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Synthesis and Characterization of Oligodeoxynucleotides Containing the Major DNA Adducts Formed by 1,6- and 1,8-Dinitropyrene

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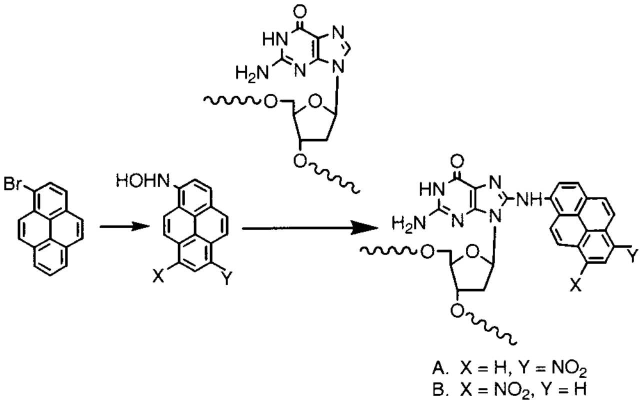
ABSTRACT



An efficient method for the synthesis of oligonucleotides containing a site-specific DNA adduct formed by the carcinogens 1,6- and 1,8-dinitropyrene has been developed. Palladium-catalyzed amination provided a straightforward route for the synthesis of aminonitropyrenes which, following separation, were reduced to the nitrosonitropyrenes. The *N*-hydroxyaminonitropyrene, generated in situ from each nitrosonitropyrene, was used successfully to introduce the dinitropyrene-derived DNA adduct at a defined site in an oligonucleotide.

Atmospheric reactions of polycyclic aromatic hydrocarbons with NO_2 or HNO_3 generate nitroaromatic compounds of which nitropyrenes are a major class.[1] They are also formed from incomplete combustions. As a result, nitropyrenes are ubiquitous in the environment and could arise from sources as diverse as grilled food and diesel exhaust.[1,2] Most of the nitropyrenes are mutagenic in bacterial[3] and mammalian[4] cells. They are also tumorigenic in experimental animals.[5] The mutagenicity and tumorigenicity of the nitropyrenes are believed to be mediated through partial nitroreduction by cellular enzymes followed by formation of covalent adducts with DNA. The partially reduced derivatives of 1-nitropyrene (1-NP) and 1,6- and 1,8-dinitropyrene (1,6- and 1,8-DNP) generate structurally similar adducts at the C8 position of guanine (Figure 1).[6] While 1-NP is the most abundant nitroaromatic compound in the environment, 1,6- and 1,8-DNP are the most potent mutagens and carcinogens in this

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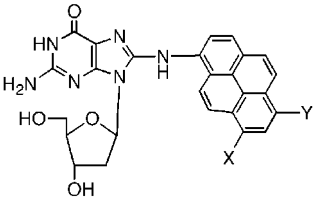

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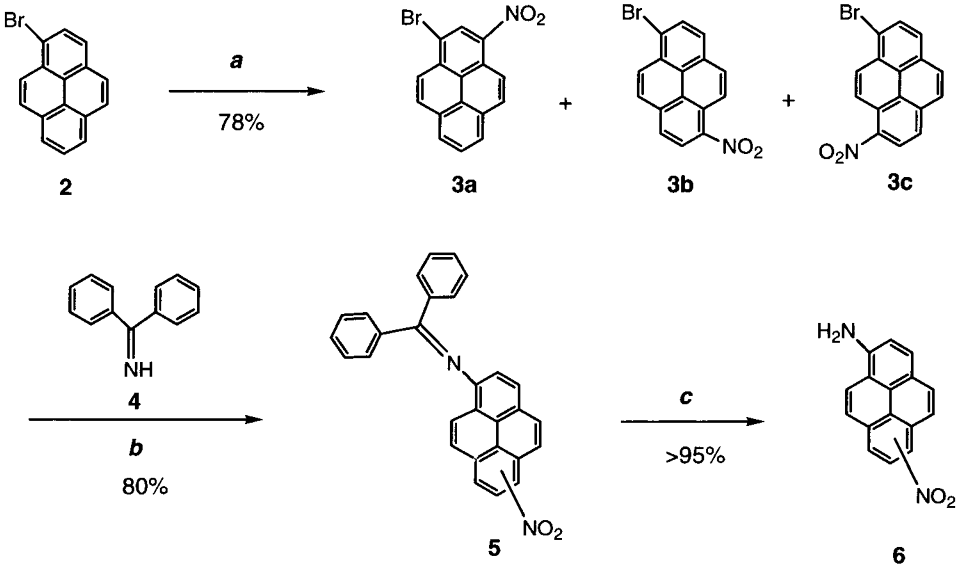
Figure 1. **a**. dG^{1-AP}: *N*-(deoxyguanosin-8-yl)-1-aminopyrene; x = y = H. **b**. dG$^{1,6-ANP}$: *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene; x = NO_2, y = H. **c**. dG$^{1,8-ANP}$: *N*-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene; x = H, y = NO_2.

