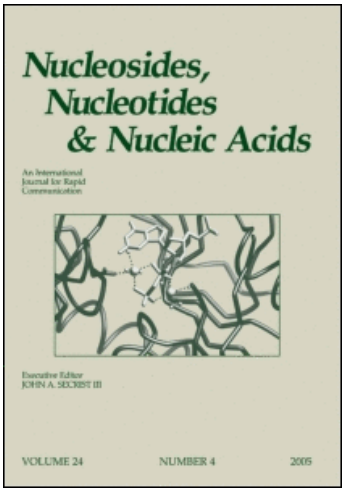

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TERMINAL FLUOROOLEFINS. THE SYNTHESIS OF NOVEL CARBOACYCLIC NUCLEOSIDES.

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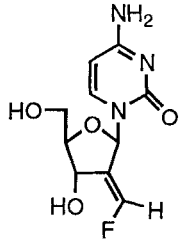
ABSTRACT:
A facile preparation of ketone **7** from butanetriol acetonide **5** is reported, and its utility for the synthesis of novel carboacyclic nucleosides **3E**, **3Z**, **4E** and **4Z** by the selective manipulation of multiple functionalities is demonstrated.

The incorporation of the terminal fluoroolefin group into substrates has been useful in the design of a number of mechanism-based enzyme inhibitors.[2] A recent report describes the stereospecific synthesis of (E)-2′-deoxy-2′-(fluoromethylene)cytidine (**1**) and its potent antiproliferative and cytotoxic activity.[3] Another report details the design and syntheses of (E)- and (Z)-5′-fluoro-4′,5′-didehydro-5′-deoxyadenosines (**2E** and **2Z**) as mechanism-based and competitive inhibitors of S-adenosyl-L-homocysteine (SAH) hydrolase.[4] In each of these cases, olefin geometry is important for optimal activity. As a continuation of these efforts, we were interested in the synthesis of carboacyclic nucleoside analogs **3E**, **3Z**, **4E** and **4Z** which incorporate the terminal fluoroolefin moiety. Our approach for obtaining these target molecules was to synthesize the functionalized acyclic side chains and then couple with heterocyclic bases through alkylation or Mitsunobu[5] reactions.



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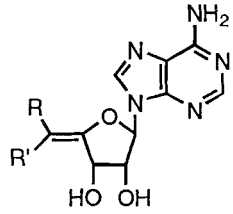
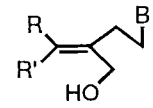


2E R=H; R'=F
2Z R=F; R'=H

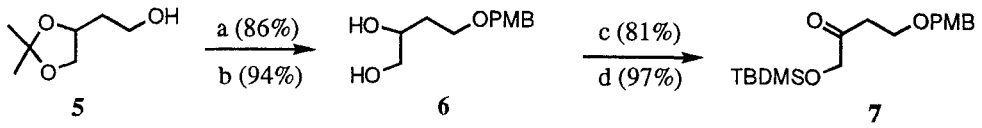


3E R=F; R'=H; B=Adenine
3Z R=H; R'=F; B=Adenine
4E R=F; R'=H; B=Cytosine
4Z R=H; R'=F; B=Cytosine

Butanetriol acetonide **5**[6] was chosen as the starting material with the terminal carbinol envisioned as the site for coupling with heterocyclic bases (Scheme I). To differentiate between the two primary alcohol functional groups, alcohol **5** was first protected as its *p*-methoxybenzyl (PMB) ether, and then treated with 80% aqueous acetic acid to afford diol **6** in 81% overall yield for the two steps. The selective protection of the primary alcohol group as the *t*-butyldimethylsilyl (TBDMS) ether was accomplished by 4-dimethylaminopyridine (DMAP) catalyzed silylation[7] in 81% yield, allowing for Swern oxidation of the secondary alcohol to furnish ketone **7** in 97% yield. This sequence provides multigram quantities of ketone **7** which is suitably protected to allow for the selective manipulation of the three functional groups.

The next phase of the synthesis involved the introduction of the terminal fluoroolefin at the carbonyl position of **7**, and the selective deprotection of the two differentially protected primary alcohols (Scheme II). Horner-Wittig reaction of ketone **7** with *in situ* generated diethyl 1-fluoro-1-(phenylsulfonyl)methane phosphonate[8] carbanion afforded fluorovinyl sulfone **8** in 84% yield as a 2:1 E/Z mixture of geometric isomers. Treatment of sulfone mixture **8** with tributyltin hydride (2 equivs.) in cyclohexane at reflux[9] afforded a 2:1 mixture of fluorovinyl stannanes **9**. Subsequent treatment of **9** with excess tetrabutylammonium fluoride (TBAF) produced both protodestannylation and desilylation, resulting in a 2:1 mixture of fluoro olefins **10** in 96% yield for the two steps. The separation of the two geometric isomers of **10** was achieved with difficulty at this stage of the synthesis. However, benzoylation of alcohol mixture **10** and removal of the *p*-methoxybenzyl group from mixture **11** with DDQ conveniently afforded a chromatographically separable mixture of **12E** and **12Z** in 75% yield for the two steps in a 2:1 ratio, respectively.

