Methylene−*gem*-Difluorocyclopropane Analogues of Nucleosides: Synthesis, Cyclopropene−Methylenecyclopropane Rearrangement, and Biological Activity[1]

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Alkylation−elimination of adenine and 2-amino-6-chloropurine with *gem*-difluorocyclopropane dibromide **10** gave *E*- and *Z*-methylene−*gem*-difluorocyclopropanes **11a**, **11b**, **12a**, and **12b** and *gem*-difluorocyclopropenes **13a** and **13b**. Debenzylation of intermediates **11a**, **11b**, **12a**, and **12b** afforded *E*- and *Z*-methylenecyclopropanes **4a**, **4b**, **5a**, and **5b**. Hydrolysis of 2-amino-6-chloropurine derivatives **4b** and **5b** afforded guanine analogues **4c** and **5c**. Composition of products (except **14b**) obtained from alkylation−elimination reflects thermodynamically controlled cyclopropene−methylenecyclopropene rearrangement. The *E*-isomer **4a** was moderately active against human cytomegalovirus and along with the *Z*-isomer **5a** was active against leukemia L1210 and solid tumors in vitro.

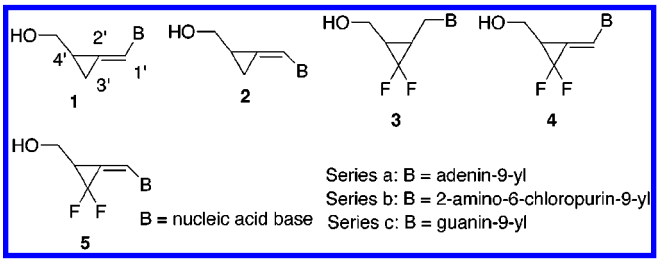
Introduction

Recently, we described a series of methylenecyclopropane analogues of nucleosides **1** and **2** (Chart 1). The *Z*-isomers **1** are broad-spectrum antiviral agents especially effective against human cytomegalovirus (HCMV).[2,3] Replacing hydrogen with fluorine led in many cases to biologically active compounds.[4] Among nucleosides fluorinated in the carbohydrate moiety,[5] *gem*-difluoro analogues play a significant role. The anticancer drug gemcitabine[6] (2′-deoxy-2′,2′-difluorocytidine) and the acyclic nucleotide 9-(5,5-difluoro-5-phosphonopentyl)guanine, an inhibitor of purine nucleoside phosphorylase,[7] are notable examples. More recently, we described[8] also *gem*-difluorocyclopropane nucleoside analogues **3**. In view of a potent antiviral activity of methylenecyclopropanes **1**, structure−activity relationships in this series of analogues are under active investigation.[9,10] As a part of this effort, *gem*-difluoro analogues **4** and **5** have become of interest.

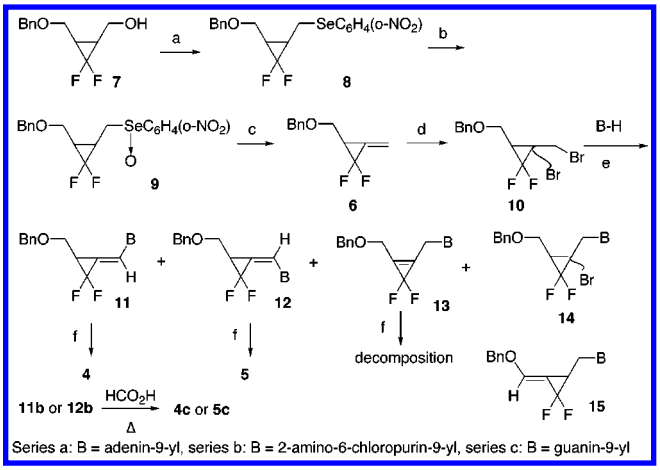
Results and Discussion

The alkylation−elimination approach that was successfully applied for synthesis of nonfluorinated analogues[2,3] **1** and **2** seemed the most promising for synthesis of **4** and **5**. The starting methylene−*gem*-difluorocyclopropane (**6**) was prepared from *gem*-difluorocyclopropane[8] **7** by a modification of the described procedure[11] via selenide **8** and selenoxide **9** (Scheme 1). **6** was converted to the vicinal dibromocyclopropane **10** in 95% yield using pyridine·HBr_3 in CH_2Cl_2. Alkylation−elimination of sodium salt of adenine with **10** in DMF was perfomed at room temperature. A mixture of three products was obtained, the *E*,*Z*-isomers **11a** and **12a** (21 and 7%, respectively) and, surprisingly, *gem*-difluorocyclopropene **13a** (31%). All three products were readily separated by chromatography on a silica gel column. Debenzylation of **11a** and **12a** using BCl_3 in CH_2Cl_2 at −78 °C was uneventful to give target analogues **4a** and **5a** in 75 and 77% yield, respectively. An

