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Stereoselective Synthesis and Antiviral Activity of Methyl-Substituted *cyclo*Sal-Pronucleotides

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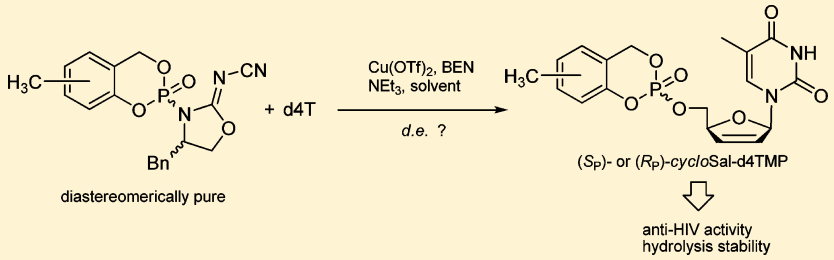
Supporting Information



ABSTRACT: Methyl-substituted *cyclo*Sal-pronucleotides of d4TMP were synthesized with high diastereoselectivities in satisfying chemical yields. The individual diastereomers were tested against HIV-1 and HIV-2 infected wild-type CEM/0 and HIV-2 infected thymidine kinase deficient CEM cells. All diastereomers tested showed significant antiviral activity in CEM/0 and strong activity in CEM/TK$^-$ cell cultures. The antiviral activities were strongly dependent on the chirality at the phosphate group and the position of the methyl-group(s) in the *cyclo*Sal moiety. In CEM/TK$^-$ cell cultures the difference in antiviral potency was found to be 7- to 20-fold. The stability of each diastereomer was studied in aqueous phosphate buffer and in CEM/0 cell extracts. Large differences in the half-lives were found. A comparison of the relative lipophilicity of the methyl-substituted *cyclo*Sal triesters was performed based on the retention times obtained by reversed phase HPLC. The results obtained clearly confirm the importance of a diastereoselective synthesis of *cyclo*Sal-pronucleotides.

INTRODUCTION

Several nucleoside analogues such as stavudine (d4T), zalcitabine (ddC), or zidovudine (AZT) are used as potent and selective reverse transcriptase inhibitors to combat human immunodeficiency virus (HIV) infections. The ultimately bioactive compounds of such nucleoside analogues are the corresponding 5′-triphosphates, which are formed intracellularly by three different cellular kinases. Often the first phosphorylation to the nucleotide is the rate-limiting step because of the specificity of the involved nucleoside kinases. If certain dideoxynucleoside analogues, e.g., 2′,3′-dideoxyuridine, are not active against HIV in cell culture under conditions in which others are, this is often due to their poor, or lack of, phosphorylation at the nucleoside kinase level.[1] However, the direct administration of the nucleoside mono-, di-, or triphosphate is not possible because of their high polarity which prevents cell membrane penetration. To bypass this limitation, several pronucleotide strategies[2] have been developed to mask nucleotides, thus enabling their passage through the membrane. Inside the cells the prodrugs need to undergo chemical or enzymatic transformation to the phosphorylated metabolite.[3] Some of these pronucleotides are P-chiral compounds.[4−6] In those cases in which the diastereomers could be stereoselectively synthesized[4a,b,5d,e] or could be separated by means of HPLC[5c,f,g] a significant difference in the antiviral activity of the single diastereomers was observed depending on the configuration of the phosphorus atom.[5d,e] In the past, the synthesis, hydrolysis, and antiviral evaluation of a large number of *cyclo*Sal-pronucleotides were reported.[4c,g,l] They were always synthesized as 1:1 diastereomeric mixtures. In a very few cases only, the individual diastereomers were obtained by means of semipreparative (RP)-HPLC. Recently, we reported on two approaches for the stereoselective synthesis of *cyclo*Sal-pronucleotides using chiral leaving groups.[4a,b] Here, we report on stereochemical syntheses, antiviral activity, and stability of several methyl-substituted *cyclo*Sal-pronucleotides.

RESULTS AND DISCUSSION

Chemistry. Since the stereoselective synthesis of 3-methyl -substituted *cyclo*Sal derivatives failed when using the linear reaction sequence reported before,[4b] the synthesis of all title compounds was carried out using a convergent synthesis pathway based on phosphorus(V) chemistry (Scheme 1). As chiral leaving groups, *N*-cyaniminooxazolidines derived from L-phenylalanine and the non-natural isomer D-phenylalanine were used. The unsubstituted *N*-cyaniminothiazolidine was found to be a good leaving group in phosphorylation reactions of

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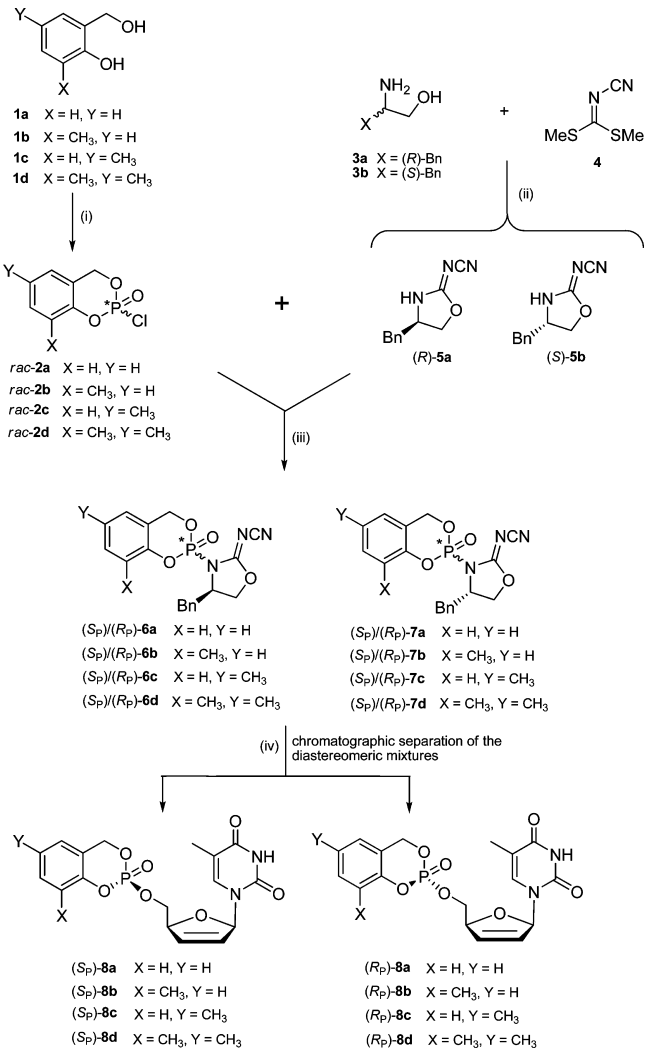
Scheme 1. Stereoselective Synthesis of *cyclo*Sal-Phosphate Triesters[a]



