5-(1-Acetoxyvinyl)-*cyclo*Saligenyl-2′,3′-dideoxy-2′,3′- didehydrothymidine Monophosphates, a Second Type of New, Enzymatically Activated *cyclo*Saligenyl Pronucleotides

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In our attempt to further develop the *cyclo*Sal pronucleotide concept, we report on 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs as a new type of enzyme-activated pronucleotides. These compounds were converted into 5-acetyl-*cyclo*Sal-d4TMPs by (carboxy)esterase cleavage inside the cells. The enzymatic reaction led to the formation of a strong electron-withdrawing substituent that strongly accelerates the chemical hydrolysis of the *cyclo*Sal nucleotide to give d4TMP. For some *cyclo*Sal-d4TMPs a separation into the diastereomers was achieved. The absolute configuration was assigned by correlation of circular dichroism spectra with similar compounds. Most of the compounds showed complete retention of antiviral activity in TK-deficient CEM/TK$^-$ cells, which proves the TK-bypass potential of this approach. Interestingly, (S_P)-isomers of *cyclo*Sal phosphate triesters showed better antiviral activity in HIV-2-infected thymidine-kinase deficient CEM/TK$^-$ cells than their (R_P)-counterparts.

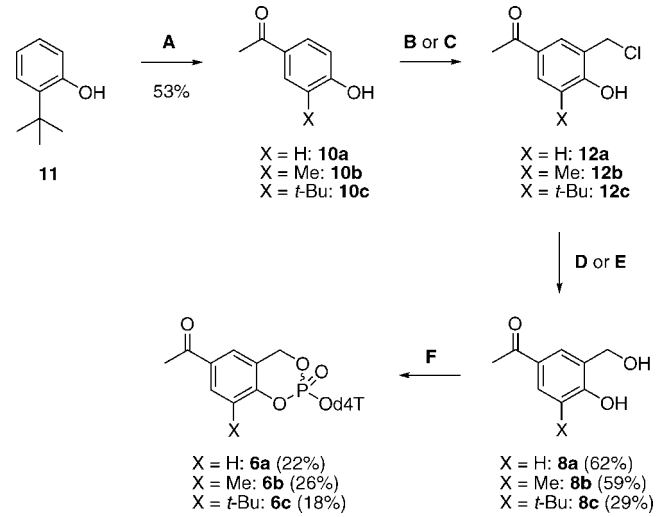
Introduction

The *cyclo*Sal[a] concept has been widely used for the delivery of nucleoside monophosphates into cells.[1,2] Because of the lipophilic character of *cyclo*Sal phosphate triesters and their chemically triggered delivery mechanism, a drug concentration equilibrium is formed through the cell membrane. In order to trap *cyclo*Sal triesters inside the cells, so-called "lock-in" *cyclo*Sal pronucleotides were designed.[3−5] These compounds contained (carboxy)esterase-cleavable esters attached to the aromatic ring. To avoid any influence on the chemical hydrolysis properties, the ester moieties have been separated from the aromatic ring by a C_2 spacer. The *Cyclo*Sal-d4TMP acid ester and *cyclo*Sal-d4TMP alcohol ester,[3] as well as AM- and POM-acylals[4] or amino acid ester functionalized *cyclo*Sal-d4TMPs,[5] have been investigated. The common motif of these compounds is that after enzymatic cleavage a highly polar carboxylate group is formed. Hydrolysis studies in phosphate buffer (PBS, pH 7.3) and in T-lymphocyte CEM cell extracts revealed that an intracellular trapping is possible when highly polar, charged *cyclo*Sal-d4TMP acids are released. The fast intracellular release of these compounds is the primary aim of the "lock-in" concept. D4TMP **1** is released from the *cyclo*Sal-d4TMP carboxylic acids by chemical hydrolysis. However, the chemical hydrolysis of the charged carboxylate bearing *cyclo*Sal phosphate triesters was considerably slower than its lipophilic precursor. Therefore, a conceptually different approach to enzymatic activation of *cyclo*Sal pronucleotides has been introduced and was described recently.[6,7]

In this approach, prodrugs contained again a lipophilic, functionalized substituent with electron-donating or only weak electron-withdrawing properties attached to the aromatic ring of the *cyclo*Sal mask. After passive transport into the cell the functionalized substituent was converted into a strong electron withdrawing substituent by enzymatic cleavage with the consequence that the so formed strong electron-withdrawing group (an aldehyde function) in the *cyclo*Sal structure caused a significant decrease in hydrolysis stability. Preferably, the enzymatic conversion does not occur in the extracellular environment or is very slow. In contrast to earlier approaches,[4] in this case the enzymatic activation resulted in a rapid delivery of the nucleotide (e.g., d4TMP **1**, Figure 1). Thus, in contrat to our earlier approach, the "lock-in" effect with these compounds happens at the nucleotide level.

As the first type of this new generation of enzymatically activated *cyclo*Sal prodrugs, different diacyloxymethyl-*cyclo*Sal-d4TMPs were studied. 5-Diacetoxymethyl- (**3a**−**c** (5-di-AM),

Scheme 1. Synthesis of 4-Acetylsalicyl Alcohols **8** and 5-Acetyl-*cyclo*Sal-d4TMPs **6**[a]



[a] Reagents and conditions. Method A: (i) $AlCl_3$, toluene, −45 °C, 2 h; (ii) acetyl chloride, −45 °C, 2.5 h. Method B: formaldehyde solution (37%), conc HCl, 50 °C, 4 h. Method C: formaldehyde solution (37%), conc HCl, 100 °C, 2.5 h. Method D: $CaCO_3$, H_2O, aq THF, room temp, 1 day. Method E: $CaCO_3$, H_2O, aq THF, 50 °C, 6 h. Method F: (i) Et_2O or THF, PCl_3, pyridine, −20 °C, 4 h; (ii) CH_3CN, DIPEA, d4T **2**, −20 °C to room temp, 2−3 h; (iii) CH_3CN, *t*-BuOOH, −20 °C to room temp, 0.5−2 h.

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[a] Abbreviations: acyl, acetoxyvinyl; CD, circular dichroism; di-AM, diacetoxymethyl; di-*i*BOM, diisobutyroxymethyl; *cyclo*Sal, *cyclo*Saligenyl.

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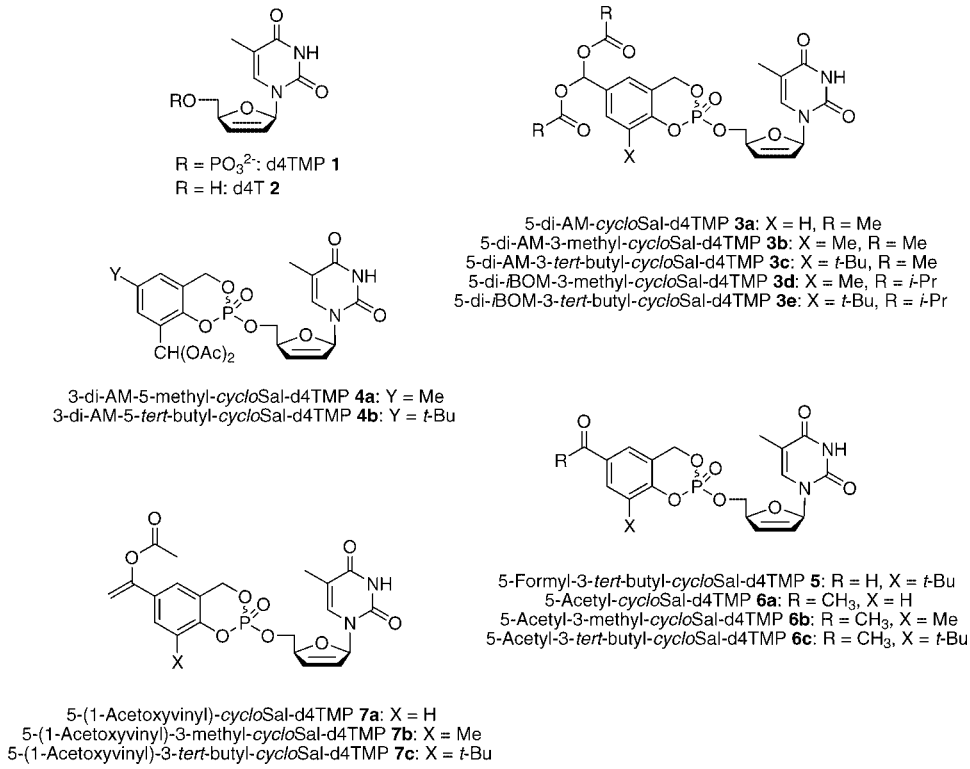


Figure 1. Structures of d4TMP **1**, d4T **2**, and *cyclo*Sal-d4TMPs **3**−**7**.

