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Isolation, Characterization, and Independent Synthesis of Guanine Oxidation Products

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Keywords: Bioorganic chemistry / DNA damage / Guanine / Nucleobases / Oxidation

Oxidatively produced DNA damage is a physiological process that has been associated with many human conditions. Of the four DNA bases, guanine is the most easily oxidized and exhibits a large number of oxidation products. The focus of this Microreview is on the structure of the proposed intermediates and final products of guanine oxidation, as determined by spectroscopic methods and independent synthetic studies. The major and minor primary and secondary oxidative DNA lesions that arise from the oxidation of guanine under a variety of conditions, as well as associated structures, are described and all the relevant information regarding the isolation, characterization, and independent synthesis or generation of these lesions in synthetic oligonucleotides is presented. Finally, information on the biological studies that have been advanced, based on the basic molecular information obtained by the aforementioned studies, is provided. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2006)

1. Introduction

The field of oxidatively produced DNA damage[1] is multidisciplinary, extending its spectrum beyond chemistry to biology and medicine. Investigations in this field have addressed the following issues: (a) determination of the underlying mechanisms of DNA cleavage through the isolation and characterization of various lesions, such as oxidized bases and sugars, DNA adducts, strand breaks, and DNA-protein cross-links; (b) detection and quantification of the observed lesions in cellular DNA and estimation of the endogenous and exogenous cellular factors that lead to damage; (c) utilization of modified oligonucleotides carrying lesions as probes for the determination of the mutagenic potential of each lesion through their interaction with repair enzymes and polymerases; and finally (d) elucidation of the biological consequences of DNA damage in the cell cycle.[2] Extensive research over the last ten years on the molecular consequences of oxidatively produced DNA damage has revealed a wealth of chemistry involving the sugar[3] and base[4] components of nucleosides, nucleotides, synthetic oligonucleotides, and natural DNA. In particular, base modification with a variety of oxidizing agents has led to the isolation and characterization of a large number of stable and unstable lesions. Of the four bases, guanine is the most easily oxidized and presents the richest chemistry.

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Thanasis Gimisis (left) was born in Athens, Greece, in 1963. He received his PhD degree from the University of Michigan, Ann Arbor, in 1993 under the supervision of Prof. Masato Koreeda. His thesis involved the synthesis of PAH diol epoxides. He then joined the group of Dr. Chrys Chatgilialoglu at the CNR of Bologna, Italy, as a postdoctoral fellow and worked on the C-1′ radical generation in nucleosides. Following a second postdoctoral fellowship in 1998 with Prof. M. Orfanopoulos at the University of Crete, Greece, he joined the Faculty of the Chemistry Department at the University of Athens in 2000 where he is currently Assistant Professor. His major research interests include the study of the mechanisms involved in DNA damage through generation of reactive intermediates and the independent synthesis and structural characterization of DNA oxidative lesions.

Crina Cismaş (née Socaci) (right) was born in Reghin (Romania) in 1977. She received her BSc, MSc, and PhD degrees in 1999, 2000, and 2004, respectively, from the "Babes-Bolyai" University in Cluj-Napoca for work on the synthesis and structural analysis of derivatives with saturated six-membered heterocycles under the supervision of Prof. Ion Grosu. During her PhD she spent 14 months as a DAAD fellow in the group of Prof. J. Liebscher at the Humboldt University in Berlin, Germany, working on the synthesis of new amphiphilic purine derivatives. In January 2005 she joined the group of Prof. T. Gimisis as a Marie Curie postdoctoral fellow and is working on developing a new methodology for the structural characterization of oxidatively damaged DNA bases.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

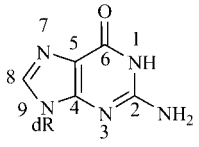
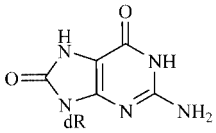
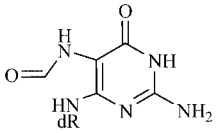
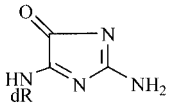
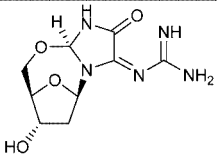
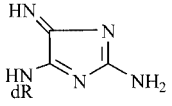
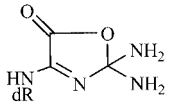
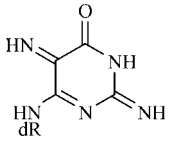
Unequivocal structure determination, stability studies, and insertion or generation of lesions in synthetic oligonucleotides has allowed for biological studies on the mutagenicity and reparability of each lesion.[5]

During the past decade a large number of mechanistic papers have appeared in which attempts have been made to clarify the structure of the intermediates and the mechanisms involved in the formation of most guanine oxidation products (Tables 1 and 2) under a variety of oxidative conditions. In many cases, the original proposals have been fine-tuned by the authors in subsequent papers in the light of more recent findings by the same or other groups working in this area. For a newcomer to the field this can be a source of confusion, as the current mechanistic proposal, or even the structure of an intermediate, might differ from that in a previous publication.

Although there have been numerous reviews covering the mechanistic[6–9] and biological aspects[1,2,5,10–13] of DNA damage, there has been no review to date covering the organic chemistry aspects of this field with respect to the isolation, characterization, and independent synthesis of DNA damage products.

Our general interest in the area of oxidatively produced DNA damage[14–19] prompted us to focus our attention in this Microreview on the structure of the proposed intermediates and final products of guanosine oxidation, as deter-

Table 1. 2′-Deoxyguanosine and primary lesions.

Comp.	Structure	Formula	Symbol	Base mass	Mass difference (L–G)
1		$C_{10}H_{13}N_5O_4$	dGuo	151	0
2		$C_{10}H_{13}N_5O_5$	8oxo dGuo	167	+16
3		$C_{10}H_{15}N_5O_5$	FaPy dGuo	169	+18
4		$C_8H_{12}N_4O_4$	dIz	112	–39
4'		$C_9H_{13}N_5O_4$	dDPy	255[a]	–12
5		$C_8H_{13}N_5O_3$	dDIz	111	–40
6		$C_8H_{14}N_4O_5$	dZ (dOz)	130	–21
7		$C_9H_{13}N_5O_4$		139	–12

[a] Nucleoside mass.

mined by spectroscopic methods and independent synthetic studies. Independent synthesis, when available, provides a succinct corroboration of the proposed structure and hints at the stability and properties of the lesion under study. When independent synthetic studies are not available, the physical and spectroscopic properties of the lesion are the next "hard" available data. After X-ray crystallography, NMR studies are "hard" data that can be unequivocally corroborated by subsequent independent synthesis studies. Last but not least, mass spectrometry with soft ionization techniques, especially when coupled with isotopic labeling and MS/MS analysis, can be a powerful tool for structure elucidation, and a large number of structural assignments have been possible because of such studies. Nevertheless, as all spectroscopic data are subject to interpretation, it should be expected that in the near future some of the proposed

Table 2. 8-Oxo-7,8-dihydro-2′-deoxyguanosine and secondary lesions.

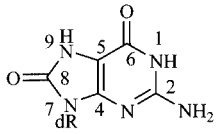
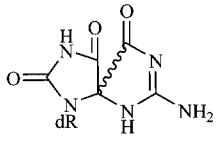
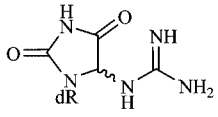
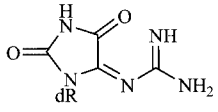
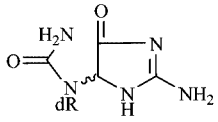
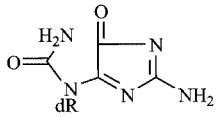
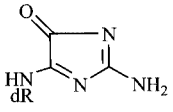
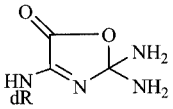
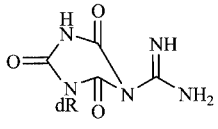
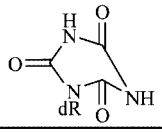
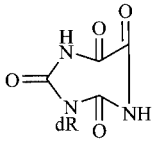
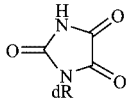
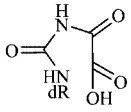
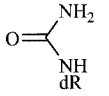
Comp.	Structure	Formula	Symbol	Base mass	Mass difference (L–8oG)
2		$C_{10}H_{13}N_5O_5$	8oxo dGuo	167	0
8		$C_{10}H_{13}N_5O_6$	dSp	183	+16
9		$C_9H_{15}N_5O_5$	dGh	157	–10
10		$C_9H_{13}N_5O_5$	dGh^{ox}	155	–12
11		$C_9H_{15}N_5O_5$	dIa	157	–10
12		$C_9H_{13}N_5O_5$	dIa^{ox}	155	–12
4		$C_8H_{12}N_4O_4$	dIz	112	–39
6		$C_8H_{14}N_4O_5$	dZ (dOz)	130	–21
13		$C_9H_{13}N_5O_6$	dCac	171	+4

Table 2. *Continued*

Comp.	Structure	Formula	Symbol	Base mass	Mass difference (L–8oG)
14		$C_8H_{11}N_3O_6$	dCa	129	–38
15		$C_9H_{11}N_3O_7$	dTt	157	–10
16		$C_8H_{10}N_2O_6$	dPa	114	–53
17		$C_8H_{12}N_2O_7$	dOa	132	–35
18		$C_6H_{12}N_2O_4$	dUa	60	–107

structures will have to be redrawn, kindling the excitement of the researchers in this field.

This review is divided into sections corresponding to structurally similar products arising from a guanine base modification. The products are divided into two families, namely those that can be formed from guanine oxidation without the intermediacy of 8-oxo-7,8-dihydroguanine (8-oxoGua; primary oxidation products), and those that are considered as products of further oxidation of 8-oxo-7,8-dihydroguanine (secondary oxidation products). However, there is no firm line even between these two families, since many proposed mechanisms are still under debate.

For each group of lesions, a short introductory note presents the reported conditions for the formation of this modification, and emphasis is given to the isolation, characterization, and stability studies of each lesion, together with any studies related to independent synthesis or generation of the lesion in synthetic oligonucleotides. Finally, a summary is given of each lesion's biological impact, as determined by studies in vitro. Since this last part is not the main purpose of the Microreview, the reader is directed to recent reviews providing extensive information regarding replication and repair.[20–23]

The stable primary and secondary lesions discussed here are summarized in Tables 1 and 2, respectively, where the compound numbering used in the schemes and figures can also be found. The original position of the atoms and the numbering in the guanine base are preserved throughout this review in order to have a clear view of the origin of each atom in the produced lesions.

2. Primary Oxidation Products

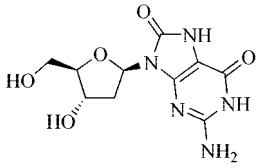
2.1. 8-Oxo-7,8-dihydro-2′-deoxyguanosine (2)

2.1.1. Formation and Characterization

This compound is the most well-studied DNA oxidative lesion. A SciFinder Scholar® search produces around 2000 hits for RN = [88847-89-6], the RN corresponding to 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo). This is an important lesion that is often employed as a biomarker of oxidative stress. Until recently, the yield of 8-oxodGuo, as well as that of other products of guanine oxidation, had been overestimated by a factor of up to three orders of magnitude. Recent research efforts, however, have determined the best analytical and biochemical methods for the accurate measurement of 8-oxodGuo.[24,25] The current estimate is that there are approximately 2000 8-oxodGua lesions generated per human cell per day.[1,26]

8-OxodGuo (**2**, Figure 1) is the main product of γ-radiolysis[27] or (photosensitized) one-electron oxidation of dGuo in DNA.[28,29] It is the major product of $^1O_2$ oxidation of dGuo in DNA when a thermolabile source of $^1O_2$ is used.[30] This contrasts the behavior of monomeric dGuo in solution and reflects the different stability of the tran-

sient intermediates involved within the double helix. NMR studies[31] and calculations[32,33] have determined that the 6,8-diketo tautomer is the predominant species in solution.

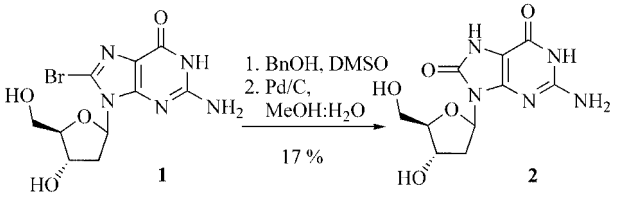


2, 8-oxodGuo

Figure 1. Structure of 8-oxodGuo.

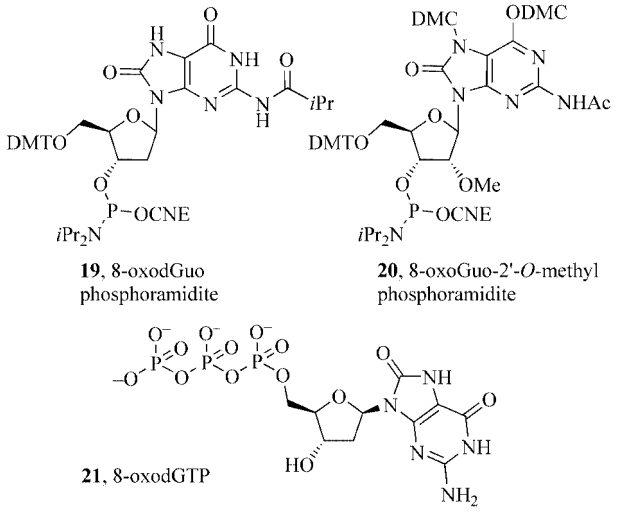
2.1.2. Synthesis/Generation

8-OxodGuo (**2**) is the only 2′-deoxyguanosine oxidation product currently commercially available as the free nucleoside and the corresponding phosphoramidite. Synthetically, this lesion is readily available from dGuo through a three-step sequence that involves bromination of the 8-position, substitution of bromine by a benzyloxy group in DMSO, and hydrogenolysis of the benzyl group (Scheme 1).[34]



Scheme 1. Synthesis of 8-oxodGuo.

The low yield of the substitution step was improved recently by increasing the BnOH/DMSO ratio to 3/2.[35] Extension of this methodology provided the 5′-DMT-phosphoramidite (**19**),[36] the corresponding 2′-*O*-methyl-ribonucleoside phosphoramidite (**20**),[37] and 8-oxodGTP (**21**)[35] (Figure 2).



19, 8-oxodGuo phosphoramidite

20, 8-oxoGuo-2′-*O*-methyl phosphoramidite

21, 8-oxodGTP

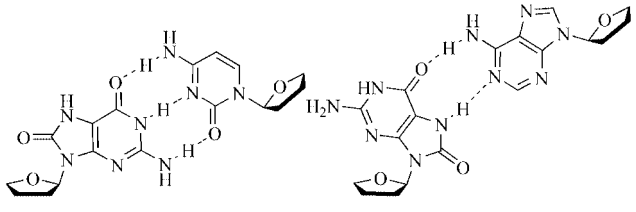
Figure 2. Structures of **19**, **20** and **21**.