[a]Reagents and conditions: (i) $POCl_3$, THF, NEt_3, −70 °C, 1 h, −50 °C, 1 h, rt, 4 h; (ii) CH_3OH, 65 °C, 2 h; (iii) CH_2Cl_2, NEt_3, rt, 7 h; (iv) **6a−d** (S_P) or (R_P), **7a−d** (S_P) or (R_P), d4T, $Cu(OTf)_2$, BEN, solvent, NEt_3, rt, 5 days.

alcohols,[7] and it was easily prepared according to the procedure of Maienfisch.[8] Starting from salicyl alcohols **1a−d**, a cyclization with phosphorus oxychloride was performed to obtain the corresponding racemic *cyclo*Sal derivatives *rac*-**2a** to *rac*-**2d**. These compounds were then reacted with the chiral leaving groups (*R*)-**5a** and (*S*)-**5b** to give the corresponding 1:1 diastereomeric mixtures (S_P)/(R_P)-**6a−d** and (S_P)/(R_P)-**7a−d** which could be easily separated by means of column chromatography into the single diastereomers. Each pure diastereomer was finally reacted with 3′-deoxy-2′,3′-didehydrothymidine (d4T) as nucleoside analogue to give compounds (S_P)- or (R_P)-**8a−d**. In all cases copper(II) triflate [$Cu(OTf)_2$] in combination with *N*,*N*′-ethylenebis(benzaldiimine) (BEN) was used as described before.[4a,9] By a change of the order of addition of the reagents in the last reaction step, each single diastereomer of (S_P)/(R_P)-**6a−d** and (S_P)/(R_P)-**7a−d** was converted to the corresponding *cyclo*Sal-phosphate triester in good to excellent diastereomeric excess and in dependence on the solvent. All reactions were stopped after 5 days even if unreacted starting material was detected by means of thin layer chromatography.

The determination of the obtained diastereospecificity was done by means of ^1H or ^{31}P NMR spectroscopy or analytical HPLC, since not all phosphate triesters showed completely separated signals in the used analytical methods. However, in those cases in which the two phosphate triesters displayed separate signals in all three methods, identical diastereomeric excesses were determined. Because of the lack of a single-crystal X-ray structure of one of the title compounds, the absolute assignment of the configuration on the phosphorus atom could not be made. However, Gisch[4k] et al. crystallized a similar *cyclo*Sal-phosphate triester of d4T and assigned the configuration by X-ray analysis, in that case the R_P configuration. In that report, the (R_P)-diastereomer showed a lower antiviral activity against HIV-1 and HIV-2 infected CEM/0 and HIV-2-infected CEM/TK^- cells compared to its (S_P)-counterpart. This may be explained by different membrane permeabilities of the single diastereomers. Evidence for that was recently published by Kortylewicz[10] et al. by in vitro uptake kinetic studies of radiolabeled cyclosaligenyl monophosphates of 5-iodo-2′-deoxyuridine in LS 174T and OVCAR-3 cancer cell lines. Remarkable differences in the inhibitory potency of both diastereomers against BChE were also found previously by us as well as in the recent report.[4h,10] According to this, we assigned the (R_P)-configuration to the less active *cyclo*Sal-phosphate triester. Assuming that the formation of the (R_P)-configurated compound took place following an addition−elimination mechanism with inversion of configuration,[11] the corresponding starting material should have (S_P)-configuration and vice versa.

In order to determine the dependence of the diastereomeric excess on the solvent, *cyclo*Sal-phosphate triesters were synthesized using the isomerically pure compounds (S_P)- or (R_P)-**6a−d** (Scheme 1). First, *cyclo*Sal triesters (S_P)- and (R_P)-**6a** were used as model compounds and reacted with d4T under reaction conditions described above (Scheme 1, step iv). The used solvents, the observed diastereomeric excess (de), and the corresponding yields are summarized in Table 1.

Table 1. Yield and solvent dependency on the diastereomeric excess

starting material	solvent	% de[a]	yield[b] (%)	product
(R_P)-**6a**	THF/CH_3CN (1:1)	94	26	(S_P)-**8a**
	THF	94	6	
	CH_3CN	92	27	
	CH_2Cl_2	84	22	
(S_P)-**6a**	THF/CH_3CN (1:1)	91	19	(R_P)-**8a**
	THF	90	15	
	CH_3CN	84	13	
	CH_2Cl_2	74	23	

[a]% de determined by ^1H NMR after purification. [b]Isolated yields.

