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Insight Into the Inhibition of Ribonucleotide Reductases by 2'-chloro-2'-deoxynucleotides and 2'-azido-2'-deoxynucleotides: Biomimetic Studies with Model Substrates

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INSIGHT INTO THE INHIBITION OF RIBONUCLEOTIDE REDUCTASES BY 2'-CHLORO-2'-DEOXYNUCLEOTIDES AND 2'-AZIDO-2'-DEOXYNUCLEOTIDES: BIOMIMETIC STUDIES WITH MODEL SUBSTRATES

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by

Mukesh Madan Mudgal

2016

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Mukesh Madan Mudgal, and entitled Insight Into the Inhibition of Ribonucleotide Reductases by 2'-chloro-2'-deoxynucleotides and 2'-azido-2'-deoxynucleotides: Biomimetic Studies with Model Substrates, having been approved in respect to style and intellectual content, is referred to you for judgment.

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

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Francisco Fernandez-Lima

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John Makemson

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Stanislaw Wnuk , Major Professor

Date of Defense: June 30, 2016

The dissertation of Mukesh Madan Mudgal is approved.

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Dean Michael R. Heithaus
College of Arts, Sciences and Education

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Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2016

DEDICATION

I would like to dedicate this dissertation to my family.Thank you very much for your understanding, encouragement, support, love and sacrifices.

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ABSTRACT OF THE DISSERTATION

INSIGHT INTO THE INHIBITION OF RIBONUCLEOTIDE REDUCTASES BY 2'-CHLORO-2'-DEOXYNUCLEOTIDES AND 2'-AZIDO-2'-DEOXYNUCLEOTIDES: BIOMIMETIC STUDIES WITH MODEL SUBSTRATES

by

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Professor Stanislaw Wnuk, Major Professor

Ribonucleotide Reductases (RNRs) are crucial enzymes that catalyze reduction of ribonucleotides to deoxyribonucleotides, required for the biosynthesis of DNA. Vital role played by RNR in the biosynthesis of DNA and its control on cell growth made it one of the main targets for anticancer therapy. Several laboratories clarified the aspects of reaction cascades at active site of RNR. Biochemical studies of RNR by Stubbe for the inactivation of RDPR by 2'-chloro-2'-deoxyuridine-5'-diphosphate emphasizes departure of chlorine as an anion, while biomimetic studies by Robins with 6'-*O*-nitro-2'-chloro-homonucleosides emphasizes the elimination of chlorine substituent from 2'-position as a radical. To clarify the ambiguity in the mechanism of inhibition of RNR by 2'-chloro-2'-deoxyuridine, biomimetic reactions with model 6-*O*-nitro-1,5-dideoxyhomosugar derivatives were investigated. The study includes several modes: (i) synthesis of 6-*O*-nitro-1,5-dideoxyhomosugar derivatives with chlorine, bromine or tosyl substituent at the C2 position with *ribo* and *arabino* configurations, (ii) biomimetic studies of 6-*O*-nitro-1,5-dideoxyhomosugar derivatives with Bu_3SnH/AIBN to provide chemical evidences to

distinguish the nature of elimination of chlorine from 2'-chloro-2'-deoxyuridine upon its incubation with enzyme, and (iii) kinetic studies to differentiate between heterolytic or homolytic C2'-chlorine bond cleavage.

In the second half of this dissertation, azido and sulfenamide modified nucleosides and 2-azidolyxofuranoside derivatives have been synthesized with the azido or sulfenamide substitution at a specific site in the sugar or in the base moiety. The electron-induced site specific formation of neutral aminyl radicals (RNH●) and their subsequent reactions have been investigated using ESR spectroscopy. In 2'-AZdC the RNH● site is attached to a 2^{o} C-atom, where as in 4'-AZdC, the RNH● site is attached to a 3^{o} C-atom, respectively. These studies elucidated how stereo and electronic environment affect formation and subsequent reactivity of various types of RNH● generated from azidonucleosides. To avoid the interaction of transient radical with nucleoside heterocyclic bases, 2-azidolyxofuranoside derivatives as a simpler abasic model were synthesized and studied with ESR spectroscopy. Aminyl radical generated from 2-azidolyxofuranoside derivatives subsequently abstracted hydrogen from C5 intramolecularly. These studies were designed to understand the mechanism of damage in various DNA model structures.

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LIST OF ABBREVIATIONS

Ac_2O	acetic anhydride
AcOH	acetic acid
Ag_2CO_3	silver carbonate
AIBN	azobisisobutyronitrile
AZT	3'-azido-2',3'-deoxythymidine
Ar	aromatic (NMR)
3'-AZG	3′-azido-2′,3′dideoxyguanosine
Bu_3SnH	tributyltin hydride
Bu_3SnD	tributyltin deuteride
BzOH	benzoic acid
β	beta
Bn	benzyl
br	broad (NMR)
$(Bu_3Sn)_2O$	tributyltin oxide
CD_3OD	deuterated methanol
CD_3CN	acetonitrile-d_3
CH_2Cl_2	methylene chloride
$(CH_3)_2CO$	acetone
CMV	cytomegalovirus
CNDAC	2′-C-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine
CuAAC	copper (I)-catalyzed azide-alkyne cycloaddition
calcd	calculated (HRMS)

^{o}C	degrees celsius
D_2O	deuterated water
DMAP	4-dimethylaminopyridine
DNA	deoxyribonucleic acid
DMF	*N*,*N*-dimethylformamide
dNTPs	deoxynucleotide triphosphates
dAdo	2'-deoxyadenosine
δ	delta
d	doublet (NMR)
DMSO	dimethylsulfoxide
EPR	electron paramagnetic resonance
ESI MS	electron spray ionization
EtOAc	ethyl acetate
FDA	food and drug administration
g	gram(s)
H_2O	water
H_2SO_4	sulfuric acid
HCl	hydrochloric acid
HMPA	hexamethylphosphoramide
HIV	human immunodeficiency virus
HBV	hepatitis B virus
HSV	herpes simplex virus
HCV	hepatitis C virus

HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	hertz
J	coupling constant in Hz (NMR)
LiCl	lithium chloride
L	liter(s)
MeCN	acetonitrile
$MgSO_4$	magnesium sulfate
MSNT	1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4- triazole
M	multiplet (NMR)
μ	micro
M	moles per liter
mol	mole(s)
MS	mass spectrometry
m/z	mass to charge ratio (MS)
$NaBH_4$	sodium borohydride
$NaHCO_3$	sodium bicarbonate
NaN_3	sodium azide
NDPs	nucleotide diphosphates
NH_3	ammonia
2'-N_3dUrd	2'-azido-2'-deoxyuridine
NMR	nuclear magnetic resonance
NDP	nucleoside diphosphate reductase

$(PhO)_2CO$	diphenyl carbonate
$PhSiH_3$	phenylsilane
q	quartet (NMR)
RNA	ribonucleic acid
RNR	ribonucleotide reductase
RDPR	ribonucleoside diphosphate reductase
rt	room temperature
SPAAC	strain-promoted azide-alkyne cycloaddition
s	singlet (NMR)
TFA	trifluoroacetic acid
TfCl	trifluoromethane sulfonyl chloride
THF	tetrahydrofuran
TMSCl	trimethylsilylchloride
$TMSN_3$	trimethylsilylazide
TMSOTf	trimethylsilyl triflate
TBDMSCl	*tert*-butyl(chloro)dimethylsilane
TBAF	*tetra*-*N*-butylammoniumfluoride
t-Bu	*tert*-butyl

1. INTRODUCTION

1.1. Anticancer and antiviral nucleoside analogues

Nucleosides consists of nitrogenous base and a sugar component (ribose or deoxyribose), whereas nucleotides contains sugar, a nucleobase, and one or more phosphate groups. Nucleosides and nucleotides are involved in numerous cellular processes such as synthesis of DNA and RNA, cell signaling, metabolism and regulation of enzymes. Analogues of nucleosides and nucleotides are prepared by chemical modifications of sugar and/or the base and are designed to mimic their physiological counterparts. These analogues affect cellular metabolism and further are incorporated into the DNA and RNA to inhibit cell division and viral replication.[1] Thus nucleoside and nucleotide analogues can serve as therapeutic agents and can be used to inhibit growth of cancer cells and inhibit viral replication. Besides being incorporated into the nucleic acids, these analogues can interact with important enzymes like DNA polymerases, RNA polymerases, kinases, ribonucleotide reductase, DNA methyltransferases, purine and pyrimidine nucleoside phosphorylase and thymidylate synthase to inhibit their activities.[1]

Mechanisms of action of nucleoside analogues

Nucleoside and nucleotide analogues follow the same metabolic pathways as of endogenous nucleosides or nucleotides in cells and also interfere with the normal metabolic processes within cells. These analogues enter the cell through a group of membrane transport proteins called nucleoside transporters.[2,3] Once these analogues enter into the cells they get phosphorylated with nucleoside kinase enzymes. Di-phosphorylated and triphosphorylated nucleoside analogues are active forms of these

drugs which get accumulated in cancer or virus-infected cells. Further these drugs act by inhibiting the enzymes such as viral polymerases, human polymerases or ribonucleotide reductase, as well as by getting incorporated into newly synthesized DNA and RNA. The incorporation of the nucleoside or nucleotide analogues into DNA leads to termination of the chain elongation or the induction of apoptosis.[1] In addition to their antiviral and anticancer activities the nucleoside and nucleotide analogues are also used in treatment of hyperuricemia (abnormally high level of uric acid in the blood), immunosuppression, phosphodiesterase inhibitions (inhibit the production of second messengers within cells, such as cyclic adenosine monophosphate), neuroprotection (regeneration of nervous system, its cells, structure and function) and cardioprotection.[1]

Antiviral nucleoside analogue

Nucleoside analogues are an important class of antiviral agents used to treat human immunodeficiency virus (HIV) infection, hepatitis B virus (HBV), cytomegalovirus (CMV) and herpes simplex virus (HSV) infection.[4] The first antiviral nucleoside analogue approved by FDA in 1969 was a thymidine analogue edoxudine, but is not currently in use. Presently, there are 25 FDA approved nucleoside and nucleotide analogues used as antiviral agents.[1]

Viral infection is broadly classified into three main classes; (i) Chronic viral infections like HIV, HBV and HCV, which has affected around 600 million people worldwide, (ii) Acute viral infections like influenza and haemorrhagic fever and (iii) Benign viral infections, e.g., common cold etc. Selected antiviral molecules available in the market depicted in Figure 1 are deoxyadenosine analogues e.g., didanosine (HIV), vidrabine (chemotherapy), adenosine analogs e.g., BCX4430 (Ebola), deoxycytidine

analogues e.g., cytarabine (chemotherapy), lamivudine (HIV, hepatitis-B), zalcitabine (HIV), guanosine and deoxyguanosine analogues e.g., abacavir (HIV), telbivudine (hepatitis-B), zidovudine (HIV), deoxyuridine analogues e.g., idoxuridin.

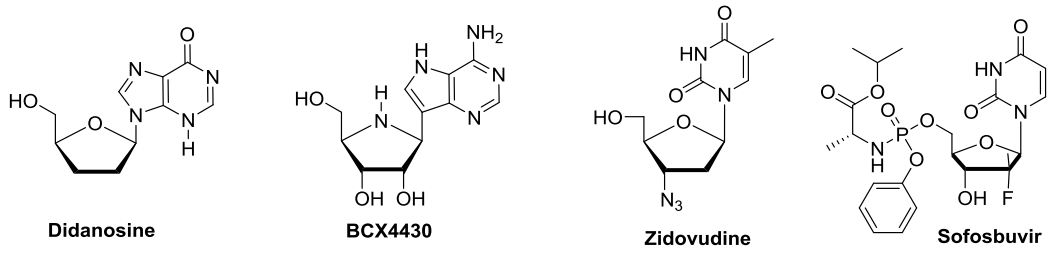


Figure 1. Selected nucleosides with antiviral activity

New anti-HCV nucleosides

Derivatives of 2'-deoxy-2'-fluorocytidine (FdC) like, methylated FdC (PSI-6130)[4], 2'-C-methylcytidine (Met-dC) derivative valopicitabine, are examples of new anti-HCV compounds. Mericitabine, another derivative of FdC has successfully completed several phase I and II studies alone or in combination with pegylated interferon (group of proteins produced and released by host cells in response to viruses, bacteria, parasites and tumor cells).

Sofosbuvir (Figure 1) is a uridine nucleotide analog and is recently approved as anti-HCV drug. It is a potent inhibitor of the NS5B polymerase (proteins essential for viral RNA replication) in the Hepatitis C virus. The NS5B has been found to be a valuable target for antiviral agents. Sofosbuvir is a phosphoramidate prodrug that has to be triphosphorylated within the cells to produce its action. Once administered into the body, sofosbuvir gets converted into the active form with the help of enzymes present in the human hepatic cells. Thus it is directly converted into the active metabolite during

metabolism in the liver. This analog further acts as competitive inhibitors by mimicking the physiological nucleotide and blocks the NS5B polymerase, thus inhibiting the HCV-RNA synthesis by RNA chain termination.[5]

New anti-HIV nucleosides

Human Immunodeficiency virus (HIV) is a retrovirus which causes acquired immunodeficiency syndrome (AIDS). Human Immunodeficiency virus inhibitors are classified into two broad categories non-nucleoside inhibitors and the nucleoside inhibitors. Festinavir is the analogue of the first generation anti-HIV nucleoside stavudine, prepared by attachment of the ethynyl group in the 4'- position of the ribose ring of stavudine (Figure 2). Festinavir is more potent than stavudine and has reduced toxicity.[6] It is now in phase II of clinical trial. Another new and potent anti-HIV molecule is apricitabine. It is a second-generation deoxycytidine analogue. Apricitabine has high barrier to resistance and no mutation detected during the in vivo studies of this molecule.[7]

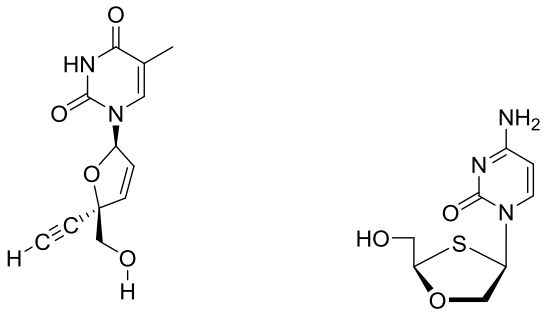


Figure 2. Selected anti-HIV nucleosides

The field of antiviral agents is active and novel anti-HCV compounds are under development. Molecules are also developed against HIV, HBV, herpes simplex virus,

cytomegalovirus, influenza and emerging viruses. However more attention is required for the development of selective antiviral agents to reduce their side effects by not getting incorporated into genomic and mitochondrial DNA of host cells.

Anticancer nucleoside analogues

Numerous anticancer molecules are synthesized and evaluated for the treatment of cancers after the approval of cytarabine in 1969 by the US FDA for the treatment of acute myeloid leukemia. Currently approved anticancer agents includes, cytarabine (acute myeloid leukaemia and acute lymphocytic leukaemia), fludarabine (chronic lymphocytic leukaemia non-Hodgkins lymphoma), cladribine (hairy cell leukaemia), gemcitabine (Figure 3), (non-small lung cancer, pancreatic cancer, bladder cancer and breast cancer), clofarabine (acute lymphocytic leukaemia), nelarabine (T-cell acute lymphoblastic leukaemia), capecitabine (metastatic breast cancer, metastatic colorectal cancer), floxuridine (advanced colon cancer, kidney cancer and stomach cancer), deoxycoformycin (hairy cell leukemia chronic lymphocytic leukaemia), azacitidine (myelodysplastic syndrome), decitabine (myelodysplastic syndrome, acute myeloid leukemia.

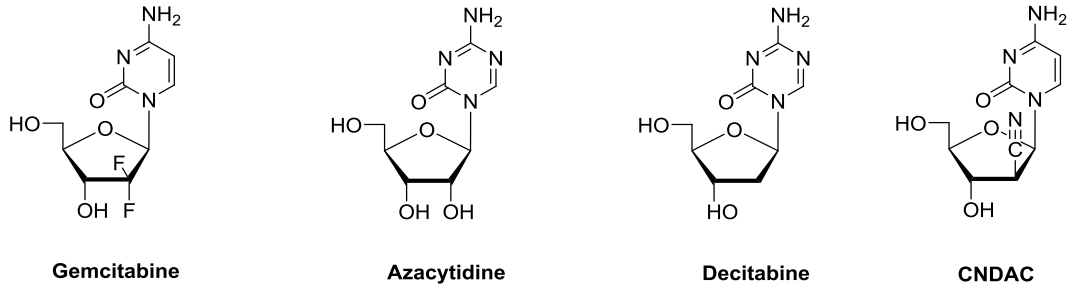


Figure 3. Selected anticancer nucleoside analogues

New anticancer nucleosides

Very few examples of anticancer nucleoside analogues are known which cure cancer in patients. Cytarabine halts myeloid leukemia (uncontrolled growth of abnormal white blood cells and its accumulation in bone marrow which interferes with production of normal blood cells) in less than 30% of all adult patients. Most of the other cytotoxic agents provide temporary relief or prolong life with no definitive cure. Thus there is a strong need to improve the cytotoxicity of currently available cytotoxic nucleoside analogues.

The Cytarabine analogue CNDAC [1-(2-C-cyano-2-deoxy-β-d-arabinopentofuranosyl) cytosine] (Figure 3), induces DNA strand breakage upon its incorporation into DNA because of the nucleophilic attack of the cyano group. It showed good cytotoxic activity *in vivo* on human cancer xenografts (graft of tissues from human). Sapacitabine is an oral nucleoside analogue prodrug. It is a derivative of CNDAC and is under phase III of clinical trials.

The 8-chloroadenosine and 8-aminoadenosine analogues decrease the concentration of the ATP intracellularly by reducing the RNA synthesis and inducing cell death.[8,9] The 8-chloroadenosine induces cytotoxicity by decreasing MET expression,[10] a tyrosine kinase receptor responsible for embryonic development, organogenesis and wound healing in myeloma cells. It also reduces the cyclin E (a member of cyclin family binds to G_1 phase of cell cycle, which further determines cell division) expression in breast cancer cells. The 8-chloroadenosine is under phase-I clinical trial with chronic lymphocytic leukemia.[1]

New cytotoxic nucleoside analogues are capable of altering the role of nucleoside analogues in cancer therapy. To accomplish this they need to have their own and original mechanism of action.

1.2. Ribonucleotide Reductase: Function and inhibitions

1.2.1. Mechanistic aspects

Ribonucleotide reductases (RNRs) convert the nucleotides to 2'-deoxynucleotides.[11] The 2'-deoxynucleotides act as monomeric precursors required for DNA replication and repair.[12] Inhibition of RNRs leads to depletion of the deoxyribonucleotides pool available for DNA synthesis and therefore is an appealing concept for rational drug design.[13,14] Number of 2'-modified pyrimidine nucleotides have been synthesized such as 2'-chloro[15] and 2'-azido 5'-di(or tri)phosphates and are found to be potent inhibitors of RNRs.[16-19] Gemcitabine is an analog of cytidine with two fluorine atoms at the 2'-position of the ribose ring. It is a first-line chemotherapeutic agent used for the treatment of pancreatic and non-small cell lung cancers.[13,14,20,21]

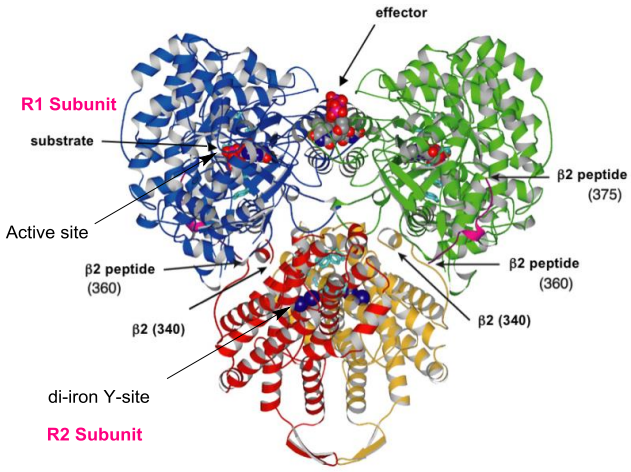


Figure 4. Structure of class I RNR[22]

Ribonucleoside 5'-diphosphate reductase (RDPR) from *Escherichia coli* (Gram negative bacteria) have been studied extensively. *Escherichia coli* have similar composition as of mammalian RDPRs.[23] It was found to contain two non-identical subunits (R1 and R2) (Figure 4). The R1 subunit of the *Escherichia coli* consists of allosteric control sites (site other than enzyme's active site where effector binds and regulates the protein) and cysteine. While the R2 subunit contains a di-iron chelate and essential tyrosine free radical which produce a proximate thiyl radical[24] on Cys439 on R1 subunit via electron and proton transfer reactions. The thiyl radical abstracts the hydrogen from 3' position of the ribonucleotide substrate to initiate nucleotide reduction.[25] This reaction cascade further leads to the elimination of water from 2' position.[25] These initial steps in the mechanism of the reduction of the ribonucleotides to deoxyribonucleotides catalyzed by RNR are consistent with the Stubbe's enzymatic studies with gemcitabine[26-30] along with theoretical modeling studies performed by Ramos and Siegbahn,[31,32] and chemical biomimetic studies by Giese[33] and Robins[34,35] as well as McCarthy's 2'-deoxy-2'-fluoromethylenecytidine.[36,37] Nevertheless the detection of the ribosyl-based radical during RNR-catalyzed deoxygenation of the natural substrates remains elusive.[38]

1.2.1.1. Enzymatic studies by Stubbe

Stubbe *et al.* proposed the mechanism for the reduction of the nucleoside diphosphate substrates (Figure 5).[12] It has been postulated that in the first step of RNR-catalyzed reduction of the ribonucleotides to deoxyribonucleotides reactions, a thiyl radical SCys439 abstract H3' of the ribosyl (**A**, X = OH) to generate the C3' radical. Base promoted (Glu441) removal of the 3'-hydroxyl proton leads to loss of water (C2'-H_2O) in a heterolytic manner (**B** → **C**) to produce 3'-keto-2'-deoxydeoxynucleotide.[24,39] Enzyme

inactivation by 2'-azido substituted nucleotides (**A**, X = N_3) are accompanied by loss of azide ions and formation of 3'-keto-2'-deoxynucleotides[12,15,17] (e.g., **D**), which dissociate further from protein and generate 2-methylene-3(2*H*)-furanone Michael acceptor, which alkylates the nucleophiles on enzyme and causes covalent enzyme inhibition. Incubation of RNR by gemcitabine results in inhibition of both R1 and R2 subunits of ribonucleotide reductase (RNR).[26-30] Transfer of the hydrogen atom from cysteine thiols gives 3-keto intermediate **D**. Further the electron and proton transfer takes place to produce **E**, cysteine and the glutamate. The original hydrogen atom (Ha) is recaptured from SCys439 by the C3' radical to complete reduction of the substrate to give 2'-deoxynucleoside 5'-diphosphate, **F** with regeneration of the SCys439 radical for the next catalytic cycle.

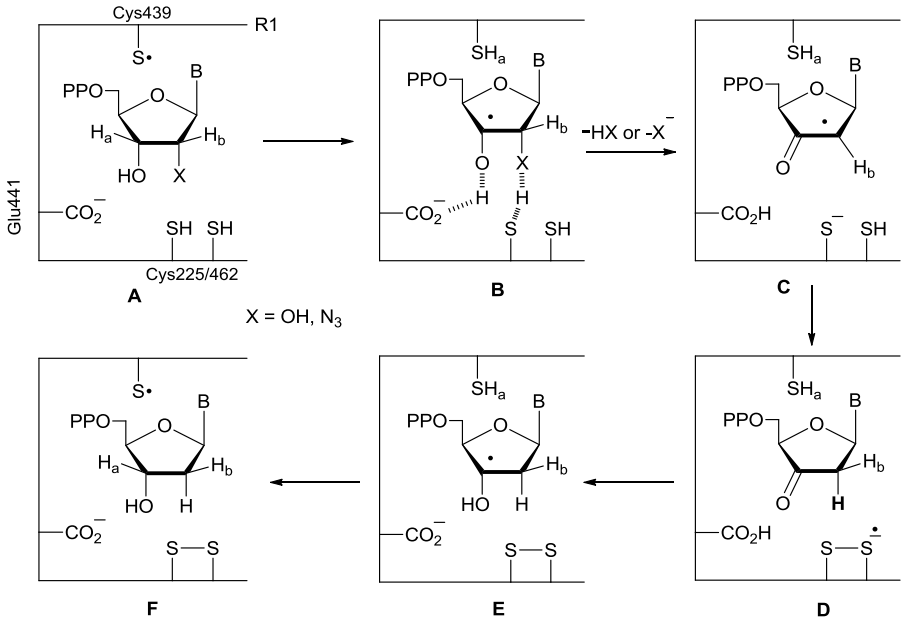


Figure 5. Proposed mechanism for the reduction of ribonucleoside diphosphates with RDPR.[12]

Stubbe, *et al.*[25] also proposed hypothesis for the inactivation of the RDPR by 2'-chloro-2'-deoxyuridine (Figure 6). Proton and electron transfer takes place from R2 subunit to R1 subunit to generate SCys439 free-radical. The SCys439 radical abstracts

hydrogen from C3' position to generate radical at C3', **H**. Hydroxyl proton from C3' position forms the hydrogen bonding with the carboxylate group of glutamate and leads to **loss of chloride as anion** from C-2' to give α-keto radical **I.** The α-keto radical **I** abstracts proton from cysteine C439 to give 2'-deoxy 3'-keto NDP **J**. Successive β-eliminations of base from H2' and iPP (pyrophosphate) from H4' gives furanone **K**. The α-keto radical **I** can alternatively abstract hydrogen atom from the dithiol pair to give intermediate **L**, which upon successive β-eliminations generates furanone **K**. But in the transformation of **L** to **K**, regeneration of cysteine C439 free radical does not take place which, in turn, avoids the initiation of another catalytic cycle. The furanone **K** is a Michael acceptor which alkylates the nucleophiles on enzyme and causes covalent enzyme inhibition.[40] This hypothesis proposed by Stubbe, *et al.* is based on the departure of **chlorine as an anion**.

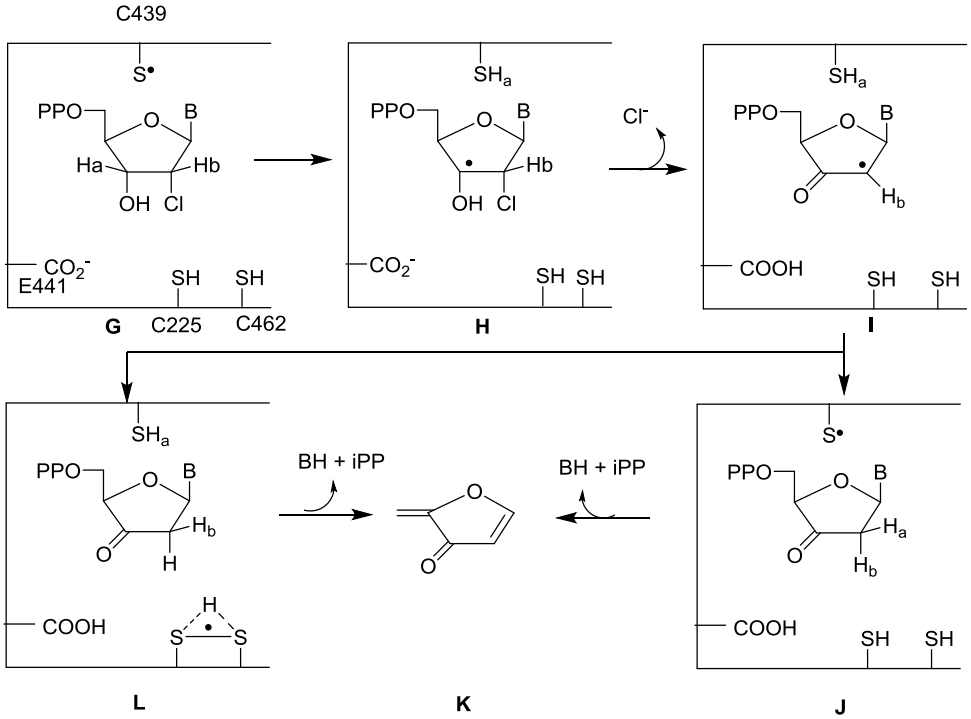


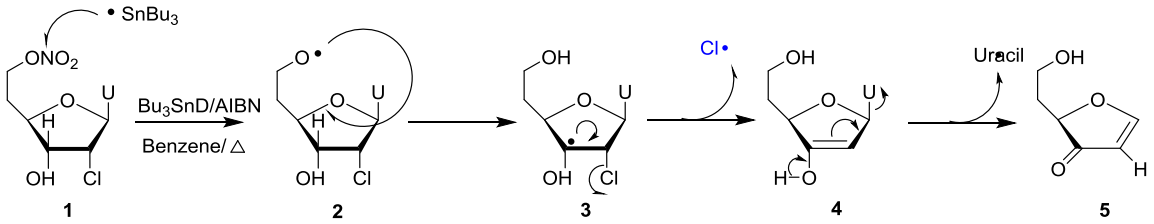
Figure 6. Proposed mechanism for inactivation of RDPR by 2'-chloro-2'-deoxy-NDPs.[25]

1.2.1.2. Biomimetic studies by Robins and Giese

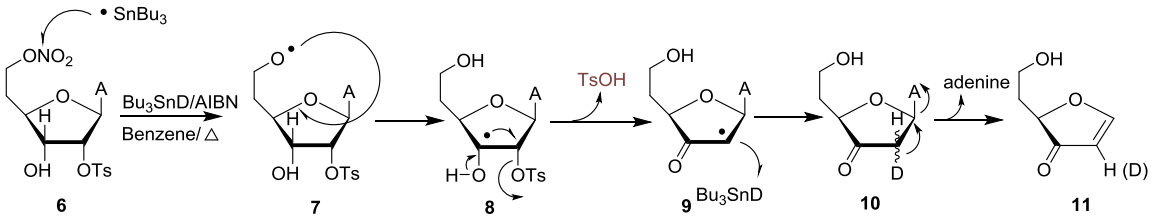
Robins *et al.*[34,35] and Giese[33] worked on the biomimetic reactions designed to simulate the initiation/elimination cascade that occurs during reductions and mechanism-based inactivation mediated by RNRs. Biomimetic reactions are performed to study the nature of elimination of substituent from C2' position upon generation of radical at C3' position.

Robins *et al.* designed the 6'-*O*-nitro-2'-(substituted) homonucleosides that upon treatment with tributyltin hydride and AIBN allow generation of 6'-oxyl radical which were positioned to abstract H3' and produce hydroxyl C3' radical. Scheme 1 demonstrates the plausible mechanism for the conversion of 2'-chloro-6'-*O*-nitro-homouridine derivative **1** into furanone **5** and Scheme 2 demonstrates the plausible mechanism for the conversion of 6'-*O*-nitro-2'-*O*-tosyl homoadenosine derivative **6** into **11**. The 6'-oxy radical, generated from the treatment of substrate **1** with Bu_3SnD/AIBN, abstracts hydrogen from the C3' (by [1,5]-hydrogen shift via a six member cyclic transition state)[41,42] and eventually leads to the loss of chlorine as a radical rather than as an anion. The β-elimination of uracil base from the enol intermediate results in furanone **5** without incorporation of deuterium. Instead, the deuterium transfer from Bu_3SnD to chlorine occurs and that propagates the radical chain reaction. Similarly, treatment of 6'-*O*-nitro-2'-*O*-tosylhomoadenosine **6** with Bu_3SnD/AIBN gives 6'-oxy radical[43] leads to the abstraction of H3' (by [1,5]-hydrogen shift via a six member cyclic transition state)[44] to give C3' radical **8**. Loss of tosylate from **8**[45] leads to a C2'-radical **9** which, after capturing a deuterium radical from Bu_3SnD and subsequent β-elimination of adenine, gives

partially deuterated furanone **11**. Thus no deuterium incorporation into **5** was observed upon treatment of 2'-chloro homouridine derivative with Bu_3SnD whereas furanone **11** with deuterium incorporation was formed upon treatment of 2'-*O*-tosyl homouridine derivative with Bu_3SnD. Proposed mechanisms prove the ionic *vs* radical cleavage of substituents from C2' position of nucleosides. However, deuterium transfer into **11** takes place selectively from the less hindered α-face[46] to give unstable 2'-deoxy-2'-deuterio-3'-ketohomoadenosine **10** [α/β~4:1]. The β-elimination of adenine from ketone **10** would give **11** with only 15-30 % deuterium incorporation at C2' position. They found that **chlorine atom** eliminates (as **radical**; **1→5**)[47] and **toluenesulfonic acid** (as **anion**, **6** → **11**)[48] from C2' upon generation of α-hydroxy radicals at C3'.



Scheme 1. Proposed mechanism for the one-electron (chlorine atom) elimination upon generation of C3' radicals[34,47,48]



Scheme 2. Proposed mechanism for the two-electron (toluenesulfonate anion) elimination upon generation of C3' radicals[34,47,48]

Robins *et al* also found that departure of the substituents from C2' upon generation of 3'-deoxy C3' radicals lacking 3'-hydroxyl group underwent elimination via different

paths.[49] Hence, treatment of 2'-(azido, bromo, chloro, iodo, and methylthio) nucleoside 3'-thionocarbonates with Bu_3SnH/AIBN produced 3'-deoxy C3' radicals that underwent loss of the 2'-substitutent to give 2',3'-didehydro-2',3'-dideoxynucleosides. On the other hand, analogous 3'-thionocarbonates with 2'-fluoro or 2'-*O*-(mesyl or tosyl) substituents (anionic leaving groups) underwent hydrogen transfer to the C3' radical to give the 3'-deoxy-2'-[fluoro or *O*-(mesyl or tosyl)] derivatives (Figure 7). Thus treatment of 2'-deoxy-3'-*O*-phenoxythiocarbonyl nucleosides, **12** with azido, bromo, chloro, iodo, or methylthio substitution at 2'-position with Bu_3SnH generates C3' radicals, **13** (Figure 7). Further the reaction cascade leads to the elimination of the substituent as a radical from the C2' with formation of 2',3'-unsaturated derivative **14**. While treatment of 2'-deoxy-3'-*O*-phenoxythiocarbonyl nucleosides, **12** with fluoro, mesyloxy, or tosyloxy substituents at the 2'-position with Bu_3SnH generates radical at 3'-position, **13** which abstracted hydrogen from the stannane to give 3'-deoxy-2'-(fluoro, mesyloxy, or tosyloxy) derivatives **15**. Since the homolytic scission to release a high energy fluorine atom or a mesyloxy or tosyloxy radical is energetically prohibitive the elimination did not occurred.

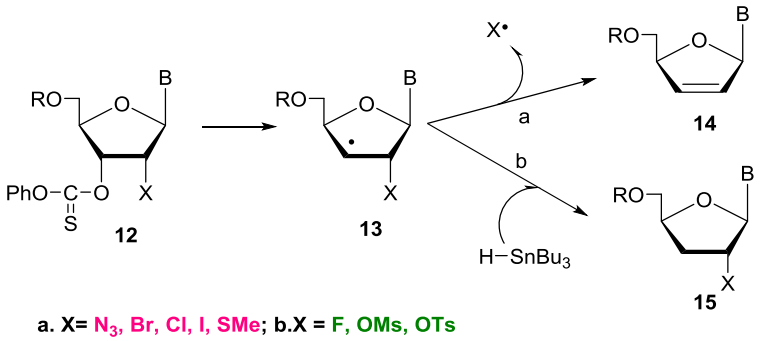
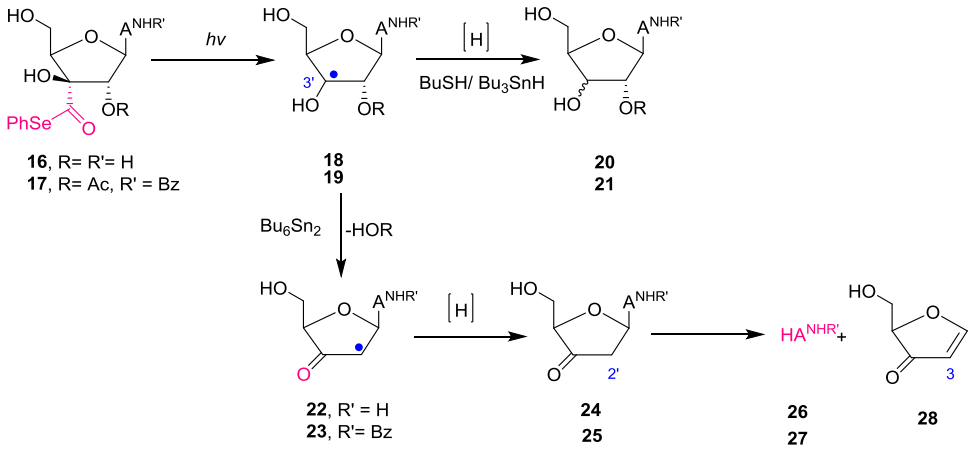


Figure 7. Fragmentation of 2'-substituted-3'-thionocarbonates upon treatment with Bu_3SnH/AIBN/Δ.

Conversion of nucleotides to 2'-deoxynucleotides catalyzed by RNR starts with generation of radical at C-3′ of nucleotide with further loss of hydroxyl from C-2'.[24,39]

Several attempts have been made to trap radical at C-3′ in the enzymatic system[50] and in the biomimetic systems[51] but the direct observation of the nucleotide radical were not possible. Giese *et al*[33] performed biomimetic studies to trap radical at C-3′ nucleoside. They developed a method to selectively generate a radical at the C-3′ of the nucleoside. They introduced the selenol ester group at the C-3′ which is chemically stable but can be cleaved off homolytically under mild conditions. The Se-C bond in the selenol ester moiety is weak and can be cleaved off easily with either by directly using UV light ($\lambda >$ 320 nm) or in a photochemically induced radical chain process using Bu_3SnH.

