Enzymatically Activated *cyclo*Sal-d4T-monophosphates: The Third Generation of *cyclo*Sal-Pronucleotides

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The third generation of *cyclo*Sal-pronucleotides, 5-diacetoxymethyl-*cyclo*Sal-d4T-monophosphates (5-di-AM-*cyclo*Sal-d4TMPs), is reported as a new class of "lock-in"-modified *cyclo*Sal-pronucleotides. These compounds bear an esterase-cleavable geminal dicarboxylate (acylal) attached to the aromatic ring of the saligenyl unit. The conversion into a strong acceptor group (aldehyde) leads to a strong decrease in hydrolytic stability. As a consequence, a fast release of a nucleoside monophosphate (i.e., d4TMP) follows. The concept of this enzymatic activation is proven by hydrolysis studies in phosphate buffer, cell extracts, and human serum. These investigations showed the conversion of the acylal group into a polar aldehyde by enzymatic cleavage. Besides, 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 (nucleotide) formation by cellular kinases. For d4T **1** the first phosphorylation step to the monophosphate catalyzed by the salvage-pathway enzyme thymidine kinase (TK) is the metabolism-limiting step.[2,3] Therefore, this nucleoside should be delivered as the monophosphate into cells. Lipophilic nucleotide-releasing systems (pronucleotides) could circumvent this limitation (TK bypass).[4] As we reported before, the *cyclo*Sal-pronucleotide technique is such a successful class of an antivirally active nucleotide delivery system.[5] These lipophilic precursors **2** (*cyclo*Sal-d4TMP; Figure 1)[a] deliver d4TMP by a purely chemical triggered cascade reaction.[6] Also, this approach has been applied successfully to improve the antiviral potency of a variety of other nucleoside analogues.[7−9] Moreover, it was shown that the technology converts nonactive nucleoside analogues like 2-ribo-fluoro-ddA[10] or BVDU[11] into potent antiviral agents, and *cyclo*Sal-ACVMP compounds entirely retain their antiviral activity against ACV-resistant HSV-1 strains.[12] The latter compounds also proved active against pox viruses while the parent is nonactive.[13]

Several structure−reactivity properties have been revealed, e.g., the more electron-withdrawing the substituent attached to the aromatic ring, the more hydrolysis labile the *cyclo*Sal-triester.[5c] Due to the lipophilic character of *cyclo*Sal-phosphate triesters such as **2** and the chemically triggered delivery mechanism, a drug concentration equilibrium is formed through the cell membrane (Figure 2, step c). For an effective antiviral effect it is necessary to reach high intracellular concentrations of the pronucleotide, which then presumably leads to also high concentrations of the nucleotide. In order to trap the lipophilic *cyclo*Sal-triester, we designed the second generation of *cyclo*Sal-pronucleotides, the so-called "lock-in"-*cyclo*Sal-pronucleotides.

These pronucleotides bear a (carboxy)esterase-cleavable ester group attached to the aromatic ring. To avoid a considerable reduction of the chemical stability because of the electron-withdrawing effect of the ester moieties, the ester moiety was separated from the aromatic ring by an alkyl spacer (C_2 or C_3). Two types of ester-bearing *cyclo*Sal-triesters have been developed: the *cyclo*Sal-d4TMP acid ester and the *cyclo*Sal-d4TMP alcohol ester. Hydrolysis studies in phosphate buffer (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-d4TMP acid esters such as **3** (Figure 1). From *cyclo*Sal-triester **4**, d4TMP was released by the designed chemically induced pathway.[5] However, these "lock-in"-compounds led to a delayed drug delivery because the charged intermediate **4** showed high chemical stability.[14,15]

In this paper we disclose new, conceptually different, enzymatically activated *cyclo*Sal-pronucleotides **5** (Figure 1). In this new approach, lipophilic electron-donating substituents or substituents with only weak electron-withdrawing properties attached to the aromatic ring are converted into a polar acceptor substituent by intracellular cleavage (step d, Figure 2). In contrast to our initial approach,[14,15] the conversion into a strong acceptor group leads to a strong decrease in hydrolysis stability and thus a rapid formation of a charged intermediate (phosphodiester). Due to the resulting polarity, the diester should be trapped. From the phosphodiester, d4TMP is released subsequently (step e, Figure 2). This concept is based on the higher intracellular concentration of esterases compared to the extracellular medium.[16] In addition it is also based on a considerable difference between the high extracellular hydrolysis stability of the *cyclo*Sal-d4TMP bearing the lipophilic ester moiety (step a, Figure 2) and the low intracellular hydrolysis stability of *cyclo*Sal-d4TMPs after enzyme-driven cleavage (step d, Figure 2). Nevertheless, the nucleotide can also be formed from the intact X-group bearing phosphate triester after membrane passage. However, the rate of release is very low.

As a suitable substituent for this concept, geminal alkyl dicarboxylates (acylals) were considered, which could be converted into aldehydes by intracellular cleavage (Figure 3).

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[a] Abbreviations: AM, acetoxymethyl; POM, pivaloxymethyl; POC, isopropyloxycarbonylmethoxy; *cyclo*Sal, *cyclo*Saligenyl.

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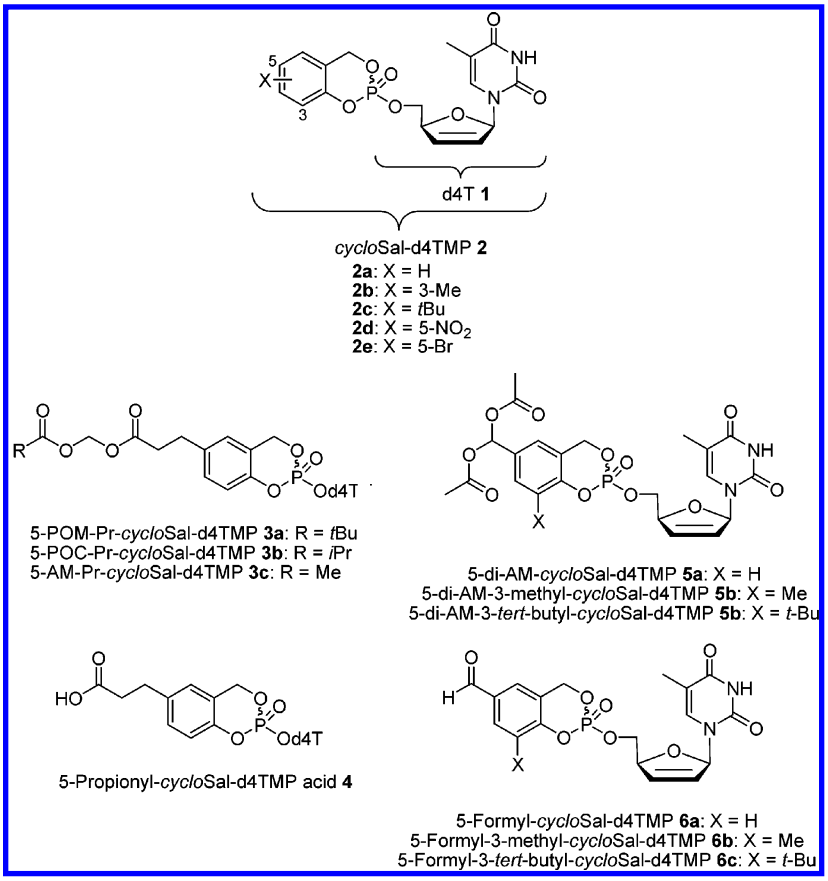


Figure 1. Structures of target compounds **5** and **6**, other *cyclo*Sal-d4TMPs **2**−**4** and d4T **1**.

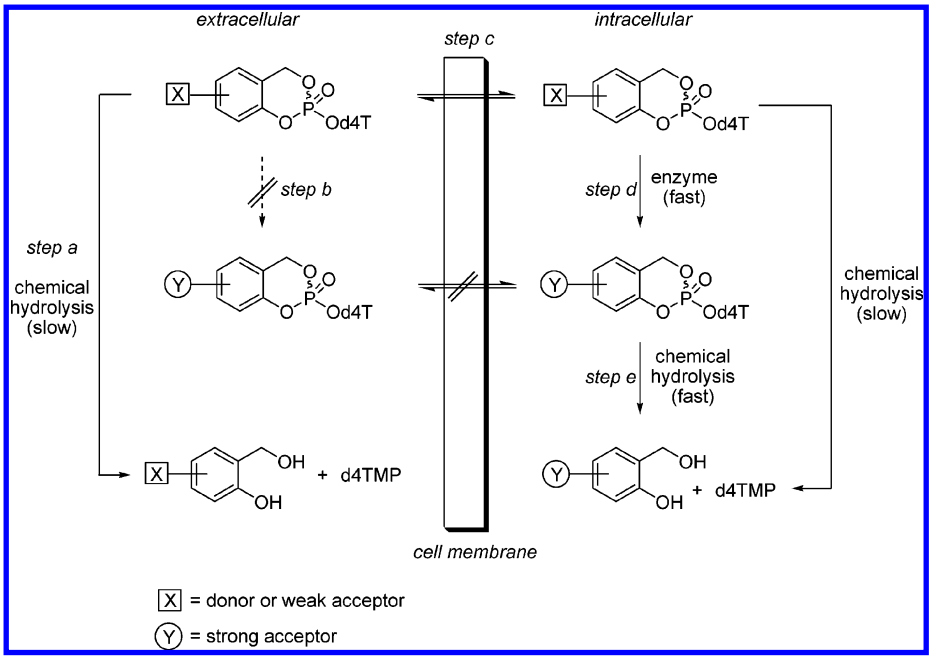


Figure 2. Concept of enzymatically activated *cyclo*Sal-d4T-monophosphates.

The strong electron-withdrawing effect of the formyl group should significantly decrease the stability of the *cyclo*Sal-triester in the same way as known for the 5-nitro-*cyclo*Sal-d4TMP (**2d**, Figure 1, $t_{1/2}$ = 0.15 h in phosphate buffer (pH = 7.3, Table 1)).[7] Thus, a sufficient difference in hydrolysis stability between triester **5** and formyl triester **6** could be reached.

