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Second-Generation *cyclo*Sal-d4TMP Pronucleotides Bearing Esterase-Cleavable Sites − The "Trapping" Concept

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An extension of the *cyclo*Sal-pronucleotide approach is presented. Attachment of an enzyme-cleavable ester/acylal group to the *cyclo*Sal-d4TMP triesters should allow these compounds to be trapped intracellularly after cleavage. The ester/acylal groups were introduced in the 3- or 5-position of the *cyclo*Sal ring system, and surprising differences were observed in hydrolysis studies in CEM cell extracts with respect to the ester/acylal moiety. While acetyl and levulinyl esters were readily cleaved, alkyl esters of *cyclo*Sal-d4TMP acids proved to be resistant to enzymatic cleavage. In contrast, AM-, POM- and POC-acylals were rapidly cleaved in the extracts, leading to *cyclo*Sal-d4TMP acids. The antiviral activity of the compounds against HIV is also presented.

Introduction

The antiviral action of nucleoside analogues like 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T, **1**) depends on their conversion into the ultimately bioactive triphosphates via the mono- and diphosphates (nucleotides) by cellular kinases. However, the first phosphorylation step to the monophosphates catalysed by the salvage-pathway enzyme thymidine kinase (TK) is often the metabolism-limiting step.[1,2] The use of nucleotides as therapeutic agents is impossible due to their high polarity and their catabolism in the blood by unspecific nucleotidases. In contrast, lipophilic nucleotide-releasing systems (pronucleotides) could circumvent these limitations (TK bypass).[3] We have developed *cyclo*Sal-pronucleotides as a new class of chemically cleavable nucleotide prodrugs of biologically active nucleoside analogues.[4] These lipophilic precursors **2** (*cyclo*Sal-d4TMP; Scheme 1) deliver d4TMP inside cells and achieve an efficient thymidine-kinase bypass.[5] *cyclo*Sal-Phosphate triesters **2** deliver the nucleotides by a chemically triggered cascade reaction (Figure 1, step a). This approach has been applied successfully to various nucleoside analogues.[6–11] Due to the lipophilic character of *cyclo*Sal-triesters like **2** and the chemically triggered delivery mechanism, a concentration equilibrium is formed through the membrane (X-prodrug, Figure 1, step b). For effective antiviral efficiency it is necessary to have high intracellular concentrations of the pronucleotide, which leads to high concentrations of the nucleotide. Thus, in order to trap the lipophilic *cyclo*Sal-triester, the attachment of a (carboxy)esterase-cleavable ester site in the *cyclo*Sal aromatic ring should lead to interesting new properties. However, ester groups are electron-withdrawing substituents and would lead to a considerable reduction of the chemical stability of the triesters. To avoid this, an alkyl spacer/isolator has to be introduced. An enzymatic cleavage reaction releases a highly polar (carboxylic acid) or at least a more polar *cyclo*Sal derivative (alcohol; Y-prodrug, Figure 1, step c).[12] The nucleotide is finally released from the polar Y-prodrug by a chemical mechanism (identical to step a).

This concept is based on the higher intracellular concentrations of esterases compared to the extracellular medium. This use of an "esterase strategy" is well known in prodrug development. Two drugs that are based on this strategy have recently been approved by the FDA as antivirals: bis{pivaloxymethyl (POM)}-PMEA (2002, Adefovir dipivoxyl, Hepsera®) and bis{isopropyloxycarbonylmethoxy (POC)}-PMPA (2001, Tenofovir disoproxil fumarate, Viread®).[13]

Two types of ester-bearing *cyclo*Sal-triesters were envisaged, namely *cyclo*sal-d4TMP acid esters **3** and **4**, which release the *cyclo*Sal-d4TMP acids **5a**,**b**, or *cyclo*Sal-d4TMP alcohol esters **6**, which liberate the *cyclo*Sal-d4TMP alcohol **7** after enzymatic cleavage (Scheme 1). In both cases, a more polar *cyclo*Sal derivative than the precursor is formed that should lead to intracellular accumulation (Y-prodrug, Figure 1; "lock-in" or "trapping" concept).

Here, the synthesis, properties and the antiviral evaluation of these new potentially enzyme-cleavable *cyclo*Sal-d4TMP triesters are reported.

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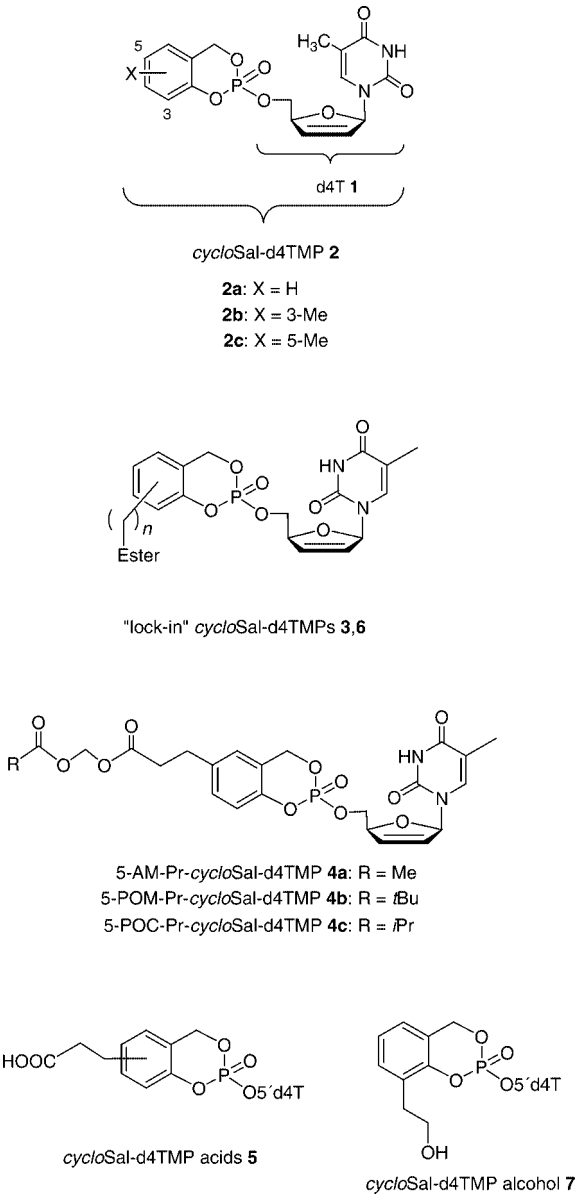


Scheme 1. Target *cyclo*Sal pronucleotides **3**–**7** of d4T **1**.

Results and Discussion

Chemistry

In order to be able to use our previous protocol[4] for the synthesis of triesters **3**–**7**, the required salicyl alcohols **8** were prepared from the corresponding phenols **9**. All synthetic steps are depicted in Schemes 2, 3 and 4. While the methyl ester **9a** was prepared by transesterification of dihydrocoumarin (**10**) with methanol in the presence of H_2SO_4 in 96% yield, the benzyl ester **9b** was synthesised by alkylation of 3-(2-hydroxyphenyl)propionic acid (**11**) with benzyl bromide in the presence of DBU in toluene (97% yield).[14] The *tert*-butyl ester **9c** was obtained by esterification of carboxylic acid **11** in the presence of DMF/dineopentylacetal in toluene (85% yield).[15] The acetyl (**9d**) and levulinyl esters (**9e**) of 2-(2-hydroxyphenyl)ethanol (**12**) were prepared by a transesterification of the acetyl or levulinyl group of ethyl acetate or ethyl levulinate in the presence of SiO_2·$NaHSO_4$ as catalyst in 95% and 82% yield, respectively.[16] The pivaloyl ester (**9f**) of **12** was synthesised from an activated pivaloyl donor by the "twisted amide" method reported previously (80% yield).[17]

Phenols **9a**–**f** were then transformed into the salicyl alcohols **8a**–**f** by treatment with paraformaldehyde and phenylboronic acid in the presence of 0.5 equiv. of propionic acid (50–85% yield).[4] Diols **8** were then treated with PCl_3 to yield cyclic chlorophosphanes **13**, which were used subsequently for the phosphitylation with d4T **1**.[18] The intermediately formed phosphite triesters were oxidized in a one-pot reaction with *tert*-butyl hydroperoxide (*t*BuOOH) to give triesters **3** and **6** (n = 2) in 30–60% yield (not optimised).[4] Interestingly, the yields here were found to be lower than reported previously for some unknown reasons. However, it was noticed that the yields depend markedly on the quality of the *t*BuOOH. Finally, *cyclo*Sal-d4TMP acid **5a** was prepared by cleavage of the *tert*-butyl ester in **3c** with TFA (87% yield), and *cyclo*Sal-d4TMP alcohol **7** was obtained after delevulinylation of **6c** with hydrazine hydrate (25% yield).

