Synthesis and Antiviral Activity of (*Z*)- and (*E*)-2,2-[Bis(hydroxymethyl)cyclopropylidene]methylpurines and -pyrimidines: Second-Generation Methylenecyclopropane Analogues of Nucleosides[1]

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The second generation of methylenecyclopropane analogues of nucleosides **5a**−**5i** and **6a**−**6i** was synthesized and evaluated for antiviral activity. The 2,2-bis(hydroxymethyl)methylenecyclopropane (**11**) was converted to dibromo derivative **7** via acetate **12**. Alkylation−elimination of adenine (**16**) with **7** afforded the *Z*/*E* mixture of acetates **17** + **18**, which was deacetylated to give analogues **5a** and **6a** separated by chromatography. A similar reaction with 2-amino-6-chloropurine (**19**) afforded acetates **20** + **21** and, after deprotection and separation, isomers **5f** and **6f**. The latter served as starting materials for synthesis of analogues **5b**, **5e**, **5g**−**5i** and **6b**, **6e**, **6g**−**6i**. Alkylation−elimination of N^4-acetylcytosine (**22**) with **7** afforded a mixture of isomers **5c** + **6c** which were separated via N^4-benzoyl derivatives **23** and **24**. Deprotection furnished analogues **5c** and **6c**. Alkylation of 2,4-bis(trimethylsilyloxy)-5-methylpyrimidine (**25**) with **7** led to bromo derivative **26**. Elimination of HBr followed by deacetylation and separation gave thymine analogues **5d** and **6d**. The guanine *Z*-isomer **5b** was the most effective against human and murine cytomegalovirus (HCMV and MCMV) with $EC_{50} = 0.27-0.49$ μM and no cytotoxicity. The 6-methoxy analogue **5g** was also active ($EC_{50} = 2.0-3.5$ μM) whereas adenine *Z*-isomer **5a** was less potent ($EC_{50} = 3.6-11.7$ μM). Cytosine analogue **5c** was moderately effective, but 2-amino-6-cyclopropylamino derivative **5e** was inactive. All *E*-isomers were devoid of anti-CMV activity, and none of the analogues was significantly active against herpes simplex viruses (HSV-1 or HSV-2). The potency against Epstein−Barr virus (EBV) was assay-dependent. In Daudi cells, the *E*-isomers of 2-amino-6-cyclopropylamino- and 2,6-diaminopurine derivatives **6e** and **6h** were the most potent ($EC_{50} \approx 0.3$ μM), whereas only the thymine *Z*-isomer **5d** was active ($EC_{50} = 4.6$ μM). Guanine *Z*-derivative **5b** was the most effective compound in H-1 cells ($EC_{50} = 7$ μM). In the *Z*-series, the 2-amino-6-methoxypurine analogue **5g** was the most effective against varicella zoster virus (VZV, $EC_{50} = 3.3$ μM) and 2,6-diaminopurine **5h** against hepatitis B virus (HBV, $EC_{50} = 4$ μM). Adenine analogues **5a** and **6a** were moderately active as substrates for adenosine deaminase.

Recently, we have described a series of nucleoside analogues **1a**−**1d** comprising a methylenecyclopropane system.[2] The *Z*-isomers of these analogues are effective in vitro against a broad spectrum of DNA viruses, especially human cytomegalovirus (HCMV) and Epstein−Barr virus (EBV).[3,4] Synguanol (**1a**, Chart 1) and 6-cyclopropylamino analogue **1e** are orally effective[5] in vivo against Balb/c mice infected with murine CMV. The latter compound is as potent as ganciclovir (**2**) but it is less toxic against proliferating bone marrow CFU-GM and BFU-E cells. Also, analogues **1a** and **1e** are effective against a number of laboratory and clinical isolates of HCMV, including those resistant to ganciclovir.[6,7] More recently, studies of the enantioselectivity of antiviral effects indicated that, in contrast to synadenol (**1a**),[8] the anti-CMV effect of 2-aminopurine methylenecyclopropanes is exclusively associated with the *S*-(+)-enantiomers.[9] The in vivo potency of the *S*-(+)-analogue **1e**, investigated in two models of HCMV infection of SCID mice, was comparable with that of ganciclovir (**2**).[10]

Analogues **1** can be derived from achiral acyclovir (**3**) by replacing the C−O−C grouping with a bioisosteric[11] methylenecyclopropane moiety (Chart 1). This operation leads to chiral *Z*- and *E*-isomers **1** and **4**. Both C−O−C and methylenecyclopropane moiety differ significantly in rigidity. Thus, acyclovir (**3**) comprises five rotatable bonds, but analogue **1a** has only three. In a similar vein, ganciclovir (**2**) can be transformed to more rigid *Z*- and *E*-methylenecyclopropane counterparts **5** and **6**. Analogues **1** and **4** are chiral, but structures **5** and **6** with two *gem*-hydroxymethyl groups are devoid of chirality.

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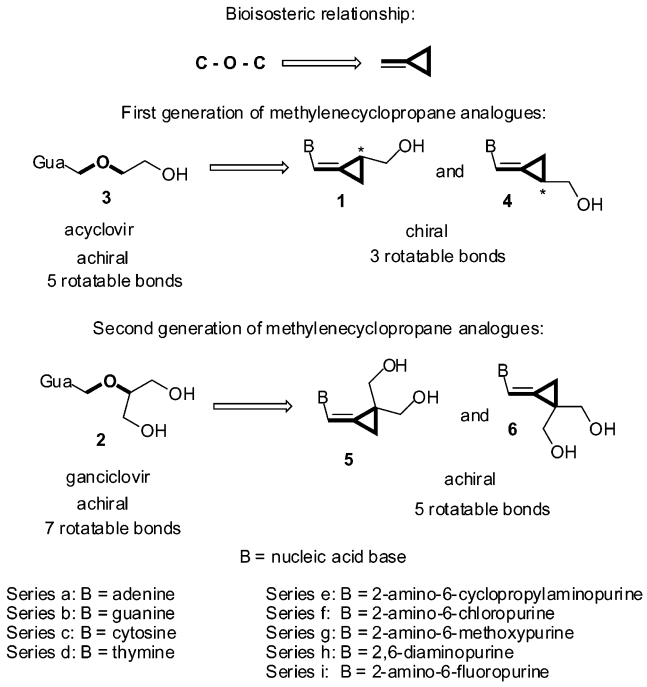
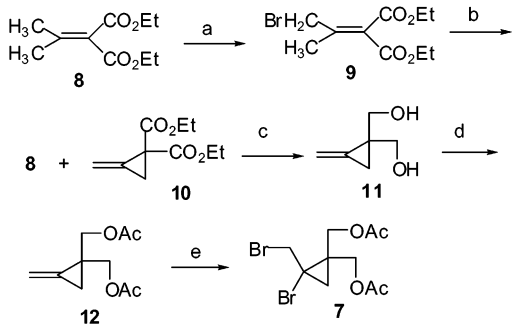
Chart 1



Scheme 1[a]

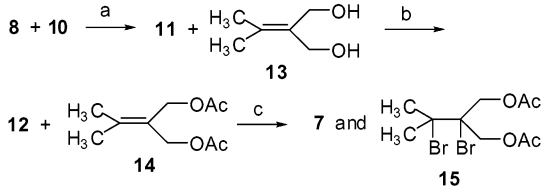


[a] (a) NBS, $(BzO)_2$, CCl_4, illumination. (b) (1) *t*-BuOK, *t*BuOH, Δ; (2) separation. (c) $LiAlH_4$, Et_2O. (d) Ac_2O, pyridine. (e) Br_2, CCl_4.

For these reasons, we became interested in synthesis and biological evaluation of 2,2-bis-hydroxymethylcyclopropane analogues **5** and **6**. Comparison of biological effects of the first- and second-generation series (**1** and **4** vs **5** and **6**) was then considered of utmost importance.

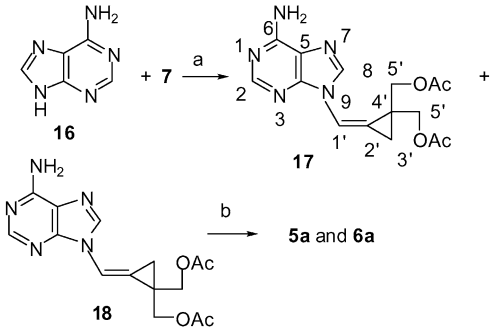
Synthesis. The alkylation−elimination procedure that proved useful[3,4,12] for the synthesis of the first-generation analogues **1a**−**1d** and **4a**−**4d** was considered the method of choice. The alkylating reagent, 1-bromo-1-bromomethyl-2,2-bis-(acetoxymethyl)cyclopropane (**7**), was prepared as shown in Scheme 1. Reaction of commercially available diethyl isopropylidenemalonate (**8**) with NBS and dibenzoyl peroxide in CCl_4 under illumination with light gave bromo derivative **9** as a crude product in quantitative yield. Compound **9** was then transformed to a 1:1 mixture of diethyl isopropylidenemalonate (**8**) and diethyl methylenecyclopropane-2,2-dicarboxylate **10** (47%) using tBuOK in tBuOH by a modification of the described procedure.[13] It should be noted that starting bromo ester **9** was free from isopropylidenemalonate **8**. Most likely, diester **8** was formed by a transfer of positive bromine from **9** to reagent or solvent. Compound **9** then can be regarded as a "vinylogue" of diethyl bromomethylmalonate, which is a known source of bromonium cation.[14] Chromatography on a silica gel column gave **10** (23% yield), which was reduced to diol **11** with $LiAlH_4$ in ether as described (78% yield).[15] Acetylation with acetic anhydride in pyridine provided 1,1-bis(acetoxymethyl)methylenecyclopropane (**12**) in 93% yield. Addition of elemental bromine in CCl_4 gave the alkylating reagent **7** (58%).

Scheme 2[a]



[a] (a) $LiAlH_4$, Et_2O. (b) Ac_2O, pyridine. (c) (1) Pyridine·HBr_3, CH_2Cl_2; (2) separation.

Scheme 3[a]

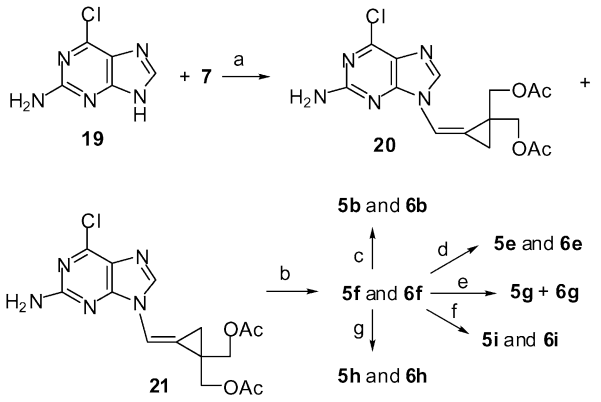


[a] (a) K_2CO_3, DMF, Δ. (b) (1) K_2CO_3, MeOH/H_2O; (2) separation.

