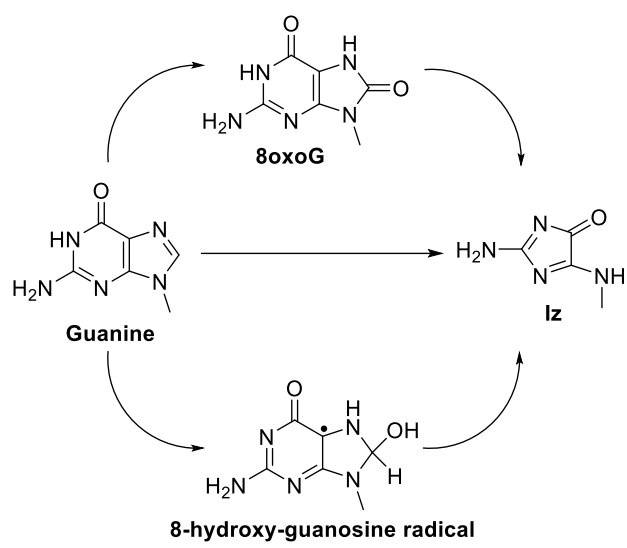


# **Computational Investigation into the Oxidation of Guanine to Form Imidazolone (Iz) and Related Degradation Products**

Sebastien P. Hebert and H. Bernhard Schlegel\*

Department of Chemistry, Wayne State University, Detroit, Michigan 48202, United States

# TOC Figure



**ABSTRACT:** Imidazolone (Iz) is one of the many products resulting from oxidative damage to DNA. Three pathways for the formation of Iz and related degradation products have been studied by density functional theory using the  $\omega$ B97XD functional with the 6-31+G(d,p) basis set and SMD implicit water solvation plus a small number of explicit water molecules positioned to help stabilize charged species and facilitate reaction steps. The first pathway starts with guanine radical and the addition of superoxide at C5. Endoperoxide formation was calculated to have slightly lower barriers than diol formation. The next steps are pyrimidine ring opening and decarboxylation. Ring migration then proceeds via an acyclic intermediate rather than a bicyclic intermediate and is followed by formamide loss to yield Iz. The second pathway starts with 8oxoG and proceeds via C5 superoxide addition and diol formation to a relatively stable intermediate, oxidized guanidinohydantoin ( $Gh_{ox}$ ). The barriers for hydroxide ion addition to  $Gh_{ox}$  are much lower than for water addition and should yield more Iz and parabanic acid at higher pH. The third pathway starts with 8-hydroxy guanine radical formed by hydroxyl radical addition to C8 of guanine or water addition to C8 of guanine radical. Superoxide addition at C5 is followed by diol formation, ring opening and decarboxylation similar to pathways 1 and 2 subsequently leading to Iz formation. The calculated pathways are in good agreement with experimental observations.

## 1. INTRODUCTION

Because guanine has relatively low oxidation potential, it is more readily subject to oxidative degradation than other nucleotides.<sup>1</sup> As such, experimental studies of oxidative damage to DNA often use guanine as a model.<sup>2-4</sup> Multiple pathways have been proposed for guanine oxidation and many have been probed in experimental and computational investigations. Over the past few years, numerous studies have probed the reaction paths leading to important guanine oxidative damage products such as 8-oxo-7,8-dihydro-guanine (**8oxoG**),<sup>5-8</sup> 2,6-diamino-4-hydroxy-5-formamidopyrimidine-guanine (**Fapy-dG**),<sup>9, 10</sup> guanidinohydantoin-2'-deoxyribonucleoside (**Gh**),<sup>8, 11-17</sup> spiroiminodihydantoin-2'-deoxyribonucleoside (**Sp**),<sup>7, 8, 11-16, 18-20</sup> guanine-lysine cross-link (**G<sup>+</sup>Lys**),<sup>21-31</sup> guanine-thymine cross-link (**G<sup>+</sup>T**),<sup>15, 32-36</sup> 5-carboxamido-5-formamido-2-iminohydantoin-2'-deoxyribonucleoside (**2lh**),<sup>8, 14-16, 37, 38</sup> 8-nitroguanine (**8nitroG**),<sup>39-46</sup> and 5-guanidino-nitroimidazole (**Nlm**)<sup>44-47</sup>. In addition to these products, oxidation of guanine and **8oxoG** is known to also produce imidazolone (**Iz**) and its hydrolysis product, oxazolone (**Z**). The mechanistic details of these pathways are still under investigation.<sup>6, 8, 12-16, 18, 20, 48-53</sup> Depending on conditions, additional oxidation products can form in competition with **Iz**. Oxidized **Gh** (**Gh<sub>ox</sub>**),<sup>12, 13, 19, 20, 51-57</sup> oxidized iminoallantoin (**Ia<sub>ox</sub>**),<sup>52</sup> parabanic acid (**Pa**),<sup>51, 52, 57</sup> and oxaluric acid (**Oa**)<sup>13, 19, 52, 54-56</sup> have been detected, and are thought to be related to the pathways for **Iz** formation.

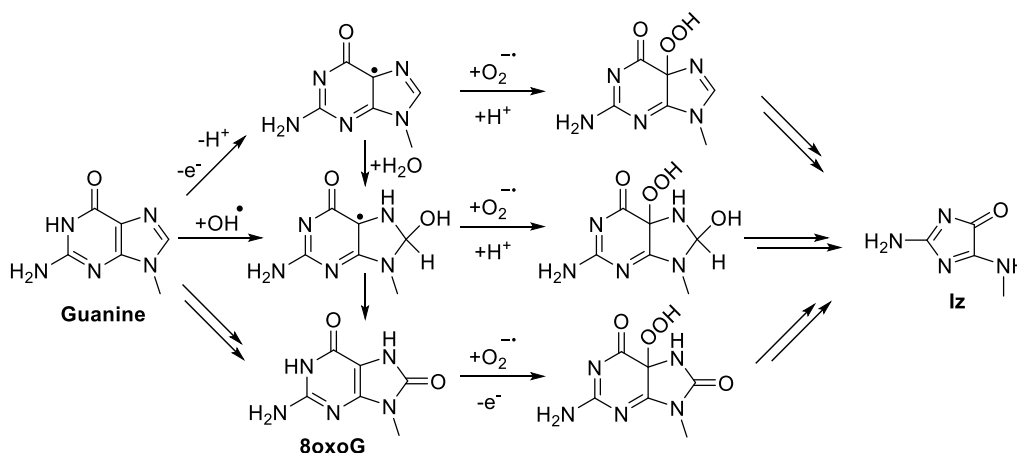
A number of experimental conditions affect the yield of **Iz** and related products of DNA oxidative damage. There is a strong pH dependence on product distribution comparing oxidation of guanine and **8oxoG**.<sup>12, 57</sup> Addition of superoxide dismutase reduced **Iz/Gh<sub>ox</sub>/Ia<sub>ox</sub>** formation by about half when starting from **8oxoG** as a reactant, compared to only small changes starting from oxidation of guanine.<sup>12, 53, 56</sup> Benzoquinone was found to have a similar effect.<sup>20</sup> Additionally, the presence or absence of reducing agents were considered to more appropriately model differing cellular conditions.<sup>8</sup> These studies showed that reducing agents resulted in more **2lh** formation over **Iz** while **Sp**, **Gh**, and **8oxoG** formation were largely unaffected. Isotopic labelling studies found incorporation of oxygen from solvent or molecular oxygen depending on conditions.<sup>13, 18, 20, 49-51, 54</sup> Along with **Iz**, many relevant experimental studies also found **Gh<sub>ox</sub>**, **Ia<sub>ox</sub>**, **Pa**, and **Oa**.<sup>2, 12, 13, 19, 20, 51-56</sup>

Cadet and coworkers found **Iz / Z** formation as the dominant product in both hydroxyl radical and riboflavin type I photooxidation of guanine.<sup>49</sup> Burrows and coworkers investigated riboflavin photooxidation of guanine and **8oxoG**, and found **Iz** as a significant product.<sup>12</sup> The product distributions were found to change significantly with pH and with the addition of superoxide dismutase (**SOD**), with oxidation of guanine and **8oxoG** showing different behavior. Cadet and coworkers investigated the mechanism for **Iz** formation via riboflavin photooxidation of guanine and <sup>15</sup>N labelled **8oxoG**, and found the conversion of guanine to **8oxoG** to oxidative products (predominantly **Iz**) as the likely pathway.<sup>18</sup> Shafirovich and coworkers used laser photolysis to oxidize 2-aminopurine in a single strand nucleobase sequence, which propagates to oxidize guanine, and found a combination of **8oxoG** and **Iz**, with no other reported products.<sup>6</sup> Similar to the work by the Burrows group, Tannenbaum and coworkers found a pH dependence for **Iz** formation in oxidation of guanine and **8oxoG** induced by peroxyxynitrite.<sup>57</sup> Fleming and Burrows work found riboflavin photooxidation of a G-quadruplex system produced **Iz** and **Sp** in yields of about 45% each.<sup>16</sup> Their experiments in this study found significant **Iz** formation in the case of riboflavin type I photooxidation, but little to none in the cases of Rose Bengal type II photooxidation, carbonate radical oxidation or Cu(II) Fenton oxidation of guanine.<sup>16</sup> In a recent work, Burrows and coworkers

obtained significant yields of **Iz** in oxidation of guanine by iron Fenton chemistry and by X-ray radiolysis of water.<sup>8</sup> This work also helped to establish the relationship between the **Iz** and **2lh** oxidative products. Introduction of reducing agents found increasing **2lh** and decreasing **Iz** yields, with ratios indicative of reduction of a common intermediate. Collectively, most works related to **Iz** assume the presence of superoxide, which may be produced from molecular oxygen reacting with a transient radical species, such as the riboflavin radical anion. Shafirovich and coworkers found **Iz** formation from carbonate radical oxidation; however, this seems to be a unique case,<sup>15</sup> as **Iz** is typically not seen in guanine oxidation by carbonate or sulfate radicals. The significance of superoxide in the production of **Iz** has led to a number of studies related to understanding guanine – peroxy adduct formation. Meunier and coworkers have explored guanine and **8oxoG** oxidation using a two-electron oxidant (Mn-TMPyP) in conjunction with a persulfate salt (KHSO<sub>5</sub>) which mimic the G-OOR intermediate found from superoxide radical combination.<sup>13, 50, 51, 55</sup> Their early results showed rapid formation of **Iz** from guanine with no **8oxoG** intermediate.<sup>50</sup> They also reported the formation of two sets of byproducts, formamide and a combination of CO<sub>2</sub> and ammonia. The oxidation state at the C8 position is believed to control the byproducts formed. Separate H<sub>2</sub><sup>18</sup>O and <sup>18</sup>O<sub>2</sub> labelling experiments found water incorporation into the formamide byproduct, but no labelled molecular oxygen incorporation into the **Iz** product. These results are likely due to nucleophilic addition of the peracid outcompeting other mechanistic pathways. Further study also found the **Gh<sub>ox</sub>** product and subsequent hydrolysis to oxaluric acid.<sup>13, 51, 55</sup> Guanine oxidation in the presence of lipid peroxy radicals also produced **Iz** and **Gh<sub>ox</sub>**.<sup>20</sup> Similar to other reports, oxidation of guanine was found to produce **Iz**, while oxidation of **8oxoG** primarily produced **Sp** and **Gh<sub>ox</sub>**. Oxidation of guanine and **8oxoG** with riboflavin also found **Gh<sub>ox</sub>** and **Oa**, with formation of **Ia<sub>ox</sub>** and **Pa** being relevant to these mechanistic pathways.<sup>12</sup>

The current work investigates various pathways for **Iz** formation along with possible side products such as **Gh<sub>ox</sub>**, **Ia<sub>ox</sub>**, **Pa** and **Oa**. Scheme 1 summarizes the three pathways considered in the present study. The first pathway starts with guanine radical, which decomposes slowly at neutral and basic pH, but readily combines with superoxide radical. The second pathway starts with **8oxoG**, a common oxidative damage product which can produce **Iz** by superoxide addition at C5. A third pathway starts with 8-hydroxy guanine radical formed by hydroxyl radical addition to C8 of guanine followed by superoxide addition at C5 leading to **Iz**. Hydroxyl radical can be produced in Fenton chemistry or radiolysis of water by ionizing radiation. 8-Hydroxy guanine radical can also be formed by water addition to guanine radical cation.

**Scheme 1.** Initial steps in three pathways for the formation of **Iz** considered in the present study.



## 2. METHODS

**2.1. Electronic Structure Calculations.** All calculations were performed using the development version of the Gaussian series of programs<sup>58</sup> and the  $\omega$ B97XD density functional<sup>59</sup> with the 6-31+G(d,p) basis set.<sup>60-63</sup> SMD implicit water solvation<sup>64</sup> was used to model aqueous conditions. Explicit waters were included as a supplement to the implicit model, as in previous studies.<sup>65, 66</sup> Guanine was capped with a methyl group in place of the N9-bound sugar moiety.

**2.2 pK<sub>a</sub> Calculations.** The pK<sub>a</sub>'s for reactants and intermediates are calculated from the free energy differences for deprotonation in solution

$$pK_a = \frac{G_{deprotonated} + G_{H^+_{(aq)}} - G_{protonated}}{2.303RT} \quad (1)$$

where  $R$  is the gas constant (1.987 cal K<sup>-1</sup> mol<sup>-1</sup>),  $T$  is the temperature (298.15 K), and the free energy of the proton in water is -270.3 kcal/mol<sup>67-70</sup>, defined as

$$G_{H^+_{(aq)}} = G_{H^+_{(g)}} + G^{1atm \rightarrow 1M} + \Delta G_{H^+_{(solv.)}} \quad (2)$$

where the gas phase free energy of a proton is  $G_{H^+_{(g)}} = -6.287$  kcal/mol, the conversion from 1 atm to 1 mol/L is  $G^{1atm \rightarrow 1M}$  is 1.89 kcal/mol<sup>71</sup>, and energy of solvation of a proton is  $\Delta G_{H^+_{(solv.)}} = -265.9$  kcal/mol.<sup>67</sup>

Experimentally, the reactions are carried out in buffered solutions. Computationally, the relative free energies of different protonation states of a compound in a buffer can be determined by calculating the free energy for the transfer of a proton to / from a proton acceptor / donor that has a pK<sub>a</sub> equal to the pH of the buffer. The relative concentrations of the different protonation states can be determined from the Boltzmann distribution and the relative free energies. Imidazole (pK<sub>a</sub> = 7.7 calc., 6.95 exp.<sup>72</sup>) was used to model pH 7 conditions.

