were tested on CaM-PDE at 1 μM cGMP in the presence of calmodulin, on cGMP-PDE at 1 μM cGMP in the presence of EGTA, and on cAMP-PDE at 1 μM cAMP in the presence of EGTA.

The concentration of the drug that inhibits 50% of the enzymatic activity (IC_{50}) was determined with a minimum of six concentrations of drugs in the linear part of the curve obtained by fitting percent enzymatic activity to the logarithmic concentration of the drug. IC_{50} was calculated by linear regression (correlation coefficient was >0.950). For the assay the drugs were dissolved in DMSO or DMF and diluted so that in the assay medium the final concentration of the organic solvent was not higher than 1%. Controls were run with the assay medium in the same conditions and did not modify enzymatic activity. Results were expressed as mean ± SEM derived from three determinations performed on different enzyme preparations.

Acknowledgment. This work was partially supported by a grant from Ministère de la Recherche et de la Technologie (No. 85-C-1161). We thank Alain Lebec and Marlyse Wernert for the excellent technical and secretarial assistance.

Synthesis and Antiviral Activity of the Nucleotide Analogue (*S*)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]cystosine

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The acyclic nucleotide analogue (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (**2**, HPMPC) was prepared on a multigram scale in 18% overall yield starting from (*R*)-2,3-*O*-isopropylideneglycerol. The key step in the nine-step synthetic route is coupling of cytosine with the side-chain derivative **8** which bears a protected phosphonylmethyl ether group. In vitro data showed that HPMPC has good activity against herpes simplex virus types 1 and 2, although it was 10-fold less potent than acyclovir [ACV, 9-[(2-hydroxyethoxy)methyl]guanine]. By comparison, HPMPC exhibited greater activity than ACV against a thymidine kinase deficient strain of HSV 1 and was more potent than ganciclovir [DHPG, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine] against human cytomegalovirus. In vivo, HPMPC showed exceptional potency against HSV 1 systemic infection in mice, having an ED_{50} of 0.1 mg/kg per day (ip) compared with 50 mg/kg per day for ACV. HPMPC was also more efficacious than ACV in the topical treatment of HSV 1 induced cutaneous lesions in guinea pigs.

De Clercq and Holy recently described the novel acyclic nucleotide analogue (*S*)-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine (**1**, HPMPA) as a potent and selective antiviral agent with activity against a broad spectrum of DNA viruses.[1,2] Biochemical studies have shown that HPMPA inhibits HSV 1 DNA synthesis at a concentration much lower than required for inhibition of cellular DNA synthesis and that HPMPA is converted by cellular kinases to its mono- and diphosphate ester derivatives.[3] HPMPA thus appears to share a common mechanism of action with the nucleoside analogue acyclovir [ACV, 9-[(2-hydroxyethoxy)methyl]guanine] in that a triphosphate equivalent is formed which can act as an inhibitor of viral DNA polymerase and consequently viral replication. However, the biosynthesis of ACV-triphosphate requires initial conversion of ACV to its monophosphate by virus-encoded thymidine kinase (TK) prior to further phosphorylation by cellular kinases. Since HPMPA acts as a metabolically stable monophosphate equivalent, it does not rely on activation by the viral enzyme and, as a consequence, has activity against viruses that do not encode a thymidine kinase such as cytomegalovirus, as well as TK-deficient strains of herpes simplex virus. On the other hand, the selective action of acyclovir as an antiviral agent results in part from its preferential phosphorylation by virus-specified TK in infected cells; monophosphorylation by host enzymes does not occur to a significant extent in uninfected cells. Although HPMPA bypasses this level of selectivity, it does exhibit a significant preference for suppression of viral over cellular DNA synthesis and is a highly selective antiviral agent.[3]

The antiviral activity of a series of (phosphonylmethoxy)alkyl purine and pyrimidine derivatives related to HPMPA has been reported by De Clercq and co-workers.[4] Among the more promising of these acyclic nucleotide analogues is the cytosine derivative (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (**2**, HPMPC), which has demonstrated in vitro activity against a wide range of DNA viruses and is particularly effective in the inhibition of cytomegalovirus.[5] Following our initial interest in HPMPA,[6] we have continued the evaluation of this class of nucleotide analogues and herein report our recent work on HPMPC. This paper provides details for a modified synthetic route[7] that allows the preparation of multigram quantities of HPMPC and describes our findings on the activity of HPMPC against DNA viruses in both in vitro and in vivo model systems. For comparison, the related acyclic cytosine derivative 1-[2-(phosphonylmethoxy)ethyl]cytosine (**3**, PMEC) was also synthesized.

Chemistry

Our strategy for the synthesis of the nucleotide analogues HPMPC and PMEC is based on alkylation of cytosine with an acyclic side-chain derivative already functionalized with a protected phosphonylmethyl ether group.

(1) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464.
(2) Holy, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2775.
(3) Votruba, I.; Bernaerts, R.; Sakuma, T.; De Clercq, E.; Merta, A.; Rosenberg, I.; Holy, A. *Mol. Pharmacol.* **1987**, *32*, 524.
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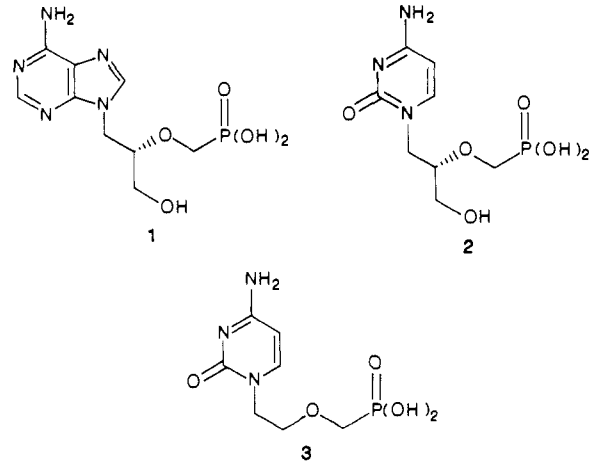
(5) Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. *Antimicrob. Agents Chemother.* **1988**, *32*, 1839.
(6) Webb, R. R.; Martin, J. C. *Tetrahedron Lett.* **1987**, *28*, 4963.
(7) Webb, R. R.; Wos, J. A.; Bronson, J. J.; Martin, J. C. *Tetrahedron Lett.* **1988**, *29*, 5475.