Photolysis of the precursors was achieved by subjecting them to radiations at 20 °C in methanol or acetonitrile/water (1:1) and in the presence of an H-donor such as Bu_3SnH or ^{t}BuSH as radical scavenger. Mixtures of acetonitrile and aqueous buffer solutions like triethylammonium acetate buffer (TEAA buffer, pH 7) were employed to mimic the reaction conditions found in an enzyme pocket.



Scheme 3. Traping C-3′ radical generated from selenol ester precursor

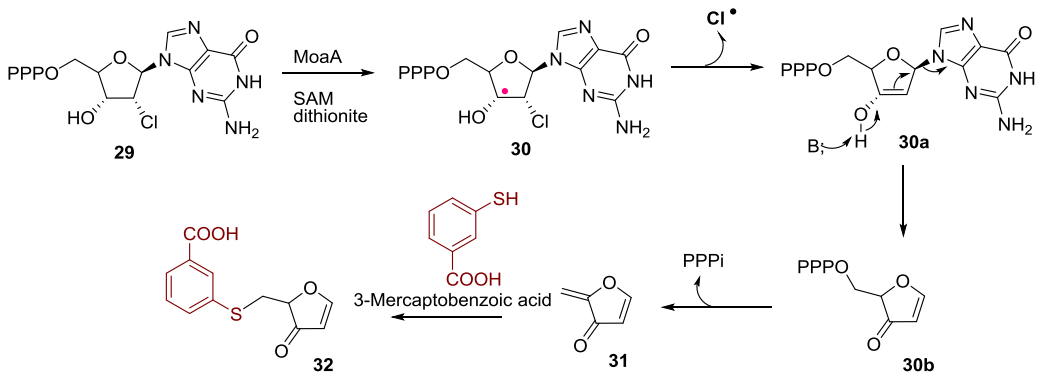
Precursor **16** led to the formation of reduction product **20** as exclusive product of the reaction (Scheme 3). Deuteration at C-3′ observed when the reaction was performed

using deuterated solvent indicating that the selenol ester **16** is an excellent radical precursor and the radical can be selectively generated at C-3′ either by photo induction chain reaction or direct photolysis. In this case since the Bu_3SnH or ^tBuSH are highly efficient in trapping the radical at C-3′ the formation of 2'-deoxygenated product could not compete with the direct reduction. In order to achieve the formation of the 2'-deoxygenated product **28** Giese *et al*[33] used Bu_6Sn_2 as a radical mediator. Because the much slower radical trapping reaction with the use of Bu_6Sn_2, 57% of adenine **26**, 10% of 2,3-dihydro-2-hydroxymethylfuran-3-one **28**, and only 35% of product **20** was produced.

Photolysis of the precursor **17** having acetate at C-2′ as a better leaving group, clarifies that formation of free nucleobase is fully related to the 2′-deoxygenation. Same reaction when performed employing Bu_3SnD as trapping reagent >90% of deuteration at C-2′ of **25** and 52% at C-3 of **28** was observed. When the highly reactive radical scavenger ^tBuSH was used direct reduction product **21** was formed. Deuterium incorporation at C-3′ (>80% by NMR) was observed when reaction was performed using CD_3OD and ^tBuSH.

1.2.1.3. Enzymatic studies by Begley

Recently Begley *et al.* proposed mechanism of the MoaA catalyzed reaction of 2'-chloroGTP, **29** to probe the mechanism of molybdoterin biosynthesis.[52] MoaA is a radical *S*-adenosylmethionine enzyme which catalyzes the first step in molybdoterin biosynthesis.[53] Interestingly the authors proposed that in the MoaA catalyzed reaction of 2'-chloroGTP, the substituent from 2'-position departs as a radical upon generation of the radical at C3'-position (Scheme 4),[52] which is in agreement with the Robins biomimetic studies (see scheme 1).



Scheme 4. Mechanism of the MoaA catalyzed reaction of 2'-chloroGTP

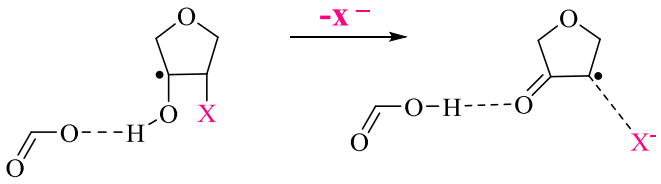
1.2.1.4. Theoretical considerations by Ramos

Ramos *et al.*[32] noted that the enzymatic and the biomimetic systems are different. In biomimetic systems the basic group capable of protonating/deprotonating the 3'-HO group is absent. The basic group is crucial for the enzymes. It allows the transfer of charge from and into the substrates as needed. In most of the enzymes E441 serve as a basic group capable of protonating/deprotonating the 3'-HO group, and is fundamental to several enzymatic reaction steps.[54] As in the enzymatic system the basic function is present, the inhibitor can eliminate an anion without generating a system with a charge separation (Scheme 5). The substituent from the 2'-position eliminates as an anion while the proton from the 3'-OH eliminates as cation thus circumventing the charge separation.

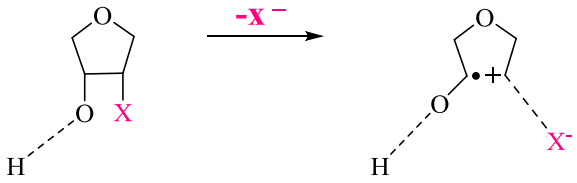
In contrast biomimetic systems do not contain the basic function and therefore the elimination of a substituent from the 2'-position as an anion will result in a system with a charge separation: a negative charge in the leaving substituent and a positive charge an unstable carbocation radical in the 2'-C position (Scheme 5). Thus according to Ramos *et al*. this is why in biomimetic experiments the substituents are not eliminated as anions

and depart as radicals. Also, biomimetic experiments were performed in benzene/toluene which is known to stabilize chlorine radicals.

System A : **With base adequate to deprotonate 3'-HO**



System B : Without base adequate to deprotonate 3'-HO



Scheme 5. Elimination of the substituents as anions with (top) and without (bottom) a basic group able to deprotonate 3'-hydroxyl group

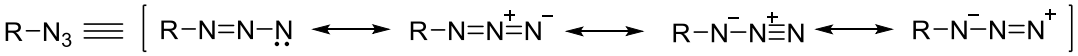
In order to check this hypothesis Ramos *et al*. designed a simple model system and performed the theoretical calculations. System-A (Scheme 5) includes a basic group (E441) capable of protonating/deprotonating the 3'-HO group and system-B does not include the basic function. They found that in the system A the basic function takes the proton from the 3'-HO group, and further the substituent from the 2'-position eliminates chlorine as anion. However in system B the substituent did not eliminate spontaneously and leaves as a radical.

Thus they concluded that the nature of the elimination of the substituents from 2'-position can be controlled. Substituents will depart as anion if the basic group is present (as in the enzymatic system) or it will depart as a radical in absence of the basic residue

capable of protonating/deprotonating the 3'-HO group. Since enzymes contain such basic functionality they eliminate anions and not radicals.

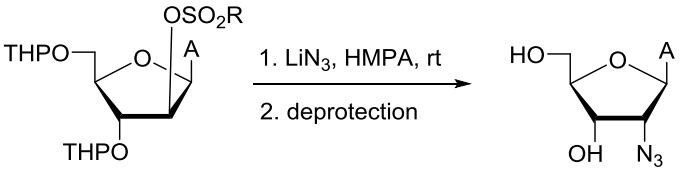
1.3. Chemistry of azido nucleosides: Synthesis and applications

The first organic azide compound, phenyl azide, was synthesized by Peter Grieb in 1864. A few years later the Curtius rearrangement was developed. Organic azide compounds gained industrial interest because of its use in the synthesis of heterocycles such as triazoles and tetrazoles as functional groups in compounds having medicinal properties. Azides are represented by following resonance structure.



Synthesis of the modified azido nucleosides gained high importance after the discovery of 3′-azido-3′-deoxythymidine for the treatment of AIDS. Following are the established methods for the synthesis of the azido modified nucleosides.

1. Nucleophilic displacement by azide: 2′-azido-2′-deoxyadenosine could be prepared by direct nucleophilic displacement of suitably protected 2′-sulfonate of arabino-furanosyladenines. The triflate **35** (Scheme 6) upon reaction with LiN_3 produced product in high yields in comparison to the mesylated, **33** or tosylated, **34** starting material.[55,56]

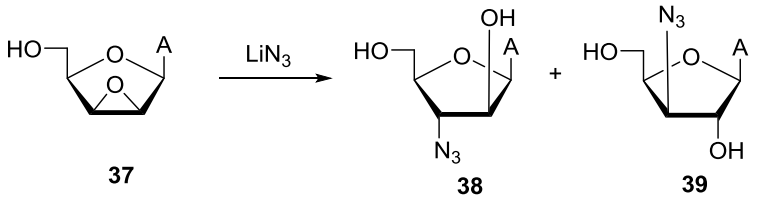


33, R = CH_3
34, R = $C_6H_4NO_2$
35, R = CF_3

36

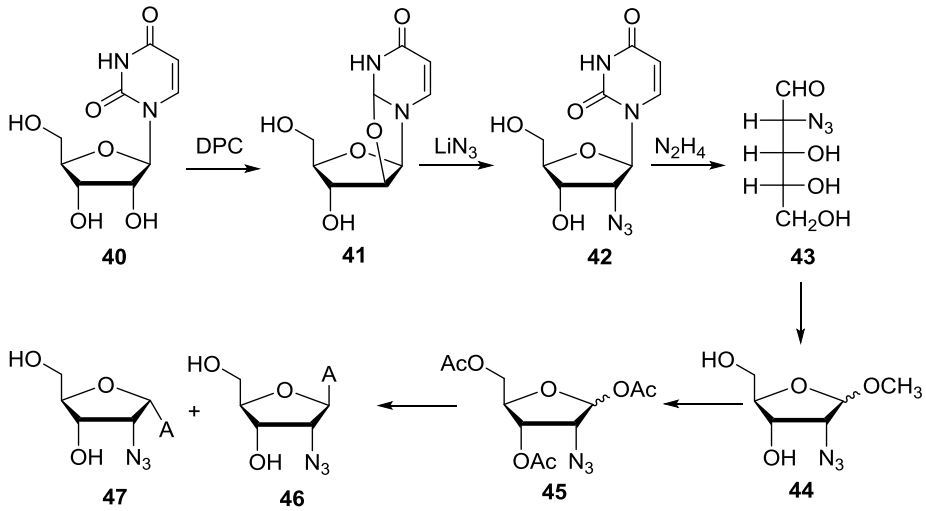
Scheme 6. Synthesis of 2′-azido-2′-deoxyadenosine

2. Opening of nucleoside epoxides by azide: Derivatives of nuclosides like, 2′-*O*-anhydro and epoxides also acts as starting material for the synthesis of azidonucleosides. LiN_3 does not cleave the epoxides preferentially at the C-3′ site and therefore this method is not suitable for the synthesis of 2′-azido-2′-deoxynucleosides. For example treatment of lyxofuranosyladenosine with LiN_3 yielded mixture of arabino, **38** (79%) and xylo, **39** (8%), respectively (Scheme 7).[57]



Scheme 7. Synthesis of azido nucleosides from epoxides

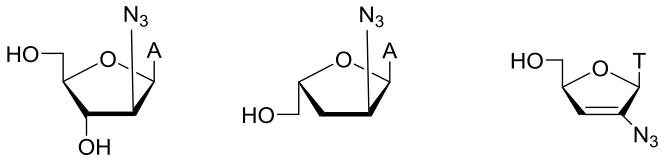
3. From azidosugars: Azidouridine, **42** was prepared from readily available uridine, **40** *via* 2,2'-*O*-anhydrouridine (Scheme 8). Further the glyosidic bond was cleaved by hydrazine hydrate to obtain 2-azido-2-deoxy-D-ribose **43**. Methyl glycoside **44** was prepared from **43** and which upon acetylation gave **45**. Diacetate, **45** subsequently coupled with adenine followed by deactylation gave anomeric mixture of **46** and **47**.[58]



Scheme 8. Synthesis of azidonucleosides via 2,2'-*O*-anhydrouridine

Applications of azido nucleosides:

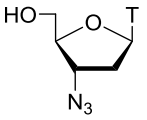
1. 2′-Azido-2′-deoxynucleosides: The 2′-azido-2′-deoxynucleosides analogue, **48** (Figure 8) displayed significant cytotoxic and antiviral activities.[59] The 2′-azido-2′,3'-dideoxynucleoside compound **49** inhibits the HBV replication in concentrations between 2.2 and 5.0 µM.[60] Similarly, the didehydronucleoside compound **50** displayed inhibitory activity against HIV-1 and HIV-2 in vitro.[61]



48 **49** **50**

Figure 8. Selected 2′-azido-2′-deoxynucleosides with biological activities

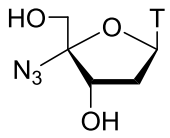
2. 3'-Azido-3'-deoxynucleosides: AZT, **51** (Figure 9) also known as Zidovudine is an antiretroviral drug,[62] approved by FDA for the treatment of HIV. It is marketed under the brand name of Retrovir. It acts by reducing the replication of virus. The AZT can also be used for the prevention of the HIV transmission from mother to child during birth.



51

Figure 9. Selected 3'-azido-3'-deoxynucleosides with biological activities

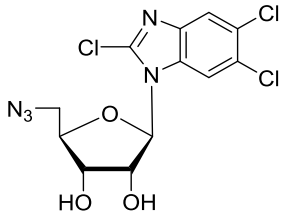
3. 4'-Azidonucleosides: 4'-azido-2'-deoxy-α-D-nucleosides displayed HIV inhibitory activity.[63] These compounds showed reduced activity upon modifications at the 2'- or 3'-position. 4'-azidothymidine, **52** (Figure 10) and were evaluated against cells infected with HIV. These compounds displayed similar inhibitory activity to that of AZT.



52

Figure 10. Selected 4'-azidonucleosides with biological activities

4. 5'-Azido-5'-deoxynucleosides: Trichlorobenzimidazole derivative, **53** (Figure 11) displayed significant activity against human cytomegalovirus in plaque.[64]



53

Figure 11. Selected 5'-azido-5'-deoxynucleosides with biological activities

1.3.1. Azido nucleosides as inhibitors of RNR

Sjoberg, Stubbe and coworkers used EPR spectroscopy to study the reaction between ribonucleotide reductase and substrates and substrate analogues. Studies with the mechanism-based inhibitors of RNR substrate, 2'-azido-2'-deoxyuridine-5'-diphosphate have provided insight into the mechanism(s) of reduction of natural NDPs into dNDPs.[16-19] They studied the characteristics of the new EPR signal and determined the localization of the unpaired electron at nucleotide molecule, which was the first direct evidence for free radical chemistry with RDPR.

Studies have been done with the various isotopically labeled derivatives: (1'-, 2'-, 3'-, or 4'- [2H])-N_3UDPs and 2'- [$^{15}N_3$, ^{13}C]-N_3UDP to examine the mechanism of inactivation of RDPR by 2'-azido-2'-deoxynucleoside 5'-diphosphate (N_3UDP).[17] Experiments with doubly labeled 2'[$^{15}N_3$]-azido-2'-[^{13}C]-UDP showed that the resulting nitrogen-centered radical was derived from the azide moiety and had no hyperfine interaction with the ^{13}C

nucleus, which required cleavage of the 2'-carbon-nitrogen bond.[17] Furthermore, EPR studies with a [β-^{2}H] cysteine-labeled RDPR have indicated that the nitrogen centered radical is covalently bound to the sulfur of a cysteine of the R1 subunit of RNR.[65]

Cys439 thiyl radical abstracts the H3' in substrate and subsequently the loss of azide anion takes place from the initial C3' radical intermediate to give the ketyl radical **B** (Figure 12). Further proton-coupled electron transfer generates the 2'-deoxy-3'-ketonucleotide **C** to generate the thiyl radical in the active site. Conversion (**B** $\rightarrow$ **C**) is analogous to the proposed mechanism for the reduction of natural NDP, which also leads to generation of the identical 3'-keto-2'-deoxynucleotide intermediate,[12,66] which make study of the inhibition of RNR by N_3UDP more significant.

Reaction of hydrazoic acid with the thiyl radical generates N_2 and a sulfinylimine radical **H.** The sulfinylimine radical **H** was not detected by EPR. It is postulated that the protonated azide is essential for this mechanism since it is unlikely that the thiyl radical would react with an azide ion.[17,19] The initial nitrogen-centered radical **H** reacts further with the oxygen or carbon atoms of a carbonyl group of the 3'-keto-2'-deoxynucleotide to generate radicals **D** or **G**, respectively.[18] The inactivation of the enzyme with 3'[^{17}O]-N_3UDP[67] **A** were most consistent with the formation of **G** and provided the first evidence for the trapping of a 3'-ketonucleotide in the reduction process by a nitrogen-centered radical **H**.[19] The chemical requirements also favor formation of **G** (over **D**) and there is precedent in literature for the analogous addition of aminyl radicals to the carbonyl[68] and imino groups.[69]

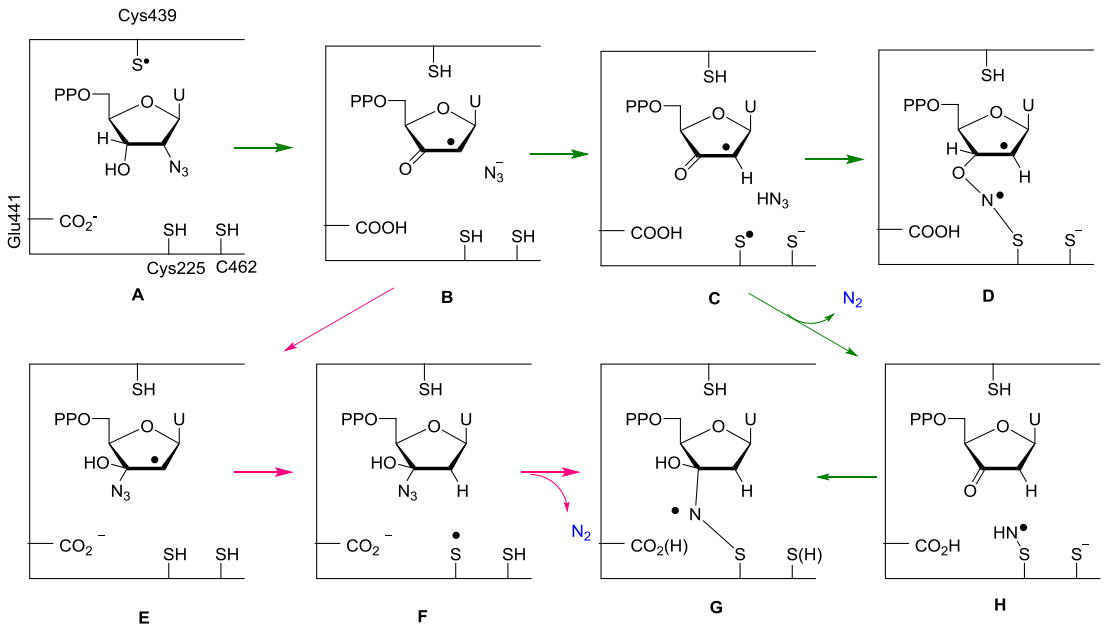


Figure 12. Proposed structures for the nitrogen centered radicals and pathways for their generation during inactivation of RDPR by N_3UDP

Pereira and coworkers performed theoretical modeling study and provided the alternative[70] hypothesis, in which the released azide ion is proposed first to add to the 2'-ketyl radical **B** with the concomitant protonation of the ketone oxygen by E441. The resulting radical **E** is then reduced at the 2'-position by C225 to generate the C225 thiyl radical **F**. Subsequent attack of the thiyl radical on an alkyl azide (instead of HN_3) would lead to the same nitrogen center radical **G**, which was detected experimentally.[19]

1.3.2. Reduction of azido nucleosides to amino nucleosides

1.3.2.1. Staudinger reduction

Staudinger and Meyer developed in 1919 a method for the reduction of azides to amines with phosphines (Figure 13).[71] Triphenylphosphine reacts with azide to produce phosphazide intermediate which subsequently loses N_2 to form iminophosphoranes. Further the aqueous workup leads to formation of the amine and phosphine oxide.

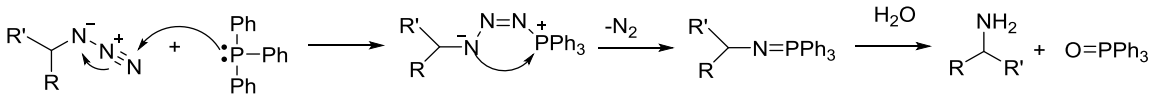
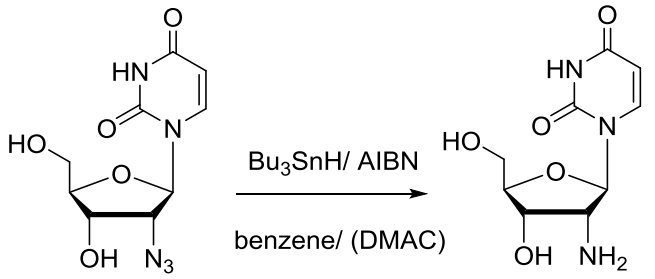


Figure 13. Reaction mechanism for the Staudinger reduction

The phosphoroamidite-based solid phase synthesis of azido modified nucleosides can be problematic because of the Staudinger reduction of the azide group to amino group with the trivalent phosphrous reagent used.

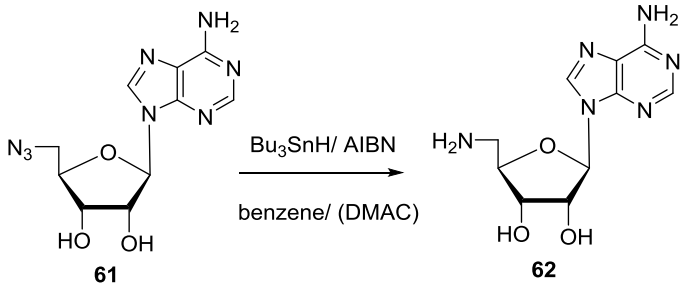
1.3.2.2. Radical reduction

Radical mediated reduction of azide to amine can be accomplished by stoichiometric Bu_3SnH (Figure 14 (1)).[72-76] This reduction of azide is an example of a Sn-N bond-forming process. The most efficient route for the synthesis of primary amines from alcohol is the activation of the alcohols to make it a good leaving group and subsequent displacement of the oxy anion by azide. Methods available for the reduction of the azide to amine are catalytic hydrogenolyses, reduction with Raney nickel with or without hydrazine, hydrogen sulfide/mecaptans, and the Staudinger phosphine/phosphite method. Robins *et al.*[74] developed and reported the Bu_3SnH mediated reduction of azide to amine from unprotected azidonucleosides. Treatment of unprotected 2'-azido-2'-deoxyuridine **42** with Bu_3SnH/AIBN in hot benzene yielded the 2'-amino-2'-deoxyuridine **60** (Scheme 9). Analogues treatment of 5'-azido-5'-deoxyadenosine, **61** with Bu_3SnH/AIBN afforded 5'-amino-5'-deoxyadenosine, **62** (Scheme 10)**.**



42 **60**

Scheme 9. Bu_3SnH-mediated reduction of 2'-azido-2'-deoxyuridine to 2'-amino-2'-deoxyuridine[74]



61 **62**

Scheme 10. Bu_3SnH-mediated reduction of 5'-azido-5'-deoxyadenosine to 5'-amino-5'-deoxyadenosine

Fu *et al.*[77] developed reaction condition where this reduction of azide to amine can be achieved by catalytic use of Bu_3SnH and a silicon hydride fills the role of stoichiometric reductant. Bu_3SnH-catalyzed, silicon hydride-mediated azide reduction process (Figure 15) has not proved to be practical and afforded the product in low yield. Fu *et al.* employed another strategy which relies upon reduction of Sn-O to Sn-H[78] (Figure 16). Addition of alcohol to the reaction transfer $SnBu_3$ group from the $RNHSnBu_3$ to the oxygen of alcohol. The silicon hydride reduces resulting tin hydride to regenerate the catalyst, Bu_3SnH.

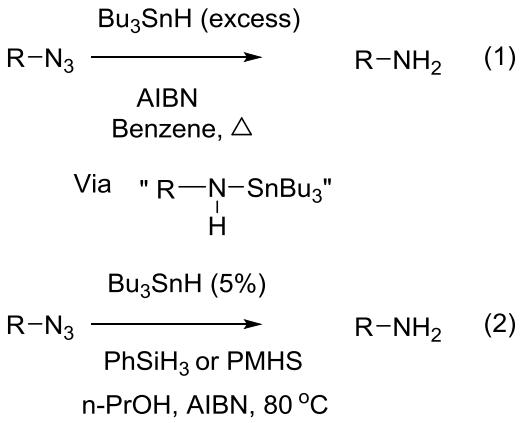


Figure 14. Bu_3SnH-mediated reduction of azide to amine.[77]

Reaction of primary, secondary, tertiary or aryl azide with Bu_3SnH (5 mol %) and $PhSiH_3$ (0.6 equiv) employing less hindered *n*-PrOH (2 equiv) gave primary amine in good yield.[77]

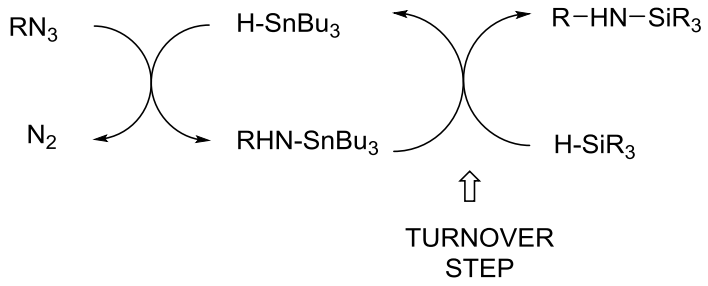


Figure 15. Bu_3SnH-catalyzed, silicon hydride-mediated azide reduction.[77]

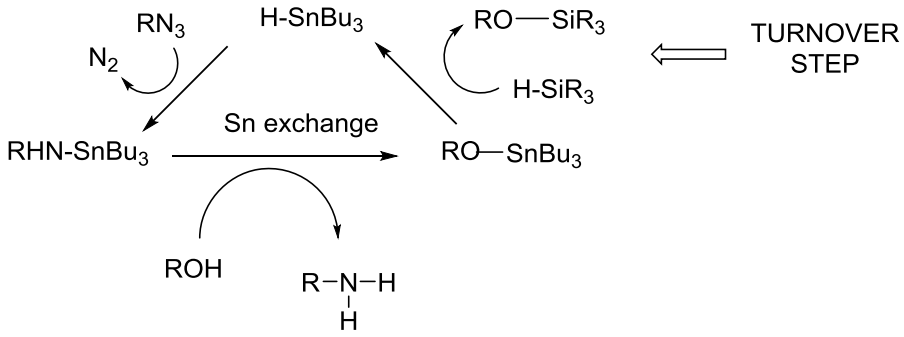
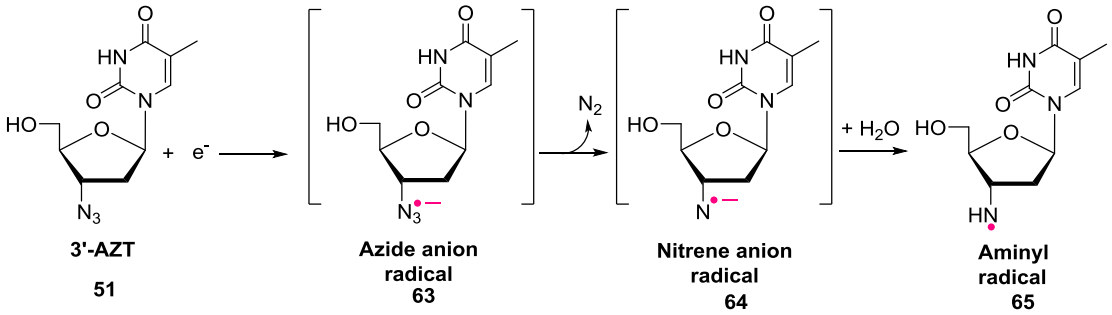


Figure 16. Bu_3SnH-catalyzed, silicon hydride-mediated azide reduction: Alternate approach[77]

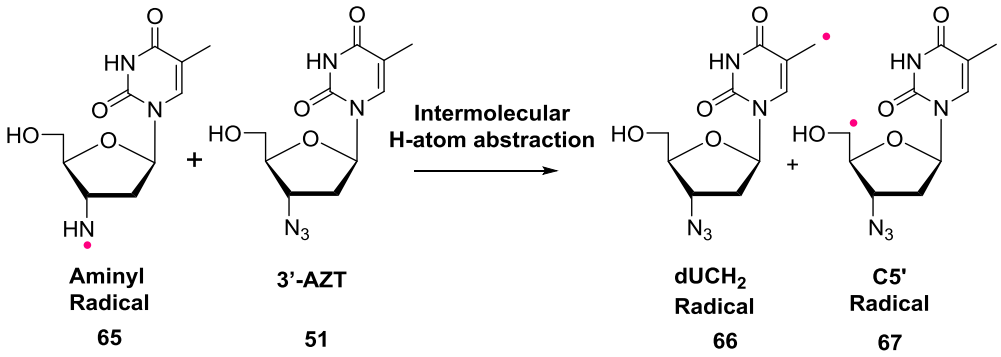
1.3.2.3. One electron reduction of 3'-azido-3'-deoxythymidine

Sevilla and coworkers reported the formation of the neutral aminyl radical (RNH•) from 3′-azido-3′-deoxythymidine (3′-AZT) and 3′-azido-2′,3′-dideoxyguanosine (3′-AZG).[79] The 3′-AZT, **51** upon electron attachment in γ-irradiated aqueous glassy (7.5 M LiCl) systems at 77K in the absence of oxygen, results in an unstable azide anion radical intermediate, **63** ($RN_3^{\bullet -}$) which was not spectroscopically detected. Loss of N_2 from ($RN_3^{\bullet -}$) forms a highly basic and unstable nitrene anion radical **64** ($RN^{\bullet -}$) which has also not been detected spectroscopically. Nitrene anion radical **64** upon subsequent protonation give neutral aminyl radical **65** (RNH•) (Scheme 11). [79]



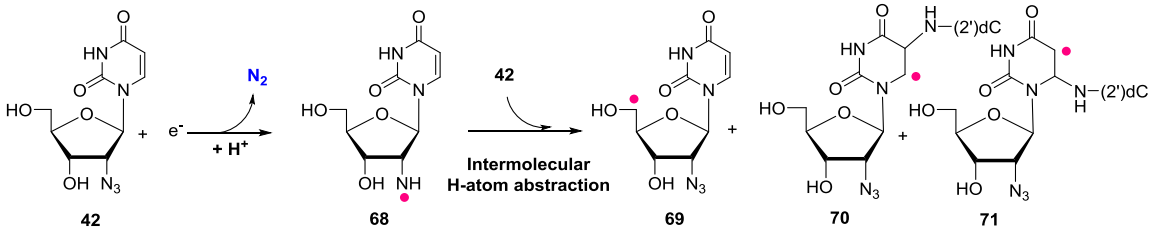
Scheme 11. Formation of aminyl radical in 3'-AZT

The aminyl radical **65** (RNH•) is highly reactive and has been found to be potent H-atom abstracting agent leading to base damage and formation of sugar radicals. On annealing to higher temperatures (ca. -113 to -103 ^{o}C), aminyl radical in 3′-AZT undergoes a bimolecular H-atom abstraction either from the methyl group at C5 in a thymine base to give $dUCH_2$• radical **66** or from the C5′-atom to give C5′• radical **67** of a proximate 3′-AZT (Scheme 13).[79] Azide group in the modified nucleoside showed more attraction towards the electron compared to the thymine moiety which is consistent with the theoretical calculations and the ESR spectral calculations.



Scheme 12. Intermolecular H-atom abstraction by 3'-AZT aminyl radical

Generation of the aminyl radical is also observed in case of the 2'-azido-2'-deoxyuridine (2'-AZdU, **42**) (Scheme 13).[80] As like 3'-AZT, subjection of the 2'-azido-2'-deoxyuridine (2'-AZdU, **42**) to γ-irradiation in homogeneous aqueous glassy (7.5 M LiCl) solutions produces the aminyl radical (U(C2')-NH•, **68)**. The aminyl radical in (2'-AZdU, **68**) undergo bimolecular hydrogen atom abstraction at C5' of 2'-AZdU to produce a carbon-centered radical or can add bimolecularly to the C5=C6 double bond yielding a 6-yl (addition to C5), **70** as well as a 5-yl (addition to C6), **71** radicals.



Scheme 13. Intermolecular H-atom abstraction by 2'-AZdU aminyl radical

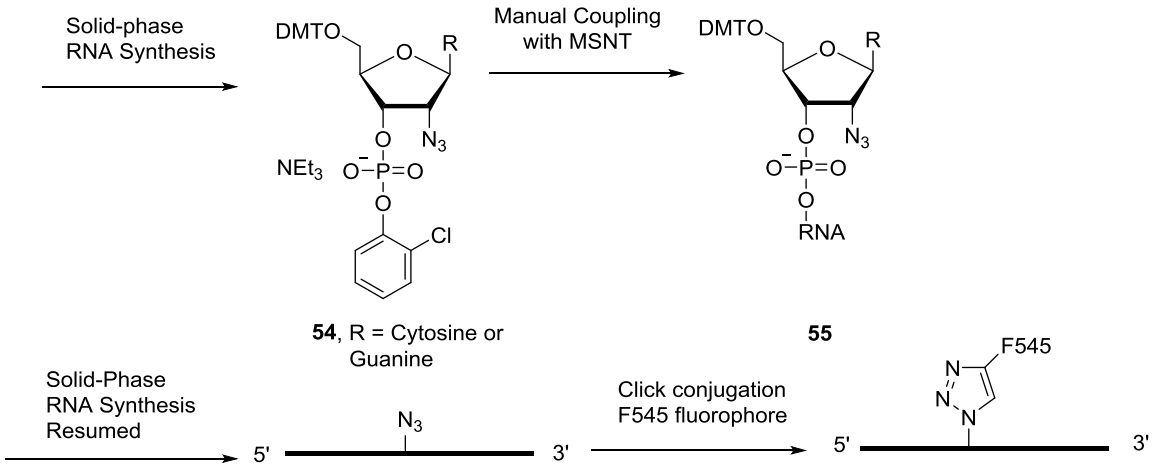
1.3.3. Azido nucleosides as substrates for Cu (I)-catalyzed reaction (CuAAC) and strain-promoted azide-alkyne cycloaddition (SPAAC).

Azido-modified oligonucleotides are widely used for the synthesis of bioconjugates employing azide-alkyne click chemistry approach.[81-87] Phosphoramidite reagents are used to introduce the terminal alkynes or cyclooctynes into the

oligonucleotides. However this approach cannot be used for the azido modified compounds[88] as the azide with P (III) in the phosphoramidite undergoes Staudinger reaction with the azide. Therefore the *H*-phosphonate[89,90] and the phosphotriester[91-94] protocols are used for the synthesis of azide-modified oligonucleotides. Other options are post synthetic incorporation of azides into oligonucleotides[95-97] and enzymatic[98-100] methods for the incorporation of the azide-modified triphosphates. The use of azido oligonucleotides gained interest with the introduction of strain-promoted azide−alkyne cycloaddition (SPAAC).[101] The azido compounds can be coupled with both terminal alkynes in a Cu (I)-catalyzed reaction (CuAAC) and strained cycloalkynes. It has been reported that the Staudinger reaction does not take place between the azide group in the support-bound growing nucleotide chain and the phosphoramidite reagents.[89] Thus automated phosphoramidite synthesis could be used for the azide-containing solid supports to produce 3′-azidemodified oligonucleotides.[102-104]

Fauster *et al.*[105] synthesized the cytidine and guanosine 2′-azido modified analogues. Synthesis of oligonucleotides using phosphoramidite chemistry is generally not possible since azides are proven to be susceptible to Staudinger reduction in the presence of P (III).[86] Inspite of this finding Fauster proved that the azido group at the C2′-position of furanose are compatible to the phosphoramidite reagent with the steps of automated oligonucleotide synthesis (Scheme 14).

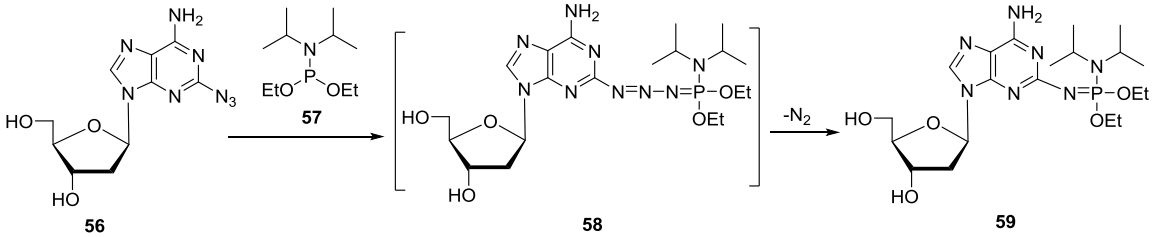
Phosphoramidites were incorporated site-specifically into the growing oligonucleotides using 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT). Automated addition of remaining residues of phosphoramidities continued without affecting the free C2'-azide group.



Scheme 14. Synthesis of azido-modified RNA fragments using phosphoramidite chemistry.[86]

Sekine *et al.*[88] prepared 2-azidodeoxyadenosine, **56** from deoxyguanosine by using combined reagent of $TMSN_3$-BuONO. Further to incorporate **56** into DNA they tried two approaches. First approach is the phosphoramidite method and the other is *H*-phosphonate approach.

