5-24-2002

Targeting of ribonucleotide reductase with 2'-Disulfide analogs of adenosine. Electronic versus steric effects in radical deoxygenation of fluorine-containing nucleosides

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DOI: 10.25148/etd.FI14060887

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TARGETING OF RIBONUCLEOTIDE REDUCTASE WITH 2'-DISULFIDE ANALOGS OF ADENOSINE. ELECTRONIC VERSUS STERIC EFFECTS IN RADICAL DEOXYGENATION OF FLUORINE-CONTAINING NUCLEOSIDES

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

in

CHEMISTRY

by

Dania Companioni

2002

To: Dean Arthur W. Herriott
College of Arts and Sciences

This thesis, written by Dania Companioni, and entitled Targeting of Ribonucleotide Reductase with 2'-Disulfide Analogs of Adenosine. Electronic versus Steric Effects in Radical Deoxygenation of Fluorine-containing Nucleosides, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Florida International University, 2002

DEDICATION

I dedicate this thesis to my husband, my father, my sisters, and especially to the memory of my mother.

ACKNOWLEDGMENTS

I wish to thank my major professor, Dr. Stanislaw Wnuk for his support, patience, and direction. I thank Dr. John T. Landrum and Dr. David Becker for helpful suggestions. I also would like to thank all my coworkers in the lab.

ABSTRACT OF THE THESIS

TARGETING OF RIBONUCLEOTIDE REDUCTASE WITH 2'-DISULFIDE ANALOGS OF ADENOSINE. ELECTRONIC VERSUS STERIC EFFECTS IN RADICAL DEOXYGENATION OF FLUORINE-CONTAINING NUCLEOSIDES

by

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Florida International University, 2002

Miami, Florida

Professor Stanislaw Wnuk, Major Professor

Inhibition of the enzyme ribonucleotide reductase is an appealing concept for the rational drug design against rapid proliferation of systems such as viruses and cancer cells. Synthesis of 2'-thionucleoside analogs as possible inhibitors of the enzyme was targeted in this research. For protecting the reactive thiol group, mixed disulfides were prepared as precursors of the sulfur-containing nucleosides. The thionucleoside obtained are currently under biological studies.

Fluorinated compounds are widely used in biochemistry, medicinal chemistry, and pharmacology. Synthesis of 2'-deoxy-2'-fluoroadenosine and its arabino epimer as well as 3'-deoxy-3'-fluoroadenosine and its xylo epimer were also targeted in order to study the electronic versus steric β-fluorine effects in radical deoxygenation of fluorine containing pentofuranose nucleosides. Our results clearly show that steric effect of the heterocyclic base from the β-face of the sugar is decisive, favoring the incorporation of deuterium from the less hindered α-face.

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1. INTRODUCTION:

1.1. GENERAL INTRODUCTION:

Studies on nucleic acids date from 1868 when Friedrich Miescher obtained a precipitate of a phosphorus-containing substance from human pus cells which he named nuclein. He also found this material to be common constituent of yeast, kidney, liver, testicular, and nucleated red blood cells. In fact, his nuclein was really a nucleoprotein and it was not until 1889 that Richard Altmann obtained the first protein-free material, to which he gave the name nucleic acid.[1]

Nucleic acids are very long, thread-like polymers, made up of a linear array of monomers called nucleotides, which are the phosphate esters of nucleosides. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are the two basic kinds of nucleic acids. RNA is made up of ribonucleotides and serves in the transcription and translation of genetic information in cells while DNA is the repository of this information and its monomers are 2'-deoxyribonucleotides.[2]

All nucleotides are constructed from three components: a nitrogen heterocyclic base, a pentose sugar, and a phosphate residue. The major bases are monocyclic pyrimidines or bicyclic purines. The major purines are adenine (A) and guanine (G) that are found in both DNA and RNA. The mayor pyrimidines are cytosine (C), thymine (T), and uracil (U) (Fig. 1).[1-4]

In nucleosides, the purine nitrogen-9 or pyrimidine nitrogen-1 are attached to the carbon-1 of a pentofuranose sugar. In ribonucleic acid, the pentose is D-ribose, which is locked into a five-membered furanose ring by a β-glycosylic linkage (Fig. 2). In DNA,

the pentose is 2-deoxy-D-ribose and the methylated pyrimidine base thymine takes the place of uracil in RNA (Fig. 3).

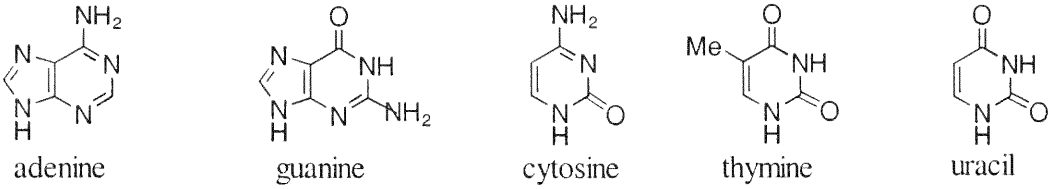


Figure 1 Structures of the five major purine and pyrimidine bases of nucleic acids

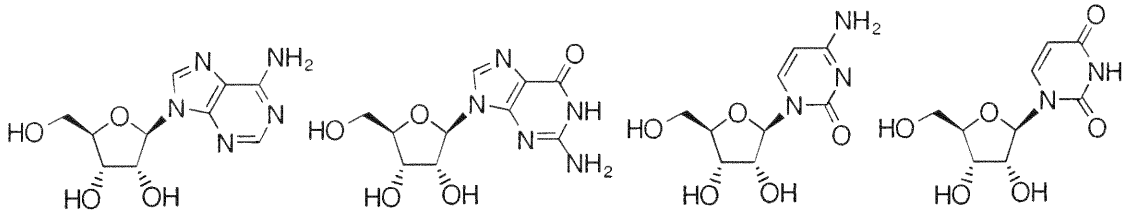


Figure 2 Structure of the four ribonucleosides

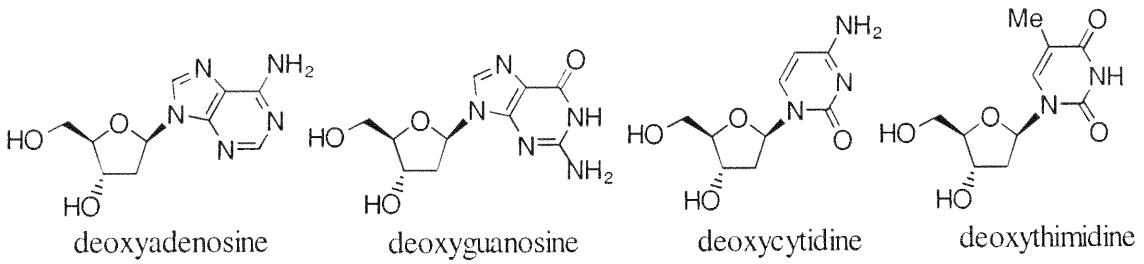
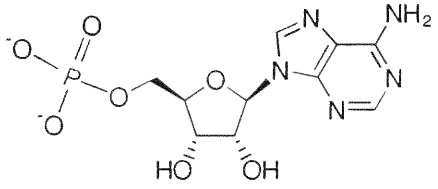
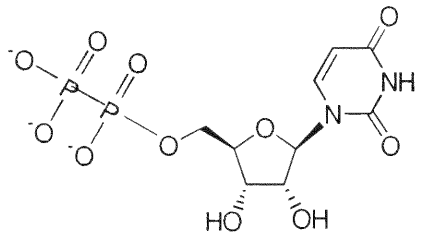


Figure 3 Structure of the four major deoxyribonucleosides

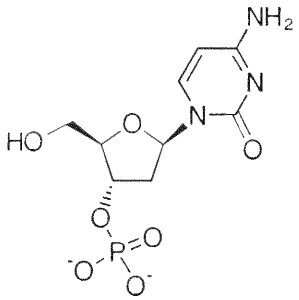
Nucleotides or nucleoside monophosphates are formed when phosphoric acid esterifies a sugar –OH group of nucleosides. Nucleosides in which the phosphoric acid esterifies two of the available ribose hydroxyl groups are known as cyclic nucleotides. Nucleoside monoesters of pyrophosphoric acid are called nucleoside diphosphates while nucleoside esters of tripolyphosphoric acid are nucleoside triphosphates (Fig. 4).[1]



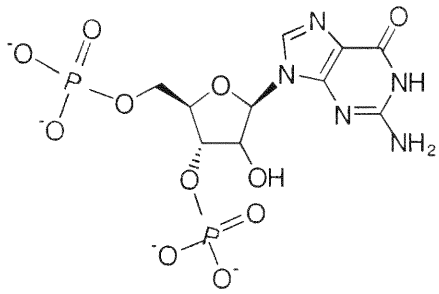
Adenosine **5'-monophosphate**



Uridine **5'-diphosphate**



2'-Deoxycytidine **3'-monophosphate**



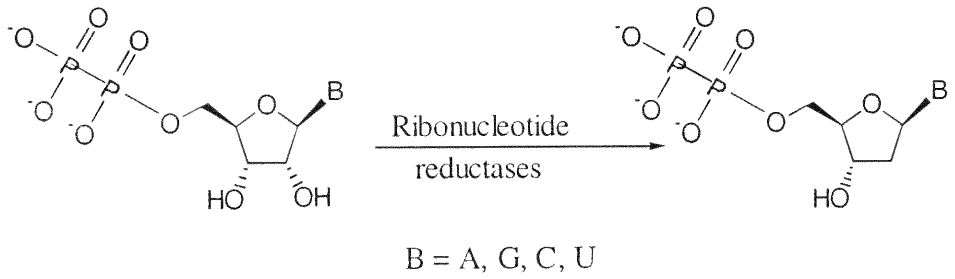
Guanosine **3',5'-bisphosphate**

Figure 4 Structure of some ribonucleotides

The first nucleoside syntheses were planned to prove the structures of adenosine and the other ribo- and deoxyribonucleosides. Modern syntheses have been aimed at producing nucleoside analogs, frequently for using them as inhibitors of nucleic acid metabolism.[5] Modifications of the sugar moiety of nucleotides at the 2'-position have resulted in a number of mechanism-base inhibitors of ribonucleotide reductase. Since nucleoside analogs have been found to show a wide and useful range of biological activity, hundreds, if not thousands, analogs have been synthesized in pharmaceutical laboratories across the world. In particular, chemistry of fluorine- and sulfur-modified nucleosides[6,7] have gained increased attention since their functionalities present in sugar moieties have led to active compounds. In recent times, industrial targets for this work have been anti-viral, anti-cancer, and chemotherapeutic agents.[1]

1.2 RIBONUCLEOTIDE REDUCTASES:

The deoxyribonucleotides have only one metabolic purpose: to serve as precursors for DNA synthesis.[8,9] In most organisms, ribonucleotide diphosphates (NDPs) are the substrates for deoxyribonucleotide formation. Reduction at the 2'-position of the ribose ring in NDPs produces 2'-deoxy forms of these nucleotides. This reaction involves replacement of the 2'-OH by a hydride ion ($H:^-$) and is catalyzed by an enzyme known as ribonucleotide reductase (Scheme I). Enzymatic ribonucleotide reduction involves a free radical mechanism, and four classes of ribonucleotide reductases are known, differing from each other in their mechanisms of free radical generation. Three of these ribonucleotide reductases have been intensively investigated.[10]



Scheme I Reduction of ribonucleotides to deoxyribonucleotides by the enzyme ribonucleotide reductase.

The RNR isolated from bacteria grown under aerobic conditions is prototypical of the Class I RNRs. This enzyme uses ribonucleotide diphosphates (RDPR) as the substrates. It is composed of two homodimeric subunits, R1 and R2. The R2 subunit contains a cofactor, which is composed of an unusual μ-oxo-bridged diferric cluster adjacent to a tyrosyl radical (•Tyr 122 in *Escherichia coli*). The tyrosyl radical is essential

for catalysis and is generated by the diferrous form of R2 in the presence of molecular oxygen.

The RNR isolated from *Lactobacillus leichmannii* (class II) requires adenosyl cobalamin (Adocbl = coenzyme B12) as a radical generated cofactor. These enzymes use ribonucleotide triphosphate (RTPR) as substrate. RTPR is a single polypeptide (M_r = 76 kda), which catalyzes the conversion of nucleotide triphosphates (NTPs) to 2'-deoxynucleotide triphosphate (dNTPs) with concomitant oxidation of two active site thiols to disulfides.

The RNRs isolated from *E. coli* grown under anaerobic conditions (class III) is distinct from the enzyme produced under aerobic conditions (class I). When isolated it is proved to have an essential glycyl radical which is generated by the second subunit of the enzyme that is equivalent to R2 in the aerobic *E. coli* RNR. The activating enzyme requires *S*-adenosylmethionine and an iron-sulfur cluster to generate the glycyl radical *via* a novel mechanism.

The RNR from *Brevibacterium ammoniagenes* (which is considered as class IV) has been recently characterized. It is thought to possess $\alpha_2\beta$ subunit structure and a dinuclear Mn^{3+} cluster analogous to the diferric cluster of aerobic *E.coli* RNR. This class of enzymes is poorly understood.

Despite the dramatic differences in these reductases (Fig. 5),[10] their mechanisms for nucleotide reduction (at least for classes I-III) are similar and unusual, involving exquisitely controlled radical base chemistry. Each of the metallo-cofactors initiates these radical-dependent nucleotide reduction processes by generating a transient protein radical. The working model is based on the two best-characterized RNRs, those from *E.*

coli and *L. leichmannii*. The tyrosyl radical (in *E. coli*) and the adenosylcobalamin radical (in *L. leichmannii*) has the same function: generation of the thiyl radical.[10,11]

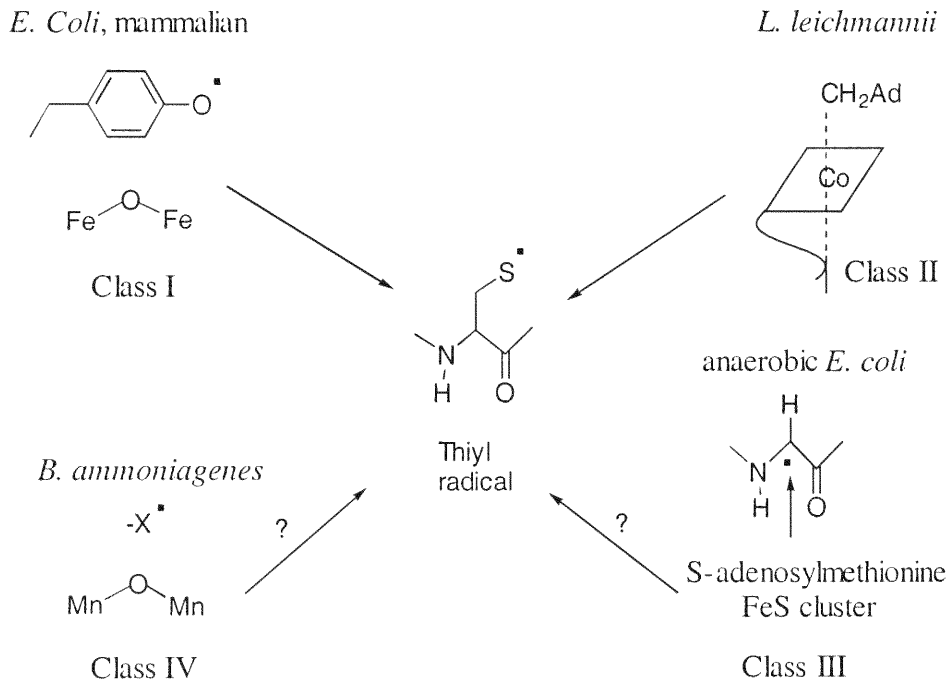


Figure 5 The cofactors used by the four major classes of RNRs.

Work in this dissertation was correlated with the mechanism of the ribonucleoside diphosphate reductases (RDPR) of *E. coli* since it closely resembles the mammalian ribonucleotide reductases. The diferric–tyrosyl radicals required by class I RNRs are potentially attractive targets for the design of antitumor and antiviral agents.

1.2.1. STRUCTURE OF RDPR FROM *Escherichia coli*:

The enzyme system for dNDP formation consists of four proteins, two of which constitute the ribonucleotide reductase, an enzyme of the $\alpha_2\beta_2$ type. The other two

proteins, thioredoxin and thioredoxin reductase,[2] function in the delivery of reducing equivalents. The two proteins of ribonucleotide reductase[12-15] are designated R1 and R2 and each is a homodimer in the haloenzyme (Fig. 6).[10]

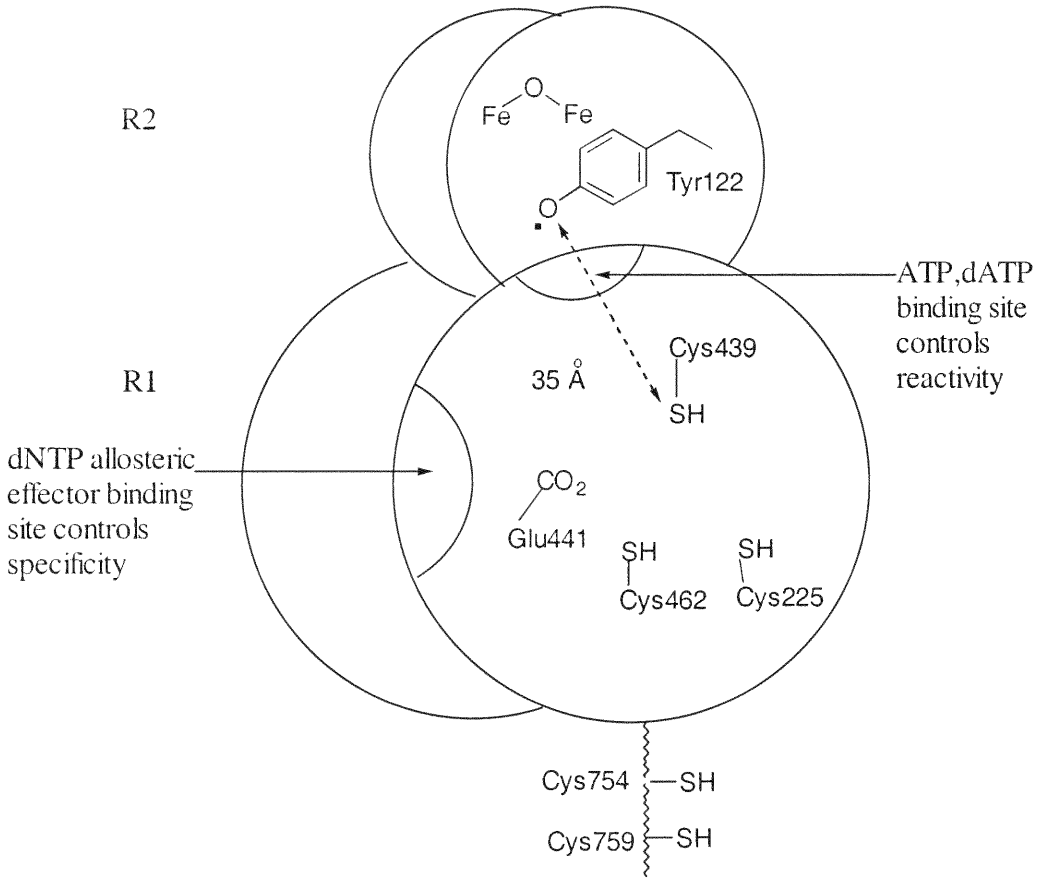


Figure 6 Ribonucleotide reductase class I

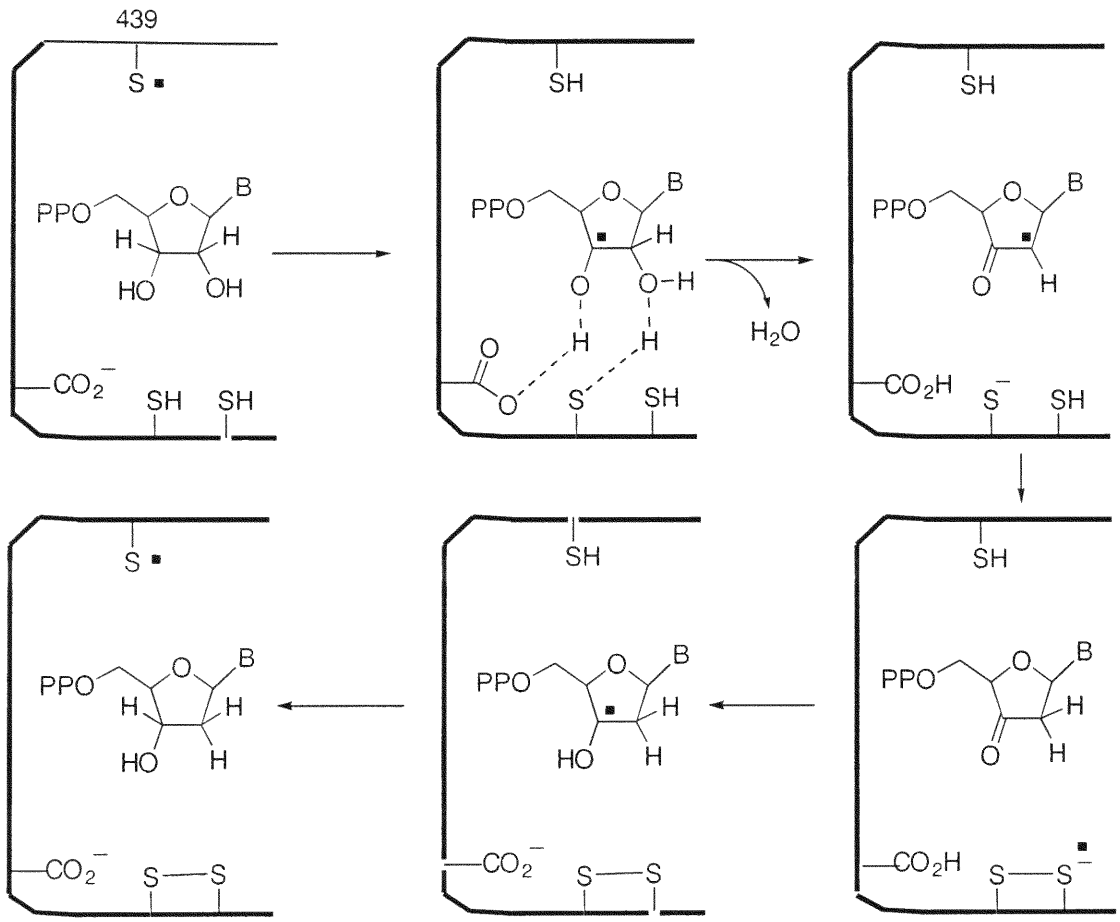
The R1 homodimer carries two types of regulatory sites in addition to the catalytic site. Purine and pyrimidine diphosphate substrates bind at the catalytic site. One regulatory site, the substrate specificity site, binds the dNTPs and ATP, and the identity of the nucleotide bound there determines which nucleoside diphosphate is reduced at the

catalytic site. If ATP is in the substrate specificity site, ribonucleotide reductase preferentially binds pyrimidine nucleosides at its active site and reduces them. With dTTP in the specificity-determining site, GDP is the preferred substrate. When dGTP binds to this specificity site, ADP becomes the favored substrate for reduction. The other regulatory site, the overall activity site, binds either the activator ATP or the negative effector dATP. If ATP is bound, the enzyme is active, while if its deoxy counterpart, dATP, occupies this site, the enzyme is inactive. In addition, it also contains the five cysteines that are essential for catalysis. The dithiol pair (Cys462/Cys225) deliver the reducing equivalent at C2' while Cys754/Cys759 reduce the resulting disulfide to the dithiol pair required for next catalytic cycle.[10,13]