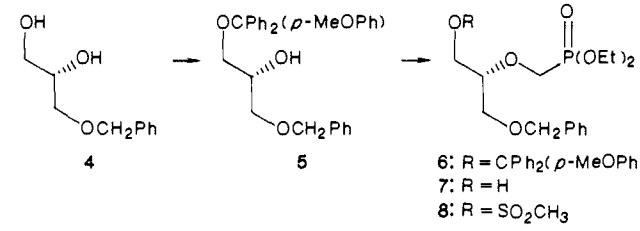
0022-2623/89/1832-1457$01.50/0



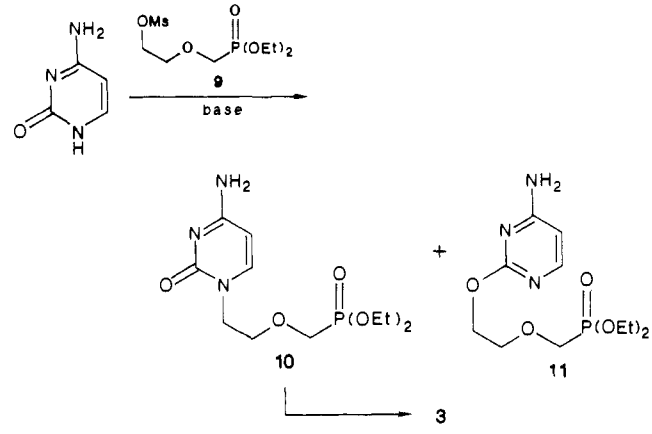
The synthetic route for preparation of the key HPMPC side-chain intermediate **8** is outlined in Scheme I. Conversion of (*R*)-2,3-*O*-isopropylideneglycerol to (*S*)-3-*O*-benzylglycerol (**4**) was accomplished according to the literature procedure[8] by phase-transfer-catalyzed benzylation followed by cleavage of the acetonide under acidic conditions. Diol **4** was isolated by distillation in 82% overall yield. The monomethoxytrityl ether group was selected for protection of the remaining primary hydroxyl function. Reaction of **4** with monomethoxytrityl chloride and triethylamine (catalytic DMAP, CH_2Cl_2) provided the bis-protected glycerol derivative **5** in 90% yield. For introduction of the phosphonylmethyl ether group, the sodium alkoxide of **5** was generated by treatment with NaH in THF at 65 °C. The resulting slurry was cooled to 0 °C and then added to a solution of diethyl [(tosyloxy)methyl]-phosphonate[9] in THF at 0 °C to provide the phosphonate **6** in 65% yield. Similar yields of the alkylated product were obtained when DMF was used as the reaction solvent. On larger scales, it was more convenient to use the monomethoxytrityl ether **5** in the alkylation reaction without prior purification; phosphonate **6** was then isolated in 55% overall yield from diol **4**. Removal of the monomethoxytrityl protecting group was effected next by heating a solution of **6** in aqueous acetic acid at 100 °C for 20 min to furnish alcohol **7** in 86% yield. Alternatively, treatment of a methanolic solution of the monomethoxytrityl ether with Amberlyst-15 ion-exchange resin[10] at room temperature smoothly provided the desired alcohol in 90% yield. Conversion of **7** to the mesylate derivative **8** was accomplished by addition of triethylamine to a cooled (0 °C) solution of the alcohol and methanesulfonyl chloride in CH_2Cl_2. Chromatographic purification furnished the mesylate in 97% yield; however, the mesylate was generally used without purification in the coupling reaction with cytosine. A similar procedure was employed for preparation of the methanesulfonate ester of 2-[(diethylphosphonyl)methoxy]ethanol[11] (**9**), the alkylphosphonate side chain required for the synthesis of PMEC.

Our studies on the alkylation of cytosine were initially directed toward the synthesis of the simpler, achiral molecule PMEC (Scheme II). Coupling of cytosine with the straight-chain mesylate **9** was effected by treatment with potassium carbonate in DMF at 90 °C. Chromatographic separation of the two major products provided the

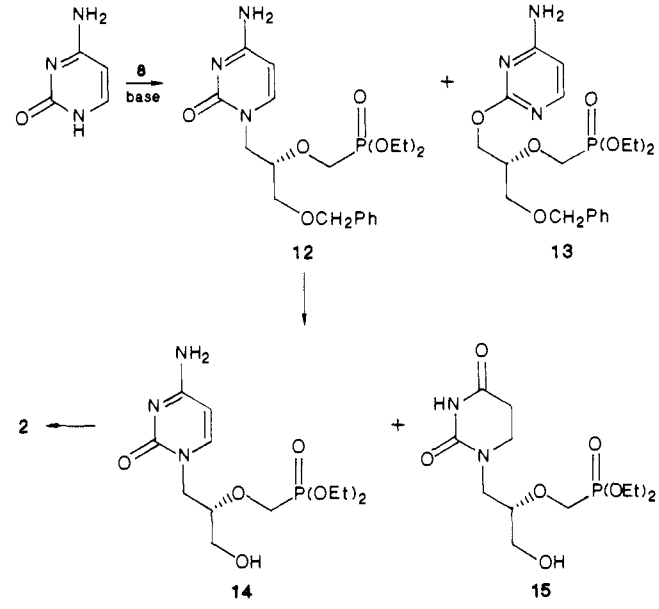
Scheme I



Scheme II



Scheme III



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(9) Holy, A.; Rosenberg, I. *Collect. Czech. Chem. Commun.* **1982**, *47*, 3447; Kluge, A. F. *Org. Synth.* **1985**, *64*, 80.
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N^1-substituted cytosine derivative **10** in 47% yield and the less polar O^2-alkylated isomer **11** in 12% yield. The initial structural assignments for the isomeric products **10** and **11** were made by comparison of their NMR (^1H and ^{13}C) spectra with data for known cytosine derivatives. For example, the chemical shifts of the pyrimidine ring carbons of **10** and cytidine are nearly identical and differ substantially from positions of these peaks in the ^{13}C NMR spectrum of **11**. In addition, the chemical shift of the methylene carbon attached to the pyrimidine base (C-1′) in **10** is upfield relative to the corresponding signal from **11** (48 vs 66 ppm). A 2D NMR ^1H–^{13}C correlation experiment was used to confirm our assignments. For the N-alkylated product **10**, a three-bond coupling interaction was observed between C-1′ and the proton at C-6 of the heterocyclic base; this was not observed for the product designated as the O-alkylated isomer. Deprotection of the phosphonate ester of diethyl-PMEC (**10**) was achieved by

Table I. In Vitro Antiviral Activities of HPMPC, HPMPA, PMEC, ACV, and DHPG against Herpes Simplex Virus and Cytomegalovirus

compd	ID_{50},[a] μg/mL			
	HSV 1	HSV 1 (TK⁻)	HSV 2	HCMV
HPMPC	5.4	0.7	2.3	0.22
HPMPA	9.3	3.2	25.2	0.27
PMEC	>100		>100	>2.7
ACV	0.5	4.3	0.3	
DHPG	0.23	>10	0.94	1.8

[a] The 50% inhibitory dose, determined by plaque-reduction assays in vero (HSV) or MRC-5 (HCMV) cells. Virus strains are as follows: HSV 1, BW^5 strain; HSV 1 (TK^-), Z826 strain; HSV 2, G strain; and MCMV, AD-169 strain.

treatment with bromotrimethylsilane[12] in DMF to afford PMEC (3) in 80% yield.

In order to complete the synthesis of HPMPC, coupling of cytosine with mesylate 8 was required first, followed by deprotection of the primary alcohol and phosphonate functionalities (Scheme III). While potassium carbonate could be employed to promote the alkylation of cytosine with 8, the yield of the N^1-coupled product was moderate (30–35%), and several minor side products were formed in addition to the isomeric O^2-alkylated product. Upon further investigation, we found that use of cesium carbonate provided higher yields of the desired product 12, even though a similar ratio (3:1) of the N- and O-alkylated isomers was obtained. Thus reaction of cytosine and mesylate 8 in the presence of cesium carbonate in DMF at 90 °C afforded the N-alkylated product 12 in 67% yield; the O-alkylated isomer 13 was isolated in 23% yield. Reaction of the sodium salt of cytosine with 8 was also examined. Treatment of cytosine with NaH (DMF, 90 °C) followed by addition of the mesylate gave a similar ratio of the N- and O-alkylated products (3.5:1), although a lower yield (45%) of the desired product 12 was obtained. The structural assignments for isomers 12 and 13 were based on a comparison with the spectroscopic data for the PME series.

