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Fluorinated methylenecyclopropane analogues of nucleosides. Synthesis and antiviral activity of (*Z*)- and (*E*)-9-{[(2-fluoromethyl-2-hydroxymethyl)-cyclopropylidene]methyl} adenine and -guanine

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Abstract

Synthesis and antiviral activity of the title fluoromethylenecyclopropane analogues **15a**, **15b**, **16a** and **16b** is described. Methylenecyclopropane carboxylate was first transformed to 2,2-bis-hydroxymethylmethylenecyclopropane. Selective monoacetylation followed by introduction of fluorine gave 2-acetoxymethyl-2-fluoromethylmethylenecyclopropane as the key intermediate. The synthesis of analogues **15a**, **15b**, **16a** and **16b** then followed alkylation-elimination procedure as described previously for other methylenecyclopropane analogues. The adenine *Z*-isomer **15a** was found to be a potent inhibitor of Epstein-Barr virus (EBV) in vitro with EC_{50}/CC_{50} (μM) 0.5/55.7. Compounds **15b**, **16a** and **16b** were also active but at higher concentrations, EC_{50}/CC_{50} (μM) 3.2–7.5/53.6–64.1. Analogue **15a** inhibited hepatitis C virus by virtue of its cytotoxicity and it moderately inhibited replication of the Towne strain of human cytomegalovirus (HCMV). The *E*-isomer **16a** was a substrate for adenosine deaminase whereas the *Z*-isomer **15a** was not deaminated.

Keywords

Methylenecyclopropanes; Nucleoside analogues; Alkylation-elimination; Methylenecyclopropane-methylenecyclobutane rearrangement; Antiviral agents; Adenosine deaminase

1. Introduction

The *Z*-methylenecyclopropane analogues of purine nucleosides **1** and **2** are effective antiviral agents whereas the *E*-isomers **3** and **4** (Chart 1) are either inactive or of limited potency.[1–3] The guanine analogue **2b** (cyclopropavir) is currently under preclinical investigation as a

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possible drug against infections caused by human cytomegalovirus (HCMV).[4,5] It is also effective in vitro[6] against Epstein-Barr virus (EBV) and human herpes viruses (HHV) 6 and 8. Structure-activity relationships (SAR) studies have indicated that introduction of fluorine into the cyclopropane moiety of **1** and **3** can also provide new antiviral agents. Thus, purine and/or pyrimidine *Z*- and *E*-2-fluoro analogues **5** and **6** were effective against HCMV, EBV or varicella zoster virus (VZV).[7] Purine 3-fluoroanalogues **7, 8, 9** and **10** had more narrow antiviral effects or they were less potent.[8] This trend was also reflected in the bis(2,2-hydroxymethyl)-3-fluoro derivatives[9] **11** and **12**.

Fluorine can mimic both a hydrogen atom and a hydroxy group because of its small van der Waals radius and polarity of the carbon-fluorine bond.[10] Although all possible monofluoromethylenecyclopropane analogues (**5** through **12**) derived by replacement of hydrogens of the cyclopropane moiety were investigated,[7–9] compounds having the hydroxy group(s) replaced with fluorine have not been described. Similar fluoro analogue of ganciclovir **13** exhibited activity[11] against herpes simplex virus 1 (HSV-1). Because cyclopropavir **2b** can be regarded as a rigid bioisostere of anti-HCMV drug ganciclovir[4] **14** it was of interest to synthesize and investigate biological activity of purine fluoromethylenecyclopropane analogues **15a**, **15b**, **16a** and **16b**.

2. Results and discussion

2.1. Synthesis

Methylenecyclopropane diol **17** was chosen as a convenient starting material for synthesis of analogues **15** and **16**. For the present purpose, compound **17** was obtained by an alternate approach. Methylenecyclopropane carboxylate[12] **18** was reduced using a less than a stoichiometric amount of diisobutylaluminum hydride (DIBALH). The intermediary aldehyde **19** was not isolated but it was subjected in situ to aldol and crossed Cannizzaro reaction with formaldehyde to give diol **17** in 64% yield. It should be noted that this is a new synthesis of an important intermediate for cyclopropavir (**2b**).[4,13,14] Acetylation of **17** via the corresponding cyclic orthoester[13] gave monoacetate **20** in 78% yield. Reaction of **20** with diethylaminosulfur trifluoride (DAST)[15,16] using pyridine in CH_2Cl_2 at −78 °C did not give the expected fluoro derivative **21** but it led instead to a ring-expanded fluorocyclobutane **22** as the only product in 87% yield. It is important to note that this is a new synthesis of methylenefluorocyclobutane skeleton. The parent compound[17] is accessible only by reaction of [1.1.1]propellane with XeF_2. Recently, ring expansion of prolinols to fluoropiperidines effected by DAST was described.[18] Nevertheless, the reaction course was not uniform and ratio of five-membered to six-membered products was about 2 : 3.

The reaction is initiated by transformation of **20** by DAST to intermediate **23** (Scheme 2). In the next step, a non-classical[19] cyclopropylmethyl carbocation **24** existing in equilibrium with cyclobutonium ion **25** reacts with fluoride ion to give methylenefluorocyclobutane **22**. The reported solvolysis[20] of methylenecyclopropylmethyl chloride and deamination[21] of methylenecyclopropylmethylamine led also to methylenecyclobutanes in addition to methylenecyclopropyl methyl derivatives.

It was then clear that avoiding formation of intermediary carbocation might lead to a successful synthesis of methylenefluorocyclopropane **21**. Therefore, monoacetate **20** was converted to methylsulfonate **26** (81%) using methylsulfonyl chloride (MsCl) which, in turn, was smoothly transformed to fluorocyclopropane **21** (72%) using tetrabutylammonium fluoride (NBu_4F) in THF. Addition of bromine via pyridinium tribromide gave dibromo derivative **27** which was used for alkylation elimination[1–3] of nucleic acid heterocycles. The reaction of **27** with adenine gave the *Z*,*E*-isomeric mixture methylenecyclopropanes **28a** in 65% yield. The yield of **28c** obtained with 2-amino-6-chloropurine was lower (46%). Deacetylation of **28a** using K_2CO_3

in 90% aqueous methanol at room temperature furnished the target analogues **15a** and **16a** after chromatographic separation in 49 and 43% yield, respectively. In a similar fashion, deacetylation of intermediate **28c** at 0 °C afforded the *Z*- and *E*-isomers **15c** and **16c** (46 and 54%). Hydrolytic dechlorination of **15c** and **16c** using 80% formic acid at 80 °C provided guanine analogues **15b** and **16b** (84 and 91%).

