Synthesis and Biological Evaluation of Purine-Containing Butenolides

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6-Chloropurine derivatives of γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **3a**, γ-(*Z*)-ethylidene-2-methoxy-3-(4-nitro)benzyloxybutenolide **3b**, γ-(*Z*)-ethylidene-2-(4-nitro)benzyloxy-3-methoxybutenolide **3c**, γ-(*Z*)-ethylidene-2,3-di(4-nitro)benzyloxybutenolide **3d**, and dimethylphosphono-γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **11** as well as the adenine derivative of γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **6** were synthesized. The key steps in the high-yield synthesis of **6** involved hydration/dehydration of the C_4═C_5 in the precursor **3a**. In the presence of NH_4OH at elevated temperature, **3a** underwent a reverse Michael-type addition with water to produce hydrate **5**. At 37 °C, **6** was also hydrated in the presence of *S*-adenosyl-L-homocysteine hydrolase to afford **5**. Butenolide **6** exhibited an inhibitory property toward the enzyme. Such type II (enzyme-mediated addition of water across C_4═C_5) mechanism is the first example of "enzyme-substrate intermediate" inactivation of *S*-adenosyl-L-homocysteine hydrolase. In contrast with type I mechanism-based inactivation, reduction of enzyme-bound $NADP^+$ to NADPH was not observed. Upon treatment with HCl, stereoselective dehydration of **5** occurred to give the target molecule **6**. At ambident temperature, **3a** was hydrated in the presence of NH_4OH or pig liver esterase to produce 6-chloropurine derivative **4**. An unambiguous proof of the structures of **3**−**5** was obtained by X-ray crystallographic analysis. For the synthesis of phosphonate derivative **11**, the key step involved chlorination of phosphonate **9** by use of CF_3SO_2Cl and 1,8-diazabicyclo[5.4.0]undec-7-ene in CH_2Cl_2. 6-Chloropurine-containing butenolide **3d**, 6-chloropurine derivative of 4-hydroxybutenolide **4**, and adenine-containing 4-hydroxybutenolide **5** did not show anticancer and antiviral activities. 6-Chloropurine-containing ethylidene-2,3-dialkoxybutenolides **3a**−**c** and phosphonate **11**, however, exhibited inhibitory activity against murine leukemias (L1210 and P388), breast carcinoma (MCF7), and human T-lymphoblasts (Molt4/C8 and CEM/0) cell lines. They were also notably active toward thymidine kinase-deficient varicella-zoster virus (TK^-VZV). Adenine-containing ethylidene-2,3-dimethoxybutenolide **6** exhibited marked selectivity in cytostatic activity against the murine leukemia (P388) cell line.

Introduction

The biological importance of unsaturated lactones is well known.[1−7] In particular, the γ-alkylidenebutenolide skeleton[8,9] is a useful entity that is present in natural products such as fibrolides,[10] dihydroxerulin,[11] and protoanemonin.[12] Protoanemonin, its analogues, and its derivatives possess antiviral, antibiotic, and anticancer activities.[12,13] Thus, unsaturated analogues of nucleosides are a focus of much attention as antiviral and antitumor agents.[13−18]

Mechanism-based inhibition of enzymes that are crucial to metabolic pathways involved in cell division is an attractive concept for the design of rational chemotherapeutic agents.[19,20] Such an enzyme in the nucleic acid manifold is *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase) (EC 3.3.1.1).[21] AdoHcy hydrolase catalyzes the hydrolysis of AdoHcy to Hcy and adenosine.[22,23] This regulatory enzyme modulates the continuous biosynthesis of nucleic acids by cleavage of AdoHcy.[23] Thus, it has attracted considerable attention as a target for the design of antiviral and/or anticancer drugs.[24−27]

Herein we report the synthesis of a new series of purine derivatives of unsaturated lactones **3a**−**d**, **4**−**6**, and **11**, their inhibitory effects on AdoHcy hydrolase, and their antiviral as well as anticancer activities.

Chemistry

Synthesis of Purine-Substituted Butenolides 3a−d and 6 (Scheme 1). We treated ditosylates **1a**−**d**[8,28] with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and *n*-Bu_4NCl in CH_3CN at reflux to produce the corresponding chloro-γ-(*Z*)-ethylidene-2,3-dialkoxybutenolides **2a**−**d** in about 80% yield.[8] The structures of **2c** and **2d** were confirmed by X-ray crystallography (data not shown). The stereochemistry of the C_4═C_5 in **2a** and **2b** was deduced from ^1H NMR characteristics of the olefin proton.[8,29] Chloro compounds **2a**−**d** were condensed, individually, with 6-chloropurine by use of Et_3N

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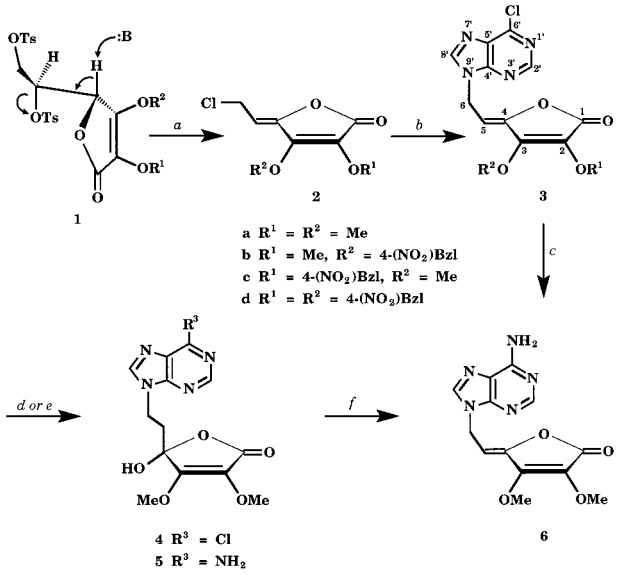
Scheme 1. Synthesis of Purine-Containing Butenolides **3**−**6**[a]



[a] Reagents: (a) DBU, CH_3CN, *n*-Bu_4NCl, reflux, 16 h (80% for **2a**, 79% for **2b**, 83% for **2c**, 81% for **2d**); (b) 6-chloropurine, DMF, Et_3N, 25 °C, 16 h, (80% for **3a**, 80% for **3b**, 70% for **3c**, 76% for **3d**); (c) NH_3 (gas), CH_3CN, pressure bottle, 80 °C, 30.0 h, 16%; (d) For **4**: NH_4OH, CH_3CN, 25 °C, 1.0 h, 84%; (e) For **5**: NH_4OH, CH_3CN, 60 °C, 3.0 h, 80%; (f) HCl (gas), EtOAc or CH_3CN, reflux, 2.0 h, 80−85%.

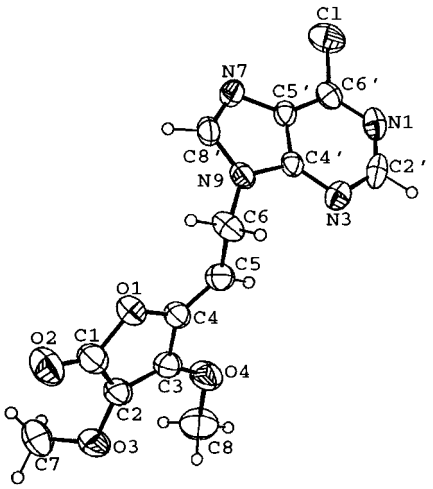


Figure 1. ORTEP view of the crystal structure of 6-chloropurin-9-yl-γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **3a**.

in DMF to afford the corresponding N_9-alkylated products **3a**−**d** (70−80% yields). Stereostructure of **3a** was determined by its X-ray crystallographic analysis (Figure 1).

