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Synthesis and Incorporation of a Cationic Pyridinium Nucleoside Mimic in Duplex DNA: Effect on Long-Distance Radical Cation Transport

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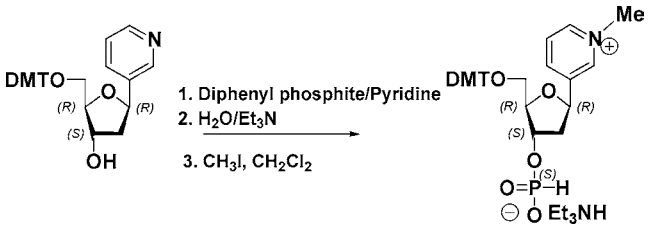
ABSTRACT



A convenient method for the synthesis and solid-phase incorporation of a cationic nucleoside mimic and its role in DNA long-range charge transfer are described.

Methylation of DNA is well-known to play an important role in mismatch repair, DNA−protein interaction, gene expression, the control of chromosome structure, and the development of mutational hotspots.[1] Methylation occurs at electron-rich nucleobase sites, especially guanine N7 and adenine N1 and N3, and it is an important natural process that converts these normally neutral entities to positively charged moieties.[2] The one-electron oxidation of DNA similarly converts an uncharged nucleobase to a positively charged species (in this case, a radical cation).[3] There has been widespread interest in examining the chemical consequences of DNA oxidation because this process can result in the creation of mutations.[4] A significant finding from the investigation of DNA one-electron oxidation is that the resulting radical cations can migrate long distances through the duplex by a hopping mechanism.[5−10] We wanted to investigate the effect of interposing a positive charge-bearing nucleobase on radical cation hopping in DNA. The naturally occurring methylated nucleobases are not suitable for this purpose because they are prone to decomposition through cleavage of their glycosidic bonds.[11] Therefore, we opted to examine oligonucleotide duplexes containing a model compound based on an unnatural *C*-nucleoside. In this case, a carbon−carbon bond forms the link between the model base and a dideoxyribose; this link will be sufficiently stable when the base is alkylated for incorporation into DNA strands by standard

(1) Barras, F.; Marinus, M. G. *Trends Genet.* **1989**, *5*, 139−143.
(2) Drablos, F.; Feyzi, E.; Aas, P. A.; Vaagbo, C. B.; Kavli, B.; Bratlie, M. S.; Pena-Diaz, J.; Otterlei, M.; Slupphaug, G.; Krokan, H. E. *DNA Repair* **2004**, *3*, 1389−1407.
(3) Schuster, G. B. *Long-Range Charge Transfer in DNA I, II*; Springer-Verlag: Heidelberg, 2004; Vol. 236, 237.
(4) Loft, S.; Poulsen, H. E. *J. Mol. Med.* **1996**, *74*, 297−312.
(5) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747−8748.
(6) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8353−8358.
(7) Giese, B.; Biland, A. *Chem. Commun.* **2002**, 667−672.
(8) O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 11471−11483.
(9) Lewis, F. D.; Zhu, H.; Daublain, P.; Fiebig, T.; Raytchev, M.; Wang, Q.; Shafirovich, V. *J. Am. Chem. Soc.* **2006**, *128*, 791−800.
(10) Joseph, J.; Schuster, G. B. *J. Am. Chem. Soc.* **2006**, *128*, 6070−6074.
(11) Bloomfield, V. A.; Crothers, D. M.; Tinoco, J. I. *Nucleic Acids: Structure, Properties, and Function*; University Science Books: Sausalito, Ca, 1999.

