Design, Synthesis, and Biological Evaluation of Novel Nucleoside and Nucleotide Analogues as Agents against DNA Viruses and/or Retroviruses

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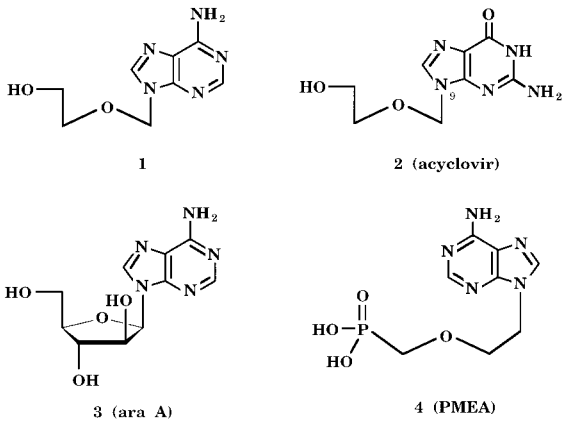
A novel strategy was developed for the synthesis of N^7-purine acyclic nucleosides **9** and **14**. The key step involved the reaction between [2-(*p*-methoxyphenyloxy)ethoxy]methyl chloride and N^9-tritylated nucleobases **6** or **11** followed by concomitant self-detritylation. N^7-Guanine acyclic nucleoside **9** exhibited antiviral activity, but was phosphorylated by both HSV and Vero cell thymidine kinases. Thus, it showed more potent cellular toxicity than acyclovir (**2**). N^7-Adenine acyclic nucleoside **14** was found to be an excellent antiviral agent as well as a good inhibitor of calf mucosal adenosine deaminase. This inhibitory property allows for a greater expression of antiviral activity of antiviral agents, such as N^9-adenine acyclic nucleoside **1** and ara-A (**3**). Compound **14** was phosphorylated neither by herpes simplex virus (HSV) thymidine kinase nor by Vero cell thymidine kinase, yet it enhanced the rate constant for the monophosphorylation of acyclovir (**2**) by HSV thymidine kinase. Consequently, the combination of acyclovir (**2**) and **14** exhibited greater antiviral activity than acyclovir alone. 7-[2-(Phosphonomethoxy)ethyl]adenine (**20**) was also synthesized. The key step involved the reaction of 9-(2-cyanoethyl)adenine (**15**) with methyl iodoacetate in the presence of lithium 2,2,6,6-tetramethylpiperidine in THF. Unlike 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, **4**), the N^7-isomer **20** was not phosphorylated effectively by 5-phosphoribosyl 1-pyrophosphate synthetase (PRPP synthetase). Thus, it did not exhibit pronounced antiviral activity. Dinucleotide 5′-monophosphate **24** and its butenolide ester **25** were also synthesized. Compound **24** showed substrate activity toward PRPP synthetase and exhibited notable activity against DNA viruses. The antiviral activity of the ester derivative **25** was found to be higher than that of the parent molecule **24**. Dinucleotide 5′-monophosphate **24** is suseptible to degradation by snake venom and spleen phosphodiesterases. However, its respective butenolide ester derivative **25** was completely resistant to snake venom and spleen enzymes. Butenolide ester derivatives **28** and **29** were also synthesized and exhibited notable anti-DNA virus and anti-retrovirus activity in vitro. Compounds **2, 4, 9, 14, 20, 24, 25,** and **28** were also evaluated for their inhibitory effect on HSV-1-induced mortality in NMRI mice. N^7-adenine acyclic nucleoside **14** [LD_{50} (intraperitoneal, ip) 950 mg/kg], nucleotide-containing butenolide **25** [LD_{50} (ip) 675 mg/kg], and butenolide **28** [LD_{50} (ip) 710 mg/kg] were found to be potent anti-HSV-1 agents in vivo. In addition, butenolide **28** efficiently decreased tumor formation induced by Moloney murine sarcoma virus (MSV) in NMRI mice while significantly increasing the survival time of MSV-infected mice.

Introduction

Antiviral agents (Chart 1) including 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, **2**),[1] 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir),[2] 9-[(2,3-dihydroxy-1-propoxy)methyl]guanine,[3] (*S*)-9-[3-hydroxy-2-(phosphonylmethoxy)propyl]adenine (HPMPA), 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, **4**), and 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG, **27**) are N^9-isomers of acyclic nucleosides.[4] The N^7-isomers of purine nucleosides constitute another class of antiviral agents.[5]

Chart 1



Under kinetic control,[6] alkylation of purines gives both the N^7-isomers and the N^9-isomers, with the latter

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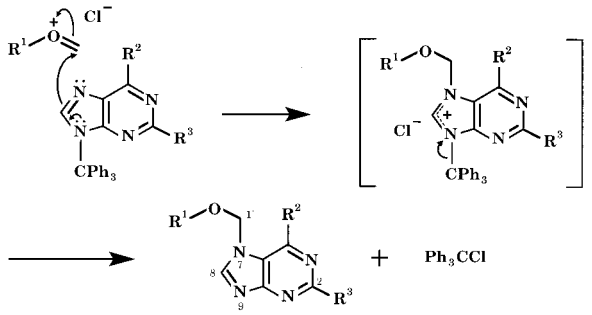
Scheme 1



being the predominant component.[7] Regioselective synthesis of N^2-acetyl-7-[(2-acetoxyethoxy)methyl]guanine (65% yield) through alkylation of N^2,N^9-diacetylguanine with 2-oxa-1,4-butanediol diacetate in the presence of $TiCl_4$ in AcOH has been reported.[5b] Unfortunately, the synthesis of N^6-acetyl-7-[(2-acetoxyethoxy)methyl]adenine via N,$^6N^9$-diacetyladenine by the above strategy failed. Herein we report a highly regioselective route toward purine nucleosides with acyclic substituents at the N-7 position. Our strategy involves the alkylation of N^9-tritylated purines, which is followed by concomitant self-detritylation to yield the desired N^7-alkylated purine nucleosides (Scheme 1). Compounds **9** and **14** synthesized from this strategy were found to possess significant activity against herpes simplex viruses (HSV).

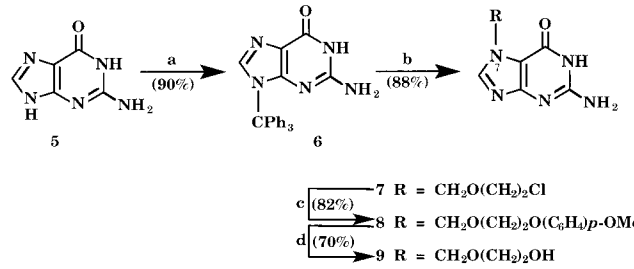
(Phosphonomethoxy)alkylpurines (i.e., PMEA, **4**) effectively inhibit a wide array of DNA viruses (herpes-, adeno-, irido-, and poxviruses) and retroviruses [Moloney murine sarcoma virus (MSV), murine leukemia virus (MuLV), and human immunodeficiency virus (HIV)].[4] We were interested in the preparation of the N^7-isomer of PMEA, compound **20**, using the above strategy. Unfortunately, the desired precursor **16** was not produced effectively. Thus, an alternative, and more efficient, method was devised as shown in Scheme 4.

Unlike PMEA (**4**), N^7-acyclic adenine phosphonate **20** was found to not be a good substrate for PRPP synthetase and did not exhibit potent antiviral activity. On the other hand, newly synthesized dinucleotide 5′-monophosphate **24**, which is an aggregate of phosphonate **20** and adenosine monophosphate (AMP, **21**), was found to be an alternate substrate for PRPP synthetase and exhibited notable antiviral activity.

One major problem often encountered in the use of nucleotide analogues as potential therapeutics for viral infections is one of low lipophilicity.[8a] We have therefore synthesized novel butenolide ester derivatives **25**, **28**, and **29** as membrane-soluble prodrugs.

Generally, phosphoesters are very stable against nonenzymatic or even enzymatic hydrolysis.[8a] The rationale behind the design of our new prodrugs is based on the known fast hydrolysis of donor-substituted benzyl phosphoesters.[8a] Similarly, the oxygen of the C-2 methoxy group of the butenolide moiety can act as a donor[8b] group to facilitate the hydrolysis of prodrugs to their respective active species (see Scheme 6). This induces the spontaneous cleavage of esters **25**, **28**, and **29** inside the infected cells to yield the corresponding bioactive nucleotides **24**, **4**, and **27** as well as (*Z*)-4-(2-hydroxyethylidenyl)-2,3-dimethoxy-$\Delta^{\alpha,\beta}$-butenolide [LD_{50} (ip) > 1.0 g/kg]. Consequently, enzymatic activation for the hydrolysis of prodrugs is not required due to the intrinsic activation provided by the anchimeric assistance of C_2-OMe. As such, compounds **25**, **28**, and **29** exhibited superior lipophilicity and notable antiviral activity.

Scheme 2[a]



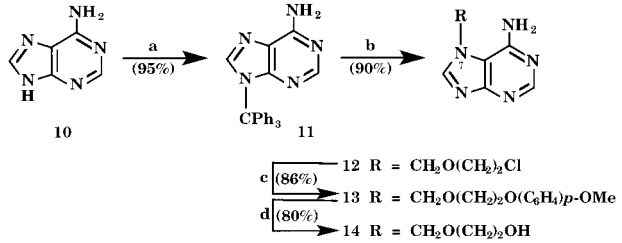
[a] Reagents and conditions: (a) (1) $Me_3SiNHSiMe_3$, $(NH_4)_2SO_4$ (cat.), Δ, 24 h; (2) Ph_3CCl, MeCN, 25 °C, 7.0 h. (b) $Cl(CH_2)_2OCH_2Cl$, DMF, 25 °C, 8.0 h. (c) *p*-$MeO(C_6H_4)O(CH_2)_2OCH_2Cl$, DMF, 25 °C, 8.0 h. (d) CAN, $MeCN/H_2O$ (3:1), 0−25 °C, 1.0 h.

