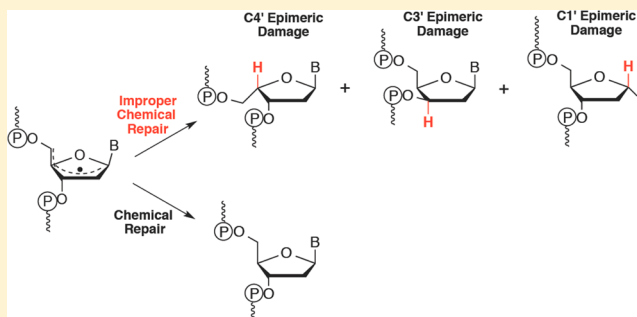# Epimeric 2-Deoxyribose Lesions: Products from the Improper Chemical Repair of 2-Deoxyribose Radicals

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ABSTRACT: Genomic integrity is constantly challenged by DNA damaging agents such as reactive oxygen species (ROS). Consequently, DNA damage can compromise the fidelity and efficiency of essential DNA metabolic processes, including replication and transcription, which may contribute significantly to the etiology of many human diseases. Here, we review one family of DNA lesions, the epimeric 2-deoxyribose lesions, which arise from the improper chemical repair of the 2-deoxyribose radicals. Unlike most other DNA lesions, the epimeric 2-deoxyribose lesions are indistinguishable from their corresponding unmodified nucleosides in both molecular mass and chemical reactivity. We placed our emphasis of discussion on the formation of these lesions, their impact on the structure and stability of duplex DNA, their biological consequences, their potential therapeutic relevance, and future research directions about these modified nucleosides.

CONTENTS

1. INTRODUCTION

Reactive oxygen species (ROS) are essential for many biological processes, including immune responses,[1] cell signaling,[2] neurotransmission,[3] etc. Despite the presence of enzymatic and nonenzymatic antioxidant defense mechanisms,[4−6] oxidative stress may persist in cells as a result of endogenously and exogenously generated ROS,[7] which may lead to damage to macromolecules including DNA, proteins and lipids.[8] Oxidative DNA damage is known to be associated with the onset of many human diseases including cancer and neurodegeneration.[9−11]

Damage to DNA may compromise genomic integrity. A variety of DNA lesions including modified nucleobases and 2-deoxyribose,[12,13] DNA strand breaks,[14] DNA−DNA cross-links,[15−19] and DNA−protein cross-links[18−20] can be produced. Some DNA lesions like phosphoglycoaldehydes and abasic sites are chemically reactive,[21−26] whereas lesions like the 8,5′-cyclopurine-2′-deoxynucleosides are not chemically reactive and exhibit elevated stabilities compared to the corresponding unmodified nucleosides.[27,28] Unrepaired DNA lesions are known to compromise the fidelity and/or efficiency of DNA-templated processes, including DNA replication and transcription.[29,30] Thus, identifying the DNA lesions generated, evaluating their impact on DNA replication and transcription, and investigating their repair are fundamental to gaining insights into their implications in human health.

Epimeric 2-deoxyribose lesions, a.k.a. "pseudorepair" or "inverted" lesions, may arise from the improper chemical repair of 2-deoxyribose radicals generated from ROS attack. Specifically, the hydroxyl radical can abstract a hydrogen atom from the 2-deoxyribose moiety to yield carbon-centered radicals.[31] In the presence of an H-atom donor, improper repair of these radicals formed at the C1′-, C3′-, and C4′-positions can result in the inversion of stereochemical configuration at these carbons (Scheme 1). In this review, we will summarize the research progress that has been made pertaining to the

Scheme 1. Generation of the Epimeric 2-Deoxyribose Lesions in DNA Resulting from Oxidatively Induced Damage
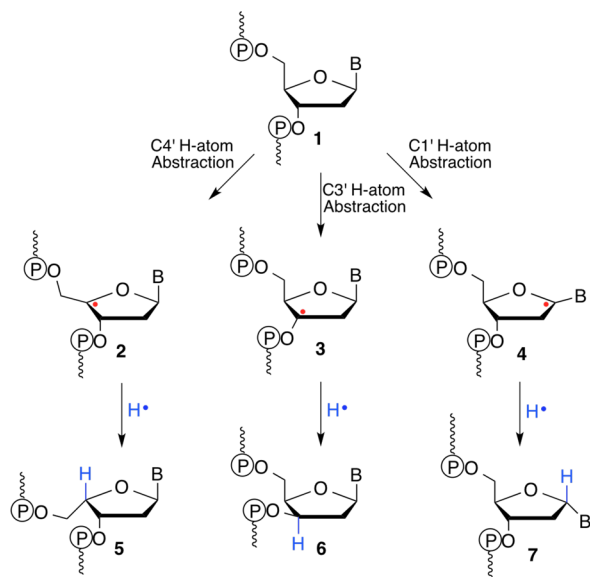
epimeric lesions of 2-deoxyribose in DNA. Areas to be addressed include the known formation of these lesions, their impact on stability and structure of duplex DNA, and their biological consequences. Additionally, we will discuss their potential therapeutic relevance, as well as future research directions about these modified nucleosides.

