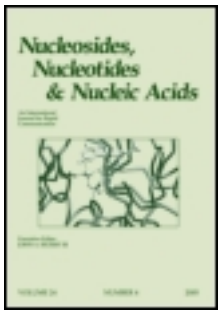

Nucleosides, Nucleotides and Nucleic Acids

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FLUOROANALOGUES OF ANTI-CYTOMEGALOVIRUS AGENT CYCLOPROPAVIR: SYNTHESIS AND ANTIVIRAL ACTIVITY OF (*E*)- AND (*Z*)-9-{[2,2-BIS(HYDROXYMETHYL)-3-FLUOROCYCLOPROPYLIDENE]METHYL}-ADENINES AND GUANINES

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□ *Synthesis of fluorinated cyclopropavir analogues* ***13a***, ***13b***, ***14a***, *and* ***14b*** *is described starting from alkene* ***15***. *Addition of carbene derived from dibromofluoromethane gave bromofluoro cyclopropane* ***16***. *Reduction (compound* ***17****) followed by desilylation gave intermediate* ***18****, which was transformed to 2-nitrophenylselenenyl derivative* ***19***. *Oxidation to selenoxide* ***20*** *was followed by β-elimination to afford methylenecyclopropane* ***21***. *Addition of bromine provided compound* ***22*** *for alkylation-elimination of adenine and 2-amino-6-chloropurine. The resultant E,Z isomeric mixtures of methylenecyclopropanes* ***23a*** + ***24a*** *and* ***23c*** + ***24c*** *were resolved and the individual isomers were deprotected to give adenine analogues* ***13a*** *and* ***14a*** *as well as compounds* ***13c*** *and* ***14c***. *Hydrolytic dechlorination of* ***13c*** *and* ***14c*** *furnished guanine analogues* ***13b*** *and* ***14b***. *The only significant antiviral effects were observed with analogue* ***13a*** *against HCMV and* ***14a*** *against VZV in cytopathic inhibition assays.*

Keywords 3-Fluoromethylenecyclopropanes; cyclopropavir; nucleoside analogues; antivirals

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INTRODUCTION

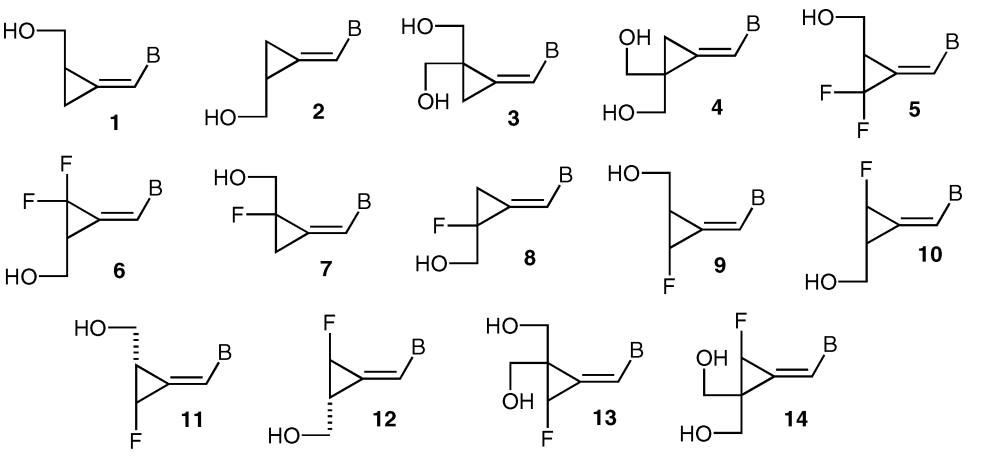
Among methylenecyclopropane analogues of nucleosides, the purine *Z*-isomers **1** and **3** were identified as effective antivirals whereas the *E*-isomers **2** and **4** were active in only a few cases.[1,2] The most potent analogue,[3,4] cyclopropavir (**3b**), is presently a subject of mechanism of action studies[5–8] and preclinical development as a therapeutic agent against human cytomegalovirus (HCMV). It also is effective against Epstein-Barr virus (EBV),[3] and human herpes virus 6 and 8 (HHV-6 and HHV-8).[3,6] A considerable attention also has been paid to fluorinated methylenecyclopropanes. The *geminal* difluoro analogues **5a**, **5b**, **6a**, and **6b** are with the exception of **5a** devoid of antiviral activity.[9] By contrast, monofluoro derivatives **7a** through **12a** and **7b** through **12b** exhibit varying degree of antiviral[10–12] effects particularly against HCMV (**7a**), Epstein-Barr virus (EBV, **7a**), varicella zoster virus (VZV, **9a**) and HIV-1 (**7a** and **9a**). Some antiviral activity has been noted with the *trans*-isomers **8b** and **12a**. Consequently, the synthesis of fluorinated 2,2-bis(hydroxymethyl)methylenecyclopropanes **13a**, **13b**, **14a**, and **14b** related to cyclopropavir (**3b**) was of interest.

RESULTS AND DISCUSSION

Synthesis

The synthesis of these analogues commenced with addition of carbene derived from $CHBr_2F$ to alkene **15** in CH_2Cl_2 under phase-transfer conditions (Bu_4NI and 50% NaOH) to give bromofluorocyclopropane **16** (72%, Scheme 1). Reduction with Bu_3SnH using AIBN as a catalyst gave fluoro derivative **17** in 83% yield. The TBDMS group was removed using Bu_4NF in THF to give hydroxymethylcyclopropane **18** in 94% yield. These procedures followed a similar sequence that started from chlorofluorocyclopropane **16** (Br = Cl).[13] Reaction with 2-nitrophenyl selenocyanate and Bu_3P in THF[9] afforded intermediate **19** (91%). Oxidation with H_2O_2 in THF furnished crude selenoxide **20** which was converted to methylenecyclopropane **21** (57%) by a thermolysis at 80°C in toluene. Addition of bromine via pyridinium perbromide in CH_2Cl_2 gave dibromo derivative **22** in 91% yield.

