Intracellular Trapping of *cyclo*Sal-Pronucleotides: Modification of Prodrugs with Amino Acid Esters‡

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A new class of d4TMP-*cyclo*Sal-pronucleotides bearing enzymatically cleavable amino acid esters is reported. These compounds are designed to trap the pronucleotide inside the cell by a fast conversion of a nonpolar ester group into a charged carboxylate. This should prevent efficient diffusion equilibrium across the cell membrane to the extracellular environment, leading to an intracellular accumulation of the compounds. This initial conversion is followed by a slow release of the nucleoside monophosphate (i.e., d4TMP). The concept is proven by hydrolysis studies in phosphate buffer, cell extracts, and human serum. These investigations revealed a high sensitivity of some amino acid ester modifications to conversion by cellular extracts, resulting in the fast release of a charged intermediate, whereas no cleavage of the modification is found in phosphate buffer. In addition, antiviral activities against HIV are presented.

Introduction

Nucleoside analogues are commonly used as antiviral or antitumor agents.[1] The antiviral effect of nucleoside analogues, such as 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T, **1**), depends on their conversion into the ultimately bioactive triphosphates via mono- and diphosphate formation by cellular kinases. In the case of d4T, the metabolism-limiting step is the first phosphorylation catalyzed by the salvage pathway enzyme thymidine kinase (TK).[2,3] As a consequence, attempts to directly deliver d4T-monophosphate into intact cells would be warranted. Different lipophilic nucleotide-releasing systems (pronucleotides) were designed to achieve a passive membrane transport of the nucleotide.[4] Among these, *cyclo*Sal-pronucleotides were successfully used in the intracellular delivery of e.g. d4TMP (TK bypass).[5] This delivery depends on a chemically triggered cascade reaction.[6] The *cyclo*Sal[a] approach has also been applied to other nucleoside analogues, resulting in improved antiviral potency of the compounds.[7−9]Moreover, it was shown that this technology converts nonactive nucleoside analogues like 2′-ribofluoro-2′,3′-dideoxyadenosine[10] or 5-(*E*)-bromovinyl-2′-deoxyuridine (BVDU)[11]into potent antivirals or could expand the antiviral spectrum for other nucleoside analogues such as BVDU.[11] Likewise, *cyclo*Sal-acyclovirMP compounds entirely retain their antiviral activity against acyclovir-resistant HSV-1 strains.[12] The latter compounds also proved active against pox viruses, while the parent compound is nonactive.[13]

As a result of the the lipophilic character of *cyclo*Sal-phosphate triesters such as **2** (Figure 1) and the chemically triggered delivery mechanism, a drug concentration equilibrium across the cell membrane is formed (Figure 2). However, for a strong antiviral effect it is necessary to achieve high intracellular concentrations of the pronucleotide, which then presumably also leads to high concentrations of the released nucleotide. In order to efficiently trap the lipophilic prodrug, we designed the second generation of *cyclo*Sal-pronucleotides ("lock-in" *cyclo*Sal triesters).[14,15] These compounds are bearing a (carboxy)esterase-cleavable ester (trigger) attached to the aromatic ring. To avoid a considerable reduction of the chemical stability due to the electron-withdrawing effect of the ester moieties, a C_2-spacer separates the trigger from the aromatic system.

Two types of ester-bearing *cyclo*Sal triesters have been developed: the *cyclo*Sal-d4TMP acid ester and the *cyclo*Sal-d4TMP alcohol ester. As simple esters were not cleaved by esterases, more elaborate acylal systems were used to release the corresponding carboxylates. This approach was based on the acetoxymethyl (AM) and pivaloyloxymethyl (POM) modification. Hydrolysis studies in phosphate buffer (PBS, pH 7.3) and in T-lymphocyte CEM cell extracts showed that an effective intracellular trapping should be possible if highly polar *cyclo*Sal-d4TMP acids such as **4** (Figure 1) are released from *cyclo*Sal acid esters such as **3** (Figure 1). Chemical hydrolysis of **4** via the cascade reaction finally led to an intracellular d4TMP delivery.[14,15] The trapping concept was further proven by enrichment studies with intrinsically fluorescent *cyclo*Sal-pronucleotides using a U-tube setup.[16] However, the AM modification suffered from a low stability in RPMI/FCS incubation media, resulting in modest antiviral data. Although the POM modification proved to be more stable under these conditions, the release of a potentially cytotoxic pivaloic acid represents the main drawback.[5]

In this paper we disclose the synthesis, properties, and antiviral evaluation of a new series of compounds, based on the modification of *cyclo*Sal acid **4** with different amino acid esters. The possible cleavage mechanism is summarized in Figure 3. All metabolites display higher polarity compared to the parent pronucleotide, in principle preventing diffusion from the cytoplasm once they are formed. In this context we assume that a high stability of the pronucleotides in phosphate buffer or human serum (step a, Figure 2) but a low stability in cell extracts (step d, Figure 2) is favorable to induce an

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[a] Abbreviations: AM, acetoxymethyl; POM, pivaloyloxymethyl; *cyclo*Sal:, *cyclo*saligenyl; DCC, dicyclohexylcarbodiimide; HOBt, hydroxybenzotriazole.

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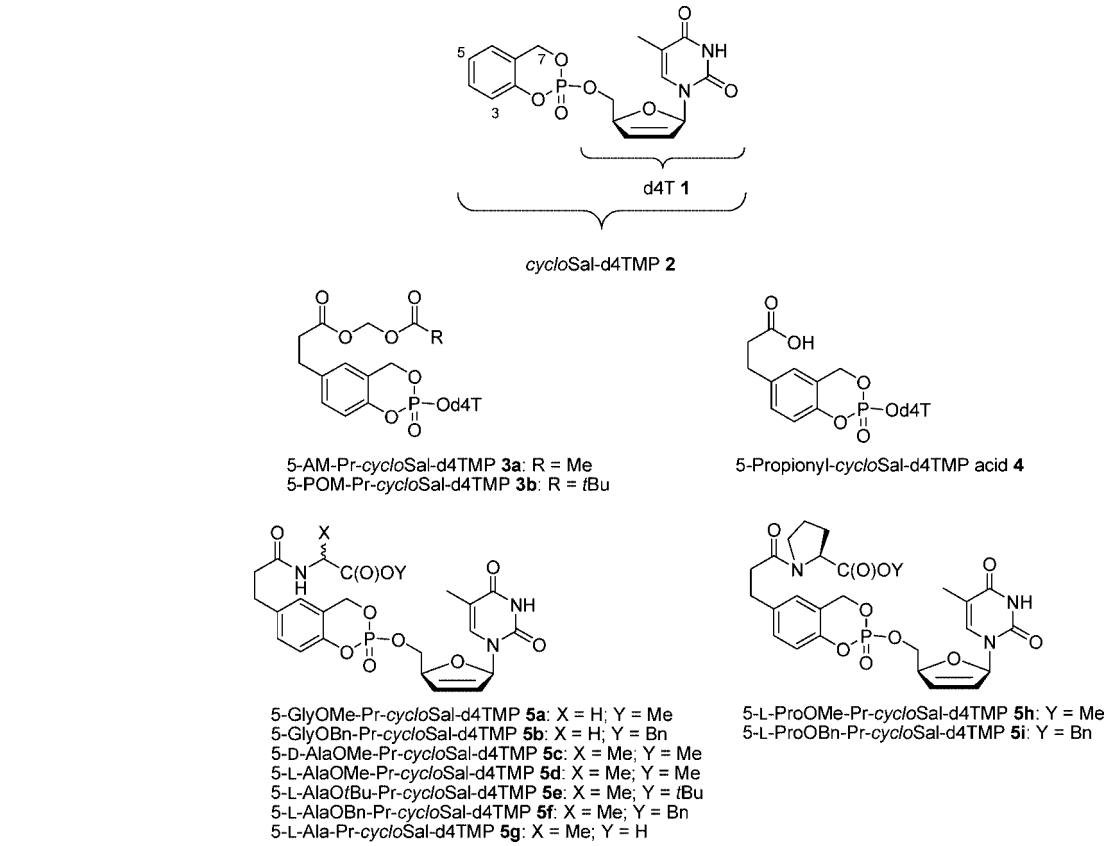


