ORGANIC LETTERS

2004
Vol. 6, No. 21
3817−3820

Oligonucleotide Incorporation of 8-Thio-2′-deoxyguanosine

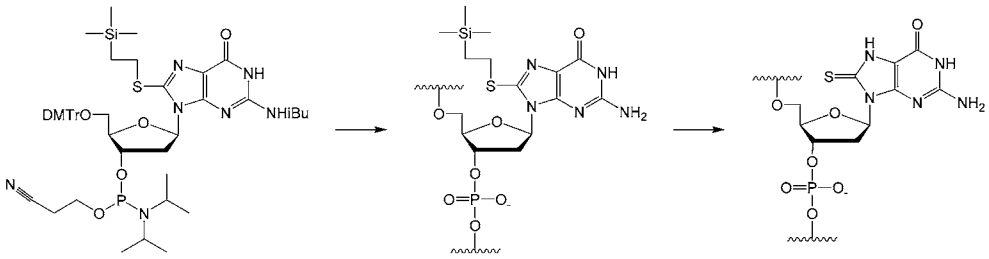
Michelle L. Hamm,* Rushina Cholera, Courtney L. Hoey, and Timothy J. Gill

Department of Chemistry, University of Richmond, Gottwald N-113, Richmond, Virginia 23173

mhamm@richmond.edu

Received August 11, 2004

ABSTRACT



8-Thio-2′-deoxyguanosine (SdG) is a useful analogue of the abundant promutagen 8-oxo-2′-deoxyguanosine (OdG). Its synthesis and DNA incorporation using standard phosphoramidite chemistry is reported. To prevent oxidation during DNA synthesis, the sulfur was protected as a 2-(trimethylsilyl)ethyl sulfide. Subsequent treatment with TBAF yielded the desired 8-thiocarbonyl functionality. Melting studies with SdG revealed almost equal stabilities of SdG:dC and SdG:dA base pairs, lending insight into the base-pairing preferences of OdG.

8-Oxo-2′-deoxyguanosine (OdG) is an abundant DNA lesion produced by the reaction of 2′-deoxyguanosine (dG) with radical oxygen species.[1] OdG leads to dG to T transversions,[2] and its presence has been linked to aging[3] and diseases such as cancer.[4] OdG differs from dG at only the N7 and C8 positions (Scheme 1); thus, these positions must be key in determining the deleterious effects of OdG. For example, both these positions are thought to play a critical role in the promutagenicity of OdG. When synthesizing DNA, polymerases incorporate only 2′-deoxycytidine (dC) opposite dG but can incorporate either dC or 2′-deoxyadenosine (dA) opposite OdG.[5] This is because while dG only forms base pairs to dC, OdG forms stable base pairs to both dC and dA (Scheme 2).[6] When base pairing to dA, OdG adopts a *syn* conformation about the glycosidic bond and uses the N7-hydrogen and C6-oxygen of the Hoogsteen edge to contact dA.[7] When base pairing to dC, however, OdG is in the *anti* base conformation and normal Watson−Crick pairing is utilized.

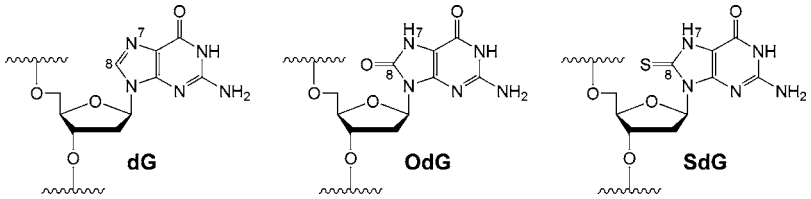
Interestingly, melting studies have shown that despite having one less hydrogen bond, OdG(*syn*):dA base pairs are only slightly less stable than OdG(*anti*):dC base pairs. This similarity is thought to be in part due to the C8-oxygen which, because of its steric bulk, causes OdG to prefer the *syn* conformation,[8] thereby destabilizing OdG(*anti*):dC base pairs.

