Bis-*cyclo*Sal-d4T-monophosphates: Drugs That Deliver Two Molecules of Bioactive Nucleotides

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Received October 10, 2006

Bis-*cyclo*Sal-d4T-monophosphates have been synthesized as potentially anti-HIV active "dimeric" prodrugs of 2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphate (d4TMP). These pronucleotides display a mask−drug ratio of 1:2, a novelty in the field of pronucleotides. Both bis-*cyclo*Sal-d4TMP **6** and bis-5-methyl-*cyclo*Sal-d4TMP **7** showed increased hydrolytic stability as compared to their "monomeric" counterparts and a completely selective hydrolytic release of d4TMP. The hydrolysis pathway was investigated *via* ^{31}P NMR spectroscopy. Moreover, due to the steric bulkiness, compound **6** already displayed strongly reduced inhibitor potency toward human butyrylcholinesterase (BChE), while compound **7** turned out to be devoid of any inhibitory activity against BChE. Partial separation of the diastereomeric mixture of **6** revealed strong dependence of the pronucleotides' properties on the stereochemistry at the phosphorus centers. Both **6** and **7** showed good activity against HIV-1 and HIV-2 in wild-type CEM cells *in vitro*. These compounds were significantly more potent than the parent nucleoside d4T **1** in HIV-2-infected TK-deficient CEM cells, indicating an efficient TK-bypass.

Introduction

A major problem in the application of nucleoside analogues as antivirally active agents is their requirement for bioactivation and conversion to the nucleoside triphosphates, the biologically active species. In the case of the anti-HIV active 3′-deoxy-2′,3′-didehydrothymidine (d4T) **1** (Figure 1), thymidine kinase (TK)-mediated phosphorylation yields d4T monophosphate (d4TMP) and is followed by two additional enzymatic steps to lead to d4T diphosphate (d4TDP) and finally d4T triphosphate (d4TTP).[1] D4TTP is antivirally active as it inhibits the reverse transcriptase (RT) of HIV. The first phosphorylation step yielding d4TMP represents the metabolic bottleneck of this bioactivation cascade. Often, nucleoside analogues have a poor affinity for the first phosphorylation reaction.[2] Hence, several attempts have been made to bypass the first phosphorylation step by application of lipophilic prodrugs of nucleoside monophosphates (NMPs).[3] These pronucleotides are designed to allow cellular uptake and intracellular release of NMPs. Among these approaches, the *cyclo*Saligenyl (*cyclo*Sal) concept was established as a nucleotide delivery system.[4] The intracellular cleavage of *cyclo*Sal pronucleotides (*cyclo*Sal-d4TMP **2**, Figure 1) is based on an entirely pH-driven chemical hydrolysis mechanism, which has been reported extensively and is briefly summarized in Figure 1.[5] It should be pointed out that *cyclo*Sal pronucleotides display a mask−drug ratio of 1:1 while all other pronucleotide approaches lead to the delivery of a relatively higher number of masking groups (mask−drug ratio usually 2−4:1).[3]

Besides d4T **1**,[5,6] the *cyclo*Sal pronucleotide approach led to remarkable improvement of the antiviral activity with several other nucleoside analogues, including 2′,3′-dideoxyadenosine (ddA),[7] 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A),[7] 2′-fluoro-2′,3′-dideoxyadenosine (2′-F-ddA),[8] and the carbocyclic nucleosides carbovir (CBV), abacavir,[9] and acyclovir (ACV).[10] Impressive is the significant anti-HIV activity of *cyclo*Sal-F-*ribo*-ddAMP while the parent nucleoside is entirely inactive.[8] A further example of such a dramatic effect is the application of the *cyclo*Sal technology to (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU): while BVDU itself is inactive against the Epstein−Barr virus (EBV), vaccinia virus, and cowpox virus, several *cyclo*Sal derivatives of BVDU showed significant potency against these viruses.[11]

As *cyclo*Sal pronucleotides represent reactive organophosphates, which may be potential cholinesterase inhibitors, investigations concerning such an unwanted effect had to be carried out.[12] Cholinesterase inhibition might potentially lead to serious side effects in a possible therapeutic application of *cyclo*Sal compounds. However, in a recent study we have clearly proven that *cyclo*Sal pronucleotides do *not* inhibit the physiologically important acetylcholinesterase (AChE, EC 3.1.1.7, neither that from calf serum nor from beef erythrocytes nor from *Electrophorus electricus* nor human AChE).[12,13] In contrast, a structure−activity relationship was found for the inhibition of human butyrylcholinesterase (BChE, EC 3.1.1.8). BChE is a serum enzyme, much less specific than AChE. Its physiological role is yet unclear. It is known though that BChE can be used for the treatment of succinylcholine apnea in humans,[14] and it is known to protect rodents from the toxic effects of cocaine.[15] However, we also could show that substitution patterns of increasing steric demand in the *cyclo*Sal moiety could prevent the *cyclo*Sal pronucleotides from displaying the unwanted BChE inhibition or at least significantly reduce their inhibitory potency, possibly due to a steric repulsion in the active site of the enzyme.[12,16] Hence, there was an interest in the development of *cyclo*Sal derivatives with increased "bulkiness".

We already described the synthesis and properties of 3-phenyl-*cyclo*Sal-d4TMP **5** (Figure 2).[17] While this derivative showed a satisfying hydrolytic profile as well as high antiviral potency, it was a surprisingly strong BChE inhibitor.[12] Nevertheless, its otherwise beneficial properties led us to the design of so-called bis-*cyclo*Sal-d4TMP **6** and bis-5-methyl-*cyclo*Sal-d4TMP **7**, "dimeric" forms of the prototype *cyclo*Sal-d4TMP **8** and 5-methyl-*cyclo*Sal-d4TMP **9** (Figure 2). Compound **6** derives from **5** by formal attachment of a second nucleotidyl moiety to

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10.1021/jm0611713 CCC: $37.00
Published on Web 03/01/2007

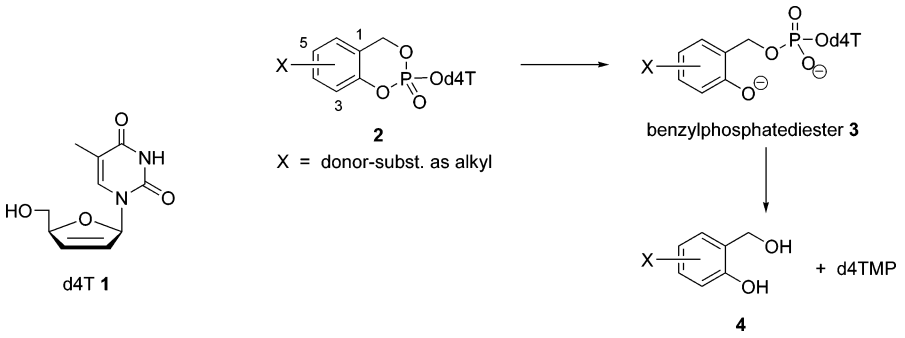


Figure 1. The chemical formula of d4T **1** and the hydrolysis pathway of *cyclo*Sal pronucleotides with *cyclo*Sal-d4TMP **2** shown as the example.

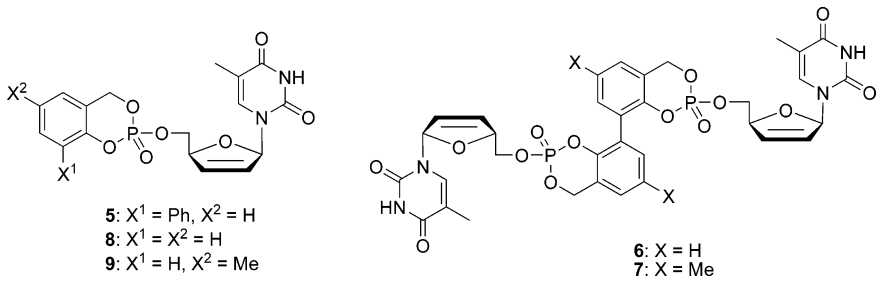


Figure 2. Structures of "monomeric" *cyclo*Sal-d4TMPs **5**, **8**, **9** and novel "dimeric" target structures **6**, **7**.

the second aromatic ring of the biphenyl system of **5**. The design of **6**, **7** was based on three rationales: first, they display a formal mask−drug ratio of 1:2, an unprecedented novelty in the field of pronucleotides. Second, their massively increased steric bulkiness should lead to a complete loss or at least strong reduction of BChE inhibitor activity. Third, the additional methyl groups in **7** as compared to **6** should lead to a higher hydrolytic stability which might be advantageous with respect to nucleotide delivery in the intact cells and thus the prodrug's antiviral activity.[5]

In this study, we describe the synthesis of **6**, **7** and investigations concerning their hydrolytic properties, hydrolysis pathways, BChE inhibitor potencies, and anti-HIV activities.

Results and Discussion

Chemistry. Generally, for the synthesis of *cyclo*Sal pronucleotides, an approach using phosphorus(III) chemistry has been established. The required cyclic saligenyl chlorophosphites can be obtained from appropriately substituted salicyl alcohol derivatives.[4]

Accordingly, for the synthesis of bis-*cyclo*Sal-d4TMP **6** the hydroxymethylated biphenyl diol ("dimeric" salicyl alcohol) **10** was prepared first. The synthesis of **10** could be achieved *via* two different routes. For the first route, dialdehyde **11** has been synthesized according to a previously published procedure[18] with few modifications regarding purification: commercially available biphenyl 2,2′-diol was protected as a methoxymethyl (MOM) ether (66% yield) and subsequently bis-formylated using DMF as a formylating agent after ortho-lithiation. However, chromatographic purification of the protected dialdehyde turned out to be extremely difficult. Hence, a crude mixture of this material was deprotected to yield dialdehyde **11** (48% over two steps). Final reduction of **11** using $LiAlH_4$ led to isolation of the desired alcohol **10** in 94% yield (30% over four steps, Scheme 1).

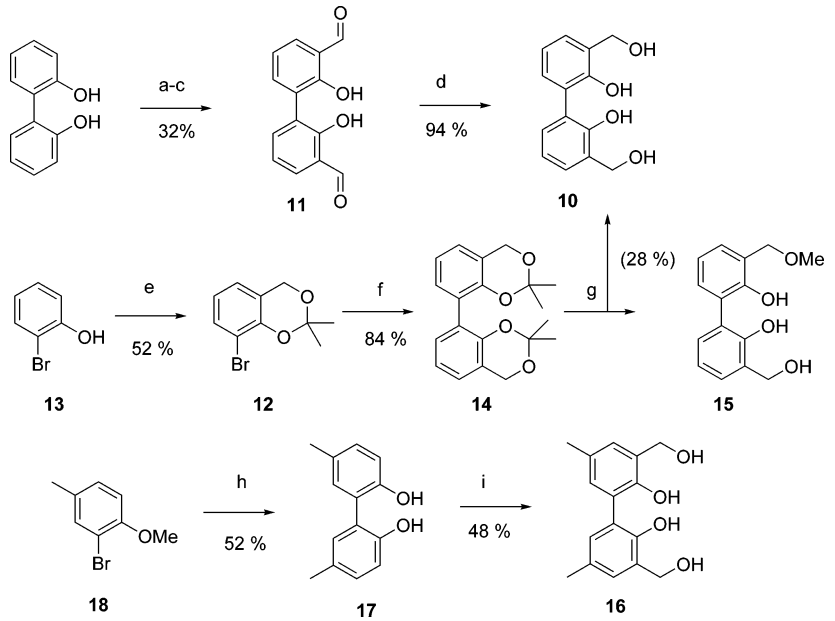
The second route for the preparation of **10** was based on 3-bromosalicyl alcohol isopropylidene acetal **12** which can be obtained from 2-bromophenol **13** in three steps (overall yield 52%).[19] Acetal **12** was lithiated and subsequently oxidatively homo-coupled using iron(III) acetylacetonate leading to **14** in 84% yield (Scheme 1).

