Synthesis of 2′,5′-Dideoxy-2-fluoroadenosine and 2′,5′-Dideoxy-2,5′-difluoroadenosine: Potent P-Site Inhibitors of Adenylyl Cyclase

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Glycosylation of 2-fluoroadenine with the appropriate protected thioglycoside derivatives, followed by deprotection and anomer separation, produced the α- and β-anomers of 2′,5′-dideoxy-2-fluoroadenosine (**1**), 2′,5′-dideoxy-2,5′-difluoroadenosine (**2**), and 2′-deoxy-2-fluoroadenosine (**3**). These were examined as P-site inhibitors of adenylyl cyclase. The presence of fluorine on the purine ring increased potency of inhibition, and the most potent compound, β-2′,5′-dideoxy-2-fluoroadenosine (**1b**), was 3 times more potent than β-2′,5′-dideoxyadenosine.

Introduction

Adenosine 3′:5′-cyclic monophosphate (cAMP) is a ubiquitous regulatory molecule that plays a critical role in intracellular signaling pathways through activation of protein kinases and of cyclic nucleotide-gated ion channels. The diverse and critical effects on cell function that result from these downstream activation events make clear the importance of precise regulation of intracellular cAMP levels. This regulation is accomplished by modulation of the activity of adenylyl cyclase, the enzyme that catalyzes the synthesis of cAMP from 5′-ATP, most notably by interaction with G-protein α subunits. These subunits mediate the influence of cell-surface receptors that are activated by such diverse moieties as peptide hormones, neurotransmitters, prostaglandins, and adenosine.[1]

The actions of adenosine on adenylyl cyclase are complex. In 1970 Sattin and Rall[2] demonstrated that adenosine stimulates cAMP accumulation in brain slices through stimulation of adenylyl cyclase. Later it was shown that adenosine also could inhibit adenylyl cyclase. Subsequently, Londos and Wolff[3] proposed two distinct sites of action for adenosine. These were the so-called R-sites (ribose moiety of adenosine strictly required) that are adenosine binding domains of cell-surface G-protein coupled receptors that activate or inhibit cyclase activity indirectly through interactions of G_s or G_i. In contrast, the proposed P-sites (purine moiety of adenosine required) proved to be directly on adenylyl cyclase and binding of adenosine to these sites was responsible for direct inhibition of the enzyme. The mechanism and possible physiological significance of modulation of adenylyl cyclase activity by the binding of endogenous adenosine and by synthetic P-site inhibitors recently have attracted much attention.[4−9]

Key structural requirements for P-site ligands include a requirement for an intact adenine moiety, enhanced inhibitory potency with 2′-deoxy- and especially 2′,5′-dideoxyribosyl moieties, a β-glycosidic linkage for the ribosyl moiety, and a notably strong preference for a 3′-phosphate. For example, examination of adenosine analogues revealed an order of potency 2′,5′-dd-3′-A4P > 2′,5′-dd-3′-ATP > 2′,5′-dd-3′-ADP > 2′,5′-dd-3′-AMP > 2′-d-3′-AMP > 3′-AMP > 2′-d-Ado > Ado.[7,8] This SAR and kinetics of inhibition have provided valuable insight into the mechanism and modes of binding of P-site ligands.[4,6−9] From structural studies, it is now known that "P-site" ligands bind within the catalytic active site at the same locus as substrate 5′-ATP, but with the post-transition configuration of the enzyme, with the possible exclusion of divalent cation at one of the two metal binding sites.[10,11]

Of the adenosine derivatives studied as P-site inhibitors, those containing 2-F-substituted adenines consistently exhibited increased inhibitory potency.[12,13] To take advantage of this observation, structural features of other known P-site ligands were combined with this enhancing 2-fluoro substitution. The initial target was 2′,5′-dideoxy-2-fluoroadenosine (**1**). With procedures developed in this synthetic work, we extended this work to include 2′5′-dideoxy-2,5′-difluoroadenosine (**2**) and the known 2′-deoxy-2-fluoroadenosine (**3**). We report herein the details of the syntheses, as well as the results of examination of their effects on adenylyl cyclase activity.

Chemistry

The development of methods to synthesize nucleosides and nucleotides fluorinated on the sugar and/or base moiety has been an active area of research in nucleoside chemistry. This research reflects the potent biological properties possessed by many of these fluorinated analogues. In particular, many members of this class have proven to be effective anticancer and antiviral agents. There have been two general approaches to the syntheses of 2-fluoroadenine nucleosides. Much of the

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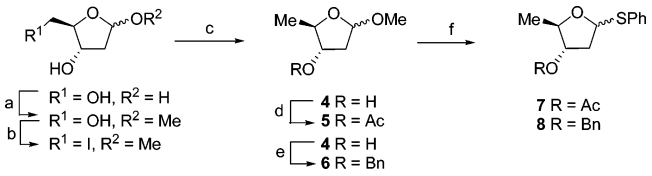
Scheme 1[a]

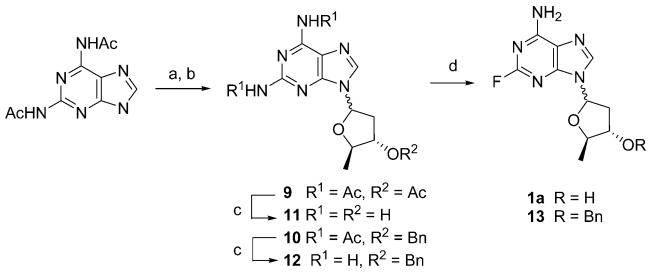


[a] Reagents and conditions: (a) Dowex H^+, MeOH; (b) I_2, Ph_3P (ref 10); (c) H_2/Pd−C (ref 10); (d) Ac_2O, pyridine; (e) BnBr, NaH, $(n\text{-Br})_4N^+I^-$; (f) PhSH, ZnI_2, $(n\text{-Bu})_4N^+I^-$.

early work was based on synthesis of an appropriate 2-aminopurine nucleoside, followed by generation of a 2-diazonium group and displacement of this group with fluoride. For example, Montgomery and Hewson[14] prepared 2-fluoroadenosine from 2-aminoadenosine in 16% yield by a Schiemann reaction in aqueous solution. Using KNO_2/HF/pyridine to effect this conversion, Krolikiewicz and Vorbrüggen[15] improved the conversion to 80%. Montgomery and Hewson[16] initially prepared 2′-deoxy-2-fluoroadenosine by a multistep sequence that included fluorination of a 2,6-diaminopurine nucleoside precursor as a key step, but the overall yield was quite low. Secrist and co-workers[17] recently developed an alternative approach to 2′-deoxy-2-fluoroadenosine based on free radical removal of the 2′-OH group from the more readily available 2-fluoroadenosine. The second general approach involved synthesis of a fluorinated base followed by coupling to the sugar. However, direct coupling of such bases with 2-deoxysugars is complicated by a tendency to form mixtures of α- and *β*-anomers, a complication lessened in the ribose series because of the participation of the sugar C-2 OH group during coupling. However, a recent report[18] describes an NBS-promoted coupling reaction of thioglycosides with silylated heterocyclic bases that favors formation of the *β*-anomer when applied to 2′-deoxy-D-*threo*-pentofuranosyl thioglycosides. Although steric factors in the glycosylation reaction undoubtedly will differ with 5′-deoxypentofuranosides in the erythro configuration, we have emphasized this coupling procedure in the present syntheses.

