*-ddA to the monophosphate F-*ribo*-ddAMP, a step that may have been impaired by the C3′-*endo*-conformation of the tetrahydrofuran ring. Most likely, F-*ribo*-ddA, as discussed above, is rapidly and quantitatively deaminated to give F-*ribo*-ddI, which fails to be readily phosphorylated to F-*ribo*-ddIMP. Further rate-limiting steps are the two enzymatic conversions that would be required to convert F-*ribo*-ddIMP into F-*ribo*-ddAMP.[43] That these two steps do not appear to be very efficient can be deduced from the significantly lower antiviral activity of 3-methyl-*cyclo*Sal-F-*ribo*-ddIMP (**9c**) (EC_{50} 75 and 32 μM) relative to 3-methyl-*cyclo*Sal-F-*ribo*-ddAMP (**5c**) (EC_{50} ca. 12 μM). In the case of 3-methyl-*cyclo*Sal-F-*ara*-ddIMP (**8c**) these conversions did not cause a decrease in bioactivity when compared to 3-methyl-*cyclo*Sal-F-*ara*-ddAMP (**4c**) which was 3.6 μM for both compounds. Finally, for **5a**,**b**, a comparable anti-MSV activity to that observed for the *ara*-configurated triesters **4a** was detected (3−5 μM).

Conclusion

In summary, the *cyclo*Sal-FddAMPs **4** and **5** provide an efficient means to deliver intracellularly the nucleoside monophosphates F-*ara*-ddAMP and F-*ribo*-ddAMP. In the first case, the anti-HIV activity of *cyclo*Sal-F-*ara*-ddAMP (**4**) in CEM cells improved by 10-fold relative to F-*ara*-ddA (**2**). Additionally, biological activity was observed against MSV in C3H/3T3 fibroblasts. In the second case, an antivirally inactive nucleoside analogue (**3**, F-*ribo*-ddA) was converted into an active compound (**5**, *cyclo*Sal-F-*ribo*-ddAMP) that showed even higher