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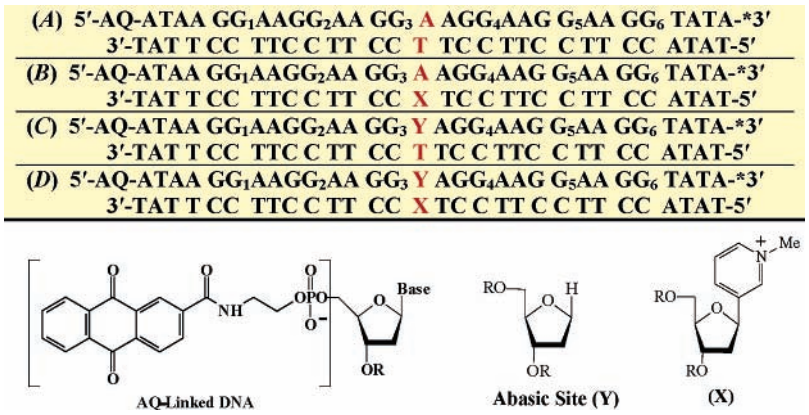


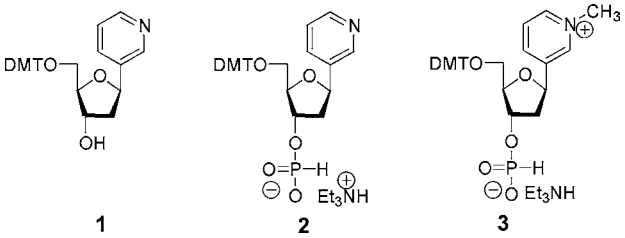
Figure 1. Structures of the DNA oligomers used in this work. X corresponds to the location of a pyridinium nucleobase, and Y corresponds to an abasic site.

solid-phase methods. To this end, we prepared the pyridinium nucleoside (**3**) and built it selectively into duplex DNA oligomers. We report herein the synthesis of **3**, its incorporation by solid-phase synthesis into oligonucleotides, an analysis of the stability of duplex DNA oligomers that contain this cationic nucleoside mimic, and the effect of the pyridinium nucleoside on long-distance charge transfer in duplex DNA.

Synthesis of the Pyridinium Nucleoside and Incorporation in DNA. Our initial synthetic strategy for the single-step preparation of a pyridinium *C*-nucleoside based on Heck coupling to form a carbon−carbon bond between 3-iodopyridine and 1,4-anhydro-2-deoxy-3-*O*-(*tert*-butyldiphenylsilyl)-D-erythro-pent-1-enitol[12] failed to yield the desired product. General methods for the multistep construction of *C*-nucleosides include the nucleophilic displacement of a halogen atom from C-1 of a protected dideoxyribose by a functionalized precursor or heteroaryl moiety[13] and the reaction of a *C*-nucleophile at C-1 of a deoxyribose followed by ring closure to reform a furanose ring. We chose the latter approach, which has been previously used to prepare close analogues of **1**.[14]

The preparation of the 4,4-dimethoxy trityl protected 1,2-dideoxy-1-(3-pyridyl)-D-ribofuranose (**1**) followed from the method reported by Eaton and Millican,[14] and its N-methylation proceeded smoothly. However, we were unable to convert the N-methylated pyridinium nucleoside to a phosphoramidite suitable for DNA synthesis because of its insolubility. Because methylation of the imine nitrogen after phosphoramidation will result in uncontrolled reactions on the trivalent phosphorus,[15] we sought other routes for forming the phosphate ester bond that would be less sensitive to the methylation reaction conditions. On the basis of these considerations, we chose a route that proceeds through pentavalent phosphorus intermediates (experimental details are provided in the Supporting Information). The reaction of **1** with 7 equiv of diphenyl phosphite in pyridine followed by hydrolyses in water and triethylamine solution afforded the H-phosphonate (**2**) in 78% yield.[16] The reaction of **2** with excess methyl iodide in dichloromethane solution gave the desired H-phosphonate **3**, which is suitable for incorporation into DNA using automated solid-phase synthesis (Chart 1).

Chart 1



Postsynthetic oxidation of the oligomers with H-phosphonate groups with iodine (4% in a pyridine−water−triethylamine mixture) converts the H-phosphonates to phosphate groups.[17] This procedure was used to prepare the oligonucleotides shown in Figure 1 where * indicates the position of a ^{32}P radiolabel and **X** represents the position of the pyridinium nucleobase.