Coupling of Diaminopurine Derivatives Followed by Fluorination. We initially attempted the synthesis of 2′,5′-dideoxy-2-fluoroadenosine (**1**) based on initial coupling of a silylated derivative of readily available 2,6-diacetamidopurine with the appropriate thioglycoside. Methyl 2,5-dideoxyribofuranoside (**4**) was prepared from 2-deoxyribofuranose as described by Soll and Seitz,[19] with minor changes in the procedure (Scheme 1). For example, we used an acidic resin (Dowex, hydrogen form, HCR-W2) to catalyze the initial formation of methyl 2-deoxyribofuranoside from the furanose, since product could be isolated by simple filtration from the resin, obviating the neutralization of acid. Replacement of the 5-hydroxyl group with iodine occurred under Mitsunobu conditions, and hydrogenolysis produced **4** as a mixture of anomers. This mixture was converted to the 3-*O*-acetyl derivative **5** with acetic anhydride and to the 3-*O*-benzyl derivative **6** by alkylation with benzyl bromide. The phenyl 2-deoxy-1-thio-D-*erythro*-pentofuranosides (**7** and **8**) were easily prepared by treating **5** or **6** with benzenethiol, zinc iodide, and tetrabutylammonium iodide in anhydrous dichloromethane (Scheme 1).[20]

Scheme 2[a]



[a] Reagents and conditions: (a) HMDS, $(NH_4)_2SO_4$, reflux, 3 h, 48−52%; (b) sugar **7** or **8,** CH_2Cl_2, molecular sieves, 4Å, NBS, room temp, 72 h; (c) NaOMe/MeOH, reflux, 3 h, ∼100%; (d) (60%) HF/pyridine, *t*-$BuNO_2$, −20 to −30 °C, ∼4 h, ∼10%.

The condensation reaction of the sugars **7** or **8** with the silylated derivative of 2,6-diacetamidopurine proceeded with poor stereoselectivity to give a chromatographically inseparable anomeric mixture of the N-9 regioisomers of 2-acetamido-N^6-acetyl-9-(3-*O*-acetyl-2,5-dideoxy-α,*β*-D-*erythro*-pentofuranosyl)adenine (**9**) in 52% yield (α:*β* = 1.25:1 by ^1H NMR) and 2-acetamido-N^6-acetyl-9-(3-*O*-benzyl-2,5-dideoxy-α,*β*-D-*erythro*-pentofuranosyl)adenine (**10**) in 48% (α:*β* = 4:1 by ^1H NMR) (Scheme 2). The anomeric configurations of **9** or **10**, and also of **11** (below), were tentatively assigned on the basis of empirical anomeric proton NMR line shape criteria described by Robins et al.[21] According to these criteria, the H-1′ proton of the *β*-anomer will appear as an "apparent triplet" whereas in the α-configuration H-1′ will appear as a doublet of doublets. The deacylation of the anomeric mixtures of **9** and **10** in 1 N methanolic sodium methoxide produced **11** and **12** (quantitative yield), respectively, in an α:*β* ratio of 1:1 of **11** and an α:*β* ratio of 4:1 of **12** (Scheme 2) (see Supporting Information).

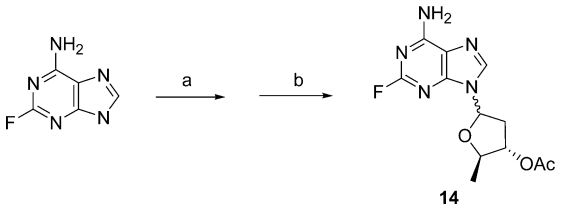
The α and *β* anomers of **11** were separated by preparative thin-layer chromatography on silica gel for further characterization. The anomeric configuration of the higher R_f product was assigned the α-configuration from a 2D-NOESY experiment (see Supporting Information) confirming the assignment based on the signal shape of the H-1′ proton.

The anomeric mixtures of compounds **11** (α:*β* = 1:1) and **12** (α:*β* = 4:1) were then treated with 60% HF/pyridine and *tert*-butyl nitrite at −10 °C to produce **1a** and **13** in unsatisfactory yields (ca. 10%). The 3-*O*-benzyl derivative **13** was obtained as an anomeric mixture (α:*β* = 1:1) instead of the α:*β* ratio of 4:1 present in **12**, and only the α-anomer **1a** was obtained from **11**. This may be due to the difficulties encountered during the separation/purification of **13** or **1** by preparative chromatography.

Glycosylation Reaction with 2-Fluoroadenine. Synthesis of 2′,5′-Dideoxy-2-fluoroadenosine (1). The low yield in the fluorination reaction and the poor stereoselectivity of the glycosylation reaction prompted us to examine a different approach, based on glycosylation of the fluorinated purine. This alternative approach was made more attractive by the commercial availability of 2-fluoroadenine.

Sugino and Sugimura[20] found that reaction of a series of silylated purines (and silylated pyrimidines) with 2-deoxy thiosugars (threo) produced excellent yields of nucleosides with good *β*-selectivity. However, condensa-

Scheme 3[a]



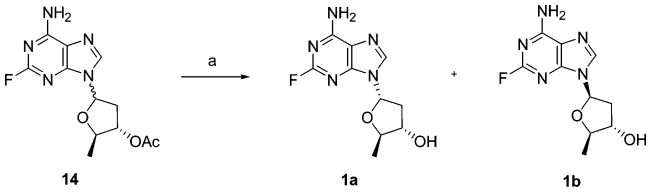
[a] Reagents and conditions: (a) HMDS, $(NH_4)_2SO_4$, reflux; (b) thioglycoside **7**, NBS, molecular sieves, 4 Å, solvent, 0°C to room temperature.

