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Biomimetic Modeling of the Nitrogen-centered Radical Postulated to occur during the Inhibition of Ribonucleotide Reductases by 2'-Azido-2'-deoxynucleotides.

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

BIOMIMETIC MODELING OF THE NITROGEN-CENTERED RADICAL POSTULATED TO OCCUR DURING THE INHIBITION OF RIBONUCLEOTIDE REDUCTASES BY 2'-AZIDO-2'-DEOXYNUCLEOTIDES

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Thao P. Dang

2010

To: Dean Kenneth Furton
College of Arts and Sciences

This dissertation, written by Thao P. Dang, and entitled Biomimetic Modeling of the Nitrogen-centered radical Postulated to occur during the Inhibition of Ribonucleotide Reductases by 2'-Azido-2'-deoxynucleotides, 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.

_____
Kathleen Rein

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Jose Almirall

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Bruce McCord

_____
DeEtta Mills

_____
Stanislaw Wnuk, Major Professor

Date of Defense: November 10, 2010

The dissertation of Thao P. Dang is approved.

_____
Dean Kenneth Furton
College of Arts and Sciences

_____
Interim Dean Kevin O'Shea
University Graduate School

Florida International University, 2010

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DEDICATION

I dedicate this dissertation to my family: Cha Me.

Thank you so much for all your love, support, and sacrifice.

Cam on su yeu thuong, khuyen khich, va hy sinh cua Cha Me da danh cho con.

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Most foremost, I would like to thank Dr. Stanislaw Wnuk for the opportunity to work in your laboratory. Thank you for your advice, support, and patience. I am also very grateful of Dr. Jose Almirall for your advice and support as well as your help with funding my trip to Bologna, which was an integral part of my dissertation. I also would like to give thanks to Dr. Kathleen Rein, Dr. Bruce McCord, and Dr. DeEtta Mills for your time, advice, and support in the past 5.5 years. I would also like to extend my gratitude towards the National Institute of Health (SC1CA138176 from NIGMS and NCI), Doctoral Evidence Acquisition Fellowship, Kauffman Doctoral Student Assistantship**,** Florida International University, and the Minority Biomedical Research Support Program for funding and their support.

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Christian Schoneich, and Dr. Olivier Mozziconacci. I am very appreciative of your time and effort.

In the past 5.5 years, my labmates have shared with me much laughter. You guys have made everyday in the lab a visit to home. More importantly, you guys have been an anchor of support during my hard days; and for that, I am forever grateful.

I cannot acheive my goals in life without the encouragement, sacrifice, love, and care of my parents either. I love you Cha Me. Thank you for being there for me every steps of my journey, never question nor doubt my decision. Also, my family of choice, your frienship and encouragement are forever engraved in my heart. This journey would never have begun without the inspiration from my 11th grade Chemistry teacher Mrs. Tarver, my Organic Chemistry lab instructor Mrs. Brenda Harmon, my heroine Ms. Emily Campbell, and my academic support Upward Bound at Atlanta Metropolitan College.

Lastly, I would also like to thank this country for the opportunity for me to live with freedom and to pursue an education, a dream, and an American dream.

ABSTRACT OF THE DISSERTATION

BIOMIMETIC MODELING OF THE NITROGEN-CENTERED RADICAL POSTULATED TO OCCUR DURING THE INHIBITION OF RIBONUCLEOTIDE REDUCTASES BY 2'-AZIDO-2'-DEOXYNUCLEOTIDES

by

Thao P. Dang

Florida International University, 2010

Miami, Florida

Professor Stanislaw Wnuk, Major Professor

Ribonucleotide reductases (RNR) are essential enzymes that catalyze the reduction of ribonucleotides to 2'-deoxyribonucleotides, which is a critical step that produces precursors for DNA replication and repair. The inactivation of RNR, logically, would discontinue producing the precursors of the DNA of viral or cancer cells, which then would consequently end the cycle of DNA replication. Among different compounds that were found to be inhibitors of RNR, 2'-azido-2'-deoxynucleotide diphosphates (N_3NDPs) have been investigated in depth as potent inhibitors of RNR. Decades of investigation has suggested that the inactivation of RNR by N_3NDPs is a result of the formation of a nitrogen-centered radical (N•) that is covalently attached to the nucleotide at C3' and cysteine molecule C225 [3'-C(R-S-N•-C-OH)]. Biomimetic simulation reactions for the generation of the nitrogen-centered radicals similar to the one observed during the inactivation of the RNR by azionuclotides was investigated. The study included several modes: (i) theoretical calculation that showed the feasibility of the ring closure reaction between thiyl radicals and azido group; (ii) synthesis of the model azido nucleosides with

a linker attached to C3' or C5' having a thiol or vicinal dithiol functionality; (iii) generation of the thiyl radical under both physiological and radiolysis conditions whose role is important in the initiation on RNR cascades; and (iv) analysis of the nitrogen-centered radical species formed during interaction between the thiyl radical and azido group by electron paramagnetic resonance spectroscopy (EPR). Characterization of the aminyl radical species formed during one electron attachment to the azido group of 2'-azido-2'-deoxyuridine and its stereospecifically labelled 1'-, 2'-, 3'-, 4'- or 5,6-[$^2H_2$]-analogues was also examined. This dissertation gave insight toward understanding the mechanism of the formation of the nitrogen-centered radical during the inactivation of RNRs by azidonucleotides as well as the mechanism of action of RNRs that might provide key information necessary for the development of the next generation of antiviral and anticancer drugs.

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1. INTRODUCTION

1.1 Ribonucleotide Reductases: Function and Structure

Ribonucleotide reductases (RNRs) are enzymes that catalyze the conversion of ribonucleotides to 2'-deoxyribonucleotides, which is essential in providing the monomeric precursors required for DNA replication and repair. [1-3] This is the only known pathway for generating deoxyribonucleotides *de novo*.[1-3] The critical role of RNRs has made them an appealing target for drug design on the basis of the concept of disrupting the primary source of DNA components. The inhibition of RNRs has been extensively studied in the past several decades for the purpose of producing antiviral and anticancer agents.

After RNRs reduce the purine and pyrimidine ribonucleotides (NDPs) to their deoxyribonucleotide substrates (dNDPs), 5'-nucleoside diphosphate kinase continues the process of DNA replication and repair by converting dNDPs to their corresponding 2'-deoxyribonucleoside-5'-triphosphates (dNTPs). The last step of the DNA biosynthesis is the incorporation of dNTPs by DNA polymerization into DNA fragments. [4,5] However, the conversion of the uridine diphosphate substrate (dUDPs) into its triphosphates substrate involves a different pathway. The dUDPs are first transformed into deoxyuridine 5'-monophosphates (dUMPs), followed by thymidylate synthase methylating at C5' of the uracil ring to produce thymidine 5'-monophosphates (TMPs). Lastly, the formation of thymidine 5'-triphosphates (TTPs) occurs via 5'-nucleoside kinases.[4,5] (Figure 1) Overall, RNRs catalyze a fundamental reaction to produce dNDPs, which are the precursors necessary for DNA replication and repair.

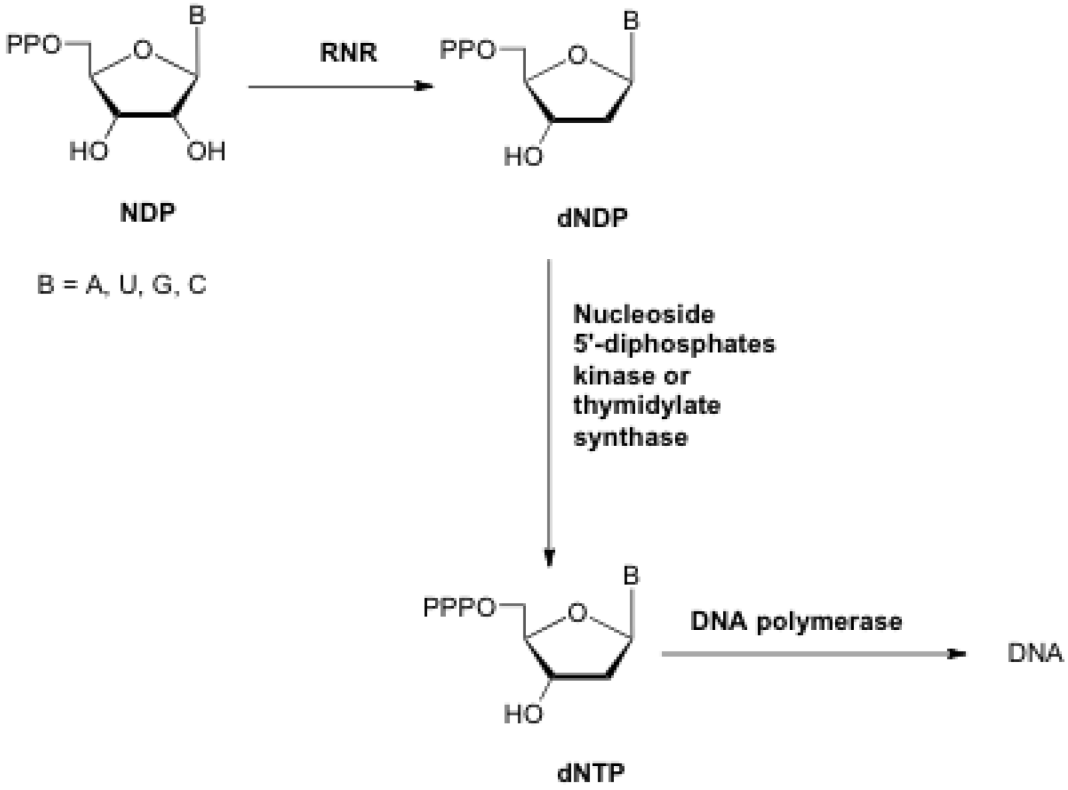


Figure 1. The process of DNA replication and repair with RNRs catalyzing the first step.

The structure of RNRs consists of two subunits, R1 and R2. The R1 subunit is composed of allosteric control sites and active sites that regulate activity and specificity. The R2 subunit contains cofactors that have radical components, which generate a thiyl radical in the R1 subunit to initiate the mechanism of ribonucleotide reduction. An important characteristic to note about the R1 and R2 subunits is that they have no enzymatic activities of their own, except for when they interact with each other.[1,6-9]

Ribonucleotide reductases are classified into four classes on the basis of the different cofactors in their R2 subunits. Class I RNRs are found in many aerobic bacteria such as *Escherichia coli (E. coli),* DNA viruses such as herpes simplex virus, and all

eukaryotic species. Their cofactors contain a tyrosyl radical and a diferric iron center, which generate a thiyl radical C439 in the R1 subunit. [1,10-12]

The prototype of Class II RNRs was isolated from *Lactobacillus leichmannii*. Adenosylcobalamin (AdoCbl), or coenzyme B_{12}, acts as a cofactor for this monomeric enzyme. It has been suggested that AdoCbl abstracts the hydrogen atom from the cysteine residue (cys408) in the R1 subunit to form a thiyl radical, generating cob(II)alamin and, supposedly, an 5'-deoxyadenosyl radical. [1,10-12]

The RNR that was isolated from the facultative anaerobic *E. coli* is the prototype of Class III RNRs. The prototypical Class III RNR has a quaternary structure containing a glycyl radical as the cofactor, which is formed from S-adenosylmethionine and an iron-sulfur cluster. Like other classes of RNRs, the glycyl radical also generates a thiyl radical in the R1 subunit. [1,10-12]

Class IV RNRs were isolated from *Brevibacterium ammoniagenes*, which in comparison to other classes of RNRs, have not been studied extensively. However, they also have the cofactor that generates a thiyl radical. It is believed that the cofactor is composed of a dinuclear Mn^{3+} cluster similar to the diferric cluster of the class I RNR.[1,10-12]

Class I RNRs, which was isolated from *E. coli* grown under aerobic conditions, are composed of two homodimeric subunits, R1 and R2 (Figure 2). The structure of the R1 protein contains five cysteine residues, a glutamic acid residue, an aspargine residue, and allosteric-effector binding sites that execute important functions in the mechanism of ribonucleotide reduction. The ribonucleotide substrates are positioned between the cys439 and the cys225-cys462. The cys225-cys462 is located at the 2'-position of the

substrate while the cys439 is located at the other side of the substrate where it has van der Waals interaction with the 3'-carbon atom. The other two cysteine residues, cys754 and cys759, are located at the end of the N-terminal of the R1 protein. The glutamic acid residue 441 is positioned in such a way that it is hydrogen-bonded to the 3'-oxygen atom, whereas asn437 is hydrogen-bonded to the 2'-oxygen atom. [6,8,12-14]

The R2 subunit is comprised of a stable tyrosyl radical (•Tyr122) and a diferric iron center (Fe—O—Fe) as its cofactors. The R2 protein possesses an unusual α-helical structure that resembles an antiparallel hairpin. The tyrosyl radical, which is generated from the diferric cluster, performs a crucial task as the initiator of ribonucleotide reduction by generating a thiyl radical (S•) on the cysteine residue (cys439) of the R1 subunit.[9,15]

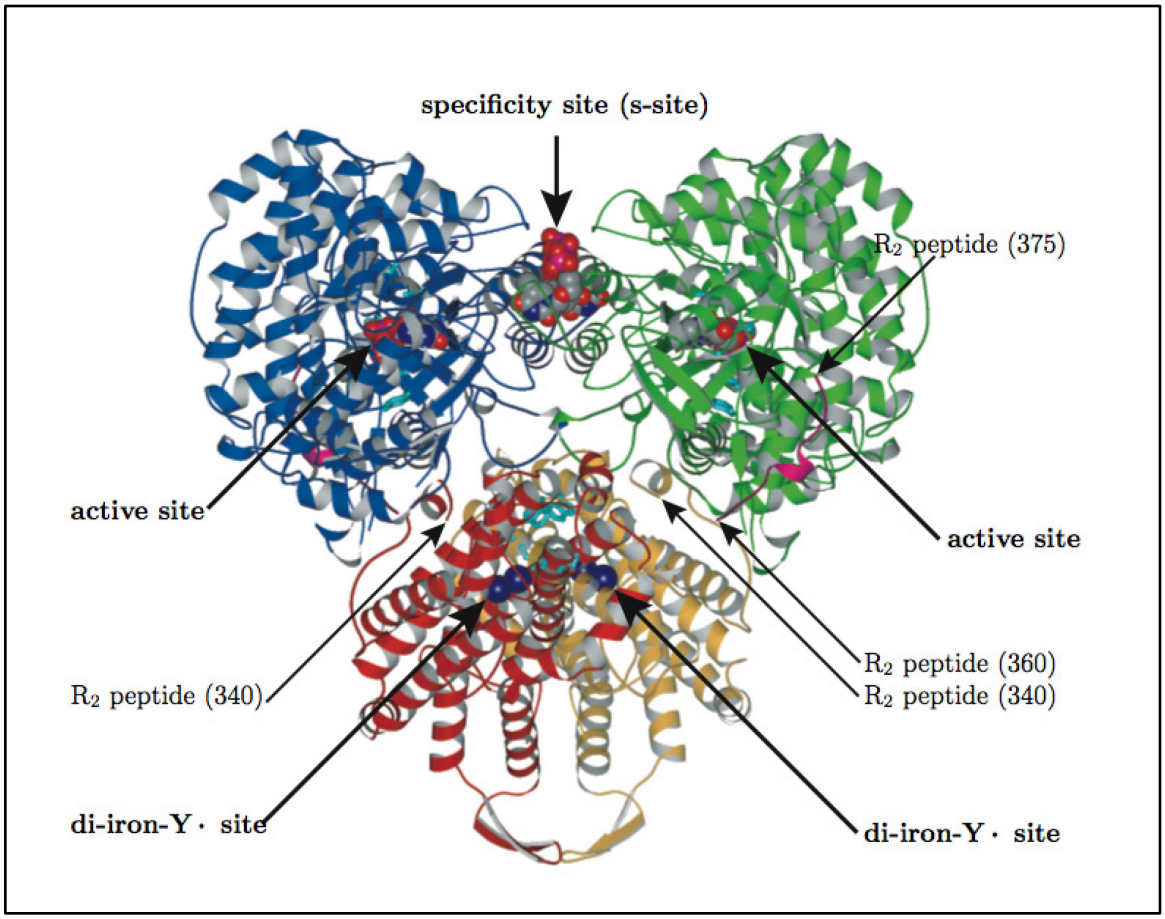


Figure 2[16]**.** Structure of Class I RNR.

1.1.1 Mechanism for conversion of RNA monomers into DNA

The mechanism of ribonucleotide reduction has been extensively investigated in the past three decades. Stubbe et al. postulated the accepted mechanism on the basis the long-range interaction between the tyrosyl radical on the R2 subunit and the cysteine residue (cys439) on the R1 unit, about 35 Å apart. Even though only the tyrosyl radical is stable enough to be characterized by electron paramagnetic resonance spectroscopy (EPR), the mechanism of radical chemistry is generally accepted.[1,8] The reduction of ribonucleotides to deoxynucleotides catalyzed by RNRs is initiated by the tyrosyl radical on Tyrosine 122 generating the reactive cysteine thiyl radical at cys439 via a long-range

electron transfer process.[17-19]. The study of the reduction of uridine 5'-diphosphate (UDP) to deoxyuridine 5'-diphosphate (dUDP) using isotopically labeled [3'-^{3}H]-UDP and [^{14}C]-UDP provided evidence of the cleavage of 3'-carbon hydrogen bond, which suggested that the first step in ribonucleotide reduction is the abstraction of 3'-hydrogen and the formation of a substrate radical **2**.[20] The radical at 3'-position (C3') of the substrate is then transferred to 2'-position (C2'). With glu441 accepting a proton from the hydrogen of the hydroxyl group on C3' and cys225 donating a proton to the oxygen atom on C2', the radical on C3' is then transferred to C2' eliminating the hydroxyl group on C2' while releasing H_2O and oxidizing the hydroxyl group on C3' to form 3'-keto-2-deoxyribonucleotides **3**.[3,7,21-23] The next step of the mechanism involves the radical on C2' abstracting the hydrogen atom on cys462, and cys225 donating the hydride ion to cys462 forming a disulfide radical anion **4**. The radical from the cys225-cys462 is then transferred to C3' reducing the keto to hydroxyl with the help of glu441. [1,18] The last step of the mechanism is the regenerating of thiyl radical on cys439 by the radical on C3' abstracting the hydrogen from cys439.[18,24] (Figure 3)

Deoxynucleotides then leave the active site allowing for other ribonucleotides to enter and to be reduced by RNRs. The cys225-cys462 disulfide unit formed during the reduction is reduced to regenerate the free sulfhydryl groups by the two cysteine residues located at the end of the N-terminal of the R1 protein, cys754 and cys759, in order to continue the mechanistic cycle. The regeneration is done by a coenzyme, nicotiamide adenine dinucleotide phosphate (NADPH), acting as a reducing agent donating a hydrogen atom to the disulfide unit. [1,18,25]

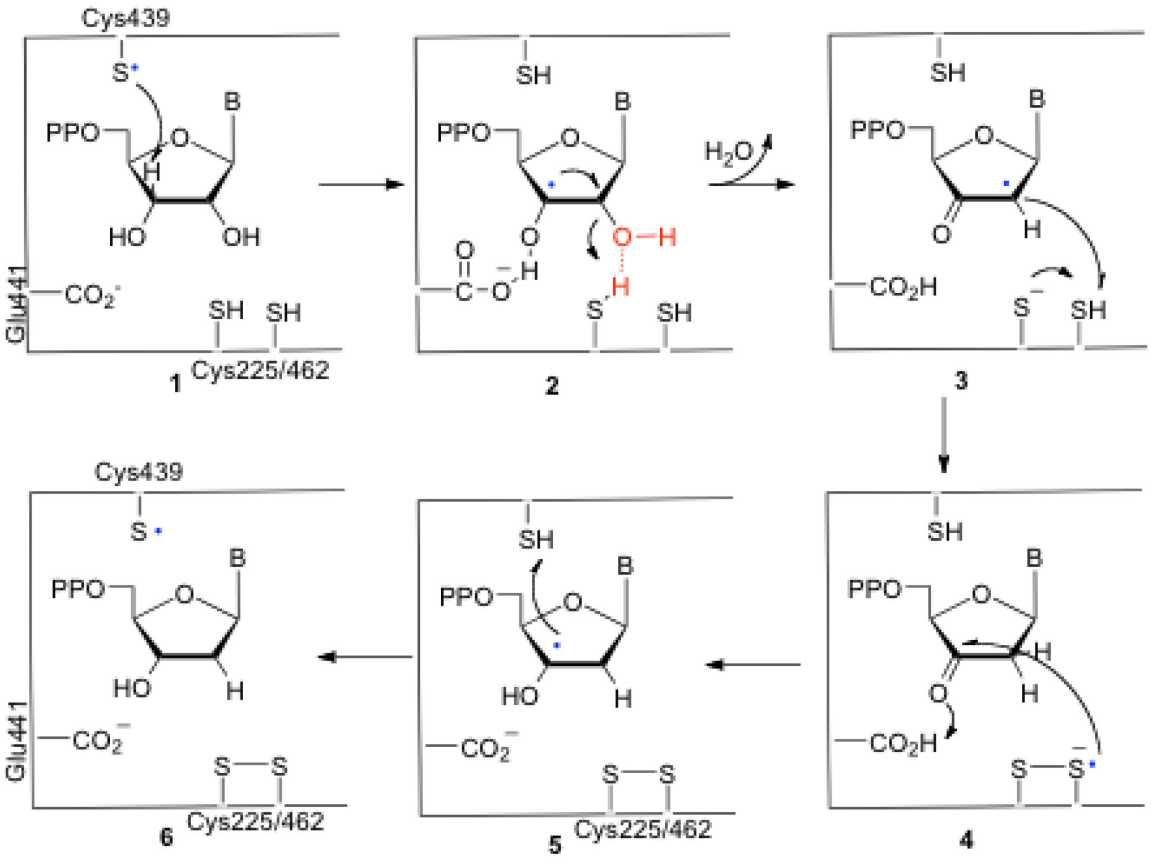
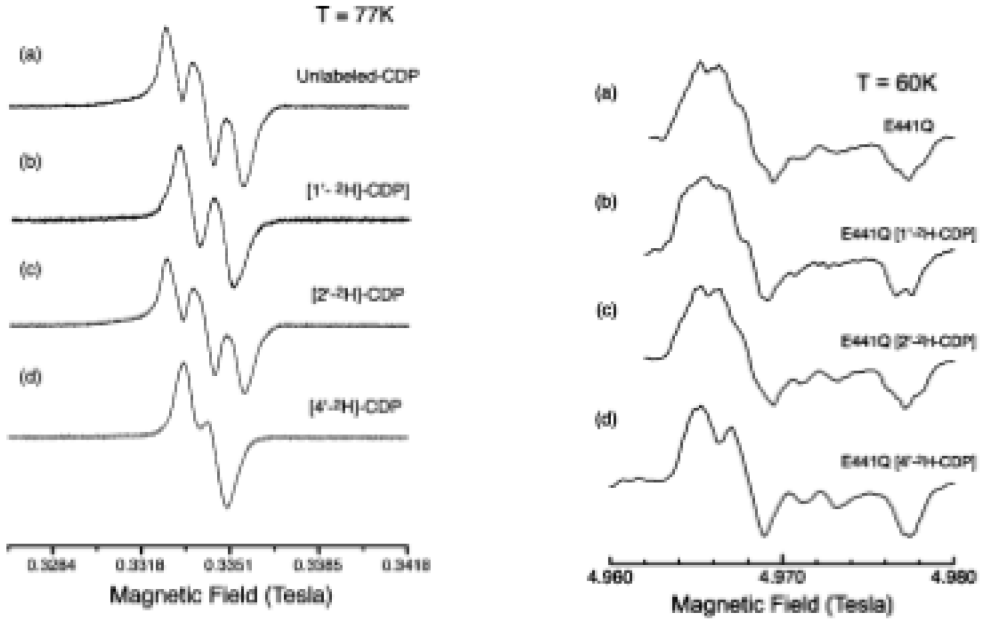


Figure 3. Acceptable mechanism of the conversion of ribonucleotide to deoxyribonucleotide catalyzed by RNR.

There is no question that the mechanism of RNR involves radical chemistry, but the structure of the natural substrate derived radical has still not been known. The Sjoberg group from Sweden and Stubbe from MIT observed an interesting protein and nucleotide-based radical formed during reaction of natural substrates, cytidine diphosphates (CDPs), with a mutant glutamate protein E441Q, in which the side chain group is an amide instead of a carboxylate.[26] They observed two radicals: one radical occured on a millisecond time scale was proposed to be a thiyl or disulfide radical anion; the second radical, observed on the three minute time scale, was substrate-derived. These studies

provided the first insight into the mechanism of reduction of the 3'-ketodeoxynucleotide. The effect of the isotopic substitution on the hyperfine couplings with 9 and 140 GHz EPR pulsed spectroscopy was studied using E441Q and a series of specifically and isotopically labeled CDPs. Surprisingly, the 9 GHz EPR spectra of the radical generated from E441Q with CDP and [1'-^{2}H], [2'-^{2}H], and [4'-^{2}H]-CDP taken at 77 K at 3 min reaction time showed that deuteration at either C1' or C4' collapsed the triplet, that was observed for unlabeled-CDP, to a doublet; while no such collapse occurred by deuteration at C2' (Figure 1.4).[26]



A **B**

Figure 4[26]. **A**) Nine GHz and **B**) 140 GHz pulsed EPR spectra of the radical generated from E441Q with CDP and [1'-^{2}H], [2'-^{2}H], and [4'-^{2}H]-CDP taken at 77 K and 60 K, respectively, and hand quenched at 3 min reaction time.

Spectroscopic evidence and quantum chemical calculations indicated that the structure of the nucleotide-derived radical species is a semidione of type **9** (Figure 5), an

off-pathway radical with additional oxygen in the nucleotide. The semidione was resulted from rapid exchange between radicals **7** and **8** through hydrogen transfer between the oxygen atoms at C2' and C3' (Figure 5). This structure was an unexpected result that gave important insight into the intermediates involved in the mechanism of RNR.[26]

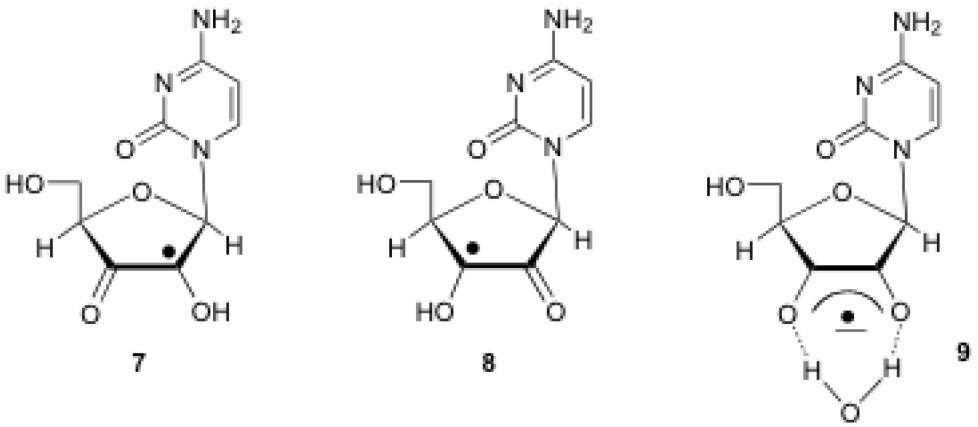


Figure 5. The structure of semidione formed during interaction of E441Q mutant of RNR with CDP

1.1.2 Inhibition of ribonucleotide reductases by 2'-azido-2'-deoxynucleotides

In 1976, Thelander *et al.* discovered that 2'-azido-2'-deoxynucleotides (N_3NDPs) can be potent inactivators of RNRs from aerobic *E. coli*, *Lactobacillus leichmannii*, and calf thymus cells. Thelander originally reported that the incubation of RNR with N_3NDPs resulted in the destruction of the tyrosyl radical at the R2 subunit without affecting the R1 subunit.[27] They also observed an increase in the absorption at 320 nm, which is linked to the dissociation of the 3'-ketonucleotides **13** via the cleavage of the base from the sugar moiety, the release of inorganic pyrophosphate, and the formation of 2-methylene-3(*2H*)-furanone **18**.[27-30] The electrophilic furanone can inactivate protein R1 by Michael alkylation and form the new chromophore with the absorption at 320 nm.[31-33]

1.1.2.1 Proposed mechanism on the basis of the experimental data

Although the mechanism of the irreversible inactivation of RNRs by azidonucleotides has been investigated in details, the mechanism is still not fully understood. In an effort to identify the substrate radical, Sjoberg used EPR spectroscopy to study the reaction between RNR and 2'-azido-2'-deoxyuriidne 5'-diphosphate (2'-N_3UDP). A new transient radical was observed, which marked the first evidence of a free radical intermediate in the reaction between a substrate-like inhibitor and the *E. coli* RNR. This new radical species had a characteristic EPR hyperfine structure of a major triplet with ~25 G splitting and a smaller doublet splitting of 6.3 G, in which the unpaired electrons were found to be localized at the proximal nitrogen of the azido group of the nucleotide molecule.[34] In 1984, Stubbe labeled 2'-azido-2'-deoxyuridine-5'-diphosphates (N_3UDPs) with ^{15}N and observed the collapse of a triplet splitting into a doublet; therefore, their data suggested that the radical was indeed derived from the azide moiety.[35]

Reactions of labeled N_3UDPs with ribonucleotide diphosphates reductases (RDPR) offered important information about the inactivation of RNR by azidonucleotides: the R1 subunit was also modified during the inhibition along with the destruction of the tyrosyl radical of the R2 subunit. The study also recorded the potency of the inactivation of N_3NDPs: 1 equivalent can completely eliminate enzymatic activity of RNR.[29] The structure of the nitrogen-centered radical (N•) was elusive during this time; it was thought that the nitrogen-centered radical was still attached at 2'-carbon of N_3NDPs. By double labeling of N_3NDPs with ^{13}C and $^{15}N_3$ and incubating with RDPR, Stubbe and Robins reported in 1993 that there was no hyperfine interaction between the

nitrogen-centered radical and the ^{13}C nucleus, which suggested the cleavage of the nitrogen bond on the 2'-carbon (**11**⇒**12**).[24] Incubation of oxidized R1 and mutated cysteine proteins C225SR1 and C462SR1 with N_3UDPs suggested the release of N_3^-, $N_3^\bullet$, or HN_3. On the basis of the results, it was proposed the thiyl radical at C225 **13** reacted with the released hydrazoic acid (HN_3) to form a sulfinylimine radical **14**. [24] In summary, the experimental data suggested that the inhibitory mechanism of RNR by N_3UDP started with the abstraction of a hydrogen atom from the carbon at C3' by cysteinyl radical C439 to give **11**. However, rather than releasing H_2O upon oxidizing the hydroxyl group on C3', azide ion was released to form the ketyl radical **12.** The released azide ion, which picked up hydrogen to form HN_3, then reacted with thiyl radical on C225 to give the sulfinylimine radical **14** (Figure 6).

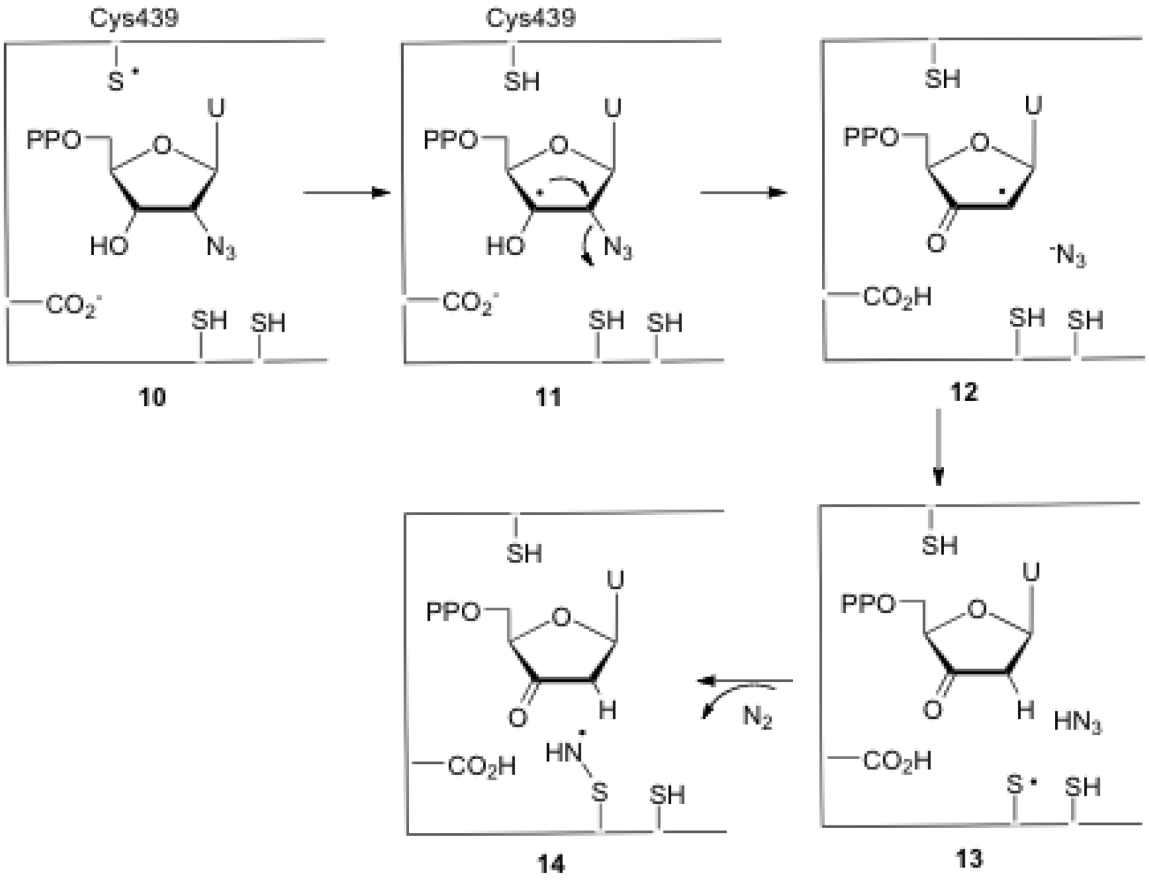


Figure 6. Proposed mechanism of inhibition of RNR by N_3UPD on the basis of the experimental data

Further EPR investigations of the [β-^{2}H]cysteine labeled RNR incubating with N_3UDPs produced a radical signal without the hyperfine splitting of 6.3 G, which gave evidence for the involvement of the thiyl radical of C225-R1. The structure of the nitrogen-centered radical was proposed to be either **16** or **18** as a result of the sulfinylimine radical adding to either the carbonyl carbon or the oxygen of the carbonyl group, respectively, of the intermediate 3'-keto-2'-deoxyuridine 5'-diphosphate **14** (Figure 7).[30]

To distinguish between the two proposed structures of **16** and **18** the inactivation of RNR was performed with 3'-[^{17}O]-N_3UDP, in which broadening of the N$^{\bullet}$ signal was detected. Density functional theory (DFT) calculations were employed to determine that structure **16** was most consistent with the EPR data. This [^{17}O]-labeled experiment also afforded the first evidence for the trapping of 2'-deoxy-3'-ketonucleotide in the reduction process of the natural substrate of RNR.[18]

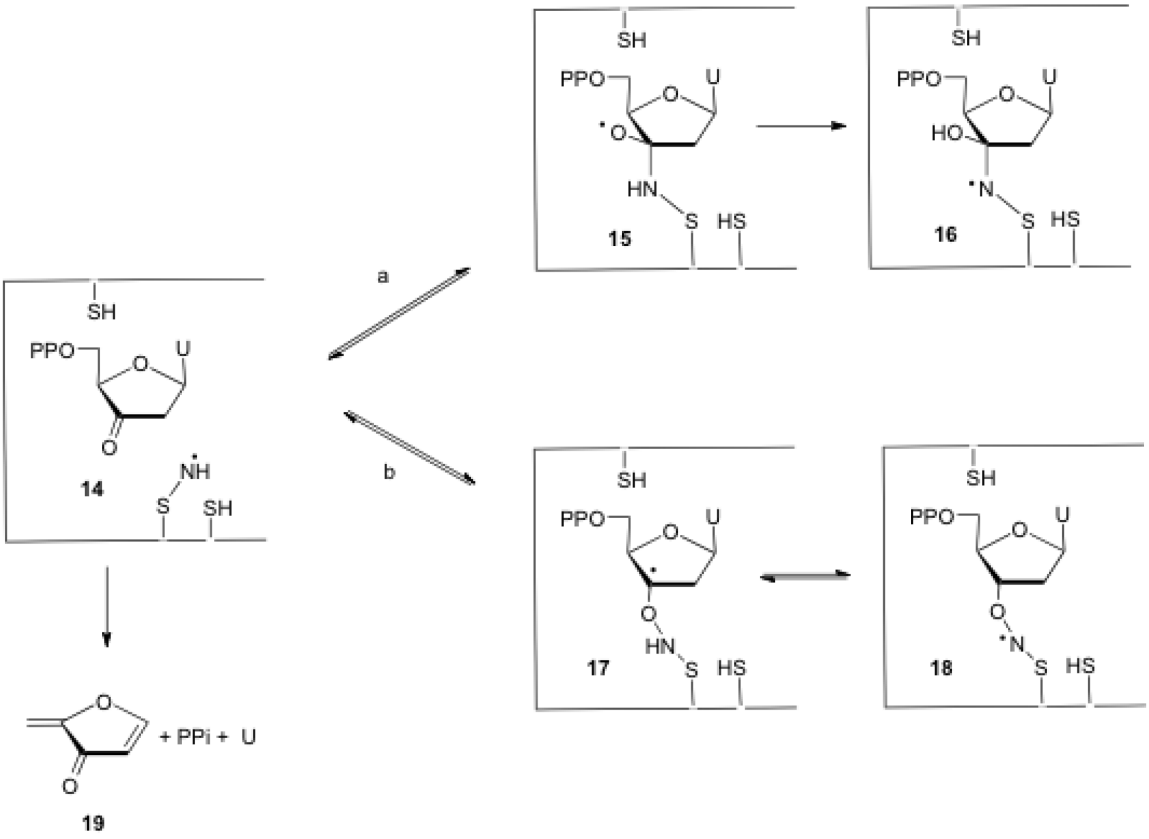


Figure 7. Proposed structure of the nitrogen-centered radical

1.1.2.2 Alternative mechanism derived from theoretical calculations

The theoretical modeling study of Pereira and coworkers[36] proposed an alternative pathway for the formation of the identical nitrogen-center radical **16**. Using DFT with the Gaussian98 suite of programs, Pereira and coworkers checked the viability of the

reactions proposed to make a distinction between the two radical intermediates and found a different and kinetically more favorable mechanism.

The putative mechanistic pathway proposed by Stubbe (Figure 6) was calculated to be plausible with an overall reaction free energy ΔG_r of -62.3 kcal/mol. Even though the formation of the sulfinylimine radical with the release of N_2 (**13**→**14**) was calculated to be greatly exothermic (ΔG_r of -52.5 kcal/mol), it also had a high activation free energy $\Delta G^{\neq}$ of 26.6 kcal/mol, which led to the exploration of alternative pathways. The last reaction to form the radical intermediate **16** involving the direct transfer of the hydrogen atom from the nitrogen to the 3'—O was reported to be acceptable in terms of feasibility with $\Delta G^{\neq}$ of 14.6 kcal/mol and a ΔG_r of -19.5 kcal/mol. Overall, the mechanistic pathway proposed by Stubbe derived from experimental data is plausible; however, the energy barriers for several reactions are high.[36]

An alternative mechanistic pathway was explored to find a route that was kinetically favored. The new mechanism proposed that the azide ion released from N_3UDP was added to the 2'-ketyl radical **12**→**20**, followed by the reduction at the 2'-position by C225 to generate the C225 thiyl radical **21**. Subsequent attack of the thiyl radical **21** on an alkyl azide led to the formation of the nitrogen-center radical **16** along with the loss of N_2, which was detected experimentally (Figure 8). [36]

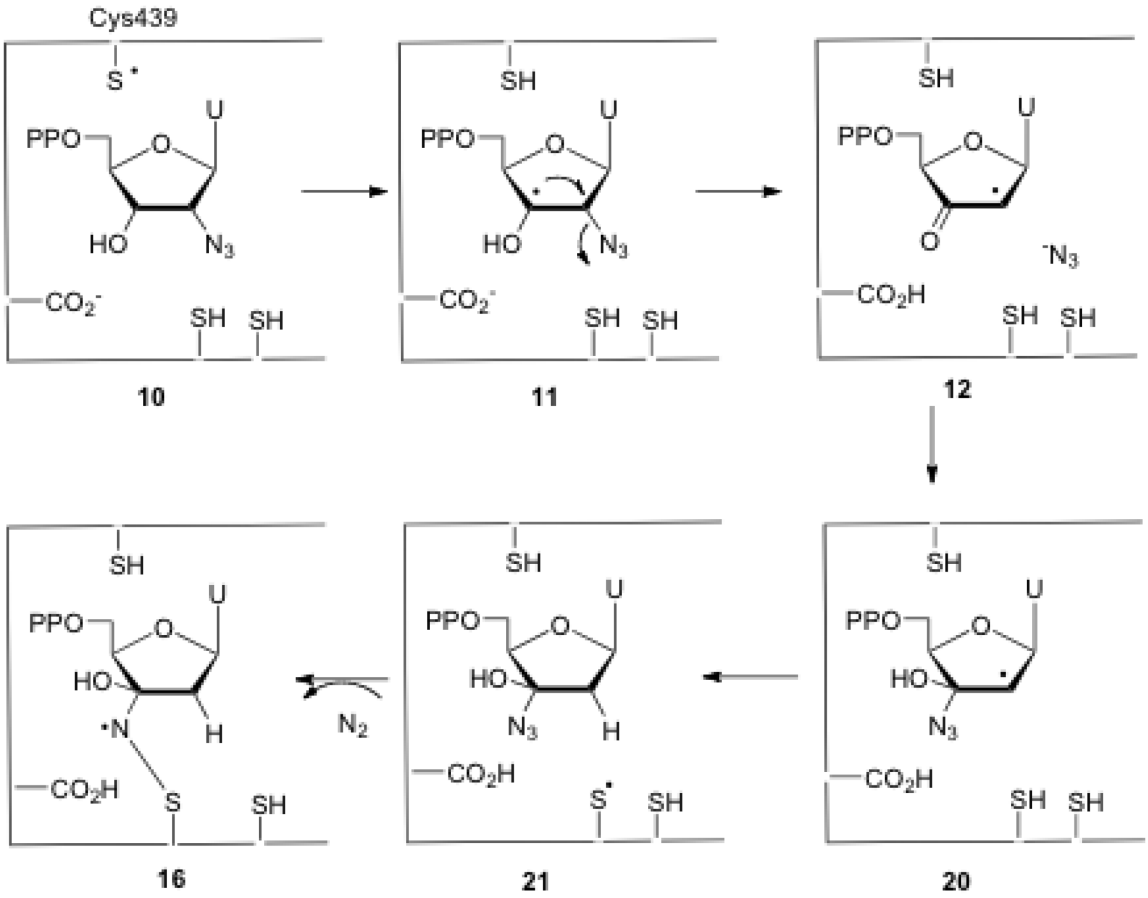


Figure 8. Alternative mechanism of the formation of N• derived from theoretical calculations

The addition of the azide to the C3' keto group was reported to be stereochemically favored by the fact that the azide became very well positioned to achieve the addition to the carbon upon leaving the ring. The abstraction of a thiol hydrogen of cysteine residue C225 by the carbon radical **20** was plausible with $\Delta G^{\neq}$ of 7.2 kcal/mol and a ΔG_r of -10.3 kcal/mol. Formation of the transient radical **16** is consisted of the newly-formed thiyl radical attacking the nitrogen of the azide bound to the C3' atom. The calculations suggested that the reaction is kinetically possible and very exoenergetic (ΔG_r = -53.8 kcal/mol and $\Delta G^{\neq}$ = 15.6 kcal/mol).[36]

1.1.3 Inhibition of ribonucleotide reductases by other 2'-substituted-nucleosides

The discovery of 2'-azido **10** and 2'-chloro **22** nucleotides inactivating the active site of *E. coli* RNR in 1976 led to the development of other 2'-substituted derivatives that were also potent inhibitors of RNR such as 2'-mercapto **23**, 2'-fluoro **24**, 2',2"-difluoro **25**, and 2'-fluoromethylene **26** nucleotides (Figure 9). Of those derivatives, 2',2'-difluoro-2'-deoxycytidine became an approved anticancer drug under the name of Gemcitabine or Gemzar for the treatment of non-small cell lung cancer , adenocarcinoma of the pancreas, bladder cancer, and murine leukemias.[37-40] Also, (*E*)-2'-fluoromethylene-2'-deoxycytidine **26** is on clinical trials as a new antitumor agent.[41]

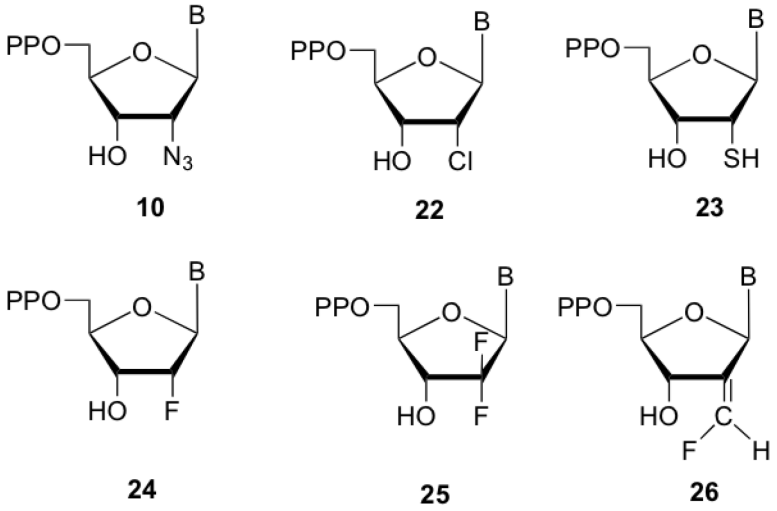


Figure 9. 2'-Substituted pyrimidine nucleosides that are potent inbititors of RNR.