group. The oligodeoxynucleotides containing these adducts at specific sites are useful probes for exploring the mechanism of mutagenesis and carcinogenesis. Indeed, site-specific introduction of the DNA adduct formed by 1-NP into an oligonucleotide allowed a series of structural and biochemical investigations.[7] However, the synthesis of oligonucleotides containing DNA adducts formed by the DNPs has not been reported. Herein we describe a convenient method for synthesis of the reductively activated derivatives of 1,6- and 1,8-DNP, their reaction with an oligonucleotide to form adducted oligonucleotides, and characterization of the latter.

1,6- and 1,8-DNP undergo a series of reductions in vivo to form nitroso, *N*-hydroxy, and amino derivatives. The ultimate carcinogen is believed to be either the *N*-hydroxy compound or an *O*-esterified derivative formed by an enzymatic conjugation. The initial DNA adduct formation is thought to proceed through an S_N2 reaction of these activated derivatives (or a nitrenium ion) with the N7 of guanine, which ultimately rearranges to the C8 adduct.[8] In a prior work we were successful in synthesizing adducted oligonucleotides containing *N*-(deoxyguanosin-8-yl)-1-aminopyrene (dG^{AP}) by allowing the unmodified oligonucleotides in duplex form to react with *N*-hydroxy-1-aminopyrene generated in situ from 1-nitrosopyrene.[9] However, the synthesis of aminonitropyrenes, the precursors of nitrosonitropyrenes, is not as straightforward. One published method of preparation involves nitration of 1-*N*-acetylaminopyrene followed by separation and hydrolysis of the products,[3c] whereas an alternate approach utilizes Zinin reduction of the dinitro compounds.[6b,10] Unfortunately, both methods gave very poor yields on our hands. Recent advances in Pd-catalyzed amination[11] provided an alternative route for the synthesis of the aminonitropyrenes in much better yield as shown in Scheme 1. Nitration of 1-bromopyrene (**2**) provided a mixture of bromonitropyrenes (**3a**,**b**,**c**) that was converted to a mixture of aminonitropyrenes (**6**) in two steps. While nitration only provided three mononitro derivatives in a 2:2:1 ratio as determined by GC-MS, their separation turned out to be difficult. The mixture of bromonitropyrenes, therefore, was converted to a mixture of benzophenone imine adducts by reflux with 1.2 equiv of benzophenone imine in the presence of $Pd(OAc)_2$, BINAP, and Cs_2CO_3 in THF for 16 h.[11a] Acidic cleavage of the mixture of benzophenone imine adducts was accomplished in high yield (>95%), and the resulting aminonitropyrenes were separated by chromatography on silica gel. The separation of the aminonitropyrenes was much more efficient than that of either the bromonitro or benzophenone imine derivatives. With this synthetic approach, from 2.1 mmol of 1-bromopyrene we were able

Scheme 1[a]



[a] CH_3COOH, HNO_3, rt, 24 h; [b] 1.2 equiv. **4**, 3 mol% $Pd(OAc)_2$, 4.5 mol% BINAP, 1.4 equiv. Cs_2CO_3, THF, reflux, 16 h; [c] 5% by vol 2 M HCl, THF, rt, 20 min.

to isolate 0.5 mmol each of 1-amino-6-nitropyrene and 1-amino-8-nitropyrene. A major advantage of this synthetic strategy is that it circumvents the selective reduction problem of the dinitro compounds.

