Nucleotides and Pronucleotides of 2,2-Bis(hydroxymethyl)methylenecyclopropane Analogues of Purine Nucleosides: Synthesis and Antiviral Activity

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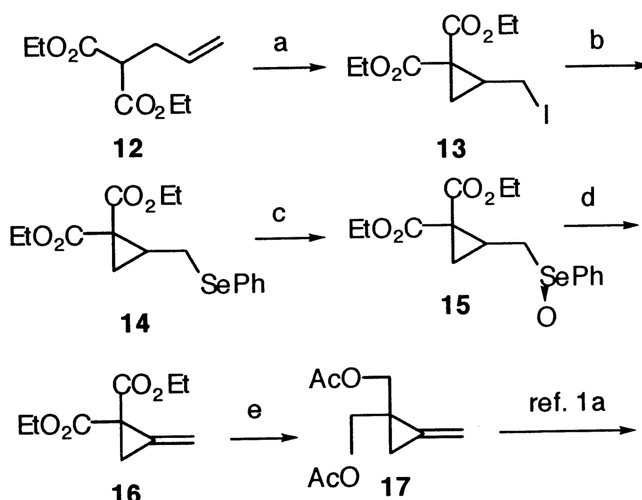
Phenylmethylphosphor-L-alaninate pronucleotides **7a**, **7b**, **8a**, and **8b**, cyclic phosphates **10a** and **10b**, and phosphates **11a** and **11b** derived from 2,2-bis(hydroxymethyl)methylenecyclopropane analogues **1a**, **1b**, **2a**, and **2b** were synthesized and evaluated for their antiviral activity. An improved protocol for the synthesis of analogues **1a**, **1b**, **2a**, and **2b** is also described. Phosphate **11a** was the most effective agent against human and murine cytomegalovirus (EC_{50} 0.25−1.1 μM). The *Z*-pronucleotides **7a** and **7b** had EC_{50} 3.6−25.2 and 3−18.4 μM, respectively. The EC_{50} of cyclic phosphate **10a** was 6.0−20 μM. The activity against Epstein−Barr (EBV) was assay-dependent. Pronucleotides **7a** and **7b** and phosphate **11a** had EC_{50} 2.3−3.4 μM against EBV/H-1, but **7b** was cytotoxic (CC_{50} 3.8 μM). Cyclic phosphate **10a** was the only compound effective against EBV/Daudi (EC_{50} 0.96 μM), but it was inactive in H-1 cells. Pronucleotide **7a** was active against varicella zoster virus with EC_{50} 6.3 and 7.3 μM, respectively, and hepatitis B virus (HBV, EC_{50} 4.1 μM). Cyclic phosphate **10a** was the most effective analogue against HBV (EC_{50} 0.8 μM).

Recently, we have described (*Z*)- and (*E*)-2,2-bis(hydroxymethyl)methylenecyclopropane analogues of nucleosides **1** and **2** as antiviral agents.[1] Several of these analogues are active in vitro at micromolar or submicromolar concentration range[1a,b] against herpesviruses such as human and murine cytomegalovirus (HCMV and MCMV), Epstein−Barr virus (EBV), varicella zoster virus (VZV), and human herpes virus 6 and 8 (HHV-6 and HHV-8). The guanine analogue **1a** (ZSM-I-62, cyclopropavir), the most potent agent against HCMV and MCMV among the methylenecyclopropane analogues known to date, is highly effective in reducing MCMV replication in visceral organs of mice and HCMV replication in implanted human tissues in SCID mice. In four experimental CMV infections, cyclopropavir (**1a**) was more active than ganciclovir.[1c]

Transformation of the first-generation purine methylenecyclopropane analogues **3** and **4** into phosphoroalaninate triester pronucleotides **5** and **6** increased antiviral potency in several instances.[2−6] It was therefore of interest to investigate the antiviral activity of prodrugs **7** and **8** of the second-generation (*Z*)- and (*E*)-2,2-bis(hydroxymethyl)methylenecyclopropane analogues **1** and **2**. Our attention focused on guanine and adenine pronucleotides **7a** and **8a** and **7b** and **8b**. In addition, ganciclovir cyclic phosphate **9** is an effective antiherpetic agent with a mechanism of action different from ganciclovir.[7−10] More recently, similar cyclic phosphates

Scheme 1[a]



[a] Reagents: (a) 1. Ti(*i*-PrO)$_4$ (cat.), pyridine, I_2, CH_2Cl_2. 2. $KMnO_4$, H_2O. (b) $(PhSe)_2$, NaOH, EtOH, $NaBH_4$. (c) $KMnO_4$, H_2O. (d) $(i\text{-}Pr)_2NEt$, toluene, 80 °C. (e) 1. $LiAlH_4$, Et_2O. 2. Ac_2O, pyridine.

derived from 2′,3′-dideoxy-4′-hydroxymethyl-3′-thioribonucleosides were reported to exhibit in vitro anti-HCMV effects.[11] Because cyclopropavir (**1a**) can be regarded as a rigid analogue[1a] of ganciclovir, we extended our investigation to cyclic phosphates **10a** and **10b**. The synthesis and antiviral activity of analogues **7a**, **7b**, **8a**, **8b**, **10a**, and **10b** are the subject of this contribution. Phosphates **11a** and **11b**, the starting materials for synthesis of **10a** and **10b**, were also included in the study. The structures of **1** and **2** as well as other nucleotides discussed below are shown in Chart 1.

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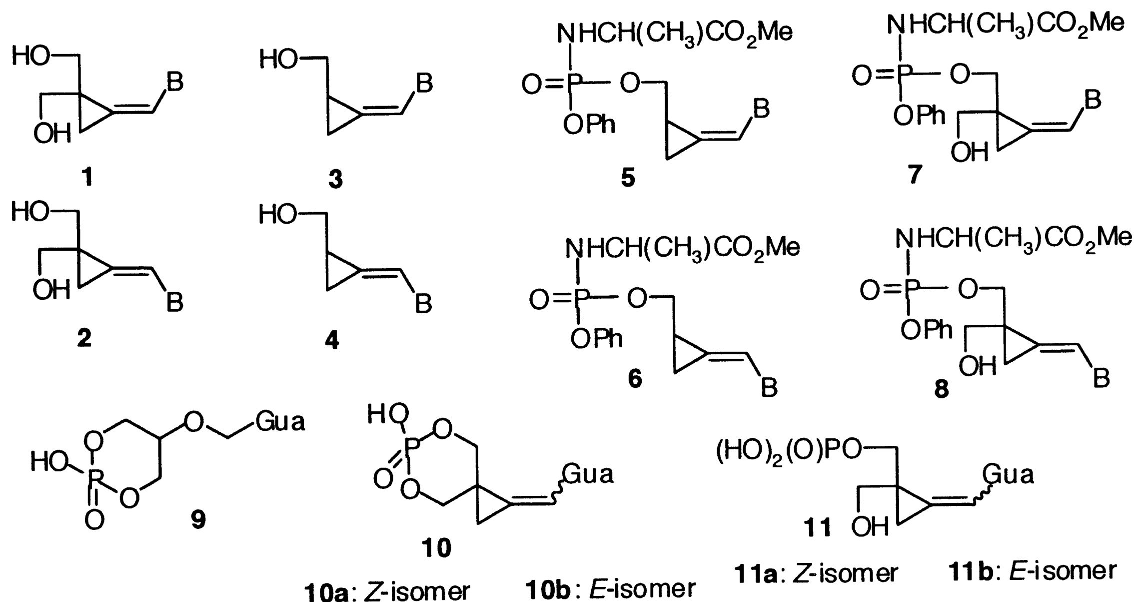
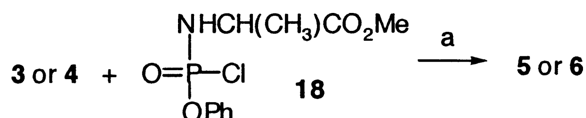
Chart 1[a]



[a] B = purine, in formulas **1**–**8**. Series a: B = Gua. Series b: B = Ade.

Scheme 2[a]

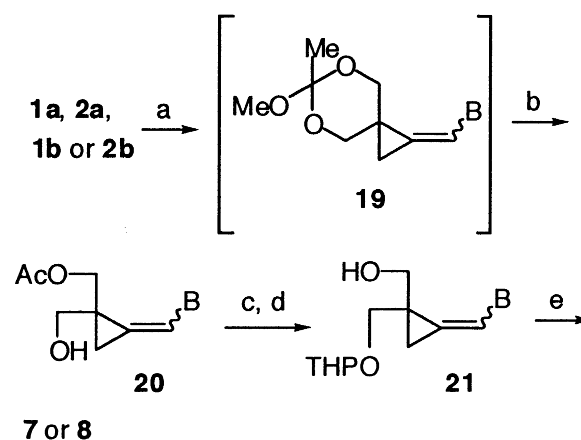


[a] Reagents: (a) 1-Methylimidazole, pyridine.

