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The elusive 8-fluoroadenosine: a simple non-enzymatic synthesis and characterization

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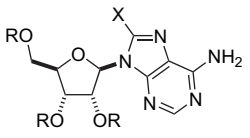
Abstract—The only successful synthesis of 8-fluoroadenosine reported until now relied on an enzymatic removal of the acetate protecting groups using thermally resistant hydrolases. In the present communication we describe the first non-enzymatic synthesis of 8-fluoroadenosine. According to this, the C_8-fluorine atom was introduced in a halogen-exchange process performed at elevated temperature. The chief obstacle in the synthesis of 8-fluoroadenosine, the removal of the protecting groups in the presence of the labile C_8–F bond, was addressed by judicious choice of acid-labile protecting groups. Their deprotection in the presence of C_8–F is described. Using this newly developed procedure, significant quantities of 8-fluoroadenosine were synthesized and, for the first time, its physicochemical properties including pH-dependent stability, examined in detail. The intermediate generation of 8-fluoroadenosines as a tool to increase the reaction rates of nucleophilic substitutions was briefly examined and successfully demonstrated with the example of 8-cyanoadenosine. The presented procedure is applicable to the synthesis of various adenosine analogs with potential pharmacological significance.

1. Introduction

8-Halogen substituted adenosines can serve as a convenient starting point in the syntheses of unnatural purine nucleosides.[1,2] The most versatile of them, 8-bromoadenosine[3] (**1d**, Fig. 1), first described in 1964, is often used in reactions involving direct displacements with nucleophiles[4,5] as well as transition metal catalyzed cross-couplings[6,7]. The synthetic utility of 8-chloroadenosine[8] (**1c**) and 8-iodoadenosine[9] (**1e**), first synthesized in 1972 and 1971, respectively, is much more limited.[10–12] In spite of numerous attempts,[13] the first successful synthesis of 8-fluoroadenosine[14] (**1b**) was not accomplished until 1998.



1a, R=H, X = H
1b, R=H, X = F
1c, R=H, X = Cl
1d, R=H, X = Br
1e, R=H, X = I
1f, R=Ac, X = NH_2
1g, R=Ac, X = Br
1h, R=Ac, X = F
1i , R=Ac, X = H
1j , R=H, , X = N_3

Figure 1.

8-Bromoadenosine (**1d**) can be easily synthesized on a large scale by direct bromination of adenosine (**1a**) in a buffered medium.[15,16] Synthesis of 8-iodoadenosine (**1e**) is more problematic and requires a lithiation prior to quenching with molecular iodine.[17] 8-Chloroadenosine (**1c**) cannot be obtained by direct chlorination at all, and alternative methods had to be developed.[18,19]

A purported synthesis[9,20] of 8-fluoroadenosine (**1b**) involved a Balz–Schiemann reaction of 2′,3′,5′-tri-*O*-acetyl-8-aminoadenosine (**1f**) followed by deprotection with methanolic ammonia that appeared to be, in retrospect, too harsh to produce the desired 8-fluoroadenosine (**1b**). In fact numerous attempts at synthesis of acetylated 8-fluoroadenosines in our laboratory using various modifications of the Balz–Schiemann reaction failed to afford detectable (LCMS) amounts of this product. Similar conclusions were drawn by others.[14,21] On the other hand, an apparently successful attempt was described by Kobayashi in 1976.[21] According to this, exposure of 2′,3′,5′-tri-*O*-acetyl-8-bromoadenosine (**1g**) to potassium fluoride in the presence of 18-crown-6 at high temperature (120 °C) for extended period of time (48 h) led to the formation of 2′,3′,5′-tri-*O*-acetyl-8-fluoroadenosine (**1h**) in moderate yield of 25%. However, no attempts at removal of the acetyl protecting groups were described in this communication. The identity of 2′,3′,5′-tri-*O*-acetyl-8-fluoroadenosine (**1h**) was confirmed in 1996 by Barrio, who was able to access the same compound by fluorination of 2′,3′,5′-tri-*O*-acetyl-adenosine (**1i**) with

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elemental fluorine.[22] However, it was not until 1998 Barrio described the first successful synthesis of 8-fluoroadenosine (**1b**) by an enzymatic deprotection of 2′,3′,5′-tri-*O*-acetyl-8-fluoroadenosine (**1h**) using thermally stable hydrolases.[14]

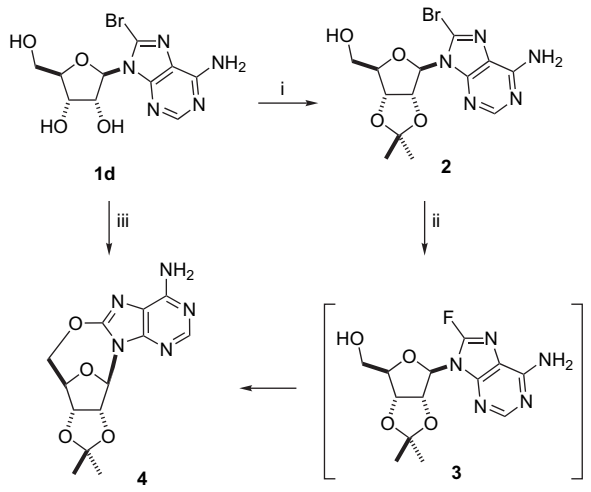
Our interest in 8-fluoroadenosines stemmed from the obvious capacity of the fluorine to attenuate the basicity of the nitrogen atom at position 7. It is well known that this nitrogen plays a crucial role in adenosine deaminase (ADA) mediated metabolic degradation of adenosine.[23] During the catalytic cycle an acid–base interaction between 7-N of adenosine and aspartate-296 of ADA increases the electrophilicity of adenosine's 6-C, which in turn leads to ADA-catalyzed nucleophilic hydroxylation at this position and eventually to the formation of inosine.[24] Therefore, 8-fluoroadenosine (**1b**) should be a metabolically more robust isostere of adenosine.

