Interaction of *cyclo*Sal-Pronucleotides with Cholinesterases from Different Origins. A Structure−Activity Relationship†

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A large number of *cyclo*Sal-nucleotide triesters **1**−**49** have been studied concerning their ability to inhibit cholinesterases of different origins as well as to inhibit HIV replication in cell culture. It was shown that *none* of the triesters showed inhibitory effects against human acetylcholinesterase (AChE; isolated enzyme) as well as against AChE from beef erythrocytes and calf serum. In contrast, inhibition of butyrylcholinesterase (BChE) has been observed for some triesters in human and mouse serum. *cyclo*Sal pronucleotides showed strong competitive inhibition with respect to the substrate acetylcholine chloride (K_i/K_m: $\sim 2 \times 10^{-5}$) and acted by time-dependent irreversible inhibition of the human serum BChE. Detailed studies demonstrated that the inhibitory effect against BChE is dependent on the nucleoside analogue, the substitution pattern of the *cyclo*Sal-moiety, and particularly on the stereochemistry at the phosphorus atom. Structural requirements to avoid the inhibition of BChE by *cyclo*Sal-nucleotide triesters have been elucidated in the reported study.

Introduction

*cyclo*Sal-phosphate triesters represent a particular class of pronucleotides.[1] These lipophilic derivatives of various antiviral and antitumor active nucleoside analogues have been developed as intracellular delivery forms of the corresponding nucleotide analogues.[2] By releasing the nucleotide, the metabolic bottleneck of the conversion of the nucleoside into the nucleotide by cellular or viral enzymes can be bypassed (chemical trojan horse concept). The *cyclo*Sal-approach relies on a chemically driven hydrolysis reaction. The initial chemical hydrolysis step intrinsically activates the remaining masking group which is then spontaneously cleaved to yield the nucleotide (tripartite prodrug). Often, nucleoside analogues, e.g. 3′-deoxy-2′,3′-didehydrothymidine (d4T),[4] have a poor affinity for the first activation (phosphorylation) step, and this may markedly impair their biological (i.e. antiviral, antitumor) efficacy.[5] The approach has been applied successfully to a number of different nucleoside analogues, e.g. d4T,[6] 2′,3′-dideoxyadenosine (ddA),[7] 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A),[7] fluorinated 2′,3′-dideoxyadenosine (2′-F-ddA),[8] carbocyclic nucleosides (CBV and abacavir),[9] and acyclovir (ACV).[10] Recently, we have reported that by attaching the *cyclo*Sal-masking group on (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU), the resulting pronucleotide showed considerable anti-Epstein−Barr-virus (EBV) activity, while the parent nucleoside BVdU was entirely inactive.[11]

However, as *cyclo*Sal-phosphate triesters belong to the class of hydrolytically labile phosphate esters, they may interfere with cholinesterases. If an interaction with acetylcholinesterase (AChE; EC 3.1.1.7) is involved, serious side effects may be expected due to the important physiological role of this enzyme.[12] Acetylcholinesterase inactivates acetylcholine and thus terminates its action at the junctions of various cholinergic nerve endings with their effector organs or postsynaptic sites. If acetylcholine is not destroyed, it can again activate the cholinergic receptor resulting in continuous stimulation of the cholinergic systems throughout the central and peripheral nervous systems, permanent contraction of skeletal muscle, and eventual death.[13] Anti-cholinesterase (anti-AChE) agents that inhibit AChE have been extensively used as agricultural insecticides and nerve gases, e.g. tabun, sarin, and VX,[14] and are also used as therapeutic agents.[15] The organophosphates represent the most characteristic type of anti-AChE agents. The mode of action of these agents is their irreversible inactivation of AChE by phosphorylating a serine residue in the active site of the enzyme. The phosphorylated adduct is hydrolytically labile but becomes extremely resistant to further hydrolysis after "aging", resulting in virtually permanent inactivation of the enzyme (suicide inhibition mechanism). The general formula of these AChE inhibitors is depicted in Figure 1A. The residue R and the leaving group X can be of a large variety of substituents including alkyl, alkoxy, aryloxy, amido, mercapto, halo, cyano, thiocyano, and phenoxy. Diisopropylfluorophosphate (DFP) and parathion (metabolized to paraoxon) are probably the most extensively studied compounds among this group of anti-AChE organophosphates. Moreover, it was previously reported that several *S*-alkyl- and *S*-benzylsaligenin cyclic phosphorothioate derivatives (Figure 1B) act as AChE inhibitors.[16] The structure−activity

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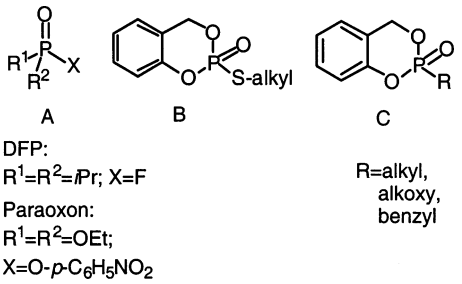


Figure 1. Structures of known acetylcholinesterase (AChE) inhibitors.

relationship for enzyme inhibition of these compounds revealed three major effects: a lipophilic effect, an electronic effect, and a steric effect. More recently, a study of substituted [1.3.2]-benzodioxaphosphorin-2-oxide derivatives containing alkyl, alkoxy, or aryloxy groups at the phosphorus atom (Figure 1C) showed that several of these compounds displayed inhibitory effects on neuropathy targeted esterase in vitro.[17] In those studies, all compounds were tested as enantiomeric mixtures.

In addition to AChE, a closely related cholinesterase is known which is present in various vertebrate tissues (e.g. liver, brain, lung) and predominately in human serum: the unspecific serum butyrylcholine esterase (BChE; EC 3.1.1.8). The physiological role of this enzyme is still unknown. However, plasma BChE is of pharmacological and toxicological importance because it hydrolyzes ester-containing drugs such as succinylcholine and cocaine. Therefore, purified BChE has been used for treatment of succinylcholine apnea in humans[18] and it is known to protect rodents from the toxic effects of cocaine.[19] Both AChE and BChE belong to the group of serine hydrolases employing a catalytic triad and so the catalytic site and mechanism of both enzymes should be comparable.

In this study, we have evaluated a large number of *cyclo*Sal-nucleotide derivatives for their inhibitory activities against acetyl- and butyrylcholinesterases from different origins. Structural features to avoid interaction with butyrylcholinesterase have been elucidated.

Results and Discussion

Chemistry. The *cyclo*Sal-triesters **1**−**49** used in this study have been prepared as previously reported[6a,7,8,11b] except triester **25** (Figure 2).

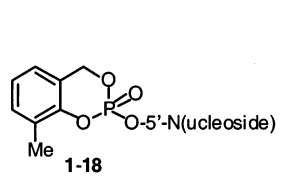
Starting from 3-fluorophenol, both *tert*-butyl groups were introduced by an acid-catalyzed Friedel−Crafts alkylation using isobutene to yield 2,4-bis-*tert*-butyl-5-fluorophenol in 70% yield. Hydroxymethylation of this material was performed using a solution of formaldehyde in water (71% yield of the salicyl alcohol derivative). Conversion into the cyclic chlorophosphite and preparation of the corresponding *cyclo*Sal-d4TMP triester **25** was carried out as described before (59% yield).[6a,7,8,11b] The triesters have been characterized by means of ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, by analytical reversed-phase high-performance liquid chromatography (RP-HPLC), and by mass spectrometry. Most of the studied *cyclo*Sal-phosphate triesters were used as diastereomeric mixtures. However, 3-methyl-*cyclo*Sal-d4TMP **4**, 3-methyl-*cyclo*Sal-ganciclovir monophosphate (3-Me-*cyclo*Sal-GCVMP) **10**, 3-*tert*-butyl-*cyclo*Sal-d4TMP **22**, 3-phenyl-*cyclo*Sal-BVdUMP **48**, and 5-methoxy-*cyclo*Sal-d4AMP **49** have been separated into their diastereomers by preparative HPLC using isocratic acetonitrile/water eluents. The metabolic fates and antiviral activities of most of these test compounds have been reported previously.[6−11,20]

Determination of the IC_{50} Values of the Triesters 1−49 against Cholinesterases from Different Origins. Studies were done using human, mouse, and calf serum. The first two sera contain butyrylcholinesterase (BChE). The latter serum is known to contain acetylcholinesterase (AChE) but no BChE. In addition, isolated enzymes were used: human AChE, human BChE, beef erythrocyte AChE, and AChE from electric eel (*electrophorus electricus*). The assay is a modified assay of Rappaport measuring the hydrolysis of acetylcholine catalyzed by these enzymes to yield acetic acid at pH 7.8.[21] As indicator, *m*-nitrophenol is used. The progression of the reaction was therefore followed photometrically (absorption at λ = 420 nm) due to the decreasing amounts of *m*-nitrophenolate.

Effect of Preincubation of Human Serum AChE with 3-Me-*cyclo*Sal-ddAMP 14 on Enzyme Activity. In a first set of experiments, human serum BChE was preincubated with 0.05 μM 3-Me-*cyclo*Sal-ddAMP **14** for different time periods (varying from 0 to 30 min). The reaction was allowed to proceed upon addition of the substrate, after which the conversion of the substrate was recorded at 5 and 15 min. Whereas no preincubation of the enzyme with the inhibitor resulted in 25 to 40% inhibition, preincubation resulted in a striking time-dependent inactivation. A preincubation time as short as 1 min resulted in 65 to 70% inhibition, and a preincubation time of 3 to 4 min afforded complete inhibition of BChE (Figure 3). *cyclo*Sal-phosphate triesters are chemically hydrolyzable in a pH-dependent manner[3]. Thus, to avoid any interference between chemical (half-lives at pH 7.3: 1−75 h) and enzyme-catalyzed hydrolysis (100% inhibition within 3−4 min) and to ascertain proper IC_{50} measurements within the linear time-scale of the reaction, IC_{50} values were determined after no longer than 5 min incubation.

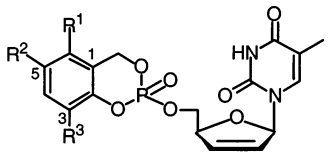
In a second set of experiments, different (human serum) BChE concentrations were exposed to fixed 3-Me-*cyclo*Sal-ddAMP concentrations, after which the remaining enzyme activity was recorded. Whereas 0.015 μM inhibitor completely inactivated 25 μL of (human serum) enzyme, the BChE reaction resumed and proceeded linearly and proportionally with control (in the absence of inhibitor) at higher serum concentrations (data not shown). The presence of 0.025 μM inhibitor inactivated 50 μL of (human serum) enzyme, but at higher enzyme concentrations, reaction again proceeded linearly and proportionally with control. The enzyme activity values in the presence of 0.02 μM inhibitor were between those recorded for 0.015 and 0.025 μM inhibitor (data not shown). These data point to a stoichiometric irreversible inhibition of BChE by the inhibitor.

Kinetic Properties of the Inhibition of 3-Me-*cyclo*Sal-ddAMP 14 against Human Serum BChE. Two concentrations of 3-Me-*cyclo*Sal-ddAMP **14** (0.05 and 0.1 μM) were combined with six different concentrations of the substrate acetylcholine chloride in a human serum BChE-catalyzed reaction, and the data

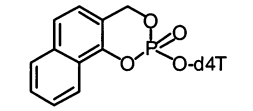


1-18

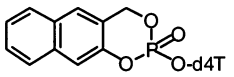
1: N = dT
2: N = AZT
3: N = ddT
4: N = d4T
5: N = 3'-*O*-Lev-dT
6: N = 5-FdU
7: N = BVdU
8: N = dG
9: N = ACV
10: N = GCV
11: N = PCV
12: N = carbovir (CBV)
13: N = dA
14: N = ddA
15: N = d4A
16: N = F-*ara*-ddA
17: N = F-*ribo*-ddA
18: N = abacavir (ABC)



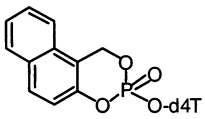
19: $R^1 = R^2 = R^3 = H$
20: $R^1 = H$; $R^2 = Me$; $R^3 = H$
21: $R^1 = H$; $R^2 = R^3 = Me$
22: $R^1 = R^2 = H$; $R^3 = t$Bu;
23: $R^1 = H$; $R^2 = t$Bu; $R^3 = H$
24: $R^1 = H$; $R^2 = R^3 = t$Bu
25: $R^1 = F$; $R^2 = R^3 = t$Bu
26: $R^1 = R^2 = H$; $R^3 = s$Bu;
27: $R^1 = H$; $R^2 = s$Bu; $R^3 = H$
28: $R^1 = R^2 = H$; $R^3 = CH_2CH_2C(O)OMe$;
29: $R^1 = H$; $R^2 = CH_2CH_2C(O)OMe$; $R^3 = H$
30: $R^1 = R^2 = H$; $R^3 = CH_2CH_2C(O)Ot$Bu
31: $R^1 = H$; $R^2 = CH_2CH_2C(O)Ot$Bu; $R^3 = H$
32: $R^1 = R^2 = H$; $R^3 = CH_2CH_2C(O)OBn$;
33: $R^1 = H$; $R^2 = CH_2CH_2C(O)OBn$; $R^3 = H$
34: $R^1 = R^2 = H$; $R^3 = CH_2CH_2COOH$;
35: $R^1 = H$; $R^2 = CH_2CH_2COOH$; $R^3 = H$
36: $R^3 = R^2 = H$; $R^3 = Ph$
37: $R^1 = H$; $R^2 = Ph$; $R^3 = H$



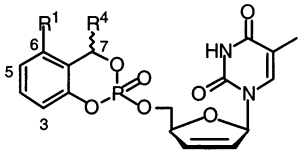
benzo(a)-*cyclo*Sal-d4TMP **38**



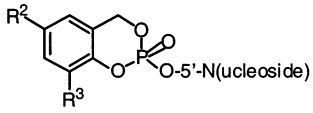
benzo(b)-*cyclo*Sal-d4TMP **39**



benzo(c)-*cyclo*Sal-d4TMP **40**



41: $R^1 = Cl$; $R^4 = H$
42: $R^1 = Cl$; $R^4 = Me$
43: $R^1 = Cl$; $R^4 = n$-Bu
44: $R^1 = Cl$; $R^4 = CH_2C(O)OEt$ (ECM)
45: $R^1 = H$; $R^4 = CH_2Cl$
46: $R^1 = H$; $R^4 = CHCl_2$
47: $R^1 = H$; $R^4 = CCl_3$



48: $R^2 = H$; $R^3 = Ph$; N = BVdU
49: $R^2 = OMe$; $R^3 = H$; N = d4A

Figure 2. Structural formulas of the studied *cyclo*Sal-phosphate triesters.

