# Studies on Enzyme-Cleavable Dialkoxymethyl-*cyclo*Saligenyl-2′,3′-dideoxy-2′,3′-didehydrothymidine Monophosphates

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Recently we reported on conceptually new enzymatically activated *cyclo*Sal-pronucleotides. Now, we developed this concept further with new compounds of this type. The basic idea is fast intracellular cleavage of a functionalized group at the *cyclo*Sal residue that results in a rapid delivery of the nucleotide and thus an intracellular enrichment of the nucleotide. The introduction of a higher alkylated acylal group, the di-*iso*-butyryloxymethyl group, to the aromatic ring led to the expected higher stability of these prodrugs against enzymatic cleavage but also entailed surprisingly a decrease in hydrolysis stabilities and solubility problems. For some compounds, a separation of the two diastereomeric forms ($R_P$ or $S_P$) was achieved. By X-ray structure analysis, the absolute configuration at the P-atom was assigned. For all separated diastereomers the ($S_P$) form showed better antiviral activity than the ($R_P$) form.

## Introduction

It has been clearly proven that chiral *cyclo*Sal[a]-d4T-monophosphates (*cyclo*Sal-d4TMPs) are highly efficient prodrugs for the delivery of d4TMP **1** (Figure 1) into cells.[1] This is of high interest because the rate-limiting phosphorylation of the approved anti-HIV drug d4T[2] **2** (Figure 1) is the formation of the monophosphate **1** by the cellular enzyme thymidine kinase (TK).[3] Recently, we presented the first compounds of the third generation of *cyclo*Sal-pronucleotides, the 5-diacetoxymethyl-*cyclo*Sal-d4TMPs (5-di-AM-*cyclo*Sal-d4TMPs; **3a**−**c**; Figure 1), as so-called enzymatically activated *cyclo*Sal-d4T-monophosphates.[4]

The concept of enzymatic activation is exemplified shown for 5-di-AM-3-*t*Bu-*cyclo*Sal-d4TMP (**3c**) in Figure 2. Compound **3c** has a hydrolysis half-life of 7.9 h at the physicological pH value 7.3 (determined in 25 mM phosphate buffer), so it should be stable enough to pass the cell membrane by passive transport (step c) without being hydrolyzed to a significant amount in the extracellular medium (step a). Inside the cell, the weak electron-withdrawing diacetoxymethyl-group is rapidly converted into a strong acceptor (a formyl group) by enzymatic cleavage, with the result that the chemical hydrolysis rate increases dramatically. We determined a half-life of 0.15 h for the conversion of compound **3c** into compound **4c** in T-lymphocyte CEM cell extracts.

The half-life of the released 5-formyl-3-*t*Bu-*cyclo*Sal-d4TMP (**4c**) is 8-fold lower (1.0 h) as compared to that of **3c**. The hydrolysis of **4c** leads rapidly to a charged benzylphosphodiester (not shown). This prevents an efflux through the cell membrane. The selective release of d4TMP **1** from **4c** has been proven by $^{31}$P NMR studies. Compound **3c** has an $EC_{50}$ value of 0.78 ± 0.0 $\mu$M against HIV-2 in mutant thymidine kinase-deficient CEM/TK$^-$ cells, so an effective delivery of d4TMP **1** can be assumed.

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[a] Abbreviations: di-AM, diacetoxymethyl; di-*i*BOM, di-*iso*-butyroxymethyl; *cyclo*Sal, *cyclo*-saligenyl; TK, thymidine kinase.

However, this antiviral activity was not as good as for some other *cyclo*Sal-d4TMPs (e.g., 3-methyl-*cyclo*Sal-d4TMP; $t_{1/2}$ = 17.5 h (PBS, pH = 7.3); $EC_{50} = 0.10 \pm 0.0$ $\mu$M[5]). This might be due to a lower cellular uptake (transport) and/or to a partial extracellular cleavage of the diacetoxymethyl function to the formyl function (step b, Figure 2) in RPMI-1640 culture medium containing 10% heat-inactivated fetal calf serum (RPMI/FCS(10%)), which is also used for the cultivation of the CEM cells in the antiviral testing.

Here, we describe a new series of enzymatically activated *cyclo*Sal-d4T-monophosphates.

## Results and Discussion

**Chemistry.** First, we moved the enzyme-labile diacetoxymethyl group from the 5- to the 3-position (compounds **5**, Figure 1) to prove the stability of the diacetoxymethyl function in this position. *Cyclo*Sal-triesters **5a**,**b** were synthesized starting from the respective 3-formyl-5-alkyl-*cyclo*Sal-d4TMP **6a** or **6b**. These compounds have been prepared in a two-step synthesis from 6-formyl-4-alkylsalicyl alcohol **7a**,**b**, respectively. The synthetic steps performed for the preparation of salicyl alcohols **7a**,**b** are summarized in Scheme 1. All reactions to yield target structures **5a**,**b** are shown in Scheme 2. 6-Formyl-4-methylsalicyl alcohol (**7a**) was synthesized starting from commercially available 5-methylsalicylic acid (**8**). First, acid **8** was converted into the methyl ester **9** with conc $H_2SO_4$ and $CH_3OH$ in 95% yield. Bromination ($Br_2$ in $CHCl_3$) led to the desired 3-bromo-5-methyl-methylsalicylate (**10**; 92% yield). Ester **10** was then reduced to 6-bromo-4-methylsalicyl alcohol **11a** ($LiAlH_4$ in THF; 91% yield).

6-Bromo-4-*tert*-butylsalicyl alcohol (**11b**) was synthesized starting from 4-*tert*-butylphenol (**12**). *Ortho*-formylation of **12** was carried out almost as described in the literature.[6] Bromination of 5-*tert*-butylsalicyl aldehyde (**13**) with $Br_2$ in acetic acid afforded 3-bromo-5-*tert*-butylsalicyl aldehyde (**14**) in 92% yield.[7] Aldehyde **14** was reduced to salicyl alcohol **11b** (91% yield) as it has been done for **10** to yield **11a**.

After protection of salicyl alcohols **11a**,**b** with 2,2-dimethoxypropane in acetone as isopropylidene acetals (**15a**: 74%, **15b**: 86% yield),[8] the resulting 6-bromo-4-alkylsalicyl alcohol iso-

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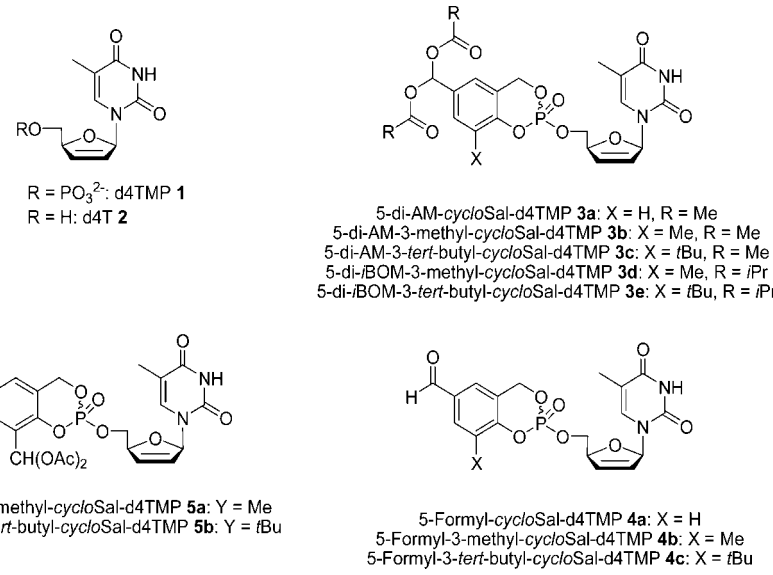