anti-HIV potency than F-*ara*-ddA (**2**). The pronounced susceptibility of **3** to rapid deamination by ADA may originate from its preferred 3′-*endo* (N) conformation which results in the accumulation of F-*ribo*-ddI. Differences in the conformation of the sugar pucker may also account for the lack of conversion of F-*ribo*-ddI (**7**) to the corresponding monophosphate. Further enzymatic studies will have to be conducted to prove this hypothesis. For F-*ara*-ddA (**2**), some limitations during the direct phosphorylation (by deoxycytidine kinase or adenosine kinase), or during the deamination/reamination steps, may be alleviated by using the *cyclo*Sal approach, thus resulting in an improved antiviral activity. An important difference between *cyclo*Sal-F-*ara*-ddAMP (**4**) and *cyclo*Sal-ddAMP is that with the former only a 10-fold increase in potency is observed relative to the parent drug, whereas with the latter the increase in potency is 100-fold. This may indicate that the activating pathways for the parent compounds are more efficient for F-*ara*-ddA (**2**) than for ddA or ddI. However, due to the considerably lower IC_{50} of ddATP to RT (0.11 μM)[44] as compared to F-*ara*-ddATP (12 μM),[42] the inefficient metabolic activation is, in part, compensated. Nevertheless, since there is considerably more ddA loss through catabolic pathways, ddA seems to benefit more than F-*ara*-ddA from the *cyclo*Sal approach. However, the last assumptions remain to be proven by metabolism studies using the *cyclo*Sal triesters in comparison to the parent nucleosides.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions (argon atmosphere). Solvents: Anhydrous dimethylformamide (DMF) and anhydrous tetrahydrofuran (THF) were obtained in a Sure/Seal bottle from FLUKA and stored over 4 Å molecular sieves; ethyl acetate, methylene chloride, and methanol for the chromatography were distilled before used. Triethylamine was distilled from CaH_2 prior to use. The solvents for HPLC were obtained from Merck (acetonitrile, HPLC grade) and Riedel-de-Haën (water, HPLC grade). Evaporation of solvents was carried out on a rotary evaporator under reduced pressure or with a high-vacuum pump. Chromatography: Chromatotron (Harrison Research 7924), silica gel 60_{PF} (Merck, gipshaltig); UV detection at 254 nm. Column chromatography: Merck silica gel 60 (40−60 μm). TLC: analytical thin-layer chromatography was performed on Merck precoated aluminum plates 60 F_{254} with a 0.2-mm layer of silica gel containing a fluorescent indicator; sugar-containing compounds were visualized with the sugar spray reagent (0.5 mL of 4-methoxybenzaldehyde, 9 mL of ethanol, 0.5 mL of concentrated sulfuric acid, and 0.1 mL of glacial acetic acid) by heating with a heat gun or a hot plate. HPLC: (Merck-Hitachi) semipreparative HPLC, LiChroCART 250-10 containing LiChrospher 100 RP-18 (10 μm); analytical HPLC, EcoCART 125-3 with LiChrospher 100 RP-18 endcapped (5 μm), gradient I (standard gradient) 18−100% CH_3CN (0−16 min), 100% CH_3CN (16−20 min), 18% CH_3CN (19−31 min), flow 0.5 mL, UV detection at 260 nm; gradient II 0−100% CH_3CN (0−18 min), 100% CH_3CN (18.1−20 min), 0% CH_3CN (20.1−33 min), flow 0.5 mL, UV detection at 265 nm; gradient III 11−100% CH_3CN (0−18 min), 100% CH_3CN (18−20 min), 11% CH_3CN (20.1−32 min), flow 0.5 mL, UV detection at 265 nm. NMR spectra were recorded using (^{1}H NMR) Bruker DMX 600 at 600 MHz, AMX 400 at 400 MHz, or Bruker WH 270 at 270 MHz (DMSO as internal standard); (^{13}C NMR) Bruker AMX 400 or DMX 600 at 100.6 or 150.9 MHz, respectively ($CDCl_3$ or DMSO as internal standard); (^{31}P NMR) Bruker AMX 400 at 162 MHz (H_3PO_4 as external standard); (^{19}F NMR) Bruker AMX 400 at 376.5 MHz ($CFCl_3$ as external standard). All ^{1}H and ^{13}C NMR chemical shifts (δ) are measured in parts per million (ppm) downfield from tetramethylsilane, $(CD_3)(CDH)SO$ being set at δ_H 2.49 as a reference. The ^{31}P NMR chemical shifts are measured in ppm using H_3PO_4 as external reference. The spectra were recorded at room temperature, and all ^{13}C and ^{31}P NMR spectra were recorded in the proton-decoupled mode. UV spectra were taken with a Varian Cary 1E UV/VIS spectrophotometer. The infrared spectra were recorded with a Perkin-Elmer 1600 series FT-IR spectrometer in KBr pellets. Mass spectra were obtained with a Fisons electrospray VG platform II spectrometer in positive mode (ESI^+). The test compounds were isolated as mixtures of diastereomers arising from the mixed stereochemistry at the phosphate center. The resulting lyophilized triesters did not give useful microanalytical data, most probably due to incomplete combustion of the compounds, but were found to be pure by rigorous HPLC analysis (three different gradients), high-field multinuclear NMR spectroscopy, and electrospray mass spectrometry.

General Procedure for the Preparation of the *cyclo*Sal-F-*ara*-ddAMPs 4a−c, *cyclo*Sal-F-*ribo*-ddAMPs 5a−c, 3-Methyl-*cyclo*Sal-F-*ara*-ddIMP (8c), and 3-Methyl-*cyclo*Sal-F-*ribo*-ddIMP (9c). The reactions were performed in an argon atmosphere under anhydrous conditions. To a solution of the nucleoside **2**, **3**, **5**, or **6** (0.20 mmol) in 2 mL of DMF and 1 mL of THF, cooled to −40 °C, was added DIPEA (2.0 equiv, 0.40 mmol). Then the chlorophosphanes[36] (2.0 equiv, 0.4 mmol) were added within 0.5 h, and the solutions were stirred for 20 min to complete the reaction (TLC analysis). For the oxidation of the intermediate cyclic phosphites, *tert*-butyl hydroperoxide (2.0 equiv, 0.4 mmol) was added to the reaction mixture at −40 °C. After stirring for 0.5 h, the reaction mixture was warmed to room temperature, and the solvent was removed under reduced pressure. The residues were purified twice by chromatography on silica gel plates on a chromatotron, first using a gradient of CH_3OH in ethyl acetate (0−30% methanol) followed by a gradient of CH_3OH in CH_2Cl_2 (0−20%), to yield the title compounds **4a**−**c**, **5a**−**c**, **8c**, and **9c**.

