*cyclo*Sal-Pronucleotides of 2′,3′-Dideoxyadenosine and 2′,3′-Dideoxy-2′,3′-didehydroadenosine: Synthesis and Antiviral Evaluation of a Highly Efficient Nucleotide Delivery System

Chris Meier,*[,†,‡] Tina Knispel,[†,‡] Erik De Clercq,[§] and Jan Balzarini[§]

Institute of Organic Chemistry, Julius-Maximilians-University Würzburg, Am Hubland, D-97074 Würzburg, Germany, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

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The synthesis, hydrolysis, and antiviral evaluation of novel, lipophilic *cyclo*Sal-ddAMP (**9a**−**d**) and *cyclo*Sal-d4AMP (**10a**−**d**) derivatives of the antiviral purine dideoxynucleoside analogues 2′,3′-dideoxyadenosine (ddA) (**2**) and 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A) (**3**) are reported. These potential pronucleotides release ddAMP (**7**) or d4AMP (**8**) selectively by a controlled, chemically induced tandem reaction. All new compounds **9** and **10a**−**d** were synthesized in good yields using our previously reported phosphorus(III) method starting from substituted salicyl alcohols **14a**−**h**. The phosphotriesters **9** and **10** were obtained with a stereochemical preference of 2:1 with respect to the configuration at the phosphorus center. In an 1-octanol/water mixture phosphotriesters **9** and **10** exhibited 7−43-fold higher lipophilicity than the parent nucleosides ddA (**2**) and d4A (**3**) as judged by their log *P* values. In hydrolysis studies, **9** and **10** decomposed under mild aqueous basic conditions releasing solely ddAMP (**7**) and d4AMP (**8**), as well as the diols **14**. Further hydrolysis studies under acidic conditions showed a marked increase in stability with respect to the acid-catalyzed cleavage of the glycosyl bond. Phosphotriesters **9** and **10** exhibited antiviral potencies against wild-type HIV-1 and HIV-2 strains in human T-lymphocyte (CEM/O) cells that were, respectively, 100- and 600-fold higher than those of ddA (**2**) and d4A (**3**). Furthermore, all triesters **9** and **10** were markedly more active than the corresponding ddI compounds **11** and **12**, which supports the concept of the delivery of the adenine nucleotides. Studies with adenosine deaminase (ADA) and adenosine monophosphate deaminase (AMPDA) showed that the triesters were not substrates for enzymatic deamination. The studies reported herein demonstrate conclusively that the *cyclo*Sal triesters deliver exclusively the nucleotides ddAMP and d4AMP, not only under chemical-simulated hydrolysis but also under intracellular conditions fulfilling the adenosine deaminase bypass premise.

Introduction

Nucleoside analogues, e.g., 3′-azido-2′,3′-dideoxythymidine (AZT) or 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T), are used as antiviral agents in the treatment for AIDS.[1,2] The only purine nucleoside analogue clinically approved to date is 2′,3′-dideoxyinosine (ddI, **1**; Didanosine, Videx), the deamination product of 2′,3′-dideoxyadenosine (ddA, **2**).[3,4] Like all nucleoside analogues that are active against human immunodeficiency virus (HIV), ddA (**2**) as well as the unsaturated 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A, **3**) need to be converted to their corresponding 5′-triphosphates intracellularly before they can express their biological activity as inhibitors of the target enzyme (reverse transcriptase, RT).[5] The first phosphorylation step is predominantly catalyzed by nucleoside kinases and/or 5′-nucleotidase, but ddA (**2**) and d4A (**3**) have very poor affinity for their activating enzymes.[5,6] Indeed, in the case of ddA (**2**), only very low intracellular levels of ddATP are detected. This metabolite shows a similar inhibitory effect of HIV-RT (K_i = 0.22 μM) to that of AZTTP (K_i = 0.1 μM).[7] Furthermore, in marked contrast with the pyrimidine 2′,3′-dideoxynucleosides (i.e., d4T, AZT), ddA (**2**) is extensively catabolized.[3,4,8−10] The main catabolic pathway is deamination into ddI (**1**) by the ubiquitous cellular enzyme adenosine deaminase (ADA).[11] The inosine derivative **1** is either inactivated by cleavage to hypoxanthine (Hx) by purine nucleoside phosphorylase (PNP) or further phosphorylated to the monophosphate ddIMP (**5**) by 5′-nucleotidase,[12] a cellular enzyme whose activity remains nearly constant during all phases of the cell cycle.[13,14] No further phosphorylation of **5** to the corresponding triphosphate has been observed. Therefore, ddIMP (**5**) enters the adenosine anabolic pathway through the action of adenylosuccinate synthetase and adenylosuccinate lyase to give 2′,3′-dideoxyadenosine monophosphate (ddAMP, **7**).[11,15] However, the enzymatic conversion of **5** into **7** is not very efficient, and both intervening enzymes become rate-limiting steps. Further phosphorylation by cellular kinases converts ddAMP to the triphosphate level (Scheme 1).[3,10] It can be assumed that d4A (**3**) may follow a similar pathway via d4I (**4**) and d4IMP (**6**) to 2′,3′-dideoxy-2′,3′-didehydroadenosine monophosphate (d4AMP, **8**). However, this has never been demonstrated.

* To whom correspondence should be addressed.
† Julius-Maximilians-University of Würzburg.
§ Katholieke Universiteit Leuven.
‡ New address: Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany.

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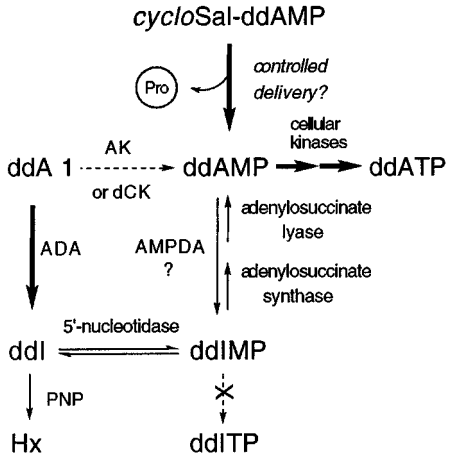
Scheme 1. Metabolism of the Purine Nucleosides ddA and d4A

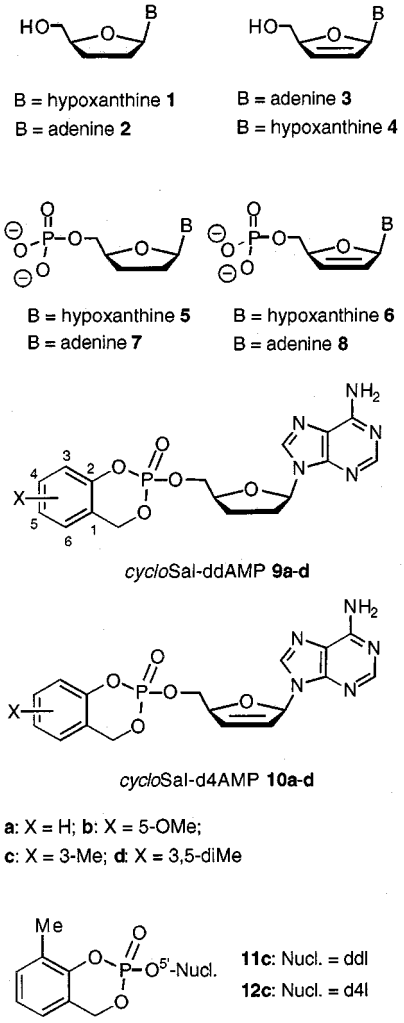


Consequently, the direct administration of ddAMP (**7**) and d4AMP (**8**) could in principle bypass the metabolic limiting processes of ADA deamination (ADA bypass) and thus improve the therapeutic potential of these drugs. However, a well-known problem in antiviral therapy is the poor bioavailability of highly polar nucleotides due to low membrane penetration. Therefore, intracellular delivery of nucleotides, i.e., ddAMP (**7**) and d4AMP (**8**), from neutral, lipophilic pronucleotides[16−18] could bypass the rate-limiting ADA → 5′-nucleotidase route to improve biological activity (pronucleotide approach, Scheme 1). Several types of masked nucleoside 5′-monophosphate prodrugs containing two phosphate blocking groups have been synthesized to achieve intracellular nucleotide delivery.[19] The major differences of these approaches are the nature of the delivery mechanisms. An additional drawback in the use of ddA (**2**), and especially d4A (**3**), is their high acid instability which complicates oral administration of the drugs. In one report, it was shown that the introduction of an electron-withdrawing phosphate ester group at the 5′-position of ddA had a stabilizing effect on the glycosyl bond.[17]

As part of our ongoing program to develop efficient pronucleotide delivery systems, we were interested in a completely new nucleotide delivery approach based on pH-driven selective chemical hydrolysis:[20] the *cyclo*saligenyl-pronucleotides (*cyclo*Sal-NMP).[21] This concept has been successfully introduced with other anti-HIV nucleosides such as d4T. Indeed, the *cyclo*Sal-d4TMP triesters proved to be successful thymidine kinase bypass agents.[22]

Here we report the synthesis, lipophilic and hydrolytic properties, and biological activity of *cyclo*Sal-2′,3′-dideoxyadenosine monophosphates (**9**, *cyclo*Sal-ddAMP) and *cyclo*Sal-2′,3′-dideoxy-2′,3′-didehydroadenosine monophosphates (**10**, *cyclo*Sal-d4AMP) which we propose function as neutral pronucleotides of ddAMP (**7**) and d4AMP (**8**), as well as efficient ADA bypass agents (Chart 1). Furthermore, the effect of the *cyclo*Sal modification on the acid stability of the pronucleotide as well as the lack of enzymatic deamination by adenosine deaminase (ADA) and adenosine monophosphate deaminase (AMPDA) are reported. The data obtained for **9** and **10** were compared to those of the corresponding ddIMP (**11c**) and d4IMP (**12c**) triesters.