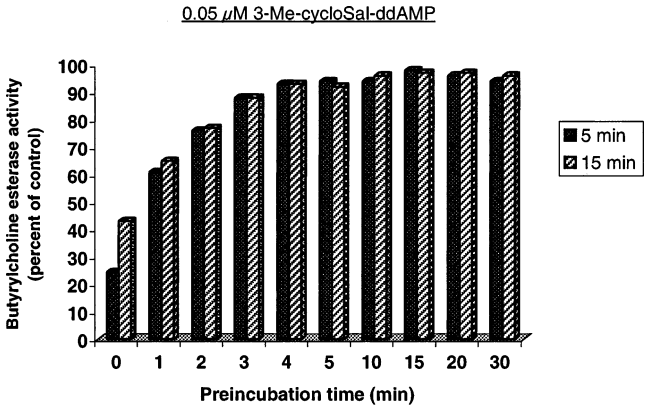


Figure 3. Effect of preincubation of human serum AChE with 3-Me-*cyclo*Sal-ddAMP **14** on enzyme activity.

were plotted as a Lineweaver−Burk diagram. The inhibition of the enzyme proved competitive with respect to the substrate. The K_i values were 0.017 and 0.027 μM (measured after 5 and 15 min incubation, respectively), whereas the K_m values ranged between 862 and 977 μM which results in a K_i/K_m ratio of $\sim 2 \times 10^{-5}$ (Figure 4). These results prove that the reaction of 3-methyl-*cyclo*Sal-ddAMP **14** took place in the active site and again point to the known suicide mechanism.

Variation of the Nucleoside Part. First, a variety of 3-methyl-*cyclo*Sal-nucleotides **1**−**18** were evaluated for their inhibitory effects on human BChE present in human serum. 3-Methyl-substituted *cyclo*Sal derivatives have been selected because these methyl-substituted triesters showed optimal antiviral activities so far. First, the use of the competitive BChE-selective inhibitor ethopropazine proved that the enzyme activity and the inhibitory effects of the compounds discussed below are solely a result of the inhibition of BChE. As expected, there was no AChE activity in human serum. The enzymatic activity observed in the serum could be suppressed by ethopropazine in a concentration-dependent

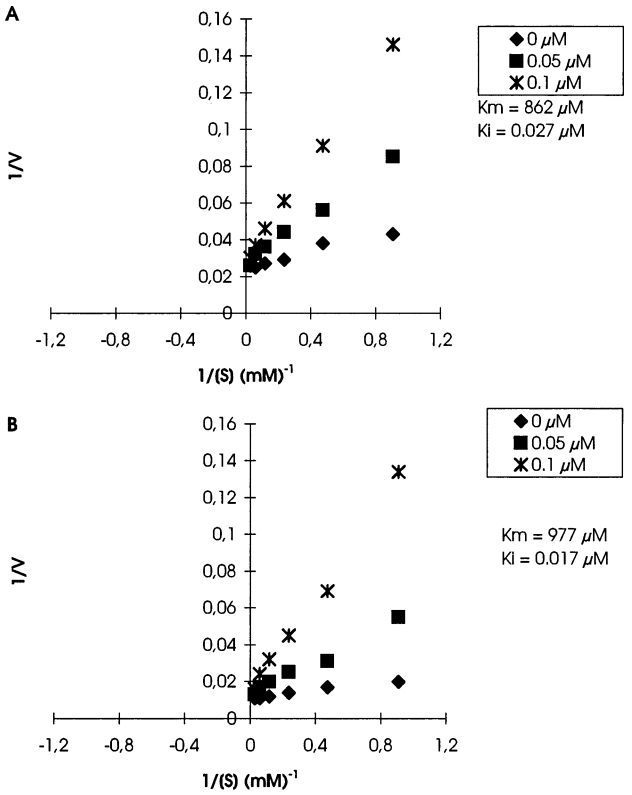


Figure 4. Lineweaver−Burk plots for the inhibitory activity of 3-Me-*cyclo*Sal-ddAMP **14** against human serum AChE (a) after 5 min and (b) after 15 min incubation.

manner. At 46 μM ethopropazine, no cleavage of acetylcholine was observed. Moreover, such experiments were also carried out with isolated human butyrylcholinesterase (Sigma). No difference was observed in the experiments using human serum or isolated human BChE. Most of the 3-methyl-*cyclo*Sal-phosphate triesters were tested as diastereomeric mixtures. The results are summarized in Table 1.

None of the tested *cyclo*Sal-triesters was found to be inhibitory to human AChE and electric eel AChE (only data from *electrophorus electricus* AChE are shown in Table 1). In human serum, clear differences can be observed within the series of pyrimidine nucleotide prodrugs. Nucleotide prodrugs bearing nucleosides with a 3′-substituent like the azido or the hydroxy group showed very weak if any inhibitory potency to BChE (entries 1, 2, 6, 7). It seems that even the nature of the heterocycle has an influence on the inhibitory effect against the enzyme. While the thymine-bearing nucleotide triester **1** showed weak inhibition (IC_{50} = 36 μM), modified uracil heterocycles bearing nucleotide triesters such as 5-fluorouracil or (*E*)-5-(2-bromovinyl)uracil were devoid of any inhibitory potency (entries 6, 7).

As the pyrimidine nucleotide prodrugs, purine nucleotide-bearing derivatives showed a broad range of IC_{50} values. The most inhibiting derivatives of all compounds tested are 3-methyl-*cyclo*Sal-triesters bearing a 2′,3′-dideoxy (d2) or a 2′,3′-dideoxy 2′,3′-didehydro (d4)-sugar residue (triesters **3**, **4**, **14**, **15**, **17**, **18**). IC_{50} values varied between 0.9 μM and 1.3 μM. However, the 2′-*ara*-fluoro counterpart **16** (entry 16) proved to be 5-fold less inhibitory as compared to the ddA triester **14** (entry 14). Interestingly, all guanine bearing triesters **8**−**11**, except the carbovir triester **12** and the modified guanine triester derivative ABC **18**, proved to be entirely noninhibitory toward BChE irrespective of having a cyclic or an acyclic "glycon" residue. 3-Methyl-*cyclo*Sal-ACVMP **9** proved also noninhibitory against BChE. This compound has no 3′-substituent and a very flexible backbone, which should be able to adopt the structural requirement in the active site of the enzyme. In contrast, carbovir triester **12** bearing a guanine moiety but a d4-sugar showed an IC_{50} of 2.5 μM. This result clearly points to the predominant importance of the presence of a lipophilic "glycon" in the nucleoside leading to high BChE inhibitory potency.

Table 1. Inhibitory Activity and Bioactivity of Diastereomeric Mixtures of 3-Methyl-*cyclo*Sal-nucleotides: Variations in the Nucleoside Part

	triester	nucleoside	IC_{50}[a] (μM) AChE	IC_{50}[a] (μM) BChE	biol. activity (μM) [ref]
1	**1**	dT	>50	36	n.a.[b]
2	**2**	AZT	>50	40	0.006[c] [19]
3	**3**	ddT	>50	1.0	4.3[c] [22]
4	**4**	d4T	>50	1.2	0.087[c] [5]
5	**5**	3′-*O*-Lev-dT	>50	>50	n.a.[b]
6	**6**	5-FdU	>50	>50	0.04[d] [21]
7	**7**	BVdU	>50	>50	4.1[e] [10]
8	**8**	dG	>50	>50	n.a.[b]
9	**9**	ACV	>50	>50	0.15[f] [9]
10	**10**	GCV	>50	>50	3.3[f] [22]
11	**11**	PCV	>50	>50	17.7[f] [23]
12	**12**	CBV	>50	2.5	0.47[c] [8]
13	**13**	dA	>50	28	n.a.[b]
14	**14**	ddA	>50	0.91	0.047[c] [6]
15	**15**	d4A	>50	1.2	0.065[c] [6]
16	**16**	F-*ara*-ddA	>50	5.0	3.67[c] [7]
17	**17**	F-*ribo*-ddA	>50	1.1	11.7[c] [7]
18	**18**	ABC	>50	1.3	0.70[c] [8]

[a] IC_{50}: 50% inhibitory concentration for the acetylcholine cleavage by the AChE (*Electrophorus electricus*) and human BChE.[b] Not available. [c] Anti-HIV-1 activity (EC_{50}): 50% effective concentration in human lymphocytic CEM cells. [d] Antitumor activity (IC_{50}): 50% inhibitory concentration in L1210 cells. [e] Anti-EBV activity (EC_{50}) 50% effective concentration blocking EBV-DNA synthesis. [f] Anti-HSV-1 activity (EC_{50}): 50% effective concentration in Vero cells.

BChE Inhibition as a Function of the Substitution Pattern in the *cyclo*Sal Moiety. Next, the effect of the different substituents in different positions of the *cyclo*Sal-group was studied. We have chosen d4T as the nucleoside analogue because 3-methyl-*cyclo*Sal-d4TMP **4** belongs to the most potent BChE-inhibiting triesters (Table 1, entry 4). Moreover, so far we have the largest number of different *cyclo*Sal-derivatives prepared with d4T available. The results are summarized in Table 2.

The nature of the substituent had a pronounced effect on the prodrug's inhibition of the enzyme. As compared to 3-methyl triester **4**, the unsubstituted *cyclo*Sal-d4TMP **19** was even more inhibitory to BChE (0.77 μM). This increased potency has been expected from previous results obtained by Casida et al.[16] However, the inhibitory effect decreases significantly by introducing bulky alkyl substituents. For instance, the 3-*tert*-butyl group resulted in a 3.5-fold decreased inhibitory activity compared to the 3-methyl-substituted prodrug **4** (Table 2). Modification of the 5-position of the *cyclo*Sal-group (triesters **20**, **23**, **27**) showed rather identical inhibitory activity as compared to corresponding modifications at the 3-position (triesters **4**, **22**, **26**). However, the inhibitory potency was most efficiently decreased by concomitant introduction of substituents in the 3- *and* the 5-position. 3,5-Dimethyl-*cyclo*Sal-d4TMP **21** and 3,5-

Table 2. Inhibitory Activity and Anti-HIV-1 Activity of Diastereomeric Mixtures of *cyclo*Sal-D4TMP Phosphate Triesters: Variations in the *cyclo*Sal Moiety

	triester	substituent	IC_{50}[a] (μM) AChE	IC_{50}[a] (μM) BChE	EC_{50} (μM)[b]
1	**19**	H	>50	0.77	0.28
2	**4**	3-Me	>50	1.2	0.10
3	**20**	5-Me	>50	4.6	0.18
4	**21**	3,5-Me	>50	46	0.10
5	**22**	3-*t*Bu	>50	4.2	0.18
6	**23**	5-*t*Bu	>50	5.6	0.14
7	**24**	3,5-*t*Bu	>50	>50	1.1
8	**25**	3,5-*t*Bu,6-F	>50	48	0.16
9	**26**	3-*s*Bu	>50	2.0	0.08
10	**27**	5-*s*Bu	>50	3.3	1.9
11	**28**	3-MeOOC Et	>50	2.7	0.16
12	**29**	5-MeOOC Et	>50	8.8	0.24
13	**30**	3-*t*BuOOC Et	>50	3.4	0.33
14	**31**	5-*t*BuOOC Et	>50	4.4	0.18
15	**32**	3-BnOOC Et	>50	2.8	0.15
16	**33**	5-BnOOC Et	>50	22	0.08
17	**34**	3-HOOC Et	>50	>50	0.19
18	**35**	5-HOOC Et	>50	>50	0.14
19	**36**	3-Ph	>50	0.35	0.13
20	**37**	5-Ph	>50	4.4	0.40
21	**38**	benzo[*a*]	>50	0.25	0.14
22	**39**	benzo[*b*]	>50	0.41	0.41
23	**40**	benzo[*c*]	>50	3.9	0.09
24	**41**	6-Cl	>50	0.57	0.09
25	**42**	6-Cl, 7-Me	>50	1.8	0.19
26	**43**	6-Cl, 7-Bu	>50	13	0.19
27	**44**	6-Cl, 7-ECM	>50	>50	0.14
28	**45**	7-CH_2Cl	>50	3.9	0.19
29	**46**	7-$CHCl_2$	>50	26	0.16
30	**47**	7-CCl_3	>50	27	0.19

[a] S_p and R_p represent the configuration of the diastereomers. R_p, S_p represents an equal mixture of both diastereomers that could not be separated. [b] IC_{50}: 50% inhibitory concentration for the acetylcholine cleavage by cholinesterase.

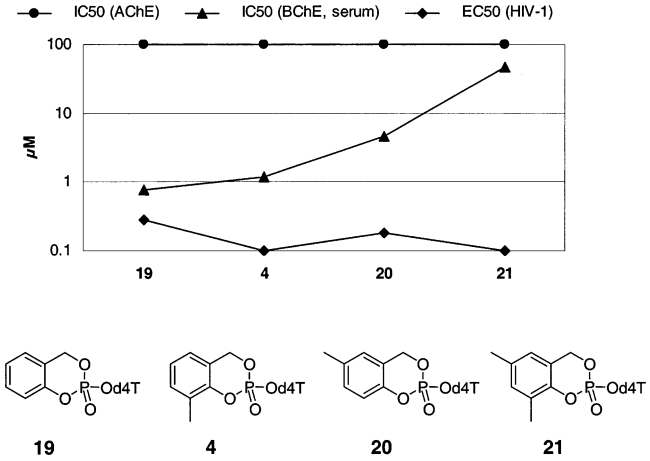


Figure 5. Graphical representation of the anti-AChE and anti-BChE potency and the antiviral activity of alkyl-substituted *cyclo*Sal-d4TMP triesters.

di-*tert*-butyl-*cyclo*Sal-d4TMP **24** proved to be virtually noninhibitory to BChE (entries 4 and 7). The varying inhibitory effects of triesters **20**−**24** due to the substitution pattern on the *cyclo*Sal part of the masking molecule has no impact on the interaction with AChE (all compounds were found to be entirely inactive) and also not on the observed anti-HIV-1 activity (all compounds showed virtually identical HIV-1 activity). This has been summarized in Figure 5.

Unfortunately, until today there is no crystal structure available from butyrylcholinesterase.[25] However, from the amino acid sequence it can be concluded that at least some resemblance exists in the overall structural features of BChE and AChE. In contrast to BChE, crystallographic structures of AChE have been resolved for different species including *Drosophila*,[26] *Torpedo californica*,[27] mouse[28] and man.[29] Nevertheless, due to the observation that *cyclo*Sal-triesters are *not* inhibitors of AChE, this enzyme has not been used as an appropriate BChE model for molecular modeling studies. However, from the observed potential for variation in the substitution pattern on the BChE inhibition it can be concluded that the *cyclo*Sal-moiety as such does not entirely fill the active site pocket.