Alternately (Scheme 2), a mixture of diethyl isopropylidene malonate (**8**) and methylenecyclopropane-2,2-dicarboxylate (**10**) obtained as shown above (Scheme 1) was reduced with $LiAlH_4$ to give diol **11** and 2-isopropylidenepropane-1,3-diol (**13**, 76%) inseparable by chromatography on a silica gel column. Acetylation provided also an inseparable mixture of acetates **12** + **14** in 92% yield. In the next step, addition of bromine using pyridinium perbromide in CH_2Cl_2 furnished 1,1-bis-(acetoxymethyl)-1,2-dibromo-2,2-dimethylethane (**15**) and 1-bromo-1-bromomethyl-2,2-bis-(acetoxymethyl)cyclopropane (**7**), which were separated by chromatography in 37% and 46% yield, respectively. Although the yield of the key intermediate **7** is somewhat lower as compared with the method described in Scheme 1, the chromatographic separation of **7** and **15** is easier than that of **8** and **10**.

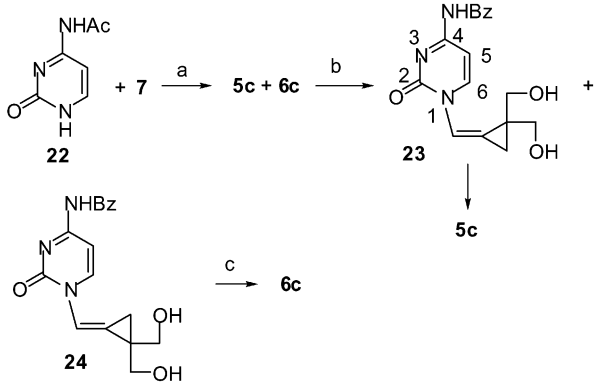
Alkylation of adenine (**16**) with reagent **7** using K_2CO_3 in DMF at 100 °C was accompanied by elimination of HBr to give an isomeric mixture of acetates **17** and **18** in 42% yield (Scheme 3). Deacetylation with K_2CO_3 in aqueous methanol furnished the *Z*- and *E*-isomers **5a** and **6a**, which were resolved directly by chromatography (38% and 29%, respectively). It should be noted that derivatization was necessary[4,12] to separate isomers **1a** and **4a**. In a similar fashion, alkylation of 2-amino-6-chloropurine (**19**) with reagent **7** using K_2CO_3 in DMF at 100 °C (Scheme 4) provided a mixture of acetates **20** and **21** (61%). Deprotection with K_2CO_3 in methanol−water (9:1) for 30 min at room temperature followed by chromatographic separation afforded compounds **5f** (53%) and **6f** (37%). Hydrolysis of **5f** and **6f** with HCO_2H

Scheme 4[a]



[a] (a) K_2CO_3, DMF, Δ. (b) (1) K_2CO_3, MeOH/H_2O; (2) separation. (c) (1) HCO_2H, Δ; (2) NH_3, MeOH. (d) Cyclopropylamine, EtOH. (e) K_2CO_3, MeOH. (f) KF, catalytic NMe_3, DMF. (g) NH_3, MeOH.

Scheme 5[a]

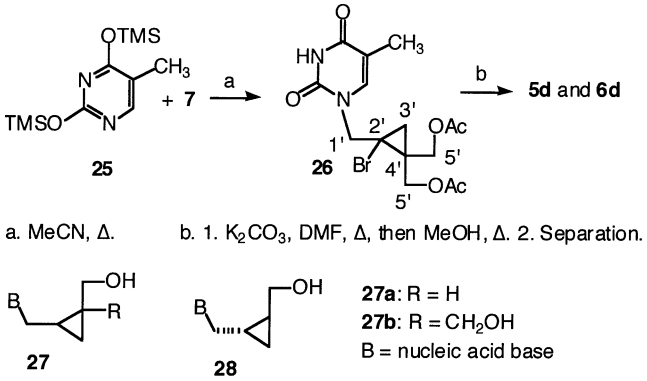


[a] (a) (1) K_2CO_3, DMF, Δ; (2) MeOH, Δ. (b) (1) Bz_2O, EtOH, Δ; (2) separation. (c) NH_3, MeOH.

gave guanine analogues **5b** and **6b** in 89 and 84% yield, respectively. Reaction of compounds **5f** and **6f** with methanol and K_2CO_3 gave 6-methoxy analogues **5g** (91%) and **6g** (92%), whereas displacement of chlorine with cyclopropylamine afforded 6-cyclopropylamino derivatives **5e** and **6e** in 92% and 86% yield, respectively. Ammonolysis of **5f** and **6f** then furnished 2,6-diaminopurine analogues **5h** (85%) and **6h** (81%). Synthesis of 2-amino-6-fluoro analogues **5i** and **6i** followed the procedure[16] used for a similar derivative of ganciclovir, but only a catalytic amount of trimethylamine was used. Compounds **5i** and **6i** were obtained in 85% and 81% yield, respectively.

In the pyrimidine series, N^4-acetylcytosine (**22**) was alkylated with reagent **7** in DMF at 100 °C to give after deacetylation an isomeric mixture of **5c** and **6c** in a ratio of 1:1.4 and 61% yield (Scheme 5). As in case of the first-generation analogues **1c** and **4c**, the isomers were inseparable by chromatography on silica gel and derivatization[4] was necessary for separation. Selective N^4-benzoylation of the mixture **5c** + **6c** with benzoic anhydride in refluxing ethanol afforded N^4-benzoyl derivatives **23** and **24**, which were separated by chromatography in 38 and 35% yield, respectively. Debenzoylation of **23** and **24** with NH_3 in methanol gave cytosine analogues **5c** (86%) and **6c** (83%). For synthesis of thymine derivatives **5d** and **6d**, 2,4-bis(trimethylsilyloxy)-5-methylpyrimidine (**25**) was used as a starting material.[4] Alkylation with dibromide **7** by prolonged

Scheme 6[a]



[a] (a) MeCN, Δ. (b) (1) K_2CO_3, DMF, Δ, then MeOH, Δ; (2) separation.

reflux in MeCN furnished the bromocyclopropane **26** in 74% yield (Scheme 6). Elimination of the elements of HBr with K_2CO_3 in DMF at 100 °C coupled with a subsequent deacetylation in situ gave after chromatographic separation the *Z*-isomer **5d** (38%) and *E*-isomer **6d** (36%).

Assignment of *Z*- and *E*-Isomers. In all cases, the *Z*-isomers were less polar, moving faster on TLC and eluting from silica gel columns prior to the *E*-isomers. These patterns were observed[3,4] also with the first-generation analogues **1a**−**1d** and **4a**−**4d**. Comparison of NMR spectra of analogues **5a**−**5d** and **6a**−**6d** with the first-generation series **1a**−**1d** and **4a**−**4d** has provided the best argument for an unambiguous isomeric assignment. The chemical shift patterns found in the ^{1}H NMR spectra of analogues **1a**−**1d** and **4a**−**4d** were also followed in compounds **5a**−**5d** and **6a**−**6d** (Table 1). In particular, purine H_8 and pyrimidine H_6 signals of the *Z*-isomers are always significantly downfield from those of *E*-isomers. In fact, the $H_8(H_6)$ chemical shifts of *E*-isomeric series **4** and **6**, where little influence of the hydroxymethyl group on the heterocyclic base is to be expected, are virtually identical. Deshielding of the *Z*-isomers is best explained[3,4] by a juxtaposition of the oxygen atom of the hydroxymethyl group toward the $H_8(H_6)$ in the *Z*-isomers **1**. This effect is preserved in analogues **5**; in fact, an additional downfield shift of the H_8 signals relative to those of **1** was observed in the *Z*-isomers **5a** and **5b** ($\Delta\delta$ 0.08−0.14 ppm). Depending on the rotameric disposition of hydroxymethyl groups, both oxygen atoms can be either juxtaposed to the $H_8(H_6)$ of heterocyclic base in an *anti* conformation, as shown for adenine analogue **5a** (Figure 1, rotamer A), or one of them may resemble the $C_{3'}$−OH in 2′-deoxynucleosides (Figure 1, rotamer B). The $H_8\cdots O_{5'}$ ($O_{5''}$) distances (2.5 Å) of rotamer A are close to the range of 2.0−2.2 Å estimated from the $C_{8(6)}\cdots H_{8(6)}\cdot\cdot\cdot O_{5'}$ distance (3.0−3.2 Å) in crystal structures of nucleosides.[18] Hydrogen bonds $H_8\cdots O_{5'}$ in guanosine residues of the anticodon loop in $tRNA^{Asp}$ are on average 2.2−3.0 Å long.[19]

An additional trend observed in both series of analogues is a downfield shift of the $H_{1'}$ signal in *E*-isomers relative to the *Z*-isomers. The OH frequencies then follow an opposite pattern. The $H_{5'}$ (hydroxymethyl) signals of both *Z*-isomers **5** and *E*-isomers **6** appear as two distinct AB systems. As in the first-generation series **1** and **4**, the differences in chemical shifts ($\Delta\delta$) of

Table 1. Comparison of Selected ^{1}H NMR Chemical Shifts (δ) of the *Z*-Isomers **5a**−**5d** and **1a**−**1d** with *E*-Isomers **6a**−**6d** and **4a**−**4d**

isomer[a]	OH	$H_{1'}$	H_8 or[b] H_6	$H_{5'}$	$\Delta\delta$ (H_{51})
1a (*Z*)	5.11	7.38	8.74	3.33, 3.73	0.40
5a (*Z*)	5.07	7.37	8.82	3.52, 3.68	0.16
				3.53, 3.67	0.14
4a (*E*)	4.82	7.48	8.48	3.41	-
6a (*E*)	4.76	7.48	8.49	3.46, 3.52	0.06
				3.48, 3.51	0.03
1b (*Z*)	5.04	7.11	8.31	3.32, 3.68	0.36
5b (*Z*)	4.99	7.07	8.41	3.48, 3.63	0.15
				3.49, 3.62	0.13
4b (*E*)	4.80	7.21	8.04	3.37	-
6b (*E*)	4.76	7.21	8.03	3.41, 3.48	0.07
				3.43, 3.47	0.04
1c (*Z*)	4.93	7.30	8.13	3.31, 3.53	0.22
5c (*Z*)	5.02	7.31	8.27	3.34, 3.57	0.23
4c (*E*)	4.75	7.37	7.96	3.32	-
6c (*E*)	4.78	7.38	7.96	3.37, 3.42	0.05
				3.37, 3.41	0.04
1d (*Z*)	5.06	7.15	8.20	3.15, 3.71	0.56
5d (*Z*)	4.99	7.17	8.32	3.40, 3.61	0.21
				3.41, 3.60	0.19
4d (*E*)	4.75	7.22	7.81	3.32	-
6d (*E*)	4.66	7.25	7.82	3.38, 3.45	0.07
				3.40, 3.43	0.03

[a] All spectra were determined in CD_3SOCD_3. The δ values for **1a**, **1b**, **4a**, and **4b** were taken from ref 3 and those for **1c**, **1d**, **4c**, and **4d** from ref 4. The δ values of $H_{5'}$ for compounds **1a**−**1d** and **4a**−**4d** reflect the centers of multiplets, whereas those for analogues **5a**−**5d** and **6a**−**6d** were calculated from the respective AB spin systems.[17] [b] H_8 of purines, H_6 of pyrimidines.

nonequivalent protons are invariably larger in the *Z*-isomers **5** than in *E*-isomers **6**. All these facts support the *Z*/*E* assignment of the isomeric series **5** and **6**.