Since diastereomers (S_P)/(R_P)-**8a** displayed two closely spaced signals in the ^{31}P NMR spectrum, the diastereomeric excess was determined by ^1H NMR spectroscopy after column chromatography. No separation of the diastereomers or enrichment of one diastereomer by means of column chromatography was possible so that the determination of the de values by ^1H NMR is reliable. Notably both diastereomers (S_P)- and (R_P)-**6a** led to the corresponding *cyclo*Sal compounds (S_P)- and (R_P)-**8a** in different de values in

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dependence on the solvent. Best results were obtained with 1:1 THF/CH_3CN. The use of this solvent mixture for stereoselective reactions was previously reported[4b,5d] but with *tert*-butylmagnesium chloride as base. However, the use of this base for the last reaction step using *N*-cyaniminooxazolidine as chiral leaving group was found to be unsuitable because of a loss of diastereomeric excess.[4a]

No final explanation for the observed isomerization can be given, but a plausible argument was made in a previously report.[4a] In order to prove the suitability of the 1:1 THF/CH_3CN mixture for the last reaction step diastereomers, (S_P)- and (R_P)-**8b** were synthesized using both this solvent mixture and CH_3CN. The reaction in THF was very slow, and a lot of unreacted starting material was detected even after 5 days of reaction time. Again, best de values were obtained in 1:1 THF/CH_3CN (Table 2). With the finding of an appropriate solvent for the stereoselective synthesis of *cyclo*Sal compounds, methyl-substituted *cyclo*Sal-phosphate triesters (S_P)- and (R_P)-**8c,d** were then synthesized (Table 2).

Table 2. Diastereomeric Excess of (S_P)- and (R_P)-8b in CH_3CN and (S_P)- and (R_P)-8b−d in THF/CH_3CN (1:1)

starting material	% de	yield[d] (%)	product
(R_P)-**6b**[a]	91[b]	nd[e]	(S_P)-**8b**
(S_P)-**6b**[a]	82[b]	nd[e]	(R_P)-**8b**
(R_P)-**6b**	95[b]	23	(S_P)-**8b**
(S_P)-**6b**	94[b]	20	(R_P)-**8b**
(R_P)-**6c**	95[c]	26	(S_P)-**8c**
(S_P)-**6c**	92[c]	21	(R_P)-**8c**
(R_P)-**6d**	94[b]	21	(S_P)-**8d**
(S_P)-**6d**	95[b]	29	(R_P)-**8d**

[a]CH_3CN as solvent. [b]% de determined by ^{31}P NMR spectroscopy of the crude mixture. [c]% de determined by ^{1}H NMR after purification. [d]Isolated yields. [e]nd: not determined.

In almost all cases very high diastereomeric excesses were obtained in THF/CH_3CN (1:1). Only in the case of (S_P)-**6c** a slightly higher isomerization was observed.

The title compounds (S_P)- and (R_P)-**8a−d** were also synthesized from the corresponding isomerically pure diastereomers (S_P)- and (R_P)-**7a−d** which are the mirror images of the used starting materials (R_P)- and (S_P)-**6a−d** (Table 3).

As expected, high diastereomeric excesses were also observed using isomerically pure compounds (S_P)- or (R_P)-**7a−d**. However, the stereospecific synthesis of the title compounds (S_P)- and (R_P)-**8a−d** could be carried out by using the appropriate isomerically pure diastereomer (S_P)- and (R_P)-**6a−d** or (S_P)- and (R_P)-**7a−d** as starting material with very high diastereoselectivities. Interestingly, it was found that the (R_P)-*cyclo*Sal triesters **8a−d** were favorably obtained with higher diastereomeric excesses using (S_P)-**7a−d** as starting materials. On the other hand (R_P)-**6a−d** isomers led to the (S_P)-*cyclo*Sal triesters **8a−d** in higher diastereoselectivities.

Table 3. Diastereomeric Excess of (S_P)- and (R_P)-8a−d in THF/CH_3CN (1:1) with (S_P)- and (R_P)-7a−d as Starting Materials

starting material	% de	yield[c] (%)	product
(R_P)-**7a**	94[a]	19	(S_P)-**8a**
(S_P)-**7a**	96[a]	12	(R_P)-**8a**
(R_P)-**7b**	90[b]	33	(S_P)-**8b**
(S_P)-**7b**	96[b]	27	(R_P)-**8b**
(R_P)-**7c**	88[a]	19	(S_P)-**8c**
(S_P)-**7c**	94[a]	24	(R_P)-**8c**
(R_P)-**7d**	86[b]	16	(S_P)-**8d**
(S_P)-**7d**	97[b]	32	(R_P)-**8d**

[a]% de determined by ^{1}H NMR after purification. [b]% de determined by ^{31}P NMR spectroscopy of the crude mixture. [c]Isolated yields.

Lipophilicity. A qualitative measure of the lipophilicity of the unsubstituted and the different methyl-substituted *cyclo*Sal-phosphate triesters (S_P)- and (R_P)-**8a−d** and the parent nucleoside d4T was done by means of reversed phase (RP)-HPLC. The obtained retention times reflect the relative lipophilicity of the various derivatives (see Supporting Information). All *cyclo*Sal triesters are markedly more lipophilic than the parent nucleoside d4T. As expected, the presence of lipophilic groups as a methyl substituent increased the lipophilicity of the corresponding *cyclo*Sal triesters. Therefore, the unsubstituted *cyclo*Sal triesters (S_P)- and (R_P)-**8a** showed the lowest lipophilicity while the 3,5-dimethyl *cyclo*Sal triesters (S_P)- and (R_P)-**8d** showed the highest lipophilicity. It was shown that the 5-methyl substituted *cyclo*Sal triesters (S_P)- and (R_P)-**8c** were more lipophilic than their 3-methyl counterparts (S_P)- and (R_P)-**8b**. Additionally, in contrast to (S_P)- and (R_P)-**8a** and (S_P)- and (R_P)-**8c**, the 3-methyl-substituted (S_P)- and (R_P)-**8b** and the 3,5-dimethyl-substituted *cyclo*Sal triesters (S_P)- and (R_P)-**8d** showed good separation of the corresponding isomers. In both cases the slow-eluting diastereomer has (S_P)-configuration.