From the studies described above, structural requirements can be derived in order to make the binding of the triester weaker. It was shown in our experiments using isolated BChE and BChE in human serum that substituents particularly in the 3- and 5-position of the *cyclo*Sal-moiety should have a pronounced effect on the binding. So, we included *cyclo*Sal-d4TMP triesters **36** and **37** (Table 2, entries 19, 20) that bear larger substituents than the *tert*-butyl group.[30] A phenyl residue in the 3- or 5-position is a rigid substituent which spans a distance of about 5 Å. Surprisingly, triester **36** was found to be one of the most inhibitory triesters among the whole d4TMP series (0.35 μM)! We conclude that due to the presence of a number of aromatic amino acids in the active site of BChE, the phenyl ring may accommodate a lipophilic aromatic pocket stabilized by $\pi-\pi$-interactions. However, as expected, introduction of the phenyl ring in the 5-position in triester **37** led to a 6-fold reduction of the inhibitory potency as compared to the unsubstituted *cyclo*Sal-ring in triester **19**. The same trend was found for the ring anullated benzo[*a*]-, benzo[*b*]-, and benzo[*c*]-*cyclo*Sal-d4TMPs **38**−**40**.[30] The more the additional aromatic ring is shifted away from the 3-position, the weaker the inhibitory activity (entries 21−23). Obviously, the highly lipophilic nature of the active site of BChE can also be deduced from the values found for propionic acid-modified *cyclo*Sal-d4TMP triesters **34** and **35**. The acid group is deprotonated under the reaction conditions used in the assay and the presence of the highly polar carboxylate is the reason for these compounds being devoid of any inhibitory activity against BChE (entries 17, 18). Interestingly, substitutions at the 7-position by methyl (**42**), butyl (**43**), or ethoxycarbonylmethyl (ECM; **44**) led to a reduction of the binding properties.[31] Moreover, large substituents such as the chlorinated 7-methyl-groups also considerably decrease the binding affinity (triesters **45**−**47**, entries 28−30).[31]

Finally, it should be added that for most of the studied cases, the described compounds bearing different substitution patterns in the *cyclo*Sal-part are still antivirally active and retained the antiviral potency in thymidine-kinase (TK)-deficient cell lines and thus achieved the TK-bypass. This can be demonstrated for example with 3,5-bis-*tert*-butyl-6-fluoro-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate **25**. This compound showed a chemical half-life of 6.2 h, only weak if any inhibitory activity against BChE (IC_{50} 48 μM; Table 2), no AChE inhibition (IC_{50} >50 μM), strong anti-HIV-1 (EC_{50} 0.13 μM, Table 2) and anti-HIV-2 activity (EC_{50} 0.33 μM) and full retention of activity in HIV-2-infected TK-deficient CEM cells (EC_{50} 0.60 μM).[32]

Table 3. Dependence of the Inhibitory Activity against AChE (*Electrophorus electricus*) and Human BChE on the Stereochemistry of the *cyclo*Sal-nucleotides

	triester	config[a]	IC_{50}[b] (μM) AChE	IC_{50}[b] (μM) BChE	antiviral act. (μM)
1	3-Me-*cyclo*Sal-d4TMP **4**	R_p	>50	>50	0.08[c]
2	3-Me-*cyclo*Sal-d4TMP **4**	S_p	>50	0.24	0.42[c]
3	3-*t*Bu-*cyclo*Sal-d4TMP **22**	R_p	>50	>50	0.13[c]
4	3-*t*Bu-*cyclo*Sal-d4TMP **22**	S_p	>50	2.6	0.6[c]
5	3-Me-*cyclo*Sal-FdUMP **6**	R_p	>50	>50	0.040[d]
6	3-Me-*cyclo*Sal-FdUMP **6**	S_p	>50	>50	0.044[d]
7	3-Ph-*cyclo*Sal-BVdUMP **48**	R_p	>50	>50	-
8	3-Ph-*cyclo*Sal-BVdUMP **48**	S_p	>50	3.7	-
9	5-OMe-*cyclo*Sal-d4AMP **49**	R_p	>50	>50	0.043[c]
10	5-OMe-*cyclo*Sal-d4AMP **49**	S_p	>50	1.8	0.5[c]
11	3-Me-*cyclo*Sal-GCVMP **10**	R_p	>50	>50	3.29[e]
12	3-Me-*cyclo*Sal-GCVMP **10**	S_p	>50	23	-

[a] IC_{50}: 50% inhibitory concentration for the acetylcholine cleavage by cholinesterase. [b] Anti-HIV-1 activity (EC_{50}): 50% effective concentration in CEM cells

Due to the fact that the active site of the enzyme is chiral, a difference in the inhibition may be observed with respect to the stereochemistry at the phosphorus atom of the *cyclo*Sal-triesters.

Dependence of the Inhibitory Potency on the P-Stereochemistry. Some of the prepared *cyclo*Sal-NMP triesters have been separated into their diastereoisomers by semipreparative HPLC. Compounds were found to be stereochemically purely separated as judged by ^{1}H NMR, ^{31}P NMR, and HPLC. These separated diastereomers then were subjected to the assays with AChE from electric eel and BChE from human serum. Again, *no* inhibitory activity has been found against AChE for *both* diastereoisomers. Much more interesting was the behavior of the triesters against human BChE (Table 3). As expected, a marked difference in the inhibition was observed. In all cases, the S_p diastereoisomer was found to be inhibitory toward the enzyme while the R_p isomers were entirely noninhibiting, e.g. the R_p diastereomer of the d4TMP derivatives **4**,**22** were devoid of appreciable inhibitory activity while their S_p derivatives showed pronounced inhibitory activity. This behavior was independent of the nature of the nucleoside (Table 3). Thus, human BChE is able to markedly discriminate between the S_p and R_p diastereomers of the *cyclo*Sal-nucleotide prodrugs. To some extent, such stereoselectivity has also been reported for *T. californica* AChE against E-2020 ((*R*,*S*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine (donepezil hydrochloride; Aricept:[33] the enzyme showed a 5-fold higher affinity for the *R*-enantiomer (K_i = 3.3 nM) than for the *S*-enantiomer (K_i = 17.5 nM). In contrast, among the *cyclo*Sal-nucleotide prodrugs, the difference in IC_{50} between the two diastereomers was found to be up to >200-fold.

Moreover, an interesting inverse correlation between the inhibitory potency and the antiviral activity was observed. As a rule, the most antivirally active diastereomer corresponded to the least BChE-inhibitory diastereomer. Thus, the R_p diastereomer of 3-Me-*cyclo*Sal-d4TMP **4**, while not being inhibitory to human BChE at 50 μM, was 5-fold more anti-HIV active (0.087 μM) than the S_p diastereomer.[6a] In contrast, the S_p diastereomer was markedly more inhibitory against BChE (0.24 μM). Also, 5-OMe-d4AMP **49** (R_p) was 11-fold more inhibitory to HIV than 5-OMe-d4AMP (S_p) (EC_{50}: 0.043 and 0.50 μM, respectively).[7] Furthermore, the R_p diastereomer was not inhibitory against human BChE at 50 μM whereas the S_p diastereomer was inhibitory at 1.8 μM (Table 3). In deciding which *cyclo*Sal anti-HIV prodrug(s) should be chosen as clinical candidate(s) the optimal phosphorus configuration is R_p, but it would be advantageous to choose *cyclo*Sal substituents with reduced anti-BChE activity of the S_p configuration to avoid the need of separating the diastereomers.

Studies with AChE from Different Sources. Finally, selected *cyclo*Sal-phosphate triesters were studied against AChE and BChE from different origins. As summarized in Table 4, *cyclo*Sal-phosphate triesters were found to show 10−30-fold lower affinities for the murine serum BChE enzyme as compared to the human serum BChE and lack of measurable inhibitory effect against the calf and erythrocyte AChE.

Thus, the results obtained with beef erythrocyte AChE were in full agreement with the results found with AChE from electric eel and man. In the case of BChE, the results clearly prove that even functionally similar enzymes may behave differently with respect to the source of the species they were derived from. This may be related to subtle differences in the structure of the enzymes.

Conclusion

In summary, we can conclude from the presented data that *cyclo*Sal-phosphate triesters of various nucleosides and nucleoside analogues are noninhibitory to human AChE. Some of the compounds show significant inhibitory activity against human serum BChE and to a minor extent against mouse serum BChE. However, several structural factors clearly determine the inhibitory potency of the compounds:

Table 4. Inhibitory Activities of *cyclo*Sal NMP Derivatives against Cholinesterases from Different Species

*cyclo*Sal-triester		configuration[a]	IC_{50}[b] (μM) human serum BChE	mouse serum BChE	calf serum AChE	beef erythrocytes AChE
3-Me-*cyclo*Sal-ddAMP	**14**	(R_p, S_p)	0.91	2.35	>50	>50
3-Me-*cyclo*Sal-d4AMP	**15**	(R_p, S_p)	1.2	10.5	>50	>50
3-Me-*cyclo*Sal-F-*ara*-ddAMP	**16**	(R_p, S_p)	5.0	>50	>50	>50
3-Me-*cyclo*Sal-d4TMP	**4**	(S_p)	0.24	≥5	>50	>50
3-Me-*cyclo*Sal-d4TMP	**4**	(R_p)	>50	>50	>50	>50
3-Me-*cyclo*Sal-AZTMP	**2**	(R_p, S_p)	>50	>50	>50	>50

[a] Stereochemistry at the phosphorus atom of the *cyclo*Sal-phosphate triester. [b] IC_{50}: 50% inhibitory concentration for the acetylcholine cleavage by the AChE (*Electrophorus electricus*) and human BChE. [c] Anti-HIV-1 activity (EC_{50}): 50% effective concentration in wild-type CEM cell cultures. [d] Cytostatic activity (IC_{50}): 50% inhibitory concentration against L1210 cells. [e] Anti-HSV-1 activity (EC_{50}): 50% effective concentration in Vero cell cultures.

(i) polar or bulky substituents in the 3′-position of the glycon ring reduce or destroy the inhibitory effect;

(ii) polar or bulky substituents in the 3-, 5-, and/or 7-position of the *cyclo*Sal-moiety reduce the inhibitory potency;

(3) the inhibitory effect depends on the stereochemistry at the phosphorus atom. While the R_p stereoisomers were devoid of any enzyme inhibition, only the S_p diastereomers showed an inhibitory effect. Importantly, the R_p diastereomer was found to be antivirally more active as compared to the S_p isomer.

Moreover, a few characteristics of the enzyme can be concluded. The active site seems to be highly lipophilic and is able to accommodate the *cyclo*Sal-triesters. However, there should be significant structural differences between the two enzymes because *cyclo*Sal-triesters are inhibitory to human and murine BChE but not to human and bovine AChE. Work in order to eliminate the inhibition of BChE by structural changes of the *cyclo*Sal-moiety seem to be possible with the help of the presented data and work along this is under current investigation in our laboratories.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions (argon or nitrogen atmosphere). Solvents: Anhydrous methylene chloride (CH_2Cl_2), anhydrous tetrahydrofuran (THF), and anhydrous acetonitrile (CH_3CN) were obtained in a Sure/Seal bottle from Fluka and stored over 4 Å molecular sieves; ethyl acetate, methylene chloride, and methanol employed in chromatography were distilled prior to use. Diisopropyethylamine (DIPEA) was distilled from sodium prior to use. Evaporation of solvents was carried out on a rotary evaporator under reduced pressure or using a high-vacuum pump. Chromatography: Chromatotron (Harrison Research 7924), silica gel 60_{Pf} (Merck, "gipshaltig"), UV detection at 254 nm; for column chromatography, Merck silica gel 60, 230−400 mesh was used. TLC: analytical thin-layer chromatography was performed on Merck precoated aluminum plates 60 F_{254} with a 0.2 mm layer of silica gel containing a fluorescence indicator; sugar-containing compounds were visualized with the sugar spray reagent (0.5 mL of 4-methoxybenzaldehyde, 9 mL of ethanol, 0.5 mL of concentrated sulfuric acid, and 0.1 mL of glacial acetic acid) by heating with a fan or on a hot plate. NMR spectra were recorded using (^{1}H NMR) Bruker AC 250 at 250 MHz, Bruker WM 400 at 400 MHz, Bruker AMX 400 at 400 MHz or Bruker DMX 500 at 500 MHz; (^{13}C NMR) Bruker WM 400 at 101 MHz, Bruker AMX 400 at 101 MHz or Bruker DMX 500 at 126 MHz (calibration was done in both cases with the solvent); (^{31}P NMR) Bruker AMX 400 at 162 MHz or Bruker DMX 500 at 202 MHz (H_3PO_4 as external standard); (^{19}F NMR) Bruker WH 270 at 254 MHz or Bruker DMX 500 at 471 MHz ($CFCl_3$ as external standard). All ^{1}H and ^{13}C NMR chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane. ^{31}P NMR chemical shifts are quoted in ppm using H_3PO_4 as external reference. ^{19}F NMR chemical shifts are quoted in ppm using $CFCl_3$ as external reference. The spectra were recorded at room temperature, and all ^{13}C and ^{31}P NMR were recorded in proton-decoupled mode. UV/vis extinction was measured with a Varian Cary 1E UV/Vis spectrometer. Mass spectra were obtained with a Finnigan electrospray MAT 95 Trap XL (ESI) or a VG Analytical VG/70−250 F spectrometer (FAB, matrix was *m*-nitrobenzyl alcohol). The test compounds were isolated as mixtures of diastereomers arising from the mixed stereochemistry at the phosphate center. In some cases, separation of diastereomers was performed using semipreparative or preparative RP-HPLC (Merck, acetonitrile/water eluents). The resulting lyophilized triesters were found to be pure by HPLC analysis, high-field multinuclear NMR spectroscopy, and mass spectroscopy. Synthesis of some of the *cyclo*Sal-nucleoside monophosphates has been described before.[6a,7,8,11b]

General Procedure for the Preparation of the *cyclo*Sal-nucleoside Monophosphates. (A) The appropriate salicyl alcohol derivatives needed for the preparation of the *cyclo*Sal phosphotriesters were obtained by established methods[2a,20,31] except of the synthesis of **51**. They were converted into the chlorophosphites (phosphitylating agents) by the procedure published before.[1,20] The highly moisture sensitive chlorophosphites were used in the subsequent synthetic procedure without further purification.

To a solution of the nucleoside analogue (0.40 mmol) in 10 mL of dry acetonitrile was added diisopropylethylamine (0.80 mmol, DIPEA). The resulting solution was cooled to −20 °C, and the appropriate chlorophosphite (0.80 mmol if calculated as hypothetically pure product) was added. The solution was allowed to warm to room temperature, and stirring was continued for 1 h (TLC monitoring). Afterward, *tert*-butyl hydroperoxide (1.4 mmol, solution in *n*-decane) was added at −20 °C. After warming up to room temperature and stirring for 1 h again (TLC monitoring), the solvent was removed under reduced pressure. The resulting residue was purified by preparative TLC (Chromatotron, 1. ethyl acetate/methanol 9:1, 2. Dichloromethane/methanol gradient). Subsequent lyophilization yielded the products as colorless foams.

