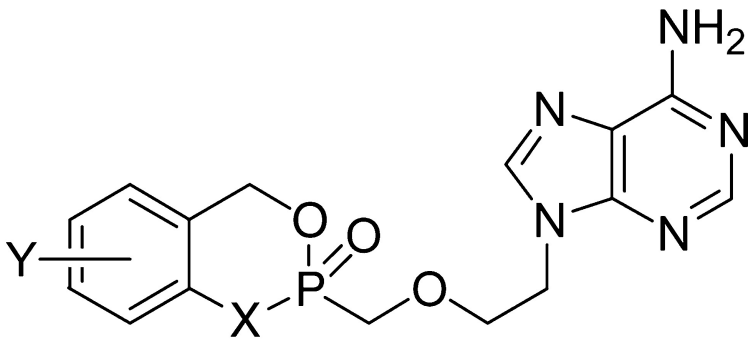
Journal of Medicinal Chemistry

Article

*cyclo*Sal-PMEA and *cyclo*Amb-PMEA: Potentially New Phosphonate Prodrugs Based on the *cyclo*Sal-Pronucleotide Approach

Chris Meier, Ulf Grbig, Christian Mller, and Jan Balzarini

J. Med. Chem., **2005**, 48 (25), 8079-8086• DOI: 10.1021/jm050641a • Publication Date (Web): 10 November 2005



X = O and NH

Y = H, 6-F, 3-Me, 3-*t*Bu, 3,5-*t*Bu

*cyclo*Sal- (**5**) and *cyclo*Amb-PMEA **6**

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

*cyclo*Sal-PMEA and *cyclo*Amb-PMEA: Potentially New Phosphonate Prodrugs Based on the *cyclo*Sal-Pronucleotide Approach

Chris Meier,*,# Ulf Görbig,# Christian Müller,# and Jan Balzarini†

Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany, and Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received July 5, 2005

Two new classes of lipophilic prodrugs of the antiviral active phosphonate 9-[2-phosphonomethoxyethyl]adenine (PMEA **1**) have been prepared and were studied with regard to their hydrolysis properties and biological activity. A first series of compounds was prepared on the basis of the *cyclo*Sal nucleotide approach. Because of the surprisingly low hydrolysis stability of these *cyclo*Sal-PMEA derivatives, more stable derivatives have to be developed. Instead of using salicyl alcohol, in *cyclo*Amb-PMEA derivatives 2-aminobenzyl alcohols were attached to PMEA **1**. The latter compounds showed a considerably higher stability compared to the *cyclo*Sal counterparts. Stability studies revealed that all lipophilic prodrugs delivered PMEA selectively by chemical means. All compounds proved to be noninhibiting to acetyl- and butyrylcholinesterase, and some of the phosphonate diesters were found to be more active against HIV compared to the parent PMEA.

Introduction

The acyclic nucleoside phosphonates 9-[2-phosphonomethoxyethyl]adenine (PMEA; Adefovir **1**; Figure 1), (*R*)-9-[2-phosphonomethoxypropyl]adenine (PMPA; Tenofovir **2**), and (*S*)-9-[3-hydroxy-2-phosphonomethoxypropyl]cytosine (HPMPC; Cidofovir **3**) show broad antiviral activity against DNA and retroviruses not only in vitro but also in-vivo.[1] For example, PMEA **1** has demonstrated antiviral activity against human immunodeficiency virus (HIV), Rauscher murine sarcoma virus (R-MuLV), herpes simplex virus (HSV), cytomegalovirus (CMV), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), Epstein−Barr virus (EBV), and hepatitis B virus (HBV).

However, the therapeutic use of these nucleoside phosphonates is limited due to their poor oral bioavailability caused by the negatively charged phosphonate moiety that is present at physiological pH. PMEA was reported to have <1% oral bioavailability in monkeys and <11% in rats.[2] Therefore, neutral and membrane-permeable prodrugs were synthesized. Bis(POM)-PMEA,[3] bis(DTE)-PMEA, and bis(SATE)-PMEA,[4] for example, can penetrate cellular membranes and deliver the parent PMEA by enzymatic activation. In the last several years, we have successfully developed the *cyclo*Sal approach for the delivery of nucleoside monophosphates (pronucleotides). The structure of the prototype *cyclo*Sal compounds **4** is shown in Figure 2. The *cyclo*Sal approach has already been applied successfully to several nucleoside analogues,[5] for example, the anti-HIV 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T)[6] and the anti-herpes-simplex virus 5-[(*E*)-2-bromovinyl]-2′-deoxyuridine (BVDU).[7] We have proven that *cyclo*Sal triesters are able to penetrate cell membranes, release, for example, d4TMP intracellularly, and thus improve the biological activity.[6] The most remarkable advantage of the *cyclo*Sal nucleotides is the use of only one masking unit (a salicyl alcohol) per nucleotide and the purely pH-driven delivery of the nucleotides. Recently, we attached esterase-cleavable side chains to the aromatic ring of the *cyclo*Sal moiety to trap the *cyclo*Sal derivatives intracellularly.[8]

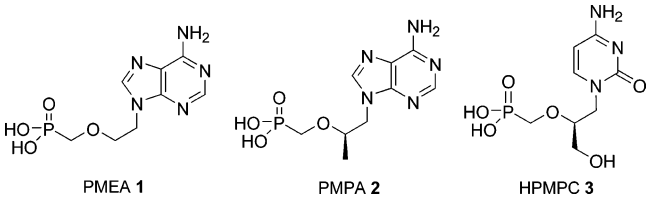


Figure 1. Structural formulas of the acyclic nucleoside phosphonate analogues PMEA, PMPA, and HPMPC.

Because of the interesting properties of nucleoside phosphonates, the *cyclo*Sal approach was applied to PMEA. Here, we report on the synthesis of two new classes of PMEA prodrugs (*cyclo*Sal-PMEA **5** and *cyclo*Amb-PMEA **6**, Figure 2), their hydrolysis properties, and their biological evaluation.

Results and Discussion

Chemistry. In contrast to prototype *cyclo*Sal-phosphate triesters of type **4**, the synthesis of the phosphonate cannot be achieved by our previously developed P(III)-route using *cyclo*Sal-chlorophosphites prepared from salicyl alcohols **7** and PCl_3. This led to the formation of *cyclo*Sal-phosphites that were subsequently oxidized by *t*-BuOOH.[5−8] For the phosphonates, diethyl ester of PMEA **8** was used as starting material. First, the exocyclic amino group was blocked with the monomethoxytrityl group in 89% yield.[9] Treatment of compound **9** with trimethylsilyl bromide and pyridine in CH_3CN gave the intermediate bis(trimethylsilyl) ester that was immediately converted into the corresponding

* To whom correspondence should be addressed. Tel: +49-40-42838-4324. Fax: +49-40-42838-2495. E-mail: chris.meier@chemie.uni-hamburg.de.
# University of Hamburg.
† Rega Institute for Medical Research.

10.1021/jm050641a CCC: $30.25
Published on Web 11/10/2005

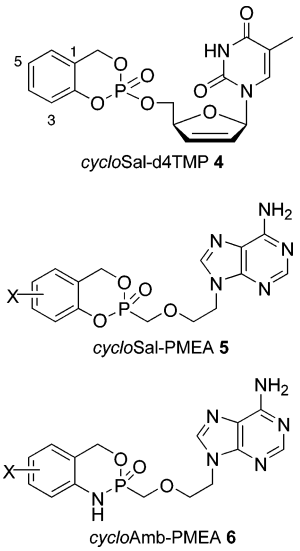
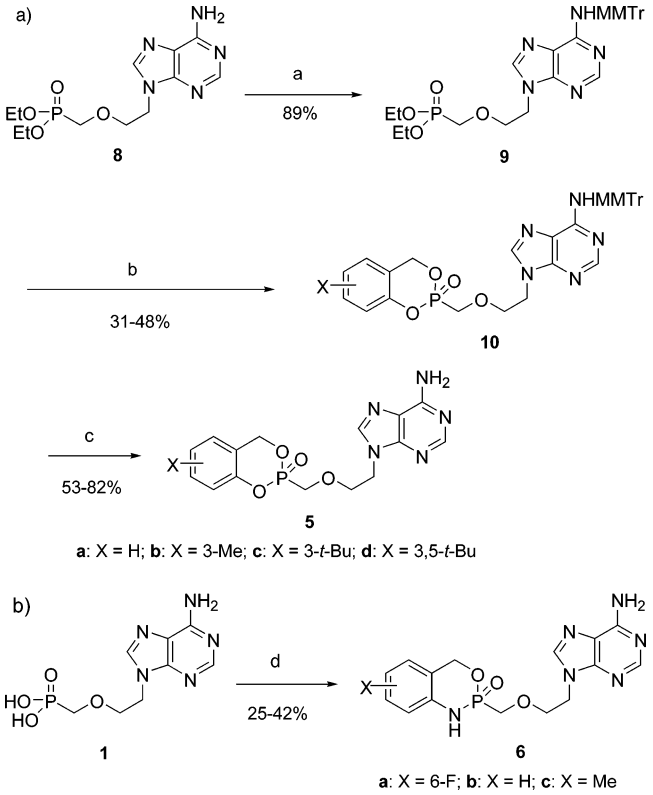


