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

Mukesh M. Mudgal Dr.
mmudg001@fiu.edu

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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

A dissertation submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in CHEMISTRY 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.

_______________________________________
Kevin O'Shea

_______________________________________
Kathleen Rein

_______________________________________
Francisco Fernandez-Lima

_______________________________________
John Makemson

_______________________________________
Stanislaw Wnuk, Major Professor

Date of Defense: June 30, 2016

The dissertation of Mukesh Madan Mudgal is approved.

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

_______________________________________
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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Firstly, I would like to express my sincere gratitude to my professor Dr. Stanislaw Wnuk for giving me opportunity to work in his research group. Thank you very much for your continuous support, advice, patience and motivation. I would also like to thanks my committee members Dr. Kevin O’Shea, Dr. Kathleen Rein, Dr. Francisco Fernandez-Lima and Dr. John Makemson for their valuable time, advice and constant support during the past 5 years. I would also like to extend my gratitude towards the department of chemistry and biochemistry at Florida International University for funding and support.

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I would like to thank my loving family for their support, love and sacrifices during my Ph.D. My mummy and papa, I have no words to acknowledge the sacrifices you made and the dreams you had let go, just to give me a shot at achieving mine. Thank you very much for everything. My brother Mangesh for invaluable support and love over the years. You are always my best friend with whom I shared all my ups and down over the years. I would also like to thanks my beloved wife Shweta for her support, encouragement, quiet patience and unwavering love.
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

Mukesh Madan Mudgal

Florida International University, 2016

Miami, Florida

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 Bu₃SnH/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° C-atom, whereas in 4'-AZdC, the RNH● site is attached to a 3° 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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<th>Description</th>
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<tr>
<td>Ac&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>acetic anhydride</td>
</tr>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>Ag&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>silver carbonate</td>
</tr>
<tr>
<td>AIBN</td>
<td>azobisisobutyronitrile</td>
</tr>
<tr>
<td>AZT</td>
<td>3'-azido-2',3'-deoxythymidine</td>
</tr>
<tr>
<td>Ar</td>
<td>aromatic (NMR)</td>
</tr>
<tr>
<td>3'-AZG</td>
<td>3'-azido-2',3'dideoxyguanosine</td>
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<td>Bu&lt;sub&gt;3&lt;/sub&gt;SnH</td>
<td>tributyltin hydride</td>
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<td>Bu&lt;sub&gt;3&lt;/sub&gt;SnD</td>
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<tr>
<td>BzOH</td>
<td>benzoic acid</td>
</tr>
<tr>
<td>β</td>
<td>beta</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
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<td>(Bu&lt;sub&gt;3&lt;/sub&gt;Sn)&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>tributyltin oxide</td>
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<td>CD&lt;sub&gt;3&lt;/sub&gt;OD</td>
<td>deuterated methanol</td>
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<td>DMAP</td>
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<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
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<td>dNTPs</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
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<tr>
<td>HSV</td>
<td>herpes simplex virus</td>
</tr>
<tr>
<td>HCV</td>
<td>hepatitis C virus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>HRMS</td>
<td>high resolution mass spectroscopy</td>
</tr>
<tr>
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<td>hertz</td>
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<tr>
<td>$J$</td>
<td>coupling constant in Hz (NMR)</td>
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<tr>
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<tr>
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<tr>
<td>NaN$_3$</td>
<td>sodium azide</td>
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</tr>
<tr>
<td>2'-N$_3$dUrd</td>
<td>2'-azido-2'-deoxyuridine</td>
</tr>
<tr>
<td>NMR</td>
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<tr>
<td>NDP</td>
<td>nucleoside diphosphate reductase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
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<td>-----------</td>
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<tr>
<td>(PhO)$_2$CO</td>
<td>diphenyl carbonate</td>
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<tr>
<td>RNR</td>
<td>ribonucleotide reductase</td>
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<tr>
<td>RDPR</td>
<td>ribonucleoside diphosphate reductase</td>
</tr>
<tr>
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</tr>
<tr>
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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. Diphosphorylated 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. 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.

**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. 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.

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
analogue 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)\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{7}

![Festinavir and Apricitabine](image_url)

**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](image)
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. The 8-chloroadenosine induces cytotoxicity by decreasing MET expression, 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 G1 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.
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. The 2'-deoxynucleotides act as monomeric precursors required for DNA replication and repair. Inhibition of RNRs leads to depletion of the deoxyribonucleotides pool available for DNA synthesis and therefore is an appealing concept for rational drug design.

Number of 2'-modified pyrimidine nucleotides have been synthesized such as 2'-chloro and 2'-azido 5'-di(or tri)phosphates and are found to be potent inhibitors of RNRs. 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.

Figure 4. Structure of class I RNR
Ribonucleoside 5'-diphosphate reductase (RDPR) from *Escherichia coli* (Gram negative bacteria) have been studied extensively. *Escherichia coli* have similar composition as of mammalian RDPRs. 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 thyl radical on Cys439 on R1 subunit via electron and proton transfer reactions. The thyl radical abstracts the hydrogen from 3' position of the ribonucleotide substrate to initiate nucleotide reduction. This reaction cascade further leads to the elimination of water from 2' position. 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 along with theoretical modeling studies performed by Ramos and Siegbahn, and chemical biomimetic studies by Giese and Robins as well as McCarthy's 2'-deoxy-2'-fluoromethylenecytidine. Nevertheless the detection of the ribosyl-based radical during RNR-catalyzed deoxygenation of the natural substrates remains elusive.

1.2.1.1. Enzymatic studies by Stubbe

Stubbe *et al.* proposed the mechanism for the reduction of the nucleoside diphosphate substrates (Figure 5). It has been postulated that in the first step of RNR-catalyzed reduction of the ribonucleotides to deoxyribonucleotides reactions, a thyl 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'-H2O) in a heterolytic manner (B → C) to produce 3'-keto-2'-deoxydeoxynucleotide. Enzyme
inactivation by 2'-azido substituted nucleotides (A, X = N₃) are accompanied by loss of azide ions and formation of 3'-keto-2'-deoxynucleotides¹²,¹⁵,¹⁷ (e.g., D), which dissociate further from protein and generate 2-methylene-3(2H)-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. 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.
1.2.1.2. Biomimetic studies by Robins and Giese

Robins et al.\textsuperscript{34,35} and Giese\textsuperscript{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$_3$SnD/AIBN, abstracts hydrogen from the C3' (by [1,5]-hydrogen shift via a six member cyclic transition state)\textsuperscript{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$_3$SnD to chlorine occurs and that propagates the radical chain reaction. Similarly, treatment of 6'-O-nitro-2'-O-tosylhomoadenosine 6 with Bu$_3$SnD/AIBN gives 6'-oxy radical\textsuperscript{43} leads to the abstraction of H3' (by [1,5]-hydrogen shift via a six member cyclic transition state)\textsuperscript{44} to give C3' radical 8. Loss of tosylate from 8\textsuperscript{45} leads to a C2'-radical 9 which, after capturing a deuterium radical from Bu$_3$SnD 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 Bu₃SnD whereas furanone 11 with deuterium incorporation was formed upon treatment of 2'-O-tosyl homouridine derivative with Bu₃SnD. 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-face⁴⁶ 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.\textsuperscript{49} Hence, treatment of 2'- (azido, bromo, chloro, iodo, and methylthio) nucleoside 3'-thionocarbonates with Bu$_3$SnH/AIBN produced 3'-deoxy C3' radicals that underwent loss of the 2'-substituent 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$_3$SnH 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 toslyoxy substituents at the 2'-position with Bu$_3$SnH generates radical at 3'-position, 13 which abstracted hydrogen from the stannane to give 3'-deoxy-2'- (fluoro, mesyloxy, or toslyoxy) derivatives 15. Since the homolytic scission to release a high energy fluorine atom or a mesyloxy or toslyoxy radical is energetically prohibitive the elimination did not occurred.