2. C1′-EPIMER

2.1. Formation. The C1′-epimers of 2′-deoxyadenosine (dA), 2′-deoxyguanosine (dG), and 2′-deoxyuridine (dU) were initially reported in nucleosides. Exposure of aqueous solutions of dA or dG to ionizing radiation under anoxic conditions resulted in the generation of 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine (α-dA) and 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)guanine (α-dG), respectively.[32,33] Apart from these purine nucleosides, 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)uracil (α-dU) was found to form from the selectively generated C1′-radical of 2′-deoxyuridine.[34] It is anticipated that both 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-

thymine (α-dT) and 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-cytosine (α-dC) can also emanate from oxidative damage.

The inversion from the β- to α-anomer at the C1′-position of dA in DNA was initially reported when poly(dA), poly(dA-dT), or salmon testis DNA was exposed to γ rays *in vitro*.[35] With the exception of the loss of adenine (which displayed a 3-fold higher level of occurrence relative to α-dA), the formation of this C1′-epimer was identified as a major lesion of dA in DNA under anaerobic conditions. Yields (based on the total number of dA residues subjected to damaging conditions) of α-dA were 1.3−1.5% for all sequences. Other lesions of dA and their respective yields are (5′R)-8,5′-cyclo-2′-deoxyadenosine (0.05−0.21%), 8-hydroxyadenine (0.03−0.10%), and 8-hydroxy-2′-deoxyadenosine (0.07%). Considering that 8,5′-cyclo-2′-deoxyadenosine has been observed previously in cells and tissues,[36−38] it is reasonable to predict that α-dNs should also be formed at appreciable levels *in vivo*. Intriguingly, Lesiak and Wheeler showed that α-dA can occur readily in the absence of thiols, which was proposed to result from either the addition of a hydrated electron followed by protonation and/or disproportionation reactions.[35]

Relative to the DNA damage induced by γ rays, exposure of dA and 2′-deoxyadenosine-5′-monophosphate with γ rays resulted in significantly less amounts of α-dA relative to ODNs,[35,39] indicating that the polynucleotide structure enhances the formation of α-dA. Apart from α-dA, α-dU was identified in ODNs as a result of selective oxidative damage.[40,41] Currently, the formation of α-dG, α-dC, or α-dT in DNA remains to be determined.

The C1′-epimers were proposed to form from 2-deoxyribose damage occurring at the C4′- and/or C1′-position (Scheme 2). Originally, Mariaggi et al.[32] proposed that α-dA results from the C4′-radical (**2**) through the hydroxyl radical-mediated hydrogen abstraction from this carbon. Through this mechanism, a Schiff base (**9**) is formed via ring opening of the sugar moiety facilitated by the protonation of the furanose oxygen. This leads to the generation of the C1′-cation (**10**), immediately followed by the reconstitution of the pentofuranosyl moiety in the presence of an H-atom donor, generating the two C1′-epimers (**1** and **7**).[32] This mechanism for α-dA formation is supported by the presence of two other dA damage products also proposed to originate from the C4′-radical, 9-(2-deoxy-α-D-*erythro*-pentopyranosyl)adenine and 9-(2-deoxy-β-D-*erythro*-pentopyranosyl)adenine, which require a free 5′-hydroxyl