Thelander *et al.* reported that 2'-chloronucleotides **22** (ClNDPs) inactivate RNR; and although less effective than the 2'-azido derivatives, ClNDPs offered a different paradigm for the inhibition. While N_3NDPs destroyed the tyrosyl radical of R2 subunit, ClNDPs only inactivated R1 without affecting R2; even though the inhibition required the presence of active R2.[27,35] The mechanism of the inhibition of RNR by ClNDPs

begins similarly to the inhibition of N_3NDPs. However, after the release of the chloro group at 3' position and forming the 3'-keto-2'-deoxynucleotide **14**, it was reported that the 3'-keto intermediate collapses to generate a reactive sugar intermediate 2-methylene-3-furanone **19**, diphosphates, and base without the formation of any transient radical (Figure 10). It was rationalized that the inactivation of the enzyme is the result of the alkylation of the R1 subunit by 2-methylene-2-furanone.[32,42]

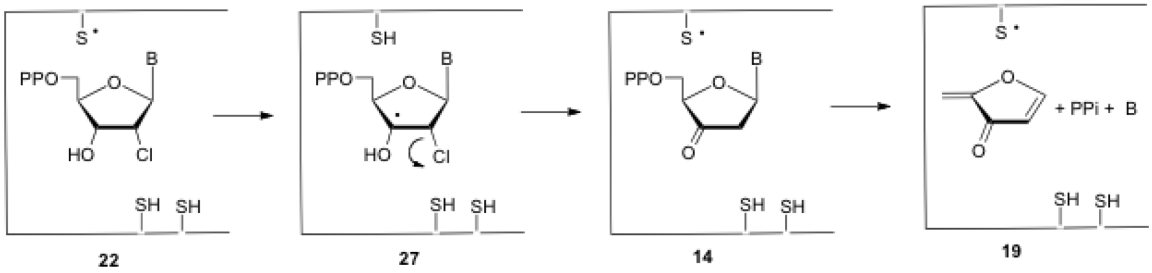


Figure 10. Mechanism of the inhibition of RNR by ClNDPs.

In 1996, Coves and coworkers showed that 2'-deoxy-2'-mercaptouridine 5'-diphosphate **23** is a very potent inactivator of RNR.[43,44] It was found that the irreversible inactivation of RDPR by **23** was achieved efficiently at the concentration of $K_i = 35$ μM and at the rate of $k_{inact} = 0.18\ s^{-1}$. Different from the azido and chloro analogs, the thiol analog **23** was reported to selectively inactivate the R2 subunit; while R1 might have been modified but did not affect the activity of RDPR. The EPR studies detected a transient organic radical during the reaction of inactivation with hyperfine structure consistent with a perthiyl $RSS^{\bullet}$ radical. Experiments with deuterium labeled R1 cysteines of the β protons show that the perthiyl radical was located on protein R1 as a result of the loss of the hyperfine structure. Even though formation of the observed perthiyl radical is still under investigation, the mechanism was proposed to first generate a disulfide **28** in

the presence of oxygen involving sulfur atoms from the substrate analog and a cysteine residue of R1. In the absence of oxygen, the inactivation was not achieved. The thiyl radical from C439, generated from the R2 tyrosyl radical, abstracts the 3'-hydrogen of the disulfide intermediate, which is followed by the homolytic cleavage of the 2'-carbon—sulfur bond, and the release of a 3'-keto derivative to form the observed perthiyl radical **30** (Figure 11). Theoretical calculations also led to a proposal of an alternative pathway for the formation of the perthiyl radical, which might be also spontaneously formed upon the 3'-H atom abstraction without the stepwise mechanism.

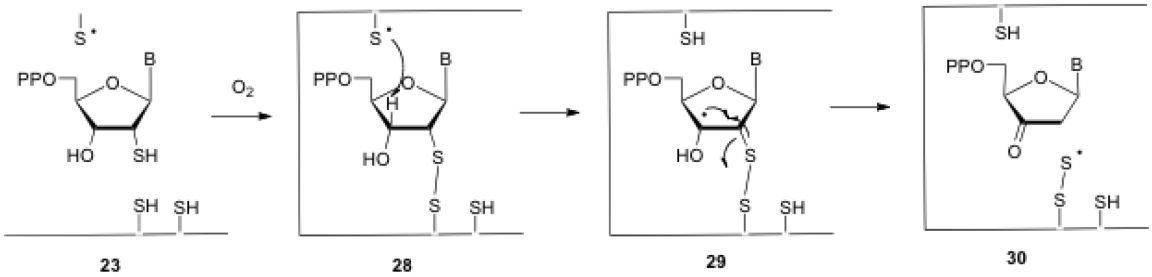


Figure 11. Proposed mechanism of the inhibition of RNR by 2'-mercaptonucleotides.

The development of 2'-halo-substituted nucleotides has resulted in some powerful anticancer and antitumor drugs. While 2'-deoxy-2'-fluoronucleotides **24** inactivate RNR, substitution of difluoro **25** and fluoromethylene **26** increases the potency.[28,41,45,46] Gemcitabine, 2',2'-dideoxydifluorocytidine, is currently being clinically used as a drug for the treatment of nonsmall cell lung carcinomas and advanced pancreatic cancer.[37,47] It was first synthesized and demonstrated to have antitumor activity by Hertel and coworkers in 1990.[48] The mode of action of Gemcitabine is cell phase specific, meaning that it kills cells undergoing DNA synthesis. Gemcitabine is metabolized by

deoxycytidine kinase to give the corresponding 5'-diphosphate (dFdCDP), which inhibits RNR and reduces the cellular concentration of the four DNA monomers. Deoxycytidine kinase further metabolized dFdCDP to form the corresponding 5'-triphosphate (dFdCTP), which competes with the natural deoxycytidine 5'-triphosphate (dCTP) for DNA replication. When a molecule of dFdCTP replaced a molecule of dCTP, DNA synthesis can no longer continue.[38,40,49,50] These two mechanism for disrupting DNA synthesis by Gemcitabine are responsible for its efficacy as an anticancer drug.

Much research has been performed to elucidate the complex mechanism of the inhibition of RNR by Gemcitabine. Incubation of Gemcitabine with RDPR from *E. coli* resulted in 1 equiv. of cytosine, 2 equiv. of fluoride, reduction of the tyrosyl radical on the R2 subunit, and inactivation of R1. However, no detection of the furanone at 320 nm was observed like most 2'-substituted nucleotides during the inactivation.[39,51] Instead, formation of a new stable substrate derived radical was detected as a triplet EPR signal at 9 GHz during the inhibition of RNR by Gemcitabine.[51] Moreover, unlike other 2'-substituted nucleotides that require the presence of reductants (namely cysteine residues C754 and C759 and NADPH) for the inhibition, the presence or absence of reductants did not affect the inhibitory action of Gemcitabine. Inhibition in the presence of the reducing system happened through covalent labeling, which increased interaction between the two subunits. In the absence of the reducting system, inhibition happened through the loss of the tyrosyl radical and the formation of the new radical species.

A complex mechanism of the inhibition of RNR by Gemcitabine was also proposed on the basis of the theoretical work by the Ramos group.[37,40] The proposed mechanism initiates as in the mechanism for the natural substrate by the abstraction of

H3' by the thiyl radical C439, which leads to the release of HF **31** and formation of fluoroketyl radical **32**. Subsequent abstraction of hydrogen led to the formation of the disulfide anion radical and fluoroketo nucleotides **33**. The cycle is then repeated resulting in releasing another HF to form the 3'-ketyl intermediate **35**. In the absence of the reductants, it was proposed that that 3'-ketyl radical abstracts the hydrogen from C439 and generates the 3'-keto intermediate **36**. The cycle of the RNR cascade was inhibited by the abstraction of 4'-hydrogen by the cysteinyl radical to form the 4'-ketyl radical **37**. In the presence of the reductants, the RNR cascade was stopped by C225 anion abstracting 2'-hydrogen and forming **39**.

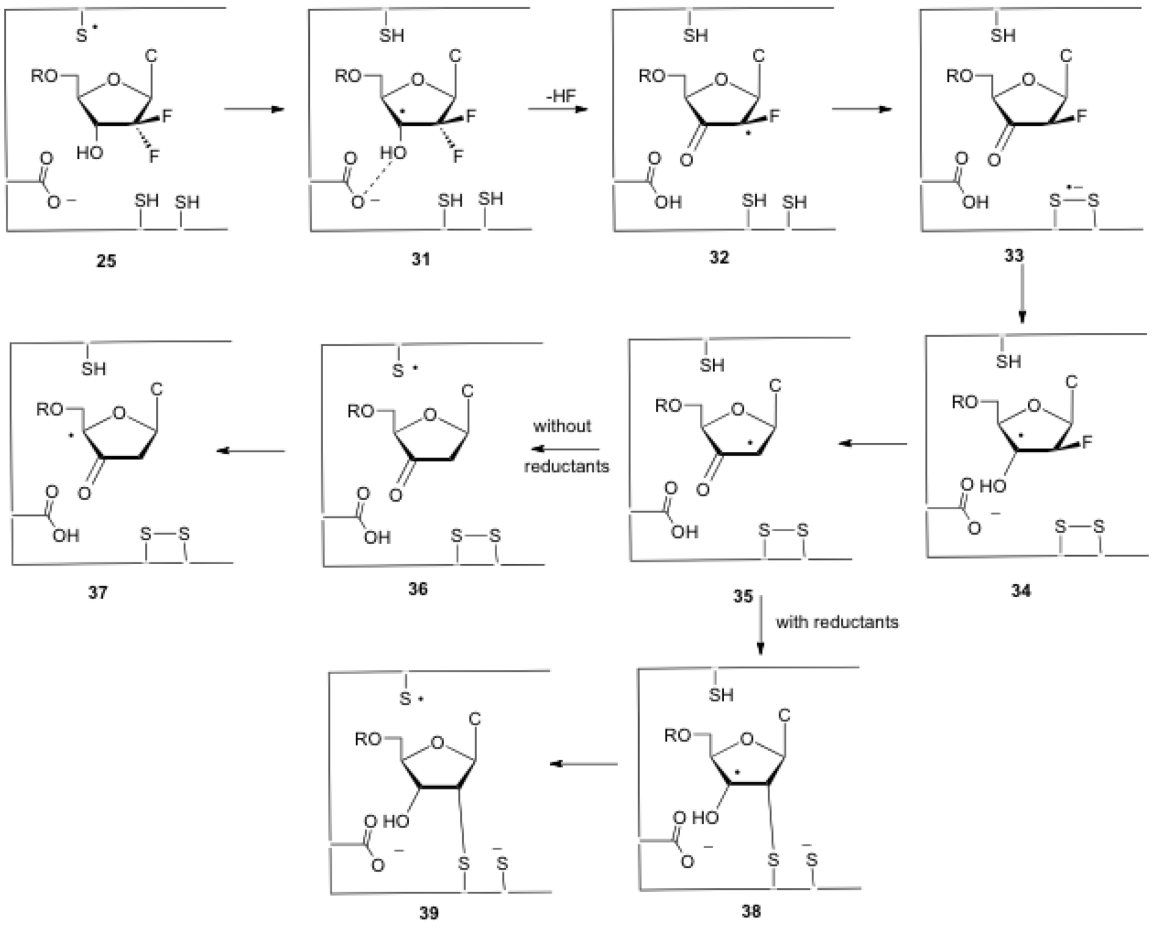


Figure 12. Proposed mechanism of RNR inhibition by Gemcitabine derived from theoretical calculations.

Recently, the Stubbe group reported new information on the inhibitory mechanism of RNR by Gemcitabine. The research provided new insight on the mechanism of RNR. To identify the new radical species that formed during inhibition, they incubated [1'-^{2}H-] **25** with wild type RNR as well as mutant C225S-RNR, and found the collapse of the 9 GHz triplet hyperfine pattern to a doublet. The EPR characteristics of this new readical species shared many similarities with the semidione **9** generated when E441Q-RNR reacting with CDP.[26,47,52] A new dual-mechanism was proposed, as shown in Figure 13.[52,53] Stubbe proposed that following H3' abstraction and elimination of HF, the 3'-fluoroketyl radical **32** is formed, which is in resonance with the fluoro enone form **40**. Addition of water to **40** and elimination of fluoride ion led to radical **41** without reductants, with EPR characteristics similar to semidione **9** (see Figure 5). In the presence of the reductant, the cysteine residue directly attached to C2' as shown in **42**. Similar EPR features of a radical species were detected during the inactivation of p53-RNR and *Lactobacillus leichmannii* RNR with Gemcitabine.[47,53,54] The detection and elucidation of this new radical species not only provided information for the mechanism of inhibition of RNR by Gemcitabine, but also suggested of a new intermediate for the mechanism of the natural substrate.

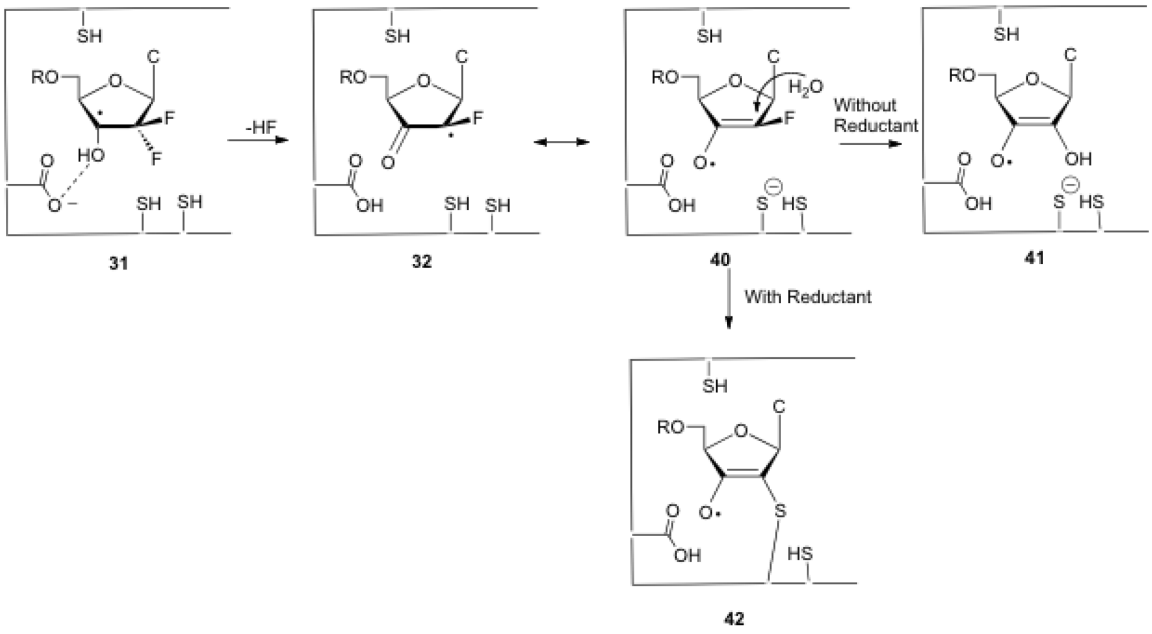


Figure 13. Proposed mechanism of RNR inhibition by Gemcitabine on the basis of the detection of the new radical species.

Another nucleotide analog, (*E*)-2'-fluoromethylene-2'-deoxycitidine 5'-diphosphates, which was synthesized by McCarthy and coworkers in 1991, is currently under clinical trials as an antitumor agent.[31,41,46] The inhibitory pathway of compound **26** is also very complex with the formation of a new chromophore at 334 nm. It was proposed that inhibition of RNR is achieved by the generation of 2'-methylene-3'-ketocytidine 5'-diphosphate after the protonation and subsequent release of fluoride.[41,55,56]

1.2 Thiyl radical in biological systems

Functionalized aliphatic thiols (RSH) are abundant in living organisms. The most common thiols in the body are glutathione (GSH) — a tripeptide made up of three amino acids: cysteine, glutamic acid, and glycine — and amino acids such as cysteine (CysH) and homocysteine (HSH).[57,58] Thiyl radicals ($RS^{\bullet}$), generated from thiols, are sulphur-

centered free radicals that can undergo electron transfer and hydrogen transfer reactions with different biological molecules.[59-61]

The generation of thiyl radicals is vital for different biological mechanisms including enzymatic functioning in ribonucleotide reductase and pyruvate formate lyase.[58,62] However, thiols in biological systems are best known for their ability to "repair" the radical-induced damage to biomolecules from free radicals.[58,60,63] Free radicals are atoms carrying an unpaired electrons that can cause damages to tissues and organs.[63] For example, damage to biomolecules (including DNA species) through enzyme activity, radiation or toxic agents can generate carbon-centered radicals by the breakage of a C—H bond, in which thiols can perform the "repair" reaction by donating a hydrogen atom (eq 1).[58] While thiols are remarkable antioxidants protecting cells from damage caused by free radicals, such processes of biochemical oxidation/reduction also produce the reactive thiyl radicals, which can have harmful effects such as enzyme inactivation and peroxidative injury.[58,60,63]

$$RSH + DNA^{\bullet} \rightarrow DNA + RS^{\bullet} \qquad \textbf{eq 1}$$

Thiyl radicals are also important intermediates during oxidative stress in living cells.[63,64] During oxidative stress, reactive oxygen species (ROS), including hydroxyl radicals ($^{\bullet}OH$), superoxide radical anion ($O_2^{\bullet-}$), and hydroperoxyl radical ($HO_2^{\bullet}$), abstract electrons from biomolecules such as proteins, nucleic acids and lipids. The exceptionally active ROS are continuously produced during oxygen metabolism. Electron abstraction by ROS of biomolecules initiates a free radical chain reaction that leads to peroxidation.[63,64] Although both proteins and nucleic acids are subjected to perioxidation, lipids are more susceptible to reactions with ROS because they are fatty acids located at the cell

membranes. The reaction between ROS and lipids yields the alkyl radical of $L^{\bullet}$ (eq 2a), which leads to the formation of lipid peroxide radicals $L\text{-}OO^{\bullet}$ by oxidation (eq 2b) and then the production of lipid peroxides LOOH (eq 2c). However, lipid peroxidation causes the formation of polar peroxidic, carbonyl and hydroxyl groups, which lowers hydrophobicity and generates toxic aldehydes and hydrocarbons. Thiols, in particular glutathione (GSH), perform detoxification by reacting with organic peroxides generated during lipid perioxidation.[63] However, the generated thiyl radicals can also cause lipid isomerization. Thiyl radicals convert the natural all-*cis* double bonds of unsaturated phospholipids to the unnatural *trans* form, which affects the membrane structures, lipid metabolism, and enzymatic reactions.[58,65-67] Furthermore, thiols are also very reactive toward ROS, generating dangerous thiyl radicals.[60,63]

$$LH + ROS \rightarrow L^{\bullet} \qquad \textbf{eq 2a}$$

$$L^{\bullet} + O_2 \rightarrow L\text{-}OO^{\bullet} \qquad \textbf{eq 2b}$$

$$LH + L\text{-}OO^{\bullet} \rightarrow LOOH + L^{\bullet} \qquad \textbf{eq 2c}$$

1.2.1 Generation of the thiyl radicals

Thiyl radicals are formed through many different means. Thiols undergo one-electron oxidation in antioxidant reactions and form thiyl radicals in the process (eq 3).[63]

$$RSH \rightarrow RS^{\bullet} + H^{+} + e \qquad \textbf{eq 3}$$

Figure 14 shows the multiple pathways by which biological oxidations produce thiyl radicals. All reactions of thiols with other free radicals afford thiyl radicals, including carbon-centered radicals ($R^{\bullet}$), reduced oxygen species such as superoxide ($O_2^{\bullet -}$), perhydroxyl radical ($^{\bullet}OOH$), hydroxyl radicals ($^{\bullet}OH$), and peroxyl radicals ($ROO^{\bullet}$).[59,60]

Peroxidases and myoglobin also can oxidize RSH to RS$^{\bullet}$ using H_2O_2 or ROOH.[68,69] Peroxynitrite (-OONO), generated from the reaction between nitric oxide ($^{\bullet}$NO) and superoxide, and nitrogen dioxide have also shown to form thiyl radicals from oxidizing thiols.[60]

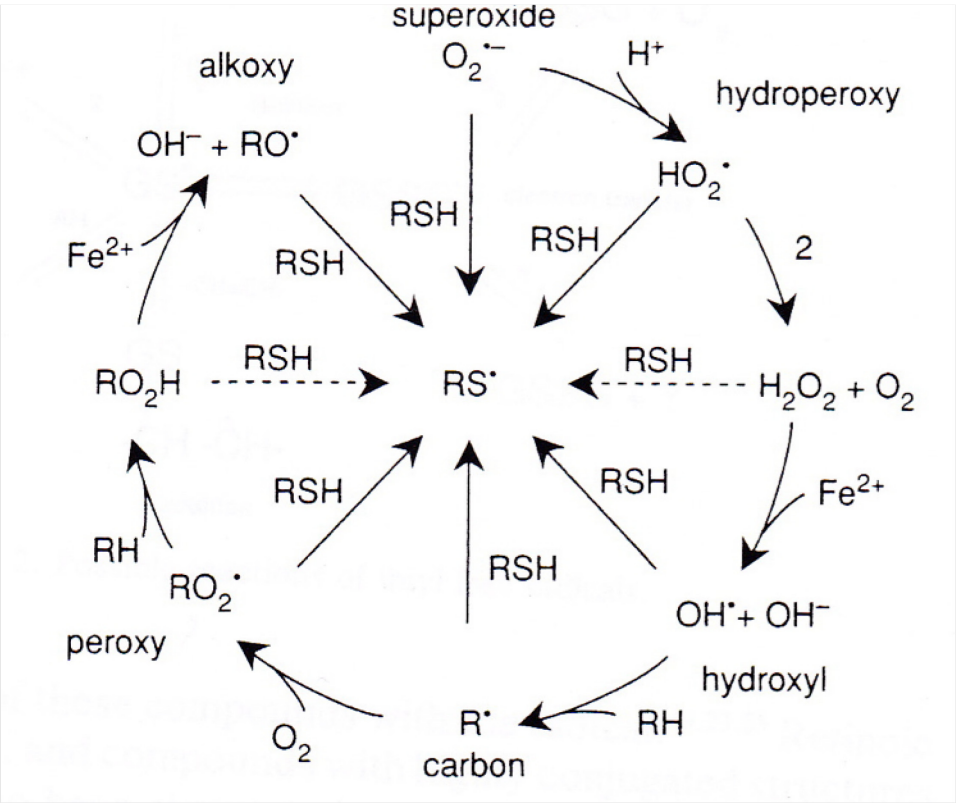


Figure 14. Formation of thiyl radicals by oxidation of thiols by other free radicals. The dashed arrows refer to enzyme-catalyzed reactions.[59]

Reactions of thiols with transition metal ions such as Fe^{3+} and Cu^{2+}, which can change their oxidation state, can also generate thiyl radicals by one-electron oxidation (eq 4a).[58,63] Thiyl radicals can be formed thermally[59] from disulfide functionalities either by directly breaking the sulfur-sulfur bond under free-radical conditions (eq 4b) or single-electron reduction processes (eq 4c).[58]

$$RSH + Fe^{3+} \rightarrow (RS^{-})\text{-}Fe^{3+} \rightarrow RS^{\bullet} + Fe^{2+} \qquad \textbf{eq 4a}$$

$$X^{\bullet} + RS\text{-}SR \rightarrow RSX + RS^{\bullet} \qquad \textbf{eq 4b}$$

$$RS\text{-}SR + e^{-} \rightarrow (RSSR)^{-\bullet} \rightarrow RS^{\bullet} + RS^{-} \qquad \textbf{eq 4c}$$

Photolysis, in addition, can cause homolysis and form $RS^{\bullet}$ from disulfides and all other functionalities.[58,59] Other techniques for the formation of thiyl radicals include radiolysis and sonolysis. Gamma or X-irradiation of aqueous solutions generates hydroxyl and hydrogen radicals, and hydrated electrons (eq 5a) that can abstract the hydrogen atom from thiols to afford thiyl radicals (eq 5c).[59,70,71]

$$H_2O \rightsquigarrow {}^{\bullet}OH + e_{aq}^{-} + H^{\bullet} \qquad \textbf{eq 5a}$$

$$e_{aq}^{-} + N_2O \rightarrow {}^{\bullet}OH + N_2 + {}^{-}OH \qquad \textbf{eq 5b}$$

$${}^{\bullet}OH/H^{\bullet} + RSH \rightarrow RS^{\bullet} + H_2O \qquad \textbf{eq 5c}$$

1.2.2 Reactions of thiyl radicals

Since the "repairing" reactions of thiol compounds and biomolecules simultaneously generates the thiyl radicals, the chemical character and reactivity of the formed $RS^{\bullet}$ are critical. The protective and repairing efficacy of thiols depend on the regenerative reduction of thiyl radicals (eq 6).

$$RS^{\bullet} + e^{-} + H^{+} \rightarrow RSH \qquad \textbf{eq 6}$$

Thiyl radicals can take part in addition reactions, hydrogen abstraction and electron transfer reactions, as well as intramolecular rearrangements of free radicals.[63,72] Thiyl radicals are presumed to be inert and disappear by dimerization, comparing to oxygen-centered analogues.[72,73] For example, the dimerization of glutathionyl radicals (eq 7)

occurs at the rate of $k = 1.5 \times 10^9\ M^{-1}s^{-1}$.[73] However, steady-state levels of $RS^\bullet$ would in general be low *in vivo* and therefore these radicals would not be likely to meet.[73]

$$GS^\bullet + GS^\bullet \rightarrow GSSG \qquad \textbf{eq 7}$$

A more likely reaction would be between the $RS^\bullet$ and the thiolate anion, which generate disulfide radical anions (eq 8b).[63,70,73] At physiological conditions, pH ≈ 7.0, about 1 to 2% of RSH exists as its thiolate form (eq 8a).[73] Disulfide radical anions are powerful reducing agents, reducing with metal ions as well as molecular oxygen.[63,70,72,73] They also react rapidly with protons ($k \approx 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$)[72,74], but collapse quickly into thiyl radicals and thiols (eq 8c) (k in the order of $10^6\ s^{-1}$)[72].

$$RSH \rightleftharpoons RS^- + H^+ \qquad \textbf{eq 8a}$$

$$RS^\bullet + RS^- \rightarrow (RSSR)^{-\bullet} \qquad \textbf{eq 8b}$$

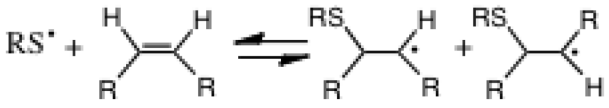
$$(RSSR)^{-\bullet} + H^+ \rightleftharpoons (RSSRH)^\bullet \rightleftharpoons RS^\bullet + RSH \qquad \textbf{eq 8c}$$

Two important addition reactions of thiyl radicals are with oxygen and unsaturated fatty acids. The reaction of thiyl radicals and oxygen produce the thiyl peroxyl radicals (eq 9a), which are unstable and undergo further reactions with thiols to form sulphinyl radicals or sulphonyl radicals (eq 9b-9c).[63,73,75] With unsaturated fatty acids, thiyl radicals added to the double bonds (eq 10) causing the *cis/trans* isomerization that affects the packing and density of bilayer lipid cell membranes and their biological functions.[63,72]

$$RS^\bullet + O_2 \rightarrow RSOO^\bullet \qquad \textbf{eq 9a}$$

$$RSOO^\bullet + RSH \rightarrow RSO^\bullet + RSOH \qquad \textbf{eq 9b}$$

$$RSOO^\bullet + RSH \rightarrow RSOOH + RS^\bullet \qquad \textbf{eq 9c}$$



eq 10

Hydrogen abstraction by thiyl radicals from activated C-H bonds constitutes the reverse "repair" reactions. Alcohols and ethers, unsaturated fatty acids, and nucleotides such as NADH or NADPH are among the compounds that undergo hydrogen transfer with thiyl radicals. Glutathionyl, cysteinyl, and cysteaminyl radicals are probably the most active and abundant thiyl radicals in biological systems that can abstract hydrogen from linoleic and arachidonic acids, or DNA bases and deoxyribose. The success of the repair reactions depends on the subsequent reactions of the resulting thiyl radicals, in which hydrogen transfer reactions with NADH (eq 11) normally facilitate repair because of its nature to regenerate. However, hydrogen abstraction by thiyl radicals of unsaturated fatty acids also causes damages to lipids.[72,73] Electron transfer by thiyl radicals is another important reaction. It can occur with molecular oxygen and reductants such as ascorbate. Oxidation of ascorbate (AH^-) by electron transfer of thiyl radicals (eq 12) to form ascorbyl radicals ($A^{\bullet -}$) plays an important role in detoxification of the thiyl radicals since the formed $A^{\bullet -}$ can enter a disproportiation reaction to regenerate ascorbate and dehydroascrobate.[63,73]

$$RS^{\bullet} + NADH \rightarrow RS^- + NAD^{\bullet} + H^+ \qquad \textbf{eq 11}$$

$$AH^- + RS^{\bullet} \rightarrow RSH + A^{\bullet -} \qquad \textbf{eq 12}$$

1.2.3 Detection of thiyl radicals

Electron paramagnetic resonance (EPR) spectroscopy can be used to detect thiyl radicals that are generated by pulse radiolysis.[60] However, thiyl radicals in most cases can hardly be directly detected by EPR spectrosopy because of line broadening as a result of

their large anisotropy *g* factor. Direct EPR can only detect thiyl radicals in condensed systems at low temperature.[76] To solve such problems, organic nitrone or nitroso compounds were applied as spin traps to catch the reactive thiyl radicals by forming more stable nitroxides. The common spin traps for thiyl radicals are 2-methyl-2-nitrosopropane (MNP), α-phenyl-*tert*-butyl-*N*-nitrone (PBN), 5,5-dimethyl-1-pyrolline-*N*-oxide (DMPO), and 5-diethoxyphosphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEMPMO). With MNP, thiyl radicals are identified by the characteristic *g*-value of the MNP/$^{\bullet}$RS adduct (**43,** Figure 15), formed by the reaction between the spin trap and thiyl radicals. The PBN/$^{\bullet}$RS adduct **44** is useful to characterize high-molecular-mass thiyl radicals, but the EPR features are not characteristic for detecting small-molecular-mass thiyl radicals.[60,77] However, Mullins and coworkers showed that adducts of PBN with thiyl radicals (**44**) of low-molecular weight thiols such as cysteine, homocysteine and glutathione can also be detected and identified. They reported that the differences in the β-proton hyperfine couplings are significant for identification of thiyl radicals. The EPR lineshapes and the radical adduct lifetimes also are individual characteristics that distinguish the nature of the trapped thiyl radical.[47]

5,5-Dimethyl-1-pyrolline-*N*-oxide is probably the most successful spin trap for thiyl radicals since EPR spectra of DMPO-cysteinyl, DMPO-gluthionyl and DMPO-penicillamine adducts **45** are easily differentiated.[78] DEMPMO is a phosphorylated spin trap that reacts with thiyl radicals to produce EPR spectra from two diastereomers (**46-47**). The phosphorus hyperfine coupling of the DEMPMO/$^{\bullet}$RS adduct gives very complex EPR spectral patterns, but the spectral features used to distinguish between thiyl and hydroxyl radical adducts are distinct.

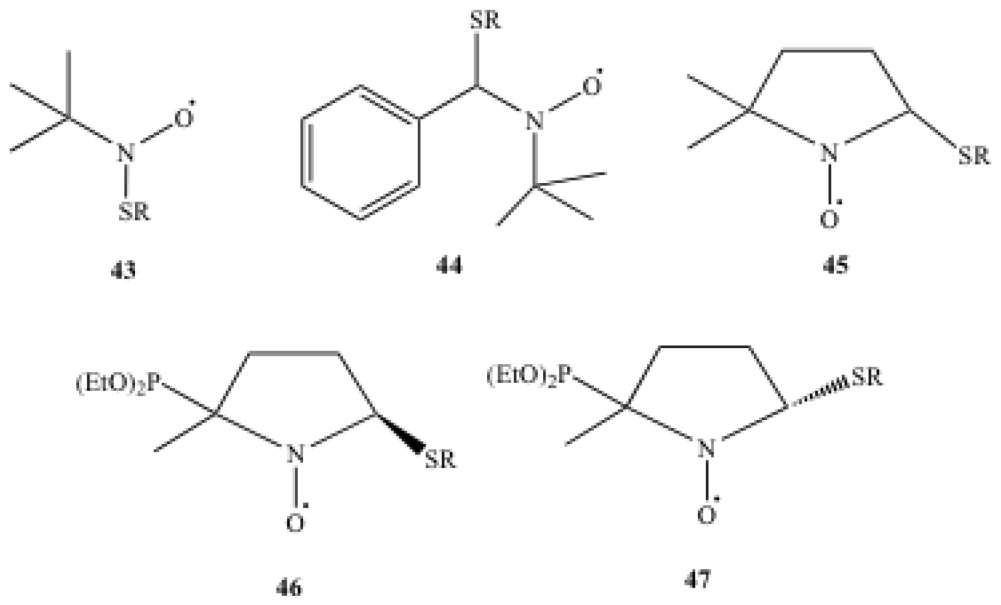


Figure 15. Structures of spin adducts of thiyl radicals.

1.3 Chemistry of alkyl azides

Since phenyl azide was first synthesized by Peter Grieβ in 1864, syntheses and application of electron-rich organic azides in organic synthesis have been widely examined.[79-81] Most organic azides are explosive; especially hydrogen azide, methyl azide, and heavy-metal azide.[79,80] However, azides have contributed greatly as substrates and/or intermediates to organic synthesis in such processes as cycloadditions, synthesis of aniline *N*-alkyl-substituted-anilines, and nitrenes.[80-84] In the last century, organic azides have also contributed to industrial uses as well as to medicine. For example, azides were employed in the synthesis of heterocycles such as triazoles and tetrazoles, and azidonucleosides for treatment of AIDS, as well as for use as detonators in explosive technology.[79,80,85]

Figure 16 shows the structure of azide as polar mesomeric structures. The dipolar structures of **48c** and **48d** suggest the facile decomposition to nitrene and dinitrogen.

From structure **48d**, the regioselectivity of reactions with nucleophiles and electrophiles is explained: attack on N^{γ} by nucleophiles and on N^{α} by electrophiles. Mesomeric structures **48b** and **48c** provide justification to some of the properties of azides: strong IR absorption at ≈ 2114 cm^{-1} for phenyl azide, UV absorption at both 287 nm and 216 nm for alkyl azides, weak dipole moment, and acidity of aliphatic azides. As a result, the azide ion behaves often as a pseudohalide, which means it resembles halide.[80].

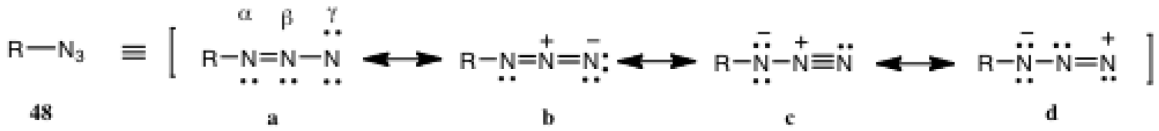
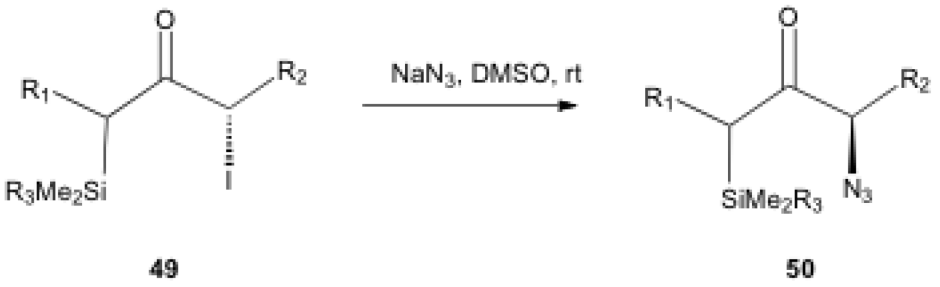


Figure 16. The resonance structure of azide[80]

Since the first synthesis of alkyl azides by Curtius, there have been many reported syntheses of alkyl azides.[80] The classic nucleophilic substitution has the most abundant usage, however many other methods have also been examined including the Mitsunobu, polar 1,2- and 1,4-addition, C-H activation, and diazo transfer reactions.[80] Sodium azide is most often used as a source of azide for nucleophilic substitution in addition to other azides such as alkali azides, tetraalkylammonium azides, polymer-bound azides, and silver azide. The substitution usually occurs by replacing a good leaving group such as triflates, halides, carboxylates, and mesylates. The reactions generally take place in DMF or DMSO at thermal conditions, even though different conditions such as ionic liquids, supercritical carbon dioxide, and even microwave have reported to be used for the nucleophilic substitution. The synthesis of α-azidoketones **50,** reported by Enders *et. al.,* is an example of the classic nucleophilic substitution to afford alkyl azides with inversion of configuration (Scheme 1).[86]



49 **50**

Scheme 1. Synthesis of α-azidoketones

The Mitsunobu reaction is frequently applied to the synthesis of alkyl azides from primary and secondary alcohols. Both primary and secondary alcohols react with hydrogen azide or more preferentially with diphenylphosporyl azide (DPPA) in the presence of diethyl azodicarboxylate (DEAD) to yield alkyl azide. Under this condition, secondary alcohols afford azide compounds with an inversion of stereochemistry.

Reduction of the azide moiety to amine is an important reaction for organic azides. There are numerous methods to perform reduction including hydrogenation, the Staudinger method, or by the use of reducing agents such as sodium borohydride, lithium aluminum hydride, or dithiolthreitol.[79] The hydrogenation method, by employing H_2 in the presence of a catalyst such as palladium or Raney nickel, has been commonly applied to reduce azides. Hydrogenation generally gives very good yield for reduction; however, it is not very selective. Hydrogenation can reduce numerous other groups such as alkene, alkyne, carbonyl, nitro, and imine. The Staudinger method, on the other hand, is one of the most selective and mildest methods for the conversion of azides to amines. The Staudinger reaction involves treatment of triphenylphosphine to azides forming the corresponding iminophosphorane, which follows by hydrolysis to afford the amines. Reducing agents also effectively convert azides to amines, but they lack selectivity.[79]

1.3.1 Reduction of alkyl azides by radicals

Radical reactions of azides have been also of great interest and importance providing valuable synthetic tools in the last thirty years.[82,87-92] Aliphatic, aromatic, and sulfonyl azides serve as good acceptors of carbon- and heteroatom-centered radicals.[82,92,93] Reported studies have suggested that homolytic addition to an azide can occur at either position N^{α} or N^{χ} of **48a**, generating 3,3-triazenyl (**51**) or 1,3-triazenyl radical (**52**), respectively.[94,95] It is unclear at which position the addition would occur preferentially because the aminyl radicals can be formed from both triazenyl radicals by the release of dinitrogen. As for sulfonyl azides, homolytic addition is more likely to take place at the terminal nitrogen N^{χ}, generating the 1,3-trianenyl radical intermediate.[82] Under the radical conditions, the aminyl radicals are further reduced to amines.

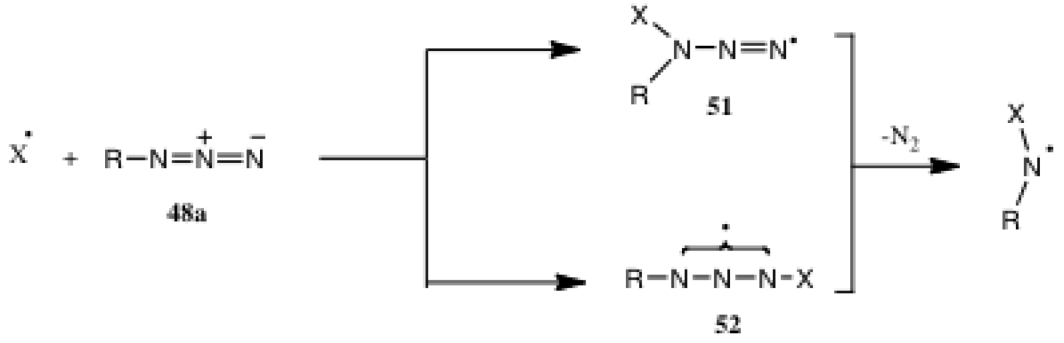


Figure 17[82]**.** Formation of aminyl radicals from homolytic addition of radicals to aliphatic and aromatic azides

Recently, reactions of aliphatic azides and alkyl radicals have been gaining interest as a result of their utility in the synthetic routes of *N*-heterocycles.[82,87,91,92] Carbon radicals are usually not very reactive species toward aliphatic and aromatic azides. However, carbon radicals including alkyl, vinyl, aryl, and acyl radicals, can undergo intramolecular additions onto aliphatic and aromatic azides affording six- and/or five-

membered cyclic aminyl radicals that can be further converted into various *N*-heterocycles. [82,93,96]

One of the very first evidence of the reaction between the carbon radical and an azide group was the intramolecular addition of 2'-azido-2-biphenylyl radical **53**, onto an aromatic azide yielding the cyclic carbazolyl radical **54**. However, despite such discovery, studies involving aryl radical cyclization with aryl azides have not gained much interest (Figure 18).[82,97]

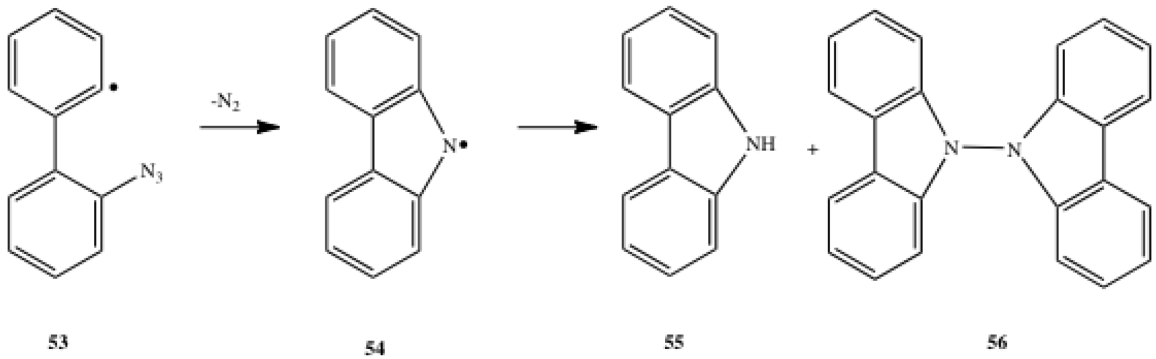
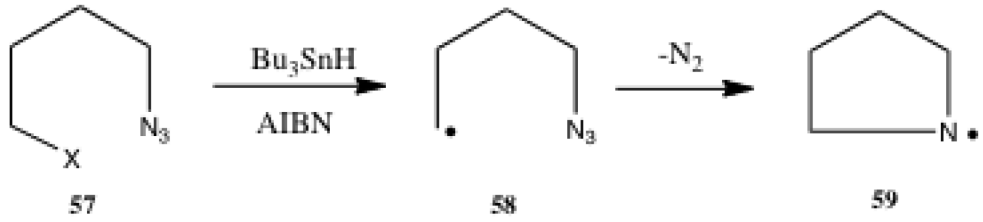


Figure 18. Cyclization of aryl radicals onto aromatic azides

In 1994, Kim reported the intramolecular addition of alkyl radicals to alkyl azides via the intermediate aminyl radical **59** (Scheme 2). The alkyl radical **58** was generated by treatment of aliphatic bromides and iodides bearing an azido substituent **57** with tributyltin hydride (Bu_3SnH) and azobisisobutyronitrile (AIBN) in refluxing benzene.[91] There have been reports of 1-hydroxy-1-methylethyl radicals ($Me_2C^{\bullet}OH$) reacting with sulphonyl, aryl, and acyl azides to form the substituted aminyl radicals.[93,98] Surprisingly, $Me_2C^{\bullet}OH$ was also found to react with primary alkyl azides to yield dialkylaminyl radicals ($H_3CN^{\bullet}CH_3$), while no reaction occured with t-butyl azide.[94]



Scheme 2. Intramolecular addition of alkyl radical to alkyl azide

The *t*-butoxyl radicals **60**, which were formed by thermolysis of di-t-butyl hyponitrite, also were found to react with alkyl azides to form iminyl radicals. On the basis of the EPR data, the iminyl radicals **61** were observed during the reaction of the *t*-butoxyl radical and alkyl azide in t-butylbenzene or cyclopropane (Figure 19).

