Nucleotide Delivery from *cyclo*Saligenyl-3′-azido-3′-deoxythymidine Monophosphates (*cyclo*Sal-AZTMP)[1]

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The application of our *cyclo*Saligenyl- (*cyclo*Sal) pronucleotide concept to the approved anti-HIV dideoxynucleoside 3′-azido-3′-deoxythymidine AZT (**1**) is reported. This pronucleotide concept has been designed to deliver the corresponding 3′-azido-3′-deoxythymidine monophosphate AZTMP (**2**) by selective chemical hydrolysis from the lipophilic precursors *cyclo*Sal-AZTMP **4a–h**. All derivatives **4a–h** were synthesized using differently substituted salicyl alcohols **7a–h** as starting materials. In hydrolysis studies, compounds **4** decomposed selectively releasing AZTMP (**2**) and the salicyl alcohols **7** following the designed tandem reaction. Furthermore, due to the electronic properties introduced by substituents, the half-lives of the triesters **4** could be ajusted over a wide range. Phosphotriesters **4** exhibited considerable biological activity in HIV-1 and HIV-2 infected wild-type human T-lymphocyte (CEM/O) cells, whereas, contrary to our expectations, nearly all activity was lost in HIV-2 infected thymidine-kinase-deficient CEM cells.

Introduction

Nucleoside analogs are among the most effective classes of antiviral agents investigated so far for the treatment of HIV infections[2]. Since the discovery of 3′-azido-3′-deoxythymidine (AZT, Zidovudine, Retrovir®, **1**)[3] as the first drug for the treatment of AIDS, considerable efforts have been made to develop more active and less toxic inhibitors of the critical virus-associated enzymes. Most of these nucleoside analogues are 2′,3′-dideoxynucleosides like AZT (**1**). The general mode of action of these drugs is the inhibition of HIV reverse transcriptase[4][5][6] or incorporation into the growing DNA chain, which results in DNA chain termination[4]. However, the anti-HIV dideoxynucleosides are not active as such. After membrane penetration, intracellular conversion of the nucleoside analogues to their 5′-mono-, 5′-di- or 5′-triphosphates is essential for the expression of the biological activity. This biotransformation is brought about by host cell kinases in three steps via the nucleoside mono- and diphosphates[7]. Although dideoxynucleosides offer great promise for the treatment of HIV infections, several shortcomings limit realization of their full therapeutic efficacy. One problem is the limited ability of certain dideoxynucleosides to undergo biotransformation into the triphosphates[8] which is at least as important as the affinity of these compounds for the target enzyme.

Furthermore, the resistance of the human immunodeficiency virus to the clinically used antiviral deoxynucleoside AZT (**1**) is, on the one hand, directly related to multiple point mutations within the HIV-1 reverse transcriptase (RT) gene of the virus and, on the other hand, to the low susceptibility of the resistant cells to the drug due to a decreased activity of the phosphorylating enzyme thymidine-kinase (TK)[9]. Consequently, the direct application of the nucleotide 3′-azido-3′-deoxythymidine monophosphate (AZTMP, **2**) would circumvent the metabolization-limiting enzyme TK, leading to TK-bypass (Scheme 1). However, due to the high polarity of AZTMP (**2**), this compound is not able to penetrate biological membranes and hence circumvent high intracellular concentrations of this phosphorylated derivative. Furthermore, **2** is rapidly dephosphorylated in blood serum. Thus, a key goal in trying to improve the therapeutic potential of AZT and other nucleoside analogues could be the delivery of the corresponding nucleotide from neutral, lipophilic prodrugs[10] of the general type **3** (*Pronucleotide Approach*[11]; Scheme 1).

To achieve this goal, generally, uncharged nucleotide triesters are used as membrane-permeable nucleotide precursors[12][13]. The major differences between the approaches are the delivery mechanisms of the nucleotides. While almost all approaches based on chemical hydrolysis reported to date have proved to be unable to deliver the *nucleotide* selectively and consequently serve only as nucleoside "depots", the concepts based on *enzymatic activation* as in the bis-POM phosphotriesters[14], the bis-SATE phosphotriesters[15] and the aryloxyphosphoramidates[16] demonstrated the successful intracellular delivery of free nucleotides from highly lipophilic precursors.

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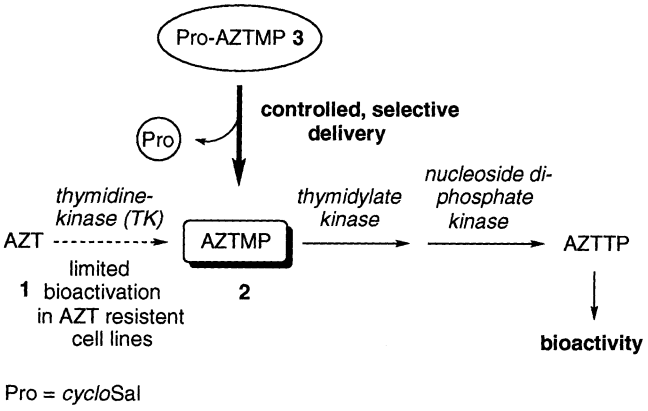
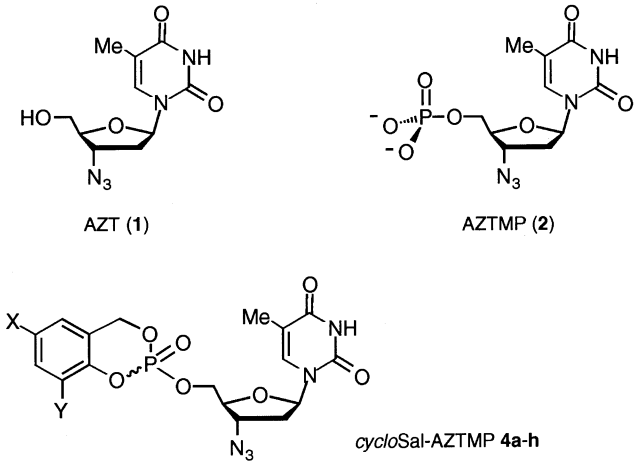
Scheme 1. Principle of an AZTMP *cyclo*Sal-pro-nucleotide approach



As part of our ongoing program to develop efficient pro-nucleotide systems[17], we have reported on the design and synthesis of an entirely new pro-nucleotide approach based on a pH-driven selective chemical hydrolysis: the ***cyclo*sali**-genyl **n**ucleoside **m**ono**p**hosphates (*cyclo*Sal-NMP)[18]. This new concept has been successfully introduced with the anti-HIV nucleosides ddT[19] and d4T[20] for the TK-bypass as well as with ddA[21] for the adenosine deaminase-bypass[22].

Here, we report on the synthesis and biological evaluation of *cyclo*Sal-3′-azido-3′-deoxythymidine monophosphates **4a−h** (*cyclo*Sal-AZTMP, Scheme 2) as potential neutral prodrugs AZTMP (**2**) of the approved drug AZT (**1**).

Scheme 2. Structures of AZT (**1**), AZTMP (**2**) and the general structure the *cyclo*Sal-AZTMP phosphotriesters **4**

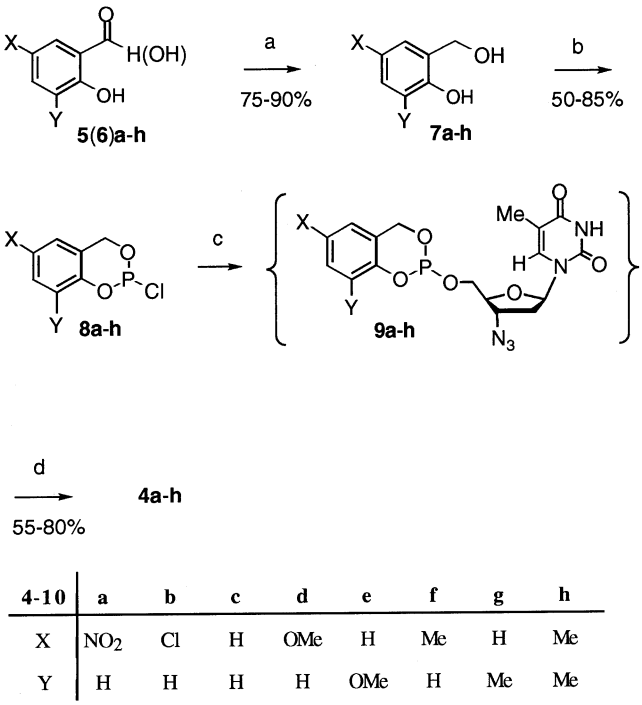


Furthermore, we report on their lipophilic properties (partition coefficients; *Pa* values), on hydrolysis studies in aqueous buffers at different pH values, as well as on hydrolysis studies under more biologically adapted conditions using RPMI-1640 culture medium containing 10% heat-inactivated fetal calf serum (FCS) at 37°C.

Results and Discussion

The title compounds **4a−h** were synthesized using reactive phosphorus(III) chemistry. The salicyl alcohols **7** were obtained by reduction of the corresponding salicylaldehydes **5** or salicylic acids **6** in 75−90% yield. 3,5-Dimethylsalicylaldehyde **5h** was prepared by *ortho*-formylation of the corresponding phenol using dichloromethyl methyl ether in the presence of $TiCl_4$. The reaction sequence to yield the *cyclo*Sal-phosphotriesters **4** is summarized in Scheme 3.

Scheme 3. Synthetic pathway to the *cyclo*Sal-AZTMP triesters **4a−h**. Reaction conditions: a) $NaBH_4$ for **5** ($LiAlH_4$ for **6**); b) PCl_3, pyridine, Et_2O, −10°C, 2 h; c) AZT **1**, DIPEA, CH_3CN, 0°C, 20 min.; d) TBHP, CH_3CN, r.t., 30 min.