Figure 1. Structures of target compounds **5**, other *cyclo*Sal-d4TMPs **2**−**4**, and d4T **1**.

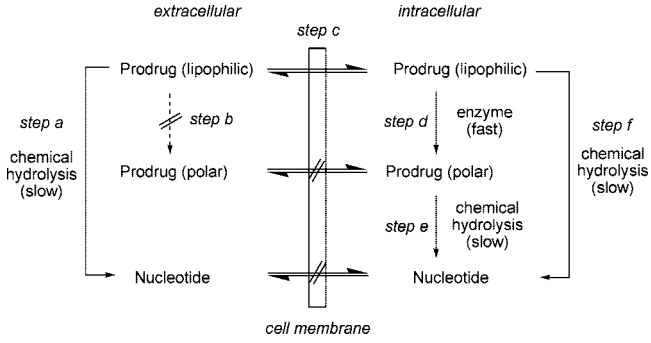


Figure 2. "Lock-in" concept.

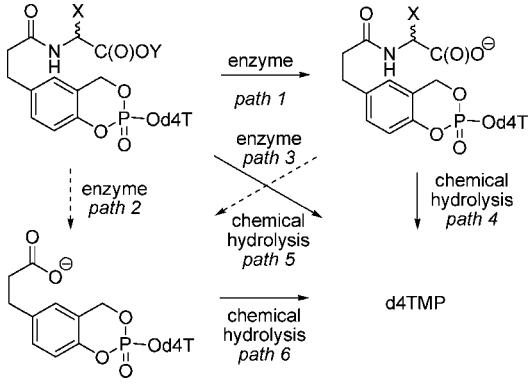
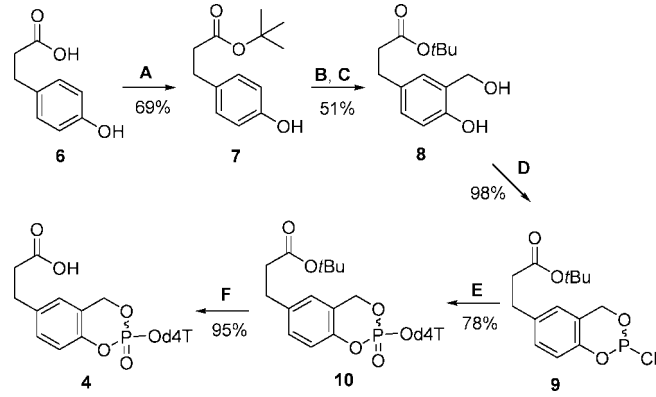


Figure 3. Possible cleavage mechanisms of amino acid ester functionalized *cyclo*Sal-d4TMPs.

accumulation of the "lock-in" pronucleotides. The main advantages of the amino acid ester modification compared to, for example, POM modification are the flexibility of possible modifications to adjust stability and polarity and the possibility to circumvent the release of pivaloic acid and formaldehyde.

Scheme 1. Synthesis of 5-Propionyl-*cyclo*Sal-d4TMP Acid **4**[a]

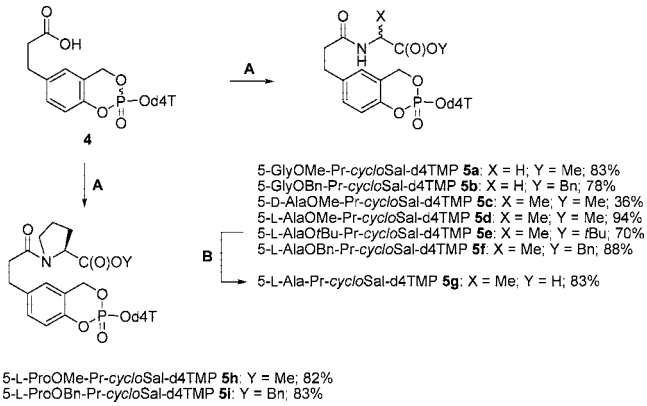


[a] Reagents and conditions. Method A: *N*,*N*-DMF-dineopentylacetale, ^{t}BuOH, toluene, reflux. Method B: phenylboronic acid, propionic acid, paraformaldehyde, toluene, reflux. Method C: H_2O_2, 0°C. Method D: PCl_3, pyridine, Et_2O, −20 °C. Method E: d4T **1**, DIPEA, CH_3CN, ^{t}BuOOH, −20 °C to room temp. Method F: TFA, CH_2Cl_2.

Chemistry

The key intermediate 5-propionyl-*cyclo*Sal-d4TMP acid **4** was synthesized using a previously published route (Scheme 1).[15] Briefly, commercially available 3-(4-hydroxyphenyl)propanoic acid **6** was converted into the corresponding *tert*-butyl ester **7** and subsequently transformed to salicylic alcohol **8** by an ortho-selective hydroxymethylation.[17] Salicylic alcohol **8** was treated with PCl_3 and pyridine in Et_2O. Pyridinium chloride was then

Scheme 2. Synthesis of Target Compounds **5**[a]



[a] Reagents and conditions. Method A: DCC, HOBt, DIPEA, salts of amino acid esters, DMF. Method B: TFA, CH_2Cl_2.

removed by filtration, yielding saligenyl chlorophosphite **9** in excellent purity. This compound was used to prepare 5-^{t}Bu-propionyl-*cyclo*Sal-d4TMP **10** in 78% yield using the standard procedure.[15] The *tert*-butyl ester was subsequently cleaved with TFA to give key intermediate **4** in a 95% yield as a 0.9:1 mixture of diastereomers.

The following coupling of 5-propionyl-*cyclo*Sal-d4TMP acid **4** with different amino acid ester salts was achieved in DMF using DCC, HOBt, and DIPEA (Scheme 2). Generally, this synthesis resulted in excellent yields except for D-alanine methyl ester **5c**. L-Alanine *tert*-butyl ester **5e** was converted to 5-L-Ala-propionyl-*cyclo*Sal-d4TMP **5g** by cleavage of the ester group with TFA. With acids **4** and **5g**, reference compounds for HPLC analysis were available to investigate the different mechanisms of hydrolysis (Figure 3). Further, both compounds were used as references in antiviral evaluation.