The two Fe atoms within the single active site by the R2 homodimer generate the Tyr122 free radical required for ribonucleotide reduction on a specific R2 residue. The Tyr122 radical in turn, generates a thiyl free radical (Cys-S•) on Cys439 on R1 unit.[16,17] It has been proposed that Cys439-S• initiates ribonucleotide reduction by abstracting the 3'-H from the ribose ring of the nucleoside diphosphate substrate and forming a free radical on C-3'. Subsequent dehydration forms the deoxyribonucleotide product.[10]

1.2.2. POSTULATED MECHANISM OF ACTION OF RDPR:

Deoxyribonucleotides are formed by reduction of the corresponding ribonucleotides. The 2'-hydroxyl group of the ribose is replaced by a hydrogen atom in a reaction that takes place at the level of the ribonucleoside 5'-diphosphate. The mechanism appears to be simple at first sight but it is in fact extremely complicated. The most accepted mechanism proposed for a generic nucleotide reduction is shown in Scheme II.



Scheme II Proposed mechanism for nucleotide reduction by RNRs.

The key steps involve cofactor-mediated formation of a transient thiyl radical which initiates the nucleotide reduction process by abstracting the 3'-hydrogen atom from the nucleoside diphosphate. H_2O is lost,[10,18] and the two cysteines on the α-face of the nucleotide (Cys462, Cys225) deliver the required reducing equivalents, generating a 3'-ketodeoxynucleotide and a disulfide radical anion. This intermediate is subsequently reduced to give dNDP and a disulfide, regenerating the thiyl radical. The essential metallo-cofactor, the interactions between the two subunits of the class I RNRs and the

unusual radical intermediates in the nucleotide reduction process have all provided proven targets for design of inhibitors of this essential enzyme.

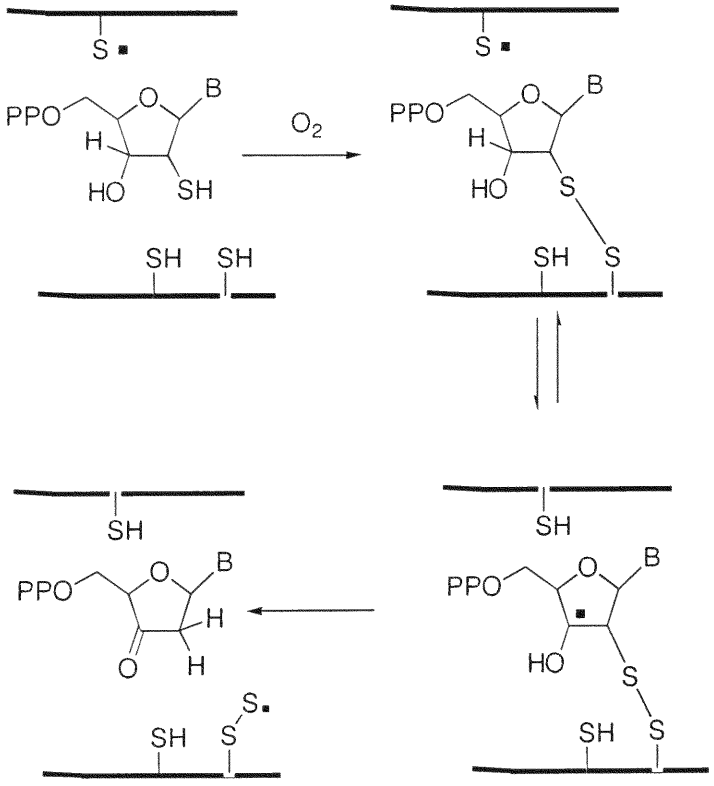
1.2.3. ENZYME INHIBITION BY 2'-THIOURIDINE DIPHOSPHATE:

DNA synthesis depends on a supply of deoxyribonucleotides. These are found at low levels within cells, and the enzymic reduction of ribonucleotides to deoxyribonucleotides is thought to be a rate-controlling step in the biosynthesis of DNA. The importance of ribonucleotide reductases in DNA synthesis has led to intensive studies on the inhibition of this enzyme. The enzyme from *Escherichia coli* is the prototype for all mammalian and viral ribonucleotide reductases. 2'-Substituted 2'-dNDP derivatives, as substrate analogs, have been shown to be very potent inactivators of this enzyme, some of them with potential applications as anticancer drugs in clinics. Furthermore, studies with these inhibitors have provided much insight into the enzyme mechanism.

In 1976, Thelander and coworkers reported that 2'-chloro-2'-deoxynucleotides (X = Cl) such as ClCDP and 2'-azido-2'-deoxunucleotides (X = N_3) such as N_3UDP or N_3CDP were potent inactivators of RDPR.[20] A detailed understanding of the mechanism by which these compounds inactivate the enzyme has evolved since their discovery.[10]

In 1996, Fontecave and his group reported that 2'-deoxy-2'-mercaptouridine 5'-diphosphate, a substrate analog, was a very efficient inactivator of RDPR (K_i = 35 μM, k_{inact} = 0.18 s^{-1}).[21,22] It was found that the inactivation was due to specific scavenging of the protein R2 tyrosyl radical. During reaction, a transient organic radical was detected by EPR spectroscopy. Its g anisotropy (g_z = 2.0620, g_y = 2.0265, and g_x = 2.0019) and its

hyperfine structure were consistent with a perthiyl RSS• radical. The loss of the hyperfine structure by deuterium labeling of the β protons of R1 cysteines unambiguously showed that the perthiyl radical was located on protein R1. This unique feature set this compound apart from those mechanism-based inhibitors such as 2'-azido- or 2'-chloro-2'-deoxyribonucleotide which induce partial or total protein R1 inactivation. These results allowed the formulation of a third type of mechanism for the inhibition of RDPR by 2'-deoxy-2'-mercaptouridine 5'-diphosphate (Scheme III).[21]



Scheme III Proposed mechanism-based inactivation of RDPR by 2'-deoxy-2'-mercaptouridine 5'-diphosphate.

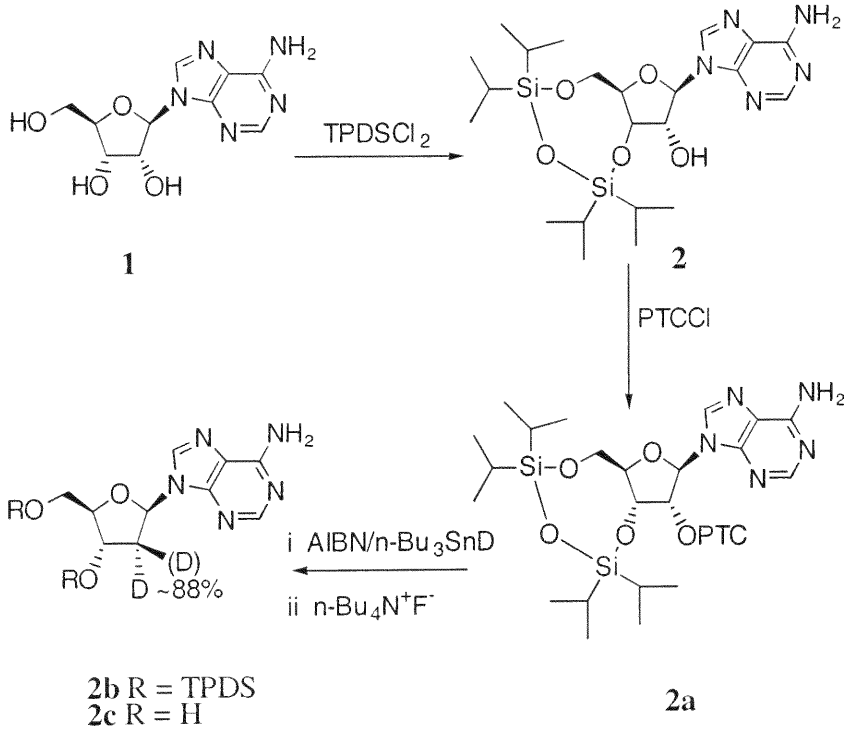
Upon binding of 2'-deoxy-2'-mercaptouridine 5'-diphosphate to the active site of RDPR, a mixed disulfide is generated, coupling the sulfur atom of the substrate to the cysteine (462 or 225) of R1, in the presence of oxygen. Cys439 thiyl radical, generated at the expense of the R2 tyrosyl radical as for the natural substrate, abstracts the 3'-hydrogen of the mixed disulfide intermediate. In the absence of oxygen, no disulfide is formed explaining why no loss of tyrosyl radical could be observed.[21]

The next step is proposed to be the homolytic cleavage of the 2'-carbon-sulfur bond, releasing a 3'-keto derivative and generating the perthiyl radical observed by EPR spectroscopy. The tyrosyl radical cannot be regenerated during reaction of the perthiyl radical with protein R2, thus explaining the inactivation of R2. The formation of the perthiyl radical requires that the active site cysteines of R1 be in the reduced form. If they are oxidized or changed to serines, the tyrosyl radical disappears as expected, but no perthiyl radical could be observed. It was observed that mutation of cys439 to serine totally blocks the electron transfer.

1.3. CHEMICAL PREPARATION OF DEOXYNUCLEOSIDES FROM NUCLEOSIDES. RADICAL DEOXYGENATION

Ribonucleosides, as their 5'-di- or triphosphates, are biosynthetically converted to their 2'-deoxy counterparts by ribonucleotide reductases. These completely retention-stereoselective free radical mediated deoxygenations utilize a complex sequence of enzymatic reactions and cofactors to provide the sole the novo pathway to the DNA components.[23] From the chemical point of view, many different methodologies have been employed for the preparation of deoxynucleosides and/or deoxynucleotides. 2'-

Deoxygenation of ribonucleosides has been pursued for many years with variable success. Regiospecific 2'-deoxygenation of ribonucleosides has been impeded by the difficulty in differentiating the secondary cis 2' and 3' hydroxyl groups. In 1983 a general procedure for the efficient deoxygenation of secondary alcohols was reported.[24] Regioselective protection of adenosine **1** with 1,1,3,3-tetraisopropyl-1,3-disiloxanediyl chloride (TPDS) gave the 3',5'-*O*-TPDS-nucleoside **2**. Phenoxythiocarbonylation (PTC) of the 2'-hydroxyl group of the protected compound, AIBN-initiated homolytic deoxygenation with tri-*n*-butyltin hydride, and deprotection with tetra-*n*-butylammonium fluoride completed the conversion of ribonucleosides to 2'-deoxyribonucleosides **2c**. Overall, conversion yields ranged from 57 to 78% (Scheme IV).



1 **2** **2a**

2b R = TPDS
2c R = H

Scheme IV Regiospecific and stereoselective conversion of ribonucleosides to 2'-deoxyribonucleoside.

Greater than 85% stereoselectivity on the deuterium transfer from the bulky tributylstannane to the less hindered α (ribo) face for the 2'-deoxygenation of adenosine derivatives,[24] reduced with tri-*n*-butyltin deuteride, in comparison with the complete retention stereoselectivity executed by ribonucleotide reductase was found.

1.4. THE STEREOSELECTIVITY OF RADICAL REACTIONS

In describing the stereochemical features of chemical reactions, we can distinguish between two types: stereospecific reactions and stereoselective reactions. A stereospecific reaction is one in which stereoisomeric starting material afford stereoisomerically different products under the same reaction conditions. A stereoselective reaction is one in which a single reactant has the capacity of forming two or more stereoisomeric products in a particular reaction but one is formed preferentially. The stereochemistry of the most fundamental reaction types such as addition, substitution, and elimination are described by terms, which specify the stereochemical relationship between the reactants and products.[25]

The stereoselectivity of free radical reactions is an area of considerable current interest.[26,27] For example, it has been already pointed out that some additions are *syn*, with both groups approaching from the same side. The others are *anti*, with the groups approaching from opposite sides of the double or triple bond during addition to carbon-carbon multiple bonds. For cyclic compounds, there are further aspects of steric orientation. In *syn* addition to an unsymmetrical cyclic olefin, the two groups can come in from the more-hindered face or from the less-hindered face in the double bond. Electronic effects can also play a part in determining which face is attacked. Studies on

five- and six-membered ring radicals as well as on vinylic radical systems have clearly demonstrated the dominance of steric influences of vicinal substituents in determining the facial *anti*-selectivity. In the case of hydrogen transfer reactions the *anti*- or *syn*-selectivity of the incoming hydrogen, or deuterium, is determined by the stereochemistry of the starting material or according to the position of a specific substituent on the molecule.[25]

1.4.1. EFFECT OF VICINAL FLUORINE ON RADICAL REACTIONS

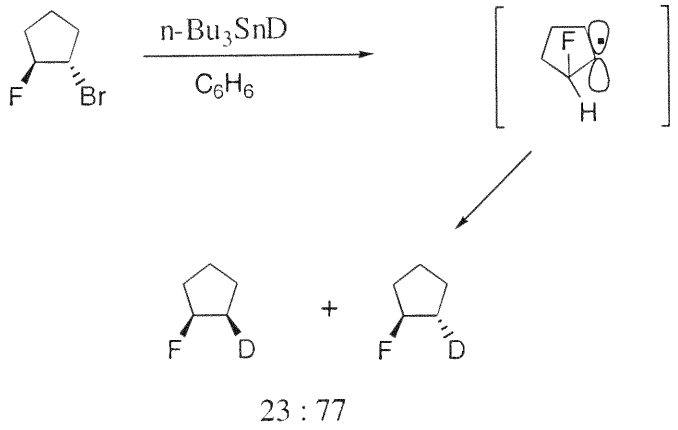
Alkyl radicals are generally pyramidal, although the inversion barrier is very small. They can however, have rigid pyramidal or planar structures. It has been suggested that two factors are of principal importance in favoring a pyramidal structure. One is a torsional effect in which the radical center tends to adopt a staggered conformation of the radical substituents. There is also a hyperconjugative interaction between the half-filled orbital and the hydrogen that is aligned with it. This hyperconjugation is stronger in the conformation in which the pyramidalization is in the same direction as to minimize eclipsing.

Radical geometry is also significantly affected by substituent groups that can act as π donors. Addition of a fluorine substituent favors a pyramidal structure. It has been reported that there is a repulsive interaction between the singly occupied *p* orbital and the filled orbitals occupied by "lone-pair" electrons on the fluorine substituent. This repulsive interaction is minimized by adoption of a pyramidal geometry.[25]

There have been many studies aimed at deducing the geometry of radical sites by examining the stereochemistry of radical reactions. A planar or rapidly inverting radical

would lead to racemization, whereas a rigid pyramidal structure should lead to product of retained configuration. Cyclic molecules permit deductions about stereochemistry without the necessity of using resolved chiral compounds.

Dolbier and Balberger observed *anti*-selectivity (77:23) in the reduction of β-fluorocyclopentenyl radicals with tributyltin deuteride (Scheme V).[28] They precluded steric effects from having been the decisive factor in determining the stereoselectivity of D-atom transfer. Influence of vicinal fluoro substituents on the diastereoselectivity of radical reactions was attributed to the effect of *anti*- versus *syn*-pyrimidalization of radicals in transition states. Marquez and coworkers reported that non-metal hydride radical deoxygenation of nucleoside was favorably influenced by the presence of a β-fluorine.[29] β-oxygen effect on radical deoxygenation of thionocarbonyl esters has also been examinated.[30]

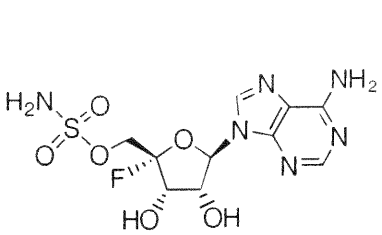


Scheme V Reduction of β-fluorocyclopentenyl radical

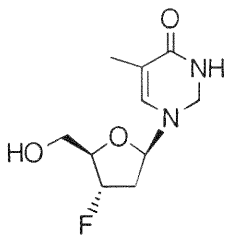
1.5. FLUORINATED NUCLEOSIDE ANALOGS

Nucleocidin (Fig. 7), one of the very few natural products containing a fluorine atom, has been isolated from *Streptomyces calvus* and is used as an antitrypanosomal antibiotic.[31] The fluorine atom is the most electronegative atom that can be introduced in an organic compound and has a van der Waals radius (1.35 Å) comparable to that of hydrogen (1.17 Å). Substitution of fluorine for hydrogen has a strong effect on the electronic configuration of a molecule, often reflected by a dramatic change in biological activity.[32]

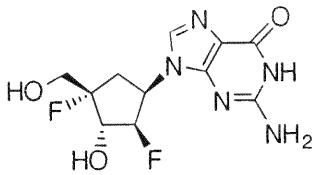
It is well established that the introduction of a fluorine atom at a sugar carbon in nucleosides alters their biological activities toward various cellular, pathogenic, and tumor-specific enzymes in various ways.[33] For example, 3'-fluoro-2',3'-dideoxythimidine was found to be a potent inhibitor of HIV-induced cytopathogenicity and 4'-fluorinated carbocyclic nucleoside displays potent antiviral activity against *Herpes simplex* viruses.



Nucleosidin



3'-fluoro-2',3'-dideoxythimidine



2'-ara-fluoro-4'-fluoro carbocyclic guanosine

Figure 7 Fluorinated nucleoside analogs

The replacement of a hydroxyl group by a fluorine atom causes only a minor change in the steric effect of the functionality, but such a substitution has profound

effects on the chemical properties. It also changes the stereoelectronic properties, which result in specific overall conformational change of the fluorinated nucleosides. The strong gauche effect of the fluorine substituent (due to its high electronegativity) has a profound stereoelectronic effect on the stereochemical orientation of the neighboring groups.[34]

1.6. OBJECTIVE OF THE RESEARCH

Synthesis of 9-(2-thio-β-D-arabinofuranosyl)adenine 5'-diphosphate **10** was targeted in this study as potential inhibitor of ribonucleotide reductases. The arabino 2'-mercapto group in inhibitor **10** might interact with crucial thiyl radical at cysteine 439, which is hypothesized to initiate nucleotide reduction by abstraction of H3' from the substrate ribonucleotide. Such interaction between these two thiols may lead to the inhibition of RDPR via formation of the R1 Cys439–2'-mercapto disulfide bridge.

