

Synthesis, oligonucleotide incorporation and base pair stability of 7-methyl-8-oxo-2'-deoxyguanosine†

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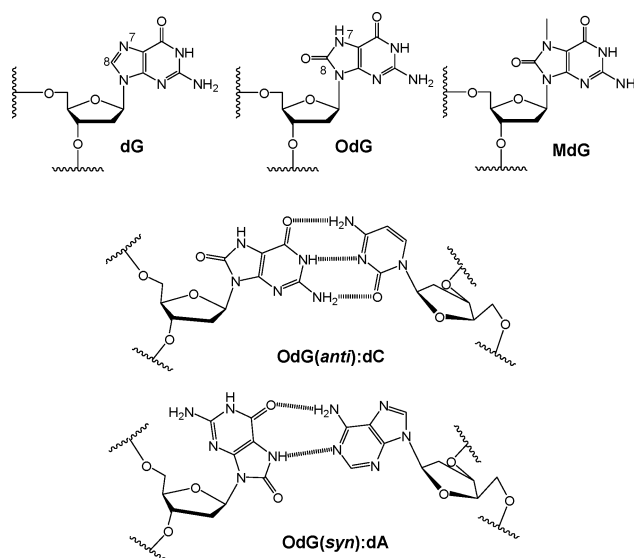
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7-Methyl-8-oxo-2'-deoxyguanosine, an analogue of the abundant promutagen 8-oxo-2'-deoxyguanosine, was incorporated into oligonucleotides and tested for its stability in various base pairs.

8-Oxo-2'-deoxyguanosine (OdG) is an abundant DNA lesion that arises through exposure of dG to chemical carcinogens, radiation and metabolic respiration.¹ It is one of the most common damaged nucleotides in mammalian cells,² and has been linked to ageing³ as well as cancer.⁴ Previous studies have shown that OdG forms stable base pairs with both dC and dA,^{5,6} and it can lead to dG → T transversions *in vivo*.⁷ Though OdG adopts an *anti* conformation and utilizes classic Watson–Crick hydrogen bonds when base pairing to dC,⁵ it adopts the *syn* conformation and uses its Hoogsteen edge for hydrogen bonding when base pairing to dA.⁶ OdG differs from dG at only the N7 and C8 positions, and both these sites are key to its promiscuous base pairing. It has been shown that the steric bulk of the oxygen at C8 destabilizes the *anti* conformation,⁸ thereby destabilizing OdG(*anti*):dC base pairs, while the N7-hydrogen can act as a hydrogen bond donor and stabilize OdG(*syn*):dA mismatches. Due to these, and possibly other reasons, OdG(*anti*):dC base pairs are only a bit more stable than OdG(*syn*):dA base pairs,⁹ despite having an additional hydrogen bond.

In response to the abundance and promutagenicity of OdG, cells have evolved repair enzymes that remove OdG from OdG:dC base pairs.¹⁰ Since none of these enzymes are active with dG:dC base pairs, the N7 and/or C8 positions must be an important element for substrate recognition by these enzymes. Several crystal structures have provided evidence for a direct interaction between the N7-hydrogen and the various enzymes,¹¹ while biochemical studies suggest the C8-oxygen may be important instead.¹⁰ To address these questions and provide a means to alter the N7 position while leaving the C8-oxygen unchanged, we set about synthesizing 7-methyl-8-oxo-2'-deoxyguanosine (MdG) and incorporating it into DNA.

Though 7-methyl-8-oxoriboguanosine has been reported previously,¹² the 2'-deoxy derivative (MdG) is not known. We first attempted to synthesize MdG using a method similar to that reported for 7-methyl-8-oxoriboguanosine by methylating a fully protected dG derivative at N7 and then oxidizing at C8.