Biological Activity. Antiviral Effects. Analogues **5a**−**5i** and **6a**−**6i** were tested in vitro against the following viruses: human and murine cytomegalovirus (HCMV and MCMV), herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Epstein−Barr virus (EBV), varicella zoster virus (VZV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV-1). The results are summarized in Tables 2 and 3. No significant activity (EC_{50} >10 μM) was seen in HIV-1 assays of analogues **5a**−**5d** and **6a**−**6d** (data not shown).

Against CMV, the guanine *Z*-derivative **5b** is clearly the most potent analogue in all three assays, with EC_{50} ranging from 0.27 to 0.49 μM (Table 2). It is not cytotoxic and is 5−19 times more efficacous than ganciclovir, surpassing also the potency of racemic[3] or *S*-(+)-synguanol[9] (**1b**). Compound **5b** was followed by 6-methoxy analogue **5g**, with EC_{50} = 2.0−3.5 μM, the efficacy of which in HCMV assays is equal to that of the corresponding first-generation analogue[9] **1g**. It is about 5 times less potent against MCMV than **1g**. Adenine analogue **5a** is effective against the Towne strain of HCMV (EC_{50} = 3.6 μM) but less potent in the other two assays. The 2,6-diaminopurine derivative **5h** was effective only in a single assay, and 2-amino-6-chloropurine analogue **5f** was inactive. The moderate activity of 2-amino-6-fluoropurine **5i** is interesting, because the respective ganciclovir analogue was inactive.[16] Among the pyrimidine derivatives, cytosine analogue **5c** was active, whereas thymine derivative **5d** did not exhibit any significant anti-CMV effect. The total lack of potency of 2-amino-6-cyclopropylamino analogue **5e** is surprising. As already mentioned, the respective first-generation compound **1e** is a promising drug candidate

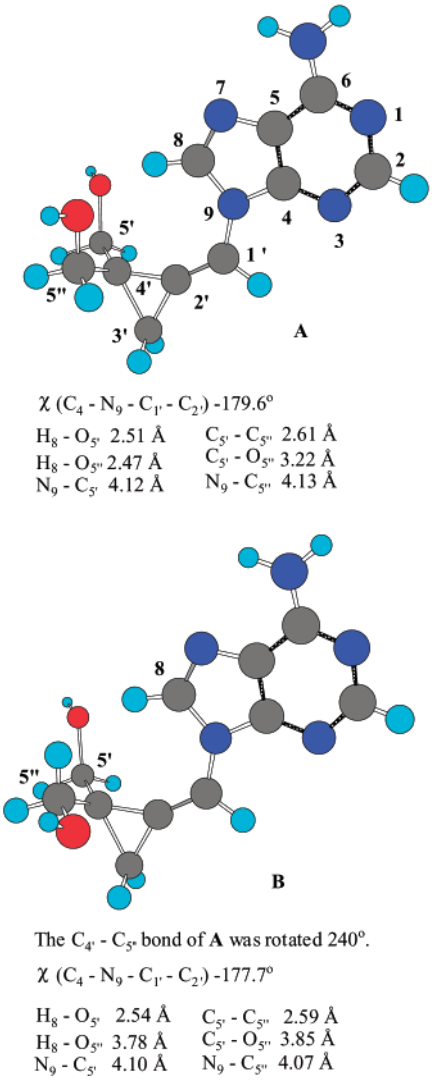


Figure 1. Two $C_{4'}$−$C_{5''}$ rotamers of adenine analogue **5a**.

for HCMV infections. Apparently, the activation mechanism available for compound **1e** is inoperative in the case of analogue **5e**. This result points to significant differences in the mechanism of action between the first- and second-generation methylenecyclopropane analogues of nucleosides. The *E*-isomers **6a**−**6i** were ineffective as anti-CMV agents.

The first generation of methylenecyclopropane analogues provided only exceptionally[3,4] active agents against HSV-1 and HSV-2, although compounds with a more lipophilic purine-6 substituent were quite potent[20] in several assays. In the second-generation series only the thymine *Z*-analogue **5d** was moderately effective in a single assay (ELISA) with EC_{50} = 10 μM. All tested compounds were noncytotoxic (CC_{50} >100 μM) in KB cells, which serve as a cytotoxicity control in ELISA assays. None of the analogues was active against HSV-1 or HSV-2 in HFF or Vero culture. In contrast, several compounds of the second generation were effective against EBV and VZV (Table 3). In the *Z*-isomeric series, thymine analogue **5d** was the most potent against EBV (EC_{50} = 4.6 and 19 μM, respectively) whereas compounds **5b** and **5g** were effective only in H-1 culture (EC_{50} = 7 and 4.8 μM, respectively). Analogue **5g** was the most potent anti-VZV agent in a plaque reduction assay (EC_{50} = 3.3 μM). Significant

Table 2. Inhibition of Human and Murine Cytomegalovirus (HCMV and MCMV) Replication by 2,2-Bis(hydroxymethyl)methylenecyclopropane Analogues

	EC_{50}/CC_{50} (μM)		
	HCMV/HFF[a]		
compd	Towne[b]	AD169[c]	MCMV/MEF[a]
5a	3.6/100	11.7/>404	9.7/>404
6a	>100/>100	>404/>404[d]	NT[e]
5b	0.46/>100	0.49/>380	0.27/>380
6b	39/>100	123/>380	42/>380
5c	32/>100	14.3/>448	54/>448
6c	>100/>100	>448/>448[d]	NT
5d	>100/>100	>420/>420[d]	NT
6d	>100/>100	>420/>420[d]	NT
5e	>100/>100	255/>331[d]	NT
6e	>100/>100	327/>331[d]	NT
5f	>100/>100	>355/>355[d]	NT
6f	>100/>100	>355/>301[d]	NT
5g	3.5/>100	2.7/>361	2.0/>361
6g	>100/>100	>361/>361[d]	NT
5h	15/>100	>381/>381[d]	NT
6h	>100/>100	313/>381[d]	NT
5i	38/>100	22.2/>377	8.7/>377
6i	33/>100	43.7/>377	18.9/176
ganciclovir	4.1/>100	2.3/>392	5.0/>35

[a] Plaque reduction assay. [b] Visual cytotoxicity. [c] Cytotoxicity by neutral red uptake. [d] Cytopathic effect (CPE) inhibition assay. [e] NT = not tested.

Table 3. Inhibition of EBV, VZV, and HBV Replication by 2,2-Bis(hydroxymethyl)methylenecyclopropane Analogues

	EC_{50}/CC_{50} (μM)			
compd	EBV/Daudi[a]	EBV/H-1[b,c]	VZV/HFF[d]	HBV/2.2.15[b,c]
5a	166/>202	16	>404	>10/>50
6a	132/>202	>20	11.7 (44.9)[e]	>10/>50
5b	45/74	7	>380	>10/>50
6b	119/>190	>20	>357[e]	>20/>50
5c	>45/70	>20	>448	>10>50
6c	152/>224	>20	>448	>10/>50
5d	4.6/>210	19	>420	>10/>50
6d	178/>210	>20	>420	>10/>50
5e	>165/>165	>20	>66	>20/>100
6e	0.30/>165 (19.9)[c]	>20	>331	>20/>100
5f	>178/>178	16.4	>71	>20/23.6
6f	142/>178	16.4	>71	>20/11.6
5g	161/>180	4.8	11.2 (3.3)[e]	>20/90
6g	>180/>180	>20	322	>20/>100
5h	>191/>191	>20	8.4 (38.9)[e]	4/48
6h	<0.31/>191(15.3)[c]	>20	270	>20/>100
5i	>189/>189	20.8	41.8 (50.1)[e]	>20/31.3
6i	>189/>189	18.5	31.7 (180)[e]	>20/56.4
control	1.1/>222[f]	5[g]	1.5/>444[f]	0.02/>100[h]

[a] Viral capsid antigen (VCA) ELISA. [b] Cytotoxicity was determined in CEM cells; see HBV data. [c] DNA hybridization assay. [d] Cytopathic effect (CPE) inhibition assay. For cytotoxicity see Table 2. [e] Plaque reduction assay. [f] Acyclovir. [g] Ganciclovir. [h] Lamivudine (3TC).

anti-HBV activity was found only with the 2,6-diaminopurine derivative **5h** ($EC_{50} = 4$ μM).

Of significant interest is the potency of purine *E*-isomers against EBV, which was seen only rarely in the first generation of methylenecyclopropane analogues.[3,4] Thus, the 2-amino-6-cyclopropylaminopurine **6e** and 2,6-diaminopurine **6h** were the most potent in Daudi cells ($EC_{50} \leq 0.3$ μM) of all second-generation analogues investigated, but they were ineffective in the H-1 culture. Both analogues were noncytotoxic. The activity of **6e** and **6h** against EBV/Daudi could not be explained as a result of conversion to guanine analogue **6b**, because the latter was inactive. It should be also noted that activity against EBV was observed in isolated cases of the *E*-isomers of the first-generation analogues[3,4] (**4a** and **4c**) as well as in some other compounds with a more extended (*E*-like) structure.[21−23] Adenine *E*-isomer **6a** was moderately effective against VZV in both assays with $EC_{50} = 11.7$ and 44.9 μM, respectively.

The increased rigidity of methylenecyclopropane analogues **1** and **5** (Chart 1) is probably an important factor in their biological effects. Thus, the anti-HSV-1 activity of analogues **27a**, **27b** and **28**, lacking a double bond, was restricted to guanine *Z*-isomer **27a** (B = Gua), whereas the *E*-isomer **28** or bis(hydroxymethyl) derivative **27b** were ineffective.[24] All these analogues are less rigid than methylenecyclopropanes **1**, **4**, **5**, or **6**.