Chemistry

Synthesis of N^7-Guanine Acyclic Nucleosides 7 and 9 (Scheme 2). N^9-tritylated guanine **6** was synthesized in two steps. Silylation of guanine (**5**) with hexamethyldisilazane (HMDS) in the presence of a catalytic amount of $(NH_4)_2SO_4$ at refluxing temperature followed by condensation of the resultant silylated guanine with trityl chloride in MeCN at 25 °C afforded the desired N^9-tritylated guanine **6** in 90% yield. Treatment of **6** with (chloroethoxy)methyl chloride[9] in DMF at room temperature gave the corresponding N^7-alkylated guanine **7** in 88% yield. Likewise, treatment of **6** with [2-(*p*-methoxyphenyloxy)ethoxy]methyl chloride[10] led to the N^7-isomer **8** in 82% yield. Removal of the *p*-methoxyphenyl moiety was then achieved by treatment with ceric ammonium nitrate (CAN)[11] in a mixture of MeCN and H_2O (3:1) at 0−25 °C to afford compound **9** in 70% yield. Incidentally, acyclovir (**2**) is the N^9-isomer of the N^7-alkylated guanine **9**. These two regioisomers exhibited characteristic differences in their ^1H and ^{13}C NMR spectra[7,12] The signals of the $H_2C(1')$ (5.81 ppm) and HC(8) (8.67 ppm) for the N^7-isomer **9** were found to be shifted downfield relative to the corresponding signals of the N^9-isomer **2**, in which the $H_2C(1')$ and HC(8) resonated at 5.35 and 7.81 ppm, respectively. On the other hand, the NH_2 signal was observed to be shifted upfield for the N^7-isomer, 5.96 ppm for compound **9**, relative to the corresponding signal for compound **2**, which was observed at 6.52 ppm. The ^{13}C NMR signals for C(1′) (75.25 ppm) and C(8) (143.92 ppm) of the N^7-isomer **9** were found to be shifted downfield relative to the corresponding signals of acyclovir (**2**), which were observed, respectively, at 71.64 and 137.89 ppm. In contrast, the signal of C(5) of compound **9** resonated at 107.16 ppm, which was upfield from that of the N^9-isomer **2** at 116.52 ppm. The UV λ_{max} of the N^7-isomer **9** appeared at 289 nm, whereas the corresponding λ_{max} of the N^9-isomer **2** appeared at 253 and 273 (sh) nm.

Synthesis of N^7-Adenine Acyclic Nucleosides 12 and 14 (Scheme 3). By the same synthetic strategy shown in Scheme 2, novel N^7-alkylated adenines **12** and **14** were obtained from adenine (**10**) via the N^9-tritylated adenine **11**. Reaction of **11** with (2-chloroethoxy)methyl

Scheme 3[a]



[a] Reagents and conditions: (a) Ph_3CCl, pyridine/DMF (3:1), 25 °C, 7.0 h. (b) $Cl(CH_2)_2OCH_2Cl$, CH_2Cl_2, 25 °C, 8.0 h. (c) *p*-$MeO(C_6H_4)$-$O(CH_2)_2OCH_2Cl$, CH_2Cl_2, 25 °C, 10 h. (d) CAN, MeCN/H_2O (3:1), 0−25 °C, 1.0 h.

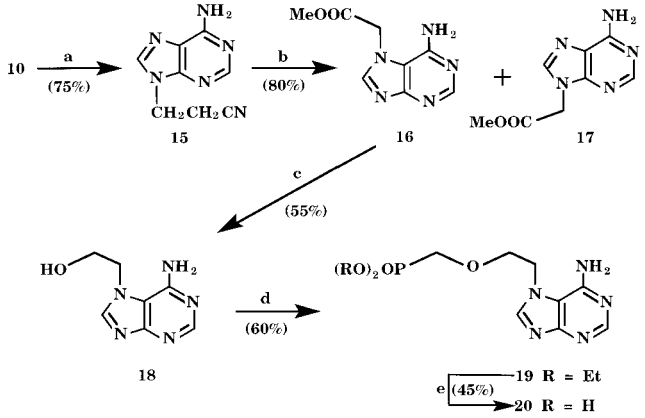
chloride in CH_2Cl_2 at 25 °C gave N^7-adenine derivative **12** in 90% yield. Likewise, reaction of **11** with [2-(*p*-methoxyphenyloxy)ethoxy]methyl chloride in CH_2Cl_2 at 25 °C gave N^7-isomer **13** in 86% yield. Treatment of **13** with CAN then produced the deprotected compound **14** in 80% yield.

The structures of the N^7-substituted adenines were assigned by ^1H and ^{13}C NMR spectra. In methanol-d_4, pronounced downfield shifts were observed for the signals resulting from the $H_2C(1')$ (5.79 ppm) and HC-(8) (8.60 ppm) of the N^7-isomer **14** when compared with those of the N^9-isomer **1**, respectively observed at 5.67 and 8.27 ppm. In DMSO-d_6, the NH_2 signals of the N^7-alkylated product **14** was shifted downfield to ∼9.2 and 10.0 ppm, whereas the corresponding signals of the N^9-isomer **1** appeared as a broad peak at ∼7.3 ppm. The ^{13}C NMR signals of the C(1′) (82.01 ppm) and C(8) (147.68 ppm) for the N^7-isomer **14** were found to be shifted downfield relative to those of the N^9-isomer **1**, which were observed respectively at 74.31 and 143.14 ppm. The signals of the C(2) (144.29 ppm) and C(6) (152.12 ppm) for isomer **14**, however, were found to be shifted upfield relative to those of the N^9-isomer **1**, observed respectively at 153.74 and 157.19 ppm. Furthermore, the H−C(2) coupling constants for the N^7- and N^9-isomers were respectively 220 and 204 Hz, whereas the corresponding H−C(8) coupling constants were respectively 224 and 217 Hz. The attachment of the side chain at the N-7 position of adenine was confirmed by heteronuclear multiple quantum coherence (HMQC) spectroscopy, in which the $H_2C(1')$ and C(6) exhibited a strong interaction in **14** whereas the corresponding N^9-isomer **1** showed long-range coupling between $H_2C(1')$ and C(4).

Synthesis of N^7-Adenine Acyclic Nucleoside Phosphonate 20 (Scheme 4). Alkylation of adenine (**10**) with 3-bromopropionitrile in the presence of NaH in DMF gave N^9-(cyanoethyl)adenine **15** in 75% yield. Reaction of **15** with methyl iodoacetate and lithium 2,2,6,6-tetramethylpiperidine (LiTMP) in THF afforded a mixture of N^7-alkylated product **16** (60% yield) and N^9-isomer **17** (20% yield). Reduction of the ester group in **16** with $NaBH_4$ in wet THF[13] gave N^7-(hydroxyethyl)-adenine **18** in 55% yield. Conversion of **18** to phosphonate **19** (60% yield) was accomplished by use of diethyl (*p*-toluenesulfonyloxymethane)phosphonate and sodium *tert*-butoxide in DMF.[14] Treatment of compound **19** with Me_3SiBr[15] then afforded phosphonic acid **20** in 45% yield.

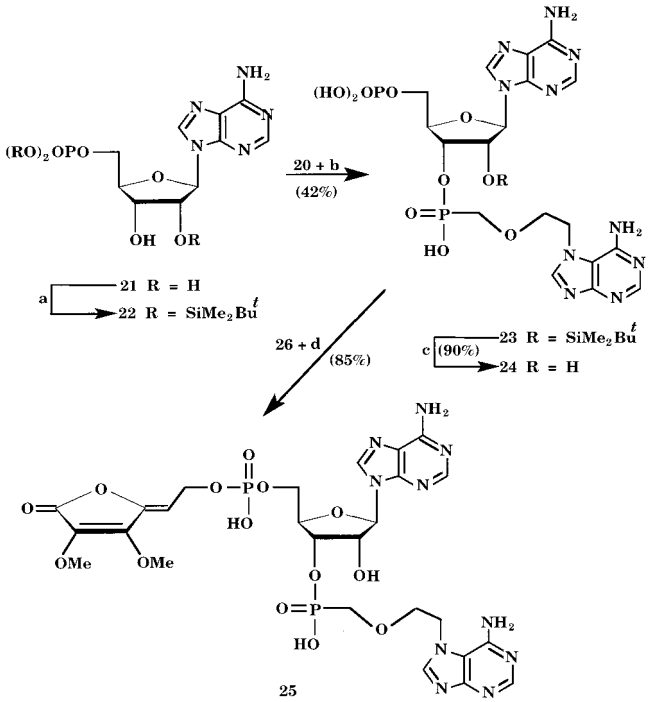
Synthesis of Dinucleotide 5′-Monophosphate 24, Its Butenolide Ester 25, PMEA-Containing Butenolide 28, and PMEG-Containing Butenolide 29 (Schemes 5 and 6). The nucleotide analogue **24** was readily obtained in three steps from adenosine 5′-monophosphate (**21**), starting with silylation of phosphate **21** in CH_3CN with *t*-$BuMe_2SiCl$ in the presence of $AgNO_3$ and pyridine.[16] The resulting trisilylated compound **22** was condensed with phosphonic acid **20** by use of trichloromethanesulfonyl chloride in collidine and THF to afford dinucleotide 5′-monophosphate **23** in 42% overall yield.[17] Desilylation of **23** with *n*-Bu_4NF in THF at 25 °C gave dinucleotide **24** in 90% yield, which was then reacted with (*Z*)-4-(2-chloroethylidenyl)-2,3-dimethoxy-$\Delta^{\alpha,\beta}$-butenolide (**26**)[8b] in the presence of $NaHCO_3$ in DMF to afford the target molecule **25** in 85% yield. Similarly, treatment of compound **26** with either

Scheme 4[a]



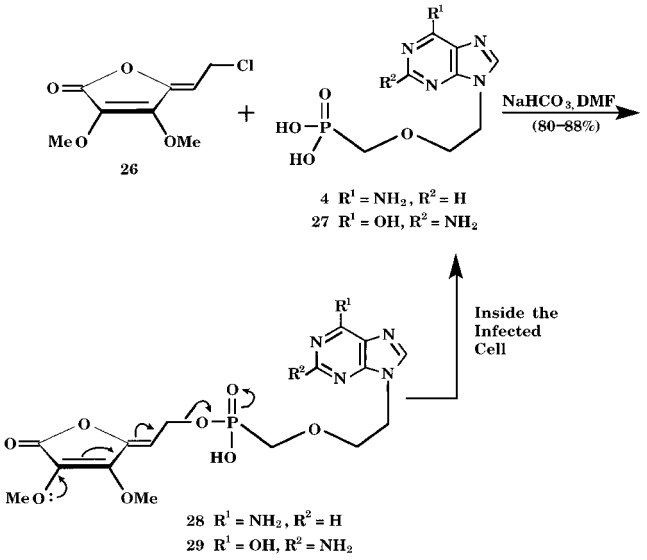
[a] Reagents and conditions: (a) $BrCH_2CH_2CN$, NaH, DMF, 65 °C, 17.0 h. (b) ICH_2COOME, LiTMP, THF, −20 to −25 °C, 16.0 h. (c) $NaBH_4$, wet THF, 25 °C, 4.0 h. (d) Diethyl (*p*-toluenesulfonyloxymethane)phosphonate, *tert*-butoxide, DMF, 25 °C, 4.0 h. (e) Me_3SiBr, CH_2Cl_2/DMF, 25 °C, 7.0 h.

