

# Reviews

## An Overview of Chemical Processes That Damage Cellular DNA: Spontaneous Hydrolysis, Alkylation, and Reactions with Radicals

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The sequence of heterocyclic bases on the interior of the DNA double helix constitutes the genetic code that drives the operation of all living organisms. With this said, it is not surprising that chemical modification of cellular DNA can have profound biological consequences. Therefore, the organic chemistry of DNA damage is fundamentally important to diverse fields including medicinal chemistry, toxicology, and biotechnology. This review is designed to provide a brief overview of the common types of chemical reactions that lead to DNA damage under physiological conditions.

### Contents

1. Introduction	1747	5.7. Tandem Lesions	1754
2. Hydrolysis of DNA	1747	6. Conclusion	1756
2.1. Spontaneous Hydrolysis of the Phosphodiester Backbone Is Very Slow	1747		
2.2. Hydrolytic Deamination of DNA Bases	1748		
2.3. Spontaneous Hydrolysis of the Glycosidic Bonds Connecting the Nucleobases to the DNA Backbone	1749		
2.4. Properties of Abasic Sites Arising from Depurination	1749		
3. Overview: Common Reactions by Which Small Organic Molecules Damage DNA	1750		
4. DNA Alkylation	1750		
4.1. Preferred Alkylation Sites in DNA	1750		
4.2. The Chemical Fate of Alkylated DNA	1750		
4.2.1. Alkylated Phosphates Are Chemically Stable	1750		
4.2.2. Alkylation at Some Positions on the DNA Nucleobases Yields Chemically Stable Adducts	1751		
4.2.3. Alkylation of Some Endocyclic Nitrogens on the Nucleobases Yields Labile Lesions That Undergo Deglycosylation or Ring Opening	1751		
4.2.4. Alkylation at Some Endocyclic Nitrogens Accelerates Deamination	1751		
5. Reactions of Radicals with DNA	1752		
5.1. Abstraction of Hydrogen Atoms from the 2'-Deoxyribose Sugars of DNA: General Features	1752		
5.2. Alkali-Labile Strand Damage Stemming from Abstraction of the C1'-Hydrogen Atom	1752		
5.3. Direct Strand Cleavage Initiated by Abstraction of the 4'-Hydrogen Atom	1753		
5.4. Reactions of Radicals with the Nucleobases	1753		
5.5. Addition of Radicals to C5 of Thymine Residues: Generation of Thymine Glycol	1753		
5.6. Addition of Radicals to Guanine Residues: Generation of FAPy-G and 8-Oxo-G Residues	1754		

### 1. Introduction

The sequence of heterocyclic bases on the interior of the DNA double helix constitutes the genetic code that drives the operation of all living organisms (Figure 1) (1–4). Accurate readout of DNA sequence is necessary for the expression of functional mRNA and, ultimately, proteins in cells (5). In addition, during cell division, faithful replication of DNA is required to produce daughter cells containing exact copies of the genome (6). In light of these facts, it is not surprising that chemical modification of cellular DNA can have profound biological consequences. DNA damage can trigger changes in gene expression, inhibit cell division, or trigger cell death (7–9). In addition, attempts to replicate damaged DNA can introduce errors (mutations) into the genetic code (10). Thus, the organic chemistry of DNA damage is fundamentally important to diverse fields including medicinal chemistry, toxicology, and biotechnology (11–13). This review is designed to provide a brief overview of the most common types of chemical reactions that lead to DNA damage under physiological conditions.

### 2. Hydrolysis of DNA

**2.1. Spontaneous Hydrolysis of the Phosphodiester Backbone Is Very Slow.** Hydrolysis of the phosphodiester groups in the backbone of DNA is thermodynamically favored ( $\Delta G^\circ = -5.3$  kcal/mol) (14) but extremely slow (Scheme 1) (15).<sup>1</sup> Work with carefully designed model compounds such as **1** indicate that the half-life of phosphodiester hydrolysis is

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<sup>1</sup> In the interest of brevity, many of the reaction mechanisms shown in this review are schematic in nature. For example, protonation, deprotonation, and proton transfer steps may not be explicitly depicted or may not distinguish specific acid–base catalysis from general acid–base catalysis. In some cases, arrows indicating electron movement are used simply to

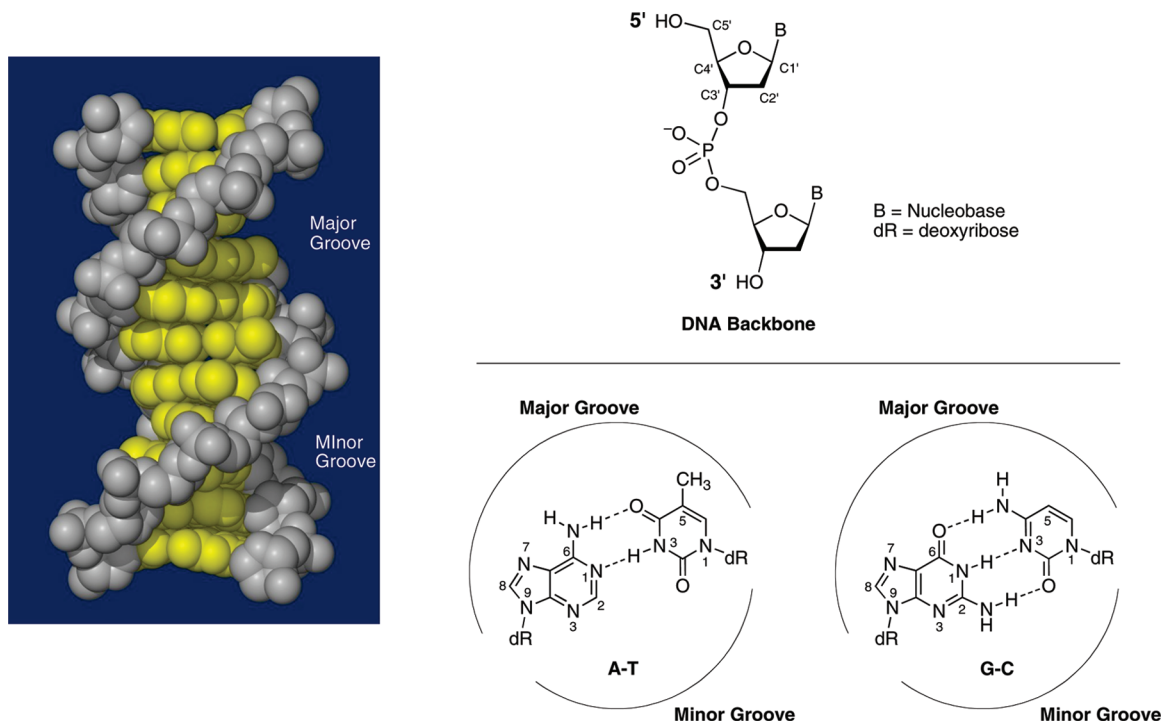
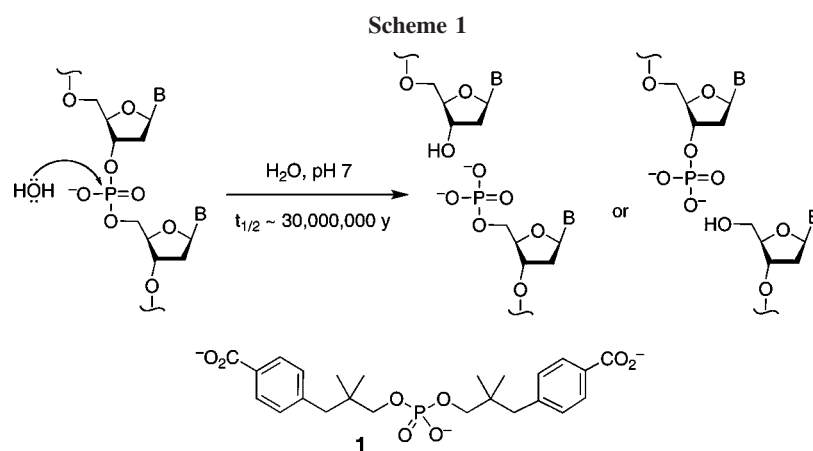
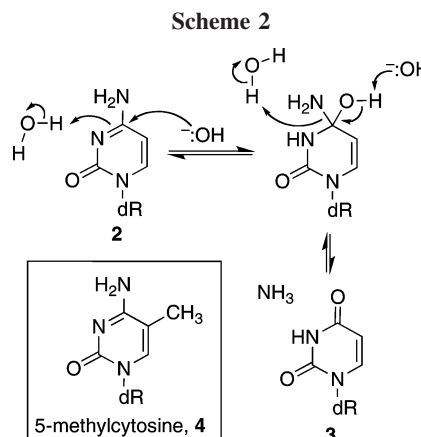