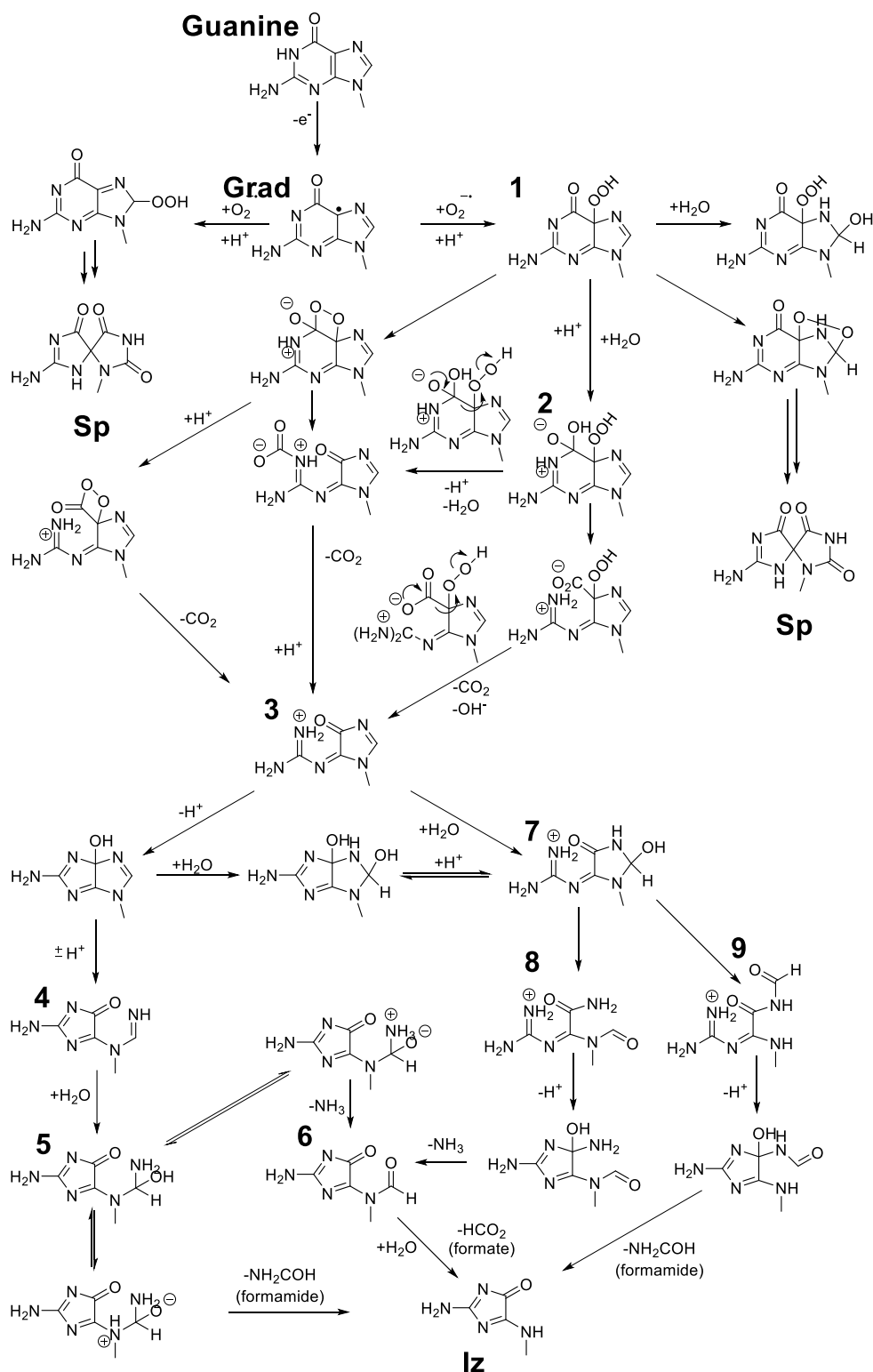
## 3. RESULTS

### 3.1 Overview of Reaction Pathways

Iz formation may proceed through a number of differing pathways involving peroxy addition at C5. Three key pathways are considered in the present study, as shown in Scheme 1. First, evidence for Iz formation by guanine oxidation and superoxide radical addition has been found in many experimental studies.<sup>6, 8, 12, 14, 16, 18, 20, 53, 56, 73</sup> C5- and C8- peroxy adducts have also been investigated in the oxidation of guanine by lipid peroxy radicals and by Mn-TMPyP/KHSO<sub>5</sub>.<sup>20, 50, 51</sup> The resulting hydroperoxy adduct, **C5OOH-G**, is the initial intermediate in the first of the pathways. A second pathway involves **8oxoG** which is prevalent in oxidative damage of DNA. Because of its lower oxidation potential, **8oxoG** was also examined experimentally as a starting point for Iz formation and showed very different reactivity compared to guanine.<sup>12, 18, 20, 52, 56, 57, 73</sup> Superoxide addition to **8oxoG** radical is taken as the initial intermediate for the second pathway in the present study. Finally, water addition to the C8 position of guanine radical or C8 hydroxyl radical addition to guanine results in 8-hydroxy guanine radical (**C8OH-Grad**) which can combine with superoxide at C5. **C5OOH-C8OH-G** is the initial intermediate in the third pathway of Iz formation considered in the present study.

### 3.2 Guanine Radical Oxidation to Iz

**Scheme 2.** Overview of Iz formation via guanine radical.

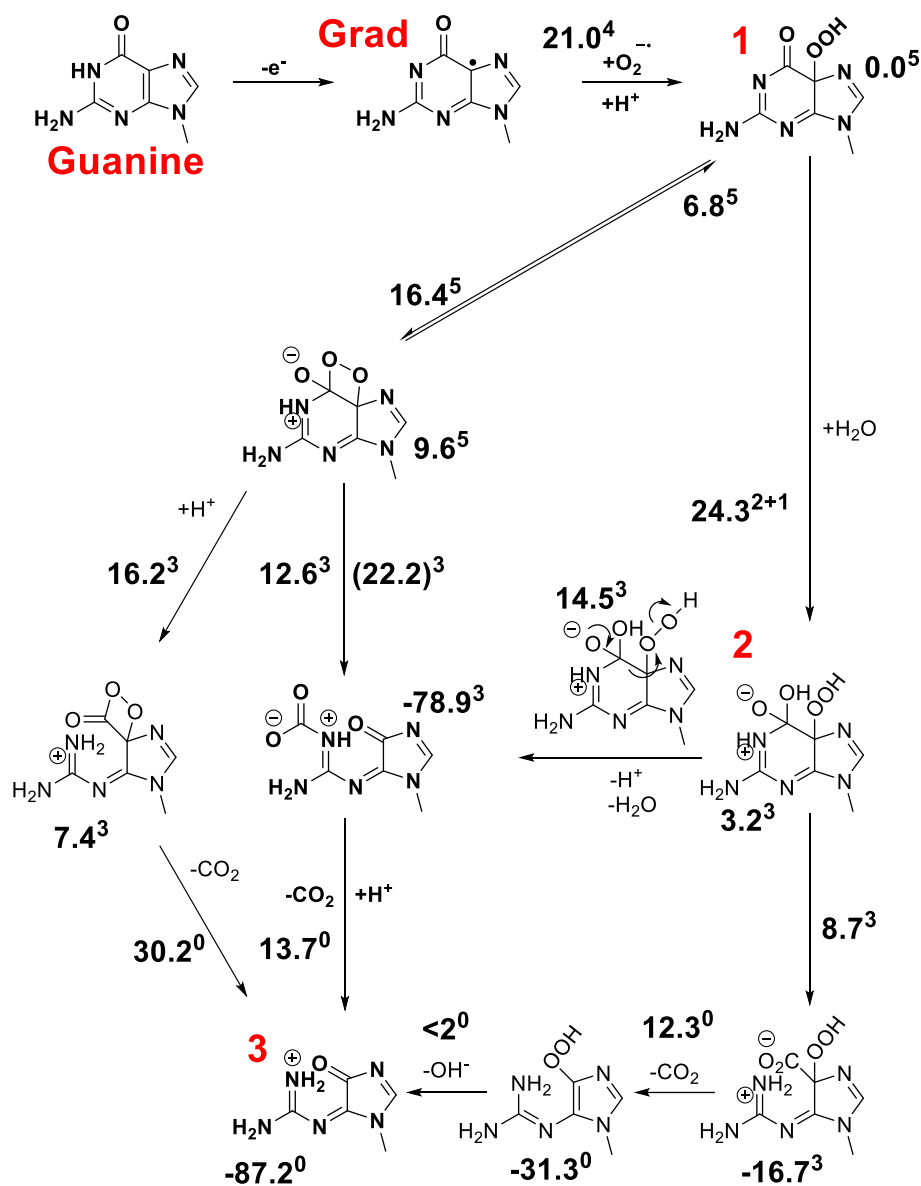


Scheme 2 shows an overview of the major steps in the formation of **Iz** starting from guanine radical. Guanine radical may be produced through a number of oxidative pathways and using a number of different oxidants. Superoxide can be formed by reaction of molecular oxygen with a transient intermediate radical, or as an intermediate in Fenton chemistry or by electron scavenging in photolysis. Guanine radical and superoxide may combine readily at C5 yielding intermediate **1**. Alternatively, radical combination at the C8 position leads to **Sp** and is discussed later. Diol formation at the C6 position may occur in the case of **Gh** formation and is considered here in an analogous fashion, resulting in intermediate **2**. C5-C6 or C6-N1 ring opening and decarboxylation produce intermediate **3**. Alternatively, while **Sp** forms by deprotonation of the C5 alcohol of **5OH-8oxoG** and acyl migration, **1** cannot easily undergo the same bond migration. Deprotonation of the hydroperoxyl group can result in a C5-C8 endoperoxide and formation of **Sp**, or in a C5-C6 endoperoxide followed by either C5-C6 or C6-N1 bond cleavage and decarboxylation forming intermediate **3**. This suggests that for the guanine radical pathway to **Iz**, intermediate **3** may be produced either via an endoperoxide or a diol.

Ring migration in **3** via bicyclic and acyclic intermediates was considered for the next steps of the pathway. Ring migration to **4** allows for water addition to the formimide moiety, producing **5**, and subsequent formamide loss to the final **Iz** product. This pathway may also produce intermediate **6**, which has not been reported in the literature. Hydrolysis at C8 would result in **Iz**; however, the formate byproduct has not been reported, whereas formamide is known to form.<sup>50</sup> Water addition to the C8 of **3** results in **7**, which may undergo C8 alcohol deprotonation and N7-C8 or C8-N9 bond cleavage. While both pathways may result in the **Iz** product, N7-C8 ring opening produces formate and ammonia, while C8-N9 opening produces the experimentally detected formamide.



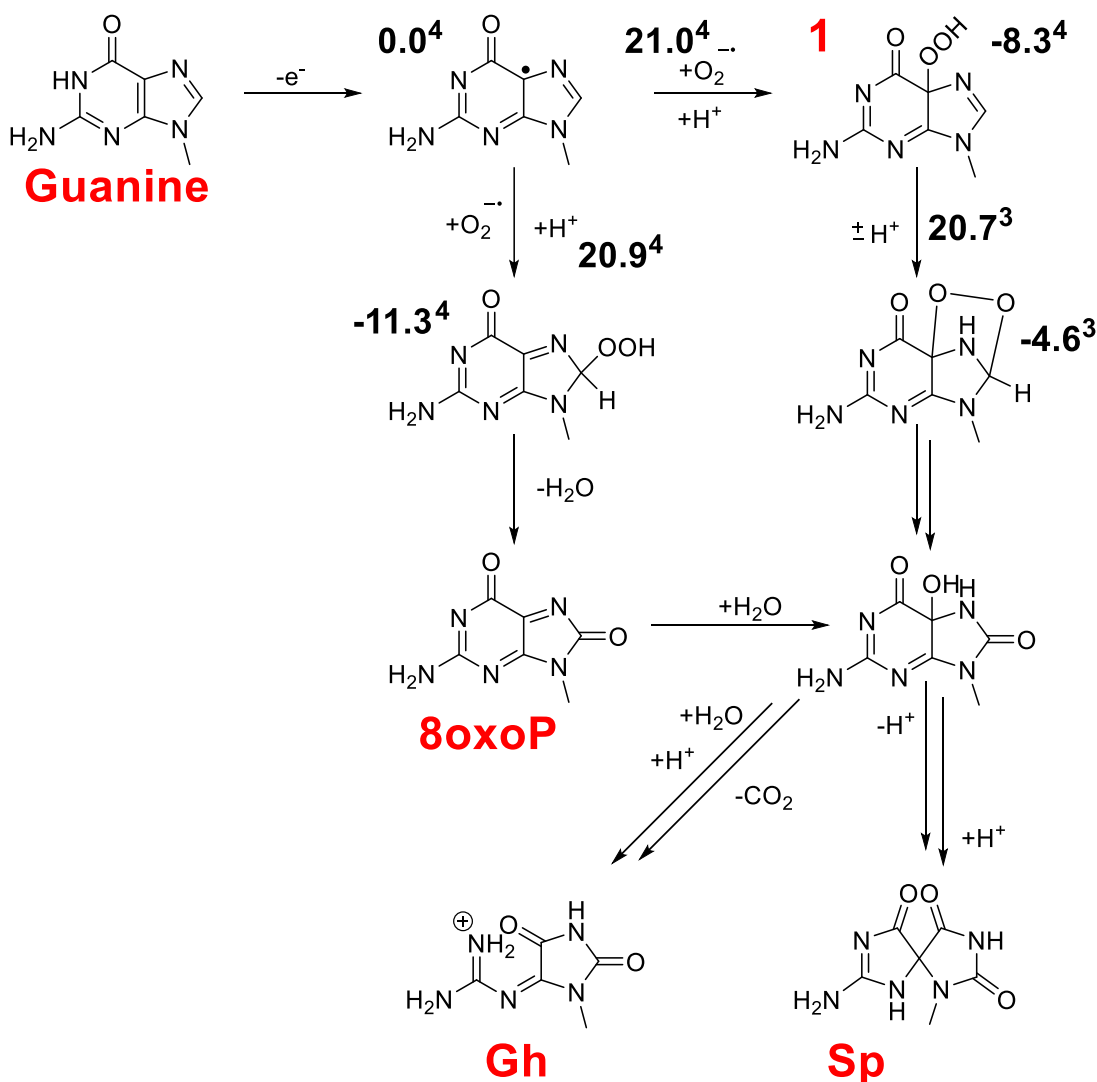
**Scheme 3.** Comparison of endoperoxide and diol formation steps in the early stages of **Iz** formation from guanine. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. Free energies in parentheses are barriers relative to **1**. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer. Structure numbers are shown in red.