***cyclo*Saligenyl-5′-*O*-(2′,3′-dideoxy-2′-fluoroarabinosyladenyl)phosphate (*cyclo*Sal-F-*ara*-ddAMP, 4a):** yield 53%; ^{1}H NMR (400.1 MHz, DMSO-d_6) δ 8.15 (s, 2H, H2); 8.13 (d, 1H, H8); 8.11 (d, 1H, H8); 7.37−7.30 (m, 6H, NH_2, H4-aryl); 7.26 (t, 1H, H6-aryl); 7.24 (t, 1H, H6-aryl); 7.18 (dt, 1H, H5-aryl); 7.17 (dt, 1H, H5-aryl); 7.13 (dd, 1H, H3-aryl); 7.07 (dd, 1H, H3-aryl); 6.36 (dd, 1H, H1′); 6.35 (dd, 1H, H1′); 5.55−5.40 (m, 5H, H2′, CH_2-benzyl); 4.45−4.27 (m, 5H, H4′, H5′); 2.77−2.60 (m, 2H, H3″); 2.31−2.17 (m, 2H, H3′); ^{13}C NMR (67.9 MHz, DMSO-d_6) δ 155.98 (C6); 152.80 (C2); 149.49 (C2-aryl); 149.10 (C4); 139.29 (d, C8); 129.70 (C6-aryl); 126.00 (C4-aryl); 124.36 (C5-aryl); 121.00 (C1-aryl); 118.19 (C5); 118.07 (C3-aryl); 90.92 (d, C2′); 84.16 (d, C1′); 75.07 (d, C4′); 69.37 (C5′); 68.51 (CH_2-benzyl); 32.56 (d, C3′); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.55; −9.66; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −187.08; −187.21; MS (ESI^+) m/z 422.0 (M + 1); UV (CH_3CN) λ_{max} 257.42 nm, 208.98 nm, 193.00 nm; λ_{min} 226.16 nm, 202.12 nm; IR (KBr) ν 3330.0; 3184.2; 1648.1; 1599.1; 1489.0; 1296.6; 1247.6; 1192.8; 1052.2; 947.0; 759.7; 651.4; 579.1; R_f value 0.42 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.03 min (95.0%, gradient I); 16.13 min (95.1%, gradient II); 20.24 min (95.0%, gradient III).

***cyclo*(5-Methoxysaligenyl)-5′-*O*-(2′,3′-dideoxy-2′-fluoroarabinosyladenyl)phosphate (5-OMe-*cyclo*Sal-F-*ara*-ddAMP, 4b):** yield 60%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 2H, H2); 8.12 (d, 1H, H8); 8.10 (d, 1H, H8); 7.30 (s, 4H, NH_2); 7.05 (d, 1H, H3-aryl); 6.98 (d, 1H, H3-aryl); 6.89 (dd, 1H, H4-aryl); 6.84 (dd, 1H, H4-aryl); 6.83 (d, 1H, H6-aryl); 6.82 (d, 1H, H6-aryl); 6.35 (dd, 1H, H1′); 6.34 (dd, 1H, H1′); 5.44 (dd, 1H, H_A-benzyl); 5.40 (dd, 1H, H_A-benzyl); 5.37 (dd, 1H, H_B-benzyl,); 5.34 (dd, 1H, H_B-benzyl); 5.40−5.28 (m, 2H, H2′); 4.43−4.24 (m, 6H, H4′, H5′); 3.70 (s, 3H, OCH_3); 3.69 (s, 3H, OCH_3); 2.76−2.58 (m, 2H, H3′); 2.32−2.15 (m, 2H, H3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 156.00 (C6); 155.59 (C5-aryl); 152.83 (C2); 149.13 (C4); 143.12 (C2-aryl); 139.33 (d, C8); 121.77 (C1-aryl); 119.14 (C3-aryl); 118.12 (C5); 116.14 (C6-