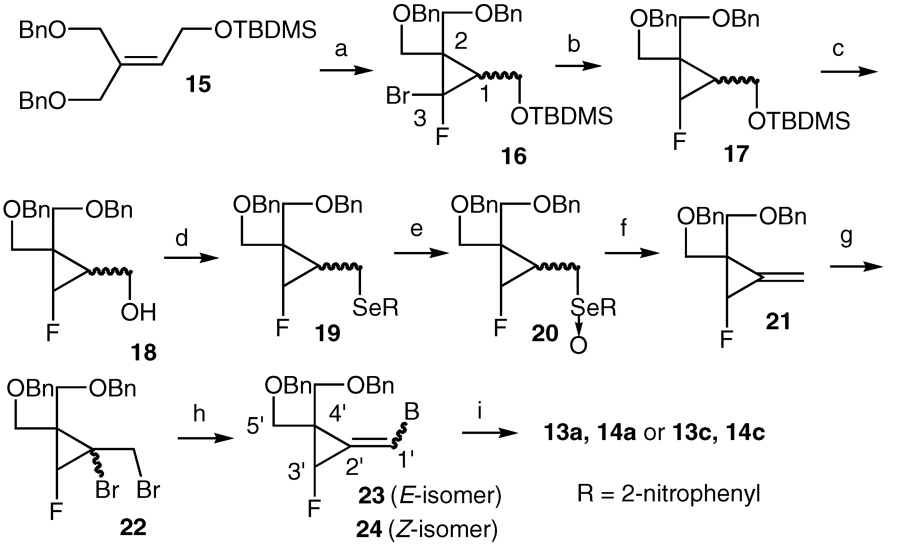
Alkylation-elimination of adenine (K_2CO_3, DMF, 100°C) with **22** provided, after chromatographic separation, the *E*- and *Z*-isomers **23a** and **24a** in 21 and 35% yield, respectively. The separated isomers **23a** and **24a** were debenzylated using $BCl_3{\cdot}SMe_2$ complex in CH_2Cl_2 to give compounds **13a** and **14a** (83 and 84% yield). In a similar fashion, alkylation-elimination of 2-amino-6-chloropurine with dibromo derivative **22** gave the *E*- and *Z*-isomers **23c** and **24c** in 17.5 and 37% yield, respectively. Somewhat surprisingly, the



B = nucleic acid base

Series a: B = Ade, series b: B = Gua, series c: B = 2-amino-6-chloropurine

Chart 1



For series a, b and c see Chart 1

a. 1. $CHFBr_2$, CH_2Cl_2.
   2. NaOH (50%), Bu_4NI.
b. Bu_3SnH, AIBN, Δ.
c. Bu_4NF, THF.
d. 2-NO_2PhSeCN, Bu_3P, THF.
e. 1. H_2O_2, THF.
f. Toluene, Δ.
g. Pyridine.HBr_3, CH_2Cl_2.
h. B-H, K_2CO_3, DMF, Δ.
i. BCl_3.$SeMe_2$, CH_2Cl_2.
j. 1. 80% HCO_2H, Δ.
   2. NH_3, MeOH.

SCHEME 1

TABLE 1 Chemical shifts (δ) of the relevant ^{1}H NMR signals of fluorinated methylenecyclopropanes **13a**, **14a**, **13b**, **14b**, **13c**, and **14c**

Compound[a]	$H_{1'}$	H_8	OH
13a	7.89	8.82	5.18, 5.22
14a	7.53	8.34	4.89
13b	7.55	8.42	5.23
14b	7.26	7.90	4.87
13c	7.69	8.79	5.20
14c	7.37	8.30	4.90

[a]CD_3SOCD_3 as solvent. For numbering of signals see Table 2.

yields of the *E(cis)* isomers **23a**, **23c** are lower than those of the *Z(trans)* isomers **24a**, **24c**. Debenzylation of **23c** and **24c** furnished compounds **13c** (86%) and **14c** (84%). Hydrolytic dechlorination using 80% formic acid at 80°C gave guanine analogues **13b** and **14b** in 90.5 and 85% yield.

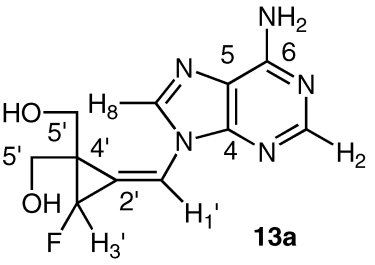
E- and *Z*-Isomeric Assignment

Methylenecyclopropane nucleoside analogues with a *cis*-configuration of the nucleobase and hydroxymethyl groups **1** and **3** are always less polar moving faster on silica gel than the *trans*-configured isomers[1,2] **2** and **4**. In the ^{1}H NMR spectra, the H_8 of purine *cis*-isomers **1** and **3** are more deshielded than the corresponding signals of the *trans*-isomers **2** and **4**. Both trends (polarity and H_8 chemical shifts) are preserved in the fluorinated analogues[9,10,12] **5** through **12**. It is then not surprising that similar patterns of the H_8 chemical shifts were also found in analogues **13** and **14** (Table 1). Interestingly, the OH groups of **13a** are not equivalent. In addition, the OH signals of the *E(cis)* isomers **13** are more deshielded than those of the *Z(trans)* isomers **14** as also found in fluorinated and nonfluorinated methylenecyclopropanes alike.[3,9,10,12] The $H_{1'}$ chemical shifts of **13** and **14** then follow the trend observed in 3′-fluorinated analogues[9,12] **5**, **6**, and **9** through **12**. The $H_{1'}$ configured *cis* relative to F in the *E*-isomer **13** is more deshielded than in *Z*-isomer **14** where the orientation of both atoms is *trans*. An unambiguous confirmation of the isomeric structure came from the nuclear Overhauser effect (NOE) experiments with adenine analogues **13a** and **14a** (Table 2). As expected, the NOE enhancements were observed between the H_8 in *anti*-like conformation and *cis*-orientated OH and $H_{5'}$ protons of the *E*-isomer **13a**. By contrast, the interactions $H_8 - H_{3'}$ and $H_{5'} - H_{1'}$ were strongest in the *Z*-isomer **14a**.