Chart 1



Scheme 1



[a] Key: (a) (o-NO_2)C_6H_4SeCN, Bu_3P, THF; (b) H_2O_2, THF; (c) toluene, Δ; (d) pyridine·HBr_3, CH_2Cl_2; (e) NaH, DMF; (f) BCl_3, CH_2Cl_2, −78 °C.

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Table 1. Antitumor Activity of Methylenecyclopropanes **1a**, **4a**, and **5a**

		mouse tumors		human colon tumors		
compd[a]	leukemia L1210	C38	M17/Adr	H15	H116	normal cells (fibroblasts)
1a	500−570	550−600	460		0−160	0−130
4a	340−420	470	370	100−300	70−200	0−130
5a	600−660	580	590	400−550	550	200−500
SR271425[b]	0−190	650−750	0−50	60−150	360	0−110

[a] Disk-diffusion assay,[14] 500 μg/disk, 200 units = 6 mm. [b] 11 μg/disk.[14]

attempted deprotection of cyclopropene analogue **13a** led only to decomposition.

Alkylation−elimination of 2-amino-6-chloropurine with reagent **10** performed in a similar fashion (NaH in DMF at room temperature) gave a mixture of four compounds that were all separated by chromatography on silica gel. The yields of the *E*- and *Z*-isomers **11b** and **12b** and cyclopropene **13b** were 12, 3.5, and 17%. The product of a simple alkylation, bromo derivative **14b**, was also obtained in 10% yield. Debenzylation of **11b** and **12b** gave compounds **4b** and **5b** in 81 and 77% yield, respectively. Hydrolysis with 80% HCO_2H afforded guanine analogues **4c** (66%) and **5c** (71%).

The UV spectra of methylene−*gem*-difluorocyclopropanes reveal significant bathochromic shift throughout the entire region of spectrum when compared with nonfluorinated analogues.[2]

The *E*,*Z*-isomeric structures were assigned by NMR spectra. The H_8 values of the isomers with the heterocyclic base juxtaposed to hydroxymethyl group (*E*-isomers **4a**−**c** and *Z*-isomers **1a**−**c**) are significantly deshielded relative to those of the *Z*-isomers **5a**−**c** and the *E*-isomers **2a**−**c**. A selective deshielding of the $H_{1'}$ alkene proton by fluorine in the cis position was observed only in the *E*-isomers **4a**−**c**. The absence of alkene protons as well as the presence of three different methylene groups excluded structures **15a** and **15b** as possible alternatives for cyclopropenes **13a** and **13b**. These assignments were confirmed by the NOE experiments with isomers **4a** and **5a**. Thus, all protons of the cyclopropane moiety of *E*-isomer **4a** located in the vicinity of H_8 exhibit NOE enhancements whereas none were found for a more distant $H_{1'}$. An opposite situation was encountered in the *Z*-isomer **5a**. In this case, all cyclopropane protons are closer to $H_{1'}$ than to H_8, and consequently, NOE was observed at $H_{1'}$ but not at H_8.

Because the *E*- and *Z*-isomers **4a** and **4c** and **5a** and **5c** were the main targets of our study, we investigated a possibility of isomerization of cyclopropene **13b** into the corresponding methylenecyclopropanes. Base-catalyzed cyclopropene−methylene−cyclopropane rearrangements have been described in both nonfluorinated[12] and *gem*-difluorocyclopropane series.[13] Isomerization of cyclopropene **13b** using DBU in DMF at 0 °C (2 min) gave *E*-isomer **11b** (37%), *Z*-isomer **12b** (11.5%), and starting material **13b** (39%) that were separated by chromatography on a silica gel column. The ratio of these components resembled that obtained by alkylation−elimination of nucleic acid bases (Scheme 1). The result indicated that isomerization of cyclopropene **13b** to the *E*- and *Z*-isomers **11b** and **12b** is possible but only to a limited extent.