2.2. The *Z,E*-Isomeric Assignment

As in previous cases of methylenecyclopropane analogues,[2,4] the NMR spectroscopy was indispensable to confirm the *Z,E*-isomeric structure of analogues **15a**, **15b** and **16a**, **16b**. The chemical shift patterns of relevant protons parallell those of analogues **2a**, **2b** and **4a**, **4b** (Table 1). Thus, the ^{1}H NMR signals of OH and H_8 of the *Z*-isomers **15a**, **15b** are more deshielded than those of the *E*-isomers **16a**, **16b** whereas an opposite pattern was found in the alkene $H_{1'}$ signals. In the ^{13}C NMR spectra, the cyclopropane $C_{4'}$ of the *Z*-isomers **15a**, **15b** is located at a lower field than in the *E*-isomers **16a**, **16b** in contrast to the corresponding $C_{3'}$ shifts. The final confirmation of the *Z.E*-isomeric assignment came from the NOE experiments performed with adenine analogues **15a** and **16a** (Table 2). In the *Z*-isomer **15a**, the NOE enhancements were found between the *cis*-arranged $H_{1'}$ and $H_{3'}$ protons as well as between the H_8 and protons of OH, CH_2F and CH_2O groups. By contrast, in the *E*-isomer **16a** a strong NOE interaction occurs between the *cis*-located $H_{3'}$ and H_8. Also, the NOE enhancements were found between the $H_{3'}$ and OH, CH_2F and CH_2O groups.

2.3. Antiviral Activity

Compounds **15a**, **15b**, **16a** and **16b** were tested against the following viruses: herpes simplex virus 1 and 2 (HSV-1 and HSV-2), human cytomegalovirus (HCMV, Towne and AD 169 strains), varicella zoster virus (VZV), Epstein-Barr virus (EBV), human immunodeficiency virus (HIV-1), hepatitis B and C virus (HBV and HCV). They were all effective against EBV in Akata cells using a DNA hybridization assay.[22] The adenine analogue **15a** was the most potent (Table 3) and least cytotoxic. It was more effective than cyclopropavir (**2b**). The *E*-isomer **16a** and guanine derivatives **15b**, **16b** were less effective than **15a**. Interestingly, a somewhat similar anti-EBV activity pattern was found with *Z*- and *E*-isomers of fluoroanalogues[7] **5a, 5b, 6a** and **6b** which can be regarded as lower homologues of **15a, 15b, 16a** and **16b**. However, an exact comparison is not possible because of the differences in assays. In the series of fluoroanalogues **7** through **12** only adenine *Z*-isomer[8] **9a** was effective against EBV. It is likely that the mechanism of anti-EBV action of analogues **15a, 15b, 16a** and **16b** includes their phosphorylation to triphosphates which then inhibit the viral DNA polymerase as suggested for other fluorinated methylenecyclopropane analogues.[7,8]

Compound **15a** also inhibited HCV in Huh7 AVA5 cells[23] (replicon assay) with EC_{50}/CC_{50} (μM) 6.5/11 using 2'-methylcytidine as a control (EC_{50}/CC_{50} 1.8/>300) but the antiviral activity was poorly separated from cytotoxicity. Compound **15a** moderately inhibited the replication of HCMV Towne strain but not AD169 strain (plaque reduction assay) in human foreskin fibroblast (HFF) cells /EC_{50}/CC_{50} (μM) 46/>100, ganciclovir (**14**) as a control exhibited EC_{50}/CC_{50} 2.5/>100. No significant activity against the rest of tested viruses was detected.

2.4. Adenosine Deaminase (ADA)

Adenine analogues **15a** and **16a** were investigated as substrates for adenosine deaminase. In agreement with the general trend in the series of methylenecyclopropane analogues,[1,2] the *E*-isomer **16a** was a moderate substrate and it was deaminated after 28 h, whereas the *Z*-isomer **15a** was resistant to deamination.

3. Conclusion

Fluoromethylenecyclopropane analogues **15a, 15b, 16a** and **16b** were synthesized and evaluated for antiviral activity. All analogues were inhibitors of replication of EBV in Akata cells with adenine derivative **15a** being the most potent with EC_{50}/CC_{50} (μM) 0.5/55.7. Against HCMV, only compound **15a** had a moderate effect whereas its potency against HCV was offset by cytotoxicity. No activity was observed against other tested viruses. The *E*-isomer **15b** was a moderate substrate for adenosine deaminase whereas *Z*-isomer **15a** was not deaminated.

4. Experimental

4.1. 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, methanol - NaCl) mode. Thin-layer chromatography (TLC) was performed on Analtech aluminum foils coated with silica gel F254.

4.2. 2,2-Bis(hydroxymethyl)methylenecyclopropane (17)

A solution of DIBALH in hexane (1 M, 26 mL, 26 mmol) was added dropwise to ethyl 8 methylenecyclopropane carboxylate[12] **18** (4.12 g, 32.7 mmol) in dichloromethane at −78 °C with stirring. The stirring was continued for 1 h. TLC (hexane - AcOEt 4 : 1) indicated the presence of aldehyde **19** as the major product accompanied by minor amounts of the faster moving starting ester **18** and slower moving methylenecyclopropylmethanol. The reaction was quenched with saturated aqueous NH_4Cl (100 mL). The mixture was stirred for 6 h, the aqueous layer was extracted with ether (2 × 100 mL), the combined organic phase was dried ($MgSO_4$) and it was concentrated to about 10 mL by distillation at <45 °C at an atmospheric pressure. A mixture of this product, aqueous formaldehyde (37%, 65 mL, 0.8 mol) and KOH (18.3 g, 0.33 mmol) in methanol (60 mL) was stirred for 5 days at room temperature. Methanol was removed in vacuo and the aqueous portion was extracted with ethyl acetate (10 × 100 mL). The organic phase was dried ($MgSO_4$) and concentrated. The precipitated paraformaldehyde was filtered off using a short silica gel column which was then washed with AcOEt - hexanes (4 : 1). The solvents were evaporated and the residue was refluxed in 1 M HCl (5 mL) for 2 h. The volatile components were evaporated and the crude product was chromatographed on a silica gel column using AcOEt - hexanes (1 : 1) to give diol 17 (1.88 g, 64% based on DIBALH) as a yellow oil. TLC (AcOEt - hexanes, 2 : 1) and ^{1}H NMR spectrum were identical with those of authentic samples.[4,13]