2.1.3. Related Structures

Addition of hydroxyl radical (˙OH) to a DNA base generates a secondary radical, which is a reactive species capable of inducing further damage to neighboring bases. Such tandem base lesions involving 8-oxoGua have been characterized and their biological features have been determined. Specifically, X-ray irradiation of oligonucleotide tetramers, e.g. d(CpGpTpA), in oxygenated aqueous solutions leads to the degradation of either thymine or cytosine to a formamido remnant with concurrent formation of 8-oxodGuo in the adjacent position.[38] The products have been analyzed by FAB mass spectrometry and ^{1}H NMR spectroscopy,[39] and oligonucleotides containing the same tandem base damage have been independently synthesized.[40] The phosphodiester bond between these vicinal lesions is stable in the presence of both snake venom phosphodiesterase (a 3′-exonuclease) and calf spleen phosphodiesterase (a 5′-exonuclease). This has allowed the development of a methodology for the quantitative release and ESI-MS/MS analysis of the tandem lesions as dinucleoside monophosphates. The detection limit in aerated aqueous solutions of γ-irradiated DNA is close to 10 fmol.[41]

2.1.4. Repair/Mutagenicity

The commercial availability of standard phosphoramidites of this lesion has also aided a number of biological studies involving 8-oxodGuo-modified oligonucleotides. A crystal structure (at 2.5-Å resolution) of a self-complementary DNA duplex containing an 8-oxodGuo·dAdo mismatch was reported as early as 1994.[42] This, together with earlier solution structures solved by NMR studies, established the existence of Hoogsteen base-pairing between a *syn*-8-oxodGuo and an *anti*-dAdo (Figure 3). Recently, a crystal structure of polymerase β containing an *anti*-8-oxodGuo·*anti*-dCTP and a *syn*-8-oxodGuo·*anti*-dATP, at 2.8- and 3.1-Å resolution, respectively, has been reported.[43] A flip of the phosphate backbone is observed in the former system in order to accommodate the C8-oxygen. A similar crystal structure of 8-oxodGuo·dCTP (at 2.8 Å) with a *pol* mutant clarified the ease with which this lesion is surpassed in replication.[44]



2·dC, 8-oxodG(*anti*)·dC(*anti*) **2·dA**, 8-oxodG(*syn*)·dA(*anti*)

Figure 3. Base pairing modes of 8-oxodGuo.

The family of base-excision repair (BER) enzymes involved in the repair of 8-oxodGuo in *Escherichia coli* is known as the "GO system" (guanine oxidase system).[45] This system consists of formamidopyrimidine DNA glycosylase (Fpg or MutM), the deoxyadenosine mismatch repair protein (MutY), and MutT. Fpg is a bifunctional enzyme

with both DNA glycosylase and AP-lyase activity that was shown to recognize purines with an open imidazole ring over 20 years ago.[46] Fpg catalyzes the removal of 8-oxodGuo paired with dC, MutT prevents its incorporation into DNA by catalyzing the hydrolysis of 8-oxodGTP to 8-oxodGMP, and MutY removes the mispaired adenine.

Repair of 8-oxodGuo when paired with dC is also effected by human 8-oxo-7,8-dihydroguanine DNA glycosylase 1 (hOGG1),[47] a eukaryotic functional homologue to Fpg that is structurally related to the Nth enzyme family (vide infra).[48] MutH glycosylase (a MutY homologue) removes mispaired dAdo opposite 8-oxodGuo.[49] Finally, the 8-oxodGTP phosphatase MTH (MutT homologue) hydrolyzes 8-oxodGTP and removes it from the nucleotide pool.[50,51]

Structures of the 8-oxodGuo DNA glycosylase hOGG1 bound to DNA provided the first look at a bifunctional mammalian DNA glycosylase/AP lyase and showed how this enzyme specifically recognizes the base that is paired with 8-oxodGuo. hOGG1 inserts a loop that contains an asparagine residue to extrude the substrate base from the DNA helix into the active-site binding pocket.[52] The cytosine base paired with 8-oxodGuo is recognized and interacts with the enzyme through hydrogen bonding. Last year the same group reported the crystal structure of a crosslinked complex of normal (at 2.5 Å) and 8-oxodG (at 2.4 Å) containing DNA with hOGG1. Crosslinking allowed the elucidation of the discrimination mechanism of the enzyme between a normal guanine and a damaged 8-oxodGuo residue and provided evidence for the mechanism of base extrusion.[53] The binding of hOGG1 to damaged 8-oxodGuo and undamaged sites in DNA has also been recently studied by atomic force microscopic imaging of single molecules.[54] As evidenced by the bending of the double strand within the enzyme, an initial weak interaction of the DNA glycosylase is followed by a transition where each base is flipped into the active site and is then released or excised, depending on whether it is a normal base or a suitable substrate.

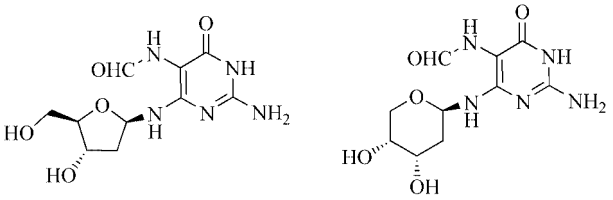
2.2. Formamidopyrimidines and Related Structures

2.2.1. Formation

The 2,6-diamino-4-hydroxy-5-formamidopyrimidine 2′-deoxyribonucleoside (**3**, FaPydGuo) is a major primary degradation product of dGuo under anoxic conditions.[4] FaPydGuo is the major product of hydroxyl radical degradation of dGuo under reducing conditions[55,56] and in the γ-radiolysis of DNA under anoxic conditions.[57,58] The related 2′-deoxyadenosine analogue FaPydAdo is the major product of γ-irradiation of dAdo in oxygen-free aqueous solutions,[59,60] but is also produced by simple alkaline treatment of dAdo in the monomeric form[61] or in single-stranded oligonucleotides.[62]

2.2.2. Isolation/Characterization

The isolation of FaPydGuo was first reported as one of the products of the exposure of dGuo to γ-rays in oxygen-free aqueous solutions.[55] It was determined that the opening of the imidazole ring in the monomeric form of the lesion induces a lability of the N-glycosidic bond, resulting in a rearrangement of the sugar moiety through the intermediacy of a Schiff base. It appears that the α and β pyranose anomers are thermodynamically more stable than the corresponding furanose isomers, with the α anomer prevailing (Figure 4). Attempts to isolate the furanoid isomers of the corresponding FaPydAdo analogue[60] were unsuccessful and led to the observation of transient signals that were assigned to the β-furanose sugar (β-*f*-FaPydAdo). It was shown that a slow conversion occurs, initially giving signals attributed to the α-furanose sugar (α-*f*-FaPydAdo) with concomitant decrease of the β anomer and, eventually, disappearance of both furanose signals and emergence of an anomeric mixture of pyranoses, with the α-*p*-FaPydAdo prevailing.[60] This process also leads, as expected, to a partial hydrolysis of the N-glycosidic bond and release of the free modified base (FaPy).



3, f-FaPydGuo **3'**, p-FaPydGuo

Figure 4. Furanoid and pyranoid isomers of FapydGuo.

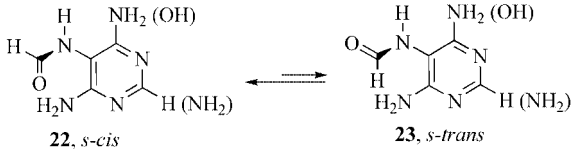
The furanose–pyranose isomerization is slow in the crude solution mixture, but it seems that the only way to characterize furanoses is through hydroxyl protection. For example, acetylation of a crude mixture of N^7-substituted FaPyGuos results in the isolation of both furanose and pyranose isomers. Therefore, all reports of *f*-FaPy-nucleosides with free hydroxyls,[63–67] and especially those reporting NMR spectroscopic data,[68,69] should be viewed with skepticism since the compounds actually isolated and characterized most probably should be assigned to the corresponding pyranoid structures.

The pyranoid derivatives can be differentiated from the furanoid isomers by considering vicinal coupling constants.[70] The diaxial orientation of specific vicinal protons on the pyranose chair conformation leads to large coupling constants – $J_{1',2'}$ and $J_{4',5''}$ in the β anomer as well as $J_{1',2''}$ and $J_{2'',3'}$ in the α anomer are above 10 Hz. The anomeric 1′-H proton is further split by the adjacent NH, and appears as a characteristic doublet in [D_6]DMSO at about δ = 7.05 ppm (major rotamer) for *p*-FaPydAdo.

2.2.3. Synthesis/Generation

Recent synthetic efforts by the groups of Greenberg[71–73] and Carell[74] have shed light on the intrinsic needs for an efficient synthesis of this lesion as well as its incorporation into synthetic oligonucleotides.

A successful synthesis of this lesion, as well as its incorporation into synthetic oligonucleotides, requires a number of hurdles to be overcome. The major obstacle is the aforementioned furanose-to-pyranose isomerization, which prevents the presence of a free 5′-hydroxy in the last stages of the synthesis and during oligonucleotide synthesis. The presence of an electron-withdrawing phosphate group at the 5′-position greatly enhances the stability of the N-glycosidic bond of the FaPy residue (vide infra). Therefore, as described below, the successful incorporation of the lesion in synthetic oligonucleotides requires the generation of the FaPy base on a nucleotide dimer. Anomeric isomerization is another issue to be addressed since it is well known that formamidopyrimidines epimerize readily. Synthetic efforts from the Carell group[74] have provided insight into the stability and conformation of FaPydGuo. The α anomer is thermodynamically more stable when in monomeric or single strand form, and a relaxation time, τ, of 6.5 h at 25 °C in water/acetonitrile (1:1) was determined for a protected 2′-deoxy derivative. Both anomers decompose by releasing the base moiety, with half-lives, $t_{1/2}$, of 37.8 and 65.2 h for the β and α anomer, respectively, in water/acetonitrile (1:1) at 50 °C.[74] Nevertheless, it was recently suggested that both FaPydGuo and FaPydAdo lie in a preferred β-conformation when in the double helix and opposite to their natural base-pairs.[75]



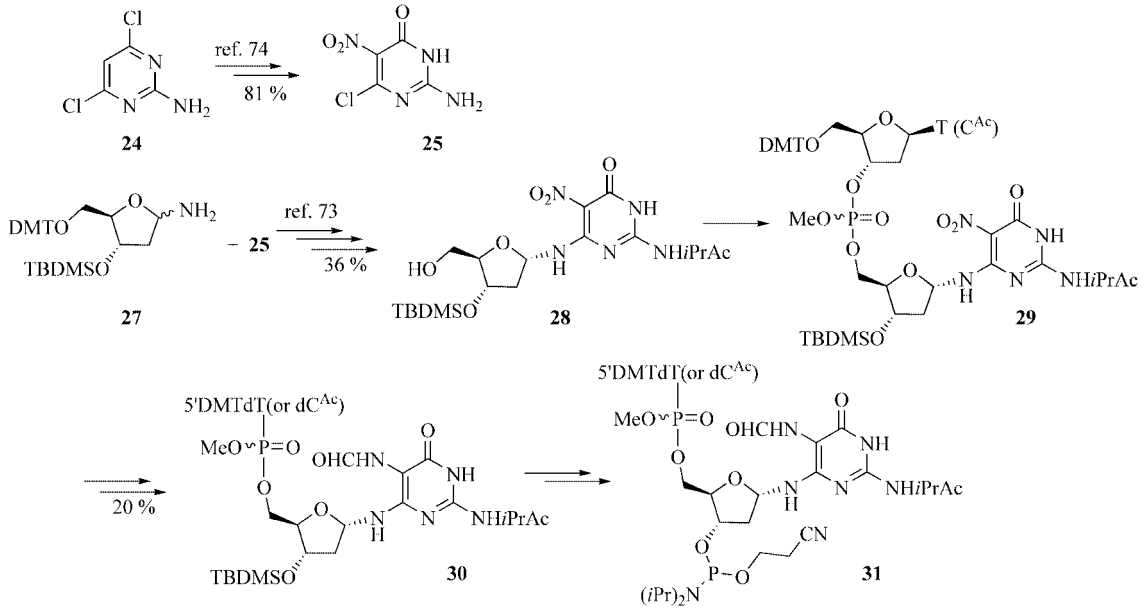
Scheme 2. *s-cis* and *s-trans* conformers of FaPyAde(Gua).

Another conformational issue to be addressed is the formamide rotamer stability. Due to the partial π character of the formylamine (N–CO) bond, there is a restricted rotation of the amide bond. The FaPyAde system exhibits two sets of peaks in the ^1H and ^{13}C spectra in [D$_6$]DMSO.[60] The major set was assigned to an *s-cis* conformation, and the minor to an *s-trans* conformation (Scheme 2). A characteristically large coupling ($J_{7,8}$ = 11.7 Hz) is observed for the *trans* tautomer ($J_{7,8}$ = 1.3 Hz for the *cis* tautomer).

The rotational barrier was estimated to be 75.5 kJ mol^{-1} (at 370 K) in accordance with previous results from open-ring N^7-methylguanine in polynucleotides.[76] A similar barrier has been estimated for the FaPydGuo lesion from temperature-dependent NMR studies, which also corroborated the presence of the same two major rotamers in [D$_6$]DMSO solutions. Interestingly, it was also observed that in $CDCl_3$, a less polar solvent that probably mimics the typical environment of a protein–DNA complex, FaPydGuo exists solely in the *cis*-amide conformation, and no rotamer equilibrium was observed even at elevated temperature. The presence of a seven-membered hydrogen-bonded ring system was thus invoked to explain the stability of the *cis* rotamer, and the importance of this fact in the recognition of this lesion by repair enzymes was discussed.[74]

Finally, the choice of protecting groups during monomer and oligonucleotide synthesis is critical for a successful sequence of reactions. These problems have been elegantly addressed by the group of Greenberg, who, in a series of papers,[71–73] managed to resolve most of these issues.

For oligonucleotide synthesis, the incorporation of FaPydGuo as part of a dinucleotide phosphoramidite proved to be successful. Initially, the instability of an intermediate nitropyrimidine under Brönsted acid 5′-detritylation led to a synthetic design directed towards oligonucleotide synthe



Scheme 3. Synthesis of FaPydGuo dinucleotide phosphoramidites.

sis using reverse phosphoramidites.[71] More recently, however, selective 5′-detritylation of a nitropyrimidine intermediate without concomitant furanose-to-pyranose rearrangement was effected in the presence of a mild Lewis acid ($FeCl_3$·$6H_2O$) and allowed the use of a 5′-dimethoxytrityl dinucleotide phosphoramidite.[73] Change of the β-cyanoethyl to an *O*-methyl phosphoramidite allowed for further manipulations without loss of this protection until the end of the oligonucleotide synthesis, where it required special treatment (disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate).[71] Finally, change of the phenoxyacetyl protection of the exocyclic amine to a 4-isopropylphenoxyacetyl ensured greater stability and lipophilicity, which aided chromatographic separation.[73] A summary of the above synthetic steps is outlined in Scheme 3.

2.2.4. Related Structures

The anomeric instability of the FaPydGuo lesion has prompted researchers to target *C*-FaPy analogues that are not only configurationally stable at the pseudo anomeric center but also provide an overall stability that can be utilized in biological studies. Two *C*-FaPy analogues have been reported (Figure 5).

