Investigation of Oxidative DNA Damage from Ionizing Radiation

By

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Submitted to the Graduate Faculty as partial fulfillment of the requirements for

the Doctor of Philosophy Degree in Medicinal Chemistry

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Oxidative damage to deoxyribonucleic acids (DNA) caused by the direct and indirect effect of ionizing radiation and endogenously produced reactive oxygen species (ROS) can lead to mutations, carcinogenesis and cell death. Damage from ionizing radiation is partially produced by secondary low energy electrons (LEEs) that form along the ionization track. Although it has been established that LEEs can result in the formation of single and double strand breaks, the mechanism of formation of these associated lesions has not been established. It has been shown that addition of low energy electrons to DNA components leads to the formation of transient radical anions that undergo bond cleavage by dissociative electron attachment. The formation of these reactive intermediates causes the generation of carbon-centered radicals on the sugar moiety. Therefore, it is believed that carbon-centered radicals play a fundamental role in this process. One of the intermediates formed is the C2',3'-dideoxythymidinyl radical (102). The goal of this project is to investigate the fate of the C2',3'-dideoxy-C3'-thymidinyl radical in DNA, an intermediate of DNA-LEE interactions. Synthesis of α- and β-C3'-deoxy-3'-pivaloylthymidine (101 and 103, respectively) as radical precursors
of this intermediate has been completed and their efficiencies in radical generation has been evaluated. Through photolysis ($\lambda \geq 320$ nm) of these modified thymidines under anaerobic conditions, the radical of interest (102) was generated in the case of $\alpha$-C3'-deoxy-3'-pivaloylthymidine (103). These findings contribute to the elucidation of the mechanistic pathways of oxidative damage to DNA by LEEs.

Oxidative damage caused by reactive oxygen species, generated from the ionization of water molecules surrounding DNA or from endogenous sources, result in formation of single and double strand breaks. The hydroxyl radical is capable of damaging the sugar phosphate backbone by abstracting hydrogen atoms from the deoxyribose moiety of DNA leading to formation of single and double strand breaks. These strand breaks are associated with the formation of electrophilic fragments capable of reaction with local nucleophiles to form DNA adducts. Since the 5'-hydrogens of deoxyribose in B-form DNA are the most accessible to solvent, they are highly vulnerable to abstraction. This event leads to the formation of the C5'-radical. Under aerobic conditions and in the presence of thiol the radical forms strand breaks terminated with a 5'-aldehyde moiety and a 3'-phosphate moiety. Oligonucleotides containing a 5'-aldehyde moiety such as 36, are unstable and undergo elimination to deliver the aromatic compound furfural (67). It was shown that furfural can form an adduct with adenine in DNA. The goal of this project is to investigate the stability and reactivity of oligonucleotides containing a 5'-aldehyde lesion and its degradation products under physiological conditions. The half-life of oligonucleotides containing a 5'-aldehyde lesion was determined to be 96 hours in single-stranded DNA. The half-life of 3',4'-didehydro-2',3'-dideoxy-5'-oxothymidine (66) was determined to be 13 hours under physiological...
conditions. These experiments help to explain the types of adducts that form as a result of oxidative damage. These adducts can be used as biomarkers for early detection of disease such as cancer.
I dedicate this dissertation to my mother, my daughters Myes and Tasneem, my sisters Buthina, Sokina, Tahani, Sumia and to my brother Alhaj-Saleh Abdallah.
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</tr>
<tr>
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</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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xxiv
$^1$H NMR  Proton Nuclear Magnetic Resonance Spectroscopy
IR  Ionizing Radiation
LEE  Secondary Low-Energy Electron
MALDI-ToF MS  Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry
MDSB  Multiple Double Strand Breaks
NMR  Nuclear Magnetic Resonance Spectroscopy
nm  Nanometer
ROS  Reactive Oxygen Species
SE  Secondary Electrons
SSB  Single Strand Breaks
ssDNA  Single Strand Deoxyribonucleic Acid
T  Thymine
TBAF  TetraButylammonium Fluoride
TBDMS  $t$-Butyldimethylsilyl
TBDMSCI  $t$-Butyldimethylsilyl Chloride
TBDPSCI  $t$-Butyldiphenylsilyl Chloride
TEAA  Triethylammonium Acetate Buffer
TEAB  Triethylammonium Bicarbonate Buffer
THF  Tetrahydrofuran
UV  Ultraviolet
List of Symbols and Formulas

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<tr>
<th>Symbol</th>
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<td>Radon</td>
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<td>sec-BuLi</td>
<td>sec-Butyllithium</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
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<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
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<tr>
<td>2',3'-unsat.T</td>
<td>2',3'-didehydro-2',3'-dideoxythymidine</td>
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<td>λ</td>
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CHAPTER ONE: Introduction and Background

1.1 Ionizing Radiation (IR)

Ionizing radiation (IR) is composed of particles or waves that have enough energy to liberate electrons from atoms or molecules. IR arises from both natural and man-made sources. Natural sources include cosmic rays, radon gas, generated from the decay of radium; and radioactive elements found in the earth’s crust such as uranium, thorium, potassium and their radioactive decay products; and various radionuclides found naturally in food and drinks. Man-made sources include medical diagnostics and therapies such as X-rays in diagnostic radiology or the use of radiation in cancer treatments, and industrial sources such as those found at research and teaching institutions, nuclear reactors; and radioactive waste.¹

The most common types of radiation emitted from natural sources or man-made radionuclides are alpha particles, beta particles, gamma rays, x-rays, cosmic radiation, and neutron radiation.

1.1.1 Alpha particles (α)

Alpha particles are highly energetic (3-7 MeV), positively charge particles consisting of two protons and two neutrons bound together forming a particle identical to a helium nucleus. Alpha particles are emitted during radioactive decay of the heaviest radioactive elements such as uranium-238, radium-226, and polonium-210. Alpha
particles move slowly through the air due to their high mass and can be stopped by a sheet of paper. Therefore, external exposure is considered not harmful because the alpha particles lack the energy to penetrate the outer dead layer of skin. However, alpha particles are very harmful when alpha particle emitting isotopes are ingested or inhaled.\(^1\,^2\)

### 1.1.2 Beta particles (β)

Beta particles are high-energy, fast moving electrons or positrons emitted from radioactive nuclei such as potassium. Beta particles can be generated from both natural and man-made sources, and are smaller than alpha particles. Beta particles are more penetrating than alpha particles into tissues or materials, but can be absorbed by plastic sheets, glass, aluminum, or layers of clothing. Beta particles are less damaging than alpha over equally traveled distances. Beta particles are most hazardous when they are inhaled or ingested.\(^1\,^2\)

### 1.1.3 Gamma rays (γ)

Gamma rays are very high-energy photons (\(>100\) keV) that are emitted from radioactive nuclei along with alpha and beta particles. Gamma rays can be emitted from natural sources as well as man-made sources. Gamma rays have no charge and no mass and therefore they are very penetrating and require several inches of lead to be stopped. Gamma rays are hazardous to the entire body since they can be easily absorbed by internal organs and tissues.\(^1\,^2\)

### 1.1.4 X-rays

X-rays are high-energy photons (100 eV-100 keV), but are lower in energy than gamma rays and are produced by the interaction of charged particles with matter. X-rays are less penetrating than γ-rays and a few millimeters of lead can stop X-rays. X-rays are
considered to be the largest source of man-made radiation exposure due to their use in medicine and industry for examinations and inspections.¹

1.1.5 Cosmic rays

Cosmic rays are energetic particles from deep space. They are a mixture of different types of radiation including alpha particles, electrons, protons and positrons. They can have energies above $10^{20}$ eV.¹

1.1.6 Neutron (n)

The neutron is emitted by unstable nuclei and has no electric charge. It can be very penetrating. It interacts with matter causing emission of gamma and beta radiation and require heavy shielding to reduce exposure.¹

1.2 Interaction of IR with Biological Systems

Ionizing radiation causes damage to all biological systems with DNA being one of the most important targets. IR induces mutagenicity, genotoxicity and cell death by damaging DNA. This damage includes base and sugar modifications, base release, single and double strand breaks (SSB, DSB), and clustered damage.³ This damage is produced from the energy deposition of the primary particles in cellular medium as well as the secondary species, i.e., ions, radicals and low energy electrons (LEEs) generated along the radiation track.⁴,⁵ These phenomena are called the direct and indirect effects of IR on DNA. In the direct effect, energy from IR deposits in the various nucleic acid components, whereas in the indirect effect energy deposition occurs in water molecules surrounding the DNA. It has been estimated that the direct effect contributes about 40% to cellular DNA damage, while water radiolysis products contribute about 60%.³ The
chemistry involved in the interaction of ionizing radiation with DNA in the direct and indirect effect will be discussed in the following sections.

1.2.1 Direct Effect

1.2.1.1 Interaction of IR with DNA

When high-energy particles or fast charged particles produced by primary radiation such as photoelectrons or Compton electrons pass near DNA molecules, the result is DNA perturbation by rapid change of the electromagnetic field produced by the moving charge. Energy transfer from IR to DNA occurs within femtoseconds of the initiating events and are divided into three groups: primary, secondary and reactive.

In primary events (Figure 1-1), deposited radiation ionizes and excites DNA constituents (reaction 1 and 2, Figure 1-1). For simplification, AB represents simple hypothetical diatomic molecule located in the cell.
During primary events 80% of the energy deposited leads to reaction 1 producing radical cations and secondary electrons (SE). The radical cation either dissociates into fragment ions and SE (reaction 4, Figure 1-1) or reacts with its solvation shell forming in the case of DNA base-OH adduct radicals or can release protons from the sugar moiety to form neutral radicals. SE generated in reaction 1 and 4 have energies less than 30 eV with a most likely energy between 9 and 10 eV. These SE are generated in large numbers (i.e., $\sim 3 \times 10^4$/ MeV of deposited energy) and represent a large portion of primary radiation energy. The rest of the absorbed radiation energy (20%) produces excited molecules (reaction 2, Figure 1-1), which dissociate to produce neutral species (reaction 5, Figure 1-1) or ions (reaction 6, Figure 1-1). Excited molecules may
autoionize if there is enough energy for its own ionization to generate more SE (reaction 3, Figure 1-1).

The formation of secondary electrons is responsible for the secondary events shown in Figure 1-1. Secondary electrons interact with other molecules found in the cells, before their thermalization, and induce more damage, which is referred to as the indirect effect of IR on biological systems. This will be discussed later in this chapter. All reactive species formed during primary and secondary events react with all biological molecules to generate new damage products.

1.2.2 Indirect Effect

1.2.2.1 Interaction of LEE with DNA

As mentioned above, secondary low energy electrons (LEEs) with kinetic energies of 0-30 eV are generated in large amounts by ionizing radiation. The interaction of LEE with DNA results in the formation of single and double strand breaks as well as base release. Secondary LEEs with energies below ~30 eV produced from the direct effect and the ionization of water molecules interact with DNA by two pathways, resonant and nonresonant scattering. In the nonresonant scattering mechanism, LEEs interact with DNA resulting in the reproduction of some or any of the reactions 1-6 (vertical arrow in Figure 1-1), depending on their energies. The outcome of nonresonant scattering is single (SSB) and double (DSB) strand breaks as well as multiple double (MDSB) strand break formations through ionization, excitation and dissociation of DNA components. In the resonant scattering, LEEs cause DNA strand breaks only through the formation of transient anions (reaction 8, Figure 1-1) leading to resonance stabilization (reaction 9, Figure 1-1), dissociative electron attachment (DEA)
(reaction 10, Figure 1-1), and vibrational and electronic excitation (reaction 11, Figure 1-1), which undergoes dissociation to provide neutral and ionic atoms (reaction 12 and 13, respectively, Figure 1-1). In addition, geminate recombination (reaction 14, Figure 1-1) is another way to produce excited molecules and can lead to degradation via reaction 5 and 6 in, Figure 1-1).\(^{4,5}\)

1.2.2.2 Interaction of IR with Water

When IR interacts with the water layers surrounding DNA, ionization and electronic excitation occur in about \(10^{-16}\) seconds resulting in the formation of radical cations and subexcitation electrons (reaction 1, Figure 1-2). Subexcitation electrons have sufficient energy to ionize or excite further water molecules until its energy is no longer enough to cause ionization or excitation of other water molecules. In addition to ionization, excitation of water molecules by the absorption of energy from IR is another pathway leading to the formation of water molecules in their electronic excited state (reaction 2, Figure 1-2).
Figure 1-2: Events induced by absorption of energy by water\textsuperscript{11}

After formation of water radical cations (H$_2$O$^{+}$), subexcitation electrons (e$^{-}_{\text{sc}}$) and electronically excited water molecules (H$_2$O*), reactions can occur within $10^{-16}$ to $10^{-12}$ seconds which are shown in Figure 1-3 (reaction 1-4)$^{11}$. The water radical cation reacts with subexcitation electrons to form water molecules with high vibrational energies, which either decompose to hydrogen atoms (H$^{\cdot}$) and hydroxyl radicals (HO$^{\cdot}$), or generate hydrogen gas (H$_2$) and hydrogen peroxide (H$_2$O$_2$) on a time scale of $10^{-13}$ sec (reaction 1, Figure 1-3). The water radical cation is a strong acid and rapidly loses a proton to surrounding water molecules forming hydronium ion (H$^{+}_{\text{aq}}$) and HO$^{\cdot}$ (reaction 2, Figure 1-3), on a time scale of $10^{-14}$ sec.
Subexcitation electrons can either undergo dissociative attachment to water resulting in the generation of hydroxyl radical, hydrogen gas and hydroxide (reaction 3, Figure 1-3), or can thermalize and become solvated electrons on a time scale of $10^{-12}$ sec. (reaction 4, Figure 1-3). Collectively, the interaction of IR with water molecules results in formation of radical cations, excited water molecules, and subexcitation electrons that further react with each other or with water molecules to produce water radiolysis products (hydrogen atom, hydroxyl radical and solvated electrons) which are capable of causing damage to all cellular components with DNA being a primary target.
1.3 DNA Damage by Hydroxyl Radicals (HO•)

1.3.1 Base Damage in DNA by Hydroxyl Radicals (HO•)

Hydroxyl radicals are generated exogenously as the result of exposure of water to IR as explained above, or endogenously through cellular metabolism, oxidative stress, chronic infection and inflammation. The hydroxyl radical is a small, highly diffusible, highly reactive species with electrophilic properties that are capable of damaging the nucleobase and the sugar moiety of DNA. The hydroxyl radical can add to the C-C and C-N double bonds of purines in DNA or C-C double bonds of pyrimidines. The addition of the hydroxyl radical to the double bonds of nucleobases is regioselective due to its electrophilic nature. For instance, the hydroxyl radical favors addition to the more electron rich C5 position of thymine over the less electron rich C6 position. The hydroxyl radical can also abstract hydrogen atoms from the methyl group of thymine.

1.3.2 Sugar Damage in DNA by Hydroxyl Radicals (HO•)

Hydroxyl radicals are highly reactive oxygen species and are capable of hydrogen atom abstraction from the five positions of the 2'-deoxyribose moiety of DNA. The resulting species are carbon-centered radicals whose reactivity depends on their environment. The order of reactivity of HO’ toward each hydrogen atom of the 2'-deoxyribose moiety was proposed based on solvent accessibility of the sugar hydrogen atoms in B-form DNA (H5' > H4' > H3' ≈ H2' ≈ H1'). The chemistry of each of these sugar radicals is discussed in the following sections.
1.3.2.1 C1'-Radical

The C1'-deoxyribosyl radical is an N-glycosyl radical that is generated through the abstraction of the C1'-hydrogen of the 2'-deoxyribose moiety in DNA by hydroxy radicals generated from exogenous and endogenous sources. The fate of this radical was investigated through the independent generation of 2'-deoxyuridin-1'-yl (1) in single and double stranded DNA (ssDNA, dsDNA).\textsuperscript{20,21} Upon reduction with \( \beta \)-mercaptoethanol (BME) under anaerobic conditions, the 2'-deoxyuridin-1'-yl (1) radical gives rise to a mixture of \( \beta \)- and \( \alpha \)-deoxyuridines (2 and 3 respectively, Scheme 1.1). The \( \beta:\alpha \) ratios are (4.1:1) in ssDNA and (6.2:1) in dsDNA with the restoration of the naturally occurring \( \beta \)-anomer dominate over the premutagenic \( \alpha \)-anomer.\textsuperscript{21} In addition to reduction products, the 2-deoxyribonolactone (7, Scheme 1.1) is formed in small amounts under these conditions. In the presence of oxygen, 2'-deoxyuridin-1'-yl (1) radical is trapped by oxygen at a diffusion-controlled rate to generate peroxyl radical 4. Reduction of 4 by thiol produces ribonolactone 7. In the absence of thiol, the peroxyl radical undergoes superoxide release to generate a carbocation which reacts with water to deliver ribonlactone 7.\textsuperscript{22,23,24} Under physiological conditions, the ribonlactone undergoes \( \beta \)-elimination to form butenolide species 9 which has a half-life of 20 hours in ssDNA and 32-54 hours in dsDNA.\textsuperscript{25} Lesion 9 can undergo a second elimination to release 5-methylene-2-furanone (10), which is a strong electrophile that has the ability to react with cellular nucleophiles. 2-Deoxyribonolactone 7 can induce formation of crosslinks with DNA repair proteins such as polymerase \( \beta \) and endonuclease III, in which the lysine at the active site of these enzymes undergoes nucleophilic attack on the lactone to produce an amide linkage. Normally, during excision of an unmodified abasic site, the lysine
forms a Schiff’s base with the C1-aldehyde in the open form of the native abasic site. The formation of an amide linkage results in the inhibition of DNA repair enzymes (12, Scheme 1.2). Lesion 9 acts as a Michael acceptor that has been shown to be reactive toward sulfur and nitrogen nucleophiles.  