Table 1. Optimization of the Coupling Reaction of Silylated 2-F-adenine with Thioglycoside **7**

entry	amount of $(NH_4)_2SO_4$, equiv	solvent	yield,[a] %	α:β of **14**[b]
1	0.1[c]	DCM	29	4.6:1
2	1.0	DCM	33	1.4:1
3	1.0	toluene	47	1.3:1
4	4.0	toluene	34	1.2:1

[a] Isolated yields. [b] Determined by ^{1}H NMR (300 MHz). [c] Small amount of DMF was added (DMF/HMDS, 5:100).

Scheme 4[a]



[a] Reagents and conditions: (a) 5% Et_3N in MeOH, room temperature.

tion of **7** (erythro) with silylated 2-fluoroadenine by NBS-promoted reaction under conditions described by Sugino and Sugimura (Scheme 3) initially gave modest yields and undesired α-selectivity (entry 1, Table 1). This was similar to our results with the diacetamidopurine. The low solubility of 2-fluoroadenine in hexamethyldisilazane seemed perhaps problematic, but addition of DMF led to lower yields. However, if DMF was excluded, silylation was not complete even after 24 h. Fortunately, with addition of 1.0 equiv of ammonium sulfate and vigorous reflux in hexamethylsilazane, the reaction was complete after several hours, as evidenced by the formation of a clear solution. The yield of **14** was increased only modestly, but there was a more significant decrease in α-selectivity (entry 2). The yield was further improved by replacing dichloromethane with toluene as solvent (entry 3). When the amount of ammonium sulfate was increased to 4 equiv, the yield was decreased with almost the same α-selectivity. We have used conditions corresponding to entry 3 for the preparation the α- and β-anomers of **14** (Scheme 3) and related nucleosides (see below) and consistently have achieved satisfactory results.

The anomeric mixture of product **14** was deacetylated to give the corresponding α and β anomers **1a** and **1b** in high yield. These were separated by chromatography on silica gel (Scheme 4).

The anomeric configuration of the higher R_f product was assigned from a 2D-NOESY experiment. An NOE between H-1′ and H-5′, H-1′ and H-2′b, and H-1′ and H-3 was observed, while there were no NOEs observed between H-1′ and H-4′ and between H-1′ and H-2a′

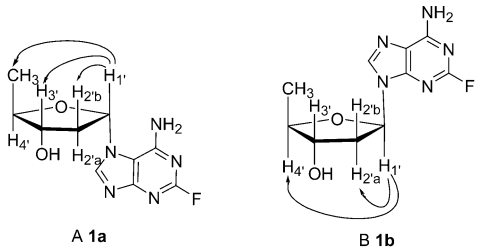


Figure 1. NOEs observed between H-1′ and other protons in compounds **5a** and **5b**.

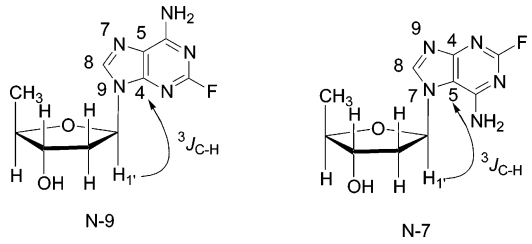


Figure 2. N-9 and N-7 regioisomers of **1b**.

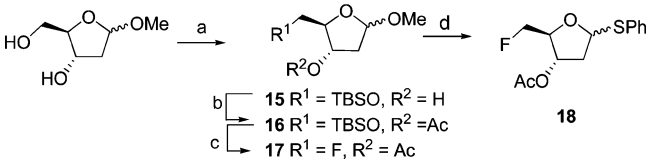
(Figure 1A). These findings indicate that the higher R_f product is the α anomer **1a**. This confirmed the previous assignment of **1a** produced by the alternative synthetic procedure described above. The 2D-NOESY experiment of the lower R_f product showed NOEs between H-1′ and H-2′a and between H-1′ and H-4′, while there was no NOE between H-1′ and H-5′ (Figure 1B). These results indicate that this is the β anomer **1b**. The spectra are provided in Supporting Information.

The assignment of the site of the attachment of the purine ring (N-7 or N-9) of **1b** was investigated by the heteronuclear multiple bond correlation (HMBC). The cross-peaks in HMBC spectra contain information on heteronuclear long-range coupling constants between protons and carbons separated by two or three chemical bonds ($^2J_{C-H}$ or $^3J_{C-H}$). As shown in Figure 2, a $^3J_{C-H}$ can be observed between H-1′and C-4 in the case of the regioisomer N-9, while a $^3J_{C-H}$ between H-1′and C-5′ can be detected in the N-7 regioisomer (C-4 distinguished from C-5 with the coupling of $^3J_{C-F}$ on ^{13}C NMR). The HMBC spectrum of compound **1b** showed a correlation between H-1′ and C-4, while there was no correlation between H-1′ and C-5. This suggested that compound **1b** was the N-9 regioisomer. The spectrum is provided in Supporting Information.

Synthesis of 2′,5′-Dideoxy-2,5′-difluoroadenosine (2). The stronger inhibition of adenylyl cyclases by 2′,5′-dideoxyadenosine compared with 2′-deoxyadenosine demonstrates the favorable influence on binding caused by replacement of the 5′-hydroxyl group with hydrogen. The ability of the 5′-deoxy sugar to have favorable hydrophobic interactions with a binding domain has been proposed as an explanation for the enhanced potency.[9] Fluorine is considered to be a good isosteric replacement for hydroxyl, but fluorine is unable to function as a hydrogen bond donor.[22] Fluorine substitution would also increase hydrophobicity relative to a 5′-OH but presumably would decrease hydrophobicity relative to the 5′-deoxy analogue.[22] To explore the effects of 5′-substitution, the 5′-deoxy-5′-fluoro analogue was prepared.

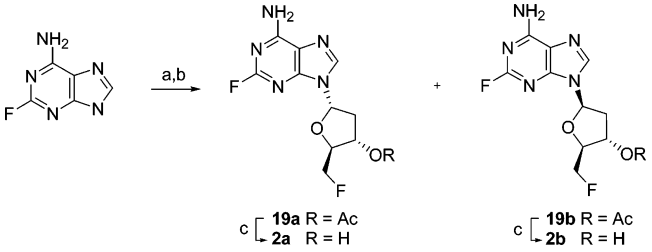
The synthesis was also based on condensation of the thioglycoside with silylated 2-fluoroadenine. For this

Scheme 5[a]



[a] Reagents and conditions: (a) ref 26; (b) Ac_2O, pyridine, DCM, 0 °C to room temperature, 98%; (c) bis[(2-methoxyethyl)amino]-sulfur trifluoride, DCM, reflux, 86%; (d) PhSH, ZnI_2, *n*-$Bu_4N^+I^-$, DCM, 0 °C to room temperature, 47%.

