(*Z*)- and (*E*)-[2-Fluoro-2-(hydroxymethyl)cyclopropylidene]methylpurines and -pyrimidines, a New Class of Methylenecyclopropane Analogues of Nucleosides: Synthesis and Antiviral Activity[1]

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The *Z*- and *E*-isomers of fluoromethylenecyclopropane analogues **11a**−**d** and **12a**−**d** were synthesized, and their antiviral activities were evaluated. The purine (*Z*,*E*)-methylenecyclopropane carboxylates **13** and **24** were selectively fluorinated using lithium diisopropylamide, LiCl, and *N*-fluorobenzenesulfonimide to give (*Z*,*E*)-fluoroesters **22** and **25**. Reduction with $LiBH_4$ or diisobutylaluminum hydride gave after chromatographic separation *Z*-isomers **11a** and **11e** and *E*-isomers **12a** and **12e**. The O-demethylation of **11e** and **12e** afforded guanine analogues **11b** and **12b**. Fluorination of (*Z*,*E*)-cytosine and thymine esters **15** and **16** afforded (*Z*,*E*)-fluoroesters **26** and **27**, which were resolved before the reduction to analogues **11c** and **11d** and **12c** and **12d**. Adenine *Z*-isomer **11a** was the most effective against Towne and AD169 strains of human cytomegalovirus (HCMV, EC_{50} 3.6 and 6.0 μM, respectively), but it was less effective against murine virus (MCMV, EC_{50} 69 μM). Thymine *Z*-isomer **11d** was effective against HSV-1 in BSC-1 cells (ELISA, EC_{50} 2.5 μM) but inactive against HSV-1 or HSV-2 in Vero or HFF cells. All of the analogues with the exception of **12d** were effective at least in one of the assays against Epstein−Barr virus (EBV) in Daudi or H-1 cells in a micromolar or submicromolar range. Cytosine and thymine *Z*-isomers **11c** and **11d** were active against varicella zoster virus (VZV) with EC_{50} 0.62 μM. Adenine *Z*- and *E*-isomers **11a** and **12a** were effective against HIV-1 in MT-2 or MT-4 cells with EC_{50} 12−22 and 2.3−7.6 μM, respectively, whereas only **12a** was effective against hepatitis B virus (HBV) with EC_{50} 15 μM. Analogues **11a** and **12a** were weak substrates for adenosine deaminase.

Recently, we described a structurally novel class of nucleoside analogues based on a methylenecyclopropane template.[2,3] The *Z*-series **1** (Chart 1) provided a number of purine analogues effective against several types of herpesviruses such as human cytomegalovirus (HCMV), Epstein−Barr virus (EBV), and human herpes virus 6 and 8 (HHV-6 and HHV-8).[4,5] The *E*-isomers **2** were active only in a few instances. These studies have led to selection of (*S*)-(+)-2-amino-6-cyclopropylaminopurine analogue **3** (QYL-1064), which is an orally effective anti-HCMV agent, as a good candidate for preclinical investigation.[4,6] More recently, a second generation of analogues **1** and **2**, compounds **4** and **5**, also yielded several active antivirals.[7] In this series, guanine analogue **4b** (cyclopropavir, ZSM-I-62), whose in vitro potency surpasses that of anti-HCMV drug ganciclovir, appears as the most promising candidate for a preclinical investigation.[8]

Previously, we reported[9] on *gem*-difluoromethylenecyclopropane analogues **6a**, **6b**, **7a**, and **7b**. In this group, only adenine *E*(*cis*)-isomer **6a** exhibited a moderate anti-HCMV effect. It is possible that biological effects of these analogues are adversely influenced by their limited stability. We anticipated that the stability will be improved by introduction of only a single fluorine atom into a different position of the cyclopropane ring. In addition, several effective antiviral agents are found among fluorinated nucleosides.[10] For example, antibiotic nucleocidin[11] **8** and antiherpetic agent 4′-fluorocarbocyclic-2′-deoxyguanosine[12] **9** (X = H) have a fluorine atom situated α to the sulfamoyloxymethyl or hydroxymethyl group of the ribofuranose moiety. Compounds **9** (X = H or F) were effective against herpes simplex virus type 1 and 2 (HSV-1 and HSV-2),[12,13] and the fluoro analogue of lobucavir **10** exhibited activity against a broad spectrum of herpes viruses.[14] On the basis of these considerations, a study of methylenecyclopropane analogues of nucleosides with a similar orientation of the hydroxymethyl group and fluorine atom appears warranted. In this contribution, we describe the synthesis and antiviral activity of analogues **11a**−**d** and **12a**−**d**. Comparison of biological effects of this series with the non-

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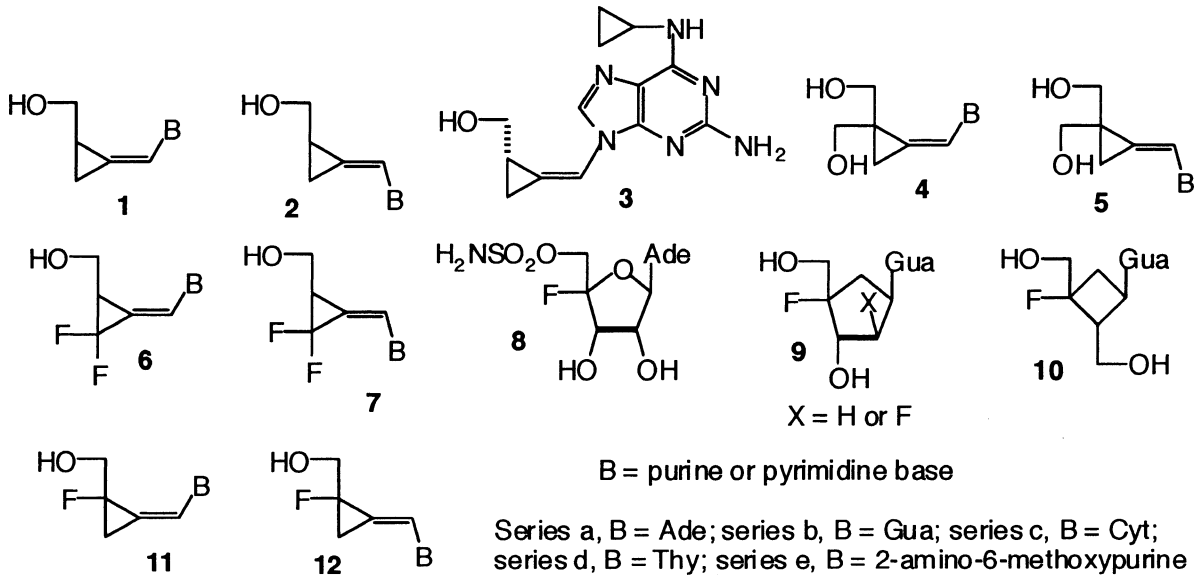
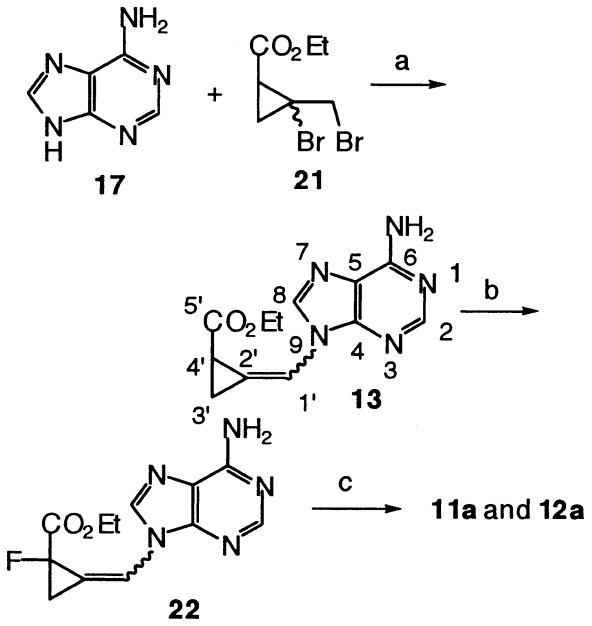
Chart 1



Scheme 1[a]

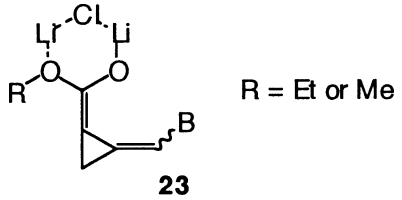


[a] Reagents: (a) K_2CO_3, DMF, Δ; (b) (1) LiCl, LDA, THF, −78 °C, (2) NFSI, THF, −78 °C; (c) $LiBH_4$, MeOH, THF.

fluorinated analogues **1a**−**d** and **2a**−**d** will also be discussed.

Synthesis. Previous results have shown that a direct electrophilic fluorination can be used for the synthesis of nucleoside analogues. Thus, a key step in the synthesis of analogue **10** was fluorination[14] of an ester intermediate with $FClO_3$ via carbanion formation. More recently, fluorination of carbanions of *tert*-butyl thymidine 3′-*O*-benzenesulfonyl-5′-carboxylate and the respective xylo derivative with a safer agent, *N*-fluorobenzenesulfonimide (NFSI), was described.[15] These results have indicated that ionizable NH functions of nucleic acid bases may not interfere with carbanion generation. Because methylenecyclopropane esters **13**−**16** (Schemes 1−4) are accessible by an alkylation−elimination of the corresponding nucleic acid bases or precursors **17**−**20** using a readily available ethyl (*Z*,*E*)-2-bromo-2-(bromomethyl)cyclopropane-1-carboxylate[16,17] (**21**, Scheme 1), direct fluorination was considered as a method for the synthesis of the desired targets **11a**−**d** and **12a**−**d**.