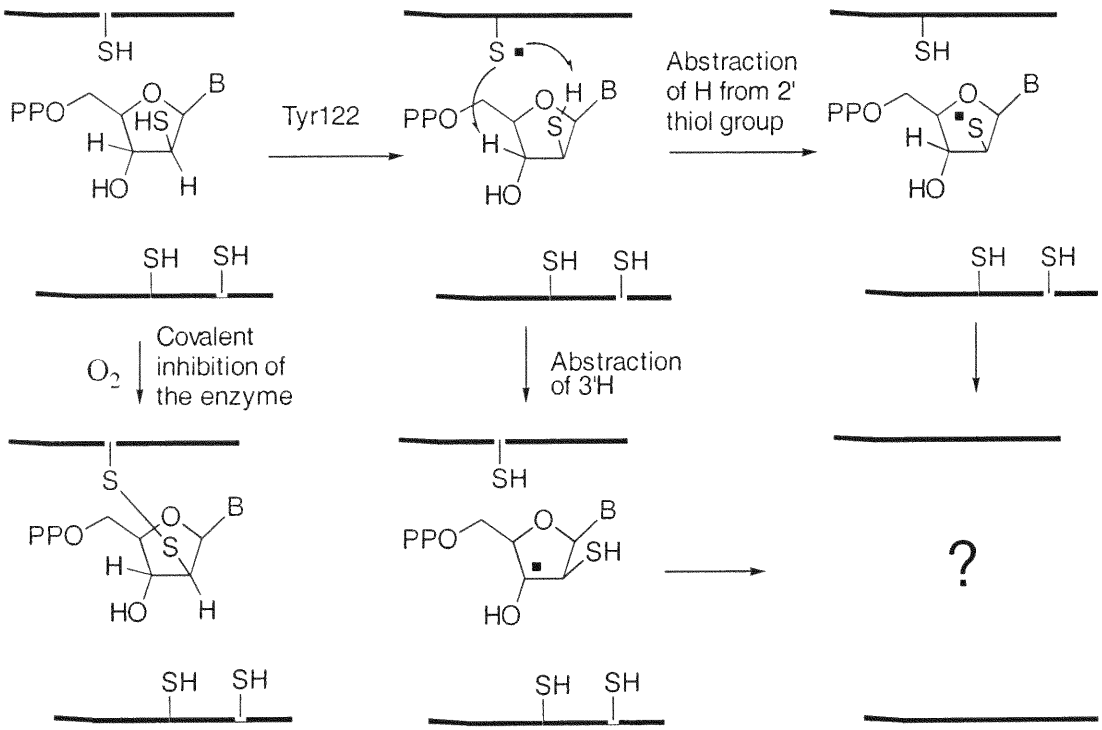
Competitive abstraction of hydrogen from the 2'-thiol function rather than regular abstraction of H3' by Cys439 have to be considered as an alternative inhibition pathway (Scheme VI). It is noteworthy that homolytic bond dissociation energies of 91 kcal/mol for S-H and 94 kcal/mol for H-C-O make hydrogen atom abstraction from 2'-thiol thermodynamically more feasible.[35]

However, binding attributes including conformation of the synthesized molecule at the active site of the enzyme might either favor or exclude such interaction from the stereochemical point of view.

The arabino thionucleotide can also serve as a valuable probe for understanding the role of the cysteinyl 439 radical during enzymatic deoxygenation with natural substrates. It is also well known that arabino nucleoside analogs are very potent,

clinically approved, anticancer agents. Therefore, the synthesized thionucleosides are being evaluated against various cancer lines.

In order to evaluate steric and stereochemical flexibility of disulfide analogs for binding at the active site of the enzyme, synthesis of the corresponding arabino methyl disulfide and the ribo n-propyl disulfide derivatives (**15** and **22** respectively) was also targeted during this work.



Scheme VI A possible inhibition of RDPR by 9-(2-thio-β-D-arabinofuranosyl)adenine 5'-diphosphate.

Preparation of 2'-deoxy-2'-fluoroadenosine **35** and its arabino epimer **37** as well as 3'-deoxy-3'-fluoroadenosine **36** and its xylo epimer **38** was the second target on this

project. Stereoselectivity of deuterium incorporation during radical deoxygenation was determined in order to study the electronic versus steric β-fluorine effects on all of these fluorine containing pentofuranose nucleosides.

Incorporation of deuterium into the sugar moiety of compounds under study was followed by H^1 NMR analysis of signals for H2',2" or H3',3", depending on the case.

2. RESULTS AND DISCUSSION

2.1. SYNTHESIS OF SULFUR MODIFIED COMPOUNDS

2.1.1. SYNTHESIS OF 9-[2-DEOXY-2-ALKYLDITHIO-β-D-ARABINOFURANOSYL]ADENINE

Incorporating a thiol function in nucleosides, nucleotides, and oligonucleotides and preparing their stable precursors is of interest in the search of bioactive compounds.[36] Mixed disulfides have been found to be interesting precursors that could be reduced efficiently and rapidly under mild conditions for generating the active species.[22] A literature search revealed that different methods have been developed in this regard.[22,37,38]

From the available thionucleosides,[36] purine 2'-mercapto analog[39,40] **6** was chosen because attempts to synthesize 1-(2-thio-β-D-arabinofuranosyl)uracil failed due to the rapid Michael addition of arabino 2'-mercapto function across the double bond of the uracil moiety to give 2'-deoxy-2',6-epithio-5,6-dihydro derivative.[41,42]

Selective 3,5-*O*-protection of adenosine[43] **(1)** was achieved by employing Markiewicz-Wiewiõrowski reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TPDSCl_2$) to give **2**. Activation of the hydroxyl group at carbon 2' of the silyl protected compound with *N*-Phenyltrifluoromethanesulfonimide $[(CF_3SO_2)_2NC_6H_5]$ in the presence of dimethylaminopyridine (DMAP) gave 3',5'-*O*-[1,1,3,3-tetraisopropyldisiloxane(TPDS)-1,3-diyl]-2'-*O*-(trifluoromethanesulfonyl)adenosine[44] **3** in 79% yield.

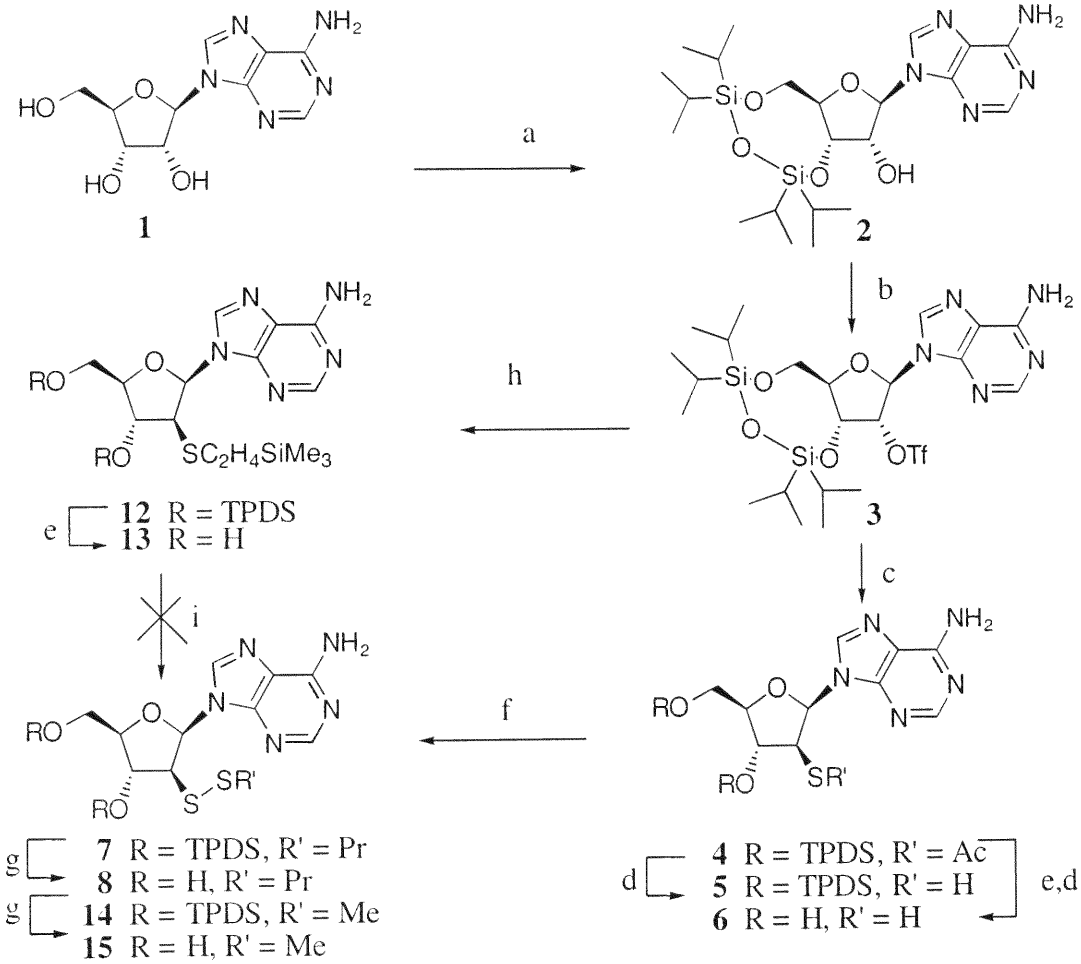
In a first approach for the preparation of a mixed disulfide, nucleophilic substitution of the triflate group on compound **3** by a thioacetate has been investigated. Thus, treatment of **3** with potassium acetate gave arabino epimer **4** of the protected 2'-*S*-acetyl-2'-thioadenosine.

Sequential removal of the 3',5'-*O*-TPDS [(tetrabutylammonium fluoride (TBAF)] and 2'-*S*-acetyl (NH_3/MeOH) groups afforded 9-(2-thio-β-D-arabinofuranosyl)adenine (**6**; ~15-30%) after purification on a Dowex 1 × 2-200 anion-exchange resin (OH^-) column.[39,40] Careful mild treatment of **4** with TBAF effected selective desilylation to give 2'-*S*- acetyl-**6** as a major product in addition to a small amount of **6**, after separation on silica gel column (Scheme IV). The 2'-mercapto compound **6** had spectroscopic data as reported[40] but slow formation of the corresponding disulfide was apparent since the treatment of the mixture with dithiothreitol (DTT) lead to a much sharper peak on TLC.

The mixed disulfide **8** with a propyl side-chain was prepared from **6** using diethyl azodicarboxylate (DEAD) as a oxidizing agent in the presence of propanethiol.[22,45] Thus, treatment of **6** with DEAD and 1-propanethiol in THF gave disulfide **8** (~35%) plus unchanged **6** (~20%). In order to overcome solubility problems which significantly lower the yield, compound **4** was treated with NH_3/MeOH to give 9-(3,5-*O*-TPDS-2-thio-β-D-arabinofuranosyl)adenine (**5**) in quantitative yield. Treatment of **5** with DEAD/C_3H_7SH/THF gave protected disulfide **7** (75%) but separation from the diethyl hydrazinedicarboxylate byproduct was tedious. Desilylation (NH_4F/MeOH)[46] gave **8** (60%) which was readily purified. The characteristic signals for the propyl group (CH_3-CH_2-CH_2-S-) were observed in ^1H (0.88, 1.53, 2.57 ppm) and ^{13}C (12.1, 22.0, 41.0 ppm) NMRs of the disulfides **7** and **8**.

In the second approach, recently developed chemistry utilizing 2'-[2-(trimethylsilyl)ethane]thiol group[37,38] was employed for the attempted preparation of the stable disulfide precursors. Thus, displacement of the triflate from **3** with 2-(trimethylsilyl)ethanethiolate in DMF proceeded smoothly at 60 °C to give 2'-*S*-[(2-trimethylsilyl)ethyl]-2-thionucleoside **12** in good yield (Scheme VII).

Scheme VII[a]



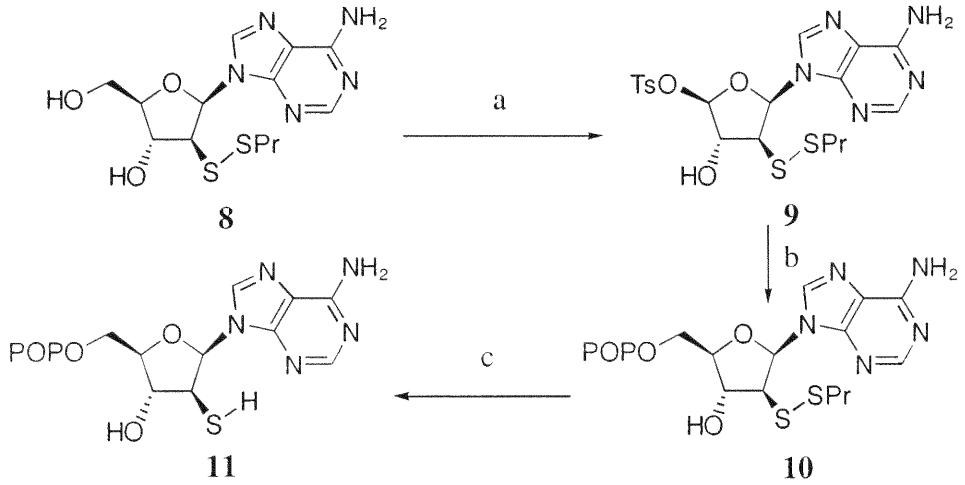
[a] Key: (a) $TPDSCl_2$/pyridine; (b) CH_2Cl_2/*N*-phenyltrifluoromethanesulfonimide/DMAP; (c) CH_3COSK/DMF; (d) NH_3/MeOH; (e) TBAF/THF; (f) DEAD/PrSH or MeSH/THF; (g) NH_4F/MeOH; (h) Me_3SiCH_2SH/K_2CO_3/DMF; (i) $(CH_3)_2S_2/(CH_3)_2S(SCH_3)BF_4$/THF.

Deprotection of **12** with TBAF gave **13** showing stability of 2-(trimethylsilyl)ethyl group towards fluoride.[47] However, reaction of **12** or **13** with dimethyl(methylthio)sulfonium tetrafluoroborate[37,38,47] in the presence of large excess of dimethyl sulfide failed to produce methyl disulfide derivatives of type **14** or **15**. Instead, unchanged **12** or **13** and a fluorescent byproduct(s) were isolated from the reaction mixtures indicating instability of the purine ring (as opposed to pyrimidine)[36,37] under reaction conditions.

2.1.2. PREPARATION OF 9-[2-DEOXY-2-PROPYLDITHIO-β-D-ARABINOFURANOSYL]ADENINE 5'-DIPHOSPHATE

From the methods available for the 5'-phosphorylation of nucleosides[48] we chose Poulter's methodology[49] which is based on the displacement of the 5'-*O*-tosylate ester with the corresponding pyrophosphate ion. Thus, disulfide **8** was converted to a 5'-*O*-tosylate **9** using standard chemistry with a bulky propyl group allowing selective 5'-tosylation. Treatment of **9** with tris(tetrabutylammonium) hydrogen pyrophosphate[49] CH_3CN effected substitution to give 5'-diphosphate **10** (Scheme VIII). The ammonium salt of the nucleotide was purified by ion-exchange chromatography on DEAE Sephadex A-25 (HCO_3^-) using linear gradient of triethylammonium bicarbonate (TEAB, 0.05 $\rightarrow$ 0.50 M) as elutant. The ^{31}P NMR spectrum showed two doublets (J = 23.5 Hz) at δ -9.75 and -5.26 ppm, diagnostic for two phosphorus atoms, and ^1H NMR spectrum confirmed the structure. This material was converted to sodium salts by passing it through a Dowex 50 (Na^+) column.

Scheme VIII [a]



[a] Key: (a) TsCl/pyridine; (b) $(Bu_4N)_3HP_2O_7/CH_3CN$; (c) DTT/H_2O

Reduction of diphosphate **10** with slight a excess of DTT immediately generates[21] the corresponding thiol **11** as observed by TLC and MS. Dithiothreitol was chosen as a reducing agent since it is one of the electron sources used in the assays for ribonucleotide reductase activity in vitro.

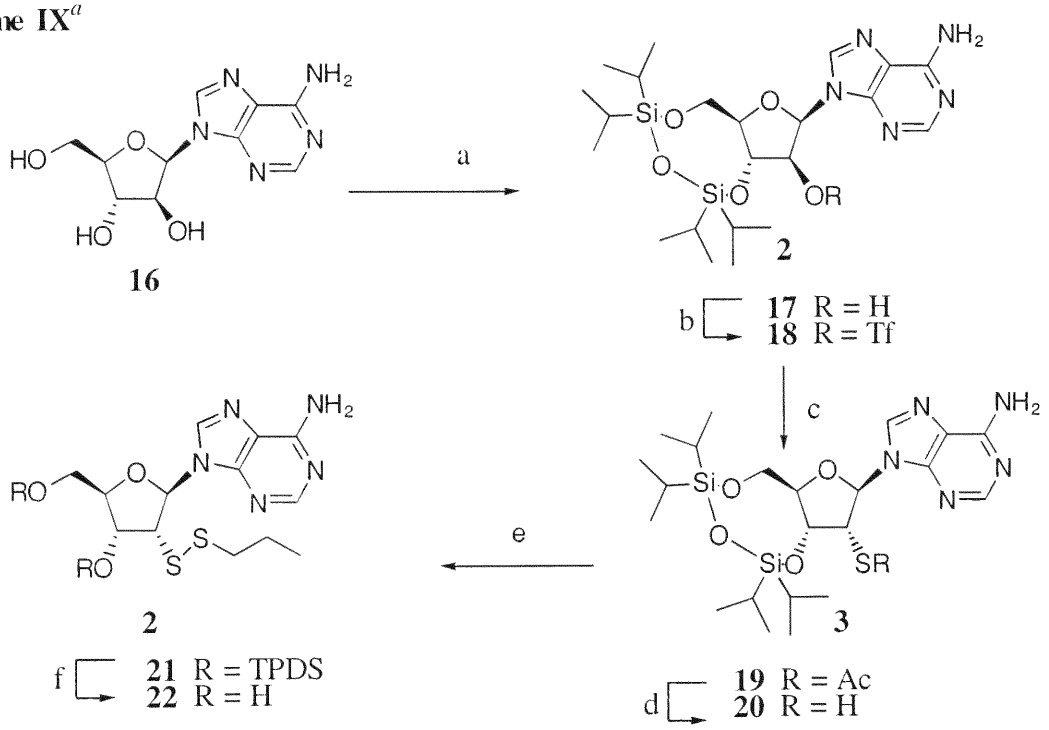
2.1.3. SYNTHESIS OF OTHER ANALOGS

In addition to 9-[2-deoxy-2-propyldithio-β-D-arabinofuranosyl]adenine **8**, the corresponding methyl disulfide analog **15** and the n-propyl ribo-derivative **22** were also obtained. These compounds were prepared in order to evaluate steric and stereochemical flexibility of disulfides analogs for binding at the active sites of the enzyme.

The methyl disulfide **15** was obtained by treatment of the thiol **6** with MeSH/DEAD/THF using the same procedure as described for **8** (Scheme VII). Synthesis of the propyl disulfide **22** started from 9-(β-D-arabinofuranosyl)adenine **16** and involved: Markiewicz 3',5'-*O*-protection, triflate activation (O2') towards nucleophilic substitution,

displacement of triflate group by thioacetate with inversion of configuration, and deacetylation to give ribo-thiol **20**. Treatment of the latter with PrSH/DEAD/THF and desilylation gave **22** (Scheme IX).

Scheme IX[a]



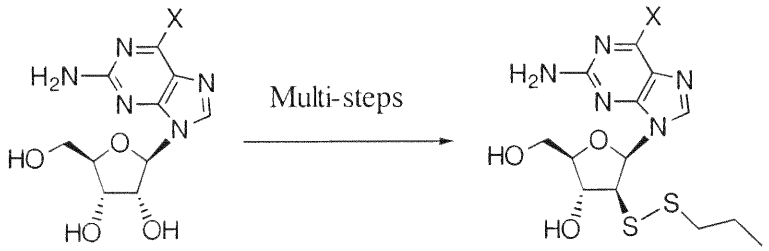
[a]Key: (a) $TPDSCl_2$/pyridine; (b) CH_2Cl_2/*N*-phenyltrifluoromethanesulfonimide/DMAP; (c) CH_3COSK/DMF; (d) NH_3/MeOH; (e) DEAD/PrSH; (f) NH_4F/MeOH

2.1.4. SYNTHESIS OF 2,6-DISUBSTITUTED PURINE THIONUCLEOSIDES

Other 2,6-disubstituted-purine thionucleoside have been designed and synthesized, using the same methodology presented above, to evaluate as anticancer analogs. Thus, 2,6-diamino- **(a)**, 2-amino-6-methoxy- **(b)** and 2-amino-6-chloro-9-(2-deoxy-2-propyldithio-β-D-arabinofuranosyl)purine **(c)** were prepared from the corresponding commercially available 2,6-amino-, 2-amino-6-methoxy- and 2-amino-6-

chloro-9-(β-D-arabinofuranosyl)purine, respectively (Scheme X). The synthesis was performed in collaboration with Dr. Elzbieta Lewandowska.

Scheme X



Series: a $X = NH_2$; **b** $X = OCH_3$; **c** $X = Cl$

It has to be pointed out that the disubstituted analogs can be converted *in vivo* and *in vitro* to the corresponding guanosine derivatives by the action of adenosine deaminase. Therefore, they can be considered as possible pro-guanosine inhibitors. Guanosine derivatives are much more difficult to manipulate and synthesize in the laboratory.