4.3. 2-Acetoxymethyl-2-hydroxymethyl-1-methylenecyclopropane (20)

A mixture of of diol **17** (1.80 g, 15.8 mmol), trimethyl orthoacetate (2.9 g, 23.7 mmol) and *p*-toluenesulfonic acid (2 mg) in CH_2Cl_2 (20 mL) was stirred for 1 h at room temperature. The reaction was quenched with Et_3N (0.1 mL) and solvent was evaporated. The residue was dissolved in 80% acetic acid (5 mL) and the solution was allowed to stand at room temperature for 30 min whereupon it was diluted with dichloromethane (200 mL). The organic phase was 9 washed with saturated $NaHCO_3$ (2 × 200 mL, **caution!**) and water (2 × 200 mL). It was dried ($MgSO_4$) and the solvent was removed to give product **20** (1.87 g, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 5.52 (t, 1H, *J* = 3.1 Hz), 5.43 (t, 1H, *J* = 1.8 Hz, CH_2=), 4.15, 4.10 (AB, 2H, *J* = 11.6 Hz, CH_2OAc), 3.56, 3.51 (AB, 2H, *J* = 11.6 Hz, CH_2OH), 2.09 (s, 3H, CH_3), 1.25 (t, 2H, *J* = 2.4 Hz, H_3). ^{13}C NMR 171.9 (C=O), 134.9 (C=), 105.2 (CH_2=), 66.7, 65.2 (CH_2O), 26.2 (C_2), 21.2 (CH_3), 13.8 (C_3). ESI-MS 179 (84.8, M + Na), 157 (26.6, M + H), 97 (100.0). Anal. Calcd for $C_8H_{12}O_3 \times 0.25\ H_2O$: C,59.80; H, 7.84 H. Found: C, 59.74; H, 7.73.

4.4. 3-Acetoxymethyl-3-fluoro-1-methylenecyclobutane (22)

DAST (0.16 mL, 0.81 mmol) was added dropwise to a stirred solution of acetate **20** (75 mg, 0.48 mmol) and pyridine (0.16 mL, 2 mmol) in CH_2Cl_2 (20 mL) at −78 °C. The temperature was allowed to raise, the solvent was evaporated and the crude product was chromatographed on a silica gel column using hexanes - ether (4 : 1) to give compound **22** (70 mg, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.97 (m, 2H, CH_2=), 4.26 (d, 2H, J = 22.8 Hz, CH_2O), 3.05 (dt, J = 19.0, 2.9 Hz, 2H), 2.84 (m, 2H, H_2, H_4), 2.11 (s, 3H, CH_3). ^{13}C NMR 171.1 (C=O), 136.9 (d, J = 15.7 Hz, C=), 109.7 (d, J = 8.2 Hz, CH_2=), 91.0 (d, J = 216.4 Hz, C_3), 66.1 (d, J = 23.1 Hz, CH_2O), 41.7 (d, J = 23.1 Hz, C_2, C_4), 21.0 (CH_3). ^{19}F NMR -149.32 (m). EI-MS 138 (34.5, M - HF), 116 (22.6, M - CH_2CO), 97 (100.0). HRMS calcd for $C_8H_{10}O_2$ (M - HF) 138.0681. Found: 138.0682. Anal. Calcd for $C_8H_{11}FO_2$: C, 60.75; H. 7.01. Found: C, 61.02; H, 7.07.

4.5. 2-Acetoxymethyl-2-methylsulfonyloxymethylmethylenecyclopropane (26)

Methylsulfonyl chloride (0.90 mL 11.5 mmol) was added dropwise with stirring and external ice cooling to a solution of acetate **20** (1.80 g, 11.5 mmol) and triethylamine (3.3 mL, 23 mmol) in CH_2Cl^2 (20 mL). The stirring was continued for 1 h, the mixture was diluted with ether (150 mL), the organic phase was washed with water (100 mL), saturated $NaHCO_3$ (2 × 100mL), water 10 (2 × 100 mL) and it was dried with $MgSO_4$. The solvent was evaporated to give compound **26** (2.2 g, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 5.59 (t, 1H, J = 3.1 Hz), 5.51 (t, 1H, J = 1.8 Hz, CH_2=), 4.20, 4.16 (AB, 2H, J = 10.5 Hz), 4.14, 4.05 (AB, 2H, J = 11.6 Hz, CH_2O), 3.01 (s, 3H, CH_3SO_2), 2.07 (s, 3H, CH_3CO), 1.42 (t, 2H, J = 1.8 Hz, H_3). ^{13}C NMR 171.1 (C=O), 132.9 (C=), 107.0 (CH_2=), 72.1 (CH_2OMs), 65.5 (CH_2OAc), 37.8 (CH_3SO_2), 23.1 (C_2), 21.1 (CH_3 of AcO), 14.7 (C_3). EI-MS (MeOH + LiCl) 241 (M + Li, 100.0), 475 (2M + Li, 48.8). Anal. Calcd for $C_9H_{14}O_5S \times H_2O$: C, 42.85; H. 6.39. Found: C 42.97; H, 6.40.

4.6. 2-Acetoxymethyl-2-(fluoromethyl)methylenecyclopropane (21)

A solution of Bu_4NF (1M, 35 mL, 35 mmol) in THF was added with stirring to compound **26** (1.65 g, 7 mmol) in THF (100 mL) under N_2 at room temperature. The stirring was continued for 6 h, the mixture was diluted with ether (200 mL), the organic phase was washed with saturated $NaHCO_3$ (2 × 200 mL), water (2 × 200 mL) and it was dried ($MgSO_4$) The solvent was removed by distillation at an atmospheric pressure. The crude product was chromatographed on a silica gel column using 1-pentane - ether (15 : 1) to give compound **21** (0.80 g, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 5.57 (t, 1H, J = 2.4 Hz), 5.49 (poorly resolved dd, 1H, CH_2=), 4.43, 4.40 and 4.30, 4.28 (2AB, 2H, $J_{H,F}$ = 48.8 Hz, J_{AB} = 9.8 Hz, CH_2F), 4.17, 4.10 (AB, 2H, J_{AB} = 12.0 Hz, CH_2OAc), 2.09 (s, 3H, CH_3), 1.38 (m, 2H, H_3). ^{13}C NMR 171.2 (C=O), 133.2 (C=), 106.3 (CH_2=), 85.5 (d, J = 172.3 Hz, CH_2F), 65.7 (CH_2OAc), 24.3 (d, J = 23.1 Hz, C_2), 21.2 (CH_3), 14.0 (d, J = 6.7 Hz, C_3). ^{19}F NMR −216.52 (poorly resolved tt, J = 48.8, 2,6 Hz). EI-MS 138 (16.7, M - HF), 116 (23.8, M - CH_2CO), 97 (100.0). HRMS calcd for $C_8H_{10}O_2$ (M - HF) 138.0681. Found 138.0687.