Fluorination of adenine (*Z*,*E*)-methylenecyclopropane ester[16] **13** using lithium diisopropylamide (LDA) and NFSI in tetrahydrofuran (THF) gave a mixture of *Z*,*E*-isomers **22** in 68% yield (Scheme 1). To achieve high yields, it was necessary to include LiCl in the protocol. The order of addition of reaction components was also important. First, excess (about 6 equiv) of LiCl and ester **13** in THF were cooled to −78 °C. The LDA in THF was then added followed by NFSI as the last component. In the absence of LiCl, yields were low or no product was obtained. Also, use of LiCl makes a large excess of strong base[15] superfluous. It is likely that LiCl stabilizes the ester enolate as observed with enolates of carbonyl compounds[18] (**23**). Additional LiCl salt bridges may occur between the heterocyclic moiety and ester enolate function.



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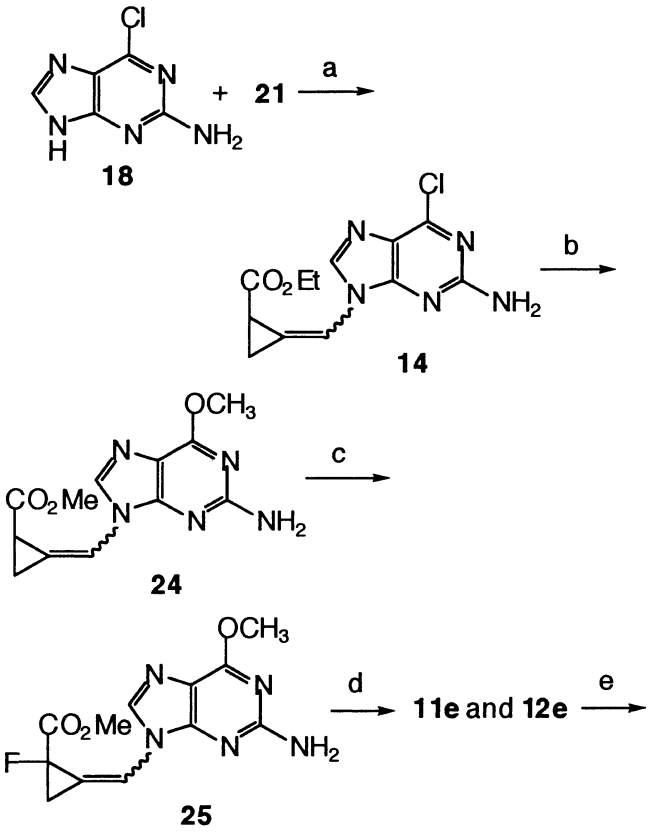
Reduction of **22** with $LiBH_4$ in the presence of methanol[19] in THF furnished, after chromatographic resolution, analogues **11a** and **12a** in 27 and 16% yields, respectively. It should be emphasized that for separation of the parent analogues **1a** and **2a** derivatization was necessary.[16,20] The *Z*- and *E*-isomers **11a** and **12a** are of sufficient stability for in vitro antiviral testing; they were unchanged at pH 7 at room temperature for 1 week.

The *Z*,*E*−2-amino-6-chloropurine esters **14** were not successfully fluorinated by this procedure. It is possible that carbanions derived from the heterocycle[21] compete with the formation of enolate. At any rate, the *Z*,*E*-ester **14** was readily transformed to the 6-methoxy derivative **24** in 95% yield (Scheme 2) using K_2CO_3 in methanol.[4] Reesterification to methyl ester occurred at the same

Scheme 2[a]



11b and 12b

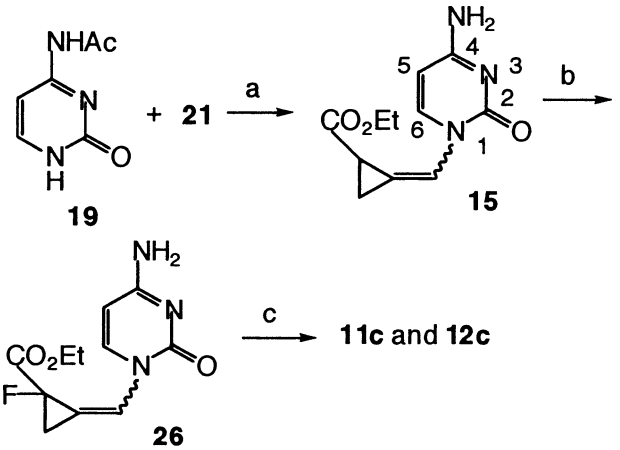
[a] Reagents: (a) K_2CO_3, DMF, Δ; (b) K_2CO_3, MeOH; (c) (1) LiCl, LDA, THF, −78 °C, (2) NFSI, THF, −78 °C; (d) DIBALH, THF; (e) KI, Me_3SiCl, MeCN.

time. Fluorination of **24** under the conditions described for adenine ester **22** gave the *Z*,*E*-ester **25** (19%). Reduction with diisobutyl aluminum lithium hydride (DIBALH) in THF furnished analogues **11e** and **12e**, which were separated by chromatography in 54 and 34% yields, respectively. The O^6-demethylation was performed with separated isomers using KI and Me_3SiCl in MeCN[22] to give the corresponding guanine analogues **11b** and **12b** in 76 and 73% yields, respectively.

Fluorination was also successful with pyrimidine methylenecyclopropane carboxylates **15** and **16**, which were not resolved by chromatography.[17] Thus, fluorination of the (*Z*,*E*)-cytosine ester[17] **15** under the conditions described for adenine analogue **22** gave the *Z*- and *E*-fluoroester **26** after chromatographic separation in 27 and 22% yields, respectively (Scheme 3). The separated isomers were reduced with $LiBH_4$ as described for adenine ester **22** to give the *Z*-isomer **11c** (57%) and *E*-isomer **12c** (71%).

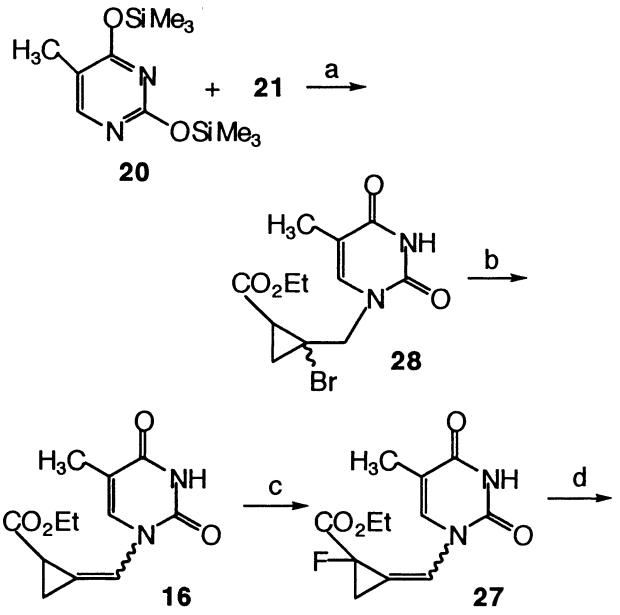
Previous synthesis[17] of (*Z*,*E*)-thymine esters **16** suffered from contamination with methyl esters due to workup of the reaction mixture with methanol. This was now avoided, and intermediate esters **28** and **16** were obtained in 67 and 56% yields, respectively (Scheme 4). Chromatographic separation of **16** was only partly successful, giving pure *Z*-isomer and a mixture of the *Z*- and *E*-isomers. Fluorination of the *Z*-isomer of **16**, using lithium hexamethyldisilazane (LiHMDS) and NFSI in THF at −78 °C, gave the *Z*-isomer of **27** in 26%

Scheme 3[a]



[a] Reagents: (a) (1) K_2CO_3, DMF, Δ. (2) K_2CO_3, EtOH/H_2O; (b) (1) LiCl, LDA, THF, −78 °C, (2) NFSI, THF, −78 °C; (c) DIBALH, THF.

Scheme 4[a]



11d and 12d

[a] Reagents: (a) MeCN, Δ; (b) K_2CO_3, DMF, Δ; (c) (1) LiCl, LDA, THF, −78 °C, (2) NFSI, THF, −78 °C or NSFI, LiHDMS, THF, −78 °C; (d) $LiBH_4$, MeOH, THF.

yield. To obtain both *Z*- and *E*-isomers of **27**, an isomeric mixture of **16** was fluorinated, using the conditions described for adenine analogue **22** (Scheme 1). The *Z*- and *E*-isomers of **27** were smoothly separated in 41 and 30% yields, respectively. Reduction of the separated isomers with $LiBH_4$, following the protocol for **22** (Scheme 1), afforded analogues **11d** and **12d** (68 and 71% yields, respectively).

