Elucidating DNA damage and repair processes by independently generating reactive and metastable intermediates

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DNA damage is a double-edged sword. The modifications produced in the biopolymer are associated with aging, and give rise to a variety of diseases, including cancer. DNA is also the target of anti-tumor agents and the most generally used nonsurgical treatment of cancer, ionizing radiation. Agents that damage DNA produce a variety of radicals. Elucidating the chemistry of individual DNA radicals is challenging due to the availability of multiple reactive pathways and complexities inherent with carrying out mechanistic studies on a heterogeneous polymer. The ability to independently generate radicals and their metastable products at defined sites in DNA has greatly facilitated understanding this biologically important chemistry.

DNA damage is a fact of life and sometimes a cause of cellular death, which in the case of a cancer cell is desirable. Exogenous reagents that alkylate or oxidize it constantly assault the biopolymer. In addition, oxidative damage is a consequence of respiration, a necessary requirement for life, due to the formation of reactive oxygen species. The modifications produced in DNA can be carcinogenic and capable of inducing cell death by triggering apoptosis. The latter is exploited by anti-cancer therapies that target DNA. Some of these agents alkylate the nucleobases, while others oxidize nucleic acids *via* radical and/or radical ion intermediates.[1–4] Determining how nucleic acids are oxidatively damaged is a challenging endeavor. DNA is a heterogeneous polymer that is typically available in smaller molar quantities than mechanistic chemists are accustomed to working with. In addition, mechanistic studies on individual damaging agents can be further complicated by the generation of multiple intermediates randomly throughout the biopolymer. On the other hand, questions arise from observations that different damaging agents can generate distinct products from common intermediates. For instance, how and why small molecules, such as $Cu(OP)_2$ and the enediyne antibiotics (*e.g.* esperamicin) produce different products from a common deoxyribosyl intermediate was unclear until a few years ago. γ-Radiolysis is the most commonly used cancer treatment that targets DNA, and the most chemically complex. Exposing DNA to γ-radiolysis can be likened to hitting fine crystal with a hammer. Radiation scientists have significantly increased our understanding of radiation induced DNA damage by using a variety of methods.[5] However, there are inherent limitations imposed by the unselective nature of the process to studying ionizing radiation induced damage directly. This complex chemistry has been unraveled over the past decade and a half by using organic chemistry to independently generate the putative reactive and metastable intermediates. This approach has provided mechanistic insight, resolved mechanistic controversies, identified new DNA damage pathways, and provided explanations for differences in DNA damage pathways that proceed *via* common intermediates.[6–12]

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Independent generation of a C1′-nucleoside radical in DNA and mechanistic studies of its reactivity

The C1′-carbon–hydrogen bond is one of the two weakest such bonds in the deoxyribose backbone.[13,14] However, it is buried in the minor groove, making it the least accessible to a diffusible species (Fig. 1). The inaccessibility of the C1′-hydrogen atom to diffusible species (*e.g.* hydroxyl radical) was believed to be reflected in the

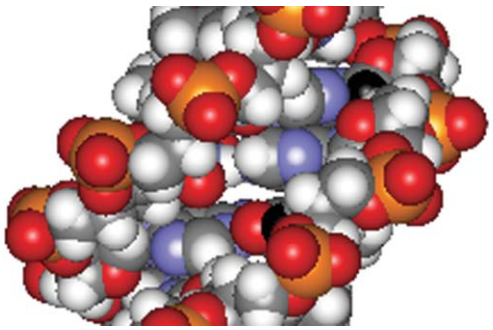
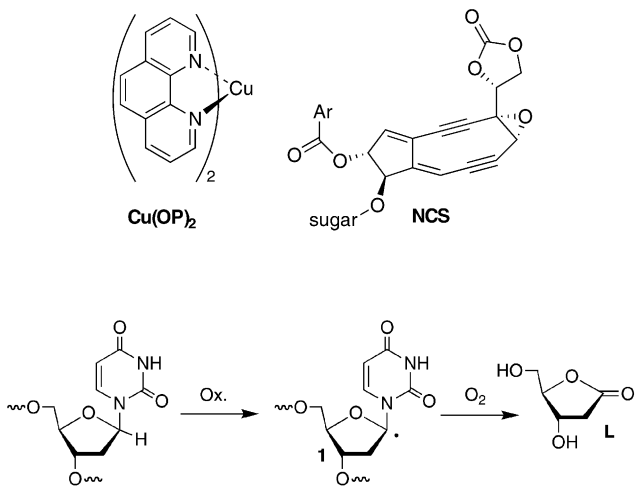


Fig. 1 CPK model of duplex DNA looking into the minor groove. The C1′-hydrogen atoms of neighboring nucleotides are indicated in black.

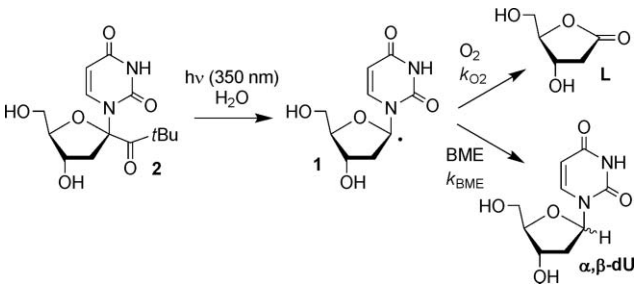
low efficiency for formation of 2-deoxyribonolactone (L) following γ-radiolysis. However, the yield of L has been shown to be considerably higher in irradiated DNA than previously thought, albeit not necessarily *via* direct hydrogen atom abstraction by a diffusible species.[15–17]

2-Deoxyribonolactone is an example of an alkali-labile lesion (one which results in a strand break upon subjection to alkaline conditions), and is a signature product of C1′-oxidation (Scheme 1). DNA damaging agents that bind in the minor groove overcome the hydrogen atom's inaccessibility by delivering their reactive component. Molecules that are believed to abstract the C1′-hydrogen atom include the very useful structural probe, $Cu(OP)_2$ and anti-tumor agents, such as the neocarzinostatin chromophore (NCS).[4,18,19] With the exception of $Cu(OP)_2$, all of these damaging agents produce 2-deoxyribonolactone. In contrast, reaction of duplex DNA with $Cu(OP)_2$ results in immediate cleavage (direct strand scission) of the DNA backbone. Although these phenomenological observations were reported in the literature in the late 1980's and early 1990's, it was unclear why $Cu(OP)_2$ produced direct strand breaks but other damaging agents yielded 2-deoxyribonolactone from the C1′-radical. In addition, the mechanism for transformation of the radical into 2-deoxyribonolactone was not understood. The ability to independently generate the C1′-radical in DNA and the metastable 2-deoxyribonolactone lesion was instrumental in addressing these questions.



Scheme 1

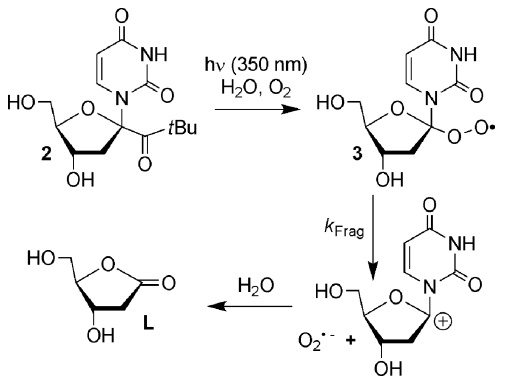
The C1′-radical of 2′-deoxyuridine (**1**) was generated *via* Norrish Type I photocleavage of **2**.[20]† The radical (**1**) is trapped by thiols with a high degree of stereoselectivity (Scheme 2). β-2′-Deoxyuridine is favored more than 6-fold over the α-nucleotide when **1** is trapped by β-mercaptoethanol (BME) in duplex DNA. Competition studies between BME and O_2 indicate that the thiol traps **1** with a bimolecular rate constant of $\sim 4 \times 10^6$ M^{-1} s^{-1} in single stranded DNA or the monomer.[20,21] The rate constant is reduced by a factor of 2–3 ($1.8 \pm 0.6 \times 10^6$ M^{-1} s^{-1}) in duplex DNA.[21] This is consistent with the decreased accessibility of the radical in the duplex.[13] Moreover, the relative rate constants ($k_{O2}/k_{BME} = 1100$) indicate that in the presence of physiological levels of thiol ($\sim$5 mM) and O_2 (63 μM) trapping by the latter will dominate (>90%).



Scheme 2

Studies on the reactivity of monomeric **1** and oligonucleotides revealed that 2-deoxyribonolactone was produced under aerobic conditions.[20,22] 2-Deoxyribonolactone (L) formation was detected *via* mass spectrometry, and subsequently using a series of chemical reactions that provided a "fingerprint" for 2-deoxyribonolactone.[23,24] Isotopic labeling and kinetic experiments revealed that the peroxyl radical (**3**) is transformed into the lactone *via* an unusual mechanism (Scheme 3).[25] The peroxyl radical undergoes heterolytic fragmentation to release a molecule of superoxide and the C1′-carbocation. The latter yields 2-deoxyribonolactone, following trapping by water. Superoxide formation was detected spectrophotometrically utilizing epinephrine oxidation to adrenochrome. Kinetic competition studies using ^{18}O incorporation into 2-deoxyribonolactone grossly underestimated the rate constant for superoxide elimination (k_{Frag}), as shown by Newcomb and Chatgilialoglu who utilized **2** in laser flash photolysis experiments to determine that the rate constant for superoxide elimination from **3** is $\sim 10^4$ s^{-1}.[26] This is similar to the rate constant for the comparable reaction of structurally similar anomeric peroxyl radicals of sugars.[27] Moreover, superoxide elimination from **3** is too fast for physiological concentrations of thiols (5–10 mM) to compete. These experiments revealed an unrecognized consequence of DNA damage by anti-tumor agents that produce L *via* C1′-hydrogen atom abstraction, such as neocarzinostatin and the enediynes.[4] When these agents oxidize DNA, 2-deoxyribonolactone formation is accompanied by superoxide generation. Superoxide does not directly react with DNA, but is transformed into species such as hydroxyl radical, which does.

