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Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribose in nucleic acids

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Abstract

A broad scientific community is involved in investigations aimed at delineating the mechanisms of formation and cellular processing of oxidatively generated damage to nucleic acids. Perhaps as a consequence of this breadth of research expertise, there are nomenclature problems for several of the oxidized bases including 8-oxo-7,8-dihydroguanine (8-oxoGua), a ubiquitous marker of almost every type of oxidative stress in cells. Efforts to standardize the nomenclature and abbreviations of the main DNA degradation products that arise from oxidative pathways are reported. Information is also provided on the main oxidative radicals, non-radical oxygen species, one-electron agents and enzymes involved in DNA degradation pathways as well in their targets and reactivity. A brief classification of oxidatively generated damage to DNA that may involve single modifications, tandem base modifications, intrastrand and interstrand cross-links together with DNA-protein cross-links and base adducts arising from the addition of lipid peroxides breakdown products is also included.

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Keywords

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Introduction

Formation of reactive oxygen species (ROS) is ubiquitous in living organisms due to the occurrence of oxidative metabolism through mitochondrial respiration [1]. The superoxide anion radical ($O_2^{\bullet -}$) thus generated and other derived species such as hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) may be implicated in intracellular cell signalling [2] while their potentially deleterious effects are usually prevented by defence systems involving specialized enzymes (superoxide dismutase, catalase) and anti-oxidants [34]. Overproduction of ROS and reactive nitrogen species (RNS) including nitric oxide has been shown to be associated with physical exercise, ischemia/reperfusion, chronic inflammation, atherosclerosis, type 2 diabetes, neurodegenerative disorders and cancer [5–7]. Ionizing radiation, solar light and various xenobiotics once metabolized are also able to induce oxidative stress [8,9]. Since DNA is the main cellular target for several of the deleterious effects in biology including mutagenesis and ultimately carcinogenesis, major efforts have been devoted during the last two decades to better understand the mechanisms of oxidative degradation of nucleobases [10–16] and 2-deoxyribose of DNA [11,17]. In addition, numerous attempts have been made to monitor the formation of oxidatively generated base and sugar damage in cellular DNA using chromatographic methods, immunoassays and enzymatic based approaches (for a recent review see [18]). Indirect assessment of oxidatively damaged DNA in humans has been achieved by the non-invasive measurement of several oxidized bases and nucleosides in urine, plasma and saliva [19,20]. Relevant information has also been gained on the removal of oxidatively damaged DNA that for single modified bases and abasic sites is predominantly mediated by base excision repair [21–24]. In addition, the mutagenic features of most oxidized bases and several clustered DNA lesions have been determined [21,24–26]. Insights into the ability for translesional synthesis polymerases to bypass blocking DNA lesions have also been gleaned [26]. These few examples demonstrate how the topic of chemical biology of DNA oxidation is broad involving both fundamental and applied aspects with increasing clinical applications. This explains, at least partly, inconsistencies that may be noted in the designation of several DNA modifications including 8-oxoGua. The inappropriate use of the term ‘oxidative’ for qualifying oxidized bases is another issue that is discussed in this article. A lack of relevant mechanistic information may be frequently noted in the discussions concerning the assessment of oxidative stress that is often based on the elevation of levels of ROS! In this respect most of the assays available for measuring ROS are not sufficiently specific [27], thus limiting the relevance of the information gained from the ROS determinations. The aim of this article is to provide recommendations to scientists involved in the chemistry, biochemistry and biology of oxidatively damaged DNA in order to ensure a better uniformity in the field. This constitutes an extension of a previous account that was mostly focused on nomenclature and terminology issues [28].

Oxidizing species and agents

Radicals (one-electron oxidants)

Superoxide radical—$O_2^{\bullet -}$ is enzymatically generated in cells by one-electron reduction of molecular oxygen and may also be produced chemically by the reaction of O_2 with either radiation-induced solvated electrons or radical anions generated by photosensitizers. $O_2^{\bullet -}$ that is in dynamic equilibrium with the conjugate acid $HO_2^{\bullet}$, a better oxidant [29], which

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predominantly exists as an anion in neutral aqueous solution ($pK_a = 4.8$) [30]. While $O_2^{\bullet -}$ does not exhibit any detectable reactivity toward DNA components in aqueous solutions, several reactions have been observed with oxidatively formed DNA base radicals. Thus, $O_2^{\bullet -}$ appears to reduce thymine peroxyl radicals [31] in model studies, giving rise to related hydroperoxides after protonation [32]. Other examples of reactions of $O_2^{\bullet -}$ that are likely to be biologically relevant concern the oxidizing guanine radical G(–H)$^\bullet$ arising from deprotonation of the guanine radical cation [33]. The strongly oxidizing G(–H)$^\bullet$ predominantly undergoes reduction in double stranded DNA fragments together with the addition of $O_2^{\bullet -}$ to the C5 of the guanyl radical [33]. Similar reactions have also been observed for the oxidizing tyrosyl radical [34].

Hydroxyl radical—$^\bullet$OH is mostly generated in cells through the Fenton reaction [29]. This implies disproportionation of $O_2^{\bullet -}$ by superoxide dismutase to give oxygen and hydroperoxide peroxide (H_2O_2), in the first step, and subsequent reduction of H_2O_2 by transition active metal ions, such as Fe^{2+} and Cu^+. Another source of $^\bullet$OH is provided by the radiolysis of water molecules, commonly referred as to the indirect effect of ionizing radiation [11]. The reactivity of $^\bullet$OH is very high with the nucleobases and 2-deoxyribose moieties of DNA by either addition to the 5, 6-double bonds of pyrimidine bases and the C8 of purine bases or hydrogen abstraction from the methyl group of thymine, 2-amino group of guanine and 2-deoxyribose [11,35].

Alkoxyl radicals—RO$^\bullet$ are usually produced by the reaction of organic hydroperoxides with reduced transition metals such as Fe^{2+} according to organic Fenton-like reactions [11]. One of the main expected reactions is one-electron abstraction.