Due to both its promutagenicity and abundance, cells have evolved specific repair enzymes that recognize and remove OdG from DNA. Since dG is not a substrate for these enzymes, they likely recognize OdG at either the C8-oxygen or N7-hydrogen. More recent crystallographic studies have implicated the N7-hydrogen and not the C8-oxygen as the

(1) Kasai, H.; Nishimura, S. in *Oxidative Stress: Oxidants and Antioxidants*; Sies, H., Ed.; Academic: London, 1991; pp 99−116.
(2) Le Page, F.; Margot, A.; Grollman, A. P.; Sarasin, A.; Gentil, A. *Carcinogenesis* **1995**, *16*, 2779−2784.
(3) Fraga, C. G.; Shigenaga, M. K.; Park, J.-W.; Degan, P.; Ames, B. N. *Proc. Natl. Acad. U.S.A.* **1990**, *87*, 4533−4537.
(4) Floyd, R. A. *Carcinogenesis* **1990**, *11*, 1447−1450.
(5) Shibutani, S.; Takeshita, M.; Grollman, A. P. *Nature* **1991**, *349*, 431−434.
(6) Plum, G. E.; Grollman, A. P.; Johnson, F.; Breslauer, K. J. *Biochemistry* **1995**, *34*, 16148−16160.
(7) McAuley-Hecht, K. E.; Leonard, G. A.; Gibson, N. J.; Thomson, J. B.; Watson, W. P.; Hunter, W. N.; Brown, T. *Biochemistry* **1994**, *33*, 10266−10270.
(8) Sarma, R. H.; Lee, C.-H.; Evans, F. E.; Yathindra, N.; Sundaralingham, M. *J. Am. Chem. Soc.* **1974**, *96*, 7337−7348.

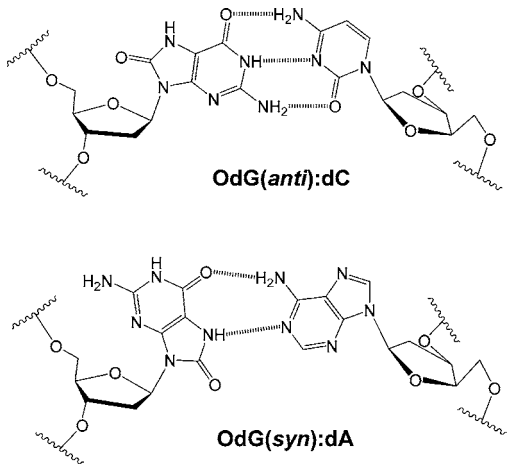
10.1021/ol0484097 CCC: $27.50
Published on Web 09/22/2004

Scheme 1. Structures dG, OdG, and SdG



mode of detection,[9] though other biochemical studies suggest the C8-oxygen may play some role.[10] To address these discrepancies and gain more insight into the exact role of the C8-oxygen in OdG mutagenicity and repair, we set about creating an analogue of OdG that would differ from it at only the C8 position.

Scheme 2. Base Pairing of OdG



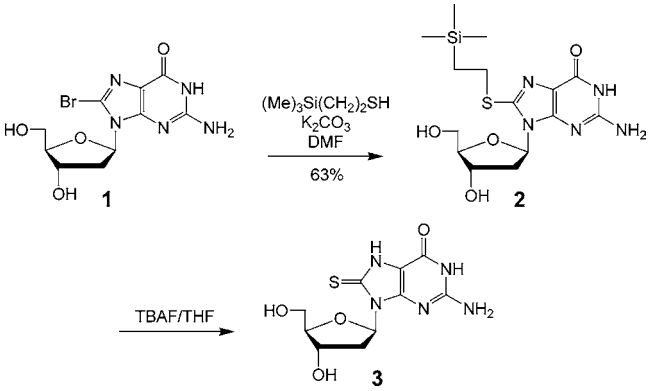
To this end, replacement of oxygen with sulfur has been used extensively to probe the role of oxygen in biomolecular recognition and catalysis. This is because though sulfur has similar bonding properties to oxygen, it differs significantly in its affinity toward metals,[11] ability to hydrogen bond,[12] and its atomic radius and bond length. For example, metal replacement studies with 3′-thio-3′-deoxyuridine have suggested a direct metal interaction to the active site 3′-oxygen during the cleavage reaction of the *Tetrahymena* ribozyme,[13] while stability studies of G-tetrads using 6-thio-2′-deoxyguanosine have demonstrated the importance of a 6-oxo moiety to tetrad formation.[14]

In a similar fashion, the study of an analogue of OdG where the 8-oxo moiety has been replaced with sulfur may be a useful tool with which to better understand the specific properties and interactions of the C8-oxygen that dictate the activity of OdG in cells. For example, if steric bulk off of C8 is important in the similar stabilities of OdG(*syn*):dA and OdG(*anti*):dC base pairs, then 8-thio-2′-deoxyguanosine (SdG; Scheme 1), which has greater bulk off C8, should form base pairs to dC and dA with even more similar stabilities. Additionally, since SdG differs from OdG at only the C8-position, biochemical studies with the aforementioned repair enzymes should lend insight into necessity of the 8-oxo moiety.

