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Invited Review

Biologically Relevant Chemical Reactions of N7-Alkylguanine Residues in DNA

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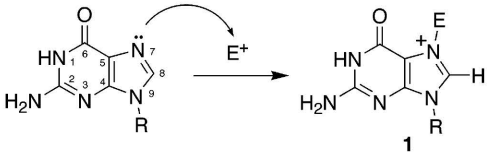
1. Introduction

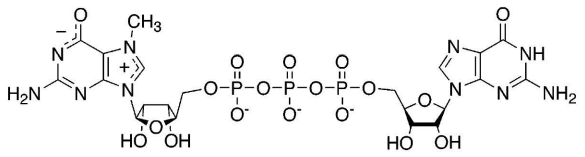
The N7 position of guanine is the most nucleophilic site within the heterocyclic bases of DNA (*1*). Accordingly, when double-stranded DNA is exposed to electrophiles, the predominant reaction often involves covalent bond formation at this site (Scheme 1) (*2*−*7*). The resulting product (**1**; Scheme 1) is often referred to as a "DNA adduct" or "DNA lesion". While small adducts such as N7-methylguanine and N7-ethylguanine have been characterized as well-tolerated by cells (*3*, *8*, *9*), it is clear that larger N7-alkylguanine lesions such as those formed by the aflatoxins, pluramycins, altromycins, azinomycins, *S*-(2-haloethyl)glutathione, and leinamycin can have potent biological activities including cytotoxicity and mutagenicity (*10*−*21*).

Scheme 1

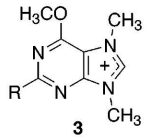


1

N7-Alkyl-2′-deoxyguanosines (**1**) were among the first covalent DNA adducts characterized (*22*, *23*), and over the years, a wealth of studies have examined the fundamental chemical properties of these lesions. The resulting information provides a foundation for understanding the biological effects of this type of DNA damage. In addition, this chemistry is relevant to naturally occurring N7-alkylguanines such as those found in the 5′-cap of eucaryotic mRNA transcripts (**2**) and in secondary metabolites such as the heteromines (**3**, **4**) and herbipoline (**5**) (*24*−*27*). During our recent studies of the N7-guanine adduct formed by the natural product leinamycin (*21*), we became aware of the need for a comprehensive review covering this important area of nucleic acid chemistry. Therefore, we set out here to provide an overview of the biologically relevant chemical reactions of N7-alkylguanine lesions.

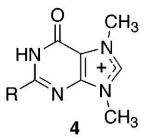


2, 5′-mRNA cap



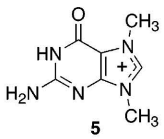
3

Heteromine A R=N(CH$_3$)$_2$
B R=NHCH$_3$
C R=NH$_2$



4

Heteromine D R=N(CH$_3$)$_2$
E R=NHCH$_3$



5

Herbipoline

In this review, we consider five fundamental reactions of N7-alkylguanine residues in DNA: (i) loss of the C-8 proton, (ii) depurination, (iii) ring opening to yield 5-*N*-alkyl-2,6-diamino-4-hydroxyformamidopyrimidine (alkyl-FAPy-G) lesions, (iv) reactions in which the intact guanine residue serves as a leaving group in decomposition of the adduct, and (v) rearrangement of N7 adducts to C-8 adducts. In 1963, Lawley and Brookes noted regarding N7-alkylguanine lesions that "destabilization of the alkylated deoxyguanosine moieties result(s) principally from the quaternization of the N7 which... deplete(s) the electrons from the guanine ring system" (*28*). Consistent with this idea, almost all known decomposition reactions of N7-alkylguanine lesions are driven by neutralization of the formal positive charge that is imposed on the purine ring system by N7-alkylation.

2. Acidity of the C-8 Proton in N7-Alkylguanine Residues

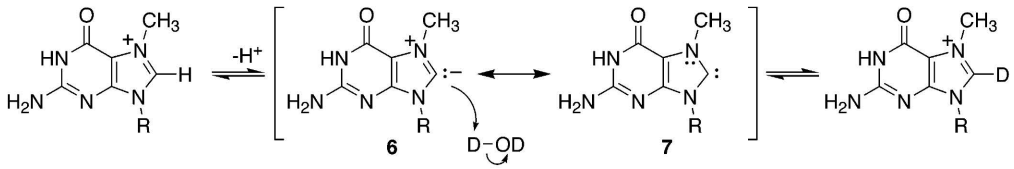
2.1. Exchange of the C-8 Proton

The C-8 proton in N7-alkylguanine residues readily undergoes exchange with solvent protons (*29*, *30*). At pH 4.1 and 28 °C, the half-life of this exchange process for N7-methylguanosine is 5 min (*30*). At pH 1, no C-8 exchange was observed over the course of 6 h, and at pH 7, the reaction was too fast to be measured by NMR. A similar exchange of the C-8 proton in unalkylated guanosine occurs much more slowly, with a rate constant of 4.15×10^{-3} h^{-1} at pH 7.1 and 37 °C ($t_{1/2} = 167$ h) (*31*). In these early studies, exchange of the C-8 proton of N7-methyladenine residues in duplex DNA was not observed at pH 6−7 (*30*), although this reaction has been reported more recently for 2′-deoxyadenosine adducts of aflatoxin B_1 and *S*-(1-acetoxymethyl)glutathione at neutral pH (*32*, *33*).

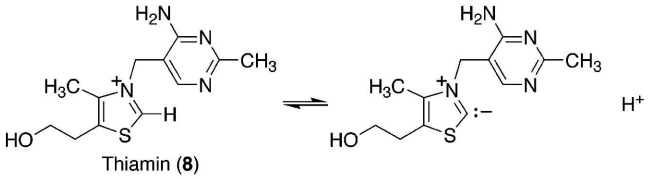
The results of mechanistic studies suggest that exchange of the C-8 proton in N7-methylguanine residues takes place via the ylid **6**, which can also be formulated as a stable, neutral carbene (**7**; Scheme 2) (*30*, *34*, *35*). A similar ylid accounts for the facile exchange of the hydrogen on the alkylthiazolium ring in thiamin (**8**) and is a catalytically important form of this enzyme cofactor (Scheme 3) (*36*, *37*). Jencks and co-workers reported that the relevant proton in thiamin has a pK_a of 18 (*38*). Interestingly, analogous to the thiamin-derived ylid (*34*, *39*), 7-methylguanosine can catalyze the benzoin condensation (Scheme 4) (*30*).

The facile exchange of the C-8 proton in N7-alkylguanine residues has been utilized for quantitative detection of N7-alkylguanine lesions in DNA (*40*). These assays measure the loss of radioactivity from 8-^{3}H-guanine-containing DNA following exposure to various alkylating agents. The yield of tritiated water that is "washed out" of the labeled DNA correlates directly with the number of 7-alkylguanine residues present in the alkylated DNA (*40*). This method has been used as a diagnostic test to

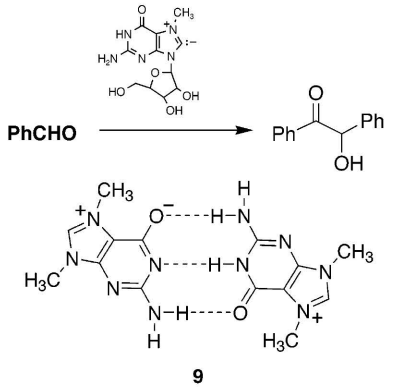
Scheme 2



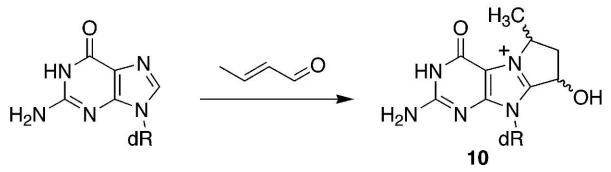
Scheme 3



Scheme 4



Scheme 5



determine whether N7-guanine adducts are formed by novel DNA-alkylating agents (*40*−*42*) and also was adapted as a means to measure the relative rates of DNA alkylation by dimethyl sulfate and several nitrogen mustard derivatives (*43*). Finally, in NMR studies, the disappearance of the C-8 hydrogen resonance due to exchange with deuterated solvent is considered characteristic of N7-alkylguanine lesions in DNA (*44*−*46*).

The alkylation of guanine residues at the N7 position also increases the acidity of the N1 proton on the base. The pK_a of the N1 proton changes from approximately 9 in an unmodified guanine residue to about 7 in an N7-alkylguanine residue (*28*, *47*−*51*). It has been suggested that deprotonated N7-alkylguanine residues could contribute to mutagenesis by mispairing with thymidine triphosphate during DNA replication (*52*, *53*). In addition, the increased acidity of N7,N9-dialkylguanines allows the formation of the stable self-complimentary base pair **9** in aqueous solution (*54*).

2.2. Formation of Cyclic C-8/N7-Guanine Adducts

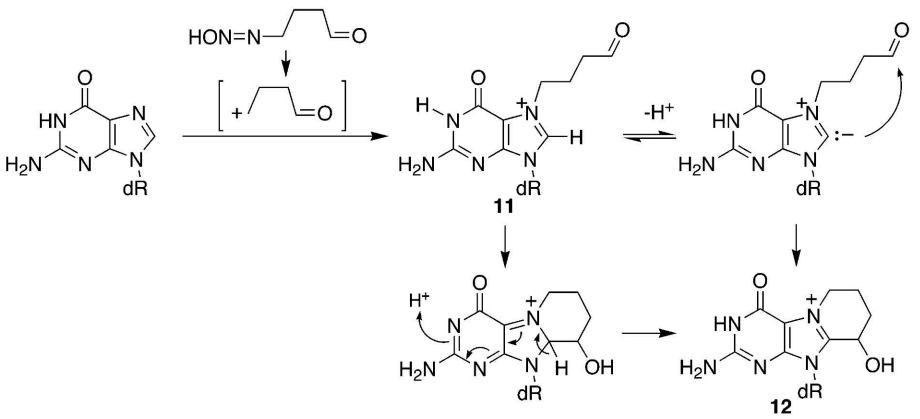
In reactions with certain bifunctional electrophiles, the propensity for N7-alkylated guanine residues to form equilibrium amounts of the corresponding C-8 anion leads to the formation of cyclic lesions spanning the N7 and C-8 positions of the base. For example, crotonaldehyde and 4-oxobutanediazohydroxide produce adducts **10** and **12**, respectively (Schemes 5 and 6) (*55*−*57*). Two reasonable mechanisms have been proposed for this process (*55*, *56*) involving intramolecular attack of C-8 on the aldehyde residue of an initially formed N7 adduct (e.g., **11**; Scheme 6) triggered by loss of either the N1 proton or the C-8 proton (Scheme 6). Interestingly, significantly higher yields of the cyclic adduct were formed in the reaction of 4-oxobutanediazohydroxide with guanosine as compared to 2′-deoxyguanosine (*56*). It was rationalized that the inductive effect of the 2′-OH group found in ribose increases the acidity of the C-8 proton. Consistent with this idea, treatment of the N7-alkyl-2′-deoxyguanosine adduct **11** with base leads to disappearance of the starting material along with a corresponding increase in the cyclic adduct **12** (*56*).

