Table 3. Activity of **3** against a Wide Spectrum of HIV-1 Variants[a]

virus	mutations[a]	IC_{50} (nM) values						
		SQV	RTV	IDV	NFV	APV	1	3
1 (ET)	L10I	17	15	30	32	23	nd	3
2 (B)	L10I,K14R,L33I,M36I,M46I,F53L,K55R,I62V,L63P,A71V, G73S,V82A,L90M,I93L	230	>1000	>1000	>1000	290	10.2	15
3 (C)	I10L,I15V,K20R,M36I,M46L,I54V,K55R,I62V,L63P,K70Q, V82A,L89M	100	>1000	500	310	300	3.5	5
4 (G)	L10I,V11I,T12E,I15V,L19I,R41K,M46L,L63P,A71T, V82A,L90M	59	>1000	500	170	310	3.7	20
5 (TM)	L10I,K14R,R41K,M46L, I54V,L63P,A71V,V82A,L90M, I93L	250	>1000	>1000	>1000	220	3.5	4
6 (EV)	L10V,K20R,L33F,M36I, M46I,I50V,I54V,D60E,L63P,A71V, V82A,L90M	>1000	>1000	>1000	>1000	>1000	nd	52
7 (ES)	L10I,M46L,K55R,I62V,L63P, I72L,G73C,V77I,I84V,L90M	>1000	>1000	>1000	>1000	>1000	nd	31
8 (K)	L10F/D30N/K45I/A71V/T74S	20	57	260	>1000	68	3	3

[a] Amino acid substitutions identified in the protease-encoding region of HIV-1_{ET} (ET), HIV-1_B (B), HIV-1_C (C), HIV-1_G (G), HIV-1_{TM} (TM), HIV-1_{EV} (EV), HIV-1_{ES} (ES), HIV-1_K (NFV_R) as compared to consensus B sequence cited from the Los Alamos database. All values were determined in triplicate. The IC_{50} values were determined by employing PHA-PBMC as target cells and the inhibition of $p24^{Gag}$ protein production as the endpoint.

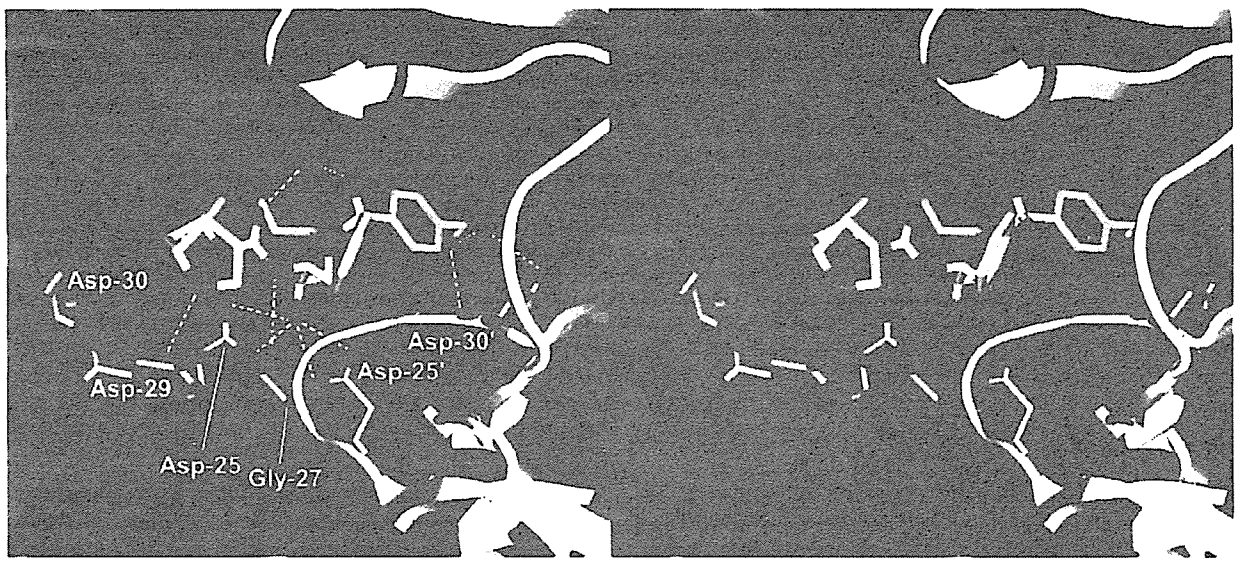

Figure 2. Stereoview of compound **3** bound to the active site of wild-type HIV-1 protease. The protein backbone is illustrated as a ribbon structure. Hydrogen bonds to the active site Asp-25 and Asp-25′, Gly-27, backbone NH of Asp-29, and Asp-30′, and to two bound water molecules are shown.

tor resistance-associated amino acid substitutions (HIV-1_B, HIV-1_C, HIV-1_G, HIV-1_{TM}, HIV-1_{EV}, and HIV-1_{ES}), isolated from patients with HIV-1 infection having received 7−11 different antiviral agents for 24 to 81 months,[5,7] was highly resistant to all the currently available protease inhibitors tested (Table 3). However, inhibitor **3** exerted highly potent activity against all of these six variants with IC_{50} values ranging 4−52 nM. An HIV-1 variant, HIV-1_K, which was selected in vitro in the presence of up to 5 μM concentrations of nelfinavir and contains five amino acid substitutions including D30N,[7] was also tested against inhibitor **3**. It was found that inhibitor **3** was highly potent against HIV-1_K with an IC_{50} value of as low as 3 nM (Table 3). These data indicate that inhibitor **3** is highly active against a wide spectrum of drug-resistant variants. Overall, the potency of inhibitor **3** against the HIV-1 strains tested in the present study was comparable to that of inhibitor **1**.

To gain molecular insight into the ligand-binding site interactions responsible for its potent antiviral activity against a wide spectrum of multi-PI-resistant HIV-1 variants, we have solved the X-ray crystal structure of the inhibitor complex with wild-type protease at 1.35 Å resolution.[28] A stereoview of the inhibitor **3**-bound structure is shown in Figure 2. The central hydroxyl group of the inhibitor forms four hydrogen bonds to the active site Asp25 and Asp25′ side chains, with the distances 2.6−3.2 Å. An oxygen of the sulfonamide group and the carbamate carbonyl oxygen form hydrogen bonds (2.8 and 2.9 Å, respectively) to the conserved water molecule that is hydrogen bonded to the backbone NH groups of Ile50 and Ile50′, as is commonly seen in HIV-1 protease/inhibitor complexes.[29] The oxygen of the P2 hexahydrocyclopentafuran forms a hydrogen bond with the backbone NH of Asp29 with the distance between heavy atoms of 2.8 Å.

This interaction cannot occur for inhibitor **4** which lacks the ring oxygen. The P2 hexahydrocyclopentafuranyl group also makes good C−H···O contacts with the main-chain carbonyl of Gly48. The amide nitrogen of the carbamate moiety has a 3.2 Å hydrogen bond with the main chain carbonyl oxygen of Gly27. The hydroxyl of the P2′ benzyl alcohol group forms a hydrogen bond to the backbone NH (3.1 Å) and a water-mediated contact with the side chain oxygen of Asp30 (OH_{inh}···H_2O···OOC distances are 2.5 and 2.3 Å, respectively). Therefore, the inhibitor **3** forms three direct hydrogen bonds and three water-mediated contacts to the protease residues, excluding contacts with catalytic aspartates. The important hydrophobic contacts include C−H···π interactions. The P1 benzyl group makes C−H···π interactions with Pro81′ and Val82′ (3.6−3.8 Å). The P1′ isobutyl group lies in a hydrophobic pocket formed by Pro81, Val82, Ile84, Gly49′, and Ile-

Table 4. Comparison of the Crystal Structure of Protease and Inhibitor **3** with Multi-PI-resistant Protease Crystal Structures[a]

PDB code	mutations	RMSD (Å)
2FDD[30]	L10I, K20R, M36I, M46I, I50V, I54V, I62V, L63P, A71V, V82A, L90M	0.7
1SGU[31]	K20R, L33F, M36I, I54V, L63P, A71V, V82A, I84V, L90M	0.5
1HSH[32]	L10V, M36I, M46I, A71V, I93L	1.1
2AZC[33]	M46I, F53L, L63P, V77I	0.8
1B6K[34]	K14R, R41K, L63P	0.5
2AVV[35]	L33I, G73S	0.5

[a] RMSD is the root-mean-square deviation of alpha-carbon positions for protease backbones.

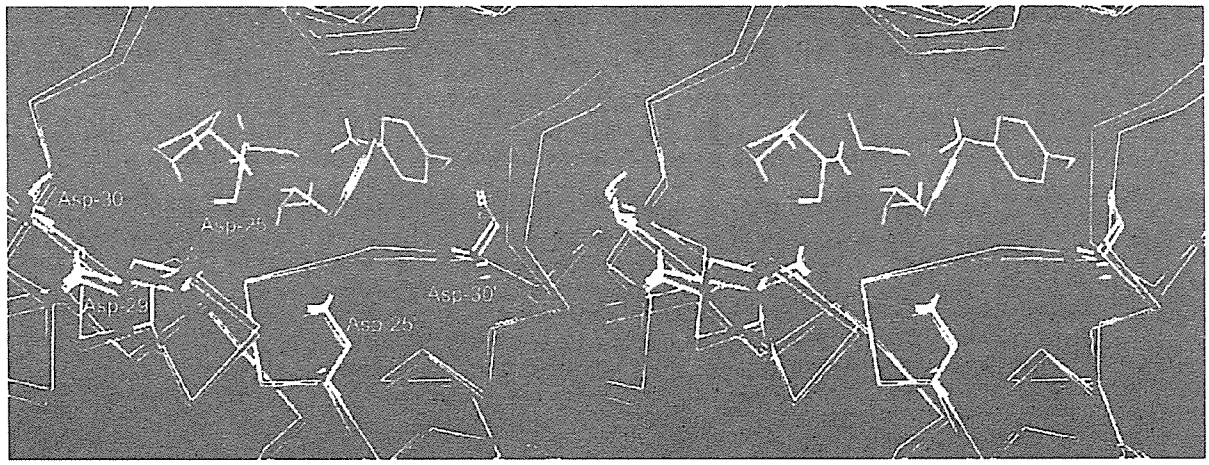

Figure 3. Stereoview of inhibitor **3** bound to the active site of wild-type HIV-1 protease, superimposed upon the structures of the three most highly mutated drug-resistant proteases of Table 3. Protein backbones are represented as alpha-carbon traces. Red = wild-type; blue = 2FDD; green = 1SGU; yellow = 1HSH. Note that, despite the multiple mutations, backbone positions change very little, especially in the active site, and hydrogen bonds to backbone NHs are not disrupted. Also, the hydroxymethyl is able to hydrogen bond to both the backbone and the side chain of Asp-30′.

50′. The P2′ group also forms a number of C−H···π contacts with Ala28′, Val32′, Ile47′, and Ile50, with distances of 3.6−3.9 Å with the closest interaction involving the side chain of Val32′. Inhibitor **3** participates in extensive interactions in the S2 to S2′ subsites, including tight hydrogen bonding with the protein backbone. Other PIs typically show fewer interactions with the main chain atoms. These interactions of inhibitor **3** with the protease backbone may explain its superior antiviral property and its potent activity against a wide spectrum of multi-PI-resistant HIV-1 strains.

To evaluate interactions of inhibitor **3** with the multi-drug-resistant variants of HIV-1 protease listed in Table 3, we have compared our crystal structure with several published crystal structures containing multiple mutations.[30−35] The structures used for comparison, and the relevant mutations, are listed in Table 4. None of these structures corresponds exactly to proteases of the multi-drug-resistant HIV-1 variants illustrated in Table 3, but these structures incorporate 21 of the 33 mutations listed in Table 3. The other mutations in Table 3 (V11I, T12E, I15V, L19I, M46L, K55R, D60E, K70Q, A71T, I72L, G73C, and L89M) have not, to our knowledge, been present in published crystal structures, with the exception of M46L. This mutation has been published only in unliganded crystal structures, in which the active site is open and distorted.[36,37] Residues 11, 12, 15, 19, 46, 55, 60, 70, 71, 72, 73, and 89 are all far from the active site, so that the effects of these mutations on drug resistance must be indirect and, therefore, difficult to model or predict. A stereoview of inhibitor **3** bound to the active site of wild-type HIV-1 protease, superimposed upon the structures of the three most highly mutated drug-resistant proteases (from Table 3), is shown in Figure 3.

In each case, a least-squares fit of protease alpha-carbons was carried out, and the interactions of inhibitor **3** with the mutant protease were examined. In all six structures, the following interactions were maintained: (a) hydrogen bonding of the secondary OH of the inhibitor to Asp-25 and Asp-25′ of the active site; (b) hydrogen bonding of the tetrahydrofuran oxygen of the inhibitor to the backbone NH of Asp-29, and a possible hydrogen bond to the backbone NH of Asp-30; (c) hydrogen bonding of the CH_2OH of the inhibitor to the backbone NH of Asp-30′; (d) hydrogen bonding of the CH_2OH of the inhibitor to the side chain carboxylate of Asp-30′ (requires ~10−20° rotation of the side chain around the C-alpha:C-beta bond to optimize hydrogen bond distance; (e) in P2, van der Waals contact with Gly-48; (f) in P1, van der Waals contact with Gly-49, Ile-50, Leu-23′, and Pro-81′; (g) in P1′, van der Waals contact with Ile-84; (h) in P2′, van der Waals contact with Ile-50, Asp-30′, Val-32′, and Ile-47′.

Furthermore, many other binding interactions were also retained. These include: (a) in P2, van der Waals contact of Ile-47 was retained in all structures except 1HSH, which has the I47V mutation; van der Waals contact of Ile-50′ was retained in four of the structures, but was reduced in 2FDD (which has the I50V mutation) and in 2AZC; (b) in P1, van der Waals contact of Val-82′ was reduced in 2FDD, 1SGU, and 2AZC, all of which have the V82A mutation; van der Waals contact of Ile-84′ was retained in all structures except 1SGU, which has the I84V mutation; (c) in P1′, van der Waals contact of Pro-81 was reduced in 1HSH, 2AZC, and 1B6K; van der Waals contact of Val-82 was reduced in 2FDD, 1SGU, and 2AZC, all of which have the V82A mutation. All six were able to maintain binding to the water molecule in the binding site, with the exception of the 2AZC structure; as reported,[33] one of the flaps of this structure is distorted and a second water molecule is required for inhibitor binding to one of the Ile50 NH groups. It is noteworthy that, even in multi-drug-resistant

proteases with a number of mutations, the hydrogen bonding interactions of inhibitor **3** with the protease backbone are well maintained.

Conclusion

In summary, we have reported here the structure-based design of novel HIV-1 protease inhibitors incorporating a stereochemically defined 5-hexahydrocyclopenta[*b*]furanyl urethane as the P2-ligand and a 4-hydroxymethylsulfonamide as the P2′-ligand. The inhibitors are designed with the purpose of making extensive interactions including hydrogen bonding with the protein backbone of HIV-1 protease active site. One such inhibitor (**3**) has exhibited exceedingly potent antiviral activity and superior activity against multi-PI-resistant variants compared to other FDA approved PIs. The synthesis of P2-ligand alcohol, (3a*S*,5*R*,6a*R*)-5-hydroxy-hexahydrocyclopenta[*b*]furan was carried out enantioselectively by an enzymatic asymmetrization with acetyl cholinesterase as one of the key steps. A protein−ligand crystal structure of **3**-bound HIV-1 protease (1.35 Å resolution) revealed extensive interactions in the enzyme active site. Of particular note, both P2 and P2′-ligands are involved in hydrogen bonding with the backbone of both S2- and S2′-subsites. Comparison of protein−ligand X-ray structure of **3** with others structures of mutant proteases clearly indicated that the backbone interactions are maintained. The design of an inhibitor to specifically interact with the backbone may serve as an important guide to combat drug resistance. Further design and chemical modifications are currently underway.

Experimental Section

General. All moisture sensitive reactions were carried out under nitrogen or argon atmosphere. Anhydrous solvents were obtained as follows: THF, diethyl ether and benzene, distilled from sodium and benzophenone; dichloromethane, pyridine, triethylamine, and diisopropylethylamine, distilled from CaH_2. All other solvents were HPLC grade. Column chromatography was performed with Whatman 240−400 mesh silica gel under low pressure of 5−10 psi. TLC was carried out with E. Merck silica gel 60-F-254 plates. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and Bruker Avance 400 and 500 spectrometers. Infrared spectra were recorded on a Mattson Genesis II FTIR instrument. Optical rotations were measured using a Perkin-Elmer 341 polarimeter.

(1*R*,4*S*)-(+)-4-(*tert*-Butyldimethylsilyloxy)-2-cyclopentenyl Acetate (6). To a stirred solution of alcohol **5** (1.1 g, 7.6 mmol) in tetrahydrofuran (20 mL) was added imidazole (779 mg, 11.4 mmol) followed by *tert*-butyldimethylsilyl chloride (1.54 g, 9.5 mmol). The reaction mixture was stirred at 23 °C for 24 h after which solids were removed by filtration, and the filtrate was concentrated to dryness. The residue was dissolved in ethyl acetate and washed with 1 N hydrochloric acid (3×), saturated aqueous sodium bicarbonate (2×), and brine solution. The organic phase was dried over anhydrous magnesium sulfate, filtered, and concentrated to give compound **6** (1.9 g, 99%) as a colorless liquid. ^1H NMR ($CDCl_3$, 300 MHz): δ 6.03 (m, 1H), 5.95 (m, 1H), 5.51 (m, 1H), 4.77 (m, 1H), 2.86 (dt, 1H, J = 7.5 Hz, J = 13.5 Hz), 2.10 (s, 3H), 1.66 (dt, 1H, J = 5 Hz, J = 14 Hz), 0.99 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.3, 139.3, 131.6, 77.4, 75.3, 41.6, 26.3, 21.6, 18.6, −4.1, −4.2.

1*R*,4*S*-[4-(2-Bromo-1-ethoxy-ethoxy)-cyclopent-2-enyloxy]-*tert*-butyl-dimethyl-silane (7). TBS-ether **6** (2 g, 7.8 mmol) was dissolved in methanol (50 mL) and treated with potassium carbonate (1.7 g, 12.5 mmol). The mixture was stirred for 20 min at 23 °C; solvent was evaporated under reduced pressure. The product was extracted with ethyl acetate (3×), dried over anhydrous sodium sulfate, and concentrated. The crude product was purified by flash column chromatography to provide hydroxyl ether (1.6 g, 97%) as a colorless oil. $[\alpha]^{20}_D$ −21.6 (*c* 1, $CHCl_3$) [lit.,[38] $[\alpha]^{20}_D$ −21.2 (*c* 0.89, $CHCl_3$)]; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.84 (dd, J = 1 Hz, J = 9.0 Hz, 1H), 5.77 (dd, J = 1 Hz, J = 9 Hz, 1H), 4.56 (t, J = 9 Hz, 1H), 4. 48 (t, J = 9 Hz, 1H), 2.59 (dt, J = 12 Hz, J = 22 Hz, 1H), 2.40 (bs, 1H), 1.42 (dt, J = 7.5 Hz, J = 22 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 137.0, 136.1, 75.6, 75.4, 44.9, 26.2, 18.5, −4.2.

To a solution of above hydroxyl ether (400 mg, 1.86 mmol) and *N*-bromosuccinimide (330 mg, 1.86 mmol) in CH_2Cl_2 (10 mL) at −10 °C was added ethyl vinyl ether (0.27 mL, 2.8 mmol). The reaction mixture was allowed to warm to 23 °C. After 12 h aqueous ammonium chloride (15 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2×). Combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and then concentrated in vacuo. The crude product was purified by flash column chromatography to afford compound **7** (660 mg, 97%) as a colorless liquid. ^1H NMR ($CDCl_3$, 300 MHz): 5.82 (d, 2H, J = 2.7 Hz), 4.70 (q, 1H, J = 5.7 Hz), 4.50−4.58 (m, 2H), 3.50−3.62 (m, 2H), 3.27−3.30 (m, 2H), 2.59−2.64 (m, 1H), 1.53−1.60 (m, 1H), 1.16−1.18 (m, 3H), 0.83 (s, 9H), 0.04 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): 138.1, 138.0, 133.4, 133.0, 101.2, 101.0, 79.7, 79.6, 75.2, 75.1, 62.1, 61.9, 42.8, 42.3, 32.5, 32.4, 26.2, 26.0, 18.5, 15.6, 15.5, −4.1, −4.2. MS (CI): *m*/*z* 365.1 $[M + H]^+$; HRMS calcd for $C_{15}H_{29}BrO_3Si$ $[M + H]^+$ 365.1148; found 365.1145.