The main concern when incorporating any sulfur-containing analogue into DNA is to prevent oxidation of the sulfur during the iodine oxidation or ammonium hydroxide deprotection steps of DNA synthesis. To prevent this, previous syntheses of DNA containing sulfur analogues have usually involved protection of the sulfur as a sulfide. For example, 6-thio-2′-deoxyguanosine, 4-thio-2′-deoxyuridine, 4-thio-2′-deoxythymidine, 4-thiouridine, and 6-thioinosine are all protected as β-cyanoethyl sulfides during DNA synthesis.[15] Though such a protection scheme may well work for SdG, this route would require the starting material 2-cyanoethanethiol which is highly toxic and not commercially available. For synthetic ease, we chose to protect the sulfur moiety as a 2-(trimethylsilyl)ethyl sulfide and use tetrabutylammonium fluoride (TBAF) treatment as a means of sulfur deprotection.

Though there is no published synthesis to 8-(2-(trimethylsilyl)ethyl)thio-2′-deoxyguanosine (TMSE-SdG), 8-(2-

Scheme 3. Synthesis of SdG



(9) (a) Bruner, S. D.; Norman, D. P. G.; Verdine, G. L. *Nature* **2000**, *403*, 859−866. (b) Fromme, J. C.; Verdine, G. L. *J. Biol. Chem.* **2003**, *278*, 51543−51548.

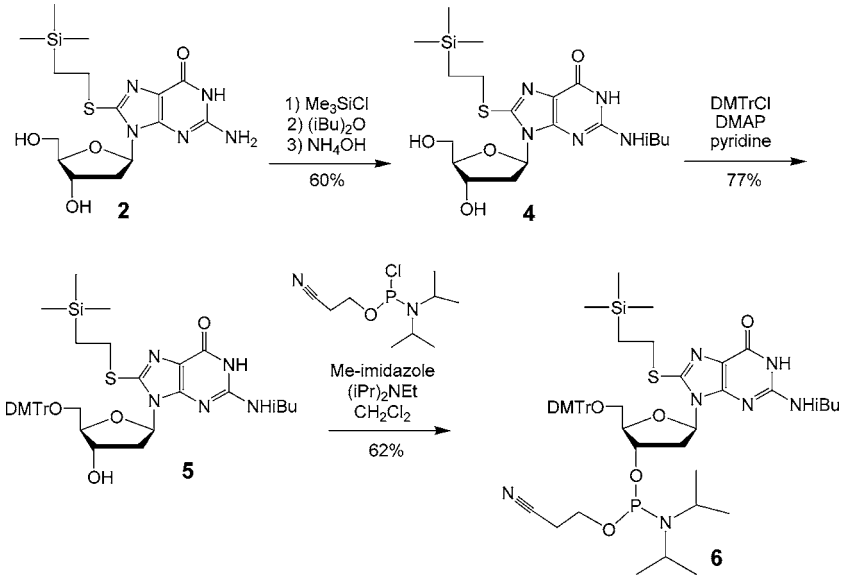
(10) Asagoshi, K.; Yamada, T.; Terato, H.; Ohyama, Y.; Monden, Y.; Arai, T.; Nishimura, S.; Aburatani, H.; Lindahl, T.; Ide, H. *J. Biol. Chem.* **2000**, *275*, 4956−4964.

(11) Pecoraro, V. L.; Hermes, J. D.; Cleland, W. W. *Biochemistry* **1984**, *23*, 5262−5271.

(12) Platts, J. A.; Howard, S. T.; Bracke, B. R. F. *J. Am. Chem. Soc.* **1996**, *118*, 2726−2733.

(13) Piccirilli, J. A.; Vyle, J. S.; Caruthers, M. H.; Cech, T. R. *Nature* **1993**, *361*, 85−88.