3. Depurination of N7-Alkylguanine Residues

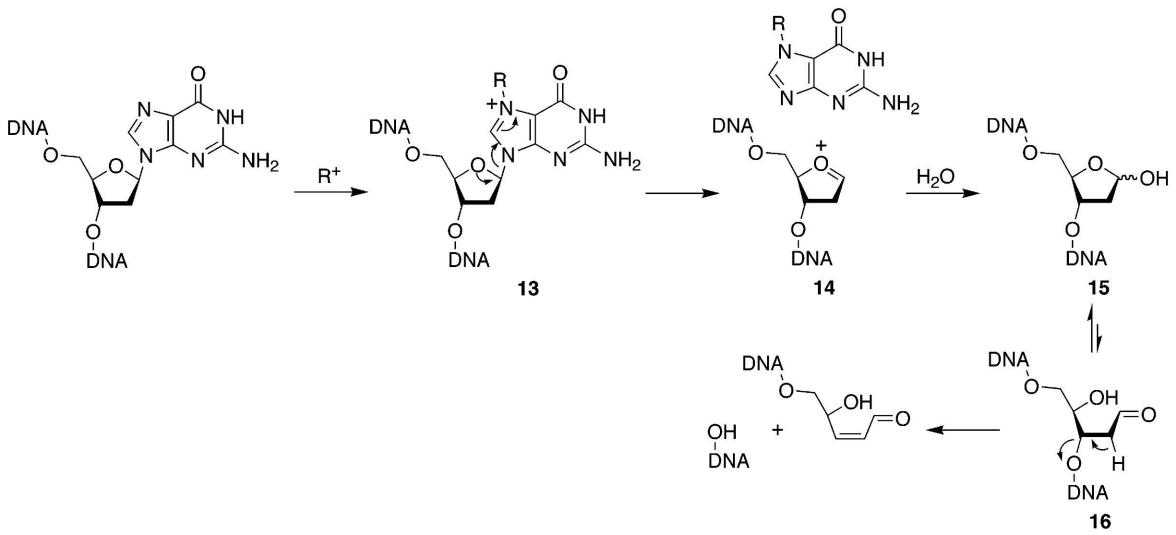
3.1. Acid-Catalyzed Depurination

The rate for spontaneous loss of unmodified purine and pyrimidine residues from duplex DNA under physiological conditions (pH 7.4, 37 °C) is quite low at 3×10^{-11} ($t_{1/2}$ = 730 years) and 1.5×10^{-12} s^{-1} ($t_{1/2}$ = 14 700 years), respectively (*58*−*60*). However, the loss of purine residues from DNA (depurination) is greatly accelerated under acidic conditions and at high temperatures (*59*−*62*). Under acidic conditions, the N7 position of guanine in DNA becomes partially protonated (the pK_a of **13** where R = H is between 2.5 and 3.5) (*50*, *63*, *64*). Protonation places a formal positive charge on the guanine residue, thus converting the base to a good leaving group for a heteroatom-assisted S_N1 type hydrolysis reaction (Scheme 7) (*50*, *65*−*67*). Formally, this is classified as a $D_N + A_N$ reaction (*68*). The abasic sites (**15**) resulting from depurination are both toxic and mutagenic (*12*, *69*, *70*). Abasic sites exist (*71*) predominantly as the cyclic acetal **15**,

Scheme 6



Scheme 7



which is in equilibrium with small amounts (~1%) of the ring-opened aldehyde form (**16**). The loss of the acidic α-proton adjacent to the carbonyl residue in the aldehydic form of the abasic site can lead to a DNA strand break via elimination of the 3′-phosphate group (Scheme 7) (*72*, *73*). Conversion of an abasic site to a strand break in duplex DNA occurs with a half-life of about 200 h in pH 7.4 buffer at 37 °C (*72*, *73*). Thermolysis (*74*), treatment with hydroxide, or treatment with amines such as putrescine, dimethylethylenediamine, or piperidine facilitates strand breakage at abasic sites (*75*−*79*).

Acid-catalyzed depurination occurs about four times faster in single-stranded DNA than in double-stranded DNA at pH 7.4 and 70 °C (*59*). Inspection of the Arrhenius plot shown in Figure 8 of ref *59* reveals that the activation energy (E_a, obtained from the slopes of the plots) for these two processes is similar and that the increased depurination rates seen in single-stranded DNA vs double-stranded DNA can be traced to differences in the preexponential value, *A* (obtained from the intercepts of the plots, the *A* value reflects $\Delta S^{\ddagger}$ for the reaction). This indicates that the entropy of activation for acid-catalyzed depurination is considerably more favorable in single-stranded DNA than it is for the reaction in double-stranded DNA. One can rationalize this observation by envisioning that as the glycosidic bond breaks, the guanine residue released from single-stranded DNA enjoys a greater increase in entropic freedom as compared to that experienced during the same reaction in the context of duplex DNA, where rotational and translational motion of the base is constrained by stacking and hydrogen bonding within the double helix. A similar trend can be seen in the data of Suzuki and co-workers in their studies of the acid-catalyzed depurination of guanine from internal and terminal positions in single-stranded oligodeoxyribonucleotides (*63*).

3.2. Chemical Mechanism for the Depurination of N7-Alkylguanine Residues

Alkylation of the N7 position of guanine residues in DNA places a formal positive charge on the guanine ring system and greatly increases the rate of depurination (Figure 1) (*28*). Alkylation at a number of other sites in DNA including N3-G, N7-A, N3-A, O^2-C, and O^2-T similarly accelerates the rate of glycosidic bond hydrolysis relative to that seen for the unalkylated bases (*2*, *4*, *69*, *80*, *81*). Analogous to acid-catalyzed depurination, the loss of N7-alkylguanine residues occurs by a unimolecular $D_N + A_N$ mechanism and the reaction rates increase at low pH and higher temperatures (*65*, *67*).

3.3. Overview of Factors That Affect the Rate of Depurination

Depurination is usually the predominant reaction observed for N7-alkylguanine residues in double-stranded DNA under physiological conditions. Nonetheless, the rate at which different N7-alkylguanine lesions undergo depurination from duplex DNA varies widely, with half-lives ranging from 3 to 150 h at neutral pH and 37 °C (Figure 1). It is difficult to provide a simple chemical rationale for all of the depurination rates shown in Figure 1, but several general trends regarding how changes in structure affect the rate of depurination are clear and can be summarized as follows: (i) electron-withdrawing groups on the N7 substituent facilitate depurination by improving the leaving group ability of the alkylated base, (ii) electron-withdrawing substitutents (such as 2′-OH, -F, and -OMe) on the sugar destabilize the oxocarbenium ion-like transition state leading to depurination, thus slowing the reaction, and (iii) depurination is slower in duplex DNA than it is in single-stranded DNA or monomeric nucleosides. In addition, in Section 3.7, we briefly consider cases where the number of adducts per DNA base pair ("adduct density") influences the rate at which N7-alkylguanine residues undergo decomposition. Finally, in Section 3.8, we will consider the speculative, but intriguing, possibility that negatively charged functional groups such as carboxylate residues on the N7 substituent or the phosphate residues of DNA may facilitate depurination by neighboring group participation.

3.4. Electron-Withdrawing Groups on the N7 Substituent Increase Depurination Rates

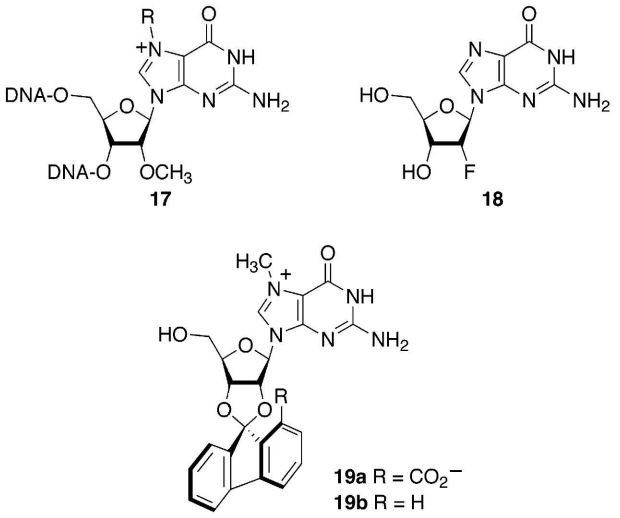
The work of Eisenbrand and Moschel showed that electron-withdrawing groups on the N7 substitutent cause an increase in the rate at which N7-alkylguanosines undergo depurination (*82*, *83*). This trend is

reflected in a Taft ρ (ρ^*) value of 0.175 for a set of substituted N7-ethylguanosine derivatives and a Hammett ρ value (ρ) value of 0.09 for a series of substituted N7-benzylguanosine derivatives. These results indicate that the sensitivity of this reaction to the electron-donating or electron-withdrawing nature of the N7 substituent is modest. For example, a substrate bearing the strongly electron-withdrawing *p*-nitro substituent will react only 1.2 times faster than a substrate bearing the strongly electron-donating *p*-methoxy substituent. For reasons of experimental convenience, these studies were conducted at low pH and high temperature (e.g., 1 N HCl, 50 °C) to accelerate the loss of the alkylated base. In addition, these studies were conducted using ribose, rather than 2′-deoxyribose, analogues. Nonetheless, it seems likely that the general conclusions regarding substituent effects are valid for depurination of N7-alkylated 2′-deoxyguanosine derivatives under physiological conditions.