Although it is known that the azide compounds react with trivalent phosphorus compounds[106-108] to yield iminophosphorane derivatives. To check the compatibility of 2-azidodeoxyadenosine with phosphoramidite reagents Sekine *et al*. performed reaction of **56** with diethyl *N*,*N*-diisopropylphosphoramidite, **57** in DMF-CD_3CN (Scheme 15). Initial product was the intermediate **58** of Staudinger reaction, and the final product was iminophosphorane **59.**



Scheme 15. Reaction of 2-azidodeoxyadenosine with *N*,*N*-diisopropylphosphoramidite reagent.[88]

Further the synthesis of the oligodeoxynucleotides containing 2-azidodeoxyadenosine was achieved by H-phosphanate approach.

1.3.4. Formation of aminyl radicals in nucleosides and its importance in DNA damage

Deoxyribonucleic acid (DNA) in the living organisms is more prone to generate radicals upon subjection to the ionizing radiations. Mostly the radicals are formed in the heterocyclic bases purine and pyrimidine compared to the sugar part of the nucleoside.[109] These radicals are responsible for the most of the damage in the nucleotides e.g., strand breaks and interstrand cross-links.[110] Studies have been done to generate the reactive intermediates of interest employing techniques like UV photolysis and γ-radiation.[111] It is difficult to study the free radical generation and its reactions in actual DNA because of its complex nature. To understand the mechanism of formation of the radical and its reaction simpler model systems are developed and the experiments were performed in aqueous solution. Formation of the hydroxyl radicals is also reported but lifetime of this radical is very short while the nitrogen centered radicals have longer lifetime.[112] Several research groups worked to study the reactivity of these radicals and these studies helped understand the reaction mechanisms and revealed complexities in damage pathways. Therefore to understand the formation and reactivity of the radicals it is important to design and synthesize the precursors that could generate aminyl radical.

1.4. Sulfenamides: Synthesis and medicinal relevance

1.4.1. Sulfenamides in organic chemistry

Sulfenamides is a class of organosulfur compounds which is represented by the general formula of $RSNR'_2$, where R and R' are H, alkyl, or aryl groups. Sulfenamides are sometimes spelled as sulphenamides and are designated by different ways such as sulfamines, sulfur amines, mercaptoamines, aminothio compounds, amino sulfides, sulfur amides, and thiohydroxylamines. They are considered as a derivative of sulfenic acids. Sulfenamides are named in the similar way as sulfonamide but the prefix "sulfon" should be changed to "sulfen". The nomenclature of the sulfenamides has not been systematized thoroughly.[113]

The chemistry of sulfenamides is much less explored. Number of workers reported the thermal instability of the sulfenamides.[114,115] Interactions between the lone pairs of electrons on sulfur and nitrogen may destabilize the S-N bond polarization.[116] Introduction of electron withdrawing group (EWG) on the aryl group attached to either N or S atom increases the stability of sulfenamide significantly.[113,117]

Sulfenamides are photolabile compounds classified as type-1 phototherapeutic agents.[117] They are reported to generate abundant free radicals upon photoexcitation with UV/Visible light. These photolyzed compounds cause cell death in a dose dependent manner. Photochemotherapy is a form of therapy which uses light sensitive compounds that are nontoxic and upon exposure to light, they become toxic to the targeted malignant and other diseased cells. Phototherapeutic agents are classified into two broad categories type-1 and type-2.[117] In type-1, absorption of light by the photosensitizer causes bond fragmentation to produce reactive intermediates such as free radicals, which induces cell

death. Type-2 processes induce cell death by generation of reactive oxygen species (ROS) like hydroxyl radical, superoxide radical anion. Photosensitizer upon excitation transfers energy to nearby molecular oxygen to produce singlet oxygen and subsequently produce ROS like, hydroxyl radical, superoxide radical anion which are responsible for the cell death. Thus type-2 process is facilitated by singlet oxygen and needs red light for optimal generation of singlet oxygen, in contrast the type-1 process does not require oxygen for activity and therefore the type-I process could be useful for removal of lesions under hypoxic conditions.

Sulfenamide modified nucleosides are underdeveloped as compared to the azido modified nucleosides. If sulfenamide modified nucleosides will generate the aminyl radical successfully, they can have advantage over azido modified nucleosides as they should be stable under the conditions required for the standard solid phase synthesis of deoxynucleosides.[105,107,118] Thus the sulfenamides can serve as more convenient substrates for the synthesis of nucleotides and oligo nucleotides.

1.4.2. Sulfenamides as potential antitherapeutics

Several sulfenamide derivatives have been developed in past few decade possessing medicinal values. The pyridinamine sulfenamide, **72** and oxazolidinone sulfenamide, **73** (Figure 17) are reported to inhibit platelet lipoxygenase and leukocyte migration.[113] Sulfenamide **73** also displayed the antiasthmatic properties. The pyridyl sulfenamide, **74** showed the antitumor activity against tumor cells in female mice. Omeprazole, **75** is used for the treatment of gastric ulcers and shows its activity by inhibiting the gastric (H+/K+)-ATPase present in the acidic compartments of the

parietal.[119,120] Omeprazole is a prodrug and is transformed into the active form in acidic media to produce the effect.

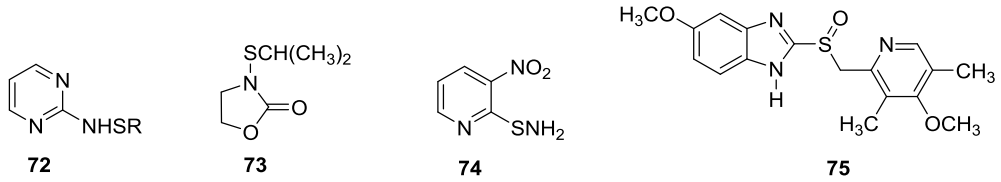
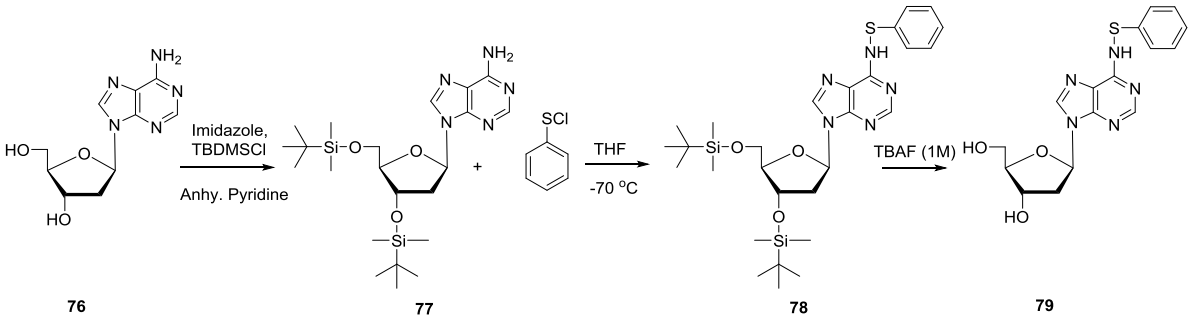


Figure 17. Structure of sulfenamide derivatives with medicinal applications

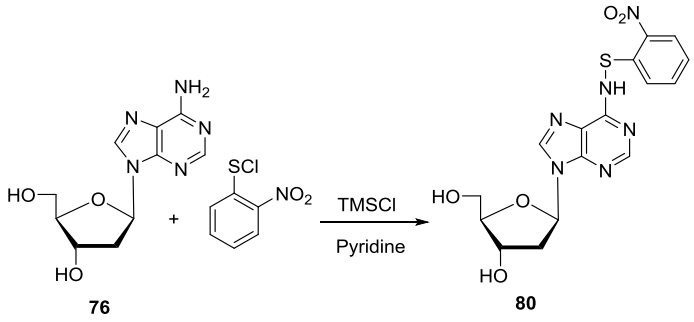
1.4.3. Nucleosides modified with sulfenamide moieties

Kuttappan and coworkers observed the formation of the nitrogen (N^6) centered aminyl radical in 2'-deoxyadenosine (dAdo).[121] They prepared two different modified 2'-deoxyadenosines, N^6-[phenylsulphenyl]-2'-deoxyadenosine, **79** (Scheme 16) and N^6-[2-nitrophenylsulphenyl]-2'-deoxyadenosine, **80** (Scheme 17) to study the photochemical properties. N^6-[phenylsulphenyl]-2'-deoxyadenosine was prepared from 2'-deoxyadenosine, **76**. Silyl protection of hydroxyl groups in the deoxyadenosine gave silylated deoxyadenosine **77**.[122,123] Nucleophilic attack of the exo 6-amino group of **77** on the phenylsulphenyl chloride, afforded protected sulfenamide **78**. Further TBAF assisted removal of silyl protection group in **78** afforded N^6-[phenylsulphenyl]-2'-deoxyadenosine, **79**.[124]



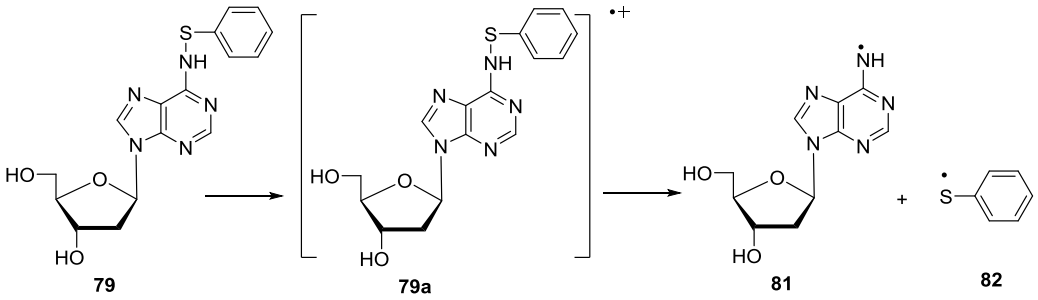
Scheme 16. Synthesis of N^6-[phenylsulphenyl]-2'-deoxyadenosine

Similarly the N^6-[2-Nitrophenylsulphenyl]-2'-deoxyadenosine, **80** was prepared from 2'-deoxyadenosines, **76** in one step. Treatment of 2'-deoxyadenosines, **76** with 2-nitrophenylsulphenyl chloride, in presence of TMSCl and pyridine afforded N^6-[2-nitrophenylsulphenyl]-2'-deoxyadenosine, **80**.[125]



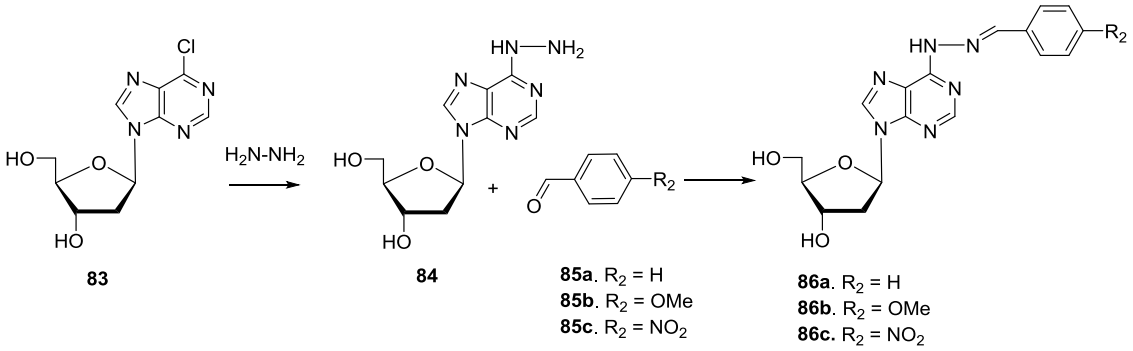
Scheme 17. Synthesis of N^6-[2-nitrophenylsulphenyl]-2'-deoxyadenosine[121]

The UV photolysis of compound **79** (Scheme 18) and **80** was performed in 20% acetonitrile/water. Both compounds displayed good absorption in the near UV region, and were irradiated at 302 nm. Cleavage of the N-S bond in **79** and **80** observed to afford dAdo as a major product. The products from the photolysis of N^6-[2-nitrophenylsulphenyl]-2'-deoxyadenosine **80** were analyzed by HPLC and characterized by mass spectrometry. They also checked the reactivity of both compounds **79** and **80** by irradiating them with γ-rays and observed that the N^6-[phenylsulphenyl]-2'-deoxyadenosine **79**, was more reactive than the compound **80**. Also the aminyl radical formation was confirmed by pulse radiolysis method.



Scheme 18. Mechanism for the formation of aminyl radical from N^6-[phenylsulphenyl]-2'-deoxyadenosine[121]

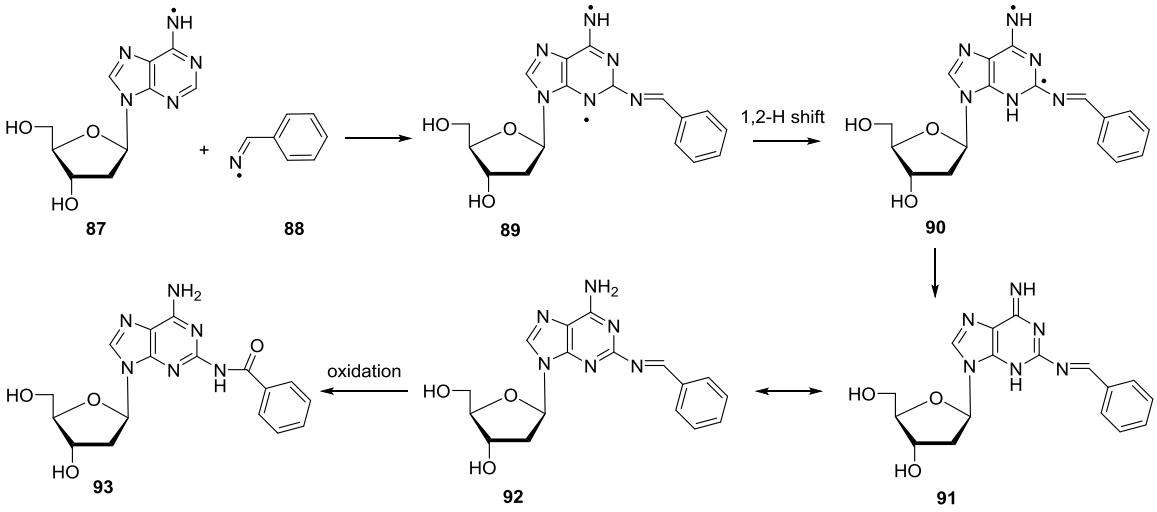
Wagner *et al*[126] reported the formation of the dAdo N^6-aminyl radicals from the 2'-deoxyadenosine (dAdo) containing photoactive hydrazone substituents (Scheme 19). They prepared three derivatives **86a**, **86b** and **86c** of 2'-deoxyadenosine containing the hydrazine substituent at N^6 to generate N^6-aminyl radicals of dAdo.



Scheme 19. Synthesis of 2'-deoxyadenosine containing the hydrazine substituent at N^6.[126]

All three derivatives of dAdo showed UV absorption between 300 and 400 nm and upon UV photolysis in presence of glutathione, H-donor produced dAdo N^6-aminyl radicals and benzylidene iminyl radicals. The photolysis studies were performed in the presence of non-modified dAdo in order to mimic the reactions taking place in DNA. N^6-aminyl radical, **87** and benzylidine radicals, **88** recombined to yield 2-(benzylideneamino)-2'-deoxyadenosine **92** (Scheme 20) alternatively the N^6-aminyl

radical also undergoes intramolecular addition to non-modified dAdo. Subsequently compound **92** undergoes autooxidation to produce **93**.

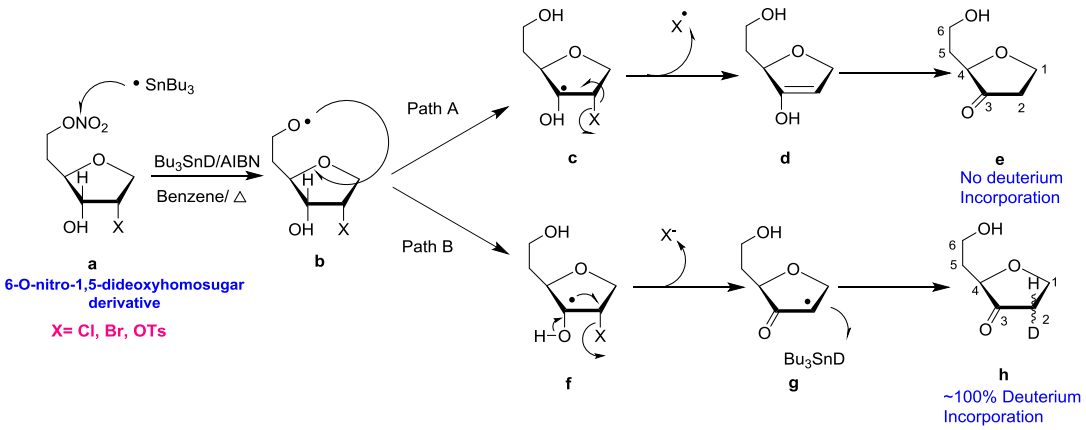


Scheme 20. Generation of the aminyl radical and its subsequent reactions from 6-*N*-hydrazine-2'-deoxyadenosine substrates.[126]

2. RESEARCH OBJECTIVES

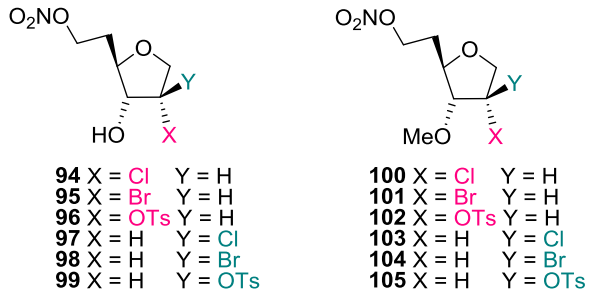
The first goal of my dissertation was to clarify the ambiguity in the previously described mechanism of inhibition of ribonucleotide reductase (RNR) enzyme by 2'-chloro-2'-deoxyuridine. The initial target was to synthesize 6-*O*-nitro-1,5-dideoxyhomosugar derivatives with chlorine, bromine or tosyl substituent at the C2 position from diacetone glucose via multistep synthetic routes. The biomimetic model reactions (intended to mimic the radical-initiated reactions postulated to occur at active sites of RNR) with these 2-substituted hexofuranoses were designed to provide chemical evidence which would distinguish the nature of the elimination of chlorine from 2'-chloro-2'-deoxyuridine upon its incubation with the enzyme.

The model 6-*O*-nitro-1,5-dideoxyhomoribofuranose derivatives with halo (chloro, **94** or bromo, **95**; Figure 18) substituent at C2 position upon treatment with Bu_3SnH/AIBN were expected to undergo radical-initiated fragmentation with elimination of the C2 halo substituent as radical to give furanone **e** with no deuterium incorporation at C2. In contrast, tosyl group from 6-*O*-nitro-2-*O*-tosyl precursors was expected to depart as anion upon subjection to radical fragmentation with Bu_3SnH/AIBN to give furanone **h** with 100% deuterium incorporation at C2 (Scheme 21).



Scheme 21. Expected results from the biomimetic fragmentation of 6-*O*-nitro-1,5-dideoxyhomoribose derivatives

To probe the effect of streochemical inversion corresponding 6-*O*-nitro-1,5-dideoxy homoarabinofuranose derivatives **97**, **98** and **99** were designed with substituents at C2 position in *anti*-orientation to the C3-hydroxyl group (Figure 18). I also designed the 3-*O*-methyl substrates with *ribo* (*syn*) and *arabino* (*anti*) configurations (**100**- **105**). These 3-*O*-methyl precursors were expected to probe the role of proton on the 3-hydroxy group. The 3-*O*-methyl precursors lack the possibility to be converted to 3-keto product, an important intermediate during the RNA-catalyzed reactions.



94 X = Cl Y = H
95 X = Br Y = H
96 X = OTs Y = H
97 X = H Y = Cl
98 X = H Y = Br
99 X = H Y = OTs

100 X = Cl Y = H
101 X = Br Y = H
102 X = OTs Y = H
103 X = H Y = Cl
104 X = H Y = Br
105 X = H Y = OTs

Figure 18. 2-Substituted hexofuranoses and 3-*O*-methyl hexofuranoses

Kinetic experiments were also planned to provide additional evidence to support the results obtained from biomimetic model reactions. Kinetic experiments would allow discrimination between heterolytic C2'-chlorine bond cleavage (with departure of chloride anion) and homolytic C2'-chlorine bond cleavage (with departure of chlorine radical) upon regioselective generation of radical at C3' (carbon-β). In order to study the kinetics, the progress of the reaction (rate) for 6-*O*-nitro-1,5-dideoxyhomohomosugar derivatives with chlorine, bromine or tosyl substituent at the C2 position were proposed to be investigated by ^{1}H NMR. The rate of reaction is expected to be lower in the case of departure of C2 chlorine as an anion when compared to the elimination of C2 tosyl group as an anion. Whereas, the rate is expected to be higher for the departure of chlorine as a radical than that of tosyl anion.

In the second objective of my dissertation I proposed to synthesize azido modified nucleosides, sulfenamide modified nucleosides and 2-azidolyxofuranoside derivatives. The nucleosides are designed to have the azido or sulfenamide substitution at a specific site in the sugar or in the base moiety. These compounds upon subjection to a radiation produced electrons, were expected to generate aminyl radical (RNH•) at a specific site. Generation of the RNH• and their subsequent reactions were proposed to be monitored

using electron spin resonance (ESR) spectroscopy. These studies were designed to understand the mechanism of damage in various DNA model structures

I planned to synthesize 2'-azido-2'-deoxycytidine (2'-AZdC, **106**) and 4'-azidocytidine (4'-AZC, **107**) to explore the possibility of formation of aminyl radical and to study its subsequent reactions (Figure 19). In 2'-AZdC the aminyl radical site is attached to a 2^{o} C-atom (2'), where as in 4'-AZdC, the aminyl radical site is attached to a 3^{o} C-atom (4') in the sugar moiety, respectively. The difference in the stereo and electronic environment of these site-specific RNH• would be expected to clarify the difference in their reactivity.

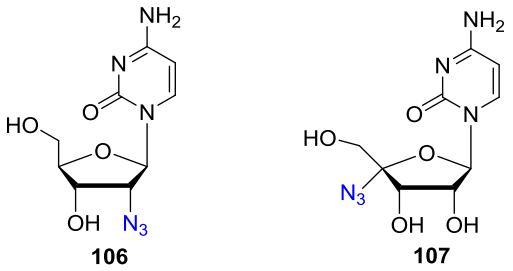
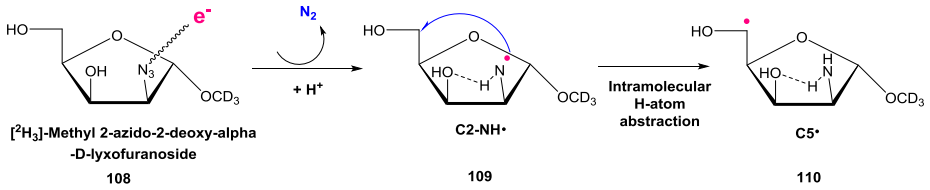


Figure 19. Structure of 2'-AZdC and 4'-AZC

To avoid the interaction of the transient radical with the nucleoside heterocyclic bases, I also designed 2-azidolyxofuranoside derivatives as a, simpler abasic models. I proposed to synthesize methyl 2-azido-2-deoxy-α-D-lyxofuranoside and its deuterium labeled analogues. The 2-azidolyxofuranoside derivatives (e.g., **108**; Scheme 22) were expected to generate aminyl radical, **109** and the reactive aminyl radical was further anticipated to participate in the intramolecular C5 hydrogen abstraction to generate 5'-sugar radical, **110**. In 2-azidolyxofuranoside derivatives the substituents at C1 and C2 have the *trans* relative configuration and with removal of base we could further drive intramolecular interaction to produce the expected 5'-sugar radical. The deuterium

analogues of the 2-azidolyxofuranoside were designed to provide additional evidences about the position of the radical species that formed from the bi- or uni-molecular abstraction of hydrogen in sugar.



Scheme 22. Formation of aminyl radical from [$^2H_3$]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside and its subsequent reaction

I was also interested to examine if sulfenamide modified nucleosides which might also serve as a suitable substrate for the site specific generation of aminyl radical via radiation produced electrons. The synthesis of nucleosides with sulfenamide substitution at specific sites, either in the sugar (**111** and **112**) or base (**113**) were planned (Figure 20). Although, nucleosides with sulfenamide modifications are less studied compared to azido analogues, the formation of RNH• from sulfenamides has been reported. If these sulfenamides would generate the aminyl radical successfully they could be incorporated into the deoxyoligonucleotides. Compared to the azido modified nucleosides the sulfenamide modified nucleosides are expected to be more stable towards the phosphoramidite reagents used for the solid phase deoxyoligonucleotides synthesis.

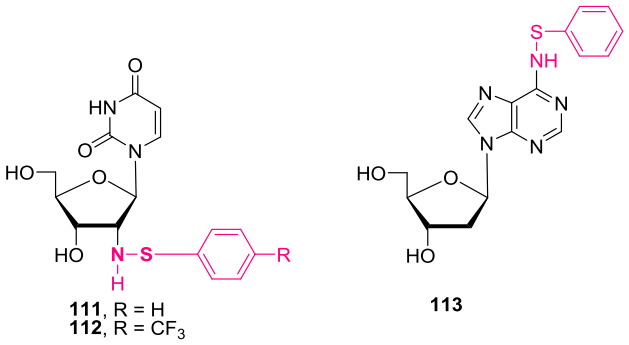


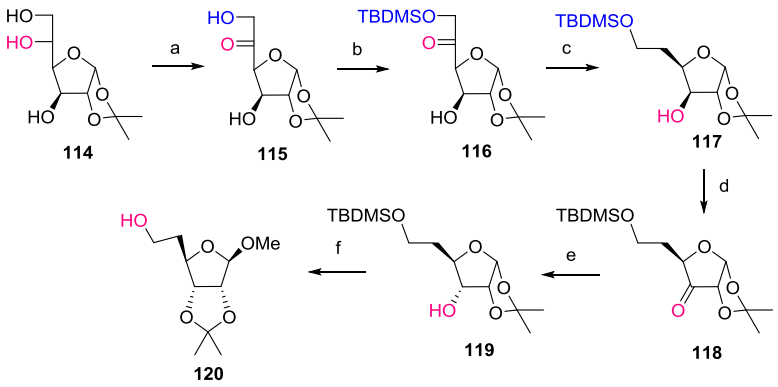
Figure 20. Sulfenamide modified nucleosides for generation of *N*-centered radical at sugar or base

3. RESULTS AND DISCUSSION

3.1. Modeling inhibition of ribonucleotide reductase by 2-substituted hexofuranoses

3.1.1. Synthesis of 2-substituted 1,5-dideoxyhomosugar analogues

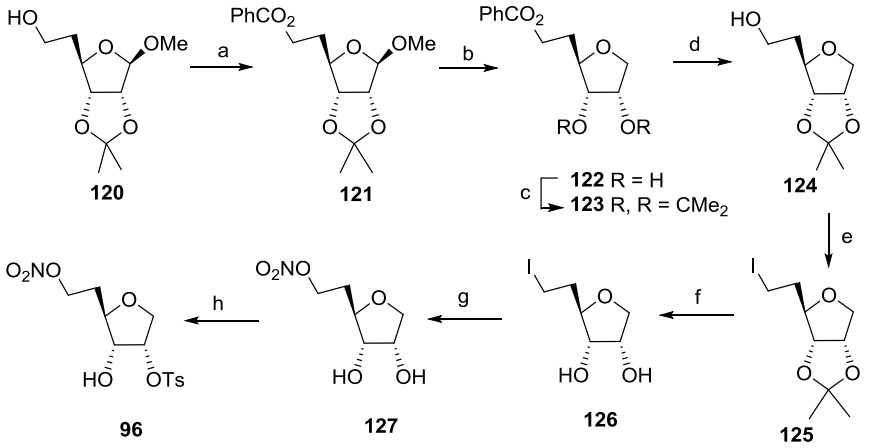
Synthesis of methyl 5-deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside **120** which served as convenient starting material for the preparation of model compounds, started with regioselective oxidation of 1,2-*O*-isopropylidene-α-D-glucose **114**. The critical steps in this synthesis of **120** were stereochemical inversion of configuration at C3 and regioselective deoxygenation at C5. Thus treatment of **114** with $(Bu_3Sn)_2O$ in chloroform and Br_2 afforded the 5-ulose **115**. Silylation of the hydroxyl group in **115** using TBDMSCl gave **116**. Deoxygenation of **116** using tosyl hydrazine via formation of tosylhydrazone yielded 5-deoxy sugar **117**. The configuration of hydroxyl group at C3 position in **117** is inverted by oxidation and further stereoselective reduction using sodium borohydride. Thus treatment of **117** with CrO_3 in pyridine afforded **118** and subsequent stereoselective reduction with sodium borohydride yielded **119**. Methanolysis of **119** and one-pot treatment with acetone gave **120** (~40% overall)[48](Scheme 23).



a. (i) $(Bu_3Sn)_2O$/ $CHCl_3$ /△ (ii) Br_2 b. TBDMSCl/ Pyridine c. (i) $TsNHNH_2$/ MeOH,4h, rt (ii) $NaBH_4$/reflux/8 h d.CrO_3/Pyridine/Ac_2O CH_2Cl_2/1 h/rt e. $NaBH_4$/EtOH/4 h/rt f. (i) HCl/MeOH (ii) Me_2CH $(OMe)_2$/Me_2CO/△

Scheme 23. Synthesis of methyl 5-deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside

Benzoylation of **120** using benzoyl chloride and selective deoxygenation of the resulting **121** at the anomeric carbon with excess (5 equiv.) boron trifluoride etherate and triethylsilane[127,128] produced a mixture of **122** and **123** due to the partial removal of the isopropylidene protection group (Scheme 24). Treatment of **122** with 1,2-dimethoxypropene/acetone/TsOH gave protected 1,4-anhydroalditol **123** (44% overall from **120**). Debenzoylation of **123** and subsequent iodination and deacetonization produced 6-iodo furanitol **126** (53% from **123**).



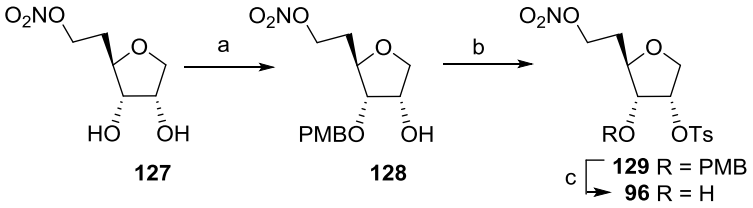
(a) $BzCl/Et_3N/CH_2Cl_2/0\ ^oC$. (b) $Et_3SiH/BF_3Et_2O/CH_2Cl_2$. (c) TsOH hydrate/acetone/2,2-dimethoxypropane. (d) KOH/MeOH. (e) Ph_3P/I_2/imidazole/toluene/Δ. (f) HCl/H_2O/MeOH. (g) $AgNO_3/CH_3CN$. (h) $Bu_2SnO/TsCl/Et_3N$/MeOH.

Scheme 24. Synthesis of 6-*O*-nitro-3-hydroxy-2-*O*-tosyl homosugar precursor

Displacement of iodide with $AgNO_3/CH_3CN$ yielded 1,4-anhydro-5-deoxy-6-*O*-nitro-D-*ribo*-hexofuranitol **127** (89%); a key substrate for further studies. Direct tin-mediated tosylation[129] of **127** produced a mixture of two isomers, from which the major 2-*O*-tosyl regioisomer **96** was isolated in 43% yield.

Alternatively **96** was obtained by tin-mediated benzylation of **127** with *p*-methoxybenzyl (PMB) chloride which also yielded a mixture of isomers from which 3-*O*-PMB regioisomer **128** was isolated (Scheme 25). Subsequent treatment of **128** with

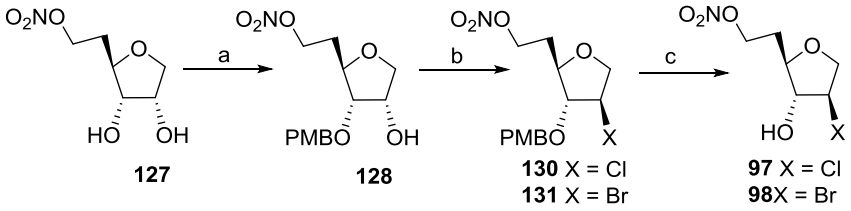
TsCl/pyridine followed by removal of PMB group from resulting **129** with ceric ammonium nitrate (CAN) afforded **96** (54% from **128**).



(a) Bu_2SnO/PMBCl/DMF/Δ. (b) TsCl/pyridine.(c) CAN/MeCN/H_2O.

Scheme 25. Alternative synthesis of 6-*O*-nitro-3-hydroxy-2-*O*-tosyl homosugar precursor

Mitsunobu-assisted chlorination of **128** with freshly prepared HCl•pyridine gave protected 2-chloro-D-*arabino*-hexofuranose **130** (62%) a substrate for further studies. Similar treatment of **128** with freshly prepared HBr•pyridine gave 2-bromo counterpart **131** (49%)**.** Debenzylation of **130** and **131** with CAN gave 2-chloro substrate **97** (78%) and 2-bromo substrate **98** (79%), respectively (Scheme 26).

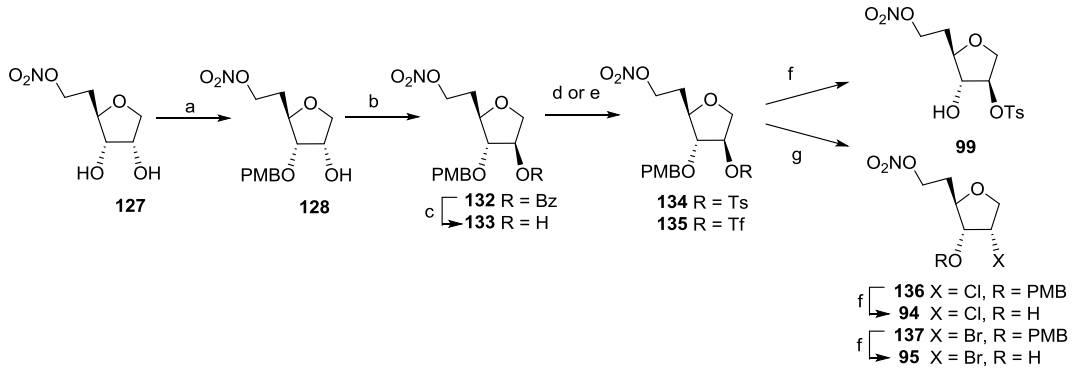


(a) Bu_2SnO/PMBCl/DMF/Δ. (b) Ph_3P/DIAD/HCl.pyr or HBr.pyr/THF.(c) CAN/MeCN/H_2O.

Scheme 26. Synthesis of 6-*O*-nitro-3-hydroxy-2-chloro/bromo homosugar precursor

The 2-chloro(or bromo)-D-*ribo*-hexofuranose was also prepared from **128** *via* double inversion approach. Thus, Mitsunobu-treatment of **128** with benzoic acid produced 2-*O*-benzoyl-D-*arabino*-hexofuranose **132.** Debenzoylation of **132** and treatment of the resulting **133** with TfCl/DMAP produced reasonably stable 2-*O*-triflate **135**. Displacement of triflate with LiCl or LiBr followed by debenzylation of the resulting **136**

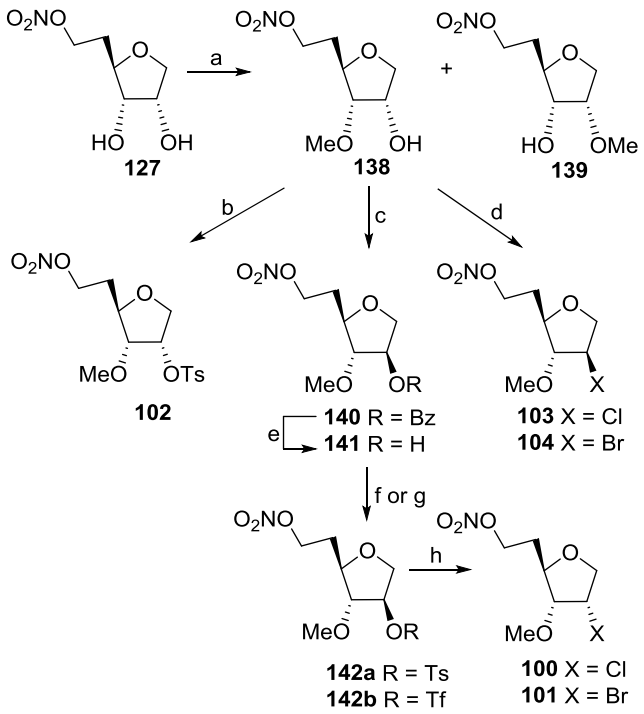
or **137** with CAN gave 2-chloro(or bromo)-D-*ribo*-hexofuranose **94** or **95.** Tosylation of **133** followed by debenzylation of the resulting **134** afforded 2-*O*-tosyl-*arabino* substrate **99** (Scheme 27).



(a) Bu_2SnO/PMBCl/DMF/Δ. (b) Ph_3P/DIAD/$PhCO_2H$/THF/-50°C.(c) KOH/MeOH. (d) TsCl/pyridine. (e) TfCl/DMAP/CH_2Cl_2/0°C. (f) CAN/MeCN/H_2O. (g) LiCl or LiBr/DMF.