Scheme 5[a]



[a] Reagents and conditions: (a) Bu^tMe_2SiCl, $AgNO_3$, pyridine/CH_3CN, 25 °C, 7.0 h. (b) CCl_3SO_2Cl, collidine, THF, 25 °C, 10.0 h. (c) *n*-Bu_4NF, THF, 25 °C, 30 min. (d) $NaHCO_3$, DMF, 25 °C, 1.0 h.

Scheme 6



26

4 $R^1 = NH_2$, $R^2 = H$
27 $R^1 = OH$, $R^2 = NH_2$

28 $R^1 = NH_2$, $R^2 = H$
29 $R^1 = OH$, $R^2 = NH_2$

PMEA (**4**) or PMEG (**27**) in the presence of $NaHCO_3$ in DMF respectively gave an 80% or 88% yield of the desired compound **28** or **29**.

Lipophilicity and Solubility Tests. Lipophilicity and water solubility were determined by the distribution between 1-octanol and water according to the methods reported by Baker et al.[18] Adenine acyclic nucleosides **1** and **14** were observed to exhibit higher lipophilicity as well as water solubility than those exhibited by N^7-guanine acyclic nucleoside **9**, acyclovir (**2**), and ara-A (**3**) (see Supporting Information, Table 7). Phosphonate **20** and butenolide ester derivatives **25**, **28**, and **29** also exhibited higher lipophilicity and water solubility as compared to PMEA (**4**) or PMEG (**27**). On the other hand, even though the solubility of nucleotide analogue **24** in water was found to be higher than that of PMEA (**4**), its lipophilicity was lower than that of **4**. Furthermore, butenolide ester derivative **25** showed higher lipophilicity as well as water solubility with respect to the parent nucleotide 5′-monophosphate **24**.

Biological Results and Discussion

Kinetic Studies of Competitive Inhibition of Adenosine Deaminase by Acyclic Nucleosides and Nucleotides. The rates of deamination of N^9-alkylated adenine **1**, 9-(β-D-arabinofuranosyl)adenine (ara-A, **3**), PMEA (**4**), N^7-alkylated adenine **14**, phosphonate **20**, dinucleotide 5′-monophosphate **24**, its butenolide ester derivative **25**, and PMEA-containing butenolide **28** in the presence of calf mucosal adenosine deaminase (ADA, EC 3.5.4.4) in buffer solutions were determined.[19] Additionally, the inhibition studies on these compounds were carried out on the basis of the Kaplan method (Table 1).[20] The results showed that both the N^9- and N^7-acyclic nucleosides **1** and **14** functioned as ADA substrates. The V_{max} of **14** was less than that of **1** by a factor of 4. Compounds **1**, **14**, and **24** showed competitive inhibition of ADA when ara-A was used as a substrate. However, N^7-isomer **14** was found to be more efficient than the N^9-isomer **1** and nucleotide analogue **24** as an inhibitor of ADA. PMEA (**4**), acyclic nucleoside phosphonate **20**, and nucleotide-containing butenolides **25** and **28** were neither a good substrate nor an inhibitor of the enzyme. Nucleotide analogue **24**, however, was a substrate for ADA, but its V_{max} was about 95% less than that of ara-A (**3**). The slow rate of deamination of compound **24** by ADA may reflect the lack of substrate activity of the acyclic nucleoside phosphonate moiety therein in the active site of the enzyme.

Table 1. Substrate Activity and Inhibitory Property against ADA[a]

substrate	K_m (10^{-5} M)	rel V_{max}	K_i (10^{-5} M)
1	14	1.5×10^{-2}	14
3 (araA)	4.3	1.0	
4 (PMEA)	43		>80
14	20	1.5×10^{-6}	0.83
20	>80		>80
24	16	9.8×10^{-2}	10
25	64		>80
28	>80		>80

[a] The reaction velocity, V, in micromoles per minute per milligram of the enzyme was determined,[19] and a plot of 1/[S] ([S] = substrate concentration) vs 1/V was made. Thus, by the method of Lineweaver and Burk, the Michaelis (K_m) and maximum velocity constants (V_{max}) were determined.[19] For inhibition studies, in addition to araA (**3**), substrate solutions contained **1**, **4**, **14**, **20**, **24**, **25**, or **28** and then K_i was measured for each substrate.[20]

Table 2. Phosphorylation of Various Nucleosides and Thymidine with HSV or Vero Cell Thymidine Kinases[a]

	HSV thymidine kinase		Vero cell thymidine kinase	
substrate	K_m (μM)	rel V_{max}	K_m (μM)	rel V_{max}
1	2.0×10^4	<3.0	$>3.0 \times 10^4$	<3.0
2 (acyclovir)	1.5	39.2	2.2×10^4	<3.0
3 (araA)	1.5×10^4	<3.0	1.3×10^4	<3.0
9	12.8	28.0	18.3	12.0
14	30.0	<3.0	$>3.0 \times 10^4$	<3.0
2 + **14** (1:1 w/w)		80.9		<3.0
thymidine	1.0	100.0	1.0	1.0×10^2

[a] Apparent K_m values were determined for HSV and Vero cell thymidine kinases from reactions containing 50 mM Tris-HCl (pH 7.5), 2.0 mM ATP, 2.0 mM $MgCl_2$, 1.0 mg/mL BSA, 1.5 μM [^{14}C]thymidine, and 198 units of enzymes/mL.[21] The results were compared with those of thymidine. All reactions were performed at 37 °C. The radiochemical nucleoside kinase coupled assay was used in the determination of the relative substrate velocities.[21]

Comparison of Phosphorylation of Nucleosides by HSV and Vero Cell Thymidine Kinases. The rates of phosphorylation of N^9-adenine acyclic nucleoside **1**, acyclovir (**2**), ara-A (**3**), N^7-guanine acyclic nucleoside **9**, and N^7-adenine acyclic nucleoside **14**, as well as a mixture of **2** and **14** (w/w = 1:1), a mixture of **1** and **2** (w/w = 1:1), and a mixture of **2** and **3** (w/w = 1:1), in the presence of HSV or Vero cell thymidine kinase were determined[21] and the results were compared with those of thymidine. It was observed that N^7-guanine nucleoside **9**, unlike acyclovir, can be phosphorylated by both the HSV thymidine kinase and the host cell kinase. On the other hand, these enzymes were found not to phosphorylate adenine nucleosides **1**, **14**, and ara-A (**3**); yet the N^7-adenine nucleoside **14** induced a 2-fold increase on the rate of phosphorylation of acyclovir (**2**) (see Table 2). N^9-adenine nucleosides **1** and **3**, however, did not induce any increase on the rate of phosphorylation of **2**. It is believed that the N^7-adenine nucleoside **14** binds (K_m = 30 μM) to a specific receptor at the active site of the enzyme to exhibit the observed activatory property toward the HSV thymidine kinase.

Enzymatic Conversion of Acyclic Nucleoside Phosphonates and Nucleotide 5′-Monophosphate

Table 3. Kinetics of the PRPP Synthetase Reaction with Acyclic Nucleoside Phosphonates **4** and **20**, Nucleotide Analogue **24**, and AMP (**21**)[a]

substrate	K_m (mM)	V_{max} (μmol unit^{-1} h^{-1})	K_i[b] (mM)
4 (PMEA)	1.5	0.096	3.0
20	4.8	0.013	17
21 (AMP)	0.24	14	
24	0.57	12	0.79

[a] The PRPP synthetase reaction mixture contained 10.0 mM potassium phosphate buffer (pH 8.0), 5.0 mM $MgCl_2$, 2.5 mM PRPP, an appropriate amount of AMP (**21**) or test compounds, and 0.04 unit of PRPP synthetase. The formation of ATP, diphosphates of PMEA (**4**), **20**, or **24** was analyzed by HPLC according to the method of Balzarini and De Clercq.[4i] [b] Inhibition of AMP phosphorylation by the enzyme was measured in the presence of different substrate (AMP) concentrations and appropriate concentrations of **4**, **20**, and **24**. The reaction mixture was incubated at 37 °C for 15 min with 0.002 unit of PRPP synthetase. The formation of ATP was followed by HPLC on a Partisphere anion-exchange column as described.[4i]

Analogue to Their Antivirally Active Diphosphates (Triphosphate Equivalents). N^9-Acyclic nucleoside phosphonate (PMEA, **4**), N^7-acyclic nucleoside phosphonate **20**, adenosine 5′-monophosphate (**21**), and nucleotide 5′-monophosphate **24** were individually incubated with PRPP and PRPP synthetase, during which time the reaction proceeded linearly. The assays were then terminated after 4 h by the addition of MeOH. HPLC on an anion-exchange Partisphere column was used to analyze the formation of ATP (**21**pp), PMEApp (**4**pp), **20**pp, and **24**pp. Inhibitory effects of PMEA (**4**), **20**, and **24** toward phosphorylation of AMP (**21**) was also evaluated according to an established procedure.[4i] Results are summarized in Table 3.

The substrate affinity of N^7-acyclic nucleoside phosphonate **20** (K_m = 4.8 mM) for the enzyme was found to be 4 times less than that of PMEA (**4**) (K_m = 1.5 mM). Similarly, the V_{max} for conversion of **20** to **20**pp is at least 7 times lower than that for the conversion of **4** to **4**pp. On the other hand, nucleotide analogue **24** was found to be a better substrate than PMEA (**4**) for PRPP synthetase (K_m = 0.57 mM). It can also be phosphorylated (V_{max} = 12) by the enzyme at a rate similar to that of AMP (**21**) (V_{max} = 14).