**Figure 1.** Structures of d4TMP **1**, d4T **2**, and *cyclo*Sal-d4TMPs **3**, **4**, and **5**.

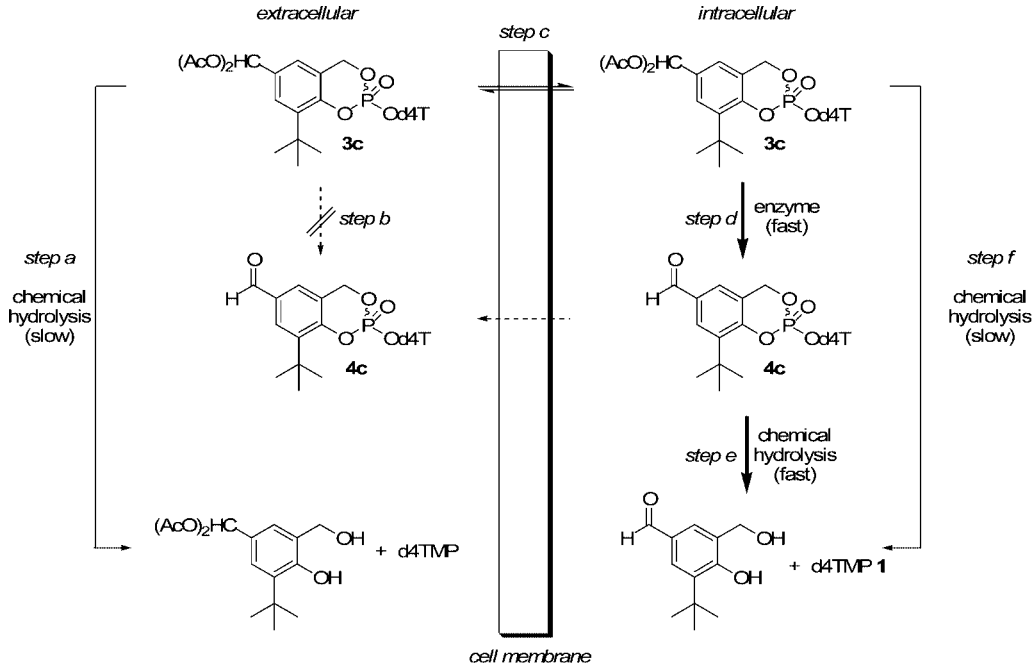


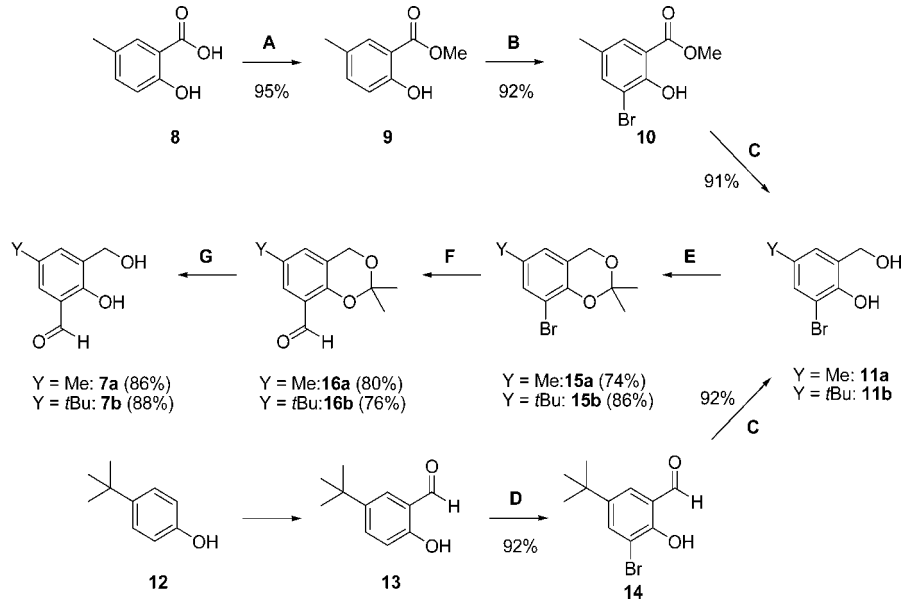

**Figure 2.** Concept of enzymatic activation exemplified by compound **3c**.

propylidene acetals **15a** and **15b** were converted by treatment with *n*-butyllithium and *N*,*N*-dimethylformamide into the corresponding carbaldehydes **16a** (80% yield) and **16b** (76% yield).[9] The cleavage of the isopropylidene group was achieved by the usage of HCl in a mixture of acetonitrile and water to yield 6-formyl-4-methylsalicyl alcohol (**7a**) in 86% and 6-formyl-4-*tert*-butylsalicyl alcohol (**7b**) in 88%, respectively. 3-Formyl-5-alkyl-*cyclo*Sal-d4TMPs **6a** and **6b** were then synthesized using our established phosphorus(III)-method via the corresponding chlorophosphites,[10] except that the reaction steps to the chlorophosphites were carried out at −55 °C instead of −20 °C because, at −20 °C, a side reaction has been observed. 3-Formyl-5-alkyl-*cyclo*Sal-d4TMPs were isolated in 44% yield (**6a**) and 36% (**6b**) yield, respectively.

Finally, triesters **6a** and **6b** were converted into the acylals 3-diacetoxymethyl-5-methyl-*cyclo*Sal-d4TMP (**5a**, 3-di-AM-5-Me-*cyclo*Sal-d4TMP; 19% yield) and 3-diacetoxymethyl-5-*tert*-butyl-*cyclo*Sal-d4TMP (**5b**, 3-di-AM-5-*t*Bu-*cyclo*Sal-d4TMP; 10% yield) with acetic anhydride and zirconium(IV) chloride.[11] The conversion of **6b** to 3-di-*iso*-butyroxymethyl-5-*tert*-butyl-*cyclo*Sal-d4TMP (3-di-*i*BOM-5-*t*Bu-*cyclo*Sal-d4TMP) with isobutyric anhydride and zirconium(IV) chloride failed. This may be caused by steric hindrance because even the yields for the conversion into the 3-diacetoxymethyl-5-alkyl-*cyclo*Sal-d4TMPs **5a** and **5b** were not as good as those obtained in the synthesis of the 5-diacetoxymethyl counterparts **3a**−**c**.[4]

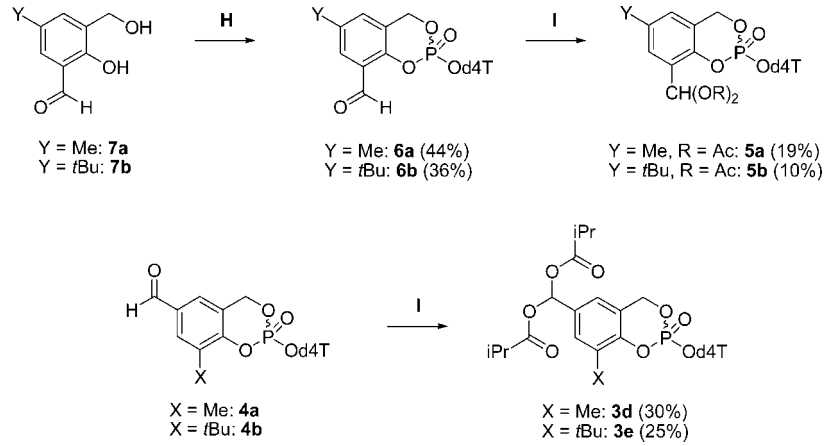
Second, two new 5-di*iso*butyroxymethyl-*cyclo*Sal-d4TMPs (**3d**,**e**, Figure 1) were prepared to study whether the acylals have more beneficial properties against the cleavage in RPMI/FCS(10%). Compounds **3d** and **3e** could be synthesized from **4b** and **4c** using the same method as for the preparation of diacetoxymethyl acylals in 30% (**3d**) and 25% (**3e**) yield, respectively. 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.

**Scheme 1.** Synthesis of the 6-Formyl-4-alkylsalicyl Alcohols **7a** and **7b**[a]




[a] Reagents and conditions: method **A**: $CH_3OH$, conc $H_2SO_4$, 0 °C to reflux, 2 d; method **B**: $CHCl_3$, $Br_2$, rt, 1 d; method **C**: THF, $LiAlH_4$, rt to reflux, 3 h; method **D**: HOAc, $Br_2$, 50 °C, 18 h; method **E**: acetone, 2,2-dimethoxypropane, *para*-toluenesulfonic acid monohydrate, $Na_2SO_4$, 40 °C, 3 d; method **F**: THF, *n*-BuLi, DMF, −78 °C, 3 h; method **G**: $CH_3CN/H_2O$, (cat.) HCl.

**Scheme 2.** Synthesis of the target *cyclo*Sal-d4TMPs **3d**, **3e**, and **5**[a]




[a] Reagents and conditions: method **H**: (i) $Et_2O$ or THF, $PCl_3$, pyridine, −55 °C, 4 h; (ii) $CH_3CN$, DIPEA, d4T **1**, −20 °C to rt, 3 h; (iii) $CH_3CN$, *t*BuOOH, −20 °C to rt, 1 h; method **I**: $CH_3CN$, acetic anhydride or isobutyric anhydride, $ZrCl_4$, rt, 0.75−3 h.

Because of the nonstereoselective synthesis, *cyclo*Sal-pronucleotides were always obtained as mixtures of two diastereomers ($R_P$ and $S_P$ configuration). For **3c**, **3e**, and **4c** the diastereomers were separated by preparative RP-HPLC using water/acetonitrile mixtures as eluent. According to their retention time, the diastereomers are distinguished into the *fast*- and the *slow*-diastereomers. An unambiguous stereochemical attribution was made following single-crystal X-ray structure of **4c-*slow***. An ORTEP diagram is shown in Figure 3.

The absolute stereochemistry of the phosphorus atom was attributed to be ($R_P$), so the ($S_P$) configuration can be assigned to **4c-*fast***. In the hydrolysis studies in CEM/0 cell extracts, it was clearly shown that **4c-*slow*** is formed by enzymatic cleavage of **3c-*slow*** and **3e-*slow***, respectively. Thus, these diastereomers have ($R_P$) configuration, too. According to this, **3c-*fast*** and **3e-*fast*** must have the ($S_P$) configuration. As an example, the HPLC chromatograms of the hydrolysis studies of **3c-*fast*** and **3c-*slow*** in CEM/0 cell extracts after 5 min are shown in Figure 4.

**Hydrolysis Studies.** *Cyclo*Sal-triesters **3**−**6** have been studied for their stability at physicological pH value 7.3 in aqueous 25 mM phosphate buffer. The half-lives refer to the cleavage of the triesters and are summarized in Table 1. The data for triester **3b** and **4b** were taken from ref 4.