(3a*R*,5*R*,6a*R*)-2-Ethoxy-5-*tert*-butyldimethylsiloxy-hexahydro-cyclopenta[*b*]furan (8). The bromo derivative **7** (600 mg, 1.64 mmol), n-tributyltin hydride (0.6 mL, 2.14 mmol), and AIBN (10 mg) in benzene (15 mL) were refluxed for 4 h. Then it was cooled to 23 °C, and benzene was removed under reduced pressure. The crude product was chromatographed on silica gel to obtain bicyclic ether **8** (410 mg, 87%) as a viscous liquid. ^1H NMR ($CDCl_3$, 300 MHz): 5.86 (dd, 0.5 H, J = 2.4 Hz, J = 9.3 Hz), 5.12 (dd, 0.5 H, J = 4.5 Hz, J = 7.2 Hz), 3.97−4.75 (m, 2H), 3.63−3.74 (m, 1H), 3.32−3.40 (m, 1H), 2.22−2.78 (m, 1H), 1.52−2.04 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): 106.1, 105.7, 83.1, 75.1, 73.6, 62.8, 62.6, 43.9, 42.6, 42.0, 41.5, 40.9, 40.2, 38.9, 38.9, 28.6, 27.1, 26.2, 26.1, 18.5, 18.3, 15.6, 15.5, −4.2, −4.4, −4.5. MS (CI): *m*/*z* 287.2 $[M + H]^+$; HRMS calcd for $C_{15}H_{30}O_3Si$ $[M + H]^+$ 287.2043: found 287.2041.

(3a*S*,5*R*,6a*R*)-5-Hydroxy-hexahydro-cyclopenta[*b*]furan (9). To a cold (0 °C) solution of bicyclic ether **8** (400 mg, 1.4 mmol) and triethylsilane (0.9 mL, 5.6 mmol) in CH_2Cl_2 (10 mL) was added $BF_3{\cdot}Et_2O$ (320 μL, 2.8 mmol), and the reaction mixture was stirred for 10 min. Saturated aqueous sodium bicarbonate solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×). The combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Purification by flash column chromatography provided cyclopentanofuran derivative (300 mg, 90%) as a colorless liquid. $[\alpha]^{20}_D$ 8.6 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.36 (m, 1H), 4.03 (m, 1H), 3.85 (m, 1H), 3.73 (m, 1H), 2.50 (m, 1H), 2.1−1.9 (m, 3H), 1.70 (m, 1H), 1.57 (m, 1H), 1.39 (m, 1H), 0.84 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 82.9, 73.6, 67.2, 42.8, 41.7, 40.9, 33.5, 26.2, 18.5, −4.3, −4.4. MS (CI): *m*/*z* 243.1 $[M + H]^+$; HRMS calcd for $C_{13}H_{26}O_2Si$ $[M + H]^+$ 243.1781; found 243.1779.

The above cyclopentanofuran (200 mg, 0.82 mmol) was dissolved in tetrahydrofuran (5 mL) and treated with tetrabutylammonium fluoride (1.2 mL, 1 M solution in tetrahydrofuran, 1.23 mmol). The mixture was stirred for 2 h at 23 °C. Solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography to afford alcohol **9** (101 mg, 97%) as a viscous oil. $[\alpha]^{20}_D$ 13.0 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.62 (dt, 1H, J = 1.2 Hz, J = 6.3 Hz), 4.47 (m, 1H), 4.27 (m, 1H), 3.85 (m, 1H), 2.90 (m, 1H), 1.5−2.6 (m, 7H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 86.0, 75.1, 68.6, 42.9, 41.8, 41.8, 35.4. MS (CI): *m*/*z* 129.1 $[M + H]^+$; HRMS calcd for $C_7H_{12}O_2$ $[M + H]^+$ 129.0916; found 129.0915.

(3a*S*,5*R*,6a*R*)-[Carbonic Acid 2′,5′-Dioxo-pyrrolidin-1-yl-ester]-hexahydro-cyclopenta[*b*]furan-5-yl Ester (10). A solution of alcohol **9** (50 mg, 0.39 mmol), *N*,*N*′-disuccinimidyl carbonate (122 mg, 0.47 mmol), and triethylamine (82 μL, 0.59 mmol) in acetonitrile (2 mL) was stirred at 23 °C for 12 h. After this period,

the reaction mixture was treated with saturated aqueous sodium bicarbonate (2 mL). The resulting mixture was extracted with ethyl acetate (3×). The combined organic extracts were dried over sodium sulfate. Evaporation of the solvent followed by flash column chromatography furnished mixed carbonate **10** (80 mg, 78%) as viscous oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.23 (dt, 1H, J = 1.5 Hz, J = 5.7 Hz), 4.58 (m, 1H), 4.08 (m, 1H), 3.84 (m, 1H), 2.93 (s, 4H), 2.84 (m, 1H), 1.9−2.39 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.1, 151.3, 84.6, 83.5, 68.1, 41.9, 39.5, 38.4, 34.1, 25.8.

(±)-4-(Benzyloxy)-octahydropentalen-2-ol (12). To a suspension of benzyl ether **11** (500 mg, 2.47 mmol) and silver acetate (495 mg, 2.96 mmol) in acetic acid (6 mL) at 23 °C was added iodine (688 mg, 2.72 mmol) slowly for a period of 10 min. After being stirred for 2 h the reaction mixture was filtered and the filter cake was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure. The dark brown solution was diluted with CH_2Cl_2 washed with water, 2 N sodium carbonate solution, 5 N sodium thiosulfate solution, water, and brine solution, and dried over anhydrous sodium sulfate. The solvent was evaporated, and the crude product was purified by flash column chromatography to afford iodoacetate (690 mg, 70%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45−7.59 (m, 5H), 5.31−5.40 (dt, 1H, J = 9.9 Hz), 4.72−4.85 (ABq, 2H, v = 20.7 Hz, J = 11.7 Hz, J = 29.7 Hz), 4.54 (t, 1H, J = 9 Hz), 4.09−4.15 (m, 1H), 3.07−3.15 (m, 1H), 2.65−2.70 (m, 1H), 2.24 (s, 3H), 2.20−2.23 (m, 1H), 1.78−1.90 (m, 2H), 1.56−1.63 (m, 1H), 1.26−1.37 (dt, 1H, J = 9.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.9, 139.1, 128.7, 127.9, 127.8, 83.6, 80.2, 72.5, 54.4, 39.8, 37.0, 31.0, 28.9, 24.8, 21.4. MS (CI): m/z 401.0 [M + H]$^+$; HRMS calcd for $C_7H_{21}IO_3$ [M + H]$^+$ 401.0614; found 401.0611.

A solution of above iodoacetate (500 mg, 1.25 mmol) in diethyl ether (10 mL) was added to a suspension of lithium aluminum hydride (95 mg, 2.5 mmol) in diethyl ether (20 mL) at 0 °C. The mixture was heated at reflux for 24 h and then quenched by the addition of aqueous 4% sodium hydroxide solution (0.4 mL). The reaction mixture was stirred at room temperature for another 2 h. The white precipitate was filtered off, and the filtrate was evaporated to dryness in vacuo to give an oily residue which was purified by silica gel column chromatography to afford alcohol **12** (210 mg, 72%) as colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.43−7.47 (m, 5H), 4.73 (ABq, 2H, v = 25.6 Hz, J = 11.7 Hz, J = 39.3 Hz), 4.32 (m, 1H), 4.11 (m, 1H), 3.92 (bs, 1H), 2.70−2.85 (m, 1H), 2.64−2.69 (m, 1H), 1.96−2.19 (m, 5H), 1.65−1.78 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.4, 128.8, 128.1, 82.0, 75.1, 71.6, 46.7, 42.4, 42.1, 35.5, 32.2, 31.2. MS (CI): m/z 233.1 [M + H]$^+$; HRMS calcd for $C_{15}H_{20}O_2$ [M + H]$^+$ found 233.1542; found 233.1540.

(±)-5-(*tert*-Butyldimethylsilyloxy)-octahydropentalen-1-ol (13). To a solution of alcohol **12** (120 mg, 0.51 mmol) and imidazole (80 mg, 1.24 mmol) in dimethylformamide (3 mL) at 0 °C was added *tert*-butyldimethylsilyl chloride (92 mg, 0.61 mmol). The resulting mixture was stirred for 12 h at 23 °C. The reaction was quenched by addition of cold water (30 mL) and extracted with diethyl ether (3×). The combined organic extracts were dried over anhydrous sodium sulfate and filtered. Evaporation of the solvent followed by flash column chromatography of the crude product furnished TBS ether (160 mg, 90%) as colorless liquid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.18−7.28 (m, 5H), 4.43 (ABq, 2H, v = 18.7 Hz, J = 12.0 Hz, J = 25.8 Hz), 3.95 (m, 1H), 4.77 (q, 1H, J = 7.2 Hz), 2.39 (m, 1H), 2.23 (m, 1H), 2.03 (m, 1H), 1.07−1.79 (m, 7H), 0.83 (s, 9H), 0.02 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 139.4, 128.7, 127.9, 127.7, 82.1, 74.8, 71.7, 43.8, 41.5, 38.1, 35.8, 29.2, 28.6, 26.3, 18.6, −4.2, −4.2. MS (CI): m/z 346.2 [M + H]$^+$; HRMS (m/z) $C_{21}H_{34}O_2Si$ [M + H]$^+$ 346.2328; found 346.2325.

To a stirred solution of sodium in liquid ammonia was added a solution of above TBS-ether (85 mg, 0.24 mmol) in THF (5 mL) dropwise for 5 min. The reaction was quenched by addition of excess solid ammonium chloride and allowed to warm to 23 °C. The mixture was diluted with water and extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over anhydrous sodium sulfate. Evaporation of the solvent and flash column chromatography of the crude product furnished alcohol **13** (61 mg, 99%) as colorless liquid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.22 (m 1H), 3.97 (bs, 1H), 3.84 (bd, 1H, J = 9.3 Hz), 2.57−2.60 (m, 1H), 2.39−2.42 (m, 1H), 1.39−1.94 (m, 8H), 0.80 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 77.0, 74.4, 48.4, 43.4, 41.4, 38.1, 35.7, 31.7, 26.1, 18.4, −4.2, −4.8. MS (CI): m/z 257.1 [M + H]$^+$; HRMS calcd for $C_{14}H_{28}O_2Si$ [M + H]$^+$ 257.1936, found 257.1933.

(±)-*tert*-Butyldimethyl-(octahydropentalen1yloxy)silane (14). A solution of alcohol **13** (61 mg, 0.24 mmol) and *N,N'*-thiocarbonyldiimidazole (128 mg, 0.72 mmol) in 2:1 toluene−pyridine (6 mL) was heated at 55 °C for 12 h. After this period, the reaction mixture was concentrated in vacuo to a yellow residue which was dissolved in CH_2Cl_2 (20 mL) and washed with 0.1 N hydrochloric acid, saturated sodium bicarbonate solution, and then with water. The organic layer was dried over anhydrous sodium sulfate. Evaporation of solvent, followed by flash column chromatography yielded 1-*O*-thiocarbonyl imidazoyl derivative (70 mg, 82%) as colorless semisolid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.32 (s, 1H), 7.61 (s, 1H), 6.99 (s, 1H), 5.52−5.57 (m, 1H), 4.02−4.08 (m, 1H), 2.73−2.79 (m, 1H), 2.36−2.40 (m, 1H), 2.02−2.12 (m, 3H), 1.18−1.80 (m, 5H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 184.2, 137.3, 131.0, 118.3, 86.8, 74.8, 43.7, 42.0, 38.8, 36.1, 29.6, 28.5, 26.3, 18.6, −4.3, −4.2.

To a refluxing solution of tributyltinhydride (288 μL, 0.99 mmol) in dry toluene (5 mL) under argon atmosphere was added above thiocarbonylimidazoyl derivative (70 mg, 0.19 mmol) in dry toluene (5 mL) in a dropwise manner over 10 min. The reaction was refluxed for an additional 30 min, and the solvent was removed in vacuo to give colorless oil. The crude product was purified on a silica gel column to afford TBS ether **14** (40 mg, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.95 (m, 1H), 2.24 (m, 2H), 1.91−1.96 (m, 2H), 1.11−1.59 (m, 8H), 0.83 (s, 9H), −0.01 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 75.0, 43.0, 40.1, 34.0, 29.0, 27.5, 26.3, 18.6, 16.2, 14.0, −4.3, −4.3. MS (CI): m/z 241.2 [M + H]$^+$; HRMS calcd for $C_{14}H_{28}OSi$ [M + H]$^+$ 241.2069; found 241.2061.

(±)-Octahydropentalen-2-ol (15). To a solution of TBS-ether **14** (30 mg, 0.11 mmol) in dry THF (1 mL) was added a 1 M solution of tetrabutylammonium fluoride (440 μL, 0.44 mmol), and the mixture was stirred at 23 °C for 10 h. After this period, solvent was removed under reduced pressure and the crude product was purified by flash chromatography to afford alcohol **15** (14 mg, 99%) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.06 (m, 1H), 2.33−2.36 (m, 2H), 2.11−2.16 (m, 2H), 1.54−1.66 (m, 4H), 1.42−1.45 (m, 2H), 1.14−1.18 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 74.3, 42.4, 40.0, 33.5, 29.6. MS (CI): m/z 127.1 [M + H]$^+$; HRMS calcd for $C_8H_{14}O$ [M + H]$^+$ 127.1124; found 127.1123.

Succinimidyl Carbonate 16. A solution of alcohol **15** (10 mg, 0.07 mmol), *N,N'*-disuccinimidyl carbonate (24.3 mg, 0.09 mmol), and triethylamine (14 μL, 0.1 mmol) in acetonitrile (2 mL) was stirred at 23 °C for 12 h. After this period, the solvent was evaporated and the residue was purified by flash column chromatography to afford the mixed carbonate **16** (15.3 mg, 89%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.88−4.95 (m, 1H), 2.76 (s, 4H), 2.35−2.37 (m, 2H), 2.13−2.23 (m, 2H), 1.59−1.66 (m, 2H), 1.35−1.49 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.1, 84.9, 40.6, 38.7, 34.1, 25.8.

Preparation of 4-Diacetoxytoluenesulfonyl Chloride. A solution of *p*-toluenesulfonyl chloride (4.02 g, 21.1 mmol) in a mixture (1:1) of acetic acid and acetic anhydride (80 mL) was treated with concentrated sulfuric acid (6.4 mL, 105.5 mmol) at 0 °C. Chromium trioxide (8 g, 84.4 mmol) was added slowly to maintain the reaction temperature below 10 °C. The mixture was stirred at 5 °C for 30 min. The reaction was quenched with ice water and filtered, and the solid filter cake was washed with water. The solid product was then suspended in saturated sodium bicarbonate solution, and the mixture was stirred for 2 h. The reaction mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over anhydrous sodium sulfate. Evaporation of the solvent followed by purification of the resulting crude product by flash column chromatography provided the title compound (2.4 g, 38%) as a solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (d, 2H, J = 8.4 Hz), 7.79 (d, 2H, J = 8.4 Hz), 7.75 (s, 1H), 2.16 (s, 6H).

Compound 19. To a stirred solution of (1-oxiranyl 2-phenylethyl)-carbamate **17** (200 mg, 0.76 mmol) in 2-propanol (6 mL) was added isobutylamine (340 μL, 4.55 mmol). The resulting mixture was heated at reflux for 6 h. After this period, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography to provide the corresponding secondary amine (268 g, 99%) as a white solid. Mp 145 °C (decomposed); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.20−7.33 (m, 5H), 4.69 (d, 1H, J = 8.8 Hz), 3.84−3.88 (m, 1H), 3.48−3.53 (m, 1H), 3.04 (dd, 1H, J = 4.5 Hz, J = 14.2 Hz), 2.90 (dd, 1H, J = 3.8 Hz, J = 7.8 Hz), 2.84 (dd, 1H, J = 3.1 Hz, J = 12.4 Hz), 2.76 (dd, 1H, J = 5.8, 12.3), 2.57 (dd, 1H, J = 6.6 Hz, J = 11.4 Hz), 2.44 (dd, 1H, J = 7 Hz, J = 11.7 Hz), 1.85−1.89 (m, 1H), 1.35 (s, 9H), 0.96 (d, 3H, J = 4.3 Hz), 0.95 (d, 3H, J = 4.3 Hz).^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 137.8, 130.0, 128.9, 126.9, 80.3, 70.7, 57.9, 54.1, 52.2, 36.9, 28.7, 28.1, 20.9, 20.9. MS (ESI): m/z 359.2 $[M + Na]^+$; HRMS calcd for $C_{19}H_{32}N_2O_3$ $[M + Na]^+$; 359.2311; found 359.2306.

To a solution of above secondary amine (97 mg, 0.29 mmol) and 4-diacetoxytoluenesulfonyl chloride (103 mg, 0.35 mmol) in THF (5 mL) at 0 °C was added *N,N'*-diisopropylethylamine (78 μL, 0.45 mmol) followed by 4-(dimethylamino)pyridine (4 mg, 0.03 mmol). The resulting mixture was stirred at 23 °C for 4 h. Evaporation of the solvent under reduced pressure, followed by flash column chromatography over silica gel yielded compound **19** (151 mg, 88%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (d, 2H, J = 8.4 Hz), 7.81 (s, 1H), 7.76 (d, 2H, J = 8.4 Hz), 7.30−7.43 (m, 5H), 4.73 (d, 1H, J = 7.5 Hz), 3.84−3.92 (m, 2H), 3.20 (d, 2H, J = 6 Hz), 2.92−3.14 (m, 4H), 2.26 (s, 6H), 1.92−2.01 (m, 1H), 1.45 (s, 9H), 1.02 (d, 3H, J = 6.6 Hz), 0.98 (d, 3H, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.0, 156.5, 140.4, 140.0, 138.1, 129.9, 128.9, 128.1, 128.0, 126.9, 88.9, 73.1, 58.8, 55.1, 54.0, 35.8, 28.6, 27.5, 21.2, 20.5, 20.2. MS (ESI): m/z 630.2 $[M + Na]^+$; HRMS calcd for $C_{30}H_{42}N_2O_9S$ $[M + Na]^+$ 630.2509: found 630.2504.

Compound 20. A solution of compound **19** (151 mg, 0.25 mmol) in methanol (10 mL) was treated with potassium carbonate (51 mg, 0.37 mmol). The mixture was stirred for 20 min at 23 °C. The solvent was evaporated under reduced pressure, and the product was extracted with ethyl acetate, dried over anhydrous sodium sulfate and concentrated. The crude product was purified by flash chromatography to provide the corresponding aldehyde (110 mg, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz): 10.1 (s, 1H), 8.05 (d, 2H, J = 8.5 Hz), 7.99 (d, 2H, J = 8.5 Hz), 7.2−7.36 (m, 5H), 4.75 (d, 1H, J = 8.5 Hz), 3.81−3.94 (m, 3H), 3.22 (t, 2H, J = 3.5 Hz), 2.94−3.06 (m, 4H), 1.93 (m, 1H), 1.40 (s, 9H), 0.93 (d, 3H, J = 6.5 Hz), 0.91 (d, 3H, J = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.2, 156.6, 144.4, 139.2, 138.1, 130.6, 129.9, 128.8, 128.4, 123.1, 80.3, 72.9, 58.3, 55.4, 53.4, 36.0, 28.6, 27.4, 20.4, 20.3. MS (ESI): m/z 527.2 $[M + Na]^+$; HRMS calcd for $C_{26}H_{36}N_2O_6S$ $[M + Na]^+$ 527.2192; found 527.2188.

To a stirred solution of above aldehyde (110 mg, 0.22 mmol) in methanol (2 mL) at 0 °C was added sodium borohydride (9.5 mg, 0.25 mmol), and the mixture was stirred for 15 min. The reaction was quenched with saturated ammonium chloride solution. The solvent was evaporated under reduced pressure, and the product was extracted with ethyl acetate (3×). The combined organic extracts were dried over anhydrous sodium sulfate, and solvent was evaporated. The crude product was purified by flash chromatography to provide compound **20** (96 mg, 90%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.67 (d, 2H, J = 8.4 Hz), 7.43 (d, 2H, J = 8.4 Hz), 7.12−7.25 (m, 5H), 4.71 (s, 2H), 4.57 (d, 1H, J = 7.8 Hz), 3.67−3.76 (m, 3H), 2.72−3.03 (m, 6H), 1.78 (m, 1H), 1.27 (s, 9H), 0.83 (d, 3H, J = 6.6 Hz), 0.79 (d, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.4, 146.5, 138.1, 137.7, 129.9, 128.9, 127.9, 127.5, 126.8, 80.1, 73.1, 64.6, 59.0, 55.1, 54.0, 35.8, 28.6, 27.5, 20.5, 20.2. MS (ESI): m/z 529.2 $[M + Na]^+$; HRMS calcd for $C_{26}H_{38}N_2O_6S$ $[M + Na]^+$ 529.2349; found 529.2344.