Beside *cyclo*Sal triesters carrying a C_2 linker between the *cyclo*Sal moiety and the ester group, a C_3 linker was introduced in order to prove the efficiency of isolating the ester group from the aromatic ring (*cyclo*Sal-d4TMP ester **3d**, n = 3). However, the starting material needed for this synthesis is not commercially available. Thus, a Suzuki cross-coupling procedure was used for the synthesis of the required salicyl alcohol **13**. The required precursors for the Suzuki coupling are isopropylidene acetal **14** and trialkylborane **15**.[19] The former compound was prepared starting from 2-bromophenol (**16**) by selective *ortho*-hydroxymethylation to yield **17** and subsequent acetalisation. The latter compound **15** is the hydroboration product of methyl butenoate **18** and 9-BBN. Trialkylborane **15** was immediately coupled with the acetal **14** to give the 3-alkylated acetal **19** in 83% yield. Cleavage of the acetal with catalytic amounts of HCl resulted in the formation of salicyl alcohol **13** (70% yield), which was converted into the *cyclo*Sal triester **3d** by the standard procedure (Scheme 3). The advantage of this Suzuki cross-coupling protocol is that it can be used as a general procedure for the synthesis of *cyclo*Sal nucleotides with variable linker lengths.

In addition to the compounds carrying the functionalised alkyl residue in the 3-position, *cyclo*Sal-d4TMP esters **4a**–**c** with the alkyl group attached at the 5-position of the *cyclo*Sal-moiety were prepared starting from 5-propionyl-*cyclo*Sal-d4TMP acid (**5b**). Carboxylic acid **5b** was prepared starting from 3-(4-hydroxyphenyl)propionic acid (**20**) by the same synthetic route as described for **5a** (Scheme 4). *cyclo*Sal-d4TMP acid **5b** was used in an alkylation reaction with bromomethyl acetate in the presence of DIPEA in CH_3CN at 10 °C for 3 h to yield acetoxymethyl (AM) acylal **4a** in 65% yield.[20] A general problem of such alkylation reactions is the concomitant alkylation of the nucleobase. To avoid this, the reaction temperature was kept as low as possible. Although the yield is only 65%, no base-alkylated

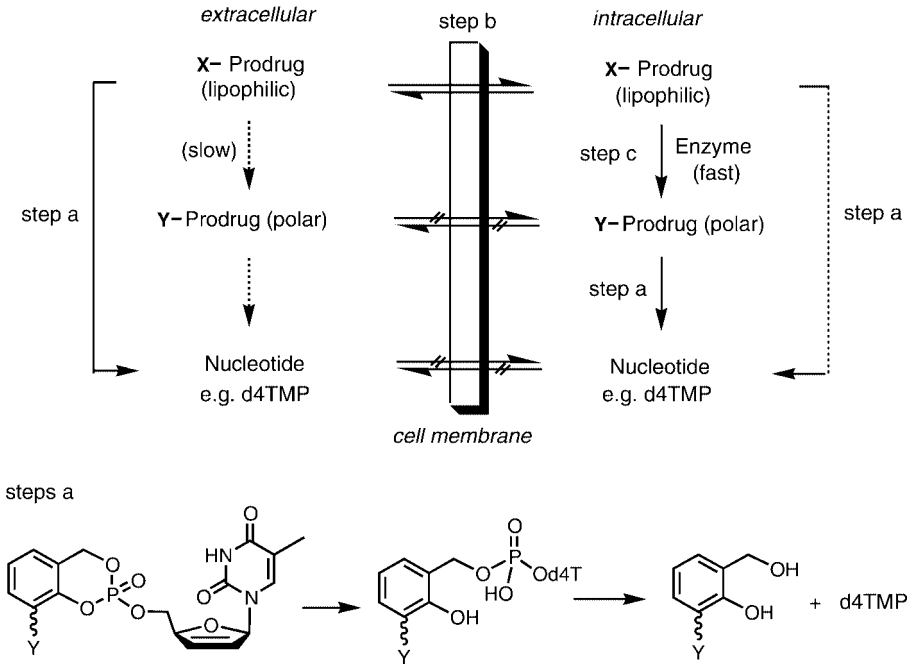
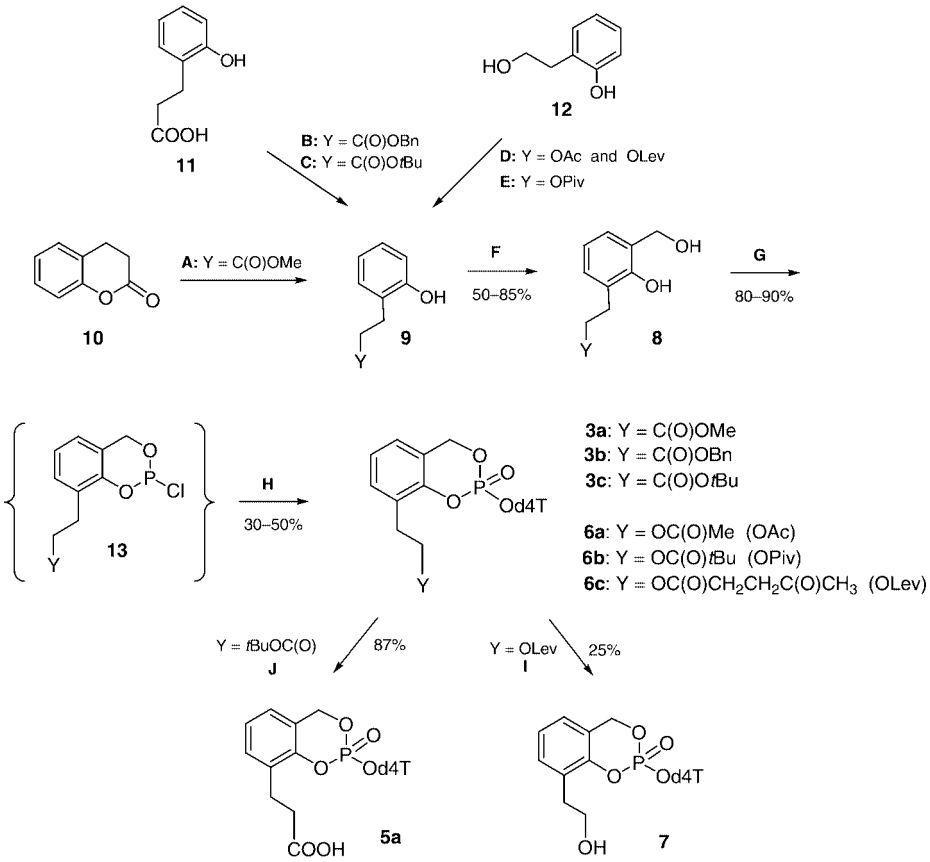
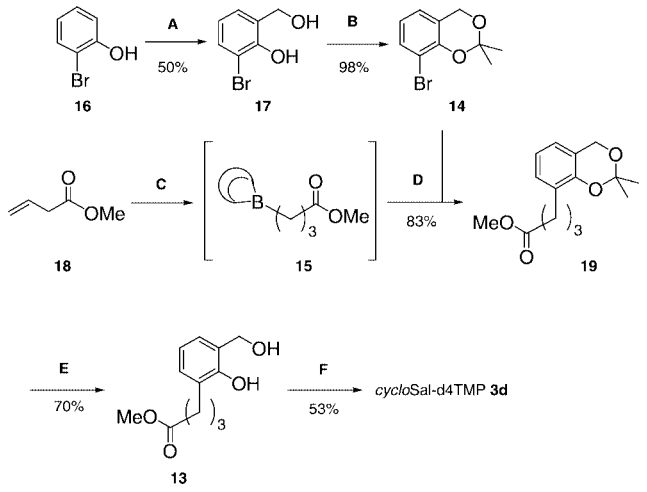


Figure 1. Trapping concept for the *cyclo*Sal-pronucleotides and the chemically induced cleavage of the triesters.



Scheme 2. Synthesis of the corresponding salicyl alcohols and the target triesters **3**, **5**, **6** and **7**. Method **A**: methanol, H_2SO_4, reflux, 5–8 h; method **B**: benzyl bromide, DBU, toluene, reflux, 7 h; method **C**: $(CH_3)_2NCH(OCH_2tBu)_2$, *tert*-butyl alcohol, toluene, reflux, 5 h; method **D**: ethyl acetate or ethyl levulinate, *n*-hexane, SiO_2·$NaHSO_4$, 67 °C, 6–18 h; method **E**: 3-pivaloyl-1,3-thiazolidine-2-thione, toluene, 65 °C, 48 h; method **F**: i) phenylboronic acid, propionic acid (cat.), *p*-formaldehyde, toluene, reflux, 6–8 h; ii) H_2O_2, THF, 0 °C, 30 min; method **G**: PCl_3, pyridine, Et_2O, 0–21 °C, 1 h; method **H**: i) d4T **1**, CH_3CN, DIPEA, 0–20 °C; ii) *t*BuOOH, CH_3CN, room temp., 30 min; method **I**: NH_2NH_2·H_2O, pyridine/HOAc (3:2), pyridine, 0 °C, 10 min; method **J**: TFA (10 equiv.), CH_2Cl_2, room temp., 1 h.