† For simplicity, all radicals and products are referred to using the same descriptor whether they are monomeric or part of a biopolymer.



Scheme 3

Independent generation of 2′-deoxyuridin-1′-yl (1). A tool for investigating the mechanism of action of the radiosensitizer, tirapazamine (Tpz)

The effectiveness of ionizing radiation at destroying tumors is compromised by the hypoxic (O_2 deficient) state that such cells can exist in. Molecular oxygen is required to trap the DNA radicals and competes with thiols (*e.g.* glutathione), which can repair the reactive intermediates produced by ionizing radiation. Radiosensitizing agents are molecules that increase the effectiveness of ionizing radiation under hypoxic conditions. Some radiosensitizing agents, such as 5-bromo-2′-deoxyuridine are incorporated in DNA by polymerases in place of the appropriate nucleotide (*e.g.* thymidine). Molecules such as tirapazamine (**Tpz**), which is being evaluated in several anti-cancer clinical trials, sensitize DNA to ionizing radiation even though they are not incorporated in the biopolymer.[28] There is evidence to suggest that tirapazamine behaves in a bimodal manner. This radiosensitizing agent is believed to produce hydroxyl radical following reductive activation.[29] However, the molecule also contains a nitroxide, and as such was postulated to react rapidly with radicals. Consequently, the possibility that tirapazamine acts as a surrogate for O_2 by trapping DNA radicals and "fixing" damage by preventing their repair by thiols was investigated.[30,31] 2′-Deoxyuridin-1′-yl (**1**) was used as a model DNA radical. Kinetic studies using β-mercaptoethanol showed that tirapazamine (and other structurally similar 1,2,4-benzotriazine-1,4-*N*-oxides, *e.g.* **4**) reacted with **1** in duplex DNA with rate constants comparable in magnitude to that measured for the nitroxide, TEMPO (**5**), but slower than its own reduction product (**4**) or the electron affinic sensitizing agent, misonidazole (**6**, Table 1). The rate constants are competitive with thiol, indicating that **Tpz** and the related molecules could act as a surrogate for O_2 and transform the DNA radicals into lesions. The formation of 2-deoxyribonolactone was additional evidence for **Tpz** acting as a surrogate for O_2.

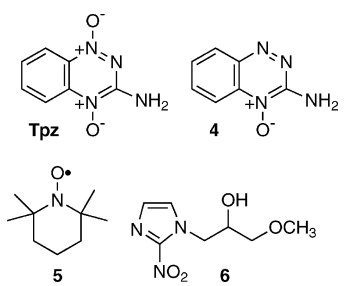


Table 1 Rate constants for the trapping of 2′-deoxyuridin-1′-yl (**1**) in double stranded DNA by tirapazamine and various nitroxide containing molecules

Compound	$k_{Trap}/M^{-1}\ s^{-1}$
Tirapazamine (Tpz)	$4.6 \pm 1.1 \times 10^6$
4	$3.1 \pm 0.1 \times 10^7$
TEMPO (**5**)	$9.1 \pm 0.8 \times 10^6$
Misonidazole (**6**)	$2.9 \pm 0.7 \times 10^7$

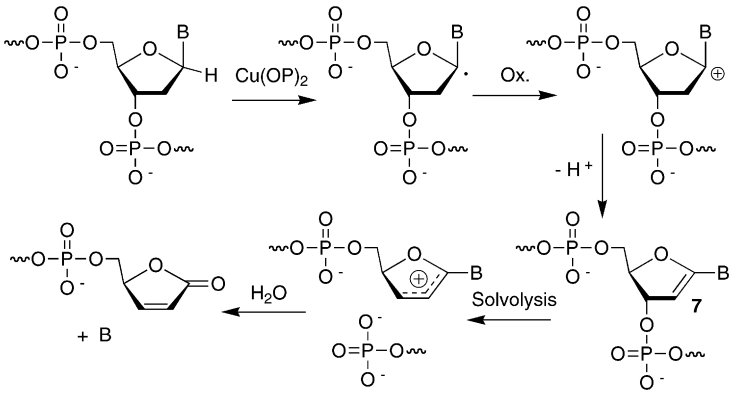
Independent generation of 2-deoxyribonolactone (L) in DNA. A useful mechanistic probe of DNA damage

Its alkaline lability prevents 2-deoxyribonolactone from being incorporated directly into chemically synthesized oligonucleotides, which are deprotected under basic conditions. Formation of **L** in high yield upon aerobic photolysis of **2** constituted the first preparative method for this lesion.[20,22] Subsequently, elegant methods for photochemically producing 2-deoxyribonolactone were reported by Kotera and Sheppard.[32–34] The ability to independently generate 2-deoxyribonolactone in DNA has proven to be useful for discovering how enzymes cope with this lesion and as a means for resolving a mechanistic question in the field of DNA damage.

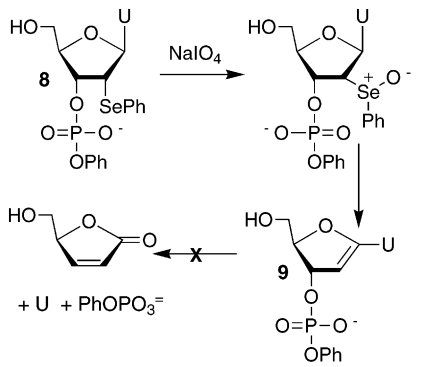
With respect to the latter, independent generation of 2-deoxyribonolactone at a defined site in oligonucleotides was a powerful tool for elucidating why C1′-oxidation by $Cu(OP)_2$ results in direct strand breaks instead of the oxidized abasic site. Sigman, who discovered and developed the use of $Cu(OP)_2$ as a DNA cleaving agent proposed that direct strand scission resulted from solvolysis of the 3′-allylic phosphate of the 1′,2′-dehydronucleotide (**7**) following oxidation of the original C1′-radical (Scheme 4).[35] Given the mechanism discussed above for O_2 dependent 2-deoxyribonolactone formation (reported after Sigman's proposal was published), which proceeds through the carbocation, it was unclear why the 1′,2′-dehydronucleotide (**7**) would be formed in DNA by $Cu(OP)_2$ and not by other damaging agents that abstract the C1′-hydrogen atom.

Model studies carried out by Chen cast further doubt on this mechanism and support for an alternative (Scheme 5).[36] Chen showed that a 1′,2′-dehydronucleotide containing an allylic phosphate (**9**) generated by mild periodate oxidation of **8** was stable in aqueous buffer and was therefore kinetically incompetent to be on the pathway for direct strand scission induced by $Cu(OP)_2$. An alternative mechanism based upon examination of the observed rate constant of β-elimination from **10** as a function of $Cu(OP)_2$ concentration was proposed (Scheme 6). The observed rate constant varied linearly with $Cu(OP)_2$ concentration, but not phenanthroline or cupric ion. Extrapolation of the observed rate constant indicated that an effective molarity of $Cu(OP)_2$ bound to DNA between 10 and 100 M was sufficient to explain the observed DNA strand scission using this mechanism. It was proposed that the noncovalently bound $Cu(OP)_2$ complex and/or one of its oxidized intermediates (*e.g.* a copper bound hydroxyl complex) deprotonated the lactone's α-carbon.

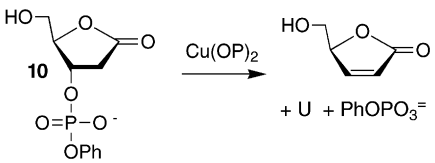
The take home message from this study was that 2-deoxyribonolactone is the last common intermediate in DNA oxidation by anti-tumor agents that abstract the C1′-hydrogen atom and $Cu(OP)_2$. The latter produces direct strand breaks because the lactone lesion is unstable in the presence of the noncovalently



Scheme 4

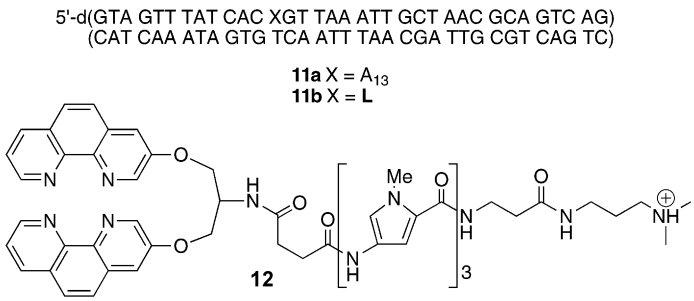


Scheme 5



Scheme 6

bound metal complex. This mechanism, and the widely accepted notion that the C1′-position is the major oxidation site by $Cu(OP)_2$ was subsequently questioned in studies on hexanucleotide duplexes.[37] However, Bales demonstrated that the conditions under which the hexanucleotide experiments were carried out did not test the β-elimination mechanism because the DNA containing 2-deoxyribonolactone dehybridized under the reaction conditions and $Cu(OP)_2$ does not bind to single stranded material.[38] Bales provided further evidence in support of the proposal that $Cu(OP)_2$ produces direct strand breaks by effecting β-elimination from 2-deoxyribonolactone by taking advantage of independent generation of the lesion in DNA and copper-phenanthroline conjugates of the minor groove binding molecule, distamycin.[38,39] A duplex containing 2-deoxyribonolactone (**11b**) that was substituted for a 2′-deoxyadenosine (**11a**), which is cleaved by **12** underwent cleavage with a half-life of 20.6 min (Scheme 7). The rate constant for elimination ($k_{Elim} = 5.6 \pm 0.7 \times 10^{-4}$ s^{-1}) was 3–4 times faster than the overall rate constant for DNA oxidation at A_{13} ($k_{Ox} = 1.9 \pm 0.6 \times 10^{-5}$ s^{-1}) that results in a direct strand break or alkali-labile lesion.



