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SYNTHESIS OF 4'- FLUOROADENOSINE AS AN INHIBITOR OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE

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Abstract: Synthesis of 4'-Fluoroadenosine was achieved via N^6-benzoyl-9-(5-deoxy-2, 3-0-dibenzoyl-β-D-erythro-pent-4-enofuranosyl)adenine as intermediate. Evaluation of its inhibitory effect on S-adenosyl-L-homocysteine hydrolase exhibited interesting properties. 4'-Fluoroadenosine caused a time-dependent inactivation of the enzyme, this inactivation was accompanied by reduction of the enzyme-bound NAD^+(E.NAD^+) to NADH.

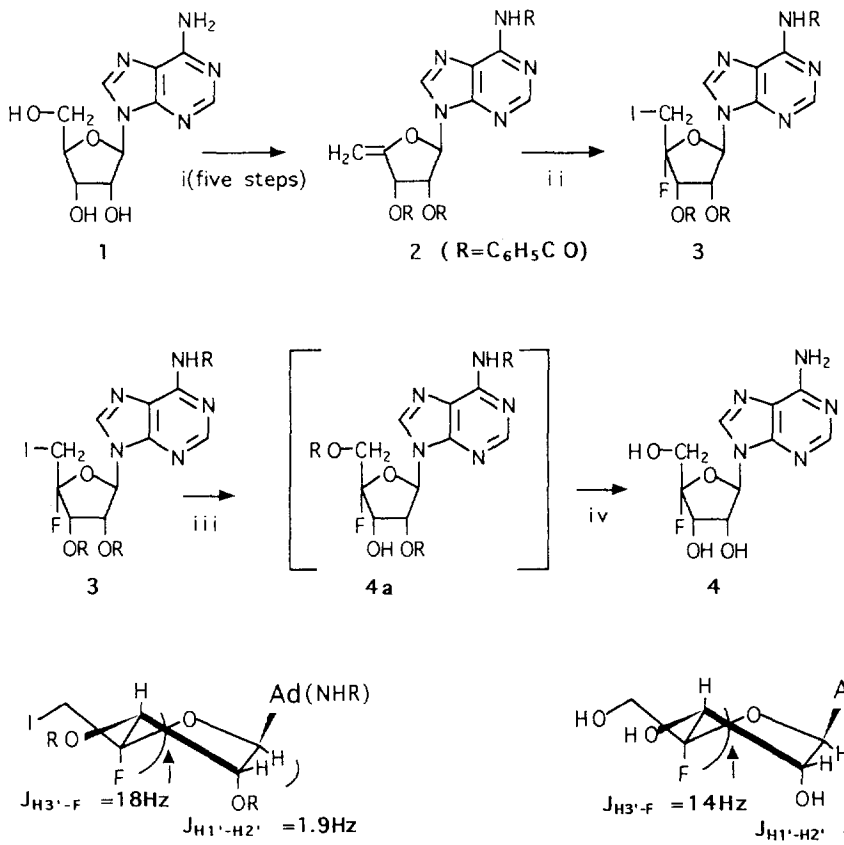
As a substituent fluorine is unique, it can serve as an isosteric replacement for hydrogen or the hydroxyl group in various metabolites such as carbohydrates or nucleosides leading to closely related analogues with a wide variety of biological activities.[1] While there has been considerable interest in fluorination at 2' and 3' positions of nucleosides much less work has been devoted to synthesis of 4'-fluorinated nucleosides .[2a, b, c, d, e]

Although protected 4'-fluoroadenosine has already been described,[2a, e] the free form of this nucleoside has been inacessible until now and thus its biological activity never investigated. 4'-Fluoroadenosine is still a nucleoside of great interest. It could be anticipated that 4'-fluoroadenosine could function as type I mechanism -based inhibitor[3] of S-adenosyl-L-homocysteine (AdoHcy) hydrolase, an important target for the design of antiviral agents. Based on the Palmer-Abeles mechanism,[4] this close structural analogue of adenosine might be a good substrate of AdoHcy hydrolase for the first oxidation step catalysed by the tighly bound NAD^+ cofactor, converting the enzyme to its reduced form, enzyme-NADH. But the lack of the 4'-proton should prevent further steps of the catalytic process, leaving the enzyme in its inactivated, reduced form E.-NADH.