As aforementioned, *cyclo*Sal triesters are diastereomeric entities. To investigate the effect of the configuration at phosphorus, we enriched both diastereoisomers of **5f** up to a ratio of 9:1 (judged from ^{31}P NMR) by preparative RP-HPLC. Because we failed to assign the absolute stereochemistry up to now, these compounds will be named as **5f fast** and **5f slow**, which refer to the mobility properties on the reversed-phase silica gel chromatography column. Compounds bearing L-proline caused a mixture of diastereomers and rotamers, which was proven by coalescence NMR experiments.

All triesters **5** were characterized by means of ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy as well as high resolution mass spectrometry.

Results and Discussion

Chemical Stability. Stability studies of *cyclo*Sal-triesters **5** were conducted in aqueous 25 mM phosphate buffer (PBS pH 7.3). The hydrolysis products were detected by analytical RP-HPLC. Additionally, hydrolysis studies of compounds **5** in a 1:1 mixture of DMSO-d_6 and PBS (pH 7.3) followed by ^{31}P NMR spectroscopy revealed d4TMP and d4T diphosphate (d4TDP) as the sole phosphorus containing products resulting from chemical hydrolysis of triesters **5** by either hydroxide (to give d4TMP) or phosphate (to give d4TDP, Figure 4). Though the detection of small amounts of d4TDP is more reliable with NMR techniques, it can also be found in chromatograms if appropriate methods are used. The HPLC-determined half-lives are summarized in Table 1. They relate to the slow chemically induced cleavage of the phosphorus triester and not to the cleavage of the trigger moiety. The average half-life of all diastereomeric *cyclo*Sal-triesters **5** is about 6 h. This shows that the C_2-spacer separates the electron withdrawing amide bond effectively from the aromatic system, resulting in a slightly increased stability compared to prototype compound **2** ($t_{1/2}$ = 4.4 h). Additionally, the chemical stability seems to be roughly independent of the amino acid and the type of ester, supporting the assumption that the chemical hydrolysis does only take place at the phosphate moiety. L-Alanine compound **5g** bearing a nonesterified amino acid differs from this behavior. It displays a 2-fold increased stability of 12 h. This increase was also found for 5-propionyl-*cyclo*Sal-d4TMP acid **4**. We assume that the negatively charged carboxylate repulsively interacts with hydroxide ions, thus enhancing stability of the compounds. The separated diastereomers of triester **5f** illustrate the strong effect of the configuration at phosphorus on the chemical hydrolysis properties. The diastereomer **5f fast** is hydrolyzed twice as fast as **5f slow**. This is in line with previous experiments.[5]

In summary, d4T monophosphate was released from all compounds within a reasonable period of time. We did not find any interaction of the amino acid ester modification with the chemical hydrolysis mechanism of *cyclo*Sal triesters. Despite the increased average half-life of compound **5g**, this derivative also selectively released d4TMP.

Hydrolysis in Cell Extracts and Human Serum. The cleavage of the different ester groups was investigated in hydrolysis studies using T-lymphocyte CEM cell extracts (CE). We were able to show that at least for L-alanine bearing compounds **5d**−**f** the hydrolysis in PBS buffer proceeds via path 5 (Figure 3) to yield d4TMP directly, whereas in cellular extracts the amino acid ester is cleaved by an esterase (path 1, Figure 3). We did not observe a cleavage of the amide bond by peptidases that may be present in the serum (paths 2 and 3, dashed lines). The stabilities of the compounds are summarized in Table 1. A very fast enzymatic cleavage of the trigger moiety was found for esters of L-alanine and L-proline. Additionally, the cleavage was found to be faster for benzyl esters compared to methyl esters. This led to the desired increase of polarity (e.g., **5f** log P = 3.37 vs **5g** log P = 1.56; Table 1). There was no dependence of the cleavage rate on the configuration at phosphorus, which can be taken from the results obtained for **5f fast** and **5f slow** ($t_{1/2}$ = 0.5 h each). These results clearly show that the rate of cleavage strongly depends on different factors, which are the amino acid, the stereochemistry of the amino acid (D-AlaOMe **5c** is not cleaved by esterases, whereas L-AlaOMe **5d** was cleaved rapidly), and the type of ester (the ^{t}Bu-ester of **5e** is not cleaved enzymatically). Such structure−activity relationship has also been observed for the arylphosphoramidate ester prodrugs.[18] If there is no enzymatic cleavage of the trigger, the measured stabilities resemble those obtained for pure chemical hydrolysis, as this is now the main pathway taking place. The most distinct difference in chemical stability and enzymatic stability was found for **5f slow**. We measured an almost 18-fold reduction of stability in cell extracts compared to phosphate buffer. This complies with our intention to have a fast enzymatic hydrolysis of, for example, **5f slow** preceding the slow chemical hydrolysis of the trapped compound **5g** to give d4T monophosphate.

Further investigations in 5% and 50% human serum diluted with phosphate buffered saline (pH 6.8) were carried out with compounds **5f fast** and **5f slow**. In 5% human serum there was no effect on the stability, and the half-lives measured slightly increased compared to those obtained at pH 7.3. However, in 50% human serum we observed a striking difference for both diastereomers. Compound **5f fast** remained stable under these conditions with hydrolysis half-lives of 4.5 h in PBS (pH 7.3)

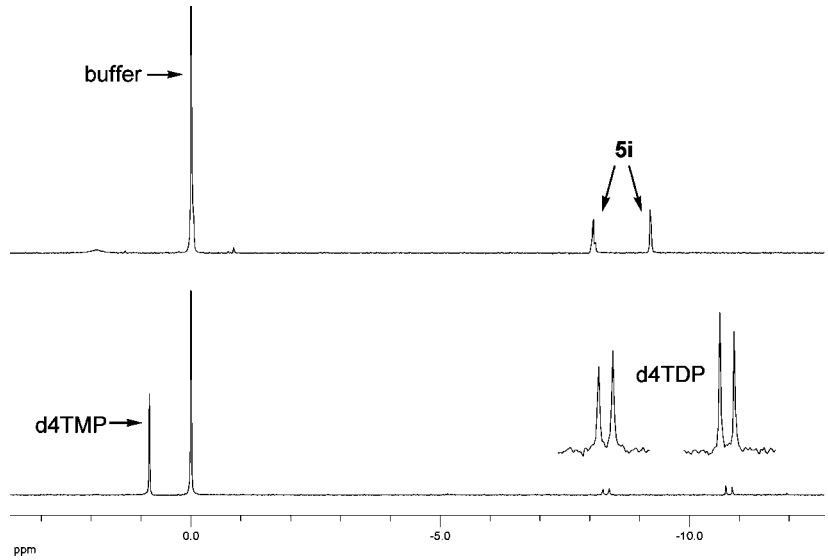


Figure 4. Chemical hydrolysis of **5i** followed by ^{31}P NMR spectroscopy {solvent, DMSO-d_6/50 mM PBS buffer (pH 7.3), 1:1 (v/v)}. Spectra were recorded at t_0 (top) and at the end of the hydrolysis (bottom), revealing the release of 85% d4TMP and 15% d4TDP. H_3PO_4 was used as external reference.