Scheme 6[a]



[a] Reagents and conditions: (a) HMDS, $(NH_4)_2SO_4$, reflux; (b) thioglycoside **18**, NBS, molecular sievers, 4 Å, DCM, 20% (**19a**) and 12% (**19b**); (c) 5% Et_3N in MeOH/H_2O (10:1), 70% (**2a**), 69% (**2b**).

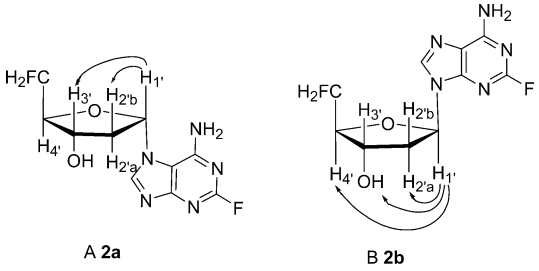


Figure 3. NOEs observed between H-1′ and other protons in anomers **2a** and **2b**.

synthesis we developed a new synthesis of 2′,5′-deoxy-5′-fluororibose, a compound previously made by aldolase condensation of 3-fluoro-2-hydroxypropanal with acetaldehyde.[23] Methyl 2-deoxyribofuranoside was converted to the 3-*O*-acetyl-5-*O*-(*tert*-butyldimethylsilyl) derivative **16** in two steps. Reaction of **16** with bis[(2-methoxyethyl)amino]sulfur trifluoride gave the 2,5-dideoxy-5-fluoro riboside **17**, from which was prepared the phenylthioriboside **18** with the usual procedure (Scheme 5).

Reaction of **18** with 2-fluoroadenine under conditions optimized for the preparation of **14** produced the anomeric mixture **19a** and **19b**. These were separated by chromatography on silica gel and deacylated to give **2a** and **2b** (Scheme 6).

The α and β anomers of **2a** and **2b** (Figure 3) were assigned by their distinguished NOESY spectra in the same manner as for **1a** and **1b** (Figure 2). The spectra are provided in Supporting Information.

It is necessary to point out that the coupling system of H-1′ of α-anomer **1a** and **2a** is doublet−doublet, while the coupling system of H-1′ of β-anomer **1b** and **2b** are triplet ($^2J_{1',2'a} = {}^2J_{1',2'b}$) in their ^{1}H NMR spectra. Thus, the "line shape" criterion observed by Robins et al. can be verified by the NOEs observed in these experiments.[21]

The N-9 regioisomer **2b** was identified by its HMBC spectrum. The HMBC spectrum of compound **2b** showed

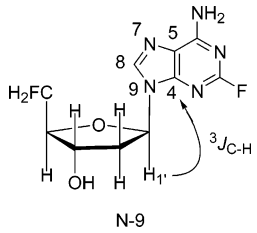


Figure 4. HMBC observed between H-1′ and C-4 of compound **2b**.

a correlation between H-1′ and C-4, while there is no correlation between H-1′ and C-5 (Figure 4). This confirms that compound **1b** was the desired N-9 regioisomer. The spectra are provided in Supporting Information.

Preparation of 2′-Deoxy-2′-fluoroadenosine (3). Previous syntheses of this analogue by Montgomery and Hewson[16] and by Secrist and co-workers[17] were discussed in the Introduction. Because of the current interest in this analogue as a prodrug for the toxic 2-fluoroadenine,[24] we have communicated in an earlier report our new synthesis of this compound using the same approach as described above for the synthesis of **1** and **20**.[25]

Biological Results

The efficacy of the nucleoside derivatives described in this report was tested with a detergent-dispersed preparation of adenylyl cyclase extracted from whole rat brain. This particular preparation has been used to characterize inhibition kinetics and inhibition by numerous ligands over many years and as such provides a reliable and remarkably reproducible means by which inhibitory potencies can be compared.[4−8] As predicted from data in an earlier report with 2F-Ado,[12] β-2′-d-2-F-Ado ($IC_{50} \approx 4.6$ μM) and β-2′,5′-dd-2-F-Ado ($IC_{50} \approx 0.9$ μM) were noticeably more potent that the corresponding unmodified nucleosides β-2′-d-Ado ($IC_{50} \approx 15$ μM) and β-2′,5′-dd-Ado ($IC_{50} \approx 2.8$ μM) (Figure 5 and Table 2). The lack of effectiveness of the cognate α-anomers, α-2′-d-2-F-Ado and α-2′,5′-dd-2-F-Ado, was consistent with an earlier observation that α-Ado was not inhibitory.[4] Somewhat unexpected were the observations that β-2′,5′-dd-2,5′-di-F-Ado ($IC_{50} \approx 1$ μM) was essentially as potent as β-2′,5′-dd-2-F-Ado. The weak activity detected for the corresponding α-anomer, α-2′,5′-dd-2,5′-di-F-Ado ($IC_{50} \approx 29$ μM), could be caused by traces of the β-anomer in the test sample, an issue under investigation. The data support the concept that 2-F-substituted adenine derivatives will provide a basis for the development of yet more potent and selective inhibitors and of this important family of enzymes. Experiments on the effects of these compounds on intact cell and tissue systems are in progress. Given the central role that adenylyl cyclases play in transmembrane signaling mechanisms, the availability of more potent, selective, and membrane-permeable inhibitors should be expected to have broad usefulness in research in areas of cell physiology and intercellular signaling.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus in sealed tubes and are uncorrected. Analytical TLC was performed on Merck silica gel

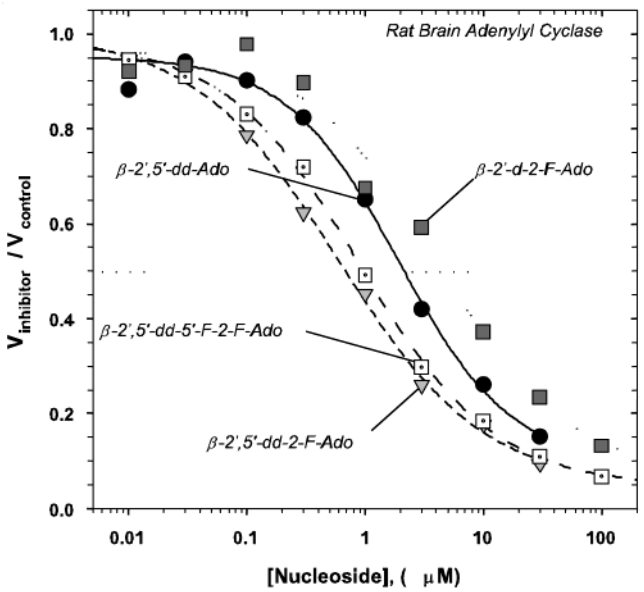


Figure 5. Inhibition of adenylyl cyclase by fluorine derivatives of adenosine. Adenylyl cyclase was extracted from whole rat brain and assayed as previously described.[4−8] The reaction mixture included 100 μM MnATP, 5 mM $MnCl_2$, 100 μM forskolin for a 15 min reaction at 37 °C. Data are from representative experiments, and values are enzyme velocities (nmol of cAMP formed/(min·mg protein)) in the presence of inhibitors relative to velocities in their absence.