These compounds were purified by HPLC and characterized by electron spray ionization mass spectrometry. DNA **A** contains only standard Watson−Crick DNA base pairs, and in **B**, a pyridinium nucleobase is opposite an adenine. In **C**, an abasic site (**Y**) is at this location opposite a thymine, and in **D**, a pyridinium group is opposite the abasic site.

(12) Farr, R. N.; Daves, G. D., Jr. *J. Carbohydr. Chem.* **1990**, *9*, 653−660.
(13) Buchanan, J. G. *Prog. Chem. Org. Nat. Prod.* **1983**, *44*, 243−299.
(14) Eaton, M. A. W.; Millican, T. A.; Mann, J. *J. Chem. Soc., Perkin Trans. 1 (1972−1999)* **1988**, (3), 545−547.
(15) Lewis, E. S.; Smith, M. J.; Christie, J. J. *J. Org. Chem.* **1983**, *48*, 2527−2531.
(16) Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355−3358.
(17) Cullis, P. M.; Lee, M. *J. Chem. Soc., Chem. Commun.* **1992**, (17), 1207−1208.

Table 1. Ratio (R_n) of a Relative Amount of Strand Cleavage at Each GG Step (1−6) to the Total Amount of Strand Cleavage at All GG Steps as Determined by Phosphorimagery

DNA	R_1	R_2	R_3	R_4	R_5	R_6
A	0.20 ± 0.002	0.20 ± 0.01	0.17 ± 0.01	0.16 ± 0.004	0.16 ± 0.01	0.11 ± 0.003
B	0.21 ± 0.01	0.19 ± 0.01	0.17 ± 0.003	0.15 ± 0.003	0.15 ± 0.002	0.13 ± 0.01
C	0.17 ± 0.004	0.20 ± 0.01	0.15 ± 0.01	0.15 ± 0.004	0.13 ± 0.01	0.12 ± 0.01
D	0.16 ± 0.01	0.17 ± 0.01	0.22 ± 0.003	0.16 ± 0.004	0.15 ± 0.003	0.15 ± 0.004

To determine the effect of introducing a positively charged moiety into duplex DNA, we compared the stability of the modified structures to those containing normal DNA bases. Thermal denaturation of duplexes **A** and **B** was monitored by UV absorption spectroscopy in 10 mM sodium phosphate buffer solution. The results (see Supporting Information) reveal that the melting temperature (T_m) of the modified duplex is lowered by 3 °C, which shows that incorporation of the pyridinium nucleobase results in only modest destabilization. Similarly, inclusion of the pyridinium group opposite an abasic[18] site (duplex **C**) results in no significant change in T_m (1 °C) compared to the case where a thymine is opposite the abasic site (duplex **D**). The circular dichroism spectra of the DNA duplexes examined in this work indicate that they all maintain overall B-form structures.[19]

We have shown that irradiation of AQ-linked DNA duplexes with UV light (350 nm where only the AQ absorbs) results in the injection of a radical cation that can migrate through the DNA by hopping until it is consumed in an irreversible chemical reaction.[20] The reaction commonly occurs at guanine or G_n ($n = 1, 2, 3$) sites because guanine has a low ionization potential and the radical cation pauses there momentarily facilitating the reaction with H_2O or O_2.[21] These reactions convert the guanine to a product that is revealed as a strand break by treatment of the irradiated DNA sample with piperidine.

The location of the strand breaks is easily determined by high-resolution polyacrylamide gel electrophoresis and autoradiography. We applied these techniques to the investigation of long-distance radical cation migration in the DNA duplexes containing pyridinium nucleobases.

DNA **A** has six GG steps that are separated by AA bases on the ^{32}P-labeled strand. We have shown previously for this arrangement that the rate of hopping from GG to GG is much faster than the rate of reaction by trapping with H_2O or O_2.[22] For this reason, all of the GG steps in DNA **A** are approximately equally reactive. Figure 2 shows autoradiograms resulting from the irradiation of the AQ-linked DNA oligomers. As expected, each of the GG steps in DNA **A** show approximately the same amount of strand cleavage; these data are summarized in Table 1.