Table 1

			$t_{1/2}$ (h)[b]			EC$_{50}$ (μM)[c] CEM/O[h]			
	substituent	log P_{calcd}[a]	PBS[e]	CE[f]	serum[g]	HIV-1	HIV-2	CEM/TK$^-$ [i] HIV-2	CC$_{50}$(μM)[d]
5a	GlyOMe-pr	1.05	6.5	4.5	nd[k]	2.0 ± 0.0	1.6 ± 0.6	4.0 ± 0.0	220
5b	GlyOBn-pr	2.83	6.2	4.8	nd[k]	0.31 ± 0.08	0.47 ± 0.12	0.4 ± 0.0	75
5c	D-AlaOMe-pr	1.59	5.0	6.2	nd[k]	0.4 ± 0.07	0.4 ± 0.07	4.2 ± 0.5	61
5d	L-AlaOMe-pr	1.59	6.4	1.1	nd[k]	0.8 ± 0.3	0.7 ± 0.0	2.5 ± 0.7	152
5e	L-AlaO^{t}Bu-pr	2.42	5.7	6.8	nd[k]	0.3 ± 0.07	0.4 ± 0.1	3.8 ± 0.0	53
5f fast	L-AlaOBn-pr	3.37	4.5	0.5	4.4	0.3 ± 0.0	0.3 ± 0.1	1.0 ± 0.5	48
5f slow	L-AlaOBn-pr	3.37	8.8	0.5	1.3	0.3 ± 0.0	0.4 ± 0.3	1.0 ± 0.3	61
5f mix	L-AlaOBn-pr	3.37	6.4	nd[k]	nd[k]	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	23
5g	L-Ala-pr	1.56[l]	12	7	nd[k]	0.5 ± 0.2	0.4 ± 0.0	4.1 ± 0.8	77
5h	L-ProOMe-pr	1.82	7.9	1.1	nd[k]	1.3 ± 0.0	1.6 ± 0.6	2.8 ± 1.1	182
5i	L-ProOBn-pr	3.60	6.1	0.4	nd[k]	0.7 ± 0.42	1.1 ± 0.44	21 ± 1.4	46
3a	5-POM-pr	4.04	5.6	0.38	nd[k]	0.23 ± 0.04	0.33 ± 0.11	0.7 ± 0.08	24
3b	5-AM-pr	2.18	4.3	0.25	nd[k]	0.20 ± 0.11	0.53 ± 0.39	25 ± 19.1	81
2	H	0.28	4.4	4.0	nd[k]	0.10 ± 0.02	0.13 ± 0.04	0.90 ± 0.28	31
4	C(O)OH-pr	2.10[l]	13	nd[k]	nd[k]	0.14 ± 0.10	0.80 ± 0.20	50 ± 30	74
1		−0.48	na[j]	na[j]	na[j]	0.48 ± 0.45	0.63 ± 0.21	47.5 ± 26.3	234

[a] Calculated partition coefficients (log *P*) calculated using log *P* function implemented in ChemDraw 6.0. [b] Hydrolysis half-lives. [c] Antiviral activity in T-lymphocytes: 50% effective concentration (shown values are the mean of two to three independent experiments). [d] Cytotoxicity: 50% cytotoxic concentration. [e] 25 mM phosphate buffer (pH 7.3). [f] CEM cell extracts (pH 6.9). [g] 50% human serum, pH 6.8. [h] Wild-type CEM cells. [i] Thymidine kinase-deficient CEM cells. [j] na: not applicable. [k] nd: not determined. [l] Calculated for protonated acid.

and 4.4 h in human serum. In contrast, the half-life of compound **5f slow** was reduced from 8.8 h in PBS (pH 7.3) to 1.3 h in 50% human serum. Thus, in human serum, stereochemistry at phosphorus is important for the enzymatic stability, which was not the case in cell extracts. We have not yet figured out the reason for this behavior, but first investigations may point toward an interaction of the triester with butyrylcholine esterase (BChE). Previous experiments have shown that always one diastereomer of different *cyclo*Sal triesters inhibited BChE by irreversible binding to the active site of the enzyme. To circumvent this problem, bulky substituents can be placed in the 3-position of the aromatic moiety.[19] However, the reduced half-life of compound **5f slow** may also originate from unspecific protein binding.

Antiviral Evaluation. All newly prepared triesters were evaluated for their anti-HIV activity in wild-type CEM/0 and mutant thymidine kinase-deficient CEM/TK$^-$ cells (Table 1). As reference compound, d4T **1** was used. *cyclo*Sal-d4TMPs **5** proved to be equipotent against HIV-1 and HIV-2 in wild-type CEM/0 cells as **1**. All triesters **5** are markedly more active against HIV-2 in CEM/TK$^-$ cells compared to the parent nucleoside **1**. The worst TK$^-$-activity was found for the L-proline benzyl ester **5i**, which may be due to its poor solubility in water. In contrast, a full retention of activity in TK$^-$-cells was found for 5-GlyOBn-Pr-*cyclo*Sal-d4TMP **5b** and 5-L-AlaOBn-Pr-*cyclo*Sal-d4TMP **5f**, indicating that a benzyl ester is a favorable modification. However, we have no explanation of why the mixture of compounds **5f** is 3-fold more active in TK-deficient cells compared to the separated diastereomers. We cannot exclude that this is within the experimental error. On the other hand, the L-alanine modified triester **5g** showed a slight loss of TK$^-$ activity, which may result from its hampered diffusion across the cell membrane. Otherwise, **5f** should not have higher TK$^-$ activity compared to **5g**, as the first one is rapidly converted into the latter in intact cells. Thus, if compound **5g** is released inside the cell, e.g., from compound

5f, an intracellular accumulation seems to be possible. The increased cytotoxicity of some of the triesters **5** may reflect the higher amount of bioactive nucleoside phosphates formed in the cells interacting with the cellular processes compared to the situation starting the metabolism from the parent nucleoside **1**. Generally, we did not find cytotoxic effects of salicylic alcohol and masks derived from it.

Conclusion

A new synthetic route to amino acid ester modified *cyclo*Sal-pronucleotides has been developed. It has been shown that some of the compounds are rapidly converted in cell extracts to compounds with increased polarity. Further, the cleavage products release d4T monophosphate in a subsequent chemical hydrolysis. Therefore, if 5-L-AlaOBn-Pr-*cyclo*Sal-d4TMP **5f** is delivered into cells, a fast hydrolysis to 5-L-Ala-Pr-*cyclo*Sal-d4TMP **5g** should enrich the prodrug and eventually lead to higher concentrations of d4TMP. The chemical stabilities of compounds **5** were independent of the amino acid ester attached to the C_2-spacer, but enzymatic stabilities strongly depended on both the amino acid and ester. In general, modifications with L-alanine benzyl esters seem to have the most promising properties, both concerning their hydrolysis and antiviral profile, which is similar to results obtained for phosphoramidate prodrugs, although the structure is considerably different.[18] As a result, the modification of *cyclo*Sal-pronucleotides with amino acid esters can be used as a trigger that is chemically inert under physiological conditions but is cleaved in cellular extracts by enzymes. This led to an increase of polarity (**5f** $\log P = 3.37$ vs **5g** $\log P = 1.56$), which may hamper diffusion of the compound into the extracellular matrix. Further, with this concept, polarity and enzymatic stability can be adjusted.