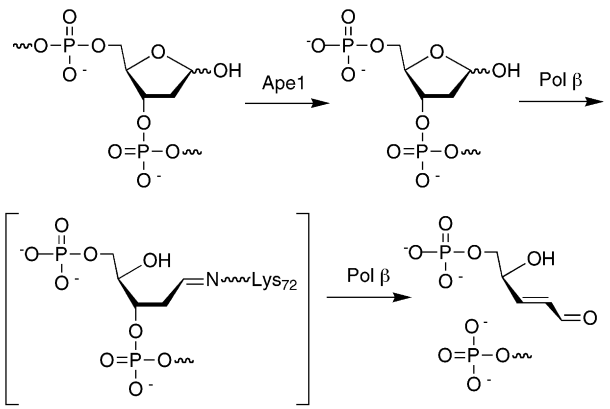
Scheme 7

These data indicated that cleavage at 2-deoxyribonolactone was kinetically competent to explain direct strand scission by copper-phenanthroline complexes. Support for the hypothesis that this pathway was a major pathway for DNA cleavage by minor groove binding conjugate **12** was obtained by measuring the growth ($k_G = 1.8 \pm 0.4 \times 10^{-5}$ s^{-1}) and decay ($k_D = 4.7 \pm 0.9 \times 10^{-4}$ s^{-1}) of direct strand scission and alkali-labile lesions in total (eqn (1)).[38] The similarity in the rate constants between k_{Elim} and k_D means that either 2-deoxyribonolactone is the major alkali-labile lesion formed or that other such lesions undergo elimination with the same rate constant. Finally, the observation that k_G and k_{Ox} are within experimental error of one another indicates that the majority of damage events induced by the $Cu(OP)_2$ conjugate proceed through an intermediate alkali-labile lesion resulting from C1′-oxidation.

$$\underset{\textbf{(12-11a)}}{Cu(OP)_2\cdot DNA} \xrightarrow{k_G} \text{Alkali labile lesion} \xrightarrow{k_D} \text{Strand break} \qquad (1)$$

Independent generation of 2-deoxyribonolactone reveals a novel inhibition pathway of DNA repair

Most DNA lesions, including abasic sites (AP), are repaired in multiple steps by a series of enzymes.[40–42] The first step in AP site repair in prokaryotes is carried out by two families of base excision repair (BER) enzymes. These repair pathways are necessarily very efficient because ~10 000 AP sites are produced in a cell per day.[43] One family of enzymes induces β-elimination *via* Schiff-base formation involving a lysine side chain or N-terminal proline. This is a minor pathway in *E. coli*, where the majority of AP sites are incised by 5′-phosphodiesterases. However, a lyase reaction is involved in the major pathway for AP site repair in eukaryotes following incision by a 5′-phosphodiesterase (Scheme 8).[44]

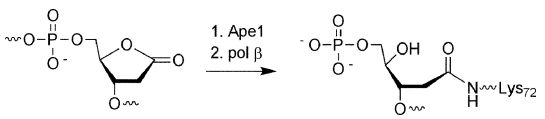


Scheme 8

2-Deoxyribonolactone is very similar in size and shape to an AP site, and it was postulated that repair enzymes containing lyase activity would recognize the lesion.[23] However, nucleophilic attack by a lysine side chain on the lactone would result in a cross-link between the DNA and protein. Indeed, cross-linking was observed when a duplex containing L and endonuclease III, a bifunctional BER enzyme from *E. coli* that contains a lyase function, were incubated.[45,46] Support for the proposed cross-linking between L and the lysine side chain involved in Schiff base formation was obtained using a mutated form of endonuclease III. Substituting alanine for lysine 120 eliminated DNA–protein cross-link formation. This was the first example of a DNA lesion that formed a cross-link to a repair enzyme. Subsequently, oxanosine was also shown to form DNA–protein cross-links.[47]

Several other base excision repair enzymes from a variety of species were assayed for cross-linking to 2-deoxyribonolactone, but none showed significant reactivity.[46] The absence of cross-linking by these other enzymes was attributed to their weaker lyase activity than endonuclease III. Interestingly, mild alkaline treatment of DNA containing 2-deoxyribonolactone produces the transiently stable α,β-unsaturated butenolide cleavage product, which is analogous to the product produced upon lyase reaction between an AP site and a Type II repair enzyme.[37] The butenolide is cross-linked by formamidopyrimidine DNA glycosylase (Fpg) and endonuclease VIII (NEIL1), but not endonuclease III. These lyase containing base excision repair enzymes are distinguished from others by utilizing an N-terminal proline to effect elimination. They are also the only enzymes capable of inducing β- and δ-elimination of AP sites. One possible explanation for their cross-linking to the butenolide is that unlike endonuclease III, Fpg and NEIL1 typically recognize the analogous α,β-unsaturated remnant of an AP site.

The biological relevance of the above cross-linking reactions could be questioned because although AP sites are intermediates formed during repair by base excision repair enzymes, most endogenously produced AP sites are repaired by 5′-phosphodiesterases. Exonuclease III and endonuclease IV are responsible for this activity in *E. coli*, whereas apurinic endonuclease 1 (Ape1) is the primary enzyme responsible for incising AP sites in human cells.[48] 2-Deoxyribonolactone is efficiently incised by each of these enzymes.[49,50] However, incision of 2-deoxyribonolactone by Ape1 presents a challenge in mammalian cells during the next step in DNA repair. Incision by Ape1 is followed by elimination of the resulting 5′-deoxyribose phosphate by DNA polymerase β (pol β), which utilizes a lysine side chain (Lys_{72}) to induce elimination *via* Schiff base formation.[44] As expected, based upon the interaction with endonuclease III (Scheme 9), DNA polymerase β and 2-deoxyribonolactone cross-link one another.[51] In addition, experiments using mutant protein implicated the requirement for the Lys_{72} side chain previously implicated in Schiff base formation.



Scheme 9

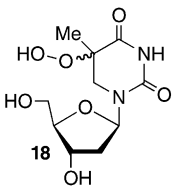
The formation of DNA–protein cross-links between 2-deoxyribonolactone and a repair enzyme provides a possible chemical basis for the cytotoxic effects of anti-tumor agents that produce this lesion. For instance, it was reported that DNA damage produced by the neocarzinostatin chromophore is refractory to repair.[52] Our experiments suggest that 2-deoxyribonolactone formation could be the source of inhibition. Failure to effect repair will prevent replication and transcription. Although a recent report in cell lysates suggests that cells have a "work around" method for lactone enzyme cross-links, one cannot discount their biological importance, particularly given the correlation described above regarding the effect of neocarzinostatin on cells.[53]

γ-Radiolysis produces DNA damage by forming nucleobase centered reactive intermediates

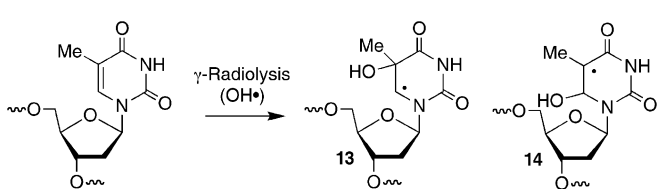
Natural products (*e.g.* bleomycin, neocarzinostatin) that oxidatively damage DNA typically bind in the minor groove and abstract one or more hydrogen atoms from the deoxyribose ring.[4,54] Exposing DNA to γ-radiolysis also results in the formation of sugar radicals. These and other reactive intermediates can be produced in DNA *via* direct ionization (the "direct effect" of γ-radiolysis) or *via* reaction with hydroxyl radical, which is generated by the ionization of water (the "indirect effect" of γ-radiolysis).[5] Hydroxyl radical adds to double bonds about an order of magnitude faster than it abstracts hydrogen atoms from carbon–hydrogen bonds. The inherent differences in rate constants are reflected in the distribution of reactive intermediates produced by hydroxyl radical ($OH^{\bullet}$) reaction with DNA. Nucleobase radicals are believed to account for as much as 93% of the reactive

intermediates formed.[5] Nucleobase radicals are also produced by the direct ionization of DNA, followed by reaction with water. However, it is not clear what fraction of this damage pathway results in nucleobase radical formation.

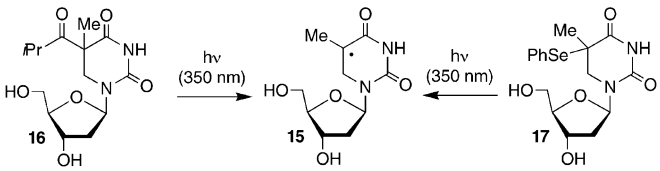
Nucleobase radical formation is a distinctive chemical pathway exhibited by γ-radiolysis. Radiation chemists have extensively studied the role of these radicals in nucleic acid strand scission using biopolymers, monomers, and short oligonucleotides substrates. The reactivity of these substrates has been examined using an elaborate battery of analytical and spectroscopic tools, including pulse radiolysis, EPR spectroscopy, and mass spectrometry.[5,55–58] Based on these studies, it has been proposed that ~40% of the reactions between OH• and nucleic acids result in direct strand scission.[59] The predominance of nucleobase radical formation requires that some of these reactive intermediates lead to strand cleavage. In order to yield a strand break the radical center must be transferred to the sugar backbone. Considering the number of possible nucleobase radicals and their respective peroxyl radicals produced by OH•, as well as the number of potential hydrogen atom abstraction sites, it is difficult to identify individual pathways using randomly generated reactive intermediates. Independent generation of reactive intermediates is well suited for exploring the feasibility of such molecular rearrangements.



