# Synthesis and Antiviral Evaluation of Alkoxyalkyl Derivatives of 9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine against Cytomegalovirus and Orthopoxviruses

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9-(*S*)-(3-Hydroxy-2-phosphonomethoxypropyl)adenine [(*S*)-HPMPA] was one of the first acyclic nucleoside phosphonates described and has been reported to have good antiviral activity against most double-stranded DNA viruses, including the herpes group viruses and the orthopoxviruses. However, (*S*)-HPMPA is not orally bioavailable and has not been developed for clinical use. We have prepared orally bioavailable lipid esters of (*S*)-HPMPA and report their synthesis and antiviral evaluation against cytomegalovirus and orthopoxviruses. These esters were evaluated in vitro in cells infected with human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), vaccinia (VV), and cowpox viruses (CV). The most active compound, oleyloxyethyl-(*S*)-HPMPA, was found to have $EC_{50}$ value of 0.003 $\mu$M against HCMV vs 1.4 $\mu$M for unmodified HPMPA. In cells infected with VV and CV, octadecyloxyethyl-(*S*)-HPMPA had $EC_{50}$ values of 0.01−0.02 $\mu$M versus 2.7−4.0 $\mu$M for unmodified HPMPA. When compared with the alkoxyalkyl esters of cidofovir, the corresponding alkoxyalkyl esters of (*S*)-HPMPA were equally active against HCMV and MCMV but were 15−20-fold more active against VV and CV in vitro. The alkoxyalkyl esters of (*S*)-HPMPA are promising new compounds worthy of further investigation for treatment of infections caused by herpes viruses and orthopoxviruses.

## Introduction

(*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)-adenine] was the first acyclic nucleoside phosphonate to show potent, broad-spectrum antiviral activity. Described by Holý and De Clerq in 1986,[1] (*S*)-HPMPA inhibits replication of many double-stranded DNA viruses, including herpes simplex virus (HSV) types 1 and 2, varicella zoster virus (VZV), thymidine kinase-deficient ($TK^-$) mutants of HSV and VZV, human cytomegalovirus (HCMV), Epstein−Barr virus, hepatitis B virus, adenoviruses, and various orthopoxviruses.[2] (*S*)-HPMPA also arrests the growth of *Plasmodium falciparum* (the causative agent of malaria)[3] and exhibits cytostatic activity against L1210 mouse leukemia cells in vitro.[4]

After the discovery of (*S*)-HPMPA, extensive studies of acyclic nucleoside phosphonates led to the development of three licensed antivirals—cidofovir (CDV; CMV retinitis), adefovir (hepatitis B), and tenofovir (HIV/AIDS)[5]—and the identification of many more compounds with potentially useful biological activity.[6] However, due to the presence of two negative charges, free acyclic nucleoside phosphonates are not absorbed well orally.[7] After intravenous injection, drugs of this class accumulate in the kidney, the site of dose-limiting toxicity.[8] CDV (Vistide) is administered intravenously, while the oral formulations of adefovir (Hepsera) and tenofovir (Viread) are the bis-pivaloyloxymethyl and bis-(isopropoxycarbonyl)oxymethyl esters, respectively.[9,10] Several other phosphonate-masking prodrug strategies, including aryl phosphonamidates[11] and cycloSal esters,[12] have been applied to acyclic nucleoside phosphonates.

Of particular interest is the potent activity of CDV, (*S*)-HPMPA, and other nucleoside phosphonates against orthopoxviruses, including cowpox, vaccinia,[13] ectromelia (monkeypox), and smallpox (variola virus).[14] If used as a weapon against an unimmunized population, variola virus has the potential to infect tens of thousands of individuals, kill 30% or more of those infected, and trigger the vaccination of millions.[15] However, large numbers of people are not good candidates for vaccination because of immunosuppression, skin diseases, and cardiovascular disease. Another concern is suggested by the discovery that recombinant poxviruses encoding interleukin-4 are lethal despite prior vaccination.[16] For these reasons, development of therapeutics for orthopoxvirus infection is an important area of research.[17] Animal studies indicate that parenteral CDV may be useful for prevention and/or treatment of vaccinia and variola infections.[14] Since intravenous agents would not be practical in an emergency situation, safe orally active antiviral drugs for orthopox infections are needed.