Here, we report on the synthesis, properties, and antiviral evaluation of the third generation *cyclo*Sal-d4TMP pronucleotides.

Chemistry

To use our established phosphorus(III)-chemistry route,[5c] 4-formylsalicyl alcohols **7a**−**c** were synthesized first. 4-Bromosalicyl alcohols **8a**−**c** were used as key intermediates for the synthesis of **7**. The synthetic steps needed for the preparation of compounds **8** are summarized in Scheme 1, and all subsequent steps are shown in Scheme 2. While 4-bromosalicyl alcohol (**8a**) was prepared by reduction of the commercially

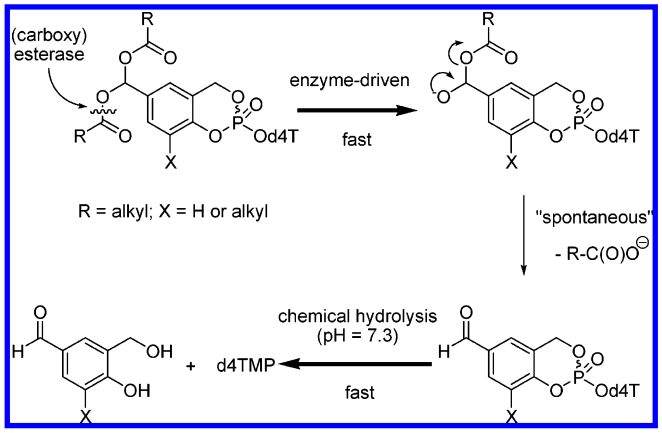


Figure 3. Enzyme-triggered hydrolysis cascade of 5-dialkyloxymethyl-*cyclo*Sal-d4TMPs.

available 5-bromosalicyl aldehyde (**9**) ($LiAlH_4$ in THF; 89% yield), 4-bromo-6-methylsalicyl alcohol (**8b**) was synthesized starting from 3-methylsalicyl acid (**10**). After conversion of salicylic acid **10** into the methyl ester **11** (concd H_2SO_4/CH_3OH; 86% yield), 5-bromo-3-methyl-methyl salicylate (**12**) was prepared by bromination (Br_2, $CHCl_3$; 83% yield).

Then, ester **12** was reduced in the same way as **9** to yield salicyl alcohol **8b** in 91%. 4-Bromo-6-*tert*-butylsalicyl alcohol (**8c**) was synthesized starting from 2-*tert*-butylphenol (**13**). *Ortho*-formylation of **13** mediated by $MgCl_2$ was carried out as described before (94% yield).[17] Bromination of the resulting 3-*tert*-butylsalicyl aldehyde (**14**) with Br_2 in acetic acid afforded 5-bromo-3-*tert*-butylsalicyl aldehyde (**15**) in 60% yield.[18] Reduction of aldehyde **15** with $NaBH_4$ yielded **8c** (71% yield).

Protection of salicyl alcohols **8a**−**c** as isopropylidene acetals was done with 2,2-dimethoxypropane, *p*-toluenesulfonic acid monohydrate, and anhydrous sodium sulfate in acetone (**16a**: 95%, **16b**: 83% yield, **16c**: 94%).[15] The resulting 4-bromosalicyl alcohol isopropylidene acetals **16a**−**c** were treated first with *n*-butyllithium in THF and afterward with *N*,*N*-dimethylformamide.[19]

This led via bromo/lithium-exchange to 4-formylsalicyl alcohol isopropylidene acetal **17a** (94% yield), to 4-formyl-6-methylsalicyl alcohol isopropylidene acetal **17b** (80% yield), and 4-formyl-6-*tert*-butylsalicyl alcohol isopropylidene acetal **17c** (71% yield), respectively. The acetals were cleaved with HCl to give 4-formylsalicyl alcohols **7a** (81% yield), **7b** (94% yield), and **7c** (68% yield). For the cleavage of the isopropylidene acetals **17a** and **17b**, only catalytic amounts of HCl were needed.[15]

4-Formylsalicyl alcohols **7a**−**c** were converted into the *cyclo*Sal triester **6a**−**c** using the standard procedure.[4,6] Because alcohols **7a** and **7b** were not soluble in Et_2O, THF was used as solvent instead. However, pyridinium chloride that is formed in the reaction is also more soluble in THF as compared to diethyl ether. Interestingly, in the synthesis of 5-formyl-3-methyl-*cyclo*Sal-d4TMP (**6b**) the formed saligenyl chlorophosphite intermediate was sufficiently soluble in Et_2O. Thus, THF was removed, Et_2O was added, and the precipitated pyridinium chloride was filtered after the reaction mixture was stored at −20 °C overnight. 5-Formyl-*cyclo*Sal-d4TMP **6a** was isolated in 31% yield, **6b** in 51%, and 5-formyl-3-*tert*-butyl-*cyclo*Sal-d4TMP **6c** in 49%, respectively. Finally, triesters **6a**−**c** were converted into the acylals 5-diacetoxymethyl-*cyclo*Sal-d4TMP **5a** (5-di-AM-*cyclo*Sal-d4TMP; 44% yield), 5-diacetoxymethyl-3-methyl-*cyclo*Sal-d4TMP **5b** (5-di-AM-3-Me-*cyclo*Sal-d4TMP; 45% yield), and 5-diacetoxymethyl-3-*tert*-butyl-*cyclo*Sal-d4TMP **5c** (5-di-AM-3-*t*Bu-*cyclo*Sal-d4TMP; 23% yield) with acetic anhydride and zirconium(IV) chloride.[20]

Characterization of triesters **5** and **6** was carried out by means of ^1H, ^{13}C, and ^{31}P NMR spectroscopy as well as high-resolution mass spectrometry.

Results and Discussion

Chemical Stability. Stability studies of the *cyclo*Sal-triesters **5** and **6** were done in aqueous 25 mM phosphate buffer (pH = 7.3). The hydrolysis products were detected by means of HPLC. The half-lives are summarized in Table 1. For comparison the data of the known triesters **2** and **3** are also given.[7,15]

The final hydrolysis products in the case of **5** and **6** as well as triesters **2** (except of **2b**,**c**) and **3** were exclusively d4TMP and the salicyl alcohol. The half-lives given in Table 1 refer to the cleavage of the triesters.

As expected, the formyl *cyclo*Sal-triester **6a** showed a very short half-life (0.18 h). The half-life is nearly identical to that of the 5-nitro-*cyclo*Sal-counterpart **2d** ($t_{1/2}$ = 0.15 h; Table 1). This is due to the nearly identical electron-withdrawing activity of the two substituents. In contrast, due to their electron-donating effect, alkyl groups in position 3 of the aromatic ring led to a higher hydrolysis stability (compare triester **2a**; $t_{1/2}$ = 4.4 h[5b] with 3-methyl-*cyclo*Sal-d4TMP **2b**; $t_{1/2}$ = 17.5 h[7] and 3-*tert*-butyl-*cyclo*Sal-d4TMP **2c**; $t_{1/2}$ = 73 h; Table 1). Therefore, the methyl substituent in triester **6b** increased the half-life 2-fold ($t_{1/2}$ = 0.35 h), while the *tert*-butyl group in triester **6c** led to a 5.3-fold higher chemical stability ($t_{1/2}$ = 0.95 h). The chemical stabilities of the 5-di-AM-*cyclo*Sal-d4TMPs **5** increased in the same order of magnitude (**5a**: $t_{1/2}$ = 1.2 h; **5b**: $t_{1/2}$ = 2.3 h; **5c**: $t_{1/2}$ = 7.0 h; Table 1). With regard to the basic concept, the difference between chemical stability of acylals **5** in comparison to chemical stability of the corresponding 5-formyl-*cyclo*Sal-d4TMPs (**6a**−**c**) is important. In all cases the difference was found to be 7-fold.

Finally, the selective release of d4TMP from 5-formyl-*cyclo*Sal-d4TMPs **6** was proven by ^{31}P NMR spectroscopy (as an example the chemical hydrolysis of **6b** is shown in Figure 4).

Hydrolysis in Cell Extracts, RPMI/FCS, and Human Serum. The cleavage of the acylal group of the 5-di-AM-*cyclo*Sal-d4TMPs **5a**−**c** was investigated in hydrolysis studies using T-lymphocyte CEM cell extracts (CE; Table 1). Half-lives were 0.08 h for compounds **5a** and **5b** and 0.12 h for compound **5c**.

Even more important, the expected 5-formyl-*cyclo*Sal-d4TMPs **6a**−**c** were selectively formed in all cases as proven by HPLC coelution experiments! The more or less identical half-lives of the three compounds led to the conclusion that the enzymatic cleavage is not affected by additional substituents in the *cyclo*Sal-moiety (methyl or *tert*-butyl) attached to the aromatic ring.