Chemical Hydrolysis Studies. The title compounds (S_P)- and (R_P)-**8a−d** were studied for their hydrolytic stability in aqueous 25 mM phosphate buffer, pH 7.3, and in CEM cell extracts (pH 6.9) as well as evaluated for their anti-HIV activity in vitro (Table 4). The final products of this pH-driven hydrolysis mechanism were exclusively d4TMP and the corresponding salicyl alcohol.[4c]

As expected, the unsubstituted *cyclo*Sal compounds (S_P)- and (R_P)-**8a** showed the lowest hydrolysis stability while the dimethyl-substituted triesters (S_P)- and (R_P)-**8d** showed the highest $t_{1/2}$, confirming the additional stabilizing effect caused by the methyl substituents in the aromatic ring due to the electron-donating properties. In addition, the individual diastereomers of 3-methyl-substituted *cyclo*Sal triesters (S_P)-**8b** and (R_P)-**8b** were found to be more stable against chemical hydrolysis than the 5-methyl-substituted counterparts (S_P)-**8c** and (R_P)-**8c**, showing that a methyl group in position 3 of the aromatic ring led to an additional significant increase in hydrolysis half-life.

Equally substituted diastereomers showed significant differences in the half-lives, e.g., $t_{1/2}$ = 6.8 h for (S_P)-**8a** and $t_{1/2}$ = 2.2 h for (R_P)-**8a**. The (S_P)-configurated isomer was the more stable one in all cases. This proves that the chemical stability of the *cyclo*Sal triesters is dependent on the absolute configuration at the phosphorus atom.

Enzymatic Hydrolysis Studies. In all cases the hydrolysis half-lives in phosphate buffer and in CEM cell extracts were found more or less comparable which confirmed the initial idea that the delivery mechanism is relatively independent of enzymatic activation (the correlation coefficient of the $t_{1/2}$ values for all eight **8a**, **8b**, **8c**, and **8d** diastereomers in PBS versus CEM cell extracts was r = 0.918, Supporting Information).[4c] The (S_P)-configurated isomers were invariably more stable compared to the (R_P)-isomers in the studies in both phosphate buffer pH 7.3 and the CEM cell extracts, pH

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Table 4. Hydrolysis Data and Antiviral Activity of (S_P)- and (R_P)-8a−d Compared to d4T

	$t_{1/2}$ (h)[a]		EC_{50} (μM)[b]			
			CEM/0[f]		CEM/TK$^-$[g]	
compd	PBS[d] pH 7.3	CE[e]	HIV-1	HIV-2	HIV-2	CC_{50} (μM)[c]
(S_P)-**8a**	6.8	9.3	0.16 ± 0.028	0.34 ± 0.092	0.35 ± 0.27	70 ± 16
(R_P)-**8a**	2.2	4.2	0.24 ± 0.078	1.1 ± 0.29	2.6 ± 3.1	140 ± 59
(S_P)-**8b**	22.1	18	0.09 ± 0.085	0.17 ± 0.028	0.16 ± 0.11	39 ± 2.1
(R_P)-**8b**	9.2	8.7	0.48 ± 0.35	1.1 ± 0.30	3.2 ± 2.6	179 ± 69
(S_P)-**8c**	11.5	14.8	0.13 ± 0.0	0.35 ± 0.26	0.21 ± 0.11	57 ± 23
(R_P)-**8c**	3.3	10.9	0.18 ± 0.071	2.1 ± 0.78	3.1 ± 2.5	143 ± 15
(S_P)-**8d**	34.3	27.6	0.12 ± 0.049	0.22 ± 0.042	0.18 ± 0.13	60 ± 3.5
(R_P)-**8d**	13.7	19.8	0.40 ± 0.18	1.1 ± 0.17	2.7 ± 0.92	120 ± 3.5
d4T	na[h]	na[h]	0.78 ± 0.16	1.3 ± 0.14	150 ± 141	>250

[a]Hydrolysis half-lives. [b]Antiviral activity in T-lymphocytes: 50% effective concentration. [c]Cytostatic activity: 50% cytostatic concentration. [d]25 mM phosphate buffer. [e]CEM cell extracts (pH 6.9). [f]Wild-type CEM/0 cells. [g]Thymidine kinase deficient CEM/TK$^-$ cells. [h]na: not applicable.

7.4. The differences in the half-lives of the isomers in each pair of diastereomers in the CEM cell extracts tended to be somewhat lower than the differences of the same compounds in the phosphate buffer, e.g., $t_{1/2}$ = 9.3 h for (S_P)-**8a** and $t_{1/2}$ = 4.2 h for (R_P)-**8a** (2-fold difference) versus $t_{1/2}$ = 6.8 h for (S_P)-**8a** and $t_{1/2}$ = 2.2 h for (R_P)-**8b** (3-fold difference) (Table 4).