We previously reported that esterification of cidofovir with certain alkanols or alkoxyalkanols enhances the antiviral activity and selectivity of CDV in vitro.[18−20] Optimal in vitro activity was found with 20-atom alkyl, alkenyl, alkylglyceryl, or alkoxyalkyl groups esterified to CDV.[21] Oral dosing of several CDV esters showed good bioavailability, greatly reduced accumulation of drug in kidney,[22] and anti-orthopoxvirus efficacy in three lethal animal models of orthopoxvirus disease, including ectromelia.[23−25] On the basis of these results, hexadecyloxypropyl cidofovir (HDP-CDV) is currently under development for possible use as an oral treatment in case of a smallpox bioterror attack.[26] CDV-resistant strains of CMV have been isolated from treated patients[27] and laboratory-induced CDV resistance has been observed in camelpox, cowpox, monkeypox, and vaccinia viruses.[28] Additional agents against

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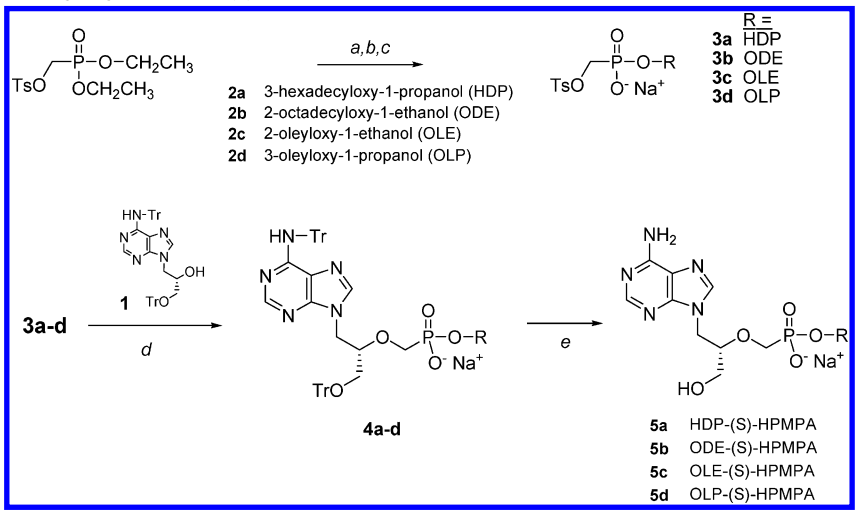
**Scheme 1.** Synthesis of Alkoxyalkyl Esters of (*S*)-HPMPA[a]




[a] (a) TMSBr, $CH_2Cl_2$; (b) oxalyl chloride, cat. *N*,*N*-DMF, $CH_2Cl_2$; (c) **2a**−**d**, ethyl ether, pyridine; (d) NaH, $Et_3N$ (solvent), 50 °C; (e) 80% aq $CH_3COOH$, 60 °C.

poxvirus disease would be useful. Since (*S*)-HPMPA was reported earlier to be more active against poxviruses than CDV,[13,14,29] we began investigating (*S*)-HPMPA derivatives to address the need for more active and selective antiviral drugs. In this report, we describe the synthesis and biological evaluation of several new alkoxyalkyl analogues of (*S*)-HPMPA.

## Results

**Chemistry.** We previously synthesized alkoxyalkyl CDV esters by either esterifying cyclic CDV (cCDV) with alkoxyalkyl bromides or, more efficiently, using the Mitsunobo reaction to couple alkoxyalkanols to cCDV.[21] Since we did not have access to premade (*S*)-HPMPA or cyclic (*S*)-HPMPA, we envisioned the route outlined in Scheme 1 as a more direct approach to the target compounds. Our strategy was based on previous (*S*)-HPMPA syntheses reported by Webb[30] and Holý.[31] Both groups prepared (*S*)-9-(2,3-dihydroxypropyl)adenine derivatives with the 3-hydroxyl and 6-amino groups protected and then etherified the 2-hydroxyl using a dialkyl *p*-toluenesulfonyloxymethylphosphonate in the presence of a strong base. Deprotection, including dealkylation of the phosphonodiesters with bromotrimethylsilane, gave (*S*)-HPMPA.

We prepared (*S*)-9-(3-trityloxy-2-hydroxypropyl)-$N^6$-trityladenine according to Webb's method, expecting that it would be more stable than the $N^6$-benzoyl derivative under the basic conditions of the impending alkylation step. Adenine, heated with (*S*)-trityl glycidyl ether in *N*,*N*-DMF/DBU, gave (*S*)-9-(3-trityloxy-2-hydroxypropyl)adenine. Further reaction of (*S*)-9-(3-trityloxy-2-hydroxypropyl)adenine with trityl chloride in refluxing pyridine afforded (*S*)-9-(3-trityloxy-2-hydroxypropyl)-$N^6$-trityladenine (**1**) in 53% yield.

Rather than alkylate **1** with a dialkyl toluenesulfonyloxymethylphosphonate, the standard method for HPMP compounds, we prepared alkoxyalkyl toluenesulfonyloxymethylphosphonates **3a**−**d**, with the lipophilic groups preattached. Initial attempts to couple toluenesulfonyloxymethylphosphonic acid to alkoxyalkanols **2a**−**d** using DCC/pyridine resulted in low yields of the monoesters due to loss of the tosyl group during the condensation. A more efficient procedure was to treat the diethyl *p*-toluenesulfonyloxymethylphosphonate with TMSBr and then oxalyl chloride. Reaction of the resulting phosphonodichloridate with **2a**−**d**, followed by hydrolysis, gave phosphonoesters **3a**−**d** in good yield.