Figure 1. Structure of DNA.



approximately 30 000 000 years under physiologically relevant conditions (15–18). In short, this means that spontaneous hydrolysis of the phosphodiester linkages in DNA does not occur to a significant extent under biological conditions, although the reaction can be vastly accelerated by various catalysts including phosphodiesterases, lanthanide ions, and transition metal ions (19–23).

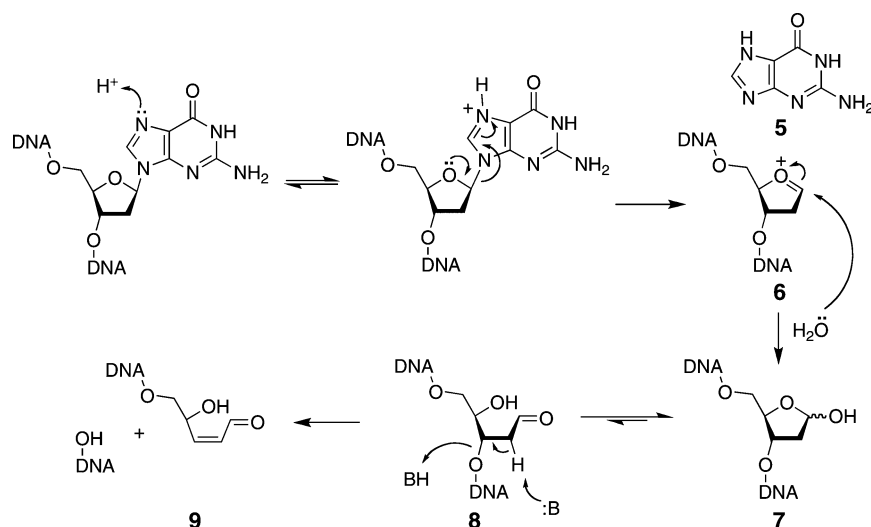
**2.2. Hydrolytic Deamination of DNA Bases.** Cytosine residues **2** in DNA can undergo hydrolytic deamination to yield uracil residues **3** (Scheme 2) (24–28). The reaction may proceed via either attack of hydroxide on the neutral nucleobase or attack of water on the N3-protonated base (Scheme 2) (24, 25, 29). Sensitive genetic reversion assays revealed that cytosine deamination occurs in duplex DNA with a half-life of 30 000–85 000 years at pH 7.4 and 37 °C (27, 28). In single-stranded DNA and at base mismatches in duplex DNA, deamination proceeds much faster ( $t_{1/2} \sim 200$  years) presumably due to increased solvent accessibility of the base (26, 30). Deamination of guanine and adenine residues in DNA is much slower, occurring at only 2–3% of the rate of cytosine deamination (31).



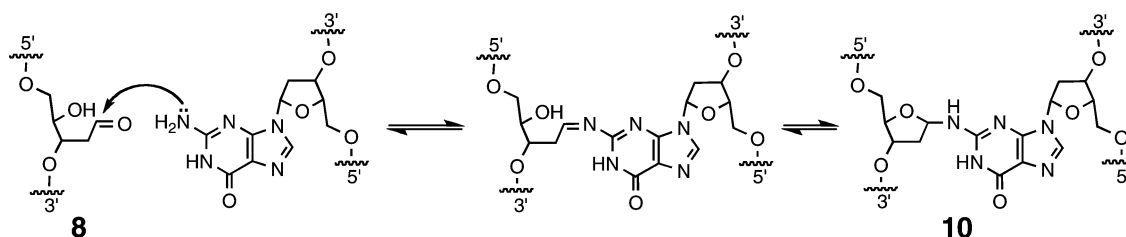
Methylcytosine residues **4** are mutation hotspots in bacterial and eukaryotic genomes (28). The deamination of 5-methylcytosine residues occurs approximately 2–3 times faster than at unmodified cytosine residues (28); however, the increased mutation frequencies observed at methylcytosine positions are believed to stem not from increased deamination at these sites but from the fact that the resulting G–T mismatches are poorly

direct the reader's attention to sites where the "action" is occurring rather than to illustrate complete sequences of bond-making and bond-breaking.

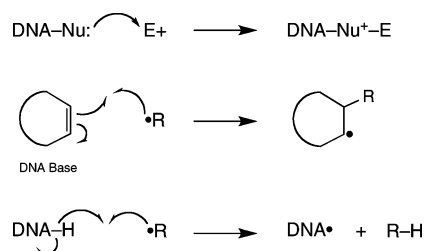
Scheme 3



Scheme 4



Scheme 5



repaired and produce G-C→A-T transitions in one of the daughter cells (28, 32, 33). Mutagenesis resulting from deamination at 5-methylcytosine residues teaches us an important, general lesson: That is, DNA damage reactions that are terribly slow and low yielding can have profound biological consequences if the resulting lesion is not efficiently repaired and is mutagenic or cytotoxic.