For this reason, the synthesis of 4'-fluoroadenosine was undertaken starting from adenosine (Scheme I). In our approach, benzoyl groups were preferred for nitrogen and 2', 3'-hydroxyl protection as it has been reported that a 4'-fluorine enhances the lability of glycosidic linkage of nucleosides in acidic media[2a,c] and furthermore benzyl protecting groups cannot be easily removed from 4'-fluoronucleoside analogues.[2c,e]

Accordingly, N^6-benzoyl-9-(5-deoxy-2-3-0-dibenzoyl-β-D-erythro-pent-4-enofuranosyl)adenine **2**[5] was prepared in five steps from adenosine in 50 % total yield. Incorporation of fluorine at the 4'-position was achieved by the general route described for the first time by Moffatt et al.[2a] in their synthesis of nucleocidin. Treated by IF, generated *in situ* from silver fluoride and iodine, **2** gave the 4'-fluoro-5'-deoxy-iodo nucleoside **3**[6] in moderate yield (30 %) after separation of the 4'-fluoro epimeric adducts .

The structure of **3** was confirmed by the presence of the characteristic high vicinal coupling constant JH3'-F (18 Hz), in both ^1H and ^{19}F NMR spectra and the lack of long distance coupling with 2'-H and 1'-H protons.[2] The expected C3' endo conformation of the furanose ring of **3** (Scheme I) was also confirmed by NMR . This conformation approaches the 3' and 5' positions and should allow an assisted displacement by the 3'-benzoate (*via* a probable benzoxonium intermediate) of the 5'-iodine neighboring group after its activation as an hypoiodate derivative,[7] as described previously by Maag et al.[8]



Reagents : (i) 1) $(C_6H_5)_3CCl$, Pyridine ; 2) C_6H_5COCl, Pyridine ; 3) HCO_2H ; 4) $CH_3P(I)(C_6H_5O)_3$, DMF ; 5) DBN, CH_3CN, 60°; (ii) AgF, I_2, CH_2Cl_2 ; (iii) MCPBA, CH_2Cl_2, H_2O ; (iv) Na_2CO_3, CH_3OH .

SCHEME I

Effectively, treatment of **3** with an excess of *m*. chloroperbenzoic acid (MCPBA) in CH_2Cl_2, saturated with water, at room temperature for 24 h revealed the presence of compound **4 a**[9] as the main component in the reaction mixture. Deprotection of **4a** was best achieved with sodium carbonate in methanol[10] which allows clean removal of 0- and N-benzoyl protecting groups and gave after purification on silicagel ($CH_3CO_2C_2H_5/CH_3OH$: 8/2) the pure **4**, fully characterised, in 40 % yield from **3**.[11]

Pure 4'-Fluoroadenosine obtained in this way was assayed for its activity on AdoHcy hydrolase, purified to homogeneity from rat livers as described by Kajander and Raina.[12] The enzyme used in our experiments had a specific activity of 4 units/mg (Km for Ado = 1 μM). AdoHcy hydrolase activity was assayed in the direction of AdoHcy synthesis using (8-^{14}C)-Ado as described previously.[13] 4'-Fluoroadenosine was stable in the buffer used for the assay over a period of four days (HPLC control).