Scheme 3 details the calculations for the initial steps for guanine oxidation to **Iz**, showing C5 addition of superoxide to guanine radical and competitive branching between diol and endoperoxide formation. Diol formation has a barrier of 24.3 kcal/mol and is slightly endothermic. Tautomerization to the N1 protonated zwitterion was found to be the most favorable protonation state. Deprotonation of the C6 alcohol facilitates both C5-C6 and C6-N1 ring opening, with barriers of 14.5 kcal/mol and 8.7 kcal/mol, respectively. Pyrimidine ring opening at C6-N1 is followed by decarboxylation. The resulting intermediate undergoes barrierless hydroxide loss, forming **3**. The competing endoperoxide path to **3**

involves the formation of a C5-C6 dioxetane (the corresponding C5-C8 endoperoxide is discussed in Scheme 4 and leads to **Sp** and **Gh**). Formation of the C5-C6 dioxetane is 9.6 kcal/mol endothermic and has 16.4 kcal/mol barrier when calculated with 5 explicit waters. The dioxetane has a low barrier of 6.8 kcal/mol for reverting back to **1**. Ring opening of the dioxetane has a barrier of 16.4 kcal/mol for the C6-N1 bond and 12.6 kcal/mol for the C5-C6 bond. The latter ring opening not only has a lower barrier but also has a very strong driving force because of the relaxation of four membered ring strain and formation of C=O double bonds. Decarboxylation to **3** has a calculated barrier of 13.7 kcal/mol. Formation of **3** from **1** via the C5-C6 endoperoxide has an overall barrier of 22.2 kcal/mol compared to 24.3 kcal/mol for the diol pathway.

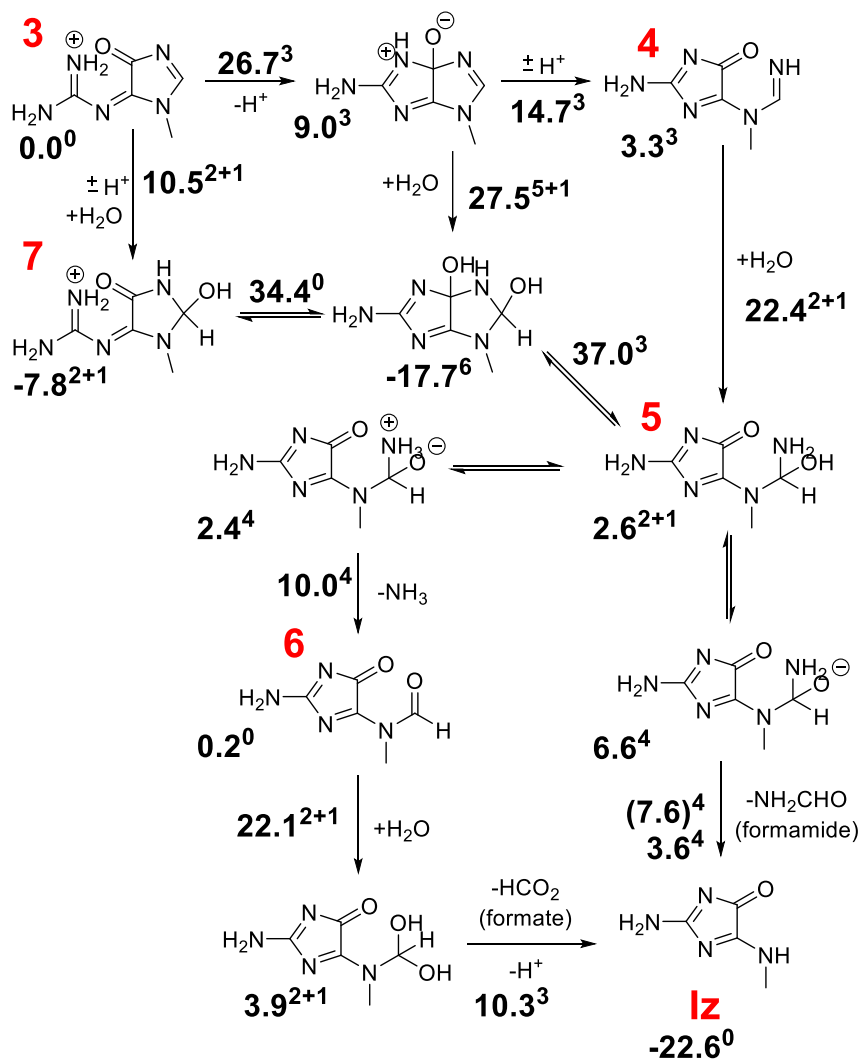
**Scheme 4.** Sp and Gh formation via superoxide addition to C5 and C8 of guanine radical.



Superoxide addition to guanine radical can also lead to the formation of **Sp** and **Gh** in competition with **Iz**, as seen in a number of investigations.<sup>2, 6, 8, 12-16, 18-20, 52, 57</sup> Scheme 4 summarizes guanine radical conversion to **Sp** and **Gh** via superoxide addition to C5 and C8. While details of this pathway are outside

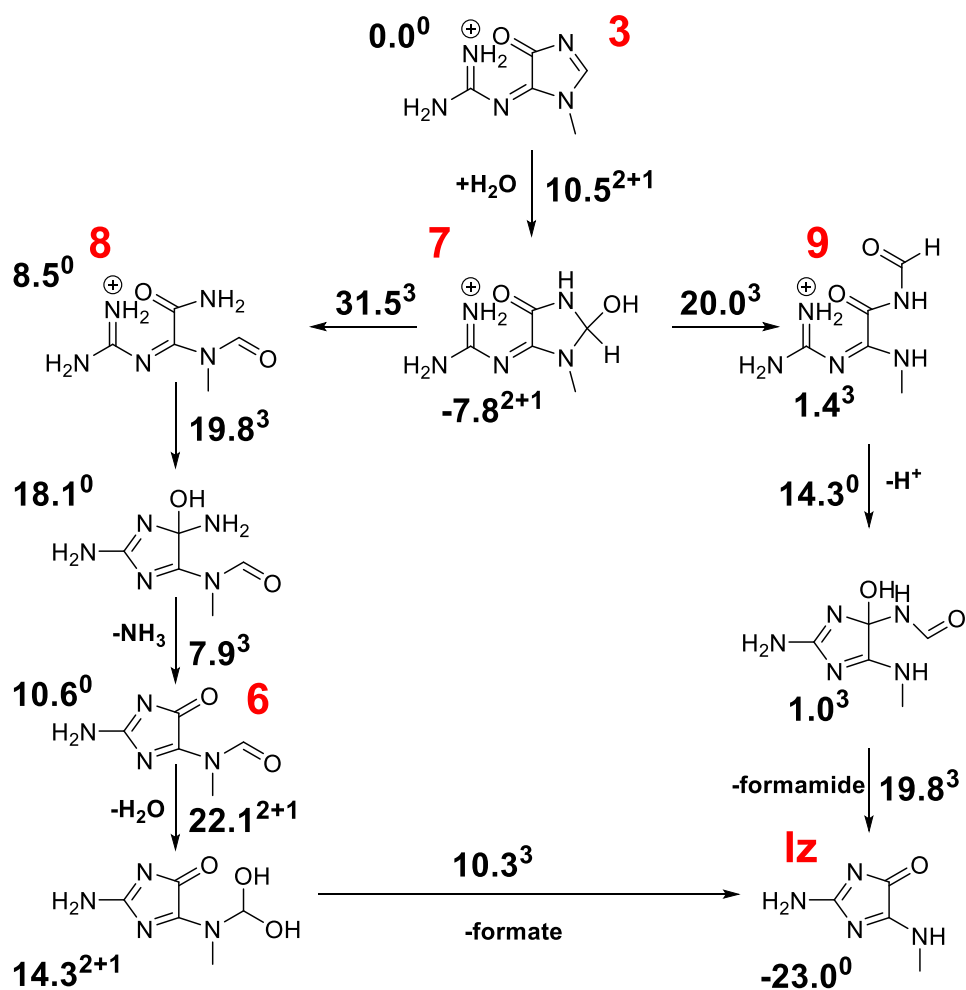
of the scope of this study, the present calculations found the barriers for superoxide addition to C5 and C8 of guanine radical to be equal in energy. For C5 addition, the barrier to form the C5-C8 endoperoxide is 20.7 kcal/mol compared to 16.4 kcal/mol to form the C5-C6 endoperoxide. Opening of the endoperoxide leads an intermediate which may produce the **Sp** / **Gh** products. For C8 addition of superoxide, transfer of the C8 hydrogen to the C8 hydroperoxyl group results in water loss and formation of **8oxoP**. C5 addition of water yields the same intermediate on the way to **Sp** / **Gh**. Given that C5 addition does not favor **Sp**, it is likely that C8 radical combination with superoxide results in one pathway of formation of **Sp** in competition with **Iz**, while C5 addition is a minor pathway to **Sp**. In studies involving Fenton chemistry, where both hydroxyl radical and superoxide are reactive intermediates, the presence or absence of reducing agents resulted in varying yields of **Iz** and **2Ih** but the yields of **Sp**, **Gh**, and **8oxoG** remained mostly unaffected.<sup>8</sup>

**Scheme 5.** Formation of **Iz** from **3** via bicyclic paths. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. Free energies in parentheses represent barriers relative to **5**. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer.



Burrows and coworkers studied conversion of **Gh** and **Ia**, in which the ring migration is believed to occur via a bicyclic intermediate.<sup>17</sup> Conversion of **3** to **4** or **5** was considered via a similar ring migration mechanism. Deprotonation of the guanidinium group followed by nucleophilic addition occurs with a barrier of 26.7 kcal/mol and results in a zwitterionic, bicyclic intermediate. Tautomerization from N1 to N7 and ring opening has a barrier of 14.7 kcal/mol, yielding **4**. Water addition to **4** produces **5**. Intermediate **5** may also be formed via water addition to **3** and similar ring migration; however, the barriers involved were calculated to be considerably higher than conversion of **3** to **5** via **4**. Tautomerization between the C8 alcohol and amine groups of **5** result in an isoenergetic zwitterion which undergoes facile loss of ammonia to produce **6**. Water addition to the C8 carbonyl followed by deprotonation and loss of formate produces **Iz**. However, experimental studies did not report formate as a byproduct, but did find formamide.<sup>50</sup> In agreement with experiment, the calculations found a low energy pathway involving deprotonation of **5** and loss of formamide to produce **Iz**.

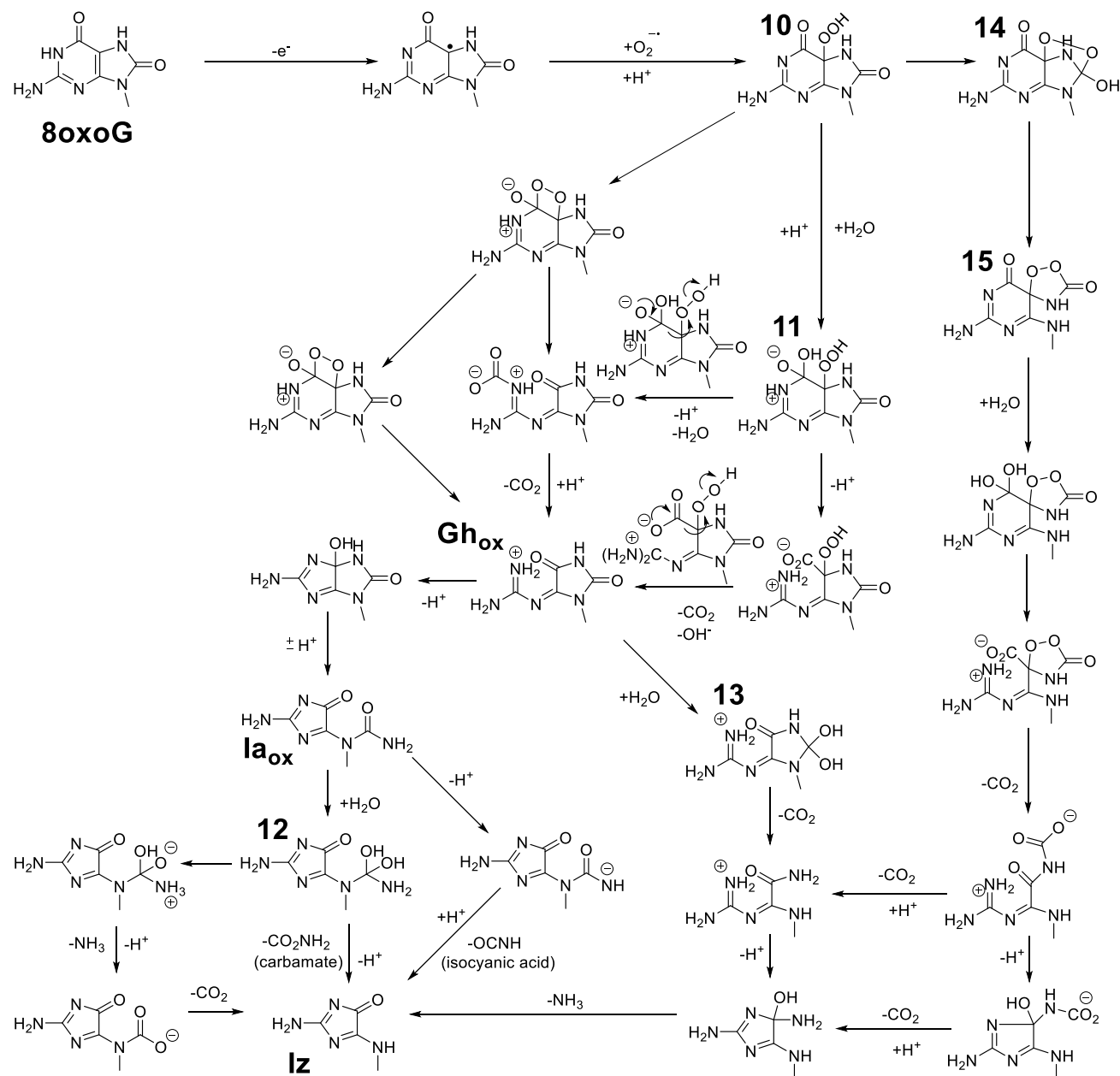
**Scheme 6.** Formation of **Iz** from **3** via acyclic paths. Free energies are given in kcal/mol. Barrier heights are given. Free energies in parentheses represent relative transition state energies. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer.



Scheme 6 shows the conversion of **3** to **Iz** through pathways involving acyclic intermediates. Water addition to the C8 position of **3** was calculated to have a barrier of 10.5 kcal/mol and was exothermic, producing intermediate **7**. N7-C8 and C8-N9 ring opening of **7** found barriers of 31.5 kcal/mol and 20.0 kcal/mol and produced **8** and **9**, respectively. C8-N9 ring opening is followed by ring closing at the N1-C5 position, resulting in ring migration. Proton transfer and formamide loss yields the final **Iz** product. N7-C8 ring opening also led to ring migration. This was followed by ammonia loss, hydration and loss of formate to yield **Iz**. While both pathways may form **Iz**, C8-N9 opening produces experimentally detected formamide,<sup>50</sup> as opposed to N7-C8 ring opening, which produces formate. The lower energy pathway involving ring migration via an acyclic intermediate and formamide loss (Scheme 6) is favored over the pathways involving bicyclic intermediates (Scheme 5). Collectively these data suggest that **Iz** may be produced from guanine via multiple branching pathways, with water addition to **3** forming **7** being favored over production of **4**, and C8-N9 ring opening to **9** rather than N7-C8 ring opening, as the lowest energy pathway.