Synthesis. The previously described procedure starting from diethyl isopropylidene malonate[1a] was adequate for the preparation of limited amounts of 2,2-bis(hydroxymethyl)cyclopropane analogues including cyclopropavir (**1a**) for initial antiviral screening. The major drawback of this protocol is a difficult chromatographic separation of a key intermediate at an early stage of synthesis. It was clear that in order to obtain the larger amounts of **1a** required for in vivo testing and further preclinical studies a more efficient procedure would have to be devised. Such a protocol is described in Scheme 1. Commercially available diethyl allylmalonate (**12**) was transformed into iodomethylcyclopropane dicarboxylate **13** in 72% yield using a modification of the reported procedure.[12,13] Intermediate **13** was then converted to phenylselenylmethylcyclopropane **14** (83%). Oxidation with $KMnO_4$ afforded phenylselenoxide **15** (70%), which, in turn, was transformed to methylenecyclopropane dicarboxylate **16** by a base-catalyzed β-elimination (63% yield). Reduction and acetylation[1a] were combined in a single step to give diacetate **17** in 92% yield. The latter was used for synthesis of analogues **1a** and **2a** and **1b** and **2b** as described earlier.[1a]

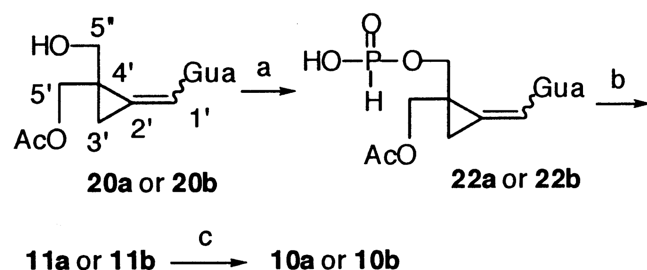
The first-generation pronucleotides **5** and **6** were obtained by a direct phosphorylation[2,6] of methylenecyclopropanes **3** and **4** with reagent **18** in pyridine using 1-methylimidazole as a catalyst (Scheme 2). To avoid formation of mixtures of mono- and diphosphorylated products, one of the hydroxy groups of analogues **1** and **2** was selectively protected as shown in Scheme 3. Analogue **1a** was transformed by an acid-catalyzed reaction with trimethyl orthoacetate in dimethylformamide (DMF) to the cyclic orthoacetate **19a**, which was smoothly hydrolyzed to monoacetate **20a** in 84% overall yield (Scheme 3). A similar method was used for synthesis of monoacyl derivatives of ganciclovir.[14] Analogues **2a**, **1b**, and **2b** gave the respective acetates **20b**, **20c**, and **20d** (via cyclic ortho esters **19b**, **19c**, and **19d**) in 82, 87, and 63% yield, respectively. Direct phosphorylation of **20a**, **20b**, **20c**, and **20d** with reagent **18** was not investigated because the subsequent removal of the *O*-acetyl group is not compatible with an alkali-labile phosphotriester alaninate moiety.[15] Therefore, acetates **20a**, **20b**, **20c**, and **20d** were converted to tetrahydropyranyl (THP) derivatives **21a**, **21b**, **21c,** and **21d** by an acid-catalyzed reaction with dihydropyran in DMF followed by deacetylation with NH_4OH (yields 77–87%). The phosphorylation of compounds **21a**, **21b**, **21c,** and **21d** with reagent **18** was uneventful, giving the pronucleotides **7a** and **8a** and **7b** and **8b** in 29–45% yield

Scheme 3[a]



[a] Series a: B = Gua, *Z*-isomer. Series b: B = Gua, *E*-isomer. Series c: B = Ade, *Z*-isomer. Series d: B = Ade, *E*-isomer. Reagents: (a) 1. $MeC(OMe)_3$, $MeSO_3H$, DMF. 2. NEt_3. (b) 80% AcOH. (c) 3,4-Dihydro-2*H*-pyran, $MeSO_3H$, DMF. (d) NH_4OH, MeOH, Δ. (e) 1. **18**, 1-methylimidazole, pyridine/THF. 2. 80% AcOH. 3. 0.1 M Na_2HPO_4, pH 7.5.

Scheme 4[a]



[a] Series a: *Z*-isomers. Series b: *E*-isomers. Reagents: (a) 1. $(PhO)_2P(O)H$, pyridine, Δ. 2. NEt_3, H_2O. (b) 1. TMSCl, imidazole, pyridine. 2. I_2, pyridine. 3. H_2O. 4. NH_4OH. 5. Dowex 50 ($H^{(+)}$). (c) *N,N'*-Dicyclohexyl-4-morpholinecarboxamidine, DCC, pyridine, Δ.

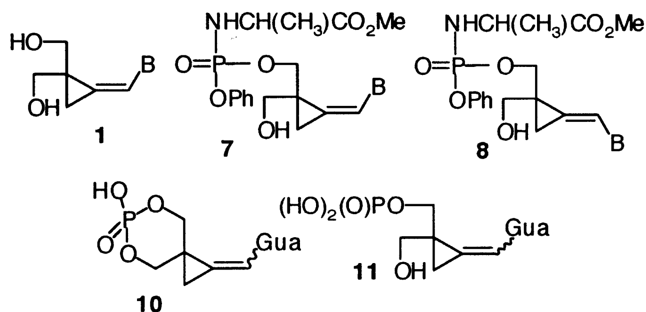
upon prolonged treatment with 80% acetic acid to remove THP groups and cleave any byproducts phosphorylated at the heterocyclic moiety.[2]

Acetates **20a** and **20b** were used as convenient starting materials for the synthesis of cyclic phosphates **10a** and **10b** (Scheme 4). Phosphitylation using diphenyl phosphite in pyridine according to the method described previously[16,17] for synadenol (**3b**) and its *E*-isomer (**4b**) gave phosphites **22a** and **22b** as triethylammonium salts in 80 and 73% yield, respectively. Silylation of **22a** and **22b** followed by oxidation with iodine in pyridine and deacetylation afforded the phosphates **11a** (86%) and **11b** (75%). Cyclization by a method previously described for ribonucleoside 3′,5′ cyclic phosphates[18] using a *N,N'*-dicyclohexylcarboxamidinium salt of **11a** and *N,N'*-dicyclohexylcarbodiimide (DCC) in pyridine furnished cyclic phosphate **10a** in 93% yield. In a similar fashion, the *E*-isomer **10b** was obtained from phosphate **11b** (89%).

Antiviral Activity. Pronucleotides **7a**, **8a**, **7b**, and **8b**, cyclic phosphates **10a** and **10b**, and phosphates **11a** and **11b** were assayed in vitro against the following viruses: human and murine cytomegalovirus (HCMV and MCMV), Epstein–Barr virus (EBV), varicella zoster virus (VZV), hepatitis B virus (HBV), and herpes simplex virus 1 and 2 (HSV-1 and HSV-2). All tested analogues were inactive against human immunodeficiency virus (HIV-1). The results are summarized in Tables 1–3.

The *Z*-isomeric pronucleotide **7a** was an inhibitor of replication of HCMV/HFF (Towne and AD169 strains), but its potency was about one log lower than that of the parent compound, cyclopropavir (**1a**, Table 1). It was less effective against MCMV/MEF. A similar decrease of activity relative to synguanol (**3a**) was observed[2] with pronucleotide **5a**. The lower activity may be the result of less effective intracellular conversion to phosphate **11a** according to the mechanism proposed for similar analogues[19] (Scheme 5), possibly in the phosphoamidase step. Regardless of the precise mechanistic details of the bioactivation of **5a** and **7a**, our results support the hypothesis that the active metabolite of cyclopropavir (**1a**) is phosphate **11a**. Indeed, other findings have indicated[1d] that viral kinase UL97 is required for the phosphorylation of **1a**. The same enzyme was implicated in the phosphorylation of synguanol (**3a**).[20] Pronucleotides **7a**, **7b**, **8a**, and **8b** are substrates for porcine liver esterase (PLE), a widely accepted[19] model of intracellular esterases. The PLE-catalyzed hydrolysis of **7a** led to formation of diastereoisomeric phosphoramidate **23**

Table 1. Inhibition of HCMV and MCMV Replication by Phosphate Derivatives of *gem*-Bis(hydroxymethyl)methylenecyclopropane Analogues



Formulas 1, 7 and 8, series a: B = Gua, series b: B = Ade
Formulas 10 and 11, series a: *Z*-isomer, series b: *E*-isomer

	EC_{50}/CC_{50} (μM)		
	HCMV/HFF[a]		MCMV/MEF[a]
compound	Towne[b]	AD169[c]	
1a[d]	0.33/100–200[e]	0.49/>380	0.27/>380
1b[d]	2.4/>100[f]	11.7/>404	9.7/>404
7a	3.6/>100	8.1/>198	25.2/>198
7b	3/32	18.4/200	NT
8a	>100/>100	>198/>198[g]	NT
8b	>100/>100	>205/>205[g]	NT
10a	20/>100	6.0/>301	7.2/>301
10b	>100/>100	>299/>299[g]	NT
11a	0.25/100	1.1/>291	0.26/175
11b	>100/>100	>291/>291[g]	NT
ganciclovir	2.1/>100	1.6/>392	2.1/>392

[a] Plaque reduction assay. NT = not tested. [b] Visual cytotoxicity. [c] Cytotoxicity by neutral red uptake. [d] Data from ref 1a. [e] Average of four experiments. [f] Average of three experiments. [g] Cytopathic effect (CPE) inhibition assay.

(via intermediates **24** and **25**) as shown by ^{31}P NMR spectra (Scheme 5). No evidence for a formation of cyclic phosphate **26** or **10a** was found. The activity of adenine *Z*-pronucleotide **7b** against HCMV was comparable with that of parent analogue **1b** in both AD169 and Towne strains of the virus. With the latter strain, however, increased cytotoxicity to the HFF host cells was observed.