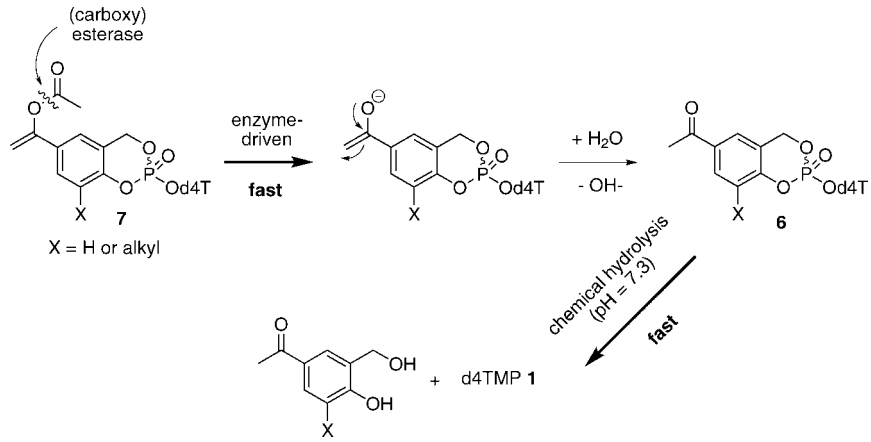


Figure 2. Enzyme assisted hydrolysis cascade of 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7**.

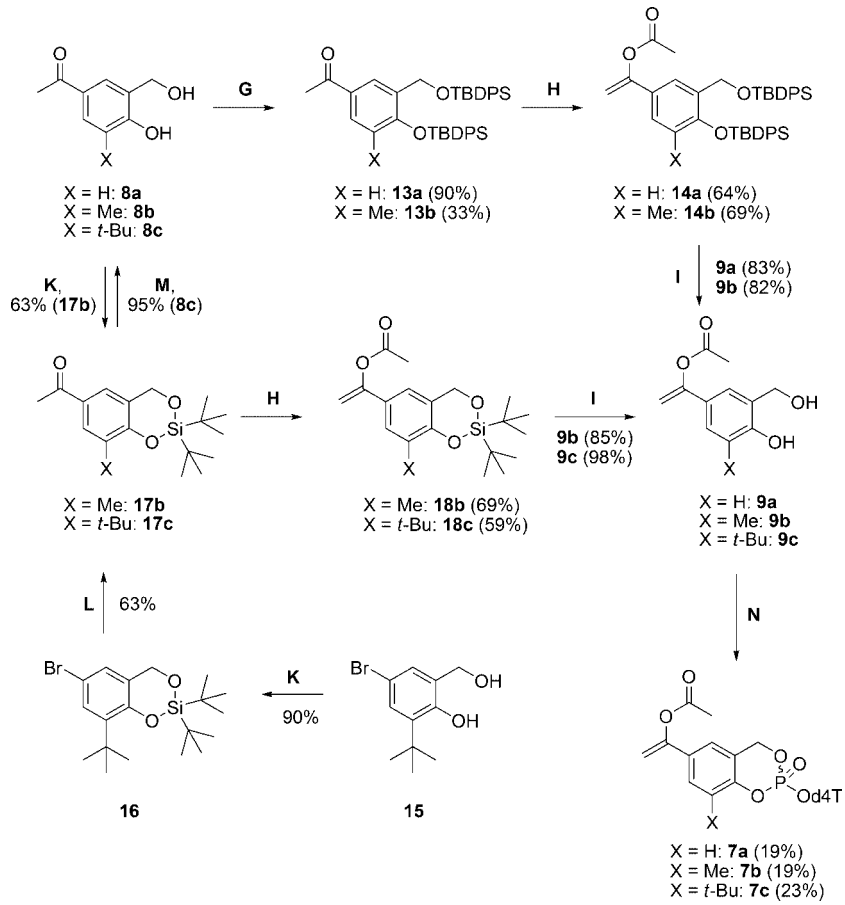
Figure 1),[6] 5-diisobutyroxymethyl-, and 3-diacetoxymethyl-*cyclo*Sal-d4TMPs (**3d**,**e** (5-di-*i*BOM) and **4a**,**b** (3-di-AM), Figure 1) were synthesized. Indeed, these compounds showed the expected hydrolytic behavior in several chemical and biological media. Their enzymatic cleavage into the corresponding formyl-substituted compounds (e.g., **5** (Figure 1) resulting from the cleavage of **3c** or **3e**) was proven, and a general relationship between the hydrolysis stability, the absolute configuration at the phosphorus atom, and the antiviral activity against HIV was found. By comparison of 5-di-AM-*cyclo*Sal-d4TMPs **3a**−**c** and 5-di-*i*BOM-*cyclo*Sal-d4TMPs **3d**,**e**, respectively, the 3-*t*-Bu-substituted compounds (**3c**,**e**) showed the highest stability against chemical hydrolysis and the best antiviral activity. Interestingly, the antiviral testing of the separated diastereomers of compounds **3c**,**e** and **5** revealed significant differences. In all cases, the (S_P)-diastereomer was more active than the (R_P)-isomer. The absolute configuration was assigned on the basis of a single-crystal X-ray structure of the slow-eluting isomer of **5**, which showed (R_P)-configuration. In CEM/0 cell extracts (R_P)-**5** was formed from **3c**-*slow* and **3e**-*slow* by enzymatic cleavage. Thus, compounds **3c**-*slow* and **3e**-*slow* have to be (R_P)-isomers as well.[7]

Here, we report the synthesis, properties, and antiviral evaluation of a second type of enzymatically activated *cyclo*Sal-d4TMPs: 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs (**7a**−**c**, 5-(1-acyl)-*cyclo*Sal-d4TMPs, Figure 1). These new compounds were expected to be cleaved to 5-acetyl-*cyclo*Sal-d4TMPs **6a**−**c** by (carboxy)esterases. The proposed hydrolysis cascade is shown in Figure 2.

Results and Discussion

Chemistry. In order to use our established method for the preparation of *cyclo*Sal-NMPs starting from saligenyl chlorophosphites,[2] 4-acetyl- (**8a**−**c**) and 4-(1-acetoxyvinyl)salicyl alcohols (**9a**−**c**) were synthesized. All synthetic steps are summarized in Schemes 1 and 2. 4-Acetylsalicyl alcohols **8a**−**c** were synthesized starting from the respective 4-hydroxyac-

Scheme 2. Synthesis of 5-(1-Acetoxyvinyl)-*cyclo*Sal-d4TMPs **7**[a]



[a] Reagents and conditions. Method G: TBDPSCl, imidazole, abs pyridine, room temp, 4 days. Method H: isopropenyl acetate, *p*-TsOH, 110 °C, 18 or 90 h. Method I: THF, $Et_3N \cdot 3HF$, room temp, 20−30 min. Method K: $t\text{-}Bu_2Si(OTf)_2$, abs CH_2Cl_2, abs pyridine, room temp, 2 h. Method L: (i) abs THF, *n*-BuLi, −78 °C, 2 h; (ii) *N*,*N*-dimethylacetamide, −50 °C, 1 h. Method M: THF, TBAF, room temp, 1 h. Method N: (i) Et_2O, PCl_3, pyridine, −20 °C, 4 h; (ii) CH_3CN, DIPEA, d4T **2**, −20 °C to room temp, 1.5 h; (iii) CH_3CN, *t*-BuOOH, −20 °C to room temp, 0.5−1.5 h.

etophenones **10a**−**c**. While **10a**,**b** were commercially available, **10c** was prepared from 2-*tert*-butylphenol **11** first. This was achieved using the method described by Shimomura et al.[8] The 4-hydroxyacetophenones **10a**−**c** were converted into salicyl alcohols **8a**−**c** in two steps (chloromethylation and nucleophilic substitution). The chloromethylation of **10a** to **12a** has been described by Yanagi et al.,[9] and the following nucleophilic substitution reaction from **12a** to **8a** was performed by a modified method from Buchanan et al.[10] So **8a** was synthesized in a two-step procedure in 62% yield. Similar conditions were used for the preparation of **8b**,**c** in 59% (**8b**) and 29% yield (**8c**), respectively. Because of an insufficient reactivity of the starting material, the reactions leading to **8c** had to be performed at a higher temperature. However, this led to a significant formation of a byproduct, which could be characterized as the methylene protected derivative of **8c**. This was formed in the first step during the chloromethylation. 5-Acetyl-*cyclo*Sal-d4TMPs **6a**−**c** were finally synthesized from salicyl alcohols **8a**−**c** using the corresponding chlorophosphites in moderate yields (18−26%).

