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Reaction Pathways Initiated by One-electron Oxidation of Guanine: Reaction Pathways Leading to Stable End Products

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Reaction Pathways Initiated by One-Electron Oxidation of Guanine: Oxidation Steps Leading to Stable End Products

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by

Zach Cutright

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ABSTRACT

Reaction Pathways Initiated by One-Electron Oxidation of Guanine: Oxidation Steps Leading to Stable End Products

by

Zach Cutright

8-hydroxyguanine (8-oxoG) is one of the most important products resulting from the oxidation of guanine in DNA. 8-oxoG is known as a biomarker of oxidative stress such as lung cancer. 2,5-diamino-4H-imidazol-4-one (Iz) and its hydrolysis product 2,2-diamino-4-[(2-deoxy-β-D-erythro-pentofuranosyl)-amino]-2,5-dihydrooxazol-5-one (Oz) are also important products of oxidation of guanine in DNA. Recently, novel types of dimer intermediates related to 8-oxoG and Iz have been identified in our research lab. Using high performance liquid chromatography (HPLC), LC-MS, and NMR coupled with educated hypotheses, it is possible to identify the products, X1 and X2, resulting from the oxidation of guanine and decipher the mechanisms leading to the products. Gaining a better understanding of these mechanisms could potentially lead to more effective medical treatment of conditions resulting from oxidative stress. X1 and X2 were isolated in large amounts from deoxyguanosine (dGuo) via photochemical oxidation. Subsequent testing included a comparison of the effects of different pH values as well as determination of the lifetimes for the X2 product during hydrolysis and reactions with amines. It was shown from the results that X1 undergoes further oxidation to produce X2.

ACKNOWLEDGEMENTS

Dr. Roginskaya, for your continuous support and working hard to ensure that I had a full understanding of the research.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ROS	Reactive oxygen species
$O_2^{\bullet-}$	Superoxide radical anion
H_2O_2	Hydrogen peroxide
$^{\bullet}OH$	Hydroxy radical
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
DGh	Dehydroguanidinohydantoin
Oa	Oxaluric acid
Pa	Parabanic acid
Ua	Urea
Sp	Spiroiminodihydantoin
Gh	Guanidinohydantoin
Iz	2,5-Diamino-4H-imidazol-4-one
8-oxoG	8-hydroxyguanine
Oz	2,2-diamino-4-[(2-deoxy- β-D-erythro-pentofuranosyl)-amino]-2,5-dihydrooxazol-5-one
$G^{\bullet}$	Guanine radical
dGuo	Deoxyguanosine
HPLC	High Performance Liquid Chromatography
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
NMR	Nuclear Magnetic Resonance
PDA	Photodiode array
RuBpy	Tris(bipyridine) ruthenium(II) chloride

CHAPTER 1. INTRODUCTION

Background

Humans are among the vast list of organisms that require oxygen in order to survive. Oxygen is a crucial component in countless metabolic processes, including mitochondrial respiration. During mitochondrial respiration, however, oxygen is reduced and dangerous reactive oxygen species (ROS) are produced[1-8]. These ROS, along with others, cause oxidative stress by overwhelming the antioxidants in the body designed to suppress and regulate ROS. The reactive oxygen species will oxidize biological organic molecules, which results in the oxidation and eventual break-down of cells known as oxidative damage[1, 14-15]. This often causes a chain reaction, promoting the activation of more ROS; among these are the superoxide radical anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxy radical ($^{\bullet}OH$). When these ROS are found at high levels, they pose a serious threat to tissues and may incite apoptosis[8]. When higher levels of ROS persist, as in chronic cases, they can result in vascular diseases like arteriosclerosis[9-10]. The oxidation of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) as a result of ROS action has been found to cause mutations and plays a large role in the onset and advancement of carcinogenesis[1,10].

DNA oxidation is intricately linked to the onset of cancer[3]. Guanine is one of four constituent bases found in nucleic acids and is an important component found within DNA sequences. Of the four DNA nucleotide bases, guanine has the lowest one-electron redox potential; therefore, it will undergo oxidation more readily than the other three bases found in DNA sequences[5-7]. Oxidation occurs if there is any transfer of electrons during a given reaction.

Guanine is even more likely to undergo oxidation when it is found to immediately repeat within a given sequence: *'GG'* is more readily oxidized than a single *'*G' found in the middle of a DNA sequence[5-7].

Table 1. Redox Potentials of DNA Nucleobases[5]

DNA Nucleotide Base	Redox Potential (E^o), V
Guanine	1.29
Adenine	1.42
Cytosine	1.60
Thymine	1.70

In the case that guanine is oxidized, it can yield many different mutagenic products such as: dehydroguanidinohydantoin (DGh), oxaluric acid (Oa), parabanic acid (Pa), urea (Ua), spiroiminodihydantoin (Sp), and guanidinohydantoin (Gh)[2]. These products cause transversion mutations in DNA; they form non-Watson-Crick base pairs with guanine, which are the hydrogen bonds found between CG/AT base pairs in a DNA helix[4]. If DNA replication produces an adenine opposite of the oxidized guanine product, this will result in a G:C-T:A DNA transversion[11-13]. Likewise, if another guanine is produced opposite of the oxidized guanine product, this will result in a G:C-G:G DNA transversion[4, 11-13].

8-oxoG, Iz and Oz

The three mutagenic products that tie into this research are 8-hydroxyguanine (8-oxoG), 2,5-Diamino-4H-imidazol-4-one (Iz), and (Oz) (Figure 1).

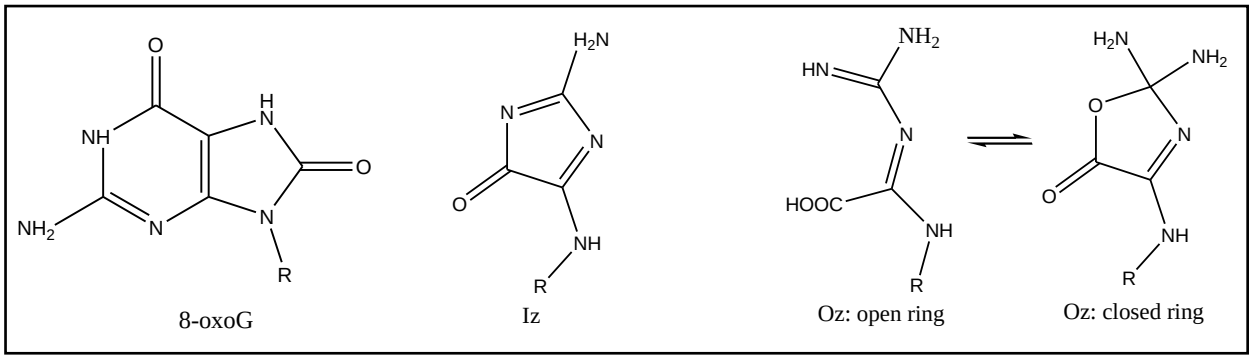


Figure 1. Structures of mutagenic products as a result of guanine oxidation[2].

Mechanisms of Iz Formation

Three different mechanisms can lead to the formation of Iz. The guanine radical ($G^{\bullet}$) will react with the superoxide radical anion ($O_2^{\bullet-}$), producing a peroxyl radical. The peroxyl radical is protonated to form an intermediate that is highly unstable, prompting it to lose CO_2, formamide ($HCONH_2$) and undergo a ring opening to yield Iz (Figure 2)[2, 17].

A second mechanism yielding Iz involves the addition of molecular oxygen (O_2) to $G^{\bullet}$ instead of the superoxide radical anion. This is more common *in vitro*, hence the molecular oxygen. The new intermediate undergoes the same ring opening process as before. However, Iz is not stable *in vivo* and is easily hydrolyzed to form Oz (Figure 2)[2, 17].