Scheme 27. Synthesis of 6-*O*-nitro-3-hydroxy-2-chloro/bromo/tosyl homosugar precursor

I also prepared 2-substituted 6-*O*-nitro-3-*O*-methylhexofuranitol precursors for comparative studies. Thus, tin-mediated methylation of **127** produced a mixture of isomers **138**/**139** from which the 3-*O*-methyl regioisomer **138** was isolated in 38% yield (Scheme 28). Treatment of **138** with TsCl in pyridine yielded *ribo* 2-*O*-tosyl sugar **102** whereas Mitsunobu-treatment of **138** with benzoic acid followed by debezoylation of resulting **140** and subsequent tosylation of **141** gave *arabino* 2-*O*-tosyl epimer **142a.** Mitsunobu-assisted halogenation of **138** with HCl•pyridine or HBr•pyridine gave 2-chloro(or 2-bromo)-3-*O*-methyl-D-*arabino*-hexofuranose **103** or **104**. Triflation of **141** and subsequent displacement of triflate in **142b** with LiCl or LiBr gave 2-chloro(or bromo)-D-*ribo* epimers **100** or **101.**



(a) Bu_2SnO/MeI/DMF/Δ. (b) TsCl/pyridine. (c) Ph_3P/DIAD/$PhCO_2H$/THF/-50°C. (d) Ph_3P/DIAD/HCl.pyr or HBr.pyr/THF. (e) KOH/MeOH. (f) TsCl/pyridine. (g) TfCl/DMAP/CH_2Cl_2/0°C. (h) LiCl or LiBr/DMF.

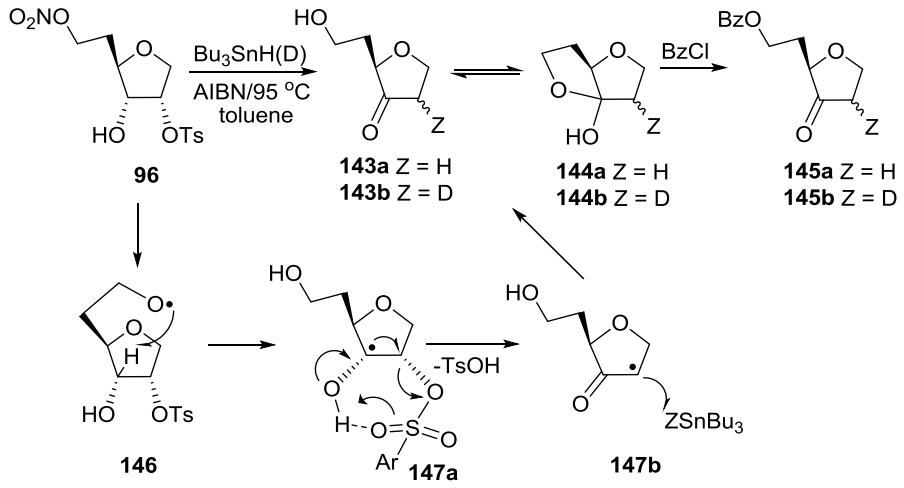
Scheme 28. Synthesis of 6-*O*-nitro-3-*O*-methylhexofuranitol precursors

3.1.2. Biomimetic studies

3.1.2.1. 6-*O*-Nitro-3-hydroxy-2-*O*-tosyl homosugar precursors

Treatment of the 6-*O*-nitro-2-*O*-tosyl *ribo* precursor **96** with Bu_3SnH/AIBN with toluene at 95 °C for 1 h gave 3-keto product **143a** [(*R*)-2-(hydroxyethyl)-3(2*H*)-dihydrofuranone] which was found to be in equilibrium with cyclic hemiacetal **144a** (66%, ~1:1; ^1H and ^{13}C NMR; Scheme 29) in addition to 6-hydroxy byproduct resulting from homolysis of nitrate ester without elimination of tosyl group (Table 1, entry 1). ^{13}C NMR shows ketone peak at 215.9 ppm for **143a** and hemiacetal peak at 114.9 for **144a**. Analogous treatment of **96** with Bu_3SnD, instead of Bu_3SnH gave 2-deuterio epimers (2*R/S*, ~1:1) of **143b** in equilibrium mixture (~ 1:1) with **144b** (71%, entry2). ^1H NMR

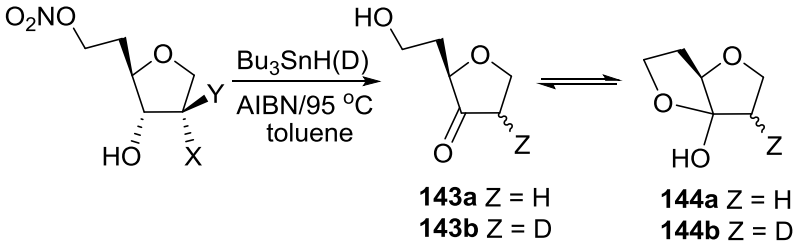
spectrum of **143b**/**144b** corresponded to that of the **143a**/**144a** with reduction of the integrated intensity for the H2/2' signal to half and simplification of the H1/1' signals. The ^{13}C NMR spectrum **143b**/**144b** showed triplets (with 1:1:1 intensity) at 36.7 and 38.9 for carbons 2 as results of splitting to deuterium while MS and HRMS showed up to 95% of isotopic ([^{2}H]) incorporation. Formation of **143a** or **144b** is consistent with generation of 6-oxyl radical **146** and abstraction[34,48] of H3 to give hydroxyl-containing C3 radical **147a**. Loss of toluenesulfonic acid from **147a**, with a concerted [1,2]-electron shift, would produce the C2-radical intermediate **147b**. Hydrogen or deuterium transfer from the stannane to **147b** would give **143a** or **143b** (Scheme 29). Treatment of the **143a**/**144a** mixture with BzCl provided a stable 6-*O*-benzoyl keto product **145a**. Analogous treatment of **143b**/**144b** with BzCl provided 6-*O*-benzoyl 2-deuterio epimer **145b**.



Scheme 29. Proposed mechanism for elimination of tosyl group from 6-*O*-nitro-3-hydroxy-2-*O*-tosyl homosugar precursor, 147a

These results are in agreement with Barton's nitrite[130] and Wagner's *δ*-substituted aryl ketone[131] photolysis studies that had shown that a six-membered transition state is favorable for abstraction of hydrogen by an oxyl radical. Also [1,5]-hydrogen shift was

observed with oxyl radicals generated from nitrate esters with Bu_3SnH in carbohydrates.[44] The radical-induced loss of toluenesulfonic acid (**147a** to **147b**) is analogous to the rearrangement which converts 2'-*O*-tosyladenosine into 9-(2-deoxy-*α*-D-*threo*-pentofuranosyl)adenine via [1,2]-hydride shift from C3' to C2' with loss of tosylate.[45,132] The present concerted [1,2]-electron shift with generation of a carbonyl group at C3' provides the driving force for expulsion of toluenesulfonic acid *via* seven-membered hydrogen-bonded intermediate **147a** is also in agreement with a theoretical studies[31,133] on RNR-catalyzed deoxygenation reactions as well as biomimetic studies.[33,48]



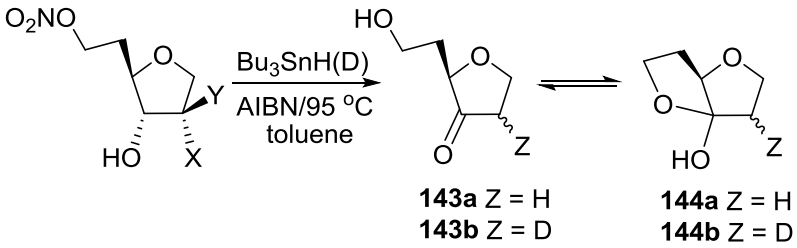
entry	substrate			reagent	product	ratio[b]	yield[c] (%)
	No	X	Y				
1	**96**	OTs	H	Bu_3SnH	**143a:144a**	1:1	66[d]
2	**96**	OTs	H	Bu_3SnD	**143b:144b**	1:1	71[d]
3	**99**	H	OTs	Bu_3SnH	**143a:144a**	1:1	93
4	**99**	H	OTs	Bu_3SnD	**143b:144b**	1:1	93

Table 1. Biomimetic studies with 6-*O*-nitro-3-hydroxy-2-*O*-tosyl homosugar precursors[a]

[a] Reactions were performed on 0.057 mmol (**96** or **99**) scale of substrates (0. 029-0.047 M) with 5 equiv. of $Bu_3SnH(D)$ and 2 equiv. of AIBN in toluene (95°C, 1-2 h.). [b] Determined by ^1H NMR. [c] Isolated yields. [d] 1,5-Dideoxy-2-*O*-tosyl-D-ribo-hexofuranose was also formed (19%).

3.1.2.2. 6-*O*-Nitro-3-hydroxy-2-chloro (or bromo) homosugar precursors

Similar treatment of 2-chloro **94** and 2-bromo **95** *ribo* precursors with Bu_3SnH also produced a 3-keto/hemiacetal mixture **143a**/**144a** (~1:1; 81%, entry 1 and 64%, entry 3; Table 2). Analogous treatment of **94** or **95** with Bu_3SnD yielded mixture of **143b**/**144b** as 2-deuterio epimers indicating elimination of chloride and bromide, as anions, rather than as chlorine or bromine radical.

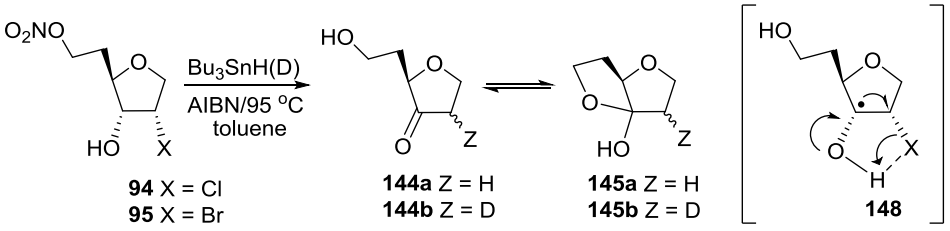


entry	substrate			reagent	product	ratio[b]	yield[c] (%)
	No	X	Y				
1	**94**	Cl	H	Bu_3SnH	**143a:144a**	1:1	81
2	**94**	Cl	H	Bu_3SnD	**143b:144b**	1:1	67
3	**95**	Br	H	Bu_3SnH	**143a:144a**	1:1	64
4	**95**	Br	H	Bu_3SnD	**143b:144b**	1:1	68
5	**97**	H	Cl	Bu_3SnH	**143a:144a**	1:1	97
6	**97**	H	Cl	Bu_3SnD	**143b:144b**	1:1	82
7	**98**	H	Br	Bu_3SnH	**143a:144a**	1:1	67[d]
8	**98**	H	Br	Bu_3SnD	**143b:144b**	1:1	54[d]

Table 2. Biomimetic studies with 6-*O*-nitro-3-hydroxy-2-chloro(or bromo)-homosugar precursors[a]

[a] Reactions were performed on 0.094 mmol (**94** or **97**), or 0.078 (**95** or **98**) scale of substrates (0. 029-0.047 M) with 5 equiv. of $Bu_3SnH(D)$ and 2 equiv. of AIBN in toluene (95°C, 1-2 h.). [b] Determined by ^1H NMR. [c] Isolated yields. [d] The reduced hydrodebrominated 2-deoxy byproduct was also isolated (~10-15%).

These results were unexpected since analogous radical cascade decomposition reactions with 2'-chloro-2'-deoxy nucleoside resulted in the elimination of chlorine radical giving sugar decomposition product without incorporation of deuterium.[34,47] Formation of hydrogen-bonded intermediates (e.g., **148**; Scheme 30), leading to intramolecular deprotonation of C3-hydroxyl group, might facilitate loss of chloro or bromo substituent as chloride or bromide anion (as HCl or HBr) via heterolytic cleavage of C2-halogen bond. These results are in agreement with theoretical calculations[32] and experimental results on inhibition of RNR by 2'-chloro-2'-deoxynucleotides[12,15] and also reinforce the importance of deprotonation of C3'-OH group by E441 during enzymatic deoxygenation reaction.[12,134] It is also noteworthy here that treatment of 2'-chloro(or bromo)-3'-thionocarbonate nucleosides with Bu_3SnH which generates 3'-deoxy C3' radical (depleted from participation in hydrogen-bonding interaction with C2' substituents) resulted in the loss of chlorine or bromine radical to give 2',3'-unsaturated nucleosides, while the analogous radical fragmentation of 2'-*O*-tosyl-3'-thionocarbonates nucleosides effected hydrogenolysis of C3' radical to give 3'-deoxy-2'-*O*-tosylnucleosides.[49]

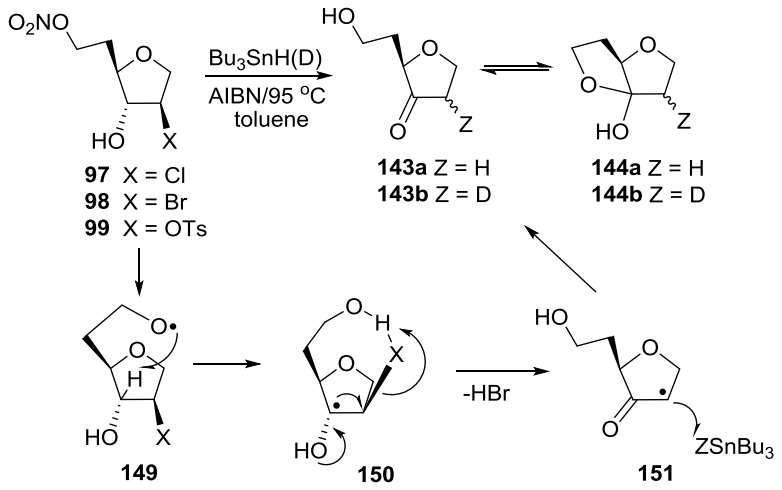


Scheme 30. Proposed mechanism for elimination of halo substituents (chloro or bromo) from 2-chloro 94 and 2-bromo 95 *ribo* precursors

Heating of compounds **94**, **95** and **96** in toluene without tin hydride and AIBN at 95 ^{o}C or even at 110 ^{o}C for up to 4 h resulted in the recovery of the intact substrates in

almost quantitative yields. This proved thermal stability of substrates **94**, **95** and **96** under conditions used for model reactions and excluded possibilities of heterolytic dissociation of C2-substituents. The substrate **96** was also stable upon heating (95 ^{o}C/2.5 h) in polar aprotic solvent such as DMF.

Since the hydrogen bonding to C2'-hydroxyl group (or other C2'-substituents) in nucleotide substrate is believed to play a critical role in the RNR-catalyzed heterolytic cleavage of C2'-heteroatom bond,[12,31] we also performed model reaction on two sets of substrates which should preclude formation of the intramolecular hydrogen bonding between C3-hydroxyl group and C2-substituent. One set was 2-substituted *arabino* precursors **97**, **98** and **99** (with *anti* relations between substituents at C2 and C3) while the second set was 3-*O*-methyl precursors with both *ribo* (*syn)* and *arabino* (*anti*) configurations (**100**, **101**, **102, 103,104** and **105**). The second set also precludes formation of the crucial 3-keto intermediate, formed during RNA-catalyzed reactions. However, treatment of 6-*O*-nitro-2-chloro **97** or 2-bromo **98** substrates with Bu_3SnH also, unexpectedly, produced a 3-keto/hemiacetal mixture **143a**/**144a** (~1:1; 97%, entry 5 and 67%, entry 7; Table 2) indicating elimination of chloride or bromide as anions rather than as chlorine or bromine radical. Analogous treatment of **97** and **98** with Bu_3SnD yielded mixture of **143b**/**144b** as 2-deuterio epimers (entries 6 and 8). These results were especially surprising since hydroxyl group at C3 and halogen substituent at C2 are in -*anti*-orientation what should preclude formation of hydrogen-bonded complexes between them. Apparently, elimination of halide anion via heterolytic cleavage of carbon-halogen bond might be facilitated by an eight-membered hydrogen-bonded intermediate of type **150** between 6-OH group and chloro or bromo substituent (Scheme 31).

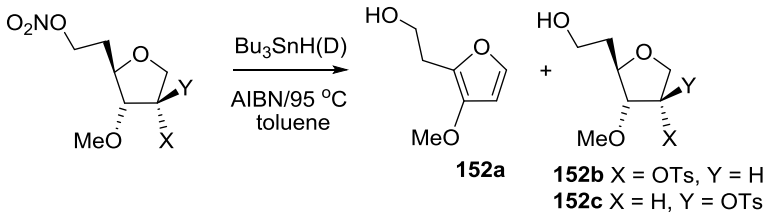


Scheme 31. Proposed mechanism for elimination of C-2 substituents from 2-substituted *arabino* precursors 97, 98 and 99.

3.1.2.3. 6-*O*-Nitro-3-*O*-methyl-2-substituted homosugar precursors

The biomimetic reactions with 3-*O*-methyl precursors lacking the 3-hydroxyl group led to different fragmentation upon treatment with tin radical emphasizing the importance of deprotonation of the 3'-hydroxyl group (and/or its participation in hydrogen bonding) during RNR-catalyzed reactions. Thus, treatment of 6-*O*-nitro-3-*O*-methyl-2-*O*-tosyl *ribo* substrate **102** with Bu_3SnH/AIBN/toluene/Δ gave 2-(hydroxyethyl)-3-methoxyfuran **152a** in 63% yield and the 6-hydroxy byproduct **152b** (22%) without the loss of tosyl group (Table 3). These results suggest that seven-membered hydrogen-bonded intermediate **147a**, which cannot be formed with 3-*O*-metyl substrate **102**, is critical for the ionic elimination of the tosylate group from C2 of **96**. Treatment of **102** with Bu_3SnD also yielded **152a** and **152b** without observed epimerization at C3 or deuterium incorporation at C3 (entry 2). The detrimental effect of the protecting groups (e.g., acetate) at C3 hydroxyl for the deuterium exchange at C3 in the analogues homoribofuranose models has been noted.[135] Fact that tosylate **102** is thermally stable while heating at 95 ^{o}C in

toluene suggest that radical or radical/ionic cascades of reaction is needed for the elimination of tosyl group (or tosylate) to form furane derivative **152a**.



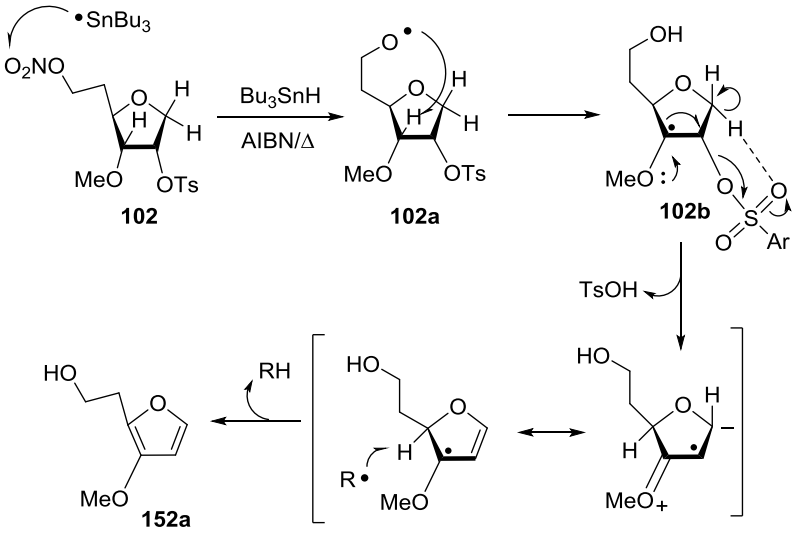
entry	substrate			reagent	product	Yield[b](%)
	No	X	Y			
1	**102**	OTs	H	Bu_3SnH	**152a**	63[c]
2	**102**	OTs	H	Bu_3SnD	**152a**	61[c]
3	**105**	H	OTs	Bu_3SnH	**152a**	51[e]
4	**105**	H	OTs	Bu_3SnD	**152a**	50[e]

Table 3. Biomimetic studies with 6-*O*-nitro-3-*O*-methyl-2-*O*-tosyl homo sugar substrates[a]

[a] Reactions were performed on 0.055 mmol (**102** or **105**) scale of substrates (0. 027-0.044 M) with 5 equiv. of $Bu_3SnH(D)$ and 2 equiv. of AIBN in toluene (95°C, 1.5 h.). [b] Isolated yields. [c] Compound **152b** (22%) was also isolated. [e] Compound **152c** (38%) was also isolated.

A six membered transition state involving the affinity of the C1 α-proton in **102** and the tosylate oxygen might lead to elimination of HOTs with generation of a 1,2-double bond (Scheme 32). Further abstraction of the C4 hydrogen from the resulting resonance structure would produce furan **152a**. Reaction of 3-*O*-methyl-2-*O*-tosyl *arabino* precursor **105** with Bu_3SnH gave 2-(hydroxyethyl)-3-methoxyfuran **152a** (51%) and the 6-hydroxy byproduct **152c** (38%) without the loss of tosyl group, as was the case with *ribo* analogue **102** (entry 1). Analogous reaction with Bu_3SnD also produced **152a** and **152c** without epimerization and no deuterium incorporation at C3 (entry 4). In this case of arabino precursor **105** the affinity of the C1 β-proton and the tosylate oxygen

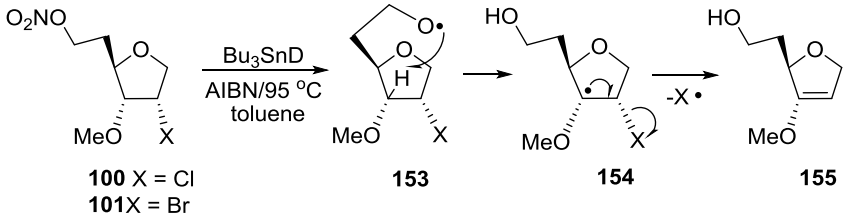
might lead to elimination of HOTs with generation of a 1,2-double bond. Further abstraction of the C4 hydrogen from the resulting resonance structure would produce furan **152a**.



Scheme 32. Proposed mechanism for elimination of TsOH from 6-*O*-nitro-3-*O*-methyl-2-*O*-tosyl *ribo* substrate 102

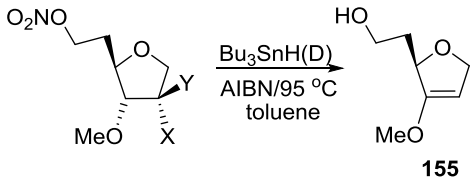
Reaction of 3-*O*-methyl-2-chloro(or bromo) *ribo* precursors **100** or **101** with Bu_3SnD resulted in the formation of vinyl ether **155** without deuterium incorporation (entry 1 and 2). ^{13}C NMR spectrum of **155** shows olefinic peak at 90.2 and 157.7 ppm while ^{1}H NMR spectrum shows olefinic hydrogen at 4.60-4.70 ppm. Formation of **155** is consistent with generation of C3 radical **154** and subsequent elimination of chlorine or bromine as radical (Scheme 33) in analogy to the β-elimination of phenylsulfinyl radical upon generation of the radical at vicinal carbon at similar model systems.[35] Lack of ability to form the hydrogen-bonded intermediate of type **148** in **100** or **101** and their inability for the conversion to 3-keto product (as in case of 3-hydroxy substrates **94** or **95**) can be

accountable for the radical elimination of the halo substituent from C2 position upon generation of the radical at C3.



Scheme 33. Proposed mechanism for elimination of C-2 substituents from 3-*O*-methyl-2-chloro(or bromo) *ribo* precursors 100 and 101.

Treatment of 3-*O*-methyl-2-chloro(or bromo) *arabino* precursors **103** or **104** with Bu_3SnD also resulted in the elimination of halo substituent as radical to give the vinyl ether **155** without deuterium incorporation (entries 3 and 4). The reactions, apparently, do not involve participation of 6-hydroxyl group as in the case of 3-hydroxy-2-halo *arabino* precursors **97** and **98** in which hydrogen-bonded intermediate of type **150** was most probably accountable for the elimination of halo substituent from C2 as chlorine or bromine anion. Also since formation of 3-keto product(s) which might be a driving force for the reaction is precluded with 3-*O*-methyl substrate **103** or **104**



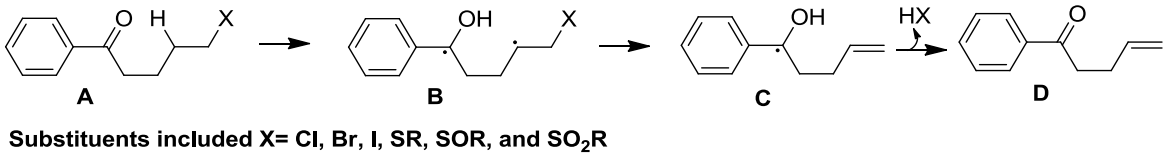
entry	substrate			reagent	product	Yield[b](%)
	No	X	Y			
1	**100**	Cl	H	Bu_3SnD	**155**	55
2	**101**	Br	H	Bu_3SnD	**155**	37[c]
3	**103**	H	Cl	Bu_3SnD	**155**	63
4	**104**	H	Br	Bu_3SnD	**155**	47[c]

Table 4. Biomimetic studies with 6-*O*-nitro-3-*O*-methyl-2-chloro(or bromo)-homosugar substrates[a]

[a] Reactions were performed on 0.088 mmol (**103** or **100**), or 0.074 (**104** or **101**) scale of substrates (0. 027-0.044 M) with 5 equiv. of Bu_3SnH(D) and 2 equiv. of AIBN in toluene (95°C, 1.5 h.). [b] Isolated yields. [c]The reduced hydrodebrominated 2-deoxy byproduct was also isolated (~15-25%).

3.1.3. Kinetic studies

Kinetic experiments were also planned to measure the rate of elimination of the substituent from the C2 position. The landmark work by Newcomb and Wagner on photochemistry of γ-substituted aryl ketones established high rates for the departure of halogen upon generation of radical at adjacent carbon.[131,136] Scheme 34 illustrates the conversion of 1,4-diradical **B** to **D** via the monoradical **C** with β-elimination of halogen as radical. Wagner *et al*. proposed that the rate constant for the radical elimination reaction increases in the order Cl < Br < I. Thus, I was expecting the higher rates of departure of halo-substituents (Cl, Br) from C2-position upon generation of radical at C3-position than for departure of tosyl anion. While rate of reaction is expected to be lower in the case of departure of C2 chlorine as an anion when compared to the elimination of C2 tosyl group as an anion.



Scheme 34. Photoactivated elimination of δ-substituent from aryl ketone

In order to study the kinetics for my reactions, the progress of the biomimetic reactions was followed by calculating disappearance of a peak for the starting material or appearance of a peak for the product in ^{1}H NMR spectra of the crude reaction mixture.

Values of **-log(C/C_0)** was plotted against time **t** (Figure 21). Where, C/C_0 is the ratio of concentration of 6-*O*-nitrohomo sugar derivative at time **t** to the initial concentration of 6-*O*-nitro homosugar derivative.

Parallel experiments of substrates **96**, **97**, **98** or **99** with Bu_3SnH in toluene have been examined. It was found that the fragmentation of tosylate **96** in toluene-d_8 with Bu_3SnH and AIBN at 75^oC was easy to follow and showed 90-95% consumption of **96** in 2.5 h with the formation of the **143a/144a** mixture as the sole product (80-85% yield based on TLC and ^1H NMR). (It is noteworthy that heating of **96** in toluene-d_8 at 55 ^oC showed only 50% consumption of substrate **96** after 2.5 h.) Experiments with *arabino* tosylate **99** and *arabino* chloro substrate **97** at 75 **^oC** revealed that the process with **99** was only slightly faster than with **97**. Interestingly fragmentation of *ribo* tosylate **96** with the formation of **143a/144a** proceeded with the rate similar to the one observed for **97**. Reaction of the bromo substrate **98** with Bu_3SnH at 75 ^oC resulted in the formation of the reasonable quantity of the 2-deoxy byproduct(s) resulting from radical-mediated hydrogenolytic cleavage of carbon-bromine bond in addition to the **143a/144a** mixture making the quantitative analysis of ^1H NMR spectra difficult.

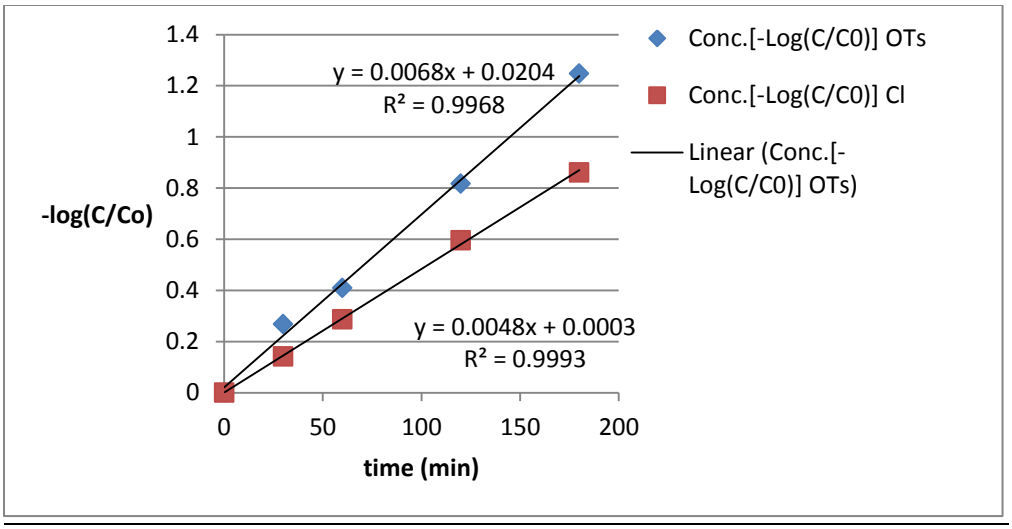


Figure 21. Pseudo-first order plots for the conversion of 97 (chloro) and 99 (tosylate) into 143a/144a

3.1.4. Mechanistic considerations

Various theoretical, chemical and biochemical mechanism have been proposed for the conversion of ribonucleoside 5'-diphosphates to 2'-deoxynucleotides by RDPRs. Stubbe, *et al*.[25]proposed mechanism for the inactivation of the RDPR by 2'-chloro-2'-deoxyuridine and emphasized on the departure of chlorine as an **anion** from the C2 position. The furanone **K** formed upon successive β-eliminations is a Michael acceptor which alkylates the nucleophiles on enzyme and causes covalent enzyme inhibition (Figure 6).[40]

In contrast to Stubbe's hypothesis, Robins *et al*. proposed departure of chlorine substituent from 2'-position of nucleoside as a **radical** rather than as an anion.[49,137] (Scheme 1) However, the drawbacks of Robins hypothesis is that, it gives the furanone product **11** with only 15-30% deuterium incorporation at C2', which makes the spectral analysis of the product difficult.

In the case of 6-*O*-nitro-3-hydroxy-2-*O*-tosyl homosugar precursors I observed that the generation of the radical at the 6-oxy position leads to abstraction of the hydrogen from the 3-position. This mechanism is in agreement with the photolysis studies by Barton's nitrite ester[42] and Wagner's δ-substituted aryl ketone[131] photolysis studies wherein they have shown that a six-membered transition state is favorable for the abstraction of hydrogen by an oxy radical. The mechanism proposed in this dissertation for the expulsion of the toluenesulfonic acid via formation of the seven-membered hydrogen-bonded intermediate is also in agreement with theoretical studies.[31,133] However theoretical modeling[133] of RDPR-catalyzed 2'-deoxygenation showed that the carboxylate group of glutamate forms a hydrogen bond with the 3'-OH and the H-donors form hydrogen bonds with 2'-OH and eliminates as water. Similarly the mechanism proposed in this dissertation for the heterolytic cleavage of the TsO–C2 linkage, is based on the attraction between the cis 3-OH in **96** and a tosylate oxygen. While in the case of the arabino tosylate **99** when there is no cis 3-OH, the **143b**/**144b** mixture was obtained as the product. In this instance the tosyl group at the C2 position might have formed a hydrogen bond with the 6-oxy radical, resulting in the heterolytic cleavage of the TsO–C2 bond.

Treatment of 6-*O*-nitro-3-hydroxy-2-(chloro or bromo) precursors in ribo or arabino configuration with Bu_3SnH also gave **143b**/**144b**. In all of the cases loss of bromide or chloride anions from an intermediate C3 radical occurred rather than homolytic loss of a halogen atom.

Elimination of halo substituent from the C2 position observed only in case of 3-*O*-methyl precursors. Thus treatment of the 2-chloro-3-*O*-methyl ribo **100** and arabino **103**

substrates with Bu_3SnH gave vinyl ether **155.** Similarly the 2-bromo-3-*O*-methyl ribo **101** and arabino **104** epimers also gave **155**. In this case since the formation of the ketone is precluded the elimination of the halogen atom occurred as a radical.

Ramos *et al.*[32] proposed that the substituent from the C2 position can leave as a radical or anion depending on the presence or absence of a basic residue capable of deprotonating the 3'-HO group. In the biomimetic studies I performed, clearly the elimination of the substituent is possible without a basic residue.

Treatment of the 3-*O*-methyl-2-*O*-tosyl ribo **102** or arabino **105** substrates with Bu_3SnH gave aromatized 2-(2-hydroxyethyl)-3-methoxyfuran (**152a**). Since in this case also the formation of the ketone is precluded the α-proton on C1 and a tosylate oxygen forms hydrogen bonding and the substituent departs as anion. Also, in the stability study with the tosylate **102** in toluene at 95 ^{o}C, no elimination of tosylate observed which indicates that generation of a C3 radical was necessary for elimination of tosylate and production of **152a**.

Thus this biomimetic studies with the 6-*O*-nitrohomosugar precursors provide explanation for the two-electron elimination of hydrogen-bonded water in the mechanism proposed for the reduction of nucleoside diphosphate substrates by Stubbe. It also provides an explanation for the one-electron dissociation of chlorine radical from substrate 2'-chloro-2'-deoxynucleoside di(or tri)phosphate *inactivators* of RNR and the MoaA enzyme.

3.2. Azido sugar and nucleosides analogues as precursors to aminyl radicals

3.2.1. 2-Azido-2-deoxy-α-D-lyxofuranosides and its labelled analogues

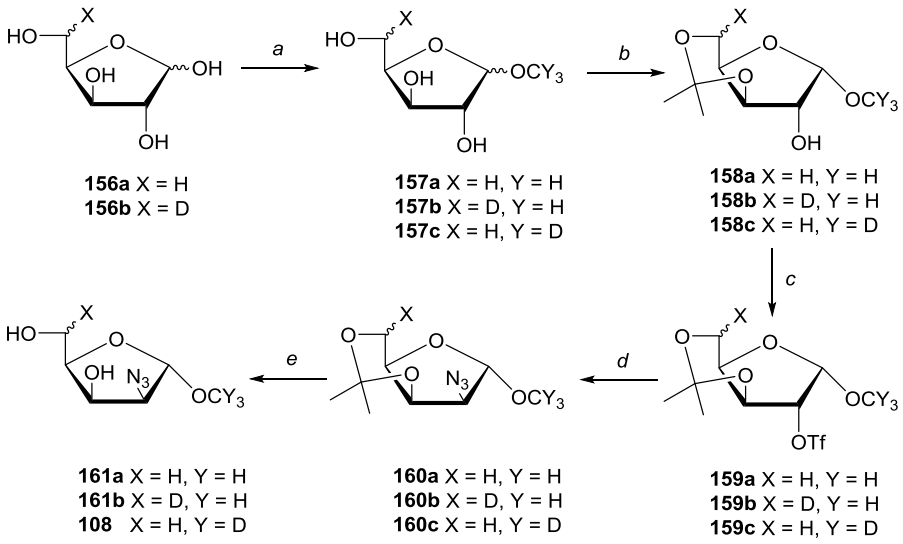
Based on Sevilla's work on 3′-azido-3′-deoxythymidine (3′-AZT) and 5′-azido-5′-deoxythymidine (5'-AZT) analogues,[79] studies with the simple abasic system to avoid the interaction of the transient radical with the nucleoside heterocyclic bases were investigated. So, I designed abasic model to investigate the H-atom abstraction pathway by RNH•. The azido group is in *trans* relative configuration to H3 and H4 in *cis* relative configuration to H1 and H5,5'. By employing the abasic model we could further drive bimolecular interaction to produce the 5'-sugar radical and the H-atom abstraction pathway by RNH• could be studied without interference.

The 2-azido-2-deoxy-α-D-lyxofuranoside, **108** and its analogues upon electron attachment in γ-irradiated aqueous glassy (7.5 M LiCl) systems at 77 K in the absence of oxygen is anticipated to generate the neutral aminyl radical (RNH•, **109**). Aminyl radical, **109** is expected to undergo intramolecular hydrogen atom abstraction to produce **110** (Scheme 22). To learn about the position of the radical species that formed from the biomolecular abstraction of hydrogen in sugar, compounds are designed with deuterium labeled at methyl group at anomeric carbon atom, C4, C5 and C5, C5'. Formation of the aminyl radical (RNH•) via one electron attachment, its participation in the intramolecular C5 hydrogen abstraction and subsequent reactions will be investigated by EPR method developed by Dr. Sevilla.