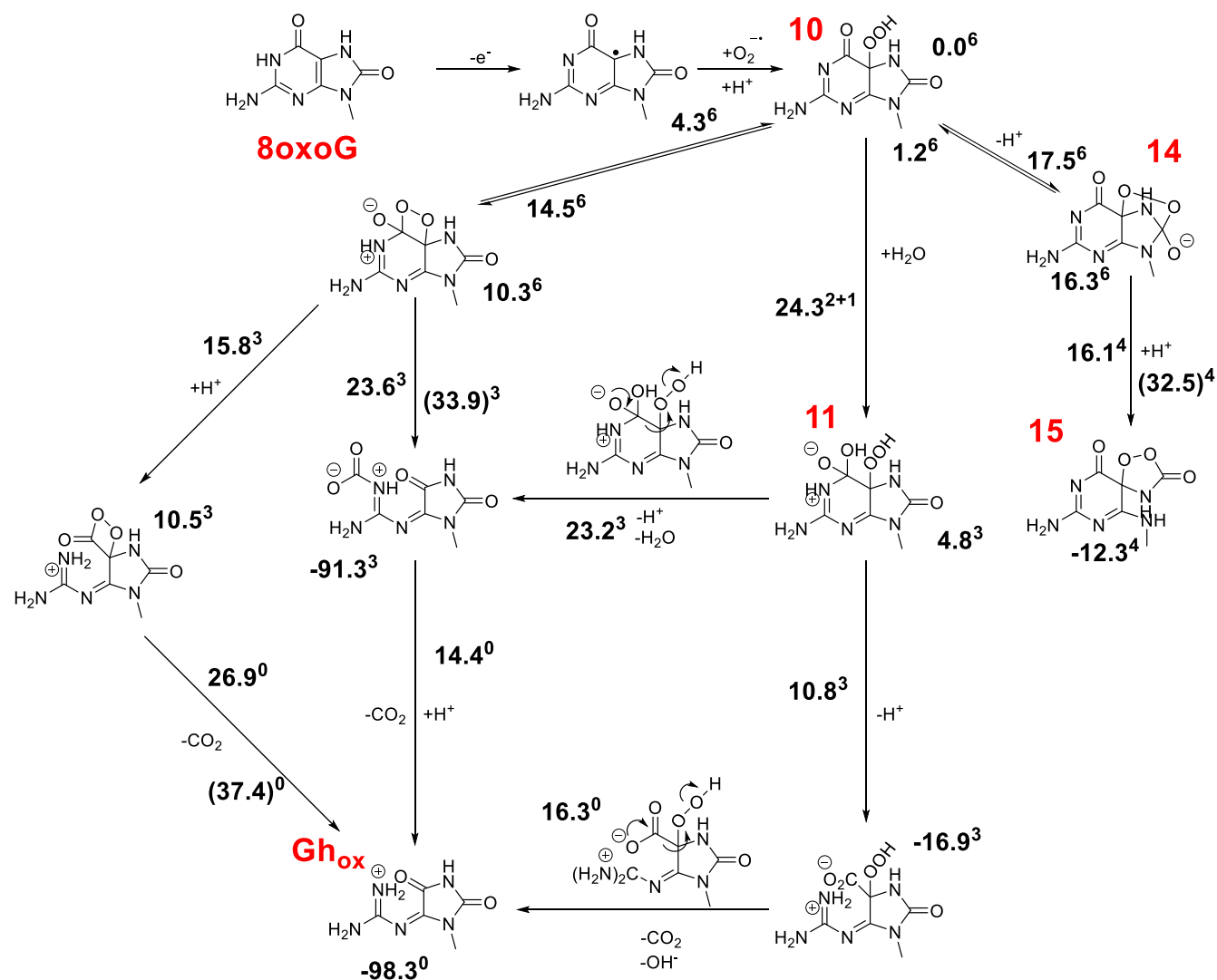
### 3.3 8oxoG oxidation to Iz

**Scheme 7.** Overview of **Iz** formation from **8oxoG**.



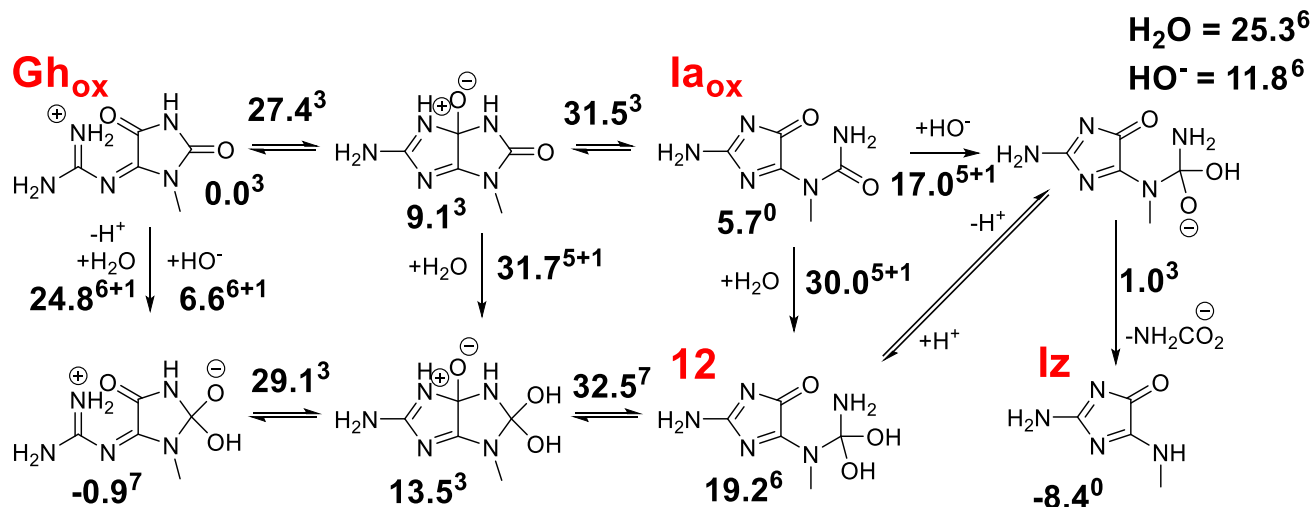
Scheme 7 shows the **Iz** formation pathways starting from **8oxoG**. Similar to the guanine oxidation pathway, both diol formation and endoperoxide formation were considered as initial steps. Stabilization related to delocalization into the N7-C8  $\pi$  bond should be absent in the **8oxoG** pathway, potentially resulting in higher barriers. The **Gh<sub>ox</sub>** intermediate has been observed in a number of experimental studies of **8oxoG** oxidation<sup>2, 12, 13, 19, 20, 51, 52, 55, 56</sup> and acts as an intermediate analogous to **3** in the pathway for guanine oxidation to **Iz** (Scheme 2). Ring migration via bicyclic or acyclic intermediates leads to **Iz** formation. In contrast to the C5-C8 endoperoxide formed from the guanine-superoxide adduct, the C5-C8 endoperoxide from the **8oxoG**-superoxide adduct can undergo C8-N9 ring opening leading to **Iz**.

**Scheme 8.** Comparison of endoperoxide and diol formation steps along early stages of **Iz** formation from **8oxoG**. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. Free energies in parentheses represent barriers relative to **10**. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer. Structure numbers are shown in red.



Scheme 8 shows the energetics for the initial steps in **8oxoG** oxidation to **Iz**, including competition between diol and endoperoxide formation. Similar to the guanine pathway (Scheme 3), endoperoxide formation was calculated to have lower barriers than diol formation. Endoperoxide formation was calculated to be endothermic with barriers of 14.5 kcal/mol for the C6 position and 17.5 kcal/mol for the C8 position. The resulting intermediates may undergo ring opening with high barriers, with the most favorable pathway being C8 endoperoxide ring opening, having a calculated composite barrier relative to **10** for endoperoxide formation and C8-N9 ring opening of 32.5 kcal/mol. Diol formation was calculated to have a barrier of 24.3 kcal/mol, similar to the guanine oxidation pathway. C6-N1 ring opening has a barrier of 10.8 kcal/mol and is followed by loss of CO<sub>2</sub> and OH<sup>-</sup> to form **Gh<sub>ox</sub>**.

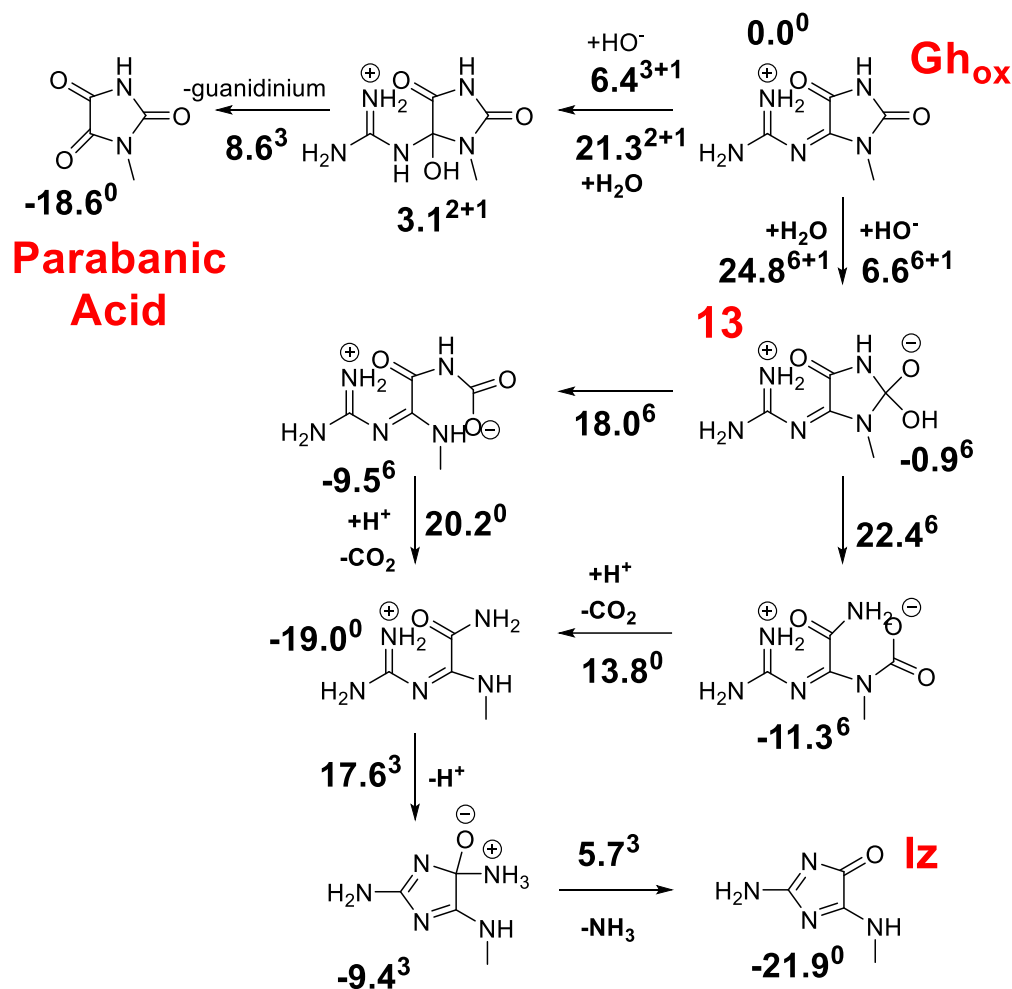
**Scheme 9.** Formation of **Iz** from **Gh<sub>ox</sub>** via bicyclic paths. Free energies are given in kcal/mol. Barrier heights are given. Free energies in parentheses represent relative transition state energies. The number of explicit waters used is denoted by superscripts for each step. The superscript n+1 denotes water addition, where one water molecule adds and n others allow for proton transfer.



Scheme 9 shows formation of **Iz** from **Gh<sub>ox</sub>** via ring migration involving a bicyclic intermediate. Water addition to C8 was calculated to have a barrier of 24.8 kcal/mol, similar to the water addition barrier in Scheme 5 for the guanine oxidation pathway. Ring migration from **Gh<sub>ox</sub>** to **la<sub>ox</sub>** has an initial barrier of 27.4 kcal/mol, followed by a ring opening barrier of 31.5 kcal/mol to the **la<sub>ox</sub>** intermediate. Hydrolysis of **la<sub>ox</sub>** was calculated to have a barrier of 30.0 kcal/mol, forming **12**. Intermediate **12** can then deprotonate and undergo carbamate loss to **Iz**. Hydroxide addition to **la<sub>ox</sub>** followed by carbamate loss is a lower energy pathway to **Iz**. Nevertheless, the calculated barriers for ring migration via a bicyclic intermediate are prohibitively high and an alternate pathway is needed.



**Scheme 10.** Formation of **Iz** from **Gh<sub>ox</sub>** via acyclic paths. Free energies are given in kcal/mol. Barrier heights are given. Free energies in parentheses represent relative transition state energies. The number of explicit waters used is denoted by superscripts for each step. The superscript n+1 denotes water addition, where one water molecule adds and n others allow for proton transfer.