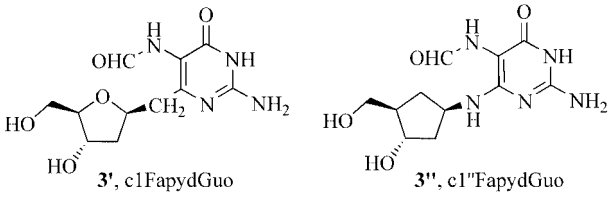


Figure 5. Synthetic *C*-FapydGuo analogues.

The synthesis of DMT-phosphoramidites of c1FaPydAdo (**36**, **37**) is outlined in Scheme 4.[72] The previously reported phosphorus ylide of the C-heterocycle is introduced in a Wittig reaction and, after suitable manipulation of the heterocycle ring, the mixture of diastereomers is separated after the DMT group has been inserted. Both diastereomers give the corresponding phosphoramidites in moderate yields.

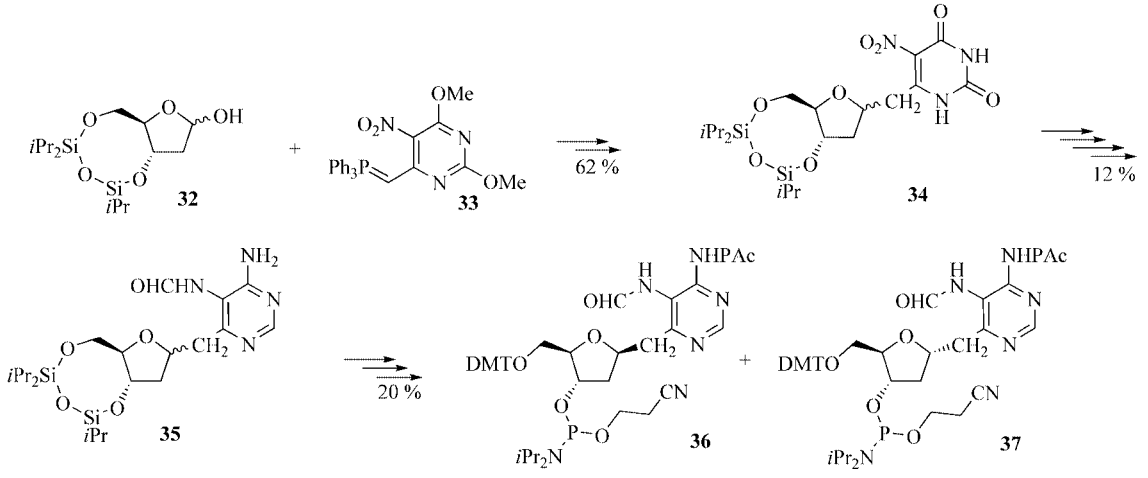
The synthesis of the corresponding c1FaPydGuo DMT-phosphoramidites **42** is considerably more complicated[77] as the starting heterocycle required different protection at the 2- and 4-positions. Even then, the rigorous conditions required for the introduction of the 2-amino group necessitate desilylation prior to the aminolysis. The rest of the sequence is straightforward, apart from the inability to introduce the phosphoramidite group on the α epimer due to steric reasons. Again, separation of the epimers was achieved by centrifugal TLC at the DMT level (Scheme 5).

A different approach has been utilized by the Carell group (Scheme 6).[78] Replacement of the 2-deoxyribosylamine with a homochiral cyclopentylamine in the reaction with 2-amino-6-chloro-5-nitro-4-oxopyrimide allowed the formation of c1′′FaPydGuo (**3′′**; Figure 5)as a clean and nonepimerizable β-isomer. The remaining transformations on the heterocycle were analogous to those previously reported[74] for the natural lesion. Deprotection and phosphoramidite elaboration gave the required phosphoramidite **47** in seven steps and 18% total yield (Scheme 6).

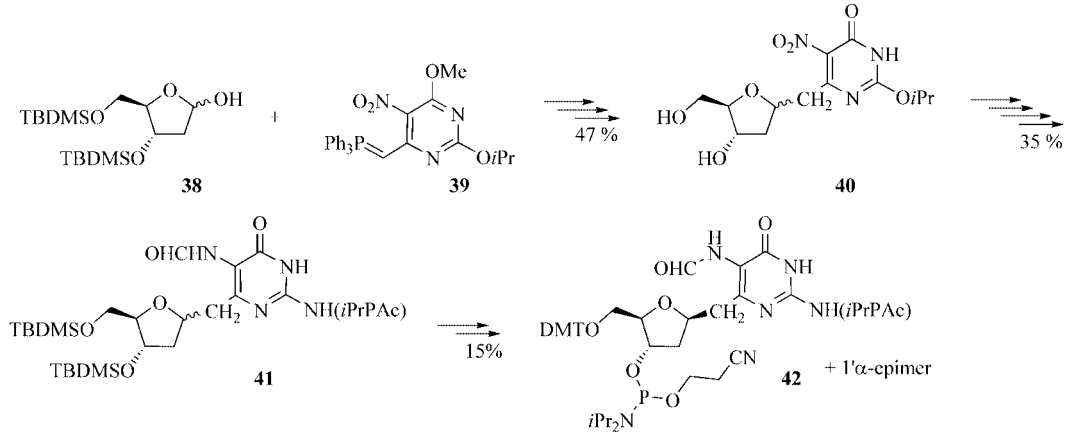
A number of N^7-substituted FaPy analogues have been isolated and characterized from the reaction of FaPydAdo[79] and FaPydGuo[80–82] as by-products of N^7-alkylated purines, and this subject has been reviewed elsewehere.[83] Three examples are given in Figure 6.

2.2.5. Repair/Mutagenicity

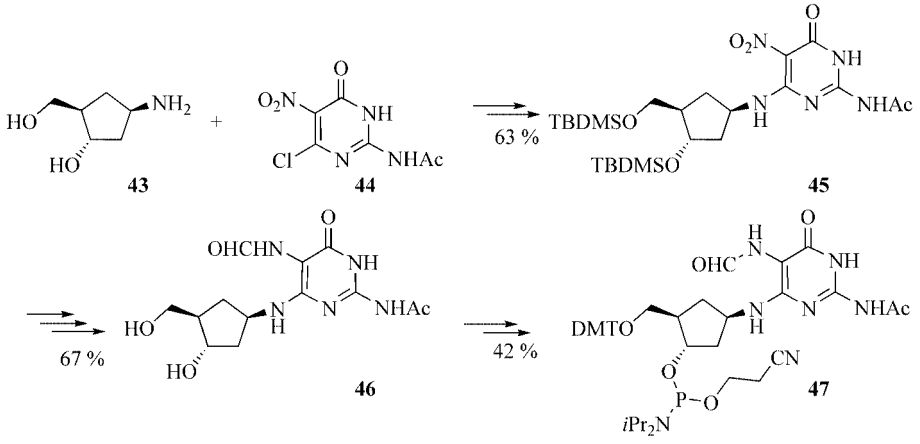
Initial duplex destabilization studies with synthetic α/β-FaPydGuo[84] and homologous β-c1FaPydGuo[77] and β-c1′′FaPydGuo[78] were contradictory with regard to the relative stability of the possible mismatches. Biological repair studies with damaged DNA showed that the FaPydGuo lesion is efficiently recognized and excized by the repair enzyme hOGG1 as well as by Fpg. This lesion is considered to be premutagenic, since the Klenow fragment of DNA polymerase I (Kf exo$^-$) misincorporates dA opposite FaPydGuo, leading to G to T transversions in a comparable degree to 8-oxodGuo.[84] The Klenow fragment (Kf and Kf exo$^+$) is generated by the removal of the 5′-3′ exonuclease activity from DNA polymerase I whilst keeping the 3′-5′



Scheme 4. Synthesis of c1FaPydAdo DMT-phosphoramidites.



Scheme 5. Synthesis of c1FaPydGuo DMT-phosphoramidites.



Scheme 6. Synthesis of c1′′FaPydGuo phosphoramidites.

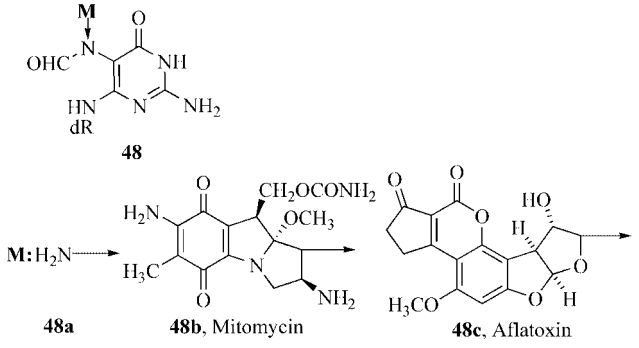


Figure 6. Example of N^7-substituted FapydGuo analogues.

exonuclease activity intact, whereas Kf exo$^-$ is a site-specific mutant of Kf with diminished 3′-5′ exonuclease activity.[85]

Nevertheless, Fpg binds duplexes and excises FaPydGuo more efficiently from FaPydGuo·dC (or β-c1FaPydGuo·dC) pairs than from FaPydGuo·dA (or β-c1FaPydGuo·dA) mispairs, in vitro. At the same time, MutY incises dAdo opposite FaPydGuo, but incision is slower than from DNA containing 8-oxodGuo opposite dAdo.[86]

In comparison, FaPydAdo is also premutagenic with Kf misincorporating mainly dAdo, as above, and dGuo to a lesser extent. It also appears to be a more potent premutagenic lesion than 8-oxodGuo in vitro.[87] Fpg excises FaPydAdo opposite T efficiently as well as from FaPydAdo·dA and FaPydAdo·dGuo mispairs. The lack of incision by MutY indicates that FaPydAdo could be deleterious to the genome. Interestingly, a duplex containing the β-c1FaPydAdo·dT base-pair inhibits repair of FaPydAdo by Fpg.[88]

Very recently, the crystal structure of the complex between the Fpg protein from *Lactococcus lactis* (*Ll*Fpg) and a c1′′FaPydGuo containing a 14-mer double stranded oligonucleotide were reported at a 1.8-Å resolution.[89] Surprisingly, despite the similarities between the FaPydGuo and 8-oxodGuo structures, the two lesions prefer opposite conformations within the enzyme binding pocket (*anti* for c1′′FaPydGuo and *syn* for 8-oxodGuo). An interaction of the C-8 carbonyl with a protein residue through a water molecule was postulated.

2.3. Imidazolone, Oxazolone, and Related Structures

2.3.1. Formation

The nucleosides 2-amino-5-[(2-deoxy-β-D-erythropentofuranosyl)amino]-4*H*-imidazol-4-one (**4**, dIz) and 2,2-di-

amino-4-[(2-deoxy-β-D-erythropentofuranosyl)amino]-5-(2*H*)-oxazolone (**6**, dZ) correspond to a well-studied family of primary oxidation products of 2′-deoxyguanosine (Figure 7). They are considered to arise from the oxidation of dGuo under a variety of conditions. For example, they are the major product of hydroxyl radical addition to dGuo[90] and also the major product from the photosensitized, one-electron oxidation of dGuo in the monomeric form,[90] or in single-stranded oligonucleotides[91] and double stranded DNA,[92] catalyzed by type-I photosensitizers such as benzophenone and riboflavin. They are also observed as minor products in the type-II photooxidation of 8-oxo-dGuo catalyzed by photosensitizers such as methylene blue,[93] conditions known to generate primarily $^1O_2$, as well as under other types of photosensitized conditions involving triplet-excited ketones.[94–97] Imidazolone (**4**, dIz) is the predominant product of $^1O_2$ oxidation of a 8-methoxydGuo residue inserted into an oligonucleotide trimer.[98] Finally, imidazolone has been reported to be the major, two-electron oxidation product of monomeric dGuo and as one of the products of dGuo in double-stranded oligonucleotides by a cationic metalloporphyrin complex activated by $KHSO_5$ ([MnTMPyP]/$KHSO_5$), a strong, two-electron oxidant that generates a high-valent metal–oxo species Mn^V=O.[99–104]

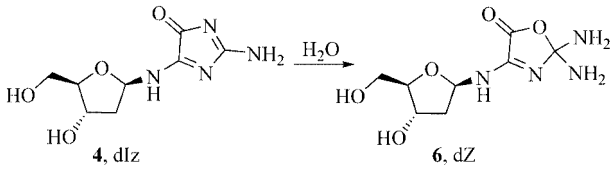
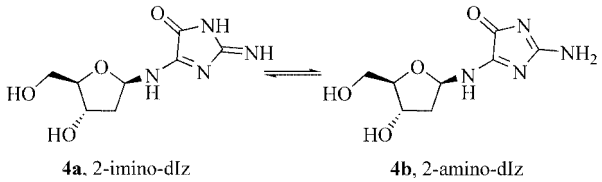


Figure 7. Structure of dIz and its hydrolysis product dZ.

2.3.2. Isolation/Characterization

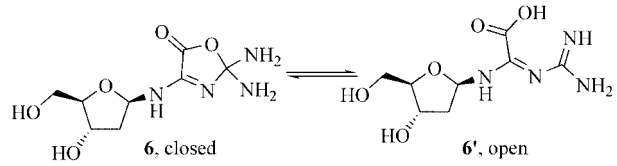
The *O*-acetyl derivatives of dIz (AcdIz) and dZ (AcdZ) were first isolated as the major products of the benzophenone-mediated photosensitization of AcdGuo.[90,105] The acetylation of the hydroxy groups facilitated the separation on a Nucleosil ODS column and prevented the formation of the $O^{5'}$,8-cyclization product characterized earlier (vide infra).[106] The slowest eluting nucleoside was assigned the structure of imidazolone (AcdIz) and was observed to be quantitatively hydrolyzed to oxazolone (AcdZ), a faster eluting nucleoside, with a half-life of hydrolysis of 2.45 h at 37 °C when left in a neutral aqueous solution.[90] The structure assignment for both structures was based on extensive ^1H, ^{13}C, ^{15}N, and ^{17}O (for dZ) 1D and 2D NMR experiments, coupled with FAB and HR mass spectrometric studies.[90,107] These latter studies have been reinforced in recent years by ^{18}O-labeling studies that shed light on the mechanism of formation of these lesions under a variety of conditions.[91,103,108]

Both compounds contain a characteristic NH proton, which couples with the anomeric 1′-H proton and appears as a doublet at δ = 9.32 (J = 7.3 Hz) and 8.18 ppm (J = 9.1 Hz) for dIz and dZ, respectively, thus indicating the breaking of the imidazole ring of the purine as in the case of FaPydAde (vide supra). Imidazolone exhibits three exchangeable NH signals in the ^1H NMR spectrum and it was initially thought to be a 2-imino-2,3-dihydro-4*H*-imidazol-4-one (Scheme 7). Nevertheless, the aromaticity of the heterocyclic ring, as evidenced by the shifts of the sugar protons and base carbons,[107] reports from similar compounds,[109] and the recent findings regarding imidazolone's preferential binding opposite dGuo in duplex DNA,[110] all indicate that a 2-amino-4*H*-imidazol-4-one tautomer is thermodynamically more populated in the double helix. The latter representation has thus been used in all recent publications.



Scheme 7. Two possible tautomers of dIz.