The chief obstacle in the successful synthesis of 8-fluoroadenosine (**1b**) is the removal of protecting groups in the presence of a quite reactive C_8–F bond. This was clearly demonstrated by Barrio, who noticed a rapid fluorine displacement in the presence of alkali, alkoxides, or ammonia and was successful in removing the acetate protecting groups only under the rather mild conditions of an enzymatic hydrolysis.[14] On the other hand, the decreased basicity of 7-N in 8-fluoroadenosine (**1b**) should make its protonation more difficult, and, therefore, it should be comparatively stable under acidic conditions. Clearly, the use of acid-labile protecting groups appeared to be a logical choice.

2. Chemistry

Even though direct C_8-fluorination[22] of a suitably protected adenosine was certainly possible, we decided to adopt the more facile halogen-exchange protocol.[21,25] The starting material, 8-bromoadenosine (**1d**) could be easily synthesized on large scale by a direct bromination of adenosine (**1a**) under controlled pH of 4.0.[15] Initial attempts at displacement of the bromine atom with fluorine in unprotected **1d** using cesium or potassium fluoride led to complete consumption of the starting material without production of any detectable amounts (HPLC condition A or B) of the desired 8-fluoroadenosine (**1b**). In the absence of the fluorine source **1d** was completely stable under otherwise identical conditions.

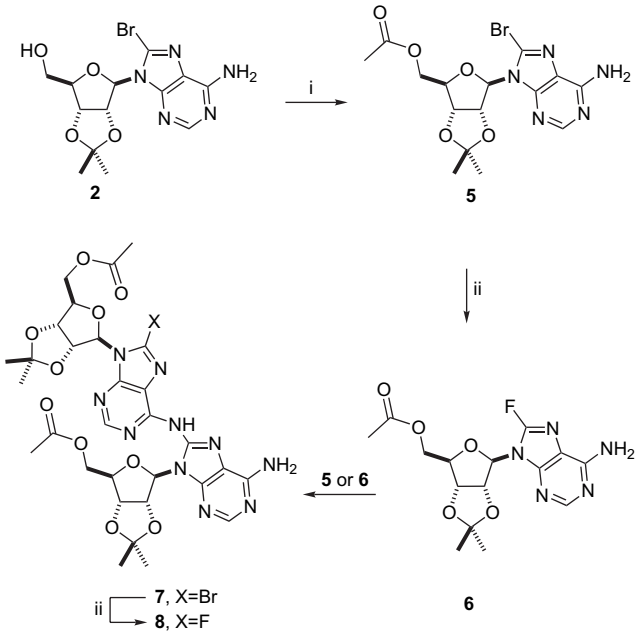
In order to improve the solubility of 8-bromoadenosine in acetonitrile it was converted under standard conditions to the known[26] 8-bromo-2′,3′-*O*-(1-methylethylidene) adenosine (**2**). When this material was exposed to cesium or potassium fluoride in dry acetonitrile at elevated temperature of 100 °C, it smoothly converted to the 5′,8-anhydro-adenosine derivative **4**, Scheme 1. Once again, under otherwise identical conditions, when the fluorine source was omitted, no cyclization took place and the starting material was recovered unchanged. This compound was previously synthesized from bromo derivative **2** under strongly basic conditions.[27] The stability of **2** in acetonitrile at 100 °C in the absence of the fluorine source suggested that the halogen exchange indeed took place (intermediate **3**) and this was followed by a secondary ring closure.



Scheme 1. Reagents and conditions: (i) 2,2-dimethoxypropane, PTSA, DMF; (ii) CsF, MeCN, 100 °C, sealed tube, 18 h; (iii) Ref. 27.

The ease by which this cyclization could be monitored (HPLC condition A) allowed for a facile optimization of the halogen-exchange conditions. It soon became obvious that the use of crown-ethers is not necessary for the exchange and either KF or CsF is an effective fluoride source. The reaction was conveniently performed at 100 °C; it became sluggish at lower temperature, and the yields diminished when the reaction temperature was increased. The careful exclusion of moisture was of crucial importance, and the fluoride salt was flame dried under high vacuum before use.

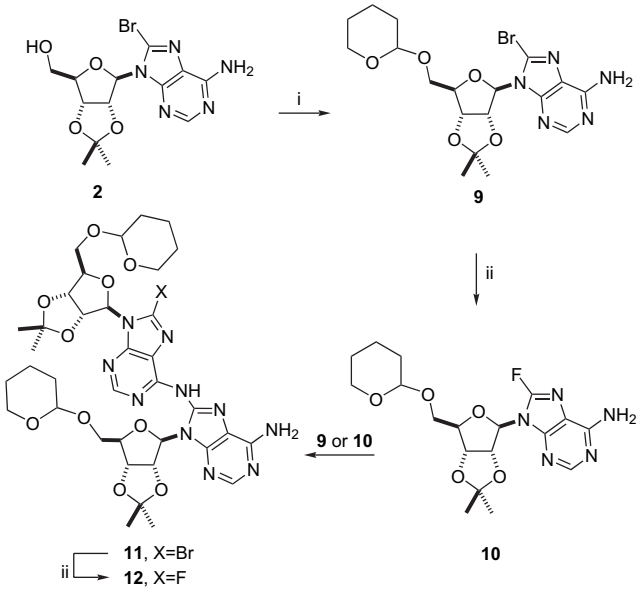
In order to prevent the ring closure after the halogen exchange, the 5′-hydroxyl was protected as an acetate **5**, Scheme 2. Exposure of this material in acetonitrile to cesium fluoride at 100 °C for 9 h led to the formation of 5′-*O*-acetyl-8-fluoro-2′,3′-*O*-(1-methylethylidene) adenosine (**6**) (about



Scheme 2. Reagents and conditions: (i) Ac_2O, pyridine, 60 °C, 18 h; (ii) CsF, MeCN, 100 °C, sealed tube, 9 h.