4.7. (*Z*,*E*)-1-Acetoxymethyl-1-fluoromethyl-2-bromo-2-bromomethylcyclopropane (27)

A mixture of of pyridinium tribromide (2.12 g, 6.6 mmol) and compound **21** (0.7 g (4.4 mmol) in CH_2Cl_2 (20 mL) was stirred at 0 °C for 1 h. The solid portion was filtered off and it was washed with CH_2Cl_2 (5 mL). The filtrate was diluted with ether (100 mL), the organic phase was washed with saturated $Na_2S_2O_3$ (2 × 100 mL) and water (2 × 100 mL) and it was dried with Na_2SO_4. The solvents were evaporated and the residue was chromatographed on a silica gel column using AcOEt - hexanes (1 : 10) to give product **27** (1.12 g, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.80 - 4.20 (cluster of m, 6H, CH_2F, CH_2Br, CH_2OAc), 2.08, 2.07 (2s, 3H, CH_3), 1.46, 1.32 (2m, 2H, H_3). ^{13}C NMR 170.9 (C=O), 86.8, 81.6 (2d, J = 173.8 Hz, CH_2F), 67.9 (d, J = 1.5 Hz), 61.7 (d, J = 1.5 Hz, CH_2OAc), 42.1 (d, J = 4.5 Hz), 42.0 (d, J =

4.3 Hz, CH_2Br), 41.3, 41.2 (C_1), 33.1 (d, J = 23.1 Hz), 32.8 (d, J = 20.9 Hz, C_2), 26.5, 26.3 (2d, J = 6.7 Hz, C_3), 21.09, 21.06 (CH_3). ^{19}F NMR −219.24 (dt, J = 48.9, 6.2 Hz), −219.92 (tt, 47.4, 9.0, 4.1 Hz). ESIMS 339, 341, 343 (53.3, 100.0, 51.8, M + Na). Anal. Calcd for $C_8H_{11}Br_2FO_2$: C, 30.22; H. 3.49. Found: C, 30.61; H, 3.50.

4.8. (*Z*,*E*)-9-[(2-Acetoxymethyl-2-fluoromethylcyclopropylidene)methyl]adenine (28a)

A mixture of of dibromide 27 (400 mg, 1.26 mmol), adenine (170 mg, 1.26 mmol) and K_2CO_3 (1.8 g, 12.6 mmol) in DMF (25 mL) was stirred for 5 h at 110–115 °C. After cooling, solids were filtered off and they were washed with DMF (5 mL). The filtrate was concentrated in vacuo and the residue was chromatographed on a silica gel column using CH_2Cl_2 - methanol (200 : 5) to give compound $_{28a}$ (240 mg, 65%) as a white solid. The *Z*/*E* ratio was 1 : 1 as determined by ^1H NMR, mp 189–196 °C. UVλ_{max} 277 nm (ε 8,400), 263 (ε 11,800), 227 (ε 24,900). ^1H NMR δ 8.50, 8.34 (1H, 2s, 1H, H_8), 8.19, 8.18 (2s, 1H, H_2), 7.58 (t, J = 2.5 Hz), 7.51 (m, 1H, $H_{1'}$), 7.39 (bs, 2H, NH_2), 4.79, 4.65 and 4.62, 4.49 (2AB, $J_{H,F}$ = 49.0 Hz, J_{AB} = 10.1 Hz), 4.46 (d, 2H, J = 49.2 Hz, CH_2F), 4.40, 4.13 and 4.20, 4.14 (2AB, 2H, $_J$ = 11.7 Hz, CH_2OAc), 2.06, 1.91 (2s, 3H, CH_3), 1.95, 1.70 (2m, 2H, H_3). ^{13}C NMR 171.1, 170.6 (C=O), 156.8 (C_6), 153.9 (C_2), 149.0, 12 148.9 (C_4), 138.0 (C_8), 119.1 (C_5), 115.6 (d, J = 8.0 Hz), 115.3 (d, J = 7.0 Hz, $C_{2'}$), 113.1, 112.9 ($C_{1'}$), 86.4 (d, J = 169.4 Hz), 85.5 (d, J = 169.2 Hz, CH_2F), 65.8, 65.2 (CH_2OAc), 26.6 (d, J = 23.2 Hz), 24.6 (d, J = 23.2 Hz, $C_{4'}$), 21.4, 21.1 (CH_3), 15.9 (d, J = 6.8 Hz), 12.8 (d, J = 7.1 Hz, $C_{3'}$). ^{19}F NMR −215.10, −214.94 (2 overlapped t, J = 48.2 Hz). ESI-MS 292 (100.0, M + H), 314 (44.4, M + Na). Anal. Calcd for $C_{13}H_{14}FN_5O_2$: C, 53.60; H, 4.84; N, 24.04. Found: C, 53.51; H, 4.89; N, 23.87.

4.9. (*Z*)-9-[(2-Fluoromethyl-2-hydroxymethylcyclopropylidene)methyl]adenine (15a) and (*E*)-9-[(2-Fluoromethyl-2-hydroxymethylcyclopropylidene)methyl]adenine (16a)

mixture of compound **28a** (220 mg, 0.76 mmol) and K_2CO_3 (200 mg, 1.45 mmol) in methanol - water (9 : 1, 20 mL) was stirred for 1 h at room temperature. The solvent was evaporated and the residue was chromatographed on a silica gel column using CH_2Cl_2 - methanol (20 : 1) to give the *Z*-isomer **15a** (93 mg, 49%), followed by *E*-isomer **16a** (80 mg, 43%).