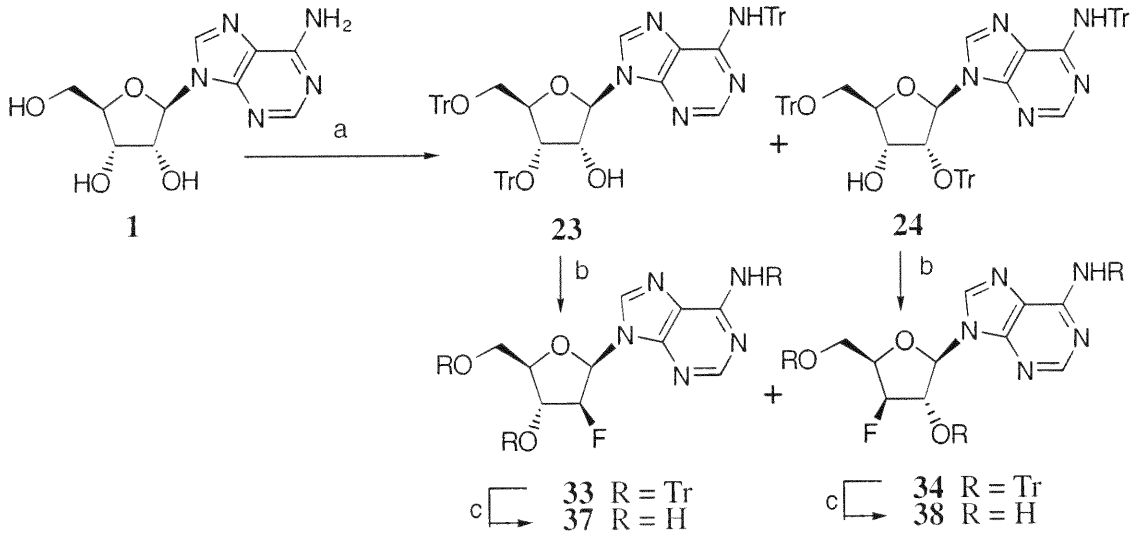
2.2. SYNTHESIS OF FLUORINE MODIFIED COMPOUNDS

2.2.1. PREPARATION OF 2'-DEOXY-2'-FLUOROADENOSINE AND 3'-DEOXY-3'-FLUOROADENOSINE

For our synthetic purposes we were interested in a shorter route to the 2'-deoxy-2'-fluoroadenosine **35** and its arabino epimer **37** as well as 3'-deoxy-3'-fluoroadenosine **36**

and its xylo epimer **38**. Taking advantage of the control of conformation by bulky protecting groups, Pankiewicz and his coworkers reported[50,51] an excellent method for fluorination of ribonucleosides at the C2'-β position. Thus, treatment of 3',5'-di-*O*-trityl-adenosine **23** and 2',5'-di-*O*-trityladenosine **24**, obtained after tritylation of adenosine **1**, with (diethylammino)sulfur trifluoride (DAST) gave **33** and **34** (Scheme XI). Detritylation with trifluoroacetic acid afforded compounds **37** and **38**, respectively in moderate overall yield.

Scheme XI[a]

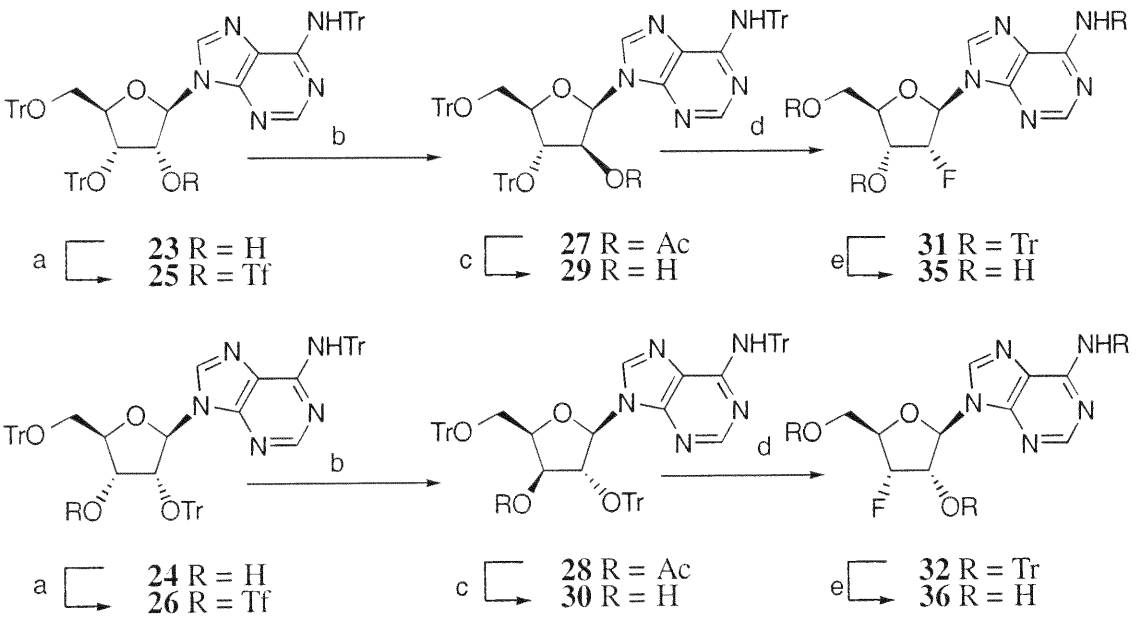


[a] Key: (a) Trityl chloride/pyridine; (b) DAST/CH_2Cl_2/pyridine; (c) $CF_3COOH/CHCl_3$ 1:9

On the other hand, inversion of the 2'- and 3'-hydroxyl groups in trityl-protected adenosines **23** and **24** was performed via *O*-triflyl derivatives **25** and **26.** Subsequent substitution with sodium acetate produced inverted esters **27** and **28**. Treatment with

$MeOH/NH_3$ effected deacetylation to give the arabino- **29** and xyloderivatives **30**. Fluorination with DAST, induced a second inversion of configuration, to give 2'-ribo-fluoro **31** and 3'-ribo-fluoro **32** in good synthetic yields. Detritylation afforded **35** and **36**, respectively (Scheme XII).

Scheme XII[a]



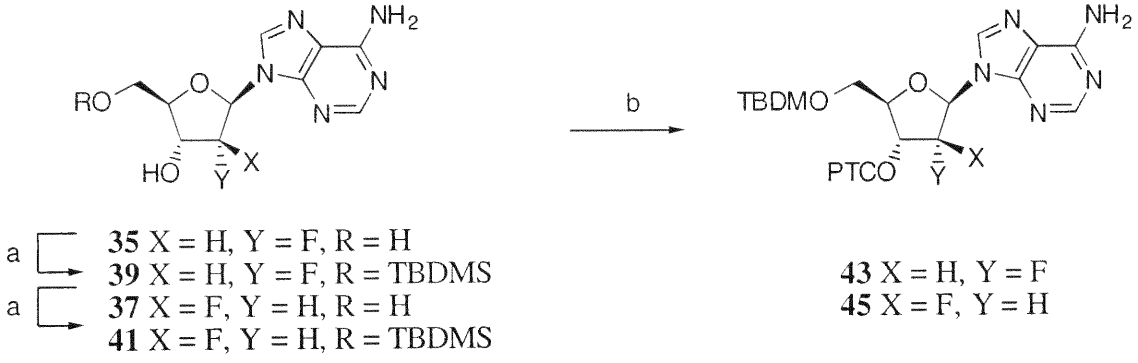
[a] Key:(a) $CF_3SO_2Cl/DMAP/CH_2Cl_2$; (b) AcONa/DMF; (c) $MeOH/NH_3$; (d) DAST/pyridine/dichloromethane; (e) $CF_3COOH/CHCl_3$ 1:9.

2.2.2. SYNTHESIS OF PHENOXYTHIOCARBONYL PRECURSORS OF DEOXYFLUORONUCLEOSIDES

Regioselective silylation (O5') of the fluoro nucleosides **35**, **36**, **37**, and **38** with *tert*-butyldimethylsilyl (TBDMS) chloride in DMF in the presence of imidazole produced **39**, **40**, **41**, and **42**. Treatment of **39** with phenoxythiocarbonyl (PTC) chloride[24] gave 5'-

O-TBDMS-3'-*O*-PTC-2'-fluoro-2'-deoxyadenosine **43**. Analogous treatment of **41** afforded the fluoro-arabino epimer **45** (Scheme XIII). The 3'-fluoro isomers **40** and **42** were converted to 5'-*O*-TBDMS-2'-*O*-PTC-3'-fluoro-3'-deoxyadenosine **44** and its fluoro-xylo epimer **46**, respectively (Scheme XIV), completing the synthesis of all four fluoro-isomer models.

Scheme XIII[a]

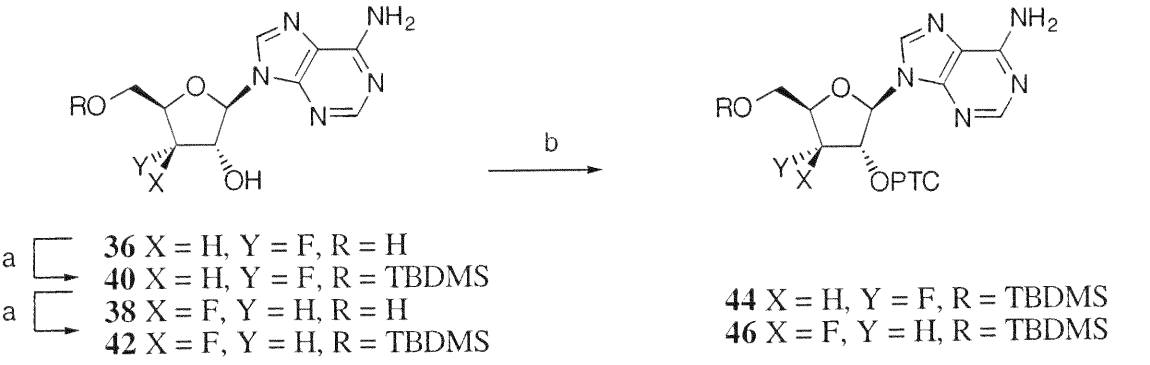


a **35** X = H, Y = F, R = H
39 X = H, Y = F, R = TBDMS
a **37** X = F, Y = H, R = H
41 X = F, Y = H, R = TBDMS

43 X = H, Y = F
45 X = F, Y = H

[a] Key: (a) TBDMSCl/imidazole/DMF; (b) PTCCl/DMAP/CH_3CN

Scheme XIV[a]



a **36** X = H, Y = F, R = H
40 X = H, Y = F, R = TBDMS
a **38** X = F, Y = H, R = H
42 X = F, Y = H, R = TBDMS

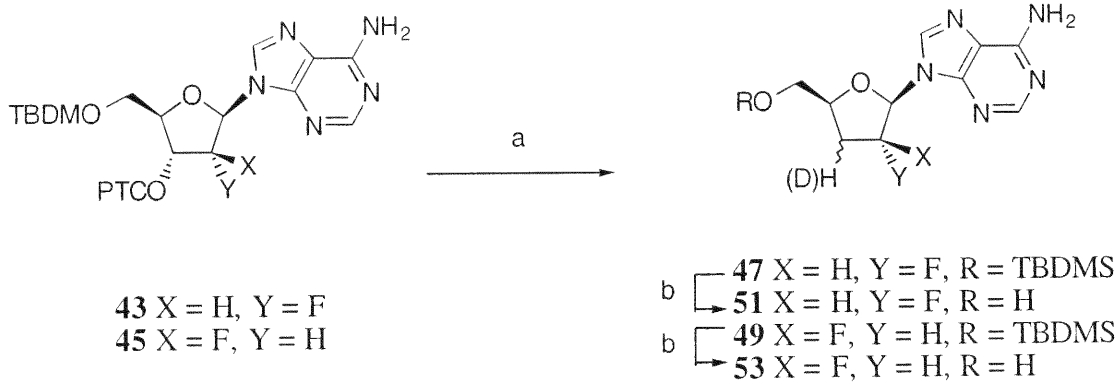
44 X = H, Y = F, R = TBDMS
46 X = F, Y = H, R = TBDMS

[a] Key: (a) TBDMSCl/imidazole/DMF; (b) PTCCl/DMAP/CH_3CN

2.2.3. RADICAL DEOXYGENATION. STERIC AND ELECTRONIC EFFECT OF A FLUORINE SUBSTITUENT ON RADICAL DEOXYGENATION

Treatment of 3'-PTC ester **43** with tributyltin deuteride effected deoxygenation to give 5'-*O*-TBDMS-2',3'-dideoxy-3'-deuterio-2'-fluoroadenosine epimers **47** (3' *R/S*, 64:36). On the other hand, reduction of 3'-PTC ester **45** (fluorine up) gave the corresponding 3'-deuterio epimers **49** (3' *R/S*, 93:7) (Scheme XV). The ratio of ribo/xylo deuterium substitution (Table 1) was determined on the bases of vicinal coupling constant analysis ($^3J_{F\text{-}H}$ and $^3J_{H\text{-}H}$) in ^{1}H NMR.

Scheme XV



43 X = H, Y = F
45 X = F, Y = H

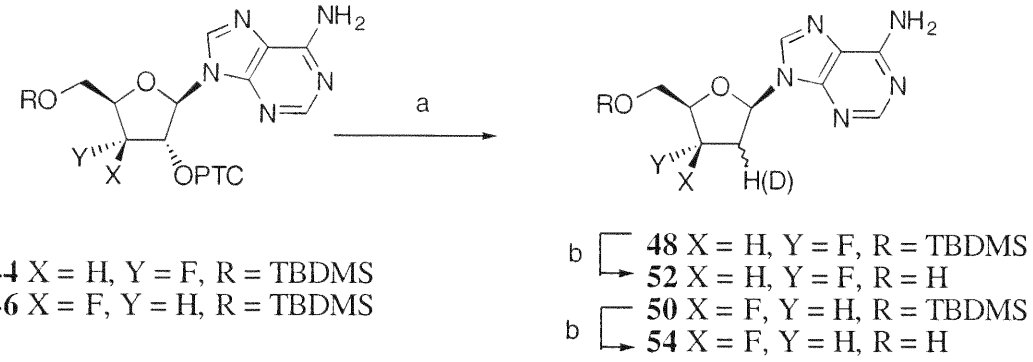
47 X = H, Y = F, R = TBDMS
51 X = H, Y = F, R = H
49 X = F, Y = H, R = TBDMS
53 X = F, Y = H, R = H

[a] Key: (a) Bu_3SnD/AIBN/toluene/85 °C; (b) NH_4F/MeOH.

Radical deoxygenation of **44** (fluorine down) gave 5'-*O*-TBDMS-2',3'-dideoxy-2'-deuterio-3'-fluoroadenosine **48** (2' *R/S*, 14:86) as 2'-deuterio epimers. Analogous treatment of **46** (fluorine up) effected reduction to give epimers **50** (2' *R/S*, 7:93) (Scheme XVI). Radical deoxygenation was performed twice on all four phenoxythiocarbonyl derivatives with all showing similar results to the NMR spectra that are presented in

Table 1 as average values. Coupling constants are in agreement with the values measured and calculated by Chattopadhyaya and coworkers[34] for the 2',3'-dideoxy-3' (or 2')-fluorouridine derivatives and for adenosine analog **54**.

Scheme XVI[a]



44 X = H, Y = F, R = TBDMS
46 X = F, Y = H, R = TBDMS

48 X = H, Y = F, R = TBDMS
52 X = H, Y = F, R = H
50 X = F, Y = H, R = TBDMS
54 X = F, Y = H, R = H

[a] Key: (a) Bu_3SnD/AIBN/toluene/85 °C; (b) NH_4F/MeOH.

Table 1. The ratio for deuterium substitution during radical deoxygenation of 2'(or 3')-*O*-PTC derivatives of fluorine-containing pentafuranose nucleosides

2'(3')-*O*-PTC-3'(2')-fluoro precursors	Ratio of 2'(3')-deuterio epimers	Diastereotopic excess
43 2'-fluoro-ribo	**47** (3'*R/S*, 64:36)	28 *anti/syn*, 36:64
45 2'-fluoro-arabino	**49** (3'*R/S*, 93:7)	86 *anti/syn*, 93:7
44 3'-fluoro-ribo	**48** (2'*R/S*, 15:85)	70 *anti/syn*, 15:85
46 3'-fluoro-xylo	**50** (2'*R/S*, 7:93)	86 *anti/syn*, 93:7

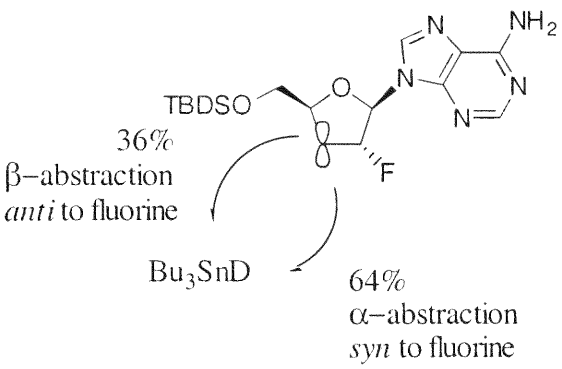
Duplicate samples were combined and deprotected. Thus, treatment of **47**, **48**, **49**, and **50** with NH_4/MeOH afforded **51**, **52**, **53**, and **54** respectively. The ratio of 2' (or 3')-deuterio epimers for the deprotected fluoronucleosides was also determined and defined as a ratio of *anti/syn* selectivity of deuterium substitution versus fluorine substituent (Table 2).

Table 2. Ratio of 2' (or 3')-deuterio epimers for deprotected fluoronucleosides

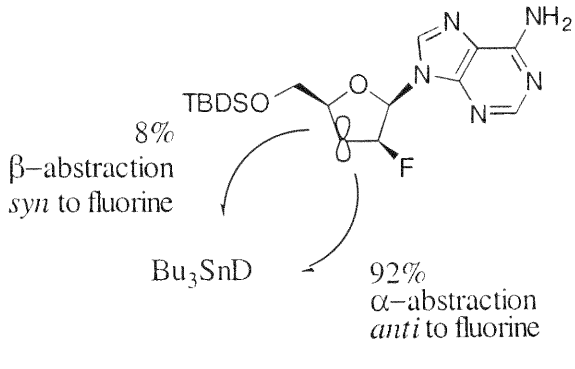
2'(3')-dideoxy-3'(2')-fluoro precursors	Ratio of 2'(3')-deuterio epimers	Diastereotopic excess
51 2'-fluoro-ribo	(3'*R/S*, 64:36)	28 *anti/syn*, 36:64
53 2'-fluoro-arabino	(3'*R/S*, 92:8)	84 *anti/syn*, 92:8
52 3'-fluoro-ribo	(2'*R/S*, 14:86)	72 *anti/syn*, 14:86
54 3'-fluoro-xylo	(2'*R/S*, 7:93)	86 *anti/syn*, 93:7

These results clearly indicate that although steric effect of the heterocyclic base is the most decisive factor in determining stereoselectivity for deuterium abstraction by pentafuranosyl radical, stereochemical position of vicinal fluorine also influences stereoselectivity for deuterium incorporation. Thus, if fluorine has no significant steric effect, stereoselectivity is enhanced for the *anti* delivery to vicinal fluorine substituent (Scheme XVII).

Scheme XVII



43 ⟶ **47**



45 ⟶ **49**

3. EXPERIMENTAL SECTION

3.1. GENERAL PROCEDURE

Melting points were determined with a capillary apparatus and are uncorrected. UV spectra were measured in methanol using a Shimadzu UV-2101 PC scanning spectrometer. ^{1}H (Me_4Si) at 400 MHz, ^{13}C (Me_4Si) at 100 MHz, ^{31}P (H_3PO_4) at 162 MHz and ^{19}F (CCl_3F) at 376.4 MHz NMR spectra were recorded at on a Bruker NMR spectrometer with solutions in $CDCl_3$, unless otherwise specified. Mass spectra were obtained by the atmospheric pressure chemical ionization (APCI) technique using a Finnigan Navigator LC/MS and MeOH as a solvent. Evaporations were effected with Buchi rotary evaporator using water aspirator or mechanical oil pump at < 35 °C. Elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. Merck silica gel 60-F_{254} precoated aluminium sheets were used for thin-layer chromatography (TLC) and products were detected with 254 nm light. Merck silica gel 60 (230-400 mesh) was used for column chromatography. Unless otherwise specified solvents used for column chromatography and TLC were as follows: **(S1)** EtOAc; **(S2)** The upper phase of EtOAc/*i*-PrOH/H_2O (4:1:2); **(S3)** EtOAc/S2 (90:10); **(S4)** EtOAc/S2 (75:25); **(S5)** 30% MeOH in S2; **(S6)** $CHCl_3$/MeOH (95:5); **(S7)** *i*-PrOH/NH_4OH/H_2O (3:1:1). Reagent grade chemicals were used and solvents were dried by reflux over and distillation from CaH_2 under argon except THF (K/benzophenone). Sonification was performed with a Branson 5200 ultrasonic bath. Ion exchange chromatography was performed with Sephadex-DEAE A-25, (40-120μ), Dowex-50W-hydrogen and Dowex-1-chloride foam (2% cross linking, 100-200 mesh) ion exchange resins.