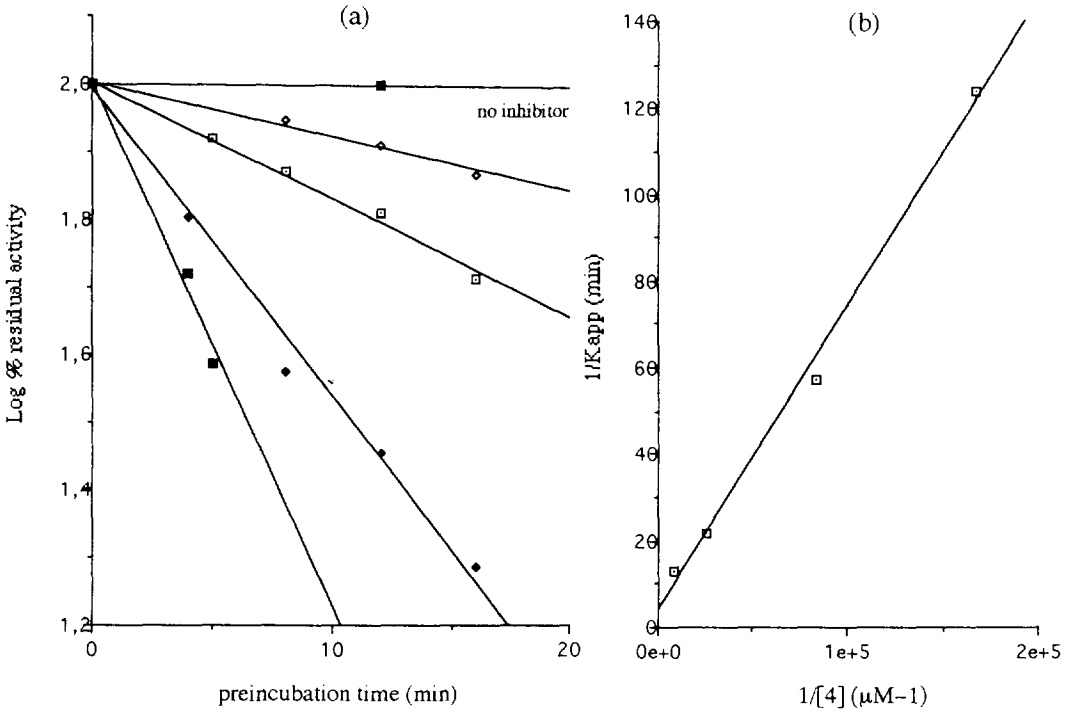


Fig 1(a) : Time and concentration dependent inactivation of AdoHcy hydrolase by **4**. 6 nM rat liver AdoHcy hydrolase was preincubated for the indicated times at 37°C with different concentrations of **4.** Assays performed with :(8-^{14}C)-Ado [10 μM], Hcy [5 mM], 20 mM potassium phosphate buffer pH 7.5, 1 mM EDTA, 1 mg/mL BSA and **4** (6, 12, 40, 120 μM). Fig 1(b): Plot of 1/Kapp vs 1/[**4**] from which the K_i and k_{inact} values were calculated.[14] (Data were the average of duplicate measurements).

Incubation experiments with AdoHcy hydrolase and **4** showed a time dependent loss of enzyme activity, the deactivation rate depending on the inhibitor concentration (Fig. 1a). Pseudo first-order kinetics were observed and from a reciprocal plot of apparent rate constants (K_{app}) against reciprocals of inhibitor concentations (Fig. 1b), according to the method of Kitz and Wilson,[14] the following inhibition parameters were estimated: k_{inact} = 0.24 min^{-1} and K_i = 166 μM. The affinity of 4'-Fluoroadenosine for the enzyme was expected to be of the same order as that of adenosine, but it turned out to be 100 fold lower. This difference can be explained by the effect of fluorine substitution, which imposes a C3' endo geometry to the ribosyl moiety of **4** (Scheme I). This observation should be helpful to the understanding of the binding mode of the substrate at the proposed active site of AdoHcy hydrolase.[15]

The formation of NADH during the inhibition process (stoichiometric with the amount of enzyme present) was observed spectrophotometrically. When buffered AdoHcy hydrolase (1,5 nmol subunits) was mixed with **4** (150 nmol) an increase of 4.10^{-2} AU was observed after 40 min in the UV absorbance profile at 340 nm (Fig. 2). In addition, after complete inactivation of the enzyme by **4**, associated NADH was released by ethanol treatment and quantatively determined by a fluorescence method (excitation at 340 nm, emission at 460 nm). A stoichiometry of 0.92 mol of NADH/mol of enzyme subunit was obtained.