(B) Analogously to procedure A, but with the use of dry DMF/THF 2:1 as solvent and 0.60 mmol chlorophosphite.

(C) Analogously to procedure A, but with the use of dry DMF/THF 1:5 as solvent and 0.60 mmol chlorophosphite. Addition of the chlorophosphite was carried out at −40 °C, and the solution was allowed to warm to 0 °C to complete the phosphitylation reaction.

(D) Analogously to procedure A, but with the use of dry DMF/THF 1:10 as solvent. The phosphitylation reaction was carried out at −70 °C for 15 min.

(E) The appropriate DMTr-protected nucleoside analogues have been converted into the corresponding *cyclo*Sal-nucleoside monophosphates according to procedure A. Afterward, deprotection was carried out using benzenesulfonic acid or trifluoroacetic acid in dichloromethane/methanol 7:3 at room temperature. After evaporation of the solvent under reduced pressure and purification by preparative TLC (Chromatotron, dichloromethane/methanol 17:3), the product was lyophilized.

3-Methyl-*cyclo*Sal-thymidine monophosphate 1: procedure B; yield 31%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 2H, NH); 7.45 (s, 1H, H6-thymine); 7.43 (s, 1H, H6-thymine); 7.29−7.24 (m, 2H, H4-aryl); 7.12−7.08 (m, 4H, H5-aryl, H6-aryl); 6.19 (dd, 1H, H1′); 6.18 (dd, 1H, H1′); 5.50 (dd, 1H, H-benzyl); 5.48 (dd, 1H, H-benzyl); 5.45 (s, 2H, OH); 5.44 (dd, 1H, H-benzyl); 5.41 (dd, 1H, H-benzyl); 4.38−4.21 (m, 6H, H4′, H5′); 3.93−3.91 (m, 2H, H3′); 2.23 (s, 3H, CH_3-aryl); 2.22 (s, 3H, CH_3-aryl); 2.18−2.08 (m, 4H, H2′); 1.75 (s, 3H, CH_3-thymine); 1.72 (s, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.8 (C4-thymine); 163.8 (C4-thymine); 150.5 (C2-thymine); 148.1 (d, C2-aryl); 148.1 (d, C2-aryl); 136.0 (C6-thymine); 135.9 (C6-thymine); 131.1 (C4-aryl); 127.0 (C3-aryl); 127.0 (d, C3-aryl); 124.1 (d, C6-aryl); 123.8 (C5-aryl); 121.2 (d, C1-aryl); 121.1 (d, C1-aryl); 110.0 (C5-thymine); 110.0 (C5-thymine); 84.3 (d, C4′); 84.3 (d, C4′); 84.2 (C1′); 84.1 (C1′); 70.1 (C3′); 70.0 (C3′); 68.6 (d, C5′); 68.5 (d, C5′); 68.0 (d, C-benzyl); 67.9 (C-benzyl); 38.8 (C2′); 38.6 (C2′); 15.0 (CH_3-aryl); 15.0 (CH_3-aryl); 12.2 (CH_3-thymine); 12.2 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.72; −7.75; R_f value 0.47 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 425.1114 (M + H$^+$), found 425.1121 (M + H$^+$).

3-Methyl-*cyclo*Sal-3′-deoxythymidine monophosphate 3: procedure A; yield 21%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.28 (s, 2H, NH); 7.45 (s, 1H, H6-thymine); 7.44 (s, 1H, H6-thymine), 7.28−7.25 (m, 2H, H4-aryl); 7.12−7.08 (m, 4H, H5-aryl, H6-aryl); 6.02 (dd, 1H, H1′); 6.00 (dd, 1H, H1′); 5.51 (dd, 1H, H-benzyl); 5.47 (dd, 1H, H-benzyl); 5.43 (dd, 1H, H-benzyl); 5.40 (dd, 1H, H-benzyl); 4.40−4.23 (m, 4H, H5′); 4.22−4.16 (m, 2H, H4′); 2.30−2.23 (m, 2H, H2′); 2.23 (s, 3H, CH_3-aryl); 2.22

(s, 3H, CH_3-aryl); 2.06−1.90 (m, 4H, H2′, H3′); 1.89−1.82 (m, 2H, H3′); 1.75 (s, 3H, CH_3-thymine); 1.72 (s, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 150.5 (C2-thymine); 150.5 (C2-thymine); 148.1 (d, C2-aryl); 148.1 (d, C2-aryl); 135.9 (C6-thymine); 135.9 (C6-thymine); 131.1 (C4-aryl); 131.0 (C4-aryl); 127.0 (d, C3-aryl); 124.1 (C6-aryl); 123.7 (C5-aryl); 121.2 (d, C1-aryl); 109.6 (C5-thymine); 85.1 (C1′); 85.0 (C1′); 78.0 (C4′); 77.9 (C4′); 69.2 (d, C5′); 69.1 (d, C5′); 68.6 (d, C-benzyl); 68.4 (d, C-benzyl); 30.5 (C2′); 30.4 (C2′); 25.4 (C3′); 25.3 (C3′); 15.0 (CH_3-aryl); 15.0 (CH_3-aryl); 12.2 (CH_3-thymine); 12.2 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.65; −7.73; R_f value 0.42 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 409.1165 (M + H$^+$), found 409.1159 (M + H$^+$).

3-Methyl-*cyclo*Sal-3′-*O*-levulinyl-thymidine monophosphate 5: procedure A; yield 33%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH); 11.37 (s, 1H, NH); 7.52 (s, 1H, H6-thymine); 7.49 (s, 1H, H6-thymine); 7.29−7.25 (m, 2H, H4-aryl); 7.13−7.09 (m, 4H, H5-aryl, H6-aryl); 6.17 (dd, 2H, H1′); 5.54−5.40 (m, 4H, H-benzyl); 5.18 (ddd, 1H, H3′); 5.16 (ddd, 1H, H3′); 4.42−4.31 (m, 4H, H5′); 4.16−4.12 (m, 2H, H4′); 2.75 (t, 2H, H3-Lev); 2.75 (t, 2H, H3-Lev); 2.51−2.49 (m, 4H, H2-Lev); 2.40−2.25 (m, 4H, H2′); 2.23 (s, 3H, CH_3-aryl); 2.21 (s, 3H, CH_3-aryl); 2.13 (s, 6H, H5-Lev); 1.76 (s, 3H, CH_3-thymine); 1.74 (s, 3H; CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 207.0 (C4-Lev); 172.1 (C1-Lev); 163.8 (C4-thymine); 163.8 (C4-thymine); 150.5 (C2-thymine); 148.1 (d, C2-aryl); 148.1 (d, C2-aryl); 135.9 (C6-thymine); 135.7 (C6-thymine); 131.1 (C4-aryl); 127.1 (d, C3-aryl); 127.0 (C3-aryl); 124.1 (d, C6-aryl); 123.7 (C5-aryl); 121.1 (d, C1-aryl); 110.2 (C5-thymine); 110.1 (C5-thymine); 84.3 (C1′); 84.2 (C1′); 81.7 (d, C4′); 81.6 (d, C4′); 73.7 (C3′); 73.6 (C3′); 68.7 (d, C-benzyl); 68.5 (d, C-benzyl); 67.8 (d, C5′); 67.7 (d, C5′); 37.6 (C3-Lev); 35.7 (C2′); 35.7 (C2′); 29.6 (C5-Lev); 27.8 (C2-Lev); 15.0 (CH_3-aryl); 14.9 (CH_3-aryl); 12.2 (CH_3-thymine); 12.1 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.87; −7.90; R_f value 0.44 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 523.1482 (M + H$^+$), found 523.1492 (M + H$^+$).

3-Methyl-*cyclo*Sal-5-fluoro-2′-deoxyuridine monophosphate 6: procedure D; yield 20%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 2H, NH); 7.85 (d, 2H, H6-thymine); 7.24 (m, 2H, H4-aryl); 7.08 (m, 4H, H5-aryl, H6-aryl); 6.12 (dd, 2H, H1′); 5.48 (dd, 2H, H-benzyl); 5.40 (m, 4H, H-benzyl, OH); 4.35 (ddd, 2H, H5′); 4.25 (ddd, 2H, H5′); 4.17 (m, 2H, H3′); 3.89 (m, 2H, H4′); 2.20 (s, 6H, CH_3-aryl); 2.11 (ddd, 4H, H2′); ^{13}C NMR (63 MHz, DMSO-d_6) δ 157.0 (C4-thymine); 149.0 (C2-thymine); 147.9 (C2-aryl); 140.1 (C5-thymine); 130.9 (C4-aryl); 126.9 (C3-aryl); 124.9 (C6-thymine); 123.9 (C5-aryl); 123.5 (C6-aryl); 121.0 (C1-aryl); 84.5 (C1′); 84.3 (C4′); 69.6 (C3′); 68.5 (C5′); 67.6 (C-benzyl); 38.5 (C2′); 14.8 (CH_3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ −7.87; −7.94; ^{19}F NMR (254 MHz, DMSO-d_6) δ −182.2; R_f value 0.33 (CH_2Cl_2/MeOH, 9:1); MS (ESI$^-$, *m*/*z*) calcd 428.1 (M), found 427.4 (M).

3-Methyl-*cyclo*Sal-2′-deoxyguanosine monophosphate 8: procedure C; yield 4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 2H, NH); 7.78 (s, 2H, H8-guanine); 7.26−7.07 (m, 6H, H4-aryl, H5-aryl, H6-aryl); 6.45 (s, 4H, NH_2); 6.13 (dd, 1H, H1′); 6.13 (dd, 1H, H1′); 5.50−5.33 (m, 6H, H-benzyl, OH); 4.42−4.20 (m, 6H, H3′, H5′); 4.01−3.96 (m, 2H, H4′); 2.62−2.55 (m, 2H, H2′); 2.30−2.25 (m, 2H, H2′); 2.17 (s, 6H, CH_3-aryl); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.9 (C6-guanine); 153.8 (C2-guanine); 151.0 (C4-guanine); 148.0 (d, C2-aryl); 135.3 (C8-guanine); 135.3 (C8-guanine); 131.0 (C4-aryl); 127.1 (d, C3-aryl); 127.0 (d, C3-aryl); 124.1 (C6-aryl); 123.7 (C5-aryl); 123.7 (C5-aryl); 121.1 (d, C1-aryl); 121.0 (d, C1-aryl); 117.0 (C5-guanine); 116.9 (C5-guanine); 84.8 (C1′); 84.7 (C1′); 82.8 (C4′); 82.7 (C4′); 70.4 (C3′); 68.6 (d, C5′); 68.5 (d, C5′); 68.2 (d, C-benzyl); 68.0 (d, C-benzyl); 40.0 (C2′); 15.1 (CH_3-aryl); 15.0 (CH_3-aryl); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.48; −7.98; R_f value 0.20 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 450.1179 (M + H$^+$), found 450.1165 (M + H$^+$).

3-Methyl-*cyclo*Sal-ganciclovir monophosphate 10: procedure **E**; yield 35% (four diasteromers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (s, 4H, NH); 7.72 (s, 4H, H8-guanine); 7.24−7.20 (m, 4H, H5-aryl); 7.09−7.02 (m, 8H, H4-aryl, H6-aryl); 6.45 (s, 8H, NH_2); 5.40 (dd, 4H, H-benzyl); 5.37 (s, 8H, H1‘); 5.28 (dd, 4H, H-benzyl); 4.82 (t, 4H, OH); 4.17 (ddd, 4H, H5‘); 4.05 (ddd, 4H, H5‘); 3.75 (ddd, 4H, H4‘); 3.34 (dd, 4H, H4‘); 3.28 (dd, 4H, H3‘); 2.19 (s, 12H, CH_3-aryl); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.9 (C6-guanine); 154.0 (C2-guanine); 151.4 (C4-guanine); 148.1 (C2-aryl); 137.7 (C8-guanine); 131.0 (C4-aryl); 127.1 (C3-aryl); 124.1 (C5-aryl); 123.6 (C6-aryl); 116.6 (C5-guanine); 77.4 (C3‘); 71.2 (C1‘); 68.4 (C-benzyl); 67.2 (C5‘); 59.7 (C4‘); 15.1 (CH_3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ -7.89; R_f value 0.18 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 438.1 (M + H$^+$), found 438.4 (M + H$^+$).

3-Methyl-*cyclo*Sal-penciclovir monophosphate 11: procedure **E**; yield 43% (four diastereomers); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 4H, NH); 7.61 (s, 2H, H8-guanine); 7.59 (s, 2H, H8-guanine); 7.21 (dd, 4H, H5-aryl); 7.07−7.02 (m, 8H, H4-aryl, H6-aryl); 6.39 (s, 8H, NH_2); 5.44 (dd, 4H, H-benzyl); 5.36 (dd, 4H, H-benzyl); 4.65 (t, 2H, OH); 4.63 (t, 2H, OH); 4.18−3.85 (m, 16H, H1′, H4‘); 3.40−3.30 (m, 8H, H5‘); 2.19 (s, 6H, CH_3-aryl); 2.17 (s, 6H, CH_3-aryl); 1.72−1.60 (m, 12H, H2‘, H3‘); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.9 (C6-guanine); 153.6 (C2-guanine); 151.2 (C4-guanine); 148.1 (C1-aryl); 148.0 (C1-aryl); 137.3 (C8-guanine); 131.0 (C4-aryl); 127.0 (C3-aryl); 127.0 (C3-aryl); 124.0 (C5-aryl); 123.6 (C6-aryl); 116.7 (C5-guanine); 68.6 (C-benzyl); 68.5 (C-benzyl); 68.1 (C4‘); 59.9 (C5‘); 59.8 (C5‘); 41.2 (C1‘); 38.9 (C3‘); 38.8 (C3‘); 28.0 (C2‘); 28.0 (C2‘); 15.1 (CH_3-aryl); 15.0 (CH_3-aryl); ^{31}P NMR (162 MHz, DMSO-d_6) δ -9.09; R_f value 0.31 (CH_2Cl_2/MeOH, 9:1); MS (ESI$^+$, *m*/*z*) calcd 436.1 (M + H$^+$), found 436.3 (M + H$^+$).