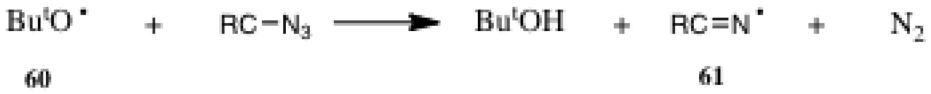


Figure 19. Reaction of t-butoxyl radical with alkyl azide

Although very few references in azide chemistry involve tin chemistry, the radical-mediated reduction of azides to amines using tributylstannane in the presence of AIBN as the radical initiator is a major contribution.[99] The method was first applied to the conversion of acyl azides to amides. However, the reaction of stannyl radicals ($Bu_3Sn\bullet$) with alkyl azides was also reported to be very efficient. The reduction was proposed to proceed via the generation of radicals **63a** or **63b**, follow by the loss of nitrogen to give the aminyl radical **64**. Further reduction of the aminyl radical afforded amines (Figure 20).[91,100]

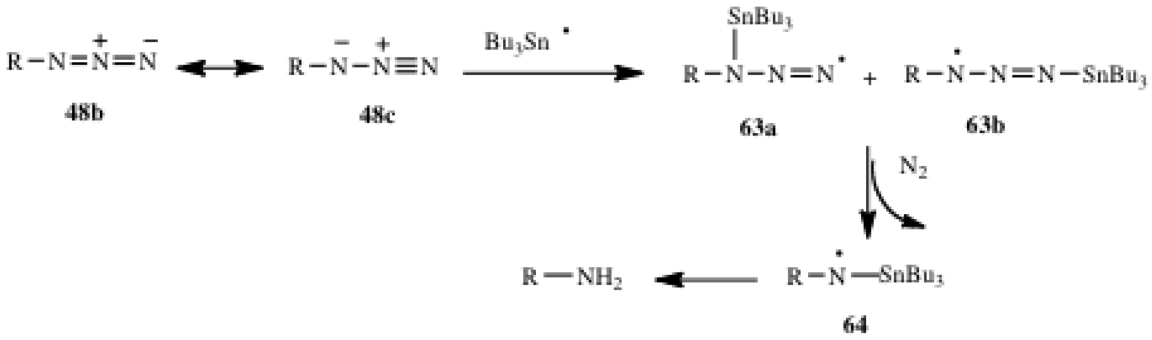
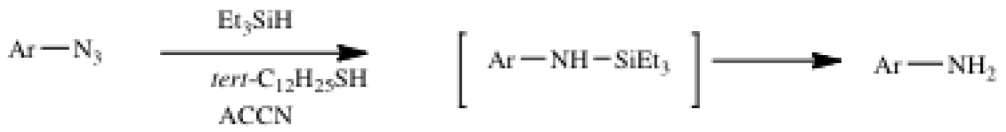


Figure 20. Reduction of alkyl azide with stannyl radicals to amines

Although radical chemistry involving alkyl azides is less developed, the reaction of silyl radicals with alkyl azides is well documented. In spite of the fact that tin chemistry using tributylstannane under radical conditions toward organic azides is inarguably successful and important, this application is limited by the known toxicity of organotin compounds, and also by the difficulty of full removal of tin residues from reaction mixtures.[87,101] Therefore, to replace the toxic tin with the nontoxic organosilicon and organogermanium is of great interest. Triorganogermanium hydride (Bu_3GeH) was discovered to efficiently reduce aryl azides;[102] though no example to our knowledge has been reported of its application toward alkyl azides. In 2006, it was reported by Benati and coworkers that aromatic azides in the presence of triethylsilane and a radical initiator with the addition of catalytic amounts of *tert*-dodecanethiol in toluene were successfully reduced to corresponding anilines in quantitative yields (Scheme 3).[87] Interestingly, without *tert*-dodecanethiol, triethylsilane alone was not capable of reducing the aryl azides under radical conditions because of the inhibition of the chain reaction caused by inefficient H-transfer from the nucleophilic silane to the intermediary *N*-silylaminyl radicals, which are nucleophilic as well. The method reported by Benati did not apply to alkyl azides.



Scheme 3. Reduction of aromatic azide with *t*-dodecanethiol in the presence of triethylsilane

In 2007, the Chatgilialoglu group reported that tris(trimethylsilyl)silyl radicals reduced alkyl azides to the corresponding amines in the presence of 2-mercaptoethanol as the radical carrier and ACCN as the radical initiator.[103] Unlike the typical radical reactions which are performed in organic solvents such as benzene and toluene, the reported methodology also was successful in water. The addition of the amphiphilic 2-mercaptoethanol was also the key to the reaction, since no reaction was observed without its presence. The mechanism, which involved the formation of a silyl-substituted aminyl radical by the addition of silyl radicals to the azide function, following by the loss of nitrogen is shown in Figure 21. The thiol acted as the hydrogen atom donor to give the silylamine and the thiyl radical, which in turn reacted with $(TMS)_3SiH$ to regenerate the silyl radical and the thiol. The mechanistic chain followed with the hydrolysis of the silylamine in water to afford the final product as a primary amine.

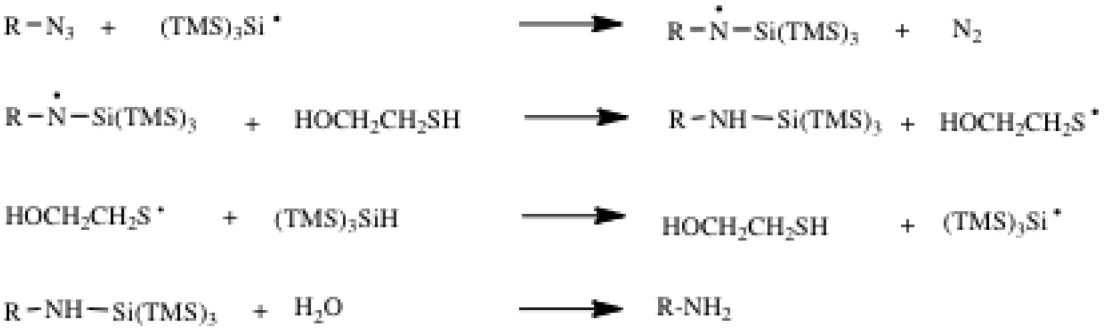


Figure 21. Reduction of alkyl azide by silyl radical

1.3.2 Reduction of alkyl azides by thiols

Thiols such as dithiolthreitol, glutathione, and 2-mercaptoethanol have demonstrated their ability to reduce alkyl azides under nonphysiological conditions.[104,105] and aryl azides under physiological conditions.[106] Furthermore, 3'-azido-3'-deoxythymidine was reduced by thiols to 3'-amino-3'-deoxythymidine under physiological conditions (pH 7.2 and at 37^{o}C in water).[104,107] The second-order rate constants for the reduction were reported to be 2.77 x 10^{-3}, 6.55 x 10^{-5}, and 6.35 x 10^{-6} $M^{-1}s^{-1}$, for the dithiothreitol, glutathione, and mercaptoethanol, respectively. According to these rate constants, the reduction happens faster with dithiothreitol, and slowly with glutathione and mercaptoethanol.

2. RESEARCH OBJECTIVES

Mechanism-based inhibitors of ribonucleotide reductases (RNRs) such as 2'-azido-2'-deoxyuridine-5'-diphosphate (N_3UDP) have provided insight into the mechanism of reduction of the natural nucleotides into 2'-deoxynucleotides. Experiments with ^{13}C, ^{15}N and ^{17}O labeled N_3UDP established a mechanism of the RNR inhibition which proposed an azide loss from the initial C3' radical to generate 2'-deoxy-3'-ketonucleotide. Subsequent addition of the initial sulfinylimine radical, formed from the reaction of HN_3 with Cys225-based thiyl radical, to 3'-keto group, generated the elusive nitrogen-center radical, and provided the first evidence for the trapping of 2'-deoxy-3'-ketonucleotides in the reduction process (Figure 6-8). Herein, I design a biomimetic modeling of the nitrogen-centered radical postulated to occur during the inactivation of the RNR by azidonucleotides, which will allow us to understand better the mechanism of the inhibition of RNR by N_3NDPs.

The purpose of this dissertation was planned to be executed in several steps. First, the design of model compounds (**65-70**) that have all the attributes which upon the generation of the thiyl radical can mimic the environment of the mechanism proposed for the inactivation of RNR by 2'-azido-2'-deoxynucleotides was completed. Model compounds as modifications of adenosine or thymidine will all share an azido group at C3', and a thiol or dithiol functionality at either C2' or C5' (Figure 22). Secondly, the theoretical calculations will be performed in order to check the feasibility of the ring closure reaction between the thiyl radical and azide group within the model compounds (Figure 23). Thirdly, the designed model compounds (**65-70**) will be synthesized.

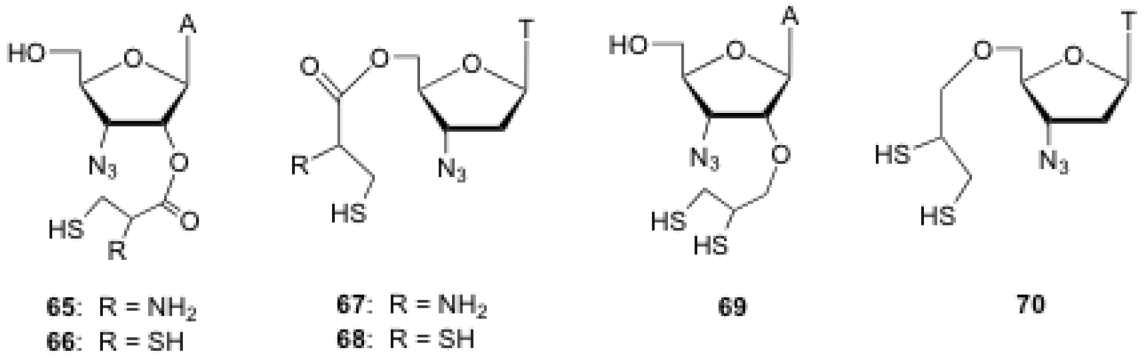


Figure 22. Selected 3'-azido nucleosides with thiol or vicinal disulfide groups attached at the 2'- or 5'-OH via an ester or ether linkage

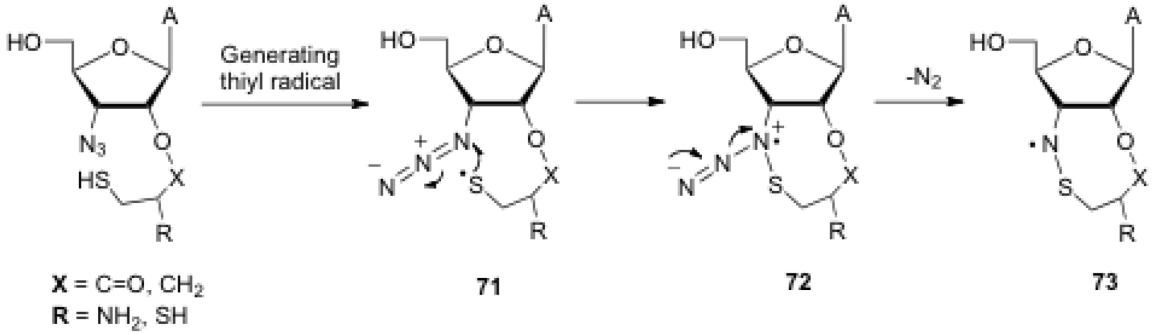


Figure 23. Plausible pathway of the intramolecular reactions between the thiyl radical and azide

Biomimetic studies involving the generation of the thiyl radical at physiological conditions will be performed to study the interaction between the generated thiyl radicals and the alkyl azide. The stability of the model compounds will first be examined, especially the azido group, under the physiological conditions, using 2,2'-azobis-(2-methyl-propionamidine)-dihydrochloride (AAPH) in water at a relatively low temperature of 37 ^{o}C, to selectively generate a thiyl radical. The interaction between the generated thiyl radical and azide will then be monitored by ^{1}H NMR spectroscopy. Also, biomimetic studies involving the generation of the alkyl thiyl radical by gamma

irradiation will be followed to examine the interaction between the alkyl thiyl radicals and the alkyl azide. Finally, the nitrogen-centered radical species postulated to occur during the reaction between the thiyl radical and azido group will be analyzed by electron paramagnetic resonance spectroscopy (EPR). Characterization of the aminyl radical species formed during one electron attachment to the azido group of 2'-azido-2'-deoxyuridine and its stereospecifically labelled 1'-, 2'-, 3'-, 4'- or 5,6-[$^2H_2$]-analogues will also be a target for study of the transient sugar-based radicals suggested to occur during the inhibition of RNR by 2'-azido-2'-deoxynucleotides. Understanding the mechanism of the formation of the nitrogen-centered radical during the inactivation of RNR by azidonucleotides may help to understand the mechanism of action of RNR and provide key information necessary for the development of the next generation antiviral and anticancer drugs.

3. RESULTS AND DISCUSSION

3.1 Design of 3'-azido-3'-deoxynucleosides with thiol or dithiol vicinal moieties

As discussed in the Introduction, experiments with ^{13}C, ^{15}N and ^{17}O labeled N_3UDP established the mechanism for the inhibition of RNR by N_3NDPs which involves an azide loss from the C3' radical to generate 2'-deoxy-3'-ketonucleotide. Subsequent addition of the initial sulfinylimine radical **14** (see Figure 6-7), formed from the reaction of HN_3 with Cys225-based thiyl radical, to 3'-keto group provided the first evidence for the trapping of 2'-deoxy-3'-ketonucleotides in the reduction process. In a theoretical modeling study by Pereira and coworkers[36] an alternative pathway for the formation of the identical nitrogen-center radical **16** was proposed via direct addition of the released azide ion to 3'-ketonucleotide (Figure 8). 3'-Azido-3'-deoxynucleosides bearing a cysteinyl or vicinal disulfide substituent attached to C2' or C5' were designed to mimic the interaction between the thiyl radical **21** and the azido group, which were hypothesized to produce the nitrogen-center radical **16**.

Taking into consideration the mechanism for the inhibition of azidonucleotides by RNR presented in Introduction (Figure 6-8), I designed a series of nucleoside derivatives with the azido group attached at C3' and thiol/vicinal dithiol attached to either C2' or C5'. I envisioned that inducing an intramolecular reaction between either the alkyl thiyl radical or radical disulfide anion and the alkyl azido group would lead to the formation of the aminyl radical, which would parallel the reaction postulated to occur during inhibition of RNR by N_3NDP. Compounds **65-70** were designed to have the azide moiety at C3' and a thiol functionality either at C2' or C5', which are modifications of adenosine or thymidine bearing a cysteinyl or dithiol functionality connected via an ester and/or ether

linkage. A cysteinyl chain with a free thiol group (**65**, **67**) might mimic the cysteine residue C225 that participates in the formation of **16**. Vicinal disulfide groups (as in **66**, **68**) are meant to mimic the two cysteine residues C225 and C462 that are proposed to play an important role in the reduction of ketonucleotides as the disulfide radical anion in the mechanism of RNR. The nitrogen centered radical was proposed to be formed from the interaction between a thiyl radical and the azide, but the involvement of another cysteine molecule is also possible. The difference in the ester and the ether linkage is purely for the stability of the compounds. The ether linkage was designed to improve the stability by avoiding a possible breakage/hydrolysis of the ester group that bears the thiol or vicinal dithiol functions.

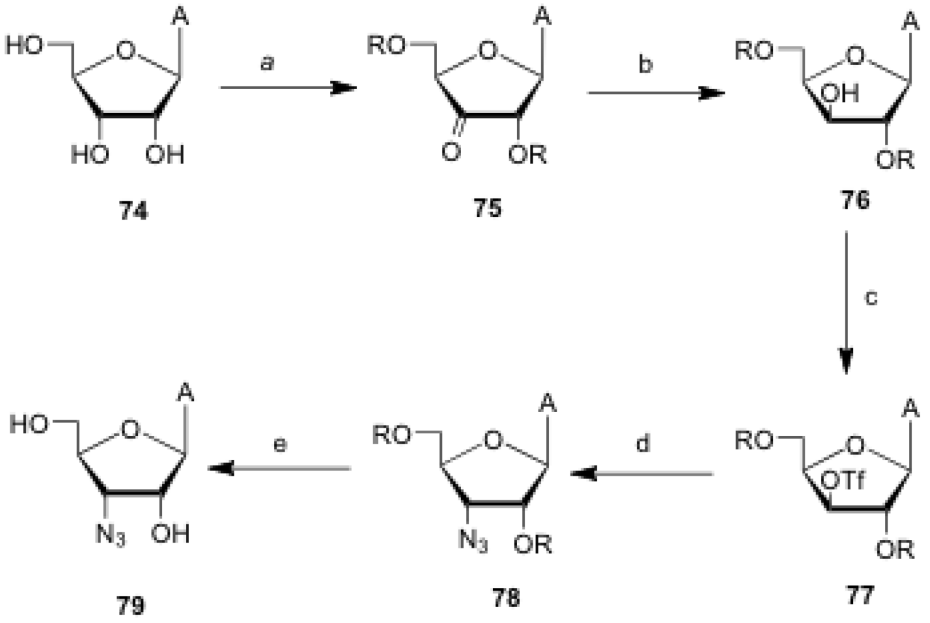
Upon generating the thiyl radical from the model compounds, it is hypothesized that an interaction between the thiyl radical and the azido moiety would occur. The plausible mechanism involves the ring closure, which would result in the loss of nitrogen and generate the nitrogen centered radical species (Figure 23).

3.1.1 Synthesis of 3'-azido-3'-deoxyadenosine with a linkage at C2'

3.1.1.1 Preparation of 3'-azido-3'-deoxyadenosine

The 3'-azido-3'-deoxyadenosine analogues with modifications at C2' were prepared starting from 3'-azido-3'-deoxyadenosine **79**. The traditional synthesis pathway[108] was followed by first selectively protecting adenosine **74** at C5' and C3' with *tert*-butyldimethylsilylchloride in pyridine (Scheme 4). Subsequent oxidation using pyridinium chromate complex afforded unstable 3'-ketoadenosine **75**. Reduction with sodium borohydride in acetic acid afforded xylofuranosyl adenosine **76** with inversion of configuration of the hydroxyl group on C2'. Subsequent triflation of **76** with

trifluorosulfonylchloride afforded **77** generating a good leaving group to undergo nucleophilic substitubtion with sodium azide in *N,N*-dimethylformamide to give protected azido nucleoside **78**. Subsequent desilylation of **78** with tetrabutylammoniumchloride in tetrahydrofuran afforded **79** as a white solid. Instead of employing this traditional pathway, which involved the time-consuming six-steps synthesis, I also attempted the synthesis of **79** in fewer steps. Thus, addition of azide to ketosugars and ketonucleosides using lithium azide in the presence of chlorotrimethylsilane was first attempted. The Mitsonobu reaction[109] was also applied to add azide directly to ribonucleosides; however the reaction yielded only undesired side products.

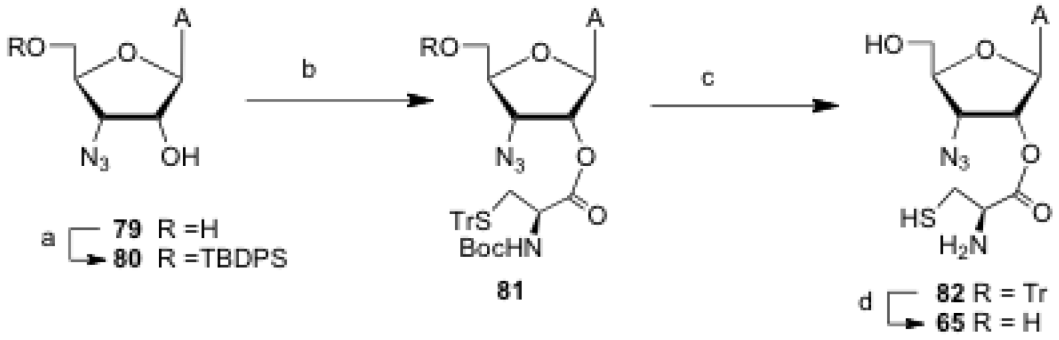


Reagents: (a) (i) t-BDMSCl/py, (ii) CrO_3, py, Ac_2O; (b) $NaBH_4$/AcOH; (c) TfCl/DMAP/CH_2Cl_2; (d) NaN_3/DMF; (e) TBAF/THF.

Scheme 4. Synthesis of 3'-azido-3'-deoxyadenosine**.**

3.1.1.2 Synthesis of 3'-azido-3'-deoxy-2'-*O*-cysteinyladenosine

Silylation of **79**[108] with *tert*-butyldiphenylsilyl chloride (TBDPSCl) gave **80** (Scheme 5)**.** Standard condensation of **80** with *N*-Boc-*S*-trityl-cysteine with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) as the activator in the presence of 4-dimethylaminopyridine (DMAP)[110] afforded **81** (80%). Treatment of **81** with TFA/H_2O[111] effectively removed the silyl and Boc protection groups to give **82**, but protic acids *per se* were found ineffective for cleavage of the trityl thioether.[112] The *S*-detritylation was also unsuccessful upon treatment with Hg salts.[112] I found, however, that treatment of **82** with TFA in the presence of Et_3SiH[113] cleanly removed the *S*-trityl group affording **65** (80%). It is noteworthy that the azido group was not affected[114] by Et_3SiH employed in the *S*-detritylation step, although reduction of the azido group to the primary amino group by more reactive radical-based reducing agent $(Me_3Si)_3SiH$ is known.[103] Compound **65** was found to be prone to oxidation to the corresponding disulfide and/or cleavage of the cysteinyl ester bond at the 2' position during silica gel column purification. The thiol group in **65** could approach the azido group from the α face via a non-constrained eight-membered ring, which might mimic the interaction between Cys225 of the enzyme and the azide group at C3' (e.g., **21** → **16**, Figure 8).

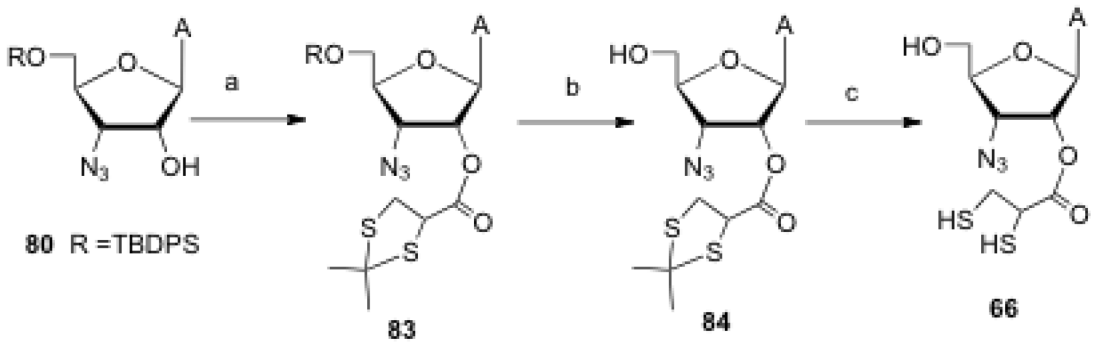


Reagents: (a) TBDPSCl/py; (b) *N*-BOC-*S*-tritylcysteine/EDCI/DMAP/CH_2Cl_2; (c) TFA/H_2O; (d) TFA/Et_3SiH/CH_2Cl_2

Scheme 5. Synthesis of 3'-azido-3'-deoxy-2'-*O*-cysteinyladenosine.

3.1.1.3 Synthesis of 3'-azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropanoyl)adenosine

Condensation of **80** and 2,3-*S*-isopropylidene-2,3-dimercaptopropionic acid (vide infra) with EDCI as the activator in the presence of DMAP[110] afforded **83** (99%, Scheme 6). The removal of the isopropylidene group from **84** to yield **66** although challenging, was achieved by the generation of the mercuric salt complex with $HgCl_2$ followed by treatment with hydrogen sulfide.[115] Thus, selective 5'-*O*-desilylation (TFA/H_2O)[111] of **83** and subsequent deacetonization ($HgCl_2$/H_2S/MeCN/H_2O)[115] of **84** afforded **66** (55%). Thiyl radicals generated from **66** might interact with the azido group via seven- (S● at Cα) or eight-membered transition states (S● at Cβ). Analogue **66** with a vicinal dithiol moiety could mimic the Cys225/Cys462 pair that is oxidized to a cysteine disulfide complex during the catalytic cycle of RNR. Also, the absence of a reactive α-amino C-H bond in **66**, as opposed to cysteine containing analogues (e.g., **65**), which were showed to be reactive with thiyl radicals,[116,117] could prove to be more selective.

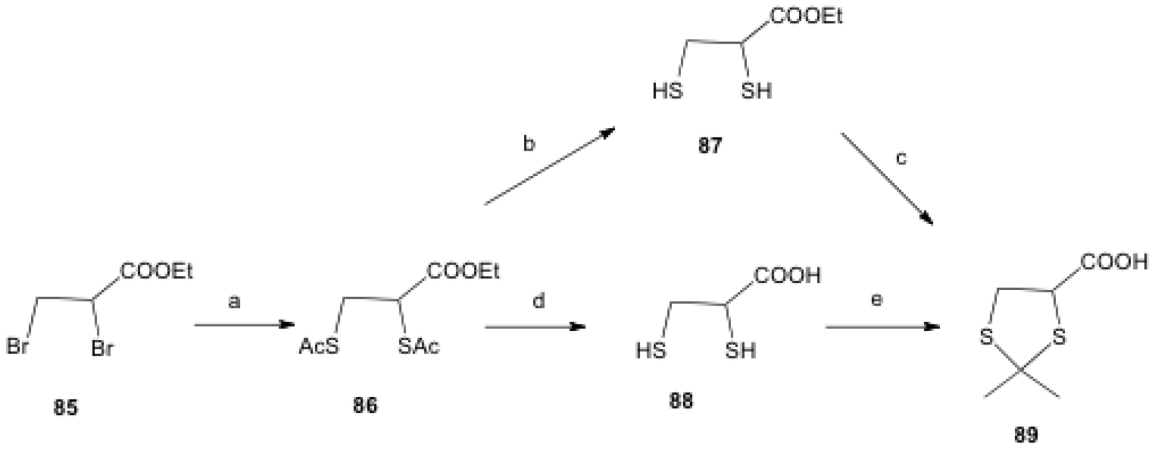


Reagents: (*a*) 2,3-S-isopropylidene-dimercaptopropionic acid/EDCI/DMAP/CH_2Cl_2; (*b*) TFA/H_2O; (*c*) (i) $HgCl_2$/MeCN/H_2O, (ii H_2S/MeOH

Scheme 6. Synthesis of 3'-azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropanoyl)adenosine

Surprisingly, practical procedures for the synthesis of 2,3-*S*-isopropylidene-2,3-dimercaptopropionic acid **89** (Scheme 7) were either not available in the literature or were lacking critical experimental data. Compound **89** was first prepared from ethyl 2,3-dibromopropionate **85** in four steps: (i) nucleophilic substitution of bromides with thiolacetic acid; (ii) acid-catalyzed deacetylation; (iii) acetonization and (iv) saponification.[118,119] Thus, the substitution of bromides with thiolacetates by the reaction of **85** with thiolacetic acid in the presence of triethylamine afforded ethyl 2,3-bis(acetylthio)propanoate **86** in good yield (98%) with sufficient purity without purification by chromatography. Acid-catalyzed *S*-deacetylation of **86** by dry HCl in anhydrous ethanol at ambient temperature provided ethyl 2,3-dimercaptopropanoate **87**; however, this reaction required 3 days for completion and gave low yield (35%). Acetonization and saponification of **87** were then performed to afford **89** in sufficient yield and purity by using (i) dry HCl and acetone at ambient temperature for 12 h and (ii) aqueous sodium hydroxide (1M) in ethanol. Later a different method to synthesize **89**

from **85** in better yield was developed, which was also less time-consuming. Thus, refluxing **86** in aqueous HCl (1 M) for 24h affected both deacetylation and hydrolysis of the ester to afford free acid **88** in very good overall yield (99%). Subsequent standard acetonization of **88** in acetone in the presence of HCl afforded **89**.



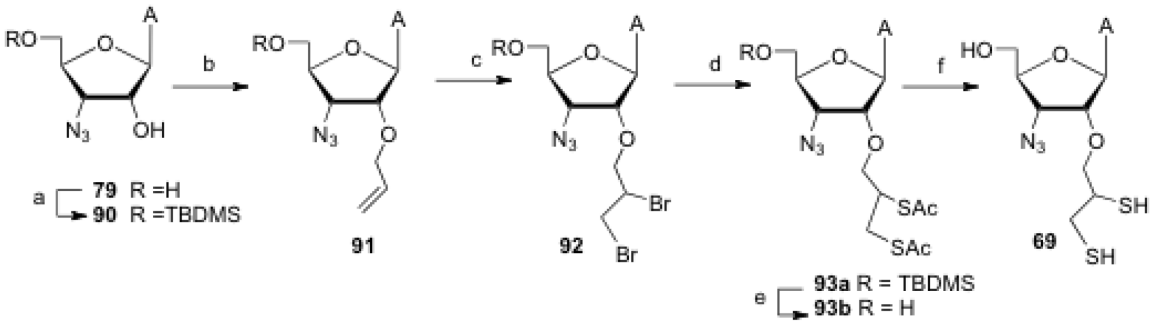
Reagents: (a) TEA/thiolacetic acid/EtOH; (b) dry HCl/EtOH; (c) (i) dry HCl/acetone, (ii) 10% NaOH/EtOH; (d) HCl (1M); (e) conc. HCl/acetone

Scheme 7. Synthesis of 2,3-*S*-isopropylidene-2,3-dimercaptopropionic acid

3.1.1.4 Synthesis of 3'-azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropyl)adenosine

To increase the stability of the linker through which the thiol group is attached to 2' hydroxyl group, model compounds with 2'-*O*-alkyl linker were also synthesized (e.q. **69**). Thus, careful treatment of **90** with allyl bromide and NaH[120] gave 2' monoallylated product **91** although only in 48% yield. Attempted allylation of **90** with allyl ethyl carbonate in the presence of Pd(0) catalyst gave, as expected,[110] a mixture of the 6-*N* and 2'-*O* allylated products. Bromination[121] of **91** (Br_2, -50°C) yielded dibromo compound **92** (90%) as a 1:1 mixture of diastereoisomers. Treatment of **92** with potassium thioacetate[119] (DMF, 3 days) gave the bis(acetylthio) derivative **93** (98%). The attempted

reaction of **92** at low temperature with NaHS resulted in the formation of the thiirane mixture(s), among other byproducts. Deprotection of the **93** was accomplished in a two-steps procedure. Thus, desilylation with TFA/H_2O[111] followed by subsequent *S*-deacylation[122] with NaOH/EtOH gave the desired dithiol **69** (Scheme 8). It is also noteworthy that attempted deacylation with NH_3/MeOH failed to yield dithiol **69** producing instead a complex reaction mixture.



Reagents: (a) TBDMSCl/py; (b) NaH/allyl bromide/DMF; (c) Br2/CHCl3; (d) KSac/DMF; (e) TFA/H_2O; (f) (i) NaOH/MeOH, (ii) HCl/H_2O.

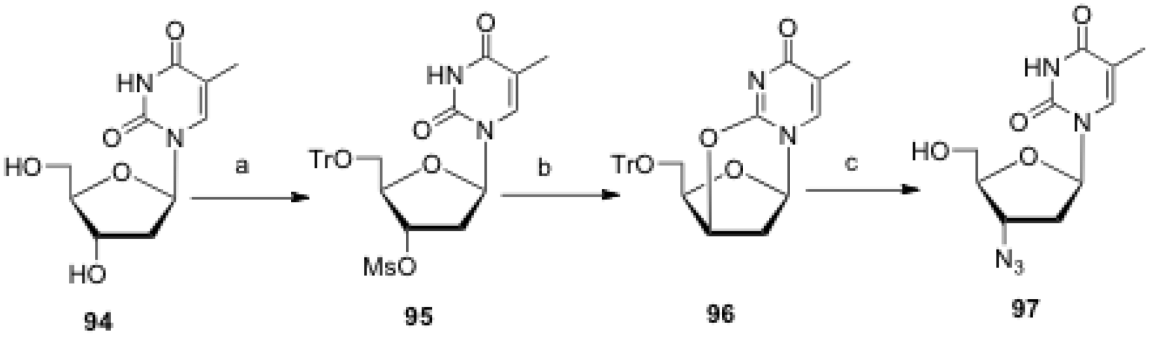
Scheme 8. Synthesis of 3'-azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropyl)adenosine

3.1.2 Synthesis of 3'-azido-3'-deoxythymidine with modifications at C5'

3.1.2.1 Preparation of 3'-azido-3'-deoxythymidine

The 3'-azido-3'-deoxythymidine **97** (AZT) was prepared from thymidine **94** in several steps following literature reports. [AZT, also called Zidovudine, is used for treatment of HIV positive patients. Its mode of action is either by its anti-human telomerase reverse transcriptase activity or being a radiosensitizer in irradiated tumor cells.[123-125]] Thus, one-pot 5'-*O*-tritylation and 3'-*O*-mesylation of **94** with trityl chloride and methanesulfonyl chloride in dry pyridine afforded protected thymidine **95**.[126] Cyclization of **95** to 2,3'-*O*-anhydrothymidine **96** was accomplished following the Fox and Miller method[127]. Hence, refluxing **95** in a mixed solution of 90% ethanol and 1 N

sodium hydroxide achieved the cyclization. However, the time required for the completion of the reaction was very critical. Exactly six minutes of reflux provided the product **96** in good yield. Less than six minutes of reflux gave unreacted starting material while more than six minutes resulted the undesired hydrolysis to xylo analogue. Azidation[128] of **96** by the addition of sodium azide in DMF in the presence of water under reflux condition and subsequent detritylation with 80% acetic acid afforded 3'-azido-3'-deoxythymidine **97**.

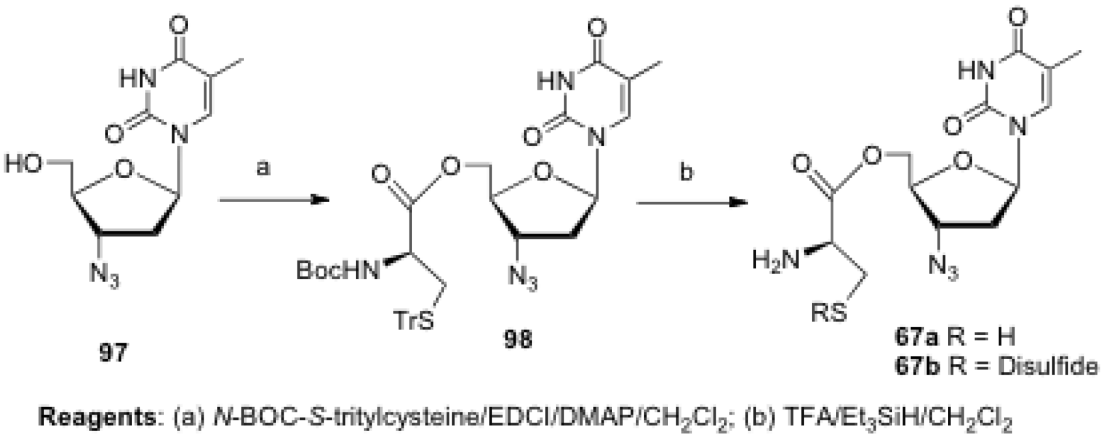


Reagents: (a) (i) TrCl/py, (ii) MsCl; (b) NaOH/EtOH; (c) (i) NaN_3/DMF, (ii) 80% AcOH

Scheme 9. Synthesis of 3'-azido-3'-deoxythymidine

3.1.2.2 Synthesis of 3'-azido-5'-*O*-cysteinyl-3'-deoxythymidine

Condensation[110] of AZT **97** with *N*-Boc-*S*-trityl-cysteine gave **98** in good yield (79%). Successive treatment with TFA/Et_3SiH[113] affected removal of the Boc and *S*-trityl protection groups to give desired nucleoside **67a** in one step (85%). Compound **67a** slowly oxidizes to the corresponding disulfide **67b** while exposing to the air during purification. [Compound **67b** is more polar than **67a** on TLC; **67b** is shifted downfield by Δ 0.18 ppm for Hβ,β' as compared to **67a** on ^{1}H NMR; see experimental]

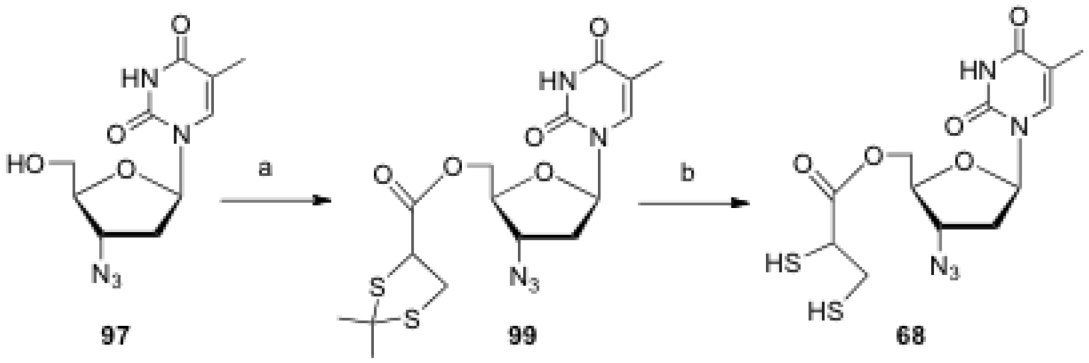


Reagents: (a) *N*-BOC-*S*-tritylcysteine/EDCI/DMAP/CH_2Cl_2; (b) TFA/Et_3SiH/CH_2Cl_2

Scheme 10. Synthesis of 3'-azido-5'-*O*-cysteinyl-3'-deoxythymidine

3.1.2.3 Synthesis of 3'-azido-3'-deoxy-5'-*O*-(2,3-dimercaptopropanoyl)thymidine

The 3'-azido-3'-deoxythymidine analogue **68** with *vicinal* disulfide function attached at C5' via an ester linker was prepared from compound **97** analogously to the procedure described above for **67a**. Shortly, condensation of **97** with 2,3-*S*-isopropylidene-2,3-dimercaptopropionic acid **88** afforded **99** in 95% yield as a mixture of diastereomers (1:1.3; Scheme 11). Deprotection of **99** with a mercuric salt complex followed by treatment with hydrogen sulfide provided **68** with good overall yield. The thiyl radical generated from **67a**/**67b** could add to the azido group via a nine-membered intermediate, whereas thiyl radical(s) from **68** could add via an eight- (S● at Cα) or nine-membered TS (S● at Cβ). These AZT analogues do not have a hydroxyl group at C2', and have a closer electronic resemblance to the 2'-deoxy-3'-ketonucleotide intermediates generated during inhibition of RNR by N_3UDP. The addition of thiyl radicals at C6 of the thymine[129,130] was not expected.

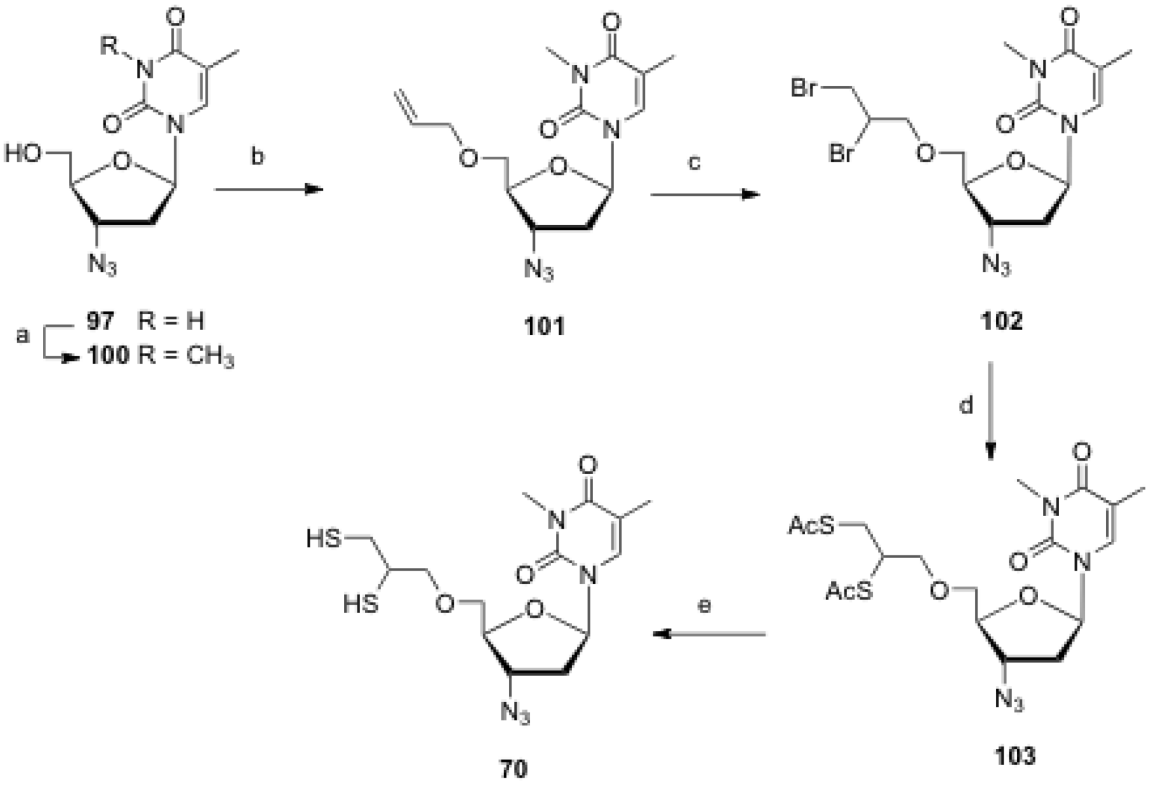


Reagents: (*a*) 2,3-S-isopropylidene-dimercaptopropionic acid/EDCI/DMAP/CH_2Cl_2; (*b*) (i) $HgCl_2$/MeCN/H_2O, (ii) H_2S/MeOH

Scheme 11. Synthesis of 3'-azido-3'-deoxy-5'-*O*-(2,3-dimercaptopropanoyl)thymidine

3.1.2.4 Synthesis of 3'-azido-5'-*O*-(2,3-dimercaptopropyl)-3'-deoxy-*N*-methylthymidine

To increase the stability of the linker through which thiol group is attached to 5' hydroxyl group, AZT analogue **70** with 5'-*O*-alkyl linker was synthesized. In principle, a four-step procedure, which was developed for the synthesis of adenosine analogue **69** was followed: allylation, bromination, thioacetylation, and *S*-deacetylation. However, attempted allylation of **97** by treatment with allyl bromide and sodium hydride produced uracil *N*-allylation instead of 5'-*O*-allylation. To avoid such competitive N-3 allylation, 3'-azido-3'-deoxythymidine **97** was 3-*N*-methylated with diazomethane to afford **100** (Scheme 12). Treatment of **100** with allyl bromide in the presence of sodium hydride as a base did not achieve allylation of at 5'-*O*-position of **100**. However, reaction of **100** with allyl bromide in the presence of 18-crown-6 and potassium hydroxide successfully afforded 5'-*O*-allyllated nucleoside **101**. Subsequent procedures, as described for **69**, were then followed to afford thymidine analogue **70**.



Reagents: (a) CH_2N_2/EtOH; (b) KOH/18-crown-6/allyl bromide; (c) $Br_2/CHCl_3$; (d) KSAc/DMF; (e) (i) NaOH/MeOH, (ii) HCl/H_2O.

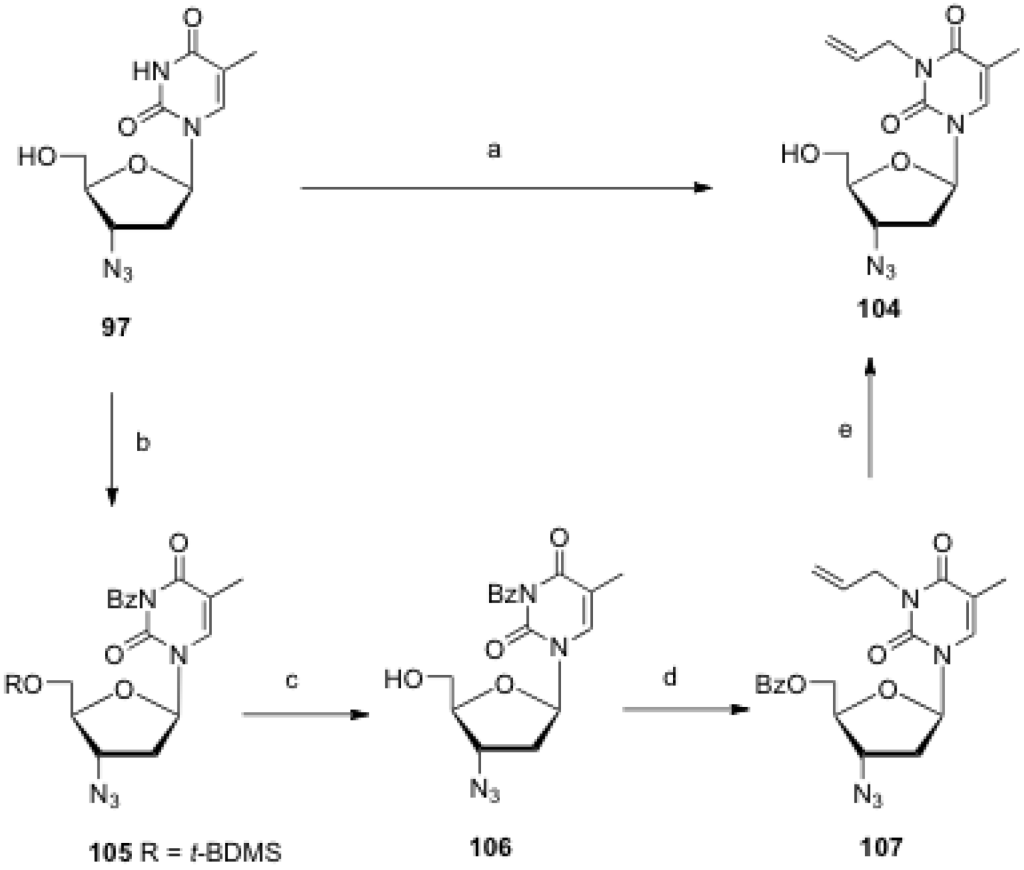
Scheme 12. Synthesis of 3'-azido-5'-*O*-(2,3-dimercaptopropyl)-3'-deoxy-*N*-methylthymidine

3.1.2.5 Developing selective *O*-allylation versus *N*-allylation for the pyrimidine nucleosides

Because our results on the selective 5'-*O*-allylation of 3'-azido-3'-deoxythymidine differ from the literature report, I had undertaken effort to further investigate regioselective allylation of pyrimidine nucleosides. As an alternative to the classical method of 5'-*O*-allylation of thymidine that involves several steps of protection and deprotection of the hydroxyl groups and the base, Zerrouki et. al.[131] reported a procedure using microwave or ultrasound activation that selectively acheive 5'-*O*-allylation in one

step with high yield. Following the method described by Zerrouki, allylation of 3'-azido-3'-deoxythymidine with allyl bromide in the presence of NaH in DMF was performed under both ultrasound and microwave (100 W) activation. However, ^{1}H NMR data showed a broad singlet peak at approximately 3.30 ppm that corresponds to a hydroxyl group. Under closer examination of the ^{13}C NMR data, the peak that corresponds to the Cα of the allyl was located at 43.34 ppm. According to literature, the Cα of the *O*-allyl should be located at approximately 73 ppm whereas the Cα of the *N*-allyl would be present at approximately 43 ppm. Therefore, it can be concluded that *N*-allylation occurred instead of 5'-*O*-allylation to give **104** (Scheme 13).