Treatment of **3a** with NH_3 gas in CH_3CN at 80 °C resulted in destruction of **3a**, and the target molecule **6** was obtained in 16% yield only. We believe that the reactivity of C_4=C_5 in **3a** was responsible for the low-yield preparation of adenine-containing γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **6**. We took advantage of this reactivity and proposed to protect the C_4=C_5 in **3a** through a reverse Michael-type addition of H_2O. To evaluate this hypothesis, we calculated bond charge (δq) for C_4=C_5 in **3a** (C_4=C_5 $\delta q = C_5q - C_4q = -0.97e$); the δqs for the carbonyl of β-lactam nucleus in a serious of carbapenems were recently found to be about $-1.50e$.[30]

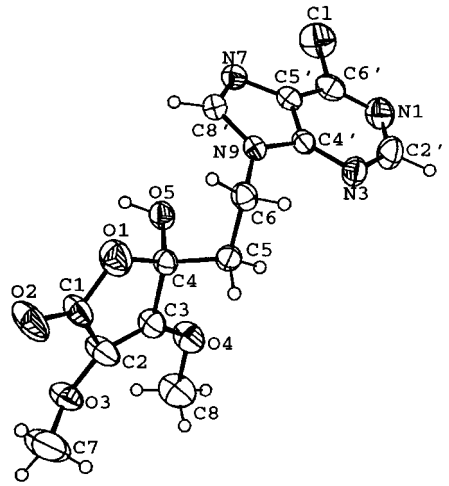


Figure 2. ORTEP view of the crystal structure of 6-chloropurin-9-yl-ethyl-4-hydroxy-2,3-dimethoxybutenolide **4**.

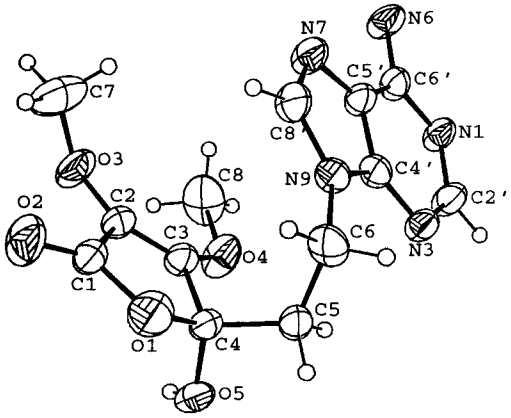
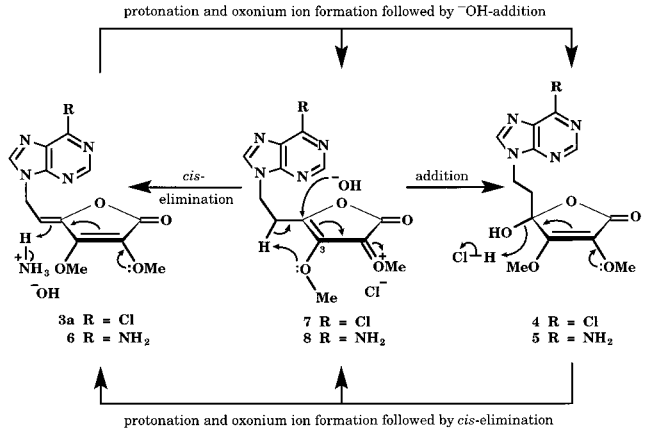


Figure 3. ORTEP view of the crystal structure of adenin-9-yl-ethyl-4-hydroxy-2,3-dimethoxybutenolide **5**.

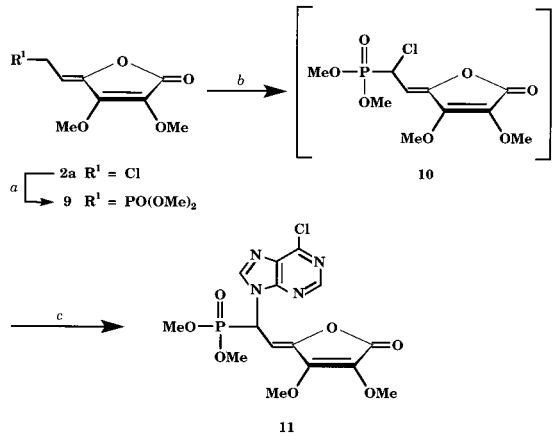
These results indicate the susceptibility of C-4 in **3a** toward nucleophilic attack by H_2O. Thus, treatment of **3a** with NH_4OH in CH_3CN at 25 °C afforded the racemic hydrated 6-chloropurine derivative **4** in 84% yield. Compound **3a** was also enantioselectively hydrated by use of pig liver esterase (PLE) at pH 6.8 to give **4**, $[\alpha]^{25}_D$ −15.40, in 95% yield. Furthermore, reaction of **3a** with NH_4OH in CH_3CN at 60 °C produced the racemic hydrated adenine derivative **5** in 80% yield. The stereostructures of **4** and **5** were determined by spectroscopic methods as well as X-ray crystallographic analyses (see Figures 2 and 3). A plausible mechanism for the hydration of **3a** is proposed in Scheme 2.

On the basis of microscopic reversibility, we hypothesized that dehydration of **4** or **5** via the corresponding intermediates **7** and **8** may produce high yields of the respective 6-chloropurine-containing γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **3a** and adenine-containing γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **6** (see Scheme 2). Thus, compounds **4** and **5** were separately treated with HCl gas in CH_3CN or EtOAc at reflux to afford **3a** (∼80% yield) and **6** (∼85% yield), respectively, in a stereoselective manner. As shown in Scheme 2, we believe that the oxygen of the methoxy group at the C-3 position of the intermediate **7** or **8** is responsible for the observed selectivity through a cis elimination process. Furthermore, we found by computer modeling that the

Scheme 2. Mechanism of the Hydration/Dehydration Reactions



Scheme 3. Synthesis of 6-Chloropurine-Containing Phosphonobutenolide **11**[a]



[a] Reagents: (a) $P(OMe)_3$, reflux, 3.0 h, 82%; (b) CF_3SO_2Cl, DBU, CH_2Cl_2, 0−25 °C, 1.5 h; (c) 6-chloropurine, DMF, Et_3N, 25 °C, 10 h, 48% overall yields.

Z-isomer **6** is thermodynamically more stable than the respective *E*-isomer by 2.18 kcal mol^{-1}.

Synthesis of 6-Chloropurine-Substituted Phosphonobutenolide 11 (Scheme 3). For the synthesis of racemic 6-chloropurine-containing dimethylphosphono-γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **11**, we reacted 6-chlorobutenolide **2a** with $P(OMe)_3$ at reflux. After 3.0 h, phosphonate **9** was obtained in 82% yield. Chlorination of **9** with CF_3SO_2Cl[31,32] and DBU in CH_2Cl_2 produced the intermediate **10**. This reactive compound without purification was subsequently treated with 6-chloropurine and Et_3N in DMF to afford **11** in 48% overall yield.

Biological Results and Discussion

Inhibition of AdoHcy Hydrolase. Using methodology previously described by Borchardt et al.,[22b,33,34] compounds **3a**−**d**, **4**−**6**, and **11** were evaluated for inhibitory property against purified bovine liver AdoHcy hydrolase. Neplanocin A, as potent inhibitor of AdoHcy hydrolase,[35] was used as a reference compound. The maximal inactivation at the highest concentration used (100 μM) was approximately 63% of the original enzyme activity with **3a**−**c** and 40% with **11** (Table 1). In contrast, adenine derivative **6** caused significant inactivation of AdoHcy hydrolase (90%) at a concentration of 100 μM. Compounds **3d**−**5** were practically inactive. 6-Chloropurine derivatives **3a**−**c** appear to have similar inhibitory property. Adenine derivative **6**, however, inactivated the enzyme more effectively than **3a**−**c** and **11**.