Figure 7. Fragmentation of 2'-substituted-3'-thionocarbonates upon treatment with Bu$_3$SnH/AIBN/\(\Delta\).

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'.\textsuperscript{24,39}
Several attempts have been made to trap radical at C-3’ in the enzymatic system\textsuperscript{50} and in the biomimetic systems\textsuperscript{51} but the direct observation of the nucleotide radical were not possible. Giese et al\textsuperscript{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$_3$SnH.

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$_3$SnH or tBuSH 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.

\begin{align*}
\text{PhSe} & \rightarrow \text{PhSe}^* \\
16, R = R' = H & \rightarrow 19, R = Ac, R' = Bz \\
17, R = H & \rightarrow 18 \\
\text{Bu}_3\text{Sn}_2 & \rightarrow \text{HOR} \\
19 & \rightarrow 20 \\
21 & \rightarrow 22, R' = H \\
23, R = Bz & \rightarrow 24 \\
\text{Bu}_3\text{SnH} & \rightarrow 25 \\
24, R' = H & \rightarrow 26 \\
27 & \rightarrow 28
\end{align*}

\textbf{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 Bu₃SnH or ¹BuSH 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 al²³ used Bu₆Sn₂ as a radical mediator. Because the much slower radical trapping reaction with the use of Bu₆Sn₂, 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’-deoxyxygenation. Same reaction when performed employing Bu₃SnD 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 ¹BuSH was used direct reduction product 21 was formed. Deuterium incorporation at C-3’ (>80% by NMR) was observed when reaction was performed using CD₃OD and ¹BuSH.

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).
1.2.1.4. Theoretical considerations by Ramos

Ramos et al.\textsuperscript{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.\textsuperscript{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**

![Diagram of System A](image)

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

![Diagram of System B](image)

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.

\[ R-N_3 \equiv \left[ R-N=N\equiv N \leftrightarrow R-N=N^+\equiv N^- \leftrightarrow R-N^-\equiv N^+ \rightarrow R-N^+\equiv N^- \right] \]

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 arabinofuranosyladenines. The triflate 35 (Scheme 6) upon reaction with LiN₃ produced product in high yields in comparison to the mesylated, 33 or tosylated, 34 starting material. 55,56

![Scheme 6. Synthesis of 2'-azido-2'-deoxyadenosine](image)
2. Opening of nucleoside epoxides by azide: Derivatives of nucleosides like, 2′-O-anhydro and epoxides also acts as starting material for the synthesis of azidonucleosides. LiN₃ 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 LiN₃ yielded mixture of arabino, 38 (79%) and xylo, 39 (8%), respectively (Scheme 7)\(^{57}\)

![Scheme 7. Synthesis of azido nucleosides from epoxides](image)

3. From azidosugars: Azidouridine, 42 was prepared from readily available uridine, 40 via 2,2′-O-anhydrouridine (Scheme 8). Further the glycosidic 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](image)
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.\(^{61}\)

![Figure 8. Selected 2′-azido-2′-deoxynucleosides with biological activities](image)

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.

![Figure 9. Selected 3′-azido-3′-deoxynucleosides with biological activities](image)

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.
4. 5’-Azido-5’-deoxyribonucleosides: Trichlorobenzimidazole derivative, 53 (Figure 11) displayed significant activity against human cytomegalovirus in plaque.64

Figure 10. Selected 4’-azidonucleosides with biological activities

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])-N3UDPs and 2’-[15N3, 13C]-N3UDP to examine the mechanism of inactivation of RDPR by 2’-azido-2’-deoxynucleoside 5’-diphosphate (N3UDP).17 Experiments with doubly labeled 2’-[15N3]-azido-2’-[13C]-UDP showed that the resulting nitrogen-centered radical was derived from the azide moiety and had no hyperfine interaction with the 13C
nucleus, which required cleavage of the 2'-carbon-nitrogen bond. Furthermore, EPR studies with a [β-²H] 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.

Cys439 thyl 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 thyl radical in the active site. Conversion (B → 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, which make study of the inhibition of RNR by N₃UDP more significant.

Reaction of hydrazoic acid with the thyl radical generates N₂ 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 thyl radical would react with an azide ion. 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. The inactivation of the enzyme with 3'[¹⁷O]-N₃UDP 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. 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 and imino groups.
Figure 12. Proposed structures for the nitrogen centered radicals and pathways for their generation during inactivation of RDPR by N$_3$UDP

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.
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 phosphorous reagent used.

1.3.2.2. Radical reduction

Radical mediated reduction of azide to amine can be accomplished by stoichiometric Bu$_3$SnH (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$_3$SnH mediated reduction of azide to amine from unprotected azidonucleosides. Treatment of unprotected 2'-azido-2'-deoxyuridine 42 with Bu$_3$SnH/AIBN in hot benzene yielded the 2'-amino-2'-deoxyuridine 60 (Scheme 9). Analogues treatment of 5'-azido-5'-deoxyadenosine, 61 with Bu$_3$SnH/AIBN afforded 5'-amino-5'-deoxyadenosine, 62 (Scheme 10).
Scheme 9. Bu$_3$SnH-mediated reduction of 2'-azido-2'-deoxyuridine to 2'-amino-2'-deoxyuridine$^{74}$

\[
\begin{align*}
\text{HO} & \quad \text{Bu$_3$SnH/ AlBN} & \quad \text{HO} \\
\text{N$_3$} & \quad \text{humidity (DMAC)} & \quad \text{NH$_2$}
\end{align*}
\]

Scheme 10. Bu$_3$SnH-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$_3$SnH and a silicon hydride fills the role of stoichiometric reductant. Bu$_3$SnH-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$_3$SnH.
Figure 14. Bu₃SnH-mediated reduction of azide to amine.⁷⁷

Reaction of primary, secondary, tertiary or aryl azide with Bu₃SnH (5 mol %) and PhSiH₃ (0.6 equiv) employing less hindered n-PrOH (2 equiv) gave primary amine in good yield.⁷⁷

Figure 15. Bu₃SnH-catalyzed, silicon hydride-mediated azide reduction.⁷⁷

Figure 16. Bu₃SnH-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). 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 (RN3•−) which was not spectroscopically detected. Loss of N2 from (RN3•−) forms a highly basic and unstable nitrene anion radical 64 (RN•−) 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 °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 dUCH2• 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). 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. 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 as the azide with P (III) in the phosphoramidite undergoes Staudinger reaction with the azide. Therefore the H-phosphonate\textsuperscript{89,90} and the phosphotriester\textsuperscript{91-94} protocols are used for the synthesis of azide-modified oligonucleotides. Other options are post synthetic incorporation of azides into oligonucleotides\textsuperscript{95-97} and enzymatic\textsuperscript{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).\textsuperscript{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.\textsuperscript{89} Thus automated phosphoramidite synthesis could be used for the azide-containing solid supports to produce 3’-azidemodified oligonucleotides.\textsuperscript{102-104}