3-Methyl-*cyclo*Sal-2′-deoxyadenosine monophosphate 13: procedure **C**; yield 14%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H, H2-adenine); 8.25 (s, 1H, H2-adenine); 8.12 (s, 1H, H8-adenine); 8.11 (s, 1H, H8-adenine); 7.27−7.21 (m, 6H, H4-aryl, NH_2); 7.10−7.03 (m, 4H, H5-aryl, H6-aryl); 6.36 (dd, 2H, H1′); 5.52 (s, 2H, OH); 5.48−5.31 (m, 4H, H-benzyl); 4.49−4.45 (m, 2H, H3′); 4.38 (ddd, 1H, H5′); 4.36 (ddd, 1H, H5′); 4.31−4.22 (m, 2H, H5′); 4.04−4.00 (m, 2H, H4′); 2.85−2.77 (m, 2H, H2′); 2.32 (ddd, 2H, H2′); 2.20 (s, 3H, CH_3-aryl); 2.14 (s, 3H, CH_3-aryl); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.2 (C6-adenine); 152.7 (C2-adenine); 149.2 (C4-adenine); 148.1 (C2-aryl); 139.6 (C8-adenine); 139.5 (C8-adenine); 131.0 (C4-aryl); 127.0 (d, C3-aryl); 124.0 (C6-aryl); 123.7 (C5-aryl); 123.6 (C5-aryl); 121.1 (d, C1-aryl); 119.4 (C5-adenine); 119.4 (C5-adenine); 84.9 (C1′); 84.8 (C1′); 83.6 (d, C4′); 70.4 (C3′); 68.5 (d, C5′); 68.5 (d, C5′); 68.0 (d, C-benzyl); 67.9 (d, C-benzyl); 38.6 (C2′); 38.4 (C2′); 15.0 (CH_3-aryl); 14.9 (CH_3-aryl); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.93; −8.09; R_f value 0.23 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 434.1229 (M + H$^+$), found 434.1232 (M + H$^+$).

3-*tert*-Butyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 22: procedure A; yield 91%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH); 11.35 (s, 1H, NH); 7−37−7.35 (m, 2H, H4-aryl); 7.25 (d, 1H, H6-thymine); 7.22 (d, 1H, H6-thymine); 7.21−7.13 (m, 4H, H5-aryl, H6-aryl); 6.83 (ddd, 1H, H1′); 6.83 (ddd, 1H, H1′); 6.44 (ddd, 1H, H3′); 6.42 (ddd, 1H, H3′); 6.07−6.03 (m, 2H, H2′); 5.48−5.35 (m, 4H, H-benzyl); 5.01−4.96 (m, 2H, H4′); 4.35−4.31 (m, 4H, H5′); 1.61 (d, 3H, CH_3-thymine); 1.60 (d, 3H, CH_3-thymine); 1.36 (s, 9H, CH_3-*t*Bu); 1.33 (s, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.0 (C4-thymine); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.9 (C2-thymine); 148.9 (d, C2-aryl); 148.9 (d, C2-aryl); 138.6 (d, C3-aryl); 138.5 (d, C3-aryl); 136.0 (C6-thymine); 135.9 (C6-thymine); 133.1 (C3′); 133.0 (C3′); 127.5 (C4-aryl); 127.5 (C4-aryl); 127.5 (C2′); 127.5 (C2′); 124.7 (C6-aryl); 124.4 (C5-aryl); 123.3 (d, C1-aryl); 123.2 (d, C1-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 89.4 (C1′); 89.3 (C1′); 84.4 (d, C4′); 84.3 (d, C4′); 69.1 (d, C5′); 69.0 (d, C5′); 68.4 (d, C-benzyl); 68.4 (d, C-benzyl); 34.5 (C-*t*Bu); 34.5 (C-*t*Bu); 29.8 (CH_3-*t*Bu); 29.7 (CH_3-*t*Bu); 12.0 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.23; −7.49; R_f value 0.36 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 471.1 (M + Na$^+$), 487.1 (M + K$^+$), found 471.4 (M + Na$^+$), 487.3 (M + K$^+$).

5-*tert*-Butyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 23: procedure A; yield 34%; ^{1}H NMR

(400 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH); 11.36 (s, 1H, NH); 7.41 (d, 1H, H4-aryl); 7.38 (d, 1H, H4-aryl); 7.32 (s, 2H, H6-aryl); 7.22 (d, 1H, H6-thymine); 7.18 (d, 1H, H6-thymine); 7.07 (d, 1H, H3-aryl); 7.04 (d, 1H, H3-aryl); 6.82 (ddd, 1H, H1′); 6.81 (ddd, 1H, H1′); 6.44 (ddd, 1H, H3′); 6.38 (ddd, 1H, H3′); 6.03 (ddd, 1H, H2′); 6.02 (ddd, 1H, H2′); 5.52 (dd, 1H, H-benzyl); 5.48 (dd, 1H, H-benzyl); 5.41 (d, 1H, H-benzyl); 5.38 (d, 1H, H-benzyl); 5.00−4.95 (m, 2H, H4′); 4.36−4.26 (m, 4H, H5′); 1.69 (d, 3H, CH_3-thymine); 1.61 (CH_3-thymine); 1.28 (s, 9H, CH_3-*t*Bu); 1.27 (s, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.9 (C2-thymine); 147.5 (d, C2-aryl); 147.5 (d, C2-aryl); 147.2 (C5-aryl); 135.9 (C6-thymine); 135.8 (C6-thymine); 133.1 (C3′); 133.0 (C3′); 127.5 (C2′); 127.5 (C2′); 126.9 (C4-aryl); 126.8 (C4-aryl); 123.1 (C6-aryl); 123.0 (C6-aryl); 120.7 (d, C1-aryl); 120.6 (d, C1-aryl); 117.8 (d, C3-aryl); 117.7 (d, C3-aryl); 109.8 (C5-thymine); 89.3 (C1′); 84.3 (d, C4′); 84.3 (d, C4′); 68.8 (d, C5′); 68.7 (d, C5′); 68.5 (d, C-benzyl); 68.4 (d, C-benzyl); 34.4 (C-*t*Bu); 31.2 (CH_3-*t*Bu); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.84; −7.86; R_f value 0.36 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 471.1 (M + Na^+), 487.1 (M + K^+), found 471.4 (M + Na^+), 487.3 (M + K^+).

3,5-Bis-*tert*-Butyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 24: procedure A; yield 50%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H, NH); 11.35 (s, 1H, NH); 7.32 (s, 2H, H4-aryl); 7.23−7.21 (m, 4H, H6-aryl, H6-thymine); 6.83 (ddd, 2H, H1′); 6.44 (ddd, 1H, H3′); 6.42 (ddd, 1H, H3′); 6.07−6.02 (m, 2H, H2′); 5.47−5.33 (m, 4H, H-benzyl); 5.01−4.96 (m, 2H, H4′); 4.41−4.29 (m, 4H, H5′); 1.58 (d, 3H, CH_3-thymine); 1.57 (d, 3H, CH_3-thymine); 1.36 (s, 9H, CH_3-*t*Bu); 1.33 (s, 9H, CH_3-*t*Bu); 1.28 (s, 18H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.9 (C2-thymine); 146.7 (C2-aryl); 146.6 (C2-aryl); 146.4 (C5-aryl); 137.7 (d, C3-aryl); 135.9 (C6-thymine); 135.8 (C6-thymine); 133.1 (C3′); 133.0 (C3′); 127.5 (C2′); 127.5 (C2′); 123.9 (C6-aryl); 122.5 (d, C1-aryl); 121.5 (C4-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 89.3 (C1′); 89.2 (C1′); 84.4 (d, C4′); 84.3 (d, C4′); 68.9 (d, C5′); 68.9 (d, C5′); 68.7 (d, C-benzyl); 68.6 (d, C-benzyl); 34.6 (C-*t*Bu); 34.6 (C-*t*Bu); 34.5 (C-*t*Bu); 31.3 (CH_3-*t*Bu); 29.8 (CH_3-*t*Bu); 29.7 (CH_3-*t*Bu); 11.9 (CH_3-thymine); 11.9 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −6.93; −7.23; R_f value 0.50 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 505.2104 (M + H^+), found 505.2116 (M + H^+).

3,5-Bis-*tert*-Butyl-6-fluoro-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 25: procedure A; yield 59%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH); 11.36 (s, 1H, NH); 7.26 (d, 2H, H4-aryl); 7.23 (d, 1H, H6-thymine); 7.22 (s, 1H, H6-thymine); 6.85−6.81 (m, 2H, H1′); 6.45−6.42 (m, 2H, H3′); 6.08−6.03 (m, 2H, H2′); 5.56 (dd, 1H, H-benzyl); 5.53 (dd, 1H, H-benzyl); 5.47 (dd, 1H, H-benzyl); 5.41 (dd, 1H, H-benzyl); 5.02−4.98 (m, 2H, H4′); 4.37−4.35 (m, 4H, H5′); 1.61 (s, 6H, CH_3-thymine); 1.35 (s, 9H, CH_3-*t*Bu); 1.34 (s, 18H, CH_3-*t*Bu); 1.32 (s, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.1 (C4-thymine); 164.1 (C4-thymine); 155.1 (d, C6-aryl); 151.1 (C2-thymine); 147.3 (C2-aryl); 136.1 (C6-thymine); 133.7 (C5-aryl); 133.2 (C3′); 133.1 (C3′); 131.7 (C2′); 131.6 (C2′); 127.7 (d, C3-aryl); 125.2 (d, C4-aryl); 111.5 (dd, C1-aryl); 111.3 (dd, C1-aryl); 110.1 (C5-thymine); 110.0 (C5-thymine); 89.7 (C1′); 89.5 (C1′); 84.5 (d, C4′); 84.4 (d, C4′); 69.5 (d, C5′); 69.3 (d, C5′); 63.9 (C-benzyl); 34.7 (C-*t*Bu); 34.6 (C-*t*Bu); 34.3 (d, C-*t*Bu); 30.0 (d, CH_3-*t*Bu); 29.9 (CH_3-*t*Bu); 29.8 (CH_3-*t*Bu); 12.1 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −8.71; −9.07; ^{19}F NMR (471 MHz, DMSO-d_6): δ −117.37 (d); −117.50 (d); R_f value 0.50 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 523.2009 (M + H^+), found 523.2092 (M + H^+).

3-*sek*-Butyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 26: procedure A; yield 56% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 2H, NH); 11.31 (s, 2H, NH); 7.27 (d, 4H, H4-aryl); 7.22 (d, 2H, H6-thymine); 7.18 (d, 2H, H6-thymine); 7.17−7.09 (m, 8H, H5-aryl, H6-aryl); 6.82−6.77 (m, 4H, H1‘); 6.41−6.36 (m, 4H, H3‘); 6.03−6.00 (m, 4H, H2‘); 5.48−5.32 (m, 8H, H-benzyl); 4.96−4.91 (m, 4H, H4‘); 4.35−4.21 (m, 8H, H5‘); 3.00−2.88 (m, 4H, CH-*s*Bu); 1.61 (d, 6H, CH_3-thymine); 1.60 (d, 6H, CH_3-thymine); 1.58−1.50 (m, 8H, CH_2-*s*Bu); 1.15 (d, 6H, CH−CH_3-*s*Bu); 1.12 (d, 6H, CH−CH_3-*s*Bu); 0.77−0.71 (m, 12 H, CH_2−CH_3-*s*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.9 (C2-thymine); 147.8 (C2-aryl); 147.8 (C2-aryl); 136.0 (C3-aryl); 135.9 (C3-aryl); 135.9 (C3-aryl); 133.1 (C3′); 133.0 (C3′); 133.0 (C3‘); 127.7 (C4-aryl); 127.6 (C4-aryl); 127.6 (C2′); 127.5 (C2‘); 124.5 (C6-aryl); 123.9 (C5-aryl); 122.1 (C1-aryl); 122.0 (C1-aryl); 121.9 (C1-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 89.4 (C1′); 89.2 (C1‘); 84.4 (C4′); 84.3 (C4′); 84.3 (C4′); 84.3 (C4‘); 68.9 (d, C5′); 68.9 (d, C5′); 68.7 (d, C5′); 68.6 (C5‘); 68.5 (C-benzyl); 68.4 (C-benzyl); 68.4 (C-benzyl); 68.4 (C-benzyl); 33.4 (CH-*s*Bu); 33.3 (CH-*s*Bu); 33.2 (CH-*s*Bu); 33.1 (CH-*s*Bu); 29.4 (CH_2-*s*Bu); 29.4 (CH_2-*s*Bu); 29.1 (CH_2-*s*Bu); 28.9 (CH_2-*s*Bu); 20.7 (CH-CH_3-*s*Bu); 20.6 (CH-CH_3-*s*Bu); 20.3 (CH-CH_3-*s*Bu); 12.1 (CH_2-CH_3-*s*Bu); 12.1 (CH_2-CH_3-*s*Bu); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −6.80; −6.89; −7.21; −7.24; R_f value 0.36 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 471.1 (M + Na^+), 487.1 (M + K^+), found 471.4 (M + Na^+), 487.3 (M + K^+).

5-*sek*-Butyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 27: procedure A; yield 27% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 4H, NH); 7.22−7.19 (m, 4H, H4-aryl); 7.17 (s, 4H, H6-thymine); 7.12 (s, 4H, H6-aryl); 7.04 (dd, 4H, H3-aryl); 6.82−6.79 (m, 4H, H1‘); 6.43 (ddd, 2H, H3‘); 6.36 (ddd, 2H, H3‘); 6.02 (ddd, 2H, H2‘); 6.00 (ddd, 2H, H2‘); 5.51−5.35 (m, 8H, H-benzyl); 4.98−4.95 (m, 4H, H4‘); 4.35−4.26 (m, 8H, H5‘, H5‘); 2.62−2.55 (m, 4H, CH-*s*Bu); 1.67 (s, 6H, CH_3-thymine); 1.60 (s, 6H, CH_3-thymine); 1.57−1.48 (m, 8H, CH_2-*s*Bu); 1.17 (d, 12H, CH−CH_3-*s*Bu); 0.76 (t, 6H, CH_2−CH_3-*s*Bu); 0.75 (t, 6H, CH_2−CH_3-*s*Bu); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.6 (C4-thymine); 164.6 (C4-thymine); 151.6 (C2-thymine); 151.6 (C2-thymine); 148.5 (d, C2-aryl); 148.4 (d, C2-aryl); 144.3 (C5-aryl); 136.6 (C6-thymine); 136.5 (C6-thymine); 133.7 (C3′); 133.7 (C3‘); 129.2 (C4-aryl); 129.2 (C4-aryl); 129.1 (C4-aryl); 128.2 (C2′); 128.2 (C2‘); 125.2 (C6-aryl); 125.2 (C6-aryl); 125.1 (C6-aryl); 125.1 (C6-aryl); 121.8 (d, C1-aryl); 121.7 (d, C1-aryl); 121.7 (d, C1-aryl); 118.7 (C3-aryl); 110.5 (C5-thymine); 110.5 (C5-thymine); 90.0 (C1′); 90.0 (C1‘); 85.0 (C4′); 85.0 (C4‘); 69.3 (d, C-benzyl); 69.3 (d, C-benzyl); 69.2 (d, C5′); 69.1 (d, C5‘); 41.0 (CH-*s*Bu); 41.0 (CH-*s*Bu); 31.4 (CH_2-*s*Bu); 31.3 (CH_2-*s*Bu); 31.3 (CH_2-*s*Bu); 31.3 (CH_2-*s*Bu); 22.5 (CH-CH_3-*s*Bu); 22.5 (CH-CH_3-*s*Bu); 22.5 (CH-CH_3-*s*Bu); 22.5 (CH-CH_3-*s*Bu); 12.9 (CH_2-CH_3-*s*Bu); 12.8 (CH_2-CH_3-*s*Bu); 12.8 (CH_3-thymine); 12.7 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.77; −7.78; −7.80; R_f value 0.36 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 471.1 (M + Na^+), 487.1 (M + K^+), found 471.3 (M + Na^+), 487.2 (M + K^+).