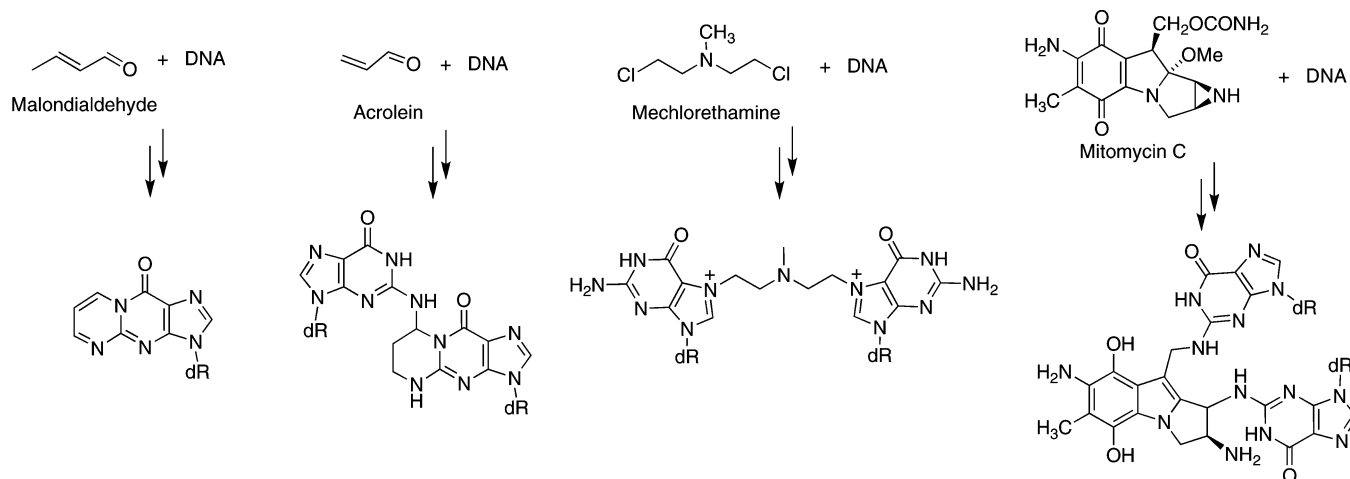
Alkylation of the N3-position of cytosine and reactions that lead to saturation of the 5,6-double bond in cytosine and 5-methylcytosine accelerate deamination (34–41). Activation-induced cytidine deaminases catalyze the conversion of cytosine residues to uracil residues in single-stranded regions of DNA (42, 43). In addition, deamination is an important reaction that is associated with exposure of DNA to nitrosating agents and nitric oxide (44–47).

**2.3. Spontaneous Hydrolysis of the Glycosidic Bonds Connecting the Nucleobases to the DNA Backbone.** With regard to hydrolytic stability, the glycosidic bonds that hold the nucleobases to the sugar–phosphate backbone are weak points in the structure of DNA (Scheme 3). The pyrimidine bases cytosine and thymine are lost with rate constants of  $1.5 \times 10^{-12} \text{ s}^{-1}$  ( $t_{1/2} = 14\,700$  years), while the reaction is faster at the purine bases guanine and adenine, occurring with rate constants of  $3.0 \times 10^{-11} \text{ s}^{-1}$  ( $t_{1/2} = 730$  years) (48, 49). Accordingly, hydrolytic cleavage of the glycosidic bonds in DNA is often referred to as “depurination” because the reaction is much more facile at purines than at pyrimidines. Hydrolysis of the glycosidic bond in 2'-deoxypurines proceeds via a specific acid-catalyzed  $S_N1$  reaction mechanism (50, 51). Equilibrium protonation increases the leaving group ability of the base and facilitates unimolecular, rate-limiting C–N bond cleavage that generates the free base 5 and an oxocarbenium ion 6 (Scheme 3). The oxocarbenium ion undergoes subsequent hydrolysis to yield an abasic site 7 (often referred to as an apurinic site or AP site). It is calculated that spontaneous depurination generates about 10 000 abasic sites

per cell per day (49). Indeed, steady state levels of 10 000–50 000 abasic sites have been detected in cells (52, 53). Depurination occurs about four times faster in single-stranded DNA than it does in duplex DNA (49).

**2.4. Properties of Abasic Sites Arising from Depurination.** Abasic sites (7, Scheme 3) generated by depurination are cytotoxic and mutagenic (54, 55). This lesion exists as an equilibrium mixture of the ring-closed acetal (7, 99%) and the ring-opened aldehyde (8, 1%) (56). Abstraction of the acidic  $\alpha$ -proton adjacent to the aldehyde group in 8 leads to  $\beta$ -elimination of the phosphate residue on the 3'-side of the abasic site. This strand-cleavage reaction occurs with a half-life of 200 h under physiological conditions (pH 7.4 and 37 °C) (57, 58). Thermal workup, sodium hydroxide, or treatment with various amines such as piperidine, dimethylethylene diamine, or putrescine facilitate strand cleavage at abasic sites (59–62). Subsequent  $\gamma,\delta$ -elimination of the phosphate on the 5'-side of the abasic site 9 is relatively slow under physiological conditions (57) but occurs readily under basic conditions (e.g., 0.05 M NaOH, 37 °C, and 2 h) to generate a strand break with 3'- and 5'-phosphate termini (63, 64).

In addition to generating single strand breaks, the aldehyde residue of the ring-opened form of the abasic site can react with the exocyclic  $N^2$ -amino group of a guanine residue on the



**Figure 2.** Reactions of bifunctional electrophiles with DNA.

opposing strand to generate an interstrand cross-link **10** at 5'-d(CAp) sites (where Ap = the abasic site; Scheme 4) (65). This is especially intriguing because interstrand DNA cross-links are highly cytotoxic lesions (66–68).

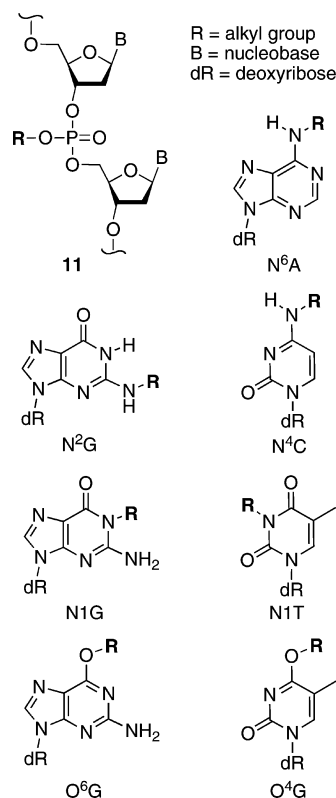
### 3. Overview: Common Reactions by Which Small Organic Molecules Damage DNA

The vast majority of DNA damage caused by bioactive molecules such as drugs, toxins, and mutagens falls into just two general categories: (1) alkylation of a DNA nucleophile by an electrophile or (2) the reaction of a  $\pi$ -bond or C–H bond in DNA with a radical intermediate (Scheme 5). In the following sections, we will consider the reactions of electrophiles and radicals with DNA and examine the fate of the chemically modified DNA that is generated in these processes.

