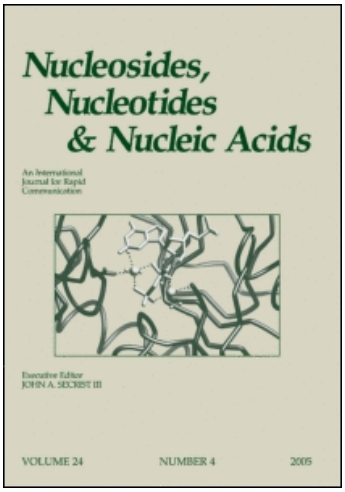

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Short Synthesis And Antiviral Evaluation Of *c*-Fluoro-Branched Cyclopropyl Nucleosides

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To cite this Article Oh, Chang Hyun and Hong, Joon Hee(2007) 'Short Synthesis And Antiviral Evaluation Of *c*-Fluoro-Branched Cyclopropyl Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 26: 4, 403 — 411
To link to this Article: DOI: 10.1080/15257770701297018
URL: http://dx.doi.org/10.1080/15257770701297018

Nucleosides, Nucleotides, and Nucleic Acids, 26:403–411, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701297018

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SHORT SYNTHESIS AND ANTIVIRAL EVALUATION OF *C*-FLUORO-BRANCHED CYCLOPROPYL NUCLEOSIDES

Chang Hyun Oh and Joon Hee Hong □ *Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul, Republic of Korea and BK21 Project Team, College of Pharmacy, Chosun University, Kwangju, Republic of Korea*

□ *A series of novel fluorocyclopropyl nucleosides were synthesized using the Simmons-Smith reaction as a key reaction starting from 1,3-dihydroxyacetone. All the nucleosides synthesized were assayed against several viruses. Among the compounds synthesized, the 5-fluorouracil analogue* ***15*** *showed significant anti-HCMV activity (9.22 μM).*

Keywords Cyclopropyl nucleoside; antiviral agent; Simmons-Smith cyclopropanation

INTRODUCTION

Introduction of a fluorine atom to the carbohydrate moiety of nucleosides was found to confer interesting biological activities, as observed with FLT,[1] L-FMAU,[2] and L-2′-F-d4N.[3] The special properties of the fluorine atom, such a strong electronegativity, small size, and low polarizability of the C-F bond, can have considerable impact on the behavior of a molecule in a biological environment. Therefore, the fluorinated drugs can deeply alter various biological steps: strong hydrogen binding with enzyme or receptor.[4]

The synthesis of designed cyclopropyl nucleoside analogues[5] have been inspired by their interesting biological activities and chemical and enzymatic stability. In particular, Sekiyama et al.[6] prepared tri-substituted cyclopropyl nucleosides with an additional hydroxymethyl group at 1′-position, A-5021 (**1**) along with other congeners. These compounds showed more potent antiviral activity against HSV-1 than acyclovir (ACV) and penciclovir, and comparable activity against VZV (Figure 1).

Received 11 August 2006; accepted 24 January 2007.
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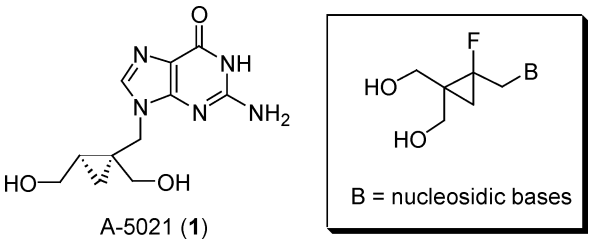
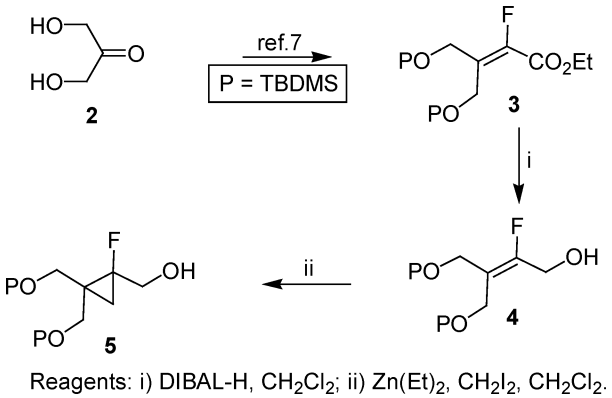


FIGURE 1 The rationale for the design of the desired nucleoside.

