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

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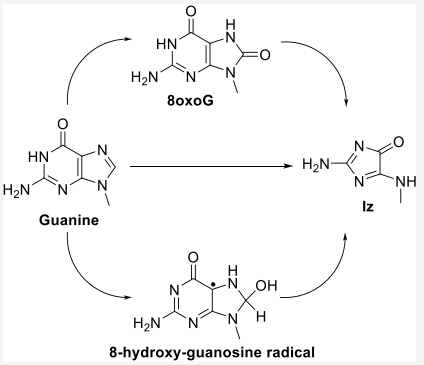
ABSTRACT: Imidazolone (Iz) is one of the many products resulting from oxidative damage to DNA. Three pathways for the formation of Iz and related degradation products have been studied by density functional theory using the ωB97XD functional with the 6-31+G(d,p) basis set and SMD implicit water solvation 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 nucleosides.[1] As such, experimental studies of oxidative damage to DNA often use guanine as a model.[2−4] 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**),[5−8] 2,6-diamino-4-hydroxy-5-formamidopyrimidine-guanine (**Fapy-dG**),[9,10] guanidinohydantoin-2′-deoxyribonucleoside (**Gh**),[8,11−17] spiroiminodihydantoin-2′-deoxyribonucleoside (**Sp**),[7,8,11−16,18−20] guanine-lysine cross-link (**G^Lys**),[21−31] guanine-thymine cross-link (**G^T**),[15,32−36] 5-carboxamido-5-formamido-2-iminohydantion-2′-deoxyribonucleoside (**2Ih**),[8,14−16,37,38] 8-nitroguanine (**8nitroG**),[39−46] and 5-guanidino-nitroimidazole (**NIm**).[44−47] 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.[6,8,12−16,18,20,48−53] Depending on conditions, additional oxidation products can form in competition with **Iz**. Oxidized **Gh** ($\mathbf{Gh_{ox}}$),[12,13,19,20,51−57] oxidized iminoallantoin ($\mathbf{Ia_{ox}}$),[52] parabanic acid (**Pa**),[51,52,57] and oxaluric acid (**Oa**)[13,19,52,54−56] 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**.[12,57] Addition of superoxide dismutase reduced $\mathbf{Iz}/\mathbf{Gh_{ox}}/\mathbf{Ia_{ox}}$ formation by about half when starting from **8oxoG** as a reactant, compared to only small changes starting from oxidation of guanine.[12,53,56] Benzoquinone was found to have a similar effect.[20] Additionally, the presence or absence of reducing agents were considered to more appropriately model differing cellular conditions.[8] These studies showed that reducing agents resulted in more **2Ih** formation over **Iz** while **Sp**, **Gh**, and **8oxoG** formation were largely unaffected. Isotopic labeling studies found incorporation of oxygen from solvent or molecular oxygen depending on conditions.[13,18,20,49−51,54] Along with **Iz**, many relevant experimental studies also found $\mathbf{Gh_{ox}}$, $\mathbf{Ia_{ox}}$, **Pa**, and **Oa**.[2,12,13,19,20,51−56]

Cadet and co-workers found **Iz**/**Z** formation as the dominant product in both hydroxyl radical and riboflavin

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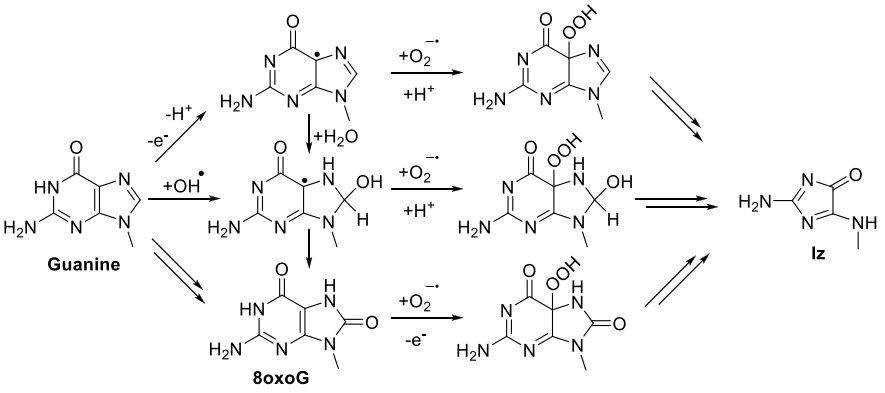
Scheme 1. Initial Steps in Three Pathways for the Formation of Iz Considered in the Present Study



type I photooxidation of guanine.[49] Burrows and co-workers investigated riboflavin photooxidation of guanine and **8oxoG** and found **Iz** as a significant product.[12] 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 co-workers investigated the mechanism for **Iz** formation via riboflavin photooxidation of guanine and ^{15}N-labeled **8oxoG** and found the conversion of guanine to **8oxoG** to oxidative products (predominantly **Iz**) as the likely pathway.[18] Shafirovich and co-workers 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.[6] Similar to the work by the Burrows group, Tannenbaum and co-workers found a pH dependence for **Iz** formation in oxidation of guanine and **8oxoG** induced by peroxynitrite.[57] In their work, Fleming and Burrows found riboflavin photooxidation of a G-quadruplex system produced **Iz** and **Sp** in yields of about 45% each.[16] 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.[16] In a recent work, Burrows and co-workers obtained significant yields of **Iz** in oxidation of guanine by iron Fenton chemistry and by X-ray radiolysis of water.[8] This work also helped to establish the relationship between the **Iz** and **2Ih** oxidative products. The introduction of reducing agents resulted in increasing **2Ih** 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 co-workers found **Iz** formation from carbonate radical oxidation; however, this seems to be a unique case[15] 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 co-workers have explored guanine and **8oxoG** oxidation using a two-electron oxidant (Mn-TMPyP) in conjunction with a persulfate salt ($KHSO_5$), which mimics the G-OOR intermediate found from superoxide radical combination.[13,50,51,55] Their early results showed rapid formation of **Iz** from guanine with no **8oxoG** intermediate.[50] They also reported the formation of two sets of byproducts, formamide and a combination of CO_2 and ammonia. The oxidation state at the C8 position is believed to control the byproducts formed. Separate $H_2{}^{18}O$ and $^{18}O_2$ labeling experiments found water incorporation into the formamide byproduct but no labeled 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 $\mathbf{Gh_{ox}}$ product and subsequent hydrolysis to oxaluric acid.[13,51,55] Guanine oxidation in the presence of lipid peroxy radicals also produced **Iz** and $\mathbf{Gh_{ox}}$.[20] Similar to other reports, oxidation of guanine was found to produce **Iz**, while oxidation of **8oxoG** primarily produced **Sp** and $\mathbf{Gh_{ox}}$. Oxidation of guanine and **8oxoG** with riboflavin also found $\mathbf{Gh_{ox}}$ and **Oa**, with formation of $\mathbf{Ia_{ox}}$ and **Pa** being relevant to these mechanistic pathways.[12]

The current work investigates various pathways for **Iz** formation along with possible side products such as $\mathbf{Gh_{ox}}$, $\mathbf{Ia_{ox}}$, **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 that 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.

2. METHODS

2.1. Electronic Structure Calculations. All calculations were performed using the development version of the Gaussian series of programs[58] and the ωB97XD density functional[59] with the 6-31+G(d,p) basis set.[60−63] SMD implicit water solvation[64] was used to model aqueous conditions. Explicit waters were included as a supplement to the implicit model, as in previous studies.[65,66] Guanine was capped with a methyl group in place of the N9-bound sugar moiety.