A similar reaction had been observed before by Katz and Cram as an unwanted side reaction in an attempted cross-coupling.[20] However, acidic deprotection of **14** employing ion-exchange resin (Dowex), a method otherwise identified to be useful for cleavage of salicyl alcohol isopropylidene acetals in our laboratories, turned out to be difficult. Multiple amounts of methylated byproduct **15** (24−60% isolated yields) were formed depending on the reaction conditions. Several attempts to avoid the formation of compound **15** by changing and optimizing the conditions (reaction time, amount of Dowex and methanol) failed. The best isolated yield for tetrol **10** upon Dowex treatment of **14** on a larger scale was 28%. Although this drawback seems to make the second route for the preparation of **10** unfavorable as compared to the first one, avoidance of the use of the highly toxic MOM-chloride and easier purification have to be mentioned as two key advantages of the second approach.

For the synthesis of bis-5-methyl-*cyclo*Sal-d4TMP **7**, alcohol **16** was needed (Scheme 1). Thus, methylated biphenyl diol **17** was synthesized starting from 2-bromo-4-methylanisole **18** according to procedures reported before[21] and employing an iron-cross coupling reaction as described above (52% yield over two steps). Subsequent hydroxymethylation of **17** using formaldehyde under basic conditions directly led to the isolation of **16**, though in moderate yield (48%). An application of this rather simple hydroxymethylation method for the preparation of **10** had been impossible as 2,2′-biphenyldiol has both an unsubstituted ortho- and para-position relative to the phenolic hydroxyl group which can react with formaldehyde, probably resulting in tedious product mixtures.

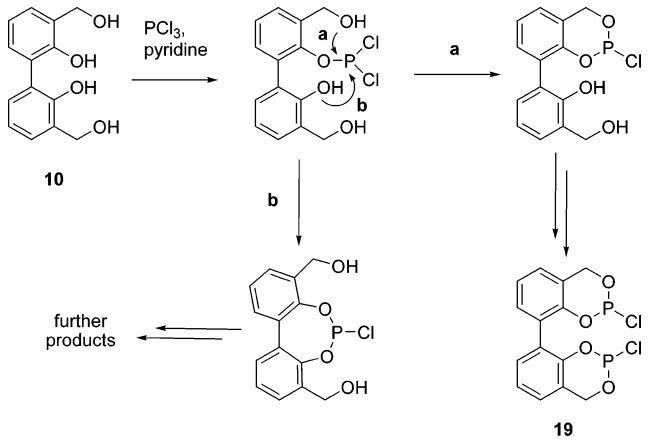
For the conversion of alcohols **10**, **16** into the corresponding phosphitylating agents **19**, **20**, tetrols **10**, **16** were treated with phosphorus(III) chloride under basic conditions. However, besides formation of the desired six-membered rings, formation of seven-membered rings leading to unwanted byproducts also seemed to be possible (shown for **10** in Scheme 2). ^{31}P NMR investigation of the crude product from the reaction of **10** with

Scheme 1. Synthesis of Biphenyl Tetrols **10**,**16**[a]



[a] Reagents and conditions: (a) (i) NaH, THF, 0 °C to r.t., 1 h; (ii) MOM-Cl, 0 °C to r.t., 16 h; (b) (i) *n*-BuLi, Et_2O, r.t., 2 h; (ii) DMF, 0 °C to r.t., 5 h; (c) 6 M HCl, CH_2Cl_2, EtOH, reflux, 16 h; (d) (i) $LiAlH_4$, THF, r.t. to reflux, 3 h; (ii) H^+/H_2O; (e) see ref 19; (f) (i) *n*-BuLi, THF, −80 °C, 1 h; (ii) $Fe(acac)_3$, THF, −80 °C to r.t., 17 h; (g) Dowex 50X8, CH_2Cl_2/MeOH 1:1, r.t., 65 h; (h) see ref 21; (i) (i) 37% HCHO/H_2O, NaOH, 40 °C, 4 d; (ii) H^+/H_2O; MOM = methoxymethyl.

Scheme 2. Possible Reaction Products for the Reaction of **10** with PCl_3



PCl_3 using the standard conditions (pyridine at −20 °C)[4] indeed indicated significant formation of seven-membered ring byproducts (integral ratio 70% of product **19** to 30% of byproducts). Further reduction of the reaction temperature helped to decrease the amount of byproducts formed significantly (−40 °C: (87:13); −60 °C: (88:12)), so that the synthesis of **19** was finally carried out at −40 °C instead of −20 °C.

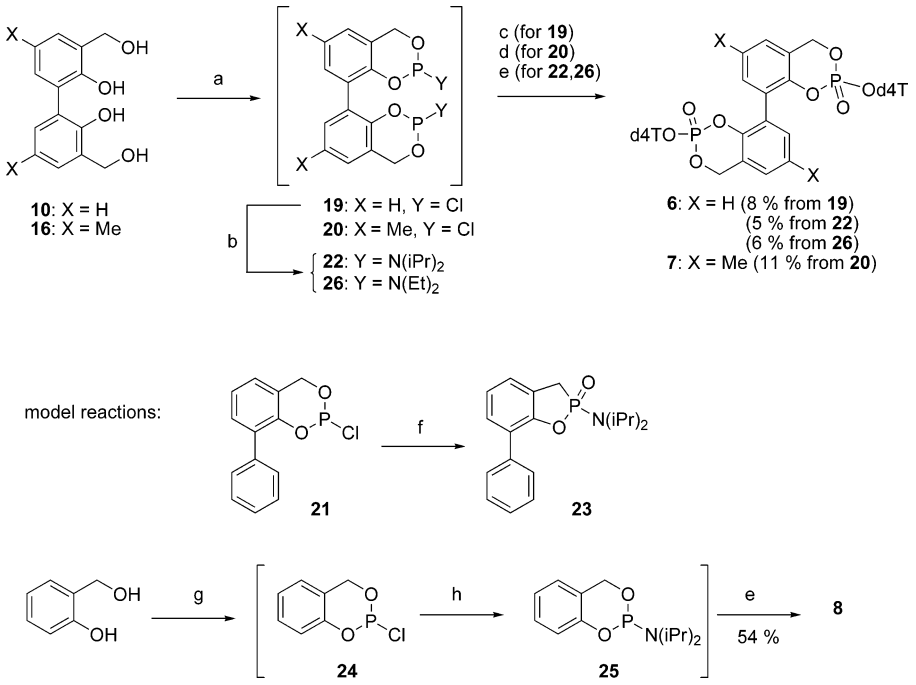
Subsequent treatment of d4T **1** with crude chlorophosphite **19** (most saligenyl chlorophosphites are too sensitive for standard purification procedures)[4] led to target pronucleotide **6**, but only after tedious chromatography and in poor yield (8%, Scheme 3).

Consequently, ways to improve the yield of nucleotide synthesis were investigated. The synthesis of *cyclo*Sal compounds *via* phosphoramidite chemistry was described before,[22] but preparation of the required phosphoramidite reagents from the saligenyl chlorophosphites was performed starting from purified chlorophosphites which were obtained in the case of low molecular weight saligenyl chlorophosphites by vacuum Kugelrohr distillation. As compound **19** was not volatile enough to be distilled *in vacuo*, a way to prepare saligenyl phosphoramidites from crude chlorophosphites had to be elaborated. In a model reaction, crude 3-phenyl saligenyl chlorophosphite **21**[17] was treated with diisopropylamine (DIPA). However, instead of saligenyl *N*,*N*-diisopropylaminophosphoramidite **22**, the air-stable ring contraction product **23** was isolated. Formation of phosphonamidate **23** probably was the result from an Arbuzov−Michaelis rearrangement of **22** catalyzed by traces of acidic pyridinium chloride present in the crude mixture of **21**. Traces of pyridinium chloride are often present because the preparation of chlorophosphites like **21** is achieved using pyridine as base.[23] In contrast, it was demonstrated that saligenyl phosphoramidites can be prepared from crude chlorophosphites if these had been prepared in the absence of pyridine, e.g., if triethylamine was used instead of pyridine. Triethylammonium chloride as a potential impurity of **24** probably is too weak as an acid to catalyze an Arbuzov−Michaelis rearrangement of **25** similar to that of **22**. The crude phosphoramidite **25** can subsequently be used for pronucleotide synthesis in satisfying yields as can be seen in the case of *cyclo*Sal-d4TMP **8** (Scheme 3).

Application of this synthetic methodology led to the successful preparation of *N*,*N*-diisopropylaminophosphoramidite **22** from chlorophosphite **19**. Treatment of d4T **1** with **22** and subsequent oxidation afforded target compound **6**. However, the yield (5%) was found to be similar to that obtained on the chlorophosphite route. In order to circumvent these purification problems, *N*,*N*-diethylaminophosphoramidite **26** was synthesized and applied on nucleotide synthesis instead. Unfortunately, this approach did not lead to a considerable improvement in the yield of **6** (6%) either. Thus, the synthesis of the second target compound **7** was performed using chlorophosphite chemistry *via* phosphitylating agent **20** (prepared analogously to **19**) finally leading to the isolation of **7** in 11% yield (Scheme 3).

*Cyclo*Sal-pronucleotides were always obtained as mixtures of two diastereomers (R_P and S_P configuration).[4] In the case of bis-*cyclo*Sal-d4TMPs **6**, **7**, two stereogenic centers are formed in the course of preparation. Hence, they should be obtained as mixtures of three stereoisomers (R_P/R_P, R_P/S_P, and S_P/S_P con-

Scheme 3. Synthesis of Pronucleotides **6**, **7** from Alcohols **10**, **16** and Model Reactions[a]



[a] Reagents and conditions: (a) (i) PCl_3, Et_2O, −40 °C, 10 min; (ii) pyridine or NEt_3 (if subsequent conversion to a phosphoramidite was envisaged), −40 °C, 2 h; (iii) r.t., 2 h; (b) $(alkyl)_2NH$, Et_2O, r.t., 3 h, no purification; (c) (i) d4T **1**, DIPEA, CH_3CN, −20 °C to r.t., 1 h; (ii) *t*-BuOOH, −20 °C to r.t., 1 h; (d) like c, but 6 h reaction period in i; (e) (i) d4T **1**, pyridine·HCl, CH_3CN, 0 °C, 3 h; (ii) *t*-BuOOH, 0 °C to r.t.,1 h; (f) $(i\text{-Pr})_2NH$, Et_2O, r.t., 20 h; (g) (i) PCl_3, Et_2O, −20 °C, 10 min; (ii) NEt_3, −20 °C, 2.5 h; (iii) r.t., 2 h; (h) like b with alkyl = *i*-Pr; DIPEA = diisopropylethylamine (Hünig's base).