## 4. DNA Alkylation

**4.1. Preferred Alkylation Sites in DNA.** Almost all of the heteroatoms in the double helix have the potential to be alkylated. The preferred sites of alkylation in duplex DNA depend strongly on the nature of the alkylating agent. For example, in the case of diethylsulfate, the preferred sites of reaction follow the order: N7G  $\gg$  P–O  $>$  N3A  $\gg$  N1A  $\sim$  N7A  $\sim$  N3G  $\sim$  N3C  $\gg$  O6G (69, 70). In contrast, the preferred sites for alkylation by ethyldiazonium ion follow the order: P–O  $\gg$  N7G  $>$  O2T  $>$  O6G  $>$  N3A  $\sim$  O2C  $>$  O4T  $>$  N3G  $\sim$  N3T  $\sim$  N3C  $\sim$  N7A (69, 70). The N7-position of the guanine residue is the most nucleophilic site on the DNA bases and is a favored site of reaction for almost all small, freely diffusible alkylating agents. The preferences observed at other sites commonly have been rationalized in terms of hard–soft reactivity principles (71). Hard alkylating agents (defined by small size, positive charge, and low polarizability) such as diazonium ions display increased reactivity with hard oxygen nucleophiles in DNA (69, 70, 72–74). On the other hand, soft (large, uncharged, and polarizable) alkylating agents like dialkylsulfates favor reactions at the softer nitrogen centers in DNA.<sup>2</sup>

Typically, small diffusible alkylating agents react with DNA at multiple sites; however, connection of a nonselective alky-



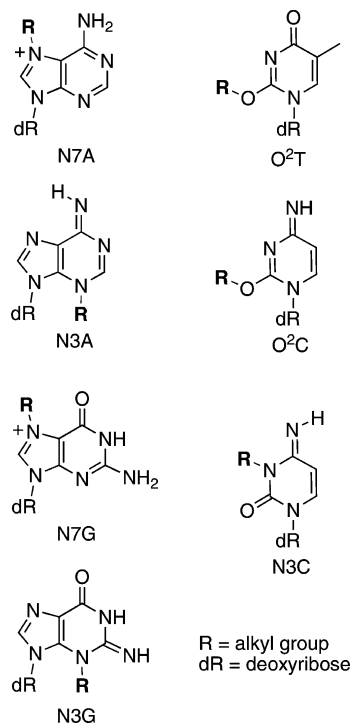
**Figure 3.** Chemically stable lesions resulting from DNA alkylation.

lating agent to noncovalent DNA-binding units can confer sequence and atom site selectivity to the reaction (75–83). Bifunctional alkylating agents (molecules containing two electrophilic centers) can cross-link two nucleophilic centers in the DNA duplex. Examples of bifunctional DNA alkylating agents shown in Figure 2 include the endogenous lipid peroxidation products malondialdehyde (84) and acrolein (85) and the anticancer drugs mitomycin C (86) and mechlorethamine (87). Cross-links may present special challenges to DNA repair systems, and agents that generate cross-links can be exceptionally bioactive (66–68). Accordingly, a significant number of clinically used anticancer drugs are DNA cross-linking agents (66).

**4.2. The Chemical Fate of Alkylated DNA. 4.2.1. Alkylated Phosphates Are Chemically Stable.** Alkylation of a DNA phosphodiester residue yields a phosphotriester (**11**, Figure 3). Phosphotriesters undergo hydrolysis about  $10^9$  times more

<sup>2</sup> Fishbein and co-workers provided an excellent discussion of the mechanistic origin of hard–soft acid base effects in DNA alkylation reactions. On page 1466 of ref 74, these authors note that “the low degree of covalency in the transition state for  $S_N2$  substitution on primary diazonium ions means that electrostatic stabilization of the transition state by the nucleophile will become a dominant interaction and this gives rise to enhanced oxygen atom alkylation by primary diazonium ions”.





**Figure 4.** Chemically labile lesions resulting from DNA alkylation.

rapidly than native DNA phosphodiester (15). Nonetheless, hydrolysis of phosphotriesters is slow under physiological conditions, and alkylated DNA phosphates are chemically stable both *in vitro* and *in vivo* (88, 89).

**4.2.2. Alkylation at Some Positions on the DNA Nucleobases Yields Chemically Stable Adducts.** Alkylation at the exocyclic nitrogen atoms N<sup>2</sup>G, N<sup>6</sup>A, and N<sup>4</sup>C, the amidic nitrogens at N1-G and N1-T, and the oxygens at O<sup>6</sup>-G and O<sup>4</sup>-T yield chemically stable adducts (Figure 3) (90–96). Special attention has been paid to the O<sup>6</sup>-G and O<sup>4</sup>-T adducts because these reactions alter the Watson–Crick hydrogen-bonding faces of these bases and cause miscoding and mutagenesis during DNA replication (97–99).

**4.2.3. Alkylation of Some Endocyclic Nitrogens on the Nucleobases Yields Labile Lesions That Undergo Deglycosylation or Ring Opening.** Alkylation of the endocyclic nitrogens N7G, N7A, N3G, N3A, N1A, and N3C can destabilize the nucleobases, facilitating deglycosylation and ring-opening reactions (Figure 4). The half-lives for deglycosylation of bases modified with simple alkyl groups in DNA generally follow the trend: N7dA (3 h) > N3dA (24 h) > N7dG (150 h) > N3dG (greater than 150 h) > O<sup>2</sup>dC (750 h) > O<sup>2</sup>dT (6300 h) > N3dC (>7700 h) (36, 96, 100–105). The mechanisms for these processes (Scheme 6) mirror that described above for acid-catalyzed deglycosylation reactions (Scheme 3) (106). Studies on the depurination of rates of N7-alkylguanine derivatives

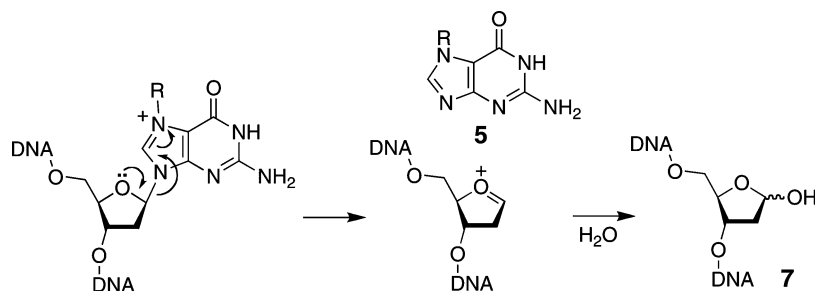
indicate that electron-withdrawing groups on the alkyl substituent increase the rate of deglycosylation (103, 107, 108).

Alternatively, attack of water at the C8-position of an N7-alkylguanine residue initiates a ring-opening reaction that yields the corresponding 5-(alkyl)formamidopyrimidine (FAPy) derivative **12** (Scheme 7) (103, 109–116). In general, the ring-opening reaction that yields alkyl-FAPy derivatives is slow compared to the competing depurination reaction under physiological conditions (103, 117), although basic conditions (e.g., pH 10, 37 °C, and 4 h) can be used to generate alkyl-FAPy lesions from N7-alkylguanine lesions in oligonucleotides (116, 118). Nonetheless, FAPy lesions are formed *in vivo* and are persistent lesions found in animals following exposure to alkylating agents (119–121). In this regard, it may be important that the glycosidic bond in alkyl-FAPy residues is stable at neutral pH (122). Spontaneous deglycosylation of FAPy residues occurs only slowly, with an estimated (103, 123) half-life of about 1500 h in single-stranded DNA at pH 7.5 and 37 °C. The deglycosylation reaction is pH-dependent, however, and at pH 6.5, complete deglycosylation of 5-methyl-FAPy in an oligonucleotide was observed over 48 h (122). The stereochemistry of the attachment between the FAPy base and the deoxyribose sugar residue undergoes anomerization via an imine intermediate **13** (Scheme 7) (122, 124, 125). The half-life of this process is 3.5 h for the FAPy derivative of deoxyguanosine in a nucleoside derivative (123, 126) and 16 h for the FAPy derivative of aflatoxin B1 in a single-stranded DNA oligomer (124).