Completion of the HPMPC synthesis required unmasking of the primary hydroxyl and the phosphonic acid moieties. Removal of the benzyl protecting group was achieved by transfer hydrogenation with 20% $Pd(OH)_2$–C in cyclohexene/ethanol to provide the diethyl ester of HPMPC (14) in 70% yield. The dihydrouracil derivative 15 was also produced in the reaction as a result of reduction of the cytosine ring; the yield of this side product was typically 5–15%. Final deprotection of 14 to give HPMPC (2) was accomplished by treatment with bromotrimethylsilane[12] in DMF or, more conveniently, CH_3CN. Concentration of the reaction mixture, followed by treatment of the residue with water and ethanol, afforded HPMPC as a crystalline solid in 95% yield. Multigram quantities of HPMPC have been synthesized by this nine-step route, which proceeds in 18% overall yield from (*R*)-2,3-*O*-isopropylideneglycerol.

Biological Results and Discussion

The phosphonate derivatives HPMPC, HPMPA, and PMEC were compared with acyclovir (ACV) and ganciclovir [DHPG, 9-[(1,3-dihydroxy-2-propoxy)methyl]-guanine] for antiviral activity against various strains of herpes simplex virus (HSV) in vero cells and human cytomegalovirus (HCMV) in MRC-5 cells (Table I). The in vitro potency is expressed as the concentration of compound required for 50% inhibition of viral growth.

(12) McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc., Chem. Commun.* **1979**, 739.

Table II. In Vivo Effect of Intraperitoneal Administration of HPMPC on HSV 1 Systemic Infection in Mice[a]

compound	dose, mg/kg per day	survival, %	mean survival time, days
	Experiment 1		
HPMPC	200	10/10 (100)[b]	21.0[b]
	100	10/10 (100)[b]	21.0[b]
	10	10/10 (100)[b]	21.0[b]
ACV	200	3/4 (75)[b]	18.5[b]
	100	7/10 (70)[b]	18.4[b]
	50	5/10 (50)[b]	15.1[b]
	10	2/10 (20)	10.4[b]
placebo control		1/20 (5)	7.4
	Experiment 2		
HPMPC	10	10/10 (100)[b]	21.0[b]
	1	9/10 (90)[b]	19.7[b]
	0.1	5/10 (50)[c]	14.9[b]
ACV	200	7/9 (78)[b]	18.2[b]
placebo control		3/19 (16)	10.1

[a] Mice were inoculated intraperitoneally with (2–6) × 10^2 PFU/0.2 mL of HSV 1 (HL-34 strain). Treatment was initiated 3 h postinfection and was given twice a day for 5 consecutive days. The experiment was terminated at day 21 postinfection. [b] Significant value as compared to the placebo control, $P < 0.05$. [c] $P < 0.08$.

HPMPC exhibited good activity against HSV 1 and HSV 2, although it was 10-fold less potent than ACV. By comparison, HPMPC was more potent against a thymidine kinase deficient strain of HSV 1 than either ACV or DHPG, indicating that the antiviral activity of HPMPC is not dependent on virus-specified TK. HPMPC was also more effective than DHPG in its ability to inhibit replication of HCMV, a virus that does not encode TK. The adenine derivative HPMPA, which bears the same side-chain appendage as HPMPC, was also evaluated in vitro and found to be comparable to or slightly less potent than HPMPC.[4] The achiral phosphonate derivative PMEC (3), which lacks the hydroxymethyl substituent present on the side chain of HPMPC, was not active against any of the virus strains tested. For each compound, no cellular toxicity was observed at concentrations up to 100 μg/mL.

The in vivo activity of HPMPC was evaluated and compared with that of ACV in a systemic HSV 1 infection model in mice (Table II). The antiviral effect of each compound was demonstrated by a significant reduction in mortality rate and increase in the mean survival time as compared with placebo control. In an initial experiment, HPMPC was administered systemically at doses of 200, 100, and 10 mg/kg per day and compared with ACV given at 200, 100, 50, and 10 mg/kg per day. HPMPC proved surprisingly potent, giving 100% protection at the lowest dose of 10 mg/kg per day. By comparison, ACV did not provide complete protection even at the 200 mg/kg per day dose. This result is in contrast with the in vitro data which suggest that HPMPC is less potent than ACV against HSV 1. In a subsequent experiment designed to determine the effect of lower doses of HPMPC, it was found that HPMPC was active even at 0.1 mg/kg per day: 50% of the treated mice survived when given HPMPC at this dose. HPMPC exhibited a similar antiviral effect in mice systemically infected with HSV 2, resulting in 100% survival when administered at a dose of 10 mg/kg per day (ip); treatment with the same dose of ACV produced no significant increase in the number of survivors compared with the placebo control group.

The effect of topical application of HPMPC on HSV 1 induced primary cutaneous lesions in guinea pigs was also measured (Table III). HPMPC was applied at concentrations of 5%, 1%, and 0.1% and compared with a 5% preparation of acyclovir. Treatment with HPMPC at all

Table III. Effect of HPMPC Treatment on Lesion Score, Duration, and Healing Time in HSV 1 Cutaneous Infection in Guinea Pigs[a]

compd	lesion formation[b] total score	lesion formation[b] duration time	healing period[b] total score	healing period[b] duration time	severity,[b] mean score
virus control	19.4	6.0	56.8	3.2	2.8
placebo	15.2	5.0	60.8	5.2	3.0
5% HPMPC	3.0[c]	2.2[c]	85.4[c]	4.2	0.8[c]
1% HPMPC	7.0[c]	3.8[c]	75.0[c]	3.8	1.0[c]
0.1% HPMPC	8.2[c]	3.8[c]	72.8[c]	4.4	1.4[c]
5% ACV	14.0	5.4	60.4	4.2	2.4

[a] Treatment was initiated 3 h postinfection and given twice a day for 5 days. Topical preparations were in a Tween 80/poly(ethylene glycol) vehicle. [b] The accumulative scores were collected from five individual animals. Two separate response variables, the lesion formation stage and the healing period stage, were analyzed statistically. A high cumulative score for the healing stage indicates an infection with short healing period. The response variable that measures the severity of the infection is equal to the highest sequential step reached in the formative stage. [c] $P < 0.01$ compared to virus control.

doses significantly reduced the lesion formation and shortened the healing period compared with the untreated control or placebo control animals. The severity of the infection was also dramatically decreased. In contrast, there was no significant difference between areas treated with a 5% ACV preparation and the placebo control treated or untreated areas.

The results clearly indicate that HPMPC is a highly effective antiviral agent for the treatment of systemic or cutaneous herpes simplex virus infection. Furthermore, HPMPC was more potent than ACV in both infection models. The in vitro data also indicate that HPMPC possesses a broader spectrum of activity than ACV, since it is active against viruses that do not encode thymidine kinase, an enzyme required for the conversion of ACV to its biologically active form.

Experimental Section

Melting points were determined on an electrothermal digital capillary apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co.; column chromatography was carried out by using Woelm silica gel (32–63 μm). Infrared spectra were obtained on a Perkin-Elmer Model 1800 FT-IR spectrophotometer. Ultraviolet spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM300 NMR spectrometer or a Varian Gemini 300 NMR spectrometer; chemical shifts are expressed in parts per million (δ) with tetramethylsilane as an internal standard. Mass spectra were measured on a Finnegan 4500 spectrometer (low resolution) or a Kratos MS50 spectrometer (high resolution). Spectroscopic data and elemental analyses were obtained by the Analytical Department, Bristol-Myers, Wallingford, CT.