Table 1. Hydrolytic Stabilities of Bis-*cyclo*Sal-d4TMPs **6**, **7** Compared to "Monomeric" Reference Compounds

*cyclo*Sal-d4TMP	$t_{1/2}$ [h][a]
6 (mix, X = H)	8.2
6 *fast* (X = H)	3.2
6 *slow* (X = H)	8.5
7 (mix, X = Me)	12
5 (mix, X = 3-Ph)	5.1
8 (mix, X = H)	4.4
9 (mix, X = 5-Me)	6.2

[a] 25 mM phosphate buffer, pH 7.3, 37 °C; $t_{1/2}$ values refer to disappearance of starting pronucleotides.

figuration, respectively) in a ratio of 1:2:1. According to ^1H and ^{31}P NMR spectroscopy, **6** indeed was obtained as a mixture of three diastereomers, but in a ratio of 1:2:2. The minor stereoisomer (**6** ***fast***) could be separated by preparative RP-HPLC from the remaining mixture of stereoisomers (**6** ***slow***), which proved to be inseparable. Interestingly, **7** was isolated as a mixture of only two diastereomers (ratio 0.7:1.0). Most probably, the expected third stereoisomer was lost from the product mixture during chromatographic purification. However, the diastereomeric mixture of **7** turned out to be inseparable *via* RP-HPLC just like **6** ***slow***. It should be pointed out that neither **6** nor **7** formed atropisomers due to potentially hindered rotation around the biphenyl axis.

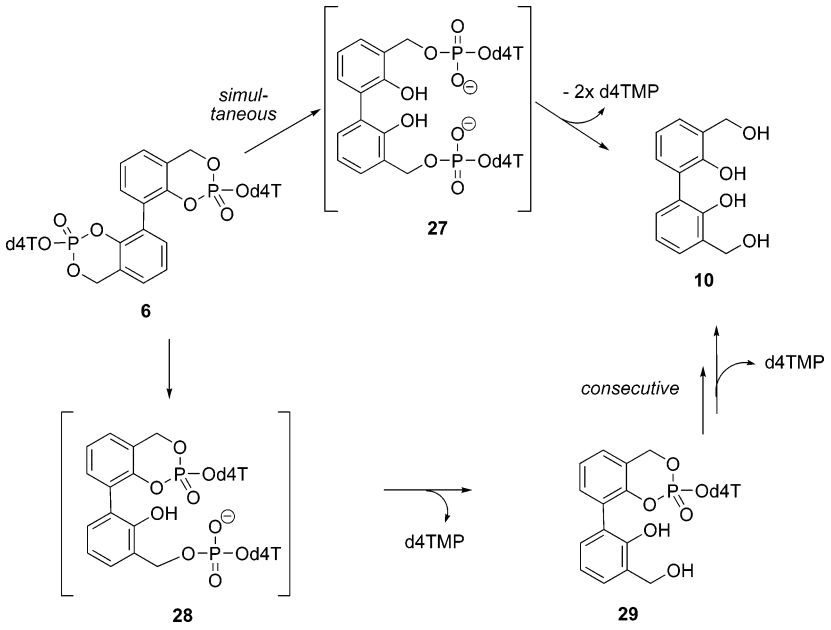
Hydrolytic Stabilities. The hydrolytic stabilities of **6**,**7** in phosphate buffer (pH 7.3, 37 °C) were investigated (release of d4TMP was measured by HPLC analysis) and compared to several reference compounds (Table 1). Both **6** (diastereomeric mixture, $t_{1/2}$ = 8.2 h) and **7** ($t_{1/2}$ = 12 h) turned out to be 2-fold more stable than their "monomeric" counterparts, *cyclo*Sal-d4TMP **8** ($t_{1/2}$ = 4.4 h) and 5-methyl-*cyclo*Sal-d4TMP **9** ($t_{1/2}$ = 6.2 h). This was clearly attributed to the presence of the second nucleotidyl moiety, as a comparison of the stabilities of **6** and 3-phenyl-*cyclo*Sal-d4TMP **5** ($t_{1/2}$ = 5.1 h) has shown. The influence of the stereochemistry at the phosphorus centers on the hydrolytic stability of **6** was significant: **6** ***slow*** ($t_{1/2}$ = 8.5 h) possessed a half-life slightly higher than that of the diastereomeric mixture. However, **6** ***fast*** ($t_{1/2}$ = 3.2 h) proved to be even more labile than the "monomeric" reference **8** (diastereomeric mixture).

Hydrolysis Pathway. It was of major importance to investigate whether the chemical hydrolysis of **6**, **7** led to d4TMP formation. Therefore, the pathway of hydrolysis of **6** was studied in detail. The prodrug **6** might either undergo simultaneous cleavage of both phosphate triester moieties according to the mechanism proven for *cyclo*Sal pronucleotides (Figure 1) leading to the bis-(benzyl phosphate diester) **27**, which then yields 2 equiv of d4TMP and alcohol **10** as the byproduct, or might be hydrolyzed consecutively, implying that product **29** is formed *via* the labile intermediate **28** prior to the liberation of the second d4TMP molecule. These theoretically possible hydrolytic routes are depicted in Scheme 4.

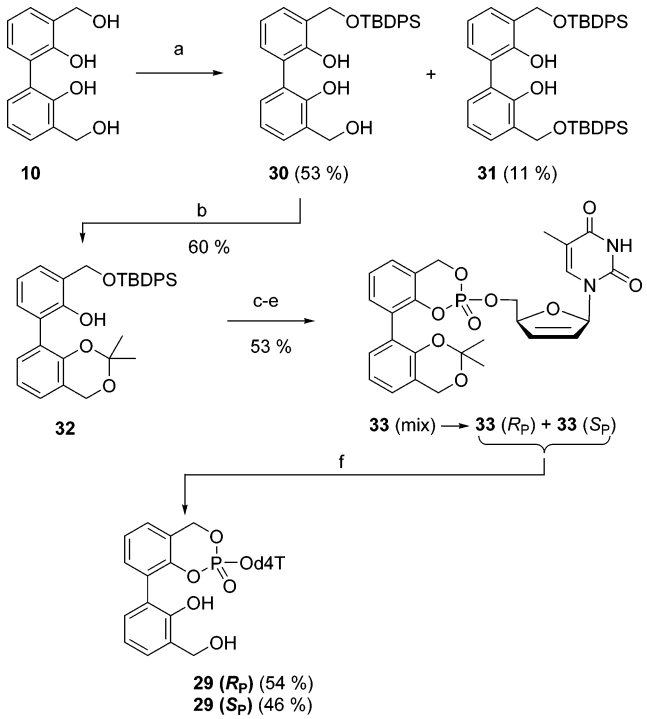
The only intermediate in this hydrolysis pattern not being intrinsically instable is pronucleotide **29** (which will be named 3-Sal-*cyclo*Sal-d4TMP). Thus, an independent synthesis of **29** was necessary in order to be used as reference for hydrolysis studies on **6**. Its individual preparation was achieved starting from alcohol **10** (Scheme 5).

After several unsuccessful attempts to selectively protect only one of the salicyl alcohol moieties of tetrol **10** as an isopropylidene acetal, one of the benzyl alcohol functions in **10** was blocked as *tert*-butyldiphenyl silyl (TBDPS) ether. The best results for this reaction were achieved using 0.75 equiv of TBDPS chloride and DMAP as an activator, allowing the isolation of monosilyl ether **30** in 53% yield with concomitant formation of the unwanted byproduct **31** in 11% yield. Subsequent protection of **30** as isopropylidene acetal (**32**) was achieved using 2,2-dimethoxypropane under acidic conditions in 60% yield. However, after desilylation of **32** to give the salicyl alcohol derivative (89% yield), isopropylidene protected pronucleotide **33** (3-Isopr-Sal-*cyclo*Sal-d4TMP) could be prepared

Scheme 4. Potential Pathways for the Hydrolysis of Bis-*cyclo*Sal-d4TMP **6**



Scheme 5. Synthesis of 3-Sal-*cyclo*Sal-d4TMP **29**, a Potential Intermediate in the Hydrolysis of **6**[a]



[a] Reagents and conditions: (a) (i) 0.75 equiv of TBDPS-Cl, NEt_3, DMAP, THF, 0 °C to r.t., 2 h; (ii) CH_3OH; (b) 2,2-dimethoxypropane, *p*-TsOH, Na_2SO_4, acetone, 40 °C, 3 d; (c) NH_4F, CH_3OH, r.t., 61 h; (d) (i) PCl_3, Et_2O, −40 °C; (ii) pyridine/Et_2O, −40 °C, 1 h; (iii) r.t., 1 h; (e) (i) d4T **1**, DIPEA, CH_3CN, −20 °C to r.t., 1 h; (ii) *t*-BuOOH, −20 °C to r.t., 1 h; (f) Dowex 50X8, CH_2Cl_2/MeOH 1:1, r.t., 8 d; TBDPS = *tert*-butyldiphenylsilyl; DMAP = 4-dimethylaminopyridine; DIPEA = diisopropylethylamine (Hünig's base).

in 60% yield using the chlorophosphite method. The diastereomeric mixture of **33** (R_P/S_P) was separated using preparative RP-HPLC, and the diastereomerically pure material was then deprotected to afford two diastereomers of **29** separately. Mild cleavage of the isopropylidene acetal was achieved by treatment of **33** with Dowex ion-exchange resin in moderate yields of 54% and 46%, respectively, due to the necessity to stop the reaction before complete conversion of the acetal in order to prevent extensive side reactions. As attempts to crystallize either **29** or **33** in order to obtain an X-ray structure failed, assignment of the stereochemistry at the phosphorus centers was performed using CD spectroscopy and comparison of the spectra to those of a *cyclo*Sal reference compound with known stereochemical configuration.[4c]

Table 2. Hydrolytic Stabilities of the *cyclo*Sal-d4TMPs **29**, **33**

*cyclo*Sal-d4TMP	$t_{1/2}$ [h][a]
29 (mix)	1.3
29 (R_P)	1.4
29 (S_P)	1.1
33 (mix)	16
33 (R_P)	21
33 (S_P)	14

[a] 25 mM phosphate buffer, pH 7.3, 37 °C; $t_{1/2}$ values refer to disappearance of starting pronucleotides.

The pronucleotides **29**, **33** were investigated for their hydrolytic stability as described above (Table 2). While **29** turned out to be remarkably labile ($t_{1/2} = 1.3$ h) as compared to both the 3-phenyl derivative **5** and "dimer" **6** ($t_{1/2} = 5.1$ h and $t_{1/2} = 8.2$ h, Table 1), its protected precursor **33** displayed a significant stability ($t_{1/2} = 16$ h) in comparison to the reference compounds, pointing to a major influence of the polarity or the steric effects of the phenyl ring substituents on the hydrolytic stabilities. Lipophilic and sterically demanding substituents may prevent a nucleophilic attack on the phosphate triester, as the most lipophilic derivative **33** showed the longest hydrolysis half-life of the pronucleotides studied. In both cases (**29** and **33**), the R_P-configured diastereomer turned out to be more stable than the S_P isomer, but while this effect was rather small for **29** ($\Delta t_{1/2} \sim 0.3$ h), it was more pronounced for **33** ($\Delta t_{1/2} \sim 7$ h).

Following the preparation of the potential hydrolytic intermediate **29**, the hydrolysis of **6** (mixture of three diastereomers) was investigated using ^{31}P NMR spectroscopy (imidazole/HCl buffer, pH 7.3, r.t.). The changes in conditions compared to the stability studies in phosphate buffer allowed the monitoring of the hydrolytic reaction over several days. In an initial experiment, the mixture of stereoisomers of **6** was incubated and only the final hydrolysis products were of interest. D4TMP was detected as the sole hydrolysis product of **6** (Figure 3).