2.2. pK_a Calculations. The pK_a's for reactants and intermediates are calculated from the free energy differences for deprotonation in solution

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

where R is the gas constant (1.987 cal K^{-1} mol^{-1}), T is the temperature (298.15 K), and the free energy of the proton in water is −270.3 kcal/mol,[67−70] defined as

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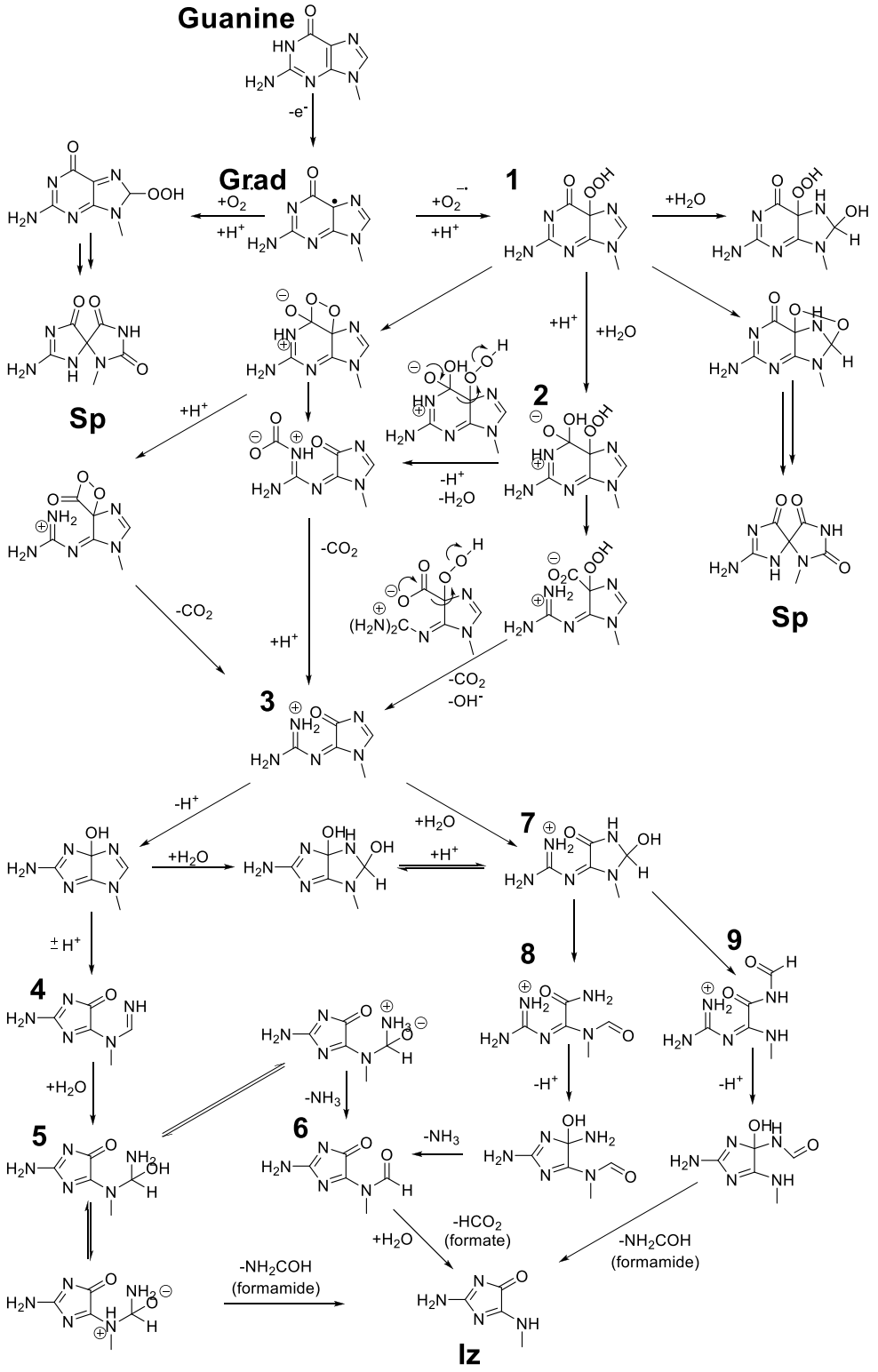
Scheme 2. Overview of Iz Formation via Guanine Radical



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

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 $G^{1\text{atm}\rightarrow 1\text{M}} = 1.89$ kcal/mol,[71] and energy of solvation of a proton is $\Delta G_{H^+_{(solv.)}} = -265.9$ kcal/mol.[67]

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_a 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_a = 7.7 calcd, 6.95 exptl)[72] was used to model pH 7 conditions.

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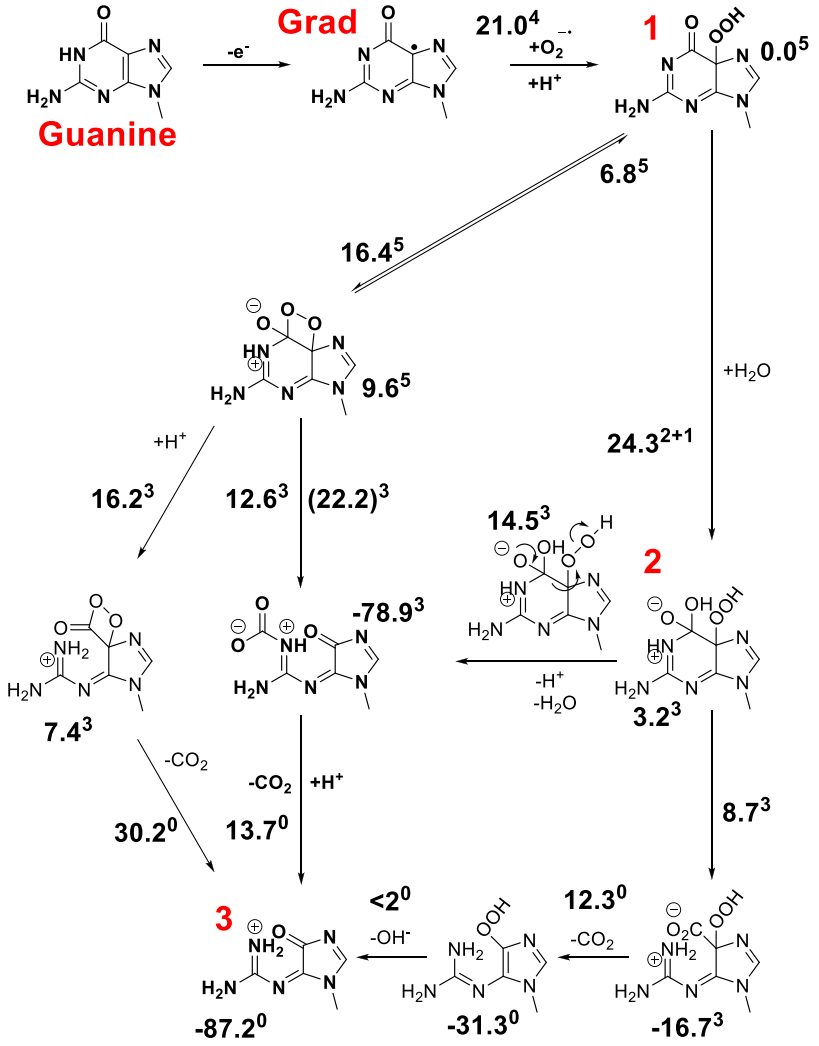
Scheme 3. Comparison of Endoperoxide and Diol Formation Steps in the Early Stages of Iz Formation from Guanine[a]



[a]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.