Chart 1. Structures of ddI (**1**), ddA (**2**), d4A (**3**), d4I (**4**), Their Corresponding Monophosphates **5**−**8**, and Title *cyclo*Sal Phosphotriesters **9**−**12**



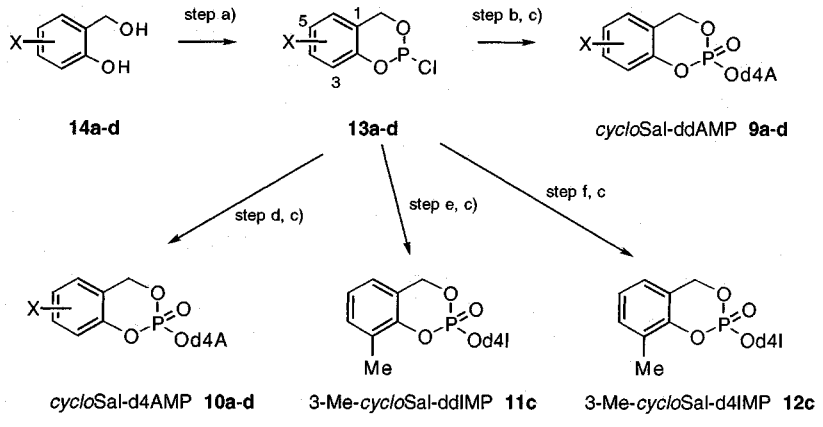
A preliminary account on the ddA derivatives was published recently.[23]

Results and Discussion

Chemistry. ddA (**2**) was synthesized from 2′-deoxyadenosine by (1) regioselective 5′-*O*-benzoylation with benzoic acid catalyzed by *N*,*N*′-bis(2-oxooxazolidin-3-yl)-phosphorodiamidic chloride (BOPDC) in pyridine[24] (65% yield), (2) regioselective 3′-*O*-esterification with thiocarbonyl diimidazole and subsequent reaction with methanol (77% yield; note: esterification using phenoxythiocarbonyl chloride was not sufficiently regioselective), and (3) Barton deoxygenation to 5′-benzoyl-2′,3′-dideoxyadenosine (88% yield), followed by a 12-h treatment with ammonia in methanol (30% overall yield).[23] d4A (**3**) was prepared from adenosine via 3′-bromo-3′-deoxy-2′,5′-di-*O*-acetyladenosine by a reductive elimination with freshly prepared Zn/Cu couple using a combination of procedures published by Walker et al.[25] Deprotection of the 5′-position with cold saturated methanolic ammonia yielded d4A (**3**) in 35% overall yield.

For comparative studies, vide infra, we also prepared in nearly quantitative yields ddI (**1**) and d4I (**4**) by enzymatic deamination of ddA (**2**) and d4A (**3**) using

Scheme 2. Synthetic Pathways to the Title *cyclo*Sal Triesters **9**−**12**[a]



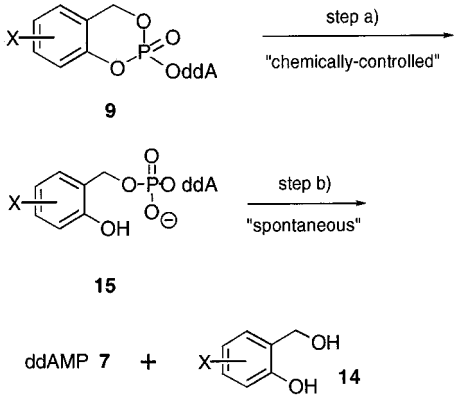
[a] Reaction conditions: (a) PCl_3, pyridine, Et_2O, −10 °C, 2 h; (b) ddA (**2**), DIPEA, DMF/THF (2:1), −40 °C, 30 min; (c) TBHP, DMF/THF (2:1), −40 °C−rt, 1 h; (d) d4A (**3**), DIPEA, DMF/THF (2:1), −40 °C, 30 min; (e) from **13c**: ddI (**1**), DIPEA, DMF/THF (2:1), −40 °C, 30 min; (f) from **13c**: d4I (**4**), DIPEA, DMF/THF (2:1), −40 °C, 30 min.

adenosine deaminase (ADA).[26] All nucleoside derivatives **1**−**4** synthesized here showed spectroscopic data identical to those reported before.[27,28]

The synthesis of the *cyclo*Sal triesters **9** and **10** was carried out without protection of the exocyclic amino group of the adenine nucleosides **2** and **3**. The commonly used base-labile protecting groups had to be avoided due to the potential instability of the target triester derivatives **9** and **10**, and acid-labile protecting groups could have resulted in the acid-catalyzed cleavage of the glycosyl bond of ddA and d4A. The *cyclo*Sal residue was introduced using our previously reported reactive chlorophosphanes **13**, which were prepared from the appropriate salicyl alcohols **14**.[29] The only differences from our previously reported reaction sequence were the solvent used and the reaction temperature. Due to low solubility of **2** or **3** in acetonitrile, a solvent mixture of dimethylformamide and tetrahydrofuran (DMF/THF, 2:1) was used. The regioselective 5′-*O*-phosphorylation reaction of **2** and **3** was achieved at −40 °C to enhance the *O*- vs *N*-selectivity in the presence of the unprotected amino group. Following this procedure, the *cyclo*Sal-ddAMP (**9a**−**d**) and *cyclo*Sal-d4AMP (**10a**−**d**) triesters were obtained in good yields and with a regioselectivity of 8:1 in favor of the 5′-*O*-modification. The reaction sequence is outlined in Scheme 2.

In contrast to our previous results with *cyclo*Sal-d4TMP, where 1:1 diastereomeric mixtures were obtained, the title compounds **9** and **10** were isolated as 1:1.6 diastereomeric mixtures with respect to the configuration at the phosphorus center after purification on a chromatotron. Most probably, this uneven ratio is due to kinetic control during the low-temperature reactions. The structure of the diastereomers was characterized by extensive ^1H, ^{13}C, and ^{31}P NMR spectroscopy, as well as by electrospray mass spectrometry (ESI, positive mode) and UV spectroscopy. As expected, the phosphotriesters displayed two closely spaced signals in the ^{31}P NMR spectra. The purity was checked by analytical reversed-phase high-performance liquid chromatography (RP-HPLC) using three distinct eluant gradients (water/acetonitrile gradients). In almost all cases, the two expected peaks of the diastereoisomers could be separated by analytical RP silica gel columns (C18), but in a few cases the two peaks overlapped partially or completely. For the biological evaluation, small amounts of the *cyclo*Sal phosphotriesters **9a**−**d** and **10a**−**d** were additionally purified by semipreparative HPLC using acetonitrile/water eluents in order to eliminate any traces of nucleoside impurities. Only in the case of the 5-methoxy-substituted *cyclo*Sal-d4AMP **10b** was the separation of the diastereomers possible by means of semipreparative HPLC. After lyophilization, all phosphotriesters **9** and **10** were obtained as white fluffy solids. To compare the properties and antiviral potencies of the *cyclo*Sal-ddAMP and *cyclo*Sal-d4AMP triesters with the corresponding inosine derivatives, the 3-methyl-*cyclo*Sal-nucleotides of ddI and d4I (**11c** and **12c**) were prepared by similar methods.

Scheme 3. Chemical Hydrolysis Pathways of *cyclo*Sal Phosphotriesters Shown for the *cyclo*Sal-ddAMP **9**



Determination of the Partition Coefficients (log *P* Values). The partition coefficients (log *P* values) of the *cyclo*Sal-NMPs **9**−**12**, as well as those of the parent nucleoside analogues **1**−**4**, were determined in 1-octanol/water by our previously reported HPLC method.[22a,29] The *P* values of all the phosphotriester derivatives were >1, and all log *P* values are positive. *cyclo*Sal-ddAMP phosphotriesters **9** exhibited *P* values that were 7.5−43-fold higher than those of the parent nucleoside ddA (**2**) (log $P = -0.28$), and *cyclo*Sal-

d4AMPs **10** were 7−28-fold more lipophilic than d4A (**3**) (log *P* −0.49). As expected, 3-methyl-*cyclo*Sal-ddAMP (**9c**) was 5-fold more lipophilic than the corresponding inosinate derivative **11c**. Compared to AZT (log *P* of 0.025),[29−31] which enters mammalian cells by passive, nonfacilitated diffusion,[32] the new ddA- and d4A-pronucleotides showed 3.6−21-fold (*cyclo*Sal-ddAMPs **9**) and 2.1−8.4-fold (*cyclo*Sal-d4AMPs **10**) increases in lipophilicity. According to the higher log *P* values, an improved cellular uptake by passive diffusion was anticipated.