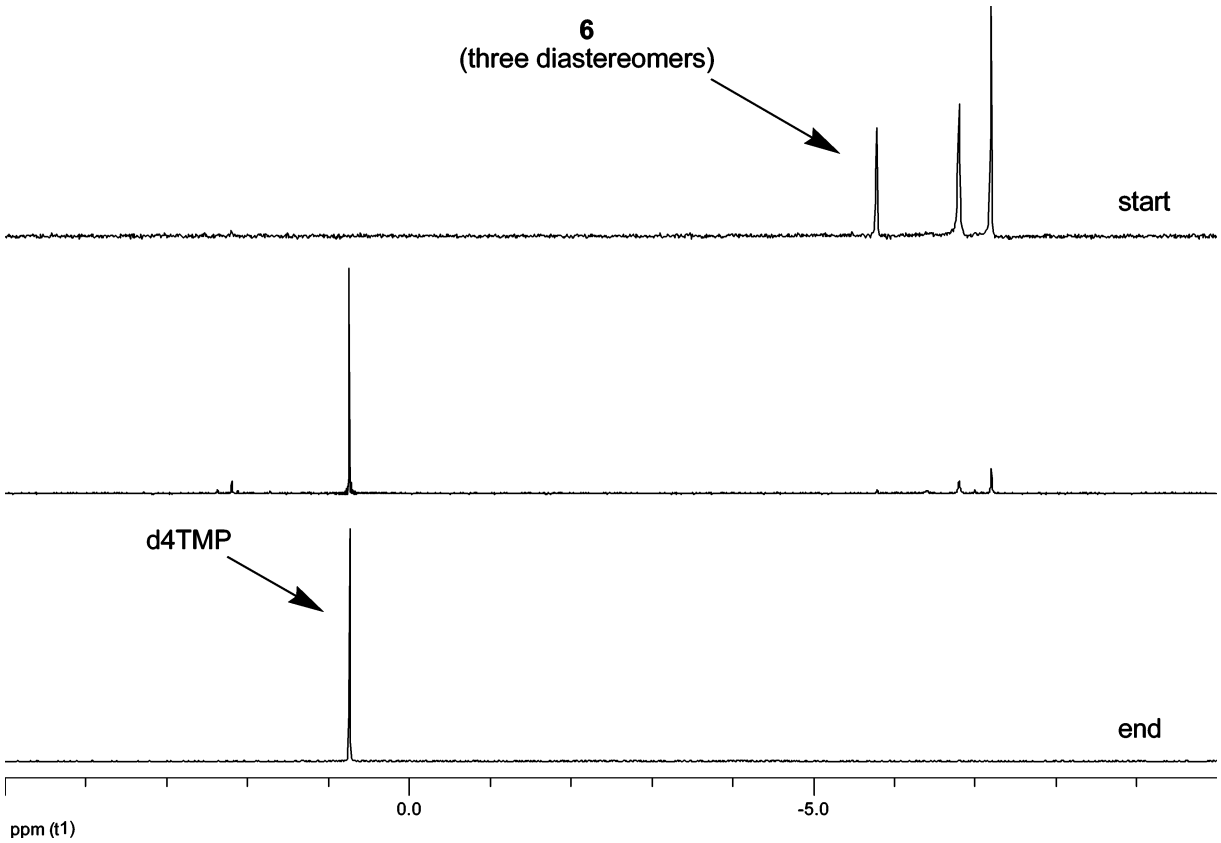


Figure 3. ^{31}P NMR hydrolysis study of pronucleotide **6** (diastereomeric mixture).

Table 3. BChE Inhibitory Potency and Anti-HIV Activity of the Novel *cyclo*Sal-d4TMPs

		antiviral activity EC_{50} [μM][b]			CC_{50} [μM][c]
		CEM/0		CEM/TK$^-$	CEM/0
compound	IC_{50} (BChE) [μM][a]	HIV-1	HIV-2	HIV-2	
6 (mix, X = H)	40	0.19 ± 0.05	0.27 ± 0.11	2.33 ± 0.58	27.8 ± 11.0
6 ***fast*** (X = H)	14	0.25 ± 0.14	0.30 ± 0.09	3.33 ± 2.31	107 ± 13
6 ***slow*** (X = H)	>50	0.21 ± 0.17	0.22 ± 0.16	2.37 ± 1.48	90.7 ± 16.1
7 (X = Me)	>50	0.37 ± 0.25	0.41 ± 0.19	1.60 ± 0.57	67.1 ± 7.1
29 (mix)	2.3	n.d.[d]	n.d.	n.d.	n.d.
29 (R_P)	>50	0.27 ± 0.05	0.50 ± 0.05	0.70 ± 0.05	69.4 ± 11.7
29 (S_P)	0.79	0.34 ± 0.10	0.40 ± 0.00	1.50 ± 0.70	78.7 ± 6.2
33 (mix)	4.7	0.35 ± 0.09	0.40 ± 0.00	1.07 ± 0.83	65.0 ± 6.7
33 (R_P)	>50	0.36 ± 0.07	0.40 ± 0.00	0.55 ± 0.21	55.3 ± 23.0
33 (S_P)	3.6	0.50 ± 0.14	0.40 ± 0.00	3.0 ± 1.4	89.3 ± 11.7
5 (X = 3-Ph)	0.35	0.13 ± 0.05	0.27 ± 0.13	0.28 ± 0.18	22.0 ± 5.0
8 (X = H)	0.77	0.15 ± 0.07	0.13 ± 0.06	0.30 ± 0.25	26.5 ± 22.4
9 (X = 5-Me)	4.6	0.46 ± 0.24	0.46 ± 0.48	1.25 ± 0.90	37.9 ± 20.0
10	>50	>150	>150	n.d.	>150
16	>50	>150	>150	n.d.	>150
d4T **1**	–	0.25 ± 0.00	0.19 ± 0.10	51.0 ± 10.4	105 ± 78

[a] Inhibitor activity: 50% inhibitory concentration against human BChE (human serum). [b] Antiviral activity: 50% effective concentration. [c] Cytostatic activity: 50% cytostatic concentration. [d] n.d. = not determined.

A more detailed investigation on the hydrolysis of partially separated diastereomers **6** ***fast***/***slow*** followed with more ^{31}P NMR spectra recorded at shorter time intervals. However, for both hydrolyses the formation of **29** (R_P/S_P) could not be observed. If consecutive hydrolysis takes place, then **29** is hydrolyzed significantly faster than it is formed, making its detection impossible. Hence, a consecutive hydrolysis pathway cannot be ruled out. Analogously proven by ^{31}P NMR spectroscopy, the hydrolysis of the methyl-substituted derivative **7** (mixture of two diastereomers) led also exclusively to d4TMP as the sole hydrolysis product.

Biological Activity

BChE Inhibitor Activity. All synthesized pronucleotides as well as the tetrols **10**, **16** were tested for their inhibitory potency toward human BChE as described before[12] using human serum as a BChE source. Their inhibitor activities were compared with several reference compounds (Table 3).

The results for the bis-*cyclo*Sal-d4TMPs **6**, **7** met the expectations, as they showed significantly reduced BChE inhibitory activity. With IC_{50} values of 40 μM for **6** (diastereomeric mixture) and out of the range of physiological importance (>50 μM) for **7**, they turned out to inhibit human

BChE more than 50-fold (**6**) and more than 10-fold (**7**) weaker than their "monomeric" counterparts *cyclo*Sal-d4TMP **8** ($IC_{50} = 0.77\ \mu M$)[12] and 5-methyl-*cyclo*Sal-d4TMP **9** ($IC_{50} = 4.6\ \mu M$).[12] Furthermore, a comparison of the IC_{50} value of the isomeric mixture of **6** to those of several other compounds reveals the crucial role of the second nucleotidyl moiety to successfully overcome BChE inhibition. While 3-phenyl-*cyclo*Sal-d4TMP **5** ($IC_{50} = 0.35\ \mu M$)[12] inhibited the enzyme even stronger than prototype **8**, 3-Sal derivative **28** (mix, $IC_{50} = 2.3\ \mu M$) and its synthetic precursor **33** (mix, $IC_{50} = 4.7\ \mu M$) displayed a ~10-fold weaker inhibitory potency. However, this effect becomes remarkably pronounced only due to the simple attachment of a second nucleotidyl residue as in **6**, leading to a further 9- to 17-fold decrease of inhibitory activity. The further reduction in inhibitory activity upon the attachment of two additional methyl substituents (**7**) is in accordance to previous results proving disubstituted "monomeric" *cyclo*Sal nucleotides to be rather weak BChE inhibitors.[12]

The essential influence of the stereochemistry at the phosphorus center on the BChE inhibitor potency of the *cyclo*Sal pronucleotides has been described before[12] and could be confirmed within this study. The R_P diastereomers of the monomeric *cyclo*Sal derivatives **29**, **33** showed no BChE inhibitory activity at all, and the effect solely occurred from the S_P isomer. Similarly, the single diastereomer **6** ***fast*** displayed weak BChE inhibition ($IC_{50} = 14\ \mu M$) while the remaining two diastereomers **6** ***slow*** turned out to be noninhibitory ($IC_{50} > 50\ \mu M$). Thus, it can be assumed that **6** ***fast*** should be the (S_P,S_P) diastereomer though this needs further confirmation in future investigations. As expected the tetrols **10**, **16** showed no inhibitory activity against BChE.

Antiviral Activity and Cytotoxicity. The synthesized pronucleotides and tetrols **10**, **16** were evaluated for their anti-HIV activity and cytotoxicity *in vitro* (Table 3). All *cyclo*Sal derivatives investigated showed activities against HIV-1 and HIV-2 in wild-type CEM cells (CEM/0) comparable to those of the parent nucleoside **1**. The efficiency to bypass TK-mediated phosphorylation of d4T as a consequence of intracellular d4TMP delivery is reflected by the prodrugs' retention of marked anti-HIV-2 activities in TK-deficient CEM cells (CEM/TK^-). Several pronucleotides (**29** (R_P), **33** (R_P) and references **5**, **8**) displayed virtually full retention of their anti-HIV-2 activities in the TK-deficient cell line, indicating a highly successful TK-bypass. Although still showing activities about 25-fold more pronounced than d4T, bis-*cyclo*Sal-d4TMPs **6**, **7** slightly lost some antiviral activity in the TK^- cells as compared to the wild-type cells. It cannot be ruled out that the cellular uptake of this new type of pronucleotide was partially hindered due to its markedly increased molecular size. Furthermore, it seems remarkable that **33** (diastereomeric mixture) displayed a slight loss of activity in CEM/TK^- cells as well since its significant hydrolytic stability should allow an efficient cell penetration prior to its cleavage. Interestingly, the stereochemistry at the phosphorus atoms also had an influence on the antiviral activities. For both **29** and **33**, the R_P diastereomer led to a successful TK-bypass while the S_P diastereomer lost some activity in the TK^- cells. It would be tempting to speculate that the R_P diastereomer could be better taken up by the intact CEM cells than the S_P diastereomer, suggesting a degree of preference or selectivity of uptake of diastereomeric compounds. However, this finding is also important since the more active R_P isomer did not show any BChE inhibitor potency, while the less active S_P isomer did. Moreover, it should be pointed out that the R_P isomer was hydrolytically more stable than the corresponding S_P isomer for both **29** and **33**. However, differences in hydrolytic stabilities alone cannot be responsible for the observed differences in antiviral activities in the CEM/TK^- cells. For example, **29** (R_P) was hydrolytically much more labile than **33** (S_P) ($t_{1/2} = 1.4$ h vs $t_{1/2} = 14$ h), but it possessed a better anti-HIV-2 activity in CEM/TK^- cells, whereas the anti-HIV-2 activities of **6** ***fast*** and **6** ***slow*** in this cell line turned out to be comparable.

The cytotoxicity of most of the *cyclo*Sal derivatives tested seemed to be slightly higher than that of d4T **1**, but a specific trend with respect to certain masking structures or a correlation to the antiviral activities could not be elucidated. Finally, as expected the tetrols **10**, **16** showed no antiviral activity against HIV and were found to be non-cytotoxic. This is in complete agreement with a lot of other salicyl alcohols that we have synthesized and tested before.