In conclusion, *Z*-analogues **5** appear to have a more narrow profile of antiviral activity than the first-generation series **1**. This is particularly apparent by comparison of adenine analogues **1a** and **5a**. Thus, compound **1a** is effective against a broad range of viruses,[3] including HIV-1 and HBV, whereas significant potency for analogue **5a** is limited to CMV. The strong anti-CMV effect of purine analogues seems to be restricted to guanine and 2-amino-6-methoxypurine analogues **5b** and **5g**. Nevertheless, the in vitro activity of **5b** against HCMV surpasses that of **1g**, and both compounds are roughly equally potent against MCMV. Analogue **5b** is a good prospect for a more detailed investigation, including in vivo studies. A lack of chirality is an additional advantage of the second-generation series of analogues **5** and **6**. Nevertheless, it should be noted that because of the pro-chirality of the $C_{4'}$ carbon atom, the analogues can be activated inside the infected cells to chiral triphosphates, in analogy to the mechanism for ganciclovir.[25,26] The triphosphates may then not act solely as simple terminators of the growing DNA chain but at least to some extent support incorporation of additional nucleotide(s) as observed with ganciclovir.[27] In fact, either of the hydroxymethyl groups (pro-*S* or pro-*R*) of analogues **5** or **6** can play an ambiguous role, either to be phosphorylated or substitute for $C_{3'}$−OH of a nucleoside (see Figure 1, rotamer A and B). Nevertheless, in view of the fact that the activity of 2-aminopurine analogues **1** against HCMV is strictly *S*-selective,[9] it is possible that the *S*-configured triphosphate is ultimately responsible for the antiviral activity of analogue **5b**.

More effective antiviral agents were found in the *E*-isomeric series **6** than among the first-generation analogues[3,4] **2**. This is shown with a potent inhibition of EBV/Daudi with compounds **6e** and **6h** as well as anti-VZV activity of adenine analogue **6a**.

Adenosine Deaminase (ADA). Adenine analogues **5a** and **6a** are substrates for ADA from calf intestine. As in case of the first-generation analogues[3] **1a** and **4a**, the *E*-isomer **6a** is a better substrate than *Z*-isomer **5a**. Thus, compound **6a** was >90% deaminated after 24 h incubation with ADA, whereas >80% of **5a** remained intact.

Experimental Section

General Methods. See ref 3. The UV spectra were measured in ethanol and NMR spectra in CD_3SOCD_3, unless stated otherwise. Mass spectra were determined in electron-impact (EI-MS), chemical ionization (CI-MS, 2-methylpropane as an ionization gas) or electrospray ionization (ESI-MS, MeOH−NaCl) mode. Conformers A and B in Figure 1 were generated

using Chem3D Pro Version 5.0 software (Cambridge Scientific Computing, Inc., Cambridge, MA).

Diethyl Bromoisopropylidenemalonate (9). Diethyl isopropylidenemalonate (**8**, 50 g, 0.25 mol) was refluxed with stirring with *N*-bromosuccinimide (44.3 g, 0.25 mol) and dibenzoyl peroxide (1.0 g, 4.1 mmol) in CCl_4 (100 mL) under illumination with Kodak Ectagraphic slide projector lamp ELH (300 W) for 1.5 h. The reaction was completed, as indicated by a negative starch−iodine test. The resulting mixture was diluted with CCl_4 (100 mL) and it was cooled in an ice-bath. The precipitated succinimide was filtered off and the filtrate was evaporated in vacuo at room temperature. The residual pale yellow oil of diethyl bromoisopropylidenemalonate (**9**, 71.1 g) was used in the next experiment without further purification.

Diethyl Methylenecyclopropane-2,2-dicarboxylate (10). Compound **9** (43.4 g, 0.156 mol) was added to a vigorously stirred, refluxing solution of tBuOK (17.5 g, 0.156 mol) in tBuOH (500 mL) under N_2. The stirring was continued for 15 min, and the mixture was immediately cooled in an ice bath. Acetic acid was then added, the solid portion was filtered off and thoroughly washed with ether. The filtrate was concentrated in vacuo and diluted with ether, and the organic layer was washed several times with water. After drying with $MgSO_4$, the solution was evaporated in vacuo and the residue was distilled, bp 93−99 °C/0.3 Torr, yielding 14.8 g (47%) of a 1:1 mixture of diesters **8** + **10**. This mixture was chromatographed on a silica gel column using first hexanes−ether (40:1) and then (20:1) to give product **10** (7.3 g, 23%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 6H, ^3J = 7.2 Hz, CH_3), 2.15 (t, 2H, J = 2.4 Hz, CH_2), 4.17 (q, 4H, ^3J = 7.2 Hz, OCH_2), 5.53 (t, 1H, J = 2.1 Hz) and 5.62 (t, 1H, J = 2.6 Hz, $=CH_2$); ^{13}C NMR 14.2 (CH_3), 18.2 (C_3), 23.3 (C_2), 61.0 (CH_2O), 105.2 ($=CH_2$), 130.5 (C=), 167.9 (CO); EI-MS 199 (1.8, M + H), 170 (M − C_2H_4, 16.8), 142 (M − 2C_2H_4, 30.8), 124 (100.0); EI-HRMS calculated for $C_{10}H_{15}O_4$ (M + H) 199.0970, found 199.0964; calculated for $C_8H_{10}O_4$ (M − C_2H_4) 170.0579, found 170.0577.

2,2-Bis(hydroxymethyl)methylenecyclopropane (11). A solution of diester **10** (6.50 g, 32 mmol) in ether (60 mL) was added to a stirred suspension of $LiAlH_4$ (1.90 g, 51 mmol) in ether (50 mL) at such a rate as to maintain a gentle reflux. The resultant mixture was refluxed for 15 h. It was then quenched carefully with water (4 mL) and 2 M NaOH (8 mL). The ether phase was separated and the aqueous portion was extracted with ether. Combined organic phases were dried ($MgSO_4$) and ether was distilled off using a Vigreux column to give diol **11** (2.84 g, 78%) as a colorless oil. The ^{1}H NMR spectrum was identical to that described by Dolbier et al.[15]

2,2-Bis(acetoxymethyl)methylenecyclopropane (12). Acetic anhydride (13 mL) was added dropwise to a stirred solution of compound **11** (2.65 g, 23 mmol) in pyridine (6 mL) at room temperature. The stirring was continued for 16 h, the reaction was quenched with water, and product was extracted with cold (4 °C) pentane (70 mL) at 4 °C. The combined organic phase was washed successively with saturated aqueous $CuSO_4$, 5% HCl, aqueous $NaHCO_3$, and brine. It was then dried with $MgSO_4$, solvent was evaporated, and the residue was chromatographed on a silica gel column (hexanes−ether, 20:1) to give compound 1**2** as a colorless liquid (4.28 g, 93%). ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 2H, J = 2.1 Hz, H_3), 2.07 (s, 6H, CH_3), 4.03 and 4.10 (AB, 4H, ^2J = 11.6 Hz, OCH_2), 5.46 (t, J = 1.8 Hz, 1H) and 5.40 (t, 1H, J = 2.7 Hz, $=CH_2$); ^{13}C NMR 14.3 (C_3), 21.1 (CH_3), 22.9 (C_2), 66.3 (CH_2O), 106.0 ($=CH_2$), 134.1 (C=), 171.3 (CO); CI-MS 199 (M + H, 0.27), 57 (100.0).

1,1-Bis(acetoxymethyl)-2-bromo-2-(bromomethyl)cyclopropane (7). A. From 2,2-bis(acetoxymethyl)methylenecyclopropane (12). Bromine (3.2 g, 20 mmol) was added dropwise to a solution of compound **12** (3.95 g, 20.0 mmol) in CCl_4 (30 mL) with stirring at 0 °C. The stirring was continued for 30 min. The reaction mixture was diluted with ethyl acetate (100 mL) and the organic phase was washed with a saturated aqueous solution of $Na_2S_2O_3$ and $NaHCO_3$ and then with water. After drying with $MgSO_4$, the solvents were evaporated, and residue was chromatographed on a silica gel column (hexanes−ethyl acetate, 10:1 and then 5:1) to give compound **7** as a white solid (4.15 g, 58%): mp 56−58 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 and 1.33 (AB, 2H, J = 7.2 Hz, H_3), 2.08 and 2.10 (2s, 6H, CH_3), 3.75, 3.96, 4.25, 4.29 and 4.20, 4.48 (3AB, 6H, J = 11.2, 13.0 and 12.4 Hz, CH_2Br + CH_2O); ^{13}C NMR 21.1 (CH_3), 27.2 (C_2), 32.1 (C_3), 41.5 (CH_2Br), 42.5 (C_1), 62.3 and 68.1 (CH_2O), 170.96 and 171.01 (CO); CI-MS 361, 359 and 357 (M + H, 21.3, 42.8 and 22.0), 299 (100.0), 277 and 279 (M − Br, 68.2 and 68.0); EI-HRMS calcd for $C_{10}H_{14}{}^{79}Br_2O_4$ − Br 277.0075, found 277.0074. Anal. ($C_{10}H_{14}Br_2O_4$) C, H, Br.

B. From a Mixture of Diesters 8 + 10. A mixture of diesters **8** + **10** (2.0 g, 10 mmol) was reduced with $LiAlH_4$ in ether as described for diol **11**. The obtained mixture of diols **11** + **13** (866 mg, 76%) was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 2H, J = 2.1 Hz, H_3 of **11**), 1.76 (6H, CH_3 of **13**), 3.65 (AB, 4H, ^2J = 10.8 Hz, CH_2O of **11**), 4.27 (s, 4H, CH_2O of **13**), 5.38 (poorly resolved t, 1H) and 5.47 (t, 1H, J = 2.1 Hz, $=CH_2$ of **11**).

A mixture of diols **11** + **13** (570 mg, 5 mmol) was acetylated using acetic anhydride in pyridine as described for diacetate **12** to give a 1:1 mixture of diacetates **12** + **14** (915 mg, 92%), which was used directly in the next step. Compound **14**: ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 6H, CH_3), 2.03 (s, 6H, CH_3 of Ac), 4.65 (s, 4H, CH_2O); ^{13}C NMR 21.1, 21.2 (CH_3), 62.7 (CH_2O), 123.1 and 141.2 (C=C), 171.4 (CO). ^{1}H NMR and ^{13}C NMR of compound **12** were identical with the product described above by acetylation of diol **11**.