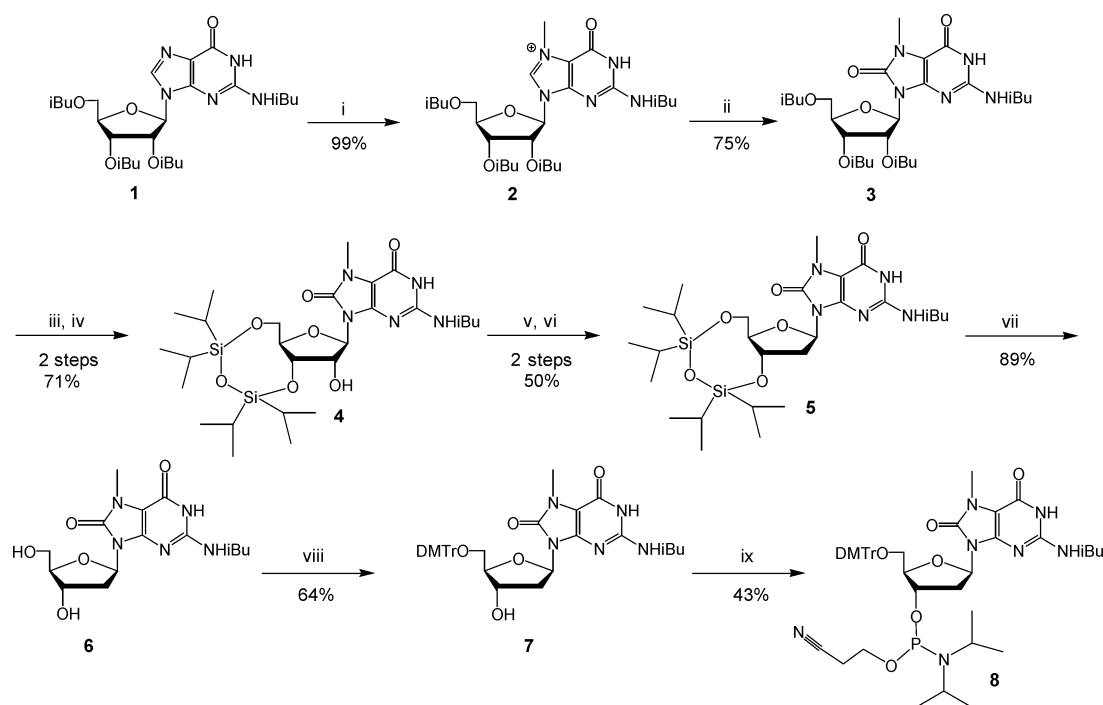


Though the methylated product could be isolated in good yield, the material consistently decomposed during the oxidation step, most likely due to depurination. Even after much investigation, no suitable procedure could be found. Thus we decided to synthesize the ribose derivative and then employ Barton–McCombie chemistry to deoxygenate at C2'. Since the standard protecting group for the exocyclic amine of dG in DNA solid-phase synthesis is an isobutyryl (iBu) group, we first synthesized the tetraisobutyrylated rG derivative (**1**) through known procedures.¹³ The nucleoside was then methylated at N7 with iodomethane before oxidation at C8 with hydrogen peroxide in acetic acid to form *N*,2',3',5'-tetraisobutyryl-7-methyl-8-oxoguanosine (**3**; Scheme 1). Selective deprotection of the sugar alcohols with sodium methoxide before selective protection of the 3'- and 5'-alcohols with a tetraisopropylidisiloxy (TIPDS) group yielded compound **4**. After derivatizing the free 2'-hydroxyl with a phenoxythiocarbonyl group, it was easily removed by reductive cleavage with tributyltin hydride and AIBN to give the 2'-deoxy derivative **5**. The TIPDS group was then removed with 0.5 M TBAF in THF and the isobutyryl-protected MdG derivative **6** was isolated. Finally, in order to prepare MdG for chemical DNA synthesis, the 5'-hydroxyl was protected as a dimethoxytrityl (DMTr) ether and the 3'-hydroxyl was activated as a phosphoramidite.

Standard synthesis and deprotection conditions were used to construct an oligonucleotide 11 nucleotides long, with the sequence 5'-dCCATCXCTACC-3', where X is MdG (**9a**). After purification by 20% PAGE and RP-HPLC, **9a** was characterized by MALDI-TOF mass spectrometry, as well as nuclease digest experiments (see Supplementary information†). To complete the

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† Electronic supplementary information (ESI) available: Synthesis, purification and characterization of **2–8**, **9a** and **10**. Procedures and full or raw data for oligonucleotide digestion and analysis, and melting and NMR studies. See DOI: 10.1039/b612597b



Scheme 1 Synthesis of the phosphoramidite derivative of MdG ready for solid-phase synthesis. *Reagents and conditions:* (i) CH_3I , DMF, 37°C , overnight; (ii) 30% H_2O_2 , AcOH, 37°C , 3.25 h; (iii) 0.2 M NaOH in 65 : 35 pyridine–MeOH, 0°C , 30 min; (iv) TIPDS–Cl, pyridine, rt, 1 h; (v) ClC(S)OPh , CH_2Cl_2 , rt, 2 h; (vi) Bu_3SnH , AIBN, toluene, 70°C , 1.5 h; (vii) 0.5 M TBAF in THF, rt, 2.5 h; (viii) DMTr–Cl, DMAP, pyridine, rt, 1.25 h; (ix) $(\text{iPr})_2\text{NEt}$, Me–imidazole, $\text{ClP}(\text{N}(\text{iPr})_2)\text{OCH}_2\text{CH}_2\text{CN}$, CH_2Cl_2 , rt, 30 min.

series, oligonucleotides where $\text{X} = \text{dG}$ (**9b**) and OdG (**9c**) were purchased and purified as well. All three oligonucleotides were then paired with complementary oligonucleotides that contained one of the four naturally occurring deoxynucleotides opposite the X position, and tested for their stability in melting studies (Table 1).¹⁴ Since all duplexes were melted under the same conditions and they all contained the same sequence except at the one base pair, the stabilities of the varying base pairs can be directly compared.