Inhibitor 3. A solution of compound **20** (30 mg, 0.06 mmol) in 30% trifluoroacetic acid in CH_2Cl_2 (3 mL) was stirred at 23 °C for 30 min. After this period, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in toluene and evaporated at reduced pressure. The residue was dissolved in acetonitrile (2 mL) and cooled to 0 °C. To this solution were added *N,N'*-diisopropylethylamine (41 μL, 0.24 mmol) and mixed carbonate **10** (16.8 mg, 0.06 mmol). The resulting mixture was stirred at 23 °C for 6 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography over silica gel to furnish inhibitor **3** (28 mg, 87%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.91 (d, 2H, J = 8.4 Hz), 7.66 (d, 2H, J = 8.4 Hz), 7.35−7.39 (m, 5H), 5.01 (t, 1H, J = 4.2 Hz), 4.93 (s, 2H), 4.89 (bs, 1H), 3.95−4.03 (m, 3H), 3.80−3.87 (m, 1H), 2.95−3.32 (m, 6H), 2.75−2.81 (m, 1H), 2.11−2.19 (m, 4H), 1.92−2.04 (m, 2H), 1.69−1.75 (m, 1H), 1.58 (dt, 1H, J = 4.5 Hz, J = 14.4 Hz), 1.08 (d, 3H, J = 6.6 Hz), 1.05 (d, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.6, 146.7, 137.9, 137.4, 129.8, 128.9, 127.9, 127.5, 126.9, 84.0, 77.4, 72.8, 68.1, 64.5, 59.1, 55.2, 54.1, 41.8, 39.7, 38.7, 36.2, 34.2, 27.6, 20.5, 20.2. MS (ESI): m/z 583.2 $[M + Na]^+$; HRMS calcd for $C_{29}H_{40}N_2O_7S$ $[M + Na]^+$ 583.2454; found 583.2465.

Inhibitor 4. A solution of compound **20** (23.4 mg, 0.05 mmol) in 30% trifluoroacetic acid in CH_2Cl_2 (3 mL) was stirred at 23 °C for 30 min. After this period, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in toluene and evaporated. The residue was dissolved in acetonitrile (2 mL) and cooled to 0 °C. *N,N'*-Diisopropylethylamine (41 μL, 0.24 mmol) and mixed carbonate **16** (10.0 mg, 0.04 mmol) were then added. The resulting mixture was stirred for 6 h at 23 °C. The reaction mixture was then concentrated under reduced pressure, and the residue was purified by chromatography over silica gel to furnish inhibitor **4** (18 mg, 80%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.00 (d, 2H, J = 8.4 Hz), 7.76 (d, 2H, J = 8.4 Hz), 7.45−7.57 (m, 5H), 5.04 (s, 2H), 4.96−5.00 (m, 1H), 4.06−4.14 (bm, 2H), 3.01−3.41 (m, 4H), 2.59 (m, 2H), 2.25−2.36 (m, 3H), 2.04−2.13 (m, 1H), 1.34−1.87 (m, 10H), 1.16 (d, 3H, J = 6.6 Hz), 1.12 (d, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.5, 146.5, 138.0, 137.6, 129.9, 128.9, 127.9, 127.5, 126.9, 73.0.4, 64.6, 59.0, 55.3, 54.0, 40.5, 39.2, 35.7, 34.1, 27.6, 25.7, 20.5, 20.2. MS (ESI): m/z 581.2 $[M + Na]^+$; HRMS calcd for $C_{30}H_{42}N_2O_6S$ $[M + Na]^+$ 581.2656; found 581.2663.

Inhibitor 2. To a solution of compound **18** (128 mg, 0.24 mmol) in ethyl acetate (15 mL) was added 10% Pd/C (10 mg). The mixture was stirred at 23 °C under an H_2-filled balloon for 11 h. The reaction mixture was filtered through a bed of Celite, and the filter cake was washed with ethyl acetate. Evaporation of solvent under reduced pressure, followed by flash chromatography on silica gel, afforded the corresponding aromatic amine (122 mg, 95%) as a white solid. M.p.: 60−63 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.54 (d, 2H, J = 8.5 Hz), 7.19−7.30 (m, 5H), 6.68 (d, 2H, J = 8.5 Hz), 4.60 (d, 1H, J = 8.4 Hz), 3.75−3.80 (m, 2H), 2.99−3.11 (m, 3H), 2.89−2.92 (m, 2H), 2.77 (dd, 1H, J = 6.7 Hz, J = 13.2 Hz), 1.80−1.86 (m, 1H), 1.34 (s, 9H), 0.89 (d, 3H, J = 6.6 Hz), 0.86 (d, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.0, 150.3, 137.9, 129.5, 128.4, 126.4, 114.3, 79.6, 72.8, 58.7, 54.6, 53.8, 35.4, 29.7, 28.2, 27.2, 20.2, 19.9. MS (ESI): m/z 514.2 $[M + Na]^+$; HRMS calcd for $C_{25}H_{37}N_3O_5S$ $[M + Na]^+$ 514.2352; found 514.2349.

A solution of above amine (22 mg, 0.04 mmol) in 30% trifluoroacetic acid in CH_2Cl_2 (3 mL) was stirred at 23 °C for 40 min. After this period, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in acetonitrile (2 mL). The solution was cooled to 0 °C, and mixed carbonate **10** (11.5 mg, 0.04 mmol) and *N,N'*-diisopropylethylamine (43.8 μL, 0.25 mmol) were added. The resulting mixture was stirred at 23 °C for 8 h. The reaction mixture was then concentrated under reduced pressure, and the residue was purified by flash column chromatography over silica gel to provide inhibitor **2** (18.5 mg, 75%) as an amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.58

(d, 2H, J = 8.6 Hz), 7.22−7.33 (m, 5H), 6.71 (d, 2H, J = 8.6 Hz), 4.91 (m, 1H), 4.79 (m, 1H), 4.43 (m, 1H), 3.87 (m, 2H), 3.71 (q, 1H, J = 7H), 3.01−3.16 (m, 2H), 2.93 (dd, 1H, J = 8.2 Hz), 2.86 (dd, 1H, J = 8.6 Hz, J = 14.1 Hz), 2.80 (dd, 1H, J = 6.1 Hz), 2.66 (m, 1H), 2.04 (m, 3H), 1.87 (m, 2H), 1.65 (m, 3H), 1.48 (m, 1H), 0.95 (d, 3H, J = 6.5 Hz), 0.90 (d, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 150.3, 137.4, 129.2, 128.1, 126.1, 125.9, 113.8, 83.4, 72.3, 67.4, 58.6, 54.5, 53.6, 41.2, 39.0, 38.1, 35.5, 33.6, 29.4, 27.0, 19.9, 19.6. MS (ESI): m/z 568.2 $[M + Na]^+$; HRMS calcd for $C_{28}H_{39}N_3O_6S$ $[M + Na]^+$ 568.2458; found 568.2461.

Cells, Viruses, and Antiviral Agents. MT-2 cells were grown in an RPMI-1640-based culture medium supplemented with 15% fetal calf serum (HyClone Laboratories, Logan, UT), 50 unit/mL penicillin, and 50 μg/mL of streptomycin. The following HIV-1 viruses were used for the drug susceptibility assay: HIV-1_{LAI}, HIV-1_{Ba-L}, and HIV-2_{EHO}.[7,9] 3′-Azido-2′,3′-dideoxythymidine (AZT or zidovudine) was purchased from Sigma (St. Louis, MO). Saquinavir (SQV) and ritonavir (RTV) were kindly provided by Roche Products Ltd. (Welwyn Garden City, U.K.) and Abbott Laboratories (Abbott Park, Ill.), respectively. Indinavir (IDV) and Nelfinavir (NFV) were kindly provided by Japan Energy Inc., Tokyo. Amprenavir (APV) was a kind gift from Glaxo-Wellcome, Research Triangle, NC.

Drug Susceptibility Assay. The susceptibility of HIV to various drugs was determined as previously described[7,9] with minor modifications. Briefly, MT-2 cells (2×10^4/mL) were exposed to 100 50% tissue culture infectious doses ($TCID_{50}$) of HIV in the presence of various concentrations of drugs in 96-well microculture plates and were incubated at 37 °C for 7 days. After 100 μL of the medium was removed from each well, 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (10 μL, 7.5 mg/mL in phosphate-buffered saline) was added to each well in the plate, followed by incubation at 37 °C for 2 h. After incubation, to dissolve the formazan crystals, 100 μL of acidified 2-propanol containing 4% (v/v) Triton X-100 was added to each well and the optical density measured in a kinetic microplate reader (Vmax, Molecular Devices, Sunnyvale, CA). All assays were performed in duplicate or triplicate. In determining the sensitivty of HIV isolates to drugs, phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM) (1×10^6/mL) were exposed to 50 $TCID_{50}$ of each isolate and cultured in the presence or absence of various concentrations of drugs in 10-fold serial dilutions in 96-well microculture plates. On day 7 of culture, the supernatant was harvested and the amount of p24 Gag protein was determined by using a fully automated chemiluminescent enzyme immunoassay system (Lumipulse F; Fujirebio Inc., Tokyo).[7,9] Drug concentrations that suppressed the production of p24 Gag protein by 50% (IC_{50}) were determined by comparison with the p24 production level in drug-free control cell culture. All assays were performed in triplicate.

Determination of X-ray Structure of Inhibitor 3-Bound HIV Protease$_{WT}$. The X-ray crystal structure of inhibitor **3** bound to HIV Protease$_{WT}$ has been deposited in the Protein Databank with access code 2HB3. The crystals of the PR_{WT} complexed with GRL-06579A, which was dissolved in dimethyl sulfoxide, were grown by the hanging-drop vapor diffusion method using 10:1 molar ratio of the inhibitor to protein. The well solution contained sodium acetate buffer (pH = 4.2) and 1.4 M NaCl. Crystals were transferred into a cryoprotectant solution containing the reservoir solution plus 20−30% (v/v) glycerol, mounted on a nylon loop, and flash-frozen in liquid nitrogen. X-ray diffraction data were collected on the ×26C beamline of the National Synchrotron Light Source, Brookhaven National Laboratory at 90 K using 0.96 Å wavelength. Data were processed using HKL2000.[39] A medium-sized platelike crystal, with dimensions of 0.2 × 0.2 × 0.1 mm, diffracted to 1.35 Å resolution with mosaicity of 0.4° and produced an R_{sym} value of 5.0% (52%) for data between 50 and 1.35 Å resolution. These data were reduced in space group $P2_12_12$ with unit cell dimensions of a = 58.1 Å, b = 86.5, c = 45.9 Å with one dimer per asymmetric unit. The CPP4i suite of programs[40,41] was used to obtain a molecular replacement solution using as the starting model the PR_{L90M} complex with TMC114 (PDB code 2F81), which is in the same space group. The structure was refined using SHELX97[42] and refitted using O 10.[43] Alternate conformations were modeled for the protease residues when obvious in the electron density maps. Anisotropic atomic displacement parameters (B-factors) were refined for all atoms including solvent molecules. Stereochemical parameters for the inhibitor were created using GAUSSIAN03 with the DFT quantum-chemical method. Hydrogen atoms were added at the final stages of the refinement. The identity of ions and other solvent molecules from the crystallization conditions was deduced based on the shape and peak height of the $2F_o - F_c$ and $F_o - F_c$ electron density, the potential hydrogen bond interactions and interatomic distances. The crystal structure was refined with three chloride anions, two sodium cations, one glycerol molecule, and 205 water molecules including partial occupancy sites. The final R_{work} (R_{free}) values were 14.7% (19.2%) for all data between 10 and 1.35 Å resolution. The rmsd values from ideal bonds and angles were 0.012 Å and 3.0°. The average B-factor for all atoms was 20.9 Å^2; the average B-factor for atoms of the inhibitor was 12.7 Å.

Acknowledgment. Financial support by the National Institutes of Health (GM 53386, A.K.G. and GM62920, I.T.W.) is gratefully acknowledged. This work was also supported in part by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health, and in part by a Grant-in-aid for Scientific Research (Priority Areas) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Monbu Kagakusho), a Grant for Promotion of AIDS Research from the Ministry of Health, Welfare, and Labor of Japan (Kosei Rohdosho: H15-AIDS-001), and the Grant to the Cooperative Research Project on Clinical and Epidemiological Studies of Emerging and Re emerging Infectious Diseases (Renkei Jigyo: No. 78, Kumamoto University) of Monbu-Kagakusho. The work was supported by the Georgia Research Alliance and the Molecular Basis of Disease program of Georgia State University. We thank Annie Heroux for measuring the X-ray diffraction data at beamline X26C of the National Synchrotron Light Source. Use of the National Synchrotron Light Source, Brookhaven National Laboratory, was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-98CH10886.

Supporting Information Available: HPLC and HRMS data for compounds **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM060561M

9-{[3-Fluoro-2-(hydroxymethyl)cyclopropylidene]methyl}adenines and -guanines. Synthesis and Antiviral Activity of All Stereoisomers[1]

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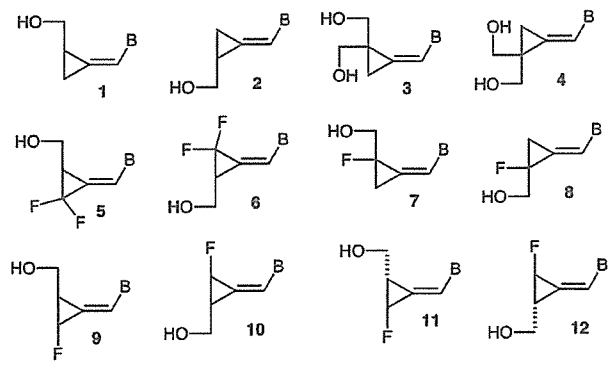
Received June 20, 2006

All stereoisomers of adenine and guanine methylene-3-fluoromethylenecyclopropane analogues of nucleosides **9a**, **9b**, **10a**, **10b**, **11a**, **11b**, **12a**, and **12b** were synthesized and their antiviral activities were evaluated. A highly convergent approach permitted the synthesis of all these analogues using a single intermediate **15**. Reaction of aldehyde **13** with fluorotrichloromethane and tri-*n*-butylphosphine gave fluoroalkenes **14a** + **14b** (83:17). Addition of carbene derived from ethyl diazoacetate gave cyclopropane **15** as the major product. Reduction (**19**), bromination (**20**), and phenylselenenylation (**21**), followed by Se oxidation and β-elimination gave *cis*-methylenecyclopropane **22**. Addition of bromine provided the reagent **23** for alkylation−elimination. Reaction of **23** with adenine led to an isomeric mixture **25a** + **26a** that after deprotection afforded analogues **9a** and **10a**. The 2-amino-6-chloropurine furnished **25e** + **26e** and after deblocking (**9e** and **10e**) and hydrolysis gave targets **9b** and **10b**. Intermediate **15** provided, after debenzylation (**27**), 2-nitrophenylselenenylation (**28**), reduction (**29**), benzylation (**30**), and oxidation−elimination *trans*-methylenecyclopropane **31**. Addition of bromine gave reagent **32**. Further transformations followed the sequence outlined for analogues **9a**, **9b**, **10a**, and **10b**. Analogue **9b** was effective against human cytomegalovirus (HCMV; Towne) with EC_{50} 2.9 μM. The *trans*-isomer **10b** inhibited AD169 strain of HCMV (EC_{50} 15 μM) and the murine virus MCMV (EC_{50} 2.5 μM). Compound **12a** was effective against Epstein−Barr virus (EC_{50} < 0.03 μM). Analogue **9a** inhibited varicella zoster virus (EC_{50} 5.9 μM) and human immunodeficiency virus type 1 (EC_{50} 5.2 μM). Analogues **9a**, **10a**, and **11a** are moderate substrates for adenosine deaminase. The structure−activity relationships will be discussed in context with other methylenecyclopropane analogues.

Methylenecyclopropane analogues of nucleosides are antiviral agents effective especially against human cytomegalovirus (HCMV) and Epstein−Barr virus (EBV).[2,3] The antiviral potency of the first generation series resides mostly in purine *Z*-(*cis*)-isomers **1** (Chart 1), whereas the *E*-(*trans*)-isomers **2** and pyrimidine analogues are active only exceptionally. The second generation *Z*-(*cis*)-isomers **3** have a more narrow antiviral effect,[4−6] but the guanine analogue cyclopropavir (**3**, B = Gua) is effective in vivo[7] and it is currently being developed as a potential drug against HCMV infections. As in the first generation series, the *E*-(*trans*)-isomers **4** lack anti-HCMV activity, but some EBV potency has been noted.[4,6]

Frequently, fluoro analogues of biologically active compounds have yielded effective agents in many areas of biology and biochemistry.[8,9] For these reasons, we focused our attention on methylenecyclopropane analogues of nucleosides fluorinated in the cyclopropane moiety. In the previous work, we reported on 3,3-difluoromethylenecyclopropane analogues[10] **5** and **6** and, more recently, 2-fluoro-substituted compounds[11] **7** and **8**. Although activity of compounds **5** and **6** was limited to a moderate potency[10] of the *E*-(*cis*)-isomer **5a** against HCMV, several *Z*-(*cis*)- and *E*-(*trans*)-isomers of the series **7** and **8** were effective[11] against HCMV and EBV. Also, the methylene-3,3-difluorocyclopropanes **5** and **6** have limited stability[10] that may have affected the biological activity, but monofluoro compounds **7** and **8** are stable.[11] It was then of interest to investigate the isomeric methylene-3-fluorocyclopropane analogues **9**, **10**, **11**, and **12**.

Chart 1. [a]



[a] B = nucleic acid base: series a, B = Ade; series b, B = Gua; series c, B = Cyt; series d, B = Thy; series e, B = 2-amino-6-chloropurine.

Synthesis. At the outset, it was clear that a convergent approach utilizing a single intermediate for synthesis of all antici-

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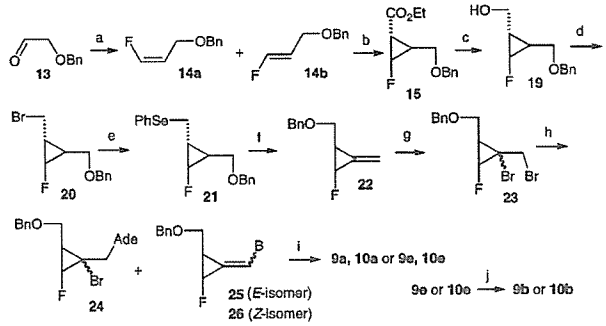
10.1021/jm0607404 CCC: $33.50
Published on Web 09/09/2006

Scheme 1[a]

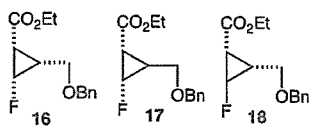


[a] Reaction conditions: (a) (1) $CFCl_3$, Bu_3P, CH_2Cl_2; (2) NaOH (10%); (b) N_2CHCO_2Et, $Cu(AcAc)_2$, CH_2Cl_2, Δ; (c) DIBALH, THF; (d) Ph_3P, Br_2, CH_2Cl_2; (e) Ph_2Se_2, NaOH, $NaBH_4$, EtOH; (f) (1) H_2O_2, THF, 0 °C; (2) (*i*-Pr)$_2$NEt, toluene, Δ; (g) pyridine·HBr_3, CH_2Cl_2; (h) B-H, K_2CO_3, DMF, Δ; (i) (1) BCl_3, CH_2Cl_2, −78 °C; (2) $NaHCO_3$, MeOH; (j) (1) 80% HCO_2H, Δ; (2) NH_3, MeOH. For series a, b, and e, see Chart 1.

pated analogues **9**, **10**, **11**, and **12** would be most convenient. Such a key compound should comprise the fluorocyclopropane ring carrying two different but modifiable substituents at the remaining cyclopropane carbon atoms. The different fluorocyclopropane stereochemistry necessary for *cis*-fluoro analogues **9** and **10** versus *trans*-isomers **11** and **12** could then be generated by manipulation of the cyclopropane substituents.

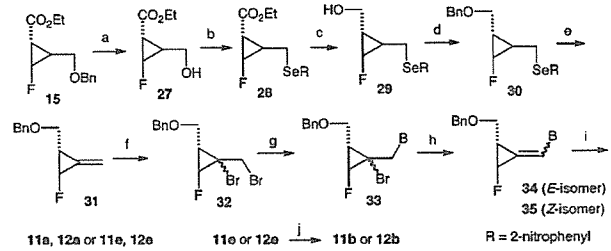
The synthesis of such a key intermediate, compound **15**, is described in Scheme 1.

Benzyloxyacetaldehyde **13** was converted to an isomeric mixture of fluoroalkenes **14a** + **14b** (*Z*/*E* = 83:17) in 68% yield by a modified Wittig reaction using fluorotrichloromethane and tri-*n*-butylphosphine in dichloromethane followed by alkaline hydrolysis.[12] Addition of carbene[13] derived from ethyl diazoacetate catalyzed by copper(II) acetylacetonate in dichloromethane gave a mixture of four cyclopropane stereoisomers **15**, **16**, **17**, and **18** (65% conversion) and two unidentified fluorine-containing components. The major stereoisomer **15** was formed by addition of carbene from a less-hindered side of the double bond of the *cis*-isomer **14a**. It was readily obtained by chromatography on a silica gel column in 42% yield. The other three stereoisomers **16**, **17**, and **18** were obtained as an unresolvable mixture identified by ^{19}F NMR spectroscopy. Reduction of **15**



with diisobutylaluminum hydride in tetrahydrofuran afforded hydroxymethylcyclopropane **19** (95%). Bromination using bromine−triphenylphosphine complex[14] in dichloromethane gave crude bromomethylcyclopropane **20** (91%), which was, in turn, converted to phenylselenenylcyclopropane **21** in 94% yield using sodium phenylselenide generated in situ from diphenyl diselenide.[5] Oxidation with 30% hydrogen peroxide was followed by β-elimination, catalyzed by diisopropylethylamine in toluene[10] at 80−85 °C to give methylenecyclopropane **22** (68%). Addition of bromine via pyridinium tribromide in dichloromethane afforded dibromide **23** (83%), obtained as a single stereoisomer of 95% isomeric purity. Alkylation-elimination protocol with adenine (K_2CO_3, DMF, 100−105 °C, 48 h) led to the alkylated product **24** and isomeric mixture of methylenecyclopropanes **25a** + **26a**. The elimination procedure was repeated with **24** to give additional **25a** + **26a** (total yield

Scheme 2[a]



[a] Reaction conditions: (a) (1) BCl_3, CH_2Cl_2, −78 °C; (2) $NaHCO_3$; (b) 2-nitrophenyl selenocyanate, Bu_3P, THF; (c) DIBALH, THF; (d) BnBr, NaH, THF; (e) (1) H_2O_2, THF, 0 °C; (2) toluene, Δ; (f) pyridine·HBr_3, CH_2Cl_2; (g) B-H, K_2CO_3, DMF; (h) K_2CO_3, DMF, Δ; (i) Me_2S·BCl_3, CH_2Cl_2; (j) (1) 80% HCO_2H, Δ; (2) NH_3, MeOH. For series a, b, and e, see Chart 1.