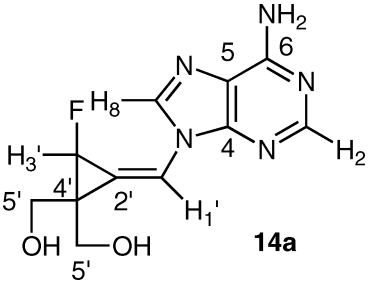
Antiviral Activity

To our surprise, introduction of a fluorine atom in the 3′ position (compound **13b**) abolished the antiviral activity of cyclopropavir (**3b**). Some

TABLE 2 The NOE enhancements of relevant ^{1}H NMR signals of fluorinated adenine methylenecyclopropanes **13a** and **14a**



13a



14a

Compound[a]	H_{iir}	δ	H_{obs}	δ	NOE (%)
13a	H_8	8.82	OH	5.18–5.22	2.37
	H_8	8.82	$H_{5'}$	3.53, 3.74	1.16, 2.37
	OH	5.18, 5.22	H_8	8.82	2.79, 2.89
	$H_{5'}$	3.74	H_8	8.82	2.13
14a	H_8	8.34	$H_{3'}$	5.34, 5.51[a]	3, 3.2
	$H_{3'}$	5.34, 5.51[b]	H_8	8.34	4.10, 2.48
	$H_{5'}$	3.53, 3.64	$H_{1'}$	7.53	1.32, 1.60

[a]Both halves of the doublet were observed separately.
[b]Both halves of the doublet were irradiated separately.

antiviral effects were seen only in adenine analogues **13a** and **14a**. Thus, the *Z*-isomer **13a** had a moderate anti-HCMV effect in Towne strain of the virus (Table 3). The *Z*-isomer **13a** and *E*-isomer **14a** were effective against AD169 strain of HCMV (EC_{50} 1.8 μM) and VZV (EC_{50} 2.7 μM), respectively, but

TABLE 3 Anti-herpesvirus activity of 2,2-bis(hydroxymethyl)-3-fluoromethylenecyclopropane analogues of nucleosides

			EC_{50}/CC_{50} (μM)	
	HCMV/HFF		EBV/Akata[a]	VZV/HFF[b]
Compound	Towne[c,d]	AD169[e,f]		
13a	51/>100	1.8/170[g]	84.5/>100	>60
14a	>100/>100	>60/>300	97.3/>100	2.7[h]
13b	>100/>100	>300/260	>100/>100	>300
14b	>100/>100	>300/197	>100/>100	238.5
Control	2.5/>100[i]	0.05/>100[i]	14/[j]	0.25[j]

[a]DNA hybridization assay.
[b]For cytotoxicity in HFF cells see HCMV/HFF (AD169).
[c]Plaque reduction assay.
[d]Visual cytotoxicity.
[e]Cytopathic effect (CPE) inhibition assay in stationary HFF cells.
[f]Cytotoxicity by neutral red uptake.
[g]The EC_{50} in plaque reduction assay was >20 μM.
[h]The EC_{50} in plaque reduction assay was >100 μM.
[i]Ganciclovir.
[j]Acyclovir.

only in cytopathic effect inhibition assays. In both cases, no activity in plaque reduction assays was seen. Little effect was also observed against EBV and all analogues were inactive against HSV-1, HSV-2, HBV, or HIV-1. It is possible that compounds **13a**, **13b**, **14a** and **14b** are not effective substrates for activation enzymes responsible for phosphorylation or their triphosphates are not capable of inhibiting the viral DNA polymerase. Isomers **12a** and **13a** were not substrates for adenosine deaminase.

CONCLUSION

3-Fluoromethylenecyclopropane analogues **13a**, **13b**, **14a**, and **14b** were prepared and their antiviral activity was investigated. In contrast to nonfluorinated analogues **3a** and **3b**, they were devoid of significant anti-herpetic activity.

EXPERIMENTAL SECTION

General Methods

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. Mass spectra were determined in electron-impact (EI-MS) or electrospray ionization (ESI-MS, methanol-NaCl) mode.

Reagents

For abbreviations of common reagents and protecting groups see the *Journal of. Organic Chemistry,* volume 70, pages 26A–27A (2005). Dibromofluoromethane,[14] diphenyl diselenide,[15] and 2-nitrophenyl selenocyanate[16] were prepared as described.

(*cis,trans*)-2,2-Bis(benzyloxymethyl)-3-bromo-1-*tert*-butyldimethylsilyloxymethyl-3-fluorocyclopropane (16).[17] The protocol for the corresponding chloro derivative[13] (**16**, Br = Cl) was modified as follows. A mixture of $CHBr_2F$ (8.64 g, 45 mmol), alkene 1**5** (12.4 g, 30 mmol) and NBu_4I (1.11 g, 3.0 mmol) in CH_2Cl_2 (22 mL) and 50% aqueous NaOH (22 mL) was stirred at room temperature for 12 hours. Water (100 mL) was then added at 0°C, the organic layer was separated and the aqueous portion was extracted with CH_2Cl_2 (5 × 40 mL). The combined organic phase was washed with saturated NaCl and it was dried ($MgSO_4$). The solvent was evaporated and the crude product was chromatographed on a silica gel column in hexanes-ether (60 : 1 to 10 : 1) to give compound **16** as a colorless oil (11.30 g, 72%). ^{1}H NMR ($CDCl_3$) δ 0.05–0.07 (m, 6H, $SiMe_2$, 0.88 and 0.89 (2s, 9 H, Me of *t*-Bu), 1.60, 1.64–1.76 (2m, 1H, H_1), 3.49–3.91