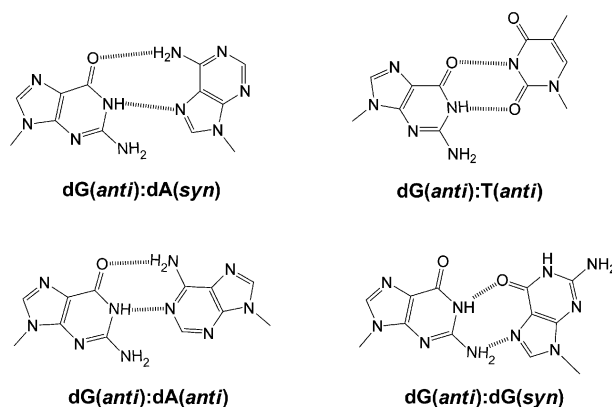
Similar to previous results, OdG:dC and OdG:dA base pairs were less and more stable, respectively, than dG:dC and dG:dA base pairs. Interestingly, MdG:dC and OdG:dC base pairs were of similar stability, indicating the absence of the N7-hydrogen and the presence of the N7-methyl are both unimportant to the stability of this base pair. Not surprisingly, MdG:dA base pairs were significantly less stable than OdG:dA base pairs. This can easily be explained not only by the absence of an N7-hydrogen, but also by the presence of the N7-methyl, which could be in steric clash with the N1 of dA if MdG is in the *syn* conformation.

Table 1 Melting temperatures ($T_m/^\circ\text{C}$) of DNA duplexes^a

	5' – CCATC X CTACC – 3' 3' – GGTAG Y GATGG – 5'			
	Y = dC	Y = dA	Y = T	Y = dG
X = dG	57.5 ± 0.5	43.2 ± 0.2	46.3 ± 0.4	45.1 ± 0.2
X = OdG	52.7 ± 0.5	48.0 ± 0.3	41.5 ± 0.2	40.9 ± 0.5
X = MdG	53.3 ± 0.2	39.0 ± 0.2	41.2 ± 0.6	38.3 ± 0.6

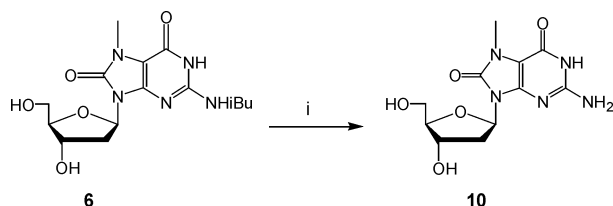
^a Average $T_m \pm$ standard deviation was calculated from three or more melting experiments.

Additionally, MdG:dA , and MdG:T and OdG:T base pairs were all less stable than dG:dA and dG:T base pairs, respectively. Since dG often adopts the *anti* conformation when base pairing to dA and T (as in dG:dC base pairs; Scheme 2),¹⁵ the steric bulk of the C8-oxygen in MdG and OdG would also destabilize these mismatches if they are also in the *anti* conformation. Finally, OdG and MdG formed less stable base pairs with dG than did dG itself. Even though dG:dG base pairs usually involve one dG in the *anti* conformation and the other dG in the *syn* conformation,¹⁶ neither of these options are suitable for OdG and MdG, since the steric clash of the C8-oxygen would destabilize the base pair if they adopted the *anti* conformation, while neither has the strong N7 hydrogen bond accepting ability required when in the *syn* conformation.



Scheme 2 Common structures for base pair mismatches with dG.

To further confirm that the C8-oxygen destabilizes the *anti* conformation in MdG, we synthesized the MdG mononucleoside from its isobutyrylated derivative (Scheme 3). Deprotection through treatment with methoxide, followed by purification through a reverse-phase C₁₈ column yielded the pure monomer **10**. It is known that an *anti*-to-*syn* nucleoside conformational change results in strong downfield and upfield shifts in the H2' and C2' signals, respectively.¹⁷ Such shifts were observed for MdG when compared to dG (see Supplementary information†), consistent with MdG preferring the *syn* conformation. These results further the argument that the presence of the C8-oxygen destabilizes the *anti* conformation, thereby destabilizing base pairs that contain MdG in the *anti* conformation.



Scheme 3 Synthesis of the MdG monomer. *Reagents and conditions:* (i) 0.3 M NaOMe in HOME, 40 °C, 5 h.

In conclusion, we have developed an efficient route toward the incorporation of the OdG analogue MdG into oligonucleotides. It should be noted that though this adduct may be produced naturally in cells, it is highly unlikely due to the already low abundance of either OdG or 7-methyl-2'-deoxyguanosine. Still, MdG is of great importance since it has the potential to help elucidate the role of the N7-hydrogen in the biological activities of OdG. We are currently testing the activity of various repair enzymes with MdG in order to better understand their modes of substrate recognition.

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