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Facile Interstrand Migration of the Hydrocarbon Moiety of a Dibenzo[*a,l*]pyrene 11,12-Diol 13,14-Epoxide Adduct at N^2 of Deoxyguanosine in a Duplex Oligonucleotide

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Abstract: When a synthesized deoxyribonucleotide duplex, 5′-CCATCG*CTACC-3′·5′-GGTAGCGATGG-3′, containing a trans 14*R* dibenzo[*a,l*]pyrene (DB[*a,l*]P) adduct, corresponding to trans opening of the (+)-(11*S*,12*R*)-diol (13*R*,14*S*)-epoxide by N^2 of the central G* residue, was allowed to stand for 2−6 days at ambient temperature in neutral aqueous solution, three new products were observed on denaturing HPLC. One of these corresponded to loss of the DB[*a,l*]P moiety from the original adducted strand to give an 11-mer with an unmodified central dG. The other two products resulted from a highly unexpected migration of the hydrocarbon moiety to either dG_5 or dG_7 of the complementary strand, 5′-GGTAG$_5$CG$_7$ATGG-3′. Enzymatic hydrolysis of the two 11-mer migration products followed by CD spectroscopy of the isolated adducted nucleosides indicated that, in both cases, the hydrocarbon moiety had undergone configurational inversion at C14 to give the cis 14*S* DB[*a,l*]P dG adduct. MS/MS and partial enzymatic hydrolysis showed that the major 11-mer had the hydrocarbon at dG_7. Two 11-mer oligonucleotides were synthesized with a single cis 14*S* DB[*a,l*]P dG adduct either at G_7 or at G_5 and were found to be chromatographically identical to the major and minor migration products, respectively. Although HPLC evidence suggested that a small extent of hydrocarbon migration from the trans 14*S* DB[*a,l*]P dG diastereomer also occurred, the very small amount of presumed migration products from this isomer precluded their detailed characterization. This interstrand migration appears unique to DB[*a,l*]P adducts and has not been observed for their fjord-region benzo[*c*]phenanthrene or bay-region benzo[*a*]pyrene analogues.

Polycyclic aromatic hydrocarbons (PAHs) are common environmental contaminants that are metabolically activated to mutagenic and carcinogenic bay- or fjord-region diol epoxides.[1−3] These diol epoxides react with DNA to form adducts via ring opening at the reactive benzylic position, primarily by the exocyclic amino groups, N^6 of deoxyadenosine (dA) and N^2 of deoxyguanosine (dG).[4] Dibenzo[*a,l*]pyrene (DB[*a,l*]P) is the most potent PAH carcinogen identified to date.[5,6] The postulated ultimate carcinogens, DB[*a,l*]P 11,12-diol 13,14-epoxides (Figure 1), possess both a pyrene ring system, analogous to that of the highly reactive and carcinogenic benzo[*a*]pyrene 7,8-diol 9,10-epoxide, and a hindered fjord region, analogous to the much less reactive,[7] but even more strongly carcinogenic[8] benzo[*c*]-phenanthrene 3,4-diol 1,2-epoxide. We wish to determine how this combination of structural features affects the reactivity of the DB[*a,l*]P diol epoxides as well as the conformations of their DNA adducts, with a view to understanding the high carcinogenicity of this hydrocarbon. Here, we report the surprising migration of the hydrocarbon moiety from a dG residue in a synthesized 11-mer oligonucleotide containing a site-specific DB[*a,l*]P dG adduct to a neighboring dG residue on its complementary strand in a DNA duplex. This migration, to date unknown for adducts from other PAHs, occurs under very mild conditions (near-neutral aqueous solution at ambient temperature) that are normally innocuous when used for handling such modified DNA duplexes. This facile migration constitutes a