The novel 5-L-AlaOBn-propionyl-*cyclo*Sal mask has already been applied to other nucleoside analogues, and results of the antiviral evaluation of the compounds will be published elsewhere. To further prove the possible enrichment of lock-in modified compounds, we are currently conducting fluorescence microscopy studies. Finally, we are running our recently developed stereoselective synthesis to obtain compounds **5** as pure isomers.

Experimental Section

NMR spectra were recorded with a Bruker AMX 400 or a Bruker DRX 500 Fourier transform spectrometer. All ^{1}H and ^{13}C NMR chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane and calibrated on solvent signals. The ^{31}P NMR chemical shifts (proton decoupled) are quoted in ppm using H_3PO_4 as the external reference. The spectra were recorded at room temperature. Mass spectra were obtained with a VG Analytical VG/70-250 F [FAB (double focusing), matrix *m*-nitrobenzyl alcohol] spectrometer. ESI mass spectra were recorded with a VG Analytical Finnigan ThermoQuest MAT 95 XL spectrometer. For thin layer chromatography (TLC) Merck precoated 60 F_{254} plates with a 0.2 mm layer of silica gel were used; sugar-containing compounds were visualized with sugar spray reagent (0.5 mL of 4-methoxybenzaldehyde, 9 mL of EtOH, 0.5 mL of concentrated sulfuric acid, and 0.1 mL of glacial acetic acid). All preparative TLC experiments were performed on a Chromatotron (Harrison Research, model 7924T) using glass plates coated with 1, 2, or 4 mm layers of Merck 60 PF_{254} silica gel containing a fluorescent indicator. For column chromatography, Merck silica gel 60, 230−400 mesh, was used. Analytical HPLC was performed on a Merck-Hitachi HPLC system (D-7000) equipped with a LiChroCART 125-3 column containing reversed phase silica gel Lichrospher 100 RP 18 (5 μM; Merck, Darmstadt, Germany). The lyophilized products **5** did not give useful microanalytical data most probably because of incomplete combustion of the compounds or varying amounts of water but were found to be pure by rigorous HPLC analysis. Diethyl ether was dried over sodium/benzophenone and distilled under nitrogen. THF was dried over potassium/benzophenone and distilled under nitrogen. Pyridine, CH_2Cl_2, and CH_3CN were distilled from calcium hydride under nitrogen. *N*,*N*-Diisopropylethylamine and triethylamine were distilled from sodium prior to use. The solvents for HPLC were obtained from Merck (CH_3CN, HPLC grade).

Compounds **4** and **7**−**10** were synthesized as reported in ref 14, and analytical data were according to those published.

General Procedure for the Preparation of Amino Acid Ester Modified *cyclo*Sal-d4TMPs 5a−i. The reactions were carried out under a nitrogen atmosphere. Amino acid ester salts (2.0 equiv) were suspended in dry DMF and solubilized by addition of dry DIPEA (Hünigs base, 2.0 equiv). 5-Propionyl-*cyclo*Sal-d4TMP acid (**4**, 1.0 equiv), HOBt (1.1 equiv), and DCC (1.3 equiv) were added successively. The solution was stirred 16 h at room temperature. The solvent was removed in vacuo. The residue was diluted with ethyl acetate and washed twice with water. The organic layer was dried and concentrated (Na_2SO_4), and the crude products were purified by preparative TLC (Chromatotron) and lyophilized from CH_3CN/H_2O, 1:1 (v/v).

5-GlyOMe-propionyl-*cyclo*Sal-d4TMP (5a). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 30 mg, 65 μmol), DIPEA (22 μL, 130 μmol), glycine methyl ester·HCl (16 mg, 130 μmol), HOBt (11 mg, 72 μmol), DCC (18 mg, 85 μmol), dissolved in 1 mL dry DMF. Purification was done by preparative TLC (Chromatotron; CH_2Cl_2/MeOH, gradient elution 0−10%). Yield: 29 mg (54 μmol, 83%) of a diastereomeric mixture (ratio 0.8:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (2 × s, 1H, NH_{het}), 8.33 (t, 1H, J = 5.8 Hz, NH-11), 7.30−7.24 (m, 1H, H-4), 7.20, 7.19 (2 × q, 1H, J = 1.2 Hz, H-6_{het}), 7.15−7.08 (m, 2H, H-6, H-3), 6.82−6.76 (m, 1H, H-1′), 6.42, 6.36 (2 × ddd, J = 1.7 Hz, J = 1.7 Hz, J = 6.0 Hz, H-3′), 6.05−5.98 (m, 1H, H-2′), 5.53−5.30 (m, 2H, H-7), 5.00−4.90 (m, 1H, H-4′), 4.40−4.21 (m, 3H, H-12, H-5′), 3.82 (2 × d, 2H, J = 5.8 Hz, H-12), 3.61 (s, 3H, OMe), 2.90−2.77 (m, 2H, H-8), 2.47−2.36 (m, 2H, H-9), 1.66, 1.59 (2 × d, 3H, J = 0.9 Hz, Me_{het}) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −8.71, −8.77 ppm.

5-GlyOBn-propionyl-*cyclo*Sal-d4TMP (5b). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 50.0 mg, 108 μmol), DIPEA (38 μL, 0.22 mmol), glycine benzyl ester·*p*-TsOH (73 mg, 0.22 mmol), HOBt (18 mg, 0.12 mmol), DCC (30 mg, 0.14 mmol), dissolved in 1 mL dry DMF. Purification was done by flash chromatography (CH_2Cl_2/MeOH, 15:1 v/v). Yield: 51 mg (84 μmol, 78%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34, 11.33 (2 × s, 1H, NH_{het}), 8.38 (2 × t, 1H, J = 5.8 Hz, NH-11), 7.40−7.30 (m, 5H, H_{aryl}), 7.28−7.23 (m, 1H, H-4), 7.20, 7.19 (2 × q, 1H, J = 1.2 Hz, H_{het}-6), 7.14−7.06 (m, 2H, H-6, H-3), 6.81−6.78 (m, 1H, H-1′), 6.41, 6.35 (2 × ddd, 1H, J = 1.7 Hz, J = 1.7 Hz, J = 6.0 Hz, H-3′), 6.05−5.97 (m, 1H, H-2′), 5.51−5.30 (m, 2H, H-7), 5.12 (s, 2H, H-14), 4.98−4.92 (m, 1H, H-4′), 4.38−4.22 (m, 2H, H-5′), 3.89, 3.88 (2 × d, 2H, J = 5.9 Hz, H-12), 2.89−2.75 (m, 2H, H-8), 2.47−2.40 (m, 2H, H-9), 1.66, 1.58 (2 × d, 3H, J = 1.1 Hz, Me_{het}) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −8.70, −8.77 ppm.