(*S*)-3-*O*-Benzylglycerol (4). A mixture of (*R*)-2,3-*O*-isopropylideneglycerol[13] (150 g, 1.13 mol), benzyl bromide (350 g, 2.04 mol), benzyltriethylammonium bromide (7.5 g, 0.021 mol), and 450 mL of 10 N aqueous NaOH solution was stirred vigorously at 90–95 °C for 15 h, then allowed to cool to room temperature, and poured into a 2-L separatory funnel. The layers were separated, and the aqueous phase was extracted with Et_2O (2 × 300 mL). The combined organic phases were washed with water (3 × 300 mL) and saturated NaCl solution (1 × 400 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give a residual yellow oil. The residue was dissolved in 600 mL of 1.5 N aqueous H_2SO_4 solution and heated at 90 °C with vigorous stirring. After 5 h the reaction mixture was allowed to cool to room temperature and extracted with petroleum ether (bp 40–60 °C, 3 × 400 mL) to remove all dibenzyl ether, some benzyl alcohol, and only a small amount of the desired product. The aqueous phase was then adjusted to pH 10–12 by addition of 15% aqueous NaOH (600 mL); the solution became milky when basic pH was reached. The aqueous phase was extracted with ethyl acetate (3 × 500 mL), and the combined organic phases were washed with saturated NaCl solution (1 × 500 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to afford a yellow-orange oil. The product was purified by distillation to provide 169 g of 4 [82% from (*R*)-2,3-*O*-isopropylideneglycerol] as a clear, pale yellow oil: bp 132–135 °C (0.2 mmHg); $[\alpha]^{20}_D$ −5.88° (neat) [lit.[14] bp 140–142 °C (0.15 mmHg), $[\alpha]^{22}_D$ −5.85° (neat)]; IR (neat) 3200 (br, OH), 3180, 3100, 3000, 2960, 1615, 1518 (s), 1260 (s), and 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H, Ar H), 4.54 (s, 2 H, benzylic), 3.82–3.90 (m, 1 H, H-2), 3.50–3.65 (m, 4 H, H-1 and H-3), 2.88 (d, J = 4 Hz, 1 H, C_2-OH), and 2.43 (br t, J = 4 Hz, 1 H, C_1-OH).

(*R*)-3-*O*-Benzyl-1-*O*-[(*p*-methoxyphenyl)diphenylmethyl]glycerol (5). Diol 4 (67.4 g, 0.370 mol) was dissolved in 600 mL of CH_2Cl_2 under argon and cooled to 0 °C. (*p*-Methoxyphenyl)diphenylmethyl chloride (137 g, 0.444 mol) and (dimethylamino)pyridine (4.0 g, 0.032 mol) were added, and then triethylamine (75.0 g, 0.740 mol) was added dropwise via addition funnel over 20 min. A precipitate formed immediately upon addition of the amine. When the addition was complete, the ice bath was removed and the resulting yellow-brown slurry was stirred at room temperature for 16 h and then was poured into water (600 mL). The layers were agitated and separated, and the aqueous phase was extracted with CH_2Cl_2 (500 mL). The combined organic layers were washed with 50% saturated $NaHCO_3$ solution (500 mL) and saturated NaCl solution (500 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give 215 g of a yellow oil. The residue was coevaporated twice with toluene and used without purification. On a separate run (0.175 mol of diol), the product was purified by chromatography on silica gel (5:1 10–20% ethyl acetate/hexane) to afford 75.5 g (90%) of 5 as a viscous, pale yellow oil: ^1H NMR ($CDCl_3$) δ 7.45 (d, J = 8 Hz, 2 H, Ar H), 7.15–7.35 (m, 15 H, Ar H), 6.82 (d, J = 6 Hz, 2 H, Ar H), 4.52 (s, 2 H, benzylic), 3.90–4.14 (m, 1 H, H-2), 3.77 (s, 3 H, OCH_3), 3.50–3.62 (m, 2 H, H-3), 3.15–3.28 (m, 2 H, H-1), and 2.40 (d, J = 4 Hz, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 158.59, 144.36, 138.03, 135.49, 130.36, 128.82, 127.82, 127.67, 126.91, 113.12, 86.38, 73.36 (OCH_2Ph), 71.59 (C-2), 69.96 (C-3), 64.48 (C-1), and 55.20 (O-CH_3); mass spectrum (methane/DCI), m/e (rel intensity) 454 (MH^+, 25), 377 (28), 273 (100).

(*R*)-3-*O*-Benzyl-2-*O*-[(diethylphosphonyl)methyl]-1-*O*-[(*p*-methoxyphenyl)diphenylmethyl]glycerol (6). A solution of 5 (215 g of crude material) in 300 mL of THF was added dropwise via cannula over 25 min to a suspension of NaH (13.3 g, 80% dispersion in oil, 0.443 mol) in 400 mL of THF at room temperature under argon in a 2-L, three-necked, round-bottomed flask equipped with a reflux condenser. The resulting brown-gray slurry was stirred at room temperature for 30 min and then heated at reflux for 5 h to give a brown, cloudy solution. The reaction mixture was allowed to cool to room temperature and then was cooled to 0 °C with an ice bath and transferred via cannula over 30 min to a solution of diethyl [(tosyloxy)methyl]phosphonate[9] (143 g, 0.443 mol) in 200 mL of THF cooled to 0 °C in a flask equipped with an overhead mechanical stirrer. Within 10 min, formation of a thick precipitate was observed; after 1 h, the ice bath was removed, and the reaction mixture was stirred at room temperature for 14 h. The thick yellow-brown slurry was treated slowly with 100 mL of absolute EtOH and then concentrated in vacuo to near dryness. The orange-brown residue was partitioned between ethyl acetate (600 mL) and water (600 mL), and the aqueous layer was extracted further with ethyl acetate (500 mL). The combined organic layers were washed with aqueous NH_4Cl solution (500 mL) and saturated NaCl solution (500 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give 300 g of a viscous orange-brown oil. Purification by column chromatography on silica gel (5:1 50–75% ethyl acetate/hexanes) gave 122 g of phosphonate 6 (55% from 4) as a viscous pale yellow oil: IR (neat) 3000, 1600, 1370 (s), and 1180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45 (d, J = 8 Hz, 2 H, Ar H), 7.15–7.35 (m, 15 H, Ar H), 6.80

(13) Purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL.

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(d, J = 6 Hz, 2 H, Ar H), 4.50 (s, 2 H, benzylic), 4.05–4.23 (m, 5 H, H-2 and 2 POCH_2), 3.98 (d, J = 8 Hz, 2 H, OCH_2P), 3.77 (s, 3 H, OCH_3), 3.55–3.65 (m, 2 H, H-3), 3.22 (d, J = 4 Hz, 2 H, H-1), and 1.20–1.30 (m, 6 H, 2 $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 159.28, 145.04, 138.79, 136.12, 130.99, 129.03, 128.93, 128.57, 128.40, 128.14, 127.47, 113.63, 86.88, 81.17 (d, J = 12 Hz, C-2), 73.71 (OCH_2Ph), 70.88 (C-3), 64.92 (d, J = 165 Hz, OCH_2P), 63.61 (C-1), 62.82 (d, J = 7 Hz, $POCH_2$), 55.52 (OCH_3), and 16.66 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (methane/DCI), m/e (rel intensity) 604 (M^+, 5), 333 (20), 301 (15), 273 (100).