Antiviral Evaluation. All diastereomerically pure *cyclo*Sal triesters (S_P)- and (R_P)-**8a−d** and the parent nucleoside d4T were evaluated for their in vitro antiviral potency against HIV-1 and HIV-2 infected CEM cells. Data for HIV-2 in a mutant cell line (CEM/TK$^-$) were also included in order to prove the TK bypass (Table 4). Remarkably all (S_P)-configurated diastereomers showed a higher antiviral activity against HIV-1 and HIV-2 in wild-type CEM/0 cells and in mutant thymidine kinase-deficient cells (CEM/TK$^-$) than the (R_P)-configurated counterparts. Additionally, the (S_P)- and (R_P)-*cyclo*Sal compounds **8a−d** generally showed higher antiviral activity against HIV-1 in wild-type T-lymphocytic CEM/0 cultures compared to the parent nucleoside analogue d4T. More importantly, all (S_P)- and (R_P)-*cyclo*Sal compounds **8a−d** showed full retention of antiviral activity in CEM/TK$^-$ cell cultures and were found to be much more antivirally active than d4T, which lost its antiviral potency completely because of the lack of its bioactivating enzyme thymidine kinase. The efficient release of d4TMP from the corresponding *cyclo*Sal triesters was therefore confirmed in this assay system. In all cases a pronounced difference in the antiviral activity in CEM/TK$^-$ cells between the individual diastereomers (S_P)-**8a−d** and (R_P)-**8a−d** was found (7-fold for (S_P)-**8a** and (R_P)-**8a**, 20-fold for (S_P)-**8b** and (R_P)-**8b**, 15-fold for (S_P)-**8c** and (R_P)-**8c**, and 15-fold for (S_P)-**8d** and (R_P)-**8d**). These results confirm the importance of a diastereoselective synthesis of *cyclo*Sal-phosphate triesters. In all cases a correlation of the biological activity with the half-lives in phosphate buffer and in CEM cell extracts was observed. The (S_P)-configurated diastereomers showed higher hydrolysis stabilities and higher antiviral activities than the (R_P)-configurated counterparts. However, it was intriguing to observe that, although a shorter half-life correlated with a lower antiviral efficacy, for each diastereomeric pair of compounds, the absolute $t_{1/2}$ values of the compounds did not correlate with their antiviral efficacy (Supporting Information). For example, (R_P)-**8a** had the lowest $t_{1/2}$ and (R_P)-**8d** the highest $t_{1/2}$ among the (R_P) diastereomers, but they showed virtually identical anti-HIV activities. Likewise, among the (S_P)-diastereomers, (S_P)-**8a** had the lowest $t_{1/2}$ and (S_P)-**8d** the highest $t_{1/2}$, and yet their antiviral activities were quite similar. Thus, compound half-lives seem to consistently affect the antiviral activity (lower half-life results in a lower antiviral activity) within paired diastereomers, but this consistent observation cannot be made between diastereomers of different compounds. It may be likely that the eventual antiviral efficacy of the compounds is the complex result of different efficiencies in cellular uptake of pairs of diastereomeric compounds, combined with different chemical/cellular half-lives.

The antivirally more active (S_P)-diastereomers were consistently also somewhat more cytostatic (2- to 3-fold) than the corresponding (R_P)-diastereomers.

Differences in the antiviral activities between (S_P)- and (R_P)-diastereomers could also be observed for (aryloxy)-phosphoramidate prodrugs (up to 66-fold),[5d,e] and both the *cyclo*Sal and the (aryloxy)phosphoramidate approaches were able to release efficiently d4T monophosphate from the corresponding prodrug inside the cell.

CONCLUSION

In summary, three methyl-substituted and the unsubstituted *cyclo*Sal-phosphate triesters of d4T (S_P)- and (R_P)-**8a−d** were stereospecifically synthesized using a convergent synthetic route.[4a] Some isomerization of the configuration at the phosphorus atom of the products was observed in dependence on the solvent.

The lipophilicity of all *cyclo*Sal triesters (S_P)- and (R_P)-**8a−d** was compared with the lipophilicity of d4T according to the retention times obtained by means of (RP)-HPLC analysis. 3,5-Dimethyl-substituted *cyclo*Sal triesters showed the highest lipophilicity. The diastereomerically pure compounds were studied concerning their stability in phosphate buffer (pH 7.3) and in CEM cell extracts. In both hydrolysis media, all (S_P)-configurated isomers were found to be more stable than the (R_P)-configurated counterparts. The *cyclo*Sal compounds were tested against HIV-1- and HIV-2-infected CEM/0 and HIV-2-infected CEM/TK$^-$ cells. All title compounds proved to be potent inhibitors of HIV-1 and HIV-2 replication in wild-type CEM/0 cell cultures, showing significant biological activity for all (S_P)-configurated isomers that was superior to that found for the corresponding other diastereomer. The differences in the antiviral potency were found to be between 7-fold and 20-fold. Most important was that whereas the parent nucleoside d4T lost complete antiviral potency in CEM/TK$^-$ cells, all *cyclo*Sal

compounds retained significant antiviral activity. These results clearly demonstrate the dependence of the biological activity and the half-lives of *cyclo*Sal compounds on the configuration at the phosphorus center and consequently the importance of diastereospecific access to these compounds. For further studies only the (S_P)-diastereomers should be used because of the favorable antiviral properties. The (S_P)-*cyclo*Sal-d4TMPs should be prepared starting from (R_P)-**6a**–**d** in solvent CH_3CN/THF, 1:1, to give optimal diastereospecificities.

EXPERIMENTAL SECTION

General. All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions and nitrogen atmosphere. All solvents were dried over an appropriate drying agent. Triethylamine, dichloromethane, and acetonitrile were dried by heating under reflux over calcium hydride for several days followed by distillation. Dichlormethane was stored over activated 4 Å molecular sieves and acetonitrile over 3 Å molecular sieves. THF was dried by heating under reflux over potassium and benzophenone followed by distillation. Ethyl acetate, petroleum ether 50–70, dichloromethane, and methanol for chromatography were distilled before used. Evaporation of solvents was carried out on a rotary evaporator under reduced pressure or using a high-vacuum pump. Column chromatography was performed by using Merck silica gel 60, 230–400 mesh. 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, 0.1 mL of glacial acetic acid, and 0.5 mL of concentrated sulphuric acid) by heating with a fan. The coupling product of the *cyclo*Sal mask and the leaving groups was visualized with a solution of potassium permanganate and potassium carbonate in sodium hydroxide (1.5 g of $KMnO_4$, 10 g of K_2CO_3, 1.5 mL of 10% NaOH, and 200 mL of H_2O). NMR spectra were recorded on a 400 or 500 MHz spectrometer (Bruker AMX 400, Bruker AV 400, or a Bruker DRX 500). All ^1H and ^{13}C NMR chemical shifts are quoted in ppm and were calibrated on solvent signals. ^{31}P NMR chemical shifts are quoted in ppm using H_3PO_4 as external reference. All ^{13}C and ^{31}P NMR spectra were recorded in the proton-decoupled mode. High resolution mass spectra were obtained with a VG Analytical VG/70-250F spectrometer (FAB, matrix was *m*-nitrobenzyl alcohol). HR-ESI spectra were obtained with an Agilent Technologies ESI-TOF 6224 spectrometer. Analytical HPLC was performed on a VWR-Hitachi LaChromElite HPLC system consisting of a VWR-Hitachi L-2130 pump, autosampler, and a VWR-Hitachi UV detector L-2455. The columns used were a LiChroCART 125-3 column containing reversed phase silica gel LiChrospher 100 RP-18e (5 μm, Merck, Darmstadt, Germany) and a Nucleodur C18 Isis, 5 μm (Macherey-Nagel).