3.2. SYNTHESIS OF 2'-DISULFIDE ANALOGS OF ADENOSINE

3',5'- *O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanediyl)adenosine (2). Procedure A

To 400 mg (1.5 mmol) of dried adenosine **1** suspended in 12 mL of pyridine was added 480 μL (474 mg, 1.5 mmol) of 1,1,3,3-tetraisopropyl-1,3-disiloxanediyl chloride. The mixture was stirred at room temperature for 3 h, pyridine was evaporated and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with ice-cold 5% HCl/H_2O, saturated $NaHCO_3$/H_2O and NaCl/H_2O, dried ($MgSO_4$), filtered and evaporated. The residue was column chromatographed (EtOAc→5% MeOH/EtOAc) to give **2** (694.5 mg, 91%) with spectroscopic and physical properties as reported.[24]

2'-*O*-(Trifluoromethanesulfonyl)-3',5'- *O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine (3). Procedure B

510 mg (1.0 mmol) of **2** and 4-dimethylaminopyridine (DMAP) (367 mg, 3.0 mmol) were suspended in anhydrous CH_2Cl_2 (5 mL) at 0 °C. 428 mg (1.2 mmol) of *N*-phenyltrifluoromethanesulfonimide were added to the cold solution and the reaction mixture was stirred for 20 min. Solvent was evaporated and residue was partitioned (ice-cold AcOH/H_2O 1:9//CH_2Cl_2). Organic phase was washed with ice-cold saturated $NaHCO_3$ and NaCl, dried ($MgSO_4$) and evaporated. Residue was column chromatographed (EtOAc/hexanes; 70:30) to afford **3** (507.3 mg, 79%): ^{1}H NMR δ 1.03-1.26 (m, 28, 4 × *i*-Pr), 4.03 (d, J = 13.3 Hz, 1, H5''), 4.11 (d, J = 9.3 Hz, 1, H4'), 4.20 (d, J = 13.1 Hz, 1, H5'), 5.31 (dd, J = 4.7 Hz, 9.2 Hz, 1, H3'), 5.82 (d, J = 4.7 Hz, 1, H2'),

6.14 (s, 1, H1'), 6.39 (br s, 2, NH_2), 7.98 (s, 1, H2), 8.26 (s, 1, H8) and other data as reported.[44]

9-[2-*S*-Acetyl-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2-thio-β-D-arabinofuranosyl]adenine (4). Procedure C

CH_3COSK (434 mg, 3.8 mmol) was added to a solution of **3** (1.53 g, 2.38 mmol) in dried DMF (10 mL) and the resulting mixture was stirred overnight at ambient temperature. Volatiles were evaporated and the residue was partitioned (EtOAc//$NaHCO_3$/H_2O). The organic layer was washed (NaCl/H_2O), dried ($MgSO_4$), evaporated and the residue was column chromatographed (EtOAc/hexanes; 70:30) to give **4**[52] (1.01 g, 75%): mp 68-76 °C; UV max 260 nm (ε 15 100), min 233 nm (ε 6 200); ^{1}H NMR δ 0.80-1.30 (m, 28, 4 × *i*-Pr), 2.19 (s, 3, Ac), 3.99-4.08 (m, 2, H4',5''), 4.28 (dd, *J* = 4.5 Hz, 12.5 Hz, 1, H5'), 4.59 (dd, *J* = 7.3 Hz, 10.3 Hz, 1, H2'), 5.06 (dd, *J* = 8.2 Hz, 10.0 Hz, 1, H3'), 6.45 (d, *J* = 7.3 Hz, 1, H1'), 7.95 (s, 1, H2), 8.29 (s, 1, H8); MS (APCI) *m/z* 568 (100, MH^+). Anal. calcd for $C_{24}H_{41}N_5O_5Si_2S$ (567.86): C, 50.76; H, 7.28; N, 12.33. Found: C, 50.39; H, 7.49; N, 11.82.

9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2-thio-β-D-arabinofuranosyl]adenine (5). Procedure D

A solution of **4** (241 mg, 0.43 mmol) in saturated NH_3/MeOH (50 ml) at 0 °C was stirred for 30 min at ~0 °C. Volatiles were evaporated, and the residue was column chromatographed ($CHCl_3$/MeOH; 95:5) to give **5** [254 mg, 97%; contaminated (^{1}H NMR) with 0.5 equiv. of CH_3CONH_2]: mp 78-82 °C; UV max 261 nm (ε 14 400), min 230 nm

(ε 3100); ^{1}H NMR δ 1.02-1.19 (m, 28, 4 × *i*-Pr), 1.47 (d, *J* = 8.1 Hz, 1, SH), 3.87 (dd, *J* = 7.6 Hz, 9.7 Hz, 1, H2'), 3.91 (dt, *J* = 8.2 Hz, 2.8 Hz, 1, H4'), 4.07 (dd, *J* = 12.9 Hz, 2.7 Hz, 1, H5'), 4.24 (dd, *J* = 12.9 Hz, 2.8 Hz 1, H5'), 4.62 (t, *J* = 8.9 Hz, 1, H3'), 6.12 (br s, 2, NH_2), 6.42 (d, *J* = 7.1 Hz, 1, H1'), 8.14 (s, 1, H2), 8.35 (s, 1, H8); ^{13}C NMR δ 12.8-17.9 (12, 4 × *i*-Pr), 48.7 (C2'), 61.4 (C5'), 75.7 (C3'), 84.2 (C4'), 85.3 (C1'), 120.1 (C5), 139.7 (C8), 150.2 (C4), 153.2 (C2), 155.9 (C6); MS (APCI) *m/z* 526 (100, MH^+). Anal. calcd for $C_{22}H_{39}N_5O_4Si_2S_2$ (525.54): C, 50.28; H, 7.43; N, 13.33. Found: C, 49.91; H, 7.68; N, 12.81.

9-(2-Thio-β-D-arabinofuranosyl)adenine (6)

Treatment of **4** (283 mg, 0.5 mmol) with TBAF (1 M solution in THF; 1 mL) in THF (15 mL) at 0 °C for 30 min. followed by NH_3/MeOH (10 mL; 2h, ambient temperature) gave crude **6**. This material was partitioned (EtOAc/H_2O) and the water layer was chromatographed [Dowex 1 × 2 (OH^-); H_2O × 25% MeOH/H_2O) to give 9-(2-thio-β-D-arabinofuranosyl)adenine[40] [**6**; 89 mg, 55%; contaminated (^{1}H NMR) by the corresponding dimer-disulfide].

9-[3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2-deoxy-2-propyldithio-β-D-arabinofuranosyl]adenine (7). Procedure E

A solution of **5** (370 mg, 0.7 mmol) and DEAD (113 μL, 0.72 mmol) in THF (7 mL) was stirred overnight at ambient temperature and then propane-1-thiol (3.68 mL, 3.09 g, 41 mmol) was added. Reaction mixture was refluxed for 8 h and volatiles were evaporated. The residue was partitionated (Na_2CO_3/H_2O//EtOAc) and the organic layer

was washed (NaCl), dried ($MgSO_4$), evaporated and column chromatographed (EtOAc/hexane; 1:1) to give **7** (182 mg, 43%): mp 110-118 °C (soften); UV max 261 nm (ε 7600), min 233 nm (ε 1400); ^{1}H NMR δ 0.94 (t, J = 7.4 Hz, 3, CH_3), 1.07-1.17 (m, 28, 4 5 *i*-Pr), 1.62 (sextet, J = 7.4 Hz, 2, CH_2), 2.61 (t, J = 7.4 Hz, 2, SCH_2), 3.91-3.97 (m, 2, H2',4'), 4.05-4.10 (m, 1, H5',5'), 4.60 (t, J = 8.5 Hz, 1, H3'), 6.54 (d, J = 6.8 Hz, 1, H1'), 8.15 (s, 1, H2), 8.33 (s, 1, H8); MS (APCI) *m/z* 600 (100, MH^+).

9-(2-Deoxy-2-propyldithio-β-D-arabinofuranosyl)adenine (8). Procedure F

NH_4F (222 mg, 6.0 mmol) was added to a stirred solution of **7** (180 mg, 0.3 mmol) in MeOH (25 ml). After 18 h, volatiles were removed *in vacuo* and the residue was column chromatographed ($CHCl_3$/MeOH; 95:5) to give **8** (60 mg, 56%): mp 120-124 °C (dec.); UV max 261 nm (ε 14 800), min 231 nm (ε 5100); ^{1}H NMR (MeOH-d_4) δ 0.87 (t, J = 7.3 Hz, 3, CH_3), 1.53 (sextet, J = 7.3 Hz, 2, CH_2), 2.57 (t, J = 7.2 Hz, 2, CH_2S), 3.86-3.95 (m, 4, H2',4',5',5''), 4.53 (t, J = 7.6 Hz, 1, H3'), 6.58 (d, J = 7.1 Hz 1, H1'), 8.21 (s, 1, H2), 8.44 (s, 1, H8); ^{13}C NMR (MeOH-d_4) δ 12.13 (CH_3), 22.03 (CH_2), 40.96 (SCH_2), 60.22 (C3'), 62.54 (C5'), 71.94 (C2'), 84.92 & 85.32 (C4' & C1'), 119.0 (C5), 140.79 (C8), 149.50 (C4), 152.68 (C2), 156.30 (C6); MS (APCI) *m/z* 358 (100, MH^+). Anal. calcd. for $C_{13}H_{19}N_5O_3S_2 \cdot H_2O$ (375.47): C, 41.59; H, 5.64; N, 18.65. Found: C, 42.21; H, 5.65; N, 18.27.

Treatment of **6** (160 mg, 0.56 mmol) with DEAD (98 mg, 0.57 mmol) and propane-1-thiol (2.9 mL, 2.4 g, 32 mmol) in THF (15 mL), as described for **7**, also gave **8** (35 mg, 17%).

9-[2-Deoxy-2-propyldithio-5-*O*-(*p*-toluenesulfonyl)-β-D-arabinofuranosyl]adenine (9)

para-Tolylsulfonyl chloride (106 mg, 0.55 mmol) was added to a solution of **8** (40 mg, 0.11 mmol) in anhydrous pyridine (2 mL) and the reaction mixture was stirred for 3 h at 0 °C. Volatiles were evaporated, and the residue was partitioned ($NaHCO_3/H_2O$//EtOAc). The organic layer was washed ($AcOH/H_2O$, $NaHCO_3/H_2O$), dried ($MgSO_4$). Residue was evaporated and column chromatographed (EtOAc → 2.5% MeOH/EtOAc) to give **9** (28 mg, 52%): ^{1}H NMR δ 0.90 (t, J = 7.2 Hz, 3, CH_3), 1.56 (sextet, J = 7.2 Hz, 2, CH_2), 2.42 (s, 3, $PhCH_3$), 2.59 (m, 2, CH_2S), 3.90 (t, J = 8.2 Hz, 1, H2'), 4.16 (m, 1, H4'), 4.42 (dd, J = 11.2 Hz, 3.1 Hz, 1, H5'), 4.45 (dd, J = 11.3 Hz, 4.5 Hz, 1, H5'), 4.81 (t, J = 8.2 Hz, 1, H3'), 6.48 (d, J = 7.2 Hz 1, H1'), 7.27 (d, J = 8.0 Hz, 2, $H_{arom.}$), 7.87 (d, J = 8.1 Hz, 2, $H_{arom.}$), 7.99 (s, 1, H2), 8.29 (s, 1, H8).

Preparation of Tris(tetra-*n*-butylammonium) hydrogen pyrophosphate

Method A. Tetrasodium pyrophosphate (1.11g, 2.5 mmol) was dissolved in deionised water (10 mL). The dissolved salt was applied to a column of Dowex 50W 2-200 (H^+) ion change resin (2% cross linking, 100-200 mesh, hydrogen form) and eluted with deionised water (~30 mL). Acidic fractions (pH ~1.5) were collected and resulting solution was neutralized (pH ~7.3) by dropwise addition of 40% (wt/wt) solution tetra-*n*-butylammonium hydroxide in H_2O. The neutralized solution was evaporated to yield viscous, clear syrup, which upon high vacuum drying gave a hydroscopic white solid (2.2 g, 98%).[49] ^{1}H NMR (D_2O) δ 0.77 (t, J = 7.3 Hz, 3H), 1.20 (m, 4H), 3.02 (t, J = 8.3 Hz, 2H); ^{31}P NMR (D_2O) δ -6.67 (s).

Note: Tetrasodium pyrophosphate can be substituted by disodium hydrogen pyrophosphate to give tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (97% yield) with identical spectroscopic characteristics.

Method B. A 10%(v/v) solution of disodium hydrogenpyrophosphate (3.33g, 15.0 mmol) in aqueous ammonium hydroxide (15 mL) was passed through a 2.5 cm x 7.0 cm column of Dowex 50WX2-200 ion exchange resin (2% cross linking, 100-200-mesh, hydrogen form). The free acid was eluted with deionised water (100 mL) and the resulting solution (pH ~1.5) was immediately neutralized to pH 7.3 with aqueous tetra-*n*-butylammonium hydroxide. The resulting solution (approximately 150 ml total volume) was dried by lyophilization to yield 13.1 g (97%) of a hydroscopic white solid.

9-(2-Deoxy-2-propyldithio-β-D-arabinofuranosyl)adenine 5'-diphosphate (10)

Tris(tetra-n-butylammonium) hydrogen pyrophosphate (31 mg, 0.035 mmol) was added to a stirred solution of **9** (12 mg, 0.023 mmol) in CH_3CN (0.5 mL) and stirring was continued at ambient temperature for 72 h. Volatiles were removed in vacuo and the residue was dissolved in water and was purified on an ion exchange resin [Sephadex-DEAE A-25, 40-120μ; gradient elution with triethylammonium bicarbonate (0.05 $\rightarrow$ 0.5 M)]. Appropriate fractions were evaporated, and the residue was dissolved in H_2O and was converted to the sodium salt by passing the solution through a Dowex (Na^+) column to yield **10** (4 mg, 29%) as trisodium salt: ^{1}H NMR (MeOH-d_4) δ 0.64 (t, J = 6.3 Hz, 3, CH_3), 1.27 (sextet, J = 6.5 Hz, 2, CH_2), 2.32 (t, J = 6.7 Hz, 2, CH_2S), 3.88 (dd, J = 7.4 Hz, 8.8 Hz, 1, H2'), 4.03-4.07 (m, 1, H4'), 4.19-4.23 (m, 2, H5',5''), 4.49 (t, J = 8.3 Hz, 1,

H3'), 6.84 (d, J = 7.1 Hz 1, H1'), 8.11 (s, 1, H2), 8.33 (s, 1, H8); ^{31}P NMR δ -9.75 (d, J = 23.5 Hz, 1, P_α), -5.26 (d, J = 23.5 Hz, 1, P_β).

Treatment of **10** (2 mg) with dithiothreoitol (2 mg) in H_2O (2 mL) gave **11** whose structure was confirmed by MS (odor of PrSH released was immediately noticed).

9-[2-*S*-(2-Trimethylsilylethyl)-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-2-thio-β-D-arabinofuranosyl]adenine (12)

2-(Trimethylsilyl)ethanethiol (0.1 mL, 90 mg, 0.7 mmol) was added to a stirred suspension of **3** (371 mg, 0.6 mmol) and K_2CO_3 (276 mg, 2 mmol) in DMF (3 mL) at ambient temperature, and the reaction mixture was heated at 60 °C overnight. Volatiles were evaporated and the residue was partitioned [$CHCl_3$//$NaHCO_3$/H_2O (ice-cold)]. The organic layer was washed (NaCl/H_2O), dried ($MgSO_4$), evaporated and column chromatographed (EtOAc/hexanes; 1:1) to give **12** (108 mg, 30%): ^{1}H NMR δ -0.07 (s, 9H, $SiMe_3$), 0.57 (dt, J = 5.2 Hz, 14.0 Hz, 1, CHH_ASi), 0.61 (dt, J = 14.0 Hz, 5.2 Hz, 1, CH_BHSi), 0.80-1.31 (m, 28, 4 × i-Pr), 2.49 (dt, J = 5.6 Hz, 12.7 Hz, 1, SCHH_A), 2.53 (dt, J = 5.6 Hz, 12.7 Hz, 1, SCH_BH), 3.72 (dd, J = 7.0 Hz, 9.7 Hz 1, H2'), 3.89 (dt, J = 7.9 Hz, 3.0 Hz, 1, H4'), 4.09 (dd, J = 2.8 Hz, 12.9 Hz, 1, H5''), 4.16 (dd, J = 3.2 Hz, 12.9 Hz, 1 H5'), 4.55 (dd, J = 8.3 Hz, 9.3 Hz, 1, H3'), 6.50 (d, J = 7.0 Hz, 1, H1'), 8.08 (s, 1, H2), 8.32 (s, 1, H8); MS (APCI) m/z 626 (100, MH^+). Anal. calcd for $C_{13}H_{19}N_5O_3SSi_2 \times H_2O$ (644.07): C, 50.35; H, 8.29; N, 10.87. Found: C, 50.39; H, 8.11; N, 10.49.

Treatment (ambient temperature to ~45 °C, 2h to overnight) of **12** (97 mg, 0.16 mmol) [or **13** (27 mg, 0.07 mmol)] with dimethyl(methylthio)sulfonium tetrafluoroborate (219 mg, 1.12 mmol) and dimethyl sulfide (0.72 mL, 496 mg, 8 mmol) in anhydrous THF

(5 mL) gave recovered **12** or **13** (~20% to 80% depends on reaction conditions) plus other byproduct(s).

9-[2-*S*-(2-Trimethylsilylethyl)-2-thio-β-D-arabinofuranosyl]adenine (13)

TBAF/THF (1M; 0.3 mL, 0.3 mmol) was added to a solution of **12** (50 mg, 0.08 mmol) in THF (2 mL) and was stirred for 2h at ambient temperature. Volatiles were evaporated and the residue was column chromatographed (EtOAc → 8% MeOH/EtOAc) to give **13** (27 mg, 90%): ^{1}H NMR δ -0.08 (s, 9H, $SiMe_3$), 0.59-0.63 (m, 2, $SiCH_2$), 2.40-2.45 (m, 2, SCH_2), 3.74 (dd, *J* = 6.9 Hz, 8.8 Hz 1, H2'), 3.80-4.01 (m, H4',5',5''), 4.32-4.38 (m, 1, H3'), 6.57 (d, *J* = 6.9 Hz, 1, H1'), 8.20 (s, 1, H2), 8.41 (s, 1, H8); MS (APCI) *m/z* 384 (100, MH^+).

9-[3,5-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanyl)-2-deoxy-2-methyldithio-β-D-arabinofuranosyl]adenine (14)

A solution of **4** (100 mg, 0.19 mmol) and DEAD (29.1 μL, 0.19 mmol) in THF (5 mL) was stirred overnight at ambient temperature and then methanethiol (183 mg, 3.8 mmol) in THF (3 mL) was added. Stirring was continued in a pressure Ace glass at ambient temperature for 96 h, and then volatiles were evaporated and the residue was column chromatographed (EtOAc/hexane; 1:1) to give **14** (40.6 mg, 37%): ^{1}H NMR δ 1.04-1.14 (m, 28, 4 × *i*-Pr), 2.32 (s, 3, SCH_3), 3.92-3.99 (m, 2, H2',4'), 4.05-4.21 (m, 2, H5',5''), 4.76 (t, *J* = 7.8 Hz, 1, H3'), 6.07 (brs, 2, NH_2), 6.53 (d, 1, *J* = 7.1 Hz, 1, H1'), 8.01 (s, 1, H2), 8.33 (s, 1, H8); MS (APCI) *m/z* 572 (100, MH^+).

9-(2-Deoxy-2-methyldithio-β-D-arabinofuranosyl)adenine (15)

Treatment of **14** (19 mg, 0.033 mmol) by procedure **F** gave **15** (7.3 mg, 67%): UV max 261 nm (ε 12 700), min 230 nm (ε 2800); ^{1}H NMR (MeOH-d_4) δ 2.33 (s, 3, CH_3S), 3.87-4.00 (m, 4, H2',4',5',5''), 4.57 (t, J = 7.7 Hz, 1, H3'), 6.57 (d, J = 7.0 Hz 1, H1'), 8.20 (s, 1, H2), 8.39 (s, 1, H8); MS (APCI) *m/z* 330 (100, MH^+); Anal. calcd. for $C_{11}H_{15}N_5O_3 \cdot 0.5$ EtOAc (373.45): C, 41.85; H, 5.13; N, 18.75. Found: C, 41.47; H, 5.38; N, 18.26.

9-[3,5- *O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanediyl)-β-D-arabinofuranosyl]adenine (17)

Treatment of 9-(β-D-arabinofuranosyl)adenine **16** (267 mg, 1.0 mmol) by procedure A afforded **17**[44] (380 mg, 77%): ^{1}H NMR δ 0.9-1.3 (m, 28, 4 × *i*-Pr), 3.85 (dt, J = 7.7 Hz, 2.6 Hz, 1, H4'), 4.02-4.13 (m, 2, H5',5''), 4.54 (t, J = 7.7 Hz, 1, H3'), 4.65 (t, J = 7.0 Hz, 1, H2'), 6.23 (d, J = 6.0 Hz, 1, H1'), 8.09 (s, 1, H2), 8.14 (s, 1, H8).

9-[2-*O*-(Trifluoromethanesulfonyl)-3,5- *O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-β-D-arabinofuranosyl]adenine (18)

Treatment of **17** (380 mg, 0.76 mmol) by procedure **B** gave **18**[44] (360.6 mg, 75%); ^{1}H NMR δ 0.99-1.23 (m, 28, 4 × *i*-Pr), 3.95-3.99 (m, 1, H4'), 4.09 (dd, J =12.36 Hz, 3.13 Hz, 1, H5''), 4.24 (dd, J = 12.31 Hz, 6.03 Hz, 1, H5'), 5.40 (t, J = 6.9 Hz, 1, H3'), 5.49 (t, J = 6.4 Hz, 1, H2'), 6.42 (d, J = 6.1 Hz, 1, H1'), 7.95 (s, 1, H2), 8.30 (s, 1, H8).