Each purified isomer of aminonitropyrene was oxidized to nitrosonitropyrene using *m*-chloroperoxybenzoic acid. The nitrosonitropyrene was converted to the *N*-hydroxy derivative in situ in the presence of ascorbic acid, which was allowed to react with the self-complementary decamer, 5′-d(AAATGCATTT), in DMF−H_2O (1:9), pH 5.0−5.5 for 16−20 h at ambient temperature. HPLC analysis of the reaction mixture showed that the adducted decamer eluted more slowly than the unmodified oligonucleotide on a reverse-phase column (Figure 2) and that the typical yields were 10−15% for both the 1,6- and 1,8-isomers. As shown in Figure 3, the modified decamers have a slower mobility than the unmodified decamer in polyacrylamide gel electrophoresis. The site of adduction was established by treatment of the modified decamer with piperidine at 90 °C for 1 h. This reaction has been shown to cleave DNA at the sites of C8- or N7-alkylated guanines, similar to the Maxam−Gilbert G reaction.[12] Indeed, polyacrylamide gel electrophoresis of the piperidine-treated modified decamers (lanes 5 and 7) showed a band parallel to the Maxam−Gilbert G reaction (lane 3) of the unmodified decamer (Figure 4). HPLC analysis of the enzymatically digested adducted decamers revealed the absence of dG as well as the presence of a late-eluting peak that cochromatographed with an authentic sample of dG$^{1,6-ANP}$

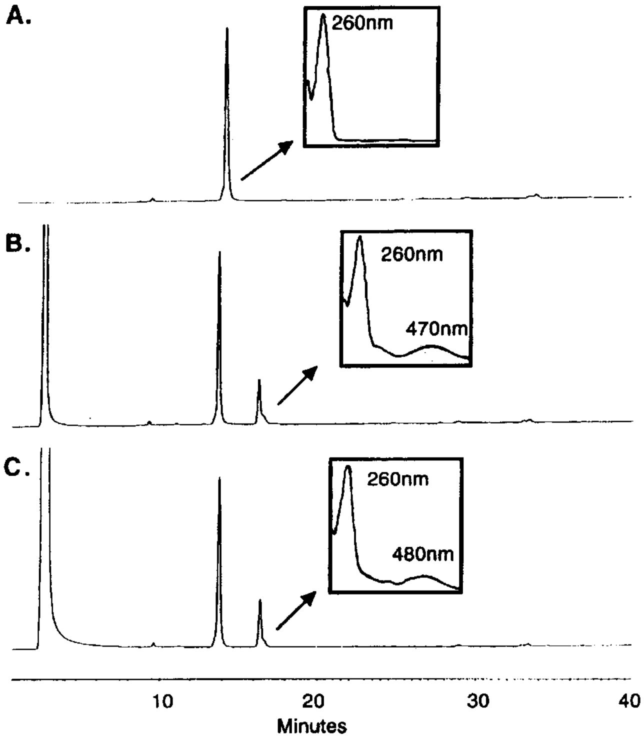


Figure 2. Reverse-phase HPLC chromatograms of oligonucleotides: (A) unreacted 10mer with the absorption spectrum in the inset; (B) crude reaction mixture of 10mer with *N*-hydroxy-1-amino-6-nitropyrene; absorption spectrum of the slower eluting adducted oligonucleotide is shown in the inset; (C) crude reaction of 10mer with *N*-hydroxy-1-amino-8-nitropyrene; absorption spectrum of the slower eluting adducted oligonucleotide is shown in the inset.

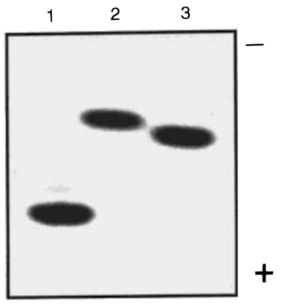


Figure 3. The ^{32}P-labeled samples were run on a 84 cm long 20% polyacrylamide gel for 26 h at 2500 V: lane 1, 5′-AAATGCATTT-3′; lane 2, 5′-AAATG$^{1,6-ANP}$CATTT-3′; lane 3, 5′-AAATG$^{1,8-ANP}$-CATTT-3′.

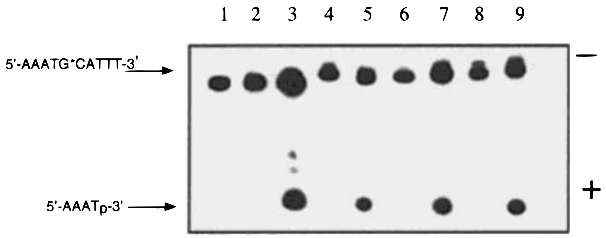


Figure 4. The ^{32}P-labeled samples were run on a 20% polyacrylamide gel for 2.5 h at 1800 V: 1, control 10mer; 2, control 10mer treated with piperidine (no DMS); 3, control 10mer treated with DMS followed by piperidine; 4, 10mer with 1,8-ANP adduct; 5, 10mer with 1,8-ANP adduct treated with piperidine; 6, 10mer with 1,6-ANP adduct; 7, 10mer with 1,6-ANP adduct treated with piperidine; 8, 10mer with 1-AP adduct; 9, 10mer with 1-AP adduct treated with piperidine.