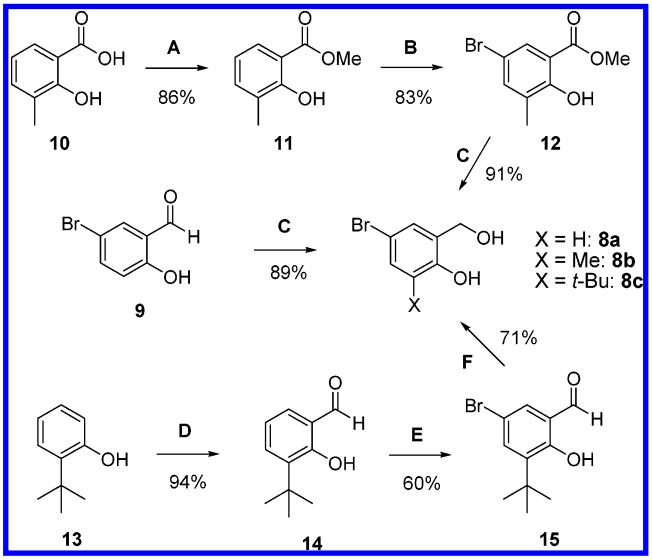
Compounds **5** were also incubated in RPMI-1640 culture medium containing 10% heat-inactivated fetal calf serum (RPMI-1640/FCS (10%)). In case of 5-di-AM-*cyclo*Sal-d4TMP **5a** the half-life was found to be 0.9 h. Thus, the stability was slightly reduced as compared to the PBS buffer, pH 7.3 ($t_{1/2}$ = 1.2 h; Table 1). The same was observed for triesters **5b** and **5c**. For triester **5b**, that showed a stability of $t_{1/2}$ = 2.3 h in phosphate buffer (Table 1), a half-life of 1.5 h was determined. For compound **5c** the half-life decreased from 7.0 to 4.7 h. This slight decrease in stability may be due to the more basic pH of the RPMI-1640 medium (pH 7.6) as compared to the PBS buffer. To get evidence for that, we incubated compounds **5b**,**c**

Table 1. Hydrolysis Data, Lipophilicity, and Antiviral Activity of **5** and **6** compared to **1**, **2**, and **3**

			$t_{1/2}$ [h][b]				EC_{50} [μM][c]			CC_{50}[μM][d]
							CEM/0[i]		*CEM/TK*$^-$[j]	
	substituent	logP_{calcd}[a]	PBS[e]	CE[f]	RPMI/FCS[g]	*serum*[h]	HIV-1	HIV-2	HIV-2	
6a	5-CHO	0.17	0.18	n.d.[l]	n.d.	n.d.	0.41 ± 0.29	0.15 ± 0.08	5.0 ± 4.6	113
6b	5-CHO-3-Me	0.67	0.35	n.d.	n.d.	n.d.	0.45 ± 0.32	1.27 ± 0.64	4.5 ± 0.7	82
6c	5-CHO-3-*t*-Bu	2.00	0.95	n.d.	n.d.	n.d.	0.45 ± 0.07	0.6 ± 0.0	17.5 ± 6.5	59
5a	5-di-AM	−0.16	1.2	0.08	0.9	1.5	0.42 ± 0.28	0.4 ± 0.0	10.5 ± 8.3	99
5b	5-di-AM-3-Me	0.34	2.3	0.08	1.5	3.6	0.50 ± 0.17	0.70 ± 0.14	6.3 ± 2.9	44
5c	5-di-AM-3-*t*-Bu	1.66	7.0	0.12	4.7	8.7	0.32 ± 0.14	0.75 ± 0.07	3.0 ± 0.0	17
3a	5-POM-Pr	4.04	5.6	0.38	n.d.	n.d.	0.23 ± 0.04	0.33 ± 0.11	0.7 ± 0.08	24
3b	5-POC-Pr	3.56	3.9	0.9	n.d.	n.d.	0.26 ± 0.20	0.60 ± 0.00	1.73 ± 1.97	74
3c	5-AM-Pr	2.18	4.3	0.25	n.d.	n.d.	0.20 ± 0.11	0.53 ± 0.39	25 ± 19.1	81
2a	H	0.28	4.4	4.0	n.d.	n.d.	0.10 ± 0.02	0.13 ± 0.04	0.90 ± 0.28	31
2b	3-Me	0.77	17.5	14.5	n.d.	n.d.	0.06	0.07	0.05	32
2c	3-*t*Bu	2.10	73	n.d.	n.d.	n.d.	0.18	0.65	0.33	35
2d	5-NO_2	0.50	0.15	n.d.	n.d.	n.d.	0.29	0.40	40	75
2e	5-Br	1.37	0.88	n.d.	n.d.	n.d.	0.14 ± 0.02	0.26 ± 0.01	15.0 ± 7.1	64
1	-	−0.48	n.a.[k]	n.a.	n.a.	n.a.	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 means of two to three independent experiments). [d] Cytostatic activity: 50% cytostatic concentration. [e] 25 mM phosphate buffer (pH = 7.3). [f] CEM cell extracts (pH = 6.9). [g] RPMI/10% heat-inactivated fetal calf serum (FCS), pH 7.6. [h] Human serum, pH 6.8. [i] Wild-type CEM cells. [j] Thymidine kinase-deficient CEM cells. [k] n.a.: not applicable. [l] n.d.: not determined.

Scheme 1. Synthesis of the 4-Bromosalicyl Alcohols **8a**−**c**[a]



[a] Reagents and conditions: Method A: CH_3OH, concd H_2SO_4, 0 °C to reflux, 20 h; method B: $CHCl_3$, Br_2, r.t., 3 d; method C: THF, $LiAlH_4$, r.t. to reflux, 3 h; method D: CH_3CN, Et_3N, $MgCl_2$, paraformaldehyde, reflux, 5 h; method E: HOAc, Br_2, r.t., 2 h; method F: EtOH, $NaBH_4$, r.t., 3 h.

in PBS at pH 7.6. As expected, the half-lives dropped from 2.3 h at pH 7.3 to 1.9 h (**5b**) and from 7.0 to 6.2 h for triester **5c**. However, the drop is not as high as observed in the RPMI/FCS medium. Thus, it cannot be excluded that although the fetal calf serum is heat-inactivated, residual esterase activity is responsible also for the further drop in stability. In this context it is interesting to mention, that for all compounds **5** some cleavage of the acylal group was observed by means of HPLC. This would again point to esterase cleavage of the acylal moiety in the RPMI/FCS. This instability of AM-acylals in culture medium containing FCS has been observed before for 5-AM-Pr-*cyclo*Sal-d4TMP **3c** (Figure 1).[15] Nevertheless, the final product in all cases was again d4TMP.

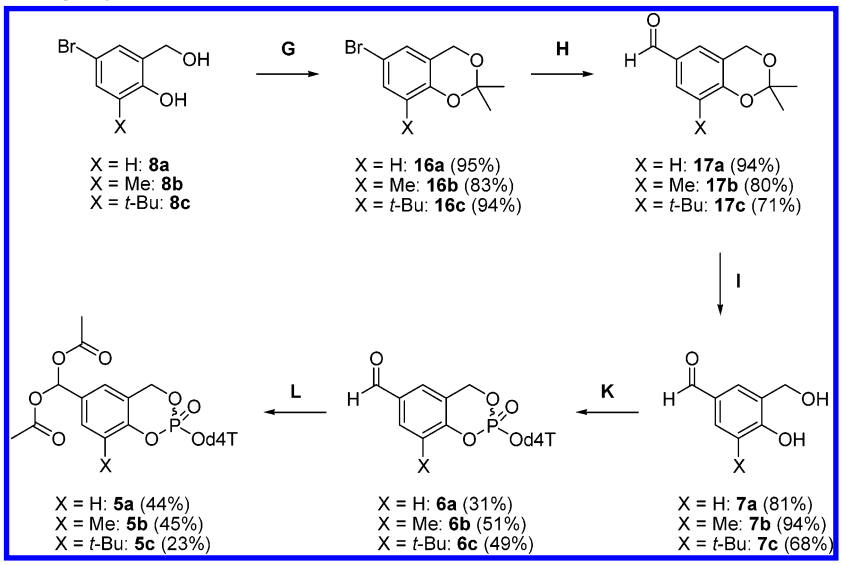
In contrast, studies in human serum, pH 6.8, revealed that no cleavage of the acylal group took place and half-lives increased as determined to be: **5a**: 1.5 h; **5b**: 3.6 h; **5c**: 8.7 h. Again, d4TMP was the sole product together in addition to the masking unit.

Antiviral Evaluation. *Cyclo*Sal-triester **5** and **6** were evaluated for their anti-HIV activity in wild-type CEM/0 and mutant thymidine kinase-deficient CEM/TK$^-$ cells (Table 1). As the reference compound d4T **1** was used. The *cyclo*Sal-d4TMPs **5** and **6** proved to be equipotent against HIV-1 and HIV-2 in wild-type CEM/0 cells as **1**. All triesters **5** and **6** are markedly more active against HIV-2 in CEM/TK$^-$ cells as compared to the parent nucleoside **1**. Nevertheless, some loss in activity in CEM/TK$^-$ cells for compounds with low hydrolysis stabilities (**5a** and **5b**, **6a**−**c**) in comparison to the activity in CEM/0 cells was observed (Table 1). This parallels the behavior of compound **2d** and 5-bromo-*cyclo*Sal-d4TMP (**2e**, Table 1), which have comparable half-lives in phosphate buffer (pH = 7.3; $t_{1/2}$ = 0.15 h (**2d**); $t_{1/2}$ = 0.88 h (**2e**)). Taking the results of the hydrolysis in RPMI-1640 culture media into account, the partial loss in activity of **5c** can be ascribed to the acylal cleavage in the culture media. Nevertheless, **5c** has nearly the same activity in CEM/TK$^-$ cells as the unsubstituted triester **2a**. Interestingly, each time compounds **5** or **6** are more active as compared to the parent nucleoside, it seems that the triesters are also more cytostatic (Table 1). This is a general phenomenon of the *cyclo*Sal-pronucleotides. However, this effect cannot be attributed to the released masking unit because for *cyclo*Sal-pronucleotides without any biological activity also no toxicity was found although the compounds released the nucleotides and the salicyl alcohols as well. We believe that this may be related to the higher amount of bioactive nucleoside phosphates formed in the cells interacting with the cellular processes as compared to the situation starting the metabolism from the parent nucleoside.

Conclusion

A synthetic route to enzymatically cleavable acylal-*cyclo*Sal-d4TMPs has been developed and the initial concept of an enzymatically driven accelerated cleavage of the *cyclo*Sal-triesters was proven. Acylal moieties in 5-diacetoxymethyl-*cyclo*Sal-d4TMPs **5** were found to be cleaved enzymatically to release 5-formyl-*cyclo*Sal-d4TMPs **6** ($t_{1/2}$ = 0.08−0.12 h) rapidly. The kinetics of this cleavage was independent of other substituents at the aromatic ring (H, methyl, or *tert*-butyl group). A significant difference (nearly 7-fold) in hydrolysis stability of compounds **5** and **6** at pH = 7.3 was found. Therefore, if a 5-diacetoxymethyl-*cyclo*Sal-d4TMP **5** is delivered into cells, a

Scheme 2. Synthesis of the target *cyclo*Sal-d4TMPs **5** and **6**[a]



[a] Method G: acetone, 2,2-dimethoxypropane, *p*TsOH, Na_2SO_4, 40 °C, 3 d; method H: THF, *n*-BuLi, DMF, −78 °C, 3 h; method I: CH_3CN/H_2O, (cat.) HCl; method K: (i) THF and/or Et_2O, PCl_3, pyridine, −20 °C to r.t., 4.5 h; (ii) CH_3CN, DIPEA, d4T **1**, −20 °C to r.t., 3 h; (iii) CH_3CN, *t*BuOOH, −20 °C to r.t., 1 h; method L: CH_3CN, acetic anhydride, $ZrCl_4$, r.t., 45 min.