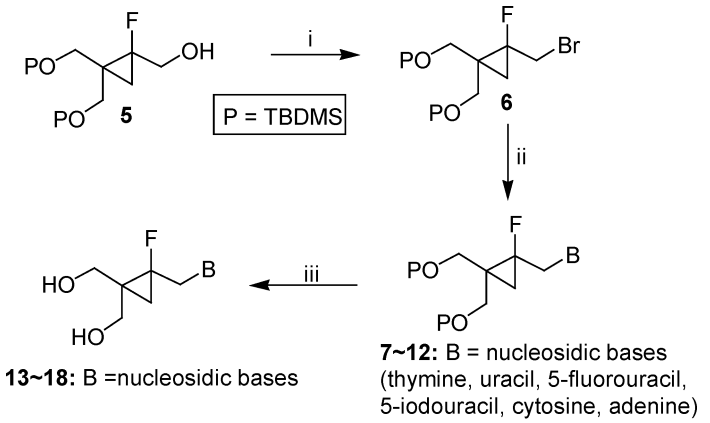
Encouraged by these interesting structures and the antiviral activities of cyclopropyl nucleosides, this study synthesized novel classes of nucleosides containing trisubstituted cyclopropane with an additional fluorine group at the 1′-position and evaluated them against various viruses because fluorine group might act as a hydrogen bonding acceptor at the active site of their target enzyme.

RESULTS AND DISCUSSION

Scheme 1 shows the synthesis of the cyclopropyl compound, which is the key intermediate for the preparation of the trisubstituted cyclopropyl nucleosides. The fluoroester **3** was prepared from commercially available 1,3-dihydroxyacetone using the reported procedure.[7] Compound **3** was subjected to reduction conditions using diisobutylaluminum hydride (DIBAL-H) to provide the fluoroallylic alcohol, which underwent Simmons-Smith reaction[8] with Et_2Zn/CH_2I_2 to give compound **4** in 80% yield. In order to alkylate the sugar moiety by a nucleophilic substitution reaction (S_N2), the allylic alcohol **4** was converted to the allylic bromide **5** in high yield by the sequential addition of NBS to a solution of the alcohol and triphenylphosphine in CH_2Cl_2 (Scheme 2).[9] The condensation of



SCHEME 1 Synthesis of fluorocyclopropane.



SCHEME 2 Synthesis of target nucleosides.

the allylic bromide **6** with various bases (thymine, uracil, 5-fluorouracil, 5-iodouracil, cytosine, adenine) in DMF with cesium carbonate as a basic catalyst (Cs_2CO_3) afforded the nucleoside derivatives **7**∼**12**. The deprotection of the *t*-butyldimethylsilyl group (TBDMS) using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave the desired fluorocyclopropyl nucleosides, **13**∼**18**.

Antiviral assays against several viruses such as HIV-1 (MT-4 cells), HCMV (AD-169), HSV-1, and HSV-2 (CCL-81 cells) were performed. As shown in Table 1, the 5-fluorouracil derivative **15** showed significant activity against anti-HCMV ($EC_{50} = 9.22$ μmol) without showing significant toxicity to the host cell up to 100 μM.

TABLE 1 The antiviral activities of the synthesized compounds

	HIV-1 EC_{50} (μM)	HSV-1 EC_{50} (μM)	HSV-2 EC_{50} (μM)	HCMV EC_{50} (μM)	Cytotoxicity CC_{50} (μM)
13	>100	47.9	>100	>100	>100
14	>100	>100	>100	>100	>100
15	32.56	>100	>100	**9.22**	>100
16	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100
18	67.5	77.97	>100	>100	>100
AZT	0.001	ND	ND	ND	1.5
GCV	ND	ND	ND	0.5	>10
ACV	ND	0.2	ND	ND	>100

ND: not determined.

EC_{50} (μM): concentration required inhibiting 50% of virus-induced cytopathicity.

CC_{50} (μM): concentration required to reduce cell viability by 50%.

In conclusion, novel cyclopropyl nucleosides **13**∼**18** were synthesized starting from 1,3-dihydroxyacetone using the Simmons-Smith reaction as a key step. The fluorouracil derivative **15** showed significant anti-HCMV activity. It is expected that the information obtained in this study will be useful for the development of novel cyclopropyl nucleosides. Studies toward this end as well as those aimed at clarifying the mechanism are currently underway.