Scheme 3. Synthesis of the ester-functionalised *cyclo*Sal-d4TMP triester **3d** with a C_3 linker. Method **A**: i) phenylboronic acid, propionic acid, $(CH_2O)_n$, toluene, reflux, 18 h; ii) H_2O_2, THF, 0 °C, 45 min; method **B**: 2,2-dimethoxypropane, *p*TsOH, acetone, Na_2SO_4; method **C**: 9-BBN, THF, 0 °C → 55 °C, 16 h; method **D**: K_2CO_3, [Pd(dppf)Cl_2], DMF, 55 °C, 4 d; method **E**: cat. HCl, CH_3CN /H_2O, 5 min; method **F**: i) PCl_3, pyridine, Et_2O, –20 °C to room temp., 2 h; ii) **1**, DIPEA, CH_3CN, –20 °C to room temp., 1 h; iii) *t*BuOOH, CH_3CN, –20 °C to room temp., 1 h.

product was isolated from the above reaction. In contrast to bromomethyl acetate, bromomethyl pivalate is not commercially available. Therefore, attempts were made to convert the less reactive chloromethyl pivalate into the iodomethyl pivalate by a Finkelstein reaction. Chloromethyl pivalate was dissolved in acetone in the presence of NaI. After 3 h, NaCl had formed and iodomethyl pivalate was used immediately for the alkylation. In a second attempt, the alkylation reagent was formed in the presence of the acid **5b**, DIPEA and NaI in CH_3CN. However, in both cases a precipitation of the sodium salt of acid **5b** was observed. Consequently, an overalkylation of the remaining acid in solution was performed leading, again, to complex and inseparable mixtures. Finally, pivaloxymethyl (POM) triester **4b** was prepared from the less reactive chloromethyl pivalate in CH_3CN at ambient temperature by leaving the reaction to proceed for 9 d. Triester **4b** was isolated in 46% yield.

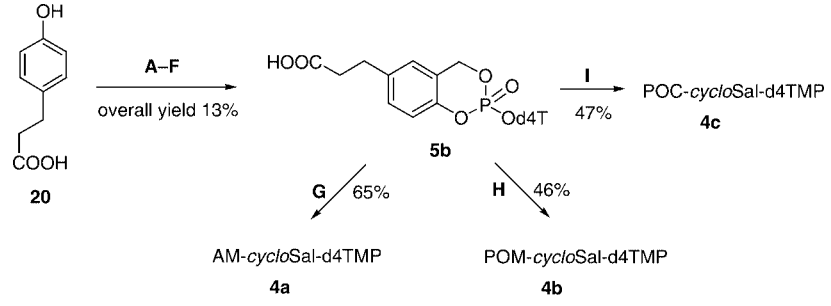
The isopropyloxycarbonylmethoxy (POC) derivative **4c** was prepared with the corresponding chloromethyl alkylation reagent. This reagent was easily synthesised from chloromethyl formate and 2-propanol. However, chloromethyl isopropyl carbonate is even less reactive than chloromethyl pivalate. Thus, at ambient temperature nearly no reaction took place even after 16 d. However, heating of the reaction mixture in CH_3CN to 50 °C for 7 d gave the target triester **4c** in 47% yield (Scheme 4).

All triesters **3**–**7** were characterised by means of ^1H, ^{13}C and ^{31}P NMR spectroscopy as well as high-resolution mass spectrometry. Experimental details are given here and in a previous report.[12]

Chemical Stability Studies

Triesters **3**–**7** were studied for their stability in aqueous 25 mM phosphate buffer (pH = 7.3). The half-lives are summarised in Table 1. As expected, the hydrolysis of the *cyclo*Sal triester was observed in these studies without additional hydrolysis of the carboxylic acid esters. Thus, the half-lives given in Table 1 refer to the cleavage of the triesters and formation of d4TMP; *cyclo*Sal-d4TMP triesters **3a**–**c** and **5a**,**b** have half-lives of between 7.3 and 13.5 h. From these values, and comparison with the half-lives of two *cyclo*Sal triesters studied before [unsubstituted triester **2a** ($t_{1/2}$ = 4.4 h) and 3-methyl-*cyclo*Sal-d4TMP **2b** ($t_{1/2}$ = 17.5 h), Table 1],[6] it was concluded that the ethylene spacer length is not entirely sufficient to separate the electron-withdrawing ester moiety from the aromatic ring. However, triester **3d**, which has a propylene linker, is more stable ($t_{1/2}$ = 17.6 h), and its stability is similar to that of 3-methyl-*cyclo*Sal-ester **2b**.

The chemical stability of *cyclo*Sal-d4TMP acids **5** was surprisingly 1.8- to 3.0-fold higher than those of esters **3** and **4**. This may be due to the overall negative charge of the deprotonated acid **5** at pH = 7.3, which avoids an efficient nucleophilic attack at the phosphorus atom. In contrast, *cyclo*Sal-d4TMP alcohol **7** shows a similar chemical stability to the esters. In all cases the products were d4TMP and the corresponding diol. This was proven by



Scheme 4. Synthesis of enzyme-cleavable 5-functionalised *cyclo*Sal-d4TMP triesters **4**. Method **A**: $(CH_3)_2NCH(OCH_2tBu)_2$, *tert*-butyl alcohol, toluene, reflux, 5 h; method **B**: i) phenylboronic acid, propionic acid (cat.), *p*-formaldehyde, toluene, reflux, 6–8 h; ii) H_2O_2, THF, 0 °C, 30 min; method **C**: PCl_3, pyridine, Et_2O, 0–21 °C, 1 h; method **D**: i) d4T **1**, CH_3CN, DIPEA, 0–20 °C; ii) *t*BuOOH, CH_3CN, room temp., 30 min; method **F**: TFA (10 equiv.), CH_2Cl_2, room temp., 1 h; method **G**: bromomethyl acetate, DIPEA, CH_3CN, 10 °C, 3 h; method **H**: chloromethyl acetate, DIPEA, CH_3CN, room temp., 9 d; method **I**: chloromethyl isopropyl carbonate, DIPEA, CH_3CN, 50 °C, 7 d.

Table 1. Lipophilicity, hydrolysis data and antiviral activity of the triesters **3**–**7** compared to the prototypes **2** and d4T **1**.

	X or Y	*n*	log$P_{calcd.}$[a]	$t_{1/2}$ [h][b]		EC_{50} [μM][c]			CC_{50} [μM][d]
				PBS[e]	CE[f]	CEM/O[g]		CEM/TK$^-$[h]	
						HIV-1	HIV-2	HIV-2	
3a	COOMe	2	0.46	7.3	7.2	0.09 ± 0.05	0.25 ± 0.15	0.40 ± 0.42	57
3b	COOBn	2	2.19	9.1	6.0	0.15 ± 0.09	1.00 ± 0.28	2.89 ± 1.96	44
3c	COO*t*Bu	2	1.65	13.5	9.0	0.33 ± 0.11	0.50 ± 0.14	1.14 ± 0.96	43
3d	COOMe	3	0.79	17.6	6.0	0.35 ± 0.09	0.47 ± 0.12	1.35 ± 0.92	85
4a	AM-Pr	–	2.18	4.3	0.25	0.20 ± 0.11	0.53 ± 0.39	25 ± 19.1	81
4b	POM-Pr	–	4.04	5.6	0.38	0.23 ± 0.04	0.33 ± 0.11	0.7 ± 0.08	24
4c	POC-Pr	–	3.56	3.9	0.9	0.26 ± 0.20	0.60 ± 0.00	1.73 ± 1.97	74
5a	COOH	2	–3.96	22.9	20.4	0.19 ± 0.08	1.4 ± 0.60	20.0 ± 0.00	100
5b	COOH	2	–3.96	12.5	12.4	0.14 ± 0.06	0.8 ± 0.08	50 ± 30	76
6a	OAc	2	0.42	13.6	1.9	0.16 ± 0.09	0.33 ± 0.24	0.15 ± 0.0	40
6b	OPiv	2	1.65	13.1	6.6	0.16 ± 0.09	0.70 ± 0.42	0.40 ± 0.96	55
6c	OLev	2	0.37	12.5	2.0	0.13 ± 0.04	0.33 ± 0.11	0.15 ± 0.1	58
7	OH	2	-0.53	12.6	15.0	0.24 ± 0.01	0.33 ± 0.11	0.49 ± 0.3	96
2a	H	–	0.28	4.4	4.0	0.10 ± 0.02	0.13 ± 0.04	0.90 ± 0.28	31
2b	3-Me	–	0.77	16	14.5	0.073 ± 0.05	0.10 ± 0.02	0.55 ± 0.21	29
2c	5-Me	–	0.77	6.2	6.0	0.46 ± 0.24	0.46 ± 0.48	1.25 ± 0.90	38
1	–	–	–0.48	n.a.	n.a.	0.23 ± 0.04	0.24 ± 0.02	15.0 ± 7.1	60

[a] Calculated partition coefficients (log*P*) calculated using the log*P* function implemented in ChemDraw 6.0. [b] Hydrolysis half-lives. [c] Antiviral activity in T-lymphocytes: 50% effective concentration, values shown are means of two to three independent experiments. [d] Cytostatic activity: 50% cytostatic concentration. [e] 25 mM phosphate buffer, pH = 7.3. [f] CEM cell extracts, pH = 6.9. [g] Wild-type CEM cells. [h] Thymidine kinase-deficient CEM cells.