46%). Finally, debenzylation with boron trichloride in dichloromethane at −78 °C furnished, after chromatographic separation, analogues **9a** and **10a** in 47% yield each. Alkylation-elimination with 2-amino-6-chloropurine and **23** under the conditions described for adenine isomers **25a** + **26a** gave isomeric mixture **25e** + **26e** (56%). Debenzylation gave the *E*- and *Z*-isomers **9e** and **10e** in 47% yield each. Hydrolysis with 80% formic acid afforded guanine analogues **9b** and **10b** (both in 95% yield).

The key intermediate **15** served also as a starting material for the synthesis of the isomeric series **11** and **12** (Scheme 2). The *O*-debenzylation of **15** with boron trichloride in dichloromethane at −78 °C furnished hydroxyester **27** (83%). Reaction with 2-nitrophenyl selenocyanate and tri-*n*-butylphosphine in tetrahydrofuran (THF) using the procedure described[10] for difluoro analogues **5** and **6** gave 2-nitrophenylselenenyl derivative **28** in 92% yield. Reduction with diisobutylaluminum hydride in THF afforded hydroxymethylcyclopropane **29** (95%). Benzylation with benzyl bromide using sodium hydride in THF led to intermediate **30** (71%). Oxidation with hydrogen peroxide in THF, followed by β-elimination (see also Scheme 1, **21** → **22**) provided methylenecyclopropane **31** (*trans*-isomer of **22** from Scheme 1) in 73% yield. Addition of bromine furnished dibromocyclopropane **32** (83%), obtained, in contrast to isomeric derivative **23**, as a mixture of *cis*- and *trans*-isomers. Further transformations followed those described in Scheme 1, but the alkylation and elimination steps were separated. Alkylation of adenine (K_2CO_3, DMF, 25−40 °C) with **32** gave alkylated product **33a** in 86% yield.

β-Elimination was effected with K_2CO_3 in DMF at 100 °C to furnish *E*- and *Z*-isomers **34a** and **35a**, which were separated by chromatography in 27 and 18% yields, respectively. The *O*-debenzylation afforded adenine analogues **11a** and **12a** (74−79%). The reaction sequence with 2-amino-6-chloropurine proceeded in a similar fashion: **32** → **33e** (87%) → **34e** and **35e** (29 and 33%) → **11e** and **12e** (74% each). Hydrolysis then provided guanine analogues **11b** and **12b** in 73−76% yield.

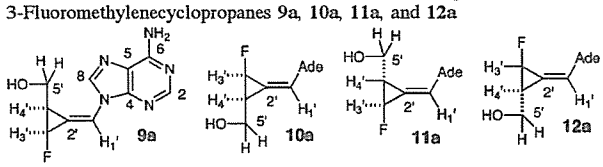
Isomeric Structure of Analogues 9, 10, 11, and 12. A preliminary isomeric assignment of *cis*- and *trans*-alkene isomers **9** versus **10** and **11** versus **12** was made on the basis of chromatographic mobility that followed the pattern observed previously[3] for other methylenecyclopropane analogues. The isomers **9** and **11** with a *cis*-configured base are faster moving than the respective *trans*-isomers **10** and **12**. Although this "rule" may be of value for distinguishing *cis*- and *trans*-isomers at the alkene bond, it has little relevance for determining the *cis*- and *trans*-configuration at the cyclopropane moiety in **9** versus **11** and **10** versus **12**. All these isomeric structures were readily established from ^1H and ^{19}F NMR spectra (Table 1). The

Table 1. Chemical Shifts (δ) and Coupling Constants $^3J_{F,H}$ of the Relevant ^{1}H NMR Signals of Fluorinated Methylenecyclopropanes **5a**, **6a**, **9a**, **10a**, **11a**, and **12a**

compound[a]	$H_{1'}$	H_8	OH	$^3J_{F,H}$ (Hz)
5a	8.19	8.68	5.39	<1, 7.5
6a	7.64	8.16	5.24	<1, 6.3
9a	7.89	8.72	5.21	<1
10a	7.55	8.35	5.03	<1
11a	7.93	8.71	5.17	12.2
12a	7.58	8.34	5.02	10.9

[a] DMSO-d_6 as solvent. Values for **5a** and **6a** were taken from ref 10.

Table 2. NOE Enhancements of Relevant ^{1}H NMR Signals of 3-Fluoromethylenecyclopropanes **9a**, **10a**, **11a**, and **12a**



compound[a]	$H_{i'r}$	δ	H_{obs}	δ	NOE (%)
9a	H_8	8.72	$H_{4'}$	2.48	2.84
	$H_{4'}$	2.48	H_8	8.72	2.20
	OH	5.21	H_8	8.72	1.98
	$H_{4'}$	2.48	$H_{3'}$	5.32, 5.49[a]	1.87, 2.40
10a	H_8	8.35	$H_{3'}$	5.53, 5.70[a]	2.47, 2.44
	$H_{3'}$	5.53, 5.70[a]	H_8	8.35	5.23, 3.67
	$H_{4'}$	2.37	$H_{3'}$	5.53, 5.70[a]	3.33, 3.76
	$H_{3'}$	5.53, 5.70[a]	$H_{4'}$	2.37	13.05, 12.57
11a	H_8	8.71	$H_{4'}$	2.60	1.54
	$H_{4'}$	2.60	H_8	8.71	3.36
	H_8	8.71	OH	5.17	0.30
	OH	5.17	H_8	8.71	2.58
12a	H_8	8.34	$H_{3'}$	5.28, 5.45[a]	1.08, 0.68
	$H_{3'}$	5.28, 5.45[a]	H_8	8.34	4.44, 2.33

[a] Doublets of the $H_{3'}$ signals of **9a** and **10a** were treated separately as two singlets. Likewise, the doublets of doublets of **11a** and **12a** were treated as two doublets.

chemical shifts of the *cis*-isomers of the purine H_8, alkene $H_{1'}$, and OH of **9a** and **11a** are all located downfield from the respective *trans*-isomers **10a** and **12a**. A similar deshielding pattern was observed previously[10] for the corresponding 3,3-difluoromethylene analogues **5a** and **6a**. The $^3J_{F,H}$ coupling constants were then instrumental for isomeric assignment of the substituents in the cyclopropane moiety. The fluorine signals of isomers **9a** and **10a** with *trans*-situated proton and fluorine appear as doublets with $^3J_{F,H} < 1$ Hz, whereas compounds **11a** and **12a** having *cis*-configured protons, exhibit $^3J_{F,H} = 12.2$ and 10.9 Hz, respectively. This is in accord with the general pattern in fluorocyclopropanes,[15] where the $^3J_{F,H-cis}$ are much larger than $^3J_{F,H-trans}$. As expected, *cis*- and *trans*-located *geminal* fluorine atoms of difluoro analogues **5a** and **6a** also follow these trends.[10] Similar relationships were observed for analogues containing bases other than adenine, compounds **9b**, **10b**, **9e**, and **10e**. The $^3J_{F,H}$ values of a similar magnitude, <1 and 13.9 Hz, were also observed for *cis*- and *trans*-methylenecyclopropanes **22** and **31** lacking the heterocyclic bases.

The NOE experiments with analogues **9a**, **10a**, **11a**, and **12a** confirmed these assignments (Table 2). As expected, in compounds **9a** and **11a**, with a *cis*-configured adenine, the NOE enhancements were observed between the H_8 and $H_{4'}$ and OH and H_8. In *trans*-isomers **10a** and **12a**, an interaction between the H_8 and $H_{3'}$ was observed. The NOE enhancements between the *cis*-configured cyclopropane protons $H_{3'}$ and $H_{4'}$ were noted for analogues **9a** and **10a**, but they were absent in **11a** and **12a**, where this relationship is *trans*.

Table 3. Inhibition of HCMV and HSV-1 Replication by 3′-Fluoromethylenecyclopropane Analogues of Nucleosides

	EC_{50}/CC_{50} (μM)		
	HCMV/HFF		
compound	Towne[a,b]	AD169[c,d]	HSV-1/BSC-1[e]
9a	31/>100	44.3/>100	80/>100
10a	>100/>100	>100/>100	>100/>100
9b	2.9/>100	>12/>300	70/>100
10b	>100/>100	15/266[a,f]	20/>100
11a	>100/>100	45/>300	20/>100
12a	>100/>100	285/>300	50/>100
11b	>100/>100	>60/255	>100/>100
12b	>100/>100	>60/183	>100/>100
control	1.8/>100[g]	0.15/>100[g]	3.5/>100[h]

[a] Plaque reduction assay. [b] Visual cytotoxicity. [c] Cytopathic effect (CPE) inhibition assay. [d] Cytotoxicity by neutral red uptake. [e] ELISA. Cytotoxicity was determined in KB cells. All listed compounds were inactive against HSV-1 or HSV-2 in Vero (EC_{50}/CC_{50} >50/>50 μM)[a] and HFF cells (EC_{50}/CC_{50} >100/>100 μM)[c] culture. [f] Against MCMV/MEF[a] the EC_{50}/CC_{50} was 2.5/>100 μM. Because of a lack of significant potency against HCMV/AD169, other compounds listed in Table 1 were not tested. [g] Ganciclovir. [h] Acyclovir.

Table 4. Inhibition of EBV, VZV, HIV-1, and HBV Replication by 3′-Fluoromethylenecyclopropane Analogues of Nucleosides

	EC_{50}/CC_{50} (μM)				
	EBV		VZV	$HIV\text{-}1_{LAI}$	HBV
compound	Daudi[a]	H-1[b,c]	HFF[d,e]	MT-2[d]	2.2.15[b,c]
9a	>50/>50	>20/>100	5.9[f]	5.2/>10	>20
10a	>50/>50	>20/>100	54.6[f]	>10/>10	>20
9b	>100/>100	13/74	>100[f]	>10/>10	>20
10b	>100/>100	>20/>100	68.3[f]	>10/>10	>20
11a	>100/>100	>10/9	33.6[f]	>10/>10	>10
12a	<0.03/>100	>10/>100	>60	>10/>10	>10
11b	>100/>100	>30/>100	>100	>10/>10	>30
12b	91.6/>100	>30/>100	>60	>10/>10	>30
control	0.33/>100[g]	5[h]	0.03[g]	0.02/>10[i]	0.02/>100[j]

[a] Viral capsid antigen (VCA) ELISA. [b] DNA hybridization assay. [c] Cytotoxicity was determined in CEM cells. [d] Cytopathic effect (CPE) assay. [e] Only the EC_{50} values are listed, for CC_{50} values, see HCMV(AD169)/HFF in Table 1. [f] Plaque reduction assay. [g] Acyclovir. [h] Ganciclovir. [i] AZT. [j] Lamivudine.

Biological Activity. Antiviral Activity. Analogues **9a**, **9b**, **10a**, **10b**, **11a**, **11b**, **12a**, and **12b** were tested against the following viruses: HCMV, herpes simplex virus 1 and 2 (HSV-1 and HSV-2), EBV, varicella zoster virus (VZV), human immunodeficiency virus type 1 (HIV-1), and hepatitis B virus (HBV). The results are summarized in Tables 3 and 4. Analogue **9b** was active against HCMV/HFF (EC_{50}/CC_{50} 2.9/>100 μM) in Towne strain of the virus (Table 3) but it had little effect against the AD169 strain. Conversely, *trans*-isomer **10b** was somewhat effective against HCMV/HFF in the AD169 strain (EC_{50}/CC_{50} 15/266 μM) and inactive against Towne strain. It was active with 2.5/>100 μM against the murine virus MCMV/MEF. Analogues **11a**, **11b**, **12a**, and **12b** were without significant effect.

Moderate antiviral effects were detected by ELISA against HSV-1/BSC-1. The most potent compounds **10b** and **11a** had EC_{50}/CC_{50} 20/>100 μM. Against EBV/Daudi (VCA-ELISA), the *trans*-isomer **12a** was the most effective analogue, with EC_{50}/CC_{50} <0.03/>100 μM, but it was devoid of potency in H-1 cells, as determined by DNA hybridization assay (Table 4).

Under the latter conditions, guanine analogue **9b** was moderately active with EC_{50}/CC_{50} 13/74 μM. It was inactive in Daudi cells. The adenine analogue **9a** was the most potent compound against VZV/HFF with EC_{50}/CC_{50} 5.9/>100 μM, and it also

inhibited HIV-1/MT-2 (EC_{50}/CC_{50} 5.2/>10 μM). The tested compounds were inactive against HBV in 2.2.15 cells, and they were noncytotoxic, with the exception of analogue **11a** with CC_{50} 9 μM in CEM cells.

The analogues described herein conclude the first generation of methylenecyclopropanes **1** and **2** with a single fluorine atom in the cyclopropane moiety. Therefore, some generalizations regarding the structure–activity relationships of antiviral activity in this whole series of compounds can be made. The antiviral activity of the purine methylenecyclopropane analogues with a *cis* configuration of the nucleobase follows approximately the order **1** > **7** > **9** > **11** > **5**. Introduction of the fluorine appears to narrow the antiviral effects.[10,11] Two *geminal* fluorines (**5a**, **5b**, **6a**, and **6b**) decrease the chemical stability of the analogues.[10] Compounds with a *trans*-configured nucleobase **10** and **12** were mostly devoid of significant antiviral activity. Strong potency, sometimes in submicromolar range, was detected against EBV/Daudi by VCA-ELISA but it was not always reproduced in DNA hybridization assays. Analogues **8a**, **8b**, **8c** (ref 11), and **12a** serve as examples. It is likely that the mechanism of action of active analogues of this series follows the conversion to the corresponding triphosphates, which then inhibit the relevant DNA polymerase or reverse transcriptase. This was documented[16] for nonfluorinated analogues **1** and **2**.

Adenosine Deaminase (ADA). Adenine fluoroanalogues **9a**, **10a**, **11a**, and **12a** were investigated as substrates for adenosine deaminase from calf intestine, whereas compounds **9a**, **10a**, and **11a** are moderate substrates, which were about 50% deaminated after 24 h; analogue **12a** was resistant to deamination up to 48 h.

Experimental Section

General Methods. The UV spectra were measured in ethanol and NMR spectra were determined at 300 or 400 MHz (^{1}H), 75 or 100 MHz (^{13}C), and 376 MHz (^{19}F). For ^{19}F NMR, $CFCl_3$ was used as a reference. For atom numbering of 3-fluoromethylenecyclopropane analogues, see Table 2, formula **9a**. Mass spectra were determined in electron-impact (EI-MS) or electrospray ionization (ESI-MS, methanol–NaCl) mode. Benzyloxyacetaldehyde[17] (**13**) and 2-nitrophenyl selenocycanate[18] were prepared as described. For the nomenclature of compounds with a double *cis–trans* isomerism in the cyclopropane ring, an *r,c,t* system[19] was adopted. The substituents were denoted as follows: *r* (reference), *c* (*cis*), and *t* (*trans*). Adenosine deaminase from calf intestine, ×6B8577, 19.8 units/mg solid, was the product of Worthington, Lakewood, New Jersey.

(*Z,E*)-1-Benzyloxymethyl-2-fluoroethene (14a + 14b). Tri-*n*-butylphosphine (205 mL, 0.825 mmol) was added dropwise with stirring at 0 °C to a mixture of CH_2Cl_2 (270 mL) and $CFCl_3$ (25.7 mL, 0.275 mol). The stirring was continued for 1 h at 0 °C and then 3 h at room temperature. Benzyloxyacetaldehyde[17] (**13**, 33.3 g, 0.22 mol) in CH_2Cl_2 (20 mL) was added at 0 °C with stirring, which was continued for 16 h at room temperature. Sodium hydroxide solution (10%, 330 mL) was then added, and the stirring was continued for 24 h. The reaction mixture was cooled to 0 °C, and the pH was adjusted to 5.0 by a careful addition of HCl. The organic phase was separated, washed with 5% HCl (5%, 2 × 150 mL), and dried over $MgSO_4$. The solvents were removed in vacuo, the residue was dissolved in hexanes, and the solution was filtered through a silica gel pad. After removal of hexanes, the crude product was chromatographed on a silica gel column in hexanes to hexanes–Et_2O (40:1) to give *Z*- and *E*-isomers **14a** and **14b** (25.2 g, yield 68%, 83% **14a**, and 17% **14b**, as determined by ^{19}F NMR) as a colorless oil, which was of sufficient purity to be used in the next step. ^{1}H NMR ($CDCl_3$) δ 3.98 (dt, J = 7.2, 1.6 Hz, **14b**), 4.22 (dt, 2H, J = 7.2, 2.0 Hz, **14a**, CH_2O), 4.54 (s, **14b**), 4.56 (s, 2H, CH_2Ph, **14a**), 5.09 (ddt, J = 42.0, 7.2, 4.8 Hz, **14a**), 5.60 (ddt, 1H, J = 18.4, 11.2, 7.2 Hz, 1H, **14b**, CH=), 6.67 (ddd, J = 84.4, 4.8, 1.6 Hz, **14a**), 6.75 (ddd, 1H, J = 83.6, 11.2, 1.6 Hz, **14b**, CHF=), 7.36 (m), 7.41 (m, 5H, Ph). ^{13}C NMR 61.9 (d, J = 6.7 Hz, **14a**), 64.8 (d, J = 14.2 Hz), **14b**, CH_2O), 72.2 (**14b**), 72.5 (**14a**, CH_2Ph), 108.5 (d, J = 3.7 Hz, **14a**), 108.9 (d, J = 9.8 Hz, **14b**, CH=), 128.0 (**14a**), 128.1 (**14b**), 128.2 (**14a**, **14b**), 128.7 (**14a**), 128.8 (**14b**), 138.2 (**14b,**) 138.4 (**14a**, Ph), 149.9 (d, J = 261.9 Hz, **14a**), 152.1 (d, J = 261.2 Hz, **14b**, CHF=). ^{19}F NMR −126.07 (dd, J = 83.9, 44.4 Hz, **14a**), −125.54 (ddt, J = 83.9, 18.1, 3.0 Hz, **14b**). EI-MS 166 (M, 3.0), 165 (M − H, 4.2), 91 ($PhCH_2$, 100.0). EI–HRMS calcd for $C_{10}H_{11}OF$, 166.0794; found, 166.0791.

Ethyl *t*-2-Benzyloxymethyl-*t*-3-fluorocyclopropane-*r*-1-carboxylate (15). Ethyl diazoacetate (11.5 g, 100 mmol) was added to a refluxing solution of **14a** + **14b** (16.0 g, 96.38 mmol) and copper acetylacetonate (0.75 g, 2.87 mmol) in CH_2Cl_2 (120 mL) using a syringe pump (0.34 mL/h) with stirring. The stirring was continued for 1 h, solvent was evaporated, and the residue was put on a silica gel column that was eluted with hexane–EtOAc (100:0 to 10:1) to give unreacted **14a** + **14b** (5.6 g, 35%), followed by a mixture of products. Solvents were evaporated, the residue was dissolved in ether (150 mL), and $KMnO_4$ (15 g) in water (60 mL) was added with external ice-cooling and stirring to remove unsaturated impurities. The stirring was continued for 6 h, and excess $KMnO_4$ was removed by addition of solid $Na_2S_2O_3$. The mixture was filtered through a short silica gel pad that was eluted with ether. The organic phase was washed successively with saturated $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL), and it was dried over $MgSO_4$. The crude product was chromatographed on a silica gel column in hexane–Et_2O, 50:1 to give a faster moving isomer **15** as a colorless oil (6.65 g, 42%). The slower moving fraction was an inseparable mixture consisting of the three remaining isomers **16**, **17**, and **18** and two unidentified fluorine-containing impurities.