Scheme 1.1: Fate of the 2'-deoxyuridin-1'-yl (1) radical
Oxidation at the C2'-deoxyribose moiety in DNA occurs through the abstraction of either one of the two hydrogen atoms. This carbon centered radical has been generated by γ-irradiation and by the abstraction of a 2'-hydrogen by the photochemically generated 5-uridinyl radical. Trapping of C2'-radical with oxygen leads to the formation of peroxyl radical. The fate of 13 depends on the conformation of DNA when its formed. For example, when 13 is formed in B-form DNA the erythrose abasic site 16 is the major product, while in Z-form DNA the primary product is ribonucleotide 17. The erythrose abasic site 16 is stable to hydrolysis in comparison to other abasic sites, with a half-life of 3 h in 0.1 M NaOH at 37 °C.
Scheme 1.3: Fate of the C2'-deoxyguanyl radical\textsuperscript{33}

1.3.2.3 C3'-Radical

The C3'-deoxyriboosyl radical (18) is an α-phosphatoxyalkyl radical generated by the abstraction of the C3'-hydrogen atom by the hydroxyl radical. The fate of this radical was investigated using Rh-phenanthrenequinone diimine complexes\textsuperscript{34,35} and site-specific radical generation using photolabile groups at the C3' position.\textsuperscript{36} Under anaerobic conditions, it has been suggested that 18 is oxidized to a carbocation 19 that undergoes solvolysis to yield 20. Compound 20 undergoes elimination to yield 3'-ketonucleotide 21 and 5'-phosphorylated oligonucleotide 8. Compound 21 can undergo a second elimination to release 3'-phosphate 11, small sugar fragment 22 and nucleobase 23. The decomposition of 18 under aerobic conditions results in formation of peroxyl radical 24 which converts to hydroperoxide 25 in the presence of thiol. The hydrogen peroxide (25) undergoes a Criegee-type rearrangement to generate intermediate 26. Subsequent
decomposition leads to the formation of base propenoate 29, 5'-Phosphate 8, and 3'-phosphoglycolaldehyde (3'-PGA, 30) (Scheme 1.4).\textsuperscript{37}

Scheme 1.4: Fate of C3'-deoxyribosyl radical proposed by Stubbe et.al.\textsuperscript{37,34}
It was shown by Bryant-Friedrich that in the presence of thiol and in the absence of oxygen, the C-3' radical in single stranded DNA is reduced to give the repaired 2'-deoxyribose 31, and possibly the pseudorepair 2-deoxyxylose 32 in oligomers (Scheme 1.5). Under aerobic conditions, trapping of 18 results in formation of isomeric peroxyl radicals 33 and 34 which subsequently degrade to give rise to strand breaks 8 and 11 as the major products. Lesions 30, 3'-phosphoglycolate (3'-PG) 35, and 5'-aldehyde 36 were also detected from experiments performed under aerobic conditions and confirmed by mass spec analysis. Formation of 30 can be explained, as mentioned above, through molecular rearrangement of the peroxyl radical formed at the C3'-position, while formation of 35 and 36 can be explained by oxidation of the C4' radicals of the same nucleotide and the oxidation of C5' radicals of the 3'-adjacent nucleotide, respectively. Compound 37 can undergo a second elimination step to release 3'-phosphate 11, and small sugar fragment (22) (Scheme 1.5).
It was observed by Awada et al. that 2-phosphoglycolaldehyde (38), a model for 3'-PGA (30), decomposes to glyoxal under biological conditions and reacts with dG (40).
in DNA to form the diasteromeric 1,N2-glyoxal adducts of dG (41 and 42, Scheme 1.6).\textsuperscript{40}

\begin{center}
\includegraphics[width=\textwidth]{Scheme1.6.png}
\end{center}

Scheme 1.6: DNA adducts derived from phosphoglycolaldehyde\textsuperscript{40}

1.3.2.4 C4'-Radical

The C4'-radical has been the most studied sugar radical in DNA. The C4'-deoxyribose radical (43) is a $\beta$-phosphatoxyalkyl radical and $\alpha$-oxy radical bearing two phosphate groups beta to the radical making the elimination of phosphate fast. C4'-deoxyribose radical 43 has been independently generated in nucleosides and oligonucleotides.\textsuperscript{41,42} The fate of this radical under anaerobic and aerobic conditions has been investigated in detail.\textsuperscript{43} Under anaerobic conditions and in the absence of reducing agent, the C4'-deoxyribose radical (43) in ss- or ds-DNA undergoes heterolytic cleavage to generate radical cation 44 and strand breaks (8) with a rate constant of about $10^3$ s$^{-1}$ in ssDNA (dsDNA is 10 times slower) (Scheme 1.7).\textsuperscript{44} Trapping of radical cation 44 by a water molecule yields radical 45, which undergoes a second $\beta$-phosphate elimination to
give 3'-phosphate containing oligonucleotide 11 and radical cation 46. Radical 45 can abstract a hydrogen from GSH to generate 47. In the presence of GSH, there is competition between heterolytic cleavage of the C4'-deoxyribosyl radical (43) and hydrogen abstraction from GSH. Trapping of 43 with GSH occurs at a rate of $1.9 \times 10^6$ M$^{-1}$s$^{-1}$ giving rise to a mixture of repaired (48) and “pseudorepaired” (49) nucleotides. The hydrogen trapping reaction is stereoselective in dsDNA resulting in formation of natural 2'-deoxyribonucleotides (ratio 48:49 = 9) while in ssDNA trapping is nonselective, producing 48 and 49 in a ratio of 1.5.

Under aerobic conditions and in the presence of GSH, C4'-radical 43 is trapped by oxygen to form isomeric peroxy radicals 50 with a rate constant of $2 \times 10^9$ M$^{-1}$s$^{-1}$. This reaction was found to be reversible. Peroxy radicals (50) abstracts a hydrogen atom from GSH to form hydroperoxide 51. The hydroperoxide undergoes a Criegee-type rearrangement to generate intermediate 52 which subsequently decomposes to yield base propenal 55, 5'-phosphorylated oligonucleotide (8), and 3'-phosphoglycolate (3'-PG) 35 (Scheme 1.7).
Scheme 1.7: Fate of C4'-thymidinyl radical
Base propenal 55 can react with deoxyguanosine in DNA to form the DNA adduct M₁dG (56) (Scheme 1.8).⁴⁷,⁴⁸

![Scheme 1.8: M₁dG adduct of base propenal with guanine](image)

1.3.2.5 C5'-Radical

The C5'-deoxyribosyl radical (57) is an α-phosphoxygenalkyl radical generated by the abstraction of one C5'-hydrogen. Under anaerobic conditions, the C5' radical (57) attacks the C8-N7 double bond in purine bases of the same nucleoside leading to cyclization at C8. The outcome of this cyclization is the formation of both R and S diastereomers of 8,5'-cyclo-2'-deoxyguanosine (cdG) or 8,5'-cyclo-2'-deoxyadenosine (cdA, 59 and 60, Scheme 1.9).⁴⁹,⁵⁰ The rate constant of intramolecular cyclization of 2'-deoxyadenosine-5'-yl radical and 2'-deoxyguanosine-5'-yl is 1.6 × 10⁵ s⁻¹ and 1.0 × 10⁶ s⁻¹, respectively.⁴⁹,⁵¹ In the presence of physiological concentrations of thiol, hydrogen trapping of the radical competes with the cyclization process. Trapping of the C5' radical with GSH results in formation of 58 and occurs at a rate of 4.9 × 10⁷ M⁻¹ s⁻¹.⁵² In the presence of oxygen, C5' radical 57 reacts with oxygen at a diffusion-controlled rate (~1.9 × 10⁹ M⁻¹ s⁻¹) to form peroxyl radical 61. The peroxyl radical decomposes by two pathways (Scheme 1.9), one yielding strand breaks with 3'-phosphate (11) and 5'-
aldehyde (63) end groups, while the other delivers 5'- (2-phosphoryl-1,4-dioxobutane) 64 along with 3'-formyl oligonucleotide (65). 54

Scheme 1.9: Fate of C5'-deoxyadenosyl radical
The 5'-aldehyde can undergo β-elimination to form unsaturated aldehyde 66 and 5'-phosphorylated oligonucleotide 8. Unsaturated aldehyde 66 undergoes base elimination to deliver furfural 67 and ree base 23 (Scheme 1.10).

Scheme 1.10: Fate of 5'-thymidinylaldehyde

It was shown that furfural can form an adduct with adenine residues in DNA. This adduct subsequently undergoes deglycosylation to form kinetin (68) and an abasic site (69) (Scheme 1.11).

Scheme 1.11: Adduct formation between furfural and an adenine residue in DNA

5'-(2-Phosphoryl-1,4-dioxobutane) (64) in its cyclic form can exist in equilibrium with the open chain form (Scheme 1.12). This dialdehydic lesion undergoes β-elimination to form trans-1,4-dioxo-2-butene (70), a highly reactive α,β unsaturated dicarbonyl species. It has been demonstrated that this electrophilic lesion can react with
nucleophiles in DNA to form stable oxadiazabicyclo (3.3.0)octamine adducts of dC, dA, and dG under biological conditions (reaction with dC, Scheme 1.13).\textsuperscript{58,59}

\begin{equation}
\begin{array}{c}
\text{HO-} \quad \text{O} \\
\text{O=P-O^-} \quad \text{OR} \\
\text{64} \\
\end{array} \quad \leftrightarrow \quad 
\begin{array}{c}
\text{O=O} \\
\text{O=P-O^-} \quad \text{OR} \\
\text{64} \\
\end{array} \quad \rightarrow \quad 
\begin{array}{c}
\text{O=O} \\
\text{R} \\
\text{70} \\
\end{array} + 
\begin{array}{c}
\text{O=P-O^-} \\
\text{OR} \\
\text{8} \\
\end{array}
\end{equation}

Scheme 1.12: Fate of 5'-\(\text{(2-phosphoryl-1,4-dioxobutane)}\)

\begin{equation}
\begin{array}{c}
\text{NH}_2 \\
\text{N} \\
\text{71} \\
\end{array} + 
\begin{array}{c}
\text{O=O} \\
\text{70} \\
\end{array} \quad \rightarrow \quad 
\begin{array}{c}
\text{H} \quad \text{N} \\
\text{N} \\
\text{72} \\
\end{array}
\end{equation}

Scheme 1.13: Adduct formation by trans-1,4-dioxo-2-buten with deoxycytidine

1.4 DNA Damage by Hydrogen Atoms (H\textsuperscript{+})

The hydrogen atom (H\textsuperscript{+}) is considered a reducing species generated from ionizing water molecules through the indirect effect of ionizing radiation.\textsuperscript{3,11} It can damage DNA through addition to the double bonds of the nucleobases resulting in formation of neutral nucleobase radicals that can react further to cause more damage.\textsuperscript{60}
1.5 DNA Damage by Solvated Electrons (e\textsuperscript{-aq})

Solvated electrons are generated from radiolysis of water molecules and are stronger reducing agents than the hydrogen atom.\textsuperscript{3} Solvated electrons are highly reactive and damage biological molecules by one-electron transfer.\textsuperscript{11} Hydrated electrons add to the nucleobases of DNA forming radical anions (73, Figure 1-4) that are easily protonated resulting in the formation of neutral radicals (74 and 75).\textsuperscript{3}

\[
\begin{align*}
\text{Figure 1-4: Reaction of a solvated electron with uracil}\textsuperscript{61}
\end{align*}
\]

1.6 Significance of the DNA Damage by IR

IR causes a variety of damage to DNA in living systems by randomly ionizing the DNA components and the surrounding water molecules producing a cascade of secondary electrons with LEE <15 eV. This damage can be in the form of base and sugar damage, single and double strand brakes, and clustered damage, which can be genotoxic, mutagenic, and lethal.\textsuperscript{5,6,63,64,65} Although ionizing radiation is used as a therapeutic method to treat cancer, it can not distinguish between the DNA of healthy cells and cancerous cells. Understanding the mechanisms involved in IR induced damage to DNA will help in the development of more efficient radiotherapeutic methods.
1.6.1 Significance of the Investigation of the C-3' dideoxy Radical Generated by LEE-DNA interaction.

It has been established that DNA damage is mostly produced by secondary species generated along the radiation track. LEEs are produced in large quantities and have been established to induce both single and double strand breaks as well as base release. This was supported through the investigation of the basic interaction of LEE with ribose derivatives, nucleobases, oligonucleotides, and plasmid DNA. It was determined that electrons below 15 eV break DNA bonds through a resonance process called dissociative electron attachment (DEA) producing stable anions and radicals fragments. Although this process induces DNA strand breaks, the chemical steps leading to the formation of the final DNA modification remain to be established.

One proposed mechanism to explain the mechanism of LEE-induced strand breaks in DNA which involves dissociative electron attachment is seen in Figure 1-5. It is believed that LEE are captured first by the π* orbital of the nucleobases in DNA followed by electron transfer to the π* orbital of the phosphate group resulting in the formation of transient molecular anions at the phosphate group, which dissociate via two possible pathways: Path A involves homolytical cleavage of the C-O σ bond at the 3' and/or 5' positions resulting in the formation of carbon-centered radicals and phosphorylated oligonucleotides (Figure 1-5), whereas path B involves homolytic cleavage of the P-O σ bond at the 3' and/or 5' positions resulting in the formation of phosphoryl radicals and alkoxyl anions (Figure 1-5). It is believed that cleavage of the phosphodiester bond takes place primarily via C-O σ bond...
cleavage as shown in pathway A. The formation of reactive intermediates 78 and 83 was confirmed through the identification of the dephosphorylated C3' sugar radical through electron spin resonance (ESR) studies of argon ion and γ-irradiated hydrated DNA.\(^{76}\) Investigation of the reactivity and fate of this sugar radical intermediate will facilitate the elucidation of the structure of the final DNA lesions and establish the mechanism of DNA damage resulting from LEE.

![Diagram of DNA cleavage pathways](image)

**Figure 1-5:** Proposed pathways for phosphodiester bond cleavage of DNA via LEE\(^{66}\)

### 1.6.2 Independent Generation of the Deoxyribosyl Radical

Many investigations have been performed to understand the alterations in DNA induced by LEE.\(^3\) However, DNA damage by IR and DNA damaging agents can be
complicated due to the generation of multiple reactive intermediates (nucleobase and sugar radicals) through random attack at biopolymers.\textsuperscript{77} To facilitate the mechanistic investigation of DNA damage, reactive intermediates have been independently generated in a controlled manner at defined sites in DNA.\textsuperscript{38,78} This strategy has allowed for the elucidation of reaction mechanisms and uncovered reaction pathways while improving the overall understanding of biologically important chemistry.\textsuperscript{79,80,81} Independent generation of reactive intermediates involved in DNA damage facilitate separation of the chemical processes that generate this species from the effect of a damaging agent on the reactivity of the intermediate.\textsuperscript{82} The use of photolabile groups such as phenylselenyl\textsuperscript{83} or acyl groups\textsuperscript{36,84} to generate the radical of interest at specific sites has been very successfully applied to the generation of pyrimidine nucleobase\textsuperscript{84} radicals and sugar radicals at various positions in nucleosides\textsuperscript{20} and nucleotides.\textsuperscript{85} Acyl derivatives of modified nucleosides and oligonucleotides have been utilized as excellent radical precursors, undergoing Norrish Type I photocleavage processes at wavelengths above those that usually damage DNA. In Norrish Type I photocleavage, photoactivation of acyl groups (isopropyl, acetyl, or pivaloyl) result in excitation of electrons to the singlet state followed by bond scission at either side of the carbonyl group (Figure 1-6). Photocleavage of acyl derivatives of oligonucleotides (86) can undergo bond scission by two pathways: Path A, in which the homolytic cleavage occurs between the C-CO bond between ketone and 2'-deoxyribose moiety of the nucleotide resulting directly in formation of sugar radical \textsuperscript{87} and acyl radical \textsuperscript{88} fragment. Acyl radical \textsuperscript{88} undergoes decarbonylation to deliver carbon monoxide (89) and a second alkyl radical (90). In path B, homolytic cleavage of R-CO results in formation of the acyl sugar radical 91 and alkyl
radical 90. Compound 91 subsequently decarbonylate to release carbon monoxide 89 and sugar radical 87.\textsuperscript{86}

![Chemical diagram showing Norrish Type I photocleavage of oligonucleotide containing acyl groups](image)

Figure 1-6: Norrish Type I photocleavage of oligonucleotide containing acyl groups

The pivaloyl group was chosen to be used as a radical precursor for the mechanistic investigation of LEE induced DNA damage. Since it is expected to undergo photocleavage through path B, forming an acyl sugar radical (91) which is expected to undergo fast decarbonylation (\( \geq 10^5 \text{ s}^{-1} \)) rather than reduction by a hydrogen atom source process.\textsuperscript{86,87,88} In addition, the tert-butyl radical (90) is believed to diffuse away from the site of radical generation without participating in secondary reactions.\textsuperscript{45} On the other
hand, methyl and isopropyl ketones were shown to produce side products and cause lower yields. The pivaloyl group has been used successfully to site-specifically generate the radical of interest at a predetermined position on the base and sugar moieties of nucleosides and oligonucleotides. **Figure 1-7** shows examples of pivaloyl radical precursors (92-100) that have been used to efficiently generate nucleobase and sugar radicals in nucleosides and oligonucleotides.\textsuperscript{89,90,91,92} These radical precursors helped in the elucidation of mechanisms involved in the fate of radicals induced from IR.
Figure 1-7: Pivaloyl radical precursors designed to generate radicals at different positions in nucleosides and nucleotides
1.7 Relevance of the Investigation of the Oxidative Damage to C-5'

ROS, with hydroxyl radical being one of the most reactive species, can abstract hydrogen atoms from the deoxyribose moiety of DNA causing the formation of sugar radicals that degrade frequently to strand breaks accompanied by end modified DNA fragments.\textsuperscript{17,53} Those fragments can degrade to small electrophilic fragments capable of reacting with cellular nucleophiles such as nucleobases and side chains of proteins forming DNA adducts and/or DNA-protein crosslinks. DNA adduct formation can be toxic, mutagenic, and carcinogenic. Therefore identification of adducts formed through 2'-deoxyribose oxidation in DNA has the potential to reveal biomarkers suitable for early detection of many diseases with cancer being one of the most important. Biomarker discovery will aid in the early detection of cancer and its treatment.

Several DNA adducts were found to form as a result of oxidative damage to the sugar moiety in DNA (\textbf{Figure 1-8}). The formation of these adducts were explained in the above sections. Oxidation at the C5' position of 2'-deoxyribose results in formation of strand breaks terminated with a 5'-aldehyde group (36).\textsuperscript{17,77} Degradation of this lesion results in formation of furfural (67), a compound known to form adducts with adenine to give kinetin (68).\textsuperscript{55,57} Investigation of the ability of 5'-aldehyde containing oligonucleotides and its degradation products 66 and 67 to form adducts with DNA at physiological conditions will be conducted. These investigations may reveal biomarkers to be used for early detection of diseases.

