Doubly Loaded *cyclo*Saligenyl-Pronucleotides – 5,5′-Bis-(*cyclo*Saligenyl-2′,3′-dideoxy-2′,3′-didehydrothymidine Monophosphates)

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Recently, we reported on 3,3′-bis-(*cyclo*Saligenyl-2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphates) (3,3′-bis-(*cyclo*Sal-d4TMPs) **4**) as the first pronucleotides with a mask-to-drug ratio of 1:2 that is still a novelty in the field of pronucleotides. Here, we report on a new set of compounds of these unique type of *cyclo*Saligenyl prodrugs **5** that bear a biaryl axis at the 5-position of the *cyclo*Sal residue. All compounds **5** showed pronounced in vitro activity against HIV-1 and HIV-2 in wild-type CEM cell cultures and better retained their antiviral activities in thymidine kinase-deficient CEM cells than the compound **4** series. Moreover, compound **5b** is the first bis-(*cyclo*Sal-d4TMP) that even showed complete retention of antiviral activity in TK-deficient CEM cells. The complex hydrolysis behavior of **5** was investigated, and the proposed hydrolysis mechanism was proven by means of ^{31}P NMR spectroscopy and HPLC analysis.

Introduction

The action of antiviral or antitumor active nucleoside analogues is highly dependent on the efficient stepwise conversion into the bioactive 5′-triphosphates via mono- and diphosphate formation mediated by cellular kinases. In the case of the anti-HIV nucleoside 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T **1**, Figure 1), the first phosphorylation to its nucleotide (d4TMP **2**, Figure 1) by the salvage pathway enzyme thymidine kinase (TK) is the anabolism-limiting step.[1] To overcome this bottleneck, pronucleotide systems for the selective intracellular delivery of nucleotides have been developed.[2] Among these approaches, the *cyclo*Sal[a] concept has been established as the only system that releases nucleoside monophosphates by a pure chemical hydrolysis mechanism.[3] Moreover, *cyclo*Sal-pronucleotides (general structure: **3**, Figure 1) are unique because they display a mask-to-drug ratio of 1:1 while all other pronucleotide systems have a higher ratio (2:1 to 4:1).[2]

To improve the number of equivalents of released nucleotide per masking unit (mask-to-drug ratio of 1:2), we recently developed "dimeric" *cyclo*Sal pronucleotides **4** (Figure 1) using the antiviral active nucleoside analogue d4T (**1**) for initial studies.[4] The 3,3′-bis-(*cyclo*Sal-d4TMPs) (**4**) showed good anti-HIV activity in wild-type CEM cells and were significantly more active than **1** in TK-deficient CEM cells (Table 2) although the antiviral activity was not fully retained.

In the synthesis of compounds **4** a major problem was the formation of a side product in considerable amounts. This compound was formed during the formation of the bis-saligenyl chlorophosphites starting from the corresponding tetrols. As a consequence, the following reaction of the crude chlorophosphites with d4T (**1**) led to compounds **4** in only poor yields (8% (**4a**), 11% (**4b**)).

Because of the nonstereoselective synthesis, the *cyclo*Sal-pronucleotides **3** were always obtained as mixtures of two diastereomers (R_P and S_P configuration).[3] Therefore, bis-(*cyclo*Sal-d4TMPs) as **4** were formed as mixtures of three diastereomers (R_P,R_P; R_P,S_P; S_P,S_P) in a theoretical ratio of 1:2:1. However, this ratio was not observed. For example, **4a** was isolated in a diastereomeric ratio of 1:2:2.[4] In conclusion, there may be a steric interaction between the two *cyclo*Sal-d4TMP units in 3,3′-bis-*(cyclo*Sal-d4TMPs) **4** that led to some extent to stereodifferentiation.

Here, we report on the synthesis, properties, and antiviral evaluation of bis-(*cyclo*Sal-d4TMPs) linked via the two 5-positions of the *cyclo*Sal-residue. These 5,5′-bis-(*cyclo*Sal-d4TMPs) **5** (Figure 1) were expected to have two sterically independent *cyclo*Sal-d4TMP units. Moreover, a detailed investigation of the complex hydrolysis pathway of **5a** was carried out.

Results and Discussion

Chemistry. For the synthesis of 5,5′-bis-(*cyclo*Sal-d4TMPs) **5** one of the synthetic routes developed for the synthesis of the 3,3′-counterpart **4a** also proved to be suitable after a few modifications.[4] Starting from 4-bromosalicylisopropylidene acetals **6a**−**c**,[5] a halogen−lithium exchange was performed, and the resulting intermediate was used for an oxidative homocoupling with iron(III) acetylacetonate to yield the respective bis-(isopropylidene)-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diols **7a**−**c** in moderate to good yields (37−75%). For the acidic deprotection of acetals **7** our previously reported ultrafast method with hydrochloric acid[5a] was used to yield biphenyl tetrols **8a**−**c**. These reactions are summarized in Scheme 1.

Target molecules **5a**−**c** were synthesized using the method via saligenyl chlorophosphites (**9a**−**c**, Scheme 2).[6] These phosphitylating agents were synthesized in yields of about 54−55% with moderate purities (purity grade, 59−68%; impurity: pyridine hydrochloride) from tetrols **8**. Subsequent treatment of crude chlorophosphites **9a**−**c** with d4T (**1**) and oxidation with *tert*-butyl hydroperoxide (*t*-BuOOH) led to the desired 5,5′-bis-(*cyclo*Sal-d4TMPs) **5a**−**c** (28−37%). Nevertheless, the yields obtained here are 4- to 5-fold higher compared to their 3,3′-bis-(*cyclo*Sal)-counterparts.

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[a] Abbreviations: *cyclo*Sal, *cyclo*Saligenyl; DTBS, di-*tert*-butylsilyl; Fe(acac)$_3$, iron(III) acetylacetonate.

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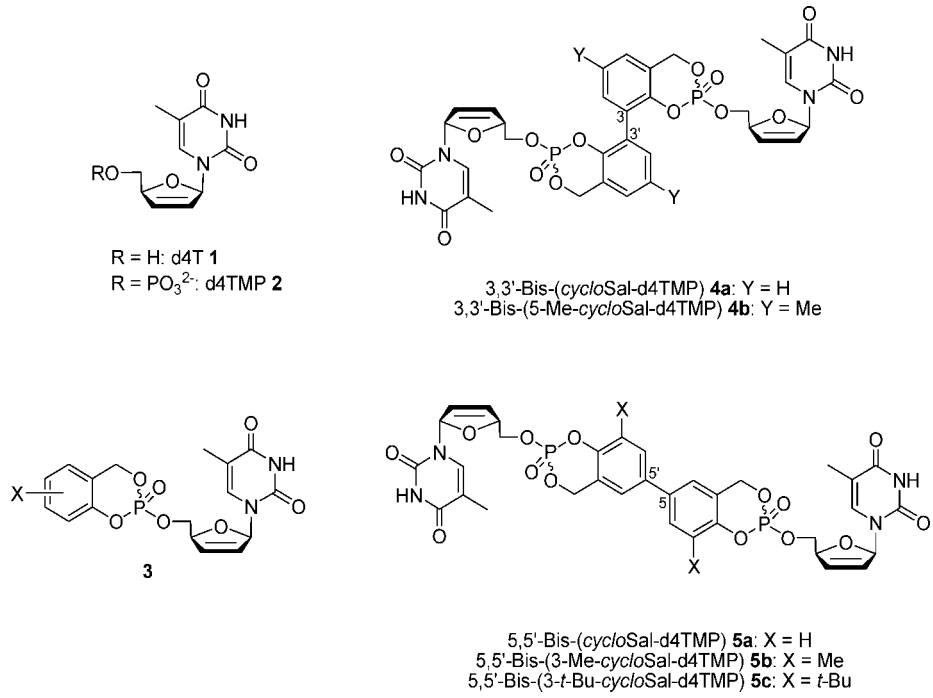
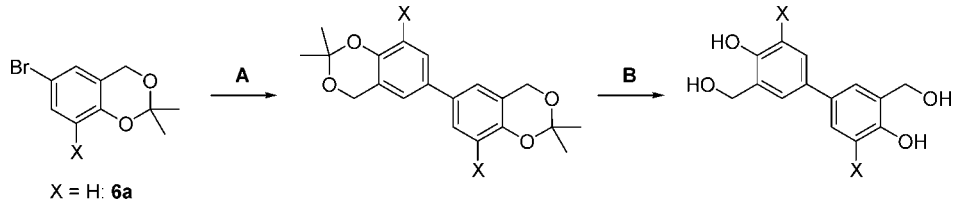


Figure 1. Structures of d4T **1**, d4TMP **2**, "monomeric" *cyclo*Sal-d4TMP **3**, and bis-(*cyclo*Sal-d4TMPs) **4** and **5**.

Scheme 1. Synthesis of Biphenyl tetrols **8** Starting from Acetals **6**[a]



[a] Reagents and conditions: Method A: (i) *n*-BuLi, THF, −80 °C, 1 h; (ii) $Fe(acac)_3$, THF, −80 °C to room temp, 36−42 h. Method B: conc HCl, CH_3CN/H_2O/THF), heat, 30 s or 3 min.

For **5c**, Oxone (2 $KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) was tested as an alternative oxidation reagent.[7] This attempt resulted in a comparable yield of **5c** of 30%. For all bis-pronucleotides **5**, final purification was achieved by preparative HPLC.