Interconversion of all three components **11b**, **12b**, and **13b** (catalysis with DBU in DMF at room temperature) was investigated by HPLC. After 1 min, the average ratio **11b**:**12b**:**13b** = 3.6:1:5.4 resembled that found in the above-mentioned preparative experiment with cyclopropene **13b**. This ratio was not changed after a prolonged reaction time or when excess of DBU was used, but decomposition of the products gradually occurred. Thus, the observed composition of products is thermodynamically controlled.

The shelf life of methylene−*gem*-difluorocyclopropanes **4a**−**c** and **5a**−**c** is limited, and a prolonged storage at low temperatures is mandatory. The least stable are the guanine analogues **4c** and **5c** where satisfactory elemental analyses could not be obtained although both compounds were fully characterized by spectroscopic methods.

Biological Activity

Analogues **4a**, **4c**, **5a**, and **5c** were tested against HCMV, HSV-1, HSV-2, EBV, HBV, VZV, and HIV-1 in previously described assays.[2] Only the *E*-isomer **4a** had activity against the Towne strain of HCMV propagated in human foreskin fibroblast (HFF) cells (EC_{50} 21 μM), and it was non-cytotoxic (CC_{50} >100 μM). More moderate effects were observed against herpes simplex virus type 1 (HSV-1, EC_{50} 70 μM) in BSC-1 cells as determined by ELISA assay and EBV in Daudi cells (EC_{50} 77 μM). A decreased antiviral potency relative to that of nonfluorinated analogue[2] **1a** can be explained by a limited capability of phosphorylation inside the infected cells. Lesser chemical stability of **4a** may also be a factor. **4a** and **5a** also exhibited antitumor activity (Table 1). The *E*-isomer **4a** was more selective than *Z*-isomer **5a**. The activity of **4a** corresponded to that of the nonfluorinated analogue **1a**, but unlike **5a**, *E*-isomer **2a** was inactive. In contrast to **1a** and **2a**, compounds **4a** and **5a** were not substrates for adenosine deaminase.

Experimental Section

General Methods. See ref 2. The UV spectra were recorded in ethanol. The NMR spectra were determined at 300 or 400 MHz (^{1}H), 75 or 100 MHz (^{13}C), and 376 MHz (^{19}F) in DMSO-d_6 unless specified otherwise. $CFCl_3$ was used as a reference for ^{19}F spectra. For FAB-MS, the thioglycerol matrix was used.

2-Benzyloxymethyl-3,3-difluoro-1-methylenecyclopropane (6). The described procedure[11] starting from *gem*-difluorocyclopropane[8] **7** was modified as follows. Selenide **8** (4.4 g, 10.4 mmol) was treated with 30% H_2O_2 (10 mL) in THF (50 mL) at 0 °C. The reaction mixture was allowed to stand at room temperature for 16 h, and then it was diluted with EtOAc (100 mL) and washed with saturated $Na_2S_2O_3$ solution and water. The organic phase was dried over Na_2SO_4, and the solvent was evaporated to give a crude selenoxide **9** as a yellow solid. A solution of **9** in toluene (50 mL) was heated at 80 °C for 48 h, and the solvent was evaporated. The residue was chromatographed on a silica gel column using hexane−EtOAc (97:3) to give product **6** (1.56 g, 71%) as a colorless oil. The ^{1}H, ^{13}C, and ^{19}F NMR spectral data corresponded to those reported.[11]

(*E,Z*)-2-Benzyloxymethyl-1-bromo-1-bromomethyl-3,3-difluorocyclopropane (10). A solution of **6** (1.05 g, 5.0 mmol) in CH_2Cl_2 (50 mL) was treated with pyridine hydrobromide perbromide (2.56 g, 8 mmol) at 0 °C. The reaction mixture was allowed to stand at room temperature for 16 h. It was diluted with EtOAc (100 mL) and washed with saturated $Na_2S_2O_3$−$NaHCO_3$ solution and water. The organic phase was dried over Na_2SO_4, and the solvent was evaporated to give a pale yellow liquid **10** (1.76 g, 95.1%), which was used directly in the next step. ^{1}H NMR ($CDCl_3$) (δ): 1.96−2.03 and 2.42−2.49 (m, 1H), 3.61−3.85 (m, 4H), 4.50−4.58 (m, 2H), 7.30−7.39 (m, 5H).