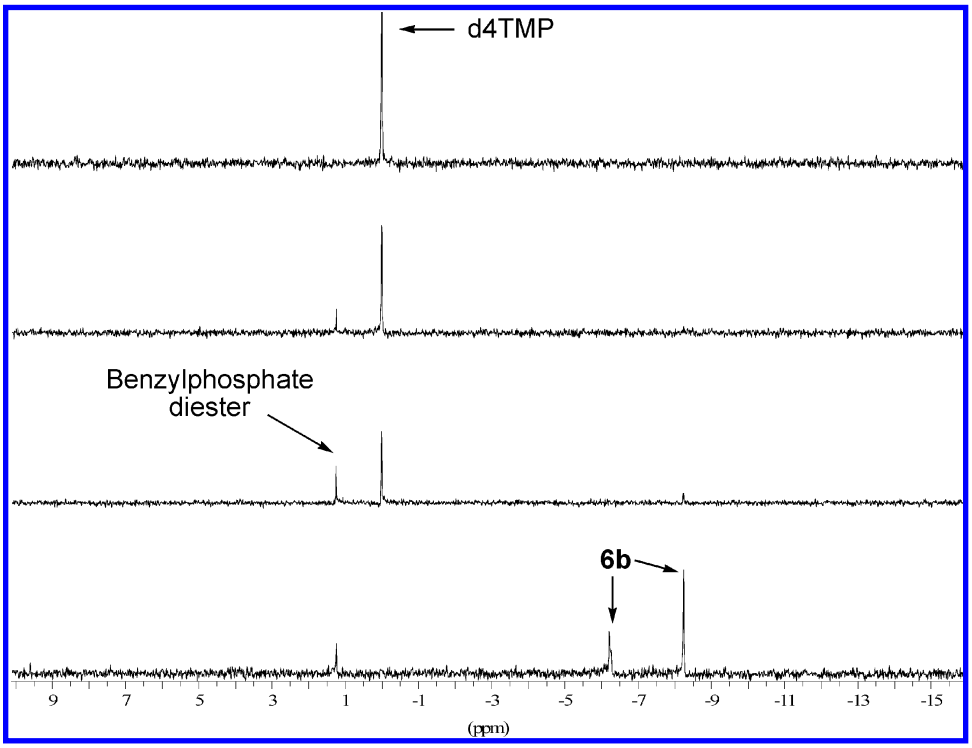


Figure 4. Chemical hydrolysis of **6b** followed by ^{31}P NMR spectroscopy {solvent: DMSO-d_6/50 mM imidazole/HCl buffer (pH = 7.3), 1:1 (v/v); spectra were recorded at t_0 (below), during hydrolysis (two middle spectra) and at the end of the hydrolysis (top); H_3PO_4 was used as external reference}.

fast cleavage to give 5-formyl-*cyclo*Sal-d4TMP **6** should proceed with the consequence of a rapid release of d4TMP. In the future, additional enzyme-cleavable groups will be studied in order to increase the chemical stability while leaving the enzymatic cleavage unchanged.

Experimental Section

NMR spectra were recorded with a Bruker AMX 400 or a Bruker DRX 500 Fourier transform spectrometer. All ^1H 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], 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 concd sulfuric acid, and 0.1 mL of glacial acetic acid). All preparative TLCs were performed on a chromatotron (Harrison Research, Model 7924T) using glass plates coated with 1 mm, 2 mm, 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** and **6** did not give useful microanalytical data most probably due to 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 destilled 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).

3-Methylsalicyl Acid Methyl Ester (11). 3-Methylsalicyl acid (**10**, 80.0 g, 0.53 mol) was dissolved in 1.3 L of CH_3OH, and concd sulfuric acid (252 mL, 4.73 mol) was added slowly at 0 °C. The reaction mixture was heated under reflux for 20 h, allowed to cool down, and then poured into 3 L of iced water. The aqueous layer was extracted three times with CH_2Cl_2, the combined organic layers were dried with sodium sulfate, and the solvent was removed in vacuo to yield a dark red oil as the crude product. Purification was achieved by distillation. Yield: 74.7 g (0.45 mol, 86%) of a colorless liquid. bp 64 °C. TLC R_f value 0.65 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.87 (s, 1H, phenol-OH), 7.67−7.63 (m, 1H, aryl-H-6), 7.46−7.41 (m, 1H, aryl-H-4), 6.90−6.83 (m, 1H, aryl-H-5), 3.90 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.27 (COO), 158.93 (aryl-C-2), 136.66 (aryl-C-4), 127.33 (aryl-C-6), 126.00 (aryl-C-3), 118.96 (aryl-C-5), 111.82 (aryl-C-1), 52.64 (OCH_3), 15.41 (CH_3) ppm. MS (FAB) *m*/*z* = calcd 167.1 [M + H$^+$], found 167.1 [M + H$^+$].

5-Bromo-3-methyl-methyl Salicylate (12). 3-Methylsalicyl acid methyl ester (**11**, 5.01 g, 30.1 mmol) was dissolved in 50 mL of $CHCl_3$. A solution of bromine (1.53 mL, 30.0 mmol) in 30 mL of $CHCl_3$ was added within 45 min at room temperature. The reaction mixture was stirred for 3 d at room temperature. After washing once with 39% sodium bisulfite solution and twice with water, the organic layer was dried with sodium sulfate and concentrated under reduced pressure. The product was purified by recrystallization from CH_3OH. Yield: 6.10 g (24.9 mmol, 83%) of colorless crystals. mp 112−114 °C. TLC R_f value 0.72 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.78 (s, 1H, phenol-OH), 7.75−7.73 (m, 1H, aryl-H-6), 7.66−7.63 (m, 1H, aryl-H-4), 3.91 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.84 (COO), 157.93 (aryl-C-2), 138.48 (aryl-C-4), 129.27 (aryl-C-3), 129.02 (aryl-C-6), 113.37 (aryl-C-1), 109.71 (aryl-C-5), 52.90 (OCH_3), 15.13 (CH_3) ppm. MS (FAB) *m*/*z* = calcd 244.0 [M], found 244.0 [M].

3-*tert*-Butylsalicyl Aldehyde (14). Under nitrogen, to a mixture of 2-*tert*-butylphenol (**13**, 15.0 g, 100 mmol) in 200 mL of dry CH_3CN were added paraformaldehyde (20.2 g, 674 mmol), anhydrous magnesium dichloride (14.3 g, 150 mmol), and dry triethylamine (52.2 mL, 374 mmol). The reaction mixture was stirred for 5 h at reflux. The mixture was then cooled to room temperature, and 5% aqueous HCl was added. The phases were separated with CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2 one more time. The combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The product was purified by flash chromatography (petroleum ether 50−70/CH_2Cl_2 gradient; 6:1). The product thus obtained was pure enough for further reaction as shown by ^{1}H NMR. Purification by preparative TLC [Chromatotron; petroleum ether 50−70] afforded an analytically pure sample (recovery: 74%). Yield: 16.7 g (93.7 mmol, 94%) of a yellow oil. TLC R_f value 0.67 (CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.79 (s, 1H, phenol-OH), 9.88 (s, 1H, CHO), 7.53 (dd, *J* = 7.7, 1.6 Hz, 1H, aryl-H-6), 7.40 (dd, *J* = 7.7, 1.6 Hz, 1H, aryl-H-4), 6.95 (dd, *J* = 7.7, 7.7 Hz, 1H, aryl-H-5), 1.42 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.28 (CHO), 161.38 (aryl-C-2), 138.43 (aryl-C-3), 134.24 (aryl-C-6), 132.12 (aryl-C-4), 120.83 (aryl-C-1), 119.35 (aryl-C-5), 35.00 (*t*-Bu-C), 29.35 (*t*-Bu-CH_3) ppm. MS (FAB) *m*/*z* = calcd 179.1 [M + H$^+$], found 179.1 [M + H$^+$].

5-Bromo-3-*tert*-butylsalicyl Aldehyde (15). To a stirred solution of 3-*tert*-butylsalicyl aldehyde (**14**, 16.4 g, 92.1 mmol) in 46 mL of HOAc was added a solution of Br_2 (5.18 mL, 102 mmol) in 20 mL of HOAc dropwise within 20 min. The reaction mixture was stirred for 3 h at room temperature and afterward diluted with CH_2Cl_2. The organic layer was washed with 39% sodium bisulfite solution, water, saturated aqueous $NaHCO_3$, and brine and was dried with sodium sulfate. The solvent was removed in vacuum. The product was purified by column chromatography on silica gel [petroleum ether 50−70/CH_2Cl_2 gradient (0−20%)]. Yield: 14.2 g (55.5 mmol, 60%) of yellow crystals. mp 58−60 °C. TLC R_f value 0.76 (CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.73 (s, 1H, phenol-OH), 9.81 (s, 1H, CHO), 7.58 (d, *J* = 2.4 Hz, 1H, aryl-H-6), 7.52 (d, *J* = 2.4 Hz, 1H, aryl-H-4), 1.40 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 196.15 (CHO), 160.37 (aryl-C-2), 141.31 (aryl-C-3), 137.16 (aryl-C-6), 133.76 (aryl-C-4), 121.86 (aryl-C-1), 111.29 (aryl-C-5), 35.30 (*t*-Bu-C), 29.17 (*t*-Bu-CH_3) ppm. MS (FAB) *m*/*z* = calcd 257.0 [M + H$^+$], found 257.0 [M + H$^+$].