Isomer 15: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 7.2 Hz, CH_3), 1.91–1.99 (m, 2H, H_2, H_1), 3.78–3.58 (2 partly overlapped AB's, 2H, CH_2OBn), 4.08 (2q, 2H, J = 7.2 Hz, CH_2 of Et), 4.52, 4.59 (AB, 2H, J = 11.7 Hz, CH_2Ph), 4.92 (ddd, 1H, J = 63.2, 5.6, 2.4 Hz, H_3), 7.28–7.36 (m, 5H, Ph). ^{13}C NMR 14.4 (CH_3), 24.9 (d, J = 12.7 Hz, C_1), 26.3 (d, J = 8.3 Hz, C_2), 61.3 (CH_2 of Et), 65.6 (d, J = 7.5 Hz, CH_2OBn), 73.0 (CH_2Ph), 76.1 (d partly overlapped with $CDCl_3$, J = 230.5 Hz, C_3), 127.95, 127.98, 128.7, 138.2 (Ph), 170.7 (C=O). ^{19}F NMR −220.05 (ddd, J = 64.0, 18.1, 7.5 Hz). EI-MS 252 (M, 5.1), 91 ($PhCH_2$, 100.0). EI–HRMS calcd for $C_{14}H_{17}FO_3$, 252.1162; found, 252.1164. Anal. $C_{14}H_{17}FO_3$ (C, H).

Ethyl *c*-2-Benzyloxymethyl-*c*-3-fluorocyclopropane-*r*-1-carboxylate (16). ^{19}F NMR ($CDCl_3$) −232.66 (dt, J = 65.5, 9.0 Hz).

Ethyl *t*-2-Benzyloxymethyl-*c*-3-fluorocyclopropane-*r*-1-carboxylate (17). ^{19}F NMR ($CDCl_3$) −220.66 (ddd, J = 64.0, 18.1, 7.5 Hz).

Ethyl *c*-2-Benzyloxymethyl-*t*-3-fluorocyclopropane-*r*-1-carboxylate (18). ^{19}F NMR ($CDCl_3$) −205.84 (dt, J = 64.0, 18.4 Hz).

***t*-2-Benzyloxymethyl-*t*-3-fluorocyclopropyl-*r*-1-methanol (19).** Diisobutylaluminum hydride (DIBALH) in hexanes (1M, 45.80 mL, 45.80 mmol) was added to a solution of ester **15** (4.62 g, 18.33 mmol) in hexane (40 mL) with stirring at 0 °C during 10 min under N_2. The stirring was continued for 1 h. The reaction was quenched by a dropwise addition of HCl (5%, 50 mL) and then it was extracted with ether (4 × 30 mL). The organic phase was washed successively with saturated $NaHCO_3$ (2 × 30 mL) and water (2 × 30 mL). The solvents were evaporated, and the residue was chromatographed on a silica gel column in hexanes–EtOAc = 10:1 to 5:1 to give compound **19** as a colorless oil (3.66 g, 95%). ^{1}H NMR ($CDCl_3$) δ 1.18 (ddd, 1H, J = 13.0, 6.6 Hz, H_2), 1.34 (dddd, 1H, J = 22.0, 13.6, 6.4, 1.6 Hz, H_1), 2.95 (br s, 1H, OH), 3.33 (m, 1H), 3.31–3.46 (m, 1H, CH_2OH), 3.57–3.71 (m, 2H, CH_2OBn), 4.50 (ddd, 1H, J = 64.0, 6.4, 2.4 Hz, H_3), 4.44, 4.60 (split AB partly overlapped with H_3, 2H, CH_2Ph), 4.52 (ddd partly overlapped with CH_2Ph, 1H, J = 63.6, 6.0, 2.1 Hz, H_3), 7.28–7.36 (m, 5H, Ph). ^{13}C NMR 21.6 (d, J = 10.5 Hz), 25.1 (d, J = 9.7 Hz, $C_{1'}$, C_2), 61.8 (CH_2OH), 67.3 (d, J = 8.2 Hz, CH_2OBn), 73.0 (CH_2Ph), 75.1 (d, J = 223.5 Hz, C_3), 128.0, 128.1, 128.7, 138.3 (Ph). ^{19}F NMR

−223.80 (ddd, J = 64.0, 21.5, 4.5 Hz). EI-MS 210 (M, 1.2), 91 ($PhCH_2$, 100.0). EI−HRMS calcd for $C_{12}H_{15}FO_2$, 210.1056; found, 210.1057. Anal. $C_{12}H_{15}FO_2$ (C, H).

_t_-2-Benzyloxymethyl-_t_-3-fluoro-_r_-1-bromomethylcyclopropane (20). Bromine (2.60 g,16.24 mmol) was added with stirring to a solution of PPh_3 (4.65 g, 17.71 mmol) in CH_2Cl_2 (20 mL) over 20 min, maintaining the temperature below −30 °C. Compound **19** (3.10 g, 14.76 mmol) in CH_2Cl_2 (8 mL) was then added dropwise, and the mixture was allowed to warm to room temperature. It was diluted with hexanes (150 mL), whereupon it was filtered through a silica gel plug (10 g). The plug was washed with hexanes−ethyl acetate (30:1, 150 mL), and the combined filtrates were evaporated to provide compound **20** as a colorless oil containing <10% PPh_3 (3.65 g, 91%). This product was used for preparation of phenylselenenyl derivative **21**.

The experiment performed on a 1-mmol scale of **19** gave after chromatography on a silica gel column in hexanes−Et_2O (50:1 to 30:1) compound **20** (250 mg, 92%). ^1H NMR ($CDCl_3$) δ 1.32 (ddd, J = 12.0, 6.0, 2.0 Hz, 1H, H_2), 1.60 (dddd, J = 21.6, 10.8, 6.4, 2.0 Hz, 1H, H_1), 3.28 (dd, 2H, J = 7.6, 2.0 Hz, CH_2Br), 3.55 (poorly resolved dd, 1H), 3.77 (poorly resolved ddd, 1H, CH_2OBn), 4.54, 4.60 (AB, 2H, J = 12.2 Hz, CH_2Ph), 4.56 (ddd, 1H, J = 63.0, 6.0, 1.6 Hz, H_3), 7.30−7.37 (m, 5H, Ph). ^{13}C NMR 25.2 (d, J = 11.2), 26.6 (d, J = 10.5 Hz, C_1, C_2), 32.8 (CH_2Br), 66.6 (d, J = 7.5 Hz, CH_2OBn), 72.9 (CH_2Ph), 77.6 (d, J = 226.8 Hz, C_3), 128.0, 128.1, 128.7, 138.4 (Ph). ^{19}F NMR −219.63 (ddd, J = 64.0, 20.0, 5.6 Hz). ESI-MS (MeOH − KOAc) 311, 313 (M + K, 92.8 and 100.0). Anal. $C_{12}H_{14}BrFO$ (C, H).

_t_-2-Benzyloxymethyl-_t_-3-fluoro-_r_-1-(phenylselenenylmethyl)-cyclopropane (21). Ph_2Se_2 (1.71 g, 5.50 mmol) was refluxed in ethanol (25 mL) until a clear solution was obtained. After cooling, NaOH (4M, 2.75 mL, 11 mmol) was added, followed by $NaBH_4$ (0.835 g, 11 mmol). The reaction mixture was refluxed for 30 min and then it was cooled again to room temperature. A solution of compound **20** (3.0 g, 11 mmol) in ethanol (10 mL) was slowly added with stirring. After 3 h, water (125 mL) was added, the mixture was extracted with EtOAc, and the organic phase was dried ($MgSO_4$) and concentrated. The residue was chromatographed on a silica gel column in hexane−EtOAc (30:1) to give compound **21** (3.63 g, 94%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.20 (dd, J = 14.0, 6.4 Hz, H_2), 1.35−1.46 (m, 1H, H_1), 2.75−2.90 (poorly resolved 2 AB's, 2H, CH_2SePh), 3.49 (poorly resolved dd, 1H), 3.72 (dd, 1H, J = 10.4, 5.6 Hz, CH_2OBn), 4.47 (poorly resolved dd, 1H, J = 63.2, 6.8 Hz, H_3), 4.52, 4.59 (AB, 2H, J = 12.0 Hz, CH_2Ph, partly overlapped with H_3), 7.29−7.34 (m, 4H), 7.37 (br d, 4H), 7.56−7.58 (m, 2H, 2 × Ph). ^{13}C NMR 23.3, 25.8 (2d, J = 10.5 Hz, C_1, C_2), 28.3 (CH_2SePh), 67.1 (d, J = 8.3 Hz, CH_2OBn), 72.8 (CH_2Ph), 77.8 (d, J = 227.6 Hz, C_3), 127.6, 127.9, 128.0, 128.7, 129.4, 129.6, 133.8, 138.6 (2 × Ph). ^{19}F NMR −219.63 (ddd, J = 64.0, 21.5 Hz, <1 Hz). EI-MS 350, 348 (M, 1.90, 0.87), 91 ($PhCH_2$, 100.0). EI−HRMS calcd for $C_{18}H_{19}FO^{80}Se$, 350.0585; found, 350.0585. Anal. $C_{18}H_{19}FOSe$ (C, H).

***cis*-2-(Benzyloxymethyl)-3-fluoro-1-methylenecyclopropane (22).** Hydrogen peroxide (30%, 11.19 mL, 98.7 mmol) was added dropwise with stirring to a solution of compound **21** (3.49 g, 10 mmol) in THF (30 mL) at −60 °C. The mixture was allowed to warm to room temperature. After 14 h, water (100 mL) and EtOAc (100 mL) were added, the organic phase was washed with $NaHCO_3$ (5%) and water, it was dried ($MgSO_4$), and the solvents were evaporated. The residue was dissolved in toluene (15 mL), diisopropylethylamine (3.50 mL, 20 mmol) was added, and the reaction mixture was stirred at 80−85 °C for 2 h. After removal of solvents, the crude product was chromatographed on silica gel in hexanes−EtOAc (30:1) to give compound **22** (1.30 g, 68%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 2.08−2.17 (m, 1H, H_2), 3.60 (poorly resolved dd, 1H), 3.82 (ddd, 1H, J = 10.7, 5.6, 1.2 Hz, CH_2OBn), 4.56, 4.63 (AB, 2H, J = 12.0 Hz, CH_2Ph), 5.08 (dd, 1H, J = 69.0, 7.5 Hz, H_3), 5.66, 5.90 (2 poorly resolved t, 2H, CH_2=), 7.30−7.39 (2m, 5H, Ph). ^{13}C NMR 23.9 (d, J = 15.1 Hz, C_2), 66.5 (d, J = 2.0 Hz, CH_2OBn), 69.3 (d, J = 231.7 Hz, C_3), 72.8 (CH_2Ph), 110.8 (J = 3.0 Hz, CH_2=), 130.9 (C_1), 127.9, 128.0, 128.6, 138.6 (Ph). ^{19}F NMR −218.27 (dd, J = 68.9, <1 Hz). EI-HRMS calcd for $C_{12}H_{13}FO$, 192.0950; found, 192.0955. Anal. $C_{12}H_{13}FO$ (C, H).

***r*-2-Benzyloxymethyl-*c*, or *t*-1-bromo-*c*, or *t*-1-Bromomethyl)-*c*-2-fluorocyclopropane (23).** Pyridinium tribromide (3.20 g, 10.24 mmol) was added with stirring to a solution of compound **22** (1.2 g, 6.25 mmol) in CH_2Cl_2 (50 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature. After 16 h, it was diluted with EtOAc (100 mL), and the resultant solution was washed sequentially with saturated $Na_2S_2O_3$, $NaHCO_3$, and water. The organic phase was dried over $MgSO_4$, and the solvents were evaporated. The crude product was chromatographed on a silica gel column in hexanes−Et_2O (40:1) to afford compound **23** (1.82 g, 83%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.52−1.60 (m, 1H, H_2), 3.6−3.80 (cluster of m, 4H, CH_2Br and CH_2OBn), 4.47 (dd, 1H, J = 64.0, 7.6 Hz, H_3), 4.58 (s, CH_2Ph), 7.30−7.37 (cluster of m, 5H, Ph). ^{13}C NMR 29.1 (d, J = 9.7 Hz, C_2), 38.8 (d, J = 9.0 Hz, C_1), 40.5 (d, J = 1.8 Hz, CH_2Br), 66.9 (d, J = 5.9 Hz, CH_2-OBn), 73.3 (CH_2Ph), 74.6 (d, J = 235.8 Hz, C_3), 128.04, 128.06, 128.7, 138.1 (Ph). ^{19}F NMR −213.89 (dd, J = 64.0, 9.0 Hz).[20] ESI-MS (MeOH + KOAc) 389, 391, 393 (M + K, 52.7, 100.0, 51.8). Anal. $C_{12}H_{13}Br_2FO$ (C, H).

(*Z,E*)-9-{[*cis*-(3-Fluoro-2-benzyloxymethyl)cyclopropylidene]-methyl}adenine (25a + 26a) and *c*- or *t*-9-{[*c*- or *t*-1-Bromo-*c*-3-fluoro-*r*-2-(benzyloxymethyl)cyclopropyl]methyl}adenine (24). A mixture of adenine (287 mg, 2.2 mmol), compound **23** (704 mg, 2.0 mmol), and K_2CO_3 (1.66 g, 12.0 mmol) in DMF (10 mL) was stirred under N_2 for 4 h at 40 °C and then at 100−105 °C for 45 min. The mixture was rapidly cooled to −78 °C and then it was allowed to warm to room temperature. The insoluble solid was filtered off using a silica gel pad (5 g) that was washed with DMF (70 mL). The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel column in EtOAc−MeOH (40:1 to 30:1) to give the faster moving *E,Z*-isomeric mixture **25a** + **26a** and slower moving intermediate **24** (490 mg, 1.21 mmol). The elimination procedure was repeated with **24** using K_2CO_3 (0.83 g, 6 mmol) and DMF (5.0 mL). The product was chromatographed as described above to give *Z,E*-mixture **25a** + **26a** and bromo derivative **24** (120 mg, 14.8%). Both portions of **25a** + **26a** were combined and they were rechromatographed in EtOAc−MeOH (50:1 to 30:1) to give *E,Z*-isomers **25a** + **26a** (300 mg, 46%, *E*/*Z* = 1:1).

***E,Z*-Isomers 25a + 26a:** Mp 166−170 °C. UV λ_{max} 237 nm (ϵ 25 700), 280 (ϵ 8500). ^1H NMR ($CDCl_3$) δ 2.46 (br m, 1H, $H_{4'}$), 3.68, 3.85 (2m, 2H, $H_{5'}$), 4.47−4.63 (m, 2H, OCH_2Ph), 5.21, 5.37 (2 partially overlapped dd, J = 69.0, 69.6, 6.4 Hz, 1H, $H_{3'}$), 6.50, 6.53 (2s, 2H, NH_2), 7.26−7.36 (m, 5H, Ph), 7.59, 7.98 (2br s, 1H, $H_{1'}$), 8.38, 8.20, 8.75 (3s, 2H, H_2 and H_8). ^{19}F NMR −214.83 and −215.88 (2d, J = 70.0, <1 Hz). EI-MS 325 (M, 0.36), 91 (100.0). ESI-MS (MeOH) 326 (M + H, 100.0). EI−HRMS calcd for $C_{17}H_{16}N_5FO$, 325.1339; found, 325.1339.

Compound 24: Mp 161−163 °C. UV λ_{max} 209 nm (ϵ 21 800), 261 (ϵ 12 200). ^1H NMR ($CDCl_3$) δ 1.79−1.87 (m, 1H, $H_{4'}$), 3.65−3.75 (m, 2H, $H_{5'}$), 4.36−4.52 (overlapped m of CH_2Ph and $H_{1'}$, 4H), 4.92 (dd, 1H, J = 64.0, 7.2 Hz, $H_{3'}$), 5.85 (s, 2H, NH_2), 7.26−7.36 (m, 5H, Ph), 8.02, 8.32 (2s, 2H, H_2, H_8). ^{13}C NMR 26.9 (d, J = 10.5 Hz, $C_{4'}$), 39.1 (d, J = 9.8 Hz, $C_{2'}$), 51.9 ($C_{1'}$), 66.5 (d, J = 5.2 Hz, $C_{5'}$), 73.1 (d, J = 234.2 Hz, $C_{3'}$), 73.2 (CH_2 of Bn), 119.7 (C_5), 127.9, 128.0, 128.7, 138.0 (Ph), 140.9 (C_8), 150.4 (C_4), 153.4 (C_2), 155.8 (C_6). ^{19}F NMR −216.85 (ddd, J = 64.0, 9.2 Hz). ESI-MS 406, 408 (M + H, 97.0, 100.0).

(*E*)-9-{[*cis*-(3-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}adenine (9a) and (*Z*)-9-{[*cis*-(3-Fluoro-2-hydroxymethyl)-cyclopropylidene]methyl}adenine (10a). Boron trichloride (1 M in CH_2Cl_2, 5.7 mL, 5.7 mmol) was added to a solution of the *Z,E*-isomers **25a** + **26a** (230 mg, 0.71 mmol) in CH_2Cl_2 (55 mL) at −78 °C under N_2 over 10 min with stirring. The stirring was continued for 3.5 h at −78 °C, whereupon the reaction was quenched with methanol (25 mL) and $NaHCO_3$ (6.0 g, 71.4 mmol). The reaction mixture was allowed to warm to room temperature and it was stirred for 4 h. The insoluble solid was filtered off using a short silica gel pad (3.5 g) that was washed with CH_2Cl_2−MeOH (2:1, 60 mL).

After removal of solvents, the residue was chromatographed on a silica gel column in EtOAc–MeOH (50:1) to give the *E*-isomer **9a** (64 mg, 38%), followed by *Z*-isomer **10a** (56 mg, 33%).

***E*-Isomer 9a:** Mp 243–245 °C. UV max λ_{max} 237 nm (ϵ 24 500), 280 (ϵ 8300). ^{1}H NMR (DMSO-d_6) δ 2.48 (overlapped with DMSO-d_5, H$_{4'}$), 3.69 (br s, 2H, H$_{5'}$), 5.21 (poorly resolved t, 1H, OH), 5.41 (dd, 1H, J = 70.8 and 6.0 Hz, H$_{3'}$), 7.42 (s, 2H, NH$_2$), 7.89 (br s, 1H, H$_{1'}$), 8.20 (s, 1H, H$_2$), 8.72 (s, 1H, H$_8$). ^{13}C NMR 27.3 (d, J = 14.1 Hz, C$_{4'}$), 58.2 (C$_{5'}$), 69.1 (d, J = 229.1 Hz, C$_{3'}$), 111.7, 116.5 (C$_{1'}$, C$_{2'}$), 119.2 (C$_5$), 138.5 (C$_8$), 149.1 (C$_4$), 154.0 (C$_2$), 156.8 (C$_6$). ^{19}F NMR −215.47 (d, J = 70.0 Hz). EI-MS 235 (M, 28.0), 218 (M − OH, 77.9), 205 (M − CH$_2$O, 24.9), 135 (adenine, 91.7), 136 (adenine + H, 100.0). EI–HRMS calcd for $C_{10}H_{10}FN_5O$, 235.0869; found, 235.0865. Anal. $C_{10}H_{10}FN_5O\cdot0.1H_2O$ (C, H, N).

***Z*-Isomer 10a:** Mp 230–232 °C. UV λ_{max} 237 nm (ϵ 25 200), 280 (ϵ 8000). ^{1}H NMR (DMSO-d_6) δ 2.35–2.38 (br m, 1H, H$_{4'}$), 3.51 (m, 1H), 3.69 (poorly resolved td, 1H, H$_{5'}$), 5.03 (t, 1H, J = 5.2 Hz, OH), 5.61 (dd, 1H, J = 69.0 and 6.6 Hz, H$_{3'}$), 7.45 (s, 2H, NH$_2$), 7.55 (s, 1H, H$_{1'}$), 8.20 (s, 1H, H$_2$), 8.35 (s, 1H, H$_8$). ^{13}C NMR 26.3 (d, J = 14.2 Hz, C$_{4'}$), 58.1 (C$_{5'}$), 70.0 (d, J = 229.9 Hz, C$_{3'}$), 112.1, 114.9 (d, J = 3.2 Hz, C$_{1'}$, C$_{2'}$), 119.1 (C$_5$), 138.0 (C$_8$), 148.9 (C$_4$), 154.2 (C$_2$), 156.8 (C$_6$). ^{19}F NMR −215.45 (d, J = 70.0 Hz). EI-MS 235 (M, 8.4), 218 (M − OH, 100.0), 135 (adenine, 22.9), 136 (adenine + H, 48.0). EI-HRMS calcd for $C_{10}H_{10}FN_5O$, 235.0869; found, 235.0870. Anal. $C_{10}H_{10}FN_5O\cdot0.1\ H_2O$ (C, H, N).

