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The Elusive 8-Fluoroadenosine

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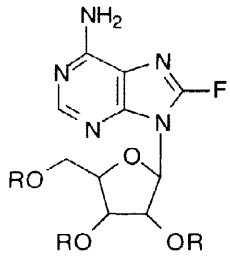
Abstract: The synthesis of 8-fluoroadenosine has been accomplished for the first time. The kinetics of deamination of 8-fluoroadenosine with the enzyme adenosine deaminase has also been measured.

Keywords: Enzymes and Enzyme reactions; Fluorine and compounds; Nucleosides; Purines



1 R = H
2 R = $COCH_3$

As an outgrowth of recent new procedures to access to 8-fluoropurines,[1] we now report the synthesis of the hitherto elusive 8-fluoroadenosine (8-FAdo, **1**). The purported synthesis of 8-FAdo involving the application of the Balz-Schiemann reaction on 8-amino-2',3',5'-tri-O-acetyladenosine with fluoroboric acid, followed by treatment with methanolic ammonia for 10 h[2] has been refuted by subsequent investigations.[3,4] It seems now clear that 2',3',5'-tri-O-acetyl-8-fluoroadenosine (**2**) is quite sensitive to deprotection conditions involving the use of alcoholic ammonia.[1,5a] Other standard conditions for nucleoside deprotection using alkali[5b] or alkoxides[5c] are equally ineffective, causing rapid defluorination.[5a]

On the other hand, an enzymatic deprotection with thermostable hydrolases[6] in organic solvents could offer an excellent alternative to the chemical approaches for sensitive derivatives such as the fluorinated purine **2**. Thus, we have successfully utilized these enzymes,[7] for the first time, to cleanly hydrolyze the fluorotriacetyl derivative **2** without causing any defluorination. The following experimental procedure is illustrative: 3.0 mg of ESL-001-02 biocatalyst[7] were added to a 2.3 mM solution of **2**[1] in MeOH (20 mL) and the reaction mixture was stirred at 45°C for 3h. The reaction mixture was ultracentrifuged at 4°C to recover the enzyme[8] and 1.0 mL 50 mM MOPS buffer, pH 7.0 was added, followed by additional ultracentrifugation to assure complete recovery of the deprotected product. After buffer removal by flash chromatography (silica gel, $HCCl_3$: MeOH: H_2O, 40:9:1), the product was purified by HPLC (Whatman Partisil 10, 9.4 x 50 mm; $HCCl_3$: MeOH: H_2O, 41:8.5:1) to afford pure 8-FAdo (yield: 5.91 mg; 44.9%).[9] Fluorine-19 NMR ($CD_3OD/CFCl_3$; δ -103.96), proton NMR[10] and carbon-13 NMR (Table 1), all confirm the assigned structure. The C-8 NMR signal in **1** was shifted downfield by more than 10 ppm in comparison with its counterpart in adenosine (Ado, Table 1). The value of the C_8-F coupling

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Table 1. Carbon-13 Chemical Shifts[a]

Nucleoside	C-1'	C-2'	C-3'	C-4'	C-5'	C-2	C-4	C-5	C-6	C-8
Adenosine	91.26	75.47	72.67	88.19	63.48	153.51	150.02	121.05	157.61	142.01
1	89.76	74.16	72.77	88.71	63.84	153.28	148.92	115.34	156.92	152.45

[a]Shifts (125 MHz) are given in parts per million relative to TMS. All determinations were made with a 3 mM solution of the nucleoside in CD_3OD at 22°C.

constant (250.7 Hz) is in general agreement with published values for 2-fluoroadenosine,[11] substituted monofluorobenzenes[12] and fluoropyridines.[13] Interestingly, the C-2' signal in **1** was shifted upfield by 1.31 ppm relative to that of Ado (Table 1), whereas, the C-3' chemical shifts in both Ado and 8-FAdo were quite similar. In other 8-substituted nucleosides, analogous changes have been attributed to conformational changes from *anti* to *syn* around the N(9)-C(1') bond. The magnitude of such relative chemical shift differences in **1**, suggests an increase in the *syn* population when compared with that of Ado. This is in agreement with the expected low steric influence of the fluorine atom on the nucleoside conformation in comparison with the results observed for 8-chloro- and 8-bromopurine nucleosides (Table 2).[14] This is also consistent with the experimental observations of a marked flexibility about the glycosydic bond of 8-substituted purine analogs with substituents having a

Table 2. Differences in Carbon-13 Chemical Shifts for C-2' and C-3[a]

Compound	$\Delta\delta$C2' - δC3'	Attributed Conformation
Adenosine[a]	+ 2.80[14c]	anti
8-Fluoroadenosine[b]	+ 1.39	see ref 14c
8-Chloroadenosine[a]	+ 0.42[14d]	syn
8-Bromoadenosine[a]	+ 0.24[14d]	syn
2-Fluoroadenosine[c]	+ 3.26[11]	anti