Scheme 2. Proposed Mechanisms for the Formation of the C1′-Epimeric Lesions[32,45]
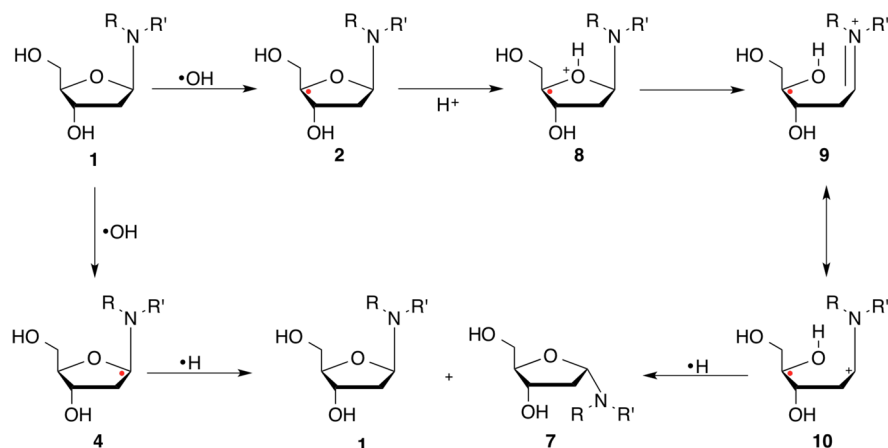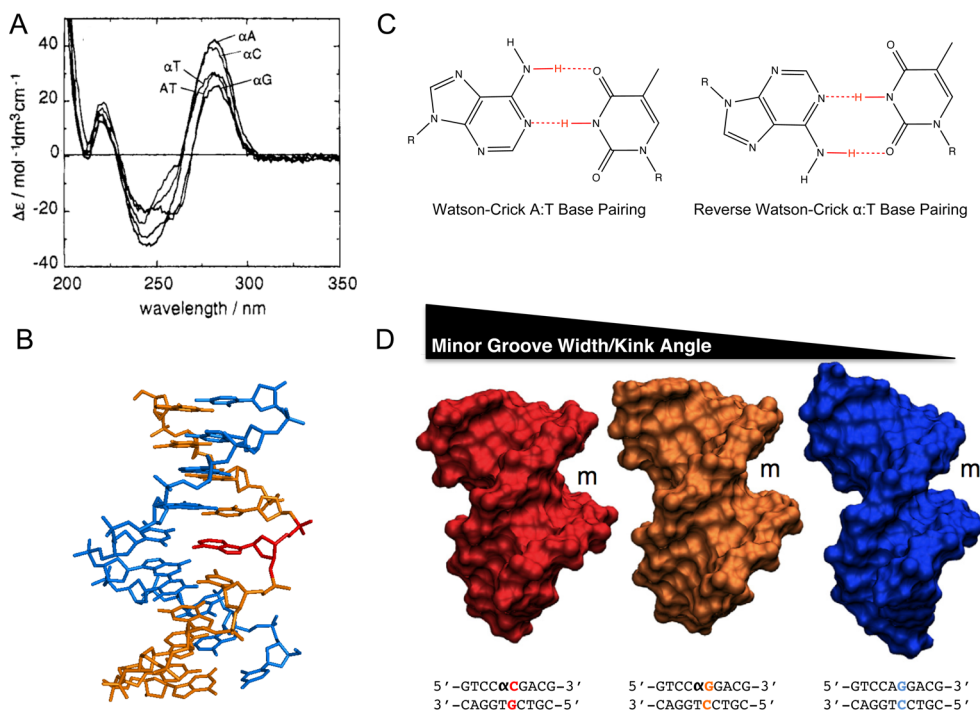
Figure 1. Structural characteristics of double-stranded DNA containing a single α-dA lesion. (A) CD spectra of double-stranded DNA revealing the impact of the α-dA on DNA secondary structure.[47] The global B-form DNA conformation observed for the unmodified duplex (AT) is maintained for duplexes containing the α-dA lesion being paired with the correct (dT) and incorrect (dA, dC, and dG) nucleosides; α-dA is represented by α. (B) NMR solution structure of an α-dA-containing 10-mer duplex DNA demonstrating the B-form duplex conformation.[48] The position of α-dA is indicated in red. PDB ID: 1S0T. (C) Comparison between Watson−Crick base pairing interactions of A and T normally observed in B-form duplex DNA and the reverse Watson−Crick base pairing interaction observed between α-dA and dT in the NMR solution structure.[48] (D) Structural comparison highlighting the impact of the nearest-neighbor base pair on the minor-groove width and kink angle introduced by α-dA.[49] Duplexes (red and orange) containing the α-dA lesion were solved by NMR, while the unmodified duplex (blue) is a model. The minor groove is indicated by m, while the position of α-dA is indicated by α. The nearest-neighbor base pair altered is highlighted in red, orange, or blue, respectively. Panel A was reprinted from ref 47. Copyright 1995 American Chemical Society. Panel D was adapted with permission from ref 49. Copyright 2012 Elsevier.

group for the pentopyranosyl ring formation.[32] However, selectively generated C4′-radicals of dA and dT did not give rise to the formation of α-dA or α-dT, respectively,[42−44] suggesting that the C4′-radical is unlikely to be a precursor for the formation of the C1′-epimers. Instead, the epimerization of the C1′-position is more likely attributed to the formation of the C1′-radical (4);[45,46] indeed, both α-dU and β-dU were observed to form from the independently generated 2′-deoxyurid-1′-yl radical.[34,41]

2.2. Impact on DNA Stability and Structure. The impact of the α-anomer on the thermodynamic stability of duplex DNA containing 2′-deoxynucleosides with the normal 3′-5′ phosphodiester linkage has only been characterized for α-dA. This was accomplished by performing UV melting temperature measurements of a 9-mer duplex with a site-specifically incorporated α-dA being paired with the correct (dT) or incorrect nucleoside (dA, dG, or dC).[47] In this report, the measured T_m values were employed to gauge the impact of α-dA on ΔG, ΔH and ΔS by assuming a two-state transition model. The impact of α-dA on duplex stability was found to vary with the pairing nucleoside of α-dA. The presence of a single α-dA·dT base pair was found to increase slightly the duplex stability (ΔT_m of 0.6 °C) relative to the canonical dA·dT base pair,[47] whereas the α-dA·dT base pair was observed to be destabilizing in other studies.[48,49] Discrepancies among these findings might be attributed to the different sequence contexts and/or duplex lengths used in these studies. This notion is supported by a 4.4 °C range (49.0−53.4 °C) in melting

temperature (i.e., duplex stability) introduced by changing the nearest neighbor base-pairs.[49] In the case of the incorrect base pairings of α-dA (α-dA·dA, α-dA·dG, and α-dA·dC), significant destabilization was observed, as reflected by a drop in T_m values by 7.2−11.0 °C and a rise in $\Delta G_{25\ °C}$ values by 1.67−2.64 kcal/mol.[47] The α-dA·dG pairing resulted in the largest degree of destabilization to duplex DNA ($\Delta T_m = -11.0$ °C, $\Delta\Delta G_{25\ °C} = 2.64$ kcal/mol), whereas lower extents of destabilization were found for the α-dA·dC ($\Delta T_m = -7.4$ °C, $\Delta\Delta G_{25\ °C} = 1.78$ kcal/mol) and α-dA·dA ($\Delta T_m = -7.2$ °C, $\Delta\Delta G_{25\ °C} = 1.67$ kcal/mol) mispairs.[47] Aside from α-dA, the impact of α-dG, α-dT, and α-dC on duplex stability was evaluated for duplexes containing two α-dNs with unconventional phosphodiester linkages (3′-3′ and 5′-5′).[50] In that study, α-dC was found to be significantly more destabilizing ($\Delta T_m = -10.0$ °C) than α-dA, α-dG, and α-dT, which all exhibit similar effects (average $\Delta T_m = -5.3$ °C).[50] The effect of α-dG, α-dC, and α-dT on duplex stability for the conventional 3′-5′ phosphodiester linkage remains to be determined.