Kinetic Studies. The *cyclo*Sal-pronucleotide concept has been designed to release selectively the nucleotides and the masking group by a controlled, chemically induced tandem reaction. In contrast to other prodrug concepts based on enzymatic hydrolysis,[19] our approach involves the successive coupled cleavage of the phenyl and benzyl esters of the *cyclo*Sal phosphotriester. The degradation pathway has been proven by different methodologies and has been applied to different nucleosides, e.g., d4T[22] and ACV.[33] In both cases, the intracellular delivery of the corresponding nucleotides has been clearly demonstrated by the observed biological activity even in the case of thymidine kinase-deficient cell lines or virus strains. Surprisingly, the approach failed to deliver AZTMP in an in vitro test system.[29] Here, we applied the concept to adenosine analogues **2** and **3** with the intent to bypass adenosine deamination by ADA and thereby avoid the rapid conversion of adenine nucleosides into the corresponding hypoxanthine derivatives.

The rates of hydrolysis of the *cyclo*Sal-ddAMP and -d4AMP derivatives **9** and **10** were measured under a variety of conditions using different aqueous buffers and pH values. Hydrolyses rates in 25 mM phosphate buffer, pH 7.3, allowed us to compare the effect of the purine base on the *cyclo*Sal motif relative to the pyrimidine nucleotide analogues described before.[22a,29] On the other hand, the effect of 5′-phosphorylation on the stability of the glycosyl bond and the acid stability of the *cyclo*Sal moiety itself were determined in a glycine/HCl buffer at pH values of 3.0 and 1.5, respectively. These studies were conducted with all *cyclo*Sal phosphotriesters **9** and **10** and showed a clear correlation between electronic properties and hydrolytic stability of the triesters. From our previous work, we selected the 3,5-dimethyl, the 3-methyl, and the 5-methoxy, as well as the unsubstituted *cyclo*Sal phosphotriesters, because these groups and substitution patterns gave the best biological results. The overall behavior of the *cyclo*Sal phosphotriester in terms of chemical hydrolysis correlated well with successful membrane penetration. The 3-methyl-*cyclo*Sal derivatives of ddIMP (**11c**) and d4IMP (**12c**) were also included in the study. The degradation of the *cyclo*Sal-ddAMPs **9** and *cyclo*Sal-d4AMPs **10** was followed by RP-HPLC analysis. The half-lives were determined by integration of the decreasing peaks of the triesters versus time. The half-lives are summarized in Table 1.

As in the case of the *cyclo*Sal-d4TMP compounds, a clear correlation between hydrolytic stability and the substituents on the aromatic ring was observed. In phosphate buffer (pH 7.3), the half-lives increased commensurate with the electron-donating ability of the substituent. However, at this pH value the half-lives were considerably longer than previously detected for the pyrimidine nucleoside *cyclo*Sal derivatives[22a,29] (e.g., about 3.8-fold (**9**) and 2.5-fold (**10**) longer than those of the corresponding d4TMP triesters) despite the common *cyclo*Sal motif. We have no explanation for this marked increase in stability. It was also apparent that the d4AMP derivatives **10** were less stable than the ddAMP derivatives **9**. However, the nucleotides ddAMP (**7**) and d4AMP (**8**), plus the salicylic alcohol, were the only products detected at 260 nm. Further hydrolysis studies of **9** and **10** were performed in RPMI culture medium with and without 10% heat-inactivated FCS. Under these conditions, a slight decrease in half-lives was observed. This is most probably due to the more basic pH of the medium used (e.g., 11.4 h (**9a**), 15.4 h (**9b**), and 33.8 h (**9c**); data not shown). As before, only the monophosphates were observed as hydrolysis products.

A very important disadvantage of purine 2′,3′-dideoxynucleosides is their high instability against acid-catalyzed cleavage of the glycosyl bond.[34] To investigate this issue, the ddAMP (**9a**−**d**) and d4AMP (**10a**−**d**) phosphotriesters, as well as the corresponding ddIMP (**11c**) and d4IMP (**12c**) derivatives, were incubated in 25 mM glycine/HCl buffer (pH 3.0 and 1.5), and their half-lives were compared to those of the parent nucleoside analogues, ddA, d4A, and ddI. Within each series, **9a**−**d** or **10a**−**d**, the $t_{1/2}$ values remained constant around 13 and 0.15 h, respectively, at pH 3.0 (Table 1). This suggests that the proton-induced cleavage of the glycosyl bond is independent of the substitution pattern in the *cyclo*Sal residue.[34] Furthermore, the 86-fold increase in lability of the d4AMP triesters **10** compared to triesters **9** reflect the higher stability of the allylic carbocation of the latter, which is formed after cleavage of the glycosyl bond. However, as can be concluded from the half-lives at pH 3.0 and 1.5, the introduction of the *cyclo*Sal moiety at the 5′-position of the dideoxyribose residue stabilized the glycosyl bond considerably (10−

Table 1. Hydrolysis Half-Lives ($t_{1/2}$) in Different Aqueous Buffers and log *P* Values of the Nucleosides **1**−**4** and the *cyclo*Sal-NMPs **9**−**12**

		hydrolysis ($t_{1/2}$, h)[b] in aqueous buffers at 37 °C			
compd	subst X	pH 7.3[a]	pH 3.0[c]	pH 1.5[d]	log *P* [e]
9a	H	17.0	12.9	0.60	0.59
9b	5-OMe	23.4	12.2	0.80	0.64
9c	3-Me	44.3	13.9	0.53	0.88
9d	3,5-diMe	58.2	10.3	0.73	1.35
2		na[f]	1.28	0.03	−0.28[31]
11c	3-Me	44.5	3.0	0.20	0.14
1		na[f]	0.2	0.03	−1.25[31]
10a	H	10.3	0.14	0.010	0.36
10b	5-OMe	14.5	0.14	0.011	0.48(f)/0.41(s)
10c	3-Me	32.3	0.15	0.0098	0.63
10d	3,5-diMe	44.7	0.14	0.010	0.95
3		na[f]	0.01	≪0.01	−0.49
12c	3-Me	29.7	0.04	≪0.01	0.078
4		na[f]	≪0.01	≪0.01	−0.89
AZT		na[f]	na[f]	na[f]	0.025[31]

[a] pH 7.3: 25 mM phosphate buffer (Sörensen). [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 (h). [c] pH 3.0: 25 mM glycine/HCl buffer. [d] pH 1.5: 25 mM glycine/HCl buffer. [e] log *P*: log of the partition coefficient. [f] na, not available.

15-fold) with respect to ddA (**2**) and d4A (**3**) (Table 1). A 10-fold increase in stability has also been observed for the bisSATE-ddAMP triesters at pH 2.0.[17] Table 1 also shows that all 2′,3′-dideoxyinosine derivatives are considerably less stable when compared to their 2′,3′-dideoxyadenosine counterparts. The higher lability of ddI relative to ddA has already been observed before; however, the *cyclo*Sal-dd4IMP triesters are 15-fold more stable than the parent nucleosides. As expected, a pH dependence was also observed. At pH 1.5, the rate of cleavage of the glycosyl bond of the *cyclo*Sal phosphotriesters **9** was ca. 23-fold higher than at pH 3.0. This was less than the 43-fold increase observed for ddA (**2**). The corresponding reaction rates of the d4AMP triesters **10** were too fast to be determined under the experimental conditions used. The decrease of the triester peak in the HPLC chromatograms was caused nearly exclusively by glycosyl bond cleavage and not by chemical hydrolysis of the phosphate esters. This was also verified for the *cyclo*Sal derivative of F-*ara*-ddA, an acid-stable analogue of ddA, which under identical reaction conditions at pH 3 showed a $t_{1/2}$ of 284 h (compared to 13 h for **9**) and at pH 1.5 a $t_{1/2}$ of 19 h (compared to 0.54 h for **9**).