Organic peroxyl radicals—Organic ROO$^\bullet$ may be formed either by O_2 addition to carbon centred radicals or one-electron oxidation of hydroperoxides by Fe^{3+}. The reaction of most organic peroxyl radicals with DNA are expected to be minor because of their low concentrations in cells and moderated reactivity compared to $^\bullet$OH. The situation is quite different for peroxyl radicals produced within the DNA chain by the initial reaction of $^\bullet$OH or one-electron oxidants with pyrimidine bases. For example, peroxyl radicals derived from pyrimidine bases have been shown to undergo addition to either the adjacent purine guanine base [36] or pyrimidine bases [37], thereby, leading to the formation of intra-strand tandem base lesions.

In addition, there is indirect evidence for the occurrence of one-electron oxidation of vicinal guanine bases by pyrimidine peroxyl radicals in isolated DNA exposed to $^\bullet$OH [38].

Non-radical oxidants

Hydrogen peroxide—H_2O_2, which arises mostly in cells by enzymic dismutation of $O_2^{\bullet -}$ does not exhibit any reactivity in the absence of reduced transition metals with DNA with the exception of a minor oxidation reaction of adenine at N1 [39]. It should be pointed out that H_2O_2, the precursor of $^\bullet$OH, may diffuse like O_2 within cells and across membranes.

Ozone—O_3 is a major environmental pollutant generated in the atmosphere by complex photoreactions. In aqueous solutions, O_3 is a highly reactive ROS that has been predominantly shown to oxidize thymine and cytosine through initial [2 + 4] cycloaddition to the 5,6-pyrimidine bond. This leads to ureides and pyrimidine rearrangement compounds such as 5-hydroxy-5-methylhydantoin and 5-hydroxyhydantoin lesions via transient Crieggée ozonide intermediates [39]. However, in lung tissues, O_3 is mainly converted through ascorbate-mediated deactivation into $^1O_2$ [40]. It is now well documented that O_3 inhalation leads to enhanced cellular iNOS-derived $^\bullet$NO formation in the lung through

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activation of NFk-B [41]. It may be added that the validity of the proposal that O_3 is generated endogenously in arteriosclerotic plaques [42] has been questioned [43,44].

Singlet oxygen—$^1O_2$ is efficiently generated in cells upon UVA irradiation through a type II mechanism involving energy transfer from the excited endogenous photosensitizers to O_2 [14]. This mechanism is relevant for *ex vivo* studies and UV-exposed parts of the body (skin and eye), whereas it seems to be unlikely as an important mechanism for ROS generation in internal organs and circulation. Chemical generation of $^1O_2$ in cells through the Russell mechanism [45] that involves the dismutation of peroxyl radicals is an unlikely process in cells. $^1O_2$ has been shown to specifically react with guanine among nucleobases giving rise to the overwhelming formation of 8-oxoGua in cellular DNA without generating detectable amounts of single strand breaks [46]. However, whether or not such reactions occur *in vivo* remains to be elucidated. Evidence has been provided for the generation of $^1O_2$ by the reaction of HOCl with H_2O_2 and lipid hydroperoxides through, in the latter case, generation of peroxyl radicals [47] followed by their dismutation according to the concerted Russell mechanism [45]. However, evidence that this reaction occurs in cells is also awaiting further experiments.

Hypochlorous acid—The potent oxidant HOCl (p*K*a 7.4) is produced in neutrophils during inflammation through the activation of myeloperoxidase, which catalyzes the reaction of chloride anion with H_2O_2 as part of the defence system against microorganisms [48]. HOCl and hypochlorite (OCl^-), its conjugate base, are efficient chlorinating agents of DNA and RNA nucleobases in both model compounds and cells [49–51]. Similar halogenation reactions to DNA are also mediated, albeit with a lower efficiency, by *N*-chloramines that arise from the reaction of HOCl with amino acids [51]. The ability for HOCl to oxidize cellular DNA remains to be established because the enhanced levels of oxidized pyrimidine bases observed by gas-chromatography-mass spectrometry measurements [52] are questionable due to the unreliability of the method of analysis [18].

Peroxynitrite—The term peroxynitrite refers to peroxynitrite anion ($ONOO^-$) and its conjugated acid form, peroxynitrous acid (ONOOH) with a p*K*a of 6.8 [53]. Peroxynitrite is a major oxidizing and nitrating biological agent [18]; it is formed at sites of inflammation by the very fast reaction between $O_2^{\bullet-}$ and macrophage-derived nitric oxide ($^\bullet NO$ with also the use of NO) [54]. The latter reaction further emphasizing the importance of SOD for detoxifying the almost poorly reactive $O_2^{\bullet-}$. There is a wide range of reactions in which $ONOO^-$ may be involved [55–57]. Under physiological conditions, only two secondary modes of action should be considered. The main one is the fast and efficient conversion of $ONOO^-$ in the presence of CO_2 or bicarbonate through transient nitrosoperoxycarbonate into the carbonate radical ($CO_3^{\bullet-}$) [58,59], a strong and selective one-electron oxidant of guanine [60,61]. In addition, nitrogen dioxide ($^\bullet NO_2$) is also generated but it slowly reacts with biomolecules [57]. One of the two main nitration products of DNA is 8-nitroguanine [62,63], which, though unstable, has been detected in a number of human tissues associated with various diseases [64–66]. A likely mechanism of formation of 8-nitroguanine involves the concerted action of $O_2^{\bullet-}$ and $^\bullet NO_2$ similar to the mechanism of formation of 3-nitrotyrosine [53,57]. Competitive addition of $^\bullet NO_2$ to the C5 position of G(-H)$^\bullet$ gives rise after rearrangement to 5-guanidino-4-nitroimidazole (Nim) [63]. Another indirect and minor decomposition reaction of $ONOO^-$ in biological systems involves the generation of $^\bullet OH$ by slow proton-catalyzed homolysis of peroxynitrous acid [53,57].

One-electron agents—Several chemical and physical agents are able to abstract one electron from DNA components in the following decreasing order of susceptibility: guanine > adenine, cytosine ~ thymine > 2-deoxyribose that parallels their reduction potential [67].