Elution was performed using a water/acetonitrile (Sigma-Aldrich, HPLC grade) eluent. Method I (for the determination of the half-lives) was used: 5–100% CH_3CN (0–25 min): 100–5% CH_3CN (25–30 min), 5% CH_3CN (30–35 min), a flow rate of 0.5 mL/min, and UV detection at 265 nm. Method II (for the determination of the lipophilicity): 5–100% CH_3CN (0–50 min), 100–5% CH_3CN (50–55 min), 5% CH_3CN (55–60 min), a flow rate of 0.5 mL/min, and UV detection at 265 nm. The purity of *cyclo*Sal-phosphate triesters (S_P)- and (R_P)-**8a**–**d** was checked by means of HPLC and was in all cases ≥95%.

General Procedure A: Preparation of 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-2-oxide Derivatives *rac*-2. A solution of the corresponding salicyl alcohol **1** (1.0 equiv) and triethylamine (2.1 equiv) in THF was added dropwise within 1 h to a stirred solution of $P(O)Cl_3$ (1.1 equiv) in THF at −70 °C. The mixture was allowed to warm to −50 °C and stirred for 1 h. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 4 h. The triethylammonium chloride was filtered. The solvent was removed under reduced pressure using a high-vacuum pump. The crude product was purified by column chromatography on silica gel (petroleum ether 50–70/ethyl acetate, 1:1).

General Procedure B: Preparation of the Leaving Groups (*R*)-5a, (*S*)-5b. The respective 2-amino alcohol (*R*)-**3a** or (*S*)-**3b** (1.0 equiv) was added to a solution of dimethyl cyanodithioiminocarbonate **4** (1.0 equiv) in methanol. The reaction mixture was heated for 3 h under reflux and stirred at room temperature for 15 h. The originating methanethiol was oxidized to methanesulfonic acid by using nitric acid. The product was filtered, washed with cold petroleum ether, and dried under reduced pressure.

General Procedure C: Preparation of the Diastereomeric Mixtures (S_P)/(R_P)-6a–d and (S_P)/(R_P)-7a–d. To a suspension of the leaving group (*R*)-**5a** or (*S*)-**5b** (1.0 equiv) and triethylamine (1.1 equiv) in dichloromethane was added a solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-2-oxide derivative *rac*-**2a**, *rac*-**2b**, *rac*-**2c**, or *rac*-**2d** (1.0–1.5 equiv) in dichloromethane at room temperature. The reaction mixture was stirred for 5–10 h at room temperature. The solvent was removed under reduced pressure using a high-vacuum pump. Ethyl acetate was added. The reaction mixture was stirred for 30 min at room temperature and stored 2 h at 0 °C. The precipitated salt was filtered. The solvent was removed and the crude product was purified by column chromatography on silica gel (petroleum ether 50–70/ethyl acetate, 1:2).

General Procedure D: Preparation of the *cyclo*Sal-Phosphate Triesters (S_P)/(R_P)-8a–d. Copper(II) triflate (1.0 equiv), BEN (1.0 equiv), and the isomerically pure diastereomer (1.0 equiv) were put in a flask under nitrogen atmosphere and dissolved in the corresponding solvent. The solution was then stirred for 30 min and added to a solution of d4T (2.5 equiv) and triethylamine (2.5 equiv) in the same solvent as before at room temperature. The reaction mixture was stirred for 5 days and quenched with saturated ammonium chloride solution and extracted with dichloromethane three times. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (dichloromethane/methanol, 19:1).

(R_P,4′R_C)- and (S_P,4′R_C)-2-(4′-Benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)/(R_P)-6a. General procedure C was used with (*R*)-4-benzyl-2-(*N*-cyanimino)oxazolidine (*R*)-**5a** (0.35 g, 1.74 mmol) in 20 mL of dichloromethane, 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (*rac*-**2a**, 0.53 g, 2.61 mmol) in 20 mL of dichloromethane, and 0.27 mL (0.19 g, 1.9 mmol) of triethylamine. The product (S_P)/(R_P)-**6a** (0.405 g, 64%) was obtained as a colorless foam as a diastereomeric mixture which was then separated by column chromatography.

(R_P)-6a. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.41–7.26 (H-aromat), 7.25–7.09 (H-aromat), 5.79 (H-4), 5.47 (H-4′), 4.81–4.71 (H-13), 4.58–4.50 (H-12), 4.50–4.45 (H-12′), 3.50 (H-14), 3.01 (H-14′). ^{31}P NMR (162 MHz, $CDCl_3$): δ = −14.35 ppm.

(S_P)-6a. ^1H NMR (400 MHz, $CDCl_3$): 7.39–7.23 (H-aromat), 7.23–7.17 (H-aromat), 7.16–7.11 (H-aromat), 7.10–7.05 (H-aromat), 5.87 (H-4), 5.43 (H-4′), 4.83–4.74 (H-13), 4.60–4.52 (H-12), 4.52–4.45 (H-12′), 3.40 (H-14), 2.99 (H-14′). ^{31}P NMR (162 MHz, $CDCl_3$): δ = −14.17 ppm.

(R_P,4′R_C)- and (S_P,4′R_C)-8-Methyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)/(R_P)-6b. General procedure C was used with (*R*)-4-benzyl-2-(*N*-cyanimino)oxazolidine (*R*)-**5a** (0.50 g, 2.48 mmol) in 20 mL of dichloromethane, 2-chloro-8-methyl-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (*rac*-**2b**, 0.810 g, 3.72 mmol) in 20 mL of dichloromethane, and 0.38 mL (0.276 g, 2.73 mmol) of triethylamine. The product (S_P)/(R_P)-**6b** (0.430 g, 45%) was obtained as a colorless foam as a diastereomeric mixture which was then separated by column chromatography.