(*E*)-9-{[2-(Benzyloxymethyl)-3,3-difluorocyclopropylidene]methyl}adenine (11a), (*Z*)-9-{[2-(Benzyloxymethyl)-3,3-difluorocyclopropylidene]methyl}adenine (12a), and 9-{[2-(Benzyloxymethyl)-3,3-difluorocyclopropenyl]methyl}adenine (13a). A mixture of adenine (1.485 g, 11 mmol) and NaH (60% oil suspension, 600 mg, 12.5 mmol) in DMF (100 mL) was stirred at room temperature for 4 h. The mixture was cooled to 0 °C, a solution of dibromide **10** (1.76 g, 4.76 mmol) in DMF (5 mL) was added dropwise, and the stirring was continued at room temperature for 16 h. The solvent was evaporated in vacuo, and the residue was chromatographed on a silica gel column (CH_2Cl_2−MeOH, 49:1) to give the products **11a** (350 mg, 21.4%), **12a** (120 mg, 7.4%), and **13a** (510 mg, 31.2%) as white solids.

11a: mp 254−255 °C; UV max 284 nm (ϵ 6,000), 245 (ϵ 24,100). ^{1}H NMR (δ): 3.23−3.28 (1H, m), 3.60 (1H, dd, J = 9.3 and 9.0 Hz), 3.85−3.91 (1H, m), 4.49 (1H, d, J = 12.3 Hz), 4.54 (1H, d, J = 12.3 Hz), 7.24−7.32 (5H, m), 7.50 (2H, s), 8.20 (1H, s), 8.25 (1H, d, J = 3.0 Hz), 8.58 (s, 1H). ^{13}C NMR: 31.0 (t, J = 12.0 Hz), 66.5, 72.7, 107.5 (t, J = 7.1 Hz), 108.0 (t, J = 285.3 Hz), 119.2, 119.4, 128.1, 128.2, 128.9, 138.5, 139.0, 149.3, 154.3, 156.8. ^{19}F NMR: 65.5 (d, J = 174.1 Hz), 77.8 (dd, J = 174.9, 6.0 Hz). EI-MS: 343 (M, 0.7), 342 (M − H, 1.1), 91 (100.0). HRMS calcd. for $C_{17}H_{14}F_2N_5O$ (M − H) 342.1166, found: 342.1164. Anal. ($C_{17}H_{15}F_2N_5O$) C, H, N.

12a: mp 232−234 °C; UV max 284 nm (ϵ 6,700), 245 (ϵ 24,200). ^{1}H NMR (δ): 3.02−3.07 (1H, m), 3.52 (1H, dd, J = 10.5, 9.6 Hz), 3.71−3.78 (1H, m), 4.54 (2H, s) 7.26−7.35 (5H, m), 7.48 (2H, s), 7.70 (1H, d, J = 2.1 Hz), 8.19 (1H, s), 8.23 (1H, s). ^{13}C NMR: 28.4 (t, J = 12.1 Hz), 66.2, 72.4, 106.7 (t, J = 247.0 Hz), 107.6 (t, J = 7.8 Hz), 118.3, 119.4, 128.3, 129.0, 138.7, 139.0, 149.1, 154.5, 156.8. ^{19}F NMR: 66.8 (d, J = 172.6 Hz), 79.2 (d, J = 173.7 Hz). EI-MS: 343 (M, 0.5), 342 (M − H, 1.0), 91 (100.0). HRMS calcd. for $C_{17}H_{14}FN_5O$ (M − HF) 323.1182, found: 323.1179. Anal. ($C_{17}H_{15}F_2N_5O$) C, H, N, F.

13a: mp 195−196 °C; UV max 261 nm (ϵ 12,500), 209 (ϵ 22,500). ^{1}H NMR (δ): 4.30 (2H, s), 4.33 (2H, s), 5.45 (2H, s), 7.12−7.14 (2H, m), 7.24−7.32 (3H, m), 7.37 (2H, s), 8.17 (1H, s), 8.21 (1H, s). ^{13}C NMR: 37.6, 61.7, 72.5, 103.6 (t, J = 269.3 Hz), 119.3, 127.3 (t, J = 11.0 Hz), 128.3, 128.4, 129.0, 129.2 (t, J = 11.0 Hz), 137.7, 141.2, 150.2, 153.5, 156.7. ^{19}F NMR: 99.7. FAB-MS: 344 (M + H, 100.0). Anal. ($C_{17}H_{15}F_2N_5O$) C, H, N, F.