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A major endogenous one-electron oxidant is the carbonate radical ($CO_3^{\bullet -}$) that exclusively reacts with guanine [60,61]. Potassium bromate ($KBrO_3$), a widely used water disinfectant, once reduced by thiols to BrO_2 [68], is able to induce the formation of 8-oxoGua in cellular DNA [69,70] via the transient formation of the guanine radical cations [68]. The immunosuppressive agent azathioprine is inserted and converted in DNA into 6-thioguanine, an efficient type I photosensitizer of guanine [71,72]. Nanosecond UVC-laser irradiation has been shown to be an efficient tool for generating, with similar efficiency, the radical cations of all four main nucleobases in isolated and cellular DNA via bi-photonic ionization [73,74]. Direct interaction of highly energetic photons of gamma- or X-rays with cellular DNA is able to ionize the 2-deoxyribose moiety in addition to the four main nucleobases [11]. It is worth noting that the initial formation of base radical cations in double-stranded DNA is usually followed by hole migration with preferential trapping of the radical cation by guanine bases [75,76]. This leads to considerable redistribution of initially damaged sites with the preferential formation of 8-oxoGua as observed in cellular DNA [74].

Enzymatic oxidation reactions of 5-methylcytosine—Evidence has recently been provided for enzymatic oxidation of the methyl group of 5-methylcytosine (5-mCyt), a minor DNA base implicated in epigenetic processes. Thus, TET (ten eleven translocation) enzymes belonging to the family of oxoglutarate and iron-dependent dioxygenases have been shown to generate 5-(hydroxymethyl)cytosine (5-HmCyt) in nuclear and mitochondrial DNA of mammalian cells; 5-HmCyt is an oxidation product previously identified as resulting from the reaction of $^{\bullet}$OH and one-electron oxidants with 5-mCyt [77,78]. Iterative TET oxidation has been found to convert 5-HmCyt into 5-formylcytosine (5-ForCyt) [79,80] and the ultimate oxidized state 5-carboxycytosine (5-CaCyt) [80,81] as further steps of a possible demethylation mechanism of 5-mCyt.

Terminology

The term ‘oxidative DNA damage’ is widely used in most publications to designate the decomposition products of DNA that are generated upon exposure to a large number of oxidizing agents. This phrase, however, suggests that the final oxidation products of DNA have the ability to further oxidize compounds in the vicinity. Clearly, this is not the case because most of the oxidation products of nucleobases and 2-deoxyribose isolated and characterized so far do not exhibit oxidizing properties. There may be a few potential exceptions that concern the relatively stable thymine hydroperoxides including 5-(hydroperoxymethyl)uracil and *cis* and *trans* 5-(6)-hydroperoxy-6-(5)-hydroxy-5,6-dihydrothymine produced by either $^{\bullet}$OH or one-electron oxidation [32]. The decomposition of thymine hydroperoxides by ferrous ions may generate highly reactive alkoxyl radicals that could readily abstract hydrogen atoms from vicinal molecules in DNA. For the above reason, we strongly encourage, as a more appropriate alternative phrase for ‘oxidative DNA damage’, the use of ‘oxidatively damaged DNA’, ‘oxidatively-generated DNA damage’, ‘oxidatively-derived damage to DNA’ or ‘oxidation-induced DNA damage’. This collective grouping of products includes 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 2,4-diamino-5-formamidopyrimidine(FapyAde).FapyGua and FapyAde are initially formed by either one-electron oxidation or addition of $^{\bullet}$OH at C8 of the purine ring followed by one-electron reduction of the 8-hydroxy-7,8-dihydropurinyl radical and opening of the imidazole ring [12,15,82].

Several thousand abasic sites are generated in each mammalian cell daily by the spontaneous hydrolysis of the *N*-glycosidic bond of mostly purine 2′-deoxyribonucleosides in DNA. Additionally, abasic sites are produced as a consequence of oxidation reactions to DNA since several oxidatively generated base lesions including FapyGua and FapyAde show an

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increase in the lability of their *N*-glycosidic bonds [82–85]. Therefore, the resulting apurinic sites should be considered as indirect oxidatively damaged DNA lesions.

Classes of oxidatively generated damage

Lesions arising from a single oxidation hit

In most cases, with the exception of ionizing radiation, oxidatively generated DNA lesions in cells are initiated by a single oxidative hit.

Single nucleobase lesions—•OH and one-electron oxidants generate a wide range of single oxidized bases by reaction with thymine, cytosine, 5-methylcytosine, adenine and guanine according to well documented mechanisms [10,12,15,16,39]. Among them one may mention 8-oxoGua, an ubiquitous DNA oxidation product because it is generated by radical reactions with •OH, one-electron oxidants [10,12] and vicinal pyrimidine peroxyl radical [36]. In contrast, $^1O_2$ exclusively reacts with guanine giving rise predominantly in cellular DNA to 8-oxoGua [14,36]. However, only 12 oxidized bases (Tables I and Figures 1, 2) from the 80 or so identified modifications in model studies have been detected in cellular DNA using the accurate HPLC-MS/MS or HPLC coupled with electrochemical detection (ECD) methods. In addition, three oxidized bases including 5-(hydroxymethyl)cytosine (5-HmCyt), 5-formylcytosine (5-ForCyt) and 5-carboxylcytosine (5-CaCyt) (Table I and Figure 3) are detected as products of the enzymatic oxidation of 5-methylcytosine in the DNA of human cells [77–81]. The three chlorinated nucleobases (Table I and Figure 4) may also be included in the list of the base oxidation products.

Single strand breaks—The formation of single strand breaks (SSBs) in cellular DNA mostly occurs from •OH-mediated hydrogen abstraction from the 2-deoxyribose moiety at C3, C4 and C5 [11,17]. In addition, several radiomimetic compounds such as bleomycin, neocarzinostatin and other enedynes are able to induce SSBs and also double strand breaks (DSBs) in DNA as the result of hydrogen abstraction at C4 and eventually C5 [17,86]. As it will be discussed later the formation of C4 and C5 centred radicals does not lead exclusively to the formation of SSBs since other competitive reactions have been found to occur.