Fauster \textit{et al.}\textsuperscript{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).\textsuperscript{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 phosphoramidites continued without affecting the free C2’-azide group.
Sekine et al. prepared 2-azidodeoxyadenosine, 56 from deoxyguanosine by using combined reagent of TMSN₃-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 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-CD₃CN (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.
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.\(^\text{109}\) These radicals are responsible for the most of the damage in the nucleotides e.g., strand breaks and interstrand cross-links.\(^\text{110}\) Studies have been done to generate the reactive intermediates of interest employing techniques like UV photolysis and \(\gamma\)-radiation.\(^\text{111}\) 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.\(^\text{112}\) 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', 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.\textsuperscript{113}

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

Sulfenamides are photolabile compounds classified as type-1 phototherapeutic agents.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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
Omeprazole is a prodrug and is transformed into the active form in acidic media to produce the effect.

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). 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. 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.
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\(^{121}\)](image)

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 $\gamma$-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.
Wagner et al.\textsuperscript{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.

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 benzylidene 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.  

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-\textit{O}-nitro-1,5-dideoxyhomoribofuranose derivatives with halo (chloro, 94 or bromo, 95; Figure 18) substituent at C2 position upon treatment with Bu\textsubscript{3}SnH/AIBN were expected to undergo radical-initiated fragmentation with elimination of the C2 halo substituent as radical to give furanone \textit{e} with no deuterium incorporation at C2. In contrast, tosyl group from 6-\textit{O}-nitro-2-\textit{O}-tosyl precursors was expected to depart as anion upon subjection to radical fragmentation with Bu\textsubscript{3}SnH/AIBN to give furanone \textit{h} with 100\% deuterium incorporation at C2 (Scheme 21).

\begin{center}
\textbf{Scheme 21. Expected results from the biomimetic fragmentation of 6-\textit{O}-nitro-1,5-dideoxyhomoribose derivatives}
\end{center}

To probe the effect of stereochemical inversion corresponding 6-\textit{O}-nitro-1,5-dideoxy homorabinofuranose derivatives 97, 98 and 99 were designed with substituents at C2 position in \textit{anti}-orientation to the C3-hydroxyl group (Figure 18). I also designed the 3-\textit{O}-methyl substrates with \textit{ribo} (\textit{syn}) and \textit{arabino} (\textit{anti}) configurations (100-105). These 3-\textit{O}-methyl precursors were expected to probe the role of proton on the 3-hydroxy group. The 3-\textit{O}-methyl precursors lack the possibility to be converted to 3-keto product, an important intermediate during the RNA-catalyzed reactions.
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° C-atom (2'), where as in 4'-AZdC, the aminyl radical site is attached to a 3° 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](image)

To avoid the interaction of the transient radical with the nucleoside heterocyclic bases, I also designed 2-azidoloxofuranoside 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-azidoloxofuranoside 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-azidoloxofuranoside 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 $[^{3}H_3]$-methyl 2-azido-2-deoxy-$\alpha$-D-lyxofuranoside and its subsequent reaction](image)

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$^\bullet$ 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](image)
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-\(\beta\)-D-\(\text{ribo}\)-hexofuranoside 120 which served as convenient starting material for the preparation of model compounds, started with regioselective oxidation of 1,2-\(O\)-isopropylidene-\(\alpha\)-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\(_3\)Sn)\(_2\)O 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. Methanolyis of 119 and one-pot treatment with acetone gave 120 (~40% overall)\(^{18}\) (Scheme 23).

**Scheme 23. Synthesis of methyl 5-deoxy-2,3-\(O\)-isopropylidene-\(\beta\)-D-\(\text{ribo}\)-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\textsuperscript{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).

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

Displacement of iodide with AgNO\textsubscript{3}/CH\textsubscript{3}CN yielded 1,4-anhydro-5-deoxy-6-O-nitro-D-ribo-hexofuranitol 127 (89%); a key substrate for further studies. Direct tin-mediated tosylation\textsuperscript{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 \textit{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).

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-arabinohexofuranose 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).

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-benzyol-D-arabinohexofuranose 132. Debenzyolation 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 debenzyolation 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).

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.
3.1.2. Biomimetic studies

3.1.2.1. 6-O-Nitro-3-O-tosyl homosugar precursors

Treatment of the 6-O-nitro-2-O-tosyl ribo precursor 96 with Bu₃SnH/AIBN with toluene at 95 °C for 1 h gave 3-keto product 143a [(R)-2-(hydroxyethyl)-3(2H)-dihydrofuranone] which was found to be in equilibrium with cyclic hemiacetal 144a (66%, ~1:1; ¹H and ¹³C NMR; Scheme 29) in addition to 6-hydroxy byproduct resulting from homolysis of nitrate ester without elimination of tosyl group (Table 1, entry 1). ¹³C NMR shows ketone peak at 215.9 ppm for 143a and hemiacetal peak at 114.9 for 144a. Analogous treatment of 96 with Bu₃SnD, instead of Bu₃SnH gave 2-deuterio epimers (2R/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 13C 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 ([2H]) incorporation. Formation of 143a or 144b is consistent with generation of 6-oxyl radical 146 and abstraction34,48 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 nitrite130 and Wagner’s δ-substituted aryl ketone131 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$_3$SnH in carbohydrates. 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. 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 on RNR-catalyzed deoxygenation reactions as well as biomimetic studies.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>entry</th>
<th>substrate</th>
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<th>product</th>
<th>ratio$^b$</th>
<th>yield$^c$ (%)</th>
</tr>
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<td>Y</td>
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<td>Bu$_3$SnH</td>
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<td>OTs</td>
<td>H</td>
<td>Bu$_3$SnD</td>
<td>143b:144b</td>
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<td>99</td>
<td>H</td>
<td>OTs</td>
<td>Bu$_3$SnD</td>
<td>143b:144b</td>
</tr>
</tbody>
</table>

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$_3$SnH(D) and 2 equiv. of AIBN in toluene (95°C, 1-2 h.).$^b$

Determined by $^1$H 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 Bu₃SnH 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 Bu₃SnD yielded mixture of 143b/144b as 2-deuterio epimers indicating elimination of chloride and bromide, as anions, rather than as chlorine or bromine radical.

![Chemical structure diagram]

<table>
<thead>
<tr>
<th>entry</th>
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<th>reagent</th>
<th>product</th>
<th>ratio</th>
<th>yield (%)</th>
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<td>Cl</td>
<td>Bu₃SnH</td>
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<td>1:1</td>
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<td>95</td>
<td>Br</td>
<td>Bu₃SnH</td>
<td>143a:144a</td>
<td>1:1</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
<td>Br</td>
<td>Bu₃SnD</td>
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<td>1:1</td>
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<td>H</td>
<td>Bu₃SnD</td>
<td>143b:144b</td>
<td>1:1</td>
</tr>
<tr>
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<td>Br</td>
<td>Bu₃SnH</td>
<td>143a:144a</td>
<td>1:1</td>
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<td>98</td>
<td>Br</td>
<td>Bu₃SnD</td>
<td>143b:144b</td>
<td>1:1</td>
</tr>
</tbody>
</table>

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

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 Bu₃SnH(D) and 2 equiv. of AIBN in toluene (95°C, 1-2 h.). Determined by ¹H NMR. Isolated yields. The reduced hydrodebraninated 2-deoxy byproduct was also isolated (~10-15%).