Figure 2. Structural formulas of the *cyclo*Sal-phosphate triesters **4** as well as the *cyclo*Sal- and *cyclo*Amb-PMEA derivatives **5** and **6**.

dichloride[10] that was reacted with four different salicyl alcohols **7** in the presence of triethylamine in CH_2Cl_2 to give *cyclo*Sal-PMEA diesters **10a**−**d** in 31−48% yield after purification on silica gel chromatography. Finally, the MMTr group was cleaved by treatment of **10** with TFA in CH_3CN, which led to the target *cyclo*Sal-PMEA derivatives **5a**−**d** in 53−82% yield (Scheme 1a). The different substitution pattern in the *cyclo*Sal moiety has been selected due to their influence on the hydrolysis properties of the *cyclo*Sal system as reported before.[5] Attempts to prepare the *cyclo*Sal-PMEAs without protection of the exocyclic amino group led to a complex mixture of reaction products. Moreover, different modifications for the above reaction sequence were tried. We tried to attach the *t*-Boc group instead of the MMTr group of the exocyclic amino function. However, the reaction of diethyl PMEA **7** with Boc anhydride or the active ester "Boc-on" in the presence of different bases and solvents failed. In contrast, the introduction of the fluorenylmethoxycarbonyl (FMOC) group could be achieved partly. Reaction of diethyl PMEA **8** with FMOC-Cl in pyridine gave a mixture of the *N*,*N*-di-FMOC and the *N*-FMOC derivative in 78 and 18%, respectively. With both compounds the above-mentioned reaction sequence (TMS-Br; oxalyl chloride or PCl_5; salicyl alcohol) led to the formation of the *N*-FMOC protected intermediate analogous to diester **10**. However, the yields were found to be only 5−12%. The cleavage of the FMOC groups was only achieved by addition of NEt_3 in pyridine in poor 20% yield. Other cleavage conditions such as DBU/CH_3CN, NEt_3/CH_3CN, piperidine/THF, morpholine/DMF, TBAF/THF, or NEt_3·HF/THF did not led to the deprotected *cyclo*Sal-PMEAs **5** most presumably due to their low hydrolytic stability (see hydrolysis section). Besides the use of the MMTr group, the more stable trityl group also was introduced. The analogous reaction sequence to the *cyclo*Sal-PMEAs gave the diesters in 9% yield. In this reaction, oxalyl chloride was used for the chlorination reaction instead of PCl_5. However, oxalyl chloride caused more acidic reaction conditions so that in addition to the tritylated *cyclo*Sal-PMEA diester **10** the cleavage of the trityl

Scheme 1. Synthetic Pathways toward *cyclo*Sal-PMEAs **5** (a) and *cyclo*Amb-PMEAs **6** (b)[a]



[a] Reaction conditions: (a) MMTrCl, NEt_3, DMAP, CH_2Cl_2, rt, 4 h; (b) 1. TMSBr, pyridine, CH_3CN, rt, 16 h; 2. PCl_5, CH_2Cl_2, rt, 2.5 h; 3. Salicyl alcohol **7a-d**, NEt_3, CH_2Cl_2, 0 °C, 1 h; (c) TFA, CH_3CN, rt, 1 h; (d) 1. $(COCl)_2$, *N*,*N*-diethylformamide, CH_2Cl_2; 2. pyridine, CH_2Cl_2; 3. 2-aminobenzyl alcohol **11a-c**, DIPEA, CH_2Cl_2, CH_3CN, rt, 1 h.

group was detected by TLC. As expected, this effect was more pronounced when the MMTr group was used instead so that PMEA diesters could not be isolated.

In addition to the use of the dichlorides of protected PMEA for the esterification, it has been published that esterification of PMEA **9** salts with strong activation agents is also a suitable route. Thus, *N*-MMTr-diethyl PMEA **9** was treated with TMSBr in CH_3CN and then quenched by addition of aqueous triethylammonium bicarbonate buffer. The formed bis(triethylammonium) salt of *N*-MMTr-PMEA was subsequently reacted with the salicyl alcohols **7** in the presence of mesitylenesulfonyl-3-nitrotriazole (MSNT) in pyridine. After purification, the target *N*-MMTr-*cyclo*Sal-PMEA diesters **10** was isolated in 23% yield. Finally, the yields obtained by this route are markedly lower as compared to those obtained starting from PMEA dichloride. Moreover, the purification was tedious.

In addition, a second group of lipophilic PMEA prodrugs has been prepared. By using 2-aminobenzyl alcohols **11** instead of salicyl alcohols, the phenol oxygen atom in the *cyclo*Sal moiety is replaced by a nitrogen atom. Thus, so-called *cyclo*Amb(*cyclo*aminobenzyl)-PMEAs **6a**−**c** were prepared. In this case, PMEA **1** was used as starting material. First, PMEA was converted into the dichloride by the reaction with oxalyl chloride, *N*,*N*-diethylformamide (DEF) in CH_2Cl_2. Under these reaction conditions, the exocyclic amino group is temporarily blocked as a formamidine group.[11] Then, 2-ami-

Table 1. Hydrolysis, BChE- and Anti-HIV Data of *cyclo*Sal- and *cyclo*Amb-PMEA Derivatives

		chem stability[a]			biological activity in CEM/O cells		
					EC_{50}[c] [μM]		
comp	X	pH 7.3 [h]	pH 2.0 [h]	BChE[b] IC_{50} [μM]	HIV-1	HIV-2	CC_{50}[d] [μM]
5a	H	0.09	n.d.[e]	>50	n.d.[e]	n.d.[e]	n.d.[e]
5b	3-Me	0.56	n.d.[e]	>50	n.d.[e]	n.d.[e]	n.d.[e]
5c	3-*t*-Bu	4.05	23.7	>50	3.0 ± 1.4	4.5 ± 0.7	29 ± 6.5
5d	3.5-*t*-Bu	3.05	9.6	>50	5.5 ± 0.7	5.3 ± 0.5	17 ± 0.6
6a	6-F	1.3	n.d.[e]	>50	28 ± 0.0	26 ± 2.1	244 ± 8.5
6b	H	4.0	9.6	>50	20 ±7.1	21 ± 9.2	183 ± 24.0
6c	3-Me	21.3	29.0	>50	29 ± 2.9	56 ± 43.4	>250
15[f]		14.7	n.d.[e]		0.18 ± 0.11	0.075 ± 0.007	0.74 ± 0.11
PMEA **1**					10 ± 6.4	10	50 ± 13

[a] Hydrolysis in 25 mM phosphate buffer at 37 °C or 25 mM citrate buffer, pH 2.0 at 37 °C; half-lives ($t_{1/2}$) were determined from the decreasing peak of the starting phosphate triester and are the mean of duplicate experiments. [b] Inhibition of human butyrylcholinesterase. [c] Anti-HIV-1 activity (EC_{50}): 50% effective concentration in wild-type CEM cell cultures. [d] CC_{50}: cytotoxic concentration. [e] Not determined due to low chemical stability. [f] Bis(POM)PMEA.

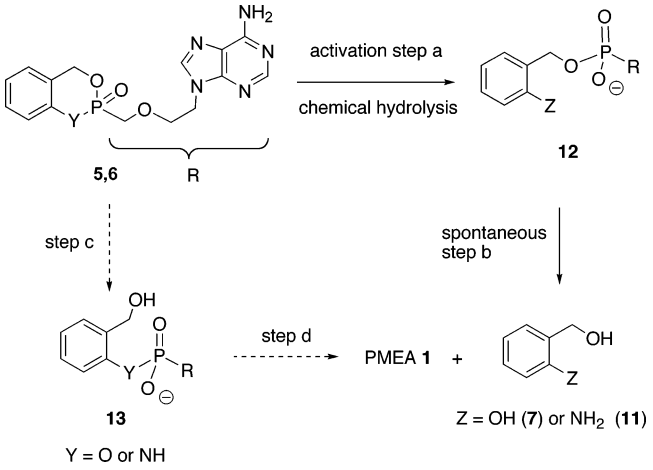
nobenzyl alcohols **11a**−**c** were used for the preparation of the target *cyclo*Amb-PMEA derivatives **6a**−**c** that were isolated in 25−42% yield. Additionally, a reaction sequence starting from diethyl PMEA **8** as described for the *cyclo*Sal-PMEAs **5** was tested. However, although the target compounds **6** can be isolated, the yields were very poor (3−7%). Exchange of the base (DIPEA instead of NEt_3) and the solvent (CH_2Cl_2 to CH_3CN) did not improve the yields. The reaction sequence is summarized in Scheme 1b.