3.2.1.1. Synthesis of 2-azido-2-deoxy-α-D-lyxofuranosides and its labelled analogues.

The azido sugar analogues **161a** and **161b** were synthesized using the modified Fleet and Smith procedure[138] (Scheme 35). Treatment of the D-xylose **156a** with 0.5%

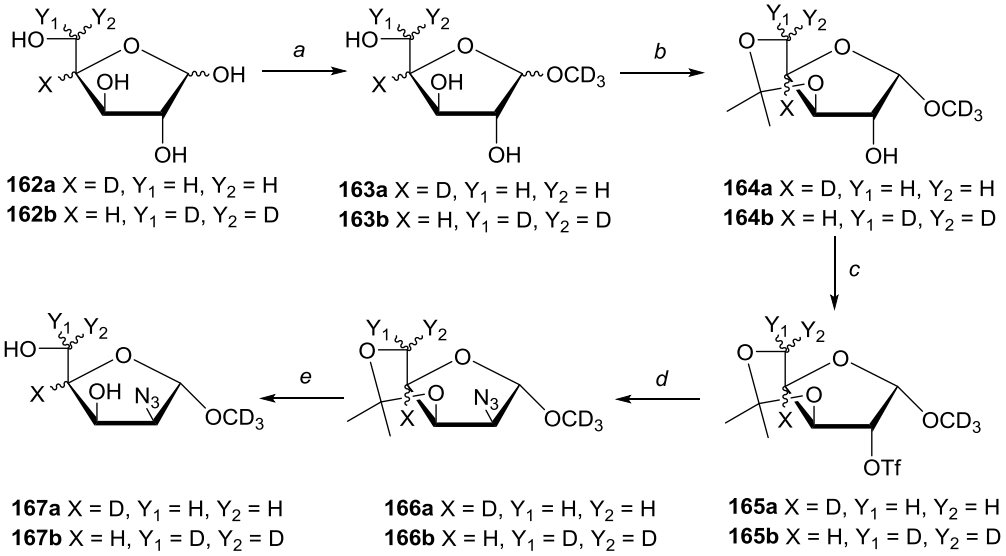
HCl/CH_3OH yielded Methyl D-xylofuranoside **157a** as reported.[139] Methyl D-xylofuranoside **157a** further treated with Conc. H_2SO_4/ acetone as reported[139] resulted in (α/β, 3:2) mixture of 3,5-*O*-isopropylidine protected D-xylofuranoside. The α-anomer **158a** was isolated using column chromatography. Compound **158a** was converted to 2-*O*-triflate ester **159a** upon treatment with trifluoromethanesulfonyl chloride (TfCl) and 4-(dimethylamino)pyridine (DMAP) in methylene chloride.[140] The yield and purity for **159a** is better than when the original procedure[138] employing the triflic anhydride in pyridine was used. Azidation of **159a** was accomplished by displacement of the triflate from **159a** with NaN_3 in DMF. In the final step of the synthesis the isopropylidene protection group was removed using AcOH to give methyl 2-azido-2-deoxy-α-D-lyxofuranoside **161a**.[138] Analogous displacement of the triflate from **159a** with [^{15}N]-NaN_3 yielded 2-[^{15}N]-azido isotopomer **160a**. Subjection of 5-[^{2}H]-D-xylose **156b** to the same synthetic sequence yielded 5-deuterium labeled azido sugar **161b.**



Reagents: (*a*) 0.5%HCl/CH_3OH or 0.5%DCl/CD_3OD; (*b*) $(CH_3)_2CO$/conc. H_2SO_4; (*c*)TfCl/DMAP/CH_2Cl_2; (*d*) NaN_3 or [^{15}N]-NaN_3/DMF; (*e*) AcOH/H_2O (7:3)

Scheme 35. Synthesis of methyl 2-azido-2-deoxy-a-D-lyxofuranoside and its deuterium labeled analogues.

Analogous treatment of D-xylose **156a** with 0.5% DCl/CD_3OD in place of 0.5% HCl/ CH_3OH produced methyl-d_3 xylofuranoside **157c** with 100% deuterium incorporation. Compound **157c** was converted to methyl-d_3 2-azido-2-deoxy-α-D-lyxofuranoside **108** following the same synthesis method mentioned above.



162a X = D, Y_1 = H, Y_2 = H
162b X = H, Y_1 = D, Y_2 = D

163a X = D, Y_1 = H, Y_2 = H
163b X = H, Y_1 = D, Y_2 = D

164a X = D, Y_1 = H, Y_2 = H
164b X = H, Y_1 = D, Y_2 = D

165a X = D, Y_1 = H, Y_2 = H
165b X = H, Y_1 = D, Y_2 = D

166a X = D, Y_1 = H, Y_2 = H
166b X = H, Y_1 = D, Y_2 = D

167a X = D, Y_1 = H, Y_2 = H
167b X = H, Y_1 = D, Y_2 = D

Reagents: (*a*) 0.5%DCl/CD_3OD; (*b*) $(CH_3)_2CO$/conc. H_2SO_4; (*c*)TfCl/DMAP/CH_2Cl_2; (*d*) NaN_3 / DMF; (*e*) AcOH/H_2O (7:3)

Scheme 36. Synthesis of deuterium labeled analogues of 2-azido-2-deoxy-α-D-lyxofuranoside.

Subjection of 4-[^{2}H]-D-xylose **162a** to the same synthetic sequence as mentioned above yielded methyl-d_3 4-deuterium labeled analogue **167a**. Similarly, 5-[$^2H_2$]-D-xylose **162b** was converted to methyl-d_3 5-dideuterium labeled azidolyxofuranoside **167b** (Scheme 36).

3.2.1.2. ESR characterization of electron-mediated formation of the aminyl radicals and their subsequent reactions.

The radiation-produced electron-induced site specific formation of neutral aminyl radical ((RNH•/RND• (1-OMe-(C2)-NH•/1-OMe-(C2)-ND•)) and their reactions in

methyl 2-azido-2-deoxy-α-D-lyxofuranoside **161a**, methyl 2-[^{15}N]-azido-2-deoxy-α-D-lyxofuranoside **[^{15}N]-161a**, methyl 5-[^{2}H]-2-azido-2-deoxy-α-D-lyxofuranoside **161b**, [$^{2}H_3$]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside **108**, [$^{2}H_3$]-methyl 2-azido-2-deoxy-α-D-4-[^{2}H]-lyxofuranoside **167a**, and [$^{2}H_3$]-methyl 2-azido-2-deoxy-α-D-5-[$^{2}H_2$]-lyxofuranoside **167b** in γ-irradiated homogeneous aqueous (D_2O or H_2O) glassy (7.5 M LiCl) system have been investigated.

3.2.1.3a Assignment of the aminyl radical formed at C2 of 2-azido-2-deoxy-α-D-lyxofuranosides and its labeled analogs

From our assignment of RNH• (T(C2′)-^{14}ND•) in 3′-AZT,[79] the major HFCC values in 1-OMe-(C2)-^{14}ND• and its ^{15}ND• isotopomer 1-OMe-(C2)-^{15}ND• are expected from three sources – the axially symmetric anisotropic HFCC hyperfine coupling from the azide N-atom, the isotropic β-hydrogen coupling due to the H2-atom, and the deuterium coupling from the exchangeable NH/ND. The 77 K ESR spectrum (green) of 1-OMe-(C2)-^{14}ND• formed via radiation-induced electron attachment in aqueous glassy (7.5 M LiCl/D_2O (pH ca. 5)) sample of **161a** obtained via γ-irradiation at 77 K is presented in Figure 22 (A). To make this assignment of 1-OMe-(C2)-^{14}ND• unequivocal, experiments were carried out using a matched sample of monoisotopically labelled ^{15}N incorporated **[^{15}N]-161a** and the results are presented in Figure 22 (B). Each experimentally recorded ESR spectrum shown in Figures 22 (A), and 22 (B) was obtained after subtraction of the 250 G $Cl_2^{\bullet-}$ spectrum.[79,141,142] The red spectrum in Figure 22 (B) is assigned to 1-OMe-(C2)-^{15}ND•. The 1-OMe-(C2)-^{14}ND• (green, Figure 22 (A)) spectrum and 1-OMe-(C2)-^{15}ND• (red, Figure 22(B)) spectrum match very well with the simulated spectra generated using typical beta hyperfine coupling constant

(HFCC) value. Thus, with the aid of **[^{15}N]-161a,** the unequivocal identification of the aminyl radical was achieved.

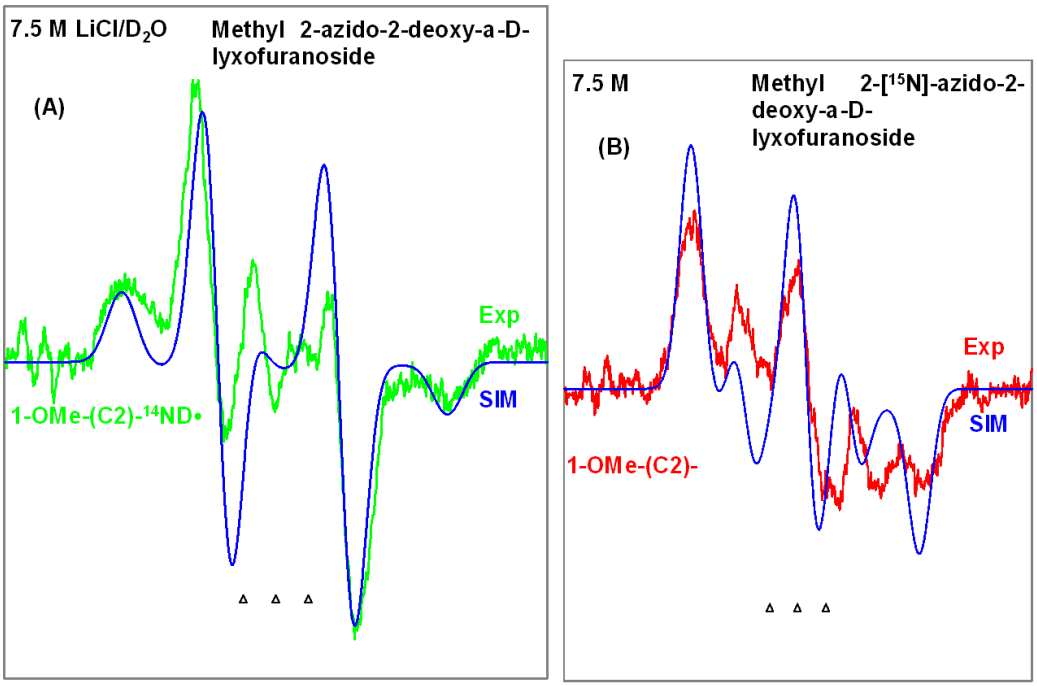


Figure 22. Comparison of ESR spectra of the aminyl radical generated from methyl 2-azido-2-deoxy-α-D-lyxofuranoside and its ^{15}N isotopomer.

Figure 22. The simulated spectra (blue) (for simulation parameters, see text) are superimposed on the top of the each experimentally recorded spectrum. The three reference markers (open triangles) in this figure and in other figures show the position of Fremy's salt resonance with the central marker at g = 2.0056. Each of these markers is separated from each other by 13.09 G.

3.2.1.3b Assignment of the radical species formed due to the reactions of 1-OMe-(C2)-^{14}ND• in 2-azido-2-deoxy-α-D-lyxofuranosides and its labeled analogs

Studies employing matched samples of [$^{2}H_3$]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside **108** and **161a** yielded very similar ESR spectra. Similarities of the total hyperfine splitting, of the center of the spectra, and of line shapes in these two spectra clearly established that the aminyl radical spectrum is not affected by the deuteration of the methyl group at the anomeric carbon atom. Moreover, loss of the line components at

the wings owing to the axially symmetric anisotropic hyperfine coupling from the azide N-atom at 150 K and onwards (spectra 23 (C) to 23 (F)) showed that the site of radical generation due to subsequent reaction of the aminyl radical in lyxofuranose is not located at the OCD_3 group or at the anomeric carbon atom C1. The site for the secondary radical is rather at C3, or at C4, or at C5 of the furanoside ring (Figure 23). This is further supported by the annealing studies (ca. 140 to ca. 165 K) of matched sample of **[^{15}N]-161a** which yielded spectra with similar total hyperfine splitting, similar center, and similar line shapes to those shown in Figures 23 (B) to 23 (F) (data not shown)

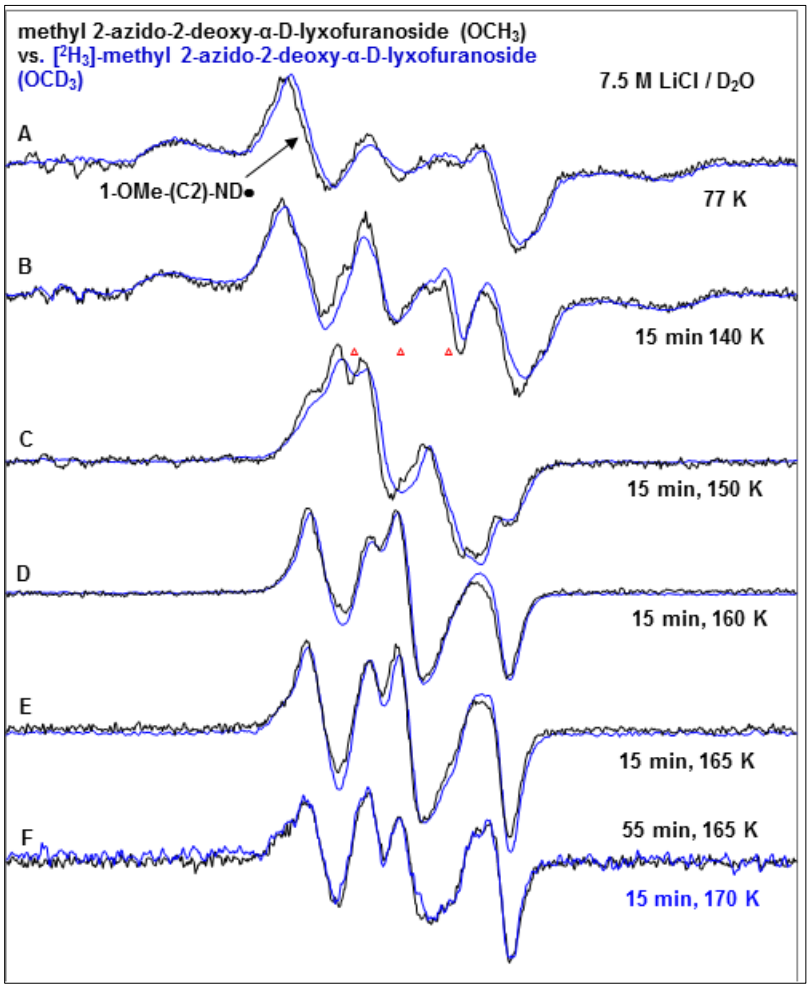


Figure 23. Comparison of ESR spectra of the aminyl radical generated from methyl 2-azido-2-deoxy-α-D-lyxofuranoside and [^{2}H$_3$]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside

Figure 23. Comparison of the aminyl radical formation via (A) electron attachment at 77 K and (B) to (F) the subsequent reactions of the aminyl radical shown via progressive annealing from 77 K to ca. 170 K in matched samples of methyl 2-azido-2-deoxy-α-D-lyxofuranoside (OCH_3) (161a black) and of [$^2H_3$]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside (OCD_3) (108 blue).

We have tested the hypothesis that 1-OMe-(C2)-^{14}ND• from **161a** could abstract H-atoms from sugar carbons by employing matched samples of **161a** (black, Figure 24) its 5-deutereo, **161b** (green, Figure 24) and 5-dideutereo lyxofuranoside, **167b** (pink) isotopomers, (Figure 25). Following the spectral results shown in Figure 22, the spectra in Figures 24 (A) and 25 (A) are assigned to 1-OMe-(C2)-^{14}ND• and 5-D-1-OMe-(C2)-^{14}ND• owing to the presence of the $\boldsymbol{A}_{zz}$ component of the anisotropic nitrogen HFCC of ca. 41 G and isotropic β HFCC value of 2-H at (49.0 G). These results further establish that 5-deuteration does not show any observable effect on the spectra of these aminyl radicals. Upon annealing from 77 K to 145 K (spectra shown in Figure 24 (B) and Figure 25 (B)), we find that owing to the softening of the glass caused by the temperature increase, 1-OMe-(C2)-^{14}ND• migrates and reacts with the 5-H atom in the 2-azidolyxofuranoside resulting in the central anisotropic doublet of ca. 20 G owing to the α-hydrogen in C5•. Collapse of this central doublet to a singlet in the matched sample of **161b** (Figure 24) and **167b** (see Figure 25) under identical conditions has led to this unequivocal assignment of the central anisotropic doublet of ca. 20 G due to C5•.

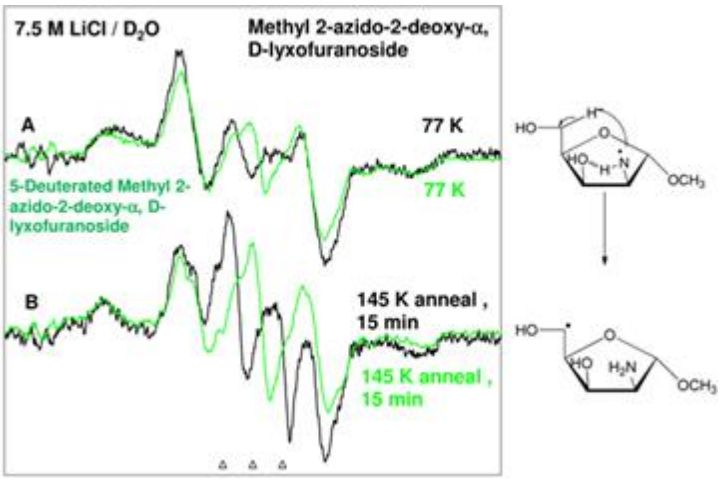


Figure 24. Comparision of the (A) electron attachment at 77 K and and (B) the subsequent reaction shown via progressive annealing at 145 K in matched samples of 161a (black) and 161b (green).

Increasing the concentration (0.5 to 10 mg/ml) of the lyxofuranoside in the solution appeared to have no effect on the extent of C5• formation from the spectra recorded under the same microwave power, modulation, and gain. From these results, we conclude that the 1-OMe-(C2)-^{14}ND• mediated C5• formation observed in these lyxofuranoside sample occur via intramolecular H-atom abstraction. This can be explained by the 6-membered transition state where the aminyl radical generated at C2 nitrogen atom abstracts the C5 hydrogen, **109** → **110** (Scheme 22).

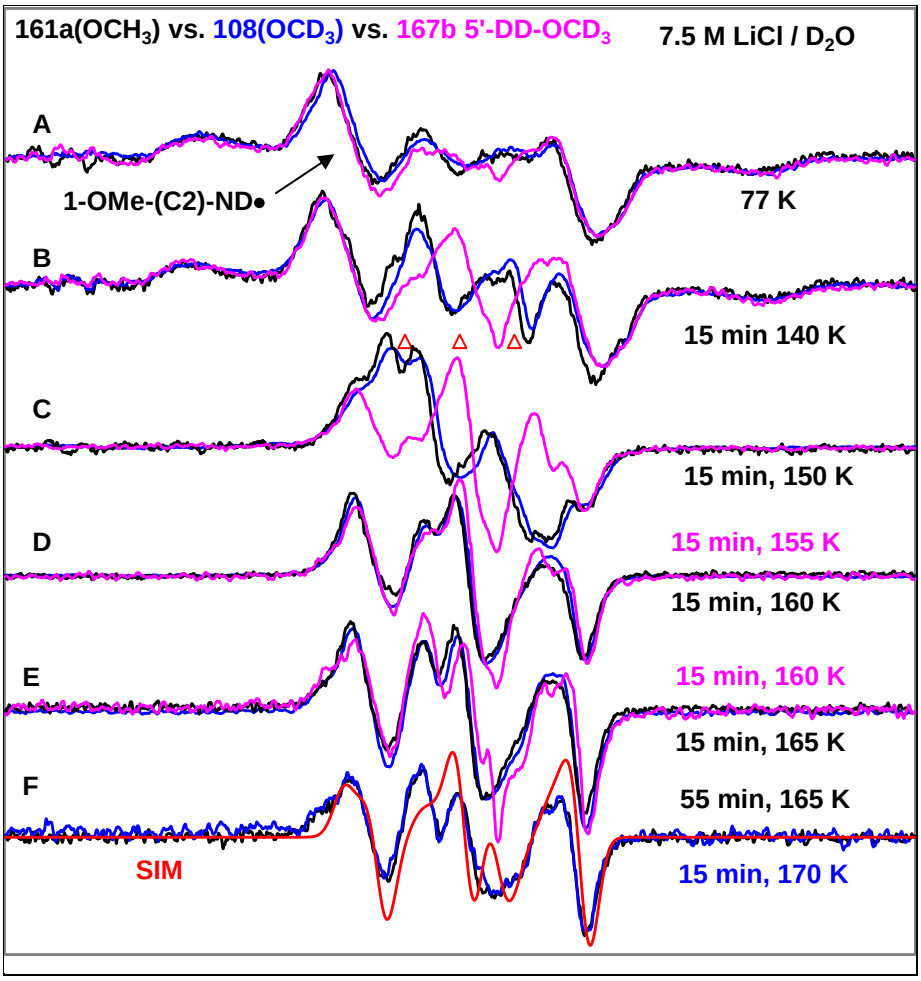
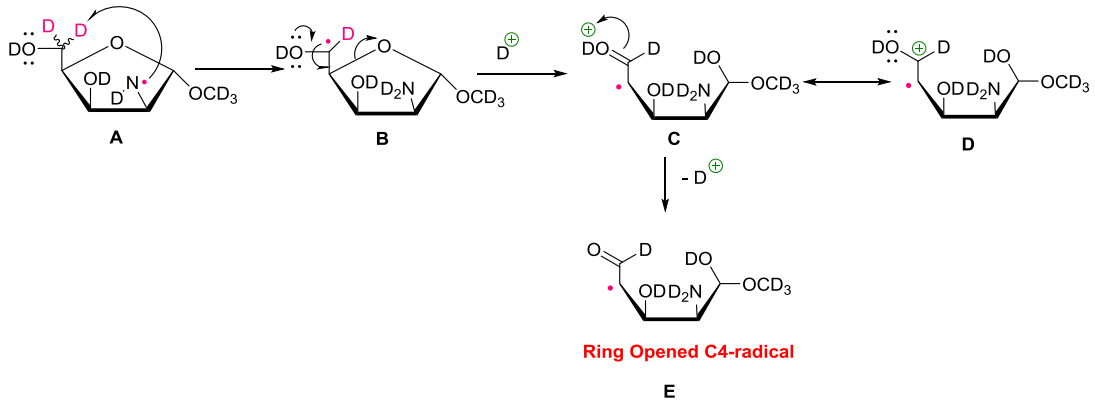


Figure 25. Formation of aminyl radical in 161a, 108 and 167b and it's subsequent reactions

Figure 25. Comparison of the aminyl radical formation via (A) electron attachment at 77 K and (B) to (F) the subsequent reactions of the aminyl radical shown via progressive annealing from 77 K to ca. 170 K in matched samples of **161a** (OCH_3) (black), of **100** (OCD_3) (blue), and of **167b** (pink). The data of **161a** and **100** were taken from Figure 23.

Analyses of spectrum F in Fig. 23 and spectrum F in Fig. 25 show that these two spectra have similar total hyperfine splitting, similar center, and similar line shapes. However, comparison of spectra from Fig. 23 (C) to 23 (F) and 25 (F) show that most probably C5• is converted to another radical. Simulation experiment, which matches experimental data, suggest that C5• **B** most probably undergo conversion to the stable

C4• **E** via ring opening. Therefore, we have concluded that in the azido furanose substrates **161b**, **100, 167a** and **167b**, the intermediary C5• underwent the rapid conversion to the ring opened C4• (see Scheme 37).



Scheme 37. Conversion of the C5• to the ring opened C4•

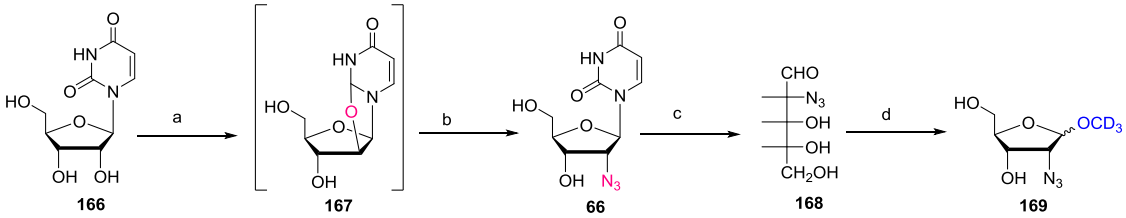
3.2.2. Design and synthesis of methyl 2-azido-2-deoxyriboside.

In lyxofuranoside derivatives **108**, **161** and **167** the azido group is in *trans* configuration to H3 and in *cis* configuration to the H5,5' and anomeric H1. It is also in the opposite geometric configuration to H4. To explore the possibility of generation of aminyl radical and its subsequent reactions when the azido group is in *cis* configuration to H4 and *trans* to H3, H5,5', I designed methyl 2-azido-2-deoxyriboside, **169** an alternative abasic model compound. Since it is abasic compound the interaction of the transient radical with the nucleoside heterocyclic bases can be also avoided.

The methyl 2-azido-2-deoxyriboside, **169** upon electron attachment in γ-irradiated aqueous glassy (7.5 M LiCl) systems at 77 K is expected to generate the neutral aminyl radical (RNH•). Further the aminyl radical could undergo intramolecular/intermolecular hydrogen abstraction. Formation of the aminyl radical (RNH•) and its subsequent

reactions in methyl 2-azido-2-deoxyriboside, **169** will be studied using the EPR method developed by Dr. Sevilla group.

The azido sugar analogue **169** can be synthesized using the method reported by Eckstein *et al.* which utilized glycosylic bond cleavage in 2'-azido-2'-deoxyuridine **66** for the preparation of 2-azido-2-deoxyribose, **168**.[58] The 2'-azido-2'-deoxyuridine, **66** was prepared according to the modified Verheyden method (Scheme 38).[143] Treatment of uridine, **166** with diphenyl carbonate in hexamethylphosphoramide and catalytic amount of sodium bicarbonate leads to *insitu* formation of O^2,2'-cyclouridine, **167**. Addition of sodium azide to the same reaction mixture yielded 2'-azido-2'-deoxyuridine, **66** in a "one-pot" reaction. Treatment of 2'-azido-2'-deoxyuridine, **66** with 15% hydrazine hydrate at 65^{o}C leads to cleavage of the glyosidic bond with the formation of 2-azido-2-deoxyribose, **168**. Further 2-azido-2-deoxyribose, **168** treated with 0.5% HCl/CH_3OH to give methyl 2-azido-2-deoxyriboside, **169**.



a. Diphenyl Carbonate/Hexamethylphosphoramide/140 ^{o}C/sodium bicarbonate b. Lithium azide c. 15% hydrazine hydrate solution/65^{o}C/H_2O/ benzaldehyde. d.0.5% DCl/CD_3OD.

Scheme 38. Possible synthesis of methyl 2-azido-2-deoxyriboside

3.2.3. 2'-Azido-2'-deoxycytidine (2'-AZdC) and 4'-azidocytidine (4'-AZC).

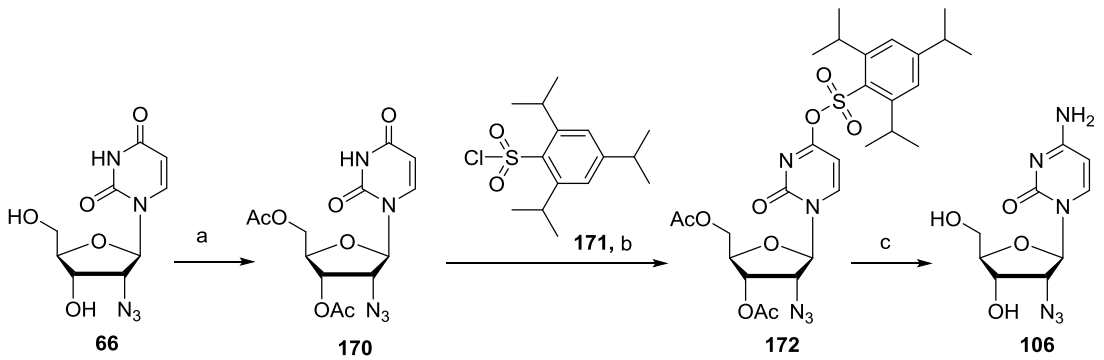
3.2.3.1. Design of 2'-AZdC and 4'-AZC.

Following the work regarding the formation of radicals in 3'-AZT and the 5′-azido-5′-deoxythymidine (5'-AZT) analogues as well as 2'-azido-2'-deoxy uridine after attachment of electron in γ-irradiated aqueous glassy (7.5 M LiCl) systems,[79] I planned to

extend my efforts to study radiation-generated electron-induced site specific formation of neutral aminyl radicals (RNH•) and their reactions in 2'-azido-2'-deoxycytidine (2'-AZdC, **106**) and 4'-azidocytidine (4'-AZC, **107**). In 2'-AZdC the aminyl radical site would be attached to a 2^{o} C-atom (2') and in 4'-AZdC, to a 3^{o} C-atom (4') in the sugar moiety. This difference in the stereo and electronic environment of these site-specific aminyl radicals is expected to give clear idea about the difference in their reactivity

3.2.3.2. Synthesis of 2'-AZdC and 4'-AZC.

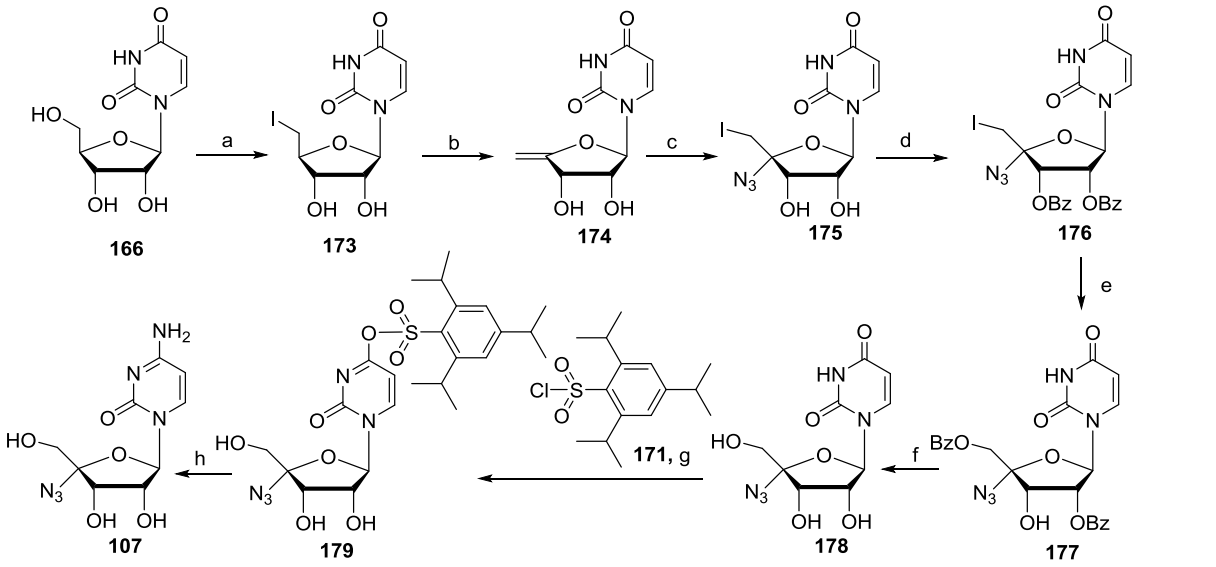
The 2'-azido-2'-deoxycytidine (2'-AZdC, **106**) was synthesized from 2'-azido-2'-deoxyuridine, **66** (Scheme 39), which was readily obtained from uridine.[144] Shortly, acetylation of free hydroxyl groups in **66** yielded 3',5'-di-O-acetyl-2'-azido-2'-deoxyuridine**, 170.** Conversion of the 3',5'-di-*O*-acetyl-2'-azido-2'-deoxyuridine**, 170** to 2'-azido-2'-deoxycytidine, **106** was achieved in good yield (85%) employing modification of the literature procedure by Fritz *et al.*[145] Thus treatment of **170** with 2,4,6-triisopropylbenzenesulfonyl chloride, **171** afforded trisylated compound **172**. Subsequent global deprotection of compound **172** with aqueous ammonium hydroxide in THF afforded 2'-AZdC **106** (85%).



(a) Acetic anhydride/Pyridine; (b) TEA/DMAP/CH_2Cl_2; (C) Aq. Ammonia/THF

Scheme 39. Synthesis of 2'-azido-2'-deoxycytidine (2'-AZdC).

The 4'-azidocytidine (4'-AZC, **107**) was also synthesized from the conversion of uridine counterpart **177**. The 4'-azidouridine was prepared as reported (Scheme 40).[146] Shortly, treatment of unprotected uridine **166** with triphenylphosphine and iodine in the presence of imidazole leads to selective iodination at 5' to produce the 5'-halonucleoside intermediate. Dehydrohalogenation of the incipient 5'-halonucleoside with the aid of NaOMe readily generates the 4'-olefin, **174**. In a critical step stereoselective addition of iodine azide (generated in situ from iodine monochloride and sodium azide in DMF) to the 4'-methylene uridine derivative **174** produced **175**. Iodine added preferentially to the β-face of the double bond to generate the iodium ion intermediate which was open on the α-side, at 4', and stereospecific addition of the azide ion at that site produced **175**. Protection of the hydroxyl groups in **175**, with benzyl protection group generated the protected compound **176**. Treatment of **176**, with *m*-chloroperbenzoic acid in dichloromethane yielded the 5'-*O*-benzoyl-3'-hydroxyl analogue **177** in which migration of the 3',5'-trans oriented benzoyl protection group occurred via formation of the 3',5'-cyclic benzoxonium ion.[146] Removal of benzoyl protection using sodium methoxide in methanol gave **178**. Conversion of 4'-azido uridine, **178** to 4'-azidocytidine, **107** was achieved by modification of the literature procedure.[147] Reaction of **178** with 2,4,6-triisopropyl benzene sulfonyl chloride in the presence of TEA and DMAP in dichloromethane resulted in the regioselective trisylation at O^4 position. The incipient trisylated derivative **179** without further purification was directly converted to the 4'-azidocytidine (4'-AZC, **107)** upon treatment with aqueous ammonium hydroxide in THF in 80% yield. [118]



a. I_2/PPh_3/Imidazole b. NaOMe/MeOH c. ICl/NaN_3/DMF d..BzCl/Pyridine e. mCPBA/CH_2Cl_2 f.$NaOCH_3$/MeOH g.TEA/DMAP/CH_2Cl_2 h.Aq. Ammonia/THF

Scheme 40. Synthesis of 4'-azidocytidine (4'-AZC)

3.2.3.3. EPR characterization during one electron oxidation of 2'-AZdC and 4'-AZC.

The electron-induced site specific formation of neutral aminyl radicals (RNH•) and their reactions in 2'-azido-2'-deoxycytidine (2'-AZdC, **106**, Figure 26) and 4'-azidocytidine (4'-AZC, **107**, Figure 27) in γ-irradiated aqueous glassy (7.5 M LiCl) systems have been studied by EPR spectroscopy.

The ESR spectra of the γ-irradiation produced radicals in matched samples of 2'-AZdU (black) and 2'-AZdC (blue) at the native pD (*ca.* 5) of the homogeneous glassy solutions of 7.5 *M* LiCl in D_2O as well as of the subsequent reactions of these radicals are shown in Figure 26. The ESR spectroscopic studies presented here have provided the evidence for the formation of neutral and localized aminyl radical at C2' in 2'-AZdU

(dU(C2')-ND•) or in 2'-AZdC (dC(C2')-ND•) owing to electron attachment in the azido group of 2'-AZdU or of 2'-AZdC at 77 K.

It is evident from Figure 26 that the ESR characteristics (e.g., overall hyperfine splitting, g-value at the center, lineshape) of the spectra obtained from the commercially available 2'-AZdU and the in-house synthesized 2'-AZdC samples are quite identical. We note here that the central doublets in Figure 26A are assigned to the uracil anion radical ($U^{\bullet -}$, black) and cytosine anion radical ($C^{\bullet -}$, blue) and the ESR characteristics (e.g., overall hyperfine splitting, g-value at the center, lineshape) of these doublets are identical to the spectra of $U^{\bullet -}$ and $C^{\bullet -}$ reported in the literature.[148] Furthermore, the spectra shown in Figure 26A (and also in 26(B)) have the line components owing to dU(C2')-ND• (or dC(C2')-ND•).

Via progressive annealing from 140 K up to 167 K (spectra shown in Figures 26(C) to 26(G)), we find that owing to the softening of the glass caused by the increase in the temperature, either dU(C2')-ND• (black) (or dC(C2')-ND• (blue)) migrates and reacts with a 2'-AZdU or 2'-AZdC molecule in its proximity. The spectra presented in Figure 26(G) show that the final radical formed via bimolecular reaction of either dU(C2')-ND• (or dC(C2')-ND•) with an unreacted 2'-AZdU or 2'-AZdC (blue) molecule leads to the formation of a C-centered radical. There are two possible assignments of this C-centered radical. The radical site could be at the C5- or C6 site of the C5-C6 double bond in uridine or in cytosine since the aminyl radical may add to the C5 or C6-site of the C5-C6 double bond. This observation is in consistent with the literature where it is reported that in cytosine, RNH• undergoes electrophilic addition to the C=C double bond.[109,126,149-151]

Alternatively, the aminyl radical might cause H-atom abstraction from the 5'-site and the resulted C5'• could have a similar spectrum owing to an anisotropic α-H coupling at C5' and owing to an isotropic β-H coupling due to C4'.[148,152]

Comparison of the ESR spectra presented in Figures 26 and 27 clearly demonstrate that unlike dU(C2')-ND• (or dC(C2')-ND•), the C(C4')-ND• does not have any isotropic β-H HFCC. Comparison of spectra in Figure 26 (G) with those in Figure 27(G) suggests that RNH• attached to the tertiary alkyl carbon (generated from **107**) could be less reactive.

Its ESR characteristics are as follows: anisotropic ^{14}N-atom HFCC (37.5, 0, 0) G, and anisotropic a-D (N-D) (4.5, 0, 6) G, a mixed Lorentzian/Gaussian (1/1) linewidth of 10 G, with g-values of (2.0020, 2.0043, 2.0043). The simulated spectrum (red) of C(C4')-ND• obtained by employing these ESR parameters match the experimental spectrum very well.