3. RESULTS

3.1. Overview of Reaction Pathways. **Iz** formation may proceed through a number of differing pathways involving peroxyl 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.[6,8,12,14,16,18,20,53,56] C5- and C8- peroxyl adducts have also been investigated in the oxidation of guanine by lipid peroxyl radicals and by Mn-TMPyP/$KHSO_5$.[20,50,51] The resulting hydroperoxyl 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 it showed very different reactivity compared to that of guanine.[12,18,20,52,56,57] 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 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, 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

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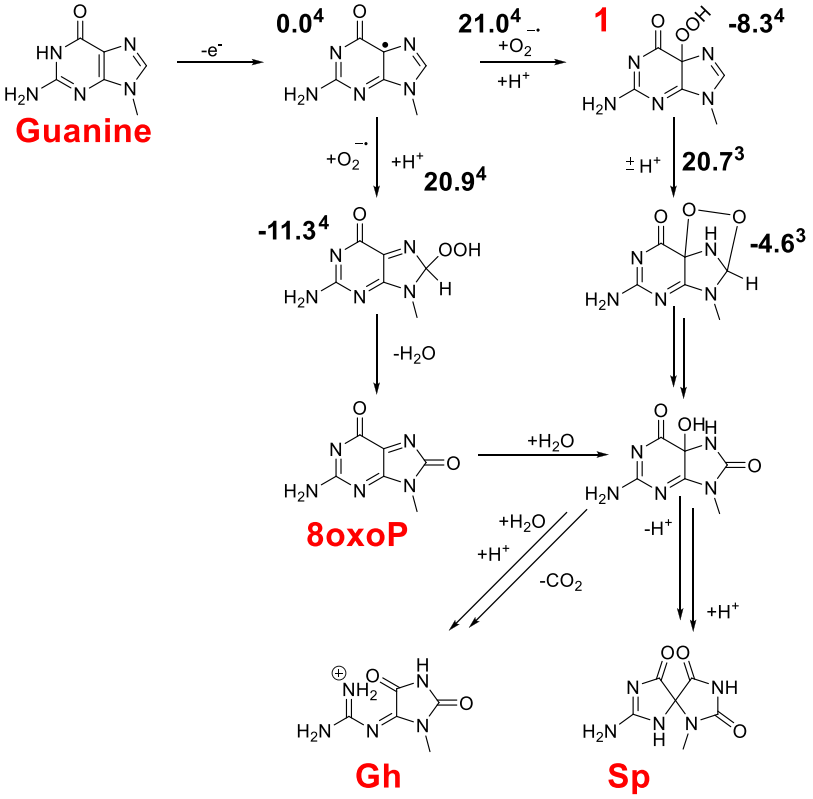
Scheme 4. Sp and Gh Formation via Superoxide Addition to C5 and C8 of Guanine Radical



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.[50] 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 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 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 five 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.

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.[2,6,8,12−16,18−20,52,57] 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 that 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

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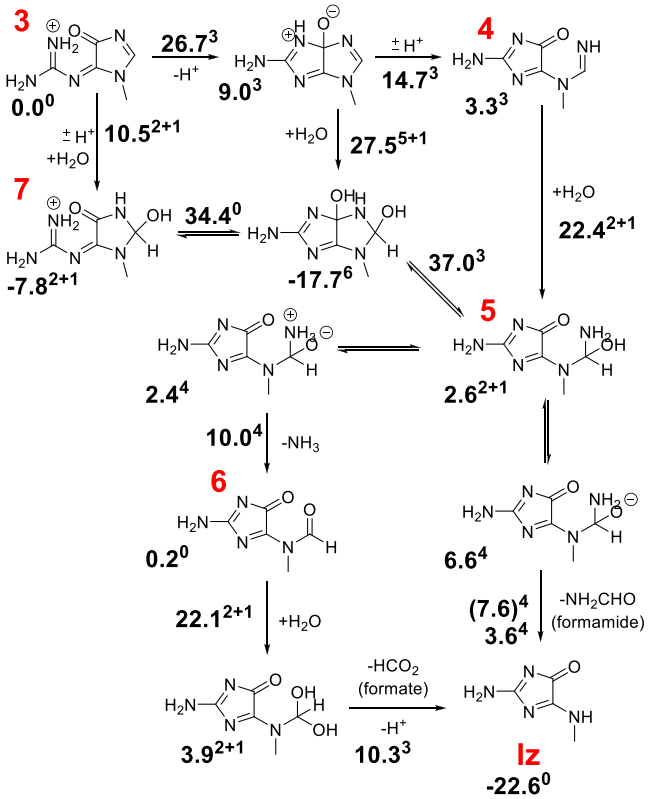
agents resulted in varying yields of **Iz** and **2Ih** but the yields of **Sp**, **Gh**, and **8oxoG** remained mostly unaffected.[8]

Burrows and co-workers studied interconversion of **Gh** and **Ia**, in which the ring migration is believed to occur via a bicyclic intermediate.[17] Conversion of **3** to **4** or **5** was considered via a similar ring migration mechanism. Deprotonation of the guanidium 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.[50] In agreement with experiment, the calculations found a low energy pathway involving deprotonation of **5** and loss of formamide to produce **Iz**.

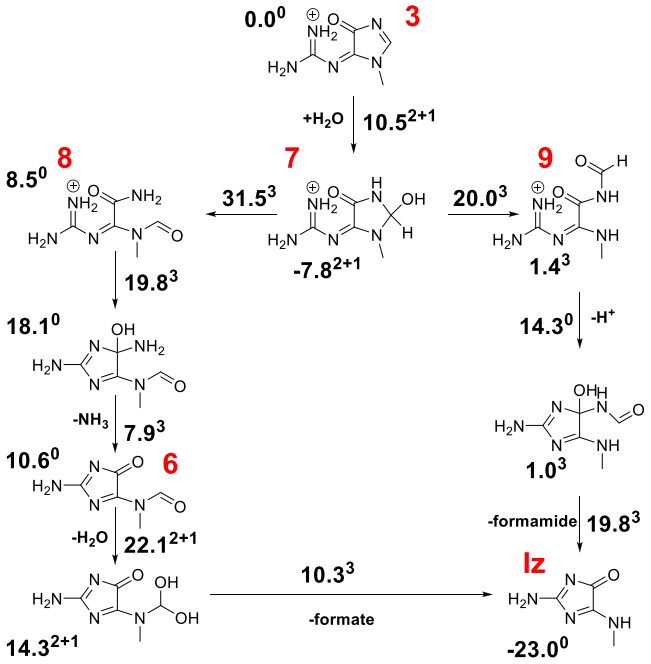
Scheme 6 shows the conversion of **3** to **Iz** through pathways involving acyclic intermediates. Water addition to the C8 position of **3** was calculated to have a barrier of 10.5 kcal/mol

Scheme 5. Formation of Iz from 3 via Bicyclic Paths[a]



[a]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.

Scheme 6. Formation of Iz from 3 via Acyclic Paths[a]



[a]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.

and was exothermic, producing intermediate **7**. N7−C8 and C8−N9 ring openings of **7** found barriers of 31.5 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,[50] 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 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 π bond should be absent in the **8oxoG** pathway, potentially resulting in higher barriers. The $\mathbf{Gh}_{ox}$ intermediate has been observed in a number of experimental studies of **8oxoG** oxidation[2,12,13,19,20,51,52,55,56] 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**.