Enzymatic Studies. As already discussed, ddA and d4A are highly susceptible to deamination by ADA which produces inactive inosine derivatives. It was assumed that this enzymatic deamination was responsible for a considerable loss in biological potency because the required reamination at the ddIMP and d4IMP levels becomes rate-limiting. Since our goal was to bypass the deamination by ADA by 5′-modification of ddA and d4A, while at the same time developing a nucleotide delivery system, it was necessary to study the stability of the *cyclo*Sal-ddAMPs **9** and *cyclo*Sal-d4AMPs **10** against ADA deamination. In addition, we studied the enzymatic stability of **9** and **10** against adenosine monophosphate deaminase (AMPDA) because this enzyme is normally responsible for the deamination of AMP.[15] The reason for also testing the AMPDA stability was to exclude the possibility that the *cyclo*Sal triesters are deaminated to give the corresponding *cyclo*Sal-dd(4)IMP counterparts. The concentration of ddA, d4A, and the respective 3-methyl-substituted *cyclo*Sal phosphotriesters was set at 185 μM, and the deamination reactions were followed by UV spectroscopy at 260 nm. The nucleosides ddA (**2**) and d4A (**3**) were completely deaminated to the inosine analogues within 2−10 min in the presence of 0.25 U of ADA (EC 3.5.4.4; from calf intestinal mucosa) in 10 mM phosphate buffer (pH 6.8) at 37 °C. The close to neutral pH of the phosphate buffer was chosen to suppress deglycosylation during the incubation time. As expected,[35] all 5′-modified *cyclo*Sal phosphotriesters were completely stable against ADA deamination after 90 min, even in the presence of a 2-fold higher concentration of the enzyme (0.5 U). In one case, we prolonged the incubation time to 12 h without detecting any deamination reaction. Both *cyclo*Sal-ddAMP and *cyclo*Sal-d4AMP triesters remained unchanged after the incubation time as shown by their characteristic UV spectra. From this observation we can clearly exclude the formation of *cyclo*Sal-ddIs and *cyclo*Sal-d4Is through deamination of their adenosine counterparts.

The stability experiments using rabbit muscle AMPDA (EC 3.5.4.6) were carried out with 2′-deoxyadenosine monophosphate (dAMP) and 2′,3′-dideoxyadenosine monophosphate (ddAMP) as reference compounds in the presence of 0.5 and 1 U of AMPDA, respectively, in 10 mM phosphate buffer (pH 6.8). The 3-methyl-*cyclo*Sal-ddAMP (**9**) and -d4AMP (**10**) were incubated with 2 U of AMPDA under the same conditions. As before, the enzymatic reactions were followed by UV spectroscopy (260 nm) at 37 °C. Under these conditions, dAMP and ddAMP were completely converted to dIMP and ddIMP within 60 min. On the other hand, 3-methyl-*cyclo*Sal-ddAMP (**9c**) and -d4AMP (**10c**) were completely stable against AMPDA deamination for 200 min, even at twice the concentration of enzyme. The presence of the intact 6-amino group in the *cyclo*Sal compounds after the incubation time was demonstrated by the characteristic UV absorption maximum at 259 nm. These experiments showed that the *cyclo*Sal derivatives described here are completely stable against both ADA and AMPDA and should therefore deliver only the adenine nucleotides ddAMP and d4AMP intracellularly, and not ddIMP or d4IMP.

Antiviral Evaluation. The successful thymidine kinase bypass by *cyclo*Sal-d4TMP[22] and *cyclo*Sal-ACVMP phosphotriesters[33] has been shown before. These results demonstrated (1) a pronounced structure−bioactivity correlation with respect to the substituents on the *cyclo*Sal moiety, (2) the ready membrane penetration of the *cyclo*Sal-d4TMP triesters, (3) the efficient intracellular delivery of d4TMP, and (4) the complete independence from TK.[22] In sharp contrast, the corresponding *cyclo*Sal-AZTMP derivatives lost nearly all the antiviral activity observed in wild-type CEM/O cells[29] when tested in TK-deficient CEM cells. The parent nucleosides ddA (**2**) and d4A (**3**), as well as the corresponding *cyclo*Sal compounds **9** and **10**, were evaluated for their ability to inhibit the replication of HIV-1 and HIV-2 in rapidly dividing human T-lymphocytic cells (CEM cells). The compounds were also tested against Moloney murine sarcoma virus (MSV)-induced transformation of C3H/3T3 embryo murine fibroblasts. Furthermore, to demonstrate the efficiency of the ADA bypass, 3-methyl-*cyclo*Sal-ddIMP (**11c**) and 3-methyl-*cyclo*Sal-d4IMP (**12c**) were included in the antiviral tests since these compounds can only deliver ddIMP and d4IMP, respectively. To get to bioactive nucleoside triphosphates from ddIMP and d4IMP, two enzyme-catalyzed conversions into ddAMP and d4AMP have to take place prior to further metabolism to the corresponding triphosphate level (Scheme 1). It is generally accepted that these conversions are not very efficient.[15] The test compounds **9a**−**d** and **10a**−**d** were free of the parent nucleosides **2** and **3**, as verified by analytical RP-HPLC (>99% purity). The antiviral activities of the clinically used nucleoside analogues, d4T and AZT, are given for comparison. The results obtained are displayed in Table 2.

Both parent nucleoside analogues ddA (**2**) and d4A (**3**) proved effective against HIV-1- and HIV-2-induced cytopathogenicity in CEM cells with a 10-fold difference in antiviral potency (**2**, EC_{50} 4.3 and 4.5 μM; **3**, 30 and 50 μM). In contrast, the *cyclo*Sal-ddAMP triesters **9** exhibited a 90−170-fold increase in antiviral potency

Table 2. Antiviral Activity and Selectivity Indices of *cyclo*Sal Phosphotriesters **9a**−**d**, **10a**−**d**, **11c**, and **12c**, as Well as the Nuleosides **1**−**4**, on the Replication of HIV-1 and HIV-2 in Wild-Type CEM/O Cells and against Transformation Induced in MSV-Infected 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)
9a	H	0.03	0.13	2.86	93	>50	3096
9b	5-OMe	0.047	0.16	3.90	87	>50	1851
9c	3-Me	0.047	0.03	1.18	28	>50	585
9d	3,5-diMe	0.025	0.025	1.14	10	>50	383
2		4.3	4.5	40	>250	>50	>57
11c	3-Me	1.1	0.7	>50	58	>50	53
1		3.5	4.0	>50	156	>50	44
10a	H	0.065	0.35	0.30	86	>50	1329
10b (mix)	5-OMe	0.08	0.065	1.21	77	>50	962
10b (f)	5-OMe	0.043	0.050	2.83	40	>50	932
10b (s)	5-OMe	0.50	0.33	12.8	62	>50	125
10c	3-Me	0.065	0.19	1.24	49	>50	746
10d	3,5-diMe	0.05	0.055	1.14	25	>50	494
3		30	50	35	96	>50	3
12c	3-Me	5.3	5.0	>50	94	>50	18
4		4.3	>50	>50	87	>50	20
d4T		0.18	0.26	15.0	56	nd[d]	311
AZT		0.007	0.006	>100	>100	nd[d]	>14000

[a] 50% effective concentration. [b] 50% cytotoxic concentration. [c] Minimal cytotoxic concentration. [d] nd, not determined. [e] Selectivity index. [f] (mix), (f), and (s) refer to diastereomeric mixture and respectively the resolved "fast-eluting" and "slow-eluting" diastereomers.

against HIV-1-induced cytopathogenicity. A similar increase in potency was also observed against HIV-2-induced cytopathogenicity. Furthermore, the selectivity indices (SI) of the *cyclo*Sal phosphotriesters **9** increased by 50-fold. As expected, due to the fast enzymatic deamination of ddA into ddI, ddI was equally antivirally potent and showed a similar SI value as ddA (**2**) in the same assay. Interestingly, 3-methyl-*cyclo*Sal-ddIMP (**11c**) was 4 times more potent than ddI. This enhancement in bioactivity could be attributed to an increased intracellular delivery of ddIMP (**5**). The significantly lower potency against HIV-1 of 3-methyl-*cyclo*Sal-ddIMP (**11c**) compared to 3-methyl-*cyclo*Sal-ddAMP (**9c**) (EC_{50}: 1.1 and 0.047 μM, respectively) clearly shows that in the former case the delivery of ddIMP (**5**) is not sufficient for optimal antiviral activity, while in the latter case the delivery of ddAMP (**7**) is responsible for the strong increase in antiviral potency. This effect was also reflected in the SI values which showed that for the ddIMP compound **11c** it was about 50, whereas for the ddAMP triester **9c** it was about 650. This 13-fold increase in SI is likely the result of a different intracellular metabolic profile for each compound, since the basic structure and delivery pathways of **9** and **10** are identical. The lowest cytotoxic concentration observed for *cyclo*Sal-ddAMP **9a** was 86.4 μM resulting in the highest SI of all the *cyclo*Sal compounds tested (SI 3096).

Although ddA (**2**) was 10 times more potent than d4A (**3**), the *cyclo*Sal-d4AMP triesters **10** exhibited comparable potencies when compared to the *cyclo*Sal-ddAMP derivatives **9**. This represents a 460−600-fold increase in antiviral potency relative to d4A. At the same time, the SI values increased from 3, for the parent nucleoside analogue d4A (**3**), to 500, 750, and 1300 for the *cyclo*Sal derivatives **10a**,**c**,**d**, respectively. Previously, we have observed that biological activity was dependent on the configuration at the asymmetric phosphorus atom.[22a] In this study we were able to separate the 5-methoxy-*cyclo*Sal-d4AMP diastereoisomers **10b**. As seen in Table 2, both diastereomers exhibited different antiviral activity with the "fast"-eluting isomer being 11- and 6-fold more potent than the "slow"-eluting diastereomer. Essentially the same difference has been observed with the *cyclo*Sal-d4TMP triesters before.[22a] However, the chemical hydrolysis of those compounds showed only a 2-fold difference in the half-lives. The antiviral potency and the SI of the diastereomeric mixture were intermediate between the values of the diastereomerically pure compounds.