4-10	a	b	c	d	e	f	g	h
X	NO_2	Cl	H	OMe	H	Me	H	Me
Y	H	H	H	H	OMe	H	Me	Me

After chromatographic purification (60−85% yield), the *cyclo*Sal-AZTMPs **4a−h** were characterized by means of ^{1}H-, ^{13}C-, and ^{31}P-NMR, UV spectroscopy, as well as by electrospray mass spectrometry (ESI, negative mode). As expected, the phosphotriesters displayed two closely spaced signals in the ^{31}P-NMR spectra (1:1 mixtures; $\delta = -8.2$ to -9.8), corresponding to the presence of diastereomers that result from mixed stereochemistry at the phosphorus center. The purity of the *cyclo*Sal-AZTMPs **4a−h** was checked by analytical HPLC. For the biological assays, small amounts of the *cyclo*Sal-AZTMP triesters were additionally purified by semi-preparative HPLC using acetonitrile/water eluents in order to ensure complete elimination of all traces of AZT (**1**). Even using semi-preparative HPLC, we were unable to separate the diastereomers. After HPLC purification, the phosphotriesters **4** were obtained by lyophilization of the fractions.

Determination of the Partition Coefficients (Pa Values): The partition coefficients (*Pa* values) of the *cyclo*Sal-AZTMPs **4a−h** were determined in 1-octanol/sodium phosphate buffer (pH 6.8). Triesters **4a−h** were partitioned between 1-octanol and phosphate buffer for 5 min. and after centrifugation the relative concentration in each solvent phase was determined by reversed-phase HPLC analysis.

This very simple method of partition coefficient determination allowed a qualitative estimation of the lipophilicities of compounds **4a−h**[23]. Unlike most nucleosides, AZT **1** can enter mammalian cells by passive, non-facilitated diffusion[24]. Consequently, the determined *Pa* value of **1** (1.06[25]) was used as a reference for the potential ability of compounds **4** to diffuse passively through membranes. As can be seen in Table 1, the measured *Pa* values were six- to sixty-fold higher (*Pa* = 6.5−62.2) as compared to the value for AZT (**1**).

Kinetic Studies: The nucleotide delivery system described here has been designed to selectively release the nucleotide and the two-component masking group by a controlled, chemically-induced tandem reaction involving successive, coupled cleavage of the phenyl and the benzyl ester groups of the *cyclo*Sal-phosphotriester. Within this concept, the coupling of the two ester hydrolyses is very important because it has previously been reported that the chemical[26] and sometimes even the enzymatic hydrolysis[27] of phosphodiesters may be extremely difficult. In our *cyclo*Sal pronucleotide concept, the lower stability of the phenyl as compared to the benzyl phosphate ester allows us to discriminate between these two phosphate ester bonds in the initial step yielding a benzylphospho**di**ester from of the *cyclo*Sal-phosphotriester. As a consequence, the initial hydrolysis of the phenyl ester causes an important activation of the remaining masking group and induces a spontaneous cleavage to yield the nucleotide[17]. The hydrolysis pathway has been verified by NMR spectroscopy[20].

In order to verify the selective delivery of AZTMP (**2**), hydrolysis experiments of the *cyclo*Sal-AZTMPs **4a−h** were carried out in different aqueous buffers and RPMI-1640 culture medium with or without heat-inactivated fetal calf serum. First, the triesters **4** were hydrolyzed in 50 mM phosphate buffer, pH 7.3, at 37°C as a model of the physiological milieu. Furthermore, the pH dependence of the hydrolysis was studied by carrying out experiments in borate buffer (50 mM, pH 8.9), TRIS buffer (50 mM, pH 6.9) and in acetate buffer (50 mM, pH 4.6). The degradation of **4a−h** was followed by means of reversed-phase HPLC analysis. The half-lives were determined by quantifying the decreasing peaks of **4** vs. time and are summarized in Table 1.

Most importantly, *cyclo*Sal-triesters **4a−g** were degraded following pseudo-first-order kinetics yielding only AZTMP **2** and the salicyl alcohols in all the studied media and the results confirmed the anticipated pH dependence of the hydrolysis. At pH < 7, only the 3,5-dimethyl-substituted derivative **4h** additionally yielded some (30%) of the ″wrong″ phenyl AZT-phosphodiester, which is stable to further hydrolysis. As can be seen in Table 1, for a given substituent the stability of the phosphotriesters **4a−h** do not differ greatly under mild acidic conditions (*cf.* the hydrolyses at pH 4.6 *vs.* pH 6.9) but are still considerably varying for the different substituents, whereas the half-lives decrease markedly at more basic pH values (hydrolysis at pH 8.9). More strikingly, we observed a clear correlation between the electronic effects of the various salicyl alcohol substituents and the hydrolysis half-lives in different aqueous buffers. The strong influence of the substituent electronic effect is demonstrated by the 3-methoxy and the 3-methyl groups: in contrast to the corresponding 5-substituted derivatives, where the methoxy derivative is equally stable as compared to the methyl derivative in all buffers, the situation is different for the 3-substituted phosphotriesters. The reason for this is that in the 5-position the mesomeric electronic effects are more important than the inductive effect, whereas in the 3-position the inductive effects of the methoxy group overcompensates the mesomeric effect, with the consequence of a considerable increase of susceptibility of this compound towards hydrolysis. These examples clearly demonstrate that we have a tool at our disposal whereby the hydrolysis kinetics of the phosphotriesters **4** can be controlled by varying the substituents on the aryl ring.

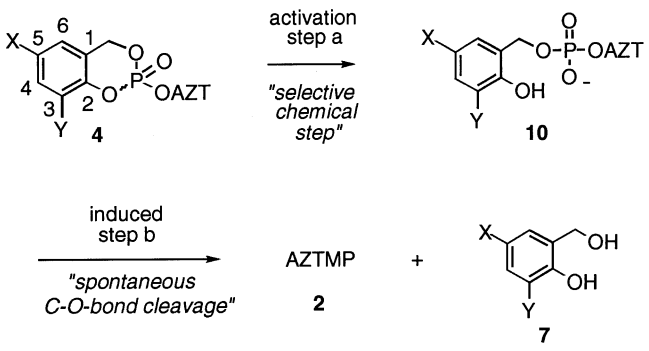
Table 1. Hydrolysis in different aqueous buffers and *Pa* values of *cyclo*Sal-AZTMPs **4a−h**

4 and **1**	Hydrolysis ($t_{1/2}$) in aqueous buffers at 37°C				Hydrolysis ($t_{1/2}$) in CM at 37°C[e]		*Pa* value[g]
	pH 4.6[a]	pH 6.9[b]	pH 7.3[c]	pH 8.9[d]	without FCS[f]	with FCS[f]	
	(h)	(h)	(h)	(h)	(h)	(h)	
4a	1.8	1.5	0.2	0.1	n.d.[h]	n.d.[h]	6.5
4b	6.7	6.4	0.7	0.25	0.3	0.3	41.5
4c	25.0	24.5	3.7	0.83	2.3	2.3	12.5
4d	28.7	28.3	4.1	0.9	3.9	4.0	13.2
4e	10.1	9.5	1.6	0.5	0.8	0.8	7.3
4f	29.0	28.3	4.1	1.0	4.1	4.1	30.7
4g	69.9	68.5	10.7	1.4	4.9	4.8	26.9
4h	102.1	98.2	14.7	2.1	9.4	9.3	62.2
1	n.a.[i]	n.a.[i]	n.a.[i]	n.a.[i]	n.a.[i]	n.a.[i]	1.09

[a] 50 mmol acetate buffer, pH 4.6. − [b] 50 mmol TRIS buffer, pH 6.9. − [c] 50 mmol phosphate buffer, pH 7.3. − [d] 50 mmol borate buffer, pH 8.9. − [e] CM: RPMI-1640 culture medium. − [f] FCS: fetal calf serum. − [g] *Pa*: partition coefficient. − [h] Not determined. − [i] Not available.

Moreover, all the results are in full agreement with the designed hydrolysis pathway of a selective cleavage of the phenyl ester to give 2-hydroxybenzylphospho**di**ester **10** (step a) and a subsequently induced spontaneous cleavage of **10**, presumably via a C−O bond cleavage following the Grob fragmentation mechanism, followed by water quenching[28] of the remaining masking group, leaving AZTMP (**2**) and salicyl alcohols **7** (step b) (Scheme 4).

Scheme 4. The hydrolysis pathway of *cyclo*Sal-AZTMP phosphotriesters **4a−h**



Furthermore, an interesting change in the rate-determining step of this tandem reaction was observed: whereas we detected the intermediate 2-hydroxybenzylphosphodiester **10** in the hydrolysis of the 5-nitro- and the 5-chloro-substituted *cyclo*Sal-AZTMP derivatives **4a** and **4b** (Figure 1), we were unable to detect this intermediate in any reaction involving the donor-substituted phosphotriesters (Figure 2). As indicated in Figure 1, the formation of AZTMP from 5-chloro-*cyclo*Sal-AZTMP (**4b**) shows a sigmoidal increase, whereas it shows an exponential increase in the hydrolysis of 5-methyl-*cyclo*Sal-AZTMP **4f**. The same was observed for the salicylic alcohol. This observation points to the transformation of the phosphodiester **10b** to AZTMP **2** being the rate-limiting step in the former case and is in accordance with the conversion of the phosphotriester **4f** to the phosphodiester **10f** being the rate-limiting step in the latter case. This behavior can again be explained by the influence of the different substituents: the more electron-donating the substituent, the faster is the degradation of the intermediate 2-hydroxybenzylphosphodiester **10**.