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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.\textsuperscript{34,47} Formation of hydrogen-bonded intermediates (e.g., 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\textsuperscript{32} and experimental results on inhibition of RNR by 2'-chloro-2'-deoxynucleotides\textsuperscript{12,15} and also reinforce the importance of deprotonation of C3'-OH group by E441 during enzymatic deoxygenation reaction.\textsuperscript{12,134} It is also noteworthy here that treatment of 2'-chloro(or bromo)-3'-thionocarbonate nucleosides with Bu\textsubscript{3}SnH 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-tosyl nucleosides.\textsuperscript{49}

\begin{center}
\textbf{Scheme 30. Proposed mechanism for elimination of halo substituents (chloro or bromo) from 2'-chloro 94 and 2-bromo 95 ribo precursors}
\end{center}

Heating of compounds 94, 95 and 96 in toluene without tin hydride and AIBN at 95 °C or even at 110 °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 °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 Bu3SnH 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 Bu3SnD yielded mixture of 143b/144b as 2-deutero 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$_3$SnH/AIBN/toluene/$\Delta$ 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$_3$SnD 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 °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.

\[\text{O}_2\text{NO} \quad \overset{\text{Bu}_3\text{SnH(D)}}{\text{Bu}_3\text{SnH(D)}} \quad \text{Bu}_3\text{SnH} \quad \text{Bu}_3\text{SnD} \quad \text{Bu}_3\text{SnH} \quad \text{Bu}_3\text{SnD} \quad \text{152a} \quad \text{152b} X = \text{OTs}, Y = \text{H} \quad \text{152c} X = \text{H}, Y = \text{OTs} \]

<table>
<thead>
<tr>
<th>entry</th>
<th>substrate</th>
<th>reagent</th>
<th>product</th>
<th>Yield(^b)(%)</th>
</tr>
</thead>
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<td>OTs</td>
<td>H</td>
<td>Bu3SnH 152a</td>
</tr>
<tr>
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<td>102</td>
<td>OTs</td>
<td>H</td>
<td>Bu3SnD 152a</td>
</tr>
<tr>
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<td>H</td>
<td>OTs</td>
<td>Bu3SnD 152a</td>
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</table>

<table>
<thead>
<tr>
<th>Table 3. Biomimetic studies with 6-O-nitro-3-O-methyl-2-O-tosyl homo sugar substrates(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Reactions were performed on 0.055 mmol (102 or 105) scale of substrates (0.027-0.044 M) with 5 equiv. of Bu3SnH(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.</td>
</tr>
</tbody>
</table>

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 Bu3SnH 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 Bu3SnD 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 Bu3SnD resulted in the formation of vinyl ether 155 without deuterium incorporation (entry 1 and 2). 13C NMR spectrum of 155 shows olefinic peak at 90.2 and 157.7 ppm while 1H 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. 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) arabinose precursors 103 or 104 with
Bu$_3$SnD 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 arabinose 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

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

<table>
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<tr>
<th>entry</th>
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<th>reagent</th>
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<th>Yield$^p$(%)</th>
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<td>Bu$_3$SnD</td>
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<td>101</td>
<td>Br</td>
<td>Bu$_3$SnD</td>
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<td>155</td>
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</table>
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 Bu₃SnH(D) and 2 equiv. of AIBN in toluene (95°C, 1.5 h.). *Isolated yields. 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. 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](image)

**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 ¹H 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$_3$SnH in toluene have been examined. It was found that the fragmentation of tosylate 96 in toluene-$d_8$ with Bu$_3$SnH and AIBN at 75°C 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 $^1$H NMR). (It is noteworthy that heating of 96 in toluene-$d_8$ at 55°C showed only 50% consumption of substrate 96 after 2.5 h.) Experiments with arabinotosylate 99 and arabinochloro substrate 97 at 75 °C 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$_3$SnH at 75 °C 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 $^1$H NMR spectra difficult.
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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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\textsuperscript{42} and Wagner’s δ-substituted aryl ketone\textsuperscript{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.\textsuperscript{31,133} However theoretical modeling\textsuperscript{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$_3$SnH 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$_3$SnH 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$_3$SnH gave aromatized 2-(2-hydroxyethyl)-3-methoxyfuran (152a). Since in this case also the formation of the ketone is precluded the $\alpha$-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 °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, 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, 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.


The azido sugar analogues 161a and 161b were synthesized using the modified Fleet and Smith procedure (Scheme 35). Treatment of the D-xylose 156a with 0.5%
HCl/CH₃OH yielded Methyl D-xylofuranoside 157a as reported.¹³⁹ Methyl D-xylofuranoside 157a further treated with Conc. H₂SO₄/acetone as reported¹³⁹ resulted in (α/β, 3:2) mixture of 3,5-O-isopropylidene protected D-xylofuranoside. The α-anomer 158a was isolated using column chromatography. Compound 158a was converted to 2-Ο-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 procedure¹³⁸ employing the triflic anhydride in pyridine was used. Azidation of 159a was accomplished by displacement of the triflate from 159a with NaN₃ 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 [¹⁵N]-NaN₃ yielded 2-[¹⁵N]-azido isotopomer 160a. Subjection of 5-[²H]-D-xylose 156b to the same synthetic sequence yielded 5-deuterium labeled azido sugar 161b.

![Diagram of the synthesis process](image)

**Scheme 35. Synthesis of methyl 2-azido-2-deoxy-α-D-lyxofuranoside and its deuterium labeled analogues.**
Analogous treatment of D-xylose 156a with 0.5% DCl/CD$_3$OD in place of 0.5% HCl/CH$_3$OH produced methyl-$d_3$ xylofuranoside 157c with 100% deuterium incorporation. Compound 157c was converted to methyl-$d_3$ 2-azido-2-deoxy-$\alpha$-D-lyxofuranoside 108 following the same synthesis method mentioned above.


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-[$^2$H$_2$]-D-xylose 162b was converted to methyl-$d_3$ 5-dideuterium labeled azidolxyofuranoside 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-[15N]-azido-2-deoxy-α-D-lyxofuranoside [15N]-161a, methyl 5-[2H]-2-azido-2-deoxy-α-D-lyxofuranoside 161b, [2H3]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside 108, [2H3]-methyl 2-azido-2-deoxy-α-D-4-[2H]-lyxofuranoside 167a, and [2H3]-methyl 2-azido-2-deoxy-α-D-5-[2H2]-lyxofuranoside 167b in γ-irradiated homogeneous aqueous (D2O or H2O) 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′)-14ND•) in 3′-AZT,79 the major HFCC values in 1-OMe-(C2)-14ND• and its 15ND• isotopomer 1-OMe-(C2)-15ND• 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)-14ND• formed via radiation-induced electron attachment in aqueous glassy (7.5 M LiCl/D2O (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)-14ND• unequivocal, experiments were carried out using a matched sample of monoisotopically labelled 15N incorporated [15N]-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 Cl2•¯ spectrum.79,141,142 The red spectrum in Figure 22 (B) is assigned to 1-OMe-(C2)-15ND•. The 1-OMe-(C2)-14ND• (green, Figure 22 (A)) spectrum and 1-OMe-(C2)-15ND• (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 $[^{15}\text{N}]$-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 $^{15}\text{N}$ 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)-$^{14}\text{ND}^\bullet$ in 2-azido-2-deoxy-α-D-lyxofuranosides and its labeled analogs

Studies employing matched samples of $[^2\text{H}_3]$-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 OCD₃ 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 [¹⁵N]-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 (OCH₃) vs. [²H₃]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside (OCD₃)
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 (OCH₃) (161a black) and of [³H₃]-methyl 2-azido-2-deoxy-α-D-lyxofuranoside (OCD₃) (108 blue).