Table 1. Inhibition of AdoHcy Hydrolase with **3a**−**d**, **4**−**6**, **11**, and Neplanocin A

	enzyme activity remaining[a,b](%)			
compd	0.1 μM	1.0 μM	10.0 μM	100 μM
neplanocin A	21.83 ± 1.72	7.91 ± 0.12	2.61 ± 0.04	<0.05
3a	>99	86.90 ± 2.46	68.20 ± 1.95	34.97 ± 3.24
3b	>99	91.03 ± 4.12	71.23 ± 2.87	37.61 ± 2.50
3c	>99	87.97 ± 3.26	69.47 ± 2.61	36.18 ± 1.96
3d	>99	>99	95.18 ± 3.08	87.45 ± 2.76
(−)-**4**	>99	>99	>99	>99
(±)-**4**	>99	>99	>99	>99
5	>99	>99	>99	>99
6	87.98 ± 1.53	78.25 ± 2.06	53.26 ± 1.18	9.63 ± 1.03
11	>99	92.78 ± 3.18	84.77 ± 2.09	61.25 ± 2.17

[a] AdoHcy hydrolase (25.0 nM)[33,34] was incubated with varying concentrations of neplanocin A, **3a**−**d**, **4**−**6**, and **11** at 37 °C for 20 min, and the remaining enzyme activity was determined by measuring rates of formation of AdoHcy from Ado and Hcy as described.[22b] [b] Data are the average of duplicate determinations.

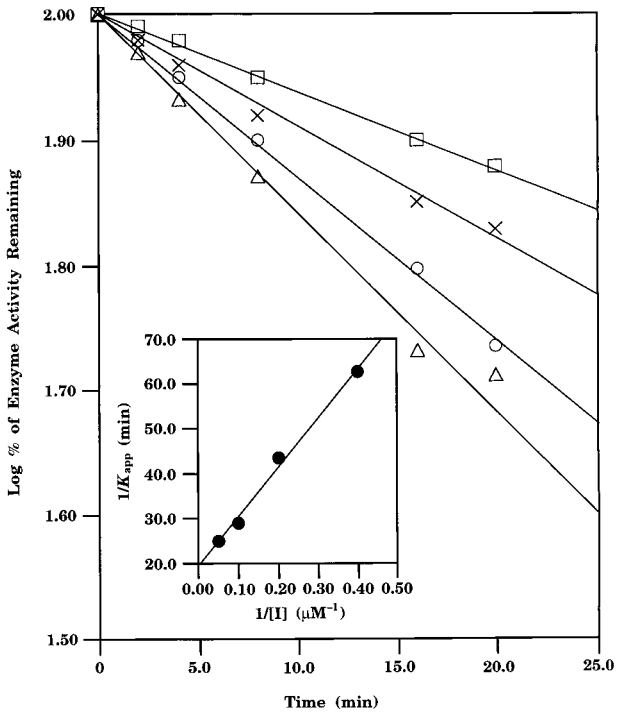


Figure 4. Time-dependent inactivation of AdoHcy hydrolase (25.0 nM) with **6** (□, 2.50 μM; ×, 5.00 μM; ○, 10.0 μM; Δ, 20.0 μM) in buffer solution (pH 7.20) at 37 °C. At the indicated time points, residual enzyme activity was determined.[22b] Inset: Plot of $1/k_{app}$ versus 1/[I] from which K_i and k_2 values were calculated.[22c] Data are the average of duplicate measurements.

Compound **6** was also shown to be a time-dependent inactivator of AdoHcy hydrolase (Figure 4). Kinetic analysis of its inactivation process by the Kitz and Wilson method[22c] gave the inactivation rate constant (k_2 = 0.052 min^{-1}), dissociation constant (K_i = 5.68 μM), and derived second-order rate constant ($k_2/K_i = 9.15 \times 10^3$ M^{-1} min^{-1}).

In contrast with type I mechanism-based inhibition, "cofactor depletion" in which enzyme-bound $NADP^+$ is reduced to NADPH, inactivation of AdoHcy hydrolase with **6** did not result in a change in the initial $NADP^+$/NADPH ratio.[22b] On the other hand, if activity of **6**

Table 2. Substrate Activities and Inhibitory Properties against Adenosine Deaminase[a]

substrate	K_m (μM)	rel V_{max}	K_i (μM)
adenosine	43.24	1.00	
5	>800		>800
6	265.63	1.97×10^{-5}	315.68

[a] Kinetic parameters of adenine derivatives as substrates or inhibitors of ADA (EC 3.5.4.4) were determined as described by Ogilvie et al.[36] and Moosavi-Movahedi et al.[37]

against AdoHcy hydrolase involves nucleophilic catalysis, it is conceivable that a covalent bond might be formed linking the enzyme and the inhibitor. As such, attack by amino acid functionalities (e.g., an amino group on Lys-426 or Arg-196) might cause type II (covalent binding) inhibition of the enzyme.[22b] In fact, compound **6** was enantioselectively hydrated to **5**, $[\alpha]^{25}_D$ −20.32, in the presence of AdoHcy hydrolase in phosphate buffer, pH 7.20, at 37 °C after 4 h, indicating that this compound is a substrate for hydrating activity of the enzyme. Such "enzyme-substrate intermediate" type inactivation of AdoHcy hydrolase would be unique.

Kinetic Studies of Competitive Inhibition of ADA. By following an established procedure,[36] we determined the rates of deamination of **5**, **6**, and adenosine in the presence of calf mucosal ADA (EC 3.5.4.4) in buffer solutions. Inhibition studies were carried out on the basis of the Kaplan method.[37] The results are shown in Table 2.

We found that compound **6** was an ADA substrate. The V_{max} of **6** was, however, 10^{-5} times less than that of adenosine. Compound **6** showed competitive inhibition of ADA when adenosine was used as a substrate. Compound **5** was neither a substrate nor an inhibitor of ADA.

Anticancer Activity. The anticancer screening experiments for compounds **3a**−**d**, **4**−**6**, **11**, as well as the reference compound 9-(β-D-arabinofuranosyl)cytosine (ara-C) were carried out in vitro against murine leukemias (L1210 and P388), breast carcinoma (MCF7), and human T-lymphocytes (Molt4/C8 and CEM/0). The activity is expressed as the concentration (μM) required to inhibit tumor cell proliferation by 50% (IC_{50}).[13a,13b] Results are listed in Table 3.

6-Chloropurine derivatives **3a**−**c** displayed more pronounced inhibition on the majority of examined cell lines. These compounds, however, are at least 10 times less active than ara-C (Table 3). On the other hand, toxicity (IC_{50}) of **3a**−**c** against uninfected cells is about 20 μM (see Table 4); yet ara-C possesses an IC_{50} value of 2.05×10^{-4} μM toward HeLa cells.[13c] Adenine-containing butenolide **6** exhibited marked selectivity in cytostatic activity against P388 leukemia. Phosphonate derivative **11** showed slight inhibitory activity against malignant murines and the human Molt4/C8. 6-Chloropurine-containing butenolide **3d** as well as the hydrated derivatives **4** and **5** were totally inactive against malignant tumor cell lines.

Antiviral Activity. We tested the synthesized compounds for their inhibition of cytopathogenicity of 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) in HeLa cell culture.[38,39] These compounds include **3a**−**d**, **4**−**6**, and **11**. Acyclovir was used as the reference compound. Toxicity of these compounds was evaluated by their ability to cause morphological changes in cells at different concentrations. The minimum inhibitory concentrations (IC_{50}), measured by use of the linear regression method,[39,40] are summarized in Table 4.