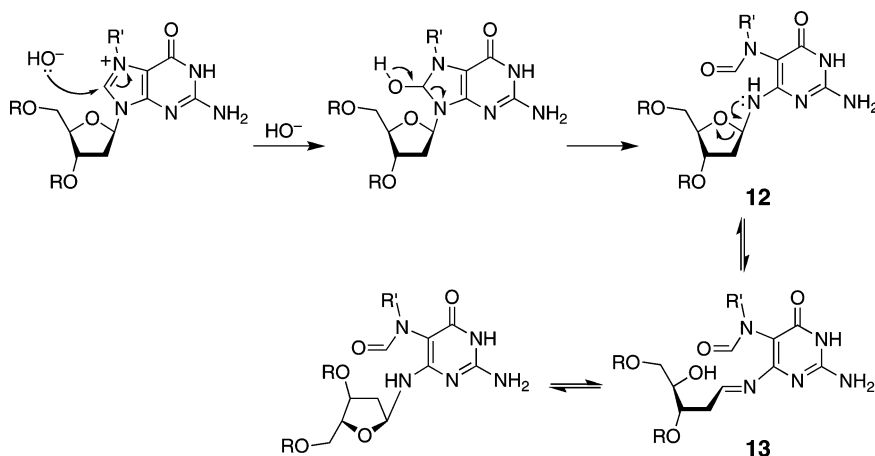
Adenine residues alkylated at the N1-position are also prone to hydrolytic ring opening. In this case, however, ring opening initiates a Dimroth rearrangement reaction that leads to an apparent migration of the alkyl group from the N1-position to the exocyclic N<sup>6</sup>-position of the nucleobase (Scheme 8) (127). The Dimroth rearrangement occurs with a half-life of approximately 150 h at pH 7 in the context of the nucleotide, 1-methyl-2'-deoxyadenosine-5'-phosphate (101). Importantly, this rearrangement can occur even when the adducted base is stacked within the DNA double helix (128).

**4.2.4. Alkylation at Some Endocyclic Nitrogens Accelerates Deamination.** Alkylation of the N3-position of cytosine (Scheme 9) vastly accelerates deamination of the nucleobase. For example, deamination of 3-methyl-2'-deoxycytidine, occurring with a half-life of 406 h, proceeds 4000 times faster than the same reaction in the native nucleoside (36). Alkylation of the endocyclic nitrogen in deoxycytidine also facilitates deglycosylation ( $t_{1/2}$  = 7700 h, lower pathway in Scheme 9), but in the context of the nucleoside, the deamination reaction is approximately 20 times faster and predominates (36). Alkylation at the N1-position of 2'-deoxyadenosine nucleosides typically leads to a Dimroth rearrangement rather than deamination (129); however, the hydroxyl group of N1-styrene adducts

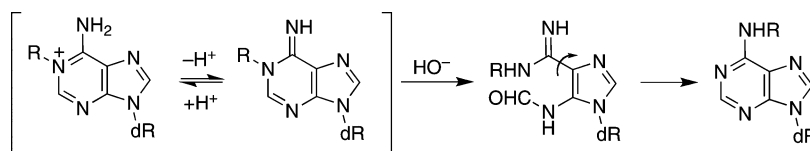
**Scheme 6**



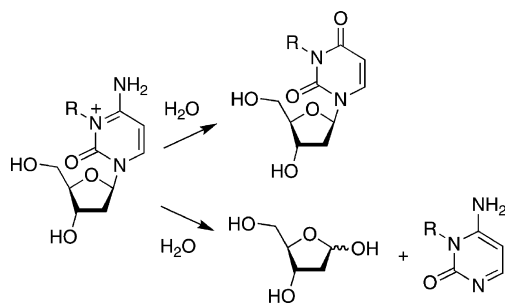
Scheme 7



Scheme 8



Scheme 9



facilitates deamination via intramolecular attack on C6 of the adducted base (Scheme 10) (130–132).

## 5. Reactions of Radicals with DNA

**5.1. Abstraction of Hydrogen Atoms from the 2'-Deoxyribose Sugars of DNA: General Features.** Abstraction of hydrogen atoms from the sugar–phosphate backbone of DNA generates 2-deoxyribose radicals that lead to final strand damage products via complex reaction cascades (133–137). In the case of highly reactive species such as the hydroxyl radical ( $\text{HO}^\bullet$ ), the relative amounts of direct strand cleavage stemming from hydrogen atom abstraction at each position of the deoxyribose sugars are dictated by steric accessibility of the deoxyribose hydrogens rather than their C–H bond strengths (133–138). The steric accessibility of the deoxyribose hydrogens in DNA follows the trend:  $\text{H5}' > \text{H4}' \gg \text{H3}' \sim \text{H2}' \sim \text{H1}'$  (138). Here, I will summarize DNA strand damage arising from hydrogen atom abstraction at the C1'- and C4'-positions of deoxyribose.

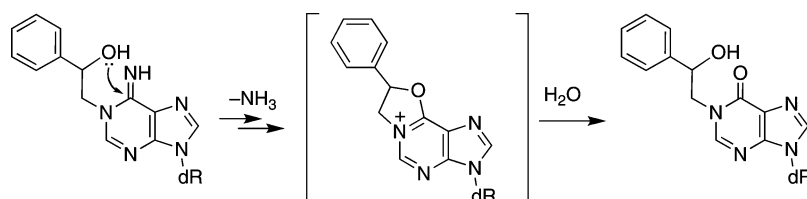
These examples were selected because they are perhaps the best-characterized pathways and are illustrative of the general types of reactions that stem from radical-mediated damage to the deoxyribose units of DNA. A number of excellent reviews provide more comprehensive coverage of radical-mediated strand damage reactions (133–137).

**5.2. Alkali-Labile Strand Damage Stemming from Abstraction of the C1'-Hydrogen Atom.** Although sequestered deep in the minor groove of duplex DNA (138), the C1'-hydrogen atom is an important reaction site for a variety of agents including  $\text{Cu}(\text{o-phenanthroline})_2$ , neocarzinostatin, espermicin, dynemicin, tirapazamine, and radiation (134, 139). In addition, some base radical adducts efficiently abstract the C1'-hydrogen atom (140).

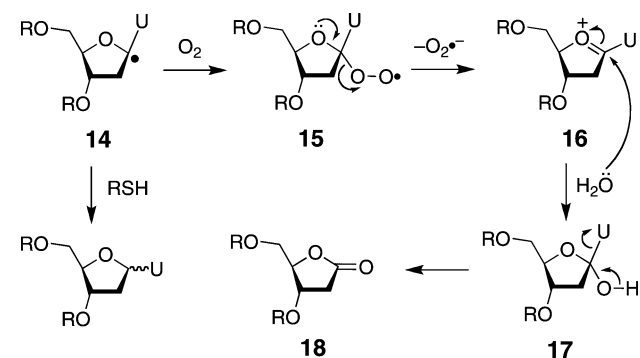
In cells, 2-deoxyribose radicals generally are expected to react with either molecular oxygen, present at 60–100  $\mu\text{M}$ , or thiols such as glutathione, which are present at 1–10 mM. The C1' radical abstracts a hydrogen atom from thiols with a rate constant of  $1.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  in duplex DNA (141). This reaction has the potential to generate either the natural  $\beta$ -anomer (a “chemical repair” reaction) or the mutagenic  $\alpha$ -anomer. Greenberg's group showed that, in duplex DNA, the C1' radical reacts with 2-mercaptoethanol to yield a 6:1 ratio of the natural  $\beta$ -anomer over the  $\alpha$ -anomer (141).