The enantiomeric mixtures of the PMEA esters **5** and **6** were characterized by means of ^1H, ^{13}C, and ^{31}P NMR spectroscopy, by analytical reversed-phase high-performance liquid chromatography (RP-HPLC), and by FAB or ESI mass spectrometry and UV spectroscopy. After lyophilization, phosphonate diesters **5** and **6** were obtained as white fluffy solids.

The lipophilicity (log*P*) of the new phosphonate derivatives can be determined experimentally by partitioning the compound between *n*-octanol/water or can be evaluated qualitatively by their retention time on the HPLC. Balzarini et al. showed some years ago that the retention time on a HPLC column using the same gradient can be correlated with the log*P* value of a compound.[12] Here, some of the compounds are too labile to be used in this experimental procedure (see below). Therefore, a combination of both methods was used. The retention times and the determined log*P* values of *cyclo*Sal-phosphonates **5** were 9.4 min (**5a**), 11.5 min (**5b**), 12.1 min and log*P* 0.47 (**5c**), and 16.4 min and log*P* 1.72 (**5d**), respectively. The log*P* values for compounds **5a**,**b** were not available due to their low chemical stability (Table 1). In comparison, *cyclo*Amb-phosphonate esters **6** showed retention times of 11.9 min and a log*P* of 0.49 (X = F; **6a**), 11.0 min and log*P* 0.23 (X = H; **6b**), and 12.1 min and log*P* 0.45 (X = 3-Me; **6c**). These data suggest that the *cyclo*Amb-phosphonate diesters are slightly more lipophilic as compared to *cyclo*Sal-phosphonates **5**. However, compared to the *cyclo*Sal-phosphate esters **4**, both phosphonates are more hydrophilic: 12.3 min (X = H; log*P* = 0.27); 13.7 min (X = 6-F; log*P* = 0.69); and 13.6 min (X = 3-Me; log*P* = 0.70). Thus, 3-*t*Bu-*cyclo*Sal-PMEA **5c** has more or less the same lipophilicity as 3-Me-*cyclo*Amb-PMEA **6c** and *cyclo*Sal-d4TMP **4**.

Hydrolysis Studies. The original *cyclo*Sal-phosphate triester approach has been designed to release the nucleotides and the masking group selectively by a chemically induced tandem or cascade reaction.[5] In contrast to other prodrug concepts that are based on enzymatically triggered activation, our approach involves the successive coupled cleavage of the phenyl and benzyl esters of the *cyclo*Sal-phosphate triester. This chemical degradation pathway has been proven by different methodologies (e.g., ^{31}P NMR or hydrolysis in labeled water). The intracellular delivery of the nucleotides has additionally been proven by the observed biological activity in different cell lines and in thymidine kinase-deficient CEM cells. Additionally, cell incubation studies with radiolabeled *cyclo*Sal-phosphate triesters demonstrated increased intracellular amounts of phosphorylated metabolites.[13] Here we investigated (i) whether the new *cyclo*Sal- or *cyclo*Amb-PMEA phosphonate diesters are able to release PMEA **1** and (ii) whether the mechanism of this release is identical to that found in the case of *cyclo*Sal-phosphate triesters (Scheme 2).

Scheme 2. Hydrolysis Mechanism of the *cyclo*Sal- and *cyclo*Amb-PMEA Derivatives



First, the chemical stability was determined in phosphate buffer, pH 7.3 (Table 1). The series of *cyclo*Sal-PMEA derivatives **5** showed an unexpected 30-fold decrease of the stability with respect to the corresponding prototype *cyclo*Sal-phosphate esters of d4T **4**. The half-lives of the unsubstituted- (**5a**) and 3-methyl-*cyclo*Sal-PMEA **5b** were 0.1 and 0.6 h only. Thus, they are too labile to be useful as prodrugs. However, due to the introduction of stabilizing substituents such as the 3-*tert*-butyl- in **5c** or the 3,5-di-*tert*-butyl groups in **5d**

the half-lives were increased to 3−4 h. The considerable lower stability points to a much higher electrophilicity of the phosphorus atom in the phosphonates as compared to the phosphates. This may be due to lack of pπ-dπ-back-bonding of the additional oxygen atom in the phosphate esters.[14] This interpretation is supported by the observation that dimethyl methylphosphonate hydrolyzed 20-fold faster than trimethyl phosphate in alkaline solution.[15] The activation barrier of the (E_{Act}) phosphate hydrolysis was found to be 2.7 kcal/mol higher compared to the E_{Act} of methylphosphonate.[16]

Nevertheless, ^{31}P NMR studies in imidazole-HCl buffer, pH 7.3 revealed that the hydrolysis product of the *cyclo*Sal-PMEA diester **5** is solely PMEA **1**. Thus, the hydrolysis mechanism via benzylphosphonate ester **12** as shown in Scheme 2 is entirely identical to that of the *cyclo*Sal-phosphates. Interestingly, the selectivity of the cleavage is much higher. In the case of 3,5-di-*tert*-butyl-*cyclo*Sal-d4TMP about 33% of the phenyl phosphate diester (analogous to **13**, Scheme 2) instead of the benzyl phosphate diester was formed as a side product. The phenyl monoester **13** was *not* formed in one of the *cyclo*Sal-PMEA hydrolyses! ^{31}P-NMR spectra in Supporting Information show the hydrolysis of 3-methyl-*cyclo*Sal-PMEA **5b** (18.5 ppm) and 3,5-di-*tert*-butyl-*cyclo*Sal-PMEA **5d** (19.8 ppm) in imidazole/HCl buffer at pH 7.3. Only in the spectra of compound **5b** can the benzyl PMEA monoester **12b** be detected in small amounts because the first hydrolysis is much faster compared to the hydrolysis of diester **5d**. At the end of the hydrolyses in both cases PMEA (15.4 ppm) was observed as the product. In the phosphate triester series, 3-methyl-*cyclo*Sal derivatives led to the formation of 6−8% of the phenyl phosphate diester.[17]

The unexpected low stability of the *cyclo*Sal-PMEA derivatives **5** led to the development of the *cyclo*Amb-PMEA derivatives **6** because the less electronegative nitrogen reduces the electrophilicity of the phosphorus atom and consequently should therefore increase the stability. In ^{31}P NMR experiments, the selective degradation to PMEA from 3-methyl-*cyclo*Amb-PMEA **6b** was observed again (Supporting Information).

More importantly, the stability of the compounds was dramatically higher compared to the *cyclo*Sal-PMEAs, for example, a 35-fold increase was observed for 3-methyl-*cyclo*Amb-PMEA **6c** compared to 3-methyl-*cyclo*Sal-PMEA **5b** (Table 1). The half-lives of the *cyclo*Amb-phosphonate esters were very similar to those of the corresponding *cyclo*Sal-d4TMP phosphate triesters. Thus, by simply exchanging the phenol oxygen by a nitrogen atom, the higher electrophilicity of the phosphonate P-atom was compensated. However, a further consequence was observed in the ^{31}P NMR studies. In the case of the *cyclo*Sal-d4TMP triesters **4** the rate-limiting step of the delivery of the d4TMP was the cleavage of the phosphate triester to the intermediate phosphate diester. However, in the case of the *cyclo*Amb-phosphonate diesters **6** the rate-limiting step is the cleavage of the intermediate benzyl phosphonate monoester **11** to PMEA. This was observed particularly for 6-fluoro-*cyclo*Amb-PMEA **6a** and *cyclo*Amb-PMEA **6b**. In the former case, the formation of PMEA was very slow. In contrast, 3-methyl-*cyclo*Amb-PMEA **6c** led to a comparatively fast formation of PMEA because in addition to the amino group a second electron-donating methyl group is present in the aromatic ring. This favors the cleavage of the benzyl-C−O-bond to release PMEA.

One major advantage of the phosphonate prodrugs of PMEA and related derivatives is their oral bioavailability. To address this property in part the stability of some of the new *cyclo*Sal-PMEA and *cyclo*Amb-PMEA derivatives in acidic media was determined in citrate-buffer at pH 2.0 (Table 1). All studied compounds showed markedly higher stability at this pH value compared to the stability values found at pH 7.3. The *cyclo*Amb-compounds **6** were found to be 1.3−3-fold more stable. Interestingly, the *cyclo*Sal-PMEA compounds **5** proved to be 3−6-fold more stable. It cannot be excluded that this difference is caused by an *N*-protonation in the case of the *cyclo*Amb-derivatives.