Figure 1. Hydrolysis of 5-chloro-*cyclo*Sal-AZTMP (**4b**) in phosphate buffer, pH 7.3

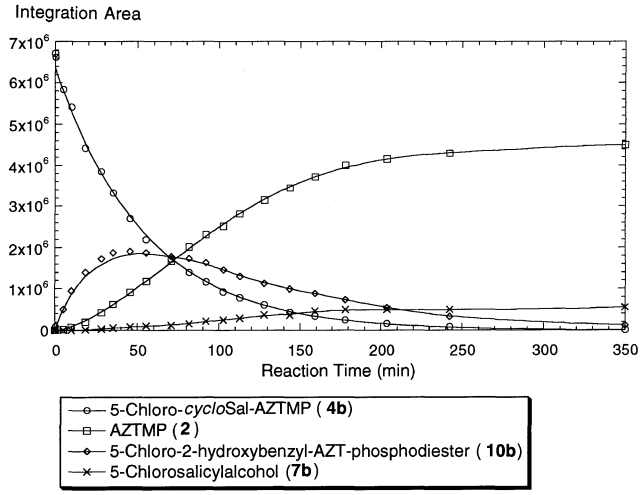
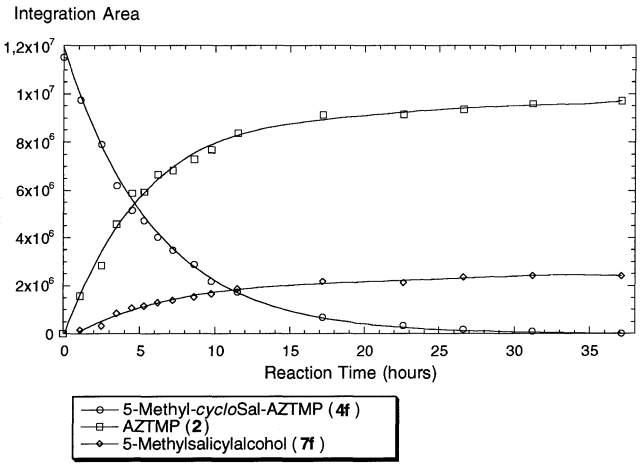


Figure 2. Hydrolysis of 5-methyl-*cyclo*Sal-AZTMP (**4f**) in phosphate buffer, pH 7.3



From the results summarized in Table 1, the donor-substituted compounds of the *cyclo*Sal-AZTMPs **4d, f−h** in particular, which exhibit half-lives of 4−14 hours in phosphate buffer at pH 7.3, seem to be interesting candidates for biological evaluation.

Further hydrolysis studies were carried out in RPMI-1640 culture medium with and without 10% heat-inactivated fetal calf serum (FCS) at 37°C. This medium is normally used for the cultivation of human T-lymphocytes used in in vitro anti-HIV tests[29]. The hydrolysis products in RPMI-1640 culture medium were again exclusively AZTMP (**2**) and the diols **7a−h**, but the half-lives were slightly shorter than in phosphate buffer. The shorter half-lives in the RPMI-1640 medium are certainly an effect of its more basic pH value (pH 7.8). In the RPMI-1640 medium supplemented with 10% FCS, we also detected the dephosphorylation of AZTMP into AZT by residual phosphatases in the serum. On the basis of the measured half-lives, the donor-substituted derivatives of **4** should be sufficiently stable to serve as intracellular "depots" of AZTMP (**2**). Finally, it should be mentioned that so far we have not found any evidence for a contribution from an enzymatic degradation of the *cyclo*Sal-phosphotriesters in the RPMI-1640/FCS hydrolyses. Thus, under the conditions used in these studies, the degradation of our pro-nucleotides seems to be purely chemical. Nevertheless, additional hydrolysis studies in sera, in plasma and in cell extracts will be the subject of further studies in our laboratory and the results will be reported in due course.

Antiviral Evaluation: The *cyclo*Sal-d4TMP and *cyclo*Sal-ddAMP phosphotriesters have already demonstrated the effectiveness of our new pro-nucleotide system in inhibiting HIV-1 and HIV-2 replication in rapidly dividing human T-lymphoblastic leukaemia cells (CEM/O cells)[20][21]. Moreover, particularly striking was the complete retention of biological activity of donor-substituted *cyclo*Sal-d4TMP triesters in thymidine kinase-deficient CEM cells, as well as the structure-bioactivity correlation: The stronger the electron-donating effect of the substituent, the better was the antiviral activity against HIV-1 and HIV-2 infected wild-type CEM/O cells and in mutant thymidine-kinase-deficient CEM/TK$^-$ cells[20]. These results confirmed the highly selective intracellular delivery of d4TMP and ddAMP and demonstrated efficient TK- and ADA-bypasses, respectively.

For these reasons, the antiviral activities of the *cyclo*Sal-AZTMPs **4** were also evaluated against HIV-1 and HIV-2 infected CEM/O cells and HIV-2 infected CEM/TK$^-$ cells (Table 2). The test compounds were proven to be free of the parent nucleoside **1** by means of analytical HPLC.

The parent nucleoside AZT (**1**) proved to be extremely active against HIV-1 and HIV-2 induced cytopathicity in CEM/O cells (EC_{50}: 0.006 μM). As anticipated, AZT (**1**) virtually loses its antiviral potential when evaluated against HIV-2 in CEM/TK$^-$ cells, owing to the absence of its bioactivating enzyme thymidine-kinase. As can be seen in Table 2, all *cyclo*Sal-AZTMPs **4a−h** proved to be as active as AZT (**1**) against HIV-1 or HIV-2 induced cytopathicity in

Table 2. Antiviral evaluation of the inhibitory effect of HIV-1 and HIV-2 replication of *cyclo*Sal-AZTMPs **4a**−**h** in CEM/O cells and in thymidine kinase-deficient CEM/TK$^-$ cell

4 and **1**	X	Antiviral Activity EC_{50} (μM)[a] CEM/O HIV-1	CEM/O HIV-2	CEM/TK$^-$ HIV-2	Cytotoxicity CC_{50} (μM)[b]
4a	5-NO_2	0.008	0.02	>100	79
4b	5-Cl	0.005	0.006	>40	34
4c	5-H	0.004	0.005	>20	70
4d	5-OCH_3	0.006	0.021	30	66
4e	3-OCH_3	0.009	0.009	>100	>100
4f	5-CH_3	0.005	0.006	21	79
4g	3-CH_3	0.006	0.013	15	40
4h	3,5-CH_3	0.007	0.017	7	41
1	−	0.006	0.005	>100	>100

[a] 50% effective concentration. − [b] 50% cytotoxic concentration.

the wild-type cell line. Nevertheless, no correlation of the antiviral activity with either the hydrolytic stability in the aqueous buffers and in RPMI-1640 culture medium, or with the lipophilicity of **4a**−**h** (*Pa* values) was observed. To our surprise, and despite the pronounced antiviral activity in the wild-type cell line, the biological activity was almost completely lost in the HIV-2 infected CEM/TK$^-$ cells. Only the donor-substituted triesters **4f**−**4h** exhibited some antiviral activity in the TK$^-$ cells (5 to 14-times more active than AZT (**1**)). These results are in complete contrast to the observed antiviral activity of the related d4TMP and ddAMP derivatives studied previously[20][21]. As yet, we have no explanation for the failure of the *cyclo*Sal-AZTMP derivatives in the CEM/TK$^-$ anti-HIV test. On the other hand, it should be mentioned that comparable effects were observed in the phosphoramidate approach of McGuigan et al.[16a] and the bis-SATE approach of Imbach et al.[15a]: the AZT-phosphoramidates and the bis-SATE-AZTMP phosphotriesters also showed a marked decrease (100-fold and 10-fold, respectively) in biological activity on going from the wild-type cells to the TK-deficient cells. As with our system, both of the approaches have been proven to give good results with d4T[15a][16a] and ddA[15c][16e]. Consequently, it seems that there is a particular metabolic limitation that prevents the retention of activity in the TK$^-$ cells. Another possibility that should be considered is a different cellular uptake of the *cyclo*Sal-d4TMP and *cyclo*Sal-ddAMP phosphotriesters as compared to the corresponding *cyclo*Sal-AZTMP derivatives. Furthermore, it has been reported that AZTMP (**2**) accumulates at a sufficiently high level for readily measurable levels of the nucleotide to diffuse back into the extracellular medium. We are currently investigating whether the activity can be recovered in AZT-resistant cells.

Conclusion

In summary, as with the previously reported *cyclo*Sal-d4TMP and the *cyclo*Sal-ddAMP triesters, hydrolysis studies clearly show that our prodrug approach is suitable for the delivery of AZTMP (**2**) from *cyclo*Sal-AZTMPs **4a**−**h** by a non-enzymatic activation at physiological pH. The half-lives of hydrolysis could be readily controlled by variation of the substitution on the aromatic ring. All compounds exhibited considerably higher partition coefficients than the parent nucleoside AZT (**1**). Surprisingly, and in contrast to the corresponding derivatives of the nucleoside analog d4T, the biological activity of **4a**−**h** found in the wild-type cells was not retained in TK-deficient cells. Further work is currently in progress aimed at elucidating the underlying reasons for this loss of activity, as this is an important question with respect to the exploration of the *pro-nucleotide* concept.

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Experimental Section

General: 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 F254 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 4-methoxybenzaldehyde, 9 ml ethanol, 0.5 ml concentrated sulfuric acid and 0.1 ml glacial acetic acid) by heating with a hot air blower or a hot plate. − HPLC: (Merck-Hitachi) semi-preparative HPLC: LiChroCART 250-10 containing LiChrospher 100 RP-18 (10 μm); analytical HPLC: LiChroCART 250-4 with LiChrospher 100 RP-18 (5 μm) standard gradient: 18−100% CH_3CN in water (0−16 min), 100% CH_3CN (16−19 min), 18% CH_3CN (19−31 min), flow rate 0.5 ml/min., UV detection at 269 nm. − Distillation: Büchi Kugelrohr apparatus. − NMR spectra were recorded using: ^1H NMR: Bruker AMX 400 at 400 MHz (tetramethylsilane or DMSO as internal standard). − ^{13}C NMR: Bruker AM 250 at 63 MHz ($CDCl_3$ or DMSO as internal standard). − ^{31}P NMR: Bruker AMX 400 at 162 MHz (H_3PO_4 as external standard). All ^1H- and ^{13}C-NMR chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane. The ^{31}P-NMR chemical shifts are quoted in ppm using H_3PO_4 as external reference. The spectra were recorded at room temperature. − UV/Vis spectroscopy: Varian Cary 1E. − IR spectroscopy: Perkin-Elmer 1600 Series FT-IR. − Elemental analyses: Heraeus CHN-O Rapid. The resulting lyophilized triesters **4** did not give acceptable microanalytical data, 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. − Mass spectrometry: electrospray MS: Fisons VG platform II, negative mode, acetonitrile as solvent; FAB-MS: VG Analytical ZAB 2 SEQ.