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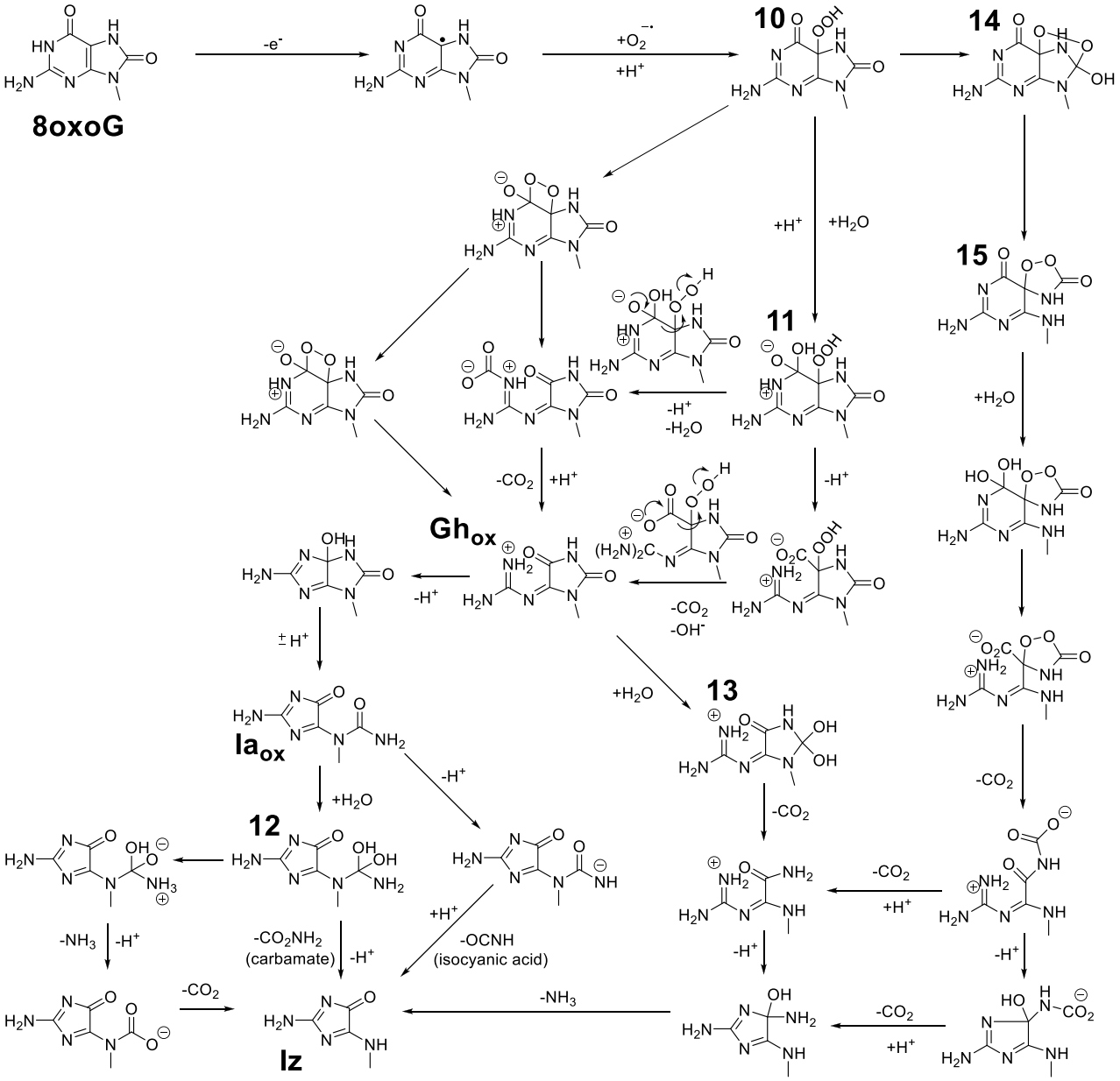
Scheme 7. Overview of Iz Formation from 8oxoG



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 those of 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_2 and OH^- to form $\mathbf{Gh_{ox}}$.

Scheme 9 shows formation of **Iz** from $\mathbf{Gh_{ox}}$ 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 $\mathbf{Gh_{ox}}$ to $\mathbf{Ia_{ox}}$ has an initial barrier of 27.4 kcal/mol, followed by a ring opening barrier of 31.5 kcal/mol to the $\mathbf{Ia_{ox}}$ intermediate. Hydrolysis of $\mathbf{Ia_{ox}}$ 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 $\mathbf{Ia_{ox}}$ 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 shows the hydrolysis pathways from $\mathbf{Gh_{ox}}$ 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 guanidium loss with a low barrier of 8.6 kcal/mol to the experimentally detected parabanic acid product. Parabanic acid

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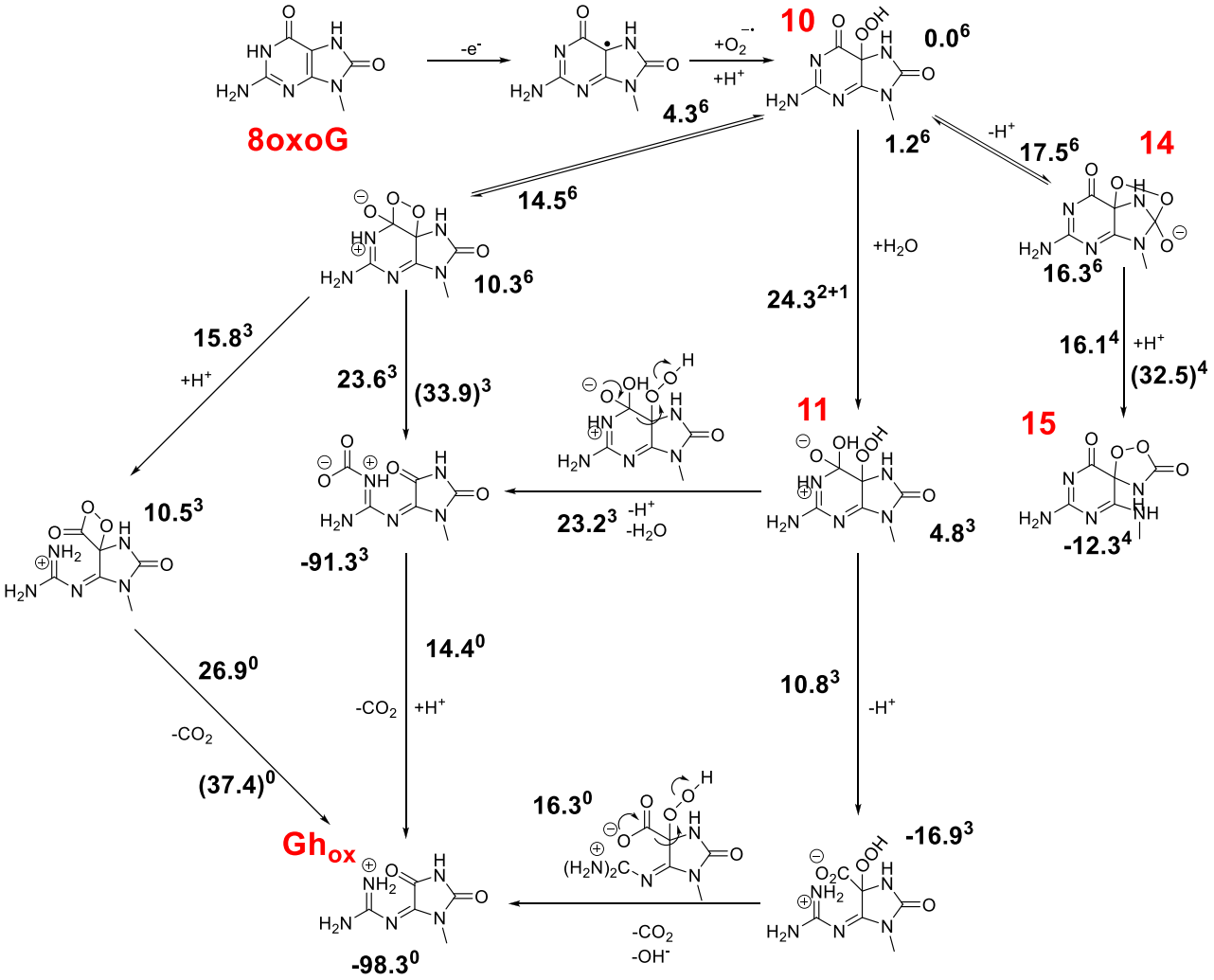
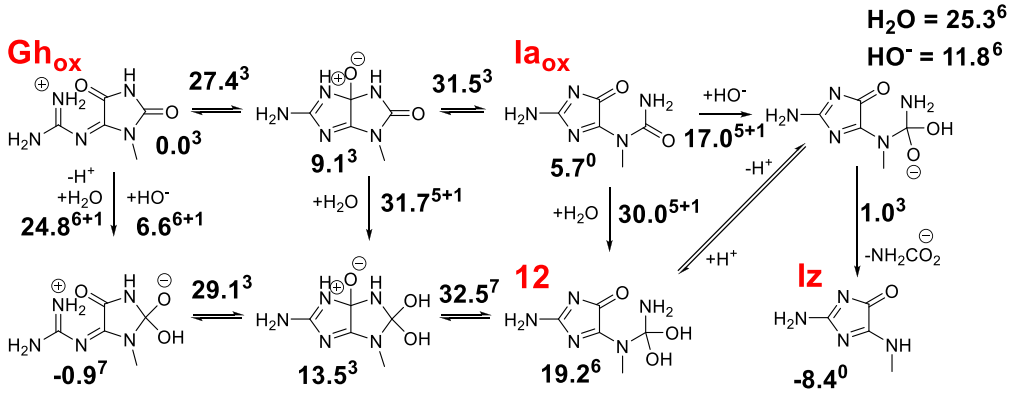
Scheme 8. Comparison of Endoperoxide and Diol Formation Steps along Early Stages of Iz Formation from 8oxoG[a]