Conclusion

In summary, the first synthesis of bis-*cyclo*Sal-d4TMPs **6**, **7** as "dimeric" pronucleotides bearing a mask−drug ratio of 1:2 was accomplished. The novel derivatives proved to be hydrolytically more stable than their "monomeric" counterparts and exclusively hydrolyzed to d4TMP. They showed significantly reduced inhibitory potency toward human BChE, making this bis-*cyclo*Sal-nucleotide approach attractive for further applications. The anti-HIV-2 activities of **6**, **7** in TK-deficient human CEM cells were significantly higher than that of the parent nucleoside d4T **1**, providing a rather efficient TK-bypass capacity of these prodrugs. Additional work to further improve the mask structure is envisaged and is currently in progress.

Experimental Section

Chemistry. All air-sensitive reactions were carried out under an atmosphere of dry nitrogen. Solvents used in the inert gas syntheses were commercially available dry solvents stored under argon and over molecular sieves (Fluka). Diethyl ether and THF were dried over sodium/benzophenone and distilled under nitrogen. 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 on a Chromatotron (Harrison Research, Model 7924T) using glass plates coated with 1 mm 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. NMR spectra were recorded using Bruker AMX 400 and Bruker DRX 500 Fourier transform spectrometers. All ^1H and ^{13}C NMR chemical shifts (δ) are quoted in ppm 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. EI mass spectra were measured on a VG Analytical VG/70-250S spectrometer (double focusing). FAB low and high resolution (HR) mass spectra were recorded on a VG Analytical 70-250S spectrometer using an MCA method and poly(ethylene glycol) as support. ESI mass spectra were obtained on a Finnigan ThermoQuest MAT 95 XL spectrometer. IR spectra were measured on a Thermo Nicolet AVATAR 370 FT-IR spectrometer, and wavenumbers ν are quoted in cm^{-1}. UV spectra were recorded on a Varian Cary 1E UV−visible spectrophotometer and absorption maximum wavelengths λ_{max} are given in nm. UV absorptions of *cyclo*Sal nucleotides were determined from their HPLC data (diode array detector). Analytical HPLC was performed on a Merck-Hitachi HPLC system (D-7000) equipped with a LiChroCART 125-3 column containing reversed phase silica gel Lichrospher 100 RP 18 (5 μm) (Merck, Darmstadt, Germany). Preparative HPLC was carried out on an HPLC system consisting of a Merck-Hitachi L-6250 Intelligent Pump, a Merck-Hitachi LaChrom UV detector L-7400, and a Merck-Hitachi D-2500A Chromato-Integrator using a Merck Hibar RT 250-25 column

containing reversed phase silica gel Lichrospher 100 RP 18 (5 μm) (Merck, Darmstadt, Germany). The flow rate was 10 mL/min, and detection was performed at a wavelength of 260 nm. The appropriate fractions were pooled and lyophilized. The lyophilized *cyclo*Sal compounds 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.

General Procedure A (Deprotection of Salicyl Alcohol Isopropylidene Acetals). The salicyl alcohol isopropylidene acetal was dissolved in DCM/MeOH 1:1 and vigorously stirred with Dowex 50X8 (H^+ form, previously washed with DCM and MeOH) at room temperature for the indicated time. The reaction was monitored *via* TLC or HPLC. After filtration, the filtrate was evaporated under reduced pressure, and the crude product was purified by chromatography.

General Procedure B (Synthesis of Saligenyl Chlorophosphites). Under a nitrogen atmosphere, freshly distilled PCl_3 was added to a solution of the salicyl alcohol derivative in Et_2O at the indicated temperature. After ca. 10 min, a solution of pyridine or NEt_3 in Et_2O was added dropwise at the same temperature over 2−3 h. The reaction mixture was warmed to room temperature and further stirred at room temperature for 1−2 h. Pyridinium or triethylammonium chloride was completely precipitated by storing the mixture at −20 °C overnight. After filtration under nitrogen, the solvent was removed *in vacuo*. The resulting crude products were used in pronucleotide synthesis without further purification.

General Procedure C (Synthesis of Saligenyl Phosphoramidites). Under a nitrogen atmosphere, a solution of dialkylamine in Et_2O was added dropwise to a solution of crude saligenyl chlorophosphite in Et_2O at room temperature. The mixture was stirred for 3 h at room temperature. After filtration under nitrogen, the solvent was removed *in vacuo*. The resulting crude products were used in pronucleotide synthesis without further purification.

General Procedure D (Synthesis of *cyclo*Sal Nucleotides Using Chlorophosphites). Under a nitrogen atmosphere, DIPEA and the crude saligenyl chlorophosphite, dissolved in MeCN, were added to a solution of d4T **1** in MeCN at −20 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. The reaction was monitored *via* TLC (DCM/MeOH 9:1). After cooling to −20 °C, a solution of *tert*-butyl hydroperoxide in *n*-decane was added. The solution was warmed to room temperature again and further stirred for 1 h (TLC monitoring). The solvent was removed *in vacuo*, and the resulting crude products were purified by preparative TLC (Chromatotron, 1. EtOAc/MeOH 9:1, 2. DCM/MeOH gradient, MeOH contained 0.1% HOAc). The oily products were lyophilized to yield colorless foams.

General Procedure E (Synthesis of *cyclo*Sal Nucleotides Using Phosphoramidites). Under a nitrogen atmosphere, crude saligenyl phosphoramidite, dissolved in MeCN, was added to a solution of d4T **1** and pyridinium chloride in MeCN at 0 °C. The reaction mixture was stirred 3 h at 0 °C. The reaction was monitored *via* TLC (DCM/MeOH 9:1). Subsequently, a solution of *tert*-butyl hydroperoxide in *n*-decane was added at 0 °C. The solution was warmed to room temperature and further stirred for 1 h (TLC monitoring). The solvent was removed *in vacuo*, and the resulting crude products were purified as described in general procedure D.

Bis-*cyclo*Sal-d4TMP (6). Method I: General Procedure D with d4T **1** (300 mg, 1.34 mmol), crude chlorophosphite **19** (938 mg), DIPEA (0.47 mL, 0.35 g, 2.7 mmol), 5.5 M solution of *tert*-butyl hydroperoxide in *n*-decane (0.86 mL, 4.8 mmol), and MeCN (30 mL). The product **6** (42 mg, 8%) was obtained as a colorless foam and mixture of three diastereomers (1.1:2.0:1.9): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 6H), 7.42−7.36 (m, 6H), 7.36−7.27 (m, 6H), 7.27−7.23 (m, 6H), 7.17 (s, 2H), 7.16 (s, 2H), 7.12 (s, 2H), 6.81−6.76 (m, 6H), 6.32−6.27 (m, 6H), 6.03−5.92 (m, 6H), 5.64−5.40 (m, 12H), 4.99−4.88 (m, 6H), 4.29−4.18 (m, 12H), 1.64 (s, 6H), 1.60 (s, 12H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.1, 151.1, 147.2, 136.0, 135.9, 133.1, 131.3, 131.1, 127.7, 126.8, 122.4, 110.0, 89.8, 84.4, 69.0, 68.3, 12.2, 12.1; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.75, −8.21, −8.47; MS (FAB) *m*/*z* calcd 783.1 (M + H^+), found 783.3 (M + H^+); HR−MS (ESI^+) *m*/*z* calcd 805.1228 (M + Na^+), found 805.1290 (M + Na^+); UV (HPLC) λ_{max} 265; TLC R_f value 0.40 (DCM/MeOH 9:1).

Method II: General Procedure E with d4T **1** (379 mg, 1.69 mmol), crude phosphoramidite **22** (1.10 g, dissolved in 25 mL MeCN), pyridinium chloride (781 mg, 6.76 mmol), 5.5 M solution of *tert*-butyl hydroperoxide in *n*-decane (0.90 mL, 4.9 mmol), and MeCN (20 mL). Preparative TLC on the Chromatotron did not provide the pure product, so final purification was achieved using preparative HPLC (MeCN/H_2O 24:76, H_2O contained 0.5% HOAc). This also allowed partial separation of the 3 diastereomers to furnish **6** ***fast*** (6 mg, 1 diastereomer) and **6** ***slow*** (28 mg, 2 diastereomers, 1.0:1.0) as colorless foams, providing an overall yield of 34 mg (5%). Analytical data of **6** ***fast***: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41−7.36 (m, 2H), 7.36−7.32 (m, 2H), 7.32−7.27 (m, 2H), 7.16 (s, 2H), 6.83−6.78 (m, 2H), 6.35−6.29 (m, 2H), 6.04−5.98 (m, 2H), 5.57 (dd, J = 14.5, 15.1 Hz, 2H), 5.46 (dd, J = 12.0, 14.5 Hz, 2H), 4.98−4.88 (m, 2H), 4.29−4.18 (m, 4H), 1.61 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.1, 151.0, 147.2, 136.0, 135.9, 133.3, 133.0, 131.8, 131.1, 127.7, 126.8, 122.3, 110.1, 89.6, 84.3, 68.9, 68.3, 12.1; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.76; Preparative HPLC t_R = 25.4 min (MeCN/H_2O 24:76, H_2O contained 0.5% HOAc). Analytical data of **6** ***slow***: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 4H), 7.41 (dd, J = 1.9, 6.9 Hz, 4H), 7.37−7.28 (m, 4H), 7.28−7.24 (m, 4H), 7.18 (s, 2H), 7.12 (s, 2H), 6.82−6.76 (m, 4H), 6.32−6.27 (m, 4H), 6.05−5.99 (m, 2H), 5.96−5.90 (m, 2H), 5.61 (dd, J = 14.5, 15.1 Hz, 2H), 5.59 (dd, J = 14.5, 15.1 Hz, 2H), 5.50 (dd, J = 13.1, 14.5 Hz, 2H), 5.46 (dd, J = 12.6, 14.5 Hz, 2H), 4.99−4.88 (m, 4H), 4.29−4.18 (m, 8H), 1.64 (s, 6H), 1.61 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.1, 151.0, 147.3, 136.7, 135.9, 133.0, 131.8, 131.6, 127.6, 126.8, 126.8, 122.1, 110.3, 89.5, 89.3, 84.4, 69.0, 68.7, 12.2, 12.1; ^{31}P NMR (202 MHz, DMSO-d_6) δ −8.22, −8.46; Preparative HPLC t_R = 27.6 min (MeCN/H_2O 24:76, H_2O contained 0.5% HOAc).

Method III: General Procedure E with d4T **1** (539 mg, 2.40 mmol), crude phosphoramidite **26** (1.40 g, dissolved in 35 mL MeCN), pyridinium chloride (1.11 g, 9.61 mmol), 5−6 M solution of *tert*-butyl hydroperoxide in *n*-decane (1.41 mL, ≥ 7.05 mmol), and MeCN (30 mL). The product **6** (53 mg, 6%) was obtained as a colorless foam and mixture of three diastereomers (1.0:2.5:2.5). Partial separation of diastereomers was carried out as described above to yield **6** ***fast*** (4 mg, 1 diastereomer) and **6** ***slow*** (24 mg, 2 diastereomers, 1.0:1.0) as colorless foams. The anaytical data were identical with those reported above.