As expected, 3-formyl-5-alkyl-*cyclo*Sal-triester **6a** and **6b** showed very short half-lives (0.25 h). These half-lives are similar to those of 5-formyl-*cyclo*Sal-d4TMP **4a** ($t_{1/2}$ = 0.18 h).[4] But in contrast to the 5-formyl-3-alkyl-*cyclo*Sal-phosphate triester derivatives ($t_{1/2}$ = 0.35 h (3-Me, **4b**), 1.0 h (3-*t*Bu, **4c**)), no difference in the hydrolysis stability depending on the alkyl group was detected. For 3-diacetoxymethyl-5-alkyl-*cyclo*Sal-d4TMPs **5a** and **5b**, the same observation was made ($t_{1/2}$ = 3.2 h (**5a**) vs 3.7 h (**5b**)). Considering the hydrolysis half-lives of 5-methyl-*cyclo*Sal-d4TMP ($t_{1/2}$ = 6.2 h)[12] and 5-*tert*-butyl-*cyclo*Sal-d4TMP ($t_{1/2}$ = 7.2 h), this has been expected. This points to the fact that the electron-donating potency of an alkyl group in the 5-position of the aromatic ring has no significant

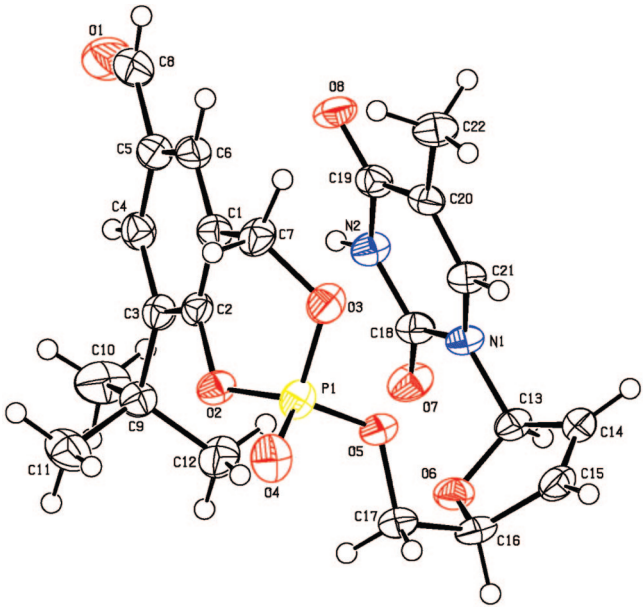



**Figure 3.** *ORTEP*-plot of the asymmetric unit of **4c**-***slow***, showing the atom-labeling scheme (atomic ellipsoids represent displacement parameters at the 50% probability level at approximately −150 °C).

effect on the hydrolysis half-life. But with regard to the concept of enzymatic activation, the difference between the chemical stability of acylals **5** in comparison to the chemical stability of the corresponding 3-formyl-*cyclo*Sal-d4TMPs (**6a** and **6b**) is important. This difference was found to be almost 13- to 15-fold.

Surprisingly, the chemical hydrolysis half-lives of 5-di-*iso*-butyroxymethyl-3-alkyl-*cyclo*Sal-d4TMPs **3d**-***mix*** ($t_{1/2}$ = 1.3 h) and **3e**-***mix*** ($t_{1/2}$ = 3.5 h) markedly decreased compared to that of their 5-diacetoxymethyl-analogues **3b**-***mix*** ($t_{1/2}$ = 2.3 h) and **3c**-***mix*** ($t_{1/2}$ = 7.9 h). In hydrolysis studies of the separated diastereomers, no remarkable difference was found. In all cases, the hydrolysis half-lives of the *fast*- and the *slow*-diastereomers were found to be almost identical.

The cleavage of the acylal group of the dialkoxymethyl-*cyclo*Sal-d4TMPs **3** and **5** was tested using crude T-lymphocyte CEM cell extracts (CE; Table 1). In the case of the 5-di-acetoxymethyl-*cyclo*Sal-d4TMPs **3a-c**, a very quick cleavage was found before.[4] The same result was obtained for the newly prepared 3-diacetoxymethyl-*cyclo*Sal-d4TMPs **5a** and **5b**. The methyl-substituted derivative **5a** showed a half-life of 0.25 h, while the *tert*-butyl-substituted compound **5b** showed a half-life that was below 0.1 h. Thus, while the *tert*-butyl group and the methyl substituent had no influence on the rate of the chemical hydrolysis, the *t*-butyl derivative was 3-fold less stable in cell extracts.

In the case of 5-diacetoxy- and 5-di-*iso*-butyroxymethyl-3-alkyl-*cyclo*Sal-d4TMPs, the contrary was observed: in the presence of the *tert*-butyl group the half-life of the cleavage decreased in comparison to the half-life of the methyl counterpart. For 5-di-*iso*-butyroxymethyl-3-alkyl-*cyclo*Sal-d4TMPs half-lives of 1.0 h (**3e**-***mix***) and 0.5 h (**3d**-***mix***) were measured in cell extracts, respectively. Almost the same difference (2-fold) was found for the 5-di-AM counterparts (0.15 h (**3c**-***mix***) vs 0.08 h (**3b**-***mix***)). More importantly, it is obvious that the 5-di-*i*BOM-*cyclo*Sal-d4TMPs **3d**-***mix*** and **3e**-***mix*** were significantly more stable against enzymatic cleavage (in both cases approximately 6-fold in comparison to their 5-di-AM counterparts **3b**-***mix*** and **3c**-***mix***).

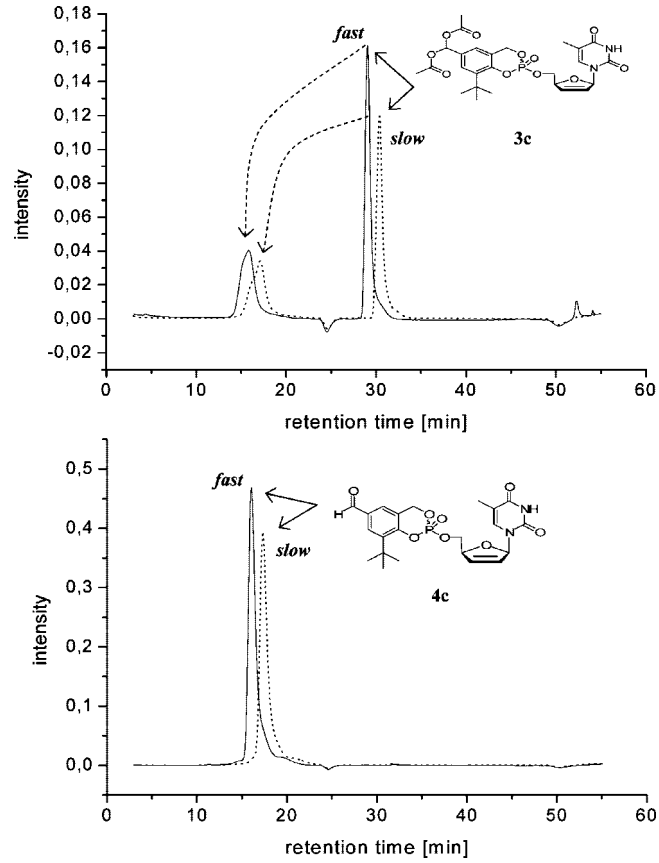



**Figure 4.** Top: HPLC chromatograms of the hydrolysis studies in CEM/0 cell extracts of **3c**-***fast*** (line) and **3c**-***slow*** (dotted line) after 5 min. Bottom: HPLC chromatograms of the synthesized references of **4c**-***fast*** (line) and **4c**-***slow*** (dotted line). HPLC method: HPLC analysis method III.

All acylal substituted compounds **3** and **5** were incubated in RPMI-1640 culture medium containing 10% heat-inactivated fetal calf serum (RPMI/FCS (10%)) to investigate their stability (Table 1). The half-lives of 3-di-AM-*cyclo*Sal-d4TMPs were determined to be $t_{1/2}$ = 1.5 h (**5a**) and $t_{1/2}$ = 1.9 h (**5b**). This is a 2-fold decrease in half-life compared to those in PBS buffer (pH 7.3). Nevertheless, this difference is in the same range as has been found before for **3b**. Only minor cleavage of the acylal function to the formyl function was observed. For compound **3c**-***mix***, the half-life decreased from 7.9 h (chemical hydrolysis) to 2.8 h (RPMI/FCS(10%)). The separated diastereomers showed the same result. Maybe the decrease of half-lives could be a result of residual esterase activity in the fetal calf serum (although it had been heat-inactivated (30 min, 56 °C)). Alternatively, the more basic pH value of the RPMI-1640 medium (pH 7.6) could also play a role. Therefore, we incubated the *cyclo*Sal-d4TMPs in PBS at pH = 7.6 (Table 1). The half-lives of the di-AM-*cyclo*Sal-d4TMPs decreased by the same extent as in the culture medium. But, when comparing the amount of formed 3- or 5-formyl-*cyclo*Sal-d4TMPs at the same time points in PBS pH = 7.6 (data taken from the HPLC chromatograms), the cleavage in RPMI/FCS(10%) (pH = 7.6) is more pronounced. So, this points to a cleavage of the diacetoxymethyl group due to the more basic pH value as well as to the presence of residual esterase activity of the FCS.