The antiviral activity of 3-methyl-*cyclo*Sal-d4IMP (**12c**) showed almost exactly the same antiviral potency as the parent nucleoside d4I (**4**) and was about 6 times more potent than d4A (**3**). Surprisingly, d4I (**4**) was also 7-fold more potent than d4A (**3**) (Table 2). Again, the results show that even the intracellular delivery of d4IMP (**6**) does not produce a comparable biological effect to that obtained with *cyclo*Sal-d4AMP **10**.

An interesting difference from our previous findings with the *cyclo*Sal-d4TMP triesters is the virtual independence of the biological activity of the *cyclo*Sal compounds studied here relative to the substitution pattern of the aromatic ring of the *cyclo*Sal moiety. Earlier, we noted a clear correlation between antiviral activity and the *cyclo*Sal substituents and, furthermore, with the hydrolysis rates which were influenced by the electronic properties of the substituents. Although a similar correlation between hydrolysis rates and the substitution pattern of the prodrugs was observed in the present study using (1) phosphate buffer (pH 7.3), (2) RPMI culture medium with 10% heat-inactivated FCS, and (3) glycine/HCl buffer, the antiviral activity was the same, within the experimental errors of the test system, for all prodrugs **9** and **10**. For the first time, these findings seem to support a different activation pathway for the ddA and d4A phosphotriesters when compared to the corresponding d4T derivatives. Although the *cyclo*Sal concept was based on a chemical hydrolysis process to give the monophosphates (which has been proven above), one possible explanation can be a concurrent enzyme-catalyzed initial step which operates before the spontaneous second step of the tandem

reaction to lead to the monophosphates. This could explain, in part, the above-mentioned independency of the antiviral activity from the substituents of the *cyclo*Sal residue. However, this assumption has been proven by separate hydrolysis studies using isolated enzymes (e.g., lysate, carboxyesterase) or cell extracts. The SI values decreased relative to the electron-donating properties of the substituent resulting in higher cytotoxicity (CC_{50} value) and lower SI values. The reason for this increase in cytotoxicity remains unclear. This trend can be seen in both series **9** and **10**. It should be added that the salicylic alcohols have been tested for comparison and showed no antiviral activity and no toxicity at concentrations >500 μM (data not shown).

In addition to their antiviral activity in HIV-infected CEM cells, the *cyclo*Sal prodrugs **9** and **10** were evaluated against MSV-induced transformation of C3H/3T3 embryo murine fibroblasts. As summarized in Table 2, both series of ddAMP and d4AMP phosphotriesters improved bioactivity up to 35-fold for the *cyclo*Sal-ddAMPs and 30-fold for the *cyclo*Sal-d4AMPs. The minimal cytotoxic concentration (MCC) in this assay was >50 μM in all cases. As in the HIV/CEM assay, no important differences in bioactivity were observed with the different substituents on the *cyclo*Sal moiety. Additionally, ddI, d4I, and the *cyclo*Sal-dd(4)IMP triesters were devoid of any biological activity in the MSV assay (EC_{50} > 50 μM).

Conclusion

From the antiviral data presented, it can be concluded that within each series the adenine nucleotides are being released intracellularly. This is due to the inability of the *cyclo*Sal triesters to serve as substrates for ADA and AMPDA. Additionally, it seems that the compounds are taken up intracellularly by passive diffusion, a property that is significantly enhanced relative to the parent nucleosides (log P < 0) by the higher log P values (log P > 0). Compared to the *cyclo*Sal-d4TMP phosphotriesters, the compounds presented here showed a marked improvement in biological activity, thus fulfilling the premise of the *cyclo*Sal concept. Moreover, the antiviral activity data are comparable to those achieved using alternative prodrug approaches.[16−18] As shown by the chemical hydrolysis studies, the difference between the *cyclo*Sal approach reported here and these other alternatives is the intrinsic independence of the *cyclo*Sal system from hydrolytic enzymes, as for example with the bisSATE[17] approach (applied to ddA) and the phosphoramidates approach[16,18] (applied to ddA and d4A). However, in contrast to the results of the corresponding *cyclo*Sal-d4TMP derivatives,[22a] the results reported here may also point to an enzymatic contribution to the triester cleavage in addition to or instead of the chemical hydrolysis.

It can be concluded that the reported *cyclo*Sal phosphotriesters are suitable to selectively deliver ddAMP and d4AMP. This leads to a marked increase (up to 600-fold) in bioactivity against HIV-1 and HIV-2 in infected CEM cell cultures. The mechanism of action of the *cyclo*Sal compounds is based on the successful bypass of ADA, an enzyme responsible for a fast deamination of adenine-containing nucleoside analogues. The reason for the observed pronounced improvement in antiviral potency can be additionally rationalized in terms of bypassing the need to convert hypoxanthine-bearing nucleosides and nucleotides into adenine nucleotides and the circumvention of enzymatic catabolism by PNP. In conclusion, at least four enzymatic reactions that are normally necessary to metabolize ddA/d4A into ddAMP/d4AMP can be circumvented by the use of the described *cyclo*Sal-pronucleotide approach. Noteworthy are the considerably higher selectivity indices of some the compounds. The phosphotriesters described here will be investigated in the future for their hydrolytic behavior in crude cell extracts. Also, cellular uptake and metabolism of the prodrug will have to be monitored with the use of radiolabeled derivatives.

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 employed in chromatography were distilled before used. Triethylamine was distilled from CaH_2 prior to use. The solvents for the 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 using 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 fan 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 0−100% CH_3CN (0−20 min), 100% CH_3CN (20−22 min), 0% CH_3CN (22.1−35 min), flow 0.5 mL, UV detection at 260 nm; gradient II (standard gradient) 11−100% CH_3CN (0−20 min), 100% CH_3CN (20−22 min), 0% CH_3CN (22.1−35 min), flow 0.5 mL, UV detection at 260 nm; gradient III 0−80% CH_3CN (0−20 min), 100% CH_3CN (20.1−22 min), 0% CH_3CN (22.1−35 min), flow 0.5 mL, UV detection at 260 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). All ^{1}H and ^{13}C NMR chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane, $(CD_3)(CDH)SO$ being set at δ_H 2.49 as a reference. ^{31}P NMR chemical shifts are quoted 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. 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-ddAMPs 9, *cyclo*Sal-d4AMPs 10, 3-Methyl-*cyclo*Sal-ddIMP (11c), and 3-Methyl-*cyclo*Sal-d4IMP (12c). The reactions were performed in an argon atmosphere under anhydrous conditions. To a solution of the nucleoside **1**, **2**, **3**, or **4** (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 **13a**−**d**[28] (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 **9**−**12**.

***cyclo*Saligenyl-5′-*O*-(2′,3′-dideoxyadenosinyl)phosphate (*cyclo*Sal-ddAMP, 9a):** yield 47%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H, H8); 8.27 (s, 1H, H8); 8.19 (s, 1H, H2); 8.18 (s, 1H, H2); 7.41 (t, 1H, H4-aryl); 7.37 (t, 1H, H4-aryl); 7.31 (s, 2H, NH_2); 7.30 (s, 2H, NH_2); 7.27 (d, 1H, H5-aryl); 7.26 (d, 1H, H5-aryl); 7.23 (d, 2H, H6-aryl); 7.16 (d, 1H, H6-aryl); 7.08 (d, 1H, H6-aryl); 6.29 (dd, 1H, H1′); 6.28 (dd, 1H, H1′); 5.51 (dd, 1H, H_A-benzyl); 5.45 (dd, 1H, H_A-benzyl); 5.43 (dd, 1H, H_B-benzyl); 5.39 (dd, 1H, H_B-benzyl); 4.44−4.27 (m, 6H, H4′, H5′); 2.61−2.45 (m, 2H, H2′); 2.22−2.10 (m, 2H, H3′); ^{13}C NMR (63 MHz, DMSO-d_6) δ 156.03 (C6); 152.53 (C2); 149.34, 149.29 (d, C2-aryl); 148.93 (C4); 138.98, 138.92 (C8); 129.68 (C6-aryl); 125.97 (C4-aryl); 124.34 (C5-aryl); 121.02, 120.86 (d, C1-aryl); 119.08 (C5); 118.18, 118.04 (C3-aryl); 84.35, 84.28 (C1′); 78.50, 78.46 (C4′); 69.15, 69.05 (C5′); 68.40, 68.30 (t, CH_2-benzyl); 30.65 (C3′); 25.80 (C2′); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.77; −9.83; (ESI$^+$) m/z 404.1; UV (CH_3CN) λ_{max} 259.62.5 nm, 210.07 nm; λ_{min} 230.24 nm, 203.10 nm; IR (KBr) ν 3325.9, 3175.7, 2954.4, 1651.3, 1598.5, 1574.1, 1488.3, 1459.5, 1415.3, 1370.0, 1296.8, 1245.4, 1222.8, 1192.0, 1093.5, 1043.8, 1019.5, 991.1, 938.2, 881.4, 842.5, 799.5, 760.0, 724.2, 696.8, 652.8, 579.4, 533.5; R_f value 0.35 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 17.11 min (99.6%, gradient I); 13.51 min (99.6%, gradient II); 19.49 min (99.5%, gradient III).