Bis-5-methyl-*cyclo*Sal-d4TMP (7). General Procedure D with d4T **1** (779 mg, 3.47 mmol), crude chlorophosphite **20** (1.18 mg, dissolved in 30 mL MeCN), DIPEA (1.22 mL, 903 mg, 7.00 mmol), 5−6 M solution of *tert*-butyl hydroperoxide in *n*-decane (2.34 mL, ≥ 11.7 mmol), and MeCN (80 mL). The time for the phosphitylation reaction was increased to 6 h, and addition of the chlorophosphite **20** was carried out portionwise. The product **7** (160 mg, 11%) was obtained as a colorless foam and mixture of two diastereomers (0.7:1.0): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 4H), 7.19−7.08 (m, 12H), 6.81−6.77 (m, 4H), 6.31−6.28 (m, 4H), 6.03−6.00 (m, 2H), 5.94−5.91 (m, 2H), 5.56 (dd, J = 14.5, 14.5 Hz, 2H), 5.53 (dd, J = 14.1, 14.1 Hz, 2H), 5.45 (dd, J = 14.5, 14.5 Hz, 2H), 5.40 (dd, J = 14.1, 14.1 Hz, 2H), 4.92−4.88 (m, 4H), 4.26−4.16 (m, 8H), 2.33 (s, 6H), 2.30 (s, 6H), 1.65 (s, 6H), 1.62 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9, 150.9, 150.8, 150.5 (d, J = 7.1 Hz), 150.4 (d, J = 7.1 Hz), 135.9, 133.7, 133.7, 132.9, 132.8, 131.8, 127.4, 126.7, 125.7, 125.4, 125.4, 109.8, 89.3, 84.2 (d, J = 7.8 Hz), 68.7 (d, J = 6.1 Hz), 68.6 (d, J = 8.8 Hz), 20.2, 12.0, 11.9; ^{31}P NMR (202 MHz, DMSO-d_6) δ −8.13, −8.26; MS (FAB-HR) *m*/*z* calcd 833.1601 (M + Na^+), found 833.1594 (M + Na^+); UV (HPLC) λ_{max} 267; TLC R_f value 0.34 (DCM/MeOH 9:1).

***cyclo*Sal-d4TMP (8).** General Procedure E with d4T **1** (84 mg, 0.37 mmol), crude phosphoramidite **25** (241 mg, dissolved in 5 mL MeCN), pyridinium chloride (179 mg, 1.51 mmol), 5.5 M solution of *tert*-butyl hydroperoxide in *n*-decane (0.23 mL, 1.3 mmol), and MeCN (10 mL). The product **8** (77 mg, 54%) was

obtained as a colorless foam and mixture of two diastereomers. The anaytical data were identical with those reported previously.[6]

3,3′-Bis(hydroxymethyl)biphenyl 2,2′-Diol (10). Method I: Under a nitrogen atmosphere, a solution of aldehyde **11** (6.03 g, 25.1 mmol) in THF (100 mL) was added dropwise over 40 min to a suspension of $LiAlH_4$ (1.58 g, 41.6 mmol) in THF (160 mL) at room temperature. The reaction mixture was stirred at room temperature for 2 h and heated under reflux for 1 h. The reaction was quenched by addition of 2 N HCl at 0 °C up to pH 4. After addition of water, the phases were separated and the aqueous phase extracted with EtOAc (5×). The combined organics were dried over anhydrous Na_2SO_4, and the solvent evaporated under reduced pressure. The resulting crude product was purified by filtration through a thin pad of silica (EtOAc) to yield **10** (5.77 g, 94%) as a yellow-brown solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (dd, J = 1.8, 7.4 Hz, 2H), 7.05 (dd, J = 1.8, 7.6 Hz, 2H), 6.89 (dd, J = 7.4, 7.6 Hz, 2H), 4.62 (s, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.1, 130.2, 129.6, 126.5, 124.2, 120.1, 60.1; MS (EI) *m*/*z* calcd 246 (M), found 246 (M, 1%), 214 (35), 200 (66), 181 (58), 152 (50), 139 (22), 128 (45), 115 (49), 102 (11), 91 (28), 77 (39), 63 (26), 51 (32), 39 (100); IR (KBr) ν 3384, 2927, 1589, 1446, 1325, 1228, 1202, 1013, 768, 744; UV (MeOH) λ_{max} 284, 243, 225; TLC R_f value 0.45 (DCM/MeOH 9:1), 0.09 (DCM/MeOH 30:1).

Method II: General Procedure A with acetal **14** (9.78 g, 30.0 mmol), Dowex 50X8 (117 g), DCM/MeOH 1:1 (400 mL) and a reaction period of 65 h. The reaction was monitored *via* TLC (DCM/MeOH 30:1). Purification was carried out by column chromatography (DCM/MeOH gradient (3−10%)) to yield **10** (2.08 g, 28%) as a yellow-brown solid. The anaytical data were identical with those reported above. Analytical data of byproduct 3-(methoxymethyl)-3′-(hydroxymethyl)biphenyl 2,2′-diol **15** (isolated from various test reactions as a yellowish solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (dd, J = 1.7, 7.5 Hz, 1H), 7.27 (dd, J = 1.6, 7.5 Hz, 1H), 7.12 (dd, J = 1.7, 7.5 Hz, 1H), 7.09 (dd, J = 1.6, 7.5 Hz, 1H), 6.94 (dd, J = 7.5, 7.5 Hz, 2H), 4.67 (s, 2H), 4.52 (s, 2H), 3.37 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.8, 151.5, 130.9, 130.1, 129.4, 128.0, 127.0, 126.8, 126.5, 126.1, 119.8, 119.8, 69.8, 59.9, 57.9; MS (EI) *m*/*z* calcd 260 (M), found 260 (M, 25%), 228 (100), 210 (78), 197 (52), 181 (58), 153 (39), 141 (12), 128 (16), 115 (20), 105 (9), 91 (7), 76 (12), 61 (7), 40 (28); IR (KBr) ν 3411, 3215, 2995, 2920, 2869, 1590, 1462, 1443, 1385, 1230, 1071, 1016, 943, 840, 776, 750; UV (MeOH) λ_{max} 282, 243, 225; TLC R_f value 0.38 (DCM/MeOH 30:1).

3,3′-Bis-formylbiphenyl 2,2′-Diol (11). The title compound was obtained according to the previously published procedure[18] with the following modifications: After the MOM protection, purification was performed using column chromatography (dichloromethane) furnishing a yellow oil, yield 66%. Attempts to fully purify the formylated MOM-protected compound (column chromatography, dichloromethane/methanol gradient) failed, so purification had to be carried out after MOM deprotection employing column chromatography (dichloromethane) and preparative TLC (Chromatotron, petroleum ether/dichloromethane gradient) to obtain a yellowish solid, yield 48% over two steps. The anaytical data were identical with those reported.[18]

Bis(isopropylidene)-3,3′-bis(hydroxymethyl)biphenyl 2,2′-Diol (14). Under a nitrogen atmosphere, a 1.6 M solution of *n*-butyllithium in *n*-hexane (120 mL, 0.192 mol) was added to a solution of 3-bromosalicyl alcohol isopropylidene acetal **12** (46.5 g, 0.191 mol, obtained from 2-bromophenol **13** in three steps with 52% overall yield[19]) in THF (400 mL) at −80 °C and stirred for 1 h at the same temperature. The resulting mixture was added to a solution of iron(III) acetylacetonate (74.2 g, 0.210 mol) in THF (160 mL) at −80 °C. The reaction mixture was warmed to room temperature and stirred for 17 h at room temperature. The solvent was evaporated under reduced pressure and the residue dissolved in EtOAc and 2 N HCl. After separation of phases, the aqueous phase was extracted with EtOAc (2×). The combined organics were washed with 2 N HCl (4×), water (2×) and brine (2×), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The resulting crude product was purified by repeated column chromatography (petroleum ether/DCM gradient (25−100%)) to yield **14** (26.2 g, 84%) as a brown oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.05 (dd, J = 1.8, 7.5 Hz, 2H), 7.02 (dd, J = 1.5, 7.4 Hz, 2H), 6.93 (dd, J = 7.4, 7.5 Hz, 2H), 4.87 (s, 4H), 1.43 (s, 12H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 148.4, 130.1, 126.3, 124.4, 119.7, 99.4, 60.4, 24.9; MS (EI) *m*/*z* calcd 326 (M), found 326 (M, 25%), 268 (29), 269 (6), 211 (32), 210 (100), 183 (10), 182 (61), 181 (28), 154 (6), 153 (25), 152 (17), 115 (5), 91 (8), 43 (6); UV (MeOH) λ_{max} 282, 226; TLC R_f value 0.12 (petroleum ether/DCM 3:1).

3,3′-Bis(hydroxymethyl)-5,5′-dimethylbiphenyl 2,2′-Diol (16). 5,5′-Dimethylbiphenyl 2,2′-diol **17** (3.00 g, 13.8 mmol, obtained from 2-bromo-4-methylanisole **18** in two steps with 52% overall yield[21]) and sodium hydroxide (18.0 g, 450 mmol) were dissolved in 37% aqeous formaldehyde solution (200 mL) at 0 °C and stirred at 40 °C for 4 d. The reaction was monitored *via* TLC (DCM/MeOH 9:1) and quenched by addition of 10% HCl up to pH 4. The aqueous solution was extracted with EtOAc (5×). The combined organics were dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The resulting crude product was purified by filtration through a thin pad of silica (DCM/MeOH 9:1) and column chromatography (DCM/MeOH 19:1) to yield **16** (1.86 g, 48%) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.10 (d, J = 1.9 Hz, 2H), 6.88 (d, J = 1.9 Hz, 2H), 4.61 (s, 4H), 2.27 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 149.0, 130.3, 129.4, 128.2, 127.2, 126.6, 59.7, 20.5; MS (EI) *m*/*z* calcd 274 (M), found 274 (M, 23%), 256 (100), 238 (60), 225 (93), 210 (70), 195 (48), 182 (14), 165 (39), 152 (23), 141 (14), 128 (20), 115 (24), 104 (17), 91 (8), 77 (20), 65 (6), 40 (100); IR (KBr) ν 3277, 2917, 1472, 1355, 1220, 1198, 1045, 862, 641, 606; UV (MeOH) λ_{max} 291, 225; TLC R_f value 0.45 (DCM/MeOH 9:1).

Bis-saligenylchlorophosphite (19). Method I: General Procedure B with alcohol **10** (1.03 g, 4.11 mmol), PCl_3 (0.84 mL, 1.3 g, 9.6 mmol), pyridine (1.50 mL, 1.47 g, 18.6 mmol, dissolved in 7.5 mL Et_2O), Et_2O (100 mL), and a reaction temperature of −40 °C. The crude product **19** (938 mg) was obtained as a yellow oil and mixture of two diastereomers: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (d, J = 7.6 Hz, 4H), 7.08 (dd, J = 7.6, 7.6 Hz, 4H), 6.86 (d, J = 7.6 Hz, 4H), 5.60−4.85 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.7, 131.1, 127.7, 125.6, 123.5, 121.7, 121.5, 61.1; ^{31}P NMR (202 MHz, $CDCl_3$) δ 140.40.

Method II: General Procedure B with alcohol **10** (990 mg, 4.00 mmol), PCl_3 (0.85 mL, 1.3 g, 9.6 mmol), NEt_3 (2.55 mL, 1.85 g, 18.3 mmol), Et_2O (50 mL), and a reaction temperature of −40 °C. The crude product **19** (1.01 mg) was obtained as a yellow oil and mixture of two diastereomers. The anaytical data were identical with those reported above.