Solutions of **1** in triethylamine were treated with sodium hydride and then phosphonoesters **3a**−**d** and the mixture was heated to 50 °C to obtain fully protected (*S*)-HPMPA esters **4a**−**d**. Interestingly, the use of triethylamine as the solvent facilitated the alkylation reaction. Lower yields resulted when alkylation was attempted in either THF or *N*,*N*-DMF solvent. Reaction of **3a** and **3b** with **1** provided satisfactory yields of fully protected (*S*)-HPMPA analogues. However, unsaturated esters **3c** and **3d** gave lower yields of alkylated product and complete separation of products **4c** and **4d** from residual **3c**−**d** by flash chromatography was difficult. However, impure **4c** and **4d**, could be deprotected (80% aq acetic acid) and then purified easily by flash chromatography on silica gel to give **5c** and **5d**. In contrast to the unsaturated esters, saturated phosphonates **3a** and **3b** reacted with (*S*)-9-(3-trityloxy-2-hydroxypropyl)-$N^6$-trityladenine in triethylamine to give fully protected intermediates **4a** and **4b**, and they were isolable in reasonable yields and high purity. Compounds **4a** and **4b** were deprotected (80% acetic acid) to provide (*S*)-HPMPA derivatives **5a** and **5b**.

**Biological Activity. In Vitro Antiviral Activity.** The (*S*)-HPMPA alkoxylalkyl esters **5a**−**d** were evaluated for their inhibitory activity in cells infected with HCMV, MCMV, VV, and CV. The unmodified phosphonates (*S*)-HPMPA and CDV were used as comparators. All four alkoxyalkyl esters were more active than (*S*)-HPMPA against HCMV and MCMV (Table 1). Against HCMV, $EC_{50}$ values of 3 nM were obtained for all four analogues, which is about 270-fold more potent than HPMPA itself. The most selective compound was OLP-(*S*)-HPMPA (**5d**), which had a selectivity index of 25 000 against HCMV due to its lower cytotoxicity. Against MCMV, the most active compound was HDP-(*S*)-HPMPA (**5a**) and the most selective was OLP-(*S*)-HPMPA (**5d**).

When the compounds were evaluated against VV and CV they were also more active than (*S*)-HPMPA (Table 2). The most active compound was OLE-(*S*)-HPMPA (**5c**), with an $EC_{50}$ value of 3 nM. Against VV and CV, the alkoxyalkyl esters were 150−900 and 117−561-fold more active, respectively, than (*S*)-HPMPA.

## Discussion

Esterification of (*S*)-HPMPA to alkoxyalkyl moieties having 20 or 21 atoms clearly increases antiviral activity and selectivity, as shown in Tables 1 and 2, extending our findings with CDV

**Table 1.** Inhibition of Human and Murine Cytomegalovirus (HCMV and MCMV) Replication by Alkoxyalkyl Esters of (*S*)-HPMPA

| compd | cytotoxicity ($CC_{50}$, $\mu$M)[a,b] | HCMV | | MCMV | |
|---|---|---|---|---|---|
| | | $EC_{50}$ ($\mu$M)[b] | selectivity[c] | $EC_{50}$ ($\mu$M)[b] | selectivity[c] |
| CDV | >317 ± 0 | 1.2 ± 0.4 | >264 | 0.04 ± 0.03 | >7925 |
| (*S*)-HPMPA | >289 ± 38 | 0.82 ± 0.23 | >352 | 0.16 ± 0.06 | >181 |
| HDP-(*S*)-HPMPA (**5a**) | 9.7 ± 6.2 | 0.003 ± 0.0007 | 3233 | 0.002 ± 0 | 485 |
| ODE-(*S*)-HPMPA (**5b**) | 1.5 ± 0.04 | 0.003 ± 0.001 | 500 | 0.003 ± 0.002 | 500 |
| OLE-(*S*)-HPMPA (**5c**) | 6.3 ± 1.6 | 0.003 ± 0.0007 | 2100 | 0.04 ± 0.007 | 158 |
| OLP-(*S*)-HPMPA (**5d**) | 74.8 ± 6.3 | 0.003 ± 0 | 25000 | 0.1 ± 0.03 | 750 |

[a] Cytotoxicity by neutral red uptake in HFF cells. [b] Values are the mean of two or more assays ± standard deviation. [c] Selectivity index (SI) = $CC_{50}/EC_{50}$.