Table 2. Inhibition of Adenylyl Cyclase by Adenine Nucleosides[a]

nucleoside	IC_{50}, μM
β-Ado	82[b]
α -Ado	>300[b]
β-2′-d-Ado	15[b]
β-2′-d-2-F-Ado	4.6
α-2′-d-2-F-Ado	>100
β-2′,5′-dd-Ado	2.8
β-2′,5′-dd-2-F-Ado	0.89
α-2′,5′-dd-2-F-Ado	>100
β-2′,5′-dd-2,5′-di-F-Ado	0.98
α-2′,5′-dd-2,5′-di-F-Ado	29

[a] Experiments were conducted as described for Figure 5. IC_{50} values are averages from at least two experiments and were determined from graphical analyses of data as shown in Figure 2. Variation in IC_{50} values is less than ±20%. [b] Values from ref 4.

plates (10 cm) with QF-254 indicator. Reactions were monitored by TLC, and spots were visualized with UV light or by spraying with a phosphomolybdic acid (20 wt % in ethyl alcohol) reagent. Preparative TLC were performed on Uniplate silica gel GF plates (20 cm × 20 cm, 2000 μm) purchased from Analtech. Flash column chromatography was performed with 230−400 mesh silica gel-60 from Merck. Solvents for extraction and chromatography were technical grade and were used as received. Reaction solvents were distilled from the indicated drying agents: dichloromethane (P_2O_5), methanol (magnesium), tetrahydrofuran (THF) (sodium benzophenone ketyl), and hexamethyldisilazane (CaH_2). Infrared spectra (IR) were obtained on an Easy IR 314 FTS/45 spectrophotometer from the BIO-RAD Digilab Division. Peaks are reported in cm^{-1} with the following relative intensities: s (strong, 67−100%), m (medium, 34−66%), w (weak, 0−33%). ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini-300 FT spectrometer. Tetramethylsilane (TMS) was used as an internal standard for ^1H and ^{13}C NMR spectra and CCl_3F for ^{19}F NMR spectra. Spectra were recorded in the following solvents: $CDCl_3$ (7.26 ppm for ^1H, 77.0 ppm for ^{13}C) and dimethyl sulfoxide (δ 2.50 ppm for ^1H, 39.5 ppm for ^{13}C). All ^{19}F NMR spectra were recorded in Me_2SO-d_6, and ^{19}F chemical shifts are expressed in δ ppm upfield (minus sign) from CCl_3F. Low-resolution electron-impact (EI) mass spectra were obtained on a Finnigan 4600 spectrometer with a typical ionization voltage of 70 eV. High-resolution chemical ionization (CI) mass spectra were obtained on a VG Analytical 7070 E spectrometer using ammonia. Data are reported in the form *m*/*e* (intensity relative to base = 100). Elemental analyses were performed by Atlantic Microlab. Inc, Norcross, GA, and Galbraith Laboratories, Inc., Knoxville, TN. Yields refer to chromatographically and spectroscopically pure compounds.

Methyl 3-*O*-Acetyl-2,5-dideoxy-D-*erythro*-pentofuranoside (5). To an ice-cold solution of methyl 2,5-dideoxy-α,β-D-*erythro*-pentofuranoside **4** (1.1 g, 8.32 mmol) in anhydrous dichloromethane (5 mL) was added pyridine (1.5 mL, 18.5 mmol) and acetic anhydride (1.7 mL, 18 mmol). The reaction mixture was stirred for 20 h at room temperature under N_2. The solution was concentrated by rotary evaporation, and the residue was coevaporated with toluene and was chromatographed on silica gel (hexane/EtOAc, 9:1) to give 1.05 g of a mixture of α and β anomers of **5** as a colorless oil (72% yield). ^1H NMR ($CDCl_3$, 300 MHz) (α and β), δ: 1.33 (d, 3H, $J_{4,5}$ = 6.6, H-5), 2.05, (s, 3H, CH_3COO−), 2.19 (ddd, 1H, $J_{2b,3}$ = 3.9 Hz, $J_{1,2b}$ = 5.5 Hz, J_{gem} = 14.5, H-2b), 2.35 (ddd, 1H, $J_{1,2a}$ = 2.7 Hz, $J_{2a,3}$ = 6.75 Hz, $J_{2a,2b}$ = 14.5 Hz, H-2a), 3.37 (s, 3H, OCH_3), 4.15 (qd, 1H, $J_{3,4}$ =2.7 Hz, $J_{4,5}$= 6.6 Hz, H-4), 5.03 (ddd, 1H, $J_{2a,3}$= 6.75 Hz, $J_{2b,3}$ = 3.9 Hz, $J_{3,4}$ =2.7 Hz, $J_{2b,3}$ = 3.9 Hz, H-3), 5.12 (dd, 1H, $J_{1,2a}$ = 2.7 Hz, $J_{1,2b}$ = 5.5 Hz, H-1). IR (CCl_4), cm^{-1}: 2970 (s), 1739 (s), 1450 (w), 1368 (w), 1260 (s), 1096 (s), 990 (s), 865 (w), 798 (s), 665 (w), 487 (w). MS (CI, NH_3), *m*/*e*: 192 ($[M + NH_4]^+$, 5.0), 160 (100), 143 (50). Anal. Calcd for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.47; H, 8.30.