3.5. Electron-Withdrawing Groups on the Sugar Residue Decrease Depurination Rates

Electron-withdrawing groups on the sugar residue markedly slow the depurination of N7-alkylguanine residues (*65*, *84*, *85*). For example, the depurination reaction of N7-methylguanosine is 150 times slower than the same reaction for the 2′-deoxyguanosine adduct in 0.1 N HCl at 70 °C (Figure 1) (*65*, *83*). Presumably, this is because the electron-withdrawing 2′-OH residue in guanosine destabilizes the oxocarbenium ion-like transition state of the depurination reaction (e.g., intermediate **14** in Scheme 7) (*65*). While it is possible to quantitatively measure the effect of the 2′-hydroxyl on depurination rates under acidic conditions, it may be difficult (or impossible) to directly compare depurination rates for N7-alkylated guanosine and 2′-deoxyguanosine substrates at neutral pH because an alternative mode of decomposition—a ring-opening reaction (discussed in Section 4)—becomes predominant in the case of N7-alkylguanosine derivatives (*86*).

The ability of 2′-electron-withdrawing groups to slow depurination has been put to practical use in at least two areas. In one study, 2′-OMe substituents (**17**) were employed to inhibit depurination of nitrogen mustard-derived N7-alkylguanine lesions in oligonucleotide substrates used in in vitro DNA repair experiments (*87*). In a separate study, Verdine and co-workers relied on the known (*88*) resistance of 2′-deoxy-2′-fluoropurine nucleosides to depurination in the design oligonucleotide inhibitors of the DNA repair enzyme alkyl-*N*-purine DNA glycosylase (*89*). These previous studies lead us to suggest that oligonucleotides containing 2′-deoxy-2′-fluoroguanosine residues (**18**) (*90*, *91*) might be used to prepare duplexes containing stable N7-guanine lesions for use in NMR, X-ray crystallographic, or DNA repair studies. The 2′-fluoro substituent may alter the conformation of the sugar residue from C-2′ endo to C-3′ endo (*92*) but has essentially the same steric requirements as the 2′-proton found in natural DNA (*88*). A potential drawback in this approach lies in the possibility that the electron-withdrawing 2′-fluoro substituent may facilitate decomposition of the N7-alkylguanine residue via an alternate pathway involving ring opening of the modified DNA base (this general reaction is discussed in Section 4).



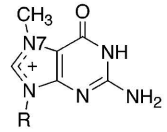
3.6. Depurination Is Slower in Duplex DNA Than in Single-Stranded DNA or Monomeric Nucleosides

The rate at which a given N7-alkylguanine residue undergoes depurination is altered by the structural context of the lesion. For a given N7 adduct, the rate of depurination becomes progressively slower as one moves from nucleosides, to single-stranded DNA, to double-stranded DNA (Figure 1) (*93*). In the few cases where data are available, it can be seen that depurination of N7-alkylguanine residues in double-stranded DNA occurs 50−100 times more slowly than in the monomeric nucleoside (Figure 1). This is an important observation because it indicates that the persistence of N7-alkylguanine lesions in duplex DNA is not controlled solely by the inherent chemical stability of the glycosidic bond in the modified nucleoside but also may be drastically affected by the three-dimensional structure of the double helix in the region surrounding the lesion. Thus, an N7-alkylguanine adduct that distorts the local structure of the DNA duplex (or occurs in a region of DNA that is distorted for other reasons) should be expected to depurinate more rapidly, with a rate approaching that seen for the adduct in a single-stranded or nucleoside context.

While the effect of sequence on the depurination of cross-linked adducts has been examined (*94*, *95*), to the best of our knowledge, the effects of flanking sequence on the depurination of noncross-linked N7-alkylguanine residues in duplex DNA remain unexplored.

3.7. Adduct Density May Alter Stability of N7-Alkylguanine Lesions in Duplex DNA

At least two studies indicate that adduct density (the number of adducts per DNA base pair) can influence the stability of N7-alkylguanine adducts in double-stranded DNA. For example, Wogan and co-workers found that the N7-guanine adduct of aflatoxin B_1 (**37**) is less stable in DNA that is more extensively modified by the carcinogen (the chemistry of this adduct is discussed further in Section 5, and the relevant structures are shown in Scheme 20). The half-life for disappearance of this N7 adduct from DNA containing 1 adduct per 60 base pairs is about 48 h, while the half-life in DNA containing 1 adduct per 1500 base pairs is 100 h (*96*). Several different reactions contribute to the disappearance of this adduct

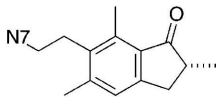


Depurination
In ds-DNA
$t_{1/2}$ = 3800 h (pH 7,10C) (170)
$t_{1/2}$ = 460 h (pH 7,22C) (170)
$t_{1/2}$ = 192 h (pH 7.2,37C) (172)
$t_{1/2}$ = 150 h (pH 7,37C) (173)
$t_{1/2}$ = 144 h (pH 7,37C) (105)
$t_{1/2}$ = 105 h (pH 7,37C) (171)
$t_{1/2}$ = 70 h (pH 7,39C) (170)
$t_{1/2}$ = 69 h (pH 7.4,37C) (169)
$t_{1/2}$ = 4.1 min (pH 7,100C) (113)
In 5'-dGMP
$t_{1/2}$ = 23 h (pH 7.4,37C) (169)
$t_{1/2}$ = 16.4 h (pH 6.9,37C) (28)
In 3'-dGMP
$t_{1/2}$ = 3.8 h (pH 7.4,37C) (198)
In 3',5'-diphosphate of dG
$t_{1/2}$ = 6.3 h (pH 7.4,37C) (198)
In dG
$t_{1/2}$ = 11.8 h (pH 7,30C) (28)
$t_{1/2}$ = 6.5 h (pH 7,37C) (4)
$t_{1/2}$ = 5.9 h (pH 7.4,37C) (169)
$t_{1/2}$ = 4.4 h (pH 4.2,24C) (93)
$t_{1/2}$ = 1.4 h (pH 7,46C) (28)
$t_{1/2}$ = 48 s (0.1M HCl,70C) (174)
In Guo
$t_{1/2}$ = 7.1 h (pH 0,37C) (28)
$t_{1/2}$ = 2.1 h (0.1M HCl,70C) (174)
$t_{1/2}$ = 1.1 h (1N HCl,50C) (83)

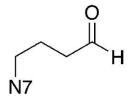
Fapy Formation
In ds-DNA
$t_{1/2}$ >>54 h (pH 8.9,37C) (48)
dG
$t_{1/2}$ = 9.8 h (pH 8.9,37C) (48)
$t_{1/2}$ = 4.3 h (pH 10,24C) (93)
$t_{1/2}$ = 3 h (pH 10,37C) (109)
$t_{1/2}$ = 22 min (pH 10,25C) (174)
In Guo
$t_{1/2}$ = 85 h (pH 7.2,25C) (86)
$t_{1/2}$ = 6.1 h (pH 8.9,37C) (48)
$t_{1/2}$ = 4 h (pH 8.9,37C) (83,175)
$t_{1/2}$ = 3.3 h (pH 9.2,37C) (169)
$t_{1/2}$ = 1.5 h (pH10.2,20C) (28)
$t_{1/2}$ = 1.2 h (pH 10,37C) (109)
$t_{1/2}$ = 30 min (pH 9.5,37C) (176)
$t_{1/2}$ = 12 min (pH 10,25C) (174)
$t_{1/2}$ = 11 min (pH 9.9,40C) (82)
In 3'-monophosphate
$t_{1/2}$ = 19.8 h (pH 8.9,37C) (48)
In 2'-monophosphate
$t_{1/2}$ = 25.6 h (pH 8.9,37C) (48)
In 5'-monophosphate
$t_{1/2}$ = 40.6 h (pH 8.9,37C) (48)
In 5'-diphosphate
$t_{1/2}$ = 46 h (pH 8.9,37C) (48)
In 5'-triphosphate
$t_{1/2}$ = 53.5 h (pH 8.9,37C) (48)

Depurination
In dG
$t_{1/2}$ = 19.3 h (pH 7,30C) (28)
$t_{1/2}$ = 2.2 h (pH 7,46.3C) (28)
In 5'-dGMP
$t_{1/2}$ = 19 h (pH 6.9,37C) (28)
In Guo
$t_{1/2}$ = 15 h (1N HCl,37C) (175)
$t_{1/2}$ = 9.9 h (pH 0,37C) (28)

Fapy Formation
In Guo
$t_{1/2}$ = 15 h (pH 8.9,37C) (83,177)
$t_{1/2}$ = 52 min (pH 9.9,40C) (82)

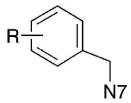


Depurination from ss-DNA
$t_{1/2}$ = 31 h (pH 7.5,32C) (178)



Depurination from ds-DNA
$t_{1/2}$ = 33 h (pH 7.5,32C) (55)

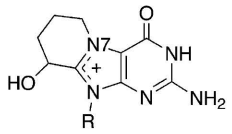
Depurination from ds-DNA
$t_{1/2}$ = 62 h (in vivo) (196)



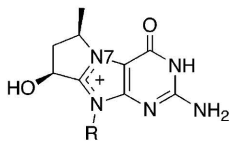
Depurination in Guo
(1N HCl, 50C) (82)
R = p-NO_2: $t_{1/2}$ = 59 min
R = p-Cl: $t_{1/2}$ = 56 min
R = H: $t_{1/2}$ = 65 min
R = p-CH_3: $t_{1/2}$ = 72 min
R = p-OCH_3: $t_{1/2}$ = 70 min

Fapy Formation in Guo
(pH 9.9, 40C) (82)
R = p-NO_2: $t_{1/2}$ = 2.7 min
R = p-Cl: $t_{1/2}$ = 7.3 min
R = H: $t_{1/2}$ = 11.5 min
R = p-CH_3: $t_{1/2}$ = 14.3 min
R = p-OCH_3: $t_{1/2}$ = 14.4 min

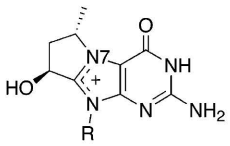
R = H, $t_{1/2}$ = 30 min
(pH 9.5, 37C) (179)



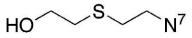
Depurination from ds-DNA
$t_{1/2}$ = 54 h (pH 7,37C) (55)
25.3 mmol adduct/mol of G
$t_{1/2}$ = 13 h (pH 7,37C) (55)
4 mmol adduct/mol of G



