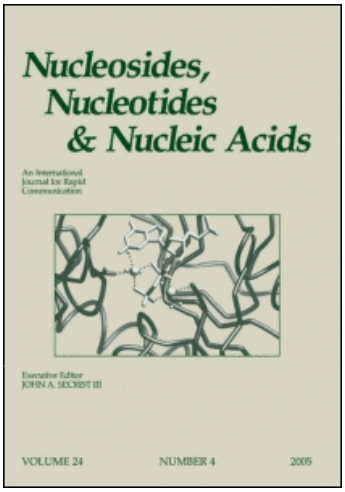

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Synthesis, Cytotoxicity and Metabolism of the 2',2'-Difluoro-Analogs of Deoxyadenosine (dFdA)and Deoxyguanosine (dFdG).

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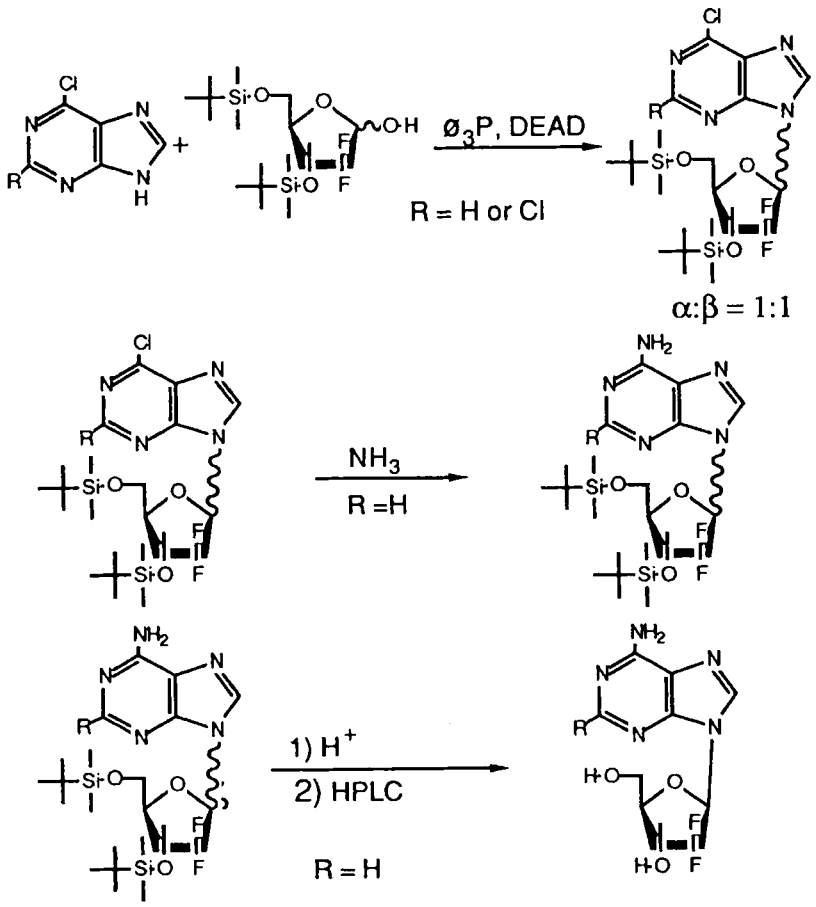
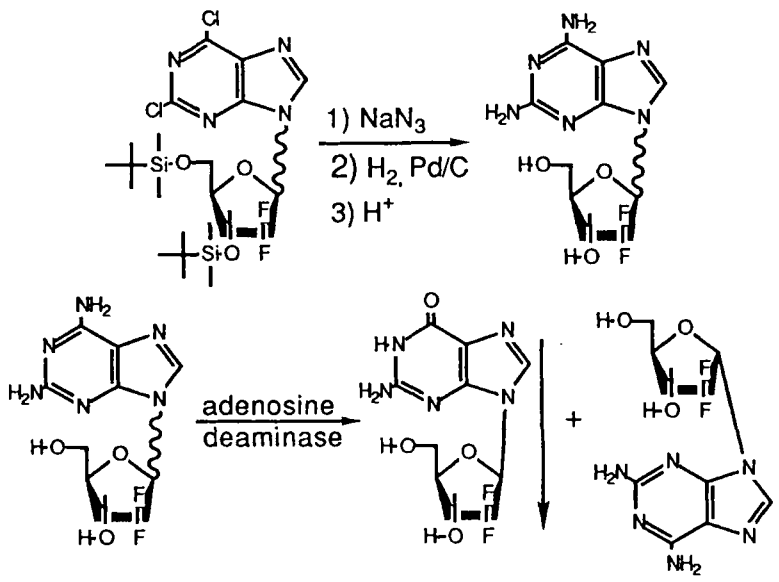
Abstract: 2',2'-Difluoro analogs of deoxyadenosine (dFdA) and deoxyguanosine (dFdG) were synthesized. The *in vitro* toxicity and metabolism of dFdA and dFdG was studied in human leukemia cell lines.

This report is on the synthesis and biological activity of a series of 1-(2-deoxy-2,2-difluororibofuranosyl) purine nucleosides as part of our program in the design and synthesis of nucleosides of potential value as anticancer and/or antiviral agents. We had previously developed a practical synthesis of 3,5-t-butyl-dimethylsilyl-2-deoxy-2,2-difluoro-D-ribose.[1] Reaction of this disilyl derivative with 6-chloropurine using Mitsunobu conditions, i.e., triphenylphosphine and DEAD2, yielded a 1:1, α/β mixture of the blocked purine nucleoside(50%). Amination with ammonia in ethanol, followed by hydrolytic cleavage of the silyl blocking groups yielded the free nucleoside in 98% yield. Separation of the anomers was accomplished using reverse phase HPLC to give 2'-deoxy-2',2'-difluoro-adenosine[3](Scheme 1).