Circular dichroism (CD) experiments demonstrated that the global conformation of duplex DNA harboring an individual α-dA remains in B-form (Figure 1A).[47] In addition, the NMR solution structure of duplex DNA containing an α-dA·dT pair revealed local changes in duplex structure (Figure 1B),[48] where α-dA participates in both reverse Watson−Crick base-pairing with dT and base-stacking interactions with the neighboring nucleobase (Figure 1C). Furthermore, α-dA was observed to introduce a kink at the modified nucleoside site and result in an

expansion of the minor groove by 18°.[48] Overall, these structural perturbations were found to vary with the sequence context (Figure 1D).[49]

2.3. Biological Consequences. So far, replication studies have been conducted only for α-dA. In this respect, α-dA was found to stall the Klenow fragment *in vitro*, though bypass could be observed after prolonged incubation.[51] Maxam–Gilbert sequencing and primer extension assays indicate that α-dA directs the incorporation of both correct (dTMP) and incorrect (dCMP, dAMP and dGMP) nucleotides, with the misincorporation of dGMP being significantly disfavored. In addition, promutagenic properties and bypass efficiency of α-dA were found to be sequence-dependent, particularly by the nearest neighbor base pair but independent of the exonuclease activity of the polymerase. In addition, similar results were obtained for T7 and Taq DNA polymerases as well as a reverse transcriptase.[51]

Replication studies with the use of α-dA-bearing, single-stranded M13 bacteriophage showed that α-dA constitutes a moderate block to DNA replication and introduces single nucleotide deletion in *E. coli* cells.[52] Although nucleotide misincorporation was not found *in vivo*, the frequency of single-nucleotide deletion was observed to be sequence-dependent, with mutation frequencies ranging from 1 to 26%.[52] A misinsertion-strand-slippage mechanism was thought to contribute to the generation of single-nucleotide deletions.[52] The impact of α-dT, α-dG, and α-dC on DNA replication, however, remains to be investigated.

Some *in vitro* repair experiments were conducted for both α-dA and α-dT. The C1′-epimeric lesions were found to be substrates for repair enzymes of bacteria, yeast, and humans. For *E. coli*, a wide range of repair enzymes were evaluated, including multiple endonucleases (i.e., endonucleases III, IV, VIII, and deoxyinosine-3′-endonuclease), exonuclease III, and formamidopyrimidine *N*-glycosylase.[53] Among these enzymes, endonuclease IV was the only repair enzyme capable of recognizing both α-dA and α-dT.[53,54] In addition, this endonuclease activity was selective for dsDNA substrates, as the corresponding ssDNA constructs could not be cleaved by the enzyme.[53] Apart from the *E. coli* enzymes, *S. cerevisiae* apurinic/apyrimidinic (AP) endonuclease 1 (Apn1) and human AP endonuclease I (Ape1) could also recognize α-dA and α-dT.[54,55] Together, these reports suggest that the repair pathway of α-dA and α-dT is conserved among species and that α-dG and α-dC are likely substrates for the same repair pathway. On the grounds that the α-dNs are not recognized by DNA glycosylases in the base excision repair (BER) pathway,[53] it has been proposed that these C1′-epimeric lesions are repaired by the nucleotide incision repair (NIR) pathway,[55] an alternative pathway to BER.[56]

3. C3′-EPIMER

3.1. Formation. The inversion of configuration at the C3′-position results in the generation of the C3′-epimeric lesions, i.e., 1-(2′-deoxy-R-L-*threo*-pentofuranosyl)-containing nucleosides, commonly referred to as xylose nucleosides. These types of lesions were first observed to form from the independently generated C3′-radicals of nucleosides in the presence of an H-atom donor.[57] The C3′-epimeric lesion was observed to form in equal amounts as the properly chemically repaired C3′-epimer of 2-deoxyribose.[57] In addition, 1-(2′-deoxy-R-L-*threo*-pentofuranosyl)thymine (dxT) was found to form in ODNs from the independently generated C3′-radical

under anaerobic conditions,[58] where dxT was produced more readily in model replication-relevant architectures (ssDNA and 5′-overhang) than in dsDNA.[58] Moreover, the formation of the C3′-epimeric lesion competes directly with strand break formation under anaerobic conditions (Scheme 3).[58,59] Future investigations about the *in vivo* formation of dxT and other dxNs will provide valuable insights into the physiological relevance of the C3′-epimeric lesions.

Scheme 3. Fate of the C3′-Radical in Anoxic Conditions[a]
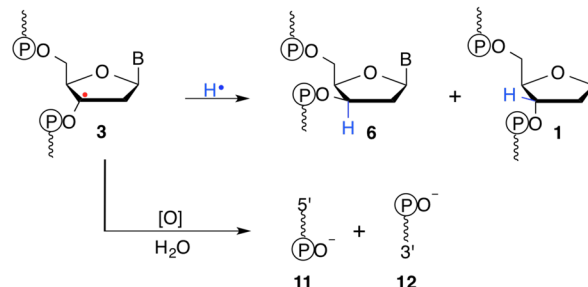
[a]Formation for the C3′-epimeric lesion and competing strand break pathway resulting from oxidation ([O]) in aqueous solution.