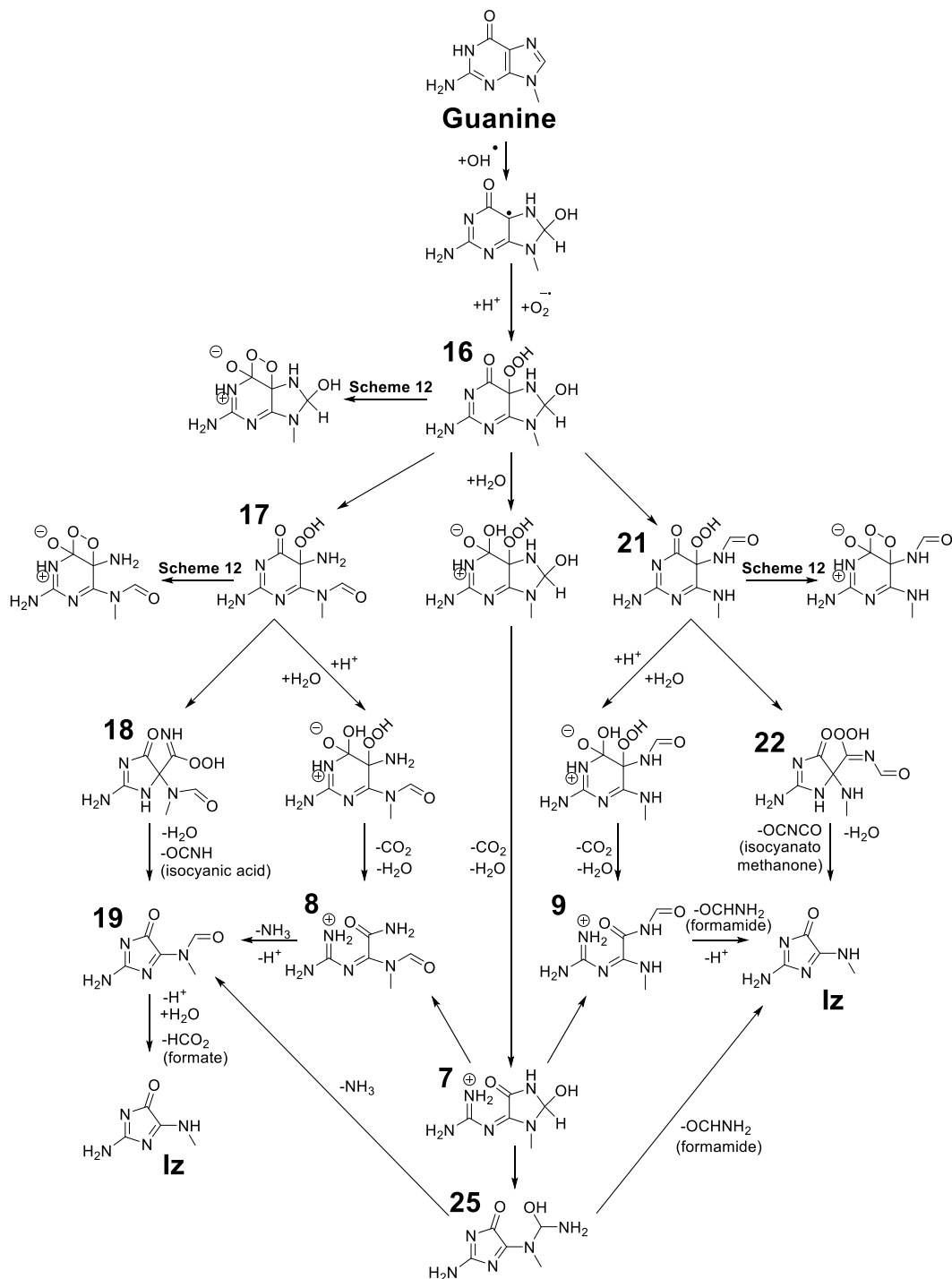
Scheme 10 shows the hydrolysis pathways from **Gh<sub>ox</sub>** to **Iz** and parabanic acid. The barriers for water addition are similar to the initial diol formation, 21.3 kcal/mol at C4 and 24.8 at C8. The C4 alcohol adduct may undergo deprotonation and guanidinium loss with a low barrier of 8.6 kcal/mol to the experimentally detected parabanic acid product. Parabanic acid may undergo further hydrolysis to oxaluric acid (discussed in section 3.5). The barriers for hydroxide addition to **Gh<sub>ox</sub>** are 15 – 18 kcal/mol lower than for water addition. C8 hydroxide addition forms a zwitterion intermediate, **13**. The barrier for C8-N9 ring opening of **11** was calculated to be about 4 kcal/mol lower than for N7-C8 ring opening. Decarboxylation of either intermediates is followed by ring closing with a barrier of 17.6 kcal/mol and ammonia loss with a small barrier of only 5.7 resulting in **Iz**. The lower overall barrier for parabanic acid formation compared to **Iz** is in agreement with the experimental observation that little or no **Iz** is formed directly from **Gh<sub>ox</sub>** below basic pH.

**Gh<sub>ox</sub>** conversion to **Iz** has not been found experimentally, except for some evidence at high pH.<sup>12</sup>  
<sup>57</sup> The barriers for OH<sup>-</sup> addition are much lower than water addition, 6.4 kcal/mol vs 21.3 kcal/mol at C4

and 6.6 kcal/mol vs 24.8 kcal/mol at C8. The low barriers of hydroxide addition may explain the significance of pH in formation of **Iz** compared to **Gh<sub>ox</sub>** and its hydrolysis products after **8oxoG** oxidation.

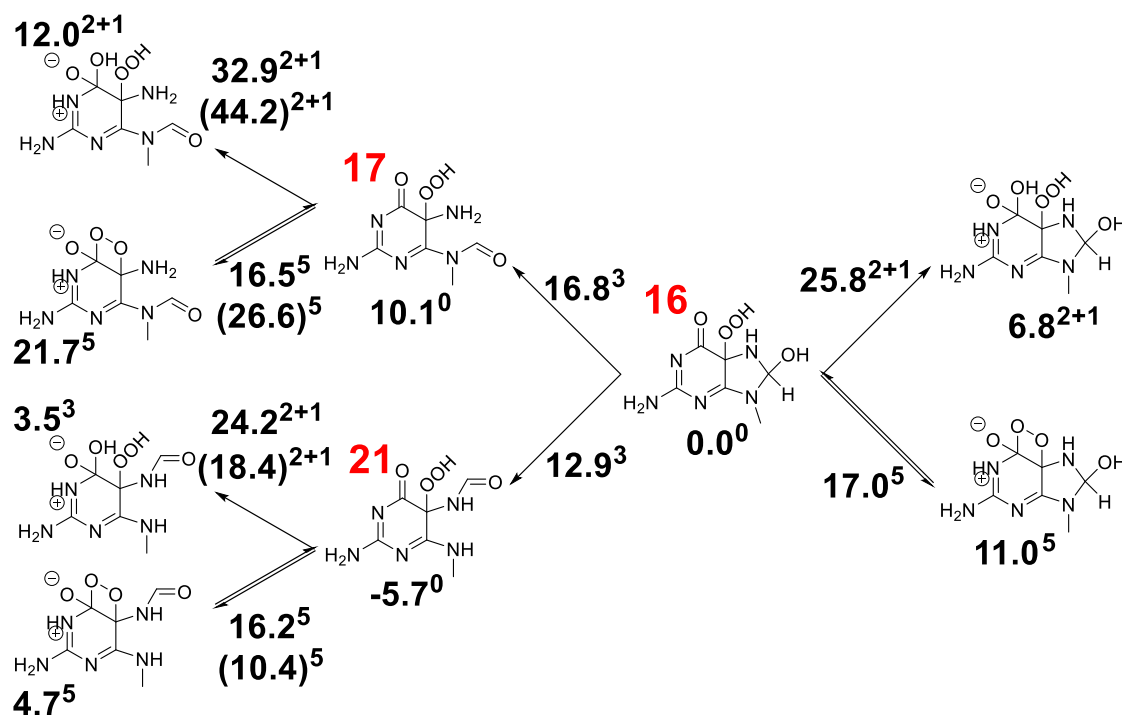
### 3.4 8 Hydroxy Guanine Radical Oxidation to **Iz**

**Scheme 11.** Overview of **Iz** formation from 8 hydroxy guanine radical



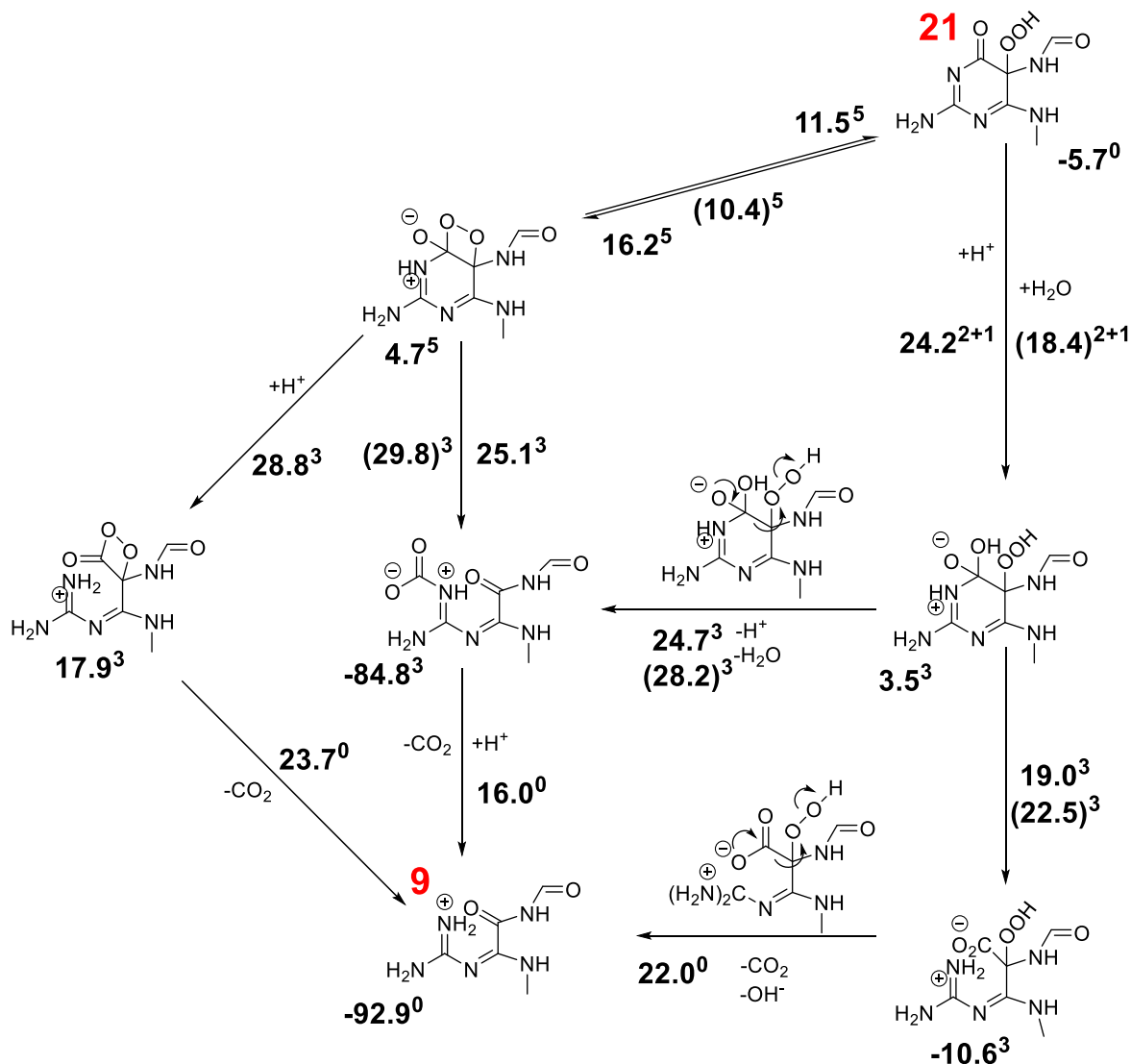
Scheme 11 summarizes **Iz** formation from 8 hydroxy guanine radical, which may arise from hydroxyl radical addition to C8 of guanine or water addition to C8 of guanine radical cation, followed by combination with superoxide at C5. Intermediate **16** has not been detected experimentally; however, this may be a result of relatively rapid conversion to a more stable intermediate.

**Scheme 12.** Comparison of diol formation, endoperoxide formation, N7-C8 and C8-N9 opening pathways from **16**. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. Free energies in parentheses represent barriers relative to **16**. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer. Structure numbers are shown in red.



Scheme 12 shows the energetics for the first few steps of the conversion of **16** to **Iz**. N7-C8 and C8-N9 ring openings were considered in addition to diol formation and endoperoxide formation as initial steps. Similar to the guanine and 8oxoG pathways, diol formation was calculated to have a barrier of 25.8 kcal/mol. Formation of the C5-C6 endoperoxide had a barrier of 17.0 kcal/mol and was 11.0 kcal/mol endothermic. N7-C8 and C8-N9 ring opening steps were calculated to have barriers of 16.8 and 12.9 kcal/mol, resulting in **17** and **21**. Formation of **17** was calculated to be 10.1 kcal/mol endothermic, while **21** was 5.7 kcal/mol exothermic. Similar to pathways starting from guanine radical or **8oxoG** radical, formation of the C5-C6 endoperoxide from **21** has a barrier of 16.2 kcal/mol while formation of the diol has a barrier of 24.2 kcal/mol.

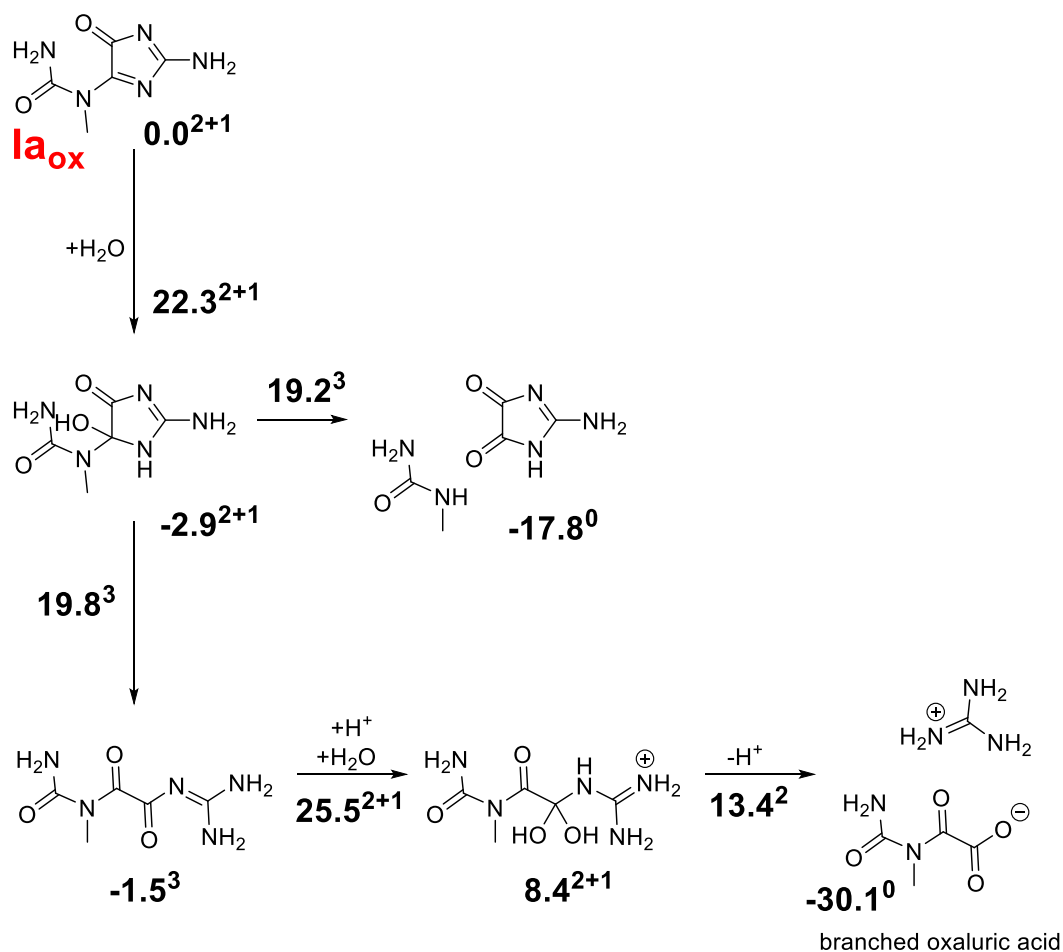
**Scheme 13.** Comparison of endoperoxide and diol formation steps along early stages of **Iz** formation from **21**. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. Free energies in parentheses represent barriers relative to **16**. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer. Structure numbers are shown in red.