(R_P)-6b. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.28 (H-aromat) 7.23 (H-7), 7.09 (H-6), 6.97 (H-5), 5.75 (H-4), 5.43 (H-4′), 4.82–4.74 (H-14), 4.55 (H-13), 4.52–4.47 (H-13′), 3.43 (H-15), 3.09 (H-15′), 2.34 (H-9) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −13.05 ppm.

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(S_P)-6b. ^{1}H NMR (400 MHz, $CDCl_3$): 7.40−7.24 (H-aromat), 7.22 (H-7), 7.09 (H-6), 6.97 (H-5), 5.85 (H-4), 5.39 (H-4′), 4.86−4.77 (H-14), 4.58 (H-13), 4.53−4.47 (H-13′), 3.43 (H-15), 2.99 (H-15′), 2.30 (H-9) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −12.87 ppm.

(R_P,4′R_C)- and (S_P,4′R_C)-6-Methyl-2-(4′-benzyl-2′-*N*-cyanimi-nooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)/(R_P)-6c. General procedure C was used with (*R*)-4-benzyl-2-(*N*-cyanimino)oxazolidine (*R*)-**5a** (0.50 g, 2.48 mmol) in 20 mL of dichloromethane, 2-chloro-6-methyl-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (*rac*-**2c**, 0.810 g, 3.72 mmol) in 20 mL of dichloromethane, and 0.38 mL (0.276 g, 2.73 mmol) of triethylamine. The product (S_P)/(R_P)-**6c** (0.503 g, 53%) was obtained as a colorless foam as a diastereomeric mixture which was then separated by column chromatography.

(R_P)-6c. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40−7.25 (H-aromat), 7.15 (H-7), 7.00 (H-8), 6.92 (H-5), 5.75 (H-4), 5.42 (H-4′), 4.79−4.70 (H-14), 4.52 (H-13), 4.50−4.44 (H-13′), 3.49 (H-15), 3.00 (H-15′), 2.35 (H-9) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −14.23 ppm.

(S_P)-6c. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40−7.21 (H-aromat), 7.14 (H-7), 7.00 (H-8), 6.92 (H-5), 5.83 (H-4), 5.38 (H-4′), 4.82−4.73 (H-14), 4.55 (H-13), 4.52−4.46 (H-13′), 3.41 (H-15), 3.40 (H-15′), 2.34 (H-9) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −14.04 ppm.

(R_P,4′R_C)- and (S_P,4′R_C)-6,8-Dimethyl-2-(4′-benzyl-2′-*N*-cya-niminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)/(R_P)-6d. General procedure C was used with (*R*)-4-benzyl-2-(*N*-cyanimino)oxazolidine (*R*)-**5a** (0.30 g, 1.49 mmol) in 12 mL of dichloromethane, 2-chloro-6,8-dimethyl-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (*rac*-**2d**, 0.52 g, 2.24 mmol) in 12 mL of dichloromethane, and 0.23 mL (0.16 g, 1.63 mmol) of triethylamine. The product (S_P)/(R_P)-**6d** (0.386 g, 65%) was obtained as a colorless foam as a diastereomeric mixture which was then separated by column chromatography.

(R_P)-6d. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40−7.26 (H-aromat), 7.02 (H-7), 6.75 (H-5), 5.70 (H-4), 5.38 (H-4′), 4.81−4.72 (H-13), 4.54 (H-12), 4.51−4.45 (H-12′), 3.43 (H-14), 3.07 (H-14′), 2.30 (H-20, H-21) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −12.97 ppm.

(S_P)-6d. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40−7.21 (H-aromat), 7.01 (H-7), 6.75 (H-5), 5.80 (H-4), 5.33 (H-4′), 4.84−4.75 (H-13), 4.57 (H-12), 4.53−4.47 (H-12′), 3.42 (H-14), 2.98 (H-14′), 2.30 (H-20), 2.25 (H-21) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −12.75 ppm.

***cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (S_P)-8a.** General procedure D was used with (4′R_C)-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (R_P)-**6a** (50 mg, 0.135 mmol), BEN (32 mg, 0.135 mmol), copper(II) triflate (49 mg, 0.135 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (76 mg, 0.34 mmol) and triethylamine (47 μL, 34 mg, 0.34 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (S_P)-**6a** (13.6 mg, 26%) was obtained as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (NH), 7.39−7.34 (H-12), 7.28 (H-14), 7.21 (H-13), 7.19−7.15 (H-6), 7.11 (H-11), 6.80−6.76 (H-1′), 6.38−6.34 (H-3′), 6.03−5.97 (H-2′), 5.55−5.34 (H-8), 4.99−4.92 (H-4′), 4.37−4.22 (H-5′), 1.62 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.37 ppm.

***cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (R_P)-8a.** General procedure D was used with (4′R_C)-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)-**6a** (50 mg, 0.135 mmol), BEN (32 mg, 0.135 mmol), copper(II) triflate (49 mg, 0.135 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (76 mg, 0.34 mmol) and triethylamine (47 μL, 34 mg, 0.34 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (R_P)-**8a** (9.8 mg, 19%) was obtained as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.35 (NH), 7.42−7.36 (H-12), 7.29 (H-14), 7.23−718 (H-13, H-6), 7.14 (H-11), 6.82−6.79 (H-1′), 6.45−6.41 (H-3′), 6.05−6.00 (H-2′), 5.55−5.35 (H-8), 4.99−4.92 (H-4′), 4.33−4.28 (H-5′), 1.68 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.34 ppm.