5-D-AlaOMe-propionyl-*cyclo*Sal-d4TMP (5c). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 40 mg, 86 μmol), DIPEA (30 μL, 0.17 mmol), D-alanine methyl ester·HCl (25 mg, 0.18 mmol), HOBt (15 mg, 0.11 mmol), DCC (24 mg, 0.11 mmol), dissolved in 1 mL dry DMF. Purification was done by preparative TLC (Chromatotron; EtOAc/MeOH, gradient elution 0−2%; second purification with pure CH_2Cl_2). Yield: 17 mg (31 μmol, 36%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.35, 11.34 (2 × s, 1H, NH_{het}), 8.27 (d, 1H, J = 6.5 Hz, NH-11), 7.23−7.15 (m, 2H, H_{het}-6, H-4), 7.11−7.09 (m, 1H, H-6), 7.03, 7.00 (2 × d, 1H, J = 8.4 Hz, H-3), 6.82−6.75 (m, 1H, H-1′), 6.42, 6.35 (2 × ddd, J = 1.6 Hz, J = 1.6 Hz, J = 6.1 Hz, H-3′), 6.04−5.98 (m, 1H, H-2′), 5.50−5.30 (m, 2H, H-7), 5.00−4.90 (m, 1H, H-4′), 4.35−4.20 (m, 3H, H-12,

H-5′), 3.60 (s, 3H, OMe), 2.78 (t, 2H, J = 7.2 Hz, H-8), 2.39 (t, 2H, J = 7.5 Hz, H-9), 1.68, 1.62 (2 × d, 3H, J = 0.9 Hz, Me_{het}), 1.23, 1.22 (2 × d, 3H, J = 7.3 Hz, Me) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −9.25, −9.28 ppm.

5-L-AlaOMe-propionyl-*cyclo*Sal-d4TMP (5d). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 50.0 mg, 108 μmol), DIPEA (38 μL, 0.22 mmol), L-alanine methyl ester·HCl (30 mg, 0.22 mmol), HOBt (18 mg, 0.12 mmol), DCC (30 mg, 0.14 mmol), dissolved in 1 mL dry DMF. Purification was done by flash chromatography (CH_2Cl_2/MeOH, 9:1 v/v). Yield: 56.0 mg (102 μmol, 94%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34, 11.33 (2 × s, 1H, NH_{het}), 8.31, 8.30 (2 × d, 1H, J = 6.9 Hz, NH-11), 7.28−7.22 (m, 1H, H-4), 7.20, 7.19 (2 × q, 1H, J = 1.2 Hz, H-6_{het}), 7.15−7.08 (m, 2H, H-6, H-3), 6.82−6.76 (m, 1H, H-1′), 6.42, 6.35 (2 × ddd, J = 1.8 Hz, J = 1.8 Hz, J = 6.0 Hz, H-3′), 6.05−5.99 (m, 1H, H-2′), 5.52−5.30 (m, 2H, H-7), 5.00−4.90 (m, 1H, H-4′), 4.40−4.20 (m, 3H, H-12, H-5′), 3.61, 3.60 (2 × s, 3H, OMe), 2.87−2.75 (m, 2H, H-8), 2.47−2.35 (m, 2H, H-9), 1.66, 1.59 (2 × d, 3H, J = 1.1 Hz, Me_{het}), 1.25, 1.23 (2 × d, 3H, J = 7.3 Hz, Me) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −8.71, −8.74 ppm.

5-L-AlaO′Bu-propionyl-*cyclo*Sal-d4TMP (5e). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 60.0 mg, 129 μmol), DIPEA (45 μL, 0.26 mmol), L-alanine *tert*-butyl ester·HCl (47 mg, 0.26 mmol), HOBt (22 mg, 0.14 mmol), DCC (36 mg, 0.17 mmol), dissolved in 1 mL dry DMF. Purification was done by preparative TLC (Chromatotron; CH_2Cl_2/MeOH, gradient elution 0−2%). Yield: 53 mg (90 μmol, 70%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.35, 11.34 (s, 1H, NH_{het}), 8.14 (d, 1H, J = 7.0 Hz, NH-11), 7.24−7.15 (m, 2H, H_{het}-6, H-4), 7.12−7.07 (m, 1H, H-6), 7.02, 7.00 (2 × d, 1H, J = 8.3 Hz, H-3), 6.82−6.75 (m, 1H, H-1′), 6.41, 6.34 (2 × ddd, J = 1.6 Hz, J = 1.6 Hz, J = 6.0 Hz, H-3′), 6.03−5.98 (m, 1H, H-2′), 5.48−5.30 (m, 2H, H-7), 4.99−4.90 (m, 1H, H-4′), 4.36−4.20 (m, 2H, H-12, H-5′), 4.09 (m, 1H, H-12), 2.82−2.70 (m, 2H, H-8), 2.38 (t, 2H, J = 7.5 Hz, H-9), 1.68, 1.62 (2 × s, 3H, Me_{het}), 1.38 (s, 9H, *t*-Bu), 1.23, 1.22 (2 × d, 3H, J = 7.1 Hz, Me) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −9.15, −9.52 ppm.

5-L-AlaOBn-propionyl-*cyclo*Sal-d4TMP (5f fast and 5f slow). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 30.0 mg, 65 μmol), DIPEA (22 μL, 0.13 mmol), L-alanine benzyl ester·*p*-TsOH (46 mg, 0.13 mmol), HOBt (11 mg, 72 μmol), DCC (18 mg, 85 μmol), dissolved in 1 mL dry DMF. Purification was done by preparative TLC (Chromatotron; EtOAc/MeOH, gradient elution 0−5%). Yield: 36 mg (58 μmol, 88%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. The diastereomers **5f fast** and **5f slow** were enriched by means of preparative RP-HPLC (CH_3CN/H_2O, 1:4) up to a ratio of 9:1 for each compound (as judged from ^{31}P NMR). **5f fast**: ^{1}H NMR (400 MHz, MeOD): δ = 7.40−7.27 (m, 6H, H_{het}-6, H_{aryl}), 7.20 (d, 1H, J = 8.4 Hz, H-4), 7.06 (s, 1H, H-6), 6.98 (d, 1H, J = 8.4 Hz, H-3), 6.90 (ddd, 1H, J = 1.6 Hz, J = 1.6 Hz, J = 3.5 Hz, H-1′), 6.41 (ddd, 1H, J = 1.4 Hz, J = 1.4 Hz, J = 6.0 Hz, H-3′), 6.01−5.96 (m, 1H, H-2′), 5.37 (dd, 2H, J = 9.4 Hz, J = 14.0 Hz, H-7), 5.15 (s, 2H, H-14), 5.02−4.96 (m, 1H, H-4′), 4.45−4.37 (q, 1H, J = 7.3 Hz, H-12), 4.40−4.30 (m, 2H, H-5′), 2.95−2.80 (m, 2H, H-8), 2.50 (t, 2H, J = 7.4 Hz, H-9), 1.77 (s, 3H, Me_{het}), 1.33 (d, 3H, J = 7.3 Hz, Me) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, MeOD): δ = −8.23 ppm. **5f slow**: ^{1}H NMR (400 MHz, MeOD): δ = 7.36−7.29 (m, 6H, H_{het}-6, H_{aryl}), 7.18 (d, 1H, J = 8.4 Hz, H-4), 7.05 (d, 1H, J = 1.7 Hz, H-6), 6.94 (d, 1H, J = 8.4 Hz, H-3), 6.87 (ddd, 1H, J = 1.7 Hz, J = 1.7 Hz, J = 3.4 Hz, H-1′), 6.35 (ddd, 1H, J = 1.7 Hz, J = 1.7 Hz, J = 6.0 Hz, H-3′), 6.00−5.96 (m, 1H, H-2′), 5.40−5.34 (m, 2H, H-7), 5.15 (s, 2H, H-14), 5.02−4.96 (m, 1H, H-4′), 4.45−4.30 (m, 3H, H-12, H-5′), 2.95−2.80 (m, 2H, H-8), 2.50 (t, 2H, J = 7.5 Hz, H-9), 1.77 (d, 3H, J = 1.1 Hz, Me_{het}), 1.34 (d, 3H, J = 7.3 Hz, Me) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, MeOD): δ = −8.55 ppm.