Normal and oxidized abasic sites—The formation of abasic sites in cells takes place by hydrolysis of the *N*-glycosidic bond of oxidatively damaged 2′-deoxyribonucleosides (*vide supra*). Hydrogen abstraction at C1 gives rise quantitatively to 2-deoxyribonolactone (dL) (Figure 5) while formation of C4 centred radical within the sugar moiety is the initial step of a sequence of reactions leading to the generation of the so-called “C′4 oxidized abasic site” (C4-AP) as a cyclic form in equilibrium with the acyclic 2-deoxypentos-4-ulose abasic site [17]. Hydrogen abstraction at C2 of the 2-deoxyribose is a minor process that gives rise to a third type of oxidized abasic site [17]. One may also note that the carbon centred radical formed by •OH-mediated hydrogen abstraction from the 5-hydroxymethyl group has been proposed to lead to the generation of 1, 4-dioxobutane abasic lesions (Figure 5) according to a still undetermined mechanism and at best as a minor process [87].

Intrastrand base cross-links—Three types of oxidatively generated intrastrand lesions between two vicinal bases have been detected in isolated DNA. One involves intramolecular addition of carbon centred pyrimidine radical with vicinal purine bases, the reaction being favoured in the 5′-purine-3′-pyrimidine sequence [88,89]. Two of these guanine containing tandem base lesions including G[8 - 5m]T and G[8 - 5]C (Figure 6) whose formation involves 5-(uracilyl) methyl and 6-hydroxy-5,6-dihydrocytosyl radicals, respectively, as the reactive intermediates have been measured in cellular DNA using an accurate and sensitive HPLC-MS^3 assay [90,91]. Following the pioneering work of Box and his collaborators [92],

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evidence has been provided that pyrimidine peroxyl radicals generated by either •OH or one-electron oxidants are able to efficiently react with adjacent purine [36] and pyrimidine bases [37] leading to the formation of tandem base lesions in isolated DNA [38]. However, none of these intra-strand cross-links has been detected so far in cellular DNA. This is also the case of guanine-thymine tandem lesion or more distant cross-links between the G and T bases that are produced upon the initial formation of guanine radical cations [63].

Purine 5′,8-cyclonucleosides—This class of lesions constitute another example of tandem modifications since both the base and the sugar moiety of the same 2′-deoxyribonucleoside unit is damaged upon •OH-mediated hydrogen abstraction from C5 of the 2-deoxyribose moiety [93,94]. Intramolecular cycloaddition of 5′-yl radicals to the C8 position of adenine or guanine must compete with efficient O_2-trapping, which explains low yields of the 5′*R* and 5′*S* diastereomers of either 5′,8-cyclo-2′-deoxyadenosine (cdAdo) or 5′,8-cyclo-2′-deoxyguanosine (cdGuo) resulting from gamma-irradiation of naked and cellular DNA under aerobic conditions [95]. In fact, only traces of the predominant (5′*R*)-cdAdo (Figure 6) were detected in the DNA of gamma-irradiated THP1 monocytes using accurate HPLC-MS/MS analysis [95]. This contrasts with single quadrupole HPLC-MS and GC-MS analyses of purine 5′,8-cyclonucleosides that determined levels of both cdAdo and cdGuo to be about two orders of magnitude greater [96,97].

DNA interstrand cross-links—Four mechanisms are available for the oxidative formation of cross-links between two opposite DNA strands. One involves the generation of a reactive unsaturated ketoaldehyde formed upon a β-elimination reaction involving the deoxypentos-4-ulose abasic site. This reaction takes place in a sequence dependence manner since the reaction is promoted by adenine and to a lesser extent by cytosine bases located on the complementary strand [98,99]. The final product involves the formation of interstrand cross-links (ICLs) with opposite cytosine and adenine [99,100]. However, so far, the only adducts detected in cellular DNA exposed to either gamma-rays or incubated with radiomimetic bleomycin have been 2′-deoxycytidine adducts consisting of the four diastereomers of 6-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-hydroxy-3(3-hydroxy-2-oxopropyl)-2,6-dihydroimidazo[1,2-c]-pyrimidin-5(3*H*)-one (Figure 6) [100]. A second type of oxidatively generated ICLs has been observed in cells pre-incubated with 6-thiopurines and exposed to UVA irradiation [101]. The formation of ICL may be rationalized in terms of the initial excitation of 6-thioguanine and subsequent one-electron oxidation of guanine to give the corresponding purine radical cation followed by addition of the latter species to nucleophilic cytosine on the opposite strand. Work is in progress to further elucidate this tentative mechanism [Angelov et al, unpublished data]. A third type of ICL that has been recently proposed on the basis of relevant model studies involves the transient formation of 5′-(2-phosphoryl-1, 4-dioxobutane (DOB, Figure 5), an oxidized abasic site arising from initial hydrogen abstraction at C5 of the 2-deoxyribose moiety [87]. The putative release of highly reactive 2-butene-1,4-dial consecutive to a β-elimination reaction has been shown to react selectively with adenine on the opposite strand to generate the interstrand cross-link. However, the mechanism of formation of DOB that may involve the transient generation of an alkoxyl radical remains to be established. A fourth type of ICL that results from independent O_2 reaction of 5-(uracilyl)methyl with opposite adenine has been identified in DNA duplexes [102]. This arises from the formation of a covalent bond between the thymine methyl group and the adenine 6-amino group. However, the formation of adenine containing ICLs arising from the reaction with either DOB or 5-(uracilyl)methyl remains to be established in cellular DNA.

DNA-protein cross-links—It has been found that the guanine radical cation generated by electron abstraction from the purine base in the TGT trinucleotide in the presence of bound

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trilysine peptide was able to undergo an efficient nucleophilic addition with the free ε-amino group of central lysine giving rise to a DNA-protein cross-link [103].

As an alternative mechanism, it was suggested that DNA-protein crosslinking formation may arise from initial one-electron oxidation of the lysine residue [104]. It remains to be established whether guanine-lysine adducts are formed in cells by a similar pathway upon UVA photolysis of DNA in which 6-thioguanine residues have been incorporated [105].