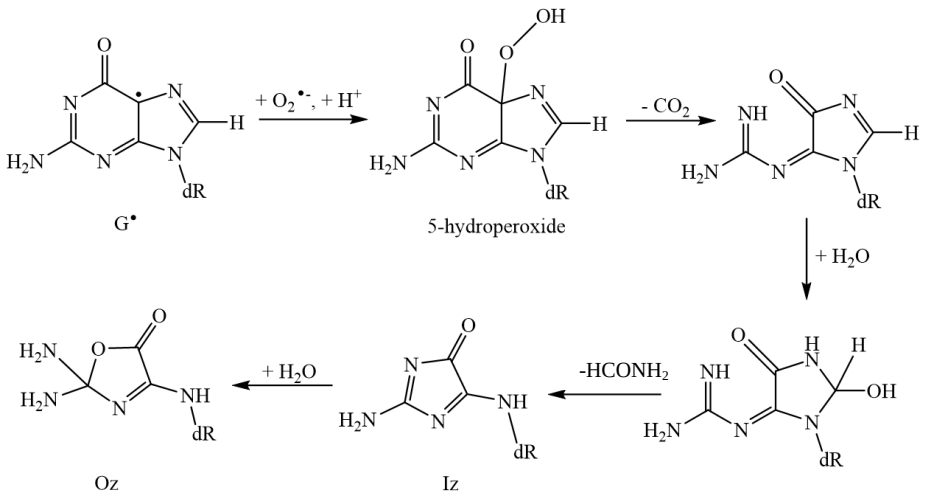


Figure 2. Formation of Iz and Oz.

The third mechanism yielding Iz is a reaction between 8-oxoG and either a singlet oxygen atom, molecular oxygen, or the superoxide radical anion[18] (Figure 3). A hyperoxide group is formed on 8-oxoG and this unstable intermediate undergoes two ring openings, two losses of CO_2, and the loss of an amine group to produce Iz[2, 18].

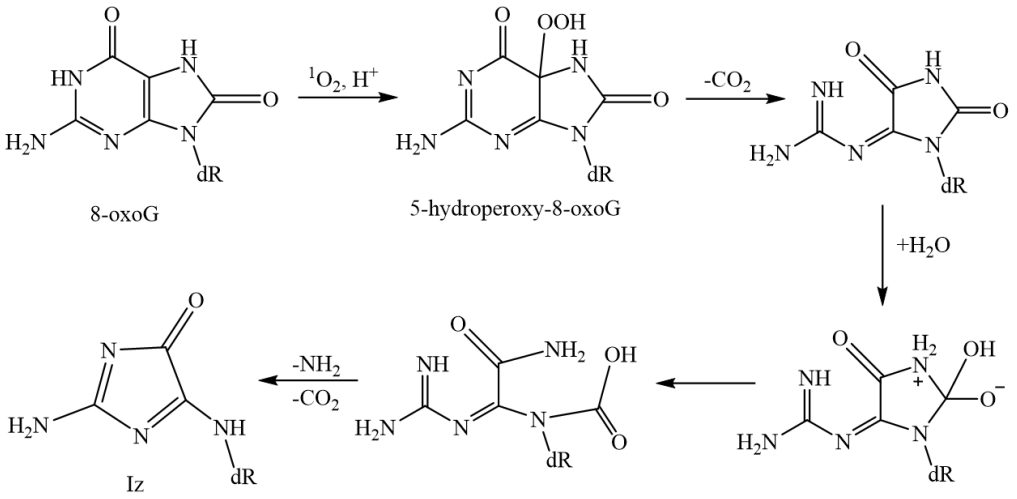


Figure 3. Formation of Iz from 8-oxoG and singlet oxygen[18].

8-oxoG is of particular interest because it serves as a biomarker for oxidatively damaged DNA[1]. The structural analysis of 8-oxoG revealed that it will be produced in either a *syn* or *anti* conformation. If 8-oxoG is produced in the *syn*-conformation, it will form a base pair with adenine by via the Hoogsteen edge found on the *syn* 8-oxoG. If produced in the *anti*-conformation, 8-oxoG will form Watson-Crick base pairs, as mentioned before, with cytosine[2]. It is prevalent in lung tissue before and during the onset of lung cancer. Therefore, it is most found in people working in areas that are saturated in diesel particles or polluted urban regions[1].

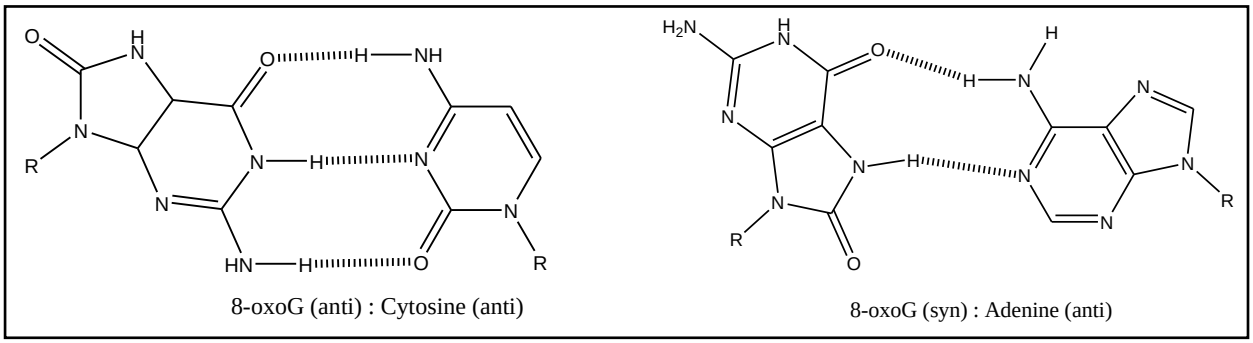
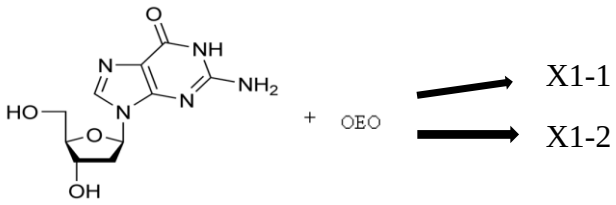


Figure 4. Anti/syn conformations of 8-OxoG binding with cytosine and adenine, respectively[2]

For experimental purposes, this research utilizes deoxyguanosine (dGuo) as it is a monomeric analog of guanine in polymeric DNA. In earlier research in Dr. Roginskaya's lab group, Guo and dGuo were used without any significant different of the products.

Discovery of X1 and X2

Two stable intermediates were detected by HPLC during the oxidation of dGuo and Guo by a number of OEOs. One intermediate was eluted as two peaks with close retention times and identical UV-vis spectra, which allows one to suggest that these are isomers. These products were named X1-1, X1-2, and the second one was named X2 (Figure 5).



Deoxyguanosine

Figure 5. One-electron oxidation of dGuo yields two intermediates.

Earlier data in Dr. Roginskaya's research group predicted that X1-1, X1-2, and X2 are all dimers with links between the G-G structural units based on the analysis performed by liquid chromatography with tandem mass spectroscopy (LC-MS/MS) (Figures 3-5). The masses of both X1-1 and X1-2 were determined to be 548 units confirming that these products are isomers (in MS/MS one unit is added to the mass of the parental peak). While both isomers were recorded to have the same mass, the difference in HPLC elution times indicates structural differences between these two oxidation products. Identical UV-vis spectra indicate that X1-1 and X1-2 are stereoisomers. The mass of X2 was found to be 520 units.