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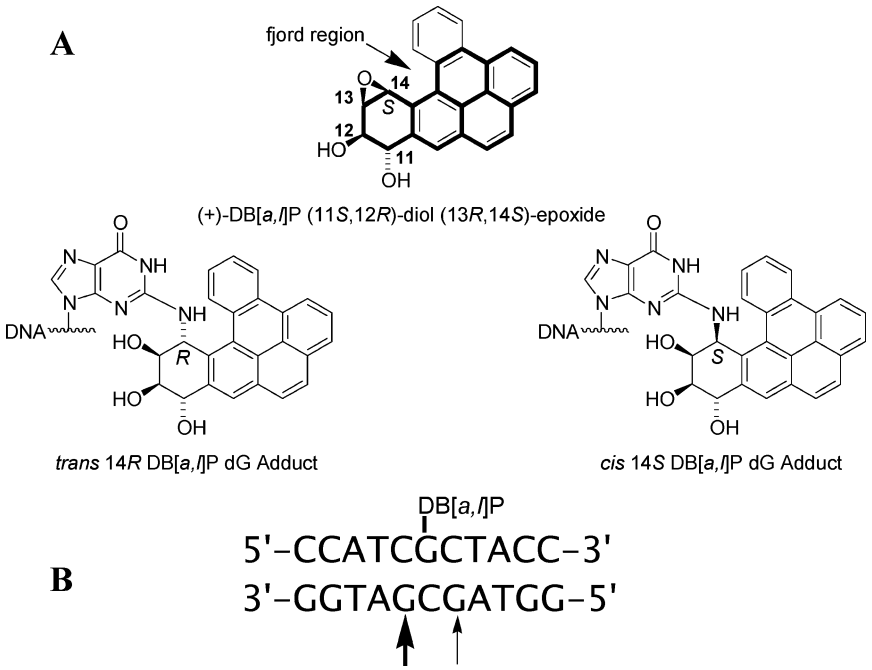

Figure 1. (A) Structures of DB[*a*,*l*]P (11*S*,12*R*)-diol (13*R*,14*S*)-epoxide and its derived trans and cis dG adducts in DNA. The benzo[*a*]pyrene ring system is shown with heavy lines, and the fjord region of DB[*a*,*l*]P is indicated by the arrow. (B) The duplex oligonucleotide showing the initial position of the adduct and the major (heavy arrow) and minor (light arrow) sites of migration of the hydrocarbon to the complementary strand.

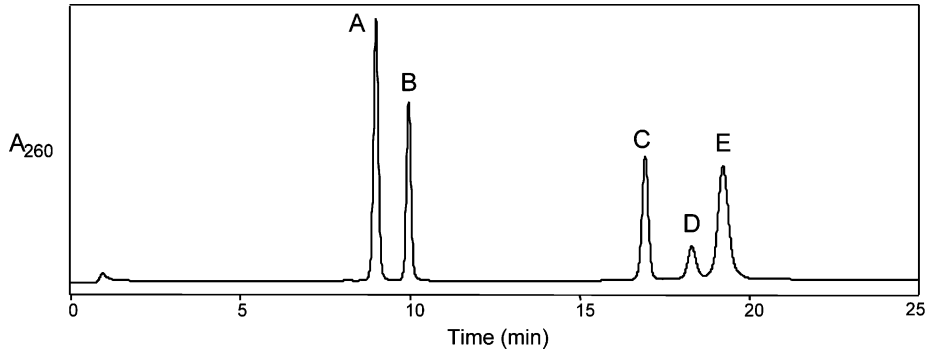

Figure 2. HPLC traces at 55 °C of the adducted 11-mer strand (E) and its complement (A) after 6 days in 10 mM 2-(*N*-morpholino)ethanesulfonic acid buffer, pH 6.5, at ambient temperature. Chromatography was run on a Waters Xterra MS C_{18} column (4.6 × 50 mm) eluted at 1 mL/min with a linear gradient that increased the proportion of solvent B in solvent A from 5% to 20% in 15 min, followed by isocratic elution with 20% B, where A is 0.1 M $(NH_4)_2CO_3$, pH 7.0, and B is a 1:1 mixture of A with CH_3CN.

remarkable new mechanism for induction of mutations by these adducts at site(s) adjacent to the initially modified base in DNA.