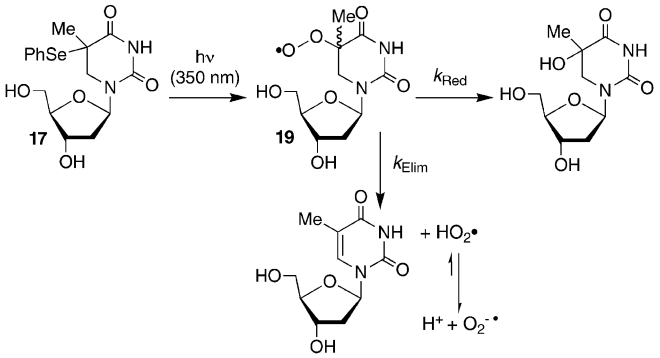
Photolabile, synthetic nucleosides have been very useful for studying the reactivity of pyrimidine nucleobase radicals. The regioisomeric OH• addition products are formed in between 2 : 1 and 4 : 1 ratio, with addition to the C5-position (**13**) favored by the electrophilic radical (Scheme 10). For the sake of synthetic expediency the formal C6-hydrogen atom addition product (**15**) was generated from **16** *via* Norrish Type I photocleavage (Scheme 11).[60] In addition, phenyl selenide **17** was employed for carrying out studies on monomeric **15** (Scheme 11).[25] The reactivity of **15** with hydrogen atom donors designed to mimic 2-deoxyribose suggested that it was highly unlikely that the radical would lead to direct strand breaks by abstracting hydrogen atom(s) from the adjacent nucleotides in DNA.[60] When generated in the presence of O_2 a diastereomeric mixture of the respective hydroperoxide (**18**) was obtained. The intermediate peroxyl radical (**19**) formally eliminates hydroperoxyl radical (OOH•), which deprotonates at physiological pH (Scheme 12).[25] Consistent with this, superoxide ($O_2^{\bullet -}$) is detected spectroscopically in the reaction. Elimination of OOH• produces thymidine and efficiently competes with reduction by hydrogen atom donor ($k_{Elim}/k_{Red} = 1.3 \times 10^{-2}$ M).



Scheme 10



Scheme 11

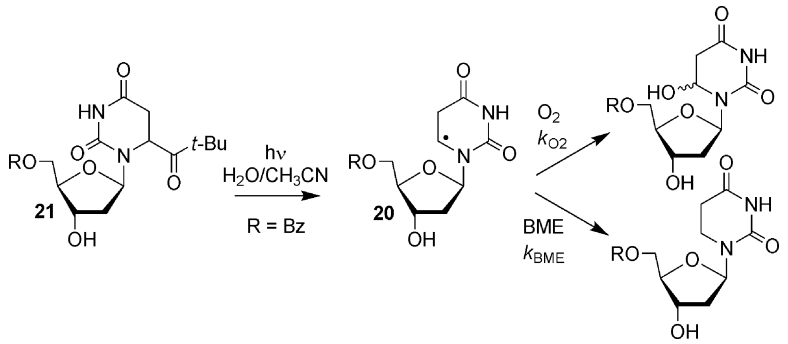


Scheme 12

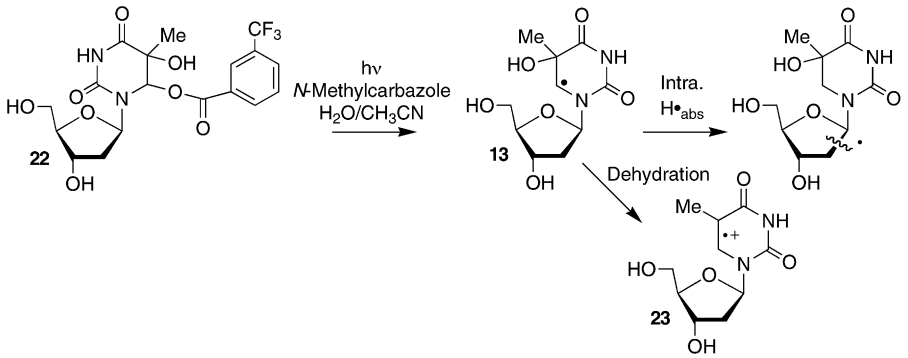
Independent generation of **15** from **16** in single stranded oligonucleotides corroborated the radical's reluctance to effect hydrogen atom abstraction from adjacent nucleotides.[61,62] This was evident by the absence of direct strand scission or alkali-labile lesions under anaerobic conditions. In contrast, strand damage was evident at the position of original radical generation and adjacent nucleotide when the radical was generated under aerobic conditions. Incorporation of synthetically deuterated thymidines at the 5′-adjacent position indicated that the peroxyl radical selectively abstracted the C1′-hydrogen atom from this site.

The dramatic effect of O_2 on strand scission and alkali-lability was also observed in studies involving the C5-radical adduct (**20**, Scheme 13).[63–65] Radical **20** was generated *via* Norrish Type I photocleavage from **21** and was also designed for synthetic expediency to model the OH• radical adduct (**13**, Scheme 10). Studies on monomeric **13** were also carried out under anaerobic conditions using **22** (Scheme 14).[66,67] The chemical stability of **22** and mechanism of radical formation (photoinduced single electron transfer) from it, prohibited producing **13** from it under aerobic conditions or within oligonucleotides. However, **22** was useful for determining the proficiency of **13** at inducing intranucleotidyl hydrogen atom abstraction, as well as its ability to generate the cation radical (**23**) *via* hydroxide elimination. The latter has also been proposed to be an intermediate in direct strand scission.[57] A combination of isotopic labeling and ^{2}H NMR experiments indicated that neither of these processes occurs at rates fast enough to compete with reaction with O_2 or hydrogen atom donors such as 1,4-cyclohexadiene or thiols.[66,67]

The Norrish Type I precursor (**21**) to **20** was compatible with aerobic conditions and solid phase oligonucleotide synthesis. Competitive kinetic studies between O_2 and β-mercaptoethanol (BME) established that monomeric **20** reacts with the thiol with a rate constant ($k_{BME} = 8.8 \pm 0.5 \times 10^6$ M^{-1} s^{-1}) typical of an alkyl radical (Scheme 13).[64,68] The reaction between **20** and BME was useful for calibrating its reactivity and establishing the verity of using O_2 as a competitor in relative rate studies. Subsequently, O_2 was used to approximate the rate constant for reaction of **20** with



Scheme 13

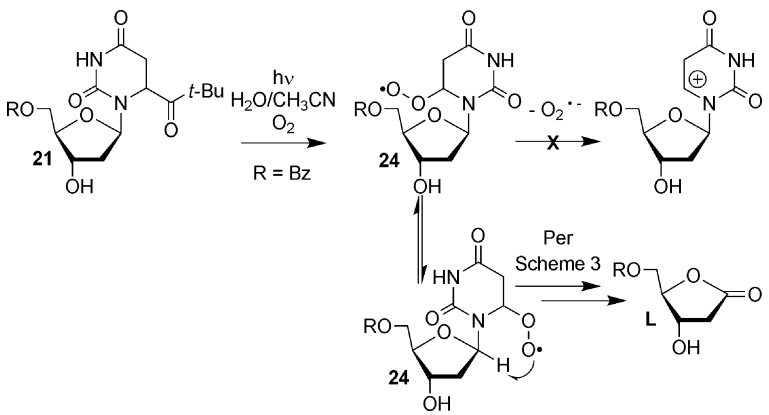


Scheme 14

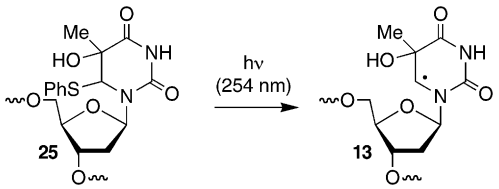
2,5-dimethyltetrahydrofuran (MTHF, $k_{MTHF} = 31.0 \pm 2.5\ M^{-1}\ s^{-1}$) where MTHF was used as a model of deoxyribose in DNA. The rate constant for hydrogen atom abstraction from MTHF (k_{MTHF}) is sufficiently slow, that even if one assumes that the effective molarity of the adjacent deoxyribose ring(s) in DNA is 10 M, radical transfer from the nucleobase to the sugar will not compete with trapping by O_2 or thiol.

Photochemical generation of **20** under aerobic conditions enabled characterization of the reactivity of the respective peroxyl radical (**24**, Scheme 15).[64] Unlike **19** (Scheme 12) or **3** (Scheme 3) there was no evidence for $O_2^{\bullet-}$ elimination from **24**. However, the formation of 2-deoxyribonolactone suggested that the peroxyl radical abstracted the C1′-hydrogen atom. Control experiments ruled out artifacts such as C1′-hydrogen atom abstraction by the precursor's excited state. Quenching of lactone formation by BME indicated that the rate constant for the product (and rate) determining abstraction step was on the order for related reactions.[69,70]

The reactivity of **20** and **24** in polymers largely paralleled the chemistry described in the monomer. There was no evidence for radical transfer from the nucleobase in **20** to its sugar or the deoxyribose of an adjacent nucleotide.[63] Initial studies on related radical **13** when it was generated from **25** (Scheme 16) in di- and trinucleotides upon 254 nm irradiation led to the proposal that the nucleobase radical added to the adjacent guanine.[71] The reaction with an adjacent nucleotide produces two contiguously damaged nucleotides, and is referred to as a tandem lesion.[72,73] Tandem lesions are a subset of well-studied clustered lesions, which are



Scheme 15



Scheme 16

defined as two damaged nucleotides within ~1.5 turns of duplex DNA.[74–77] Although subsequent experiments in duplex DNA did not corroborate the initial studies, it is possible that such lesions are formed, but were not detectable under the reported conditions.[78]