aryl); 110.61 (C4-aryl); 90.96 (d, C2′); 84.15 (d, C1′); 75.08 (d, C4′); 69.25 (CH_2-benzyl); 68.47 (C5′); 55.57 (OCH_3); 32.59 (d, C3′); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.38; −9.48; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −187.07; −187.19; MS (ESI$^+$) *m/z* 452.4 (M + 1); UV (CH_3CN) λ_{max} 257.13 nm, 195.94 nm; λ_{min} 235.61 nm; IR (KBr) ν 3393.5; 1621.7; 1551.5; 1456.4; 1396.3; 1328.4; 1182.2; 970.8; 849.0; 744.8; R_f value 0.42 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.44 min (99.0%, gradient I); 16.21 min (99.0%, gradient II); 20.16 min (99.0%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′-fluoro-arabinosyladenyl)phosphate (3-Me-*cyclo*Sal-F-*ara*-ddAMP, 4c):** yield 58%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 2H, H2); 8.11 (d, 1H, H8); 8.10 (d, 1H, H8); 7.31 (s, 4H, NH_2); 7.24−7.20 (m, 2H, H5-aryl); 7.08−7.03 (m, 4H, H4-aryl, H6-aryl); 6.35 (dd, 1H, H1′); 6.34 (dd, 1H, H1′); 5.46 (dd, 1H, H_A-benzyl); 5.44 (dd, 1H, H_A-benzyl); 5.40 (dd, 1H, H_B-benzyl); 5.34 (dd, 1H, H_B-benzyl); 5.44−5.36 (m, 2H, H2′); 4.42−4.27 (m, 6H, H4′, H5′); 2.77−2.60 (m, 2H, H3′); 2.31−2.19 (m, 2H, H3′); 2.20 (s, 3H, CH_3); 2.12 (s, 3H, CH_3); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 155.98 (C6); 152.80 (C2); 149.11 (C4); 147.8 (C2-aryl); 139.38 (d, C8); 130.84 (C4-aryl); 126.94 (C3-aryl); 123.91 (C5-aryl); 123.51 (C6-aryl); 120.96 (C1-aryl); 118.12 (C5); 90.96 (d, C2′); 84.17 (d, C1′); 75.07 (C4′); 69.79 (CH_2-benzyl); 68.44 (C5′); 32.52 (d, C3′) 14.90 (CH_3); ^{31}P NMR (162 MHz, DMSO-d_6) δ −8.92; −8.96; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −187.08; −187.28; MS (ESI$^+$) *m/z* 436.1 (M + 1); UV (CH_3CN) λ_{max} 257.68 nm, 209.76 nm, 194.97 nm; λ_{min} 228.10 nm, 205.10 nm; IR (KBr) ν 3331.0; 3213.8; 2954.9; 1644.5; 1602.6; 1474.9; 1292.9; 1191.1; 1089.5; 943.0; 819.9; 651.7; R_f value 0.42 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.59 min (96.8%, gradient I); 16.89 min (96.8%, gradient II); 21.72 min (96.8%, gradient III).

***cyclo*Saligenyl-5′-*O*-(2′,3′-dideoxy-2′-fluororibosyladenyl)phosphate (*cyclo*Sal-F-*ribo*-ddAMP, 5a):** yield 56%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (s, 1H, H2); 8.17 (s, 1H, H2); 8.12 (s, 1H, H8); 8.11 (s, 1H, H8); 7.34 (t, 1H, H4-aryl); 7.32 (s, 2H, NH_2); 7.31 (s, 2H, NH_2); 7.30 (t, 1H, H4-aryl); 7.21 (dd, 1H, H6-aryl); 7.20 (dd, 1H, H6-aryl); 7.16 (dt, 1H, H5-aryl); 7.15 (dt, 1H, H5-aryl); 7.08 (dd, 1H, H3-aryl); 7.01 (dd, 1H, H3-aryl); 6.26 (d, 1H, H1′); 6.25 (d, 1H, H1′); 5.74 (dd, 1H, H2′); 5.72 (dd, 1H, H2′); 5.44 (dd, 1H, H_A-benzyl); 5.42 (dd, 1H, H_A-benzyl); 5.37 (dd, 1H, H_B-benzyl); 5.34 (dd, 1H, H_B-benzyl); 4.58−4.54 (m, 2H, H4′); 4.45 (ddd, 1H, H_B5′); 4.40 (ddd, 1H, H_B5′); 4.33 (ddd, 1H, H_A5′); 4.29 (ddd, 1H, H_A5′); 2.65−2.62 (m, 4H, H2′); 2.41−2.99 (m, 4H, H3′); ^{13}C NMR (100.61 MHz, DMSO-d_6) δ 155.97 (C6); 155.55 (C2); 149.17, 147.11 (d, C2-aryl); 148.54 (C4); 138.86 (C8); 129.52 (C4-aryl); 125.83 (C5-aryl); 124.22 (C6-aryl); 120.69 (C1-aryl); 118.95 (C5); 117.94, 117.89 (C3-aryl); 96.14 (d, C2′); 88.66 (d, C1′); 78.39, 78.33 (d, C4′); 68.30 (C5′); 68.15, 68.08 (CH_2-benzyl); 32.46 (d, C3′); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.832; −9.845; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −178.08; −178.16; MS (ESI$^+$) *m/z* 422.4 (M + 1); 443.9 (M + Na); UV (CH_3CN) λ_{max} 259.01 nm, 208.99 nm, 192.99 nm; λ_{min} 227.36 nm, 203.19 nm; IR (KBr) ν 3331.9; 3189.7; 1645.1; 1600.1; 1488.6; 1460.3; 1417.8; 1371.1; 1298.1; 1244.6; 1192.3; 1106.7; 1021.1; 949.3; 842.7; 798.3; 758.6; 720.9; 651.5; 581.8; 532.4; R_f value 0.40 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.31 min (94.0%, gradient I); 16.45 min (94.0%, gradient II); 19.52 min (94.2%, gradient III).