Phenyl 3-*O*-Acetyl-2,5-dideoxy-1-thio-α,β-D-*erythro*-pentofuranoside (7). Zinc iodide (3.66 g, 11.47 mmol), benzenethiol (1.25 mL, 11.48 mmol), and tetrabutylammonium iodide (0.5 g, 1.35 mmol) were added to a solution of **5** (1.0 g, 5.74 mmol) in dry CH_2Cl_2 (60 mL). This suspension was refluxed with stirring under N_2. After 7 h, the reaction mixture was filtered. The filtrate was washed with saturated $NaHCO_3$ (10 mL) and was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed on silica gel with hexane/EtOAc (9:1) to give 1.02 g (70%) of a mixture of α and β anomers (1.8:1) of **7** as a colorless oil. ^1H NMR ($CDCl_3$, 300 MHz) (α and β), δ: 1.30 (d, 3H, $J_{4,5}$ = 6.6 Hz, H-5), 1.33 (d, 3H, $J_{4,5}$ = 6.3 Hz, H-5), 1.99−2.05 (m, 4H, CH_3COO−, H-2a), 2.10 (s, 3H, CH_3COO−), 2.28 (ddd, 1H, $J_{2a,3}$ = 5.7 Hz, $J_{1,2a}$ = 8.7 Hz, $J_{2a,2b}$ = 14.4 Hz, H-2b), 2.39 (ddd, 1H, $J_{1,2a}$ = 6.3 Hz, $J_{2a,3}$ = 1.8 Hz, $J_{2a,2b}$ = 14.4 Hz, H-2a), 2.88 (ddd, 1H, $J_{1,2b}$ = 7.8 Hz, $J_{2b,3}$ = 8.0 Hz, $J_{2a,2b}$ = 14.7 Hz, H-2b), 4.17 (qd, 1H, $J_{3,4}$ = 1.8 Hz, $J_{4,5}$ = 6.6 Hz, H-4), 4.31−4.40 (m, 1H, H-4), 4.77 (ddd, 1H, J = 3.9 Hz, J = 5.0, $J_{2b,3}$ = 8.0 Hz, H-3), 4.93 (dt, 1H, $J_{3,4}$ = $J_{2b,3}$ = 1.8 Hz, $J_{2a,3}$ = 5.7 Hz, H-3), 5.54 (dd, 1H, $J_{1,2b}$ = 6.3 Hz, $J_{1,2a}$ = 8.7 Hz, H-1), 5.69 (dd, 1H, $J_{1,2a}$ = 3.3 Hz, $J_{1,2b}$ = 7.8 Hz, H-1), 7.20−7.54 (m, 5H, H-arom). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 7.96 and 19.81 (C-5), 20.84 (CH_3COO−,), 37.75 and 38.97 (C-2), 78.67 and 77.91 (C-4), 81.79 (C-3), 85.32 and 86.35 (C-1), 126.96−135.92 (C-arom), 170.94 (−*C*O−). IR (CCl_4), cm^{-1}: 2962 (s), 2906 (w), 1737 (s), 1583 (w), 1481 (w), 1439 (w), 1242 (s), 1178 (w), 1091 (s), 1020 (s), 937 (w), 798 (s), 741 (w), 691 (w), 513 (w). MS (EI, 70 eV), *m*/*e*: 252 (M^+, 10), 208 (10), 143 (100), 109 (100), 83 (100), 55 (100). MS (CI, NH_3), *m*/*e*: 270 ([M + NH4]$^+$, 90), 160 (90), 143 (100). HRMS (CI, NH_3) Calcd for ($C_{13}H_{16}O_3S$): 252.33. Found: 252.08.

2-Fluoro-9-(3-*O*-acetyl-2, 5-dideoxy-α,β-D-*erythro*-pentofuranosyl)adenine (14). A suspension of 2-fluoroadenine (766 mg, 5.0 mmol) and $(NH_4)_2SO_4$ (661 mg, 5.0 mmol) in hexamethyldisilazane (10 mL) was heated at rigorous reflux under N_2 until the solution became clear. The excess of hexamethyldisilazane was removed under reduced pressure. The residue and thioglycoside **7** (1.26 g, 5.0 mmol) were dissolved in 20 mL of dry toluene under N_2, and then 2.5 g of powered molecular sieves, 4 Å, was added. After being stirred at room temperature for 30 min, the reaction mixture was

cooled to 0 °C and NBS (979 mg, 5.5 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred for 18 h. The addition of aqueous $Na_2S_2O_3$ was followed by filtration and then extraction with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (dichloromethane/methanol, 40:1) to give 694 mg (47%) of **14** as a mixture of α and β anomers (α:β = 1.3:1) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 8.06 (1H, s, H-8 (α)), 7.94 (1H, s H-8 (β)), 6.39 (1H, dd, 7.2, 2.1 Hz H-1′ (α)), 6.30 (1H, dd, 8.1, 6.0 Hz, H-1′ (β)), 6.03 (br, NH_2 (α and β)), 5.15 (1H, dt, 6.0, 2.1 Hz, H-3′ (β)), 5.09 (1H, dt, 6.6, 1.5 Hz, H-3′ (α)), 4.56 (1H, dq, 2.1, 6.6 Hz, H-4′ (α)), 4.28 (1H, dq, 2.4, 6.6 Hz, H-4′ (β)), 3.0−2.5 (4H, m, H-2′ (α and β)), 2.13 (3H, s, OAc (β)), 2.00 (3H, s, OAc (α)), 1.47 (3H, d, 6.6 Hz, H-5′(β)), 1.36 (3H, d, 6.6 Hz, H-5′ (α)). MS (CI, NH_3), *m/e*: 296 (MH^+).

2-Fluoro-9-(2,5-dideoxy-D-*erythro*-pentofuranosyl)-adenine (1a and 1b). To a solution of **14** (694 mg, 1.89 mmol) in 15 mL of methanol was added triethylamine (0.75 mL). The solution was stirred at room temperature for 48 h, and then a small amount of silica gel was added to quench the reaction. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (dichloromethane/methanol, 9:1) to give **1a** (210 mg, 44%) as a white solid and then **1b** (171 mg, 36%) as a white solid. The ^{1}H NMR spectrum of the α anomer **1a** was identical to that of **1a** as prepared above (see Supporting Information). The following data are for the the β-anomer **1b**. ^{1}H NMR (CD_3OD, 300 MHz), δ: 8.17 (1H, s, H-8), 6.26 (t, 6,6 Hz H-1′), 4.4−4.2 (1H, m, H-4′), 4.1−4.0 (1H, m, H-3′), 2.9−2.8 (1H, m, H-2′b), 2.5−2.4 (1H, m, H-2′a), 1.37 (3H, d, 6.3 Hz, H-5). ^{13}C NMR (DMSO, 75 MHz), δ: 158.1 (d, $^1J_{CF}$ = 202.1 Hz, C2), 157.6 (d, $^3J_{CF}$ = 21.1 Hz, C6), 150.4 (d, $^3J_{CF}$ = 20.5 Hz, C4), 139.9 (C8), 117.6 (d, $^4J_{CF}$ = 4.0, C5), 82.92 (C1′), 82.54 (C4′), 74.58 (C3′), 38.40, (C5′), 19.07, (C2′). MS (CI, NH_3), *m/e*: 254 (MH^+).