Nucleobase adducts with lipid peroxidation breakdown products—An abundant literature is available on the formation of addition products mostly involving guanine and, to a lesser extent, adenine and cytosine with the breakdown products of lipid peroxides including electrophile malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) [106,107]. This reaction gives rise, among other products, to the predominant formation of pyrimido[1,2-α]purine-10(3*H*)one (M_1Gua) and 1,N^2-propanoguanine (Figure 7). In addition, several exocyclic ethenobase adducts including 1,N^2-ethenoguanine (1,N^2-εGua), 1,N^6-ethenoadenine (1,N^6-εAde) and 1,N^4-ethenocytosine (1,N^4-εCyt) that arise from the reaction of HNE and 4-hydroxy-2,3-epoxynonenal, its epoxidation product, with amino-substituted nucleobases have been measured in cellular DNA (Figure 7) under oxidative conditions [106–112]. It has also been found that M_1Gua may arise from the reaction of guanine with MDA and/or base propenal that may be generated by •OH-mediated hydrogen abstraction at C4 of the 2-deoxyribose moiety [17].

Alkali-labile lesions—This broad class of so-called ‘alkali-labile lesions’ consists of several oxidatively damaged sites that may be converted into DNA SSBs either upon hot piperidine treatment or during the conditions of alkaline gel electrophoresis [113,114] and alkaline elution technique [115]. Under usual conditions of piperidine treatment (0.2M–1M, 90°C, 30 minutes), several DNA modifications can be mapped at nucleotide resolution using polyacrylamide gel electrophoresis (PAGE) analysis. In addition to highly labile normal and all oxidized abasic sites [113], this analysis reveals several oxidatively generated base modifications, which are at least partly con-vertedintoSSBs,suchas2,5-diamino-4*H*-imidazolone (Iz) [116], 2,2,4-triamino-5-(2*H*)-oxazolone (Oz) [117], 5,6-dihydroxy-5,6-dihydrothymine (ThyGly) [118], FapyAde and, to lesser extent, FapyGua [119]. In contrast to previous claims, 8-oxoGua has been found to be stable under conditions of hot piperidine treatment [117,120]. The strongly alkaline conditions (0.3M NaOH, pH > 13) that are used for the measurement of DNA strand breaks using either the alkaline version of the comet assay or the alkaline eluting technique are incompatible with the instability at high pH of abasic sites, Iz and Oz. However, it is likely that under these conditions the other alkali-labile base lesions including ThyGly, FapyAde, Fapy-Gua and also 5-formyluracil (5-ForUra) are only partly degraded [121]. This should lead to the generation of secondary SSBs that prevent accurate assessment of directly generated DNA nicks, which is otherwise possible by lowering the pH to less than 12.5 during the alkaline unwinding step and electrophoresis. It is clear that there is a need of additional information concerning the alkaline stability of several oxidized bases under the conditions of PAGE analysis or comet assay measurements.

Enzyme-sensitive sites—The alkaline comet assay and alkaline elution technique have been rendered more specific toward oxidatively damaged DNA bases by pre-incubation of the released DNA by bacterial repair enzymes including endonuclease III (endo III) and formamidopyrimidine DNA *N*-glycosylase (Fpg) [113–116,122]. These enzymes mostly excise oxidized pyrimidine bases (ThyGly and 5,6-dihydroxy-5,6-dihydrouracil) and purine modifications (8-oxoGua, FapyGua, FapyAde) [123]. More recently, human 8-oxoguanine DNA glycosylase I (OGG1), which is more specific for 8-oxoGua than its bacterial homologue Fpg [123], has also been used to measure 8-oxoGua and FapyGua in cells [124]

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and human populations [125]. The abasic sites generated by the repair enzymes are converted into SSBs under the alkaline conditions of either electrophoresis or elution technique analysis. A major limitation of these highly sensitive enzymatic based assays is their poorer ability to act on some tandem base lesions. This may concern lesions composed of 8-oxoGua and another oxidized base, which have been shown to be generated in isolated DNA under radical oxidations and refractory to repair glycosylase-mediated base excision [38]. This explains, at least partly, the lower steady-state levels of Fpg-sensitive sites measured in the DNA of human lymphocytes with respect to the yield of 8-oxoGua that was assessed by HPLC methods [126].

Lesions arising from multiple simultaneous oxidation hits

Typically high energy photons delivered by x- and gamma-ray sources are capable of generating several radicals and excited state molecules along the energy deposit track within one or two DNA helix turns [127]. The multiplicity and density of these potentially damaging events is known to increase with the linear energy transfer (LET) of ionizing radiation and heavy ions [127,128].

Double strand breaks—The formation of nicks on both DNA strands that are separated by less than 15 base pairs are the most representative types of DNA damage induced by two simultaneous and independent radical events. It may be added that DSBs constitute an heterogeneous class of DNA damage not only due to differences in the size of the clustered lesions but also to the presence of chemical modifications of nucleobases and sugar moieties. Several methods including neutral comet assay, pulsed-field gel electrophoresis and immunofluorescence detection of γ-H2AX foci are available for measuring DSBs in cells [129–131]. However, the specificity of γ-H2AX formation as an indicator of DSBs has been questioned [132,133].

Oxidatively generated clustered damage—A second, broad class of complex DNA lesions that may be generated by several simultaneous radical events mediated by $^{\bullet}OH$, ionization and secondary electrons consist of the presence of SSBs, oxidized bases and/or abasic sites within one or two helix turns [134,135]. Several sub-classes of bi-stranded clustered lesions, which may involve at least one SSB on one DNA strand and base modifications/and or abasic sites on the opposite strand, have been measured in cellular DNA using enzymatic based assays [136,137]. However, the yields of radiation-induced clustered DNA damage thus measured appear to be overestimated [18].

Secondary oxidation products

It has been proposed that highly oxidizable 8-oxoGua may be subjected to further oxidation by a second independent radical hit involving either $^1O_2$ or one-electron oxidants. It should be reminded that 8-oxoGua exhibits a much higher susceptibility than guanine to the two latter oxidants, and an abundant literature is available on the characterization and mechanism of formation of secondary oxidation products of 8-oxoGua [138–141]. Spiroimino-dihydantoin (Sp), a major one-electron oxidation product of 8-oxoGua, was measured in the DNA of *Escherichia coli* cells that were exposed to chromate [142] using a HPLC-MS assay that suffers from a lack of accuracy when a high sensitivity of detection is required [18]. It has also been shown that the formation of Sp is at best a minor process in double-stranded DNA [13]. Furthermore the secondary oxidation of 8-oxoGua in cellular DNA has been questioned [143]. One may wonder whether 8-oxoGua can efficiently compete with the overwhelming number of guanines in DNA for one-electron oxidation even if the reaction is more favorable by about one-hundred times for 8-oxoGua and also taking into consideration the fact that electron transfer may occur in DNA. An exception may involve systems that lead to the efficient formation of guanine radical cations within

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cellular DNA such as 6-thioguanine, a UVA type I photosensitizer [71], which upon incorporation into DNA is likely to favour further oxidation of initially formed 8-ox-oGua in the vicinity. Another example of the high susceptibility of certain modified nucleobases to one-electron oxidants is given by 5-hydroxycytosine [16] but again the same remarks apply as above for 8-oxoGua.