General Procedure for the Synthesis of the Cyclic Salicylchlorophosphanes **8a**−**h**: The cyclic chlorophosphanes were prepared according to a procedure published by Wissner et al.[31]. Briefly, the salicyl alcohols **7**, which have been prepared by reduction from the corresponding salicylaldehydes or the salicylic acids using $NaBH_4$ or $LiAlH_4$ in multi-gram scales (ca. 10 g), were dissolved in dry diethyl ether at −10°C. Then, 1.2 equiv. of phosphorus trichloride was added. To this mixture, a solution of pyridine in diethyl ether was added dropwise over a period of 30 min. After stirring for 1 h, the reaction mixture was allowed to warm to room temperature

and stirring was continued for further 2 h. The mixture was stored for about 12 h at 0°C and the precipitated pyridinium hydrochloride was filtered off under argon using a Schlenk flask. The solvent was evaporated under reduced pressure and the residue was subjected to Kugelrohr distillation in high vacuum. The chlorophosphanes **8** were isolated as colorless liquids, which solidified on standing at −20°C. Compounds **8** were too unstable to allow the measurement of infrared spectra or analysis of their elemental compositions.

5-Nitrosalicyl Alcohol (**7a**): Yield: 45% (yellow solid). − M.p. 129°C. − R_f (TLC) = 0.50 (CH_2Cl_2/CH_3OH, 9:1); 0.44 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.07 (s, br, 1 H, OH_{aryl}), 8.21 (td, 1 H, $^4J_{HH}$ = 2.9 Hz, $^4J_{HH}$ = 0.8 Hz, H-6$_{aryl}$), 8.02 (dd, 1 H, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 2.9 Hz, H-4$_{aryl}$), 6.94 (d, 1 H, $^3J_{HH}$ = 8.9 Hz, H-3$_{aryl}$), 5.34 (s, br, 1 H, OH_{benzyl}), 4.51 (s, 2 H, H_{benzyl}). − ^{13}C NMR ([D$_6$]DMSO): δ = 160.69 (C-2$_{aryl}$), 139.85 (C-5$_{aryl}$), 130.51 (C-1$_{aryl}$), 124.20 (C-4$_{aryl}$), 122.89 (C-6$_{aryl}$), 114.86 (C-3$_{aryl}$), 57.62 (C_{benzyl}). − IR (KBr): ṽ = 3459 cm^{-1}, 3077, 1592, 1487, 1334, 815, 757, 701. − $C_7H_7NO_4$ (169.3): calcd. C 49.62, H 4.13; found C 49.57, H 4.09.

5-Nitrosalicylchlorophosphane (**8a**): Yield: 45% (yellow oil). − B.p. 155−163°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 8.17 (dd, 1 H, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 0.9 Hz, H-4$_{aryl}$), 7.98 (d, 1 H, $^4J_{HH}$ = 0.9 Hz, H-6$_{aryl}$), 7.13 (d, 1 H, $^3J_{HH}$ = 7.9 Hz, H-3$_{aryl}$), 5.55 (dd, 1 H, $^2J_{HH}$ = 14.2 Hz, $^3J_{HP}$ = 2.2 Hz, H_{benzyl}), 5.14 (dd, 1 H, $^3J_{HH}$ = 14.2 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}). − ^{31}P NMR ($CDCl_3$): δ = 140.01.

5-Chlorosalicyl Alcohol (**7b**): Yield: 73% (colorless plates). − M.p. 91°C. − R_f (TLC) = 0.60 (CH_2Cl_2/CH_3OH, 9:1); 0.52 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 9.61 (s, br, 1 H, OH_{aryl}), 7.27 (d, 1 H, $^4J_{HH}$ = 2.7 Hz, H-6$_{aryl}$), 7.06 (dd, 1 H, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 2.7 Hz, H-4$_{aryl}$), 6.77 (d, 1 H, $^3J_{HH}$ = 8.5 Hz, H-3$_{aryl}$), 5.09 (t, 1 H, $^3J_{HH}$ = 5.5 Hz, OH_{benzyl}), 4.46 (d, 2 H, $^3J_{HH}$ = 5.0 Hz, H_{benzyl}). − ^{13}C NMR ([D$_6$]DMSO): δ = 152.84 (C-2$_{aryl}$), 131.03 (C-1$_{aryl}$), 126.72 (C-4$_{aryl}$), 126.54 (C-6$_{aryl}$), 122.41 (C-5$_{aryl}$), 116.00 (C-3$_{aryl}$), 57.71 (C-$_{benzyl}$). − IR (KBr): ṽ = 3442 cm^{-1}, 3183, 1615, 1515, 1480, 1458, 1436, 1384, 1315, 1255, 1229, 1208, 1164, 1040, 1000, 999, 984, 930, 864, 818, 757, 701. − $C_7H_7ClO_2$ (158.6): calcd. C 52.99, H 4.41; found C 52.85, H 4.36.

5-Chlorosalicylchlorophosphane (**8b**): Yield: 85% (pale yellow oil). − B.p. 171−180°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 7.21 (dd, 1 H, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 2.5 Hz, H-4$_{aryl}$), 6.97 (d, 1 H, $^4J_{HH}$ = 2.5 Hz, H-6$_{aryl}$), 6.91 (d, 1 H, $^3J_{HH}$ = 8.7 Hz, H-3$_{aryl}$), 5.40 (dd, 1 H, $^2J_{HH}$ = 14.4 Hz, $^3J_{HP}$ = 2.3 Hz, H_{benzyl}), 4.98 (dd, 1 H, $^3J_{HH}$ = 14.4 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}). − ^{13}C NMR ($CDCl_3$): δ = 144.68 (C-2$_{aryl}$), 130.34 (C-1$_{aryl}$), 129.31 (C-5$_{aryl}$), 125.67 (C-6$_{aryl}$), 122.79 (C-4$_{aryl}$), 120.98 (C-3$_{aryl}$), 65.95 (C-$_{benzyl}$). − ^{31}P NMR ($CDCl_3$): δ = 139.83.

Salicylchlorophosphane (**8c**): Yield: 65% (colorless oil). − B.p. 160−175°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 7.16 (dt, 1 H, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 0.9 Hz, H_{aryl}), 7.00 (dt, 1 H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.2 Hz, H_{aryl}), 6.88 (2 d, 2 H, $^3J_{HH}$ = 8.3 Hz, H_{aryl}), 5.50 (dd, 1 H, $^3J_{HH}$ = 14.3 Hz, $^3J_{HP}$ = 2.3 Hz, H_{benzyl}), 4.92 (dd, 1 H, $^3J_{HH}$ = 14.3 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}). − ^{13}C NMR ($CDCl_3$): δ = 129.11 (C_{aryl}), 125.66 (C_{aryl}), 123.86 (C_{aryl}), 121.46 (C_{aryl}), 121.26 (C_{aryl}), 119.28 (C_{aryl}), 60.95 (C_{benzyl}). − ^{31}P NMR ($CDCl_3$): δ = 140.67.

5-Methoxysalicyl Alcohol (**7d**): Yield: 85% (colorless solid). − M.p. 79°C. − R_f (TLC) = 0.55 (CH_2Cl_2/CH_3OH, 9:1); 0.49 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 8.80 (s, br, 1 H, OH_{aryl}), 6.88 (d, 1 H, H_{aryl}), 6.57−6.69 (m, 2 H, H_{aryl}), 4.95 (s, br, 1 H, OH_{benzyl}), 4.45 (s, 2 H, H_{benzyl}), 3.65 (s, 3 H, OCH_3). − ^{13}C NMR ([D$_6$]DMSO): δ = 152.17 (C_{aryl}), 147.77 (C_{aryl}), 129.55 (C_{aryl}), 115.05 (C_{aryl}), 112.76 (C_{aryl}), 112.11 (C_{aryl}), 58.25 (C_{benzyl}), 55.25 (OCH_3). − IR (KBr): ṽ = 3442 cm^{-1}, 3183, 1615, 1515, 1480, 1458, 1436, 1384, 1315, 1255, 1229, 1208, 1164, 1040, 1000, 999, 984, 930, 864, 818, 757, 701. − $C_8H_{10}O_3$ (154.2): calcd. C 62.21, H 6.53; found C 62.44, H 6.68.

5-Methoxysalicylchlorophosphane (**8d**): Yield: 50% (colorless oil). − B.p. 160−175°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 6.80 (d, 1 H, $^3J_{HH}$ = 8.3 Hz, H-3$_{aryl}$), 6.71 (dd, 1 H, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 2.9 Hz, H-4$_{aryl}$), 6.38 (d, 1 H, $^4J_{HH}$ = 2.9 Hz, H-6$_{aryl}$), 5.34 (dd, 1 H, $^3J_{HH}$ = 14.3 Hz, $^3J_{HP}$ = 2.3 Hz, H_{benzyl}), 4.97 (dd, 1 H, $^3J_{HH}$ = 14.3 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}), 3.67 (s, 3 H, OCH_3). − ^{13}C NMR ($CDCl_3$): δ = 155.79 (C_{aryl}), 122.02 (C_{aryl}), 120.37 (C_{aryl}), 114.82 (C_{aryl}), 110.30 (C_{aryl}), 107.85 (C_{aryl}), 61.17 (C_{benzyl}), 55.67 (OCH_3). − ^{31}P NMR ($CDCl_3$): δ = 139.38.