Observation of Figures 6, 7, and 8 reveals that X1 and X2 contain two equivalents of deoxyribose[20-22]. These multiple peaks are a result of fragmentation and their amplitude is indicative of mass. Looking at Figures 3 and 4, the difference between the 549 peak and the 433 peak is 116, the mass of deoxyribose. Again, the difference between 433 and 317 is 116. This discovery led to the hypothesis that the X1 isomer is a dimer consisting of two deoxyribose units. This same trend is followed in the LC-MS/MS spectra of X2 (Figure 8), supporting this hypothesis that X2 also contains two deoxyribose units[20-22].

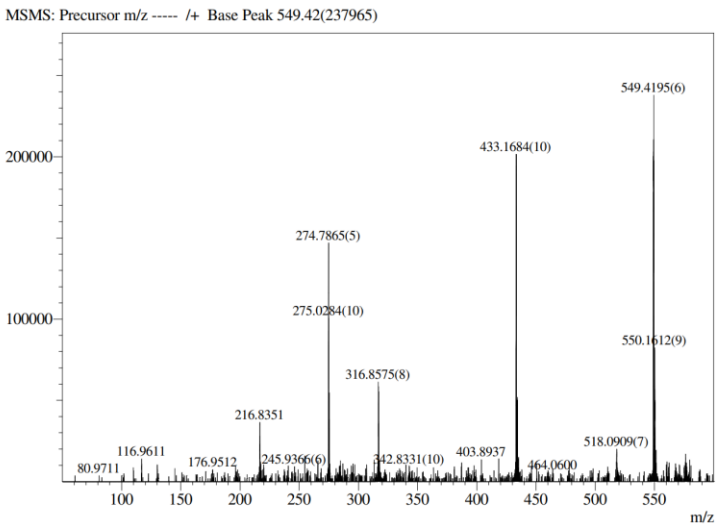


Figure 6. LC-MS/MS spectrum of X1-1[20-22]

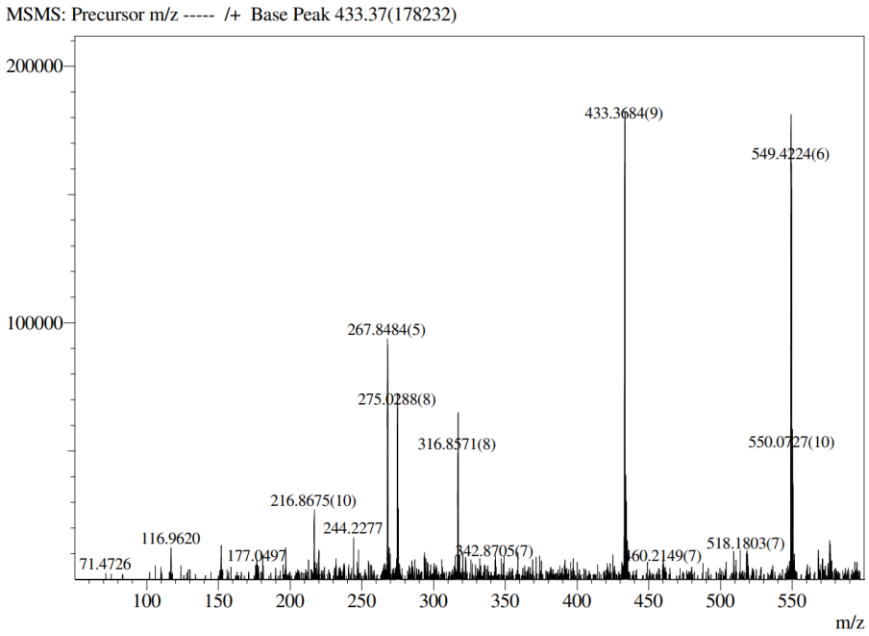


Figure 7. LC-MS/MS spectrum of X1-2[20-22]

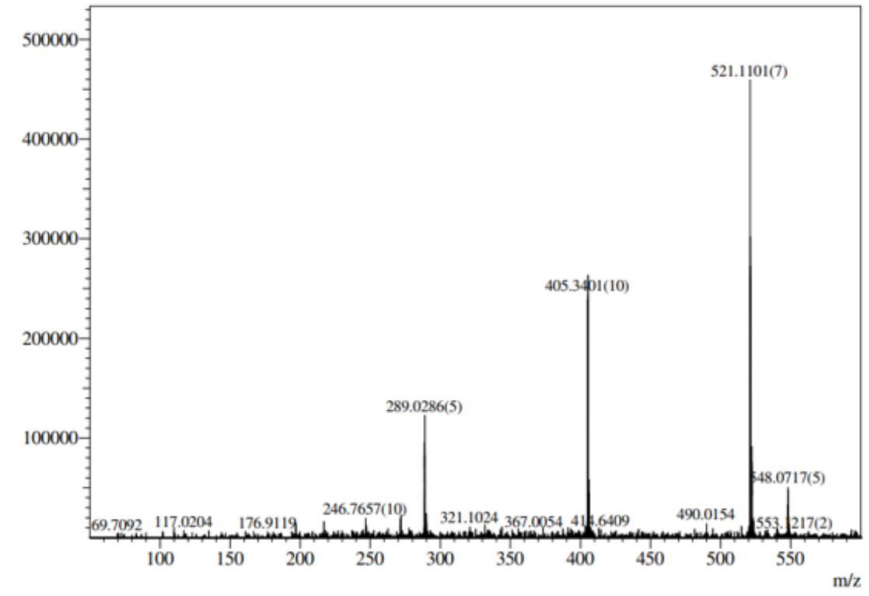


Figure 8. LC-MS/MS spectrum of X2[20-22]

These intermediates can potentially serve as biomarkers of oxidative stress. Therefore, knowing more about their structure and the reaction mechanism leading to these stable intermediates is crucial to the understanding of the chemistry of guanine oxidation in DNA.

Specific Aims

The tasks assigned to me entailed the synthesis of both X1 and X2 in large amounts, testing samples of X1 and X2 in various conditions, and deciphering data to confirm the structures of X1 and X2.

The first several months of research were consumed in the synthesis of X1 and X2. The products were collected in large amounts and isolated for NMR analysis. X2 was then subjected to additional testing to investigate its properties. The first test was concerned with finding the half-life of X2 during hydrolysis reactions in the presence of different pH buffers. The reactions were carried out in an HPLC autosampler unit, and the data points were used to calculate the half-lives of X2 in each buffer solution. The half-life at physiological pH was determined to be 7.25 h. The second test involved reacting X2 with different amines and again calculating the half-life of X2 during these reactions.

CHAPTER 2. EXPERIMENTAL METHODS

Instrumentation, Glassware, and other Materials

Instrumentation:

High Performance Liquid Chromatography (HPLC) was a pivotal method in experiments as it was used for the separation and analysis of low-molecular-weight compounds. It is equipped

with an autosampler, a degasser, a column oven, and a photodiode array detector (PDA). Other instruments used in the research included an illumination apparatus (light-emitting diode, LED, operating at 470 nm), dry-oven, vortex mixer, microcentrifuge, spin-vacuum system, and electronic laboratory scales.

Glassware and Other Materials:

Micropipettes, plastic pipettes, beakers, graduated cylinders, centrifuge tubes, stir rods, HPLC glass inserts, and glass vials were all used during the synthesis and experimentation that took place in the lab.

Reagents

Deoxyguanosine (dGuo):

The structure of dGuo is very similar to that of guanosine, but dGuo is missing a hydroxyl group at the 2´ position of the sugar ring, therefore making it deoxyribose. dGuo is one of four deoxyribonucleosides that form DNA.

Potassium persulfate ($K_2S_2O_8$):

Potassium persulfate was used to generate sulfate (SO_4^{2-}) and a sulfate radical anion ($SO_4^{\bullet-}$) via illumination.