(*S*)-3-*O*-Benzyl-2-*O*-[(diethylphosphonyl)methyl]glycerol (7). Procedure A. Phosphonate **6** (42.0 g, 0.070 mol) was treated with 400 mL of 80% aqueous acetic acid, and the mixture was placed on a steam bath for 20 min. The resulting bright yellow-orange solution was concentrated in vacuo, and the yellow residue was coevaporated with 5% ethanol in toluene (2 × 200 mL) to give 45 g of a yellow oil. Purification by column chromatography on silica gel (10:1 75% ethyl acetate/hexane to 8% ethanol/ethyl acetate) provided 19.9 g of **7** (86%) as a clear, colorless oil.

Procedure B. A solution of **6** (64.7 g, 0.107 mol) in 300 mL of methanol was treated with Amberlyst-15 ion-exchange resin (5.5 g, prewashed twice with 50 mL of methanol). The reaction mixture was stirred at room temperature for 16 h and then was filtered through a 1-in. pad of Celite. The filtrate was concentrated in vacuo to give 63 g of a pale yellow oil, which was purified by column chromatography as in procedure A to provide 32.0 g of **7** (90%) as a clear, colorless oil: $[\alpha]^{20}_D$ −13.57° (c = 1.57, $CHCl_3$); IR (neat) 3400 (s), 2960, 2920, 2860, 1240, 1100, 1050, 1030, and 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28–7.40 (m, 5 H, Ar H), 4.52 (s, 2 H, benzylic), 4.03–4.24 (m, 5 H, H-2 and 2 POCH_2), 3.85 (dd, J = 5, 8 Hz, 1 H, OCHP), 3.48–3.75 (m, 5 H, H-1 and H-3 and OCHP), 3.23 (t, J = 4 Hz, 1 H, OH), 1.29 (t, J = 6 Hz, 3 H, $POCH_2CH_3$), and 1.27 (t, J = 6 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 138.53, 129.02, 128.33, 128.22, 83.05 (d, J = 9 Hz, C-2), 73.89 (OCH_2Ph), 70.62 (C-3), 64.99 (d, J = 165 Hz, OCH_2P), 63.20 (d, J = 6 Hz, $POCH_2$), 62.77 (C-1), and 16.61 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (methane/DCI), m/e (rel intensity) 333 (MH^+, 100), 91 (10). Anal. ($C_{15}H_{25}O_6P$) C, H.

(*R*)-3-*O*-Benzyl-2-*O*-[(diethylphosphonyl)methyl]-1-*O*-(methylsulfonyl)glycerol (8). A solution of **7** (32.0 g, 0.096 mol) in 350 mL of CH_2Cl_2 was cooled to 0 °C under argon. Methanesulfonyl chloride (13.2 g, 0.116 mol) was added rapidly via syringe, and after 10 min, triethylamine (19.5 g, 0.193 mol) was added dropwise via addition funnel over 15 min. The resulting pale yellow slurry was allowed to warm to room temperature over 16 h and then was poured into water (300 mL). The layers were agitated vigorously and separated, and the aqueous layer was back-extracted with CH_2Cl_2 (300 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (300 mL) and saturated NaCl solution (400 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to afford 39.9 g (101%) of crude **8** as a yellow oil which was used without purification. On a separate run (0.089 mol of alcohol), the mesylate was purified by column chromatography on silica gel (10:1 75% ethyl acetate/hexane to 100% ethyl acetate) to afford 36.6 g (97%) of **8**: $[\alpha]^{20}_D$ −10.87° (c = 1.73, $CHCl_3$); IR (neat) 2960, 2940, 2920, 2860, 1360, 1250, 1180, 1100, 1050, 1030, 980, and 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.25–7.38 (m, 5 H, Ar H), 4.52 (s, 2 H, benzylic), 4.38 (dd, J = 5, 14 Hz, 1 H, H-1), 4.25 (dd, J = 8, 14 Hz, 1 H, H-1), 4.13 (quintet, J = 6 Hz, 2 H, $POCH_2$), 4.11 (quintet, J = 6 Hz, 2 H, $POCH_2$), 3.82–3.96 (m, 3 H, C-2 and OCH_2P), 3.50–3.63 (m, 2 H, H-3), 3.02 (s, 3 H, OSO_2CH_3), 1.28 (t, J = 6 Hz, 3 H, $POCH_2CH_3$), and 1.27 (t, J = 6 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 137.32, 128.34, 127.79, 127.59, 78.45 (d, J = 11 Hz, C-2), 73.49 (OCH_2Ph), 69.01 (C-3), 68.21 (C-1), 64.44 (d, J = 165 Hz, OCH_2P), 62.44 (d, J = 6 Hz, $POCH_2$), 37.39 (OSO_2CH_3), and 16.36 (d, J = 5 Hz, $POCH_2CH_3$); mass spectrum (methane/DCI), m/e (rel intensity) 411 (MH^+, 100), 333 (5), 315 (20), 91 (25). Anal. ($C_{16}H_{27}O_8PS$) C, H.

2-[(Diethylphosphonyl)methoxy]ethyl Methanesulfonate (9). A solution of 2-[(diethylphosphonyl)methoxy]ethanol[11] (15.5 g, 0.073 mol) in 300 mL of CH_2Cl_2 was treated with methanesulfonyl chloride (9.20 g, 0.080 mol) and triethylamine (11.1 g, 0.110 mol) according to the procedure used for the preparation of **8**. Purification by column chromatography on silica gel (10:1 2–5% methanol/methylene chloride) provided 20.3 g (96%) of **9** as a clear, colorless oil: IR (neat) 2990, 2940, 2910, 1350, 1245, 1180, 1020, 975, and 925 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.36–4.50 (m, 2 H, CH_2OMs), 4.26 (quintet, J = 7 Hz, 4 H, $POCH_2$), 3.92–3.99 (m, 4 H, CH_2OCH_2P), 3.20 (s, 3 H, OSO_2CH_3), and 1.40 (t, J = 7 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 70.86 (d, J = 11 Hz, CH_2OCH_2P), 68.66 ($MsOCH_2$), 65.43 (d, J = 170 Hz, OCH_2P), 62.51 (d, J = 6 Hz, $POCH_2$), 37.71 (OSO_2CH_3), and 16.46 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (CI), m/e (rel intensity) 291 (MH^+, 100), 263 (50), 235 (15), 195 (50), 167 (95), 139 (90). Anal. ($C_8H_{19}O_7PS$) C, H.