***cyclo*(5-Methoxysaligenyl)-5′-*O*-(2′,3′-dideoxyadenosinyl)phosphate (5-OMe-*cyclo*Sal-ddAMP, 9b):** yield 67%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H, H8); 8.30 (s, 1H, H8); 8.23 (s, 1H, H2); 8.21 (s, 1H, H2); 7.34 (s, 2H, NH_2); 7.33 (s, 2H, NH_2); 7.12 (d, 1H, H3-aryl); 7.04 (d, 1H, H3-aryl); 6.98 (dd, 1H, H4-aryl); 6.93 (dd, 1H, H4-aryl); 6.89 (d, 1H, H6-aryl); 6.87 (d, 1H, H6-aryl); 6.32 (m, 2H, H1′), 5.50 (dd, H_A-benzyl); 5.43 (dd, H_A-benzyl); 5.41 (dd, H_B-benzyl); 5.37 (dd, H_B-benzyl); 4.44−4.27 (m, 6H, H4′, H5′); 3.81 (s, 3H, OCH_3); 3.41 (s, 3H, OCH_3); 2.61−2.49 (m, 4H, H2′); 2.27−2.11 (m, 4H, H2′); ^{13}C NMR (63 MHz, DMSO-d_6) δ 156.02 (C6); 155.51 (C5-aryl); 152.55 (C2); 148.93 (C4); 142.92, 142.85 (d, C2-aryl); 138.94 (C8); 121.75, 121.71 (d, C1-aryl); 119.07 (C3-aryl); 118.98 (C5); 115.09, 115.00 (C6-aryl); 110.54, 110.46 (C4-aryl); 84.28 (C1′); 78.62, 78.52 (C4′); 69.13 (C5′), 68.37, 68.25 (d, CH_2-benzyl); 55.55 (OCH_3), 30.73 (C3′); 25.80 (C2′); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.57; −9.63; (ESI$^+$) m/z 434.1; UV (CH_3CN) λ_{max} 259.39 nm, 195.02 nm; λ_{min} 236.18 nm; IR (KBr) ν 3331.2, 3177.6, 2954.0, 2837.9, 1647.7, 1598.7, 1575.3, 1497.9, 1472.9, 1431.2, 1364.5, 1297.8, 1244.1, 1198.4, 1159.9, 1094.0, 1024.7, 948.9, 914.9, 866.8, 798.9, 759.6, 726.8, 695.9, 650.0, 568.6, 524.3, 480.6; R_f value 0.36 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 16.53 min, 16.75 min (98.6%, gradient I); 12.33 min, 12.79 min (98.6%, gradient II); 19.01 min, 19.21 min (98.6%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxyadenosinyl)-phosphate (3-Me-*cyclo*Sal-ddAMP, 9c):** yield 58%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H, H8); 8.21 (s, 1H, H8); 8.14 (s, 1H, H2); 8.13 (s, 1H, H2); 7.27 (s, 2H, NH_2); 7.26 (s, 2H, NH_2); 7.21−7.16 (m, 2H, H-aryl); 7.07−7.00 (m, 4H, H-aryl); 6.26−6.21 (m, 2H, H1′); 5.43 (dd, 1H, H_A-benzyl); 5.36 (dd, 1H, H_A-benzyl); 5.33 (dd, 1H, H_B-benzyl); 5.30 (dd, 1H, H_B-benzyl); 4.39−4.19 (m, 6H, H4′, H5′); 2.50−2.42 (m, 4H, H2′); 2.18 (s, 3H, CH_3); 2.12 (s, 3H, CH_3), 2.20−2.06 (m, 4H, H3′); ^{13}C NMR (63 MHz, DMSO-d_6) δ 155.98 (C6); 152.46 (C2); 148.86 (C4); 147.87, 147.77 (d, C2-aryl); 138.91, 138.82 (C8); 130.75 (C4-aryl); 126.84, 126.72 (C3-aryl); 123.81 (C5-aryl); 123.40 (C6-aryl); 120.90, 120.84 (d, C1-aryl); 119.07 (C5); 84.31, 84.21 (C1′); 78.60, 78.49 (C4′); 69.23, 69.14 (C5′); 68.38, 68.27 (d, CH_2-benzyl); 30.71, 30.63 (C3′); 25.81, 25.73 (C2′); 14.81, 14.72 (CH_3−C3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.11; −9.21; (ESI$^+$) m/z 418.1; UV (CH_3CN) λ_{max} 259.87 nm, 195.98 nm; λ_{min} 230.58 nm; IR (KBr) ν 3332.2, 3179.0, 2955.2, 1647.9, 1598.4, 1575.7, 1473.8, 1415.1, 1367.9, 1296.7, 1190.2, 1090.6, 1017.3, 939.8, 883.3, 823.0, 774.5, 723.9, 696.8, 651.6, 532.7; R_f value 0.38 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 16.15 min, 16.29 min (99.4%, gradient I); 12.09 min, 12.48 min (99.4%, gradient II); 18.59 min, 18.77 min (99.4%, gradient III).

***cyclo*(3,5-Dimethylsaligenyl)-5′-*O*-(2′,3′-dideoxyadenosinyl)phosphate (3,5-diMe-*cyclo*Sal-ddAMP, 9d):** yield 50%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (s, 1H, H2); 8.19 (s, 1H, H2); 8.11 (s, 1H, H8); 8.10 (s, 1H, H8); 7.24 (s, 2H, NH_2); 7.22 (s, 1H, NH_2); 7.00 (s, 1H, H4-aryl); 6.96 (s, 1H, H4-aryl); 6.81 (s, 1H, H6-aryl); 6.78 (s, 1H, H6-aryl); 6.22 (dd, 1H, H1′); 6.20 (dd, 1H, H1′); 5.36 (dd, 1H, H_A-benzyl); 5.29 (dd, 1H, H_A-benzyl); 5.28 (dd, 1H, H_B-benzyl); 5.23 (dd, 1H, H_B-benzyl); 4.34−4.15 (m, 6H, H4′, H5′); 2.48−2.43 (m, 2H, H2′); 2.20 (s, 6H, CH_3−C5-aryl); 2.12 (s, 6H, CH_3−C3-aryl); 2.13−2.08 (m, 4H, H3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 155.92 (C6); 152.41, 152.39 (C2); 148.82, 148.78 (C4); 145.67, 145.63 (d, C2-aryl); 138.86, 138.78 (C8); 132.89, 132.85 (C5-aryl); 131.17, 131.15 (C6-aryl); 126.39, 126.34 (C4-aryl); 123.41, 123.39 (C3-aryl); 120.37, 120.32 (d, C1-aryl); 119.00, 118.96 (C5); 84.22, 84.13 (C1′); 78.51, 78.46 (d, C4′); 69.04, 69.01 (C5′); 68.32, 68.20 (d, CH_2-benzyl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.076; −9.18; (ESI$^+$) m/z 432.4, 453.9 (M + Na$^+$); UV (CH_3CN) λ_{max} 259.16 nm, 197.96 nm; λ_{min} 231.02 nm; IR (KBr) ν 3329.3, 3185.7, 2927.7, 1647.0, 1600.3, 1573.1, 1483.4, 1415.4, 1363.7, 1331.2, 1299.7, 1245.1, 1202.1, 1150.0, 1096.8, 1045.8, 993.4, 931.9, 858.9, 799.1, 698.2, 665.4, 582.7, 487.9; R_f value 0.39 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 117.95 min, 18.09 min (98.8%, gradient I); 15.16 min, 15.40 min (98.8%, gradient II); 20.53 min, 20.69 min (98.9%, gradient III).

