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Base Pair Stability of 8-Chloro- and 8-Iodo-2′-deoxyguanosine Opposite 2′-Deoxycytidine: Implications Regarding the Bioactivity of 8-Oxo-2′-deoxyguanosine

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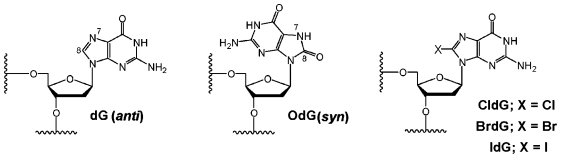
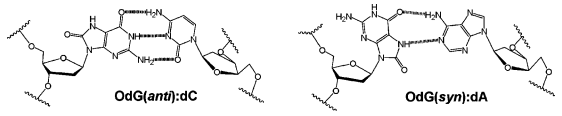
Reactive oxygen species, which are formed by solar radiation, chemical carcinogens, and normal aerobic metabolism, produce a number of complex lesions in DNA, the most common of which is oxidation at the C8 position of 2′-deoxyguanosine (dG) to form 8-oxo-2′-deoxyguanosine (OdG).[1] OdG is known to be mutagenic,[1] and it has been linked to aging[2] and several diseases, including cancer.[3]



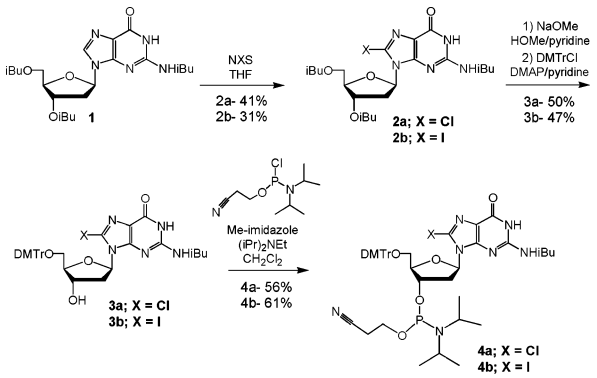
Though OdG can exist in alternate tautomeric states, the 6,8-diketo form predominates at physiological pH.[4] In this form, N7 becomes a hydrogen bond donor; C8 becomes a hydrogen bond acceptor, and the most stable conformation about the glycosidic bond changes from *anti* (as in dG) to *syn*.[5] When incorporated into oligonucleotides, OdG retains its 6,8-diketo structure, but its glycosidic bond conformation depends on the identity of its base pair partner. Unlike unmodified dG, OdG can form stable base pairs with both dC and dA. When pairing to dC, the unfavored *anti* orientation is required,[4a] and the base pair is destabilized as compared to a dG:dC base pair.[6]



When paired to dA, however, OdG adopts the favored *syn* conformation and uses its Hoogsteen edge to hydrogen bond with the Watson−Crick face of dA.[7] It has been shown that OdG(*syn*):dA base pairs are only slightly less stable than OdG(*anti*):dC base pairs, despite having one less hydrogen bond.[6] Since the dual base pairing ability of OdG is believed to be responsible for mutation and possibly the link between OdG and aging and disease, it is of great importance to fully understand the exact structural and electronic properties of OdG that lead to the relatively similar stabilities of OdG(*anti*):dC and OdG(*syn*):dA base pairs.

To this end, it has been proposed that the instability of OdG:dC base pairs relative to dG:dC base pairs may be due, at least in part, to the steric bulk of the 8-oxygen. It has been reasoned that when in the *anti* conformation the 8-oxygen is in steric clash with the connected deoxyribose sugar, thus destabilizing the base pair overall.[4] However, this rationale has its critics,[8] and the question remains somewhat controversial. To address the effect of C8 steric bulk on the stability of dG:dC base pairs, we synthesized analogues of dG that contained halogens of increasing size (chlorine, bromine, and iodine) at the C8 position. We then incorporated these analogues into DNA and determined their relative base pair stabilities when opposite dC.

Scheme 1



Though 8-bromo-2′-deoxyguanosine (BrdG) has been known for some time,[9] efficient syntheses of 8-chloro-2′-deoxyguanosine (CldG) and 8-iodo-2′-deoxyguanosine (IdG) derivatives have not been reported. Previous methods of chlorination and iodination at the C8 position of purines have included reactions with lithium diisopropylamine and tosyl chloride[10] and *N*-iodosuccinamide (NIS) in DMSO,[11] respectively. Unfortunately, none of these procedures proved effective with dG. However, since the iodination procedure was similar to the well-established route to BrdG[9] (where dG is reacted with *N*-bromosuccinamide in water), modifications of the procedure were tested for the synthesis of CldG and IdG. After much examination, good reactivity was found when THF was used as the solvent and a protected dG derivative was used as the reagent. Thus, the triisobutyrylated dG derivative **1** was reacted with *N*-chlorosuccinamide (NCS) in THF for 2 days at room temperature to yield the CldG derivative **2a**, or NIS in THF for 3 days at 35 °C to yield the IdG derivative **2b** (Scheme 1). The triisobutyrylated dG derivative was used not only because it was soluble in THF but also because isobutyryl (iBu) groups are the standard protecting group for the exocyclic amine during solid phase synthesis of DNA. To further prepare CldG and IdG for DNA synthesis, the isobutyryl ester protecting groups were selectively removed with sodium methoxide before protection of the 5′-oxygen as a dimethoxytrityl (DMTr) ether. Finally, the 3′-oxygen was activated as a phosphoramidite to produce the DNA synthesis-ready nucleosides **4a** and **4b**.