It has been hypothesized[4i] that the primary amino group at the C-6 position of purines is essential for hydrogen bonding with the enzyme. The NH_2 group in **20** is sterically hindered by an adjacent side chain at the N-7 position. As such, it cannot as effectively interact with the active site of the enzyme when compared to PMEA (**4**), natural substrate AMP (**21**), or nucleotide analogue **24**.

Activity of Snake Venom and Spleen Phosphodiesterases against Nucleotide Analogues. Compound **24**, possessing the skeletons of both phosphonate **20** and 5′-adenosine monophosphate (**21**), was dephosphorylated at the 5′-position by snake venom in 80% yield after 8 h.[17] The resultant 9-(β-D-furanosyl)adenine 3′-[1-(adenin-7-ylethoxy)methyl]phosphonate was hydrolyzed further in the presence of spleen phosphodiesterase to afford adenosine and phosphonate **20** in about 40−60% yield after 8 h.[17] Spleen phosphodiesterase also degraded compound **24** to give 5′-adenosine monophosphate (**21**) and phosphonate **20** in an overall yield of 60% after 8 h. These results indicate that the phosphodiesterases recognized dinucleotide analogue **24** as a normal substrate. In addition, we found that nucleotide-containing butenolide **25** was completely resistant to snake venom and spleen enzymes.

Anti-DNA Virus Activity in Vitro. The newly synthesized compounds were tested for inhibition of cytopathogenicity of the herpes simplex type 1 virus (HSV-1), herpes simplex type 2 virus (HSV-2), thymidine kinase-positive (TK^+) and thymidine kinase-deficient (TK^-) strains of varicella-zoster virus (VZV), and human cytomegalovirus in Vero cell culture up to a level as high as 128 μg/mL.[22,23] Compounds tested include N^7-alkylated purines **7**, **9**, **12**, **14**, and **20**, as well as mixtures of **1** and **14** (w/w = 1:1), **2** and **14** (w/w = 1:1), **3** and **14** (w/w = 1:1), in addition to N^9-substituted adenine **1**,[24] acyclovir (**2**),[25,26] ara-A (**3**),[27] PMEA (**4**),[4i] PMEG (**27**),[4i] nucleotide analogue **24**, and nucleotide-containing butenolides **25**, **28**, and **29**. Toxicity of these compounds was evaluated by their ability to cause morphological changes in HeLa and Vero cells at different concentrations. The minimum inhibitory concentrations (IC_{50}) were measured by use of the linear regression method (see Supporting Information, Table 8).[28,29]

The pronounced anti-DNA virus activity of **14** with respect to the corresponding chloro derivative **12** showed that the presence of a hydroxyl group is essential for antiviral activity. Results from the biological tests also indicated that adenine nucleoside **14** was not effectively deaminated by ADA; yet it inhibited the deactivating property of the enzyme and led to the observed increase in the antiviral activity of **1** and ara-A (**3**). Furthermore, use of adenine acyclic nucleoside **14** resulted in a 2-fold increment in the rate of phosphorylation of acyclovir (**2**) by HSV thymidine kinase. As such, a combination of **14** and **2** exhibited profound antiviral activity. N^7-Adenine nucleoside **14** was found to be less toxic than the corresponding N^9-isomer **1**. On the other hand, both HSV and cellular thymidine kinases can phosphorylate N^7-guanine nucleoside **9**. As a result, this nucleoside exhibited more toxicity than acyclovir (**2**).

The rate of phosphorylation of N^7-acyclic nucleoside phosphonate **20** to its antivirally active anabolite **20**pp by PRPP synthetase is 7 times less than that of the PMEA (**4**). Thus in comparison to compound **4**, compound **20** exhibited less activity against DNA viruses. On the other hand, nucleotide 5′-monophosphate analogue **24**, possessing a natural AMP moiety, was converted to its diphosphate (triphosphate form) **24**pp at a rate comparable to that of AMP (**21**), which is about 120 times faster than the rate of conversion of PMEA (**4**) to PMEApp (**4**pp). Consequently, nucleotide 5′-monophosphate **24** exhibited higher anti-DNA virus activity than PMEA (**4**).

The ability of a drug to penetrate a membrane and exhibit biological activity can be correlated to its lipophilicity.[8a,17] Consequently, we prepared compounds **25**, **28**, and **29** possessing a butenolide ester functionality as lipophilic prodrugs. These compounds displayed superior antiviral activity relative to their respective parent compounds nucleotide 5′-monophosphate **24**, PMEA (**4**), and PMEG (**27**). In addition, spleen phosphodiesterase can recognize and at least partly hydrolyze nucleotide analogue **24** to the biologically less active phosphonate **20** inside the infected cells; whereas its

Table 4. Antiviral Effects of Compounds **2, 4, 9, 14, 20, 24, 25,** and **28** against HSV-1-Induced Mortality in NMRI Mice upon Intraperitoneal Administration[a]

compound	dose (mg kg^{-1} day^{-1})	no. of mice	mean day of symptom initiation[b] [%]	mean day of animal death[c] [%]
2 (acyclovir)	250	20	>21 [100]	>21 [100]
	150	20	19.1 ± 1.3 [86]	>21 [100]
	100	15	15.6 ± 1.6 [65]	18.9 ± 2.1 [80]
4 (PMEA)	250	20	>21 [100]	>21 [100]
	150	20	18.5 ± 1.9 [80]	>21 [100]
	100	15	14.7 ± 1.4 [56]	17.0 ± 1.1 [77]
9	250	20	15.1 ± 1.2 [67]	18.5 ± 1.0 [90]
	150	20	13.0 ± 1.1 [57]	16.0 ± 1.5 [78]
	100	15	10.9 ± 0.6 [48]	14.1 ± 0.8 [66]
14	250	20	>21 [100]	>21 [100]
	150	20	19.9 ± 1.3 [88]	>21 [100]
	100	15	17.5 ± 2.1 [80]	19.8 ± 1.7 [95]
20	250	20	16.8 ± 2.4 [75]	20.0 ± 1.7 [95]
	150	20	15.0 ± 0.9 [62]	17.8 ± 1.5 [80]
	100	15	12.8 ± 1.1 [51]	15.4 ± 1.3 [70]
24	250	20	>21 [100]	>21 [100]
	150	20	19.3 ± 1.6 [85]	>21 [100]
	100	15	16.0 ± 1.7 [70]	19.5 ± 1.2 [90]
25	250	20	>21 [100]	>21 [100]
	150	20	>21 [100]	>21 [100]
	100	15	19.6 ± 1.5 [94]	>21 [100]
28	250	20	>21 [100]	>21 [100]
	150	20	>21 [100]	>21 [100]
	100	15	>21 [100]	>21 [100]
saline	0	20	3.38 ± 0.7 [0]	9.4 ± 0.6 [0]

[a] Mice were inoculated intraperitoneally with HSV-1 (KOS). Treatment was initiated 4 h postinfection and continued for 6 consecutive days. Experiments were terminated at day 21. [b] Values in brackets represent percentage of HSV-1-infected mice without symptoms at day 21 postinfection. [c] Values in brackets represent percentage of HSV-1-infected mice that were alive at day 21 postinfection.

butenolide ester derivative **25** was found to be stable toward phosphodiesterases. Thus, in comparison with nucleotide analogue **24**, its prodrug **25** possesses superior bioavailability and greater stability both in vitro and in vivo.

Anti-HSV-1 Activity in Vivo and Determination of LD_{50} for N^7-Adenine Acyclic Nucleoside 14, Nucleotide-Containing Butenolide 25, and PMEA-Containing Butenolide 28 in Mice. Acyclovir (**2**), PMEA (**4**), N^7-guanine acyclic nucleoside **9**, N^7-adenine acyclic nucleoside **14**, N^7-acyclic nucleoside phosphonate **20**, nucleotide 5′-monophosphate **24**, and butenolide ester derivatives **25** and **28** were evaluated for their inhibitory effects on HSV-1-induced mortality in NMRI mice (Table 4).[4a] The butenolide derivative of PMEA, **28,** appeared to be the most potent anti-HSV-1 agent in vivo, followed by nucleotide-containing butenolide **25**, nucleoside analogue **14**, nucleotide analogue **24**, acyclovir (**2**), PMEA (**4**), phosphonate **20**, and nucleoside analogue **9**. Since compound **28** is less active in vitro against HSV-1 when compared to compounds **25** and **14**, the in vitro potency does not directly translate to in vivo potency. These results confirms previous findings.[4c,4d,4h]

All compounds were administered intraperitoneally (ip, 100−250 mg kg^{-1} day^{-1}) for 6 consecutive days. Compounds **2**, **4**, **14**, **24**, and **25** gave full protection against HSV-induced mortality at the 150 mg/kg dose level. The same level of protection was provided by compound **28** at a dose of 100 mg/kg. Survival times of all treated groups were found to be significantly different from the placebo-treated control group (see Table 4). The potent anti-HSV-1 activity exhibited by compounds **2**, **4**, **14**, **24**, **25**, and **28** clearly demonstrated that they are taken up effectively by cells to exert in vivo activity. None of the compounds were toxic to the mice at the highest dose tested.

Table 5. Inhibitory Effects of Nucleotide Analogues on the Cytopathogenicity of HIV-1 and HIV-2 in MT4 Cells, as Well as on the Cytopathogenicity of MSV in CEM Cells and Cellular Toxicity

	IC_{50}[a] (μg/mL)				
compound	HIV-1 (IIIB)	HIV-2 (LAV-2)	MSV	MT4 cell[b]	CEM cell[b]
4 (PMEA)	4.1	3.8	2.0	274	285
20	7.8	9.1	27	>300	>300
24	5.9	6.4	17	298	>300
25	4.9	4.2	13	>300	>300
27 (PMEG)	16	18	0.19	16	12
28	1.4	1.0	0.93	265	280
29	6.0	7.1	0.020	14	13

[a] Inhibitory concentrations (IC_{50}) were determined by use of an established procedure[28,30] and represent the average of duplicate determinations. [b] Concentration of the compound required to reduce the number of viable uninfected cells by 50%.