In the case of **5c**, all three diastereomers could be separated and were isolated in the theoretically expected ratio of 1:2:1. The first (**5c**-*fast*) and the slowest eluting diastereomer (**5c**-*slow*) showed only one signal in the ^{31}P NMR spectra (−8.83 ppm (*fast*) and −8.59 ppm (*slow*)), while for **5c**-*middle* two signals were observed in the spectra. This points to the fact that **5c**-*middle* is the (R_P,S_P)-isomer. Moreover, both diastereomers of **5c** with identically configurated phosphorus atoms also showed identical signals in the ^1H NMR for both *cyclo*Sal-d4TMP units. ^1H NMR spectra of **5c**-*middle* appears like the formal "sum" of the spectra of **5c**-*fast* and **5c**-*slow*. Diastereomers of **5a** could only be partially separated; the first eluting peak contained two diastereomers, while the slow-eluting compound was identical to **5a**-*slow*. NMR spectra of these diastereomers led to the same conclusions as made for **5c**. The separation of the diastereomers of **5b** was not possible.

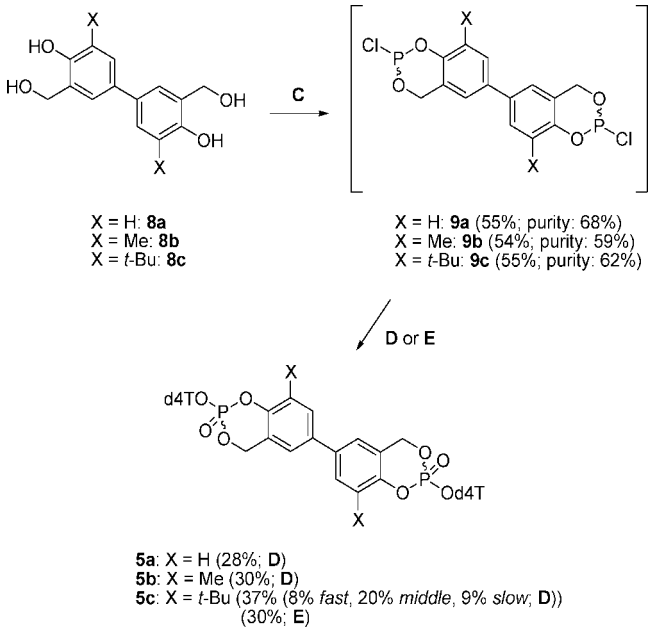
Hydrolysis Studies. Compounds **5a**−**c** were studied for their hydrolytic stability at pH 7.3 (physiological) and pH 7.6 in aqueous 25 mM phosphate buffer (PBS) and in the incubation medium for the antiviral tests (RPMI/heat-inactivated FCS (10%), pH 7.6). All given half-lives refer to the disappearance of starting pronucleotides and are summarized in Table 1. For comparison, the corresponding data of triesters **4a**,**b** are given as well.

Hydrolysis studies at physiological pH 7.3 revealed that 5,5′-bis-(*cyclo*Sal-d4TMPs) **5a**−**c** are less stable against chemical hydrolysis than their 3,3′-counterparts **4a**,**b**. This has been expected from the earlier reported data of phenyl- or methyl-substituted *cyclo*Sal-d4TMPs (for X (in **3**, Figure 1) = 3-Ph, $t_{1/2}$ = 5.1 h; for X = 5-Ph, $t_{1/2}$ = 3.1 h; for X = 3-Me, $t_{1/2}$ = 15 h; for X = 5-Me, $t_{1/2}$ = 5.1 h).[8]

Additional alkyl substituents in the aromatic rings of **5** led to a marked increase of the hydrolysis half-life with the *tert*-butyl group having a significantly stronger influence than the methyl group. For the unsubstituted compounds **4a** and **5a** a remarkable influence of the stereochemistry at the phosphorus centers was observed.

Interestingly, the unsubstituted compound **5a** showed a decrease in hydrolysis stability in RPMI/FCS (10%). While the half-lives of **5a**-*mix* and **5a**-*fast* dropped 2-fold, the half-life of **5a**-*slow* decreased from 3.3 to 1.0 h. This can be clearly attributed to the more basic pH 7.6 as can be seen from the values determined in PBS (pH 7.6). In contrast, the half-life of the methyl-substituted **5b** was identical in all three media ($t_{1/2}$ = 2.2−2.3 h). The half-lives of **5c** in PBS (pH 7.6) were only slightly shorter than in PBS (pH 7.3) but are significantly increased in RPMI/FCS (10%). For all samples of **5c** (X= *t*-Bu)

Scheme 2. Synthesis of 5,5′-Bis-(*cyclo*Sal-d4TMPs) **5**[a]



[a] Reagents and conditions. Method C: (i) PCl_3, Et_2O(THF), −40 °C, 10 min; (ii) Et_2O, pyridine, −40 °C, 3 h; (iii) room temp, 1 h. Method D: (i) d4T (**1**), DIPEA, CH_3CN, −20 °C to room temp, 1−1.5 h; (ii) *t*-BuOOH, −20 °C to room temp, 1 h. Method E: (i) d4T (**1**), DIPEA, CH_3CN, −20 °C to room temp, 1 h; (ii) Oxone in H_2O, −10 °C to room temp, 10 min.

Table 1. Hydrolysis Data of **5a**−**c** and **13** Compared to **4a**,**b**

		$t_{1/2}$ (h)[a]		
		PBS[b]		
triester		pH 7.3	pH 7.6	RPMI/FCS[c]
5a (*mix*)	5,5′-(H)	1.7	0.5	0.8
5a (*fast*)	5,5′-(H)	1.0	0.4	0.6
5a (*slow*)	5,5′-(H)	3.3	0.7	1.0
5b (*mix*)	5,5′-(Me)	2.2	2.3	2.3
5c (*mix*)	5,5′-(*t*-Bu)	4.7	4.1	8.3
5c (*fast*)	5,5′-(*t*-Bu)	4.3	3.9	7.9
5c (*middle*)	5,5′-(*t*-Bu)	4.7	4.1	8.0
5c (*slow*)	5,5′-(*t*-Bu)	5.3	4.4	9.1
13 (*mix*)	5-Sal[d]	2.2	2.0	2.1
4a (*mix*)	3,3′-(H)	8.2	nd[e]	nd[e]
4a (*fast*)	3,3′-(H)	3.2	nd[e]	nd[e]
4a (*slow*)	3,3′-(H)	8.5	nd[e]	nd[e]
4b (*mix*)	3,3′-(Me)	12	nd[e]	nd[e]

[a] Hydrolysis half-lives. [b] 25 mM phosphate buffer. [c] RPMI/10% heat-inactivated fetal calf serum (FCS), pH 7.6. [d] 5-(Saligen-5′-yl)-*cyclo*Sal-d4TMP. [e] nd: not determined.

the half-life in cell culture medium is approximately doubled compared to the half-life in PBS (pH 7.6). It is important to mention that the final products in all hydrolyses were exclusively d4TMP and the respective tetrol **8**.

Hydrolysis Pathway. To get further insight in the hydrolysis behavior of such 5,5′-bis-(*cyclo*Sal-d4TMPs) **5**, the chemical hydrolysis pathway of **5a** was studied in detail. The hydrolysis of **5a** may proceed via different pathways. Triester **5a** can be hydrolyzed, either leading to bis(benzyl phosphate diester) **10** or leading to benzyl phosphate ester **11**. From the labile intermediate **10**, 2 equiv of d4TMP **2** can be released either simultaneously (direct release of **2** and **8a**) or consecutively (release of **2** and **8a** via benzyl phosphate ester **12**). If the hydrolysis takes place via **11**, 5-(saligen-5′-yl)-*cyclo*Sal-d4TMP **13** has to be formed. Then **2** and **8a** would be released via **12**. All these theoretically possible hydrolytic routes are shown in Scheme 3.

The only stable intermediate in this hydrolysis cascade is compound **13**. In order to use it as reference, a route for its preparation was developed. Analogous studies have already been made for the hydrolysis of **4a**. However, there the corresponding hydrolysis intermediate was neither detectable in the ^{31}P NMR spectra nor detectable in HPLC analysis.[4]

Synthesis of **13** was achieved starting from 4-bromosalicyl alcohol **14**.[5a] Compound **14** was protected using the di-*tert*-butylsilyl protecting group (DTBS) to give **15** (81% yield).[9] Then **15** was reacted with 4-bromosalicyl alcohol isopropylidene acetal **6a** (1:1 mixture) using the conditions of the oxidative coupling described above. This reaction resulted in heterocoupling product **16** in 31% yield as well as in both homocoupling products **7a** (31% yield) and **17** (46% yield). The last two compounds can be deprotected in excellent yields (**7a**, 89%; **17**, 88%) to tetrol **8a** and then can be used as starting material in further syntheses, for example, for compound **5a** (Scheme 2). These reactions are depicted in Scheme 4.

The DTBS group of **16** was removed selectively with triethylamine trihydrofluoride ($NEt_3 \cdot 3HF$) to give salicyl alcohol **18** (96% yield). Subsequently, isopropylidene-protected pronucleotide **19** (5-(isopropylidenesaligen-5′-yl)-*cyclo*Sal-d4TMP, 45% yield) was prepared via the chlorophosphite. Finally, acidic deprotection of **19** led to reference compound **13** (85% yield). These steps are also summarized in Scheme 4.

The hydrolysis stability of **13** was analyzed as described above for bis-(*cyclo*Sal-d4TMPs) **5** (Table 1). At the physiological pH, reference compound **13** was found to be slighly more stable ($t_{1/2}$ = 2.2 h; Table 1) in comparison to **5a**-*mix* ($t_{1/2}$ = 1.7 h). Interestingly, for compound **13** no influence of the more basic pH was found here ($t_{1/2}$(PBS, pH 7.6) = 2.0 h; $t_{1/2}$(RPMI/FCS) = 2.1 h). Again, as with triester **5**, compound **13** hydrolyzed selectively to d4TMP **2**. The formation of **13** during the hydrolysis of bis-(*cyclo*Sal-d4TMP) **5a**-*mix* was clearly proven by HPLC analysis. In Figure 2 the HPL-chromatograms of the hydrolysis of **5a**-*mix* at the beginning (top) and after an incubation time of 1 h at 37 °C as well as the reference chromatogram of **13** (bottom) are shown. Obviously, after 1 h a significant amount of **13** was formed. With the used HPLC method (method I; Supporting Information) polar compounds like d4TMP **2** (with two negative charges) are eluted together with the injection peak from the RP column. A signal for the possible bis(benzyl phosphate diester) **10** would appear here, too. But because of a possible overlapping with the signals of **2** and DMSO, it cannot be clearly determined whether this intermediate is formed or not. The intermediates **11** and **12** with only one negative charge should elute later compared to **10**. Signals a−c in Figure 2 are increasing at the beginning but are disappearing during the course of hydrolysis (data not shown). So, these signals should result from such intermediates. Signal a could be attributed to triol **12** (Scheme 3) because this intermediate was present in the hydrolysis of **13,** too (see C in Figure S1 in Supporting Information). Accordingly, signals b and c should be attributable to the two formed diastereomers (R_P and S_P) of intermediate **11**. The small signal d corresponds to the formed tetrol **8a** (see A in Figure S1 in Supporting Information).