Tris(bipyridine) ruthenium(II) chloride,-$[Ru(bpy)_3]^{2+}$ $2Cl^-$ (RuBpy):

RuBpy was used as a photosensitizer in the process of creating OEO's through illumination.

Buffers

To make potassium phosphate buffer (pH 6.9), 1 M K_2HPO_4 and 1 M $K_2H_2PO_4$ were combined in a 1:1 ratio to produce this solution. pH was further adjusted by addition of HCl solution and measuring pH with the pH-meter. Sodium phosphate buffer (pH 6.9) was used to simulate pH conditions during half-life determination of X2. Acetate buffer (pH 4.6) was used to simulate pH conditions during half-life determination of X2. Borate buffer (pH 8.9) was used to simulate pH conditions during half-life determination of X2.

HPLC Solvents

40 mM ammonium acetate (CH_3COONH_4) was used as the mobile phase in HPLC during most experiments. 0.1% acetic acid was used as a mobile phase in HPLC to separate optical isomers X1-1 and X1-2. 80% v/v acetonitrile/water was used as the mobile organic phase in HPLC.

Other Reagents

Ethanolamine ($OHCH_2CH_2NH_2$) and ethylamine ($CH_3CH_2NH_2$) were used in experiments observing the reactivity of X2 with primary amines. Methanol (CH_3OH) was used as a solvent to extract X2 and remove it from the solid phase.

HPLC Preparation and Use

For HPLC analysis, a two solvent system was utilized. 40 mM ammonium acetate buffer (solvent A) is pumped through the HPLC column upon initialization and 30 min of equilibration of the device. Once this is done, a conditioning run injection is started with no sample loaded. The temperature of the column was held at 30 ^oC and the sample tray at 10 ^oC, minus a few exceptions.

Once real samples were placed in the tray and inserted, 20% v/v aqueous acetonitrile is pumped through the column. Linear acetonitrile gradients were utilized to elute the products from 0% to 20% over a time range of 15 min; this occurred in tandem with an increase of acetonitrile from 0% to 16%. During this time frame, a two-lamp photodiode array (PDA) takes optical measurements of the eluted solution containing the various products[16]. Once the HPLC machine has concluded its measurements of the products and a representative chromatogram has been developed, the column is washed for two minutes with 40% acetonitrile solution.

Synthesis of X1 and X2

Some small amount of solid dGuo was dissolved in 10 mL of phosphate buffer, pH 6.9. A prepared sample of dGuo underwent a 1:10 dilution using HPLC water. The UV-Vis spectrum of the resulting solution was measured. From this spectrum, the exact concentration of the stock dGuo sample was determined using the extinction coefficient of dGuo (13700 M^{-1} cm^{-1}). The saturated solution of 0.2 M $K_2S_2O_8$ was prepared by adding some amount of $K_2S_2O_8$ to 600 mL of water and mixing the resultant solution. The sample of dGuo was combined with a 1:40 ratio of $K_2S_2O_8$ (5 mM final concentration), 1:20 ratio of 0.950 mM RuBpy (0.0475 M final concentration), and a modified amount of phosphate buffer to yield a 3 mM dGuo solution. The persulfate was added last during the experiments, just before illumination to ensure the reaction can occur under accurate timing.

For the mass production of X1 and X2, 10 mL of dGuo mixture solution was illuminated for 40 min in order to optimize the yield of X1. The reason behind this logic is that X1 can undergo further oxidation to yield X2. Therefore, X2 is produced much easier than X1. Using optimum conditions for the yield of X1 by illuminating the 10 mL dGuo reaction solution for

approximately 40 min, a roughly 1:2 yield of X1:X2 resulted; a 10 µL injection of the solution was run through HPLC to ensure optimum X1 yield was achieved (Figure 9).

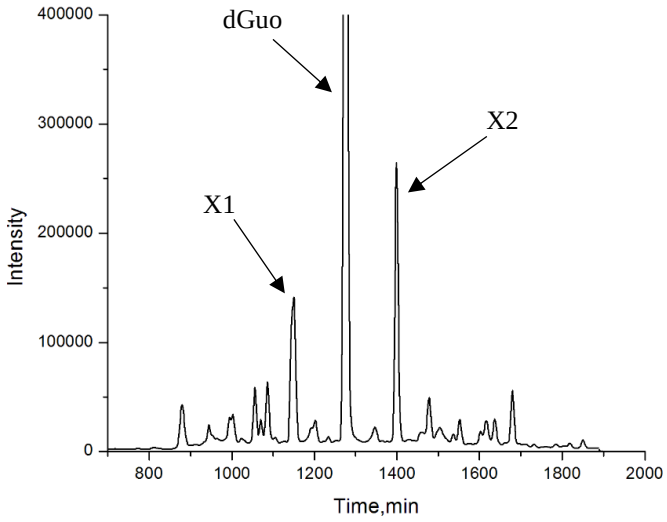


Figure 9. Yield of X1 and X2 after 40 min of illumination of 3 mM dGuo reaction solution.

In order to separate X2 from dGuo and X1, 200-300 mg of ion exchange resin were added in order to purify the sample from the reacted ruthenium complexes. The solution was stirred for approximately 5 min. Then, 50 µL of 1M phosphate buffer was added in order to neutralize the solution.

A miniature preparation column containing a Strata-X SPE cartridge (Figure 10) was conditioned by running 1 mL of methanol through the column followed by 1 mL of HPLC water. The mixture of methanol and water that was collected in the bottom of the column was removed. Then, the illuminated 10 mL of the solution containing X1, X2, and dGuo along with other products was run through the Strata-X SPE cartridge. X2 was absorbed on the methanol/HPLC water-soaked cartridge while X1 and dGuo flowed through and were collected in the bottom of the column. X1 and dGuo were removed from the bottom of the column and the retained X2 was forced through the column by adding a 1:1 mixture of methanol and 0.1% acetic acid to the

cartridge. Separate centrifuge tubes, one containing the X1/dGuo mixture and the other holding X2, were placed in a Centrivap to spin-dry the solutions and purify each of the samples.

Once the samples have been dried, 40 mM ammonium acetate was added to the tube containing X2 and stirred until the solid product was fully dissolved. The X2 solution was run through HPL using a 40mM ammonium acetate mobile phase at a flow rate of 4 mL/min. During the incidence of the X2 peak on the chromatogram, the elute was immediately collected into a clean 15-mL centrifuge tube. This was repeated through 80 µL injections until the X2 was collected.

This procedure was carried out with the X1 mixture using 0.1% acetic acid as the solvent and mobile phase in HPLC. Using 0.1% acetic acid produces separate peaks for the X1 isomers X1-1 and X1-2. This allowed for the collection of these products separately as they eluted during different peaks along the chromatogram. dGuo was also collected so that it could be reused (Figure 10).