We have tested the hypothesis that 1-OMe-(C2)-¹⁴ND• from 161a could abstract H-atoms from sugar carbons by employing matched samples of 161a (black, Figure 24) its 5-deutero, 161b (green, Figure 24) and 5-dideutero 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)-¹⁴ND• and 5-D-1-OMe-(C2)-¹⁴ND• owing to the presence of the Azz 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)-¹⁴ND• 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. Comparison 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)-14ND• 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, $^{109} \rightarrow ^{110}$ (Scheme 22).
Figure 25. Formation of aminyl radical in 161a, 108 and 167b and its 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 (OCH₃) (black), of 100 (OCD₃) (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•](image)

### 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 glycosyl bond cleavage in 2'-azido-2'-deoxyuridine 66 for the preparation of 2-azido-2-deoxyribose, 168. The 2'-azido-2'-deoxyuridine, 66 was prepared according to the modified Verheyden method (Scheme 38). Treatment of uridine, 166 with diphenyl carbonate in hexamethylphosphoramide and catalytic amount of sodium bicarbonate leads to insitu formation of O₂,2'-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°C leads to cleavage of the glycosidic bond with the formation of 2-azido-2-deoxyribose, 168. Further 2-azido-2-deoxyribose, 168 treated with 0.5% HCl/CH₃OH to give methyl 2-azido-2-deoxyriboside, 169.

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, 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° C-atom (2') and in 4'-AZdC, to a 3° 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%).

![Scheme 39. Synthesis of 2'-azido-2'-deoxycytidine (2'-AZdC).](image-url)
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). 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. 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. 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 O4 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.
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 D₂O 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•−, black) and cytosine anion radical (C•−, 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•− and C•− reported in the literature. 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.
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 pH (ca. 5) of the homogeneous glassy solution of 7.5 M LiCl in D₂O. (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•¯) 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 D2O. (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•¯) 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 N6-
[Phenylsulphenyl]-2'-deoxyadenosine and \(N^6\)-[2-nitrophenylsulphenyl]-2'-deoxyadenosine analogues,\(^ {121}\) and studied the formation of aminyl radicals by UV photolysis and \(\gamma\)-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-Saryl 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-Saryl 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.\textsuperscript{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, \textbf{111} and 2'-N-(4-trifluoromethylphenyl)-sulphenyl-2'-amino-2'-deoxyuridine, \textbf{112} were prepared
employing the general method reported by Davis and co-workers\textsuperscript{116,154} as depicted in Scheme 41. Thus, treatment of diphenyl disulfide, 183 at 45\(^\circ\)C with readily available 2'-deoxy 2'-aminouridine,\textsuperscript{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](image)

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).
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$_4$)$_6$Mo$_7$O$_{24}$∙4H$_2$O/H$_2$SO$_4$/H$_2$O 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$_3$N (8.9 g, 12.3 mL, 88.2 mol) in
CH₂Cl₂ (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%): ¹H 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, 2H), 4.98 (s, 1H), 7.42–7.57 (m, 3H), 8.04–8.06 (m, 2H); ¹³C 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 C₁₇H₂₂O₆Na [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 Et₃SiH (19.4 g, 26.7 mL, 167.7 mmol) in CH₂Cl₂ (10 mL) was added BF₃•OEt₂ (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 NaHCO₃/H₂O solution (200 mL) was added slowly with vigorous stirring. CH₂Cl₂ was added (50 mL), aqueous phase was separated and washed with CH₂Cl₂ (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: ¹H NMR (CD₃OD) 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,
$^{13}$C NMR (CD$_3$OD) $\delta$ 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$_5$Na [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 $\times$ 50 mL) and evaporation to dryness provided oil that was filtered through silica to give 123 (6.4 g, 91%): $^1$H NMR $\delta$ 1.33, 1.52 (2 $\times$ s, 2 $\times$ 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 $\delta$ 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$_5$Na [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 $\rightarrow$ EtOAc) to give 124 (8.0 g, 87%): $^1$H NMR $\delta$ 1.33, 1.51 (2 $\times$ s, 2 $\times$ 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 $\delta$ 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$_9$H$_{17}$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$_3$P (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 $\rightarrow$ hexanes/EtOAc, 6:1) to give 125 (13.03 g, 85%): $^1$H NMR $\delta$ 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 $\delta$ 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$_9$H$_{15}$IO$_3$Na [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$_2$O (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 $\rightarrow$ EtOAc) to give 126 (8.73 g, 72%): $^1$H NMR $\delta$ 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 $\delta$ 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$_6$H$_{11}$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$_3$CN (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 C₆H₁₁NO₆Na [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 Bu₂SnO (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, Et₃N (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 C₁₃H₁₈NO₈SNa [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/H₂O
(1:99, 30 mL) and CHCl₃ (30 mL). The aqueous layer was extracted with CHCl₃ and the combined organic phase was washed with ice-cold saturated NaHCO₃/H₂O (30 mL), brine (30 mL) and dried (Na₂SO₄). 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%): ¹H 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 = 11.2 Hz, 1H), 5.13 (dt, J = 4.7, 7.0 Hz, 1H), 6.88 and 7.21 (2 x d, J = 9.5 Hz, 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 Bu₂SnO (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 PMBC1 (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 \(\delta\) 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), 3.82 (dd, \(J = 3.9, 7.3\) 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 \(\delta\) 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\(_7\)Na [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\(_3\)P (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\(_2\)Cl\(_2\) (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\(_6\)Na [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\(_3\)CN (7 mL) and H\(_2\)O (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 \(\delta\) 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 \(\delta\) 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\(_6\)H\(_{11}\)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\(_3\)P (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\(_2\)Cl\(_2\) (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\(_3\)CN (14 mL) and H\(_2\)O (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 \(\delta\) 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 \(\delta\) 31.2, 51.5, 69.9, 73.6, 81.5, 83.5. HRMS ESI/DART \(m/z\) calcd for
C$_6$H$_{14}^{79}$BrN$_2$O$_5$ [M+NH$_4$]$^+$ 273.0081, found 273.0084; calcd for C$_6$H$_{14}^{81}$BrN$_2$O$_5$ [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$_3$P (2.71 g, 10.35 mmol) and PhCO$_2$H (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 $\delta$ 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 $\delta$ 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), 5.00–5.02 (m, 2H); $^{13}$C NMR $\delta$ 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_{7}Na [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_{2}O (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_{2}O (30 mL), brine (30 mL) and dried (Na_{2}SO_{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_{2}O (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$_2$O$_8$S [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$_2$Cl$_2$ (2 mL) at 0°C (ice-bath). After 1 h, the reaction mixture was partitioned between ice-cold AcOH/H$_2$O (1:99, 30 mL) and CH$_2$Cl$_2$ (30 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (30 mL) and the combined organic phase was washed with ice-cold saturated NaHCO$_3$/H$_2$O (30 mL), brine (30 mL) and dried (Na$_2$SO$_4$) 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$_2$O (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (30 mL), dried (Na$_2$SO$_4$) 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,
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$_2$O (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$_{14}$H$_{18}$ClNO$_3$Na [M+Na]$^+$ 354.0715, found 354.0718; calcd for C$_{14}$H$_{18}$ClNO$_3$Na [M+Na]$^+$ 356.0691, found 356.0694.