In contrast, tandem lesions were the major types of damage resulting from 5,6-dihydro-2′-deoxyuridin-6-yl radical (**20**) in duplex DNA under aerobic conditions *via* the respective peroxyl radical (**24**).[63] Tandem lesions involving the 5′-adjacent and 3′-adjacent nucleotides were detected by gel electrophoresis and account for at least 65% of the alkali-labile lesions derived from **24**. Tandem lesions involving the 5′-adjacent nucleotide resulted from addition to the pyrimidine ring and hydrogen atom abstraction. Deuterium isotope effects indicated that C1′-hydrogen atom abstraction occurred selectively. Based upon previous studies, the C1′-radical was expected to result in 2-deoxyribonolactone formation.[25,26] This hypothesis was confirmed using a series of fingerprint reactions diagnostic for this oxidized abasic site.[23] Further confirmation for this product was obtained using MALDI-TOF MS analysis of photolyzed single stranded DNA. Gel electrophoresis analysis indicates that 2-deoxyribonolactone containing tandem lesions account for ~15–25% of the tandem lesions derived from **24**. Selective C1′-hydrogen atom abstraction by **24** was consistent with predicted carbon–hydrogen bond strengths and the proximity of the peroxyl radical oxygen atom, which is able to reach into the minor groove from its position in the major groove (Fig. 2).[13,14] The peroxyl radical oxygen of **24** can approach to within 1.5 Å of the C1′-hydrogen atom of the 5′-adjacent nucleotide without distorting the duplex. Formation of 2-deoxyribonolactone *via* **24** provides a mechanism to explain formation of this lesion from diffusible species, despite the hydrogen atom's poor accessibility.[13,15]

Molecular modeling reveals that the deoxyribose hydrogen atoms of the 3′-adjacent nucleotide are considerably further (>5 Å) from the diastereomeric peroxyl radical due to the helical twist of the duplex. Consistent with this picture, tandem lesions ascribable to hydrogen atom abstraction from this component are not observed. However, tandem lesions resulting from peroxyl radical addition to the pyrimidine double bond of the 5′- and 3′-adjacent nucleotides are detected *via* gel electrophoresis following piperidine treatment, and more explicitly using MALDI-TOF MS.

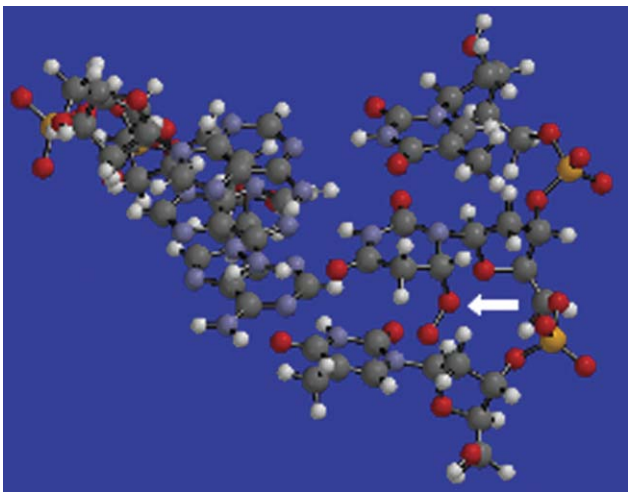
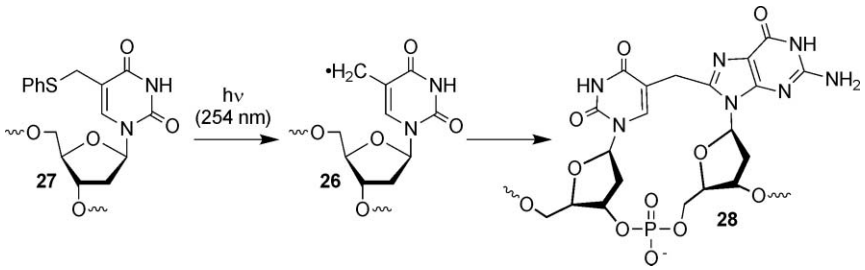


Fig. 2 Ball and stick model showing proximity of the peroxyl radical oxygen of **24** (note: white arrow) to the C1′-hydrogen atom of the 5′-adjacent nucleotide.

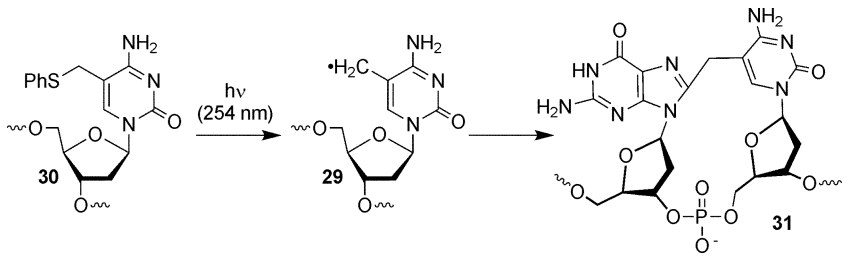
Overall, these studies reveal that tandem lesions are produced in higher yield than previously recognized. They also suggest that direct strand breaks may be formed less efficiently than is generally accepted if nucleobase radicals are the major family of reactive species produced by ionizing radiation.

Interstrand cross-links *via* a nucleotide radical

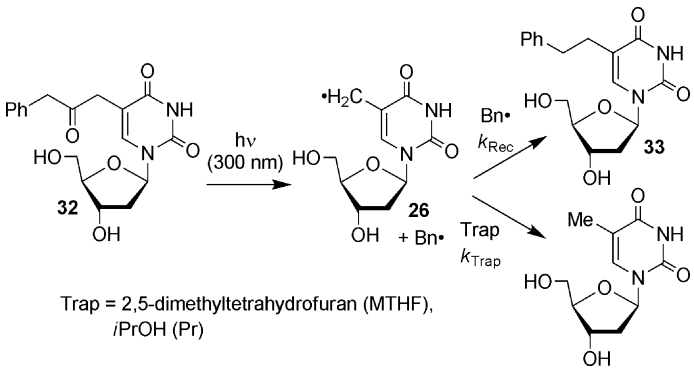
Molecular modeling of the 5-(2′-deoxyuridinyl)methyl radical (**26**) also indicated that this radical and its respective peroxyl radical was well positioned to react with the sugar and nucleobase moiety of the 5′-adjacent nucleotide, but only the latter of the 3′-nucleotide. Addition of **26** to a 5′-adjacent 2′-deoxyguanosine (**28**) upon 254 nm irradiation of **27** was observed in single stranded oligonucleotides (Scheme 17).[79–81] Similarly, tandem lesions involving addition of the respective radical derived from 5-methyl-2′-deoxycytidine (**29**) into 2′-deoxyguanosine bonded to either phosphate (*e.g.* **31**) were also detected when **30** was photolyzed at 254 nm (Scheme 18).[12,82] Anderson used the Norrish Type I reaction to generate **26** from the benzyl ketone (**32**).[83] Kinetic competition studies were carried out under anaerobic conditions using 2,5-dimethyltetrahydrofuran (MTHF, k_{MTHF}) and 2-propanol (Pr, k_{Pr}) as deoxyribose models (Scheme 19). The yield of the recombination product (**33**) and its assumed formation rate constant ($k_{Rec} = 2 \times 10^9$ M^{-1} s^{-1}) were used to determine the rate



Scheme 17



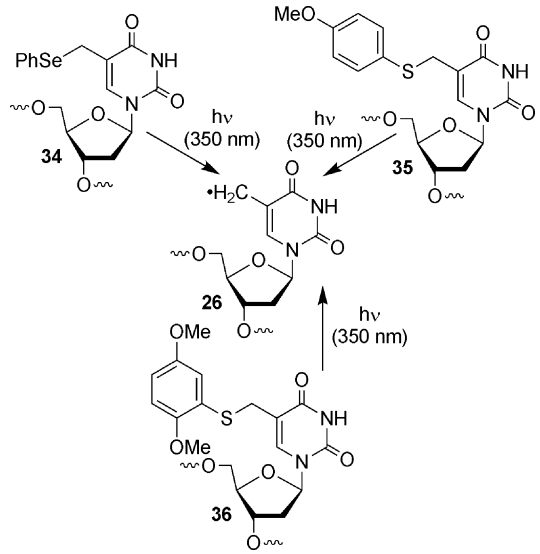
Scheme 18



Scheme 19

constants for hydrogen atom abstraction by **26**. The estimated bimolecular rate constants (k_{MTHF} = 46.1 ± 15.4 M^{-1} s^{-1}, k_{Pr} = 13.6 ± 3.5 M^{-1} s^{-1}) are too slow to compete with O_2 trapping or thiol quenching of **26**, but suggest that the radical could abstract hydrogen atoms from an adjacent nucleotide in DNA in the absence of these reactants.