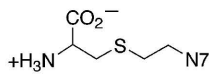
Depurination from ds-DNA
$t_{1/2}$ = 32 h (pH 7,37C) (55)



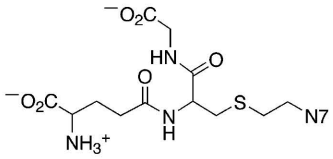
Depurination from ds-DNA
$t_{1/2}$ = 31 h (pH 7,37C) (55)



Depurination
In ds-DNA
$t_{1/2}$ = 52 h (pH 7,37C) (180)
In 5'-dGMP
$t_{1/2}$ = 8 h (pH 7,37C) (28)

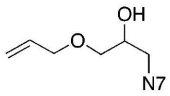


Depurination in dG
$t_{1/2}$ = 6.5 h (pH 7.4,27C) (181)

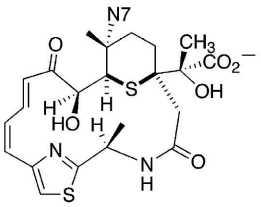


Depurination
In ds-DNA
$t_{1/2}$ = 150 h (pH6 or 7,37C) (182)
$t_{1/2}$ = 70-100 h, In vivo (182)
In dG
$t_{1/2}$ = 7.4 h (pH 7.4,27C) (181)
Fapy Formation
In ds-DNA
No Fapy observed pH 7 (183)
In dG
$t_{1/2}$ = 8.7 h (pH 10,37C) (109)
In Guo
$t_{1/2}$ = 2.4 h (pH 10,37C) (109)

Depurination in dG
$t_{1/2}$ = 1.3 h (pH 6,37C) (175)



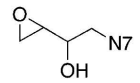
Depurination in ds-DNA
$t_{1/2}$ = 38 h (pH 7.4,37C) (184)
In 3'-dGMP
$t_{1/2}$ = 2.1 h (pH 7,37C) (184)
$t_{1/2}$ = 1.6 h (pH 6,37C) (184)



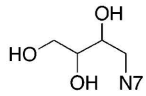
Depurination
In ds-DNA
$t_{1/2}$ = 442 h (pH 7,4C) (21)
$t_{1/2}$ = 17.4 h (pH 7,25C) (21)
$t_{1/2}$ = 3.5 h (pH 7,37C) (21)
$t_{1/2}$ = 59 min (pH 7,50C) (21)
$t_{1/2}$ = 12 min (pH 7,60C) (21)
In dG
$t_{1/2}$ = 92 h (pH 7,4C) (21)
$t_{1/2}$ = 2.2 h (pH 7,25C) (21)
$t_{1/2}$ = 61 min (pH 7,37C) (21)

Depurination
In ds-DNA
$t_{1/2}$ = 52.7 h (pH 7.4,37C) (185)
In 3'-dGMP
$t_{1/2}$ = 5.7 h (pH7.4,37C) (198)
In dG
$t_{1/2}$ = 116 min (pH 6,37C) (175)
In Guo
$t_{1/2}$ = 66 min (1N HCl,50C) (83)

Depurination
In ds-DNA
$t_{1/2}$ = 120 h (pH 7.4,37C) (197)
In 5'-dGMP
$t_{1/2}$ = 38 h (pH 7.4,37C) (197)
$t_{1/2}$ = 8.4 h (pH 5.2,37C) (197)
In 3'-dGMP
$t_{1/2}$ = 1.5 h (pH 7.4,37C) (197)
$t_{1/2}$ = 1.5 h (pH 5.2,37C) (197)

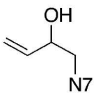


Depurination in ds-DNA
$t_{1/2}$ = 31 h (pH 7.4,37C) (186)

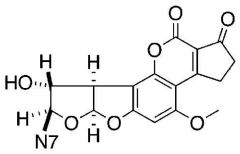


Depurination in ds-DNA
$t_{1/2}$ = 30 h (pH 7.4,37C) (186)

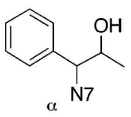
Depurination
In ds-DNA
$t_{1/2}$ = 50 h (pH 7.2,37C) (187)
In Guo
$t_{1/2}$ = 90 h (pH 7.4,37C) (188)



Depurination
In ds-DNA
$t_{1/2}$ = 50 h (pH 7.2,37C) (187)
In Guo
$t_{1/2}$ = 50 h (pH 7.4,37C) (188)

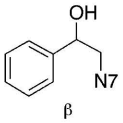


Depurination in ds-DNA
$t_{1/2}$ = 77 h (pH 7.3,37C) (125)
$t_{1/2}$ = 63 h (pH 7,37C) (125)
$t_{1/2}$ = 60 h (pH 7,37C) (96)
$t_{1/2}$ = 58 h (pH 6.7,37C) (125)
$t_{1/2}$ = 8 h (in vivo) (96)
Fapy Formation in ds-DNA
$t_{1/2}$ = 99 h (pH 7,37C) (125)
$t_{1/2}$ = 69 h (pH 7.3,37C) (125)
$t_{1/2}$ = 8.5 h (pH 8.5,37C) (96)



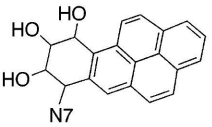
α

Depurination in ds-DNA
$t_{1/2}$ = 22 h (pH 7,37C) (189)
In 3'-dGMP
$t_{1/2}$ = 7.3 h (pH 7.4,37C) (198)
$t_{1/2}$ = 7.3 h (pH 6,37C) (198)
(20mM Na succinate,8mM $CaCl_2$)
$t_{1/2}$ = 4.4 h (pH 6,37C) (198)
(10mM NaP_i, 1M NaCl)
In Guo
$t_{1/2}$ = 3.2 h (0.2M HCl,63C) (189)
$t_{1/2}$ = 75 min (pH 1,70C) (93)
Fapy Formation in Guo
$t_{1/2}$ = 4.3 h (pH 9.8,37C) (189)
$t_{1/2}$ = 2.2 h (pH 10,37C) (189)
$t_{1/2}$ = 1.5 h (pH 10,24C) (93)

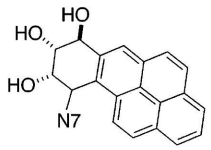


β

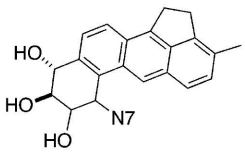
Depurination in ds-DNA
$t_{1/2}$ = 248 h (pH 4.2,24C) (93)
$t_{1/2}$ = 22 h (pH 7,37C) (189)
In ss-DNA
$t_{1/2}$ = 70 h (pH 4.2,24C) (93)
(two isomers)
In 3'-dGMP
$t_{1/2}$ = 4.7 h (pH 7.4,37C) (198)
$t_{1/2}$ = 4.6 h (pH 6,37C) (198)
(20mM Na succinate,8mM $CaCl_2$)
$t_{1/2}$ = 2.6 h (pH 6,37C) (198)
(10mM NaP, 1M NaCl)
In dG
$t_{1/2}$ = 4.7 h (pH 4.2,24C) (93)
$t_{1/2}$ = 1.4 min (pH 1,70C) (93)
In Guo
$t_{1/2}$ = 3.2 h (0.2M HCl,63C) (189)
$t_{1/2}$ = 34.5 min (pH 1,70C) (93)
Fapy Formation in dG
$t_{1/2}$ = 317 min (pH 10,24C) (93)
Fapy Formation in Guo
$t_{1/2}$ = 4 h (pH 9.8,37C) (189)
$t_{1/2}$ = 2.2 h (pH 10,37C) (189)
$t_{1/2}$ = 58 min (pH 10,24C) (93)



Depurination in ds-DNA
$t_{1/2}$ = 24 h (pH 7.4,37C) (190)

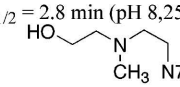


Depurination in ds-DNA
$t_{1/2}$ = 3 h (pH 4-7,37C) (191)
$t_{1/2}$ = 3 h (pH 7.5,37C) (192)

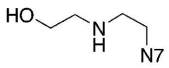


Depurination in ds-DNA
$t_{1/2}$ ~ 2 h (pH 7,37C) (193)
(presumed adduct structure)

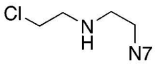
Depurination in ds-DNA
$t_{1/2}$ = 1.5 min (pH 7,100C) (113)
In dG
$t_{1/2}$ = 42 min (pH 6,25C) (145)
In Guo
$t_{1/2}$ = 50 min (1N HCl,50C) (83)
Fapy Formation in dG
$t_{1/2}$ = 21 min (pH 7.7,25C) (145)
Fapy Formation Guo
$t_{1/2}$ = 11 min (pH 7,25C) (145)
$t_{1/2}$ = 5 min (pH 7.7,25C) (145)
$t_{1/2}$ = 2.8 min (pH 8,25C) (145)



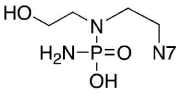
Depurination in ds-DNA
$t_{1/2}$ = 9.1 h (pH 7,37C) (95)
$t_{1/2}$ = 1.8 h (pH 7,70C) (95)
In dG
$t_{1/2}$ = 1.6 h (pH 4.2,25C) (194)
Fapy Formation in dG
$t_{1/2}$ = 3.6 h (pH 8.5,25C) (194)
Fapy Formation in Guo
$t_{1/2}$ = 1.7 h (pH 7.4,24C) (194)
$t_{1/2}$ = 47 min (pH 7.4,37C) (194)



Fapy Formation in Guo
$t_{1/2}$ = 6.3 h (pH 7.4,22C) (176)
$t_{1/2}$ = 5 h (pH 9,22C) (176)



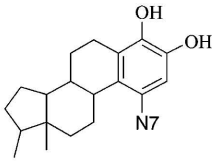
Fapy Formation in Guo
$t_{1/2}$ = 6.8 h (pH 7.4,22C) (176)
$t_{1/2}$ = 5.6 h (pH 9,22C) (176)
$t_{1/2}$ = 2.1 h (pH 7.4,37C) (176)
$t_{1/2}$ = 1.2 h (pH 9,37C) (176)



Depurination in dG
$t_{1/2}$ = 8.5 h (pH 4.2,25C) (194)
Fapy Formation in dG
$t_{1/2}$ = 6.8 h (pH 8.8,37C) (194)
Fapy Formation in Guo
$t_{1/2}$ = 24 h (pH 7.4,25C) (194)
$t_{1/2}$ = 9.5 h (pH 7.4,37C) (194)
$t_{1/2}$ = 3.1 h (pH 8.8,37C) (194)