For the synthesis of 4-(1-acetoxyvinyl)salicyl alcohols (**9a**−**c**) the hydroxyl groups of 4-acetylsalicyl alcohols **8a**,**b** were protected as TBDPS ethers first. Compound **8a** was converted into **13a** in an excellent yield of 90%, while protection of salicyl alcohol **8b** gave **13b** in only 33% yield. The reaction of **8c** resulted in the mono-TBDPS ether at the benzylhydroxyl group. Even the use of the sterically less demanding TBDMS protecting group again only yielded the monoprotected derivative. So obviously steric reasons counteract this "double" protection. Compounds **13a**,**b** were subsequently converted into the corresponding 4-(1-acetoxyvinyl) derivatives **14a**,**b** using isopropenyl acetate and *p*-toluenesulfonic acid in good yields (**14a**, 64%; **14b**, 69%).[11] Finally, deprotection with triethylamine trihydrofluoride ($NEt_3 \cdot 3HF$) gave salicyl alcohols **9a** (83%) and **9b** (82%), respectively.

The preparation of 4-(1-acetoxyvinyl)-6-*tert*-butylsalicyl alcohol **9c** was achieved starting from 4-bromo-6-*tert*-butylsalicyl alcohol **15**, which has already been used in the synthesis of 5-formyl-3-*tert*-butyl-*cyclo*Sal-d4TMP **5**.[6,12] The hydroxy functions of salicyl alcohol **15** were protected as a cyclic di-*tert*-butylsilyl ether to yield **16** in 90%.[13] Then the acetyl function was introduced via bromo/lithium exchange using *N*,*N*-dimethylacetamide.[14] Compound **17c** was obtained in 63% yield. As an alternative, compound **17c** can also be deprotected using TBAF to yield 4-acetyl-6-*tert*-butylsalicyl alcohol **8c** in 95%. On the other hand, conversion of **17c** into enolic ester **18c** (59% yield) and deprotection (98% yield) finally led to **9c**. 4-(1-Acetoxyvinyl)-6-methylsalicyl alcohol **9b** was synthesized using the cyclic silyl protecting group as well. Protection of salicyl alcohol **8b** gave **17b** (63% yield), and the enolic ester **18b** was then obtained in 69% yield. Deprotection with $NEt_3 \cdot 3HF$ gave **9b** in 85% yield. By this route, salicyl alcohol **9b** was synthesized in an overall yield of 37% from **8b**. The overall yield using TBDPS as the protecting group was only 19%. Salicyl alcohols **9a**−**c** were then converted into 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7a**−**c** in moderate yields (19−23%).

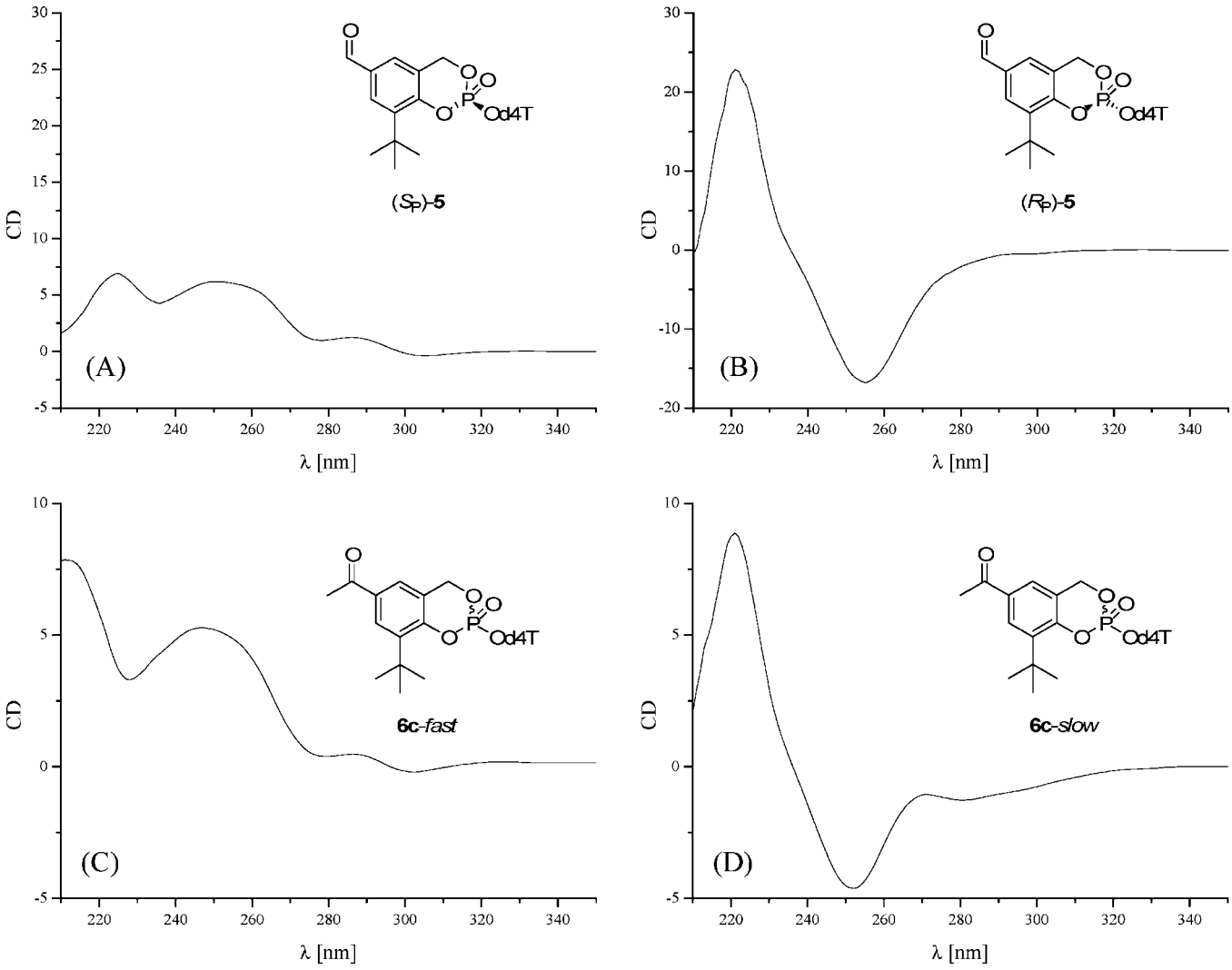


Figure 3. CD spectra of (S_P)-**5** (A) and (R_P)-**5** (B) compared to **6c**-*fast* (C) and **6c**-*slow* (D).

Characterization of all triesters was carried out by means of ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy as well as high-resolution mass spectrometry (HR-MS).