Although oxidative stress causes damage to DNA components resulting in the formation of modified nucleobases and 2-deoxyribose lesions as seen above, but most of this damage can be repaired by DNA repair enzymes such as base excision repair
enzymes (BER) which can remove modified nucleotides including various types of abasic sites whereas bulkier lesions and cross-links are removed by nucleotide excision repair enzymes (NER).

Figure 1-8: DNA adducts derived from 2'-deoxyribose oxidation
CHAPTER TWO

2.1 RESULTS and DISCUSSION: Synthesis and Photolysis of C3'-Modified Nucleosides

DNA damage caused by ionizing radiation and DNA damaging agents is a complex process involving the generation of multiple reactive intermediates through random attack at biopolymers.\(^77\) In order to overcome multiple radical formation and a lack of control over the site of radical generation, organic chemistry has been used to independently generate reactive intermediates in a controlled manner at defined sites in biopolymers.\(^82\) This approach has clarified reaction mechanisms and identified new DNA damage pathways.\(^38,78\) These studies often use photolabile groups such as phenylselenyl\(^83\) or acyl groups\(^36\) to generate the radical of interest. Pivaloyl derivatives of modified nucleosides\(^82,84\) and oligonucleotides\(^39,28\) have been utilized as precursors for site-specific generation of nucleobase and sugar radicals. With the goal to investigate the fate of the C2',3'-dideoxy-C3'-thymidinyl radical in DNA, a proposed intermediate of DNA-LEE interactions,\(^66\) C2',3'-dideoxy-C3'-pivaloyl modified thymidines (101 and 103) were synthesized and their suitability as precursors of the C2',3'-dideoxy-C3'-thymidinyl radical (102) investigated (Figure 2-1).
Designing efficient photoactive or photolabile radical precursors requires consideration of a set of factors. The stability of photolabile groups during synthesis, and the ability of these groups to generate the radical of interest upon exposure to ultraviolet (UV) light must be utilized. Norrish Type I photochemical reactions have proven to be very effective for generating DNA radicals at different positions at the monomer and polymer levels.\(^{38, 78, 84}\) The pivaloyl moiety was chosen as a photolabile group based upon its demonstrated successes in the generation of nucleoside-based radical intermediates.\(^{93-90}\) The pivaloyl group has a carbonyl moiety that absorbs light outside the range that usually damages DNA and subsequently undergoes Norrish Type I photocleavage. This photochemical process involves the generation of a tert-butyl and an acyl radical. The acyl radical subsequently undergoes decarbonylation to form a second alkyl radical. The tert-butyl radical is believed to diffuse away from the site of radical generation without participating in secondary reactions.\(^{45}\) To investigate the mechanistic pathway of DNA damage through the formation of the C2',3'-dideoxy-C3'-thymidinyl radical (102), \(\alpha\)- and

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Figure 2-1: Photochemical generation of C2',3'-dideoxy-C3'-thymidinyl radical (102) from precursors 101 and 103
β-C2',3'-dideoxy-C3'-pivaloylthymidines (101 and 103) were synthesized. The isomeric precursors were synthesized to investigate the effect of the stereochemistry of the C3'-radical precursors on product formation.

The synthesis β-C2',3'-dideoxy-C3'-pivaloylthymidine (101) started with the conversion of commercially available thymidine (104) to C3'-methylene thymidine (107) according to published literature procedures (Scheme 2.1).94

Scheme 2.1: Synthesis of compound 10794

Compound 107 was subjected to hydroboration-oxidation to afford primary alcohol 108 as the β-isomer in 89% yield. Oxidation of the hydroxymethyl using the Dess-Martin periodinane at low temperature gives only the β-isomer of aldehyde 109 in 100% yield. The aldehyde was then subjected to nucleophilic addition using tert-butyl lithium in the presence of cerium (III) chloride to deliver alcohol 110 in 67% yield as a diasteromeric mixture. Oxidation of secondary alcohol 110 using the Dess-Martin Reagent afforded the β-isomer of ketone 111 in 96% yield. Subsequent deprotection of
the 5'-hydroxyl with THF/TFA/H₂O (8:1:1) afforded nucleoside 101 in 91% yield. (Scheme 2.2).⁹⁵

Scheme 2.2: Synthesis of β-C2',3'-dideoxy-C3'-pivaloylthymidine 101⁹⁵

In order to obtain α-isomer 103 a different strategy was employed. Commercially available thymidine (104) was converted to α-aldehyde (115) according to published protocols (Scheme 2.3).⁹⁶,⁹⁷ The key step in the stereoselective formation of the α-isomer was the conversion of tert-butylidiphenylsilyl protected C3'-O-phenoxythiocarbonylthymidine (113) to tert-butylidiphenylsilyl protected C3'-styrylthymidine (114). The reason for stereoselectivity is attributed to the presence of the
C5' bulky protecting group that stericly blocks the top face of the nucleoside forcing the incoming styryl group to approach from the least hindered $\alpha$-face of the C3'-radical.

Scheme 2.3: Synthesis of compound 115$^{96,97}$

Conversion of compound 115 to tert-butyl alcohol 116 as a diasteromeric mixture employing the same synthetic strategic as used in the case of the $\beta$-isomer proceeded in low yield (13%). Extending the reaction time to 8 hours, however increased the yield to 30%. The lower yield of this reaction compared to the same reaction for the $\beta$-isomer may be due to the presence of the bulky 5'-terty-butyldiphenylsilyl protecting which decreases the reactivity of the formyl group to nucleophilic addition. Oxidation of 116 using the Dess-Martin periodinane delivered the desired ketone (117) in 100% yield. Removal of the TBDPS with TBAF resulted in the formation of $\alpha$-isomer 103 in 44% yield (Scheme 2.4)$^{95}$ The absolute configuration of these substrates was confirmed through Nuclear Overhauser Effect Spectroscopy (NOESY NMR) analysis. In NOESY experiments the correlation between nuclei that are physically close to each other in space regardless of whether there is a bond between them are detected. Thus, atoms that
are in close proximity to each other display an NOE effect. In our experiments, a strong NOE effect between the 3'-H, 4'-H and 2'-H were used to determine the absolute configuration of these modified substrates. Spectral data for (108-111) and (115-103) is available in (Figure 1-14, Appendix C), and NOESY spectra is available in supporting information of the cited reference. 

Scheme 2.4: Synthesis of α-C2',3'-dideoxy-C3'-pivaloylthymidine (103)

2.3 Investigation of the suitability of α and β-Pivaloylketone Precursors 101 and 103 for the Generation of C3' Radical 102.

The use of photolabile precursors to independently generate the C2',3'-deoxy-C3'-thymidinyl radical in nucleic acids will help to elucidate the reactivity of the radical and facilitate the identification of the damage products generated from this intermediate in nucleosides and nucleotides. The suitability of α and β-pivaloylketone precursors 101 and 103 for site specific generation of C2',3'-dideoxy-C3'-thymidinyl radical 102 under
anaerobic conditions and in the presence of different hydrogen atom donors was
explored.

2.3.1 Photochemical Generation of the C2',3'-Dideoxy-C3' Radical in the Presence of
tri-nButyltin Hydride.

Initial photolysis experiments were carried out in order to determine whether the
C2',3'-dideoxy-α-C3'-pivaloylthymidine (103) and C2',3'-dideoxy-β-C3'-pivaloylthymidine (101) are capable of generating the C2',3'-dideoxy-C3'-thymidinyl radical (102). Their efficiencies as radical precursors were determined through trapping
of 102 with a hydrogen atom donor. The formation of reduction product 118 provides
proof for the suitability of 101 and 103 as precursors of the C2',3'-dideoxy-C3'-thymidinyl radical (102) Figure 2-2.
Figure 2-2: Strategic approach to determine the feasibility of 101 and 103 as photochemical precursors of the C2',3'-dideoxy-C3'-thymidinyl radical

Photolysis of 103 (1 mM) was performed, using a 500 W high pressure mercury arc lamp fitted with an IR filter, focusing lens, and 320 nm cutoff filter, in 1:1 acetonitrile/water containing excess tri-nbutyltin hydride (Scheme 2.5).

Scheme 2.5: Photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) from 103 in the presence of tri-nbutyltin hydride

The photolysate was analyzed directly without workup using high performance liquid chromatography (HPLC) employing a C18 reversed-phase column with detection.
at 254 nm. The products were isolated and identified by electrospray ionization mass spectrometry (ESI-MS) and by comparison with authentic samples. Figure 2-3 shows the reversed-phase HPLC chromatogram of the crude photolysate (green), the control (red), and the standards (blue). Irradiation of 103 under the above conditions resulted in 62% conversion of 103 to reduction product 118 as the sole product. The formation of the reduction product indicates that 103 is a viable precursor of radical 102. In order to confirm formation of the reduction product, a standard was synthesized and injected along with the precursor 103 and their retention times were compared to the photolysate (blue). In addition, the identities of photoproduct 118 and precursor 103 were confirmed with ESI-MS. The mass spectrum shows a molecular weight of 249.2 m/z corresponding to the sodium adduct of 118 and a molecular weight of 333.4 m/z corresponding to the sodium adduct of 103 (Appendix A, Figure 1 and 2, respectively). A control experiment was done in which the precursor (103) was prepared as described with the exclusion of exposure to UV light. From the HPLC chromatogram, we see only the presence of 103 indicating the formation of the reduction product in the photolysate results only from trapping of 102 with a hydrogen atom confirming the viability of 103 as a precursor of 102.
Figure 2-3: Reversed-phase HPLC analyses of the photolysate resulting from photolysis of 103 (green), Controls (red), Standard 118 along with radical precursors 103 (blue) were injected separately.

In contrast, β-C3’-pivaloylthymidine (101) was photolyzed under the same conditions as used for α-precursor 103 (Scheme 2.6).

Scheme 2.6: Product identification from photochemical generation of the C2’,3’-dideoxy-C3’-thymidinyl radical (102) from 101 in 1:1 acetonitrile/water in the presence of tri-n-butyltin hydride

The crude sample was analyzed by HPLC on a C8 reversed-phase column without any workup and detection at 254 nm. Unfortunately, reduction product 118 was detected in very small amount (peak at 13.580 min, Figure 2-4). Fractions eluting at 13.580 min...
and 13.740 min were collected together and concentrated. Analysis by MALDI-ToF MS indicated \( m/z \) values of 249.092 and 265.058 corresponding to the monoisotopic masses of the sodium and potassium adducts of 118, respectively, and a monoisotopic mass of 277.090 corresponding to the sodium adduct of aldehyde 119 (Appendix A, Figure 3). Aldehyde 119 is a side product resulting from homolytic cleavage of the tert-butyl-acyl bond followed by subsequent trapping with tri-nbutyltin hydride. This implies that there is competition between trapping of the acyl radical and liberation of carbon monoxide to generate the C2',3'-dideoxy-C3'-thymidinyl radical (102). In addition to MALDI-ToF analysis, the retention time of the aldehyde was compared to that of independently synthesized aldehyde (119) and both were identical. The major component of the mixture eluting at 14.860 min could not be analyzed by either ESI-MS or MALDI-ToF MS. Analysis of fractions eluting at 9.900 and 12.787 min with MALDI-ToF also failed. The fraction eluting at 20.533 min was confirmed to be the precursor 101 by MALDI-ToF MS (333.151 and 349.114 monoisotopic masses of sodium and potassium adducts, respectively (Appendix A, Figure 4).
Figure 2-4: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of 101 (1 mM) in 1:1 acetonitrile/water in the presence tri-nbutyltin Hydride

Since the photolysis of the β-C3'-pivaloylthymidine 101 in 1:1 CH$_3$CN/H$_2$O did not afford the reduction product as the major product, the photolysis solvent was changed to absolute acetonitrile, as a polar aprotic organic solvent, to determine if elimination of solute-solvent hydrogen bonding would have any effect on product distribution. Photolysis of β-C3'-pivaloylthymidine 101 was performed in acetonitrile containing an excess of tri-nbutyltin hydride for 1 h at 15 °C after degassing with argon for 20 minutes (Scheme 2.7).

Scheme 2.7: Photochemical generation of the C2',3'-dideoxy-C3'-acylthymidinyl radical (120) from 101 in acetonitrile in the presence of tri-nbutyltin hydride
The crude sample was analyzed by reversed-phase HPLC (Figure 2-5). The chromatogram shows the presence of fractions eluting at 13.887 and 20.593 min which were collected, concentrated, and analyzed by MALDI-ToF MS. The MALDI data shows monoisotopic masses equal to 255.235 and 277.073 which corresponds to the hydrogen and sodium adducts of aldehyde (119), respectively and a monoisotopic mass of 333.109 which corresponds to the sodium adduct of recovered starting material 101, the potassium adduct of 101 was also observed (m/z 349.080) (Appendix A, Figure 5 and 6, respectively). This result indicates that acetonitrile as solvent makes trapping of the acyl radical (120) by a hydrogen atom faster than decarbonylation. This may be due to the formation of a hydrogen bond between the oxygen of the acyl group and the hydrogen of the 5′-hydroxyl group forming a seven member ring causing the decarbonylation to be slow and the trapping with hydrogen atom donor faster. Additionally, it was reported that decarbonylation of acyl radicals decreases with increasing solvent polarity due to a decrease in the dipole moment during the bond scission process. This causes the exothermicity of the cleavage to decrease. Thus, the activation energy increases which cause slower decarbonylation.98
Figure 2-5: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of \textbf{101} (1 mM) in acetonitrile in the presence \textit{tri-n}butyltin hydride.

Since acetonitrile resulted in acyl radical trapping, we investigated the photochemical reactivity of \textbf{101} in the presence of a polar protic solvent. Photolysis of \textbeta-C3'-pivaloylthymidine (\textbf{101}) was performed in water with 0.1 % acetonitrile containing an excess \textit{tri-n}butyltin hydride for 1 h at 15 °C after degassing with argon for 20 minutes (Scheme 2.8). Additionally, the same experiment was performed in methanol with 0.1 % acetonitrile containing an excess \textit{tri-n}butyltin hydride for 1 h at 15 °C after degassing with argon for 20 minutes (Scheme 2.9).

Scheme 2.8: Photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (\textbf{102}) from \textbf{101} in water in the presence of \textit{tri-n}butyltin hydride.
Scheme 2.9: Photochemical generation of the C2',3'-dideoxy-C3'-acylthymidinyl radical (120) from 101 in methanol in the presence of tri-n-butyltin hydride

The crude sample from the photolysis in water was analyzed as described above (Figure 2-6). The chromatograph shows no signs of reduction product 118. The chromatograph shows the presence of components at 9.967, 14.987, and 20.587 minutes, which were collected, concentrated, and analyzed by MALDI-ToF MS. Unfortunately, compounds eluting at 9.967 and 14.987 min were not identified using this technique. A substance eluting at 20.587 min gave a monoisotopic mass of 333.161 which corresponds to the sodium adduct of starting material 101 (Appendix A, Figure 7).

Figure 2-6: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of 101 (1 mM) in water in the presence tri-n-butyltin hydride
In contrast, the analysis of the photolysate in methanol indicated the presence of a major component at 13.673 min, which was determined to be aldehyde 119 (Figure 2-7). These results indicate that both protic and aprotic polar solvents do not facilitate the generation of the C2',3'-Dideoxy-C3'-thymidinyl Radical (102) from precursor 101. We conclude that organic polar solvents facilitate the trapping of the acyl radical generating aldehyde 119 and inorganic solvents result in formation of unidentified products. In the case of the photolysis of 101 in water, the unidentified products may be the result of the involvement of the Norrish Type II instead of Type I photocleavage in which the excited carbonyl compound undergoes intramolecular abstraction of a γ-hydrogen to produce a 1,4-biradical as a primary photoproduct 121, followed by either intramolecular recombination of the two radicals to form cyclic compound 122 or fragmentation to form 123 which may undergo subsequent degradation to form 124 and 39 (Figure 2-8).

Figure 2-7: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of 101 (1 mM) in methanol in the presence tri-nbutyltin hydride
Figure 2-8: Proposed mechanism for Norrish Type II photocleavage for 101

Based on the results obtained from the photolysis of both C2',3'-dideoxy-α-C3'-pivaloylthymidine (103) and C2',3'-dideoxy-β-C3'-pivaloylthymidine (101) in the presence of a strong hydrogen atom donor, we conclude that precursor 103 is suitable to generate the C2',3'-dideoxy-C3'-thymidinyl Radical (102). In order to fully evaluate these precursors, we examined their photochemical conversion in the presence of the more biologically relevant hydrogen atom donor glutathione (GSH).

2.3.2 Photochemical Generation of the C2',3'-Dideoxy-C3'-Radical in the Presence of Glutathione.

Glutathione is a reducing agent produced by cells as a defense mechanism against radicals. The physiological concentration of GSH in cells is 6 mM. Therefore photolysis of precursor 103 (1 mM) in 1:1 acetonitrile/water in the presence of 6 mM GSH as a hydrogen atom donor (Scheme 2.10).

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Scheme 2.10: Photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) from 103 in 1:1 acetonitrile/water in the presence of GSH

The crude sample was analyzed directly by HPLC and MALDI-ToF MS as described above (Figure 2-9). The chromatogram shows the presence of substances eluting at 3.973, 6.647, 8.560, 9.733, 10.367, 12.320, 13.740, and 20.327 minutes. The compound with a retention time of 3.973 min corresponds to the sodium adduct of GSH. Compounds eluting at 6.647, 9.733, 10.367, 12.320 min could not be identified. The component eluting at 8.560 min corresponds to thymine (23). The component eluting at 13.740 min gives monoisotopic masses of 249.085 and 265.059 corresponding to the sodium and potassium adducts of reduction product 118, respectively (Appendix A, Figure 8) and that at 20.327 min gives monoisotopic masses of 333.153 and 349.125 corresponding to the sodium and potassium adducts of starting material 103 (Appendix A, Figure 9).
Figure 2-9: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of 103 (1 mM) in 1:1 acetonitrile/water in the presence 6 mM GSH.

In contrast the photolysis of precursor 101 (1 mM) was carried out using the same methodology as described for 103 (Scheme 2.11).