***cyclo*(5-Methoxysaligenyl)-5′-*O*-(2′,3′-dideoxy-2′-fluororibosyladenyl)phosphate (5-OMe-*cyclo*Sal-F-*ribo*-ddAMP, 5b):** yield 59%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (s, 1H, H2); 8.17 (s, 1H, H2); 8.13 (s, 1H; H8); 8.11 (s, 1H, H8); 7.32 (s, 2H, NH_2); 7.31 (s, 2H, NH_2); 7.01 (d, 1H, H3-aryl); 6.94 (d, 1H, H3-aryl); 6.88 (dd, 1H, H4-aryl); 6.83 (dd, 1H, H4-aryl); 6.79 (d, 1H, H6-aryl); 6.78 (d, 1H, H6-aryl); 6.27 (d, 1H, H1′); 6.25 (d, 1H, H1′); 5.73 (dd, 1H, H2′); 5.72 (dd, 1H, H2′); 5.39 (dd, 1H, H_A-benzyl); 5.37 (dd, 1H, H_A-benzyl); 5.31 (dd, 1H, H_B-benzyl); 5.32 (dd, 1H, H_B-benzyl); 4.57−4.53 (m, 2H, H4′); 4.42 (ddd, 1H, H_B5′); 4.37 (ddd, 1H, H_B5′); 4.31 (ddd, 1H, H_A5′); 4.26 (ddd, 1H, H_A5′); 3.72 (s, 3H, OCH_3); 3.71 (s, 3H, OCH_3); 2.62−2.53 (m, 2H, H3′); 2.39−2.30 (m, 2H, H3′); ^{13}C NMR (100.61 MHz, DMSO-d_6) δ 156.01 (C6); 155.46 (C5-aryl); 152.60 (C2); 148.59 (C4); 142.87 (C2-aryl); 138.89 (C8); 121.59, 121.50 (C1-aryl); 118.97, 118.87 (C3-aryl); 115.03, 114.94 (C6-aryl); 110.48 (C4-aryl); 96.19 (d, C2′); 88.70 (d, C1′); 78.41 (C4′); 68.35, 68.28 (d, C5′); 67.99 (CH_2-benzyl); 55.59, 55.50 (OCH_3); 32.90 (d, C3′); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.625; −9.647; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −178.05; −178.19; MS (ESI$^+$) *m/z* 452.2 (M$^+$ + 1); 474.4 (M + Na); UV (CH_3CN) λ_{max} 258.98 nm, 195.52 nm; λ_{min} 235.98 nm; IR (KBr) ν 3448.0; 3190.1; 2938.0; 1647.4; 1600.5; 1498.1; 1477.0; 1433.2; 1364.2; 1329.0; 1297.4; 1241.3; 1199.2; 1104.3; 1025.4; 952.7; 916.9; 868.6; 820.4; 759.0; 726.1; 646.6; 528.8; R_f value 0.40 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.41 min, 18.51 min (96.0%, gradient I); 16.32 min, 16.48 min (96.1%, gradient II); 20.08 min, 20.23 min (96.0%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′-fluororibosyladenyl)phosphate (3-Me-*cyclo*Sal-F-*ribo*-ddAMP, 5c):** yield 65%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (s, 1H, H2); 8.17 (s, 1H, H2); 8.103 (s, 1H, H8); 8.100 (s, 1H, H8); 7.31 (s, 4H, NH_2); 7.20−7.17 (m, 2H, aryl H); 7.05−7.01 (m, 4H, aryl H); 6.26 (d, 1H, H1′); 6.25 (d, 1H, H1′); 5.72 (dd, 1H, H2′); 5.71 (dd, 1H, H2′); 5.41 (dd, 1H, H_B-benzyl); 5.39 (dd, 1H, H_B-benzyl); 5.33 (dd, 1H, H_A-benzyl); 5.32 (dd, 1H, H_A-benzyl); 4.58−4.53 (m, 2H, H4′); 4.44 (ddd, 1H, H_B5′); 4.38 (ddd, 1H, H_B5′); 4.31 (ddd, 1H, H_A5′); 4.27 (ddd, 1H, H_A5′); 2.62−2.53 (m, 2H, H3′); 2.39−2.30 (m, 2H, H3′); 2.15 (s, 3H, CH_3); 2.11 (s, 3H, CH_3); ^{13}C NMR (100.61 MHz, DMSO-d_6) δ 156.02 (C6); 152.57 (C2); 148.59 (C4); 147.78, 147.72 (d, C2-aryl); 138.91 (C8); 130.71 (C4-aryl); 126.76, 126.69 (C3-aryl); 123.78 (C5-aryl); 123.35 (C6-aryl); 120.79, 120.70 (C1-aryl); 119.01 (C5); 96.34 (d, C2′); 88.72 (d, C1′); 78.48, 78.44 (C4′); 68.31 (C5′); 68.22 (CH_2-benzyl); 32.64, 32.43 (d, C3′) 14.72, 14.63 (CH_3-C3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.23; −9.24; ^{19}F NMR (376.43 MHz, DMSO-d_6) δ −178.06; −178.08; MS (ESI$^+$) *m/z* 435.9 (M + 1); 457.9 (M + Na); UV (CH_3CN) λ_{max} 258.96 nm, 209.46 nm, 195.93 nm, λ_{min} 229.10 nm, 205.98 nm; IR (KBr) ν 3331.2; 3186.1; 1646.2; 1598.9; 1473.8; 1418.3; 1369.0; 1330.5; 1296.4; 1190.2; 1091.4; 1015.7; 942.1; 822.8; 775.8; 649.3; 532.6; R_f value 0.40 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.89 min (96.1%, gradient I); 16.99 min (96.2%, gradient II); 20.63 min (96.1%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′-fluoro-arabinosylhypoxanthyl)phosphate (3-Me-*cyclo*Sal-F-*ara*-ddIMP, 8c):** yield 67%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.44 (s, 2H, NH); 8.09 (s, 1H, H2); 8.08 (s, 1H, H2); 8.06 (d, 1H, H8); 8.05 (d, 1H, H8); 7.25−7.22 (m, 2H, H5-aryl); 7.10−7.06 (m, 4H, H4-aryl, H6-aryl); 6.37 (dd, 1H, H1′); 6.38 (dd, 1H, H1); 5.49 (dd, 1H, H_A-benzyl); 5.44 (dd, 1H, H_A-benzyl); 5.41 (dd, 1H, H_B-benzyl); 5.40 (dd, 1H, H_B-benzyl); 5.43 (m, 1H, H2′); 5.32 (m, 1H, H2′); 4.43−4.31 (m, 6H, H4′, H5′); 3.34 (s, 3H, CH_3); 3.31 (s, 3H, CH_3); 2.74−2.66 (m, 2H, H3′); 2.27−2.19 (m, 2H, H3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) 155.47 (C6); 147.93 (C2-aryl); 147.88, 147.85 (C4); 146.22 (C2); 138.80, 138.76 (d, C8); 130.87, 130.85 (C4-aryl); 126.90, 126.85 (C3-aryl); 123.95 (C5-aryl); 123.59 (C5), 123.55, 123.53 (C6-aryl); 120.90 (C1-aryl); 90.93 (d, C2′); 84.39, 84.28 (d, C1′); 75.38, 75.31 (d, C4′); 69.28, 69.24 (C5′); 68.44, 68.39 (CH_2-benzyl); 32.50 (d, C3′); 14.93, 14.77 (CH_3-C3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −8.93; −8.96; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −187.41; −187.51; MS (ESI$^+$) *m/z* 437.4 (M + 1); UV (CH_3CN) λ_{max} 249.01 nm, 243.17 nm, 197.02 nm; λ_{min} 246.82 nm, 225.29 nm; IR (KBr) ν 3466.2; 3120.2; 2956.5; 2904.7; 1702.4; 1587.8; 1548.1; 1512.8; 1472.6; 1377.5; 1293.5; 1191.0; 1124.4; 1025.0; 942.3 817.8; 650.3; 602.4; 528.6; R_f value 0.33 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 17.88 min (99.3%, gradient I); 16.44 min (99.3%, gradient II); 19.87 min (99.3%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′-fluororibosylhypoxanthyl)phosphate (3-Me-*cyclo*Sal-F-*ribo*-ddIMP, 9c):** yield 58%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.37 (s, 2H, NH); 8.10 (s, 1H, H2); 8.09 (s, 1H, H2); 8.01 (d, 1H, H8); 8.00 (d, 1H, H8); 7.20−7.18 (m, 2H, H5-aryl); 7.05 (dd, 2H, H6-aryl); 7.02 (dd, 2H, H6-aryl); 6.24 (d, 1H, H1′); 6.23 (d, 1H, H1′); 5.67 (dd, 2H, H2′); 5.43 (dd, 2H, H_A-benzyl); 5.35 (dd, 1H, H_B-benzyl); 5.34 (dd, 1H, H_B-benzyl,); 4.58−4.53 (m, 2H, H4′); 4.44 (ddd, 1H, H_B5′); 4.39 (ddd, 1H, H_B5′); 4.31 (ddd,