As for 5-formyl- (**5**) and 5-diacyloxymethyl-3-*t*-Bu-*cyclo*Sal-d4TMPs **3c**,**e**,[7] a separation of the diastereomers of 5-acetyl- (**6c**) and 5-(1-acetoxyvinyl)-3-*t*-Bu-*cyclo*Sal-d4TMP **7c** was achieved using preparative RP-HPLC. The slow-eluting diastereomer of **6c** crystallized under the same conditions as the slow-eluting (R_P)-**5**. Unfortunately, the obtained crystals were not suitable for X-ray analysis. But because of the structural similarity of compounds **5** and **6c**, circular dichroism (CD) was used for the attribution of the absolute configuration at the phosphorus atom. The CD spectra of (S_P)-**5** and (R_P)-**5** were compared with those of **6c**-*fast* and **6c**-*slow* (Figure 3). Because of the similar spectra, it is obvious that the (S_P)-configuration can be attributed to **6c**-*fast* and the (R_P)-configuration to **6c**-*slow*. Analytical HPLC studies in CEM/0 cell extracts showed the enzymatic cleavage of **7c**-*fast* to (S_P)-**6c** and of **7c**-*slow* to (R_P)-**6c**, respectively (see SI). According to this, **7c**-*fast* should be in the (S_P)-configuration while **7c**-*slow* is the (R_P)-isomer.

Hydrolysis Studies. Target compounds **6** and **7** have been studied for their hydrolytic stability at physiological pH 7.3 in aqueous 25 mM phosphate buffer (PBS) and in the incubation medium for the antiviral test (RPMI/heat-inactivated FCS (10%), pH 7.6). The given half-lives refer to the disappearance of the triesters. The enzymatic cleavage of 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7** was examined in CEM/0 cell extracts (CE). All half-lives are summarized in Table 1. For comparison, the corresponding data of triesters **3c**,**e** and **5** are given as well.

Chemical Hydrolysis. As expected from our work on 5-formyl- (as **5**) and 5-di-AM-*cyclo*Sal-d4TMPs **3a**−**c**,[7] alkyl groups in position 3 of the aromatic ring also led to a higher hydrolysis stability in the case of the 5-acetyl triester (**6a**) or 5-(1-acetoxyvinyl) triester (**7a**). According to the studies of the diastereomeric mixtures in phosphate buffer (PBS), pH 7.3, the methyl group increases the stability of the acetyl compound 2.5-fold (**6b**) and the *tert*-butyl group led to an 8-fold increase in hydrolytic stability (**6c**). In the case of 5-(1-acetoxyvinyl) compounds **7**, the methyl group increases the hydrolysis half-life 4.5-fold, while the *tert*-butyl group led to a 9-fold increase. Similar changes were found for 5-di-AM compounds **3a**−**c** ($t_{1/2}$ = 1.2 h (3-H, **3a**), 2.3 h (3-Me, **3b**), 7.9 h (3-*t*-Bu, **3c**)) and the 5-formyl-*cyclo*Sal-d4TMPs ($t_{1/2}$ = 0.18 h (3-H), 0.35 h (3-Me), 0.95 h (3-*t*-Bu, **5**)).[7] Surprisingly, the separated diastereomers of triester **6c** showed no difference in the chemical stability and the difference in half-lives of the fast-eluting 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMP (S_P)-**7c** ($t_{1/2}$ = 13.5 h) and the slow-eluting (R_P)-**7c** ($t_{1/2}$ = 11.3 h) was unusually small.

Hydrolysis studies of compounds **6** in a 1:1 mixture of DMSO-d_6 and imidazol/HCl buffer (pH 7.3) followed by ^{31}P NMR spectroscopy revealed that d4TMP **1** was the exclusive phosphorus containing hydrolysis product. Analogous studies

Table 1. Hydrolysis Data and Antiviral Activity of **6a**−**c** and **7a**−**c** Compared to **2**, **3c**,**e**, and **5**

		$t_{1/2}$ [h][a]			EC_{50} [μM][b]			
					CEM/0[g]			
	substituent	PBS,[d] pH 7.3	CE[e]	RPMI/FCS[f]	HIV-1	HIV-2	CEM/TK$^-$,[h] HIV-2	CC_{50}[μM][c]
6a *(mix)*	5-acetyl	0.3	nd[j]	0.6	1.3 ± 0.071	3.0 ± 3.0	>50	148
6b *(mix)*	5-acetyl-3-Me	0.75	nd[j]	1.0	0.54 ± 0.19	2.2 ± 2.3	36 ± 0.0	96
6c *(mix)*	5-acetyl-3-*t*-Bu	2.3	nd[j]	2.5	0.45 ± 0.31	0.87 ± 0.12	1.7 ± 0.42	49
6c *(fast)*	5-acetyl-3-*t*-Bu	2.3	nd[j]	3.2	0.18 ± 0.0071	0.51 ± 0.39	0.55 ± 0.36	56
6c *(slow)*	5-acetyl-3-*t*-Bu	2.3	nd[j]	2.0	0.75 ± 0.049	1.2 ± 1.1	2.8 ± 1.1	64
7a *(mix)*	5-(1-acyl)	1.4	0.13	1.0	0.65 ± 0.35	0.80 ± 0.13	>50	111
7b *(mix)*	5-(1-acyl)-3-Me	6.1	0.07	1.8	0.18 ± 0.071	0.40 ± 0.23	0.73 ± 0.042	18
7c *(mix)*	5-(1-acyl)-3-*t*-Bu	12.3	0.04	5.2	0.47 ± 0.099	0.88 ± 0.45	1.7 ± 2.0	49
7c *(fast)*	5-(1-acyl)-3-*t*-Bu	13.5	0.04	7.6	0.21 ± 0.035	0.80 ± 0.57	0.27 ± 0.12	20
7c *(slow)*	5-(1-acyl)-3-*t*-Bu	11.3	0.04	3.7	0.47 ± 0.34	1.1 ± 0.44	1.7 ± 0.42	67
3c *(mix)*	5-di-AM-3-*t*-Bu	7.9	0.15	2.8	0.65 ± 0.35	0.80 ± 0.13	0.78 ± 0.0	19 ± 0.0
3c *(fast)*	5-di-AM-3-*t*-Bu	7.9	0.3	2.9	0.54 ± 0.51	0.75 ± 0.49	0.29 ± 0.16	17 ± 2.1
3c *(slow)*	5-di-AM-3-*t*-Bu	8.3	0.15	2.8	1.1 ± 1.3	0.84 ± 0.085	3.9 ± 2.7	51 ± 21
3e *(mix)*	5-di-*i*BOM-3-*t*-Bu	3.5	1.0	5.9	0.53 ± 0.45	2.0 ± 2.3	2.4 ± 2.3	20 ± 3.5
3e *(fast)*	5-di-*i*BOM-3-*t*-Bu	3.3	1.0	5.7	0.72 ± 0.68	0.85 ± 0.64	1.4 ± 0.91	14 ± 1.4
3e *(slow)*	5-di-*i*BOM-3-*t*-Bu	3.8	0.8	4.5	0.89 ± 0.16	1.9 ± 2.1	3.6 ± 0.42	40 ± 1.4
5 *(mix)*	5-CHO-3-*t*-Bu	1.0	nd[j]	1.5	0.39 ± 0.40	1.0 ± 0.22	15 ± 6.4	33 ± 7.8
5 *(fast)*	5-CHO-3-*t*-Bu	1.3	nd[j]	1.7	0.16 ± 0.071	0.69 ± 0.41	2.6 ± 1.9	27 ± 11
5 *(slow)*	5-CHO-3-*t*-Bu	1.0	nd[j]	1.6	0.81 ± 0.042	1.4 ± 0.81	10 ± 0.0	41 ± 1.4
2		na[i]	na[i]	na[i]	0.85 ± 0.64	1.1 ± 0.49	69 ± 70	220

[a] Hydrolysis half-lives. [b] Antiviral activity in T-lymphocytes: 50% effective concentration (shown values are mean values of two to three independent experiments). [c] Cytostatic activity: 50% cytostatic concentration. [d] 25 mM phosphate buffer. [e] CEM cell extracts (pH 6.9). [f] RPMI/10% heat-inactivated fetal calf serum (FCS), pH 7.6. [g] Wild-type CEM cells. [h] Thymidine kinase-deficient CEM cells. [i] na: not applicable. [j] nd: not determined.

in a DMSO-d_6/PBS mixture (pH 7.3) showed an identical picture (except the appearance of a small amount of d4TDP resulting from the hydrolysis of triesters **6** by phosphate).