HPLC co-elution experiments or ^{31}P NMR spectroscopy (step c, Figure 1).

As expected, the chemical stability half-lives of the 5-modified *cyclo*Sal-d4TMP triesters **4** dropped to 4–5.3 h. This reduced stability was expected because 5-methyl-*cyclo*Sal-d4TMP **2c** was also found to be less stable than 3-methyl-*cyclo*Sal-d4TMP **2b** (Table 1). Again, only d4TMP was released from triesters **4**, whereas the AM, POM or POC moieties proved to be stable under these conditions (Figure 2). Due to the potentially acid-labile acylal structure, the stability of triesters **4a**–**c** was determined in citrate/HCl buffer at pH = 2.0. However, compounds **4** were not cleaved either at the acylal function or at the phosphate ester moiety within 30 min.

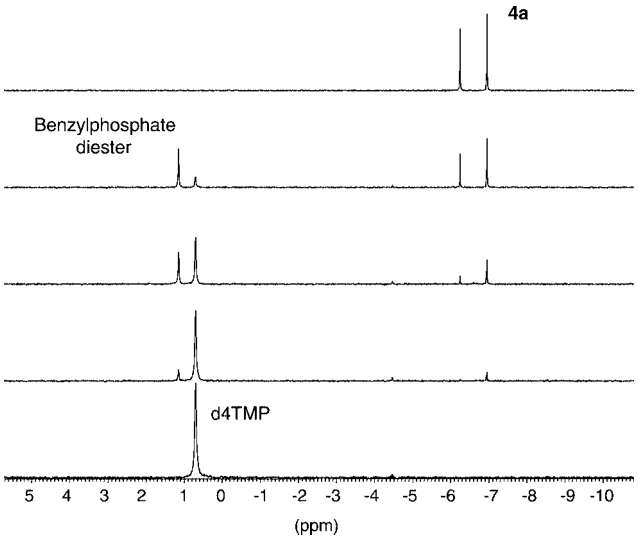


Figure 2. Chemical hydrolysis of the AM-*cyclo*Sal-d4TMP triester **4a** followed by ^{31}P NMR spectroscopy {solvent: [D_6]DMSO/50 mM imidazole·HCl buffer (pH = 7.3), 1:1 (v/v); spectra were recorded once a week; H_3PO_4 was used as external reference}.

Cell Extract Studies

Hydrolysis studies in T-lymphocyte cell extracts (CE; Table 1) proved the cleavage of the ester groups in the case of triesters **6a** (OAc ester; $t_{1/2}$ = 1.9 h vs. 13.6 h in the chemical hydrolysis) and **6c** (OLev ester; $t_{1/2}$ = 2.0 h vs. 12.5 h). Thus, triester alcohol **7** was formed (HPLC co-elution experiments). The Piv triester **6b** is more stable due to its branched alkyl group. Thus, an about 9-fold more polar product [log*P* = –0.53 (**7**) vs. 0.42 (**6a**) to 0.37 (**6c**)] is delivered compared to the precursors. However, although a more polar compound (**7**) is liberated, it cannot be excluded that the alcohol group is still not polar enough to achieve the desired intracellular trapping.

Surprisingly, triesters **3**, which were used to release the much more polar carboxylate group (*cyclo*Sal-d4TMP acid **5a**, log*P* = –3.96), were found to be inert to enzymatic cleavage. Although the half-lives measured in the cell extracts were lower than those found in the chemical hydrolysis studies, no formation of the free acid **5a** was observed by means of HPLC. This resistance to the cell extract enzymes was unexpected because carboxyl groups in drugs are often blocked bioreversibly by esterification.[20] Moreover, in previous test experiments methylpropionyl salicyl alcohol (**8a**) was incubated with pig liver esterase (PLE).[21] In those experiments, the methyl ester was cleaved extremely rapidly.

In contrast to the unsuccessful delivery of *cyclo*Sal triester **5a** from triesters **3**, the AM, POM and POC acylals **4** were efficiently cleaved in cell extracts with formation of *cyclo*Sal triester **5b**. Thus, the carboxylate group can be liberated (Figure 3).

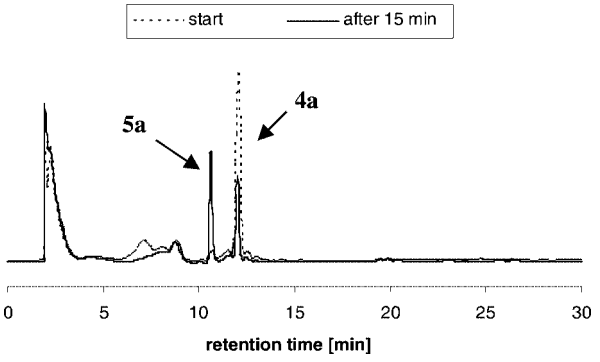


Figure 3. Cell extract incubation of AM-*cyclo*Sal-d4TMP triester **4a** followed by HPLC (HPLC method: HPLC analysis method II was performed as reported in ref.[7]).

For AM acylals **4a** the half-life drops 17-fold ($t_{1/2}$ = 0.25 h) with respect to the chemical stability. Subsequently, triester **5b** delivered d4TMP. The POM acylal **4b** is slightly more stable than the AM acylal, but a 15-fold reduction of the half-life was still observed. The most stable compound was the POC-acylal **4c**, which only shows a fourfold reduction in stability, although the acylal cleavage is still the predominant reaction. In addition, *cyclo*Sal triesters **4** were also studied for their stability in human serum. The stability in serum was found to be identical to that found in the chemical hydrolysis studies (**4a**: $t_{1/2}$ = 3.9 h; **4b**: 5.2 h; **4c**: 5.1 h). Finally, these compounds were also incubated with RPMI culture medium containing 10% fetal calf serum. Surprisingly, AM-acylal **4a** was cleaved significantly in this medium. In contrast, the POM and POC acylals proved to be resistant to the calf serum enzymes (**4a**: $t_{1/2}$ = 2.4 h; **4b**: 4.2 h; **4c**: 3.7 h).

Antiviral Evaluation

Finally, triesters **3**–**7** were tested for their anti-HIV activity in wild-type CEM and mutant thymidine-kinase-deficient CEM/TK$^-$ cells (Table 1). D4T (**1**) was used as the reference compound as it is active against HIV-1 and HIV-2 in wild-type CEM/O but inactive in TK-deficient CEM/TK$^-$ cells. The same activity has been found for *cyclo*Sal-d4TMP acids **5**, which can be attributed to a lack of membrane penetration due to the charged carboxylate. The remaining activity in the wild-type CEM cells should be due to an extracellular delivery of d4TMP, dephosphorylation to d4T **1** and subsequent cellular uptake of d4T, which is then rephosphorylated and consequently shows activity in CEM/O cells (but not in CEM/TK$^-$ cells due to lack of TK). As assumed, *cyclo*Sal-d4TMP triester **7**, which contains the hydroxyethyl group, proved to be antivirally active in the wild-type CEM cell culture but also in the mutant TK-deficient cells. Thus, the compound is still lipophilic enough to cross the cell membrane. Consequently, a passive efflux from the cells cannot be excluded. Nevertheless, enzyme-degradable *cyclo*Sal-triester alcohol esters **6a** and **6c** showed full retention of antiviral activity in the CEM/TK$^-$ cells. However, enzyme-stable triester **3a** also retained its antiviral activity entirely (Table 1).

A striking difference was observed for *cyclo*Sal-d4TMP acylals **4**. In contrast to triesters **4b**,**c**, the AM acylal **4a** lost its activity against HIV (Table 1). This compound shows the same antiviral activity profile as *cyclo*Sal-d4TMP acid **5b**. Taking the results of the hydrolysis in RPMI/FCS culture medium into account, the AM acylal is already significantly cleaved in this medium to the acid, which is unable to pass the membrane. In contrast, triesters **4b** and **4c** were found to be stable in the RPMI/FCS medium and therefore led to full retention of antiviral activity in the mutant CEM/TK$^-$ cells.

Conclusions

The results disclosed here prove that the delivery of highly polar *cyclo*Sal-d4TMP acids such as **5** is possible in principal. However, it seems that the delivery of *cyclo*Sal-d4TMP alcohols **7** may not lead to sufficient "trapping", although the compounds proved to be entirely independent of the presence of thymidine kinase in the cells. Assuming an efficient intracellular trapping of the *cyclo*Sal-d4TMP acids, one should expect a considerably higher intracellular concentration of these compounds. The reason why the bioactivity of the precursors is only comparable to that of the *cyclo*Sal-d4TMP alcohol **7** remains unclear and will be studied further in our laboratories. Maybe this is due to the metabolism profile of the nucleoside d4T itself or to the higher chemical stability of the intermediate *cyclo*Sal-d4TMP acid **5**, which prevents the fast build-up of higher d4TMP concentrations in the cells during the incubation period. Further experiments will be conducted in order to obtain more labile ester sites and enzyme-cleavable precursors of *cyclo*Sal-d4TMP acid **5**.