Depurination in dG
$t_{1/2}$ = 69 min (pH 6,37C) (175)
Depurination in Guo
$t_{1/2}$ = 60 min (1N HCl,50C) (83)

Depurination in Guo
$t_{1/2}$ = 58 min (1N HCl,50C) (83)



Depurination in dG
$t_{1/2}$ = 5 h (195)

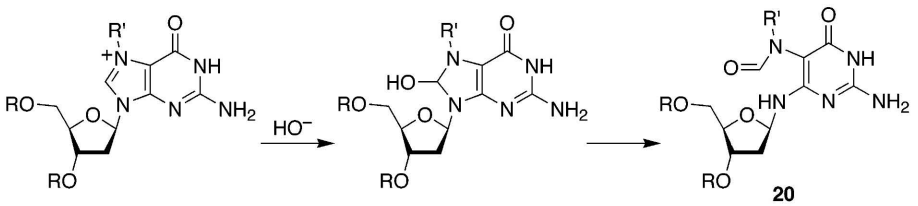
Figure 1. Chemical stability of N7-alkylguanine adducts.

from double-stranded DNA including depurination, ring opening (Section 4), and hydrolysis (Section 5). While the effect of adduct density on the rate of depurination was not explicitly examined, it was shown that at higher adduct densities, conversion of this lesion to the ring-opened (FAPy) derivative and 2,3-dihydro-2,3-dihydroxyaflatoxin B_1 (**39**) was more rapid. These researchers suggested that distortion of the DNA duplex in heavily adducted DNA might be responsible for the decreased stability of the aflatoxin lesion (*96*). In a more recent example, Hecht and co-workers noted that adduct density alters the half-life for depurination of the cyclic N7/C-8-guanine adduct (**12**; Scheme 6) resulting from treatment of DNA with *N*-nitrosopyrrolidine (*55*). In this case, the stability of the lesion is increased when higher levels of adduct are present in the DNA (Figure 1).

3.8. Negatively Charged Residues May Facilitate Depurination by Neighboring Group Assistance

Several recent studies indicate that negatively charged functional groups can accelerate depurination reactions by neighboring group assistance. For example, certain

Scheme 8



base excision repair enzymes are thought to catalyze depurination reactions by positioning a negatively charged carboxylate residue near the endocyclic O4′-oxygen of the nucleotide residue that is destined for excision. This arrangement may accelerate the reaction through electrostatic stabilization of the oxocarbenium ion-like transition state (e.g., see **14** in Scheme 7) (*97*, *98*). Evidence supporting the ability of a carboxylate residue to increase the rate at which an N7-alkylguanine residue undergoes depurination is found in the model reactions of Van Arman and Czarnik, which showed that depurination of the carboxylate-substituted nucleoside **19a** occurs at least 43 times faster than an analogue (**19b**) lacking the carboxylate residue (*99*). Accordingly, one can imagine that some carboxylate-containing N7-alkylguanine substituents have the potential to accelerate their own depurination via neighboring group assistance.

Similarly, recent theoretical and experimental results indicate that catalysis of glycosidic bond hydrolysis by the enzyme uracil DNA glycosylase involves electrostatic stabilization of the oxocarbenium ion intermediate by DNA phosphate residues near the excised uracil residue (*98*, *100*, *101*). On the basis of this precedent, it is interesting to consider whether some N7-alkylguanine lesions may distort the structure of the DNA double helix in a manner that allows neighboring phosphate residues to faciliate depurination.

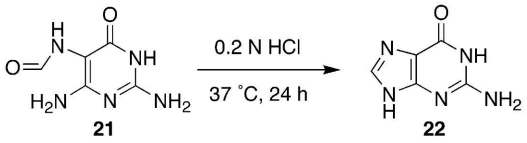
Overall, it is not possible to rationalize all of the observed depurination rates for various N7-alkylguanine residues shown in Figure 1 based solely upon the electronic properties of the N7 substituent. This observation leads us to conclude that differences in the local structure of the DNA duplex surrounding N7-alkylguanine residues may play an important role in determining the rate at which these lesions undergo depurination in double-stranded DNA via mechanisms such as those discussed above in Sections 3.6 and 3.8.

4. Imidazolium Ring Opening of N7-Alkylguanine Residues to Yield Alkyl-FAPy-G Lesions

4.1. Basic Conditions Favor Opening of the Imidazolium Ring in N7-Alkylguanosines

Early work showed that N7-alkylguanine residues are subject to attack of hydroxide at the C-8 position of the base. This reaction leads to the production of alkyl-FAPy-G lesions (**20**; Scheme 8) in which the imidazolium ring of the alkylated guanine has been opened (*47*, *102*–*108*). The reaction is favored by basic conditions. For example, the ring-opening reaction of 7-methyl-2′-deoxyguanosine is reported to be very slow at neutral pH but has a half-life of 9.8 h in pH 8.9 buffer at 37 °C (*48*) and a half-life of 3.0 h in pH 10 buffer at 37 °C (*109*). The ring-opening reaction in duplex DNA or 2′-deoxy-

Scheme 9



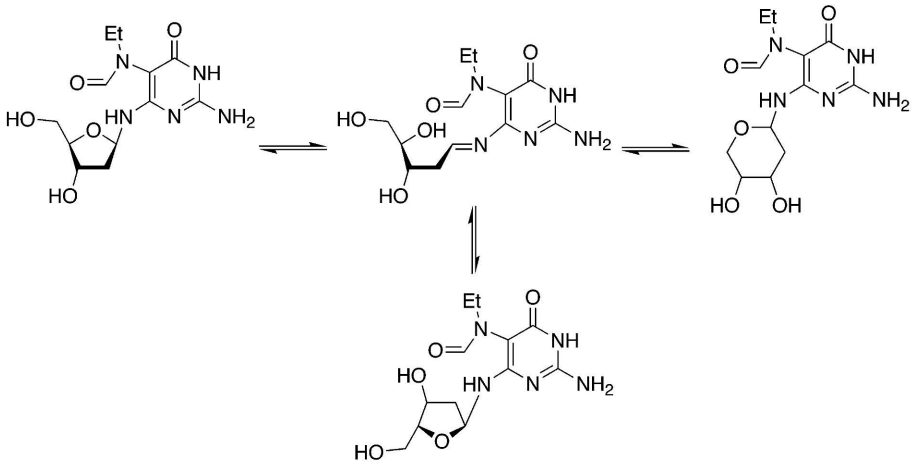
oligonucleotides is typically carried out using conditions such as pH 9.0/Na_2CO_3/25 °C/6 h, pH 10/Na_2HPO_4/37 °C/4 h, or 0.1 M NaOH/25 °C/1 h (*11*, *109*–*111*). The ring-opening reaction is probably irreversible under physiological conditions, although efficient reclosure of the imidazole ring in the unalkylated FAPy-G free base (**21**) to yield guanine (**22**) has been reported under acidic conditions (0.2 N HCl, 37 °C, 24 h) (Scheme 9) (*112*). Both Chetsanga and Hemminki noted that cyclization of FAPy lesions to the corresponding guanine free base is not effective under more acidic conditions (0.4−1 N HCl) (*112*, *113*). Nonetheless, low yields of a ring-closed N7-alkylguanine derivative were seen following treatment of the FAPy lesion derived from aflatoxin B_1 epoxide with 0.6 N HCl at 100 °C (*114*).

Early researchers proposed that ring opening occurs to place the formyl group (−CHO) on N7 of the purine ring system (*47*, *102*, *103*), and recent NMR studies have rigorously confirmed this structural assignment in one case (*109*, *110*). Presumably, this regiochemistry is favored because the developing negative charge on N9 during the ring-opening reaction can be delocalized into the α,β-unsaturated carbonyl moiety of the pyrimidine ring. Note that the N7-nitrogen of the purine ring system becomes N-5 in the numbering system of the newly formed pyrimidine ring in **20**; however, for convenience, we refer to FAPy lesions in this article using the atom numbering system of the parent purine residue. At least two groups originally suggested that ring opening can yield a mixture of N7- and N9-formyl derivatives (*106*, *115*); however, other theories have been offered more recently to explain these observations (discussed further in Section 4.3).

4.2. The Glycosidic Bond in Alkyl-FAPy-G Lesions Is Chemically Stable

The glycosidic bond in FAPy lesions is chemically stable under physiological conditions (*105*, *113*). Importantly, this means that, unlike the parent N7-alkylguanine lesions, FAPy residues are not spontaneously lost from cellular DNA. Indeed, it has been found that alkyl-FAPy-G lesions can persist for long periods of time in the liver of rats exposed to alkylating agents (*116*–*119*). The persistence of FAPy residues in cellular DNA may be biologically significant because these lesions are both mutagenic and cytotoxic (*11*, *120*, *121*).

Scheme 10

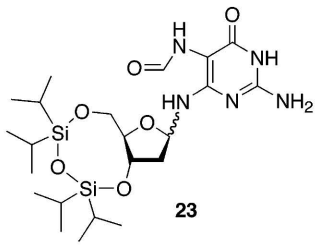


Evidence for the stability of the glycosidic bond in alkyl-FAPy lesions has been provided by several different studies. Chetsanga et al. showed that only about 4% of the FAPy derivative of 7-methylguanine was released from double-stranded DNA after 240 h in pH 7.4 buffer at 37 °C (*105*). Furthermore, recent studies with the unalkylated derivative (**20**, R′ = H) have provided useful information regarding the stability of the glycosidic bond in FAPy residues. The rate constants measured for deglycosylation of this lesion in single-stranded DNA (10 mM phosphate buffer, pH 7.5) at 55 ($t_{1/2}$ = 514 h) and 90 °C ($t_{1/2}$ = 91 h) by Greenberg and co-workers (*122*) allow us to estimate that the half-life for the loss of FAPy-G at 37 °C would be approximately 1500 h. In addition, these researchers reported that FAPy-G (**20**, R′ = H) sites in 2′-deoxyoligonucleotides are only partially cleaved by exposure to 1 M piperidine at 90 °C for 20 min (Maxam−Gilbert workup) (*123*). In contrast, earlier workers concluded that the Maxam−Gilbert G-reaction proceeds via a methyl-FAPy intermediate (**20**, R = CH_3) that yields strand cleavage in 1 M piperidine at 90 °C for 20 min (*77*). Similarly, footprinting protocols using diethyl pyrocarbonate rely upon piperidine-mediated cleavage of ring-opened purine residues (*124*).