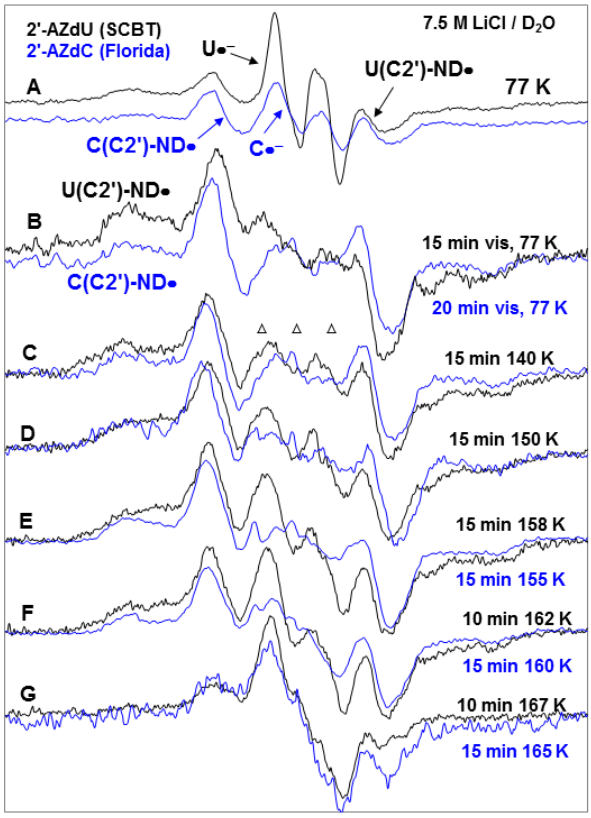


Figure 26. The electron-induced site specific formation of neutral aminyl radicals (RNH•) and their reactions in 2'-azido-2'-deoxycytidine

Figure 26. Shows first derivative ESR spectra of matched samples of commercially available (from Santa Cruz Biotechnology, CA, USA) 2'-AZdU (black) and the in-house synthesized 2'-AZdC (blue) at the native pD (*ca.* 5) of the homogeneous glassy solution of 7.5 *M* LiCl in D_2O. (A) After g-irradiation (absorbed dose = 500 Gy) produced one-electron addition at 77 K. (B) After visible illumination of samples employing a photoflood lamp at 77 K for 15 to 20 minutes. The central doublet in both spectra due to the cytosine anion radical ($C^{\bullet-}$) is removed by photoejection of the excess electron. Spectra (C) to (G) are obtained after subsequent annealing for 10 to 15 min in the dark from 150 to 167 K. All ESR spectra shown in Figures A to E were recorded at 77 K. The three reference markers in this figure and in subsequent figures are Fremy's salt resonances with central marker is at g= 2.0056 and each of three markers is separated from one another by 13.09 G.

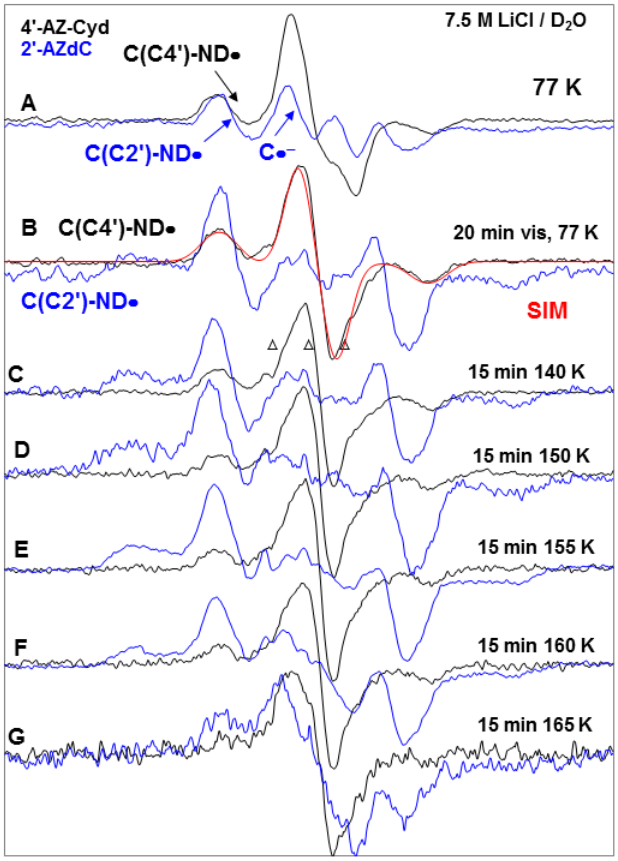


Figure 27. The electron-induced site specific formation of neutral aminyl radicals (RNH•) and their reactions in 4'-azidocytidine

Figure 27. Shows first derivative ESR spectra of matched samples of the in-house synthesized 4'-AZCyd (black) and 2'-AZdC (blue, also shown in Figure 26) at the native pD (*ca.* 5) of the homogeneous glassy solution of 7.5 *M* LiCl in D_2O. (A) After g-irradiation (absorbed dose = 500 Gy) produced one-electron addition at 77 K. (B) After visible illumination of samples employing a photoflood lamp at 77 K for 20 minutes. The central doublet in both spectra due to the cytosine anion radical ($C^{\bullet-}$) is removed by photoejection of the excess electron. The simulated spectrum of C(C4')-ND• is also shown (for simulation parameters, see below). Spectra (C) to (G) are obtained after subsequent annealing for 10 to 15 min in the dark from 140 to 165 K. All ESR spectra shown in Figures A to G were recorded at 77 K.

3.3. Sulfenamide modified nucleosides

Formation of neutral aminyl radical (RNH•) has also been speculated from nucleoside modified with sulfenamide moiety. Kuttappan synthesized N^6-

[Phenylsulphenyl]-2'-deoxyadenosine and N^6-[2-nitrophenylsulphenyl]-2'-deoxyadenosine analogues,[121] and studied the formation of aminyl radicals by UV photolysis and γ-irradiation. The formation of dAdo N^6-aminyl radical was observed by pulse radiolysis.[121]

Sulfenamide modified nucleosides analogues with the sulfenamido substitution at specific sites either in the sugar (**111**and **112**) or base (**113**) have been prepared (Figure 28). To see if the nucleoside(sugar)-based aminyl radical can be generated from sulfenamide derivatives, I designed 2'-deoxy-2'-sulfenamide uridine (2'-SAdU) analogue **111** and **112** with the alkyl-NH-S-aryl frame. To study the effect of the substitution in the aromatic ring attached to sulfur, 2'-deoxy-2'-sulfenamide uridine analogue **112** with EWG substituents in the aryl ring has been synthesized.

Also, to investigate the sulfenamide derivative with the aryl-NH-S-aryl frame which is expected to be more stable and more reliable precursor to the aminyl radicals, the 2'-deoxyadenosine (dAdo) analogue N^6-[phenylsulphenyl]-2'-deoxyadenosine **113** (Figure 28) has been prepared. These sulfenamide modified nucleosides are expected to serve as precursors to site-specific generation of RNH• via radiation-produced electrons (Figure 29).

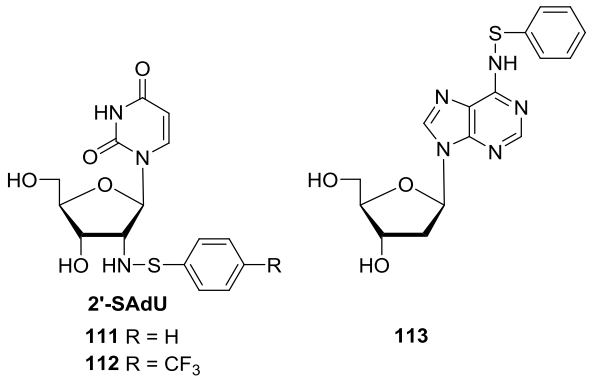


Figure 28. Sulfenamide modified nucleosides as possible substrate for the generation of *N*-centered radical at sugar or base

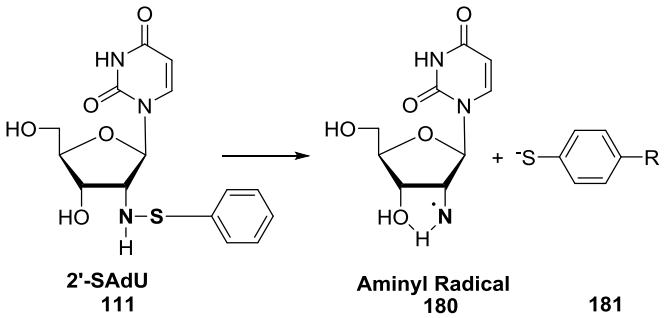


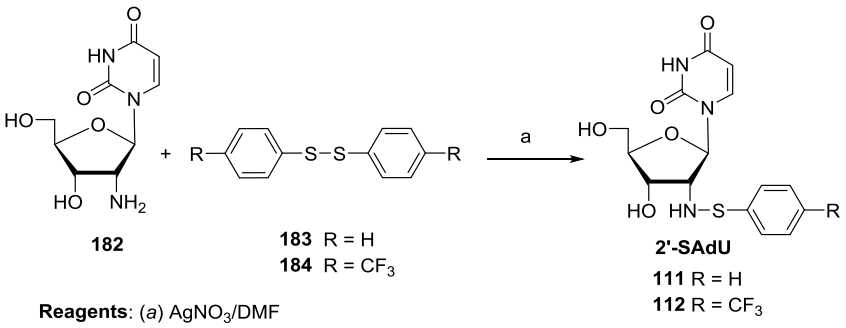
Figure 29. Plausible formation of aminyl radical in 2'-*N*-phenylsulphenyl-2'-amino-2'-deoxyuridine

Characterization of the reactive RNH• and investigation of the DNA damage it promotes will be carried out using electron spin resonance (ESR) spectroscopy. If these sulfenamide modified nucleosides would generate aminyl radical successfully I anticipate that they would be more stable compared to the azido modified nucleosides towards the P (III)-base phosphorylation protocols. Azido group in the modified nucleosides reacts intramolecularly with the phosphoramidite and greatly affects the oligo coupling.[94,107,118,153]

3.3.1. Synthesis of 2'-*N*-phenylsulphenyl-2'-amino-2'-deoxyuridine and its analogues.

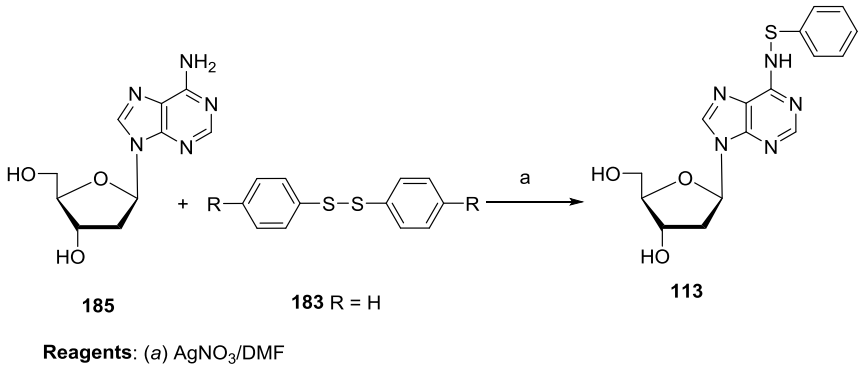
The 2'-*N*-phenylsulphenyl-2'-amino-2'-deoxyuridine, **111** and 2'-*N*-(4-trifluoromethylphenyl)-sulphenyl-2'-amino-2'-deoxyuridine, **112** were prepared

employing the general method reported by Davis and co-workers[116,154] as depicted in Scheme 41. Thus, treatment of diphenyl disulfide, **183** at 45ºC with readily available 2'-deoxy 2'-aminouridine,[154] **182** in presence of AgOAc afforded 2'-*N*-phenylsulphenyl-2'-amino-2'-deoxyuridine **111** as a white solid in 38% yield. Analogues treatment of 4,4'-bis(trifluoromethyl)-2,2'-diphenyl disulfide, **184** with 2'-deoxy-2'-aminouridine afforded 2'-*N*-(4-trifluoromethylphenyl)-sulphenyl-2'-amino-2'-deoxyuridine **112** as a white solid in 12% yield.



Scheme 41. Synthesis of 2'-*N*-phenylsulphenyl-2'-amino-2'-deoxyuridine

Similarly, synthesis of N^6-[phenylsulphenyl]-2'-deoxyadenosine **113** was accomplished by employing the general method reported by Davis and co-workers. Thus, treatment of diphenyl disulfide, **183** with readily available 2'-deoxyadenosine, **182** in the presence of AgOAc afforded N^6-[phenylsulphenyl]-2'-deoxyadenosine, **113** as a white solid in 12% yield (Scheme 42).



Scheme 42. Synthesis of N^6-[phenylsulphenyl]-2'-deoxyadenosine

3.3.2. 2'-*N*-Phenylsulphenyl-2'-amino-2'-deoxyuridine and its analogues as substrate for the aminyl radicals

4. EXPERIMENTAL PROCEDURES

4.1. General procedures

The ^{1}H (400 or 500 MHz) and ^{13}C (100 or 125 MHz) NMR spectra were determined with solutions in $CDCl_3$ unless otherwise noted. HRMS were obtained in TOF-ESI mode unless otherwise noted. TLC was performed with Merck kieselgel 60-F_{254} sheets and products were detected with 254 nm light or by visualization with $Ce(SO_4)_2/(NH_4)_6Mo_7O_{24}{\cdot}4H_2O/H_2SO_4/H_2O$ reagent. Merck kieselgel 60 (230-400 mesh) was used for column chromatography. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 (except for THF/potassium) under argon or by passing the solvents through the activated alumina cartridges using solvent purification system.

4.2. Synthesis

Methyl 6-*O*-Benzoyl-1,5-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (121). To a solution of methyl 1,5-dideoxy-2,3-isopropylidene-β-*ribo*-hexofuranoside[48] (**120**; 13.0 g, 58.8 mmol) and Et_3N (8.9 g, 12.3 mL, 88.2 mol) in

CH_2Cl_2 (50 mL) was added dropwise BzCl (12.4 g, 10.2 mL, 88.2 mmol) at 0 °C. The cooling bath was removed and after 1 h the reaction mixture was quenched by addition of MeOH (2 mL). After additional 30 min the volatiles were evaporated and the residue was column chromatographed (hexanes → hexanes/EtOAc, 6:1) to give **121** as pale yellow oil (17.3 g, 92%): ^{1}H NMR δ 1.32, 1.49 (2 × s, 2 × 3H), 2.01–2.04 (m, 2H), 3.38 (s, 3H), 4.39–4.52 (m, 3H), 4.62–4.65 (m, 2H), 4.98 (s, 1H), 7.42–7.57 (m, 3H), 8.04–8.06 (m, 2H); ^{13}C NMR δ 24.6, 26.2, 33.9, 54.8, 61.6, 83.6, 84.0, 85.2, 109.6, 112.0, 128.1, 129.3, 129.9, 132.6, 166.0; MS FAB *m/z* 345 (3, $[M+Na]^+$), 291 (100, $[M-OMe]^+$); HRMS ESI *m/z* calcd for $C_{17}H_{22}O_6Na$ $[M+Na]^+$ 345.1314, found 345.1317.

6-*O*-Benzoyl-1,5-dideoxy-D-*ribo*-hexofuranose (**122)** and **6-*O*-Benzoyl-1,5-dideoxy-2,3-*O*-isopropylidene-D-*ribo*-hexofuranose (123).** To a stirred solution of **121** (18.0 g, 55.9 mmol) and Et_3SiH (19.4 g, 26.7 mL, 167.7 mmol) in CH_2Cl_2 (10 mL) was added BF_3•OEt_2 (23.8 g, 21.3 mL, 167.7 mmol). Mildly exothermic reaction ensued after approx. 15 min and the reaction mixture was allowed to stir for an additional 3h. The reaction flask was placed in an ice slush and a saturated $NaHCO_3/H_2O$ solution (200 mL) was added slowly with vigorous stirring. CH_2Cl_2 was added (50 mL), aqueous phase was separated and washed with CH_2Cl_2 (50 mL). Organic fractions were combined and evaporated to dryness. Column chromatography (hexanes/EtOAc, 6:1 → EtOAc) gave contaminated (~10%) **123** (1.5 g, 9%) and **122** (6.1 g, 43%) as syrup. Compound **122** had: ^{1}H NMR (CD_3OD) δ 1.87–1.94 (m, 1H), 2.12–2.19 (m, 1H), 3.70 (dd, *J* = 2.9, 9.8 Hz, 1H), 3.79 (dd, *J* = 5.4, 7.3 Hz, 1H), 3.85–3.89 (m, 1H), 4.07 (dd, *J* = 4.9, 9.8 Hz, 1H), 4.16 (td, *J* = 2.9, 4.9 Hz, 1H), 4.38–4.50 (m, 2H), 7.45–7.61 (m, 3H), 8.02–8.04 (m,

2H); ^{13}C NMR (CD_3OD) δ 32.4, 61.9, 70.8, 72.2, 75.9, 78.9, 128.3, 129.5, 129.9, 133.0, 166.8; MS FAB *m/z* 275 (100, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_{13}H_{16}O_5Na$ $[M+Na]^+$ 275.1531, found 275.0902.

Diol **122** (6.1 g, 24.2 mmol) and TsOH hydrate (0.5 g, 2.6 mmol) were dissolved in a mixture of acetone (40 mL) and 2,2-dimethoxypropane (10 mL) and left to stir at room temperature for 30 min. Neutralization with sat. $NaHCO_3$ (100 mL) followed by EtOAc extraction (2 × 50 mL) and evaporation to dryness provided oil that was filtered through silica to give **123** (6.4 g, 91%): ^{1}H NMR δ 1.33, 1.52 (2 × s, 2 × 3H), 1.86–1.92 (m, 2H), 3.86 (dd, *J* = 4.4, 10.7 Hz, 1H), 3.97 (dd, *J* = 1.5, 10.7 Hz, 1H), 4.23–4.26 (m, 1H), 4.36–4.49 (m, 2H), 4.52 (dd, *J* = 1.5, 6.3 Hz, 1H), 4.80–4.83 (m, 1H), 7.42–7.57 (m, 3H), 8.03–8.05 (m, 2H); ^{13}C NMR δ 24.9, 26.5, 29.7, 61.5, 71.6, 80.8, 81.2, 85.0, 112.8, 128.3, 129.5, 130.1, 132.9, 166.4. MS FAB *m/z* 293 (100, $[M+H]^+$), 315 (15, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_{16}H_{20}O_5Na$ $[M+Na]^+$ 315.1208, found 315.1209.

1,5-Dideoxy-2,3-*O*-isopropylidene-D-*ribo*-hexofuranose (124). To a stirred solution of **123** (14.0 g, 47.9 mmol) in MeOH (50 mL) was added a solution of KOH (2.0 g, 35.7 mol) in MeOH (50 mL). After 1 h the volatiles were evaporated and the residue was column chromatographed (hexanes/EtOAc, 2:1→ EtOAc) to give **124** (8.0 g, 87%): ^{1}H NMR δ 1.33, 1.51 (2 × s, 2 × 3H), 1.63–1.76 (m, 2H), 3.74–3.82 (m, 2H), 3.89 (dd, *J* = 4.4, 10.4 Hz, 1H), 3.94 (d, *J* = 10.3 Hz, 1H), 4.19–4.22 (m, 2H), 4.48 (dd, *J* = 1.5, 5.8 Hz, 1H), 4.81 (t, *J* = 5.1 Hz, 1H); ^{13}C NMR δ 24.5, 26.2, 32.4, 59.2, 71.1, 80.4, 82.2, 84.6, 112.3. MS FAB *m/z* 189 (100, $[M+H]^+$); HRMS ESI *m*/z calcd for $C_9H_{17}O_4$ $[M+H]^+$ 189.1127, found 189.1123.

6-Iodo-1,5,6-trideoxy-2,3-*O*-isopropylidene-D-*ribo*-hexofuranose (125). To a stirred solution of **124** (9.0 g, 47.9 mmol), Ph_3P (15.1 g, 57.6 mmol) and imidazole (7.9 g, 116.2 mmol) in toluene (225 mL) was added iodine (14.6 g, 57.5 mmol) at 70 °C. The suspension was vigorously stirred another 2 h, then allowed to cool to ambient temperature and the solution decanted to another flask. Volatiles were evaporated and the residue was column chromatographed (hexanes → hexanes/EtOAc, 6:1) to give **125** (13.03 g, 85%): ^{1}H NMR δ 1.33, 1.52 (2 × s, 2 × 3H), 1.88–1.97 (m, 2H), 3.14–3.27 (m, 2H), 3.78 (dd, *J* = 4.4, 10.7 Hz, 1H), 3.95 (dd, *J* = 1.5, 10.7 Hz, 1H), 4.10 (ddd, *J* = 1.5, 4.9, 9.2 Hz, 1H), 4.23 (dd, *J* = 2.0, 6.3 Hz, 1H), 4.78 (m, 1H); ^{13}C NMR δ 1.0, 24.7, 26.2, 34.0, 71.2, 80.4, 83.6, 84.1, 112.3; MS FAB *m/z* 321 (12, $[M+Na]^+$), 319 (100); HRMS ESI *m/z* calcd for $C_9H_{15}IO_3Na$ $[M+Na]^+$ 320.9964, found 320.9970.

6-Iodo-1,5,6-trideoxy-D-*ribo*-hexofuranose (126). A solution of **125** (14.0 g, 47.0 mmol) in a mixture of MeOH (100 mL), H_2O (25 mL) and conc. HCl (25 mL) was stirred at room temperature 3 h. Volatiles were evaporated and the residue was column chromatographed (hexanes/EtOAc, 2:1 → EtOAc) to give **126** (8.73 g, 72%): ^{1}H NMR δ 2.00–2.07 (m, 1H), 2.16–2.25 (m, 1H), 3.22–3.45 (m, 4H), 3.74–3.77 (m, 2H), 3.84 (br s, 1H), 4.10 (dd, *J* = 4.9, 10.3 Hz, 1H), 4.27 (br s, 1H); ^{13}C NMR δ 1.7, 37.3, 70.9, 72.8, 75.6, 81.6; MS FAB *m/z* 258 (10, $[M]^+$), 74 (100); HRMS ESI *m/z* calcd for $C_6H_{11}IO_3$, 257.9753, found 257.9740.

1,5-Dideoxy-6-*O*-nitro-D-ribo-hexofuranose (127). A suspension of **126** (12.0 g, 46.5 mmol) and $AgNO_3$ (15.8 g, 93.0 mmol) in CH_3CN (150 mL) was stirred at room temperature for 2 days. The yellow precipitate was filtered off, washed with EtOAc and

the volatiles were evaporated. Column chromatography (hexanes/EtOAc, 1:1 → EtOAc) gave **127** (7.99 g, 89%): ^{1}H NMR δ 1.88–1.96 (m, 1H), 2.08–2.15 (m, 1H), 3.43 (br s, 2H), 3.73–3.83 (m, 3H), 4.12 (dd, *J* = 4.9, 10.3 Hz, 1H), 4.23–4.26 (m, 1H), 4.56–4.66 (m, 2H); ^{13}C NMR δ 30.2, 70.1, 70.8, 72.6, 75.9, 77.8; MS FAB *m/z* 216 (100, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_6H_{11}NO_6Na$ $[M+Na]^+$, 216.0484 found 216.0497.

1,5-Dideoxy-6-*O*-nitro-2-*O*-tosyl-D-ribo-hexofuranose (96). Method A. A solution of diol **127** (370 mg, 1.92 mmol) and Bu_2SnO (477 mg, 1.92 mmol) in anhydrous MeOH (40 mL) was heated in a sealed flask at 75 °C for 1 h. After the flask was cooled to 0 °C, Et_3N (1.15 g, 1.6 mL, 11.4 mmol) was added with stirring followed by TsCl (2.17 g, 11.4 mmol) dissolved in a minimum volume of acetone. The volatiles were evaporated, and the residue was suspended in acetone and deposited on silica. Column chromatography (hexanes/EtOAc, 6:1 → 3:1) gave a 5:2 mixture of two isomers (670 mg) from which the main product **96** (320 mg, 48%) was isolated after second column chromatography: ^{1}H NMR δ 1.85–1.92 (m, 1H), 2.09–2.17 (m, 1H), 2.46 (s, 3H), 2.76 (d, *J* = 7.8 Hz, 1H), 3.75 (dt, *J* = 3.9, 8.3 Hz, 1H), 3.80 (dd, *J* = 3.1, 11.2 Hz, 1H), 3.89 (dt, *J* = 5.4, 7.8 Hz, 1H), 4.06 (dd, *J* = 4.9, 11.2 Hz, 1H), 4.52–4.62 (m, 2H), 4.93 (dt, *J* = 3.0, 5.3 Hz, 1H), 7.38, 7.82 (2 × d, *J* = 8.3 Hz, 2 × 2H); ^{13}C NMR δ 21.6, 30.2, 69.8, 70.1, 74.9, 77.8, 79.4, 127.8, 130.1, 132.8, 145.6; MS FAB *m/z* 370 (100, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_{13}H_{17}NO_8SNa$ $[M+Na]^+$ 370.0573, found 370.0587.

Method B. Step *a*. TsCl (66 mg, 0.35 mmol) was added to a stirred solution of **128** (100 mg, 0.32 mmol) in anhydrous pyridine (1mL) at ambient temperature. After 16 h, the volatiles were evaporated and residue was partitioned between ice-cold $AcOH/H_2O$

(1:99, 30 mL) and $CHCl_3$ (30 mL). The aqueous layer was extracted with $CHCl_3$ and the combined organic phase was washed with ice-cold saturated $NaHCO_3/H_2O$ (30 mL), brine (30 mL) and dried (Na_2SO_4). Column chromatography (EtOAc/hexane, 5:95 → 35:65) gave 1,5-dideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-2-*O*-tosyl-D-*ribo*-hexofuranose (**129;** 82 mg, 55%): ^{1}H NMR δ 1.78-1.81 (m, 1H), 2.00-2.02 (m, 1H), 2.47 (s, 3H), 3.59 (dd, *J* = 5.0, 8.6 Hz, 1H), 3.83 (s, 3H), 3.86 (dt, *J* = 2.2, 9.6 Hz, 2H), 4.04 (dd, *J* = 4.5, 11.2 Hz, 2H) , 4.26 (d, *J* = 11.2 Hz, 1H), 4.47 (m, 2H), 4.58 (d, *J* = 11.2Hz, 1H), 5.13 (dt, *J* = 4.7, 7.0 Hz, 1H), 6.88 and 7.21 (2 x d, *J* = 9.5Hz, 2 x 2H), 7.34 and 7.84 (2 x d, *J* = 8.3 Hz, 2 x 2H). Step *b*. A solution of CAN (176 mg, 0.32 mmol) and **129** (75 mg, 0.16 mmol) in MeCN (1.5 mL) and water (0.15 mL) was stirred at ambient temperature for 22 h. Volatiles were evaporated and the residue was column chromatographed (EtOAc/hexane, 3:97 → 30:70) to give **96** (54 mg, 98%) with data as above.

1,5-Dideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-D-*ribo*-hexofuranose (128). A suspension of diol **127** (1.20 g, 6.22 mmol) and Bu_2SnO (1.70 g, 6.84 mmol) in anhydrous MeOH (10 mL) was heated in a sealed flask at 75 °C for 1 h. After the flask was cooled to ambient temperature, the volatiles were evaporated. DMF (5 mL) and PMBCl (1.95 g, 1.7 mL, 12.44) were added, and the reaction mixture was heated at 90 °C for 18 h. Volatiles were evaporated, and the residue was chromatographed (hexanes/EtOAc, 6:1 → 1:1) to give 1,5-dideoxy-2,3-di-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-D-*ribo*-hexofuranose (1.55 g; 20% contaminated) followed by 1,5-dideoxy-2-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-D-*ribo*-hexofuranose (0.47 g, 24%) and **128** (0.60 g; 31%) of sufficient purity for use in subsequent reaction. Sample of **128** was repurified on column

chromatography for spectroscopic characterization: ^{1}H NMR δ 1.77–1.85 (m, 1H), 1.93–2.00 (m, 1H), 3.20 (br s, 1H), 3.60 (dd, *J* = 5.4, 7.3 Hz, 1H), 3.76 (dd, *J* = 2.9, 10.3 Hz, 1H), 3.81 (s, 3H), 3.85 (dt, *J* = 3.9, 7.3 Hz, 1H), 4.01 (dd, *J* = 4.9, 10.3 Hz, 1H), 4.18 (m, 1H), 4.47–4.61 (m, 4H), 6.88–6.92 (m, 2H), 7.24–7.30 (m, 2H); ^{13}C NMR δ 30.5, 55.2, 69.1, 70.0, 72.6, 73.3, 76.1, 82.4, 114.0, 128.8, 129.7, 159.7; MS FAB *m/z* 336 (100, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_{14}H_{19}NO_7Na$ $[M+Na]^+$ 336.1059, found 336.1072.

2-Chloro-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-1,2,5-trideoxy-D-*arabino*-hexofuranose (130). To a solution of Ph_3P (2.18 g, 8.31 mmol) and DIAD (1.26 g, 1.22 mL, 6.23 mmol) in THF (14 mL) was added a solution of **128** (0.65 g, 2.08 mmol) in THF (6 mL) followed by freshly prepared HCl•pyridine (0.72 g, 6.23 mmol; prepared by slow addition of TMSCl (0.68 g, 0.8 mL, 6.31 mmol) to a solution of pyridine (0.98 g, 1.0 mL, 12.4 mmol) in MeOH (0.32 g, 0.4 mL, 9.9 mmol) and CH_2Cl_2 (10 mL) at 0 °C. Volatiles were evaporated and the crystalline residue was dried at 80 °C under vacuum overnight). The suspension was stirred overnight at room temperature. Volatiles were evaporated, and the residue was column chromatographed (hexanes/EtOAc, 10:1 → 6:1) to give **130** (0.43 g, 62%): ^{1}H NMR 2.05–2.10 (m, 2H), 3.82 (br s, 4H), 3.81–3.86 (m, 1H), 3.90 (br d, *J* = 3.4 Hz, 1H), 4.02 (d, *J* = 10.3 Hz, 1H), 4.13 (dd, *J* = 4.4, 10.7 Hz, 1H), 4.47–4.65 (m, 4H), 6.90–6.93 (m, 2H), 7.25-7.28 (m, 2H); ^{13}C NMR 30.9, 55.2, 60.1, 70.0, 72.1, 74.2, 80.9, 89.8, 113.9, 128.8, 129.6, 159.5; MS FAB *m/z* 209 (100, $[M]^+$); HRMS ESI *m/z* Calcd for $C_{14}H_{18}ClNO_6Na$ $[M+Na]^+$ 354.0720, found 354.0706.

2-Chloro-1,2,5-trideoxy-6-*O*-nitro-D-*arabino*-hexofuranose (97). A solution of **130** (0.43 g, 1.30 mmol) and CAN (1.42 g, 2.59 mmol) in CH_3CN (7 mL) and H_2O (0.7 mL) was stirred for 1.5 h. The reaction mixture was concentrated, and the residue was

deposited on silica gel and column chromatographed (hexanes/EtOAc, 10:1 → 3:1) to give **97** (0.21 g, 78%): ^{1}H NMR δ 2.10–2.22 (m, 2H), 2.69 (br s, 1H), 3.80–3.84 (m, 1H), 4.02 (br d, *J* = 10.8 Hz, 1H), 4.14–4.25 (m, 3H), 4.58–4.68 (m, 2H); ^{13}C NMR δ 30.9, 62.3, 70.0, 73.3, 81.7, 83.2; MS FAB *m/z* 212 (5, $[M+H]^+$), 120 (100); HRMS ESI *m/z* calcd for $C_6H_{11}ClNO_5[M+H]^+$ 212.0326, found 212.0327.

2-Bromo-1,2,5-trideoxy-6-*O*-nitro-D-arabino-hexofuranose (98). Step *a*. To a solution of Ph_3P (2.85 g, 10.09 mmol) and DIAD (1.65 g, 8.15 mmol) in THF (30 mL) was added a stirred solution of **10** (1.75 g, 5.43 mmol) in THF (10 mL) followed by freshly prepared HBr•pyridine (1.30 g, 8.15 mmol; prepared by slow addition of TMSBr (0.93 g, 0.8 mL, 6.06 mmol) to a solution of pyridine (0.98 g, 1.0 mL, 12.4 mmol) in MeOH (0.32 g, 0.4 mL, 9.9 mmol) and CH_2Cl_2 (10 mL) at 0 °C. Volatiles were evaporated and the crystalline residue was dried at 80 °C under vacuum overnight). The suspension was stirred at room temperature for 22 h. Volatiles were evaporated, and the residue was column chromatographed (hexanes/EtOAc, 10:1 → 6:1) to give crude 2-bromo-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-1,2,5-trideoxy-D-arabino-hexofuranose (**131**; 1.0 g, 49%) which was directly used in next step. Step *b*. A solution of crude **131** (1.0 g, 2.66 mmol) and CAN (2.91 g, 5.32 mmol) in a mixture of CH_3CN (14 mL) and H_2O (1.4 mL) was stirred for 1.5 h. The resulting mixture was concentrated and the residue was deposited on silica and column chromatographed (hexanes/EtOAc, 10:1 → 4:1) to give **98** (0.54 g, 79%): ^{1}H NMR δ 2.14-2.26 (m, 2H), 3.80-3.86 (dt, *J* = 5.2, 8.8 Hz, 1H), 4.10 (dd, *J* = 4.0, 10.6 Hz, 1H), 4.16-4.22 (m, 1H), 4.26-4.33 (m, 2H), 4.58-4.72 (m, 2H); ^{13}C NMR δ 31.2, 51.5, 69.9, 73.6, 81.5, 83.5. HRMS ESI/DART *m/z* calcd for

$C_6H_{14}{}^{79}BrN_2O_5$ $[M+NH_4]^+$ 273.0081, found 273.0084; calcd for $C_6H_{14}{}^{81}BrN_2O_5$ $[M+NH_4]^+$ 275.0061, found 275.0061.

1,5-Dideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-*D*-*arabino*-hexofuranose (133). *Step a.***.** A solution of compound **128** (2.70 g, 8.63 mmol) in THF (8 mL) followed by a solution of DIAD (2.09 g, 2.03 mL, 10.35 mmol) in THF (3 mL).were slowly (12 min) added to a stirred solution of Ph_3P (2.71 g, 10.35 mmol) and $PhCO_2H$ (1.26 g, 10.35 mmol) in THF (40 mL) at -50 °C under nitrogen atmosphere. The resulting mixture was allowed to warm to ambient temperature within 1 h (it became colorless at -20 °C). Volatiles were evaporated, and the residue was column chromatographed (hexanes → hexanes/EtOAc, 3:1) to give 2-*O*-benzoyl-1,5-dideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-*D*-*arabino*-hexofuranose (**132** ;2.87 g, 80%): ^{1}H NMR δ 2.0-2.07 (m, 2H), 3.79 (s, 3H), 3.85 (td, *J* = 1.0, 4.6 Hz, 1H), 3.90 (td, *J* = 5.0, 7.6 Hz, 1H), 4.08-4.12 (m, 2H), 4.51-4.59 (m, 3H), 4.75 (d, *J* = 11.7 Hz, 1H), 5.43 (td, *J* = 1.4, 2.7 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 7.27 (d, *J* = 8.7, 2H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.61 (tt, *J* = 1.5, 7.5 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 2H). *Step b.* A suspension of KOH (0.40 g, 7.14 mol) in MeOH (20 mL) was added to a stirred solution of **132** (2.82 g, 6.76 mmol) in MeOH (20 mL) at ambient temperature. After 1 h, the solution was neutralized with AcOH to pH 7. The volatiles were evaporated and the residue was column chromatographed (hexanes/EtOAc, 3:1 → 2:1) to give **133** (1.64 g, 77%): ^{1}H NMR δ 1.26 (d, *J* = 2.9 Hz, 1H), 2.00–2.04 (m, 2H), 3.64 (d, *J* = 2.9 Hz, 1H), 3.80 (br s, 4H), 3.83 (d, *J* = 10.3 Hz, 1H), 3.91 (dd, *J* = 3.9, 10.3 Hz, 1H), 4.27–4.29 (m, 1H), 4.48–4.58 (m, 4H), 6.88–6.91 (m, 2H), 7.24–7.27 (m, 2H); ^{13}C NMR δ 30.9, 55.2, 70.2, 71.8, 74.1, 76.1 80.3, 89.2, 113.9, 128.4, 129.5, 159.4;

MS FAB *m/z* 336 (100, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_{14}H_{19}NO_7Na$ $[M+Na]^+$ 336.1059, found 336.1047.

1,5-Dideoxy-6-*O*-nitro-2-*O*-tosyl-*D*-*arabino*-hexofuranose (99). *Step a*. TsCl (134 mg, 0.70 mmol) was added to a stirred solution of **133** (100 mg, 0.32 mmol) in anhydrous pyridine (1mL) at ambient temperature. After 16 h, the volatiles were evaporated and residue was partitioned between ice-cold $AcOH/H_2O$ (1:99, 30 mL) and $CHCl_3$ (30 mL). The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (30 mL). Combined organic phase was washed with ice-cold saturated $NaHCO_3/H_2O$ (30 mL), brine (30 mL) and dried (Na_2SO_4), concentrated *in vacuo* and column chromatographed (EtOAc/hexane, 5:95 → 35:65) to give 1,5-dideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-2-*O*-tosyl-*D*-*arabino*-hexofuranose (**134a**; 128 mg, 86%) as a colorless oil: ^{1}H NMR δ 1.90-1.96 (m, 2H), 2.46 (s, 3H), 3.72-3.78 (m, 1H), 3.82 (s, 3H), 3.84 (dt, *J* = 1.2, 4.6 Hz, 1H), 3.87 (d, *J* = 4.0 Hz, 1H), 3.92 (d, *J* = 11.3 Hz, 1H), 4.33 (d, *J* = 11.3 Hz, 1H), 4.42-4.49 (m, 3H), 4.93 (dt, *J* = 1.2, 4.0 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.81 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR δ 21.6, 30.3, 55.3, 69.8, 71.4, 71.8, 80.2, 83.9, 86.3, 114.0, 127.8, 128.8, 129.5, 130.1, 133.4, 145.4, 159.6. *Step b*. A solution of CAN (352 mg, 0.64 mmol) and **134a** (100 mg, 0.21 mmol) in MeCN (1mL) and H_2O (0.1 mL) was stirred at ambient temperature for 22 h. Volatiles were evaporated and the residue was column chromatographed (EtOAc/hexane, 3:97 → 30:70) to give **99** (73 mg, 98%) as a colorless oil: ^{1}H NMR δ 1.97-2.07 (m, 1H), 2.07-2.17 (m, 1H), 2.47 (s, 3H), 3.69-3.76 (m, 1H), 3.85-3.90 (m, 1H), 3.95 (dd, *J* = 5.5, 11.3 Hz, 1H), 4.17 (dd, *J* = 2.5, 6.0 Hz, 1H), 4.51-4.63 (m, 2H), 4.76 (dt, *J* = 2.5, 5.5 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 8.5 Hz,

2H); ^{13}C NMR δ 21.6, 30.2, 69.7, 70.3, 80.5, 80.9, 86.5, 127.9, 130.1, 132.8, 145.6. HRMS ESI/DART *m/z* calcd for $C_{13}H_{21}N_2O_8S$ $[M+NH_4]^+$ 365.1013, found 365.1031.