Scheme 2.11: Photochemical generation of the C2',3'-dideoxy-C3'-acylthymidinyl radical (120) from 101 in 1:1 acetonitrile/water in the presence of GSH.

The crude photolysate was analyzed in the same manner as described above (Figure 2-10). Through HPLC analysis aldehyde 119 (13.653 min) and the starting material (20.553 min) were identified (Appendix A, Figure 10). The absence of reduction product 118 indicates that GSH traps the acyl radical preventing decarbonylation.
2.3.3 Photochemical Generation of the C2',3'-Dideoxy-C3'-Radical in the Absence of Hydrogen Atom Donor.

Photolysis of both 103 and 101 was performed using the same method as described above except no hydrogen atom donor was added. The crude photolysates were injected without any workup and the photoproducts were detected at 254 nm (Figure 2-11 and 2-12, respectively).

Figure 2-11: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of 103 (1 mM) in 1:1 acetonitrile/water in the absence of hydrogen atom donor.
The analysis of the crude photolysate 103 (Figure 2-11) indicated the formation of the reduction product 118 (13.753 min) (Appendix A, Figure 11, 12), C2',3'-didehydro-2',3'-dideoxythymidine (125) (12.927 min) (Appendix A, Figure 13, 14) and thymine (23) (8.720 min) plus other unidentified peaks (Scheme 2.12).

Scheme 2.12: Product identification from photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) from 103 in 1:1 acetonitrile/water in the absence of hydrogen atom donor

In contrast, the HPLC chromatogram of the photolysate of 101 indicates the presence of a very small amount of photoproducts as seen by Figure 2-12. This indicates that precursor 101 is not efficient at generation of the C2',3'-dideoxy-C3'-radical (102).

Figure 2-12: Reversed-phase HPLC chromatogram of the photolysate resulting from photolysis of 101 (1 mM) in 1:1 acetonitrile/water in the absence of hydrogen atom donor
In conclusion, photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) from β-C2',3'-dideoxy-C3'-pivaloylthymidine 101 and α-C2',3'-dideoxy-C3'-pivaloylthymidine 103 precursors were investigated under different conditions and based on the results obtained, precursor 103 was found to generate the reduction product 118 under all conditions. In the absence of a hydrogen atom donor the formation of C2',3'-didehydro-2',3'-dideoxythymidine (125) was detected which indicates a radical disproportionation mechanism between two alkyl radicals of either the C2',3'-dideoxythymidinyl radical 102 and tert-butyl radical or the two C2',3'-dideoxythymidinyl radicals to generate compounds 118 and 125 (Scheme 2.13 and 2.14, respectively).84

![Scheme 2.13: Disproportionation between the C2',3'-dideoxy-C3'-thymidinyl radical (102) and tert-butyl radical, a proposed mechanism for formation of the reduction product 118 and the unsaturated product 125 in the absence of a hydrogen atom donor](image-url)
Scheme 2.14: Disproportionation between the C2',3'-dideoxy-C3'-thymidinyl radical (102), a proposed mechanism for formation of the reduction product 118 and the unsaturated product 125 in the absence of a hydrogen atom donor.

On the other hand precursor 101 was unable to generate reduction product 118 under all conditions and resulted in the formation of products that could not be identified using our present methods. Therefore, based on the results obtained precursor 103 will be used to investigate the fate of the C2',3'-deoxy-C3'-thymidinyl radical in oligonucleotides.
3 CHAPTER THREE:

3.1 Results and Discussion: Investigation of the 5'-Aldehyde Lesion

Oxidative damage to DNA caused by the indirect effect of ionizing radiation leads to the formation of single and double strand breaks.\(^3\) At sites of strand cleavage, oxidized electrophilic fragments\(^17\) are formed that are capable of reacting with local nucleophiles to form adducts.\(^40,58,48\) Oxidation of 2-deoxyribose at the 5'-position in DNA leads to the formation of strand breaks containing 3'-phosphate (11) and 5'-aldehyde residues (36) under aerobic conditions. Oligonucleotides containing a 5'-aldehyde moiety such as 36 are unstable and are proposed to undergo β-elimination to form electrophilic fragment 66 followed by loss of the nucleobase 23 to deliver the aromatic compound furfural and free base (67, Scheme 1.10)\(^56\) as shown in chapter one. It was shown that furfural can form an adduct with adenine in DNA which is undergoes subsequent deglycosylation to deliver kinetin (Scheme 1.11).\(^100\) With the goal to investigate the stability and reactivity of oligonucleotides containing a 5'-aldehyde lesion, and its degradation products, fragments believed to be produced upon C5'-oxidation, and oligonucleotides containing the 5'-aldehyde moiety were independently synthesized.
3.2 Overview of DNA synthesis

Oligonucleotides were synthesized using standard automated solid phase techniques. Two types of chemistries are employed in automated DNA synthesis, the phosphoramidite and H-phosphonate methods. Automated DNA synthesis is a cyclic, stepwise process carried out by the addition of nucleotide residues to the 5'-terminus of the growing chain until the desired sequence is assembled. The first step of the synthetic cycle is treatment of the derivatized solid support (126) with acid to remove the DMT group to yield the 5'-hydroxyl nucleotide (127, step 1, Figure 3-1). The next step is coupling the free 5'-hydroxyl group of 127 with the incoming 5'-protected 3'-phosphoramidite (128) using an activator such as tetrazole or 0.25 M 5-ethylthio-1H-tetrazole (step 2, Figure 3-1). The phosphite triester linkage (129) usually carries a protecting group that prevents any side reactions that could result in branched oligomers, which would interfere with coupling yields. A capping step is introduced following the coupling reaction to terminate any chains that did not couple (130); therefore it is designed to minimize the presence of oligomeric impurities and facilitate the purification of the final product (step 3, Figure 3-1). Finally, the phosphite triester linkage in 129 is oxidized to a stable phosphate triester (132) using iodine as an oxidizing agent and water as the oxygen donor, producing a chain that has been lengthened by one nucleotide 132 (step 4, Figure 3-1). The 5'-end protecting group of the added nucleotide is then removed, and the cycle is repeated until the desired length is achieved. Final deprotection of the bases and the phosphate groups and cleavage of the oligonucleotides from the solid support liberates the desired oligonucleotide with a DMTr group on its 5'-end 133 (step 5, Figure 3-1).
Figure 3-1: Steps involved in solid-phase oligonucleotide synthesis
3.3 Oligonucleotides Synthesis

Syntheses of standard oligonucleotides were carried out using the phosphoramidite method as described in Figure 3-1. Modified oligonucleotides were partially synthesized with the phosphoramidite method, followed by manual coupling of the 5'-modified nucleoside using H-phosphonate chemistry. The feasibility of this methodology for incorporation of modified nucleosides into DNA oligomers has been demonstrated previously in our laboratory employing modified 3'-H-phosphonate derivatives. This approach allows the use of two types of chemistries in one synthesis providing the flexibility needed for optimization of the synthesis of modified oligonucleotides.

3.3.1 Synthesis of 5'-Modified Thymidine Building Blocks for Oligonucleotide Synthesis

Oligonucleotides containing 5'-aldehyde nucleosides at their 5'-termini are labile to alkaline conditions and temperatures normally used to deprotect oligonucleotides synthesized on solid supports; therefore, we introduce the aldehyde into the oligonucleotide postsynthetically through oxidative cleavage of the diol containing oligonucleotides. The 5'-aldehyde moiety in nucleotides is unstable due to its electrophilic character and acidic proton at the α-position to the carbonyl group. These factors contribute to the ease of β-elimination to form electrophilic fragment followed by loss of the nucleobase (23) to deliver the aromatic compound furfural (67). This aldehyde is also reactive towards many nucleophiles, therefore, the need to mask its reactivity is required. This was achieved by introducing a diol at the 5'-position of the nucleoside. The synthesis of oligonucleotides containing a 5'-aldehyde was previously...
reported by Greenberg through the oxidative cleavage of the oligonucleotides containing a 5'-dil. Greenberg introduced the 5'-dil nucleoside into oligonucleotides using phosphoramidite chemistry. In this work, the synthesis of the 5'-dil nucleoside was done based on methods developed by Greenberg and introduced into oligonucleotides was using the H-phosphonate method (135). We determined that the dill was compatible with the use of this methodology (134). The synthesis of 3'-H-phosphonate derivative 135 began with the synthesis of compound 134 in 92% using published literature protocols. Compound 134 was converted to 135 using a published procedure in which the phosphorus tris(imidazole) was generated in-situ by reacting phosphorus trichloride with imidazole and triethylamine in dichloromethane at -10 °C (Scheme 3.1). After the completion of the reaction, the product was hydrolyzed using 1 M triethylammonium bicarbonate to give 135 in 34% yield as a diasteromeric mixture. Purity and identity were confirmed by NMR and MS (Appendix B, Figure 1-4).

Scheme 3.1: Synthesis of building block 135 for DNA synthesis

3.3.2 Incorporation of 135 into Oligonucleotides

Oligonucleotides containing 5'-modified nucleosides at their 5'-termini were synthesized utilizing monomer building block 135. The syntheses were performed using
semi-automated techniques in which the unmodified part of the oligomer was synthesized on an Applied Biosystems 391 DNA synthesizer using the phosphoramidite method on a 0.2 µmol scale, followed by manual coupling of 5'-modified nucleoside 135 using H-phosphonate chemistry. **Scheme 3.2** shows the synthesis of modified oligonucleotide 138 (T₄CTATCTATCT, where T₄ = 134) as an example of the strategy used in the manual incorporation of H-phosphonate 135 into oligonucleotides. The 5'-hydroxyl of 136 was deprotected during automated synthesis in order to facilitate coupling with the incoming H-phosphonate. Modified nucleoside 135 was then incorporated into oligonucleotides using a manual syringe technique. Briefly, 135 was dissolved in a mixture of acetonitrile/pyridine (1:1) to a final concentration of 0.1 M. The solution was transferred into a 1 mL syringe purged with argon. In another purged syringe was placed 5 µL of freshly distilled pivaloyl chloride in 305 µL of acetonitrile/pyridine (1:1). Each syringe was then attached to either end of the CPG column containing the polymer-bound unmodified oligomer 136.
Scheme 3.2: Incorporation of 135 into oligonucleotide using a manual coupling strategy

The activator was delivered first followed by the monomer and after repetitive mixing for 20 minutes, the reagents were then removed and the oligomer was washed with 5 mL acetonitrile. The H-phosphonate linkage was oxidized to the corresponding more stable phosphodiester linkage by attaching the column to two 1 mL purged syringes, one containing 300 µL of 4% iodine in pyridine/H₂O/THF (1:1:8) and the other containing 300 µL of THF/H₂O/triethylamine (8:1:1) for 20 minutes. The column was then washed with 10 mL of anhydrous acetonitrile. Oligonucleotides were deprotected and cleaved from the solid support using concentrated ammonium hydroxide for 15-18 hours at 55 °C. The cleaved oligonucleotides have DMT groups on the 5'-termini, which allows the use of Oligonucleotide Purification Cartridges (OPC) for purification to yield...
Purity and identity of all oligonucleotides was confirmed by analytical ion-exchange HPLC (Figure 3-2) and MALDI-MS (Figure 3-3) respectively.

Figure 3-2: Ion-exchange HPLC chromatogram of modified oligonucleotide 138

Figure 3-3: MALDI-ToF MS of modified oligonucleotide 138
Attempts to use 1-Adamantanecarbonyl chloride as an activator in manual coupling failed to incorporate modified nucleoside 135 into oligonucleotides, which may be due to the bulkiness of this activator and the presence of bulky groups on the 5' end of 135.

Modified oligonucleotides with different base context were synthesized and analyzed by analytical ion-exchange HPLC and MALDI-ToF MS (Table 3.1) (Appendix B, Figure 5-14). Quantification of these oligonucleotides was performed using UV absorbance ($\lambda_{\text{max}} = 260$ nm). Collectively, the results from these analyses confirmed the formation of the desired modified oligonucleotides with high purity and high coupling yields.

### Table 3.1: Modified oligonucleotides containing diol and MALDI-ToF analyses

<table>
<thead>
<tr>
<th>Sequence #</th>
<th>Sequences</th>
<th>Calculated [M+H]$^+$</th>
<th>Measured [M+H]$^+$</th>
<th>nmole</th>
</tr>
</thead>
<tbody>
<tr>
<td>138</td>
<td>$5^\prime$CTATCTATCT</td>
<td>3286.588</td>
<td>3286.607</td>
<td>202</td>
</tr>
<tr>
<td>139</td>
<td>$5^\prime$ATCTCTCTCT</td>
<td>3262.577</td>
<td>3262.485</td>
<td>214</td>
</tr>
<tr>
<td>140</td>
<td>$5^\prime$GTCTCTCTCT</td>
<td>3278.572</td>
<td>3278.403</td>
<td>202</td>
</tr>
<tr>
<td>141</td>
<td>$5^\prime$ATCTCTGCTT</td>
<td>3302.583</td>
<td>3302.449</td>
<td>215</td>
</tr>
<tr>
<td>142</td>
<td>$5^\prime$TTCTCTCTCT</td>
<td>3253.565</td>
<td>3253.461</td>
<td>213</td>
</tr>
<tr>
<td>143</td>
<td>$5^\prime$CTCTCTCTCT</td>
<td>3238.566</td>
<td>3238.286</td>
<td>230</td>
</tr>
</tbody>
</table>

#### 3.3.3 Synthesis of Modified Oligonucleotides Containing a 5'-Aldehyde Lesion (144)

The vicinal diol at the 5'-terminus in oligonucleotide 138 was converted to aldehyde 144 via oxidative cleavage using sodium periodate$^{103}$ (Scheme 3.3). The 5'-aldehyde containing oligomer was isolated by desalting using a G-25 sephadex column.
Analyses were performed using analytical ion-exchange HPLC (Figure 3-4) and MALDI-ToF MS (Figure 3-5). The product was quantified using UV absorbance measurements at $\lambda_{\text{max}} = 260$ nm.

Scheme 3.3: Synthesis of 5'-aldehyde containing oligomer 144

![Scheme 3.3: Synthesis of 5'-aldehyde containing oligomer 144](image)

Figure 3-4: Ion-exchange HPLC chromatogram of 5'-aldehyde containing oligomer 144
Figure 3-5: MALDI-ToF MS of 5'-aldehyde containing oligomer 144

3.3.3.1 Storage of Modified Oligonucleotide Containing 5'-Aldehyde (144)

As mentioned above, oligonucleotides containing a 5'-aldehyde are unstable, therefore, the need to determine the proper conditions to isolated and investigate its reactivity is important. In order to determine the stability of 144 during long term storage, the aldehyde was synthesized and stored in water at 4 °C in pH = 7 after desalting with a G-25 sephadex column. The decomposition of the 5'-aldehyde to the corresponding 5'-phosphorylated ODN was monitored by MALDI-ToF over 10 days (Scheme 3.4). The presence of small amount of the elimination product 145 was observed after 10 days of storage.
Scheme 3.4: Decomposition of 144 to 145

3.3.3.2 Stability of 5'-Aldehyde Containing Oligomer (144) under Physiological Conditions

To determine the half-life of the 5'-aldehyde in single-stranded oligonucleotides, oligonucleotide 144 was incubated in 100 mM phosphate buffer (pH = 7.4) at 37 °C and the conversion of 144 to 145 was measured by analytical ion-exchange chromatography. The half-life was determined to be 96 hours (Figure 3-6), which is similar to that found in the literature (100.7 hours) for ternary complexes (10 mM phosphate buffer at pH = 7.2 at 37 °C).

Figure 3-6: Ion-exchange HPLC chromatogram of 5'-aldehyde containing oligomer 144 after 96 hrs at pH 7.4 and 37 °C
3.4 Synthesis of the Nucleoside Resulting from β-Elimination of 5'-Aldehyde Containing Oligomers

Oligonucleotides containing 5'-aldehyde moiety 36 (Scheme 1.10) are unstable and can undergo β-elimination to form highly reactive electrophilic fragments 3',4'-didehydro-2',3'-dideoxy-5'-oxothymidine (66), followed by loss of nucleobase (23) to deliver aromatic compound furfural (67, Scheme 1.10) under both basic and acidic conditions. Compound 66 was synthesized to investigate its stability and reactivity under physiological conditions.

Scheme 3.3.5: Mechanism for the decomposition of 36 to 67

3.4.1 Synthesis of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66)

The synthesis of 66 started with the conversion of commercially available thymidine (104) to 5'-O-tert-butylidimethylsilyl-3'-O-benzoyl-thymidine (146)¹⁰⁴-¹⁰⁵ according to published literature procedures. Although compound 146 was previously synthesized, full characterization was not reported. Compound 147 is also known, however the synthesis was reported through regioselective acylation of thymidine (104) using an enzymatic reaction in the presence of an oxime ester. In our approach, the deprotection of the 5'-hydroxyl was done using a modified published literature protocol¹⁰⁴
in which THF/TFA/H$_2$O was used to afford 147 in 95% yield compared to the reported yield of 59%. The reaction was over night as compared to 72 hours.$^{106}$ Oxidation of primary alcohol 147 with the Dess-Martin Reagent afforded 3'-O-benzoyl-5'-oxothymidine (148) which was used directly in the next reaction. Subsequent benzoyloxy elimination leading to 66 was performed under mildly basic conditions by treatment of 148 with 4 molar equivalents of triethylamine (TEA) at room temperature to afford 66 in 21% yield. The lower yield is due to the low solubility of 147 in solvents used for the Dess-Martin reaction and the fast elimination of thymine when treating 148 with TEA (Scheme 3.5). Compound 148 was obtained in the literature using a modified Moffat procedure in higher yield.$^{107}$ However, due to the convenience of using the Dess-Martin reagent, the reaction was accomplished using this reagent. Purity and identity were confirmed by NMR and ESI-MS, respectively (Appendix B, Figure 15-23).

Scheme 3.6: Synthesis of 3',4'-didehydro-2',3'-dideoxy-5'-oxothymidine (66)
3.4.1.1 Stability of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66)

Modified nucleoside 66 was incubated in 100 mM phosphate buffer (pH = 7.4) at 37 °C (Figure 3-7). The decomposition of 66 to furfural and thymine was monitored by analytical reversed-phase HPLC chromatography (Figure 3-8).