FAPy-G lesions are typically characterized and quantitated following their removal from DNA using acid treatment (e.g., 0.1 N HCl or 75% formic acid, 24−100 °C, 15 min to 72 h) (*96*, *105*, *114*, *125*−*127*), neutral thermal hydrolysis (2 h, 100 °C) (*126*), or a repair enzyme such as FAPy glycosylase (FPG) (*128*−*132*). Interestingly, the acidic conditions often used for the release of alkyl-FAPy-G from DNA are similar to the optimal conditions (0.2 N HCl, 37 °C, 24 h) reported for the cyclization of the FAPy-G free base to guanine (*112*) (Scheme 9). It is not clear whether acid-catalyzed cyclization of alkyl-FAPy-G lesions to N7-alkylguanines interferes with the accurate determination of the levels of these adducts in DNA.

4.3. Anomerization and Conformational Isomerism in Alkyl-FAPy-G Lesions

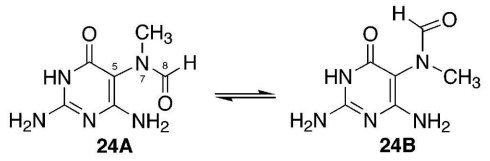
The FAPy lesions derived from ring opening of N7-alkylguanine nucleosides exist as an equilibrating mixture of isomers as shown in Scheme 10. Sugar ring opening proceeds via a C1′-N9 imine intermediate. Reclosure of the sugar ring can occur by intramolecular attack of either the C4′- or the C5′-hydroxyl residues on the imine intermediate to yield the pyranose derivative, the original FAPy lesion, or the anomeric isomer of the original lesion (*133*, *134*). The six-membered pyranose form of the sugar is favored (*133*, *134*). Rearrangement to the pyranose derivative is, of course, not possible when the 5′-hydroxyl is masked by a protecting group or incorporated into polymeric DNA (*135*). Importantly, the sugar ring-opening reaction may allow epimerization (anomerization) of FAPy lesions in double-stranded DNA. The rate constant for anomerization of a 5′-phosphorylated FAPy-dA nucleoside is 6 × 10^{-5} s^{-1} ($t_{1/2}$ = 3.2 h) in phosphate buffer (*122*). Bergdorf and Carell found the relaxation time ($\tau = 1/[k_1 + k_{-1}]$) for the anomerization of a protected FAPy-dG nucleoside **23** in 1:1 acetonitrile/water to be 6.5 h, which translates to a half-life ($t_{1/2} = \ln 2/[k_1 + k_{-1}]$) of 4.5 h for the reaction (*136*).



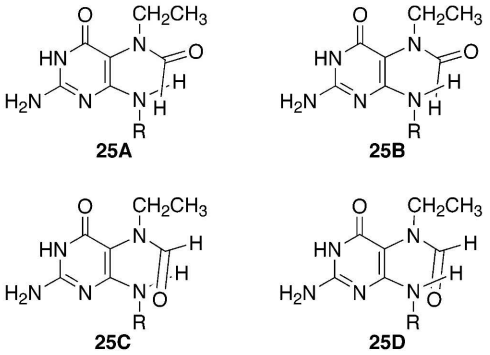
23

Even in cases where isomerization of the sugar residue discussed above is not possible (i.e., in the deglycosylated free base), alkyl-FAPy-G lesions exist as equilibrating mixtures that are separable by HPLC and appear as distinct species in the NMR (*104*−*106*, *109*, *126*, *134*). Early researchers speculated that this mixture might consist of regioisomers in which the formyl group is attached at either N9 or N7 of the ring-opened guanine or equilibrating mixtures of formylated and deformylated analogues (*106*). However, as noted above, recent work firmly established that the formyl group in FAPy derivatives is attached at the N7-nitrogen of the purine ring system (*109*). In fact, the isomerism observed in FAPy-G derivatives results from slow rotation about the N7/C-8 amide bond and/or the C-5/N7 bond in the adduct (*104*, *109*, *119*, *126*, *134*). In the deglycoslylated Me-FAPy-G free base (**24**), isomerism was attributed to slow rotation about the C-5/N7 bond (Scheme 11) (*104*, *126*). On the other hand, in a β-ribofuranose triacetate derivative (**25**), Tomasz and co-workers reported that the isomers arise

Scheme 11



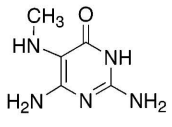
Scheme 12



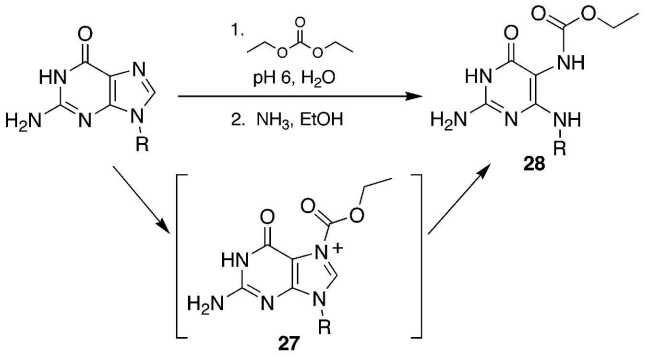
from hindered rotation about the N7/C-8 bond as shown in Scheme 12 (*134*). Note that the isomers (**25A**/**25B** and **25C**/**25D**) shown in Scheme 12 are diastereomeric if chiral centers are present in the N9 substitutent (e.g., in glycosylated derivatives) but are enantiomeric in the deglycosylated free base (*134*). The distribution of conformers appears to be somewhat different in the nonalkylated FAPy-dG analogue **23** (*136*). The half-life for the interconversion of conformational isomers in Me-FAPy-G free base is approximately 8 min at 37 °C (*126*). As one might expect, the position of conformational equilibria and the rate of equilibration for alkyl-FAPy-G lesions are altered in the DNA duplex. For example, the FAPy-G adduct of aflatoxin B_1 in duplex DNA shows a single ^{1}H NMR resonance for the −CHO residue, indicating that the adduct exists as a single conformational isomer due to hindered rotation about the formyl bond within the context of the double helix (*110*). Similarly, the distribution of conformational isomers observed for Me-FAPy-G is altered when the adduct is generated within duplex DNA (*126*).

4.4. Loss of the Formyl Group from Alkyl-FAPy Lesions

The formyl group can be lost from alkyl-FAPy lesions under some conditions. Chetsanga and co-workers assigned a minor product resulting from the treatment of N7-methylguanosine with 0.2 N NaOH at 37 °C for 4 h as deformylated Me-FAPy-G derivative **26** (*105*). Leonard and co-workers isolated a ring-opened, deformylated product (**28**) after treatment of guanosine with diethyl pyrocarbonate in pH 6 water for several days, followed by workup with ethanolic ammonia (Scheme 13) (*137*). It is likely that the ammonia workup causes deformylation in this reaction (*138*). Consistent with the idea that basic conditions can remove the formyl group from a FAPy lesion, solvolysis of adenine nucleosides in 1 M



Scheme 13



NaOH produces the ring-opened, deformylated, free base 4,5,6-triaminopyrimidine (*85*).

Acidic conditions also can deformylate FAPy lesions. Boiteux et al. reported that treatment of poly-(dGC) containing Me-FAPy-G residues with 0.1 N HCl at 37 °C for 17 h produced deformylated Me-FAPy free base as a side product along with the desired Me-FAPy free base as the major product (*126*). Barak et al. showed that 1 N HCl at 35 °C for 72 h converts Me-FAPy-guanosine to the deformylated, deglycosylated, ring-opened free base (**26**) (*139*). Kohn and Spears reported that ring-opened guanine adducts of nitrogen mustards in DNA containing 8-^{14}C-guanine residues release the radiolabeled formyl group from the FAPy lesion when subjected to acid hydrolysis using 13% $HClO_4$ at 70 °C for 16 h (*111*). The resulting ^{14}C-labeled formic acid was quantitatively detected as $^{14}CO_2$ following oxidation with 50 mM $Hg(OAc)_2$ at 50 °C for 20 h (*111*). Interestingly, at least two groups have reported that deformylated Me-FAPy can be reformylated by incubation with formic acid (*105*, *126*). Accordingly, removal of FAPy lesions from DNA using formic acid does not generate the deformylated FAPy free base, while treatment with hydrochloric acid can produce the deformylated side product (*105*, *126*, *139*).

4.5. Effects of the N7 Substitutent, the Sugar Residue, and Structural Context on the Rate of Ring Opening

The ring-opening reaction of N7-alkylguanine residues, like the depurination reaction discussed in Section 3, is favored by the presence of electron-withdrawing groups on the N7 substitutent (*82*, *83*). The substituent effects on this reaction are markedly larger than those seen for the depurination reaction. For example, Müller and Eisenbrand reported a Taft ρ^* value of 1.89 for the ring-opening reaction in a series of substituted N7-ethylguanosines and Moschel reported a Hammett ρ value of 0.80 for a series of substituted N7-benzylguanosine adducts (*82*, *83*). For experimental convenience, the reactions in both of these studies were conducted under basic conditions (e.g., pH 8.9, 37 °C), which markedly accelerated the ring-opening reaction. In addition, the reactions were conducted on alkylated guanosine, rather than 2′-deoxyguanosine nucleosides. Nonetheless, it seems likely that the general conclusions of these studies regarding substituent effects on ring opening are relevant to the reaction of 2′-deoxyguanosine derivatives under physiological conditions.

The conversion of N7-alkylguanine residues to FAPy lesions is also favored by electron-withdrawing substit-

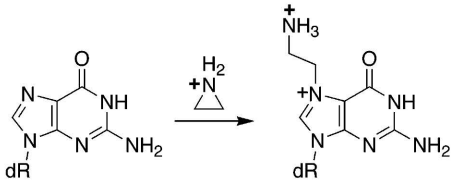
uents on the sugar residue. The ring-opening reaction in RNA nucleosides is about 2−3 times faster than in DNA nucleosides (*48*) (Figure 1), presumably as a result of the electron-withdrawing effects exerted by the 2′-hydroxyl group of the ribose residue. Note that the 2′-hydroxyl group in RNA accelerates conversion of N7-methylguanosine to the FAPy derivative while slowing the competing depurination reaction (as discussed in Section 3.5). The net result of the opposing effects exerted by the 2′-OH substituent on these two potential decomposition pathways is that FAPy formation is vastly favored over depurination for the N7-methylguanosine nucleoside at physiological pH (*86*). A half-life of 85 h was measured for the ring opening of 7-methylguanosine at pH 7.2 and 25 °C using an NMR assay (*86*). Free ribose was not observed in this experiment even after 1 month, suggesting that depurination does not occur to a significant extent under these conditions.