3.2. Impact on DNA Stability and Structure. Alterations in both DNA stability and structure have been reported for ODNs containing a single dxN lesion. Primarily, these studies have been performed using UV melting analysis, circular dichroism (CD) measurements, and molecular modeling. It has been reported that the presence of a single dxT in double-stranded DNA affects both DNA stability and structure, though the duplex maintains B-DNA geometry.[60,61] In model DNA replication-relevant architectures (fork, 5′-overhang, and 3′-overhang), dxT was more destabilizing and introduced greater structural alterations when located closer to the 3′-terminus of the damaged ODN.[60] The presence of an individual dxC or dxG in duplex DNA also led to a significant decrease in duplex stability.[62,63] Initial structural characterizations of all four dxNs individually, as well as the structural analysis of fully xylose duplex DNA, indicate that the sugar pucker of the dxNs adopts the C3′-endo conformation, resembling that of ribonucleosides (Figure 2).[62,64] Together, these reports suggest that the presence of an isolated dxN may perturb the stability and structure of duplex DNA *in vivo*.

3.3. Biological Consequences. Investigations about the impact of the C3′-epimeric lesions on biological processes are limited. Along this line, primer extension assays were conducted
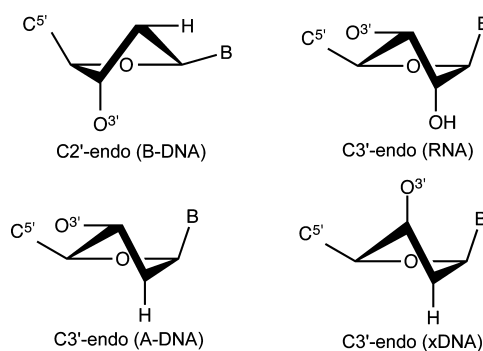

Figure 2. 2-Deoxyribose sugar puckers observed in duplex DNA (B and A form),[93,94] RNA,[94] and for dxNs.[62,64]

to assess the ability of DNA polymerases to incorporate 2′-deoxyxyloadenosine triphosphate (dxATP) and/or 2′-deoxyxylothymidine triphosphate (dxTTP) into DNA *in vitro*.[64] It was found that several different DNA polymerases were capable of incorporating dxNTPs into the elongating strand. Notably, complete primer extension was not observed,[64] suggesting that the presence of the dxN may significantly inhibit DNA replication *in vivo*. Provided that the sugar pucker of the dxNs prefers the C3′-endo conformation, it is likely that the lack of complete primer extension is a result of the structural effects of the dxN lesion. Future investigations seeking to obtain detailed structural data, as well as experiments measuring the bypass efficiencies of the dxN lesions, will significantly expand the current knowledge about the impact of these lesions on DNA replication.

The effects of dxNs on restriction endonuclease activity was also examined.[65] Endonuclease activity on DNA substrates containing dxC and/or dxT was observed to vary among enzymes and positions of the dxN. *Eco*RII was incapable of cleaving the DNA backbone with the presence of dxNs at the restriction recognition site, while the activity of *Mva*I was variable and overall significantly more tolerant of the presence of the lesion than *Eco*RII.[65]

4. C4′-EPIMER

4.1. Formation. The C4′-epimeric lesions of dT and dA were found to be generated *in vitro* in single- and double-stranded DNA from independently generated radicals,[66] while the C4′-epimer of dG could be induced in an aqueous solution of dG upon exposure to γ-rays.[33] Similar to the C3′-epimeric lesion, the formation of the C4′-epimer competes with strand break generation (Scheme 4).[66] The stereoselectivity of **5**

Scheme 4. Fate of the C4′-Radical in Anoxic Conditions[a]
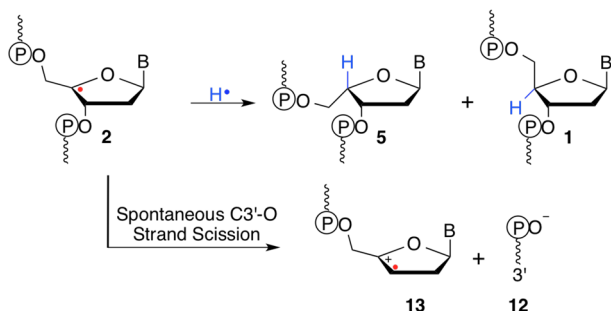
[a]The formation for the C4′-epimeric lesion (**5**) and competing strand break pathway arising from spontaneous strand scission. Formation of the radical cation (**13**) was previously confirmed in nucleosides.[43]

versus **1** was observed to be directly influenced by the DNA structure, with significantly greater levels of **5** being generated in ssDNA than dsDNA. Nevertheless, the levels of **5** were reported not to be affected by either the source of H-atom donor or the identity of the nucleoside (dT or dA) from which the radical is generated,[66] suggesting that the C4′-epimeric lesions may be produced in DNA at similar levels for the four canonical 2′-deoxynucleosides. Oxidative damage in ssDNA in the presence of oxygen resulted in the epimeric mixtures of **5** and **1**, with yields (1−51%) varying with the concentrations of H-atom donor.[67] Currently, the formation of these C4′-epimeric lesions *in vivo* remains to be determined.