Scheme 4. Synthesis of the Fully Protected Phosphoramidite Derivative of SdG

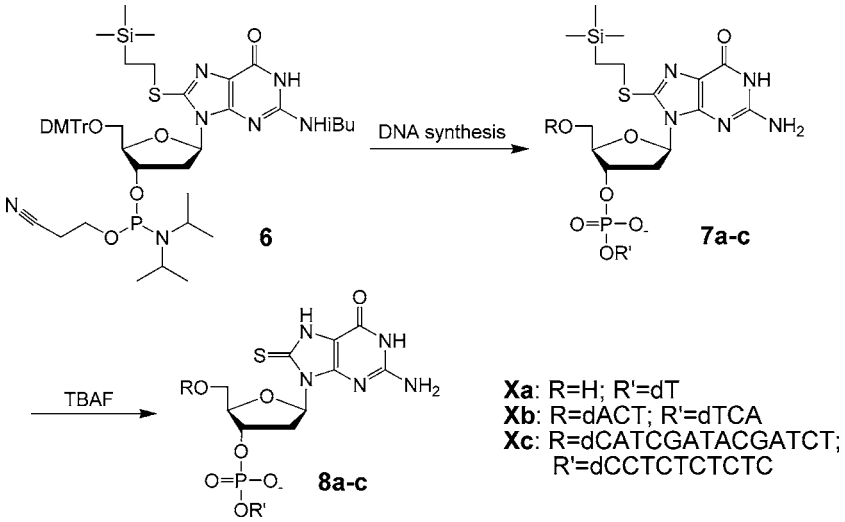


(trimethylsilyl)ethyl)thio-2′-deoxyadenosine has been reported previously.[16] In a reaction analogous to the adenosine derivative, TMSE-SdG (**2**) was synthesized through treatment of 8-bromo-2′-deoxyguanosine[17] (**1**) with commercially available 2-(trimethylsilyl)ethanethiol (Scheme 3). To confirm that no decomposition of the sulfur would occur during solid-phase DNA synthesis, compound **2** was incubated separately for 1 h at room temperature in the DNA synthesis oxidizing reagent (0.02 M I_2) or 20 h in aqueous ammonia at 55 °C. After both incubations no decomposition was observed, indicating that the trimethylsilylethyl (TMSE) protection scheme is suitable for solid-phase phosphoramidite chemistry. Further, to prove TBAF treatment would be an appropriate means for sulfur deprotection, we treated nucleoside **2** with 1.0 M TBAF in tetrahydrofuran (THF) for 30 min. After HPLC purification, pure SdG (**3**; Scheme 3) was obtained.

The synthesis to the phosphoramidite derivative of **2** was continued by protecting the exocyclic amine as an isobutyryl (iBu) amide (Scheme 4) using the well-known transient protection procedure.[18] The 5′-hydroxyl was then protected as a dimethoxytrityl (DMTr) ether to produce **5**. Finally, the 3′-hydroxyl was activated as a phosphoramidite to create the DNA synthesis ready 8-thio-2′-deoxyguanosine derivative **6**.

Amidite **6** was then utilized in DNA synthesis using a DNA synthesizer and standard procedures. Coupling yields were over 95% according to HPLC or gel purification run after ammonium hydroxide deprotection. Three different oligonucleotides (2, 7, and 25 nucleotides long; Scheme 5) were synthesized; their sequences were 5′-d^{SiS}GT-3′ (**7a**), 5′-dACT^{SiS}GTCA-3′ (**7b**), and 5′-dCATCGATACGATCT^{SiS}GCCTCTCTCTC-3′ (**7c**), respectively, where ^{SiS}G is the TMSE-protected SdG derivative. The oligonucleotides

Scheme 5. DNA Incorporation and Sulfur Deprotection of SdG



were then purified by preparative HPLC (**7a**) or gel purification and preparative HPLC (**7b**,**c**) before treatment with 1.0 M TBAF in THF for 30 min to reveal the free sulfur and produce oligonucleotides **8a**−**c**.

To further prove the 8-sulfur moiety was not compromised during oligonucleotide synthesis, deprotection, or purification, oligonucleotide **8b** was digested to its individual nucleotides and analyzed by analytical HPLC.[19] As seen in Figure 1A, only four peaks (corresponding to dC, T, dA, and SdG) were observed. To further characterize the supposed SdG peak, we compared digested **8b** to an authentic sample of compound **3** (Figure 1B). The supposed SdG peak and **3** had identical retention times (16.0 min) and coeluted under the same reaction and chromatography conditions. Furthermore, the supposed SdG peak is not dG or OdG which have retention times of 6.2 and 7.1 min, respectively, when treated under the same reaction and chromatography conditions. It should be noted that longer TBAF deprotection treatments (4 h) did result in some decomposition of SdG to form dG (as characterized by HPLC and mass spectrometry), presumably by oxidative desulfurization.

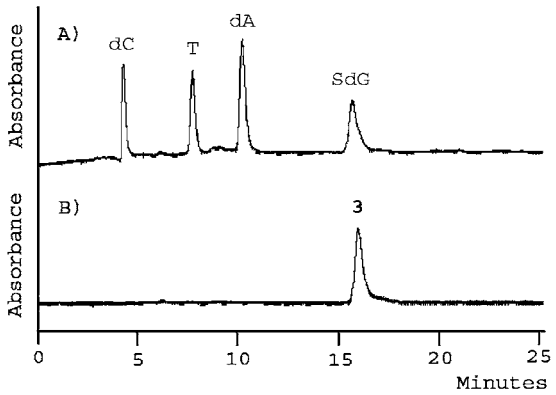


Figure 1. Analytical HPLC analysis of (A) nuclease-digested **8b** and (B) mock nuclease-digested **3** (an authentic standard of SdG).