In addition to hydrolysis studies under pure chemical conditions, the compounds were also incubated with CEM/O cell extracts and in 20% human serum. Because of the very low stability of *cyclo*Sal-PMEAs **5a**,**b** only the more stable *cyclo*Sal-PMEA derivatives **5c** and **5d** were studied. Under both conditions the two compounds showed half-lives of 3 to 4 h and the rapid release of PMEA. Thus, the stability was nearly identical to that observed in the aqueous system. This result points to a pure chemically driven hydrolysis mechanism. Such a behavior has been observed already for the *cyclo*Sal-phosphate triesters.[5] Additionally, *cyclo*Amb-PMEAs **6** were incubated in CEM cell extracts. As shown by means of HPLC, compounds **6** proved to be much more stable than *cyclo*Sal-diesters **5** and they release the benzyl phosphonate ester of type **12** (Z = NH_2; Scheme 2) first. From this intermediate PMEA is slowly released. After 8 h of incubation of *cyclo*Amb-PMEA **6a** in the extracts, 74% of the starting material, 16% of the intermediate **13**, and 10% of PMEA was detected. It cannot be excluded that intermediate **12** is or can also be cleaved enzymatically by a phosphoramidase present in the CEM cell extracts. This enzyme has been suggested for the cleavage of nucleoside phosphoramidite monoesters formed from phosphoramidites pronucleotides.[18]

Anti-Cholinesterase Activity. As the *cyclo*Sal-PMEAs, all three *cyclo*Amb-PMEAs were found to be noninhibitory to human acetyl- (data not shown)[19] and butyryl cholinesterase (Table 1). Obviously, independently from the chemical stability of the lipophilic PMEA compounds, these structures are not substrates for this class of enzymes. In more detailed previous studies, it was shown that nucleosides bearing an adenine heterocycle had even better substrate properties compared to guanine or pyrimidines.[20] Thus, the acyclic "glycon" structure of PMEA seems to determine the lack of cholinesterase-inhibiting properties.

Antiviral Evaluation. The phosphonate prodrugs have been tested for their antiviral potency against HIV-1 and HIV-2 in CEM/O cells. The results of these tests are summarized in Table 1. PMEA **1** was found to be active against both virus strains at 10 μM. Interestingly, although *cyclo*Sal-PMEA derivatives **5c**,**d** have only half-lives of 3−4 h in the hydrolyses studies, both of them proved to be slightly more antivirally potent compared to the parent compound (3−5 μM). *Cyclo*Sal-PMEAs **5a**,**b** were not tested because of their extreme

lability. For comparison, bis(POM)PMEA **15** was also tested. The antiviral activity was found to be about 0.075−0.18 μM in all three assays. Thus, compound **15** is 16-fold more active compared to 3-*tert*-butyl-*cyclo*Sal-PMEA **5c**. On the other hand, the SI value of the same compounds are 9.7 for 3-*tert*-butyl-*cyclo*Sal-PMEA **5c** while that of bis(POM)PMEA was 4.1! Thus, the higher antiviral activity of the bis(POM) derivative was counteracted by a markedly increased cytotoxicity. This parallel increase in toxicity with increased antiviral potency has also been observed in the case of the bis(SATE)-PMEAs[4] and the phosphoramidates of PMEA.[18] The higher antiviral activity of the *cyclo*Sal-PMEAs **5** may be attributed to the low chemical stability ($t_{1/2}$ 3−4 h, releasing quickly the parent drug) and/or the lower lipophilicity as compared to, for example, bis(POM)-PMEA.

Although the chemical stability has been increase considerably, *cyclo*Amb-PMEAs **6** surprisingly exhibited antiviral activity at an EC_{50} of 20−29 μM only. This is 2−3-fold lower compared to the parent and 10-fold lower than the *cyclo*Sal-PMEAs. However, the three *cyclo*Amb-derivatives had CC_{50} values as high as 186 μM to >250 μM. Thus, these compounds are even less toxic than PMEA **1** (CC_{50}: 50 μM) and the SI value is as before about 10. The lower antiviral activity may be attributed to the slow release of PMEA from the *cyclo*Amb-PMEAs. As described in the hydrolysis studies section, the rate-limiting step in the PMEA delivery of the *cyclo*Amb-derivatives is the cleavage of the intermediate benzyl phosphonate ester.

The interesting different behavior of the two groups of prodrugs will be studied in antiviral assays of different DNA viruses, for example, HSV-1 and HBV.

Conclusion

In summary, from the hydrolytic and antiviral data disclosed here, *cyclo*Sal-PMEAs **5** and *cyclo*Amb-PMEAs **6** provide a method to deliver the nucleoside phosphonate PMEA intracellularly. It was proven that the delivery mechanism is the same as for *cyclo*Sal-d4TMP **4**. Interestingly, the selectivity of the degradation pathway was even better as compared to the *cyclo*Sal-phosphate triesters because in the hydrolysis studies none of the new derivatives led to the formation of the benzyl phosphonate ester **12**. The stability was modulated considerably by replacing the phenol oxygen by a nitrogen atom. The antiviral evaluation showed interesting properties among the two different groups of lipophilic prodrugs. While *cyclo*Sal-PMEAs improved the antiviral activity as compared to PMEA, the *cyclo*Amb-derivatives reduced the cytotoxicity noticeable. Further work to tune the properties of these two interesting classes of prodrug compounds are currently underway in our laboratories.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions (argon atmosphere). Solvents: anhydrous methylene chloride (CH_2Cl_2), anhydrous tetrahydrofurane (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 before used. Ethyldi*iso*propylamine (DIPEA) was distilled from Na prior to use. The solvents for the HPLC were obtained from Merck (acetonitrile, HPLC grade). Ion pairing buffer solution was prepared by mixing 6.6 mL of tetrabutylammonium hydroxide with 1000 mL of water. The pH value was adjusted to 3.8 by adding concentrated phosphoric acid (buffer I). To 60 mL of buffer I solution 1000 mL of water was added (buffer II). 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. 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 acectic acid) by heating with a fan or a hot plate. HPLC: (Merck-Hitachi) analytical HPLC, LiChroCART 250-3 with LiChrospher 100 RP-18 endcapped (5 μm), gradient I 12−80% CH_3CN (0−20 min), 12% CH_3CN (20−35 min), flow 0.6 mL, UV detection at 250 nm; gradient II 8−100% CH_3CN (0−22 min), 100% CH_3CN (22−27 min), 8% CH_3CN (27−33 min), flow 0.6 mL, UV detection at 250 nm; gradient III same as II, instead of water the ion pairing buffer solution was used. 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 ($CDCl_3$ or DMSO as internal standard); (^{13}C NMR) Bruker WM 400 at 101 MHz, Bruker AMX 400 at 101 MHz or Bruker DMX 500 at 123 MHz ($CDCl_3$ or DMSO as internal standard); (^{31}P NMR) Bruker AMX 400 at 162 MHz or Bruker DMX 500 at 202 MHz (H_3PO_4 as external standard). All ^{1}H and ^{13}C NMR chemical shifts (δ) are quoted in parts per million (ppm) downfield from tetramethylsilane, $(CD_3)(CD_2H)SO$ being set at δ_H 2.49 as a reference. ^{31}P NMR chemical shifts are quoted in ppm using H_3PO_4 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 spectra were taken with a Varian Cary 1E UV/Vis spectrometer. Infrared spectra were recorded with a Perkin-Elmer 1600 Series FT-IR or a ATI Mattson Genesis Series FT-IR spectrometer in KBr pellets. 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 enantiomers arising from the mixed stereochemistry at the phosphate center. The resulting lyophilized triesters did not gave useful microanalytical data most probably due to incomplete combustion of the compound, but were found to be pure by HPLC analysis, high-field multinuclear NMR spectroscopy, and mass spectroscopy.

9-[2-Diethylphosphonylmethoxyethyl]adenine 7. Diethyl-PMEA **8** was prepared according to a protocol published by Holy et al.[21] and the modifications introduced by Bronson et al.[22]

***N*^{6}-4-Monomethoxytrityl-9-[2-diethylphosphonylmethoxyethyl]adenine 9.** The compound was prepared according to Puech et al.[9] Yield: 2.13 g (3.54 mmol, 89%, colorless foam); the analytical data were found to be identical to those published.