Although **32** was compatible with solid phase oligonucleotide synthesis, the requirement that it be photolyzed at 300 nm encouraged us to search for a photochemical precursor(s) that was labile to 350 nm irradiation, in order to minimize random DNA damage. The phenyl selenide (**34**) and methoxy substituted aryl sulfides (**35**, **36**) met this criterion (Scheme 20).[84,85] Generation



Scheme 20

of **26** in duplex DNA did not give rise to any direct strand breaks, or alkali-labile lesions. Instead, photolysis of **34** produced interstrand cross-links in high yield.[85,86] Cross-links are produced in very low yield when DNA is exposed to γ-radiolysis, indicating that 5-(2′-deoxyuridinyl)methyl radical (**26**) must also be formed in low yield by this damaging agent. Nonetheless, interstrand cross-link formation from a DNA radical is chemically novel and potentially biologically significant, as they are strongly associated with the cytotoxic effects of anti-tumor agents such as mitomycin C.[87,88]

Extensive studies were undertaken to verify that the interstrand cross-links were due to **26**.[85] Quenching of interstrand cross-links by the hydrogen atom donor, glutathione (GSH) was consistent with a radical mediated process. That the cross-links were produced by all three photochemical precursors (**34**–**36**) was also considered to be strong evidence for a common intermediate. However, cross-link formation was independent of O_2. This is an unusual attribute for a radical reaction because rate constants for O_2 trapping of radicals are typically close to the diffusion controlled limit (k_{O2} = 2 × 10^9 M^{-1} s^{-1}). This seemingly anomalous observation was reconciled with a radical mechanism by experiments utilizing the monomeric radical precursor (**36**), which established that O_2 trapping of **26** was reversible (Scheme 21). Reversible trapping of **26** was evident from the nonlinear dependence of the product ratio as a function of GSH concentration (Fig. 3). The rate constant determined for loss of O_2 (k_{-O2} = 3.4 s^{-1}) from **37** was consistent with that determined

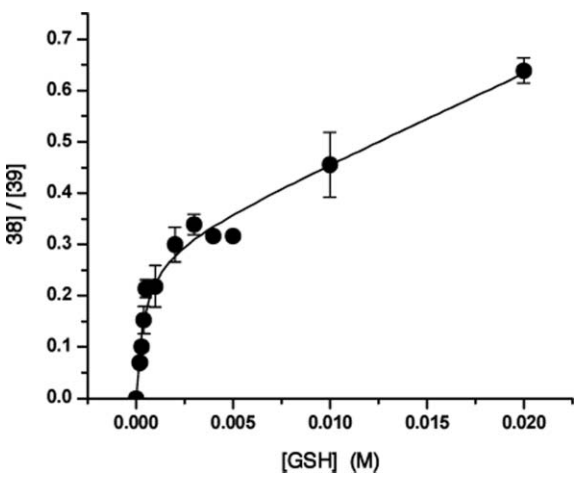
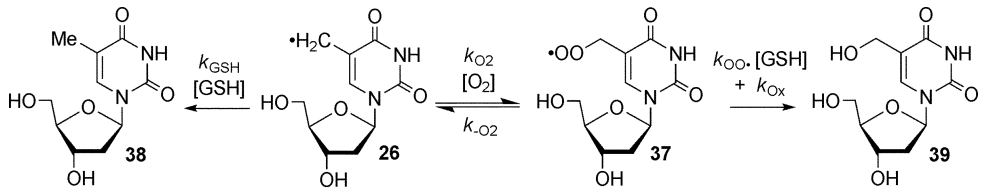


Fig. 3 Nonlinear dependence of product ratio derived from 5-(2′-deoxyuridinyl)methyl radical (**26**) derived from **36** on GSH concentration as evidence for reversible peroxyl radical (**37**) formation.



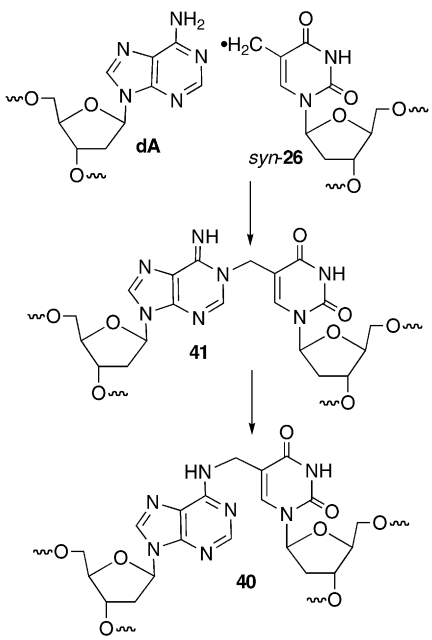
$$\frac{[38]}{[39]} = \frac{k_{GSH}\,[26]\,[GSH]}{k_{OO\bullet}\,[37]\,[GSH] + k_{Ox}\,[37]}$$

$$\frac{[38]}{[39]} = \left(\frac{k_{GSH}\,[GSH]}{k_{O2}\,[O_2]}\right)\left(\frac{k_{-O2} + k_{OO\bullet}\,[GSH] + k_{Ox}}{k_{OO\bullet}\,[GSH] + k_{Ox}}\right)$$

Scheme 21

for other peroxyl radicals of similar structure.[9,27] The verity of this rate constant was supported by that estimated for GSH trapping of **26** ($k_{GSH} = 6.9 \times 10^6$ M^{-1} s^{-1}), which was what one would expect for an alkyl radical reacting with a thiol.[68]

ESI-MS and the hydroxyl radical cleavage method developed by Hopkins were initially employed to investigate the structure of the cross-linked product.[86,89] The former affirmed the O_2 independent nature of the reaction. Analysis of hydroxyl radical cleavage revealed another novel aspect of cross-linking by **26**. In contrast to alkylating agents, which typically react with nucleotides that are 1–2 base pairs apart, the radical reacted exclusively with the opposing deoxyadenosine.[88] The atomic level structure of the cross-link was determined following enzymatic digestion of the duplex and reverse phase HPLC purification of the remaining dinucleoside. The product (**40**) obtained was the result of formal addition to the *N6*-amino group of 2′-deoxyadenosine (Scheme 22). The identical product was isolated when monomeric **26** was generated in the presence of 2′-deoxyadenosine. It was proposed, and later confirmed, that **40** was not the primary product.[85] The initial product (**41**), which has not been isolated, is believed to result from addition of *syn*-**26** to the *N1*-position of the opposing deoxyadenosine (Fig. 4).



Scheme 22

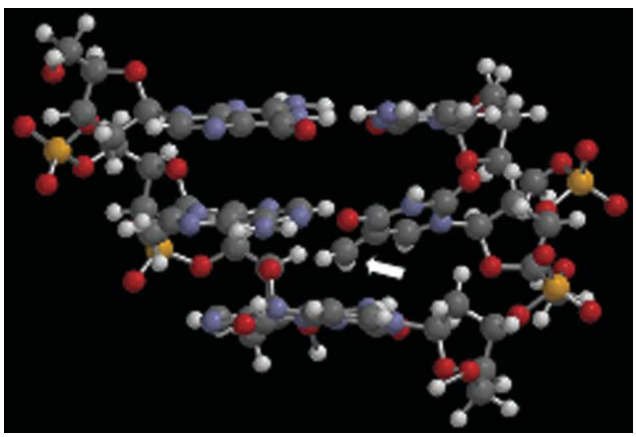


Fig. 4 Molecular modeling illustrates the proximity of *syn*-**26** to the opposing 2′-deoxyadenosine (~2.3 Å) in a trinucleotide duplex (5′-d(C**26**C·GAG)). The white arrow points towards the radical center in **26**.

Potential applications of interstrand cross-link formation

Interstrand cross-linking by a nucleotide radical under anaerobic conditions is potentially biologically useful. As mentioned previously, the efficacy of radiation therapy is often compromised because tumors are hypoxic.[90] We realized that if γ-radiolysis generated **26** from the phenyl selenide (**34**) this molecule would be useful as a radiosensitizing agent, provided its nucleotide triphosphate is accepted as a substrate for DNA polymerase. 5-(2′-Deoxyuridinyl)methyl radical (**26**) would have an advantage over other radiosensitizers, such as 5-bromo- and 5-iodo-2′-deoxyuridine because cross-links are more deleterious than the single strand breaks and alkali-labile lesions produced by the 5-halopyrimidines.[91–95] Irradiation (^{137}Cs) of **42** produced cross-links in good yield.[96] However, their dependence on O_2 indicated a change in mechanism from UV-irradiation to one involving reactive oxygen species (ROS) (Fig. 5). Subsequent experiments that probed for $OH^{\bullet}$, superoxide, and H_2O_2 revealed that the

latter, produced from water by ionizing radiation, was necessary for cross-linking. It was proposed that H_2O_2 oxidized **34** to the selenoxide, which underwent a [2,3]-sigmatropic rearrangement to form a highly electrophilic quinone methide like species (**43**, Scheme 23). This *syn*-conformation of this molecule produces the identical primary product generated from **26** (Scheme 22) by alkylating N1 of the opposing deoxyadenosine.

5'-d(AGA TGG AC**34** CAG GTA C)
3'-d(TCT ACC TG A GTC CAT G)
42

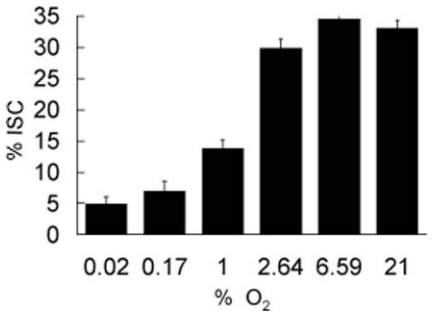
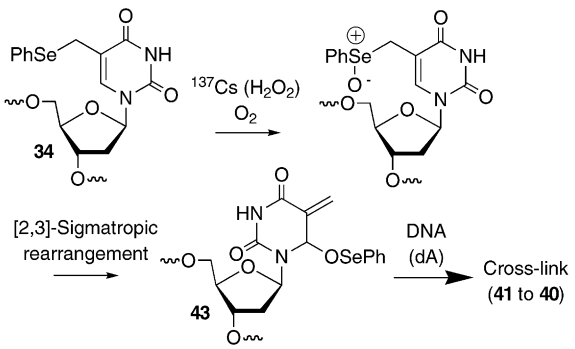
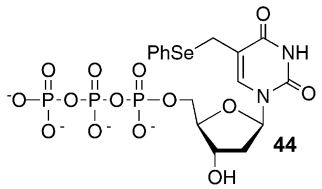


Fig. 5 Interstrand cross-link (ISC) formation from ^{137}Cs irradiation of **42** as a function of O_2 concentration.