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$_2$O (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (30 mL), dried (Na$_2$SO$_4$) 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$_{18}$BrNO$_6$Na [M+Na]$^+$ 398.0210, found 398.0203; calcd for C$_{14}$H$_{18}$BrNO$_6$Na [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.

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$_2$SnO (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 C₇H₁₃NO₆Na [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); ¹³C 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 C₇H₁₄NO₆[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/H₂O (1:99, 30 mL) and CHCl₃ (30 mL). The organic layer was separated and the aqueous layer was extracted with CHCl₃ (30 mL). Combined organic phase was washed with ice-cold NaHCO₃/H₂O (30 mL), brine (30 mL), dried (Na₂SO₄), 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 = 2.4, 11.5 Hz, 1H), 4.07 (dd, J = 4.4, 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); ¹³C NMR δ 21.6, 30.5, 30.5, 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 C₁₄H₂₃N₂O₈S [M+NH₄]⁺ 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
Ph₃P (0.31 g, 1.16 mmol) and PhCO₂H (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%): ¹H 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); ¹³C 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 C₁₄H₁₇O₇N[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/H₂O. Volatiles were evaporated, and the residue was column chromatographed (hexanes/EtOAc, 3:1 → 1:1) to give 140 (0.87 g, 72%): ¹H 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); ¹³C 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 C₇H₁₄NO₆[Na+ 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
Ph₃P (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%): ¹H 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); ¹³C NMR δ 31.3, 58.1, 59.4, 70.0, 74.2, 80.9, 92.8; HRMS ESI/DART m/z calcd for C₇H₁₆₃₅ClN₂O₅ [M+NH₄]⁺ 243.0742, found 243.0752; calcd for C₇H₁₆₃₇ClN₂O₅ [M+NH₄]⁺ 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 Ph₃P (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%): ¹H 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); ¹³C NMR δ 31.5, 48.5, 58.0, 70.0, 74.6, 81.2, 93.0. HRMS ESI/DART m/z calcd for C₇H₁₆₇⁹BrN₂O₅ [M+NH₄]⁺ 287.0237, found 287.0252; calcd for C₇H₁₆₈¹BrN₂O₅ [M+NH₄]⁺ 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/H₂O (1:99, 30 mL) and CHCl₃ (30 mL). The organic layer was separated and the aqueous layer was extracted with CHCl₃ (30 mL). Combined organic phase was washed with ice-cold saturated NaHCO₃/H₂O (30 mL), brine (30 mL) and dried (Na₂SO₄), concentrated in vacuo and column chromatographed (EtOAc/hexane, 5:95 → 35:65) to give 141 (240 mg, 69%) as a colorless oil: ¹H 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). ¹³C 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 C₁₄H₂₃N₂O₈S [M+NH₄]⁺ 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 CH₂Cl₂ (2 mL) at 0°C (ice-bath). After 1 h, the reaction mixture was partitioned between ice-cold AcOH/H₂O (1:99, 30 mL) and CH₂Cl₂ (30 mL). The aqueous layer was extracted with CH₂Cl₂ (30 mL) and the combined organic phase was washed with ice-cold saturated NaHCO₃/H₂O (30 mL), brine (30 mL) and dried (Na₂SO₄) 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: ¹H 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\textsubscript{2}. Volatiles were evaporated and residue was partitioned between ice-cold saturated NaHCO\textsubscript{3}/H\textsubscript{2}O (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (15 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and column chromatographed (EtOAc/hexane, 5:95 \rightarrow 15:85) to give 100 (49 mg, 74\%) as a colorless oil: $^1$H NMR $\delta$ 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 $\delta$ 30.7, 57.6, 58.1, 69.9, 73.9, 76.1, 84.7. HRMS ESI/DART m/z calcd for C\textsubscript{7}H\textsubscript{16}\textsuperscript{35}ClN\textsubscript{2}O\textsubscript{5} [M+NH\textsubscript{4}]$^+$ 243.0742, found 243.0747; calcd for C\textsubscript{7}H\textsubscript{16}\textsuperscript{37}ClN\textsubscript{2}O\textsubscript{5} [M+NH\textsubscript{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\textsubscript{2}. Volatiles were evaporated and residue was partitioned between ice-cold saturated NaHCO\textsubscript{3}/H\textsubscript{2}O (15 mL) and EtOAc (15 mL). The separated organic phase was washed with brine (15 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and column chromatographed (EtOAc/hexane, 5:95 \rightarrow 15:85) to give 101 (41 mg, 52\%) as a colorless oil: $^1$H NMR $\delta$ 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 $\delta$ 30.7, 49.5, 58.2, 69.9, 73.9, 76.1, 84.4; HRMS ESI/DART m/z calcd for C\textsubscript{7}H\textsubscript{16}\textsuperscript{79}BrN\textsubscript{2}O\textsubscript{5}[M+NH\textsubscript{4}]$^+$ 287.0237, found 287.0239; calcd for C\textsubscript{7}H\textsubscript{16}\textsuperscript{81}BrN\textsubscript{2}O\textsubscript{5} [M+NH\textsubscript{4}]$^+$ 289.0222, found 289.0218.
**Biomimetic studies with the 3-hydroxyl substrates. Typical Procedure.** A solution of 96 (20 mg, 0.06 mmol), Bu₃SnH (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 °C for 1 h. Volatiles were evaporated carefully (at 25 °C 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 C₆H₁₁O₃ [M+H]+ 131.0703, found 131.0707. Ketone 143a had: ¹H 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); ¹³C NMR δ 32.9, 38.9, 59.8, 68.9, 78.6, 215.9. Hemiacetel 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); ¹³C 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%): ¹H 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 Bu₃SnD (77 μL, 83 mg, 0.28 mmol), instead of Bu₃SnH gave 2-deutero epimers (2R/S, ~1:1) of 143b in equilibrium mixture (~ 1:1) with 144b (5.2 mg, 71%) as a colorless oil: ¹H 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). ¹³C
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$_6$H$_{10}$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 (2R/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 $J_{C2:D} = 20.1$ Hz) and 144b two sets of triplets of equal intensity at 36.35 and 3.36 with $J_{C2:D} = 20.1$ 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$_2$Cl$_2$ (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$_3$SnH (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$_7$H$_{11}$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$_6$S [M+H]$^+$ 317.1053, found 317.1055.

Analogous treatment of 102 with Bu$_3$SnD 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 Bu₃SnH, 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: ¹H 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); ¹³C 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 C₁₄H₂₁O₆S [M+H]+ 317.1053, found 317.1050.