Preparation of 4-Bromosalicyl Alcohols 8. The 4-bromosalicyl carbonyl compounds **9**, **12**, and **15** were reduced by standard procedures with $LiAlH_4$ or $NaBH_4$ as previously described in reference[21] to yield the respective 4-bromosalicyl alcohols **8a**−**c**. The products **8a** and **8b** were purified by column chromatography on silica gel with EtOAc and by preparative TLC [Chromatotron; CH_2Cl_2/CH_3OH gradient (0−5%)]. Compound **8c** was purified by column chromatography on silica gel with CH_2Cl_2.

4-Bromosalicyl Alcohol (8a). Quantities: 5-Bromosalicyl aldehyde (**9**, 8.00 g, 39.8 mmol) dissolved in 60 mL of dry THF, $LiAlH_4$ (1.35 g, 35.5 mmol) as a suspension in 100 mL of dry THF. Yield: 7.15 g (35.2 mmol, 89%) of slightly yellow crystals. mp 111−114 °C. TLC R_f value 0.13 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.69 (s, 1H, phenol-OH), 7.39 (d, *J* = 2.5 Hz, 1H, aryl-H-3), 7.19 (dd, *J* = 8.4, 2.5 Hz, 1H, aryl-H-5), 6.72 (d, *J* = 8.4 Hz, 1H, aryl-H-6), 5.11 (s, 1H, benzyl-OH), 4.44 (s, 2H, benzyl-H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 150.90 (aryl-C-1), 131.40 (aryl-C-2), 131.02 (aryl-C-5), 126.89 (aryl-C-3), 121.04 (aryl-C-4), 110.76 (aryl-C-6), 59.48 (benzyl-C) ppm. MS (FAB) *m*/*z* = calcd 202.0 [M], found 202.0 [M].

4-Bromo-6-methylsalicyl Alcohol (8b). Quantities: 5-Bromo-3-methyl-methyl salicylate (**12**, 2.00 g, 8.16 mmol) dissolved in 30 mL of dry THF, $LiAlH_4$ (620 mg, 16.3 mmol) as a suspension in 50 mL of dry THF. yield: 1.62 g (7.46 mmol, 91%) of colorless crystals. mp 67−69 °C. TLC R_f value 0.15 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.64 (s, 1H, phenol-OH), 7.24−7.21 (m, 1H, aryl-H-3), 7.17−7.13 (m, 1H, aryl-H-5), 5.30 (s, 1H, benzyl-OH), 4.51 (s, 2H, benzyl-H), 2.14 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 151.42 (aryl-C-1), 131.37 (aryl-C-2), 130.88 (aryl-C-5), 127.10 (aryl-C-6), 127.03 (aryl-C-3), 110.48 (aryl-C-4), 58.69 (benzyl-C), 15.98 (CH_3) ppm. MS (FAB) *m*/*z* = calcd 216.0 [M], found 216.0 [M].

4-Bromo-6-*tert*-butylsalicyl Alcohol (8c). Quantities: 5-Bromo-3-*tert*-butylsalicyl aldehyde (**15**, 14.0 g, 54.7 mmol) dissolved in 275 mL of EtOH and $NaBH_4$ (1.85 g, 48.9 mmol). Yield: 10.0 g (38.8 mmol, 71%) of an orange oil. TLC R_f value 0.39 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.77 (s, 1H, phenol-OH), 7.23 (d, *J* = 2.5 Hz, 1H, aryl-H-3), 7.14 (d, *J* = 2.5 Hz, 1H, aryl-H-5), 5.75 (s, 1H, benzyl-OH), 4.58 (s, 2H, benzyl-H), 1.34 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 152.81 (aryl-C-1), 139.36 (aryl-C-6), 131.20 (aryl-C-2), 127.62 (aryl-C-3), 127.34 (aryl-C-5), 110.94 (aryl-C-4), 60.03 (benzyl-C), 34.59 (*t*-Bu-C), 29.31 (*t*-Bu-CH_3) ppm. MS (FAB) *m*/*z* = calcd 258.0 [M], found 258.0 [M].

Preparation of 4-Bromosalicyl Alcohol Isopropylidene Acetals 16. The 4-bromosalicyl alcohol isopropylidene acetals **16a**−**c** were synthesized by standard procedures as previously described in reference.[15] Deviating from that **16b** and **16c** were purified by preparative TLC [Chromatotron; petroleum ether 50−70/ CH_2Cl_2 gradient (0−30%)].

4-Bromosalicyl Alcohol Isopropylidene Acetal (16a). Quantities: 4-Bromosalicyl alcohol (**8a**, 4.80 g, 23.6 mmol) dissolved in 95 mL of acetone, 2,2-dimethoxypropane (15.0 mL, 0.121 mol), *p*-toluenesulfonic acid monohydrate (450 mg, 2.37 mmol), anhydrous sodium sulfate (9.6 g). Yield: 5.45 g (22.4 mmol, 95%) of a yellow oil. TLC R_f value 0.60 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.33−7.28 (m, 2H, aryl-H-3, aryl-H-5), 6.76 (d, J = 7.6 Hz, 1H, aryl-H-6), 4.81 (s, 2H, benzyl-H), 1.46 (s, 6H, acetal-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 150.06 (aryl-C-1), 130.67 (aryl-C-5), 127.76 (aryl-C-3), 122.11 (aryl-C-2), 118.74 (aryl-C-6), 111.56 (aryl-C-4), 99.68 (acetal-C), 59.62 (benzyl-C), 24.45 (acetal-CH_3) ppm. MS (FAB) m/z = calcd 242.0 [M], found 242.0 [M].

4-Bromo-6-methylsalicyl Alcohol Isopropylidene Acetal (16b). Quantities: 4-Bromo-6-methylsalicyl alcohol (**8b**, 3.00 g, 13.8 mmol) dissolved in 60 mL acetone, 2,2-dimethoxypropane (8.70 mL, 70.2 mmol), *para*-toluenesulfonic acid monohydrate (262 mg, 1.38 mmol), anhydrous sodium sulfate (6.0 g). Yield: 2.78 g (10.8 mmol, 83%) of a yellow oil. TLC R_f value 0.67 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.22−7.20 (m, 1H, aryl-H-5), 7.12−7.10 (m, 1H, aryl-H-3), 4.78 (s, 2H, benzyl-H), 2.08 (s, 3H, CH_3), 1.46 (s, 6H, acetal-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 148.16 (aryl-C-1), 131.14 (aryl-C-5), 127.99 (aryl-C-6), 125.03 (aryl-C-3), 121.36 (aryl-C-2), 111.09 (aryl-C-4), 99.63 (acetal-C), 59.64 (benzyl-C), 24.59 (acetal-CH_3), 14.81 (CH_3) ppm. MS (FAB) m/z = calcd 256.0 [M], found 256.0 [M].

4-Bromo-6-*tert*-butylsalicyl Alcohol Isopropylidene Acetal (16c). Quantities: 4-Bromo-6-*tert*-butylsalicyl alcohol (**8c**, 5.17 g, 20.0 mmol) dissolved in 90 mL of acetone, 2,2-dimethoxypropane (12.6 mL, 102 mmol), *para*-toluenesulfonic acid monohydrate (379 mg, 2.00 mmol), anhydrous sodium sulfate (8.7 g). Yield: 5.63 g (18.8 mmol, 94%) of a yellow oil. TLC R_f value 0.78 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.17−7.15 (m, 1H, aryl-H-5), 7.15−7.12 (m, 1H, aryl-H-3), 4.81 (s, 2H, benzyl-H), 1.48 (s, 6H, acetal-CH_3), 1.30 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 148.56 (aryl-C-1), 139.68 (aryl-C-6), 127.30 (aryl-C-5), 125.50 (aryl-C-3), 122.11 (aryl-C-2), 111.68 (aryl-C-4), 99.08 (acetal-C), 59.91 (benzyl-C), 34.44 (*t*-Bu-C), 29.09 (*t*-Bu-CH_3), 24.46 (acetal-CH_3) ppm. MS (FAB) m/z = calcd 298.1 [M], found 298.1 [M].

General Procedure for the Preparation of 4-Formylsalicyl Alcohol Isopropylidene Acetals 17. Under nitrogen, the respective 4-bromosalicyl alcohol isopropylidene acetal (**16a-c**, 1.0 equiv.) was dissolved in dry THF, cooled down to −78 °C and a 1.6 M solution of *n*-butyl lithium (2.0 equiv.) in hexane was added dropwise. The reaction was stirred at −78 °C for 2 h and then treated with dry *N*,*N*-dimethylformamide (10.0−15.0 equiv.) as a 1:1 solution in dry THF. The reaction mixture was stirred at −78 °C for 0.75 h and warmed slowly up to room temperature. Then the mixture was diluted with ethyl ether, washed (3 X H_2O, 1 X brine) and dried with magnesium sulfate. The solvent was removed in vacuo and the residue was purified by preparative TLC [Chromatotron; petroleum ether 50−70 (**17a**, **17b**) or petroleum ether 50−70/CH_2Cl_2 gradient (0−50%; **17c**)].

4-Formylsalicyl Alcohol Isopropylidene Acetal (17a). Quantities: 4-Bromosalicyl alcohol isopropylidene acetal (**16a**, 1.61 g, 6.62 mmol) dissolved in 40 mL of dry THF, *n*-butyl lithium (8.80 mL, 1.6 M in hexane, 14.1 mmol), *N*,*N*-dimethylformamide (7.50 mL, 97.3 mmol) dissolved in 7.50 mL of dry THF. Yield: 1.13 g (5.88 mmol, 94%) of yellow crystals. mp 56−58 °C. TLC R_f value 0.19 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.84 (s, 1H, formyl-H), 7.73 (dd, J = 8.4, 2.0 Hz, 1H, aryl-H-5), 7.69−7.66 (m, 1H, aryl-H-3), 6.98 (d, J = 8.4 Hz, 1H, aryl-H-6), 4.92 (s, 2H, benzyl-H), 1.51 (s, 6H, acetal-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.42 (formyl-C), 156.12 (aryl-C-1), 129.97 (aryl-C-5), 129.18 (aryl-C-4), 127.46 (aryl-C-3), 120.12 (aryl-C-2), 117.26 (aryl-C-6), 100.65 (acetal-C), 59.90 (benzyl-C), 24.60 (acetal-CH_3) ppm. MS (FAB) m/z = calcd 193.1 [M + H^+], found 193.1 [M + H^+].