The two phosphoramidite derivatives were then utilized in DNA synthesis using a DNA synthesizer and all standard procedures, except that with the iodinated derivative, ammonium hydroxide deprotection was carried out at room temperature for 22 h (to lessen deiodination). Two 11 nucleotide long oligonucleotides were synthesized, each with the sequence 5′-dCCATCXCTACC-3′, but where X was CldG (**5b**) or IdG (**5d**). To complete the analysis,

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Table 1. Melting Temperatures (T_m) of DNA Duplexes (°C)[a]

5'-dCCATC**X**CTACC-3'
3'-dGGTAGCGATGG-5'

X = dG	X = OdG	X = CldG	X = BrdG	X = IdG
57.5 ± 0.5	52.7 ± 0.5	51.1 ± 0.3	49.6 ± 0.4	47.6 ± 0.4

[a] Conditions: 1 M NaCl, 0.1 mM EDTA, and 100 mM sodium phosphate, pH 7.0. Average T_m values ± standard deviation were calculated from three or more melts.

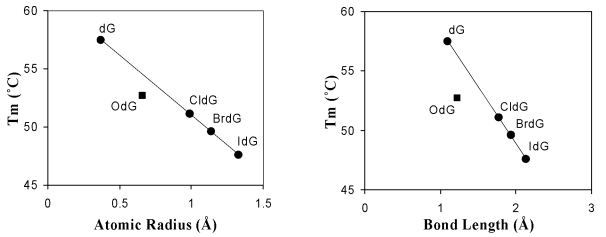

Figure 1. Graphs of melting temperature versus atomic radius (left) and bond length (right) at C8 with **5a**−**d** (circles) and **5e** (squares).

Table 2. Chemical Shifts of Nuclei of dG, CldG, BrdG, and IdG[a]

	C4′	C1′	C3′	C2′	2′-H
dG	87.5	82.5	70.6	39.5	2.50
CldG	87.8	83.9	70.9	36.6	3.09
BrdG	87.8	85.0	70.9	36.4	3.16
IdG	88.0	87.3	71.2	36.7	3.18

[a] Conditions: 0.04 M in DMSO-d_6. All shifts are relative to TMS.

oligonucleotides, where X was dG (**5a**), BrdG (**5c**), or OdG (**5e**), were purchased. The oligonucleotides were then purified by gel electrophoresis and reverse-phase HPLC before their purity and identity were confirmed by nuclease digest experiments (see Supporting Information).

The five oligonucleotides were paired with complimentary DNA strands and tested for their stabilities using melting studies (Table 1). Similar to previous work, OdG:dC[6] and BrdG:dC[12] base pairs were found to be less stable than dG:dC base pairs. CldG:dC and IdG:dC base pairs, which were also less stable than dG:dC base pairs, had stabilities that directly associate to atomic radius and bond length; the greater the atomic radius or bond length, the less stable the analogue in a base pair opposite dC. Interestingly, the correlation between melting temperature and either atomic radius or bond length at C8 with **5a**−**d** was entirely uniform with this duplex (Figure 1). Though an 8-halogen also destabilized dG base pairs to dA and T, as well as slightly stabilized dG base pairs to dG, the uniform correlation was unique for base pairs to dC (see Supporting Information).

To address whether steric bulk off of C8 may destabilize dG:dC base pairs by destabilizing the *anti* conformation of dG, the deprotected nucleosides dG, CldG, BrdG, and IdG were synthesized (CldG (**6a**) and IdG (**6b**)) or purchased (dG and BrdG) and tested for their inherent glycosidic bond conformation using NMR spectroscopy. It is known that an *anti* to *syn* conformational change results in a downfield shift of the C1′, C3′, C4′, and H2′ signals, as well as an upfield shift of the C2′ signal.[5,14] As seen in Table 2, such shifts were observed for all three halogenated nucleosides as compared to that of dG, indicating their preference for the *syn* conformation. These results are similar to previous results for OdG[5] and are consistent with the argument that steric bulk off of C8 destabilizes the *anti* conformation of dG.

In conclusion, these data stand in strong agreement with the premise that steric bulk of C8 destabilizes dG:dC base pairs by destabilizing the *anti* conformation of dG. Accordingly, it stands to reason that the steric bulk of the C8-oxygen plays some role in the destabilization of OdG:dC base pairs and thus the overall similar stabilities of OdG:dC and OdG:dA base pairs. It is interesting to note that the melting temperature with OdG (**5e**) varied from the trends for the halogens (Figure 1). These differences may be due, at least in part, to the increased nonpolarity and polarizability of the halogenated bases since both properties are known to increase helix stability.[13]

Finally, although methods were developed for the syntheses of oligonucleotides containing CldG and IdG because of their relevance to OdG, they are likely to find much wider application since halogenated nucleotides are commonly used in nucleic acid research. For example, halogenated nucleotides have found a great deal of use in crystallographic[15] and photo-cross-linking experiments[16] and as convertible nucleosides[17] and structural probes.[18]

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Supporting Information Available: Experimental procedures for the synthesis and purification of **2a**,**b**, **3a**,**b**, **4a**,**b**, **5b**, **5d**, and **6a**,**b**. ^{1}H and ^{13}C for **2a**,**b**, **3a**,**b**, and **6a**,**b**. ^{31}P NMR for **4a**,**b**. HPLC traces for **5b** and **5d**. Oligonucleotide digestion and analysis, and melting and NMR studies. Raw and/or complete data for Tables 1 and 2 and Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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