Enzymatic Hydrolysis. In contrast to chemical hydrolysis, studies in T-lymphocyte CEM cell extracts showed an extremely fast cleavage ($t_{1/2}$ = 0.04−0.13 h) of all 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7** ($t_{1/2}$ = 1.4−13.5 h; up to a 300-fold increase) to the corresponding 5-acetyl-*cyclo*Sal-d4TMP **6**. Thus, enolic esters **7** are suitable compounds for the concept of enzymatic activation. The absolute configuration at the phosphorus atom had no influence on the enzymatic cleavage reaction (compare compounds **7c**). In contrast, the corresponding tests for 5-diacyloxymethyl-*cyclo*Sal-d4TMPs **3c**,**e** showed a slower cleavage of the (S_P)-isomers. The half-lives of triesters **7** in RPMI-1640 culture medium containing 10% heat-inactivated fetal calf serum (RPMI/FCS (10%)) were determined to be $t_{1/2}$ = 1.0 h (**7a**), $t_{1/2}$ = 1.8 h (**7b**), and $t_{1/2}$ = 5.2 h (**7c**). These values are lower than the stabilities found in the PBS studies. A similar decrease of hydrolysis stability has previously been found for the 5-di-AM-*cyclo*Sal-d4TMPs **3a**−**c**, especially for **3c**.[7] The cause of the reduced stability in RPMI culture medium seems to be the same in both series of compounds: on one hand a partial cleavage of the enolic ester to the acetyl group has been observed that may be a result of remaining esterase activity (although the calf serum is heat-inactivated); on the other hand the RPMI/FCS medium has a more basic pH value (7.6).

Antiviral Evaluation. The *cyclo*Sal triesters **6** and **7** were evaluated for their in vitro anti-HIV activity (Table 1). All compounds showed comparable or even higher antiviral potency against HIV-1 and HIV-2 in wild-type CEM/0 cells than d4T **2**. More importantly, 5-(1-acetoxyvinyl)-3-alkyl-*cyclo*Sal-d4TMPs **7b** and **7c**-*fast* showed full retention of antiviral activity in CEM/TK$^-$ cells. Surprisingly, 5-acetyl-*cyclo*Sal-d4TMP **6c** showed full retention of the activity as well, while 5-acetyl-3-methyl-*cyclo*Sal derivative **6b** entirely lost its antiviral potency. This may be due to a higher lipophilicity of **6c** compared to **6a**,**b** in addition to a sufficient hydrolysis stability allowing the transport into cells. Interestingly, although compound **7c** is significantly more stable against chemical hydrolysis in RPMI/FCS culture medium than **7b** ($t_{1/2}$ = 5.2 h (**7c**) vs $t_{1/2}$ = 1.8 h (**7b**)), the anti-HIV activity of these two compounds is similar. So an effective trapping of the released acetyl compound seems to be just as important as sufficient hydrolysis stability. In CEM/TK$^-$ cells a complete loss of activity was only observed for compounds showing very low hydrolysis stabilities (**6a**,**b** and **7a**). According to the low chemical stabilities, these compounds seem to hydrolyze almost completely in the extracellular medium releasing d4TMP **1**. D4TMP **1** is dephosphorylated to d4T **2**, which then acts in its usual way in CEM/0 cells. In summary, the idea of our concept is most obvious when compounds **6b** and **7b** are compared. Compound **6b** is hardly active in CEM/TK$^-$-deficient cells most probably because of its low chemical stability (0.75 h), whereas derivative **7b**, which is the lipophilic precursor of **6b**, is fully active in this cell line (chemical stability, 6.1 h). All data presented show that **7b** is cleaved intracellularly after membrane passage into **6b** that rapidly releases d4TMP.

Interestingly, the separated diastereomers of **6c** and **7c** showed the same result as the previously reported compounds **3c**,**e** and **5**:[7] for all derivatives the fast-eluting (S_P)-isomer is significantly more antivirally active than its (R_P)-counterpart.

Conclusion

A synthetic route to various 5-acetyl- (**6**) and 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7** has been developed successfully. These compounds were analyzed with regard to their hydrolytic and antiviral behavior. It was shown that the enzymatic cleavage of the acetoxyvinyl group to the acetyl group occurred rapidly in CEM/0 cell extract in contrast to chemical hydrolysis. Moreover, a selective release of d4TMP **1** from 5-acetyl triesters **6** was also proven in ^{31}P NMR studies. All compounds proved to be potent inhibitors of HIV-1 and HIV-2 replication in wild-type CEM/0 cells, and at least 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7b** and **7c** retained entirely their antiviral potency in CEM/TK$^-$ cells. In addition, compounds **6c** and **7c** were separated into their stereoisomers by preparative RP-HPLC and the absolute configuration at the phosphorus was assigned. The antiviral activity of the fast-eluting (S_P)-diastereomers consis-

tently proved to be superior to the antiviral activity of the (R_P)-diastereomers. In conclusion, 5-(1-acetoxyvinyl)-*cyclo*Sal-d4TMPs **7** can be used as enzymatically activated prodrugs. We are now focusing our attention to enolic esters in which the ester moiety is stabilized against hydrolytic cleavage in the RPMI/FCS (10%).

Experimental Section

NMR spectra were recorded with a Bruker AMX 400, Bruker AV 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 spectrometer [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) VWR precoated 60 F_{254} plates with a 0.2 mm layer of silica gel (VWR no. 5554) 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 TLCs were performed on a chromatotron (Harrison Research, model 7924T) using glass plates coated with 1, 2, or 4 mm layers of VWR 60 PF_{254} silica gel containing a fluorescent indicator (VWR no. 7749). For column chromatography, Merck silica gel 60, 230−400 mesh, was used. UV spectra were recorded on a Varian Cary 1E UV−visible spectrophotometer, and absorption maximum wavelengths λ_{max} are given in nm. UV absorptions of *cyclo*Sal nucleotides were determined from their HPLC data (diode array detector). Circular dichroism spectra were recorded on an AVIV CD instrument model 215. 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). Preparative HPLC was carried out on an HPLC system consisting of a Merck-Hitachi L-6250 Intelligent Pump, a Merck-Hitachi LaChrom UV detector L-7400, and a Merck Hitachi D-2500A Chromato-Integrator using a Merck Hibar RT 250-25 column containing reversed phase silica gel Lichrospher 100 RP 18 (5 μM; Merck, Darmstadt, Germany). The flow rate was 10 mL/min, and detection was performed at a wavelength of 260 nm. 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 destilled from sodium prior to use. Isopropenyl acetate was distilled under nitrogen prior to use. The solvent for HPLC (CH_3CN) was obtained from Acros (HPLC grade).

General Procedure A: Preparation of 4-Acetylsalicyl Alcohols (8). The respective 4-hydroxyacetophenone (**10**) was suspended in concentrated hydrochloric acid, and formaldehyde solution (37%) was added. The reaction mixture was stirred at the temperature mentioned below until the reaction was completed. In case of 4-hydroxyacetophenone (**10a**), the crude chloromethylated product **12a** was obtained by filtration as a red solid. In case of compounds **10b**,**c** ethyl acetate was added to dissolve the generated violet precipitates, and the phases were separated. The aqueous layer was extracted with ethyl acetate twice, and the combined organic layers were dried with sodium sulfate. The solvent was removed in vacuum to yield violet crystals (**12b**,**c**). The crystals of **12a**−**c** were dissolved in aqueous THF, and additional water and calcium carbonate were added. The reaction mixture was stirred at the temperature mentioned below until the reaction was completed. Concentrated hydrochloric acid was added dropwise to adjust the pH to less than 7, and the phases were separated with ethyl acetate. The aqueous layer was extracted with ethyl acetate twice, and the combined organic layers were dried with magnesium sulfate. The products were purified by column chromatography (petroleum ether 50−70/ethyl acetate 1:1 (**8b**) or 4:1 (**8c**) or CH_2Cl_2/CH_3OH 19:1 (**8a**).

General Procedure B: Preparation of Bis-*O*-(*tert*-butyldiphenylsilyl)-4-acetylsalicyl Alcohols (13). Under nitrogen, the respective 4-acetylsalicyl alcohol (**8**, 1.0 equiv) was dissolved in dry pyridine. After the addition of imidazole (7.0 equiv) and *tert*-butyldiphenylsilyl chloride (4.0 equiv), the solution was stirred at room temperature until the reaction was completed. At 0 °C the reaction mixture was diluted with water and the phases were separated with ethyl acetate. The aqueous layer was extracted with ethyl acetate three times. The combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The products were purified by column chromatography (petroleum ether 50−70/CH_2Cl_2 2:1 (**13b**) or CH_2Cl_2 (**13a**)).