(cluster of m, 6 H, CH_2O), 4.43–4.57 (m, 4 H, CH_2Ph), 7.26–7.35 (m, 10H, Ph). ^{13}C NMR −5.2, −5.1, −5.0 (3s, $SiMe_2$, 18.4 (quaternary C of *t*-Bu), 26.1 (Me of *t*-Bu), 34.4, 34.9 (2d, J = 8.2, 8.6 Hz), 35.7, 37.4 (2d, J = 9.7 Hz, C_1, C_2), 57.2 (d, J = 7.5 Hz), 60.9, 64.0 (d, J = 9.0 Hz), 68.2, 68.27, 68.34 (CH_2OSi, CH_2OBn), 72.5, 72.8, 73.1, 73.3, 73.5 (CH_2Ph), 91.3 (d, J = 304.4 Hz, C_3), 127.78, 127.83, 127.9, 128.0, 128.5, 128.6, 138.3, 138.5 (Ph). ^{19}F NMR −132.68 (d, $^3J_{F,H\text{-}cis}$ = 20.0 Hz, *cis*-isomer), −149.57 (s, *trans*-isomer). ESI-MS 523, 525 (M + H, 5.1, 5.3), 545, 547 (M + Na, 95.0, 100.0).

(*cis, trans*)-2,2-Bis(benzyloxymethyl)-3-fluoro-1-(2-nitrophenylselenenylmethyl) cyclopropane (17). The procedure used for reduction of the corresponding chloro derivative[13] (**16**, Br = Cl) was modified as follows. Compound **16** (11.0 g, 21.0 mmol) was heated with a catalytic amount of AIBN (110 mg, 0.67 mmol) under N_2 at 90°C. Bu_3SnH (6.13 mL, 23.1 mmol) was then added and the mixture was stirred for 4 hours. After cooling, silica gel (4 g) was added and the mixture was put on the top of a silica gel column. Chromatography was performed in hexanes-ether (100: 1 to 30 : 1) to give compound **17** as a colorless oil (7.74 g, 83%). The ^1H, ^{13}C and ^{19}F NMR ($CDCl_3$) corresponded to the mixture of *E* and *Z*-isomers **7** described[13] as individual components. ESI-MS 445 (M + H, 5.6), 467 (M + Na, 100.0).

(*cis, trans*)-2,2-Bis(benzyloxymethyl)-3-fluoro-1-(hydroxymethyl) cyclopropane (18). The described procedure[13] was modified as follows. A solution of compound **17** (7.6 g, 17.11 mmol) and Bu_4NF (1.0 M in THF, 20 mL, 20 mmol) in THF (40 mL) was stirred at room temperature for 8 hours. The solvent was evaporated and the residue was chromatographed on a silica gel column using hexanes-ether (10 : 1 to 1 : 1) to give compound **18** as a colorless oil (5.31 g, 94%). The ^1H, ^{13}C and ^{19}F NMR spectra corresponded to those described.[13] ESI-MS 331 (M + H, 6.3), 353 (M + Na, 100.0).

(*cis, trans*)-2,2-Bis(benzyloxymethy)l-3-fluoro-1-(2-nitrophenylselenenylmethyl)-cyclopropane (19). A mixture of compound **18** (5.0 g, 15.2 mmol), 2-nitrophenyl selenocyanate (4.14 g, 18.24 mmol) and Bu_3P (4.5 mL, 18.24 mmol) in THF (30 mL) was stirred for 4 hours at room temperature. The solvents were removed and the residue was chromatographed on a silica gel column using hexanes-EtOAc (10 : 1 to 8 : 1) to give the selenide **19** (7.1g, 91%) as a pale yellow oil. ^1H NMR ($CDCl_3$) δ 1.38, 1.55 (m, 1H, H_3), 2.85–3.21 (m, 2H, CH_2Se), 3.33–3.56, 3.77–3.89 (2m, 4H, CH_2OBn, 4.45–4.73 (m, 5H of Bn, H_1), 7.27–7.36 (m, 11H, C_6H_5), 7.43–7.54m, 2H), 8.29 (d, 1H, J = 8.0 Hz, $NO_2C_6H_4$, aromatic H's). ^{19}F NMR −215.33 (dd, J = 64.0, 21.5 Hz), −232.11 (dd, J = 64.0 Hz, 6.0 Hz). ESI-MS 538 (M + Na, 100.0).

2,2-Bis(benzyloxymethyl)-3-fluoro-1-methylenecyclopropane (21). A mixture of selenide **19** (7.0 g, 14.0 mmol) and 30% H_2O_2 (9.3 mL, 79.1 mmol) in THF (42 mL) was stirred for 14 hours at room temperature. Ethyl