Methyl 3-*O*-Acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α,β-D-*erythro*-pentofuranoside (16). Pyridine (5.78 g. 73.16 mmol), acetic anhydride (7.48 g, 73.16 mmol), and 4-dimethylaminopyrdine (0.22 g, 1.8 mmol) were added to a solution of methyl 5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α,β-D-*erythro*-pentofuranoside[26] (9.66 g, 36.58 mmol) in anhydrous dichloromethane (100 mL) at 0 °C. The reaction mixture was stirred for 6 h at room temperature under N_2. The solution was concentrated under reduced pressure and then was coevaporated with toluene. The residue was purified by chromatography on silica gel (hexane/EtOAc, 9:1) to give 10.9 g (98%) of a mixture of α and β anomers of **16** as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 5.3−5.1 (2H, m, H-3 and H-1), 4.1−4.0 (1H, m, H-4), 4.0−3.6 (2H, m, H-5), 3.39 and 3.36 (3H, s, OMe), 2.2−2.0 (2H, m, H-2), 2.09 and 2.07 (3H, m, CH_3CO), 0.89 (9H, s, Si(C(CH_3)$_3$), 0.08 (6H, s, Si(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 170.91 and 170.26 (CH_3*C*O), 105.48 and 105.37 (C1), 84.38 and 84.21 (C5), 75.39 and 74.64 (C4), 64.29 and 63.45 (C3), 55.17 and 54.97 (OMe), 39.38 and 39.09 (C2), 25.86 and 25.84 (SiC(*C*H$_3$)$_3$), 21.36 and 20.99 (*C*H$_3$CO), 18.27 (Si*C*(CH$_3$)$_3$), −5.4 (Si(CH$_3$)$_2$).

Methyl 3-*O*-Acetyl-5-fluoro-2-deoxy-α,β-D-*erythro*-pentofuranoside (17). To a solution of furanoside **16** (4.57 g, 15.0 mmol) in dichloromethane (10 mL) was added bis[(2-methoxyethyl)amino]sulfur trifluoride (6.64 g, 30.0 mmol) at room temperature. After the reaction mixture was heated at reflux for 16 h, it was cooled to 0 °C and methanol (5 mL) was added dropwise. Triethylamine (5 mL) was added to neutralize the resulting hydrofluoride. The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel (hexane/EtOAc, 4:1) to give 2.48 g of **17** as a yellow oil (86% yield). Small amounts of pure α and β anomers were isolated for characterization).

α Anomer. ^{1}H NMR ($CDCl_3$, 300 MHz) 5.25−5.19 (1H, m, H-3), 5.18−5.15 (1H, m, H-1), 4.65−4.35 (2H, dm, $^1J_{HF}$ = 46.8 Hz, H-5), 4.30−4.20 (1H, dm, $^2J_{HF}$ = 18.0 Hz, H-4), 3.37 (3H, s, OMe), 2.42−2.10 (2H, m, H-2), 2.07 (3H, s, CH_3CO). ^{13}C NMR ($CDCl_3$, 75 MHz),δ 170.76 (*C*O), 105.69 (C1), 83.76 ($^1J_{CF}$ = 171.8 Hz, C5), 82.58 ($^2J_{CF}$ = 19.9 Hz, C4), 74.26 ($^3J_{CF}$ = 5.7 Hz, C3), 55.35 (OMe), 39.18 (C2), 21.09 (*C*H$_3$CO). ^{19}F NMR ($CDCl_3$, 282 MHz), δ −226.7 (dt, *J* = 47.4, 18.3 Hz).

β Anomer. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 5.16−5.11 (2H, m, H-1 and H-3), 4.62 (2H, dm, $^1J_{HF}$ = 47.4 Hz, H-5), 4.21 (1H, dm, $^2J_{HF}$ = 28.8 Hz H-4), 3.41 (3H, s, OMe), 2.42−2.00 (2H, m, H-2), 2.10 (3H, s, CH_3CO). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 171.24 (CO), 105.42 (C1), 83.93 ($^1J_{CF}$ = 171.3 Hz, C5), 82.34 ($^2J_{CF}$ = 18.2 Hz, C4), 73.60 ($^3J_{CF}$ = 6.8 Hz, C3), 55.32 (O*C*H$_3$), 39.16 (C2), 21.18 (*C*H$_3$CO). ^{19}F NMR ($CDCl_3$, 282 MHz), δ: −226.7 (dt, *J* = 47.1, 27.3 Hz).

Phenyl 3-*O*-Acetyl-5-fluoro-2-deoxy-1-thio-α,β-D-*erythro*-pentofuranoside (18). To the solution of furanoside (**17**) (2.46 g, 12.8 mmol) in dry dichloromethane (100 mL) at 0 °C were added benzenethiol (2.82 g, 25.6 mmol), zinc iodide (7.30 g, 22.9 mmol), and tetrabutylammonium iodide (0.946 g, 2.56 mmol). After being stirred for 1 h at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was filtered, and the filtrate was washed with saturated aqueous $NaHCO_3$ and was extracted with dichloromethane. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane/EtOAc, 9:1) to give 1.62 g (47%) of **18** as a colorless oil (small amounts of pure α and β anomers were isolated for characterization).

α Anomer. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 7.60−7.50 (2H, m), 7.4−7.2 (3H, m), 5.80 (1H, dd, 7.8, 2.4 Hz, H-1), 5.21−5.17 (1H, m, H-3), 4.78−4.51 (2H, dm, $^1J_{HF}$ = 46.8 Hz, H-5), 4.48−4.35 (1H, dm, $^2J_{HF}$ = 29.1 Hz, H-4), 2.9−2.7 (1H, m, H-2), 2.32−2.1 (1H, m, H-2′), 2.12 (3H, s, CH_3CO). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 170.91 (CO), 135.57, 130.95, 129.05, 127.17, 87.78 (C1), 82.56 ($^1J_{CF}$ = 171.9 Hz, C5), 82.27 ($^2J_{CF}$ = 18.2 Hz, C4), 73.60 ($^3J_{CF}$ = 6.3 Hz, C3), 39.47 (C2), 21.10 (*C*H$_3$CO).