Experimental Section

General Remarks: NMR spectra were recorded with Bruker AMX 400 and Bruker DRX 500 Fourier-transform spectrometers. All ^{1}H and ^{13}C NMR chemical shifts (δ) are quoted in ppm and calibrated with respect to 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. EI mass spectra were measured with a VG Analytical VG/70-250S spectrometer (double focussing), ESI mass spectra with a Finnigan ThermoQuest MAT 95 XL spectrometer and FAB high-resolution (HR) mass spectra with a VG Analytical 70-250S spectrometer using an MCA method and poly(ethylene glycol) as support. Merck precoated 60 F_{254} plates with a 0.2 mm layer of silica gel were used for thin layer chromatography (TLC). All preparative TLCs were performed with a chromatotron® (Harrison Research, Model 7924T) using glass plates coated with 1- or 2-mm layers of Merck 60 PF_{254} silica gel containing a fluorescent indicator. For

column chromatography, Merck silica gel 60 (230–400) mesh was used. Analytical HPLC was performed with a Merck–Hitachi HPLC system (D-7000) equipped with a LiChroCART 125-3 column containing reversed-phase silica gel Lichrospher 100 RP 18 (5 μM; Merck, Darmstadt, Germany). The lyophilized products **2**–**7** 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 (gradient of 5–100% CH_3CN in water within 25 min., flow 0.5 mL min^{-1}). All reactions were carried out under dry nitrogen, except for the synthesis of **13**, **14** and **17**. Solvents used in the inert-gas syntheses were commercially available dry solvents stored under argon and over molecular sieves (Fluka). *N*,*N*-Dimethylformamide was additionally degassed and stored under nitrogen and over molecular sieves. Diethyl ether was dried with sodium/benzophenone and distilled under nitrogen. Potassium carbonate (water-free, Merck) was dried and stored under nitrogen.

3-Bromosalicyl Alcohol (17): A solution of 2-bromophenol (**16**; 50.0 mL, 81.5 g, 0.471 mol), phenylboronic acid (68.9 g, 0.565 mol), paraformaldehyde (28.3 g, 0.942 mol) and propionic acid (17.6 mL, 17.5 g, 0.236 mol) in 1 L of toluene was heated under reflux in a Dean–Stark apparatus. Every 3 h, the same amount of paraformaldehyde was added. This procedure was continued until a total amount of paraformaldehyde (170 g, 5.66 mol) and a reaction period of 18 h were reached. The reaction was monitored by TLC (CH_2Cl_2/MeOH, 9:1). The solvent was removed under reduced pressure and the resulting residue was dissolved in CH_2Cl_2 and water. The aqueous phase was then extracted twice with CH_2Cl_2. The combined organic phases were washed with water, dried with sodium sulfate and concentrated under reduced pressure to yield the dioxaborine as a crude product (98.2 g, dark red oil) which was dissolved in 340 mL of THF. After cooling to 0 °C, 340 mL of 30% hydrogen peroxide was added and the reaction was continued at that temperature for 45 min. After completion of the oxidation, the mixture was diluted with water and extracted five times with diethyl ether. The combined organic phases were washed with 39% sodium bisulfite solution and brine, dried with sodium sulfate and concentrated under reduced pressure. The product was purified by column chromatography on silica gel [CH_2Cl_2/MeOH gradient (0–2%)]. Yield: 50.5 g (0.248 mol, 53% over 2 steps) of a yellow-brown oil which crystallised at –20 °C after several days. R_f = 0.13 (CH_2Cl_2). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 4.58 (s, 2 H, benzyl-H), 5.38 (s, 1 H, OH), 6.80 (dd, $^3J_{H,H}$ = 7.9, $^3J_{H,H}$ = 7.6 Hz, 1 H, 5-H), 7.25–7.32 (m, 1 H, 6-H), 7.40 (dd, $^3J_{H,H}$ = 7.9, $^4J_{H,H}$ = 1.2 Hz, 1 H, 4-H), 9.13 (s, 1 H, OH) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 59.48 (benzyl-C), 110.76 (C-3), 121.04 (C-5), 126.89 (C-6), 131.02 (C-4), 131.40 (C-1), 150.90 (C-2) ppm. MS (EI): *m*/*z* (%) = calcd. 202 [M]; found 202 (25), 184 (100), 156 (14), 105 (83), 94 (17), 77 (47), 63 (12), 51 (20).

3-Bromosalicyl Alcohol Isopropylidene Acetal (14): A mixture of 3-bromosalicyl alcohol (**17**; 45.4 g, 0.224 mol), 2,2-dimethoxypropane (139 mL, 117 g, 1.12 mol), *para*-toluenesulfonic acid monohydrate (4.26 g, 22.4 mmol) and 89.6 g of anhydrous sodium sulfate in 900 mL of acetone was heated at 40 °C for 3 d. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and water. The aqueous phase was extracted twice with ethyl acetate. The combined organic phases were washed twice with 1 M sodium hydroxide and water, dried with sodium sulfate and concentrated under reduced pressure. The product was purified by column chromatography on silica gel (CH_2Cl_2). Yield: 54.0 g (0.222 mol, 99%) of a brown oil. R_f = 0.64 (CH_2Cl_2/MeOH, 30:1). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.52 (s, 6 H, acetal-CH_3), 4.86 (s, 2 H, benzyl-H), 6.87 (dd, $^3J_{H,H}$ = 7.9, $^3J_{H,H}$ = 7.4 Hz, 1 H, 5-H), 7.11 (d, $^3J_{H,H}$ = 7.4 Hz, 1 H, 6-H), 7.46 (dd, $^3J_{H,H}$ = 7.9, $^4J_{H,H}$ = 0.8 Hz, 1 H, 4-H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 24.75 (acetal-CH_3), 60.09 (benzyl-C), 100.81 (acetal-C), 110.02 (C-3), 121.52 (C-5), 121.81 (C-1), 124.74 (C-6), 131.40 (C-4), 147.60 (C-2) ppm. MS (EI): *m*/*z* (%) = calcd. 242 [M]; found 242 (25), 184 (100), 156 (6), 105 (45), 77 (26), 51 (16).

3-[3-(Methoxycarbonyl)propyl]salicyl Alcohol Isopropylidene Acetal (19): 9-BBN (22 mL, 11 mmol; 0.5 M in THF) was added dropwise to methyl 3-butenoate (1.00 g, 10.0 mmol) at 0 °C. After removing the cooling bath, the solution was stirred at 55 °C overnight. Afterwards, acetal **14** (1.86 g, 8.00 mmol), potassium carbonate (2.49 g, 16.0 mmol), [Pd(dppf)Cl$_2$] (198 mg, 0.27 mmol) and 50 mL of DMF were added and the mixture stirred at 55 °C for 4 d. The reaction was monitored by TLC (petroleum ether 50–70/ethyl acetate, 4:1). The solution was allowed to cool to room temp. and then poured into 1 M HOAc/NaOAc buffer (pH = 5) and stirred for 10 min. The aqueous layer was extracted twice with Et_2O, the combined organic layers were dried with sodium sulfate and the solvents removed in vacuo. The brown crude was purified by column chromatography on silica gel (petroleum ether 50–70/ethyl acetate, 4:1). Yield: 1.25 g (4.70 mmol, 59%) of a colourless oil. R_f = 0.36 (petroleum ether 50–70/ethyl acetate, 4:1). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.76 (quint, $^3J_{H,H}$ = 7.4 Hz, 2 H, 9-H), 1.43 (s, 6 H, acetal-CH_3), 2.24 (t, $^3J_{H,H}$ = 7.4 Hz, 2 H, 10-H), 2.49 (t, $^3J_{H,H}$ = 7.4 Hz, 2 H, 8-H), 3.55 (s, 3 H, 12-H), 4.77 (s, 2 H, 7-H), 6.73 (dd, $^3J_{H,H}$ = 7.2 Hz, 1 H, aryl-5-H), 6.87 (dd, $^3J_{H,H}$ = 7.2 Hz, 1 H, aryl-6-H), 6.97 (dd, $^3J_{H,H}$ = 7.2 Hz, 1 H, aryl-4-H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 24.56 (acetal-CH_3), 24.63 (C-9), 28.05 (C-8), 32.67 (C-10), 51.18 (C-12), 60.14 (C-7), 99.08 (acetal-C), 119.22 (C-1), 119.78 (C-5), 122.81 (C-6), 125.37 (aryl-C-4), 127.90 (aryl-C-1), 128.21 (aryl-C-4), 128.65 (aryl-C-3), 148.91 (aryl-C-2), 172.18 (C-11) ppm. MS (ESI$^+$): *m*/*z* = calcd. 287.13 [M + Na$^+$]; found 287.23.