1H, H_A5'); 4.27 (ddd, 1H, H_A5'); 2.47−2.30 (m, 4H, H3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 156.39, 156.37 (C6); 147.79 (C2-aryl); 147.37 (C4); 145.88 (C2); 138.06, 138.01 (C8); 130.72 (C4-aryl); 126.87 (C3-aryl); 124.48 (C5); 123.80 (C5-aryl); 123.36, 123.35 (C6-aryl); 120.90 (C1-aryl); 96.36 (d, C2′); 88.73 (d, C1′); 78.70, 78.66 (d, C4′); 68.31 (CH_2-benzyl); 68.26 (C5′); 32.29, 32.15 (d, C3′) 14.74, 14.68 (CH_3); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.16; −9.20; ^{19}F NMR (376.43 MHz, DMSO-d_6) δ −178.03; −178.16; MS (ESI$^+$) m/z 437.4 (M + 1); UV λ_{max} (CH_3-CN) 250.60 nm, 249.18 nm; λ_{min} 249.18 nm, 226.46 nm; IR (KBr) ν 3418.9; 1692.0; 1584.1; 1446.6; 1290.3; 1190.4; 1090.5; 1024.1; 949.5; 814.3, 643.9; R_f value 0.39 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 18.04 min (97.4%, gradient I); 15.39 min (97.4%, gradient II); 19.37 min (97.5%, gradient III).