4.2. Impact on DNA Stability and Structure. Investigations about the impact of the C4′-epimeric lesions on DNA stability are minimal, with no reports characterizing structural changes. This is likely due to the lack of facile synthetic routes to obtaining these C4′-epimers. The presence of the C4′-epimer of dA in the duplex portion of a dangling end substrate was reported to be destabilizing, as reflected by a decrease in melting temperature of 1.2 °C.[68] The C4′-epimer of dA may not disrupt Watson−Crick base-pairing interactions based on the fidelity of nucleotide incorporation opposite this lesion, as discussed in section 4.3 below;[68,69] nevertheless, it remains to be assessed whether the same findings can be extended to the C4′-epimers of other nucleosides.

4.3. Biological Consequences. Not much is known about the biological consequences of the C4′-epimeric lesions with the exception of the studies assessing how this lesion affects DNA replication. Primer extension assay with the use of the Klenow fragment revealed that the C4′-epimer of dA introduced a stop-site one nucleotide downstream of the lesion, though it could be bypassed after prolonged incubation.[69] In addition, this bypass was independent of the exonuclease activity of the polymerase. Intriguingly, the levels of incorporation of the incorrect dNTPs were found to be decreased relative to the undamaged substrate.[69] Thus, the presence of the C4′-epimeric lesion of dA was found not to be mutagenic *in vitro* but may be cytotoxic as reflected by the stalling of the Klenow fragment of DNA polymerase I.[69]

With respect to DNA repair, the ability of the NER pathway to repair the C4′-epimer of dA was investigated *in vitro* with the use of human cell extracts.[68] In duplex DNA, the lesion was found not to be a substrate for human NER when it is paired with a dT; however, it could be recognized by the human NER machinery when it is present in mispairs.[68] Therefore, these results suggest that the C4′-epimer of dA formed in A·T base pairs is unlikely a substrate for the NER pathway. It remains to be examined whether the C4′-epimers of this and other nucleosides can be repaired by other pathways including BER and NIR.

5. PHYSIOLOGICAL RELAVANCE

Currently, none of the epimeric 2-deoxyribose lesions have been detected *in vivo*. Given the literature precedence in identifying the formation of these lesions *in vitro* at all three stereocenters (C1′, C3′, and C4′) of 2-deoxyribose, it is highly likely that these purely structural lesions can be generated *in vivo* from oxidative DNA damage.

The presence of an H-atom donor source facilitates the formation of these epimeric DNA lesions. As observed at both the C1′- and C4′-positions, the nature of the H-atom donor does not impact lesion formation.[40,66] In cells, there are a variety of potential H-atom donors that are readily available, with glutathione (GSH) being the major antioxidant in the nucleus, cytosol, and mitochondria.[70] Upon the formation of the 2-deoxyribose radicals, the presence of O_2 readily competes with H-atom donors via generating peroxyl radicals of 2-deoxyribose, which can also be reduced by H-atom donors. Thus, it is anticipated that the greatest levels of these epimeric lesions are generated under conditions and/or microenvironments where O_2 levels are low and H-atom donors are prevalent. For example, the nucleus is known to contain relatively low concentrations of O_2[71] and adequate concentrations (3−15 mM) of GSH,[70] which was previously demonstrated to facilitate the formation of these lesions.[58,66,67]
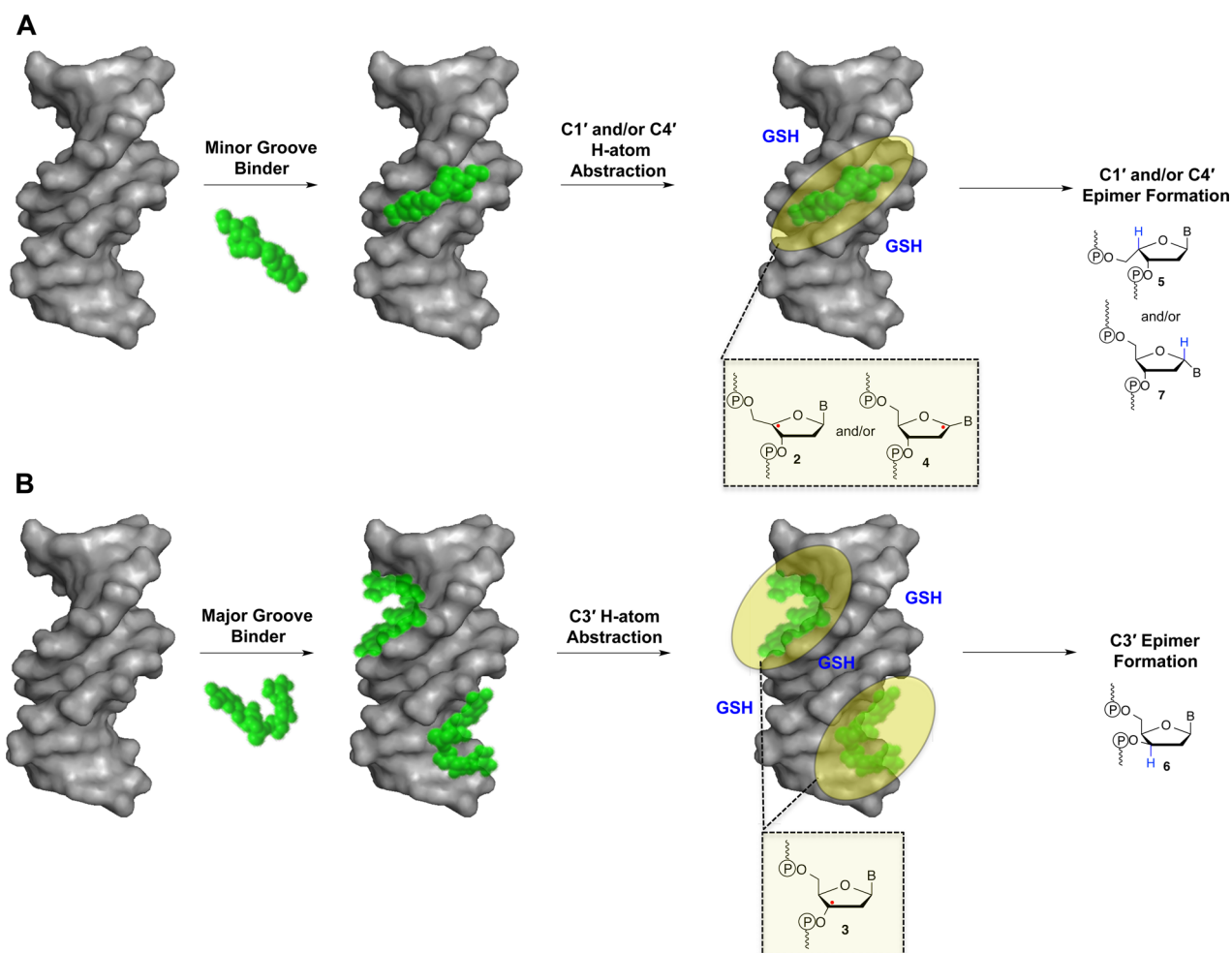
Figure 3. Potential formation of the epimeric 2-deoxyribose lesions resulting from DNA binding agents. (A) Proposed formation of the C1′- and C4′-epimers resulting from the H-atom abstraction by minor-groove binders. (B) Proposed formation of the C3′-epimers resulting from the C3′-radical generation by major-groove binders.