![Chemical Structure](image)

Figure 3-7: Incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C

The chromatogram shows the presence of fractions eluting at 5.380, 10.140, and 12.073 min corresponding to thymine, furfural, and starting material 66, respectively. The identity of each component was confirmed by comparison of the elution times as compared to the elution times of authentic samples. The half-life of 66 was found to be 13 hours based on the amount of 66 recovered (Figure 3-9).
Figure 3-8: Reversed-phase HPLC chromatogram of incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C at 12 hours

![HPLC Chromatogram](image)

Figure 3-9: Incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C

Compound 66 is a Michael acceptor and is a highly reactive electrophilic. It has been shown in the literature that the related compound, ribonolactone 9, generated as a result of C1'-oxidation undergoes β-elimination and can produce an electrophile capable of reaction with sulfur and nitrogen nucleophiles. Based on these observation the stability of 66 was determined in the presence of glutathione. Compound 66 was incubated in 100 mM phosphate buffer (pH = 7.4) at 37 °C in the presence of 6 mM GSH. An aliquot of the reaction mixture were analyzed by analytical reversed-phase HPLC chromatography. Figure 3-10 shows a chromatogram after 30 minutes of incubation. All fractions were...
collected and analyzed by MALDI-ToF or ESI MS. MALDI data from compounds eluting at 3.320 min shows monoisotopic masses equal to 308.108 and 346.048 which corresponds to the protonated and sodium adducts of GSH, respectively (Figure 24, Appendix B), while the fraction eluting at 5.380 min has a monoisotopic mass of 127.038 corresponding to protonated thymine (Figure 25, Appendix B). Unfortunately, all other fractions could not be identified by MALDI-ToF MS. The presence of glutathione accelerates the degradation of 66 and results in the formation of new compounds. Furfural (67) was not a major product although thymine release was observed. This may be due either to the formation of adducts between furfural and GSH or the formation of adducts between GSH and 66 that also facilitate thymine release. Figure 3-11 shows the consumption of more than 75% of 66 in 30 minutes.

Figure 3-10: Reversed-phase HPLC chromatogram after incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C in the presence of 6 mM GSH after 30 minutes
Figure 3-11: Incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C in the presence of 6 mM GSH

3.4.1.2 Reactivity of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66) in the Presence of Deoxyadenosine

3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66) is an unsaturated aldehyde that is a highly reactive electrophile which may result in the formation of DNA adducts. To explore the reactivity of this electrophilic fragment towards the nucleobases found in DNA, incubation of 66 in 100 mM phosphate buffer (pH = 7.4) at 37 °C in the presence of excess 2-deoxyadenosine (dA) (149) was performed. An aliquot of the reaction mixture was analyzed by analytical reversed-phase HPLC (Figure 3-12). The chromatogram shows the decomposition of 66 to furfural (67) and thymine (23). The formation of adducts was however not observed, although the degradation of 66 to furfural and thymine was accelerated. The half-life of 66 in the presence of dA was found to be 3 hours based on recovered 66 compared to 13 hours in the absence of dA under the same conditions of incubation (Figure 3-13).
3.4.1.3 Reactivity of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66) in the Presence of a dA Containing Oligonucleotide

To investigate the reactivity of 66 towards adenine in oligonucleotides, the incubation of 66 in 100 mM phosphate buffer (pH = 7.4) at 37 °C in the presence of
excess unmodified oligonucleotide (150) was performed (Figure 3-14). An aliquot of the reaction mixture was analyzed by analytical reversed-phase HPLC (Figure 3-15). As was the case with dA (149), the chromatogram shows the decomposition of 66 to furfural (67) and thymine (23). The formation of adducts was not observed.

Figure 3-14: Incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C in the presence of excess unmodified oligonucleotide 5'-TCTATCTATCT-3'

Figure 3-15: Reversed-phase HPLC chromatogram after the incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C in the presence of an excess of unmodified oligonucleotide (TCTATCTATCT) for 24 hours

3.5 Reactivity of Furfural

Oxidation at the 5' position of DNA has been shown to lead to the formation of furfural, which is believed to form adducts with the amino group of adenine in oligonucleotides. Upon heating or treatment with base, an intramolecular rearrangement occur. Under acidic conditions the resulting adduct is undergoes deglycosylation to form
kinetin (Scheme 1.11). To investigate the reactivity of furfural toward 2-deoxyadenosine, incubation of furfural with dA was performed under non-physiological (pH 2 and 50 °C) conditions to mimic the conditions for the proposed mechanism of kinetin formation, as well as under physiological conditions (pH 7.4 and 37 °C) to determine its reactivity toward 2-deoxyadenosine.

3.5.1 Reactivity of Furfural (67) in the Presence of an Excess of dA at pH = 7.4 and 37 °C

To explore the reactivity of 67 toward 2-deoxyadenosine under physiological conditions, incubation of 67 in 100 mM phosphate buffer (pH=7.4) at 37 °C in the presence of excess deoxyadenosine (dA) (149) was performed. An aliquot of the reaction mixture were analyzed by analytical reversed-phase HPLC (Figure 3-16). The chromatogram shows no sign of adduct formation under these conditions.

![Figure 3-16: Reversed-phase HPLC chromatogram after incubation of 67 in 100 mM phosphate buffer pH = 7.4 at 37 °C in the presence of excess deoxyadenosine (dA) for 65 hours](image)

Since incubation of furfural under these conditions did not yield adduct formation, the incubation of furfural with dA was performed under acidic conditions and at elevated temperatures to mimic the conditions reported for the formation of kinetin.
3.5.2 Reactivity of Furfural (67) in the Presence of Excess dA at pH = 2 and 37 °C

To investigate the reactivity of 67 toward 2-deoxyadenosine under acidic conditions, 67 was incubated in 100 mM phosphate buffer (pH = 2.0) at 37 °C in the presence of excess deoxyadenosine (dA) (Figure 3-17). An aliquot of the reaction mixture were analyzed by analytical reversed-phase HPLC (Figure 3-18). The chromatogram shows, the deglycosylation of deoxyadenosine (149) resulting in the formation of adenine. The formation of adducts was not observed. The same result was obtained in the case of incubation of dA with an excess of furfural at the same pH of 2. Increasing the temperature of incubation from 37 °C to 50 °C did not yield to any adduct formation (Figure 3-19).

![Chemical structures](image)

Figure 3-17: Incubation of 23 in 100 mM phosphate buffer pH = 2.0 at 37 °C in the presence of excess deoxyadenosine (dA, 149)
In conclusion, oligonucleotides containing a 5'-aldehyde moiety were successfully synthesized and stored at 4 °C for 10 days before β-elimination to form a 5'-phosphate oligonucleotide could be detected. The half-life was determined to be 96 hours in ssDNA under physiological conditions which is similar to what was reported in the literature for ternary complexes (100.7 h). 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66), a proposed product resulting from the β-elimination of the oligonucleotide containing 5'-aldehyde, was successfully synthesized and the half-life of this lesion was determined to
be under physiological conditions (pH 7.4 and 37 °C) 13 hours. The presence of GSH has a great impact on the stability of 66 and resulted in the formation of new compounds that are still under investigation. 2-Deoxyadenosine accelerated the degradation of 66 to thymine and furfural with a half-life of 3 hours compared to 13 hours in the absence of dA. No adduct formation was observed in the incubation of 66 with unmodified oligonucleotides containing dA. Moreover, incubation of furfural with dA did not result in adduct formation.
CHAPTER FOUR

4.1 Conclusion and Future Research Directions

4.1.1 Synthesis and Photolysis of C3'-Modified Nucleosides

Oxidative damage to DNA by IR induces mutagenesity, genotoxicity and cell death, which contributes to the development of many diseases with cancer being one of the most important. DNA damage has also been implicated in aging. IR produces large amounts of LEEs with energy $< 15$ eV. These species have been established to cause SSBs and DSBs as well as base release. It was determined that LEEs break DNA bonds through a resonance process called dissociative electron attachment producing stable anions and radical fragments (Figure 1-5). One of the intermediates postulated to form is the C2',3'-dideoxy-C3'-thymidinyl radical (102). The goal of this work was to synthesize stable and efficient photolabile radical precursors for the generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) to elucidate the mechanism involved in LEE induced DNA damage. In order to understand the mechanisms involved in LEE induced damage to DNA, the fate of 102 was determined through its independent generation and elucidation of the structures of final DNA damage products. To facilitate this investigation, site-specifically modified nucleosides containing acyl photolabile groups were synthesized and proven to selectively generate the reactive intermediate of interest in a controlled manner at defined sites.
The synthesis of α- and β-C2',3'-dideoxy-C3’-pivaloylthymidine (103 and 101, respectively) was successfully achieved employing organic chemistry. The efficiency of 103 and 101 for the generation of C2',3'-dideoxy-C3'-thymidinyl radical (102) photochemically was investigated under anaerobic conditions using excess tri-nbutyltin hydride as a hydrogen atom donor. The formation of reduction product 118 provides proof for the suitability of 103 and 101 as precursors of the C2',3'-dideoxy-C3'-thymidinyl radical (102). The results showed that when α-isomer 103 is photolyzed in the presence of excess tri-nbutyltin hydride the reduction product was obtained as the sole product, which indicates the suitability of this precursor for radical generation. On the other hand, photolysis of the β-isomer in the presence of excess tri-nbutyltin hydride did not generate the reduction product 118 but instead resulted in the formation of products that could not be identified using our present methods. Attempts of changing the solvents in the case of β-isomer 101 were made to determine if the solvent plays any role in product formation. When the photolysis was done in absolute acetonitrile, as a polar aprotic organic solvent, aldehyde 119 was observed indicating trapping of the acyl radical (120) with a hydrogen atom faster than liberating carbon monoxide, this is may be due to the formation of a hydrogen bond between the oxygen of the acyl group and the hydrogen of the 5'-hydroxyl group forming a seven membered ring causing the decarbonylation to be slow and the trapping with a hydrogen atom donor to be faster. A second attempt using methanol as a polar protic organic solvent was carried out, Aldehyde 119 was also the only product observed. This also can be explained in the same way as seen in the case of acetonitrile. Additionally, it was reported that decarbonylation of acyl radicals decreases with increasing solvent polarity due to a decrease in the dipole moment during
the bond scission process since the exothermicity of the cleavage decreases with increasing solvent polarity. Thus the activation energy increases which causes slower decarbonylation.\textsuperscript{98} Using water as a solvent for photolysis was examined and it resulted in formation of products that could not be identified which could be due to the involvement of a Norrish Type II reaction as seen in Figure 2-8.

In order to fully evaluate these precursors, we examined their photochemical conversion in the presence of the more biologically relevant hydrogen atom donor glutathione (GSH). Photolysis of $\alpha$-isomer 103 was performed in the presence of 6 mM GSH and resulted in the formation of reduction product 118 in addition to other products that could not be identified using our present methods. In contrast, photolysis of $\beta$-isomer 101 in the presence of 6 mM GSH resulted in the formation of aldehyde 119 as the sole product. When the photolysis of both isomers was performed in the absence of a hydrogen atom donor, the $\alpha$-isomer 103 resulted in formation of the reduction product 118 and 2',3'-didehydro-2',3'-dideoxythymidine (125), which indicates the involvement of a radical disproportionation mechanism between two alkyl radicals. These radicals are either the C2',3'-dideoxy-C3'-thymidinyl radical (102) and the tert-butyl radical or two C2',3'-dideoxy-C3'-thymidinyl radical (102) to generate compounds 118 and 125 (Scheme 2.13 and 2.14, respectively). Additional products were formed such as free base and small amounts of unidentified products, but in case of the $\beta$-isomer 101 the photolysis resulted in formation of a very small amount of photoproducts that could not be identified.

Collectively, photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) from $\beta$-C2',3'-dideoxy-C3'-pivaloylthymidine 101 and $\alpha$-C2',3'-
dideoxy-C3'-pivaloylthymidine 103 precursors were investigated under different conditions and based on the results obtained, precursor 103 was found to generate the reduction product 118 under all conditions. In the absence of a hydrogen atom donor the formation of C2',3'-didehydro-2',3'-dideoxythymidine (125) was detected which indicates the involvement of a radical disproportionation mechanism as explained. On the other hand precursor 101 was unable to generate the reduction product 118 under any conditions and resulted in the formation of products that could not identified using our present methods in addition to aldehyde 119 resulting from trapping of the acyl radical. Therefore, based on the results obtained precursor 103 will be used to investigate the fate of the C2',3'-dideoxy-C3'-thymidinyl radical (102) in oligonucleotides.

In order to incorporate 103 into oligonucleotides, the phosphoramidite or the H-phosphonate building block of α-C2',3'-dideoxy-C3'-pivaloylthymidine 103 will be synthesized and incorporated into DNA using automated reversed DNA synthesis in the case of the phosphoramidite building block, or semi-automated synthesis in the case of the H-phosphonate building block. In the case of the H-phosphonate monomer the unmodified portion of the oligonucleotides will be synthesized using automated reversed DNA synthesis. The identity and purity of both modified and unmodified oligonucleotides will be established using MALDI-ToF and IEX- and RP-HPLC. Quantification will be done using UV-Vis spectroscopy. The photochemical generation of the C2',3'-dideoxy-C3'-thymidinyl radical (102) in oligonucleotides will be conducted under anaerobic and aerobic conditions in the presence of GSH at physiological conditions. The identification of the damage products will be performed using MALDI-
ToF and IEX- and RP-HPLC. This will help elucidate the mechanistic pathway in which LEE induce DNA damage.

Analysis of the unknown products could be carried out by LC-MS spectrometry which otherwise were difficult to be observed by ESI and MALDI MS techniques. Changing the photolysis conditions for the β-isomer 101 from neutral to mild basic may result in the formation of the radical of interest 102. This prevents the formation of intramolecular hydrogen bonding between the acyl and the 5'-hydroxy groups that can helps in faster decarbonylation.

4.1.2 5'-Oxidative Damage

Oxidative damage to DNA caused by ROS leads to the formation of single and double strand breaks. Oxidation of 2-deoxyribose at the 5'-position in DNA leads to the formation of strand breaks containing 3'-phosphate (11) and 5'-aldehyde residues (36) under aerobic conditions. Oligonucleotides containing a 5'-aldehyde moiety such as 36 are unstable and are proposed to undergo β-elimination to form electrophilic fragment 66 followed by loss of the nucleobase 23 to deliver the aromatic compound furfural and free base (67, Scheme 1.10). With the goal to investigate the stability and reactivity of oligonucleotides containing a 5'-aldehyde lesion, and its degradation products, fragments believed to be produced upon C5'-oxidation, and oligonucleotides containing the 5'-aldehyde moiety were independently synthesized. The synthesis of the H-phosponate of the modified 5'-nucleoside 135 was successfully achieved and used toward the synthesis of modified oligonucleotides (138-143) with high coupling yields. The 5'-diol containing oligonucleotides were successfully converted to the corresponding 5'-aldehyde through oxidative cleavage. Storage of the 5'-aldehyde containing oligonucleotide (144) at 4 °C
was stable for 10 days before β-elimination to form a 5'-phosphate oligonucleotide could be detected. The half-life of the 5'-aldehyde lesion in single-stranded DNA under physiological conditions (pH 7.4, 37 °C) was determined to be 96 hours which is agreement to what is reported in the literature\textsuperscript{102} for ternary complexes (100.7 h). The 3',4'-didehydro-2',3'-dideoxy-5'-oxothymidine (66), a proposed product resulting from the β-elimination of the oligonucleotide containing 5'-aldehyde, was successfully synthesized and the half-life of this lesion was determined to be under physiological conditions (pH 7.4 and 37 °C) 13 hours. The presence of GSH has a great impact on the stability of 66 and resulted in the formation of new compounds that are still under investigation. 2-Deoxyadenosine accelerated the degradation of 66 to thymine and furfural with a half-life of 3 hours compared to 13 hours in the absence of dA. No adduct formation was observed in the incubation of 66 with unmodified oligonucleotides containing dA. Moreover, incubation of furfural with dA under physiological condition (pH 7.4 and 37 °C) did not result in adduct formation. Moreover, incubation of furfural with dA under (pH 2 and 37 °C) resulted in deglycosylation of dA.

In order to investigate stability and reactivity of oligonucleotides containing a 5'-aldehyde toward cellular nucleophile, incubation with GSH, nucleosides such as dA, dG, and dC as well as small DNAs and amino acids such as lysine and arginine and small peptides be investigated to determine adduct formation.

In order to identify electrophilic fragment such as 66, we could try to use derivatization methods to convert the aldehyde to more stable compound that can be detected by MS or NMR techniques. For example, compound 66 can be reacted with
ethyl thiol in the similar way as was performed by stubbe to identify the 2-Methylene-3(2H)-furanone\textsuperscript{109}. 
5 CHAPTER FIVE

5.1 EXPERIMENTAL PROCEDURES

All experiments were performed with standard laboratory instruments under an argon atmosphere using oven-dried glassware where water and oxygen exclusion were necessary.

5.2 Materials

All chemicals, reagents and solvents were purchased from the following commercial suppliers: Acros Organics, Sigma-Aldrich, EMD Chemicals, PHARMCO-AAPER and Fisher Scientific, and used without further purification unless otherwise stated. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories. For chromatographic separation, HPLC grade solvents were used. Deionized water was purified with a PURELAB® Ultra Water Purification System. Anhydrous THF was dried over activated alumina. Triethylammonium acetate buffer (1 M) was purchased from Calbiochem. Pivaloyl chloride (Acros Organic) was distilled according to standard techniques. The Dess-Martin Reagent was synthesized according to published literature.\textsuperscript{110} \(\beta\text{-tri-Butylstannylstyrrene}\) was synthesized according to published literature.\textsuperscript{111-112}

5.3 Structural Analysis
All synthesized products were characterized by NMR spectroscopy and mass spectrometry.

5.3.1 NMR Spectroscopy

5.3.1.1 $^1$H-NMR

All $^1$H-NMR spectra were acquired on a Varian VXR-400, Varian Unity Inova-600 NMR, or Bruker-Avance III-600 Nuclear Magnetic Resonance spectrometer in CDCl$_3$, CD$_3$CN, or DMSO. Chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS) in CDCl$_3$, residual CD$_3$CN, or DMSO as internal references. Coupling constants ($J$) are reported in hertz (Hz). Multiplicity is as follows: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet. The protons of the carbons of the furanose ring and the nucleobase thymine are designated as shown in Figure 5-1.