General Procedure C: Preparation of Di-*tert*-butylsilyl Protected Salicyl Alcohols (16, 17b). Under nitrogen, the respective salicyl alcohol (**8b** or **15**, 1.0 equiv) was dissolved in dry CH_2Cl_2. After the addition of dry pyridine (4.4 equiv) and di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (1.3 equiv), the solution was stirred at room temperature for 2 h. At 0 °C the reaction mixture was diluted with water and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 twice, and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The products were purified by flash column chromatography (petroleum ether 50−70/CH_2Cl_2 7:1 (**16**) or preparative TLC [Chromatotron; petroleum ether 50−70/CH_2Cl_2 1:2 (**17b**)].

General Procedure D: Preparation of Silyl Protected 4-(1-Acetoxyvinyl)salicyl Alcohols (14, 18). Under nitrogen, the corresponding silyl protected 4-acetylsalicyl alcohol (**13** or **17**, 1.0 equiv) was dissolved in isopropenyl acetate, and *p*-toluenesulfonic acid (0.3 equiv) was added. The solution was stirred at 110 °C for the given time. After the addition of water the phases were separated with diethyl ether and the aqueous layer was extracted with diethyl ether twice. The combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The products were purified by column chromatography (petroleum ether 50−70/CH_2Cl_2 1:1 (**14a**) or 1:2 (**18b**), petroleum ether 50−70/CH_2Cl_2 gradient (25−50%, **14b**) or CH_2Cl_2 (**18c**)).

General Procedure E: Cleavage of Silyl Protecting Groups. The corresponding silyl protected 4-acetylsalicyl alcohol (**17b**, 1.0 equiv) or 4-(1-acetoxyvinyl)salicyl alcohol (**14**, **18**, 1.0 equiv) was dissolved in THF, and triethylamine trihydrofluoride (3.0 equiv) was added. The solution was stirred at room temperature for the given time, and afterward the phases were separated with diethyl ether and water. The aqueous layer was extracted with diethyl ether once, the combined organic layers were washed with a 5% sodium bicarbonate solution, and the solvent was removed in vacuum. The products were purified by preparative TLC [Chromatotron; petroleum ether 50−70/ethyl acetate 6:1 (**8c**), CH_2Cl_2 (**9c**) or CH_2Cl_2/CH_3OH gradient (0−2%, **9b** (starting from **18b**)) or (0−3%, **9a**) or (0−10%, **9b** (starting from **14b**))].

General Procedure F: Preparation of *cyclo*Sal-d4T Monophosphates (6, 7). Method A. Under nitrogen, a solution of the respective salicyl alcohol (**8**, **9**) in dry diethyl ether was cooled to −20 °C. After dropwise addition of freshly distilled phosphorus(III) chloride and stirring at −20 °C for 10 min, a solution of dry pyridine in dry diethyl ether 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 the best possible precipitation of pyridinium chloride. Filtration under nitrogen and concentration of the filtrate under reduced pressure yielded the phosphitylating agent (saligenyl chlorophosphite) as a crude product, which was directly used for the synthesis of the *cyclo*Sal-d4T monophosphate without further purification.

Under nitrogen, d4T (**2**, 1.0 equiv) was dissolved in dry CH_3CN and cooled to −20 °C. Then DIPEA (1.6 equiv) and the respective

saligenyl chlorophosphite (2.0 equiv) in dry CH_3CN were added. The reaction mixture was stirred at room temperature until the conversion of d4T **2** was completed. Subsequently, *tert*-butyl hydroperoxide (5.5 M in *n*-nonane, 3.0 equiv) was added at −20 °C. The solution was stirred at room temperature until the oxidation was completed and then poured into water or a suitable buffer solution. The phases were separated with ethyl acetate, the aqueous layer was extracted with ethyl acetate three times, and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The resulting 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.

Method B. Method B was performed as described for method A, but a 3:1 mixture of dry diethyl ether and dry THF was used as a solvent in the synthesis of the saligenyl chlorophosphite.

5-Acetyl-*cyclo*Sal-d4T Monophosphate (6a). General procedure F (method B) was used, with 500 mg (3.01 mmol) of 4-acetylsalicyl alcohol (**8a**) dissolved in 30 mL of dry diethyl ether and 10 mL of dry THF, 0.25 mL (2.9 mmol) of phosphorus(III) chloride, and 0.44 mL (5.5 mmol) of dry pyridine in 2.5 mL of dry diethyl ether. Yield: 616 mg. Quantities for *cyclo*Sal-d4T monophosphate synthesis were as follows: 305 mg of crude saligenyl chlorophosphite dissolved in 4 mL of dry CH_3CN, 195 mg (0.870 mmol) of d4T (**2**) dissolved in 9 mL of dry CH_3CN, 0.22 mL (1.3 mmol) of DIPEA, and 0.48 mL (5.5 M in *n*-nonane, 2.7 mmol) of *tert*-butyl hydroperoxide. Reaction time: 3 h. Oxidation time: 1 h. Buffer: 1 M HOAc/NaOAc (pH 5). Yield: 83 mg (0.19 mmol, 22%) of a diastereomeric mixture (ratio 0.4:1) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.36−11.33 (m, 2H, 2 × NH), 7.97−7.91 (m, 4H, 2 × aryl-H-4, 2 × aryl-H-6), 7.26 (d, J = 8.5 Hz, 1H, 1 × aryl-H-3), 7.22 (d, J = 8.5 Hz, 1H, 1 × aryl-H-3), 7.17 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.16 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 6.81−6.78 (m, 1H, 1 × 1′-H), 6.78−6.75 (m, 1H, 1 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.37 (ddd, J = 6.0, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.04−6.00 (m, 2H, 2 × 2′-H), 5.62−5.53 (m, 2H, 2 × benzyl-H), 5.49−5.37 (m, 2H, 2 × benzyl-H), 4.98−4.94 (m, 2H, 2 × 4′-H), 4.37−4.24 (m, 4H, 4 × 5′-H), 2.55 (s, 6H, 2 × $COCH_3$,), 1.67 (d, J = 1.3 Hz, 3H, 1 × thymine-CH_3), 1.62 (d, J = 1.3 Hz, 3H, 1 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.69, −9.89 ppm.

5-Acetyl-3-methyl-*cyclo*Sal-d4T Monophosphate (6b). General procedure F (method B) was used, with 500 mg (2.77 mmol) of 4-acetyl-6-methylsalicyl alcohol (**8b**), dissolved in 30 mL of dry diethyl ether and 10 mL of dry THF, 0.23 mL (2.6 mmol) of phosphorus(III) chloride, and 0.40 mL (5.0 mmol) of dry pyridine in 3 mL of dry diethyl ether. Yield: 610 mg. Quantities for *cyclo*Sal-d4T monophosphate synthesis were as follows: 605 mg of crude saligenyl chlorophosphite dissolved in 5 mL dry CH_3CN, 283 mg (1.26 mmol) of d4T (**2**) dissolved in 25 mL of dry CH_3CN, 0.34 mL (2.0 mmol) of DIPEA, and 0.70 mL (5.5 M in *n*-nonane, 3.9 mmol) of *tert*-butyl hydroperoxide. Reaction time: 2 h. Oxidation time: 30 min. Buffer: 1 M HOAc/NaOAc (pH 5). Yield: 143 mg (0.319 mmol, 26%) of a diastereomeric mixture (ratio 0.5:1) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.33 (s, 1H, 1 × NH), 11.31 (s, 1H, 1 × NH), 7.87 (s, 2H, 2 × aryl-H-4), 7.75 (s, 2H, 2 × aryl-H-6), 7.19 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.15 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 6.81−6.77 (m, 2H, 2 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.38 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.05−6.00 (m, 2H, 2 × 2′-H), 5.61−5.49 (m, 2H, 2 × benzyl-H), 5.48−5.37 (m, 2H, 2 × benzyl-H), 5.00−4.92 (m, 2H, 2 × 4′-H), 4.36−4.23 (m, 4H, 4 × 5′-H), 2.54 (s, 6H, 2 × $COCH_3$), 2.27 (s, 3H, 1 × CH_3), 2.24 (s, 3H, 1 × CH_3), 1.66−1.60 (m, 6H, 2 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.87, −9.33 ppm.