ether (200 mL) was added, the organic phase was washed with water (5 × 40 mL) and it was dried ($MgSO_4$). The solvent was evaporated to give the crude selenoxide **20** as a yellow syrup. This product was heated in toluene (30 mL) at 80°C for 4 hours. The solvent was evaporated and the residue was chromatographed on a silica gel column in hexanes-Et_2 O (50 : 1 to 30 : 1) to give methylenecyclopropane **21** (2.93 g, 67%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 3.52, 3.84 (split AB, 2H, J = 10.0 Hz), 3.73 (poorly resolved dd, 2H, J = 8.0), CH_2OBn), 4.56 (s, 2H), 4.59 (AB, 2H, J = 11.6 Hz, CH_2 of Bn), 4.95 (d, 1H, J = 68 Hz, H_1), 5.77, 5.95 (2s, 2H, = CH_2), 7.31–7.40 (m, 10H, Ph). ^{13}C NMR 32.6 (d, J = 14.2 Hz, C_2), 66.9, 69.6 (d, J = 4.5 Hz, CH_2OBn), 72.5 (d, C_3, J = 236.5 Hz), 73.0, 73.2 (CH_2 of Bn), 111.4 (d, J = 2.2 Hz, CH_2 =), 134.1 (C_1), 127.8, 127.9, 128.6, 128.7, 138.5, 138.7 (Ph). ^{19}F NMR −214.02 (d, J = 67.4 Hz). ESI-MS 313 (M + H, 12.0), 335 (M + Na, 100.0). Anal. Calcd for $C_{20}H_{21}FO_2$: C, 76.90; H, 6.78. Found: C, 76.85; H, 6.98.

(*cis, trans*)-2,2-Bis(benzyloxymethyl)-1-bromo-1-bromomethyl-3-fluorocyclopropane (22). Pyridinium tribromide (4.3 g, 13.5 mmol) was added to a solution of compound **21** (2.8 g, 8.97 mmol) in CH_2Cl_2 (40 mL) with stirring at −20°C. The reaction mixture was allowed to warm to room temperature and the stirring was continued for 10 hours. The solvent was evaporated and the crude product was chromatographed in hexane-Et_2 O (50 : 1 to 10 : 1) to afford compound **22** as a colorless oil (3.92 g, 90.7%, isomeric ratio 1.6/1). ^{1}H NMR ($CDCl_3$) δ 3.75–4.07 (m, 6H, CH_2OBn, CH_2 Br), 4.53–4.60 (m, 4 H, CH_2 of Bn), 4.58, 4.83 (2d, 1H, J = 64.0, 63.2 Hz, H_3), 7.35–7.43 (m, 10 H, Ph). ^{13}C NMR 35.7 (d, J = 6.7 Hz), 36.5 (d, J = 8.6 Hz), 37.6 (d, J = 10.4 Hz), 39.1 (C_2, C_1), 42.6 (d, J = 9.0 Hz), 43.7 (d, J = 9.8 Hz, CH_2 Br), 63.6 (d, J = 8.3 Hz), 66.6, 69.8 (d, J = 5.9 Hz), 71.7 (CH_2OBn), 73.2, 73.3, 73.6, 73.7 (CH_2Ph), 76.7 (d, J = 241.1 Hz), 81.6 (d, J = 245.5 Hz, C_3), 128.0, 128.1, 128.2, 128.3, 128.7, 128.8, 128.9, 137.6, 138.0, 138.2, 138.3 (Ph). ^{19}F NMR −211.55 (d, J = 64.0 Hz), −217.20 (d, J = 64.4 Hz). ESI-MS (MeOH + KOAc) 509, 511, 513 (M + K, 28.6, 100.0, 33.3).

(*E*)- and (*Z*)-9-{[2,2-Bis(benzyloxymethyl)-3-fluorocyclopropylidene] methyl}adenine (23a) and (24a). A mixture of adenine (594 mg, 4.4 mmol), compound **22** (1.92 g, 4.0 mmol) and K_2CO_3 (3.31 g, 24 mmol) in DMF (20 mL) was stirred for 6 hours at room temperature under N_2 and then at 100–105°C for 4 hours. The insoluble solid was filtered off using a silica gel pad (5 g) which was washed with DMF (70 mL). The solvent from the filtrate was evaporated in vacuo and the residue was chromatographed in EtOAc-hexanes (1 : 3 to 2 : 1) to give the faster moving *E*-isomer **23a** (380 mg, 21.3%) followed by Z-isomer **24a** (630 mg, 35.4%).

E-Isomer **23a**: m.p. 152–153°C. UV λ_{max} 242 nm (ε 24,400), 281 (ε 8,200). ^{1}H NMR ($CDCl_3$) δ 3.53, 3.93 (AB, 2H, J_{AB} = 10.2Hz), 3.79, 3.84 (AB, 2H, J = 9.8 Hz, 10.0 Hz, $H_{5'}$), 4.53, 4.54, 4.55 (3s, 4H, CH_2 of Bn), 5.05 (d, 1H, J = 69.6 Hz, $H_{3'}$), 6.54 (s, 2H, NH_2), 7.25–7.34 (m, 10H, Ph), 8.00

(s, 1H, $H_{1'}$), 8.37 (s, 1H, H_2), 8.91 (s, 1H, H_8). ^{13}C NMR 34.1 (d, J = 12.7 Hz, $C_{4'}$), 66.6, 69.4 (d, J = 3.7 Hz, $C_{5'}$), 71.29, 71.34 (2d, J = 237.3, 238.0 Hz, $C_{3'}$), 73.5, 73.6 (CH_2 of Bn), 112.2 (d, J = 3.0 Hz, $C_{2'}$), 117.4 ($C_{1'}$), 119.5 (C_5), 127.95, 127.97, 128.09, 128.18, 128.70, 128.75, 137.74, 137.96 (Ph), 138.6 (C_8), 149.2 (C_4), 153.7 (C_2), 156.1 (C_6). ^{19}F NMR −210.95 (d, J = 70.0 Hz). ESI-MS (MeOH + KOAc) 446 (M + H, 100.0), 484 (M + K, 100.0).