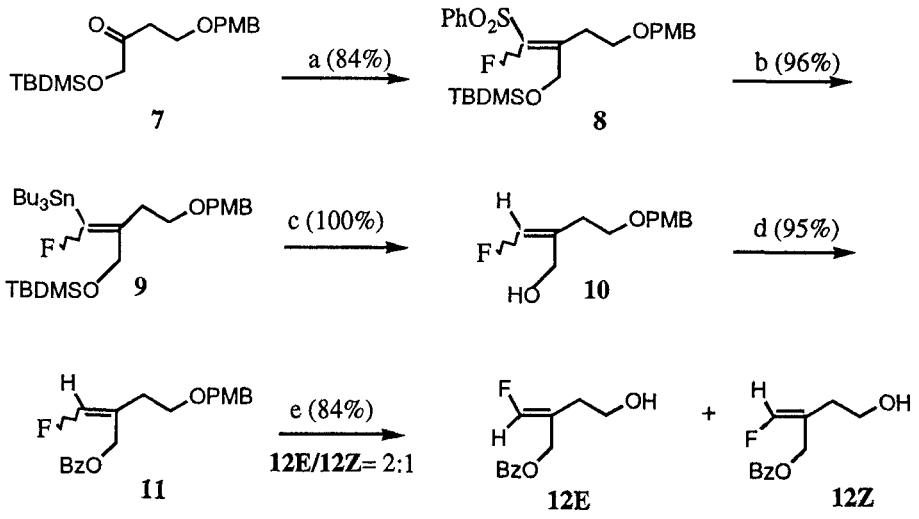
The coupling reactions of readily available acyclic side chains **12E** and **12Z** with heterocyclic bases were next examined (Scheme III). For the introduction of purine bases, the Mitsunobu reaction of 6-chloropurine[10] with alcohols **12E** and **12Z** produced the N^9-substituted purines **13E** and **13Z** in 59% and 49% yields, respectively. Debenzoylation and conversion of **13E** and **13Z** to adenosines **3E** and **3Z** was conveniently accomplished with methanolic ammonia in 54% and 49% yields, respectively. NOESY and RELAY ^1H



5 6 7

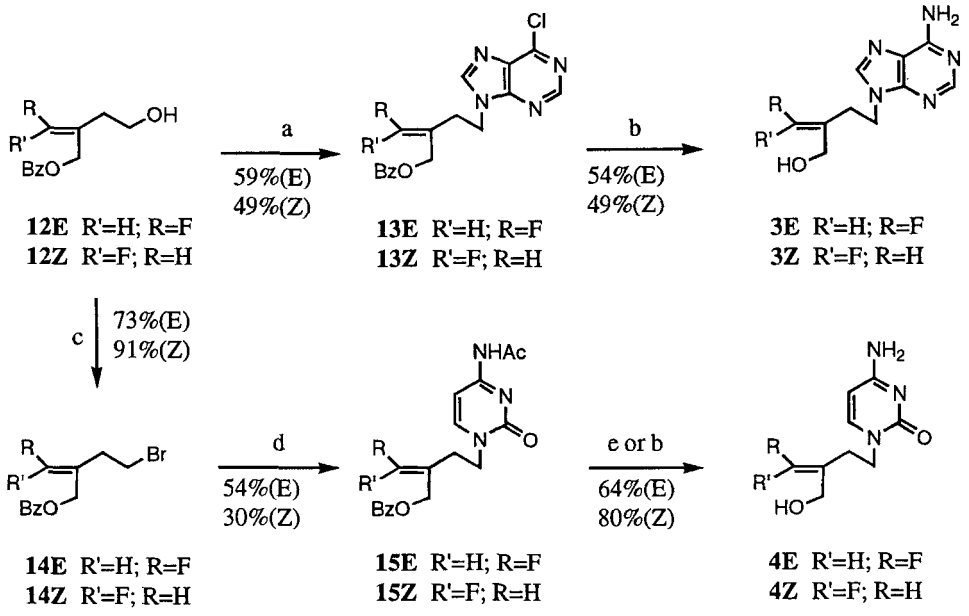
Reagents: (a) *p*-$CH_3OC_6H_4CH_2Cl$, NaH, DMF, rt(16h); (b) 80% aq. HOAc, rt(6h); (c) TBDMS-Cl, Et_3N, DMAP, DMF, rt(16h); (d) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -60°C-rt.

Scheme I



Reagents: (a) $PhSO_2CH_2F$, $(EtO)_2P(O)Cl$, $LiN(Si(CH_3)_3)_2$, THF, -60°C-rt; (b) Bu_3SnH, AIBN, cyclohexane, 80 °C; (c) 2.5eq. TBAF, THF, 0°C(1h); (d) benzoyl chloride, pyridine; (e) DDQ, CH_2Cl_2-H_2O, 16:1, rt.

Scheme II



Reagents: (a) 6-chloropurine, DEAD, Ph_3P, THF, rt; (b) NH_3(g), MeOH, 50°C(16h); (c) NBS, Ph_3P, CH_2Cl_2, rt; (d) K_2CO_3, DMF, N^6-acetylcytosine, 80°C; (e) 1% aq. NaOH, MeOH, rt.

Scheme III

NMR experiments confirmed olefin geometries and N^9-substitution of the final products.[11] For the synthesis of cytidines **4E** and **4Z**, a two-step sequence of Mitsunobu coupling of **12E** and **12Z** with 4-ethoxypyrimidin-2-one[10] followed by treatment with methanolic ammonia was planned. Although Mitsunobu coupling was successful, repeated attempts at conversion to cytidine final products with methanolic ammonia at elevated temperatures (sealed tube) failed in our hands. The successful synthesis of cytidines **4E** and **4Z** commenced with the conversion of alcohols **12E** and **12Z** to electrophilic bromides **14E** and **14Z** in 73% and 91% yields, respectively. Alkylation of **14E** and **14Z** with N^6-acetylcytosine[12] afforded **15E** and **15Z** in fair yields, and hydrolysis or aminolysis completed the synthesis of cytidines **4E** and **4Z** .