**Table 2.** Inhibition of Vaccinia and Cowpox Virus Replication by Alkoxyalkyl Esters of (*S*)-HPMPA

| compd | cytotoxicity ($CC_{50}$, $\mu$M)[a,b] | vaccinia Copenhagen | | cowpox Brighton | |
|---|---|---|---|---|---|
| | | $EC_{50}$ ($\mu$M)[b] | selectivity[c] | $EC_{50}$ ($\mu$M)[b] | selectivity[c] |
| CDV | >317 ± 0 | 25.3 ± 6.1 | >12.5 | 30.7 ± 6.3 | >10.3 |
| (*S*)-HPMPA | >289 ± 38 | 2.7 ± 2.4 | >107 | 4.0 ± 3.8 | >72 |
| HDP-(*S*)-HPMPA (**5a**) | 9.7 ± 6.2 | 0.01 ± 0.004 | 970 | 0.02 ± 0.006 | 485 |
| ODE-(*S*)-HPMPA (**5b**) | 1.5 ± 0.4 | 0.01 ± 0.003 | 150 | 0.02 ± 0.017 | 75 |
| OLE-(*S*)-HPMPA (**5c**) | 6.3 ± 1.6 | 0.003 ± 0.001 | 2100 | 0.007 ± 0 | 900 |
| OLP-(*S*)-HPMPA (**5d**) | 74.8 ± 6.3 | 0.018 ± 0.005 | 4156 | 0.034 ± 0.004 | 2200 |

[a] Cytotoxicity by neutral red uptake. [b] Values are the mean of two or more assays ± standard deviation. [c] Selectivity index (SI) = $CC_{50}/EC_{50}$.

**Table 3.** Inhibition of Viral Replication by Alkoxyalkyl Esters of CDV and HPMPA[a]

| compd | $EC_{50}$ ($\mu$M) | | | |
|---|---|---|---|---|
| | HCMV | MCMV | vaccinia | cowpox |
| cidofovir (HPMPC) | 1.20 | 0.04 | 46.2 | 44.7 |
| HDP-CDV | 0.0009 | 0.0009 | 0.80 | 0.60 |
| ODE-CDV | 0.0009 | 0.0010 | 0.20 | 0.30 |
| (*S*)-HPMPA | 0.82 | 0.16 | 2.70 | 4.00 |
| HDP-(*S*)-HPMPA (**5a**) | 0.003 | 0.002 | 0.01 | 0.02 |
| ODE-(*S*)-HPMPA (**5b**) | 0.003 | 0.003 | 0.01 | 0.02 |

[a] Data abstracted from Tables 1 and 2 and from refs 18, 19, and 21.

(HPMPC) to (*S*)-HPMPA, a related acyclic nucleoside phosphonate.[18−21] HDP-CDV gains its increased antiviral activity because of its greatly enhanced cell uptake and conversion to CDV by cellular phospholipases C and/or phosphodiesterases.[32] The same mechanisms are presumed to increase the antiviral activity of HDP-(*S*)-HPMPA compared with (*S*)-HPMPA. In vitro studies with $^{14}$C-labeled HDP-(*S*)-HPMPA are currently in progress.

We compared the antiviral activity of HDP and ODE esters of CDV and HPMPA against HCMV and MCMV and with VV and CV using previously published data for the CDV analogues (Table 3).[18,19,21] Against HCMV and MCMV the HDP and ODE esters of CDV and HPMPA differed little in activity, the CDV esters being slightly more active (2−3-fold) than the HPMPA analogues. Unmodified CDV and (*S*)-HPMPA had similar antiviral activity against the cytomegaloviruses. However, against both poxviruses, (*S*)-HPMPA was much more active than CDV (11−16-fold). Strikingly, the HDP and ODE esters of HPMPA were substantially more active than the corresponding esters of CDV: 80- and 20-fold for VV and 30- and 15-fold for CV. The increased activity of HDP- and ODE-(*S*)-HPMPA versus HDP- and ODE-CDV against poxviruses but no difference in activity versus CMV is not likely to be due to cellular uptake and metabolism of the alkoxyalkyl esters of CDV and HPMPA to their respective diphosphates, because both the CMV and poxvirus studies were done in the same HFF cell line. Therefore, the marked increase in (*S*)-HPMPA analogue activity against the poxviruses may be due to differences in their effect on the vaccinia E9L DNA polymerase. Evans and coworkers[33] found that the mechanism of inhibition of the VV DNA polymerase by CDV-diphosphate was slightly different than that reported for the CMV DNA polymerase.[34] With the VV DNA polymerase, CDV can be incorporated into the growing DNA strand opposite template G's; however, the enzyme exhibits a lower catalytic efficiency compared with dCTP. CDV-terminated primers are good substrates for the next dNMP addition, but these CDV + 1 base reaction products are not good substrates for further DNA synthesis. Although CDV can be excised from the primer 3′-terminus by the 3′-to-5′ proofreading exonuclease activity of the VV DNA polymerase, DNAs bearing CDV as the penultimate 3′-residue are completely resistant to exonuclease attack.[33] It will be interesting to determine the mechanism of (*S*)-HPMPA diphosphate inhibition of the vaccinia DNA polymerase in view of the multiple log increase in antiviral activity noted with HPMPA and its alkoxyalkyl esters as compared with CDV.