4-Formyl-6-methylsalicyl Alcohol Isopropylidene Acetal (17b). Quantities: 4-Bromo-6-methylsalicyl alcohol isopropylidene acetal (**16b**, 2.20 g, 8.56 mmol) dissolved in 50 mL of dry THF, *n*-butyl lithium (10.7 mL, 1.6 M in hexane, 17.1 mmol), *N*,*N*-dimethylformamide (6.60 mL, 85.7 mmol) dissolved in 6.60 mL of dry THF. Yield: 1.40 g (6.79 mmol, 80%) of a brown oil. TLC R_f value 0.49 (CH_2Cl_2/ CH_3OH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.81 (s, 1H, formyl-H), 7.63−7.60 (m, 1H, aryl-H-5), 7.53−7.50 (m, 1H, aryl-H-3), 4.92 (s, 2H, benzyl-H), 2.17 (s, 3H, CH_3), 1.52 (s, 6H, acetal-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.50 (formyl-C), 154.30 (aryl-C-1), 130.06 (aryl-C-5), 128.48 (aryl-C-4), 126.15 (aryl-C-6), 125.35 (aryl-C-3), 119.40 (aryl-C-2), 100.62 (acetal-C), 59.93 (benzyl-C), 24.73 (acetal-CH_3), 15.10 (CH_3) ppm. MS (FAB) m/z = calcd 207.1 [M + H^+], found 207.1 [M + H^+].

4-Formyl-6-*tert*-butylsalicyl Alcohol Isopropylidene Acetal (17c). Quantities: 4-Bromo-6-*tert*-butylsalicyl alcohol isopropylidene acetal (**16c**, 2.83 g, 9.46 mmol) dissolved in 55 mL of dry THF, *n*-butyl lithium (11.8 mL, 1.6 M in hexane, 18.9 mmol), *N*,*N*-dimethylformamide (10.9 mL, 142 mmol) dissolved in 10.9 mL of dry THF. Yield: 1.66 g (6.68 mmol, 71%) of a yellow oil. TLC R_f value 0.27 (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.84 (s, 1H, formyl-H), 7.69−7.66 (m, 1H, aryl-H-5), 7.54−7.51 (m, 1H, aryl-H-3), 4.93 (s, 2H, benzyl-H), 1.54 (s, 6H, acetal-CH_3), 1.36 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.72 (formyl-C), 154.75 (aryl-C-1), 137.70 (aryl-C-6), 128.36 (aryl-C-4), 126.22 (aryl-C-5), 125.54 (aryl-C-3), 120.09 (aryl-C-2), 100.09 (acetal-C), 60.18 (benzyl-C), 34.36 (*t*-Bu-C), 29.10 (*t*-Bu-CH_3), 24.59 (acetal-CH_3) ppm. MS (FAB) m/z = calcd 249.1 [M + H^+], found 249.1 [M + H^+].

4-Formylsalicyl Alcohol (7a). Two drops of concd hydrochloric acid were added to a solution of **17a** (1.08 g, 5.62 mmol) in 30 mL of CH_3CN/H_2O (7:3) and the reaction mixture was brought to boil with a heat gun for 30 s. This procedure was repeated until the deprotection has been completed (monitoring by TLC, CH_2Cl_2/CH_3OH, 9:1). The reaction mixture was diluted with CH_2Cl_2 and water. The organic layer was extracted three times with water. The combined aqueous layers were extracted five times with EtOAc. Afterward the combined organic layers were dried with sodium sulfate and the solvent was removed under reduced pressure. Yield: 690 mg (4.54 mmol, 81%) of yellow crystals. mp 142−145 °C. TLC R_f value 0.45 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.62 (s, 1H, phenol-OH), 9.80 (s, 1H, formyl-H), 7.89−7.86 (m, 1H, aryl-H-3), 7.64 (dd, J = 8.4, 2.0 Hz, 1H, aryl-H-5), 6.93 (d, J = 8.4 Hz, 1H, aryl-H-6), 5.17 (s, 1H, benzyl-OH), 4.50 (s, 2H, benzyl-H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.27 (formyl-C), 160.07 (aryl-C-1), 130.51 (aryl-C-5), 129.76 (aryl-C-2), 128.73 (aryl-C-3), 128.23 (aryl-C-4), 114.80 (aryl-C-6), 57.86 (benzyl-C) ppm. MS (FAB) m/z = calcd 153.1 [M + H^+], found 153.1 [M + H^+].

4-Formyl-6-methylsalicyl Alcohol (7b). Three drops of concd hydrochloric acid were added to a solution of **17b** (1.22 g, 5.92 mmol) in 30 mL of CH_3CN/H_2O (7:3) and the reaction mixture was brought to boil with a heat gun for 30 s. This procedure was repeated until the deprotection was complete (monitoring by TLC, CH_2Cl_2/CH_3OH, 9:1). The reaction mixture was diluted with CH_2Cl_2 and water and the aqueous layer was extracted two times with CH_2Cl_2. Then the combined organic layers were dried with sodium sulfate and the solvent was removed under reduced pressure. The product was purified by preparative TLC [Chromatotron; CH_2Cl_2/CH_3OH gradient (0−2%)]. Yield: 922 mg (5.55 mmol, 94%) of yellow crystals. mp 108−110 °C. TLC R_f value 0.42 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.78 (s, 1H, formyl-H), 7.71 (d, J = 1.7 Hz, 1H, aryl-H-3), 7.56 (d, J = 1.7 Hz, 1H, aryl-H-5), 4.59 (s, 2H, benzyl-H), 2.23 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.41 (formyl-C), 158.32 (aryl-C-1), 131.07 (aryl-C-5), 129.07 (aryl-C-4), 128.20 (aryl-C-2), 127.07 (aryl-C-3), 124.69 (aryl-C-6), 58.89 (benzyl-C), 16.17 (CH_3) ppm. MS (FAB) m/z = calcd 167.1 [M + H^+], found 167.1 [M + H^+].

4-Formyl-6-*tert*-butylsalicyl Alcohol (7c). To a solution of **17c** (1.59 g, 6.40 mmol) in 30 mL of CH_3CN/H_2O (7:3) 3 mL of concd hydrochloric acid were added. The reaction mixture was then

brought to boil with a heat gun for 30 s. This procedure was repeated until the deprotection was complete (monitoring by TLC, CH_2Cl_2). The reaction mixture was diluted with CH_2Cl_2 and water and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water twice, dried with sodium sulfate and concentrated under reduced pressure. The product was purified by preparative TLC [Chromatotron; petroleum ether 50−70/CH_2Cl_2 gradient (50−100%) and CH_2Cl_2/CH_3OH gradient (0−5%)]. Yield: 903 mg (4.34 mmol, 68%) of an orange oil. TLC R_f value 0.43 (CH_2Cl_2/CH_3OH, 19:1). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.81 (s, 1H, formyl-H), 7.68 (d, J = 2.0 Hz, 1H, aryl-H-5), 7.65 (d, J = 2.0 Hz, 1H, aryl-H-3), 4.69 (s, 2H, benzyl-H), 1.39 (s, 9H, *t*-Bu) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.61 (formyl-C), 159.92 (aryl-C-1), 136.97 (aryl-C-6), 128.37 (aryl-C-2), 127.97 (aryl-C-4), 127.58 (aryl-C-3), 127.32 (aryl-C-5), 60.59 (benzyl-C), 34.45 (*t*-Bu-C), 29.25 (*t*-Bu-CH_3) ppm. MS (FAB) m/z = calcd 209.1 [M + H$^+$], found 209.1 [M + H$^+$].

5-Formyl-*cyclo*Sal-d4T-monophosphate (6a). Under nitrogen, a solution of the salicyl alcohol derivate **7a** (300 mg, 1.97 mmol) in 15 mL of dry THF was cooled to −20 °C. After addition of freshly destilled phosphorus(III)chlorid (0.21 mL, 2.4 mmol) and stirring at −20 °C for 10 min, a solution of dry pyridine (0.37 mL, 4.6 mmol) in dry THF was added at the same temperature over a period of 3 h. After completion of the addition the reaction mixture was allowed to warm up to room temperature and stirred for 1.5 h. It was kept at −20 °C overnight for best possible precipitation of pyridinium chloride. Filtration under nitrogen and concentration of the filtrate under reduced pressure afforded the phosphitylating agent (saligenyl chlorophosphite) as a crude product to be directly used for the synthesis of the *cyclo*Sal phosphate triester **6a** without further purification. The general synthesis of *cyclo*Sal-d4T-monophosphates has been published before.[4,6] To a solution of d4T (**1**, 180 mg, 0.804 mmol) in 15 mL of dry CH_3CN was added DIPEA (0.22 mL, 1.3 mmol). The resulting solution was cooled to −20 °C and the saligenyl chlorophosphite (396 mg, 1.83 mmol) dissolved in dry CH_3CN was added. The reaction mixture was allowed to warm up to room temperature and stirring was continued for 3 h. Subsequently, *tert*-butyl hydroperoxide (5.5 M in *n*-nonane; 0.45 mL, 2.5 mmol) was added at −20 °C. After warming up to room temperature and stirring for 1 h the reaction mixture was poured over 1 M HOAc/ NaOAc buffer (pH = 5). The aqueous layer was extracted with EtOAc four times. The combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by TLC [Chromatotron; CH_2Cl_2/CH_3OH (0.1% HOAc) gradient (0−5%)]. The isolated product was lyophilized from CH_3CN/H_2O 1:1 (v/v). Yield: 105 mg (0.250 mmol, 31%) of a diastereomeric mixture (ratio 0.8:1.0) as a colorless foam. TLC R_f value 0.50 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.34−11.32 (m, 2H, 2 X NH), 9.95 (s, 2H, 2 X formyl-H), 7.97−7.90 (m, 2H, 2 X aryl-H-4), 7.89−7.85 (m, 2H, 2 X aryl-H-6), 7.35 (d, J = 8.2 Hz, 1H, 1 X aryl-H-3), 7.32 (d, J = 8.4 Hz, 1H, 1 X aryl-H-3), 7.18 (s, 1H, 1 X thymine-H-6), 7.17 (s, 1H, 1 X thymine-H-6), 6.81−6.79 (m, 1H, 1 X 1′-H), 6.78−6.76 (m, 1H, 1 X 1′-H), 6.44−6.41 (m, 1H, 1 X 3′-H), 6.39−6.36 (m, 1H, 1 X 3′-H), 6.05−6.00 (m, 2H, 2′-H), 5.63 (dd, J = 14.8, 6.9 Hz, 1H, 1 X benzyl-H), 5.60 (dd, J = 14.8, 6.3 Hz, 1H, 1 X benzyl-H), 5.53−5.46 (m, 2H, 2 X benzyl-H), 4.99−4.94 (m, 2H, 2 X 4′-H), 4.40−4.27 (m, 4H, 2 X 5′-H), 1.67 (s, 3H, 1 X thymine-CH_3), 1.63 (s, 3H, 1 X thymine-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.47 (2 X formyl-C), 163.67 (2 X thymine-C-4), 163.66 (2 X aryl-C-2), 150.73 (2 X thymine-C-2), 134.96 (2 X thymine-C-6), 131.90 (2 X C-3′), 131.03 (2 X aryl-C-4), 127.29 (2 X aryl-C-5), 126.91 (2 X aryl-C-6), 126.69 (2 X C-2′), 122.16 (2 X aryl-C-1), 118.45 (2 X aryl-C-3), 109.70 (2 X thymine-C-5), 88.73 (2 X C-1′), 84.53 (2 X C-4′), 68.34 (2 X C-5′), 67.47 (2 X benzyl-C), 11.51 (2 X thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.84, −10.03. HRMS (ESI$^+$) m/z = calcd 443.0620 [M + Na$^+$], found 443.0585 [M + Na$^+$]. UV/vis (water/CH_3CN): λ_{max} = 258 nm.