Compounds **3a**−**c**, **6**, and **11** were found to be more active than acyclovir against TK^-VZV. 6-Chloropurine

Table 3. Inhibitory Effects of Butenolides **3a**−**d**, **4**−**6**, **11**, and ara-C on the Growth of Malignant Tumor Cell Lines

	IC_{50}[a] (μM)				
compd	L1210	P388	MCF7	Molt4/C8	CEM/0
ara-C	0.17 ± 0.03	0.14 ± 0.01	1.03 ± 0.34	0.65 ± 0.02	0.78 ± 0.01
3a	4.52 ± 0.78	8.12 ± 1.03	16.87 ± 1.79	5.97 ± 0.92	4.71 ± 0.97
3b	5.81 ± 0.71	6.49 ± 0.96	15.43 ± 2.04	7.35 ± 1.01	3.98 ± 0.49
3c	6.03 ± 0.97	7.42 ± 1.01	14.21 ± 1.85	6.16 ± 0.74	5.04 ± 0.66
3d	>120	>120	>120	>120	>120
(±)-**4**	>120	>120	>120	>120	>120
5	>120	>120	>120	>120	>120
6	93.35 ± 3.25	2.67 ± 0.58	>120	78.46 ± 2.71	84.51 ± 3.12
11	16.25 ± 2.16	14.31 ± 1.08	90.11 ± 3.78	12.47 ± 0.96	96.05 ± 4.01

[a] The IC_{50} values were estimated from dose−response curves compiled from at least two independent experiments and represent the compound concentration (μM) required to inhibit tumor cell proliferation by 50%.

Table 4. Anti-Herpes and Anticellular Activities of **3a**−**d**, **4**−**6**, **11**, and Acyclovir in Tissue Culture

	IC_{50}[a] (μM)				
compd	HSV-1 (KOS)	HSV-2 (G)	TK^+VZV (YS)	TK^-VZV (YS/R)	HeLa cell[b]
acyclovir	0.11 ± 0.01	0.19 ± 0.02	8.71 ± 0.13	26.43 ± 1.02	253.64 ± 2.12
3a	10.91 ± 0.10	13.33 ± 0.12	5.92 ± 0.07	1.62 ± 0.11	23.37 ± 1.14
3b	9.83 ± 0.24	14.82 ± 0.23	4.40 ± 0.14	1.03 ± 0.02	19.32 ± 1.13
3c	7.24 ± 0.53	12.61 ± 0.53	6.32 ± 0.03	0.89 ± 0.31	20.18 ± 2.02
3d	28.45 ± 1.21	40.47 ± 0.87	37.49 ± 0.43	34.74 ± 0.38	16.97 ± 2.06
(±)-**4**	>128	>128	>128	>128	148.93 ± 2.35
(−)-**4**	>128	>128	>128	>128	132.43 ± 1.68
5	>128	>128	>128	>128	156.73 ± 3.16
6	14.54 ± 0.94	13.54 ± 0.94	12.82 ± 0.12	6.07 ± 0.29	42.35 ± 2.09
11	13.15 ± 1.02	16.17 ± 2.10	10.21 ± 0.16	5.03 ± 0.08	28.14 ± 1.14

[a] Inhibitory concentrations (IC_{50}) represent the mean of duplicate determinations.[38−40] [b] Concentration of the compound required to cause microscopically visible change or disruption in about 50% of the cell sheet.

derivatives **3a**−**c**, however, showed the most significant antiviral activity against TK⁻VZV. The antiviral activity of adenine derivative **6** and phosphonate **11** was also more against TK⁻VZV than other viruses. 6-Chloropurine derivative **3d** exhibited very weak antiviral activity. On the other hand, the hydrated derivatives **4** and **5** did not inhibit cytopathogenicity of the herpes viruses.

Summary and Conclusions

A series of new purines-containing alkylidenebutenolides were synthesized. These compounds include 6-chloropurine derivatives of γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **3a**, γ-(*Z*)-ethylidene-2-methoxy-3-(4-nitro)benzyloxybutenolide **3b**, γ-(*Z*)-ethylidene-2-(4-nitro)benzyloxy-3-methoxybutenolide **3c**, γ-(*Z*)-ethylidene-2,3-di(4-nitro)benzyloxybutenolide **3d**, and dimethylphosphono-γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **11** as well as adenine derivative of γ-(*Z*)-ethylidene-2,3-dimethoxybutenolide **6**. Compounds **3a**−**c** are weak inhibitors of AdoHcy hydrolase. Nevertheless, they showed notable antiviral and anticancer activities. On the other hand, **3d** possessing 2,3-di(4-nitro)benzyloxy groups is not an effective inhibitor of AdoHcy hydrolase. It was a weak antiviral agent and did not inhibit the growth of tumor cell lines. An ethylidene unit appears to be essential for biological activity. The C_4═C_5 in **11** is sterically hindered by an adjacent phosphonate functionality at C-6 position. As such, phosphonate **11** is an extremely weak inhibitor for AdoHcy hydrolase. Its antiviral and anticancer properties were also weak. Adenine derivative **6** inhibited AdoHcy hydrolase through an "enzyme-substrate intermediate" mechanism. This is the first example of type II nucleophilic catalysis inhibitor that catalyzed hydration of inhibitor **6** to **5**. In contrast with type I mechanism-based inactivation, reduction of enzyme-bound $NADP^+$ to NADPH was not observed in the presence of **6**. This compound inhibited specifically P388 leukemia cells but not the other ones. Compound **6** exhibited weak activity against herpes viruses. The lack of significant biological activity of **6** may be due to its deamination by ADA in the cellular system. Results from biological tests indicate that the hydrated derivatives **4** and **5** were totally inactive. Consequently, the biological properties of **3a**−**c**, **6**, and **11** may have its origin in the C_4═C_5.

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 Sigma or Fluka 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. For ^{31}P NMR, acetone-d_6 was used as solvent; $PO(Me)_3$ (δ 1.59 ppm) was used as an internal standard. UV−vis spectroscopy was carried out using 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 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.

Natural Bond Orbital (NBO) Partial Atomic Charges. Ab initio calculations were carried out by using the Gaussian 98 program.[41] Full geometry optimization of **3a** was performed at the HF/3-21G* level. Single-point HF/6-31G* calculations were carried out to evaluate the natural bond orbital (NBO) partial atomic charges.[42] The results were analyzed in terms of a bond charge (δq), which is defined as the difference between the atomic charges of the terminal and initial atoms of a given bond, $\delta q = q_t - q_i$. The bond charge can be regarded as a measure of the chemical bond polarity.

$$\text{For } \mathbf{3a}: \quad C_4{=}C_5\ \delta q = C_5 q - C_4 q = -0.97e$$

Crystallographic Analysis. Crystals of **3a** for X-ray structure analysis were prepared by growth under slow evaporation at room temperature of the dilute solution of EtOAc. For **4** and **5**, crystallization was performed in a dilute solution of MeOH. Specimens of suitable quality were mounted in a thin walled glass capillary and used for measurement of precise cell constants and intensity data collection. Diffraction measurements were made on an Enraf-Nonius CAD-4 diffractometer by use of graphite-monochromatized Mo Kα radiation ($\lambda = 0.71069$ Å). Unit cell parameters were obtained by a least-squares fit to the automatically centered settings for 25 reflections. Intensity data collected by use of $\omega/2\theta$ scan mode. All intensity data were collected for Lorentz polarization and absorption (empirical ψ corrections). The structures of **3a**, **4**, and **5** were solved by direct methods (Solver).[43] All non-hydrogen atoms were located from the difference Fourier maps, and were refined by full-matrix least squares procedures as well as with anisotropic displacement factors. Calculations and full matrix least-squares refinements were performed by utilization of the *NRCVAX* program package.[43,44]

Crystal data for **3a**: $M_r = 322.05$, space group $P2_1/C$, $a = 7.0560$ (4) Å, $b = 28.0260$ (4) Å, $c = 7.1563$ (8) Å, $\beta = 95.190$ (3)°, $V = 1409.3$ (8) Å^3, $Z = 4$, $F(000) = 668$, $d_\alpha = 1.526$ g cm^{-3}, μ(Mo Kα) = 0.290 mm^{-1}, $S = 0.9808$, $R_f/R_w = 0.044/0.057$ for 200 parameters and 1765 reflections, and $R_f/R_w = 0.069/0.058$ for all 2490 independent reflections measured in the range 2.50−2Θ-50.00°.