1-[2-[(Diethylphosphonyl)methoxy]ethyl]cytosine (10). Mesylate **9** (4.07 g, 0.014 mol) was dissolved in 140 mL of DMF and treated with cytosine (1.89 g, 0.017 mol) and K_2CO_3 (3.89 g, 0.028 mol). The reaction mixture was heated at 90 °C for 16 h and then was allowed to cool to room temperature and filtered to remove insoluble material. The filtrate was concentrated to give 3.67 g of a viscous oil, which was purified by column chromatography on silica gel (30:1 5–10% methanol/methylene chloride) to afford 2.02 g (47%) of **10** as a white solid along with 0.52 g (12%) of **11** as a colorless foam. For **10**: mp 143–144 °C; UV_{max} (MeOH) 274 nm (ϵ = 7500); ^1H NMR (Me_2SO-d_6) δ 7.47 (d, J = 7 Hz, 1 H, H-6), 7.09 (br s, 2 H, NH_2), 5.63 (d, J = 7 Hz, 1 H, H-5), 4.02 (quintet, J = 7 Hz, 4 H, 2 POCH_2), 3.79–3.83 (m, 4 H, H-1′, OCH_2P), 3.70 (t, J = 5 Hz, 2 H, H-2′), and 1.23 (t, J = 7 Hz, 6 H, 2 $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 166.05 (C-4), 155.68 (C-2), 146.45 (C-6), 92.77 (C-5), 70.24 (d, J = 12 Hz, C-2′), 64.08 (d, J = 165 Hz, OCH_2P), 61.67 (d, J = 5 Hz, $POCH_2$), 48.05 (C-1′), and 16.13 (d, J = 5 Hz, $POCH_2CH_3$); mass spectrum (FAB), m/e (rel intensity) 306 (MH^+, 100), 278 (10), 138 (15). Anal. ($C_{11}H_{20}N_3O_5P$) C, H, N. For **11**: UV_{max} (MeOH) 272 nm (ϵ = 8300), 228 nm (ϵ = 7000); ^1H NMR (Me_2SO-d_6) δ 7.91 (d, J = 6 Hz, 1 H, H-6), 6.08 (d, J = 6 Hz, 1 H, H-5), 5.64 (br s, 2 H, NH_2), 4.38–4.41 (m, 2 H, H-1′), 4.13 (quintet, J = 7 Hz, 4 H, 2 POCH_2), 3.87–3.89 (m, 4 H, H-2′, OCH_2P), and 1.23 (t, J = 7 Hz, 6 H, 2 $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 164.99 (C-4), 164.86 (C-2), 157.04 (C-6), 99.58 (C-5), 71.40 (d, J = 12 Hz, C-2′), 65.64 (C-1′), 65.44 (d, J = 165 Hz, OCH_2P), 62.39 (d, J = 5 Hz, $POCH_2$), and 16.27 (d, J = 5 Hz, $POCH_2CH_3$); mass spectrum (FAB), m/e (rel intensity) 306 (MH^+, 95), 195 (100).

1-[2-(Phosphonylmethoxy)ethyl]cytosine (3). Bromotrimethylsilane (5.01 g, 0.033 mol) was added dropwise via syringe over 5 min to a solution of **10** (1.00 g, 0.003 mol) in 25 mL of DMF at room temperature under argon. The resulting clear orange solution was stirred for 14 h and then concentrated in vacuo. The residual orange oil was placed under high vacuum for 1 h and then was treated with 2 mL of water followed by 20 mL of acetone. The solid that formed was collected by filtration and recrystallized from water/ethanol to give 0.657 g of **3** (81%) as a white solid: mp 174–177 °C; UV_{max} (H_2O) 278 nm (ϵ = 9300), (0.1 N HCl) 282 nm (ϵ = 11 500), (0.1 N NaOH) 274 nm (ϵ = 9700); ^1H NMR (Me_2SO-d_6) δ 7.70 (br s, 2 H, NH_2), 7.60 (d, J = 7 Hz, 1 H, H-6), 5.68 (d, J = 7 Hz, 1 H, H-5), 3.82 (t, J = 5 Hz, 2 H, H-1′), 3.65 (t, J = 5 Hz, 2 H, H-2′), and 3.50 (d, J = 8 Hz, 2 H, OCH_2P); ^{13}C NMR (Me_2SO-d_6) δ 161.24 (C-4), 150.05 (C-2), 149.24 (C-6), 92.88 (C-5), 69.21 (d, J = 10 Hz, C-2′), 66.61 (d, J = 160 Hz, OCH_2P), and 48.02 (C-1′). Anal. ($C_7H_{12}N_3O_5P{\cdot}H_2O$) C, H, N.

(*S*)-1-[3-(Benzyloxy)-2-[(diethylphosphonyl)methoxy]propyl]cytosine (12). A solution of **8** (10.0 g, 0.024 mol) in 50 mL of DMF was vigorously stirred and heated at 85–90 °C in a 500-mL, three-necked, round-bottomed flask equipped with an overhead mechanical stirrer. Cytosine (3.25 g, 0.029 mol) was added in one portion followed by addition of cesium carbonate (15.9 g, 0.049 mol). The reaction mixture was stirred at 90 °C for 2.5 h, allowed to cool to room temperature, and then filtered to remove insoluble material. The filtrate was concentrated to give 15 g of a yellow oil. Purification by column chromatography on silica gel (15:1 5–10% methanol/methylene chloride) provided 6.65 g (67%) of the desired N-alkylated product **12** along with 2.36 g (23%) of the O-alkylated isomer **13**. For **12**: UV_{max} (MeOH) 274 nm (ϵ = 7800); ^1H NMR (Me_2SO-d_6) δ 7.40 (d, J = 7 Hz, 1 H, H-6), 7.25–7.40 (m, 5 H, Ar H), 6.97 (br s, 2 H, NH_2), 5.60 (d, J = 7 Hz, 1 H, H-5), 4.48 (s, 2 H, benzylic), 3.70–4.08 (m, 8 H, H-1′, H-2′, OCHP, and 2 POCH_2), 3.50–3.65 (m, 2 H, OCHP and H-3′), 3.44 (dd, J = 5, 11 Hz, 1 H, H-3′), and 1.10–1.25 (t, J = 8 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 166.63 (C-4),

156.40 (C-4), 147.38 (C-6), 138.71, 128.69, 127.98, 127.90, 93.13 (C-5), 78.54 (d, J = 11 Hz, C-2′), 72.69 (OCH_2Ph), 69.47 (C-3′), 63.10 (d, J = 165 Hz, OCH_2P), 61.89 (d, J = 6 Hz, $POCH_2$), 50.02 (C-1′), and 16.23 (d, J = 5 Hz, $POCH_2CH_3$); mass spectrum (methane/DCI), m/e (rel intensity) 426 (MH^+, 100), 318 (5). Anal. ($C_{19}H_{28}N_3O_6P\cdot0.25H_2O$) C, H, N. For **13**: UV_{max} (MeOH) 272 nm (ϵ = 7550), 228 nm (ϵ = 8700); ^1H NMR (Me_2SO-d_6) δ 7.82 (d, J = 7 Hz, 1 H, H-6), 7.24–7.37 (m, 5 H, Ar H), 6.82 (br s, 2 H, NH_2), 6.07 (d, J = 7 Hz, 1 H, H-5), 4.50 (s, 2 H, benzylic), 4.28 (dd, J = 5, 14 Hz, 1 H, H-1′), 4.19 (dd, J = 8, 14 Hz, 1 H, H-1′), 3.95–4.05 (m, 6 H, H-2′, OC*H*P, and 2 $POCH_2$), 3.85–3.95 (m, 1 H, OC*H*P), 3.53–3.65 (m, 2 H, H-3′), and 1.18 (t, J = 6 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 165.99 (C-4), 165.17 (C-2), 156.71 (C-6), 138.75, 128.67, 127.87, 99.88 (C-5), 78.82 (d, J = 11 Hz, C-2′), 72.61 (OCH_2Ph), 69.45 (C-3′), 65.50 (C-1′), 63.54 (d, J = 160 Hz, OCH_2P), 61.98 (d, J = 6 Hz, $POCH_2$), and 16.26 (d, J = 5 Hz, $POCH_2CH_3$); mass spectrum (methane/DCI), m/e (rel intensity) 426 (MH^+, 100), 315 (20), 140 (25).