***cyclo*Saligenyl-5′-*O*-(2′,3′-dideoxy-2′,3′-didehydroadenosinyl)phosphate (*cyclo*Sal-d4AMP, 10a):** yield 54%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.141 (s, 1H, H2); 8.143 (s, 1H, H2); 7.92 (s, 1H, H8); 7.93 (s, 1H, H8); 7.29 (s, 2H, NH_2); 7.28 (s, 2H, NH_2); 7.35 (t, 1H, H4-aryl); 7.27 (t, 1H, H4-aryl); 7.23 (dd, 1H, H6-aryl); 7.19 (dd, 1H, H6-aryl); 7.16 (dt, 1H, H5-aryl); 7.15 (dt, 1H, H5-aryl); 7.11 (dd, 1H, H3-aryl); 6.97 (dd, 1H, H3-aryl); 6.94 (ddd, 1H, H1′); 6.92 (ddd, 1H, H1′); 6.48 (ddd, 1H, H3′) 6.43 (ddd, 1H, H3′); 6.25 (ddd, 1H, H2′); 6.22 (ddd, 1H, H2′); 5.46 (dd, 1H, H_A-benzyl); 5.38 (dd, 1H, H_A-benzyl); 5.35 (dd, 1H, H_B-benzyl); 5.32 (dd, 1H, H_B-benzyl); 5.09−5.06 (m, 2H, H4′); 4.36−4.26 (m, 4H, H5′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 155.96 (C6); 152.64, 152.61 (C2); 149.28, 149.23 (d, C2-aryl); 149.13, 149.12 (C4); 138.51, 138.44 (C8); 132.16, 132.09 (C3′); 129.67, 129.58 (C6-aryl), 126.64 (C2′); 125.93 (C4-aryl); 124.34, 124.28 (C5-aryl); 120.99, 120.81 (d, C1-aryl); 118.76 (C5); 118.08, 118.03 (d, C3-aryl); 87.79, 87.77 (C1′); 84.85, 84.83 (d, C4′); 68.39, 68.22 (d, C5′); 68.29, 68.27 (d, CH_2-benzyl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.65; −9.84; MS (ESI$^+$) m/z 401.9, 423.9 (M + Na$^+$); UV (CH_3CN) λ_{max} 259.06 nm, 194.00 nm; λ_{min} 228.10 nm; IR (KBr) ν 3333.0, 3182.9, 1650.0, 1598.9, 1488.5, 1418.2, 1371.0, 1293.1, 1242.7, 1193.3, 1093.7, 1021.0, 949.7, 846.2, 828.5, 761.3, 720.8, 650.3, 578.2; R_f value 0.35 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 15.72 min (98.6%, gradient I); 9.43 min, 9.87 min (98.5%, gradient II); 18.03 min (98.6%, gradient III).

***cyclo*(5-Methoxysaligenyl)-5′-*O*-(2′,3′-dideoxy-2′,3′-didehydroadenosinyl)phosphate (5-OMe-*cyclo*Sal-d4AMP, 10b):** yield 50%; ^{1}H NMR fast diastereoisomer (600 MHz, DMSO-d_6) δ 8.16 (s, 1H, H2); 7.97 (s, 1H, H8); 7.30 (s, 2H,

NH_2); 6.94 (ddd, 1H, H1′); 6.91(dd, 1H, H4-aryl); 6.82−6.81 (m, 2H, H3-aryl., H6-aryl); 6.45 (dt, 1H, H3′); 6.24 (ddd, 1H, H2′); 5.43 (dd, 1H, H_A-benzyl); 5.31 (dd, 1H, H_B-benzyl); 5.08 (m, 1H, H4′); 4.33 (ddd, 1H, H_B5′); 4.29 (ddd, 1H, H_A5′); 3.73 (s, 3H, OCH_3); ^{31}P NMR fast diastereoisomer (162 MHz, DMSO-d_6) δ −9.44; ^{1}H NMR slow diastereoisomer (600 MHz, DMSO-d_6) δ 8.16 (s, 1H, H2); 7.99 (s,1H, H8); 7.31 (s, 2H, NH_2); 7.07 (d, 1H, H3-aryl); 6.96 (ddd, 1H, H1′); 6.92 (dd, 1H, H4-aryl); 6.79 (d, 1H, H6-aryl); 6.50 (dt, 1H, H3′); 6.26 (ddd, 1H, H2′); 5.35 (ddd, 1H, H_A-benzyl); 5.28 (ddd, 1H, H_B-benzyl); 5.08 (m, 1H, H4′); 4.30 (m, 2H, H5′); 3.74 (s, 3H, OCH_3); ^{31}P NMR slow diastereoisomer (162 MHz, DMSO-d_6) δ −9.65; ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 155.97 (C6); 155.50, 155.43 (C5-aryl); 152.69, 152.66 (C2); 149.15, 149.13 (C4); 142.85, 142.83 (d, C2-aryl); 138.52, 138.48 (C8); 132.17, 132.10 (C3′); 126.64 (C2′); 121.68, 121.56 (d, C1-aryl); 118.95, 118.89 (C3-aryl); 118.74 (C5); 115.08, 114.85 (C6-aryl); 110.57, 110.42 (C4-aryl); 87.77, 87.75 (C1′); 84.88, 84.83 (C4′); 68.36, 68.23 (d, C5′); 68.29, 68.19 (d, CH_2-benzyl); 55.50, 55.47 (OCH_3); MS (ESI$^+$) *m*/*z* 431.9, 453.9 (M + Na^+); UV (CH_3CN) λ_{max} 258.99 nm, 195.88 nm; λ_{min} 236.23 nm; IR (KBr) ν 3336.2, 3185.9, 2946.8, 1647.5, 1598.8, 1576.8, 1497.9, 1473.3, 1420.2, 1293.2, 1241.5, 1200.5, 1125.9, 1094.2, 1026.1, 994.3, 951.5, 911.1, 827.9, 799.3, 760.4, 721.1, 649.4; R_f value 0.35 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 16.25 min, 16.39 min (98.7%, gradient I); 11.88 min, 12.12 min (98.7%, gradient II); 18.44 min (98.7%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′,3′-didehydroadenosinyl)phosphate (3-Me-*cyclo*Sal-d4AMP, 10c):** yield 56%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.133 (s, 1H, H2); 8.130 (s, 1H, H2); 7.96 (s, 1H, H8); 7.95 (s, 1H, H8); 7.26 (s, 2H, NH_2); 7.25 (s, 2H, NH_2); 7.22−7.01 (m, 6H, aryl. H); 6.94 (m, 2H, H1′); 6.48 (dt, 1H, H2′); 6.43 (dt, 1H, H2′); 6.24 (ddd, 1H, H3′); 5.42 (dd, 1H, H_A-benzyl); 5.34 (dd, 1H, H_A-benzyl); 5.33 (dd, 1H, H_B-benzyl); 5.28 (dd, 1H, H_B-benzyl); 5.08 (m, 2H, H4′); 4.31−4.26 (m, 4H, H5′); 2.19 (s, 3H, CH_3); 2.10 (s, 3H, CH_3); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 155.96 (C6); 152.65 (C2); 149.13 (C4); 147.83, 147.73 (d, C2-aryl); 138.48, 138.39 (C8); 132.14, 132.07 (C3′); 130.78 (C4-aryl); 126.82 (C3-aryl); 126.64 (C2′); 123.82 (C5-aryl); 123.43, 123.37 (C6-aryl); 120.92, 120.71 (d, C1-aryl); 118.77, 118.74 (C5); 87.80, 87.77 (C1′); 84.91, 84.84 (d, C4′); 68.44, 68.30 (d, C5′); 68.24, 68.19 (d, CH_2-benzyl); 14.83, 14.65 (C3-aryl-CH_3); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.44; −9.65; MS (ESI$^+$) *m*/*z* 415.9, 437.9 (M + Na^+); UV (CH_3CN) λ_{max} 258.96 nm, 194.38 nm; λ_{min} 228.12 nm; IR (KBr) ν 3328.4, 3182.1, 2944.0, 2886.7, 1649.9, 1597.9, 1574.8, 1472.9, 1418.7, 1368.0, 1328.9, 1293.7, 1238.8, 1203.8, 1190.4, 1126.2, 1090.7, 1023.7, 993.4, 945.0, 880.8, 828.7, 775.4, 720.8, 648.3, 614.1, 583.8, 505.1; R_f value 0.35 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 16.21 min, 16.32 min (97.1%, gradient I); 11.99 min, 12.35 min (97.2%, gradient II); 19.03 min (97.0%, gradient III).