Crystal data for **4**: $M_r = 340.06$, space group Pm^{-1}, $a = 7.7690$ (8) Å, $b = 9.8250$ (3) Å, $c = 10.1511$ (12) Å, $\alpha = 79.172$ (17)°, $\beta = 78.350$ (9)°, $\gamma = 82.412$ (18)°, $V = 741.8$ (3) Å^3, $Z = 2$, $F(000) = 352$, $d_\alpha = 1.526$ g cm^{-3}, μ (Mo Kα) = 0.290 mm^{-1}, $S = 0.9860$, $R_f/R_w = 0.050/0.061$ for 217 parameters and 2077 reflections, and $R_f/R_w = 0.064/0.062$ for all 2603 independent reflections measured in the range 2.50−2Θ-50.00°.

Crystal data for **5**: $M_r = 321.11$, space group Pm^{-1}, $a = 8.1060$ (4) Å, $b = 9.6871$ (21) Å, $c = 13.0600$ (4) Å, $\alpha = 98.636$ (21)°, $\beta = 90.490$ (3)°, $\gamma = 109.080$ (3)°, $V = 956.3$ (6) Å^3, $Z = 2$, $F(000) = 400$, $d_\alpha = 1.324$ g cm^{-3}, μ (Mo Kα) = 0.100 mm^{-1}, $S = 0.8005$, $R_f/R_w = 0.076/0.089$ for 244 parameters and 2190 reflections, and $R_f/R_w = 0.112/0.090$ for all 3375 independent reflections measured in the range 2.50−2Θ-50.00°.

Purification of AdoHcy Hydrolase and Evaluation of the Effectiveness of Potential Inhibitors. AdoHcy hydrolase was purified from bovin liver as described by Borchardt et al.[33,34] Incubation of AdoHcy hydrolase (25.0 nM) with varying concentrations of neplanocin A, **3a**−**d**, **4**−**6**, and **11**

in potassium phosphate buffer (500 μL; 50.0 mM, pH 7.20) containing EDTA (1.00 mM) at 37 °C for 20.0 min gave the data shown in Table 1. The remaining enzyme activity was determined by the method of Robins et al.[22b] This method involves measuring rates of formation of AdoHcy from Ado and Hcy in phosphate buffer containing EDTA at 37 °C for 15.0 min.

Determination of AdoHcy Hydrolase Inhibition Constants. Incubation of AdoHcy hydrolase (25.0 nM) with various concentrations of **6** (2.50−20.0 μM) for different times (0−20 min) gave the data shown in Figure 4.[22b] Time-dependent inactivation data were used to calculate the pseudo-first-order rate constants (k_{app}) by plotting the logarithm of the remaining enzyme activity versus time. The kinetic constants k_2 and K_i were obtained from a plot of $1/k_{app}$ versus 1/[inhibitor] using the equation $1/k_{app} = 1/k_2 + (K_i/k_2)/[I]$.[22c]

Analysis of E-NADP$^+$ and E-NADPH. To AdoHcy hydrolase (50 μM) in 1.0 mL of 50.0 mM phosphate buffer (pH 7.20) containing 1.0 mM EDTA was added **6** (15.0 mM), with mixing for 20 s. The UV spectrum (280−500 nm) was recorded periodically at 25 °C. The reference cell contained the same enzyme solution to which had been added 100 μL of water.[22b] No increase in the absorbance at 320−340 nm was observed within 20 min. Thus, the conversion of E-NADP$^+$ to E-NADPH did not occur.

Adenosine Deaminase Studies. The reported procedures were used for ADA.[36,37] Results are summarized in Table 2.

Anticancer Test Procedure in Vitro. Murine leukemias (L1210 and P388), breast carcinoma (MCF7), and human T-lymphoblasts (Molt4/C8 and CEM/0) cell lines were cultured in DMEM supplemented with 10% FBS, 2.0 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.[13] Under this condition, the generation time for L1210, P388, MCF7, Molt4/C8, and CEM/0 cells was about 13, 12, 17, 18, and 21 h, respectively. Compounds **3a**−**d**, **4**−**6**, **11**, and ara-C, at various concentrations, were added to L1210, P388, MCF7, Molt4/C8, and CEM/0 cells (200 cells/mL) in their exponential phase of growth. The cell numbers of the control cultures, as well as that of the cultures supplemented with the test compounds, were determined after 24, 48, and 72 h of growth. The IC_{50} values were estimated from dose−response curves compiled from two independent experiments and represent the compound concentration (μM) required to inhibit proliferation of the respective malignant tumor cell lines by 50% after 72 h incubation (Table 3).

Antiviral and Anticellular Evaluations. The methods for measuring viruses-induced cytopathogenicity as well as the toxicity of **3a**−**d**, **4**−**6**, and **11** toward HeLa cell have been described previously.[38−40] Results are summarized in Table 4.

***(Z)*-4-(2-Chloroethylidene)-2,3-dimethoxy-$\Delta^{\alpha,\beta}$-butenolide (2a).** To a solution of ditosylate **1a** (5.12 g, 9.99 mmol) in CH_3CN (100 mL) and DBU (1.53 g, 10.0 mmol) was added *n*-Bu_4NCl (2.78 g, 10.0 mmol). The reaction mixture was stirred at reflux for 16 h. The solution was concentrated under reduced pressure, and EtOAc (160 mL) was added. The EtOAc solution was washed with water (3 × 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 (hexanes/EtOAc = 8.5:1.5) afforded **2a** (1.63 g, 7.99 mmol) as an oil in 80% yield: R_f (hexanes/EtOAc = 1:1) 0.68; IR (CH_2Cl_2) ν 2920 (C_5H), 1782 (C=O), 1668 (C=C) cm^{-1}; UV (EtOH) λ_{max} 210 (log ϵ 5.10); ^1H NMR ($CDCl_3$) δ 3.88 (s, 3 H, C_2OCH_3), 4.08 (s, 3 H, C_3OCH_3), 4.30 (d, J = 7.50 Hz, 2 H, CH_2), 5.38 (t, J = 7.50 Hz, 1 H, =CH); ^{13}C NMR ($CDCl_3$) δ 36.32 (C_6), 59.21 (2-OCH_3), 60.10 (3-OCH_3), 103.41 (C_5), 125.20 (C_4), 143.52 (C_2), 148.01 (C_3), 164.51 (C=O); MS *m/z* 204 (M^+, Cl-cluster). Anal. ($C_8H_9O_4Cl$) C, H, Cl.

***(Z)*-4-(2-Chloroethylidene)-2-methoxy-3-(4-nitro)benzyloxy-$\Delta^{\alpha,\beta}$-butenolide (2b).** Compound **2b** (2.56 g, 7.86 mmol) was prepared in 79% yield from **1b** (6.30 g, 9.95 mmol), DBU (1.53 g, 10.0 mmol), and *n*-Bu_4NCl (2.78 g, 10.0 mmol) in CH_3CN (100 mL) by the method used for the synthesis of **2a** from **1a**: mp 134−136 °C; R_f (hexanes/EtOAc = 1:1) 0.48; IR (CH_2Cl_2) ν 2947 (C_5H), 1780 (C=O), 1659 (C=C),1527, 1344, 850 (NO_2) cm^{-1}; UV (EtOH) λ_{max} 206 (log ϵ 4.10), λ_{max} 275 (log ϵ 5.03); ^1H NMR ($CDCl_3$) δ 3.85 (s, 3 H, C_2OCH_3), 4.27 (d, J = 8.40 Hz, 2 H, CH_2), 5.53 (t, J = 8.40 Hz, 1 H, =CH),5.52 (s, 2 H, CH_2), 7.51 (AB q, J = 8.10 Hz, 2 H, Ph), 8.24 (AB q, J = 8.10 Hz, 2 H, Ph); ^{13}C NMR ($CDCl_3$) δ 36.30 (C_6), 59.81 (2-OCH_3), 71.70 (3-OCH_2), 103.93 (C_5), 123.92 (C_4), 143.31 (C_2), 147.96 (C_3), 162.83 (C=O), 125.60−145.68 (Ph); MS *m/z* 325 (M^+, Cl-cluster). Anal. ($C_{14}H_{12}$ NO_6Cl) C, H, N, Cl.