(*S*)-1-[3-Hydroxy-2-[(diethylphosphonyl)methoxy]propyl]cytosine (14). A mixture of **12** (12.5 g, 0.029 mol) and $Pd(OH)_2$ on carbon (12 g, 20%) in 160 mL of 1:1 ethanol/cyclohexene was heated at reflux. Thin-layer chromatography showed a constant ratio of starting material/product (~1:2) after 4 h, so the reaction mixture was filtered through a 1-in. pad of Celite, and the pad was washed with hot EtOH. The filtrate was concentrated in vacuo and the pale yellow, glassy residue was dissolved in 160 mL of 1:1 ethanol/cyclohexene. $Pd(OH)_2$ on carbon (12 g, 20%) was added, and the reaction mixture was heated at reflux for 8 h, at which time TLC showed little remaining starting material. The mixture was filtered while hot through a 1-in. pad of Celite, and the collected solid was rinsed with hot EtOH. The filtrate was concentrated in vacuo to give 9.8 g of a clear, pale yellow oil. Purification by column chromatography on silica gel (10:1 7.5–10% methanol/methylene chloride) afforded 6.93 g (70%) of **14** as a white foam, along with 0.49 g (5%) of **15**. For **14**: $[\alpha]^{20}_D$ −79.4° (c = 0.96, MeOH); UV_{max} (MeOH) 274 nm (ϵ = 6800); ^1H NMR (Me_2SO-d_6) δ 7.83 (br s, 1 H, exch, NH), 7.50 (d, J = 7 Hz, 1 H, H-6), 7.34 (br s, 1 H, exch, NH), 5.72 (d, J = 7 Hz, 1 H, H-5), 4.91 (br s, 1 H, exch, OH), 3.88–4.02 (m, 6 H, H-1′, H-2′, 2 $POCH_2$), 3.77 (dd, J = 5, 14 Hz, 1 H, H-1′), 3.53–3.64 (m, 2 H, OCH_2P), 3.36–3.47 (m, 2 H, H-3′), 1.02 (t, J = 6 Hz, 3 H, $POCH_2CH_3$), and 1.01 (t, J = 6 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 165.83 (C-4), 155.89 (C-2), 147.04 (C-6), 92.96 (C-5), 80.08 (d, J = 12 Hz, C-2′), 62.75 (d, J = 170 Hz, OCH_2P), 61.76 (d, J = 6 Hz, $POCH_2$), 60.22 (C-3′), 49.43 (C-1′), and 16.30 (d, J = 6 Hz, $POCH_2CH_3$); mass spectrum (methane DCI), m/e (rel intensity) 336 (MH^+, 100), 318 (15), 290 (25). Anal. ($C_{12}H_{22}N_3O_6P\cdot0.75H_2O$) C, H, N. For **15**: UV_{max} (MeOH) 210 nm (ϵ = 8400); ^1H NMR (Me_2SO-d_6) δ 10.07 (s, 1 H, exch, NH), 4.71 (t, J = 5 Hz, 1 H, OH), 3.92–4.05 (m, 5 H, 2 $POCH_2$, OC*H*P), 3.85 (dd, J = 9, 14 Hz, 1 H, OC*H*P), 3.52–3.62 (m, 1 H, H-2′), 3.21–3.50 (m, 6 H, H-1′, H-3′, H-6), 2.53 (t, J = 5 Hz, 2 H, C-5), and 1.20 (t, J = 6 Hz, 6 H, 2 $POCH_2CH_3$); ^1H NMR ($CDCl_3$) δ 8.16 (br s, 1 H, exch, NH), 3.91–4.02 (m, 4 H, 2 $POCH_2$), 3.70 (d, J = 8 Hz, 2 H, OCH_2P), 3.34–3.58 (m, 7 H, H-6, H-1′, H-2′, H-3′), 2.47 (t, J = 6 Hz, 2 H, H-5), 2.03 (br s, 1 H, exch, OH), 1.14 (t, J = 6 Hz, 3 H, $POCH_2CH_3$), and 1.13 (t, J = 6 Hz, 3 H, $POCH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 169.89 (C-4), 153.35 (C-2), 81.43 (d, J = 12 Hz, C-2′), 63.73 (d, J = 165 Hz, OCH_2P), 62.53 (d, J = 6 Hz, $POCH_2$), 62.45 (d, J = 6 Hz, $POCH_2$), 60.30 (C-3′), 47.91 (C-1′), 44.17 (C-6), 30.94 (C-5), and 16.30 (d, J = 5 Hz, $POCH_2CH_3$); mass spectrum (methane/DCI), m/e (rel intensity) 339 (MH^+, 100), 321 (20); exact mass spectrum (FAB), calcd for $C_{12}H_{24}N_2O_7P$ (MH^+) 339.1321, found 339.1316.

(*S*)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]cytosine (2). A solution of **14** (9.20 g, 27.4 mmol) in 100 mL of anhydrous CH_3CN was treated with bromotrimethylsilane (42.0 g, 274 mmol) dropwise via syringe over 5 min at room temperature under argon. The resulting yellow solution was stirred at room temperature for 14 h, and then the reaction mixture was concentrated in vacuo. The residual oil was placed under high vacuum (0.2 mmHg) for 2 h and then was treated with water (20 mL). After 1 h, 200 mL of EtOH was added, and within 0.5 h a solid precipitated from the solution. The mixture was allowed to stand at 0 °C for 12 h and then was filtered to provide 7.27 g (95%) of **2** as a white powder. In order to remove trace amounts of EtOH, the solid was dissolved in 125 mL of hot water and the solution was lyophilized to give 7.30 g of **2** as a fluffy white solid: mp 260 °C dec; $[\alpha]^{20}_D$ −87.7° (c = 1.052, H_2O); UV_{max} (0.1 N NaOH) 282 nm (ϵ = 8800), (0.1 N HCl) 274 nm (ϵ = 5500); ^1H NMR (D_2O/NaOD) δ 7.85 (d, J = 7 Hz, 1 H, H-6), 6.13 (d, J = 7 Hz, 1 H, H-5), 4.15 (dd, J = 3, 14 Hz, 1 H, H-1′), 3.73–3.89 (m, 4 H, H-1′, H-2′, and OCH_2P), and 3.53–3.63 (m, 2 H, H-3′); ^{13}C NMR (D_2O/NaOD) δ 164.76 (C-4), 155.53 (C-2), 151.30 (C-6), 96.68 (C-5), 81.69 (d, J = 11 Hz, C-2′), 68.15 (d, J = 165 Hz, OCH_2P), 62.10 (C-3′), and 52.15 (C-1′); mass spectrum (FAB), m/e (rel intensity) 279 (M^+, 100), 173 (85), 171 (90), 154 (20), 112 (40). Anal. ($C_8H_{14}N_3O_6P\cdot H_2O$) C, H, N.

The sodium salt of HPMPC was prepared for in vivo studies. A solution of **2** (7.25 g, 24.4 mmol, monohydrate form) in 75 mL of water was treated with 1.00 N NaOH (41.0 mL, 41.0 mmol) until neutral pH was reached as indicated by a pH meter. The resulting solution was lyophilized to provide 8.29 g of the 1.7 sodium salt of **2** as a fluffy white powder: ^1H NMR (D_2O) δ 7.63 (d, J = 7 Hz, 1 H, H-6), 5.96 (d, J = 7 Hz, 1 H, H-5), 4.00 (dd, J = 4, 14 Hz, 1 H, H-1′), 3.65–3.83 (m, 4 H, H-1′, H-2′, and OCH_2P), and 3.46–3.59 (m, 2 H, H-3′). Anal. ($C_8H_{12,3}N_3O_6PNa_{1,7}\cdot2.5H_2O$) C, H, N.