In the case of oxazolone (dZ), apart from the doublet assigned to the anomeric 9-NH, all the other exchangeable protons appear as two broad singlets[90] or a coalesced broad singlet, and only three ^{15}N signals are detected in ^{15}N NMR, thus indicating the equivalency of two NH_2 groups and leading to the proposed structure of a 2,2-diamino-5(2*H*)-oxazolone ring. Nevertheless, the possibility of an equilibrium with an originally proposed[105] open-ring tautomer could not be ruled out.[90] The ^{17}O NMR spectrum of an isotopically enriched oxazolone exhibits two resonance signals,[107] but the small chemical shift difference of 13.8 ppm) cannot be accounted for by a closed-ring lactone-type structure.[91] In any case, a strong intramolecular hydrogen bonding of the carboxyl hydrogen or the use of polar NMR solvents could lead to a splitting of the oxygen resonances in the ^{17}O NMR spectrum of carboxylic acids.[111] Although an equilibrium between the closed and open structures has been invoked to explain these results (Scheme 8), oxazolone's spectra indicate the presence of a single species.



Scheme 8. Possible equilibrium between the closed and open structures of dZ.

Another interesting property of the oxazolone lesion is the loss of CO_2 observed in its ESI mass spectrum. The corresponding peak is significant and is observed in the spectrum of the monomer[90,91,93] as well as when dZ is inserted in oligonucleotides.[103] It relates to a well-characterized decarboxylation of imidazole-2-carboxylic acid-type products[112] and provides further evidence of the proposed structure. This observed fragment rules out a third possible

open structure where the N^3=C^4 bond of imidazolone has been hydrolyzed instead.[91]

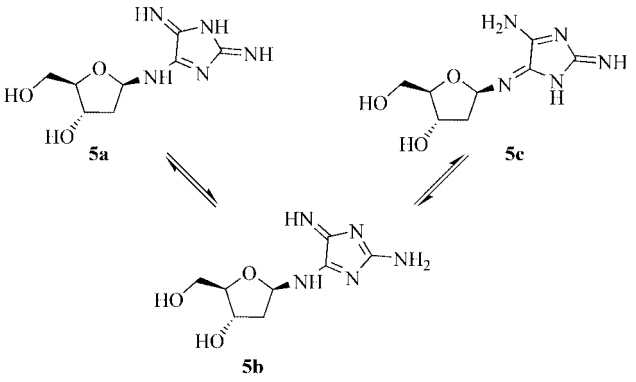
2.3.3. Related Structures

2.3.3.1. α-Anomers

As mentioned above, the dIz and dZ lesions are similar to the FaPydGuo in the presence of an NH group in the anomeric position, and one could expect that these lesions would be prone to instability and epimerization of the anomeric center. Such an epimerization has been observed during the photosensitized oxidation of dGuo catalyzed by benzophenone,[102] although it was shown that this was not due to the anomeric instability of the product dIz. The presence of the α-anomer in the reaction mixture was attributed to the anomeric instability of a precursor, namely of a commonly postulated C8-hydroxylated intermediate.

2.3.3.2. Diimino Imidazole (dDIz)

A structure related to imidazolone has been reported by Ohshima and co-workers (Scheme 9).[113–115] It was observed as a product of hypochlorous acid or a myeloperoxidase/H_2O_2/Cl^- oxidation of 2′-dGuo[113] or 3′,5′-di-*O*-acetyl-2′-dGuo[115] after *N*-acetyl cysteine (*N*-AcCys) termination. It was shown to arise from a not fully characterized dichloro derivative after *N*-AcCys treatment. The dDIz lesion also formed in the reaction was shown to arise from a different monochloro derivative, also not fully characterized. It was slowly hydrolyzed to dIz and Iz free-base ($t_{1/2}$ = 4.9 h, pH 7.0, 37 °C).



Scheme 9. Possible tautomers of dDIz.

Interestingly, the same product was also observed during the photosensitized, type-II oxidation of dGuo (but not 8-oxodGuo) by $^1O_2$ together with dIz, dZ, dSp, and 8-oxodGuo.[114] This product was isolated by RP-HPLC and was characterized by ^{1}H NMR, ^{13}C NMR ([D_6]DMSO, D_2O), and UV spectroscopy and ESI-MS/MS and HRMS. It was assigned to a 2,5-diimino-2*H*,5*H*-imidazole (dDIz) structure. Three exchangeable NH proton signals are observed in [D_6]DMSO but none exhibit coupling with the anomeric proton.[113] Since both the proposed (**5a**) and the analogous to dIz (**5b**) tautomeric forms would be expected to possess such a coupling, we propose that a third tautomeric form (like **5c**) that does not possess an anomeric NH predominates in solution (Scheme 9).

2.3.3.3. Cyclic Oxoimidazolidine (4′)

Another structure related to the postulated C-8-hydroxylated intermediate was isolated in earlier studies.[106] It was characterized by ^{1}H NMR, ^{13}C NMR, and UV spectroscopy as well as FAB-MS and assigned the structure (2*S*)-2′,5′-anhydro-1-(2-deoxy-β-D-erythropentofuranosyl)-5-guanidinylidene-2-hydroxy-4-oxoimidazolidine (**4′**, Figure 8). This previously characterized product provided structural evidence for the postulated precursor of dIz.[90]

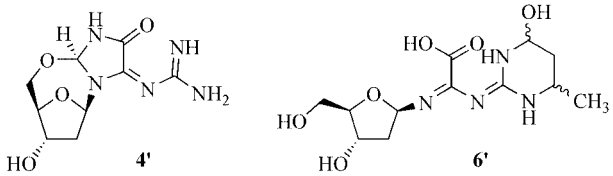


Figure 8. Structures of **4′** and **6′**.

2.3.3.4. Open Oxazolone Ring (6′)

A structure relating to oxazolone was recently reported as the product of $SO_4^{\bullet-}$ oxidation of 1,N^2-propano-2′-deoxyguanosine, a known dGuo adduct with crotonaldehyde (**6′**, Figure 8).[116] The product was characterized by ^{1}H NMR, ^{13}C NMR (D_2O), IR, and UV spectroscopy and ESI-MS. Although it corresponds to a mixture of two *trans* isomers, only one species is described without any stereochemical information. The structure exhibits an [M – CO_2 + H]$^+$ peak in the ESI mass spectrum characteristic of the oxazolone lesion. No information was provided for the exchangeable protons since the ^{1}H NMR spectrum was recorded in D_2O, but the carbonyl moiety shows an absorption at $\tilde{\nu}$ = 1730 cm^{-1} in the IR spectrum (cf. two peaks at 1781 and 1737 for dZ).[107]

2.3.3.5. Vicinal Lesion (49)

In a different report, a vicinal lesion was observed upon photo-irradiation of a TpdG nucleotide dimer in the presence of a type-I photosensitizer (benzophenone, riboflavin, or menadione).[117] The lesion was characterized by ^{1}H and ^{13}C NMR spectroscopy (D_2O) as well as ESI-MS, coupled with labeling and glycosidic bond hydrolysis studies, and was assigned the structure of a cyclic dimer (Figure 9).[117] A mechanism was proposed that involves the addition of a

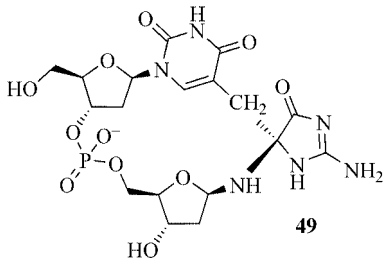


Figure 9. Structure of vicinal lesion **49**.

thymine-methyl radical to the C-4 of the adjacent guanine base.

For comparison purposes, the ^{13}C chemical shifts reported for the base carbons of three dIz-type and three dZ analogues are summarized in Table 3.

Table 3. Summary of ^{13}C NMR signals (δ, ppm) for reported imidazolone and oxazolone analogues.

Entry	Symbol	C2	C4	C5	Solvent	Ref.
1	dIz	184.8	166.5	176.7	[D_6]DMSO	[107]
2	AcdIz	184.1	165.9	175.3	[D_6]DMSO	[90]
3	dDIz	179.9	162.0	171.7	[D_6]DMSO	[113]
4	dZ	166.3	156.1	160.1	[D_6]DMSO	[107]
5	AcdZ	166.4	157.1	159.3	[D_6]DMSO	[90]
6	Crot dZ	163.2	157.8	162.9	D_2O	[116]

2.3.4. Synthesis/Generation

Although no independent synthesis has been reported to date for either dIz or dZ, both lesions have been generated successfully in oligonucleotides. In the case of dZ this was done by the riboflavin-mediated photosensitization of a central guanine residue in DNA fragments, followed by spontaneous hydrolysis of the initially formed Iz to Z (20 h at room temp., 15%).[91] In a similar procedure, and avoiding the hydrolysis step, Iz-containing oligonucleotides were prepared in 38% yield based on the consumed starting oligonucleotide.[110]

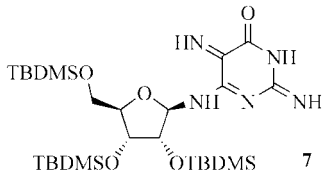
2.3.5. Repair/Mutagenicity

The reported alkaline-lability of these lesions differentiates them from 8-oxodGuo, which is not completely cleaved by hot piperidine treatment.[118] dAMP nucleotide insertion opposite oxazolone is mainly observed during in vitro DNA synthesis catalyzed by Kf exo$^-$ and *Taq (Thermus aquaticus)* polymerase. This suggests that oxazolone may lead to G to T transversions. At the same time, oxazolone blocks DNA synthesis when catalyzed by polymerase β.[119] Oxazolone is a substrate for both Fpg and endonuclease III (endo III) repair enzymes, which excise this lesion with efficiencies similar to that of 8-oxodGuo and better than that of 5-hydroxycytidine (5-OHC).[119] Endonuclease III (endo III or Nth) is an oxidized base specific *E. coli* DNA glycosylase/AP lyase, which removes primarily oxidized pyrimidines and is structurally different from Fpg.[20]

On the other hand, only dGTP is specifically incorporated opposite dIz during primer extension experiments by DNA polymerase I (pol I) and, in the reverse experiment, dIzTP is incorporated opposite dGuo catalyzed by Kf.[110]

2.4. 2,5-Diimino-2,5-dihydro-3*H*-pyrimidin-4-one (7)

A new guanosine primary oxidation product was reported recently by Foote and co-workers in the low-temperature, photoinduced singlet oxygen oxidation of a protected Guo.[120] It was the major product when the photooxidation was performed at –78 °C, although 8-oxoGuo and other secondary products were also observed. This product was isolated by low-temperature (0 °C) chromatographic separation and was characterized by ^1H and ^{13}C NMR spectroscopy (CD_2Cl_2, [D_6]acetone, –20 °C) as well as by HRMS, ESI-MS, and MS/MS. It proved unstable in solution, decomposing to a mixture of unknown products after standing at room temp. for a day. The proposed structure (Figure 10) was well corroborated by its 1D and 2D NMR spectroscopic data. Additional support for its structure, as well as the mechanism of its formation, was obtained by studying the transient intermediates formed between –100 and –43 °C during the photooxidation of an 8-^{13}C-guanosine derivative.[121] It was determined that the proposed diiminopyrimidone **7** shares a common intermediate with 8-oxoGuo, namely an 8-hydroperoxide, which decomposes at low temperature by releasing $^{13}CO_2$ through the formation of two transient isomeric carbamic acids (see mechanism in Scheme 21). It was determined by $^{18}O_2$ labeling and a model study[122] that both oxygen atoms of CO_2 come from a single oxygen molecule.



7

Figure 10. Structure of **7**.

3. Secondary Oxidation Products

It has been established today that 8-oxodGuo, the most well studied and ubiquitous product of oxidatively produced DNA damage, is very susceptible to oxidation and far more reactive than dGuo itself towards one-electron oxidants and singlet oxygen. During the last ten years a large number of studies have appeared in the literature reporting the major and minor lesions formed from the oxidation of 8-oxodGuo analogues under a variety of oxidation conditions. These secondary lesions will be described in detail in the following sections. It should, nevertheless, be clarified that in some cases there is a fine line between primary and secondary oxidation products. Imidazolone, a "primary", and spiroiminodihydantoin, a "secondary" oxidation product, for example, have both been identified as products arising from either dGuo or 8-oxodGuo oxidation, and the intermediacy of 8-oxodGuo in their oxidation mechanism has neither been proven or ruled out.

3.1. Spiroiminodihydantoin, Guanidinohydantoin, Their Precursor, and the Original Misassignments

The four compounds of Figure 11 are closely related to each other and their interesting story is currently rather well understood. Initially, Cadet and workers isolated a new, stable lesion in the methylene blue photosensitized oxidation of an acetylated dGuo and assigned to it the struc-

ture of 4-OH-8-oxodGuo.[123] Almost at the same time, Foote and co-workers,[124] during low-temperature studies on the rose bengal photooxidation of a silyl-protected 8-oxoGuo derivative, observed a short-lived species, stable at or below –40 °C, that was assigned to the same 4-OH-8-oxoGuo structure, based on the similarities with the spectra reported by Cadet. Burrows and co-workers[125] initially assigned the same structure to a product arising from the one-electron oxidation of 8-oxodGuo inserted in oligonucleotides, but later reported a new stable lesion as the product of the one-electron oxidation of an 2′,3′,5′-tri-*O*-acetylated 8-oxoGuo and assigned it the structure of spiroiminodihydantoin (Sp).[126] Their assignment was corroborated by independent synthesis of the aglycon (vide infra). Soon after, Tannenbaum and co-workers[127] correlated the latter product reported by Burrows and the original compound reported by Cadet with a lesion they isolated from the peroxynitrite oxidation of an acetylated 8-oxoGuo, and found them to be identical. The final corroboration of the spiro structure came from a specific NMR experiment by Adam and co-workers,[128] which unequivocally differentiated the two structures (vide infra).

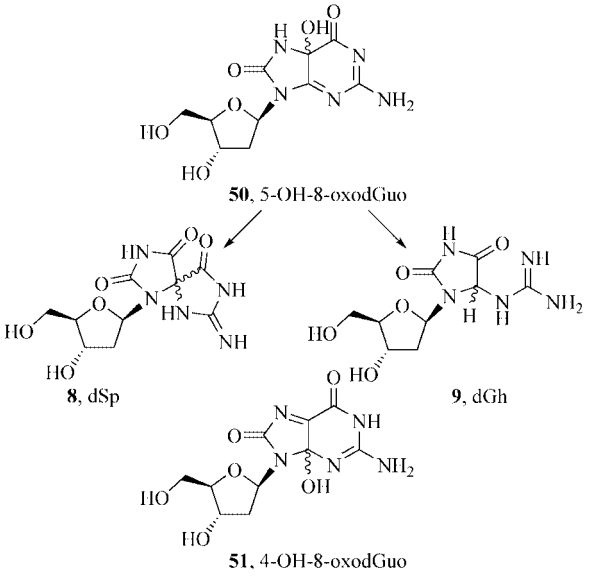


Figure 11. Structures of dSp, dGh, 5- & 4-OH-8-oxodGuo.