Phosphate **11a** is almost equally effective as cyclopropavir (**1a**) against HCMV and MCMV (Table 1), but similar to other nucleotides,[21] it is most likely dephosphorylated at the cell membrane to cyclopropavir (**1a**), which is therefore rephosphorylated inside the cells. The only advantage of phosphate **11a** is then an increased solubility at pH 7 over that of the parent analogue **1a**. Of interest is the potency of cyclic phosphate **10a** against HCMV (AD169) and MCMV in the micromolar range even though it was somewhat less effective against the Towne strain of HCMV. The efficacy of **10a** in the AD169 assay (EC_{50} 6.0 μM) does not differ much from that of ganciclovir cyclic phosphate **9** (EC_{50} 2 and 5.9 μM, respectively).[7,8]

As observed previously with methylenecyclopropane analogues **3** and pronucleotides **5**, the activity against EBV is quite often assay-dependent.[1a,6,22] Thus, pronucleotides **7a** and **7b** showed greater antiviral activity in H-1 cells than **1a** and **1b**, although cytoxicity also increased (Table 2). In contrast, **7a** and **7b** had no significant activity in either viral or cellular proliferation when Daudi cells were used. The parent analogue **1a** and phosphate **11a** had a similar level of potency in H-1 cells, but they lacked a significant effect in Daudi

Scheme 5

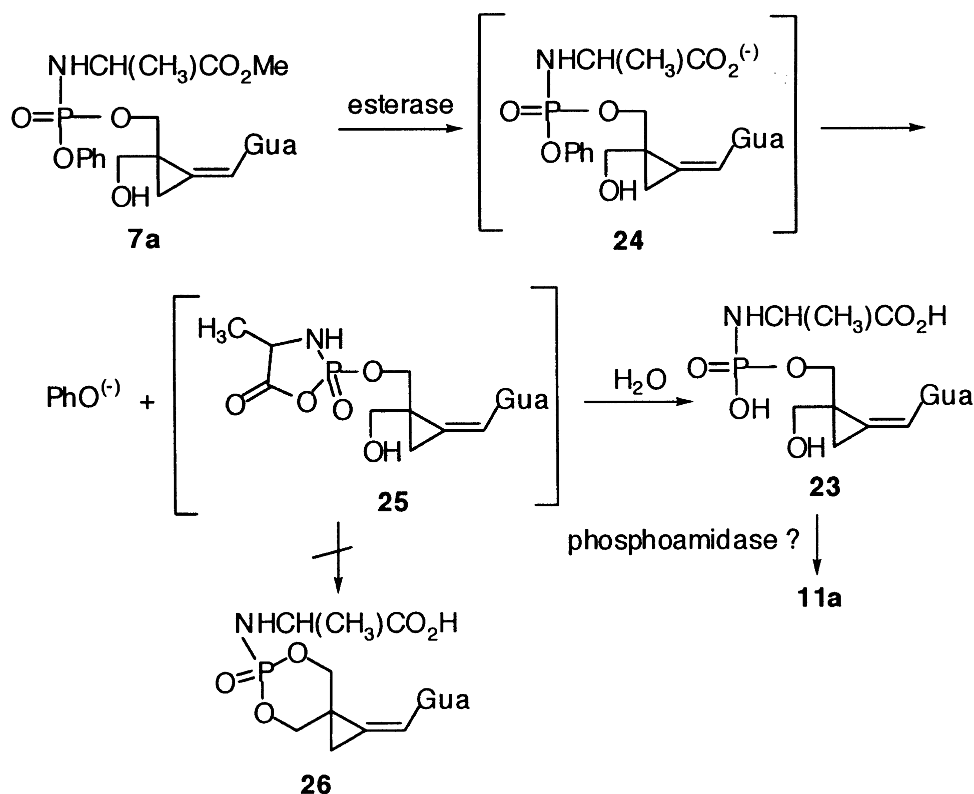
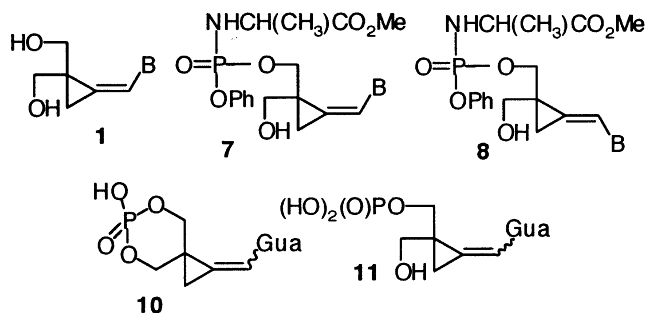


Table 2. Inhibition of EBV, VZV, and HBV Replication by Phosphate Derivatives of *gem*-Bis(hydroxymethyl)methylenecyclopropane Analogues



Formulas **1**, **7** and **8**, series a: B = Gua, series b: B = Ade
Formulas **10** and **11**, series a: *Z*-isomer, series b: *E*-isomer

	EC_{50}/CC_{50} (μM)			
	EBV		VZV	HBV
compound	Daudi[a]	H-1[b,c]	HFF[d,e]	2.2.15[b,f]
1a	45/74	7/>50	>380	>10
1b	166/>202	>20/>50	>404	>10
7a	>99/>198	3.4/35	6.3(7.3)[g]	4.1
7b	>102/>102	3/3.8	>41	12.8
8a	>99/>198	>20/>100	>198	>10
8b	>102/>102	>20/>25	>205	>20
10a	0.96/>150	>20/>100	>301	0.8
10b	>150/>150	>20/>100	>299	>20
11a	>146/>146	2.3/62	>291	15
11b	>146/>146	>20/>100	>291	>20
control	1.1/>222[h]	5[i]	1.6/>444[h]	0.05/>100[j]

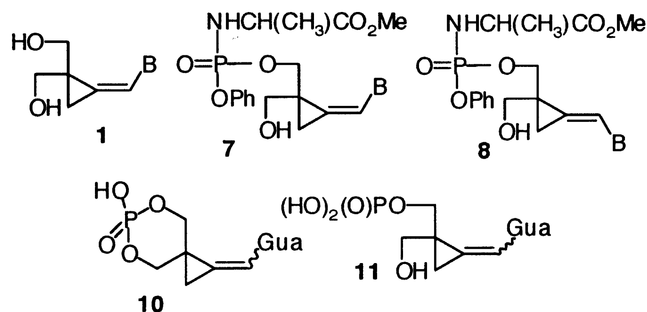
[a] Viral capsid immunofluorescence (VCA) ELISA. [b] DNA hybridization assay. [c] Cytotoxicity was determined in CEM cells. [d] Cytopathic effect (CPE) inhibition assay. [e] For CC_{50} values, see Table 1, footnote *c*. [f] For CC_{50} values, see EBV/H-1 assays. [g] Plaque reduction assay. [h] Acyclovir. [i] Ganciclovir. [j] Lamivudine.

cells. By contrast, cyclic phosphate **10a**, the most potent and noncytotoxic anti-EBV agent listed in Table 2 (EC_{50} 0.96/>150, Daudi), was ineffective in H-1 culture. The result in Daudi culture is not compatible with a partial or total enzymatic hydrolysis to **11a** or **1a** because, as already mentioned, the latter analogues lacked significant activity. Instead, the mechanism of action of cyclic phosphate **10a** may resemble that of ganciclovir cyclic phosphate **9**. Compound **9** is an antiviral analogue per se; it is converted to neither ganciclovir nor ganciclovir phosphate.[7−10] The intramolecular distance between the heterocyclic moiety (N-9) and phosphorus atom determined from MM2 minimized model of **10a** is very close to that observed in a crystal structure of ganciclovir cyclic phosphate[23,24] (**9**, 5.944 versus 5.893 Å). This further stresses the similarity between **10a** and **9**.

Against VZV/HFF, pronucleotide **7a** was the only potent analogue as shown in both the cytopathic and plaque reduction assay (EC_{50} 6.3 and 7.3 μM, respectively). Cyclopropavir (**1a**) was inactive. A similar potentiating effect of the adenine pronucleotide **7b** relative to **1b** was also seen, albeit to a lesser extent. The other analogues lacked significant potency. Interestingly, cyclic phosphate **10a** was a potent anti-HBV agent (EC_{50} 0.8 μM), surpassing both phosphate **11a** (EC_{50} 15 μM) and cyclopropavir (**1a**, EC_{50} >10 μM). This result is also indicative that the mechanism of action of cyclic phosphate **10a** is different from cyclopropavir (**1a**). The potency of both pronucleotides **7a** and **7b** was also lower than that of **10a** (EC_{50} 4.1 and 12.8 μM, respectively) but higher than that of parent analogues **1a** and **1b**.

Although the efficacy of the tested analogues in HSV-1 and HSV-2 assays was only moderate and assay-dependent, some aspects of their antiviral properties deserve discussion. Thus, the EC_{50} values of **10a** were 16−22 μM in BSC-1 and Vero cells, whereas no activity

Table 3. Inhibition of HSV-1 and HSV-2 Replication by Phosphate Derivatives of *gem*-Bis(hydroxymethyl)methylenecyclopropane Analogues



Formulas **1**, **7** and **8**, series a: B = Gua, series b: B = Ade
Formulas **10** and **11**, series a: *Z*-isomer, series b: *E*-isomer

	EC50/CC50 (μM)				
	HSV-1			HSV-2	
compound	BSC-1[a]	HFF[b]	Vero[c,d]	HFF[b,d]	Vero[c]
1a	>100/>100	>380	>50	>380	>50
1b	50/>100	>404	>50	>404	>50
7a	>100/>100	>198	>50	>198	>50
7b	55/3.5	21.1[c]	>40	22.1[c]	>40
8a	70/>100	>198	>50	>198	>50
8b	>100/>100	>205	>30	>205	>30
10a	20/>100	>301	16/>100	242	22
10b	>100/>100	>299	>50	>299	>50
11a	>100/>100	9.9[c]	50	(23.3)[c]	>50
11b	>100/>100	>291	>50	>291	>50
acyclovir	1.5/>100	0.9[e]	13.5[f]	6.7[e]	32.3[f]

[a] ELISA. Cytotoxicity was determined in KB cells. [b] Cytopathic effect (CPE) assay. For cytotoxicity see Table 1, footnote *c*. [c] Plaque reduction assay. [d] For cytotoxicity, see Table 2, footnote *c*. [e] The CC_{50} in HFF cells was >444 μM. [f] The CC_{50} in CEM cells was >200 μM.

was observed in HFF cells (Table 3). Even more interesting is the activity of phosphate **11a** against HSV-1 and HSV-2 in HFF (EC_{50} 9.9 and 23.3 μM, respectively) although the parent analogue **1a** was inactive. This may indicate that phosphate **11a** is capable under certain circumstances of penetrating the cellular membrane. The only moderately effective pronucleotide was adenine derivative **7b** with EC_{50} 21.1 and 22.1 μM against HSV-1 and HSV-2 in HFF culture. Compound **7b** was cytotoxic in KB cells with CC_{50} 3.5 μM. The *E*-isomers **8a**, **8b**, **10b**, and **11b** were inactive.

