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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.

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 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 HHV-8. Structure–activity relationship (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**–**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**.

Keywords: Methylenecyclopropanes; Nucleoside analogues; Alkylation–elimination; Methylenecyclopropane–methylenecyclobutane rearrangement; Antiviral agents; Adenosine deaminase.
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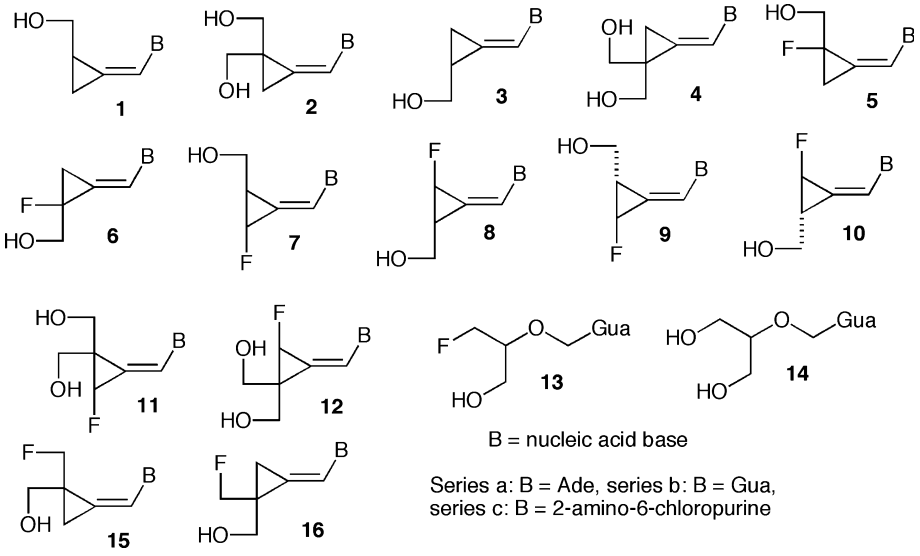


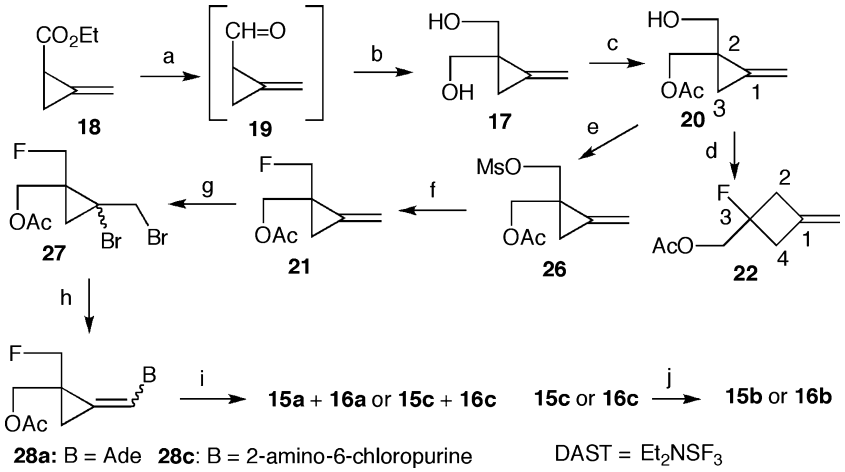
Chart 1.

2. Results and discussion

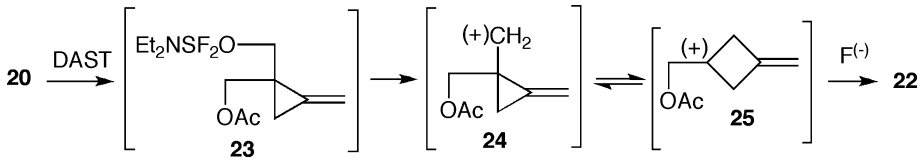
2.1. Synthesis

Methylenecyclopropane diol **17** was chosen as a convenient starting material for synthesis of analogues **15** and **16** (Scheme 1). 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.



Scheme 1. Reagents and conditions: (a) DIBALH, CH_2Cl_2, −78 °C; (b) 1—37% CH_2O, MeOH; 2—1 M HCl, Δ; (c) 1—$MeC(OMe)_3$, cat. TSOH, CH_2Cl_2; 2—NEt_3; 3—80% AcOH; (d) DAST, pyridine, CH_2Cl_2, −78 °C to rt; (e) MsCl, NEt_3, CH_2Cl_2; (f) Bu_4NF, THF; (g) Pyridine·HBr_3, CH_2Cl_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_2H, Δ; 2—NH_3, MeOH, 0 °C.