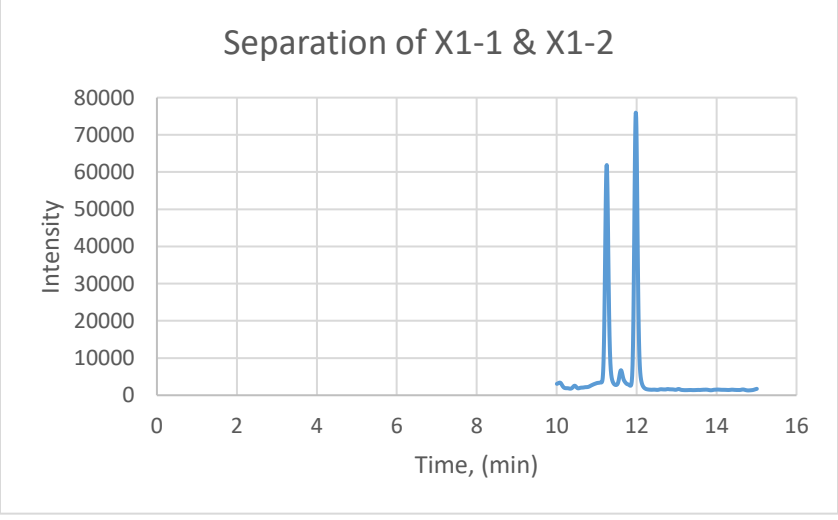


Figure 10. Collection of X1-1 (left peak) and X1-2 (right peak)

Oxidation of X1 to X2

A sample of pure X1 was dissolved in 0.1% acetic acid and a 10 µL-aliquot was run through HPLC. Once the chromatogram was produced, the same solution of X1 was mixed with some amount of 10 mM persulfate and was illuminated at 470 nm in the presence of 0.0475 M RuBpy for approximately 1 min. Once this was done, the sample (10 µL) was run through HPLC for the second time. The resulting chromatogram was compared with the previous results and analyzed for a conversion of X1 to X2 as a result of one-electron oxidation.

Testing the Stability of X2 in Different pH Conditions

X2 was dissolved in variable pH buffers to test its stability under these conditions. The first step of the experiment involved dissolving the dried purified X2 product in acetate buffer with a pH value of 4.6; the amount of acetate added to X2 was just sufficient to dissolve all of the solid product. At time zero and every subsequent 30 min for a total of 270 min, or 4.5 h, the reaction solution was measured via HPLC. The solution containing X2 and acetate buffer was run through HPLC using acetate buffer (pH 4.6) as the mobile phase with a flow rate of 1 mL/min held at a temperature of 37 ^{o}C. The intensity of the X2 peaks at each time recording were tabulated and the half-life of X2 was calculated. This was repeated using sodium phosphate (pH 6.9) and borate buffer (pH 8.9), each serving as the solvent and mobile phase in each experiment. However, when borate was used as the solvent and mobile phase, the reaction between X2 and borate proceeded too quickly at 37 ^{o}C and accurate results were not obtained. The experiment was modified by lowering the temperature conditions to 4 ^{o}C in order to decrease the rate of the reaction; these yielded data that were easier to interpret.

Reaction of X2 with Primary Amines

X2 was mixed with a sufficient amount of 0.1 M ethanolamine solution able to dissolve all of the solid product. The reaction solution was run through HPLC using an ethanolamine mobile phase at a flow rate of 1 mL/min. The temperature conditions for the reaction with ethanolamine was set to 15 ^{o}C in order to obtain accurate data. The sample was run through HPLC at time zero and every subsequent 30 min for a total of 270 min, or 4.5 h. The plotted data points were used to calculate the half-life of X2 in the presence of this primary amine. This experiment was repeated using ethylamine, but the temperature conditions were set to 37 ^{o}C as this yielded the best representative data.

CHAPTER 3. RESULTS AND DISCUSSION

Preparation of X1 and X2 from dGuo

During preparation of X1 and X2 from dGuo, the time of illumination was important to the yield of each compound. When illuminating 10 mL of the dGuo prep solution to produce OEO's (Figure 11), an illumination time of 40 min was optimal for the yield of X1.

$$Ru(II)(bpy)_3^{2+} + h\nu \rightarrow Ru(II)(bpy)_3^{2+*}$$
$$Ru(II)(bpy)_3^{2+*} + S_2O_8^{2-} \rightarrow Ru(III)bpy_3^{3+} + SO_4^{\bullet -} + SO_4^{2-}$$

Figure 11. Reaction scheme of production of OEOs: $Ru(III)bpy_3^{3+}$ *and* $SO_4^{\bullet -}$

It has been later proven that X1 can undergo a one-electron oxidation to form X2. The illumination of 10 mL of the dGuo solution was monitored via HPLC every 10 min, and the collected data has been illustrated in Figure 12.

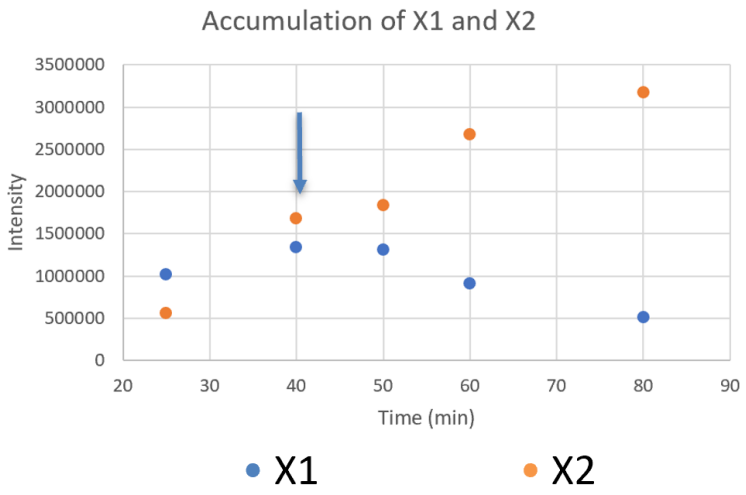


Figure 12. Intensity of X1 and X2 under different illumination times

In Figure 12, it is seen that X1 accumulation reaches a peak at a timed illumination of 40 min. After this point, the concentration of X1 decreases and X2 accumulation is seen to rise at a higher rate, suggesting that X1 is oxidized to form X2.

An experiment was carried out to further prove this process: pure X1 was run through HPLC (Figure 13) then exposed to illumination in the presence of 5 mM persulfate and 0.475 mM RuBpy for 1 min. Then, the sample was run through HPLC the second time (Figure 14).

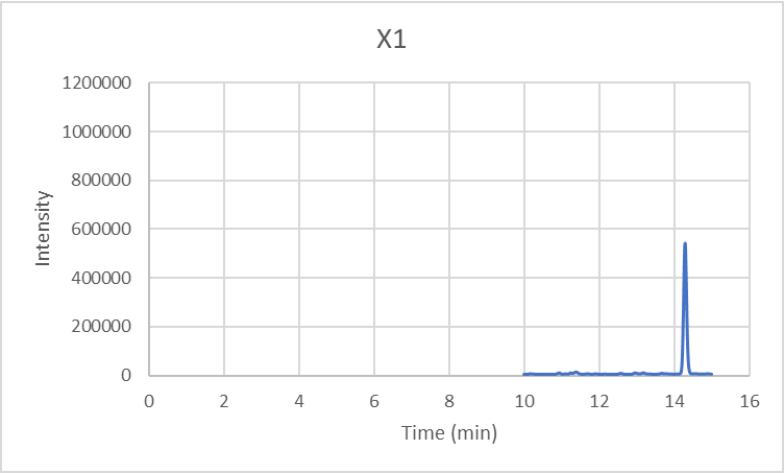


Figure 13. HPLC Chromatogram of pure X1 sample.

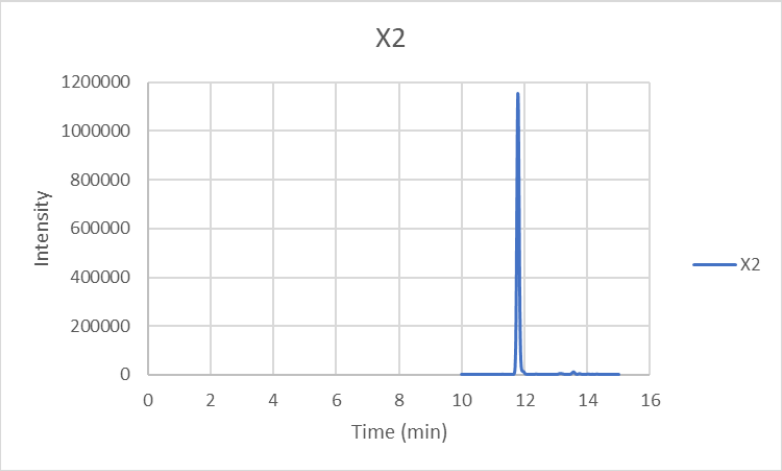


Figure 14. HPLC Chromatogram after 1 min illumination of X1 in presence of 5 mM persulfate.