***(Z)*-4-(2-Chloroethylidene)-2-(4-nitro)benzyloxy-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (2c).** Compound **2c** (2.69 g, 8.26 mmol) was prepared in 83% yield from **1c** (6.30 g, 9.95 mmol), DBU (1.53 g, 10.0 mmol), and *n*-Bu_4NF (2.78 g, 10.0 mmol) in CH_3CN (100 mL) by the method used for the synthesis of **2a** from **1a**: mp 126−128 °C; R_f (hexanes/EtOAc = 1:1) 0.51; IR (CH_2Cl_2) ν 2949 (C_5H), 1777 (C=O), 1655 (C=C), 1523, 1347, 853 (NO_2) cm^{-1}; UV (EtOH) λ_{max} 205 (log ϵ 4.14), λ_{max} 276 (log ϵ 5.11); ^1H NMR ($CDCl_3$) δ 3.97 (s, 3 H, C_3OCH_3), 4.19 (d, J = 8.19 Hz, 2 H, CH_2), 5.42 (t, J = 8.19 Hz, 1 H, =CH),5.26 (s, 2 H, CH_2), 7.49 (AB q, J = 8.60 Hz, 2 H, Ph), 8.17 (AB q, J = 8.60 Hz, 2 H, Ph); ^{13}C NMR ($CDCl_3$) δ 36.23 (C_6), 59.50 (3-OCH_3), 72.38 (2-OCH_2), 104.36 (C_5), 123.77 (C_4), 142.83 (C_2), 147.80 (C_3), 163.46 (C=O), 123.18−148.64 (Ph); MS *m/z* 325 (M^+, Cl-cluster). Anal. ($C_{14}H_{12}$ NO_6Cl) C, H, N, Cl.

***(Z)*-4-(2-Chloroethylidene)-2,3-di(4-nitro)benzyloxy-$\Delta^{\alpha,\beta}$-butenolide (2d).** Compound **2d** (3.60 g, 8.06 mmol) was prepared in 81% yield from **1d** (7.50 g, 9.95 mmol), DBU (1.53 g, 10.0 mmol), and *n*-Bu_4NF (2.78 g, 10.0 mmol) in CH_3CN (100 mL) by the method used for the synthesis of **2a** from **1a**: mp 149−151 °C; R_f (hexanes/EtOAc = 1:1) 0.38; IR (CH_2Cl_2) ν 2950 (C_5H), 1777 (C=O), 1655 (C=C),1528, 1347, 844 (NO_2) cm^{-1}; UV (EtOH) λ_{max} 204 (log ϵ 4.26), λ_{max} 275 (log ϵ 6.95); ^1H NMR ($CDCl_3$) δ 4.27 (d, J = 8.27 Hz, 2 H, CH_2), 5.28 (s, 2H, C_2-OCH_2), 5.47 (s, 2 H, C_3-OCH_2), 5.56 (t, J = 8.27 Hz, 1 H, =CH), 7.45, 7.46 (2AB q, J = 8.54 Hz, 4 H, Ph), 8.18, 8.22 (2AB q, J = 8.54 Hz, 4 H, Ph); ^{13}C NMR ($CDCl_3$) δ 36.15 (C_6), 71.89 (2-OCH_2) 72.05 (3-OCH_2), 104.79 (C_5), 123.83 (C_4), 142.60 (C_2), 147.93 (C_3), 162.84 (C=O), 123.76−146.55 (Ph); MS *m/z* 446 (M^+, Cl-cluster). Anal. ($C_{20}H_{15}N_2O_8Cl$) C, H, N, Cl.

***(Z)*-4-[2-(6-Chloropurin-9-yl)ethylidene]-2,3-dimethoxy-$\Delta^{\alpha,\beta}$-butenolide (3a).** To a solution of 6-chloropurine (1.55 g, 10.0 mmol) and Et_3N (5.16 g, 51.0 mmol) in DMF (50 mL) was added **2a** (2.05 g, 10.0 mmol). The reaction mixture was stirred at 25 °C for 16 h. It was then diluted with EtOAc (150 mL) and H_2O (100 mL). The organic layer was separated and then washed with H_2O (4 × 100 mL). It was then dried over $MgSO_4$-(s) and concentrated under reduced pressure to yield an oil. Purification was carried out by use of column chromatography (hexanes/EtOAc = 1:1) to afford compound **3a** (2.58 g, 8.01 mmol) in 80% yield: mp 105−107 °C; R_f (EtOAc) 0.34; IR (CH_2Cl_2) ν 3115 ($C_{8'}H$), 3050 ($C_{2'}H$), 2920 (C_5H), 1778 (C=O), 1690 (C=C) cm^{-1}; UV (EtOH) λ_{max} 204 (log ϵ 4.60), λ_{max} 264 (log ϵ 4.30); ^1H NMR ($CDCl_3$) δ 3.90 (s, 3 H, C_2OCH_3), 4.09 (s, 3 H, C_3OCH_3), 5.11 (d, J = 7.66 Hz, 2 H, CH_2), 5.54 (t, J = 7.66 Hz, 1 H, =CH), 8.14, 8.74 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR ($CDCl_3$) δ 38.40 (C_6), 59.41 (2-OCH_3), 60.20 (3-OCH_3), 99.71 (C_5), 125.21 (C_4), 131.40 ($C_{5'}$), 144.80 (C_2), 144.91 ($C_{2'}$), 147.70 ($C_{4'}$), 151.01 (C_3), 151.52 ($C_{6'}$), 152.01 ($C_{8'}$), 163.22 (C=O); MS *m/z* 322 (M^+, Cl-cluster). Anal. ($C_{13}H_{11}N_4O_4Cl$) C, H, N, Cl.

***(Z)*-4-[2-(6-Chloropurin-9-yl)ethylidene]-2-methoxy-3-(4-nitro)benzyloxy-$\Delta^{\alpha,\beta}$-butenolide (3b).** Compound **3b** (3.54 g, 7.98 mmol) was prepared in 80% yield from **2b** (3.25 g, 9.98 mmol), 6-chloropurine (1.55 g, 10.0 mmol), and Et_3N (5.16 g, 51.0 mmol) in DMF (50 mL) by the method used for the synthesis of **3a** from **2a**: mp 139−141 °C; R_f (EtOAc) 0.23; IR (CH_2Cl_2) ν 3115 ($C_{8'}H$), 3050 ($C_{2'}H$), 2920 (C_5H), 1780 (C=O), 1659 (C=C) 1523, 1344, 844 (NO_2) cm^{-1}; UV (EtOH) λ_{max} 204 (log ϵ 4.60), λ_{max} 264 (log ϵ 4.30), 273 sh; ^1H NMR ($CDCl_3$) δ 3.86 (s, 3 H, C_2OCH_3), 5.12 (d, J = 7.60 Hz, 2 H, CH_2), 5.49 (s, 2H, C_3OCH_2), 5.59 (t, J = 7.60 Hz, 1 H, =CH), 7.46 (AB q, J = 8.57 Hz, 2 H, Ph), 8.17 (AB q, J = 8.57 Hz, 2 H, Ph) 8.18, 8.70 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR ($CDCl_3$) δ 38.51 (C_6), 59.83 (2-OCH_3), 71.80 (3-OCH_2), 100.07 (C_5), 123.82 (C_4), 127.82 ($C_{5'}$), 144.68 (C_2), 144.92 ($C_{2'}$), 145.38 ($C_{4'}$), 147.93 (C_3),

151.43 ($C_{6'}$), 151.63 ($C_{8'}$), 162.56 (C═O), 123.87−144.68 (Ph); MS *m*/*z* 443 (M^+, Cl-cluster). Anal. ($C_{19}H_{14}N_5O_6Cl$) C, H, N, Cl.