General Procedure for the Preparation of the *N*^{6}-MMTr-*cyclo*Sal-PMEAs 10a−d. One equivalent of diethyl PMEA **9** was coevaporated twice with dry CH_3CN and subsequently solubilized in CH_3CN in an inert atmosphere. After addition of 50 μL of pyridine, 2.5 equiv of bromotrimethylsilane (TMSBr) were added slowly and the resulting mixture was stirred for 16 h at ambient temperature. The volatiles were distilled off in a high vacuum and the residual was coevaporated twice with toluene. The remaining foam was solubilized in dry CH_2Cl_2 and 2.1 equiv of PCl_5 were added. After 2.5 h of stirring at room temperature, the solvent was removed in high vacuum. The remaining yellowish foam was again coevaporated with toluene and then CH_2Cl_2 was added. The solution was cooled to 0 °C and within 15 min a solution of 2 equiv of

the salicyl alcohol **7** and 3.8 equiv of NEt_3 in dry CH_2Cl_2 were added dropwise. After complete addition, the solution was warmed to room temperature and the reaction was followed by TLC (CH_2Cl_2/MeOH 9:1 v/v; 0.1% HOAc). After complete evaporation of the solvent, the residues were purified by chromatography on a Chromatotron on silica gel plates using a gradient of MeOH (0−4%) in CH_2Cl_2.

N^6-4-Monomethoxytrityl-9-[2-*cyclo*Sal-phosphonylmethoxyethyl]adenine (N^6-MMTr-*cyclo*Sal-PMEA) 10a. Reagents: 500 mg (0.83 mmol) of N^6-4-monomethoxytrityl-9-[2-diethylphosphonylmethoxyethyl]adenine **9**, 269 μL (318 mg, 2.08 mmol) of bromotrimethylsilane (TMSBr), 5 mL of dry CH_3CN; 5 mL of dry CH_2Cl_2, 364 mg (1.75 mmol) of PCl_5; 2.5 mL of dry CH_2Cl_2, 207 mg (1.66 mmol) of salicyl alcohol **7a**, 440 μL (319 mg, 3.16 mmol) of dry NEt_3, 2.5 mL of dry CH_2Cl_2. Yield: 244 mg (0.39 mmol, 46%; colorless foam); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.3 (s, 1H), 7.32−7.26 (m, 9H), 7.22−7.19 (m, 5H), 7.14 (d, 1H), 7.01 (dd, 1H), 7.01 (d, 1H), 6.84 (d, 2H), 5.27 (dd, 1H), 5.23 (dd, 1H), 4.22−4.15 (m, 3H), 4.13 (dd, 1H), 3.83 (dd, 1H), 3.79 (dd, 1H), 3.70 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.9, 153.6, 151.2, 149.6, 148.8, 145.3, 141.6, 137.3, 130.0, 129.9, 128.6, 127.8, 126.7, 126.3, 124.2, 122.6, 120.4, 118.0, 113.2, 70.5, 70.1, 67.1, 64.0, 55.2, 42.5; ^{31}P NMR (202 MHz, DMSO-d_6) δ 16.56; MS (FAB) *m/z* calc. 633.21, found 634.5 (M + H$^+$).

N^6-4-Monomethoxytrityl-9-[2-(3-methyl-*cyclo*Sal)-phosphonylmethoxyethyl]adenine (N^6-MMTr-3-Me-*cyclo*Sal-PMEA) 10b. Reagents: 300 mg (0.50 mmol) of N^6-4-monomethoxytrityl-9-[2-diethylphosphonylmethoxyethyl]adenine **9**, 161 μL (191 mg, 1.25 mmol) of bromotrimethylsilane (TMSBr), 3 mL of dry CH_3CN; 4 mL of dry CH_2Cl_2, 302 mg (1.05 mmol) of PCl_5; 2.0 mL of dry CH_2Cl_2, 138 mg (0.99 mmol) of 3-methylsalicyl alcohol **7b**, 264 μL (192 mg, 1.89 mmol) of dry NEt_3, 2.0 mL of dry CH_2Cl_2. Yield: 102 mg (0.16 mmol, 32%; colorless foam); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (s, 1H), 7.84 (s, 1H), 7.31−7.19 (m, 13H), 7.15−7.12 (m, 1H), 7.00−6.94 (m, 2H), 6.86−6.83 (m, 2H), 5.25 (dd, 1H), 5.26 (dd, 1H), 4.20−4.13 (m, 3H), 4.09 (dd, 1H), 3.81 (dd, 1H), 3.77 (dd, 1H), 3.71 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.9, 153.5, 151.2, 148.7, 148.1, 145.4, 141.5, 137.3, 131.1, 130.0, 128.6, 127.8, 126.8, 126.7, 123.8, 123.7, 122.8, 120.4, 113.2, 70.5, 70.0, 67.0, 64.0, 55.1, 42.5, 15.0; ^{31}P NMR (202 MHz, DMSO-d_6) δ 17.46; MS (FAB) *m/z* calc. 647.23, found 648.4 (M + H$^+$).

N^6-4-Monomethoxytrityl-9-[2-(3-*tert*-butyl-*cyclo*Sal)-phosphonylmethoxyethyl]adenine (N^6-MMTr-3-*t*-Bu-*cyclo*Sal-PMEA) 10c. Reagents: 500 mg (0.83 mmol) of N^6-4-monomethoxytrityl-9-[2-diethylphosphonylmethoxyethyl]-adenine **9**, 269 μL (318 mg, 2.08 mmol) of bromotrimethylsilane (TMSBr), 5 mL of dry CH_3CN; 5 mL of dry CH_2Cl_2, 364 mg (1.75 mmol) of PCl_5; 2.5 mL of dry CH_2Cl_2, 300 mg (1.66 mmol) of 3-*tert*-butylsalicyl alcohol **7c**, 440 μL (319 mg, 3.16 mmol) of dry NEt_3, 2.5 mL of dry CH_2Cl_2. Yield: 274 mg (0.40 mmol, 48%; colorless foam); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (s, 1H), 7.81 (s, 1H), 7.31−7.26 (m, 9H), 7.22−7.19 (m, 5H), 7.07−7.02 (m, 2H), 6.84 (d, 2H), 5.24 (dd, 1H), 5.20 (dd, 1H), 4.19 (dd, 2H), 4.19 (dd, 1H), 4.13 (dd, 1H), 3.83 (dd, 1H), 3.80 (dd, 1H), 3.70 (s, 3H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.9, 153.5, 151.2, 148.7, 145.3, 141.6, 138.3, 137.3, 129.9, 128.6, 127.8, 127.3, 126.6, 124.5, 123.8, 120.4, 120.3, 113.2, 70.5, 70.4, 67.0, 64.0, 55.1, 42.7, 34.4, 29.7; ^{31}P NMR (202 MHz, DMSO-d_6) δ 16.93; MS (FAB) *m/z* calc. 689.28, found 690.5 (M + H$^+$).

N^6-4-Monomethoxytrityl-9-[2-(3,5-Di-*tert*-butyl-*cyclo*Sal)-phosphonylmethoxyethyl]adenine (N^6-MMTr-3,5-di-*t*-Bu-*cyclo*Sal-PMEA) 10d. Reagents: 500 mg (0.83 mmol) of N^6-4-monomethoxytrityl-9-[2-diethylphosphonylmethoxyethyl]-adenine **9**, 269 μL (318 mg, 2.08 mmol) of bromotrimethylsilane (TMSBr), 5 mL of dry CH_3CN; 5 mL of dry CH_2Cl_2, 364 mg (1.75 mmol) of PCl_5; 2.5 mL of dry CH_2Cl_2, 392 mg (1.66 mmol) of 3,5-di-*tert*-butylsalicyl alcohol **7d**, 440 μL (319 mg, 3.16 mmol) of dry NEt_3, 2.5 mL of dry CH_2Cl_2. Yield: 193 mg (0.26 mmol, 31%; colorless foam); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (s, 1H), 7.76 (s, 1H), 7.30−7.26 (m, 9H), 7.22−7.19 (m, 4H), 7.07−7.02 (m, 2H), 6.84 (d, 2H), 5.27 (dd, 1H), 5.22 (dd, 1H), 4.19 (dd, 2H), 4.17 (dd, 1H), 4.10 (dd, 1H), 3.83 (dd, 1H), 3.80 (dd, 1H), 3.71 (s, 3H), 1.26 (s, 9H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.9, 153.5, 151.2, 148.7, 148.2, 145.9, 145.3, 141.6, 137.5, 137.3, 129.9, 128.6, 127.8, 126.7, 123.9, 123.5, 121.5, 120.3, 113.1, 70.5, 70.1, 67.3, 63.9, 55.2, 42.6, 34.5, 34.4, 31.3, 29.7; ^{31}P NMR (202 MHz, DMSO-d_6) δ 16.98; MS (FAB) *m/z* calc. 745.34, found 746.2 (M + H$^+$).