2'-*S*-Acetyl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2'-thioadenosine (19)

Treatment of **18** (290 mg, 0.45 mmol) by procedure **C** gave **19** (200 mg, 78%): ^{1}H NMR δ 1.05-1.12 (m, 28, 4 × *i*-Pr), 2.31 (s, 3, Ac), 4.02-4.09 (m, 3, H4',5',5''), 4.70 (dd, *J* = 7.4 Hz, 4.8 Hz, 1, H2'), 5.26 (dd, *J* = 7.2 Hz, 5.4 Hz, 1, H3'), 6.07 (d, *J* = 4.8 Hz, 1, H1'), 7.97 (s, 1, H2), 8.29 (s, 1, H8); MS (APCI) *m/z* 568 (100, MH^+).

3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2'-thioadenosine (20)

Treatment of **19** (180 mg, 0.31 mmol) by procedure **D** gave **20** (160 mg, 96%): ^{1}H NMR δ 1.03-1.15 (m, 28, 4 × *i*-Pr), 2.19 (d, *J* = 6.4 Hz, 1, SH), 4.08 (d, *J* = 4.7 Hz, 1, H2'), 4.17-4.25 (m, 3, H4',5',5''), 4.88 (t, *J* = 6.4 Hz, 1, H3'), 5.98 (d, *J* = 7.1 Hz, 1, H1'), 6.22 (br s, 2, NH_2), 8.01 (s, 1, H2), 8.34 (s, 1, H8); MS (APCI) *m/z* 526 (100, MH^+).

3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanyl)-2'-deoxy-2'-propyldithioadenosine (21)

Treatment of **20** (140 mg, 0.27 mmol) by procedure **E** gave **21** (67 mg, 42%): ^{1}H NMR δ 0.89 (t, *J* = 7.3 Hz, 3, CH_3), 1.07-1.12 (m, 28, 4 × *i*-Pr), 1.59 (sextet, *J* = 7.2 Hz, 2, CH_2), 2.56 (t, *J* = 7.4 Hz, 2, SCH_2), 4.05-4.07 (m, 2, H2',4'), 4.19-4.26 (m, 2, H5',5''), 5.20 (t, *J* = 7.3 Hz, 1, H3'), 6.07 (br s, 2, NH_2), 6.34 (d, *J* = 3.5 Hz, 1, H1'), 8.03 (s, 1, H2), 8.29 (s, 1, H8); MS (APCI) *m/z* 600 (100, MH^+).

2'-Deoxy-2'-propyldithioadenosine (22)

Treatment of **21** (60 mg, 0.1 mmol) by procedure **F** gave **22** (12.4 mg, 35%): UV max 260 nm (ε 14 200), min 229 nm (ε 2 600); ^{1}H NMR (MeOH-d_4) δ 0.74 (t, *J* = 7.3 Hz,

3, CH_3), 1.36 (sextet, J = 7.05 Hz, 2, CH_2), 2.18-2.24 (m, 2, CH_2S), 3.80-3.89 (m, 2, H5',5''), 4.19-4.25 (m, 1, H4'), 4.27-4.31 (m, 1, H2'), 4.55 (d, J = 7.0 Hz, 1, H3'), 6.20 (d, J = 9.2 Hz 1, H1'), 8.22 (s, 1, H2), 8.35 (s, 1, H8); MS (APCI) *m/z* 358 (100, MH^+).

3.3. SYNTHESIS OF FLUORO-CONTAINING COMPOUNDS

Tritylation of Adenosine

A mixture of dried adenosine (**1**, 5 g, 18.7 mmol), DMAP (1.92 g, 15.7 mmol), and TrCl (17.5 g, 62.6 mmol) in 150 mL of pyridine was heated at 80 °C. An additional amount of TrCl (5 g) was added after 18 h. The reaction was quenched with EtOH (50 mL) after 40 h. Solvent was evaporated, and the residue was partitioned ($NaHCO_3/H_2O$//EtOAc). The organic layer was washed ($NaCl/H_2O$), dried ($MgSO_4$), filtered, and evaporated. This crude material was chromatographed on a silica gel column. Gradient elution (15% EtOAc/Hexanes → 30% EtOAc/Hexanes) and evaporation of the appropriate fractions gave compounds $O^{3'}$, $O^{5'}$, N^6-Tritrityladenosine (**23**) and $O^{2'}$, $O^{5'}$, N^6-Tritrityladenosine (**24**).

Compound **23** (3.6 g, 19%), mp 152-155 °C, was eluted first: ^{1}H NMR δ 2.59 (dd, $J_{4'-5''}$ = 6.1 Hz, $J_{5'-5''}$ = 10.9 Hz, 1, H5''), 3.25 (dd, $J_{4'-5'}$ = 1.8 Hz, 1, H5'), 3.33-3.39 (m, 1, H4'), 4.25-4.27 (m, 1, H3'), 4.62-4.71 (m, 1, H2'), 6.15 (d, $J_{1'-2'}$ = 6.2 Hz, 1, H1'), 7.02-7.41 (m, 45, 3 × Tr), 8.05 (s, 1, H2), 8.09 (s, 1, H8) and other data as reported.[50]

Compound **24** (4.1 g, 22%), mp 152-154 °C, was eluted next: ^{1}H NMR δ 2.87-2.88 (m, 1, H4'), 3.03 (dd, $J_{4'-5''}$ = 4.4, $J_{5'-5''}$ = 10.4 Hz, 1, H5''), 3.30 (dd, $J_{4'-5'}$ = 3.4 Hz, 1,

H5'), 3.99-4.01 (m, 1, H3'), 5.15-5.18 (m, 1, H2'), 6.37 (d, $J_{1'-2'}$ = 7.4 Hz, 1, H1'), 7.08-7.44 (m, 45, 3 × Tr), 7.90 (s, 1, H2), 7.99 (s, 1, H8) and other data as reported.[50]

2'-*O*-Triflyl-3',5'-di-*O*-trityl-N^6-trityladenosine (25). Procedure G

To a mixture of **23** (1.4 g, 1.41 mmol), DMAP (169 mg, 1.41 mmol), and Et_3N (392 μL, 2.8 mmol) in CH_2Cl_2 (12 mL) was added dropwise a solution of CF_3SO_2Cl (300 μL). The reaction mixture was stirred at room temperature for 30 min. The mixture was concentrated *in vacuo*, and the residue was partitioned (H_2O//$CHCl_3$). The organic layer was washed (NaCl/H_2O), dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on a column of silica gel with $CHCl_3$ to give **25**[50] (1.4 g, 90%) as a foam: ^{1}H NMR δ 2.92 (dd, $J_{4'-5''}$ = 3.7 Hz, $J_{5'-5''}$ = 11.1 Hz, 1, H5''), 3.33 (d, 1, H5'), 3.68-3.72 (m, 1, H4'), 4.64-4.66 (m, 1, H3'), 5.92 ("t", $J_{2'-1'/3'}$ = 5.5 Hz, 1, H2'), 6.73 (d, $J_{1'-2'}$ = 6.4 Hz, 1, H1'), 7.28-7.48 (m, 45, 3 × Tr), 8.05 (s, 1, H2), 8.1 (s, 1, H8).

3'-*O*-Triflyl-2',5'-di-*O*-trityl-N^6-trityladenosine (26)

Treatment of **24** (1.5 g, 1.51 mmol) by procedure **G** gave **26**[53] (1.5 g, 88%): ^{1}H NMR δ 3.17 (dd, $J_{4'-5''}$ = 4.9 Hz, $J_{5'-5''}$ = 10.2 Hz, 1, H5''), 3.35 (dd, $J_{4'-5'}$ = 7.6 Hz, 1, H5'), 4.32-4.35 (m, 2, H3',4'), 5.97 (dd, $J_{2'-3'}$ = 4.2 Hz, $J_{1'-2'}$ = 7.9 Hz, 1, H2'), 6.14 (d, $J_{1'-2'}$ = 7.9 Hz, 1, H1'), 7.22-7.37 (m, 45, 3 × Tr), 7.56 (s, 1, H2), 7.83 (s, 1, H8).

9-(3,5-Di-*O*-trityl-β-D-arabinofuranosyl)-N^6-trityladenine (29). Procedure H

A mixture of **25** (1 g, 0.89 mmol) and AcONa (1 g, dried at 60 °C under vacuum) in 20 mL of DMF was stirred for 3 days at room temperature. Solvent was evaporated and the residue was partitioned between H_2O and EtOAc. Organic layer was washed with H_2O twice, dried ($MgSO_4$), filtered, and evaporated. Residue was suspended in MeOH/NH_3 (50 mL), stirred 24 h, and concentrated *in vacuo*. The resulting solid was column chromatographed on silica gel with $CHCl_3$ to give **29**[50] (644 mg, 73%): ^{1}H NMR δ 2.96 (dd, $J_{4'-5''}$ = 3.5 Hz, $J_{5'-5''}$ = 10.5 Hz, 1, H5''), 3.42 (dd, $J_{4'-5'}$ = 1.9 Hz, 1, H5'), 3.61-3.66 (m, 1, H2'), 3.94-3.98 (m, 1, H4'), 4.07 (s, 1, H3'), 6.31 (d, $J_{1'-2'}$ = 2.3 Hz, 1, H1'), 7.01 (s, 1, NH), 7.19-7.39 (m, 45, 3 × Tr), 8.05 (s, 1, H2), 8.35 (s, 1, H8).

9-(2,5-Di-*O*-trityl-β-D-xylofuranosyl)-N^6-trityladenine (30)

Treatment of **26** (1 g, 0.89 mmol) by procedure **H** gave **30**[53,54] (660 mg, 75%): ^{1}H NMR δ 3.47 (dd, $J_{4'-5''}$ = 3.6 Hz, $J_{5'-5''}$ = 10.7 Hz, 1, H5''), 3.57 (dd, $J_{4'-5'}$ = 7.5 Hz, 1, H5'), 3.95-4.02 (m, 1, H3'), 4.29-4.36 (m, 1, H4'), 4.58 (s, 1, H2'), 5.47 (s, 1, H1'), 6.96-7.43 (m, 47, H2, NH, 3 × Tr), 8.01 (s, 1, H8).

2'-Deoxy-2'-fluoro-3',5'-di-*O*-trityl-N^6-trityladenosine (31). Procedure I

To an ice-cold solution of **29** (550 mg, 0.6 mmol) and pyridine (0.6 mL, 6 mmol) in CH_2Cl_2 (7.5 mL) were added 0.38 mL of DAST (443.3 mg, 3 mmol). Ice was removed after 5 min. Mixture of reaction was stirred overnight at room temperature, then diluted with of CH_2Cl_2 (5.5 mL) and quenched by addition of 5% $NaHCO_3$ (4 mL). The organic

layer was separated, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$ to afford compound **31**[50] (260 mg, 47%): ^{1}H NMR δ 2.89 (dd, $J_{4'\text{-}5''}$ = 5.2 Hz, $J_{5'\text{-}5''}$ = 10.9 Hz, 1, H5''), 3.30 (d, 1, H5'), 3.92-3.95 (m, 1, H4'), 4.46 ("dt", $J_{3'\text{-}2'/4'}$ = 5.0 Hz, $J_{3'\text{-}F}$ = 13.1 Hz, 1, H3'), 4.85 (dt, $J_{2'\text{-}1'/3'}$ = 4.0 Hz, $J_{2'\text{-}F}$ = 51.6 Hz, 1, H2'), 6.27 (dd, $J_{1'\text{-}2'}$ = 3.5 Hz, $J_{1'\text{-}F}$ = 14.7 Hz, 1, H1'), 6.98 (s, 1, NH), 7.12-7.39 (m, 45, 3 × Tr), 7.80 (s, 1, H2), 7.96 (s, 1, H8); ^{19}F NMR δ –201.8 (dt, $J_{1'/3'\text{-}F}$ = 15 Hz, $J_{2'\text{-}F}$ = 50 Hz).

3'-Deoxy-3'-fluoro-2',5'-di-*O*-trityl-N^6-tritylad enosine (32)

Treatment of **30** (650 mg, 0.65 mmol) by procedure **I** gave compound **32**[53,54] (550 mg, 84%): ^{1}H NMR δ 3.05 (dd, $J_{4'\text{-}5''}$ = 3.5 Hz, $J_{5'\text{-}5''}$ = 10.4 Hz, 1, H5''), 3.33 (dd, $J_{4'\text{-}5'}$ = 4.6 Hz, $J_{5'\text{-}5''}$ = 10.5 Hz, 1, H5'), 3.60 (dd, $J_{3'\text{-}2'}$ = 3.8 Hz, $J_{3'\text{-}F}$ = 53.1 Hz, 1, H3'), 4.24 (dt, $J_{4'\text{-}5'/5''}$ = 3.7 Hz, $J_{4'\text{-}F}$ = 35.0 Hz, 1, H4'), 4.85 (ddd, $J_{2'\text{-}1'}$ = 7.6 Hz, $J_{2'\text{-}3'}$ = 3.8 Hz, $J_{2'\text{-}F}$ = 21.6 Hz, 1, H2'), 6.27 (d, $J_{1'\text{-}2'}$ = 7.7 Hz, 1, H1'), 7.08-7.44 (m, 46, NH, 3 × Tr), 7.81 (s, 1, H2), 7.89 (s, 1, H8); ^{19}F NMR δ –192.55 (dt, $J_{2'/4'\text{-}F}$ = 27 Hz, $J_{3'\text{-}F}$ = 52 Hz).

9-(2-Deoxy-2-fluoro-3,5-di-*O*-trityl-β-D-arabinofuranosyl)-N^6-tritylad enine (33)

Treatment of **23** (1 g, 1.01 mmol) by procedure **I** gave compound **33**[50] (309.7 mg, 31%): ^{1}H NMR δ 3.28 (dd, $J_{4'\text{-}5''}$ = 2.7 Hz, $J_{5'\text{-}5''}$ = 10.0 Hz, 1, H5''), 3.34 (dd, $J_{4'\text{-}5'}$ = 7.6 Hz, $J_{5'\text{-}5'}$ = 9.9 Hz, 1, H5'), 3.73 (dd, $J_{2'\text{-}1'}$ = 2.2 Hz, $J_{2'\text{-}F}$ = 50.1 Hz, 1, H2'), 4.31 (dd, $J_{3'\text{-}4'}$ = 2.5 Hz, $J_{3'\text{-}F}$ = 16.1 Hz, 1, H3'), 4.60 (dt, $J_{4'\text{-}3'/5''}$ = 2.7 Hz, $J_{4'\text{-}5'}$ = 7.1 Hz, 1, H4'), 6.45 (dd, $J_{1'\text{-}2'}$ = 2.2 Hz, $J_{1'\text{-}F}$ = 24.7 Hz, 1, H1'), 7.03 (s, 1, NH), 7.24-7.46 (m, 45, 3 × Tr), 7.89 (s,

1, H2), 8.09 (s, 1, H8); ^{19}F NMR δ –195.74 (ddd, $J_{1'\text{-F}}$ = 16 Hz, $J_{3'\text{-F}}$ = 23 Hz, $J_{2'\text{-F}}$ = 50 Hz).

9-(3-Deoxy-3-fluoro-2,5-di-*O*-trityl-β-D-xylofuranosyl)-N^6-trityladenine (34)

Treatment of **24** (1 g, 1.01 mmol) by procedure **I** gave compound **34** (505.2 mg, 51%): ^{1}H NMR δ 3.33 (dd, $J_{4'\text{-}5''}$ = 6.3 Hz, $J_{5'\text{-}5''}$ = 9.7 Hz, 1, H5''), 3.53 (dd, $J_{4'\text{-}5'}$ = 6.7 Hz, $J_{5'\text{-}5''}$ = 9.0 Hz, 1, H5'), 3.76 (dd, $J_{3'\text{-}4'}$ = 1.7 Hz, $J_{3'\text{-F}}$ = 50.2 Hz, 1, H3'), 4.27 (dtd, $J_{4'\text{-}3'}$ = 1.7 Hz, $J_{4'\text{-}5'/5''}$ = 6.3 Hz, $J_{4'\text{-F}}$ = 31.2 Hz, 1, H4'), 4.44 (dd, $J_{2'\text{-}1'}$ = 1.1 Hz, $J_{2'\text{-F}}$ = 14.1 Hz, 1, H2'), 6.53 (d, $J_{1'\text{-}2'}$ = 1.1 Hz, 1, H1'), 6.99 (s, 1, NH), 7.21-7.45 (m, 45, 3 × Tr), 7.69 (s, 1, H2), 8.18 (s, 1, H8); ^{19}F NMR δ –200.02 (ddd, $J_{2'\text{-F}}$ = 14 Hz, $J_{4'\text{-F}}$ = 31 Hz, $J_{3'\text{-F}}$ = 50 Hz).

2'-Deoxy-2'-fluoroadenosine (35). Procedure J

Compound **31** (250 mg, 0.25 mmol) was dissolved in a mixture (7 mL) of CF_3COOH-$CHCl_3$ (1:9, v/v) and kept at room temperature for 24 h, then partitioned with H_2O. The aqueous layer was first concentrated, then applied to a column of Dowex 1x2-200 (OH^-) ion-exchange resin and eluted with 30% MeOH/H_2O. Evaporation of the appropriated fraction gave compound **35**[50] (45.2 mg, 49%): ^{1}H NMR (MeOH-d_4) δ 3.79 (dd, $J_{4'\text{-}5''}$ = 3.0 Hz, $J_{5'\text{-}5''}$ = 12.6 Hz, 1, H5''), 3.96 (dd, $J_{4'\text{-}5'}$ = 2.0 Hz, $J_{5'\text{-}5''}$ = 12.6 Hz, 1, H5'), 4.45-4.82 (m, 1, H4'), 4.64 (ddd, $J_{3'\text{-}2'}$ = 4.7 Hz, $J_{3'\text{-}4'}$ = 5.7 Hz, $J_{3'\text{-F}}$ = 15.9 Hz, 1, H3'), 5.45 (ddd, $J_{2'\text{-}1'}$ = 3.5 Hz, $J_{2'\text{-}3'}$ = 4.3 Hz, $J_{2'\text{-F}}$ = 52.7 Hz, 1, H2'), 6.32 (dd, $J_{1'\text{-}2'}$ = 3.3 Hz, $J_{1'\text{-F}}$ = 15.7 Hz, 1, H1'), 8.22 (s, 1, H2), 8.41 (s, 1, H8); ^{19}F NMR δ –208.41 (dt, $J_{1'/3'\text{-F}}$ = 15 Hz, $J_{2'\text{-F}}$ = 53 Hz).

3'-Deoxy-3'-fluoroadenosine (36)

Treatment of **32** (540 mg, 0.54 mmol) by procedure **J** gave compound **36**[55] (145.9 mg, 86%): ^{1}H NMR (MeOH-d_4) δ 3.81 (dd, $J_{4'\text{-}5''}$ = 2.1 Hz, $J_{5'\text{-}5''}$ = 12.7 Hz, 1, H5''), 3.88 (dd, $J_{4'\text{-}5'}$ = 2.1 Hz, $J_{5'\text{-}5''}$ = 12.7 Hz, 1, H5'), 4.46 (dt, $J_{4'\text{-}5'/5''}$ = 2.1 Hz, $J_{4'\text{-}F}$ = 27.7 Hz, 1, H4'), 4.99 (ddd, $J_{2'\text{-}1'}$ = 8.0 Hz, $J_{2'\text{-}3'}$ = 4.3 Hz, $J_{2'\text{-}F}$ = 25.2 Hz, 1, H2'), 5.13 (dd, $J_{3'\text{-}2'}$ = 4.2 Hz, $J_{3'\text{-}F}$ = 54.5 Hz, 1, H3'), 6.02 (d, $J_{1'\text{-}2'}$ = 8.0 Hz, 1, H1'), 8.2 (s, 1, H2), 8.3 (s, 1, H8); ^{19}F NMR δ –200.39 (dt, $J_{2'/4'\text{-}F}$ = 27 Hz, $J_{3'\text{-}F}$ = 54 Hz).

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (37)

Treatment of **33** (280 mg, 0.28 mmol) by procedure **J** gave compound **37** (50.3 mg, 66%): ^{1}H NMR (MeOH-d_4) δ 3.81 (dd, $J_{4'\text{-}5''}$ = 7.1 Hz, $J_{5'\text{-}5''}$ = 12.2 Hz, 1, H5''), 3.83 (dd, $J_{4'\text{-}5'}$ = 4.0 Hz, $J_{5'\text{-}5''}$ = 12.2 Hz, 1, H5'), 4.02 ("q", $J_{4'\text{-}3'/5'/5''}$ = 4.7 Hz, 1, H4'), 4.54 (ddd, $J_{3'\text{-}2'}$ = 3.1 Hz, $J_{3'\text{-}4'}$ = 4.6 Hz, $J_{3'\text{-}F}$ = 18.1 Hz, 1, H3'), 5.15 (ddd, $J_{2'\text{-}1'}$ = 4.0 Hz, $J_{2'\text{-}3'}$ = 3.1 Hz, $J_{2'\text{-}F}$ = 52.2 Hz, 1, H2'), 6.49 (dd, $J_{1'\text{-}2'}$ = 4.1 Hz, $J_{1'\text{-}F}$ = 16.0 Hz, 1, H1'), 8.23 (s, 1, H2), 8.36 (s, 1, H8); ^{19}F NMR δ –200.01 (dt, $J_{1'/3'\text{-}F}$ = 18 Hz, $J_{2'\text{-}F}$ = 53 Hz) and other data as reported.[50,56]

9-(3-Deoxy-3-fluoro-β-D-xylofuranosyl)adenine (38)

Treatment of **34** (480 mg, 0.48 mmol) by procedure **J** gave compound **38**[57] (50.1 mg, 39%): ^{1}H NMR (MeOH-d_4) δ 3.96 (dd, $J_{4'\text{-}5''}$ = 6.1 Hz, $J_{5'\text{-}5''}$ = 11.7 Hz, 1, H5''), 3.98 (dd, $J_{4'\text{-}5'}$ = 5.6 Hz, $J_{5'\text{-}5''}$ = 11.7 Hz, 1, H5'), 4.49 (dtd, $J_{4'\text{-}3'}$ = 3.2 Hz, $J_{4'\text{-}5'/5''}$ = 5.8 Hz, $J_{4'\text{-}F}$ = 27.9 Hz, 1, H4'), 4.73 (dt, $J_{2'\text{-}1'/3'}$ = 1.7 Hz, $J_{2'\text{-}F}$ = 13.7 Hz, 1, H2'), 5.12 (ddd, $J_{3'\text{-}2'}$ = 1.8

Hz, $J_{3'-4'}$ = 2.8 Hz , $J_{3'-F}$ = 51.7 Hz, 1, H3'), 6.10 (d, $J_{1'-2'}$ = 1.7 Hz, 1, H1'), 8.18 (s, 1, H2), 8.23 (s, 1, H8); ^{19}F NMR δ –204.12 (ddd, $J_{2'-F}$ = 14 Hz, $J_{4'-F}$ = 28 Hz, $J_{3'-F}$ = 52 Hz).