Nucleoside analogs **3E**, **3Z**, **4E** and **4Z** were evaluated as cytotoxic agents in a HeLa cell assay (see experimental for procedure). The best activities were obtained with adenosine **3E** and cytidine **4E** ($IC_{50} \cong 200$ nM), whereas **3Z** and **4Z** were approximately five times less potent ($IC_{50} \cong 1000$ nM). The difference in the biological activities of **3E** and **4E** relative to **3Z** and **4Z** may be attributed to olefin geometry, and is consistent with data reported for **1** ($IC_{50} = 58$ nM) and its Z-isomer ($IC_{50} = 3870$ nM).[3b]

In conclusion, a convenient route to a novel series of carboacyclic fluoroolefin nucleosides is reported and preliminary cytotoxic data is presented. Future work will define parameters for optimum activity within these series.

EXPERIMENTAL

All melting points are uncorrected. ^1H NMR spectra were recorded on a Varian VXR-300 (300 MHz, multinuclear probe) in $CDCl_3$. ^{13}C NMR were recorded on a VXR-300 (75 MHz) in $CDCl_3$. ^{19}F NMR spectra were recorded at 282 MHz in $CDCl_3$ on the Varian VXR-300 with $CFCl_3$ as an external standard. Mass spectra were obtained with a Finnigan MAT Model 4600 (electron impact and chemical ionization) mass spectrometer. Combustion analyses for C, H, and N were performed by Marion Merrell Dow Analytical Laboratories, Cincinnati, OH. The silica gel used in flash chromatography was 40-63 mm size.

4-(4-Methoxybenzyloxy)butan-1,2-diol (6). A solution of butane-1,2,4-triol-1,2-acetonide **5**[5] (9.54g, 65.3 mmol) in anhydrous DMF (30 mL) was added dropwise to a rapidly stirred suspension of hexane washed NaH (4.0g of 60% w/w suspension, 100 mmol, 1.5 eq) in anhydrous DMF (50 mL) at ambient temperature. A cloudy yellow solution resulted and after 1h, a solution of 4-methoxybenzyl chloride (11.25g, 71.8 mmol, 1.1 eq) in anhydrous DMF (30 mL) was added dropwise. After the addition was complete, the reaction was allowed to stir for 16 h at ambient temperature. The reaction was slowly quenched with saturated aqueous NH_4Cl (10 mL) then partitioned between ethyl ether (200 mL) and water (400 mL). The aqueous layer was extracted with ether (3 x 200 mL) and the combined extracts were washed with brine (2 x 100 mL), dried

($MgSO_4$), filtered and concentrated under reduced pressure to give 4-(4-methoxybenzyloxy)butan-1,2-diol-1,2-acetonide as a cloudy, dark yellow oil. The product was purified by flash chromatography (silica gel, 9:1 hexane/ethyl acetate, $R_f = 0.16$) to a clear, pale yellow oil (15 g, 86% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (s,3H), 1.40 (s,3H), 1.86 (m,2H), 3.53 (q, J=2.0 Hz, 2H), 3.56 (q, J=4.0 Hz, 2H), 3.80 (s, 3H), 4.04 (d, J=8.3 Hz, 1H), 4.06 (d, J=8.3 Hz, 1H), 4.20 (q, J=6.7 Hz, 1H), 4.43 (s, 2H), 6.87 (dt, J=6.8, 1.1 Hz, 2H), 7.25 (dt, J=6.8, 1.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.77, 26.92, 33.84, 55.25, 66.74, 69.63, 72.72, 73.89, 108.49, 113.77, 129.19, 130.40, 159.16; HRMS calc $C_{15}H_{22}O_4$) 266.1518, found, 266.1511.

4-(4-methoxybenzyloxy)butan-1,2-diol-1,2-acetonide (15 g, 56.3 mmol) was stirred in 80% aqueous acetic acid (100 mL) for 18 h at ambient temperature. The solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane (300 mL) and then washed quickly with saturated aqueous $NaHCO_3$ (2 x 50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Residual acetic acid was removed on a rotary evaporator under high vacuum with heating to afford **6** (11.94 g, 52.92 mmol, 94%) as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ 1.73 (m, 2H), 2.87 (bs, 1H), 3.46 (dd, J = 11.3, 6.5 Hz, 2H), 3.58 (q, J = 3.6 Hz, 1H), 3.63 (m, 1H), 3.79 (s, 3H), 3.87 (m, 1H), 4.44 (s, 2H), 6.87 (dt, J = 8.2, 1.8 Hz, 2H), 7.24 (dt, J = 8.2, 1.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.75, 55.21, 66.49, 67.75, 71.16, 72.89, 113.82, 129.38, 159.25; HRMS calc ($C_{12}H_{18}O_4$) 226.1205, found , 226.1202.