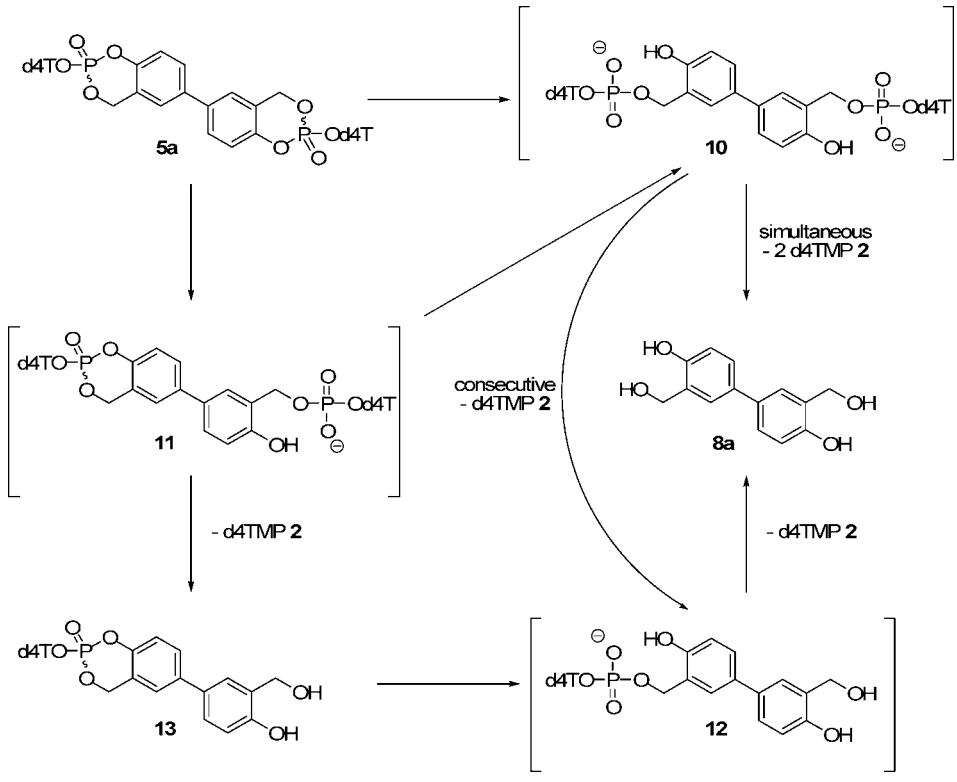
Because a simultaneous hydrolysis pathway could not be ruled out completely by this method, further analysis using ^{31}P NMR spectroscopy (NMR hydrolysis in imidazole/HCl buffer, pH 7.3, 37 °C) was performed. In an initial experiment reference compound **13** was hydrolyzed (Figure 3a). During hydrolysis the formation of the benzyl phosphate diester **12** (Figure 3b) and d4TMP **2** (Figure 3c) was observed. At the end, d4TMP **2**

Table 2. Antiviral Activity and Cytotoxicity of Pronucleotides **5a**−**c**, **13**, and **4a**,**b** and Tetrols **8** Compared to d4T **1**

compound		EC_{50} (μM)[a]			
		CEM/0[c]		CEM/TK$^-$[d]	CEM/0[c]
		HIV-1	HIV-2	HIV-2	CC_{50} (μM)[b]
5a (*mix*)	5,5′-(H)	0.51 ± 0.21	0.62 ± 0.51	7.5 ± 2.5	115 ± 58
5a (*fast*)	5,5′-(H)	0.46 ± 0.27	1.2 ± 0.0	4.4 ± 2.1	107 ± 39
5a (*slow*)	5,5′-(H)	0.47 ± 0.20	1.2 ± 0.21	3.3 ± 1.8	78 ± 2.1
13	5-Sal[e]	0.73 ± 0.042	0.91 ± 0.28	3.7 ± 1.1	82 ± 1.4
5b (*mix*)	5,5′-(Me)	0.40 ± 0.0	0.83 ± 0.24	0.85 ± 0.61	64 ± 7.1
5c (*mix*)	5,5′-(*t*-Bu)	0.88 ± 0.18	1.3 ± 0.071	2.9 ± 1.2	20 ± 0.71
5c (*fast*)	5,5′-(*t*-Bu)	0.81 ± 0.25	1.6 ± 0.95	2.8 ± 1.7	18 ± 1.4
5c (*middle*)	5,5′-(*t*-Bu)	0.49 ± 0.45	1.0 ± 0.085	3.2 ± 0.64	19 ± 0.0
5c (*slow*)	5,5′-(*t*-Bu)	0.86 ± 0.31	2.0 ± 0.0	2.6 ± 0.85	20 ± 1.4
8a		99 ± 4.9	117 ± 41	18 ± 0.71	>250
8b		20 ± 2.8	>50	>50	96 ± 0.71
8c		7.9 ± 3.0	7.9 ± 3.0	6.9 ± 2.7	20 ± 0.71
d4T **1**		0.56 ± 0.20	0.79 ± 0.19	29 ± 3.8	>250
4a (*mix*)[f]	3,3′-(H)	0.19 ± 0.05	0.27 ± 0.11	2.33 ± 0.58	27.8 ± 11.0
4a (*fast*)[f]	3,3′-(H)	0.25 ± 0.14	0.30 ± 0.09	3.33 ± 2.31	107 ± 13
4a (*slow*)[f]	3,3′-(H)	0.21 ± 0.17	0.22 ± 0.16	2.37 ± 1.48	90.7 ± 16.1
4b (*mix*)[f]	3,3′-(Me)	0.37 ± 0.25	0.41 ± 0.19	1.60 ± 0.57	67.1 ± 7.1

[a] Antiviral activity in T-lymphocytes: 50% effective concentration (shown values are the mean of two to three independent experiments). [b] Cytostatic activity: 50% cytostatic concentration. [c] Wild-type CEM cells. [d] Thymidine kinase-deficient CEM cells. [e] 5-(saligen-5′-yl)-*cyclo*Sal-d4TMP. [f] Data taken from ref 4.

Scheme 3. Theoretical Pathways of Chemical Hydrolysis of 5,5′-Bis-(*cyclo*Sal-d4TMP) **5a**



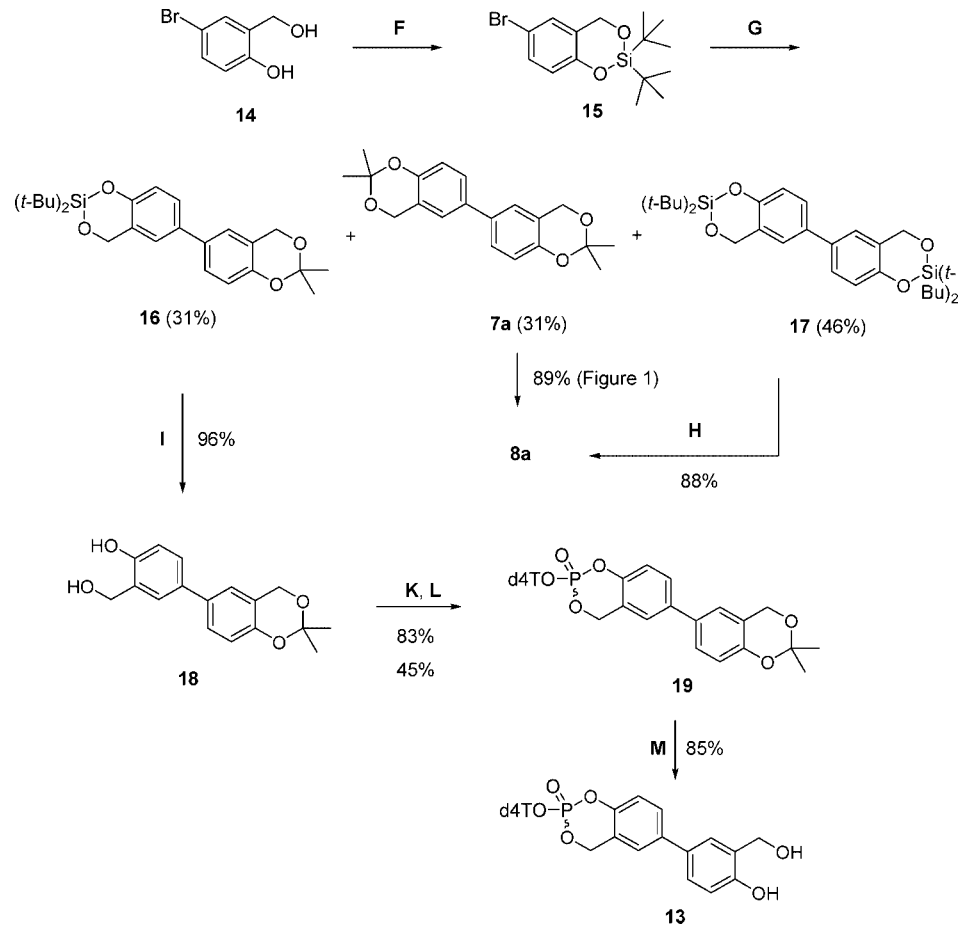
was found to be the sole phosphorus containing hydrolysis product (Figure 3d).