Experimental Section

General Methods. See ref 1a. The UV spectra were measured in ethanol, and NMR spectra were determined at 400 MHz (^{1}H), 100 MHz (^{13}C), and 162 MHz (^{31}P) NMR in CD_3SOCD_3 unless stated otherwise. Mass spectra were obtained in an electrospray ionization mode (ESI-MS) in methanol−NaCl. A stock solution of reagent **18** in THF instead of CH_2Cl_2 was prepared as described.[2] Porcine liver esterase (lyophilized powder) was a product of Sigma, St. Louis, MO. The *O*-substituted compounds **11a**, **11b**, and **20a**−**d** are racemic whereas **7a**, **7b**, **8a**, **8b**, and **21a**−**d** are mixtures of four diastereoisomers each. The alanine moiety in **7a**, **7b**, **8a**, and **8b** is of L-configuration.

Diethyl 1-(Iodomethyl)cyclopropane-2,2-dicarboxylate (13). The procedure described for the corresponding dimethyl ester[12] was modified as follows. To a solution of a diethyl allylmalonate (**12**, 144.6 g, 0.72 mol) in CH_2Cl_2 (500 mL) were added Ti(*i*-PrO)$_4$ (43 mL, 0.15 mol), pyridine (117 mL, 1.44 mol), and iodine (550 g, 2.16 mol) under N_2 at room temperature with stirring. The stirring was continued for 45 h. The resultant solution was washed with 4 M HCl (1 L), saturated aqueous $Na_2S_2O_3$ (2 × 1 L), brine (1 L), 4 M HCl (1 L), and brine (1 L). The organic phase was dried ($MgSO_4$) and evaporated to give crude product **13** (220 g) of which 15% was unchanged **12** according to the ^{1}H NMR and the rest was **13**. The starting material **12** was removed by slowly adding $KMnO_4$ (100 g, 0.63 mol) with external ice cooling to a stirred suspension of crude product **13** in water (300 mL). After 50 min of being stirred at 0 °C, the solution was filtered, and the filter cake was washed with hexane/ethyl acetate 2:1 mixture (4 × 150 mL). The layers were separated, and the organic phase was washed with water (300 mL), saturated Na_2SO_3 solution (300 mL), and brine (2 × 500 mL). After being dried over $MgSO_4$ and evaporation of the solvent, 170 g (72%) of pure **13** was obtained. The ^{1}H and ^{13}C NMR spectra corresponded to those reported in the literature.[25]

Diethyl 1-(Phenylselenyl)methylcyclopropane-2,2-dicarboxylate (14). Caution! Because selenium compounds are toxic, contact with skin should be avoided. All operations should be carried out in a well-ventilated hood. Diphenyl diselenide[26] (78 g, 0.25 mol) was refluxed in ethanol (300 mL) until a clear solution was obtained. After being cooled to room temperature, 4 M NaOH (125 mL, 0.5 mol) was added followed by solid $NaBH_4$ (19 g, 0.5 mol) in several portions. The resultant mixture was refluxed for 30 min, brought back to room temperature, and stirred while adding dropwise to it a solution of compound **13** (163 g, 0.5 mol) in ethanol (200 mL). After 15 min, water (500 mL) was added, and the mixture was extracted with hexane/ethyl acetate 2:1 (1 L). The aqueous layer was saturated with NaCl and reextracted with the same solvent mixture (2 × 500 mL). The combined extracts were washed with brine (500 mL) and dried over Na_2SO_4. Evaporation of the solvents provided a residue that was purified by column chromatography (950 g of silica gel) in hexane/ethyl acetate (1:0 to 10:1) to give 147 g (83%) of product **14** as an oil containing 5% of malonate **12** according to the ^{1}H NMR spectra. This product was directly used in the next step. An analytical sample was obtained by column chromatography of a 100-mg sample of **14** on silica gel using hexane/ethyl acetate (25:1) to give 90 mg of compound **14**. ^{1}H NMR ($CDCl_3$) δ: 7.53 (m, 2H) and 7.24 (m, 3H, phenyl), 4.09−4.28 (m, 4H, CH_2O), 3.04 (dd, J = 12.0 and 6.4 Hz, 1H) and 2.71 (dd, J = 12.4 and 9.2 Hz, 1H, CH_2Se), 2.22 (m, 1H, CH of cyclopropane), 1.44 (dd, J = 8.8 and 6.0 Hz,1H) and 1.38 (dd, J = 8.8 and 6.8 Hz, 1H, CH_2 of cyclopropane), 1.28 and 1.25 (2t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 169.8, 168.0 (CO), 134.0, 129.5, 129.3, 127.6 (phenyl), 61.9, 61.8 (CH_2O), 36.0 (quarternary C of cyclopropane), 28.7, 26.8 (CH_2Se, CH of cyclopropane), 22.3 (CH_2 of cyclopropane), 14.4, 14.3 (CH_3). EI-MS: 354, 356 (M, 7.4, 15.1), 153 (100.0). Anal. ($C_{16}H_{20}O_4Se$) C, H.

Diethyl 1-(Phenylselenyl)methylcyclopropane 2,2-dicarboxylate Selenium Oxide (15). $KMnO_4$ (169 g, 1.07 mol) was added slowly with stirring and ice cooling to a suspension of compound **14** (147 g, 0.42 mol) in water (400 mL). After being stirred for 2 h, the reaction mixture was filtered, and the filter cake was washed with ethyl acetate (6 × 300 mL). The layers were separated, and the organic phase was washed with water (500 mL) and brine (2 × 500 mL). It was dried over $MgSO_4$. Evaporation of the solvent provided crude product **15** (134 g) containing traces[27] of $KMnO_4$. This material was dissolved in THF (300 mL), and H_2O_2 (30%, 40 mL) was added dropwise within 1 h with stirring. After an additional 10 min of being stirred, ethyl acetate (300 mL) was added, and the organic phase was washed with water (500 mL), saturated $NaHCO_3$ solution (300 mL), and brine (300 mL). It was dried ($MgSO_4$) and evaporated to give pure oxide **15** (109 g, 70%, two diastereoisomers) as an oil. ^{1}H NMR ($CDCl_3$) δ: 7.70 (m, 2H) and 7.50 (m, 3H, phenyl), 4.16 (m, 4H, CH_2O), 3.08 (dd, J = 12.4 Hz, J = 7.6 Hz) and 2.75 (m, 2H, CH_2SeO), 2.20 (m) and 1.97 (m, 1H, CH of cyclopropane), 1.49 (m), 1.39 (dd, J = 8.0 Hz, J = 7.0 Hz) and 1.23 (m, 8H, CH_2 of cyclopropane and CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 169.1, 169.0, 168.1, 167.8 (CO), 140.0, 139.7, 131.9, 131.7, 129.91, 129.89, 126.4, 126.1 (phenyl), 62.3, 62.13, 62.09 (CH_2O), 52.8, 52.7 (CH_2SeO), 34.4, 33.8 (quarternary C of cyclopropane), 21.8, 21.6, 21.31, 21.27

(CH and CH_2 of cyclopropane), 14.3, 14.2 (CH_3). EI-MS: 371, 373 (M + H, 1.2, 2.3), 57 (100.0). Anal. ($C_{16}H_{20}O_5Se$) C, H.

Diethyl Methylenecyclopropane-2,2-dicarboxylate (16). A solution of compound **15** (217 g, 0.58 mol) and *i*-Pr_2NEt (201 mL, 1.15 mol) in toluene (800 mL) was heated at 85−89 °C for 24 h. After being cooled, the solution was washed with water (400 mL). The aqueous layer was extracted with ethyl acetate (3 × 400 mL). The combined organic phase was washed with saturated $NaHCO_3$ solution (500 mL) and brine (500 mL). It was dried over $MgSO_4$, and the solvents were evaporated to give crude product **16** containing a small amount of selenide **14** that could not be removed by chromatography. To obtain pure **16**, the crude product **16** was dissolved in THF (1 L), and H_2O_2 (30%, 400 mL) was added dropwise with stirring and ice cooling. The stirring was then continued for 14 h at room temperature. Ethyl acetate (500 mL) was added, the layers were separated, and the organic phase was washed with water (500 mL). The aqueous layer was saturated with NaCl and extracted with ethyl acetate (2 × 400 mL). The combined organic phase was washed with saturated $NaHCO_3$ solution (700 mL) and brine (700 mL). It was dried over $MgSO_4$, the solvents were evaporated, and the residue was chromatographed on a silica gel column using hexane/ethyl acetate (10:1) to afford compound **16** (73 g, 63%) identical with the product obtained previously[1a] by a different procedure.

2,2-Bis(acetoxymethyl)methylenecyclopropane (17). A solution of diester **16** (75 g, 0.37 mol) in ethyl ether (100 mL) was added dropwise with stirring and external ice cooling to a suspension of $LiAlH_4$ (18.2 g, 0.47 mol) in ethyl ether (500 mL). The mixture was refluxed for 17 h. After being cooled to 0 °C, water (36 mL) and 20% NaOH (73 mL) were added dropwise. Stirring was continued for 10 min, the solids were filtered off, and the filter cake was washed with ethyl acetate (10 × 150 mL). Combined washings were dried over Na_2SO_4, and the solvent was evaporated to give 2,2-bis(hydroxymethyl)-methylenecyclopropane (50 g) that was dissolved in pyridine. Acetic anhydride (225 mL, 2.04 mol) was added slowly with stirring and ice cooling to a solution of the above obtained crude product (50 g) in pyridine (200 mL, 2.47 mol). Stirring was then continued at room temperature for 14 h. The mixture was cooled again to 0 °C, and water (500 mL) was added slowly. The resulting mixture was extracted with ethyl ether (2 × 500 mL). The combined extracts were washed with 10% $CuSO_4$ (500 mL), 2 M HCl (500 mL), saturated $NaHCO_3$ solution (500 mL), and brine (500 mL). They were dried over $MgSO_4$, and the solvent was evaporated to give diacetate **17** (69 g, 92% in two steps) identical with the product described previously.[1a]