1-(t-Butyldimethylsilyloxy)-4-(4-methoxybenzyloxy)butan-2-one (7). Diol **6** (42.43g, 187.5 mmol) was dissolved in anhydrous DMF (700 mL) and cooled in an ice bath. *t*-Butyldimethylsilyl chloride (31.11g, 206 mmol, 1.1 eq), triethylamine (31.4 mL, 225 mmol, 1.2 eq), and a catalytic amount of 4-(dimethylamino)pyridine (5.8 g, 4.7 mmol, 2.5 mol %) were then added. Stirring was continued for 10 min. and the bath was removed and stirring was continued at room temperature for 18 h. Solids were filtered off and the filtrate was partitioned between ethyl ether (500 mL) and water (100 mL). The aqueous layer was extracted with ether (2 x 500 mL), and the combined ether extracts were washed with brine (2 x 500 mL), and dried ($MgSO_4$), filtered and concentrated

under reduced pressure to yield a cloudy colorless oil. The mono-protected diol was purified by flash chromatography (silica gel, 4:1 hexane/ethyl acetate, $R_f = 0.25$) to a clear colorless oil (51.4 g, 81% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.90 (s, 9H), 1.69 (m, 2H), 2.77 (d, J=4.0 Hz, 1H), 3.50 (dd, J=11, 7 Hz, 2H), 3.59 (qd, J=7, 4 Hz, 2H), 3.75 (s, 3H), 4.40 (s, 2H), 6.82 (dt, J=8, 2 Hz, 2H), 7.20 (dt, J=8, 2 Hz, 2H).

To a solution of oxalyl chloride (1 mL, 11.45 mmol, 1.3 eq), in CH_2Cl_2 (35 mL) was added DMSO (1.69 mL, 23.79 mmol, 2.7 eq) dropwise at -78°C never allowing the temperature of the reaction mixture to exceed -55°C. After 10 min., a solution of the mono-protected diol (3.00 g, 8.81 mmol) in CH_2Cl_2 (10 mL) was added dropwise never allowing the internal temperature to exceed -65°C. After 20 min., triethylamine (4.9 mL, 35.24 mmol, 4 eq) was added and the cooling bath was then removed and the mixture was warmed to ambient temperature and stirred for 1.5h. The reaction was diluted to 200 mL with CH_2Cl_2 and washed with water (2 x 100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to a yellow oil which was purified by flash chromatography (silica gel, 9:1 hexane/ethyl acetate, $R_f = 0.18$) to afford **7** (2.90 g, 8.55 mmol, 97%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.074 (s, 6H), 0.91 (s, 9H), 2.76 (t, J=6.4 Hz, 2H), 3.73 (t, J=6.4 Hz, 1H), 3.80 (s, 3H), 3.89 (t, J=5.2 Hz, 1H), 4.20 (s, 2H), 4.44 (s, 2H), 6.87 (dt, J= 8.7, 1.7 Hz, 2H), 7.24 (dt, J=8.7, 1.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ -5.55, 25.74, 38.85, 55.24, 64.73, 69.69, 72.88, 113.76, 129.33, 130.13, 159.20, 209.81: Anal. Calcd for $C_{18}H_{30}SiO_4$; %C, 63.87, %H, 8.93. Found; %C, 63.69, %H, 8.82.

1-(*t*-Butyldimethylsilyloxy)-3-(benzenesulfonyl)-3-fluoro-2-[2-(4-methoxybenzyloxy)ethyl]prop-2-ene (8). Lithium bis(trimethylsilyl)amide (70 mL, 1 M in THF, 70 mmol, 2.0 eq) was added to a solution of fluoromethyl phenyl sulfone (6.10 g, 34.96 mmol, 1.1 eq) and diethyl chlorophosphate (6.3 g, 34.96 mmol, 1.1 eq) in anhydrous THF (100 mL) at -78°C. After 1 h, a solution of ketone **7** (10.76 g, 31.78 mmol) in anhydrous THF (50 mL) was added at -78°C. After 5 min, the bath was removed and the reaction was warmed to room temperature and stirred for 18 h. The reaction was quenched with saturated aqueous NH_4Cl (30 mL) and then partitioned between ethyl ether (200 mL) and

water (200 mL). The aqueous layer was extracted with ether (2 x 200 mL) and the combined ether layers were washed with water (200 mL) and brine (200 mL), then dried ($MgSO_4$), filtered and concentrated under reduced pressure to a dark brown oil. The crude product was purified by flash chromatography (silica gel, 9:1 hexane/ethyl acetate, R_f = 0.20) to afford **8** (13.17 g, 29.40 mmol, 84%) as an oil. ^1H NMR (300 MHz, $CDCl_3$) mixture of isomers, δ 0.01 (s, 6H), 0.11 (s, 6H), 0.87 (s, 9H), 0.92 (s, 9H), 2.56 (td, J=6.4, 3.6 Hz, 2H), 2.67 (td, J=6.4, 3.6 Hz, 2H), 3.00 (td, J=6.4, 1.2 Hz, 2H), 3.12 (td, J=6.4, 1.2 Hz, 2H), 3.59 (t, J=6.4 Hz, 2H), 3.69 (t, J=6.4 Hz, 2H), 4.20 (d, J=5.0 Hz, 2H), 4.31 (d, J=3.5 Hz, 2H), 4.40 (s, 2H), 4.49 (s, 2H), 4.70 (d, J=2.3 Hz, 2H), 7.18 (dt, J=8.5, 2.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) mixture of isomers, δ 18.28, 25.70, 27.50 (d, J=12 Hz), 55.22, 57.68 (d, J=5 Hz), 58.75 (d, J=12 Hz), 67.00 (d, J=2 Hz), 68.21 (d, J=4 Hz), 72.38, 72.45, 113.75, 128.21 (d, J=21 Hz), 128.28 (d, J=21 Hz), 129.22 (d, J=10 Hz), 129.25 (d, J=15 Hz), 134.12, 134.22, 138.53, 138.65, 148.81 (d, J=284 Hz), 149.28 (d, J=284 Hz), 159.14; ^{19}F NMR (282 MHz, $CDCl_3$) mixture of isomers, δ -119.20, -119.60 (1:2 ratio); HRMS calc ($C_{25}H_{36}O_5SiSF$) 495.2037, found, 495.2013.