When analyzing the chromatogram depicted in Figure 13, one major peak is seen resulting from the injection of X1. This sample subsequently underwent a 1 min illumination in the presence of 5 mM persulfate, which served the purpose of creating the one-electron oxidant sulfate radical anion. Figure 14 shows the chromatogram of the resulting solution, which shows one major peak characterized as X2. This led to the conclusion that X1 undergoes one-electron oxidation to form X2.

The mechanism for the one-electron oxidation of X1 is not clear, but a hypothetical reaction scheme has been proposed (Figure 15). X1 will undergo the initial step of one-electron oxidation, in this case by sulfate radical anion, and will lose a proton as a result. The intermediate produced from this oxidation is unstable, causing a ring-opening to form a second intermediate. The second intermediate contains a carbon-centered radical which undergoes reduction to evolve CO gas.

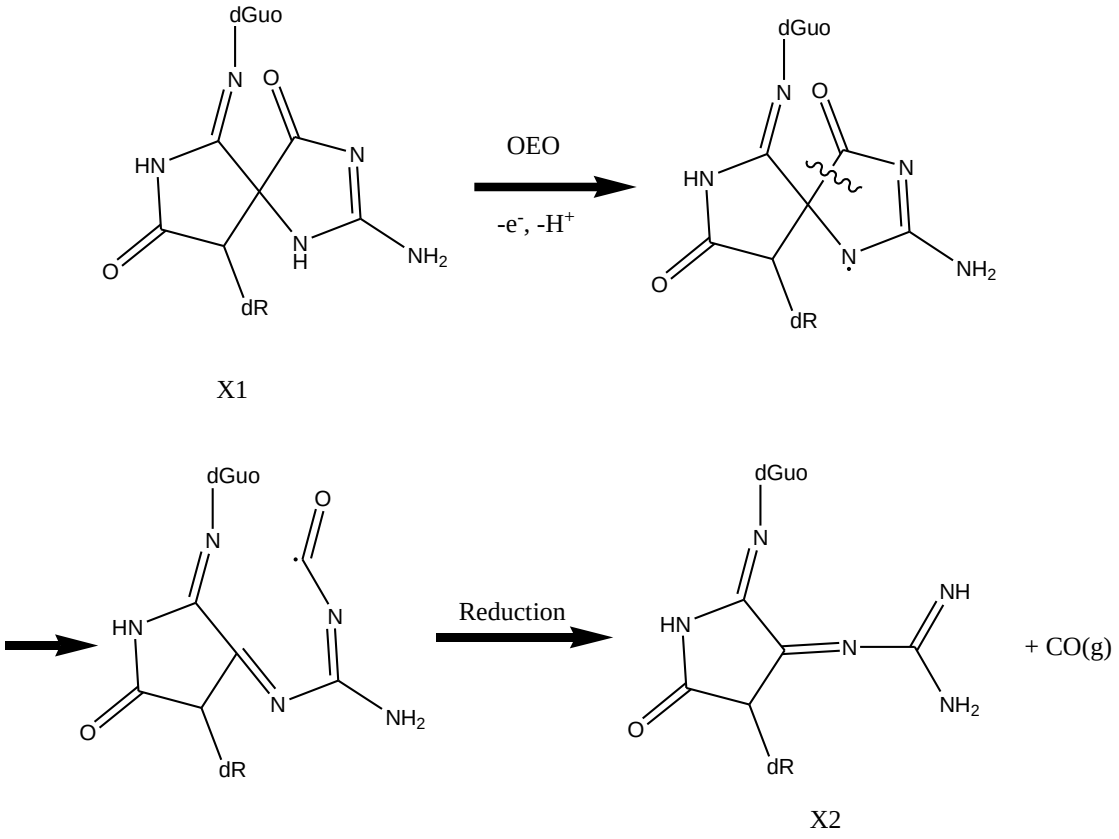


Figure 15. Proposed reaction mechanism for one-electron-oxidation of X1 to form X2

Stability of X2 in Varying pH Conditions

This experiment was run on X2 alone. While X1 is known to be unstable upon one-electron oxidation, previous research found its stability was not affected by different pH conditions.

The purpose of this experiment was to determine the the effect of pH on the stability of X2. The stability of X2 was determined through calculating the half-life for each of three pH values: pH 4.6, pH 6.9, and pH 8.9 (Figure 16).

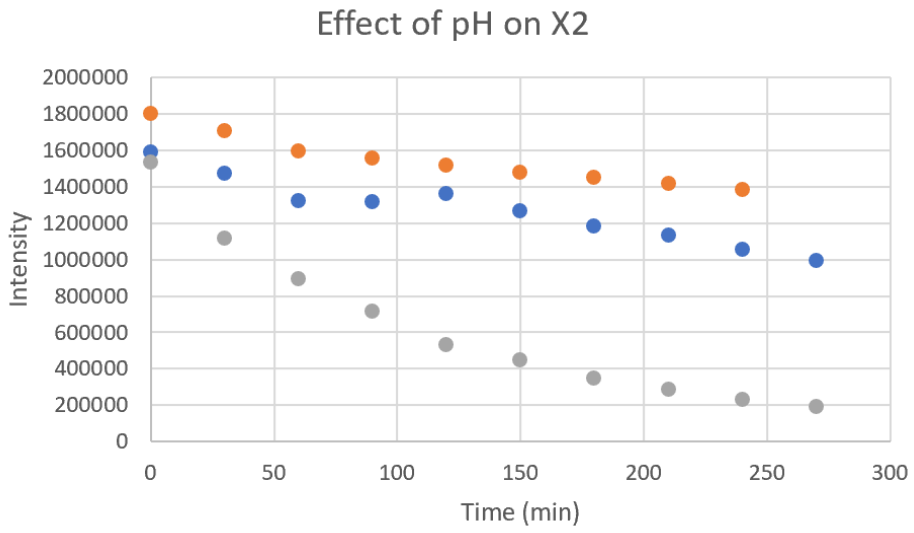


Figure 16. Plot of X2 lifetime in presence of acetate buffer (pH 4.6, 37 ºC, orange plot), sodium phosphate buffer (pH 6.9, 37 ºC, blue plot) and borate buffer (pH 8.9, 4 ºC, grey plot)

The temperature of X2 in the presence of acetate and sodium phosphate was kept at 37ºC. When X2 was run through HPLC in the presence of borate buffer, the reaction proceeded too rapidly and the half-life of X2 at this temperature could not be accurately determined from the resultant data. X2 was run through HPLC in the presence of borate buffer again, but this time at a temperature of 4 ºC to slow down the reaction. Therefore, the half-life of X2 is significantly less in comparison to the other two pH conditions than what is depicted by Figure 16. A summary of the conditions and half-life of X2 is given in Table 2.

Table 2. Summary of Results and Conditions Testing X2 Stability at Varying pH

Buffer Used	pH	Temperature	Half-life of X2
Acetate	4.6	37ºC	11.5 h
Sodium Phosphate	6.9	37ºC	7.25 h
Borate	8.9	4ºC	1.5 h

The sodium phosphate conditions at a pH of 6.9, 37°C are a good depiction of human physiological pH and temperature levels; human pH ranges within 7.35-7.45 and the average body temperature of the human body is 37°C. Therefore, when dGuo undergoes one-electron oxidation within human body tissue to produce the mutagenic product X2, it is predicted to have a half-life of roughly 7.25 h.