MATERIALS AND METHODS

All the chemicals were of reagent grade and were used as purchased. All the moisture-sensitive reactions were carried out in an inert atmosphere with either N_2 or Ar using distilled dry solvents. The melting points were determined using a Mel-temp II laboratory device and were uncorrected. The NMR spectra were recorded on a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan); the chemical shifts are reported in parts per million (δ) and the signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). The UV spectra were obtained using a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). The elemental analysis was carried out using an Elemental Analyzer System (Leco Corp., St. Joseph, MI, USA). The mass spectra were obtained on a Finnigan MAT SSQ 7000 spectrometer (Thermo Electron Corp., Bremen, Germany). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Dry THF was obtained by distillation from Na and benzophenone when the solution became purple.

4-(tert-Butyldimethylsilanyloxy)-3-(tert-butyldimethylsilanyloxymethyl)-2-fluoro-but-2-en-1-ol (4): DIBALH (14.96 mL, 1.0 M solution in hexane) was added slowly to a solution of compound **3** (2.76 g, 6.8 mmol) in CH_2Cl_2 (100 mL) at 0°C with constant stirring. The resulting mixture was stirred for 3 hours at the same temperature, which was followed by the addition of methanol (15 mL). The mixture was then stirred at room temperature for 3 hours to allow for the precipitation of a solid. The resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum, and the residue was purified by silica gel column chromatography (EtOAc/n-hexane, 1:30) to give compound **4** (2.18 mg, 88%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.24–4.15 (m, 6H), 0.87 (m, 18H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 158.31, 154.91, 119.24, 119.08, 63.41, 58.91, 58.43, 56.54, 25.76, 18.28, -5.37; MS (EI) for $C_{17}H_{37}FO_3Si_2$: m/z 365 (M^+).

[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1-fluoro-cyclopropyl]-methanol (5): A diethylzinc solution (1 M in hexanes, 24.67 mL, 24.67 mmol) was added to a solution of compound **4** (2.52 g, 6.92 mmol) in CH_2Cl_2 (50 mL) at −30°C under argon. Diiodomethane (4.46 mL, 55.33 mmol) was

then added and the mixture was stirred for 1 hour at 0°C. The reaction was quenched by adding a saturated NH_4Cl solution. The reaction mixture was extracted with chloroform, and the combined extracts were washed with a saturated NaCl solution, dried ($NaSO_4$), filtered, and evaporated under reduced pressure. The residue was chromatographed on silica gel column eluting with hexane-EtOAc (35:1) to give compound **5** (2.09 g, 80%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.25–4.15 (m, 2H), 3.52–3.29 (m, 3H), 3.01 (dd, J = 11.1, 0.9 Hz, 1H), 1.09 (dd, J = 20.4, 7.5 Hz, 1H), 0.82 (m, 18H), 0.72 (m, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 86.97, 84.01, 64.95, 64.66, 63.75, 63.73, 60.82, 60.69, 33.35, 33.23, 25.71, 19.77, 19.64, 18.18, -5.52; MS (EI) for $C_{18}H_{39}FO_3Si_2$: m/z 379 (M^+).

1-Bromomethyl-2,2-bis-(tert-butyldimethylsilanyloxymethyl)-1-fluoro-cyclopropane (6): *N*-bromosuccinimide (1.84 g, 5.19 mmol) was added slowly to a solution of compound **5** (0.98 g, 2.59 mmol) and triphenylphosphine (1.35 g, 5.19 mmol) in CH_2Cl_2 (20 mL) at 0°C with constant stirring. The resulting mixture was stirred for 5 hours at room temperature, and diluted with CH_2Cl_2. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by quick flash silica gel column chromatography (EtOAc/n-hexane, 1:40) to give the bromide derivative **6** (766 mg, 67%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.88–3.54 (m, 6H), 1.20–0.94 (m, 2H), 0.83 (s, 18H), 0.01 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 84.06, 81.12, 63.52, 61.67, 61.53, 60.71, 54.88, 36.43, 36.32, 34.53, 34.23, 25.82, 19.36, 19.36, 18.22, -5.60; MS (EI) for $C_{18}H_{38}BrFO_2Si_2$: m/z 442 (M^+).