(*E*)-2-Amino-6-chloro-9-{[*cis*-(3-fluoro-2-hydroxymethyl)-cyclopropylidene]methyl}purine (9e) and (*Z*)-2-Amino-6-chloro-9-{[*cis*-(3-fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}-purine (10e). A mixture of 2-amino-6-chloropurine (170 mg, 1.0 mmol), compound **23** (352 mg, 1.0 mmol), and K_2CO_3 (0.83 g, 6.0 mmol) in DMF (5 mL) was stirred at room temperature for 48 h and then at 100–105 °C for 45 min under N_2. The reaction mixture was worked up as described for the isomeric mixture **25a** + **26a**, but the elimination procedure was not repeated. The crude product was chromatographed in EtOAc–hexanes (1:1) to give the (*Z*,*E*)- isomeric mixture **25e** + **26e** (200 mg, 56%). Boron trichloride (1 M in CH_2Cl_2, 4.44 mL, 4.44 mmol) was added dropwise to a solution of compound **25e** + **26e** (200 mg, 0.56 mmol) in CH_2Cl_2 (40 mL) at −78 °C under N_2 over 10 min with stirring, which was continued for 5 h. The reaction was quenched with methanol (20 mL) and $NaHCO_3$ (4 g, 47.6 mmol). After 20 min, the mixture was allowed to warm to room temperature, and the stirring was continued for 4 h. The insoluble solid was filtered off using a silica gel pad (3.5 g), which washed with CH_2Cl_2–MeOH (2:1, 60 mL). The solvents were evaporated, and the residue was chromatographed in hexanes–EtOAc = 1.5:1 to 1:1 to give the faster moving *E*-isomer **9e** (70 mg, 46.5%), followed by *Z*-isomer **10e** (70 mg, 46.5%).

***E*-Isomer 9e:** Mp 209–211 °C. UV λ_{max} 239 nm (ϵ 26 300), 310 (ϵ 7400). ^{1}H NMR (DMSO-d_6) δ 2.44–2.48 (1H, H$_{4'}$, overlapped with DMSO-d_5), 3.66 (t, 2H, J = 6.0 Hz, H$_{5'}$), 5.15 (t, 1H, J = 5.4 Hz, OH), 5.41 (dd, 1H, J = 70.4, 6.4 Hz, H$_{3'}$), 7.10 (s, 2H, NH$_2$), 7.71 (s, 1H, H$_{1'}$), 8.66 (s, 1H, H$_8$). ^{13}C NMR 27.6 (d, J = 13.5 Hz, C$_{4'}$), 58.2 (C$_{5'}$), 69.1 (d, J = 229.1 Hz, C$_{3'}$), 112.5, 116.0 (2d, J = 2 Hz, C$_{1'}$, C$_{2'}$), 123.8 (C$_5$), 140.5 (C$_8$), 150.5 (C$_4$), 153.4 (C$_2$), 160.9 (C$_6$). ^{19}F NMR −215.58 (d, J = 71.9 Hz). EI-MS 269, 271 (M, 17.6, 6.1), 252, 254 (M − OH, 16.2, 5.6), 169, 171 (2-amino-6-chloropurine, 37.9, 21.4), 170, 172 (2-amino-6-chloropurine + H, 100.0, 31.5). EI-HRMS calcd for $C_{10}H_9{}^{35}ClFN_5O$, 269.0480; found, 269.0476.

***Z*-Isomer 10e:** Mp 193–195 °C. UV λ_{max} 239 nm (ϵ 27 200), 310 (ϵ 7200). ^{1}H NMR (DMSO-d_6) δ 2.35 (1H, poorly resolved dd, H$_{4'}$), 3.51 (dt, 1H, J = 11.6, 7.4 Hz, H$_{5'}$) and 3.66 (dt, 1H, J = 11.2, 5.6 Hz, H$_{5'}$), 5.0 (t, 1H, J = 5.8 Hz, OH), 5.62 (dd, 1H, J = 69.0, 6.6 Hz, H$_{3'}$), 7.12 (s, 2H, NH$_2$), 7.37 (s, 1H, H$_{1'}$), 8.31 (s, 1H, H$_8$). ^{13}C NMR 26.4 (d, J = 14.2 Hz, C$_{4'}$), 58.0 (C$_{5'}$), 70.0 (d, J = 229.8 Hz, C$_{3'}$), 113.0, 114.5 (d, J = 3.5 Hz, C$_{1'}$, C$_{2'}$), 123.8 (C$_5$), 140.2 (C$_8$), 150.6 (C$_4$), 153.2 (C$_2$), 161.0 (C$_6$). ^{19}F NMR −216.25 (d, J = 68.9 Hz). EI-MS 269, 271 (M, 14.9, 5.2), 252, 254 (M − OH, 14.9, 4.7), 169, 171 (2-amino-6-chloropurine, 39.0, 21.1), 170, 172 (2-amino-6-chloropurine + H, 100.0, 32.4). EI-HRMS calcd for $C_{10}H_9{}^{35}ClFN_5O$, 269.0480; found, 269.0484.

(*E*)-9-{[*cis*-(3-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}guanine (9b). A solution of the *E*-isomer **9e** (90 mg, 0.33 mmol) in 80% HCO_2H (80%, 10 mL) was heated at 80 °C with stirring for 4 h. After cooling, formic acid and water were evaporated in vacuo, the crude product was dissolved in methanol (30 mL), and NH_3 (20% in methanol, 10 mL) was added at 0 °C. The reaction mixture was stirred for 4 h at 0 °C. The solvents were removed to give the *E*-isomer **9b** (80 mg, 95%), mp > 300 °C. UV λ_{max} 242 λ_{nm} (ϵ 26 300), 273 (ϵ 10 200). ^{1}H NMR (DMSO-d_6) δ 2.39 (m, 1H, H$_{4'}$), 3.65 (d, 2H, J = 5.6 Hz, H$_{5'}$), 5.16 (s, 1H, OH), 5.36 (dd, 1H, J = 71.2, 6.2 Hz, H$_{3'}$), 6.62 (s, 2H, NH$_2$), 7.58 (s, 1H, H$_{1'}$), 8.29 (s, 1H, H$_8$), 10.69 (br s, 1H, NH). ^{13}C NMR 27.1 (d, J = 14.2 Hz, C$_{4'}$), 58.1 (C$_{5'}$), 69.0 (d, J = 229.0 Hz, C$_{3'}$), 111.3 (d, J = 3.0 Hz), 116.2 (d, J = 2.2 Hz, C$_{1'}$, C$_{2'}$), 117.0 (C$_5$), 134.8 (C$_8$), 150.8 (C$_4$), 154.8 (C$_2$), 157.3 (C$_6$). ^{19}F NMR −215.43 (d, J = 71.9 Hz). ESI-MS (MeOH) 252 (M + H, 100.0). Anal. $C_{10}H_{10}FN_5O_2$ (C, H, N).

(*Z*)-9-{[*cis*-3-Fluoro-2-(hydroxymethyl)cyclopropylidene]-methyl}guanine (10b). The procedure described above for **9b** was performed with the *Z*-isomer **10e** (90 mg, 0.33 mmol) to give compound **10b** (80 mg, 95%), mp > 300 °C. UV λ_{max} (EtOH) 242 nm (ϵ 27 900), 274 (ϵ 10 800). ^{1}H NMR (DMSO-d_6) δ 2.31 (m, 1H, H$_{4'}$), 3.49 (dd, 1H, J = 10.8, 8.8 Hz), 3.65 (dd, 1H, J = 10.4, 5.6 Hz, H$_{5'}$), 4.99 (br s, 1H, OH), 5.58 (dd, 1H, J = 68.8, 5.6 Hz, H$_{3'}$), 6.67 (s, 2H, NH$_2$), 7.26 (s, 1H, H$_{1'}$), 7.91 (s, 1H, H$_8$), 10.88 (br s, 1H, NH). ^{13}C NMR 26.2 (d, J = 15.2 Hz, C$_{4'}$), 58.0 (C$_{5'}$), 69.0 (d, J = 231.7 Hz, C$_{3'}$), 111.8, 114.8 (d, J = 3.0 Hz, C$_{1'}$, C$_{2'}$), 117.1 (C$_5$), 134.3 (C$_8$), 150.6 (C$_4$), 155.0 (C$_2$), 157.3 (C$_6$). ^{19}F NMR −216.82 (d, J = 68.5 Hz). ESI-MS (MeOH − KOAc) 252 (M + H, 90.0), 290 (M + K, 100.0), 503 (2M + H, 33.0), 541 (2M + K, 50.0). Anal. $C_{10}H_{10}FN_5O_2$ (C, H, N).

Ethyl *t*-3-Fluoro-*t*-2-hydroxymethylcyclopropane-*r*-1-carboxylate (27). Boron trichloride (1.0 M in CH_2Cl_2, 40 mL, 40 mmol) was added dropwise with stirring to ester **15** (5.0 g, 19.8 mmol) in CH_2Cl_2 at −78 °C. The reaction mixture was stirred for 1.5 h at −78 °C, 15 min at 0 °C, it was then re-cooled to −78 °C, and $NaHCO_3$ (6.72 g, 80 mmol) was added. The reaction mixture was warmed to room temperature and it was stirred for 4 h. Water (200 mL) was added, and the mixture was extracted with CH_2Cl_2 (5 × 50 mL). The organic phase was dried over $MgSO_4$, solvent was evaporated, and the crude product was chromatographed on a silica gel column in hexanes/Et_2O = 10:1 to 3:1 to give ester **27** (2.66 g, 83%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, J = 7.4 Hz, CH$_3$), 1.85 (dd, 1H, J = 14.0, 7.6 Hz, H$_2$), 1.94 (ddd, 1H, J = 18.4, 6.4, 1.6 Hz, H$_1$), 2.79 (br s, 1H, OH), 3.84, 3.69 (J_{AB} = 11.8 Hz), 3.83, 3.71 (J_{AB} = 11.4 Hz, 2 overlapped AB's, 2H, CH$_2$OH), 4.08 (2q, 2H, J = 7.6 Hz, CH$_2$ of Et), 4.88 (ddd, 1H, J = 64.0, 6.4, 1.6 Hz, H$_3$). ^{13}C NMR 14.3 (CH$_3$ of Et), 24.9 (d, J = 12.7 Hz, C$_1$), 28.6 (d, J = 8.2 Hz, C$_1$, C$_2$), 58.6 (d, J = 9.0 Hz, CH$_2$OH), 61.4 (CH$_2$ of Et), 76.4 (d, J = 229.8 Hz, C$_3$), 171.0 (C=O). ^{19}F NMR −221.34 (ddd, J = 64.4, 18.3, 7.7 Hz). EI-MS 163 (M + H, 0.4), 145 (M − OH, 1.2), 131 (M − CH$_2$OH, 44.5), 73 (100.0). EI-HRMS calcd for $C_7H_{10}FO_2$ (M − OH), 145.0665; found, 145.0665. Anal. $C_7H_{10}FO_2$ (C, H).

Ethyl *t*-2-Fluoro-*t*-3-(2-nitrophenylselenenyl)cyclopropane-*r*-1-carboxylate (28). Tributylphosphine (2.49 g, 14.8 mmol) was added to a mixture of ester **27** (2.0 g, 12.33 mmol) and 2-nitrophenyl selenocyanate[18] (3.36 g, 14.8 mmol) in THF (40 mL) at room temperature with stirring, which was continued for 2 h. The solvent was evaporated, and the crude product was chromatographed on a silica gel column using hexanes/Et_2O (10:1 to 5:1) to give product **28** (3.93 g, 92%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7.2 Hz, CH$_3$), 1.91 (dd, 1H, J = 13.8, 6.6 Hz, H$_2$), 1.98 (ddd, 1H, J = 18.2, 5.6, 2.4 Hz, H$_1$), 3.06, 3.11 (J_{AB} = 12.2 Hz), 3.08, 3.14 (J_{AB} = 12.0 Hz, 2 overlapped AB, 2H, CH$_2$Se), 4.12 (2 overlapped q, 2H, J = 7.2 Hz, CH$_2$ of Et), 4.94 (ddd, 1H, J = 64.4, 6.4, 1.6 Hz, H$_3$), 7.33 (ddd, 1H, J = 8.0, 5.6, 2.4 Hz), 7.52 (br s, 1H), 7.54 (dd, 1H, J = 8.0, 1.6 Hz), 8.27 (d, 1H, J = 8.4 Hz, 2-NO$_2$Ph). ^{13}C NMR 14.4 (CH$_3$), 21.4 (d, J = 7.5 Hz, CH$_2$Se),

25.3 (d, J = 8.2 Hz), 28.0 (d, J = 11.8 Hz), 61.5 (CH_2 of Et), 76.8 (d, J = 231.3 Hz, C_3), 126.0, 126.8, 129.2, 133.0, 134.1 (2-NO_2-Ph), 170.3 (C=O). ^{19}F NMR −220.22 (ddd, J = 64.0, 18.1, 6.0 Hz). EI-MS 347 (M + H, 5.4), 346 (M, 1.4), 125 (100.0). EI-HRMS calcd for $C_{13}H_{14}FNO_4{}^{80}Se$, 347.0072; found, 347.0066.

***t*-3-Fluoro-*t*-2-(2-nitrophenylselenenylmethyl)cyclopropane-*r*-1-methanol (29).** DIBALH in hexane (1 M, 21.67 mL, 21.67 mmol) was added to a solution of ester **28** (3.0 g, 8.67 mmol) in hexane (30 mL) at 0 °C over a period of 10 min under N_2. The stirring was continued for 1 h. The reaction was quenched by a dropwise addition of HCl (5%, 50 mL) and then it was extracted with Et_2O (4 × 30 mL). The combined organic phase was washed successively with saturated $NaHCO_3$ (2 × 30 mL) and water (2 × 30 mL). The solvent was evaporated, and the crude product was chromatographed on a silica gel column using hexane/EtOAc (10:1 to 5:1) to give product **29** as a colorless oil (2.49 g, 95%). ^{1}H NMR ($CDCl_3$) δ 1.27 (ddd, 1H, J = 13.6, 6.8, 1.6 Hz, H_2), 1.44 (ddd, 1H, J = 21.2, 12.8, 6.4 Hz, H_1), 1.68 (br s, 1H, OH), 3.08, 3.12 (J_{AB} = 11.6 Hz), 3.06, 3.14 (J_{AB} = 12.0 Hz, 2 overlapped AB, 2H, CH_2Se), 3.51−3.60 (m, 2H, CH_2O), 4.65 (ddd, 1H, J = 64.0, 6.4, 2.4 Hz, H_3), 7.32 (ddd, 1H, J = 8.0, 5.6, 2.4 Hz), 7.54 (dd, 2H, J = 8.0, 1.6 Hz), 8.29 (d, 1H, J = 8.8 Hz, 2-NO_2Ph). ^{13}C NMR 19.8 (d, J = 10.4 Hz, C_1), 23.1 (d, J = 7.5 Hz, CH_2Se), 28.3 (d, J = 9.0 Hz, C_2), 61.8 (CH_2O), 76.0 (d, J = 235.4 Hz, C_3), 125.8, 126.7, 129.4, 134.0 (2-NO_2Ph). ^{19}F NMR −223.87 (ddd, J = 64.0, 21.5, 3.0 Hz). EI-MS 303 (M + H, 4.9), 186 (100.0). EI-HRMS calcd for $C_{11}H_{12}FNO_3{}^{78}Se$, 302.9974; found, 302.9966.

***r*-1-Benzyloxymethoxymethyl-*t*-2-fluoro-*t*-3-(2-nitrophenylselenenylmethyl)cyclopropane (30).** Sodium hydride (50%, 0.63 g, 13.1 mmol) was added to a solution of compound **29** (2.0 g, 6.6 mmol) in THF (30 mL) at 0 °C. The reaction mixture was stirred for 5 h and then 1 h at room temperature. Benzyl bromide (2.57 g, 15 mmol) was added at 0 °C, the reaction mixture was slowly warmed to room temperature, and it was stirred for 16 h. The solvent was evaporated, and the crude product was chromatographed on a silica gel column in hexane/Et_2O (30:1 to 5:1) to give compound **30** (1.84 g, 71%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.26 (m, 1H, H_2), 1.46 (ddd, 1H, J = 20.9, 6.5, 2.5 Hz, H_1), 3.10 (d, 2H, J = 7.6 Hz, CH_2Se), 3.41 (poorly resolved dd, 1H, J = 6.8, 2.0 Hz, CH_2O), 4.51, 4.48 (AB, 2H, J_{AB} = 11.8 Hz, CH_2Ph), 4.63 (ddd, 1H, J = 64.0, 6.4, 2.4 Hz, H_3), 7.28−7.36 (m, 6H), 7.48−7.56 (m, 2H), 8.30 (dd, 1H, J = 8.0, 1.6 Hz, Ph + 2-NO_2Ph). ^{13}C NMR 20.0 (d, J = 11.3 Hz, C_1), 23.1 (d, J = 6.6 Hz, CH_2Se), 25.9 (d, J = 9.8 Hz, C_2), 68.6 (CH_2O), 72.9 (CH_2Ph), 76.3 (d partly overlapped with $CDCl_3$, J = 240.0 Hz, C_3), 125.7, 126.7, 127.8, 128.0, 128.7, 129.4, 133.9 (Ph + 2-NO_2Ph). ^{19}F NMR −223.69 (ddd, J = 65.0, 21.1, 4.5 Hz). EI-MS 395 (M, 0.29), 91 (100.0). EI-HRMS calcd for $C_{18}H_{18}FNO_3{}^{80}Se$, 395.0436; found, 395.0434.

***trans*-3-Benzyloxymethyl-2-fluoromethylenecyclopropane (31).** Hydrogen peroxide (30%, 1.6 mL, 15.66 mmol) was added dropwise to a solution of compound **30** (1.8 g, 4.58 mmol) in THF (20 mL) at 0 °C. The reaction mixture was stirred for 1 h and then 12 h at room temperature, whereupon it was partitioned between water (50 mL) and Et_2O (100 mL). The organic phase was washed with water (2 × 50 mL), $Na_2S_2O_3$ (5%, 2 × 20 mL), and $NaHCO_3$ (5%, 2 × 50 mL), it was dried over $MgSO_4$, and the solvent was evaporated. A solution of the crude product in toluene (25 mL) was heated at 80−85 °C for 6 h. The solvent was evaporated, and the residue was chromatographed on a silica gel column in hexanes/Et_2O (50:1) to give compound **31** (640 mg, 73%) as an oil. ^{1}H NMR ($CDCl_3$) δ 2.20−2.22 (m, 1H, H_2), 3.35−3.39 (poorly resolved dd, 1H), 3.37 (poorly resolved dd, 1H), 3.49 (m, 1H, CH_2O), 4.55 (s, 2H, $PhCH_2$), 4.76 (d, 1H, J = 67.2 Hz, H_3), 5.70, 5.92 (2s, 2H, CH_2=), 7.30−7.36 (m, 5H, Ph). ^{13}C NMR 25.0 (d, J = 13.0 Hz, C_2), 68.7 (CH_2O), 70.6 (d, J = 230.3 Hz, C_3), 72.8 ($PhCH_2$), 111.4 (CH_2=), 127.9, 128.0, 128.7, 131.8, 138.2 (C_1 + Ph). ^{19}F NMR −203.67 (dd, J = 67.4, 13.9 Hz). ESI-MS 215 (M + Na, 42.3), 91 ($PhCH_2$, 100.0).

***r*-2-Benzyloxymethyl-*c,t*-1-bromo-*c,t*-1-bromomethyl-*t*-fluorocyclopropane (32).** Pyridinium tribromide (2.0 g, 6.27 mmol) was added to a solution of compound **31** (600 mg, 3.13 mmol) in CH_2Cl_2 (15 mL) at −20 °C with stirring. The reaction mixture was warmed to room temperature, and the stirring was continued for 10 h. After removal of the solvent, the crude mixture was chromatographed on a silica gel column in hexanes/Et_2O (50:1 to 20:1) to give product **32** (910 mg, 83%). ^{1}H NMR ($CDCl_3$) δ 1.73, 2.21 (2m, 1H, H_1), 3.58−3.72 (m, 2H, CH_2Br), 3.76−3.97 (m, 2H, CH_2O), 4.45 (dd, J = 63.8, 4.1 Hz), 4.82 (dd, J = 63.0, 3.6 Hz, 1H, H_3), 4.51, 4.52, 4.59 (3s, 2H, CH_2Ph), 7.33−7.40 (m, 5H, Ph). ^{13}C NMR 33.2, 35.8 (2d, J = 11.1, 10.0 Hz, C_2), 38.0−38.1 (2 overlapped d, CH_2Br), 38.5, 40.3 (2d, J = 10.1 Hz, C_1), 64.37, 68.90, 73.2, 73.5 (CH_2O), 76.1, 81.3 (2d, J = 241.7, 243.7 Hz, C_3), 77.0, 77.4, 77.8 (CH_2Ph), 128.1, 128.14, 128.3, 128.8, 128.85, 137.5, 138.0 (Ph). ^{19}F NMR −201.56 (dd, J = 64.0, 20.0 Hz), −207.76 (dd, J = 62.9, 22.8 Hz). ESI-MS 373, 375, 377 (M + Na, 49.0, 100.0, 48.0).