Assignment of *Z*- and *E*-Isomers. As in previous cases,[7,16,17] the *Z*-isomers were always less polar, moving faster on TLC and silica gel columns than *E*-isomers. Comparison of relevant ^1H and ^{13}C NMR chemical shifts of the fluorinated analogues **11a**−**d** and **12a**−**d** with those of the nonfluorinated series[16,17] **1a**−**d** and **2a**−**d** confirmed the isomeric assignments (Table 1). Differences between the *Z*-isomers and *E*-isomers

Table 1. Comparison of Selected ^1H (δ) and ^{13}C NMR (ppm) Chemical Shifts of the *Z*-Isomers **11a**−**d** and **1a**−**d** with *E*-Isomers **12a**−**d** and **2a**−**d**

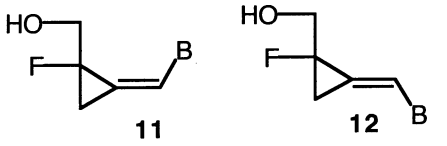
isomer[a]	OH	$H_{1'}$	H_8 or H_6[b]	$H_{5,5''}$	$C_{3'}$
1a (Z)	5.11	7.38	8.74	3.33, 3.73	6.7
11a (Z)	5.69	7.51	8.61	3.71, 4.17	15.6
2a (*E*)	4.82	7.48	8.48	3.41	7.9
12a (*E*)	5.31	7.88	8.56	3.79	17.9
1b (*Z*)	5.04	7.11	8.31	3.32, 3.68	6.6
11b (*Z*)	5.58	7.17	8.20	3.63, 4.12	15.6
2b (*E*)	4.80	7.21	8.04	3.37	9.6
12b (*E*)	5.29	7.59	8.11	3.72, 3.79	17.7
1c (*Z*)	4.93	7.30	8.13	3.31, 3.53	5.6
11c (*Z*)	5.50	7.44	7.93	3.61, 4.01	14.5
2c (*E*)	4.75	7.37	7.96	3.32	9.0
12c (*E*)	5.29	7.45	8.00	3.70	16.5
1d (*Z*)	5.06	7.15	8.20	3.15, 3.71	5.5
11d (*Z*)	5.65	7.32	8.05	3.51, 4.17	14.6
2d (*E*)	4.75	7.22	7.81	3.32	9.2
12d (*E*)	5.24	7.65	7.86	3.72	17.2

[a] All of the spectra were determined in CD_3SOCD_3. The δ and ppm values for **1a**, **1b**, **2a,** and **2b** were taken from ref 16 and those for **1c**, **1d**, **2c**, and **2d** from ref 17. [b] H_8 of purines, H_6 of pyrimidines.

observed in the nonfluorinated series **1a**−**d** and **2a**−**d** were largely preserved in the 4′-fluoro derivatives **11a**−**d** and **12a**−**d**. Thus, the H_8 of the *Z*-purines **11a** and **11b** and the H_6 of *Z*-pyrimidine **11d** are located at a lower field than those of the *E*-isomers **12a**, **12b**, and **12d**. The $\Delta\delta$ values of $H_8(H_6)$ chemical shifts of *Z*- and *E*-isomers were somewhat smaller in the fluorinated compounds **11a**, **11b**, and **11d** versus **12a**, **12b**, and **12d** than those of the nonfluorinated isomers. The cytosine analogues **11c** and **12c**, where $\delta H_6(Z) < \delta H_6(E)$, were the only exceptions. The chemical shifts of OH and $H_{1'}$ also followed the patterns observed in nonfluorinated analogues, the OH signal of the *Z*-isomers being always downfield from those of the *E*-isomers, whereas the opposite is true for the $H_{1'}$. In cytosine analogues **11c** and **12c**, both $\delta H_{1'}$ are equivalent. Another important feature for distinguishing between the *Z*- and *E*-isomers are the $H_{5',5''}$ signals of the hydroxymethyl group. In the *Z*-isomers, both hydrogen atoms are invariably nonequivalent whereas in the *E*-isomers they are either equivalent or $\Delta\delta H_{5',5''}$ is significantly smaller.[2,3,7] This characteristic pattern was fully preserved in the fluorinated analogues **11a**−**d** and **12a**−**d**. In the ^{13}C NMR spectra of methylenecyclopropanes **1** and **2**, the C_3 chemical shifts of the *Z*-isomers are upfield from those of the *E*-isomers. This was also observed in the fluorinated series **11** and **12**. Taken together, these facts support the *Z*/*E* isomeric assignment of compounds **11a**−**d** and **12a**−**d**. In addition, the *Z*-fluoroester of **22**, prepared from the *Z*-isomer of **13**, was reduced to analogue **11a**, identical with the product obtained from the *Z*,*E*-isomeric mixture **23**. In a similar fashion, fluorination of the *Z*-ester **16** gave the *Z*-fluoroester **27**. Reduction of ***Z*-27** then afforded the Z-analogue **11d**.

Biological Activity. Antiviral Effects. Analogues **11a**−**d** and **12a**−**d** 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 type 1 (HIV-1). The results are summarized in Tables 2 and 3.

Table 2. Inhibition of HCMV and HSV-1 Replication by Fluoromethylenecyclopropane Analogues of Nucleosides



11a, **12a**: B = Ade **11c**, **12c**: B = Cyt
11b, **12b**: B = Gua **11d**, **12d**: B = Thy

	EC_{50}/CC_{50} (μM)		
	HCMV/HFF		
compound	Towne[b,c]	AD169[d,e]	HSV-1/BSC-1[a]
11a	3.6/> 100[f]	6.0/>425[b,g]	>100/>100
11b	>100/>100	>79.6/>398	>100/>100
11c	>100/>100	>474/>474	>100/>100
11d	>100/>100	>442/>442	2.5/>100
12a	20/>100[f]	>16.7/>417	30/>100
12b	>100/>100	>79.6/>398	90/>100
12c	>100/>100	>474/>474	>100/>100
12d	>100/>100	>442/>442	40/>100
control	2.1/>100[h]	2.2/>392[b,h]	10/>10[i]

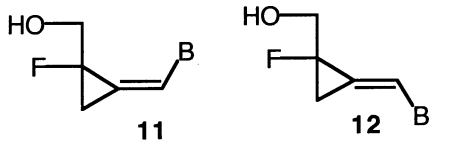
[a] ELISA. Cytotoxicity was determined in KB cells. All of the listed compounds were inactive against HSV-1 or HSV-2 in Vero (EC_{50}/CC_{50} >50/>100 μM)[b] and HFF (EC_{50}/CC_{50} >80/>400 μM)[d] culture. [b] Plaque reduction assay. [c] Visual cytotoxicity. [d] Cytopathic effect (CPE) inhibition assay. [e] Cytotoxicity by neutral red uptake. [f] Average of duplicate experiments. [g] Against MCMV/MEF[b] the EC_{50}/CC_{50} was 69.3/>425 μM. Because of a lack of potency against HCMV, other compounds listed in Table 2 were not tested. [h] Ganciclovir. [i] Acyclovir.

The only compound potent against HCMV (Towne and AD169 strains) in the micromolar range was the *Z*-adenine isomer **11a** (EC_{50} 3.6 and 6.0 μM, respectively) followed by the *E*-isomer **12a** (EC_{50} 20 and >16.7 μM, Table 2). No cytotoxicity was observed. Although potency of **11a** against HCMV corresponded to that of the nonfluorinated counterpart[16] **1a**, it was much lower against MCMV with EC_{50} 69 μM. Nucleoside analogues active against HCMV but lacking significant effect against MCMV are known.[23] The rest of the compounds were inactive including, somewhat surprisingly, the analogue of synguanol **11b**. It is then clear that the anti-HCMV effect of this class of methylenecyclopropanes is more narrow than in nonfluorinated series where both adenine and guanine *Z*-isomers **1a** and **1b** were potent anti-CMV agents.[16]

Activity against HSV-1 and HSV-2 was tested in the following host cells: BSC-1 (HSV-1), Vero (HSV-1 and HSV-2), and HFF (HSV-1 and HSV-2). Antiviral effect was seen only in BSC-1 cells (ELISA). Thymine *Z*-analogue **11d** was the most effective with EC_{50} 2.5 μM, which almost coincides with that of the parent compound[17] **1d** (EC_{50} 2.0 μM).

More interesting results were obtained in EBV assays (Table 3). Only a single analogue **12d** was inactive in both Daudi and H-1 cells. Thus, the *Z*-isomers **11a**, **11b**, **11c**, and, surprisingly, the corresponding *E*-isomers **12a**, **12b**, and **12c** were effective against EBV in Daudi cells (VCA ELISA assay) in a micromolar or submicromolar range. Efficacy of the *E*-isomers against EBV was noted before,[16,17] especially in the second-generation methylenecyclopropane analogues.[7] Potency in DNA hybridization assays was generally lower with the exception of **11b** and **11d**. Activity against EBV in H-1 cells is more

Table 3. Inhibition of EBV, VZV, HIV-1, and HBV Replication by Fluoromethylenecyclopropane Analogues of Nucleosides



11a, **12a**: B = Ade **11c**, **12c**: B = Cyt
11b, **12b**: B = Gua **11d**, **12d**: B = Thy

	EC_{50}/CC_{50} (μM)				
	EBV		VZV	HIV-1_{LAI}	HBV
compound	Daudi[a]	H-1[b,c]	HFF[d,e]	MT-2[d]	2.2.15[b,c]
11a	1.45/>213 (6.8)	13.8/>100	14.0	12/>100[f]	>20
11b	4.8/>199 (8.0)	>20/>100	>79.6	>100/>100	>20
11c	<0.38/>237 (28.4)	>20/>100	0.62	>100/>100	>20
11d	51.7/>221 (4.4)	2.5/>100	0.62	>100/>100	>20
12a	2.3/>209 (167)	3.6/>100	>83.4	2.3/>100[g]	15
12b	<0.32/>199 (29.1)	>20/>100	>79.6	>100/>100	>20
12c	0.76/>237 (94.8)	>20/>100	14.7	>100/>100	>20
12d	>221/>221	>20/>100	>442	>100/>100	>20
control	1.1/>222 (5.3)[h]	5[i]	1.6/>444[h]	0.02/>10[j]	0.02/>100[k]