In a second experiment **5a**-*mix* was hydrolyzed under the same conditions (Figure 4). As in the HPLC analysis the formation of **13** was detected (Figure 4ii). Furthermore, the formation of two different benzyl phosphate diesters **11** and **12** was observed. The later appearing one (Figure 4iii) is the known intermediate **12** that was also formed in the hydrolysis of **13** (for a detailed comparable assignment, see Figure S2 in Supporting Information). Consequently, the benzyl phosphate diester in Figure 4ii should be intermediate **11** (Scheme 3). Almost at the end of the hydrolysis (Figure 4iv) only signals for compounds **12** and **2** were present. Finally, the ^{31}P NMR spectrum was almost identical to the spectrum d in Figure 3. In conclusion, for bis-(*cyclo*Sal-d4TMP) **5a** a consecutive hydrolysis pathway leading to a selective release of 2 equiv of d4TMP **2** as the exclusive phosphorus containing product can be concluded.

Antiviral Evaluation and Cytotoxicity. *Cyclo*Sal-triesters **5** and **13** and tetrols **8** were evaluated for their in vitro anti-HIV activity and cytostatic activity (Table 2). For comparison, the values of d4T **1** and 3,3′-bis-(*cyclo*Sal-d4TMPs) **4** are given as well.

All pronucleotides **4**, **5**, and **13** showed comparable antiviral potency against HIV-1 and HIV-2 in wild-type CEM/0 cells to d4T **1** within the experimental error. Almost all compounds

Scheme 4. Synthesis of 4,7-*O*-di-*tert*-Butylsilyl-4′,7′-*O*-isopropylidene-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol **16** and 5-(Saligen-5′-yl)-*cyclo*Sal-d4TMP **13**[a]



[a] Reagents and conditions. Method F: $(t\text{-Bu})_2Si(OTf)_2$, pyridine, CH_2Cl_2, room temp, 2.5 h. Method G: (i) **6a**, *n*-BuLi, THF, −80 °C, 1 h; (ii) $Fe(acac)_3$, THF, −80 °C to room temp, 60 h. Method H: $Et_3N \cdot 3HF$, THF, room temp, 20 min. Method I: $Et_3N \cdot 3HF$, THF, room temp, 10 min. Method K: (i) PCl_3, Et_2O, −20 °C, 10 min; (ii) Et_2O, pyridine, −20 °C, 3 h; (iii) room temp, 1 h. Method L: (i) d4T **1**, DIPEA, CH_3CN, −20 °C to room temp, 1 h; (ii) Oxone in H_2O, −10 °C to room temp, 10 min. Method M: conc HCl, CH_3CN/H_2O/THF, heat, 15 s.

showed a loss in antiviral potency in the HIV-2-infected CEM/TK-deficient cells. Interestingly, the decrease of anti-HIV-2 activity for 5,5′-bis-(*cyclo*Sal-d4TMPs) **5** in CEM/TK$^-$ cell cultures is significantly less than in the case of the 3,3′-counterparts **4**. Most striking is the full retention of anti-HIV-2 activity in the CEM/TK$^-$ cell line for **5b**-*mix*. This is the first example showing that this kind of "high-loaded" pronucleotides can act as successful nucleotide delivery systems. For all separated diastereomers (**4a**, **5a**, **5c**) no dependence of the antiviral activity to the configuration of the phosphorus atom was observed.

The cytostatic activity of prodrugs **5** was found to be higher than for d4T **1** and increased with the electron-donating effect of the alkyl substituents. Taking the cytostatic effects of the tetrols **8** into account, the toxicity of **5c**-*mix* ($CC_{50} = 20\ \mu M$ for the diastereomeric mixture) can definitely be attributed to the toxicity of tetrol **8c** ($CC_{50} = 20\ \mu M$) that is released upon hydrolysis. However, tetrol **8b** that is hydrolytically released from pronucleotide **5b** was less cytostatic. Consequently, *cyclo*Sal-pronucleotide **5b** showed clearly the most selective antiviral and modest antiproliferative properties and deserves further studies.

Conclusion

An efficient synthetic route to 5,5′-bis-(*cyclo*Sal-d4TMPs) **5** as new members of the class of *cyclo*Sal-pronucleotides having a mask-to-drug ratio of 1:2 has been successfully developed. These compounds were analyzed with regard to their hydrolytic and antiviral behavior. Although compounds **5** were chemically less stable compared to their 3,3′-counterparts **4**, they showed anti-HIV-1 and HIV-2 activity in wild-type CEM cells. Moreover, the 5,5′-bis-(*cyclo*Sal-d4TMPs) **5** were only slightly less active in CEM/TK$^-$ than in CEM/0 cells. In particular, 5,5′-bis(3-methyl-*cyclo*Sal-d4TMP) **5b** has to be highlighted because it is the first member of this class that completely retained its anti-HIV-2 activity in CEM/TK$^-$ cells. Obviously, this compound is able to penetrate cell membranes and release d4TMP inside cells, which makes the compound independent of the presence of thymidine kinase. The complex hydrolysis pathway of these compounds to release 2 equiv of d4TMP **2** was studied in detail. For these studies, mono-d4TMP-loaded intermediate **13** was synthesized as a reference compound. The formation of compound **13** during the hydrolysis of **5a** was definitely proven by HPLC analysis and ^{31}P NMR hydrolysis studies, which demonstrated a consecutive hydrolysis pathway of **5a**.

Experimental Section

NMR spectra were recorded with a Bruker AMX 400, Bruker AV 400, or a Bruker DRX 500 Fourier transform spectrometer. All ^1H and ^{13}C NMR chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane and calibrated on solvent signals. The ^{31}P NMR chemical shifts (proton decoupled)

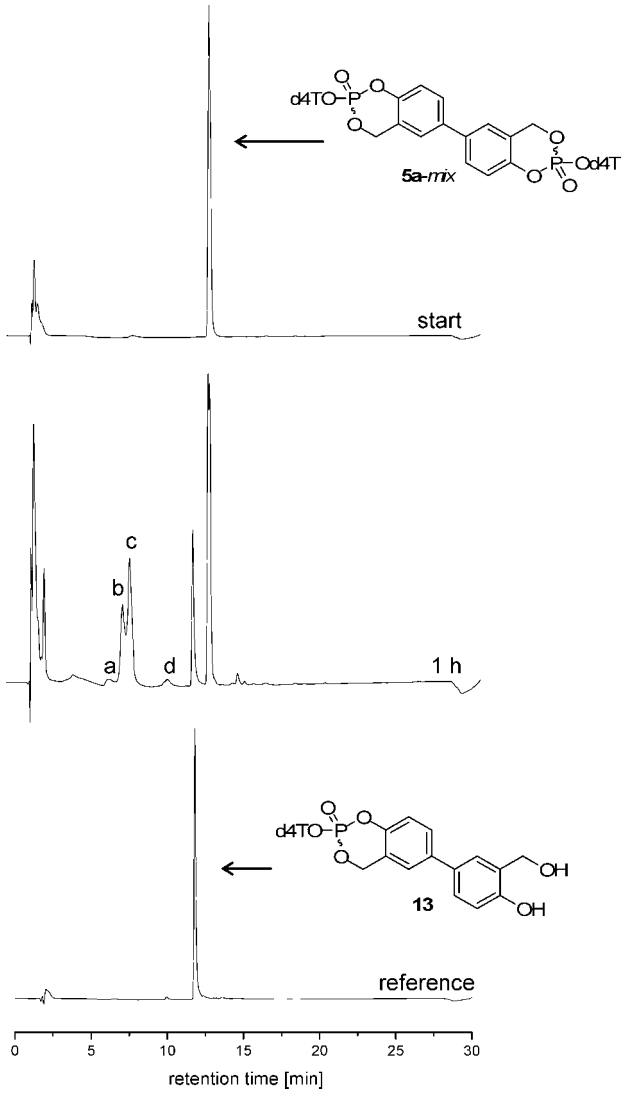


Figure 2. HPLC analytical proof of the formation of **13** during the hydrolysis of **5a**-*mix*.

are quoted in ppm using H_3PO_4 as the external reference. The spectra were recorded at room temperature. Mass spectra were obtained with a VG Analytical VG/70-250 F spectrometer [FAB, (double focusing), matrix *m*-nitrobenzyl alcohol], and ESI mass spectra were recorded with a VG Analytical Finnigan ThermoQuest MAT 95 XL spectrometer. For thin layer chromatography (TLC) VWR precoated 60 F_{254} plates with a 0.2 mm layer of silica gel (VWR no. 5554) were used; sugar-containing compounds were visualized with sugar spray reagent (0.5 mL of 4-methoxybenzaldehyde, 9 mL of EtOH, 0.5 mL of concentrated sulfuric acid, and 0.1 mL of glacial acetic acid). All preparative TLC were performed on a Chromatotron (Harrison Research, model 7924T) using glass plates coated with 1, 2, or 4 mm layers of VWR 60 PF_{254} silica gel containing a fluorescent indicator (VWR no. 7749). For column chromatography, Merck silica gel 60, 230−400 mesh, was used. UV spectra were recorded on a Varian Cary 1E UV−visible spectrophotometer, and absorption maximum wavelengths λ_{max} are given in nm. UV absorptions of *cyclo*Sal nucleotides were determined from their HPLC data (diode array detector). 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. Et_2O was dried over sodium/benzophenone and distilled under nitrogen. The purity of the new compounds **5** was checked by means of HPLC using two different gradients. Purity was in all cases >96% (details are given in the Supporting Information). THF was dried over potassium/benzophenone and distilled under nitrogen. Pyridine, CH_2Cl_2, and CH_3CN were destilled from calcium hydride under nitrogen. *N*,*N*-Diisopropylethylamine was destilled from sodium prior to use. The solvent for HPLC (CH_3CN) was obtained from Acros (HPLC grade).