5-L-Ala-propionyl-*cyclo*Sal-d4TMP (5g). 5-L-AlaO′Bu-propionyl-*cyclo*Sal-d4TMP (**5e**, 28 mg, 47 μmol) was dissolved in 10 mL of CH_2Cl_2, and an amount of 2 mL of TFA was added. After 2.5 h at room temperature, the solvent was removed in vacuo. The residue was purified by preparative TLC (Chromatotron; CH_2Cl_2 [+0.5% HOAc]/MeOH, gradient elution 1−10%) and lyophilized from CH_3CN/H_2O, 1:1 (v/v). Yield: 21 mg (39 μmol, 83%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.39 (s, 1H, COOH), 11.35, 11.34 (2 × s, 1H, NH_{het}), 8.14 (d, 1H, J = 7.2 Hz, NH-11), 7.24−7.15 (m, 2H, H_{het}-6, H-4), 7.12−7.07 (m, 1H, H-6), 7.02, 7.00 (2 × d, 1H, J = 8.4 Hz, H-3), 6.81−6.77 (m, 1H, H-1′), 6.41, 6.35 (2 × ddd, J = 1.6 Hz, J = 1.6 Hz, J = 6.0 Hz, H-3′), 6.03−5.98 (m, 1H, H-2′), 5.48−5.30 (m, 2H, H-7), 4.99−4.90 (m, 1H, H-4′), 4.36−4.14 (m, 3H, H-12, H-5′), 2.82−2.70 (m, 2H, H-8), 2.38 (t, 2H, J = 7.6 Hz, H-9), 1.68, 1.62 (2 × d, 3H, J = 1.0 Hz, Me_{het}), 1.23, 1.22 (2 × d, 3H, J = 7.3 Hz, Me) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −9.27, −9.30 ppm.

5-L-ProOMe-propionyl-*cyclo*Sal-d4TMP (5h). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 50.0 mg, 108 μmol), DIPEA (38 μL, 0.22 mmol), L-proline methyl ester·HCl (37 mg, 0.22 mmol), HOBt (18 mg, 0.12 mmol), DCC (30 mg, 0.14 mmol), dissolved in 1 mL dry DMF. First purification was done by flash chromatography (CH_2Cl_2/MeOH, 9:1 v/v), then by preparative TLC (Chromatotron; CH_2Cl_2/MeOH, gradient elution 0−5%). Yield: 51 mg (89 μmol, 82%) of a diastereomeric and rotameric mixture as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (s, 1H, NH_{het}), 7.35−7.25 (m, 1H, H-4), 7.22, 7.19 (2 × q, 1H, J = 1.2 Hz, H-6_{het}), 7.16−7.07 (m, 2H, H-6, H-3), 6.82−6.78 (m, 1H, H-1′), 6.42, 6.35 (2 × ddd, J = 1.6 Hz, J = 1.6 Hz, J = 6.0 Hz, H-3′), 6.06−5.98 (m, 1H, H-2′), 5.54−5.30 (m, 2H, H-7), 5.00−4.90 (m, 1H, H-4′), 4.60−4.20 (m, 3H, H-12, H-5′), 3.67, 3.54, 3.61, 3.60 (4 × s, 3H, OMe), 3.55−3.37 (m, 2H, H-11a), 2.81, 2.80 (2 × t, 2H, J = 7.5 Hz, H-8), 2.55 (t, 2H, J = 7.6 Hz, H-9), 2.34−2.08 (m, 1H, H-11c), 2.06−1.70 (m, 3H, H-11b, H-11c), 1.65, 1.62, 1.60, 1.59 (4 × d, 3H, J = 0.8 Hz, Me_{het}) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −8.47, −8.75 ppm.

5-L-ProOBn-propionyl-*cyclo*Sal-d4TMP (5i). Quantities were as follows: 5-propionyl-*cyclo*Sal-d4TMP acid (**4**, 40 mg, 86 μmol), DIPEA (30 μL, 0.17 mmol), L-proline benzyl ester·HCl (42 mg, 0.17 mmol), HOBt (15 mg, 0.11 mmol), DCC (24 mg, 0.11 mmol), dissolved in 2 mL dry DMF. Purification was done by preparative TLC (Chromatotron; EtOAc/MeOH, gradient elution 0−2%). Yield: 46 mg (71 μmol, 83%) of a diastereomeric and rotameric mixture as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.35, 11.34 (2 × s, 1H, NH_{het}), 7.40−7.30 (m, 5H, H_{aryl}), 7.27−7.19 (m, 1H, H-4), 7.19, 7.16 (2 × q, 1H, J = 1.2 Hz, H-6_{het}), 7.15−7.10 (m, 1H, H-6), 7.05−6.95 (m, 1H, H-3), 6.82−6.76 (m, 1H, H-1′), 6.41, 6.35 (2 × ddd, J = 1.8 Hz, J = 1.8 Hz, J = 6.0 Hz, H-3′), 6.05−5.98 (m, 1H, H-2′), 5.50−5.27 (m, 2H, H-7), 5.11 (s, 2H, H-14), 4.99−4.91 (m, 1H, H-4′), 4.40−4.20 (m, 3H, H-12, H-5′), 3.55−3.37 (m, 2H, H-11a), 2.78 (t, 2H, J = 7.5 Hz, H-8), 2.57 (t, 2H, J = 7.6 Hz, H-9), 2.25−2.10 (m, 1H, H-11c_1), 1.94−1.78 (m, 3H, H-11b, H-11c_2), 1.68, 1.61 (2 × d, 3H, J = 1.1 Hz, Me_{het}) ppm. ^{31}P NMR (162 MHz, ^{1}H decoupled, DMSO-d_6): δ = −9.23, −9.29 ppm.

Hydrolysis Studies of *cyclo*Sal Phosphate Triesters. Hydrolysis studies of *cyclo*Sal nucleotides (phosphate buffer, pH 7.3 and pH 7.6) by HPLC analysis have been described before.[20] Studies in cell extracts and human serum (obtained from pooled blood samples from the university hospital in Hamburg) were performed as reported in ref 15 with different incubation times but without using acetic acid to stop the reaction. Method 1 parameters were as follows: 0−25 min, H_2O/CH_3CN gradient (5−100%); 25−35 min, H_2O/CH_3CN (5%); flow rate 0.5 mL/min. Method 2 parameters were as method 1 parameters except TBAH ion buffer (0.55 mM) was used instead of water.

^{31}P NMR Hydrolysis Studies of *cyclo*Sal Phosphate Trimesters. ^{31}P NMR hydrolysis studies of *cyclo*Sal nucleotides **5** and **6** were carried out as described before.[20]

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

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Supporting Information Available: ^{13}C NMR and IR spectroscopic data, R_f values, and analytical HPLC data of new *cyclo*Sal compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) De Clercq, E. Strategies in the Design of Antiviral Drugs. *Nat. Rev. Drug Discovery* **2002**, *1*, 13–25.