Figure 5-1: The proton assignment of the deoxyribose sugar and the nucleobase moieties in thymidine

5.3.1.2 $^{13}$C-NMR

All $^{13}$C-NMR were performed on a Varian VXR-400, Varian Unity Inova-600, or Bruker-Avance III-600 Nuclear Magnetic Resonance spectrometer in CDCl$_3$ using the triplet centered at $\delta$ 77.23 for CDCl$_3$ as an internal standard. The spectra are $^1$H-broadband-decoupled.
5.3.1.3 $^{31}$P-NMR

$^{31}$P NMR data was obtained on a Varian VXRS-400 Nuclear Magnetic Resonance spectrometer. All chemical shifts were reported in ppm relative to the external reference 5% H$_3$PO$_4$ in CDCl$_3$. The spectra are $^1$H-broadband-decoupled.

5.3.1.4 $^1$H-NOESY-NMR

$^1$H-NOESY NMR spectra were acquired on a Varian Unity Inova-600 Nuclear Magnetic Resonance spectrometer.

5.3.2 Mass Spectrometry

Mass spectrometric analyses of the synthesized products were performed using ESI and MALDI-ToF MS.

5.3.2.1 ESI-MS

Mass spectra were performed on an Esquire electrospray ionization (ESI) mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in the positive ion mode and equipped with a quadrupole ion-trap mass analyzer. The samples were dissolved in Methanol to make final concentration of 0.1 µg/µL. The signals obtained correspond to the [M+H]$^+$ or [M+Na]$^+$ ions of the analyte.

5.3.2.2 MALDI-ToF MS

MALDI-ToF MS analyses were conducted using a MALDI-TOF UltrafleXtreme (Bruker Daltonics, Billerica, MA) operating in positive-ion, reflectron mode. Ions were formed by a pulsed UV laser beam, (Nd-YAG laser, wavelength 355 nm), with pulse duration of 1-5 ns. Spectra were recorded by accumulating 500-2500 shots per individual measurement. The pulse ion extraction delay was 100 ns. The acceleration voltage was varied from 22 to 25 kV. The samples were desalted either by reversed-phase HPLC or
by solid phase extraction using C18 ZipTip™. Sample spotting procedures are as follows:

A: for small molecules, the matrix solution employed was 2,5-dihydroxybenzoic acid (DHB) (20 mg/mL in 30% acetonitrile: 0.1% TFA in H₂O). The matrix (1 µL) was spotted on the MALDI plate. After evaporation to dryness, 1 µL of sample (1 µL each, ≥100 pmole) was spotted on top of the matrix and let dry at room temperature. The signals obtained correspond to the [M+H]⁺ or [M+Na]⁺ ions of the analyte, and isotopic peaks were recorded as well.

B: For oligonucleotides, samples, the matrix solution employed was composed of 90% of 3-hydroxypicolinic acid (HPA) (50 mg/mL in 50% acetonitrile: H₂O) and 10% of ammonium citrate (50 mg/mL in H₂O). The matrix (1 µL) was spotted on the MALDI plate. After evaporation to dryness, 1 µL of sample (1 µL each, ≥30 pmole) was spotted on top of the matrix and let dry at room temperature. The signals obtained correspond to the [M+H]⁺ ions of the analyte, and isotopic peaks were recorded as well. Calibration was conducted before each measurement using external standards. For small molecules, the standard mass range was from 0-500 m/z. The reference mixture contained 1 µL of 10 µM clonidine, 1 µL of 10 µM propranolol, 1 µL of 10 µM hexamethoxyphosphazene, 1 µL of 10 µM papaverine, 1 µL of 10 µM verapamil, 10 µL of Electrospray tuning mix (Hewlett packard product # G2431A), and 26 µL of H₂O. For oligonucleotides the standard mass range was from 1000-4000 Da. To calibrate the MALDI-Tof MS instrument in this mass range, calibration mixture (Bruker, oligonucleotide calibration standard low molecular weight) was used.
5.3.2.3 High Resolution Mass Spectrometry

High resolution mass spectrometry (HRMS) was performed on a Micromass Q-ToF II mass spectrometer located at the Mass Spectrometry and Proteomics Facility, The Ohio State University.

5.3.3 Chromatographic Methods

5.3.3.1 Thin Layer Chromatography

Thin layer chromatography (TLC) was used to monitor all organic reactions, and to monitor the elution of compounds from flash chromatography. TLC was performed using Silicycle silica gel 60 F254 aluminum backed plates. Compound spots were visualized by UV light (254 nm) and stained with an anisaldehyde dip composed of the following: 180 mL of absolute ethanol, 10 mL concentrated sulfuric acid, 2 mL glacial acetic acid, and a few drops of p-anisaldehyde.

5.3.3.2 Flash Chromatography

Purification of all modified nucleosides was performed using an automated flash chromatography system unless otherwise stated. Purification was performed on a Biotage SP4 chromatography system equipped with an in-line variable wavelength detector. The nucleoside products were detected at 254 nm and monitored at 260 nm. TLC was used to determine the purity of chromatography eluents, and fractions containing pure compound were combined and evaporated under reduced pressure using a Heidolph Collegiate Brinkmann rotary evaporator.

5.3.3.3 HPLC

All high performance liquid chromatography (HPLC) analyses were performed on a Dionex Ultimate 3000 HPLC system equipped with an in-line variable wavelength
detector. Reversed-phase HPLC was used in the analyses of the modified nucleoside in monomer and oligo experiments. The analyses include identification and quantification of the products. Two types of reversed-phase columns were used:

Column 1: Thermo Hypersil-Keystone BDS Hypersil C-18 column, 4.6 x 250 cm, 5 µm.
Column 2: Agilent ZORBAX SB-C8 column, 4.6 x 250 cm, 5 µm.

The solvent systems used were:

Solvent A: 50 mM TEAA buffer pH = 7.0.
Solvent B: Acetonitrile.

Ion-exchange HPLC was used in the analyses of the modified oligonucleotides. The analytes are separated based on charge. In contrast to RP-HPLC, a n-1 resolution is obtained with this method. Ion-exchange column used: Dionex Pac PA-100 (13 µm), 4.6 x 250 cm.

The solvent systems used were:

Solvent A: 25 mM sodium acetate in 5% acetonitrile, pH = 6.0.
Solvent B: 25 mM sodium acetate in 5% acetonitrile, pH = 6.0, 1 M NaCl.

5.3.4 Other Equipment and Devices

Centrifuge - Thermo Electron Sorall Legend Micro21 centrifuge
High vacuum pump – Edwards RV3
pH meter – Fisher Accumet Basic AB15
Pipettes- Eppendorf Series 2100
Rotary evaporator – Heidolph Collegiate Brinkmann rotary evaporator
Solvent purification system – Innovative Technology PS-MD-2 Pure Solvent system
SpeedVac concentrator – Thermo Electron Savant DNA120
Thermal mixer – Eppendorf Thermomixer
Vortex mixer – Fisher Scientific

5.4 Synthesis of Nucleoside Radical Precursors

5.4.1 Synthesis of 3'-β-(pivaloyl)-3'-deoxythymidine (101)

5.4.1.1 5'-O-(tert-Butyldimethylsilyl)-3'-deoxy-C-methylenethymidine (107):

C3'-methylene thymidine was synthesized as reported by Wengel et al.\textsuperscript{94}

5.4.1.2 1-[(5-O-tert-Butyldimethylsilyl)-3-C-(hydroxymethyl)-2,3-dideoxy-b-D-threo-
pentofuranosyl]thymine (108):

To a solution of 107 (0.94 g, 2.66 mmol) in anhydrous THF (5.4 mL) under nitrogen, was added BH\textsubscript{3}:1,4-oxathiane (0.33 mL of a 7.8 M solution in oxathiane, 2.87 mmol) at room temperature. After cooling of this mixture to 0 °C a 2 M solution of NaOH (1.5 mL) was slowly added followed by the dropwise addition of 30% aqueous H\textsubscript{2}O\textsubscript{2} (0.38 mL). Stirring was continued for 1 h at room temperature. The reaction mixture was poured into ice-water (68 mL) and extracted with diethylether (80 mL). The combined organic phase was washed with water (68 mL) and saturated aqueous NaHCO\textsubscript{3} (2 x 68 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and evaporated to dryness \textit{in vacuo}. The product was purified on a silica gel column (50:50 ethylacetate:pentane) to give 108 as a clear colorless oil. Yield: 0.88 g (89%). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 400 MHz) δ: 0.15 (6H, s, Si(CH\textsubscript{3})\textsubscript{2}), 0.94 (9H, s, SiC(CH\textsubscript{3})\textsubscript{3}), 1.81 (1H, m, 2'-H\textsubscript{b}), 1.93 (3H, s, CH\textsubscript{3}), 2.44 (1H, m, 2'-H\textsubscript{a}), 2.72 (1H, m, 3'-H), 3.34 (1H, t, J = 6.4 Hz, OH), 3.73-3.89 (3H, m, CH\textsubscript{2}OH, 5'-H), 4.00 (1H, m, 5'-H), 4.16 (1H, m, 4'-H), 6.06 (1H, dd, J = 8.2, 6.2 Hz, 1'-H\textsubscript{a}), 7.48 (1H, s, 6-H), 9.44 (1H br s, NH). \textsuperscript{13}C (CDCl\textsubscript{3}, 100 MHz) δ: −5.3, 12.8, 18.4, 26.0, 34.0, 42.6,
61.8, 62.6, 79.8, 84.3, 111.0, 135.6, 150.8, 164.2. HRMS [M + H]^+: calc. for C\textsubscript{17}H\textsubscript{31}O\textsubscript{5}N\textsubscript{2}Si 371.2002, found 371.2001.

5.4.1.3 1-[(5-O-tert-Butyldimethylsilyl)-3-C-(formyl)-2,3-dideoxy-β-D-threo-
pentofuranosyl]thymine (109):

A solution of 108 (0.83 g, 2.24 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (8 mL) was cannulated into a solution of Dess-Martin periodinane (1.43 g, 3.37 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (16 mL) at 0 °C. After stirring overnight at room temperature, diethyl ether (70 mL) was added and the solution was poured slowly into a solution of saturated NaHCO\textsubscript{3} (45 mL) containing Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}• 5H\textsubscript{2}O (5.56 g). The organic layer was removed and the aqueous layer extracted with diethyl ether (95 mL). The combined organic phase was washed with saturated NaHCO\textsubscript{3} (95 mL) followed by H\textsubscript{2}O (95 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and evaporated to dryness in vacuo. The product was isolated as a white amorphous solid. Yield: 0.83 g (100%).

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 600 MHz) δ: 0.10 (6H, s, Si(CH\textsubscript{3})\textsubscript{2}), 0.91 (9H, s, SiC(CH\textsubscript{3})\textsubscript{3}), 1.94 (3H, d, J = 1.2 Hz, CH\textsubscript{3}), 2.43 (2H, m, 2'-H), 3.27 (1H, m, 3'-H), 3.95 (2H, m, 5'-H), 4.40 (1H, m, 4'-H), 6.14 (1H, dd, J = 7.2, 6.60 Hz, 1'-H), 7.44 (1H, d, J = 1.2 Hz, 6-H), 8.27 (1H, br s, NH), 9.85 (1H, s, CHO). \textsuperscript{13}C (CDCl\textsubscript{3}, 150 MHz) δ: –5.5, 12.7, 18.3, 26.0, 31.4, 51.0, 61.8, 80.3, 83.8, 111.4, 135.4, 150.9, 164.2, 199.5. HRMS [M + H]^+: calc. for C\textsubscript{17}H\textsubscript{29}O\textsubscript{5}N\textsubscript{2}Si calc. 369.1846, found 369.1846.

5.4.1.4 1-[(5-O-tert-Butyldimethylsilyl)-3-C-(2,2-dimethyl-1-hydroxypropyl)-2,3-
dideoxy-β-D-threo-pentofuranosyl]thymine (110):

Cerium chloride was dried as described by Kamiya.\textsuperscript{113} To a suspension of dry CeCl\textsubscript{3} (16.7 g, 44.8 mmol) in THF (105 mL) at –78 °C was added a 1.6 M solution of t-butyllithium (28.0 ml, 44.8 mmol) in pentane. This was allowed to stir for 1.5 h before a
solution of 109 (0.83 g, 2.24 mmol) in THF (41 mL) was added dropwise at the same temperature. The reaction stirred at this temperature for 3.5 h, after which it was quenched by the addition of aqueous NH₄Cl (200 mL) and allowed to warm to room temperature. The product was then extracted with CH₂Cl₂ (6 x 250 mL), the organic layer dried over Na₂SO₄, and the solvent removed in vacuo. The crude product was purified on a silica gel column (40% ethyl acetate in hexane) to give 110 as a diasteromeric mixture of a clear colorless oil. Yield: 0.37 g (67%).

**1H-NMR (CDCl₃, 400 MHz)** δ: 0.16 (6H, s), 0.94 (18H, 4s), 1.93 (3H, d, J = 1.2 Hz), 2.15 (1H, m), 2.27 (1H, m), 2.69 (1H, m), 3.47 (1H, d, J = 3.2), 3.60 (1H, d, J = 2.8 Hz), 3.90 (1H, m), 4.03 (2H, m), 6.08 (1H, dd, J = 9.0, 5.8 Hz), 7.50 (1H, d, J = 1.2 Hz), 8.8 (1H, br s).

**13C (CDCl₃, 100 MHz)** δ: -5.2, 12.8, 18.5, 26.0, 27.0, 31.5, 35.5, 40.8, 63.0, 75.9, 80.5, 83.9, 111.1, 135.9, 150.8, 163.9. HRMS [M + H]⁺: calc. for C₂₁H₃₉O₅N₂Si 427.2628, found 427.2629.

5.4.1.5 1-[(5-O-tert-Butyldimethylsilyl)-3-C-(2,2-dimethyl-1-oxopropyl)-2,3-dIDEOXY-β-D-threo-pentofuranosyl]thymine (111):

To a solution of Dess-Martin periodinane (0.30 g, 0.71 mmol) in anhydrous CH₂Cl₂ (3 mL) was cannulated a solution of 110 (0.20 g, 0.47 mmol) in CH₂Cl₂ (2 mL) at 0 °C. Stirring was continued at 0 °C for 15 minutes then at room temperature for 4 h. Diethylether (14 mL) was added and the solution was poured slowly into a solution of (1.16 g, 4.69 mmol) Na₂S₂O₅.5H₂O in sat. NaHCO₃ (9 mL). The organic phase was washed with saturated NaHCO₃ (20 mL) followed by H₂O (20 mL), and saturated NaCl (20 mL), dried with MgSO₄ and evaporated to dryness in vacuo. The product was isolated as a white solid. Yield: 0.19 g (96%).

**1H-NMR (CDCl₃, 400 MHz)** δ: 0.06 (6H, s, Si(CH₃)₂), 0.88 (9H, s, SiC(CH₃)₃), 1.16 (9H, s, COC(CH₃)₃), 1.96 (3H, d, J = 0.8 Hz,
CH₃), 2.06 (1H, m, 2'-Hₐ), 2.49 (1H, m, 2'-Hₐ), 3.68 (2H, m, 5'-H), 3.81 (1H, m, 3'-H), 4.20 (1H, m, 4'-H), 6.14 (1H, dd, J = 8.0, 6.0 Hz, 1'-H), 7.51 (1H, d, J = 0.8 Hz, 6-H), 9.23 (1H, br s, NH). ¹³C (CDCl₃, 100 MHz) δ: −5.1, 12.9, 18.7, 26.0, 26.2, 36.7, 44.2, 45.0, 62.6, 80.8, 84.3, 111.4, 135.6, 150.8, 164.0, 214.1. HRMS [M + H]⁺: calc. for C₂₁H₃₇O₅N₂Si 425.2472, found 425.2473.