[a]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 9. Formation of Iz from Gh_{ox} via Bicyclic Paths[a]



[a]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.

may undergo further hydrolysis to oxaluric acid (discussed in Section 3.5). The barriers for hydroxide addition to Gh_{ox} are 15−18 kcal/mol lower than those 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 that for N7−C8 ring opening.

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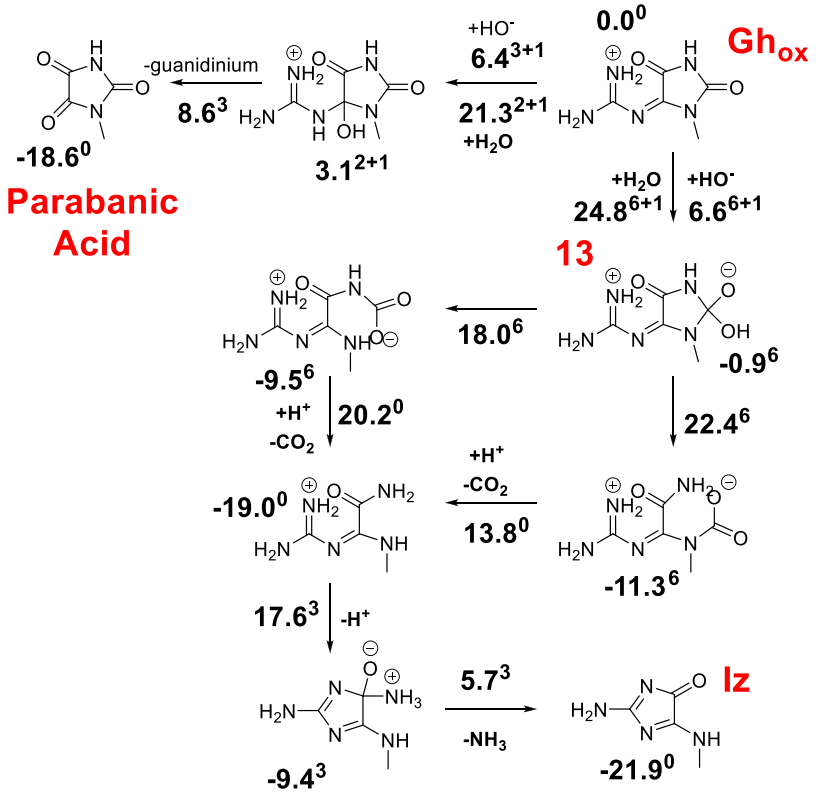
Scheme 10. Formation of Iz from Gh_{ox} via Acyclic Paths[a]



[a]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.

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_{ox} below basic pH.

Gh_{ox} conversion to **Iz** has not been found experimentally, except for some evidence at high pH.[12,57] The barriers for OH^- addition are much lower than those for water addition, 6.4 vs 21.3 kcal/mol at C4 and 6.6 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_{ox} and its hydrolysis products after **8oxoG** oxidation.

3.4. 8-Hydroxy Guanine Radical Oxidation to Iz. 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 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 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, respectively). 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. Numerous studies have found parabanic and oxaluric acid as hydrolysis products, in addition to Gh_{ox} and **Iz**. Water addition to Gh_{ox} and Ia_{ox} has been proposed as the path to these products. Scheme 14 shows oxaluric acid formation from Ia_{ox}. 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