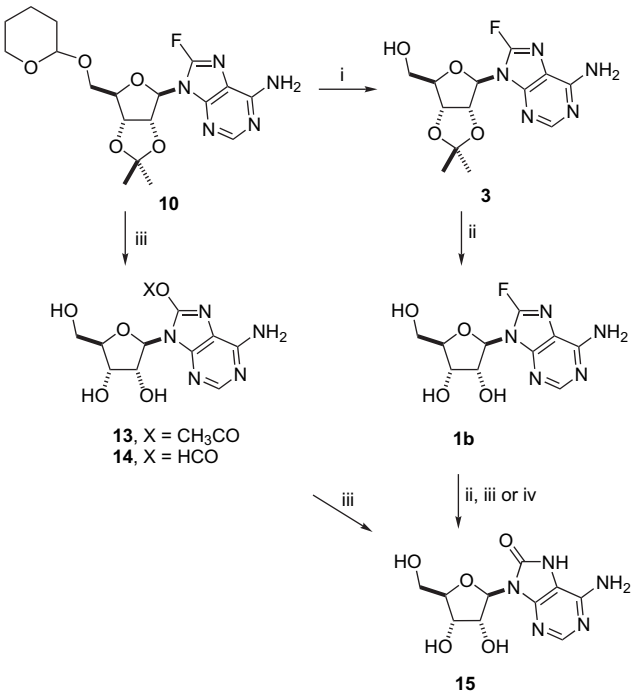
50% conversion) in a 24% isolated yield. Extending the reaction time to 18 h led to full consumption of the starting material, unfortunately the yield of **6** rapidly decreased. Careful LCMS analysis of the reaction mixtures indicated formation of dimers (**7**, $[M+H]^+$=775.3 and **8**, $[M+H]^+$=715.3) resulting from reaction of the formed 5′-*O*-acetyl-8-fluoro-2′,3′-*O*-(1-methylethylidene) adenosine (**6**) with unreacted **5** (compound **7**) followed by an additional halogen exchange (compound **8**).

Since an acid-induced removal of the acetate in **6** was potentially difficult, this protecting group was replaced by the acid-labile tetrahydropyranyl, Scheme 3. Exposure of the bromo derivative **9** to cesium fluoride in acetonitrile at 100 °C for 12 h resulted in a clean halogen exchange and the 8-fluoro derivative **10** could be isolated in 28% yield as a mixture of THP-derived diastereoisomers. Extension of the reaction time, and/or elevation of the temperature led to full consumption of the starting material, but increased the formation of dimers **11** ($[M+H]^+$=859.6) and **12** ($[M+H]^+$=799.7) and considerably lowered the yield. Eventually, the formation of these nucleoside oligomers could be greatly suppressed by performing the reaction under high dilution conditions.



Scheme 3. Reagents and conditions: (i) 3,4-dihydro-2*H*-pyran, camphor sulfonic acid, MeCN, DMF, 0 °C, 2 h; (ii) CsF, MeCN, 100 °C, 12 h.

Exposure of the protected fluoro derivative **10** to even weakly acidic medium, such as 1% TFA, led to rapid loss of the THP protecting group, Scheme 4. Under these conditions the acetonide remained unchanged, and the fluoro derivative **3** could be easily intercepted. Surprisingly, the fluoride in **3** was found to be perfectly stable, and no 8-oxo analogs were detectable (LCMS). The removal of the acetonide protecting group, however, required somewhat more forceful conditions. Increasing the concentration of trifluoroacetic acid led to rapid removal of both the THP and the acetonide, however, considerable amounts of 8-oxoadenosine (**15**), the chief breakdown product of 8-fluoroadenosine (**1b**), were formed, Scheme 4. The use of weaker but more nucleophilic formic or acetic acid led to the formation of acetate intermediate **13** and formate **14**. Quite surprisingly, the nucleophilic displacement of the fluorine did not take place until both protecting groups were removed. At later stages these reaction mixtures contained only the 8-fluoro derivative **3**, ester **13** or **14**, the desired 8-fluoroadenosine **1b**, and the final product of hydrolysis, 8-oxoadenosine **15**. Even though isolation of the desired 8-fluoroadenosine (**1b**) was possible, the yield was rather disappointing. Monitoring of these hydrolyses was further complicated by the fact that under most HPLC conditions, the desired 8-fluoroadenosine (**1b**) and the 8-oxoadenosine (**15**) were undistinguishable and conclusions were initially drawn from MS data only. Later it was discovered that omission of the modifier (TFA, formic acid, or ammonium formate) from the mobile phase resulted in excellent peak separation and this observation was applicable for preparative separations as well.

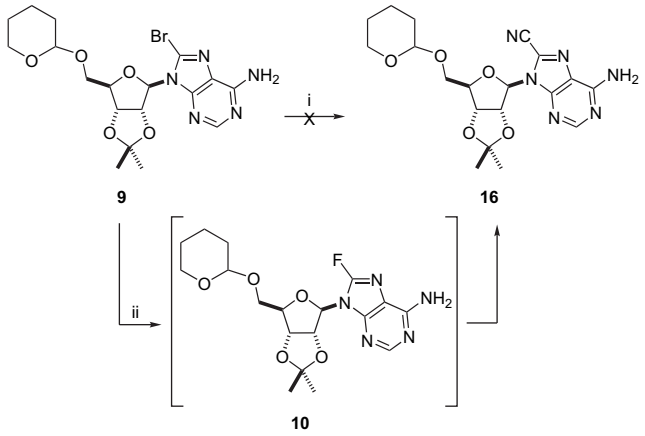


Scheme 4. Reagents and conditions: (i) 1% TFA, water, 45 min; (ii) 10% aqueous perchloric acid, 1 h; (iii) aqueous acetic or formic acid, 20–40 °C; (iv) 20–80% TFA, water.

Finally, the clean removal of the acetonide from the fluoro derivative **3** was accomplished by the use of perchloric acid, an extremely weak nucleophile. Exposure of **3** to a 10% aqueous solution of perchloric acid resulted in full cleavage of the acetonide in approximately 1 h at which time only traces of **15** were detectable. The product was then easily purified by preparative HPLC (condition D) and solid samples were obtained by lyophilization. No appreciable decomposition was noticeable during these operations and significant quantities of 8-fluoroadenosine (**1b**) were synthesized.