Classical methods as described in the literature[131] were then employed to produce the 5'-*O*-allyl-3'-azido-3'-deoxythymidine. The first step was the protection of the 5' primary hydroxyl group and of the N-3 aminyl group of thymine by selective silylation and benzylation, respectively. The silylated group was then removed by treatment with carbon tetrabromide in dry methanol to afford **106**. The structure of **106** was confirmed on the basis of the ^{1}H NMR and ^{13}C NMR data. The ^{1}H NMR data showed a broad singlet peak at approximately 3.20 ppm that corresponds to a hydroxyl group. The peak that corresponds to C5' in ^{13}C NMR spectra was located at 110.95 ppm, which is consistent with literature value for a carbon attached to a hydroxyl group. Allylation of **106** with NaH and allyl bromide in DMF was then performed. Interestingly, instead of 5'-*O*-allylation, compound **107** was produced as judged by our ^{13}C NMR data (the peak at 43.3 ppm for Cα of the allyl group attached to nitrogen atom was observed). Deacylation of **107** gave the idendical *N*-allylated product **104** according to ^{1}H and ^{13}C NMR data.



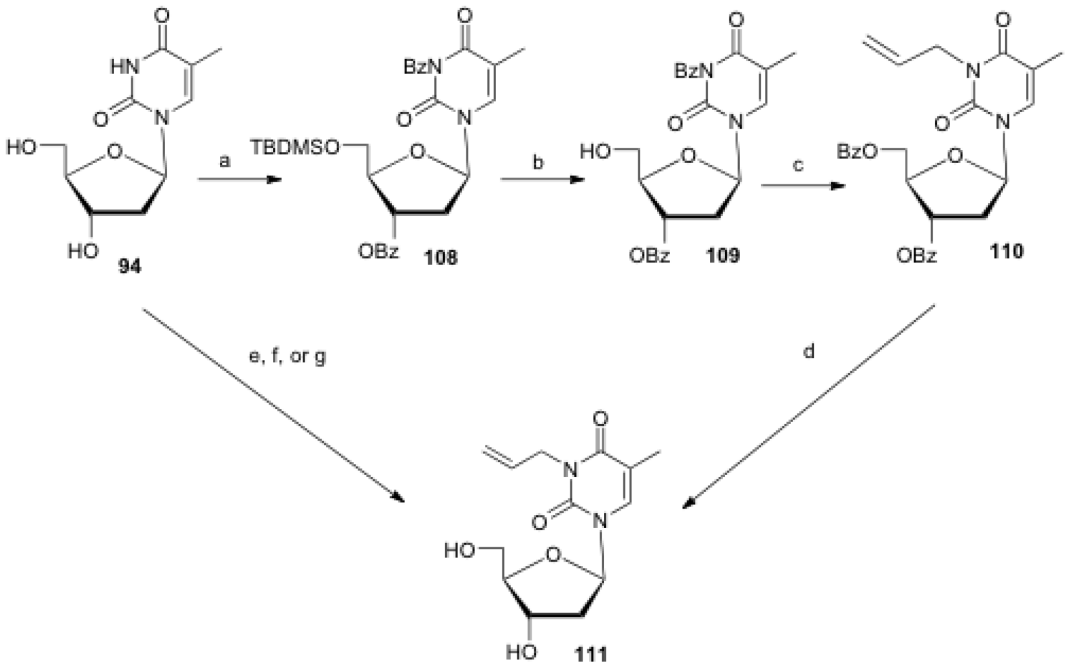
Reagents: (a) NaH/DMF/allyl bromide/ultrasound bath or microwave 100W; (b) (i) *t*-BDMSiCl/py, (ii) BzCl/DMAP; (c) CBr_4/MeOH; (d) NaH/DMF/allyl bromide; (e) NH_3/MeOH

Scheme 13. Allylation of 3'-azido-3'-deoxythymidine

Recently, Zhong et al.[132] reported successfully reproduction of Zerrouki's method of 5'-*O*-allylation using microwave activation at 40 ^{o}C with 2'-3'-*O*-isopropylideneuridine. Therefore, applications of ultrasound or microwave activation for the selective 5'-*O*-allylation was also studied. In order to understand the complete story of the regioselective 5'-*O*-allylation, allylation of thymidine **94**, 3'-azido-3'-deoxythymidine **97**, and 2'-3'-*O*-isopropylideneuridine **113** using both the classical method and the newly

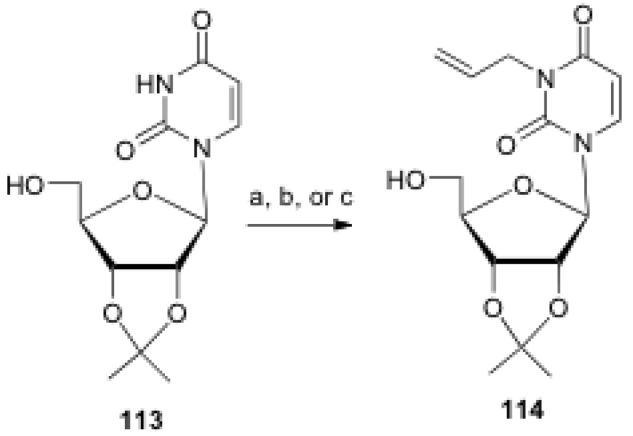
reported ultrasound and microwave procedures were performed following the methologies described by Zhong and Zerrouki.

Thus, regioselective 5'-silylation of thymidine, followed by benzoylation afforded 5'-tBDMS-3',N-dibenzoyl nucleoside **108**. Selective desilylation using carbon tetrabromide (CBr_4) in methanol gave thymidine analogue **109** with free 5'-hydroxyl. Allylation of **109** by classical method using stirring at ambient temperature for 30 min upon adding NaH and another hour after adding allyl bromide afforded **110**, which according to both ^{1}H and ^{13}C NMR data suggested *N*-allylation. Debenzoylation uisng methanoic ammonina afforded *N*-allyl-thymidine **111**. On the other hand, direct allylation of thymidine **94** was also studied using classical stirring, ultrasound and microwave activation. Allylation of **94** using classical stirring was accomplished by stirring the reaction mixture of thymidine and NaH in DMF at ambient temperature under N_2 for 30 min, following by stirring for another 4h after adding allyl bromide. Ultrasound methodology was followed by sonication of the reaction mixture for 30 min at ambient temperature before adding allyl bromide, in which stirring was continued in the ultrasound bath for another 4h. Microwave activation was performed at 100 W for 2 min upon adding NaH, and then continued for another 2 min after adding allyl bromide. All three conditions afforded identical product, which corresponded to *N*-allylated **111** by NMR data (Scheme 14). Allylation of 2'-3'-*O*-isopropylideneuridine **113** under classical stirring, ultrasound and microwave conditions also gave the *N*-allylated product **114** (Scheme 15).



Reagents: (a) (i)TBDMSCl/py, (ii) BzCl/DMAP; (b) CBr_4/MeOH; (c) (i) NaH/DMF, (ii) allyl bromide; (d) NH_3/MeOH; (e) (i) NaH/DMF/stirring; (ii) allyl bromide; (f) (i) NaH/DMF/ultrasound bath; (ii) allyl bromide; (g) (i)NaH/DMF/MW 100W; (ii) allyl bromide.

Scheme 14. Allylation of thymidine by classical methods, ultrasound and microwave activation.



Reagents: NaH/DMF/allyl bromide; (a) classical stirring; (b) ultrasound bath; (c) microwave 100W

Scheme 15. Allylation of 2'-3'-*O*-isopropylideneuridine

3.1.3 Attempted synthesis of other nucleoside derivatives

I have also attempted to synthesize nucleoside derivatives that have both the azido and hydroxyl group at C3'. For example, compound **115** is the closest analog to mimic the proposed intermediate in Ramos' mechanism for the inhibition of RNR by N_3UPD (**21**, Figure 8). This compound can be considered as an azidohydrin (at C3') and might be comparable to a cyanohydrin functional group. Cyanohydrins are normally formed by treatment of a ketone or aldehyde with hydrogen cyanide (HCN) in the presence of sodium cyanide as a catalyst.[133] [Cyanohydrins are stable, and act as important metabolites in cyanide detoxification processes in our body.[134]] In theory, analogous treatment of ketones with hydrazoic acid (HN_3) in the presence of sodium azide should produce the azidohydrin. However, such reaction is rather speculative and so far unknown in literature. The azidohydrin might be less stable than the cyanohydrin. Different pka values of HCN (9.3)[133] and HN_3 (4.6)[135] might be the reason for the sharp difference in reactivity.and stability between them. Attempts to react sodium azide with the simplified models such as 3-ketoisopropylidineglucose and protected 3'-ketothymidine were tried, but failed to produce the corresponding azidohydrin.

Synthesis of compounds **116** and **117**, which have the O-Si bond as a linker to the thiol moiety, were also attempted. Presumably, derivatives **116** and **117** would require 7 and 6-membered ring closure transition states, respectively, during the interaction between the generated thiyl radical and azide group. The procedure for the attempted synthesis of **116** or **117** involved several steps such as: (i) silylation of 3'-azidoadenosine with chloro(chloromethyl)dimethyl silane or dichlorodimethyl silane; (ii) treatment with potassium thioacetate; (iii) and *S*-deacetylation (Scheme 16). Instead

of testing such approaches with laborous 3'-azido-adenosine and AZT, the synthesis on 3',5'-disilylated adenosine was attempted. Following a standard silylation[108] procedure using pyridine as the solvent in the presence of 4-dimethylaminopyridine (DMAP) did not yield the desired products with the chloromethyldimethyl silane or chlorodimethyl silane attached to 2'-*O*-carbon. However, the treatment of chloro(chloromethyl)dimethyl silane with diethylamine in ether[136] was found to give the $ClCH_2SiMe_2NEt_2$, in which in its presence along with chloro(chloromethyl)dimethyl silane in ether successfully added the chlorosilyl chain to 2'-position. Treatment with potassium thiolacetate[119] effectively substituted chloro with thiolacetate. However, *S*-deacetylation[122,137] cleaved the O-Si bond and yielded the original protected adenosine.

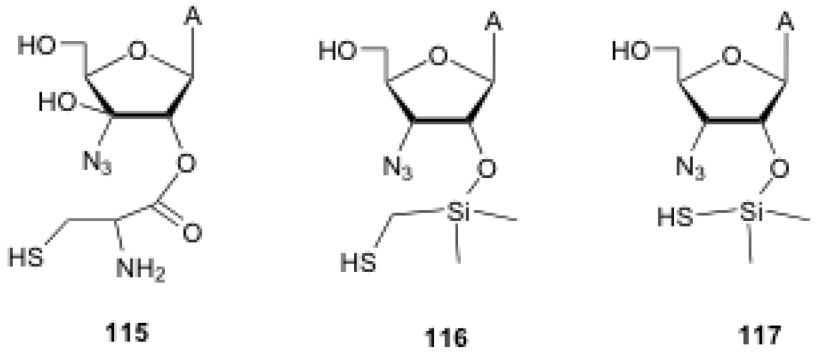
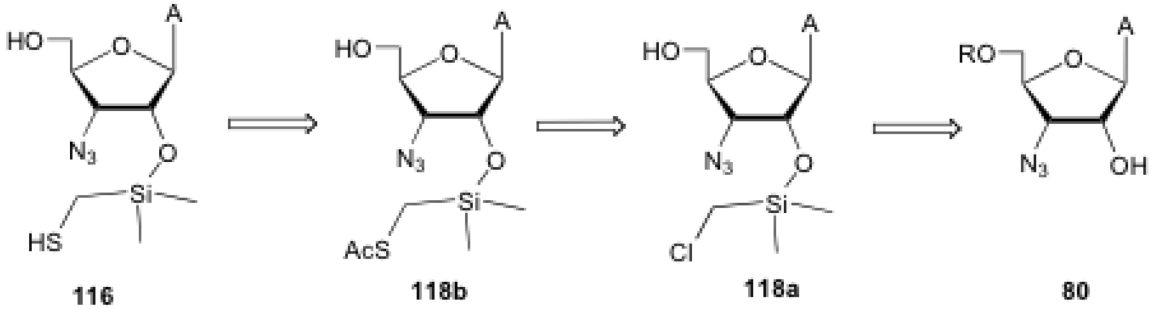


Figure 24. Other attempted model derivatives

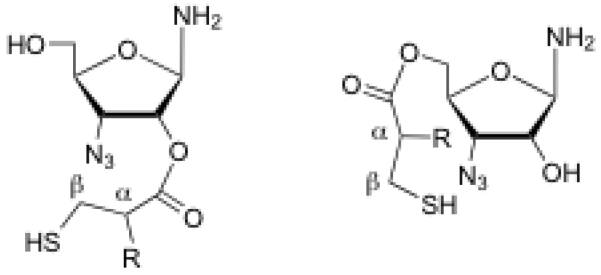


Scheme 16. Approach for the synthesis of 3'-azido nucleosides with the thiol moiety attached via O-Si bond linkage

3.2 Theoretical feasibility studies

3.2.1 General method

Density functional calculations of the reaction pathways were performed to understand the reaction mechanism of the ring closure accompanied by N_2 elimination (presented earlier in Figure 23). The method of calculations was the hybrid DFT B3LYP with the 6-31G* basis set, which provided a semiquantitative accuracy within 3-5 kcal/mol for the reaction energies and barrier heights. Model molecules obtained by replacement of the bulky heterocyclic ring with NH_2 group were used (Figure 25). The strategy involved: (a) finding an appropriate conformation of the open structure with a minimal S...N distance, (b) calculation the radical structures obtained by removal of H from the S-H group, (c) searching for transition states for the ring closure, and (d) calculation the closed-ring structures formed after elimination of N_2.



119 R = NH_2
121 R = SH

120 R = NH_2
122 R = SH

Figure 25. Model azido sugars with NH_2 at C1 positions instead of the heterocyclic ring

3.2.2 Feasibility of the ring-closure reaction between cysteine-derived thiyl radical and azido group

Nucleosides **65** and **67**, bearing the cysteinyl moeity at 2' and 5', respectively, were assummed to partake in a ring-closure reaction between the cysteine-derived thiyl

radical and azido group, via an 8 and 9-membered transition states, respectively (see Figure 23). The feasibility of such ring-closure reaction was studied using replaced cysteinate substrates **119** and **120** (Figure 25). The heterocyclic rings of adenine and thymine were replaced with the amine group to be cost effective. The calculations suggested that intramolecular reactions between thiyl radical and azido group are facile for both 2'- and 5'-*O*-cysteinate subtrates **119** and **120**. As shown in Table 1, the reaction was calculated to be exothermic by 33.6 to 40.6 kcal/mol and to have a low energy barrier of only 9.3 to 12.1 kcal/mol, which can be overcome at ambient temperature. The effect of the stereochemistry difference of the amino group on the cysteinyl moiety was also investigated. The transition state energy barrier and the total energy of the reaction were calculated to be very similar regardless of the stereochemistry at Cα.

	TS energy barrier (kcal/mol)	Energy (kcal/mol)
119 (*R* at C_α)	12.1	-35.6
119 (*S* at C_α)	10.4	-33.6
120 (*R* at C_α)	13.5	-37.8
120 (*S* at C_α)	11.7	-40.6

Table 1. Optimized (DFT B3LYP with the 6-31G*set) energies for the ring closure energy calculated for model substrates bearing a cysteinyl moiety

On the basis of the results, the ring closure reaction between the thiyl radical and azide of compounds **65** and **67** that required an 8 and 9-membered transition states was confirmed to be feasible. Figure 26 shows a molecular model of the ring closure reaction of **119** and **120** between the thiyl radical and azide through an 8 and 9-membered transition states with release of nitrogen, which represents the best approximation of the geometry of the transition state as well as the ring-closured product. Not only that the

reaction was exothermic with low activation energy ($E^{\#}$, see Table 1), but the distance between the N^{α} atom and the thiyl radical was also small (for example, 4.33 Å for **120**).

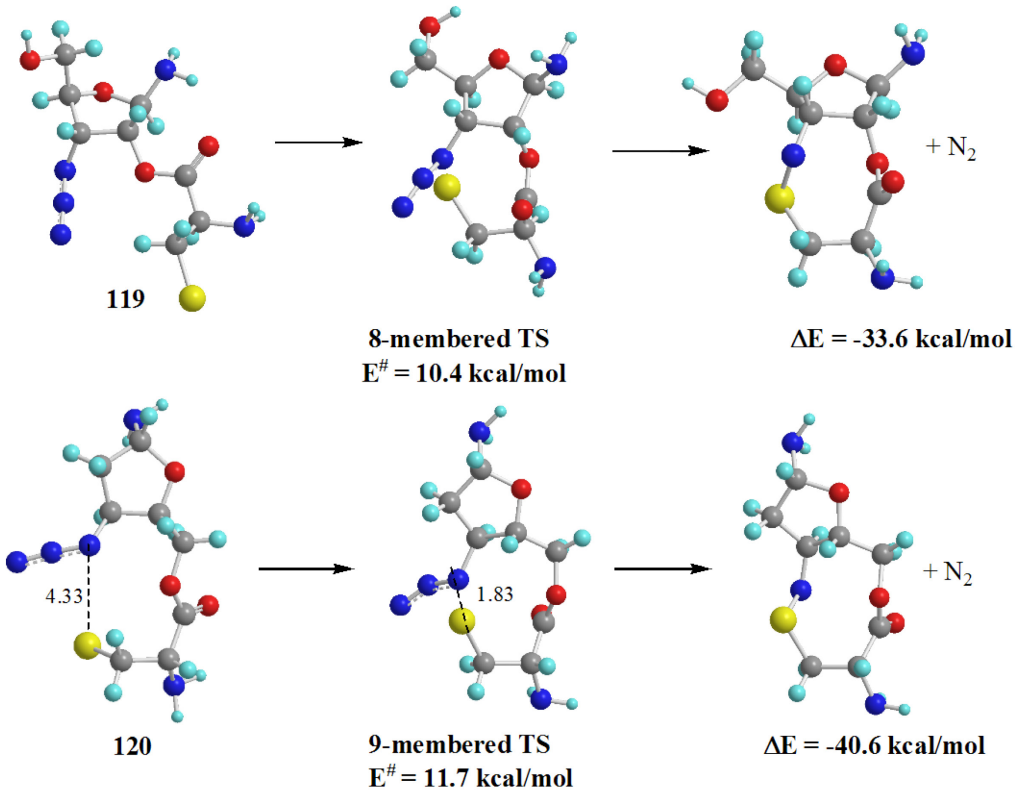


Figure 26. Ring closure reaction between a thiyl radical from a cysteinyl moiety and azide through 8 and 9-membered transition states

3.2.3 Feasibility of the ring-closure reaction between vicinal dithiols-derived thiyl radical and azido group

For substrates **121** and **122** bearing the vicinal disulfide, the calculation also suggested that the ring-closure reactions were exothermic (ΔE = from -35.7 to -38.4 kcal/mol) with low transition state energy barriers (from 9.1 to 17.8 kcal/mol; Table 2). Figure 27 shows a molecular model of the ring closure reaction of **121** and **122** between the thiyl radical from vicinal disulfide and azide through an 8 and 9-membered transition

states with the best approximation of geometry of the transition state and the ring-closured product. However, the position of the thiyl radical seemed to affect the ring-closure reaction greatly. With the primary thiyl radical at the β position, the ring closure reaction between the thiyl radical and the azido group was feasible through both 8 and 9-membered transition states for both *S* and *R* diastereomers at Cα. However, the reaction between a secondary α thiyl radical and the azido group, which required a 7 and 8-membered transition states, did not occur or occurred at a high energy barrier of 43.4 kcal/mol. It was also noted that the stereochemistry at Cα did not affect the ring closure reaction for substrate **121**, which would require an 8-membered transition state. However, for substrate **122**, which required 9-membered transition state, the energy barrier increased when the stereochemistry is R at Cα (17.8 kcal/mol as compared to 9.1 kcal/mol for S at Cα). Although there was a difference in the energy barriers, the calculated data still suggested that the ring-closure reactions for substrate **122** for both *S* and *R* diastereomers at Cα were facile.

	TS energy barrier (kcal/mol)	Energy (kcal/mol)
121 (*R* at C_α), S_β	11.4	-38.1
121 (*S* at C_α), S_β	12.6	-36.7
121 (*R* at C_α), S_α	does not occur	-
121 (*S* at C_α), S_α	43.4	-35.7
122 (*R* at C_α), S_β	17.8	-34.5
122 (*S* at C_α), S_β	9.1	-38.4
122 (*R* at C_α), S_α	does not occur	-
122 (*S* at C_α), S_α	does not occur	-

Table 2. Optimized (DFT B3LYP with the 6-31G*set) energies for the ring closure energy calculated for model substrates bearing a vicinal disulfide

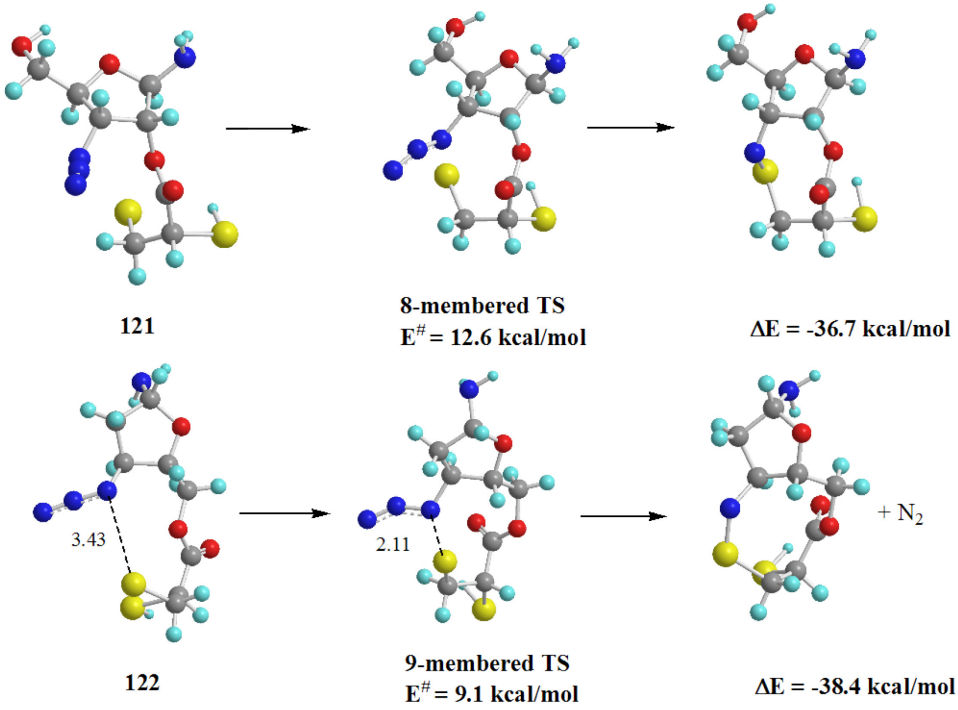


Figure 27. Ring closure reaction between a thiyl radical from vicinal disulfide and azide through 8 and 9-membered transition states

3.3 Biomimetic studies of alkyl thiyl radicals and alkyl azides using AAPH as radical initiator

In order to study the generation of thiyl radicals and their interaction with the 3'-azido group, the first criterion was to examine the stability of the azido group under both thermal and photolytic conditions used for the generation of thiyl radicals. Instead of testing the conditions on the "expensive" model compounds, commercially available 3'-azido-3'-deoxythymidine (AZT, **97**) was used to study the stability of the azido group.

Dr. Schoneichdeveloped a method for generating thiyl radicals by first producing carbon-centered radicals **124** from the radical initiator, 2,2’-azobis-(2-methyl-

propionamidine)-dihydrochloride (AAPH) **123**, using deuterated water as the solvent at a relatively low temperature of 37 ^{o}C.[138] The thiyl radical **126** is then generated by the reaction between **124** and a thiol (cysteamine **125**, Figure 28). We found this method to be very suitable for the generation of thiyl radicals from our model compounds because the structure of cysteamine is comparable to the thiol functionality of our model compounds. Also, the method uses relatively low-temperature under the physiological condition in water, which is important in maintaining the stability of our model nucleosides, especially the azido group. For these reasons, this methodology is more appealing than γ-radiolysis[139] and experiments that require di-*tert*-butyl hyponitrite as the radical initiator in organic solvents.[140-142] Generation of thiyl radicals employing stannyl radicals[143] is inapplicable because azides undergo reduction to amines with tin radicals.[144] However, silane radicals [with exception of $(Me_3Si)_3Si\bullet$][145] might provide an alternative approach for generation of thiyl radicals since they do not affect reduction of azido groups.[146]

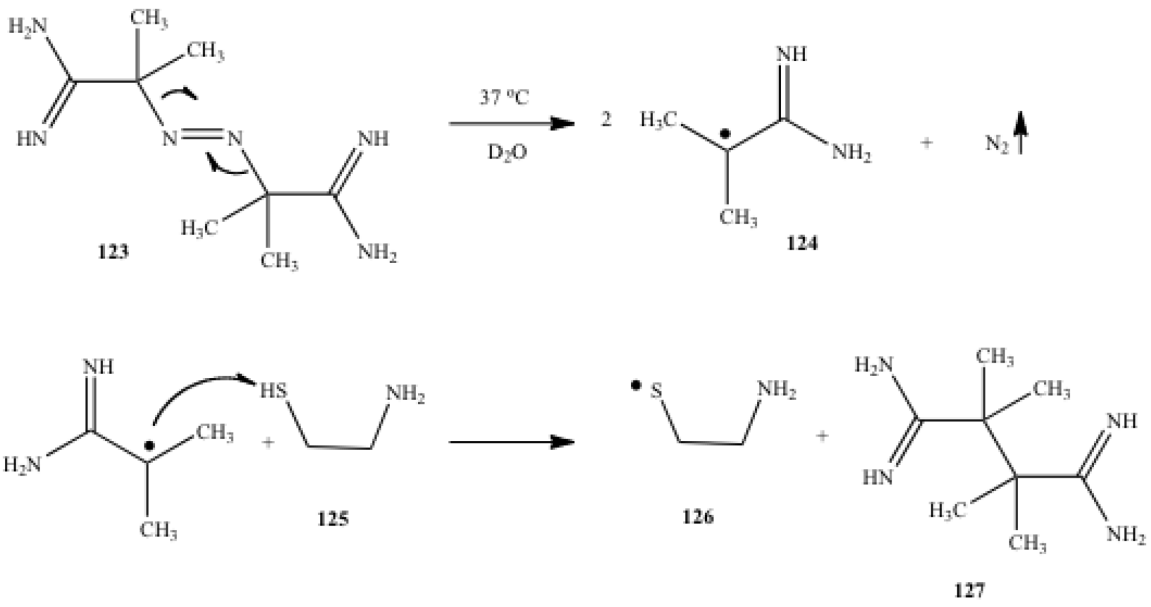


Figure 28. Thermal conditions for the generation of thiyl radicals uisng AAPH as radical initiator.

The fragmentation of AAPH **123** in D_2O at 37, 47, and 56 ^oC was examined using ^1H NMR. To evaluate the decomposition of AAPH, the ratio of the integration between the two singlets at δ 1.54 and δ 1.29 was monitored, which corresponded to the methyl groups of AAPH **123** and decomposed AAPH **124**, respectively. From the results (Table 3), that the best temperature to generate thiyl radicals from the model compounds was concluded to be at 50 ^oC.

Entry	Time (h)	Temp (^oC)	AAPH (mM)	% of AAPH fragmentation
1	15	37	18	5.0
2	17	47	18	20.8
3	17	56	18	73.2

Table 3. Decomposition of AAPH in D_2O at different temperature

3.3.1 Stability of azido group in the presence of AAPH

The stability of AZT in the presence of AAPH was investigated at 47 and 57 ^{o}C (Table 4). The stability of AZT was examined by comparing the integration between the singlet peak at δ 7.65, which corresponds to hydrogen at position 6 from the pyrimidine ring of AZT, and the triplet peak at δ 6.23, which corresponds to the anomeric proton at H1' of the sugar ring of AZT. At the two temperatures tested, the ratio of the integration between H1' and H6 peaks and their chemical shifts did not change. There were also no other peaks in the area of 5.5 to 6.5 ppm present, which is the expected area for the anomeric proton H1' for almost all nucleosides including 3'-amino-3'-deoxythymidine. The two peaks at δ 5.76 and δ 5.91 correlating to the minor products formed from decomposition of AAPH was also observed. These results suggested that the azido group is stable under the studied conditions that are required to generate thiyl radicals. Moreover, no additions to the pyrimidine ring and/or substitution of H5 or H6 with deuterium, or rearrangement within AZT was observed, which indicated that the condition is suitable for generating the thiyl radical from our model compounds.

Because the solubility of the model nucleosides in D_2O was found to be limited, the fragmentation of AAPH in deuterated methanol (MeOH-d_4) was also investigated. During the decomposition, the ratio of the integration between the two singlets at δ 1.47 and δ 1.20 was monitored, which corresponded to the methyl groups of AAPH **123** and decomposed AAPH **124** in MeOH-d_4, respectively. I found that using MeOH-d_4 instead of D_2O did not affect the rate of AAPH decomposition. AZT was also found to be stable in MeOH-d_4 in the presence of AAPH.

3.3.2 Model studies of 2-(2-azidocyclohexyl)ethanethiol with AAPH

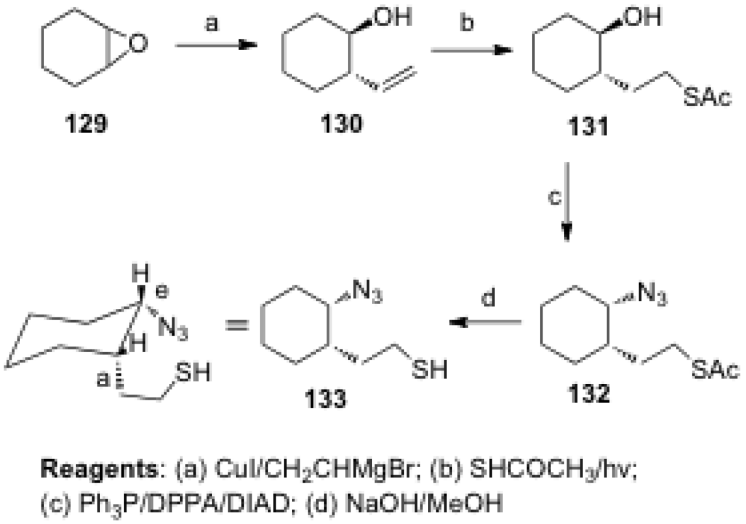
Since the purpose of our study was to investigate the ring closure reaction between the thiyl radical and azide of the model nucleosides, compounds such as 2-(2-azidocyclohexyl)ethanethiol **133** (Scheme 17) was also designed to simplify the ring closure reaction via 6-membered transition state. The formation of the 6-membered ring was assumed to be a driving force for such reaction to occur.

3.3.2.1 Synthesis and biomimetic study of 2-(2-azidocyclohexyl)ethanethiol

Addition of Grignard reagent, vinylmagnesium bromide at the low temperature of -30 ^{o}C, to the commercially available cyclohexene oxide **129**, along with catalytic amount of CuI salts, resulted in the ring opening of the epoxide to afford *trans*-2-ethenylcyclohexanol **130.** Although according to literature, the method[147] was not stereoselective, but I observed only the *trans* isomer. Free radical addition[148] of thiolacetic acid to **130** afforded *trans*-2-ethylthiolacetatecyclohexanol **131.** The Mitsonobu[149] reaction was applied to **131** to convert the hydroxyl group to azide directly with inversion of configuration, affording *cis* product **132**. Subsequent *S*-deacetylation[122] of **132** with NaOH/MeOH gave the desired 2-(2-azidocyclohexyl)ethanethiol **133** in good yield (87%).

Treatment of 2-(2-azidocyclohexyl)ethanethiol with AAPH in MeOH-d_4 did not result in the hypothesized 6-membered ring closure. Instead, only the oxidation of **133** to its disulfide form was observed on the basis of the 'H NMR downfield shift of the Hα. Perhaps constraint conformation and energy barriers due to the relative orientation of substituents: one substituted functional group is located at the axial position while

another group is located at the equitorial position precluded the formation of the planar 6-membered transition state.



Scheme 17. Synthesis of 2-(2-azidocyclohexyl)ethanethiol

3.3.3 Intermolecular interaction between AZT and 2,3-dimercaptopropionic acid or cysteine

Intermolecular interaction between the thiyl radical and azido group was studied using AZT **97** and 2,3-dimercaptopropionic acid **134** or cysteine **136** (Figure 29). Surprisingly, heating of AZT and **134** or **136** in the presence of AAPH resulted in deuterium exchange for both $H_{\beta}H_{\beta'}$ and H_{α} in 2,3-dimercaptopropionic acid and cysteine to give the fully deuterated analogues **135** and **137**, respectively. This result, however, also implied that the generated thiyl radical(s) did not interact intermolecularly with the

azido group since intact AZT was observed from the reaction mixtures.

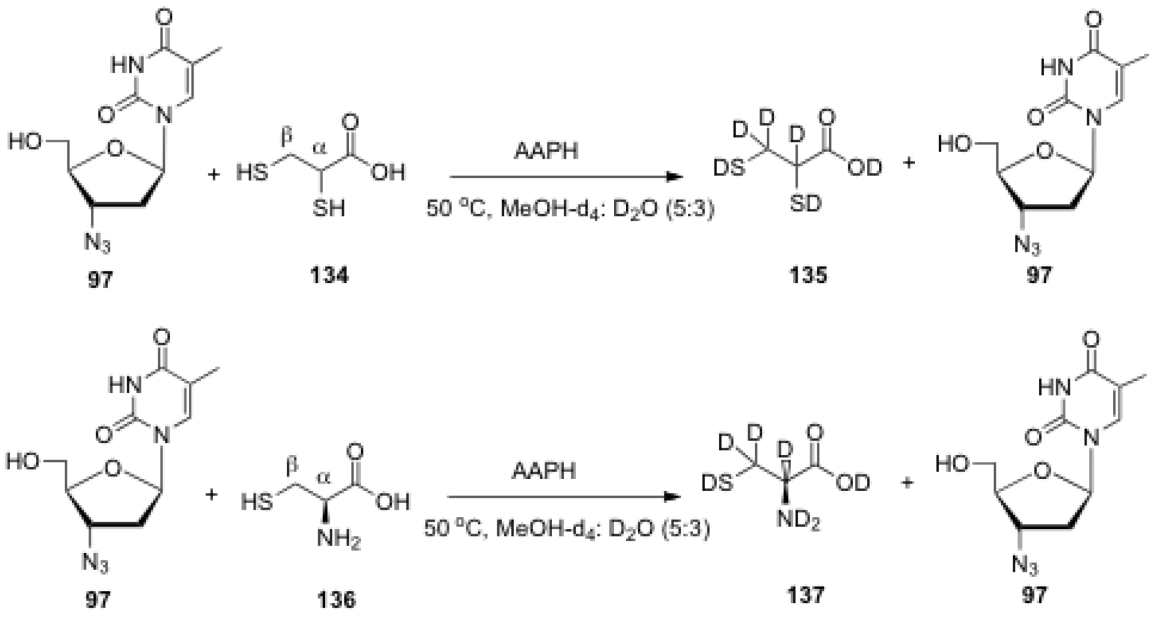


Figure 29. Intermolecular interaction between AZT and 2,3-dimercaptopropionic acid or cysteine

3.3.4 Intramolecular reaction of 3'-azido-nucleosides bearing thiol or vicinal-dithiol functionalities attached at C5' or C2' via ester linkage

The intramolecular reaction of 3'-azido-nucleosides **65-68** (see Figure 22) bearing thiol or vicinal-dithiol functionalities attached at C5' or C2' via an ester linkage was studied in the presence of AAPH as the thiyl radical initiator. However, treatment of **65-68** with AAPH in a mixture of MeOH-d_4: D_2O for 24 h at 50 ^oC also affected the hydrolysis of the ester bond from C5' or C2' to give mainly either 3'-azido-3'-deoxyadenosine **79** or 3'-azido-3'-deoxythymidine **97** as the product.

I did, however, observe a new nucleoside product **X** from the reaction of 3'-azido-3'-deoxy-5'-*O*-(2,3-dimercaptopropionyl)thymidine **68** with AAPH along with AZT (~4:1, X:AZT). This result indicated that there was interaction between the generated

thiyl radical and the azido group. From our ^{1}H NMR spectra (Figure 30), I observed that new nucleoside-based product **X** has distinctive chemical shifts of H3' and H4' when compared to their respective protons of **68** and AZT **97**. The ^{1}H NMR data of **X** was also not comparable to 3'-amino-deoxythymidine (AminoT) as different chemical shifts of H3' and H4' were observed. From these ^{1}H NMR data, it was safe to deduce that compound **X** has (i) a frame of thymine and all sugar protons as the proton peaks for H6 and CH_3 of the thymine base as well as for all protons for the ribose ring were clearly observed, (ii) no modification at C2' position since proton peak for H1' remains a triplet comparing to the starting material **68**, (iii) and the deprotection of the 5'-*O*-vicinal dithiol linkage as implied by the upfield chemical shifts of H5' and H5". Compound **X** could be formed by either intramolecular reaction of thiyl radical or thiolate and the azido group, followed by the cleavage of the ester bond. Alternatively, the ester bond at C5' could be hydrolyzed first, followed by the intermolecular reaction between mercaptopropionic acid and azide. However, as reported in section 3.3.3 above, there was no intermolecular reaction between 2,3-dimercaptopropionic acid and AZT.

I first suspected the structure of **X** to be 3'-*N*-acylamino-3'-deoxythymidine (3AcNHT) analog (Figure 31). The 3'-amino-3'-deoxythymidine was acetylated for comparison; however, according to the resulted ^{1}H NMR data for 5'-*O*-acetyl-3'-*N*-acetyl-3'-deoxythymidine (5'-Ac-3'-AcNHT), as presented in Table 4, as well as the literature values for 3'-*N*-acetyl-3'-deoxythymidine[150] (3'-AcNHT), it can be concluded from the chemical shifts of H3' and H4' that compound **X** does not have the acylamino group at C3'. Comparing to the spectroscopical data to 3'-hydroxylamino-2'-3'-dideoxythymidine[151] (HONHT), I observed that H4' chemical shift is more downfield

than H3' for both compounds **X** and HONHT even though the structure of **X** is not HONHT. Tentatively, the structure of **X** was assigned to have a 3'-hydroxylamino frame, probably acetylated further at the hydroxy group. Furthermore, the structure of X could also have a hydrazine group attached at C3' ($NHNH_2T$) due to the reaction between fragments from AAPH with AZT. It has to be noted that **X** was obtained clean by partioning between $CHCl_3$ and H_2O. Attempts to purify compound X using HPLC resulted in isolation of AminoT, which indicated that the hydroxyl amino fragment is unstable. The unstability of the hydroxyl amino fragment was also indicated by the mass of X was observed at m/z 242 $(M + H)^+$, which is comparable to AminoT.

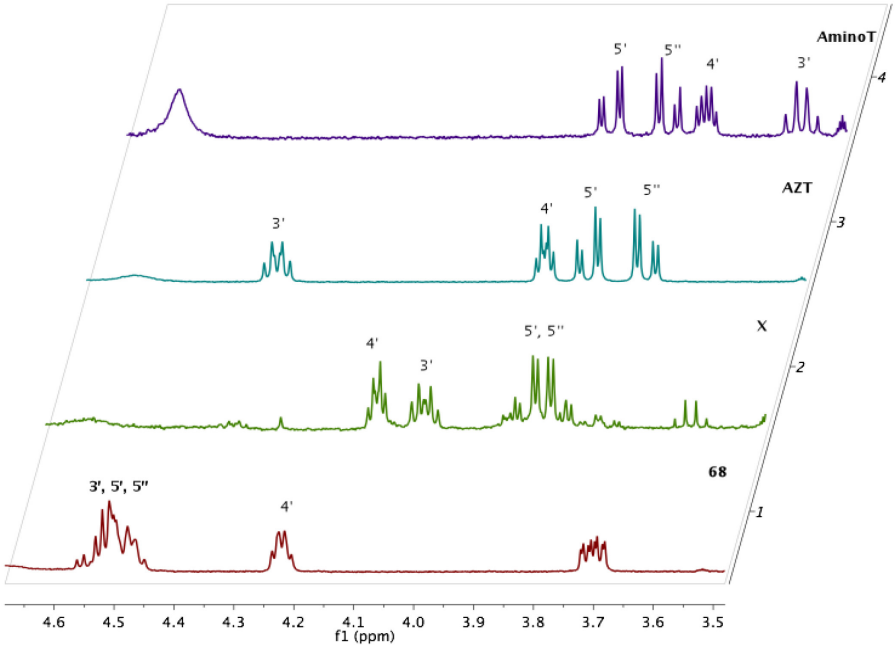


Figure 30. ^{1}H NMR in MeOH-d_4 showing H3', 4', 5' and 5'' of compounds **68, X**, AZT, and AminoT.

	1' (δ)	3' (δ)	4' (δ)	5',5'' (δ)	2',2'' (δ)
X	6.32 (t)	4.05	4.12	3.88, 3.82	2.49-2.57
68	6.20 (t)	4.49	4.22	4.54, 4.47	2.89-3.04
AZT	6.18 (t)	4.36	3.92	3.84, 3.74	2.35-2.46
AminoT	6.19 (dd)	3.55	3.71	3.85, 3.77	2.17-2.32
5'-Ac-3'-AcNHT	6.21 (dd)	4.46	4.02	4.35, 4.31	2.89-3.04
3'-AcNHT*	6.21 (t)	4.50	3.88	3.82, 3.75	2.28-2.43
OHNHT	6.22 (t)	3.72	4.03	3.84, 3.70	2.18-2.35

Table 4. ^{1}H NMR chemical shifts of the sugar-based protons of **X, 68,** AZT, AminoT, 5'-Ac-3'-AcNHT, 3'-AcNHT (*in acetone-d_6), and OHNHT.

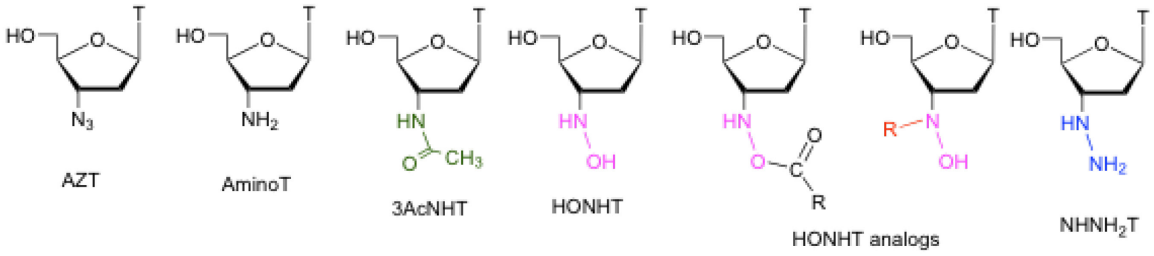


Figure 31. Tentative proposal of the structure of compound **X**.

3.3.5 Intramolecular reaction of 3'-azido-nucleotides bearing a vicinal-dithiol functionality attached at C5' or C2' via ether linkage

Because of the instability of the ester bond linkage bearing the thiol moiety attached to C5' and C2' of our model 3'-azido nucleosides, compounds **69** and **70** were also synthesized having thiol functionality attached to C2' or C5' via an ether linkage in effort to improve the stabililty. The 3'-azido model nucleosides **69** and **70** were heated at 50 ^{o}C in the presence of AAPH in MeOH-d_4. A mixture of products were observed after 24 h, and extensive purification by HPLC allowed us to isolate new products. For compound **69** with vicinal disulfide attached at C2', one of the observed products was 3'-azido-3'-deoxyadenosine along with another nucleoside product **Y**. On the basis of the ^{1}H NMR and MS data, I tentatively proposed that the structure of **Y** might have a 1,3,2-

dithiazolidine ring.[152] Dithiazolidines has been reported in literature although their chemistry has not been studied in depth. Figure 32 shows the proposed structure of **Y** and its plausible generation through the intramolecular interaction between the thiyl radical and azido group.