3-Methoxysalicyl Alcohol (**7e**): Yield: 65% (colorless solid). − M.p. 79°C. − R_f (TLC) = 0.54 (CH_2Cl_2/CH_3OH, 9:1); 0.51 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 8.49 (s, br, 1 H, OH_{aryl}), 6.91 (dd, 1 H, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.7 Hz, H_{aryl}), 6.83 (dd, 1 H, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 1.7 Hz, H_{aryl}), 6.74 (t, 1 H, $^3J_{HH}$ = 7.8 Hz, H_{aryl}), 4.92 (s, br, 1 H, OH_{benzyl}), 4.48 (s, 2 H, H_{benzyl}), 3.77 (s, 3 H, OCH_3). − ^{13}C NMR ([D$_6$]DMSO): δ = 146.95 (C-3$_{aryl}$), 142.94 (C-2$_{aryl}$), 129.04 (C-6$_{aryl}$), 119.39 (C-1$_{aryl}$), 118.45 (C-5$_{aryl}$), 110.18 (C-4$_{aryl}$), 58.15 (C_{benzyl}), 55.78 (OCH_3). − IR (KBr): ṽ = 3442 cm^{-1}, 3183, 1615, 1515, 1480, 1458, 1384, 1315, 1255, 1229, 1208, 1164, 1040, 984, 930, 757. − $C_8H_{10}O_3$ (154.2): calcd. C 62.33, H 6.54; found C 62.58, H 6.66.

3-Methoxysalicylchlorophosphane (**8e**): Yield: 65% (colorless oil). − B.p. 169−180°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 7.03 (t, 1 H, $^3J_{HH}$ = 8.0 Hz, H-5$_{aryl}$), 6.85 (d, 1 H, $^3J_{HH}$ = 8.2 Hz, H-4$_{aryl}$), 6.56 (d, 1 H, $^3J_{HH}$ = 7.8 Hz, H-6$_{aryl}$), 5.42 (dd, 1 H, $^2J_{HH}$ = 14.2 Hz, $^3J_{HP}$ = 2.2 Hz, H_{benzyl}), 4.99 (dd, 1 H, $^3J_{HH}$ = 14.2 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}), 3.87 (s, 3 H, OCH_3). − ^{13}C NMR ($CDCl_3$): δ = 149.8 (C_{aryl}), 136.15 (C_{aryl}), 124.1 (C_{aryl}), 122.7 (C_{aryl}), 117.6 (C_{aryl}), 111.9 (C_{aryl}), 61.07 (C_{benzyl}), 56.54 (OCH_3). − ^{31}P NMR ($CDCl_3$): δ = 140.36.

5-Methylsalicyl Alcohol (**7f**): Yield: 68% (colorless solid). − M.p. 104.5°C. − R_f (TLC) = 0.62 (CH_2Cl_2/CH_3OH, 9:1); 0.56 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 9.03 (s, br, 1 H, OH_{aryl}), 7.09 (d, 1 H, $^4J_{HH}$ = 1.6 Hz, H-6$_{aryl}$), 6.83 (dd, 1 H, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.7 Hz, H-4$_{aryl}$), 6.65 (d, 1 H, $^3J_{HH}$ = 8.1 Hz, H-3$_{aryl}$), 4.46 (s, 2 H, H_{benzyl}), 3.51 (s, br, 1 H, OH_{benzyl}), 2.18 (s, 3 H, CH_3). − ^{13}C NMR ($CDCl_3$): δ = 151.87 (C-2$_{aryl}$), 128.17 (C-5$_{aryl}$), 127.92 (C-4$_{aryl}$), 127.58 (C-6$_{aryl}$), 126.93 (C-1$_{aryl}$), 114.42 (C-3$_{aryl}$), 58.32 (C_{benzyl}), 20.33 (CH_3). − IR (KBr): ṽ = 3320 cm^{-1}, 1595, 1469, 1438, 1343, 1212, 1085, 995, 778, 772, 679, 611. − $C_8H_{10}O_2$ (138.2): calcd. C 69.55, H 7.29; found C 69.29, H 7.22.

5-Methylsalicylchlorophosphane (**8f**): Yield: 75% (colorless oil). − B.p. 167−182°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 7.20 (d, 1 H, $^3J_{HH}$ = 8.0 Hz, H-4$_{aryl}$), 7.01 (d, 1 H, $^3J_{HH}$ = 8.0 Hz, H-3$_{aryl}$), 6.92 (s, 1 H, H-6$_{aryl}$), 5.55 (d, 1 H, $^3J_{HP}$ = 14.3 Hz, H_{benzyl}), 5.12 (d, 1 H, $^3J_{HP}$ = 14.1 Hz, H_{benzyl}), 2.45 (s, 3 H, CH_{3aryl}). − ^{13}C NMR ($CDCl_3$): δ = 143.71 (C-2$_{aryl}$), 133.58 (C-5$_{aryl}$), 129.74 (C-1$_{aryl}$), 125.94 (C-4$_{aryl}$), 120.96 (C-6$_{aryl}$), 119.10 (C-3$_{aryl}$), 61.07 (C_{benzyl}), 20.65 (CH_3). − ^{31}P NMR ($CDCl_3$): δ = 139.87.

3-Methylsalicyl Alcohol (**7g**): Yield: 80% (colorless solid). − M.p. 72.5°C. − R_f (TLC) = 0.69 (CH_2Cl_2/CH_3OH, 9:1); 0.62 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 8.38 (s, br, 1 H, OH_{aryl}), 7.04 (d, 1 H, $^3J_{HH}$ = 7.6 Hz, H_{aryl}), 6.95 (d, 1 H, $^3J_{HH}$ = 7.6 Hz, H_{aryl}), 6.71 (t, 1 H, $^3J_{HH}$ = 7.6 Hz, H_{aryl}), 5.27 (s, br, 1 H, OH_{benzyl}), 4.56 (s, 2 H, H_{benzyl}), 2.15 (s, 3 H, CH_3). − ^{13}C NMR

([D$_6$]DMSO): δ = 152.57 (C_{aryl}), 129.01 (C_{aryl}), 127.95 (C_{aryl}), 125.01 (C_{aryl}), 124.07 (C_{aryl}), 118.97 (C_{aryl}), 59.87 (C_{benzyl}), 16.14 (CH_3). − IR (KBr): $\tilde{\nu}$ = 3320 cm^{-1}, 1595, 1469, 1438, 1343, 1212, 1085, 995, 778, 772, 679, 611. − $C_8H_{10}O_2$ (138.2): calcd. C 69.40, H 7.28; found C 69.64, H 7.37.

3-Methylsalicylchlorophosphane (**8g**): Yield: 70% (colorless oil). − B.p. 165−178°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 7.10 (dd, 1 H, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 0.7 Hz, H-6$_{aryl}$), 6.96 (t, 1 H, $^3J_{HH}$ = 7.6 Hz, H-5$_{aryl}$), 6.77 (dd, 1 H, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 0.6 Hz, H-4$_{aryl}$), 5.40 (dd, 1 H, $^2J_{HH}$ = 14.1 Hz, $^3J_{HP}$ = 2.6 Hz, H_{benzyl}), 4.98 (dd, 1 H, $^3J_{HH}$ = 14.1 Hz, $^3J_{HP}$ = 9.4 Hz, H_{benzyl}), 2.22 (s, 3 H, CH_3). − ^{13}C NMR ($CDCl_3$): δ = 130.48 (C_{aryl}), 123.35 (C_{aryl}), 123.19 (C_{aryl}), 123.17 (C_{aryl}), 61.26 (C_{benzyl}), 15.52 (CH_3). − ^{31}P NMR ($CDCl_3$): δ = 141.45.

3,5-Dimethylsalicyl Alcohol (**7h**): Yield: 57% (colorless crystalls). − M.p. 52.2°C. − R_f (TLC) = 0.60 (CH_2Cl_2/CH_3OH, 9:1); 0.52 (ethyl acetate/CH_3OH, 7:3). − ^{1}H NMR ([D$_6$]DMSO): δ = 8.15 (s, br, 1 H, OH_{aryl}), 6.84 (s, 1 H, H-6$_{aryl}$), 6.75 (s, 1 H, H-4$_{aryl}$), 5.23 (s, br, 1 H, OH_{benzyl}), 4.53 (s, 2 H, H_{benzyl}), 2.15 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3). − ^{13}C NMR ([D$_6$]DMSO): δ = 150.27 (C-2$_{aryl}$), 129.52 (C-4$_{aryl}$), 127.74 (C-5$_{aryl}$), 127.26 (C-1$_{aryl}$), 125.50 (C-6$_{aryl}$), 123.84 (C-3$_{aryl}$), 59.93 (C_{benzyl}), 20.14 (CH_3), 16.12 (CH_3). − IR (KBr):$\tilde{\nu}$ = 3394 cm^{-1}, 2920, 1469, 1486, 1213, 1085, 995, 853, 772, 679, 611. − $C_9H_{12}O_2$ (152.2): calcd. C 71.03, H 7.95; found C 71.26, H 7.84.

3,5-Dimethylsalicylchlorophosphane (**8h**): Yield: 75% (colorless oil). − B.p. 167−182°C (2.5 mbar). − ^{1}H NMR ($CDCl_3$): δ = 6.93 (s, 1 H, H-4$_{aryl}$), 6.60 (s, 1 H, H-6$_{aryl}$), 5.37 (br, 1 H, H_{benzyl}), 4.97 (br, 1 H, H_{benzyl}), 2.26 (s, 3 H, CH_3), 2.20 (s, 3 H, CH_3). − ^{13}C NMR ($CDCl_3$): δ = 142.34 (C-2$_{aryl}$), 132.92 (C-5$_{aryl}$), 132.81 (C-1$_{aryl}$), 131.18 (C-3$_{aryl}$), 129.42 (C-4$_{aryl}$), 123.39 (C-6$_{aryl}$), 61.28 (C_{benzyl}), 20.61 (5-CH_3), 15.34 (3-CH_3). − ^{31}P NMR ($CDCl_3$): δ = 141.58.