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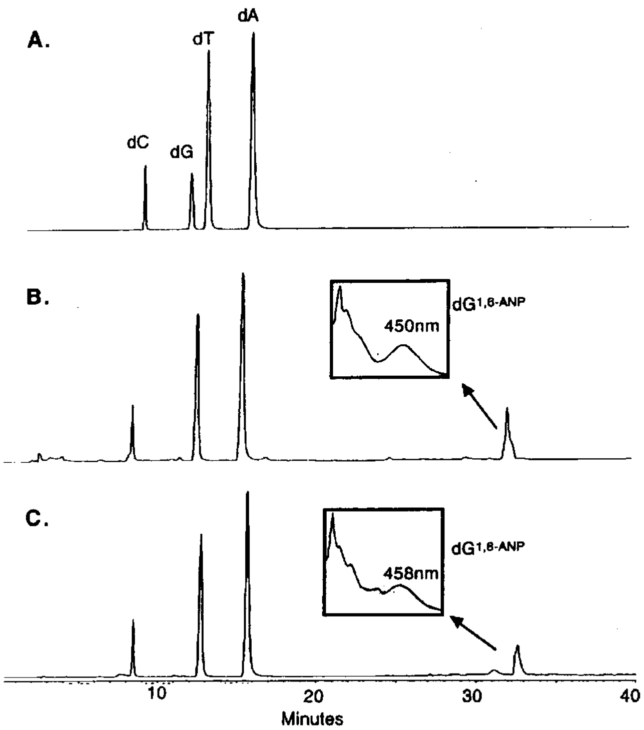

Figure 5. HPLC profile of enzymatically digested oligonucleotides: (A) digest of unmodified 5′-AAATGCATTT; (B) digest of 1,6-ANP-modified 5′-AAATG*CATTT with the inset showing the absorption spectrum of dG$^{1,6-\mathrm{ANP}}$; (C) digest of 1,8-ANP-modified 5′-AAATG*CATTT with the inset showing the absorption spectrum of dG$^{1,8-\mathrm{ANP}}$.

and dG$^{1,8-\mathrm{ANP}}$ as shown in Figures 5B and 5C, respectively.[13] The absorption spectrum of the adducted decamers with the $\lambda_{\max}$ at 470 and 480 nm showed a 20−25 nm red shift from 450 and 458 nm, respectively, for dG$^{1,6-\mathrm{ANP}}$ and dG$^{1,8-\mathrm{ANP}}$, suggesting a stacking interaction of the DNA bases with the pyrenyl residue. Additional evidence for the structure of the decamers was obtained by mass spectral analysis with electrospray ionization. Analysis of the adducted oligonucleotides gave a monoisotopic mass of 3284.63 and 3284.71 for the dG$^{1,6-\mathrm{ANP}}$- and dG$^{1,8-\mathrm{ANP}}$-containing decamers, respectively, which are in excellent agreement with the theoretical monoisotopic mass of 3284.62. Comparison of unmodified (*m*/*z* 3024.63) and adducted decamers gave a mass difference of 260, which corresponds to the expected value for the mass of the aminonitropyrene adduct.

In conclusion, we have developed a simple strategy to synthesize oligonucleotides containing the major DNA adducts formed by 1,6- and 1,8-DNP. These modified oligonucleotides will be valuable tools for the investigation of structural and biological properties of the carcinogen−DNA adducts.

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Supporting Information Available: Experimental procedures for the synthesis of **6**, ^{1}H NMR of 1,6- and 1,8-ANP, and the superimposed absorption spectra of ANP, dG$^{\mathrm{ANP}}$, and the adducted decamer. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The dG$^{\mathrm{ANP}}$ standards were isolated by reverse-phase HPLC following adduction of calf thymus DNA and subsequent enzymatic digestion. The dG adducts were characterized by ^{1}H NMR, which was found to be in agreement with the reported data.[6b,c] The absence of the singlet representing the C8 proton of guanine at ~7.9 ppm indicated that this position is the site of adduction. The MS analyses of both standards by ESI gave the correct mass of *m*/*z* 528 as well as fragmentation peaks of 412 and 366, corresponding to the loss of a sugar and a sugar plus the nitro group, respectively.