On the other hand, in duplex DNA under physiological conditions, depurination is the predominant mode of decomposition for most N7-alkylguanine adducts (Figure 1). For example, it has been noted that ring opening does not compete favorably with depurination in the case of the 7-methylguanine residue in double-stranded DNA (*140*, *141*). Similarly, Guengerich and co-workers noted that the *S*-[2-(N7-guanyl)ethyl]glutathione adduct in double-stranded DNA undergoes depurination but not ring opening (*142*). Interestingly, despite the fact that ring opening of the 7-methylguanine lesion in duplex DNA appears to be slow in vitro, significant amounts of the ring-opened product have nonetheless been detected in DNA isolated from rats treated with methylating agents (*118*, *119*).

There are conflicting reports regarding the relative rates of imidazolium ring opening and depurination for the guanine adduct formed by aflatoxin B_1-*exo*-8,9-epoxide (**37**; Scheme 20). Both the FAPy lesion and the N7-G adduct were observed in early in vitro studies (*114*, *143*). Wogan and co-workers reported that the yields of FAPy are low relative to depurination near neutral pH (*96*). As expected, these researchers found that ring opening becomes more significant at high pH. For example, at pH 8, they found that the yield of FAPy lesions after 48 h at 37 °C is about three times higher than the yield of the depurination product. Similarly, later studies by Stone and Harris reported that ring opening of the aflatoxin-guanine adduct in a 2′-deoxyoligonucleotide did not occur rapidly in pH 8 buffered solution at room temperature, but gentle warming of this mixture for 19.5 h allowed efficient conversion to the FAPy derivative (*144*). On the other hand, Wang and Cerutti reported that at pH 7.3 and 37 °C FAPy formation was faster than depurination (*125*). Interestingly, biological studies have shown that 20% of the guanine adducts formed in the liver of rats exposed to aflatoxin are converted to FAPy lesions that persist for significant periods of time (*116*, *117*). Recent results suggest that this lesion could contribute to the mutagenic properties of aflatoxin B_1 (*11*).

As noted above, electron-withdrawing groups on the N7 substituent of alkylguanine residues significantly accelerate ring opening, while only modestly facilitating the competing depurination reaction (*82*, *83*). Therefore, in the case of some strongly electron-withdrawing N7 substituents, ring-opening rates may become comparable to depurination rates under physiologically relevant conditions. For example, the half-life for ring opening of the N7-deoxyguanosine nucleoside adduct of ethyleneimine (N7-$CH_2CH_2NH_2$) is 21 min at pH 7.7 and 25 °C, while the half-life for depurination of this adduct is 42 min at pH 6 and 25 °C (*145*). The $-CH_2CH_2NH_2$ adduct derived from attack of N7-guanine on the cyclic ethyleneimine electrophile acts as a strong electron-withdrawing group because the amine residue presumably exists as a protonated, cationic ammonium group at physiological pH (Scheme 14). The rate of ring opening for this type of lesion in duplex DNA is not known but should be of significant interest because of the possibility that the resulting FAPy lesions could contribute to the cytotoxicity of clinically used nitrogen mustards such as cyclophosphamide and mechlorethamine. Consistent with the idea that the aminoethyl-FAPy-G adduct can, in fact, be produced in duplex DNA under physiological conditions and that it is biologically important, Cussuc and Laval reported that the *Escherichia coli* base excision repair enzyme FPG, which removes this type of ring-opened lesion from DNA (*146*), can protect mammalian cells against the toxicity and mutagenicity of ethyleneimine (*147*). There is at least one other example where ring opening appears to be favored over depurination for a strongly electron-withdrawing N7 adduct. In reactions of diethyl pyrocarbonate with purine ribonucleosides, a presumed N7-carboxyethyl intermediate **27** undergoes hydrolysis to the corresponding FAPy derivative, followed by deformylation to give **28**, while products arising from depurination are not observed (Scheme 13) (*137*).

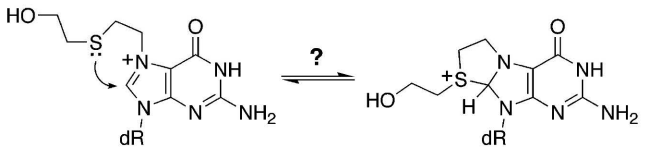
Scheme 14



Like the depurination reaction discussed in Section 3.6, the rate at which N7-alkylguanines undergo ring opening to afford alkyl-FAPy lesions is dependent upon the structural environment of the lesion. For example, the rate of imidazolium ring opening decreases markedly when 3′- or 5′-phosphate, 5′-diphosphate, or 5′-triphosphate groups are present on the sugar residue (*48*, *108*). The effect is especially marked for 5′-phosphorylated nucleosides where the negatively charged phosphate residue is positioned to electrostatically repel the approach of the hydroxide ion toward C-8 of the alkylated base (*108*). The half-life for ring opening of 7-methylguanosine in pH 8.9 buffer at 37 °C is 6.1 h whereas the corresponding half-life for the 3′-monophosphate is 19.8 h, the 2′-monophosphate is 25.6 h, the 5′-monophosphate is 40.6 h, the 5′-diphosphate is 46.0 h, and the 5′-triphosphate is 53.5 h (*48*). Of special importance, the polymeric substrates, poly-7-methyl-G and poly-7-methyl-dGdC, were described as "remarkably resistant to ring opening at pH 8.9 and 37 °C" (*48*). Similarly, Tomasz and co-workers found that conversion of the N7-guanine adduct of mitomycin C to its FAPy lesion is fast in the 2′-deoxyguanine nucleoside but is completely suppressed in duplex DNA (*134*).

Interestingly, it has been noted that N7-hydroxyethyl-(thioethyl)guanine residues in DNA are resistant to ring

Scheme 15

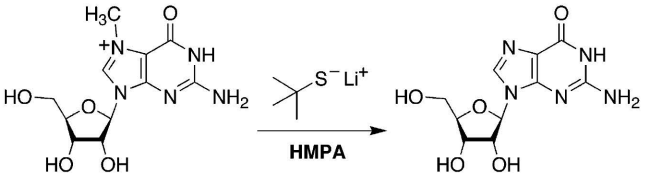


opening under conditions (pH 11.4, 37 °C, 3 days) where N7-methylguanine residues are efficiently opened (*131*). It is tempting to speculate that the attack of hydroxide at C-8 required for ring opening is inhibited by reversible intramolecular addition of the sulfide residue as proposed in Scheme 15. A similar equilibrium can be envisioned for N7-hydroxyethyl adducts resulting from the reaction of epoxides with guanine residues in DNA.

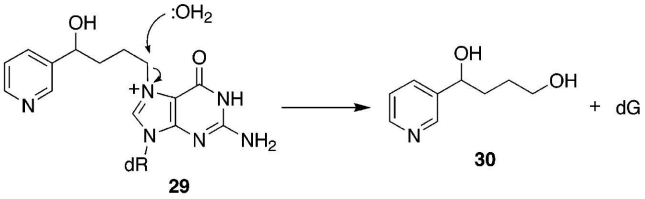
5. Guanine Residues as Leaving Groups in the Decomposition of N7-Alkylguanine Adducts: Substitution, Elimination, and Reversible Alkylation

In a few interesting cases, covalent DNA adducts have been observed to decompose via reactions where intact DNA serves as the leaving group and the original form of the electrophile is regenerated. For example, the antibiotics duocarmycin and CC-1065 reversibly alkylate the N3 position of adenine residues in duplex DNA (*148*–*150*). Similarly, the imine-forming antibiotic ecteinascidin 743 reversibly alkylates the exocyclic N^2-nitrogen atom of guanine residues in DNA (*151*). Rokita's group has studied reversible alkylation at the N1 position of 2′-deoxyadenosine by a quinone methide (*152*). Finally, recent evidence indicates that malondialdehyde-guanine adducts can be reversibly transferred between different DNA nucleophiles (*153*, *154*). Reversible DNA alkylation allows equilibration ("shuffling") of adducts to thermodynamically favored locations in DNA (*151*) and, in principle, might allow the transfer of adducts from DNA to other biological nucleophiles such chromatin proteins and DNA-binding transcription factors.