In conclusion, the alkoxyalkyl esters of HPMPA exhibit multiple log increases in antiviral activity versus HCMV, MCMV, VV, and CV versus unmodified HPMPA. HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA are particularly active against the poxviruses with antiviral activity 15−30-fold greater than the corresponding CDV analogue against VV and CV, respectively. Compounds of this class are potentially useful against infections caused by the orthopoxviruses and are worthy of further development.

## Experimental Section

**Chemistry Methods.** Elemental microanalyses were performed by Atlantic Microlabs of Norcross, GA. $^1$H and $^{31}$P NMR spectra were recorded on a Varian HG-400 NMR spectrophotometer with tetramethylsilane (internal) and 85% $D_3PO_4$ in $D_2O$ (external) as references for $^1$H and $^{31}$P (0.00 ppm), respectively. Electospray ionization mass spectra (MS ES) were obtained by HT Laboratories (San Diego, CA). Analytical thin-layer chromatography (TLC) was performed on Analtech 250 $\mu$m silica gel GF Uniplates visualized under UV light, with Phospray (Supelco, Bellafonte, PA) and charring at 400 °C. Chromatographic purification was done by the flash method using Merck silica gel (240−400 mesh). All commercially obtained reagents were used as received unless otherwise noted.

Diethyl toluenesulfonyloxymethylphosphonate was synthesized from diethyl hydroxymethylphosphonate.[35] (*S*)-9-[3-Trityloxy-2-hydroxypropyl]-$N^6$-trityladenine was prepared from adenine and (*S*)-trityl glycidyl ether (Daiso Co., Ltd.) following the method of Webb.[30] Lipophilic alkoxyalkanols (**2a**−**d**, Scheme 1) were synthesized according to procedures we previously described.[36]

**General Procedure for the Synthesis of Alkoxyalkyl Toluenesulfonyloxymethylphosphonates (3a−d).** Bromotrimethylsilane (27 g, 175 mmol) was added to a solution of diethyl toluenesulfonyloxymethylphosphonate (9.5 g, 29.5 mmol) in dichloromethane (anhydrous, 150 mL). The mixture was stirred at room temperature under a $N_2$ atmosphere for 18 h. The mixture was then concentrated under vacuum to remove solvent and excess TMSBr and then redissolved in dichloromethane (150 mL) and cooled to 0 °C with an ice bath. *N*,*N*-DMF (0.5 mL) was added, a solution of oxalyl chloride (22 g, 175 mmol) in $CH_2Cl_2$ (50 mL) was added dropwise over 30 min, and then the mixture was stirred for an additional 5 h. The mixture was evaporated to an oil which was then redissolved in $Et_2O$ (100 mL). A solution of the alkoxyalkanol (**2a−d**, 21.5 mmol) and pyridine (10 mL) in $Et_2O$ (50 mL) was added, and stirring was continued for about 3 h or until TLC analysis (1:1 hexanes/ethyl acetate) indicated complete phosphonylation of the alcohol. The reaction mixture was then added to cold, saturated $NaHCO_3$ and vigorously stirred for 1 h. After hydrolysis was complete, the organic layer was separated, dried over $MgSO_4$, and evaporated under vacuum to give the crude esters, which were purified by flash chromatography (elution solvent: 15% EtOH/$CH_2Cl_2$).

**3-(Hexadecyloxy)propyl toluenesulfonyloxymethylphosphonate, sodium salt (3a),** was prepared from 3-hexadecyloxy-1-propanol (**2a**) in 67% yield: $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, 3H, $-CH_3$), 1.26 (br s, 26H, $OCH_2CH_2(CH_2)_{13}CH_3$), 1.54 (m, 2H, $OCH_2CH_2(CH_2)_{13}CH_3$), 1.83 (qt, 2H, $-OCH_2CH_2CH_2O-$), 2.46 (s, 3H, toluyl $CH_3$), 3.38 (t, 2H, $-CH_2-$), 3.47 (t, 2H, $-CH_2-$), 3.91 (dt, 2H, $-P(O)OCH_2-$), 4.02 (d, 2H, $-CH_2P(O)O-$), 7.37 (d, 2H, aromatic), 7.80 (d, 2H, aromatic); MS (ES) *m*/*z* 571.32 $[M + Na]^+$, 593.27 $[M - H + 2Na]^+$, 547.02 $[M - H]^-$. Anal. ($C_{27}H_{48}O_7NaPS\cdot0.5H_2O$) C, H, N.