The reason X2 is more unstable at a higher pH is because its structure is prone to base-catalyzed hydrolysis. When in the presence of a base, the negatively charged $[OH]^-$ is prone to attack either of the carbonyl carbons marked in the proposed structure of X2 (Figure 17).

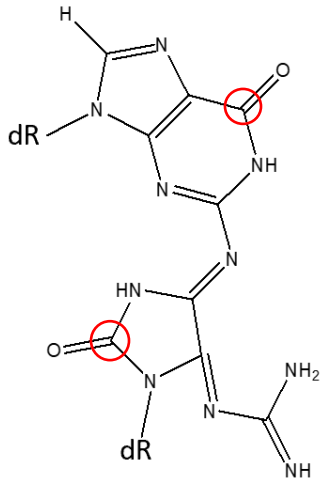


Figure 17. Proposed structure for X2.

The conclusions drawn from this experiment are that X2 is more stable in more acidic environments and that the half-life of X2 in physiological conditions is predicted to be similar to the half-life of X2 in the presence of sodium phosphate, 7.25 h.

Reaction of X2 with Primary Amines

The purpose of this experiment was to determine whether X2 is more likely to undergo hydrolysis or to react with primary amine groups. When X2 reacts with primary amines (Figure 18), it yields mutagenic Iz.

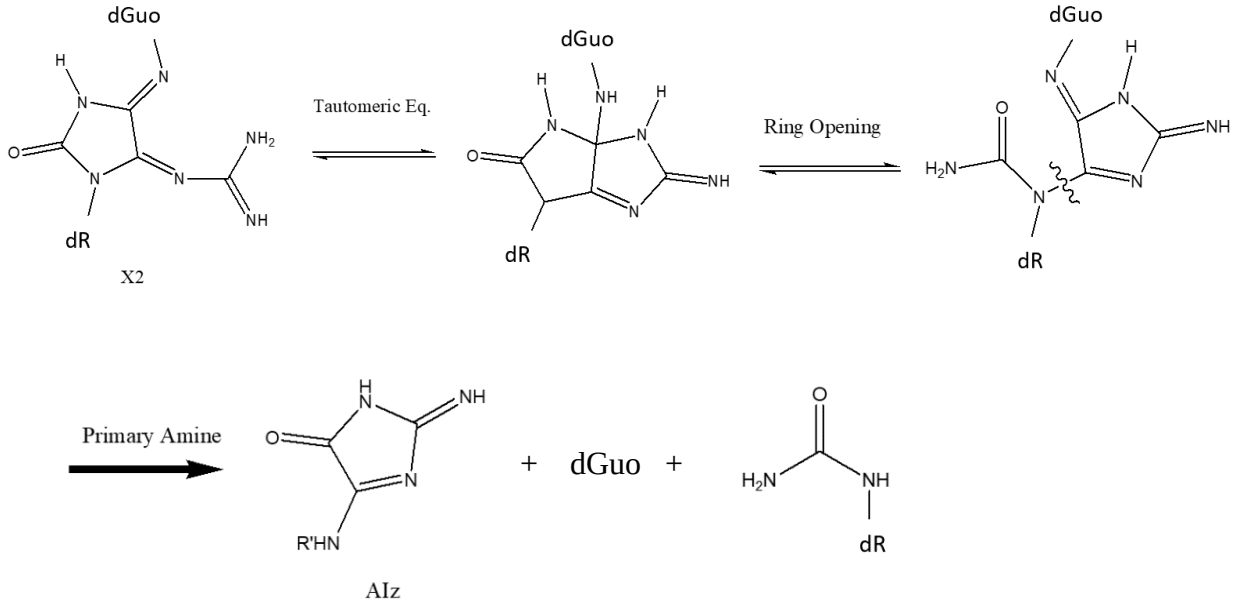


Figure 18. Reaction scheme of X2 with a primary amine[19].

In this experiment, the reaction of X2 with either ethanolamine or ethylamine yields a mutagenic product Iz. The progress of the reaction was monitored through progressive HPLC chromatograms (Figures 19).

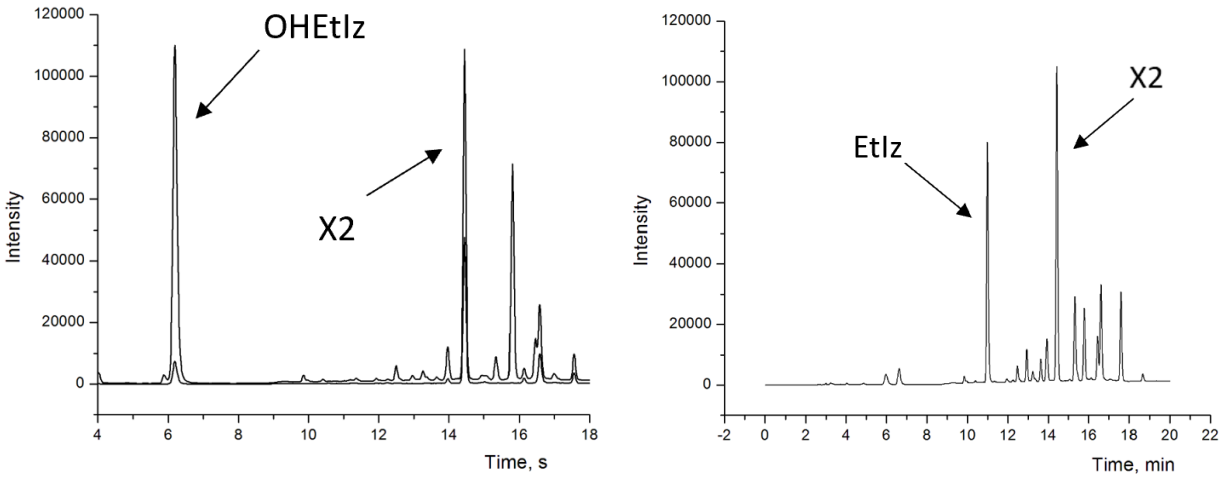


Figure 19. Representative HPLC chromatograms showing X2, OHEtIz, and EtIz.

The reaction between X2 and these primary amines was monitored through HPLC and plots were developed to calculate the half-life of X2 in the presence of each ethanolamine and ethylamine (Figures 20 and 21).