1-[[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1′-fluoro-1′-methyl] cycloprop-1′-yl]thymine (7): A solution of the cyclopropyl bromide derivative **6** (468 mg, 1.06 mmol), thymine (206 mg, 1.61 mmol) and cesium carbonate (523 mg, 1.61 mmol) in anhydrous DMF (10 mL) was stirred overnight at room temperature. The mixture was quenched by adding water and diluted with ethyl acetate. The organic layer was separated and washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc/n-hexane, 4:1) to give compound **7** (201 mg, 39%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (br s, 1H), 7.21 (s, 1H), 4.10–4.02 (m, 4H), 3.41–3.33 (m, 2H), 1.11 (dd, J = 20.2, 7.6 Hz, 1H), 0.88 (s, 18H), 0.75 (dd, J = 10.8, 2.8 Hz, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 164.65, 151.55, 142.91, 109.45, 85.19, 84.21, 63.84, 63.54, 62.61, 62.54, 49.54, 49.76, 32.54, 32.37, 25.23, 19.89, 19.80, 18.61, -5.72; MS (EI) for $C_{23}H_{43}FN_2O_4Si_2$: m/z 488 ($M+1^+$).

1-[[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1′-fluoro-1′-methyl] cycloprop-1′-yl]uracil (8): The uracil derivative was prepared from compound **6** using a similar procedure as that described for compound **7**:

yield 33%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (br s, 1H), 7.25 (d, J = 7.2 Hz, 1H), 5.49 (d, J = 7.2 Hz, 1H), 4.15–4.09 (m, 4H), 3.32–3.21 (dd, J = 16.6, 6.8 Hz, 2H), 1.05 (dd, J = 18.6, 7.8 Hz, 1H), 0.87 (m, 18H), 0.70 (m, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 164.03, 152.12, 146.23, 102.65, 84.32, 83.51, 63.69, 63.51, 62.97, 62.87, 48.51, 48.43, 33.18, 33.04, 25.79, 19.63, 19.77, 18.65, -5.51; MS (EI) for $C_{22}H_{41}FN_2O_4Si_2$: m/z 474 ($M+1^+$).

1-[[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1′-fluoro-1′-methyl]-cycloprop-1′-yl]5-fluorouracil (9): The 5-fluorouracil derivative was obtained from compound **6** using a similar procedure as that described for compound **7**: yield 29%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.89 (br s, 1H), 7.52 (d, J = 5.2 Hz, 1H), 4.18–4.08 (m, 4H), 3.19–3.10 (m, 2H), 1.00 (dd, J = 16.8, 7.2 Hz, 1H), 0.86 (s, 18H), 0.73 (dd, J = 10.2, 2.4 Hz, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 161.76, 161.50, 150.45, 142.78, 139.45, 125.76, 125.68, 85.18, 85.03, 63.56, 62.64, 62.54, 47.65, 47.56, 33.67, 33.49, 25.69, 19.29, 19.13, 18.91, -5.70; MS (EI) for $C_{22}H_{40}F_2N_2O_4Si_2$: m/z 492 ($M+1^+$).

1-[[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1′-fluoro-1′-methyl]-cycloprop-1′-yl]5-iodouracil (10): The 5-iodouracil derivative was synthesized from compound **6** using a similar procedure to that described for compound **7**: yield 32%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (br s, 1H), 7.49 (s, 1H), 4.28–4.17 (m, 4H), 3.16–3.07 (dd, J = 16.8, 6.8 Hz, 2H), 1.06 (m, 1H), 0.89 (m, 18H), 0.75 (dd, J = 12.6, 2.8 Hz, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 166.82, 152.10, 147.32, 84.76, 69.43, 63.76, 63.61, 62.43, 62.31, 47.89, 34.01, 33.90, 25.71, 19.47, 18.67, -5.47; MS (EI) for $C_{22}H_{40}FIN_2O_4Si_2$: m/z 600 ($M+1^+$).

1-[[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1′-fluoro-1′-methyl]-cycloprop-1′-yl]cytosine (11): The cytosine derivative was synthesized from compound **6** using a similar procedure to that described for compound **7**: yield 26%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, J = 7.6 Hz, 1H), 5.77 (d, J = 7.4 Hz, 1H), 4.20–4.10 (m, 4H), 3.08–2.99 (dd, J = 18.0, 7.2 Hz, 2H), 1.13 (m, 1H), 0.87 (m, 18H), 0.73 (dd, J = 10.8, 2.6 Hz, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 165.45, 155.78, 145.71, 93.84, 84.34, 84.21, 63.29, 63.06, 62.32, 48.51, 48.39, 33.29, 33.13, 25.65, 19.56, 19.41, 18.62, -5.50; MS (EI) for $C_{22}H_{42}FN_3O_3Si_2$: m/z 473 ($M+1^+$).