***Z*-isomer 15a—**Mp 234–236 °C. UV λ_{max} 278 nm (ε 7,700), 261 (ε 10,700), 227 (ε 22,800). ^1H NMR ε 8.57 (s, 1H, H_8), 8.17 (s, 1H, H_2), 7.45 (s, 1H, $H_{1'}$), 7.37 (bs, 2H, NH_2), 5.37 (t, 1H, J = 5.4 Hz, OH), 4.70, 4.56 and 4.58, 4.44 (2 partly overlapped AB, 1H, $J_{H,F}$ = 47.8 Hz, J_{AB} = 8.8 Hz, CH_2F), 3.80 (dd, 1H, J = 10.4, 4.8 Hz), 3.48 (dd, 1H, J = 11.6, 5.6 Hz, CH_2OH), 1.54 (m, 2H, $H_{3'}$). ^{13}C NMR 156.7 (C_6), 153.8 (C_2), 148.7 (C_4), 138.0 (C_8), 119.1 (C_5), 116.2 (d, J = 9.0 Hz, $C_{2'}$), 112.2 ($C_{1'}$), 85.5 (d, J = 168.6 Hz, CH_2F), 62.6 (CH_2OH), 29.4 (d, J = 23.1 Hz, $C_{4'}$), 12.0 (d, J = 7.5 Hz, $C_{3'}$). ^{19}F NMR −216.38 (t, J = 48.2 Hz). ESI-MS 250 (100.0, M + H), 272 (13.7, M + Na). Anal. Calcd for $C_{11}H_{12}FN_5O$: C, 53.01; H, 4.85; N, 28.10. Found: C, 52.99; H, 4.82; N, 27.81.

***E*-isomer 16a—**Mp 251–253 °C. UV λ_{max} 277 nm (ε 7,800), 262 (ε 11,000), 226 (ε 24,200). ^1H NMR δ 8.49 (s, 1H, H_8), 8.16 (s, 1H, H_2), 7.52 (, 1H, $H_{1'}$), 7.37 (bs, 2H, NH_2), 5.02 (t, 1H, J = 5.6 Hz, OH), 4.47 (d, 2H, J = 47.8 Hz, CH_2F), 3.54 (dd, 1H, J = 11.0, 6.2 Hz), 3.46 (dd, 1H, J = 11.2, 5.8 Hz, CH_2O), 1.76 (m, 2H, $H_{3'}$). ^{13}C NMR 156.7 (C_6), 153.8 (C_2), 149.0 (C_4), 137.9 (C_8), 119.1 (C_5), 117.1 (d, J = 9.0, $C_{2'}$), 112.0 ($C_{1'}$), 85.1 (d, J = 168.6 Hz, CH_2F), 62.5 (CH_2O), 27.6 (d, J = 23.1 Hz, $C_{4'}$), 15.0 (d, J = 7.5 Hz, $C_{3'}$). ^{19}F NMR −215.42 (poorly resolved dt, J = 48.8, 3.0 Hz). ESI-MS 250 (100.0, M + H), 272 (29.8, M + Na). Anal. Calcd for $C_{11}H_{12}FN_5O$: C, 53.01; H, 4.85; N, 28.10. Found: C, 53.25; H, 4.89; N, 28.18.

4.10. (*Z*,*E*)-2-Amino-6-chloro-9-[(2-acetoxymethyl-2-fluoromethylcyclopropylidene)- methyl] purine (28c)

A mixture of dibromide **27** (470 mg, 1.48 mmol), 2-amino-6-chloropurine (256 mg, 1.48 mmol) and K_2CO_3 (2.08 g, 15 mmol) in DMF (25 mL) was stirred for 5 h at 110–115 °C. After cooling, the solid portion was filtered off and it was washed with DMF (5 mL). Filtrate was concentrated in vacuo and the residue was chromatographed on a silica gel column using CH_2C_{l2} - methanol (200 : 1) to give product **28c** (220 mg, 46%) as a white solid. The *Z*/*E* ratio was 1 : 1 as determined by ^{1}H NMR, mp 153–170 °C. UV λ_{max} 311 nm (ε 7,900), 231 (ε 29,900). ^{1}H NMR δ 8.45, 8.25 (2s, 1H, H_8), 7.42 (d, *J* = 2.4 Hz), 7.34 (bs, 1H, $H_{1'}$), 7.06, 7.05 (2bs, 2H, NH^2), 4.76 – 4.33 (cluster of m, 4H, CH_2F, CH_2OAc), 2.05, 1.89 (2s, 3H, CH_3), 1.94 (poorly resolved t), 1.70 (bs, 2H, $H_{3'}$). ^{13}C NMR 171.0, 170.7 (C=O), 160.8 (C_6), 153.3, 153.2 (C_2), 150.4 (C_4), 140.5, 140.3 (C_8), 123.78, 123.75 (C_5), 116.7 (d, *J* = 8.2 Hz), 116.6 (d, *J* = 9.7 Hz, $C_{2'}$), 112.8, 112.6 ($C_{1'}$), 86.2 (d, *J* = 168.6 Hz), 85.5 (d, *J* = 170.1 Hz, CH_2F), 65.7, 65.2 (CH_2OAc), 26.6, 24.7 (2d, *J* = 23.1 Hz, $C_{4'}$), 21.4, 21.1 (CH_3), 16.1, 12.9 (2d, *J* = 6.7 Hz, $C_{3'}$). ^{19}F NMR −214.99 (2 overlapped dt). ESI-MS 191 (100.0), 326, 328 (6.5, 2.0, M + H), 348, 350 (5.9, 2.0, M + Na). Anal. Calcd for $C_{13}H_{13}$ $ClFN_5O_2$: C, 47.94; H, 4.02: N. 21.50. Found: C, 47.93; H, 4.08; N, 21.23.

4.11. (*Z*)-2-Amino-6-chloro-9-[(2-hydroxymethyl-2-fluoromethylcyclopropylidene)methyl]-purine (15c) and (*E*)-2-Amino-6-chloro-9-[(2-hydroxymethyl-2-fluoromethylcyclopropylidene) methyl]purine (16c)

A mixture of compound **28c** (210 mg, 0.65 mmol) and K_2CO_3 (178 mg, 1.30 mmol) in methanol - water (9 : 1, 30 mL) was stirred for 1 h at 0 °C. The solvent was evaporated and the residue was chromatographed on a silica gel column using CH_2Cl_2 - methanol (100 : 3) to give the *Z*-isomer **15c** (85 mg, 46%), followed by *E*-isomer **16c** (100 mg, 54%).