(2) Balzarini, J. Metabolism and Mechanism of Antiretroviral Action of Purine and Pyrimidine Derivatives. *Pharm. World Sci.* **1994**, *16*, 113–126.

(3) Herdewijn, P.; Balzarini, J.; De Clercq, E. 2′,3′-Dideoxynucleoside Analogues as Anti-HIV Agents. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press Inc.: Greenwich, London, 1993; Vol. 1, pp 233−318.

(4) (a) Meier, C. Pro-Nucleotides. Recent Advances in the Design of Efficient Tools for the Delivery of Biologically Active Nucleoside Monophosphates. *Synlett* **1998**, 233–242. (b) Wagner, C. R.; Iyer, V. V.; McIntee, E. J. Pronucleotides: Towards the in Vivo Delivery of Antiviral and Anticancer Nucleotides. *Med. Res. Rev.* **2000**, *20*, 417–451.

(5) (a) Meier, C. *cyclo*Sal-Pronucleotides. Design of Chemical Trojan Horses. *Mini-Rev. Med. Chem.* **2002**, *2*, 219–234. (b) Meier, C. *cyclo*Sal Phosphates as Chemical Trojan Horses for Intracellular Nucleotide and Glycosylmonophosphate Delivery. Chemistry Meets Biology. *Eur. J. Org. Chem.* **2006**, *108*, 1–1102. (c) Meier, C. *cyclo*Sal-Pronucleotides. Design of the Concept, Chemistry, and Antiviral Activity. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; Elsevir: Amsterdam, 2004; Vol. 4, pp 147−213.

(6) (a) Balzarini, J.; Aquaro, S.; Knispel, T.; Rampazzo, C.; Bianchi, V.; Perno, C.-F.; De Clercq, E.; Meier, C. Cyclosaligenyl-2′,3′-didehydro-2′,3′-dideoxythymidine Monophosphate: Efficient Intracellular Delivery of d4TMP. *Mol. Pharmacol.* **2000**, *58*, 928–935. (b) Balzarini, J.; Naesens, L.; Aquaro, S.; Knispel, T.; Perno, C.-F.; De Clercq, E.; Meier, C. Intracellular Metabolism of *cyclo*Saligenyl-3′-azido-2′,3′-dideoxythymidine Monophosphate, a Prodrug of 3′-Azido-2′,3′-dideoxythymidine (Zidovudine). *Mol. Pharmacol.* **1999**, *56*, 1354–1361.

(7) Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. *cyclo*Sal-2′,3′-dideoxy-2′,3′-didehydrothymidine Monophosphate (*cyclo*Sal-d4TMP): Synthesis and Antiviral Evaluation of a New d4TMP Delivery System. *J. Med. Chem.* **1998**, *41*, 1417–1427.

(8) Meier, C.; Habel, L.; Haller-Meier, F.; Lomp, A.; Herderich, M.; Klöcking, R.; Meerbach, A.; Wutzler, P. Chemistry and Anti-Herpes Simplex Virus Type-1 Evaluation of *cyclo*Sal-Nucleotides of Acyclic Nucleoside Analogs. *Antiviral Chem. Chemother.* **1998**, *9*, 389–402.

(9) Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J. *cyclo*Sal-Pronucleotides of 2′,3′-Dideoxyadenosine and 2′,3′-Dideoxy-2′,3′-didehydroadenosine: Synthesis and Antiviral Evaluation of a Highly Efficient Nucleotide Delivery System. *J. Med. Chem.* **1999**, *42*, 1604–1614.

(10) Meier, C.; Knispel, T.; Marquez, V. E.; De Clercq, E.; Balzarini, J. *cyclo*Sal-Pronucleotides of 2′-Fluoro-ara- and 2′-Fluoro-ribo-2′,3′-dideoxyadenosine as a Strategy To Bypass a Metabolic Blockade. *J. Med. Chem.* **1999**, *42*, 1615–1624.

(11) Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P. *cyclo*Sal-BVDUMP Pronucleotides: How To Convert an Antiviral-Inactive Nucleoside Analogue into a Bioactive Compound against EBV. *J. Med. Chem.* **2002**, *45*, 5157–5172.

(12) Balzarini, J.; Haller-Meier, F.; De Clercq, E.; Meier, C. Antiviral Activity of *cyclo*Saligenyl Prodrugs of Acyclovir, Carbovir and Abacavir. *Antiviral Chem. Chemother.* **2001**, *12*, 301–306.

(13) Sauerbrei, A.; Meier, C.; Meerbach, A.; Schiel, M.; Helbig, P.; Wutzler, P. In Vitro Activity of *cyclo*Sal-Nucleoside Monophosphates and Polyhydroxycarboxylates against Orthopoxviruses. *Antiviral Res.* **2005**, *67*, 147–154.

(14) Meier, C.; Ruppel, M. F. H.; Vukadinoviæ, D.; Balzarini, J. Second Generation of *cyclo*Sal-Pronucleotides with Esterase-Cleavable Sites: The "Lock-In"-Concept. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 89–115.

(15) Meier, C.; Ducho, C.; Jessen, H.; Vukadinoviæ-Tenter, D.; Balzarini, J. Second-Generation *cyclo*Sal-d4TMP Pronucleotides Bearing Esterase-Cleavable Sites. The "Trapping" Concept. *Eur. J. Org. Chem.* **2006**, 197–206.

(16) Jessen, H.; Fendrich, W.; Meier, C. Synthesis and Properties of Fluorescent *cyclo*Sal-Nucleotides Based on the Pyrimidine Nucleoside m5K. *Eur. J. Org. Chem.* **2006**, *92*, 4–931.

(17) Nagata, W.; Okada, K.; Aoki, T. Ortho-Specific α-Hydroxyalkylation of Phenols with Aldehydes. An Efficient Synthesis of Saligenol Derivatives. *Synthesis* **1979**, 365–368.

(18) (a) McGuigan, C.; Thiery, J. C.; Daverioa, F.; Jiang, W. G.; Davies, G.; Mason, M. Anti-Cancer ProTides: Tuning the Activity of BVDU Phosphoramidates Related to Thymectacin. *Bioorg. Med. Chem.* **2005**, *13* (9), 3219–3227. (b) Cahard, D.; McGuigan, C.; Balzarini, J. Aryloxy Phosphoramidate Triesters as Pro-Tides. *Mini-Rev. Med. Chem.* **2004**, *4* (4), 371–381.

(19) Meier, C.; Ducho, C.; Görbig, U.; Esnouf, R.; Balzarini, J. Interaction of *cyclo*Sal-Pronucleotides with Cholinesterases from Different Origins. A Structure−Activity Relationship. *J. Med. Chem.* **2004**, *47*, 2839–2852.

(20) Ducho, C.; Balzarini, J.; Naesens, L.; De Clercq, E.; Meier, C. Aryl-Substituted and Benzo-Annulated *cyclo*Sal-Derivatives of 2′,3′-Dideoxy-2′,3′-didehydrothymidine Monophosphate. Correlation of Structure, Hydrolysis Properties and Anti-HIV Activity. *Antiviral Chem. Chemother.* **2002**, *13*, 129–141.

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