[a] Viral capsid immnunofluorescence (VCA) ELISA. Values in parentheses are for DNA hybridization assay. [b] DNA hybridization assay. [c] Cytotoxicity was determined in CEM cells. [d] Cytopathic effect (CPE) assay. [e] Only the EC_{50} values are listed, for CC_{50}'s see HCMV(AD169)/HFF in Table 2. [f] EC_{50}/CC_{50} 22/>100 μM in MT-4 culture. [g] EC_{50}/CC_{50} 7.6/>100 μM in MT-4 culture. [h] Acyclovir. [i] Ganciclovir. [j] AZT. [k] Lamivudine.

limited. Only a single analogue, *E*-isomer **12a**, was strongly active against EBV in both Daudi and H-1 culture with EC_{50} 2.3 and 3.6 μM, respectively. Thymine *Z*-isomer **11d** was effective in DNA hybridization assays in both Daudi and H-1 cells (EC_{50} 4.4 and 2.5 μM, respectively) but less so in VCA ELISA assay (EC_{50} 51.7 μM). Roughly, the activity pattern follows that of the nonfluorinated analogues[16,17] although some differences were noted. For example, the *Z*-analogue **11b** was effective against EBV in Daudi cells but inactive in H-1 culture, whereas an opposite situation was encountered with synguanol (**1b**). Also, syncytol (**1c**) was a potent anti-EBV agent in both types of host cells[17] but analogue **11c** only in Daudi culture. No cytotoxicity effects were noted throughout both series. Again, as shown before for other methylenecyclopropane analogues,[4,7,24] anti-EBV activity is cell-culture- and assay-dependent. The pyrimidine *Z*-analogues **11c** and **11d** were effective against VZV in HFF culture in a submicromolar range whereas compounds **11a** and **12c** exhibited a moderate potency.

Surprisingly, activity of the *E*-isomer **12a** against HIV-1 in MT-2 and MT-4 cells (EC_{50} 2.3 and 7.6 μM, respectively) surpassed that of *Z*-isomer **11a** (EC_{50} 12 and 22 μM). In contrast, the *E*-isomer **2a** was inactive whereas synadenol (**1a**) had EC_{50} 0.75 μM (MT-2).[25] Against HBV, only the *E*-isomer **12a** had a moderate effect (EC_{50} 15 μM).

Mechanism of action studies of the methylenecyclopropane analogues strongly indicate that they are "true" nucleoside analogues activated by phosphorylation[26,27] and displaying their antiviral effects by inhibition of viral DNA polymerases (reverse transcriptase) at a triphosphate level.[28] It is then likely that the fluorinated analogues reported herein follow a similar pattern of intracellular activation.

Adenosine Deaminase (ADA). Adenine analogues **11a** and **12a** were substrates for adenosine deaminase from calf intestine. The *Z*-isomer **11a** was deaminated more slowly than *E*-isomer **12a**. Thus, deamination of analogue **12a** was 72% complete after 24 h whereas only 37% of compound **11a** was deaminated. As a rule, the *Z*-isomers of adenine methylenecyclopropane analogues are less reactive toward deamination than *E*-isomers.[2,3]

Experimental Section

General Methods. See ref 7. The UV spectra were measured in ethanol, and NMR spectra were determined at 300 or 400 MHz (^{1}H), 75 or 100 MHz (^{13}C), and 376 MHz (^{19}F) in CD_3SOCD_3 unless stated otherwise. For ^{19}F NMR, $CFCl_3$ was used as a reference. The ^{13}C NMR assignments were verified by DEPT spectra. Mass spectra were determined in electron-impact (EI-MS), chemical ionization (CI-MS, 2-methylpropane as an ionization gas), or electrospray ionization (ESI-MS, methanol−NaCl) mode.

(*Z*,*E*)-9-[(2-Carbethoxycyclopropylidene)methyl]-adenine (13). The previously described procedure[16] was modified as follows. A mixture of adenine (**17**, 0.81 g, 6.0 mmol), ethyl (*Z*,*E*)-2-bromo-2-(bromomethyl)cyclopropane-1-carboxylate (**21**, 1.74 g, 6.06 mmol), and K_2CO_3 (4.98 g, 36 mmol) in dimethylformamide (DMF) (30 mL) was heated at 100 °C with stirring under N_2 for 17 h. DMF was evaporated, the residue was dissolved in EtOAc/EtOH (10:1), and the solution was filtered using a Celite bed, which was then repeatedly washed with the same solvent. The filtrate was concentrated, and crude product was chromatographed (EtOAc/EtOH = 20:1 to 10:1) to give a mixture of the *Z*- and *E*-isomers **13** (0.99 g, 63%) in the ratio of 1.4:1 as a white solid.

(*Z*)-9-[(2-Carbethoxycyclopropylidene)methyl]-adenine (*Z*-13). The mixture of isomers (545 mg, 2.11 mmol) described above was chromatographed in EtOAc/MeOH = 40:1 to give the *Z*-isomer **13** (294 mg, 54%), isomeric purity 94−95% as determined by ^{1}H NMR. The ^{1}HNMR spectrum was identical with that of the *Z*-isomer portion of (*Z*,*E*)-isomer mixture described previously.[16] The nuclear Overhauser effect enhancements were shown between H_8 and $H_{4'}$ (5.5 and 10.8%, respectively) whereas none were observed between H_8 and $H_{3'}$.

(*Z*,*E*)-9-[(2-Carbethoxy-2-fluorocyclopropylidene)-methyl]adenine (22). A suspension of LiCl (147 mg, 3.46 mmol, dried at room temperature, 0.05−0.07 Torr for 48 h, and 80−90 °C, 0.2 Torr for 3 h) and *Z*/*E*-isomers **13** (1.4:1,

150 mg, 0.58 mmol) in THF (20 mL) was cooled to −78 °C. After 10 min, lithium diisopropylamide (LDA, 1.8 M in THF, 1.06 mL, 1.9 mmol) was added dropwise with stirring, which was continued for 45 min. *N*-Fluorobenzenesulfonimide (NFSI, 600 mg, 1.90 mmol) in THF (2 mL) was then added, and after 15 min the reaction was quenched with aqueous NH_4Cl/EtOH (1:1, 2 mL). The reaction mixture was then warmed to room temperature whereupon the solvents were evaporated, and the residue (Z/E = 1.3:1) was first chromatographed on a silica gel column using EtOAc and then using hexanes/EtOAc (1:1). The product was partitioned between CH_2Cl_2 (30 mL) and water (5 × 10 mL).[29] The organic phase was dried ($MgSO_4$), and solvent was evaporated to give the Z/E isomers **22** (2:1, 110 mg, 68%). Mp: 196−200 °C. UV λ_{max}: 238 nm (ϵ 29 100), 282 nm (ϵ 9100). ^{1}H NMR δ: 1.15 (*Z*-isomer) and 1.20 (*E*-isomer, 2t, 3H, ^3J = 7.2 Hz, CH_3), 2.42 (*Z*-isomer) and 2.64 (*E*-isomer, 2m, 2H, $H_{3'}$, and $H_{3''}$), 4.18 (q, 2H, ^3J = 7.2 Hz, OCH_2), 7.43 (*Z*-isomer) and 7.47 (*E*-isomer, 2bs, 2H, NH_2), 7.68 (*Z*-isomer, bs) and 8.07 (*E*-isomer, m, 1H, $H_{1'}$), 8.08 and 8.16 (*Z*-isomer), 8.20 and 8.59 (*E*-isomer, 4s, 1H, H_2 and H_8). ^{13}C NMR: 14.6, 14.7 (CH_3), 19.1, 21.6 (2d, $^2J_{3',F}$ = 13.5 Hz, $C_{3'}$), 62.2, 62.5 (OCH_2), 72.5, 73.4 (2d, $^1J_{4',F}$ = 235.8 Hz, $C_{4'}$), 110.6, 110.9 (2d, $^2J_{2',F}$ = 4.3 and 4.7 Hz, $C_{2'}$), 115.8, 116.3 ($C_{1'}$), 119.2, 119.6 (C_5), 138.5, 139.6 (C_8), 149.2, 149.4 (C_4), 154.0, 154.2 (C_2), 156.9 (C_6), 168.0, 168.3 (CO). ^{19}F NMR: −182.25 and −182.61 (d, $^3J_{3',F}$ = 6.0 Hz). EI-MS: 277 (M, 95.4), 205 (100.0), 135 (adenine, 21.0). HRMS calcd for $C_{12}H_{12}N_5O_2F$: 277.0975. Found: 277.0979.

(*Z*)-9-[(2-Carbethoxy-2-fluorocyclopropylidene)methyl]adenine (*Z*-22). The *Z*-isomer **13** (260 mg, 1.0 mmol) was fluorinated as described above for the (*Z*,*E*)-isomeric mixture to give the fluoro analogue *Z*-**22** (180 mg, 65%) whose ^{1}H NMR spectrum was identical with the *Z*-isomer portion of the mixture.