The LD_{50} values of the most active compounds **14**, **25**, and **28** in mice were also determined. As such, N^7-acyclic nucleoside **14** and butenolide ester derivatives **25** and **28** were administered at different doses intraperitoneally. They did not show any toxicity up to a concentration level as high as 400 mg/kg. All mice were controlled in good conditions after 6 months of administration. Nevertheless, LD_{50} (ip) values of 950, 675, and 710 mg/kg were determined for **14**, **25**, and **28**, respectively. Moreover, no discernible abnormality was observed in the histological appearance of the viscera of either the control or tested groups of mice that received the drugs ip (250 mg kg^{-1} day^{-1}) for 10 days. Furthermore, there were no physiological changes in their cardiovascular or central nerve systems.

Anti-Retrovirus Activity in Vitro. Compounds **4**, **20**, **24**, **25**, **27**, **28**, and **29** were tested for inhibition of cytopathogenicity against the human immunodeficiency viruses HIV-1 (III-B) and HIV-2 (LAV-2) in MT4 cells. These compounds were also screened for their antiviral activity against Moloney murine sarcoma virus (MSV) in CEM cells in a cell-protection assay.[30] Toxicity of these compounds was evaluated by their ability to cause morphological changes in MT4 or CEM cells at different concentrations. The minimum inhibitory concentrations (IC_{50}) were measured by the use of the linear regression method (Table 5).[28]

In comparison to the rate of phosphorylation of PMEA (**4**) to PMEApp (**4**pp) by PRPP synthetase, the conversion of dinucleotide **24** to its anabolically active form **24**pp is 120 times faster; yet PMEA (**4**) exhibited higher activity than **24** as well as the butenolide ester derivative **25** against retroviruses. Thus, the HIV and MSV reverse transcriptases may have higher affinity for PMEA (**4**) than dinucleotide analogue **24**. PMEA (**4**) was also found to be more active than its N^7-isomer **20** against retroviruses. On the other hand, butenolide ester derivatives **28** and **29** displayed superior antiviral activity relative to their respective parent molecules **4** and **27**. Thus in comparison to PMEA (**4**) and PMEG (**27**), their respective lipophilic prodrugs **28** and **29** possess superior bioavailability and greater anti-retrovirus activity. As shown in Scheme 6, we believe that

Table 6. Inhibitory Effects of Acyclic Nucleoside Phosphonate **4** and Its Prodrug **28** on MSV-Induced Tumor Formation and Associated Death in NMRI Mice upon Intraperitoneal Administration[a]

compound	dose (mg kg^{-1} day^{-1})	no. of mice	mean day of tumor initiation[b] [%]	mean day of animal death[c] [%]
PMEA (**4**)	50	15	12.5 ± 1.6 [84]	17.8 ± 2.0 [96]
	20	15	12.0 ± 1.3 [58]	15.9 ± 1.7 [76]
	10	10	9.6 ± 1.5 [19]	13.0 ± 1.1 [42]
prodrug (**28**)	50	15	18.4 ± 1.4 [95]	>21 [100]
	20	15	17.9 ± 1.7 [80]	>21 [100]
	10	10	14.0 ± 2.1 [60]	18.9 ± 1.8 [97]
control	0	30	3.82 ± 0.95 [0]	7.8 ± 1.3 [0]
untreated control[d]	0	40	>21 [100]	>21 [100]

[a] All mice received two injections within 2 days. [b] Values in brackets represent percentage of MSV-infected mice without tumors at day 21 postinfection [c] Values in brackets represent percentage of MSV-infected mice that were alive at day 21 postinfection. [d] Untreated control group was neither treated with MSV nor with the drugs.

the oxygen of the methoxy group at the C-2 position of the butenolide moiety is responsible for the ease of conversion of these novel prodrugs **28** and **29** to their corresponding potential drugs PMEA (**4**) and PMEG (**27**) inside the infected cells.

Inhibitory Effects of PMEA (4) and Its Butenolide Ester Derivative 28 on MSV-Induced Tumor Formation in Vivo. Compounds **4** and **28** were evaluated for their inhibitory effect on MSV-induced tumor formation in NMRI mice (Table 6).[31] The compounds were administered intraperitoneally (50 mg kg^{-1} day^{-1}) for 2 consecutive days. Prodrug **28** exhibited much higher anti-MSV activity than PMEA (**4**) in vivo. At a dose of 10 mg kg^{-1} day^{-1}, compound **28** prevented tumor formation in 60% of the MSV-infected mice, whereas with compound **4** at the same dosage level, only a 19% prevention was observed. In surviving animals treated with **28**, about 2 g of weight loss was observed. In the case of PMEA-treated mice, the weight loss of the surviving animals was at least 2 times more.

Summary and Conclusions

A series of new compounds were synthesized and their biological activities were determined. These compounds include N^7-guanine acyclic nucleosides **7** and **9**, N^7-adenine acyclic nucleosides **12** and **14**, N^7-acyclic nucleoside phosphonate **20**, dinucleotide 5′-monophosphate analogue **24**, nucleotide-containing butenolide **25**, PMEA-containing butenolide **28**, and PMEG-containing butenolide **29**. Unlike acyclovir (**2**), which was phosphorylated by HSV-specified thymidine kinase, its N^7-isomer **9** was recognized by both HSV and cellular thymidine kinases. As such, compound **9** was found to be less active and more toxic than compound **2** in vitro. Compound **14** was not phosphorylated in the presence of thymidine kinases. Nevertheless, with respect to the lack of activity of chloro derivatives **7** and **12**, it is reasonable to conclude that the hydroxy group in **14** is essential for its antiviral activity. Consequently, either phosphorylation of this compound is not essential for biological activity or the phosphorylation is performed by other enzymes. Moreover a combination of N^7-adenine acyclic nucleoside **14** and N^9-adenine acyclic nucleoside **1**, acyclovir (**2**), and ara-A (**3**) in a ratio of 1:1 (w/w) was found to possess profound activity against DNA viruses. PMEA (**4**), its N^7-isomer **20**, and nucleotide 5′-monophosphate **24** were converted directly to their antivirally active anabolites in the presence of PRPP synthetase, whereby a pyrophosphate group is transferred from PRPP to their phosphonate (i.e., **4** and **20**) or phosphate (i.e., **24**) moieties. Indeed, the substrate affinity for PRPP synthetase seemed to be dependent on the availability of the primary amine group at the C-6 position of purines. With respect to this structural requirement, the ability of compounds **4**, **20**, and **24** to inhibit cytopathogenicity of the DNA viruses was found to correlate well with their rate of phosphorylation by PRPP synthetase. Finally, prodrugs **25**, **28**, and **29**, possessing a butenolide moiety, were designed. These butenolide ester derivatives showed superior bioavailability and profound antiviral activity relative to their respective precursors. In comparison to nucleotide **24**, prodrug **25** also exhibited higher stability toward phosphodiesterases. Moreover, compounds **2**, **4**, **9**, **14**, **20**, **24**, **25**, and **28** were evaluated for their inhibitory effect on HSV-1-induced mortality in NMRI mice. Nucleotide-containing butenolide **25** and PMEA-containing butenolide **28** were found to be remarkable anti-HSV-1 agents in vivo. Survival times of all treated groups were determined to be significantly longer than that of the control group. Compounds **4**, **20**, **24**, **25**, **28**, and **29** were also tested against HIV-1 (III-B), HIV-2 (LAV-2), and MSV. Prodrugs **28** and **29** were found to possess notable anti-retrovirus activity in vitro as well as in vivo.

Experimental Section

General. For anhydrous reactions, glassware was dried overnight in an oven at 120 °C and cooled in a desiccator over anhydrous $CaSO_4$ or silica gel. Reagents were purchased from Fluka and enzymes from Sigma Chemical Co. Solvents, including dry ether and tetrahydrofuran (THF), were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Other solvents, including chloroform, dichloromethane, ethyl acetate, and hexanes, were distilled over CaH_2 under nitrogen. Absolute methanol and ethanol were purchased from Merck and used as received.

Melting points were obtained with a Büchi 510 melting point apparatus. Infrared (IR) spectra were recorded on a Beckman IR-8 spectrophotometer. The wavenumbers reported are referenced to the 1601 cm^{-1} absorption of polystyrene. Proton NMR spectra were obtained on a Varian XL-300 (300 MHz) spectrometer. Chloroform-*d* and dimethyl sulfoxide-d_6 were used as solvent; Me_4Si (δ 0.00 ppm) was used as an internal standard. All NMR chemical shifts are reported as δ values in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, unresolved multiplet due to the field strength of the instrument; and dd, doublet of doublets. UV spectroscopy was carried out on an HP8452A diode-array spectrophotometer. Mass spectra were carried out on a VG 70−250 S mass spectrometer. Microanalyses were performed on a Perkin-Elmer 240-B microanalyzer.

Purification on silica gel refers to gravity column chromatography on Merck silica gel 60 (particle size 230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated plates purchased from Merck (silica gel 60 F_{254}). Compounds were visualized by use of UV light, I_2 vapor, or 2.5% phosphomolybdic acid in ethanol with heating.

Determination of Solubility. Each compound (70 mg) listed in Table 7 was agitated in a 25-mL volumetric flask with phosphate buffer (0.10 M, pH 6.8, 5.0 mL) for 20 h. This solution was filtered from undissolved solid through a sintered glass funnel (4.0−5.5 mesh ASTM) and the concentration of the solution was determined by UV absorbance (see Table 7, Supporting Information).

Determination of Partition Coefficients (Lipophilicity). A solution of each compound (10 mL) in Table 7 (see Supporting Information) in phosphate buffer (0.10 M) possessing an UV absorbance of 2.2−3.3 at 258−267 nm, for adenine, or at 270−290 nm, for guanine, was shaken with 1-octanol (10 mL) in a separatory funnel for 1.5 h. The layers were separated and their concentrations were determined by an UV spectrophotometer. The partition coefficient was calculated as $P = [S]_{1-octanol}/[S]_{H_2O}$.

Enzyme Assays: (A) Adenosine Deaminase. The reported procedures[19,20] were used for ADA, and the results are summarized in Table 1.