5-Formyl-3-methyl-*cyclo*Sal-d4T-monophosphate (6b). The preparation of **6b** was carried out as described above for **6a**, but for further purification the saligenyl chlorophosphite was not storred in dry THF overnight at −20 °C but in dry Et_2O for a complete precipitation of pyridinium chloride. Quantities (for saligenyl chlorophosphite synthesis): 4-Formyl-6-methylsalicyl alcohol (**7b**, 750 mg, 4.51 mmol) dissolved in 35 mL of dry THF, freshly destilled phosphorus(III)chlorid (0.46 mL, 5.3 mmol), dry pyridine (0.81 mL, 10 mmol) in dry THF. Yield: 623 mg (2.72 mmol). Quantities (for *cyclo*Sal-d4T-monophosphate synthesis): Saligenyl chlorophosphite (620 mg, 2.71 mmol) dissolved in dry CH_3CN, d4T (**1**, 285 mg, 1.27 mmol) dissolved in 24 mL of dry CH_3CN, DIPEA (0.35 mL, 2.0 mmol), *tert*-butyl hydroperoxide (5.5 M in *n*-nonane; 0.70 mL, 3.9 mmol). Yield: 282 mg (0.649 mmol, 51%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. TLC R_f value 0.44 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.37 (s, 1H, 1 X NH), 11.34 (s, 1H, 1 X NH), 9.91 (s, 1H, 1 X formyl-H), 9.90 (s, 1H, 1 X formyl-H), 7.84−7.81 (m, 2H, 2 X aryl-H-4), 7.72−7.68 (m, 2H, 2 X aryl-H-6), 7.19 (d, J = 1.0 Hz, 1H, 1 X thymine-H-6), 7.16 (d, J = 1.0 Hz, 1H, 1 X thymine-H-6), 6.81−6.76 (m, 2H, 2 X 1′-H), 6.44−6.41 (m, 1H, 1 X 3′-H), 6.40−6.36 (m, 1H, 1 X 3′-H), 6.05−6.00 (m, 2H, 2′-H), 5.60 (dd, J = 14.8, 9.8 Hz, 1H, 1 X benzyl-H), 5.57 (dd, J = 15.1, 8.8 Hz, 1H, 1 X benzyl-H), 5.48 (dd, J = 13.9, 6.9 Hz, 1H, 1 X benzyl-H), 5.45 (dd, J = 14.2, 8.2 Hz, 1H, 1 X benzyl-H), 4.98−4.93 (m, 2H, 2 X 4′-H), 4.38−4.25 (m, 4H, 2 X 5′-H), 2.29 (s, 3H, 1 X CH_3), 2.26 (s, 3H, 1 X CH_3), 1.67 (s, 6H, 2 X thymine-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 191.49 (1 X formyl-C), 191.46 (1 X formyl-C), 163.63 (1 X thymine-C-4), 163.60 (1 X thymine-C-4), 152.24 (2 X aryl-C-2), 150.62 (2 X thymine-C-2), 135.62 (2 X thymine-C-6), 132.71 (1 X C-3′), 132.61 (1 X C-3′), 132.36 (2 X aryl-C-4), 131.94 (2 X aryl-C-5), 127.45 (2 X aryl-C-3), 127.30 (2 X C-2′), 125.17 (1 X aryl-C-6), 125.14 (1 X aryl-C-6), 121.92 (2 X aryl-C-1), 109.62 (1 X thymine-C-5), 109.53 (1 X thymine-C-5), 89.26 (1 X C-1′), 89.21 (1 X C-1′), 84.05 (1 X C-4′), 83.98 (1 X C-4′), 68.84 (1 X C-5′), 68.77 (1 X C-5′), 67.95 (1 X benzyl-C), 67.87 (1 X benzyl-C), 14.80 (1 X CH_3), 14.71 (1 X CH_3), 11.79 (1 X thymine-CH_3), 11.75 (1 X thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.07, −9.53. HRMS (ESI$^+$) m/z = calcd 457.0777 [M + Na$^+$], found 457.0787 [M + Na$^+$]. UV/vis (water/CH_3CN): λ_{max} = 260 nm.

5-Formyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (6c). The preparation of **6c** was carried out as described above for **6a**, but dry Et_2O was used as solvent. Quantities (for saligenyl chlorophosphite synthesis): 4-Formyl-6-*tert*-butylsalicyl alcohol (**7c**, 550 mg, 2.64 mmol) dissolved in 18 mL of dry Et_2O, freshly destilled phosphorus(III)chlorid (0.28 mL, 3.2 mmol), dry pyridine (0.50 mL, 6.2 mmol) in dry Et_2O; yield: 308 mg (1.13 mmol). Quantities (for *cyclo*Sal-d4T-monophosphate synthesis): Saligenyl chlorophosphite (130 mg, 0.477 mmol) dissolved in dry CH_3CN, d4T (**1**, 50 mg, 0.22 mmol) dissolved in 4 mL of dry CH_3CN, DIPEA (60 μL, 0.34 mmol), *tert*-butyl hydroperoxide (5.5 M in *n*-nonane; 0.12 mL, 0.67 mmol). Yield: 52 mg (0.11 mmol, 49%) of a diastereomeric mixture (ratio 0.7:1.0) as a colorless foam. TLC R_f value 0.56 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.35 (s, 1H, 1 X NH), 11.32 (s, 1H, 1 X NH), 9.96 (s, 2H, 2 X formyl-H), 7.94−7.91 (m, 2H, 2 X aryl-H-4), 7.78−7.75 (m, 2H, 2 X aryl-H-6), 7.22 (s, 1H, 1 X thymine-H-6), 7.19 (s, 1H, 1 X thymine-H-6), 6.84−6.77 (m, 2H, 2 X 1′-H), 6.45−6.39 (m, 2H, 2 X 3′-H), 6.06−6.01 (m, 2H, 2′-H), 5.61−5.44 (m, 4H, 2 X benzyl-H), 5.01−4.95 (m, 2H, 2 X 4′-H), 4.39−4.33 (m, 4H, 2 X 5′-H), 1.61 (s, 3H, 1 X thymine-CH_3), 1.57 (s, 3H, 1 X thymine-CH_3), 1.38 (s, 9H, 1 X *t*-Bu), 1.35 (s, 9H, 1 X *t*-Bu) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 192.23 (1 X formyl-C), 192.19 (1 X formyl-C), 163.75 (1 X thymine-C-4), 163.70 (1 X thymine-C-4), 153.07 (2 X aryl-C-2), 150.72 (2 X thymine-C-2), 139.39 (1 X aryl-C-3), 139.32 (1 X aryl-C-3), 135.88 (2 X thymine-C-6), 132.94 (1 X C-3′), 132.90 (1 X C-3′), 131.96 (2 X aryl-C-5), 129.33 (2 X aryl-C-4), 127.47 (1 X C-2′), 127.32 (1 X C-2′), 125.77 (2 X aryl-C-6), 123.89 (1 X aryl-C-1), 123.80 (1 X aryl-C-1), 109.70 (1 X thymine-C-5), 109.59 (1 X thymine-C-5), 89.35 (1 X C-1′), 89.22 (1 X C-1′), 84.11 (2 X C-4′), 69.28 (1 X C-5′), 69.23 (1 X C-5′), 67.89 (1 X benzyl-C), 67.83 (1 X benzyl-C), 34.57 (1 X *t*-Bu-C), 34.50 (1 X

t-Bu-C), 29.28 (1 X *t*-Bu-CH_3), 29.24 (1 X *t*-Bu-CH_3), 11.82 (2 X thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): $\delta = -9.07$, -9.43. HRMS (ESI^+) m/z = calcd 499.1246 [M + Na^+], found 499.1252 [M + Na^+]. UV/vis (water/CH_3CN): $\lambda_{max} = 260$ nm.