***Z*-isomer 15c—**Mp 206–208 °C. UV λ_{max} 310 nm (ε 7,000), 232 (ε 28,600). ^{1}H NMR δ 8.53 (s, 1H, H_8), 7.26 (s, 1H, $H_{1'}$), 7.04 (2H, bs, NH_2), 5.33 (t, 1H, *J* = 5.2 Hz, OH), 4.69, 4.53 and 4.57, 4.41 (2AB, 2H, $J_{H,F}$ = 48.1 Hz, J_{AB} = 9.8 Hz, CH_2F), 3.80 (dd, 1H, *J* = 11.2, 4.8 Hz), 3.44 (dd, 1H, *J* = 11.2, 5.6 Hz, CH_2O), 1.54 (m, 2H, $H_{3'}$). ^{13}C NMR 160.8 (C_6), 153.1 (C_2), 150.4 (C_4), 140.1 (C_8), 123.7 (C_5), 117.1 (d, *J* = 8.2 Hz, $C_{2'}$), 111.7 ($C_{1'}$), 85.4 (d, *J* = 167.9 Hz, CH_2F), 62.6 (CH_2OH), 29.4 (d, *J* = 23.1 Hz, $C_{4'}$), 12.1 (d, *J* = 6.7 Hz, $C_{3'}$). ^{19}F NMR −216.29 (t, *J* = 48.0 Hz). ESI-MS (MeOH + KOAc) 123 (100.0), 284, 286 (M + H, 11.0, 2.7), 322, 324 (18.2, 6.9, M + K) 605, 607 (14.9, 11.0, 2M + K). Anal. Calcd for $C_{11}H_{11}ClFN_5O$: C, 46.57; H, 3.91; N, 24.69. Found: C, 46.54; H, 3.91; N, 24.45.

***E*-isomer 16c—**Mp 216 °C (decomp). UV λ_{max} 311 nm (ε6,700), 232 (ε 27,000). ^{1}H NMR δ 8.45 (s, 1H, H_8), 7.36 (poorly resolved d, 1H, $H_{1'}$), 7.04 (2H, bs, NH_2), 5.04 (poorly resolved t, 1H, OH), 4.52, 4.39 (2 poorly resolved ddd, 2H, $J_{H,F}$ = 48.6 Hz, CH_2F), 3.54 – 3.52, 3.46 – 3.44, (2m, 2H, CH_2O), 1.76 (m, 2H, $H_{3'}$). ^{13}C NMR 160.8 (C_6), 153.3 (C_2), 150.4 (C_4), 140.2 (C_8), 123.7 (C_5), 118.1 (d, *J* = 9.7 Hz, $C_{2'}$), 111.6 ($C_{1'}$), 85.0 (d, *J* = 168.6 Hz, CH_2F), 62.4 (CH_2OH), 27.8 (d, *J* = 23.9 Hz, $C_{4'}$), 15.1 (d, *J* = 6.7 Hz, $C_{3'}$). ^{19}F NMR −215.47 (t, *J* = 48.2 Hz). ESI-MS (MeOH + KOAc) 123 (100.0), 322, 324 (22.9, 8.0, M + K), 605, 607 (6.0, 4.8, 2M + K). Anal. 15 Calcd for $C_{11}H_{11}ClFN_5O \times 0.5H_2O$: C, 45.13; H, 4.13; N, 23.93. Found: C, 45.28; H, 4.14; N, 23.57.

4.12. (*Z*)-9-[(2-hydroxymethyl-2-fluoromethylcyclopropylidene)methyl]guanine (15b)

A solution of the *Z*-isomer **15c** (85 mg, 0.3 mmol) in formic acid (80%, 15 mL) was heated at 80 °C for 4 h. The solvent was removed and the crude product was dissolved in methanolic NH_3 (20%, 30 mL) at 0 °C with stirring which was continued for 5 h. The volatile components were evaporated and methanol was evaporated from the residue (3 times). The resultant solid

was washed with methanol (5 mL) to give product **15b** (67 mg, 84%) as a white solid, mp >280 °C. UV λ_{max} 273 nm (ε 10,200), 230 (ε 25,200). ^{1}H NMR δ 10.68 (s, 1H, CONH), 8.15 (s, 1H, H_8), 7.16 (s, 1H, $H_{1'}$), 6.54 (bs, 2H, NH^2), 5.31 (t, 1H, *J* = 5.0 Hz, OH), 4.64 – 4.42 (2 overlapped AB, CH_2F), 3.74 (poorly resolved dd, 1H), 3.46 (dd, 2H, *J* = 11.2, 4.8 Hz, CH_2O), 1.49 (m, 2H, $H_{3'}$). ^{13}C NMR 157.3 (C_6), 154.7 (C_2), 150.4 (C_4), 134.5 (C_8), 116.9 (C_5), 115.7 (d, *J* = 7.1 Hz, $C_{2'}$), 112.0 ($C_{1'}$), 85.3 (d, *J* = 168.6 Hz, CH_2F), 62.4 (CH_2O), 29.2 (d, *J* = 23.1 Hz, $C_{4'}$), 11.9 (d, *J* = 6.7 Hz, $C_{3'}$). ^{19}F NMR −216.48 (t, *J* = 47.9 Hz). ESI-MS 266 (100.0, M + H), 288 (48.2, M + Na). Anal. Calcd for $C_{11}H_{12}FN_5O_2 \times 0.2\ H_2O$: C, 49.14; H, 4.65; N, 26.04. Found: C, 49.13; H, 4.54; N, 25.83.

4.13. (*E*)-9-[(2-hydroxymethyl-2-fluoromethylcyclopropylidene)methyl]guanine (16b)

The procedure described for the *Z*-isomer **15b** was followed with *E*-isomer **16c** (128 mg, 0.45 mmol). The product was recrystallized from methanol (20 mL) to give compound **16b** (109 mg, 91%) as a white solid, mp >300 °C. UV λ_{max} 272 nm (ε 10,200), 229 (ε 26,700). ^{1}H NMR δ 10.70 (s, 1H, CONH), 8.04 (s, 1H, H_8), 7.26 (s, 1H, $H_{1'}$), 6.53 (bs, 2H, NH_2), 5.01 (t, 1H, *J* = 5.8 Hz, OH), 4.52, 4.47 and 4.40, 4.35 (2AB, 2H, $J_{H,F}$ = 48.1 Hz, J_{AB} = 9.8, 8.8 Hz, CH_2F), 3.51, 3.43 (2dd, 2H, *J* = 11.0, 5.6 Hz, CH_2O), 1.71 (m, 2H, $H_{3'}$). ^{13}C NMR 157.4 (C_6), 154.6 (C_2), 16 150.6 C_4), 134.4 (C_8), 117.0 (C_5), 116.7 (d, *J* = 8.9 Hz, $C_{2'}$), 111.9 ($C_{1'}$), 85.1 (d, *J* = 167.6 Hz, CH_2F), 62.4 (CH_2OH), 27.5 (d, *J* = 23.0 Hz, $C_{4'}$), 14.8 (d, *J* = 6.7 Hz, $C_{3'}$). ^{19}F NMR −215.38 (t, *J* = 48.9 Hz). ESI-MS 266 (100.0, M + H), 288 (73.2, M + Na). Anal. Calcd for $C_{11}H_{12}N_5O_2F \times 0.2\ H_2O$: C, 49.14; H, 4,65; N, 26.04. Found: C, 49.35; H, 4.57; N, 25.96.