(*Z*)-9-{[2-(Acetoxymethyl)-2-(hydroxymethyl)cyclopropylidene]methyl}guanine (20a). A suspension of diol **1a** (1.82 g, 6.92 mmol) in DMF (100 mL) was sonicated for 10 min. Trimethyl orthoacetate (1.23 mL, 9.68 mmol) and methanesulfonic acid (67 μL, 1.04 mmol) were then added, and the mixture was stirred at room temperature for 3 h. After addition of triethylamine (3 mL), the solvent was evaporated, and the crude cyclic ortho ester **19a** was dissolved in 80% acetic acid (100 mL). The solution was stirred at room temperature for 10 h, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel CH_2Cl_2/MeOH (10:1 to 6:1) to give *O*-acetate **20a** (1.78 g, 84%). Mp: 258−260 °C. UV λ_{max}: 273 nm (ϵ 11 000), 230 (ϵ 27 500). ^{1}H NMR δ: 10.69 (s, 1H, NH), 8.21 (s, 1H, H_8), 7.14 (d, 1H J = 1.6 Hz, $H_{1'}$), 6.55 (s, 2H, NH_2), 5.25 (t, J = 5.2 Hz, 1H, OH), 4.16, 4.11 (AB, 2H, J_{AB} = 11.4 Hz, $H_{5'}$), 3.71 and 3.44 (dAB, J = 11.4 and 5.1 Hz, 2H, $H_{5''}$), 1.94 (s, 3H, CH_3), 1.45 (m, 2H, $H_{3'}$). ^{13}C NMR: 170.8 (CO), 157.4 (C_6), 154.8 (C_2), 150.4 (C_4), 134.8 (C_8), 116.9, 116.7 ($C_{2'}$, C_5), 111.9 ($C_{1'}$), 65.6 ($C_{5'}$), 63.2 ($C_{5''}$), 28.1 ($C_{4'}$), 21.2 (CH_3), 12.3 ($C_{3'}$). ESI-MS: 306 (100.0, M + H), 328 (39.8, M + Na). Anal. ($C_{13}H_{15}N_5O_4$) C, H, N.

(*E*)-9-{[2-(Acetoxymethyl)-2-(hydroxymethyl)cyclopropylidene]methyl}guanine (20b). The procedure described for the *Z*-isomer **20a** was followed with *E*-isomer **2a** (2.40 g, 9.12 mmol) to afford acetate **20b** (2.18 g, 82%) via cyclic ortho ester **19b**. Mp: 261−262 °C. UV (EtOH) λ_{max}: 272 nm (ϵ 10 500), 229 (ϵ 29 600). ^{1}H NMR δ: 10.89 (s, 1H, NH), 8.02 (s, 1H, H_8), 7.24 (d, 1H, $H_{1'}$), 6.74 (s, 2H, NH_2), 4.95 (t, J = 5.6 Hz, 1H, OH), 4.12 and 4.04 (AB, J_{AB} = 11.2 Hz, 2H, $H_{5'}$), 3.44−3.34 (m, overlapped with H_2O, $H_{5''}$), 2.02 (s, 3H, CH_3), 1.65 (m, 2H, $H_{3'}$). ^{13}C NMR: 171.1 (CO), 157.4 (C_6), 154.7 (C_2), 150.6 (C_4), 134.3 (C_8), 117.3, 116.9 ($C_{2'}$, C_5), 111.7 ($C_{1'}$), 65.6 ($C_{5'}$), 63.1 ($C_{5''}$), 26.3 ($C_{4'}$), 21.4 (CH_3), 15.0 ($C_{3'}$). ESI-MS: 306 (100.0, M + H), 328 (72.5, M + Na) 633 (16.2, 2M + Na). Anal. ($C_{13}H_{15}N_5O_4$) C, H, N.

(*Z*)-9-{[2-(Acetoxymethyl)-2-(hydroxymethyl)cyclopropylidene]methyl}adenine (20c). Trimethyl orthoacetate (3.0 mL, 23.5 mmol) and methanesulfonic acid (187 μL, 3.0 mmol) were slowly added to an ice-cooled suspension of analogue (**1b**, 2.89 g, 11.7 mmol) in DMF (120 mL). The workup including hydrolysis of intermediary ortho ester **19c** followed the protocol described for compound **20a**. After chromatography on silica gel CH_2Cl_2/MeOH (20:1), acetate **20c** (2.95 g, 87%) was obtained. Mp: 196−199 °C. UV λ_{max}: 277 nm (ϵ 8 700), 256 (ϵ 12 400), 227 (ϵ 25 500). ^{1}H NMR δ: 8.63 (s, 1H, H_8), 8.16 (s, 1H, H_2), 7.42 (poorly resolved d, 1H, $H_{1'}$), 7.35 (bs, 2H, NH_2), 5.29 (t, J = 5.2 Hz, 1H, OH), 4.24, 4.13 (AB, 2H, J = 11.4 Hz, $H_{5'}$), 3.75, 3.48 (2AB, 2H, J = 11.2 and 5.0 Hz, $H_{5''}$), 1.92 (s, 3H, CH_3 of Ac), 1.50 (m, 2H, $H_{3'}$). ^{13}C NMR: 170.8 (CO), 156.7 (C_6), 153.7 (C_2), 148.7 (C_4), 138.3 (C_8), 119.0 (C_5), 117.1 ($C_{2'}$), 112.0 ($C_{1'}$), 65.7 ($C_{5'}$), 63.4 ($C_{5''}$), 28.2 ($C_{4'}$), 21.2 (CH_3), 12.3 ($C_{3'}$). ESI-MS: 290 (M + H, 100.0), 312 (M + Na, 54.7). Anal. ($C_{13}H_{15}N_5O_3$) C, H, N.

(*E*)-9-{[2-(Acetoxymethyl)-2-(hydroxymethyl)cyclopropylidene]methyl}adenine (20d). The procedure described for compound **20c** was followed with *E*-isomer **2b** (1.90 g, 7.69 mmol) to give compound **20d** (1.40 g, 63%) via ortho ester **19d**. Mp: 191−193 °C. UV λ_{max}: 277 nm (ϵ 9 000), 257 (ϵ 12 200), 226 (ϵ 28 500). ^{1}H NMR δ: 8.49 (s, 1H, H_8), 8.18 (s, 1H, H_2), 7.53 (d, 1H, J = 2.4 Hz, $H_{1'}$), 7.39 (s, 2H, NH_2), 4.95 (t, J = 5.6 Hz, 1H, OH), 4.15, 4.10 (AB, 2H, J = 11.5 Hz, 2H, $H_{5'}$), 3.49, 3.44 (partly overlapped 2AB, J = 11.2 and 6.0 Hz, 2H, $H_{5''}$), 2.04 (s, 3H, CH_3), 1.72 (m, 2H, $H_{3'}$). ^{13}C NMR: 171.1 (CO), 156.7 (C_6), 153.8 (C_2), 148.9 (C_4), 137.9 (C_8), 119.1 (C_5), 117.8 ($C_{2'}$), 111.8 ($C_{1'}$), 65.6 ($C_{5'}$), 63.1 ($C_{5''}$), 26.5 ($C_{4'}$), 21.4 (CH_3), 15.2 ($C_{3'}$). ESI-MS (MeOH): 290 (M + H, 100.0). Anal. ($C_{13}H_{15}N_5O_3$) C, H, N.

(*Z*)-9-{[2-(Hydroxymethyl)-2-(2-tetrahydropyranyloxymethyl)cyclopropylidene]methyl}guanine (21a). A solution of methanesulfonic acid (0.14 mL, 2.23 mmol) in DMF (5 mL) was added to a stirred mixture of compound **20a** (680 mg, 2.23 mmol) and 3,4-dihydro-2*H*-pyran (3.25 mL, 35.68 mmol) in DMF (35 mL) at room temperature. The stirring was continued for 5.5 h whereupon the reaction was quenched by addition of triethylamine (0.3 mL). The solvent was evaporated in vacuo (oil pump) at room temperature. The residue was dissolved in MeOH (100 mL) and NH_4OH (30%, 20 mL), and the mixture was heated at 40−50 °C for 10 h. The volatile components were evaporated, and the crude product was chromatographed on a silica gel column using CH_2Cl_2 and CH_2Cl_2/MeOH (15:1 to 12:1 to 6:1) to give compound **21a** (672 mg, 87%). Mp: 295−298 °C. UV λ_{max}: 273 nm (ϵ 10 500), 231 (ϵ 27 300). ^{1}H NMR δ: 10.64 (s, 1H, NH), 8.36 and 8.31 (2s, 1H, H_8), 7.11 (s, 1H, $H_{1'}$), 6.53 (s, 2H, NH_2), 5.06 (m, 1H, OH), 4.60 and 4.51 (2bs, 1:1, CHO of THP), 3.88 (d) and 3.70−3.28 (cluster of m, partially overlapped with H_2O, 6H, CH_2O of THP, $H_{5''}$, $H_{5''}$), 1.70−1.31 (cluster of m, 8H, CH_2 of THP, $H_{3'}$). ^{13}C NMR: 157.3 (C_6), 154.7 (C_2), 150.3 (C_4), 134.9 (C_8), 117.6, 117.4, 116.9 (C_5, $C_{2'}$), 111.4 ($C_{1'}$), 98.4 (CHO of THP), 68.9, 68.7 (CH_2O of THP), 62.8, 62.6, 61.7, 61.3 ($C_{5'}$, $C_{5''}$), 30.64, 30.58, 29.2, 29.0, 25.67, 25.6, 19.6, 19.3 (3 × CH_2 of THP, $C_{4'}$), 12.2, 12.1 ($C_{3'}$). ESI-MS: 348 (100.0, M + H), 370 (M + Na). Anal. ($C_{16}H_{21}N_5O_4$) C, H, N.