Unlikely oxidatively generated lesions in cellular DNA

Several $^{\bullet}$OH-mediated degradation base products have not been detected in cellular DNA although they are produced by oxidation of isolated DNA or nucleosides. This is the case for 2-hydroxyadenine [144] and 5-hydroxyuracil [74] that have not been detected in cells even after exposure to doses of ion-izing radiation up to 200 Gy, indicating that at best the two oxidized bases if formed are rather minor degradation products. This also applies to the formation of three purine 5′,8-cyclonucleosides including (5′*S*)-cdAdo and the 5′*R* and 5′*S* diastereomers of cdGuo whose radiation-induced formation was not observed even after a dose of 2 kGy [95]. The formation of FapyAde that has been reported in UVB-irradiated cellular DNA [145] is questionable because it is well documented that UVB radiation is a very poor oxidizing agent and because only traces of 8-oxoGua, the preferential product of $^{1}O_{2}$ or one-electron oxidation to DNA, are detected using appropriate methods [146].

Nomenclature

As a general remark, it may be pointed out that the nomenclature used for describing oxidatively modified nucleobases, 2′-deoxyribonucleosides, ribonucleosides and sugar moieties of nucleic acids is sometimes confusing, incorrect and often lacks consistency. Even if a single letter is frequently utilized to represent each of the nucleobases in DNA (e.g. G, C, A, T), it is preferable to write the complete abbreviation when referring to monomers, for example, Gua for the nucleobase, dGuo for the 2′-deoxyribonucleoside and Guo for the ribonucleoside (see Table I and Figure 1). It is recommended to use at least the full abbreviation for the nucleobases when the bases, 2′-deoxyribonucleosides and ribonucleosides, are all mentioned in a document. The oxidatively-generated DNA lesion that has received, by far, the most attention is the ubiquitous guanine lesion, called in full 8-oxo-7,8-dihydroguanine (with 8-oxoGua as the abbreviation; Figure 1). This is the recommended name, in agreement with the International Union of Pure and Applied Chemistry (IUPAC) that considers prefixes such as (di)hydro- are non-detachable, and therefore, they should always be placed immediately before the parent name [147]. On this basis, 7, 8-dihydro-8-oxoguanine and 7-hydro-8-oxoguanine and 8-oxoguanine that are found in the literature are incorrect designations of 8-oxo-7,8-dihydroguanine. Furthermore, 8-oxo-2′-deoxyguanine is a confusing name derived from a species between a nucleobase and a 2′-deoxyribonucleoside. For clarification, the 7,8-dihydro description is used to indicate saturation of the double bond between N7 and C8 atoms of the parent unmodified guanine, from which the damaged nucleobase is derived. Hence, 8-oxo-7, 8-dihydroguanine is the 8-oxo-substituted derivative of 7,8-dihydroguanine, and the same remark applies as well to 8-oxo-7,8-dihydroadenine (8-oxoAde) and 5,6-dihydroxy-5,6-dihydrothymine (ThyGly). 8-Hydroxyguanine, which is frequently used, and indeed one used by Chemical Abstracts, is in fact a rather minor tautomer that is in dynamic equilibrium at physiological pH [148] with the predominant 6,8-diketo form. This was inferred from NMR studies of the lesion in duplex DNA [149,150] and as the

2′-deoxyribonucleoside [151], information that was further confirmed by theoretical calculations [152–154]. By implication, 8-oxo, rather than 8-hydroxy, would be the form present in the greatest amounts in biological systems, and hence, the more accurate term to use when describing this lesion *in vivo*. There is precedent for the proposed nomenclature in other molecules: the malondialdehyde-, or base propenal-derived modification of guanine,

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known as M_1Gua pyrimido[1,2-α]-purin-10(3*H*)-one (M_1Gua) is a ring-closed compound as a free nucleobase at pH 7. However, it undergoes ring-opening at alkaline pH [155], and also when base-paired with dCyd in duplex DNA [156], thereby, becoming the N^2-(3-oxo-1-propenyl)-dGuo (OPD) adduct. Nevertheless, the names remain M_1Gua (or M_1dGuo, for the 2′-deoxribonucleoside equivalent), since this is the structure that predominately exists at pH 7 [155,156]. Hence, the recommendation is to propose the names, based upon the major structure at pH 7, and biological context. The corresponding 2′-deoxyribonucleoside of 8-oxoGua is 8-oxo-7,8-dihydro-2′-deoxyguanosine (Figure 1), abbreviated as 8-oxodGuo or 8-oxodG. For completeness, it is worth noting that the ribonucleoside equivalent is 8-oxo-7,8-dihydroguanosine, abbreviated as 8-oxoGuo.

Another common error is the use of ‘2′-deoxyribose’ that should be ‘2-deoxyribose’. This applies as well for designating the sugar moiety of 2′-deoxyribonucleosides that contain an unusual base, which should be written as (2-deoxy-β-D-*erythro*-pentofuranosyl) and not (2′-deoxy-β-D-*erythro*-pentofuranosyl) when the brackets are used. One may add that corrected nomenclature for the C1 oxidized abasic site is ‘2-deoxyribonolac-tone’ and not ‘2′-deoxyribonolactone’. As a final remark, one may also point out that the preferred nomenclature for nitric oxide and nitrogen dioxide radical, that are both radical species, is $^{\bullet}NO$ and $^{\bullet}NO_2$ [156,157] respectively although NO is commonly found in the literature.