Z-isomer **24a**: m.p. 160–162°C. UV λ_{max} 244 nm (ε 24,900), 281 (ε 8,300). ^{1}H NMR ($CDCl_3$) δ 3.62–3.81 (m, 4H, $H_{5'}$), 4.52, 4.55, 4.56 (3s, 4H, CH_2Ph), 5.26 (d, 1 H, J = 68.0 Hz, $H_{3'}$), 6.77 (s, 2H, NH_2), 7.26–7.32 (m, 10 H, Ph), 7.63 (s, 1H, $H_{1'}$), 8.26 (s, 1H, H_2), 8.39 (s, 1H, H_8). ^{13}C NMR 33.4 (d, J = 13.7 Hz, $C_{4'}$), 66.7, 69.2 (d, J = 3.7 Hz, $C_{5'}$), 72.1 (d, J = 238.9 Hz, $C_{3'}$), 73.3, 73.5 (CH_2 of Bn), 113.9 ($C_{2'}$), 116.1 ($C_{1'}$), 119.6 (C_5), 127.89, 127.93, 128.03, 128.6, 128.7, 138.0, 138.1, 138.2 (C_8), 149.1 (C_4), 153.7 (C_2), 155.6 (C_6). ^{19}F NMR −211.88 (d, J = 68.5 Hz). ESI-MS 446 (M + H, 44.3), 468 (M+ Na, 100.0).

(*E*)-9-{[2,2-Bis(hydroxymethyl)-3-fluorocyclopropylidene]methyl}adenine (13a). A solution of the $BCl_3{\cdot}SMe_2$ complex (2.0 M in CH_2Cl_2, 3.35 mL, 6.73 mmol) was added dropwise to the *E*-isomer (**23a**, 300 mg, 0.673 mmol) in CH_2Cl_2 (30 mL) at room temperature under N_2 over 10 minutes with stirring which was then continued for 10 hours. $NaHCO_3$ (2.8 g, 33.3 mmol) and methanol (30 mL) were added to quench the reaction and the reaction mixture was stirred for 4 hours. The insoluble solid was filtered off using a silica gel pad (3.5 g) which was washed with CH_2Cl_2-MeOH (2 : 1, 60 mL). The solvents from the filtrate were evaporated and the residue was chromatographed using EtOAc-MeOH (10 : 1) to give product **13a** (147 mg, 82.7%), m.p. 243–245°C. UV λ_{max} 237 nm (ε 23,100), 280 (ε 8,100). ^{1}H NMR δ 3.51–3.55, 3.69–3.76 (2m, 4H, $H_{5'}$), 5.18, 5.22 (2t, 2H, J = 5.4, 6.6 Hz, OH), 5.21 (d, 1H, J = 70.4 Hz, $H_{3'}$), 7.41 (s, 2H, NH_2), 7.89 (s, 1H, $H_{1'}$), 8.19 (s, 1H, H_2), 8.82 (s, 1H, H_8). ^{13}C NMR 37.9 (d, J = 12.0 Hz, $C_{4'}$), 58.2, 60.8 (d, J = 4.5 Hz, $C_{5'}$), 72.3 (d, J = 232.1 Hz, $C_{3'}$), 114.2 (d, J = 3.0 Hz, $C_{2'}$), 116.8 (d, J = 3.0 Hz, $C_{1'}$), 119.2 (C_5), 138.5 (C_8), 148.9 (C_4), 153.9 (C_2), 156.8 (C_6). ^{19}F NMR −212.07 (d, J = 71.0 Hz). EI-MS 266 (M + H, 100.0), 288 (M + Na, 73.4), 553 (2M + Na, 20.3). Anal. Calcd for $C_{11}H_{12}FN_5O_2{\times}0.1\ H_2O$: C, 50.67; H, 4.34; N, 29.55. Found: C, 50.54; H, 4.30; N, 29.31.

(*Z*)-9-{[2,2-Bis(hydroxymethyl)-3-fluorocyclopropylidene]methyl}adenine (14a). The procedure described above for the *E*-isomer **13a** was repeated with the Z-isomer **24a** to give analogue **14a** (150 mg, 84.4%), m.p. 230–232°C. UV λ_{max} 237 nm (ε 23,400), 280 (ε 8,200). ^{1}H NMR δ 3.53 (d, 2H, J = 5.6 Hz), 3.64 (m, 2H, $H_{5'}$), 4.89 (t, 2 H, J = 5.6 Hz, OH), 5.42 (d, 1 H, J = 68.8 Hz, $H_{3'}$), 7.44 (s, 2 H, NH_2), 7.53 (s, 1 H, $H_{1'}$), 8.20 (s, 1 H, H_2), 8.34 (s, 1 H, H_8). ^{13}C NMR 36.8 (d, J = 11.9 Hz, $C_{4'}$), 58.2, 60.9 (d, J = 5.2 Hz), 73.2 (d, J = 233.6 Hz, $C_{3'}$), 115.0 (d, J = 3.7 Hz, $C_{2'}$), 119.1

(C_5), 119.2 ($C_{1'}$), 137.9 (C_8), 148.9 (C_4), 154.2 (C_2), 156.8 (C_6). ^{19}F NMR −212.87 (d, J = 68.5 Hz). ESI-MS (MeOH + KOAc) 266 (M + H, 14.3), 304 (M + K, 100.0). Anal. Calcd for $C_{11}H_{12}FN_5O_2 \times 0.1\ H_2O$: C, 50.67; H, 4.34; N, 29.55. Found: C, 50.89; H, 4.43; N, 29.33.

(*E*)- and (*Z*)-2-Amino-6-chloro-9-{[2,2-(bis(benzyloxymethyl)-3-fluorocyclopropylidene-methyl}purine (23c) and (24c). A mixture of 2-amino-6-chloropurine (430 mg, 2.55 mmol), K_2CO_3 (1.05 g, 7.62 mmol) and compound **22** (1.2 g, 2.54 mmol) in DMF (7.5 mL) was stirred under N_2 for 7 hours at room temperature, then at 60°C for 1 hour and, finally, at 100–105°C for 2.5 hours. After cooling, the insoluble portion was filtered off using a silica gel pad (5 g) which was washed with DMF (70 mL). DMF was evaporated in vacuo and the residue was chromatographed in hexanes-EtOAc (10 : 1 to 3 : 1) to give the faster moving *E*-isomer **23c** (210 mg, 17.5%) and slower moving *Z*-isomer **24c** (440 mg, 36.7%) as white solids.