5.4.1.6 1-[2,3-dideoxy-3-C-(2,2-dimethyl-1-oxopropyl)-2,3-dideoxy-β-D-threo-pentofuranosyl]thymine (101):

To a stirred solution of 111 (0.12 g, 0.283 mmol) in THF (3 mL), was added aqueous trifluoroacetic acid (0.74 mL, 1:1, TFA/H₂O) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was stirred at room temperature until completion. It was then neutralized with saturated aqueous NaHCO₃ (5 mL) and diluted with 10 mL of ethyl acetate. The aqueous layer was then extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄ and evaporated to dryness in vacuo. The crude product was purified on silica gel column (0-2% methanol in dichloromethane) to give 101 as a colorless foam. Yield: 0.08 g (91%). ¹H-NMR (CDCl₃, 400 MHz) δ: 0.99 (9H, s, COC(CH₃)₃), 1.11 (3H, s, CH₃), 1.27 (1H, m, 2'-Hₐ), 1.40 (1H, m, 2'-Hₐ), 2.30 (1H, m, 3'-H), 3.29 (1H, s, OH), 3.36 (2H, m, 5'-H), 3.35 (1H, m, 4'-H), 4.67 (1H, t, J = 7.2 Hz, 1'-H), 6.87 (1H, s, 6-H), 9.66 (1H, br s, NH). ¹³C (CDCl₃, 100 MHz) δ: 12.8, 24.9, 32.3, 38.6, 47.0, 63.8, 71.3, 83.4, 87.1, 110.0, 110.8, 137.3, 150.9, 164.5, 216.9. ESI [M + Na]⁺: calc. for C₁₅H₂₂O₅N₂Na 333.1, found 333.2.
5.4.2 Synthesis of 3'-α-(pivaloyl)-3'-deoxythymidine (103)

5.4.2.1 1-[5-(tert-Butyldiphenylsilyl)-2,3-dideoxy-3-C-formyl-β-D-erythro-
        pentofuranosyl]thymine (115):

        Compound 115 was synthesized as reported by Sanghvi, et al.\textsuperscript{97}

5.4.2.2 1-[5-(tert-Butyldiphenylsilyl)-2,3-dideoxy-3-C-(2,2-dimethyl-1-hydroxypropyl) -
        β-D-erythro-pentofuranosyl]thymine (116):

        Cerium chloride was dried as described by Kamiya.\textsuperscript{113} To a suspension of dry
        CeCl\textsubscript{3} (8.32 g, 22.34 mmol) in THF (52 mL), was added dropwise 1.7 M t-butyllithium in
        pentane (13.14 mL, 22.34 mmol) at -78 °C. The solution was allowed to stir at this
        temperature for 1.5 hours. Aldehyde 115 (0.55 g, 1.117 mmol) in THF (20 mL), which
        was co-evaporated with dry THF, was added over 25 min and allowed to stir at the same
        temperature for 8 hours, before quenching by the addition of saturated NH\textsubscript{4}Cl (101 mL)
        at -78 °C. The reaction mixture was extracted with dichloromethane (x6), the organic
        layer was dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and the solvent was removed under reduced
        pressure to afford a brownish foam. The crude mixture was purified by column
        chromatography with 1:1 ethyl acetate:hexane to afford 116 as a diastereomeric mixture
        in the form of a colorless foam. Yield: 0.17 g (30%). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 400 MHz) δ:
        0.88-0.92 (18H, 2s), 1.1 (18H, s), 1.61 (6H, s), 1.85 (1H, m), 2.02 (1H, d, \textit{J} = 5.6), 2.24
        (1H, m), 2.45 (1H, d, \textit{J} = 5.6 Hz), 2.71 (2H, m), 3.19 (1H, d, \textit{J} = 5.2 Hz), 3.33 (1H, t, \textit{J} =
        4.99 Hz), 3.81, 3.88 (2H, 2 dd, \textit{J} = 11.4, 2.8 Hz), 3.98 (1H, m), 4.07 (2H, m), 4.33 (1H, m),
        6.13 (1H, t, \textit{J} = 6.8 Hz), 6.23 (1H, t, \textit{J} = 6.4 Hz), 7.36-7.69 (22H, m), 9.08 (1H, br s),
        9.17 (1H, br s). \textsuperscript{13}C (CDCl\textsubscript{3}, 100 MHz) δ: 12.3, 19.6, 26.6, 26.7, 27.2, 32.8, 35.8, 36.1,
        38.5, 40.1, 40.6, 63.3, 66.0, 77.4, 82.2, 82.9, 84.3, 85.4, 85.6, 111.0, 128.03, 128.07,
5.4.2.3 1-[5-(tert-Butyldiphenylsilyl)-2,3-dideoxy-3-C-pivaloyl-β-D-erythro-pentofuranosyl]thymine (117):

A solution of 116 (0.17 g, 0.309 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) was added to a solution of the Dess-Martin periodinane (0.2 g, 0.472 mmol) in anhydrous CH$_2$Cl$_2$ (2.2 mL) at 0 ºC. Stirring was continued at 0 ºC for 15 minutes then at room temperature overnight. The reaction mixture was diluted with diethyl ether (10 mL), poured into ice-cold aqueous saturated NaHCO$_3$ (6.2 mL) containing Na$_2$S$_2$O$_3$·5H$_2$O (0.77 g, 3.09 mmol), and stirred for 10 minutes. The organic layer was washed with sat. NaHCO$_3$, H$_2$O, and sat. NaCl, dried over Na$_2$SO$_4$, and the solvent was removed under reduced pressure to afford 117 as a colorless foam. Yield: 0.17 g (100%). $^1$H-NMR (CDCl$_3$, 400 MHz) δ: 1.11 (18H, s, SiC(CH$_3$)$_3$ & COC(CH$_3$)$_3$), 1.62 (3H, d, $J$ = 0.8, CH$_3$), 2.24 (1H, m, 2'-H$_b$), 2.43 (1H, m, 2'-H$_a$), 3.66 (1H, dd, $J$ = 11.8, $J$ = 2.6, 5'-H), 3.84 (1H, m, 3'-H), 4.09 (1H, dd, $J$ = 12, $J$ = 2, 5'-H), 4.25 (1H, dt, $J$ = 6.8, $J$ = 2.4, 4'-H), 6.22 (1H, dd, $J$ = 6.8 Hz, $J$ = 5.2 Hz, 1'-H), 7.37-7.47 (6H, m, Ar), 7.53 (1H, d, $J$ = 0.8, 6-H), 7.64-7.68 (4H, m, Ar), 8.88 (1H, br s, NH). $^{13}$C (CDCl$_3$, 100 MHz) δ: 12.3, 19.6, 26.0, 27.3, 30.5, 39.0, 43.7, 44.9, 63.5, 83.8, 85.6, 111.1, 128.2, 130.3, 132.8, 135.6, 150.3, 164.1, 215.3. HRMS [M + Na]$^+$: calc. for C$_{31}$H$_{40}$O$_5$N$_2$SiNa 571.2604, found 571.2617.

5.4.2.4 1-(2,3-dideoxy-3-C-pivaloyl-β-D-erythro-pentofuranosyl)thymine (103):

To a solution of 117 (0.15 g, 0.27 mmol) in THF (2.3 mL) was added a 1M solution of TBAF in THF (0.41 mL) at room temperature. Stirring was continued for 1 h.
The solvent was removed in vacuo and the crude mixture was purified by column chromatography with ethyl acetate to afford 103 as a colorless foam. Yield: 0.04 g (44%).

\[^1\text{H-NMR (CDCl}_3, 400 \text{ MHz) \delta: 1.18 (9H, s, COC(CH}_3}_3)\), 1.93 (3H, s, CH\text{\textsubscript{3}}), 2.44 (2H, m, 2'-H), 2.65 (1H, dd, \(J= 6.2 \text{ Hz, } J = 3.4, 5'\text{-OH}\)), 3.62 (1H, m, 3'-H), 3.97 (2H, m, 5'-H), 4.29 (1H, dt, \(J = 8 \text{ Hz, } J = 2.4 \text{ Hz, } 4'\text{-H}\)), 6.0 (1H, dd, \(J = 7.2 \text{ Hz, } J = 4.4 \text{ Hz, } 1'\text{-H}\)), 7.48 (1H, d, \(J = 0.8 \text{ Hz, } 6\text{-H}\)), 8.67 (1H, br s, NH). \]^1\text{C (CDCl}_3, 100 \text{ MHz) \delta: 12.8, 25.8, 38.7, 43.3, 45.2, 61.9, 84.7, 88.2, 110.9, 137.6, 150.4, 164.1, 215.8. HRMS [M + Na]^+: calc. for C\text{\textsubscript{15}}H\text{\textsubscript{22}}O\text{\textsubscript{5}}N\text{\textsubscript{2}}Na 333.1426, found 333.1419.

5.5 Synthesis of Damage products

5.5.1 Synthesis of C2',3'-Dideoxythymidine (118)

Compound 118 was synthesized as reported by Audat, et al.\textsuperscript{95}

5.5.2 Synthesis of C2',3'-Didehydro-2',3'-dideoxythymidine (125)

Compound 125 was synthesized according to published literature.\textsuperscript{114}

5.6 Photolysis Experiments

Photolysis experiments were performed in 4x10 mm quartz cuvettes (Sigma Aldrich, St. Louis, MO) using an Oriel 500 W High Pressure Mercury Arc Lamp (Newport, Irvine CA) fitted with an IR filter, focusing lens, and a 320 nm cut-off filter. The temperature of the photoreactions was maintained at 15 \(^0\text{C}\) using a Peltier PTP-1 single cell temperature controller system (Varian, Palo Alto, CA). The irradiation mixtures were analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm. HPLC analyses were carried out on either column mentioned in section 5.3.3.3 using solvent systems described in the same section. A stepwise gradient
was applied. 0-20% B over 15 min, 20-75% B over 3 min, 75-90% B over 4 min. Flow rate, 1.0 mL/min

5.6.1 Photochemical Generation of the C2',3'-Dideoxy-C3'-thymidinyl Radical from 103

5.6.1.1 Photolysis of 103 in the Presence of tri-nButyltin hydride as a Hydrogen Atom Donor

A solution of radical precursor 103 (600 nmole, 1 mM in 1:1 CH3CN/H2O) was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. tri-nButyltin hydride (1000 equivalents) was added under an argon atmosphere. The mixture was immediately photolyzed for 60 min at 15 °C. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C18 column and the solvent system described above. Products were identified by ESI-MS and by comparison with authentic samples. Product yields were determined using standard curves.

5.6.1.2 Photolysis of 103 in the Presence of Glutathione as a Hydrogen Atom Donor

A solution of radical precursor 103 (600 nmole, 1 mM) and glutathione (6 mM in 1:1 CH3CN/H2O) was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. The mixture was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. After the photolysis was completed, the crude volume was adjusted to 600 µL by addition of acetonitrile. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. The products were identified by ESI-MS and by spiking the photolysate with authentic samples.
5.6.1.3 Photolysis of 103 in the Absence of a Hydrogen Atom Donor

A photolysis sample containing 103 (600 nmol, 1 mM) in CH$_3$CN/H$_2$O (1:1 v/v) was transferred to a quartz cuvette and purged with argon for 20 minutes. The sample was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. The products were identified by ESI-MS and by spiking the photolysate with authentic samples.

5.6.2 Photochemical Generation of the C2',3'-Dideoxy-C3'-thymidinyl Radical from 101

5.6.2.1 Photolysis of 101 in the Presence of tri-$n$Butyltin hydride as a Hydrogen Atom Donor

5.6.2.1.1 Photolysis of 101 in 1:1 CH$_3$CN/H$_2$O

A solution of radical precursor 101 (600 nmole, 1 mM in 1:1 CH$_3$CN/H$_2$O) was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. tri-$n$Butyltin hydride (1000 equivalents) was added under an argon atmosphere. The mixture was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. Products were identified by ESI-MS and by comparison with authentic samples.
5.6.2.1.2 Photolysis of 101 in H₂O

A solution of radical precursor 101 (600 nmole, 1 mM in H₂O containing 0.1% acetonitrile) was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. tri-nButyltin hydride (1000 equivalents) was added under an argon atmosphere. The mixture was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. Products were identified by ESI-MS and by comparison with authentic samples.

5.6.2.1.3 Photolysis of 101 in CH₃CN

A solution of radical precursor 101 (600 nmole, 1 mM in CH₃CN) was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. tri-nButyltin hydride (1000 equivalents) was added under an argon atmosphere. The mixture was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. Products were identified by ESI-MS and by comparison with authentic samples.

5.6.2.2 Photolysis of 101 in the Presence of Glutathione as a Hydrogen Atom Donor

A solution of radical precursor 101 (600 nmole, 1 mM) and glutathione 6 mM in 1:1 CH₃CN/H₂O was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. The mixture was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. After the photolysis was completed, the crude volume was adjusted to 600 µL by addition of acetonitrile. The irradiation mixture was analyzed
directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. The products were identified by ESI-MS and by spiking the photolysate with authentic samples.

5.6.2.3 Photolysis of 101 in the Absence of a Hydrogen Atom Donor

A photolysis sample containing 101 (600 nmol, 1 mM) in CH$_3$CN/H$_2$O (1:1 v/v) was transferred to a quartz cuvette and purged with argon for 20 minutes. The sample was immediately photolyzed for 60 min at 15 °C under an argon atmosphere. The irradiation mixture was analyzed directly without workup by analytical reversed-phase HPLC with UV detection at 254 nm using C8 column and the solvent system described above. The products were identified by ESI-MS and by spiking the photolysate with authentic samples.

5.7 Synthesis of C5'-modified thymidine

5.7.1 1-[5-O-(acetyl)-5-C-((4,4'-dimethoxytrityl)oxymethyl)-2-deoxy-β-D-erythro-
pentofuranosyl]thymine (134)

Synthesis of 134 was performed as reported by Kodama, et al.$^{115}$

5.7.2 1-[5-O-(acetyl)-5-C-((4,4'-dimethoxytrityl)oxymethyl)-3-H-
phosphonateTriethylammonium Salt, 2-deoxy-β-D-erythro-
pentofuranosyl]thymine (135)

Imidazole (0.30 g, 4.41 mmol) was coevaporated twice with anhydrous acetonitrile and dissolved in anhydrous CH$_2$Cl$_2$ (15 mL). While stirring at -10 °C, phosphorous trichloride (0.11 mL, 1.26 mmol) followed by a solution of anhydrous triethylamine (0.62 mL, 4.45 mmol) in CH$_2$Cl$_2$ (0.62 ml) was added dropwise with vigorous stirring. The mixture was allowed to stir for 30 min at this temperature. The
modified nucleoside (134) (0.23 g, 0.37 mmol) was coevaporated twice with anhydrous pyridine and dissolved in anhydrous CH₂Cl₂ (9.2 mL). The solution was added to the reaction mixture over 30 min. The reaction mixture was slowly warmed to room temperature and stirrred for 8 hrs. The mixture was then hydrolyzed with 1 M triethylammonium bicarbonate TEAB (27 mL). The organic layer was separated and washed with an equal volume of 1 M TEAB. The organic layer was then concentrated under reduced pressure. The crude mixture was purified by column chromatography using a stepwise gradient of methanol (0-10%) in CH₂Cl₂ containing 1% triethylamine to afford 135 as a diasteromeric mixture in the form of a yellow foam. Yield: 0.10 g (34%).

1H-NMR (CDCl₃, 400 MHz) δ: 1.27 (18H, t, J = 7.2 Hz, N(CH₂CH₃)₃), 1.84-1.87 (6H, 2s, CH₃), 1.96-2.04 (2H, m, 2'-H), 2.127-2.13 (6H, 2s, COCH₃), 2.52-2.62 (2H, m, 2'-H), 2.95-3.01 (12H, q, J = 7.2 Hz, N(CH₂CH₃)₃), 3.22-3.51 (4H, m, 3', 5'-H), 3.77 (12H, s, OCH₃), 4.30-4.33 (2H, m, 4'-H), 4.70-4.86 (2H, m, 6'-H), 5.26-5.41 (2H, m, 6'-H), 6.04 (1H, d, J = 620.3 Hz, P-H), 6.09 (1H, d, J = 622.7 Hz, P-H), 6.24 (1H, dd, J = 8.4, 5.6 Hz, 1'-H), 6.31 (1H, dd, J = 7.2, 6.0 Hz, 1'-H), 6.80-7.42 (28H, m, Ar-H and 6-H). 13C (CDCl₃, 100 MHz) δ: 7.8, 9.1, 12.7, 12.8, 21.2, 21.3, 39.3, 39.6, 45.7, 52.7, 55.3, 62.4, 62.5, 71.9, 72.5, 73.18, 73.2, 77.4, 84.0, 84.1, 84.17, 84.2, 84.5, 84.7, 86.3, 110.9, 111.2, 113.15, 113.2, 126.9, 127.87, 127.9, 128.1, 130.0, 134.6, 135.1, 135.7, 135.76, 135.8, 144.6, 150.6, 158.5, 164.0, 164.1, 169.6, 170.3. 31P NMR (CDCl₃, 400 MHz) δ: 3.84, 4.20. HRMS [M + Et₃NH]⁺: calc. for C₄₆H₆₈O₁₁N₄P 883.46, found 883.54.

5.7.3 5'-O-(tert-Butyldimethylsilyl)-thymidine (105)

Compound 105 was synthesized according to published procedure.¹⁰⁴
5.7.4 5'-O-(tert-Butyldimethylsilyl)-3'-O-benzoyl-thymidine (146)$^{105}$

To a solution of 105 (4.96 g, 13.91 mmol) in anhydrous pyridine (14.3 mL) was added benzoyl chloride (1.9 mL, 16.38 mmol) at room temperature. Stirring was continued overnight. The solvent was removed *in vacuo* and the moist residue was triturated with water (100 mL). The resulting precipitate was filtered and air dried. The crude product was azeotropically dried with acetonitrile and then recrystallized from acetonitrile to afford 146 as white crystals. Yield: 5.77 g (90%). $^1$H-NMR (CDCl$_3$, 600 MHz) δ: 0.18 (6H, s, Si(CH$_3$)$_2$), 0.96 (9H, s, SiC(CH$_3$)$_3$), 1.95 (3H, d, J = 0.6 Hz, CH$_3$), 2.23 (1H, m, 2'-H), 2.60 (1H, dd, J = 13.8, 5.4 Hz, 2'-H), 3.98 (1H, dd, J = 11.4, 1.8 Hz, 5'-H), 4.04 (1H, dd, J = 11.4, 1.8 Hz, 5'-H), 4.27 (1H, d, J = 1.8 Hz, 4'-H), 5.51 (1H, d, J = 6.0 Hz, 3'-H), 6.50 (1H, dd, J = 9.6, 5.4 Hz, 1'-H), 7.48 (2H, t, J = 7.8 Hz, 4'-Ph), 7.61 (2H, m, 3-Ph and 6-H), 8.06 (2H, dd, J = 8.4, 1.2 Hz, 2-Ph), 8.86 (1H, br s, NH). $^{13}$C (CDCl$_3$, 150 MHz) δ: -5.4, -5.3, 12.6, 18.4, 26.0, 38.2, 63.8, 76.2, 84.9, 85.7, 111.4, 128.6, 129.3, 129.8, 133.6, 135.2, 150.9, 164.4, 166.3. ESI [M + Na]$^+$: calc. for C$_{23}$H$_{32}$O$_6$N$_2$SiNa 483.59, found 483.60.

5.7.5 3'-O-benzoyl-thymidine (147)$^{106}$

To a stirred solution of 146 (3.73 g, 8.10 mmol) in THF (37 mL), was added aqueous trifluoroacetic acid (13.8 mL, 1:1, TFA/H$_2$O) at 0 °C. After stirring for 30 minutes at 0 °C, the reaction mixture was stirred at room temperature overnight. It was then neutralized with saturated aqueous NaHCO$_3$ (60 mL) and diluted with 46 mL of ethyl acetate. The resulting precipitate was formed then filtered to give 147 as white powder. Yield: 2.66 g (95%). $^1$H-NMR (DMSO-d$_6$, 400 MHz) δ: 1.80 (3H, s, CH$_3$), 2.41 (2H, m, 2'-H), 3.70 (2H, m, 5'-H), 4.16 (1H, m, 4'-H), 5.28 (1H, t, J = 5.4 Hz, 5'-OH),
5.48 (1H, m, 3'-H), 6.29 (1H, t, J = 7.2 Hz, 1'-H), 7.56 (2H, t, J = 7.8 Hz, 4-Ph), 7.68 (1H, m, 3-Ph), 7.8 (1H, s, 6-H), 8.02 (2H, dd, J = 8.4, 1.2 Hz, 2-Ph), 11.38 (1H, br s, NH). $^{13}$C (DMSO-d$_6$ 100 MHz) δ: 12.4, 36.7, 61.4, 75.7, 83.8, 84.5, 109.8, 128.8, 129.4, 133.7, 135.9, 150.5, 163.7, 165.3. ESI [M + Na]$^+$: calc. for C$_{17}$H$_{18}$O$_6$N$_2$Na 369.33, found 369.50.