General Procedure A: Homocoupling of 4-Bromosalicyl Alcohol Isopropylidene Acetals. A solution of the 4-bromosalicyl alcohol isopropylidene acetal (1.0 equiv) in dry THF was cooled to −80 °C under nitrogen, and a 1.6 M solution of *n*-butyllithium in hexane (1.0 equiv) was added. The reaction mixture was stirred at this temperature for 1 h and then added to a solution of iron(III) acetylacetonate (1.1 equiv) in dry THF (−80 °C). After 5 min the cooling was removed. The reaction mixture was allowed to warm up to room temperature and then stirred for the time given below. The reaction mixture was diluted with 2 N HCl, and the phases were separated with ethyl acetate. The aqueous layer was extracted with EtOAc twice. Then the combined organic layers were washed with 2 N HCl (3×), water (2×), and brine (2×) and dried with sodium sulfate. The solvent was removed in vaccuo, and the resulting crude products were purified by column chromatography (CH_2Cl_2).

General Procedure B: Deprotection of Isopropylidene Acetals. Concentrated HCl was added to a solution of the respective isopropylidene acetal in CH_3CN/H_2O and THF. The reaction mixture was heated to reflux with a heat gun for the given time. This procedure was repeated until the deprotection has been completed (monitoring by TLC, CH_2Cl_2). The reaction mixture was diluted with water, and the phases were separated with ethyl acetate. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried with sodium sulfate, and the solvent was removed under reduced pressure. The crude products were purified by column chromatography (CH_2Cl_2/CH_3OH, 9:1).

General Procedure C: Preparation of *cyclo*Sal-d4T-monophosphates. Variant A. Synthesis of the Saligenyl Chlorophosphite. A solution of the respective salicyl alcohol in dry Et_2O (and dry THF) was cooled to −40 °C in an inert atmosphere. After dropwise addition of freshly distilled PCl_3 and stirring at −40 °C for 10 min, a solution of dry pyridine in dry diethyl ether was added at the same temperature within 3 h. After completion the reaction mixture was allowed to warm up to room temperature and stirred for 1 h. It was kept at −20 °C overnight to precipitate the formed pyridinium chloride almost completely. Filtration under nitrogen and concentration of the filtrate under reduced pressure yielded the saligenyl chlorophosphite as a crude product which was directly used for the synthesis of the *cyclo*Sal-d4T-monophosphate without further purification. The grade of purity was determined by integration of ^{1}H NMR signals (two protons of $C_5H_5N\cdot HCl$ vs two aromatic protons of the saligenyl chlorophosphite).

Synthesis of the *cyclo*Sal Nucleoside Monophosphate. Under nitrogen, d4T (**1**, 1.2 equiv per 1.0 equiv of saligenyl chlorophosphite unit) was dissolved in dry CH_3CN and cooled to −20 °C. Then DIPEA (1.9 equiv per 1.0 equiv of saligenyl chlorophosphite unit) and the respective saligenyl chlorophosphite in dry CH_3CN were added. The reaction mixture was stirred at room temperature for the given time. Subsequently, *tert*-butyl hydroperoxide (5.5 M in *n*-nonane, 3.6 equiv per 1.0 equiv of saligenyl chlorophosphite unit) was added at −20 °C. The solution was stirred at room temperature until the oxidation was completed and then poured into water. The phases were separated with EtOAc, the aqueous layer was extracted with EtOAc three times, and the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The resulting residues were purified by flash chromatography (CH_2Cl_2/CH_3OH, 9:1) and preparative RP-HPLC. The isolated products were lyophilized from CH_3CN/H_2O (1:1).

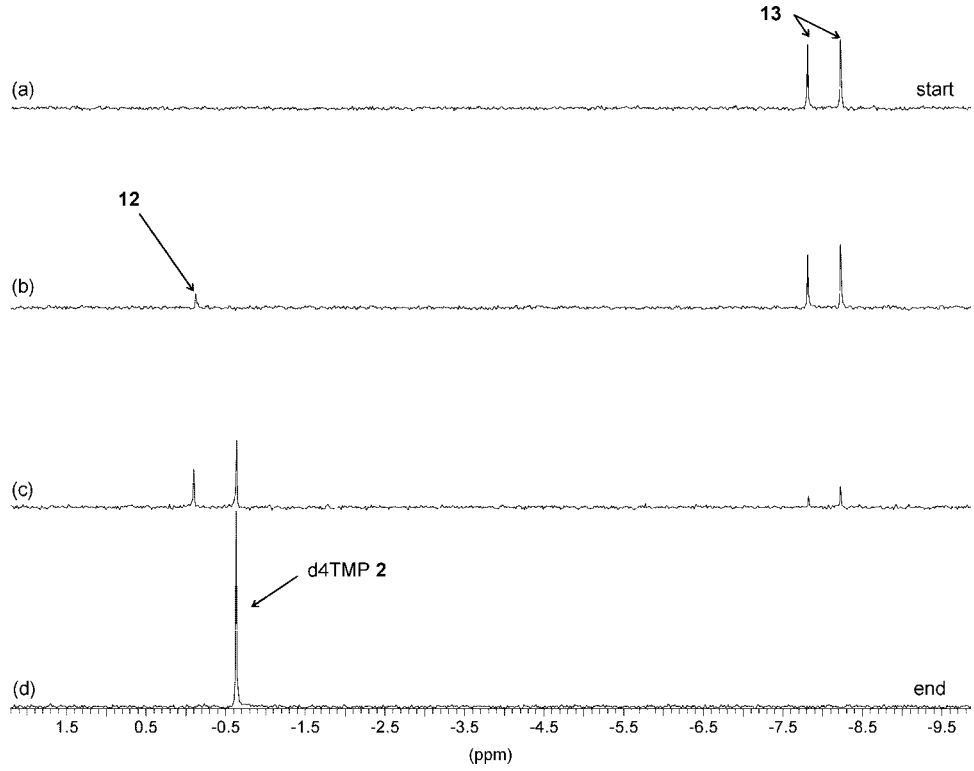


Figure 3. ^{31}P NMR hydrolysis study of **13** (diastereomeric mixture).

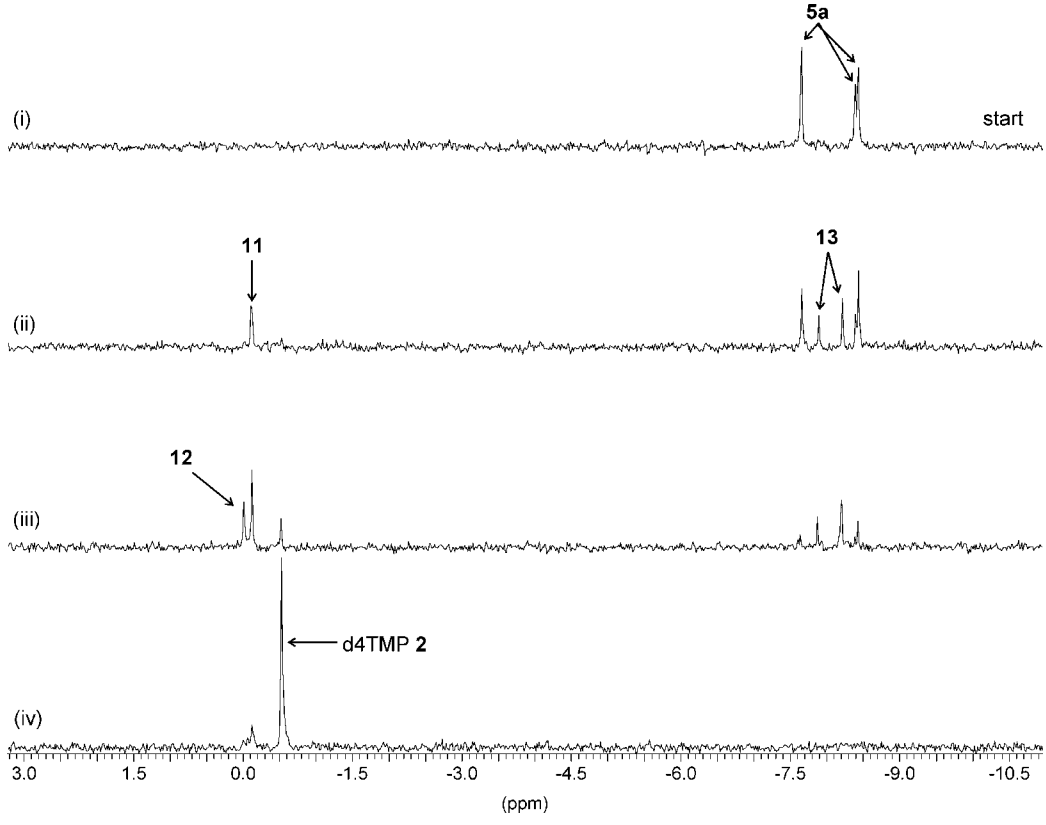


Figure 4. ^{31}P NMR hydrolysis study of **5a**-*mix*.

Variant B. Variant B was performed as described for variant A, but the preparation of the saligenyl chlorophosphite was performed at −20 °C and addition of the mixture of dry pyridine and dry Et_2O was carried out in only 1.5 h.

General Procedure D: Cleavage of DTBS Protecting Group. The corresponding DTBS-protected salicyl alcohol derivative was dissolved in THF, and $NEt_3 \cdot 3HF$ was added. The solution was stirred at room temperature for the given time, and afterwards the phases were separated with ethyl acetate and water. The aqueous layer was extracted with EtOAc once, the combined organic layers were washed with a 5% sodium bicarbonate solution, and the solvent was removed in vacuo. The products were purified by flash chromatography (CH_2Cl_2/CH_3OH, 9:1 (**8a**) or CH_2Cl_2/CH_3OH gradient 0−7%, **18**).