4.14. Adenosine deaminase (ADA) Assay[7]

The *Z*- and *E*-isomers **15a** and **16a** (4.2 – 4.4 μmol) were incubated with ADA from calf intestine (Worthington, Lakewood, New Jersey, USA, 1.5 unit/mL) in 0.05 M Na_2HPO_4 (pH 7.5, 1.2 mL) with magnetic stirring at room temperature. Aliquots were withdrawn, they were diluted with the buffer (0.2 mL/10 mL) and the UV spectra were recorded. The UV maximum of **16a** at 260 nm completely disappeared after 28 h, whereas the spectrum of **15a** was unchanged (UV_{max} 260 nm).

4.15. Antiviral Assays

The antiviral assays, with the exception of EBV and HCV, were described previously.[7]

4.15.1. EBV DNA Hybridization Assay.[22]—Akata cells were maintained in RPMI 1640, (Mediatech, Inc, Herndon, Va.) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah), L-glutamine, penicillin and gentamicin at 37 °C in a humidified 5% CO_2 atmosphere. Latently infected cells were induced to undergo a lytic infection by adding a F(ab′)2 fragment of goat anti-human IgG antibody (MP Biomedicals, Aurora, OH). Total DNA from the cells was purified and genome copy number was quantified by Real-Time PCR. The primers 5'-CGG AAG CCC TCT GGA CTT C-3' and 5'-CCC TGT TTA TCC GAT GGA ATG-3' were used with the fluorescent probe, 6FAM-TGT ACA CGC ACG AGA AAT GCG CC-TAMRA corresponding to coordinates 155959-155981 in the EBV genome (Applied Biosystems). The PCR was performed in an optical 96 well plate using an ABI 7300 Real-Time PCR system. The PCR reaction contained 900 nM primers, 200 nM probe, 12.5 μL Taqman Universal Master Mix (Applied Biosystems, Foster City, CA), and 5 μL target DNA in a final volume of 25 μL. Each sample was run in duplicate and EC_{50} values were calculated by standard methods.

4.15.2. HCV Studies—Antiviral activity of test compounds was assessed in the stably-expressing HCV replicon cell line, AVA5 (subgenomic CON1, genotype 1b)[23] maintained at sub-confluent cultures on 96-well plates as previously described.[24] Antiviral activity was

determined by blot hybridization analysis of intracellular HCV RNA and cytotoxicity was assessed by neutral red red dye uptake after 3 days of treatment. Compounds were added each day in fresh medium. Intracellular RNA levels and cytotoxicity were assessed 24 h after the last dose of compound.

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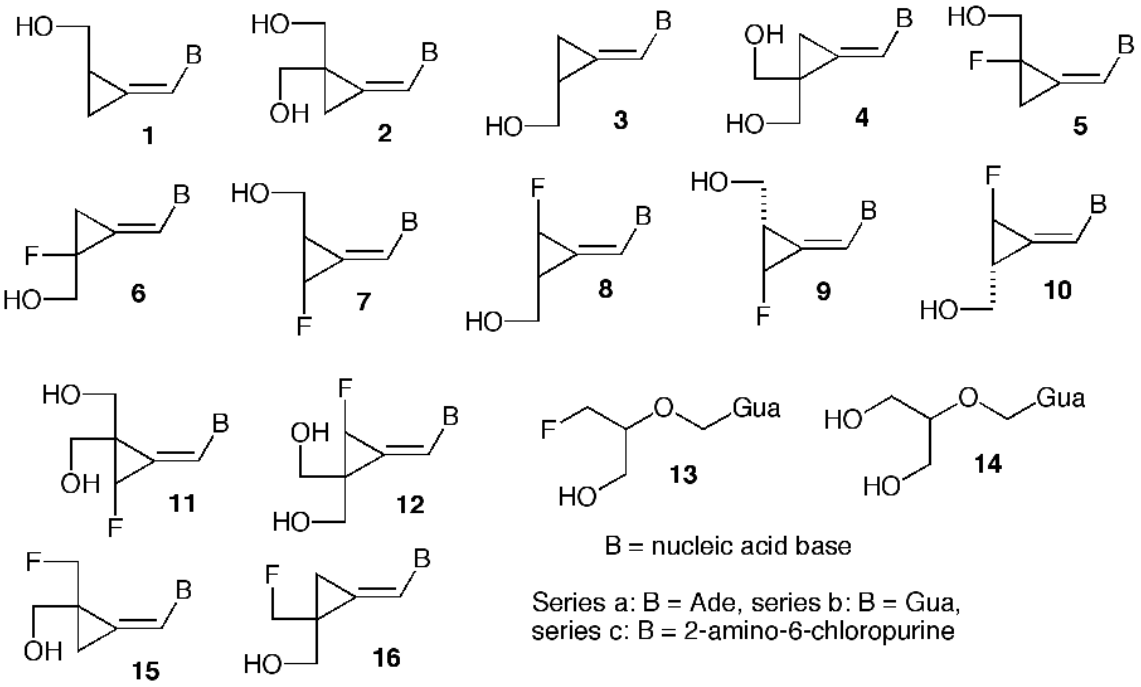
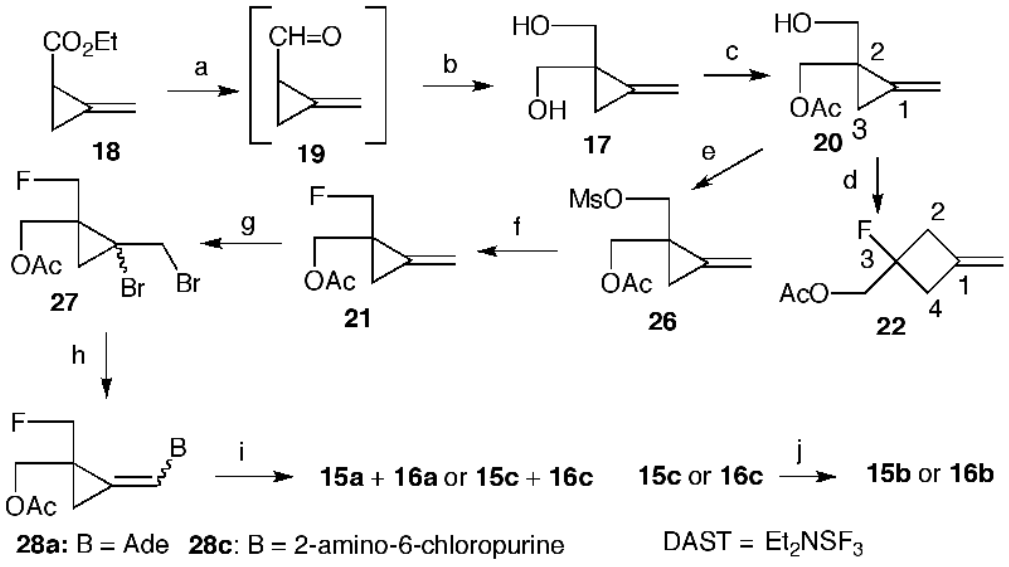
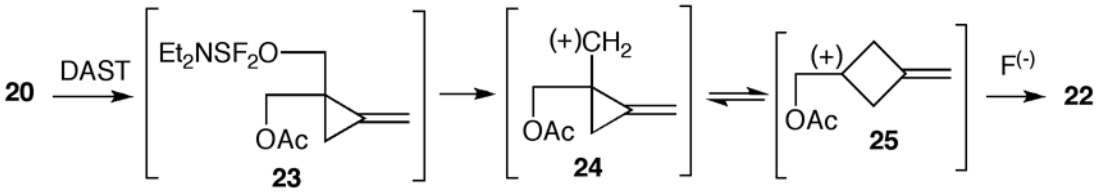