3-[3-(Methoxycarbonyl)propyl]salicyl Alcohol (13): Two drops of concd. hydrochloric acid were added to a solution of **19** (940 mg, 3.60 mmol) in 30 mL of CH_3CN/H_2O (7:1), and the reaction mixture was heated with a heat gun until it was boiling. The heating was continued until the deprotection was complete (monitoring by TLC, CH_2Cl_2/MeOH, 19:1, 0.5–5 min) and then the mixture was diluted with CH_2Cl_2 and water. The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic layers were washed subsequently with satd. ammonium hydrogencarbonate solution and water, dried with sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 14:1). Yield: 524 mg (2.30 mmol, 65%) of a colourless oil. R_f = 0.53 (CH_2Cl_2/MeOH, 19:1). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.77 (quint, $^3J_{H,H}$ = 7.5 Hz, 2 H, 9-H), 2.30 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, 10-H), 2.56 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, 8-H), 3.58 (s, 3 H, 13-H), 4.57 (d, $^3J_{H,H}$ = 5.3 Hz, 2 H, benzyl-H), 5.37 (t, $^3J_{H,H}$ = 5.3 Hz, 1 H, benzyl-OH), 6.74 (dd, $^3J_{H,H}$ = 7.5 Hz, 1 H, aryl-5-H), 6.95 (dd, $^3J_{H,H}$ = 7.5, $^4J_{H,H}$ = 1.5 Hz, 1 H, aryl-6-H) 7.06 (dd, $^3J_{H,H}$ = 7.5, $^4J_{H,H}$ = 1.5 Hz, 1 H, aryl-4-H), 8.42 (s, 1 H, phenol-OH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 24.85 (C-9), 28.82 (C-8), 32.97 (C-10), 51.21 (C-12), 60.04 (benzyl-C), 119.10 (aryl-C-5), 125.34 (aryl-C-4), 127.92 (aryl-C-3), 128.07 (aryl-C-1), 128.36 (aryl-C-6), 152.39 (aryl-C-2), 173.29 (C-11) ppm. HRMS (FAB): *m*/*z* = calcd. 224.1049 [M]; found 224.1054.

3-[3-(Methoxycarbonyl)propyl]-*cyclo*Sal-d4T-monophosphate (3-MeBu-*cyclo*Sal-d4TMP) (3d): A solution of the salicyl alcohol derivative **13** (451 mg, 2.00 mmol) in dry Et_2O was cooled to –20 °C. After addition of freshly distilled phosphorus(III) chloride (330 mg,

2.40 mmol) and stirring at –20 °C for 10 min, a solution of dry pyridine (364 mg, 4.60 mmol) 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 to room temperature and stirred for 2 h. It was kept at –20 °C overnight for a complete precipitation of pyridinium chloride. Filtration under nitrogen and concentrated of the filtrate under reduced pressure afforded the phosphitylating agent (saligenyl chlorophosphite) as a crude product to be directly used for the synthesis of the *cyclo*Sal phosphate triester **3d** without further purification. The general synthesis of *cyclo*Sal-d4T-monophosphates has been published before.[3,5,13] Diisopropylethylamine (DIPEA; 194 mg, 1.50 mmol) was added to a solution of d4T **1** (224 mg, 1.00 mmol) in dry CH_3CN. The resulting solution was cooled to –20 °C and the chlorophosphite (350 mg, 1.20 mmol) was added. The solution was allowed to warm to room temperature, and stirring was continued for 3 h. Subsequently, *tert*-butyl hydroperoxide (6 M in *n*-decane; 0.50 mL, 3.00 mmol) was added at –20 °C. After warming to room temperature and stirring for 1 h, the solvent was removed under reduced pressure. The resulting residue was purified by preparative TLC [Chromatotron®; 1. ethyl acetate/MeOH (0.1% HOAc), 9:1; 2. CH_2Cl_2/MeOH gradient (0–5%)]. Lyophilisation yielded 277 mg (0.56 mmol, 56%) of a diastereomeric mixture (ratio 1.0:1.0) as a colourless foam. R_f = 0.47 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.59 (d, $^4J_{H,H}$ = 1.0 Hz, 3 H, thymine-CH_3), 1.62 (d, $^4J_{H,H}$ = 1.0 Hz, 3 H, thymine-CH_3), 1.75–1.83 (m, 4 H, 2×9-H), 2.30 (t, $^3J_{H,H}$ = 7.4 Hz, 2 H, 10-H), 2.32 (t, $^3J_{H,H}$ = 7.4 Hz, 2 H, 10-H), 2.54–2.66 (m, 4 H, 2×8-H), 3.57 (s, 3 H, 12-H), 3.58 (s, 3 H, 12-H), 4.24–4.34 (m, 4 H, 2×5′-H), 4.97–4.93 (m, 2 H, 2×4′-H), 5.34 (dd, $^2J_{H,H}$ = 16.9, $^3J_{H,P}$ = 6.9 Hz, 1 H, benzyl-H), 5.38 (dd, $^2J_{H,H}$ = 13.2, $^3J_{H,P}$ = 6.3 Hz, 1 H, benzyl-H), 5.44 (dd, $^2J_{H,H}$ = 16.4, $^3J_{H,P}$ = 5.7 Hz, 1 H, benzyl-H), 5.48 (dd, $^2J_{H,H}$ = 14.5, $^3J_{H,P}$ = 6.3 Hz, 1 H, benzyl-H), 5.99–6.05 (m, 2 H, 2×2′-H), 6.35–6.38 (m, 1 H, 3′-H), 6.78–6.82 (m, 2 H, 2×1′-H), 7.10–7.28 (m, 8 H, 2×aryl 4-H 2×aryl 5-H, 2×aryl 6-H, 2×thymine 6-H), 11.32 (s, 1 H, NH), 11.34 (s, 1 H, NH) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 11.75 (thymine-CH_3), 11.85 (thymine-CH_3), 24.56 (C-10), 24.59 (C-10), 27.89 (2×C-8), 32.64 (C-9), 32.66 (C-9), 68.19 (d, $^2J_{C,P}$ = 3.2 Hz, benzyl-C), 68.26 (d, $^2J_{C,P}$ = 3.5 Hz, benzyl-C), 68.51 (d, ^2J = 4.0 Hz, C-5′), 68.61 (d, $^2J_{C,P}$ = 4.5 Hz, C-5′), 84.10 (C-4′), 84.18 (C-4′), 89.11 (C-1′), 89.19 (C-1′), 109.64 (thymine-C-5), 109.69 (thymine-C-5), 124.18 (2×C-aryl), 127.38 (2×C-2′), 130.30 (C-aryl), 130.35 (C-aryl), 132.74 (C-3′), 132.81 (C-3′), 135.62 (C-aryl), 135.68 (C-aryl), 150.70 (2×C-2), 163.71 (2×thymine-C-4), 172.97 (2×C-11) ppm. ^{31}P NMR (162 MHz, [D$_6$]DMSO): δ = –8.37, –8.51 ppm. HRMS (FAB): *m*/*z* = calcd. 493.1376 [M + H]; found 493.1377. HPLC: t_R = 14.5 min (method I); t_R = 11.1, 11.2 min (method II). UV/Vis (water/CH_3CN): λ_{max} = 267 nm.

5-[2-(Acetoxymethoxycarbonyl)ethyl]-*cyclo*Sal-d4T-monophosphate [(5-AM-Pr)-*cyclo*Sal-d4TMP] (4a): Under nitrogen, 2.7 mL of a solution of DIPEA in dry CH_3CN (1:100; 0.17 mmol of DIPEA) and 1.7 mL of a solution of bromomethyl acetate in dry CH_3CN (1:100; 0.17 mmol of bromomethyl acetate) were added dropwise to a solution of 5-(2-carboxyethyl)-*cyclo*Sal-d4TMP (**5b**; 70 mg, 0.15 mmol) in 22 mL of dry CH_3CN at 10 °C. The reaction mixture was stirred at 10 °C for 3 h and the reaction monitored by TLC (CH_2Cl_2/MeOH, 9:1). The solvent was removed in vacuo and the residue was purified by preparative TLC [Chromatotron®; CH_2Cl_2/MeOH gradient (0–3%)]. The isolated product was lyophilized. Yield: 53 mg (99 µmol, 65%) of a colourless solid as a mixture of two diastereomers in a 1:1 ratio. R_f = 0.36 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.62 (s, 3 H, thymine-CH_3), 1.69 (s, 3 H, thymine-CH_3), 2.05 (s, 3 H, acetyl-CH_3), 2.06 (s, 3 H, acetyl-CH_3), 2.70 (t, $^3J_{H,H}$ = 7.3 Hz, 4 H, 2×9-H), 2.84 (t, $^3J_{H,H}$ = 7.3 Hz, 4 H, 2×8-H), 4.24–4.35 (m, 4 H, 2×5′-H), 4.94–5.00 (m, 2 H, 2×4′-H), 5.34–5.58 (m, 4 H, 2×benzyl-H), 5.66 (s, 2 H, 11-H), 5.67 (s, 2 H, 1×11-H), 6.00–6.04 (m, 2 H, 2×2′-H), 6.36 (ddd, $^3J_{H,H}$ = 6.0, $^3J_{H,H}$ = 1.6, $^4J_{H,H}$ = 1.6 Hz, 1 H, 3′-H), 6.42 (ddd, $^3J_{H,H}$ = 6.0, $^3J_{H,H}$ = 1.6, $^4J_{H,H}$ = 1.6 Hz, 1 H, 3′-H), 6.78 (dd, $^3J_{H,H}$ = 2.6, $^4J_{H,H}$ = 1.6 Hz, 1 H, 1′-H), 6.80 (dd, $^3J_{H,H}$ = 2.6, $^4J_{H,H}$ = 1.6 Hz, 1 H, 1′-H), 7.02 (d, $^3J_{H,H}$ = 8.5 Hz, 1 H, aryl-3-H), 7.05 (d, $^3J_{H,H}$ = 8.5 Hz, 1 H, aryl 3-H), 7.15–7.26 (m, 63 H, 2×aryl 4-H, 2×aryl 6-H, 2×thymine 6-H), 11.34 (s, 2 H, 2×NH) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 12.00 (thymine-CH_3), 12.09 (thymine-CH_3), 20.64 (2×acetyl-CH_3), 29.16 (2×C-8), 34.59 (2×C-9), 68.38 (d, $^2J_{C,P}$ = 6.1 Hz, 2×benzyl-C), 68.52 (d, $^2J_{C,P}$ = 6.1 Hz, 2×C-5′), 79.15 (2×C-11), 84.28 (d, $^3J_{C,P}$ = 8.0 Hz, 2×C-4′), 89.35 (2×C-1′), 109.82 (2×thymine-C-5), 118.14 (d, $^3J_{C,P}$ = 8.0 Hz, 2×aryl-C-3), 125.94 (aryl-C-6), 126.00 (aryl-C-6), 127.46 (2×C-2′), 127.97 (2×aryl-C-1), 129.78 (aryl-C-4), 129.96 (aryl-C-4), 132.96 (C-3′), 133.03 (C-3′), 135.82 (thymine-C-6), 135.86 (thymine-C-6), 136.65 (2×aryl-C-5), 148.08 (d, $^2J_{C,P}$ = 7.1 Hz, 2×aryl-C-2), 150.85 (thymine-C-2), 150.88 (thymine-C-2), 163.88 (thymine-C-4), 163.90 (thymine-C-4), 169.44 (2×C-12), 171.22 (2×C-10) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = –8.00, –8.07 ppm. HRMS (ESI$^+$): *m*/*z* = calcd. 559.1094 [M + Na$^+$]; found 559.1113. HPLC: t_R = 14.0 min (method I); t_R = 11.9 min (method II). UV/Vis (water/CH_3CN): λ_{max} = 265 nm.