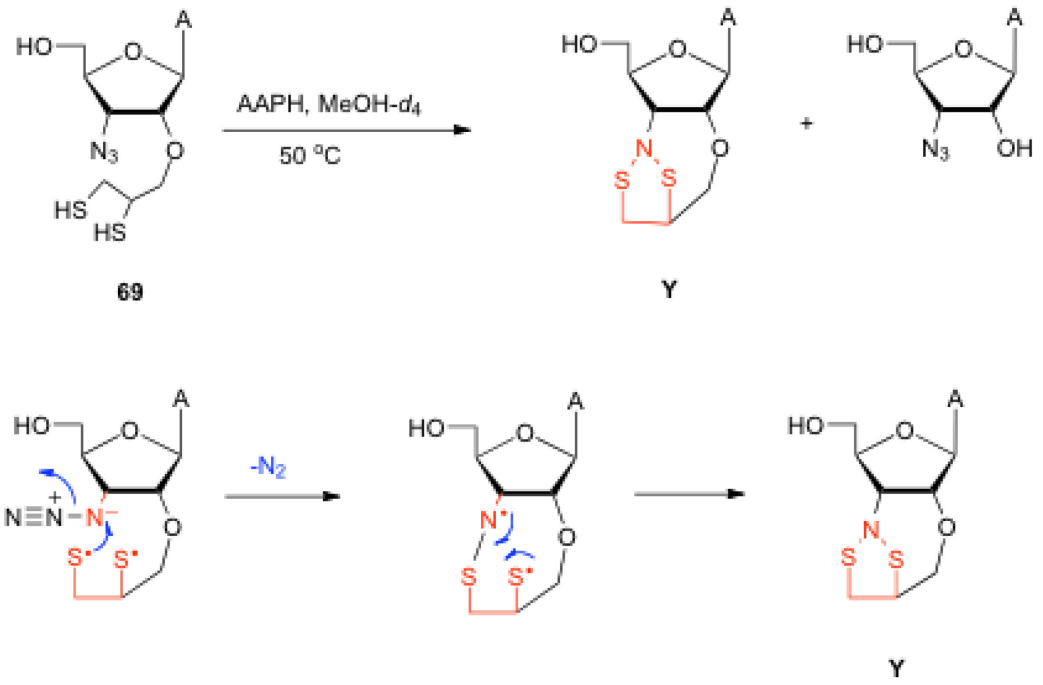


Figure 32. Formation of adenosine-based 1,3,2-dithioazolidine product Y.

Analogous reaction of AZT-derived dithiol **70** with AAPH resulted in a formation of a complex mixture of products. Laborious purfication led to the separation of few products whose tentative structures are presented in Figure 33 on the basis of their ^{1}H NMR and HRMS data.

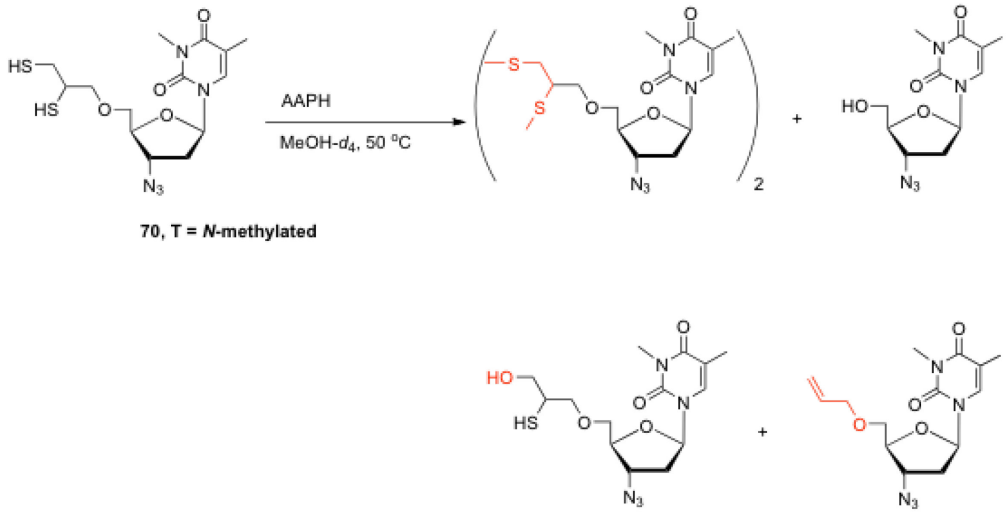


Figure 33. Reaction of 3'-azido-5'-*O*-(2,3-dimercaptopropyl)-3'-deoxy-*N*-methylthymidine and AAPH

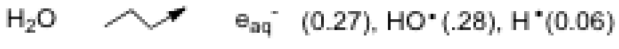
3.4 Studies of the interactions between alkyl thiyl radicals generated by gamma irradiation and alkyl azides

Because the biomimetic simulation studies, which utilized AAPH for the generation of thiyl radicals, did not provide conclusive results, I turned my attention to gamma radiation as a method for generation of thiyl radicals. Before studying the interaction between the thiyl radical and azide of 3'-azido nucleosides, the preliminary study of the reaction between aliphatic thiyl radicals and alkyl azides was examined first. As mentioned in the Introduction, there has not been clear evidence of such reaction in literature.

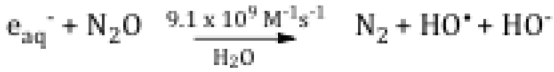
Briefly, gamma radiation is electromagnetic radiation emitted in the process of nuclear transformation or particle annihilation. Gamma rays have high frequency above 10^{19} Hz, energy above 100 keV, and wavelength less than 10 pm. Emission of gamma radiation occurs when the nucleus of a radioactive atom has too much energy. For

example, one of the most widely used source of gamma radiation emission is radionuclide Cobalt-60, which is produced by Cobalt-59 via neutron activation. Cobalt-60 decays by beta decay to the stable isotope nickel-60, in which its activated nickel nucleus emits two gamma rays with energies of 1.17 and 1.33 MeV.[153]

A cobalt-60 gamma cell was used to generate thiyl radicals via radiolysis of water under N_2O-saturated condition. As shown in eq 13, radiolyis of neutral water led to solvated electrons (e_{aq}^-), HO•, and H•, in which the values in parentheses represent the radiation chemical yields (*G*) in units of $\mu molJ^{-1}$.[154] Under a N_2O-saturated condition producing around 0.02 M of N_2O, e_{aq}^- reacted with N_2O and efficiently transformed into HO• at the rate of 9.1 x 10^9 $M^{-1}s^{-1}$ (eq 14). This means that the radiolysis of water afforded G(HO•) = 0.55 $\mu molJ^{-1}$, and that HO• and H• atoms make up 90 and 10%, respectively, of the reactive species.[154-156] The produced hydroxyl and hydrogen radicals then reacted with thiols to generate thiyl radicals (eq 15).



eq 13



eq 14

eq 15

3.4.1 Gamma irradiation of cysteine in the presence of AZT

As mentioned above, a preliminary study between alkyl thiyl radicals and alkyl azide was first conducted. Cysteine (CySH) was the chosen alkyl thiol while 3'-azido-3'-deoxythymidine (AZT) was the chosen alkyl azide. As reported in literature[157], the rate of the addition of HO• and H• to the methyl group of the pyrimidine ring of thymidine

are 4.7 x 10^9 and 3.2 x 10^8 $M^{-1}s^{-1}$, respectively; and the rate of the similar addition of HO• to AZT is reported[158] to be 9.0 x 10^9 $M^{-1}s^{-1}$. On the basis of the reported rates, we estimated that the rate for the reaction of H• and AZT would be 6.1 x 10^8 $M^{-1}s^{-1}$. As shown in eq 16 and 17, the rates[159] for the formation of thiyl radical by reaction of HO• and H• with cysteine are 4.7 x 10^{10} and 1.0 x 10^9 $M^{-1}s^{-1}$, respectively.

$$HO^{\bullet} + CySH \xrightarrow{4.7 \times 10^{10}\, M^{-1}s^{-1}} CyS^{\bullet} + H_2O \qquad \textbf{eq 16}$$

$$H^{\bullet} + CySH \xrightarrow{1.0 \times 10^{9}\, M^{-1}s^{-1}} CyS^{\bullet} + H_2 \qquad \textbf{eq 17}$$

On the basis of the reported kinetics, we had chosen to use 1.0 mM of AZT and 10 mM of cysteine to ensure that hydroxyl and hydrogen radicals would react with cysteine to generate thiyl radicals at a faster rate than their reactions with AZT (Table 5).

	HO• (s^{-1})	H• (s^{-1})
[AZT] = 1.0 mM	9.0 x 10^6	6.1 x 10^5
[CySH] = 10 mM	4.7 x 10^8	1.0 x 10^7

Table 5. The rate HO• and H• radicals would react with cysteine and AZT at the chosen concentrations.

Gamma irradiation of 1.0 mM of AZT and 10 mM of cysteine at doses of 2, 4, and 6 kGy with a dose rate of ca. 6.5 Gy/min at pH 7.0 (by using a 10 mM phosphate buffer) was carried out under deaerated (N_2O-saturated) conditions. The experiment resulted in the formation of 3'-amino-3'-deoxythymidine (t_R = 15 min), thymine (t_R = 12 min), H_2S (t_R = 5 min), cysteine (t_R = 4.9 min) and cystine (t_R = 4.8 min) on the basis of

the HPLC retention time of the standard samples and MS analysis as well as the consumption of AZT (t_R = 22 min (Figure 34).

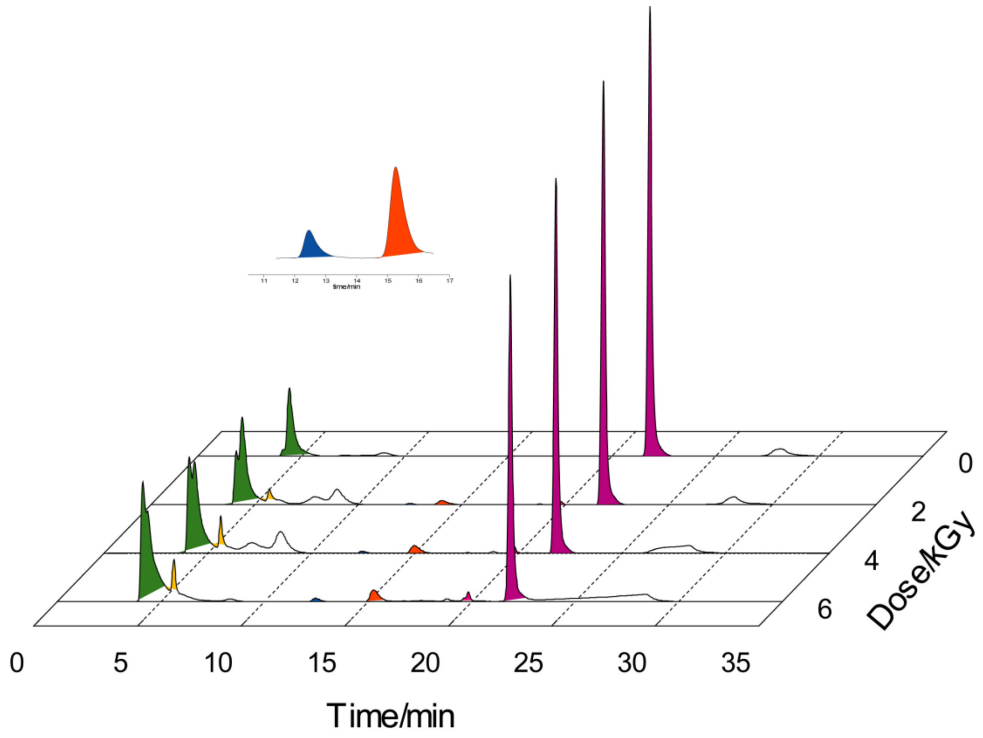


Figure 34. HPLC analysis of the γ-irradiation of a N_2O-purged solution containing 1.0 mM AZT and 10 mM cysteine.

The *G* values, which quantify the number of molecules of reactant consumed or product formed per 100eV of energy absorbed, for the consumption of AZT and for the formation of products were calculated (Figure 35). The literature reported G_{total} value for the generation of radicals is 0.61 μmolJ^{-1} (see eq 13).[155,156] In this experiment, the *G* value for the consumption of AZT was 0.05 μmolJ^{-1}. Of the AZT consumed by the reaction, the *G* value for the formation of 3'-amino-3'-deoxythymidine (aminoT) was 0.03 μmolJ^{-1} while the *G* value for the formation of thymine was 0.01 μmolJ^{-1}. The *G* value for the formation of cystine (0.46 μmolJ^{-1}) suggested that a large percentage

(~75%) of generated cysteinyl radicals recombined to form the disulfide in the termination step. Also, the reaction generated the noticeable formation of H_2S (estimated ~0.10 μmolJ^{-1}). Even though only ~10 % of radicals reacted with AZT in our system, it was gratifying to see that our data corresponded to the total value of radiation chemical yield in terms of the total amount of chemical reactions (G = 0.05, 0.46, 0.10 μmolJ^{-1}) equals the total value of radiation chemical yield (G_{total} = 0.61 μmolJ^{-1}). Since the reaction resulted in the formation of aminoT, thymine, and hydrogen sulfide, it was still uncertain whether thiyl radicals reacted with AZT to generate both aminoT and thymine or thiyl radicals reacted with AZT to give only thymine while hydrogen sulfide reacted via ionic pathway with the azido group of AZT to generate the reduced aminoT.

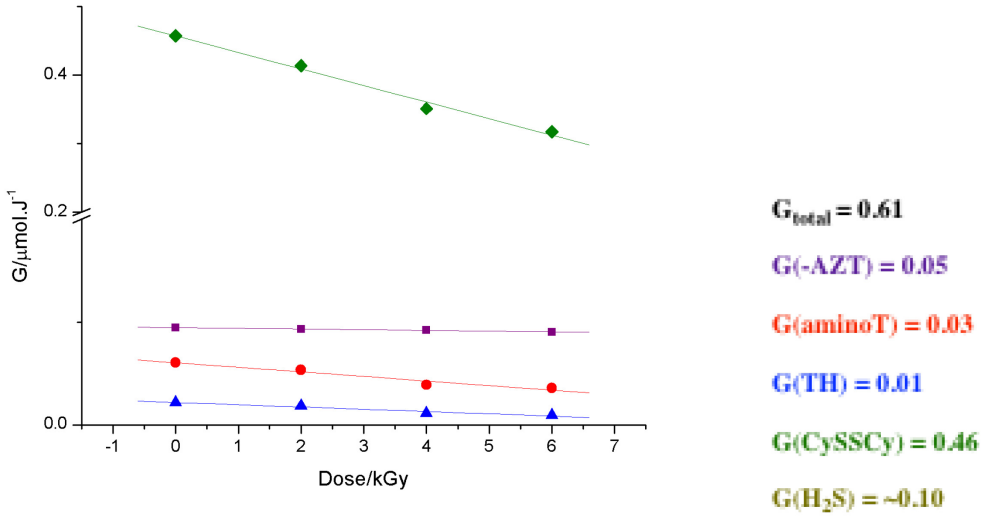


Figure 35. G values vs. dose for the γ-irradiation of a N_2O-purged solution of 1.0 mM AZT and 10 mM cysteine.

To understand the pathway of the formation of aminoT, thymine and other species through gamma-irradiation of cysteine and AZT, pH-dependent and O_2-dependent

experiments were studied. In the pH-dependent experiments, an increase or decrease formation of aminoT was expected to be observed as the changes in pH (7.00, 8.34, and 9.65) corresponded to the increase of disulfide radical anions and decrease of thiyl radicals. At pH 8.34, it can be assumed that 50% of thiyl radicals and disulfide radical anions are formed on the basis of the pK_a value[159] of cysteine (pK_a = 8.3). Hence, at pH 7.00 and 9.65, we can also assume an increase formation of thiyl radicals and disulfide radical anions, respectively. However, the results showed that the formation of both aminoT and thymine remained relatively constant, which suggested that the reduction of AZT to its amino counterpart was not due to the reactions with thiyl radicals or disulfide radical anions.

Gamma irradiation of a 10% oxygen-saturated solution of AZT and cysteine was also performed at dosage of 2 kGy at the pH of 7.0 in 10 mM phosphate buffer. Figure 36 shows that the gamma irradation reaction of a 9:1 N_2O:O_2-purged solution containing 1.0 mM of AZT and 10 mM of cysteine yielded a similar amount of 3'-amino-3'-deoxythymidine as in the N_2O-saturated reaction. More interestingly, in the presence of O_2, a noticeable increase formation of thymine was observed. According to the results, a pathway of the formation of thymine via γ-irradiation of a 9:1 N_2O:O_2-purged solution containing AZT and cysteine was proposed. The reaction of cysteinyl radicals (CyS•) with oxygen is reversible and the equilibrium is strongly on the left (Figure 37). The CyS• are suspected to abstract hydrogen from the ribose ring of AZT to form various alkyl radical (sugar radicals), which then add to the oxygen to form alkyl peroxyl radicals. These alkyl peroxyl radicals are known to facilitate the formation of thymine. [31-33]

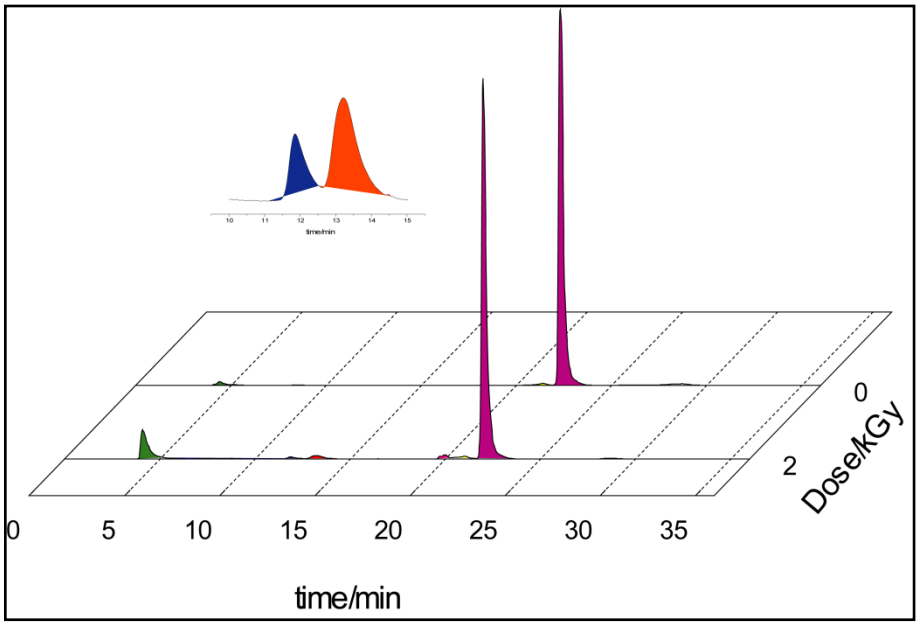


Figure 36. HPLC analysis of the γ-irradiation of a 9:1 N_2O:O_2-purged solution containing 1.0 mM of AZT and 10 mM of cysteine

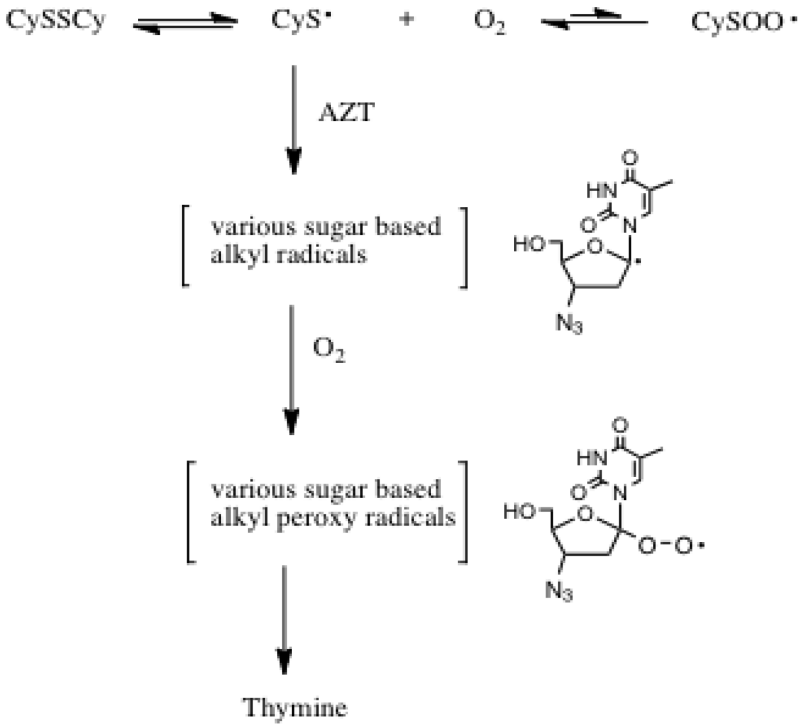


Figure 37. Proposed mechanistic pathway of the formation of thymine via gamma irradiation of a 9:1 N_2O:O_2-purged solution containing AZT and cysteine

3.4.2 Proposed mechanism

Figure 38 shows a proposed mechanistic pathway for the reaction between cysteinyl radicals and AZT at pH 7.0 under described conditions. It is assumed that cysteinyl radicals reacted with AZT to form thymine while the reduction of azide was the result of the ionic reaction with hydrogen sulfide. In literature, gamma irradiation of cysteine has been reported to generate hydrogen sulfide;[160-163] and H_2S, as a reducing agent, can spontaneously reduce AZT to its amino counterpart, which was also confirmed experimentally.[80,82,164] Under the described condition, I did not find evidence of the interaction between alkyl thiyl radicals and alkyl azide. It is also worth to mention that reactions of γ-irradiation of 2-mercaptoethanol and AZT was studied. However, both our experiments and literature showed that the reduction of AZT occurred from the thiyl radical generated by γ-irradiation of 2-mercaptoethanol happened spontaneously rather than through radical stress. [104,105] These results suggested that alkyl thiyl radicals are unlikely to react with alkyl azide.

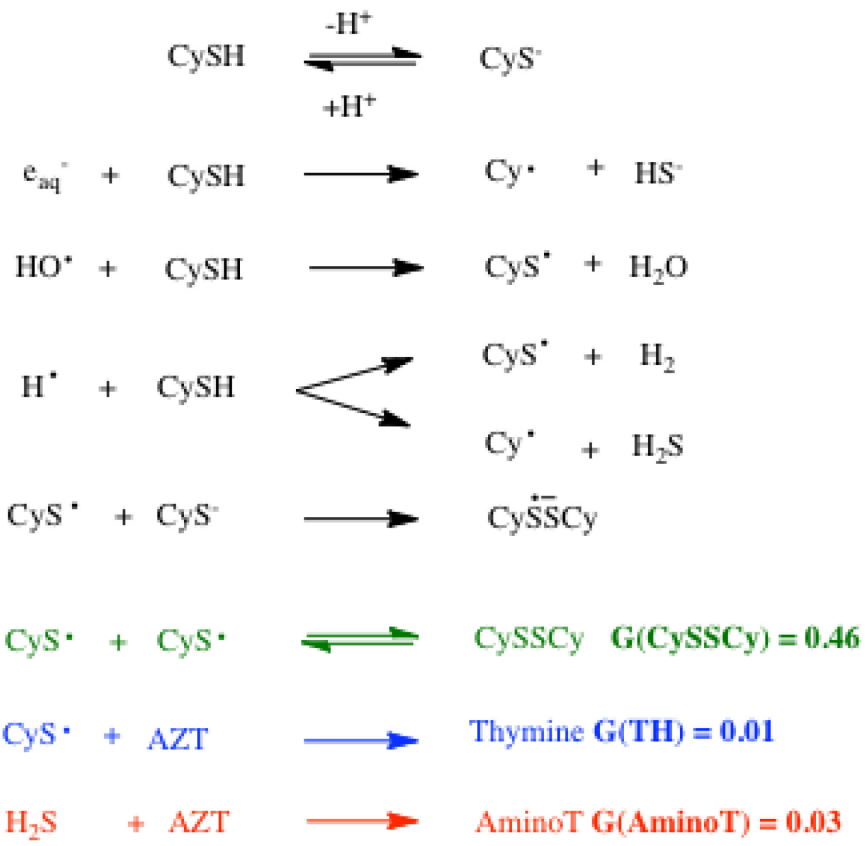


Figure 38. Proposed mechanistic pathway for the reaction between cysteinyl radicals and AZT

3.4.3 Gamma-irradiation of cysteamine and AZT in aqueous (H_2O or D_2O) glassy (7.5 M LiCl) systems

Under a different condition of γ-irradiation in aqueous (H_2O or D_2O) glassy (7.5 M LiCl) systems an absorbed dose of 525-700 Gy at 77 K[123] (see chapter 3.5.1), the reactions between the thiyl radicals generated from cysteamine and the azido group of AZT and 2'-azido-2'-deoxyuridine (2'-AZdU) were examined. Our ESR specroscopic data (Figure 39) indicated cysteaninyl radicals, appeared as broad singlets, were generated but subsequent reaction with the azido group of AZT and 2'-AZdU was not

observed. This result strongly supported the above proposed pathway (Figure 38) that alkyl thiyl radicals are not likely to react chemically with the alkyl azide.

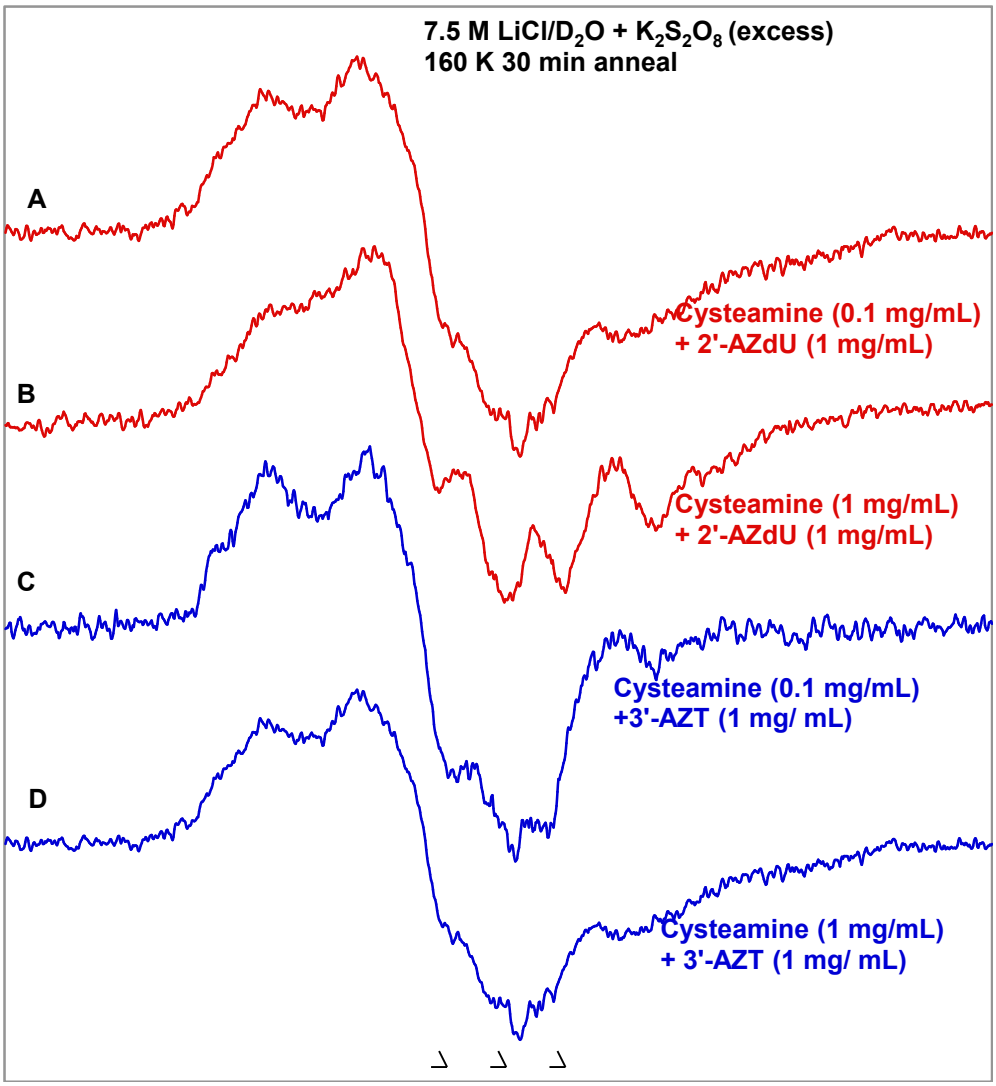


Figure 39. ESR spectral studies of cysteamine and 3'-AZT and 2'-AZdU in D_2O glasses (7.5 M LiCl/D_2O)

3.5 Structure of the aminyl radicals formed from single-electron attachment to 2'-azido-2'-deoxyuridine

3.5.1 Characterization of aminyl radicals formed from one-electron attachment to 3'-azido-3'-deoxythymidine and its analogues.

Recently, Sevilla's group[123] reported the formation of the aminyl radical via electron attachment to 3'-azido-3'-deoxythymidine (3'-AZT), as well as 5'-azido-5'-deoxythymidine (5'-AZT) and 3'-azido-2',3'-dideoxyguanosine (3'-AZG). They were able to characterize the aminyl radical derived from azido group and sugar-based radicals using electron spin resonance (ESR) spectroscopy. Gamma-irradiation was performed in aqueous (H_2O or D_2O) glassy (7.5 M LiCl) systems at an absorbed dose of 525-700 Gy at 77 K with the aid of a 109-GR 9 irradiator, which has a shielded ^{60}Co source.

The generally accepted mechanism[123] for reduction of organic azides to amines initiated by one-electron attachment involved the formation of azide anion radical (eq 18a). Next, the nitrene anion radical is formed after expulsion of N_2 (eq 18b), which captured a proton to give the aminyl radical (eq 18c). Finally, the plausible pathways for the formation of amine were proposed to be either by H-atom abstraction (eq 18d) or one-electron reduction and proton capture (eq 18e). However, up until this report, no evidence of the proposed radical intermediates (eq 18a to eq 18e) has been demonstrated for organoazides. Trapping the azide anion radical or the aminyl radical might provide key information for better understanding of several mechanistic pathways including the mechanism for the inhibition of RNRs by N_3NDPs and the radiosensitization effects of AZT.

$RN_3 + e^- \rightarrow RN_3\bullet^-$ **eq 18a**

$RN_3\bullet^- \rightarrow RN\bullet^- + N_2$ **eq 18b**

$RN\bullet^- + H_2O \rightarrow RNH\bullet$ **eq 18c**

$RNH\bullet + R'\text{-}H \rightarrow RNH_2 + R'\bullet$ **eq 18d**

$RNH\bullet + e^- + H_2O \rightarrow RNH_2 + OH^-$ **eq 18e**

Sevilla and coworkers[123] reported several important observations regarding the mechanism for the formation of amines from organic azides initiated by one-electron reduction. They first showed that the predominant site of electron attachment to AZT is at the azide group and not at the thymine moiety by ESR spectral data and theoretical calculation. Although they did not observe the unstable azide anion radical intermediate ($RN_3\bullet^-$), they did observe the one-electron additon to the azide group resulting in the formation of a neutral aminyl radical ($RNH\bullet$) in both acidic and basic media by ESR spectroscopy. The observed hyperfine splitting of *ca.* 116G and 117G (3' and 5'-AZT, respectively) and *g*-values for the new radical species were nearly identical to the simulated data. On annealing to higher temperature (ca. 160-170 K), they also observed the bimolecular hydrogen abstraction reactions between the aminyl radical and the thymine methyl group and the sugar moiety resulted in the formation of the stable thymine allyl radical and a sugar-based radical (Figure 40). Even though it is unclear at which position of ribose that the bimolecular hydrogen abstraction occurred, the proposed structure of the sugar-based radical is indeed very similar to the radical species formed during inhibition of RNRs by N_3UPDs (see structure 11, Figure 8).

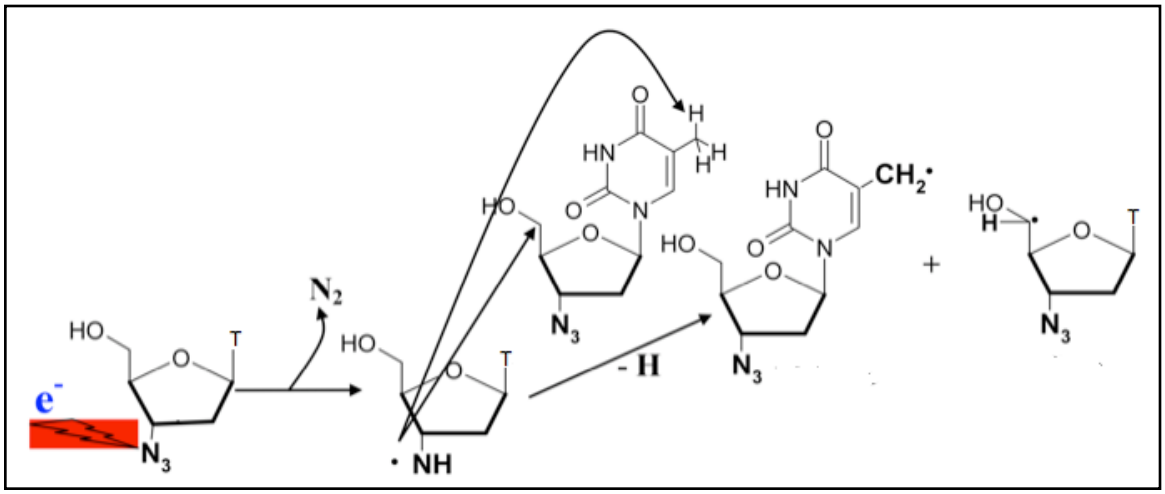


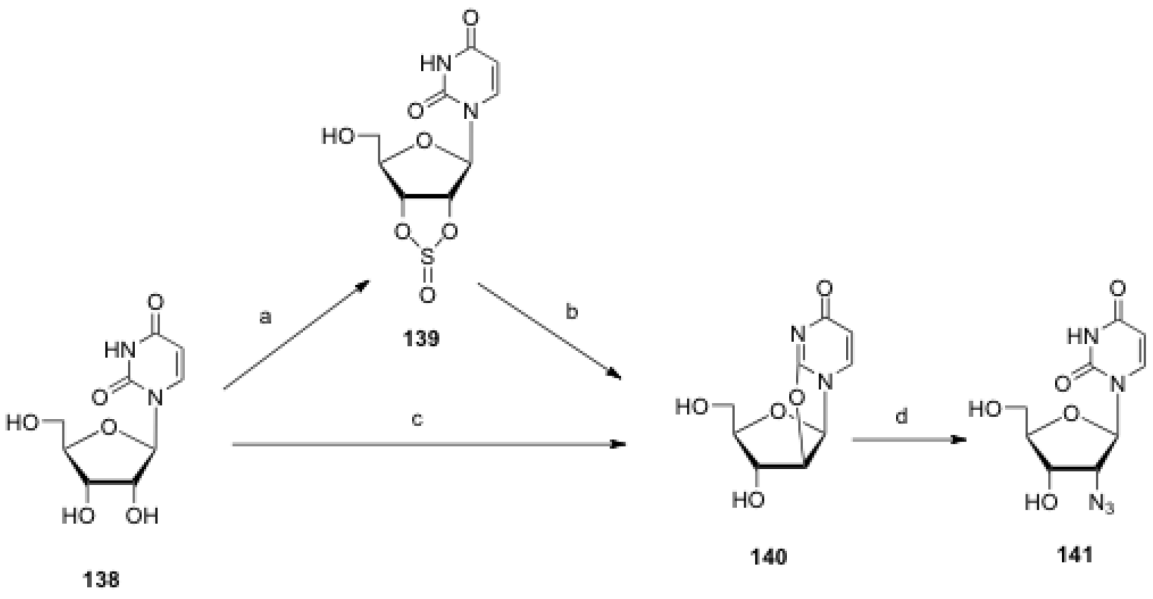
Figure 40. Bimolecular hydrogen abstraction reactions between the aminyl radical and the thymine methyl group and the sugar moiety of AZT

Although Sevilla's work demonstrated the formation of an aminyl radical from azido group, which then underwent bimolecular hydrogen abstraction from the sugar ring in nucleosides, its subsequent chemistry has not yet been elucidated. To further study the structure and chemistry of the aminyl radical formed during RNR inhibition by 2'-azido-2'-deoxyuridine (2'-N_3-dUrd), labeled analogues of 2'-N_3-dUrd at ribose 1', 2', 3', and 4' positions, as well as 5,6 position of the uracil base were prepared. Elucidation of the structure of the aminyl radical might provide key mechanistic information for the inhibition of RNRs since 2'-N_3-dUrds are one of the most powerful inhibitor of the enzyme.

3.5.2 Synthesis of 2'-azido-2'-deoxyuridine

Synthesis of 2'-azido-2'-deoxyuridine **141** was initially accomplished by conversion of uridine to 2',3'-*O*-sulfinyluridine **139** with thionyl chloride.[165] Subsequent cyclization[165,166] in the presence of sodium acetate yielded 2',2-anhydrouridine **140**.

Nucleophilic ring opening with azide anion in **140** afforded the desired 2'-azido-2'-deoxyuridine **141** according to the earlier literature reports (Scheme 18).[167,168] As a result of the observed instability of the sulfinyl group in **139**, I also synthesized 2',2-anhydrouridine convienniently by treatment of uridine with diphenyl carbonate[169] in DMF with catalytic amount of sodium bicarbonate at 150 ^{o}C. Azidation[167] of **140** with trimethylsilylazide ($TMSN_3$) in the presence of lithium fluoride (LiF) and tetramethylethylenediamine (TMEDA) for 48h afforded **141** in low yield of 26%. Addition of azide[165,168] was also achieved using sodium azide in hexamethylphosphoramide (HMPA) at 150 ^{o}C in the presence of benzoic acid, which provided **141** in 30 min in a better yield of 56%. The latter method of azidation, however, also yielded the undesired 2'-arabinouridine byproduct (~40%).



Reagents: (*a*) $SOCl_2$/ACN; (b) NaOAC/DMF; (c) diphenylcarbonate/$NaHCO_3$/DMF; (*d*) LiF/TMEDA/$TMSN_3$/DMF or NaN_3/BzOH/HMPA.

Scheme 18. Synthesis of 2'-azido-2'-deoxyuridine

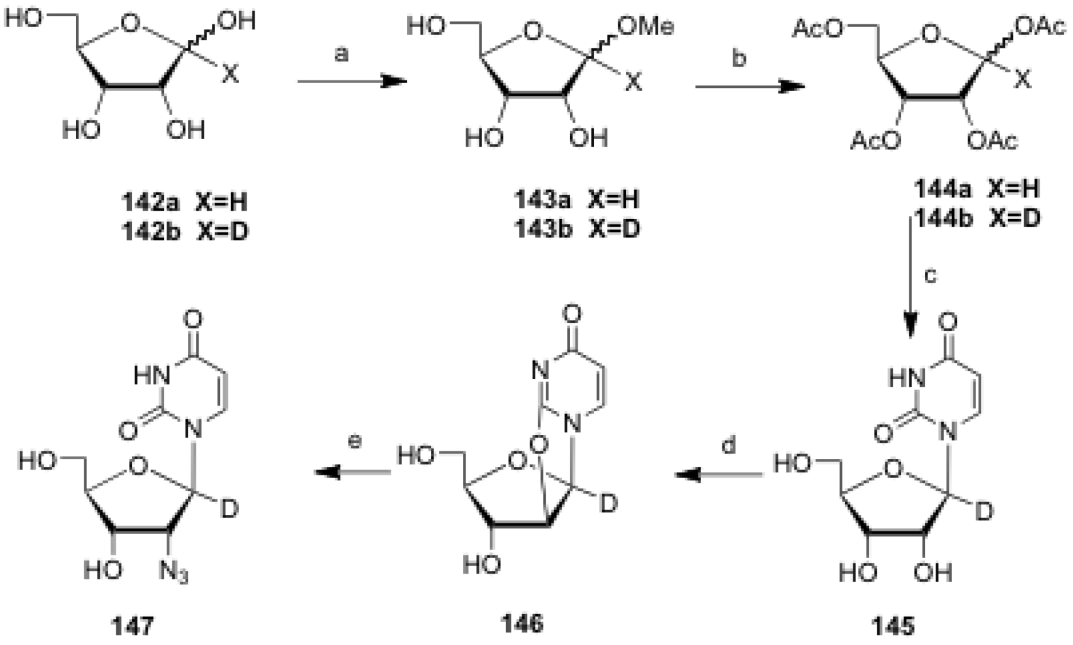
3.5. Synthesis of 1'[^{2}H]-, 2'[^{2}H]-, 3'[^{2}H]-, and 4'[^{2}H]-2'-azido-2'- deoxyuridine

In order to fully characterize and identify radicals produced during gamma radiation with 2'-azido-2'-dexoyuriidine (2'-N_3-dUrd), I have undertaken synthesis of the selectively labeled 2'-N_3-dUrd with deuterium at position C1', C2', C3', and C4'. Synthesis of these compounds was accomplished by the coupling of the suitable labeled ribofuranoses with uracil, followed by the well-established incorporation of the azido group at C2' position via the ring opening of 2',2-*O*-anhydrouridine. Synthesis of the 1'-[^{2}H], 2'-[^{2}H], 3'-[^{2}H], and 4'-[^{2}H]-N_3-Urd will be discussed below separately since the preparation of the deuterium labeled sugar precursors required different approaches.

3.5.3.1 Synthesis of 1'-[^{2}H]-2'-azido-2'-deoxyuridine

The 1-[^{2}H]-1,2,3,5-tetra-*O*-acetyl-D-ribofuranose **144b** was prepared[10] by methylation of commercially available 1-[^{2}H]-ribose **142b**, followed by standard acetylation and acetolysis (Scheme 19). Several methods were employed for conversion of **142b** into methyl ribofuranose **143b**. Thus, treatment of ribose with HCl gas in MeOH or AcCl/MeOH afforded the desired methylated product. However, the yield and purity were irreproducible because it was difficult to control to amount of HCl gas that was generated in the reaction mixture. Neutralization with both Dowex OH^- and pyridine was also inconvennient and did not give desired product in high yield nor purity. Finally, treatment of ribose with catalytic amount of sulfuric acid in MeOH at 0 ^oC, followed by neutralization with small amount of pyridine, afforded the methylated product **143b** in high yield and purity. Acetylation of **143b** followed by acetolysis provided the desired fully acetylated 1-[^{2}H]-ribose precursor **144b**. Coupling[24] of **144b** with persilylated uracil in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) according to the

Vorbugen procedure afforded acetylated 1'-[^{2}H]-uridine in 69% yield. Subsequent standard deacetylation with NH_3/MeOH yielded 1'-[^{2}H]-uridine **145** in 85% yield. Level of deuteriation was above 98% as judged by MS and ^{1}H NMR. Cyclization of **145** with diphenyl carbonate afforded 1'-[^{2}H]-2',2-anhydrouridine **146**. Introduction of the azido group at 2' position of uridine was accomplished by ring opening of **146** with sodium azide in HMPA in the presence of benzoic acid to yield 1'-[^{2}H]-2'-N_3-dUrd **147**.



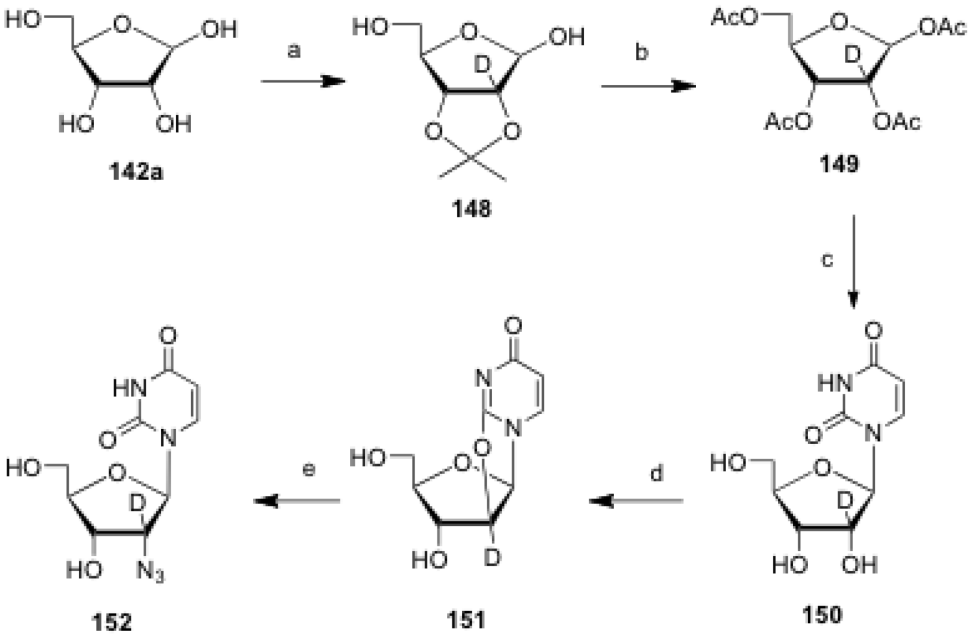
Reagents: (*a*) H_2SO_4/MeOH; (*b*) (i) Ac_2O/py, (ii) H_2SO_4/Ac_2O/AcOH; (*c*) (*i*) uracil/HMDS/TMSSiCl, (ii) TMSOTf/ACN, (iii) NH_3/MeOH; (*d*) diphenylcarbonate/$NaHCO_3$/DMF; (*e*) NaN_3/BzOH/HMPA.

Scheme 19. Synthesis of 1'-[^{2}H]-2'-azido-2'-deoxyuridine

3.5.3.2 Synthesis of 2'-[^{2}H]-2'-azido-2'-deoxyuridine

The deuterium incorporation[170] at C2 position was accomplished by acid-catalyzed acetonation of D-ribose at C2 and C3, followed by deuterium incorporation at C2 position by base-catalyzed hydrogen/deuterium exchange of H2 via enolization

process. Thus, heating of 2,3-*O*-isopropylidineribose with triethylamine in D_2O, dioxane ,and tetrahydrofuran at 90 ^oC for 6 days afforded **148** in over 98% deuterium incorporation. Subsequent standard acetylation and acetolysis yielded 2-[^{2}H]-1,2,3,5-tetra-*O*-acetyl-D-ribofuranose **149**. Coupling to uracil provided 2'-[^{2}H]-2',2-anhydrouridine, which upon cylization and azidation afforded 2'-[^{2}H]-2'-azido-2'-deoxyuridine **152** (Scheme 20).