It is reasonable to anticipate that a temporary introduction of the fluorine, as depicted in Scheme 1, would mediate not only an intramolecular cyclization, but also in fact it could

facilitate any nucleophilic displacement and examples of such acceleration have been reported.[25] When 8-bromoadenosine (**1d**) is reacted with sodium azide (80 °C, DMF) the bromide is readily displaced to yield 8-azidoadenosine (**1j**).[5] However, any attempt to produce 8-cyanoadenosine using this simple procedure fails. In fact, 8-cyanoadenosine is accessible from the corresponding iodide and zinc cyanides only by a tetrakis[tri(2-furyl)phosphine]palladium(0) catalyzed cross-coupling.[28] According to expectations, when the protected 8-bromoadenosine **9** was heated with sodium cyanide in acetonitrile at 100 °C, the starting material remained unchanged. However, addition of cesium fluoride resulted in smooth conversion of the starting material to 8-cyanoadenosine derivative **16** and we attribute this acceleration to an intermediate formation of the 8-fluoro derivative **10**, Scheme 5. The scope and limitations of such reactions for preparation of 8-substituted adenosine derivatives are currently being examined.



Scheme 5. Reagents and conditions: (i) NaCN, acetonitrile, 100 °C, 12 h; (ii) NaCN, CsF, acetonitrile, 100 °C, 12 h.

3. Properties and stability of 8-fluoroadenosine

The significant quantities of 8-fluoroadenosine (**1b**) available through the above described procedure enabled us to examine its physicochemical properties in closer detail.

Its spectral characteristics, including ^{1}H, ^{13}C, and ^{19}F NMR spectra were in perfect agreement with the structure 8-fluoroadenosine, and even though some of the signals were previously assigned incorrectly,[14] the reported values closely matched those recorded by us. Their correct assignment is summarized in Table 1.

The UV spectrophotometric profile of **1b** (λ_{max}=252.0 nm) showed a small hypsochromic shift in comparison with adenosine (λ_{max}=260.0 nm). Also the optical rotation of **1b** $[\alpha]_D$ −54.7 (*c* 0.92 g/100 mL, water) was close to that recorded under identical conditions for adenosine (−59.7).

In agreement with calculated values, the basicity of **1b** (p*K*a=3.18) is lower than that of adenosine (**1a**, p*K*a=3.61), Table 2. According to this, substantial protonation (>50%) can be expected at pH values less than 3.2, and this in turn should translate to markedly increased rate of hydrolysis only at very low pH values.

Table 1. Proton and carbon chemical shifts of 8-fluoroadenosine (CD_3OD, 24 °C)

	8-Fluoroadenosine	
	^{1}H δ ppm, (Hz)	^{13}C δ ppm (Hz)
1′	5.84 (s, 6.9)	89.74
2′	4.91 (t, 6.2)	64.15
3′	4.33 (dd, 5.0, 1.8)	72.74
4′	4.14 (br d, 2.3)	88.67
5″	3.84 (dd, 12.6, 2.5)	63.80
5′	3.70 (dd, 12.6, 3.0)	
2	8.16 (s)	153.06
4		148.85
5		115.28
6		156.72
8		152.45 (d, 250.9)

For details see Section 5 as well as Supplementary data.

Table 2

Sample	pK_a	
	Experimental (ISA H_2O at 25 °C)	Calculated (ACD/Labs 8.00)
1a	3.61±0.01	3.40
1b	3.18±0.01	2.72

Table 3

pH	Composition	$t_{1/2}$ (days)
2.0	0.1 M Perchloric acid/1 M NaOH	9.7
3.0	0.1 M Perchloric acid/1 M NaOH	22.6
5.0	0.1 M Perchloric acid/1 M NaOH	186.3
7.0	0.1 M Perchloric acid/1 M NaOH	>250[a]
9.0	0.1 M NaOH/1 M HCl	13.8
9.5	0.1 M NaOH/1 M HCl	7.0
10.0	0.1 M NaOH/1 M HCl	1.4
10.5	0.1 M NaOH/1 M HCl	0.12
11.0	0.1 M NaOH/1 M HCl	0.029

[a] Estimated value. No significant hydrolysis noticed at this pH.

The pH-dependent hydrolytic stability of **1b** was examined in close detail and the results are summarized in Table 3. An estimated half-life value in excess of 250 days indicates that 8-fluoroadenosine is remarkably stable at neutral pH. In agreement with its predicted behavior, **1b** is considerably more stable under acidic conditions ($t_{1/2}$=9.7 days, pH=2.0) than under basic conditions ($t_{1/2}$=42 min, pH=11.0). The half-life values suggest that under physiological conditions 8-fluoroadenosine might be in fact hydrolytically quite stable.

4. Conclusion

A facile non-enzymatic synthesis of 8-fluoroadenosine (**1b**) was developed through which significant quantities of this simple nucleoside became available for the first time. This allowed for a detailed examination of its physicochemical properties, including basicity and pH-dependent stability. The obtained data suggest that 8-fluoroadenosine, or its derivatives, might be more stable at physiological conditions than expected.

5. Experimental

5.1. General

All non-hydrolytic reactions, unless indicated otherwise, were carried out in dry solvents purchased from Aldrich. NMR spectra were recorded on a Varian Inova 500 or 600 MHz spectrometer and the shifts are positive in the low field direction. The ^{19}F chemical shifts were referenced indirectly to $CFCl_3$ using the deuterium signal of the solvent (CD_3OD). Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ.