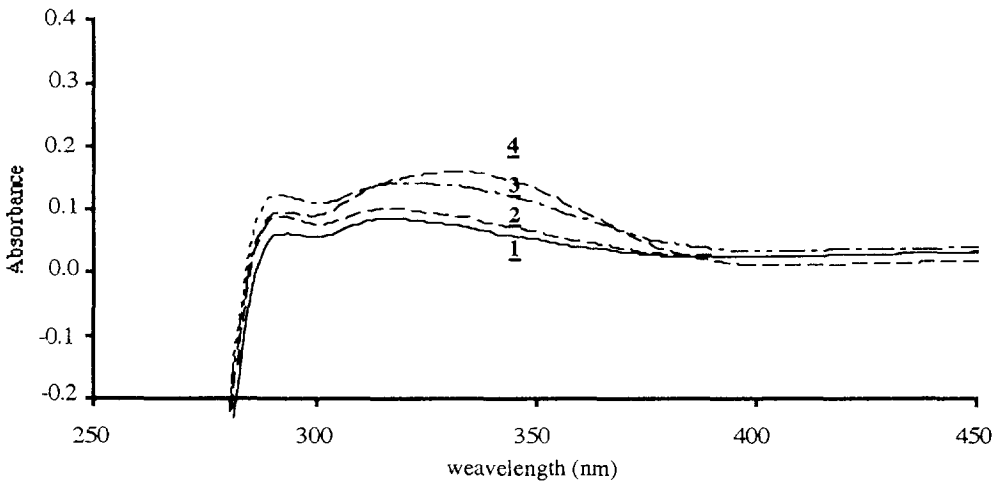


Fig 2 : UV Spectra of mixtures of AdoHcy hydrolase and **4**. AdoHcy hydrolase (2.5 μM subunit) in Phosphate Buffer 20 mM, pH 7 was mixted with **4** (250 μM) in a cuvette and incubated at 37°C. At each time point (1 : enzyme before addition of **4**; 2 : 0.5 min; 3 : 15 min; 4 : 45 min), the spectra were recorded. The instrument was referenced against the contents of the cell; before mixture the absolute absorbance versus air was 0.148 AU.

Although no putative 3'-keto-4'-fluoroadenosine (or metabolites) have yet been characterised, our results suggest a "cofactor depletion mechanism" for the inactivation of AdoHcy hydrolase by 4'-Fluoroadenosine.

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5. **2** ^{1}H NMR, δ ppm, $CDCl_3$: 4.67 and 4.82, 2H, H5', d, J=3.8Hz; 6.40, 1H, H2', dd, J=5.5Hz; 6.59, 1H, H3', d, J=5.5Hz; 6.69, 1H, H1', d, J=5.5Hz; 7.30-7.60 and 7.85-8.10, 15H; 8.25 , 8.82, 2H, H2,H8, s.

6. **3** ^{19}F NMR, δ ppm, $CDCl_3$: -106.40, $JH_{3'-F}$=18Hz.
   ^{1}H NMR, δ ppm, $CDCl_3$: 3.70-3.75, 2H, H5', m ; 6.29, 1H, H2',dd, $JH_{1'-2'}$=1.9Hz, $JH_{2'-3'}$=6.85Hz; 6.53, 1H, H1', d, J=1.9 Hz; 6.72, 1H, H3', dd, $JH_{3'-F}$=18Hz, J=6.85 Hz; 7.35-7.60 and 7.95-8.05, 15H; 8.20 , 8.90, 2H, H2, H8; 8.60, NH.

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9. **4a** . MS (DCI/NH_3) 598$(M,H)^+$.
   ^{19}F NMR, δ ppm, $CDCl_3$: -122.5.

^{1}H NMR, δ ppm, $CDCl_3$: 4.74, 2H, H5', ddd, J=3.06, 2.3, 6.47 Hz; 5.78, 1H, H3', dd, $J_{2'-3'}$=7Hz, $J_{H3'-F}$=20Hz; 6.07,1H, H2', d, J=7Hz; 6.39, 1H, H1', s; 7.35-8.05,15H, m; 8.15 , 8.56, 2H, H2,H8, s; 8.65 NH.

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11. **4** $[\alpha]_D^{20°}$ -27°(c 0.104 in H_2O)
MS (DCI/NH_3) 286$(M,H)^+$(152)(135).
^{19}F NMR, δ ppm, $CDCl_3$ -CD_3OD: -122.8 .
^{1}H NMR, δ ppm, $CDCl_3$-CD_3OD:3.70, 2H, H5', m; 4.42, 1H, H2', dd, $J_{1'-2'}$=3Hz, $J_{2'-3'}$=6.1Hz; 4.56, 1H, H3', dd, J=6.1Hz, $J_{3'-F}$=14Hz; 6.10,1H, H1', d, J=3Hz; 8.04 , 8.08, 2H, H2,H8, s.
HPLC (Nucleosil SA, 5μ , 200mm- Buffer $NH_4H_2PO_4$ 0.25M, pH4, p=15Bar) Retention time 4.4min.

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