2-Chloro-1,2,5-trideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-D-*ribo*-hexofuranose (135). Step *a*. TfCl (160 mg, 0.95 mmol) was added to a stirred solution of **133** (250 mg, 0.80 mmol) and DMAP (295 mg, 2.4 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0°C (ice-bath). After 1 h, the reaction mixture was partitioned between ice-cold AcOH/H_2O (1:99, 30 mL) and CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (30 mL) and the combined organic phase was washed with ice-cold saturated $NaHCO_3$/H_2O (30mL), brine (30mL) and dried (Na_2SO_4) to give 1,5-dideoxy-3-*O*-(*p*-methoxybenzyl)-2-*O*-(trifluoromethanesulfonyl)-6-*O*-nitro-D-*arabino*-hexofuranose as a colorless oil (**134b**; 313 mg, 88%) of sufficient purity to be used in next step. Column chromatography (EtOAc/hexane, 5:95 → 30:70) gave pure sample of **134b**: ^{1}H NMR δ 1.93-2.02 (m, 2H), 3.80-3.84 (m, 1H), 3.82 (s, 3H), 3.92 (dt, *J* = 1.2, 4.6 Hz, 1H), 4.02 (dd, *J* =3.5, 12.0 Hz,1H), 4.18 (br d, *J* = 12.1 Hz, 1H), 4.47-4.52 (m, 3H), 4.66 (d, *J* = 11.5 Hz, 1H), 5.32 (d, *J* = 3.5 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 8.8 Hz, 2H). Step *b*. A solution of crude **134b** (50 mg, 0.11 mmol; from step *a*) and dried LiCl (23 mg, 0.54 mmol) in DMF (1 mL) was stirred for 5 h at ambient temperature under N_2. Volatiles were evaporated and residue was partitioned between ice-cold saturated $NaHCO_3$/H_2O (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (30 mL), dried (Na_2SO_4) and column chromatographed (EtOAc/hexane, 5:95 → 15:85) to give **135** (30 mg, 80.6%) as a colorless oil: ^{1}H NMR δ 1.82-1.91 (m , 1H), 2.01-2.1 (m,1H), 3.72 (dd, *J* = 5.2, 8.2 Hz, 1H), 3.82 (s, 3H), 3.99 (dt, *J* = 4.0, 8.2 Hz, 1H), 4.06 (dd, *J* = 2.8,10.7 Hz, 1H), 4.30 (dd, *J* = 5.07,10.7 Hz,1H), 4.40 (d, *J* = 11.2 Hz, 1H), 4.44 (dt, *J* = 2.2, 5.2 Hz,

2H), 4.48-4.59 (m, 2H), 4.69 (d, 11.3 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR δ 30.4, 55.2, 57.8, 69.9, 72.1, 74.0, 76.3, 81.5, 114.0, 128.8, 129.8, 159.7. HRMS ESI *m/z* calcd for $C_{14}H_{18}{}^{35}ClNO_6Na$ $[M+Na]^+$ 354.0715, found 354.0718; calcd for $C_{14}H_{18}{}^{37}ClNO_6Na$ $[M+Na]^+$ 356.0691, found 356.0694.

2-Chloro-1,2,5-trideoxy-6-*O*-nitro-D-*ribo*-hexofuranose (94). A solution of CAN (74 mg, 0.13 mmol) and **19** (15 mg, 0.04 mmol) in MeCN (1mL) and H_2O (0.1 mL) was stirred at ambient temperature for 22 h. Volatiles were evaporated and the residue was column chromatographed (EtOAc/hexane, 3:97 → 30:70) to give **94** (6.2 mg, 66%) as a colorless oil: ^{1}H NMR δ 1.87-1.98 (m,1H), 2.11-2.20 (m,1H), 2.25 (d, *J* = 8.7 Hz, 1H), 3.82-3.88 (m,1H), 3.93-4.00 (m, 1H), 4.02 (dd, *J* = 4.0, 10.7 Hz, 1H), 4.33 (dd, *J* = 5.4, 10.5 Hz,1H), 4.44-4.48 (m, *J* = 4.2, 5.3 Hz, 1H), 4.55-4.67 (m, 2H); ^{13}C NMR δ 30.5, 61.1, 69.8, 72.9, 75.7, 78.3; HRMS ESI/DART *m/z* calcd for $C_6H_{14}{}^{35}ClN_2O_5$ $[M+NH_4]^+$ 229.0586, found 229.0588; calcd for $C_6H_{14}{}^{37}ClN_2O_5$ $[M+NH_4]^+$ 231.0562, found 231.0561.

2-Bromo-1,2,5-trideoxy-3-*O*-(*p*-methoxybenzyl)-6-*O*-nitro-D-*ribo*-hexofuranose (136). A solution of **134b** (30 mg, 0.06 mmol; prepared as described for **135**, step *a*) and dried LiBr (29 mg, 0.33 mmol) in DMF (1 mL) was stirred for 7 h at ambient temperature under N_2. Volatiles were evaporated and the resulting residue was partitioned between ice-cold saturated $NaHCO_3/H_2O$ (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (30 mL), dried (Na_2SO_4) and column chromatographed (EtOAc/hexane, 5:95 → 15:85) to give **136** (18 mg, 71%) as a colorless oil: ^{1}H NMR δ 1.83-1.94 (m, 1H), 2.02-2.12 (m,1H), 3.6 (dd, *J* = 5.0, 7.6 Hz, 1H), 3.84 (s, 3H), 4.03 (dt, *J* = 4.0, 8.0 Hz, 1H), 4.22 (dd, *J* = 2.8,10.6 Hz, 1H), 4.44 (d, *J* = 11.1 Hz, 1H), 4.43-4.51

(m, 2 H), 4.51-4.61 (m, 2H), 4.71 (d, *J* = 11.1 Hz, 1H) 6.92 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR δ 30.4, 49.7, 55.3, 69.9, 72.1, 74.1, 76.6, 81.2, 114.0, 128.81, 129.8, 159.71. HRMS ESI *m/z* calcd for $C_{14}H_{18}{}^{79}BrNO_6Na$ $[M+Na]^+$ 398.0210, found 398.0203; calcd for $C_{14}H_{18}{}^{81}BrNO_6Na$ $[M+Na]^+$ 400.0191, found 400.0183.

2-Bromo-1,2,5-trideoxy-6-*O*-nitro-D-*ribo*-hexofuranose (95). A solution of CAN (78 mg, 0.14 mmol) and **136** (18 mg, 0.05 mmol) in MeCN (1 mL) and water (0.1 mL) was stirred at ambient temperature for 22 h. Volatiles were evaporated and the residue was column chromatographed (EtOAc/hexane, 3:97 → 30:70) to give **95** (12 mg, 96%) as a colorless oil: ^{1}H NMR δ 1.89-1.98 (m, 1H), 2.11-2.20 (m, 2H), 3.78-3.84 (m, 1H), 3.85-3.92 (m, 1H), 4.13 (dd, *J* = 4.6 Hz, 1H), 4.43 (dd, *J* = 5.4 Hz, 1H), 4.48 (dd, *J* = 5.0 Hz, 1H), 4.55-4.70 (m, 2H); ^{13}C NMR δ 31.6, 53.7, 69.8, 72.9, 75.3, 78.8. HRMS ESI/DART *m/z* calcd for $C_6H_{14}{}^{79}BrN_2O_5$ $[M+NH_4]^+$ 273.0081, found 273.0077; calcd for $C_6H_{14}{}^{81}BrN_2O_5$ $[M+NH_4]^+$ 275.0066, found 275.0058.

1,5-Dideoxy-3-*O*-methyl-6-*O*-nitro-D-*ribo*-hexofuranose (137) and 1,5-Dideoxy-2-*O*-methyl-6-*O*-nitro-D-*ribo*-hexofuranose (138) and: A suspension of **127** (0.34 g, 1.76 mmol) and Bu_2SnO (0.44 g, 1.76 mmol) in anhydrous MeOH (8 mL) was refluxed for 30 min. Volatiles were evaporated after the flask was cooled to ambient temperature. DMF (1 mL) and MeI (1.14 g, 0.5 mL, 8.03 mmol) were added, the flask was sealed and the solution was stirred at 40 °C for 12 h. Volatiles were evaporated and the residue was column chromatographed (hexanes/EtOAc, 4:1 → 1:1) to give **137** (145 mg, 38%) and **138** (134 mg, 35 %). Compound **137** had: ^{1}H NMR δ 1.85–1.92 (m, 1H), 2.09–2.16 (m, 1H), 2.79 (d, *J* = 8.8 Hz, 1H), 3.44 (s, 3H), 3.67 (ddd, *J* = 3.9, 7.8, 8.8 Hz, 1H), 3.74–3.84 (m, 3H), 4.06 (dd, *J* = 4.4, 5.4 Hz, 1H), 4.56–4.65 (m, 2H); ^{13}C NMR δ 30.5, 57.7, 70.0,

70.1, 75.4, 78.6, 79.4; MS FAB *m/z* 230 (100, $[M+Na]^+$); HRMS ESI *m/z* calcd for $C_7H_{13}NO_6Na$ $[M+Na]^+$ 230.0641, found 230.0651. Compound **138** had: ^1H NMR δ 1.89–1.96 (m, 1H), 2.04–2.11 (m, 1H), 2.70 (d, *J* = 3.9 Hz, 1H), 3.45 (dd, *J* = 4.9, 6.8 Hz, 1H), 3.47 (s, 3H), 3.79 (dd, *J* = 2.9, 9.8 Hz, 1H), 3.86 (ddd, *J* = 3.9, 6.8, 8.3 Hz, 1H), 3.79 (dd, *J* = 4.4, 10.2 Hz, 1H), 4.31 (m, 1H), 4.55–4.65 (m, 2H); ^{13}C NMR δ 30.8, 58.3, 68.9, 70.0, 73.2, 75.9, 85.2; MS FAB *m/z* 208 (100, $[M+H]^+$); HRMS ESI *m/z* calcd for $C_7H_{14}NO_6$ $[M+H]^+$ 208.0821, found 208.0819.

1,5-Dideoxy-2-*O*-tosyl-3-*O*-methyl-6-*O*-nitro-D-*ribo*-hexofuranose (102). TsCl (101 mg, 0.53 mmol) was added to a stirred solution of **137** (100 mg, 0.48 mmol) in anhydrous pyridine (1 mL) at ambient temperature. After 16 h, the volatiles were evaporated and residue was partitioned between ice-cold $AcOH/H_2O$ (1:99, 30 mL) and $CHCl_3$ (30 mL). The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (30 mL). Combined organic phase was washed with ice-cold $NaHCO_3/H_2O$ (30 mL), brine (30 mL), dried (Na_2SO_4), concentrated *in vacuo* and column chromatographed (EtOAc/hexane, 5:95 → 35:65) to give **102** (110 mg, 63%) as a colorless oil. ^1H NMR δ 1.86-1.96 (m, 1H), 2.05-2.15 (m, 1H), 2.48 (s, 3H), 3.31 (s, 3H), 3.47 (dd, *J* = 5.0, 8.6 Hz, 1H), 3.85 (dd, *J* = 4.2, 8.6 Hz, 1H), 3.89 (dd, *J* = 2.44, 11.5 Hz, 1H), 4.07 (dd, *J* = 4.44, 11.5 Hz, 1H), 4.51-4.63 (m, 2H), 5.12 (dt, *J* = 2.2, 4.7 Hz, 1H), 7.39 (d, *J* = 8.15 Hz, 2H), 7.85 (d, *J* = 8.15 Hz, 2H); ^{13}C NMR δ 21.6, 30.5, 58.3, 69.8, 70.9, 75.9, 76.7, 83.7, 127.8, 129.9, 133.7, 145.2. HRMS ESI/DART *m/z* calcd for $C_{14}H_{23}N_2O_8S$ $[M+NH_4]^+$ 379.1170, found 379.1172.

2-*O*-Benzoyl-1,5-dideoxy-3-*O*-methyl-6-*O*-nitro-D-*arabino*-hexofuranose (139). Compound **137** (0.20 g, 0.97 mmol) in THF (6 mL) was added to a stirred solution of

Ph_3P (0.31 g, 1.16 mmol) and $PhCO_2H$ (0.14 g, 1.16 mmol) in THF (5 mL) at -50 °C. After 5 min. DIAD (0.23 g, 0.22 mL, 1.16 mmol) in THF (2 mL) was added slowly over 12 min. The reaction mixture was allowed to warm to room temperature within 1 h (it became colorless at -20 °C). Volatiles were evaporated, and the residue was column chromatographed (hexanes → hexanes/EtOAc, 10:1) to give **139** (0.23 g, 76%): ^{1}H NMR δ 2.07–2.17 (m, 2H), 3.50 (s, 3H), 3.71 (d, *J* = 3.4 Hz, 1H), 3.86–3.90 (m, 1H), 4.03–4.07 (m, 2H), 4.57–4.66 (m, 2H), 5.38–5.40 (m, 1H), 7.44–7.62 (m, 3H) , 8.02–8.14 (m, 2H); ^{13}C NMR δ 30.8, 58.0, 70.0, 71.7, 77.9, 80.4, 89.6, 128.4, 129.4, 130.1, 133.4, 165.7; MS FAB *m/z* 334 (15, $[M+Na]^+$), 312 (100, $[M+H]^+$); HRMS ESI *m/z* calcd for $C_{14}H_{17}O_7NNa$ $[M+Na]^+$ 334.0903, found 334.0904.

1,5-Dideoxy-3-*O*-methyl-6-*O*-nitro-D-*arabino*-hexofuranose (140). KOH (1.62 g, 28.9 mmol) in MeOH (80 mL) was added to a stirred solution of **139** (1.82 g, 5.85 mmol) in MeOH (80 mL). The reaction mixture was left to stir at room temperature for 1 h, then was neutralized with 5% HCl/H_2O. Volatiles were evaporated, and the residue was column chromatographed (hexanes/EtOAc, 3:1 → 1:1) to give **140** (0.87 g, 72%): ^{1}H NMR (300 MHz) δ 2.07–2.14 (m, 2H), 2.80 (br s, 1H), 3.42 (s, 3H), 3.48 (td, *J* = 1.1, 3.9 Hz, 1H), 3.79 (ddd, *J* = 3.9, 6.1, 7.3 Hz, 1H), 3.85 (br d, *J* = 10.3 Hz, 1H), 3.90 (dd, *J* = 3.7, 10.3 Hz, 1H), 4.27–4.29 (m, 1H), 4.54–4.67 (m, 2H); ^{13}C NMR δ 30.9, 57.5, 70.2, 73.9, 75.2, 80.2, 91.6; MS FAB *m/z* 208 (5, $[M+H]^+$), 71 (100); HRMS ESI *m/z* calcd for $C_7H_{14}NO_6$ $[M+H]^+$ 208.0821, found 208.0807.

2-Chloro-3-*O*-methyl-6-*O*-nitro-1,2,5-trideoxy-D-*arabino*-hexofuranose (103). Solution of **137** (0.60 g, 2.90 mmol) in THF (15 mL) was added to a stirred solution of

Ph_3P (1.52 g, 5.78 mmol) and DIAD (0.89 g, 0.86 mL, 4.38 mmol) in THF (15 mL) followed by addition of freshly prepared HCl•pyridine (0.50 g, 4.33 mmol). The suspension was stirred overnight at room temperature. Volatiles were evaporated, and the residue was chromatographed (hexanes → hexanes/EtOAc, 6:1) to give **103** (0.38 g, 61%): ^{1}H NMR δ 2.14–2.18 (m, 2H), 3.44 (s, 3H), 3.73 (d, *J* = 3.9 Hz, 1H), 3.78–3.82 (m, 1H), 4.02 (d, *J* = 10.7 Hz, 1H), 4.10 (dd, *J* = 4.4, 10.7 Hz, 1H), 4.24–4.27 (m, 1H), 4.56–4.67 (m, 2H); ^{13}C NMR δ 31.3, 58.1, 59.4, 70.0, 74.2, 80.9, 92.8; HRMS ESI/DART *m/z* calcd for $C_7H_{16}{}^{35}ClN_2O_5$ $[M+NH_4]^+$ 243.0742, found 243.0752; calcd for $C_7H_{16}{}^{37}ClN_2O_5$ $[M+NH_4]^+$ 245.0716, found 245.0715.

2-Bromo-3-*O*-methyl-6-*O*-nitro-1,2,5-trideoxy-D-*arabino*-hexofuranose (104). Solution of **137** (0.65 g, 3.13 mmol) in THF (15 mL) was added to a stirred solution of Ph_3P (1.64 g, 6.25 mmol) and DIAD (0.95 g, 0.92 mL, 4.69 mmol) in THF (15 mL) followed by addition of freshly prepared HBr•pyridine (0.75 g, 4.69 mmol). The suspension was stirred overnight at room temperature. Volatiles were evaporated, and the residue was column chromatographed (hexanes/EtOAc, 10:1 → 6:1) to give **104** (0.39 g, 46%): ^{1}H NMR δ 2.19-2.27 (m, 2H), 3.49 (s, 3H), 3.82 (dt, *J* = 4.2, 6.8 Hz, 1H), 3.92 (d, *J* = 3.8 Hz, 1H), 4.14 (dd, *J* = 1.86, 11.0 Hz, 1H), 4.21 (dd, *J* = 4.47, 11.0 Hz, 1H), 4.27-4.31 (m, 1H), 4.58-4.70 (m, 2H); ^{13}C NMR δ 31.5, 48.5, 58.0, 70.0, 74.6, 81.2, 93.0. HRMS ESI/DART *m/z* calcd for $C_7H_{16}{}^{79}BrN_2O_5$ $[M+NH_4]^+$ 287.0237 ,found 287.0252; calcd for $C_7H_{16}{}^{81}BrN_2O_5$ $[M+NH_4]^+$ 289.0218, found 289.0233.

1,5-Dideoxy-2-*O*-tosyl-3-*O*-methyl-6-*O*-nitro-D-*arabino*-hexofuranose (141). TsCl (116 mg, 1.057 mmol) was added to a stirred solution of **140** (200 mg, 0.965 mmol) in anhydrous pyridine (1 mL) at ambient temperature. After 16 h, the volatiles were

evaporated and residue was partitioned between ice-cold AcOH/H_2O (1:99, 30 mL) and $CHCl_3$ (30 mL). The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (30 mL). Combined organic phase was washed with ice-cold saturated $NaHCO_3/H_2O$ (30 mL), brine (30 mL) and dried (Na_2SO_4), concentrated *in vacuo* and column chromatographed (EtOAc/hexane, 5:95 → 35:65) to give **141 (**240 mg, 69%) as a colorless oil: ^{1}H NMR δ 1.96-2.11 (m, 2H), 2.47 (s, 3H), 3.29 (s, 3H), 3.65 (td, *J* = 1.2, 4.4 Hz, 1H), 3.69-3.75 (m, 1H) , 3.81 (dd, *J* = 4.1, 11.4 Hz, 1H), 3.92 (d, *J* = 11.4 Hz, 1H), 4.48-4.60 (m, 2H), 4.86 (td, *J* = 1.2, 4.1 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H). ^{13}C NMR δ 21.6, 30.5, 58.0, 69.8, 71.3, 80.1, 83.1, 89.4, 127.8, 130.0, 133.4, 145.4. HRMS ESI/DART *m/z* calcd for $C_{14}H_{23}N_2O_8S$ $[M+NH_4]^+$ 379.1170, found 379.1169.

2-Chloro-1,2,5-trideoxy-3-*O*-methyl-6-*O*-nitro-D-*ribo*-hexofuranose (100). *Step a*. TfCl (123 μL, 195 mg, 1.16 mmol) was added to a stirred solution of **140** (200 mg, 0.96 mmol) and DMAP (354 mg, 2.9 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0°C (ice-bath). After 1 h, the reaction mixture was partitioned between ice-cold AcOH/H_2O (1:99, 30 mL) and CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (30 mL) and the combined organic phase was washed with ice-cold saturated $NaHCO_3/H_2O$ (30 mL), brine (30 mL) and dried (Na_2SO_4) to give **142** as a colorless oil (243 mg, 74%) of sufficient purity to be used in next step. Column chromatography (EtOAc/hexane, 5:95 → 30:70) gave pure sample of **142**: ^{1}H NMR δ 2.01-2.09 (m, 1H), 2.09-2.19 (m, 1H), 3.46 (s, 3H), 3.75-3.82 (m, 2H), 3.98 (dd, *J* =3.6, 12.1 Hz,1H), 4.17 (d, *J* = 12.1 Hz, 1H), 4.53-4.65 (m, 2H), 5.26 (d, *J* = 3.5 Hz, 1H); *Step b*. A solution of crude **142** (100 mg, 0.29 mmol; from step *a*) and dried LiCl (62.5 mg, 1.47 mmol) in DMF (1 mL) was stirred

for 5 h at ambient temperature under N_2. Volatiles were evaporated and residue was partitioned between ice-cold saturated $NaHCO_3/H_2O$ (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (15 mL), dried (Na_2SO_4) and column chromatographed (EtOAc/hexane, 5:95 → 15:85) to give **100** (49 mg, 74%) as a colorless oil: ^{1}H NMR δ 1.89-2.02 (m , 1H), 2.06-2.18 (m, 1H), 3.44 (s, 3H), 3.58 (dd, *J* = 5.1, 8.0 Hz, 1H), 3.95 (td, *J* = 4.1, 8.3 Hz, 1H), 4.05 (dd, *J* = 2.7, 10.8 Hz, 1H), 4.32 (dd, *J* = 4.8, 10.8 Hz, 1H), 4.49 (td, *J* = 2.8, 5.0 Hz, 1H), 4.53-4.64 (m, 2H); ^{13}C NMR δ 30.7, 57.6, 58.1, 69.9, 73.9, 76.1, 84.7. HRMS ESI/DART *m/z* calcd for $C_7H_{16}{}^{35}ClN_2O_5$ $[M+NH_4]^+$ 243.0742, found 243.0747; calcd for $C_7H_{16}{}^{37}ClN_2O_5$ $[M+NH_4]^+$ 245.0718, found 245.0716.

2-Bromo-1,2,5-trideoxy-3-*O*-methyl-6-*O*-nitro-D-*ribo*-hexofuranose (101). A solution of crude **142** (100 mg, 0.29 mmol; prepared as described for **100**, step *a*) and dried LiBr (77 mg, 0.88 mmol) in DMF (1 mL) was stirred for 5 h at ambient temperature under N_2. Volatiles were evaporated and residue was partitioned between ice-cold saturated $NaHCO_3/H_2O$ (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (15 mL), dried (Na_2SO_4) and column chromatographed (EtOAc/hexane, 5:95 → 15:85) to give **101** (41 mg, 52 %) as a colorless oil: ^{1}H NMR δ 1.90-2.0 (m , 1H), 2.06-2.16 (m , 1H), 3.39-3.45 (m , 4H), 3.97 (td, *J* = 4.2, 8.0 Hz, 1H), 4.20 (dd, *J* = 3.0, 11 Hz, 1H), 4.45 (dd, *J* = 4.8, 11.0 Hz, 1H), 4.50 (td, *J* = 2.9, 5.0 Hz, 1H), 4.53-4.64 (m, 2H); ^{13}C NMR δ 30.7, 49.5, 58.2, 69.9, 74.0, 76.5, 84.4; HRMS ESI/DART *m/z* calcd for $C_7H_{16}{}^{79}BrN_2O_5[M+NH_4]^+$ 287.0237, found 287.0239; calcd for $C_7H_{16}{}^{81}BrN_2O_5$ $[M+NH_4]^+$ 289.0222, found 289.0218.

Biomimetic studies with the 3-hydroxyl substratess. Typical Procedure. A solution of **96** (20 mg, 0.06 mmol), Bu_3SnH (77 μL, 83 mg, 0.28 mmol), and AIBN (18 mg, 0.12 mmol) in dried toluene (2 mL) was deoxygenated (Ar) for 20 min and then heated at 95 ºC for 1 h. Volatiles were evaporated carefully (at 25 ºC and diminished pressure ~ 40 mmHg) and the residue was purified by column chromatography (EtOAc/hexane, 10:90 → 70:30) to give 1,2,5-trideoxy-D-*glycero*-hexofuranose-3-ulose **143a** in equilibrium mixture (~ 1:1) with cyclic hemiacetal **144a** (5 mg, 67%) as a colorless oil: HRMS ESI/DART *m/z* calcd for $C_6H_{11}O_3$ $[M+H]^+$ 131.0703, found 131.0707. Ketone **143a** had: ^{1}H NMR δ 1.84-1.93 (m, 1H), 1.95-2.06 (m, 2H), 2.15-2.27 (m, 2H), 3.74-3.85 (m, 2H), 3.88 (dd, *J* = 4.9, 7.4 Hz, 1H), 4.04-4.16 (m, 2H); ^{13}C NMR δ 32.9, 38.9, 59.8, 68.9, 78.6, 215.9. Hemiacetal **144a** had: 1.95-2.06 (m, 1H), 2.15-2.27 (m, 1H), 2.46-2.64 (m, 3H), 3.93-4.03 (m, 2H), 4.04-4.16 (m, 1H) 4.28 (dd, *J* = 2.27, 5.6 Hz, 1H), 4.37 (dt, *J* = 4.1, 9.2 Hz, 1H); ^{13}C NMR δ 32.1, 36.7, 64.6, 68.3, 85.7, 114.9.

Also isolated from the reaction mixture was 1,5-dideoxy-2-*O*-tosyl-*D*-*ribo*-hexofuranose (3.3 mg, 19%): ^{1}H NMR δ 1.81-1.94 (m, 2H), 2.46 (s, 3H), 3.74-3.85 (m, 4H), 3.86-3.93 (m, 1H), 4.12 (dd, *J* = 4.9, 11.2 Hz, 1H), 4.95 (dt, *J* = 3.0, 5.3 Hz, 1H), 7.38 & 7.82 (2 × d, *J* = 8.3 Hz, 2 × 2H);

Analogous treatment of **9** (20 mg, 0.06 mmol) with Bu_3SnD (77 μL, 83 mg, 0.28 mmol), instead of Bu_3SnH gave 2-deuterio epimers (2*R/S*, ~1:1) of **143b** in equilibrium mixture (~ 1:1) with **144b** (5.2 mg, 71%) as a colorless oil: ^{1}H NMR spectrum of **143b**/**144b** corresponded to this of the above **143a**/**144a** with reduction of the integrated intensity for the H2/2' signal at δ 2.15-2.27 and 2.46-2.64 to approximately half and simplification of the H1/1' signals at δ 4.04-4.16 (m, 1H) and 4.33-4.40 (m, 1H). ^{13}C

NMR spectrum of **143b**/**144b** showed triplets at δ 36.7 and 38.9 (J = 20.1 Hz) for C2 carbons because of splitting to deuterium and two close peaks of equal intensity for each hemiacetals carbons. HRMS ESI/DART *m/z* calcd for $C_6H_{10}DO_3$ $[M+H]^+$ 132.0765, found 132.0768. Isotopic incorporation (MS) was calculated to be 85-95% for [^{2}H] isotopomers of **143b**/**144b** depends on the experiments. The ^{13}C NMR spectrum for the sample of **143b**/**144b** (2*R/S*, ~1:1, [^{2}H] incorporation ~85%) showed residual peaks at 38.9 ppm and 36.7 for the unlabeled **143a** and **144a**, respectively and isotopically *upfield* shifted carbon signals for **143b** (two sets of triplets of equal intensity at 38.60 and 38.63 ppm with $J_{C2\text{-}D}$ = 20.1 Hz) and **144b** two sets of triplets of equal intensity at 36.35 and 3.36 with $J_{C2\text{-}D}$ = 20.1 Hz), respectively.

6-*O*-Benzoyl-1,2,5-trideoxy-D-*glycero*-hexofuranose-3-ulose (**145a)** BzCl (23 µL, 28 mg, 0.2 mmol), pyridine (44 µL, 43 mg, 0.54 mmol), and DMAP (4 mg, 0.032 mmol) were added to a stirred solution of **143a**/**144a** (30 mg, 0.23 mmol), in CH_2Cl_2 (2 mL). Stirring was continued at ambient temperature for 3 h and MeOH (0.3 mL) was added. Volatiles were evaporated and the residue was chromatographed (EtOAc/hexane, 5:95 →15:85) to give **145a** (22 mg, 81%) as an colorless oil: ^{1}H NMR δ 2.06-2.16 (m, 1H), 2.18-2.28 (m, 1H), 2.51 (dd, J = 6.50, 8.26 Hz, 2H), 3.90 (dd, J = 4.71, 7.0 Hz, 1H), 4.05-4.13 (m,1H), 4.28-4.36 (m, 1H), 4.39-4.46 (m,1H), 4.47-4.54 (m, 1H), 7.41-7.47(m, 2H), 7.56 (tt, J = 1.5, 7.4 Hz, 1H), 8.0 (d, J = 8.57 Hz, 2H); ^{13}C NMR δ 29.9, 36.8, 60.9, 64.7, 76.8, 128.5, 129.7, 130.2, 133.1, 166.5, 215.5. HRMS TOF/DART *m/z* calcd for $C_{13}H_{18}NO_4$ $[M+NH_4]^+$ 252.1230, found 252.1234.

6-*O*-Benzoyl-2-deuterio-1,2,5-trideoxy-D-*glycero*-hexofuranose-3-ulose (**145b).** Treatment of **143b**/**144b** (30 mg, 0.23 mmol) with BzCl, as described for **145a**, gave

145b (12 mg, 67%) as an colorless oil: ^{1}H NMR spectrum of **144b** corresponded to this of the above **145a** with reduction of the integrated intensity for the H2/2' signal at δ 2.51 to half and simplification of the H1/1' signals at 4.05-4.13 and 4.28-4.36. ^{13}C NMR spectrum showed triplet at δ 36.4 (J = 20.1 Hz) for C2 because of splitting to deuterium. HRMS ESI/DART *m/z* calcd for $C_{13}H_{13}DNaO_4$ $[M+Na]^+$ 258.0847, found 258.0836.

Biomimetic studies with 3-*O*-methyl precursors. 2-(Hydroxyethyl)-3-methoxyfuran (152a). A solution of **102** (25 mg, 0.069 mmol), Bu_3SnH (92 μL, 100 mg, 0.34 mmol), and AIBN (22.7 mg, 0.14 mmol) in dried toluene (2 mL), was deoxygenated (Ar) for 20 min and then heated at 95 ºC for 1.5 h. Volatiles were evaporated and the residue was purified by column chromatography (EtOAc/hexane, 10:90 → 75:25) to give **152a** (6.2 mg, 63%) followed by 1,5-dideoxy-2-*O*-tosyl-3-*O*-methyl-D-*ribo*-hexofuranose (**152b**, 4.8 mg, 22%) as a colorless oils. Compound **152a** had: ^{1}H NMR δ 2.89 (t, J = 6.0 Hz, 2H), 3.76 (s, 3H), 3.86 (t, J = 6.0 Hz, 2H), 6.31 (d, J = 2.1 Hz, 1H), 7.16 (d, J = 2.1 Hz, 1H); ^{13}C NMR δ 29.3, 59.4, 60.9, 102.9, 136.7, 139.7, 144.3. HRMS ESI/FT-ICR *m/z* calcd for $C_7H_{11}O_3$ $[M+H]^+$ 143.0702, found 143.0701. Compound **152b** had: ^{1}H NMR δ 1.74-1.84 (m, 1H), 1.85-1.94 (m, 1H), 2.29 (t, J = 5.9 Hz, 1H), 2.46 (s, 3H), 3.32 (s, 3H), 3.49 (dd, J = 4.9, 8.3 Hz, 1H), 3.72-3.79 (m, 2H), 3.85-3.94 (m, 2H), 4.07 (dd, J = 4.7, 11.3 Hz, 1H), 5.11 (dt, J = 2.4, 4.8 Hz, 1H), 7.36 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.1 Hz, 2H); ^{13}C NMR δ 21.6, 35.6, 58.3, 60.6, 70.9, 76.5, 79.1, 83.8, 127.8, 129.9, 133.8, 145.1. HRMS ESI/DART *m/z* calcd for $C_{14}H_{21}O_6S$ $[M+H]^+$ 317.1053, found 317.1055.

Analogous treatment of **102** with Bu_3SnD gave **152a** (6.0 mg, 61%) and (**152b**, 4.8 mg, 22%) with spectroscopic data as above.

Treatment of **141** (25 mg, 0.069 mmol) with Bu_3SnH, as described for **152a**, gave **152a** (5.0 mg, 51%) with data as above and 1,5-dideoxy-2-*O*-tosyl-3-*O*-methyl-D-*arabino*-hexofuranose **152c** (8.3 mg, 38%) as a colorless oil: ^{1}H NMR δ 1.86-1.94 (m, 2H), 2.04-2.11 (m, 1H), 2.47 (s, 3H), 3.31 (s, 3H), 3.70 (dt, *J* = 1.2, 5.0 Hz, 1H), 3.73-3.82 (m, 3H), 3.83 (d, *J* = 4.3 Hz, 1H), 3.93 (d, *J* = 11.7 Hz, 1H), 4.88 (dt, *J* = 1.3, 4.2 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR δ 21.6, 35.3, 58.0, 60.5, 71.3, 82.7, 83.4, 89.7, 127.8, 130.0, 133.5, 145.3. HRMS ESI/DART *m/z* calcd for $C_{14}H_{21}O_6S$ $[M+H]^+$ 317.1053, found 317.1050.

Analogous treatment of **141** with Bu_3SnD gave **152a** (4.9 mg, 50%) and **152c** (8.2 mg, 38%) with spectroscopic data as above.

1,2,5-Trideoxy-3-*O*-methyl-D-*glycero*-hex-2-enofuranose (155). Typical Procedure. A solution of **104** (20 mg, 0.074 mmol), Bu_3SnD (99 μL, 108 mg, 0.369 mmol), and AIBN (24 mg, 0.146 mmol) in dried toluene (2 mL), was deoxygenated (Ar) for 20 min and then heated at 95 ºC for 2 h. Volatiles were evaporated and the residue was purified by column chromatography (EtOAc/hexane, 10:90 → 70:30) to give **155**[35] (5.0 mg, 47%) as a colorless oil: ^{1}H NMR δ 1.73-1.87 (m, 1H), 1.94-2.09 (m, 1H), 2.58 (t, *J* = 5.6 Hz, 1H), 3.68 (s, 3H), 3.76-3.82 (m, 2H), 4.60-4.70 (m, 3H), 4.73-4.79 (m, 1H); ^{13}C NMR δ 35.6, 57.6, 60.6, 72.8, 81.2, 90.2, 157.7. HRMS ESI/DART *m/z* calcd for $C_7H_{16}NO_3$ $[M+NH_4]^+$ 162.1125, found 162.1131

Comparison of Reaction Rates of 96, 97 or 99 with Bu_3SnH. Independent solutions of 0.057 mmol samples of **96, 97** and **99** in toluene–d_8 (2.0 mL) were treated with 5 molar equiv of Bu_3SnH and 2 molar equiv of AIBN at 75°C. Aliquots of the individual reaction mixtures (0.3 mL) were diluted in toluene–d_8 (0.2 mL) and directly analyzed by ^{1}H NMR.

The **143a/144a** (1:1)/starting material ratios were obtained by integrating disappearance of the peak at 4.55 ppm for H2 of **96** or **97** or at 3.96 ppm for H6 of **99** and appearance of the peak at 4.10 ppm for the H4 of **143a**. The determinations were conducted under the pseudo-first-order conditions:

$$k_1 t = -2.303 \log(C/C_o) + a$$

Where C/C_o is the ratio of the concentration of starting material **96, 97,** or **99** in the mixture at time *t* to the initial concentration of starting material. Values of the term [$-\log(C/C_o)$] were plotted against [$t(\text{min})k(\text{s}^{-1}) = k_1$ ($\text{min}^{-1)}$/3600].

Methyl D-xylofuranoside (157a). D-Xylose **156a** (504 mg, 3.36 mmol) was dissolved in 0.5% HCl/MeOH (3 mL) and stirred at ambient temperature overnight. Reaction mixture was neutralized with Ag_2CO_3 (272 mg), stirred for an additional 30 min and filtered. The filtrate was evaporated to give **157a** (474 mg, 86%; α/β, 3:2) with data as reported:[155] ^{1}H NMR δ 3.44 (s, 1.2H, OMe-β), 3.49 (s, 1.8H, OMe-α), 3.84-3.94 (m, 4H, H5-α, H5'-α, H5-β, H5'-β), 4.09 (t, *J* = 4.7 Hz, 1H, H2-α), 4.18-4.24 (m, 3H, H4-α, H4-β, H2-β), 4.32 (dd, *J* = 5.1, 6.2 Hz, 1H, H3-α), 4.43 (dd, *J* = 4.9, 9.9Hz, 1H, H3-β), 4.87 (s, 0.4H, H1-β), 4.98 (d, *J* = 4.4 Hz, 0.6H, H1-α).