3-(Methylpropionyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 28: procedure A; yield 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H, NH); 11.32 (s, 1H, NH); 7.26 (dd, 2H, H4-aryl); 7.20 (d, 1H, H6-thymine); 7.18 (d, 1H, H6-thymine); 7.16−7.08 (m, 4H, H5-aryl, H6-aryl); 6.79 (ddd, 1H, H1′); 6.79 (ddd, 1H, H1′); 6.40 (ddd, 1H, H3′); 6.36 (ddd, 1H, H3′); 6.02 (ddd, 1H, H2′); 6.00 (ddd, 1H, H2′); 5.48 (dd, 1H, H-benzyl); 5.44 (dd, 1H, H-benzyl); 5.38 (dd, 1H, H-benzyl); 5.35 (dd, 1H, H-benzyl); 4.98−4.93 (m, 2H, H4′); 4.34−4.24 (m, 4H, H5′); 3.57 (s, 3H, OCH_3); 3.56 (s, 3H, OCH_3); 2.90−2.75 (m, 4H, CH_2-propinoyl); 2.59 (t, 2H, CH_2-propionyl); 2.57 (t, 2H, CH_2-propionyl); 1.63 (d, 3H, CH_3-thymine); 1.59 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5 (CO-propionyl); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.8 (C2-thymine); 148.1 (d, C2-aryl); 147.9 (d, C2-aryl); 135.8 (C6-thymine); 135.7 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 130.3 (C4-aryl); 130.2 (C4-aryl); 129.6 (C1-aryl); 129.5 (C1-aryl); 127.6 (C2′); 127.5 (C2′); 124.5 (C6-aryl); 124.3 (C5-aryl); 121.7 (C3-aryl); 121.6 (C3-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 89.4 (C1′); 89.3 (C1′); 84.3 (C4′); 68.8 (d, C5′); 68.8 (d, C5′); 68.4 (d, C-benzyl); 51.5 (OCH_3); 33.3 (CH_2-propionyl); 33.2 (CH_2-propionyl); 24.3 (CH_2-propionyl); 24.2 (CH_2-propionyl); 12.0 (CH_3-thymine); 11.9 (CH_3-thymine); ^{31}P NMR (202

MHz, DMSO-d_6) δ −7.53; −7.60; R_f value 0.57 (CH_2Cl_2/MeOH, 9:1); MS (ESI$^+$, *m*/*z*) calcd 479.1 (M + H$^+$), found 478.9 (M + H$^+$).

5-(Methylpropionyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 29: procedure A; yield 39%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 1H, NH); 11.32 (s, 1H, NH); 7.23−7.18 (m, 2H, H4-aryl); 7.18 (d, 1H, H6-thymine); 7.15 (d, 1H, H6-thymine); 7.13−7.11 (m, 2H, H6-aryl); 7.03 (d, 1H, H3-aryl); 7.00 (d, 1H, H3-aryl); 6.79 (ddd, 1H, H1′); 6.78 (ddd, 1H, H1′); 6.40 (ddd, 1H, H3′); 6.34 (ddd, 1H, H3′); 6.01 (ddd, 1H, H2′); 5.99 (ddd, 1H, H2′); 5.45 (dd, 1H, H-benzyl); 5.41 (dd, 1H, H-benzyl); 5.35 (d, 1H, H-benzyl); 5.33 (d, 1H, H-benzyl); 4.96−4.91 (m, 2H, H4′); 4.34−4.22 (m, 4H, H5′); 3.57 (s, 6H, OCH_3); 2.81 (t, 4H, CH_2-propionyl); 2.60 (t, 4H, CH_2-propionyl); 1.66 (d, 3H, CH_3-thymine); 1.59 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.6 (CO-propionyl); 163.9 (C4-thymine); 150.8 (C2-thymine); 148.0 (d, C2-aryl); 137.0 (C5-aryl); 135.8 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 129.8 (C4-aryl); 129.7 (C4-aryl); 129.0 (C1-aryl); 128.9 (C1-aryl); 127.5 (C2′); 127.4 (C2′); 125.9 (C6-aryl); 118.2 (C3-aryl); 118.1 (C3-aryl); 109.8 (C5-thymine); 89.3 (C1′); 84.3 (C4′); 84.2 (C4′); 68.5 (d, C5′); 68.5 (d, C5′); 68.4 (d, C-benzyl); 51.5 (OCH_3); 34.8 (CH_2-propionyl); 29.5 (CH_2-propionyl); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.98; −8.04; R_f value 0.52 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 479.1 (M + H$^+$), found 479.4 (M + H$^+$).

3-(*tert*-Butylpropionyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 30: procedure A; yield 73%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.32 (s, 1H, NH); 11.31 (s, 1H, NH); 7.27−7.24 (m, 2H, H4-aryl); 7.20 (d, 1H, H6-thymine); 7.18 (d, 1H, H6-thymine); 7.15−7.08 (m, 4H, H5-aryl, H6-aryl); 6.80−6.78 (m, 2H, H1′); 6.40 (ddd, 1H, H3′); 6.36 (ddd, 1H, H3′); 6.02 (ddd, 1H, H2′); 6.00 (ddd, 1H, H2′); 5.47 (dd, 1H, H-benzyl); 5.44 (dd, 1H, H-benzyl); 5.37 (dd, 1H, H-benzyl); 5.35 (dd, 1H, H-benzyl); 4.97−4.93 (m, 2H, H4′); 4.34−4.25 (m, 4H, H5′); 2.87−2.73 (m, 4H, CH_2-propionyl); 2.47 (t, 2H, CH_2-propionyl); 2.46 (t, 2H, CH_2-propionyl); 1.64 (d, 3H, CH_3-thymine); 1.57 (d, 3H, CH_3-thymine); 1.35 (s, 9H, CH_3-*t*Bu); 1.34 (s, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.4 (CO-propionyl); 171.3 (CO-propionyl); 163.9 (C4-thymine); 150.8 (C2-thymine); 148.0 (d, C2-aryl); 148.0 (d, C2-aryl); 135.8 (C6-thymine); 135.7 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 130.3 (C4-aryl); 129.7 (C1-aryl); 129.6 (C1-aryl); 127.5 (C2′); 124.4 (C6-aryl); 124.2 (C5-aryl); 121.7 (C3-aryl); 121.6 (C3-aryl); 109.8 (C5-thymine); 89.3 (C1′); 84.3 (C4′); 80.0 (OC-*t*Bu); 68.8 (d, C5′); 68.7 (d, C5′); 68.4 (d, C-benzyl); 68.4 (d, C-benzyl); 34.6 (CH_2-propionyl); 34.5 (CH_2-propionyl); 27.9 (CH_3-*t*Bu); 24.4 (CH_2-propionyl); 12.0 (CH_3-thymine); 11.9 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.50; −7.53; R_f value 0.46 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 521.2 (M + H$^+$), found 521.2 (M + H$^+$).

5-(*tert*-Butylpropionyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 31: procedure A; yield 44%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 1H, NH); 11.32 (s, 1H, NH); 7.23−7.18 (m, 2H, H4-aryl); 7.18 (d, 1H, H6-thymine); 7.15 (d, 1H, H6-thymine); 7.12 (d, 2H, H6-aryl); 7.03 (d, 1H, H3-aryl); 7.01 (d, 1H, H3-aryl); 6.79 (ddd, 1H, H1′); 6.77 (ddd, 1H, H1′); 6.40 (ddd, 1H, H3′); 6.34 (ddd, 1H, H3′); 6.00 (ddd, 1H, H2′); 5.99 (ddd, 1H, H2′); 5.44 (dd, 1H, H-benzyl); 5.41 (dd, 1H, H-benzyl); 5.35 (dd, 1H, H-benzyl); 5.32 (dd, 1H, H-benzyl); 4.96−4.92 (m, 2H, H4′); 4.33−4.22 (m, 4H, H5′); 2.77 (dd, 4H, CH_2-propionyl); 2.48 (dd, 4H, CH_2-propionyl); 1.67 (d, 3H, CH_3-thymine); 1.60 (d, 3H, CH_3-thymine); 1.34 (s, 9H, CH_3-*t*Bu); 1.33 (s, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5 (CO-propionyl); 163.9 (C4-thymine); 163.8 (C4-thymine); 150.8 (C2-thymine); 148.0 (C2-aryl); 137.1 (C5-aryl); 135.8 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 132.2 (C1-aryl); 129.8 (C4-aryl); 127.5 (C2′); 127.4 (C2′); 126.0 (C6-aryl); 125.9 (C6-aryl); 118.1 (C3-aryl); 118.0 (C3-aryl); 109.8 (C5-thymine); 89.3 (C1′); 84.3 (C4′); 84.2 (C4′); 79.9 (OC-*t*Bu); 68.5−68.3 (m, C5′, C-benzyl); 36.2 (CH_2-propionyl); 29.8 (CH_2-propionyl); 27.9 (CH_3-*t*Bu); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −8.02; R_f value 0.49 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 521.2 (M + H$^+$), found 521.3 (M + H$^+$).

3-(Benzylpropionyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 32: procedure A; yield 43%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 1H, NH); 11.31 (s, 1H, NH); 7.36−7.28 (m, 10H, H-phenyl); 7.26−7.23 (m, 2H, H4-aryl); 7.18 (d, 1H, H6-thymine); 7.17 (d, 1H, H6-thymine); 7.15−7.12 (m, 2H, H6-aryl); 7.09 (dd, 1H, H5-aryl); 7.08 (dd, 1H, H5-aryl); 6.79−6.77 (m, 2H, H1′); 6.37 (ddd, 1H, H3′); 6.32 (ddd, 1H, H3′); 5.99 (ddd, 1H, H2′); 5.97 (ddd, 1H, H2′); 5.47 (dd, 1H, H-benzyl); 5.44 (dd, 1H, H-benzyl); 5.37 (dd, 1H, H-benzyl); 5.34 (dd, 1H, H-benzyl); 5.07 (s, 2H, OCH_2); 5.06 (s, 2H, OCH_2); 4.94−4.90 (m, 2H, H4′); 4.32−4.22 (m, 4H, H5′); 2.92−2.80 (m, 4H, CH_2-propionyl); 2.68−2.62 (m, 4H, CH_2-propionyl); 1.64 (d, 3H, CH_3-thymine); 1.58 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.4 (CO-propionyl); 172.3 (CO-propionyl); 163.9 (C4-thymine); 150.8 (C2-thymine); 150.7 (C2-thymine); 148.0 (d, C2-aryl); 135.8 (C6-thymine); 135.7 (C6-thymine); 132.9 (C3′); 131.2 (C1-phenyl); 130.3 (C4-aryl); 129.5 (C1-aryl); 128.6 (C3-phenyl, C5-phenyl); 128.1 (C2-phenyl, C6-phenyl); 128.1 (C4-phenyl); 127.5 (C2′); 124.5 (C6-aryl); 124.3 (C5-aryl); 121.7 (C3-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 89.4 (C1′); 89.3 (C1′); 84.3 (C4′); 84.2 (C4′); 68.7 (C5′); 68.4 (d, C-benzyl); 68.4 (d, C-benzyl); 65.7 (OCH_2); 33.4 (CH_2-propionyl); 24.3 (CH_2-propionyl); 24.2 (CH_2-propionyl); 12.0 (CH_3-thymine); 11.9 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.57; R_f value 0.64 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 555.1532 (M + H$^+$), found 555.1532 (M + H$^+$).

5-(Benzylpropionyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 33: procedure A; yield 59%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (s, 1H, NH); 11.32 (s, 1H, NH); 7.36−7.26 (m, 10H, H-phenyl); 7.23−7.18 (m, 2H, H4-aryl); 7.18 (d, 1H, H6-thymine); 7.15 (d, 1H, H6-thymine); 7.11−7.09 (m, 2H, H6-aryl); 7.01 (d, 1H, H3-aryl); 6.99 (d, 1H, H3-aryl); 6.79 (ddd, 1H, H1′); 6.77 (ddd, 1H, H1′); 6.40 (ddd, 1H, H3′); 6.34 (ddd, 1H, H3′); 6.01 (ddd, 1H, H2′); 5.99 (ddd, 1H, H2′); 5.41 (dd, 1H, H-benzyl); 5.38 (dd, 1H, H-benzyl); 5.33 (d, 1H, H-benzyl); 5.30 (d, 1H, H-benzyl); 5.06 (s, 4H, OCH_2); 4.96−4.92 (m, 2H, H4′); 4.34−4.22 (m, 4H, H5′); 2.83 (t, 4H, CH_2-propionyl); 2.67 (t, 4H, CH_2-propionyl); 1.66 (d, 3H, CH_3-thymine); 1.60 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1 (CO-propionyl); 171.9 (CO-propionyl); 163.9 (C4-thymine); 150.9 (C2-thymine); 148.1 (C2-aryl); 136.9 (C1-phenyl); 136.3 (C5-aryl); 135.9 (C6); 135.8 (C6); 133.0 (C3′); 129.9 (C4-aryl); 129.8 (C4-aryl); 128.6 (C3-phenyl, C5-phenyl); 128.5 (C1-aryl); 128.2 (C4-phenyl); 128.1 (C1-phenyl, C6-phenyl); 127.5 (C2′); 126.0 (C6-aryl); 125.9 (C6-aryl); 118.2 (C3-aryl); 118.1 (C3-aryl); 109.8 (C5-thymine); 89.3 (C1′); 84.3 (C4′); 84.2 (C4′); 68.5 (d, C5′); 68.4 (d, C5′); 68.4 (d, C-benzyl); 68.3 (d, C-benzyl); 65.6 (OCH_2); 35.0 (CH_2-propionyl); 29.6 (CH_2-propionyl); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.98; −8.05; R_f value 0.63 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 555.2 (M + H$^+$), found 555.2 (M + H$^+$).