(*Z*)-{[2-Fluoro-2-(hydroxymethyl)cyclopropylidene]methyl}adenine (11a) and (*E*)-9-{[2-Fluoro-2-(hydroxymethyl)cyclopropylidene]methyl}adenine (12a). A 2:1 mixture of the *Z*- and *E*-esters **22** (530 mg, 1.93 mmol) was dissolved in THF (30 mL). The solution was cooled to 0 °C, and methanol (0.078 mL, 1.94 mmol) was added followed by $LiBH_4$ (2.0 M in THF, 1.93 mL, 3.86 mmol). The reaction mixture was stirred for 1 h at 0 °C whereupon aqueous methanol (50%, 12 mL) was added and the stirring was continued at room temperature for 24 h. The solvents were evaporated, and the crude product was chromatographed in EtOAc/MeOH (50:1) to give after recrystallization from methanol the *Z*-isomer **11a** (144 mg, 32%) and *E*-isomer **12a** (84 mg, 18.5%).

***Z*-Isomer 11a.** Mp: 238−239 °C. UV δ_{max}**:** 233 nm (ϵ 24 700), 278 nm (ϵ 9300). ^{1}H NMR δ: 1.92 (poorly resolved dd, 1H, $^3J_{3',F}$ = 12.0 Hz, $H_{3'}$) and 2.00 (poorly resolved dt, 1H, $^3J_{3'',F}$ = 11.2 Hz, $H_{3''}$), 3.71 (ddd, 1H, J = 28.8, 12.8, and 6.0 Hz, $H_{5'}$) and 4.17 (dt, 1H, J = 14.8 and 4.8 Hz, $H_{5''}$), 5.69 (t, 1H, $^3J_{OH,5'}$ = 5.4 Hz, OH), 7.45 (bs, 2H, NH_2), 7.51 (bs, 1H, $H_{1'}$), 8.20 (s, 1H, H_2), 8.61 (s, 1H, H_8). ^{13}C NMR: 15.6 (d, $^2J_{3',F}$ = 13.4 Hz, $C_{3'}$), 63.8 (d, $^2J_{5',F}$ = 24.7 Hz, $C_{5'}$), 78.1 (d, $^1J_{4',F}$ = 231.3 Hz, $C_{4'}$), 111.7 (d, $^2J_{2',F}$ = 4.5 Hz, $C_{2'}$), 114.0 ($C_{1'}$), 119.1 (C_5), 138.3 (C_8), 148.7 (C_4), 154.0 (C_2), 156.8 (C_6). ^{19}F NMR: −179.41 (poorly resolved ddd, J = 29.0, 13.9, and 11.3 Hz). EI-MS: 235 (15.2, M), 218 (77.3, M − OH), 135 (100.0, adenine). HRMS calcd for $C_{10}H_{10}FN_5O$: 235.0869. Found: 235.0865. Anal. ($C_{10}H_{10}FN_5O$) C, H, N.

***E*-Isomer 12a**. Mp: 233−235 °C. UV λ_{max}: 232 nm (28 100), 278 (9500). ^{1}H NMR δ: 2.05 (dd, 1H, J = 12.2 and 2.6 Hz, $H_{3'}$) and 2.20 (dt, 1H, J = 10.8 and 3.2 Hz, $H_{3''}$), 3.79 (dd, 2H, J = 20.8 and 6.0 Hz, $H_{5'}$), 5.31 (t, 1H, $^3J_{5',OH}$ = 5.6 Hz, OH), 7.44 (bs, 2H, NH_2), 7.88 (poorly resolved m, 1H, $H_{1'}$), 8.19 (s, 1H, H_2), 8.56 (s, 1H, H_8). ^{13}C NMR: 17.9 (d, $^2J_{3',F}$ = 14.2 Hz, $C_{3'}$), 63.3 (d, $^2J_{5',F}$ = 26.1 Hz, $C_{5'}$), 77.6 (d, $^1J_{4',F}$ = 230.6 Hz, $C_{4'}$), 112.5 (d, $^2J_{2',F}$ = 5.2 Hz, $C_{2'}$), 115.1 ($C_{1'}$), 119.1 (C_5), 138.0 (C_8), 149.2 (C_4), 154.0 (C_2), 156.8 (C_6). ^{19}F NMR: −178.87 (td, J = 21.3 and 10.2 Hz). EI-MS: 235 (7.9, M), 218 (100.0, M − OH), 135 (25.0, adenine). HRMS calcd for $C_{10}H_9FN_5$ (M − OH): 218.0842. Found: 218.0845. Anal. ($C_{10}H_{10}FN_5O$) C, H, N.

(*Z*,*E*)-2-Amino-6-chloro-9-[(2-carbethoxycyclopropylidene)methylpurine (14). The previously described procedure[16] was modified as follows. A mixture of 2-amino-6-chloropurine (**18**, 0.81 g, 6.0 mmol), dibromo ester **21** (1.74 g, 6.06 mmol), and K_2CO_3 (4.98 g, 36 mmol) in DMF (30 mL) was heated at 100 °C with stirring under N_2 for 17 h to give after chromatography on silica gel using EtOAc/EtOH (20:1 to 10:1) product **14** (0.99 g, 56%), Z/E = 1.5:1 as a white solid.

(*Z*,*E*)-2-Amino-6-methoxy-9-[(2-carbomethoxycyclopropylidene)methyl]purine (24). A mixture of isomers **14** (Z/E = 1.5:1, 590 mg, 2.0 mmol) was dissolved in methanol (30 mL), and K_2CO_3 (276 mg, 2.0 mmol) was added. The reaction mixture was stirred at 50 °C for 30 min. After removal of solvents in vacuo at room temperature, the residue was chromatographed on a silica gel column using hexane/EtOAc (1:1) to give the *Z*- and *E*-isomeric mixture **24** in the ratio of 1.5:1 (526 mg, 95%). Mp: 158−162 °C. UV λ_{max}: 224 nm (ϵ 27 200), 279 (ϵ 11 000). ^{1}H NMR δ: 1.91, 1.98, 2.06, and 2.15 (3m + t, 2H, $H_{3'}$), 2.63 (*Z*-isomer) and 2.89 (*E*-isomer, 2 poorly resolved t, 1H, $H_{4'}$), 3.60 (*E*-isomer) and 3.62 (*Z*-isomer, 2s, 3H, CH_3O, ester), 3.96 (s, 3H, CH_3O, purine), 6.51 (*E*-isomer) and 6.58 (*Z*-isomer, 2bs, 2H, NH_2), 7.35 and 7.40 (2bs, 1H, $H_{1'}$), 7.95 (*E*-isomer) and 8.24 (*Z*-isomer, 2s, 1H, H_8). ^{13}C NMR: 11.1, 13.6 ($C_{3'}$), 17.6, 19.7 ($C_{4'}$), 52.7, 52.8 (CH_3O, ester), 53.97, 54.02 (OCH_3, purine), 111.7, 112.2, 112.4, 112.7, 114.2 ($C_{1'}$, $C_{2'}$, C_2), 136.6, 136.9 (C_8), 153.4, 153.5 (C_4), 160.9, 161.4, 161.5 (C_6 and C_2), 171.6, 172.0 (CO). EI-MS: 275 (100.0, M), 244 (20.8, M − CH_3O), 260 (17.2, M − CH_3). HRMS calcd for $C_{12}H_{13}N_5O_3$ 275.1018. Found: 275.1021.

(*Z*,*E*)-2-Amino-6-methoxy-9-[(2-fluoro-2-carbomethoxycyclopropylidene)methyl]purine (25). The *Z*,*E*-isomeric mixture **24** (Z/E = 1.5:1, 0.42 g, 1.53 mmol) and LiCl (394 mg, 6.0 equiv) was dissolved in THF (42 mL). The stirred reaction mixture was cooled to −78 °C. After 10 min, LDA (1.8 M in THF, 2.55 mL, 4.59 mmol) was added dropwise over 5 min. The reaction mixture was stirred for 30 min at −78 °C. NFSI (1.45 g, 4.59 mmol) in THF (5 mL) was then added. After 5 min, methanol (5 mL) was added to quench the reaction. After an additional 10 min, insoluble solid was filtered off through a silica gel pad that was washed with EtOAc (4 × 30 mL). The crude isomeric mixture **25** (120 mg, 18.7%) was 70% pure according to the ^{19}F NMR spectra. The Z/E ratio of was 1:1. This product was used as such in the next step.

(*Z*)-2-Amino-6-methoxy-9-{[2-fluoro-2-(hydroxymethyl)cyclopropylidenelmethyl}purine (11e) and (*E*)-2-Amino-6-methoxy-9-{[2-fluoro-2-(hydroxymethyl)-cyclopropylidene]methyl}purine (12e). A mixture of isomers **25** (134 mg, 0.32 mmol) from the preceding experiment was dissolved in THF (7 mL). The solution was cooled to 0 °C, and DIBALH (1.0 M in hexanes, 2.2 mL, 1.76 mmol) was added dropwise with stirring. After 5 h, another portion of DIBALH (1.76 mmol) was added, and the stirring was continued for additional 2 h. Methanol (2 mL) and water (1 mL) were added, and the mixture was stirred for 16 h at room temperature. The solvents were evaporated, and crude product was purified by chromatography using EtOAc/MeOH (60:1 to 40:1) to give the *Z*-isomer **11e** (46 mg, 54%) and *E*-isomer **12e** (29 mg, 34%) as white solids.