The irradiation of DNA **B**, which incorporates a pyridinium nucleobase between GG steps 3 and 4, shows that the pyridinium group does not affect charge transport along the DNA duplex. That is, the relative amount of strand cleavage observed at GG steps 5 and 6 (after the pyridinium group) in DNA **B** is essentially the same as that observed in DNA **A**. Also, the amount of strand cleavage observed at GG_3 and GG_4 in DNA **B**, which neighbor the pyridinium base, is unaffected by this change. Evidently, the pyridinium base does affect the rate of radical cation hopping or that of its reaction with H_2O or O_2. A related result is found from analysis of DNA **C** and **D**. As we have previously reported, an abasic site in an $(A)_n$ segment has little effect on radical cation transport between GG steps.[23] This finding is reproduced in the analysis of DNA **C**, which contains an abasic site between GG_3 and GG_4. Incorporation of the pyridinium nucleotide opposite the abasic site in DNA **D** does not affect the charge-transport efficiency (the amount of reaction at GG_5 and GG_6 is unaffected), but it does result in a somewhat

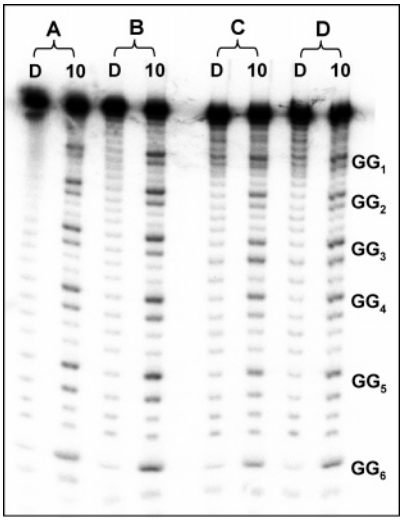


Figure 2. Phosphorimages from the irradiation of DNA oligomers **A**, **B**, **C**, and **D**. There are two lanes for each duplex: lane D is the dark control (the sample was not irradiated but treated in all ways identically to those samples that were irradiated), and lane 10 stands for samples that were irradiated for 10 min at 350 nm in a Rayonet photoreactor. All samples were treated with piperidine and then electrophoresed on a denaturing polyacrylamide gel for 2 h at 70 W.

(18) Vesnaver, G.; Chang, C. N.; Eisenberg, M.; Grollman, A. P.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3614−3618.
(19) Gray, D. M.; Hung, S.-H.; Johnson, K. H. *Methods Enzymol.* **1995**, *246*, 19−36.
(20) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(21) Joy, A.; Ghosh, A. K.; Schuster, G. B. *J. Am. Chem. Soc.* **2006**, *128*, 5346−5347.
(22) Liu, C.-S.; Hernandez, R.; Schuster, G. B. *J. Am. Chem. Soc.* **2004**, *126*, 2877−2884.
(23) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762−12771.

greater amount of strand cleavage at GG_3, which is adjacent to the modification.

Radical cation migration in DNA is a robust process that is understood most clearly as an emergent property of nucleobase sequence.[10] The results reported here reveal that embedding a modified nucleobase bearing a positive charge in an $(AAGG)_n$ segment has only a slight effect on radical cation hopping and reaction. The precise structure of the DNA in the region of the modified nucleobase is not known in detail, but the melting and CD data indicate only modest perturbation from standard B-form DNA. One possibility is that the modified nucleobase is extrahelical, which could account for its lack of influence on radical hopping. Studies to determine the structure of the modified DNA duplex are ongoing and will be reported elsewhere.

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Supporting Information Available: NMR spectra, ESI mass spectra of N-methylated nucleosides, and a detailed description of experimental procedures for the synthesis of the H-phosphonate. This material is available free of charge via the Internet at http://pubs.acs.org.

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