(*Z*)-4-[2-(6-Chloropurin-9-yl)ethylidene]-2-(4-nitro)benzyloxy-3-methoxy-Δ$^{\alpha,\beta}$-butenolide (3c). Compound **3c** (1.55 g, 3.49 mmol) was prepared in 70% yield from **2c** (1.63 g, 4.99 mmol), 6-chloropurine (0.78 g, 5.0 mmol), and Et_3N (25.8 g, 25.5 mmol) in DMF (26 mL) by the method used for the synthesis of **3a** from **2a**: mp 145−147 °C; R_f (EtOAc) 0.28; IR (CH_2Cl_2) *ν* 3105 ($C_{8'}H$), 2954 ($C_{2'}H$), 2922 (C_5H), 1774 (C═O), 1658 (C═C) 1523, 1344, 894 (NO_2) cm^{-1}; UV (EtOH) λ_{max} 206 (log ϵ 4.66), λ_{max} 265 (log ϵ 4.28), 272 sh; ^{1}H NMR ($CDCl_3$) δ 4.06 (s, 3 H, C_3OCH_3), 5.11 (d, J = 7.70 Hz, 2 H, CH_2), 5.34 (s, 2H, C_2OCH_2), 5.57 (t, J = 7.70 Hz, 1 H, ═CH), 7.54 (AB q, J = 8.82 Hz, 2 H, Ph), 8.17 (AB q, J = 8.82 Hz, 2 H, Ph) 8.25, 8.74 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR ($CDCl_3$) δ 38.52 (C_6), 59.62 (3-OCH_3), 72.48 (2-OCH_2), 100.59 (C_5), 123.37 (C_4), 128.68 ($C_{5'}$), 144.66 (C_2), 144.81 ($C_{2'}$), 147.82 ($C_{4'}$), 148.03 (C_3), 151.47 ($C_{6'}$), 152.19 ($C_{8'}$), 163.25 (C═O), 123.93−143.60 (Ph); MS *m*/*z* 443 (M^+, Cl-cluster). Anal. ($C_{19}H_{14}N_5O_6Cl$) C, H, N, Cl.

(*Z*)-4-[2-(6-Chloropurin-9-yl)ethylidene]-2,3-di(4-nitro)benzyloxy-Δ$^{\alpha,\beta}$-butenolide (3d). Compound **3d** (4.29 g, 7.60 mmol) was prepared in 76% yield from **2d** (4.46 g, 10.0 mmol), 6-chloropurine (1.55 g, 10.0 mmol), and Et_3N (51.6 g, 51.0 mmol) in DMF (50 mL) by the method used for the synthesis of **3a** from **2a**: mp 164−166 °C; R_f (EtOAc) 0.16; IR (CH_2Cl_2) *ν* 3025 ($C_{8'}H$), 2965 ($C_{2'}H$), 2928 (C_5H), 1782 (C═O), 1660 (C═C) 1528, 1349, 898 (NO_2) cm^{-1}; UV (EtOH) λ_{max} 205 (log ϵ 4.57), λ_{max} 266 (log ϵ 4.28), 277 sh; ^{1}H NMR ($CDCl_3$) δ 5.14 (d, J = 7.50 Hz, 2 H, CH_2), 5.31 (s, 2H, C_2OCH_2), 5.46 (s, 2H, C_3OCH_2), 5.63 (t, J = 7.50 Hz, 1 H, ═CH), 7.38, 7.48 (2AB q, J = 8.50 Hz, 4 H, Ph), 8.19, 8.23 (2AB q, J = 8.50 Hz, 4 H, Ph); 8.24, 8.76 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR ($CDCl_3$) δ 38.56 (C_6), 72.04 (2-OCH_2), 72.30 (3-OCH_2), 101.04 (C_5), 123.97 (C_4), 127.76 ($C_{5'}$), 144.37 (C_2), 144.85 ($C_{2'}$), 146.27 ($C_{4'}$), 148.04 (C_3), 151.97 ($C_{6'}$), 152.27 ($C_{8'}$), 162.64 (C═O), 128.64−142.42 (Ph); MS *m*/*z* 564 (M^+, Cl-cluster). Anal. ($C_{25}H_{17}N_6O_8Cl$) C, H, N, Cl.

(±)-**4-Hydroxy-4-[2-(6-Chloropurin-9-yl)ethyl]-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (4).** To a solution of **3a** (1.67 g, 5.17 mmol) in CH_3CN (50 mL) was added 25% of aqueous NH_4-OH solution (40 mL). The mixture was stirred at 25 °C for 1.0 h. The solution was concentrated under reduced pressure to afford a solid. Purification by use of column chromatography (EtOAc/MeOH = 9:1) gave compound (±)-**4** (1.48 g, 4.35 mmol) in 84% yield: mp 112−114 °C; R_f (EtOAc) 0.21; IR (KBr) *ν* 3219 (OH), 3100 ($C_{8'}H$), 3070 ($C_{2'}H$), 1775 (C═O), 1670 (C═C) cm^{-1}; UV (EtOH) λ_{max} 263 (log ϵ 4.21); ^{1}H NMR ($CDCl_3$) δ 2.41−2.55 (m, 2 H, C_5H_2), 3.73 (s, 3 H, C_2OCH_3), 3.99 (s, 3 H, C_3OCH_3), 4.48 (m, 2 H, C_6H_2), 6.98 (s, 1 H, OH), 8.41, 8.70 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR ($CDCl_3$) δ 35.70 (C_5), 39.81 (C_6), 59.24 (2-OCH_3), 60.85 (3-OCH_3), 82.74 (C_4), 124.45 (C_2), 131.36 ($C_{5'}$), 146.12 ($C_{4'}$), 150.73 (C_3), 151.71 ($C_{2'}$), 151.80 ($C_{6'}$), 155.13 ($C_{8'}$), 170.50 (C═O); MS *m*/*z* 322 (M^+-H_2O, Cl-cluster). Anal. ($C_{13}H_{13}N_4O_5Cl$) C, H, N, Cl.

(−)-4-Hydroxy-4-[2-(6-Chloropurin-9-yl)ethyl]-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (4). To a solution of **3a** (0.837 g, 2.59 mmol) in acetone (14.0 mL) was added 0.10 M phosphate buffer solution (pH 6.80, 13.0 mL) containing PLE (EC 3.1.1.1, 150 mg). After the mixture was stirred for 48 h at 25 °C, the acetone was evaporated, and the aqueous solution was kept at 10 °C to afford (−)-**4** (0.840 g, 2.46 mmol) as white crystals in 95% yield: mp 112−114 °C; $[\alpha]^{25}_D$ −15.40 (*c* 1.02, MeOH).

(±)-**4-Hydroxy-4-[2-(adenin-9-yl)ethyl]-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (5).** To a solution of **3a** (0.193 g, 0.599 mmol) in CH_3CN (5.0 mL) was added 25% of aqueous NH_4OH solution (6.0 mL) in a pressure bottle. The mixture was stirred at 60 °C for 3.0 h. The solution was concentrated under reduced pressure, and the residue was purified by use of column chromatography (EtOAc/MeOH = 7:3) to afford (±)-**5** (0.154 g, 0.479 mmol) in 80% yield: mp 144−146 °C; R_f (EtOAc) 0.08; IR (KBr) *ν* 3245−3100 (OH, NH_2), 3110 ($C_{8'}H$), 3050 ($C_{2'}H$), 1774 (C═O), 1669 (C═C) cm^{-1}; UV (EtOH) λ_{max} 259 (log ϵ 4.31); ^{1}H NMR (CD_3OD) δ 2.29−2.49 (m, 2 H, C_5H_2), 3.69 (s, 3 H, C_2OCH_3), 3.88 (s, 3 H, C_3OCH_3), 4.28 (m, 2 H, C_6H_2), 8.07, 8.19 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR (CD_3OD) δ 36.80 (C_5), 40.38 (C_6), 59.56 (2-OCH_3), 61.41 (3-OCH_3), 83.35 (C_4), 119.91 (C_2), 126.12 ($C_{5'}$), 142.84 ($C_{4'}$), 150.64 (C_3), 153.63 ($C_{2'}$), 155.91 ($C_{6'}$), 157.19 ($C_{8'}$), 171.41 (C═O); MS *m*/*z* 303 (M^+ − H_2O). Anal. ($C_{13}H_{15}N_5O_5$) C, H, N.