(B) HSV and Vero Cell Thymidine Kinases. Phosphorylation of nucleosides with HSV or Vero cell thymidine kinase was studied as described previously.[21] Results are summarized in Table 2.

(C) 5-Phosphoribosyl 1-Pyrophosphate Synthetase. Substrate affinities of acyclic nucleoside phosphonates **4**, **20**, **21**, and dinucleotide analogue **24** for PRPP synthetase as well as their inhibitory effect against the enzyme were evaluated according to the established procedures.[4i] Results are illustrated in Table 3.

(D) Snake Venom Phosphodiesterase. Snake venom phosphodiesterase (200 units) was dissolved in tris(hydroxymethyl)aminomethane buffer (1.0 mL), which was adjusted to pH 9.2 with 0.1 N HCl. The enzyme solution (0.10 mL) was added to the nucleotide **24** or **25** (0.70 mg) and the mixture was incubated at 37 °C for 8 h. The solution was then applied to Whatman 3-MM paper as a band, which was developed with a mixture of *i*-PrOH, concentrated NH_4OH, and H_2O (9:1:2). Degradation products or unreacted starting materials were separated. Dinucleotide 5′- monophosphate **24** gave 9-(β-D-furanosyl)adenine 3′-[1-(adenin-7-ylethoxy)methyl]phosphonate in about 80% yield. On the other hand, dinucleotide-containing butenolide **25** was completely resistant to the enzyme.

(E) Spleen Phosphodiesterase. Spleen phosphodiesterase (20 units) was dissolved in sodium pyrophosphate buffer (0.01 M, 1.0 mL), which was adjusted to pH 6.5 with phosphoric acid. Nucleotide **24**, **25**, or 9-(β-D-furanosyl)adenine 3′-[1-(adenin-7-yl-ethoxy)methyl]phosphonate (0.70 mg) was dissolved in ammonium acetate buffer (0.05 M, 0.20 mL), which was adjusted to pH 6.5 with acetic acid. An aliquot of the enzyme solution (0.1 mL) was added to the nucleotide solution and the mixture was incubated at 37 °C for 8 h. The solution was then applied to Whatman 3-MM paper as a band and developed with a mixture of *i*-PrOH, concentrated NH_4OH, and H_2O (9:1:2). Bands containing products were cut out, which were eluted with H_2O, and the resultant mixture was freeze-dried. The isolated products were characterized by comparison with authentic samples. Dinucleotide **24** afforded phosphonate **20** and adenosine 5′-monophosphate (**21**) in about 60% yield. 9-(β-D-Furanosyl)adenine 3′-[1-(adenin-7-ylethoxy)methyl]phosphonate gave adenosine (40% yield) and **20** (60% yield). Dinucleotide analogue **25**, having a butenolide ester unit, was found to be stable to the enzyme.

Anti-DNA Virus Activity and Anticellular Evaluations. The methods for measuring viruses-induced cytopathogenicity in Vero cell culture, as well as the toxicity of the tested compounds toward HeLa and Vero cells, have been described previously.[22,23,28] Results are summarized in Table 8 (see Supporting Information).

Anti-Retrovirus Activity and Anticellular Evaluations. The methods for measuring viruses-induced cytopathogenicity in MT4 cells or CEM cells, as well as the toxicity of the tested compounds toward MT4 and CEM cells, have been described previously.[30] Results are summarized in Table 5.

Animal Studies: (A) Anti-HSV-1 Activity. Two-week-old NMRI mice (15−20 animals/group), weighing ca. 7 g each, were infected ip with 4×10^4 units of HSV-1 (KOS).[4a] Compounds in Table 4 were administered ip once a day for 6 consecutive days, starting 4 h postinfection. Percentage of HSV-1-infected mice without symptoms and those that were alive at day 21 postinfection were observed (see Table 4). Deaths were recorded for 21 days after infection.

(B) Anti-MSV Activity in Vivo. The inhibitory effects of the compounds **4** and **27** on the initiation of MSV-induced tumor formation and survival of MSV-induced mice (10−15 animals/group) were evaluated as previously described.[31] Results are summarized in Table 6.

9-[(2-Hydroxyethoxy)methyl]adenine (1). Compound **1** was prepared by a standard procedure:[23] mp 198−199 °C; R_f (hexanes/EtOAc = 1:2) 0.23; UV (EtOH) λ_{max} 259 (ε 14 000); ^{1}H NMR (CD_3OD) δ 3.62 (s, 4 H, $O(CH_2)_2O$), 5.67 (s, 2 H, $H_2C_{1'}$), 8.22 (s, 1 H, HC_2), 8.27 (s, 1 H, HC_8); ^{13}C NMR (CD_3OD) δ 61.86 (CH_2OH), 72.08 (OCH_2) 74.31 ($C_{1'}$), 119.98 (C_5), 143.14 (C_8), 150.90 (C_4), 153.74 (C_2), 157.19 (C_6); MS *m*/*z* 209 (M^+).

9-(Triphenylmethyl)guanine (6). Guanine **5** (1.51 g, 9.99 mmol) and $(NH_4)_2SO_4$ (100 mg) were suspended in hexamethyldisilazane (HMDS) (150 mL) and refluxed for 24 h. The solvent was evaporated under reduced pressure and the residue was dissolved in CH_3CN (150 mL). Triphenylmethyl chloride (2.79 g, 10.0 mmol) was added and the reaction mixture was stirred at 25 °C for 7.0 h. The solution was concentrated under reduced pressure and the residue was purified by use of column chromatography (hexanes/EtOAc = 1.5:8.5) to afford **6** (3.54 g, 8.99 mmol) in 90% yield: mp 268−270 °C; R_f (hexanes/EtOAc = 1:2) 0.34; UV (EtOH) λ_{max} 254 (ε 13 870), 278 (sh); ^{1}H NMR (DMSO-d_6) δ 5.97 (s, 2 H, NH_2), 7.09−7.39 (m, 16 H, HC_8 + $C(C_6H_5)_3$), 10.45 (br s, 1H, NH); MS *m*/*z* 393 (M^+). Anal. Calcd ($C_{24}H_{19}N_5O$): C, H, N.

7-[(2-Chloroethoxy)methy]guanine (7). To a solution of **6** (1.77 g, 4.49 mmol) in DMF (30 mL) was added (2-chloroethoxy)methyl chloride (0.65 g, 5.0 mmol). The reaction mixture was stirred at 25 °C for 8.0 h. The solution was then partitioned between EtOAc (100 mL) and water (100 mL). The EtOAc solution was washed with water (4 × 100 mL); then it was dried over $MgSO_4$ (s) and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography (EtOAc) afforded **7** (1.07 g, 4.39 mmol) in 88% yield: mp >280 °C (decomp); R_f (hexanes/EtOAc = 1:2) 0.20; UV (EtOH) λ_{max} 288 (ε 15 100); ^{1}H NMR (DMSO-d_6) δ 3.67 (t, *J* = 6.10 Hz, 2 H, CH_2Cl), 3.75 (t, *J* = 6.10 Hz, 2 H, OCH_2), 5.62 (s, 2 H, $H_2C_{1'}$), 6.15 (s, 2 H, NH_2), 7.58 (s, 1 H, NH), 8.16 (s, 1 H, HC_8); ^{13}C NMR (DMSO-d_6) δ 43.39 (CH_2Cl), 68.50 (OCH_2), 74.71 ($C_{1'}$), 107.71 (C_5), 143.87 (C_8), 153.15 (C_4), 154.37 (C_2), 159.12 (C_6); MS *m*/*z* 243 (M^+, Cl cluster). Anal. Calcd ($C_8H_{10}ClN_5O_2$): C, H, N, Cl.

7-[[2-(*p*-Methoxyphenyloxy)ethoxy]methyl]guanine (8). Compound **8** (2.72 g, 8.20 mmol) was prepared in 82% yield from **6** (3.64 g, 9.25 mmol) and [2-(*p*-methoxyphenyloxy)ethoxy]-methyl chloride (2.18 g, 10.1 mmol) in DMF (100 mL) by the method used for the synthesis of **7**: mp >250 °C (decomp); R_f (hexanes/EtOAc = 1:2) 0.18; UV (EtOH) λ_{max} 290 (ε 16,500); ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3 H, OCH_3), 4.02−4.10 (m, 4 H, $O(CH_2)_2O$), 5.74 (s, 2 H, $H_2C_{1'}$), 5.88 (s, 2 H, NH_2), 6.67, 6.70 (AA′BB′, *J* = 9.30 Hz, 4 H, C_6H_4), 7.50 (s, 1 H, NH), 8.85 (s,1 H, HC_8); ^{13}C NMR (DMSO-d_6) δ 56.12 (CH_3), 68.74 (OCH_2), 70.20 (CH_2OPh), 75.31 ($C_{1'}$), 107.25 (C_5), 144.02 (C_8), 153.25 (C_4), 154.48 (C_2), 159.26 (C_6), 115.55, 116.38, 153.51, 155.56 (C_6H_4); MS *m*/*z* 331 (M^+). Anal. Calcd ($C_{15}H_{17}N_5O_4$): C, H, N.

7-[(2-Hydroxyethoxy)methyl]guanine (9). To a solution of **8** (1.36 g, 4.10 mmol) in a mixture of CH_3CN (30 mL) and water (10 mL) was added CAN (2.25 g, 4.10 mmol) at 0 °C. The stirred reaction mixture was allowed to warm up to 25 °C within 1.0 h. Water (30 mL) was added to afford a solid. Filtration and crystallization of the solid from EtOH/water (4:1) gave **9** (0.65 g, 2.88 mmol) in 70% yield: mp >280 °C (decomp); R_f (hexanes/EtOAc = 1:2) 0.08; UV (EtOH) λ_{max} 289 (ε 16 000); ^{1}H NMR (DMSO-d_6) δ 3.61−3.82 (m, 4 H, $O(CH_2)_2O$), 4.85 (br s, 1H, OH), 5.81 (s, 2 H, $H_2C_{1'}$), 5.96 (br s, 2 H, NH_2), 6.61 (s, 1 H, NH), 8.67 (s, 1 H, HC_8); ^{13}C NMR (DMSO-d_6) δ 60.91 (CH_2OH), 70.01 (OCH_2) 75.25 ($C_{1'}$), 107.16 (C_5), 143.92 (C_8), 153.20 (C_4), 154.40 (C_2), 159.30 (C_6); MS *m*/*z* 225 (M^+). Anal. Calcd ($C_8H_{11}N_5O_3$): C, H, N.