The half-lives of the 3-di-*i*BOM-*cyclo*Sal-d4TMPs **3d** and **3e** in PBS pH 7.6 were slightly shorter than the half-lives at pH 7.3 (**3d**-***mix***: 1.1 h (pH 7.6) vs 1.3 h (pH 7.3); **3e**-***mix***: 2.7 h (pH 7.6) vs 3.5 h (pH 7.3)), but more importantly, no significant cleavage to the corresponding formyl compound (**4b** or **4c**) was

**Table 1.** Hydrolysis Data and Antiviral Activity of **3b**−**e**, **4b**−**c**, **5a**−**b**, and **6a**−**b** compared to **2**

| | | $t_{1/2}$ [h][a] | | | | $EC_{50}$ [$\mu$M][b] | | | $CC_{50}$[$\mu$M][c] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | CEM/0[g] | | CEM/TK$^-$[h] | |
| | substituent | PBS[d] pH = 7.3 | CE[e] | RPMI/FCS[f] | PBS[d] pH = 7.6 | HIV-1 | HIV-2 | HIV-2 | |
| **4b-*mix*** | 5-CHO-3-Me | 0.35 | n.d.[j] | 0.7 | 0.35 | 0.45 ± 0.32 | 1.27 ± 0.64 | 4.5 ± 0.7 | 82 ± 20 |
| **3b-*mix*** | 5-di-AM-3-Me | 2.3 | 0.08 | 1.5 | 1.9 | 0.50 ± 0.17 | 0.70 ± 0.14 | 6.3 ± 2.9 | 44 ± 26 |
| **3d-*mix*** | 5-di-*i*BOM-3-Me | 1.3 | 0.5 | 1.6 | 1.1 | 1.60 ± 0.57 | 0.95 ± 0.49 | >10 | 20 ± 0.8 |
| **4c-*mix*** | 5-CHO-3-*t*Bu | 1.0 | n.d.[j] | 1.5 | 1.1 | 0.39 ± 0.40 | 1.0 ± 0.22 | 15 ± 6.4 | 33 ± 7.8 |
| **4c-*fast*** | 5-CHO-3-*t*Bu | 1.3 | n.d.[j] | 1.7 | 1.3 | 0.16 ± 0.071 | 0.69 ± 0.41 | 2.6 ± 1.9 | 27 ± 11 |
| **4c-*slow*** | 5-CHO-3-*t*Bu | 1.0 | n.d.[j] | 1.6 | 0.3 | 0.81 ± 0.042 | 1.4 ± 0.81 | 10 ± 0.0 | 41 ± 1.4 |
| **3c-*mix*** | 5-di-AM-3-*t*Bu | 7.9 | 0.15 | 2.8 | 2.6 | 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 | 2.7 | 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 | 2.4 | 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 | 2.7 | 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 | 2.5 | 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 | 2.9 | 0.89 ± 0.16 | 1.9 ± 2.1 | 3.6 ± 0.42 | 40 ± 1.4 |
| **6a-*mix*** | 3-CHO-5-Me | 0.25 | n.d.[j] | 0.3 | 0.3 | 3.6 ± 2.2 | 1.4 ± 0.78 | 29 ± 18 | 29 ± 4.9 |
| **5a-*mix*** | 3-di-AM-5-Me | 3.2 | 0.25 | 1.5 | 1.1 | 0.52 ± 0.41 | 1.0 ± 0.37 | 6.5 ± 3.1 | 19 ± 3.5 |
| **6b-*mix*** | 3-CHO-5-*t*Bu | 0.25 | n.d.[j] | 0.3 | 0.1 | 0.99 ± 0.30 | 1.0 ± 0.54 | 5.1 ± 1.9 | 8.8 ± 1.3 |
| **5b-*mix*** | 3-di-AM-5-*t*Bu | 3.7 | <0.1 | 1.9 | 1.2 | 0.72 ± 0.007 | 1.0 ± 0.57 | 7.3 ± 4.6 | 7.9 ± 1.9 |
| **2** | | n.a.[i] | n.a.[i] | n.a.[i] | n.a.[i] | 0.86 ± 0.057 | 1.9 ± 1.8 | 62 ± 0.48 | 182 ± 16 |

[a] Hydrolysis half-lives. [b] Antiviral activity in T-lymphocytes: 50% effective concentration (shown values are means 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/0 cells. [h] Thymidine kinase-deficient CEM TK$^-$ cells. [i] n.a.: not applicable. [j] n.d.: not determined.

observed. This showed the expected higher stability of the di-*iso*-butyroxymethyl group compared to the diacetoxymethyl group.

The hydrolysis studies of **3d** and **3e** in RPMI/FCS (10%) showed increased half-lives compared to those in phosphate buffer (PBS, pH 7.6). This may be attributed to a poorer solubility of these lipophilic pronucleotides in RPMI/FCS (10%) as compared to triesters **3a**−**c** and **4b**,**c**.

**Antiviral Evaluation.** *Cyclo*Sal-triesters **3**−**6** were evaluated for their anti-HIV activity in vitro (Table 1). All compounds showed activity against HIV-1 and HIV-2 in wild-type CEM/0 cells and are (except **6a**) considerably more active against HIV-2 in CEM/TK$^-$ cells when compared to d4T **2**.

Both 3-diacetoxymethyl-*cyclo*Sal-d4TMPs (**5a**,**b**) showed similar antiviral activity in CEM/TK$^-$ cells. This was expected due to their almost identical hydrolysis behavior in all media. In contrast, for 3-formyl-5-methyl-*cyclo*Sal-d4TMP (**6a**), a significant loss of activity in CEM/TK$^-$ cells has been observed (29 ± 18 $\mu$M), whereas 3-formyl-5-*tert*-butyl-*cyclo*Sal-d4TMP (**6b**) exhibited only a slight activity loss (5.1 ± 1.9 $\mu$M).

Although the di-*iso*-butyroxymethyl group was significantly more stable against enzymatic cleavage compared to the diacetoxymethyl group 5-di-*i*BOM-*cyclo*Sal-d4TMPs **3d** (X = Me; $EC_{50}$ >10 $\mu$M) and **3e-*mix*** (X = *t*Bu; $EC_{50}$ = 2.4 ± 2.3 $\mu$M) had a lower antiviral activity in CEM/TK$^-$ cells than their 5-di-AM counterparts **3b** (X = Me; $EC_{50}$ = 6.3 ± 2.9 $\mu$M) and **3c-*mix*** (X = *t*Bu; $EC_{50}$ = 0.78 ± 0.0 $\mu$M), respectively. One reason may be again the poorer solubility of the 5-di-*i*BOM-*cyclo*Sal-d4TMPs in RPMI/FCS(10%). Taking the hydrolysis half-lives in PBS (pH = 7.6) into account, at least for **3e**, similar antiviral activities to those of **3c** have been expected.

Interestingly, the antiviral data for the separated diastereomers of **3c**,**e** and **4c** showed a clear trend. The ($S_P$)-configurated *fast*-eluting diastereomer was always more active than the *slow*-diastereomer. In contrast to previous work, the hydrolysis data of the separated diastereomers were essentially identical here. Thus, the difference in antiviral activity may be due to a difference in membrane permeability.

## Conclusion

In summary, 5-di-*i*BOM-3-alkyl- (**3d**,**e**), 3-di-AM-5-alkyl- (**5a**,**b**), and 3-formyl-5-alkyl-*cyclo*Sal-d4TMPs (**6a**,**b**) have been synthesized successfully. Their complex hydrolytic and antiviral behavior has been analyzed and was compared with the data known for the 5-diacetoxymethyl-3-alkyl- (**3b**,**c**) and 5-formyl-3-alkyl-*cyclo*Sal-d4TMPs (**4b**,**c**). All compounds were proven to be potent inhibitors of HIV-1 and HIV-2 replication, and most of them retained strong antiviral potency in TK-deficient CEM/TK cells.

The di-*iso*-butyroxymethyl group showed the expected higher stability against enzymatic cleavage but showed also some solubility problems due to high lipophilicity. For 3-diacetoxymethyl-5-alkyl-*cyclo*Sal-d4TMPs (**6a**,**b**) and 3-formyl-5-alkyl-*cyclo*Sal-d4TMPs (**5a**,**b**), a significant difference in half-lives of the chemical hydrolysis was found (13−15 fold), as conceptually intended.

The analysis of the separated diastereomers of **3c**,**e** and **4c** points to a generally better antiviral activity for the *fast*-diastereomers than for the *slow*-diastereomers. The absolute configuration at the phosphorus atom of **4c-*slow*** was determined as ($R_P$) by single-crystal X-ray structure. According to HPLC studies, the selective release of **4c-*slow*** from **3c-*slow*** and **3e-*slow*** by enzymatic cleavage was proven. Hence, the better antiviral activity against HIV-2 in CEM/TK$^-$ cells can be correlated with the ($S_P$)-configuration of the *cyclo*Sal-triesters.

## 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 ($\delta$) 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), 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 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, 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 $\lambda_{max}$ are given in nm. UV absorptions of *cyclo*Sal nucleotides were determined from their HPLC data (diode array detector). 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 $\mu$M; 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 $\mu$M; Merck, Darmstadt, Germany). The flow rate was 10 mL/min, and detection was performed at a wavelength of 260 nm. The lyophilized products **3**−**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*-di*iso*propylethylamine and triethylamine were distilled from sodium prior to use. Acetic anhydride, isobutyric anhydride, and phosphorus(III) chloride were distilled under nitrogen prior to use. $CH_3CN$ for HPLC was obtained from Acros (HPLC grade).

**General Procedure A: Preparation of 6-Bromo-4-alkylsalicyl Alcohols (11).** The 6-bromo-4-alkylsalicyl alcohols **11a** and **11b** were synthesized by reduction of a respective 6-bromosalicyl carbonyl compound (**10**, **14**) with $LiAlH_4$. Therefore, $LiAlH_4$ (1.0 equiv for **14**, 2.0 equiv for **10**) was suspended under nitrogen in dry THF and a solution of the respective carbonyl compound (1.0 equiv) in THF was added dropwise within 30−45 min. After stirring for 2 h at room temperature, the reaction mixture was refluxed for 1 h. At 0 °C, the reaction mixture was acidified to pH 4−5 with 2 N HCl, then diluted with water and the phases were separated with ethyl acetate. The aqueous layer was extracted with ethyl acetate four times, and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The products were purified by column chromatography (ethyl acetate), whereas in the case of **11a**, an additional purification by preparative TLC [Chromatotron; $CH_2Cl_2$/$CH_3OH$ 19:1 v/v] was necessary.