Scheme 1

When 2,6-dichloropurine was used in the Mitsunobu reaction, the coupling proceeded as previously. Treatment of the α:ß mixture with sodium azide followed by catalytic hydrogenation yielded the 2,6-diaminopurine adduct (97%).[4] Hydrolytic removal of the silyl blocking groups proceeded as before. The α:ß mixture was then subjected to enzymatic hydrolysis using adenosine deaminase type 1 (Sigma). The hydrolysis proceeded rapidly and specifically on the ß nucleoside. The ß guanosine analog[5] precipitated from the reaction mixture and was collected by filtration, analytically pure.(Scheme 2). To our



Scheme 2

knowledge, this is the first reported enzymatic hydrolysis of an α:ß mixture of 2,6-diaminopurine nucleosides that has proceeded with kinetic resolution.[6]

The toxicity and metabolism of dFdA and dFdG was studied in human leukemia cell lines. dFdA was deaminated by adenosine deaminase (ADA) (Km = 23μM), but neither the deamination product nor dFdG was a substrate for purine nucleoside phosphorylase. Growth of CEM, K562, and HL60 cells was inhibited 50% (IC50) by a 3-day incubation with 0.35, 1.2, and 3.6 μM dFdA and 0.01, 0.03, and 0.28 μM dFdG, respectively. Addition of the ADA inhibitor, deoxycoformycin (dCF), decreased the IC50 of dFdA in CEM cells to 0.018 μM. Co-incubation with up to 2 mM deoxycytidine failed to reverse the toxicity of 1 μM dFdA, and 500 μM deoxycytidine did not reverse the toxicity of 5 μM dFdG. Cells deficient in deoxycytidine kinase retained the sensitivity of wild-type cells to each analog suggesting that this enzyme does not play a major role in the activation of dFdA or dFdG. The growth inhibitory action of 1 μM

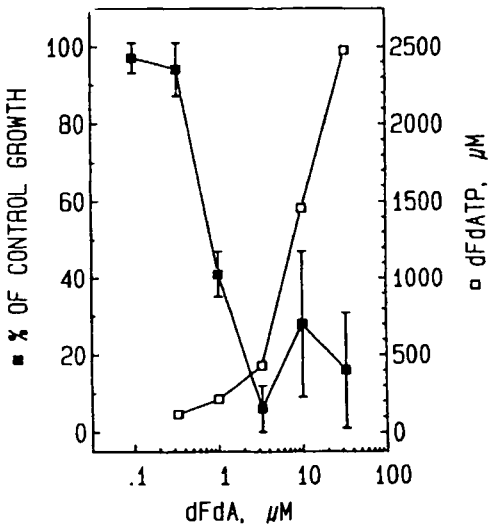


Figure 1.[8] Accumulation of dFdATP in CEM cells and cell viability determined by cloning in soft agar.

dFdA was reversed 75% by 5 μM deoxyadenosine and 50% by deoxyguanosine. Both 20 μM deoxyguanosine and 200 μM deoxyadenosine spared the toxicity of 1 μM dFdG. DNA synthesis was selectively inhibited by both dFdA and dFdG.[7]

HPLC was used to detect and quantitate the 5'-triphosphates (TP) (Figure 1). The results demonstrate the potent cytotoxicity elicited by dFdA. An IC_{50} of 0.7 μM after a 2-hr incubation is among the most toxic of purine nucleoside analogs reported. There was a substantial intracellular accumulation of the active triphosphate dFdATP. Comparison of the loss of viability and with the accumulation of dFdATP suggests a correlation between these parameters. After washing into drug-free medium, dFdATP was eliminated from CEM cells with a half-life of approximately 3.0 hr. Investigations of the cellular metabolism and the basis for differential cytotoxicity are in progress.

ACKNOWLEDGMENTS

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3. dFdA: ^{1}H NMR (CD_3OD, 300mHz), δ 3.80-4.10 (m, 4H, H-3',4',&5'), 6.33 (dd. 1, H-1'), 8.22 (s, 1, H-2), 8.40 (s, 1, H-8). MS m/z 287. UV λ_{max}, nm[10%MeOH/H_2O], 259.

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5. dFdG: ^{1}H NMR (Me_2SO-d_6, 300mHz) δ 3.65 (m, 2, 5'-CH_2), 3.85 (m, 1, H- 4'), 4.39 (m, 1, H-3'), 5.19 (apparent t, 1, 5'-OH), 5.98 (dd, 1, H-1'), 6.28 (d, 1, 3'-OH), 6.55 (bs, 2, 2-NH_2), 7.90 (s, 1, H-8). MS, m/z 303. UV λ_{max}, nm(ε_H 10-3)[pH 7] 273 (sh), 252 (8.36).

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7. Determined by ^{3}H-thymidine/uridine incorporation studies.

8. CEM were maintained in exponential growth in RPMI-1640 medium supplemented with 5% heat inactivated fetal bovine serum. To inhibit adenosine deaminase activity, cells were incubated with deoxycoformycin (1 μM) for 30 min prior to the addition of dFdA. After a 2-hr incubation with the indicated concentrations of dFdA, cells were either prepared for clonogenicity assays or nucleotide pools were analyzed. For cloning studies, cells were washed twice and diluted in pre-warmed fresh medium, and portions of the cell suspension estimated to contain between 50 to 200 colony forming units were plated in medium containing 0.26% agar. After 7 to 10 days, colonies of greater than 50 cells were counted with the aid of a dissecting microscope. A separate portion of the culture incubated with dFdA was washed and cellular nucleotides were extracted with $HClO_4$. Following neutralization, dFdATP was quantitated after separation from normal nucleotides by anion-exchange HPLC.