Bis-(isopropylidene)-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (7a). Corresponding to Method A (Scheme 1). General procedure A was carried out with 1.1 g (4.5 mmol) of 4-bromosalicyl alcohol isopropylidene acetal (**6a**), dissolved in 9.5 mL of dry THF, 2.8 mL (1.6 M solution in hexane, 4.5 mmol) of *n*-BuLi, and 1.78 g (5.03 mmol) of iron(III) acetylacetonate in 4 mL of dry THF. Reaction time: 42 h. Yield: 558 mg (1.71 mmol, 75%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38 (dd, J = 8.5, 2.4 Hz, 2H, aryl-H-6), 7.31 (d, J = 2.4 Hz, 2H, aryl-H-2), 6.83 (d, J = 8.5 Hz, 2H, aryl-H-5), 4.86 (s, 4H, benzyl-H), 1.48 (s, 12H, acetal-CH_3) ppm.

Bis-(isopropylidene)-3,3′-bis(hydroxymethyl)-5,5′-dimethylbiphenyl-4,4′-diol (7b). Corresponding to Method A (Scheme 1). General procedure A was carried out with 451 mg (1.75 mmol) of 4-bromo-6-methylsalicyl alcohol isopropylidene acetal (**6b**), dissolved in 4 mL of dry THF, 1.1 mL (1.6 M solution in hexane, 1.8 mmol) of *n*-BuLi, and 686 mg (1.94 mmol) of iron(III) acetylacetonate in 1.5 mL of dry THF. Reaction time: 36 h. Yield: 120 mg (0.339 mmol, 37%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20−7.18 (m, 2H, aryl-H-6), 6.97−6.94 (m, 2H, aryl-H-2), 4.88 (s, 4H, benzyl-H), 2.22 (s, 6H, aryl-CH_3), 1.57 (s, 12H, acetal-CH_3) ppm.

Bis-(isopropylidene)-3,3′-bis(hydroxymethyl)-5,5′-di-*tert*-butylbiphenyl-4,4′-diol (7c). Corresponding to Method A (Scheme 1). General procedure A was carried out with 1.1 g (3.7 mmol) of 4-bromo-6-*tert*-butylsalicyl alcohol isopropylideneacetal (**6c**), dissolved in 9.5 mL of dry THF, 2.3 mL (1.6 M solution in hexane, 3.7 mmol) of *n*-BuLi, and 1.44 g (4.07 mmol) of iron(III) acetylacetonate in 4 mL of dry THF. Reaction time: 36 h. Yield: 593 mg (1.23 mmol, 67%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29 (d, J = 2.2 Hz, 2H, aryl-H-6), 6.97−6.94 (m, 2H, aryl-H-2), 4.92 (s, 4H, benzyl-H), 1.59 (s, 12H, acetal-CH_3), 1.41 (s, 18H, *t*-Bu-CH_3) ppm.

3,3′-Bis(hydroxymethyl)biphenyl-4,4′-diol (8a). Method I (Corresponding to Method B (Scheme 1). General procedure B was carried out with 969 mg (2.97 mmol) of bis-(isopropylidene)-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (**7a**), dissolved in 90 mL of CH_3CN/H_2O (7:3) and 25 mL of THF, and 20 drops of concentrated HCl. Reaction time: 30 s. Yield: 651 mg (2.64 mmol, 89%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.32 (s, 2H, phenol-OH), 7.51 (d, J = 2.3 Hz, 2H, aryl-H-2), 7.24 (dd, J = 8.3, 2.5 Hz, 2H, aryl-H-6), 6.80 (d, J = 8.3 Hz, 2H, aryl-H-5), 5.01 (t, J = 5.7 Hz, 2H, benzyl-OH), 4.52 (d, J = 5.7 Hz, 4H, benzyl-H) ppm.

Method II (Corresponding to Method H (Scheme 4). General procedure D was carried out with 610 mg (1.15 mmol) of bis-di-*tert*-butylsilyl-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (**17**) dissolved in 50 mL of THF, and 1.1 mL (6.5 mmol) of $NEt_3 \cdot 3HF$. Reaction time: 20 min. Yield: 250 mg (1.01 mmol, 88%) as a slightly yellow solid. The analytical data were identical with those reported above.

3,3′-Bis(hydroxymethyl)-5,5′-dimethylbiphenyl-4,4′-diol (8b). Corresponding to Method B (Scheme 1). General procedure B was carried out with 162 mg (0.457 mmol) of bis-(isopropylidene)-3,3′-bis(hydroxymethyl)-5,5′-dimethyl-biphenyl-4,4′-diol (**7b**), dissolved in 20 mL of CH_3CN/H_2O (7:3) and 5 mL of THF, and 5 drops of concentrated HCl. Reaction time: 30 s. Yield: 115 mg (0.419 mmol, 92%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (s, 2H, phenol-OH), 7.30 (d, J = 2.3 Hz, 2H, aryl-H-2), 7.20 (d, J = 2.0 Hz, 2H, aryl-H-6), 5.30 (t, J = 5.5 Hz, 2H, benzyl-OH), 4.60 (d, J = 5.3 Hz, 4H, benzyl-H), 2.21 (s, 6H, aryl-CH_3) ppm.

3,3′-Bis(hydroxymethyl)-5,5′-di-*tert*-butylbiphenyl-4,4′-diol (8c). Corresponding to Method B (Scheme 1). General procedure B was carried out with 206 mg (0.407 mmol) of bis-(isopropylidene)-3,3′-bis(hydroxymethyl)-5,5′-di-*tert*-butylbiphenyl-4,4′-diol (**7c**), dissolved in 50 mL of CH_3CN/H_2O (5:1) and 5 mL of THF, and 3.5 mL of concentrated HCl. Reaction time: 3 min. Yield: 120 mg (0.335 mmol, 71%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.67 (s, 2H, phenol-OH), 7.25−7.22 (m, 2H, aryl-H-6), 7.22−7.19 (m, 2H, aryl-H-2), 5.84 (t, J = 5.2 Hz, 2H, benzyl-OH), 4.69 (d, J = 4.8 Hz, 4H, benzyl-H), 1.40 (s, 18H, *t*-Bu-CH_3) ppm.

5,5′-Bis-(*cyclo*Sal-d4TMP) (5a). Synthesis of Saligenyl Chlorophosphite 9a. (Corresponding to Method C (Scheme 2)). General Procedure C (variant A) was carried out with 105 mg (0.426 mmol) of 3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (**8a**) dissolved in 22 mL of dry Et_2O/dry THF (1:1), 0.10 mL (1.1 mmol) of PCl_3, and 0.17 mL (2.1 mmol) of dry pyridine in 0.85 mL of dry Et_2O. Yield: 130 mg (55%; purity, 68%).

Synthesis of *cyclo*Sal Nucleoside Monophosphate 5a (Corresponding to Method D (Scheme 2)). The synthesis involved 130 mg of crude saligenyl chlorophosphite **9a**, dissolved in 4 mL of dry CH_3CN, 125 mg (0.557 mmol) of d4T (**1**), dissolved in 13 mL of dry CH_3CN, 0.15 mL (0.86 mmol) DIPEA, and 0.30 mL (5.5 M in *n*-nonane, 1.7 mmol) of *tert*-butyl hydroperoxide. Reaction time: 1.5 h. Oxidation time: 1 h. Preparative RP-HPLC: CH_3CN/H_2O (1:2). Yield: 51 mg (65 μmol, 28%) of a diastereomeric mixture as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.36−11.32 (m, 6H, 6 × NH), 7.67−7.59 (m, 6H, 6 × aryl-H-4), 7.59−7.54 (m, 6H, 6 × aryl-H-6), 7.26−7.17 (m, 12H, 6 × aryl-H-3, 6 × thymine-H-6), 6.84−6.77 (m, 6H, 6 × 1′-H), 6.46−6.36 (m, 6H, 6 × 3′-H), 6.06−6.00 (m, 6H, 6 × 2′-H), 5.61−5.41 (m, 12H, 12 × benzyl-H), 4.98−4.95 (m, 6H, 6 × 4′-H), 4.40−4.26 (m, 12H, 12 × 5′-H), 1.69 (s, 9H, 3 × thymine-CH_3), 1.63 (s, 9H, 3 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.48, −9.52 ppm. The diastereomers were partially separated by preparative RP-HPLC (CH_3CN/H_2O 2:5).

Analytical Data of 5a-*fast* (Two Diastereomers). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.32 (s, 4H, 4 × NH), 7.64−7.59 (m, 4H, 4 × aryl-H-4), 7.59−7.57 (m, 4H, 4 × aryl-H-6), 7.26−7.17 (m, 8H, 4 × aryl-H-3, 4 × thymine-H-6), 6.84−6.77 (m, 4H, 4 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.5 Hz, 3H, 3 × 3′-H), 6.37 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.06−6.00 (m, 4H, 4 × 2′-H), 5.61−5.41 (m, 8H, 8 × benzyl-H), 4.99−4.94 (m, 4H, 4 × 4′-H), 4.40−4.25 (m, 8H, 8 × 5′-H), 1.69 (d, J = 0.7 Hz, 9H, 3 × thymine-CH_3), 1.63 (d, J = 1.3 Hz, 3H, 1 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.48, −9.52 ppm.

Analytical Data of 5a-*slow* (One Diastereomer). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.33 (s, 2H, 2 × NH), 7.64−7.59 (m, 2H, 2 × aryl-H-4), 7.59−7.54 (m, 2H, 2 × aryl-H-6), 7.22−7.17 (m, 4H, 2 × aryl-H-3, 2 × thymine-H-6), 6.80−6.77 (m, 2H, 2 × 1′-H), 6.38 (ddd, J = 6.0, 1.8, 1.8 Hz, 2H, 2 × 3′-H), 6.04−6.00 (m, 2H, 2 × 2′-H), 5.61−5.50 (m, 2H, 2 × benzyl-H), 5.50−5.41 (m, 2H, 2 × benzyl-H), 4.98−4.95 (m, 2H, 2 × 4′-H), 4.41−4.25 (m, 4H, 4 × 5′-H), 1.63 (d, J = 1.3 Hz, 6H, 2 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.52 ppm.