***c,t*-9-{[*c,t*-1-Bromo-*t*-3-fluoro-*r*-2-(benzyloxymethyl)cyclopropyl]methyl}adenine (33a).** A mixture of K_2CO_3 (280 mg, 2.22 mmol), adenine (50 mg, 0.37 mmol), and compound **32** (120 mg, 0.34 mmol) was stirred in DMF (2.0 mL) at room temperature for 8 h and at 40 °C for 2 h under N_2. The insoluble solid was filtered off, and DMF was evaporated in vacuo. The residue was chromatographed on a silica gel column using EtOAc−MeOH (100:0 to 20:1) to give compound **33a** (0.12 g, 86%), mp 112−115 °C. UV λ_{max} 261 nm (ϵ 11 700), 203 (ϵ 23 400). ^{1}H NMR ($CDCl_3$) δ 2.19−2.33 (1H, m, $H_{4'}$), 3.46−3.51, 3.57−3.67 (2m, 2H, $H_{5'}$), 4.43−5.00 (overlapped m, 5H, CH_2Ph, $H_{1'}$, $H_{3'}$), 5.84 (s, 2H, NH_2), 7.21−7.34 (m, 5H, Ph), 8.03, 8.06, 8.33 and 8.37 (4s, 1H, H_2, H_8). ^{19}F NMR −203.35 (ddd, J = 64.0, 19.6, 4.5 Hz), −206.81 (dd, J = 62.5, 23.0 Hz). ESI-MS 406, 408 (M + H, 94.0, 100.0), 428, 430 (M + Na, 24.3, 27.3).

***(E)*-{[*trans*-(3-Fluoro-2-benzyloxymethyl)cyclopropylidene]methyl}adenine (34a) and *(Z)*-{[*trans*-(3-Fluoro-2-benzyloxymethyl)cyclopropylidene]methyl}adenine (35a).** A mixture of compound **33a** (0.40 g, 0.98 mmol) and K_2CO_3 (410 mg, 3 mmol) in DMF (5 mL) was stirred for 55 min at 100 °C. The mixture was cooled to 0 °C, and the insoluble portion was filtered off using a silica gel (3.5 g) pad that was washed with DMF (10 mL). The solvent was evaporated, and the residue was chromatographed on a silica gel column using hexanes/EtOAc (1:4 to 100% EtOAc) to give the *E,Z*-isomeric mixture **34a** + **35a** (50 mg, 16%), followed by starting material **33a** (260 mg, 59%). The latter was subjected to another two cycles of elimination and chromatography to give **34a** + **35a** (105 mg, 33%). The isomeric mixture **34a** + **35a** (390 mg, 1.2 mmol, *E*/*Z* = 1.5:1) combined from several experiments was chromatographed on silica gel using hexanes−EtOAc = 1:1 to 1:2 to 100% EtOAc to give the faster moving *E*-isomer **34a** (220 mg, 56%), followed by the *Z*-isomer **35a** (150 mg, 38%).

***E*-Isomer 34a:** Mp 173−175 °C. UV λ_{max} 280 nm (ϵ 9500), 238 (ϵ 26 600). ^{1}H NMR ($CDCl_3$) δ 2.62 (m, 1H, $H_{4'}$), 3.30 (t, 1H, J = 9.2 Hz), 3.91 (dd, 1H, J = 9.6, 5.6 Hz, $H_{5'}$), 4.55 (s, 2H, CH_2-Ph), 4.97 (d, 1H, J = 68.8 Hz, $H_{3'}$), 6.08 (s, 2H, NH_2), 7.26−7.33 (m, 5H, Ph), 7.99 (s, 1H, $H_{1'}$), 8.38, 8.76 (2s, 2H, H_2, H_8). ^{13}C NMR ($CDCl_3$) δ 26.6 (d, J = 13.4 Hz, $C_{4'}$), 68.8 (d, J = 234.3 Hz, $C_{3'}$), 68.9 (d, J = 3.7 Hz, $C_{5'}$), 73.6 (CH_2Ph), 111.4 (d, J = 4.4 Hz), 117.0 (d, J = 3.7 Hz, $C_{1'}$, $C_{2'}$), 119.6 (C_5), 128.0, 128.3, 128.8, 137.5 (Ph), 138.3 (C_8), 149.3 (C_4), 153.7 (C_2), 155.8 (C_6). ^{19}F NMR −201.70 (dd, J = 68.9, 10.5 Hz). ESI-MS 326 (M + H, 100.0), 348 (M + Na, 31.6).

***Z*-Isomer 35a:** Mp 159−162 °C. UV λ_{max} 279 nm (ϵ 10 000), 237 (ϵ 29 400). ^{1}H NMR ($CDCl_3$) δ 2.58 (m, 1H, $H_{4'}$), 3.49 (poorly resolved dd, 1H), 3.60 (m, 1H, $H_{5'}$), 4.56 (s, 2H, CH_2Ph), 5.09 (d, 1H, J = 68.0 Hz, $H_{3'}$), 6.11 (s, 2H, NH_2), 7.29−7.34 (m, 5H, phenyl), 7.63 (s, 1H, $H_{1'}$), 8.19, 8.38 (2s, 2H, H_2, H_8). ^{13}C NMR 25.2 (d, J = 12.7 Hz, $C_{4'}$), 68.1 (d, J = 3.7 Hz, $C_{5'}$), 70.0 (d, J = 229.8 Hz, $C_{3'}$), 73.2 (CH_2Ph), 111.9, 116.1 ($C_{1'}$, $C_{2'}$), 119.6 (C_5), 128.0, 128.2, 128.8, 137.8 (Ph), 138.0 (C_8), 149.0 (C_4), 153.9 (C_2), 155.8 (C_6). ^{19}F NMR −202.17 (dd, J = 68.3, 10.0 Hz). ESI-MS 326 (M + H, 100.0), 348 (M + Na, 48.2).

***(E)*-{[*trans*-(3-Fluoro-2-hydroxymethyl)cyclopropylidene]methyl}adenine (11a).** A solution of BCl_3·SMe_2 complex in CH_2Cl_2 (2.0 M, 1.37 mL, 2.74 mmol) was added dropwise to a solution

of compound **34a** (150 mg, 0.46 mmol) in CH_2Cl_2 (10 mL) at room temperature with stirring, which was continued for 5 h. The reaction was quenched by adding $NaHCO_3$ (4.0 g, 47.6 mmol) and methanol (15 mL) at −78 °C and then it was stirred for 2 h at room temperature. The insoluble solid was filtered off through a silica gel (2.5 g) pad, and it was washed with CH_2Cl_2−MeOH (2:1, 50 mL). The filtrate was concentrated, and the residue was chromatographed on a silica gel column to give product **11a** (80 mg, 74%), mp 218−220 °C. UV λ_{max} 280 nm (ϵ 11 000), 237 (ϵ 32 600). ^{1}H NMR (DMSO-d_6) δ 2.60−263 (m, 1H, $H_{4'}$), 3.48, 3.67 (2m, $H_{5'}$), 5.17 (t, 1H, J = 5.6 Hz, OH), 5.21 (d, 1H, J = 70.4 Hz, $H_{3'}$), 7.41 (s, 2H, NH_2), 7.93 (s, 1H, $H_{1'}$), 8.19 (s, 1H, H_2), 8.71 (s, 1H, H_8). ^{13}C NMR 29.5 (d, J = 11.2 Hz, $C_{4'}$), 60.2 (d, J = 3.7 Hz, $C_{5'}$), 70.0 (d, J = 229.8 Hz, $C_{3'}$), 112.5 (d, J = 2.9 Hz), 116.8 (d, J = 3.7 Hz, $C_{1'}$, $C_{2'}$), 119.2 (C_5), 138.4 (C_8), 149.1 (C_4), 154.0 (C_2), 156.8 (C_6). ^{19}F NMR −200.81 (dd, J = 70.2, 12.2). EI-MS 235 (M, 14.9), 218 (M − OH, 100.0), 136 (Ade + H, 48.4), 135 (Ade, 36.0). EI-HRMS calcd for $C_{10}H_{10}N_5FO$, 235.0869; found, 235.0871. Anal. $C_{10}H_{10}FN_5O$ (C, H, N).

(*Z*)-9-{[*trans*-(3-Fluoro-2-hydroxymethyl)cyclopropylidene]methyl}adenine (12a). The procedure described above for the *E*-isomer **11a** was repeated with the *Z*-isomer **35a** to give compound **12a** (85 mg, 79%), mp 239−242 °C. UV λ_{max} 280 nm (ϵ 11 300), 237 (ϵ 31 100). ^{1}H NMR (DMSO-d_6) δ 2.42−2.45 (m, 1H, $H_{4'}$), 3.37 (poorly resolved dd, 1H), 3.51−3.54 (m, 1H, $H_{5'}$), 5.02 (br s, 1H, OH), 5.36 (d, 1H, J = 68.8 Hz, $H_{3'}$), 7.44 (s, 2H, NH_2), 7.58 (s, 1H, $H_{1'}$), 8.21 (s, 1H, H_2), 8.34 (s, 1H, H_8). ^{13}C NMR 27.6 (d, J = 11.1 Hz, $C_{4'}$), 60.3 (d, J = 4.4 Hz, $C_{5'}$), 70.8 (d, J = 229.8 Hz, $C_{3'}$), 112.7 (d, J = 2.2 Hz), 115.7 (d, J = 2.3 Hz, $C_{1'}$, $C_{2'}$), 119.2 (C_5), 138.1 (C_8), 148.9 (C_4), 154.0 (C_2), 156.7 (C_6). ^{19}F NMR −201.15 (dd, J = 68.9, 10.9 Hz). EI-MS 235 (M, 17.1), 218 (M − OH, 100.0), 136 (adenine + H, 37.7), 135 (adenine, 28.1). EI-HRMS calcd for $C_{10}H_{10}N_5FO$, 235.0869; found, 235.0871. Anal. $C_{10}H_{10}FN_5O$ (C, H, N).

2-Amino-6-chloro-*c*,*t*-9-[*c*,*t*-1-bromo-*t*-3-fluoro-*r*-2-(benzyloxymethyl)cyclopropyl]methyl}purine (33e). A mixture of 2-amino-6-chloropurine (580 mg, 3.43 mmol), compound **32** (1.2 g, 3.41 mmol) in DMF (20 mL), and K_2CO_3 (1.4 g, 10.1 mmol) was stirred for 5 h at 40 °C under N_2. The insoluble solid was filtered off using a short silica gel pad, which was washed with DMF (100 mL). The solvent was evaporated in vacuo at room temperature, and the residue was chromatographed on a silica gel column in hexanes−EtOAc (3:1 to 2:1) to give compound **33e** (1.30 g, 87%), mp 75−79 °C. UV λ_{max} 311 nm (ϵ 10 700), 244 (ϵ 11 100), 223 (ϵ 34 700). ^{1}H NMR ($CDCl_3$) δ 2.14−2.27 (cluster of m, 1H, $H_{4'}$), 3.46, 3.65 and 3.90 (3m, 2H, $H_{5'}$), 4.42−4.57 (s and m), 4.69−4.91 (m, 5H, $H_{1'}$, $H_{3'}$, CH_2Ph), 5.16, 5.24 (2s, 2H, NH_2), 7.21−7.37 (2m, 5H, Ph), 7.98, 8.00 (2s, 1H, H_8). ^{19}F NMR −203.69 (dd, J = 64.0, 20.0 Hz), −206.74 (dd, J = 62.5, 24.5 Hz). ESI-MS 440, 442, 444 (M + H, 76.9, 100.0, 24.3), 462, 464, 466 (M + Na, 65.7, 87.6, 18.3).

(*E*)-2-Amino-6-chloro-9-{[*trans*-(3-fluoro-2-(benzyloxymethyl)-cyclopropylidene]methyl}purine (34e) and (*Z*)-2-Amino-6-chloro-9-{[*trans*-(3-fluoro-2-benzyloxymethyl)-cyclopropylidene]methyl}-purine (35e). A mixture of compound **33e** (700 mg, 1.59 mmol) and K_2CO_3 (660 mg, 4.78 mmol) in DMF (8 mL) was stirred for 45 min at 100 °C. After the workup (see **33e**), the crude product was chromatographed on a silica gel column using hexanes−EtOAc (3:1 to 2:1) to give the *E*-isomer **34e** (70 mg, 18%) followed by *Z*-isomer **35e** (110 mg, 29%) and unreacted starting material **33e** (230 mg, 33%).

***E*-Isomer 34e:** Mp 93−95 °C. UV λ_{max} 307 nm (ϵ 9000), 226 (ϵ 25 800). ^{1}H NMR ($CDCl_3$) δ 2.59−2.61 (m, 1H, $H_{4'}$), 3.20 (t, 1H, J = 9.0 Hz, $H_{5'}$), 3.87 (dd, 1H, J = 10.0, 1.6 Hz, H_5), 4.55 (s, 2H, CH_2Ph), 4.94 (d, J = 68.8 Hz, $H_{3'}$), 5.24 (s, 2H, NH_2), 7.26−7.34 (m, 5H, phenyl), 7.81 (s, 1H, $H_{1'}$), 8.73 (s, 1H, H_8). ^{13}C NMR 26.7 (d, J = 12.7 Hz, $C_{4'}$), 68.5 (d, J = 3.0 Hz, $C_{5'}$), 68.8 (d, J = 235.1 Hz, $C_{3'}$), 73.5 (CH_2Ph), 111.7 (d, J = 3.7 Hz), 116.5 (d, J = 2.9 Hz, $C_{1'}$, $C_{2'}$), 125.5 (C_5), 128.1, 128.3, 128.8, 137.4 (Ph), 139.9 (C_8), 151.8 (C_4), 152.8 (C_2), 159.6 (C_6). ^{19}F NMR −201.82 (dd, J = 68.5, 12.1 Hz). ESI-MS 360, 362 (M + H, 54,2, 29.2), 382, 384 (M + Na, 100.0, 32.7), 741, 743 (2M + Na, 49.4, 35.7).

***Z*-Isomer 35e:** Mp 99−101 °C. UV λ_{max} 307 nm (ϵ 8500), 226 (ϵ 24 300). ^{1}H NMR ($CDCl_3$) δ 2.54−2.60 (m, 1H, $H_{4'}$), 3.50−3.60 (m, 2H, $H_{5'}$), 4.56 (s, 2H, CH_2Ph), 5.07 (d, J = 68.4 Hz, $H_{3'}$), 5.21 (s, 2H, NH_2), 7.30−7.38 (m, 5H, phenyl), 7.45 (s, 1H, $H_{1'}$), 8.12 (s, 1H, H_8). ^{13}C NMR 25.2 (d, J = 12.7 Hz, $C_{4'}$), 68.1 (d, J = 4.5 Hz, $C_{5'}$), 69.9 (d, J = 235.7 Hz, $C_{3'}$), 73.2 (CH_2Ph), 112.2 (d, J = 2.2 Hz), 115.6 (d, J = 2.9 Hz, $C_{1'}$, $C_{2'}$), 125.5 (C_5), 128.0, 128.2, 128.8, 137.8 (Ph), 139.5 (C_8), 152.0 (C_4), 152.5 (C_2), 159.7 (C_6). ^{19}F NMR −202.05 (dd, J = 65.5, 12.2 Hz). ESI-MS 360, 362 (M + H, 54.2, 28.6), 382, 384 (M + Na, 100.0, 32.7), 741, 743 (2M + Na, 49.1, 35.7).

(*E*)-2-Amino-6-chloro-9-{[*trans*-(3-fluoro-2-hydroxymethyl)-cyclopropylidene]methyl}purine (11e). The procedure described for adenine analogue **11a** was followed with the *E*-isomer **34e** (200 mg, 0.56 mmol) to give compound **11e** (110 mg, 74%), mp 209−211 °C. UV λ_{max} 311 nm (ϵ 8300), 239 (ϵ 29 400). ^{1}H NMR (DMSO-d_6) δ 2.60−2.66 (m, 1H, $H_{4'}$), 3.42−3.48 (m, 1H), 3.63−3.69 (m, 1H, $H_{5'}$), 5.11 (t partly overlapped with $H_{3'}$, 1H, J = 5.6 Hz, OH), 5.20 (d, 1H, J = 71.2 Hz, $H_{3'}$), 7.10 (s, 2H, NH_2), 7.74 (s, 1H, $H_{1'}$), 8.67 (s, 1H, H_8). ^{13}C NMR 29.6 (d, J = 11.9 Hz, $C_{4'}$), 60.1 (d, J = 3.8 Hz, $C_{5'}$), 70.0 (d, J = 229.1 Hz, $C_{3'}$), 113.2 (d, J = 3.7 Hz), 116.3 (d, J = 3.0 Hz, $C_{1'}$, $C_{2'}$), 123.8 (C_5), 140.5 (C_8), 150.5 (C_4), 153.4 (C_2), 160.9 (C_6). ^{19}F NMR −201.13 (dd, J = 68.9, 12.4 Hz). ESI-MS 270, 272 (M + H, 100.0, 31.0), 292, 294 (M + Na, 50.0, 15.2).

(*Z*)-2-Amino-6-chloro-9-{[*trans*-(3-fluoro-2-hydroxymethyl-cyclopropylidene]methyl}purine (12e). The procedure described for compound **11a** was performed with the *Z*-isomer **35e** (360 mg, 1.0 mmol) to give **12e** (200 mg, 74%), mp 201−203 °C. UV λ_{max} 311 nm (ϵ 7900), 239 (ϵ 28 700). ^{1}H NMR (DMSO-d_6) δ 2.40−2.46 (m, 1H, $H_{4'}$), 3.34−3.40 (overlapped with water), 3.47−3.53 (m, 1H, $H_{5'}$), 5.01 (t, 1H, J = 5.8 Hz, OH), 5.36 (d, 1H, J = 67.2 Hz, $H_{3'}$), 7.11 (s, 2H, NH_2), 7.40 (s, 1H, $H_{1'}$), 8.28 (s, 1H, H_8). ^{13}C NMR 27.7 (d, J = 11.2 Hz, $C_{4'}$), 60.2 (d, J = 4.4 Hz, $C_{5'}$), 70.9 (d, J = 229.8 Hz, $C_{3'}$), 113.5 (d, J = 2.2 Hz), 115.3 (d, J = 2.2 Hz, $C_{1'}$, $C_{2'}$), 123.8 (C_5), 140.1 (C_8), 150.6 (C_4), 153.1 (C_2), 161.0 (C_6). ^{19}F NMR −200.70 (dd, J = 68.9, 12.4 Hz). ESI-MS 270, 272 (M + H, 100.0, 3.6), 292, 294 (M + Na, 50, 15.8).

(*E*)-9-{[(*trans*-(3-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}guanine (11b). The procedure described for compound **9b** was followed using the *E*-isomer **11e** (120 mg, 0.45 mmol) to give guanine analogue **11b** (82 mg, 73%), mp > 300 °C. UV λ_{max} 273 nm (ϵ 10 200), 242 (ϵ 26 300). ^{1}H NMR (DMSO-d_6) δ 2.48−2.53 (m, 1H, $H_{4'}$), 3.61−3.65 (m overlapped with H_2O, $H_{5'}$), 5.16 (t overlapped with $H_{3'}$, 1H, OH), 5.35 (d, 1H, J = 68.2 Hz, $H_{3'}$), 6.59 (s, 2H, NH_2), 7.61 (s, 1H, $H_{1'}$), 8.28 (s, 1H, H_8), 10.69 (br s, 1H, NH). ^{13}C NMR 29.3 (d, J = 11.2 Hz, $C_{4'}$), 60.1 (d, J = 3.7 Hz, C_5), 69.9 (d, J = 229.9 Hz, $C_{3'}$), 112.2 (d, J = 3.7 Hz), 116.5 (d, J = 2.9 Hz, $C_{1'}$, $C_{2'}$), 117.1 (C_5), 134.8 (C_8), 150.8 (C_4), 154.8 (C_2), 157.3 (C_6). ^{19}F NMR −200.84 (dd, J = 70.2, 10.7 Hz). ESI-MS (MeOH − KOAc) 252 (M + H, 100.0), 290 (M + K, 20.9), 503 (2M + H, 16.1), 541 (2M + K, 6.0). Anal. $C_{10}H_{10}FN_5O_2$ (C, H, N).

(*Z*)-9-{[(*trans*-(3-Fluoro-2-hydroxymethyl)cyclopropylidene]-methyl}guanine (12b). Procedure described for compound **9b** was followed with the *Z*-isomer **12e** (200 mg, 0.74 mmol) to give *E*-isomer **12b** (140 mg, 75.5%), mp > 300 °C. UV λ_{max} 274 nm (ϵ 9900), 241 nm (ϵ 26 000). ^{1}H NMR (DMSO-d_6) δ 2.36−2.40 (m, 1H, $H_{4'}$), 3.46−3.53 (m, 1H), 3.64−3.66 (m, 1H, $H_{5'}$), 4.96 (t, 1H, 5.2 Hz, OH), 5.32 (d, 1H, J = 68.0 Hz, $H_{3'}$), 6.88 (s, 2H, NH_2), 7.29 (s, 1H, $H_{1'}$), 7.86 (s, 1H, H_8), 10.74 (s, 1H, NH). ^{13}C NMR 27.5 (d, J = 11.2 Hz, $C_{4'}$), 60.2 (d, J = 4.4 Hz, $C_{5'}$), 70.8 (d, J = 229.9 Hz, $C_{3'}$), 112.3 (d, J = 1.5 Hz), 115.6 (d, J = 2.3 Hz, $C_{1'}$, $C_{2'}$), C_5 (117.0), 134.2 (C_8), 150.6 (C_4), 155.2 (C_2), 157.2 (C_6). ^{19}F NMR −201.23 (dd, J = 67.9, 11.5 Hz). ESI-MS (MeOH + KOAc) 252 (M + H, 100.0), 290 (M + K, 11.9), 503 (2M + H, 11.3), 541 (2M + K, 3.0). Anal. $C_{10}H_{10}FN_5O_2$ (C, H, N).