In the reaction of singlet oxygen with AcGuo, Burrows and co-workers[129] identified AcSp as the major product at pH > 7, whereas at pH < 7 a guanidinohydantoin (AcGh) was formed instead. A common intermediate, specifically a 5-hydroxy-8-oxo-7,8-dihydroguanosine, was postulated for the formation of the two lesions. After additional experimentation corroborated by theoretical calculations, Foote and co-workers[130] determined that the spectroscopic data of the unstable intermediate, which they originally assigned as the 4-OH-8-oxoGuo, fit better to the postulated 5-OH-8-oxoGuo, and observed that upon warming a solution of this intermediate to room temperature a rearrangement occurred, leading to a compound assigned to the Sp structure. The aforementioned studies completed the picture for this system and clarified that 4-OH-8-oxoGuo is, to date, an unobserved species.

In the following paragraphs, details of the isolation, characterization, and biological studies involving these secondary lesions and related structures will be provided. All references referring to a stable 4-OH-8-oxoGuo and derivatives will be treated as possessing information relating to Sp.[93–96,123,131–135]

3.1.1. Spiroiminodihydantoin

3.1.1.1. Formation

Spiroiminodihydantoin (AcdSp,[123] AcSp,[128] TBDMS-Sp[130]) is the major final $^1O_2$ oxidation product of the corresponding protected Guo. dSp (**8**) is the major lesion of the photogenerated triplet state of hydroxyacetophenone oxidation of dGuo,[94–97,131,133] or of the fluoroquinolone photoinduced oxidation of dGuo (Figure 12).[134,135] The same main product was reported from the oxidation of 2′-deoxyguanosine with peroxyl radicals, generated in situ from hydroperoxides by one-electron oxidation with peroxidases.[133]

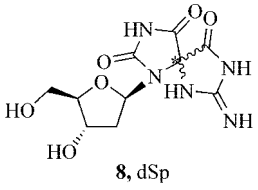


Figure 12. Structure of dSp.

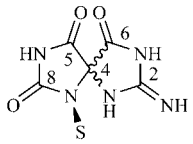
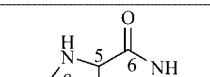
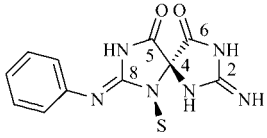
AcSp, together with AcGh (vide infra), were found to be the decomposition products in the one-electron oxidation of acetylated 8-oxoGuo by different systems [Na_2IrCl_6, $K_3Fe(CN)_6$, $KHSO_5/CoCl_2$][126,136,137] and by peroxynitrite oxidation, either at low fluxes[121] or in the presence of thiols.[127] A pH-dependent distribution of the two products (AcSp/AcGh) was observed,[115,126,136,138] indicating that there is a common intermediate that partitions in a pH-dependent manner. AcSp was the major product at physiological pH (> 6) in the above-mentioned studies. dSp is formed from 8-oxodGuo oxidation by treatment with a high-valent chromium complex[139,140] or HOCl and myeloperoxidase/H_2O_2/Cl^-.[115,141,142]

This lesion has also been observed in the self-sensitized photooxidation of dGMP[143] and in single- and double-stranded oligonucleotides, when dGuo- and 8-oxodGuo-containing oligonucleotides are oxidized by carbonate radical anion,[144] by nitrogen dioxide radicals,[145] and chromium(V)[139] or the chromium(VI)/ascorbate complex.[146]

3.1.1.2. Isolation/Characterization

AcSp was first characterized as one of the one-electron oxidation products of acetylated 8-oxoGuo.[126] HPLC separation followed by ESI-MS and ^{13}C NMR characterization showed an inseparable 1:1 mixture of (*R**) and (*S**) diastereoisomers whose spiro quaternary carbons appear as one signal at δ = 85 ppm (entry 1, Table 4).

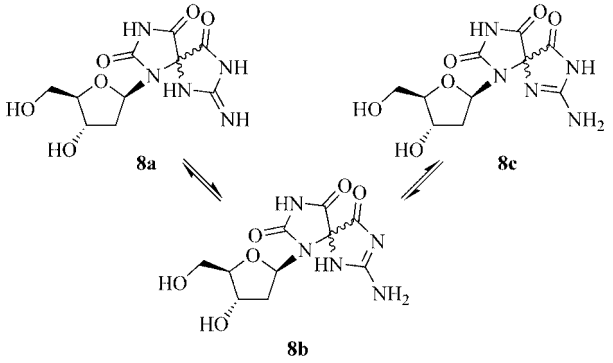
Table 4. Summary of ^{13}C NMR signals (δ, ppm) for reported spiroiminodihydantoin analogues.

Entry	S:	C-5	C-2	C-6	C-8	C-4	Solvent	Ref.
1			172.70 171.67	169.84	156.87 156.41	85.05	CD_3OD	[126]
2	 R = Ac, X = OAc	179.9 179.8	172.3 172.1	170.5 170.4	155.8 155.5	80.1 80.0	[D$_6$]DMSO	[127]
3	 R = Ac, X = H	180.5 180.0	172.3 171.7	169.5 169.2	156.0 155.3	80.5 79.5	[D$_6$]DMSO	[123]
4	 R = H, X = OH	180.57	172.21	1166.75	137.48	83.23	[D$_6$]DMSO	[147]

A similar ^{13}C NMR spectrum was later reported for the methylene blue mediated $^1O_2$ photooxidation product of AcGuo (entry 2, Table 4) and it was assigned to the same AcSp structure.[127] This assignment corrected that of the compound previously isolated under the same conditions that was given the structure of 2′,5′-di-*O*-acetyl-4-hydroxy-8-oxodGuo (entry 3, Table 4).[123] Furthermore, the authors performed an HPLC analysis of the mixtures isolated from the oxidation of 2′,3′,5′-tri-*O*-acetyl-8-oxoGuo by $KHSO_5$/$CoCl_2$ and peroxynitrite/thiol as well as from the type-II photooxidation of AcGuo. All three isolated products co-eluted, and their fragmentation pattern in the ESI-MS and MS/MS studies was identical to that of AcSp. The above-mentioned experimental evidence was later further corroborated by labeling studies with ^{18}O-labeled peroxynitrite, $H_2{}^{18}O$, and $^{18}O_2$.[138]

A specific NMR experiment (SELINQUATE) allowed for an unequivocal structural assignment of this photooxidative lesion of dGuo. This technique permits the differentiation between spirocyclic and annelated structures from the characteristic coupling patterns of the nearest carbon atoms. In the SELINQUATE spectrum,[128] the selective ^{13}C-^{13}C coherence transfer of the resonance at δ = 80 ppm shows two pairs of doublets centered at δ = 169.4 and 169.5 ppm (corresponding to C-6) and δ = 180.9 and 181.5 ppm (corresponding to C-5) for the two diastereoisomers. The magnitude of the splitting of the C-4 and C-5 signals (42 and 52 Hz, respectively) is indicative of a one-bond C–C coupling. The resonance at δ = 80 ppm was then attributed to the spiranic carbon atom (C-4) of the dSp structure, which has two carbon atom neighbors, as opposed to 4-hydroxy-8-oxodGuo, in which C-4 has only one.

Structural studies and theoretical calculations revealed that the Sp base possesses near-perpendicular and planar rings. It was calculated that the dSp lesion has stereoisomer-dependent geometric, steric, and hydrogen-bonding properties, which suggests different interactions of the two diastereoisomers with the replicative and repair enzymes. Furthermore, both (*R*) and (*S*) isomers may exist in several tautomeric forms, and orbital molecular geometry optimization calculations revealed that there is a very small difference in energy between the amino and imino forms (of the order of 1 kcal mol^{-1}; Scheme 10).[148]



Scheme 10. Possible dSp tautomeric structures.

Based on previous observations, Kino and Sugiyama recently proposed a possible base pairing of the spiroiminodihydantoin amino form with guanine in a DNA strand.[149] Considering the small calculated energy difference between the two tautomers and the observed G to T transversions, it is possible to suggest a base pairing of the imino form of dSp with the dA in DNA (Figure 13).

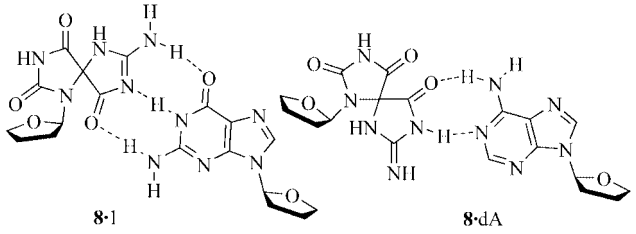
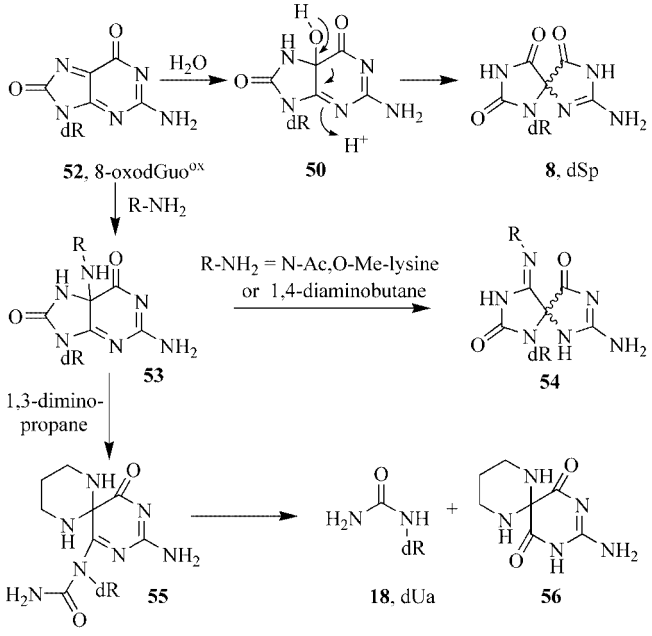


Figure 13. Proposed dSp·dG and dSp·dA pairing.

3.1.1.3. Related Structures

5-Hydroxy-8-oxo-guanosine: As previously mentioned, 5-OH-8-oxoGuo is the precursor of the Sp lesion (Figure 11 and Scheme 11).[130] The structure of 5-OH-8-oxoGuo was determined by studies where the predicted values for the ^{13}C NMR resonances estimated by theoretical calculations exhibited a smaller deviation from the experimental values (^{13}C NMR in [D_6]acetone at –40 °C) of *O*-TBDMS-protected 5-OH-8-oxoGuo than that of the corresponding estimated value for 4-OH-8-oxoGuo. In addition, a clear correlation between the anomeric proton and two sp^2-hybridized carbon atoms (C-4 and C-8) was observed in the 2D HMBC NMR spectra. At room temperature, the 5-OH-8-oxoGuo derivative slowly rearranges to give spiroiminodihydantoin (within two hours), as determined from the ^{13}C NMR spectrum in [D_6]acetone, thus confirming the structure of the previously postulated precursor of Sp.[126,127]



Scheme 11. Formation of dSp and dSp adducts and their proposed intermediates.

Spiroiminodihydantoin Adducts: Furthermore, a quinonoid intermediate (**52**, Scheme 11) has been postulated as a precursor of 5-OH-8-oxodGuo. Since the formation of 5-OH-8-oxodGuo entails a hydration step, it is possible that other nucleophiles could also participate to give DNA adducts. This is biologically significant and indicates the possible formation of DNA–protein adducts during DNA oxidation. Such a phenomenon has been observed in a model study of the oxidation of 8-oxodGuo in double-stranded DNA in the presence of either *N*-acetyl-*O*-methyllysine[150] or 1,3-diaminopropane (spermine) and 1,4-diaminobutane.[151] The spermine adduct was converted into a stable six-membered spiroaminal, which underwent hydrolysis to the urea lesion. This conversion was not observed for 1,4-diaminobutane, and the Sp adduct was formed instead as the most stable lesion (Scheme 11).

8-Aryliminospiroiminodihydantoin Analogues: The aerobic oxidation of arylamine adducts at C-8 of guanosine leads to the formation of related spirodihydantoin analogues.[147] The spiro structure was unequivocally determined by the X-ray analysis of the crystal formed for one of the two diastereoisomers of the *N*-phenyl derivative, with an (*S*)-configuration at the spiro center (Figure 14). From the crystal structure, it was observed that the guanidine groups at both five-membered rings unexpectedly prefer the exocyclic imino form, a fact that was explained by the expected ring strain of the five-membered rings, which should destabilize the amino form.

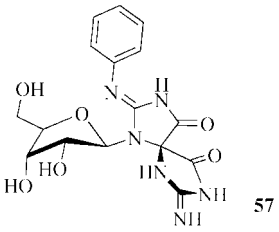
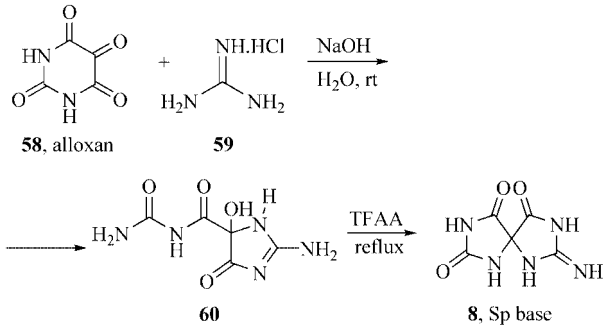


Figure 14. Crystal structure of a 8-arylimino-spiroiminodihydantoin.

3.1.1.4. Synthesis/Generation

When the structure of spiroiminodihydantoin was initially proposed, the spectroscopic data of the new compound were corroborated by independent synthesis of the aglycon.[126] Specifically, reaction of alloxan with guanidinium hydrochloride gave an ureido intermediate whose structure was established by X-ray crystallography. Acid-induced cyclization provided the Sp base, although yields were not reported (Scheme 12). The synthesized spiroiminodihydantoin heterocycle was characterized by ESI MS/MS, and the fragmentation pattern was found to be identical to that of AcSp.

The synthesis of the phosphoramidites of the *N*,*O*-diacetyl-protected diastereoisomers of dSp and their incorporation into synthetic oligonucleotides was reported before the correct assignment of the spiro structure was made.[132] This is the only reported separation of the two diastereoisomers, which was achieved for the 3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-dimethoxytrityl-protected compounds. Acetylation of the exocyclic amino and one of the amidic oxygens followed by selective deprotection of the TBDMS group allowed the introduction of the phosphoramidite in the 3′-position. The products were fully characterized by ^1H NMR spectroscopy ([D_6]DMSO) and FAB mass spectrometry. Each diastereoisomer was individually inserted into oligonucleo-



Scheme 12. Synthesis of the spiroiminodihydantoin heterocycle.

tides. The absence of significant epimerization at C-4 upon incorporation into the oligonucleotides was confirmed by HPLC and ESI MS studies.