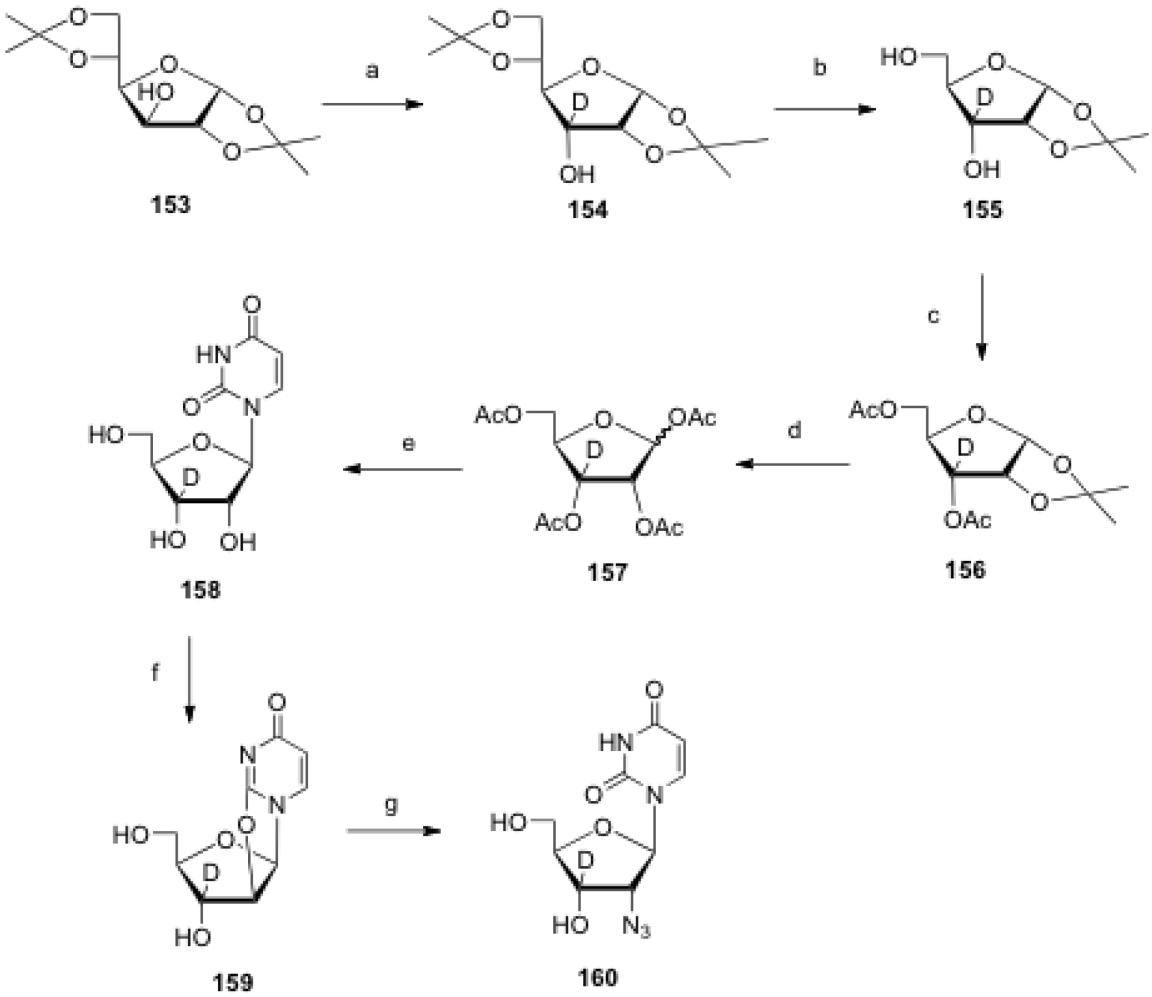
Reagents: (*a*) (i) H_2SO_4,acetone, (ii) Dioxane/THF/Et_3N/D_2O; (*b*) (i) Ac_2O/py, (ii) TFA/H_2O, (iii) Ac_2O/py; (*c*) (*i*) uracil/HMDS/TMSSiCl, (ii) TMSOTf/ACN, (iii) NH_3/MeOH; (*d*) diphenylcarbonate/$NaHCO_3$/DMF; (*e*) NaN_3/BzOH/HMPA.

Scheme 20. Synthesis of 2'-[^{2}H]-2'-azido-2'-deoxyuridine

3.5.3.3 Synthesis of 3'-[^{2}H]-2'-azido-2'-deoxyuridine

The 3-[^{2}H]-1,2,3,5-tetra-*O*-acetyl-D-ribofuranose **157** was prepared from commercially available diacetone-D-glucose **153** through several steps.[26] The deuterium incorporation at C3 position (>98%) with inversion of configuration was achieved by oxidation of **153** with chromium complex, followed by reduction of the resulting 3-

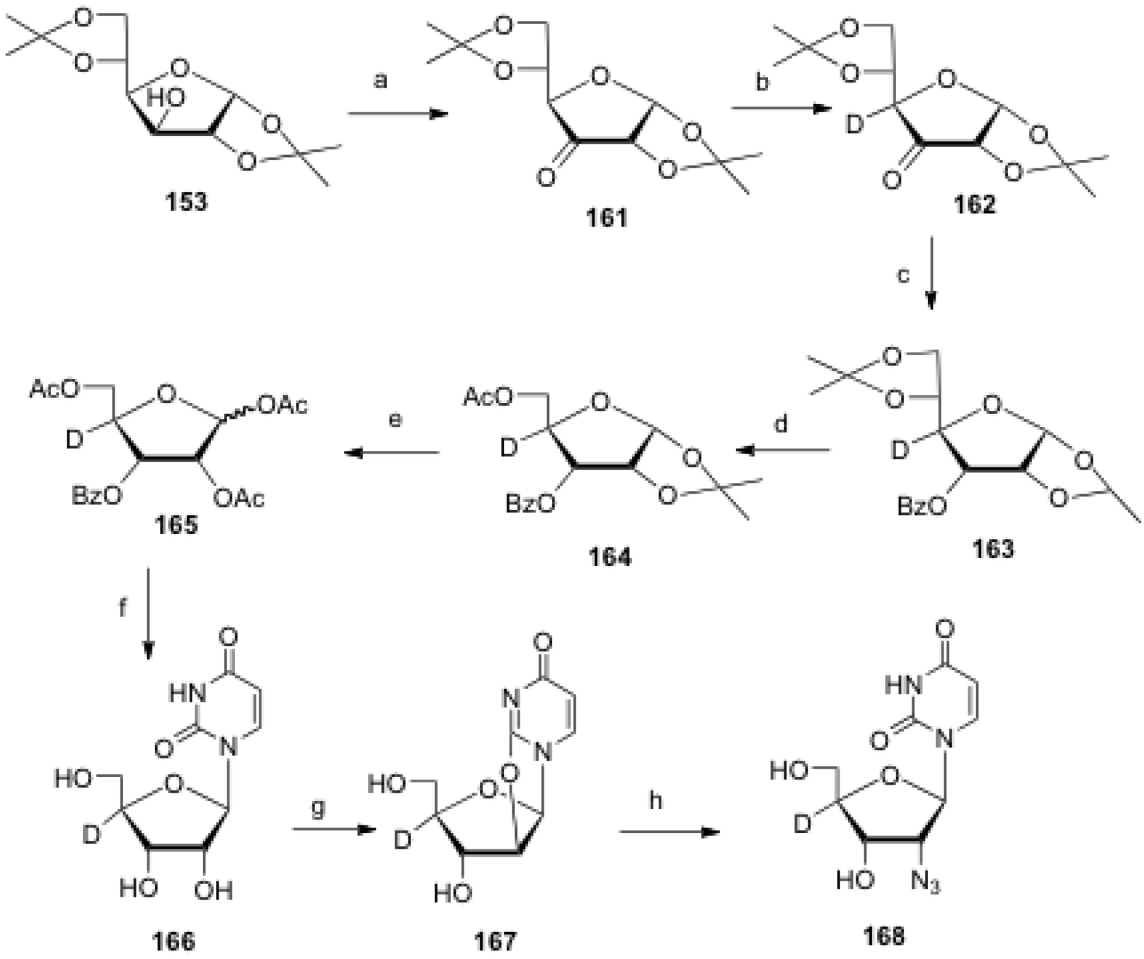
ketoglucose with $NaBD_4$ in acetic acid to afford **154**. Dehomologation of **154** with periodic acid (H_5IO_6) in ethyl acetate afforded the ribofuranose **155** after reduction of the intermediate 5-aldehyde with $NaBH_4$. The tetra-*O*-acetyl ribofuranose **157** was then prepared by standard acetylation, deacetonization, and acetolysis. Subsequent coupling of **157** with persilylated uracil afforded 3'-[^{2}H]-uridine **158**, which was then converted to 3'-[^{2}H]-N_3-dUrd **160**.



Scheme 21. Synthesis of 3'-[^{2}H]-2'-azido-2'-deoxyuridine

3.5.3.4 Synthesis of 4'-[^{2}H]-2'-azido-2'-deoxyuridine

The protected 4'-[^{2}H]-ribofuranoside **165** was also prepared from diacetone-D-glucose precursor with incorporation of deuterium at C4 position by hydrogen/deuterium exchange process by heating 3-ketoglucose **161** in D_2O/pyridine mixture for 3 days.[26] Subsequent reduction and benzoylation afforded 4-[^{2}H]-allose **163**. Dehomologation of **163**, followed by acetolysis yielded the desired precursor **164**. Removal of isopropylidine protection group and standard acetylation provided the 4-[^{2}H]-labeled ribofuranose **165.** Subsequent coupling of **165** with persilylated uracil gave 4'-[^{2}H]-uridine **166**, which was further converted to 4'-[^{2}H]-2'-N_3-dUrd **168**.

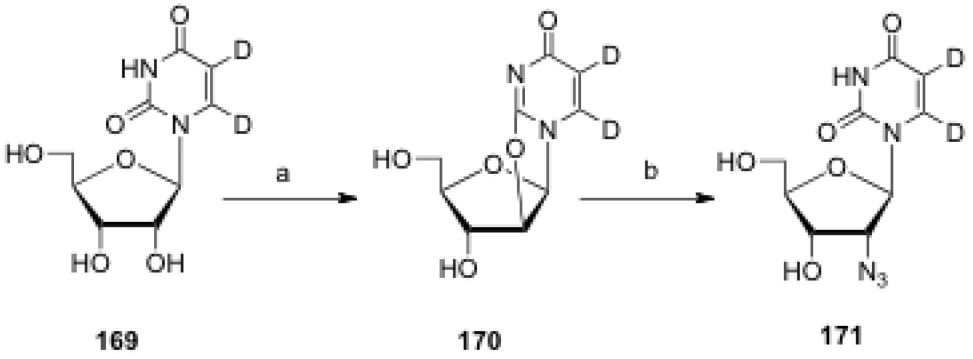


Reagents: (*a*) CrO_3/ py/Ac_2O; (*b*) D_2O/py; (*c*) (i) $NaBH_4$/AcOH, (ii) BzCl/py; (*d*) (i) H_5IO_6/EtOAc, (ii) $NaBH_4$/EtOH, (iii) Ac_2O/py; (*e*) (i) TFA/H_2O, (ii) Ac_2O/py (*f*) (i) uracil/HMDS/TMSSiCl, (ii) TMSOTf/ACN, (iii) NH_3/MeOH; (*f*) diphenylcarbonate/DMF; (*g*) NaN_3/HMPA

Scheme 22. Synthesis of 4'-[^{2}H]-2'-azido-2'-deoxyuridine

3.5.4 Synthesis of 5,6-[$^2H_2$]-2'-azido-2'-deoxyuridine

The 5,6-[$^2H_2$]-2'-azido-2'-deoxyuridine **171** was prepared by analogous procedure as described above. Thus, cyclization of the commerically available 5,6-[$^2H_2$]-uridine and subsequent azidation of the 5,6-[$^2H_2$]-2',2-anhydrouridine **170** afforded 5,6-[$^2H_2$]-N_3-dUrd **171**.



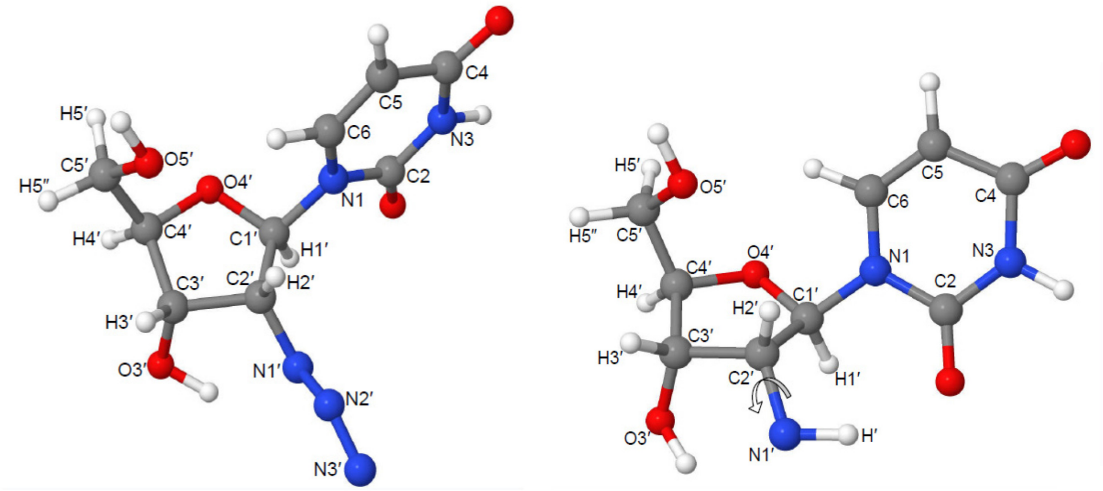
169 170 171

Reagents: (*a*) diphenylcarbonate/$NaHCO_3$/DMF; (*b*) NaN_3/BzOH/HMPA

Scheme 23. Synthesis of 5,6-[$^2H_2$]-2'-azido-2'-deoxyuridine

3.5.5 Establishing the structure of the aminyl radicals formed from 2'-azido-2'-deoxyuridine and its deuterium-labeled analogues

Gamma radiation experiments with 2'-azido-2'-deoxyuridine (2'-N_3-dUrd) and its deuterium labeled analogues were carried out similarly as the experiment performed for AZT using ESR detection for characterization of the aminyl radical species. Before the actual experiment, simulated structure of the aminyl radical and the azide anion radical of 2'-N_3-dUrd was calculated. Figure 41 shows the B3LYP/6-31G* optimized structure of the azide anion radical (dU(C2')-N_3•$^-$) and the aminyl radical (dU(C2')-NH•) of 2'-N_3-dUrd.



dU(C2')-N_3•$^-$ **dU(C2')-NH•)**

Figure 41. B3LYP/6-31G* optimized structure of the azide anion radical (dU(C2')-N_3•$^-$) and the aminyl radical (dU(C2')-NH•) of 2'-N_3-dUrd

Single-electron attachment in 2'-N_3-dUrd at 77 K under the aqueous (H_2O or D_2O) glassy (7.5 M LiCl) systems led to the formation of the neutral aminyl radical (dU(C2')-ND•). In D_2O solutions, for dU(C2')-ND•, the major hyperfine coupling were expected from two sources – the anisotropic hyperfine coupling owing to the N-atom from azide and the isotropic beta-hydrogen coupling due to the H2'- atom (Figure 42, spectrum A). The collapse of the ca. 51.5 G doublet because of the electron attachment in 2'-D-2'-N_3-dUrd at 77 K unequivocally established the presence of the H2'- beta hyperfine coupling in dU(C2')-ND• (see spectrum E vs A). The 51.5 G doublet present in non-labelled 2'-N_3-dUrd did not collapse with deuterium labelled analogs of 2'-N_3-dUrd at positions 3', 4', and 5,6 (see spectra B, C, and D). As expected, ESR spectra characteristic for the aminyl radical derived from 2'-N_3-Urd has similar characteristic to the radical generated from AZT (spectrum D).

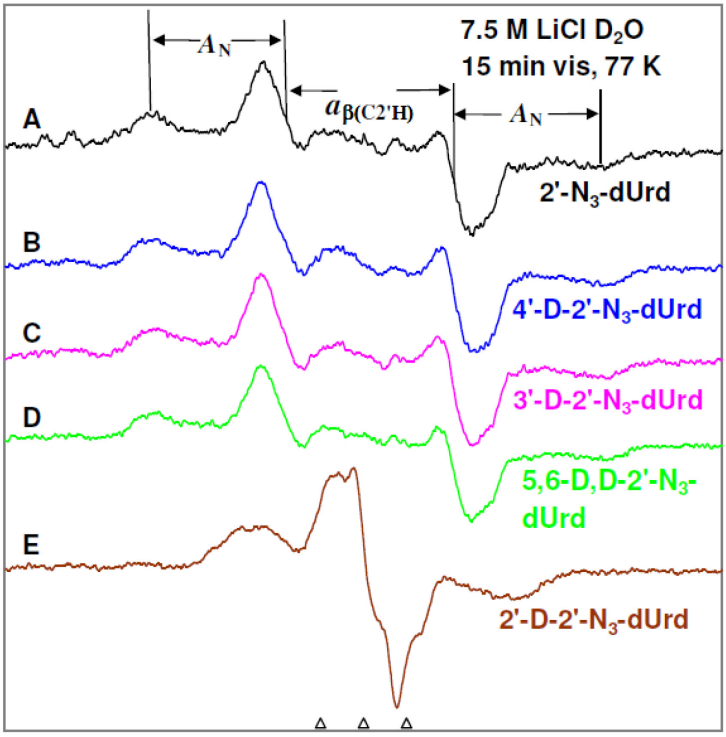


Figure 42. ESR spectral studies of electron attachment to 2'-N_3-dUrd in D_2O glasses (7.5 M LiCl/D_2O)

The ESR spectral studies of one-electron attachment to 2'-N_3-dUrd in H_2O glasses (7.5M LiCl/H_2O) were also performed and compared with the results obtained in D_2O glasses (7.5 M LiCl/D_2O) (Figure 43). Comparison of widths of these two spectra clearly showed that the central doublet due to an extra proton hyperfine coupling in the aminyl radical in H_2O (dU(C2')-NH•) (red color) was present in the H_2O glasses which was missing in the D_2O glasses (green color).

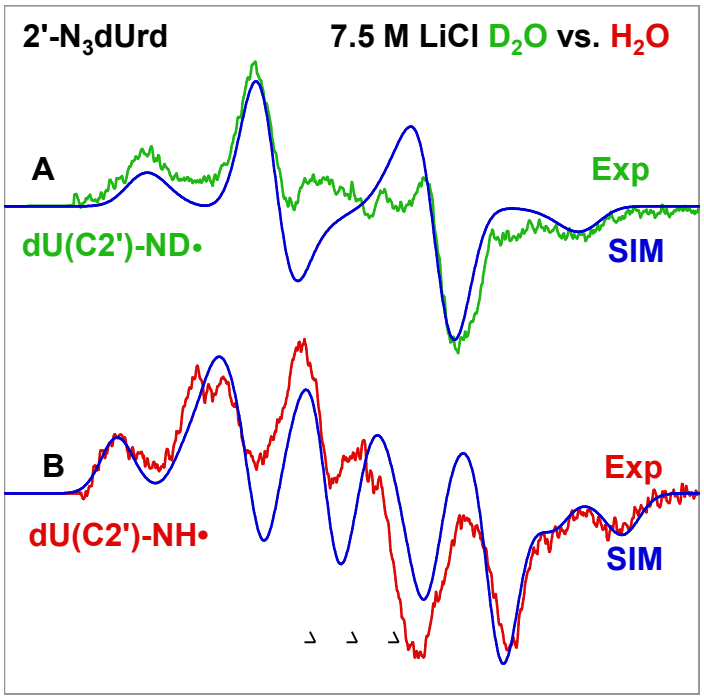


Figure 43. ESR spectral studies of electron attachment to 2'-N_3-dUrd in H_2O glasses (7.5 M LiCl/H_2O)

Figure 44 shows the comparision among the ESR spectra of the RND• generated from 2'-N_3-dUrd, 3'-N_3-dUrd, 3'-AZT, and in 5'-AZT via one-electron attachment at 77 K. Analyses of these spectra along with theoretical calculations showed that the conformational flexibility of the RND• larger when the substitution of the OH group by the azido group takes place at C2' and C5'.

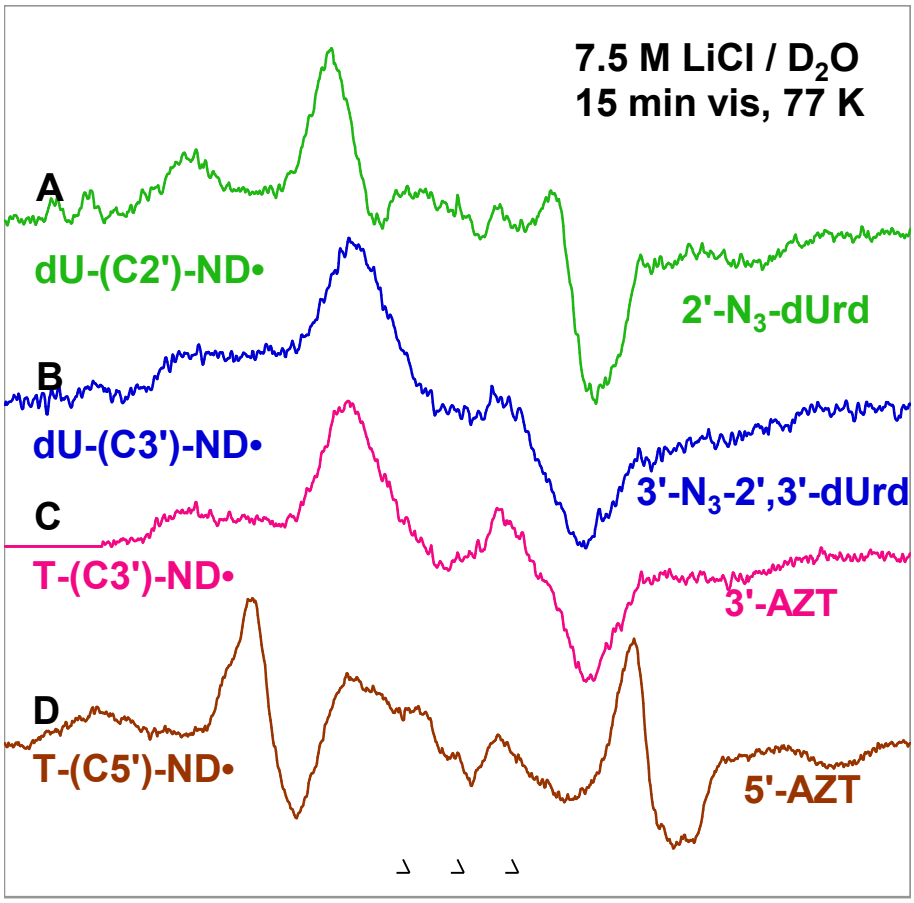


Figure 44. Spectra of the RND• found in 2'-N_3-dUrd, 3'-N_3-dUrd, 3'-AZT, and in 5'-AZT via one-electron attachment at 77 K.

These preliminary ESR results clearly established structure of the aminyl radical with the nitrogen bearing an unpaired electron still attached to the ribose ring. No elimination of azido group via heterolytic cleavage of C2'-N_3 bond, as proposed in RNR inhibition assays, was observed. Other experiments are planned with ^{13}C labeled analogues of 2'-N_3-Urd to characterize the transient sugar-derived radical at ribose ring since its analysis in present spectra were inconclusive because of the strong signal of the nitrogen-centered radical.

4. EXPERIMENTAL

The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were determined with solutions in $CDCl_3$ unless otherwise noted. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) technique and HRMS in AP-ESI mode. TLC was performed with Merck kieselgel 60-F_{254} sheets with products detected with 254 nm light or by development of color with I_2 or Ellman reagent. Merck kieselgel 60 (230-400 mesh) was used for column chromatography. HPLC purifications were performed using XTerra® preparative RP_{18} OBD^{TM} column (5µm 19 x 150 mm) with gradient program using CH_3CN/H_2O as a mobile phase. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 (except THF/potassium) under argon.

Theoretical calculations were performed by Professor Alexander Mebel at Florida International University in Miami, FL. Allylation experiments, activated by microwave (100W), was carried out in the laboratory of Professor Hyun Min Jung at the Korea Research Institute of Chemical Technology (KRICT) in Daejeon, Korea Republic. The study of the interaction between alkyl azide and alkyl thiyl radical, generated via gamma irradiation, was performed in the laboratory of Dr. Chryssostomos Chatgilialoglu at the Consiglio Nazionale delle Ricerche in Bologna, Italy. The study of the interaction between alkyl azide and alkyl thiyl radical, generated via gamma irradiation in glassy (7.5 M $LiCl/D_2O$) systems, and gamma radiation experiments with 2'-azido-2'-deoxyuridine and its deuterium labeled analogues were investigated by Dr. Amitava Adhikary in the laboratory of Professor Michael Sevilla at Oakland University in Rochester, Michigan.

3'-Azido-5'-*O*-(*tert*-Butyldiphenylsilyl)adenosine (80). TBDPSiCl (0.18 mL, 190 mg, 0.69 mmol) was added to a stirred solution of 3'-azido-3'-deoxyadenosine **79**[108] (0.10 g, 0.34 mmol) in anhydrous pyridine (1.5 mL) under N_2 atmosphere at ambient temperature. After 48 h, volatiles were evaporated and the resulting residue was partitioned ($CHCl_3$//HCl/H_2O). The organic layer was washed ($NaHCO_3$/H_2O, brine), dried ($MgSO_4$), and evaporated and the residue was column chromatographed (1 → 6% MeOH/$CHCl_3$) to give **80** (161 mg, 89%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (s, 9, *t*-Bu), 3.82 (dd, *J* = 11.8, 3.0 Hz, 1, H5"), 3.98 (dd, *J* = 11.8, 3.4 Hz, 1, H5'), 4.28 ("q", *J* = 4.3 Hz, 1, H4'), 4.36 (t, *J* = 5.0 Hz, 1, H3'), 4.92 (t, *J* = 5.2 Hz, 1, H2'), 5.99 (d, *J* = 4.9 Hz, 1, H1'), 6.20 (br s, 2, NH_2), 7.37-7.64 (m, 10, Ar), 8.10 (s, 1, H2), 8.24 (s, 1, H8); MS (APCI) *m/z* 531 (MH^+); HRMS (AP-ESI) calcd for $C_{26}H_{31}N_8O_3Si$ $[M+H]^+$ 531.6615.

3'-Azido-3'-deoxy-5'-*O*-TBDPS-2'-*O*-(2-*N*-*tert*-butylcarboxyl-3-*S*-tritylcysteinyl)adenosine (81). Compound **80** (30 mg, 0.057 mmol) was added to a stirred solution of *N*-*t*-butylcarboxyl-*S*-tritylcysteine (0.052 g, 0.113 mmol), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI; 0.030 mL, 0.30 g, 0.170 mmol), and DMAP (0.014 g, 0.113 mmol) in CH_2Cl_2 (5 mL) under N_2 atmosphere at 0^{o}C (ice bath). After 4 h, aqueous 5% HCl (20 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layer was washed with $NaHCO_3$/H_2O, brine, dried ($MgSO_4$), and evaporated. Purification on silica gel column (EtOAc/hexane, 1:1) gave **81** (44 mg, 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (s, 9, *t*-Bu), 1.34 (s, 9, *t*-Bu), 2.54 (dd, *J* = 4.5, 12.6 Hz, 1, Hβ"), 2.63 (dd, *J* = 7.5, 12.4 Hz, 1, Hβ'), 3.71 (dd, *J* = 3.6, 11.4 Hz, 1, H5"), 3.89-3.95 (m, 2, H4',5'), 4.09 ("q", *J* = 6.5 Hz, 1, Hα), 4.83 (t, *J* = 6.3 Hz, 1, H3'),

5.05 (d, J = 8.0 Hz, 1, NH), 5.88-5.92 (m, 2, H1',2'), 5.97 (s, 2, NH_2), (7.10-7.54 (m, 25, Ar), 7.80 (s, 1, H8), 8.11 (s, 1, H2); MS (APCI) *m/z* 976 $[M + H]^+$.

3'-Azido-3'-deoxy-2'-*O*-(*S*-tritylcysteinyl)adenosine (82) The solution of **81** (90 mg, 0.092 mmol) and TFA/H_2O (9:1, 3 mL) was stirred at 0 ^oC for 2h. The volatiles were evaporated and coevaporated with toluene under high vacuum. Purification on silica gel column ($CHCl_3$/MeOH, 9:1) gave **82** (45 mg, 76%): ^1H NMR (MeOH-d_4) δ 2.91 (dd, J = 5.9, 13.9 Hz, 1, Hβ"), 2.96 (dd, J = 8.5, 13.9 Hz, 1, Hβ'), 3.23 (dd, J = 5.3, 8.4 Hz, 1, Hα), 3.76 (dd, J = 2.8, 12.7 Hz, 1, H5"), 3.92 (dd, J = 2.6, 12.7 Hz, 1, H5'), 4.03 ("p", J = 2.8 Hz, 1, H4'), 4.80 (t, J = 5.9 Hz, 1, H3'), 5.93 (dd, J = 4.1, 5.5 Hz, 1, H2'), 6.16 (d, J = 3.9 Hz, 1, H1'), 7.27-7.49 (m, 15, Tr), 8.21 (s, 1, H8), 8.39 (s, 1, H2); ^{13}C NMR (MeOH-d_4): δ 33.36 (Cβ), 53.08 (Cα), 61.59 (C3'), 69.06 (C5'), 78.31 (C2'), 85.17 (C4'), 88.11 (C1'), 120.56 (C5), 128.43, 129.40, 130.67, 145.17 (Tr), 141.39 (C8), 149.98 (C6), 153.42 (C2), 157.14 (C4), 168.43 (CO). MS (APCI) *m/z* 638 $[M + H]^+$.

3'-Azido-3'-deoxy-2'-*O*-cysteinyladenosine (65). TFA (1.0 mL) and Et_3SiH (TES; 0.040 mL, 0.029 g, 0.25 mmol), was added to the solution of **82** (10 mg, 0.016 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at ambient temperature. After 1 h, the volatiles was evaporated and coevaporated with toluene under high vacuum. The residual solid was washed with ethyl ether to give **65** (5 mg, 80%): ^1H NMR (MeOH-d_4) δ 3.19 (dd, J = 4.5, 15.1 Hz, 1, Hβ"), 3.25 (dd, J = 5.4, 15.2 Hz, 1, Hβ'), 3.83 (dd, J = 2.8, 12.7 Hz, 1, H5"), 3.99 (dd, J = 2.8, 12.7 Hz, 1, H5'), 4.22 (dt, J = 2.8, 6.6 Hz, 1, H4'), 4.54 (dd, J = 4.5, 5.3 Hz, 1, Hα), 4.92 (t, J = 6.5 Hz, 1, H3'), 6.09 (dd, J = 3.6, 5.5 Hz, 1, H2'), 6.32 (d, J = 3.5 Hz, 1, H1'), 8.32 (s, 1, H8), 8.52 (s, 1, H2); ^{13}C NMR (MeOH-d_4) δ 25.34 (Cβ), 55.71 (Cα), 61.61 (C3'), 66.93 (C5'), 78.70 (C1'), 85.17 (C4'), 88.47 (C2'), 120.51 (C5), 142.32

(C8), 145.69 (C6), 150.41 (C2), 151.91 (C4), 172.30 (CO); MS (APCI) *m/z* 396 [M + H]$^+$; HRMS (AP-ESI) *m/z* 396.1195 [M + H]$^+$.

3'-Azido-3'-deoxy-5'-*O*-TBDPS-2'-*O*-(2,3-*S*-isopropylidene-2,3-dimercaptopropyl)adenosine (83). Compound **80** (0.12 g, 0.23 mmol) was added to a stirred solution of 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid (0.080 g, 0.45 mmol), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI; 0.16 mL, 0.16 g, 0.90 mmol), and DMAP (0.084 g, 0.68 mmol) in CH_2Cl_2 (10 mL) under N_2 atmosphere at 0^{o}C (ice bath). After 4 h, aqueous 5% HCl (30 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated. Purification on silica gel column (EtOAc/hexane, 1:1) gave **83** (80 mg, 53%; 4:1): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 9, 0.8, *t*-Bu), 1.07 (s, 9, 0.2, *t*-Bu), 1.81, 1.82 (2 × s, 4.8, CH_3), 1.82 (2 × s, 1.2, CH_3), 3.51 (dd, *J* = 6.2, 12.6 Hz, 0.2, Hβ"), 3.55 (dd, *J* = 6.1, 12.6 Hz, 0.8, Hβ"), 3.76-3.78 (m, 0.2, Hβ'), 3.79 (dd, *J* = 5.9, 12.6 Hz, 0.8, Hβ'), 3.85 (dd, *J* = 3.4, 11.8 Hz, 1, H5"), 4.04 (dd, *J* = 3.2, 11.8 Hz, 0.8, H5'), 4.06 (dd, *J* = 3.2, 11.8 Hz, 0.2, H5'), 4.16 (dd, *J* = 3.4, 6.7 Hz, 1, H4'), 4.61 ("q", *J* = 4.0 Hz, 1, Hα), 4.87 (t, *J* = 6.5 Hz, 0.67, H3'), 4.78 (t, *J* = 6.5 Hz, 0.33, H3'), 6.03 ("q", *J* = 2.7 Hz, 0.67, H2'), 5.97 ("q", *J* =2.7 Hz, 0.33, H2'), 6.10 (d, *J* = 3.8 Hz, 0.67, H1'), 6.13 (d, *J* = 3.8 Hz, 0.33, H1'), 6.29 (s, 2, NH_2), 7.35-7.66 (m, 10, Ar), 7.99 (s, 0.67, H8), 8.07 (s, 0.33, H8), 8.27 (s, 1, H2); ^{13}C NMR Major isomer: δ 19.19 (*t*-Bu), 26.79 (C-*t*-Bu), 33.17 (CH_3), 40.17 (Cβ), 56.58 (Cα), 59.87 (C3'), 62.64 (C5'), 63.47 (CCH_3), 76.39 (C1'), 82.61 (C4'), 86.89 (C2'), 120.06 (C5), 127.83, 127.86, 129.95, 130.03, 132.46, 132.53, 135.49, & 135.59 (Ar), 139.26 (C8), 149.51 (C6), 153.31 (C2), 155.57 (C4), 169.94 (CO); Minor isomer: 19.19 (*t*-Bu), 26.84 (C-*t*-Bu), 33.30 (CH_3), 40.21 (Cβ), 56.50 (Cα),

60.00 (C3'), 62.64 (C5'), 63.52 ($\underline{C}CH_3$), 76.31 (C1'), 82.78 (C4'), 86.71 (C2'), 120.06 (C5), 127.86,127.91, 129.98, 130.07, 132.41, 132.50, 135.49, & 135.59 (Ar), 139.07 (C8), 149.51 (C6), 153.31 (C2), 155.57 (CNH_2), 169.99 (CO); MS (APCI) *m/z* 691 $[M + H]^+$.

3'-Azido-3'-deoxy-2'-*O*-(2,3-*S*-isopropylidene-2,3-dimercaptopropyl)adenosine (84). A solution of **83** (0.050 g, 0.072 mmol) in TFA/H_2O (9:1, 1.5 mL) was stirred at 0 ^oC for 2 h. The volatiles were evaporated and coevaporated (toluene) *in vacuo*. Purification on silica gel column (EtOAc) gave **84** (10 mg, 31%, 9:1): ^1H NMR (400 MHz, $CDCl_3$) δ 1.75 (s, 3 x 0.9, CH_3), 1.82 (s, 3 x 0.1, CH_3), 1.80 (s, 3 x 0.9, CH_3), 1.83 (s, 3 x 0.1, CH_3), 3.47 (dd, *J* = 6.2, 12.6, Hz, 0.9, Hβ"), 3.45 (dd, *J* = 6.2, 12.6, Hz, 0.1, Hβ"), 3.68 (dd, *J* = 5.8, 12.6 Hz, 0.9, Hβ'), 3.70 (dd, *J* = 5.8, 12.6 Hz, 0.1, Hβ'), 3.76 (dd, *J* = 0.9, 13.2 Hz, 1, H5"), 4.02 (dd, *J* = 1.3, 13.2 Hz, 1, H5'), 4.30 ("d", *J* = 1.5 Hz, 0.9, H4'), 4.33 ("d", *J* = 1.5 Hz, 0.1 H4'), 4.57 (t, *J* = 6.0 Hz, 0.9, Hα), 4.53 (t, *J* = 6.0 Hz, 0.1, Hα), 4.74 (dd, *J* = 1.8, 5.7 Hz, 0.9, H3'), 4.76 (dd, *J* = 1.8, 5.7 Hz, 0.1, H3'), 5.97-6.00 (m, 3, H2', NH_2), 6.08 (dd, *J* = 1.3, 7.1 Hz, 1, H1'), 7.83 (s, 1, H8), 8.33 (s, 1, H2); ^{13}C NMR major isomer: δ 32.91 and 33.17 (CH_3), 39.64 (Cβ), 55.95 (Cα), 61.67 (C3'), 62.99 (C5'), 63.55 ($\underline{C}CH_3$), 75.38 (C1'), 86.13 (C4'), 88.59 (C2'), 121.08 (C5), 140.25 (C8), 148.47 (C6), 152.64 (C2), 156.00 (C4), 169.79 (CO); Minor isomer: δ 32.98 and 33.26 (CH_3), 39.72 (Cβ), 56.32 (Cα), 62.03 (C3'), 62.99 (C5'), 63.55 ($\underline{C}CH_3$), 75.38 (C1'), 86.30 (C4'), 88.45 (C2'), 121.08 (C5), 140.25 (C8), 148.47 (C6), 152.64 (C2), 156.00 (C4), 169.79 (CO); MS (APCI) *m/z* 453 $[M + H]^+$.

3'-Azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropyl)adenosine (66). $HgCl_2$ (0.078 g, 0.28 mmol) was added to a stirred solution of **84** (10 mg, 0.022 mmol) in MeCN/H_2O

(3:1, 2 mL) at room temperature. After 3 h, the resulting mixture was concentrated to dryness and the residue solid was washed with water, and dried. Hydrogen sulfide, generated from HCl/NaSH, was bubbled through a capillary into the stirring suspension of the dried residue in MeOH (8 mL). After 30 min, the resulting black solid was filtered, and the filtrate was concentrated. Purification on a silica gel column gave **66** (5 mg, 55%): ^{1}H NMR (MeOH-d_4) δ 2.88 (dd, J = 9.6, 13.7 Hz, 1, Hβ"), 2.96 (dd, J = 5.2, 13.7 Hz, 1, Hβ'), 3.65 ("q", J = 4.8 Hz, 1, Hα), 3.83 (dd, J = 3.0, 12.6 Hz, 1, H5"), 3.96 (dd, J = 2.8, 12.6 Hz, 1, H5'), 4.23 ("p", J = 2.8 Hz, 1, H4'), 4.78 (t, J = 7.3 Hz, 1, H3'), 5.99 (dd, J = 4.3, 5.6 Hz, 1, H2'), 6.35 (d, J = 4.3 Hz, 1, H1'), 8.45 (s, 1, H8), 8.70 (s, 1, H2); ^{13}C NMR (MeOH-d_4) 29.78 (Cβ), 44.35 (Cα), 61.77 (C3'), 61.84 (C5'), 78.15 (C1'), 85.52 (C4'), 88.36 (C2'), 120.51 (C5), 143.94 (C8), 145.69 (C6), 149.75 (C2), 151.91 (C4), 172.30 (CO); MS (APCI) *m/z* 413 $[M + H]^+$; HRMS (AP-ESI) *m/z* 413.0816 $[M + H]^+$.

2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid (89). Method A. (**Step A).** TEA (6.6 mL, 4.81 g, 47.48 mmol) and thiolacetic acid (3.38 mL, 3.62 g, 47.48 mmol) were added dropwise to a stirred solution of ethyl 2,3-dibromopropanoate **85** (3.13 mmol, 5.61 g, 21.58 mmol) in EtOH (60 mL) at 0 °C under Ar atmosphere. After 4 h, the volatiles were evaporated and the yellowish solid residue was dissolved in CH_2Cl_2 and was washed with 1 M HCl/H_2O (2×), saturated $NaHCO_3$/H_2O, dried (Na_2SO_4), and evaporated to give ethyl 2,3-bis(acetylthio)propanoate **86**[118] (5.29 g, 98%) of sufficient purity (^{1}H NMR) for direct use in the subsequent reaction: ^{1}H NMR δ 1.28 (t, J = 7.1 Hz, 3, Et), 2.35 (s, 3, Ac_α), 2.39 (s, 3, Ac_β), 3.31 (dd, J = 6.2, 13.8 Hz, 1, H_β), 3.42 (dd, J = 7.7, 13.8 Hz, 1, $H_{\beta'}$), 4.20 (q, J = 7.1 Hz, 2, Et), 4.37 (dd, J = 6.3, 7.6 Hz, 1, H_α). (**Step B).** A dry HCl was bubbled through a vigorously stirred solution of **86** (2.54 g, 10.14

mmol; from step a) in anhydrous EtOH (80 mL) for 3 h at ambient temperature and the resulting solution was continued to stir for 3 days. The reaction mixture was diluted with water (20 mL) and was concentrated. The resulting aqueous solution over yellow oil was extracted with CH_2Cl_2 (2×). The combined organic layer was dried (Na_2SO_4) and was evaporated to give 1.62 g of the brownish oil, which was column chromatographed (EtOAc/hexane, 5:95) to give ethyl 2,3-dimercaptopropanoate **87**[118] (631 mg, 35%): ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.34 (t, *J* = 7.1 Hz, 3, Et) 1.82 (t, *J* = 8.9 Hz, 1, SH), 2.25 (d, *J* = 10.1 Hz, 1, SH), 2.86-3.04 (m, 2, CH_2), 3.47 and 3.49 (ddd, *J* = 5.5, 9.1, 10.0 Hz, 1, CH) 4.27 (q, *J* = 7.1 Hz, 2, Et). (**Step C).** A dry HCl was bubbled through a stirred solution of **87** (93 mg, 0.56 mmol; from step b) in anhydrous acetone (40 mL) for 80 min. at ambient temperature, and the resulting mixture was allowed to stir for 12 h. The mixture was neutralized ($NaHCO_3/H_2O$) and concentrated, and the aqueous residue was extracted with CH_2Cl_2 (2×). The combined organic layer was dried (Na_2SO_4) and was evaporated to give ethyl 2,3-*S*-isopropylidene-2,3-dimercaptopropanoate[171] (101 mg, 88%) as a colorless oil of sufficient purity (^{1}H NMR) for direct use in next step: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (t, *J* = 7.1 Hz, 3, Et), 1.83 (s, 3, CH_3), 1.87(s, 3, CH_3), 3.51 (dd, *J* = 6.1, 12.4 Hz, 1, H_β), 3.77 (dd, *J* = 6.9, 12.4 Hz, 1, $H_{\beta'}$), 4.23 (q, *J* = 7.2 Hz, 2, Et), 4.52 (t, *J* = 6.5 Hz, 1, H_α); ^{13}C NMR δ 14.11 (Et), 33.07 (CH_3), 33.67 (CH_3), 40.22 (CH_2S), 57.09 (CHS), 61.85 (Et), 63.08 (CMe_2), 170.70 (CO). Step (d). An aqueous solution of NaOH (1 M, 3 mL) was added to a solution of ester (100 mg, 0.48 mmol; from step c) in EtOH (3 mL) and the turbid mixture was stirred at ambient temperature for 3 h. The clear solution was concentrated and the aqueous residue was extracted with CH_2Cl_2. The water phase was acidified with diluted HCl (0.5 M), and extracted with

CH_2Cl_2 (2×). The combined organic layer was dried (Na_2SO_4) and was evaporated to give 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid **89**[171] (69 mg, 80%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (s, 3, CH_3), 1.88 (s, 3, CH_3), 3.53 (dd, *J* = 6.1, 12.6 Hz, 1, H_β), 3.76 (dd, *J* = 5.9, 12.6 Hz, 1, $H_{\beta'}$), 4.57 (t, *J* = 6.0 Hz, 1, H_α), 9.31 (br s, 1, COOH); ^{13}C NMR δ 33.21 (CH_3), 33.34 (CH_3), 40.13 (CH_2), 57.08 (CH), 63.50 (CMe_2), 176.40 (COOH); MS (APCI) *m/z* 179 (MH^+).

Method B. (**Step D) 2,3-Dimercaptopropanoic acid (88).** HCl/H_2O (1 M, 4 mL) was added to ethyl 2,3-bis(acetylthio)propanoate **86** (120 mg, 0.51 mmol; step a from Method A described above) and the resulting mixture was heated at reflux for 24 h. The cooled reaction mixture was extracted with CH_2Cl_2 (2×). The combined organic layer was dried (Na_2SO_4) and evaporated to give 2,3-dimercaptopropanoic acid **88**[171] (70 mg, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88 (dd, 1H, *J* = 9.1, 8.6 Hz, 1, SH), 2.35 (d, *J* = 10.1 Hz, 1, SH), 2.93-3.00 (m, 2, CH_2), 3.54 (ddd, *J* = 10.1, 8.4, 5.9 Hz, 1, CH); MS (APCI) *m/z* 139 (MH^+).

Step (E). The 2,3-dimercaptopropanoic acid **88** (538 mg, 3.89 mmol) was heated in acetone (20 mL) in the presence of concentrated HCl (0.1 mL) at 50 ^{o}C for 16 h. Volatiles were evaporated and the residue was partitioned between HCl/H_2O//$CHCl_3$. The organic layer was separated, dried ($MgSO_4$), and evaporated to give 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid **89**[171] (593 mg, 86%) with identical spectroscopic data as reported above.