(*E*)-9-{[2-(Hydroxymethyl)-2-(2-tetrahydropyranyloxymethyl)cyclopropylidene]methyl}guanine (21b). The procedure described for the *Z*-isomer **21a** was followed with *E*-isomer **20b** (280 mg, 0.91 mmol) to give compound **21b** (270 mg, 85%). Mp: 233−235 °C. UV (EtOH) λ_{max}: 272 nm (ϵ 11 700), 229 (ϵ 31 900). ^{1}H NMR δ: 10.67 (s, 1H, NH), 8.04 (s,

1H, H_8), 7.22 and 7.20 (s, 1H, $H_{1'}$), 6.52 (s, 2H, NH_2), 4.78 and 4.59 (2m, 1H, 1:1, CHO of THP, OH), 3.72−3.60 (m, 2H), 3.48−3.40 (2 clusters of m, 6H, CH_2O of THP, $H_{5'}$, $H_{5''}$), 1.71, 1.58, and 1.45 (3m, 8H, CH_2 of THP, $H_{3'}$). ^{13}C NMR: 157.4 (C_6), 154.6 (C_2), 150.5 (C_4), 134.3 (C_8), 118.5, 118.2, 116.9 (C_5, $C_{2'}$), 111.1 ($C_{1'}$), 98.4, 98.3 (CHO of THP), 68.4 (CH_2O of THP), 63.1, 62.0, 61.9 ($C_{5'}$, $C_{5''}$), 31.0, 27.3, 27.2, 25.7, 19.8 (3 × CH_2 of THP, $C_{4'}$), 14.8, 14.6 ($C_{3'}$). ESI-MS: 348 (100.0, M + H), 370 (64.3, M + Na). Anal. ($C_{16}H_{21}N_5O_4$) C, H, N.

(*Z*)-9-{[2-(Hydroxymethyl)-2-(2-tetrahydropyranyloxymethyl)cyclopropylidene]methyl}adenine (21c). A solution of methanesulfonic acid (1.18 mL, 18 mmol) in DMF (5 mL) was added to a stirred mixture of compound **20c** (2.60 g, 8.99 mmol) and 3,4-dihydro-2*H*-pyran (9.1 mL, 90 mmol) in DMF (120 mL) at 0 °C. The experiment was performed as described for guanine derivative **21a** except that the reaction was quenched with triethylamine (6 mL). The crude product was chromatographed on a silica gel column using CH_2Cl_2/MeOH (30:1), and the resultant solid was recrystallized from ethyl acetate (20 mL) to give compound **21c** (2.3 g, 77%). Mp: 212−214 °C. UV λ_{max}: 278 nm (ϵ 8800), 256 (ϵ 12 000), 226 (ϵ 25 500). ^{1}H NMR δ: 8.79 and 8.76 (2s, 1H, H_8), 8.17 (s, 1H, H_2), 7.41 and 7.39 (2 overlapped s, 3H, $H_{1'}$ and NH_2), 5.11 (m, 1H, OH), 4.59 and 4.50 (2bs, 1H, CHO of THP), 3.97 (d, J = 9.6 Hz), 3.77−3.57, 3.52−3.47, and 3.36−3.29 (3 clusters of m, 6H, $H_{5'}$, $H_{5''}$, CH_2O of THP), 1.70−1.26 (cluster of m, 8H, CH_2 of THP, $H_{3'}$). ^{13}C NMR: 156.7 (C_6), 153.7 (C_2), 148.7 (C_4), 138.2 (C_8), 119.0, 119.1, 117.91, 117.85 (C_5, $C_{2'}$), 111.5 ($C_{1'}$), 98.5, 98.3 (CHO of THP), 69.2, 68.8 (CH_2O of THP), 62.6, 61.6, 61.4 ($C_{5'}$, $C_{5''}$), 30.6, 29.2, 29.1, 25.6, 25.5, 19.5, 19.4 (3 × CH_2 of THP, $C_{4'}$), 12.3, 12.2 ($C_{3'}$). ESI-MS: 248 (M + H − dihydropyran, 100.0), 332 (M + H, 37.2), 354 (M + Na, 61.3). Anal. ($C_{16}H_{21}N_5O_3$) C, H, N.

(*E*)-9-{[2-(Hydroxymethyl)-2-(2-tetrahydropyranyloxymethyl)cyclopropylidene]methyl}adenine (21d). The procedure for *Z*-isomer **21c** was followed with *E*-isomer **20d** (1.20 g, 4.15 mmol) to afford product **21d** (1.10 g, 80%). Mp: 212−216 °C. UV λ_{max}: 278 nm (ϵ 10 800), 261 (ϵ 14 200), 226 (ϵ 32 400). ^{1}H NMR δ: 8.49 (s, 1H, H_8), 8.17 (s, 1H, H_2), 7.49 (2 overlapped d, 1H, J = 1.6 Hz, $H_{1'}$), 7.38 (s, 2H, NH_2), 4.82 (m, 1H, OH), 4.60 (bs, 1H, CHO of THP), 3.75−3.64 (m, 2H) and 3.56−3.40 (cluster of m partly overlapped with H_2O, 4H, CH_2O of THP, $H_{5''}$, $H_{5'}$), 1.71−1.59 (m, 4H) and 1.44 (bd, 4H, CH_2 of THP, $H_{3'}$). ^{13}C NMR: 156.7 (C_6), 153.8 (C_2), 148.9 (C_4), 137.8 (C_8), 119.1, 118.9, 118.6 (C_5, $C_{2'}$), 111.2 ($C_{1'}$), 98.4, 98.3 (CHO of THP), 68.4 (CH_2O of THP), 63.1, 61.93, 61.87 ($C_{5'}$, $C_{5''}$), 30.9, 27.5, 27.4, 25.7, 19.82, 19.77 (3 x CH_2 of THP, $C_{4'}$), 15.0, 14.8 ($C_{3'}$). ESI-MS: 248 (M + H − dihydropyran, 48.8), 332 (M + H, 90.5), 354 (M + Na, 100.0). Anal. ($C_{16}H_{21}N_5O_3$) C, H, N.

(*Z*)-9-{[(2,2-Hydroxymethyl)cyclopropylidene]methyl}guanine (Methylphenylphosphoryl) P → N−L-Alaninate (7a). A suspension of the *Z*-isomer **21a** (410 mg, 1.18 mmol) in pyridine (80 mL) was sonicated for 10 min whereupon a solution of methyl chlorophenylphosphoryl P → N−L-alaninate (**18**) in THF (0.18 M, 40 mL, 7.2 mmol) and 1-methylimidazole (1.1 mL, 14.2 mmol) were added at room temperature. The resultant mixture was stirred at this temperature for 4 h. The solvents were evaporated in vacuo, and the residue was chromatographed on a silica gel column using CH_2Cl_2/MeOH (20:1) to give a crude product which was dissolved in acetic acid (80%, 20 mL). After the solution was stirred at room temperature for 4 days, the solvent was evaporated, and the residue was dissolved in MeOH (20 mL) and 0.1 M Na_2HPO_4 (pH 7.5, 10 mL) was added. The resultant suspension was stirred at room temperature for 22 h. The solvent was evaporated at room temperature, and the residue was dissolved in CH_2Cl_2/MeOH (8:1, 100 mL). The solids were filtered off, and they were washed with the same solvent (2 × 20 mL). The combined organic layer was concentrated, and the residue was chromatographed on a silica gel column using CH_2Cl_2/MeOH (20:1 to 15:1 to 10:1). Evaporation of solvents afforded a gum that solidified after trituration with ethyl ether (10 mL) to give *Z*-phosphoralaninate **7a** (200 mg, 33%). UV λ_{max}: 270 nm (ϵ 11 800), 230 (ϵ 28 400). ^{1}H NMR δ: 10.71 (s, 1H, NH), 8.24, 8.23, and 8.18 (4s, 1H), 7.31 and 7.14 (2m, 6H, Ph, $H_{1'}$), 6.57 (s, 2H, NH_2), 6.01 (m, 1H, NH of Ala), 5.25 (m, 1H, OH), 4.26, 4.05, 3.80, and 3.39 (4m, 5H, $H_{5'}$, $H_{5''}$, CH of Ala), 3.57, 3.533, and 3.529 (3s, 3H, OCH_3), 1.46 (m, 2H, $H_{3'}$), 1.17 (m, 3H, CH_3 of Ala). ^{13}C NMR: 174.4 (CO), 157.4 (C_6), 154.6 (C_2), 151.3 (C_{ipso}, PhO), 150.4 (C_4), 134.7 (C_8), 130.2 (C_{para}, PhO), 125.2 (C_{ortho}, PhO), 120.9 (C_{meta}, PhO), 116.9, 116.1 (C_5, $C_{2'}$), 112.0 ($C_{1'}$), 67.4 ($C_{5''}$), 62.6, 62.5 ($C_{5'}$), 52.6, 52.5 (OCH_3), 50.4, 50.3 (CH of Ala), 29.1, 29.0 ($C_{4'}$), 20.3 (CH_3 of Ala), 11.9 ($C_{3'}$). ^{31}P NMR: 4.73, 4.57, 4.52, 4.33 (1.8:1.2:1.9:1.0). ESI-MS: 246 (100.0, M − OP(O)(OPh)NHCHMeCO$_2$Me), 505 (47.8, M + H), 527 (88.4, M + Na). Anal. ($C_{21}H_{25}N_6O_7P$) C, H, N.