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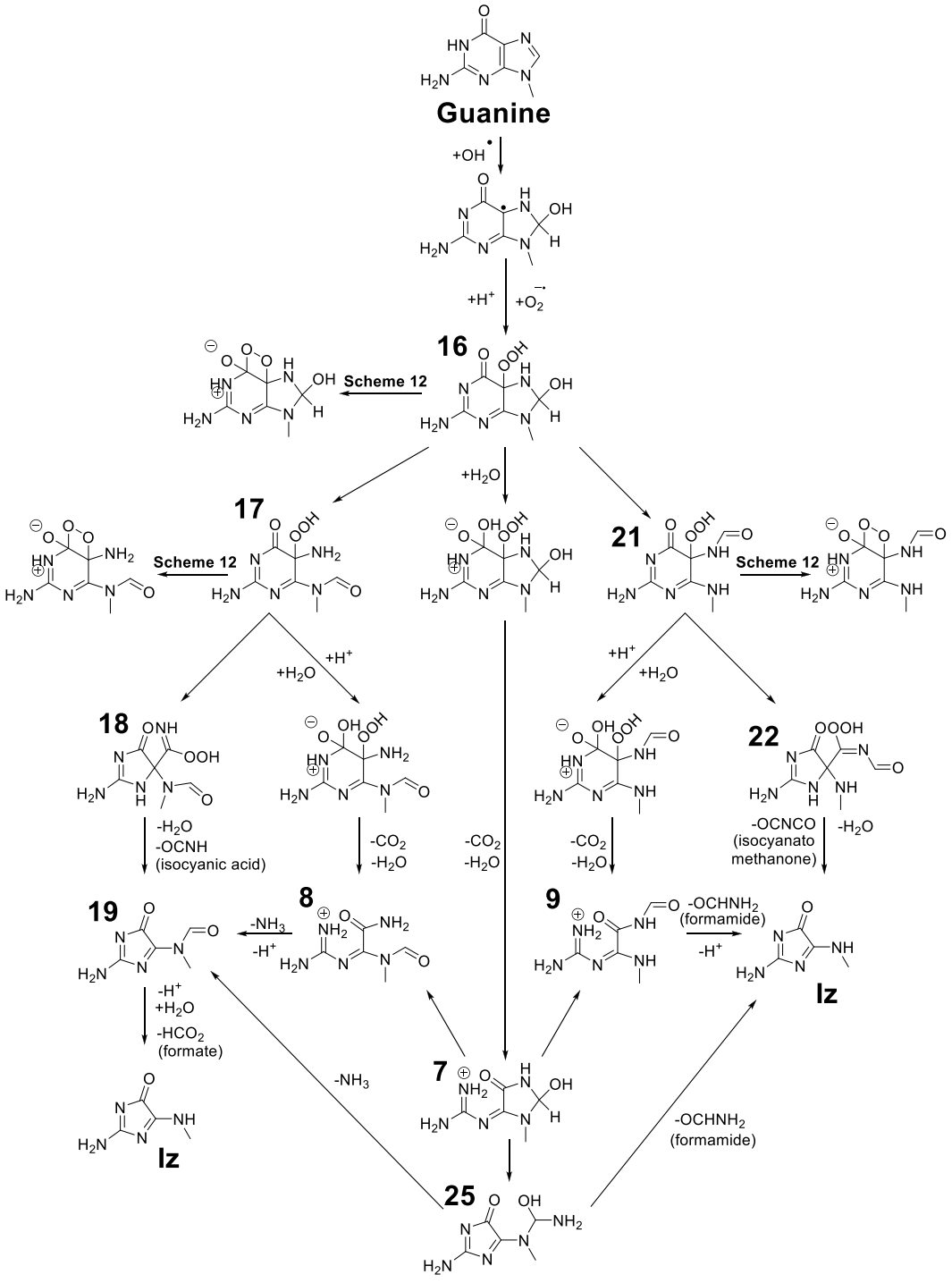
Scheme 11. Overview of Iz Formation from 8-Hydroxy Guanine Radical



to $\mathbf{Ia_{ox}}$. 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 $\mathbf{Ia_{ox}}$.[51,52] This, combined with high barriers of conversion between $\mathbf{Gh_{ox}}$ and $\mathbf{Ia_{ox}}$ (Scheme 9), suggests that $\mathbf{Ia_{ox}}$ formation and its hydrolysis may not occur readily.

Scheme 15 shows the pathway for $\mathbf{Gh_{ox}}$ hydrolysis to parabanic and oxaluric acid. The pathway for $\mathbf{Gh_{ox}}$ hydrolysis proceeds with a barrier similar to $\mathbf{Ia_{ox}}$ hydrolysis (21.3 and 22.3 kcal/mol, respectively) and produces a similarly exothermic intermediate. For $\mathbf{Gh_{ox}}$, 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.[13]

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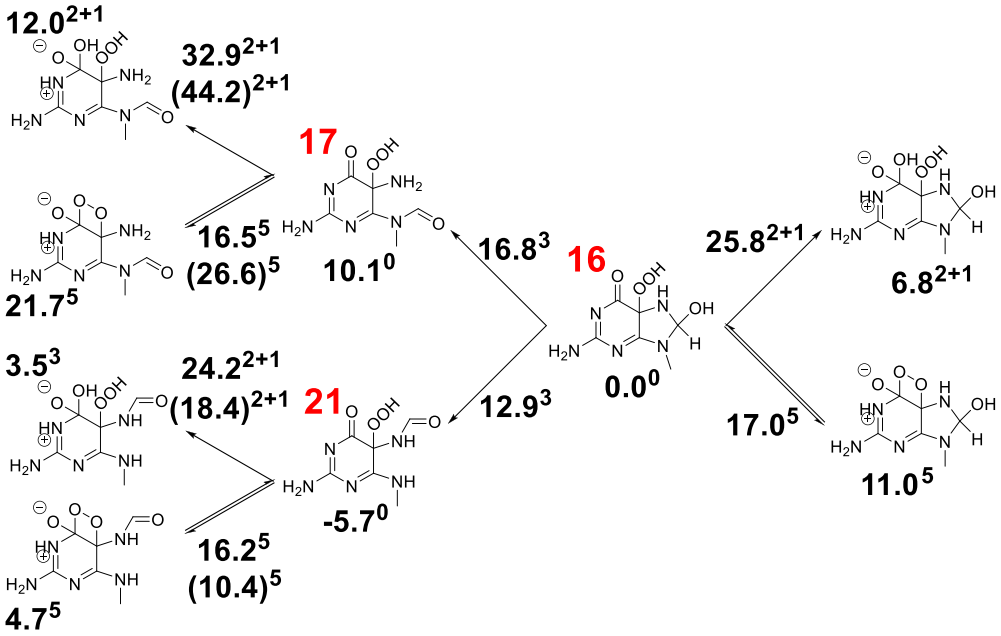
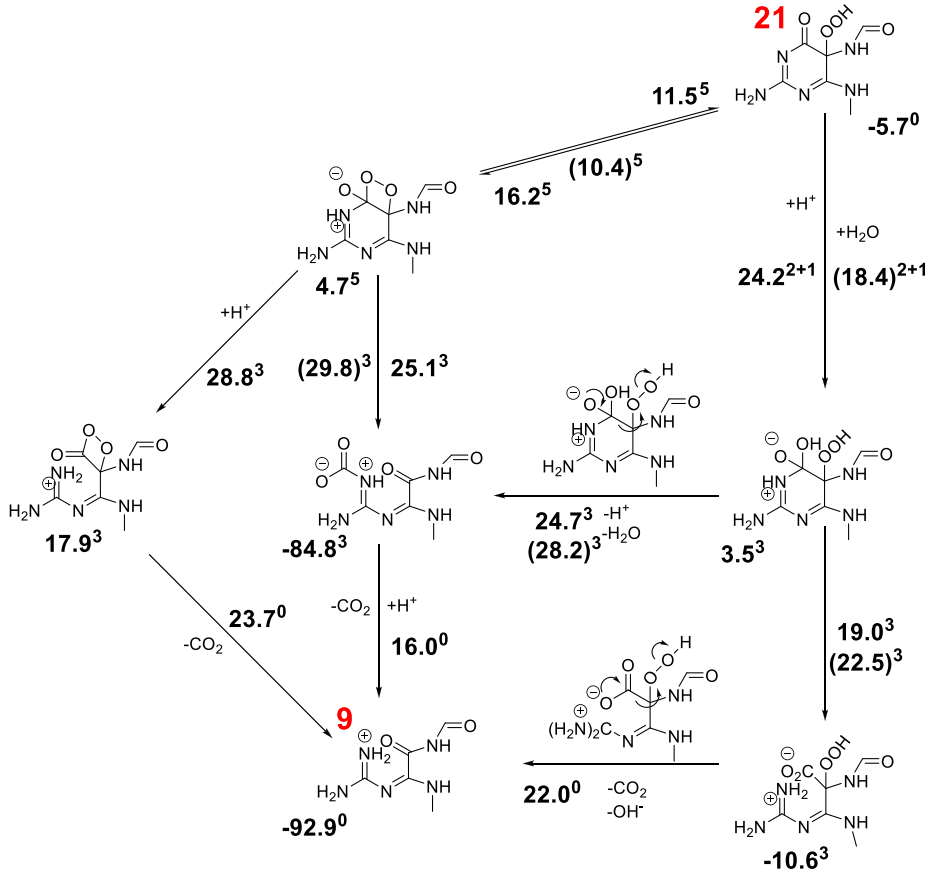
Scheme 12. Comparison of Diol Formation, Endoperoxide Formation, and N7−C8 and C8−N9 Opening Pathways from 16[a]



[a]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. Comparison of Endoperoxide and Diol Formation Steps along Early Stages of Iz Formation from 21[a]



[a]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.