In the case of cancer tissues, which are often hypoxic[72] and resistant to radiation and chemotherapeutic treatments,[73] these lesions could also be generated. The potential for these epimeric lesions to form under aerobic conditions *in vivo* is also likely (but expected to be produced at significantly lower levels) as demonstrated *in vitro* at the C4′-position.[67]

6. POTENTIAL THERAPEUTIC IMPLICATIONS

Several anticancer and antiviral drugs, as well as artificial metal nucleases, function by inducing strand breaks via H-atom abstraction from the 2-deoxyribose moiety.[74,75] The same 2-deoxyribose radicals were previously found to yield the epimeric lesions of 2-deoxyribose. The specific 2-deoxyribose radicals generated are dictated by both DNA structure and DNA-oxidizer-binding interactions.[75] In B-DNA, both the C1′ and C4′ H-atoms reside in the minor groove, whereas the C3′ H-atom is located in the major groove.[76] Therefore, drugs that bind to the minor groove and generate the C1′- and/or C4′-radical, as demonstrated by some enediynes and bleomycins,[75,77,78] may foster the inversion of stereochemistry at the C1′- and C4′-positions (Figure 3A). Given that these drugs bind to the minor groove of DNA, accessibility of the H-atom donor to the α face of the C1′- and C4′-radicals via the minor groove is likely inhibited, potentially facilitating the improper chemical repair of the 2-deoxyribose radicals by cellular thiols.

To our knowledge, the formation of the C1′- and/or C4′-epimers from enediynes or bleomycins in the presence of thiols has not been reported. Similarly, major-groove binders known to generate the C3′-radical, such as rhodium complexes,[75,79] may induce the preferential formation of the C3′-epimeric lesions (Figure 3B). In this case, the H-atom donor may only have access to the α face of the C3′-radical through the minor groove, while the proper chemical repair from the β face may be inhibited by the presence of the DNA-binding agent targeting the major groove.

In considering the potential generation of these lesions by chemotherapy or radiation therapy, a probable scenario may reside in hypoxic environments. In this regard, hypoxia is known to confer resistance to chemotherapeutics and radiation therapy, where depleted O_2 levels could decrease directly the toxicity of many therapeutics and serve as a signal for hypoxia-inducible transcription factors.[80−82] We reason that the drug resistance manifested during hypoxia could also be attributed, in part, to the formation of these epimeric 2-deoxyribose lesions facilitated by cellular thiols, which competes directly with the formation of the intended DNA strand breaks. In this regard, the formation of these lesions would prevent the generation of the DNA strand breaks, which is a toxic effect of many therapeutic agents targeting cancer cells. Consequently, any biological activity associated with the generation of the epimeric

2-deoxyribose lesions may be masked by the already abnormal activity of the cancer cells. As a result, the formation of these epimeric lesions may be generated as an alternative route for cancer cell survival during hypoxia, preventing lethal strand scissions at the expense of the biological consequences introduced by the epimeric lesions. Thus, the formation of these epimeric 2-deoxyribose lesions in cells upon exposure to chemotherapeutic agents and ionizing radiation under normoxic and hypoxic conditions merits future investigations. The outcome of such studies may provide molecular insights into hypoxia-induced resistance to chemotherapy and radiation therapy. Additionally, such studies may unveil the impact of the cellular environment on the generation of these lesions.