3-Methyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (S_P)-8b. General procedure D was used with (4′R_C)-8-methyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (R_P)-**6b** (50 mg, 0.13 mmol), BEN (31 mg, 0.13 mmol), copper(II) triflate (47 mg, 0.13 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (73 mg, 0.33 mmol) and triethylamine (46 μL, 33 mg, 0.33 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (S_P)-**8b** (11.6 mg, 23%) was obtained as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.35 (NH), 7.25 (H-12), 7.21−7.17 (H-6), 7.12−7.05 (H-13, H-14), 6.82−6.77 (H-1′), 6.38−6.33 (H-3′), 6.03−5.99 (H-2′), 5.47 (H-8a), 5.35 (H-8b), 4.97−4.91 (H-4′), 4.33−4.19 (H-5′), 2.18 (H-15), 1.62 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.77 ppm.

3-Methyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (R_P)-8b. General procedure D was used with (4′R_C)-8-methyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)-**6b** (50 mg, 0.13 mmol), BEN (31 mg, 0.13 mmol), copper(II) triflate (47 mg, 0.13 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (73 mg, 0.33 mmol) and triethylamine (46 μL, 33 mg, 0.33 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (R_P)-**8b** (10.6 mg, 20%) was obtained as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.33 (NH), 7.25 (H-12), 7.20−7.17 (H-6), 7.11−7.05 (H-13, H-14), 6.82−6.77 (H-1′), 6.43−6.39 (H-3′), 6.03−5.98 (H-2′), 5.45 (H-8a), 5.35 (H-8b), 4.98−4.91 (H-4′), 4.31−4.24 (H-5′), 2.21 (H-15), 1.65 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.57 ppm.

5-Methyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (S_P)-8c. General procedure D was used with (4′R_C)-6-methyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (R_P)-**6c** (50 mg, 0.13 mmol), BEN (31 mg, 0.13 mmol), copper(II) triflate (47 mg, 0.13 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (73 mg, 0.33 mmol) and triethylamine (46 μL, 33 mg, 0.33 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (S_P)-**8c** (13.8 mg, 26%) was obtained as a colorless foam.^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.32 (NH), 7.17−7.14 (H-6), 7.17−7.12 (H-12), 7.07 (H-14), 6.98 (H-11), 6.79−6.75 (H-1′), 6.38−6.33 (H-3′), 6.03−5.97 (H-2′), 5.44 (H-8a), 5.35 (H-8b), 4.98−4.91 (H-4′), 4.36−4.28 (H-5′), 2.26 (H-15), 1.62 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.32 ppm.

5-Methyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (R_P)-8c. General procedure D was used with (4′R_C)-6-methyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)-**6c** (32 mg, 0.084 mmol), BEN (20 mg, 0.084 mmol), copper(II) triflate (30 mg, 0.084 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (47 mg, 0.21 mmol) and triethylamine (29 μL, 21 mg, 0.21 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (R_P)-**8c** (7.1 mg, 21%) was obtained as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (NH), 7.20−7.17 (H-6), 7.19−7.15 (H-12), 7.07 (H-14), 7.01 (H-11), 6.81−6.77 (H-1′), 6.44−6.39 (H-3′), 6.04−5.98 (H-2′), 5.43 (H-8a), 5.33 (H-8b), 4.97−4.91 (H-4′), 4.30−4.25 (H-5′), 2.27 (H-15), 1.68 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.25 ppm.

3,5-Dimethyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (S_P)-8d. General procedure D was used with (4′R_C)-6,8-dimethyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (R_P)-**6d** (80 mg, 0.20 mmol), BEN (47 mg, 0.20 mmol), copper(II) triflate (72 mg, 0.20 mmol) in 4 mL of THF/CH_3CN (1:1 v/v) and d4T (0.11 g, 0.50 mmol) and triethylamine (69 μL, 50 mg, 0.50 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (S_P)-**8d** (17.2 mg, 21%) was obtained as a colorless foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.34 (NH), 7.20−7.18 (H-6), 7.04 (H-12), 6.89 (H-14), 6.81−6.77 (H-1′), 6.38−6.33 (H-3′), 6.03−5.98 (H-2′), 5.41 (H-8a), 5.31 (H-8b), 4.97−4.91(H-4′), 4.31−4.16 (H-5′), 2.22 (H-15), 2.14 (H-16), 1.62 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.75 ppm.

3,5-Dimethyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate (R_P)-8d. General procedure D was used with (4′R_C)-6,8-dimethyl-2-(4′-benzyl-2′-*N*-cyaniminooxazolidin-3′-yl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (S_P)-**6d** (32 mg, 0.081 mmol), BEN (19 mg, 0.081 mmol), copper(II) triflate (29 mg, 0.081 mmol) in

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4 mL of THF/CH_3CN (1:1 v/v) and d4T (45 mg, 0.20 mmol) and triethylamine (28 μL, 20 mg, 0.20 mmol) in 4 mL of THF/CH_3CN (1:1 v/v). The product (R_P)-**8d** (9.7 mg, 29%) was obtained as a colorless foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.33 (NH), 7.18−7.16 (H-6), 7.05 (H-12), 6.88 (H-14), 6.80−6.76 (H-1′), 6.43−6.38 (H-3′), 6.03−5.99 (H-2′), 5.40 (H-8a), 5.28 (H-8b), 4.97−4.91 (H-4′), 4.28−4.22 (H-5′), 2.22 (H-15), 2.17 (H-16), 1.65 (H-7) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.43 ppm.

Hydrolysis Studies of *cyclo*Sal-Phosphate Triesters. Hydrolysis studies of *cyclo*Sal triesters (phosphate buffer, pH 7.3) by reversed phase HPLC analysis have been done as described in ref 12. Studies in cell extracts were also performed as reported in ref 12 but with a 3.0 mM solution of the *cyclo*Sal-phosphate triester in DMSO and with different incubation times. The HPLC analysis was performed in both cases using method I described above.

Antiretroviral Evaluation. The method of antiviral evaluation has already been described in ref 4a and was based on the microscopical examination of virus-induced cytopathicity (giant cell formation) in CEM cell cultures after 4 days of virus and drug exposure.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC chromatogram of **8**, detailed analytical data for **2**, **6**, and **8**, and correlations of stability data in different media and anti-HIV-activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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