5-Acetyl-3-*tert*-butyl-*cyclo*Sal-d4T Monophosphate (6c). General procedure F (method A) was used, with 500 mg (2.25 mmol) of 4-acetyl-6-*tert*-butylsalicyl alcohol (**8c**), dissolved in 30 mL of dry diethyl ether, 0.19 mL (2.2 mmol) of phosphorus(III) chloride, and 0.32 mL (4.0 mmol) of dry pyridine in 3 mL of dry diethyl ether. Yield: 629 mg. Quantities for *cyclo*Sal-d4T monophosphate synthesis were as follows: 620 mg of crude saligenyl chlorophosphite dissolved in 10 mL of dry CH_3CN, 246 mg (1.10 mmol) of d4T (**2**) dissolved in 25 mL of dry CH_3CN, 0.30 mL (1.7 mmol) of DIPEA, and 0.60 mL (5.5 M in *n*-nonane, 3.3 mmol) of *tert*-butyl hydroperoxide. Reaction time: 3 h. Oxidation time: 2 h. Prior to purification by preparative TLC, flash chromatography (ethyl acetate/CH_3OH 9:1) was performed. Yield: 97.3 mg (0.198 mmol, 18%) of a diastereomeric mixture (ratio 0.8:1) as a colorless foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.35 (s, 1H, 1 × NH), 11.32 (s, 1H, 1 × NH), 7.90−7.85 (m, 4H, 2 × H-4, 2 × H-6), 7.22 (d, J = 1.0 Hz, 1H, 1 × thymine-H-6), 7.19 (d, J = 1.0 Hz, 1H, 1 × thymine-H-6), 6.86−6.78 (m, 2H, 2 × 1′-H), 6.46−6.39 (m, 2H, 2 × 3′-H), 6.07−6.01 (m, 2H, 2 × 2′-H), 5.59−5.39 (m, 4H, 4 × benzyl-H), 5.01−4.96 (m, 2H, 2 × 4′-H), 4.38−4.30 (m, 4H, 4 × 5′-H), 2.56 (s, 6H, 2 × OCH_3), 1.60 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.59 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.37 (s, 9H, 1 × t-Bu-CH_3), 1.34 (s, 9H, 1 × t-Bu-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.12, −9.42 ppm.

The diastereomers were separated by preparative RP-HPLC (CH_3CN/H_2O 1:2) in which an amount of 45.0 mg of the diastereomeric mixture of **6c** was used to yield 21.2 mg of **6c**-*fast* and 17.4 mg of **6c**-*slow*. Analytical data of **6c**-*fast* are as follows. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.35 (s, 1H, NH), 7.88−7.85 (m, 2H, aryl-H-4, aryl-H-6), 7.22 (s, 1H, thymine-H-6), 6.83−6.79 (m, 1H, 1′-H), 6.46−6.40 (m, 1H, 3′-H), 6.06−6.01 (m, 1H, 2′-H), 5.58−5.42 (m, 2H, benzyl-H), 5.01−4.96 (m, 1H, 4′-H), 4.38−4.30 (m, 2H, 5′-H), 2.56 (s, 3H, H-9), 1.60 (s, 3H, thymine-CH_3), 1.34 (s, 9H, t-Bu) ppm. ^{31}P NMR (161 MHz, DMSO-d_6): δ = −9.42 ppm. Analytical data of **6c**-*slow* are as follows. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.32 (s, 1H, NH), 7.90−7.85 (m, 2H, aryl-H-4, aryl-H-6), 7.19 (s, 1H, thymine-H-6), 6.83−6.78 (m, 1H, 1′-H), 6.43−6.39 (m, 1H, 3′-H), 6.07−6.02 (m, 1H, 2′-H), 5.59−5.39 (m, 2H, benzyl-H), 5.01−4.96 (m, 1H, 4′-H), 4.39−4.31 (m, 2H, 5′-H), 2.56 (s, 3H, $COCH_3$), 1.59 (s, 3H, thymine-CH_3), 1.37 (s, 9H, t-Bu-CH_3) ppm. ^{31}P NMR (161 MHz, DMSO-d_6): δ = −9.12 ppm.

5-(1-Acetoxyvinyl)-*cyclo*Sal-d4T Monophosphate (7a). General procedure F (method A) was used, with 393 mg (1.89 mmol) of 4-(1-acetoxyvinyl)salicyl alcohol (**9a**) dissolved in 20 mL of dry diethyl ether, 0.20 mL (2.3 mmol) of phosphorus(III) chloride, and 0.35 mL (4.4 mmol) of dry pyridine in 1.75 mL of dry diethyl ether. Yield: 340 mg. Quantities for *cyclo*Sal-d4T monophosphate synthesis were as follows: 335 mg of crude saligenyl chlorophosphite dissolved in 5 mL of dry CH_3CN, 149 mg (0.665 mmol) of d4T (**2**) dissolved in 15 mL of dry CH_3CN, 0.17 mL (0.98 mmol) of DIPEA, and 0.35 mL (5.5 M in *n*-nonane; 2.0 mmol) of *tert*-butyl hydroperoxide. Reaction time: 1.5 h. Oxidation time: 30 min. Buffer: phosphate (pH 7.3). Yield: 61.0 mg (0.128 mmol, 19%) of a diastereomeric mixture (ratio 0.6:1) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (s, 2H, 2 × NH), 7.56−7.51 (m, 2H, 2 × aryl-H-4), 7.50−7.46 (m, 2H, aryl-H-6), 7.20 (d, J = 1.1 Hz, 1H, 1 × thymine-H-6), 7.18 (d, J = 1.1 Hz, 1H, 1 × thymine-H-6), 7.17−7.11 (m, 2H, 2 × aryl-H-3), 6.84−6.76 (m, 2H, 2 × 1′-H), 6.42 (ddd, J = 6.2, 1.7, 1.7 Hz, 1H, 1 × 3′-H), 6.36 (ddd, J = 6.2, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.05−5.95 (m, 2H, 1 × 2′-H), 5.65−5.64 (m, 2H, 2 × CH_AH$_B$), 5.58−5.35 (m, 4H, 4 × benzyl-H), 5.03 (d, J = 2.3 Hz, 2H, 2 × CH$_A$H$_B$), 4.99−4.93 (m, 2H, 2 × 4′-H), 4.38−4.25 (m, 4H, 4 × 5′-H), 2.26 (s, 6H, 2 × $OCOCH_3$), 1.68 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.63 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.46, −9.49 ppm.

5-(1-Acetoxyvinyl)-3-methyl-*cyclo*Sal-d4T Monophosphate (7b). General procedure F (method A) was used, with 415 mg (1.87 mmol) of 4-(1-acetoxyvinyl)-6-methylsalicyl alcohol (**9b**), dissolved in 20 mL of dry diethyl ether, 0.20 mL (2.3 mmol) of phosphorus(III) chloride, and 0.35 mL (4.3 mmol) of dry pyridine in 1.8 mL of dry diethyl ether. Yield: 435 mg. Quantities for *cyclo*Sal-d4T monophosphate synthesis were as follows: 205 mg of crude saligenyl chlorophosphite dissolved in 5 mL of dry CH_3CN, 68 mg (0.30 mmol) of d4T (**2**) dissolved in 6 mL of dry CH_3CN, 80 μL (0.45 mmol) of DIPEA, and 0.16 mL (5.5 M in *n*-nonane, 0.88 mmol) of *tert*-butyl hydroperoxide. Reaction time: 1.5 h. Oxidation