2′,3′-Dideoxy-2′-fluororibosylhypoxanthine (F-*ribo*-ddI, 7): yield 85%; ^{1}H NMR (400.14 MHz, DMSO-d_6) δ 12.36 (s, br, 1H, NH); 8.31 (s, 1H, H2); 8.07 (s, 1H, H8); 6.21 (d, 1H, H1′); 5.56 (dd, 1H, H2′); 6.60 (dddd, 1H, H4′); 3.76 (dd, 1H, H5′); 3.57 (dd, 1H, H5′); 2.41 (ddd, 1H, H3′); 2.24 (ddd, 1H, H3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 156.88 (C6); 147.42 (C4); 146.26 (C2); 137.70 (C8); 124.35 (C5); 96.98 (d, C2′); 88.46 (d, C1′); 81.52 (C4′); 61.26 (C5′); 31.54 (d, C3′); ^{19}F NMR (376.43 MHz, DMSO-d_6) δ −178.79; MS (ESI$^+$) m/z 255.0 (M + 1); UV λ_{max} (CH_3CN) nm; 249.95 nm, 224.06 nm, 197.01 nm; λ_{min} 249.95 nm, 220.87 nm; IR (KBr) ν 3421.1; 2923.7; 1656.4; 1597.8; 1446.4; 1370.4; 1185.6; 1117.5; 1024.0; 818.8; 752.5; 698.1; R_f value 0.19 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 9.49 min (97.4%, gradient I); 5.02 min (97.4%, gradient II); 11.65 min (97.5%, gradient III).

Determination of the Partition Coefficients (log *P* values). log *P* values were determined as follows: A sample of the compounds (**4**, **5**, **8**, **9**) was dissolved in 0.3 mL of 1-octanol. To this solution was added 0.3 mL of water. After the phases were mixed extensively for 10 min (vortex) and separated by centrifugation (2 min at 9000 rpm), aliquots of each phase were analyzed by analytical HPLC (Merck EcoCART column filled with Lichrospher 100 reversed-phase silica gel RP-18 endcapped (5 μm), gradient 0−100% CH_3CN in water (0−20 min), 100% CH_3CN (20−22 min), 0−100% CH_3-CN in water (22.1−35 min), flow 0.5 mL, UV detection at 260 nm). The *P* values were calculated by integration of the peak areas of the aqueous and organic phases.