General Procedure of the Deprotection of N^6-MMTr-*cyclo*Sal-PMEAs 10. The MMTr-protected *cyclo*Sal-PMEA derivative was solubilized in dry CH_2Cl_2 or CH_3CN in an inert atmosphere. Then 20 vol % of trifluoroacetic acid (TFA) was added dropwise. After 1 h of stirring at room temperature, the reaction mixture was diluted with water, and the resulting mixture was freeze-dried. The residual was purified by chromatography on a Chromatotron on silica gel plates using a gradient of MeOH (0−12%) in CH_2Cl_2 containing 0.1% of HOAc. The *cyclo*Sal-PMEAs **5** were isolated as their TFA salts.

9-[2-*cyclo*Sal-phosphonylmethoxyethyl]adenine (*cyclo*Sal-PMEA) 5a. Reagents: 71.0 mg (0.11 mmol) of *N*-4-monomethoxytrityl-9-[2-*cyclo*Sal-phosphonylmethoxyethyl]adenine **10a**, 250 μL of TFA, 1 mL of dry CH_2Cl_2. Yield: 28.4 mg (0.06 mmol, 53%; colorless solid); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (br. s, 1H), 8.43 (br. s, 1H), 8.39 (s, 1H), 8.17 (s, 1H), 7.45 (dd, 1H), 7.34 (d, 1H), 7.27 (dd, 1H), 7.16 (d, 1H), 5.47 (dd, 1H), 5.43 (dd, 1H), 4.42−4.39 (m, 1H), 4.32 (dd, 1H), 4.29 (dd, 1H), 4.01 (dd, 1H), 3.97 (dd, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.2, 152.8, 149.6, 148.0, 142.6, 129.9, 126.2, 124.2, 122.5, 120.5, 117.9, 70.4, 67.1, 63.5; ^{31}P NMR (202 MHz, DMSO-d_6) δ 16.62; ^{19}F NMR (471 MHz, DMSO-d_6) δ −74.40; MS (ESI) *m/z* calc. 361.09, found 362.26 (M + H$^+$), 384.28 (M + Na$^+$).

9-[2-(3-Methyl-*cyclo*Sal)-phosphonylmethoxyethyl]adenine (3-Me-*cyclo*Sal-PMEA) 5b. Reagents: 88.0 mg (0.14 mmol) of *N*-4-monomethoxytrityl-9-[2-(3-methyl-*cyclo*Sal-phosphonylmethoxyethyl]adenine **10b**, 500 μL of TFA, 2 mL of dry CH_2Cl_2. Yield: 44.6 mg (91.1 μmol, 67%; colorless solid); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (br. s, 1H), 8.28 (br. s, 1H), 8.24 (s, 1H), 7.98 (s, 1H), 7.17 (d, 1H), 7.03−6.98 (m, 2H), 5.28 (dd, 1H), 5.23 (dd, 1H), 4.27 (dd, 1H), 4.22 (dd, 1H), 4.16 (dd, 1H), 4.12 (dd, 1H), 3.83 (t, 2H), 2.11 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.1, 152.5, 149.6, 148.2, 140.9, 131.1, 126.8, 123.8, 123.8, 122.8, 118.7, 70.6, 67.0, 64.1, 42.5, 15.0; ^{31}P NMR (202 MHz, DMSO-d_6) δ 17.49; ^{19}F NMR (471 MHz, DMSO-d_6) δ −74.46; MS (FAB) *m/z* calc. 375.11, found 376.2 (M + H$^+$).

9-[2-(3-*tert*-Butyl-*cyclo*Sal)-phosphonylmethoxyethyl]-adenine (3-*t*-Bu-*cyclo*Sal-PMEA) 5c. Reagents: 191 mg (0.28 mmol) *of N*-4-monomethoxytrityl-9-[2-(3-*tert*-butyl-*cyclo*Sal-phosphonylmethoxyethyl]adenine **10c**, 750 μL of TFA, 2 mL of dry CH_3CN. Yield: 120 mg (227 μmol, 82%; colorless solid); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.81 (s, 1H), 7.46 (s, 2H), 7.28 (d, 1H), 7.11−7.05 (m, 2H), 5.27 (dd, 1H), 5.23 (dd, 1H), 4.21 (dd, 2H), 4.20−4.17 (m, 1H), 4.14 (dd, 1H), 3.86 (dd, 1H), 3.82 (dd, 1H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.1, 151.3, 149.4, 148.6, 141.4, 138.3, 127.3, 124.6, 124.0, 123.9, 118.6, 70.6, 67.0, 64.0, 42.7, 34.4, 29.7; ^{31}P NMR (202 MHz, DMSO-d_6) δ 17.06; ^{19}F NMR (471 MHz, DMSO-d_6) δ −74.32; MS (HR-ESI) *m/z* calc. 440.1464, found 440.1469 (M + Na$^+$).

9-[2-(3,5-Di-*tert*-butyl-*cyclo*Sal)-phosphonylmethoxyethyl]adenine (3,5-Di-*t*-Bu-*cyclo*Sal-PMEA) 5d. Reagents: 145 mg (0.19 mmol) of *N*-4-monomethoxytrityl-9-[2-(3,5-di-*tert*-butyl-*cyclo*Sal-phosphonylmethoxyethyl]adenine **10d**, 500 μL of TFA, 2 mL of dry CH_3CN. Yield: 75.0 mg (0.13 mmol, 66%; colorless solid); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.78 (s, 1H), 7.29 (d, 1H), 7.26 (s, 2H), 7.16 (d, 1H), 5.29 (dd, 1H), 5.24 (dd, 1H), 4.19 (dd, 2H), 4.18 (dd, 1H), 4.11 (dd, 1H), 3.86 (dd, 1H), 3.81 (dd, 1H), 1.29 (s, 9H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.7, 152.0, 149.5, 146.0, 146.0, 141.2, 137.6, 123.9, 123.6, 121.5, 118.7, 70.6, 67.4, 64.0, 42.6, 34.5, 34.4, 31.3, 29.7; ^{31}P NMR (202 MHz, DMSO-d_6) δ 17.49; ^{19}F NMR (471 MHz, DMSO-d_6) δ −74.46; MS (HR-ESI) *m/z* calc. 496.2090, found 496.2082 (M + Na$^+$).

2-Amino-6-fluoro-benzyl alcohol 11a. 1.00 g (6.40 mmol) of 2-amino-6-fluoro-benzoic acid dissolved in 30 mL of dry THF was added dropwise to a suspension of 0.73 g (19.0 mmol) of lithiumaluminum hydride in 60 mL of dry THF. The resulting mixture was stirred for 1 h at room temperature and then refluxed for 2 h. After cooling, the mixture was neutralized with 2 N HCl and extracted with EtOAc four times. The combined organic phases were filtered through Celite and then concentrated under reduced pressure. The crude product was recrystallized from petrolether/EtOAc 99:1. Yield 64%; mp 89−90 °C,^{1}H NMR (400 MHz, DMSO-d_6) δ 7.04 (ddd, 1H), 6.49−6.41 (m, 2H), 4.79 (d, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 148.4, 129.9, 112.9, 111.6, 104.8, 55.4; ^{19}F NMR (471 MHz, $CDCl_3$) δ −120.8; Anal. Calcd for C_7H_8FNO: C 59.57 H 5.71 N 9.92, found C 59.67 H 5.81 N 9.89.

General Procedure for the Preparation of the *cyclo*Amb-nucleoside Phosphonates 6a−c. 3.5 equiv of oxalyl chloride were added slowly to a slurry of PMEA **1** (1.0 equiv) and *N,N*-diethylformamide (1.1 equiv) in dry CH_2Cl_2 and the resulting mixture was refluxed for 3 h. The reaction was cooled to room temperature and concentrated in vacuo. The resulting foam was dissolved in dry CH_2Cl_2, cooled to 0 °C, and treated slowly with 2 equiv of pyridine. The resulting solution was then added slowly to a solution of 2-aminobenzyl alcohol (1.0 equiv) and DIPEA (6.3 equivalents) in dry CH_2Cl_2 at −78 °C. The reaction mixture was slowly warmed to room temperature, stirred overnight and was then concentrated in vacuo. The crude product was dissolved in EtOH, treated with HOAc (7.0 equiv), and warmed to reflux. After 2 h, the reaction was cooled to room temperature and concentrated. The residue was purified by silica gel chromatography three times [(1) 10% MeOH in EtOAc on a column; (2) on a Chromatotron on silica gel plates using a gradient of MeOH (3−15%) in CH_2Cl_2; (3) on a Chromatotron on silica gel plates using a gradient of MeOH (3−12%) in EtOAc]. Finally, the products were freeze-dried.