Analogous treatment of 141 with Bu₃SnD gave 152a (4.9 mg, 50%) and 152c (8.2 mg, 38%) with spectroscopic data as above.

**1,2,5-‐Dideoxy-3-O-methyl-D-‐glycero-hex-2-‐enofuranose (155). Typical Procedure.** A solution of 104 (20 mg, 0.074 mmol), Bu₃SnD (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 °C for 2 h. Volatiles were evaporated and the residue was purified by column chromatography (EtOAc/hexane, 10:90 → 70:30) to give 155 (5.0 mg, 47%) as a colorless oil: ¹H 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); ¹³C NMR δ 35.6, 57.6, 60.6, 72.8, 81.2, 90.2, 157.7. HRMS ESI/DART m/z calcd for C₇H₁₆NO₃ [M+NH₄]+ 162.1125, found 162.1131

**Comparison of Reaction Rates of 96, 97 or 99 with Bu₃SnH.** Independent solutions of 0.057 mmol samples of 96, 97 and 99 in toluene–d₈ (2.0 mL) were treated with 5 molar equiv of Bu₃SnH and 2 molar equiv of AIBN at 75°C. Aliquots of the individual reaction mixtures (0.3 mL) were diluted in toluene–d₈ (0.2 mL) and directly analyzed by ¹H 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:

\[ k_1t = -2.303 \log(C/C_0) + a \]

Where \( C/C_0 \) 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 \(-\log(C/C_0)\) 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\(_2\)CO\(_3\) (272 mg), stirred for an additional 30 min and filtered. The filtrate was evaporated to give 157a (474 mg, 86%; \( \alpha/\beta \), 3:2) with data as reported:\(^{155}\) \(^1\)H NMR \( \delta \) 3.44 (s, 1.2H, OMe-\( \beta \)), 3.49 (s, 1.8H, OMe-\( \alpha \)), 3.84-3.94 (m, 4H, H5-\( \alpha \), H5'-\( \alpha \), H5-\( \beta \), H5'-\( \beta \)), 4.09 (t, \( J = 4.7 \) Hz, 1H, H2-\( \alpha \)), 4.18-4.24 (m, 3H, H4-\( \alpha \), H4-\( \beta \), H2-\( \beta \)), 4.32 (dd, \( J = 5.1, 6.2 \) Hz, 1H, H3-\( \alpha \)), 4.43 (dd, \( J = 4.9, 9.9 \)Hz, 1H, H3-\( \beta \)), 4.87 (s, 0.4H, H1-\( \beta \)), 4.98 (d, \( J = 4.4 \) Hz, 0.6H, H1-\( \alpha \)).

**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\(_2\)CO\(_3\) (500 mg) as described above for 157a to give 157b (271 mg, 99%; \( \alpha/\beta \), 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.

**[^3]H\]-Methyl D-xylofuranoside (157c).** Commercially available D-xylose 156a (150 mg, 0.1mmol) was treated with 0.5% DCl/MeOD (1 mL) and Ag\(_2\)CO\(_3\) (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.

[^2H3]-Methyl D-4[^2H]-xylofuranoside (163a). Commercially available D-4[^2H]-xylose 162a (45 mg, 0.29 mmol) was treated with 0.5% DCl/MeOD (1 mL) and Ag2CO3 (49 mg), as described above for 157a to give 163a (46 mg, 92%; α/β, 3:2) with ^1H 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.

[^2H3]-Methyl D-5,5'-[^2H2]-xylofuranoside (163b). Commercially available D-[5,5'-[^2H2]-xylose 162b (45 mg, 0.29 mmol) was treated with 0.5% DCl/MeOD (1 mL) and Ag2CO3 (49 mg), as described above for 157a to give 163b (47mg, 93%; α/β, 3:2) with ^1H 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). H2SO4 (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 NH4OH (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. The α anomer had: ^1H 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-\( \alpha \)-D-xylofuranoside (158b).** Treatment of 157b (294 mg, 1.78 mmol) with acetone/H\(_2\)SO\(_4\), as described for 158a, gave \( \alpha \) 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).

\( \text{[\( ^2 \)H}\text{_3}] \)-Methyl 3,5-O-isopropylidene-\( \alpha \)-D-xylofuranoside (158c).** Treatment of 157c (155 mg, 0.92 mmol, \( \alpha / \beta \), 3:2) with acetone/H\(_2\)SO\(_4\), as described for 158a, gave \( \alpha \) anomer of 158c (42 mg, 22%) and \( \beta \) anomer of 158c (39 mg, 21%). The \( \alpha \) anomer had data as described for 158a except for the absence of the peak for OMe at 3.55 ppm.

\( \text{[\( ^2 \)H}\text{_3}] \)-Methyl 3,5-O-isopropylidene-\( \alpha \)-D-4-[\( ^2 \)H]-xylofuranoside (164a).** Treatment of 163a (46 mg, 0.27 mmol) with acetone/ H\(_2\)SO\(_4\), as described for 158a, gave \( \alpha \) anomer of 164a (14 mg, 25%) and \( \beta \) anomer of 164a (13 mg, 23%). The \( \alpha \) 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).

\( \text{[\( ^2 \)H}\text{_3}] \)-Methyl 3,5-O-isopropylidene-\( \alpha \)-D-5-[\( ^2 \)H\(_2\)]-xylofuranoside (164b).** Treatment of 163b (46 mg, 0.3 mmol) with acetone/ H\(_2\)SO\(_4\), as described for 158a, gave \( \alpha \) anomer of 164b (13 mg, 23%) and \( \beta \) anomer of 164b (10 mg, 18%). The \( \alpha \) 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 °C, ice-bath) stirred solution of 158a (54 mg, 0.26 mmol) and DMAP (97 mg, 0.79 mmol) in dry CH₂Cl₂ (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/H₂O (1:99, 25 mL) and CH₂Cl₂. The organic layer was washed with ice-cold saturated NaHCO₃/H₂O, brine, and dried (MgSO₄), 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:¹³⁸¹H 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. NaN₃ (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 °C for 18 h. The volatiles were evaporated and the residue was partitioned between CH₂Cl₂ and H₂O. The organic layer was washed (brine), dried (MgSO₄) and evaporated. The oily residue was chromatographed (25 → 40% EtOAc/hexane) to give 160a (38 mg, 80%) with data as reported:¹³⁸¹H 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-[¹⁵N]-azido-2-deoxy-3,5-O-isopropylidene-α-D-lyxofuranoside ([¹⁵N]-160a). Treatment of 159a (64 mg, 0.19 mmol) with [¹⁵N]-NaN₃ (60 mg, 0.91 mmol) by
procedure B provided $[^{15}\text{N}]-\textbf{160a}$ (31 mg, 71%) with data as described for \textbf{160a}, except for additionally broadened signal for H2 at 3.63 ppm ("t", $J = 4.7$ Hz).

**Methyl 5-$[^2\text{H}]-2$-azido-2-deoxy-3,5-$O$-isopropylidene-$\alpha$-D-lyxofuranoside (160b).**

Step \textit{a}. Triflation of \textbf{158b} (50 mg, 0.24 mmol) by procedure A gave \textbf{159b} (60 mg, 75%) with identical spectroscopical properties as \textbf{159a} except for H4, H5 and H5' signals: $^1\text{H}$ NMR $\delta$ 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 \textit{b}. Azidation of \textbf{159b} (58 mg, 0.17 mmol) by procedure B provided \textbf{160b} (26 mg, 66%) with data as described for \textbf{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.