1-(*t*-Butyldimethylsilyloxy)-3-fluoro-2-[2-(4-methoxybenzyloxy)ethyl]-3-tri-*n*-butylstannylprop-2-ene (9). Sulfone mixture **8** (13.0 g, 26.28 mmol), tributyltin hydride (15.29 g, 52.56 mmol, 2 eq), and a catalytic amount of AIBN (100 mg) in cyclohexane (300 mL) were refluxed for 6 h, then cooled to ambient temperature and concentrated under reduced pressure to a viscous oil. The crude product was purified by flash chromatography (silica gel, 3% ethyl acetate/hexane, R_f = 0.29 and 0.25) to afford **9** (16.22 g, 25.23 mmol, 2:1 E/Z mixture of isomers, 96%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 0.036 (s, minor, 6H), 0.043 (s, major, 6H), 0.878 (m, minor, 9H), 0.881 (m, major, 9H), 1.01 (m, overlap major and minor, 6H and 6H), 1.30 (m, overlap 6H and 6H), 2.28 (m, minor, 2H), 2.56 (m, major, 2H), 3.47 (m, minor, 2H), 3.51 (m, major, 2H), 3.80 (s, overlap of major and minor, 2H and 2H), 3.93 (d, J= 3.2 Hz, major, 2H), 4.32 (d, J= 3.5 Hz, minor, 2H), 4.42 (s, minor, 2H), 4.44 (s, major, 2H), 6.86 (d, J= 8.6 Hz, overlap of major and minor, 2H and 2H), 7.25 (d, J= 8.6 Hz, overlap of major and minor, 2H and 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -101.55 (minor), -101.89 (major).

E,Z-3-Fluoro-2-[2-(4-methoxybenzyloxy)ethyl]prop-2-en-1-ol (10). To a solution of fluorovinyl stannanes **9** (1.94 g, 3.01 mmol) in THF (5 mL) was added a solution of 1M TBAF/THF (3 mL, 3 mmol) at 0°C. After 15 min, the bath was removed and the reaction proceeded until the starting material was consumed as indicated by TLC (about 15 min.). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (silica gel, 3:2 hexane/ethyl acetate, R_f = 0.40 and 0.32) to afford **10** as an oil in quantitative yield. *Z*-isomer (minor): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85 (quin d, J=3.6 Hz, 1.6 Hz, 2H), 2.29 (tdd, J=6.0, 3.5, 1.3 Hz, 2H), 3.56 (td, J= 6.0, 1.0 Hz, 2H), 3.75 (d, J= 6.5 Hz, 2H), 3.87 (s, 3H), 4.23 (dd, J= 3.1, 1.0 Hz, 2H), 4.47 (s, 2H), 6.44 (dt, J= 84.5, 1.4 Hz, 1H), 6.88 (dt, J= 8.5, 1.8 Hz, 2H), 7.24 (dt, J= 8.5, 1.8 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -131.388 (d, J= 84.5 Hz); *E*-isomer (major): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (td, J= 5.6, 2.6 Hz, 2H), 3.05 (bs, 1H), 3.59 (t, J= 5.7 Hz, 2H), 3.80 (s, 3H), 3.93 (dd, J= 5.6, 1.0 Hz, 2H), 4.48 (s, 2H), 6.55 (d, J= 84.3 Hz, 1H), 6.88 (dt, J= 8.8, 2.0 Hz, 2H), 7.25 (dt, J= 8.8, 2.0 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -131.530 (d, J= 84.3 Hz).

Benzoic Acid E,Z-3-Fluoro-2-[2-(4-methoxybenzyloxy)ethyl]allyl Ester (11). Benzoyl chloride (1.83 mL, 15.7 mmol, 1.1 eq) was added dropwise at room temperature to a solution of alcohol **10** (3.44 g, 14.3 mmol) in pyridine (15 mL) and the resulting white slurry was allowed to stir at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate (75 mL) and filtered. The filter cake was washed with ethyl acetate (25 mL) and combined with the filtrate which was then washed with saturated aqueous sodium bicarbonate 2 x 50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to an oil which was purified by flash chromatography (silica gel, 9:1 hexane/ethyl acetate) to afford **11** (4.69 g, 13.59 mmol, 95%) as a 2:1 mixture of isomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (td, J= 6.5, 1.1 Hz, 2H), 3.56 (t, J=6.5 Hz, 2H), 3.79 (s, 3H), 4.43 (s, 2H), 4.96 (dd, J= 2.8, 0.8 Hz, 2H), 6.88 (dt, J= 83.6, 1 Hz, 1H), 6.85 (dt, J= 9.0, 2.0 Hz, 2H), 7.28 (dt, J= 9.0, 2.0 Hz, 2H), 7.43 (tt, J= 7.0, 2.0 Hz, 2H), 7.56 (tt, J= 7.0, 2.0 Hz, 1H), 8.02 (dt, J= 7.0, 2.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.25 (d, J= 5.4 Hz), 55.24, 58.88 (d, J= 8.9 Hz), 67.75 (d, J= 3.0 Hz), 113.79, 115.21 (d, J= 3.5 Hz), 128.36, 129.22, 129.60, 130.07 (d, J= 13.0 Hz), 133.01, 145.97,

149.45, 159.20, 166.32; ^{19}F NMR (282 MHz, $CDCl_3$) δ -128.39 (dt, J= 83.6, 1.0 Hz); HRMS; calc ($C_{20}H_{21}O_4F$) 344.1424, found, 344.1425.