9-[2-(6-Fluoro)-*cyclo*Amb-phosphonylmethoxyethyl]-adenine (6-F-*cyclo*Amb-PMEA) 6a. Reagents: 200 mg (0.73 mmol) of PMEA **1**, 88 μL (80 mg, 0.79 mmol) of *N,N*-diethylformamide, 0.23 mL (0.35 g, 2.5 mmol) of oxalyl chloride, 5.5 mL of CH_2Cl_2; 0.13 mL (0.13 g, 1.5 mmol) of pyridine, 5 mL of CH_2Cl_2; 103 mg (0.73 mmol) of 2-amino-6-fluorobenzyl alcohol **11a**, 0.79 mL (0.60 g, 4.6 mmol) of DIPEA, 4 mL of CH_2Cl_2. Yield: 68 mg (0.18 mmol, 25%; colorless foam); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, 1H), 8.08 (s, 1H), 7.79 (s, 1H), 7.24−7.14 (m, 3H), 6.71 (dd, 1H), 6.66 (d, 1H), 5.26 (dd, 1H), 5.12 (dd, 1H), 4.18 (dt, 2H), 3.95 (dd, 1H), 3.89 (d, 1H), 3.79 (t, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.1, 155.9, 152.3, 149.4, 141.8, 140.8, 130.0, 118.5, 112.7, 110.0, 107.1, 70.3, 65.8, 60.9, 42.3; ^{19}F NMR (471 MHz, DMSO-d_6) δ −120.6; ^{31}P NMR (202 MHz, DMSO-d_6) δ 16.68; MS (HR-ESI) *m/z* calc. 378.1006, found 401.0897 (M + Na^+).

9-[2-*cyclo*Amb-phosphonylmethoxyethyl]adenine (*cyclo*Amb-PMEA) 6b. Reagents: 200 mg (0.73 mmol) of PMEA **1**, 88 μL (80 mg, 0.79 mmol) of *N,N*-diethylformamide, 0.23 mL (0.35 g, 2.5 mmol) of oxalyl chloride, 5.5 mL of CH_2Cl_2; 0.13 mL (0.13 g, 1.5 mmol) of pyridine, 5 mL of CH_2Cl_2; 90 mg (0.73 mmol) of 2-aminobenzyl alcohol **11b**, 0.79 mL (0.60 g, 4.6 mmol) of DIPEA, 4 mL of CH_2Cl_2. Yield: 86 mg (0.24 mmol, 33%; colorless foam); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (d, 1H), 8.09 (s, 1H), 7.73 (s, 1H), 7.22−7.09 (m, 3H), 7.12 (d, 1H), 6.88 (dd, 1H), 6.85 (d, 1H), 5.18−5.03 (m, 2H), 4.14 (t, 2H), 3.87 (d, 1H), 3.85 (d, 1H), 3.76 (t, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.9, 152.3, 149.4, 140.9, 139.9, 128.9, 125.6, 122.5, 120.6, 118.5, 116.7, 70.2, 66.5, 65.9, 42.3; ^{31}P NMR (202 MHz, DMSO-d_6) δ 17.49; MS (HR-ESI) *m/z* calc. 360.1100, found 361.1177 (M + H^+).

9-[2-(3-Methyl)-*cyclo*Amb-phosphonylmethoxyethyl]-adenine (3-Me-*cyclo*Amb-PMEA) 6c. Reagents: 200 mg (0.73 mmol) of PMEA **1**, 88 μL (80 mg, 0.79 mmol) of *N,N*-diethylformamide, 0.23 mL (0.35 g, 2.5 mmol) of oxalyl chloride, 5.5 mL of CH_2Cl_2; 0.13 mL (0.13 g, 1.5 mmol) of pyridine, 5 mL of CH_2Cl_2; 101 mg (0.73 mmol) of 2-amino-3-methylbenzyl alcohol **11c**, 0.79 mL (0.60 g, 4.6 mmol) of DIPEA, 4 mL of CH_2Cl_2. Yield: 117 mg (0.31 mmol, 42%; colorless foam); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.82 (d, 1H), 7.66 (s, 1H), 7.16 (s, 2H), 7.08 (d, 1H), 7.00 (d, 1H), 6.82 (dd, 1H), 5.13−5.97 (m, 2H), 4.16−4.07 (m, 2H), 3.85 (d, 2H), 3.73 (t, 2H), 2.16 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.9, 152.3, 149.3, 140.8, 138.1, 130.3, 129.2, 125.5, 123.4, 120.7, 118.5, 70.1, 66.1, 65.9, 42.4, 17.1; ^{31}P NMR (202 MHz, DMSO-d_6) δ 19.59; MS (HR-ESI) *m/z* calc. 374.1256, found 397.1151 (M + Na^+).

Kinetic Data. (a) Aqueous Buffers. 11.4 μL of DMSO stock solutions (50 mM) of the triesters were diluted in 300 μL of water or water/DMSO (c = 1.9 mM). 0.3 mL of this solution were added to 0.3 mL of aqueous buffer (50 mM phosphate buffer, pH 7.3 or 50 mM citrate buffer, pH 2.0) containing 5 μL of an aqueous AZT solution (AZT as internal standard) at 37 °C. The final concentrations were 0.96 mM for the triesters and 24.8 mM for the aqueous buffer. Aliquots of 60 μL of the hydrolysis mixture were taken, and the hydrolysis was stopped by addition of 5 μL of glacial acetic acid and frozen in liquid air. After thawing, samples were analyzed by analytical HPLC [Merck LiChroCART column, LiChrospher 100 reversed-phase silica gel RP-18 endcapped (5 μm); UV detection at 250 nm]. The hydrolysis of the compounds **5** and **6** was followed by integration of the decreasing peak areas in the HPLC chromatograms. The rate constants k were determined from slope of the logarithmic degradation curve. The half-lives ($t_{1/2}$) were calculated by using the rate constants k.

(b) CEM Cell Extract. 1.5 mM stock solution of the triesters in DMSO were prepared. 20 μL of this stock solution was mixed with 100 μL of cell extract and 20 μL of a 70 mM magnesium chloride solution. The hydrolysis process was stopped after 8 h by addition of 300 μL of acidic methanol and storage for 5 min at 0 °C. The mixtures were centrifuged by 13000 rpm for 10 min, filtered (Schleicher-Schuell Spartan 13/30, 0.2 μm), and the supernatant was analyzed as mentioned above.

(c) Human Sera. The studies were performed as described in (b) but instead of cell extracts 20% of human serum in phosphate buffer, pH 6.8 was used, and the data were collected in the same way.

^{31}P NMR Hydrolysis Studies of the *cyclo*Sal-Phosphate Triesters. Approximately 7 μmol of the *cyclo*Sal- or *cyclo*Amb-phosphonates were dissolved in 300 μL of deuterated DMSO and 700 μL of a 50 mM imidazole/HCl buffer (pH 7.3). The resulting solutions were transferred immediately into a NMR tube and investigated by ^{31}P NMR spectroscopy (proton decoupled, 202 MHz, 1024 scans each sample). All samples were stored at room temperature. Furthermore, some proton coupled ^{31}P NMR spectra (202 MHz, 1024 scans each sample) were recorded for the identification of the hydrolysis products.

Cholinesterase Assay. The assay has been carried out as published previously[19] using isolated human acetyl- and butyrylcholinesterase as well as human serum.

Determination of the Partition Coefficients (log*P* Values). log*P* values were determined as described in ref 6a.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 (HIV-1) was originally obtained from a persistently HIV-infected H9 cell line, as described previously, and was kindly provided by Dr. R. C. Gallo (then at the National Institutes of Health, Bethesda, MD). Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells. HIV-2 (strain ROD) was kindly provided by Dr. L. Montagnier then at the Pasteur Institute, Paris, France, and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells. CEM cells were obtained from the American Tissue Culture Collection (Rockville, MD). CEM cells were infected with HIV as previously described.[23] Briefly, 4×10^5 cells/mL were infected with HIV-1 or HIV-2 at ∼100 $CCID_{50}$ (50% cell culture infective dose) per mL of cell suspension. Then, 100 μL of the infected cell suspension was transferred into 96-well microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4−5 days, giant cell

formation was recorded microscopically in the HIV-infected cell cultures.

Acknowledgment. We gratefully acknowledge the support of the *Deutsche Forschungsgemeinschaft*, the Vth Framework Program of the European Commission, the Fonds voor Wetenschappelijk Onderzoek (FWO)-Vlaanderen and the Belgian Geconcerteerde Onderzoecksacties. We thank Mrs. Ann Absillis and Mrs. Lizette van Berckelaer for excellent technical help.