Bis-5-methyl-saligenylchlorophosphite (20). General Procedure B with alcohol **16** (1.72 g, 6.27 mmol), PCl_3 (1.32 mL, 2.07 g, 15.1 mmol), pyridine (2.33 mL, 2.28 g, 28.8 mmol, dissolved in 12 mL of Et_2O), Et_2O (155 mL), and a reaction temperature of −40 °C. The crude product **20** (1.54 g) was obtained as a colorless solid and mixture of two diastereomers. The compound was extremely poorly soluble in $CDCl_3$ and was therefore used for pronucleotide synthesis without further characterization.

Bis-saligenyl-*N*,*N*-diisopropylaminophosphoramidite (22). General Procedure C with crude chlorophosphite **19** (1.01 g, prepared using NEt_3 according to method II), diisopropylamine (1.80 mL, 1.30 g, 12.8 mmol, dissolved in 4 mL of Et_2O), and Et_2O (20 mL). The crude product **22** (1.14 g) was obtained as a colorless oil and mixture of two diastereomers (0.7:1.0): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (dd, J = 1.9, 7.3 Hz, 2H), 7.32 (dd, J = 2.0, 7.2 Hz, 2H), 6.97−6.91 (m, 8H), 5.18 (dd, J = 5.4, 13.4 Hz, 2H), 5.15 (dd, J = 5.3, 13.6 Hz, 2H), 4.93 (dd, J = 3.5, 13.4 Hz, 2H), 4.90 (dd, J = 3.9, 13.6 Hz, 2H), 3.63−3.53 (m, 8H), 1.19 (d, J = 6.9 Hz, 24H), 1.08 (d, J = 6.4 Hz, 12H), 1.07 (d, J = 6.7 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.1, 150.8, 131.8, 131.4, 128.3, 128.0, 124.8, 124.7, 120.4, 120.3, 65.1, 64.8 (d, J = 4.1 Hz), 44.6, 44.5, 25.1, 24.7; ^{31}P NMR (202 MHz, $CDCl_3$) δ 137.12, 136.28.

***N,N*-Diisopropyl-7-phenyl-2,3-dihydro-1,2-benzoxaphosphol-2-amine-2-oxide (23).** General Procedure C with crude chlorophosphite **21**[17] (1.13 g, prepared using pyridine according to method I), diisopropylamine (1.20 mL, 866 mg, 8.56 mmol, dissolved in 5 mL of Et_2O), Et_2O (20 mL), and a reaction period of 20 h. The crude product was purified by preparative TLC (Chromatotron, DCM). Instead of the desired product **22**, rearrangement product **23** (1.26 g, 3.83 mmol) was obtained as a colorless crystalline solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 7.6 Hz, 2H), 7.42 (dd, J = 7.6, 7.6 Hz, 2H), 7.35−7.33 (m, 2H), 7.21 (d, J = 7.6 Hz, 1H), 7.08 (dd, J = 7.6 Hz, 1H), 3.40−3.30 (m, 2H), 3.22 (dd, J = 18.3, 18.3 Hz, 1H), 3.01 (dd, J = 11.4, 18.3 Hz, 1H), 1.29 (d, J = 6.9 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.7, 129.2, 128.8, 128.2, 127.4, 126.1, 125.9, 122.7, 46.5 (d, J = 5.6 Hz), 28.0 (d, J = 111.9 Hz), 22.9, 22.3, 22.3; ^{31}P NMR (202 MHz, $CDCl_3$) δ 47.78; MS (EI) *m*/*z* calcd 329 (M), found 329 (M, 26%), 314 (54), 286 (14), 272 (100), 229 (19), 183 (15), 165 (9), 43 (7); IR (KBr) ν 2968, 2933, 1468, 1451, 1429, 1412, 1391, 1369, 1260, 1218, 1201, 1186, 1153, 1127, 1031, 1010, 888, 854, 778, 762, 703, 565; UV (MeOH) λ_{max} 282, 246; TLC R_f value 0.73 (DCM/MeOH 9:1).

Saligenylchlorophosphite (24). General Procedure B with salicyl alcohol (1.01 g, 8.10 mmol), PCl_3 (0.85 mL, 1.3 g, 9.5 mmol), NEt_3 (2.60 mL, 1.89 g, 18.7 mmol), Et_2O (30 mL), and a reaction temperature of −20 °C. The crude product **24** (1.30 g) was obtained as a yellow oil. The anaytical data were identical with those reported previously.[24]

Saligenyl-*N,N*-diisopropylaminophosphoramidite (25). General Procedure C with crude chlorophosphite **24** (757 mg, prepared using NEt_3 as described above), diisopropylamine (1.20 mL, 866 mg, 8.56 mmol, dissolved in 4 mL of Et_2O), and Et_2O (9 mL). The crude product **25** (1.04 g) was obtained as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11−7.06 (m, 1H), 6.89−6.79 (m, 3H), 5.05 (dd, J = 4.1, 14.5 Hz, 1H), 4.78 (dd, J = 4.2, 14.5 Hz, 1H), 3.60−3.50 (m, 2H), 1.19−1.13 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.2 (d, J = 4.2 Hz), 129.2, 128.5, 125.2, 121.0, 119.0, 64.2 (d, J = 4.7 Hz), 44.2, 44.1, 24.7 (d, J = 7.6 Hz), 24.5 (d, J = 8.2 Hz); ^{31}P NMR (202 MHz, $CDCl_3$) δ 137.04.

Bis-saligenyl-*N,N*-diethylaminophosphoramidite (26). General Procedure C with crude chlorophosphite **19** (1.37 g, prepared using NEt_3 according to method II), diethylpropylamine (1.77 mL, 1.25 g, 17.1 mmol, dissolved in 5 mL of Et_2O), and Et_2O (27 mL). The crude product **26** (1.46 g) was obtained as a colorless oil and mixture of 2 diastereomers (1.0:0.7): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.30 (m, 4H), 7.02−6.97 (m, 8H), 5.11−5.04 (m, 4H), 4.97−4.89 (m, 4H), 3.11−3.04 (m, 16H), 1.01 (t, J = 6.9 Hz, 12H), 1.01 (t, J = 6.9 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.9, 149.8 (d, J = 6.1 Hz), 131.3, 131.2, 127.8, 127.7, 125.6, 125.5, 124.6 (d, J = 6.1 Hz), 120.5, 120.4, 64.0, 63.7, 38.0, 37.8, 14.8, 14.8; ^{31}P NMR (202 MHz, $CDCl_3$) δ 131.03, 130.55.

(R_P)-3-Sal-*cyclo*Sal-d4TMP (29 (R_P)). General Procedure A with pronucleotide **33** (R_P) (166 mg, 0.299 mmol), Dowex 50X8 (8.3 g), DCM/MeOH 1:1 (14 mL), and a reaction period of 8 d. The reaction was monitored *via* analytical HPLC (see Supporting Information). Purification was carried out by preparative HPLC ($MeCN/H_2O$ 2:5) to yield **29** (R_P) (84 mg, 54%) as a colorless foam: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.35 (s, 1H), 8.61 (s, 1H), 7.31−7.24 (m, 4H), 7.18 (q, J = 1.1 Hz, 1H), 7.02 (dd, J = 1.5, 7.5 Hz, 1H), 6.89 (dd, J = 7.5, 7.5 Hz, 1H), 6.82−6.80 (m, 1H), 6.31 (ddd, J = 1.6, 1.6, 6.0 Hz, 1H), 5.97−5.95 (m, 1H), 5.55 (dd, J = 14.3, 14.3 Hz, 1H), 5.51 (dd, J = 5.4, 5.4 Hz, 1H), 5.45 (dd, J = 14.3, 14.3 Hz, 1H), 4.96−4.93 (m, 1H), 4.65 (d, J = 5.4 Hz, 2H), 4.28 (ddd, J = 2.5, 6.4, 11.2 Hz, 1H), 4.22 (ddd, J = 4.1, 6.9, 11.2 Hz, 1H), 1.60 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9, 152.2, 150.9, 148.4, 135.8, 132.9, 132.0, 129.6, 128.7, 127.6, 127.5, 125.4, 124.1, 123.7, 121.8, 121.7, 119.2, 109.9, 89.2, 84.3 (d, J = 8.1 Hz), 68.5 (d, J = 7.1 Hz), 68.5 (d, J = 7.0 Hz), 60.2, 11.9; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.88; MS (FAB) calcd 515.1 ($M + H^+$), found 515.2 ($M + H^+$); HR−MS (ESI^+) *m*/*z* calcd 537.1039 ($M + Na^+$), found 537.1048 ($M + Na^+$); UV (HPLC) λ_{max} 269; TLC R_f value 0.38 (DCM/MeOH 9:1); Preparative HPLC t_R = 13.8 min ($MeCN/H_2O$ 2:5).

(S_P)-3-Sal-*cyclo*Sal-d4TMP (29 (S_P)). General Procedure A with pronucleotide **33** (S_P) (181 mg, 0.326 mmol), Dowex 50X8 (9.1 g), DCM/MeOH 1:1 (15 mL), and a reaction period of 8 d. The reaction was monitored *via* analytical HPLC (see Supporting Information). Purification was carried out by preparative HPLC ($MeCN/H_2O$ 2:5) to yield **29** (S_P) (76 mg, 46%) as a colorless foam: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 1H), 8.60 (s, 1H), 7.32−7.23 (m, 4H), 7.21 (q, J = 0.9 Hz, 1H), 7.03 (d, J = 7.5 Hz, 1H), 6.91 (dd, J = 7.5, 7.5 Hz, 1H), 6.83−6.81 (m, 1H), 6.31−6.28 (m, 1H), 6.02−6.00 (m, 1H), 5.53 (dd, J = 14.4, 14.4 Hz, 1H), 5.49 (dd, J = 5.7, 5.7 Hz, 1H), 5.44 (dd, J = 14.4, 14.4 Hz, 1H), 4.94−4.91 (m, 1H), 4.65 (d, J = 5.7 Hz, 2H), 4.28−4.20 (m, 2H), 1.65 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9, 152.1, 150.9, 147.5 (d, J = 7.1 Hz), 135.9, 133.0, 132.0, 129.6, 128.9, 127.6, 127.5, 125.4, 124.1, 123.7, 121.8, 121.7, 119.3, 109.9, 89.3, 84.3 (d, J = 7.6 Hz), 68.6 (d, J = 6.3 Hz), 68.3 (d, J = 6.5 Hz), 60.1, 12.0; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.98; MS (FAB) calcd 515.1 ($M + H^+$), found 515.2 ($M + H^+$); UV (HPLC) λ_{max} 269; TLC R_f value 0.38 (DCM/MeOH 9:1); Preparative HPLC t_R = 14.1 min ($MeCN/H_2O$ 2:5).