General Procedure for the Preparation of 5-Diacetoxymethyl-*cyclo*Sal-d4T-monophosphates 5. Under nitrogen, to a mixture of the respective 5-Formyl-*cyclo*Sal-d4T-monophosphate (**6a-c**; 1.0 equiv.) dissolved in dry CH_3CN and freshly distilled acetic anhydride (25.0 equiv.) was added zirconium(IV)chloride ($ZrCl_4$; 0.5 equiv.) in one portion. The reaction mixture was stirred for 45 min at room temperature and monitored by TLC (CH_2Cl_2/CH_3OH 9:1). Then the mixture was diluted with phosphate buffer (pH = 7.3) and EtOAc and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried with sodium sulfate and the solvent was removed in vacuo. The residue was purified by preparative TLC [Chromatotron; CH_2Cl_2/CH_3OH gradient (0−5%)]. The isolated product was lyophilized from CH_3CN/H_2O 1:1 (v/v).

5-Diacetoxymethyl-*cyclo*Sal-d4T-monophosphate (5a). Quantities: 5-Formyl-*cyclo*Sal-d4T-monophosphate (**6a**, 40 mg, 95 μmol) dissolved in 0.5 mL of dry CH_3CN, freshly distilled acetic anhydride (0.23 mL, 2.4 mmol), $ZrCl_4$ (11 mg, 48 μmol). Yield: 22 mg (42 μmol, 44%) of a diastereomeric mixture as a colorless foam. TLC R_f value 0.55 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.37−11.31 (m, 2H, 2 X NH), 7.58−7.49 (m, 4H, 2 X aryl-H-4, 2 X H-8), 7.49−7.45 (m, 2H, 2 X aryl-H-6), 7.25−7.14 (m, 4H, 2 X aryl-H-3, 2 X thymine-H-6), 6.85−6.76 (m, 2H, 2 X 1′-H), 6.44−6.40 (m, 1H, 1 X 3′-H), 6.39−6.35 (m, 1H, 1 X 3′-H), 6.06−5.98 (m, 2H, 2′-H), 5.55 (dd, J = 14.8, 7.9 Hz, 1H, 1 X benzyl-H), 5.52 (dd, J = 16.4, 7.9 Hz, 1H, 1 X benzyl-H), 5.47−5.39 (m, 2H, 2 X benzyl-H), 4.99−4.93 (m, 2H, 2 X 4′-H), 4.39−4.25 (m, 4H, 2 X 5′-H), 2.11 (s, 12H, 2 X H-10), 1.67 (s, 3H, 1 X thymine-CH_3), 1.61 (s, 3H, 1 X thymine-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.57 (2 X C-9), 163.68 (2 X thymine-C-4), 162.92 (2 X aryl-C-2), 149.99 (2 X thymine-C-2), 134.58 (2 X thymine-C-6), 132.76 (2 X C-3′), 132.48 (2 X aryl-C-5), 127.32 (2 X aryl-C-4), 126.37 (2 X C-2′), 124.62 (1 X aryl-C-6), 124.58 (1 X aryl-C-6), 122.62 (2 X aryl-C-1), 117.67 (2 X aryl-C-3), 109.66 (2 X thymine-C-5), 89.17 (1 X C-1′), 89.15 (1 X C-1′), 88.30 (2 X C-8), 84.04 (2 X C-4′), 68.13 (2 X C-5′), 68.00 (2 X benzyl-C), 20.53 (2 X C-10), 11.88 (1 X thymine-CH_3), 11.81 (1 X thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): $\delta = -9.52$. HRMS (ESI^+) m/z = calcd 545.0937 [M + Na^+], found 545.0939 [M + Na^+]. UV/vis (water/CH_3CN): $\lambda_{max} = 265$ nm.

5-Diacetoxymethyl-3-methyl-*cyclo*Sal-d4T-monophosphate (5b). Quantities: 5-Formyl-3-methyl-*cyclo*Sal-d4T-monophosphate (**6b**, 50.0 mg, 115 μmol) dissolved in 0.5 mL of dry CH_3CN, freshly distilled acetic anhydride (0.27 mL, 2.8 mmol), $ZrCl_4$ (13 mg, 56 μmol). Yield: 28 mg (52 μmol, 45%) of a diastereomeric mixture (ratio 0.8:1.0) as a colorless foam. TLC R_f value 0.51 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.36−11.31 (m, 2H, 2 X NH), 7.48 (s, 2H, 2 X H-8), 7.43−7.40 (m, 2H, 2 X aryl-H-4), 7.30−7.27 (m, 2H, 2 X aryl-H-6), 7.19 (s, 2H, 2 X thymine-H-6), 6.82−6.78 (m, 2H, 2 X 1′-H), 6.43−6.40 (m, 1H, 1 X 3′-H), 6.39−6.35 (m, 1H, 1 X 3′-H), 6.05−5.99 (m, 2H, 2′-H), 5.53 (dd, J = 14.8, 8.8 Hz, 1H, 1 X benzyl-H), 5.49 (dd, J = 15.5, 8.8 Hz, 1H, 1 X benzyl-H), 5.40 (dd, J = 13.2, 4.4 Hz, 1H, 1 X benzyl-H), 5.38 (dd, J = 14.2, 4.4 Hz, 1H, 1 X benzyl-H), 4.98−4.93 (m, 2H, 2 X 4′-H), 4.35−4.22 (m, 4H, 2 X 5′-H), 2.24 (s, 3H, 1 X CH_3), 2.21 (s, 3H, 1 X CH_3), 2.10 (s, 12H, 2 X H-10), 1.64 (s, 3H, 1 X thymine-CH_3), 1.62 (s, 3H, 1 X thymine-CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.53 (2 X C-9), 163.65 (2 X thymine-C-4), 152.94 (2 X aryl-C-2), 150.65 (2 X thymine-C-2), 135.61 (2 X thymine-C-6), 132.75 (1 X C-3′), 132.71 (1 X C-3′), 129.12 (2 X aryl-C-4), 127.41 (2 X aryl-C-3), 127.31 (1 X C-2′), 127.29 (1 X C-2′), 122.75 (2 X aryl-C-1), 121.99 (2 X aryl-C-6), 109.63 (2 X thymine-C-5), 89.19 (1 X C-1′), 89.12 (1 X C-1′), 88.34 (2 X C-8), 84.06 (1 X C-4′), 84.03 (1 X C-4′), 68.63 (1 X C-5′), 68.58 (1 X C-5′), 68.08 (1 X benzyl-C), 68.01 (1 X benzyl-C), 20.51 (2 X C-10), 14.84 (1 X CH_3), 14.71 (1 X CH_3), 11.81 (1 X thymine-CH_3), 11.77 (1 X thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): $\delta = -8.82$, -8.93. HRMS (ESI^+) m/z = calcd 559.1094 [M + Na^+], found 559.1103 [M + Na^+]. UV/vis (water/CH_3CN): $\lambda_{max} = 265$ nm.

5-Diacetoxymethyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (5c). Quantities: 5-Formyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (**6c**, 39.0 mg, 81.9 μmol) dissolved in 1.0 mL of dry CH_3CN, freshly distilled acetic anhydride (0.20 mL, 2.1 mmol), $ZrCl_4$ (9.4 mg, 40 μmol). Yield: 11 mg (19 μmol, 23%) of a diastereomeric mixture (ratio 0.7:1.0) as a colorless foam. TLC R_f value 0.61 (CH_2Cl_2/CH_3OH, 9:1). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.35 (s, 1H, 1 X NH), 11.33 (s, 1H, 1 X NH), 7.52 (s, 2H, 2 X H-8), 7.47−7.44 (m, 2H, 2 X aryl-H-4), 7.39−7.36 (m, 2H, 2 X aryl-H-6), 7.22 (d, J = 1.3 Hz, 1H, 1 X thymine-H-6), 7.18 (d, J = 1.3 Hz, 1H, 1 X thymine-H-6), 6.84−6.78 (m, 2H, 2 X 1′-H), 6.44−6.38 (m, 2H, 2 X 3′-H), 6.06−6.01 (m, 2H, 2′-H), 5.54−5.36 (m, 4H, 2 X benzyl-H), 5.00−4.94 (m, 2H, 2 X 4′-H), 4.40−4.29 (m, 4H, 2 X 5′-H), 2.11 (s, 12H, 2 X H-10), 1.59 (d, J = 1.3 Hz, 3H, 1 X thymine-CH_3), 1.57 (d, J = 1.3 Hz, 3H, 1 X thymine-CH_3), 1.35 (s, 9H, 1 X *t*-Bu), 1.31 (s, 9H, 1 X *t*-Bu) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.53 (2 X C-9), 163.69 (1 X thymine-C-4), 163.64 (1 X thymine-C-4), 150.66 (2 X thymine-C-2), 149.21 (2 X aryl-C-2), 138.72 (2 X aryl-C-3), 135.66 (1 X thymine-C-6), 135.59 (1 X thymine-C-6), 132.70 (2 X C-3′), 127.36 (1 X C-2′), 127.31 (1 X C-2′), 125.59 (2 X aryl-C-4), 123.40 (1 X aryl-C-1), 123.32 (1 X aryl-C-1), 122.78 (2 X aryl-C-6), 109.68 (1 X thymine-C-5), 109.61 (1 X thymine-C-5), 89.19 (1 X C-1′), 89.06 (1 X C-1′), 88.60 (2 X C-8), 84.16 (1 X C-4′), 84.09 (1 X C-4′), 68.95 (1 X C-5′), 68.89 (1 X C-5′), 68.01 (1 X benzyl-C), 67.96 (1 X benzyl-C), 34.41 (1 X *t*-Bu-C), 34.35 (1 X *t*-Bu-C), 29.36 (1 X *t*-Bu-CH_3), 29.32 (1 X *t*-Bu-CH_3), 20.51 (2 X C-10), 11.70 (2 X thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): $\delta = -8.56$, -8.92. HRMS (ESI^+) m/z = calcd 601.1563 [M + Na^+], found 601.1594 [M + Na^+]. UV/vis (water/ CH_3CN): λ_{max} = 265 nm.

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 (method I) have been described before.[22,23] Studies in cell extracts and human serum (obtained from pooled blood samples from the university hospital in Hamburg) were performed as reported in reference[15] with different incubation times but without using acetic acid to stop the reaction.

^{31}P NMR hydrolysis studies of *cyclo*Sal phosphate triesters: ^{31}P NMR hydrolysis studies of *cyclo*Sal nucleotides **5** and **6** were carried out as described before.[23]

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.G. 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: Analytical HPLC data of new *cyclo*Sal compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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