Benzoic Acid E,Z-3-Fluoro-2-(2-hydroxyethyl)allyl Ester (12E and 12Z). To a solution of ether **11** (4.69 g, 13.6 mmol) in dichloromethane (16 mL) and water (1 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (3.40 g, 14.9 mmol, 1.1 eq). A deep green solution formed immediately upon addition of DDQ then gave way to a brown colored suspension in 20 min. The starting material was consumed in 1 h as determined by TLC (silica gel, 9:1 hexane/ethyl acetate). The reaction was then filtered and the filtrate concentrated under reduced pressure to a red oil. The crude product was purified by flash chromatography (silica gel, 2:1 hexane/ethyl acetate, R_f= 0.29(**12E**), R_f= 0.20(**12Z**)) to yield **12E** (1.60 g, 7.14 mmol, 55%), and **12Z** (0.88 g, 3.92 mmol, 29%) as oils. **12Z**: ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 (bs, 1H), 2.32 (td, J= 6.0, 1.0 Hz, 2H), 3.66 (dd, J= 12.0, 6.0 Hz, 2H), 5.00 (dd, J= 3.0, 1.0 Hz, 2H), 6.63 (dt, J= 83.0, 1.0 Hz, 1H), 7.45 (tt, J= 7.0, 2.0 Hz, 2H), 7.58 (td, J= 7.0, 2.0 Hz, 1H), 8.03 (dt, J= 7.0, 2.0 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -126.34 (d, J= 83.0 Hz). **12E**: ^1H NMR (300 MHz, $CDCl_3$) δ 1.59 (bs, 1H), 2.55 (td, J=7.0, 1.0 Hz, 2H), 3.82 (t, J= 7.0 Hz, 2H), 4.12 (dd, J= 7.0, 1.0 Hz, 2H), 6.87 (dt, J= 83.0, 1.0 Hz, 1H), 7.45 (tt, J= 7.0, 2.0 Hz, 2H), 7.58 (td, J= 7.0, 2.0 Hz, 1H), 8.03 (dt, J= 7.0, 2.0 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -127.99 (d, J= 83.0 Hz).

Benzoic Acid E-2-[2-(6-Chloropurin-9-yl)ethyl]-3-fluoroallyl Ester (13E). Triphenylphosphine (883 mg, 3.37 mmol), 6-chloropurine (520 mg, 3.37 mmol), and alcohol **12E** (775 mg, 3.37 mmol) were combined in anhydrous THF (10 mL) at room temperature. Diethylazodicarboxylate (DEAD) (587 mg, 3.37 mmol) was added slowly, dropwise and the reaction mixture became homogenous. After 48 h, starting material was consumed as determined by TLC (silica gel, 1:1 ethyl acetate/hexane). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, 1:1 ethyl acetate/hexane, R_f = 0.33). The coupled product **13E** was collected as an oil containing DEAD-H_2 which was taken on to the next step without further purification.^1H NMR (300 MHz, $CDCl_3$) δ 2.91 (t, J= 1.9 Hz, 2H), 4.53 (t, J= 7.0 Hz, 2H), 4.79 (d, J= 3.6 Hz, 2H), 6.79 (d, J= 82.1 Hz, 1H), 7.45 (dd, J= 7.3, 1.6 Hz, 2H),

7.60 (m, 1H), 8.05 (t, J= 1.1 Hz, 1H), 8.69 (s, 1H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -125.64 (d, J= 82.1 Hz).

E-2-[2-(6-Aminopurin-9-yl)ethyl]-3-fluoroprop-2-en-1-ol (3E). Crude **13E** (576 mg, 1.60 mmol) containing some DEAD-H_2 was dissolved in saturated methanolic ammonia (15 mL) and heated to 50°C in a sealed tube for 16 h. The reaction mixture was then concentrated under reduced pressure and the product purified by flash chromatography (silica gel, 9:1 dichloromethane/methanol, R_f = 0.45) to give **3E** (205 mg, 0.86 mmol, 54%) as an off white powder. m. p.= 168-169°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.63 (dd,, J= 6.7, 1.4 Hz, 2H), 3.37 (bs, 1H), 3.87 (dd, J= 4.1, 1.0 Hz, 2H), 4.28 (t, J= 7.0 Hz, 2H), 4.95 (bs, 1H), 6.74 (d, J= 85.9 Hz, 1H), 7.35 (s, 1H), 8.15 (d, J= 12.2 Hz, 1H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -135.37 (d, J= 85.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.08 (d, J=4.3 Hz), 40.86 (d, J= 2.5 Hz), 58.66 (d, J= 11.0 Hz), 119,43 (d, J= 2.8 Hz), 121.56, 141.05, 145.52, 148.92, 149.34, 151.53, 155.29; HRMS calc ($C_{10}H_{13}N_5OF$), 238.1104; found, 238.1102.