***Z*-Isomer 11e**. Mp: 208−210 °C. UV λ_{max}: 280 nm (ϵ 11 900), 222 nm (ϵ 24 600). ^{1}H NMR δ: 1.93 (dd, 1H, J = 11.6 and 1.6 Hz, $H_{3'}$) and 1.97 (dt, 1H, J = 11.6 Hz and J = 1.6 Hz, $H_{3''}$), 3.66 (ddd, 1H, J = 29.2, 13.2, and 5.6 Hz, $H_{5'}$), 4.16 (td, 1H, J = 13.6 and 4.9 Hz, $H_{5''}$), 3.96 (s, 3H, OCH_3), 5.65 (t, 1H, $^3J_{OH,5'}$ = 5.6 Hz, OH), 6.64 (s, 2H, NH_2), 7.30 (s, 1H, $H_{1'}$), 8.37 (s, 1H, H_8). ^{13}C NMR: 15.5 ($^2J_{3',F}$ = 13.4 Hz, $C_{3'}$), 54.04 (OCH_3), 63.7 ($^2J_{5'F}$ = 24.7 Hz, $C_{5'}$), 78.0 ($^1J_{4',F}$ = 231.3 Hz, $C_{4'}$), 110.9 ($^2J_{2',F}$ = 4.4 Hz, $C_{2'}$), 113.8 ($C_{1'}$), 114.1 (C_5), 136.9 (C_8), 153.2 (C_4), 161.1, 161.5 (C_6, C_2). ^{19}F NMR: −179.48 (partially overlapped ddd, J = 30.9 Hz, 16.2, and 11.2 Hz). EI-MS: 265 (41.0, M), 248 (100.0, M − OH), 166 (46.2, purine base + H), 165 (40.7, purine base). HRMS calcd for $C_{11}H_{12}N_5O_2F$ 265.0975. Found: 265.0976.

***E*-Isomer 12e**. Mp: 190−192 °C. UV λ_{max}: 280 nm (ϵ 12 300), 222 nm (ϵ 25 700). ^{1}H NMR δ: 2.00 (dd, 1H, J = 12.0

and 2.4 Hz, $H_{3'}$), 2.16 (td, lH, J = 11.6 and 2.4 Hz, $H_{3''}$), 3.76 (dt, 2H, J = 20.4 and 6.4 Hz, $H_{5'}$), 3.96 (s, 3H, OCH_3), 5.29 (t, 1H, $^3J_{OH,5'}$ = 5.8 Hz), 6.63 (s, 2H, NH_2), 7.69 (s, 1H, $H_{1'}$), 8.28 (s, 1H, H_8). ^{13}C NMR: 15.5 ($^2J_{2',F}$ = 13.5 Hz, $C_{3'}$), 54.0 (OCH_3), 63.4 ($^2J_{5',F}$ = 26.2 Hz, $C_{5'}$), 77.6 ($^1J_{4',F}$ = 229.8 Hz, $C_{4'}$), 111.8 ($^2J_{2',F}$ = 4.5 Hz, $C_{2'}$), 114.1 ($C_{1'}$), 114.9 (C_5), 136.7 (C_8), 153.6 (C_4), 161.0, 161.4 (C_6, C_2). ^{19}F NMR: −178.75 (td, J = 21.5 and 10.5 Hz). El-MS: 265 (39.9, M), 248 (100.0, M − OH), 166 (45.1, purine base + H), 165 (38.7, purine base).

(*Z*)-9-{[2-Fluoro-2-(hydroxymethyl)cyclopropylidene]-methyl}guanine (11b). A mixture of the *Z*-isomer **11e** (132 mg, 0.50 mmol) and KI (116 mg, 0.70 mmol, dried at 0.05−0.07 Torr and room temperature for 24 h) was dissolved in MeCN (10 mL). Me_3SiCl (0.09 mL, 0.765 mmol) was added, and the reaction mixture was stirred at room temperature for 16 h. The solvents were evaporated, and crude product was chromatographed on a silica gel column using CH_2Cl_2/MeOH (10:1 to 6:1) to give the *Z*-isomer **11b** (95 mg, 76%). Mp: >300 °C. UV λ_{max}: 270 nm (ϵ 11 900), 233 (ϵ 28 600). ^1H NMR δ: 1.91 (m, 2H, $H_{3'}$), 3.63 (ddd, 1H, J = 28.8, 12.8, and 6.0 Hz, $H_{5'}$), 4.12 (td, 1H, J = 14.2 and 5.3 Hz, $H_{5''}$), 5.58 (t, 1H, $^3J_{OH,5'}$ = 5.6 Hz, OH), 6.62 (bs, 2H, NH_2), 7.17 (s, 1H, $H_{1'}$), 8.20 (s, 1H, H_8), 10.72 (s, 1H, NH). ^{13}C NMR: 15.6 (d, $^2J_{3',F}$ = 14.2 Hz, $C_{3'}$), 63.6 (d, $^2J_{5',F}$ = 24.6 Hz, $C_{5'}$), 77.9 (d, $J_{4',F}$ = 231.4 Hz, $C_{4'}$), 111.5 (d, $^2J_{1',F}$ = 4.5 Hz, $C_{2'}$), 113.7 ($C_{1'}$), 116.9 (C_5), 134.8 (C_8), 150.4 (C_4), 154.8 (C_2), 157.3 (C_6). ^{19}F NMR: −179.63 (ddd, J = 29.0, 15.4, and 10.7 Hz). ESI-MS: 252 (100.0, M + H), 274 (88.7, M + Na). Anal. $C_{10}H_{10}N_5O_2F$ (C, H, N).

(*E*)-9-{[2-Fluoro-(2-hydroxymethyl)cyclopropylidene]-methyl}guanine (12b). The procedure described for the *Z*-isomer **11b** was followed using the *E*-isomer **12e** (263 mg, 0.99 mmol). After the workup, crude product was chromatographed in CH_2Cl_2/MeOH (10:1 to 5:1) to give the title compound **12b** (183 mg, 73.4%). Mp >300 °C. UV λ_{max}: 270 nm (ϵ 12 200), 234 nm (ϵ 29 200). ^1H NMR δ: 1.99 (dd, 1H, J = 12.4 and 2.8 Hz, $H_{3'}$), 2.14 (td, 1H, J = 11.0 and 2.6 Hz, $H_{3''}$), 3.72 (dt, 2H, J = 12.4 and 6.4 Hz, $H_{5'}$), 3.79 (td, 2H, J = 12.8 and 6.4 Hz, $H_{5''}$), 5.29 (t, 1H, $^3J_{OH,5'}$ = 6.2 Hz), 6.62 (bs, 2H, NH_2), 7.59 (poorly resolved t, 1H, $H_{1'}$), 8.11 (s, 1H, H_8), 10.76 (s, 1H, NH). ^{13}C NMR: 17.7 (d, $^2J_{3',F}$ = 13.5 Hz, $C_{3'}$), 63.4 (d, $^2J_{5',F}$ = 25.5 Hz, $C_{5'}$), 77.5 (d, $^1J_{4',F}$ = 229.8 Hz, $C_{4'}$), 112.1 (d, $^2J_{2',F}$ = 6.7 Hz, $C_{2'}$), 114.9 ($C_{1'}$), 117.0 (C_5), 134.4 (C_8), 150.9 (C_4), 154.8 (C_2), 157.3 (C_6). ^{19}F NMR: −178.82 (td, J = 21.5 Hz and 10.5 Hz). ESI-MS: 252 (64.9, M + H), 274 (100.0, M + Na). Anal. $C_{10}H_{10}N_5O_2F$ (C, H, N).

(*Z*,*E*)-1-[(2-Carbethoxycyclopropylidene)methyl])-cytosines (15). The described procedure[17] was modified as follows. A mixture of N^4-acetylcytosine (**19**, 9.18 g, 60 mmol), dibromoester **21** (20.67 g, 72 mmol), and K_2CO_3 (49.68 g, 360 mmol) in DMF (300 mL) was heated at 100 °C with stirring under N_2 for 7 h. DMF was evaporated, and the residue was dissolved in EtOAc/EtOH (10:1). The solution was filtered using a Celite bed, which was then washed repeatedly with the same solvent, and the filtrate was concentrated. A mixture of crude product (8.34 g, 30 mmol), EtOH/H_2O (9:1, 300 mL), and K_2CO_3 (8.28 g, 60 mmol) was stirred overnight at room temperature. The solvents were evaporated, and the residue was chromatographed on a silica gel column using CH_2Cl_2/EtOH = 100:0 to 10:1 to give the title isomeric mixture **15** (6.06 g, 86%), *Z*/*E* ratio of 1.5:1.

(*Z*)- and (*E*)-1-[(2-Fluoro-2-carbethoxycyclopropylidene)methyl]cytosine (26). A protocol for adenine analogues **22** was followed with an isomeric mixture **15** described above (270 mg, 2.0 mmol). The product was chromatographed on a silica gel column using EtOAc/EtOH (80:1 to 30:1 then 20:1) to give *Z*-isomer (137 mg, 27%) and *E*-isomer of **26** (110 mg, 22%).