***cyclo*(3,5-Dimethylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′,3′-didehydroadenosinyl)phosphate (3,5-diMe-*cyclo*Sal-d4AMP, 10d):** yield 51%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (s, 2H, H2); 7.96 (s, 1H, H2); 7.95 (s, 1H, H8); 7.27 (s, 2H, NH_2); 7.25 (s, 2H, NH_2); 7.00 (s, 1H, H4-aryl); 6.97 (s, 1H, H4-aryl); 6.92 (m, 2H, H1′); 6.82 (s, 1H, H6-aryl); 6.77 (s, 1H, H6-aryl); 6.47 (ddd, 1H, H2′); 6.43 (ddd, 1H, H2′); 6.24 (ddd, 1H, H3′); 6.22 (ddd, 1H, H3′); 5.38 (dd, 1H, H_A-benzyl), 5.28 (dd, 1H, H_A-benzyl); 5.27 (dd, 2H, H_B-benzyl), 5.22 (dd, 2H, H_B-benzyl); 5.06 (m, 2H, H4′); 4.31 (ddd, 1H, H5′); 4.28 (ddd, 1H, H5′); 4.25 (ddd, 1H, H5″); 4.22 (ddd, 1H, H5″); 2.20 (s, 3H, CH_3−C5-aryl); 2.19 (s, 3H, CH_3−C5-aryl); 2.14 (s, 3H, CH_3−C3-aryl); 2.05 (s, 3H, CH_3−C3-aryl); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 155.95 (C6); 152.63 (C2); 149.11, 149.09 (C4); 145.63, 145.58 (d, C2-aryl); 138.47, 138.38 (C8); 132.94, 132.90 (C3′); 132.16, 132.05 (C5-aryl); 131.22 (C6-aryl); 126.62, 126.60 (C2′); 126.38, 126.35 (C4-aryl); 123.46, 123.40 (C3-aryl); 120.48, 120.30 (d, C1-aryl); 118.75, 118.73 (C5); 87.76 (C1′); 84.91, 84.82 (d, C4′); 68.30, 68.25 (d, C5′); 68.35, 68.10 (d, CH_2-benzyl); 20.05, 20.01 (CH_3−C5-aryl); 14.75, 14.59 (CH_3−C3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −8.96; −9.17; (ESI$^+$) *m*/*z* 430.2; UV (CH_3CN) λ_{max} 258.98 nm, 197.25 nm; λ_{min} 230.98 nm; IR (KBr) ν 3334.2, 3177.8, 2917.5, 1642.2, 1597.9, 1573.8, 1483.8, 1417.9, 1364.4, 1328.9, 1287.0, 1238.8, 1202.8, 1150.0, 1125.9, 1094.9, 1028.0, 993.4, 970.4, 951.6, 932.9, 850.2, 828.7, 798.9, 721.3, 665.4, 583.2, 495.4; R_f value 0.35 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 17.48 min (98.0%, gradient I); 14.51 min (98.0%, gradient II); 20.03 min (98.1%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxyinosinyl)-phosphate (3-Me-*cyclo*Sal-ddIMP, 11c):** yield 35%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.33 (s, 2H, NH); 8.17 (s, 1H, H2); 8.14 (s, 1H, H2); 8.01 (s, 1H, H8); 8.00 (s, 1H, H8); 7.20 (t, 1H, H4-aryl); 7.18 (m, 1H, H4-aryl); 7.06−7.02 (m, 4H, H5-aryl, H6-aryl); 6.20 (dd, 1H, H1′); 6.19 (dd, 1H, H1′); 5.43 (dd, 1H, H_A-benzyl); 5.38 (dd, 1H); 5.33 (dd, 1H, H_B-benzyl); 5.31 (dd, 1H, H_B-benzyl); 4.35−4.17 (m, 6H, H5′, H4′); 2.48−2.37 (m 4H, H2′); 2.18 (s, 3H, CH_3); 2.16 (s, 3H, CH_3); 2.12−2.01 (m, 4H, H3′); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 156.48 (C6); 147.82, 147.78 (C2-aryl); 147.62 (C4); 145.55 (C2); 138.01, 137.96 (C8); 130.74 (C4-aryl); 126.81, 126.73 (C3-aryl); 12441, 124.37 (C5), 123.79 (C5-aryl); 123.40 (C6-aryl); 120.81, 120.74 (d, C1-aryl); 84.47, 84.38 (C1′); 78.87, 78.79 (C4′); 69.06, 69.00 (C5′); 68.31, 68.22 (d, CH_2-benzyl); 31.10, 31.07 (C3′); 25.59, 25.56 (C2′); 14.78, 14.70 (CH_3−C3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.14; −9.20; (ESI$^+$) *m*/*z* 419.3; 441.1 (M + Na^+); UV (CH_3-CN) λ_{max} 250.53 nm, 196.02 nm; λ_{min} 225.97 nm; IR (KBr) ν 3437.5, 3055.8, 2955.5, 1697.4, 1622.1, 1586.5, 1545.7, 1511.4, 1471.9, 1412.9, 1370.0, 1346.4, 1295.7, 1213.0, 1190.2, 1125.3, 1090.3, 1025.0, 1028.1, 990.9, 939.0, 881.6, 818.1, 789.6, 719.8, 688.0, 651.1, 606.6, 528.0; R_f value 0.29 (CH_2Cl_2/MeOH, 9:1); analytical HPLC t_R 16.35 min (96.7%, gradient I); 12.21 min (96.7%, gradient II); 18.92 min (96.7%, gradient III).

***cyclo*(3-Methylsaligenyl)-5′-*O*-(2′,3′-dideoxy-2′,3′-didehydroinosinyl)phosphate (3-Me-*cyclo*Sal-d4IMP, 12c):** yield 35%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.36 (s, 2H, NH); 8.05 (s, 1H, H2); 8.04 (s, 1H, H2); 7.87 (s, 1H, H8); 7.86 (s, 1H, H8); 7.23−7.12 (m, 2H, H-aryl); 7.07−7.02 (m, 4H, H-aryl); 6.91−6.88 (m, 2H, H1′); 6.50 (ddd, 1H, H3′); 6.45 (ddd, 1H, H3′); 6.24−6.21 (m, 2H, H2′); 5.45 (dd, 1H, H_A-benzyl); 5.41 (dd, 1H, H_A-benzyl); 5.29 (dd, 1H, H_B-benzyl); 5.28 (dd, 1H, H_B-benzyl); 4.33−4.22 (m, 4H, H5′); ^{13}C NMR (150.1 MHz, DMSO-d_6) δ 156.46, 156.44 (C6); 147.88 (C4); 147.71, 147.68 (d, C2-aryl); 145.86 (C2); 137.80, 137.74 (C8); 132.41, 132.36 (C3′): 130.76, 130.75 (C4-aryl); 126.79, 126.72 (d, C3-aryl); 126.37, 126.35 (C2′); 124.13, 124.11 (C5); 123.80 (C6-aryl); 123.41, 123.36 (C5-aryl); 120.78, 120.58 (d, C1-aryl); 88.07, 88.06 (C1′); 85.14, 85.06 (d, C4′); 68.29. 68.22 (C5′); 68.18, 68.03 (d, CH_2-benzyl,); 14.79, 14.64 (CH_3−C3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −9.01; −9.23; (ESI$^+$) *m*/*z* 417.1; UV (CH_3-CN) λ_{max} 250.00 nm, 245.04 nm; 203.04 nm; λ_{min} 248.59 nm; 226.84 nm; IR (KBr) ν 3447.8, 1686.1, 1588.4, 1544.6, 1474.2, 1289.6, 1207.3, 1189.7, 1090.9, 1025.4, 998.3, 954.2, 827.6, 784.4, 649.7; R_f value (CH_2Cl_2/MeOH, 9:1) 0.33; analytical HPLC t_R 16.20 min, 16.07 min (95.7%, gradient I); 10.80 min, 11.29 min (95.7%, gradient II); 18.47 min, 18.57 min (95.8%, gradient III).

Determination of the Partition Coefficients (log *P* Values). log *P* values were determined as follows: a sample of the compounds **9**−**12** 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 end-capped (5 μm), gradient 0−100% CH_3CN in water (0−20 min), 100% CH_3CN (20−22 min), 0−100% CH_3CN in water (22.1−35 min), flow 0.5 mL, UV detection at 260 nm). The *P* values were calculated by integration of the peaks 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 by analytical HPLC after thawing (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), 100% CH_3CN (20−22 min), 11% CH_3CN (22.1−35 min), 0.5 mL flow, UV detection at 260 nm). The degradation of **9**−**12** 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 using the rate constants k. Values are means of duplicate experiments.

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-ddAMP (**9c**) and 3-methyl-*cyclo*Sal-d4AMP (**10c**) 0.5 U of enzyme and a 10 mM phosphate buffer (pH 6.8) were used. The concentration of the compounds in the above-mentioned buffers was 185 μM. To 1.3 mL of this preincubated solution (37 °C) was added ADA. The enzymatic deamination was followed by UV spectroscopy (Varian, Cary 1E) at 260 nm for 90 min. The integrity of **9c** and **10c** was demonstrated after comparison of the recorded UV spectra before (t_0) and after 90 min (t_{90}) incubation with ADA for 90 min.

The deamination of the monophosphates, dAMP and ddAMP, by adenosine monophosphate deaminase (AMPDA; EC 3.5.4.6; from rabbit muscle) was carried out using 1 and 2 U of AMPDA, respectively, in a 10 mM phosphate buffer (pH 6.8, Sörensen) at 37 °C, whereas for both triesters 3-methyl-*cyclo*Sal-ddAMP (**9c**) and 3-methyl-*cyclo*Sal-d4AMP (**10c**) 2 U of enzyme was used. The concentration of the compounds in the above-mentioned buffers was 185 μM. To 1.3 mL of this preincubated solution (37 °C) was added AMPDA. The deamination reactions were followed by UV spectroscopy (Varian, Cary 1E) at 260 nm for 200 min. The integrity of **9c** and **10c** was demonstrated after comparison of the UV spectra before (t_0) and after 200 min (t_{200}) incubation with AMPDA for 200 min.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 [HIV-1 ($HTLV\text{-}III_B$)] was originally obtained from a persistently HIV-infected H9 cell line, as described previously,[36] which was kindly provided by Dr. R. C. Gallo (then 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 kindly 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.[37] 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: ^1H, ^{13}C, and ^{31}P NMR spectra and detailed tables of coupling constants for the title compounds **9a**−**d**, **10a**−**d**, **11c**, and **12c**. This information is available free of charge via the Internet at http://pubs.acs.org.

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