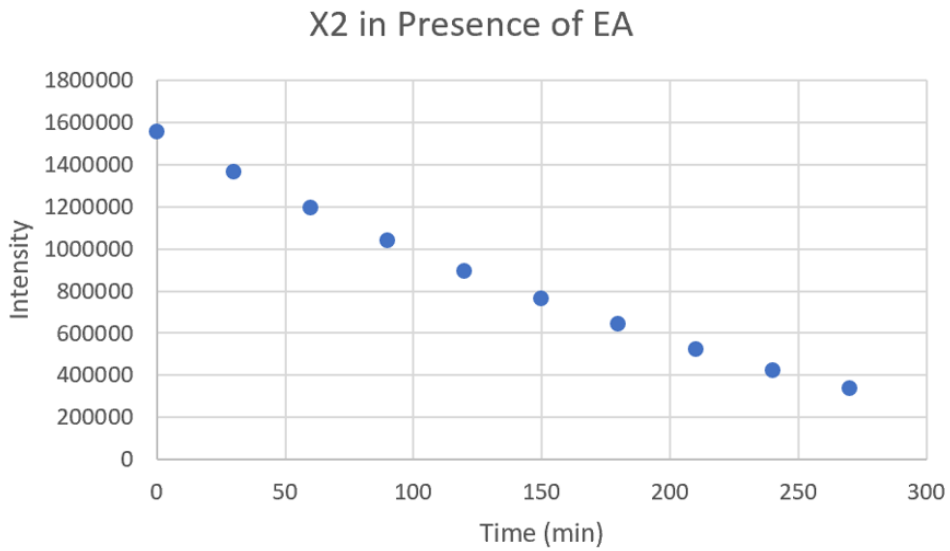


Figure 20. Lifetime of X2 in the presence of 0.1 M ethanolamine at 15°C

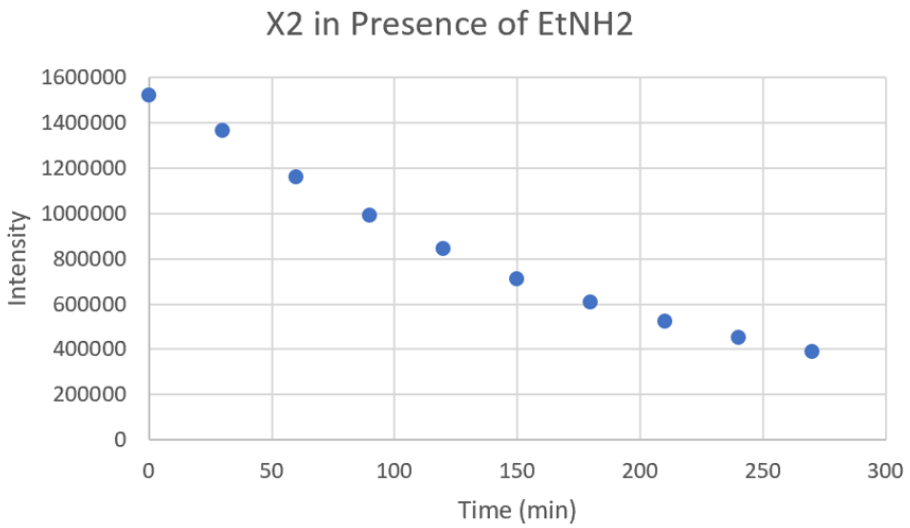


Figure 21. Lifetime of X2 in the presence of 0.1 M ethylamine at 37°C

The half-life of X2 in the presence of ethanolamine was determined to be 2 hours, while its half-life in the presence of ethylamine was determined to be 2.2 hours. While these data may seem closely related, note that the experiments were carried out at different temperatures. The reaction between X2 and ethanolamine proceeded too quickly at 37°C to allow high-quality data to be collected. Therefore, the experiment reacting X2 with ethanolamine was repeated at a lower temperature, 15°C. The conclusion that can be drawn is that X2 reacts in a significantly more rapid manner with ethanolamine than it does with ethylamine.

This information is significant because it may be applied *in vivo*: X2 is more likely to react with primary amines than it is to undergo hydrolysis by DNA. In the body, nucleosomes contain primary amine groups such as lysine. When X2 reacts with these primary amines on nucleosomes yielding a mutagenic Iz product (Figure 22), the result is DNA-protein crosslinks. These adducts might interfere with cellular functions and prompt the cell to die.

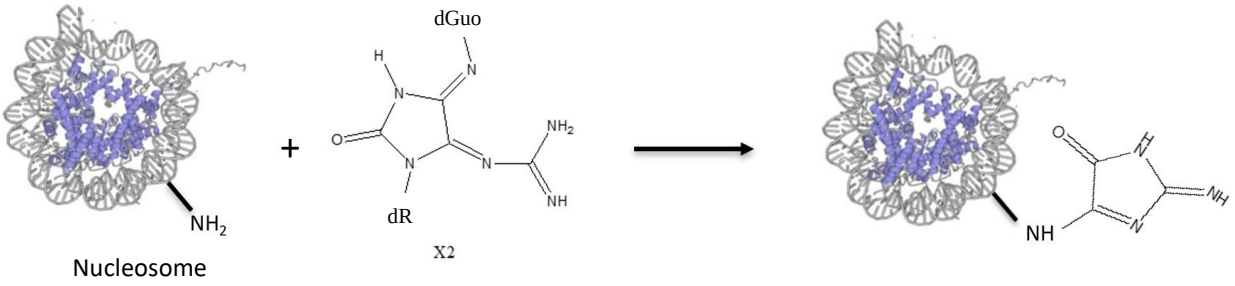


Figure 22. X2 reacts with primary amine groups on nucleosomes

Therefore, if X2 is found within body tissue as a result of the one-electron oxidation of guanine, it will likely react with nucleosomes and cause significant tissue damage via cell DNA destruction.

CHAPTER 4. FUTURE WORK

After the conclusion of this research, there is still more to be done. While there are strong predictions for the structures of X1 and X2, these structures are not certain. ^{1}H NMR and ^{13}C NMR as well as LC/MS/MS will most likely be primary methods in further research to decipher the structures of the X1 and X2 isomers.

As seen in the experimental results, the hydrolysis of X2 yields mutagenic products such as Iz. Further investigation of the different products resulting from the hydrolysis of X2 will be important to the understanding of this reaction mechanism and will allow for a better understanding of how these products affect the body.

Lastly, the analysis of X1 and X2 in double-stranded DNA is especially important. Other products resulting from the one-electron oxidation of guanine such as Iz and Oz have been proven to cause DNA mutations. The effects of X1 and X2 within short self-complimentary oligonucleotides such as CGCG and CGATCG could help lead to a better understanding of their

role in *vivo*. It would also prove useful to study the formation of X1 and X2 in highly polymerized DNA, although there are problems with detections of these lesions in highly polymerized DNA since X1 and X2, if formed, would still be attached to DNA.

CHAPTER 5. CONCLUSIONS

Previous research discovered that X1 could undergo further one-electron oxidation to yield X2. In this research, experiments were conducted to further prove this phenomenon. It was demonstrated that one-electron oxidation of X1 by 5 mM persulfate and 0.475 mM RuBpy with illumination at 470 nm results in a nearly 100% conversion of X1 into X2

Progress made within this project prior to my assigned research led to the belief that X1-1, X1-2 and X2 are dimers with links between the G-G structural units. Dr. Roginskaya's research group also predicted that X1-1 and X1-2 are stereoisomers as a result of UV-Vis analysis.

X1 and X2 were able to be synthesized in large amounts through the one-electron oxidation of dGuo. dGuo was placed in the presence of $Ru(II)bpy_3^{2+}$ and $S_2O_8^{2-}$ and illuminated to produce OEOs $Ru(III)bpy_3^{3+}$ and $SO_4^{\bullet-}$. These OEOs act on dGuo to produce X1 and X2, while the yield of each product depends on the time of the reaction solution under illumination. This is because X1 can undergo further one-electron oxidation to X2.

The stability of X2 in different pH conditions was tested. X2 reacted most rapidly in the presence of the borate buffer which had the highest pH of 8.9. The conclusion of this experiment is that X2 is more stable in acidic conditions; this is because X2 is prone to base-catalyzed hydrolysis. The half-life of X2 *in vivo* was predicted to be close to 7.25 h in the phosphate buffer (pH 6.9) at 37 ^{o}C, closely mimicking human physiological conditions.

The half-life of X2 was determined in the presence of different primary amines. The conclusion of this experiment is that X2 is more likely to react with primary amines than it is to undergo hydrolysis. This may be applied *in vivo*: X2 is more likely to react with primary amines found on nucleosomes than it is to be hydrolyzed by DNA. The result of the reaction between X2 and a primary amine is the mutagenic product Iz. The mutagenic product may cause DNA-histone protein crosslinks that will ultimately trigger cell death.

Future research is required to determine the structures of X1-1, X1-2, and X2. Further analysis using ^{1}H NMR and ^{13}C NMR, and LC-MS/MS, the investigations of X2 hydrolysis products, and the studies of the effects of X1 and X2 in double-stranded DNA will help lead researchers in this aim.

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