β Anomer. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.55−7.50 (2H, m), 7.52−7.25 (3H, m), 5.58 (1H, dd, 9.6, 6.3 Hz, H-1), 5.25−5.22 (1H, m, H-3), 4.67−4.38 (2H, dm, $^1J_{HF}$ = 46.8 Hz, H-5), 4.30−4.10 (1H, dm, $^2J_{HF}$ = 24.6 Hz, H-4), 2.5−2.0 (2H, m, H-2), 2.08 (3H, s, CH_3CO). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 170.58 (CO), 133.95, 131.96, 129.02, 127.63, 86.31 (C1), 84.14 ($^2J_{CF}$ = 19.4 Hz, C4), 82.78 ($^1J_{CF}$ = 172.5 Hz, C5), 75.04 ($^3J_{CF}$ = 4.5 Hz, C3), 39.49 (C2), 21.06 (CH3CO).

2-Fluoro-9-(3-*O*-acetyl-2-deoxy-5-fluoro-D-α-*erythro*-pentofuranosyl)adenine (19a) and 2-Fluoro-9-(3-*O*-acetyl-2-deoxy-5-fluoro-D-β-*erythro*-pentofuranosyl)adenine (19b). A suspension of 2-fluoroadenine (918 mg, 6.0 mmol) and ammonium sulfate (793 mg, 6.0 mmol) in hexamethyldisilazane (6 mL) was heated at rigorous reflux under N_2 until the solution became clear. The excess of hexamethyldisilazane was removed under reduced pressure. The residue and thioglycoside **18** (1.62 g, 6.0 mmol) were dissolved in 20 mL of dry toluene under N_2, and then 3 g of powered molecular sieves, 4 Å, was added. After being stirred at room temperature for 30 min, the reaction mixture was cooled to 0 °C and NBS (1.17 g, 6.6 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred for 15 h. The addition of aqueous $Na_2S_2O_3$ was followed by filtration and then extraction with dichloromethane. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (100% ethyl acetate) to give 218 mg (12%) of β-anomer **19b** and then 317 mg (20%) of α-anomer **19a**.

α Anomer 19a. ^{1}H NMR (CD_3OD, 300 MHz), δ: 8.24 (1H, s, H-8), 6.42 (1H, dd, *J* = 7.2, 2.1 Hz, H-1′), 4.39 (1H, dt, *J* = 6.6, 1.8 Hz, H-3′), 4.9−4.5 (3H, m, H-4′ and H-5′), 3.0−2.8 (2H, m, H-2′), 1.98 (3H, s, OAc). ^{19}F NMR (CD_3OD, 282 MHz), δ: −53.01(s, F-2), −234.60 (dt, *J* = 30.7, 46.0 Hz, F-5′). FAB-MS, *m/e*: 314.2 (MH^+).

β Anomer 19b. ^{1}H NMR (CD_3OD, 300 MHz), δ: 8.18 (1H, s, H-8), 6.38 (1H, t, *J* = 6.0 Hz H-1′), 5.52−4.48 (1H, m, H-3′), 4.69 (2H, dm, $^1J_{HF}$ = 47.1 Hz, H-5′), 4.34 (1H, dm, $^2J_{HF}$ = 27.6 Hz, H-4′), 3.0−2.6 (2H, m, H-2′), 2.13 (3H, s, OAc). ^{19}F NMR (CD_3OD, 282 MHz), δ: −52.81 (s, F-2), −232.52 (dt, 27.4, 45.7 Hz, F-5′). FAB-MS, *m/e*: 314.2 (MH^+).

2-Fluoro-9-(2-deoxy-5-fluoro-D-α-*erythro*-pentofuranosyl)adenine (2a). To a suspension of **19a** (230 mg, 0.734

mmol) in a mixture of methanol and water (30 mL, 10:1) was added triethylamine (1.5 mL). The mixture was stirred at room temperature for 24 h, and then a small amount of silica gel was added to quench the reaction. The solvent was removed under reduced pressure. The residue was purified by chromatography to give **2a** (140 mg, 70%) as a white solid. ^{1}H NMR (Me_2SO-d_6, 300 MHz), δ: 8.36 (1H, s, H-8), 7.84 (br, NH_2), 6.24 (1H, dd, J = 7.2, 3.9 Hz H-1′), 5.70 (1H, d, J = 3.0 Hz, O*H*), 4.51 (2H, dm, $^1J_{HF}$ = 47.1 Hz, H-5′), 4.4−4.3 (2H, m, H-3′ and H-4′), 2.8−2.6 (1H, m, H-2′), 2.5−2.4 (1H, m, H-2′). ^{13}C NMR (Me_2SO-d_6, 75 MHz), δ: 158.7 (d, $^1J_{CF}$ = 202.6 Hz, C2), 157.7 (d, $^3J_{CF}$ = 21.1 Hz, C6), 150.4 (d, $^3J_{CF}$ = 20.0 Hz, C4), 139.9 (C8), 117.4 (C5), 85.3 (d, 17.6 Hz, C4′), 83.5 (C1′), 82.9 (d, 167.9 Hz, C5′), 69.6 (d, J = 6.3 Hz, C3′), 39.5 (C2′). FAB-MS, *m*/*e*: 272.1 (MH^+). HRMS (FAB) Calcd for (MH^+, $C_{10}H_{12}F_2N_5O_2$): 272.0959. Found: 272.0959.

2-Fluoro-9-(2-deoxy-5-fluoro-D-*β*-*erythro*-pentofuranosyl)adenine (2b). The same procedure as above used **19b** to give **2b** (54 mg, 69%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 8.25 (1H, s, H-8), 7.86 (br, NH_2), 6.27 (1H, t, J = 6.8 Hz H-1′), 5.53 (1H, d, J = 4.5 Hz, O*H*), 4.58 (2H, dm, $^1J_{HF}$ = 47.7 Hz, H-5′), 4.5−4.4 (1H, m, H-3′), 4.03 (1H, dm, J = 22.2 Hz, H-4′), 2.8−2.6 (1H, m, H-2′), 2.5−2.4 (1H, m, H-2′). ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 158.6 (d, $^1J_{CF}$ = 202.6 Hz, C2), 157.7 (d, $^3J_{CF}$ = 21.0 Hz, C6), 150.4 (d, $^3J_{CF}$ = 20.0 Hz, C4), 139.6 (C8), 117.6 (C5), 84.9 (d, $^2J_{CF}$ = 18.2 Hz, C4′), 83.4 (C1′), 82.9 (d, $^1J_{CF}$ = 167.4 Hz, C5′), 69.7 (d, $^3J_{CF}$ = 6.3 Hz, C3′), 39.7 (C2′).

Supporting Information Available: Experimental details for the synthesis and data for characterization of compounds **6**, **8**−**13**, and **1a**; NOESY spectra for **1a**, **1b**, **2a**, and **2b**; and HMBC spectra of **1b** and **2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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