Chart 1.



Scheme 1.

Reagents: a. DIBALH, CH_2Cl_2, −78 °C, b. 1. 37% CH_2O, MeOH, 2. 1M HCl, Δ, c. 1. MeC $(OMe)_3$, cat. TsOH, CH_2CI_2. 2. NEt_3. 3. 80% AcOH, d. DAST, pyridine, CH_2CI_2. −78 °C → rt. E. MsCI, NEt_3, CH_2CI_2. f. Bu_4NF, THF. g. Pyridine, HBr_3, CH_2CI_2, 0 °C. h. B-H, K_2CO_3, DMF, Δ. i. K_2CO_3, MeOH - H_2O (9 : 1), rt or 0 °C. j. 1. 80% HCO_2, Δ. 2. NH_3, MeOH, 0 °C.



Scheme 2.

Table 1

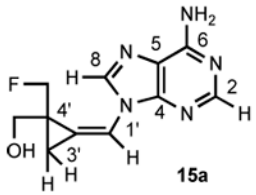
Comparison of chemical shifts (δ) of the relevant ^{1}H and ^{13}C NMR signals of the (*Z*)-and (*E*)-2,2-bis(hydroxymethyl)- and 2-fluoromethyl-2-hydroxymethylmethylenecyclopropanes **2a, 4a, 2b, 4b, 15a**, **16a**, **15b** and **16b**

Compound[a]	Isomer	OH	H_1,	H_8	C_3,	C_4,
2a	*Z*	5.07	7.37	8.82	11.7	31.4
4a	*E*	4.76	7.48	8.49	14.4	29.7
2b	*Z*	4.99	7.07	8.41	11.5	31.3
4b	*E*	4.76	7.21	8.03	14.3	29.5
15a	*Z*	5.37	7.45	8.57	12.0	29.4
16a	*E*	5.02	7.52	8.49	15.0	27.6
15b	*Z*	5.31	7.16	8.15	11.9	29.2
16b	*E*	5.01	7.26	8.04	14.8	27.5

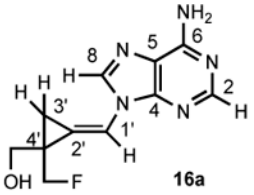
[a] CD_3SOCD_3 as solvent. For numbering of signals see Table 2. Values for **2a**, **4a**, **2b** and **4b** were taken from ref.[4]

Table 2

The NOE enhancements of relevant ^{1}H NMR signals of (*Z*)- and (*E*)-2-fluoromethyl-2-hydroxymethyltmethylenecyclopropanes **15a** and **16a**



15a



16a

Compound	H_{irr}	δ	H_{obs}	δ	NOE (%)
15a	$H_{1'}$	7.45	$H_{3'}$	1.54	1.83
	$H_{3'}$	1.54	$H_{1'}$	7.45	2.17
	OH	5.37	H_8	8.57	4.0
	H_8	8.57	OH	5.37	1.71
	CH_2F	4.44–4.70	H_8	8.57	3.16
	CH_2O	3.48–3.80	H_8	8.57	3.84
16a	$H_{3'}$	1.76	H_8	8.49	2.75
	OH	5.02	$H_{1'}$	7.52	1.42
	CH_2F	4.47	$H_{1'}$	7.52	1.34
	CH_2O	3.46	$H_{1'}$	7.52	1.87

Table 3

Inhibition of Replication of EBV with Fluoromethyl Methylenecyclopropane Nucleoside Analogues[a]

Compound	EC_{50}/CC_{50} (μM)[b]	Selectivity Index
2b	2.5/>100[c]	>40
5a	6.8/>213	>31.3
5b	8.0/>199	>24.9
6a	167/>209[d]	>1.25
6b	29.1/>199[e]	>6.8
15a	0.5/55.7	111
15b	7.5/59.7	8
16a	3.4/53.6	15.8
16b	3.2/64.1	20

[a] Akata cells, DNA hybridization assay. For details see Experimental. Acyclovir as a control had EC_{50} 1.7 μM.

[b] Results for analogues **5a - 6b** were taken from from ref.7 (DNA hybridization assay in Daudi cells).

[c] Data from ref.19

[d] EC_{50} 2.3 μM in viral capsid immunofluorescence (VCA) ELISA and 3.6 μM in H-1 cells (DNA hybridization).

[e] EC_{50} <0.32 μM in VCA ELISA.