Molecular oxygen reacts with the C1' radical **14** with a rate constant of  $1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  to yield the peroxy radical **15** (Scheme 11) (142). The peroxy radical ejects superoxide radical anion (the  $\text{pK}_\text{a}$  of the relevant conjugate acid  $\text{HOO}^\bullet$  is 5.1) with a rate constant of  $2 \times 10^4 \text{ s}^{-1}$  to generate the carbocation **16** (142) that, in turn, is attacked by water to give the presumed C1'-alcohol intermediate **17**. Loss of the DNA base yields the

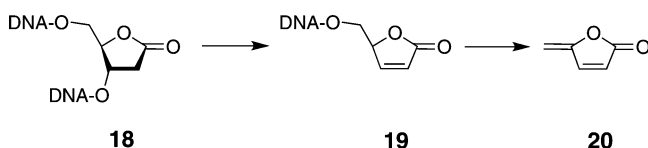
Scheme 10



Scheme 11



Scheme 12

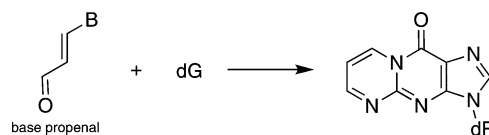


2-deoxyribonolactone abasic site **18**. The ratio of rate constants  $k_{O_2}/k_{RSH} = 1100$  (143). This means that at normal cellular concentrations of oxygen ( $\sim 70 \mu\text{M}$ ) and thiol (5 mM) more than 90% of the radical will be trapped by molecular oxygen.

The 2-deoxyribonolactone lesion **18** does not rapidly yield direct strand cleavage ("frank strand breaks") under physiological conditions (144–146). Rather, **18** is a base-labile lesion that is efficiently converted into a strand break upon treatment with piperidine (0.1 M, 20 min, and 90 °C) or hydroxide (at pH 10 and 37 °C, the  $t_{1/2}$  for the cleavage reaction is 15 min in a single-stranded oligomer) (144, 145). Under physiologically relevant conditions,  $\alpha,\beta$ -elimination of the 3'-phosphate residue slowly yields strand cleavage with a half-life of between 38 and 54 h in double-stranded DNA, depending on the identity of the base that opposes the lactone abasic site (Scheme 12) (144–146). The resulting 3'-ene-lactone intermediate **19** subsequently undergoes rapid  $\gamma,\delta$ -elimination to give the methylene lactone byproduct **20** and an oligonucleotide with a 3'-phosphate residue (145, 146). The transient ene-lactone **19** has been trapped by a variety of chemical reagents (144). In addition, the 2-ribonolactone and the ene-lactone form covalent cross-links with DNA-binding proteins (147, 148).

**5.3. Direct Strand Cleavage Initiated by Abstraction of the 4'-Hydrogen Atom.** Abstraction of the 4'-hydrogen atom from the sugar–phosphate backbone of duplex DNA is a major reaction for hydroxyl radical ( $\text{HO}^\bullet$ ), tirapazamine, bleomycin, and several enediynes (136, 139, 149). The C4' radical **21** abstracts hydrogen atoms from biological thiols with a rate constant of about  $2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  in single-stranded DNA (150). In duplex DNA, this chemical repair reaction yields a

Scheme 14

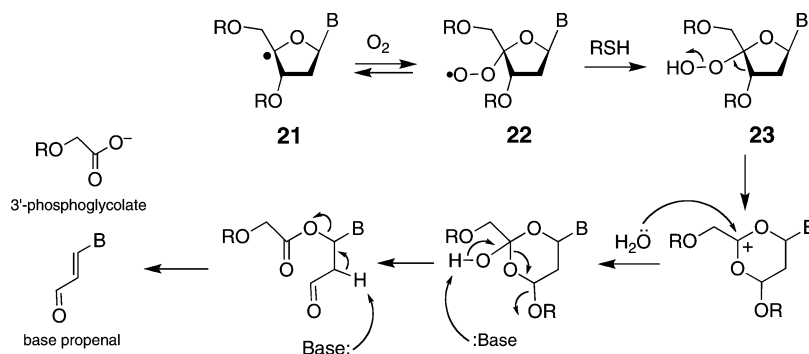


10:1 ratio of natural:unnatural stereochemistry at the C4' center (151). Reaction of the C4' radical **21** with molecular oxygen yields the peroxy radical **22**, with a presumed rate constant of about  $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (Scheme 13) (150). Reaction of the peroxy radical with thiol, occurring with a rate constant of  $k \leq 200 \text{ M}^{-1} \text{ s}^{-1}$  (152), yields the hydroperoxide **23**. This intermediate has been observed directly by MALDI mass spectroscopy in a single-stranded 2'-deoxyribonucleotide in which a single C4' radical was generated at a defined site (153). In the case of agents such as bleomycin, peroxyxynitrite, and the enediynes, the hydroperoxide **23** is thought to yield direct strand cleavage via a Criegee rearrangement, followed by hydrolysis and fragmentation reactions that generate the 5'-phosphate, 3'-phosphoglycolate, and base propenal end products (Scheme 13) (153). The base propenals generated in this reaction act as "malondialdehyde equivalents" that can go forward to cause further damage to DNA via covalent modification of guanine residues (Scheme 14) (154, 155).

**5.4. Reactions of Radicals with the Nucleobases.** Radicals react readily with the heterocyclic bases of DNA. For example, over 90% of the hydroxyl radicals generated by  $\gamma$ -radiolysis react at the nucleobases in polyU (see page 340 of ref 156). A large number of base damage products arising from the reaction of hydroxyl radical with DNA have been characterized (Figure 5) (157–162). These products arise via hydrogen atom abstraction or, more commonly, addition of hydroxyl radical to the  $\pi$ -bonds of the bases (Scheme 5). Here, we consider the formation of several common DNA base damage products arising from reactions at thymidine and deoxyguanosine. The pathways described here produce some of the most prevalent oxidative base damage products and also illustrate the general types of reactions that commonly occur following the attack of radicals on the nucleobases.

**5.5. Addition of Radicals to C5 of Thymine Residues: Generation of Thymine Glycol.** Radicals such as  $\text{HO}^\bullet$  readily add to the carbon–carbon  $\pi$ -bond of thymine residues (163). The addition of hydroxyl radical to the C5-position thymine residues generates the C6-thymidine radical (**24**, Scheme 15) (133). (To a lesser extent, hydroxyl radical also adds to the C6-position of thymine residues (133), but reactions stemming from this pathway will not be discussed here.) The base radical adduct **24** resulting from the addition of  $\text{HO}^\bullet$  at C5 may be reduced by reagents such as thiols or superoxide radical to yield the 5-hydroxy-6-hydrothymine residue (**25**, Scheme 15) (133, 164).