Scheme 16



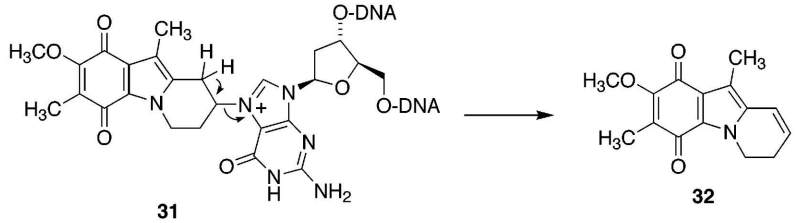
Scheme 17



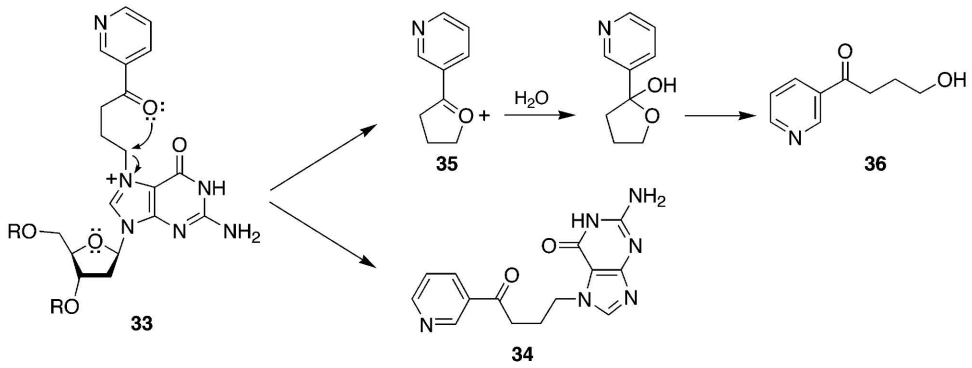
The guanine residue in N7-alkylguanine DNA adducts is expected to be a reasonably good leaving group (the pK_a of the conjugate acid is 2.5−3.5) (*50*, *63*, *64*, *155*). Accordingly, examples can be found where intact DNA serves as a leaving group in the decomposition of N7-alkylguanine residues. For example, Sidney Hecht and Kozarich showed that N7-methylguanosine could be quantitatively demethylated by treatment with excess lithium 2-methylpropane-2-thiolate in hexamethylphosphoramide (Scheme 16) (*156*). In another example, Stephen Hecht and co-workers reported that the N7-pyridylhydroxybutyl adduct **29** decomposes by the expected depurination reaction and also by hydrolytic attack on the N7 substituent to yield 4-hydroxy-1-(3-pyridyl)-1-butanol (**30**) and dG when subjected to acidic or neutral thermal hydrolysis (Scheme 17) (*157*). Skibo and co-workers found that the guanine adduct **31** in a 2′-deoxyoligonucleotide decomposes via elimination to yield **32** and the unmodified DNA oligonucleotide (Scheme 18) (*158*). Recently, Hecht and co-workers reported that adduct **33**, which is formed by reaction of the tobacco carcinogen 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone with DNA, decomposes during neutral thermal hydrolysis to produce a 1:1 mixture of **34** and **36** (Scheme 19) (*159*). Compound **34** arises from a straightforward depurination reaction (Scheme 19). More striking is the observation that the adduct also decomposes by a pathway proposed to involve neighboring group participation of the carbonyl oxygen to generate the electrophilic intermediate **35**, which, in turn, hydrolyzes to yield **36** (as shown by the arrows in Scheme 19). Loeppky and co-workers observed a similar decomposition reaction for the analogous O^6-adduct in 2′-deoxyguanosine (*160*).

One pathway for decomposition of the N7-guanine adduct of aflatoxin B_1-*exo*-8,9-epoxide (**37**) in DNA involves the release of 8,9-dihydro-8,9-dihydroxyaflatoxin B_1 (**39**) (*96*, *114*, *125*). It is possible that this aflatoxin hydrolysis product is formed by the initial liberation of aflatoxin B_1-*exo*-8,9-epoxide (**37**) from the DNA adduct as proposed in Scheme 20. In many respects, this process is analogous to the depurination of N7-alkylguanine residues discussed earlier (Scheme 7), in which the positively charged imidazolium ring of an alkylguanine residue serves as a leaving group in a reaction that generates an oxocarbenium ion (**38**). Similar to the deglycosylation of alkylpurine nucleosides, conversion of the aflatoxin B_1 adduct to 8,9-dihydro-8,9-dihydroxyaflatoxin B_1 is likely to proceed via an S_N1 mechanism rather than by direct S_N2 attack on the adduct. Thus, the literature suggests that aflatoxin may be a reversible DNA-alkylating agent. There is, however, some dispute regarding the contribution of this hydrolysis reaction to the decomposition of aflatoxin B_1 adduct. Wang and Cerutti reported that formation of 8,9-dihydro-8,9-dihy-

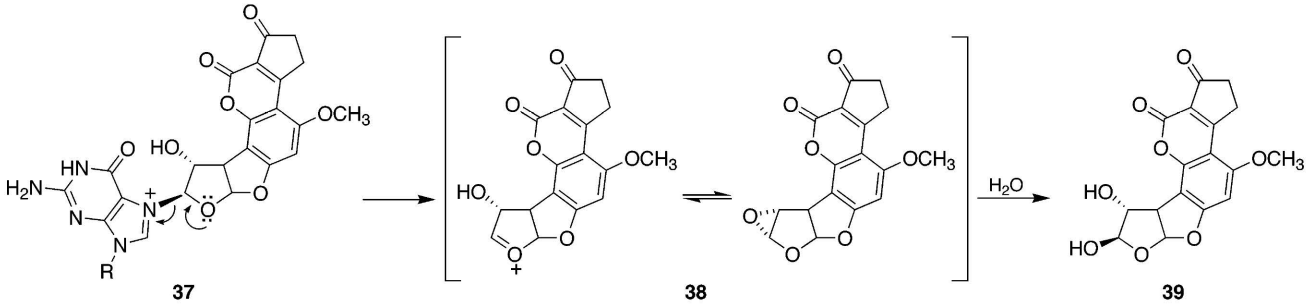
Scheme 18



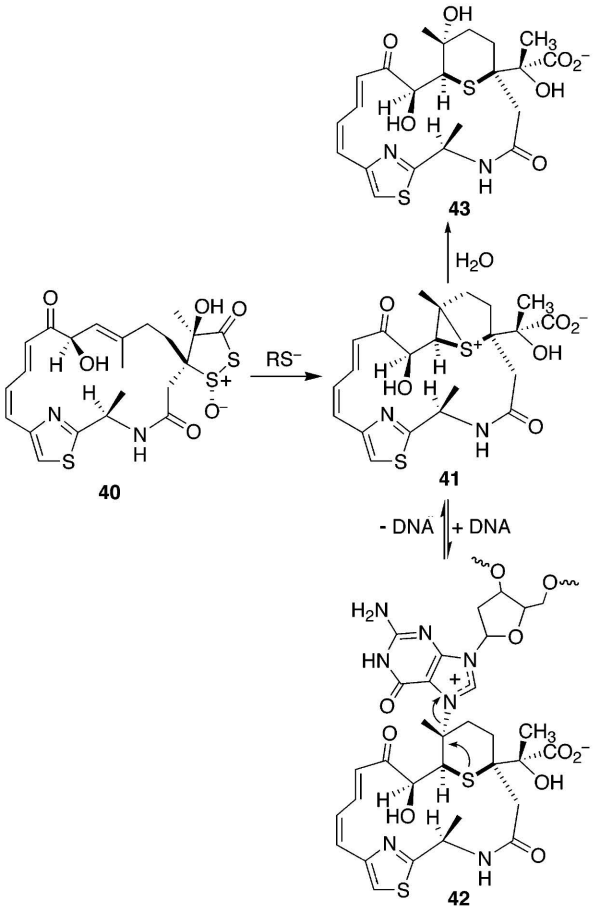
Scheme 19



Scheme 20



Scheme 21



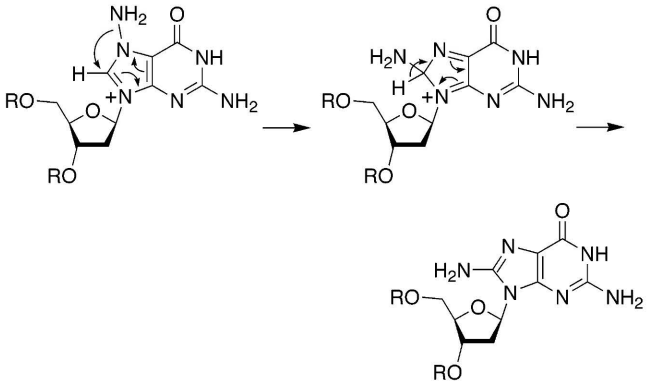
droxyaflatoxin B_1 is the major route for decomposition of the N7-guanine-aflatoxin B_1 adduct (*125*). In contrast, Wogan and co-workers reported that the contribution of this reaction was relatively modest (*96*). Similarly, Loechler and co-workers observed negligible amounts of 8,9-dihydro-8,9-dihydroxyaflatoxin B_1 arising from oligonucleotides bearing the aflatoxin-guanine adduct following treatment with 0.25 N HCl (*161*). It has been noted that conversion of the aflatoxin adduct to 2,3-dihydro-2,3-dihydroxyaflatoxin B_1 is dependent upon the density of adducts in double-stranded DNA, with the reaction occurring more rapidly in DNA containing higher levels of the adduct (*96*).

Finally, there is evidence that the natural product leinamycin (**40**) may reversibly alkylate double-stranded DNA (*20*, *21*). Following reaction with thiols, leinamycin is converted to an episulfonium ion (**41**) that efficiently alkylates the N7 position of guanine residues to yield adduct **42** (Scheme 21). The recent observation that hydrolyzed leinamycin (**43**) is released from leinamycin-modified DNA suggests that the N7-alkylguanine adduct (**42**) may decompose to regenerate activated leinamycin (**41**). In principle, the product **43** could be formed by either direct S_N2 attack of water (or hydroxide) on a leinamycin-DNA adduct or via an S_N1 reaction. However, S_N2 reactions generally do not occur at tertiary centers (*162*), so it is likely that this reaction follows an S_N1 mechanism involving neighboring group participation of the sulfide moiety to regenerate activated leinamycin **41**, as shown in Scheme 21.

6. Rearrangement of N7 Adducts to C-8 Adducts

Recent studies indicate that C-8-guanine adducts formed by reaction of DNA with electrophilic nitrogen compounds may arise via rearrangement of an initially formed N7-guanine intermediate as shown in Scheme 22 (*163*, *164*). This mechanistic proposal invokes initial attack of DNA's most nucleophilic center (N7-G) on the electrophilic species and, thus, may be more satisfying than alternate possibilities involving direct attack of the C-8 carbon. While direct addition of radicals to the C-8

Scheme 22



position of guanine is common (*165*−*167*), this center is not thought to be highly nucleophilic (*1*). In addition, the proposed N7 intermediate can explain the origin of not only the C-8-guanine adducts but also depurination products, FAPy products, and 8-oxo-dG that result from exposure of DNA to nitrenium ions (*164*). Potential mechanisms for the rearrangement of N7-dG adducts of nitrenium ions to C-8-guanine adducts have recently been examined computationally (*168*). No N7→C-8 rearrangements involving N7-alkylguanine adducts have been reported.

7. Conclusions

N7-alkylguanine residues are the major products formed in the reaction of DNA with many electrophiles. Thus, there is little doubt that future studies will continue to identify novel N7-alkylguanine adducts formed by electrophiles that are of medicinal and toxicological importance. The chemistry summarized in this review provides a foundation for considering the biological effects of N7-alkylguanine residues in DNA because these reactions ultimately determine the structural nature of the lesion(s) that will confront the cell's repair, transcription, replication, and signal transduction machinery.

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