3.1.1.5. Repair/Mutagenicity

The dSp lesion is recognized and repaired[152] by the *E. coli* BER glycosylase Fpg, by the yeast enzymes yOGG1 and yOGG2,[153] and by the mammalian glycosylase NEIL1[154] when paired with any of the four natural DNA bases in a duplex. NEIL1 belongs to the "Nei-like" family of BER enzymes[155] and is one of the mammalian homologues of Nei (or endonuclease VIII, endo VIII), the third *E. coli* DNA glycosylase.[156]

Subsequent studies[157,158] have shown that DNA polymerases are blocked by dSp, but G→C and G→T transversion mutations occur. It was found that this lesion is more effective at blocking the primer extension than a number of other lesions (dGh, dIa, dCa, dIz, dZ, 8-oxodGuo), but when bypassed is highly mutagenic.[159]

3.1.2. Guanidinohydantoin/Iminoallantoin

3.1.2.1. Formation

5-Guanidinohydantoin (**9**, dGh) is the major product of the one-electron oxidation of 8-oxodGuo in duplex DNA.[125,160] The formation of dGh in single-stranded oligonucleotides is favored by low temperatures.[157,161] At the nucleoside level, AcGh has been found to be the major product of the oxidation triggered by one-electron oxidants (e.g. IrX_6^-,[126,136,137] peroxynitrite at low fluxes,[138] HOCl, or the myeloperoxidase/H_2O_2/Cl^- system[115]) under mildly acidic conditions (vide infra). In a recent report, the nucleoside dGh was found to be the major product at room temperature, regardless of pH, in the one-electron oxidation of 8-oxodGuo (Figure 15).[162]

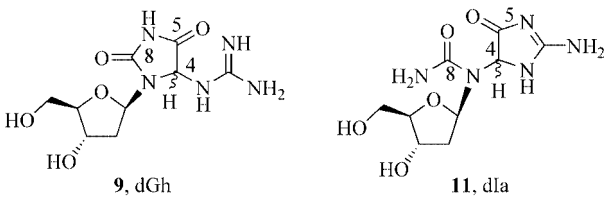


Figure 15. Structures of dGh and its associated dIa isomer.

dGh has also been observed in 8-oxodGuo-modified single- and double-stranded oligonucleotides treated with a chromium(v)–salen complex.[139,140] It was also the main product of the oxidation of a poly(GC) by a peroxochromium(v) species.[163]

3.1.2.2. Isolation/Characterization

A 5-guanidinohydantoin ribonucleoside was first reported as the two-electron, electrochemical oxidation product of 8-oxoGuo.[164] The assignment was based on GC-MS analysis of the hexasilylated derivative of the aglycon.

Gh is always reported as an inseparable mixture of diastereoisomers, which are considered to be in fast equilibrium due to enolization of the C-5 carbonyl function.[125,165,166] The possibility that the two closely observed HPLC peaks correspond to an inseparable mixture of Gh and Ia was initially proposed.[136] Specifically, the Burrows group considered that AcGh is involved in a pH-dependent equilibrium with its isomer iminoallantoin (Ia), each of them representing an approximately 1:1 mixture of two C-4 epimers.[136] They found that the interconversion of AcGh and AcIa is slow enough to permit HPLC separation, but too fast to allow complete characterization of the separate species. The 500 MHz ^{1}H NMR spectrum of the AcGh/AcIa mixture (CD_3OD) exhibits four singlets for the 4-H proton of the hydantoin base (entry 1, Table 5).

Recently, the same authors performed the singlet-oxygen oxidation of AcGuo isotopically labeled with ^{13}C at C-2 and with ^{15}N at N-1, N-2, and N-7 (Scheme 13).[129] The spectroscopic data revealed the formation of the two diastereoisomers of AcGh, with no evidence of its isomer AcIa.

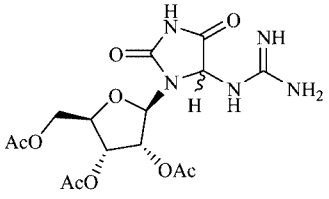
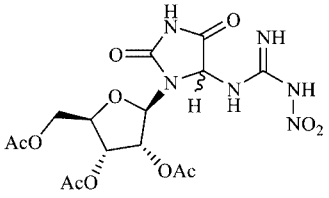
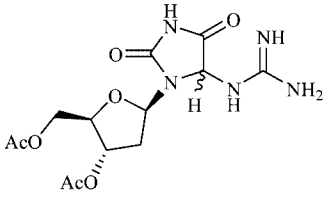
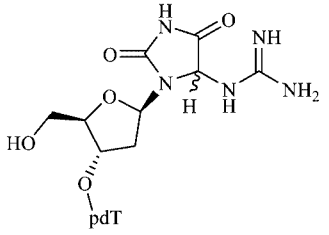
The labeled AcGh was characterized by ESI-MS/MS and ^{13}C and ^{15}N NMR spectroscopy in 10% D_2O at pH 4.5 and 7. The ^{13}C NMR spectrum exhibits two triplets (due to ^{13}C–^{15}N coupling) for the labeled C-2 atom of the guanidinium group, corresponding to the two diastereoisomers of the labeled AcGh (δ = 154.8 and 154.9 ppm). The splitting pattern of these signals proves the equivalency of the two labeled nitrogen atoms of the guanidinohydantoin structure.

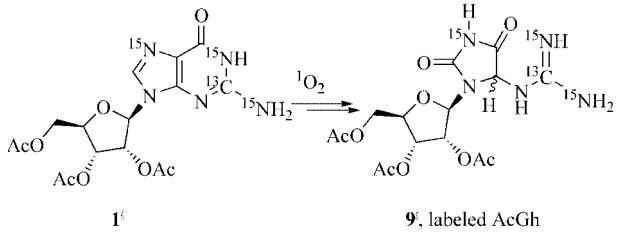
The same lesion can be obtained by $NaBH_4$ reduction of dehydroguanidinohydantoin (Gh^{ox}), an oxidized analogue of Gh. AcdGh and a nitro derivative NO_2-AcGh were isolated as diastereoisomeric mixtures from the corresponding AcdGh^{ox} and NO_2-AcGh^{ox} analogues. Initially, the AcdGh structure was assigned to a different structure where hydrolytic opening at N^9–C^4 was suggested (Scheme 14).[167]

AcdGh was characterized by ^{1}H and ^{13}C NMR spectroscopy, ESI-MS/MS, and hydrolysis to the aglycon, coupled with GC-MS analysis of the trimethylsilylated derivative. Both release the same aglycon, which corresponds to a pair of diastereoisomers (entry 3, Table 5). In a more recent paper the authors reassigned the structure of the intermediate to that of a normal dehydroguanidinohydantoin AcdGh^{ox}.[138]

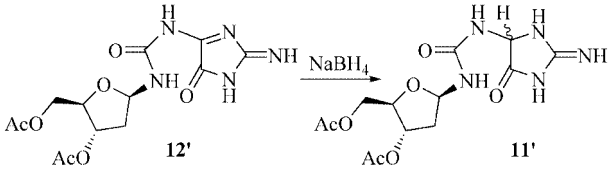
The stable reduction products of NO_2-AcGh^{ox} (entry 2, Table 5) have also been characterized[168] by ^{1}H and ^{13}C NMR spectroscopy. Additionally, the CD spectrum of the two peaks in HPLC exhibits mirror-image symmetry, which proves their diastereoisomeric relationship.

Table 5. Summary of ^{13}C and ^{1}H NMR signals (δ, ppm) at C-4 for reported Gh analogues.

Entry	Structure	H-4	C-4	Solvent	Ref.
1		5.739/5.760 5.736/5.750	66.23	CD_3OD	[136]
2		5.510 5.350	69.78 69.38	[D$_6$]DMSO	[168]
3		5.300 5.190	–	[D$_6$]DMSO	[167]
4		5.720 5.650	64.9 63.3	[D$_6$]DMSO	[165]



Scheme 13. Oxidation of a labeled AcGuo to AcGh.



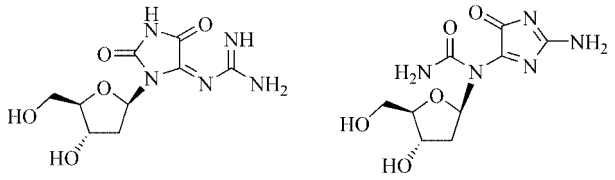
Scheme 14. Originally proposed AcdGh analogues.[167]

In a dinucleotide model, dGhpdT was isolated as a mixture of two isomers (entry 4, Table 5) from the reduction reaction of the corresponding dGh^{ox}pdT.[165] Attempted separation of the two diastereoisomers by HPLC was unsuccessful, since two different sets of singlets for the hydrogen and the carbon atom at the C-4 position of the base moiety were observed in the ^{1}H and ^{13}C NMR spectra, respectively ([D$_6$]DMSO). The ^{1}H NMR analysis determined that both C-4 hydrogen atoms exchange with D_2O, thus indicating a keto–enol equilibrium. A characteristic fragment found in the ESI-MS/MS of this lesion corresponds to the loss of the guanidinium group.

3.1.2.3. Related structures

Dehydroguanidinohydantoin/dehydroiminoallantoin: The 5-dehydroguanidinohydantoin (**10**, Gh^{ox}) lesion has been observed in the oxidation of dGuo with the Mn-TMPyP/$KHSO_5$ complex,[103,165,166] and of 8-oxoGuo by peroxynitrite[127,138,168] and singlet oxygen (Figure 16).[7,129,137,169,170]

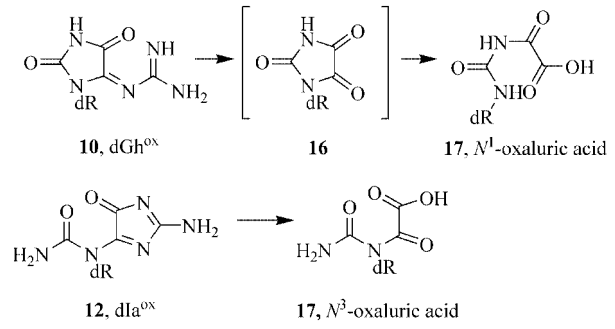
This product is not particularly stable under physiological conditions; its half-life in water is 5 h at 37 °C[170] and 8 h at 0 °C.[165] Meunier and co-workers have reported the first mass spectrometric characterization at the nucleoside level[100,103] as well as ESI-MS/MS and $H_2{}^{18}O$ labeling studies at the oligonucleotide level.[103]



10, dGh^{ox} **12**, dIa^{ox}

Figure 16. Structures of dGh^{ox} and dIa^{ox}.

A mild and fast experimental procedure has allowed the isolation of a dGh^{ox}-containing dinucleotide.[165,166] The lesion was characterized by ^{1}H NMR spectroscopy ([D$_6$]-DMSO), where three NH resonances were observed for the base at δ = 7.85, 8.22, and 9.08 ppm. Clear evidence in favor of the Gh^{ox} over the possible Ia^{ox} structure was that mild hydrolysis (Scheme 15) leads to the N^1- linear and not the "branched" N^3-oxaluric acid (see also Section 3.1.4.).[166] This experiment also corrected a previous assignment of the precursor of linear oxaluric acid.[136]

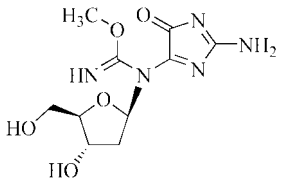


10, dGh^{ox} **16** **17**, N^1-oxaluric acid

12, dIa^{ox} **17**, N^3-oxaluric acid

Scheme 15. Possible hydrolysis of Gh^{ox} and Ia^{ox} leading to different isomers of oxaluric acid.

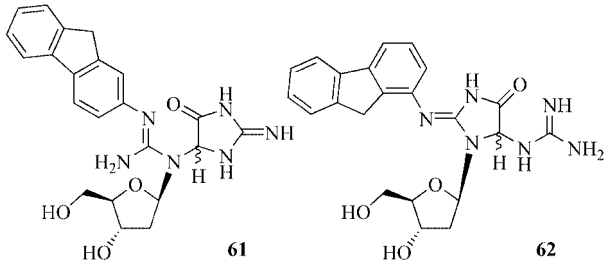
Interestingly, 8-methoxydIa^{ox} was recently detected by mass spectrometry as a product of the $^1O_2$ oxidation of 8-methoxydGuo (Figure 17).[98] The assignment was based on an MS/MS experiment, which lacked a previously reported characteristic fragment for dGh^{ox} ([BH + H – 42]$^+$ corresponding to $(H_2N)_2C{=}NH + O$).[170] Instead, a [BH + H – 57]$^+$ fragment was observed that can be accounted for by the loss of a $CH_3OC{=}NH$ group, a fragmentation possible only from the 8-MeO-dIa^{ox} isomer (Figure 17).



12'', 8-MeO-dIa^{ox}

Figure 17. Structure of 8-MeO-dIa^{ox}.

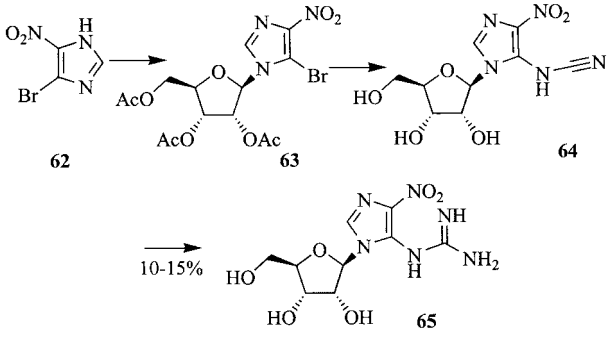
8-Aryliminoguanidinohydantoin/iminoallantoin Analogues: An iminoallantoin-related structure (**61**) was first proposed for the product of aerobic oxidation of an *N*-fluorene adduct of guanine.[147,171] The ^{1}H NMR spectrum exhibits a mixture of two diastereoisomers with two signals for the C-4 protons (δ = 5.44 and 5.48 ppm, D_2O). The first structure was considered based on the analogy with allantoin, the oxidation degradation product of uric acid,[172] although the guanidinohydantoin-related structure **62** could not be excluded (Figure 18).



61 **62**

Figure 18. Structures of 8-arylimino analogues of dIa (**61**) and dGh (**62**).

4-Guanidino-5-nitroimidazole: A 4-guanidino-5-nitroimidazole lesion has been isolated and characterized as one of the products of the peroxynitrite oxidation of AcGuo. The compound was characterized by ESI mass spectrometry and labeling studies with $O^{15}NOO^-$. To prove the assigned structure, the authors synthesized the authentic free riboside by an independent route (Scheme 16).[173]



62 **63** **64**

10-15%

65

Scheme 16. Reported synthesis of 4-guanidino-5-nitroimidazole.

3.1.2.4. Synthesis/Generation

To date, no independent synthesis of dGh-containing oligonucleotides has been reported. Therefore, alternative routes involving the specific oxidation of 8-oxodGuo-containing DNA by one-electron oxidants, followed by HPLC purification of the modified oligonucleotides, have been utilized.[125,161,174,175] To generate the dGh lesion, the temperature of the reaction mixture of the stock DNA solution in the presence of Na_2IrCl_6 was kept at 4 °C. The direct oxidation of dGuo in DNA by a high-valent chromium complex also leads to the formation of dGh, together with the dSp lesion.[139,154]

3.1.2.5. Repair/Mutagenicity

The dGh lesion can be recognized and efficiently removed by the *E. coli* Fpg[161] and Nei,[174] as well as by the mammalian BER glycosylases NEIL1 and NEIL2.[154] Biological studies in vitro have indicated that dG and dA are inserted opposite to dGh,[125,157] leading to G→T and G→C mutations. Furthermore, in vivo replication assays[158] have shown that the dGh lesion is highly mutagenic in *E. coli*, even more so than the parent 8-oxodG lesion, and causes G→C transversions almost exclusively.