Conclusions

Attempts have been made in this manuscript to provide relevant information on the main reactive species, chemicals, physical agents and enzymes that are likely involved in the oxidation reactions of cellular DNA. A description of oxidatively generated damage to DNA without certain precision about the oxidative conditions and mechanism of formation is meaningless because of the broad spectrum of ROS and chemical oxidants. There are also often oversimplifications concerning the formation of oxidatively generated damage to DNA with usually a paucity of mechanistic insights. For example, 8-oxoGua is a ubiquitous DNA oxidation product that can be generated by at least four well-studied pathways in both cellular and multicellular organisms including humans. In this respect, a complete overview of the main classes of DNA lesions produced by one or several oxidative hits is provided with emphasis on the base and sugar modifications that have been detected so far in cells. Our recommendations about the terminology and nomenclature concerning DNA oxidation products should lead to greater standardization and coherence, thus, facilitating literature searches, meaningful exchanges between scientists in this field and other fields. Therefore, while trivial or common names may continue to be used, we strongly recommend a progressive move towards consensus on the use of 8-oxo-7,8-dihydroguanine, as indicated by IUPAC, and supported by others in the field (for example, see [158]). As a final comment, we emphasize the major discovery that was made in the field of oxidatively generated damage almost 28 years ago with the characterization of 8-oxodGuo, a major radical oxidation product of 2′-deoxyguanosine [159,160] which was closely followed by another, describing the formation of 8-oxoGua in DNA [161]. A few years later the appearance of HPLC coupled with electrochemical detection [162] allowed the accurate measurement of 8-oxodGuo in cellular DNA and gave a strong impetus to more biochemically orientated studies including DNA repair and the use of 8-oxoGua and 8-oxodGuo as a biomarkers of oxidative stress.

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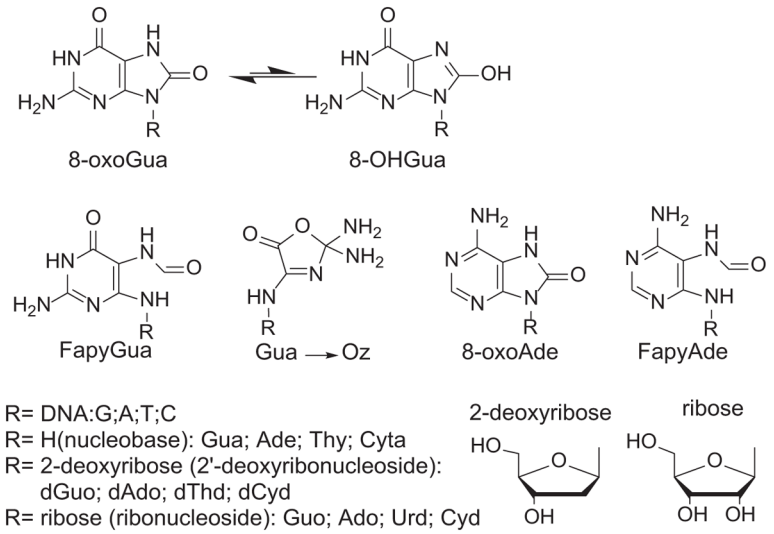

Figure 1.

Structure of oxidatively damaged purine bases that have been detected so far in cellular DNA. (8-oxoGua in dynamic equilibrium with 8-OHGua, 8-oxoAde, FapyGua, FapyAde, Oz).

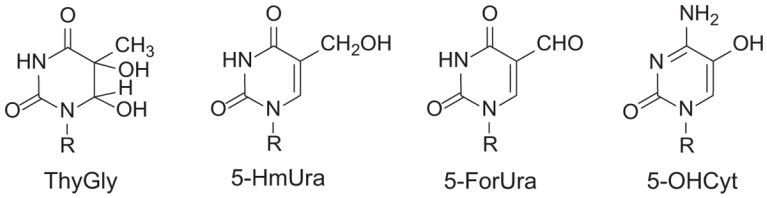

Figure 2.
Structure of oxidatively damaged pyrimidine bases that have been detected so far in cellular DNA (ThyGly, 5-ForUra, 5-HmUra, 5-OHCyt).

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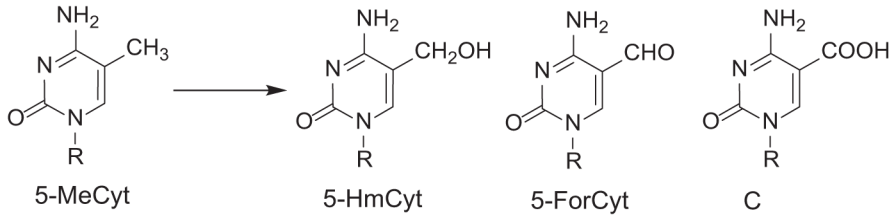

Figure 3.
Structure of enzymatically 5-methylcytosine oxidation products in cellular DNA (5-HmCyt, 5-ForCyt, 5-CaCyt).

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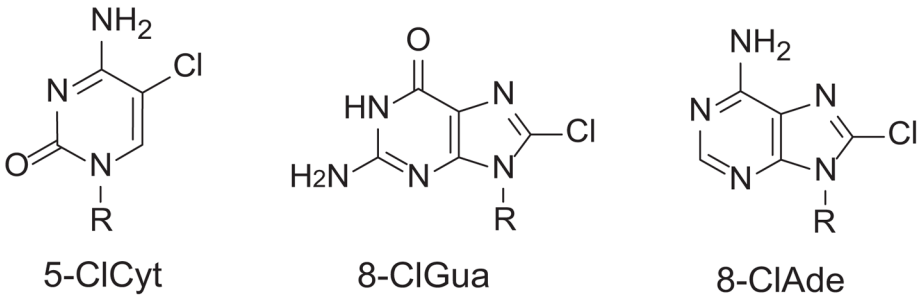

Figure 4.
Structure of chlorinated purine and pyrimidine bases that have been detected in cellular DNA (5-ClCyt, 5-ClGua, 5-ClAde).