**2-(Octadecyloxy)ethyl toluenesulfonyloxymethylphosphonate, sodium salt (3b),** was prepared from 2-octadecyloxy-1-ethanol (**2b**) in 71% yield: $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, 3H), 1.26 (br s, 30H), 1.54 (m, 2H), 2.60 (s, 3H), 3.51 (t, 2H), 3.65 (t, 2H), 4.03 (dt, 2H), 4.16 (d, 2H), 7.35 (d, 2H), 7.78 (d, 2H); MS (ES) *m*/*z* 563 $[M + H]^+$, 585 $[M + Na]^+$, 561 $[M - H]^-$. Anal. ($C_{28}H_{50}NaO_7PS\cdot1.5H_2O$) C, H, N.

**2-(Oleyloxy)ethyl toluenesulfonyloxymethylphosphonate, sodium salt (3c),** was prepared from 2-oleyloxy-1-ethanol (**2c**) in 55% yield: $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, 3H), 1.27 (br d, 18H), 1.58 (m, 2H), 2.15 (m, 4H), 2.44 (s, 3H), 3.37 (t, 2H), 3.49 (t, 2H), 4.03 + 4.13 (dt, 2H), 4.23 (d, 2H), 5.30 (t, 2H), 7.41 (d, 2H), 7.77 (d, 2H); MS (ES) *m*/*z* 583 $[M + Na]^+$, 559 $[M - H]^-$. Anal. ($C_{28}H_{48}NaO_7PS$) C, H, N.

**3-(Oleyloxy)propyl toluenesulfonylmethylphosphonate, sodium salt (3d),** was prepared from 3-oleyloxy-1-propanol (**2d**) in 54% yield: $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, 3H), 1.27 (br d, 18H), 1.56 (m, 2H), 1.81 (qt, 2H), 2.02 (m, 4H), 2.45 (s, 3H), 3.41 (t, 2H), 3.49 (t, 2H), 3.93 + 3.91 (dt, 2H), 4.00 (d, 2H), 5.33 (t, 2H), 7.42 (d, 2H), 7.81 (d, 2H); MS (ES) *m*/*z* 597 $[M + Na]^+$, 573 $[M - H]^-$. Anal. ($C_{29}H_{50}NaO_7PS\cdot0.5H_2O$) C, H, N.

**General Procedure for Alkylation of (*S*)-9-[3-Trityloxy-2-hydroxypropyl]-*N*$^6$-trityladenine (1) with 3a−d.** Sodium hydride (24 mg, 1.0 mmol) was added to a stirred solution of (*S*)-9-[3-trityloxy-2-hydroxypropyl]-*N*$^6$-trityladenine (640 mg, 0.62 mmol) in dry triethylamine (10 mL). After 15 min, the appropriate alkoxyalkyl toluenesulfonyloxymethylphosphonate (**3a−d**, 0.65 mmol) was added, and the reaction mixture was heated to 50 °C and kept there overnight. After cooling, the mixture was quenched with brine and extracted with ethyl acetate (3 × 15 mL). The organic extracts were dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash chromatography, where the products were eluted with 10% EtOH/$CH_2Cl_2$.

**3-(Hexadecyloxy)propyl (*S*)-9-[3-trityloxy-2-(phosphonomethoxy)propyl]-*N*$^6$-trityladenine (4a)** was prepared from **3a** in 66% yield: $^1$H NMR ($CDCl_3$) $\delta$ 0.88 (t, 3H), 1.33 (br s, 26H), 1.46 (m, 2H), 1.77 (qt, 2H), 3.37−3.8 (m, 9H), 3.86 (m, 2H), 4.03 (m, 2H), 7.2−7.5 (br m, 30H), 7.82 (s, 1H), 8.20 (s, 1H); MS (ES) *m*/*z* 1071 $[M + H]^+$, 1069 $[M - H]^-$. Anal. ($C_{66}H_{79}NaN_5O_6P\cdot0.5H_2O$) C, H, N.

**2-(Octadecyloxy)ethyl (*S*)-9-[3-trityloxy-2-(phosphonomethoxy)propyl]-*N*$^6$-trityladenine (4b)** was prepared from **3b** in 56% yield: $^1$H NMR ($CDCl_3$) $\delta$ 0.90 (t, 3H), 1.26 (br s, 30H), 1.41 (m, 2H), 3.3−3,7 (m, 7H), 3.8 (m, 2H), 4.21 (m, 2H), 7.2−7.6 (m, 30H), 7.93 (s, 1H), 8.10 (s, 1H); MS (ES) *m*/*z* 1085 $[M + H]^+$, 1083 $[M - H]^-$. Anal. ($C_{67}H_{81}NaN_5O_6P\cdot H_2O$) C, H, N.