7. PROSPECTS AND LIMITATIONS

The potential formation of the aforementioned epimeric DNA lesions *in vivo* is well prefaced with the reported *in vitro* literature. Demonstrating the formation of these lesions *in vivo* is essential for expanding the current scope of their investigations. This endeavor is challenged by the lack of alterations in chemical functional groups introduced by these lesions, necessitating a structural and/or enzymatic approach for their identification *in vivo*. Antibodies targeting ODNs containing only the C1′ α-anomers were previously reported.[83] These antibodies, however, may not recognize a single isolated C1′ α-anomer as generated from oxidative DNA damage. Thus, it would be interesting to develop antibodies that can be employed for recognizing an individual C1′ α-anomer or the C3′ or C4′ 2-deoxyribose epimers in DNA. Another approach would be to utilize enzymatic digestions coupled with LC-MS/MS analysis. This would necessitate optimized methods to facilitate the chromatographic separation and/or mass spectrometric differentiation of these epimeric lesions from their unmodified counterparts. Regardless of the approach, method development for assessing the formation of these lesions *in vivo* will require them to be synthetically available. The syntheses of the C1′- and C3′-epimers have been reported for all four dNs.[63,84−92] In the case of the C4′-epimers, available synthetic routes are currently limited to dA, which is generated utilizing a modified radical precursor.[69] It should be noted that the generation of epimeric nucleosides via radical intermediates is accompanied with a high risk of byproduct formation. As a result, this approach may offer the limited yield for the desired epimer. Thus, the development of facile synthetic methods for generating the full spectrum of C4′-epimeric lesions will directly broaden the experimental scope of future investigations and enable the assessment about the *in vivo* formation of these epimeric lesions.

Future studies evaluating the impact of these lesions on processes such as DNA replication, transcription, and repair will yield insightful results on the biological consequences for the formation of these lesions. Currently, *in vivo* studies are limited to the C1′-epimeric lesion of dA in the *E. coli* system.[52] If these lesions are in fact formed *in vivo*, understanding the repair of these lesions and the effects of these lesions on DNA replication and transcription in mammalian cells should be pursued. Additionally, it is possible that these lesions are formed *in vivo* under hypoxic conditions, in which case they may be utilized as biomarkers of hypoxia.

8. CONCLUSIONS

In this review, we summarized our current knowledge of the epimeric 2-deoxyribose lesions in DNA, which are the improper chemical repair products of the C1′-, C3′-, and C4′-radicals of 2-deoxyribose. Particularly, we have reviewed relevant literature highlighting the known formation of these lesions, their impact on the stability and structure of the DNA double helix, and their biological consequences. As discussed above, some of these epimeric 2-deoxyribose lesions have been observed to be generated in DNA from either γ radiation and/or independently generated radicals *in vitro*, while their *in vivo* presence remains elusive. In addition, it has been observed that the presence of an individual epimeric 2-deoxyribose lesion in DNA destabilizes the DNA double helix and introduces local structural alterations in DNA, which has been demonstrated to vary with DNA sequence context and architecture. Some of these lesions also compromise DNA replication, being promutagenic or potentially cytotoxic.

Existing literature has offered significant precedence for the potential formation of the epimeric 2-deoxyribose lesions *in vivo*, as well as an initial understanding of the potential biological consequences that they may exert, while much remains to be learned for this family of DNA lesions. In particular, determining if these DNA lesions form for all four nucleosides and measuring their respective levels of formation *in vitro* and *in vivo* will not only broaden our scope of knowledge for these DNA lesions but also unveil whether and how the fate of the 2-deoxyribose radicals is influenced by the nature of the nucleobase. Moreover, future studies addressing the impact of these lesions on DNA replication and transcription and how they are repaired in mammalian cells will offer important knowledge for understanding the implications of these lesions in human diseases.

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Notes

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■ ABBREVIATIONS

BER, base excision repair; CD, circular dichroism; dA, 2′-deoxyadenosine; α-dA, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)-adenine; dC, 2′-deoxycytidine; α-dC, 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)cytosine; dxC, 1-(2′-deoxy-R-L-*threo*-pentofuranosyl)cytosine; dG, 2′-deoxyguanosine; α-dG, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)guanine; dxG, 1-(2′-deoxy-R-L-*threo*-pentofuranosyl)guanine; dNs, 2′-deoxyribonucleosides; dT, thymidine; α-dT, 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-thymine; dxT, 1-(2′-deoxy-R-L-*threo*-pentofuranosyl)thymine; dxN, 1-(2′-deoxy-R-L-*threo*-pentofuranosyl)-containing nucleoside; dU, 2′-deoxyuridine; α-dU, 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)uracil; dsDNA, double-stranded DNA; dNTPs, 2′-deoxyribonucleoside triphosphates; dxATP, 2′-deoxyxyloadenosine triphosphate; dxTTP, 2′-deoxyxylothymidine triphosphate; dxNTPs, 2′-deoxyxylonucleoside triphosphates; DNA, deoxyribonucleic acid; GSH, glutathione; NIR,

nucleotide incision repair; ROS, reactive oxygen species; ssDNA, single-stranded DNA; UV, ultraviolet

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