***Z*-Isomer of 26**. Mp: 214−215 °C. UV λ_{max}: 299 nm (ϵ 12 300), 235 nm (ϵ 8900). ^1H NMR δ: 1.16 (t, 3H, ^3J = 7.2 Hz, CH_3), 2.31 (s, 2H, $H_{3'}$), 4.19 (m, 2H, OCH_2), 5.94 (d, 1H, $^3J_{5,6}$ = 8.0 Hz, H_5), 7.32 (d, 1H, $J_{6,5}$ = 7.2 Hz, H_6), 7.53 (s, 1H, $H_{1'}$), 7.61 and 7.65 (2s, 2H, NH_2). ^{13}C NMR: 14.5 (CH_3), 17.9 (d, $^2J_{3',F}$ = 14.2 Hz, $C_{3'}$), 62.7 (CH_2 of Et), 72.8 (d, $^1J_{4',F}$ = 236.5 Hz, $C_{4'}$), 97.9 (C_5), 106.3 ($C_{2'}$), 120.5 ($C_{1'}$), 139.7 (C_6), 153.8 (C_4), 166.1 (C_2), 167.5 (CO, ester). ^{19}F NMR: −182.98 (d, J = 4.5 Hz). EI-MS: 253 (60.1, M), 224 (54.2, M − Et), 180 (100.0, M − CO_2Et), 181 (95.8, M + H − CO_2Et), 111 (cytosine, 34.1), 110 (cytosine − H, 38.5). HRMS calcd for $C_{11}H_{12}N_3O_3F$: 253.0863. Found: 253.0863.

***E*-Isomer of 26.** Mp: 216−217 °C. UV λ_{max}: 299 nm (ϵ 11 200), 234 nm (ϵ 8000). ^1H NMR δ: 1.16 (t, 3H, ^3J = 7.2 Hz, CH_3), 2.31 (s, 2H, $H_{3'}$), 4.18 (m, 2H, OCH_2), 5.90 (d, 1H, $^3J_{5,6}$ = 8.4 Hz, H_5), 7.58 (2s, 2H, NH_2), 7.87 (dm, 1H, $H_{1'}$), 8.03 (d, 1H, $^3J_{6,5}$ = 7.6 Hz, H_6). ^{13}C NMR: 14.6 (CH_3), 20.6 (d, $^2J_{3',F}$ = 13.4 Hz, $C_{3'}$), 62.4 (OCH_2), 71.2 (d, $^1J_{4',F}$ = 235.8 Hz, $C_{4'}$), 97.3 (C_5), 106.3 ($^2J_{2',F}$ = 5.9 Hz, $C_{2'}$), 120.9 ($C_{1'}$), 140.2 (C_6), 154.2 (C_4), 166.2 (C_2), 168.2 (CO, ester). ^{19}F NMR: −185.47 (d, J = 6.0 Hz). EI-MS: 253 (36.8, M), 224 (31.1, M − Et), 180 (57.4, M − CO_2Et), 181 (57.7, M + H − CO_2Et), 141 (100.0), 111 (39.3, cytosine), 110 (38.2, cytosine − H). HRMS calcd for $C_{11}H_{12}N_3O_3F$: 253.0863. Found: 253.0860.

(*Z*)-1-{[(2-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}cytosine (11c). The *Z*-isomer of **26** (510 mg, 2.0 mmol) was reduced with DIBALH in THF (70 mL) as described for 2-amino-6-methoxypurine analogue **25**. The crude product was chromatographed on a silica gel column using CH_2Cl_2/MeOH (30:1 to 10:1 then 6:1) to give the *Z*-isomer **11c** (240 mg, 57%). Mp: 235−237 °C. UV λ_{max}: 299 nm (ϵ 12 200), 234 nm (ϵ 8500). ^1H NMR δ: 1.71−1.82 (m, 2H, $H_{3'}$), 3.61 (ddd, 1H, J = 25.3, 10.4, and 5.6 Hz, $H_{5'}$), 4.02 (ddd, 1H, J = 16.5, 12.8, and 4.0 Hz, $H_{5''}$), 5.50 (t, 1H, $^3J_{OH,5'}$ = 4.8 Hz, OH), 5.85 (d, 1H, $^3J_{5,6}$ = 7.2 Hz, H_5), 7.44 (t, 1H, J = 2.4 Hz, $H_{1'}$), 7.49 and 7.52 (2bs, 2H, NH_2), 7.93 (d, 1H, $^3J_{6,5}$ = 7.2 Hz, H_6). ^{13}C NMR: 14.5 (d, $^2J_{3',F}$ = 13.5 Hz, $C_{3'}$), 63.7 (d, $^2J_{5',F}$ = 25.5 Hz, $C_{5'}$), 78.3 (d, $^1J_{4',F}$ = 231.1 Hz, $C_{4'}$), 96.8 (C_5), 107.6 (d, $^2J_{2',F}$ = 3.7 Hz, $C_{2'}$), 119.8 ($C_{1'}$), 140.7 (C_6), 154.1 (C_4), 166.2 (C_2). ^{19}F NMR: −176.05 (ddd, J = 27.7, 16.8 and 10.5 Hz). EI-MS: 211 (M, 16.8), 194 (100.0, M − OH), 112 (78.3, cytosine + H). HRMS calcd for $C_9H_{10}N_3O_2F$: 211.0757. Found: 211.0761. Anal. $C_9H_{10}N_3O_2F$ (C, H, N).

(*E*)-1-{[(2-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}cytosine (12c). The experiment was performed with the *E*-isomer of **26** (255 mg, 1.0 mmol) in THF (30 mL) as described for the *Z*-isomer. Chromatography (CH_2Cl_2/MeOH (10:1 to 5:1) afforded the *E*-isomer **12c** (151 mg, 71%). Mp: 224−226 °C. UV λ_{max}: 299 nm (ϵ 13 200), 234 nm (ϵ 9000). ^1H NMR δ: 1.89 (dd, 1H, J = 11.6 and 2.4 Hz, $H_{3'}$), 2.00 (td, J = 11.2 and 2.4 Hz, $H_{3''}$), 3.70 (poorly resolved dd, 1H, J = 21.2 and 5.6 Hz, $H_{5'}$), 5.29 (t, 1H, $^3J_{OH,5'}$ = 6.0 Hz, OH), 7.45 (bs, 1H, $H_{1'}$), 7.65 and 7.76 (2bs, 2H, NH_2), 8.00 (d, 1H, $^3J_{6,5}$ = 7.2 Hz, H_6). ^{13}C NMR: 16.5 ($^2J_{3',F}$ = 13.4 Hz, $C_{3'}$), 63.4 (d, $^2J_{5',F}$ = 26.9 Hz, $C_{5'}$), 76.2 (d, $^1J_{4',F}$ = 229.1 Hz, $C_{4'}$), 96.7 (C_5), 108.2 (d, $^2J_{2',F}$ = 5.1 Hz, $H_{2'}$), 119.8 ($C_{1'}$), 140.2 (C_6), 154.6 (C_4), 166.2 (C_2). ^{19}F NMR: −178.84 (td, J = 21.3 and 9.2 Hz). El-MS: 211 (17.4, M), 194 (100.0, M − OH), 112 (81.8, cytosine + H). HRMS calcd for $C_9H_{10}N_3O_2F$: 211.0757. Found: 211.0757. Anal. $C_9H_{10}N_3O_2F$ (C, H, N).

(*E*,*Z*)-1-[(2-Carbethoxycyclopropylidene)methyl]-thymines (16). The procedure described[17] for a mixture of ethyl and methyl esters was streamlined as follows. The 2,4-bis(trimethylsilyl)-5-methylpyrimidine (**20**, 10.8 g, 40 mmol) and dibromoester **21** (17.2 g, 60 mmol) in MeCN (40 mL) were refluxed with stirring under N_2 for 148 h. After being cooled, EtOH (50 mL) was added. The reaction mixture was filtered using a Celite bed, which was repeatedly washed with EtOAc/EtOH (10:1). The solvents were evaporated, and the crude product was chromatographed (100% CH_2Cl_2 to CH_2Cl_2/EtOH (100:1) to give a mixture of (*Z*,*E*)-1-{[(1-bromo-2-carbethoxy)-cyclopropyl]methyl}thymine (**28**, 8.88 g, 67%).

Compound **28** (6.0 g, 18 mmol) and K_2CO_3 (7.5 g, 55.5 mmol) in DMF (100 mL) were stirred at 100 °C for 4.5 h under N_2. The solvent was evaporated, and the residue was chromatographed in hexanes/EtOAc (2:1 to 1:1) to give a mixture of the (*Z*,*E*)-isomers **16** (2.56 g, *56%, Z*/*E* = 1.7:1).

(*Z*)-1-[(2-Fluoro-2-carbethoxycyclopropylidene)methyl]-thymine (Z-27). The *Z*-isomer **16** (140 mg, 0.56 mmol) and NFSI (176 mg, 0.56 mmol) were dissolved in THF (3 mL), and lithium hexamethyldisilizane (LiHMDS, 1.0 M in THF, 0.93

mL, 0.93 mmol) was added with stirring at −78 °C during 1 h. The stirring was continued for another 2 h whereupon ethanol (3 mL) was added to quench the reaction. The solvents were evaporated, and the crude product was chromatographed on a silica gel column using hexanes/EtOAc (3:1) to give the *Z*-isomer of **27** (40 mg, 26%) identical with the compound obtained from the (*Z*,*E*) mixture **16**.

(*Z*)-1-[(2-Fluoro-2-carbethoxycyclopropylidene)methyl]-thymine and (*E*)-1-[(2-Fluoro-2-carbethoxycyclopropylidene)methyl]thymine (27). The reaction was performed as described for adenine analogue **22** with isomeric mixture **16** (500 mg, 2.0 mmol) described above. The crude product (*Z*/*E* = 1.8:1) was chromatographed on a silica gel column using hexanes/EtOAc (4:1 to 3:1) to give *Z*-**27** (224 mg, 41%) and *E*-**27** (162 mg, 30%).