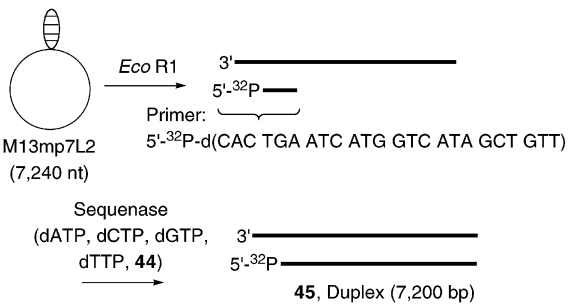


Scheme 23

This mechanism was supported by several independent experiments.[85,96] The intermediacy of the selenoxide was consistent with the ability to utilize $NaIO_4$, a common reagent for producing this functional group, to induce cross-linking. This reagent was also used to directly observe the monomeric quinone methide intermediate (**43**) by ^{1}H NMR, which was independently shown to react with azide and slowly with water.[97] The [2,3]-sigmatropic rearrangement mechanism also provided a rationale for γ-radiolysis' inability to produce cross-links from the aryl sulfide substituted nucleotides (**35**, **36**), because allylic sulfoxides face significantly higher rearrangement barriers.[98,99] Indeed, the independently synthesized monomeric allylic sulfoxides did not rearrange even upon heating. Finally, kinetic analysis provided the mechanistic connection between $NaIO_4$ induced rearrangement observed at the monomeric level and DNA interstrand cross-link formation following γ-radiolysis. The rate constants measured for cross-link formation from the methods were within experimental error of one another (γ-radiolysis: $k_{Obsd} = 3.6 \pm 0.5 \times 10^{-4}$ s^{-1}, $t_{1/2} =$ 32.1 min; $NaIO_4$: $k_{Obsd} = 4.1 \pm 0.3 \times 10^{-4}$ s^{-1}, $t_{1/2} = 28.2$ min).



Despite the change in mechanism from photolysis to γ-radiolysis conditions, the viability of **34** as a radiosensitizing agent was pursued further because some cross-linking was observed when O_2 was present at concentrations comparable to those present in severely hypoxic tumors.[90,96] One requirement was fulfilled by demonstrating that the nucleotide triphosphate (**44**) was accepted as a substrate by the Klenow exo$^-$ fragment of DNA polymerase I from *E. coli.* The modified nucleotide was incorporated ~1% as efficiently as the cognate native nucleotide, thymidine. The acceptance of **44** by a DNA polymerase was utilized to produce a DNA substrate that is more similar to what one would expect to find in a cell. The more promiscuous polymerase, sequenase, was used to prepare a 7200 nt duplex (**45**) containing an estimated 10–12 molecules of **34** randomly incorporated in one of the strands (Scheme 24). Exposure of the duplex to radiation doses on par with what a cancer patient would receive resulted in up to 71.8 ± 5.4% of interstrand cross-links under normal oxygenated aqueous solution (Fig. 6). Although the level of cross-linked DNA under hypoxic conditions (0.17% O_2) lagged behind this at lower doses, almost 60% of the substrate was cross-linked at the highest dose (10 Gy) administered.



Scheme 24

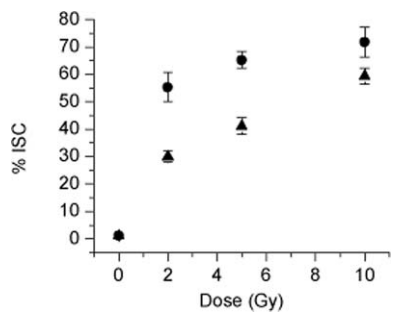


Fig. 6 Interstrand cross-link (ISC) formation in 7200 bp DNA (**45**) containing randomly incorporated **34** upon ^{137}Cs irradiation under aerobic (●, 21% O_2) and hypoxic (▲, 0.17% O_2) conditions.

The oxidative mechanism for cross-linking through **34** suggested that $^1O_2$ may also initiate this reaction, because this ROS oxidizes phenyl selenides to selenoxides.[100] Consistent with these

precedents, photosensitization by Rose Bengal produced high yields of cross-links.[97] Singlet oxygen is known to selectively oxidize deoxyguanosine in native DNA to produce alkali-labile lesions.[101–104] However, DNA interstrand cross-links are potentially more deleterious to the DNA. These observations suggest that **34** incorporated in DNA could be a useful adjuvant in photodynamic therapy.[105,106] Ultimately, whether **34** and molecules like it are active in cells will be determined by their ability to permeate the cell and its nucleus and their subsequent incorporation in DNA. The interesting reactivity of these molecules in DNA is an incentive to investigate this possibility.

Summary and future directions

Significant technological advances in the areas of solid-phase oligonucleotide synthesis and mass spectrometry facilitated combining synthetic and physical organic chemistry with biochemistry and molecular biology to investigate DNA chemistry. The ability to independently generate reactive intermediates and the labile products derived from them at defined sites in synthetic oligonucleotides has contributed to the increased understanding of a diverse range of problems in DNA damage and repair. Many fundamental questions remain to be addressed. These studies will undoubtedly uncover opportunities to apply this science and technology to systems of increasing biological significance, with the ultimate goal of understanding the roles of reactive intermediates in cells.

Acknowledgements

I thank my past and present coworkers, many of whose names are listed in the reference list, without whose contributions the research described here would not be possible. I also want to thank Professors Kent S. Gates and Yoke W. Kow for their collaboration and stimulating discussions. We are grateful for support of this research by the National Institute of General Medical Sciences (GM-054996, GM-063028).

References

1 J. D. Trzupek, J. M. Gottesfeld and D. L. Boger, *Nat. Chem. Biol.*, 2006, **2**, 79–82.
2 D. L. Boger and R. M. Garbaccio, *Acc. Chem. Res.*, 1999, **32**, 1043–1052.
3 J. Chen and J. Stubbe, *Nat. Rev. Cancer*, 2005, **5**, 102–112.
4 Z. Xi and I. H. Goldberg, in *Comprehensive Natural Products Chemistry, Volume 7, DNA and Aspects of Molecular Biology*, ed. E. T. Kool, Elsevier, Amsterdam, 1999, pp. 553–592.
5 C. Von Sonntag, *Free-Radical-Induced DNA Damage and Its Repair*, Springer-Verlag, Berlin, 2006.
6 M. M. Greenberg, *Chem. Res. Toxicol.*, 1998, **11**, 1235–1248.
7 M. M. Greenberg, in *Comprehensive Natural Products Chemistry, Volume 7, DNA and Aspects of Molecular Biology*, ed. E. T. Kool, Elsevier, Amsterdam, 1999, pp. 371–426.
8 B. Giese, A. Dussy, E. Meggers, M. Petretta and U. Schwitter, *J. Am. Chem. Soc.*, 1997, **119**, 11130–11131.
9 A. Dussy, E. Meggers and B. Giese, *J. Am. Chem. Soc.*, 1998, **120**, 7399–7403.
10 B. Giese, *Acc. Chem. Res.*, 2000, **33**, 631–636.
11 A. C. Bryant-Friedrich, *Org. Lett.*, 2004, **6**, 2329–2332.
12 Q. Zhang and Y. Wang, *J. Am. Chem. Soc.*, 2003, **125**, 12795–12802.
13 K. Miaskiewicz and R. Osman, *J. Am. Chem. Soc.*, 1994, **116**, 232–238.
14 A. O. Colson and M. D. Sevilla, *J. Phys. Chem.*, 1995, **99**, 3867–3874.
15 M. Dizdaroglu, D. Schulte-Frohlinde and C. Von Sonntag, *Int. J. Radiat. Biol.*, 1977, **32**, 481–483.
16 M. Roginskaya, W. A. Bernhard, R. T. Marion and Y. Razskazovskiy, *Radiat. Res.*, 2005, **163**, 85–89.
17 M. Roginskaya, Y. Razskazovskiy and W. A. Bernhard, *Angew. Chem., Int. Ed.*, 2005, **44**, 6210–6213.
18 D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180–186.
19 D. S. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295–2316.
20 B. K. Goodman and M. M. Greenberg, *J. Org. Chem.*, 1996, **61**, 2–3.
21 J.-T. Hwang and M. M. Greenberg, *J. Am. Chem. Soc.*, 1999, **121**, 4311–4315.
22 C. Tronche, B. K. Goodman and M. M. Greenberg, *Chem. Biol.*, 1998, **5**, 263–271.
23 J.-T. Hwang, K. A. Tallman and M. M. Greenberg, *Nucleic Acids Res.*, 1999, **27**, 3805–3810.
24 Y. Zheng and T. L. Sheppard, *Chem. Res. Toxicol.*, 2004, **17**, 197–207.
25 K. A. Tallman, C. Tronche, D. J. Yoo and M. M. Greenberg, *J. Am. Chem. Soc.*, 1998, **120**, 4903–4909.
26 C. J. Emanuel, M. Newcomb, C. Ferreri and C. Chatgilialoglu, *J. Am. Chem. Soc.*, 1999, **121**, 2927–2928.
27 C. von Sonntag and H.-P. Schuchmann, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1229–1253.
28 J. M. Brown and L.-H. Wang, *Anti-Cancer Drug Des.*, 1998, **13**, 529–537.
29 D. J. Scott and K. S. Gates, *J. Am. Chem. Soc.*, 1996, **118**, 3380–3385.
30 J. S. Daniels, K. S. Gates, C. Tronche and M. M. Greenberg, *Chem. Res. Toxicol.*, 1998, **11**, 1254–1257.
31 J. T. Hwang, M. M. Greenberg, T. Fuchs and K. S. Gates, *Biochemistry*, 1999, **38**, 14248–14255.
32 M. Kotera, A.-G. Bourdat, E. Defrancq and J. Lhomme, *J. Am. Chem. Soc.*, 1998, **120**, 11810–11811.
33 C. Crey-Desbiolles, J. Lhomme, P. Dumy and M. Kotera, *J. Am. Chem. Soc.*, 2004, **126**, 9532–9533.
34 H. J. Lenox, C. P. McCoy and T. L. Sheppard, *Org. Lett.*, 2001, **3**, 2415–2418.
35 M. M. Meijler, O. Zelenko and D. S. Sigman, *J. Am. Chem. Soc.*, 1997, **119**, 1135–1136.
36 T. Chen and M. M. Greenberg, *J. Am. Chem. Soc.*, 1998, **120**, 3815–3816.
37 T. Oyoshi and H. Sugiyama, *J. Am. Chem. Soc.*, 2000, **122**, 6313–6314.
38 B. C. Bales, M. Pitié, B. Meunier and M. M. Greenberg, *J. Am. Chem. Soc.*, 2002, **124**, 9062–9063.
39 B. C. Bales, T. Kodama, Y. N. Weledji, M. Pitie, B. Meunier and M. M. Greenberg, *Nucleic Acids Res.*, 2005, **33**, 5371–5379.
40 O. D. Schärer, *Angew. Chem., Int. Ed.*, 2003, **42**, 2946–2974.
41 S. S. Parikh, C. D. Mol, D. J. Hosfiield and J. A. Tainer, *Curr. Opin. Struct. Biol.*, 1999, **9**, 37–47.
42 S. S. David and S. D. Williams, *Chem. Rev.*, 1998, **98**, 1221–1261.
43 T. Lindahl, *Nature*, 1993, **362**, 709–715.
44 Y. K. K. Matsumoto, *Science*, 1995, **269**, 699–702.
45 M. Hashimoto, M. M. Greenberg, Y. W. Kow, J.-T. Hwang and R. P. Cunningham, *J. Am. Chem. Soc.*, 2001, **123**, 3161–3162.
46 K. M. Kroeger, M. Hashimoto, Y. W. Kow and M. M. Greenberg, *Biochemistry*, 2003, **42**, 2449–2455.
47 K. Makino and H. Ide, *J. Biol. Chem.*, 2003, **278**, 25264–25272.
48 E. C. Friedberg, G. C. Walker and W. Siede, *DNA Repair and Mutagenesis*, American Society for Microbiology, D. C. Washington, 1995.
49 Y.-j. Xu, M. S. DeMottt, J. T. Hwang, M. M. Greenberg and B. Demple, *DNA Repair*, 2003, **2**, 175–185.
50 M. M. Greenberg, Y. N. Weledji, J. Kim and B. C. Bales, *Biochemistry*, 2004, **43**, 8178–8183.
51 M. S. DeMott, E. Beyret, D. Wong, B. C. Bales, J.-T. Hwang, M. M. Greenberg and B. Demple, *J. Biol. Chem.*, 2002, **277**, 7637–7640.
52 L. F. Povirk and I. H. Goldberg, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**, 3182–3186.
53 J. S. Sung, M. S. Demott and B. Demple, *J. Biol. Chem.*, 2005, **280**, 39095–39103.
54 J. Stubbe and J. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107–1136.
55 M. Dizdaroglu, P. Jaruga, M. Birincioglu and H. Rodriguez, *Free Radical Biol. Med.*, 2002, **32**, 1102–1115.
56 A. Adhikary, D. Becker, S. Collins, J. Koppen and M. D. Sevilla, *Nucleic Acids Res.*, 2006, **34**, 1501–1511.