(−)-**4-Hydroxy-4-[2-(adenin-9-yl)ethyl]-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (5).** To a solution of **6** (0.012 g, 0.040 mmol) in 50 mM phosphate buffer solution (pH 7.20, 5.0 mL) containing 5.0 mM EDTA was added AdoHcy hydrolase (EC 3.3.1.1, 250 nM). After being stirred for 4 h at 37 °C, the reaction mixture was applied to a Sephadex G-25 spin column to remove (−)-**5** (0.011 g, 0.034 mmol) as white crystals in 85% yield: mp 145−147 °C; $[\alpha]^{25}_D$ −20.32 (*c* 1.02, MeOH).

(*Z*)-4-[2-(Adenin-9-yl)ethylidene]-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (6). Method 1. To a solution of **3a** (0.167 g, 0.517 mmol) in CH_3CN (10 mL) was added a saturated solution of CH_3CN/NH_3 (gas) (70 mL). The mixture was heated in a pressure bottle at 80 °C for 30 h. The solution was concentrated under reduced pressure to afford a solid. Purification by use of column chromatography (EtOAc/MeOH = 8:2) gave **6** (0.024 g, 0.080 mmol) in 16% yield: mp 138−140 °C; R_f (EtOAc) 0.14; IR (KBr) *ν* 3210−3150 (NH_2), 3115 ($C_{8'}H$), 3045 ($C_{2'}H$), 2930 (C_5H), 1775 (C═O), 1668 (C═C) cm^{-1}; UV (EtOH) λ_{max} 209 (log ϵ 4.51), λ_{max} 260 (log ϵ 4.20); ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3 H, C_2OCH_3), 3.99 (s, 3 H, C_3OCH_3), 4.91 (d, J = 7.80 Hz, 2 H, CH_2), 5.32 (t, J = 7.80 Hz, 1 H, ═CH), 7.20 (br, 2 H, NH_2), 8.07, 8.14 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR (DMSO-d_6) δ 30.67 (C_6), 59.23 (2-OCH_3), 60.48 (3-OCH_3), 101.36 (C_5), 128.24 (C_4), 133.31 ($C_{5'}$), 140.52 (C_2), 144.40 ($C_{2'}$), 149.46 ($C_{4'}$), 152.75 (C_3), 156.09 ($C_{6'}$), 156.14 ($C_{8'}$), 166.54 (C═O); MS *m*/*z* 303 (M^+). Anal. ($C_{13}H_{13}N_5O_4$) C, H, N.

Method 2. To a solution of **5** (0.120 g, 0.373 mmol) in EtOAc (10 mL) was added a saturated solution of EtOAc/HCl (gas) (50 mL). The suspension was heated at reflux for 2.0 h. Evaporation and purification of the residue by use of column chromatography (EtOAc/MeOH = 8:2) afforded **6** (96.3 mg, 0.317 mmol) in 85% yield.

Method 3. To a solution of **5** (0.360 g, 1.12 mmol) in CH_3CN (20 mL) was added a saturated solution of EtOAc/HCl (gas) (60 mL). The solution was heated at reflux for 2.0 h. Evaporation and purification of the residue by use of column chromatography (EtOAc/MeOH = 8:2) afforded **6** (0.272 g, 0.896 mmol) in 82% yield.

(*Z*)-4-(2-Dimethylphosphonoethylidene)-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (9). A solution of **2a** (2.20 g, 10.8 mmol) in $P(OMe)_3$ (13 mL) was heated at reflux for 3.0 h. Then, it was evaporated, and the residue was dissolved in EtOAc (80 mL). The EtOAc solution was washed with H_2O (2 × 50 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc) gave the product **9** (2.45 g, 8.82 mmol) as an oil in 82% yield: R_f (EtOAc) 0.38; IR (CH_2Cl_2) *ν* 3003 (C_5H), 1770 (C═O), 1650 (C═C), 1255 (P═O) cm^{-1}; UV (EtOH) λ_{max} 217 (log ϵ 5.81); ^{1}H NMR ($CDCl_3$) δ 2.79 (dd, J = 9.10, 21.01 Hz, 2 H, CH_2), 3.69 (d, J = 12.10 Hz, 6 H, $P(OCH_3)_2$), 3.85 (s, 3 H, C_2OCH_3), 4.07 (s, 3 H, C_3OCH_3), 5.25 (dt, J = 7.21, 12.56 Hz, 1 H, ═CH); ^{13}C NMR ($CDCl_3$) δ 30.88 (C_6), 36.33 ($P(OMe)_2$), 59.83 (2-OCH_3), 68.01 (3-OCH_3), 103.98 (C_5), 125.67 (C_4), 143.34 (C_2), 148.04 (C_3), 163.51 (C═O); MS *m*/*z* 278 (M^+, phosphorus cluster). Anal. ($C_{10}H_{15}O_7P$) C, H, P.

(±)-**(*Z*)-4-[2-Dimethylphosphono-2-(6-chloropurin-9-yl)-ethylidene]-2,3-dimethoxy-Δ$^{\alpha,\beta}$-butenolide (11).** To a stirred solution of **9** (2.78 g, 9.99 mmol) in CH_2Cl_2 (100 mL) and DBU (1.52 g, 9.98 mmol) under argon was added dropwise CF_3SO_2-Cl (1.68 g, 9.99 mmol) at 0 °C. The reaction mixture was allowed to warm to 25 °C within 1.5 h. The solvent was evaporated under reduced pressure to afford the crude intermediate **10**. Then, a solution of 6-chloropurine (1.54 g, 9.96 mmol) in DMF (50 mL) and Et_3N (3.03 g, 29.9 mmol) was added to the intermediate **10** and stirred at 25 °C for 10 h. The solution was partitioned between EtOAc (150 mL) and water (120 mL). The organic layer was separated and washed

with H_2O (4 × 100 mL). After it was dried over $MgSO_4$ (s), the solution was concentrated, and the residue was purified by use of column chromatography (EtOAc as eluant) to afford (±)-**11** (2.06 g, 4.80 mmol) as a foam in 48% overall yields: R_f (EtOAc) 0.24; IR (CH_2Cl_2) ν 3020 ($C_{8'}H$), 3004 ($C_{2'}H$), 2954 (C_5H), 1789 (C═O), 1697 (C═C), 1224 (P═O) cm^{-1}; UV (EtOH) λ_{max} 214 (log ϵ 5.62), λ_{max} 264 (log ϵ 4.31); ^1H NMR ($CDCl_3$) δ 3.77 (d, J = 12.02 Hz, 3 H, $P(OCH_3)$), 3.80 (d, J = 12.02 Hz, 3 H, $P(OCH_3)$), 3.81 (s, 3 H, C_2OCH_3), 4.17 (s, 3 H, C_3OCH_3), 6.54 (dd, J = 16.51, 32.98 Hz, 1 H, PCH), 7.15 (dd, J = 16.80, 21.25 Hz, 1 H, ═CH), 8.08, 8.70 (2 s, 2 H, $C_{2'}H$ + $C_{8'}H$); ^{13}C NMR ($CDCl_3$) δ 52.92 ($POCH_3$), 53.03 ($POCH_3$), 60.14 (2-OCH_3), 60.26 (3-OCH_3), 85.47 (C_6), 85.79 (C_5), 122.42 (C_4), 125.43 ($C_{5'}$), 132.12 (C_2), 140.26 (C_3), 140.38 ($C_{4'}$), 143.04 ($C_{2'}$), 152.36 ($C_{6'}$), 153.04 ($C_{8'}$), 164.40 (C═O); ^{31}P NMR (acetone-d_6) δ 15.08; MS m/z 430 (M^+, phosphorus cluster). Anal. ($C_{15}H_{16}N_4O_7ClP$) C, H, N, Cl, P.

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

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