**2-(Oleyloxy)ethyl (*S*)-9-[3-trityloxy-2-(phosphonomethoxy)propyl]-*N*$^6$-trityladenine (4c)** was prepared from **3c** in 12% yield. After flash chromatography, TLC analysis indicated that the product was contaminated with **3c** (<5%). The crude material was deprotected without further purification. Analysis by mass spectroscopy indicated that **4c** was the major product: MS (ES) *m*/*z* 1083 $[M + H]^+$, 1081 $[M - H]^-$.

**3-(Oleyloxy)propyl (*S*)-9-[3-trityloxy-2-(phosphonomethoxy)propyl]-*N*$^6$-trityladenine (4d)** was prepared from **3d** in 28% yield. After flash chromatography, TLC analysis indicated that the product was contaminated with **3d** (<5%). The crude material was deprotected without further purification. Analysis by mass spectroscopy indicated that **4d** was the major product: MS (ES) *m*/*z* 1097 $[M + H]^+$, 1095 $[M - H]^-$.

**General Procedure for Deprotection and Isolation of (*S*)-HPMPA Alkoxyalkyl Esters (5a−d).** Derivatives **4a−d** were suspended in 80% aqueous acetic acid (20 mL/mmol) and heated to 60 °C for 1 h or until detritylation was complete as determined by TLC analysis. After cooling, the solvent was evaporated, and the products were purified by flash chromatography. Elution with 30% MeOH/$CH_2Cl_2$ provided compounds **5a−d** as white powdery solids.

**3-(Hexadecyloxy)propyl (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine, sodium salt (HDP-HPMPA, 5a),** was prepared from **4a** in 81% yield: $^1$H NMR ($CD_3OD$) $\delta$ 0.84 (t, 3H), 1.20 (br s, 18H), 1.23 (m, 2H), 1.63 (qt, 2H), 3.67 (m, 2H), 3.20−3.55 (m, 11H), 4.05−4.34 (pair dd, 2H), 8.13 (s, 1H), 8.17 (s, 1H); $^{31}$P NMR $\delta$ 15.18; MS (ES) *m*/*z* 586 $[M + H]^+$, 608 $[M + Na]^+$, 585 $[M - H]^-$. Anal. ($C_{28}H_{52}NaN_5O_6P\cdot0.5H_2O$) C, H, N.

**2-(Octadecyloxy)ethyl (*S*)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]adenine, sodium salt (ODE-HPMPA, 5b).** Deprotection of **4b** gave ODE-HPMPA in 73% yield: $^1$H NMR ($CD_3OD$) $\delta$ 0.85 (t, 3H), 1.22 (br s, 30H), 1.43 (m, 2H), 1.89 (t, 2H), 3.35−3.80 (m, 11H), 4.00−4.15 (m, 2H), 8.11 (s, 1H), 8.15 (s, 1H); $^{31}$P NMR $\delta$ 15.06; MS (ES) *m*/*z* 600 $[M + H]^+$, 598 $[M - H]^-$. Anal. ($C_{29}H_{53}NaN_5O_6P\cdot1.5H_2O$) C, H, N.

**2-(Oleyloxy)ethyl (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine, sodium salt (OLE-HPMPA, 5c),** was prepared from **4c** in 85% yield: $^1$H NMR ($CD_3OD$) $\delta$ 0.88 (t, 3H), 1.29 (br s, 18H), 1.47 (m, 2H), 2.00 (d, 4H), 3.35 (t, (2H), 3.45 (t, 2H), 3.52−3.85 (m, 4H), 3.78 (m, 1H), 3.90 (dd, 2H), 4.45−4.34 (pair dd, 2H), 5.38 (t, 2H), 8.22 (s, 1H), 8.23 (s, 1H); $^{31}$P NMR $\delta$ 15.12; MS (ES) *m*/*z* 598 $[M + H]^+$, 596 $[M - H]^-$. Anal. ($C_{29}H_{51}NaN_5O_6P\cdot2.0H_2O$) C, H; N: calcd, 10.68; found, 9.96.

**3-(Oleyloxy)propyl (*S*)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]adenine, sodium salt (OLP-HPMPA, 5d).** Deprotection of **4d** yielded OLP-HPMPA in 77% yield: $^1$H NMR ($CD_3OD$) $\delta$ 0.88 (t, 3H), 1.27 (br s, 18H), 1.50 (m, 2H), 1.79 (qt, 2H), 2.00 (d, 4H), 3.35 (t, 2H), 3.45 (t, 2H), 3.48−3.73 (m, 4H), 3.78 (m, 1H), 3.88 (dd, 2H), 4.45−4.34 (pair dd, 2H), 5.32 (t, 2H), 8.21 (s, 1H), 8.25 (s, 1H); $^{31}$P NMR $\delta$ 15.20; MS (ES) *m*/*z* 612 $[M + H]^+$, 634 $[M + Na]^+$, 611 $[M - H]^-$. Anal. ($C_{30}H_{53}NaN_5O_6P\cdot1.25H_2O$) C, H, N.