Scheme 13 summarizes the pathways to **Iz** after diol and endoperoxide formation from **21**. N1-C6 and C5-C6 ring opening from the diol intermediate proceed through barriers of 22.5 and 28.2 kcal/mol, respectively, relative to **21**. Decarboxylation from the N1-C6 ring opening intermediate has a calculated barrier of 22.0 kcal/mol, which is also significantly higher than the cases of guanine and **8oxoG** oxidation (Schemes 3 and 8, resp.). While endoperoxide formation has lower barrier, consistent with guanine and **8oxoG** oxidation pathways, the corresponding ring opening barriers are about 30 kcal/mol, indicating the diol pathway is the favored route to intermediate **9**. Conversion of **9** to **Iz** is given in Scheme 6.

### 3.5 Parabanic and Oxaluric Acid

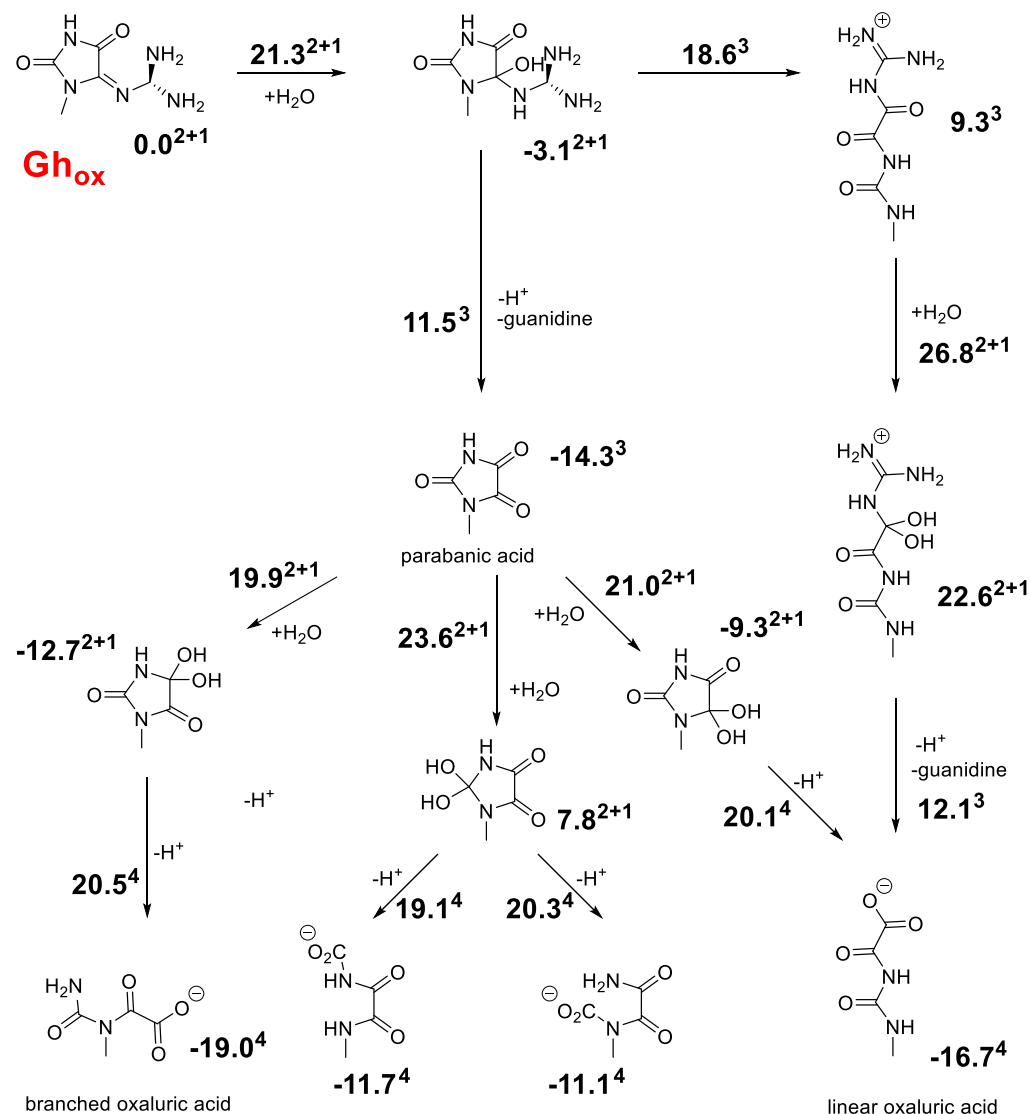
**Scheme 14.** Formation of **Pa** and **Oa** from **Ia<sub>ox</sub>**. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer. Structure numbers are shown in red.



Numerous studies have found parabanic and oxaluric acid as hydrolysis products, in addition to **Gh<sub>ox</sub>** and **Iz**. Water addition to **Gh<sub>ox</sub>** and **Ia<sub>ox</sub>** has been proposed as the path to these products. Scheme 14 shows oxaluric acid formation from **Ia<sub>ox</sub>**. C4 water addition was calculated to have a barrier of 22.3 kcal/mol and is 2.9 kcal/mol exothermic. Deprotonation of the alcohol allows for either N3-C4 or C4-N9 bond cleavage. These pathways proceed with similar barriers, 19.8 and 19.2 kcal/mol, respectively. C4-N9 bond cleavage results in loss of an amino-imidazole-dione, which was calculated to be 17.8 kcal/mol exothermic relative to **Ia<sub>ox</sub>**. N3-C4 bond cleavage results in a precursor to branched oxaluric acid. C4 water addition and guanidinium loss results in the oxaluric acid product. This pathway proceeds via a 25.5 kcal/mol barrier for water addition, followed by a 13.4 kcal/mol barrier for guanidinium loss to the final oxaluric acid product. While both parabanic acid and oxaluric acid are detected experimentally, parabanic

acid cannot form from **la<sub>ox</sub>**.<sup>51, 52</sup> This, combined with high barriers of conversion between **Gh<sub>ox</sub>** and **la<sub>ox</sub>** (Scheme 9), suggests that **la<sub>ox</sub>** formation and its hydrolysis may not occur readily.

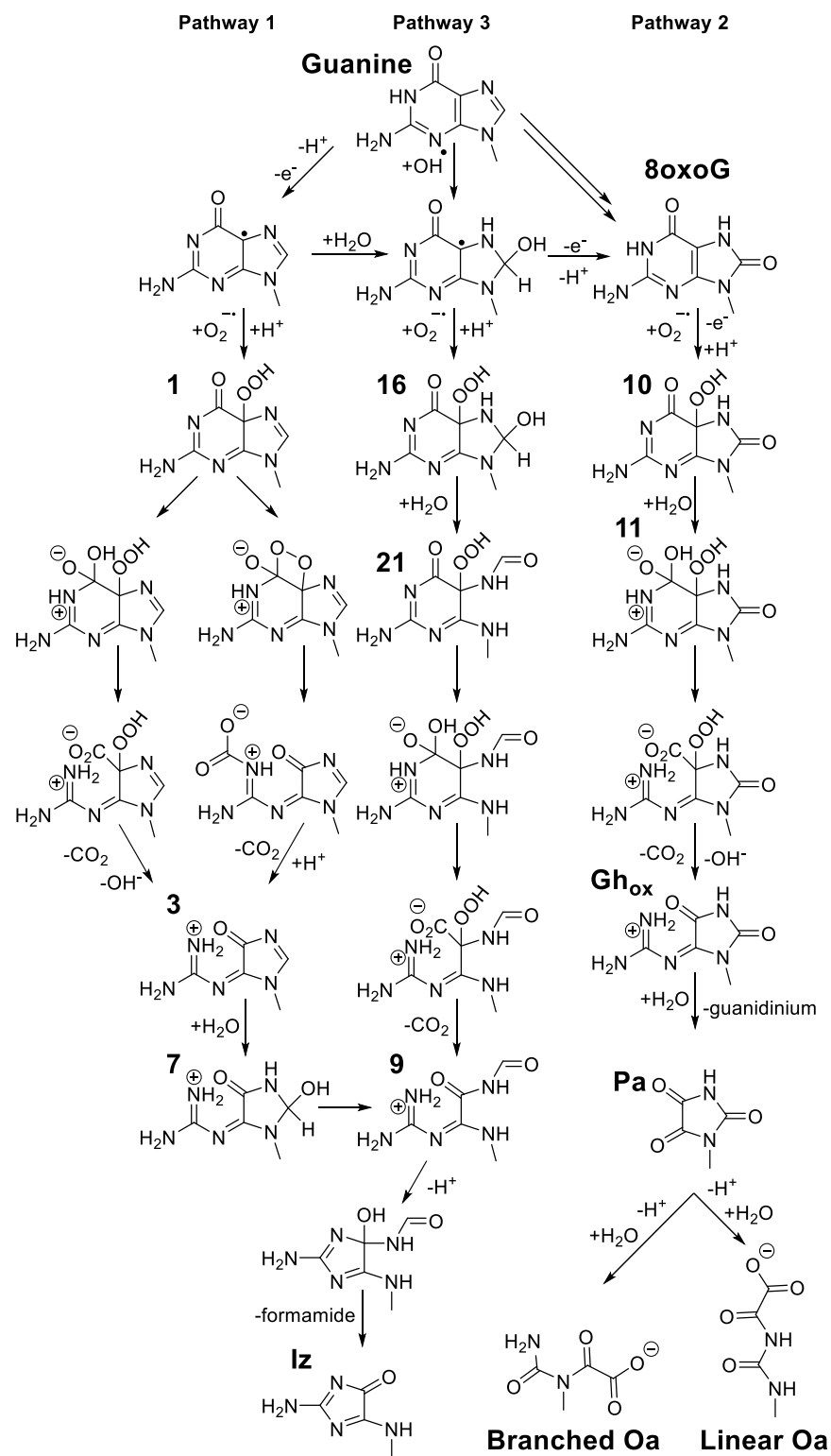
**Scheme 15.** Formation of **Pa** and **Oa** from **Gh<sub>ox</sub>**. Free energies are given in kcal/mol. Barrier heights are given relative to the reactant for the indicated step. The number of explicit waters used is denoted by superscripts for each step. The superscript 2+1 denotes water addition, where one water molecule adds and two others allow for proton transfer. Structure numbers are shown in red.



Scheme 15 shows the pathway for **Gh<sub>ox</sub>** hydrolysis to parabanic and oxaluric acid. The pathway for **Gh<sub>ox</sub>** hydrolysis proceeds with a barrier similar to **la<sub>ox</sub>** hydrolysis (21.3 kcal/mol and 22.3 kcal/mol, respectively) and produces a similarly exothermic intermediate. For **Gh<sub>ox</sub>**, the barrier for ring opening at N3-C4 to produce parabanic acid was calculated to be 11.5 compared to 18.6 kcal/mol for ring opening at C4-N9. Water addition to C4, C5, and C8 of parabanic acid were calculated to have barriers of 21.0, 19.9, and 23.6 kcal/mol, respectively. Each of these intermediates may undergo ring opening with similar barriers of 19.0 – 20.5 kcal/mol. The final products are linear and branched forms of oxaluric acid. NMR data indicates linear oxaluric acid is the dominant form.<sup>13</sup>

#### 4. SUMMARY

**Scheme 16.** Summary of lowest energy oxidation pathways for the formation of Iz from guanine radical, 8oxoG, and 8 hydroxy guanine radical.



Computational investigations into the formation of **Iz** and related products from oxidation of guanine, **8oxoG**, and 8-hydroxyl guanine radical have revealed a number of branching pathways. The lowest energy pathways are summarized in Scheme 16. In Pathway 1, after the addition of superoxide to guanine radical, endoperoxide formation was calculated to have slightly lower barriers than diol formation; however, the reaction can proceed by either path (see Scheme 3 for details of the energetics). C5-C6 pyrimidine ring opening is then facilitated by the imidazole ring  $\pi$  stabilization. Decarboxylation is followed by C8 water addition and C8-N9 ring opening. Ring migration proceeds via an acyclic intermediate rather than a bicyclic intermediate (Scheme 5 vs 6) and is followed by formamide loss to yield **Iz**. Pathways 2 and 3 do not allow for similar imidazole ring stabilization of endoperoxide ring opening and go exclusively through a diol path. In the case of **8oxoG** (Scheme 8), diol formation formed a relatively stable intermediate, **Gh<sub>ox</sub>**. Water addition to C8 of **Gh<sub>ox</sub>** was found to be higher energy than for **3** in Pathway 1, which may explain why **Gh<sub>ox</sub>** has been observed<sup>12, 13, 19, 20, 51-57</sup> but **3** has not. While water addition to **Gh<sub>ox</sub>** can produce **Iz**, parabanic acid formation is more favorable (Scheme 10), in agreement with experimental work.<sup>20, 54, 56</sup> The barriers for hydroxide ion addition to **Gh<sub>ox</sub>** are much lower than for water addition (Scheme 10), and the formation of **Iz** and parabanic acid should be competitive. Thus, at high pH, **8oxoG** oxidation should produce both **Iz** and parabanic acid / oxaluric acid. Pathway 3 for 8-hydroxy guanine radical is similar to Pathway 2 for **8oxoG**, proceeding via diol formation and C6-N1 bond cleavage before decarboxylation (Scheme 12 and 13). This leads to an acyclic intermediate in common with Pathway 1 and results in **Iz** formation.

Burrows and coworkers reported riboflavin photooxidation of guanine favored a combination of **Iz** and **Sp**.<sup>12</sup> This can easily be understood by considering the nearly identical barriers for C5 and C8 superoxide radical addition to guanine radical (Scheme 3 and 4). C8 radical combination forms either **Gh** or **Sp** depending on pH, while the C5 position favors **Iz**, but may also form **Sp/Gh** as a minor product via C5-C8 endoperoxide formation (Scheme 4). Burrows and coworkers also found that **Sp/Gh** formation did not contain radiolabeled oxygen from solvent, which further suggests a mechanism bypassing **8oxoG** formation. Our calculations find that oxidation of guanine should not produce **Gh<sub>ox</sub>** / **Ia<sub>ox</sub>**; however, riboflavin oxidation of guanine consistently produces detectable amounts of these products. One possibility is that oxidation of guanine to **8oxoG** is a minor pathway, which allows for further oxidation to **Gh<sub>ox</sub>**. In considering **8oxoG** conversion to **Iz**, **Gh<sub>ox</sub>**/**Ia<sub>ox</sub>** is the dominant species below pH 8.6 in the experimental study. Our current calculations show **Gh<sub>ox</sub>** as the most likely intermediate (Scheme 8 and 10), with hydrolysis allowing conversion to parabanic and oxaluric acids (Scheme 14 and 15). Superoxide addition to C5 of **8oxoG** can proceed to **Iz** but not to **Sp** or **Gh** (Scheme 8). This suggests that water addition at C5 to form **Sp** / **Gh** competes with superoxide addition to form **Iz**. This may further be seen in the effects of superoxide dismutase (SOD); guanine oxidation to **Iz** is suppressed by about 10% compared to nearly 50% suppression in the case of **8oxoG** to **Iz**. Assuming SOD is equally efficient in both cases, the alternative explanation would be less efficient radical combination comparing guanine and **8oxoG**. **Iz** was only found in significant yields at basic pH, which is in accord with the lower calculated barrier for hydroxide addition compared to water addition in conversion of **Gh<sub>ox</sub>** to **Iz**. Experimentally, parabanic acid was not found, but oxaluric acid was; calculations show that oxaluric acid can be formed from either **Ia<sub>ox</sub>** or **Gh<sub>ox</sub>** (Scheme 14 and 15).