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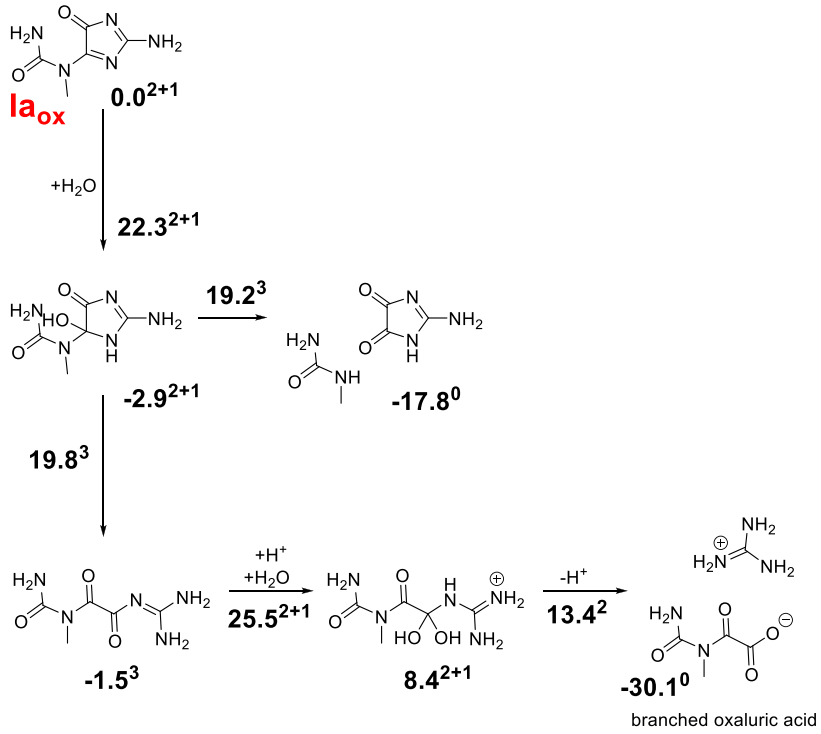
Scheme 14. Formation of Pa and Oa from Ia_{ox}[a]



[a]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.

4. SUMMARY

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 those of 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 π 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 (Schemes 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, $\mathbf{Gh}_{ox}$. Water addition to C8 of $\mathbf{Gh}_{ox}$ was found to be higher energy than for **3** in Pathway 1, which may explain why $\mathbf{Gh}_{ox}$ has been observed[12,13,19,20,51−57] but **3** has not. While water addition to $\mathbf{Gh}_{ox}$ can produce **Iz**, parabanic acid formation is more favorable (Scheme 10), in agreement with experimental work.[20,54,56] The barriers for hydroxide ion addition to $\mathbf{Gh}_{ox}$ 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 (Schemes 12 and 13). This leads to an acyclic intermediate in common with Pathway 1 and results in **Iz** formation.

Burrows and co-workers reported riboflavin photooxidation of guanine favored a combination of **Iz** and **Sp**.[12] This can easily be understood by considering the nearly identical barriers for C5 and C8 superoxide radical addition to guanine radical (Schemes 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 co-workers also found that **Sp**/**Gh** formation did not contain isotopically labeled oxygen from solvent, which further suggests a mechanism bypassing **8oxoG** formation. Our calculations find that oxidation of guanine should not produce $\mathbf{Gh}_{ox}/\mathbf{Ia}_{ox}$; 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 $\mathbf{Gh}_{ox}$. In considering **8oxoG** conversion to **Iz**, $\mathbf{Gh}_{ox}/\mathbf{Ia}_{ox}$ is the dominant species below pH 8.6 in the experimental study. Our current calculations show $\mathbf{Gh}_{ox}$ as the most likely intermediate (Schemes 8 and 10), with hydrolysis allowing conversion to parabanic and oxaluric acids (Schemes 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

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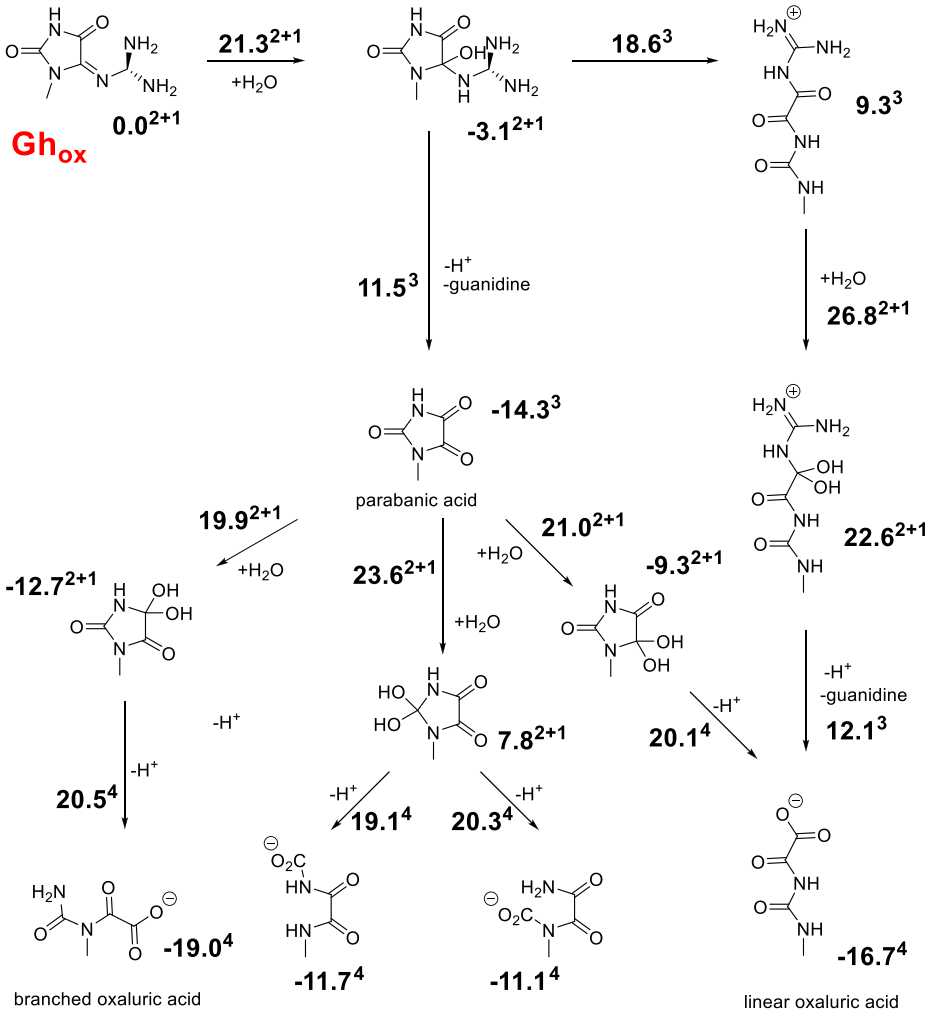
Scheme 15. Formation of Pa and Oa from Gh_{ox}[a]



[a]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.

significant yields at basic pH, which is in accord with the lower calculated barrier for hydroxide addition compared to that of water addition in conversion of Gh_{ox} to **Iz**. Experimentally, parabanic acid was not found, but oxaluric acid was; calculations show that oxaluric acid can be formed from either Ia_{ox} or Gh_{ox} (Schemes 14 and 15).