9-(Triphenylmethyl)adenine (11). To a solution of **10** (1.35 g, 9.99 mmol) and DMF (10 mL) in pyridine (30 mL) was added Ph_3Cl (2.79 g, 10.0 mmol). The reaction mixture was stirred at 25 °C for 7.0 h. It was then diluted with EtOAc (150 mL) and water (100 mL). The organic layer was separated and then washed with water (4 × 100 mL). It was dried over $MgSO_4$ (s) and concentrated under reduced pressure to yield a foam. Purification was carried out by use of column chromatography (EtOAc/hexanes = 8:2) to afford compound **11** (3.58 g, 9.48 mmol) in 95% yield: mp 260−262 °C; R_f (hexanes/EtOAc = 1:2) 0.38; UV (EtOH) λ_{max} 260 (ϵ 14 600); ^{1}H NMR ($CDCl_3$) δ 5.60 (s, 2 H, NH_2), 7.15−7.31 (m, 15 H, $C(C_6H_5)_3$), 7.74 (s, 1 H, HC_2), 8.05 (s, 1 H, HC_8); MS *m*/*z* 377 (M^+). Anal. Calcd ($C_{24}H_{19}N_5$): C, H, N.

7-[(2-Chloroethoxy)methyl]adenine (12). To a solution of **11** (1.79 g, 4.74 mmol) in CH_2Cl_2 (70 mL) was added (2-chloroethoxy)methyl chloride (0.65 g, 5.0 mmol). The reaction mixture was stirred at 25 °C for 8.0 h to afford a solid. Filteration and crystallization from MeOH gave **12** (1.66 g, 4.27 mmol) in 90% yield: mp 184−186 °C; R_f (hexanes/EtOAc = 1:2) 0.28; UV (EtOH) λ_{max} 267 (ϵ 14 600); ^{1}H NMR (DMSO-d_6) δ 3.73 (t, J = 4.5 Hz, 2 H, CH_2Cl), 3.86 (t, J = 4.5 Hz, 2 H, OCH_2), 5.76 (s, 2 H, $H_2C_{1'}$), 8.49 (s, 1 H, HC_2), 8.74 (s, 1 H, HC_8), 9.32, 10.05 (2 br s, 2 H, NH_2); ^{13}C NMR (DMSO-d_6) δ 43.13 (CH_2Cl), 68.82 (OCH_2), 78.10 ($C_{1'}$), 118.20 (C_5), 143.53 (C_2), 146.50 (C_8), 149.39 (C_4), 151.60 (C_6); MS *m*/*z* 227 (M^+, Cl cluster). Anal. Calcd ($C_8H_{10}ClN_5O$): C, H, N, Cl.

7-[[2-(*p*-Methoxyphenyloxy)ethoxy]methyl]adenine (13). After 10 h, compound **13** (2.79 g, 8.86 mmol) was synthesized in 86% yield from **11** (3.90 g, 10.3 mmol) and [2-(*p*-methoxyphenyloxy)ethoxy]methyl chloride (2.25 g, 10.4 mmol) in CH_2Cl_2 (100 mL) by the method used for the synthesis of **12**: mp 129−130 °C; R_f (hexanes/EtOAc = 1:2) 0.21; UV (EtOH) λ_{max} 270 (ϵ 15 100); ^{1}H NMR (CD_3OD) δ 3.68 (s, 3 H, OCH_3), 4.04 (br s, 4 H, $O(CH_2)_2O$), 5.83 (s, 2 H, $H_2C_{1'}$), 6.58, 6.68 (AA′BB′, J = 9.0 Hz, 4 H, C_6H_4), 8.29 (s, 1 H, HC_2), 8.61 (s,1 H, HC_8); ^{13}C NMR (CD_3OD) δ 56.03 (CH_3), 68.70 (OCH_2), 70.18 (CH_2OPh), 82.27 ($C_{1'}$), 119.59 (C_5), 144.17 (C_2), 147.57 (C_8), 150.25 (C_4), 151.97 (C_6), 115.50, 116.35, 153.44, 155.53 (C_6H_4); MS *m*/*z* 315 (M^+). Anal. Calcd ($C_{15}H_{17}N_5O_3$): C, H, N.

7-[(2-Hydroxyethoxy)methyl]adenine (14). Compound **14** (0.74 g, 3.54 mmol) was prepared in 80% yield from **13** (1.39 g, 4.43 mmol) and CAN (2.43 g, 4.43 mmol) in CH_3CN (30 mL) and water (10 mL) by the method used for the synthesis of **9**: mp 122−123 °C; R_f (hexanes/EtOAc = 1:2) 0.12; UV (EtOH) λ_{max} 265 (ϵ 14 800); ^{1}H NMR (CD_3OD) δ 3.69−3.79 (m, 4 H, $O(CH_2)_2O$), 5.79 (s, 2 H, $H_2C_{1'}$), 8.32 (s, 1 H, HC_2), 8.60 (s, 1 H, HC_8); ^{13}C NMR (CD_3OD) δ 61.80 (CH_2OH), 72.27 (OCH_2) 82.01 ($C_{1'}$), 119.62 (C_5), 144.29 (C_2), 147.68 (C_8), 150.32 (C_4), 152.12 (C_6); MS *m*/*z* 209 (M^+). Anal. Calcd ($C_8H_{11}N_5O_2$): C, H, N.

9-(2-Cyanoethyl)adenine (15). To a suspension of 57% NaH in mineral oil (0.962 g, 22.8 mmol) in dry DMF (100 mL) was added **10** (3.40 g, 24.9 mmol) under nitrogen, and the mixture was heated at 80 °C for 1.0 h. A solution of 3-bromopropionitrile (2.81 g, 21.0 mmol) in DMF (5.0 mL) was added at 25 °C, and the reaction was heated at 65 °C for 17 h. It was then diluted with EtOAc (250 mL) and 5% aqueous HCl solution (150 mL). The organic layer was separated and then washed with water (4 × 100 mL). It was dried over $MgSO_4$ (s) and concentrated under reduced pressure. Purification of the residue was carried out by use of column chromatography (EtOAc/hexanes = 7.5:2.5) to afford compound **15** (3.510 g, 18.67 mmol) in 75% yield: mp 148−150 °C; R_f (hexanes/EtOAc = 1:2) 0.38; UV (EtOH) λ_{max} 260 (ϵ 13 900); ^{1}H NMR (CD_3OD) δ 2.92 (t, J = 5.8 Hz, 2 H, CH_2CN), 3.43 (t, J = 5.8 Hz, 2 H, CH_2N), 8.35 (s, 1 H, HC_2), 8.69 (s, 1 H, HC_8); MS *m*/*z* 188 (M^+).

7-[(Methoxycarbonyl)methyl]adenine (16) and 9-[(Methoxycarbonyl)methyl]adenine (17). To a stirred solution containing **15** (0.134 g, 1.00 mmol) and methyl iodoacetate (0.30 g, 1.5 mmol) in dry THF (20 mL) was added a THF solution of LiTMP (2.8 mL, 1.2 mmol) dropwise under an argon atmosphere at −20 °C. The reaction mixture was warmed to 25 °C within 1.0 h; then it was stirred at room temperature for 15 h. The solution was partitioned between EtOAc (40 mL) and water (40 mL). The organic layer was dried over $MgSO_4$ (s) and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc/hexanes = 8:2) gave compound **17** (0.041 g, 0.20 mmol) in 20% yield. Further elution of the column with EtOAc/hexanes (9:1) afforded **16** (0.123 g, 0.594 mmol) in 60% yield. For **16**: mp 112−114 °C; R_f (hexanes/EtOAc = 1:2) 0.16; UV (EtOH) λ_{max} 267 (ϵ 14 000); ^{1}H NMR (CD_3OD) δ 3.83 (s, 3H, CH_3), 4.37 (s, 2 H, $H_2C_{1'}$), 8.41 (s, 1 H, HC_2), 8.75 (s, 1 H, HC_8); MS *m*/*z* 207 (M^+). Anal. Calcd ($C_8H_9N_5O_2$): C, H, N. For **17**: mp 148−149 °C; R_f (hexanes/EtOAc = 1:2) 0.29; UV (EtOH) λ_{max} 260 (ϵ 13 750); ^{1}H NMR (CD_3OD) δ 3.75 (s, 3H, CH_3), 4.15 (s, 2 H, $H_2C_{1'}$), 8.29 (s, 1 H, HC_2), 8.38 (s, 1 H, HC_8); MS *m*/*z* 207 (M^+).

7-(2-Hydroxyethyl)adenine (18). To a stirred solution containing **16** (0.207 g, 0.999 mmol) and water (0.50 mL) in THF (12 mL) was added $NaBH_4$ (0.38 g, 10.0 mmol). After being stirred for 4 h at 25 °C, the reaction mixture was neutralized to pH = 7.0 by use of 10% HCl aqueous solution. Solvent was evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc/hexanes = 9:1) to give **18** (0.10 g, 0.55 mmol) in 55% yield: mp 132−133 °C; R_f (hexanes/EtOAc = 1:2) 0.09; UV (EtOH) λ_{max} 265 (ϵ 14 110); ^{1}H NMR (CD_3OD) δ 3.68 (t, J = 5.9 Hz, 2 H, CH_2O), 3.66 (t, J = 5.9 Hz, 2 H, CH_2N), 8.34 (s, 1 H, HC_2), 8.68 (s, 1 H, HC_8); MS *m*/*z* 179 (M^+). Anal. Calcd ($C_7H_9N_5O$): C, H, N.