3'-Azido-5'-*O*-(*tert*-butyldimethylsilyl)-3'-deoxyadenosine (90). A solution of **79**[108] (190 mg, 0.65 mmol) in anhydrous pyridine (3 mL) containing TBDMSCl (128 mg, 0.85 mmol) was stirred overnight at ambient temperature. Next, the volatiles were

evaporated, coevaporated with toluene, and residue was column chromatographed to separate some intact starting material, and next (hexane/EtOAc, 2:8) to give **90** (201 mg, 76%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3, SiMe), 0.07 (s, 3, SiMe), 0.81 (s, 9, C*Me*$_3$), 3.81 (dd, *J* = 2.7, 11.5 Hz, 1, H5'), 3.89 (dd, *J* = 3.5, 11.5 Hz, 1, H5''), 4.29 ('q', *J* = 3.2 Hz, 1, H4'), 4.37 (dd, *J* = 3.4, 5.6 Hz, 1, H3'), 4.85 ('t', *J* = 5.3 Hz, 1, H2'), 5.56 (br., 1, OH), 5.62 (br., 2, NH_2), 5.94 (d, *J* = 5.3 Hz, 1, H1'), 8.07 (s, 1, H8), 8.33 (s, 1, H2); ^{13}C NMR δ -5.57 (SiMe), -5.49 (SiMe), 18.27 (*C*Me$_3$), 25.78 (C*Me*$_3$), 61.62 (C3'), 62.63 (C5'), 76.51 (C2'), 83.88 (C4'), 90.17 (C1'), 119.84 (C5), 138.83 (C8), 148.90 (C4), 152.44 (C2), 155.50 (C6); MS *m/z* (APCI) 407 [MH^+];

2'-*O*-Allyl-3'-azido-5'-*O*-(*tert*-butyldimethylsilyl)-3'-deoxyadenosine (91). NaH (20 mg, 0.492 mmol, 60% dispersion in oil) was added to a stirred solution of **90** (100 mg, 0.246 mmol, vacuum dried at 110ºC for 3h) in DMF (2.5 mL) at room temperature and after 15 min allyl bromide (64 μL, 90 mg, 0.37 mmol) was added. Almost no starting material was detected by TLC, and the volatiles were evaporated after 2h. Residue was partitioned (sat. $NaHCO_3$/H_2O//$CHCl_3$), organic layer was washed (brine), dried (Na_2SO_4), evaporated, and column chromatographed (hexane/EtOAc, 2:8 to EtOAc/ MeOH, 95:5) to give **91** (52 mg, 47%) as a transparent oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14 (s, 3, SiMe), 0.15 (s, 3, SiMe), 0.95 (s, 9, C*Me*$_3$), 3.85 (dd, *J* = 2.5, 11.7 Hz, 1, H5'), 4.10 (dd, *J* = 2.8, 11.7 Hz, 1, H5''), 4.16 (dd, *J* = 5.2, 6.9 Hz, 1, H3'), 4.26 (tdd, *J* = 1.3, 6.0, 12.8 Hz, 1, OC*H*$_2$CH=CH$_2$), 4.26- 4.29 (m, 1, H4'), 4.33 (tdd, *J* = 1.3, 5.5, 12.8 Hz, 1, OC*H*$_2$CH=CH$_2$), 4.58 (dd, *J* = 3.1, 5.1 Hz, 1, H2'), 5.21 (dq, *J* = 1.3, 10.4 Hz, 1, CH=C*H*$_a$H$_b$), 5.31 (dq, *J* = 1.5, 17.2 Hz, 1, CH=CH$_a$*H*$_b$), 5.90 (ddt, *J* = 5.7, 10.4, 17.2 Hz, 1, C*H*=CH$_a$H$_b$), 6.15 (br., 2, NH_2), 6.18 (d, *J* = 3.1 Hz, 1, H1'), 8.21 (s, 1, H8),

8.35 (s, 1, H2); ^{13}C NMR δ -5.50 (SiMe), -5.35 (SiMe), 18.46 (*C*Me$_3$), 25.96 (C*Me*$_3$), 58.68 (C3'), 61.88 (C5'), 71.88 (O*C*H$_2$CH=CH2), 81.85 (C2'), 82.20(C4'), 87.42 (C1'), 118.65 (OCH$_2$CH=*C*H2), 120.06 (C5), 133.29 (OCH$_2$*C*H=CH2), 138.77 (C8), 149.40 (C4), 153.09 (C2), 155.59 (C6); MS (APCI) *m/z* 447 [MH$^+$].

3'-Azido-5'-*O*-(*tert*-butyldimethylsilyl)-2'-*O*-(2,3-dibromopropyl)-3'-deoxyadenosine (92). A solution of bromine (6 uL, 19 mg, 0.117 mmol) in $CHCl_3$ (1.0 mL) was added to a stirred solution of **91** (52 mg, 0.117 mmol) in $CHCl_3$ (1.5 mL) dropwise, at -50ºC, under Ar atmosphere. The reaction mixture was stirred for 20 min at that temperature. Trace of starting material was detected by TLC, and reaction was allowed to warm to 0ºC for another 20 min. Next, after the volume of solution was reduced, reaction mixture was directly applied to a silica gel column, and chromatographed (hexane/EtOAc, 2:8 to EtOAc) to give **92** (31.5 mg, 44%) as a 1:1 mixture of diastereomers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16 (s, 3, Me), 0.17 (s, 3, Me) 0.96 (s, 9, C*Me*$_3$), 3.83-3.86 (m, 2, CH$_2$Br), 3.879 & 3.885 (2×d, *J* = 11.8 Hz, 1, H5'), 4.13 & 4.14(2×d, *J* = 11.8 Hz, 1, H5''), 4.14-4.25 (m, 3, H3', OC*H*$_2$CHBr), 4.27-4.40 (m, 2, H4', CHBr), 4.66 (dd, *J* = 2.7, 5.1 Hz, 0.5, H2'), 4.67 (dd, *J* = 2.7, 5.1 Hz, 0.5, H2'), 5.76 (br., 2, NH$_2$), 6.21 (d, *J* = 2.7 Hz, 0.5, H1'), 6.22 (d, *J* = 2.7 Hz, 0.5, H1'), 8.22 (s, 1, H8), 8.36 (s, 0.5, H2), 8.37 (s, 0.5, H2); ^{13}C NMR δ -5.49, -5.34 (SiMe$_2$), 18.47 (*C*Me$_3$), 25.97 (C*Me*$_3$), 32.25, 32.38 (CH$_2$Br), 47.99, 48.08 (CHBr), 58.70, 58.76 (C3'), 61.66, 61.68 (C5'), 72.02, 72.16 (O*C*H$_2$CHBr), 81.94, 81.98 (C4'), 83.98 (C2'), 87.45, 87.48 (C1'), 120.22 (C5), 138.77 (C8), 149.34 (C4), 153.17, 153.20 (C2), 155.38 (C6); MS (APCI) *m/z* 605 (50), 607 (100), 609 (50) [MH$^+$].

3'-Azido-5'-*O*-(*tert*-butyldimethylsilyl)-3'-deoxy-2'-*O*-[(2,3-diacethylmercapto)propyl]adenosine (93a). KSAc (106 mg, 0.93 mmol) was added to a solution of **92** (47 mg, 0.078 mmol) in DMF (3 mL) stirred under Ar atmosphere, at ambient temperature. No starting material was observed by TLC and ^{1}H NMR after 18h, and the volatiles were evaporated. The residue was partitioned (sat. $NaHCO_3/H_2O//CH_2Cl_2$), the organic layer was washed ($NaHCO_3/H_2O$, brine), dried (Na_2SO_4), evaporated to give 48 mg of colorless oil. The crude product was column chromatographed (Hexane/EtOAc, 1:4 to EtOAc/MeOH, 95:5) to give **93a** (37.5 mg, 81%) as a 1:1 mixture of diastereomers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14 (s, 3, Me), 0.15 (s, 3, Me), 0.94 (s, 9, *t*-Bu), 2.31 (s, 1.5, Ac), 2.33 (s, 1.5, Ac), 2.34 (s, 3, Ac), 3.17 (dd, *J* = 6.6, 13.9 Hz, 0.5, C*H*$_2$SAc), 3.20 (dd, *J* = 6.6, 13.9 Hz, 0.5, C*H*$_2$SAc), 3.36 (dd, *J* = 7.1, 13.9 Hz, 0.5, C*H*$_2$SAc), 3.37 (dd, *J* = 6.6, 13.9 Hz, 0.5, C*H*$_2$SAc), 3.81 (dd, *J* = 5.4, 10.0 Hz, 0.5, OC*H*$_2$CHSAc), 3.858 (d, *J* = 11.8 Hz, 0.5, H5'), 3.864 (d, *J* = 11.8 Hz, 0.5, H5'), 3.86-3.91 (m, 1.5, OC*H*$_2$CHSAc, C*H*SAc), 3.92-3.97 (m, 0.5, OC*H*$_2$CHSAc) 4.01 (dd, *J* = 4.2, 10 Hz, 0.5, OC*H*$_2$CHSAc), 4.10 (br. d, *J* = 11.8 Hz, 0.5, H5''), 4.11 (br. d, *J* = 11.8 Hz, 0.5, H5''), 4.17 (dd, *J* = 2.5, 5.1 Hz, 0.5, H3'), 4.19 (dd, *J* = 2.5, 5.1 Hz, 0.5, H3'), 4.27 ('t', *J* = 2.7 Hz, 0.5, H4'), 4.29 ('t', *J* = 2.7 Hz, 0.5, H4'), 4.57 (dd, *J* = 2.7, 5.5 Hz, 0.5, H2'), 4.58 (dd, *J* = 2.7, 5.5 Hz, 0.5, H2'), 5.99 (br. s, 2, NH_2), 6.15 (d, *J* = 2.7 Hz, 0.5, H1'), 6.16 (d, *J* = 2.7 Hz, 0.5, H1'), 8.20 (s, 0.5, H8), 8.21 (s, 0.5, H8), 8.33 (s, 0.5, H2), 8.34 (s, 0.5, H2); ^{13}C NMR δ -5.36 (Me), -5.07 (Me), 18.46 (*C*Me$_3$), 25.96 (C*Me*$_3$), 30.16, 30.39 (*C*H$_2$SAc), 30.48, 30.57, 30.60 (Ac), 43.59, 43.70 (*C*HSAc), 58.73, 58.77 (C3'), 61.75, 61.76 (C5'), 71.79, 71.82 (O*C*H$_2$CHSAc), 81.94 (C4'), 83.43, 83.64 (C2'), 87.32, 87.45 (C1'), 120.11, 120.14 (C5), 138.83, 138.87 (C8), 149.32, 149.35 (C4),

153.11, 153.12 (C2), 155.48 (C6), 194.10, 194.36, 194.52, 194.55 (SAc); MS (APCI) *m/z* 597 [MH^+];

3'-Azido-3'-deoxy-2'-*O*-[(2,3-diacetylmercapto)propyl]adenosine (93b). A solution of **93a** (37.5 mg, 0.063 mmol) in TFA/H_2O (9:1, 1 mL) was stirred at 0 ^oC for 1h. Next volatiles were evaporated and coevaporated with toluene in to give **93b** (37 mg, 99%) as a 1:1 mixture of diastereomers: ^1H NMR (400 MHz, $CDCl_3$) δ 2.291 (s, 1.5, Ac), 2.293 (s, 1.5, Ac), 2.32 (s, 1.5, Ac), 2.33 (s, 1.5, Ac), 2.97 (dd, *J* = 6.6, 14.1 Hz, 0.5, C*H*$_a$H$_b$SAc), 3.12 (dd, *J* = 6.4, 14.1 Hz, 0.5, CH$_a$*H*$_b$SAc), 3.29 (dd, *J* = 6.4, 14.1 Hz, 0.5, CH$_a$*H*$_b$SAc), 3.34 (dd, *J* = 6.6, 14.1 Hz, 0.5, C*H*$_a$H$_b$SAc), 3.65 (dd, *J* = 5.6, 9.6 Hz, 0.5, OC*H*$_2$CHSAc), 3.68-3.78 (m, 1.5, OCH$_2$C*H*SAc, OC*H*$_2$CHSAc), 3.79-3.88 (m, 1, OCH$_2$C*H*SAc, OC*H*$_2$CHSAc), 3.88 (d, *J* = 12.6 Hz, 1, H5'), 4.12 (d, *J* = 12.6 Hz, 1, H5''), 4.34-4.37 (m, 1, H4'), 4.37-4.40 (m, 1, H3'), 4.68-4.72 (m, 1, H2'), 6.12 (d, *J* = 4.8 Hz, 0.5, H1'), 6.17 (d, *J* = 4.8 Hz, 0.5, H1'), 8.41 (s, 1, H2), 8.72 (s, 0.5, H8), 8.74 (s, 0.5, H8), 8.89 (br., 1, OH); ^{13}C NMR δ 29.87, 30.16 (*C*H$_2$SAc), 30.39, 30.43, 30.53, 30.59 (Ac), 43.50, 43.57 (*C*HSAc), 59.91, 60.00 (C3'), 61.57 (C5'), 71.93, 72.20 (O*C*H$_2$CHSAc), 83.35, 83.41 (C2'), 84.31, 84.33 (C4'), 88.66, 88.79 (C1'), 119.42 (C5), 137.87 (C4), 142.89, 142.92 (C8), 145.05, 145.07 (C2), 151.68 (C6), 194.22, 194.65, 194.93, 194.94 (SAc); MS (APCI) *m/z* 483 (100), 484 (19), [MH^+];

3'-Azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropyl)adenosine (69). A saturated solution of NaOH in MeOH (2.0 mL) was added to a stirred solution of crude **93b** (yielded from 37.5 mg, 0.063 mmol of 19) in MeOH (1.0 mL) under Ar atmosphere, at -30˚C. The stirring was continued for 30 min, and no starting material was detected by TLC. The temperature was diminished to -50 ˚C, reaction mixture was acidified with HCl

(1:1) and allowed to stir for 20 min. Next, reaction mixture was extracted with CH_2Cl_2, extracts were washed with $NaHCO_3/H_2O$, brine, and dried (Na_2SO_4) to give **69** (25 mg, 99%; 1:1 mixture of diastereomers) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (t, *J* = 8.7 Hz, 0.5, CH_2SH), 1.50 (dd, *J* = 7.9, 9.5 Hz, 0.5, CH_2SH), 1.67 (d, *J* = 9.2, 0.5, CHS*H*), 1.76 (d, *J* = 8.9, 0.5, CHS*H*), 2.64-2.80 (m, 1, CH_2SH), 2.77 (dd, *J* = 5.7, 8.7 Hz, 1, CH_2SH), 2.87-2.95 (m, 0.5, C*H*SH), 2.97-3.05 (m, 0.5, C*H*SH), 3.41 (dd, *J* = 6.9, 9.5 Hz, 0.5, OCH_aH_bCHSH), 3.55 (dd, *J* = 5.2, 9.6 Hz, 0.5, OCH_aH_bCHSH), 3.70 (dd, *J* = 6.7, 9.7 Hz, 0.5, OCH_aH_bCHSH), 3.73 (br. 'd', *J* =13 Hz, 1, H5'), 3.82 (dd, *J* = 4.7, 9.5 Hz, 0.5, OCH_aH_bCHSH), 3.98 (br. d, *J* =13.2 Hz, 1, H5''), 4.24 & 4.26 (2× 'q', *J* = 1.0 Hz, 1, H4'), 4.51 (d, *J* = 5.4 Hz, 1, H3'), 5.08 & 5.09 (2×dd, *J* = 5.4, 7.6 Hz, 1, H2') 5.87 & 5.89 (2×d, *J* = 7.6 Hz, 1, H1'), 6.23 (br. 's', 2, NH_2), 6.77 (br., 1, OH), 7.90 & 7.91 (2×s, 1, C8), 8.33 (s, 1, C2); ^{13}C NMR δ 29.37, 29.68 (CH_2SH), 41.49, 41.71 (CHSH), 62.10, 62.12 (C3'), 63.36, 63.38 (C5'), 73.48, 73.73 (OCH_2CHSH), 81.33, 81.69 (C2'), 85.67, 85.70 (C4'), 89.29, 89.32 (C1'), 121.22 (C5), 140.63 (C8), 148.42 (C4), 152.64, 152.66 (C2), 156.19 (C6); MS (APCI) *m/z* 399 [MH^+]

3'-Azido-5'-*O*-(2-*N*-*tert*-butylcarboxyl-3-*S*-tritylcysteinyl)-3'-deoxythymidine (98). 3'-Azido-3'-deoxythymine (**97**; 100 mg, 0.37 mmol) was added to a stirred solution of *N*-*t*-butylcarboxyl-*S*-tritylcysteine (0.35 g, 0.75 mmol), EDCI (0.17 mL, 0.17 g, 1.1 mmol), and DMAP (0.090 g, 0.75 mmol) in CH_2Cl_2 (7.0 mL) under N_2 atmosphere at 0 ^oC (ice bath). After 4 h, the resulting mixture was added to aqueous 5% HCl (30 mL) and was extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated. Purification on a silica gel column (EtOAc/hexane, 1:1) gave **98** (211 mg, 79%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9, *t*-Bu), 2.02 (s, 3, CH_3),

2.33 (dt, *J* = 6.9, 13.9 Hz, 1, H2"), 2.50 (dt, *J* = 6.0, 13.9 Hz, 1, H2'), 2.71 (dd, *J* = 5.5, 12.3 Hz, 1, Hβ"), 2.76 (dd, *J* = 6.2, 12.3 Hz, 1, Hβ'), 4.14 ("q", *J* = 3.7 Hz, 1, H4'), 4.28 (dt, *J* = 4.8, 7.4 Hz, 1, H3'), 4.38 ("q", *J* = 7.0 Hz, 1, Hα), 4.44 (dd, *J* = 3.3, 12.3 Hz, 1, H5"), 4.52 (dd, *J* = 3.6, 12.3 Hz, 1, H5'), 5.31 (d, *J* = 7.7 Hz, 1, NH), 6.23 (t, *J* = 6.3 Hz, 1, H1'), 7.31-7.50 (m, 15, Tr); ^{13}C NMR δ 12.67 (CH_3), 28.29 (*t*-Bu), 33.90 (CH_2S), 37.43 (C2'), 52.71 (C3'), 60.28 (CHNH), 64.22 (C5'), 67.08 (Tr), 80.44 (*t*-Bu), 81.48 (C4'), 85.18 (C1'), 111.50 (C5), 127.05, 128.11, 129.39, & 144.05 (Tr), 135.13 (C6), 150.35 (C2), 154.97 (Boc), 163.98 (C4), 170.73 (CO).

3'-Azido-5'-*O*-cysteinyl-3'-deoxythymidine (67a). TFA (9 mL) and Et_3SiH (0.062 mL, 0.046 mg, 0.393 mmol) were added to a stirring solution of **98** (80 mg, 0.112 mmol) in anhydrous CH_2Cl_2 (9 mL) at 0 ^{o}C. After 3 h, the volatiles were evaporated and the residue was purified on silica gel column (EtOAc → SSE) to give **67a** (35 mg, 85%): ^{1}H NMR (MeOH-d_4) δ 1.80 (s, 3, CH_3), 2.34 (ddd, *J* = 6.1, 7.7, 13.9 Hz, 1, H2"), 2.48 (ddd, *J* = 5.6, 6.9, 12.8 Hz, 1, H2'), 3.05 (dd, *J* = 6.7, 14.2 Hz, 1, Hβ"), 3.11 (dd, *J* = 5.4, 14.3 Hz, 1, Hβ'), 3.94-3.99 (m, 2, H4',α), 4.30-4.38 (m, 3, H3',5",5'), 6.0 (t, *J* = 6.5 Hz, 1, H1'), 7.36 (s, 1, H6); ^{13}C NMR (MeOH-d_4) δ 12.59 (CH_3), 37.39 (Cβ), 38.27 (C2'), 61.70 (C5'), 54.17 (Cα), 65.87 (C3'), 82.70 (C4'), 86.03 (C1'), 111.93 (C5), 138.11 (C6), 152.14 (C2), 166.33 (C4); MS (APCI) *m/z* 371 $[M + H]^+$.

Disulfide of 67a (67b): During purification and/or manipulation in open air, compound **67a** (R_f 0.45 in SSE) slowly oxidized to the corresponding disulfide **67b** (R_f 0.40 in SSE): ^{1}H NMR (MeOH-d_4) δ 1.80 (s, 1, CH_3), 2.34 (ddd, *J* = 5.8, 7.3, 13.8 Hz, 1, H2"), 2.54 (ddd, *J* = 5.8, 8.0, 13.9 Hz, 1, H2'), 3.22 (dd, *J* = 7.0, 14.9 Hz, 1, Hβ"), 3.29 (dd, *J* = 4.9, 14.9 Hz, 1, Hβ'), 3.98 ("p", *J* = 7.3, 13.2 Hz, 1, H4') 4.34-4.44 (m, 4, H3',α,

5", 5'), 5.97 (t, J = 5.9 Hz, 1, H1'), 7.36 (s, 1, H6); ^{13}C NMR (MeOH-d_4) δ 12.47 (CH_3), 36.94 (Cβ), 38.31 (C2'), 53.04 (Cα), 64.39 (C3'), 66.76 (C5'), 82.50 (C4'), 87.91 (C1'), 112.00 (C5), 139.07 (C6), 152.17 (C2), 166.30 (C4), 169.16 (CO). MS (APCI) *m/z* 371 $[M + H]^+$; HRMS (AP-ESI) *m/z* 371.1134 $[M + H]^+$.

3'-Azido-3'-deoxy-5'-O-(2,3-*S*-isopropylidene-2,3-dimercaptopropyl)thymidine (99). Under an argon atmosphere at 0°C, a stirred solution of 2,3-*S*-isopropylidene-2,3-dimercaptopropionic acid (67 mg, 0.376mmol, 2eq) in CH_2Cl_2 (4 mL) was added DMAP (69 mg, 0.562 mmol, 3 eq), and EDCI (131µl, 0.749 mmol, 4 eq). After 5 min AZT (**97**; 50 mg, 0.187 mmol) was added to the reaction mixture, and after 50 min the only product was detected by TLC (progress 30%, after another 50 min – 80%). No starting material was observed after 3h, and RM was diluted with CH_2Cl_2, washed with saturated, aqueous $NaHCO_3$. Next, the organic phase was treated with water, and the pH was adjusted to < 7 with diluted HCl (1:9). Acidic water layer was extracted with CH_2Cl_2, extracts were combined with organic phase, washed with $NaHCO_3$, followed by brine, dried with Na_2SO_4 to give after concentration 105 mg of yellow, oily residue. Silica gel chromatography (EtOAc/hexane, 6:4) gave pure **99** (80 mg, 99%; 0.45:0.55 mixture of diastereomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (s, 1.35, CH_3), 1.82 (s, 1.65, CH_3), 1.83 (s, 1.35, CH_3), 1.84 (s, 1.65, CH_3), 1.97 (s, 3, CH_3), 2.31-2.52 (m, 2, H2',2"), 3.512 (dd, J = 6.1, 12.6 Hz, 0.45, CH_aH$_b$S), 3.515 (dd, J = 6.1, 12.6 Hz, 0.55, CH_aH$_b$S), 3.76 (dd, J = 6.5, 12.6 Hz, 0.45, CH$_a$$H_b$S), 3.80 (dd, J = 6.0, 12.6 Hz, 0.55, CH$_a$$H_b$S), 4.06-4.11 (m, 1, H4'), 4.23-4.29 (m, 1, H3'), 4.31 (dd, J = 3.1, 12.4 Hz, 0.55, H5'), 4.39 (dd, J = 4.2, 12.2 Hz, 0.45, H5'), 4.45 (dd, J = 3.5, 12.2 Hz, 0.45, H5"), 4.556 (t, J = 6.0 Hz, 0.55, CHS), 4.560 (t, J = 6.3 Hz, 0.45, CHS), 4.59 (dd, J

= 3.4, 12.4 Hz, 0.55, H5''), 6.12 (t, *J* = 6.6 Hz, 0.45, H1'), 6.20 (t, *J* = 6.6 Hz, 0.55, H1') 7.21 ('q', *J* = 1.1 Hz, 0.45, H6), 7.27 ('q', *J* = 1.1 Hz, 0.55, H6), 9.54 (br., 1, NH); ^{13}C NMR δ 12.61 (0.45, C5-*C*H$_3$), 12.66 (0.55, C5-*C*H$_3$), 33.19 (0.45, SCC$_a$H$_3$), 33.20 (0.55, SCC$_a$H$_3$), 33.22 (0.55, SCC$_b$H$_3$), 33.24 (0.45, SCC$_b$H$_3$), 37.38 (0.45, C2'), 37.48 (0.55, C2'), 39.83 (0.55, CH$_2$S), 39.86 (0.45, CH$_2$S), 56.51 (0.45, CHS), 56.59 (0.55, CHS), 60.35 (0.55, C3'), 60.60 (0.45, C3'), 63.56 (0.45, SCS), 63.69 (0.55, SCS), 64.12 (0.55, C5'), 64.43 (0.45, C5'), 81.41 (0.45, C4'), 81.69 (0.55, C4'), 85.02 (0.55, C1'), 85.38 (0.45, C1'), 111.49 (1, C5), 135.27 (0.55, C6), 135.31 (0.45, C6), 150.24 (0.45, C2) 150.29 (0.55, C2), 163.78 (0.55, C4), 163.80 (0.45, C4), 170.30 (0.45, COO), 170.32 (0.55, COO).

3'-Azido-3'-deoxy-5'-*O*-(2,3-dimercaptopropyl)thymidine (68). HgCl$_2$ (760 mg, 2.8 mmol, 15 eq) was added in one portion to a stirred solution of **99** (80 mg, 0.187 mmol) in MeCN/H$_2$O (3:1, 8 mL) at ambient temperature. The resulting white suspension was continued to stir for 60 min. and only trace of starting material was detected. The reaction mixture was decanted, and the white solid was washed with water (twice), and dried (evaporated). The mercury complex was suspended in MeOH (25 mL), and H$_2$S was bubbled into the stirring suspension (through a capillary). Almost immediately, the white solid was dissolved, and the black HgS precipitated. After 15 min the reaction mixture was evaporated to dryness, and the black residue was treated with CHCl$_3$ giving a slurry, which was directly chromatographed (60 → 70 %, EtOAc/hexane) to give pure **68** (33.5 mg, 46%; 0.45:0.55 mixture of diasteromers) as an colorless oil: ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.86 (t, *J* = 8.9 Hz, 0.45, SH), 1.88 (t, *J* = 8.9 Hz, 0.55, SH), 1.969 (d, *J* = 1.2 Hz, 1.35, CH$_3$), 1.973 (d, *J* = 1.2 Hz, 1.65, CH$_3$), 2.32 (d, *J* = 10.4 Hz, 0.45, SH), 2.33

(d, *J* = 10.5 Hz, 0.55, SH), 2.38-2.54 (m, 2, H2',2"), 2.92-3.04 (m, 2, C*H*$_2$SH), 3.51 (ddd, *J* = 5.7, 8.7, 10.5 Hz, 0.45, C*H*SH), 3.53 (ddd, *J* = 5.7, 8.7, 10.5 Hz, 0.55, C*H*SH), 4.12 ('q', *J* = 4.3 Hz, 1, H4'), 4.27-4.33 (m, 1, H3'), 4.41 (dd, *J* = 3.6, 12.2 Hz, 0.55, H5'), 4.46 (dd, *J* = 3.6, 12.2 Hz, 0.45, H5'), 4.52 (dd, *J* = 4.3, 12.2 Hz, 0.45, H5") 4.58 (dd, *J* = 4.2, 12.2 Hz, 0.55, H5"), 6.12 (t, *J* = 6.6 Hz, 0.45, H1'), 6.14 (t, *J* = 6.6 Hz, 0.55, H1'), 7.24 (q, *J* = 1.2 Hz, 0.45, H6), 7.25 (q, *J* = 1.2 Hz, 0.55, H6), 8.81 (br., 1, NH); ^{13}C NMR δ 12.67 (0.45, CH_3), 12.71 (0.55, CH_3), 29.43 (1, CH_2SH), 37.44 (1, C2'), 43.56 (0.45, CHSH), 43.62 (0.55, CHSH), 60.38 (0.55, C3'), 60.51 (0.45, C3'), 64.06 (0.55, C5'), 64.26 (0.45, C5'), 81.53 (0.45, C4'), 81.69 (0.55, C4'), 85.56 (0.55, C1'), 85.62 (0.45, C1'), 111.46 (1, C5), 135.43 (1, C6), 149.98 (1, C2), 163.44 (1, C4), 171.50 (0.55, COO), 171.55 (0.45, COO); MS (APCI) *m/z* 388 (MH^+). HRMS (AP-ESI) *m/z* calcd for $C_{13}H_{18}N_5O_5S_2$ $[M + H]^+$ 388.0744; found 388.0748.

3'-Azido-3'-deoxy-*N*-methylthymidine (100). Diazomethane solution in ether (10 mL), generated from a solution of Diazald (3.0g, 14.0 mmol) in ether (25 mL), ethanol (20 mL), and KOH (10 M, 10 mL), was added dropwise to a stirred solution of 3'-azido-3'-deoxythymidine (**97**; 200 mg, 0.75 mmol) in ethanol (15 mL) at 0 ^{o}C. After 30 min, the volatiles were evaporated to give **100** (208 mg, 99 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3, CH_3), 2.38 (ddd, *J* = 13.8, 6.4, 5.4 Hz, 1, H2"), 2.45 (dt, *J* = 13.5, 6.8 Hz, 1, H2'), 3.35 (s, 1, *N*CH_3), 3.83 (dd, *J* = 11.0, 1.3 Hz, 1, H5"), 3.97-4.04 (m, 2, H4' and H5'), 4.43 (dt, *J* = 7.2, 5.0 Hz, H3'), 6.06 (t, *J* = 6.5 Hz, H1'), 7.37 (s, 1, H6); ^{13}C NMR ($CDCl_3$) δ 13.20 (CH_3), 27.82 (*N*CH_3), 37.58 (C2'), 59.96 (C3'), 61.77 (C5'), 84.64 (C4'), 86.81 (C1'), 109.98 (C5), 134.58 (C6), 150.95 (C2), 163.70 (C4); MS (APCI) *m/z* 282 $[M + H]^+$.

5'-*O*-Allyl-3'-azido-3'-deoxy-*N*-methylthymidine (101). A stirred solution of **100** (130 mg, 0.46 mmol) in dry THF (3 mL) at ambient temperature were added KOH (78 mg, 1.39 mmol), 18-crown-6 (5 mg, 0.020 mmol), and allyl bromide (0.12 mL, 168 mg, 1.39 mmol). After 2 h, the volatiles were evaporated and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was concentrated and purified on silica gel column (EtOAc/hexane, 3:7) to afford **101** (120 mg, 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (s, 3, CH_3), 2.31 (dt, *J* = 13.6, 6.6 Hz, 1, H2"), 2.41 (ddd, *J* = 13.8, 6.3, 5.1 Hz, 1, H2'), 3.31 (s, 3, *N*CH_3), 3.63 (dd, *J* = 10.8, 2.5 Hz, 1, H5"), 3.78 (dd, *J* = 10.8, 2.5 Hz, H5'), 4.02 ("quint", *J* = 2.5 Hz, 1, H4'), 4.06 ("q", *J* = 1.4 Hz, 1, OCH_2CH*CH_2*"), 4.08 ("q", *J* = 1.4 Hz, 1, OCH_2CH*CH_2*"), 4.31 (dt, *J* = 6.9, 4.8 Hz, H3'), 5.23 (dq, *J* = 10.4, 1.2 Hz, 1, O*CH_2"*CHCH2), 5.29 (dq, *J* = 17.2, 1.6 Hz, 1, O*CH2'*CHCH2), 5.90 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1, OCH_2*CH*CH_2), 6.27 (t, *J* = 6.2 Hz, 1, H1'), 7.60 (s, 1, H6); ^{13}C NMR ($CDCl_3$) δ 13.28 (CH_3), 27.75 (*N*CH_3), 38.09 (C2'), 60.49 (C3'), 69.38 (C5'), 72.44 (O*CH_2*$CHCH_2$), 83.37 (C4'), 85.47 (C1'), 109.89 (C5), 117.87 (OCH_2CH*CH_2*), 133.40 (OCH_2*CH*CH_2), 133.73 (C6), 150.96 (C2), 163.56 (C4); MS (APCI) *m/z* 322 $[M + H]^+$.

3'-Azido-5'-*O*-(2,3-dibromopropyl)-3'-deoxy-*N*-methylthymidine (102). To a stirred solution of **101** (90 mg, 0.28 mmol) in $CHCl_3$ (2 mL) maintained at -50 °C, a solution of bromine (0.014 mL, 0.045g, 0.28 mmol) in $CHCl_3$ (1 mL) was added dropwise. After 40 min, the reaction mixture was concentrated and purified on a silica gel (EtOac/hexane, 3:7) to give **102** (80 mg, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3, CH_3), 2.31 (ddd, *J* = 13.8, 7.8, 0.92 Hz, 1, H2"), 2.40-2.47 (m, 1, H2'), 3.32 (s, 3, *N*CH_3), 3.69-3.76 (m, 2, H4' and H5"), 3.80 (dd, *J* = 10.4, 4.5 Hz, H5'), 3.92 ("dq", *J* = 11.1, 2.3 Hz, 2, O*CH_2*$CHCH_2$), 4.01- 4.06 (m, 2, OCH_2CH*CH_2*), 4.26-4.32 (m, 1, OCH_2*CH*CH_2),

4.36 ("dt", *J* = 11.5, 4.5 Hz, 1, H3'), 6.25 ("dt", *J* = 10.3, 6.5 Hz, 1, H1'), 7.38 (s, 0.47, H6), 7.42 (s, 0.53, H6); ^{13}C NMR ($CDCl_3$) δ 13.48 (CH_3), 27.85 (NCH_3), 31.77 and 32.04(C4'), 37.80 (C2'), 48.50 and 48.61 (OCH_2CHCH_2), 60.55 and 60.68 (C3'), 70.76 (C5'), 72.61 and 72.66 (OCH_2CHCH_2), 82.97 and 82.98 (OCH_2CHCH_2), 85.48 and 85.50 (C1'), 110.21 and 110.24 (C5), 133.01 and 133.10 (C6), 150.91 and 150.93 (C2), 163.47 and 163.50 (C4); MS (APCI) *m/z* 322 $[M + H]^+$.

3'-Azido-5'-*O*-[(2,3-diacethylmercapto)propyl]-3'-deoxy-*N*-methylthymidine (103). Potassium thioacetate (KSAc, 0.18g, 1.56 mmol) was added to a stirred solution of **102** (75 mg, 0.156 mmol) in DMF (5 mL) at ambient temperature under N_2 atmosphere. After 24 h, the volatiles were evaporated and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was concentrated and purified on silica gel (EtOac/hexane, 4:6) to give **103** (55 mg, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (d, *J* = 1.1 Hz, 1.5, CH_3), 1.98 (d, *J* = 1.1 Hz, 1.5, CH_3), 2.27-2.38 (m, 7, 2Ac and H2"), 2.41-2.48 (m, 1, H2'), 3.03 (dd, *J* = 13.9, 6.6 Hz, 0.5, $SCH_{2"}$), 3.11 (dd, *J* = 13.9, 7.1 Hz, 0.5, SCH_2"), 3.34-3.43 (m, 4, NCH_3 and $SCH_{2'}$), 3.53-3.75 (m, 3, H5", 5', CHSAc), 3.79-3.87 (m, 2, OCH_2CHSAc), 4.03 ("dt", *J* = 7.0, 2.6 Hz, 1, H4'), 4.37 (dt, *J* = 7.0, 4.4 Hz, 0.5, H3'), 4.43 (dt, *J* = 7.0, 4.1 Hz, 0.5, H3'), 6.28 ("q", *J* = 7.0 Hz, 1, H1'), 7.44 (d, *J* = 1.2 Hz, 0.5, H6), 7.45 (d, *J* = 1.2 Hz, 0.5, H6); ^{13}C NMR ($CDCl_3$) δ 13.36 and 13.44 (CH_3), 27.83 and 27.85 (NCH_3), 30.44, 30.49, 30.51, and 30.54 (2Ac), 30.67 and 30.68 ($CHCH_2SAc$), 37.89 (C2'), 43.73 and 43.78 (OCH_2CH), 60.64 and 60.75 (C3'), 70.50 and 70.60 (C5'), 71.95 and 72.18 (*CH*SAc), 83.05 and 83.08 (C4'), 85.40 and 85.50 (C1'), 110.09 and 110.14 (C5), 133.12 and 133.21 (C6), 150.99 (C2), 163.57 and 163.58 (C4), 193.97, 194.09, 194.48, and 194.53 (2*CO*SAc); MS (APCI) *m/z* 472 $[M + H]^+$.

3'-Azido-5'-*O*-(2,3-dimercaptopropyl)-3'-deoxy-*N*-methylthymidine (70). A saturated solution of NaOH in MeOH (4 mL) was added to a stirred solution of **103** (55 mg, 0.117 mmol) in MeOH (2 mL) at -30 ^{o}C under N_2 atmosphere. After 2 h, the reaction mixture was acidified with aqueous HCl (1:1) at -50 ^{o}C and allowed to stir for another 30 min. Next, the reaction mixture was extracted with $CHCl_3$, and the organic layer was washed with aqueous $NaHCO_3$, brine, and dried over $MgSO_4$ to give crude **70** (45 mg, 99 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (ddd, *J* = 12.7, 8.6, 4.0 Hz, 1, CH_2*SH*), 1.84 (dd, *J* = 8.1, 2.0 Hz, 1, CH*SH*), 1.96 ("t", *J* = 1.1 Hz, 3, CH_3), 2.28-2.36 (m, 1, H2"), 2.43 (dd, *J* = 6.4, 5.2 Hz, 0.6, H2'), 2.46 (dd, *J* = 6.4, 5.2 Hz, 0.4, H2'), 2.84 (ddd, *J* = 8.6, 5.8, 2.3 Hz, 2, *CH*$_2$SH), 3.12-3.21 (m, 1, *CH*SH), 3.34 (s, 3, *N*CH_3), 3.65-3.72 (m, 2, O*CH*$_2$CHSH), 3.75 (dd, *J* = 5.6, 1.6 Hz, 0.6, H5"), 3.77 (dd, *J* = 6.2, 2.1 Hz, 0.4, H5"), 3.83 (dd, *J* = 4.9, 2.9 Hz, 0.6, H5'), 3.86 (dd, *J* = 5.0, 2.9 Hz, 0.4, H5'), 4.00-4.03 (m, 1, H4'), 6.22 ("dt", *J* = 8.9, 3.8 Hz, 1, H1'), 7.38 (dd, *J* = 4.0, 1.2 Hz, 1, H6); ^{13}C NMR ($CDCl_3$) δ13.44 and 13.48 (CH_3), 27.84 (*N*CH_3), 29.91 and 29.93 (*CH*$_2$SH), 37.80 and 37.87 (C2'), 41.93 and 42.13 (*CH*SH), 60.36 and 60.44 (C3'), 70.31 and 70.41 (C5'), 74.11 and 74.16 (O*CH2*CHSH), 82.97 (C4'), 85.62 and 85.72 (C1'), 110.18 and 110.22 (C5), 133.08 and 133.14 (C6), 150.90 (C2), 163.47 and 163.48 (C4); MS (APCI) *m/z* 388 $[M + H]^{+}$.

General procedure for reactions of nucleosides 65-70 in the presence of AAPH. To Ar-saturated MeOH-d_4:D_2O (5:3, 4 mL) solution, 3'-azido nucleosides **(65-70)** (~10 mg, 0.026 mmol) and AAPH (~42 mg, 0.16 mmol) were added. The reaction mixture was then heated at 50 ^{o}C. Aliquots were taken at 6h, 12h, 18h, and 24h to monitor the process of the reaction via 'H NMR.

Compound X : After heating compound **68** in the presence of AAPH for 24h, the resulting mixture was evaporated, dissolved in $CHCl_3$, and decanted. The resulting solid was dried to give compound **X**: ^{1}H NMR D_2O δ 1.80 (s, 3, Me), 2.62-2.67 (m, 2, H2',2"), 3.83 (dd, *J* = 4.8, 12.7 Hz, 1, H5'), 3.92 (dd, *J* = 3.4, 12.6 Hz, 1, H5"), 4.08 ("td", *J* = 4.1, 8.3 Hz, 1, H3'), 4.25 (q, *J* = 4.5 Hz, 1, H4'), 6.31 (t, *J* = 6.7 Hz, 1, H1'), 7.65 (s, 1, H6); ^{1}H NMR MeOH-d_4 δ 1.92 (s, 3, Me), 2.49-2.57 (m, 2, H2',2"), 3.82 (dd, *J* = 3.5, 12.0 Hz, 1, H5'), 3.88 (dd, *J* = 3.2, 12.0 Hz, 1, H5"), 4.05 (td, *J* = 4.8, 7.9 Hz, 1, H3'), 4.12 (q, *J* = 3.8 Hz, 1, H4'), 6.32 (t, *J* = 6.8 Hz, 1, H1'), 7.84 (s, 1, H6); MS (APCI) *m/z* 242 [M + H]$^+$.

3'-Azido-3'-deoxythymine (97). Commerically available **97**: ^{1}H NMR D_2O δ 2.00 (s, 3, Me), 2.60 (t, *J* = 6.52, H2',2"), 3.88 (dd, *J* = 4.6, 12.6 Hz, 1, H5'), 3.96 (dd, *J* = 3.5, 12.6 Hz, 1, H5"), 4.11 ("td", *J* = 3.6, 6.4 Hz, 1, H4'), 4.45 (q, *J* = 6.2 Hz, 1, H3'), 6.31 (t, *J* = 6.4 Hz, 1, H1'), 7.74 (s, 1, H6); ^{1}H NMR MeOH-d_4 δ 1.90 (s, 3, Me), 2.35-2.46 (m, 2, H2',2"), 3.74 (dd, *J* = 3.4, 12.2 Hz, 1, H5'), 3.92 (dd, *J* = 3.2, 12.2 Hz, 1, H5"), 3.92 (td, *J* = 3.3, 4.8 Hz, 1, H4'), 4.36 (q, *J* = 5.7, Hz, 1, H3'), 6.18 (t, *J* = 6.4 Hz, 1, H1'), 7.82 (s, 1, H6).

3'-Amino-3'-deoxythymine (AminoT). A solution of **97** (50 mg, 0.19 mmol) in dry DMAC (0.75 ml) and dry benzene (4.5 ml) was treated with Bu_3SnH (0.01 ml, 0.11 g, 0.38 mmol) and a few crystals of AIBN. The solution was deoxygenaed (N_2, 1h) and heated at reflux for 1h. The volatiles were evaporated and the resulting residue was partitioned between $CHCl_3$ and H_2O. The aqueous layer was washed with $CHCl_3$ (3x) and concentrated to give **AminoT** (42 mg, 93 %): ^{1}H NMR D_2O δ 1.79 (s, 3, Me), 2.13-2.35 (m, 2, H2',2"), 3.48 (q, *J* = 7.4 Hz, 1, H3'), 3.69 (dd, *J* = 4.5, 12.1 Hz, 1, H5'), 3.75

("quint", J = 3.5 Hz, 1, H4'), 3.80 (dd, J = 2.8, 12.1 Hz, 1, H5"), 6.12 (dd, J = 4.6, 7.3 Hz, 1, H1'), 7.58 (s, 1, H6); ^{1}H NMR MeOH-d_4 δ 1.90 (s, 3, Me), 2.17-2.32 (m, 2, H2',2"), 3.55 (q, J = 7.1 Hz, 1, H3'), 3.71 ("quint", J = 3.3 Hz, 1, H4'), 3.77 (dd, J = 3.6, 12.2 Hz, 1, H5'), 3.85 (dd, J = 3.0, 12.2 Hz, 1, H5"), 6.19 (dd, J = 4.7, 6.9 Hz, 1, H1'), 7.89 (s, 1, H6).

5'-*O*-acetyl-3'-*N*-acetyl-3'-deoxythymine (5'-Ac-3'-AcNHT). AminoT (10 mg, 0.04 mmol) was dissolved in dry pyridine (0.5 ml) and added Ac_2O (0.03 ml, 0.14 mmol). The reaction mixture was stirred at 0 ^{o}C under N_2 overnight. The volatiles were evaporated and co-evaporated with toluene to give **5'-Ac-3'-AcNHT** (12 mg, 92%): ^{1}H NMR MeOH-d_4 δ 1.91 (s, 3, Ac), 1.97 (s, 3, Ac), 2.10 (s, 3, Me), 2.28-2.44 (m, 2, H2',2"), 4.02 (td, J = 3.6, 6.4 Hz, 1, H4'), 4.31 (dd, J = 3.5, 12.4 Hz, 1, H5'), 4.34 (dd, J = 4.7, 12.4 Hz, 1, H5"), 4.46 (q, J = 7.2 Hz, 1, H3'), 6.21 (dd, J = 5.7, 6.8 Hz, 1, H1'), 7.55 (s, 1, H6).

Compound Y: After heating compound **69** in the presence of AAPH for 24h, the resulting mixture was concentrated, extracted in $H_2O/CHCl_3$, evaporated, and dried. The aqueous layer was purified on a silica gel ($CHCl_3$/MeOH, 8:2) and reverse phase HPLC (H_2O/ACN, 85:15) to give compound **Y**: ^{1}H NMR MeOH-d_4 δ 2.73-2.84 (m, 1, CHS), 3.01-3.13 (m, 2, CH_2S), 3.64-3.72 (m, 1, OCH_2), 3.77 (dd, J = 2.1, 12.7 Hz, 1, H5'), 3.90 (dd, J = 2.0, 12.6 Hz, 0.8, H5"), 4.17-4.22 (m, 1, H4'), 4.52-4.56 (m, 1, H3'), 6.09 (m, 1, H1'), 8.23 (s, 1, H2), 8.38 (s, 1, H6); MS (ESI) *m/z* 369 $[M + H]^+$.