3.1.3. Cyanuric and Carboximidamide Cyanuric Acid

3.1.3.1. Formation

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)cyanuric acid (**14**, dCa) is the major product isolated from the photosensitized oxidation of 8-oxodGuo under conditions where a type-II ($^1O_2$) mechanism operates (Figure 19).[93] The formation of this lesion has also been observed in the peroxynitrite oxidation of 8-oxodGuo[127,167] and 8-NO_2-Guo[176] and of 8-oxodGuo in oligonucleotides (single- and double-stranded) treated with high (>500 μM) concentrations of peroxynitrite.[177,178]

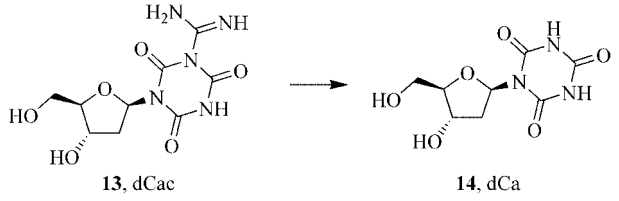


Figure 19. Structures of dCac and dCa.

3.1.3.2. Isolation/Characterization

The formation of a *tert*-butyldimethylsilyl-protected ribose derivative of cyanuric acid was first observed by Foote as a minor product in the $^1O_2$ photooxidation of a protected 8-oxoGuo.[124] The isolation and structural analysis of dCa and its precursor, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)tetrahydro-2,4,6-trioxo-1,3,5-triazine-1(2*H*)-carboximidamide (dCac) were later reported by Cadet and co-workers (Figure 19).[93]

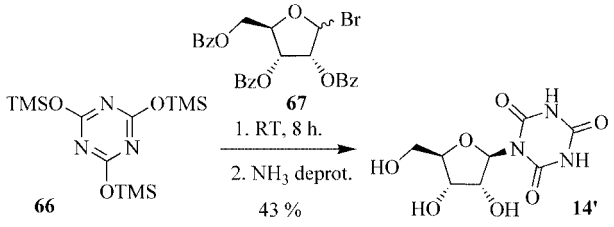
An interesting feature in the ^1H NMR spectrum of these lesions is the multiplicity of the anomeric proton (H-1′), which appears as a doublet of doublets, of unequal coupling constants. This is characteristic of the ^1H NMR spectra of 2′-deoxynucleosides that adopt a *syn*-like conformation and is evidence for a conformation with one of the carbonyl group leaning over the ribose ring.[179]

Cadet and co-workers identified an unstable precursor in the formation of dCa, although the structural assignment of this precursor was not so straightforward. Its ^1H NMR spectrum (D_2O) is almost identical to that of dCa, with only a small shift (<0.1 ppm) of the anomeric proton. On the other hand, the spectrum registered in [D_6]DMSO exhibits a very broad resonance at δ = 9.35 ppm that integrates for four protons. In addition, a broad ^{13}C resonance is observed at δ = 155.3 ppm, assigned as the guanidino C=NH. The signal broadening was accounted for by the electric quadrupole moment of the three vicinal nitrogen atoms. The FAB mass spectrum of this lesion was only possible after a short acetylation step and the insertion of two acetyl groups on the base moiety.

This compound is rather unstable and decomposes to dCa (a few days in water solution at pH 7, 15 h in [D_6]-DMSO and 1 min in buffer pH 13 solution). This was considered as additional evidence for the six-membered ring structure of dCac. Furthermore, the ESI-MS/MS experiments on AcdCac[167] showed a fragment corresponding to the ion expected for the stable derivative of cyanuric acid.

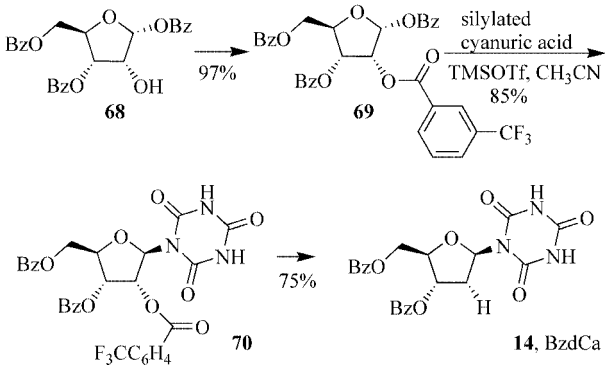
3.1.3.3. Synthesis/Generation

1-(β-D-Ribofuranosyl)cyanuric acid (**14′**, Ca) was first synthesized by Winkley and Robins[180] by treatment of silylated cyanuric acid with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (Scheme 17). Conformational studies by NMR spectroscopy were reported in a series of papers.[181–183]



Scheme 17. Independent synthesis of Ca nucleoside.

To investigate the biological and structural significance of the dCa residue in DNA, the corresponding modified 2′-deoxynucleoside was prepared chemically[184] and then site-specifically incorporated into oligoribonucleosides. A 1:2 mixture of α and β anomers was obtained after the glycosylation reaction of silylated cyanuric acid with 3,5-di-*O*-toluoyl-D-ribofuranosyl chloride in the presence of potassium nonafluorobutanesulfonate. The separation of the two anomers was achieved after deprotection of the two hydroxy groups followed by dimethoxytritylation of the 5′-OH function. Both anomers were characterized by ^1H and ^{13}C NMR spectroscopy. The oligonucleotides were synthesized on solid supports using phosphoramidite chemistry.



Scheme 18. Independent synthesis of dCa nucleoside.

Rizzo and co-workers have described the synthesis of *N*-(3,5-dibenzoyl-2-deoxy-β-D-erythropentofuranosyl) cyanuric acid by photoinduced electron-transfer deoxygenation of a 2′-(*m*-trifluoromethylbenzoyl) derivative (Scheme 18). The ^{1}H and ^{13}C NMR and FAB-HR mass spectra corroborated the structure.[185]

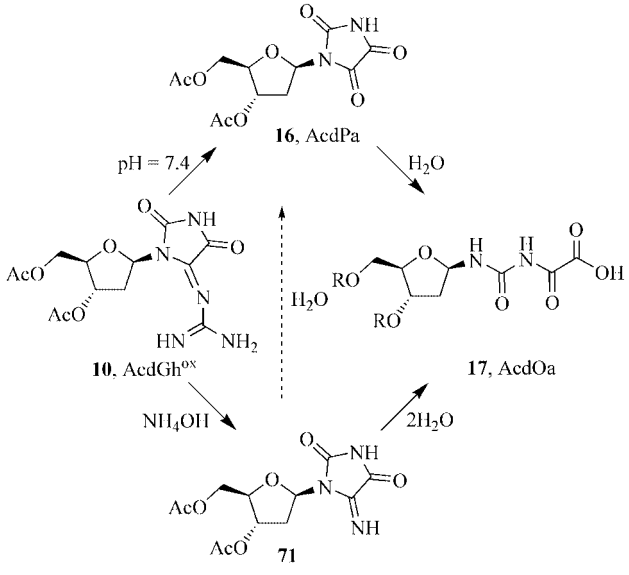
3.1.3.4. Repair/Mutagenicity

Biological repair studies with damaged DNA show that the cyanuric acid lesion is not recognized by either Fpg[186] or endo III.[184] Although the lesion is cleaved by nuclease P_1, it is resistant in the presence of both snake-venom phosphodiesterase (SVPDE, 3′-exo) and calf-spleen phosphodiesterase (CSPDE, 5′-exo), in combination with bacterial alkaline phosphatase. Cyanuric acid does not block in vitro DNA synthesis, and primer extension experiments with Kf by the "one-nucleotide extension assay" determined that dAMP (86%),[178] as well as dGMP to a lesser extent, are incorporated opposite to cyanuric acid, leading to mutagenic G to T and G to C transversions.[184] Recently, it has been found that human methylpurine DNA N-glycosylase (Mpg) can remove cyanuric acid from DNA duplexes.[187]

3.1.4. Parabanic and Oxaluric Acid

3.1.4.1. Formation

Parabanic (**16**, trioxoimidazolidine) acid and its hydrolysis product oxaluric acid (**17**, tetrazolylacetic acid) nucleosides are considered secondary oxidation products arising primarily from 8-oxoGuo oxidation (Figure 20). Parabanic acid was first identified by Sheu and Foote[124] as one of the major products of the dye-sensitized photooxidation of an 8-oxoGuo derivative. Parabanic acid has also been observed in the peroxynitrite oxidation of an 8-oxodGuo derivative[167] and 8-NO_2-Guo,[176] as well as in the oxidation of dGuo base in oligonucleotides by Mn-TMPyP/$KHSO_5$.[103] After 15 min incubation at 90 °C parabanic acid is converted into oxaluric acid.



Scheme 19. Hydrolysis of AcdGh^{ox} to AcdOa through AcdPa or 4-iminoAcdPa.

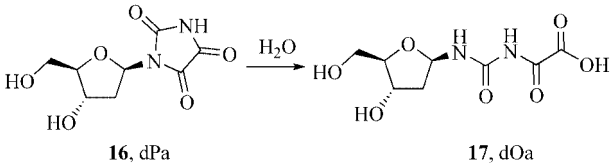


Figure 20. Structures of dPa and dOa.

Oxaluric acid is generated as a final, stable product when 8-oxodGuo-containing oligonucleotides (single- and double-stranded) are treated with low (<100 μM) concentrations of peroxynitrite[177,188] or photo-oxidized under singlet-oxygen generation conditions.[170,189] It is also observed as a product of superoxide radical addition to the oxidizing purine radical that arises from the deprotonation of initially produced radical cations of guanine.[190] The same stable lesion is also formed in the oxidation of a dGuo base by Mn^V=O in a dinucleotide.[165,166,191]

3.1.4.2. Isolation/Characterization.

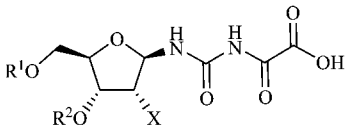
Parabanic Acid Derivatives: The parabanic acid nucleoside is considered to be a labile lesion that quickly hydrolyzes to oxaluric acid. The only example where a parabanic acid has been fully characterized by FAB mass spectrometry as well as ^{1}H and ^{13}C NMR spectroscopy in [D_6]acetone at 0 °C (entry 1, Table 7) is in the case of a *tert*-butyldimethylsilyl-protected ribonucleoside of parabanic acid that was isolated by Foote and co-workers.[124] More recently, the same authors[120] reported the same oxidation product as the result of singlet-oxygen photooxidation of a guanosine derivative and showed that, as in the previous case, this product forms through further oxidation of an initially formed 8-oxoGuo derivative. The product was characterized by comparison with the previously acquired data, although the ^{1}H NMR signal of H-1′ in CD_2Cl_2 was also reported.

The group of Tannenbaum has characterized the structure of AcdPa by ESI mass spectrometry and UV spectroscopy, as well as by hydrolysis to the aglycon and comparison with authentic samples. AcPa has also been reported as an intermediate in the hydrolysis of dehydroguanidinohydantoin (Gh^{ox}) upon incubation in 150 mM KH_2PO_4, pH 7.4 buffer. The final product of the hydrolysis under the same conditions was oxaluric acid.[167]

Oxaluric Acid Derivatives: As previously mentioned, oxaluric acid nucleosides have been observed as the hydrolysis products of either the major, less-stable intermediate Gh^{ox} or its aminolysis product 5-iminoimidazolidine-2,4-dione, and also by hydrolysis of the minor parabanic acid (Scheme 19). The 5-iminoimidazolidine-2,4-dione intermediate was identified by HRMS-MS experiments, although its hydrolytic instability precluded its characterization by NMR spectroscopy.

AcOa has been characterized by ^{1}H and ^{13}C NMR spectroscopy in [D_6]DMSO, (entry 1, Table 6) as well as UV spectroscopy (H_2O), ESI mass spectrometry, and MS/MS.[167,168]

Table 6. Summary of the ^{1}H NMR resonances (δ, ppm) of the base protons for the reported oxaluric acid analogues.

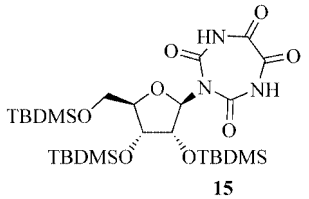
Entry		COOH	NH	NH	Ref.
1	$R^1 = R^2 = Ac$, X = OAc	9.81	7.04	8.87 (J = 9.3 Hz)	[167,168]
2	R^1 = H, R^2 = pdT, X = H	9.4	9.61	8.59 (9.4 Hz)	[191]
3	$R^1 = R^2 = Ac$, X = OAc	10.01	7.12	8.87 (J = 9.0 Hz)	[192]

In a dinucleotide model, the oxaluric acid produced from the hydrolysis of a Gh^{ox} precursor (65 °C, 30 min) has been characterized by ^{1}H NMR spectroscopy in [D$_6$]DMSO (entry 2, Table 6) as well as by ESI mass spectrometry and MS/MS. The $^3J_{H,NH}$ coupling between the anomeric proton and one of the NH groups confirmed the linear structure.[166] It was also possible to distinguish between the two possible linear forms (by N^9–C^8 or N^9–C^4 bond cleavage of the parabanic heterocycle) based on the MS/MS results, which favored the latter structure. Specifically, one fragment accounts for the loss of CO-COOH and the other for the amino-substituted nucleoside.[191]

The oxaluric acid structure has been detected in single-stranded oligonucleotides by MALDI-TOF, ESI-MS, and MS/MS, as well as by isotope labeling with $^{18}O_2$-enriched singlet oxygen and $H_2{}^{18}O$ hydrolysis.[170] It was shown that the conversion of Gh^{ox} to oxaluric acid is slower (24 h) in a 15-mer than in a 3-mer (10 h) at 37 °C.

3.1.4.3. Related Structures

1,3,5-Triazepane-2,4,6,7-tetraone (15): This seven-membered nucleoside (Figure 21) was isolated by Foote and co-workers in the $^1O_2$ oxidation of a protected 8-oxoGuo.[124] It was characterized by ^{1}H NMR spectroscopy and FAB mass spectrometry. Although the compound has been isolated more recently by the same group under similar conditions,[120] it has not been observed in any oxidation conditions in aqueous solution.

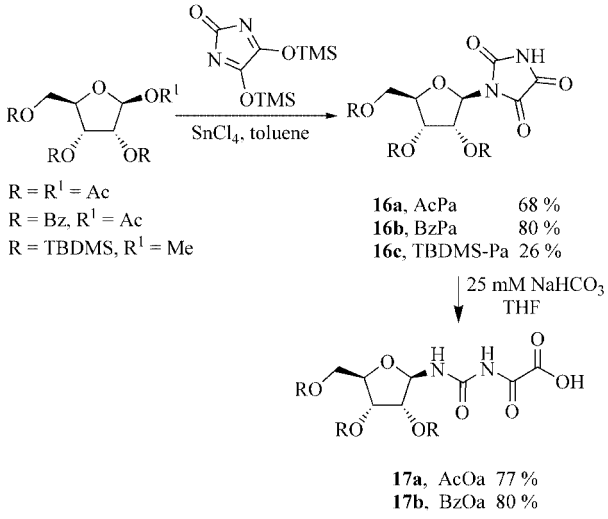


15

Figure 21. Structure of **15**.

3.1.4.4. Synthesis/Generation

The first independent synthesis of Pa and Oa derivatives[192] entailed Vorbrüggen-type *N*-glycosylation of benzoylated and acetylated riboses with a silylated derivative of the parabanic acid heterocycle (Scheme 20). The conditions for the silylation of parabanic acid were found to be critical as this heterocycle has the tendency to polymerize. At the same time, care has to be taken during the *N*-glycosylation step in order to avoid the formation of the product of double addition to the parabanic moiety. The desired nucleoside products could be differentiated from the dimer spectroscopically and chemically by *N*-methylation in the presence of CH_2N_2.