Analytical HPLC condition A: Agilent 1100, Waters Atlantis dC_{18}, 5 μm, 4.6×50 mm at 37 °C; eluant: water and MeCN without a modifier; gradient (% organic): 0.0 min (0), 5.0 min (100), 6.0 min (100); detector: PDA UV Detector/Waters Micromass ZQ Mass Spectrometer. Analytical HPLC condition B: identical to A except column: Waters SunFire C_{18}, 5 μm, 4.6×100 mm. Preparative HPLC condition C: Waters 2525 pump, 2767 injector/collector 2996 Waters Sunfire (50×100 mm, 5 μm); eluant: water and MeCN without a modifier; gradient (% organic): 0.0 min (25), 1.5 min (25), 10.0 min (65), 50 mL/min; detector: PDA UV Detector/Micromass ZQ Single Quad Mass Spectrometer, mass-triggered collection. Preparative HPLC condition D: same as condition C except gradient (% organic): 0.0 min (3), 2.0 min (3), 10.5 min (15), 10.7 min (100), 13.7 min (100).

The optical rotation was determined at 20 °C and 589 nm on a Perkin–Elmer 341 polarimeter, using a 1 dm cell.

pH-dependent stability: a stock solution of **1b** (1 mg/mL in MeCN) was prepared. An aliquot (0.1 mL) was added to a 4 mL vial and blown dry with N_2 and mild heat. Once dried, 1 mL of buffer (see Table 2) was added and gently shaken to mix. The solution was then transferred to an HPLC vial and analyzed at time by HPLC/DAD/MS analysis under the following conditions: isocratic [A=water, B=acetonitrile, 5% B for 10 min, 1 mL/min] on the column Sunfire C_{18} (4.6×100 mm, 5 μm) at 40 °C with diode array detection at 252 nm and injection volume of 10 μL. Each sample was prepared in duplicate and equilibrated at room temperature (~22 °C).

The pK_a values were determined by potentiometric titrations with spectrophotometric analysis. The titrations were performed with the Sirius GLp*K*a/D-PAS using a double junction electrode. The electrode was standardized from pH 1.8 to 12.2. The KOH titrant was standardized against potassium hydrogen phthalate and was approximately 0.5 M. The sample was dissolved in ionic strength adjusted (ISA) H_2O (0.15 M KCl) to create a stock solution of 4.0 mg/mL. An aliquot of the stock solution (0.075 mL for adenosine and 0.05 mL for L-001855881) was added to the titration vial and diluted up to 15 mL of ISA H_2O. The starting pH of the solution was adjusted with 0.5 M HCl. The solution was titrated from pH 1.8 to 7.0 at 25 °C in ISA H_2O. Titrations were repeated several times. UV spectra were recorded from 200 to 700 nm and the data were analyzed from 250 to 360 nm. The pK_a values were calculated using Refinement Pro v.1.0 software.

5.2. 8-Bromoadenosine (1d)[15]

An aqueous solution of sodium acetate (0.5 M, 200 mL) was treated with glacial acetic acid until the pH reached 4.00 (pH electrode, Accumet AP62, 13-620-AP50). To this buffer adenosine (**1a**, 5.15 g, 19.3 mmol) was added and stirred with gentle heating until the solid has fully dissolved. The solution was allowed to cool to ambient temperature and a mixture of bromine in water (4.60 g, 57.6 mmol in 100 mL of water) was added. Vigorous stirring was continued for 4 h, after which time no starting material was detectable by HPLC (condition B). The reaction mixture was decolorized by the addition of solid sodium bisulfite (spatula tip) and the pH was adjusted to 7.00 with 50% aqueous solution of sodium hydroxide. The suspension was allowed to stand at +5 °C overnight, and the solid was collected by filtration. The filter cake was washed with water (3×20 mL) and acetone (1×20 mL), and dried on high vacuum to yield 5.30 g (79%) of the pure product. $t_{R(A)}$=2.67 min, $t_{R(B)}$=2.73 min. ^1H NMR (500 MHz, DMSO-d_6), δ: 8.12 (s, 1H), 7.60 (br s, 2H), 5.83 (d, J=6.9, 1H), 5.46 (br s, 2H), 5.24 (br s, 2H), 5.08 (t, J=6.0, 1H), 4.20 (dd, J=5.0, 2.0 Hz, 1H), 3.98 (dd, J=6.2, 3.7 Hz, 1H), 3.68 (dd, J=12.1, 3.9 Hz, 1H), 3.52 (dd, J=12.1, 4.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 155.1, 152.3, 149.9, 127.2, 119.7, 90.4, 86.7, 71.1, 70.9, 62.1. HRMS for $C_{10}H_{12}BrN_5O_4$ calculated $[M+H]^+$: 346.0151, found: 346.0141. For $C_{10}H_{12}BrN_5O_4$ calculated: 34.70% C, 3.49% H; found: 34.48% C, 3.35% H.

5.3. 8-Bromo-2′,3′-*O*-(1-methylethylidene) adenosine (2)

A solution of 8-bromoadenosine (**1d**, 5.28 g, 15.3 mmol), *para*-toluenesulfonic acid (monohydrate, 3.24 g, 16.8 mmol), and 3,3-dimethoxypropane (30 mL) in dimethylformamide (5 mL) was stirred at ambient temperature for 30 h. The solvent was removed in vacuo and the residue was purified by gradient chromatography [silica gel, 2-propanol/chloroform (2-propanol: 0–10%)] to yield 3.60 g (61%) of the pure product. $t_{R(A)}$=3.63 min, $t_{R(B)}$=3.94 min. ^1H NMR (500 MHz, DMSO-d_6), δ: 8.14 (s, 1H), 6.01 (d, J=2.8, 1H), 5.59 (m, 1H), 5.02 (dd, J=6.2, 2.8 Hz, 1H), 4.16 (m, 1H), 3.51 (dd, J=11.7, 5.7 Hz, 1H), 3.43 (dd, J=11.5, 5.7 Hz, 1H), 1.54 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 155.0, 152.8, 149.7, 126.4, 119.3, 113.2, 91.0, 87.1, 81.9, 81.6, 61.5, 27.1, 25.2. HRMS for $C_{13}H_{16}BrN_5O_4$ calculated $[M+H]^+$: 386.0464, found: 368.0466. For $C_{13}H_{16}BrN_5O_4$ calculated: 40.43% C, 4.18% H; found 40.42% C, 4.16% H.