5.7.6 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66)$^{107}$

To a solution of the Dess-Martin periodinane (0.92 g, 2.169 mmol) in anhydrous CH$_3$CN (17 mL) was added 147 (0.3 g, 0.866 mmol) at 0 ºC. Stirring was continued at 0 ºC for 15 minutes then at room temperature overnight. The reaction mixture was diluted with diethyl ether (26 mL), filtered through a pad of celite, which was rinsed with acetonitrile. The organic layer was dried over Na$_2$SO$_4$, and the solvent was removed under reduced pressure to afford 3'-O-benzoyl-5'-oxothymidine (148) as a crude white powder 0.22 g. To a solution of the crude sample 148 in anhydrous CH$_2$Cl$_2$ (10 mL) was added triethylamine (0.36 mL), the reaction mixture was stirred at room temperature for 10 minutes, then the solvent was removed under reduced pressure at ambient temperature. The crude mixture was purified by column chromatography with ethyl acetate to afford 66 as white powder. Yield: 0.04 g (21%). $^1$H-NMR (CD$_3$CN, 400 MHz) δ: 1.82 (3H, d, J = 0.8 Hz, CH$_3$), 2.92 (1H, ddd J = 20.4, 5.2, 3.6 Hz, 2'-H), 3.38 (1H, ddd J = 20.4, 10.4, 2.8 Hz, 2'-H), 6.30 (1H, t, J = 2.8 Hz, 3'-H), 6.75 (1H, dd, J = 10.4, 5.2 Hz, 1'-H), 7.06 (1H, d, J = 0.8 Hz, 6-H), 9.01 (1H, br s, NH), 9.43 (1H, s, CHO). $^{13}$C (CD$_3$CN 100 MHz) δ: 12.5, 36.7, 86.8, 112.7, 121.3, 136.3, 151.1, 156.2, 164.5, 182.3. MALDI-Tof [M + Na]$^+$: calc. for C$_{10}$H$_{10}$O$_4$N$_2$Na 245.054, found 245.051
5.8 Oligonucleotide Synthesis

All oligonucleotide synthesis was carried out on an ABI 391 DNA synthesizer (Applied Biosystem, Carlsbad, CA). All reagents for automated oligonucleotide synthesis were purchased from Glen Research. Pivaloyl chloride (Acros Organic) was distilled according to the standard techniques. All syntheses were performed on a 0.2 µmole scale using controlled pore glass (CPG) columns (500 Å pore size). Syntheses of the unmodified oligonucleotides were carried out in the 3′→5′ direction using the phosphoramidite method. Modified oligonucleotides were partially synthesized in the 3′→5′ direction with standard phosphoramidite chemistry, followed by manual coupling of the modified nucleoside with H-phosphonate chemistry. Deblocking was accomplished using 3% trichloroacetic acid in dichloromethane. 5-Ethylthio-1H-tetrazole (0.25 M) in acetonitrile was used as an activator in the coupling steps of unmodified oligonucleotides, and pivaloyl chloride was used as an activator for the manual coupling of the H-phosphonate. Capping of the unreacted nucleotides was performed using acetic anhydride in THF/pyridine and 10% 1-methylimidazole in THF. Unmodified oligonucleotides were oxidized with 0.02 M I₂ in pyridine/THF/H₂O, while the oxidation of modified oligonucleotides were executed through simultaneous exposure to 4% I₂ in pyridine/H₂O/THF (1:1:8) and THF/H₂O/triethylamine (8:1:1) for 20 min.

5.8.1 Purification of Oligonucleotides

Oligonucleotides were deprotected and cleaved from the solid support by treating the resin with 1 mL of concentrated ammonium hydroxide (28-30% in H₂O) for 15-18 hrs at 55 °C, followed by purification.
5.8.1.1 Oligonucleotide Purification Cartridges (OPC) (Trityl-on Method)

Oligonucleotides sequences were synthesized with trityl on at the 5' end in order to use OPC purification. Columns were activated by flushing with 2 mL acetonitrile, followed by 2 mL 2 M triethylammonium acetate (TEAA) buffer. The ammonia solution of the crude oligonucleotide was diluted (1:3) and applied directly to the column. All impurities and failure sequences were eluted with 3 mL of (1:20) ammonium hydroxide. Excess ammonium hydroxide was then removed with 2 mL water. Direct detritylation on the column occurred by passing 2 mL of 3% TFA through the cartridge. Excess acid is then removed with 2 mL water. The pure oligonucleotide is eluted with 1 mL 20% acetonitrile in water containing 0.5% ammonium hydroxide.

5.8.1.2 Desalting of Oligonucleotide

Oligonucleotides were desalted using either OPC or size exclusion G-25 Sephadex columns.

5.8.1.2.1 Oligonucleotide Purification Cartridges (OPC)

The OPC column was activated by flushing with 2 mL acetonitrile, followed by 2 mL 2 M TEAA buffer. The oligonucleotide sample was loaded onto the column followed by flushing the column with 3 mL of 0.1 M TEAA to remove the salt from the cartridge. The desalted oligonucleotide was then eluted using 1 mL 50% aqueous acetonitrile.

5.8.1.2.2 Size Exclusion G-25 Sephadex columns.

G-25 Sephadex columns were manually prepared through packing the Macro Spin column with activated G-25 Sephadex. The column was then equilibrated with water (x 4). Centrifuging at 800 x g for 3 min to remove the storage solution. The oligonucleotides
were then loaded onto the column. The desalted oligos were eluted by centrifugation at the same speed and time.

5.8.2 Quantification of Oligonucleotides

Oligonucleotide quantification was performed on an Agilent 8453 UV-Vis spectrometer. The oligonucleotides were diluted with water and transferred into a quartz cuvette and the absorbance was measured at \( \lambda_{\text{max}} = 260 \) nm. The millimolar extinction coefficient at \( \lambda_{\text{max}} = 260 \) nm for each oligo was obtained from the online oligonucleotide properties calculator. Modified oligonucleotides were attributed the extinction coefficient of the corresponding unmodified oligos. Using Beer’s law, the concentration of the solutions and the absolute quantity of the oligos was calculated.

5.8.3 Synthesis of Modified Oligonucleotides

5.8.3.1 Modified Oligonucleotides Containing monomer (134)

A CPG-bound unmodified oligonucleotide was first synthesized on an ABI 391 DNA synthesizer using the phosphoramidite method on a 0.2 \( \mu \)mole scale. Incorporation of modified nucleoside 135 was achieved manually using H-phosphonate chemistry. Modified H-phosphonate 135 was coevaporated in anhydrous pyridine three times then dissolved in a mixture of acetonitrile: pyridine (1:1) to a final concentration of 0.1 M. This was transferred into a 1 mL syringe purged with argon. In another 1 mL purged syringe was placed a solution of 5 \( \mu \)L freshly distilled pivaloyl chloride in 305 \( \mu \)L acetonitrile: pyridine (1:1). Each syringe was then attached to either end of the CPG column containing the polymer-bound unmodified oligonucleotide. The activator was delivered first followed by the monomer and after repetitive mixing for 20 minutes, the reagents were then removed and the column was washed with 5 mL anhydrous
acetonitrile. The H-phosphonate linkage was oxidized to the corresponding more stable phosphodiester linkage by exposing the column to two 1 mL purged syringes, one containing 300 µL of 4% I₂ in pyridine/H₂O/THF (1:1:8) and the other contained 300 µL of THF/H₂O/triethylamine (8:1:1) for 20 min. The column was then washed with 10 mL anhydrous acetonitrile. Oligonucleotides were deprotected and cleaved from the solid support by treating the resin with 1 mL of concentrated ammonium hydroxide (28-30% in H₂O) for 15-18 hrs at 55 °C, followed by purification of oligonucleotides using OPC to afford the 5'-vicinal diol oligonucleotides (138-143).

5.8.3.2 Modified Oligonucleotides

To a solution of oligonucleotides containing either one of (138-143) (10 nmol) in 100 µL H₂O was added 13.58 µL of 0.2 M sodium periodate (NaIO₄) at 0 ºC. The reaction mixture was stirred for 3 hours at this temperature. The reaction was then quenched at 0 ºC by addition of methionine (0.2 M, 19.56 µL) and stirred for an additional 30 minutes. The oligonucleotides were desalted using a G-25 Sephadex column as described above. The oligomers were quantified as described in section 5.8.2. The purity of the 5'-aldehyde oligos was determined using analytical ion-exchange (IEX) and reversed-phase (RP) HPLC.

5.9 Stability of Modified Oligonucleotides

Oligonucleotides (1 nmol) were incubated in (500 µL) of 100 mM phosphate buffer pH = 7.4 at 37 ºC. Aliquots of the reaction mixture of equal amounts were removed at specific time intervals and injected onto the IEX chromatography. Decomposition of the 5'-aldehyde to the 5'-phosphorylated ODNs was monitored to determine the half-life of the lesion.
5.10 Stability of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66) at pH = 7.4 at 37 °C

Modified nucleoside 66 (1008 nmol) was incubated in 1000 µL of 100 mM phosphate buffer pH = 7.4 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times and analyzed by analytical RP-HPLC. Decomposition of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine was monitored to determine the half-life of the lesion.

5.11 Stability and Reactivity of 66 in Presence of GSH

Modified nucleoside 66 (4000 nmol) and 6 mM GSH were incubated in 1000 µL of 100 mM phosphate buffer pH = 7.4 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times and analyzed by analytical RP HPLC.

5.12 Reactivity of 3',4'-Didehydro-2',3'-dideoxy-5'-oxothymidine (66)

5.12.1 Reactivity of 66 in Presence of deoxyadenosine

Modified nucleoside 66 (12000 nmol) and deoxyadenosine (18000 nmol) were incubated in 1000 µL of 100 mM phosphate buffer pH = 7.4 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times and analyzed by analytical RP HPLC.

5.12.2 Reactivity of 66 in Presence of Oligonucleotides Containing dA

Modified nucleoside 66 (1008 nmol) was incubated in presence of (20 nmol) of unmodified oligonucleotide (TCT ATC TAT CT) in 1000 µL of 100 mM phosphate buffer pH = 7.4 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times were analyzed by analytical RP-HPLC.
5.13 Reactivity of Furfural

5.13.1 Reactivity of Furfural in Presence of dA at pH = 7.4 and 37 °C

Furfural (4000 nmol) and deoxyadenosine (8000 nmol) were incubated in 1000 µL of 100 mM phosphate buffer pH = 7.4 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times and analyzed by analytical RP HPLC.

5.13.2 Reactivity of Furfural in Presence of Excess dA at pH = 2 and 37 °C

Furfural (4000 nmol) and deoxyadenosine (8000 nmol) were incubated in 1000 µL of 100 mM phosphate buffer pH = 2 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times and analyzed by analytical RP HPLC.

5.13.3 Reactivity of Excess Furfural in Presence of dA at pH = 2 and 37 °C

Furfural (8000 nmol) and deoxyadenosine (4000 nmol) were incubated in 1000 µL of 100 mM phosphate buffer pH = 2 at 37 °C. Aliquots of the reaction mixture of equal amounts were removed at specific times and analyzed by analytical RP HPLC.
APPENDIX A

CHAPTER TWO
Figure 1: ESI-MS analysis of 118 from photolysis of 103 in presence of excess tri-nbutyltin hydride under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C

Calculated \([M+Na]^+\) 249.2
Measured \([M+Na]^+\) 249.2

Figure 2: ESI-MS analysis of 103 from photolysis of 103 in presence of excess tri-nbutyltin hydride under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C

Calculated \([M+Na]^+\) 333.3
Measured \([M+Na]^+\) 333.4

Calculated \([M+K]^+\) 349.4
Measured \([M+K]^+\) 349.4
Figure 3: MALDI-ToF mass spectrum analysis of compounds eluted at 13.580 and 13.740 min from photolysis of 101 in presence of excess tri-n-butylin hydride under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C.

Figure 4: MALDI-ToF mass spectrum analysis of compound eluted at 20.533 min from photolysis of 101 in presence of excess tri-n-butylin hydride under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C.
Figure 5: MALDI-ToF mass spectrum analysis of compound eluted at 13.887 min from photolysis of 101 in presence of excess tri-nbutyltin hydride under anaerobic conditions in acetonitrile for one hour at 15 ºC

Figure 6: MALDI-ToF mass spectrum analysis of compound eluted at 20.593 min from photolysis of 101 in presence of excess tri-nbutyltin hydride under anaerobic conditions in acetonitrile for one hour at 15 ºC
Figure 7: MALDI-ToF mass spectrum analysis of compound eluted at 20.587 from photolysis of 101 in presence of excess tri-n-butyltin hydride under anaerobic conditions in water for one hour at 15 °C.

Figure 8: MALDI-ToF mass spectrum analysis of peak 13.740 from photolysis of 103 in presence of 6 mM GSH under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C.
Figure 9: MALDI-ToF mass spectrum analysis of peak 20.327 from photolysis of 103 in presence of 6 mM GSH under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C

Figure 10: Reversed-phase HPLC chromatogram of spiking the photolysate sample with aldehyde 119 resulted in enhancement of peak at 13.680 min from photolysis of 101 in presence of 6 mM GSH under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 °C
Figure 11: ESI-MS analysis of 118 from photolysis of 103 in absence of hydrogen atom donor under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 ºC

Figure 12: Reversed-phase HPLC chromatogram of spiking the photolysate sample with reduction product 118 resulted in enhancement of peak at 13.833 min from photolysis of 103 in presence of 6 mM GSH under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 ºC
Figure 13: ESI-MS analysis of 125 from photolysis of 103 in absence of hydrogen atom donor under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 ºC.

Figure 14: Reversed-phase HPLC chromatogram of spiking the photolysate sample with 2',3'-didehydro-2',3'-dideoxythymidine (125) resulted in enhancement of peak at 12.953 min from photolysis of 103 in presence of 6 mM GSH under anaerobic conditions in 1:1 acetonitrile/water for one hour at 15 ºC.
APPENDIX B

CHAPTER THREE
Figure 1: $^1$H-NMR of compound 135 in CDCl$_3$ (400 MHZ)

Figure 2: $^{13}$C-NMR of compound 135 in CDCl$_3$ (100 MHZ)
Figure 3: $^{31}$P-NMR of compound 135 in CDCl$_3$ (400 MHZ)

Figure 4: HRMS of compound 135
Figure 5: Ion-exchange HPLC chromatogram of modified oligonucleotide 139

Figure 6: MALDI-ToF MS of modified oligonucleotide 139
Figure 7: Ion-exchange HPLC chromatogram of modified oligonucleotide 140

Figure 8: MALDI-ToF MS of modified oligonucleotide 140
Figure 9: Ion-exchange HPLC chromatogram of modified oligonucleotide 141

Figure 10: MALDI-ToF MS of modified oligonucleotide 141
Figure 11: Ion-exchange HPLC chromatogram of modified oligonucleotide 142

Figure 12: MALDI-ToF MS of modified oligonucleotide 142
Figure 13: Ion-exchange HPLC chromatogram of modified oligonucleotide 143

Figure 14: MALDI-ToF MS of modified oligonucleotide 143
Figure 15: $^1$H-NMR of compound 146 in CDCl$_3$ (600 MHZ)

Figure 16: $^{13}$C-NMR of compound 146 in CDCl$_3$ (100 MHZ)
Figure 17: ESI-MS of compound 146

Figure 18: $^1$H-NMR of compound 147 in DMSO (400 MHZ)
Figure 19: $^{13}$C-NMR of compound 147 in DMSO (100 MHZ)

Figure 20: ESI-MS of compound 147

**Calculated** [M+Na]$^+$: 369.33
**Measured** [M+Na]$^+$: 369.50
Figure 21: $^1$H-NMR of compound 66 in CD$_3$CN (400 MHZ)

NMR of compound 66 in CD$_3$CN (400 MHZ)

Figure 22: $^{13}$C-NMR of compound 66 in CD$_3$CN (100 MHZ)
23: MALDI-ToF MS of compound 66

Figure 24: MALDI-ToF MS of compound eluted at 3.320 min after incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C in presence of 6 mM GSH after 30 minutes
Figure 25: MALDI-ToF MS of compound eluted at 5.380 min after incubation of 66 in 100 mM phosphate buffer pH = 7.4 at 37 °C in presence of 6 mM GSH after 30 minutes.
Figure 1: $^1$H NMR spectrum of 108 in CDCl$_3$ (400 MHz)

Figure 2: $^{13}$C NMR spectrum of 108 in CDCl$_3$ (100 MHz)
Figure 3: $^1$H NMR spectrum of 109 in CDCl$_3$ (600 MHz)

Figure 4: $^{13}$C NMR spectrum of 109 in CDCl$_3$ (150 MHz)
Figure 5: $^1$H NMR spectrum of 110 in CDCl$_3$ (400 MHz)

Figure 6: $^{13}$C NMR spectrum of 110 in CDCl$_3$ (100 MHz)
Figure 7: $^1$H NMR spectrum of 111 in CDCl$_3$ (400 MHz)

Figure 8: $^{13}$C NMR spectrum of 111 in CDCl$_3$ (100 MHz)
Figure 9: $^1$H NMR spectrum of 101 in CDCl$_3$ (400 MHz)

Figure 10: $^{13}$C NMR spectrum of 101 in CDCl$_3$ (100 MHz)
Figure 11: $^1$H NMR spectrum of **116** in CDCl$_3$ (400 MHz)

Figure 12: $^{13}$C NMR spectrum of **116** in CDCl$_3$ (100 MHz)
Figure 13: $^1$H NMR spectrum of $^{117}$ in CDCl$_3$ (400 MHz)

Figure 14: $^{13}$C NMR spectrum of $^{117}$ in CDCl$_3$ (100 MHz)
Figure 15: $^1$H NMR spectrum of $\mathbf{103}$ in CDCl$_3$ (400 MHz)

Figure 16: $^{13}$C NMR spectrum of $\mathbf{103}$ in CDCl$_3$ (100 MHz)
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