Scheme 2.

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*- and *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*- and *E*-isomeric assignment

As in previous cases of methylenecyclopropane analogues,[2,4] the NMR spectroscopy was indispensable to confirm the *Z*- and *E*-isomeric structure of analogues **15a**, **15b**, **16a**, and **16b**. The chemical shift patterns of relevant protons parallel those of analogues **2a**, **2b**, **4a**, and **4b** (Table 1). Thus, the ^1H NMR signals of OH and H_8 of the *Z*-isomers **15a** and **15b** are more deshielded than those of the *E*-isomers **16a** and **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** and **15b** is located at a lower field than in the *E*-isomers **16a** and **16b** in contrast to the corresponding $C_{3'}$ shifts. The final confirmation of the *Z*- and *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.

Table 1. Comparison of chemical shifts (δ) of the relevant ^1H 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**

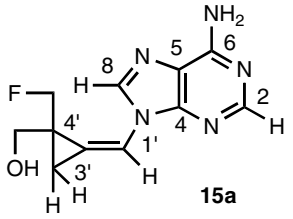
Compound[a]	Isomer	OH	$H_{1'}$	H_8	$C_{3'}$	$C_{4'}$
2a	*Z*	5.07	7.37	8.82	11.7	31.4
4a	*E*	4.76	7.48	8.49	14.4	29.7
2b	*Z*	4.99	7.07	8.41	11.5	31.3
4b	*E*	4.76	7.21	8.03	14.3	29.5
15a	*Z*	5.37	7.45	8.57	12.0	29.4
16a	*E*	5.02	7.52	8.49	15.0	27.6
15b	*Z*	5.31	7.16	8.15	11.9	29.2
16b	*E*	5.01	7.26	8.04	14.8	27.5

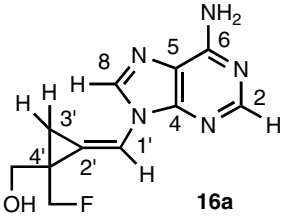
[a] CD_3SOCD_3 as solvent. For numbering of signals, see Table 2. Values for **2a**, **4a**, **2b**, and **4b** were taken from Ref. 4.

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** and **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

Table 2. The NOE enhancements of relevant ^1H NMR signals of (*Z*)- and (*E*)-2-fluoromethyl-2-hydroxymethylmethylenecyclopropanes **15a** and **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	0.22/>46[c]	209
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 4. Acyclovir as a control had EC_{50} 1.7 μM.
[b] Results for analogues **5a**–**6b** were taken from Ref. 7 (DNA hybridization assay in Daudi cells).
[c] Data from Ref. 22.
[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.

15a, **15b**, **16a**, and **16b**. However, an exact comparison is not possible because of the differences in assays. In the series of fluoroanalogues **7**–**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 with 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 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 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 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. ^1H 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. 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 rise, 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. ^1H NMR ($CDCl_3$) δ 4.97 (m, 2H, CH_2=), 4.26 (d, 2H, J = 22.8 Hz, CH_2O), 3.05 (dt, 2H, J = 19.0, 2.9 Hz), 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 × 100 mL), water (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. ^1H 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). ESI-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 (1 M, 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. ^1H 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 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. ^1H 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). ESI-MS 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 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. UV λ_{max} 277 nm (ε 8400), 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, 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)

A 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 7700), 261 (ε 10,700), 227 (ε 22,800). ^1H NMR δ 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 (two 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 7800), 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 (s, 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_2Cl_2–methanol (200:1) to give product **28c** (220 mg, 46%) as a white solid. The *Z*/*E* ratio was 1:1 as determined by ^1H NMR, mp 153–170 °C. UV λ_{max} 311 nm (ε 7900), 231 (ε 29,900). ^1H 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 7000), 232 (ε 28,600). ^1H 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 (ε 6700), 232 (ε 27,000). ^1H 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 (two 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. Calcd for $C_{11}H_{11}ClFN_5O\times0.5H_2O$: 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 (three 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 (two 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 $C_{11}H_{12}FN_5O_2\times0.2H_2O$: 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), 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 $C_{11}H_{12}FN_5O_2\times0.2H_2O$: 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, NJ, USA, 1.5 U/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 155,959–155,981 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 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 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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