(*E*)-9-{[2-(Hydroxymethyl)-3,3-difluorocyclopropylidene]methyl}adenine (4a). Boron trichloride (1 M in CH_2Cl_2, 5.8 mL, 5.8 mmol) was added to a solution of **11a** (200 mg, 0.58 mmol) in CH_2Cl_2 (50 mL) at −78 °C under N_2 over 10 min with stirring. The stirring was continued for 5 h at −78 °C. The reaction was quenched by a cautious addition of MeOH (10 mL), $NaHCO_3$ (5 g) was then added, and the mixture was stirred at room temperature for 4 h. The insoluble portion was filtered off, and it was washed with CH_2Cl_2−MeOH (4:1, 100 mL). The combined organic phase was evaporated, and the residue was chromatographed on a silica gel column (CH_2Cl_2−MeOH, 20:1) to give a white solid **4a** (110 mg, 74.8%), mp 268−270 °C; UV max 283 nm (ϵ 6,800), 243 (ϵ 27,300), 214 (ϵ 18,000). ^{1}H NMR (δ): 3.05−3.15 (1H, m), 3.62−3.68 (1H, m), 3.71−3.78 (1H, m), 5.39 (1H, t, J = 5.6 Hz), 7.48 (2H, s), 8.19 (1H, d, J = 2.4 Hz), 8.21 (1H, s), 8.68 (1H, s). ^{13}C NMR: 33.4 (t, J = 11.0 Hz), 58.2, 108.2 (t, J = 285.4 Hz), 108.3 (t, J = 7.0 Hz), 118.6, 119.2, 138.9, 149.3, 154.3, 156.8. ^{19}F NMR: 64.7 (d, J = 170.7 Hz), 78.2 (dd, J = 170.7 and 7.5 Hz). EI-MS: 253 (M, 41.9), 252 (M − H, 10.9), 236 (M − OH, 100.0). HRMS calcd. for $C_{10}H_9F_2N_5O$ 253.0775, found: 253.0781. Anal. ($C_{10}H_9F_2N_5O$) C, H, N.

(*Z*)-9-{[2-(Hydroxymethyl)-3,3-difluorocyclopropylidene]methyl}adenine (5a). The reaction was performed as described for **4a** (60 mg, 0.17 mmol of **12a**) to give a white solid **5a** (33 mg, 76.7%), mp 256−260 °C; UV max 283 nm (ϵ 5,700), 243 (ϵ 22,200), 214 (ϵ 14,100). ^{1}H NMR: 2.82−2.90 (1H, m), 3.44−3.53 (1H, m), 3.63−3.70 (1H, m), 5.24 (1H, brs), 7.48 (2H, s), 7.64 (1H, d, J = 2.0 Hz), 8.16 (1H, s), 8.22 (1H, s). ^{13}C NMR: 31.2 (t, J = 11.0 Hz), 58.2, 107.1 (t, J = 286.4 Hz), 108.4 (t, J = 6.0 Hz), 117.5, 119.4, 138.8, 149.1, 154.5, 156.8. ^{19}F NMR: 65.4 (d, J = 175.2 Hz), 79.3 (dd, J = 175.4, 6.3 Hz). EI-MS 253 (M, 12.3), 252 (M − H, 6.7), 236 (M − OH, 100.0). HRMS calcd. for $C_{10}H_9F_2N_5O$ 253.0775, found: 253.0781. Anal. ($C_{10}H_9F_2N_5O$) C, H, N.