Scheme 13



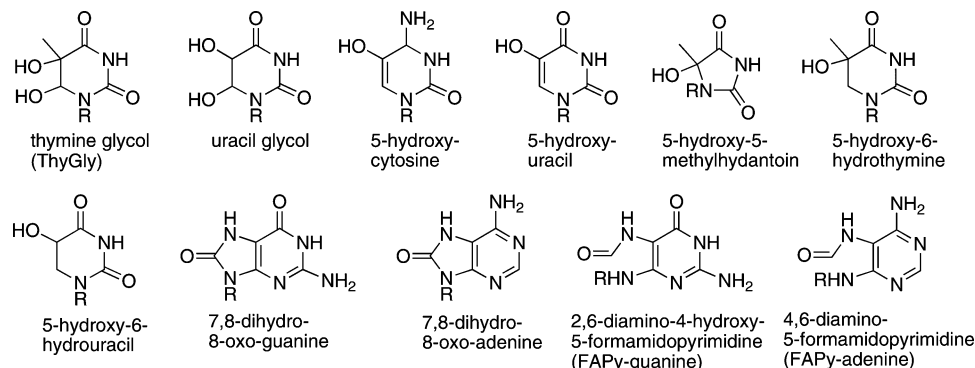
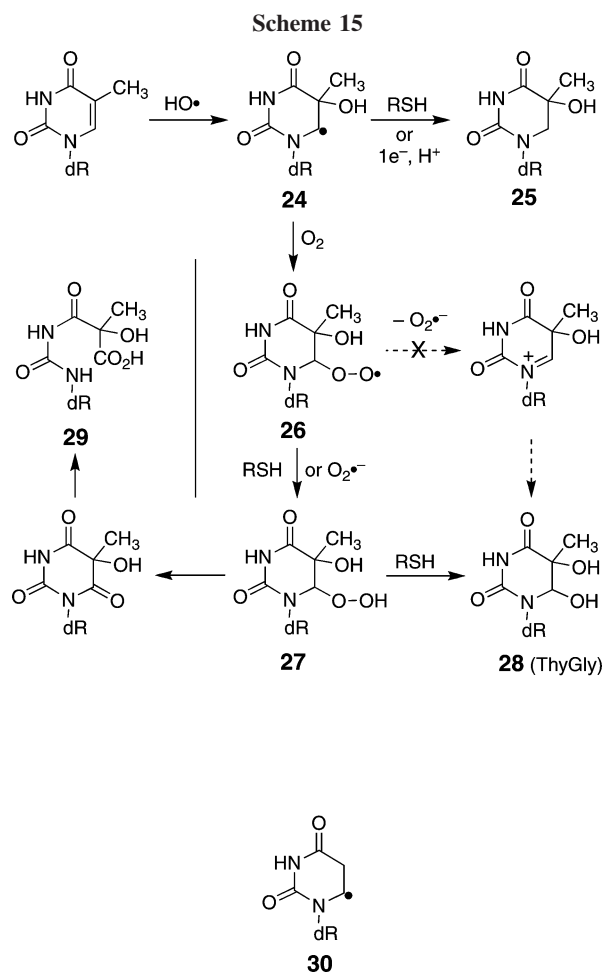


Figure 5. Oxidatively damaged nucleobases.



Alternatively, the reaction with molecular oxygen produces the peroxyl radical **26**. On the basis of model reactions involving the uracil free base (165), it has been proposed that ejection of superoxide radical anion from **26**, followed by subsequent attack of water, generates thymine glycol **28** (133). However, recent studies did not detect production of superoxide from the structurally related peroxyl radical derived from 5,6-dihydro-2'-deoxyuridin-6-yl **30**. It was concluded that the rate for superoxide elimination is less than  $10^{-3} \text{ s}^{-1}$  (166). This suggests that, in biological systems, the peroxyl radical **26** may instead abstract a hydrogen atom from thiols to give the hydroperoxide **27**, which, in turn, is expected to undergo further thiol-mediated reduction to yield thymine glycol **28**. Thymine glycol is one of the major products stemming from oxidative damage of DNA (157–161). In the absence of thiol, the hydroperoxide **27** undergoes slow decomposition ( $t_{1/2} = 1.5\text{--}10 \text{ h}$  for the nucleoside in water, with the trans isomer decomposing more

rapidly than the cis) to generate the ring-opened lesion **29** as the major product and thymine glycol **28** as a minor product (Scheme 15) (167).

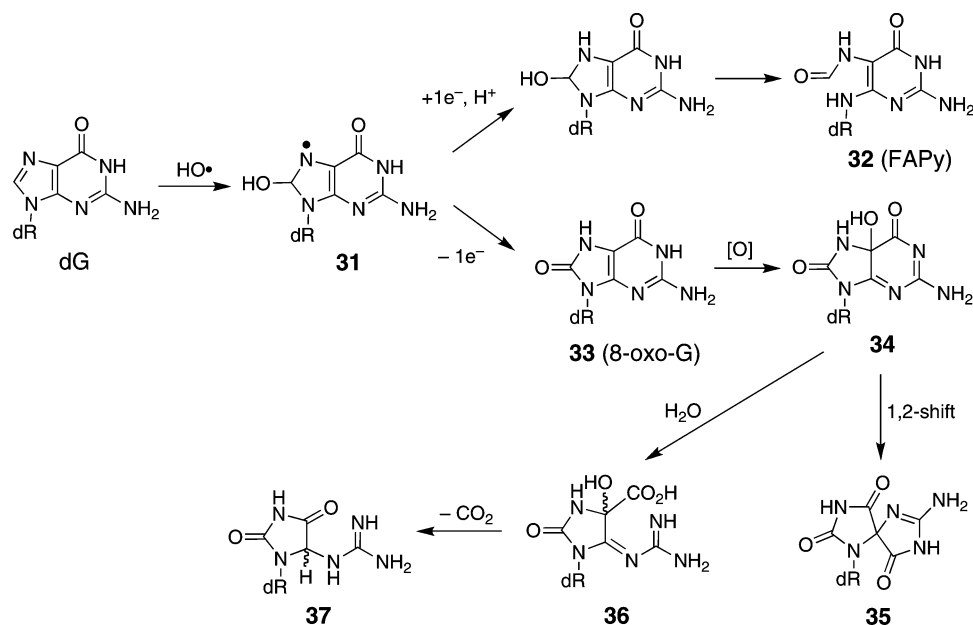
**5.6. Addition of Radicals to Guanine Residues: Generation of FAPy-G and 8-Oxo-G Residues.** Guanine is a major target for oxidative damage by radicals (157, 168, 169). A primary pathway for the reaction of radicals with guanine residues involves addition to the C8-position (Scheme 16) (157, 170, 171). This process yields a “redox ambivalent” nucleobase radical **31** that can undergo either one-electron reduction or oxidation (157, 164, 171). Reduction leads to a ring-opened formamidopyrimidine (FAPy) lesion (**32**, Scheme 16) that is chemically stable in DNA under physiological conditions and is mutagenic (172–175). On the other hand, oxidation of the base radical adduct **31** yields 8-oxo-7,8-dihydroguanosine (**33**, 8-oxo-G). The 8-oxo-G lesion has been incorporated into synthetic 2'-deoxyoligonucleotides (176) but is prone to oxidative decomposition under biologically relevant conditions (157, 177). For example, two-electron oxidation of 8-oxo-G (**33**) yields 5-hydroxy-8-oxo-7,8-dihydroguanosine **34** (178). This compound can decompose to yield the spiroamino-dihydantoin **35** via a 1,2-shift (178). Alternatively, hydrolysis of **34** generates the ureidoimidazoline **36**, which undergoes decarboxylation to the guanidinohydantoin **37** (178). In nucleosides, guanidinohydantoin formation is favored at pH values  $<7$ , while formation of the spiroaminohydantoin **35** is favored at pH values  $\geq 7$  (178–180). In the context of duplex DNA, oxidative degradation of 8-oxo-G residues yields the guanidinohydantoin residue **37** as the major product (177), although this lesion may be prone to further decomposition to products such as imidazolone and oxazolone (181, 182). Guanidinohydantoin **37**, spiroaminodihydantoin **35**, and 8-oxo-G **33** are mutagenic lesions in duplex DNA (182–186).