**General Procedure B: Preparation of Salicyl Alcohol Isopropylidene Acetals (15).** Salicyl alcohols (**11**, 1.0 equiv) were dissolved in acetone and 2,2-dimethoxypropane (5 equiv), *para*-toluenesulfonic acid monohydrate (0.1 equiv), and anhydrous sodium sulfate (3 equiv) were added. The reaction mixture was stirred for 3 d (**15b**) or 4 d (**15a**) at 40 °C and then concentrated under reduced pressure. The residue was dissolved in water and ethyl acetate, and the phases were separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with 1 M NaOH (2×) and with water. After drying with sodium sulfate, the solvent was removed in vacuo. The products were purified by preparative TLC [Chromatotron; petroleum ether 50−70/$CH_2Cl_2$ 9:1 v/v (**15a**) or petroleum ether 50−70/$CH_2Cl_2$ gradient (0−30%, **15b**)].

**General Procedure C: Preparation of 6-Formyl-4-alkylsalicyl Alcohols (16).** Under nitrogen, the 6-bromo-4-alkylsalicyl alcohol isopropylidene acetal (**15a**,**b**, 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 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 diethyl ether, washed (3 × $H_2O$, 1 × 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/$CH_2Cl_2$ gradient (0−40%; **15a**) or petroleum ether 50−70/$CH_2Cl_2$ 1:2 v/v (**15b**)].

**General Procedure D: Deprotection of Salicyl Alcohol Isopropylidene Acetals (16).** To a solution of salicyl alcohol isopropylidene acetals **16** (1.0 equiv) in $CH_3CN$/$H_2O$ (7:3), three drops (**16a**) or 3 mL (**16b**) of concd hydrochloric acid were added. The reaction mixture was brought to boil with a heat gun for 30 s. This procedure was repeated until the deprotection was complete (monitored via TLC). The phases were separated 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 products were purified by column chromatography ($CH_2Cl_2$/$CH_3OH$ gradient (0−3%)).

**General Procedure E: Preparation of 3-Formyl-5-alkyl-*cyclo*Sal-d4T-monophosphates (6).** The saligenyl chlorophosphites have been synthesized from the respective 3-formyl-5-alkylsalicyl alcohols **7** as described in ref 4, but the reactions were carried out at −55 °C instead of −20 °C. The grade of purity was determined by integration of $^1$H NMR signals (single proton Pyr·HCl vs one aromatic proton of the saligenyl chlorophosphite).

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 for 3 h. 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 for 1 h and then poured into a 1 M HOAc/NaOAc buffer (pH = 5). 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 residues were purified by preparative TLC [Chromatotron; $CH_2Cl_2$/$CH_3OH$ gradient ((0−5%; **6a**) or (0−2%; **6b**)) + 0.1% glacial acetic acid]. The isolated products were lyophilized from $CH_3CN$/$H_2O$ 1:1.

**General Procedure F: Preparation of Dialkoxymethyl-*cyclo*Sal-d4T-monophosphates 3 and 5.** Under nitrogen, to a solution of the respective formyl-*cyclo*Sal-d4T-monophosphate (**4**, **6**) in dry $CH_3CN$ and freshly distilled acetic or isobutyric anhydride zirconium(IV) chloride ($ZrCl_4$) was added in one portion. The reaction mixture was stirred at room temperature and monitored via TLC ($CH_2Cl_2$/$CH_3OH$ 9:1 v/v (**3d**, **5a**) or ethyl acetate/$CH_3OH$ 9:1 v/v (**3e**, **5b**)). If necessary, additional $ZrCl_4$ was added. Then the mixture was diluted with phosphate buffer (pH = 7.3) and ethyl acetate, and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried with sodium sulfate, and the solvent was removed in vacuo. Purification was achieved by chromatography. The isolated products were lyophilized from $CH_3CN$/$H_2O$ 1:1 or directly from the eluent used for preparative RP-HPLC.

**5-Methylsalicylic Acid Methyl Ester (9).** 5-Methylsalicylic acid (**8**, 5.00 g, 32.9 mmol) was dissolved in 80 mL of $CH_3OH$ and concd sulfuric acid (16 mL, 0.30 mol) was added slowly at 0 °C. The reaction mixture was heated under reflux for 2 d. After cooling down to room temperature, the reaction mixture was poured into 250 mL 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. Purification was achieved by preparative TLC [Chromatotron; petroleum ether 50−70/$CH_2Cl_2$ 1:1 v/v]. Yield: 5.18 g (31.2 mmol, 95%) of a colorless liquid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 10.30 (s, 1H, phenol-OH), 7.59−7.56 (m, 1H, aryl-H-6), 7.34 (dd, $J$ = 8.5, 2.3 Hz, 1H, aryl-H-4), 6.88 (d, $J$ = 8.5 Hz, 1H, aryl-H-3), 3.88 (s, 3H, $OCH_3$), 2.24 (s, 3H, $CH_3$) ppm.

**3-Bromo-5-methyl-methylsalicylate (10).** 5-Methylsalicylic acid methyl ester (**9**, 6.61 g, 39.7 mmol) was dissolved in 65 mL of $CHCl_3$, and a solution of $Br_2$ (2.02 mL, 39.5 mmol) in 40 mL of $CHCl_3$ was added within 45 min at room temperature. The reaction mixture was stirred for 1 d at room temperature and then washed with 39% sodium bisulfite solution and water (2×). The organic layer was dried with sodium sulfate and concentrated under reduced pressure. The product was purified by recrystallization from $CH_3OH$. Product remaining in $CH_3OH$ was purified by preparative

TLC [Chromatotron; petroleum ether 50−70/$CH_2Cl_2$ 3:1 v/v]. Yield: 8.93 g (36.5 mmol, 92%) of a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 10.92 (s, 1H, phenol-OH), 7.72−7.69 (m, 1H, aryl-H-4), 7.64−7.60 (m, 1H, aryl-H-6), 3.92 (s, 3H, $OCH_3$), 2.25 (s, 3H, $CH_3$) ppm.

**3-Bromo-5-*tert*-butylsalicyl Aldehyde (14).** To a stirred solution of 5-*tert*-butylsalicyl aldehyde (**13**, 4.35 g, 24.4 mmol) in 5 mL of HOAc a solution of $Br_2$ (1.29 mL, 25.2 mmol) in 12 mL of HOAc was added dropwise within 15 min. The reaction mixture was stirred for 3 h at 50 °C and was monitored via TLC (petroleum ether 50−70/ethyl acetate 4:1 v/v). As the conversion of **13** was not complete, 0.43 mL (8.4 mmol) $Br_2$ in 1.7 mL of HOAc were added dropwise and the reaction solution was stirred for additional 15 h at 50 °C. Subsequently, the reaction mixture was diluted with $CH_2Cl_2$ and the organic layer was washed with 39% sodium bisulfite solution, water, saturated aqueous $NaHCO_3$, and brine. After drying with sodium sulfate, the organic layer was concentrated under reduced pressure. The product was purified by preparative TLC [Chromatotron; petroleum ether 50−70]. Yield: 5.79 g (22.5 mmol, 92%) of a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 11.41 (s, 1H, phenol-OH), 9.85 (s, 1H, CHO), 7.81 (d, $J$ = 2.3 Hz, 1H, aryl-H-6), 7.51 (d, $J$ = 2.3 Hz, 1H, aryl-H-4), 1.33 (s, 9H, *t*Bu) ppm.

**6-Bromo-4-methylsalicyl Alcohol (11a).** General procedure A with 3-bromo-5-methyl-methylsalicylate (**10**, 3.80 g, 15.5 mmol) dissolved in 50 mL of dry THF and $LiAlH_4$ (1.18 g, 31.1 mmol) as suspension in 80 mL of dry THF. Yield: 3.06 g (14.1 mmol, 91%) of a yellow oil. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 8.85 (s, 1H, phenol-OH), 7.22−7.18 (m, 1H, aryl-H-5), 7.09−7.03 (m, 1H, aryl-H-3), 5.31 (s, 1H, benzyl-OH), 4.52 (d, $J$ = 3.3 Hz, 2H, benzyl-H), 2.20 (s, 3H, $CH_3$) ppm.

**6-Bromo-4-*tert*-butylsalicyl Alcohol (11b).** General procedure A with 3-bromo-5-*tert*-butylsalicyl aldehyde (**14**, 5.69 g, 22.1 mmol) dissolved in 40 mL of dry THF and $LiAlH_4$ (750 mg, 19.8 mmol) as suspension in 60 mL of dry THF. Yield: 5.25 g (20.3 mmol, 92%) of a yellow oil. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 8.89 (s, 1H, phenol-OH), 7.22−7.18 (m, 2H, aryl-H-3, aryl-H-5), 5.34 (t, $J$ = 5.4 Hz, 1H, benzyl-OH), 4.55 (d, $J$ = 4.8 Hz, 2H, benzyl-H), 1.24 (s, 9H, *t*Bu) ppm.

**6-Bromo-4-methylsalicyl Alcohol Isopropylidene Acetal (15a).** General procedure B with 6-bromo-4-methylsalicyl alcohol (**11a**, 2.16 g, 10.0 mmol) dissolved in 45 mL of acetone, 2,2-dimethoxypropane (6.32 mL, 51.0 mmol), *para*-toluenesulfonic acid monohydrate (191 mg, 1.11 mmol), and anhydrous sodium sulfate (4.3 g). Yield: 1.90 g (7.39 mmol, 74%) of a colorless oil. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 7.29−7.25 (m, 1H, aryl-H-5), 6.90−6.86 (m, 1H, aryl-H-3), 4.78 (s, 2H, benzyl-H), 2.21 (s, 3H, $CH_3$), 1.47 (s, 6H, acetal-$CH_3$) ppm.