General Procedure for the Synthesis of the cycloSal-AZTMP Phosphotriesters **4a−h**: The reactions were performed under anhydrous conditions in an argon atmosphere. To a solution of AZT (**1**) (0.10 g, 0.37 mmol) in 6.0 ml CH_3CN, cooled to 0°C in ice/water, was added DIPEA (0.097 g, 0.75 mmol). Then, the appropriate chlorophosphane **8** (0.75 mmol) was added over a period of 5 min. and the solutions were stirred for a further 15 min. to ensure complete reaction (TLC analysis). For oxidation of the intermediate cyclic phosphites, *t*-butyl hydroperoxide (0.072 g, 0.80 mmol; as a 5.0-6.0 M solution in decane commercially available from Aldrich) was added to the reaction mixtures at 0°C. After stirring for 30 min. and warming to room temperature, 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 and then a gradient of CH_3OH in CH_2Cl_2, to yield the title compounds **4**. The compounds were isolated as mixtures of diastereomers as a result of the mixed stereochemistry at the phosphorus atom.

cyclo-(5-Nitrosaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)-phosphate (**4a**): 0.175 g of 5-nitrosalicylchlorophosphane (**8a**) was used. − Yield: 0.125 g (70%). − R_f (TLC) = 0.38 (CH_2Cl_2/CH_3OH, 9:1); 0.55 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.30, 11.29 (2 s, 1 H, N-H), 8.30 (d, 1 H, $^4J_{HH}$ = 2.7 Hz, H-6$_{thymine}$), 8.24 (d, 1 H, $^3J_{HH}$ = 9.2 Hz, $^4J_{HH}$ = 2.7 Hz, $^5J_{HP}$ = 1.1 Hz, H-4$_{aryl}$), 7.42 (2 d, 1 H, $^4J_{HH}$ = 1.1 Hz, H-6), 7.38 (dd, 1 H, $^3J_{HH}$ = 9.1 Hz, $^5J_{HH}$ = 1.3 Hz, H-3$_{aryl}$), 6.09 (2 t, 1 H, $^3J_{HH}$ = 6.5 Hz, 2× H-1′), 5.66 (dd, 1 H, $^2J_{HH}$ = 14.9 Hz, $^3J_{HH}$ = 6.5 Hz, $^3J_{HP}$ = 17.6 Hz, H_{benzyl}), 5.56 (dd, 1 H, $^2J_{HH}$ = 14.9 Hz, $^3J_{HH}$ = 5.0 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}), 4.45 (m, 1 H, H-3′), 4.43 (dd, 1 H, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 4.6 Hz, $^3J_{HP}$ = 2.6 Hz, H-5″), 4.36 (dd, 1 H, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 5.2 Hz, $^3J_{HP}$ = 7.5 Hz, H-5′), 3.98 (m, 1 H, H-4′), 2.45 (m, 1 H, H-2″), 2.33 (m, 1 H, H-2′), 1.74 (2 d, 3 H, $^4J_{HH}$ = 1.1 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.99 (C-4), 154.1 (2 d, C-2$_{aryl}$), 150.7 (C-2), 143.8 (C-5$_{aryl}$), 136.7 (C-6), 125.4 (C-4$_{aryl}$), 122.7, 122.8 (C-6$_{aryl}$), 122.2 (2 d, C-1$_{aryl}$), 119.8 (2 d, C-3$_{aryl}$), 110.31, 110.28 (C-5), 83.34, 83.31 (C-1′), 81.35, 81.28 (C-4′), 68.2 (2 d, C_{benzyl}), 67.3 (2 d, C-5′), 59.8, 60.0 (C-3′), 35.9, 35.8 (C-2′), 12.2, 12.3 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −9.49 and −9.70 [2 s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 265 nm (10100), λ_{min} (ε) = 235 nm (4100). − IR (KBr): $\tilde{\nu}$ = 3580 cm^{-1}, 3190, 3010, 2136, 1686, 1560, 1489, 1458, 1379, 1350, 1294, 1189, 1104, 998, 946, 917. − MS (*m/z*): 478 (M − 1). − HPLC t_R: 20.24 min.

cyclo-(5-Chlorosaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)-phosphate (**4b**): 0.167 g of 5-chlorosalicylchlorophosphane (**8b**) was used. − Yield: 0.145 g (84%). − R_f (TLC) = 0.44 (CH_2Cl_2/CH_3OH, 9:1); 0.65 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.33 (s, 1 H, N-H), 7.45 (m, 1 H, H-6$_{thymine}$), 7.42 (m, 2 H, H-4$_{aryl}$ and H-6), 7.17 (d, 1 H, $^3J_{HH}$ = 9.1 Hz, H-3$_{aryl}$), 6.10, 6.09 (2 t, 1 H, $^3J_{HH}$ = 6.6 Hz, 2× H-1′), 5.52 (dd, 1 H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 5.8 Hz, $^3J_{HP}$ = 17.9 Hz, H_{benzyl}), 5.56 (dd, 1 H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 4.9 Hz, $^3J_{HP}$ = 9.3 Hz, H_{benzyl}), 4.45 (m, 1 H, H-3′), 4.38 (dd, 1 H, $^2J_{HH}$ = 11.6 Hz, $^3J_{HH}$ = 5.1 Hz, $^3J_{HP}$ = 2.4 Hz, H-5″), 4.32 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 5.2 Hz, $^3J_{HP}$ = 7.6 Hz, H-5′), 3.98 (m, 1 H, H-4′), 2.41 (m, 1 H, H-2″), 2.32 (m, 1 H, H-2′), 1.75 (2 d, 3 H, $^4J_{HH}$ = 1.0 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.61 (C-4), 150.3 (C-2), 148.23, 148.16 (2 d, C-2$_{aryl}$), 135.9 (C-6), 129.5 (C-6$_{aryl}$), 128.3 (C-5$_{aryl}$), 125.99 (C-4$_{aryl}$), 123.00 (2 d, C-1$_{aryl}$), 122.95, 122.84 (2 s, C-1$_{aryl}$), 120.14, 120.00 (2 s, C-3$_{aryl}$), 110.31, 110.28 (C-5), 83.80, 83.31 (C-1′), 81.02, 80.92 (C-4′), 68.11, 68.00 (2 d, C_{benzyl}), 67.03 (m, C-5′), 59.73, 59.52 (C-3′), 35.56, 35.46 (C-2′), 12.1, 12.0 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −9.07 and −9.18 [2 s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 266 nm (9800), λ_{min} (ε) = 234 nm (4400). − IR (KBr): $\tilde{\nu}$ = 3480 cm^{-1}, 3210, 3020, 2130, 1686, 1489, 1458, 1379, 1294, 1189, 1104, 1020, 998, 946. − MS (*m/z*): 468.5/470.5 (M − 1, ^{35}Cl/M − 1, ^{37}Cl; ratio 3:1). − HPLC t_R: 21.31 min.

cyclo-Saligenyl-5′-O-(3′-azido-3′-deoxythymidinyl)phosphate (**4c**): 0.141 g of salicylchlorophosphane (**8c**) was used. − Yield: 0.112 g (69%). − R_f (TLC) = 0.36 (CH_2Cl_2/CH_3OH, 9:1); 0.51 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.36 (s, 1 H, N-H), 7.45, 7.43 (2 d, 1 H, $^4J_{HH}$ = 1.0 Hz, H-6$_{aryl}$), 7.37 (tt, 1 H, $^3J_{HH}$ = 7.3 Hz, $^4J_{HH}$ = 1.0 Hz, H-4$_{aryl}$), 7.27 (d, 1 H, $^3J_{HH}$ = 7.6 Hz, H-6$_{thymine}$), 7.19 (tt, 1 H, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 1.0 Hz, H-5$_{aryl}$), 7.12 (d, 1 H, $^3J_{HH}$ = 7.8 Hz, H-3$_{aryl}$), 6.10 (t, 1 H, $^3J_{HH}$ = 6.7 Hz, H-1′), 5.51 (ddd, 1 H, $^2J_{HH}$ = 14.7 Hz, $^3J_{HH}$ = 5.3 Hz, $^3J_{HP}$ = 17.3 Hz, H_{benzyl}), 5.46 (ddd, 1 H, $^2J_{HH}$ = 14.7 Hz, $^3J_{HH}$ = 5.1 Hz, $^3J_{HP}$ = 9.6 Hz, H_{benzyl}), 4.45 (m, 1 H, H-3′), 4.38 (dd, 1 H, $^2J_{HH}$ = 11.1 Hz, $^3J_{HH}$ = 7.06 Hz, $^3J_{HP}$ = 4.6 Hz, H-5″), 4.31 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 5.15 Hz, $^3J_{HP}$ = 9.6 Hz, H-5′), 3.98 (m, 1 H, H-4′), 2.38 (m, 1 H, H-2″), 2.32 (m, 1 H, H-2′), 1.75, 1.72 (2 d, 3 H, $^4J_{HH}$ = 0.9 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.61 (C-4), 150.3 (C-2), 145.50 (2 s, $^3J_{CP}$ = 7.2 Hz, C-2$_{aryl}$), 135.9 (C-6), 126.2 (C-6$_{aryl}$), 124.6 (C-5$_{aryl}$), 129.92 (C-4$_{aryl}$), 121.11 (2 s, $^3J_{CP}$ = 9.9 Hz, C-1$_{aryl}$), 118.31 (2 s, $^3J_{CP}$ = 8.8 Hz, C-3$_{aryl}$), 110.31, 110.28 (C-5), 83.9 (C-1′), 81.0 (C-4′), 68.11, 68.60 (2 d, C_{benzyl}), 68.2 (m, C-5′), 59.7 (C-3′), 35.56, 35.6 (C-2′), 12.0 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −8.59 and −8.62 [2× s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 265 nm (9800), λ_{min} (ε) = 234 nm (4600). − IR (KBr): $\tilde{\nu}$ = 3520 cm^{-1}, 3230, 3020, 2140, 1684, 1489, 1458, 1379, 1290,