3-(*O*-(*tert*-Butyldiphenylsilyl)hydroxymethyl)-3′-(hydroxymethyl)biphenyl 2,2′-Diol (30). Under a nitrogen atmosphere, NEt_3 (2.41 mL, 1.75 g, 17.3 mmol), a catalytic amount of DMAP, and TBDPS chloride (2.25 mL, 2.38 g, 8.65 mmol) were added to a solution of alcohol **10** (2.84 g, 11.5 mmol) in THF (50 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred at room temperature for 2 h. The reaction was monitored *via* TLC (DCM/MeOH 30:1) and quenched by addition of MeOH (10 mL) and stirring at room temperature for 5 min. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and water. After separation of the phases, the aqueous solution was extracted with EtOAc (3×). The combined organics were washed with water (1×) and dried over anhydrous Na_2SO_4, and the solvent evaporated under reduced pressure. The resulting crude product was purified by column chromatography (DCM/MeOH gradient (0−10%)) to yield **30** (2.98 g, 53%) as a yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72−7.69 (m, 4H), 7.54−7.45 (m, 7H), 7.28 (dd, J = 1.7, 7.6 Hz, 1H), 7.10 (dd, J = 1.8, 7.6 Hz, 1H), 7.07 (dd, J = 1.5, 7.6 Hz, 1H), 7.03 (dd, J = 7.4, 7.6 Hz, 1H), 6.93 (dd, J = 7.6, 7.6 Hz, 1H), 4.85 (s, 2H), 4.63 (s, 2H), 1.09 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.5, 150.5, 135.2, 133.1, 130.2, 130.1, 130.1, 129.4, 128.9, 128.2, 126.9, 126.7, 126.4, 120.1, 119.9, 119.8, 61.5, 59.9, 26.9, 19.2; UV (MeOH) λ_{max} 284, 224; TLC R_f value 0.57 (DCM/MeOH 30:1). Byproduct 3,3′-bis-(*O*-(*tert*-butyldiphenylsilyl)-hydroxymethyl)biphenyl 2,2′-diol **31** (904 mg, 11%) could also be isolated as a yellowish oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71−7.68 (m, 8H), 7.56−7.52 (m, 2H), 7.52−7.44 (m, 12H), 7.10 (dd, J = 1.6, 7.5 Hz, 2H), 7.02 (dd, J = 7.5, 7.5 Hz, 2H), 4.83 (s, 4H), 1.08 (s, 18H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 150.7, 135.2, 133.1, 130.2, 130.1, 129.4, 128.2, 127.7, 125.5, 120.0, 61.5, 26.9, 19.1; UV (MeOH) λ_{max} 282, 244, 224; TLC R_f value 0.77 (DCM/MeOH 30:1).

3-(*O*-(*tert*-Butyldiphenylsilyl)-hydroxymethyl)-3′-(hydroxymethyl)biphenyl 2,2′-Diol 2′,3′-*O*-Isopropylideneacetal (31). A mixture of salicyl alcohol **30** (2.96 g, 6.11 mmol), 2,2-dimethoxypropane (3.9 mL, 3.3 g, 32 mmol), *p*-TsOH (120 mg, 0.631 mmol), and anhydrous Na_2SO_4 (2.5 g) in acetone (120 mL) was stirred at 40 °C for 3 d. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and water. After separation of the phases, the aqueous solution was extracted with EtOAc (2×). The combined organics were washed with water (1×) and dried over anhydrous Na_2SO_4, and the solvent evaporated under reduced pressure. The resulting crude product was purified by column chromatography (petroleum ether/DCM 1:1) to yield **32** (1.92 g, 60%) as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.71−7.68 (m, 4H), 7.52−7.44 (m, 7H), 7.09−7.02 (m, 3H), 6.97 (dd, J = 7.4, 7.6 Hz, 1H), 6.95 (dd, J = 7.4, 7.6 Hz, 1H), 4.87 (s, 2H), 4.83 (s, 2H), 1.42 (s, 6H), 1.08 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 150.9, 148.5, 135.2, 133.1, 130.3,

130.2, 130.1, 128.5, 128.2, 126.6, 125.7, 124.4, 120.1, 120.0, 119.5, 99.6, 61.7, 60.4, 26.9, 24.8, 19.1; MS (ESI$^+$) *m*/*z* calcd 547.2 (M + Na$^+$), found 547.4 (M + Na$^+$); UV (MeOH) λ_{max} 284, 244, 224; TLC R_f value 0.83 (DCM/MeOH 30:1).

3-Isopr-Sal-*cyclo*Sal-d4TMP (33). To a solution of silylated alcohol **32** (1.89 g, 3.60 mmol) in MeOH (60 mL) was added NH_4F (1.34 g, 36.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 61 h and the reaction monitored *via* TLC (DCM/MeOH 30:1). The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and water. After separation of the phases, the aqueous solution was extracted with EtOAc (3 ×). The combined organics were washed with water (1×) and dried over anhydrous Na_2SO_4, and the solvent evaporated under reduced pressure. The resulting crude product was purified by preparative TLC (Chromatotron, petroleum ether/DCM gradient (0−100%)) to yield the deprotected salicyl alcohol derivative (915 mg, 89%) as a yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.24−7.22 (m, 1H), 7.10−7.08 (m, 1H), 7.07−7.04 (m, 1H), 7.01 (dd, J = 1.8, 7.4 Hz, 1H), 6.95 (dd, J = 7.3, 7.6 Hz, 1H), 6.86 (dd, J = 7.4, 7.6 Hz, 1H), 5.41 (s, 1H), 4.88 (s, 2H), 4.63 (s, 2H), 1.44 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.0, 148.4, 130.3, 130.0, 128.9, 126.9, 126.7, 125.4, 124.1, 119.9, 119.9, 119.0, 99.5, 60.4, 60.1, 24.8; MS (EI) *m*/*z* calcd 286 (M), found 286 (M, 4%), 228 (100), 210 (54), 198 (40), 182 (55), 153 (28), 139 (6), 128 (9), 115 (14), 91 (8), 77 (6), 43 (20); UV (MeOH) λ_{max} 283, 242, 225; TLC R_f value 0.51 (DCM/MeOH 30:1).

Chlorophosphite synthesis was carried out using General Procedure B with the deprotected salicyl alcohol derivative (896 mg, 3.13 mmol), PCl_3 (0.32 mL, 0.50 g, 3.6 mmol), pyridine (0.56 mL, 0.55 g, 7.0 mmol, dissolved in 2.5 mL of Et_2O), Et_2O (30 mL), and a reaction temperature of −40 °C. The crude chlorophosphite (696 mg) was obtained as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28−7.25 (m, 1H), 7.16−7.11 (m, 2H), 7.02−6.95 (m, 3H), 5.54 (d, J = 13.2 Hz, 1H), 5.12−5.04 (m, 1H), 4.96−4.89 (m, 2H), 1.55 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.8, 143.8 (d, J = 8.1 Hz), 131.5, 130.1, 129.2 (d, J = 2.0 Hz), 125.4, 125.0 (d, J = 2.0 Hz), 124.5, 123.3, 121.3 (d, J = 13.2 Hz), 119.7, 119.5, 99.9, 61.4, 61.0, 25.3, 24.2; ^{31}P NMR (202 MHz, $CDCl_3$) δ 142.42.

Pronucleotide synthesis was carried out using General Procedure D with d4T **1** (354 mg, 1.58 mmol), crude chlorophosphite (665 mg), DIPEA (0.41 mL, 0.30 g, 2.3 mmol), 5−6 M solution of *tert*-butyl hydroperoxide in *n*-decane (0.95 mL, ≥4.8 mmol), and MeCN (36 mL). The product **33** (525 mg, 60%) was obtained as a colorless foam and mixture of two diastereomers (1.0:0.9): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 2H), 7.31−7.24 (m, 6H), 7.17−7.06 (m, 6H), 6.99 (dd, J = 7.5, 7.5 Hz, 1H), 6.97 (dd, J = 7.5, 7.5 Hz, 1H), 6.81−6.79 (m, 2H), 6.32−6.30 (m, 1H), 6.26−6.24 (m, 1H), 6.02−5.99 (m, 1H), 5.95−5.92 (m, 1H), 5.57−5.51 (m, 2H), 5.47−5.41 (m, 2H), 4.94−4.86 (m, 6H), 4.30−4.17 (m, 4H), 1.58 (s, 3H), 1.52 (s, 3H), 1.46 (s, 3H), 1.45 (s, 3H), 1.42 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9, 163.9, 150.9, 148.4, 148.4, 147.3 (d, J = 2.0 Hz), 147.2, 135.9, 135.7, 132.9, 132.9, 131.9, 131.9, 129.8, 127.6, 127.5, 125.7, 125.7, 125.3, 124.2−124.1 (m), 121.7, 121.6, 121.5, 120.1, 120.0, 119.9, 110.0, 109.9, 99.8, 89.3, 89.3, 84.3, 68.6 (d, J = 6.1 Hz), 68.3 (d, J = 5.1 Hz), 68.2 (d, J = 6.1 Hz), 60.3, 25.1, 24.8, 24.3, 24.0, 11.9, 11.8; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.62, −7.71; MS (FAB) *m*/*z* calcd 555.2 (M + H$^+$), found 555.2 (M + H$^+$); HR−MS (ESI$^+$) *m*/*z* calcd 577.1352 (M + Na$^+$), found 577.1359 (M + Na$^+$); UV (HPLC) λ_{max} 267, 248; TLC R_f value 0.52 (DCM/MeOH 9:1). Preparative HPLC (MeCN/H_2O 2:5) allowed separation of the two diastereomers to furnish **33 (R_P)** (192 mg) and **33** (S_P) (206 mg) as colorless foams. Analytical data of **33 (R_P)**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.35 (s, 1H), 7.30−7.24 (m, 3H), 7.13−7.06 (m, 3H), 6.97 (dd, J = 7.5, 7.5 Hz, 1H), 6.81−6.79 (m, 1H), 6.32−6.30 (m, 1H), 5.95−5.92 (m, 1H), 5.54 (dd, J = 14.3, 17.9 Hz, 1H), 5.43 (dd, J = 10.6, 14.3 Hz, 1H), 4.96−4.92 (m, 1H), 4.88 (s, 2H), 4.30−4.25 (m, 1H), 4.19 (ddd, J = 4.9, 6.1, 11.0 Hz, 1H), 1.52 (s, 3H), 1.45 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.8, 150.9, 148.4, 147.2, 135.7, 132.9, 131.9, 129.8, 127.5, 125.7, 125.3, 124.2, 124.2, 121.7, 121.6, 120.0, 119.9, 109.9, 99.8, 89.3, 84.2 (d, J = 7.7 Hz), 68.5 (d, J = 7.0 Hz), 68.3 (d, J = 7.3 Hz), 60.3, 24.8, 24.3, 11.8; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.62; Preparative HPLC t_R = 38.2 min (MeCN/H_2O 2:5). Analytical data of **33 (S_P)**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 1H), 7.31−7.24 (m, 3H), 7.17−7.08 (m, 3H), 6.99 (dd, J = 7.5, 7.5 Hz, 1H), 6.81−6.79 (m, 1H), 6.26−6.24 (m, 1H), 6.02−5.99 (m, 1H), 5.54 (d, J = 14.2, 14.2 Hz, 1H), 5.45 (dd, J = 14.2, 14.2 Hz, 1H), 4.93−4.86 (m, 3H), 4.21 (dd, J = 4.3, 7.1 Hz, 2H), 1.58 (s, 3H), 1.47 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9, 150.8, 148.4, 147.2 (d, J = 7.1 Hz), 135.8, 132.8, 131.9, 129.8, 127.5, 125.7, 125.3, 124.2, 124.1, 121.6, 121.5, 120.1, 120.0, 109.9, 99.8, 89.3, 84.2 (d, J = 7.2 Hz), 68.6 (d, J = 6.2 Hz), 68.2 (d, J = 6.1 Hz), 60.3, 25.1, 24.0, 11.8; ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.72; Preparative HPLC t_R = 41.1 min (MeCN/H_2O 2:5).

Hydrolysis Studies. Hydrolytic stabilities were determined and ^{31}P NMR hydrolysis experiments carried out as described previously.[17]

BChE Assay. The procedure for the BChE assay using human serum has been described before.[12]

Antiviral Activity and Cytotoxicity. Antiviral activities and cytotoxicities were determined as published previously.[6]

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft, Germany, and the "Geconcerteerde Onderzoeksacties (GOA nr. 019/05) for financial support. C. D. is grateful to a Ph. D. fellowship from the Fonds der Chemischen Industrie/BMBF, Germany. The authors also thank Mrs. Ann Absillis for excellent technical assistance.

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

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JM0611713