5'-*O*-(*tert*-Butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine (39). Procedure K

TBDMSCl (63 mg, 0.44 mmol) and imidazole (43 mg, 0.66 mmol) were added to **35** (59 mg, 0.22 mmol) in dried DMF (3.0 ml) and the solution was stirred overnight at ambient temperature. H_2O (1.0 ml) was added, volatiles were evaporated, and the residue was partitioned (EtOAc//NH_4Cl/H_2O). The organic layer was washed (brine), dried (Na_2SO_4), evaporated and chromatographed (5 → 10% MeOH/$CHCl_3$) to give **39** (39 mg, 46%): ^{1}H NMR δ 0.14 (s, 6, $(CH_3)_2Si$), 0.93 (s, 9, $(CH_3)_3CSi$), 3.93 (dd, $J_{4'-5''}$ = 2.5 Hz, $J_{5'-5''}$ = 11.7 Hz, 1, H5''), 4.09 (dd, $J_{4'-5'}$ = 2.4 Hz, $J_{5'-5''}$ = 11.7 Hz, 1, H5'), 4.19-4.25 (m, 1, H4'), 4.72 (ddd, $J_{3'-2'}$ = 4.4 Hz, $J_{3'-4'}$ = 6.5 Hz, $J_{3'-F}$ = 17.5 Hz, 1, H3'), 5.45 (ddd, $J_{2'-1'}$ = 2.2 Hz, $J_{2'-3'}$ = 4.2 Hz, $J_{2'-F}$ = 52.9 Hz, 1, H2'), 6.02 (dd, $J_{1'-2'}$ = 2.2 Hz, $J_{1'-F}$ = 15 Hz, 1, H1'), 8.23 (s, 1, H2), 8.38 (s, 1, H8); ^{19}F NMR δ –204.33 (dt, $J_{1'/3'-F}$ = 16 Hz, $J_{2'-F}$ = 53 Hz); MS (CI) *m/z* 384 (100, MH^+).

5'-*O*-(*tert*-Butyldimethylsilyl)-3'-deoxy-3'-fluoroadenosine (40)

Treatment of **36** (80 mg, 0.3 mmol) by procedure **K** gave compound **40** (56.5 mg, 50%): ^{1}H NMR δ -0.02 (s, 3, CH_3Si), 0.04 (s, 3, CH_3Si), 0.79 (s, 9, $(CH_3)_3CSi$), 3.85 (m, 2, H5',5''), 4.58 (dt, $J_{4'-5'/5'}$ = 2.8 Hz, $J_{4'-F}$ = 26.4 Hz, 1, H4'), 4.74 (ddd, $J_{2'-1'}$ = 7.0, $J_{2'-3'}$ = 4.5 Hz, $J_{2'-F}$ = 24.7 Hz, 1, H2'), 5.19 (dd, $J_{3'-2'}$ = 4.4 Hz, $J_{3'-F}$ = 54.5 Hz, 1, H3'), 6.02 (d, $J_{1'-}$

$_{2'}$ = 7.2 Hz, 1, H1'), 8.08 (s, 1, H2), 8.33 (s, 1, H8); ^{19}F NMR δ –199.45 (dt, $J_{2'/4'\text{-}F}$ = 26 Hz, $J_{3'\text{-}F}$ = 54 Hz); MS (CI) *m/z* 384 (100, MH^+).

9-[5-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]adenine (41)

Treatment of **37** (40 mg, 0.15 mmol) by procedure **K** gave compound **41**[56] (36.5 mg, 64%): ^{1}H NMR δ 0.14 (s, 6, $(CH_3)_2Si$), 0.95 (s, 9, $(CH_3)_3CSi$), 3.88 (dd, $J_{4'\text{-}5''}$ = 6.1 Hz, $J_{5'\text{-}5''}$ = 10.8 Hz, 1, H5''), 3.95 (dd, $J_{4'\text{-}5'}$ = 3.5 Hz, $J_{5'\text{-}5''}$ = 10.8 Hz, 1, H5'), 4.02 ("q", $J_{4'\text{-}3'/5'/5''}$ = 4.4 Hz, 1, H4'), 4.69 (dt, $J_{3'\text{-}2'/4'}$ = 3.2 Hz, $J_{3'\text{-}F}$ = 17.6 Hz, 1, H3'), 5.15 (dt, $J_{2'\text{-}1'/3'}$ = 3.6 Hz, $J_{2'\text{-}F}$ = 51.6 Hz, 1, H2'), 6.55 (dd, $J_{1'\text{-}2'}$ = 3.7 Hz, $J_{1'\text{-}F}$ = 18.0 Hz, 1, H1'), 8.13 (s, 1, H2), 8.37 (s, 1, H8); ^{19}F NMR δ –198.02 (dt, $J_{1'/3'\text{-}F}$ = 17 Hz, $J_{2'\text{-}F}$ = 51 Hz).

9-[5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-fluoro-β-D-xylofuranosyl]adenine (42)

Treatment of **38** (45 mg, 0.17 mmol) by procedure **K** gave compound **42** (44.5 mg, 69%): ^{1}H NMR δ 0.11 (s, 6, $(CH_3)_2Si$), 0.91 (s, 9, $(CH_3)_3CSi$), 4.03 (dd, $J_{4'\text{-}5''}$ = 6.1 Hz, $J_{5'\text{-}5''}$ = 10.2 Hz, 1, H5''), 4.08 (dd, $J_{4'\text{-}5'}$ = 6.4 Hz, $J_{5'\text{-}5''}$ = 10.5 Hz, 1, H5'), 4.56 (dtd, $J_{4'\text{-}3'}$ = 3.3 Hz, $J_{4'\text{-}5'/5''}$ = 5.9 Hz, $J_{4'\text{-}F}$ = 26.9 Hz, 1, H4'), 4.66 (dt, $J_{2'\text{-}1'/3'}$ = 1.7 Hz, $J_{2'\text{-}F}$ = 15.4 Hz, 1, H2'), 5.16 (ddd, $J_{3'\text{-}2'}$ = 2.0 Hz, $J_{3'\text{-}4'}$ = 2.9 $J_{3'\text{-}F}$ = 51.2 Hz, 1, H3'), 6.01 (d, $J_{1'\text{-}2'}$ = 1.5 Hz, 1, H1'), 7.99 (s, 1, H2), 8.31 (s, 1, H8); ^{19}F NMR δ –203.72 (ddd, $J_{2'\text{-}F}$ = 16 Hz, $J_{4'\text{-}F}$ = 26 Hz, $J_{3'\text{-}F}$ = 50 Hz); MS (CI) *m/z* 384 (100, MH^+).

5'-*O*-(*tert*-Butyldimethylsilyl)-2'-deoxy-2'-fluoro-3'-*O*-(phenoxythiocarbonyl)adenosine (43). Procedure L

PTCCl (21.5 μL, 27 mg, 0.15 mmol) was added dropwise to a stirred solution of **39** (39 mg, 0.1 mmol) and DMAP (55 mg, 0.45 mmol) in acetonitrile (3.0 mL). Stirring was continued for 5 h, and volatiles were evaporated. The residue was partitioned (EtOAc/H_2O), and the organic layer was washed (0.1 M HCl/H_2O, $NaHCO_3/H_2O$,brine), dried (Na_2SO_4), evaporated, and column chromatographed (30% hexane/EtOAc → EtOAc) to give **43** (35 mg, 66%): ^{1}H NMR δ 0.15 (s, 6, $(CH_3)_2Si$), 0.95 (s, 9, $(CH_3)_3CSi$), 3.98 (dd, $J_{4'-5''}$ = 2.0 Hz, $J_{5'-5''}$ = 11.8 Hz, 1, H5''), 4.11 (dd, $J_{4'-5'}$ = 1.7 Hz, $J_{5'-5''}$ = 11.7 Hz, 1, H5'), 4.61 (m, 1, H4'), 5.77 ("dt", $J_{2'-1'/3'}$ = 3.9 Hz, $J_{2'-F}$ = 51.7 Hz, 1, H2'), 6.04 ("dt", $J_{3'-2'/4'}$ = 5.6 Hz, $J_{3'-F}$ = 13.1 Hz, 1, H3'), 6.47 (dd, $J_{1'-2'}$ = 3.2 Hz, $J_{1'-F}$ = 15 Hz, 1, H1'), 7.15 (d, J = 8.5 Hz, 2, H_{arom}), 7.35 (t, J = 7.3 Hz, 1, H_{arom}), 7.47 (t, J = 7.8 Hz, 2, H_{arom}), 8.2 (s, 1, H2), 8.39 (s, 1, H8); ^{19}F NMR δ –205.26 (dt, $J_{1'/3'-F}$ = 14 Hz, $J_{2'-F}$ = 51 Hz); MS (CI) *m/z* 520 (100, MH^+).

5'-*O*-(*tert*-Butyldimethylsilyl)-3'-deoxy-3'-fluoro-2'-*O*-(phenoxythiocarbonyl)adenosine (44)

Treatment of **40** (24 mg, 0.062 mmol) by procedure **L** gave compound **44**[56] (24 mg, 74%): ^{1}H NMR δ 0.16 (s, 6, $(CH_3)_2Si$), 0.96 (s, 9, $(CH_3)_3CSi$), 3.93 (dd, $J_{4'-5''}$ = 2.5 Hz, $J_{5'-5''}$ = 11.5 Hz, 1, H5''), 4.01 (dd, $J_{4'-5'}$ = 1.6 Hz, $J_{5'-5''}$ = 11.4 Hz, 1, H5'), 4.59 (dt, $J_{4'-5'/5'}$ = 1.7 Hz, $J_{4'-F}$ = 25.8 Hz, 1, H4'), 5.61 (dd, $J_{3'-2'}$ = 4.3 Hz, $J_{3'-F}$ = 54.1 Hz, 1, H3'), 6.28 (ddd, $J_{2'-1'}$ = 7.6 Hz, $J_{2'-3'}$ = 4.3 Hz, $J_{2'-F}$ = 20.8 Hz, 1, H2'), 6.6 (d, $J_{1'-2'}$ = 7.2 Hz, 1, H1'), 7.07 (d, J = 7.6 Hz, 2, H_{arom}), 7.3 (t, J = 7.3 Hz, 1, H_{arom}), 7.41 (t, J = 7.6 Hz, 2, H_{arom}),

8.2 (s, 1, H2), 8.42 (s, 1, H8); ^{19}F NMR δ –199.24 (ddd, $J_{2'\text{-F}}$ = 21 Hz, $J_{4'\text{-F}}$ = 26, $J_{3'\text{-F}}$ = 54 Hz); MS (CI) *m/z* 520 (100, MH^+).

9-[5-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-2-fluoro-3-*O*-(phenoxythiocarbonyl)-β-D-arabinofuranosyl]adenine (45)

Treatment of **41** (36 mg, 0.094 mmol) by procedure **L** gave compound **45** (19 mg, 38%): ^{1}H NMR δ 0.15 (s, 6, $(CH_3)_2Si$), 0.96 (s, 9, $(CH_3)_3CSi$), 3.95 (dd, $J_{4'\text{-}5''}$ = 4.8 Hz, $J_{5'\text{-}5''}$ = 10.7 Hz, 1, H5''), 4.02 (dd, $J_{4'\text{-}5'}$ = 4.7 Hz, $J_{5'\text{-}5''}$ = 11.0 Hz, 1, H5'), 4.39-4.44 (m, 1, H4'), 5.57 (dd, $J_{2'\text{-}1'}$ = 2.8 Hz, $J_{2'\text{-F}}$ = 49.6 Hz, 1, H2'), 6.01 (dd, $J_{3'\text{-}4'}$ = 2.6 Hz, $J_{3'\text{-F}}$ = 15.6 Hz, 1, H3'), 6.59 (dd, $J_{1'\text{-}2'}$ = 2.6 Hz, $J_{1'\text{-F}}$ = 22.0 Hz, 1, H1'), 7.16 (d, J = 7.6 Hz, 2, H_{arom}), 7.36 (t, J = 7.3 Hz, 1, H_{arom}), 7.47 (t, J = 7.6 Hz, 2, H_{arom}), 8.16 (s, 1, H2), 8.40 (s, 1, H8); ^{19}F NMR δ –199.19 (dt, $J_{1'/3'\text{-F}}$ = 18 Hz, $J_{2'\text{-F}}$ = 51 Hz); MS (CI) *m/z* 520 (100, MH^+).

9-[5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-fluoro-2-*O*-(phenoxythiocarbonyl)-β-D-xylofuranosyl]adenine (46)

Treatment of **42** (44 mg, 0.12 mmol) by procedure **L** gave compound **46** (15 mg, 25%): ^{1}H NMR δ 0.14 (s, 6, $(CH_3)_2Si$), 0.95 (s, 9, $(CH_3)_3CSi$), 3.99-4.09 (m, 2, H5',5''), 4.49 (dtd, $J_{4'\text{-}3'}$ = 3.0 Hz, $J_{4'\text{-}5'/5''}$ = 7.5 Hz, $J_{4'\text{-F}}$ = 28.5 Hz, 1, H4'), 5.39 (dd, $J_{3'\text{-}4'}$ = 2.6 Hz, $J_{3'\text{-F}}$ = 49.8 Hz, 1, H3'), 6.09 (d, $J_{2'\text{-F}}$ = 13.3 Hz, 1, H2'), 6.5 (s, 1, H1'), 7.15 (d, J = 7.5 Hz, 2, H_{arom}), 7.36 (t, J = 7.8 Hz, 1, H_{arom}), 7.46 (t, J = 7.8 Hz, 2, H_{arom}), 8.09 (s, 1, H2), 8.4 (s, 1, H8); ^{19}F NMR δ –204.49 (ddd, $J_{2'\text{-F}}$ = 13 Hz, $J_{4'\text{-F}}$ = 28 Hz, $J_{3'\text{-F}}$ = 50 Hz); MS (CI) *m/z* 520 (100, MH^+).

9-(5-*O*-(tert-Butyldimethylsilyl)-3(*R/S*)-deuterio-2-fluoro-2,3-dideoxy-β-D-*erythro*-pentafuranosyl)adenine (47). Procedure M

A solution of **43** (17 mg, 0.032 mmol), AIBN (1.2 mg, 0.007 mmol) and Bu_3SnD (17.4 μL, 18 mg, 0.064 mmol) in toluene (1.0 mL) was deoxygenated (Ar) for 30 min and then was gently heated for 3 h at 85 °C. Volatiles were evaporated and the residue was column chromatographed (EtOAc) to afford **47** (3'*R/S*, ~64:36; 8.5 mg, 67%): ^{1}H NMR δ 0.13 (s, 6, $(CH_3)_2Si$), 0.96 (s, 9, $(CH_3)_3CSi$), 2.23 (dd, $J_{3'b\text{-}4'}$ = 5.1 Hz, $J_{3'b\text{-}F}$ = 19.3 Hz, 0.36, H3'b), 2.49 (ddd, $J_{3'a\text{-}2'}$ = 4.0 Hz, $J_{3'a\text{-}4'}$ = 10.7 Hz, $J_{3'a\text{-}F}$ = 42.3 Hz, 0.64, H3'a), 3.8 (dd, $J_{4'\text{-}5''}$ = 2.7 Hz, $J_{5'\text{-}5''}$ = 11.7 Hz, 1, H5''), 4.15 (dd, $J_{4'\text{-}5'}$ = 2.5 Hz, $J_{5'\text{-}5''}$ = 11.7 Hz, 1, H5'), 4.6 (dt, $J_{4'\text{-}5'/5''}$ = 2.6 Hz, $J_{4'\text{-}3a'}$ = 10.6 Hz, 1, H4'), 5.42 (dd, $J_{2'\text{-}3a'}$ = 3.7 Hz, $J_{2'\text{-}F}$ = 51.5 Hz, 1, H2'), 6.33 (d, $J_{1'\text{-}F}$ = 16.5 Hz, 1, H1'), 8.29 (s, 1, H2), 8.34 (s, 1, H8); ^{19}F NMR δ –181.04 (ddd, $J_{1'\text{-}F}$ = 16.5 Hz, $J_{3'a\text{-}F}$ = 42 Hz, $J_{2'\text{-}F}$ = 51.5 Hz) and other data as reported.[56]

9-[5-*O*-(tert-Butyldimethylsilyl)-2(*R/S*)-deuterio-3-fluoro-2,3-dideoxy-β-D-*erythro*-pentafuranosyl]adenine (48)

Treatment of **44** (17.5 mg, 0.034 mmol) by procedure **M** gave compound **48** (2'*R/S*, ~16:84; 4 mg, 33%): ^{1}H NMR δ 0.13 (s, 6, $(CH_3)_2Si$), 0.93 (s, 9, $(CH_3)_3CSi$), 2.73 (ddd, $J_{2'b\text{-}1'}$ = 8.7 Hz, $J_{2'b\text{-}3'}$ = 4.8 Hz, $J_{2'b\text{-}F}$ = 39.1 Hz, 0.84, H2'b), 2.82 (dd, $J_{2'a\text{-}3'}$ = 4.2 Hz, $J_{2'a\text{-}F}$ = 18.8 Hz, 0.16, H2'a), 3.88 (dd, $J_{4'\text{-}5''}$ = 3.0 Hz, $J_{5'\text{-}5''}$ = 11.2 Hz, 1, H5''), 3.93 (dd, $J_{4'\text{-}5'}$ = 3.7 Hz, $J_{5'\text{-}5''}$ = 11.4 Hz, 1, H5'), 4.44 (dt, $J_{4'\text{-}5'/5'}$ = 3.3 Hz, $J_{4'\text{-}F}$ = 26.4 Hz, 1, H4'), 5.36 (dd, $J_{3'\text{-}2'b}$ = 4.6 Hz, $J_{3'\text{-}F}$ = 53.5 Hz, 1, H3'), 6.55 (d, $J_{1'\text{-}2'b}$ = 8.9 Hz, 1, H1'), 8.18 (s, 1, H2),

8.37 (s, 1, H8); ^{19}F NMR δ –176.95 (ddd, $J_{4'\text{-}F}$ = 26.6 Hz, $J_{2'b\text{-}F}$ = 37.7 Hz, $J_{3'\text{-}F}$ = 52.7 Hz) and other data as reported.[31]

9-[5-*O*-(tert-Butyldimethylsilyl)-3(*R/S*)-deuterio-2-fluoro-2,3-dideoxy-β-D-*threo*-pentafuranosyl]adenine (49)

Treatment of **45** (10 mg, 0.02 mmol) by procedure **M** gave compound **49** (3'*R/S*, ~93:7; 6.5 mg, 90%) with data as reported[56] except: ^{1}H NMR δ 0.13 (s, 6, $(CH_3)_2Si$), 0.95 (s, 9, $(CH_3)_3CSi$), 3.85-3.87 (m, 2, H5',5"), 4.30 ("q", $J_{4'\text{-}3'/5'/5"}$ = 5.09 Hz, 1, H4'), 2.46 (dd, $J_{3'\text{-}4'}$ = 3.4 Hz, $J_{3'\text{-}F}$ = 26.8 Hz, 0.93, H3'), 5.30 (dt, $J_{2'\text{-}1'/3'}$ = 2.56 Hz, $J_{2'\text{-}F}$ = 53.6 Hz, 1, H2'), 6.34 (dd, $J_{1'\text{-}2'}$ = 3.2 Hz, $J_{1'\text{-}F}$ = 18.1 Hz, 1, H1'), 8.18 (s, 1, H2), 8.37 (s, 1, H8); ^{19}F NMR δ –188.04 (dt, $J_{1'/3'\text{-}F}$ = 23 Hz, $J_{2'\text{-}F}$ = 53 Hz).