Cadet and co-workers compared riboflavin-mediated photooxidation of guanine and **8oxoG**.[18] A mixture of $^{15}N_5$-labeled **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** (Schemes 3 and 5). The fact that Cadet found low yields of **Sp** compared to those of **Iz** and did not report any Gh_{ox} or Ia_{ox} is also compatible with Pathway 1.

Shafirovich and co-workers[6,56] 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 that reacted to form **Iz**, **Z**, **Oa**, **Sp**, **8oxoG**, and Gh_{ox}. 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 co-workers. Oxidation of guanine-containing oligonucleotides produced mainly **Iz**,(20%) with some **8oxoG** (1%). Oxidation of **8oxoG** containing oligonucleotides produced a combination of Gh_{ox} and **Oa** (21.5%), as well as some **Sp** (2%). Labeling studies with $H_2{}^{18}O$ showed radical combination with superoxide was favored over hydration. **8oxoG** oxidation yielded primarily Gh_{ox}, which can

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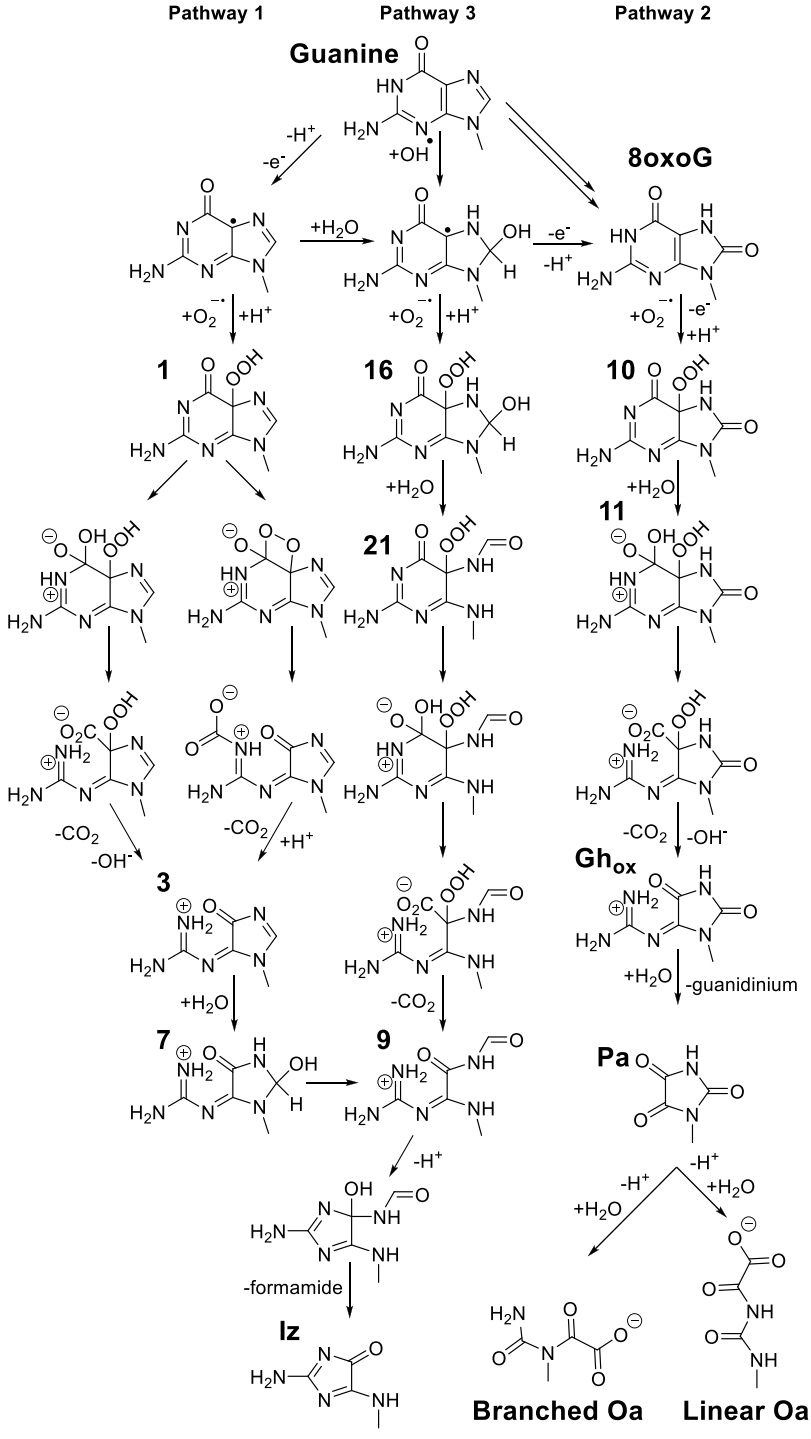
Scheme 16. Summary of Lowest Energy Oxidation Pathways for the Formation of Iz from Guanine Radical, 8oxoG, and 8-Hydroxy Guanine Radical



hydrolyze to **Oa**. These experimental results are in agreement both with riboflavin induced oxidation[12] and the present calculations.

Meunier and co-workers[13,50,51] examined guanine oxidation by Mn-TMPyP/$KHSO_5$ 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 that 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/$KHSO_5$ 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 photo-oxidation 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$_{ox}$**, which in turn produced **Iz** with ammonia and carbon dioxide as

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byproducts. Our calculations find formamide loss from guanine oxidation to **Iz** and ammonia/carbon dioxide loss from **8oxoG** oxidation (Schemes 6 and 10) in agreement with the experimentally observed byproducts.

Shafirovich and co-workers[20] investigated guanine oxidation by peroxyl 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_{ox}**, **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_{ox}**. 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 peroxyl 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), which 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 peroxyl 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 co-workers[19] investigated iron- and copper-mediated Fenton oxidation of **8oxoG** nucleobase and nucleoside. Oxidation of the **8oxoG**-free base results in predominantly **Gh_{ox}** and hydrolysis to oxaluric acid. Oxidation of the **8oxoG** nucleoside produced exclusively **Sp**. Burrows and co-workers[8,14] 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[66] are in agreement with these experimental results. C5 hydroperoxyl formation explains the production of **2Ih**/**Iz** depending on presence or absence of reducing agents (Schemes 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_{ox}** 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_{ox}** 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 at https://pubs.acs.org/doi/10.1021/acs.chemrestox.0c00039.

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

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Notes

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■ ABBREVIATIONS

2Ih	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_{ox}	oxidized guanidinohydantoin
Ia_{ox}	oxidized iminoallantoin
Iz	imidazolone
Z	oxazolone
G^Lys	guanine-lysine cross-link
8nitroG	8-nitroguanine
NIm	5-guanidino-nitroimidazole
Pa	parabanic acid
Oa	oxaluric acid
SOD	superoxide dismutase

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