Cadet and coworkers compared riboflavin-mediated photooxidation of guanine and **8oxoG**.<sup>18</sup> A mixture of <sup>15</sup>N<sub>5</sub>-labelled **8oxoG** and unlabeled guanine was oxidized, allowing for the tracking of oxidation products that arise from guanine and **8oxoG**. Their observations indicated that **8oxoG** was oxidized to



completion before any guanine oxidation products formed. Oxidation of the labeled **8oxoG** predominantly produced **Iz**, with **Sp** as a minor product. Oxidation of guanine after the labeled **8oxoG** was expended produced unlabeled **8oxoG**, which was consumed in the same manner as the labeled **8oxoG**. Oxidation of guanine also produced a cyclic intermediate arising from nucleophilic attack of the sugar alcohol to the C8 of guanine radical, which would not be present in studies using the triacetyl protected form of the nucleoside. Based on the formation of this product and the known slow water addition to neutral guanine radical, it is likely that Pathway 1 (superoxide – guanine radical combination forming **3**) is responsible for the formation of part of the observed unlabeled **Iz** (Scheme 3 and 5). The fact that Cadet found low yields of **Sp** compared to **Iz** and did not report any **Gh<sub>ox</sub>** nor **Ia<sub>ox</sub>** is also compatible with Pathway 1.

Shafirovich and coworkers<sup>6, 56</sup> investigated the reaction of superoxide with guanine radical and **8oxoG** radical. Two photon photoionization of 2-aminopurine in an oligonucleotide was used to generate superoxide and guanine or **8oxoG** radicals which reacted to form **Iz**, **Z**, **Oa**, **Sp**, **8oxoG** and **Gh<sub>ox</sub>**. Addition of SOD showed significant reduction in reaction rate, suggesting superoxide radical combination as a primary degradation pathway. SOD was found to have a more pronounced effect on **8oxoG** radical compared to guanine radical superoxide combination, which is in agreement with the findings of Burrows and coworkers. Oxidation of guanine-containing oligonucleotides produced mainly **Iz**, (20%) with some **8oxoG** (1%). Oxidation of **8oxoG** containing oligonucleotides produced a combination of **Gh<sub>ox</sub>** and **Oa** (21.5%), as well as some **Sp** (2%). Labeling studies with H<sub>2</sub><sup>18</sup>O showed radical combination with superoxide was favored over hydration. **8oxoG** oxidation yielded primarily **Gh<sub>ox</sub>** which can hydrolyze to **Oa**. These experimental results are in agreement both with riboflavin induced oxidation<sup>12</sup> and the present calculations.

Meunier and coworkers<sup>13, 50, 51</sup> examined guanine oxidation by Mn-TMPyP/KHSO<sub>5</sub> and found that **Iz** can be formed without going through **8oxoG** as an intermediate. Mn(V) two-electron oxidation of guanine forms guanine cation and persulfate can add rapidly to C5, yielding an intermediate analogous to superoxide adding to guanine radical. Labeled molecular oxygen was not incorporated in **Iz** in Mn-TMPyP/KHSO<sub>5</sub> oxidation but was incorporated in benzophenone-mediated photooxidation of guanine, suggesting persulfate adds in the former case and superoxide adds in the latter case. The reaction proceeds to form **Iz** with formamide as a byproduct, similar to benzophenone and riboflavin-mediated photooxidation of guanine. Labeling studies found water incorporation in the formamide byproduct, indicating C8 water addition. They also found that further oxidation of a transient intermediate along the path formed **Gh<sub>ox</sub>**, which in turn produced **Iz** with ammonia and carbon dioxide as byproducts. Our calculations find formamide loss from guanine oxidation to **Iz** and ammonia / carbon dioxide loss from **8oxoG** oxidation (Scheme 6 and 10) in agreement with the experimentally observed byproducts.

Shafirovich and coworkers<sup>20</sup> investigated guanine oxidation by peroxy radicals derived from the one-electron oxidation of polyunsaturated fatty acids. Arachidonic acid was oxidized by sulfate radical and addition of molecular oxygen produced a peroxy radical that added to guanine radical. This yielded a combination of **Iz**, **Gh<sub>ox</sub>**, **Oa** and **Sp**, with **Iz** being the dominant product. Peroxyl radical oxidation of **8oxoG** did not produce any **Iz** but did yield **Sp** and **Gh<sub>ox</sub>**. Labeling studies showed **Sp** formed from guanine incorporated one or two labeled waters, suggesting at least two relevant mechanistic paths. **Sp** formed from **8oxoG** incorporated only one labeled water. **Iz** formation showed no labeled water addition, suggesting exclusively formation of a C5 peroxy intermediate. The calculations are in agreement with these findings. Guanine oxidation and C8 radical combination results in **8oxoP**, followed by water addition at C5 (Scheme 4) yields **Sp** and **Gh** with one labeled oxygen. Alternatively, hydration of guanine radical to

**8oxoG** and subsequent oxidation would incorporate two labeled oxygens, while unlabeled **8oxoG** oxidation results in hydration at C5 and only one labeled oxygen. In the formation of **Iz** from guanine and peroxy radical, the labeled oxygen is lost in the decarboxylation step (Scheme 3).

Experimental studies of Fenton chemistry and X-ray radiation induced oxidation of guanine and **8oxoG** probed the role of hydroxyl radical in guanine oxidation and are directly related to Pathway 3. Smyth and coworkers<sup>19</sup> investigated iron and copper mediated Fenton oxidation of **8oxoG** nucleobase and nucleoside. Oxidation of the **8oxoG** free base results in predominantly **Gh<sub>ox</sub>** and hydrolysis to oxaluric acid. Oxidation of the **8oxoG** nucleoside produced exclusively **Sp**. Burrows and coworkers<sup>8, 14</sup> investigated Fenton chemistry oxidation and X-ray radiation induced oxidation of guanine and included the effects of reducing agents in the context of cellular systems. Oxidation in the presence of reducing agents, such as ascorbic acid, resulted in **2Ih** as the dominant product. This has been shown to be the result of C5-hydroperoxyl reduction. In the absence of reducing agents, **Iz** dominates. **Sp** and **8oxoG** formation is mostly unaffected by the presence or absence of reducing agents. The calculations in the present study and our previous study of **2Ih** formation<sup>66</sup> are in agreement with these experimental results. C5 hydroperoxyl formation explains the production of **2Ih** / **Iz** depending on presence or absence of reducing agents (Scheme 3 and 6). **Sp** formation may occur via C8 hydroperoxyl formation, conversion to **8oxoP**, and subsequent C5 water addition, or C8 hydroperoxyl reduction to **8oxoG** and hydroxyl radical conversion to **Sp** (Scheme 4). The absence of **Gh<sub>ox</sub>** and oxaluric acid in these studies may be explained by a lack of oxidation of **8oxoG** via a hydroperoxyl intermediate. An alternative explanation for both the lack of **Gh<sub>ox</sub>** formation and lack of **8oxoG** consumption is competition between hydroperoxyl reduction and C8-hydroperoxyl conversion to **8oxoP**. C5-C8 endoperoxide formation would also produce **Sp** without **8oxoG** via C8 deprotonation. This would also explain the low yields of **8oxoG** but relatively high yields of **Sp** at low reducing agent concentrations.

In the present computational study, three pathways have been examined for the formation of **Iz** resulting from the oxidation of guanine, **8oxoG** and 8-hydroxy guanine radical. The calculations are in good agreement with the numerous experimental studies of the formation of **Iz** resulting from oxidative damage to DNA.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website

Optimized molecular geometries in Cartesian coordinates and free energies (in Hartree) for all the reactants, intermediates, transition states, and products in PDF format.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: hbs@chem.wayne.edu. Phone: 313-577-2562. Fax: 313-577-8822

### Orcid

H. Bernhard Schlegel: 0000-0001-7114-2821 871

## Funding

This work was supported by a grant from National Science Foundation (CHE1856437)

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGEMENTS

The authors thank Prof. Cynthia Burrows for stimulating discussions and Wayne State University computing grid for the computational time.

## ABBREVIATIONS

2lh, 5-carboxyamido-5-formamido-2-iminohydantoin; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8oxoG, 8-oxo-7,8-dihydroguanine; 8oxoP, 2-amino-6,8-dioxo-9-methylpurine; Sp, spiroiminodihydantoin; Gh, guanidinohydantoin; Ia, iminoallantoin; Gh<sub>ox</sub>, oxidized guanidinohydantoin; Ia<sub>ox</sub>, oxidized iminoallantoin; Iz, imidazolone; Z, oxazolone; G<sup>+</sup>Lys, guanine-lysine cross-link; 8nitroG, 8-nitroguanine; NIm, 5-guanidino-nitroimidazole; Pa, parabanic acid; Oa, oxaluric acid; SOD, superoxide dismutase

## REFERENCES

- (1) Steenken, S., and Jovanovic, S. V. (1997) How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. *J. Am. Chem. Soc.* **119**, 617-618.
- (2) Neeley, W. L., and Essigmann, J. M. (2006) Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chem. Res. Toxicol.* **19**, 491-505.
- (3) Pratviel, G., and Meunier, B. (2006) Guanine oxidation: one- and two-electron reactions. *Chem. Eur. J.* **12**, 6018-6030.
- (4) Fleming, A. M., and Burrows, C. J. (2017) Formation and processing of DNA damage substrates for the hNEIL enzymes. *Free Radical Biol. Med.* **107**, 35-52.
- (5) Shukla, L. I., Adhikary, A., Pazdro, R., Becker, D., and Sevilla, M. D. (2004) Formation of 8-oxo-7,8-dihydroguanine-radicals in  $\gamma$ -irradiated DNA by multiple one-electron oxidations. *Nucleic Acids Research* **32**, 6565-6574.
- (6) Misiaszek, R., Crean, C., Joffe, A., Geacintov, N. E., and Shafirovich, V. (2004) Oxidative DNA damage associated with combination of guanine and superoxide radicals and repair mechanisms via radical trapping. *J. Biol. Chem.* **279**, 32106-32115.
- (7) Crean, C., Geacintov, N. E., and Shafirovich, V. (2005) Oxidation of guanine and 8-oxo-7,8-dihydroguanine by carbonate radical anions: Insight from oxygen-18 labeling experiments. *Angew. Chem. Int. Ed.* **44**, 5057-5060.
- (8) Alshykhly, O. R., Fleming, A. M., and Burrows, C. J. (2015) 5-Carboxamido-5-formamido-2-iminohydantoin, in addition to 8-oxo-7,8-dihydroguanine, is the major product of the iron-Fenton or X-ray radiation-induced oxidation of guanine under aerobic reducing conditions in nucleoside and DNA contexts. *J. Org. Chem.* **80**, 6996-7007.

- (9) Jaruga, P., Kirkali, G., and Dizdaroglu, M. (2008) Measurement of formamidopyrimidines in DNA. *Free Radical Biol. Med.* 45, 1601-1609.
- (10) Munk, B. H., Burrows, C. J., and Schlegel, H. B. (2007) Exploration of mechanisms for the transformation of 8-hydroxy guanine radical to FAPyG by density functional theory. *Chem. Res. Toxicol.* 20, 432-444.
- (11) Munk, B. H., Burrows, C. J., and Schlegel, H. B. (2008) An exploration of mechanisms for the transformation of 8-oxoguanine to guanidinohydantoin and spiroiminodihydantoin by density functional theory. *J. Am. Chem. Soc.* 130, 5245-5256.
- (12) Luo, W. C., Muller, J. G., and Burrows, C. J. (2001) The pH-dependent role of superoxide in riboflavin-catalyzed photooxidation of 8-oxo-7,8-dihydroguanosine. *Org. Lett.* 3, 2801-2804.
- (13) Chworos, A., Seguy, C., Pratviel, G., and Meunier, B. (2002) Characterization of the dehydro-guanidinohydantoin oxidation product of guanine in a dinucleotide. *Chem. Res. Toxicol.* 15, 1643-1651.
- (14) Fleming, A. M., Muller, J. G., Ji, I. S., and Burrows, C. J. (2011) Characterization of 2'-deoxyguanosine oxidation products observed in the Fenton-like system Cu(II)/H<sub>2</sub>O<sub>2</sub>/reductant in nucleoside and oligodeoxynucleotide contexts. *Org. Biomol. Chem.* 9, 3338-3348.
- (15) Rokhlenko, Y., Geacintov, N. E., and Shafirovich, V. (2012) Lifetimes and reaction pathways of guanine radical cations and neutral guanine radicals in an oligonucleotide in aqueous solutions. *J. Am. Chem. Soc.* 134, 4955-4962.
- (16) Fleming, A. M., and Burrows, C. J. (2013) G-quadruplex folds of the human telomere sequence alter the site reactivity and reaction pathway of guanine oxidation compared to duplex DNA. *Chem. Res. Toxicol.* 26, 593-607.
- (17) Zhu, J., Fleming, A. M., Orendt, A. M., and Burrows, C. J. (2016) pH-dependent equilibrium between 5-guanidinohydantoin and iminoallantoin affects nucleotide insertion opposite the DNA lesion. *Journal of Organic Chemistry* 81, 351-359.
- (18) Ravanat, J. L., Saint-Pierre, C., and Cadet, J. (2003) One-electron oxidation of the guanine moiety of 2'-deoxyguanosine: Influence of 8-oxo-7,8-dihydro-2'-deoxyguanosine. *J. Am. Chem. Soc.* 125, 2030-2031.
- (19) White, B., Tarun, M. C., Gathergood, N., Rusling, J. F., and Smyth, M. R. (2005) Oxidised guanidinohydantoin (Ghox) and spiroiminodihydantoin (Sp) are major products of iron- and copper-mediated 8-oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine oxidation. *Molecular BioSystems* 1, 373-381.
- (20) Crean, C., Geacintov, N. E., and Shafirovich, V. (2008) Pathways of arachidonic acid peroxy radical reactions and product formation with guanine radicals. *Chem. Res. Toxicol.* 21, 358-373.
- (21) Morin, B., and Cadet, J. (1995) Chemical aspects of the benzophenone-photosensitized formation of two lysine-2'-deoxyguanosine cross-links. *J. Am. Chem. Soc.* 117, 12408-12415.
- (22) Morin, B., and Cadet, J. (1995) Type I benzophenone-mediated nucleophilic reaction of 5'-amino-2',5'-dideoxyguanosine. A model system for the investigation of photosensitized formation of DNA-protein cross-links. *Chem. Res. Toxicol.* 8, 792-799.
- (23) Johansen, M. E., Muller, J. G., Xu, X., and Burrows, C. J. (2005) Oxidatively induced DNA-protein cross-linking between single-stranded binding protein and oligodeoxynucleotides containing 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Biochemistry* 44, 5660-5671.
- (24) Perrier, S., Hau, J., Gasparutto, D., Cadet, J., Favier, A., and Ravanat, J. L. (2006) Characterization of lysine-guanine cross-links upon one-electron oxidation of a guanine-containing oligonucleotide in the presence of a trylisine peptide. *J. Am. Chem. Soc.* 128, 5703-5710.
- (25) Silerme, S., Bobyk, L., Taverna-Porro, M., Cuier, C., Saint-Pierre, C., and Ravanat, J.-L. (2014) DNA-polyamine cross-links generated upon one electron oxidation of DNA. *Chem. Res. Toxicol.* 27, 1011-1018.