**$[^2\text{H}_3]$-Methyl 2-azido-2-deoxy-3,5-$O$-isopropylidene-$\alpha$-D-lyxofuranoside (160c).**

Step \textit{a}. Triflation of \textbf{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$_2$Cl$_2$ (1 mL) by procedure A gave \textbf{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 \textit{b}. Treatment of \textbf{159c} (36.9 mg, 0.56 mmol) with NaN$_3$ (55 mg, 0.16 mmol) in DMF (1.5 mL) by procedure B gave \textbf{160c} (20 mg, 54 %) with data as reported for \textbf{160a} except for the absence of the peak for OMe at 3.55 ppm.

**$[^2\text{H}_3]$-Methyl 2-azido-2-deoxy-3,5-$O$-isopropylidene-$\alpha$-D-4-$[^2\text{H}]$-lyxofuranoside (166a).** Step \textit{a}. Triflation of \textbf{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$_2$Cl$_2$ (1 mL) by procedure A gave \textbf{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 NaN₃ (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).

[^2]H₃]-Methyl 2-azido-2-deoxy-3,5-O-isopropylidene-α-D-5[^2]H₂-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 CH₂Cl₂ (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 NaN₃ (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/H₂O (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 161a¹³⁸ (18 mg, 56%): ¹H 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); ¹³C NMR δ 55.44 (OCH₃), 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%): ^1H NMR was as described for 161a except for broadening the signals at 4.92 ppm (H1) and 4.68 ppm (H3). On ^13C NMR additional spin-spin splitting was observed for C2 signal at 67.88 ppm (d, J_{15Nα-C2} = 2.3 Hz; 50% intensity of the C2 peak) in agreement with literature value for ^15N 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%): ^1H 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).

[^2H₃]-Methyl 2-azido-2-deoxy-α-D-lyxofuranoside (108). Deprotection of 160c (20 mg, 0.09 mmol) with AcOH/H₂O (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 ^1H NMR and ^13C NMR spectrum showed small intensity broad singlet at δ 54.61 for OCD, because of splitting to deuterium.

[^2H₃]-Methyl 2-azido-2-deoxy-α-D-4-[^2]H-lyxofuranoside (167a). Deprotection of 166a (7 mg, 0.03 mmol) with AcOH/H₂O (7:3, 1 mL) as described for 161a gave 167a (4.2 mg, 73%) with ^1H 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). ^13C NMR spectrum showed small intensity broad singlet at δ 55.44 ppm for
OCD₃ and triplet at δ 75.54 ppm (J = 22.1 Hz) for C4 peak because of splitting to deuterium.

[²H₃]-Methyl 2-azido-2-deoxy-α-D-5-[²H₂]-lyxofuranoside (167b). Deprotection of 166b (9 mg, 0.05 mmol) with AcOH/H₂O (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). ¹³C NMR spectrum showed small intensity broad singlet at δ 55.38 for OCD₃ 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 CH₂Cl₂ (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 CH₂Cl₂, extracted with saturated sodium bicarbonate solution, dried over Na₂SO₄ and evaporated to yield 2'-azido-3',5'-O-diacetyl-O⁵-(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 Na₂SO₄ and evaporated. The residue was column chromatographed (MeOH/CHCl₃, 2:8) to give 106 (51 mg, 85%) with data as reported.¹⁴⁵ ¹H NMR δ (CD₃OD)
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 CH₂Cl₂ (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 CH₂Cl₂, extracted with saturated sodium bicarbonate solution, dried over Na₂SO₄ and evaporated to yield 4'-azido-O₆-(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 Na₂SO₄ and evaporated. The residue was column chromatographed (MeOH/CHCl₃, 2:8) to give 107 (41 mg, 80%) with data as reported.¹⁵⁷

¹H NMR δ (CD₃OD) 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, NH₂), 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/CHCl₃, 1:9) to give 111 as a white solid (22 mg, 38%): ¹HNMR δ (DMSO-d₆) 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); ¹³C 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 AgNO₃ (34.31 mg), as described for 111, afforded 112 as a white solid (3.8 mg, 12%): ¹HNMR δ (CD₃OD) 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).

N⁶-[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 55° 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/CHCl₃, 1:9) to give 113 as a white solid (6.9 mg, 10%): ¹HNMR δ (DMSO-d₆) 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_{5}O_{3}S [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.

2. Preparation of Glassy Sample and their Storage

Following our works, 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, these glassy samples were γ-irradiated with the aid of 109-GR a irradiator containing shielded ⁶⁰Co 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 (H₂O or D₂O) 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 Cl₂•⁻.

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, 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, Aₐₙ= 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, 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 Bu3SnH/AIBN in toluene/Δ gave (R)-(2-hydroxyethyl)-3(2H)dihydrofuranone, 143a and its cyclic hemiacetal, 144a as 1:1 mixture. Similarly, reaction of 96 with Bu3SnD 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-oxy 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 Bu3SnD 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.\textsuperscript{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.](image)

Interestingly, analogous studies with 6-\textit{O}-nitro homoarabino derivatives also showed that the substituents from the C2 position are departing as anions. Thus, reaction of 6-\textit{O}-nitro-\textit{D-arabino}-hexofuranoses with chloro, bromo, or \textit{O}-tosyl substituents at C2 with \textit{Bu}_3\text{SnD} 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 \textit{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$_3$SnD lead to formation of the (R)-2-(2-hydroxyethyl)-3-O-methyl-2,3-dihydropuran-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$_3$SnH (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$_3$SnH/ AIBN) at 75°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-[15N]-azido and deuterium labeled at C4 or C5 were prepared by the substitution of the corresponding 2'-O-triflate substrates with NaN₃ or Na¹⁵N₃. 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 (RN₃•⁻) in the absence of oxygen. The RN₃•⁻, underwent a rapid loss of N₂ and formed a highly unstable nitrene anion radical (RN•⁻) at 77 K, which upon subsequent rapid protonation at 77 K, produced the neutral aminyl radical (RNH•).

2-[¹⁵N]-azido isotopomer clearly showed the formation of the neutral aminyl radical (RNH•). In case of methyl 5-[²H]-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, [²H₃]-methyl 2-azido-2-deoxy-α-D-5-[²H₂]-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-d₃ xylofuranoside, 5-deuterium labeled azido sugar, methyl-d₃ 5-dideuterium labeled azidolyxofuranoside and methyl-d₃ 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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VITA

MUKEH MADAN MUDGAL

1998-2002  B.S. in Pharmacy, Pharmaceutical Sciences
            Amravati University, Amravati, Maharashtra, India

2003-2005  M.S. in Pharmacy, Pharmaceutical Chemistry
            Manipal Academy of Higher Education, Manipal, Karnataka, India

2005-2008  Research Scientist
            Dabur Research Foundation, Sahibabad, Uttar Pradesh, India

2008-2011  Senior Research Scientist
            Fresenius Kabi Oncology Ltd., Gurgaon, India

2011-2016  Doctoral Candidate
            Florida International University, Miami, FL, USA

2013-2014  "Outstanding Organic Chemistry Teaching Assistant" Award

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