Benzoic Acid Z-2-[2-(6-Chloropurin-9-yl)ethyl]-3-fluoroallyl Ester (13Z). Triphenylphosphine (150 mg, 0.572 mmol, 1.0 eq), 6-chloropurine (88.5 mg, 0.572 mmol, 1.0 eq) and alcohol **12Z** (128 mg, 0.572 mmol) were combined in anhydrous THF (2 mL) at room temperature. Diethylazodicarboxylate (99.6 mg, 0.572 mmol, 1.0 eq) was added and the reaction became homogenous. After 19 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by radial chromatography (2 mm silica gel plate, 1:1 hexane/ethyl acetate, R_f = 0.24) to afford **13Z** (122 mg, 0.34 mmol, 59%) as a white solid. m.p. = 87-88°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (td, J=7.0, 3.0 Hz, 2H), 4.48 (t, J= 7.0 Hz, 2H), 5.11 (s, 2H), 6.37 (d, J=82.2 Hz, 1H), 7.47 (t, J=8.0 Hz, 2H), 7.60 (td, J=8.0, 1.0 Hz, 1H), 8.06 (dd, J=8.0, 1.0 Hz, 2H), 8.08 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.62 (d, J= 5.6 Hz), 42.86 (d, J= 3.9 Hz), 58.15 (d, J= 8.1 Hz), 76.82 (d, J= 23.9 Hz), 114.08 (d, J= 4.1 Hz), 128.58, 129.66, 133.41, 144.91, 147.18, 149.83, 151.59 (d, J= 34.5 Hz), 152.01, 166.46; ^{19}F NMR (282 MHz, $CDCl_3$) δ -123.55 (dt, J= 82.8, 2.0 Hz); HRMS calc ($C_{17}H_{15}N_4O_2ClF$), 361.0868; found, 361.0890.

Z-2-[2-(6-Aminopurin-9-yl)ethyl]-3-fluoroprop-2-en-1-ol (3Z). Compound **13Z** (122 mg, 0.34 mmol) was dissolved in saturated methanolic ammonia (10 mL) and heated to 50°C in a sealed tube for 18 h. Volatile materials were removed under reduced pressure and the residue was purified by radial chromatography (silica gel, 9:1 dichloromethane/methanol, R_f = 0.23) to give **3Z** (32 mg, 0.16 mmol, 49%) as a white solid. m.p.=173-174°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.11 (qd, J= 2.8, 1.0 Hz, 2H), 4.27 (t, J= 7.0 Hz, 2H), 4.95 (t, J= 5.7 Hz, 2H), 6.35 (d, J= 85.7 Hz, 1H), 7.17 (s, 2H), 8.09 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.43 (d, J= 7.0 Hz), 40.34 (d, J= 3.1 Hz), 54.04 (d, J=7.6 Hz), 118.61, 119.50 (d, J= 4.13 Hz), 140.80, 143.21, 146.60, 149.43, 152.30, 155.92; ^{19}F NMR (282 MHz, DMSO-d_6) d -131.04 (dt, J= 85.7, 2.8 Hz); HRMS; calc ($C_{10}H_{13}N_5OF$) 238.1104, found, 238.1102.

Benzoic Acid 2-(2-Bromoethyl)-3E-fluoroallyl Ester (14E). N-Bromosuccinimide (391 mg, 2.20 mmol, 1.1 eq) was added portionwise to a solution of alcohol **12E** (448 mg, 2.00 mmol) and triphenylphosphine (576 mg, 2.20 mmol, 1.1 eq) in dichloromethane (20 mL) at room temperature. After 18 h, the reaction mixture was diluted to 50 mL with dichloromethane and then washed with water (50 mL) and brine (50 mL) then dried $MgSO_4$) and concentrated under reduced pressure. The residue was triturated with 9:1 hexane/ethyl acetate and filtered through a plug of silica gel, eluting with the same solvent mixture. The product containing fractions were combined and evaporated to give **14E** (417 mg, 1.46 mmol, 73%), as a clear colorless oil (silica gel,19:1 hexane/ethyl acetate, R_f = 0.27). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.85 (t, J= 7.0 Hz, 2H), 3.53 (t, J= 7.0 Hz, 2H), 4.76 (dd, J= 3.6, 1.0 Hz, 2H), 6.88 (dt, J= 82.5, 1.0 Hz, 1H), 7.46 (tt, J= 7.0, 2.0 Hz, 2H), 7.56 (tt, J= 7.0, 2.0 Hz, 1H), 8.40 (dt, J= 7.0, 2.0 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -125.89 (d, J=82.5 Hz); MS (CI/CH_4) $[M+H]^+$ 287.

Benzoic Acid 2-(2-Bromoethyl)-3Z-fluoroallyl Ester (14Z). The procedure used to prepare **14E** was applied to **13Z** (455 mg, 2.03 mmol) to give **14Z** (528 mg, 1.85 mmol, 91%) as a clear colorless oil (R_f = 0.38, silica gel, 19:1 hexane/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62 (td, J= 6.8, 1.0 Hz, 2H), 3.52 (t, J= 6.8 Hz, 2H), 4.99 (dd, J= 28, 1.0 Hz, 2H), 6.64 (dt, J= 85.2, 1.0 Hz, 1H), 7.46 (tt, J= 7.0, 2.0 Hz, 2H), 7.59

(tt, J= 7.0, 2.0 Hz, 1H), 8.04 (dt, J= 7.0, 2.0 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -125.90 (d, J= 82.5 Hz); MS (CI/CH_4) $[M+H]^+$ 287.