To gain insight into the base pairing preference of OdG, we incorporated SdG into an 11 nucleotide long oligonucleotide and tested its base-pairing stabilities opposite dC and dA (Table 1). We also tested the base pairing of dG and OdG in the same duplex and under the same conditions. Similar to previous studies,[6] we found OdG:dC and OdG:dA base pairs to be of much more similar stability than dG:dC and dG:dA base pairs (T_m differences of 4.6 and 14.6 °C, respectively). Interestingly, we found SdG:dC and SdG:dA base pairs to be of almost equal stability (T_m difference of 1.2 °C). When compared to OdG, the similar stabilities of the SdG base pairs appear to arise from additional destabilization of SdG:dC since SdG:dA and OdG:dA base pairs are of equal stability, while SdG:dC base pairs are much less stable than OdG:dC base pairs (T_m difference of 6.1 °C). Considering sulfur has a larger atomic radius and longer bonding distance than oxygen, this finding is in agreement with the theory that additional steric bulk off of dG-C8 destabilizes the *anti* base conformation,[8] thereby destabilizing base pairs to dC (Scheme 2). Other possible explanations involving distortion of the purine base are not likely as crystal structure studies with other sulfur-containing purines have confirmed that the a thio substitution causes no significant structure change.[20] The finding that SdG:dA and OdG:dA base pairs are of equal stability is also of interest since it suggests that added steric bulk off of C8 does not play a significant role in the stabilities of these base pairs.

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

5'-CCATC**X**CTACC-3'
3'-GGTAG**Y**GATGG-5'

	Y = dC	Y = dA
X = dG	57.6 ± 0.5	43.0 ± 0.2
X = OdG	52.6 ± 0.5	48.0 ± 0.4
X = SdG	46.5 ± 0.3	47.7 ± 0.3

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

In summary, we have developed a rapid and efficient synthesis to oligonucleotides containing SdG using the phosphoramidite method. Ready access to such products will enable experiments that should lend further insight into the exact role of the C8-oxygen in the bioactivity of OdG. We are currently testing SdG with different repair enzymes to determine whether the 8-oxo moiety is required for OdG recognition.

Acknowledgment. We thank P. Nyffeler for many helpful discussions and P. Nyffeler and E. Fenlon for reviewing the manuscript. This work was partially supported by the Thomas F. Jeffress and Kate Miller Jeffress Memorial Trust and the NSF-CAREER program. Additionally, acknowledgment is made to Donors of the American Chemical Society Petroleum Research Fund for partial support of this research. M.L.H. is a Camille and Henry Dreyfus Start-up Awardee.

Supporting Information Available: Experimental procedures for the synthesis and purification of **2**−**6**, **7a**−**c**, and **8a**−**c**, oligonucleotide digestion and analysis, and melting studies; ^{1}H and ^{13}C for **2**−**5**; ^{31}P NMR for **6**; HPLC traces for **7a**−**c** and **8a**−**c**; raw melting data for Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0484097

(14) Rao, T. S.; Durland, R. H.; Seth, D. M.; Myrick, M. A.; Bodepudi, V.; Revankar, G. R. *Biochemistry* **1995**, *34*, 765−772.
(15) (a) Adams, C. J.; Murray, J. B.; Farrow, M. A.; Arnold, J. R. P.; Stockley, P. G. *Tetrahedron Lett.* **1995**, *36*, 5421−5424. (b) Kadokura, M.; Wada, T.; Seio, K.; Sekine, M. *J. Org. Chem.* **2000**, *65*, 5104−5113. (c) Glen Research Catalog, Sterling, VA.
(16) Chambert, S.; Gautier-Luneau, I.; Fontecave, M.; Decout, J.-L. *J. Org. Chem.* **2000**, *65*, 249−253.
(17) Gannett, P. M.; Sura, T. P. *Synth. Commun.* **1993**, *23*, 1611−1615.
(18) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.
(19) (a) Eadie, J. S.; McBride, L. J.; Efcavitch, J. W.; Hoff, L. B.; Cathcart, R. *Anal. Biochem.* **1987**, *165*, 442−447. (b) Kuimelis, R. G.; Nambiar, K. P. *Nucl. Acids Res.* **1994**, *22*, 1429−1436.
(20) Thewalt, U.; Bugg, C. E. *J. Am. Chem. Soc.* **1972**, *94*, 8892−8898.