57 K. Hildenbrand and D. Schulte-Frohlinde, *Int. J. Radiat. Biol.*, 1989, **55**, 725–738.
58 K. Hildenbrand, G. Behrens and D. J. Schulte-Frohlinde, *J. Chem. Soc., Perkin Trans. 2*, 1989, 283–289.
59 D. G. E. Lemaire, E. Bothe and D. Schulte-Frohlinde, *Int. J. Radiat. Biol.*, 1984, **45**, 351–358.
60 M. R. Barvian and M. M. Greenberg, *J. Org. Chem.*, 1995, **60**, 1916–1917.
61 M. R. Barvian and M. M. Greenberg, *J. Am. Chem. Soc.*, 1995, **117**, 8291–8292.
62 M. M. Greenberg, M. R. Barvian, G. P. Cook, B. K. Goodman, T. J. Matray, C. Tronche and H. Venkatesan, *J. Am. Chem. Soc.*, 1997, **119**, 1828–1839.
63 K. N. Carter and M. M. Greenberg, *J. Am. Chem. Soc.*, 2003, **125**, 13376–13378.
64 K. N. Carter and M. M. Greenberg, *J. Org. Chem.*, 2003, **68**, 4275–4280.
65 I. S. Hong, K. N. Carter and M. M. Greenberg, *J. Org. Chem.*, 2004, **69**, 6974–6978.
66 M. R. Barvian, R. M. Barkley and M. M. Greenberg, *J. Am. Chem. Soc.*, 1995, **117**, 4894–4904.
67 M. R. Barvian and M. M. Greenberg, *Tetrahedron Lett.*, 1992, **33**, 6057–6060.
68 M. Newcomb, *Tetrahedron*, 1993, **49**, 1151–1176.
69 I. Janik, P. Ulanski, J. M. Rosiak and C. Von Sonntag, *J. Chem. Soc., Perkin Trans.*, 2000, **2**, 2034.
70 M. N. Schuchmann and C. Von Sonntag, *Z. Naturforsch.*, 1986, **42b**, 495.
71 Q. Zhang and Y. Wang, *J. Am. Chem. Soc.*, 2004, **126**, 13287–13297.
72 H. C. Box and J. C. Wallace, *Radiat. Res.*, 1995, **141**, 91.
73 H. C. Box, H. B. Patrzyc, J. B. Dawidzik, J. C. Wallace, H. G. Freund, H. Iijima and E. E. Budzinski, *Radiat. Res.*, 2000, **153**, 442–446.
74 G. L. Dianov, P. O'Neill and D. T. Goodhead, *Bioessays*, 2001, **23**, 745–749.
75 M. E. Lomax, M. K. Gulston and P. O'Neill, *Radiat. Prot. Dosim.*, 2002, **99**, 63–68.
76 M. Weinfeld, A. ResouliNia, M. A. Chaudhry and R. A. Britten, *Radiat. Res.*, 2001, **156**, 584–589.
77 T. J. Jenner, J. Fulford and P. O'Neill, *Radiat. Res.*, 2001, **156**, 590–593.
78 Q. Zhang and Y. Wang, *Chem. Res. Toxicol.*, 2005, **18**, 1897–1906.
79 H. Hong, H. Cao and Y. Wang, *Chem. Res. Toxicol.*, 2006, **19**, 614–621.
80 A. Romieu, S. Bellon, D. Gasparutto and J. Cadet, *Org. Lett.*, 2000, **2**, 1085–1088.
81 S. Bellon, D. Gasparutto, A. Romieu and J. Cadet, *Nucleosides, Nucleotides Nucleic Acids*, 2001, **20**, 967–971.
82 Q. Zhang and Y. Wang, *Nucleic Acids Res.*, 2005, **33**, 1593–1603.
83 A. S. Anderson, J. T. Hwang and M. M. Greenberg, *J. Org. Chem.*, 2000, **65**, 4648–4654.
84 I. S. Hong and M. M. Greenberg, *Org. Lett.*, 2004, **6**, 5011–5013.
85 I. S. Hong, H. Ding and M. M. Greenberg, *J. Am. Chem. Soc.*, 2006, **128**, 485–491.
86 I. S. Hong and M. M. Greenberg, *J. Am. Chem. Soc.*, 2005, **127**, 3692–3693.
87 M. Tomasz and Y. Palom, *Pharmacol. Ther.*, 1997, **76**, 73–87.
88 D. M. Noll, T. M. Mason and P. S. Miller, *Chem. Rev.*, 2006, **106**, 277–301.
89 J. T. Millard, M. F. Weidner, J. J. Kirchner, S. Ribeiro and P. B. Hopkins, *Nucleic Acids Res.*, 1991, **19**, 1885–1892.
90 J. M. Brown and W. R. Wilson, *Nat. Rev. Cancer*, 2004, **4**, 437–447.
91 H. Sugiyama and I. Saito, *J. Am. Chem. Soc.*, 1990, **112**, 6720.
92 G. P. Cook and M. M. Greenberg, *J. Am. Chem. Soc.*, 1996, **118**, 10025–10030.
93 H. Sugiyama, K. Fujimoto and I. Saito, *Tetrahedron Lett.*, 1996, **37**, 1805–1808.
94 N. A. P. Franken, C. V. Bree, M. A. T. Veltmaat, H. M. Rodermond, J. Haveman and G. W. Barendsen, *J. Radiat. Res.*, 2001, **42**, 179–190.
95 L. L. Ling and J. F. Ward, *Radiat. Res.*, 1990, **121**, 76–83.
96 I. S. Hong, H. Ding and M. M. Greenberg, *J. Am. Chem. Soc.*, 2006, **128**, 2230–2231.
97 I. S. Hong and M. M. Greenberg, *J. Am. Chem. Soc.*, 2005, **127**, 10510–10511.
98 R. Tang and K. Mislow, *J. Am. Chem. Soc.*, 1970, **92**, 2100–2104.
99 H. J. Reich, K. E. Yelm and S. Wollowitz, *J. Am. Chem. Soc.*, 1983, **105**, 2503–2504.
100 A. Krief and F. Lonez, *Tetrahedron Lett.*, 2002, **43**, 6255–6257.
101 J. Ye, J. G. Muller, W. Luo, C. L. Mayne and A. J. Shallop, *J. Am. Chem. Soc.*, 2003, **125**, 13926–13927.
102 R. P. Hickerson, F. Prat, J. G. Muller, C. S. Foote and C. J. Burrows, *J. Am. Chem. Soc.*, 1999, **121**, 9423–9428.
103 J.-L. Ravanat, P. Di Mascio, G. R. Martinez, M. H. G. Medeiros and J. Cadet, *J. Biol. Chem.*, 2000, **275**, 40601–40604.
104 P. C. C. Lee and M. A. J. Rodgers, *Photochem. Photobiol.*, 1987, **45**, 79–86.
105 W. M. Sharman, C. M. Allen and J. E. van Lier, *Methods Enzymol.*, 2000, **319**, 376–400.
106 D. E. Dolmans, D. Fukumura and R. Jain, *Nat. Rev. Cancer*, 2003, **3**, 380–387.