9-[5-*O*-(tert-Butyldimethylsilyl)-2(*R/S*)-deuterio-3-fluoro-2,3-dideoxy-β-D-*threo*-pentafuranosyl]adenine (50)

Treatment of **46** (8.6 mg, 0.017 mmol) by procedure **M** gave compound **50** (2'*R/S*, ~6:94; 2.5 mg, 54%): ^{1}H NMR δ 0.11 (s, 6, $(CH_3)_2Si$), 0.93 (s, 9, $(CH_3)_3CSi$), 2.72 (d, $J_{2'\text{-}F}$ = 15.4 Hz, 0.93, H2'), 3.99 (dd, $J_{4'\text{-}5"}$ = 5.9 Hz, $J_{5'\text{-}5"}$ = 10.2 Hz, 1, H5"), 4.04 (dd, $J_{4'\text{-}5'}$ = 7.1 Hz, $J_{5'\text{-}5"}$ = 10.1 Hz, 1, H5'), 4.20 (dddd, $J_{4'\text{-}3'}$ = 2.5 Hz, $J_{4'\text{-}5"}$ = 6.0 Hz, $J_{4'\text{-}5'}$ = 7.2 Hz, $J_{4'\text{-}F}$ = 28.6 Hz, 1, H4'), 5.36 (dd, $J_{3'\text{-}4'}$ = 2.0 Hz, $J_{3'\text{-}F}$ = 53.4 Hz, 1, H3'), 6.56 (s, 1, H1'), 8.13 (s, 1, H2), 8.34 (s, 1, H8); ^{19}F NMR δ –193.82 (ddd, $J_{4'\text{-}F}$ = 27 Hz, $J_{2'\text{-}F}$ = 22 Hz, $J_{3'\text{-}F}$ = 54 Hz).

9-[3(*R/S*)-deuterio-2-fluoro-2,3-dideoxy-β-D-*erythro*-pentafuranosyl]adenine (51)

Procedure N

NH_4F (100 mg, 2.7 mmol) was added to **47** (15 mg, 0.04 mmol) in MeOH (2 mL), and stirring was continued overnight at reflux. Volatiles were evaporated and the residue was chromatographed (5 → 10% MeOH/$CHCl_3$) to give compound **51** (3'*R/S*, ~62:38; 5.5 mg, 55%): ^{1}H NMR (MeOH-d_4) δ 2.3 (dd, $J_{3'b\text{-}4'}$ = 5.4 Hz, $J_{3'b\text{-}F}$ = 19.5 Hz, 0.36, H3'b), 2.53 (ddd, $J_{3'a\text{-}2'}$ = 4.3 Hz, $J_{3'a\text{-}4'}$ = 9.7 Hz, $J_{3'a\text{-}F}$ = 38.2 Hz, 0.64, H3'a), 3.72 (dd, $J_{4'\text{-}5''}$ = 3.2 Hz, $J_{5'\text{-}5''}$ = 12.5 Hz, 1, H5''), 4.01 (dd, $J_{4'\text{-}5'}$ = 2.2 Hz, $J_{5'\text{-}5''}$ = 12.4 Hz, 1, H5'), 4.53-4.57 (m, 1, H4'), 5.52 (dd, $J_{2'\text{-}3'a}$ = 4.2 Hz, $J_{2'\text{-}F}$ = 52.0 Hz, 1, H2'), 6.3 (d, $J_{1'\text{-}F}$ = 16.8 Hz, 1, H1'), 8.22 (s, 1, H2), 8.45 (s, 1, H8); ^{19}F NMR (MeOH-d_4) δ –182.62 (ddd, $J_{1'\text{-}F}$ = 16 Hz, $J_{3'a\text{-}F}$ = 38 Hz, $J_{2'\text{-}F}$ = 52 Hz); MS (CI) *m/z* 255 (100, MH^+); and other properties as reported for unlabeled analog.[33,56]

9-[2(*R/S*)-deuterio-3-fluoro-2,3-dideoxy-β-D-*erythro*-pentafuranosyl]adenine (52)

Treatment of **48** (5 mg, 0.014 mmol) by procedure **N** gave compound **52** (2'*R/S*, ~13.5:86.5; 2.8 mg, 81%): ^{1}H NMR (MeOH-d_4) δ 2.12 (dd, $J_{2'b\text{-}3'}$ = 5.4 Hz, $J_{2'b\text{-}F}$ = 20.6 Hz, 0.14, H2'b), 2.4 (ddd, $J_{2'a\text{-}3'}$ = 4.3 Hz, $J_{2'a\text{-}1'}$ = 9.3 Hz, $J_{2'a\text{-}F}$ = 40.8 Hz, 0.86, H2'a), 3.25 (dd, $J_{4'\text{-}5''}$ = 2.7 Hz, $J_{5'\text{-}5''}$ = 12.5 Hz, 1, H5''), 3.3 (dd, $J_{4'\text{-}5'}$ = 2.7 Hz, $J_{5'\text{-}5''}$ = 12.4 Hz, 1, H5'), 3.84 (dt, $J_{4'\text{-}5'/5'}$ = 2.7 Hz, $J_{4'\text{-}F}$ = 27.3 Hz, 1, H4'), 4.86 (dd, $J_{3'\text{-}2'}$ = 4.6 Hz, $J_{3'\text{-}F}$ = 53.6 Hz, 1, H3'), 5.89 (d, $J_{1'\text{-}2'a}$ = 9.42 Hz, 1, H1'), 7.73 (s, 1, H2), 7.62 (s, 1, H8); ^{19}F NMR (MeOH-d_4) δ –173.44 (ddd, $J_{4'\text{-}F}$ = 28 Hz, $J_{2'a\text{-}F}$ = 41 Hz, $J_{3'\text{-}F}$ = 53 Hz); MS (CI) *m/z* 255 (100, MH^+); and other properties as reported for unlabeled analog.[33]

9-[3(*R/S*)-deuterio-2-fluoro-2,3-dideoxy-β-D-*threo*-pentafuranosyl]adenine (53)

Treatment of **49** (12.5 mg, 0.035 mmol) by procedure **N** gave compound **53** (3'*R/S*, ~92.5:7.5; 4.5 mg, 54%): ^{1}H NMR (MeOH-d_4) δ 1.8 ("dt", $J_{3'b\text{-}2'/4'}$ = 4.2 Hz, $J_{3'b\text{-}F}$ = 27.2 Hz, 0.93, H3'b), 2.05 ("dt", $J_{3'a\text{-}2'/4'}$ = 6.8 Hz, $J_{3'a\text{-}F}$ = 31.0 Hz, 0.07, H3'a), 3.2 (dd, $J_{4'\text{-}5''}$ = 6.5 Hz, $J_{5'\text{-}5''}$ = 12.0 Hz, 1, H5''), 3.27 (dd, $J_{4'\text{-}5'}$ = 3.7 Hz, $J_{5'\text{-}5''}$ = 12.0 Hz, 1, H5'), 3.77 ("q", $J_{4'\text{-}3'b/5'/5''}$ = 5.2 Hz, 1, H4'), 4.79 (dt, $J_{2'\text{-}1'/3'b}$ = 2.7 Hz, $J_{2'\text{-}F}$ = 54.1 Hz, 1, H2'), 5.76 (dd, $J_{1'\text{-}2'}$ = 3.5 Hz, $J_{1'\text{-}F}$ = 16.8 Hz, 1, H1'), 7.66 (s, 1, H2), 7.81 (s, 1, H8); ^{19}F NMR (MeOH-d_4) δ –182.62 (ddd, $J_{1'\text{-}F}$ = 17 Hz, $J_{3'b\text{-}F}$ = 27 Hz, $J_{2'\text{-}F}$ = 54 Hz); MS (CI) *m/z* 255 (100, MH^+); and other properties as reported for unlabeled analog.[33,56]

9-[2(*R/S*)-deuterio-3-fluoro-2,3-dideoxy-β-D-*threo*-pentafuranosyl]adenine (54)

Treatment of **50** (4 mg, 0.011 mmol) by procedure **N** gave compound **54** (2'*R/S*, ~8:92; 2.7 mg, 98%): ^{1}H NMR (MeOH-d_4) δ 2.75 (dd, $J_{2'b\text{-}1'}$ = 1.4 Hz, $J_{2'b\text{-}F}$ = 21.3 Hz, 0.9, H2'b), 2.96 (dd, $J_{2'a\text{-}1'}$ = 7.1 Hz, $J_{2'a\text{-}F}$ = 45.9 Hz, 0.1, H2'a), 3.89 (dd, $J_{4'\text{-}5''}$ = 6.6 Hz, $J_{5'\text{-}5''}$ = 11.6 Hz, 1, H5''), 3.96 (dd, $J_{4'\text{-}5'}$ = 5.9 Hz, $J_{5'\text{-}5''}$ = 11.6 Hz, 1, H5'), 4.26 (dtd, $J_{4'\text{-}3'}$ = 2.7 Hz, $J_{4'\text{-}5'/5''}$ = 6.4 Hz, $J_{4'\text{-}F}$ = 29.2 Hz, 1, H4'), 5.42 (dd, $J_{3'\text{-}4'}$ = 2.6 Hz, $J_{3'\text{-}F}$ = 54.1 Hz, 1, H3'), 6.59 (d, $J_{1'b\text{-}2'}$ = 1.4 Hz, 1, H1'), 8.23 (s, 2, H2,8); ^{19}F NMR (MeOH-d_4) δ –194.91 (ddd, $J_{4'\text{-}F}$ = 27 Hz, $J_{2'b\text{-}F}$ = 22 Hz, $J_{3'\text{-}F}$ = 54 Hz); MS (CI) *m/z* 255 (100, MH^+); and other properties as reported for unlabeled analog.[33]

CONCLUSION

Ribonucleotide reductases are the enzymes responsible for generating 2'-deoxyribonucleotides, the monomeric precursors required for DNA biosynthesis and DNA repair. For more than four decades, the structure of the enzymes, mechanisms of action and design and synthesis of inhibitors have been extensively studied.

Since 1992, the most accepted mechanism for the nucleotide reduction by RNRs is the one proposed by Stubbe (*Biochemistry*, **1992**, *31*, 9752-9729). Abstraction of 3'-hydrogen atom from the nucleoside diphosphate by a transient thiyl radical at Cys439 was proposed to initiate the radical reduction/cascade.

In 1996, Fontecave and coworkers showed that a substrate analog of the enzyme, 2'-deoxy-2'-mercaptouridine 5'-diphosphate, was a very efficient inactivator of RNR. They proposed that in the presence of oxygen a mixed disulfide is produced between the substrate analog and the protein and that homolytic cleavage of 2'-carbon-sulfur bond generates the perthiyl radical observed by EPR spectroscopy.

Our first target in this thesis was design and synthesis of 9-(2-thio-β-D-arabinofuranosyl)adenine 5'-diphosphate as a valuable probe to study the mechanism of inhibition of ribonucleotide reductases. We predict that the thiyl radical formed at cysteine 439 might be trapped by the arabino 2'-mercapto group via formation of R1 Cys439-2'-mercapto disulfide bridge leading to the inhibition of RDPR.

Efficient multi-step preparation of 9-(2-thio-β-D-arabinofuranosyl)adenine included: (i) protection of adenosine using Markiewicz methodology; (ii) triflation of 2'-hydroxyl group; (iii) incorporation of a thioacetyl group with inversion of configuration by nucleophilic displacement of 2'-*O*-triflate and (iv) deacetylation. Two different

approaches were evaluated for the preparation of mixed disulfides, which were used for protecting the reactive thiol group.

In the first approach, protected mixed disulfide was prepared by treatment of 2'-mercapto compound with DEAD and 1-propanethiol in THF followed by desilylation. We found that protection groups at 3' and 5'-hydroxyl groups were necessary in order to overcome solubility problems.

In the second approach, preparation of disulfide was attempted employing a new method recently developed in which 2-(trimethylslilyl)ethanethiol is used as a source of sulfur. Displacement of the triflate group afforded 2'-*S*-[(2-trimethylsilyl)ethyl]-2'-thinucleoside in good yield. However, reaction of the latter with dimethyl(methylthio)sulfonium tetrafluoroborate failed probably due to instability of the purine ring under reaction condition.

The 5'-phosphorylation was performed using Poulter's methodology, which is based on the displacement of 5'-*O*-tosylate ester with the corresponding pyrophosphate ion. The thionucleotide obtained is currently under biological studies. In addition to the arabino-2'-thioadenosine obtained, other thionucleoside analogs were also prepared using the same multi-step procedure to be evaluated against different cancer lines.

The second target of this thesis was the synthesis of four fluorine-containing nucleoside analogs in order to study the stereoelectronic effect of a β-fluorine substituent in radical deoxygenation reactions. It is well known that the presence of a fluorine atom in the sugar moiety of nucleoside analogs not only changes the conformation of the molecule, but also its biological activity.

Employing Pankiewicz's methodology we prepared the 2'-deoxy-2'-fluoroadenosine and its arabino epimer as well as 3'-deoxy-3'-fluoroadenosine and its xylo epimer. Regioselective silylation (O5') of the fluoro nucleosides and radical deoxygenation of the corresponding phenoxythiocarbonyl esters gave four deuterium labeled 2',3'-dideoxy-2' (or 3') fluoronucleoside analogs. The ratio of deuterium incorporation was determined by using ^{1}H NMR on the bases of vicinal coupling constant analysis ($^{3}J_{F\text{-}H}$ and $^{3}J_{H\text{-}H}$).

Our results clearly show that the steric effect of the heterocyclic base from the β-face of the sugar is decisive, favoring the incorporation of deuterium from the less hindered α-face. However, the presence of a β-fluorine substituent enhances the *anti*-selectivity of deuterium abstraction relative to fluorine position in the sugar ring.

All the intermediates and the products were characterized by using ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and ^{19}F NMR. Isotopic enhancement was confirmed using MS.

REFERENCES:

1. Blackburn, M. G.; Gait, J. M. *Nucleic Acids in Chemistry and Biology* 1997, 2nd edition, Oxford University Press.

2. Voet, D.; Voet, G. J. *Biochemistry* 1990, John Willey & Sons.

3. Solomon G. Organic Chemistry 1994, 6th edition, John Wiley & Sons.

4. Carey A. Francis, *Organic Chemistry* 1987, McGraw-Hill Book Company.

5. Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*, CRC Press: Boca Raton, Fl, 1988; pp. 3-30.

6. Chambert, S.; Decout, J. -L. *Org. Preparation and Procedures Int.* **2002**, *34*, 27-85.

7. Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balsarini, J.; De Clerk, E. *J. Med. Chem.* **1991**, *34*, 2195-2202.

8. Jordan, A.; Reichard, P. *Annu. Rev. Biochem.* **1998**, *67*, 71-98.

9. Ashley, G. W.; Stubbe, J. In *Inhibitors of Ribonucleoside Diphosphate Reductase activity*, International Encyclopedia of Pharmacology and Therapeutics, Section 128, Cory, J. G.; Cory, A. H., Eds.; Pergamon Press: New York 1989; pp. 55-87.

10. Stubbe, J.; Vander Donk, A. W. *Chem. Biol.* **1995**, *2*, 793-801.

11. Stubbe, J. *J. Biol. Chem.* **1990**, *265*, 5329-5332.

12. Thelander, L.; Reichard, P. *Ann. Rev. Biochem.* **1979**, *48*,133-158.

13. Nordlund, P.; Sjöberg, B.-M.; Eklund, H. *Nature* **1990**, *345*, 593-598.

14. Uhlin, U.; Eklund, H. *Nature* **1994**, *370*, 533-539.

15. Eriksson, M.; Uhlin, U.; Ramaswamy, S.; Ekberg, M.; Regnstrom, K.; Sjöberg, B. M.; Eklund, H. *Structure* **1997**, *5*, 1077-1092.

16. Reichard, P.; Ehrenberg, A. *Science* **1983**, *221*, 514-519.

17. Ehrenberg, A; Reichard, P. *J. Biol. Chem.* **1972**, *247*, 3485-3488.

18. Persson, A. L.; Eriksson, M.; Katterle, B.; Pötsch, S.; Sahlin, M.; Sjöberg, B. M. *J. Biol. Chem.* **1997**, *272*, 31533-31541.

19. Persson, A. L.; Sahlin, M.; Sjöberg, B. M. *J. Biol. Chem.* **1998**, *273*, 31016-31020.

20. Thelander, L.; Larson, B.; Hobbs, J.; Eckstein, F. *J. Biol. Chem.* **1976**, *251*, 1938-1405.

21. Coves, J.; Le Hir de Fallois, L.; Le Pape, L.; Decout, J.-L.; Fontecave, M. *Biochemistry* **1996**, *35*, 8595-8602.

22. Le Hir de Fallois, L.; Decout, J.-L.; Fontecave, M. *J. Chem. Soc.; Perkin Trans 1.* **1997**, 2587-2595.

23. Stubbe, J. A.; Ackels, D.; Segal, R.; Blakley, R. L. *J. Biol. Chem.* **1981**, *256*, 4843-4846.

24. Robins, M. J.; Wilson, J.; Hansske, F. *J. Am. Chem. Soc* **1983**, *105*, 4059-4065.

25. Carey, F. A. and Sundberg, R. J. *Advanced Organic Chemistry* 2000, 4th edition, Kluwer Academic/Plenum Publishers, New York.

26. Curran, D.; Porter, N. A.; Giese, B. *Stereoselectivity of radical reactions. Concepts, Guidelines, and Synthetic Applications*; VHC: New York, 1966.

27. Boubier, J. P.; Jung, G.; Liu, Z.; Guerin, B.; Guindon, Y. *Org. Lett.* **2001**, *3*, 1391-1394.

28. Dolbier, W. R., Jr.; Bartberger, M. D. *J. Org. Chem.* **1995**, *60*, 4984-4885.

29. Siddiqui, M. A.; Driscoll, J. S.; Abushanab, E.; Kelley, J. A.; Barchi, J. J., Jr.; Marquez, V. E. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 1-12.

30. Crich, D.; Beckwith, A. L. J.; Chen, C.; Yao, Q.; Davison, I. G. E.; Longmore, R. W.; Anaya de Parrodi, C.; Quintero-Cortez, L.; Sandoval-Ramirez, J. *J. Am. Chem. Soc.* **1995**, *117*, 8757-8768.

31. Maguire, A. R.; Meng, W.; Roberts, S. M.; Willetts, A. J. *J. Chem. Soc. Perkin Trans. 1* **1993**, *15*, 1795-1808.

32. Uesigi, S.; Miki, H.; Ikehara, M.; Iwahashi, H.; Kyogoku, Y. *Tetrahedron Lett.* **1979**, *42*, 4073-4076.

33. Herdewijn, P.; Pauwels, R.; Baba, M.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1987**, *30*, 2131-2137.

34. Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. *J. Org. Chem.* **1998**, *63*, 4967-4984.

35. Mao, S. S.; Yu, G. X.; Chalfoun, D.; Stubbe, J. *Biochemistry* **1992**, *31*, 9733-9743.

36. Wnuk, S. F. *Tetrahedron* **1993**, *49*, 9877-9936.

37. Chambert, S.; Gautier-Luneau, I.; Fontecave, M.; Decout, J. -L. *J. Org. Chem.* **2000**, *65*, 249-253.

38. Chambert, S.; Thomasson, F.; Decout, J. –L. *J. Org. Chem.* **2002**, *67*, 1898-1904.

39. Ranganathan, R.; Larwood, D. *Tetrahedron Lett.* **1978**, 4341-4344.

40. Marriott, J. H.; Mottahedeh, M.; Reese, C. B. *Carbohydr. Res.* **1991**, *216*, 257-269.

41. Imazawa, M.; Ueda, T,; Ukita, T. *Chem. Pharm. Bull.* **1975**, *23*, 604-610.

42. Shibuya, S.; Ueda, T. *J. Carbohydr. Nucleosides Nucleotides* **1980**, *7*, 49-56.

43. Nair, V.; Purdy, D. F. *Tetrahedron* **1991**, *47*, 365-382.

44. Robins, M. J.; Hawrelak, S. D.; Hernandez, A. E.; Wnuk, S. F. *Nucleosides Nucleotides* **1992**, *11*, 821-834.

45. Mukaiyama, T.; Takahashi, K. *Tetrahedron Lett.* **1968**, 5907-5908.

46. Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177-1180.

47. Anderson, M. B.; Ranasinghe, M. G.; Palmer, J. T.; Fuchs, P. L. *J. Org. Chem.* **1988**, *53*, 3127-3129.

48. Hutchinson, D. W. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B. Eds. Plenum Press: New York. 1991, Vol. 2.

49. Davisson, J. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794-1801.

50. Pankiewicz, K. W.; Krzeminski, J.; Ciszewski, L. A.; Ren, W. –Y.; Watanabe, K. A. *J. Org. Chem.* **1992**, *57*, 553-559.

51. Pankiewicz, K W. *Carbohydr. Res.* **2000**, *327*, 87-105.

52. Fukukawa, K.; Ueda, T.; Hirano, T. *Chem. Pharm. Bull.* **1983**, *31*, 1582-92.

53. Pankiewicz, K. W.; Krzeminski, J.; Watanabe, K. A. *J. Org. Chem.* **1992**, *57*, 7315-7321.

54. Herdewijn, P.; Van Aerschot, A.; Kerremans, L. *Nucleosides Nucleotides* **1989**, *8*, 65-96.

55. Battistini, C.; Giordani, A.; Ermoli, A.; Franceschi, G. *Synthesis* **1990**, 900-905.

56. Marquez, V.; Tseng, C. K. –H.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H. Jr; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S. *J. Med. Chem.* **1990**, *33*, 978-985.

57. Robins, M. J.; Fauron, Y.; Mengel, R. *J. Org. Chem.* **1974**, *39*, 1564-1569.