Pyridinium perbromide (1.60 g, 5 mmol) was added to a solution of a mixture of compounds **12** + **14** (796 mg, 4 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was allowed to stand at room temperature for 15 h. Ethyl acetate (100 mL) was then added and the organic phase was washed with a saturated solution of $NaHCO_3$ and $Na_2S_2O_3$ followed by water. After drying with Na_2SO_4, the solvents were evaporated and the crude product was chromatographed on a silica gel column using hexanes−ethyl acetate (10:1).

1,1-Bis(acetoxymethyl)-1,2-dibromo-2,2-dimethylethane (**15**) obtained as a colorless liquid was eluted first (529 mg, 37%) followed by compound **7** (white solid, 659 mg, 46%). Compound **15**: ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 6H, CH_3 of Ac), 2.15 (s, 6H, CH_3), 4.690 and 4.694 (2s, 4H, CH_2O); ^{13}C NMR 21.2 (CH_3 of Ac), 33.1 (CH_3), 65.9 (CH_2O), 67.1 and 73.3 (C−Br), 170.2 (CO). Compound **7** was identical with the product prepared by method A.

(*Z*)-9-{[2,2-Bis(acetoxymethyl)cyclopropylidene]methyl}adenine (17) and (*E*)-9-{[2,2-Bis(acetoxymethyl)cyclopropylidene]methyl}adenine (18). A mixture of adenine (**16**, 3.17 g, 2.35 mmol), dibromide **7** (0.84 g, 2.35 mmol), and flame-dried potassium carbonate (1.95 g, 14.1 mmol) in DMF (20 mL) was stirred at 100 °C under N_2 for 24 h. After cooling, the insoluble portion was filtered off and washed with DMF, and the filtrate was evaporated. The residue was chromatographed on a silica gel column using CH_2Cl_2−methanol (20:1) to give a mixture of *E*- and *Z*-isomers **17** + **18** (330 mg, 42%) in the ratio of 1:1 as a white solid: mp 155−157 °C; UV λ_{max} 276 nm (ϵ 7400), 256 (ϵ 10 800), 228 (ϵ 20 500); ^{1}H NMR ($CDCl_3$) δ 1.61 (s, 2H) and 1.79 (s, 2H, $H_{3'}$), 2.07 (s, 6H) and 2.10 (s, 6H, CH_3), 4.07 (d, 2H, ^2J = 8 Hz), 4.10 (d, 2H, ^2J = 8 Hz), 4.28 (d, 2H, ^2J = 11.2 Hz) and 4.43 (d, 2H, ^2J = 11.2 Hz, $H_{5'}$), 6.05 (s, 2H) and 6.13 (s, 2H, NH_2), 7.56 (s, 1H) and 7.70 (s, 1H, $H_{1'}$), 8.24 (s, 1H), 8.38 (s, 2H) and 8.46 (s, 1H, H_2 + H_8); ^{13}C NMR 13.3 and 15.8 ($C_{3'}$), 21.0 and 21.2 (CH_3), 23.4 and 25.1 ($C_{4'}$), 66.1 and 66.5 ($C_{5'}$), 113.0 ($C_{1'}$), 114.7 and 114.8 ($C_{2'}$ and C_5) 137.0 and 137.9 (C_8), 149.1 (C_4), 153.8 (C_2), 155.8 (C_6), 170.7 and 171.1 (CO); EI-MS 331 (M, 10.1), 136 (adenine + H, 100.0); EI-HRMS calcd for $C_{15}H_{17}N_5O_4$ 331.12805, found 331.12806. Anal. ($C_{15}H_{17}N_5O_4$) C, H, N.

(*Z*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}adenine (5a) and (*E*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}adenine (6a). A mixture of compounds **17** + **18** (309 mg, 0.93 mmol) and K_2CO_3 (0.83 g, 6 mmol) in methanol−water (9:1, 30 mL) was stirred at room temperature for 12 h. Acetic acid was carefully added and the mixture was

evaporated. The residue was chromatographed in CH_2Cl_2−methanol (10:1) to give *Z*- and *E*-isomers **5a** and **6a**.

Z-Isomer **5a** (87 mg, 38%): mp 239−242 °C; UV λ_{max} 276 nm (ϵ 8200), 262 (ϵ 11 700), 227 (ϵ 25 200); ^{1}H NMR δ 1.34 (s, 2H, $H_{3'}$), 3.52, 3.68 and 3.53, 3.67 (2AB, ^2J = 11.0 Hz, 4H, $H_{5'}$), 5.07 (t, 2H, ^3J = 4.0 Hz, OH), 7.37 (s, 1H, $H_{1'}$), 7.36 (s, 2H, NH_2), 8.17 (s, 1H, H_2), 8.82 (s, 1H, H_8); ^{13}C NMR (11.7, $C_{3'}$), 31.4 ($C_{4'}$), 62.84 ($C_{5'}$), 111.1 ($C_{1'}$), 118.5 ($C_{2'}$), 119.1 (C_5), 138.5 (C_8), 148.6 (C_4), 153.6 (C_2), 156.7 (C_6); EI-MS 247 (M, 9.1), 136 (adenine + H, 100.0); EI-HRMS calcd for $C_{11}H_{13}N_5O_2$ 247.1069, found 247.1069. Anal. ($C_{11}H_{13}N_5O_2$) C, H, N.

E-Isomer **6a** (66 mg, 29%): mp 250−252 °C; UV λ_{max} 276 nm (ϵ 7100), 260 (ϵ 10 000), 227 (ϵ 21 300); ^{1}H NMR δ 1.56 (d, 2H, 2H, J = 2 Hz, $H_{3'}$), 3.46, 3.52 and 3.48, 3.51 (partially overlapped 2AB, 4H, ^2J = 11.0 and 11.2 Hz, $H_{5'}$), 4.76 (t, 2H, ^3J = 4.8 Hz, OH), 7.48 (s, 1H, $H_{1'}$), 7.37 (s, 2H, NH_2), 8.17 (s, 1H, H_2), 8.49 (s, 1H, H_8); ^{13}C NMR 14.4 ($H_{3'}$), 29.7 ($C_{4'}$), 63.1 ($C_{5'}$), 110.9 ($C_{1'}$), 119.4 ($C_{2'}$ + C_5), 137.8 (C_8), 148.9 (C_4), 153.7 (C_2), 156.7 (C_6); EI-MS 247 (M, 9.1), 136 (adenine + H, 100.0); EI-HRMS calcd for $C_{11}H_{13}N_5O_2$ 247.1069, found 247.1070. Anal. ($C_{11}H_{13}N_5O_2$) C, H, N.

(*Z*)-2-Amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}purine (20) and (*E*)-2-Amino-6-chloro-9-{[2,2-bis(acetoxymethyl)cyclopropylidene]methyl}purine (21). The experiment was performed as described for compounds **17** + **18** using 2-amino-6-chloropurine (**19**, 0.34 g, 2 mmol), dibromide **7** (0.83 g, 2.32 mmol), K_2CO_3 (1.66 g, 12 mmol), and DMF (15 mL, 100 °C, 24 h). The crude product was chromatographed in CH_2Cl_2−methanol (49:1) to give a mixture of *Z*- and *E*-isomers **20** + **21** (447 mg, 61%) in the ratio of 1:0.7 as a white solid: mp 215−216 °C; UV λ_{max} 311 nm (ϵ 5100), 230 (ϵ 21 800), 204 (ϵ 13 700); ^{1}H NMR δ 1.64 (s, 1.4H) and 1.89 (d, 2H, J = 2.4 Hz, $H_{3'}$), 1.94 (s, 4.2H) and 2.04 (s, 6H, CH_3), 4.06−4.15 (m, 2.8H) and 4.28 (d, 4H, J = 11.2 Hz, $H_{5'}$), 7.02 (s, 1.4H) and 7.06 (s, 2H, NH_2), 7.30 (s, 0.7H, $H_{1'}$) and 7.40 (s, 1H, $H_{1'}$), 8.32 (s, 0.7H), 8.43 (s, 1H, H_8); ^{13}C NMR 13.2 and 16.2 ($C_{3'}$), 21.1 and 21.3 (CH_3), 23.7 and 25.5 ($C_{4'}$), 65.9 and 66.3 ($C_{5'}$), 112.4 and 112.7 ($C_{1'}$), 117.1 and 117.2 ($C_{2'}$), 123.7 (C_5), 140.1 and 140.6 (C_8), 150.4 (C_4), 153.2 and 153.1 (C_2), 160.8 (C_6), 170.7 and 171.0 (CO); EI-MS 365 and 367 (M, 9.3 and 3.3), 43 (100.0); EI-HRMS calcd for $C_{15}H_{16}{}^{35}ClN_5O_4$ 365.0891, found 365.0888.

(*Z*)-2-Amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5f) and (*E*)-2-Amino-6-chloro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6f). A mixture of *Z*- and *E*-isomers **20** + **21** (260 mg, 0.71 mmol) and K_2CO_3 (78 mg, 0.57 mmol) in methanol−water (9:1, 10 mL) was stirred for 30 min at room temperature. Acetic acid was carefully added and the mixture was evaporated. The residue was chromatographed on a silica gel column in CH_2Cl_2−methanol (10:1) to give *Z*-isomer **5f** (106 mg, 53%) and *E*-isomer **6f** (75 mg, 37%).

Z-Isomer **5f**: mp 207−208 °C; UV λ_{max} 310 nm (ϵ 7900), 234 nm (ϵ 27 800); ^{1}H NMR δ 1.34 (s, 2H, $H_{3'}$), 3.47, 3.67 and 3.49, 3.66 (2AB, 4H, ^2J = 10.8 and 11.2 Hz, $H_{5'}$), 5.04 (poorly resolved t, 2H, OH), 7.03 (s, 2H, NH_2), 7.18 (s, 1H, $H_{1'}$), 8.81 (s, 1H, H_8); ^{13}C NMR 11.7 ($C_{3'}$), 31.4 ($C_{4'}$), 62.8 ($C_{5'}$), 110.6 ($C_{1'}$), 119.2 ($C_{2''}$), 123.8 (C_5), 140.6 (C_8), 150.2 (C_4), 152.9 (C_2), 160.7 (C_6); ESI-MS 282 and 284 (M + H, 100.0 and 33.3), 304 and 306 (M + Na, 40.5 and 13.7), 585 and 587 (2M + Na, 32.7 and 24.4). Anal. ($C_{11}H_{12}ClN_5O_2$) C, H, Cl, N.