1191, 1106, 1020, 998, 946. − MS (*m/z*): 434.5 (M − 1). − HPLC t_R: 19.03 min.

cyclo-(5-Methoxysaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)-phosphate (**4d**): 0.164 g of 5-methoxysalicylchlorophosphane (**8d**) was used. − Yield: 0.125 g (73%). − R_f (TLC) = 0.36 (CH_2Cl_2/CH_3OH, 9:1); 0.51 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.35 (s, 1 H, N-H), 7.42, 7.44 (2 d, 1 H, $^4J_{HH}$ = 1.1 Hz, H-6$_{thymine}$), 7.07 (d, 1 H, $^3J_{HH}$ = 9.0 Hz, H-4$_{aryl}$), 6.90 (m, 1 H, H-3$_{aryl}$), 6.86 (d, 1 H, $^4J_{HH}$ = 3.0 Hz, H-6$_{aryl}$), 6.10 (t, 1 H, $^3J_{HH}$ = 6.7 Hz, H-1′), 5.44 (ddd, 1 H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 6.1 Hz, $^3J_{HP}$ = 16.9 Hz, H$_{benzyl}$), 5.46 (ddd, 1 H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 4.5 Hz, $^3J_{HP}$ = 9.7 Hz, H$_{benzyl}$), 4.44 (m, 1 H, H-3′), 4.36 (dd, 1 H, $^2J_{HH}$ = 11.1 Hz, $^3J_{HH}$ = 7.06 Hz, $^3J_{HP}$ = 4.6 Hz, H-5″), 4.31 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 5.15 Hz, $^3J_{HP}$ = 9.6 Hz, H-5′), 3.98 (m, 1 H, H-4′), 3.72 (2 s, 3 H, OCH_3), 2.39 (m, 1 H, H-2″), 2.31 (m, 1 H, H-2′), 1.75, 1.70 (2 d, 3 H, $^4J_{HH}$ = 1.1 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.63 (C-4), 155.64 (C-5$_{aryl}$), 150.35 (C-2), 143.20, 142.93 (2 s, $^3J_{CP}$ = 7.2 Hz, C-2$_{aryl}$), 135.87 (C-6), 129.92 (C-4$_{aryl}$), 121.81, 121.65 (2 s, $^3J_{CP}$ = 9.9 Hz, C-1$_{aryl}$), 119.11, 118.97 (2 s, $^3J_{CP}$ = 8.8 Hz, C-3$_{aryl}$), 115.11 (C-6$_{aryl}$), 110.65, 109.94 (C-5), 83.81, 83.70 (C-1′), 81.15, 81.00 (C-4′), 68.63, 68.55 (2 d, C$_{benzyl}$), 66.87 (m, C-5′), 59.83, 59.60 (C-3′), 55.56 (OCH_3), 35.60, 35.48 (C-2′), 12.04, 12.00 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −8.41 and −8.45 [2 s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 267 nm (10200), λ_{min} (ε) = 232 nm (4000). − IR (KBr): $\tilde{\nu}$ = 3620 cm^{-1}, 3250, 3030, 2140, 1686, 1497, 1466, 1363, 1287, 1266, 1199, 1101, 1024, 994, 948, 915. − MS (*m/z*): 464.5 (M − 1). − HPLC t_R: 19.75 min.

cyclo-(3-Methoxysaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)-phosphate (**4e**): 0.164 g of 3-methoxysalicylchlorophosphane (**8e**) was used. − Yield: 0.109 g (63%). − R_f (TLC) = 0.52 (CH_2Cl_2/CH_3OH, 9:1); 0.61 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.33 (s, 1 H, N-H), 7.44, 7.40 (2 d, 1 H, $^4J_{HH}$ = 1.2 Hz, H-6$_{thymine}$), 7.12 (2 t, 1 H, $^4J_{HH}$ = 8.1 Hz, H-5$_{aryl}$), 7.09 (d, 1 H, $^3J_{HH}$ = 8.3 Hz, H-4$_{aryl}$), 6.81 (2 d, 1 H, H-6$_{aryl}$), 6.10 (t, 1 H, $^3J_{HH}$ = 6.6 Hz, H-1′), 5.49 (ddd, 1 H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 6.7 Hz, $^3J_{HP}$ = 17.7 Hz, H$_{benzyl}$), 5.43 (ddd, 1 H, $^2J_{HH}$ = 14.4 Hz, $^3J_{HH}$ = 4.7 Hz, $^3J_{HP}$ = 9.8 Hz, H$_{benzyl}$), 4.44 (m, 1 H, H-3′), 4.36 (dd, 1 H, $^2J_{HH}$ = 11.4 Hz, $^3J_{HH}$ = 3.5 Hz, $^3J_{HP}$ = 7.0 Hz, H-5″), 4.31 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 4.8 Hz, $^3J_{HP}$ = 7.6 Hz, H-5′), 3.98 (m, 1 H, H-4′), 3.81 (2 s, 3 H, OCH_3), 2.37 (m, 1 H, H-2″), 2.33 (m, 1 H, H-2′), 1.74, 1.70 (2 d, 3 H, $^4J_{HH}$ = 1.1 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.62 (C-4), 150.36 (C-2), 148.29, 148.18 (2 s, $^3J_{CP}$ = 7.2 Hz, C-2$_{aryl}$), 138.44 (C-3$_{aryl}$), 135.85, 135.72 (C-6), 124.61 (C-4$_{aryl}$), 121.95, 121.88 (2 s, $^3J_{CP}$ = 9.9 Hz, C-1$_{aryl}$), 119.11, 118.97 (2 s, $^3J_{CP}$ = 8.8 Hz, C-3$_{aryl}$), 116.95 (C-6$_{aryl}$), 112.58 (C-5$_{aryl}$), 109.98, 109.93 (C-5), 83.66 (C-1′), 81.12, 81.00 (C-4′), 68.47, (d, C$_{benzyl}$), 67.06 (m, C-5′), 59.75, 59.58 (C-3′), 55.85 (OCH_3), 35.65, 35.45 (C-2′), 11.94 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −8.12 and −8.16 [2 s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 266 nm (9200), λ_{min} (ε) = 233 nm (4200). − IR (KBr): $\tilde{\nu}$ = 3480 cm^{-1}, 3210, 3020, 2130, 1686, 1489, 1458, 1379, 1294, 1189, 1104, 1020, 998, 946. − MS (*m/z*): 464.4 (M − 1). − HPLC t_R: 18.87 min.

cyclo-(5-Methylsaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)-phosphate (**4f**): 0.152 g of 5-methylsalicylchlorophosphane (**8f**) was used. − Yield: 0.135 g (81%). − R_f (TLC) = 0.58 (CH_2Cl_2/CH_3OH, 9:1); 0.66 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.27 (s, 1 H, N-H), 7.43, 7.41 (2 d, 1 H, $^4J_{HH}$ = 1.1 Hz, H-6$_{thymine}$), 7.15 (d, 1 H, $^4J_{HH}$ = 8.4 Hz, H-3$_{aryl}$), 7.05 (s, 1 H, H-6$_{aryl}$), 7.00 (d, 1 H, $^4J_{HH}$ = 8.3 Hz, H-4$_{aryl}$), 6.09 (t, 1 H, $^3J_{HH}$ = 6.6 Hz, H-1′), 5.46 (ddd, 1 H, $^2J_{HH}$ = 14.2 Hz, $^3J_{HH}$ = 3.7 Hz, $^3J_{HP}$ = 17.6 Hz, H$_{benzyl}$), 5.40 (ddd, 1 H, $^2J_{HH}$ = 14.3 Hz, $^3J_{HH}$ = 5.1 Hz, $^3J_{HP}$ = 9.2 Hz, H$_{benzyl}$), 4.45 (m, 1 H, H-3′), 4.36 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 3.5 Hz, $^3J_{HP}$ = 4.6 Hz, H-5″), 4.31 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 5.0 Hz, $^3J_{HP}$ = 7.5 Hz, H-5′), 3.98 (m, 1 H, H-4′), 2.37 (m, 1 H, H-2″), 2.32 (m, 1 H, H-2′), 2.26 (s, 3 H, CH_3), 1.75, 1.71 (2 d, 3 H, $^4J_{HH}$ = 1.1 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.62 (C-4), 150.33 (C-2), 147.35, 147.22 (2 s, $^3J_{CP}$ = 7.2 Hz, C-2$_{aryl}$), 135.87, 135.80 (C-6), 133.70 (C-5$_{aryl}$), 130.08 (C-4$_{aryl}$), 126.10 (C-6$_{aryl}$), 120.61, 120.46 (2 s, $^3J_{CP}$ = 9.9 Hz, C-1$_{aryl}$), 117.94, 117.81 (2 s, $^3J_{CP}$ = 8.8 Hz, C-3$_{aryl}$), 109.93 (C-5), 83.77, 83.70 (C-1′), 81.10, 80.99 (C-4′), 68.58, 68.47, (d, C$_{benzyl}$), 66.95, 66.87 (m, C-5′), 59.75, 59.57 (C-3′), 35.60, 35.47 (C-2′), 20.13 ($CH_{3\text{-aryl}}$), 12.02, 11.97 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −8.52 and −8.55 [2 s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 265 nm (11200), λ_{min} (ε) = 236 nm (3500). − IR (KBr): $\tilde{\nu}$ = 3580 cm^{-1}, 3250, 3037, 2126, 1680, 1489, 1458, 1379, 1297, 1189, 1114, 1020, 998, 946. − MS (*m/z*): 448.5 (M − 1). − HPLC t_R: 20.77 min.