**6-Bromo-4-*tert*-butylsalicyl Alcohol Isopropylidene Acetal (15b).** General procedure B with 6-bromo-4-*tert*-butylsalicyl alcohol (**11b**, 5.00 g, 19.3 mmol) dissolved in 90 mL of acetone, 2,2-dimethoxypropane (12.2 mL, 98.4 mmol), *para*-toluenesulfonic acid monohydrate (370 mg, 1.92 mmol), and anhydrous sodium sulfate (8.4 g). Yield: 4.96 g (16.5 mmol, 86%) of a colorless solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 7.41 (d, $J$ = 2.5 Hz, 1H, aryl-H-5), 7.12−7.08 (m, 1H, aryl-H-3), 4.82 (s, 2H, benzyl-H), 1.48 (s, 6H, acetal-$CH_3$), 1.24 (s, 9H, *t*Bu) ppm.

**6-Formyl-4-methylsalicyl Alcohol Isopropylidene Acetal (16a).** General procedure C with 6-bromo-4-methylsalicyl alcohol isopropylidene acetal (**15a**, 1.81 g, 7.04 mmol) dissolved in 40 mL of dry THF, *n*-butyl lithium (8.80 mL, 1.6 M in hexane, 14.1 mmol), and *N*,*N*-dimethylformamide (5.43 mL, 69.8 mmol) dissolved in 5.43 mL of dry THF. Yield: 1.15 g (5.58 mmol, 80%) of a yellow oil. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 10.26 (s, 1H, formyl-H), 7.41−7.38 (m, 1H, aryl-H-5), 7.24−7.20 (m, 1H, aryl-H-3), 4.86 (s, 2H, benzyl-H), 2.26 (s, 3H, $CH_3$), 1.54 (s, 6H, acetal-$CH_3$) ppm.

**6-Formyl-4-*tert*-butylsalicyl Alcohol Isopropylidene Acetal (16b).** General procedure C with 6-bromo-4-*tert*-butylsalicyl alcohol isopropylidene acetal (**15b**, 2.35 g, 7.85 mmol) dissolved in 45 mL of dry THF, *n*-butyl lithium (9.02 mL, 1.6 M in hexane, 14.4 mmol), and *N*,*N*-dimethylformamide (6.04 mL, 78.7 mmol) dissolved in 6.04 mL of dry THF. Yield: 1.48 g (5.96 mmol, 76%) of a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 10.27 (s, 1H, formyl-H), 7.57 (d, $J$ = 2.5 Hz, 1H, aryl-H-5), 7.47 (d, $J$ = 2.5 Hz, 1H, aryl-H-3), 4.89 (s, 2H, benzyl-H), 1.53 (s, 6H, acetal-$CH_3$), 1.26 (s, 9H, *t*Bu) ppm.

**6-Formyl-4-methylsalicyl Alcohol (7a).** General procedure D with 6-formyl-4-methylsalicyl alcohol isopropylidene acetal (**16a**, 1.87 g, 9.06 mmol) dissolved in 50 mL of $CH_3CN/H_2O$ (7:3 v/v). Yield: 1.30 g (7.82 mmol, 86%) of a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 10.76 (s, 1H, phenol-OH), 10.00 (s, 1H, formyl-H), 7.53−7.48 (m, 1H, aryl-H-3), 7.46−7.41 (m, 1H, aryl-H-5), 5.21 (t, $J$ = 5.5 Hz, 1H, benzyl-OH), 4.53 (d, $J$ = 5.0 Hz, 2H, benzyl-H), 2.29 (s, 3H, $CH_3$) ppm.

**6-Formyl-4-*tert*-butylsalicyl Alcohol (7b).** General procedure D with 6-formyl-4-*tert*-butylsalicyl alcohol isopropylidene acetal (**16b**, 1.30 g, 5.24 mmol) dissolved in 50 mL of $CH_3CN/H_2O$ (7:3 v/v). Yield: 960 mg (4.61 mmol, 88%) of an orange oil. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 10.82 (s, 1H, phenol-OH), 10.05 (s, 1H, formyl-H), 7.75 (d, $J$ = 2.5 Hz, 1H, aryl-H-3), 7.64 (d, $J$ = 2.5 Hz, 1H, aryl-H-5), 5.25 (t, $J$ = 5.4 Hz, 1H, benzyl-OH), 4.55 (d, $J$ = 4.8 Hz, 2H, benzyl-H), 1.29 (s, 9H, *t*Bu) ppm.

**3-Formyl-5-methyl-*cyclo*Sal-d4T-monophosphate (6a).** General procedure E with 6-formyl-4-methylsalicyl alcohol (**7a**, 500 mg, 3.01 mmol) dissolved in 18 mL of dry diethyl ether, phosphorus(III) chloride (0.25 mL, 2.9 mmol), and dry pyridine (0.46 mL, 5.7 mmol) in 2.3 mL of dry diethyl ether. Yield: 415 mg (purity: 96%). Quantities for *cyclo*Sal-d4T-monophosphate synthesis: 410 mg of crude saligenyl chlorophosphite dissolved in 14 mL of dry $CH_3CN$, d4T (**2**, 170 mg, 0.758 mmol) dissolved in 21 mL of dry $CH_3CN$, DIPEA (0.22 mL, 1.3 mmol), and *tert*-butyl hydroperoxide (0.43 mL, 5.5 M in *n*-nonane, 2.4 mmol). Yield: 145 mg (0.332 mmol, 44%) of a diastereomeric mixture (ratio 0.65:1) as a colorless foam. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 11.32 (s, 1H, 1 × NH), 11.30 (s, 1H, 1 × NH), 10.26 (s, 1H, 1 × formyl-H), 10.21 (s, 1H, 1 × formyl-H), 7.58−7.55 (m, 2H, 2 × aryl-H-4), 7.42−7.39 (m, 2H, 2 × aryl-H-6), 7.16 (d, $J$ = 1.3 Hz, 1H, 1 × thymine-H-6), 7.15 (d, $J$ = 1.5 Hz, 1H, 1 × thymine-H-6), 6.78−6.74 (m, 2H, 2 × 1′-H), 6.42 (ddd, $J$ = 6.0, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.35 (ddd, $J$ = 6.0, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.04−5.97 (m, 2H, 2 × 2′-H), 5.61−5.50 (m, 2H, 2 × benzyl-H), 5.48−5.36 (m, 2H, 2 × benzyl-H), 4.98−4.93 (m, 2H, 2 × 4′-H), 4.42−4.28 (m, 4H, 2 × 5′-H), 2.32 (s, 6H, 2 × $CH_3$), 1.66 (d, $J$ = 1.0 Hz, 3H, 1 × thymine-$CH_3$), 1.62 (d, $J$ = 1.0 Hz, 3H, 1 × thymine-$CH_3$) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): $\delta$ = −9.62, −10.19 ppm.

**3-Formyl-5-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (6b).** General procedure E with 6-formyl-4-*tert*-butylsalicyl alcohol (**7b**, 230 mg, 1.10 mmol) dissolved in 8 mL of dry THF, phosphorus(III) chloride (0.11 mL, 1.3 mmol) and dry pyridine (0.21 mL, 2.6 mmol) in 1.1 mL of dry THF. Yield: 295 mg (purity: 55%). Quantities for *cyclo*Sal-d4T-monophosphate synthesis: 290 mg of crude saligenyl chlorophosphite dissolved in 10 mL of dry $CH_3CN$, d4T (**2**, 110 mg, 0.495 mmol) dissolved in 15 mL of dry $CH_3CN$, DIPEA (0.14 mL, 0.80 mmol), and *tert*-butyl hydroperoxide (0.26 mL, 5.5 M in *n*-nonane, 1.4 mmol). Yield: 86 mg (0.18 mmol, 36%) of a diastereomeric mixture (ratio 0.85:1.0) as a colorless foam. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 11.32 (s, 2H, 2 × NH), 10.27 (s, 1H, 1 × formyl-H), 10.26 (s, 1H, 1 × formyl-H), 7.77−7.72 (m, 2H, 2 × aryl-H-4), 7.71−7.68 (m, 2H, 2 × aryl-H-6), 7.20 (d, $J$ = 1.0 Hz, 1H, 1 × thymine-H-6), 7.16 (d, $J$ = 1.0 Hz, 1H, 1 × thymine-H-6), 6.82−6.77 (m, 2H, 2 × 1′-H), 6.45−6.41 (m, 1H, 1 × 3′-H), 6.38−6.34 (m, 1H, 1 × 3′-H), 6.05−6.01 (m, 1H, 1 × 2′-H), 6.01−5.97 (m, 1H, 1 × 2′-H), 5.61 (dd, $J$ = 14.8, 6.0 Hz, 1H, 1 × benzyl-H), 5.57 (dd, $J$ = 15.1, 5.0 Hz, 1H, 1 × benzyl-H), 5.51−5.43 (m, 2H, 2 × benzyl-H), 4.99−4.94 (m, 2H, 2 × 4′-H), 4.43−4.21 (m, 4H, 2 × 5′-H), 1.65 (s, 3H, 1 × thymine-$CH_3$), 1.60 (s, 3H, 1 × thymine-$CH_3$), 1.29 (s, 18H, 2 × *t*Bu) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): $\delta$ = −9.68, −10.03 ppm.