(*E*)-2-Amino-9-{[2-(benzyloxymethyl)-3,3 difluorocyclopropylidene]methyl}-6-chloropurine (11b), (*Z*)-2-Amino-9-{[2-(benzyloxymethyl)-3,3-difluorocyclopropylidene]-methyl}-6-chloropurine (12b), 2-Amino-9-{[2-(benzyloxymethyl)-3,3-difluorocyclopropenyl]methyl}-6-chloropurine (13b), and (*E,Z*)-2-Amino-9-{[1-bromo-2-(benzyloxymethyl)-3,3-difluorocyclopropyl]methyl}-6-chloropurine (14b). The procedure described for adenine analogues was followed with 2-amino-6-chloropurine (4.25 g, 25 mmol), NaH (1.08 g, 22.5 mmol) in DMF (200 mL), and dibromide **10** (2.81 g, 8.00 mmol). The products were separated by column chromatography on silica gel using hexane−EtOAc (3:1) to give products **11b** (350 mg, 11.6%), **12b** (105 mg, 3.5%), **14b** (350 mg, 9.6%), and **13b** (510 mg, 16.9%).

11b: white solid, mp 138−139 °C; UV max 308 nm (ϵ 7,800), 285 (ϵ 8,100), 240 (ϵ 26,000), 205 (ϵ 21,500). ^{1}H NMR ($CDCl_3$ δ): 2.85−2.96 (1H, m), 3.51−3.58 (1H, m), 3.88 (1H, ddd, J = 9.6, 6.0 and 1.8 Hz), 4.57 (2H, AB, J = 12.9 and 12.9 Hz), 5.36 (2H, s, 7.27−7.36 (5H, m), 7.91 (1H, d, J = 3.0 Hz), 8.78 (1H, s). ^{13}C NMR: 30.4 (t, J = 12.0 Hz), 66.0, 73.7, 105.8 (t, J = 285.3 Hz), 108.5 (t, J = 8.1 Hz), 117.1, 125.2, 128.2, 128.4, 128.8, 137.2, 139.8, 152.0, 153.0, 159.8. ^{19}F NMR: 60.6 (dd, J = 175.6 and 3.0 Hz), 72.2 (dd, J = 175.4 and 9.4 Hz). EI-MS: 379 (M, 1.2), 377 (M, 3.1), 91 (100.0). HRMS calcd. for $C_{17}H_{14}{}^{35}ClF_2N_5O$ 377.0855, found: 377.0856. Anal. ($C_{17}H_{14}ClF_2N_5O$) C, H, N, Cl, F.

12b: white solid, mp 120−122 °C; UV max 307 nm (ϵ 7,900), 284 (ϵ 7,700), 241 (ϵ 24,700), 206 (ϵ 22,900). ^{1}H NMR ($CDCl_3$ δ): 2.85−2.92 (1H, m), 3.68 (2H, d, J = 7.2 Hz), 4.54 (1H, d, J = 11.7 Hz), 4.60 (1H, d, J = 12.3 Hz), 5.64 (2H, s), 7.27−7.35 (6H, m), 7.97 (1H, s). ^{13}C NMR: 29.0 (t, J = 12.0 Hz), 65.8, 73.2, 105.6 (t, J = 288.4 Hz), 108.7 (t, J = 7.1 Hz), 116.0, 125.2, 128.0, 128.2, 128.8, 137.7, 139.2, 152.1, 152.5, 160.1. ^{19}F NMR: 60.1 (d, J = 178.6 Hz), 72.2 (dd, J = 176.9 and 7.5 Hz). EI-MS: 379 (M, 0.7), 377 (M, 1.6), 91 (100.0). HRMS calcd. for $C_{17}H_{14}{}^{35}ClF_2N_5O$ 377.0855, found: 377.0851.

13b: white solid, mp 133−134 °C; UV max 310 nm (ϵ 8,000), 247 (ϵ 7,400), 220 (ϵ 28,200). ^{1}H NMR: 4.26 (2H, s), 4.33 (2H, s), 5.34 (2H, s), 7.00 (2H, s), 7.08−7.10 (2H, m), 7.24−7.32 (3H, m), 8.17 (1H, s). ^{13}C NMR: 37.8, 61.8, 72.5, 103.5 (t, J = 269.3 Hz), 123.9, 127.0 (t, J = 12.0 Hz), 128.1, 128.4, 129.0, 129.4 (t, J = 12.0 Hz), 137.7, 143.4, 150.2, 154.7, 160.6. ^{19}F NMR: 99.9. EI-MS: 379 (M, 1.5), 377 (M, 4.0), 91 (100.0). HRMS calcd. for $C_{17}H_{14}{}^{35}ClF_2N_5O$ 377.0855, found: 377.0851.