- (26) Xu, X., Muller, J. G., Ye, Y., and Burrows, C. J. (2008) DNA-protein cross-links between guanine and lysine depend on the mechanism of oxidation for formation of C5 vs C8 guanosine adducts. *J. Am. Chem. Soc.* **130**, 703-709.
- (27) Nguyen, K. L., Steryo, M., Kurbanyan, K., Nowitzki, K. M., Butterfield, S. M., Ward, S. R., and Stemp, E. D. A. (2000) DNA-protein cross-linking from oxidation of guanine via the flash-quench technique. *J. Am. Chem. Soc.* **122**, 3585-3594.
- (28) Kurbanyan, K., Nguyen, K. L., To, P., Rivas, E. V., Lueras, A. M. K., Kosinski, C., Steryo, M., Gonzalez, A., Mah, D. A., and Stemp, E. D. A. (2003) DNA-protein cross-linking via guanine oxidation: Dependence upon protein and photosensitizer. *Biochemistry* **42**, 10269-10281.
- (29) Sun, G., Fecko, C. J., Nicewonger, R. B., Webb, W. W., and Begley, T. P. (2006) DNA-protein cross-linking: Model systems for pyrimidine-aromatic amino acid cross-linking. *Org. Lett.* **8**, 681-683.
- (30) Uvaydov, Y., Geacintov, N. E., and Shafirovich, V. (2014) Generation of guanine-amino acid cross-links by a free radical combination mechanism. *Phys. Chem. Chem. Phys.* **16**, 11729-11736.
- (31) Solivio, M. J., Joy, T. J., Sallans, L., and Merino, E. J. (2010) Copper generated reactive oxygen leads to formation of lysine-DNA adducts. *Journal of Inorganic Biochemistry* **104**, 1000-1005.
- (32) Yun, B. H., Geacintov, N. E., and Shafirovich, V. (2011) Generation of guanine-thymidine cross-links in DNA by peroxyxynitrite/carbon dioxide. *Chem. Res. Toxicol.* **24**, 1144-1152.
- (33) Crean, C., Uvaydov, Y., Geacintov, N. E., and Shafirovich, V. (2008) Oxidation of single-stranded oligonucleotides by carbonate radical anions: generating intrastrand cross-links between guanine and thymine bases separated by cytosines. *Nucleic Acids Research* **36**, 742-755.
- (34) Crean, C., Geacintov, N. E., and Shafirovich, V. (2008) Intrastrand G-U cross-links generated by the oxidation of guanine in 5'-d(GCU) and 5'-r(GCU). *Free Radical Biol. Med.* **45**, 1125-1134.
- (35) Crean, C., Lee, B. A., Yun, B. H., Geacintov, N. E., and Shafirovich, V. (2008) Oxidation of guanine by carbonate radicals derived from photolysis of carbonatotetrammincobalt(III) complexes and the pH dependence of intrastrand DNA cross-links mediated by guanine radical reactions. *Chembiochem* **9**, 1985-1991.
- (36) Crean, C., Geacintov, N. E., and Shafirovich, V. (2008) Oxidation of DNA by carbonate radical anions in DNA results in the formation of novel intrastrand cross-links. *Chem. Res. Toxicol.* **21**, 2452-2452.
- (37) Ye, W., Sangaiah, R., Degen, D. E., Gold, A., Jayaraj, K., Koshlap, K. M., Boysen, G., Williams, J., Tomer, K. B., and Ball, L. M. (2006) A 2-iminohydantoin from the oxidation of guanine. *Chem. Res. Toxicol.* **19**, 506-510.
- (38) Fleming, A. M., Kannan, A., Muller, J. G., Liao, Y., and Burrows, C. J. (2011) Copper/H<sub>2</sub>O<sub>2</sub>-mediated oxidation of 2'-deoxyguanosine in the presence of 2-naphthol leads to the formation of two distinct isomeric adducts. *J. Org. Chem.* **76**, 7953-7963.
- (39) Shafirovich, V., Mock, S., Kolbanovskiy, A., and Geacintov, N. E. (2002) Photochemically catalyzed generation of site-specific 8-nitroguanine adducts in DNA by the reaction of long-lived neutral guanine radicals with nitrogen dioxide. *Chem. Res. Toxicol.* **15**, 591-597.
- (40) Shukla, P. K., and Mishra, P. C. (2008) Catalytic involvement of CO<sub>2</sub> in the mutagenesis caused by reactions of ONOO<sup>-</sup> with guanine. *J. Phys. Chem. B* **112**, 4779-4789.
- (41) Yermilov, V., Rubio, J., and Ohshima, H. (1995) Formation of 8-nitroguanine in DNA treated with peroxyxynitrite in vitro and its rapid removal from DNA by depurination. *FEBS Lett.* **376**, 207-210.
- (42) Yermilov, V., Yoshie, Y., Rubio, J., and Ohshima, H. (1996) Effects of carbon dioxide/bicarbonate on induction of DNA single-strand breaks and formation of 8-nitroguanine, 8-oxoguanine and base-propenal mediated by peroxyxynitrite. *FEBS Lett.* **399**, 67-70.
- (43) Douki, T., Cadet, J., and Ames, B. N. (1996) An adduct between peroxyxynitrite and 2'-deoxyguanosine: 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2'-deoxyguanosine. *Chem. Res. Toxicol.* **9**, 3-7.

- (44) Joffe, A., Mock, S., Yun, B. H., Kolbanovskiy, A., Geacintov, N. E., and Shafirovich, V. (2003) Oxidative generation of guanine radicals by carbonate radicals and their reactions with nitrogen dioxide to form site specific 5-guanidino-4-nitroimidazole lesions in oligodeoxynucleotides. *Chem. Res. Toxicol.* **16**, 966-973.
- (45) Misiaszek, R., Crean, C., Geacintov, N. E., and Shafirovich, V. (2005) Combination of nitrogen dioxide radicals with 8-oxo-7,8-dihydroguanine and guanine radicals in DNA: Oxidation and nitration end-products. *J. Am. Chem. Soc.* **127**, 2191-2200.
- (46) Liu, N., Ban, F. Q., and Boyd, R. J. (2006) Modeling competitive reaction mechanisms of peroxynitrite oxidation of guanine. *J. Phys. Chem. A* **110**, 9908-9914.
- (47) Niles, J. C., Wishnok, J. S., and Tannenbaum, S. R. (2001) A novel nitroimidazole compound formed during the reaction of peroxynitrite with 2',3',5'-tri-O-acetyl-guanosine. *J. Am. Chem. Soc.* **123**, 12147-12151.
- (48) Kino, K., and Sugiyama, H. (2001) Possible cause of G·C→C·G transversion mutation by guanine oxidation product, imidazolone. *Chemistry & Biology* **8**, 369-378.
- (49) Cadet, J., Berger, M., Buchko, G. W., Joshi, P. C., Raoul, S., and Ravanat, J. L. (1994) 2,2-diamino-4-(3,5-di-o-acetyl-2-deoxy-beta-d-erythropentofuranosyl) amino -5-(2h)-oxazolone - A novel and predominant radical oxidation-product of 3',5'-di-o-acetyl-2'-deoxyguanosine. *J. Am. Chem. Soc.* **116**, 7403-7404.
- (50) Vialas, C., Pratviel, G., Claparols, C., and Meunier, B. (1998) Efficient oxidation of 2'-deoxyguanosine by Mn-TMPyP/KHSO<sub>5</sub> to imidazolone dlz without formation of 8-oxo-dG. *J. Am. Chem. Soc.* **120**, 11548-11553.
- (51) Vialas, C., Claparols, C., Pratviel, G., and Meunier, B. (2000) Guanine oxidation in double-stranded DNA by Mn-TMPyP/KHSO<sub>5</sub>: 5,8-dihydroxy-7,8-dihydroguanine residue as a key precursor of imidazolone and parabanic acid derivatives. *J. Am. Chem. Soc.* **122**, 2157-2167.
- (52) Luo, W. C., Muller, J. G., Rachlin, E. M., and Burrows, C. J. (2001) Characterization of hydantoin products from one-electron oxidation of 8-oxo-7,8-dihydroguanosine in a nucleoside model. *Chem. Res. Toxicol.* **14**, 927-938.
- (53) Morikawa, M., Kino, K., Oyoshi, T., Suzuki, M., Kobayashi, T., and Miyazawa, H. (2013) Product analysis of photooxidation in isolated quadruplex DNA; 8-oxo-7, 8-dihydroguanine and its oxidation product at 3'-G are formed instead of 2, 5-diamino-4 H-imidazol-4-one. *RSC Advances* **3**, 25694-25697.
- (54) Duarte, V., Gasparutto, D., Yamaguchi, L. F., Ravanat, J. L., Martinez, G. R., Medeiros, M. H. G., Di Mascio, P., and Cadet, J. (2000) Oxaluric acid as the major product of singlet oxygen-mediated oxidation of 8-oxo-7,8-dihydroguanine in DNA. *J. Am. Chem. Soc.* **122**, 12622-12628.
- (55) Seguy, C., Pratviel, G., and Meunier, B. (2001) Characterization of an oxaluric acid derivative as a guanine oxidation product. *Chemical Communications*, 2116-2117.
- (56) Misiaszek, R., Uvaydov, Y., Crean, C., Geacintov, N. E., and Shafirovich, V. (2005) Combination reactions of superoxide with 8-oxo-7,8-dihydroguanine radicals in DNA - Kinetics and end products. *J. Biol. Chem.* **280**, 6293-6300.
- (57) Niles, J. C., Wishnok, J. S., and Tannenbaum, S. R. (2006) Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: structures and mechanisms of product formation. *Nitric Oxide* **14**, 109-121.
- (58) Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Petersson, G. A., Nakatsuji, H., Li, X., Caricato, M., Marenich, A. V., Bloino, J., Janesko, B. G., Gomperts, R., Mennucci, B., Hratchian, H. P., Ortiz, J. V., Izmaylov, A. F., Sonnenberg, J. L., Williams, Ding, F., Lipparini, F., Egidi, F., Goings, J., Peng, B., Petrone, A., Henderson, T., Ranasinghe, D., Zakrzewski, V. G., Gao, J., Rega, N., Zheng, G., Liang, W., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai,

- H., Vreven, T., Throssell, K., Montgomery Jr., J. A., Peralta, J. E., Ogliaro, F., Bearpark, M. J., Heyd, J. J., Brothers, E. N., Kudin, K. N., Staroverov, V. N., Keith, T. A., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A. P., Burant, J. C., Iyengar, S. S., Tomasi, J., Cossi, M., Millam, J. M., Klene, M., Adamo, C., Cammi, R., Ochterski, J. W., Martin, R. L., Morokuma, K., Farkas, O., Foresman, J. B., and Fox, D. J. (2019) Gaussian 16 Rev. C.01, Wallingford, CT.
- (59) Chai, J.-D., and Head-Gordon, M. (2008) Long-range corrected hybrid density functionals with damped atom–atom dispersion corrections. *Phys. Chem. Chem. Phys.* **10**, 6615-6620.
- (60) Hehre, W. J., Ditchfield, R., and Pople, J. A. (1972) Self-consistent molecular orbital methods. XII. Further extensions of Gaussian-type basis sets for use in molecular orbital studies of organic molecules. *J. Chem. Phys.* **56**, 2257-2261.
- (61) Ditchfield, R., Hehre, W. J., and Pople, J. A. (1971) Self-consistent molecular-orbital methods. IX. An extended Gaussian-type basis for molecular-orbital studies of organic molecules. *J. Chem. Phys.* **54**, 724-728.
- (62) Clark, T., Chandrasekhar, J., Spitznagel, G. W., and Schleyer, P. V. R. (1983) Efficient diffuse function-augmented basis sets for anion calculations. III. The 3-21+G basis set for first-row elements, Li-F. *J. Comput. Chem.* **4**, 294-301.
- (63) Francl, M. M., Pietro, W. J., Hehre, W. J., Binkley, J. S., Gordon, M. S., DeFrees, D. J., and Pople, J. A. (1982) Self-consistent molecular orbital methods. XXIII. A polarization-type basis set for second-row elements. *J. Chem. Phys.* **77**, 3654-3665.
- (64) Marenich, A. V., Cramer, C. J., and Truhlar, D. G. (2009) Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B* **113**, 6378-6396.
- (65) Hebert, S. P., and Schlegel, H. B. (2019) Computational study of the pH-dependent competition between carbonate and thymine addition to the guanine radical. *Chem. Res. Toxicol.* **32**, 195-210.
- (66) Hebert, S. P., and Schlegel, H. B. (2019) Computational study of the oxidation of guanine to form 5-carboxyamido-5-formamido-2-iminohydantoin (2lh). *Chem. Res. Toxicol.*
- (67) Camaioni, D. M., and Schwerdtfeger, C. A. (2005) Comment on “Accurate experimental values for the free energies of hydration of  $H^+$ ,  $OH^-$ , and  $H_3O^{+}$ ”. *J. Phys. Chem. A* **109**, 10795-10797.
- (68) Kelly, C. P., Cramer, C. J., and Truhlar, D. G. (2006) Aqueous solvation free energies of ions and ion–water clusters based on an accurate value for the absolute aqueous solvation free energy of the proton. *J. Phys. Chem. B* **110**, 16066-16081.
- (69) Isse, A. A., and Gennaro, A. (2010) Absolute potential of the standard hydrogen electrode and the problem of interconversion of potentials in different solvents. *J. Phys. Chem. B* **114**, 7894-7899.
- (70) Marenich, A. V., Ho, J., Coote, M. L., Cramer, C. J., and Truhlar, D. G. (2014) Computational electrochemistry: prediction of liquid-phase reduction potentials. *Phys. Chem. Chem. Phys.* **16**, 15068-15106.
- (71) Ben-Naim, A., and Marcus, Y. (1984) Solvation thermodynamics of nonionic solutes. *J. Chem. Phys.* **81**, 2016-2027.
- (72) Kirby, A. H. M., and Neuberger, A. (1938) Glyoxalines: the determination of their pK values and the use of their salts as buffers. *Biochemical Journal* **32**, 1146-1151.
- (73) Misiaszek, R., Crean, C., Joffe, A., Geacintov, N. E., and Shafirovich, V. (2004) Oxidative DNA damage derived from a combination of superoxide with guanine and 8-oxo-7,8-dihydroguanine radicals. *Chem. Res. Toxicol.* **17**, 1765-1765.