E-Isomer **6f**: mp 230−234 °C (dec). UV λ_{max} 310 nm (ϵ 8000), 234 (ϵ 28 800); ^{1}H NMR δ 1.54 (s, 2H, $H_{3'}$), 3.41, 3.49 and 3.43, 3.47 (2AB, 4H, ^2J = 11.6 and 11.0 Hz, $H_{5'}$), 5.42 (bs, 2H, OH), 7.02 (s, 2H, NH_2), 7.30 (s, 1H, $H_{1'}$), 8.43 (s, 1H, H_8); ^{13}C NMR 14.5 ($C_{3'}$), 29.8 ($C_{4'}$), 63.0 ($C_{5'}$), 110.4 ($C_{1'}$), 120.5 ($C_{2'}$), 123.7 (C_5), 140.1 (C_8), 150.3 (C_4), 153.2 (C_2), 160.7 (C_6); ESI-MS 282 and 284 (M + H, 100.0 and 32.1), 304 and 306 (M + Na, 27.4 and 8.9), 585 and 587 (2M + Na, 6.7 and 11.3). Anal. ($C_{11}H_{12}ClN_5O_2$) C, H, Cl, N.

(*Z*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}guanine (5b). A solution of the *Z*-isomer **5f** (100 mg, 0.36 mmol) in formic acid (95−97%, 8 mL) was heated at 80 °C with stirring for 4 h. After cooling, formic acid was evaporated in vacuo and the crude product was dissolved in methanol (30 mL). A precipitated white solid was stirred in methanolic ammonia (20%, 10 mL) at 0 °C for 4 h. After evaporation of volatile components, a suspension of the residue in methanol (100 mL) was refluxed for 2 h. The mixture was kept overnight at 0 °C to give product **5b** (83 mg, 89%): mp >300 °C. UV λ_{max} 271 nm (ϵ 11 500), 231 nm (ϵ 26 400); ^{1}H NMR δ 1.29 (s, 2H, $H_{3'}$), 3.48, 3.63 and 3.49, 3.62 (2AB, 4H, ^2J = 10.8 and 11.2 Hz, $H_{5'}$), 4.99 (t, 2H, ^3J = 5.6 Hz, OH), 6.52 (s, 2H, NH_2), 7.07 (s, 1H, $H_{1'}$), 8.41 (s, 1H, H_8), 10.64 (s, 1H, NH); ^{13}C NMR 11.5 ($C_{3'}$), 31.3 ($C_{4'}$), 62.8 ($C_{5'}$), 111.0 ($C_{1'}$), 116.9 ($C_{2'}$), 118.1 (C_5), 135.1 (C_8), 150.3 (C_4), 154.6 (C_2), 157.4 (C_6); ESI-MS 264 (M + H, 5.1), 286 (M + Na, 100.0), 549 (2M + Na, 41.1). Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

(*E*)-9-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}guanine (6b). The procedure described for the *Z*-isomer **5b** was used with compound **6f** as a starting material on 0.27 mmol scale to give the *E*-isomer **6b** (59 mg, 84%): mp >300 °C; UV λ_{max} 271 nm (ϵ 12 500), 229 (ϵ 31 800); ^{1}H NMR δ 1.49 (s, 2H, $H_{3'}$), 3.41, 3.48 and 3.43, 3.47 (2AB, 4H, ^2J = 11.6 and 11.0 Hz, $H_{5'}$), 4.76 (t, 2H,^3J = 5.6 Hz, OH), 6.58 (s, 2H, NH_2), 7.21 (s, 1H, $H_{1'}$), 8.03 (s, 1H, H_8), 10.77 (s, 1H, NH); ^{13}C NMR 14.3 ($C_{3'}$), 29.5 ($C_{4'}$), 63.0 ($C_{5'}$), 110.8 ($C_{1'}$), 116.9 ($C_{2'}$), 118.9 (C_5), 134.3 (C_8), 150.5 (C_4), 154.6 (C_2), 157.4 (C_6); ESI-MS 264 (M + H, 3.6), 286 (M + Na, 100.0), 549 (2M + Na, 33.0). Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

(*Z*)-2-Amino-6-cyclopropylamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5e). A solution of the *Z*-isomer **5f** (140 mg, 0.5 mmol) and cyclopropylamine (0.14 mL, 12.0 mmol) in ethanol (25 mL) was stirred at room temperature for 40 h. After cooling, the volatile components were evaporated, and the residue was chromatographed using CH_2Cl_2−methanol (10:1) to give compound **5e** (139 mg, 92%): mp 195−196 °C; UV λ_{max} 286 nm (ϵ 16 600), 224 (ϵ 40 200); ^{1}H NMR δ 0.54−0.57 (m, 2H) and 0.62−0.66 (m, 2H, CH_2 of cyclopropyl), 1.28 (d, 2H, J = 2.1 Hz, $H_{3'}$), 3.01 (s, 1H, CH of cyclopropyl), 3.49, 3.63 and 3.51, 3.62 (2AB, 4H, ^2J = 11.0 and 10.8 Hz, $H_{5'}$), 5.00 (t, 2H, ^3J = 4.8 Hz, OH), 5.94 (s, 2H, 2-NH_2), 7.16 (s, 1H, $H_{1'}$), 7.36 (poorly resolved d, 1H, 6-NH), 8.40 (s, 1H, H_8); ^{13}C NMR 7.12 (CH_2 of cyclopropyl), 11.6 ($C_{3'}$), 24.6 (CH of cyclopropyl), 31.3 ($C_{4'}$), 62.9 ($C_{5'}$), 111.2 ($C_{1'}$), 113.7 ($C_{2'}$), 116.8 (C_5), 135.0 (C_8), 156.6 (C_2), 161.1 (C_6); ESI-MS 303 (M + H, 100.0), 605 (2M + H, 17.3), 627 (2M + Na, 5.4). Anal. ($C_{14}H_{18}N_6O_2$) C, H, N.

(*E*)-2-Amino-6-cyclopropylamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6e). The procedure described for *Z*-isomer **5e** was followed with the *E*-isomer **6f** and (cyclopropylamine (0.70 mL, 5 mmol, 50 °C, 20 h) to give compound **6e** (130 mg, 86%): mp 164−165 °C; UV λ_{max} 286 nm (ϵ 16 300), 224 (ϵ 37 900); ^{1}H NMR δ 0.57 (s, 2H) and 0.61−0.66 (m, 2H, CH_2 of cyclopropyl), 1.48 (s, 2H, $H_{3'}$), 3.00 (bs, 1H, CH of cyclopropyl), 3.43, 3.50 and 3.45, 3.48 (2AB, 4H, ^2J = 11.4 and 11.0, $H_{5'}$), 4.71 (t, ^3J = 5.9 Hz, 2H, OH), 5.91 (s, 2H, 2-NH_2), 7.30 (poorly resolved t, 1H, $H_{1'}$), 7.41 (bs, 1H, 6-NH), 8.04 (s, 1H, H_8); ^{13}C NMR 7.1 (CH_2 of cyclopropyl), 14.3 ($C_{3'}$), 24.5 (CH of cyclopropyl), 29.4 ($C_{4'}$), 63.2 ($C_{5'}$), 111.0 ($C_{1'}$), 113.7 ($C_{2'}$), 117.3 (C_5), 133.9 (C_8), 150.7 (C_4), 156.6 (C_2), 161.2 (C_6). EI-MS 302 (M, 92.2), 285 (M − OH, 35.0), 191 (100.0); EI-HRMS calcd for $C_{14}H_{18}N_6O_2$ 302.1491, found 302.1491. Anal. ($C_{14}H_{18}N_6O_2$) C, H, N.

(*Z*)-2-Amino-6-methoxy-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5g). A mixture of compound **5f** (95 mg, 0.34 mmol) and K_2CO_3 (94 mg, 0.68 mmol) in methanol (15 mL) was refluxed for 4 h. After cooling, the solvent was evaporated and the residue was chromatographed on a silica gel column using CH_2Cl_2−methanol (10:1) to give the title compound **5g** (86 mg, 91%): mp 188−189 °C; UV λ_{max} 278 nm (ϵ 10 400), 225 (ϵ 26 900), 203 (ϵ 17 200); ^{1}H NMR δ 1.31 (s, 2H, $H_{3'}$), 3.49, 3.66 and 3.51, 3.65 (2AB, 4H, ^2J = 11.0 and 10.4 Hz, $H_{5'}$), 3.95 (s, 3H, OCH_3), 5.03 (t, 2H,^3J = 4.8 Hz, OH), 6.53 (s, 2H, NH_2), 7.19 (s, 1H, $H_{1'}$), 8.56 (s, 1H, H_8); ^{13}C NMR 11.6 ($C_{3'}$), 31.3 ($C_{4'}$), 54.0 (OCH_3), 62.8 ($C_{5'}$), 111.0 ($C_{1'}$),

114.1 ($C_{2'}$), 117.7 (C_5), 137.3 (C_8), 153.1 (C_4), 160.8 (C_2), 161.4 (C_6); EI-MS 277 (M, 23.1), 166 (100.0). Anal. ($C_{12}H_{15}N_5O_3$) C, H, N.

(*E*)-2-Amino-6-methoxy-9-{[2,2-bis(hydroxymethyl)-cyclopropylidene]methyl}purine (6g). A mixture of *E*-isomer **6f** (140 mg, 0.50 mmol) and K_2CO_3 (276 mg, 2.0 mmol) in methanol (20 mL) was refluxed for 2 h. The workup followed the procedure for the *Z*-isomer **5g** to give compound **6g** (127 mg, 92%): mp 179−180 °C; UV λ_{max} 279 nm (ϵ 10 000), 224 (ϵ 28 200), 201 (ϵ 21 200); ^{1}H NMR δ 1.51 (s, 2H, $H_{3'}$), 3.43, 3.50 and 3.45, 3.49 (2AB, 4H, ^2J = 11.2 and 11.0 Hz, $H_{5'}$), 3.95 (s, 3H, OCH_3), 4.71 (t, ^3J = 5.6 Hz, 2H, OH), 6.51 (s, 2H, NH_2), 7.31 (s, 1H, $H_{1'}$), 8.20 (s, 1H, H_8); ^{13}C NMR 14.3 ($C_{3'}$), 29.6 ($C_{4'}$), 53.9 (OCH_3), 63.1 ($C_{5'}$), 111.0 ($C_{1'}$), 114.1 ($C_{2'}$), 118.6 (C_5), 136.5 (C_8), 153.4 (C_4), 160.8 (C_2), 161.4 (C_6); EI-MS 277 (M, 3.0), 260 (M −OH, 8.7), 179 (100.0); EI-HRMS calcd for $C_{12}H_{15}N_5O_3$ 277.1175, found 277.1174. Anal. ($C_{12}H_{15}N_5O_3$) C, H, N.