Antiviral Assays. The antiviral assays were performed as described previously.[11] The HCMV assays were performed with Towne and AD169 strains of the virus in HFF culture by plaque

reduction or cytopathic effect (CPE) inhibition assay. The HSV-1 was run in BSC-1 cells by ELISA. In addition, HSV-1 and HSV-2 assays were performed in HFF (CPE inhibition) and Vero cells (plaque reduction). The EBV assays were run in Daudi culture (viral capsid antigen, VCA-ELISA) and in H-1 culture (DNA hybridization). The VZV was assayed in HFF cells (CPE inhibition or plaque reduction), HIV-1 in MT-2 cells (CPE inhibition), and HBV in 2.2.15 cells. The cytotoxicity assays were performed in HFF, KB, or CEM cells. The results are summarized in Tables 3 and 4.

Adenosine Deaminase (ADA) Assay.[11] Compounds **9a**, **10a**, **11a**, and **12a** (2.0−2.4 μmol) were incubated with ADA (1.1 unit/mL) in 0.05 M Na_2HPO_4 (pH 7.5, 0.47−0.54 mL). Aliquots were periodically withdrawn and examined by TLC in CH_2Cl_2−MeOH (9:1, multiple development, **10a**, **11a**) and EtOAc−MeOH (10:1, multiple development, **9a**, **12a**). The extent of deamination of **9a**, **10a**, and **11a** was approximately 50% after 24 and 48 h, whereas **12a** was not deaminated after 48 h.

Acknowledgment. We thank L. M. Hrihorczuk from the Central Instrumentation Facility, Department of Chemistry, Wayne State University (D. M. Coleman, Director) for mass spectra. We also thank research assistants and associates in Drs. Drach and Kern laboratories for expert performance of antiviral and cytotoxicity assays. The work described herein was supported by U. S. Public Health Service Grants RO1-CA32779 (J.Z.) and RO1-CA44358 (Y.-C.C.) from the National Cancer Institute, Contracts NO1-AI85347 and NO1-AI30049 (E.R.K.), and program project PO1-AI46390 (J.C.D.) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0607404

Resistance profile of a neutralizing anti-HIV monoclonal antibody, KD-247, that shows favourable synergism with anti-CCR5 inhibitors

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Background: The high-affinity humanized monoclonal antibody (MAb) KD-247 reacts with a tip region in gp120-V3 and cross-neutralizes primary isolates with a matching neutralization sequence motif.

Methods: We induced an HIV-1 variant that was resistant to KD-247 by exposing the JR-FL virus to increasing concentrations of KD-247 in PM1/CCR5 cells, which expressed high levels of CCR5 *in vitro*. We determined the amino acid sequence of the gp120-encoding region of the JR-FL escape mutant from KD-247. To confirm that this substitution was responsible for the KD-247-resistance, a single-round replication assay was performed. We further evaluated the anti-HIV-1 interactions between KD-247 and various CCR5 inhibitors *in vitro*.

Results: At passage 8 of the culture in the presence of 1000 μg/ml KD-247, one amino acid substitution, Gly to Glu at position 314 (G314E), was identified in the V3-tip of gp120. A pseudotyped virus with the G314E mutation was highly resistant to KD-247. Unexpectedly, this mutant virus was sensitive to CCR5 inhibitors, RANTES, recombinant human soluble CD4 (rsCD4) and an anti-CCR5 MAb, but resistant to an anti-CD4 MAb, compared with the wild-type virus. We also found that combinations of KD-247 and CCR5 inhibitors were highly synergistic.

Conclusions: The present data suggest that KD-247 has certain advantages for possible passive immunotherapy. They are: high concentrations of KD-247 are needed for viral acquisition of KD-247 resistance; the escape variants are more sensitive to CCR5 inhibitors and rsCD4; and there are high levels of synergism between KD-247 and CCR5 inhibitors at all concentrations tested.

AIDS 2006, **20**:2065–2073

Keywords: HIV-1, KD-247, anti-V3 monoclonal antibody, broadly neutralizing, CCR5 inhibitor, synergism

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These data were presented at the *Thirteenth Conference on Retroviruses and Opportunistic Infections*. Denver, CO, February 2006 [abstract 506].

Note: K. Yoshimura and J. Shibata contributed equally to this work.

Received: 21 February 2006; accepted: 27 July 2006.

ISSN 0269-9370 © 2006 Lippincott Williams & Wilkins

Introduction

In a recent paper, we described a cross-neutralizing anti-V3 antibody, KD-247, against primary isolates via sequential immunization with six peptides from V3 that contained a neutralizing epitope of HIV-1 [1]. The ability of KD-247 to neutralize HIV-1 may be dependent on site-specific binding to an epitope on the viral envelope glycoprotein. The complementarity determining regions of KD-247 were transferred from the mouse monoclonal antibody (MAb) C25, which was designed to have broad neutralization activity against HIV-1 clade B isolates. The recognition site of KD-247 was mapped to five or six amino acids around the PGR core sequence at the tip of the V3 region of gp120. The shortest reactive peptide recognized by KD-247 was determined to be IGPGR, although the epitope was stabilized by the addition of one or more supplemental amino acids. The GPGR sequence in the V3 tip is highly conserved among HIV-1 strains [2]. In a recent study, we showed that the reshaped MAb KD-247 was suitable for use not only as an antibody for passive immunization for the prevention of HIV infection but also as an antibody for passive transfer immunotherapy for infected individuals [3].

In the present study, we induced HIV-1 variants that escaped from neutralization by KD-247 *in vitro* by continuously exposing the R5 virus JR-FL to increasing concentrations of KD-247 and defined the virological properties of a pseudotyped HIV-1 clone carrying the KD-247 escape-associated *env* gene mutation. We also evaluated the anti-HIV-1 interactions between KD-247 and various CCR5 inhibitors *in vitro*.

Materials and methods

Cells, culture conditions and reagents

The CD4-positive T-cell line PM1 was maintained in RPMI 1640 (Sigma, St. Louis, Missouri, USA) supplemented with 10% heat-inactivated foetal calf serum (Hyclone, Logan Utah, USA), 50 U/ml penicillin and 50 μg/ml streptomycin. PM1/CCR5 cells were generated by standard retrovirus-mediated transduction of PM1 cells with pBABE-CCR5 provided by the National Institutes of Health AIDS Research and Preference Reagent Program [4]. PM1 and PM1/CCR5 cells were analysed for their surface expressions of CD4, CCR5 and CXCR4 using a FACSCalibur (Becton Dickinson, Franklin Lakes, New Jersey, USA). 293T cells were maintained in Dulbecco's modified Eagle medium (DMEM; Sigma) supplemented with 10% heat-inactivated FCS. The CD4 human osteogenic sarcoma cell line GHOST was maintained in DMEM supplemented with 10% FCS, 200 μg/ml G418 (Gibco BRL, Rockville, Maryland, USA) and 100 μg/ml hygromycin B (Sigma). The GHOST derivatives GHOST-hi5 and GHOST-CXCR4 stably expressed CCR5 and CXCR4, respectively, as described elsewhere [5], and were selected with 1 μg/ml puromycin (Sigma).

17b, a CD4-induced (CD4i) MAb, was a kind gift from J. Robinson (Department of Pediatrics, Tulane University Medical Center, New Orleans, Louisiana, USA). 447-52D, an anti-gp120 V3 MAb, was a kind gift from S. Zolla-Pazner (Department of Pathology, New York University School of Medicine, New York, USA). 2D7, an anti-CCR5 MAb, and RPA-T4, an anti-CD4 MAb, were purchased from BD Biosciences Pharmingen (San Jose, California, USA). 2′,3′-dideoxyinosine (ddI, didanosine) was from Calbiochem, San Diego, California, USA. 3′-thiacytidine (3TC, lamivudine) was a kind gift from R. F. Schinazi (Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, USA). Saquinavir (SQV) was kindly provided by Roche Products Ltd., Welwyn Garden City, UK. Amprenavir (APV) was a kind gift from GlaxoSmithKline, Middlesex, UK. Nelfinavir (NFV) and indinavir (IDV) were kindly provided by Japan Energy Inc., Tokyo, Japan. Recombinant human soluble CD4 (rsCD4), MIP-1α, MIP-1β and RANTES were from R&D Systems Inc., Abingdon, UK. The CCR5 inhibitors TAK-779 [6] and SCH-351125 (SCH-C) [7] were synthesized as previously described. AK-602, a CCR5 inhibitor, was kindly provided by Ono Pharmaceutical Co. Ltd., Osaka, Japan [8].

Isolation of a KD-247-resistant mutant from JR-FL *in vitro*

For the selection of a KD-247 escape virus, JR-FL [9] was treated with various concentrations of KD-247 and then infected into PM1/CCR5 cells as previously described with minor modifications [10]. Viral replication was monitored by observation of any cytopathic effects in PM1/CCR5 cells. The culture supernatant was harvested on day 7 and used to infect fresh PM1/CCR5 cells for the next round of culture in the presence of increasing concentrations of KD-247. After the virus was passaged in the presence of up to 1000 μg/ml KD-247 in PM1/CCR5 cells, a KD-247-resistant virus, JR-FL(1000)8P, was recovered from the cell culture supernatant. JR-FL was also passaged for the same time periods in PM1/CCR5 cells in the absence of KD-247 to exclude any effects of the long-term culture of eight passages, and the resulting virus was designated JR-FL(−)8P.

The sensitivities of the passage 8 JR-FL viruses in the presence or absence of KD-247 to various drugs or MAb were determined as previously described with minor modifications [11]. Briefly, PM1-CCR5 cells (2×10^3/well) were exposed to 100 50% tissue culture inhibitory doses ($TCID_{50}$) of the JR-FL(1000)P8 or JR-FL(−)P8 in the presence of various concentrations of drugs or MAb in 96-well round-bottom plates. The 50% inhibitory concentration (IC_{50}) values were determined using the MTT {3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide} (MTT) assay on day 7 of culture. All assays were performed in duplicate.

Viral RNA (0.5 μg) extracts from cell culture supernatants at several concentrations of KD-247 were reverse-transcribed using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, California, USA). The cDNA obtained were subjected to PCR amplification using *Taq* polymerase. After cloning the amplified products into pCR2.1, the Env regions in both the passaged and selected viruses were sequenced using an ABI PRISM 310 automated DNA sequencer (Applied Biosystems).

Construction of mutant envelope expression vectors and production of pseudovirions

For the construction of mutant envelope expression vectors, we used pCXN2, which contains a chicken actin promoter. Briefly, the JR-FL *env* region was cloned by PCR and ligated into pCR2.1, generating pCR2-FL_{wt}. The *Eco*RI fragment of pCR2-FL_{wt} containing the entire *env* region was ligated into pCXN2 to give pCXN-FL_{wt} [9]. A mutant Env (G314E) expression vector was generated from pCXN-FL_{wt} using a QuikChange site-directed mutagenesis kit (Stratagene, Chedar Creek, Texas, USA) and the primers JR-FLv3G/Efw (5′–TACATA-TAGGACCAGAGAGAGCATTTTATAC–3′) and JR-FLv3G/Erv (5′–GTATAAAATGCTCTCTCTGGTCC TATATGTA–3′) according to the manufacturer's protocol, and designated pCXN-FLG314E.

Plasmids pNL4-3.Luc.R^-E^- and pRSV-Rev [12], supplied by the NIH AIDS Research and Reference Reagent Program, and plasmid pCXN2, expressing wild-type or G314E Env, were cotransfected into 293T cells using the Effectene Transfection Reagent (Qiagen, Valencia, California, USA). At 24 h after the transfection, the pseudovirus-containing supernatants were harvested, filtered through a 0.2-μm pore-size filter and stored at −150°C. For measurement of the pseudovirus activities, a luminescence assay with GHOST-hi5 cells was used as previously described [13].

Neutralization assays

A single-cycle infectivity assay was used to measure the neutralization of JR-FL_{wt} or JR-FL_{GPER} pseudovirions as described previously [13]. Briefly, MAb at various concentrations and a pseudovirus suspension corresponding to 200 $TCID_{50}$ were preincubated for 15 min on ice. The virus–antibody mixtures were added to GHOST-hi5 cells, which had been seeded in a 96-well plate (1.5×10^4 cells/well) on the previous day. The cultures were incubated for 2 days at 37°C, washed with phosphate-buffered saline and lysed with lysis buffer (Luc PGC-50; PicaGene, Tokyo, Japan). Following transfer of the cell lysates to luminometer plates (Costar 3912), the luciferase activity (in relative light units) in each well was measured using Luciferase Substrate (100 μl/well; PicaGene) in a TR717 microplate luminometer (Applied Biosystems). The reduction in infectivity was determined by comparing the relative light units in the presence and absence of MAb and expressed as the percent neutralization. The same assay was repeated two to three times.

In vitro binding assay to the JR-FL_{wt} or JR-FL_{GPER} envelope

The JR-FL gp160 coding sequence was amplified from the infectious clone vector (pJR-FL) using the primers ENVA-*Eco*RI (5′–CGGAATTCGGCTTAGGCATCT CCTATGGCAGGAAGAA–3′) and ENVN-*Bam*HI (5′–CGGGATCCCGCTGCCAATCAGGGAAGTAG CCTTGTGT–3′). The product was digested with *Eco*RI and *Bam*HI and subcloned into the corresponding sites in pDNR-1r (Clontech) for sequencing and subsequent manipulation. A JR-FL_{GPER} Env expression vector was generated from pDNR-JR-FL_{wt} using a QuikChange site-directed mutagenesis kit and the primers JR-FLv3G/Efw and JR-FLv3G/Erv according to the manufacturer's protocol. The wild-type and mutated *env* gene fragments were then subcloned into pLP-IRES2-EGFP (Clontech) using Cre-recombinase (Clontech) according to the manufacturer's instructions, and designated pLP-EGFP-JR-FL_{wt} and pLP-EGFP-JR-FL_{GPER}, respectively.

293T cells were cotransfected with pRSV-Rev (0.5 μg) and pLP-IRES2-EGFP, pLP-EGFP-JR-FL_{wt} or pLP-EGFP-JR-FL_{GPER} (9.5 μg) using the Effectene Transfection Reagent (Qiagen). After 36 h, the cells were harvested, incubated with each anti-HIV-1 MAb with or without rsCD4 (0.5 μg/ml) in combination with biotin-conjugated anti-human IgG and peridinin chlorophyll-a protein-conjugated streptavidin (BD Biosciences Pharmingen), gated for the GFP-positive area and analysed using a FACSCalibur.

Data analysis and evaluation of synergy

Analysis of the synergistic, additive or antagonist effects of the antiviral agents was first performed according to the median effect principle using the CalcuSyn version 2 computer program [14,15] to provide estimates of the IC_{50} values of the antiviral reagents in different combinations. Combination indices (CI) were estimated from the data and reflected the nature of the interactions between KD-247 and the CCR5 inhibitors against JR-FL(−)8P in PM1/CCR5 cells, as evaluated using the MTT assay. Specifically, $CI < 0.9$ indicated synergy, $0.9 < CI < 1.1$ indicated additivity and $CI > 1.1$ indicated antagonism. The value of CI was directly proportional to the amount of synergy in the combination regimen. For example, values of $CI < 0.5$ represented a high degree of synergy, whereas values of $CI > 1.5$ represented significant antagonism. This approach has been widely used in analyses of antiviral interactions and was chosen to allow comparability with published literature.

Statistical analysis

Statistical correlations were analysed using Student's t test. P values < 0.05 were considered statistically significant.

Results

Selection of a KD-247 escape variant

For the isolation of a KD-247 escape mutant from R5 HIV *in vitro*, PM1 cells expressing high levels of CCR5, designated PM1/CCR5 cells, which were highly sensitive to both X4 and R5 HIV infection and accompanied by prominent syncytia [4] were used as the target cells. An R5 HIV strain, JR-FL, which uses CCR5 as its coreceptor was used for the selection of a KD-247 escape virus.

In order to select an HIV-1 variant that can escape from neutralization by KD-247 *in vitro*, we exposed PM1/CCR5 cells to JR-FL, and serially passaged the virus in the presence of increasing concentrations of KD-247, or in the absence of the MAb as a control. The selected virus was initially propagated in the presence of 1 μg/ml KD-247, and during the course of the selection procedure, the MAb concentration was increased to 1000 μg/ml. At passage 8, the supernatants containing the passaged viruses in the presence or absence of KD-247, designated JR-FL(1000)8P and JR-FL(−)8P, respectively, were harvested and titrated for their infectivities and sensitivities to KD-247, CCR5 inhibitors (TAK-779, SCH-C and AK-602), nucleoside reverse transcriptase inhibitors (NRTI; ddI and 3TC) and protease inhibitors (PI; NFV, IDV, APV and SQV), as evaluated by the MTT assay (Table 1). The IC_{50} values of KD-247 against JR-FL(−)8P and JR-FL(1000)8P were 6.3 and > 100 μg/ml, respectively. The fold difference between these IC_{50} values was > 16. JR-FL(1000)8P was sensitive to all the NRTI and PI, similar to JR-FL(−)8P. Unexpectedly, JR-FL(1000)8P was more sensitive to the three CCR5 inhibitors (TAK-779, SCH-C and AK-602), rsCD4, anti-CCR5 MAb 2D7 and RANTES than JR-FL(−)8P. However, JR-FL(1000)8P was threefold more resistant to anti-CD4 MAb RPA-T4 than JR-FL(−)8P. These data suggest that the escape variant with a highly resistant phenotype against KD-247 becomes more sensitive to CCR5 inhibitors and rsCD4, and needs higher concentration of anti-CD4 antibody for entry blocking, compared with JR-FL(−)8P.

Sequencing of the envelope region of the KD-247 escape mutant

To determine the region responsible for the reduced sensitivity of the escape mutant to KD-247, the C1–C4 regions of the envelope were sequenced after cloning of the PCR product of each region using cDNA synthesized from viral RNA obtained from the supernatants of infected cells as templates. A total of 12–16 clones for each PCR product were isolated and sequenced. Analyses of the *env* sequences of these products revealed that the selected virus had a Gly→Glu substitution at codon 314 (G314E) in the V3 region of the envelope at passage 7 (600 μg/ml; 10/12 clones) and passage 8 (1000 μg/ml; 12/16 clones) (Fig. 1). Some changes in the envelope sequence in other regions, including C1, V1, V2, C2, C3, V4 and C4 of the escape mutant were found as well as in V3 around the IGPGR sequence even at early time points in the presence of the selective pressure. It is possible that these mutations also confer resistance to KD-247 but lead to virus of decreased fitness and thus they did not expand in a next passage except for the G314E. On the other hand, the virus passaged in PM1/CCR5 cells

Table 1. Anti-HIV-1 activities of various MAb and inhibitors toward KD247-resistant JR-FL.

Antibody or inhibitor	$IC_{50} \pm SD$[a]		Fold change	P[b]
	JR-FL(−)8P	JR-FL(1000)8P		
KD-247 (μg/ml)	6.3 ± 5.0	> 100	< 16	**< 0.01**
TAK-779 (nM)	217 ± 50.3	54.7 ± 29.5	0.3	**< 0.01**
SCH-C (nM)	27.5 ± 3.5	8.0 ± 1.4	0.3	**0.02**
AK-602 (nM)	7.1 ± 4.5	0.15 ± 0.08	0.02	**0.02**
Didanosine (μM)	1.0 ± 0.57	1.0 ± 0.27	1.0	0.98
Lamivudine (μM)	0.33 ± 0.01	0.29 ± 0.04	0.9	0.30
Nelfinavir (μM)	0.033 ± 0.001	0.036 ± 0.006	1.1	0.56
Indinavir (μM)	0.017 ± 0.004	0.016 ± 0.005	0.9	0.85
Amprenavir (μM)	0.022 ± 0.001	0.017 ± 0.008	0.8	0.47
Saquinavir (μM)	0.0038 ± 0.0004	0.0034 ± 0.0004	0.9	0.42
rsCD4 (μg/ml)	3.3 ± 0.07	0.57 ± 0.48	0.2	**0.02**
Anti-CD4 MAb (RPA-T4) (μg/ml)	0.01 ± 0.004	0.03 ± 0.004	3.0	**0.01**
Anti-CCR5 MAb (2D7) (μg/ml)	0.19 ± 0.03	0.066 ± 0.005	0.3	**0.03**
MIP-1α (μg/ml)	0.006 ± 0.002	0.0029 ± 0.001	0.5	0.11
MIP-1β (μg/ml)	0.39 ± 0.08	0.23 ± 0.18	0.6	0.22
RANTES (μg/ml)	0.045 ± 0.0007	0.005 ± 0.001	0.1	**0.02**

[a]PM1/CCR5 cells (2×10^3) were exposed to 100 TCID50 of JR-FL(−)8P or JR-FL(1000)8P and then cultured in the presence of various concentrations of MAb or inhibitors. The IC_{50} values were determined using the MTT assay on day 7 of culture. Data shown represent values derived from the results of two or three independent experiments conducted in duplicate.
[b]P values < 0.05 were considered statistically significant (shown in bold type). IC, Inhibitory concentration.