[a]In DMSO-d_6; [b]CD_3OD; [c]H_2O/D_2O

van der Waals radius of less than 2Å.[15,16]

Having prepared pure 8-FAdo, we also measured the kinetics of deamination with adenosine deaminase. Calf spleen adenosine deaminase (EC 3.5.4.4.), an enzyme that requires substrate binding in the *anti* conformation,[17] hydrolyzed 8-FAdo at a slower rate than Ado.[18] 8-FAdo exhibited a markedly increased Km value and a K_{cat}/Km of 0.87 x 10^6, about 10% of that reported and also measured in our laboratory for Ado[19] (Table 3).

Table 3. Kinetic Parameters of Wild-Type and Mutant Adenosine Deaminase

Enzyme	Substrate	Km (μM)	K_{cat} S^{-1}	K_{cat}/Km (M^{-1} S^{-1})	Reference
Wild-type (mouse)	Ado	21	240	11 x 10^6	23
Wild-type (calf spleen)	Ado	38	342	9 x 10^6	This work
Wild-type (calf spleen)	8F-Ado	74	64	0.87 x 10^6	This work

It has been postulated that the hydrolysis catalyzed by adenosine deaminase involves protonation of N_1 of the purine ring by an active sulfhydryl group of the enzyme[20] followed by nucleophilic hydroxylation on C_6 leading to the formation of a tetrahedral intermediate.[21] It has also been noted that Asp 296, well conserved in bacterial as well as mammalian enzymes,[22] contributes to substrate binding by forming a hydrogen bond between its terminal carboxylate and N_7 of the purine ring.[23] Beyond the conformational effects,[14a] the strong electronegative effects of fluorine in **1** could potentially affect N_1 protonation[24] [as reflected by the measured pKa of **1** (2.95), a 0.6 pH unit drop when compared with that of Ado][25] and hydrogen bond formation between Asp 296 and N_7.[23] Also these results support predictions based on global purine charges (ΣQ) and orbital frontier parameters.[26]

The first successful procedure developed for the synthesis of 8-FAdo described herein provides a useful methodology to access highly sensitive nucleosides which are otherwise difficult to prepare. With their general accessibility, efficient means to probe enzyme reactions,[27] to produce new antiviral and anticancer drugs and to develop in-vivo non-invasive procedures for imaging gene expression[28] have now become possible.

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7. Esterase/lipase clonezyme™ library from Diversa Corp. (formerly Recombinant BioCatalysis, Inc., San Diego, CA). We have screened esterase/lipase libraries from a variety of sources [Diversa Corp. (i.e., CloneZyme™ library) and Boehringer Mannheim (i.e., Chirazyme® screening set)] as suitable biocatalyst for deprotection of sugar acylated nucleosides in neutral aqueous/organic solutions. Biocatalysts tested have varying activities and specifities for a range of different purine, acyclopurine and pyrimidine nucleosides dependent on solvent and temperature.
8. Two centriplus-10 (10,000 MW cut off; Amicon Corp.) devices were washed with 1.0 mL 18 Mohm water to remove any residual preservatives prior to loading sample. Each centriplus-10 was loaded with the reaction mixture and spun at 6,000 rpm in an

ultracentrifuge for 12 h at 4°C to dryness. An additional 1.0 mL 50 mM MOPS buffer, pH 7.0 was added to each centriplus-10 and spun at 6,000 rpm for 4h at 4°C. The ESL-001-02 biocatalyst was then recovered from the retentate and stored at 4°C in the 50 mM MOPS, pH 7.0 for reuse. The ESL-001-02 biocatalyst was reused many times (>6) in the deprotection of **2** with little or no apparent loss in activity.

9. Slow decomposition of **1** may occur during chromatography for which a rapid purification procedure is necessary.
10. 8-Fluoroadenosine: ^{1}H NMR (CD_3OD/TMS): δ: 3.69 (dd, J = 12.6 and 2.7 Hz, 1H, H-5'), 3.84 (dd, J = 12.4 and 2.6 Hz, 1H, H-5'), 4.11-4.17 (m, 1H, H-3'), 4.31 (broad d, H-2'), 4.90 (t, J = 6.3 Hz, 1H, H-4'), 5.82 (d, J = 7.0 Hz, 1H, H-1'), 8.14 (s, 1H, H-2) ppm. Anal Calcd for $C_{10}H_{12}N_5O_4F$. 0.5 H_2O: C, 40.82; H, 4.45; N, 23.80. Found C, 40.82; H, 4.65; N, 22.61. FAB HRMS ($M+H^+$), calcd for $C_{10}H_{13}N_5O_4F$ = 286.0952; Found: 286.0955.
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