Results

An oligonucleotide 11-mer containing an adduct (Figure 1A) corresponding to trans opening of the (+)-(11*S*,12*R*)-diol (13*R*,-14*S*)-epoxide of DB[*a*,*l*]P by N^2 of the central dG residue (trans 14*R* DB[*a*,*l*]P dG) was prepared and converted to the 1:1 duplex (Figure 1B) with its fully complementary, unmodified strand. The correct duplex stoichiometry was determined by chromatography on hydroxyapatite of the annealed duplex (see Experimental Section). When the duplex was allowed to remain at ambient temperature and near-neutral pH for 2−6 days and then analyzed by HPLC under denaturing conditions (55 °C), three peaks in addition to the original two were reproducibly observed from several different samples of the duplex (Figure 2). Two of these newly formed products (peaks C and D) showed long-wavelength (344 nm) UV spectra similar to that of the adducted top strand (Figure 3) indicative of a pyrene chromophore. Our initial assumption was that the new peaks were a result of decomposition of the top (adducted) strand after duplex formation, with peak B resulting from loss of the

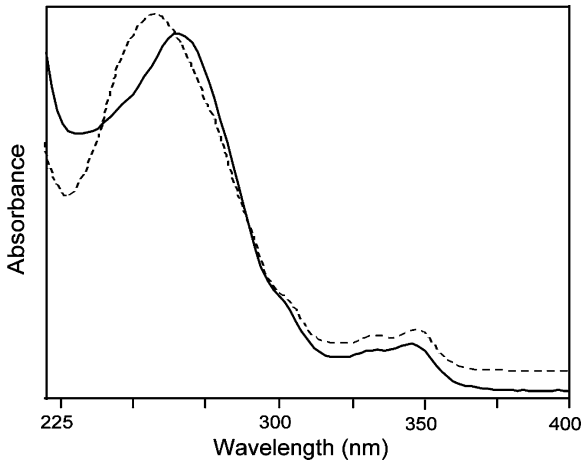

Figure 3. Comparison of the UV spectra from HPLC diode-array detection of C (broken line) and the parent adducted oligonucleotide E (solid line).

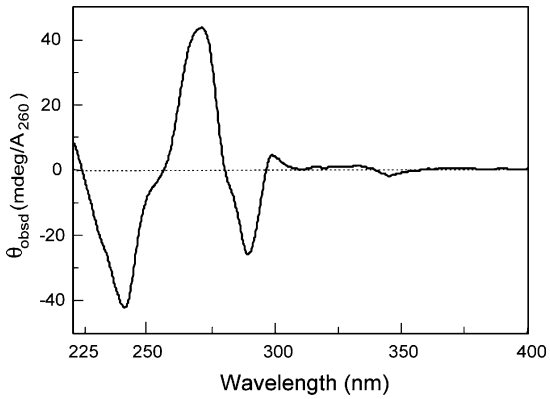

Figure 4. CD spectrum (MeOH, normalized to 1.0 A_{260}) of the nucleoside adduct derived from oligonucleotide C.

hydrocarbon modification and peaks C and D resulting from loss of base(s) or residue(s) not containing the hydrocarbon. The effect of varying pH between 5.5 and 8.0 on the extent of formation of these new products was investigated. Optimal formation of the major, adducted product C occurred at pH 6−7 (~40% of total peaks containing the A_{344} pyrene chromophore after 6 days at room temperature). The relative yield of C decreased about 2-fold at pH 8.0 and also appeared to fall off slightly at pH 5.5. Consequently, further reactions to isolate the products for characterization were routinely run at pH 6.5. Peaks B, C, and D were isolated by HPLC for further characterization. Peak B, which did not contain the hydrocarbon chromophore, gave a parent ion on low resolution MALDI-TOF MS with *m*/*z* = 3237.7, which corresponded to the expected mass (3237.2) for the top strand of the duplex with a central dG lacking the hydrocarbon moiety. Its HPLC retention time and UV spectrum were identical to those of the authentic unadducted top strand.