E-Isomer **23c**: m.p. 135–137°C. UV λ_{max} 242 nm (ε 32,800), 310 (ε 7,800). ^{1}H NMR ($CDCl_3$) δ 3.47, 3.85 (split AB, 2H, J = 12.0, 2.0 Hz), 3.72, 3.79 (AB, 2 H, J = 10.2 Hz, $H_{5'}$), 4.51, 4.53 (2s, 4H, CH_2 of Bn), 5.01 (d, 1 H, J = 68.8 Hz, $H_{3'}$), 5.22 (bs, 2H, NH_2), 7.22–7.33 (m, 10H, Ph), 7.78 (s, 1H, $H_{1'}$), 8.89 (s, 1 H, H_8). ^{13} C NMR δ 34.2 (d, J = 12.7 Hz, $C_{4'}$), 66.4, 69.2 (J = 5.2 Hz, $C_{5'}$), 71.2 (d, J = 238.9 Hz, $C_{3'}$), 73.5, 73.6 (CH_2 of Bn), 112.8 (d, J = 3.7 Hz, $C_{2'}$), 116.9 (d, J = 3.0 Hz, $C_{1'}$), 125.4 (C_5), 127.97, 128.05, 128.2, 128.67, 128.72, 137.6, 137.8 (Ph), 140.5 (C_8), 151.8, 152.8 (C_4, C_2), 159.6 (C_6). ^{19}F NMR −211.22 (d, J = 70.4 Hz). ESI-MS (MeOH) 480, 482 (M + H, 100.0, 41.1). Anal. Calcd for $C_{25}H_{23}ClFN_5O_2$: C, 62.56; H, 4.83; N, 14.59. Found: C, 62.38; H, 4.77; N, 14.56.

Z-Isomer **24c**: m.p. 145–146°C. UV λ_{max} 242 nm (ε 34,100), 310 (ε 7,900). ^{1}H NMR ($CDCl_3$) δ 3.59–3.81, m, 4 H, $H_{5'}$), 4.52 (s), 4.53, 4.58 (AB, 4 H, J = 12.0 Hz, CH_2 of Bn), 5.21 (d, 1H, J = 68.8 Hz, $H_{3'}$), 5.31 (s, 2H, NH_2), 7.26–7.34 (m, 10H, Ph), 7.46 (s, 1 H, $H_{1'}$), 8.11 (s, 1 H, H_8). ^{13}C NMR 33.4 (d, J = 12.7 Hz, $C_{4'}$), 66.6, 69.2 (J = 4.5 Hz, $C_{5'}$), 72.1 (d, J = 237.3 Hz, $C_{3'}$), 73.3, 73.5 (CH_2 of Bn), 114.0, 115.6 (d, J = 3.0 Hz, $C_{1'}$, $C_{2'}$), 125.4 (C_5), 127.9, 127.97, 128.06, 128.6, 128.7, 138.0, 138.2 (Ph), 139.5 (C_8), 152.0, 152.5 (C_4, C_2), 159.8 (C_6). ^{19}F NMR −211.29 (d, J = 67.4 Hz). ESI-MS 480, 482 (M, 100.0, 38.1), 502, 504 (M + Na, 97.6, 36.9). Anal. Calcd for $C_{25}H_{23}ClFN_5O_2$: C, 62.56; H, 4.83; N, 14.59. Found: C, 62.51; H, 4.83; N, 14.58.

(*E*)-2-Amino-6-chloro-9-{[2,2-bis(hydroxymethyl)-3-fluorocyclopropylidene]methyl}purine (13c). A solution of $BCl_3{\cdot}SMe_2$ complex (2.0 M in CH_2Cl_2, 2.1 mL, 4.2 mmol) was added dropwise to a solution of the *E*-isomer **23c** (200 mg, 0.418 mmol) in CH_2Cl_2 (8 mL) at room temperature under N_2 over 10 minutes with stirring. The stirring was continued for 6 hours. The reaction was quenched with methanol (20 mL) and $NaHCO_3$ (4.0 g, 47.6 mmol). After 10 hours of stirring, the insoluble portion was

filtered off through a silica gel pad (3.5 g) which was washed with CH_2Cl_2–MeOH (2 : 1, 60 mL). After removal of solvents from the filtrate, the residue was chromatographed using hexane-EtOAc (1 : 1 to 100% EtOAc) to afford compound **13c** (107 mg, 86%), m.p. 238–239°C. UV λ_{max} 241 nm (ε 25,700), 309 (ε 7,400). ^{1}H NMR δ 3.49–3.53, 3.70–3.77 (2m, 4H, $H_{5'}$), 5.11 (t, 1H, J = 5.6 Hz), 5.16 (t, 1H, J = 4.8 Hz, OH), 5.20 (d partly overlapped with δ 5.11, J = 70.4 Hz, $H_{3'}$), 7.08 (s, 2H, NH_2), 7.69 (d, 1H, J = 1.6 Hz, $H_{1'}$), 8.79 (s, 1H, H_8). ^{13}C NMR 37.9 (d, J = 12.0 Hz, $C_{4'}$), 58.2, 60.8 (d, J = 3.7 Hz, $C_{5'}$), 72.2 (d, J = 232.8 Hz, $C_{3'}$), 114.8 (d, J = 3.0 Hz), 116.2 (d, J = 2.9 Hz, $C_{1'}$, $C_{2'}$), 123.8 (C_5), 140.3 (C_8), 150.4 (C_4), 153.3 (C_2), 160.9 (C_6). ^{19}F NMR −212.79 (d, J = 70.0 Hz). ESI-MS 300, 302 (M + H, 90.1, 37.0), 322, 324 (M + Na, 100.0, 33.1).