(*E*)-9-{[(2,2-Hydroxymethyl)cyclopropylidene]methyl}guanine (Methylphenylphosphoryl) P → N−L-Alaninate (8a). The procedure for the *Z*-isomer **7a** was followed with *E*-isomer **21b** (230 mg, 0.66 mmol) to furnish compound **8a** (142 mg, 42%). UV λ_{max}: 270 nm (ϵ 14 000) 229 (ϵ 34 100). ^{1}H NMR δ: 10.70 (s, 1H, NH), 8.05 (s, 1H, H_8), 7.32 and 7.17 (2m, 6H, PhO, $H_{1'}$), 6.52 (s, 2H, NH_2), 5.95 (m, 1H, NH of Ala), 4.91 (m, 1H, OH), 4.07, 3.84, and 3.45 (4m, 5H, $H_{5'}$, $H_{5''}$, CH of Ala), 3.58, 3.56, 3.55, and 3.52 (4s, 3H, OCH_3), 1.68 (m, 2H, $H_{3'}$), 1.21 (m, 3H, CH_3). ^{13}C NMR: 174.4 (CO), 157.4 (C_6), 154.6 (C_2), 151.4 (C_{ipso}, PhO), 150.6 (C_4), 134.3 (C_8), 130.3 (C_{para}, PhO), 125.2 (C_{ortho}, PhO), 120.9 (C_{meta}, PhO), 117.0 (C_5, $C_{2'}$), 111.8 ($C_{1'}$), 67.9 ($C_{5''}$), 62.6 ($C_{5'}$), 52.5 (OCH_3), 50.5, 50.4 (CH of Ala), 27.4, 27.3 ($C_{4'}$), 20.3 (CH_3 of Ala), 14.8 ($C_{3'}$). ^{31}P NMR: 4.69, 4.52, 4.27, 4.10 (1.4:1.2:1.4:1.0). ESI-MS: 505 (100.0, M + H), 527 (36.6, M + Na). Anal. ($C_{21}H_{25}N_6O_7P$) C, H, N.

(*Z*)-9-{[(2,2-Hydroxymethyl)cyclopropylidene]methyl}adenine (Methylphenylphosphoryl) P → N−L-Alaninate (7b). A solution of reagent **18** (4.2 g, 15 mmol) and 1-methylimidazole (2.4 mL, 30 mmol) in pyridine (20 mL) was added slowly with stirring to compound **21c** (0.99 g, 3 mmol) in pyridine (90 mL) at room temperature. Stirring was continued for 14 h. The solvent was evaporated, and the oily residue was chromatographed using CH_2Cl_2/MeOH (30:1). After being dried at 0.1 Torr and room temperature, the crude product was dissolved in 80% acetic acid (100 mL), and the solution was stirred at room temperature for 4 days. The solvent was evaporated in vacuo at room temperature, and the residue was purified by column chromatography in CH_2Cl_2/MeOH (30:1 to 20:1). Appropriate fractions were concentrated, and ether (50 mL) was added to give *Z*-phosphoralaninate **7b** (550 mg, 35%) as a solid. UV λ_{max}: 278 nm (ϵ 8500), 256 (ϵ 12 100), 228 (ϵ 24 300), 210 (ϵ 25 800). ^{1}H NMR δ: 8.63 and 8.61 (2s, 1H, H_8), 8.17 and 8.16 (2s, 1H, H_2), 7.44 (m, 1H, $H_{1'}$), 7.35 (s), 7.30 (m), and 7.12 (m, 7H, PhO, NH_2), 5.98 (m, 1H, NH of Ala), 5.31 (m, 1H, OH), 4.36−4.29 (m), 4.08, 3.99 (2dd) and 3.47−3.38 (cluster of m partly overlapped with H_2O, 5H, $H_{5'}$, $H_{5''}$, CH of Ala), 3.56, 3.52 (2bs, 3H, CH_3O), 1.54−1.47 (m, 2H, $H_{3'}$), 1.18, 1.15 (2d, 3H, CH_3 of Ala). ^{13}C NMR: 174.4 (CO), 156.7 (C_6), 153.7 (C_2), 151.3 (C_{ipso}, PhO), 148.7 (C_4), 138.2 (C_8), 130.2 (C_{para}, PhO), 125.2 (C_{ortho}, PhO), 120.90, 120.85, 120.8 (C_{meta}, PhO), 119.1 (C_5), 116.62, 116.58 ($C_{2'}$), 112.1 ($C_{1'}$), 67.6 ($C_{5''}$), 62.9, 62.8 ($C_{5'}$), 52.6, 52.5 (CH_3O), 50.4, 50.3, 50.2 (CH of Ala), 29.3, 29.2 ($C_{4'}$), 20.4, 20.3, 20.2 (CH_3 of Ala), 11.9 ($C_{3'}$). ^{31}P NMR: 4.74, 4.58, 4.49, 4.31 (3.7:1.3:3.4:1.0). ESI-MS: 230 (M − OP(O)-(OPh)NHCHMeCO$_2$Me), 489 (M + H, 39.8), 511 (42.1, M + Na). Anal. ($C_{21}H_{25}N_6O_6P$) C, H, N.

(*E*)-9-{[(2,2-Hydroxymethyl)cyclopropylidene]methyl}adenine (Methylphenylphosphoryl) P → N−L-Alaninate (8b). A protocol for the preparation of *Z*-isomer **7b** was followed with compound **21d** (900 mg, 2.72 mmol). The crude product was first chromatographed using CH_2Cl_2/MeOH (30:1 to 20:1 to 10:1) as an eluent and, after treatment with 80% acetic acid, in ethyl acetate/MeOH (10:1) to give *E*-phosphoroalaninate **8b** (380 mg, 29%) as a solid. UV λ_{max}: 278 nm (ϵ 9300), 262 (ϵ 12 900), 227 (ϵ 28 900), 210 (ϵ 27 900). ^{1}H NMR δ: 8.48 (s, 1H, H_8), 8.18 (s, 1H, H_2), 7.52 (bs, 1H, $H_{1'}$), 7.35 and 7.18 (2m, 7H, PhO, NH_2), 5.99 (m, 1H, NH of Ala), 4.94 (m, 1H, OH), 4.13 and 4.04 (2m), 3.85 (m), 3.59−3.48 (m overlapped with 4s, 8H, $H_{5'}$, $H_{5''}$, CH of Ala, CH_3O), 1.71 (m, 2H, $H_{3'}$), 1.21 (m, 3H, CH_3 of Ala). ^{13}C NMR: 174.4 (CO), 156.7 (C_6), 153.8 (C_2), 151.5 (C_{ipso}, PhO), 149.0 (C_4), 137.8 (C_8), 130.2

(C_{para}, PhO), 125.2 (C_{ortho}, PhO), 121.0, 120.9 (C_{meta}, PhO), 119.1 (C_5), 117.4 ($C_{2'}$), 111.9 ($C_{1'}$), 67.9 ($C_{5''}$), 62.6 ($C_{5'}$), 52.5 (CH_3O), 50.5, 50.4 (CH of Ala), 27.5, 27.4 ($C_{4'}$), 20.4, 20.3 (CH_3 of Ala), 15.0 ($C_{3'}$). ^{31}P NMR: 4.66, 4.61, 4.26, 4.22 (1.3:1.0:1.6:1.1). ESI-MS: 230 (M − OP(O)(OPh)NHCHMeCO$_2$Me, 57.7), 489 (M + H, 34.9), 511 (M + Na, 100.0). Anal. ($C_{21}H_{25}N_6O_6P$) C, H, N.

(*Z*)-9-{[2-(Acetoxymethyl)-2-(phosphinyloxymethyl)-cyclopropylidene]methyl}guanine (22a). A suspension of compound **20a** (1.0 g, 3.27 mmol) in pyridine (90 mL) was sonicated for 10 min. Diphenyl phosphite (85−90%, 4.5 mL, 20 mmol) was then added at room temperature. The resulting mixture was stirred at 60°C for 2 h. After the mixture was cooled, triethylamine (15 mL) and water (10 mL) were added, and the stirring was continued for 30 min. The volatile components were then evaporated in vacuo and the crude product was chromatographed on a silica gel column CH_2Cl_2/MeOH = 3:1 to 1.5:1) to furnish 1.13 g of phosphite **22a** (1.13 g, 80%) containing 0.63 mol of triethylamine according to ^{1}H NMR spectra.[28] UV (EtOH) λ_{max}: 272 nm (ϵ 9400), 231 nm (ϵ 23 200). ^{1}H NMR (D_2O) δ: 8.01 (s, 1H, H_8), 6.94 (s, 1H, $H_{1'}$), 6.68 (d, 1H, $^1J_{H,P}$ = 635.7 Hz, P−H), 4.40 and 3.93 (AB, 2H, J = 12.0 Hz, CH_2OAc), 3.97 (d, 2H, $^3J_{P-H}$ = 7.2 Hz, $CH_2OPO_2H_2$), 3.12 (q, J = 7.3 Hz, CH_2 of NEt$_3$), 1.93 (s, 3H, Ac), 1.62 and 1.56 (AB, 2H, J_{AB} = 8.6 Hz, $H_{3'}$), 1.56 (d, J = 7.8 Hz, CH_3 of NEt$_3$). ^{13}C NMR: 173.8 (CO), 158.4 (C_6), 153.8 (C_2), 150.0 (C_4), 136.6 (C_8), 118.6 (C_5), 115.5 ($C_{2'}$), 111.9 ($C_{1'}$), 66.6, 65.7 ($C_{5'}$, $C_{5''}$), 46.8 (CH_2 of NEt$_3$), 26.0 (d, $^3J_{4',P}$ = 8.9 Hz, $C_{4'}$), 20.4 (CH_3), 12.7 ($H_{3'}$), 8.5 (CH_3 of NEt$_3$). ^{31}P NMR: 6.96. ESI-MS: 370 (100.0, M + H), 392 (28.0, M + Na).