(*Z*)-2,6-Diamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5h). A mixture of the *Z*-isomer **5f** (140 mg, 0.5 mmol) and NH_3 in methanol (saturated at 0 °C, 60 mL) was heated in a stainless steel bomb at 100 °C for 20 h. After cooling, the volatile components were evaporated, and the residue was chromatographed on silica gel using CH_2Cl_2−methanol (4:1) to give the title compound **5h** (111 mg, 85%): mp 249−250 °C; UV λ_{max} 280 nm (ϵ 13 400), 220 (ϵ 35 500); ^{1}H NMR δ 1.27 (s, 2H, $H_{3'}$), 3.49, 3.62 and 3.50, 3.61 (2AB, 4H, ^2J = 10.8 and 10.4 Hz, $H_{5'}$), 5.03 (t, 2H, ^3J = 4.8 Hz, OH), 5.85 (s, 2H, 2-NH_2), 6.74 (s, 2H, 6-NH_2), 7.13 (s, 1H, $H_{1'}$), 8.39 (s, 1H, H_8); ^{13}C NMR 11.6 ($C_{3'}$), 31.3 ($C_{4'}$), 62.9 ($C_{5'}$), 111.2 ($C_{1'}$), 113.5 ($C_{2'}$), 116.7 (C_5), 135.2 (C_8), 150.9 (C_4), 156.8 (C_2), 161.2 (C_6); EI-MS 262 (M, 19.6), 150 (purine base, 100.0); EI-HRMS calcd for $C_{11}H_{14}N_6O_2$ 262.1178, found 262.1175. Anal. ($C_{11}H_{14}N_6O_2$) C, H, N.

(*E*)-2,6-Diamino-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6h). The procedure described for the *Z*-isomer **5h** was perfomed on a 0.34 mmol scale of compound **6f** to give the *E*-isomer **6h** (72 mg, 81%): mp 219−220 °C; UV λ_{max} 280 nm (ϵ 13 700), 220 (ϵ 42 700); ^{1}H NMR δ 1.48 (d, 2H, J = 2.4 Hz, $H_{3'}$), 3.43, 3.50 and 3.45, 3.48 (2AB, 4H, ^2J = 11 Hz, $H_{5'}$), 4.68 (t, 2H, ^3J = 5.9 Hz, OH), 5.85 (s, 2H, 2-NH_2), 6.77 (s, 2H, 6-NH_2), 7.27 (t, 1H, J = 2.4 Hz, $H_{1'}$), 8.04 (s, 1H, H_8); ^{13}C NMR 14.3 ($C_{3'}$), 29.4 ($C_{4'}$), 63.2 ($C_{5'}$), 111.0 ($C_{1'}$), 113.4 ($C_{2'}$), 117.4 (C_5), 134.2 (C_8), 151.2 (C_4), 156.8 (C_2), 161.3 (C_6); EI-MS 262 (M, 26.9), 151 (purine base + H, 100.0); EI-HRMS calcd for $C_{11}H_{14}N_6O_2$ 262.1178, found 262.1172. Anal. ($C_{11}H_{14}N_6O_2$) C, H, N.

(*Z*)-2-Amino-6-fluoro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (5i). A mixture of the *Z*-isomer **5f** (140 mg, 0.5 mmol), a 1 M solution of trimethylamine in DMF (0.21 mL, 0.21 mmol), and KF (400 mg, 6.9 mmol, dried at room temperature and 0.05−0.07 Torr for 12 h) in DMF (5 mL) was vigorously stirred at room temperature for 24 h. The solids were filtered and washed with DMF, and the filtrate was evaporated in vacuo. The crude product was chromatographed on silica gel with EtOAc−methanol (50:1 to 30:1) to give the title compound **5i** (113 mg, 85%): mp 185−188 °C; UV λ_{max} 289 nm (ϵ 8400), 268 (ϵ 8700), 229 (ϵ 37 000); ^{1}H NMR δ 1.34 (s, 2H, $H_{3'}$), 3.35, 3.67 and 3.49, 3.66 (2AB, 4H, J_{AB} = 10.2 Hz, $H_{5'}$), 5.02 (poorly resolved t, 2H, OH), 7.01 (s, 2H, NH_2), 7.20 (s, 1H, $H_{1'}$), 8.77 (s, 1H); ^{13}C NMR[28] 11.7 ($C_{3'}$), 31.4 ($C_{4'}$), 62.8 ($C_{5'}$), 110.8 ($C_{1'}$), 111.9 (d, $^2J_{C,F}$ = 31.3 Hz, C_5), 119.0 ($C_{2'}$), 140.2 (C_8), 156.4 (d, $^3J_{C,F}$ = 12.0 Hz, C_4), 159.9 (d, $^1J_{C,F}$ = 250.7 Hz, C_6), 160.7 (d, $^3J_{C,F}$ = 17.9 Hz, C_2); ^{19}F NMR −72.8 (s); EI-MS 265 (M, 3.8), 248 (M − OH, 5.3), 154 (purine base + H, 100.0); EI-HRMS calcd for $C_{11}H_{12}FN_5O_2$ 265.0975, found 265.0974. Anal. $C_{11}H_{12}FN_5O_2$ (C, H, N).

(*E*)-2-Amino-6-fluoro-9-{[2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (6i). The procedure described above was perfomed with the *E*-isomer **6f** (0.5 mmol scale) to give the title compound **6i** (107 mg, 81%): mp 214−216 °C; UV λ_{max} 289 nm (ϵ 8600), 271 (ϵ 8800), 229 (ϵ 38 400); ^{1}H NMR δ 1.53 (d, 2H, J = 1.6 Hz), 3.44, 3.55 and 3.45, 3.49 (2AB, 4H, J_{AB} = 11.2 Hz, $H_{5'}$), 4.74 (t, 2H, OH, J = 5.6 Hz), 7.01 (s, 2H, NH_2), 7.33 (s, 1H, $H_{1'}$), 8.42 (s, 1H, H_8); ^{13}C NMR[28] 14.5 ($C_{3'}$), 29.8 ($C_{4'}$), 63.0 ($C_{5'}$), 110.6 ($C_{1'}$), 111.9 (d, $^2J_{C,F}$ = 31.4 Hz, C_5), 120.3 ($C_{2'}$), 139.8 (C_8), 156.6 (d, $^3J_{C,F}$ = 12.1 Hz, C_4), 159.9 (d, $^1J_{C,F}$ = 250.7 Hz, C_6), 160.7 (C_2, d, $^3J_{C,F}$ = 17.9 Hz); ^{19}F NMR −72.6 (s); EI-MS 265 (M, 2.8), 248 (M − OH, 4.0), 154 (purine base + H, 100.0); EI-HRMS calcd for $C_{11}H_{12}FN_5O_2$ 265.0975, found 265.0972. Anal. $C_{11}H_{12}FN_5O_2$ (C, H, N).

(*Z*)-1-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}cytosine (5c) and (*E*)-1-{[2,2-Bis(hydroxymethyl)-cyclopropylidene]methyl}cytosine (6c). A mixture of N^4-acetylcytosine (**22**, 1.80 g, 5.0 mmol), dibromide **7** (766 mg, 5.0 mmol), and K_2CO_3 (4.75 g, 30 mmol) in DMF (100 mL) was stirred at 100 °C under nitrogen for 12 h. The mixture was cooled to 50 °C and methanol (5 mL) was added with stirring, which was continued for 2 h. After cooling, the insoluble portion was filtered off and it was washed with DMF. The filtrate was evaporated in vacuo and the residue was chromatographed on a silica gel column in CH_2Cl_2−methanol (20:1 and then 4:1) to give a mixture of products **5c** + **6c** (680 mg, 61%) in a ratio of 1:1.4 (determined from the ^{1}H NMR spectra) as a white solid.

(*Z*)- and (*E*)-N^4-Benzoyl-1-{[2,2-bis(hydroxymethyl)-cyclopropylidene]methyl}cytosine (23 and 24). A mixture of **5c** + **6c** from the preceding experiment was dissolved in refluxing ethanol (100 mL). Benzoic anhydride (689 mg, 3.05 mmol) was added with stirring, and the refluxing was continued for 1 h. Five more 3.05-mmol portions of benzoic anhydride were added every hour. After cooling, the solvent was evaporated and the crude product was chromatographed on a silica gel column in CH_2Cl_2−methanol (20:1) to give the *Z*-isomer **23** (380 mg, 38%) and *E*-isomer **24** (350 mg, 35%) as white solids, after recrystallization from ethanol.

Z-Isomer **23**: mp 222−223 °C; UV λ_{max} 329 nm (ϵ 14 200), 270 (ϵ 19 200), 203 (ϵ 23 900); ^{1}H NMR δ 1.27 (s, 2H, $H_{3'}$), 3.46, 3.66 and 3.48, 3.64 (2AB, 4H, J = 10.8 and 11.4 Hz, $H_{5'}$), 5.00 (t, 2H, ^3J = 5.4 Hz, OH), 7.35 (poorly resolved d, 1H, H_5), 7.39 (bs, 1H, $H_{1'}$), 7.49 (t, 2H, ^3J = 7.2 Hz, H_{meta} of Bz), 7.60 (t, 1H, ^3J = 7.0 Hz, H_{para} of Bz), 7.99 (d, 2H, ^3J = 7.2 Hz, H_{ortho} of Bz), 8.69 (d, ^3J = 7.2 Hz, 1H, H_6), 11.31 (s, 1H, NH); ^{13}C NMR 11.0 ($C_{3'}$), 31.5 ($C_{4'}$), 62.9 ($C_{5'}$), 97.3 (C_5), 116.4 ($C_{1'}$), 120.2 ($C_{2'}$), 129.1 and 129.2 (C_{ortho} and C_{meta} of Bz), 133.5 and 133.8 (C_{ipso} and C_{para} of Bz), 145.3 (C_6), 154.1 (C_4), 163.7 (C_2), 168.0 (CO of Bz); ESI-MS 328 (M + H, 100.0), 350 (M + Na, 71.9), 677 (2M + Na, 52.1). Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