(*Z*)-2-Amino-6-chloro-9-{[3-fluoro-2,2-bis(hydroxymethyl)cyclopropylidene]methyl}purine (14c). The procedure described above for the *E*-isomer **13c** was followed using the *Z*-isomer **24c** (400 mg, 0.83 mmol) to give compound **14c** (210 mg, 84%), m.p. 247–249°C. UV λ_{max} 242 nm (ε 27,400), 310 (ε 7,200). ^{1}H NMR δ 3.49–3.52, 3.58–3.69 (2 m, 4 H, $H_{5'}$), 4.90 (t, 2 H, J = 5.6 Hz, OH), 5.43 (d, J = 68.0 Hz, $H_{3'}$), 7.09 (s, 2 H, NH_2), 7.37 (s, 1 H, $H_{1'}$), 8.30 (s, 1 H, H_8). ^{13}C NMR 37.0 (d, J = 12.7 Hz, $C_{4'}$), 58.1, 60.9 (d, J = 5.2 Hz, $C_{5'}$), 73.3 (d, J = 233.5 Hz, $C_{3'}$), 114.6 (d, J = 3.0 Hz), 115.8 ($C_{1'}$, $C_{2'}$), 123.8 (C_5), 140.0 (C_8), 150.6 (C_4), 153.1 (C_2), 161.0 (C_6). ^{19}F NMR −212.37 (d, J = 68.5 Hz). ESI-MS 300, 302 (M + H, 100.0, 29.8), 322, 324 (M + Na, 88.4, 23.5).

(*E*)-9-{[2,2-Bis(hydroxymethyl)-3-fluorocyclopropylidene]methyl}guanine (13b). A solution of the *E*-isomer **13e** (100 mg, 0.33 mmol) in 80% formic acid (5 mL) was heated at 80°C with stirring for 4 hours. After cooling, formic acid and water were evaporated in vacuo and the crude product was dissolved in methanol (10 mL). Ammonia in methanol (20%, 10 mL) was added at 0°C and the reaction mixture was stirred for 4 hours. The volatile components were evaporated, the crude product was dried at 0.01–0.03 torr at room temperature whereupon it was recrystallized from methanol to give compound **13b** (85 mg, 90.5%), m.p. >300°C. UV 243 (ε 22,300), 274 nm (ε 7,700). ^{1}H NMR δ 3.44–3.49, 3.64–3.74 (2 m, 4 H, $H_{5'}$), 5.16 (d partly overlapped with δ 5.23, 1 H, J = 70.8 Hz, H_3), 5.23 (t, 2 H, OH), 6.89 (s, 2 H, NH_2), 7.55 (s, 1 H, $H_{1'}$), 8.42 (s, 1 H, H_8), 11.01 (bs, 1 H, NH). ^{13} C NMR 37.7 (d, J = 11.3 Hz, $C_{4'}$), 58.0, 60.7 (d, J = 4.5 Hz, $C_{5'}$), 72.3 (d, J = 232.0 Hz, $C_{3'}$), 113.8 (d, J = 3.0 Hz), 116.4 (d, J = 2.2 Hz, $C_{1'}$, $C_{2'}$), 117.0 (C_5), 134.7 (C_8), 150.7 (C_4), 155.1 (C_2), 157.2 (C_6). ^{19}F NMR −212.63 (d, J = 71.9 Hz). ESI-MS 282 (M + H, 17.0), 304 (M + Na, 100.0). Anal. Calcd for $C_{11}H_{12}N_5FO_3$: C, 46.98; H, 4.30; N, 24.90. Found: C, 47.07; H, 4.50; N, 25.03.

(*Z*)-9-{[2,2-Bis(hydroxymethyl)-3-fluorocyclopropylidene]methyl}guanine (14b). The procedure described above for the *E*-isomer **13b** was followed using the *Z*-isomer **14e** (150 mg, 0.5 mmol) to give compound **14b**

(120 mg, 85%), m.p. >300°C. UV 243 (ε 22,800), 274 nm (ε 7,900). ^{1}H NMR δ 3.50 (poorly resolved d, 2 H), 3.56–3.67 (m, 2 H, $H_{5'}$), 4.87 (t, 2 H, J = 5.6 Hz, OH), 5.38 (d, 1 H, J = 69.2 Hz, $H_{3'}$), 6.56 (s, 2 H, NH_2), 7.26 (s, 1 H, $H_{1'}$), 7.90 (s, 1 H, H_8), 10.74 (bs, 1 H, NH). ^{13}C NMR 36.7 (d, J = 12.0 Hz, $C_{4'}$), 58.1, 60.9 (d, J = 5.2 Hz, $C_{5'}$), 73.1 (d, J = 232.8 Hz, $C_{3'}$), 114.7 (d, J = 1.4 Hz), 114.9 d, J = 2.9 Hz, $C_{1'}$, $C_{2'}$), 117.1 (C_5), 134.3 (C_8), 150.6 (C_4), 154.9 (C_2), 157.3 (C_6). ^{19}F NMR −212.86 (d, J = 68.9 Hz). ESI-MS 282 (M + H, 32.1), 304 (M + Na, 100.0), 585 (2M + Na, 32.0). Anal. Calcd for $C_{11}H_{12}N_5FO_3$: C, 46.98; H, 4.30; N, 24.90. Found: C, 46.76; H, 4.50; N, 24.90.

Antiviral Assays

The antiviral assays were performed as described previously.[10] 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 EBV assays were performed in Akata instead of Daudi cells using DNA hybridization. The VZV was assayed in HFF cells by CPE inhibition or plaque reduction. For cytotoxicity assays, HFF and Akata cells were employed.

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