**3-Diacetoxymethyl-5-methyl-*cyclo*Sal-d4T-monophosphate (5a).** General procedure F with 3-formyl-5-methyl-*cyclo*Sal-d4T-monophosphate (**6a**, 60.0 mg, 138 $\mu$mol) dissolved in 0.6 mL of dry $CH_3CN$, freshly distilled acetic anhydride (0.32 mL, 3.4 mmol),

and $ZrCl_4$ (16 mg, 67 μmol). Reaction time: 45 min. Purification: preparative TLC [Chromatotron; ethyl acetate/$CH_3OH$ 9:1 v/v] and preparative RP-HPLC (water/$CH_3CN$ 3:2 v/v). Yield: 14.4 mg (26.8 μmol, 19%) of a diastereomeric mixture (ratio 0.6:1.0) as a colorless foam. $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.33 (s, 2H, 2 × NH), 7.78 (s, 1H, 1 × H-8), 7.74 (s, 1H, 1 × H-8), 7.39−7.35 (m, 2H, 2 × aryl-H-4), 7.21−7.16 (m, 4H, 2 × aryl-H-6, 2 × thymine-H-6), 6.82−6.77 (m, 2H, 2 × 1′-H), 6.41 (ddd, *J* = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.34 (ddd, *J* = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.05−5.98 (m, 2H, 2 × 2′-H), 5.54−5.45 (m, 2H, 2 × benzyl-H), 5.42−5.32 (m, 2H, 2 × benzyl-H), 4.98−4.91 (m, 2H, 2 × 4′-H), 4.35−4.19 (m, 4H, 2 × 5′-H), 2.31 (s, 3H, 1 × $CH_3$), 2.31 (s, 3H, 1 × $CH_3$), 2.12 (s, 3H, 1 × $OC(O)CH_3$), 2.11 (s, 3H, 1 × $OC(O)CH_3$), 2.11 (s, 3H, 1 × $OC(O)CH_3$), 2.10 (s, 3H, 1 × $OC(O)CH_3$), 1.66 (d, *J* = 1.3 Hz, 3H, 1 × thymine-$CH_3$), 1.60 (d, *J* = 1.3 Hz, 3H, 1 × thymine-$CH_3$) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −9.56, −9.67 ppm.

**3-Diacetoxymethyl-5-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (5b).** General procedure F with 3-formyl-5-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (**6b**, 42.4 mg, 89.0 μmol) dissolved in 2 mL of dry $CH_3CN$, freshly distilled acetic anhydride (0.22 mL, 2.3 mmol), and $ZrCl_4$ (19 mg, 82 μmol + 15 mg, 84 μmol after 30 min). Reaction time: 2.5 h. Purification: preparative TLC [Chromatotron; $CH_2Cl_2$/$CH_3OH$ gradient (0−2%)] and preparative RP-HPLC (water/$CH_3CN$ 1:1 v/v). Yield: 5.0 mg (8.6 μmol, 10%) of a diastereomeric mixture (ratio 0.9:1.0) as a colorless foam. $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.34 (s, 2H, 2 × NH), 7.79 (s, 1H, 1 × H-8), 7.76 (s, 1H, 1 × H-8), 7.51−7.47 (m, 2H, 2 × aryl-H-4), 7.46−7.42 (m, 2H, 2 × aryl-H-6), 7.19 (d, *J* = 1.3 Hz, 1H, 1 × thymine-H-6), 7.17 (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, 1.8 Hz, 1H, 1 × 3′-H), 6.34 (ddd, *J* = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.07−5.97 (m, 2H, 2 × 2′-H), 5.59−5.48 (m, 2H, 2 × benzyl-H), 5.42 (dd, *J* = 14.1, 5.8 Hz, 1H, 1 × benzyl-H), 5.39 (dd, *J* = 14.3, 6.5 Hz, 1H, 1 × benzyl-H), 4.99−4.93 (m, 2H, 2 × 4′-H), 4.37−4.22 (m, 4H, 2 × 5′-H), 2.12 (s, 3H, 1 × $OC(O)CH_3$), 2.12 (s, 3H, 1 × $OC(O)CH_3$), 2.11 (s, 3H, 1 × $OC(O)CH_3$), 2.10 (s, 3H, 1 × $OC(O)CH_3$), 1.63 (d, *J* = 1.0 Hz, 3H, 1 × thymine-$CH_3$), 1.55 (d, *J* = 1.0 Hz, 3H, 1 × thymine-$CH_3$), 1.28 (s, 9H, 1 × *t*Bu), 1.27 (s, 9H, 1 × *t*Bu) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −9.52, −9.57 ppm.

The synthesis of compounds **3c**, **4b**, and **4c** has already been published in ref 4. The separation of the diastereomers was achieved by preparative RP-HPLC with water/$CH_3CN$ 2:1 v/v (**3c**) and water/$CH_3CN$ 2:1 v/v containing 0.5% HOAc (**4c**) as eluent.

**5-Formyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (4c). Analytical Data of 4c-*fast*.** $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.35 (s, 1H, NH), 9.96 (s, 1H, formyl-H), 7.92 (s, 1H, aryl-H-4), 7.79−7.75 (m, 1H, aryl-H-6), 7.22 (d, *J* = 1.0 Hz, 1H, thymine-H-6), 6.84−6.80 (m, 1H, 1′-H), 6.44−6.40 (m, 1H, 3′-H), 6.05−6.01 (m, 1H, 2′-H), 5.61−5.45 (m, 2H, 2 × benzyl-H), 5.00−4.96 (m, 1H, 4′-H), 4.40−4.31 (m, 2H, 5′-H), 1.61 (s, 3H, thymine-$CH_3$), 1.35 (s, 9H, *t*Bu) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −9.43 ppm.

**Analytical Data of 4c-*slow*.** $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.33 (s, 1H, NH), 9.97 (s, 1H, formyl-H), 7.93 (s, 1H, aryl-H-4), 7.79−7.76 (m, 1H, aryl-H-6), 7.20 (d, *J* = 1.0 Hz, 1H, thymine-H-6), 6.82−6.79 (m, 1H, 1′-H), 6.45−6.41 (m, 1H, 3′-H), 6.07−6.03 (m, 1H, 2′-H), 5.62−5.40 (m, 2H, 2 × benzyl-H), 5.01−4.97 (m, 1H, 4′-H), 4.41−4.34 (m, 2H, 5′-H), 1.58 (d, *J* = 1.0 Hz, 3H, thymine-$CH_3$), 1.39 (s, 9H, *t*Bu) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −9.06 ppm.

**5-Diacetoxymethyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (3c). Analytical Data of 3c-*fast*.** $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.35 (s, 1H, NH), 7.52 (s, 1H, H-8), 7.47−7.44 (m, 1H, aryl-H-4), 7.39−7.36 (m, 1H, aryl-H-6), 7.22 (d, *J* = 1.0 Hz, 1H, thymine-H-6), 6.84−6.78 (m, 1H, 1′-H), 6.42 (ddd, *J* = 6.0, 1.8, 1.5 Hz, 1H, 3′-H), 6.05−6.01 (m, 1H, 2′-H), 5.53−5.38 (m, 2H, 2 × benzyl-H), 4.99−4.94 (m, 1H, 4′-H), 4.38−4.28 (m, 2H, 5′-H), 2.11 (s, 6H, 2 × $OC(O)CH_3$), 1.59 (d, *J* = 1.0 Hz, 3H, thymine-$CH_3$), 1.31 (s, 9H, *t*Bu) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −9.06 ppm.

**Analytical Data of 3c-*slow*.** $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.31 (s, 1H, NH), 7.52 (s, 1H, H-8), 7.47−7.44 (m, 1H, aryl-H-4), 7.39−7.36 (m, 1H, aryl-H-6), 7.18 (d, *J* = 1.3 Hz, 1H, thymine-H-6), 6.82−6.79 (m, 1H, 1′-H), 6.42 (ddd, *J* = 6.0, 1.8, 1.8 Hz, 1H, 3′-H), 6.06−6.02 (m, 1H, 2′-H), 5.53−5.36 (m, 2H, 2 × benzyl-H), 5.00−4.94 (m, 1H, 4′-H), 4.40−4.28 (m, 2H, 5′-H), 2.11 (s, 6H, 2 × $OC(O)CH_3$), 1.57 (d, *J* = 1.3 Hz, 3H, thymine-$CH_3$), 1.35 (s, 9H, *t*Bu) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −8.71 ppm.