E-Isomer **24**: mp 221−223 °C; UV λ_{max} 329 nm (ϵ 14 200), 269 (ϵ 18 900), 203 (ϵ 23 400); ^{1}H NMR δ 1.52 (s, 2H, $H_{3'}$), 3.44, 3.50 and 3.45, 3.48 (2AB, 4H, ^2J = 11.2 and 11.4 Hz, $H_{5'}$), 4.76 (s, 2H, OH), 7.49 (overlapped t and d, 3H, H_{meta} + H_5), 7.40 (poorly resolved d, 1H, $H_{1'}$), 7.61 (t, 1H, J = 7.2 Hz, H_{para} of Bz), 7.99 (d, 2H, J = 7.2 Hz, H_{ortho} of Bz), 8.46 (d, 1H, J = 7.2 Hz, H_6), 11.33 (s, 1H, NH); ^{13}C NMR 13.7 ($C_{3'}$), 28.2 ($C_{4'}$), 63.0 ($C_{5'}$), 97.7 (C_5), 115.5 ($C_{1'}$), 120.8 ($C_{2'}$), 129.1 and 129.2 (C_{ortho} and C_{meta} of Bz), 133.5 and 133.8 (C_{para} and C_{ipso} of Bz), 145.1 (C_6), 154.3 (C_4), 163.7 (C_2), 168.0 (CO of Bz); ESI-MS 328 (M + H, 100.0), 350 (M + Na, 97.6), 677 (2M + Na, 100.0); EI-MS 327 (M, 0.3), 105 (Bz, 100.0); EI-HRMS calcd for $C_{17}H_{17}N_3O_4$ 327.1219, found 327.1222. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

(*Z*)-1-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}cytosine (5c). The *Z* isomer **23** (297 mg, 0.91 mmol) was stirred with NH_3 in methanol (20%, 30 mL) at room temperature for 12 h. The volatile components were evaporated, and the crude product was chromatographed on a silica gel column in CH_2Cl_2−methanol (4:1) to give the *Z*-isomer **5c** (174 mg, 86%) as a white solid: mp 250−253 °C (ethanol); UV λ_{max} 297 (ϵ 11 900), 230 (ϵ 12 700), 206 nm (ϵ 13 700); ^{1}H NMR δ 1.14 (s, 2H, $H_{3''}$), 3.34 and 3.57 (AB, 4H, ^2J = 11.0 Hz, $H_{5'}$), 5.02 (broad s, 2H, OH), 5.82 (d, 1H, J = 7.2 Hz, H_5), 7.31 (s, 1H, $H_{1'}$), 7.43 and 7.55 (2s, 2H, NH_2), 8.27 (d, 1H, J = 7.2 Hz, H_6); ^{13}C NMR 10.8 ($C_{3'}$), 31.1 ($C_{4'}$), 63.0 ($C_{5'}$), 95.7 (C_5), 115.0 ($C_{1'}$), 116.5 ($C_{2'}$), 141.2 (C_6), 154.9 (C_4), 166.1 (C_2); ESI-MS 224 (M + H, 2.7), 246 (M + Na, 100.0), 469 (2M + Na, 81.0); EI-HRMS calcd for $C_{10}H_{13}N_3O_3$ 223.0957, found 223.0953. Anal. ($C_{10}H_{13}N_3O_3$) C, H, N.

(*E*)-1-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}cytosine (6c). The *E* isomer **24** (263 mg, 0.80 mmol) was

debenzoylated using the procedure described for the *Z*-isomer **5c** to give compound **6c** (149 mg, 83%): mp 249−251 °C (ethanol); UV λ_{max} 298 (ϵ 12 200), 229 (ϵ 12 300), 206 nm (ϵ 11 900). ^{1}H NMR δ 1.39 (s, 2H, $H_{3'}$), 3.37, 3.42 and 3.37, 3.41 (2AB, 4H, ^2J = 10.6 and 11.2 Hz, $H_{5'}$), 4.78 (poorly resolved t, 2H, OH), 5.89 (d, 1H, J = 8 Hz, H_5), 7.38 (s, 1H, $H_{1'}$), 7.40 and 7.57 (2s, 2H, NH_2), 7.96 (d, 1H, J = 7.4 Hz, H_6); ^{13}C NMR 13.7 ($C_{3'}$), 27.5 ($C_{4'}$), 63.2 ($C_{5'}$), 95.9 (C_5), 115.5, 115.7 ($C_{1'}$, $C_{2'}$) 140.8 (C_6), 155.0 (C_4), 166.1 (C_2); ESI-MS 224 (M + H, 2.7), 246 (M + Na, 100.0), 469 (2M + Na, 81.0). Anal. ($C_{10}H_{13}N_3O_3$) C, H, N.

1-{[1-Bromo-2,2-bis(acetoxymethyl)cyclopropyl]methyl}thymine (26). A mixture of 2,4-bis(trimethylsilyloxy)-5-methylpyrimidine (**25**, 680 mg, 2.50 mmol) and dibromide **7** (0.90 g, 2.5 mmol) was refluxed in acetonitrile (20 mL) for 148 h. After cooling, ethanol (20 mL) was added, and solvents were evaporated. The residue was triturated with CH_2Cl_2 (50 mL), the insoluble portion was filtered off using a bed of silica gel, and it was washed with CH_2Cl_2−methanol (30:1). The combined filtrate and washings were evaporated. The crude product was chromatographed on a silica gel column in CH_2Cl_2−methanol starting from 100% CH_2Cl_2 and increasing the amount of methanol to 40:1 to give compound **26** (750 mg, 74.4%) as a white solid: mp 197−198 °C; UV λ_{max} 268 (ϵ 10 400), 210 nm (ϵ 8900); ^{1}H NMR ($CDCl_3$) δ 1.76 and 1.33 (AB, 2H, J = 7.6 Hz, H_3), 1.94 (bs, 3H, 5-CH_3), 2.08 (bs, 3H, CH_3 of Ac), 4.06, 3.97 and 4.42, 4.27 (2AB, 6H, J = 11.6 Hz, $H_{5'}$), 4.59 and 4.42 (AB, 2H, J = 13.6 Hz, $H_{1'}$), 7.54 (s, 1H, H_6), 11.35 (s, 1H, NH); ^{13}C NMR 12.7 (5-CH_3), 21.2 and 21.1 (CH_3 of Ac), 25.4 ($C_{3'}$), 19.2 ($C_{4'}$), 41.6 ($C_{2'}$), 54.7 ($C_{1'}$), 63.9 and 68.4 ($C_{5'}$), 110.6 (C_5), 141.2 (C_6), 151.8 (C_2), 164.8 (C_4), 170.7 and 171.0 (CO of Ac); EI-MS 404 and 402 (M, 0.7 and 0.7), 43 (100.0); EI-HRMS calcd for $C_{15}H_{19}{}^{79}BrN_2O_6$ 402.0426, found 402.0427. Anal. ($C_{15}H_{19}BrN_2O_6$) C, H, Br, N.

(*Z*)-1-{[2,2-Bis(hydroxymethyl)cyclopropylidene]methyl}thymine (5d) and (*E*)-9-{[2,2-Bis(hydroxymethyl)-cyclopropylidene]methyl}thymine (6d). A mixture of compound **26** (0.60 g, 1.49 mmol) and K_2CO_3 (616 mg, 4.47 mmol) in DMF (50 mL) was stirred at 100 °C under N_2 for 3 h. After cooling, methanol−water (9:1, 10 mL) was added with stirring, which was continued at room temperature for 1 h. The insoluble portion was filtered off and it was washed with DMF. The filtrate was evaporated in vacuo and the residue was chromatographed on a silica gel column which was first eluted with ethyl acetate and then CH_2Cl_2−methanol (20:1) to give the *Z*-isomer **5d** (70 mg, 38%) and *E*-isomer **6d** (65 mg, 36%) as white solids.

Z-Isomer **5d**: mp 177−179 °C; UV λ_{max} 289 (ϵ 11 700), 232 nm (ϵ 12 800); ^{1}H NMR δ 1.17 (s, 2H, $H_{3'}$), 1.76 (s, 3H, 5-CH_3), 3.40, 3.61 and 3.41, 3.60 (2AB, 4H, ^2J = 10.6 and 11.2 Hz, $H_{5'}$), 4.99 (t, ^3J = 6.0 Hz, 2H, OH), 7.17 (s, 1H, $H_{1'}$), 8.32 (s, 1H, H_6), 11.42 (s, 1H, NH); ^{13}C NMR 10.9 ($C_{3'}$), 12.7 (5-CH_3), 31.2 ($C_{4'}$), 63.0 ($C_{5'}$), 111.4 ($C_{1'}$), 114.4 ($C_{2'}$), 115.2 (C_5), 136.8 (C_6), 115.0 (C_2), 164.4 (C_4); EI-MS 238 (M, 10.4), 113 (100.0); EI-HRMS calcd for $C_{11}H_{14}N_2O_4$ 238.0954, found 238.0953. Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

E-Isomer **6d**: mp 197−199 °C; UV λ_{max} 289 (ϵ 11 000), 233 nm (ϵ 12 100); ^{1}H NMR δ 1.47 (d, 2H, J = 1.6 Hz, $H_{3'}$), 1.82 (s, 3H, 5-CH_3), 3.38, 3.45 and 3.40, 3.43 (2AB, 4H, ^2J = 11.2 and 11.4 Hz, $H_{5'}$), 4.66 (t, 2H, ^3J = 5.6 Hz, OH), 7.25 (s, 1H, $H_{1'}$), 7.82 (s, 1H, H_6), 11.46 (s, 1H, NH); ^{13}C NMR 12.8 ($C_{3'}$), 13.9 (5-CH_3), 27.8 ($C_{4'}$), 63.1 ($C_{5'}$), 110.9 ($C_{1'}$), 113.8 ($C_{2'}$), 116.3 (C_5), 136.1 (C_6), 150.2 (C_2), 164.4 (C_4); EI-MS 238 (M, 12.8), 127 (100.0); EI-HRMS calcd for $C_{11}H_{14}N_2O_4$ 238.0954, found 238.0955. Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

Adenosine Deaminase (ADA) Assay.[3] Compound **5a** or **6a** (2.6 μmol) was incubated with ADA from calf intestine (0.45 units) in 0.05 M Na_2HPO_4 (pH 7.4, 0.4 mL) at room temperature with magnetic stirring. Aliquots were periodically withdrawn and examined by TLC in CH_2Cl_2–MeOH (5:1). The spots of starting materials and deamination products were eluted with ethanol, and UV spectra were recorded. After 24 h of incubation, the extent of deamination of compounds **5a** and **6a** was 17% and 92%, respectively.

Biological Assays. The antiviral assays were performed as described previously.[3,20,23] The HCMV assays were run in HFF culture with two strains of virus, Towne and AD169, in a plaque reduction or cytopathic effect inhibition (CPE) assay. The MCMV was assayed in MEF by plaque reduction. The HSV-1 was run in BSC-1 cells by ELISA. In addition, HSV-1 and HSV-2 assays were perfomed in HFF (CPE assay) and Vero cells (plaque reduction assay). The VZV was assayed in HFF (CPE and plaque reduction), and hepatitis B virus (HBV) in 2.2.15 cells by DNA hybridization. The EBV assays were performed in Daudi cells by viral capsid antigen (VCA) ELISA and in H-1 cells by DNA hybridization assay. The cytotoxicity assays were performed in HFF, KB, and CEM cells. For further details, see Tables 2 and 3.

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