5.4. (3a*R*,4*R*,13*R*,13a*R*)-2,2-Dimethyl-4,5,13,13a-tetrahydro-3a*H*-4,13-epoxy[1,3]dioxolo[5,6][1,3]oxazocino[3,2-*e*]purin-8-amine (4)

A solution of 8-bromo-2′,3′-*O*-(1-methylethylidene) adenosine (**2**, 200 mg, 0.52 mmol) and cesium fluoride (790 mg, 5.2 mmol) in dry acetonitrile (60 mL) was heated to 100 °C in a closed thick walled glass reaction vessel for 18 h. The reaction mixture was allowed to cool to ambient temperature, the solid was filtered off, and the solvent was evaporated in vacuo. The residue (198 mg) was purified by preparative TLC ($CHCl_3$/^{i}PrOH 9:1) to yield 109.4 mg (69%) of the pure product. $t_{R(A)}$=3.32 min, $t_{R(B)}$=3.56 min.

^{1}H NMR (500 MHz, DMSO-d_6), δ: 8.11 (s, 1H), 7.10 (s, 1H), 5.94 (s, 1H), 5.10 (d, J=5.5 Hz, 1H), 4.90 (d, J=5.7 Hz, 1H), 4.74 (s, 1H), 4.64 (dd, J=13.0, 1.6 Hz, 1H), 4.13 (d, J=13.1 Hz, 1H), 1.46 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (500 MHz, DMSO-d_6), δ: 154.7, 153.1, 152.0, 147.6, 114.4, 111.9, 85.8, 85.2, 84.8, 80.1, 74.3, 25.9, 24.3. HRMS for $C_{13}H_{15}FN_5O_4$ calculated $[M+H]^+$: 306.1202, found: 306.1214. For $C_{13}H_{15}N_5O_4$ calculated: 51.14% C, 4.95% H; found: 51.08% C, 5.05% H.

5.5. 5′-*O*-Acetyl-8-bromo-2′,3′-*O*-(1-methylethylidene) adenosine (5)

A solution of 8-bromo-2′,3′-*O*-(1-methylethylidene) adenosine (**2**, 3.00 g, 7.77 mmol) in anhydrous pyridine (40 mL) was treated with acetic anhydride (1.10 mL, 11.0 mmol) and the reaction mixture was stirred at 60 °C overnight. The solvent was removed in vacuo and the residue was purified by gradient chromatography [silica gel, 2-propanol/chloroform (^{i}PrOH: 0–10%)] to afford 3.15 g (95%) of the pure material. $t_{R(A)}$=4.10 min, $t_{R(B)}$=4.48 min. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 8.50 (s, 1H), 7.52 (br s, 1H), 6.07 (s, 1H), 5.70 (d, J=6.2 Hz, 1H), 5.12 (dd, J=6.0, 3.7 Hz, 1H), 4.31 (m, 1H), 4.22 (dd, J=11.7, 5.0 Hz, 1H), 4.06 (dd, J=11.7, 7.3 Hz, 1H), 1.93 (s, 3H), 1.52 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (500 MHz, DMSO-d_6), δ: 170.0, 154.9, 152.9, 149.8, 126.3, 119.2, 113.5, 90.3, 84.5, 82.3, 81.3, 63.3, 27.0, 25.2, 20.4. HRMS for $C_{15}H_{18}BrN_5O_5$ calculated $[M+H]^+$: 428.0569, found: 428.0562. For $C_{15}H_{18}BrN_5O_4$ calculated: 42.07% C, 4.24% H; found: 41.96% C, 4.33% H.

5.6. 5′-*O*-Acetyl-8-fluoro-2′,3′-*O*-(1-methylethylidene) adenosine (6)

A solution of 5′-*O*-acetyl-8-bromo-2′,3′-*O*-(1-methylethylidene) adenosine (**5**, 200 mg, 0.47 mmol) and cesium fluoride (710 mg, 4.70 mmol) in dry acetonitrile (60 mL) was heated to 100 °C in a closed thick walled glass reaction vessel, until HPLC analysis (condition B) indicated about 50% conversion, for about 9 h. The solid was filtered off and the filtrate was concentrated in vacuo (210 mg). The crude product was purified by preparative HPLC (condition C) to afford 41.6 mg (24%) of the pure product alongside with 36.7 mg of recovered starting material. $t_{R(A)}$=3.94 min, $t_{R(B)}$=4.30 min. ^{1}H NMR (500 MHz, CD_3OD), δ: 8.36 (s, 1H), 6.13 (d, J=1.8 Hz, 1H), 5.60 (dd, J=6.2, 1.6 Hz, 1H), 5.09 (dd, J=6.2, 3.4 Hz, 1H), 4.41 (m, 1H), 4.20 (dd, J=8.1, 6.9 Hz, 2H), 1.96 (s, 3H), 1.57 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (500 MHz, CD_3OD), δ: 172.3, 153.9, 153.1, 151.8, 148.6, 115.7, 90.2, 86.9, 84.6, 82.7, 64.6, 27.3, 25.4, 20.5. ^{19}F NMR (CD_3OD) δ −104.0. HRMS for $C_{15}H_{18}FN_5O_5$ calculated $[M+H]^+$: 368.1370, found: 368.1383. For $C_{15}H_{18}FN_5O_5$ (dihydrate) calculated: 44.66% C, 5.50% H; found: 44.24% C, 4.41% H.