cyclo-(3-Methylsaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)-phosphate (**4g**): 0.152 g of 3-methylsalicylchlorophosphane (**8g**) was used. − Yield: 0.142 g (86%). − R_f (TLC) = 0.58 (CH_2Cl_2/CH_3OH, 9:1); 0.66 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.36 (s, 1 H, N-H), 7.43 (d, 1 H, $^4J_{HH}$ = 1.0 Hz, H-6$_{thymine}$), 7.24 (m, 1 H, H-4$_{aryl}$), 7.08 (m, 2 H, H-6 and 5$_{aryl}$), 7.00 (d, 1 H, $^4J_{HH}$ = 8.3 Hz, H-4$_{aryl}$), 6.10 (t, 1 H, $^3J_{HH}$ = 6.3 Hz, H-1′), 5.50 (ddd, 1 H, $^2J_{HH}$ = 14.4 Hz, $^3J_{HH}$ = 3.7 Hz, $^3J_{HP}$ = 17.3 Hz, H$_{benzyl}$), 5.43 (ddd, 1 H, $^2J_{HH}$ = 14.4 Hz, $^3J_{HH}$ = 5.1 Hz, $^3J_{HP}$ = 9.2 Hz, H$_{benzyl}$), 4.44 (m, 1 H, H-3′), 4.38 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 3.5 Hz, $^3J_{HP}$ = 4.6 Hz, H-5″), 4.31 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 5.0 Hz, $^3J_{HP}$ = 7.5 Hz, H-5′), 3.97 (m, 1 H, H-4′), 2.37 (m, 1 H, H-2″), 2.32 (m, 1 H, H-2′), 2.21, 2.20 (2 s, 3 H, CH_3), 1.73, 1.72 (2 d, 3 H, $^4J_{HH}$ = 1.0 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.63 (C-4), 150.35 (C-2), 147.95, 147.84 (2 s, $^3J_{CP}$ = 7.2 Hz, C-2$_{aryl}$), 135.90 (C-6), 130.89 (C-4$_{aryl}$), 126.91, 126.78 (2 s, $^3J_{CP}$ = 8.8 Hz, C-3$_{aryl}$), 123.98, 123.57 (C-5$_{aryl}$), 121.01 (C-6$_{aryl}$), 120.61, 120.46 (2 s, $^3J_{CP}$ = 9.9 Hz, C-1$_{aryl}$), 109.95 (C-5), 83.78, 83.68 (C-1′), 81.12, 81.00 (C-4′), 68.58, 68.50, (d, C$_{benzyl}$), 67.04 (m, C-5′), 59.71, 59.63 (C-3′), 35.58, 35.49 (C-2′), 14.84 ($CH_{3\text{-aryl}}$), 12.02 ($CH_{3\text{-thymine}}$). − ^{31}P NMR ([D$_6$]DMSO): δ = −7.92 and −7.99 [2 s, diastereomeric mixture]. − UV (acetonitrile): λ_{max} (ε) = 266 nm (10500), λ_{min} (ε) = 237 nm (4500). − IR (KBr): $\tilde{\nu}$ = 3380 cm^{-1}, 3240, 3020, 2133, 1686, 1489, 1458, 1379, 1291, 1268, 1191, 1106, 1020, 998, 946, 843, 809, 760, 721, 656. − MS (*m/z*): 448.4 (M − 1). − HPLC t_R: 20.31 min.

cyclo-(3,5-Dimethylsaligenyl)-5′-O-(3′-azido-3′-deoxythymidinyl)phosphate (**4h**): 0.163 g of 3,5-dimethylsalicylchlorophosphane (**8h**) was used. − Yield: 0.148 g (86%). − R_f (TLC) = 0.62 (CH_2Cl_2/CH_3OH, 9:1); 0.69 (ethyl acetate/CH_3OH, 8:2). − ^{1}H NMR ([D$_6$]DMSO): δ = 11.31, 11.30 (2 s, 1 H, N-H), 7.42 (d, 1 H, $^4J_{H,H}$ = 1.0 Hz, H-6$_{thymine}$), 7.03 (s, 1 H, H-4$_{aryl}$), 6.86 (s, 1 H, H-6$_{aryl}$), 6.09 (t, 1 H, $^3J_{HH}$ = 6.6 Hz, H-1′), 5.42 (ddd, 1 H, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 3.7 Hz, $^3J_{HP}$ = 17.5 Hz, H$_{benzyl}$), 5.37 (ddd, 1 H, $^2J_{HH}$ = 14.4 Hz, $^3J_{HH}$ = 5.1 Hz, $^3J_{HP}$ = 9.3 Hz, H$_{benzyl}$), 4.44 (m, 1 H, H-3′), 4.38 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 3.5 Hz, $^3J_{HP}$ = 4.6 Hz, H-5″), 4.27 (dd, 1 H, $^2J_{HH}$ = 11.5 Hz, $^3J_{HH}$ = 5.0 Hz, $^3J_{HP}$ = 7.5 Hz, H-5′), 3.97 (m, 1 H, H-4′), 2.38 (m, 1 H, H-2″), 2.31 (m, 1 H, H-2′), 2.22 (s, 3 H, CH_3), 2.167, 2.161 (2 s, 3 H, CH_3), 1.74, 1.72 (2 d, 3 H, $^4J_{H,H}$ = 1.0 Hz, $CH_{3\text{-thymine}}$). − ^{13}C NMR ([D$_6$]DMSO): δ = 163.62 (C-4), 150.35 (C-2), 145.95, 145.84 (2 s, $^3J_{CP}$ = 7.2 Hz, C-2$_{aryl}$), 135.89 (C-6), 130.87 (C-4$_{aryl}$), 126.91, 126.78 (2 s, $^3J_{CP}$ = 8.8 Hz, C-3$_{aryl}$), 123.98, 123.57 (C-5$_{aryl}$), 121.01 (C-6$_{aryl}$), 120.61, 120.46 (2 s, $^3J_{CP}$ = 9.9 Hz, C-1$_{aryl}$), 109.95 (C-5), 83.78, 83.68 (C-1′), 81.11, 81.02 (C-4′), 68.56, 68.48, (d, C$_{benzyl}$),

67.04 (m, C-5′), 59.73, 59.66 (C-3′), 35.58, 35.49 (C-2′), 20.56 ($CH_{3\text{-aryl}}$), 14.84 ($CH_{3\text{-aryl}}$), 12.02 ($CH_{3\text{-thymine}}$). – ^{31}P NMR ([D_6]DMSO): δ = −7.86 and −7.96 [2 s, diastereomeric mixture]. – UV (acetonitrile): λ_{max} (ε) = 266 nm (10500), λ_{min} (ε) = 237 nm (4500). – IR (KBr): $\tilde{\nu}$ = 3580 cm^{-1}, 3240, 3120, 2126, 1686, 1489, 1466, 1364, 1287, 1265, 1199, 1100, 1024, 994, 948, 914. – MS (*m/z*): 462.4 (M − 1). – HPLC t_R: 21.89 min.

Determination of the Partition Coefficients: Pa values were determined as follows: A sample of one of the compounds **4a−h** was dissolved in 1.0 ml of 1-octanol. To this solution, 1.0 ml of aqueous phosphate buffer solution (10 mmol, pH 6.8) was added. After mixing the phases extensively for 10 min. (vortex) and separation by centrifugation (3 min. at 9000 rpm), aliquots of each phase were analyzed by analytical HPLC [Merck Lichrocart column filled with Lichrospher 100 reversed-phase silica gel RP-18 (5 μm), gradient: 18−100% CH_3CN in water (0−16 min.), 100% CH_3CN (16−19 min.), 18% CH_3CN (19−31 min), flow rate 0.5 ml/min., UV detection at 269 nm]. By quantification of the peak areas, the *Pa* values were obtained from the quotient.

Kinetic Data: a) All aqueous buffers: 1.0 ml aqueous buffer (50 mM acetate buffer, pH 4.6; 50 mM TRIS buffer, pH 6.9; 50 mM phosphate buffer, pH 7.3 and 50 mM borate buffer, pH 8.9) was equilibrated at 37°C and mixed with 0.5 ml of a solution of 1.0 mg of compound **4a−h** in 1.2 ml H_2O (1.9 mM), also equilibrated at 37°C (final concentration of the hydrolysis mixture: 0.63 mM). To obtain the kinetic data, aliquots (100 μl) were removed from the hydrolysis mixture, the reaction was terminated by the addition of 5.0 μl acetic acid, and subsequently analyzed by analytical HPLC [Merck Lichrocart column filled with Lichrospher 100 reversed-phase silica gel RP-18 (5 μm), gradient: 18−100% CH_3CN in tetrabutylammonium phosphate, pH 3.8 (0−16 min.), 100% CH_3CN (16−20 min.), 18% CH_3CN (20−31 min.), 0.5 ml/min. flow rate, UV detection at 269 nm]. The degradation of **4** was followed by quantification of the peak areas in the HPLC chromatograms. The rate constants *k* were determined from the slopes of the logarithmic degradation curves of the title compounds. The half-lives ($t_{1/2}$) were calculated from the rate constants *k*.

b) RPMI-1640 (*R*oswell *P*ark *M*emorial *I*nstitute) culture medium containing 10% of heat-inactivated fetal calf serum (FCS): this medium was used instead of aqueous buffers and the data were collected in the same way.

Antiretroviral Evaluation: Human immunodeficiency virus type 1 [HIV-1 ($HTLV\text{-}III_B$)] was obtained from persistently HIV-infected H9 cells as described previously[32]. Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells. HIV-2 (ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France). CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD), and CEM/TK^- cells were a gift from Prof. S. Eriksson and Prof. A. Karlsson (Karolinska Institute, Stockholm, Sweden). CEM cells were infected with HIV as described previously[33]. Briefly, 4×10^5 cells/ml were infected with HIV-1 or HIV-2 at 100 $CCID_{50}$ (50% cell culture infective dose)/ml of cell suspension. Then, 100 μl of the infected cell suspension was transferred to a 96-well microtitre plate and mixed with 100 μl of appropriate diluted solutions of the test compounds. After 4 days, giant cell formation was recorded microscopically in the HIV-infected cell cultures. The 50% effective concentration (EC_{50}) and the 50% cytotoxic concentration (CC_{50}) of the test compounds were defined as the compound concentrations required to inhibit virus-induced cytopathicity by 50% or to reduce the number of viable cells in mock-infected cell cultures by 50%, respectively.

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