***Z*-Isomer 27.** Mp: 190−191 °C (EtOAc). UV λ_{max}: 287 nm (ϵ 13 300), 237 nm (ϵ 9500). ^{1}H NMR ($CDCl_3$) δ: 1.30 (t, 3H, ^3J = 7.2 Hz, CH_3 of Et), 1.98 (s, 3H, 5-CH_3), 2.19 (td, 1H, *J* = 8.1 Hz and 2.4 Hz, $H_{3'}$), 2.44 (poorly resolved dd, 1H, *J* = 11.2 Hz, $H_{3''}$), 4.32 (m, 2H, CH_2 of Et), 7.33 (s, 1H, H_6), 7.52 (t, 1H, *J* = 2.4 Hz, $H_{1'}$), 9.38 (s, 1H, NH). ^{13}C NMR (CD_3SOCD_3): 13.0 (CH_3 of Et), 14.4 (5-CH_3), 17.4 (d, $^2J_{3',F}$ = 13.5 Hz, $C_{3'}$), 62.9 (CH_2 of Et), 72.2 (d, $^1J_{4',F}$ = 240.3 Hz, $C_{4'}$), 107.2 (d, $^2J_{2',F}$ = 3.8 Hz, $C_{2'}$), 113.7, 117.5 (C_5, $C_{1'}$), 134.7 (C_6), 149.3 (C_2), 163.4 (C_4). ^{19}F NMR ($CDCl_3$): −184.00 (poorly resolved dd, *J* = 9.0 Hz). EI-MS: 268 (69.5, M), 124 (100.0). HRMS calcd for $C_{12}H_{13}N_2O_4F$: 268.0859. Found: 268.0865.

***E*-Isomer 27.** Mp: 184−186 °C. UV λ_{max}: 287 nm (ϵ 12 900), 238 (ϵ 8700). ^{1}H NMR ($CDCl_3$) δ: 1.32 (t, 3H, CH_3 of Et), 2.01 (d, 3H, 5-CH_3, *J* = 1.6 Hz), 2.33 (partly overlapped ddd, 1H, *J* = 10.8, 7.6, and 3.2 Hz, $H_{3'}$) and 2.53 (dt, 1H, partly overlapped dt, *J* = 10.4 and 1.8 Hz, $H_{3''}$), 4.30 (m, 2, CH_2 of Et), 7.66 (s, 1H, H_6), 7.88 (poorly resolved dd, 1H, $H_{1'}$), 8.78 (bs, 1H, NH). ^{13}C NMR (CD_3SOCD_3) δ: 12.8 (CH_3 of Et), 14.6 (5-CH_3), 20.9 (d, $^2J_{3',F}$ = 13.5 Hz, $C_{3'}$), 62.5 (CH_2 of Et), 71.3 (d, $J_{4',F}$ = 235.8 Hz, $C_{4'}$), 107.6 (d, $^2J_{2',F}$ = 5.2 Hz, $C_{2'}$), 112.3, 119.1 (C_5, $C_{1'}$), 135.1 (C_6), 150.1 (C_2), 164.2 (C_4). ^{19}F NMR ($CDCl_3$): −184.00 (d, *J* = 7.5 Hz). EI-MS: 268 (58.1, M), 124 (100.0). HRMS calcd for $C_{12}H_{13}N_2O_4F$: 268.0859. Found: 268.0858.

(*Z*)-1-{[(2-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}thymine (11d). The experiment followed the protocol used for adenine analogues **11a** and **12a** with the *Z*-fluoroester **27** (270 mg, 1.0 mmol) in THF (30 mL). The crude product was chromatographed on silica gel using CH_2Cl_2/MeOH (50:1 to 30:1) to give *Z*-isomer **11d** (155 mg, 68%). Mp: 173−175 °C. UV λ_{max}: 287 nm (ϵ 12 300), 236 nm (ϵ 10 400). ^{1}H NMR δ: 1.98 (s and m, 5H, 5-CH_3 overlapped with $H_{3'}$), 3.51 (ddd, 1H, *J* = 29.4, 12.6, and 6.2 Hz, $H_{5'}$) and 4.17 (dt, 1H, *J* = 13.4 and 5.2 Hz, $H_{5''}$), 5.65 (t, 1H, ^3J = 6.0 Hz, OH), 7.32 (s, 1H, $H_{1'}$), 8.05 (d, 1H, $^3J_{6,5-CH3}$ = 1.6 Hz, H_6), 11.58 (s, 1H, NH). ^{13}C NMR: 12.8 (5-CH_3), 14.6 (d, $^2J_{3',F}$ = 14.2 Hz, $C_{3'}$), 63.8 (d, $^2J_{5',F}$ = 25.4 Hz, $C_{5'}$), 78.0 (d, $^1J_{4',F}$ = 231.3 Hz, $C_{4'}$), 108.6 (d, $^2J_{2',F}$ = 4.4 Hz, $C_{2'}$), 111.6 (C_5), 117.3 (d, $^3J_{1',F}$ = 2.2 Hz, $C_{1'}$), 136.0 (C_6), 149.8 (C_2), 164.2 (C_4). ^{19}F NMR: −176.05 (ddd, *J* = 30.5, 13.7 and 10.7 Hz). EI-MS: 226 (M, 14.4), 209 (M − OH, 10.5), 127 (thymine + H, 100.0). HRMS calcd for $C_{10}H_{11}N_2O_3F$: 226.0754. Found: 226.0754. Anal. $C_{10}H_{11}N_2O_3F$ (C, H, N).

(*E*)-1-{[(2-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}thymine (12d). The procedure used for the *Z*-isomer **11d** was employed with the *E*-isomer of **27** (270 mg, 1.0 mmol) and THF (30 mL). The crude product was chromatographed on silica gel using CH_2Cl_2/MeOH (50:1 to 30:1) to give the *E*-isomer **12d** (162 mg, 71%). Mp: 166−167 °C. UV λ_{max}: 287 nm (ϵ 11 700), 237 nm (ϵ 10 500). ^{1}H NMR δ: 1.83 (d, 3H, $^3J_{CH3,6}$ = 1.6 Hz), 1.96 (dd, 1H, *J* = 12.4 and 2.4 Hz, $H_{3'}$), 2.10 (td, 1H, *J* = 13.2 and 2.4 Hz), 3.72 (dd, 2H, *J* = 21.2 and 6.4 Hz), 5.24 (t, 1H, $^3J_{OH,5'}$ = 5.6 Hz, OH), 7.65 (poorly resolved q, 1H, $^4J_{1',3'}$ = 1.6 Hz, $H_{1'}$), 7.86 (poorly resolved d, 1H, $^3J_{6,5-CH3}$ = 1.6 Hz, H_6), 11.59 (s, 1H, NH). ^{13}C NMR: 12.8 (5-CH_3), 17.2 (d, $^2J_{3',F}$ = 14.2 Hz, $C_{3'}$), 63.3 (d, $^2J_{5',F}$ = 26.2 Hz), 76.2 ($^1J_{4',F}$ = 229.8 Hz, $C_{4'}$), 109.2 (d, $^2J_{2',F}$ = 6.1 Hz, $C_{2'}$), 111.7 (C_5), 118.0 ($C_{1'}$), 135.4 (C_6), 150.2 (C_2), 164.3 (C_4). ^{19}F NMR: −179.34 (td, *J* = 21.5 and 10.5 Hz). EI-MS: 226 (M, 2.8), 209 (M − OH, 10.4) 127 (thymine + H, 100.0). HRMS calcd for $C_{10}H_{11}N_2O_3F$: 226.0754. Found: 226.0753. Anal. $C_{10}H_{11}N_2O_3F$ (C, H, N).

Stability of *Z*- and *E*-Isomers 11a and 12a. The *Z*- or *E*-isomer **11a** or **12a** (1 mg each) were dissolved in 0.02 M Na_2HPO_4, pH 7.0 (0.7 mL) by sonication (5 min). The solutions were kept at room temperature for a period of 1 week. Both compounds were unchanged as indicated by TLC (EtOAc/MeOH, 10:1).

Biological Assays. The antiviral assays were performed as described previously.[4,16,30] The HCMV assays were run in HFF culture with two strains of virus, Towne and AD169, in a plaque reduction or cytopathic effect (CPE) inhibition assay. The MCMV was assayed in MEF cells by plaque reduction. The HSV-1 was run in BSC-1 cells by ELISA. In addition, HSV-1 and HSV-2 assays were performed in HFF (CPE assay) and Vero cells (plaque reduction assay). The VZV was assayed in HFF (CPE or plaque reduction), and hepatitis B virus (HBV) in 2.2.15 cells by DNA hybridization. The EBV assays were performed in Daudi cells by viral capsid antigen (VCA) ELISA and in H-1 cells by DNA hybridization assay. The cytotoxicity assays were performed in HFF, KB, and CEM cells. For further details, see Tables 2 and 3.

The HIV-1 assays were conducted as described previously.[25] The MT-2 (2×10^3) or MT-4 (3×10^4) cells were exposed to 100 $TCID_{50}$ (50% tissue culture infectious dose) of HIV-1_{LAI}, and they were cultured in the presence of tested analogues. The EC_{50} values were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after day 7 (MT-2) or day 5 (MT- 4). The same assay was used for determination of cytotoxicity in uninfected cells. All of the assays were perfomed in duplicate.

Adenosine Deaminase (ADA) Assay.[16] Compound **11a** or **12a** (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 **11a** and **12a** was 37 and 72%, respectively.

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Supporting Information Available: Elemental analyses of **11a**−**d** and **12a**−**d**. This information is available free of charge via the Internet at http://pubs.acs.org.

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