7-[2-(Diethylphosphonomethoxy)ethyl]adenine (19). To a solution of **18** (0.18 g, 0.10 mmol) in DMF (15 mL) was added sodium *tert*-butoxide (0.150 g, 1.56 mmol). After 5 min, diethyl (*p*-toluenesulfonyloxymethane)phosphonate (0.42 g, 1.3 mmol) was added and the reaction mixture was stirred at 35 °C for 4.0 h. The reaction was then quenched with acetic acid (5.0 mL) and the mixture was partitioned between EtOAc (50 mL) and water (50 mL). The organic layer was separated and then washed with water (5 × 60 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. Purification by use of column chromatography (EtOAc/MeOH = 9:1) gave **19** (0.20 g, 0.60 mmol) in 60% yield as a white foam: R_f (hexanes/EtOAc = 1:2) 0.16; UV (EtOH) λ_{max} 266 (ϵ 15 321); ^{1}H NMR (CD_3OD) δ 1.39 (t, J = 6.7 Hz, 6H, 2 CH_3), 3.74 (t, J = 6.3 Hz, 2 H, CH_2N), 3.76 (d, J = 9.0 Hz, 2 H, CH_2P), 3.97−4.29 (m, 6 H, CH_2O + 2 CH_2OP), 8.37 (s, 1 H, HC_2), 8.77 (s, 1 H, HC_8); MS *m*/*z* 329 (M^+). Anal. Calcd ($C_{12}H_{20}N_5O_4P$): C, H, N.

7-[2-(Phosphonomethoxy)ethyl]adenine (20). To a solution of **19** (3.29 g, 10.0 mmol) in CH_2Cl_2 (130 mL) and DMF (10 mL) was added Me_3SiBr (4.95 g, 30.0 mmol); then the solution was stirred at 25 °C for 7.0 h. A mixture of MeOH and water (5:1, 40 mL) was added, and solvents were evaporated. Purification by use of column chromatography (EtOAc/MeOH = 6:4) afforded **20** (1.23 g, 4.50 mmol) in 45% yield: mp 253 °C (decomp); R_f (hexanes/EtOAc = 1:2) 0.05; UV (EtOH) λ_{max} 265 (ϵ 14 700); ^{1}H NMR (CD_3OD) δ 3.66 (t, J = 6.4 Hz, 2 H, CH_2N), 3.71 (d, J = 8.7 Hz, 2 H, CH_2P), 4.19 (t, J = 6.4 Hz, 2 H, CH_2O), 8.36 (s, 1 H, HC_2), 8.78 (s, 1 H, HC_8); MS *m*/*z* 273 (M^+). Anal. Calcd ($C_8H_{12}N_5O_4P$): C, H, N.

9-[2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(phosphono)-β-D-furanosyl]adenine 3′-[1-(Adenin-7-ylethoxy)methyl]phosphonate (23). To a solution of adenosine 5′-monophosphate (**21**) monohydrate (3.65 g, 9.99 mmol) in a mixture of pyridine (150 mL) and CH_3CN (160 mL) was added $AgNO_3$ (6.63 g, 39.0 mmol). After 10 min, *tert*-butyldimethylsilyl chloride (5.70 g, 37.8 mmol) was added. The mixture was stirred at 25 °C for 7.0 h and then filtered to remove AgCl. The filtrate was evaporated and the resultant crude product **22** was dissolved in dry THF (40 mL). In another flask, collidine (3.66 g, 30.0 mmol) was added to a solution of THF (45 mL) containing **20** (2.73 g, 10.0 mmol) at −10 °C. To this solution was added CCl_3SO_2Cl (2.20 g, 10.0 mmol) in THF (15 mL) dropwise. After crude **22** in THF was added to the mixture, it was stirred at 25 °C for 10 h. The solvents were removed, and the residue was dissolved in AcOEt (100 mL) and washed with water (3 × 100 mL). The organic layer was concentrated, and the residue was purified by use of column chromatography (EtOAc/

MeOH = 6:4) to afford **23** (3.0 g, 4.2 mmol) in 42% overall yield: mp 223−225 °C; UV (EtOH) λ_{max} 264 (ϵ 17 300); ^{1}H NMR (CD_3OD) δ 0.16 (br s, 6 H, $(CH_3)_2Si$), 1.05 (s, 9 H, $(CH_3)_3C$), 3.67−4.27 (m, 8 H, $CH_2N + CH_2O + CH_2OP + CH_2P$), 4.32−4.5 (m, 3H, $HC_{2'} + HC_{3'} + HC_{4'}$), 6.58 (d, J = 4.9 Hz, 1 H, $HC_{1'}$), 8.12, 8.42 (2s, 2 H, 2 HC_2), 8.27, 8.89 (2s, 2 H, 2 HC_8). Anal. Calcd ($C_{24}H_{38}N_{10}O_{10}P_2Si$): C, H, N.

9-[5′-*O*-(phosphono)-β-D-furanosyl]adenine 3′-[1-(Adenin-7-ylethoxy)methyl]phosphonate (24). To a solution of **23** (0.36 g, 0.50 mmol) in THF (5.0 mL) was added *n*-Bu_4NF (1.0 M solution in THF, 0.31 g, 1.2 mmol). Acetic acid (0.50 mL) was added to the mixture after it was stirred at 25 °C for 30 min. The solvents were removed, and the residue was purified by use of Whatman 3-MM paper with a mixture of *i*-PrOH, NH_4OH, and H_2O (9:1:2) as the eluent. The band at ca. R_f 0.35 was eluted with H_2O and collected by lyophilization to give **24** (0.27 g, 0.45 mmol) in 90% yield: mp >250 °C (decomp); UV (EtOH) λ_{max} 264 (ϵ 18 200); ^{1}H NMR (CD_3OD) δ 3.75−4.18 (m, 8 H, $CH_2N + CH_2O + CH_2OP + CH_2P$), 4.29−4.70 (m, 3H, $HC_{2'} + HC_{3'} + HC_{4'}$), 6.48 (d, J = 4.5 Hz, 1 H, $HC_{1'}$), 7.99, 8.39 (2s, 2 H, 2 HC_2), 8.26, 8.83 (2s, 2 H, 2 HC_8). Anal. Calcd ($C_{18}H_{24}N_{10}O_{10}P_2$): C, H, N.

9-[[(*Z*)-4-(Ethylidenyl)-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide]-5′-*O*-phosphono-β-D-furanosyl]adenine 3′-[1-(Adenin-7-ylethoxy)methyl]phosphonate (25). To a solution of **24** (0.300 g, 0.499 mmol) in DMF (20 mL) was added $NaHCO_3$ (0.30 g, 3.6 mmol). The reaction mixture was stirred at 25 °C under N_2 for 10 min. Then, butenolide **26** (0.10 g, 0.50 mmol) was added and the mixture was stirred under N_2 for 1.0 h. The solution was diluted with EtOAc (50 mL) and aqueous HCl solution (1%, 40 mL). The organic layer was separated and washed with H_2O (50 mL). Then, it was dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. Purification by use of silica gel column chromatography with EtOAc/MeOH (6:4) as eluant afforded **25** (0.32 g, 0.42 mmol) in 85% yield: mp >237 °C (decomp); UV (EtOH) λ_{max} 215 (ϵ 16 000), λ_{max} 264 (ϵ 18 540); ^{1}H NMR (CD_3OD) δ 3.69−4.12 (m, 16 H, $CH_2N + CH_2O + 2\ CH_2OP + CH_2P + C_2OCH_3 + C_3OCH_3$), 4.31−4.78 (m, 3H, $HC_{2'} + HC_{3'} + HC_{4'}$), 5.38 (t, J = 7.0 Hz, 1 H, =CH), 6.51 (d, J = 4.8 Hz, 1 H, $HC_{1'}$), 8.02, 8.40 (2s, 2 H, 2 HC_2), 8.28, 8.86 (2s, 2 H, 2 HC_8). Anal. Calcd ($C_{26}H_{32}N_{10}O_{14}P_2$): C, H, N.

[1-(Adenin-9-ylethoxy)methyl]phosphono-(*Z*)-4-(ethylidenyl)-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (28). Compound **28** (3.90 g, 8.80 mmol) was prepared in 88% yield from **4** (2.73 g, 9.99 mmol) and **26** (2.20 g, 10.0 mmol) in the same manner that **25** was prepared from **24**: mp >241 °C (decomp); R_f (hexanes/EtOAc = 1:2) 0.12; UV (EtOH) λ_{max} 218 (ϵ 13 097), λ_{max} 259 (ϵ 14 700); ^{1}H NMR (CD_3OD) δ 3.57(t, J = 6.0 Hz, 2 H, CH_2N), 3.69 (d, J = 9.0 Hz, 2 H, CH_2P), 3.89 (m, 5 H, $C_2OCH_3 + CH_2OP$), 4.06, (t, J = 6.0 Hz, 2 H, CH_2O), 4.13 (s, 3 H, C_3OCH_3), 5.41 (t, J = 7.0 Hz, 1H, =CH), 8.12 (s, 1 H, HC_2), 8.21 (s, 1 H, HC_8); MS m/z 441 (M^+). Anal. Calcd ($C_{16}H_{20}N_5O_8P$): C, H, N.

[1-(Guanin-9-ylethoxy)methyl]phosphono-(*Z*)-4-(ethylidenyl)-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (29). Compound **29** (3.7 g, 8.0 mmol) was prepared in 80% yield from **27** (2.89 g, 9.99 mmol) and **26** (2.20 g, 10.0 mmol) in the same manner that **25** was prepared from **24**: mp >260 °C (decomp); R_f (hexanes/EtOAc = 1:2) 0.05; UV (EtOH) λ_{max} 252 (ϵ 11 097), λ_{max} 273 (ϵ 8100); ^{1}H NMR (CD_3OD) δ 3.71(t, J = 7.2 Hz, 2 H, CH_2N), 3.79 (d, J = 9.1 Hz, 2 H, CH_2P), 3.92 (m, 5 H, $C_2OCH_3 + CH_2OP$), 4.10, (t, J = 7.2 Hz, 2 H, CH_2O), 4.18 (s, 3 H, C_3OCH_3), 5.50 (t, J = 6.8 Hz, 1 H, =CH), 8.76 (s, 1 H, HC_8); MS m/z 457 (M^+). Anal. Calcd ($C_{16}H_{20}N_5O_9P$): C, H, N.

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Supporting Information Available: Two tables showing results of the solubility and lipophilicity of nucleoside and nucleotide analogues and anti-DNA virus and anticellular activities of nucleoside and nucleotide analogues in tissue culture. This material is available free of charge via the Internet at http://pubs.acs.org.

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