**5-Diisobutyroxymethyl-3-methyl-*cyclo*Sal-d4T-monophosphate (3d).** General procedure F with 5-formyl-3-methyl-*cyclo*Sal-d4T-monophosphate (**4b**, 51.0 mg, 117 μmol) dissolved in 1 mL of dry $CH_3CN$, freshly distilled isobutyric anhydride (0.40 mL, 2.5 mmol), and $ZrCl_4$ (30.0 mg, 129 μmol). Reaction time: 3 h. Purification: preparative TLC [Chromatotron; $CH_2Cl_2$/$CH_3OH$ gradient (0−5%)]. Yield: 21.0 mg (35.4 μmol, 30%) of a diastereomeric mixture (ratio 0.8:1.0) as a colorless foam. $^1$H NMR (500 MHz, DMSO-$d_6$): δ = 11.35−11.32 (m, 2H, 2 × NH), 7.49 (s, 2H, 2 × H-8), 7.42−7.39 (m, 2H, 2 × aryl-H-4), 7.30−7.28 (m, 2H, 2 × aryl-H-6), 7.21−7.17 (m, 2H, 2 × thymine-H-6), 6.82−6.78 (m, 2H, 2 × 1′-H), 6.43−6.40 (m, 1H, 1 × 3′-H), 6.39−6.35 (m, 1H, 1 × 3′-H), 6.04−5.99 (m, 2H, 2 × 2′-H), 5.56−5.46 (m, 2H, 2 × benzyl-H), 5.45−5.35 (m, 2H, 2 × benzyl-H), 4.98−4.93 (m, 2H, 2 × 4′-H), 4.34−4.24 (m, 4H, 2 × 5′-H), 2.61 (hept, *J* = 6.9 Hz, 4H, 4 × C*H*$(CH_3)_2$), 2.24 (s, 3H, 1 × $CH_3$), 2.21 (s, 3H, 1 × $CH_3$), 1.63−1.61 (m, 3H, 1 × thymine-$CH_3$), 1.61−1.59 (m, 3H, 1 × thymine-$CH_3$), 1.11 (d, *J* = 7.1 Hz, 12H, 2 × CH$(CH_3)_2$), 1.08 (d, *J* = 6.9 Hz, 12H, 2 × CH$(CH_3)_2$) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −8.73, −8.82 ppm.

**5-Diisobutyroxymethyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (3e).** General procedure F with 5-formyl-3-*tert*-butyl-*cyclo*Sal-d4T-monophosphate (**4c**, 97.5 mg, 0.205 mmol) dissolved in 2 mL of dry $CH_3CN$, freshly distilled isobutyric anhydride (0.85 mL, 5.3 mmol) and $ZrCl_4$ (23.9 mg, 103 μmol + 23.9 mg, 103 μmol after 1 h). Reaction time: 2.5 h. Purification: preparative TLC [Chromatotron; $CH_2Cl_2$/$CH_3OH$ gradient (0−5%)] and preparative RP-HPLC (water/$CH_3CN$ 2:5 v/v or water/$CH_3CN$ 2:3 v/v, respectively). Yield: 32.4 mg (51.1 μmol, 25%); 15.3 mg (24.1 μmol) of **3e-*mix*** (ratio 0.8:1.0), 7.7 mg (12 μmol) of **3e-*fast***, and 9.4 mg (15 μmol) of **3e-*slow*** as colorless foams. $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.35 (s, 2H, 2 × NH), 7.54 (s, 2H, 2 × H-8), 7.44 (s, 2H, 2 × aryl-H-4), 7.36 (d, *J* = 2.0 Hz, 2H, 2 × aryl-H-6), 7.21 (d, *J* = 1.3 Hz, 1H, 1 × thymine-H-6), 7.18 (d, *J* = 1.0 Hz, 1H, 1 × thymine-H-6), 6.83−6.79 (m, 2H, 2 × 1′-H), 6.42 (ddd, *J* = 6.0, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.40 (ddd, *J* = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.05−6.01 (m, 2H, 2 × 2′-H), 5.53−5.38 (m, 4H, 4 × benzyl-H), 5.00−4.96 (m, 2H, 2 × 4′-H), 4.41−4.29 (m, 4H, 2 × 5′-H), 2.62 (hept, *J* = 7.0 Hz, 4H, 4 × C*H*$(CH_3)_2$), 1.57 (d, *J* = 1.3 Hz, 3H, 1 × thymine-$CH_3$), 1.54 (d, *J* = 1.0 Hz, 3H, 1 × thymine-$CH_3$), 1.34 (s, 9H, 1 × *t*Bu), 1.31 (s, 9H, 1 × *t*Bu), 1.11 (d, *J* = 7.0 Hz, 12H, 2 × CH$(CH_3)_2$), 1.09 (d, *J* = 7.0 Hz, 12H, 2 × CH$(CH_3)_2$) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −8.57, −8.96 ppm.

**Analytical Data of 3e-*fast*.** $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.34 (s, 1H, NH), 7.54 (s, 1H, H-8), 7.44 (s, 1H, aryl-H-4), 7.36 (d, *J* = 1.6 Hz, 1H, aryl-H-6), 7.21 (d, *J* = 1.3 Hz, 1H, thymine-H-6), 6.83−6.80 (m, 1H, 1′-H), 6.42 (ddd, *J* = 6.0, 1.6, 1.6 Hz, 1H, 3′-H), 6.04−6.01 (m, 1H, 2′-H), 5.53−5.39 (m, 2H, 2 × benzyl-H), 5.00−4.96 (m, 1H, 4′-H), 4.37−4.31 (m, 2H, 5′-H), 2.62 (hept, *J* = 6.9 Hz, 2H, 2 × C*H*$(CH_3)_2$), 1.57 (d, *J* = 1.0 Hz, 3H, thymine-$CH_3$), 1.31 (s, 9H, *t*Bu), 1.11 (d, *J* = 7.3 Hz, 6H, 1 × CH$(CH_3)_2$), 1.10 (d, *J* = 7.0 Hz, 6H, 1 × CH$(CH_3)_2$) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): δ = −8.96 ppm.

**Analytical Data of 3e-*slow*.** $^1$H NMR (400 MHz, DMSO-$d_6$): δ = 11.32 (s, 1H, NH), 7.54 (s, 1H, H-8), 7.44 (s, 1H, aryl-H-4), 7.35 (d, *J* = 1.6 Hz, 1H, aryl-H-6), 7.18 (d, *J* = 1.0 Hz, 1H, thymine-H-6), 6.82−6.79 (m, 1H, 1′-H), 6.40 (ddd, *J* = 6.0, 1.9, 1.6 Hz, 1H, 3′-H), 6.05−6.02 (m, 1H, 2′-H), 5.53−5.38 (m, 2H,

2 × benzyl-H), 5.00−4.96 (m, 1H, 4′-H), 4.40−4.30 (m, 2H, 5′-H), 2.62 (hept, $J$ = 6.9 Hz, 2H, 2 × C*H*($CH_3$)$_2$), 1.55 (d, $J$ = 1.0 Hz, 3H, thymine-$CH_3$), 1.34 (s, 9H, *t*Bu), 1.11 (d, $J$ = 7.3 Hz, 6H, 1 × CH($CH_3$)$_2$), 1.10 (d, $J$ = 7.0 Hz, 6H, 1 × CH($CH_3$)$_2$) ppm. $^{31}$P NMR (162 MHz, DMSO-$d_6$): $\delta$ = −8.57 ppm.

**Single-Crystal X-ray Diffraction Analysis of 4c-*slow*.**[13] $C_{22}H_{25}N_2O_8P$, FW: 476.41, monoclinic space group $P2_1$, $a$ = 8.3061(11) Å, $b$ = 15.864(2) Å, $c$ = 9.0212(12) Å, α = 90°, $\beta$ = 110.58°, $\gamma$ = 90°. $V$ = 1112.8(3) Å$^3$, $Z$ = 2, $\rho_{calcd}$ = 1.422 g cm$^{-3}$, $\lambda$(Mo Kα) = 0.71073 Å, $\mu$ = 0.176 mm$^{-1}$, F(000) = 500, $T$ = 153(2) K. Flack parameter[14] = −0.29(11). Crystals were obtained by diffusion method. Therefore, 3.0 mg (6.3 mmol) of **4c-*slow*** were dissolved in 0.5 mL of ethyl acetate, as unpolar solvent petroleum ether 50−70 was used. After 24 h at room temperature, colorless crystals were formed. A 0.3 mm × 0.2 mm × 0.2 mm crystal was used for data collection with Bruker Smart APEX CCD area detector diffractometer. A set of 13064 reflections was collected in the $\omega$ scan mode. There were 4914 unique reflections. Lattice parameter were determined using SAINT[15] from 428 reflections within 2.4° < 2$\theta$ < 54°. The structure was determined from 3458 reflections with $F^2 > 2\sigma(F^2)$ and was solved with SHELXS97 and refined using the SHELXL97 system of programs.[16] Refinement of $F^2$ against ALL reflections. The weighted $R$-factor w$R$ and goodness of fit $S$ ($S$ = 0.92) are based on $F^2$, conventional $R$-factors $R$ are based on $F$, with $F$ set to zero for negative $F^2$. The threshold expression of $F^2 > \sigma(F^2)$ is used only for calculating $R$-factors(gt) etc. and is not relevant to the choice of reflections for refinement. $R$-factors based on $F^2$ are statistically about twice as large as those based on $F$, and $R$-factors based on all data will be even larger. The full-matrix least-squares refinement of $F^2$ varied 304 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms. H atoms were included using a riding model, coordinate shifts of C applied to attached H atoms, C−H distances were set to 1.00 to 0.95 Å, H angles idealized, $U_{iso}$(H) were set to 1.2−1.5 $U_{eq}$(C), except for the hydrogen (NH) involved in hydrogen bonding, which coordinates were refined. Final residuals were $R1$ = 0.053 for the 3458 observed data with $F^2 > 2\sigma(F^2)$ and 0.115 for all data. Final difference Fourier excursions of 0.41 and −0.28 eÅ$^{-3}$.

**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.[17,18] Studies in cell extracts were performed as reported in ref 8 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 extracts.

**Antiretroviral Evaluation.** The method of antiviral evaluation has already been described in ref 4.

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**Supporting Information Available:** $^{13}$C NMR and UV spectroscopic data, mass spectrometric data, $R_f$ values, melting points, analytical HPLC data of new *cyclo*Sal compounds; methods for HPLC analysis; general nomenclature of salicyl alcohols and *cyclo*Sal-d4TMPs. This material is available free of charge via the Internet at http://pubs.acs.org.

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