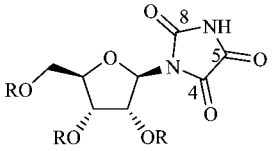


Scheme 20. Independent synthesis of Pa and Oa nucleosides.

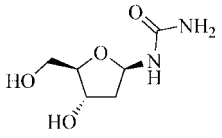
The structure **16c** (TBDMS-Pa, Scheme 20) corresponds to the compound isolated and characterized by Foote and co-workers.[193] The spectroscopic data of the originally reported compound were in complete agreement with the independently synthesized species, thus corroborating the proposed structure.

Hydrolysis of the parabanic heterocycle under mildly basic conditions provided the corresponding oxaluric acid nucleosides. Structure **16a** (AcPa) corresponds to the product isolated by Tannenbaum and co-workers.[168] The ^{1}H NMR, ^{13}C NMR, and ESI-MS data of the reported compound were in complete agreement with the data obtained from the independently synthesized species. Hydrolysis of the parabanic nucleoside under more vigorous conditions provided the corresponding urea nucleosides (Table 7).[192]

Table 7. Summary of the ^{13}C NMR signals (δ, ppm) of the parabanic base for the reported parabanic acid analogues.

Entry		C-4	C-5	C-8	Solvent	Ref.
1	R = TBDMS	158.07		153.95	[D$_6$]acetone	[124]
2	R = TBDMS	157.24	157.35	153.04	[D$_6$]acetone	[192]
3	R = Ac	154.13		150.52	[D$_6$]DMSO	[192]
4	R = Bz	156.24	156.16	151.66	[D$_6$]DMSO	[192]

Authentic oligonucleotides containing oxaluric acid have been generated by peroxynitrite[178,188] or singlet-oxygen[170,189] treatment of 8-oxodGuo-containing DNA. The HPLC purification of the DNA mixture afforded the pure lesion-modified oligonucleotides.



18, dUa

Figure 22. Structure of dUa.

3.1.4.5. Repair/Mutagenicity

Cadet and co-workers have studied the repair mechanisms and mutagenic potential of oxaluric acid adducts in vitro.[189] Nucleotide insertion opposite oxaluric acid, catalyzed by Kf exo$^-$ and *Taq* polymerase, indicated that oxaluric acid can induce G→T and G→C transversions, but blocks the primer extension in the case of DNA polymerase β. Repair experiments showed that oxaluric acid is efficiently cleaved by Fpg and endo III. Biological studies[159] in vivo have shown that oxaluric acid is bypassed efficiently by *E. coli* DNA polymerase and causes mainly G→T transversions.

3.1.5. Ureido Nucleosides

3.1.5.1. Formation

The urea lesion is a well-known degradation species of thymine oxidation via thymine glycols.[194–196] In the oxidation of guanosine derivatives it is formed during electrochemical oxidation of guanosine[164,197] and hydrolysis of oxaluric acid by addition of Mg^{2+} and Ca^{2+} salts with HCO_3^-.[159] In oligonucleotides, dUa (**18**, Figure 22) has been observed after short thermal treatment of a pre-oxidized DNA fragment by Mn-TMPyP/$KHSO_5$.[103] This lesion was also observed after hydrolysis of a spiroaminal adduct formed by oxidation of 8-oxodGuo-containing oligodeoxynucleotides in the presence of spermine (vide supra).[151]

3.1.5.2. Isolation/Characterization

As a guanosine degradation lesion, urea riboside was first isolated from the electrooxidation of guanosine at pH 3.[164] The characterization was performed by mass spectrometry and silylation followed by GC-MS analysis of the tetrasilylated urea riboside, respectively. Oligodeoxynucleotides containing dUa from guanosine oxidation have been characterized by mass spectrometry.[103,151]

3.1.5.3. Independent Synthesis/Generation

This lesion was successfully generated by thymine oxidation, and complete ^1H and ^{13}C NMR spectroscopic data for dUa and its 5′-*O*-monomethoxytrityl derivative were recorded. Since no NMR characterization has been reported for urea from guanosine oxidation, these results are presented here.

5′-*O*-MMT-dUa was isolated from the alkaline hydrolysis of the corresponding thymidine glycol as a mixture of α- and β-anomers, with the α-anomer predominating (approx. 70%) in the freshly prepared mixture (Table 8).[195,196,198] An early work erroneously assigned the isomers as a mixture of *cis* and *trans* amides.[199] The ^1H NMR spectra show two sets of signals for the sugar moiety and for the OH, NH, and NH_2 protons, thereby indicating the presence of two isomers. The characteristic coupling between the anomeric proton and the NH group was also observed (J = 10 Hz for both anomers).[198] Complete characterization of the minor isomer was achieved by warming the mixture to 55 °C to increase the percentage of the minor isomer (50:50). The ^{13}C NMR spectrum clearly exhibits two sets of carbon resonances for the sugar moiety (e.g. C-1′: δ = 81.71 and 81.63 ppm). The urea carbon atom is found in the δ = 114–159 ppm region together with carbon signals corresponding to the MMT group.[198]

Interestingly, the α-anomer was identified as the major product by different synthetic methods, even when starting from β-thymidine. The two anomers were separated but each compound led back to the original mixture within two hours at 45 °C.[196]

Table 8. Summary of the ^{1}H NMR resonances for the urea analogues.

		H-1'	H-2',2''	H-3'	H-4'	H-5',5''	NH	NH_2	Ref.
1	α	5.75	1.78 2.37	4.25	4.10	3.11	–	–	[195] [a]
	β		1.93 2.08		3.92		–	–	
2	α	5.68–5.88	1.74–2.43	4.18–4.30	4.05-4.15	3.07-3.18	–	–	[196] [a]
	β				3.82-3.92		–	–	
3	α	5.62[b]	2.33 1.72	4.14	4.02	3.03	6.65	5.90	[198] [c]
	β	5.58[b]	1.95 1.83	4.14	3.80	3.03	6.67	5.74	

[a] Spectrum reported in CD_3OD. [b] Chemical shifts in [D_6]DMSO +D_2O at 55 °C. [c] Spectrum reported in [D_6]DMSO.

The ^{1}H NMR spectrum of free dUa[198] shows a complex mixture since the free 5′-OH group induces an isomerization to a pyranosyl sugar, as has been described already for the FaPydAdo and FaPydGuo systems (vide supra). Consequently, the α- and β-anomers of both ribofuranosyl and ribopyranosyl urea are observed in the spectrum. Specifically, the spectrum exhibits four singlets for the NH_2 group and three doublets for the NH proton. A similar mixture was first reported from an attempt to synthesize dUa independently by the acid-catalyzed condensation of 2-deoxyribose with urea.[200]

It is rather unexpected that no isomeric mixture of dUa was reported by Saladino et al. after the isolation of this lesion by column chromatography from the formamide degradation of 2′-deoxycytidine.[201]

All the above evidence indicates that the oligonucleotides reported to contain *cis*/*trans* amide isomers of deoxyribosyl urea residues[195,199] actually contain an α/β anomeric mixture and, in addition, their synthesis must have been hampered by the furanose/pyranose isomerization after 5′-MMT deprotection.

3.1.5.4. Repair/Mutagenicity

Studies in vivo have demonstrated that Ua efficiently blocks *E. coli* DNA polymerase-mediated replication.[159] Nearly all mutations caused by this lesion are in the form of G→T transversions. A different distribution of mutation types is observed in SOS-induced cells, with 43% G→T, 46% G→C, and 10% G→A transversions.

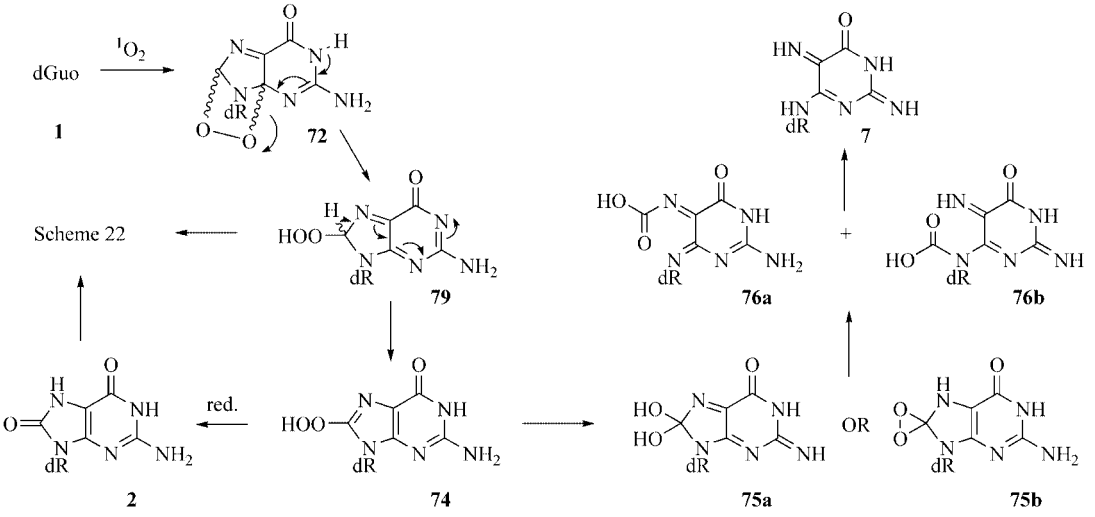
4. Conclusions

We have described the major and minor, primary and secondary oxidative DNA lesions that arise from the oxidation of guanine under a variety of conditions and have presented all the relevant information regarding the isolation, characterization, and independent synthesis or generation of these lesions in synthetic oligonucleotides. Finally, we have provided information on the biological studies that have been advanced based on the basic molecular information obtained by the aforementioned studies. It is clear that solid characterization of the proposed intermediates, precursors, and final stable structures is necessary in order to be able to draw substantive conclusions on their possible biological significance.

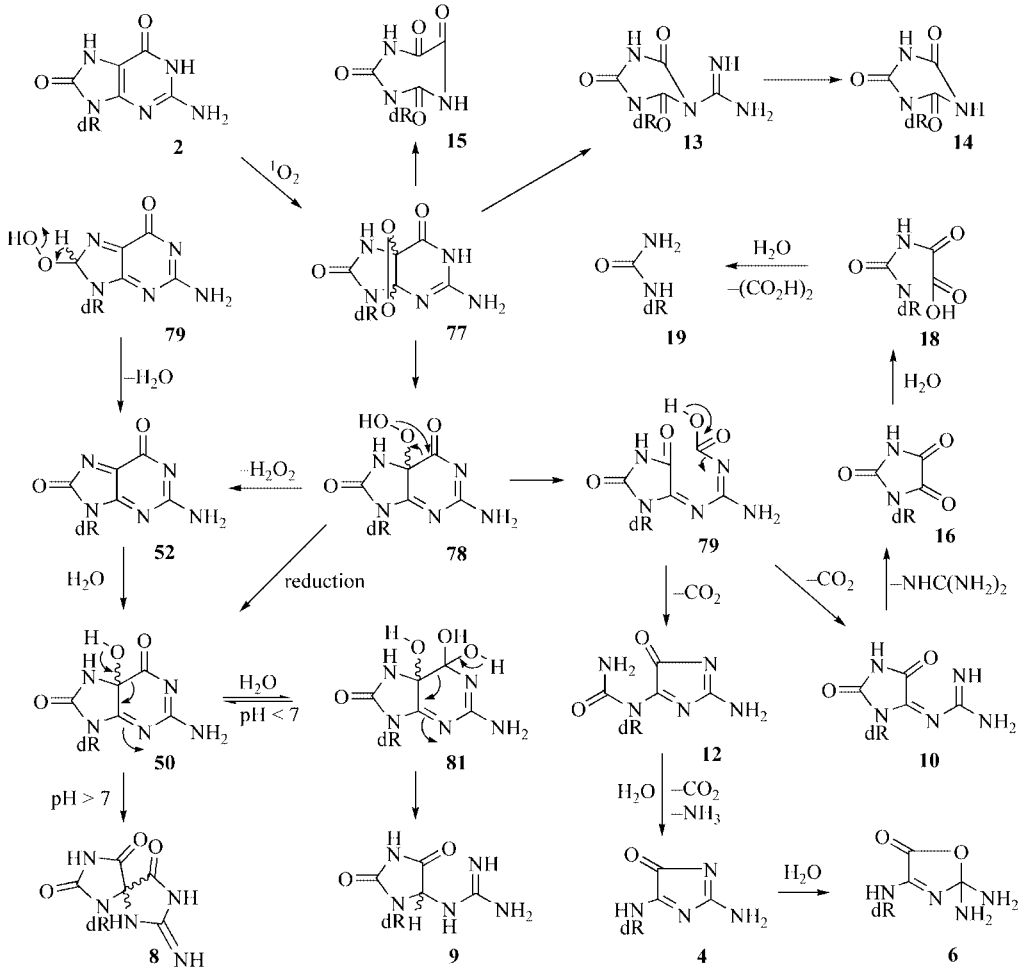
We believe that we still lack the information that will allow us to draw a comprehensive mechanistic scheme that will interconnect all observed intermediates with their appropriate precursors. This is one of the reasons, apart from lack of space, that we have not provided all the mechanistic proposals that have appeared in the literature. As each mechanistic scheme depends on the oxidation agent applied, a comprehensive scheme is further complicated by the plethora of reported oxidative conditions and the intricacies of the reagents used in each case. At the same time, it is

now clear that the microenvironment of the double helix favors and induces certain mechanistic pathways over others, further complicating the total picture.

Of all oxidative conditions described, the reaction of singlet oxygen with dGuo appears to be the system best understood to date. Through detailed mechanistic studies[120–122,124,130,193] and the utilization of clean chemical sources of $^1O_2$,[7] it has been determined that this species exhibits only two modes of reactivity, specifically a [4+2] cycloaddition onto the imidazole ring of guanine and a



Scheme 21. Proposed mechanism for the formation of the primary products of $^1O_2$ oxidation of dGuo.



Scheme 22. Proposed mechanism for the formation of secondary products of $^1O_2$ oxidation of 8-oxodGuo.

[2+2] cycloaddition with the 4,5-C=C bond of 8-oxoGua. At the same time, through low-temperature studies, Foote and co-workers have characterized a number of postulated intermediates. Therefore, a comprehensive mechanistic scheme can be drawn for the singlet-oxygen oxidation of dGuo, as presented in Schemes 21 and 22.

It should be mentioned that the intermediates leading to product **7**, as well as other intermediates (such as **77**), have only been observed in low-temperature studies in organic solvents. Also, it appears that the fate of species **79**, as shown in Scheme 22, is mainly its rearrangement to dSp, which is the major product of $^1O_2$ oxidation of dGuo in a number of systems.[123,128,130] Nevertheless, it is difficult to quantify the yield of each route as this is highly dependent upon the specific system that undergoes oxidation.

As more lesions become readily accessible and their properties clearly defined, we can expect that their biological significance will be revealed. The tools for future biological studies will be provided by progress in the area of characterization and independent synthesis or generation of these lesions and their precursors.

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