5-[2-(Pivaloyloxymethoxycarbonyl)ethyl]-*cyclo*Sal-d4T-monophosphate [5-(POM-Pr)-*cyclo*Sal-d4TMP] (4b): Under nitrogen, 1.53 mL of a solution of DIPEA in dry CH_3CN (1:100; 94.7 µmol of DIPEA) and 1.37 mL of a solution of chloromethyl pivalate in dry CH_3CN (1:100; 94.7 µmol of chloromethyl pivalate) were added dropwise to a solution of 5-(2-carboxyethyl)-*cyclo*Sal-d4TMP (**5b**; 40 mg, 86 µmol) in 10 mL of dry CH_3CN at room temperature. The reaction mixture was stirred at room temperature for 9 d and the reaction monitored by TLC (CH_2Cl_2/MeOH, 9:1). The solvent was removed in vacuo and the residue was purified by preparative TLC [Chromatotron®; CH_2Cl_2/MeOH gradient (0–3%)]. The isolated product was lyophilized. Yield: 23 mg (39 mmol, 45%) of a colourless solid as a 1.0:0.8 mixture of two diastereomers. R_f = 0.43 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.11 (s, 9 H, *t*Bu), 1.12 (s, 9 H, *t*Bu), 1.63 (s, 3 H, thymine-CH_3), 1.70 (s, 3 H, thymine-CH_3), 2.71 (t, $^3J_{H,H}$ = 7.6 Hz, 4 H, 2×9-H), 2.84 (t, $^3J_{H,H}$ = 7.6 Hz, 4 H, 2×8-H), 4.25–4.37 (m, 4 H, 2×5′-H), 4.96–5.00 (m, 2 H, 2×4′-H), 5.33–5.48 (m, 4 H, 2×benzyl-H), 5.69 (s, 2 H, 11-H), 5.70 (s, 2 H, 11-H), 6.02–6.05 (m, 2 H, 2×2′-H), 6.36 (ddd, $^3J_{H,H}$ = 5.7, $^3J_{H,H}$ = 2.5, $^4J_{H,H}$ = 2.5 Hz, 1 H, 3′-H), 6.42 (ddd, $^3J_{H,H}$ = 5.7, $^3J_{H,H}$ = 2.5, $^4J_{H,H}$ = 2.5 Hz, 1 H, 3′-H), 6.79–6.82 (m, 2 H, 2×1′-H), 7.02 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, aryl 3-H), 7.05 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, aryl 3-H), 7.16–7.25 (m, 6 H, 2×aryl 4-H, 2×aryl 6-H, 2×thymine 6-H), 11.35 (s, 2 H, 2×NH) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 12.02 (thymine-CH_3), 12.10 (thymine-CH_3), 26.61 (2×*t*Bu-CH_3), 29.16 (2×C-8), 34.52 (2×C-9), 38.33 (2×*t*Bu-C), 68.41–68.52 (m, 2×C-5′, 2×benzyl-C), 79.48 (2×C-11), 84.29 (d, $^3J_{C,P}$ = 8.1 Hz, C-4′), 84.53 (C-4′), 89.35 (2×C-1′), 109.84 (2×thymine-C-5), 118.06 (d, $^3J_{C,P}$ = 3.1 Hz, 2×aryl-C-3), 125.94 (aryl-C-6), 126.00 (aryl-C-6), 127.48 (C-2′), 127.51 (C-2′), 128.43 (2×aryl-C-1), 129.77 (aryl-C-4), 129.86 (aryl-C-4), 132.95 (C-3′), 133.02 (C-3′), 135.86 (2×thymine-C-6), 136.64 (2×aryl-C-5), 147.41 (2×aryl-C-2), 150.67 (thymine-C-2), 150.85 (thymine-C-2), 163.89 (thymine-C-4), 163.91 (thymine-C-4), 167.17 (C-12), 167.32 (C-12), 171.19 (C-10), 171.24 (C-10) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = –8.01, –8.08 ppm. HRMS (ESI$^+$): *m*/*z* = calcd. 601.1533 [M +

Na^+]; found 601.1559. HPLC: t_R = 17.6 min (method I); t_R = 14.8 min (method II). UV/Vis (water/CH_3CN): λ_{max} = 267 nm.

5-[2-(Isopropyloxycarbonyloxymethoxycarbonyl)ethyl]-*cyclo*Sal-d4T-monophosphate [5-(POC-Pr)-*cyclo*Sal-d4TMP] (4c): Under nitrogen, 1.47 mL of a solution of DIPEA in dry CH_3CN (1:50; 0.17 mmol of DIPEA) and 0.76 mL of a POC chloride solution in dry CH_3CN (33 mg/mL, 0.17 mmol POC chloride) were added dropwise to a solution of 5-(2-carboxyethyl)-*cyclo*Sal-d4TMP (**5b**; 70 mg, 0.15 mmol) in 18 mL of dry CH_3CN at room temperature. The reaction mixture was stirred at 50 °C for 7 d and the reaction monitored by TLC (CH_2Cl_2/MeOH, 9:1). The solvent was removed in vacuo and the residue was purified by preparative TLC [Chromatotron®; CH_2Cl_2/MeOH gradient (0–5%)]. The isolated product was lyophilized. Yield: 41 mg (71 μmol, 47%) of a colourless solid as a 0.5:1.0 mixture of two diastereomers. R_f = 0.53 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.25 (d, $^3J_{H,H}$ = 6.2 Hz, 12 H, 2×*i*Pr-CH_3), 1.63 (d, $^4J_{H,H}$ = 0.6 Hz, 3 H, thymine-CH_3), 1.70 (s, 3 H, thymine-CH_3), 2.73 (t, $^3J_{H,H}$ = 7.4 Hz, 4 H, 2×9-H), 2.85 (t, $^3J_{H,H}$ = 7.4 Hz, 4 H, 2×8-H), 4.25–4.36 (m, 4 H, 2×5′-H), 4.82 (sept, $^3J_{H,H}$ = 6.2 Hz, 2 H, 2×*i*Pr-CH), 4.95–4.99 (m, 2 H, 2×4′-H), 5.35–5.39 (m, 2 H, 2×benzyl-H), 5.43 (dd, $^2J_{H,H}$ = 15.6, $^3J_{H,P}$ = 5.8 Hz, 1 H, benzyl-H), 5.47 (dd, $^2J_{H,H}$ = 15.6, $^3J_{H,P}$ = 5.9 Hz, 1 H, benzyl-H), 5.69 (s, 4 H, 2×11-H), 6.01–6.05 (m, 2 H, 2×2′-H), 6.35–6.38 (m, 1 H, 3′-H), 6.42–6.45 (m, 1 H, 3′-H), 6.79–6.81 (m, 1 H, 1′-H), 6.81–6.83 (m, 1 H, 1′-H), 7.03 (d, $^3J_{H,H}$ = 8.4 Hz, 1 H, aryl-3-H), 7.05 (d, $^3J_{H,H}$ = 8.4 Hz, 1 H, aryl-3-H), 7.16–7.27 (m, 6 H, 2×aryl 4-H, 2×aryl 6-H, 2×thymine 6-H), 11.35 (s, 1 H, 1×NH), 11.36 (s, 1 H, 1×NH) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 12.00 (thymine-CH_3), 12.09 (thymine-CH_3), 21.49 (2×*i*Pr-CH_3), 29.12 (2×C-8), 34.57 (2×C-9), 68.44 (d, $^2J_{C,P}$ = 7.1 Hz, 2×benzyl-C), 68.55 (d, $^2J_{C,P}$ = 3.8 Hz, 2×C-5′), 72.78 (2×*i*Pr-CH), 81.86 (2×C-11), 84.27 (d, $^3J_{C,P}$ = 8.1 Hz, 2×C-4′), 89.35 (2×C-1′), 109.81 (thymine-C-5), 109.83 (thymine-C-5), 118.16 (d, $^3J_{C,P}$ = 2.0 Hz, 2×aryl-C-3), 125.92 (aryl-C-6), 125.99 (aryl-C-6), 127.46 (C-2′), 127.49 (C-2′), 129.73 (d, $^3J_{C,P}$ = 8.1 Hz, 2×aryl-C-1), 129.80 (aryl-C-4), 129.85 (aryl-C-4), 133.02 (C-3′), 133.04 (C-3′), 135.80 (thymine-C-6), 135.85 (thymine-C-6), 136.59 (aryl-C-5), 136.62 (aryl-C-5), 148.06 (d, $^2J_{C,P}$ = 3.4 Hz, 2×aryl-C-2), 150.93 (2×thymine-C-2), 152.87 (2×C-12), 163.86 (thymine-C-4), 163.89 (thymine-C-4), 171.19 (2×C-10) ppm. ^{31}P NMR (202 MHz, [D$_6$]DMSO): δ = –8.01, –8.09 ppm. HRMS (FAB): *m*/*z* = calcd. 581.1536 [M + H^+]; found 581.1530. HPLC: t_R = 14.1 min (method I); t_R = 13.3 min (method II). UV/Vis (water/CH_3CN): λ_{max} = 265 nm.