Kinetic Data. DMSO stock solution of the compound (9 μL, 50 mM) was diluted in 312 μL of water (c = 1.44 mM); 0.3 mL of this solution was added to 0.3 mL (37 °C) of aqueous buffer (50 mM phosphate buffer (Sörensen mixture), pH 7.3; 50 mM glycine/HCl, pH 3.0; 50 mM glycine/HCl, pH 1.5) containing 5 μL of an aqueous AZT solution (AZT as internal standard). The final concentration of the test compounds was 0.71 mM in 24.8 mM aqueous buffer. For kinetic data, aliquots of 40 μL of the hydrolysis mixture were stopped by freezing in liquid air and subsequently analyzed after thawing by analytical HPLC (Merck EcoCART column filled with Lichrosphere 100 reverse-phase silica gel RP-18 endcapped (5 μm), gradient 11−100% CH_3CN in water (0−20 min) and 11% CH_3CN (20−30 min), 0.5 mL flow, UV detection at 260 nm). The degradation of **4**, **5**, **8**, and **9** was followed by integration of the peak areas in the HPLC chromatograms. The rate constants k were determined from the slope of the logarithmic degradation curve of the title compounds. The half-lives ($t_{1/2}$) were calculated by using the rate constants k.

Enzymatic Studies. The experiments with the parent nucleosides were carried out using 0.25 U of adenosine deaminase (ADA; EC 3.5.4.4; from calf intestinal mucosa) and a 10 mM phosphate buffer, pH 7.3 (Sörensen) at 37 °C, whereas for 3-methyl-*cyclo*Sal-F-*ara*-ddAMP (**4c**) and 3-methyl-*cyclo*Sal-F-*ribo*-ddAMP (**5c**) 0.5 U of enzyme and a 10 mM phosphate buffer, pH 6.8, were used. The concentration of the compounds in the previously mentioned buffers was 185 μM. To 1.3 mL of this preincubated solution, at 37 °C, was added ADA. The enzymatic deamination was followed in a UV spectrometer (Varian, Cary 1E) at 260 nm for 90 min. A comparison of the recorded UV spectra before ADA (t_0) and after the 90-min incubation period demonstrated the integrity of **4c** and **5c**.

The deamination of the monophosphates F-*ara*-ddAMP by adenosine monophosphate deaminase (AMPDA; EC 3.5.4.6; from rabbit muscle) was carried out using 2 U of AMPDA in a 10 mM phosphate buffer, pH 6.8 (Sörensen) at 37 °C. For the triesters 3-methyl-*cyclo*Sal-F-*ara*-ddAMP (**4c**) and 3-methyl-*cyclo*Sal-F-ribo-ddAMP (**5c**), 2 U of enzyme was used. The concentration of the compounds in the previously mentioned buffers was 185 μM. To 1.3 mL of this preincubated solution, at 37 °C, was added AMPDA. The deamination reactions were followed in a UV spectrometer (Varian, Cary 1E) at 260 nm for 200 min. As before, a comparison of the recorded UV spectra before AMPDA (t_0) and after the 200-min incubation period demonstrated the integrity of **4c** and **5c**.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 [HIV-1 (HTLV-III$_B$)] was originally obtained from a persistently HIV-infected H9 cell line as described previously,[45] which was kindly provided by Dr. R. C. Gallo when at the National Institutes of Health, Bethesda, MD. Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells. HIV-2 (strain ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France), and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells. CEM cells were obtained from the American Tissue Culture Collection (Rockville, MD). CEM cells were infected with HIV as previously described.[46] Briefly, 4 × 10^5 cells/mL were infected with HIV-1 or HIV-2 at ∼100 $CCID_{50}$ (50% cell culture infective dose) per mL of cell suspension. Then 100 μL of the infected cell suspension was transferred into 96-well microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4−5 days giant cell formation was recorded microscopically in the HIV-infected cell cultures.

C3H/3T3 cells were seeded at 20 000 cells/mL into wells of tissue culture cluster plates (48 wells/plate). Following a 24-h incubation period, cell cultures were infected with 80 focus-forming units of MSV during 120 min, whereafter the culture medium was replaced by 1 mL of fresh medium containing appropriate concentrations of the test compounds. After 6 days, transformation of the cells was examined microscopically. The EC_{50} was defined as the compound concentration required to inhibit MSV-induced cell transformation by 50%. The MCC was defined as the minimum cytotoxic concentration required to cause a microscopically visible morphological alteration of the C3H/3T3 fibroblast cultures.

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Supporting Information Available: ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectra and detailed tables of coupling constants for the title compounds **4a**−**c**, **5a**−**c**, **8c**, and **9c**. This information is available free of charge via the Internet at http://pubs.acs.org.

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