**5.7. Tandem Lesions.** In some cases, initially generated DNA radicals can undergo secondary reactions within the duplex. The resulting “tandem lesions”, comprised of cross-links or multiple adjacent damage sites, may present special challenges to DNA replication and repair systems (187, 188). For example, 5,6-dihydrothymidine radical (**38**, Scheme 17), in the presence of molecular oxygen, generates the corresponding peroxyl radical **39** that abstracts the C1'-hydrogen atom from the sugar residue on the 5'-side to produce a tandem lesion **40**, consisting of a damaged pyrimidine base adjacent to a 2-deoxyribonolactone residue (189, 190).

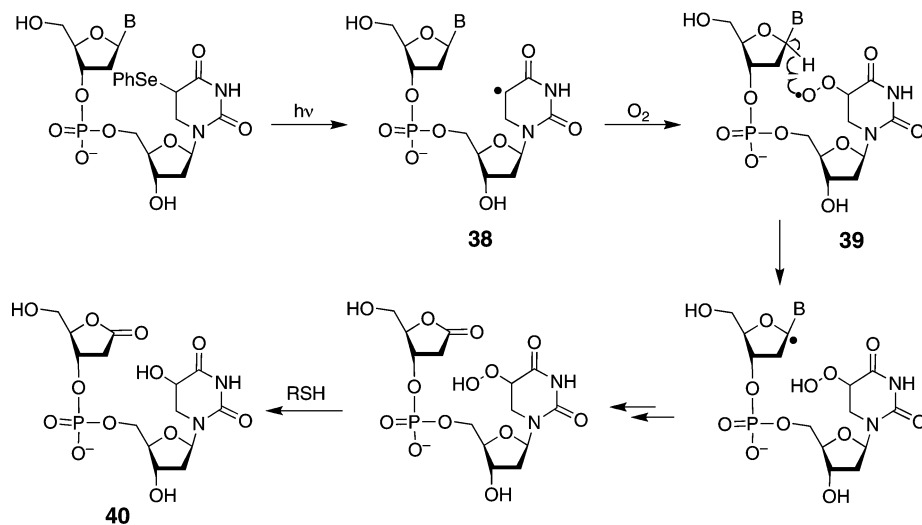
The 5-(2'-deoxyuridinyl)methyl radical (**41**, Scheme 18) generates intrastrand cross-links with adjacent purine residues (191, 192). This reaction is favored at 5'-GT sites (191). The 5-(2'-deoxycytidinyl)methyl radical can cross-link with guanine residues in a similar manner (193). The 5-(2'-deoxyuridinyl)-



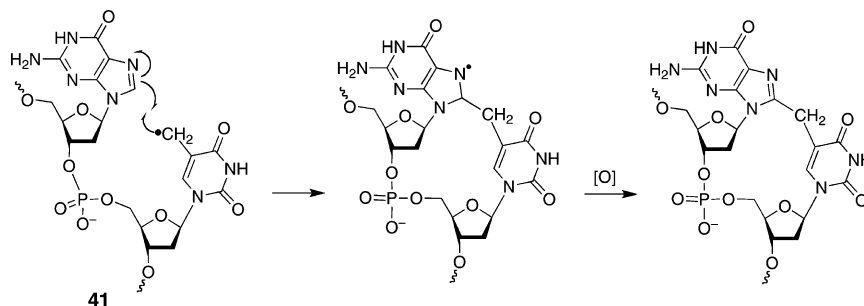
Scheme 16



Scheme 17



Scheme 18

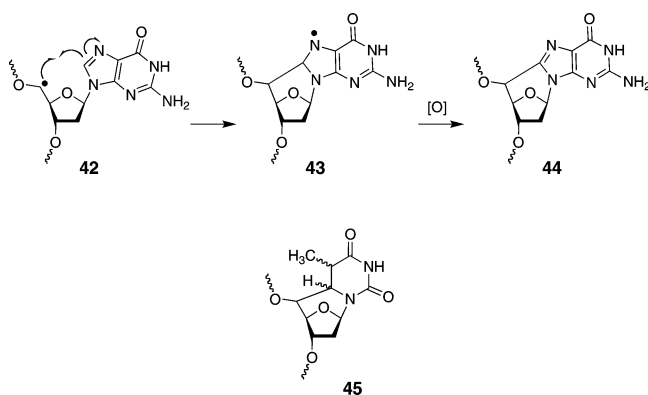


methyl radical **41** has also been shown to forge an interstrand cross-link with the opposing deoxyadenosine (194, 195).

Under low oxygen conditions, C5'-sugar radicals can react with the base residue on the same nucleotide. In purine nucleotides, the carbon-centered radical **42** can add to the C8-position of the nucleobase (Scheme 19). Oxidation of the intermediate nucleobase radical **43** yields the 8,5'-cyclo-2'-deoxypurine lesion **44** (161, 164, 187, 188, 196–199). Similarly, in pyrimidine nucleotides, the C5' radical can add to the C6-

position of nucleobase. Reduction of the resulting radical intermediate yields the 5',6-cyclo-5,6-dihydro-2'-deoxypyrimidine lesion **45** (200–202). These cyclolesions are chemically stable in DNA and may present special challenges to cellular repair systems (161, 164, 187, 188, 196–202).

Scheme 19



## 6. Conclusion

Francis Crick wrote, "DNA is such an important molecule that is almost impossible to learn too much about it" (203). Indeed, today, more than 50 years after the primary chemical structure of DNA was established (1), there is a continuing need to develop our understanding of biologically relevant DNA chemistry. Characterization of the chemical reactions of endogenous cellular chemicals, anticancer drugs, and mutagens with cellular DNA is important because the biological responses engendered by any given DNA-damaging agent are ultimately determined by the chemical structure of the damaged DNA. Improvements in methodologies for the detection and characterization of DNA damage such as LC/MS, high-field NMR, and the synthesis of non-natural 2'-deoxyoligonucleotides have continued to drive the field forward (143, 204–207). DNA-damaging agents can elicit a wide variety of cellular responses including cell cycle arrest, up-regulation of DNA repair systems, apoptosis, or mutagenesis (7–13); however, the relationships between the structure of damaged DNA and the biological responses that ensue are not yet well understood. Advances in this area have the potential to yield both fundamental and practical advances in cell biology, predictive toxicology, and anticancer drug development.

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