5.7. 8-Bromo-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (9)

A solution of 8-bromo-2′,3′-*O*-(1-methylethylidene) adenosine (**2**, 4.76 g, 12.3 mmol), camphor sulfonic acid (6.3 g, 27.1 mmol) in a mixture of acetonitrile (50 mL) and dimethylformamide (5 mL) was treated at 0 °C with 3,4-dihydro-2*H*-pyran (11.2 mL, 123 mmol). Stirring at 0 °C was continued for 2 h, and the reaction was quenched with 10% $NaHCO_3$ (40 mL). The product was extracted with chloroform (2×250 mL), the combined extracts were dried (anhydrous sodium sulfate), and concentrated in vacuo. The residue was purified by gradient chromatography [silica gel, ethyl acetate/hexanes (ethyl acetate: 0–100%)] to yield 3.83 g (66%) of the product as a mixture of diastereoisomers. $t_{R(A)}$=4.54 min, $t_{R(B)}$=5.02 min. ^{1}H NMR (500 MHz, DMSO-d_6): major isomer, δ: 8.14 (m, 1H), 6.05 (m, 1H), 5.76 (m, 2H), 4.48 (m, 1H), 4.26 (m, 1H), 3.68 (m, 1H), 3.64 (m, 2H), 3.36 (m, 1H), 3.29 (m, 1H), 1.61 (m, 2H), 1.52 (m, 3H), 1.37 (m, 4H), 1.32 (m, 3H); minor isomer, δ: 8.14 (m, 1H), 6.05 (m, 1H), 5.08 (m, 2H), 4.37 (m, 1H), 4.26 (m, 1H), 3.58 (m, 1H), 3.48 (m, 2H), 3.31 (m, 1H), 3.18 (m, 1H), 1.52 (m, 3H), 1.49 (m, 2H), 1.37 (m, 4H), 1.32 (m, 3H). ^{13}C NMR (500 MHz, DMSO-d_6), major isomer, δ: 155.0, 152.9, 149.9, 128.9, 126.5, 119.1, 113.3, 98.0, 90.5, 86.0, 82.3, 81.7, 66.5, 61.1, 29.9, 27.0, 25.3, 18.8; minor isomer, δ: 155.0, 152.9, 149.8, 128.1, 126.5, 119.1, 113.2, 97.7, 90.5, 85.6, 82.1, 81.6, 66.3, 60.8, 29.8, 27.0, 24.9, 18.7. HRMS for $C_{18}H_{24}BrN_5O_5H$ calculated: 470.1039, found: 470.1036. For $C_{18}H_{24}BrN_5O_4$ (CH_3CN) calculated: 46.97% C, 5.32% H; found: 47.40% C, 5.08% H.

5.8. 8-Fluoro-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (10)

A thick walled glass pressure reaction vessel, equipped with a magnetic stirring bar was charged with cesium fluoride (650 mg, 4.30 mmol), closed under a septum, and flame dried at high vacuum. The vessel was flooded with nitrogen and allowed to cool to ambient temperature. A solution of 8-bromo-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (**9**, 200 mg, 0.43 mmol) in acetonitrile (60 mL) was added via syringe, the vessel was closed with a solid Teflon bushing, and stirred at 100 °C. After 12 h when the HPLC analysis indicated a conversion of about 50%, the reaction mixture was allowed to cool to room temperature. The solid was filtered off, and the solvent was removed in vacuo. The crude product was purified by preparative HPLC (condition C) to yield 48.0 mg (28%) of the pure product and 12.0 mg (6%) of unreacted starting material. $t_{R(A)}$=4.11 and 4.36 min, $t_{R(B)}$=4.52 and 4.84 min. Faster eluting isomer: ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.30 (br s, 1H), 6.06 (s, 1H), 5.90 (br s, 2H), 5.60 (d, J=6.0 Hz, 1H), 5.08 (m, 1H), 4.54 (m, 1H), 4.40 (m, 1H), 3.50 (m, 1H), 3.82 (dd, J=11.0, 5.7 Hz, 1H), 3.70 (m, 1H), 3.54 (dd, J=11.0, 4.7 Hz, 1H), 3.38 (m, 1H), 1.60 (s, 3H), 1.40 (s, 3H), 1.8–1.4 (br m, ∼5H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 154.2, 152.2, 152.1, 150.1, 148.3, 114.4, 98.9, 88.6, 85.9, 82.8, 81.5, 66.8, 62.0, 30.1, 27.2, 25.4, 25.2, 19.2. ^{19}F NMR (CD_3OD), δ: −105.94. Slow eluting isomer: ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.30 (br s, 1H), 6.06 (d, J=1.6 Hz, 1H), 5.90 (br s, 2H), 5.56 (d, J=6.2 Hz, 1H), 5.06 (m, 1H), 4.52 (m, 1H), 4.40 (m, 1H), (dd, J=10.8, 5.0 Hz, 1H), 3.90 (m, 1H), 3.76 (m, 1H), 3.54 (dd, J=10.7, 7.1 Hz, 1H), 3.42 (m, 1H), 1.60 (s, 3H), 1.40 (s, 3H), 1.8–1.4 (br m, ∼5H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 154.3, 152.2, 152.0, 150.0, 148.1, 114.5, 98.9, 88.3, 86.4, 83.1, 81.7, 67.2, 62.0, 30.2, 27.2, 25.5, 25.2, 19.1. ^{19}F NMR (CD_3OD), δ: −105.47. HRMS (isomers not resolved) for $C_{18}H_{24}FN_5O_5$ calculated: 410.1837, found: 410.1840. For $C_{18}H_{24}FN_5O_5$ (trihydrate) calculated: 46.65% C, 6.52% H; found: 46.74% C, 6.26% H.

5.9. 8-Fluoro-2′,3′-*O*-(1-methylethylidene) adenosine (3)

A solution of 8-fluoro-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (**10**, 30 mg, 0.073 mmol) in acetonitrile (2 mL) was treated with five drops of trifluoroacetic acid and stirred at ambient temperature for 45 min. The entire reaction mixture was injected onto a preparative HPLC and purified under condition D. The fractions containing the product were pooled and lyophilized to afford 14.9 mg (63%) of the desired product. $t_{R(A)}$=3.43 min, $t_{R(B)}$=3.71 min. ^{1}H NMR (500 MHz, CD_3OD), δ: 8.16 (s, 1H), 7.13 (s, 2H), 5.96 (s, 1H), 5.58 (d, J=5.4 Hz, 1H), 4.96 (d, J=3.4 Hz, 1H), 4.14 (br s, 1H), 1.52 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD), δ: 155.2, 152.6, 151.0, 149.3, 147.6, 113.3, 88.2, 87.4, 82.2, 81.3, 61.3, 27.0, 25.2. ^{19}F NMR (CD_3OD), δ −104.95. HRMS for $C_{13}H_{16}FN_5O_4$ calculated $[M+H]^+$: 326.1266, found: 306.1245. For $C_{13}H_{16}FN_5O_4$ (hemihydrate) calculated: 46.71% C, 5.13% H; found: 46.69% C, 4.84% H.