5,5′-Bis(3-methyl-*cyclo*Sal-d4TMP) (5b). Synthesis of Saligenyl Chlorophosphite 9b (Corresponding to Method C (Scheme 2)). General procedure C (variant A) was carried out with 90 mg (0.33 mmol) of 3,3′-bis(hydroxymethyl)-5,5′-dimethylbiphenyl-4,4′-diol (**8b**) dissolved in 18 mL of dry Et_2O/dry THF (1:1), 70 μL (0.79 mmol) of PCl_3, and 0.14 mL (1.7 mmol) of dry pyridine in 0.70 mL of dry Et_2O. Yield: 125 mg (54%; purity: 59%).

Synthesis of *cyclo*Sal Nucleoside Monophosphate 5b (Corresponding to Method D (Scheme 2)). The synthesis involved 122 mg of crude saligenyl chlorophosphite **9b**, dissolved in 4 mL of dry CH_3CN (+0.8 mL dry THF), 96 mg (0.43 mmol) of d4T (**1**), dissolved in 9 mL of dry CH_3CN, 0.14 mL (0.82 mmol) of DIPEA, and 0.23 mL (5.5 M in *n*-nonane, 1.3 mmol) of *tert*-butyl hydroperoxide. Reaction time: 1 h. Oxidation time: 1 h. Preparative RP-HPLC: CH_3CN/H_2O (2:3). Yield: 43 mg (53 μmol, 30%) of a diastereomeric mixture as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.33 (s, 6H, 6 × NH), 7.56−7.52 (m, 6H, 6 × aryl-H-4), 7.41−7.37 (m, 6H, 6 × aryl-H-6), 7.23−7.18 (m, 6H, 6 × thymine-H-6), 6.83−6.79 (m, 6H, 6 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.8 Hz, 3H, 3 × 3′-H), 6.38 (ddd, J = 6.0, 1.8, 1.5 Hz, 3H, 3 × 3′-H), 6.06−6.00 (m, 6H, 6 × 2′-H), 5.56−5.47 (m, 6H, 6 × benzyl-H), 5.45−5.36 (m, 6H, 6 × benzyl-H), 5.00−4.95 (m, 6H, 6 × 4′-H), 4.37−4.23 (m, 12H, 12 × 5′-H), 2.28 (s, 9H, 3 × aryl-CH_3), 2.25 (s, 9H, 3 × aryl-CH_3), 1.66 (d, J = 0.8 Hz, 9H, 3

× thymine-CH_3), 1.63 (d, J = 1.0 Hz, 9H, 3 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.75, −8.88 ppm.

5,5′-Bis(3-*tert*-butyl-*cyclo*Sal-d4TMP) (5c). Synthesis of Saligenyl Chlorophosphite 9c (Corresponding to Method C (Scheme 2)). General procedure C (variant A) was carried out with 170 mg (0.474 mmol) of 3,3′-bis(hydroxymethyl)-5,5′-di-*tert*-butylbiphenyl-4,4′-diol (**8c**) dissolved in 36 mL of dry Et_2O, 0.10 mL (1.1 mmol) of PCl_3, and 0.19 mL (2.4 mmol) of dry pyridine in 0.95 mL of dry Et_2O. Yield: 206 mg (55%; purity: 62%).

Synthesis of *cyclo*Sal Nucleoside Monophosphate 5c. Method I (Corresponding to Method E (Scheme 2)). The synthesis involved 96 mg of crude saligenyl chlorophosphite **9c**, dissolved in 4 mL of dry CH_3CN, 70 mg (0.31 mmol) of d4T (**1**), dissolved in 7 mL of dry CH_3CN, and 84 μL (49 μmol) of DIPEA. Deviating from the general procedure C for the oxidation reaction, Oxone was used. Therefore, the reaction mixture was cooled to −20 °C and a suspension of 0.32 g (0.52 mmol) of Oxone in 1.5 mL of cold water was added. The cooling was removed immediately, and the reaction mixture was stirred for 10 min. Subsequently, the mixture was extracted with EtOAc twice. The combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. Preparative RP-HPLC: CH_3CN/H_2O (4:5). Yield: 33 mg (37 μmol, 30%) of a diastereomeric mixture as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.34 (s, 6H, 6 × NH), 7.50−7.44 (m, 12H, 6 × aryl-H-4, 6 × aryl-H-6), 7.24 (d, J = 1.3 Hz, 3H, 3 × thymine-H-6), 7.21 (d, J = 1.0 Hz, 3H, 3 × thymine-H-6), 6.85−6.79 (m, 6H, 6 × 1′-H), 6.46−6.39 (m, 6H, 6 × 3′-H), 6.07−6.01 (m, 6H, 6 × 2′-H), 5.56−5.38 (m, 12H, 12 × benzyl-H), 5.01−4.96 (m, 6H, 6 × 4′-H), 4.41−4.28 (m, 12H, 12 × 5′-H), 1.66−1.59 (m, 18H, 6 × thymine-CH_3), 1.39 (s, 27H, 3 × t-Bu-CH_3), 1.36 (s, 27H, 3 × t-Bu-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.59, −8.84 ppm.

Method II (Corresponding to Method D (Scheme 2). The synthesis involved 110 mg of crude saligenyl chlorophosphite **9c**, dissolved in 4 mL of dry CH_3CN, 70 mg (0.31 mmol) of d4T (**1**), dissolved in 7 mL of dry CH_3CN, 84 μL (49 μmol) of DIPEA, and 0.17 mL (5.5 M in *n*-nonane, 0.94 mmol) of *tert*-butyl hydroperoxide. Reaction time: 1 h. Oxidation time: 1 h. Preparative RP-HPLC: CH_3CN/H_2O (2:3). Yield: 9 mg of **5c**-*fast* 25 mg of **5c**-*middle* and 12 mg of **5c**-*slow* (in total, 46 mg, 51 μmol, 37%) as colorless foams.

Analytical Data of 5c-*fast*. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.36 (s, 2H, 2 × NH), 7.50−7.47 (s, 4H, 2 × aryl-H-4, 2 × aryl-H-6), 7.24 (d, J = 1.3 Hz, 2H, 2 × thymine-H-6), 6.84−6.80 (m, 2H, 2 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.5 Hz, 2H, 2 × 3′-H), 6.05−6.01 (m, 2H, 2 × 2′-H), 5.55−5.39 (m, 4H, 4 × benzyl-H), 5.03−4.95 (m, 2H, 2 × 4′-H), 4.40−4.28 (m, 4H, 4 × 5′-H), 1.61 (d, J = 1.0 Hz, 6H, 2 × thymine-CH_3), 1.36 (s, 18H, 2 × t-Bu-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.83 ppm.

Analytical Data of 5c-*middle*. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.37−11.32 (s, 2H, 2 × NH), 7.50−7.44 (m, 4H, 2 × aryl-H-4, 2 × aryl-H-6), 7.24 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.21 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 6.84−6.80 (m, 2H, 2 × 1′-H), 6.43 (ddd, J = 5.8, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.42 (ddd, J = 5.8, 1.8, 1.5 Hz, 1H, 1 × 3′-H), 6.07−6.02 (m, 2H, 2 × 2′-H), 5.55−5.39 (m, 4H, 4 × benzyl-H), 5.01−4.96 (m, 2H, 2 × 4′-H), 4.40−4.28 (m, 4H, 4 × 5′-H), 1.61 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.60 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.39 (s, 9H, 1 × t-Bu-CH_3), 1.36 (s, 9H, 1 × t-Bu-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.59, −8.84 ppm.

Analytical Data of 5c-*slow*. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.33 (s, 2H, 2 × NH), 7.49−7.46 (m, 2H, 2 × aryl-H-4), 7.46−7.44 (m, 2H, 2 × aryl-H-6), 7.21 (d, J = 1.3 Hz, 2H, 2 × thymine-H-6), 6.83−6.79 (m, 2H, 2 × 1′-H), 6.42 (ddd, J = 5.8, 1.8, 1.5 Hz, 2H, 2 × 3′-H), 6.07−6.03 (m, 2H, 2 × 2′-H), 5.55−5.38 (m, 4H, 4 × benzyl-H), 5.01−4.96 (m, 2H, 2 × 4′-H), 4.41−4.29 (m, 4H, 4 × 5′-H), 1.60 (d, J = 1.0 Hz, 6H, 2 × thymine-CH_3), 1.39 (s, 18H, 2 × t-Bu-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −8.59 ppm.

Di-*tert*-butylsilyl-4-bromosalicyl Alcohol (15). Corresponding to Method F (Scheme 4). Under nitrogen, an amount of 3.06 g (15.1 mmol) of 4-bromosalicyl alcohol (**14**) was dissolved in 100 mL of dry CH_2Cl_2. After the addition of 5.3 mL (66 mmol) of dry pyridine and 6.4 mL (20 mmol) of di-*tert*-butylsilyl bis(trifluoromethanesulfonate), the solution was stirred at room temperature for 2.5 h. At 0 °C the reaction mixture was diluted with water and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 twice. Then the combined organic layers were dried with sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petroleum ether 50−70/CH_2Cl_2, 5:1). Yield: 4.15 g (12.1 mmol, 81%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26 (dd, J = 8.6, 2.5 Hz, 1H, aryl-H-5), 7.07 (d, J = 2.5 Hz, 1H, aryl-H-3), 6.80 (d, J = 8.6 Hz, 1H, aryl-H-6), 4.95 (s, 2H, benzyl-H), 1.03 (s, 18H, Si-t-Bu-CH_3) ppm.