9-[[2,2-Bis-(tert-butyldimethylsilanyloxymethyl)-1′-fluoro-1′-methyl]-cycloprop-1′-yl]adenine (12): The cytosine derivative was prepared from compound **6** using a similar procedure to that described for compound **7**: yield 30%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.82 (s, 1H), 6.08 (br d, 2H), 4.27–4.19 (m, 4H), 3.15–3.06 (dd, J = 18.6, 7.4 Hz, 2H), 1.09 (m, 1H), 0.86 (m, 18H), 0.71 (m, 1H), 0.02 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 156.01, 152.76, 150.72, 142.59, 118.45, 88.87, 88.71, 63.67, 63.54, 62.81, 62.77, 48.76, 33.87, 33.64, 25.53, 19.78, 18.49, -5.57; MS (EI) for $C_{23}H_{42}FN_5O_2Si_2$: m/z 497 ($M+1^+$).

1-[[2,2-Bis-(hydroxymethyl)-1′-fluoro-1′-methyl]cycloprop-1′-yl]thymine (13): TBAF (2.21 mL, 1.0 M solution in THF) at 0°C was added to a solution of compound **7** (326 mg, 0.67 mmol) in THF (10 mL). The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:4) to give compound **13** (145 mg, 84%) as a white solid: m.p. 156–158°C; UV (H_2O) λ_{max} 268.0 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.45 (br s, 1H), 7.35 (s, 1H), 5.01 (m, 2H), 4.21–4.12 (m, 4H), 3.24–3.13 (dd, J = 16.8, 6.8 Hz, 1H), 2H), 1.08 (m, 1H), 0.72 (t, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 165.45, 152.12, 141.21, 108.54, 84.38, 63.72, 63.59, 62.32, 62.17, 48.76, 33.81, 33.78, 19.61; MS (EI): m/z 258 (M^+); Anal calc for $C_{11}H_{15}FN_2O_4$: C, 51.16; H, 5.85; N, 10.85. Found: C, 51.27; H, 5.71, N, 10.75.

1-[[2,2-Bis-(hydroxymethyl)-1′-fluoro-1′-methyl]cycloprop-1′-yl]uracil (14): The uracil nucleoside was synthesized from compound **8** using a similar procedure to that described for compound **13**: yield 79%; m.p. 159–162°C; UV (H_2O) λ_{max} 261.5 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.51 (br s, 1H), 7.42 (d, J = 7.4 Hz, 1H), 5.51 (d, J = 7.2 Hz, 1H), 4.98 (t, J = 5.2 Hz, 1H), 4.89 (t, J = 5.2 Hz, 1H), 4.21–4.15 (m, 4H), 3.19–3.10 (m, 2H), 1.01 (m, 1H), 0.74 (t, J = 12.4, 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 164.21, 151.32, 145.76, 103.65, 83.65, 83.49, 63.76, 63.55, 62.43, 47.76, 47.60, 33.76, 19.65, 19.51; MS (EI): m/z 245 ($M+1^+$); Anal calc for $C_{10}H_{13}FN_2O_4$: C, 49.18; H, 5.37; N, 11.47. Found: C, 49.30; H, 5.25, N, 11.39.

1-[[2,2-Bis-(hydroxymethyl)-1′-fluoro-1′-methyl]cycloprop-1′-yl]5-fluorouracil (15): The 5-Fluorouracil derivative was obtained from compound **9** using a similar procedure to that described for compound **13**: yield 80%; m.p. 164–166°C; UV (H_2O) λ_{max} 272.5 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.51 (br s, 1H), 7.49 (d, J = 5.4 Hz, 1H), 4.96 (t, J = 5.4 Hz, 1H), 4.82 (t, J = 5.2 Hz, 1H), 4.28–4.19 (m, 4H), 3.35–3.26 (m, 2H), 1.07 (m, 1H), 0.76 (t, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 162.54, 162.36, 151.65, 151.50, 141.32, 141.19, 124.62, 124.44, 85.62, 85.54, 63.78, 63.41, 62.72, 48.81, 48.73, 33.91, 33.80, 19.12, 19.01; MS (EI): m/z 263 ($M+1^+$); Anal calc for $C_{10}H_{12}F_2N_2O_4(+0.2H_2O)$: C, 45.18; H, 4.89; N, 10.54. Found: C, 45.37; H, 4.50, N, 10.59.