Antiviral Activity. a. In Vitro Antiviral Evaluation. The compounds were evaluated for antiviral activity in vitro by the standard plaque reduction assay. Experiments were conducted with vero cells infected with HSV 1 (BW^s strain),[15] HSV 1 (Z826, a TK$^-$ strain obtained from Dr. William H. Burns, Johns Hopkins Oncology Center, Baltimore, MD),[16] and HSV 2 (G strain, obtained from American Tissue Culture Collection, Rockville, MD) or MRC-5 cells infected with HCMV (AD169 strain, obtained from American Tissue Culture Collection). Briefly, confluent cell monolayers in 24-well plates were infected with 30–50 plaque-forming units of virus in 100 μL of phosphate-buffered saline. After a 1-h adsorption period, residual inoculum was replaced with 1 mL of the appropriate dilution of the test compound which had been freshly prepared in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum. After a 48-h incubation period at 37 °C in a 5% CO_2 atmosphere, cell monolayers were fixed and stained with Carbol fuchsin and plaques were counted. The antiviral potency of the drug was determined by ID_{50}, the drug concentration necessary to reduce the number of plaques by 50% of those in the virus control cultures.

b. In Vivo Systemic HSV 1 Infection in Mice. Female Swiss mice weighing 16–18 g (Charles River, Wilmington, MA) were infected intraperitoneally with (2–6) × 10^2 plaque-forming units of HSV 1 (HL-34 strain, obtained from Prof. Earl R. Kern, University of Alabama, Birmingham, AL). Mice were then randomized into groups of 10 mice each. Intraperitoneal (ip) treatment with the drug was initiated 3 h postinfection and given twice daily for 5 consecutive days. Infected placebo control mice were injected with the vehicle [0.2% (carboxymethyl)cellulose with 0.2% Tween 80 in water (CMC/T)]. Mice were observed for 21 days for mortality. Statistical analysis and the probability (P) value for the percent survival and the mean survival time were determined by Fisher's exact and Gehan–Wilcoxon tests, respectively.

c. Cutaneous HSV 1 Infection in Guinea Pigs. The method used in these experiments was described previously.[17] Briefly, hairless guinea pigs [Crl:IAF(HA)BR, Charles River, Wilmington, MA] were anesthetized (Ketaset, Bristol-Myers Co., Syracuse, NY). The back areas were divided into six squares, and 20 μL of HSV 1, HL-34 strain with a titer of 6.5 × 10^7 PFU/mL, was applied with a multiple puncture apparatus (Downs Surgical, Inc., Wilmington, MA). Treatment was initiated 3 h postinfection and given twice daily for 5 consecutive days. Four areas on each animal were treated topically with preparations of either 5% HPMPC, 1% HPMPC, or 0.1% HPMPC [each prepared fresh daily in

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CMC/T in poly(ethylene glycol) (PEG)], or with 5% acyclovir ointment (Zovirax, Burroughs Wellcome Co., Research Triangle, NC). One area on each animal was treated with PEG (placebo control), and one area was not treated (untreated control). Fifty microliters of the compound solution was applied at each treatment and spread over the infected site. Each drug was tested in five animals, with one area/animal for each compound.

For statistical analysis and interpretation of data, accumulative scores were collected from individual animals. Two separate response variables, the lesion formation stage and the healing period stage, were analyzed statistically. A high cumulative score for the healing stage indicates an infection with a short healing period. The response variable that measures the severity of the infection is equal to the highest sequential step reached in the formative stage.

Acknowledgment. We express our appreciation to Cathy A. Bartelli, Christine Franco, Robert Salvagno, and Kathleen Woods for technical assistance in obtaining biological data. We thank Dr. Antonin Holy for helpful discussions, and for providing us with a sample of HPMPC.

Registry No. **2**, 113852-37-2; **2**·Na, 120362-37-0; **3**, 117087-39-5; **4**, 17325-85-8; **5**, 120362-27-8; **6**, 120362-28-9; **7**, 120362-29-0; **8**, 120362-30-3; **9**, 117087-22-6; **10**, 120362-31-4; **11**, 120362-32-5; **12**, 120362-33-6; **13**, 120362-34-7; **14**, 120362-35-8; **15**, 120362-36-9; $TsOCH_2P(O)(OEt)_2$, 31618-90-3; $HOCH_2CH_2OCH_2P(O)(OEt)_2$, 116384-55-5; (*R*)-2,3-*O*-isopropylideneglycerol, 14347-78-5; (*R*)-1-*O*-benzyl-2,3-*O*-isopropylideneglycerol, 14347-83-2; cytosine, 71-30-7.

3,7-Dideazapurine Nucleosides. Synthesis and Antitumor Activity of 1-Deazatubercidin and 2-Chloro-2′-deoxy-3,7-dideazaadenosine

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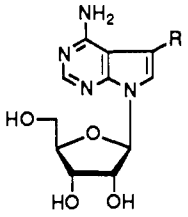
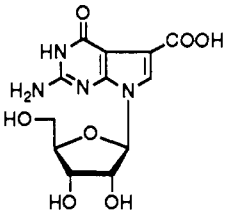
1-Deazatubercidin (**5**) has been synthesized by glycosylation of the anion of 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (**9**) with 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose (**12**). The reaction gave a mixture of blocked nucleosides with β- and α-configuration (**13a** and **13b**). Deprotection of **13a** provided 4,6-dichloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**14**), which on treatment with hydrazine, followed by reduction of the resulting 4-hydrazino compound with Raney nickel, gave 4-amino-6-chloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**15**), 1-deazatubercidin, and a small quantity of 4,6-diamino-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**16**). Dehalogenation of **15** provided another route to **5**. 2-Chloro-2′-deoxy-3,7-dideazaadenosine (**6**) together with 2′-deoxy-3,7-dideazaadenosine (**18**) was obtained by hydrazinolysis of 4,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo-[3,2-*c*]pyridine (**17**), followed by reduction of the resulting 4-hydrazino compound. Nucleosides **5**, **6**, **15**, and **18** are devoid of any significant antitumor activity in vitro. Compound **16** showed significant activity against P388 leukemia in cell culture.

After the discovery that several naturally occurring nucleoside antitumor antibiotics are derivatives of pyrrolo[2,3-*d*]pyrimidine, i.e., tubercidin (**1**), toyocamycin (**2**), sangivamycin (**3**), and cadeguomycin (**4**),[1] several structurally related deazapurine nucleosides have been synthesized. Among these all the four possible monodeazaadenosines, 1-deaza-,[2] 3-deaza-,[3] 9-deaza-,[4] and 7-deazaadenosine (tubercidin),[5] have been synthesized.



1, R = H
2, R = CN
3, R = $CONH_2$



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1-Deaza- and 9-deazaadenosine have shown pronounced growth inhibitory activity against several mouse and human leukemic cell lines,[2,4] whereas 3-deazaadenosine is an antiviral agent.[6] Of the six possible dideazaadenosines only 1,3-dideaza-[7] and 1,7-dideazaadenosine (3-deazatubercidin)[8] have been synthesized. 3-Deazatubercidin was found inactive as antitumor agent against murine leukemia L1210 and murine sarcoma S-180 cells in culture.[8] This result suggests that the nitrogen atom at position 3 of the pyrimidine ring of tubercidin is important for antitumor activity. In order to verify whether also the nitrogen atom at position 1 is essential for antitumor activity, we decided to synthesize the 1-deaza analogue of tubercidin (**5**). We now report the synthesis of compound **5** and certain other pyrrolo[3,2-*c*]pyridine nucleosides including the 3,7-dideaza analogue of the antitumor and immunosuppressive agent 2-chloro-2′-deoxyadenosine.[9]

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