Benzoic Acid 2-[2-(4-Acetylamino-2-2*H*-pyrimidin-1-yl)ethyl]-3E-fluoroallyl Ester (15E). N^6-acetylcytosine (106 mg, 0.69 mmol, 1 eq), anhydrous potassium carbonate (105 mg, 0.77 mmol, 1.1 eq), and bromide **14E** (198 mg, 0.69 mmol) were combined in anhydrous DMF (10 mL) and heated to 80°C in an oil bath for 18 h. The reaction mixture was cooled to room temperature and partitioned between dichloromethane (50 mL) and saturated aqueous sodium bicarbonate (50 mL). The organic layer was separated, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was triturated with ethyl acetate (10 mL) and allowed to stand for 20 min, The resulting precipitate was collected by filtration to give a portion of the desired product. The filtrate was concentrated under reduced pressure and the remainder of the product was recovered after flash chromatography (silica gel, 49:1 dichloromethane/methanol, R_f = 0.20) to yield **15E** (200 mg, 0.56 mmol, 81%) as a white powder. m.p.= 191-193°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.22 (s, 3H), 2.72 (td, J= 6.6, 1.8 Hz, 2H), 4.08 (t, J= 6.6 Hz, 2H), 4.78 (d, J= 4.0 Hz, 2H), 6.83 (d, J= 82.2 Hz, 1H), 7.31 (d, J= 8.0 Hz, 1H), 7.46 (tt, J= 7.0, 2.0 Hz, 2H), 7.56 (tt, J= 7.0, 2.0 Hz, 2H), 8.82 (t, J= 7.3 Hz, 1H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -126.30 (d, J= 82.2 Hz); HRMS calc ($C_{18}H_{18}FN_3O_4$) 360.1369, found 360.1369.

Benzoic Acid 2-[2-(4-Acetylamino-2-oxo-2*H*-pyrimidin-1-yl)ethyl]-3Z-fluoroallyl Ester (15Z). The procedure used to prepare **15E** was applied to **14Z** (528 mg, 1.84 mmol) to give **15Z** (179 mg, 0.55 mmol, 30%) as a white powder. m.p.= 203-204 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26 (s, 3H), 2.51 (td, J= 6.1, 0.5 Hz, 2H), 4.03 (t, J= 66.1 Hz, 2H), 5.05 (d, J= 3.0 Hz, 2H), 6.49 (d, J= 82.6 Hz, 1H), 7.37 (d, J= 7.2 Hz, 1H), 7.46 (tt, J= 7.0, 2.0 Hz, 2H), 7.46 (tt, J= 7.0, 2.0 Hz, 1H), 8.06 (dt, J= 7.0, 2.0 Hz, 2H), 9.49 (bs, 1H); ^{19}F NMR (282 MHz, CDCl3) δ -124.10 (d, J= 82.6 Hz); HRMS calc ($C_{18}H_{18}FN_3O_4$) 360.1369, found 360.1367.

4-Amino-1-(4-fluoro-3-hydroxymethylbut-3E-enyl)-1*H*-pyrimidin-2-one (4E). A mixture of **15E** (284 mg, 0.79 mmol) in methanol (20 mL) was saturated with ammonia

at 5°C, and then sealed and heated in a 50°C oil bath for 18 h. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography (silica gel, 9:1 dichloromethane/methanol, $R_f = 0.27$) to give **4E** (107 mg, 0.51 mmol, 64%) as an off-white powder. m.p.= 136-138°C; ^{1}H NMR (300 MHz, CD_3OD) δ 2.56 (td, J=6.6, 2.0 Hz), 3.29 (t, J= 6.6 Hz, 2H), 4.00 (dd, J= 4.0, 1.0 Hz, 2H), 5.81 (d, J= 7.1 Hz, 1H), 6.72 (d, J= 84.6 Hz, 1H), 7.53 (d, J= 7.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 26.09 (d, J= 4.1 Hz), 49.43, 61.81, 95.49, 120.84 (d, J= 4.6 Hz), 148.08, 151.51, 159.26, 168.44; ^{19}F NMR (282 MHz, CD_3OD) δ -133.35 (d, J= 84.6 Hz); HRMS calc ($C_9H_{13}FN_3O_2$) 214.0992, found 214.0984.

4-Amino-1-(4-fluoro-3-hydroxymethylbut-3Z-enyl)-1*H*-pyrimidin-2-one (4Z). 1% (w/v) Aqueous NaOH (2 mL) was added to **15Z** (100 mg, 0.55 mmol) in methanol (8 mL) at room temperature. After 1.5 h, the mixture was concentrated under reduced pressure and crude product was purified by flash chromatography (silica gel, 17:3 dichloromethane/methanol, $R_f = 0.23$) to give **4Z** (94.2 mg, 0.39 mmol, 70%) as a white powder. (Note: This is the preferred method for deprotection of **15Z** since the methanolic ammonia procedure requires careful chromatography to yield pure product). m.p. 121-125°C; ^{1}H NMR (300 MHz, CD_3OD) δ2.39 (td, J= 6.8, 3.0 Hz, 2H), 3.89 (t, J= 6.8 Hz, 2H), 4.24 (dd, J= 3.0, 0.7 Hz, 2H), 5.83 (d, J= 7.2 Hz, 1H), 6.40 (d, J= 85.2 Hz, 1H), 7.52 (d, J= 7.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 28.30 (d, J= 6.9 Hz), 49.07, 56.45 (d, J= 8.1 Hz), 95.56, 120.07 (d, J= 4.1 Hz), 146.19; HRMS calc ($C_9H_{13}FN_3O_2$) 214.0992, found 214.0990

Cytotoxicity Determinations in HeLa Cells. Exponentially growing HeLa cells plated at a density of 1 x 10^5 cells/35 mm dish were treated with different concentrations of compounds. The cells were allowed to grow in a 5% CO_2 incubator at 37°C for 72 h. At the end of the incubation, cells were collected by trypsinization and counted by a Coulter counter and IC_{50} for growth inhibition was determined.

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