4,7-*O*-Di-*tert*-butylsilyl-4′,7′-*O*-isopropylidene-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (16). Corresponding to Method G (Scheme 4). This reaction was carried out analogously to the homocoupling of 4-bromosalicyl alcohol isopropylidene acetals **6** (general procedure A). An equimolar mixture of 4-bromosalicyl alcohol isopropylidene acetal (1.28 g, 5.27 mmol, **6a**) and di-*tert*-butylsilyl-4-bromosalicyl alcohol (1.81 g, 5.27 mmol, **15**), dissolved in 25 mL of dry THF, 6.6 mL (1.6 M solution in hexane, 11 mmol) of *n*-BuLi, and 4.15 g (11.8 mmol) of $Fe(acac)_3$ in 10 mL of dry THF, was used. Reaction time: 60 h. Yield: 701 mg (1.64 mmol, 31%) as well as **17** (642 mg, 1.22 mmol, 46%) and **7a** (267 mg, 0.818 mmol, 31%) as colorless solids. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38−7.30 (m, 2H, aryl-H-6, aryl-H-6′), 7.15−7.12 (m, 1H, aryl-H-2′), 7.09 (d, J = 2.2 Hz, 1H, aryl-H-2), 6.96 (d, J = 8.2 Hz, 1H, aryl-H-5), 6.86 (d, J = 8.5 Hz, 1H, aryl-H-5′), 5.05 (s, 2H, benzyl-H), 4.89 (s, 2H, benzyl-H′), 1.57 (s, 6H, acetal-CH_3), 1.06 (s, 18H, Si-t-Bu-CH_3) ppm.

Analytical Data of Bis-di-*tert*-butylsilyl-3,3′-bis(hydroxymethyl)-biphenyl-4,4′-diol (17). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 (dd, J = 8.2, 2.2 Hz, 2H, aryl-H-6), 7.10 (d, J = 2.2 Hz, 2H, aryl-H-2), 6.95 (d, J = 8.2 Hz, 2H, aryl-H-5), 5.05 (s, 4H, benzyl-H), 1.06 (s, 36H, Si-t-Bu-CH_3) ppm.

4′,7′-*O*-Isopropylidene-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (18). Corresponding to Method I (Scheme 4). General procedure D was carried out with 0.35 g (0.82 mmol) of 4,7-*O*-di-*tert*-butylsilyl-4′,7′-*O*-isopropylidene-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (**16**) dissolved in 20 mL of THF and 0.38 mL (2.3 mmol) of $NEt_3 \cdot 3HF$. Reaction time: 10 min. Yield: 226 mg (0.789 mmol, 96%) as a slightly yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.41 (s, 1H, phenol-OH), 7.52 (d, J = 2.3 Hz, 1H, aryl-H-2′), 7.35 (dd, J = 8.7, 2.3 Hz, 1H, aryl-H6), 7.27 (dd, J = 8.1, 2.3 Hz, 1H, aryl-H6′), 7.26 (d, J = 2.3 Hz, 1H, aryl-H-2), 6.82 (d, J = 8.4 Hz, 1H, aryl-H-5′), 6.81 (d, J = 8.1 Hz, 1H, aryl-H-5), 5.00 (t, J = 5.6 Hz, 1H, benzyl-OH), 4.87 (s, 2H, benzyl-H′), 4.52 (d, J = 5.6 Hz, 2H, benzyl-H), 1.48 (s, 6H, acetal-CH_3) ppm.

5-(Isopropylidenesaligen-5′-yl)-*cyclo*Sal-d4TMP (19). Synthesis of Saligenyl Chlorophosphite (Corresponding to Method K (Scheme 4). General procedure C (variant B) was carried out with 188 mg (0.657 mmol) of 4′,7′-*O*-isopropylidene-3,3′-bis(hydroxymethyl)biphenyl-4,4′-diol (**18**) dissolved in 10 mL of dry Et_2O, 69 μL (79 μmol) PCl_3 and 0.10 mL (1.3 mmol) of dry pyridine in 0.5 mL of dry Et_2O. Yield: 192 mg.

Synthesis of *cyclo*Sal Nucleoside Monophosphate 19 (Corresponding to Method L (Scheme 4). The synthesis involved 129 mg of crude saligenyl chlorophosphite, dissolved in 3 mL of dry CH_3CN, 101 mg (0.446 mmol) of d4T (**1**), dissolved in 14 mL of dry CH_3CN, and 0.13 mL (0.75 mmol) of DIPEA. Reaction time: 1 h. Deviating from the general procedure C for the oxidation reaction, Oxone was used. Therefore, the reaction mixture was cooled to −10 °C and a suspension of 1.1 g (1.8 mmol) of Oxone in 5 mL of cold water was added. The cooling was removed immediately, and the reaction mixture was stirred for 10 min. Subsequently, the mixture was extracted with EtOAc twice. The combined organic layers were washed with water (2×), dried with sodium sulfate, and concentrated under reduced pressure. Yield:

93 mg (0.17 mmol, 45%) of a diastereomeric mixture as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.36 (s, 1H, 1 × NH), 11.35 (s, 1H, 1 × NH), 7.62−7.55 (m, 2H, 2 × aryl-H-4), 7.54−7.52 (m, 2H, 2 × aryl-H-6), 7.46−7.42 (m, 2H, 2 × aryl-H-4′), 7.38−7.35 (m, 2H, 2 × aryl-H-6′), 7.22 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.19 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.18 (d, J = 8.5 Hz, 1H, 1 × aryl-H-3), 7.15 (d, J = 8.5 Hz, 1H, 1 × aryl-H-3), 6.87 (d, J = 8.5 Hz, 2H, 2 × aryl-H-3′), 6.83−6.77 (m, 2H, 2 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.37 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.05−5.99 (m, 2H, 2 × 2′-H), 5.56 (dd, J = 14.6, 5.3 Hz, 1H, 1 × benzyl-H), 5.52 (dd, J = 14.6, 5.3 Hz, 1H, 1 × benzyl-H), 5.44 (dd, J = 14.6, 10.5 Hz, 2H, 2 × benzyl-H), 4.99−4.95 (m, 2H, 2 × 4′-H), 4.88 (s, 4H, 4 × benzyl-H′), 4.42−4.25 (m, 4H, 4 × 5′-H), 1.69 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.63 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.49 (s, 12H, 4 × acetal-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.37 ppm.

5-(Saligen-5′-yl)-*cyclo*Sal-d4TMP (13). Corresponding to Method M (Scheme 4). Fifteen drops of concentrated hydrochloric acid were added to a solution of 35 mg (63 μmol) of 5-(isopropylidenesaligen-5′-yl)-*cyclo*Sal-d4TMP (**19**), dissolved in 20 mL of CH_3CN/H_2O (7:3). The reaction mixture was heated to reflux with a heat gun for 15 s. The reaction mixture was diluted with water, and the phases were separated with EtOAc. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water (2×) and dried with sodium sulfate, and the solvent was removed under reduced pressure. The residue was lyophilized from CH_3CN/H_2O (1:1). Yield: 28 mg (53 μmol, 85%) of a diastereomeric mixture (ratio 1.0:1.0) as a colorless foam. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.37 (s, 2H, 2 × NH), 9.58 (s, 2H, 2 × phenol-OH), 7.60−7.47 (m, 6H, 2 × aryl-H-4, 2 × aryl-H-6, 2 × aryl-H-6′), 7.33 (dd, J = 8.3, 1.8 Hz, 2H, 2 × aryl-H-4′), 7.22 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.19 (d, J = 1.3 Hz, 1H, 1 × thymine-H-6), 7.17 (d, J = 8.5 Hz, 1H, 1 × aryl-H-3), 7.14 (d, J = 8.5 Hz, 1H, 1 × aryl-H-3), 6.84 (d, J = 8.3 Hz, 2H, 2 × aryl-H-3′), 6.83−6.77 (m, 2H, 2 × 1′-H), 6.43 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.38 (ddd, J = 6.0, 1.8, 1.8 Hz, 1H, 1 × 3′-H), 6.05−5.99 (m, 2H, 2 × 2′-H), 5.58 (dd, J = 14.6, 6.0 Hz, 1H, 1 × benzyl-H), 5.53 (dd, J = 14.6, 6.0 Hz, 1H, 1 × benzyl-H), 5.45 (dd, J = 14.6, 10.5 Hz, 2H, 2 × benzyl-H), 5.04 (t, J = 5.5 Hz, 2H, 2 × benzyl-OH), 4.99−4.94 (m, 2H, 2 × 4′-H), 4.52 (d, J = 5.5 Hz, 4H, 4 × benzyl-H′), 4.38−4.26 (m, 4H, 4 × 5′-H), 1.69 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3), 1.63 (d, J = 1.0 Hz, 3H, 1 × thymine-CH_3) ppm. ^{31}P NMR (162 MHz, DMSO-d_6): δ = −9.24, −9.29 ppm.

Hydrolysis Studies of *cyclo*Sal Phosphate Triesters. Hydrolysis studies of *cyclo*Sal nucleotides (phosphate buffer, pH 7.3) by HPLC analysis (method I, Supporting Information) have been described before.[8,10] Studies in RPMI/FCS(10%) were carried out as described in ref 5b.

Antiretroviral Evaluation. The method of antiviral evaluation has been described in ref 5a and is based on the virus-induced cytopathic effect (giant cell formation) in the CEM cell cultures.

^{31}P NMR Hydrolysis Studies of *cyclo*Sal Phosphate Triesters. ^{31}P NMR hydrolysis studies of *cyclo*Sal nucleotides **5a**-*mix* and **13** were carried out as described before.[8]

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Supporting Information Available: ^{13}C NMR and UV spectroscopic data, mass spectrometric data, R_f values, melting points, analytical HPLC data of new compounds; methods for HPLC analysis; general nomenclature of 3,3′-bis(hydroxymethyl)-5,5′-biphenyl-4,4′-diol derivatives and 5-(saligen-5′-yl)-*cyclo*Saligenyl derivatives; two figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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