time: 30 min. Prior to purification by preparative TLC, flash chromatography (ethyl acetate/CH_3OH 9:1) was performed. Preparative TLC on the Chromatotron did not provide the pure product (49 mg as a red foam), so final purification was achieved using preparative RP-HPLC (CH_3CN/H_2O 1:1). Yield: 28.1 mg (57.3 μmol, 19%) of a diastereomeric mixture (ratio 0.8:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.32 (s, 2H, 2 × NH), 7.47−7.43 (m, 2H, 2 × aryl-H-4), 7.30−7.27 (m, 2H, 2 × aryl-H-6), 7.21 (d, J = 1.0 Hz, 1H, 1 × thymine-H-6), 7.20 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 6.83−6.79 (m, 2H, 2 × 1′-H), 6.41 (ddd, J = 6.0, 1.8, 1.7 Hz, 1H, 1 × 3′-H), 6.36 (ddd, J = 6.0, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.06−5.99 (m, 2H, 2 × 2′-H), 5.62 (d, J = 2.3 Hz, 2H, 2 × CH_AH$_B$), 5.54−5.33 (m, 4H, 4 × benzyl-H), 5.01 (d, J = 2.3 Hz, 2 × 1H, 2 × CH$_A$$H_B$), 4.98−4.92 (m, 2H, 2 × 4′-H), 4.34−4.22 (m, 4H, 4 × 5′-H), 2.26 (s, 6H, 2 × $OCOCH_3$), 2.23 (s, 3H, 1 × CH_3), 2.20 (s, 3H, 1 × CH_3), 1.66 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.64 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3) ppm. ^{31}P NMR (161 MHz, DMSO-d_6): δ = −8.98, −9.02 ppm.

5-(1-Acetoxyvinyl)-3-*tert*-butyl-*cyclo*Sal-d4T Monophosphate (7c). General procedure F (method A) was used, with 500 mg (1.89 mmol) of 4-(1-acetoxyvinyl)-6-*tert*-butylsalicyl alcohol (**9c**), dissolved in 20 mL of dry diethyl ether, 0.20 mL (2.3 mmol) of phosphorus(III) chloride, and 0.35 mL (4.3 mmol) of dry pyridine in 1.8 mL of dry diethyl ether. Yield: 543 mg. Quantities for *cyclo*Sal-d4T monophosphate synthesis were as follows: 534 mg of crude saligenyl chlorophosphite dissolved in 8 mL of dry CH_3CN, 145 mg (0.647 mmol) of d4T (**2**) dissolved in 14 mL of dry CH_3CN, 0.17 mL (0.96 mmol) of DIPEA, and 0.34 mL (5.5 M in *n*-nonane, 1.9 mmol) of *tert*-butyl hydroperoxide. Reaction time: 1.5 h. Oxidation time: 1.5 h. Prior to purification by preparative TLC, flash chromatography (ethyl acetate/CH_3OH 9:1) was performed. Preparative TLC on the Chromatotron did not provide the pure product (117 mg as a slightly fawn foam), so final purification was achieved using preparative RP-HPLC. The diastereomeric mixture was obtained by the use of CH_3CN/H_2O 1:1 as solvent for the preparative RP-HPLC. The separated diastereomers were obtained by the use of CH_3CN/H_2O 2:3. Yield: 79.2 mg (0.149 mmol, 23%); 39.6 mg of the diastereomeric mixture (ratio 0.8:1), as well as 17.7 mg of **7c**-*fast* and 21.9 mg of **7c**-*slow* as colorless foams. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (s, 2H, 2 × NH), 7.43−7.39 (m, 2H, 2 × aryl-H-4), 7.37−7.32 (m, 2H, 2 × aryl-H-6), 7.23 (d, J = 1.0 Hz, 1H, 1 × thymine-H-6), 7.20 (d, J = 1.0 Hz, 1H, 1 × thymine-H-6), 6.84−6.79 (m, 2H, 2 × 1′-H), 6.44−6.38 (m, 2H, 2 × 3′-H), 6.06−6.00 (m, 2H, 2 × 2′-H), 5.67−5.63 (m, 2H, 2 × CH_AH$_B$), 5.50−5.35 (m, 4H, 4 × benzyl-H), 5.05−5.01 (m, 2H, 2 × CH$_A$$H_B$), 5.00−4.94 (m, 2H, 2 × 4′-H), 4.39−4.28 (m, 4H, 4 × 5′-H), 2.26 (s, 6H, 2 × $OCOCH_3$), 1.61 (s, 3H, 1 × thymine-CH_3), 1.59 (s, 3H, 1 × thymine-CH_3), 1.35 (s, 9H, 1 × *t*-Bu), 1.32 (s, 9H, 1 × *t*-Bu) ppm. ^{31}P NMR (161 MHz, DMSO-d_6): δ = −8.80, −9.03 ppm. Analytical data of **7c**-*fast* are as follows: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.35 (s, 1H, NH), 7.43−7.39 (m, 1H, aryl-H-4), 7.37−7.33 (m, 1H, aryl-H-6), 7.23 (d, J = 1.3 Hz, 1H, thymine-H-6), 6.84−6.80 (m, 1H, 1′-H), 6.44−6.39 (m, 1H, 3′-H), 6.05−6.00 (m, 1H, 2′-H), 5.65 (d, J = 2.3 Hz, 1H, CH_AH$_B$), 5.50−5.35 (m, 2H, 2 × benzyl-H), 5.03 (d, J = 2.5 Hz, 1H, CH$_A$$H_B$), 5.00−4.94 (m, 1H, 4′-H), 4.35−4.29 (m, 2H, 2 × 5′-H), 2.26 (s, 3H, $OCOCH_3$), 1.61 (d, J = 0.8 Hz, 3H, thymine-CH_3), 1.32 (s, 9H, *t*-Bu) ppm. ^{31}P NMR (161 MHz, DMSO-d_6): δ = −9.03 ppm. Analytical data of **7c**-*slow* are as follows: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (s, 1H, NH), 7.44−7.38 (m, 1H, aryl-H-4), 7.37−7.31 (m, 1H, aryl-H-6), 7.20 (s, 1H, thymine-H-6), 6.84−6.77 (m, 1H, 1′-H), 6.44−6.36 (m, 1H, 3′-H), 6.07−5.99 (m, 1H, 2′-H), 5.65 (d, J = 1.5 Hz, 1H, CH_AH$_B$), 5.51−5.34 (m, 2H, 2 × benzyl-H), 5.03 (d, J = 1.5 Hz, 1H, CH$_A$$H_B$), 5.00−4.93 (m, 1H, 4′-H), 4.39−4.28 (m, 2H, 2 × 5′-H), 2.26 (s, 3H, $OCOCH_3$), 1.59 (s, 3H, thymine-CH_3), 1.35 (s, 9H, *t*-Bu) ppm. ^{31}P NMR (161 MHz, DMSO-d_6): δ = −8.80 ppm.

Hydrolysis Studies of *cyclo*Sal Phosphate Triesters. Hydrolysis studies of *cyclo*Sal nucleotides (phosphate buffer, pH 7.3) by HPLC analysis (method I) have been described before.[15,16] Studies in cell extracts were performed as reported in ref 4 with different incubation times but without using acetic acid to stop the reaction for *cyclo*Sal nucleotides with acid sensitive substituents. Studies in RPMI/FCS (10%) were carried out in the same way using culture medium instead of cell extract.

Antiretroviral Evaluation. The method of antiviral evaluation has been described in ref 6 and is based on the virus-induced cytopathic effect (giant cell formation) in the CEM cell cultures.

^{31}P NMR Hydrolysis Studies of *cyclo*Sal Phosphate Triesters. ^{31}P NMR hydrolysis studies of *cyclo*Sal nucleotides **6** were carried out as described before.[16]

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Supporting Information Available: Full characterization of compounds **8a**−**c**, **9a**−**c**, **13a**,**b**, **14a**,**b**, **16**, **17b**,**c**, and **18b**,**c**; ^{13}C NMR and UV spectroscopic data, mass spectrometric data, R_f values, melting points, analytical HPLC data of new compounds; methods for HPLC analysis; general nomenclature of salicyl alcohols and *cyclo*Sal-d4TMPs; HPLC chromatograms of kinetic studies of **7c**-*fast* and **7c**-*slow* in CEM/0 cell extracts; ^{31}P NMR hydrolysis of **6c** in imidazole/HCl buffer (pH 7.3). This material is available free of charge via the Internet at http://pubs.acs.org.

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