(*Z*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}guanine Phosphate (11a). A suspension of partial triethylammonium salt **22a** (193 mg, 0.445 mmol) and imidazole[29] (151 mg, 2.22 mmol) in pyridine (30 mL) was sonicated for 10 min. Trimethylsilyl chloride (0.56 mL, 4.40 mmol) was added, and stirring at room temperature was continued for 10 min. After addition of iodine (227 mg, 0.89 mmol) in pyridine (2 mL), the stirring was continued for 10 min. Water (10 mL) was then added, and after 40 min, the volatile components were evaporated in vacuo and the residue was dissolved in water (150 mL). The aqueous solution was extracted with CH_2Cl_2 (5 × 40 mL), NH_4OH (30%, 20 mL) was added to the aqueous layer, and the mixture was kept for 24 h at room temperature. The volatile components were evaporated, the residue was dissolved in water (5 mL), the pH was adjusted to 9−9.5 with NH_4OH, and the solution was loaded onto the top of a Dowex 50 column (WX2, 200 mesh, $H^{(+)}$ form, 40 mL). Elution with water followed by concentration of the appropriate fractions gave phosphate **11a** (131 mg, 86%). Mp: 260 °C (dec). UV (H_2O) λ_{max}: 269 nm (ϵ 10 600), 229 (ϵ 26 700). ^{1}H NMR (D_2O, sodium salt) δ: 8.40 (s, 1H, H_8), 7.06 (s, 1H, $H_{1'}$), 4.17, 3.79 and 4.16, 3.81 (2AB, J = 10.6 Hz, 2H, $H_{5'}$), 3.87 and 3.64 (AB, J_{AB} = 12.2 Hz, 2H, $H_{5''}$), 1.53 and 1.50 (AB, 2H, J_{AB} = 8.7 Hz, $H_{3'}$). ^{13}C NMR: 158.5 (C_6), 153.9 (C_2), 149.4 (C_4), 136.7 (C_8), 118.7 (C_5), 115.4 ($C_{2'}$), 110.8 ($C_{1'}$), 66.2 ($C_{5''}$), 63.2 ($C_{5'}$), 29.0 ($^3J_{4',P}$ = 8.2 Hz, $C_{4'}$), 11.8 ($C_{3'}$). ^{31}P NMR: 4.75. ESI-MS: 344 (100.0, M + H), 366 (32.1, M + Na). Anal. ($C_{11}H_{14}N_5O_6P$) C, H, N.

(*Z*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}guanine Cyclic Phosphate (10a). A mixture of phosphate **11a** (140 mg, 0.40 mmol), DCC (560 mg, 2.7 mmol), and *N,N'*-dicyclohexyl-4-morpholinecarboxamidine (140 mg, 0.47 mmol) in pyridine (100 mL) was refluxed under N_2 with stirring for 10 h. Pyridine was evaporated, and the crude product was worked up as described for phosphate **11a** to give cyclic phosphate **10a** (123 mg, 93%). For analysis, it was recrystallized from H_2O. Mp: 250 °C (dec). UV (H_2O) λ_{max}: 266 nm (ϵ 13 500), 228 (ϵ 29 000). ^{1}H NMR (D_2O, sodium salt) δ: 7.88 (s, 1H, H_8), 6.90 (s, 1H, $H_{1'}$), 4.22 and 4.13 (2 apparent t, J = 11.0 and 12.2 Hz, 4H, $H_{5'}$, $H_{5''}$), 1.51 (s, 2H, $H_{3'}$). ^{13}C NMR: 165.0 (C_6), 158.9 (C_2), 150.2 (C_4), 135.9 (C_8), 116.8, 116.5 (C_5, $C_{2'}$), 111.7 ($C_{1'}$), 71.94 (d, $^2J_{5',5'',P}$ = 5.2 Hz, $C_{5'}$, $C_{5''}$), 24.0 (d, $^3J_{4',P}$ = 5.9 Hz, $C_{4'}$), 11.5 ($C_{3'}$). ^{31}P NMR: − 2.23. ESI-MS: 326 (78.1, M + H), 348 (100.0, M + Na). Anal. ($C_{11}H_{12}N_5O_5P$·0.4H_2O) C, H, N.

(*E*)-9-{[2-(Acetoxymethyl)-2-(phosphinyloxymethyl)-cyclopropylidene]methyl}guanine (22b). The procedure described for the *Z*-isomer **22a** was followed with *E*-isomer **20b** (1.50 g, 4.91 mmol) to give phosphite **22b** (1.59 g, 73% containing 0.73 mol of triethylamine[28]). UV (EtOH) λ_{max}: 271 nm (ϵ 9200), 231 (ϵ 25 600). ^{1}H NMR (D_2O) δ: 7.94 (s, 1H, H_8), 7.19 (d, 1H, J = 2.4 Hz, $H_{1'}$), 6.74 (d, 1H, $^1J_{H,P}$ = 636.5 Hz, P−H), 4.22 and 4.18 (AB, 2H, J = 12.0 Hz, CH_2OAc), 3.94 (m, 2H, $CH_2OPO_2H_2$), 3.12 (q, J = 7.3 Hz, CH_2 of NEt$_3$), 2.10 (s, 3H, Ac), 1.76 (poorly resolved m, 2H, $H_{3'}$), 1.19 (t, J = 7.4 Hz, CH_3 of NEt$_3$). ^{13}C NMR: 174.5 (CO), 158.2 (C_6), 153.9 (C_2), 149.8 (C_4), 135.9 (C_8), 117.3 (C_5), 115.2 ($C_{2'}$), 111.9 ($C_{1'}$), 66.3, 65.7 ($C_{5'}$, $C_{5''}$), 46.8 (CH_2 of NEt$_3$), 24.15 ($^3J_{4',P}$ = 8.1 Hz, $C_{4'}$), 20.6 (Ac), 15.1 ($C_{3'}$), 8.4 (CH_3 of NEt$_3$). ^{31}P NMR: 7.12. Negative ESI-MS: 368 (100.0, M − H).

(*E*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}guanine Phosphate (11b). The procedure described for the *Z*-isomer **11a** was followed with the *E*-isomer **22b** (235 mg, 0.53 mmol) to afford phosphate **11b** (137 mg, 75%). Mp: 220 °C (dec). UV (H_2O) λ_{max}: 267 nm (ϵ 12 700), 228 (ϵ 34 000). ^{1}H NMR (D_2O, sodium salt) δ: 7.97 (s, 1H, H_8), 7.29 (poorly resolved d, 1H, $H_{1'}$), 4.00, 3.75, 359 (poorly resolved AB systems, 4H, $H_{5'}$, $H_{5''}$), 1.62 and 1.54 (AB, J_{AB} = 8.8 Hz, 2H, $H_{3'}$). ^{13}C NMR: 158.2 (C_6), 153.7 (C_2), 149.2 (C_4), 135.4 (C_8), 118.1 (C_5), 115.0 ($C_{2'}$), 110.5 ($C_{1'}$), 66.0 (poorly resolved d, $C_{5'}$), 63.4 ($C_{5''}$), 26.8 ($^3J_{4',P}$ = 9.7 Hz, $C_{4'}$), 14.3 ($C_{3'}$). ^{31}P NMR: 5.25. ESI-MS: 344 (100.0, M + H). Anal. ($C_{11}H_{14}N_5O_6P$) C, H, N.

(*E*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}guanine Cyclic Phosphate (10b). The procedure described for the *Z*-isomer **10a** was followed with *E*-isomer **11b** (240 mg, 0.70 mmol) to give cyclic phosphate **10b** (202 mg, 89%). For analysis, it was recrystallized from H_2O. Mp: 265 °C (dec). UV (H_2O) λ_{max}: 268 nm (ϵ 12 400), 228 (ϵ 33 900). ^{1}H NMR (D_2O, sodium salt, 400 MHz) δ: 8.03 (s, 1H, H_8), 7.40 (s, 1H, $H_{1'}$), 4.31 and 4.08 (2 apparent t, J = 10.2 and 12.6 Hz, 4H, $H_{5'}$, $H_{5''}$), 1.84 (s, 2H, $H_{3'}$). ^{13}C NMR: (D_2O, sodium salt, 100 MHz) 168.1 (C_6), 161.3 (C_2), 150.0 (C_4), 134.2 (C_8), 117.2 (C_5), 113.8 ($C_{2'}$), 112.0 ($C_{1'}$), 71.8 (d, $^2J_{5',5'',P}$ = 4.8 Hz, $C_{5''}$, $C_{5''}$), 22.0 (d, $^3J_{4',P}$ = 5.9 Hz, $C_{4'}$), 15.2 ($C_{3'}$). ^{31}P NMR (D_2O, sodium salt): −2.16. Negative ESI-MS: 325 (100.0, M − H). Anal. ($C_{11}H_{12}N_5O_5P$·0.5H_2O) C, H, N.

Hydrolysis of Pronucleotides with Porcine Liver Esterase (PLE). 1. TLC Analysis. Pronucleotide **7a**, **7b**, **8a**, or **8b** (0.75 mg, 1.5 μmol) was stirred with PLE (200 units) in 0.02 M Na_2HPO_4 (pH 7.4, 1.0 mL) at room temperature. The reaction was periodically checked by TLC in CH_2Cl_2/MeOH = 5:1 (**7a**, **8a**) or 7:1 (**7b**, **8b**). Quantitative hydrolysis was observed after 24 h (**7a**, **8a**), 40 h (**7b**), and 3 h (**8b**).

2. Analysis by ^{31}P NMR Spectra.[30] Pronucleotide **7a** (7.5 mg, 15 μmol) was incubated with PLE (2,000 units) in 0.5 M TRIZMA·HCl in water (pH 7.6, 0.8 mL) containing acetone (0.1 mL) at room temperature. After 72 h, three signals in the ^{31}P NMR spectrum were observed: 3.53 (corresponding to a peak observed in the enzyme solution alone), 7.92, and 8.04 (diastereoisomers of phosphoramidate **23**).

Biological Assays. The antiviral assays were performed as described previously.[31] The HCMV assays were run in HFF culture with Towne and AD169 strains of virus in a plaque reduction or cytopathic effect (CPE) inhibition assay. The MCMV was assayed in MEF cells by plaque reduction. The HSV-1 was run in BSC-1 cells by ELISA. In addition, HSV-1 and HSV-2 assays were performed in HFF (CPE and plaque reduction) and Vero cells (plaque reduction assay). The VZV was assayed in HFF (CPE or plaque reduction) and hepatitis B virus (HBV) in 2.2.15 cells by DNA hybridization. The EBV assays were performed in Daudi cells by viral capsid antigen (VCA) ELISA and in H-1 cells by DNA hybridization assay. The cytotoxicity assays were performed in HFF, KB, and CEM cells. For further details, see Tables 1−3. All assays are results of single experiments unless specified otherwise.

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Supporting Information Available: Elemental analyses. This information is available online free of charge via the Internet at http://pubs.acs.org.

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