1-[[2,2-Bis-(hydroxymethyl)-1′-fluoro-1′-methyl]cycloprop-1′-yl]5-iodouracil (16): The 5-iodouracil derivative was synthesized from compound **10** using a similar procedure to that described for compound **13**: yield 83%; m.p. 160–163°C; UV (H_2O) λ_{max} 283.5 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.60 (br s, 1H), 7.42 (s, 1H), 5.03 (br s, 1H), 4.0 (t, J = 5.2 Hz, 1H), 4.20–4.11 (m, 4H), 3.21–3.15 (m, 2H), 1.12 (m, 1H), 0.79 (dd, J = 12.8, 2.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 166.71, 151.49, 146.21, 84.66, 84.49, 69.43, 63.32, 62.65, 48.38, 48.29, 33.65, 33.52, 19.47, 19.34; MS (EI): m/z 370 (M^+); Anal calc for $C_{10}H_{12}FIN_2O_4$: C, 32.45; H, 3.27; N, 7.57. Found: C, 32.28; H, 3.16, N, 7.40.

1-[[2,2-Bis-(hydroxymethyl)-1′-fluoro-1′-methyl]cycloprop-1′-yl]cytosine (17): The cytosine derivative was prepared from compound **11** using a similar procedure to that described for compound **13**: yield 77%; m.p. 159–161°C; UV (H_2O) λ_{max} 270.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.38 (d, $J = 7.4$ Hz, 1H), 5.64 (d, $J = 7.4$ Hz, 1H), 4.93 (t, $J = 5.2$ Hz, 1H), 4.80 (t, $J = 5.$ 4 Hz, 1H), 4.22–4.15 (m, 4H), 3.15–2.09 (m, 2H), 1.03 (m, 1H), 0.71 (dd, $J = 14.2$, 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 165.81, 154.34, 145.82, 92.27, 83.65, 63.29, 63.13, 62.12, 61.99, 48.86, 33.76, 19.78, 19.67; MS (EI): m/z 244 ($M+1^+$); Anal calc for $C_{10}H_{14}FN_3O_3$(+0.5MeOH): C, 48.64; H, 6.22; N, 16.21. Found: C, 48.52; H, 5.99, N, 16.41.

9-[[2,2-Bis-(hydroxymethyl)-1′-fluoro-1′-methyl]cycloprop-1′-yl]adenine (18): The adenine derivative was obtained from compound **12** using a similar procedure to that described for compound **13**: yield 76%; m.p. 181–183°C; UV (H_2O) λ_{max} 263.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.19 (s, 1H), 8.04 (s, 1H), 7.24 (br s, 2H), 4.91 (t, $J = 5.2$ Hz, 1H), 4.88 (t, $J = 5.4$ Hz, 1H), 4.30–4.21 (m, 4H), 3.11–3.01 (m, 2H), 1.02 (m, 1H), 0.70 (dd, $J = 12.6$, 2.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 155.65, 151.76, 150.11, 141.76, 119.21, 89.09, 63.87, 62.79, 62.65, 48.91, 48.80, 34.05, 19.43, 19.21; MS (EI): m/z 268 ($M+1^+$); Anal calc for $C_{11}H_{14}FN_5O_2$(+0.5H_2O): C, 47.82; H, 5.47; N, 25.35. Found: C, 47.68; H, 5.38, N, 25.13.

Anti-HCMV Activity Test

The HEL cells in the stationary phase were infected with the virus at a multiplicity of infection of 2–4 $CCID_{50}$ per well of 96-well plates. After 2 hours adsorption at 37°C, the liquid was aspirated off to remove the unabsorbed viruses, and 100 μL of MEM/2% FBS containing a compound was applied to each well in duplicate for each concentration and incubated for further 6 days. The antiviral activity was measured either microscopically or fluorometrically. For the microscopical observations, the cells were fixed with 70% ethanol, stained with a 2.5% Giemsa solution for 2 hours, rinsed with distilled water and then air-dried. The antiviral activity is expressed as the EC_{50}, or the concentration required to inhibit the virus-induced CPE by 50%. The EC_{50} values were estimated from the semi logarithmic graphic plots of the percentage of CPE as a function of the concentration of the test compound used. For the fluorometric assay, the cells were washed twice with 100 μL of phosphate-buffered saline (PBS). 100 μL of 5 μg/mL fluorescein diacetate (FDA, Sigma, St. Louis, MO, USA) was added to each well and the plates were incubated for 30 minutes at 37°C. The FDA solution was removed by aspiration and each well was washed with 100 μL PBS. The fluorescence intensity (as absolute fluorescent units, AFU) in each well was measured using a fluorescent microplate reader equipped with a 485-nm excitation and 538-nm emission filter.

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