5.10. 8-Fluoroadenosine (1b)

A solution of 8-fluoro-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (**10**, 43 mg, 0.105 mmol) was dissolved in 10% aqueous perchloric acid and stirred at ambient temperature. The reaction progress was monitored by HPLC. The reaction was complete after approximately 1 h, and 8-oxo-adenosine started to appear. The reaction mixture was terminated by injection into a preparative HPLC (condition D). The fractions containing the product were pooled, and the solvent was removed by lyophilization. In this fashion, 12.4 mg (46%) of clean product was obtained. $t_{R(A)}$=2.48 min, $t_{R(B)}$=2.41 min. $[\alpha]_D$ −54.7 (*c* 0.92 g/100 mL, water). ^{1}H NMR (500 MHz, CD_3OD), δ: 8.16 (s, 1H, 2-H), 5.84 (d, J=6.9 Hz, 1H, 1′-H), 4.91 (t, J=6.2 Hz, 1H, 2′-H), 4.33 (dd, J=5.0, 1.8 Hz, 1H, 3′-H), 4.14 (br d, J=2.3 Hz, 1H, 4′-H), 3.84 (dd, J=12.6, 2.5 Hz, 1H, 5″-H), 3.70 (dd, J=12.6, 3.0 Hz, 1H, 5′-H). ^{13}C NMR (150 MHz, CD_3OD), δ: 156.72 (6-C), 153.06 (2-C), 152.45 (d, J=250.9 Hz, 8-C), 148.85 (4-C), 115.28 (5-C), 89.74 (1′-C), 88.67 (4′-C), 64.15 (2′-C), 72.74 (3′-C), 63.80 (5′-C). ^{19}F NMR (CD_3OD), δ −107.6. HRMS for $C_{10}H_{12}FN_5O_4$ calculated $[M+H]^+$: 286.0952, found: 286.0971. For $C_{10}H_{12}FN_5O_4$ calculated: 44.15% C, 4.72% H, 23.40% N; found: 43.18% C, 4.11% H, 22.10% N.

5.11. 8-Cyano-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (16)

A flame-dried mixture of potassium cyanide (280 mg, 4.30 mmol) and cesium fluoride (196 mg, 1.29 mmol) was treated with a solution of 8-bromo-2′,3′-*O*-(1-methylethylidene)-5′-*O*-(tetrahydro-2*H*-pyran-2-yl) adenosine (**9**, 200 mg, 0.43 mmol) in 100 mL of acetonitrile. The mixture was heated in a thick walled pressure reaction vessel at 100 °C overnight. The mixture was allowed to cool to ambient temperature, the solid was filtered off, and the residue was purified by preparative HPLC, condition C. Fast eluting isomer: 58.2 mg (33%). $t_{R(A)}$=4.43 min, $t_{R(B)}$=4.88 min. ^{1}H NMR (500 MHz, CD_3OD), δ: 8.28 (s, 1H), 6.24 (d, J=2.1 Hz, 1H), 5.80 (dd, J=6.2, 1.8 Hz, 1H), 5.07 (dd, J=6.2, 2.7 Hz, 1H), 4.49 (m, 2H), 3.78 (dd, J=10.8, 5.5 Hz, 1H), 3.64 (dd, J=11.0, 4.6 Hz, 1H), 3.60 (dd, J=8.5, 3.0 Hz, 1H), 3.3 (m, 1H), 1.60 (s, 3H), 1.2–1.6 (br m, 6H), 1.40 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD), δ: 158.3, 156.7, 150.1, 124.9, 115.3, 112.1, 99.9, 93.3, 88.3, 85.2, 83.4, 68.0, 63.0, 31.2, 27.4, 26.4, 25.5, 20.1. For $C_{19}H_{24}N_6O_5$ calculated $[M+H]^+$: 416.18, found: 417.23. Slow eluting isomer: 20.1 mg (12%). $t_{R(A)}$=4.46 min, $t_{R(B)}$=4.92 min. ^{1}H NMR (500 MHz, CD_3OD), δ: 8.28 (s, 1H), 6.24 (d, J=5.79 Hz), 5.69 (dd, J=6.4, 1.8 Hz, 1H), 5.09 (dd, J=6.2, 3.2 Hz, 1H), 4.44 (m, 2H), 3.84 (dd, J=11.0, 5.3 Hz, 1H), 3.73 (ddd, J=8.5, 8.2, 2.7 Hz, 1H), 3.51 (dd, J=11.0, 6.6 Hz, 1H), 3.38 (m, 1H), 3.30 (m, 1H), 1.59 (s, 3H), 1.39 (s, 3H), 1.32–1.62 (br m, 6H). ^{13}C NMR (125 MHz, CD_3OD), δ: 15.8.4, 156.8, 150.1, 125.0, 121.1, 116.4, 115.4, 111.9, 100.4, 92.6, 88.3, 84.8, 83.4, 68.2, 63.2, 31.3, 27.4, 26.4, 25.6, 20.1. For $C_{19}H_{24}N_6O_5$ calculated $[M+H]^+$: 416.18, found: 417.20. HRMS (isomers not resolved) for $C_{19}H_{24}N_6O_5$ calculated $[M+H]^+$: 417.1886, found: 417.1896. For $C_{19}H_{24}N_6O_5$ (hemihydrate) calculated: 53.54% C, 5.92% H; found: 53.14% C, 5.71% H.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.02.053.

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