***N*-Allyl-3'-azido-3'-deoxythymidine (104).** The reaction mixture containing **97** (0.67 mmol, 180 mg) and NaH (60%, 0.81 mmol, 32 mg) in DMF (3 mL) was stirred at room temperature under N_2 atmosphere in an ultrasound bath. After 30 min, allyl

bromide (0.81 mmol, 98 mg, 0.070 mL) was added to the reaction mixture and was allowed to stir for another 3h. The volatiles were evaporated, and the residue was purified on silica gel column (EtOac/hexane, 6:4) to give **104** (177 mg, 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (s, 3, CH_3), 2.36-2.49 (m, 2, H2" and H2'), 3.12-3.48 (br, 1, OH), 3.79 (dd, *J* = 12.6, 3.3 Hz, 1, H5"), 3.93-3.96 (m, 2, H5' and H4'), 4.37 ("dt", *J* = 7.0, 5.1 Hz, 1, H3'), 4.51 (d, *J* = 4.8, 2, N*CH*$_2$CHCH$_2$), 5.18 (qd, *J* = 17.2, 1.3 Hz, 2, NCH$_2$CH*CH*$_2$), 5.78-5.88 (m, 1, NCH$_2$*CH*CH$_2$), 6.12 (t, *J* = 6.4 Hz, 1, H1'), 7.55 (d, *J* = 1.0 Hz, 1, H6); ^{13}C NMR ($CDCl_3$) δ 13.18 (CH_3), 37.63 (C2'), 43.34 (N*CH*$_2$CHCH$_2$), 59.93 (C3'), 61.76 (C5'), 84.58 (C4'), 86.60 (C1'), 110.16 (C5), 118.03 (NCH$_2$CH*CH*$_2$), 131.49 (NCH$_2$*CH*CH$_2$), 134.79 (C6), 150.56 (C2), 163.21 (C4); MS (APCI) *m/z* 308 [M + H]$^+$.

3'-Azido-*N*-benzoyl-5'-*O*-*t*BDMSi-3'-deoxythymidine (105). A solution of *tert*-Butyldimethylsilylchloride (0.79 mmol, 119 mg) and **97** (0.75 mmol, 200 mg) in anhydrous pyridine (5 mL) was stirred overnight at ambient temperature. DMAP (1.13 mmol, 137 mg) and benzoyl chloride (2.25 mmol, 316 mg, 0.26 mL) were added to the reaction mixture and was allowed to stir for another 24h at room temperature. After the volatiles were evaporated, the residue was dissolved in EtOAc and washed with aqueous HCl, $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. Purification on silica gel column (EtOAc/hexane, 1:1) afforded **105** (300 mg, 82%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16 (s, 6, CH_3), 0.96 (s, 9, *t*-Bu), 1.97 (s, 3, CH_3), 2.29 (dt, *J* = 13.7, 7.0 Hz, 1, H2"), 2.46 (dq, *J* = 13.6, 4.2 Hz, 1, H2'), 3.82 (dd, *J* = 11.1, 2.0 Hz, 1, H5"), 3.97 (dd, *J* = 11.1, 2.5 Hz, 1, H5'), 3.98 ("q", *J* = 3.1 Hz, 1, H4'), 4.26 (quint, *J* = 3.8 Hz, 1, H3'), 6.22 (t, *J* = 6.5 Hz, 1, H1'), 7.50 (t, *J* = 8.2 Hz, 2, Bz), 7.57 (d, *J* = 1.2 Hz, H6), 7.65 (tt, *J* = 7.4, 1.2

Hz, 1, Bz), 7.93 (dd, *J* = 8.5, 1.4 Hz, 2, Bz); ^{13}C NMR ($CDCl_3$) δ -5.41 (CH_3), -5.33 (CH_3), 12.61 (CH_3), 18.39 (*C*-*t*Bu), 25.96 (*t*Bu), 38.00 (C2'), 60.53 (C3'), 62.98 (C5'), 84.68 (C4'), 86.98 (C1'), 110.92 (C5), 129.15, 130.42, 131.61 and 135.10 (Bz), 135.05 (C6), 149.19 (C2), 162.75 (C4), 168.90 (CO); MS (APCI) *m/z* 486 $[M + H]^+$.

3'-Azido-*N*-benzoyl-3'-deoxythymidine (106). The solution of **105** (0.60 mmol, 290 mg) and CBr4 (0.060 mmol, 20 mg) in dry MeOH was heated at reflux for 1.5h. The volatiles were evaporated. Purification on silica gel column (EtOAc/hexane, 1:1) afforded **106** (200 mg, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3, CH_3), 2.30-2.45 (m, 2, H2" and H2'), 3.14-3.41 (br, 1, OH), 3.71 (dd, *J* = 12.0, 2.5 Hz, 1, H5"), 3.87 (dd, *J* = 12.1, 2.6 Hz, 1H5'), 3.91 ("quint", *J* = 2.4 Hz, 1, H4'), 4.30 ("dt", *J* = 6.8, 5.0 Hz, 1, H3'), 6.12 (t, *J* = 6.4 Hz, 1, H1'), 7.50 (t, *J* = 8.0 Hz, 2, Bz), 7.66 (t, *J* = 7.4 Hz, 1, Bz), 7.73 (d, *J* = 0.92 Hz, 1, H6), 7.91 (dd, *J* = 8.5, 1.3 Hz, 2, Bz); ^{13}C NMR ($CDCl_3$) δ 12.52 (CH_3), 37.70 (C2'), 60.01 (C3'), 61.77 (C5'), 84.76 (C4'), 85.98 (C1'), 110.95 (C5), 129.26, 130.44, 131.40 and 136.66 (Bz), 135.33 (C6), 149.32 (C2), 163.03 (C4), 169.03 (CO); MS (APCI) *m/z* 372 $[M + H]^+$.

***N*-Allyl-3'-azido-5'-*O*-benzoyl--3'-deoxythymidine (107).** The reaction mixture containing **106** (0.54 mmol, 200 mg) and NaH (60%, 0.65 mmol, 26 mg) in DMF (3 mL) was stirred at room temperature under N_2 atmosphere. After 30 min, allyl bromide (0.65 mmol, 78 mg, 0.056 mL) was added to the reaction mixture and was allowed to stir for another 1h. The volatiles were evaporated, and the residue was purified on silica gel column (EtOac/hexane, 3:7) to give **107** (70 mg, 32%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.71 (s, 3, CH_3), 2.38 (ddd, *J* = 13.9, 7.5, 6.3 Hz, 1, H2"), 2.56 (dq, *J* = 14.0, 5.1 Hz, 1, H2'), 4.21 (dt, *J* = 5.1, 3.5 Hz, 1, H4'), 4.35 (dt, *J* = 7.6, 5.1 Hz, 1, H3'), 4.52 (dd, *J* = 5.9,

1.7 Hz, 2, N*CH*$_2$CHCH$_2$), 4.57 (dd, *J* = 12.4, 3.8 Hz, 1, H5''), 4.69 (dd, *J* = 12.4, 3.3 Hz, 1, H5'), 5.18 (qd, *J* = 17.2, 1.4 Hz, 2, NCH$_2$CH*CH$_2$*), 5.81-5.90 (m, 1, NCH$_2$*CH*CH$_2$), 6.21 (t, *J* = 6.4 Hz, 1, H1'), 7.20 (d, *J* = 1.2 Hz, 1, H6), 7.47 (t, *J* = 7.9 Hz, 2, Bz), 7.62 (tt, *J* = 7.5, 1.2 Hz, 1, Bz), 8.04 (dd, *J* = 8.4, 1.4 Hz, 2, Bz) ; ^{13}C NMR (CDCl$_3$) δ 12.99 (CH$_3$), 37.89 (C2'), 43.33 (N*CH$_2$*CHCH$_2$), 60.54 (C3'), 63.54 (C5'), 81.97 (C4'), 85.91 (C1'), 110.58 (C5), 118.09 (NCH$_2$CH*CH$_2$*), 128.72, 129.21, 129.57 and 133.71 (Bz), 131.58 (NCH$_2$*CH*CH$_2$), 133.00 (C6), 150.39 (C2), 162.80 (C4), 166.00 (CO); MS (APCI) *m/z* 412 [M + H]$^+$.

***N*-Allyl-3'-azido-3'-deoxythymidine (104).** The solution of **107** (50 mg, 0.12 mmol) in NH$_3$/MeOH (3 mL, 7M) was stirred at ambient temperature for 5h. After the evaporation of the volatiles, the residue was partitioned between CHCl$_3$ and aqueous HCl, washed with aqueous NaHCO$_3$ and brine, dried over MgSO$_4$, and concentrated to give **104** (35 mg, 95%): NMR data are described above.

Methyl D-ribofuranoside (143a). Procedure A. Concentrated H$_2$SO$_4$ (10 μL) was added to a solution of D-ribose (**142a**, 125 mg, 0.83 mmol) in anhydrous MeOH (2 mL) under N$_2$ at 0 ^{o}C (ice bath). The reaction mixture was maintained at 4 ^{o}C overnight. Neutralization with dry pyridine (0.38 mL) and evaporation of the resulting mixture afforded **143a**[172] as (α/β, 2:3; 160 mg, 85%) of sufficient purity to be used in the next step: ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.41 (s, 1.8H, Me), 3.49 (s, 1.2H, Me), 3.70 (dd, *J* = 4.0, 12.0 Hz, 0.6H, H5), 3.73 (dd, *J* = 4.1, 12.0 Hz, 0.4H, H5'), 3.83 (dd, *J* = 3.2, 11.6 Hz, 0.6H, H5), 3.87 (dd, *J* = 3.2, 11.7 Hz, 0.4H, H5'), 3.98 (dd, *J* = 3.6, 6.4 Hz, 0.4, H3), 4.07-4.14 (m, 2, H2,4), 4.37 (t, *J* = 5.5 Hz, 0.6H, H3), 4.90 (s, 0.6H, H1$_\beta$), 4.99 (d, *J* = 4.4 Hz, 0.4H, H1$_\alpha$); MS (ESI) m/z 165 (MH$^+$).

Methyl 1-[^{2}H]-D-ribofuranoside (143b). Methylation of **142b** (125 mg, 0.83 mmol) by procedure A gave **143b**[172] (160 mg, 85%) as a 3:5 mixture of α and β anomers. ^{1}H NMR was as described for **143a** except for disappearance of H1 peaks at 4.90 and 4.99 ppm and simplification of H2 proton splitting within 4.07-4.14 ppm multiplet: MS (ESI) *m/z* 166 (MH^+).

1,2,3,5-Tetra-*O*-acetyl-D-ribofuranose (144a). Procedure B. (Step A) Acetic anhydride (0.37 mL) was added to the solution of **143a** (160 mg, 0.71 mmol) in dry pyridine (1 mL) under N_2 at 0 ^oC. The reaction mixture was allowed to warm up to rt and continued to stir overnight. EtOAc was added and the solution was washed with water and brine. Evaporation and co-evaporation with toluene gave a 1:5 mixture of α and β anomers of methyl 2,3,5,-tri-O-acetyl-D-ribofuranoside[172] (190 mg, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 3H, Ac), 1.98 (s, 3.6H, Ac), 2.00 (s, 3H, Ac), 2.02 (s, 1.3H, Ac), 3.26 (s, 3, Me), 3.33 (s, 0.6, Me), 3.99 (dd, *J* = 5.6, 11.6 Hz, 1, H5'), 4.10 (dd, *J* = 4.2, 11.7 Hz, 0.2, H5'), 4.15-4.28 (m, 2.4, H5, H4), 4.79 (s, 1, $H1_\beta$), 4.87 (dd, *J* = 4.5, 7.3 Hz, 0.2, H2), 5.01 (d, *J* = 4.5 Hz, 0.2, $H1_\alpha$), 5.06 (dd, *J* = 3.7, 7.4 Hz, 0.2, H3), 5.09 (d, *J* = 4.8 Hz, 1, H2), 5.20 (dd, *J* = 4.9, 6.7 Hz, 1, H3). (**Step B)**. The crude material from acetylation (Step A) was dissolved in acetic acid (1.2 mL) under N_2 in an ice bath. Acetic anhydride (0.27 mL) and concentrated H_2SO_4 (0.070 mL) were added and the resulting mixture was allowed to stir overnight. Ice (2.0g) was added and stirring was continued for 10 min. The product was extracted with $CHCl_3$, and the combined organic layers were washed with water and aqueous $NaHCO_3$, and concentrated. Purification on silica gel column (EtOAc/hexane, 3:7) yielded **144a**[172] (200 mg, 96%) as a 2:5 mixture of α and β anomers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 3, Ac), 1.99 (s, 1.2, Ac), 2.00 (s, 3, Ac),

2.01 (s, 4.2, Ac), 2.03 (s, 1.2, Ac), 2.04 (s, 4.2, Ac), 4.05 (dd, *J* = 5.2, 11.9 Hz, 1, H5'), 4.11 (dd, *J* = 4.0, 12.1 Hz, 0.4, H5'), 4.21-4.30 (m, 2.4, H5, H4), 4.35 ("q", *J* = 3.0 Hz, 0.4, H4), 5.14 (dd, *J* = 4.1, 6.8 Hz, 0.4, H2), 5.16 (dd, *J* = 3.0, 6.6 Hz, 0.4, H3), 5.24 (d, *J* = 4.0 Hz, 1, H2), 5.25 ("q", *J* = 4.8 Hz, 1, H3), 6.06 (s, 1, $H1_\beta$), 6.32 (d, *J* = 4.1 Hz, 0.4, $H1_\alpha$).

1-[^{2}H]-1,2,3,5-Tetra-*O*-acetyl-D-ribofuranose (144b). Acetylation of **143b** (160, 0.71 mmol) by procedure B (step A) gave 1:5 mixture of α and β anomers of methyl 1-[^{2}H]-2,3,5-tri-O-acetyl-D-ribofuranoside[172] (200 mg, 96%). ^{1}H NMR was as described above except for disappearance of H1' peaks at 5.01 and 4.79 ppm and simplification of the proton splitting for H2' at 4.87 ppm (d, *J* = 7.4 Hz). Subsequent acetolysis by procedure B (step B) afforded a 2:5 mixture of α and β anomers of **144b**[172] (215 mg, 98%). ^{1}H NMR was as described above for **144a** except for disappearance of H1 peaks at 6.06 and 6.32 ppm and simplification of proton splitting for H2 at 5.14 ppm (d, *J* = 6.8 Hz) and 5.24 ppm (s).

2-[^{2}H]-2,3-*O*-Isopropylidene-D-ribose (148). Conc. H_2SO_4 (0.075 mL) was added to a suspension of D-ribose (3 g, 0.02 mol) in dry acetone (30 mL) at rt. After stirring for 1.5 h, the reaction mixture was washed with aqueous $NaHCO_3$, dried with $MgSO_4$, and concentrated. The crude residue (~0.40 g, 2.1 mmol) was coevaporated in D_2O and then was dissolved in a mixture of dioxane/tetrahydrofuran/triethylamine/D_2O (2.4:2.4:1.2:1.6 mL). After 6 days of heating the solution at 90 ^{o}C, the reaction mixture was evaporated to give **148**[170] (0.40 g, 10%) with NMR data as reported in literature.

2-[^{2}H]-1,2,3,5-Tetra-*O*-acetyl-D-ribofuranose (149). Procedure C.The solution of **148** (0.40 g, 2.1 mmol) in Ac_2O:pyridine (1:1, 4.0 mL) was stirred at rt overnight. The

concentrated residue was dissolved in EtOAc and washed with aqueous HCl, $NaHCO_3$, and NaCl. Purification on silica gel column (EtOAc/hexane, 3:7) gave [2-^{2}H]-1,5-*O*-diacetyl-2,3-*O*-isopropylidene-D-ribofuranose as an oil residue (150 mg, 26%).

Procedure D. The above crude material (60 mg, 0.23 mmol) was dissolved in TFA:H_2O at 0 ^oC and stirred for 30 min. The reaction mixture was concentrated, coevaporated with toluene, and dried to give [2-^{2}H]-1,5-*O*-diacetyl-D-ribofuranose (50 mg, 98%) as an oily residue. Acetic anhydride (0.5 mL) and pyridine (2 mL) were added and stirring was continued overnight at rt. The reaction mixture was concentrated, dissolved in EtOAc, washed with aqueous HCl, $NaHCO_3$, and brine, and dried. Purification on silica gel column (EtOAc/hexane, 3:7) gave only the β anomer of **149**[173] (56 mg, 82%) as a clear oil. ^{1}H NMR was as described above for **144a** except for disappearance of H2 peak at 5.24 ppm and simplification of proton splitting for H3 at 5.25 ppm (d, J = 6.9 Hz).

3-[^{2}H]-diacetone-D-allose (154). Diacetone-D-glucose **153** (2.0 g, 7.7 mmol) was added to a premixed solution of chromium oxide (3.08 g, 30.8 mmol), pyridine (5.0 mL, 61.5 mmol), and CH_2Cl_2 (20 mL) at 0 ^oC. Acetic anhydride (2.9 mL, 30.7 mmol) was then added slowly to the reaction mixture and continued to stir for 30 min. The reaction mixture was concentrated to half its volume and added into the silica-gel column packed in EtOAc. Elution with EtOAc afforded crude 3-ketoglucose (1.6 g, 81%). The 3-ketoglucose was then dissolved in absolute ethanol (23 mL) and $NaBD_4$ was added (1.14 g, 30.7 mmol). The reaction mixture was first stirred at 0 ^oC and then at rt overnight. Purification on flash chromatography (EtOac) gave **154**[174] (1.2 g, 75%). The NMR data were as reported.

3-[^{2}H]-1,2-*O*-Isopropylidene-D-ribofuranose (155). Procedure E. A solution of **154** (0.50g, 1.9 mmol) in ethyl acetate (12 mL) and periodic acid (0.57 g, 2.5 mmol) was stirred at rt for 3 h. The precipitate was filtered off and washed with EtOAc. The combined filtrate was concentrated, and was dissolved in EtOH (12 mL). $NaBH_4$ was added (146 mg, 3.83 mmol) and stirring was continued for 12 h. Acetic acid (0.5 mL) was added and the volatiles were evaporated. Purification on silica gel column ($CHCl_3$/MeOH, 9:1) afforded **155**[172] (280 mg, 60%) with NMR data were as reported.

3-[^{2}H]-1,2-*O*-Isopropylidine-3,5-diacetyl-D-ribofuranose (156). Acetylation of **155** (0.28 g, 1.47 mmol) as described in procedure C (3h) afforded **156**[175] (300 mg, 75%) with NMR data were as reported.

3-[^{2}H]-1,2,3,5-Tetra-*O*-acetyl-D-ribofuranose (157). Removal of isopropylidine and acetylation of **156** (0.30 g, 1.10 mmol) by procedure D afforded **157**[173] (310 mg, 89%) as a 1:3 mixture of α and β anomers. ^{1}H NMR was as described above for **144a** except for disappearance of H3 peaks at 5.25 and 5.16 ppm, simplification of $H4_\beta$ splitting within 4.21-4.30 ppm multiplet and the collapse of the quartet to triplet of $H4_\alpha$ peak at 4.35 ppm (t, J = 3.7 Hz), as well as simplification of proton splitting for H2 at 5.24 (s) and 5.14 (d, J = 4.6 Hz).

4-[^{2}H]-5-*O*-acetyl-3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose (164). Dehomologation of **163** (0.30 g, 0.82 mmol) as described in procedure E afforded **164**[26] (259 mg, 95%) with NMR data were as reported.

4-[^{2}H]-1,2,5-Tri-*O*-acetyl-3-*O*-benzoyl-α-D-ribofuranose (165). Removal of isopropylidene and standard acetylation of **164** (257 mg, 0.76 mmol) as described in procedure D afforded **165**[26] (200 mg, 68%) as a 1:4 mixture of α and β anomers. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 0.75, Ac), 2.10 (s, 3, Ac), 2.11 (s, 3, Ac), 2.12 (s, 0.75, Ac), 2.17 (s, 0.75, Ac), 2.15 (s, 3, Ac), 4.25 (d, *J* = 12.0 Hz, 1, H5), 4.32 (d, *J* = 12.1 Hz, 0.25, H5), 4.39 (d, *J* = 12.1 Hz, 0.25, H5'), 4.42 (d, *J* = 12.0 Hz, 1, H5'), 5.36 (dd, *J* = 4.5, 6.4 Hz, 0.25, H2), 5.50 (dd, *J* = 1.3, 5.0 Hz, 1, H2), 5.55 (d, *J* = 6.4 Hz, 0.25, H3), 5.61 (d, *J* = 4.9 Hz, 1, H3), 6.25 (d, *J* = 1.3 Hz, 1, $H1_\beta$), 6.53 (d, *J* = 4.5 Hz, 0.25, $H1_\alpha$), 7.47-7.50 (m, 3, Ar), 7.61-7.65 (m, 0.75, Ar), 8.01-8.11 (m, 2.5, Ar).

Uridine (138). Procedure F. (Step A) Uracil (32 mg, 0.28 mmol) and chlorotrimethylsilane (0.047 mL, 0.040 g, 0.37 mmol) were dissolved in hexamethyldisilazane (1.5 mL) at 120 ^oC under N_2, and stirred for 3 h to give the presilylated uracil. The clear solution of presilylated uracil was evaporated and immediately added to the solution of **144a** (50 mg, 0.16 mmol) in acetonitrile (4 mL). Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.035 mL, 0.043 g, 0.20 mmol) was added and allowed to stir at rt for 15 min. The reaction mixture was heated at 70 ^oC under N_2 for 2h. Evaporation and standard work up (EtOAC//aq. $NaHCO_3$, brine) gave 2',3',5'-*O*-triacetyl-uridine (56 mg, 96%). (**Step B**) NH_3/MeOH (2 mL) was added to the 2',3',5'-*O*-triacetyl-uridine in MeOH (2mL) at 0 ^oC. The reaction mixture was stirred for 6 h, evaporated, washed with ether, and dried to give **138**[24] (35 mg, 95%).^{1}H NMR (400 MHz, D_2O) δ 3.70 (dd, *J* = 12.8, 4.4 Hz, 1, H5''), 3.80 (dd, *J* = 12.8, 3.0 Hz, 1, H5'), 4.03 ("q", *J* = 4.1 Hz, 1, H4'), 4.13 (t, *J* = 5.4 Hz, 1, H3'), 4.25 (t, *J* = 5.1 Hz, 1, H2'), 5.89 (d, *J* = 8.1 Hz, 1, H5), 5.81 (d, *J* = 4.8 Hz, 1, H1'), 7.77 (d, *J* = 8.1 Hz, 1, H6); MS (ESI) *m/z* 245 (MH^+).

1'-[^{2}H]-Uridine (145). Coupling of **144b** (50 mg, 0.16 mmol) with uracil by procedure F (step A) gave 2',3',5'-*O*-triacetyl-[1'-^{2}H]-uridine (40 mg, 69%). Subsequent

deacetylation by procedure F (step B) gave **145** (22 mg, 85%). ^{1}H NMR was as described above for **138** except for disappearance of H1' peak at 5.81 ppm and simplification of proton splitting for H2' at 4.25 ppm (d, J = 5.4 Hz). MS (ESI) *m/z* 246 (MH^+).

2'-[^{2}H]-Uridine (150). Coupling of **149** with uracil by procedure F (step A) gave 2',3',5'-*O*-triacetyl-[2'-^{2}H]-uridine (56 mg, 96%). Subsequent deacetylation by procedure F (step B) gave **150**[170] (35 mg, 95%).^{1}H NMR was as described for **138** except for disappearance of H2' peak at 4.25 ppm and simplification of proton splitting for H1' at 5.81 ppm (s) and H3' at 4.13 ppm (d, J = 4.6 Hz). MS (ESI) *m/z* 246 (MH^+).

3'-[^{2}H]-Uridine (158). Coupling of **157** (0.20 g, 0.63 mmol) with uracil by procedure F (step A) gave 2',3',5'-*O*-triacetyl-[3'-^{2}H]-uridine (230 mg, 99%). Subsequent deacetylation by procedure F (step B) gave **158** (140 mg, 97 %).^{1}H NMR was as described for **138** except for disappearance of H3' peak at 4.13 ppm and simplification of proton splitting for H2' at 4.25 ppm (d, J = 4.6 Hz) and H4' at 4.03 ppm (t, J = 2.8 Hz). MS (ESI) *m/z* 246 (MH^+).

4'-[^{2}H]-Uridine (166). Coupling of **165** (148 mg, 0.39 mmol) with uracil by procedure F (step A) gave 2',5'-*O*-diacetyl-3'-*O*-benzoyl-[4'-^{2}H]-uridine (110 mg, 58 %). Subsequent deacetylation by procedure F (step B) gave **166** (55 mg, 60%).^{1}H NMR was as described for **138** except for disappearance of H4' peak at 4.03 ppm and simplification of proton splitting for H3' at 4.13 ppm (d, J = 5.3 Hz), H5' at 3.80 ppm (d, J = 12.2 Hz), and H5" at 3.70 ppm (d, J = 12.2 Hz). MS (ESI) *m/z* 246 (MH^+).

2',3'-*O*-Sulfinyl Uridine (139). Uridine (**138**, 0.50 g, 2.0 mmol) was dissolved into a mixed solution of thionyl chloride (0.65 mL) and ACN (5 mL) with vigourously stirring. The reaction mixture was maintained at 5 ^{o}C for 5 h. The white precipitate

formed after the addition of water (0.2 mL) was filtered, washed with cold ether, and then dried in vacuum. Purification on silica gel column ($CHCl_3$/MeOH, 8:2) gave **139**[165,166] as diastereomers (0.42 g, 67%, 4:1). The NMR data were as reported.

1-(2,2'-Anhydro-β-D-arabinofuranosyl)uracil (140). Method A. The solution of **139** (0.30 g, 1.03 mmol) and NaOAc (0.42 g, 5.2 mmol) was heated in DMF at 85 ^oC, stirred for 4 h, and then cooled. The volatiles were evaporated and the residue was purified on silica gel column (SSE) to afford **140**[165,166] (210 mg, 90%).

Method B. **Procedure G** .Uridine **138** (50 mg, 0.21 mmol) was dissolved in DMF (1.0 mL) and treated with diphenyl carbonate (57 mg, 0.27 mmol) and $NaHCO_3$ (5 mg). The reaction mixture was heated at 150 ^oC for 20 min, cooled down and poured into cold ether (5 mL), and concentrated. Purification by silica gel column ($CHCl_3$/MeOH, 8:2) afforded **140** (20 mg, 43%).^{1}H NMR (400 MHz, D_2O) δ 3.47 (dd , *J* = 4.2, 12.6 Hz, 1, H5'), 3.51 (dd , *J* = 3.6, 12.6 Hz, 1, H5''), 4.31 ("dt", *J* = 1.8, 3.8 Hz, 1, H4'), 4.58 ("br s", 1, H3'), 5.38 (d, *J* = 5.9 Hz, 1, H2'), 6.11 (d, *J* = 7.4 Hz, 1, H5), 6.45 (d, *J* = 5.9 Hz, 1, H1'), 7.83 (d, *J* = 7.4 Hz, 1, H6); MS (ESI) *m/z* 227 ($M + H^+$).

1'-[^{2}H]-1-(2,2'-Anhydro-β-D-arabinofuranosyl)uracil (146). Treatment of 2'-[^{2}H]-uridine **145** (25 mg, 0.10 mmol) by procedure G gave **146** (17 mg, 75 %) with identical physical, chemical, and spectroscopical properties as **140** except except for the disappearance of H1' peak at 6.45 ppm and simplification of proton splitting for H2' at 5.38 ppm (s). MS (ESI) *m/z* 228 ($M + H^+$).

2'-[^{2}H]-1-(2,2'-Anhydro-β-D-arabinofuranosyl)uracil (151). Treatment of 2'-[^{2}H]-uridine **150** (35 mg, 0.14 mmol) by procedure G gave **151** (22 mg, 69 %) with identical physical, chemical, and spectroscopical properties properties as **140** for presence

of residual for H2' peak (~ <5%) and simplification of proton splitting for H1' at 6.45 ppm (s).

3'-[^{2}H]-1-(2,2'-Anhydro-β-D-arabinofuranosyl)uracil (159). Treatment of 3'-[^{2}H]-uridine **158** (50 mg, 0.20 mmol) by procedure G gave **159** (20 mg, 43%) with identical physical, chemical, and spectroscopical properties as **140** except for the disappearance of H3' at 4.58 ppm and simplification of proton splitting for H4' at 4.31 ppm ("t", J = 3.9 Hz).

4'-[^{2}H]-1-(2,2'-Anhydro-β-D-arabinofuranosyl)uracil (167). Treatment of 4'-[^{2}H]-uridine **166** (44 mg, 0.18 mmol) by procedure G gave **167** (25 mg, 63 %) with identical physical, chemical, and spectroscopical properties as **140** except for the disappearance of H4' peak at 4.31 ppm and simplification of proton splitting for H5' at 3.47 ppm (d, J = 12.8 Hz) and H5" at 3.51 (d, J = 12.8 Hz).

5,6-[^{2}H]-1-(2,2'-Anhydro-β-D-arabinofuranosyl)uracil (113). Treatment of 5,6-[^{2}H]-uridine **169** (50 mg, 0.20 mmol) by procedure G gave **170** (20 mg, 43 %) with identical physical, chemical, and spectroscopical properties as **140** except for the disappearance of H5 and H6 peaks at 6.11 ppm and 7.83 ppm, respectively.

2'-Azido-2'-deoxyuridine (141). **Method A**. The stirred suspension of LiF (36 mg, 1.33 mmol) in DMF (1.2 mL) was heated to 105 ^{o}C for 10 min. Then, *N,N,N,N*-tetramethylethylenediamine (TMEDA, 0.66 mL) and trimethylsilylazide (1.8 mL, 0.15 g, 1.33 mmol) were added. After 30 min of stirring at 105 ^{o}C, 2,2'-O-anhydro-1-(β-D-arabinofuranosyl)uracil **140** (0.15 g, 0.66 mmol) was added and allowed to stir at 110 ^{o}C for 48h. The volatiles were evaporated, and MeOH was added to the residue and evaporated (3x). The oily residue was then dissolved in MeOH and EtOAc. The

precipitate salts were filtered, and the filtrate was concentrated and purified on silica gel column (EtOAc/MeOH, 9:1) to afford **141**[165,168] (47 mg, 26%).

Method B. Procedure H. The solution of **140** (30 mg, 0.13 mmol) and NaN_3 (60 mg, 0.93 mmol) in 0.5 mL of HMPA was heated at 150 ^oC. After 30 min of stirring, benzoic acid (16 mg, 0.13 mmol) was added and continued stirring for another 15 min. The reaction mixture was diluted with H_2O and washed with $CHCl_3$. The organic layer was back-extracted with H_2O. The combined aqueous layers were concentrated and purified on silica gel column (EtOAC/MeOH, 9:1) to afford **141** (20 mg, 56%).^{1}H NMR (400 MHz, D_2O) δ 3.70 (dd, *J* = 4.3, 12.8 Hz, 1, H5"), 3.82 (dd, *J* = 2.8, 12.8 Hz, 1, H5'), 4.00 ("ddd", *J* = 2.9, 4.2, 6.0 Hz, 1, H4'), 4.22 ("dd", *J* = 4.5, 5.7 Hz, 1, H2'), 4.35 (t, *J* = 5.9 Hz, 1, H3'), 5.77 (d, *J* = 8.1 Hz, 1, H5), 5.80 (d, *J* = 4.4 Hz, 1, H1'), 7.75 (d, *J* = 8.1 Hz, 1, H6); MS (ESI$^-$) *m/z* 268 (MH^-).

1'-[^{2}H]-2'-Azido-2'-deoxyuridine (147). Azidation of **146** (20 mg, 0.09 mmol) by procedure H afforded **147** (10 mg, 42%) with identical physical, chemical, and spectrosopical properties as **141** except for the disappearance of H1' peak at 5.80 ppm and simplification of proton splitting for H2' at 4.22 ppm (d, *J* = 5.8 Hz); MS (ESI) *m/z* 269 ($M + H^-$).

2'-[^{2}H]-2'-Azido-2'-deoxyuridine (152). Azidation of **151** (22 mg, 0.10 mmol) by procedure H afforded **152** (7 mg, 27%) with identical physical, chemical, and spectrosopical properties as **141** except for the disappearance of H2' at 4.22 ppm and simplification of proton splitting for H1' at 5.80 ppm (s) and H3' at 4.35 ppm (d, *J* = 6.0 Hz); MS (ESI) *m/z* 269 ($M + H^-$).

3'-[^{2}H]-2'-Azido-2'-deoxyuridine (160). Azidation of **159** (20 mg, 0.09 mmol) by procedure H afforded **160** (15 mg, 63%) with identical physical, chemical, and spectrosopical properties as **141** except for the disappearance of H3' peak at 4.35 ppm and simplification of proton splitting for H2' at 4.22 ppm (d, J = 4.5 Hz) and H4' at 4.00 ppm ("dd", J = 2.9, 4.2 Hz); MS (ESI) *m/z* 269 (M + H$^-$).

4'-[^{2}H]-2'-Azido-2'-deoxyuridine (168). Azidation of **167** (25 mg, 0.11 mmol) by procedure H afforded **168** (20 mg, 67%) with identical physical, chemical, and spectrosopical properties as **141** except for the disappearance of H4' peak at 4.00 ppm and simplification of proton splitting for H3' at 4.35 ppm (d, J = 5.8 Hz), H5' at 3.82 ppm (d, J = 12.8 Hz), and H5" at 3.70 ppm (d, J = 12.8 Hz); MS (ESI) *m/z* 269 (M + H$^-$).

5,6-[$^2H_2$]-2'-Azido-2'-deoxyuridine (171). Azidation of **170** (18 mg, 0.08 mmol) by procedure H afforded **171** (11 mg, 52%) with identical physical, chemical, and spectrosopical properties as **141** except for the disappearance of H6 peak at 7.75 ppm and the presence of the residual peak (~ 30%) for H5 at 5.77 ppm (s); MS (ESI) *m/z* 270 (M + H$^-$).

***trans*-2-Ethenylcyclohexanol (130).** Cyclohexene oxide (5.05 mL, 0.050 mol) was added to a stirred solution of CuI (0.95 g, 5.0 mmol) in dry ether (20 mL) at -30 ^{o}C,. Vinyl magnesium bromide (60 mL, 1.0 mmol) was then added dropwise over a period of 1h and the resulting mixture was subsequently stirred for another 1.5h while maintaining the temperature at -30 ^{o}C. Saturated ammonium chloride was added to the red-brown reaction mixture. Washing with aqueous NaCl, drying of the organic layer with Mg_2SO_4, and evaporation afforded **130**[176] (6.1 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65-2.10

(m, 9H), 3.67-3.75 (m, 1, C*H*OH), 5.10-5.19 (m, 2, CH_2 alkene), 5.68 ("quintet", 1, J = 8.5, 17.1 Hz, CH alkene); bp = 40 °C [lit.[176,177] bp 44 °C].

***trans*-2-Ethylthioatecyclohexanol (131).** Thiolacetic acid (0.050 mol, 3.84 g, 3.59 mL) was added slowly to **130** (5.33 g, 0.042 mol) while irradiating with a 250 W bulb under a reflux condenser at ambient temperature. After 24h, the reaction was diluted with ether and washed with aqueous $NaHCO_3$ and brine, dried, and evaporated. Purification on column chromatography (hexane: EtOAc, 85:15) afforded **131** (7.7 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95-1.02 (m, 1H), 1.15-1.34 (m, 4H), 1.40-1.49 (m, 1H), 1.63-1.91 (m, 3H), 1.92-2.01 (m, 2H), 2.32 (s, 3, CH_3), 2.80-2.87 (m, 1, C*Ha*HbSAc), 2.98-3.04 (m, 1 CHa*Hb*SAc), 3.23 (dt J = 5.4, 9.8 Hz, 1, C*H*OH); ^{13}C NMR ($CDCl_3$) δ 24.82 (CH_2), 25.44 (CH_2), 26.89 (CH_2), 30.26 (CH_2SAc), 30.59 (CH_3), 32.70 (CH_2), 44.50 (CH), 74.35 (COH), 196.49 (CO).

***cis*-2-(2-Azidocyclohexyl)ethanethioate (132).** Triphenylphosphine (4.44 mmol, 1.17 g), diphenylphosphoryl azide (DPPA, 4.44 mmol, 1.22 g, 0.96 mL), and diisopropyl azodicarboxylate (DIAD, 4.44 mmol, 0.90 g, 0.86 mL) were added sequentially to the solution of **131** (0.030 mmol, 6.0 g) in dry THF (20 mL). The reaction mixture was stirred at 0 °C for 1h. Evaporation and purification on column chromatography (hexane: EtOAc, 95:5) afforded **132** (176 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23-1.32 (m, 3H), 1.45-1.69 (m, 7H), 1.95-1.98 (m, 1H), 2.33 (s, 3, CH_3), 2.83-2.92 (m, 2, CH_2SAc), 3.77 (s, 1, CHN_3); ^{13}C NMR ($CDCl_3$) δ 20.91 (CH_2), 24.83 (CH_2), 26.62 (CH_2SAc), 26.86 (CH_2), 29.54 (CH_2), 30.63 (CH_3), 32.19 (CH_2), 39.58 (CH), 61.63 (CHN_3), 195.81 (CO); MS (ESI) *m/z* 203 $[M + H]^+$.

***cis*-2-(2-Azidocyclohexyl)ethanethiol (133).** Saturated NaOH in MeOH (10 mL) was added to a solution of **132** (176 mg, 0.77 mmol) in MeOH (5 mL), and stirred under N_2 at -30 ^oC for 3h. HCl solution (1:1) was added to neutralize he reaction mixture and stirred at -50 ^oC for another 30 min. The volatiles were evaporated and washed with cold water//chloroform. The organic layer was washed with cold aqueous $NaHCO_3$ and brine to afford **133** (125 mg, 87%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25-1.35 (m, 3H), 1.43-1.72 (m, 8H), 1.96-2.00 (m, 1H), 2.54-2.60 (m, 2, CH_2SH), 3.77 (s, 1, CHN_3); ^{13}C NMR ($CDCl_3$) δ 20.94 (CH_2), 22.06 (CH_2), 24.85 (CH_2SH), 26.84 (CH_2), 29.57 (CH_2), 36.66 (CH_2), 38.93 (CH), 61.71 (CHN_3); [Turned yellow upon applying Elleman's reagent]. **Disulfide** ^1H NMR (400 MHz, $CDCl_3$) δ 1.19-1.36 (m, 3H), 1.47-1.72 (m, 8H), 1.96-1.98 (m, 1H), 2.68-2.72 (m, 2, CH_2SS), 3.77 (s, 1, CHN_3); [No changes was observed when applying Elleman's reagent].

General method for gamma irradiation. AZT (14.1 mg, 1.0 mM) and l-cysteine (60.7 mg, 10 mM) were dissolved in 50 mL of phosphate buffer solution at pH 7.08. [Buffer solution was made by dissolving 1.56 g and 3.58 g of $NaH_2PO_4 \bullet 2H_2O$ and $Na_2HPO_4 \bullet 12\ H_2O$, respectively, in 1 L of Milli-Q water.] The solution was divided into 5 vials of 9 mL each. The 5 vials of solution were saturated with N_2O (or 9:1 N_2O: O_2) for 30 min before irradiated at 2 kGy, 4 kGy, and 6 kGy, where were equivalent to 313.3, 622.7, and 919.5 min in the gamma cell respectively. At the equivalent time of the dosage, the sample was taken for HPLC and LCMS analysis.

5. CONCLUSION

In this dissertation, I presented a biomimetic simulation reaction for the generation of the nitrogen-centered radicals similar to the one observed during the inactivation of the RNR by azionuclotides. First, density functional calculations disclosed that intramolecular reactions between thiyl radical and azido group resulting in ring closure are facile for both 5'- and 2'-*O*-cysteinate substrates (~40 kcal/mol). For substrates having vicinal disulfide linkage, the calculations also indicated that the reactions are exothermic with low transition state energy barriers (~12.9 kcal/mol) for the β thiyl radical requiring 9-membered transition state for both *S* and *R* diastereomers at Cα. However, the reaction between the α thiyl radical and the azido group did not occur.

Following the encouraging results from theoretical calculations, 3'-azido-3'-deoxynucleosides bearing a cysteinyl or vicinal disulfide substituent attached to C2' or C5' via an ester linkage were successfully synthesized by condensation of the 3'-azido-3'-deoxynucleosides with the cysteine or dimercaptopropionic acid. The more robust analogues having vicinal dithiol group attached at the 2'-OH or 5'-OH groups of the 3'-azido nucleosides via *O*-alkyl linkage were also prepared to improve stability. To avoid competitive allylation of thymine, the nitrogen at position 3 of the heterocyclic base of AZT was methylated with diazomethane prior to the allylation of the 5'-hydroxyl group with allyl bromide. Subsequent bromination and replacement of bromides with thioacetate afforded the AZT analogue with a vicinal dithiol substituent attached through a 5'-*O*-alkyl linkage.

Control experiments of AZT in the presence of AAPH as a radical initiator demonstrated that the azido group of AZT was stable under the studied conditions.

Reactions between cysteine or dimercaptopropionic acid and AZT in the presence of AAPH also indicated that there was also no intermolecular interaction between the generated thiyl radicals and the azido group of AZT. The results from the intramolecular interaction studies between the thiyl radical and azido group of the model nucleosides proved to be a challenging endeavor. Both the ester and ether linkages that attached the thiol or dithiol functionalities to C5' or C2' of the 3'-azido nucleosides were either unstable under the conditions required for the generation of thiyl radicals or underwent other side reactions. However, from heating of 3'-azido-3'-deoxy-2'-*O*-(2,3-dimercaptopropyl)adenosine in the presence AAPH, the data showed the formation of a new nucleoside product that is tentatively assigned as an adenosine derivative with a 1,3,2-dithiazolidine ring attached at C3'.

The aim to study the intramolecular interaction between the thiyl radical and azide was continued with using gamma radiation as a source of radical generator. Both literature and my experiments showed that the reduction of AZT occured from the thiyl radical generated by γ-irradiation of mercaptoethanol happened spontaneously rather than through radical stress. The reaction between cysteine and AZT under γ-irradiation at different conditions provided the plausible conclusion that thiyl radicals from cysteine reacted with AZT to give thymine while the hydrogen sulfide formed during the radical stress from cysteine reduced AZT to its amino counterpart. Under a different condition of γ-irradiation in aqueous (H_2O or D_2O) glassy (7.5 M LiCl) systems, the reaction between the thiyl radicals generated from cysteamine and the azido group of AZT was also shown to be unfeasible. My results suggested that alkyl thiyl radicals are not likely to

react chemically with the alkyl azide to form the nitrogen-centered radical as proposed in the mechanism of the inactivation of the RNR by azidonucleotides.

Although evidence for the reaction between the alkyl thiyl radical and alkyl azide remains elusive, preliminary ESR results from 2'-azido-2'-deoxyuridine and its deuterium-labeled analogues clearly established the structure of the aminyl radical with the nitrogen bearing an unpaired electron still attached to the ribose ring. Furthermore, a transient sugar-derived radical at ribose ring was detected as proposed in RNR inhibition assays. These results give insight toward understanding the mechanism of the formation of the nitrogen-centered radical during the inactivation of RNRs by azidonucleotides as well as the mechanism of action of RNRs that might provide key information necessary for the development of the next generation antiviral and anticancer drugs.

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Ph.D Candidate, Chemistry (Forensic Track), Fall 2010, Florida International University, Dissertation: Biomimetic Modeling of the Nitrogen-centered Radical Postulated to occur during the Inhibition of Ribonucleotide Reductases by 2'-Azido-2'-deoxynucleotides. Advisor: Professor Stanislaw Wnuk

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- Certificate of Excellence as Organic Chemistry Laboratory Teaching Assistant, Emory University, 2004

Publications

1. Dang, Thao; Sobczak, Adam; Mebel, Alexander; Wnuk, Stanislaw. "Biomimetic simulation of reactions postulated to occur during inhibition of ribonucleotide reductases by 2'-azido-2'-deoxynucleotides." *Tetrahedron*. In Preparation
2. Dang, Thao; Adhikary, Amitava; Sevilla, Michael; Wnuk, Stanislaw. "Probing the formation of aminyl radical on electron attachment to 2'-azido-2'-deoxy uridine." *Journal of Physical Chemistry*. In Preparation

Presentations

1. Sacasa, Pablo; Andrei, Daneila; Dang, Thao; Wnuk, Stanislaw "*S*-Adenosylhomocysteine analogues with carbon-5' and sulfur atoms replaced by halovinyl unit". 233th ACS National Meeting, Chicago, IL, March 2007; Division of Carbohydrate Chemistry (lecture).
2. Dang, Thao."Synthesis of model compounds for the biomimetic simulation of reactions postulated to occur during inhibition of ribonucleotide reductases by 2'-azido-2'-deoxynucleotides". Graduate Student Association Scholarly Forum. Chemistry and Physics Oral Presentation. Florida International University. Miami, Florida. March 7th, 2008

3. Dang, Thao; ″Biomimetic simulation of reactions postulated to occur during inhibition of ribonucleotide reductases by 2'-azido-2'-deoxynucleotides″. Graduate Student Association Scholarly Forum. Chemistry and Physics Oral Presentation. Florida International University. Miami, Florida. April 1st, 2009.
4. Dang, Thao; Sobczak, Adam; Rapp, Magda; Mebel, Alexander; Wnuk, Stanislaw. "Biomimetic simulation of reactions postulated to occur during inhibition of ribonucleotide reductases by 2'-azido-2'-deoxynucleotides" 237th ACS National Meeting, Salt Lake City, UT, March 2009; Division of Medicinal Chemistry.
5. Wnuk, Stanislaw; Dang, Thao; Sobczak, Adam; Zayas, Jessica; Mebel, Alexander; "Probing the nucleosides radicals formed during inactivation of ribonucleotide reductase". 19th International Round Table Symposium: Nucleosides, Nucleotides and their Biological Applications. Lyon, France; September 2010.

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