Supporting Information Available: Detailed characterization including high-resolution mass spectrometry of compounds **10a**−**d**, **5a**−**d**, **11a** and **6a**−**c**. Additionally, ^{31}P NMR spectra are given showing the selective release of PMEA from *cyclo*Sal- and *cyclo*Amb-phosphonates. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Naesens, L.; Balzarini, J.; De Clercq, E. Therapeutical potential of PMEA as an antiviral drug. *Med. Virol*. **1994**, *4*, 147−159. (b) De Clercq, E. Broad-spectrum anti-DNA virus and anti-retrovirus activity of phosphonylmethoxyalkylpurines and -pyrimidines. *Biochem. Pharmacol.* **1991**, *42*, 963−972. (c) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* **1986**, *323*, 464−467. (d) De Clercq, E. Cidofovir in the treatment of poxvirus infections. *Antiviral Res.* **2002**, *55*, 1−13. (e) Arimilli, M. N.; Dougherty, J. P.; Cundy K. C.; Bischofberger, N. Orally bioavailable acyclic nucleoside phosphonate prodrugs: Adefovir dipivoxil and bis(POC)PMPA. In *Advances in Antiviral Drug Design*; De Clercq E., Ed.; JAI Press: Stamford, CT, 1999; pp 69−91. (f) Robbins, B. L.; Srinivas, R. V.; Kim, C.; Bischofberger, N.; Fridland, A. Anti-Human Immunodeficiency Virus Activity and Cellular Metabolism of a Potential Prodrug of the Acyclic Nucleoside Phosphonate 9-*R*-(2-Phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl)PMPA. *Antimicrob. Agents Chemother*. **1998**, *42*, 612−617.

(2) Srinivas, R. V.; Robbins, B. L.; Connelly, M. C.; Gong, Y. F.; Bischofberger, N.; Fridland, A. Metabolism an in vitro Antiretroviral Activities of Bis(Pivaloyloxymethyl)-Prodrugs of Acyclic Nucleoside Phosphonates. *Antimicrob. Agents Chemother*. **1993**, *37*, 2247−2250.

(3) Starrett Jr., J. E.; Tortolani, D. R.; Hitchcock, M. J. M.; Martin, J. C.; Mansuri, M. M. Synthesis and in vitro evaluation of a phosphonate prodrug: bis(pivaloylosymethyl) 9-(2-phosphonylmethoxyethyl)adenine. *Antiviral Res.* **1992**, *19*, 267−273.

(4) Benzaria, S.; Pélicano, H.; Johnson, R.; Maury, G.; Imbach, J. L.; Aubertin, A. M.; Obert, G.; Gosselin, G. Synthesis, in vitro antiviral evaluation, and stability studies of bis(*S*-acyl-2-thioethyl) ester derivatives of 9-(2-phosphonometoxy)ethyladenine (PMEA) as potential PMEA prodrugs with improved oral bioavailability. *J. Med. Chem*. **1996**, *39*, 4958−4965.

(5) (a) Meier, C. *Cyclo*Sal-pronucleotides − design of chemical trojan horses. *Mini Rev. Med. Chem.* **2002**, *2*, 219−234. (b) Meier, C.; Ruppel, M.; Vukadinovic, D.; Balzarini, J. "Lock-in"-*cyclo*Sal-pronucleotides − a new generation of chemical trojan horses. *Mini Rev. Med. Chem*. **2004**, *4*, 383−394. (c) Meier, C. *Cyclo*Sal-pronucleotides − design of the concept, chemistry and antiviral acticity. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press: Stamford, CT, 2004; Vol. 4, pp 147−213.

(6) (a) Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. *Cyclo*Sal-2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphate (*cyclo*Sal-d4TMP): Synthesis and antiviral evaluation of a new d4TMP delivery system. *J. Med. Chem.* **1998**, *41*, 1417−1427. (b) Ducho, C.; Wendicke, S.; Görbig, U.; Balzarini, J.; Meier, C. 3,5-*tert*-Butyl-6-fluoro-*cyclo*Sal-d4TMP − a pronucleotide with a highly optimized masking group. *Eur. J. Org. Chem*. **2003**, 4786−4791.

(7) Meier, C.; Lomp, A.; Meerbach, A.; P. Wutzler, P. *Cyclo*Sal-BVDUMP pronucleotides − How to convert an anti-EBV-inactive nucleoside analogue into a bioactive compound. *J. Med. Chem*. **2002**, *45*, 5157−5172.

(8) Meier, C.; Ruppel, M.; Vukadinovic, D.; Balzarini, J. Second generation of *cyclo*Sal-pronucleotides with esterase-cleavable sites − the "lock-in"-concept. *Nucl., Nucl., Nucleic Acids* **2004**, 89−115.

(9) Puech, F.; Gosselin, G.; Lefebvre, I.; Pompom, A.; Aubertin, A.-M.; Kirn, A.; Imbach, J.-L. Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process. *Antiviral Res.* **1993**, *22*, 155−174.

(10) (a) Morita, T.; Okamoto, Y.; Sakurai, H. A mild and facile synthesis of alkyl- and arylphosphonyl dichlorides under neutral conditions. Reaction of bis(trimethylsilyl) phosphonates with PCl_5. *Chem. Lett*. **1980**, 435−438; (b) Serafinowska, H. T.; Ashton, A. J.; Bailey, S.; Harnden, M. R.; Jackson, S. M.; Sutton, D. Synthesis and in vivo evaluation of prodrugs of 9-[2-(phosphonomethoxy)ethoxy]adenine. *J. Med. Chem.* **1995**, *38*, 1372−1379.

(11) Erion, M. D.; Reddy, K. R.; Boyer, S. H.; Matelich, M. C.; Gomez-Galeno, J.; Lemus, R. H.; Ugarkar, B. G.; Colby, T. J.; Schanzer, J.; Poelje, P. D. Design, synthesis and characterization of a series of cytochrome P_{450} 3A-activated prodrugs (HepDirect prodrugs) useful for targeting phosph(on)ate-based drugs to the liver. *J. Am. Chem. Soc*. **2004**, *126*, 5154−5163.

(12) Balzarini, J.; Cools, J. M.; De Clercq, E. Estimation of the lipophilicity of anti-HIV nucleoside analogues by determination of the partition coefficient and retention time on a LiChrosphere 60 RP-8 HPLC column. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 413−422.

(13) (a) Balzarini, J.; Aquaro, S.; Knispel, T.; Rampazzo, C.; Bianchi, V.; Perno, C.-F.; De Clercq, E.; Meier, C. Cyclosaligenyl-2′,3′-didehydro-2′,3′-dideoxythymidine monophosphate: Efficient intracellular delivery of d4TMP. *Mol. Pharmacol*. **2000**, *58*, 928−935. (b) Balzarini, J.; Naesens, L.; Aquaro, S.; Knispel, T.; Perno, C.-F.; De Clercq, E.; Meier, C. Intracellular metabolism of *cyclo*Saligenyl 3′-azido-2′,3′-dideoxythymidine monophosphate, a prodrug of 3′-azido-2′,3′-dideoxythymidine (Zidovudine). *Mol. Pharmacol*. **1999**, *56*, 1354−1361.

(14) Cox, Jr., J. R.; Ramsay, B. Mechanisms of nucleophilic substitution in phosphate esters. *Chem. Rev.* **1964**, *64*, 317−352.

(15) Hudson, R. F.; Keay, L. The hydrolysis of phosphonate esters. *J. Chem. Soc.* **1956**, 2463−2469.

(16) Westheimer, F. H.; Huang, S.; Covitz, F. Rates and mechanisms of hydrolysis of esters of phosphorous acid. *J. Am. Chem. Soc.* **1988**, *110*, 181−185.

(17) Meier, C.; Renze, J.; Ducho, C.; Balzarini, J. *Cyclo*Sal-d4TMP pronucleotides − Structural variations, mechanistic insights and antiviral activity. *Curr. Top. Med. Chem.* **2002**, *2*, 1111−1121.

(18) Balatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J. Synthesis and evaluation of novel amidate prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1053−1056.

(19) Ducho, C.; Balzarini, J.; Meier, C. Noninhibition of acetylcholinesterase by *cyclo*Sal-nucleotides. *Nucl., Nucl., Nucleic Acids* **2003**, *22*, 841−843.

(20) Meier, C.; Ducho, C.; Görbig, U.; Esnouf, R.; Balzarini, J. Interaction of *cyclo*Sal-pronucleotides with cholinesterases from different origins − A structure−activity relationship. *J. Med. Chem.* **2004**, *47*, 2839−2852.

(21) Holy, A.; Rosenberg, I. Synthesis of 9-[2-(phosphonylmethoxy)-ethyl]adenine and related compounds. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2801−2809.

(22) Bronson, J. J.; Ghazzouli, I., Hitchcock, M. J. M.; Webb, R. R., II.; Martin, J. C. Synthesis and antiviral activity of the nucleotide analogue (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]-cytosine. *J. Med Chem.* **1989**, *32*, 1457−1463.

(23) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. Detection, Isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **1984**, *224*, 497−500.

JM050641A