**Biological Methods. Cells and Viruses.** Human foreskin fibroblast (HFF) cells were prepared as primary cultures from freshly obtained newborn human foreskins (UAB or Brookwood Hospital, Birmingham, AL) as soon as possible after circumcision. Vero cells were obtained from the American Type Culture Collection (Manassas, VA). Mouse embryo fibroblast (MEF) cells were also prepared as primary cultures. Cells were propagated in minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 U/mL), and

gentamicin (25 $\mu$g/mL) in T-175 cm$^2$ tissue culture flasks (BD Falcon, Bedford, MA).

HCMV strain AD-169 and MCMV strain Smith were propagated in HFF or MEF cells using standard virological techniques. Vaccinia virus (VV) strain Copenhagen and cowpox virus (CV) strain Brighton stock pools were obtained from Dr. John Huggins of the U.S. Army Medical Research Institute for Infectious Diseases, Frederick, MD. These pools had been prepared in Vero cells and were diluted 1:50 in our laboratory to provide working stocks.

**Activity against HCMV and MCMV.** The antiviral assays were performed as described previously.[37] HFF or MEF cells were grown in 6- or 12-well plates for 2 days (HFF) or 1 day (MEF) before virus was added (diluted in MEM containing 10% FBS to 20−30 plaque forming units per well). The medium was aspirated, and 0.2 mL of virus was added to each well in triplicate with 0.2 mL of medium added to drug toxicity wells. Ganciclovir and CDV were used as positive controls. The plates were incubated for 1 h with shaking every 15 min. The infected cells were incubated with MEM containing varying concentrations of the test compounds. Compounds were serially diluted 1:5 in MEM with 2% FBS starting at 100 $\mu$M. Following incubation at 37 °C (7 days for MCMV or 8 days for HCMV), the cells were stained for 6 h with 2 mL of 0.02% neutral red in phosphate-buffered saline (PBS). After the stain was aspirated, viral plaques were enumerated with the aid of a 10× stereomicroscope. The antiviral activity was expressed as $EC_{50}$, which represents the compound concentration required to reduce virus plaque formation by 50%.

**Activity against VV and CV.** Two days prior to use, HFF cells were plated in six-well plates and incubated at 37 °C with 5% $CO_2$ and 90% humidity. On the day of assay, the drugs were made up at twice the desired concentration in 2× MEM containing 10% FBS and antibiotics and diluted serially 1:5 in 2× MEM to provide six concentrations of test compound. The initial concentration was usually 200 $\mu$M and ranged down to 0.06 $\mu$M. The virus to be used was diluted in MEM containing 10% FBS to the desired concentration that would give 20−30 plaques per well. The medium was then aspirated from the wells, and 0.2 mL of virus was added to each well in triplicate, with 0.2 mL of medium being added to drug toxicity wells. The plates were incubated for 1 h with shaking every 15 min. After the incubation period, an equal amount of 1% agarose was added to an equal volume of each drug dilution. This gave final drug concentrations beginning with 100 $\mu$M and ending with 0.03 $\mu$M and a final agarose overlay concentration of 0.5%. The drug agarose mixture was added to each well in 2 mL volumes, and the plates were incubated for 3 days, after which the cells were stained with a 0.02% solution of neutral red in PBS. After a 5−6 h incubation period, the stain was aspirated, and the plaques were counted using a stereomicroscope at 10× magnification. The MacSynergy II, version 1 computer program was used to calculate the 50% effective concentration ($EC_{50}$).

**Cytotoxicity Assays.** HFF cells were seeded into 96-well microtiter plates ($2.5 \times 10^4$ cells per well) and allowed to proliferate for 24 h. After the medium was replaced with MEM containing 2% FBS, different concentrations of the test compounds (diluted serially 5-fold from 100 to 0.03 $\mu$M) were added. The plates were incubated for 7 days, the media aspirated, and the cells stained with 0.01% neutral red in PBS, incubated for an additional hour, and then shaken for 15 min before the neutral red was solubilized with 1% glacial acetic acid−50% ethanol. The optical density was read at 540 nm to determine the number of viable cells. Cytotoxicity is expressed as the concentration of drug that reduced cell viability by 50% ($CC_{50}$) and was calculated using the MacSynergy Software.

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**Supporting Information Available:** Elemental analysis data for compounds **3a**−**d**, **4a**−**d**, and **5a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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