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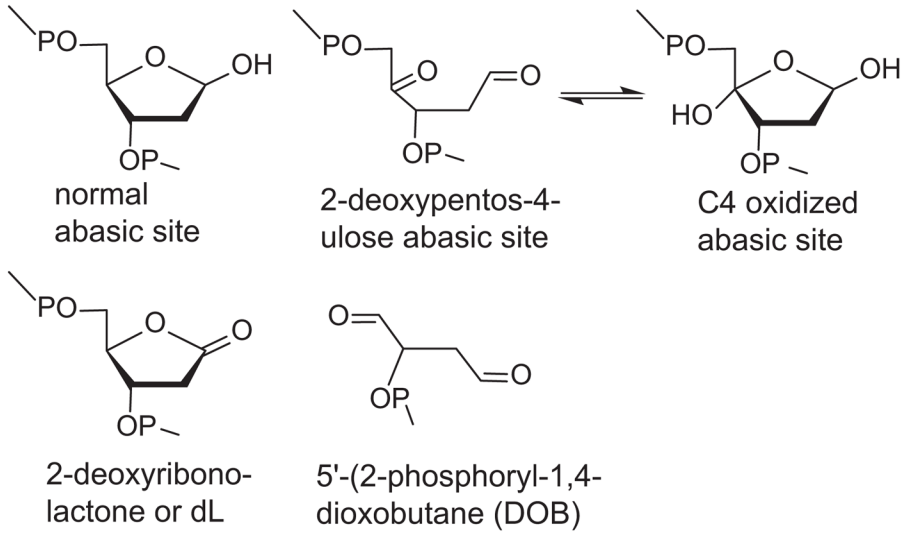

Figure 5.
Structure of the main oxidized a basic sites identified in isolatedDNA(C4-APinequilibriumwiththeacyclic2-deoxypentos-4-ulose abasic site, DOB, 2-deoxyribonolactone or dL).

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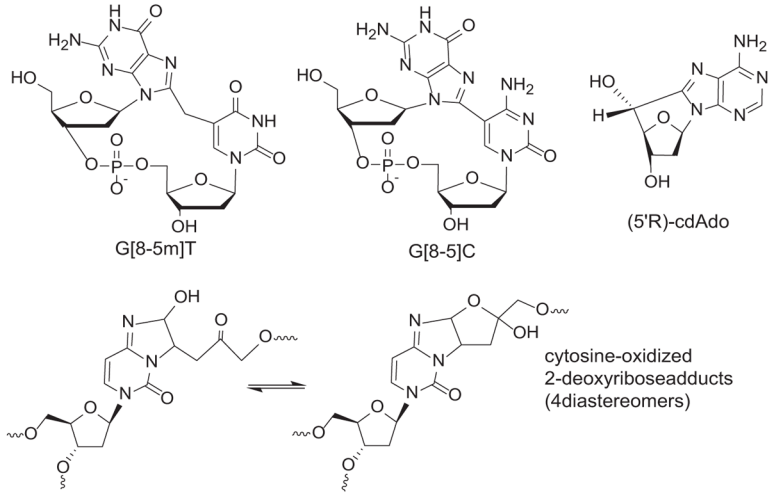

Figure 6.

Structure of oxidatively generated tandem base lesions, 5′,8-cyclo-2′-deoxyadenosine and cytosine-aldehyde interstrand cross-links that have been detected in cellular DNA (G[8 - 5m]T, G[8 - 5]C, (5′*R*)-cdAdo, cytosine adduct).

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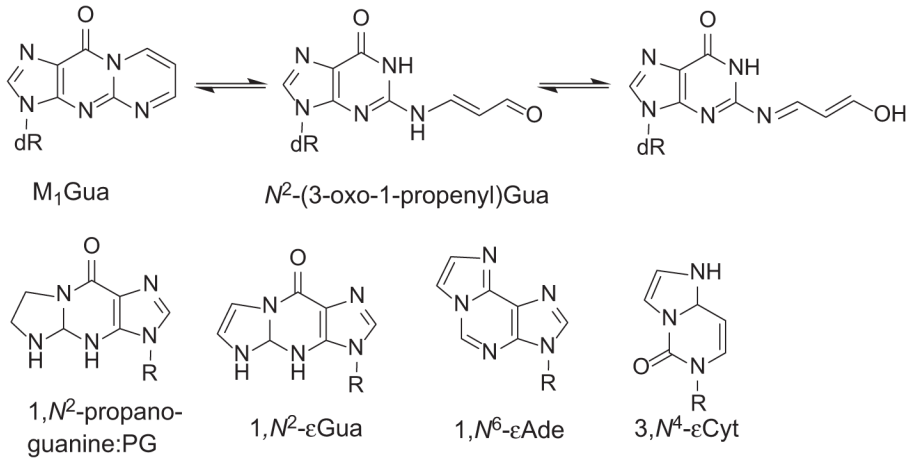

Figure 7.

Structure of the main ethenobase adducts, MDA and 4HNE guanine adducts that have been detected in cellular DNA. (M_1Gua and the open form N^2(3-oxo-1-propenyl) Guo (OPD), 1,N^2-propanoguanine, 1,N^2-εGua, 1,N^6-εAde, 1,N^4-εCyt).

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Table I

Name and abbreviation of common oxidatively damaged nucleobases of DNA.

Name	Abbreviation	Structure (see Figure)
8-Oxo-7,8-dihydroguanine	8-oxoGua	1
2,6-Diamino-4-hydroxy-5- formamidopyrimidine	FapyGua	1
8-Oxo-7,8-dihydroadenine	8-oxoAde	1
4,6-Diamino-5- formamidopyrimidine	FapyAde	1
2,2,4-Triamino-5(2*H*)oxazolone	Oz	1
5,6-Dihydroxy-5,6-dihydrothymine	Thy-Gly	2
5-(Hydroxymethyl)uracil	5-HmUra	2
5-Formyluracil	5-ForUra	2
5-Hydroxycytosine	5-OHCyt	2
5-(Hydroxymethyl)cytosine	5-HmCyt	3
5-Formylcytosine	5-ForCyt	3
5-Carboxylcytosine	5-CaCyt	3
5-Chlorocytosine	5-ClCyt	4
5-Chloroadenine	5-ClAde	4
5-Chloroguanine	5-ClGua	4
(5′*R*)-5′,8-Cyclo-2′-deoxyadenosine	(5′*R*)-cdAdo	6