3-(2-Carboxyethyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate 34. The title compound was synthesized by treatment of ester **30** (15 mg) with trifluoroacetic acid (2.4 mL) in dichloromethane (15 mL) for 1 h at room temperature. Afterward, the solvent was removed in vacuo and the residue was purified by preparative TLC (Chromatotron, dichloromethane/methanol gradient). Subsequent lyophilization yielded the product (12 mg, 71%) as a colorless foam; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.17 (s, 2H, COOH); 11.31 (s, 2H, NH); 7.27−7.25 (m, 2H, H4-aryl); 7.19 (d, 2H, H6-thymine); 7.14−7.09 (m, 4H, H5-aryl, H6-aryl); 6.80−6.78 (m, 2H, H1′); 6.40 (ddd, 1H, H3′); 6.35 (ddd, 1H, H3′); 6.02 (ddd, 1H, H2′); 6.00 (ddd, 1H, H2′); 5.47 (dd, 1H, H-benzyl); 5.44 (dd, 1H, H-benzyl); 5.37 (dd, 1H, H-benzyl); 5.34 (dd, 1H, H-benzyl); 4.96−4.92 (m, 2H, H4′); 4.34−4.24 (m, 4H, H5′); 2.85−2.75 (m, 4H, CH_2-ethyl); 2.51 (t, 2H, CH_2-ethyl); 2.50 (t, 2H, CH_2-ethyl); 1.65 (d, 3H, CH_3-thymine); 1.58 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.6 (COOH); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.8 (C2-thymine);

147.9 (d, C2-aryl); 147.9 (d, C2-aryl); 135.8 (C6-thymine); 135.7 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 130.2 (C4-aryl); 130.0 (C1-aryl); 129.9 (C1-aryl); 127.5 (C2′); 124.4 (C6-aryl); 124.3 (C5-aryl); 121.7 (C3-aryl); 121.6 (C3-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 89.4 (C1′); 89.3 (C1′); 84.3 (C4′); 68.7 (d, C5′); 68.7 (d, C5′); 68.4 (d, C-benzyl); 68.4 (d, C-benzyl); 33.5 (CH_2-ethyl); 24.2 (CH_2-ethyl); 12.0 (CH_3-thymine); 11.9 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.51; −7.56; R_f 0.16 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 464.1 (M), found 464.2 (M).

5-(2-Carboxyethyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine Monophosphate 35. The title compound was synthesized by treatment of ester **31** (14 mg) with trifluoroacetic acid (2.2 mL) in dichloromethane (15 mL) for 1 h at room temperature. Afterward, the solvent was removed in vacuo and the residue was purified by preparative TLC (Chromatotron, dichloromethane/methanol gradient). Subsequent lyophilization yielded the product (13 mg, 93%) as a colorless foam; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 2H, COOH); 11.33 (s, 1H, NH); 11.32 (s, 1H, NH); 7.24−7.19 (m, 2H, H4-aryl); 7.18 (d, 1H, H6-thymine); 7.15 (d, 1H, H6-thymine); 7.13−7.11 (m, 2H, H6-aryl); 7.03 (d, 1H, H3-aryl); 7.01 (d, 1H, H3-aryl); 6.79 (ddd, 1H, H1′); 6.77 (ddd, 1H, H1′); 6.40 (ddd, 1H, H3′); 6.33 (ddd, 1H, H3′); 6.01 (ddd, 1H, H2′); 5.99 (ddd, 1H, H2′); 5.45 (dd, 1H, H-benzyl); 5.41 (dd, 1H, H-benzyl); 5.36 (d, 1H, H-benzyl); 5.33 (dd, 1H, H-benzyl); 4.96−4.92 (m, 2H, H4′); 4.33−4.22 (m, 4H, H5′); 2.78 (t, 4H, CH_2-ethyl); 2.51 (t, 4H, CH_2-ethyl); 1.67 (d, 3H, CH_3-thymine); 1.61 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.7 (COOH); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.8 (C2-thymine); 146.5 (d, C2-aryl); 146.2 (d, C2-aryl); 137.4 (C5-aryl); 135.8 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 129.8 (C4-aryl); 129.7 (C4-aryl); 128.7 (C1-aryl); 127.5 (C2′); 127.4 (C2′); 125.9 (C6-aryl); 125.8 (C6-aryl); 118.1 (C3-aryl); 109.8 (C5-thymine); 89.3 (C1′); 84.3 (C4′); 84.2 (C4′); 68.5 (d, C5′); 68.5 (d, C5′); 68.4 (d, C-benzyl); 35.1 (CH_2-ethyl); 29.6 (CH_2-ethyl); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −8.01; −8.06; R_f value 0.16 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 464.1 (M), found 464.3 (M).

6-Chloro-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 41: procedure A; yield 51%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H, NH); 11.26 (s, 1H, NH); 7.32 (m, 2H, H4-aryl); 7.24 (m, 2H, H5-aryl); 7.13−7.00 (m, 4H, H3-aryl, H6-thymine); 6.71 (m, 1H, H1′); 6.68 (m, 1H, H1′); 6.33 (ddd, 1H, H3′); 6.27 (ddd, 1H, H3′); 5.93 (m, 2H, H2′); 5.39 (m, 4H, H-benzyl); 4.87 (m, 2H, H4′); 4.24 (m, 4H, H5′); 1.62 (d, 3H, CH_3-thymine); 1.56 (d, 3H, CH_3-thymine); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.5 (C4-thymine); 164.1 (C4-thymine); 151.8 (C2-thymine); 151.1 (C2-aryl); 136.1 (C6-thymine); 136.0 (C6-thymine); 134.0 (C6-aryl); 133.1 (C3′); 133.0 (C3′); 131.2 (C4-aryl); 131.1 (C4-aryl); 128.5 (C1-aryl); 127.7 (C2′); 125.7 (C5-aryl); 117.9 (C3-aryl); 108.1 (C5-thymine); 89.7 (C1′); 89.6 (C1′); 84.4 (C4′); 84.4 (C4′); 69.0 (C-benzyl); 67.1 (C5′); 67.0 (C5′); 12.3 (CH_3-thymine); 12.2 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −9.74; −9.77; R_f value 0.68 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 427.046 (M + H^+), found 427.043 (M + H^+).

6-Chloro-7-methyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 42: procedure A; yield 50% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.30 (s, 4H, NH); 7.38 (m, 8H, H4-aryl, H5-aryl); 7.23 (d, 1H, H6-thymine); 7.19 (dd, 1H, H3-aryl); 7.18 (d, 1H, H6-thymine); 7.18 (dd, 2H, H3-aryl); 7.16 (dd, 1H, H3-aryl); 7.13 (d, 2H, H6-thymine); 6.79 (m, 4H, H1′); 6.44 (ddd, 2H, H3′); 6.35 (ddd, 1H, H3′); 6.33 (ddd, 1H, H3′); 6.05 (ddd, 1H, H2′); 6.03 (ddd, 1H, H2′); 5.98 (ddd, 1H, H2′); 5.96 (ddd, 1H, H2′); 5.83 (m, 4H, H-benzyl); 4.90 (m, 4H, H4′); 4.30 (m, 8H, H5′); 1.67 (d, 6H, CH_3-thymine); 1.67 (d, 6H, CH_3-benzyl); 1.65 (d, 3H, CH_3-benzyl); 1.64 (d, 3H, CH_3-thymine); 1.62 (d, 3H, CH_3-benzyl); 1.52 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.0 (C4-thymine); 157.4 (C2-aryl); 151.0 (C2-thymine); 136.5 (C6-thymine); 133.9 (C4-aryl); 130.9 (C6-aryl); 129.0 (C3′); 127.2 (C1-aryl); 126.9 (C2′); 120.3 (C5-aryl); 115.8 (C3-aryl); 109.8 (C5-thymine); 89.0 (C1′); 85.0 (C4′); 67.1 (C-benzyl); 65.9 (C5′); 22.3 (CH_3-benzyl); 12.1 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −9.18; −9.21; −9.50; −9.62; R_f value 0.36 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 441.062 (M + H^+), found 441.062 (M + H^+).

6-Chloro-7-butyl-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 43: procedure A; yield 83% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.36 (s, 1H, NH); 11.35 (s, 1H, NH); 11.34 (s, 1H, NH); 11.31 (s, 1H, NH); 7.43 (m, 4H, H4-aryl); 7.35 (m, 4H, H5-aryl); 7.22 (d, 1H, H6-thymine); 7.21−7.15 (m, 4H, H3-aryl); 7.16 (d, 1H, H6-thymine); 7.14 (d, 1H, H6-thymine); 7.13 (d, 1H, H6-thymine); 6.82 (m, 1H, H1′); 6.80 (m, 1H, H1′); 6.77 (m, 2H, H1′); 6.46 (ddd, 1H, H3′); 6.42 (ddd, 1H, H3′); 6.35 (ddd, 1H, H3′); 6.31 (ddd, 1H, H3′); 6.05 (m, 2H, H2′); 5.99 (m, 1H, H2′); 5.56 (m, 1H, H2′); 5.71−5.57 (m, 4H, H-benzyl); 5.00 (m, 2H, H4′); 4.87 (m, 2H, H4′); 4.47−4.40 (m, 2H, H5′); 4.37−4.29 (m, 2H, H5′); 4.23−4.14 (m, 4H, H5′); 2.03−1.91 (m, 4H, CH_2-butyl); 1.89−1.65 (m, 4H, CH_2-butyl); 1.68 (d, 3H, CH_3-thymine); 1.65 (d, 3H, CH_3-thymine); 1.63 (d, 3H, CH_3-thymine); 1.41 (d, 3H, CH_3-thymine); 1.55−1.21 (m, 16H, CH_2-butyl, CH_2-butyl); 0.88 (t, 3H, CH_3-butyl); 0.87 (t, 3H, CH_3-butyl); 0.85 (t, 3H, CH_3-butyl); 0.82 (t, 3H, CH_3-butyl); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 163.8 (C4-thymine); 163.8 (C4-thymine); 150.9 (C2-thymine); 150.8 (C2-thymine); 150.8 (C2-thymine); 150.1 (d, C2-aryl); 150.1 (d, C2-aryl); 149.6 (d, C2-aryl); 136.0 (C6-thymine); 135.9 (C6-thymine); 133.2 (C3′); 132.9 (C3′); 131.2 (C4-aryl); 131.2 (C4-aryl); 131.1 (C4-aryl); 130.3 (C6-aryl); 130.2 (C6-aryl); 127.5 (C2′); 127.5 (C2′); 126.0 (C5-aryl); 126.0 (C5-aryl); 120.8 (C1-aryl); 120.8 (C1-aryl); 120.7 (C1-aryl); 120.7 (C1-aryl); 118.3 (C3-aryl); 118.2 (C3-aryl); 118.2 (C3-aryl); 118.1 (C3-aryl); 109.9 (C5-thymine); 109.7 (C5-thymine); 109.5 (C5-thymine); 89.3 (C1′); 89.2 (C1′); 89.2 (C1′); 84.4 (C4′); 84.3 (C4′); 84.2 (C4′); 84.2 (C4′); 80.1 (d, C-benzyl); 80.0 (d, C-benzyl); 79.7 (d, C-benzyl); 69.5 (d, C5′); 68.9 (d, C5′); 68.8 (C5′); 68.7 (d, C5′); 34.7 (CH_2-butyl); 34.0 (CH_2-butyl); 33.7 (CH_2-butyl); 27.1 (CH_2-butyl); 26.9 (CH_2-butyl); 26.7 (CH_2-butyl); 21.5 (CH_2-butyl); 21.4 (CH_2-butyl); 21.4 (CH_2-butyl); 13.8 (CH_3-butyl); 13.8 (CH_3-butyl); 13.7 (CH_3-butyl); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); 12.0 (CH_3-thymine); 11.8 (CH_3-thymine); ^{31}P NMR: (202 MHz, DMSO-d_6) δ −8.47; −8.50; −9.59; R_f value 0.59 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 483.109 (M + H^+), found 483.107 (M + H^+).

6-Chloro-7-(ethoxycarbonylmethyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 44: procedure A; yield 49% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.36 (s, 1H, NH); 11.35 (s, 1H, NH); 11.34 (s, 1H, NH); 11.31 (s, 1H, NH); 7.49−7.43 (m, 4H, H4-aryl); 7.40−7.36 (m, 4H, H5-aryl); 7.22 (d, 1H, H6-thymine); 7.21 (d, 1H, H6-thymine); 7.19 (d, 1H, H6-thymine); 7.18 (d, 1H, H6-thymine); 7.17 (m, 2H, H3-aryl); 7.12 (m, 2H, H3-aryl); 6.82 (m, 2H, H1′); 6.76 (m, 2H, H1′); 6.44 (m, 2H, H3′); 6.33 (ddd, 1H, H3′); 6.26 (ddd, 1H, H3′); 6.08−5.94 (m, 8H, H-benzyl, H2′); 5.00 (m, 2H, H4′); 4.87 (m, 2H, H4′); 4.43−4.29 (m, 4H, H5′); 4.21−4.09 (m, 4H, H5′); 4.18 (q, 2H, OCH_2); 4.13 (q, 2H, OCH_2); 3.98 (q, 2H, OCH_2); 3.96 (q, 2H, OCH_2); 3.05−2.93 (m, 8H, CH_2-ECM); 1.70 (d, 3H, CH_3-thymine); 1.68 (d, 3H, CH_3-thymine); 1.67 (d, 3H, CH_3-thymine); 1.53 (d, 3H, CH_3-thymine); 1.20 (t, 3H, CH_3-ECM); 1.19 (t, 3H, CH_3-ECM); 1.18 (t, 3H, CH_3-ECM); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.6 (CO-ECM); 168.6 (CO-ECM); 168.4 (CO-ECM); 168.4 (CO-ECM); 163.9 (C4-thymine); 163.9 (C4-thymine); 163.9 (C4-thymine); 150.9 (C2-thymine); 150.9 (C2-thymine); 150.8 (C2-thymine); 150.2 (d, C2-aryl); 150.2 (d, C2-aryl); 149.6 (d, C2-aryl); 135.9 (C6-thymine); 135.8 (C6-thymine); 135.8 (C6-thymine); 133.0 (C3′); 132.9 (C3′); 132.8 (C3′); 132.8 (C3′); 131.7 (C4-aryl); 131.7 (C4-aryl); 131.7 (C4-aryl); 131.6 (C4-aryl); 130.4 (C6-aryl); 130.3 (C6-aryl); 130.3 (C6-aryl); 127.6 (C2′); 127.6 (C2′); 127.5 (C2′); 127.5 (C2′); 126.3 (C5-aryl); 126.2 (C5-aryl); 126.2 (C5-aryl); 122.0 (d, C1-aryl); 121.4 (d, C1-aryl); 121.3 (d, C1-aryl); 118.4 (C3-aryl); 118.3 (C3-aryl); 118.2 (C3-aryl); 118.1 (C3-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 109.8 (C5-thymine); 89.5 (C1′); 89.3 (C1′); 89.3 (C1′); 89.2 (C1′); 84.2 (C4′); 84.1 (C4′); 84.1 (C4′); 76.5 (d, C-benzyl); 76.3 (d, C-benzyl); 76.2 (d, C-benzyl);

76.2 (d, C-benzyl); 69.5 (d, C5′); 69.1 (d, C5′); 69.1 (d, C5′); 69.0 (d, C5′); 61.1 (OCH_2); 61.0 (OCH_2); 61.0 (OCH_2); 61.0 (OCH_2); 38.8 (CH_2-ECM); 14.1 (CH_3-ECM); 12.2 (CH_3-thymine); 12.0 (CH_3-thymine); 12.0 (CH_3-thymine); 11.9 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −9.90; −9.95; −10.46; −10.49; R_f value 0.48 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 513.083 (M + H^+), found 513.080 (M + H^+).