Antiretroviral Evaluation: The experimental setup for the antiviral testing has been described previously.[6]

Hydrolysis Studies of the *cyclo*Sal Phosphate Triesters: Hydrolysis studies of *cyclo*Sal nucleotides (phosphate buffer, pH = 7.3) by HPLC analysis (method I) have been described before.[14,22] Studies in cell extracts were performed as reported in ref.[7] Thus, 100 μL of human CEM/O cell extracts was mixed with 20 μL of a 70 mM aqueous magnesium chloride solution. The reaction was started by addition of 20 μL of a 1.5 mM solution of the *cyclo*Sal phosphate triester in DMSO. Four separate samples of the above-mentioned mixtures were incubated for 0, 2, 4 and 8 h, respectively. This was done to avoid contamination of the biological media by taking multiple samples from only one solution. All solutions were incubated at 37 °C. To stop the reaction and precipitation of protein, 300 μL of acidified methanol (1 mL glacial acetic acid in 20 mL methanol) was added and the cap was kept at 0 °C for 5 min. After centrifugation (13000 rpm, 10 min) and filtration of the supernatant, the sample was subjected to HPLC analysis [0–50 min TBAH/phosphoric acid buffer (0.55 mM, pH = 3.8) with an acetonitrile gradient from 0 to 70%, flow 0.6 mL min^{-1}]. Two separate samples for each time point and test compound were prepared and analysed. Determination of $t_{1/2}$ was performed analogously to that for the chemical hydrolysis studies except that the absolute integral values were used instead of standardised IU. Identification of the hydrolysis products was based on the retention times of the reference compounds and co-elution experiments under identical analytical conditions.

Chemical Hydrolysis in Citrate Buffer: 10 μL of a 5 mM solution of the prodrug in DMSO was mixed with 20 μL of DMSO and 30 μL of citrate/HCl buffer (pH = 2.0) and incubated at 37 °C for 30 min. An additional reference sample using water instead of buffer was also prepared. Both samples were analysed by RP-HPLC as described previously.[7]

^{31}P NMR Hydrolysis Studies of the *cyclo*Sal Phosphate Triesters: Approximately 7 μmol of the *cyclo*Sal triesters was dissolved in 500 μL of deuterated DMSO and 500 μL of a 50 mM imidazole/hydrochloric acid buffer (pH = 7.3). The resulting kinetic solutions were transferred into an NMR tube and investigated by ^{31}P NMR spectroscopy (proton-decoupled, 202 MHz, 256 scans each sample). All samples were stored at room temperature. Furthermore, some proton-coupled ^{31}P NMR spectra (202 MHz, 512 scans each sample) were recorded for the identification of the hydrolysis products.[22]

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[1] J. Balzarini, M. Baba, R. Pauwels, P. Herdewijn, *Biochem. Pharmacol.* **1988**, *37*, 2847–2856.
[2] J. Balzarini, E. De Clercq, *Biochem. Pharmacol.* **1994**, *49*, 751–772.
[3] a) C. Meier, *Synlett* **1998**, 233–242; b) C. R. Wagner, V. V. Iyer, E. J. McIntee, *Med. Res. Rev.* **2000**, *20*, 417–451.
[4] a) C. Meier, *Mini Rev. Med. Chem.* **2002**, *2*, 219–234; b) C. Meier, J. Renze, C. Ducho, J. Balzarini, *Curr. Top. Med. Chem.* **2002**, *2*, 1111–1121; c) C. Meier, *Advances in Antiviral Drug Design* (Ed.: E. De Clercq), Elsevier, Amsterdam, **2004**, vol. 4, p. 147–213.
[5] a) J. Balzarini, S. Aquaro, T. Knispel, C. Rampazzo, V. Bianchi, C.-P. Perno, E. De Clercq, C. Meier, *Mol. Pharmacol.* **2000**, *58*, 928–935; b) J. Balzarini, L. Naesens, S. Aquaro, T. Knispel, C.-F. Perno, E. De Clercq, C. Meier, *Mol. Pharmacol.* **1999**, *56*, 1354–1361.
[6] C. Meier, M. Lorey, E. De Clercq, J. Balzarini, *J. Med. Chem.* **1998**, *41*, 1417–1427.
[7] C. Meier, C. A. Lomp, A. A. Meerbach, A. P. Wutzler, *J. Med. Chem.* **2002**, *45*, 5157–5172.
[8] J. Balzarini, F. Haller-Meier, E. De Clercq, C. Meier, *Antiviral Chem. Chemother.* **2002**, *12*, 301–306.
[9] C. Meier, L. Habel, F. Haller-Meier, A. Lomp, M. Herderich, R. Klöcking, A. Meerbach, P. Wutzler, *Antiviral Chem. Chemother.* **1998**, *9*, 389–402.
[10] C. Meier, T. Knispel, E. De Clercq, J. Balzarini, *J. Med. Chem.* **1999**, *42*, 1604–1614.
[11] C. Meier, T. Knispel, V. E. Marquez, E. De Clercq, J. Balzarini, *J. Med. Chem.* **1999**, *42*, 1615–1624.
[12] C. Meier, M. Ruppel, D. Vukadinovic, J. Balzarini, *Nucleosides Nucleotides* **2004**, *23*, 89–115.
[13] M. N. Arimilli, C. U. Kim, J. Dougherty, A. Mulato, R. Oliyai, J. P. Shaw, K. C. Cundy, N. Bischofberger, *Antiviral Chem. Chemother.* **1997**, *8*, 557–564.

[14] A. J. Pearson, P. Zhang, K. Lee, *J. Org. Chem.* **1996**, *61*, 6581–6586.
[15] H. Brechbühler, H. Büchi, E. Hatz, J. Schreiber, A. Eschenmoser, *Helv. Chim. Acta* **1965**, *48*, 1746–1771.
[16] G. W. Breton, *J. Org. Chem.* **1997**, *62*, 8952–8954.
[17] S. Yamada, T. Sugaki, K. Matsuzaki, *J. Org. Chem.* **1996**, *61*, 5932–5938.
[18] C. Meier, E. De Clercq, J. Balzarini, *Eur. J. Org. Chem.* **1998**, 837–846.
[19] J. C. Boehm, J. G. Gleason, I. Pendrak, H. M. Sarau, D. B. Schmidt, J. J. Foley, W. D. Kingsbury, *J. Med. Chem.* **1993**, *36*, 3333–3340.
[20] W. von Daehne, E. Frederiksen, E. Gundersen, F. Lund, P. Morch, H. J. Petersen, K. Roholt, L. Tybring, W. O. Godtfredsen, *J. Med. Chem.* **1970**, *13*, 607–612.
[21] C. Meier, M. Ruppel, D. Vukadinovic, J. Balzarini, *Mini Rev. Med. Chem.* **2004**, *4*, 383–394.
[22] C. Ducho, J. Balzarini, L. Naesens, E. De Clercq, C. Meier, *Antiviral Chem. Chemother.* **2002**, *13*, 129–141.

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