14b: pale yellow solid, mp 51−54 °C; UV max 310 nm (ϵ 7,900), 248 (ϵ 7,300), 221 (ϵ 27,200). ^{1}H NMR ($CDCl_3$ δ): 2.57−2.65 (1H, m), 3.50−3.55 (1H, m), 3.68 (1H, dd, J = 11.4 and 6.4 Hz), 4.37 (1H, d, J = 12.0 Hz), 4.42 (1H, d, J = 12.4 Hz), 4.45 (1H, d, J = 15.2 Hz), 4.63 (1H, d, J = 15.6 Hz), 5.40 (brs, 2H), 7.15−7.17 (2H, m), 7.24−7.32 (3H, m), 7.95 (1H, s). ^{13}C NMR: 32.9 (t, J = 7.8 Hz), 39.3 (t, J = 8.4 Hz), 48.6, 65.9, 73.1, 110.2 (dd, J = 222.0 and 217.0 Hz), 125.0, 127.7, 128.2, 128.7, 137.4, 142.3, 151.7, 154.2, 159.5. ^{19}F NMR: 61.1 (d, J = 161.7 Hz), 64.9 (dd, J = 160.0 and 15.0 Hz). EI-MS: 459 (M, 2.7), 457 (M, 2.1), 91 (100.0). HRMS calcd. for $C_{17}H_{15}{}^{79}Br^{35}ClF_2N_5O$ 457.0117, found: 457.0116.

(*E*)-2-Amino-9-{[2-(hydroxymethyl)-3,3-difluorocyclopropylidene]methyl}-6-chloropurine (4b). This reaction was performed as described for adenine analogue **4a** with **11b**

(360 mg, 0.95 mmol). The crude product was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 30:1) to give a white solid **4b** (220 mg, 80.7%), mp 239−241 °C; UV max 309 nm (ϵ 7,000), 284 (ϵ 7,300), 240 (ϵ 24,000), 204 (ϵ 13,800). ^{1}H NMR (δ): 3.06−3.13 (1H, m), 3.58−3.64 (1H, m), 3.66−3.72 (1H, m), 5.30 (1H, t, J = 5.6 Hz), 7.14 (2H, s), 8.00 (1H, d, J = 2.4 Hz), 8.59 (1H, s). ^{13}C NMR: 33.8 (t, J = 11.9 Hz), 58.3, 108.3 (t, J = 284.8 Hz), 109.2 (t, J = 8.0 Hz), 118.2, 123.9, 140.8, 150.6, 153.6, 161.0. ^{19}F NMR: 64.7 (d, J = 170.7 Hz), 78.2 (dd, J = 171.4 and 7.5 Hz). EI-MS: 289 (M, 16.9), 287 (M, 52.3), 270 (M − OH, 100.0). HRMS calcd. for $C_{10}H_8{}^{35}ClF_2N_5O$ 287.0385, found: 287.0383. Anal. ($C_{10}H_8ClF_2N_5O$) C, H, N, Cl, F.

(*Z*)-2-Amino-9-{[2-(hydroxymethyl)-3,3-difluorocyclopropylidene]methyl}-6-chloropurine (5b). The procedure described above was performed with **12b** (130 mg, 0.35 mmol). The crude product was purified by chromatography (CH_2Cl_2−MeOH, 20:1) to give a white solid **5b** (77 mg, 76.7%), mp 227−228 °C; UV max 307 nm (ϵ 7,700), 284 (ϵ 7,400), 240 (ϵ 24,500), 202 (ϵ 13,200). ^{1}H NMR (δ): 2.85−2.93 (1H, m), 3.48−3.55 (1H, m), 3.62−3.69 (1H, m), 5.23 (1H, brs), 7.07 (2H, s), 7.50 (1H, s), 8.06 (1H, s). ^{13}C NMR: 31.4 (t, J = 11.9 Hz), 58.1, 106.9 (t, J = 287.0 Hz), 108.8 (t, J = 7.3 Hz), 116.8, 123.9, 140.1, 150.7, 153.2, 161.1. ^{19}F NMR: 65.1 (d, J = 175.2 Hz), 79.0 (d, J = 175.2 Hz). EI-MS: 289 (M, 9.6), 287 (M, 28.5), 270 (M − OH, 100.0). HRMS calcd. for $C_{10}H_8{}^{35}ClF_2N_5O$ 287.0385, found: 287.0380. Anal. ($C_{10}H_8ClF_2N_5O$) C, H, N, Cl, F.