7-(Monochloromethyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 45: procedure A; yield 11% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (br, 4H, NH); 7.45−7.36 (m, 8H, H4-aryl, H6-aryl); 7.26−7.22 (m, 4H, H5-aryl); 7.22−7.20 (m, 2H, H3-aryl); 7.17−7.15 (m, 2H, H3-aryl); 7.14 (d, H6-thymine); 7.12 (d, H6-thymine); 7.11 (d, H6-thymine); 7.09 (d, H6-thymine); 6.82 (m, 1H, H1′); 6.80 (m, 1H, H1′); 6.77 (m, H, H1′); 6.75 (m, 1H, H1′); 6.42 (ddd, 1H, H3′); 6.40 (ddd, 1H, H3′); 6.36 (ddd, 1H, H3′); 6.26 (ddd, 1H, H3′); 6.05−5.89 (m, 8H, H-benzyl, H2′); 4.98−4.95 (m, 2H, H4′); 4.92−4.88 (m, 2H, H4′); 4.36−4.17 (m, 16H, H5′, CH_2Cl); 1.71 (d, 3H, CH_3-thymine); 1.68 (d, 3H, CH_3-thymine); 1.67 (d, 3H, CH_3-thymine); 1.63 (d, 3H, CH_3-thymine);^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 150.9 (C2-thymine); 149.3 (C2-aryl); 135.9 (C6-thymine); 132.9 (C6-aryl); 130.8 (C3′); 128.9 (C4-aryl); 127.5 (C1-aryl); 127.4 (C1-aryl); 127.0 (C2′); 126.9 (C2′); 126.8 (C2′); 124.9 (C5-aryl); 124.9 (C5-aryl); 118.8 (C3-aryl); 118.7 (C3-aryl); 109.9 (C5-thymine); 89.2 (C1′); 88.0 (C1′); 86.4 (C4′); 84.2 (C4′); 80.9 (C-benzyl); 78.7 (C-benzyl); 70.1 (C5′); 69.4 (C5′); 68.7 (C5′); 51.7 (CH_2Cl); 51.3 (CH_2Cl); 12.1 (CH_3-thymine); 12.1 (CH_3-thymine); 12.0 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −10.05; −10.08; −10.51; −10.60; R_f value 0.49 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 441.062 (M + H^+), found 441.077 (M + H^+).

7-(Dichloromethyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 46: procedure A; yield 57% (four diastereomers); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.35 (s, 1H, NH); 11.34 (s, 2H, NH); 11.32 (s, 1H, NH); 7.51−7.40 (m, 8H, H4-aryl, H6-aryl); 7.30−7.27 (m, 4H, H5-aryl); 7.26−7.23 (m, 4H, H3-aryl); 7.19 (d, H6-thymine); 7.18 (d, H6-thymine); 7.16 (d, H6-thymine); 7.15 (d, H6-thymine); 7.01 (m, 4H, $CHCl_2$); 6.84 (m, 2H, H1′); 6.76−6.72 (m, 2H, H1′); 6.44 (m, 4H, H-benzyl); 6.30 (m, 1H, H3′); 6.24 (m, 2H, H3′); 6.19 (m, 1H, H3′); 6.07 (m, 1H, H2′); 6.04 (m, 2H, H2′); 5.96 (m, 1H, H2′); 5.01 (m, 3H, H4′); 4.88 (m, 1H, H4′); 4.43−4.15 (m, 8H, H5′); 1.74 (d, 3H, CH_3-thymine); 1.73 (d, 3H, CH_3-thymine); 1.67 (d, 3H, CH_3-thymine); 1.66 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 150.9 (C2-thymine); 149.3 (C2-aryl); 136.0 (C6-thymine); 133.0 (C6-aryl); 131.4 (C3′); 130.5 (C1-aryl); 127.7 (C4-aryl); 127.6 (C4-aryl); 127.4 (C2′); 127.4 (C2′); 125.2 (C5-aryl); 125.1 (C5-aryl); 119.2 (C3-aryl); 119.1 (C3-aryl); 110.0 (C5-thymine); 109.9 (C5-thymine); 89.4 (C1′); 89.3 (C1′); 84.2 (C4′); 84.1 (C4′); 81.8 (C-benzyl); 81.8 (C-benzyl); 73.4 ($CHCl_2$); 73.2 ($CHCl_2$); 70.2 (C5′); 70.1 (C5′); 12.2 (CH_3-thymine); 12.2 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −10.11; −10.16; −11.10; R_f value 0.42 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 475.023 (M + H^+), found 475.020 (M + H^+).

7-(Trichloromethyl)-*cyclo*Sal-3′-deoxy-2′,3′-didehydrothymidine monophosphate 47: procedure A; yield 57% (four diastereomers); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H, NH); 11.34 (s, 2H, NH); 11.33 (s, 1H, NH); 7.69−7.63 (m, 4H, H6-aryl); 7.61−7.50 (m, 4H, H4-aryl); 7.35 (m, 4H, H5-aryl); 7.30−7.25 (m, 3H, H3-aryl); 7.26 (d, 1H, H6-thymine); 7.24 (d, 1H, H6-thymine); 7.21 (dd, 1H, H3-aryl); 7.15 (d, 1H, H6-thymine); 7.14 (d, 1H, H6-thymine); 6.85 (m, 2H, H1′); 6.81 (m, 1H, H1′); 6.74 (m, 1H, H1′); 6.58−6.48 (m, 4H, H-benzyl); 6.45 (m, 2H, H3′); 6.25 (ddd, 1H, H3′); 6.17 (ddd, 1H, H3′); 6.08 (ddd, 1H, H2′); 6.06 (ddd, 1H, H2′); 5.95 (m, 1H, H2′); 5.89 (m, 1H, H2′); 5.04 (m, 1H, H4′); 4.98 (m, 1H, H4′); 4.84 (m, 1H, H4′); 4.76 (m, 1H, H4′); 4.48 (m, 4H, H5′); 4.25−4.09 (m, 4H, H5′); 1.76 (d, 3H, CH_3-thymine); 1.75 (d, 3H, CH_3-thymine); 1.69 (d, 3H, CH_3-thymine); 1.68 (d, 3H, CH_3-thymine); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.9 (C4-thymine); 150.9 (C2-thymine); 149.3 (C2-aryl); 136.1 (C6-thymine); 132.7 (C6-aryl); 132.6 (C6-aryl); 131.3 (C3′); 130.4 (C1-aryl); 127.7 (C4-aryl); 127.6 (C4-aryl); 127.5 (C2′); 127.4 (C2′); 124.9 (C5-aryl); 124.8 (C5-aryl); 119.8 (C3-aryl); 119.8 (C3-aryl); 109.9 (C5-thymine); 109.8 (C5-thymine); 99.4 (CCl_3); 89.3 (C1′); 86.8 (C-benzyl); 86.8 (C-benzyl); 84.1 (C4′); 70.1 (C5′); 70.0 (C5′); 12.1 (CH_3-thymine); ^{31}P NMR (202 MHz, DMSO-d_6) δ −9.55; −11.06; −11.32; R_f value 0.46 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 508.984 (M + H^+), found 509.975 (M + H^+).

3-Phenyl-*cyclo*Sal-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine monophosphate 48: procedure B; yield 57%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.60 (s, 2H, NH); 7.77 (s, 1H, H6−BVU); 7.76 (s, 1H, H6−BVU); 7.50−7.26 (m, 18H, H-aryl, H8−BVU); 6.85 (d, 1H, H7−BVU); 6.82 (d, 1H, H7−BVU); 6.17 (dd, 1H, H1′); 6.14 (dd, 1H, H1′); 5.60−5.51 (m, 4H, H-benzyl); 5.47 (d, 2H, OH); 4.37−4.27 (m, 4H, H5′); 4.23−4.22 (m, 2H, H3′); 3.96−3.91 (m, 2H, H4′); 2.20−2.09 (m, 4H, H2′); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.7 (C4−BVU); 149.3 (C2−BVU); 149.3 (C2−BVU); 139.3 (C6−BVU); 135.6 (C2-aryl); 131.0 (C4-aryl); 131.0 (C1′-aryl); 130.9 (C1′-aryl); 129.9 (C7−BVU); 129.2 (C3′-aryl, C5′-aryl); 128.6 (C2′-aryl, C6′-aryl); 128.5 (C2′-aryl, C6′-aryl); 128.0 (C6-aryl); 127.9 (C6-aryl); 125.7 (C4′-aryl); 124.8 (C5-aryl); 124.8 (C5-aryl); 122.4 (C1-aryl); 121.5 (C3-aryl); 110.3 (C5−BVU); 110.2 (C5−BVU); 107.1 (C8−BVU); 107.1 (C8−BVU); 84.8 (C1′); 84.8 (C1′); 84.7 (C4′); 84.5 (d, C4′); 69.8 (C3′); 69.7 (C3′); 68.6 (d, C5′); 68.2 (d, C-benzyl); 40.0 (C2′); ^{31}P NMR (202 MHz, DMSO-d_6) δ −7.51; −7.54; R_f value 0.45 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 577.0375 (M + H^+), found 577.0403 (M + H^+).

2,4-Bis-*tert*-butyl-5-fluorophenol. Isobutene gas was bubbled through 3-fluorophenol (4.10 g) for 5 min at 40 °C. Afterward, concentrated sulfuric acid (0.34 g) was added and the inflow of isobutene was continued for 1 h. After the addition of water, dichloromethane was added and the phases were separated. The organic phase was neutralized with sat. sodium bicarbonate solution and dried (Na_2SO_4). The solvent was removed in vacuo, and the resulting residue was purified by column chromatography (petroleum ether/CH_2Cl_2, 4:1) yielding the title compound (5.73 g, 70%) as a colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (d, 1H, H5); 6.39 (d, 1H, H2); 4.76 (s, 1H, OH); 1.42 (s, 9H, CH_3-*t*Bu); 1.37 (s, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.9 (d, C3); 152.7 (d, C1); 130.8 (d, C6); 128.1 (d, C4); 125.4 (d, C5); 104.9 (d, C2); 34.4 (C-*t*Bu); 33.9 (d, C-*t*Bu); 30.2 (d, CH_3-*t*Bu); 29.8 (CH_3-*t*Bu); ^{19}F NMR (471 MHz, $CDCl_3$) δ −113.34 (dd); R_f value 0.14 (petroleum ether/CH_2Cl_2, 4:1); MS (FAB, *m*/*z*) calcd 224.1576 (M), found 224.1559 (M).

3,5-Bis-*tert*-butyl-6-fluorosalicyl Alcohol. 2,4-Bis-*tert*-butyl-5-fluorophenol (5.55 g) and sodium hydroxide (1.18 g) were dissolved in methanol (15 mL). To this solution was added an aqueous formaldehyde solution (37%, 5.9 mL), and the resulting mixture was stirred at room temperature for 3 d. The reaction was monitored by TLC (CH_2Cl_2/MeOH, 9:1) and finally quenched by the addition of water and concentrated hydrochloric acid (resulting pH 4−5). The aqueous mixture was extracted five times with dichloromethane. The combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo. The resulting crude product was recrystallized from petroleum ether to yield the title compound (4.49 g, 71%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H, OH); 7.14 (d, 1H, H4); 5.02 (s, 2H, H-benzyl); 1.41 (s, 9H, CH_3-*t*Bu); 1.34 (d, 9H, CH_3-*t*Bu); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.9 (d, C6); 154.7 (d, C2); 131.9 (d, C3); 126.9 (d, C5); 124.6 (d, C4); 112.9 (d, C1); 58.1 (d, C-benzyl); 35.0 (C-*t*Bu); 34.3 (d, C-*t*Bu); 30.5 (d, CH_3-*t*Bu); 30.0 (CH_3-*t*Bu); ^{19}F NMR (471 MHz, $CDCl_3$) δ −119.74 (d); R_f value 0.74 (CH_2Cl_2/MeOH, 9:1); MS (FAB, *m*/*z*) calcd 254.1682 (M), found 254.1681 (M).

Cholinesterase Assay. With BChE (human serum). A modification of the Rappaport procedure was applied.[21] Commercially available solutions and reagents were used (Sigma, kit 420-MC): sodium chloride solution (0.15 M in water), *m*-nitrophenol (0.75 g/L in phosphate buffer, pH 7.8), acetylcholine chloride (preweighted solid, water was added for a concentration of 150 g/L). All solutions were stored at 4 °C. Human serum was freshly prepared from blood and stored at

−80 °C in small portions. For the enzyme assay, 40 μL of sodium chloride solution, 370 μL of *m*-nitrophenol solution, 40 μL of acetylcholine chloride solution, 20 μL of a solution of the appropriate *cyclo*Sal nucleotide in DMSO, and 430 μL of water were transferred into a quarz cuvette. The enzymatic reaction was started by the addition of 100 μL of human serum. The cuvette was transferred into the UV/vis spectrometer immediately, and the extinction (λ = 420 nm, 25 °C) was recorded at 0 and after 5 min. Different concentrations of the *cyclo*Sal nucleotides in DMSO were used in order to achieve final inhibitor concentrations of 0.1, 0.5, 1, 5, 10, 30, and 50 μM. For every inhibitor concentration, ΔE values were calculated and plotted. The IC_{50} values were determined from the plot by interpolation in the pseudolinear region.

With BChE (mouse and calf serum). Analogously to the experiments with human serum, but using mouse or calf serum instead.

With AChE from *Electrophorus electricus*. As described before, but using 100 μL of a 10 U/mL solution of purified AChE from *Electrophorus electricus* (purchased from Sigma) in water instead of human serum. The enzyme solution was stored at 0 °C in order to avoid denaturation of the enzyme. Only the experiments with 50 μM inhibitor concentration were performed, as none of the tested *cyclo*Sal nucleotides exploited any inhibitor activity.

With human AChE. Analogously to the experiments with AChE from *E. electricus*, but using purified human AChE instead (purchased from Sigma).

With AChE from beef erythrocytes: Analogously to the experiments with AChE from *E. electricus*, but using purified AChE from beef erythrocytes instead (purchased from Sigma).

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