Methyl 5-[^{2}H]-D-xylofuranoside (157b). Commercially available 5-[^{2}H]-D-Xylose **156b** (250 mg, 1.66 mmol) was treated with 0.5% HCl/MeOH (3.6 mL) and Ag_2CO_3 (500 mg) as described above for **157a** to give **157b** (271 mg, 99%; α/β, 3:2): ^{1}H NMR was as described above for **157a** except for the 50% reduction in intensities for the H5 and H5' peaks at 3.84-3.94 ppm.

[$^2H_3$]-Methyl D-xylofuranoside (157c). Commercially available D-xylose **156a** (150 mg, 0.1mmol) was treated with 0.5% DCl/MeOD (1 mL) and Ag_2CO_3 (163 mg), as

described above for **157a** to give **157c** (155 mg, 92%; α/β, 3:2) with data as reported for **157a** except for the absence of the peak for OMe-β at 3.44 ppm and OMe-α at 3.49 ppm.

[$^2H_3$]-Methyl D-4-[^{2}H]-xylofuranoside (163a). Commercially available D-4-[^{2}H]-xylose **162a** (45 mg, 0.29 mmol) was treated with 0.5% DCl/MeOD (1 mL) and Ag_2CO_3 (49 mg), as described above for **157a** to give **163a** (46 mg, 92%; α/β, 3:2) with ^{1}H NMR as described above for **157a** except for the absence of the peak for OMe-β at 3.44 ppm, OMe-α at 3.49 ppm and H4-α, H4-β at 4.18-4.24 ppm and simplification of peaks for H5-α, H5'-α, H5-β, H5'-β at 3.84-3.94 ppm, H3-α at 4.32 ppm and H3-β at 4.43 ppm.

[$^2H_3$]-Methyl D-5,5'-[$^2H_2$]-xylofuranoside (163b). Commercially available D-[5,5'-$^2H_2$]-xylose **162b** (45 mg, 0.29 mmol) was treated with 0.5% DCl/MeOD (1 mL) and Ag_2CO_3 (49 mg), as described above for **157a** to give **163b** (47mg, 93%; α/β, 3:2) with ^{1}H NMR as described above for **157a** except for the absence of the peaks for OMe-β at 3.44 ppm, OMe-α at 3.49 ppm, H5 and H5' at 3.84-3.94 ppm and simplification of H4 peak at 4.18-4.24 ppm.

Methyl 3,5-*O*-isopropylidene-α-D-xylofuranoside (158a). H_2SO_4 (18 M, 1 drop) was added to a solution of **157a** (474 mg, 2.89 mmol; α/β, 3:2) in acetone (3 mL) and the resulting mixture was stirred at ambient temperature for 60 min. The reaction mixture was neutralized NH_4OH (14.8 M) to a slightly basic pH and was filtered and evaporated. The oily residue was column chromatographed (EtOAc/hexane, 3:7) to give α anomer of **158a** (185 mg, 31%) and β anomer of **158a** (90 mg, 16%) with spectral data as reported.[156] The α anomer had: ^{1}H NMR δ 1.38 (s, 3H, Me), 1.42 (s, 3H, Me), 2.94 (br. d, *J* = 2.0 Hz, 1H, OH), 3.55 (s, 3H, OMe), 3.97 (dd, *J* = 2.8, 13.7 Hz, 1H, H5), 4.04 (dd, *J*

= 2.8, 10.0 Hz, 1H, H5'), 4.04-4.07 (m, 1H, H4), 4.14 (br. s, 1H, H2), 4.22 (br. d, *J* = 1.8 Hz, 1H, H3), 5.21 (d, *J* = 4.2 Hz, 1H, H1).

Methyl 5-[^{2}H]-3,5-*O*-isopropylidene-α-D-xylofuranoside (158b). Treatment of **157b** (294 mg, 1.78 mmol) with acetone/H_2SO_4, as described for **158a,** gave α anomer of **158b** (53 mg, 15%) with data as described for **158a** except for the 50% reduction in intensities of H5 and H5' peaks at 3.93 ppm (br. s, 0.7H) and 4.00 ppm (br. s, 0.3H) and simplification of proton splitting for H4 at 4.12 ppm (br. t, *J* = 3.6 Hz).

[$^2H_3$]-Methyl 3,5-*O*-isopropylidine-α-D-xylofuranoside (158c). Treatment of **157c** (155 mg, 0.92 mmol, α/β, 3:2) with acetone/H_2SO_4, as described for **158a**, gave α anomer of **158c (**42 mg, 22%) and β anomer of **158c** (39 mg, 21%).The α anomer had data as described for **158a** except for the absence of the peak for OMe at 3.55 ppm.

[$^2H_3$]-Methyl 3,5-*O*-isopropylidine-α-D-4-[^{2}H]-xylofuranoside (164a). Treatment of **163a** (46 mg, 0.27 mmol) with acetone/ H_2SO_4, as described for **158a,** gave α anomer of **164a** (14 mg, 25%) and β anomer of **164a** (13 mg, 23%).The α anomer had data as described for **158a** except for absence of the peak for OMe at 3.55 ppm and H4 at 4.04-4.07 ppm and simplification of H5 peak at 3.97 ppm (d, *J* = 13.2 Hz, 1H, H5), H5' peak at 4.04 ppm (d, *J* = 13.2 Hz, 1H, H5') and H3 peak at 4.22 ppm (s, 1H, H3).

[$^2H_3$]-Methyl 3,5-*O*-isopropylidine-α-D-5-[$^2H_2$]-xylofuranoside (164b). Treatment of **163b** (46 mg, 0.3 mmol) with acetone/ H_2SO_4, as described for **158a,** gave α anomer of **164b** (13 mg, 23%) and β anomer of **164b** (10 mg, 18%). The α anomer had data as described for **158a** except for the absence of the peak for OMe at 3.55 ppm, H5 at 3.97 ppm and H5' at 4.04 ppm and simplification of H4 peak 4.05 ppm (d, *J* = 2.7 Hz, 1H, H4).

Methyl 2-azido-2-deoxy-3,5-*O*-isopropylidene-α-D-lyxofuranoside (159a). Step *a*. **Procedure A.** TfCl (37 µL, 58.64 mg, 0.35 mmol) was added to a cold (0 ^{o}C, ice-bath) stirred solution of **158a** (54 mg, 0.26 mmol) and DMAP (97 mg, 0.79 mmol) in dry CH_2Cl_2 (5 mL). After 15 min the ice-bath was removed and the resulting solution was stirred at ambient temperature for an additional 15 min and was partitioned between ice-cold AcOH/H_2O (1:99, 25 mL) and CH_2Cl_2. The organic layer was washed with ice-cold saturated $NaHCO_3$/H_2O, brine, and dried ($MgSO_4$), filtered and evaporated. The residual oil was column chromatographed (EtOAc/hexane, 1:1) to give methyl 3,5-*O*-isopropylidene-2-*O*-trifluromethanesulfonyl-α-D-xylofuranoside (**159a**; 70 mg, 80%) with spectroscopic data as reported:[138] ^{1}H NMR δ 1.36 (s, 3H, Me), 1.40 (s, 3H, Me), 3.48 (s, 3H, OMe) 3.85 (dd, *J* = 3.6, 12.6 Hz, 1H, H5), 4.00 (dd, *J* = 3.6, 12.6 Hz, 1H, H5'), 4.12 ("q", *J* = 3.8 Hz, 1H, H4), 4.44 (dd, *J* = 1.9, 3.7 Hz, 1H, H3), 5.01 (dd, *J* = 1.7, 4.2 Hz, 1H, H2), 5.22 (d, *J* = 4.3 Hz, 1H, H1).

Step *b*. **Procedure B**. NaN_3 (65 mg, 1.01 mmol) was added to a solution of **159a** (70 mg, 0.21 mmol) in DMF (4 mL) and the mixture was stirred at 100 ºC for 18 h. The volatiles were evaporated and the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed (brine), dried ($MgSO_4$) and evaporated. The oily residue was chromatographed (25 → 40% EtOAc/hexane) to give **160a** (38 mg, 80%) with data as reported:[138] ^{1}H NMR δ 1.46 (s, 6H, 2 × Me), 3.48 (s, 3H, OMe), 3.63 (t, *J* = 4.7 Hz, 1H, H2), 3.98 (dd, *J* = 2.1, 13.6 Hz, 1H, H5), 3.98-3.99 (m, 1H, H4), 4.06 (dd, *J* = 3.2, 13.7 Hz,1H, H5'), 4.44 (dd, *J* = 2.2, 4.5 Hz, 1H, H3), 5.22 (d, *J* = 4.8 Hz, 1H, H1).

Methyl 2-[^{15}N]-azido-2-deoxy-3,5-*O*-isopropylidene-α-D-lyxofuranoside ([^{15}N]-160a). Treatment of **159a** (64 mg, 0.19 mmol) with [^{15}N]-NaN_3 (60 mg, 0.91 mmol) by

procedure B provided [^{15}N]-**160a** (31 mg, 71%) with data as described for **160a**, except for additionally broadened signal for H2 at 3.63 ppm ("t", *J* = 4.7 Hz).

Methyl 5-[^{2}H]-2-azido-2-deoxy-3,5-*O*-isopropylidene-α-D-lyxofuranoside (160b). Step *a*. Triflation of **158b** (50 mg, 0.24 mmol) by procedure A gave **159b** (60 mg, 75%) with identical spectroscopical properties as **159a** except for H4, H5 and H5' signals: ^{1}H NMR δ 3.85 (br. "d", *J* = 3.6 Hz, 0.7H, H5), 4.00 (br. "d", *J* = 3.6 Hz, 0.3H, H5'), 4.12 (t, *J* = 3.9 Hz, 1H, H4). Step *b*. Azidation of **159b** (58 mg, 0.17 mmol) by procedure B provided **160b** (26 mg, 66%) with data as described for **160a** except for the 50% reduction in the intensities for the H5 and H5' peaks at 3.98 ppm and 4.06 ppm and simplification of the H4 peak at 3.98-3.99 ppm.

[^{2}H$_3$]-Methyl 2-azido-2-deoxy-3,5-*O*-isopropylidene-α-D-lyxofuranoside (160c). Step *a*. Triflation of **158c** (42 mg, 0.20 mmol) with TfCl (33 µL, 51 mg, 0.3 mmol) and DMAP (74.3 mg, 0.6 mmol) in dry CH_2Cl_2 (1 mL) by procedure A gave **159c** (55 mg, 80%) with identical spectroscopical properties except for the ~100% reduction in the intensity of OMe peak at 3.48 ppm. This material was sufficiently pure to be used directly in next step without column chromatography. Step *b*. Treatment of **159c** (36.9 mg, 0.56 mmol) with NaN_3 (55 mg, 0.16 mmol) in DMF (1.5 mL) by procedure B gave **160c** (20 mg, 54 %) with data as reported for **160a** except for the absence of the peak for OMe at 3.55 ppm.

[^{2}H$_3$]-Methyl 2-azido-2-deoxy-3,5-*O*-isopropylidene-α-D-4-[^{2}H]-lyxofuranoside (166a). Step *a*. Triflation of **164a** (13mg, 0.06 mmol) with TfCl (10 µL, 15.8 mg, 0.09 mmol) and DMAP (22.9 mg, 0.19 mmol) in dry CH_2Cl_2 (1 mL) by procedure A gave **165a** (14.6 mg, 69%). This material was sufficiently pure to be used directly in next step

without column chromatography. Step *b*. Treatment of **165a** (14.6 mg, 0.04 mmol) with NaN_3 (9.8 mg, 0.15 mmol) in DMF (1 mL) by procedure B gave **166a** (7 mg, 70%) with data as reported for **160a** except for absence of peaks for OMe at 3.55 ppm and H4 at 3.98-3.99 ppm and simplification of H5 peak at 3.98 ppm (d, *J* = 13.3 Hz, 1H, H5), H5' peak at 4.06 ppm (d, *J* = 13.3 Hz, 1H, H5') and H3 peak at 4.44 ppm (d, *J* = 4.5 Hz, 1H, H3).

[$^2H_3$]-Methyl 2-azido-2-deoxy-3,5-*O*-isopropylidene-α-D-5-[$^2H_2$]-lyxofuranoside (166b). Step *a*. Triflation of **164b** (13mg, 0.06 mmol) with TfCl (10 µL, 15.8 mg, 0.09 mmol) and DMAP (22.7 mg, 0.18 mmol) in dry CH_2Cl_2 (1 mL) by procedure A gave **165b** (20 mg, 94%). This material was sufficiently pure to be used directly in next step without column chromatography. Step *b*. Treatment of **165b** (20 mg, 0.06 mmol) with NaN_3 (13.4 mg, 0.2 mmol) in DMF (1 mL) by procedure B gave **166b** (9 mg, 66 %) with data as reported for **160a** except absence of peaks for H5 at 3.98 ppm and H5' at 4.06 ppm and simplification of H4 peak at 3.98-3.99 ppm (d, *J* = 2.4 Hz, 1H, H4).

Methyl 2-azido-2-deoxy-α-D-lyxofuranoside (161a). Procedure C. A solution of **160a** (38 mg, 0.17 mmol) in AcOH/H_2O (7:3, 5 mL) was stirred at 50 ºC for 60 min. The volatiles were evaporated and the crude product was column chromatographed (50 → 75% EtOAc/hexane) to give **161a**[138] (18 mg, 56%): ^{1}H NMR δ 3.38 (s, 3H, OMe), 3.88 (dd, *J* = 2.8, 12.4 Hz, 1H, H5), 3.94 (dd, *J* = 1.7, 5.4 Hz, 1H, H2), 3.95 (dd, *J* = 3.4, 12.4 Hz, 1H, H5'), 4.15 (td, *J* = 3.3, 6.3 Hz, 1H, H4), 4.68 (t, *J* = 5.8 Hz, 1H, H3), 4.92 (d, *J* = 1.7 Hz, 1H, H1); ^{13}C NMR δ 55.44 (OCH_3), 61.39 (C5), 67.89 (C2), 73.34 (C3), 78.19 (C4), 105.57 (C1).

Methyl 2-[^{15}N]-azido-2-deoxy-α-D-lyxofuranoside ([^{15}N]-161a). Deprotection of [^{15}N]-**160a** (30 mg, 0.13 mmol) by procedure C gave [^{15}N]-**161a** (15 mg, 61%): ^{1}H NMR was as described for **161a** except for broadening the signals at 4.92 ppm (H1) and 4.68 ppm (H3). On ^{13}C NMR additional spin-spin splitting was observed for C2 signal at 67.88 ppm (d, $J15_{N\alpha\text{-}C2}$ = 2.3 Hz; 50% intensity of the C2 peak) in agreement with literature value for ^{15}N labeled 2'-azido-2'-deoxyuridine.[16]

Methyl 5-[^{2}H]-2-azido-2-deoxy-α-D-lyxofuranoside (161b). Deprotection of **160b** (25 mg, 0.11 mmol) by procedure C gave **161b** (10 mg, 48%): ^{1}H NMR was as described for **161a** except for the simplification and 50% reduction in the intensities of H5 and H5' peaks at 3.85-3.95 ppm and simplification of proton splitting for H4 at 4.15 ppm (dd, *J* = 3.3, 6.3 Hz).

[$^{2}H_3$]-Methyl 2-azido-2-deoxy-α-D-lyxofuranoside (108). Deprotection of **160c** (20 mg, 0.09 mmol) with AcOH/H_2O (7:3, 1 mL) as described for **161a** gave **108** (12 mg, 73%) with data as reported for **161a** except for the absence of the peak for OMe at 3.38 ppm in ^{1}HNMR and ^{13}C NMR spectrum showed small intensity broad singlet at δ 54.61 for OCD_3 because of splitting to deuterium.

[$^{2}H_3$]-Methyl 2-azido-2-deoxy-α-D-4-[^{2}H]-lyxofuranoside (167a). Deprotection of **166a** (7 mg, 0.03 mmol) with AcOH/H_2O (7:3, 1 mL) as described for **161a** gave **167a** (4.2 mg, 73%) with ^{1}H NMR data as reported for **161a** except for absence of peaks for OMe at 3.38 ppm and H4 at 4.15 ppm and simplification of H5 peak at 3.88 (d, *J* = 12.5 Hz, 1H, H5), H5' peak at 3.95 (d, *J* = 12.4 Hz, 1H, H5') and H3 peak at 4.68 (d, *J* = 5.7 Hz, 1H, H3). ^{13}C NMR spectrum showed small intensity broad singlet at δ 55.44 ppm for

OCD_3 and triplet at δ 75.54 ppm (J = 22.1 Hz) for C4 peak because of splitting to deuterium.

[$^2H_3$]-Methyl 2-azido-2-deoxy-α-D-5-[$^2H_2$]-lyxofuranoside (167b). Deprotection of **166b** (9 mg, 0.05 mmol) with AcOH/H_2O (7:3, 1 mL) as described for **161a** gave **167b** (5.2 mg, 71 %) with data as reported for **161a** except for absence of peaks for OMe at 3.38 ppm, H5 at 3.88 ppm, and H5' at 3.95 ppm and simplification of H4 peak at 4.15 ppm (d, J = 6.3Hz, 1H, H4). ^{13}C NMR spectrum showed small intensity broad singlet at δ 55.38 for OCD_3 and at δ 61.39 ppm for C5 peak because of splitting to deuterium.

2'-Azido-2'-deoxycytidine (106). *Step a*. To a solution of 2'-azido-3',5'-*O*-diacetyl-2'-deoxyuridine **170** (85 mg, 0.24 mmol), in anhydrous CH_2Cl_2 (3 mL) under argon added DMAP (3.5 mg, 0.02 mmol) and triethylamine (50.37 µL, 0.36 mmol). 2,4,6-triisopropylbenzenesulfonyl chloride (110 mg, 0.36 mmol) was slowly added and the solution stirred for 2 h at room temperature. Reaction mixture diluted with CH_2Cl_2, extracted with saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated to yield 2'-azido-3',5'-*O*-diacetyl-O^6-(2,4,6-triisopropylbenzenesulfonyl)-2'-deoxyuridine, **172** (140.2 mg, 93.7%) as cream foam. The crude compound was used for next step without further purification; *Step b*. Crude compound **172** (140.2 mg, 0.22 mmol) dried under vacuum, was dissolved in THF (2 mL) and treated with aqueous ammonium hydroxide (2.62 mL). The solution was stirred at room temperature for 14 h. Solvent evaporated, the reaction mixture was diluted with dichloromethane, extracted with water, dried over Na_2SO_4 and evaporated. The residue was column chromatographed (MeOH/ $CHCl_3$, 2:8) to give **106** (51 mg, 85%) with data as reported.[145] ^1H NMR δ (CD_3OD)

3.69-3.86 (m, 2H, H5',5"), 3.92-4.07 (m, 2H, H2', H4'), 4.43 (t, *J* = 5.9 Hz, 1H, H3'), 5.9 (d, *J* = 3.7 Hz, 1H, H1'), 5.9 (d, *J* = 3.7 Hz, 1H, H5), 8.05 (d, *J* = 7.5 Hz, 1H, H6).

4'-Azidocytidine (107). *Step a*. To a solution of 4'-azidouridine, **178** (60 mg, 0.14 mmol), in anhydrous CH_2Cl_2 (3 mL) under argon added DMAP (2.12 mg, 0.02 mmol) and triethylamine (30 µL, 0.21 mmol). 2,4,6-triisopropylbenzenesulfonyl chloride (110 mg, 0.36 mmol) was slowly added and the solution stirred for 2 h at room temperature. Reaction mixture diluted with CH_2Cl_2, extracted with saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated to yield 4'-azido-O^6-(2,4,6-triisopropylbenzenesulfonyl)uridine, **179** (100 mg, 85%) as cream foam. The crude compound was used for next step without further purification; *Step b*. Crude compound **178** (100 mg, 0.14 mmol) dried under vacuum, was dissolved in THF (4 mL) and treated with aqueous ammonium hydroxide (2 mL). The solution was stirred at room temperature for 14 h. Solvent evaporated, the reaction mixture was diluted with dichloromethane, extracted with water, dried over Na_2SO_4 and evaporated. The residue was column chromatographed (MeOH/$CHCl_3$, 2:8) to give **107** (41 mg, 80%) with data as reported.[157] ^{1}H NMR δ (CD_3OD) 3.40-3.53 (m, 2H, H5', 5"), 4.15-4.21 (m, 2H, H2', H3'), 5.5 (d, *J* = 6.14 Hz, 1H, OH), 5.60-5.64 (m, 2H, 2XOH), 5.78 (d, *J* = 7.44 Hz, 1H, H5), 6.1 (d, *J* = 5.17 Hz, 1H, H1'), 7.2-7.4 (m, 2H, NH_2), 7.75 (d, *J* = 7.47 Hz, 1H, H6).

2'-*N*-Phenylsulphenyl-2'-amino-2'-deoxyuridine (111). Silver acetate (55 mg, 0.32 mmol) was added to a solution of diphenyl disulfide, **183** (72 mg, 0.32 mmol) in anhydrous DMF (2 mL) followed by 2'-amino-2'-deoxyuridine **182** (40 mg, 0.16 mmol). The resulting solution was stirred at 55º C for 16 h in dark. The silver mercaptide was removed by filtration. The filtrate was evaporated under reduced pressure and the residue

was column chromatographed (MeOH/$CHCl_3$, 1:9) to give **111** as a white solid (22 mg, 38%): ^{1}HNMR δ (DMSO-d_6) 3.38-3.46 (m, 1H, H2'), 3.52-3.56 (m, 2H, H5',5"), 3.88-3.92 (m, 1H, H4'), 4.08-4.12 (m, 1H, H3'), 4.61 (d, *J* = 8.5 Hz, 1H, NH), 5.10 (br. s, 1H, OH), 5.60 (d, *J* = 8.0 Hz, 1H, H5), 5.69 (br s, 1H, OH), 5.94 (d, *J* = 8.2 Hz, 1H, H1'), 7.04-7.09 (m, 1H, Ar), 7.15-7.19 (m, 2H, Ar), 7.21-7.27 (m, 2H, Ar), 7.78 (d, *J* = 8.1 Hz, 1H, H6); ^{13}C NMR δ 61.2 (C5'), 67.9 (C2'), 70.2 (C3'), 78.9 (C4'), 86.1 (C1'), 102.1 (C5), 121.8 (Ar), 124.9 (Ar), 128.6 (Ar), 140.6 (Ar-S), 142.3 (C6), 150.8 (C2), 163.0 (C4). ESI-MS (*m/z*): 350 $[M-H]^+$.

2'-*N*-(4-trifluoromethylphenyl)sulphenyl-2'-amino-2'-deoxyuridine (112). Treatment of the 4,4'-bis(trifluoromethyl)-2,2'-diphenyl disulfide (**184**;73 mg, 0.20 mmol) with 2'-amino-2'-deoxyuridine **182** (25 mg, 0.10 mmol) and $AgNO_3$ (34.31 mg), as described for **111**, afforded **112** as a white solid (3.8 mg, 12%): ^{1}HNMR δ (CD_3OD) 3.72-3.82 (m, 2H, H5',5"), 4.04-4.07 (m, 1H, H4'), 4.24-4.31 (m, 2H, H2', H3'), 5.7 (d, *J* = 8.1 Hz, 1H, H5), 6.03 (d, *J* = 7.5 Hz, 1H, H1'), 7.33-7.38 (m, 2H, Ar), 7.51-7.57 (m, 2H, Ar), 8.07 (d, *J* = 8.1 Hz, 1H, H6).

N^6-[Phenylsulphenyl]-2'-deoxyadenosine (113). Silver acetate (66 mg, 0.4 mmol) was added to a solution of diphenyl disulfide **183** (87 mg, 0.4 mmol) in anhydrous DMF (1 mL) followed by 2'-deoxyadenosine **182**(50 mg, 0.2 mmol). The resulting solution was stirred at 55º C for 16 h in dark. The silver mercaptide was removed by filtration. The filtrate was evaporated under reduced pressure and the residue was column chromatographed (MeOH/$CHCl_3$, 1:9) to give **113** as a white solid (6.9 mg, 10%): ^{1}HNMR δ (DMSO-d_6) 2.28-2.31 (m, 1H, H2'), 2.72-2.81 (m, 1H, H2"), 3.50-3.57 (m, 1H, H5'), 3.60-3.68 (m, 1H, H5"), 3.87-3.92 (m, 1H, H4'), 4.41-4.46 (m, 1H, H3'), 5.08 (t, *J* =

5.8 Hz, 1H, OH), 5.35 (d, *J* = 4.1 Hz, 1H, OH), 6.41 (t, *J* = 7.1 Hz, 1H, H1), 7.1-7.2 (m, 3H, Ar), 7.28-7.33 (m, 2H, Ar), 8.39 (s, 1H, H8), 8.55 (s, 1H, H2); ^{13}C NMR δ 40.0 (C2'), 61.7 (C5'), 70.8 (C3'), 83.9 (C4'), 88.0 (C1'), 121.4 (C5), 122.6 (Ar), 125.4 (Ar), 128.8 (Ar), 140.6 (Ar), 141.2 (C8), 149.6 (C6), 152.1 (C4), 155.12 (C2). HRMS TOF *m/z* calcd for $C_{16}H_{17}N_5O_3S$ $[M+H]^+$ 360.1125, found 360.1151.

4.3 EPR protocols

1. Preparation of Solutions of Nucleosides and Sugars:

Homogenous solutions of nucleosides or sugars were separately prepared by dissolving approximately 0.5 to 10 mg of each compound in 1mL of 7.5 M LiCl in H_2O or D_2O.[79,141,142]

2. Preparation of Glassy Sample and their Storage

Following our works,[79,141,142] these homogenous solutions were thoroughly bubbled with nitrogen gas and then immediately were drawn into 4 mm Suprasil quartz tubes (cat. No 734-PQ-8 WILMAD Glass co., Inc., Buena, NJ). These quartz tubes were rapidly immersed into liquid N_2 (77 K). Due to the rapid cooling, the homogeneous liquid solutions formed transparent homogeneous glassy solutions. These homogeneous glassy solutions were used for γ-irradiation and subsequent progressive annealing experiments. All glassy samples were stored at 77 K in Teflon containers in the dark.

3. γ-irradiation and the storage of Irradiated Samples

Following our works,[79,141,142] these glassy samples were γ-irradiated with the aid of 109-GR a irradiator containing shielded ^{60}Co source with an absorbed dose 525 – 700 Gy (45 min to 1 hr) at 77 K and were in Teflon containers in the dark before recording EPR spectra of these irradiated samples at 77 K in the dark. Owing to the irradiation of

the 7.5M LiCl glass (H_2O or D_2O) with only 0.5 to 10 mg sample per mL, nearly all the initial ionization takes place in the solution there by creating matrix electrons and holes. The electrons are scavenged by the solute (sugars) and the holes remain in the glass as $Cl_2•^-$.

4. Thermal Annealing and storage of these samples

The γ-irradiated samples were annealed in the dark in a variable temperature assembly (Air Products) in the temperature range of ca. 140 K to 175 K employing cooled nitrogen gas. These transparent glassy samples are not crystalline solids. They are actually supercooled homogeneous solutions. These supercooled solutions soften on warming via progressive thermal annealing at higher temperatures; this softening facilitates molecular migration in these solutions thereby allowing solution phase chemistry. After annealing these samples were immediately immersed in liquid nitrogen (77 K) for ESR analysis.

5. Electron Paramagnetic Resonance

Following our works,[79,141,142] the EPR spectra of samples were recorded in suprasil quartz dewar (cat. No WG-850(-Q), WILMAD Glass co., Inc., Buenas, NJ) at 77 K and 45dB (6.3μW) using a Varian Century series EPR spectrometer operating at 9.2 GHz with an E-4531 dual cavity, 9in. magnet and a 200mW klystron. For field calibration, Fremy's salt (g(center of the spectrum)= 2.0056, A_N= 13.096)

6. Analyses of EPR Spectra

Each EPR spectrum was stored in a 1000 point array along with field calibration marks from the three EPR lines of the Fremy's salt. Following our works on DNA and RNA-radicals,[79,141,142,148,152,158-166] the anisotropic simulations of the experimentally

recorded ESR spectra were carried out by employing the Bruker programs (WIN-EPR and SimFonia). The ESR parameters (e.g., hyperfine coupling constant (HFCC) values, linwidth, etc.) were adjusted to obtain the "best fit" simulated spectrum that matched the experimental ESR spectrum well. A small singlet "spike" was subtracted from an irradiated quartz at g=2.0006 from the recorded spectra for our analyses.

5. CONCLUSIONS

In this dissertation, I have successfully synthesized the 6-*O*-nitro-1-deoxyhomosugar derivatives with chlorine, bromine or tosyl substituent at the C2 position from diacetone glucose via multistep synthetic routes. Biomimetic studies with these 6-*O*-nitro homoribose derivatives revealed that the substituents (chloro, bromo, or *O*-tosyl) are departing from the C2 position in a heterolytic manner which is enhanced by the intramolecular hydrogen bonding between C3-hydroxyl group and chloro, bromo, or *O*-tosyl at C2 position (Figure 30). Reaction of the 1,5-dideoxy-6-*O*-nitro-2-*O*-tosyl-D-*ribo*-hexofuranose, **96** with Bu_3SnH/AIBN in toluene/Δ gave (*R)*-(2-hydroxyethyl)-3(2*H*)dihydrofuranone, **143a** and its cyclic hemiacetal, **144a** as 1:1 mixture. Similarly, reaction of **96** with Bu_3SnD yielded **143a** and **144a** with up to 95% of deuterium incorporation at C2 position. The first step in the proposed mechanism is the generation of the 6-oxyl radical with subsequent abstraction of H3 to generate C3 radical leading to the elimination of the tosylate anion. Proposed mechanism is in agreement with the mechanism proposed by Stubbe and is also consistent with the Robins biomimetic findings with 6'-*O*-nitro-2'-*O*-tosylhomoadenosine substrates.[167,168] Analogous treatment of 2-chloro(or bromo)-1,2,5-trideoxy-6-*O*-nitro-D-*ribo*-hexofuranose with Bu_3SnD also leads to formation of **143a/144a**. Chloride or bromide departed as anion with subsequent

deuterium incorporation at C2. This proposed mechanism in which the substituents from the C2 position are departing as anion is contradictory to the biomimetic studies done by Robins with 2-chloro substituted homouridine in which β-elimination of chlorine atom was observed upon generation of α-hydroxyl radical at C3.[137,167] Steric and/or stereoelectronic as well as conformational effects may be responsible for this difference in the radical promoted fragmentation of my 1-deoxy homoribofuranoses (lacking possibilities of anomeric effects) and adenine and uracil heterocyclic bases.

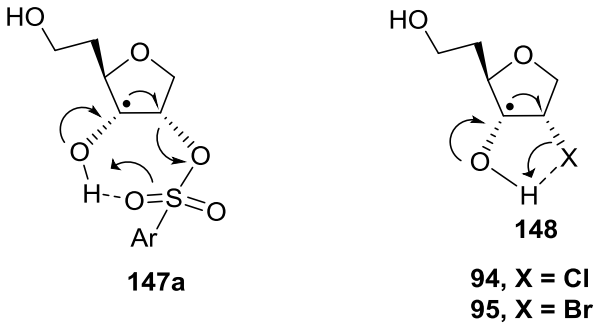


Figure 30. Hydrogen bonding facilitated elimination of the C-2 substituents as anions upon generation of C3 radical.

Interestingly, analogous studies with 6-*O*-nitro homoarabino derivatives also showed that the substituents from the C2 position are departing as anions. Thus, reaction of 6-*O*-nitro-D-*arabino*-hexofuranoses with chloro, bromo, or *O*-tosyl substituents at C2 with Bu_3SnD also resulted in the formation of 3-keto/hemiacetal mixture **143a**/**144a** with deuterium incorporation at C2 indicating departure of substituents from C2 as anions. In the arabino substrates the substituents at C2 and the hydroxyl at C3 are in *anti*-orientation which prevents the formation of the hydrogen bonding between them. Plausibly the substituent at C2 might form the hydrogen bonding with 6-hydroxyl group and subsequently departs as anion.

I also performed biomimetic studies with the 6-*O*-nitro homosugar derivatives having 3-*O*-methyl substituent. Such 3-*O*-methyl substrates lack the possibility of being hydrogen bonding donor and been not converted to the 3-keto product. Subjection of the 3-*O*-methyl-2-halo substrates to the free radical-induced relay reactions with Bu_3SnD lead to formation of the (*R*)-2-(2-hydroxyethyl)-3-*O*-methyl-2,3-dihydrofuran-3-ol, **155** without deuterium incorporations indicating that the halo substituents departed as radical. Thus the results with 3-*O*-methyl-2-halo substrates were consistent with β-elimination of the chlorine or bromine atom upon generation of α-oxy radicals at C3 leading to the formation of **155**. However, treatment of 3-*O*-methyl-2-*O*-tosyl substrates with Bu_3SnH (D) led to elimination of the toluenesulfonic acid from C2 and abstraction of the hydrogen from C1 to produce 2-(hydroxyethyl)-3-methoxyfuran, **152**.

Kinetic studies with the substrates arabino tosylate **99** and arabino chloride **97** in toluene-d_8 (Bu_3SnH/ AIBN) at 75º C revealed that the fragmentation of tosylate **99** was slightly faster than the chloro substituted substrate **97**. Biomimetic and kinetic studies with 6-*O*-nitro-1-deoxy homosugar derivatives provided plausible mechanistic explanation of the heterolytic elimination of hydrogen-bonded water from the substrate nucleoside di (or tri)phosphate inactivators of ribonucleotide reductases and the MoaA enzyme. Thus, results obtained from my studies add clarity to the previously described ambiguous hypotheses postulated for radical chemistry-based inactivation of the RNRs and MoaA.

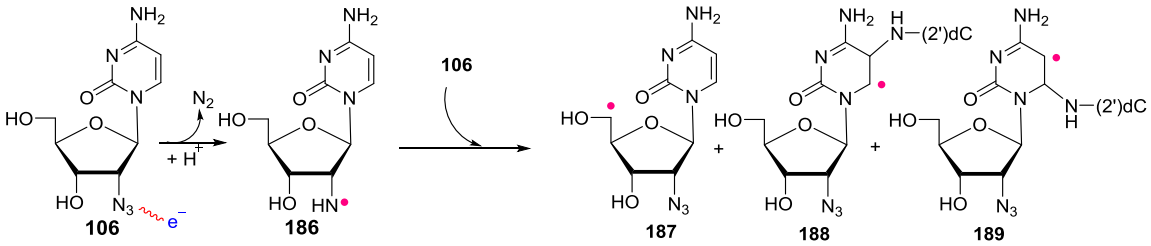
In the second goal of my dissertation, I have successfully synthesized the nucleosides with azido and sulfenamide modifications at specific sites in the sugar or in the base moieties to study generation and the subsequent reactions of the aminyl radical produced

upon one electron reductive conditions. Thus, the 2-azido-2-deoxy-α-D-lyxofuranoside derivatives with 2-[^{15}N]-azido and deuterium labeled at C4 or C5 were prepared by the substitution of the corresponding 2'-*O*-triflate substrates with NaN_3 or $Na^{15}N_3$. Upon subjection of 2-azido-2-deoxy-α-D-lyxofuranoside derivatives to radiation-produced electrons in homogeneous aqueous glassy (7.5 M LiCl) solutions lead to the formation of unstable azide anion radical intermediate ($RN_3^{\bullet -}$) in the absence of oxygen. The $RN_3^{\bullet -}$, underwent a rapid loss of N_2 and formed a highly unstable nitrene anion radical ($RN^{\bullet -}$) at 77 K, which upon subsequent rapid protonation at 77 K, produced the neutral aminyl radical (RNH•).

2-[^{15}N]-azido isotopomer clearly showed the formation of the neutral aminyl radical (RNH•). In case of methyl 5-[^{2}H]-2-azido-2-deoxy-α-D-lyxofuranoside we observed formation of the aminyl radical and subsequently this aminyl radical abstracted hydrogen from the C5. Similarly, [$^2H_3$]-methyl 2-azido-2-deoxy-α-D-5-[$^2H_2$]-lyxofuranoside upon electron attachment at 77 K produced aminyl radical followed by the abstraction of the hydrogen from C5 to generate C5-radical. Concentration dependent studies with these labeled azido sugar unequivocally identify fast unimolecular conversion of the aminyl radical to the C5-radical.

Studies with the methyl-d_3 xylofuranoside, 5-deuterium labeled azido sugar, methyl-d_3 5-dideuterium labeled azidolyxofuranoside and methyl-d_3 4-deuterium labeled azido sugar provided also data which helped us explicitly to determine a subsequent fast conversion of C5-radical to the ring opened C4-radical which is in agreement with the theoretical calculations. These observations suggest that the ring opened C4-radical might exist in a variety of conformations.

The 2'-azido-2'-deoxycytidine (2'-AZdC) and 4'-azidocytidine (4'-AZC) produced the electron-induced site specific neutral aminyl radicals (RNH•). This aminyl radical (dC(C2')-ND•) in 2'-AZdC showed the abstraction of the H-atom from the C5′-atom of a proximate 2'-AZdC to produce carbon-centered radical, C5′•, **187** which is a strand break precursor. However the aminyl radical also added bimolecularly to the C5=C6 double bond yielding a 6-yl (addition to C5), **188** as well as a 5-yl (addition to C6), **189** (Scheme 43).



Scheme 43. Formation of aminyl radical in 2'-AZdC and its reaction

Comparison of the ESR spectra of the 2'-AZdC and 4'-AZC clearly suggests that RNH• attached to the tertiary alkyl carbon generated from 4'-AZC is less reactive as compared to the RNH• attached to the secondary carbon generated from 2'-AZdC.Thus our radiation-chemical results demonstrate that: (a) stereo and electronic environment affect the formation and reactivity of various types of RNH• generated from azidonucleosides, and (b) electron-induced site specific formation of RNH• augments radiation damage in DNA-model systems either in the base or in the sugar moiety. Therefore, these studies may have potential for improvement of cancer radiotherapy as this reaction mechanism would be effective in anoxic conditions.

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