(*E*)-9-{[2-(Hydroxymethyl)-3,3-difluorocyclopropylidene]methyl}guanine (4c). A solution of **4b** (210 mg, 0.73 mmol) in formic acid (80%, 30 mL) was heated at 90 °C for 6 h. After cooling, the solution was evaporated, and the residue was dissolved in NH_3/MeOH (20%, 10 mL). The mixture was stirred at room temperature for 30 min. The volatile components were evaporated, and the crude product was crystallized from MeOH−H_2O (1:2, active carbon) to afford a white solid **4c** (180 mg, 92%), mp 304−307 °C (decomp.); UV max 292 nm (ϵ 5,500), 272 (ϵ 7,500), 243 (ϵ 28,100), 213 (ϵ 16,000). ^{1}H NMR (δ): 3.01−3.09 (1H, m), 3.60−3.70 (2H, m), 5.31 (1H, t, J = 5.6 Hz), 6.64 (2H, s), 7.86 (1H, d, J = 2.4 Hz), 8.27 (1H, s), 10.83 (1H, s). ^{13}C NMR: 33.2 (t, J = 11.9 Hz), 58.1, 108.1 (t, J = 6.6 Hz), 108.2 (t, J = 285.7 Hz), 117.1, 118.2, 135.1, 151.1, 155.0, 157.2. ^{19}F NMR: 64.9 (d, J = 170.7 Hz), 78.2 (dd, J = 171.5 and 9.0 Hz). FAB-MS: (+ KCl) 308 (M + K, 6.9), 270 (M + H, 14.8), 253 (M + H − OH, 100.0).

(*Z*)-9-{[2-(Hydroxymethyl)-3,3-difluorocyclopropylidene]methyl}guanine (5c). The procedure described above was performed with **5b** (60 mg, 0.21 mmol) to give a white solid **5c** (40 mg, 71%), mp >350 °C (decomp.); UV max 292 nm (ϵ 5,400), 272 (ϵ 8,000), 244 (ϵ 28,400), 211 (ϵ 19,300). ^{1}H NMR (δ): 2.84−2.93 (1H, m), 3.47−3.53 (1H, m), 3.60−3.68 (1H, m), 5.20 (1H, t, J = 5.6 Hz), 6.60 (2H, s), 7.38 (1H, s), 7.64 (1H, s), 10.83 (1H, s). ^{13}C NMR: 31.5 (t, J = 11.9 Hz), 58.1, 107.2 (t, J = 287.0 Hz), 107.8 (t, J = 6.4 Hz), 117.0, 117.3, 134.0, 150.8, 155.1, 157.2. ^{19}F NMR: 64.1 (d, J = 172.6 Hz), 78.1 (dd, J = 174.1 and 7.9 Hz). FAB-MS: 270 (M + H, 67.7), 269 (M, 16.3), 232 (100.0).

Conversion of 13b to 11b and 12b. DBU (20 μL, 0.13 mmol) was added to a solution of **13b** (410 mg, 1.09 mmol) in DMF (5 mL) at 0 °C. After 2 min the solvent was evaporated, and the residue was chromatographed on a silica gel column to give products **11b** (150 mg, 36.6%) and **12b** (47 mg, 11.5%) in addition to starting material **13b** (160 mg, 39%).

Cyclopropene−Methylenecyclopropane Rearrangement of 11b, 12b, and 13b. A solution of **11b**, **12b**, or **13b** (0.24 μM) in DMF (50 μL) was treated with DBU (0.03 μM) in DMF (5 μL) at room temperature. After 1 min, a sample was analyzed by a reverse-phase HPLC (Waters μBondapak C_{18}, 300 × 3.9 mm column, 45% MeCN in water, flow rate 0.9 mL/min, detection at 310 nm). The average ratio **11b**:**12b**:**13b** from these experiments was 3.6:1:5.4. An excess of DBU or extension of the reaction time did not affect this ratio, but gradual decomposition was observed.

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Supporting Information Available: Comparison of NMR chemical shifts of fluorinated and nonfluorinated methylenecyclopropanes (Table 1), NOE data (Table 2), HPLC (cyclopropene−methylenecyclopropane rearrangement, Table 3), and UV spectra of **4a** and **1a** (Figure 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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