Characterization of Major Adducted Product C. Both products C and D gave the same molecular ion (*m*/*z* 3790) on MALDI-TOF MS, whose mass was greater than that of the original pyrimidine-rich top strand including the adduct (*m*/*z* 3589.6) and thus could not have been a result of any loss from this adducted strand. This mass did, however, match the value of 3789.7 calculated for the purine-rich complementary strand containing a DB[*a*,*l*]P adduct. This observation was unlikely to have resulted from a simple, physical association of the hydrocarbon with the complementary strand, because such physical complexes were not expected to survive either high temperature HPLC or mass spectrometry without dissociation. Complete enzymatic digestion of oligonucleotide C and characterization of the resultant nucleoside adduct unambiguously confirmed the conclusion that the adduct is covalently bound to the bottom strand. Oligonucleotide C was digested[9,10] with snake venom phosphodiesterase (VPD) and alkaline phosphatase, and the nucleoside digestion product containing a 344 nm chromophore was isolated by HPLC and characterized by ESI MS (calcd for a DB[*a*,*l*]P dG adduct [cf., Figure 1], $C_{34}H_{30}N_5O_7$, 620.4; found, 620.3) and circular dichroism (CD) spectrometry. The observed CD spectrum shown in Figure 4 was identical to that of an independently synthesized sample of the cis opened 14*S* DB[*a*,*l*]P dG adduct. Note that this represents an inversion of absolute configuration at C14 such as would be expected from an S_N2 substitution reaction at C14 of the original trans 14*R* adduct by the exocyclic N^2-amino group of a bottom-strand guanine.

Because there are two guanines on the complementary strand flanking the adducted guanine on the top strand, either one was a candidate for the position of migration. A previous study had demonstrated the utility of MS/MS[11] to determine the exact location of an adduct. As shown in Figure 5, the oligonucleotide can fragment into two pieces, (a_n−B_n) (derived from the 5′-end) and w_m (derived from the 3′-end). Note that each fragment derived from the 5′-end has also undergone loss of the adducted or nonadducted base at its 3′-end. The major fragments observed from oligonucleotide C are listed in Figure 5. The fragmentation immediately 3′ to the adducted residue to give (a_7−B_7) and w_4, neither of which contained the hydrocarbon, was most diagnostic because the (a_7−B_7) fragment must have lost the adducted G_7 via depurination prior to its 3′-cleavage. The presence of the PAH moiety in the w_5 fragment also provided evidence that the hydrocarbon must be on a residue 3′ to position C_6. Thus, it was concluded that the PAH is bonded to the guanine (G_7) closer to the 3′-end of oligonucleotide C.

The location of the adduct was confirmed by partial enzyme digestion. It has been shown that oligonucleotides containing bulky PAH adducts are resistant to exonucleolytic hydrolysis at or before the adducted bases by both VPD and bovine spleen (SPD) phosphodiesterases.[10,12−15] By minimizing the amount of enzyme used as well as reaction time, partially digested oligonucleotides can be isolated and the site of PAH attachment deduced. Thus, oligonucleotide C was subjected to partial hydrolysis by limiting amounts of SPD, which digests single-stranded oligonucleotides from the 5′-end, sequentially liberating nucleoside 3′-monophosphates. On the basis of the MS/MS data, it was predicted that stalling of the enzyme between C_6 and the modified G_7 would produce a 5-mer that contained the adduct at G_7. Identification of such a partial digestion product from oligonucleotide C would confirm that the adduct is not at position 5. Digestion with a limited amount of SPD was quenched by heating after 5 min of reaction, and the major reverse-phase HPLC peak with absorbance at 344 nm was collected and analyzed by ESI-mass spectrometry. The major signal observed was at *m*/*z* = 946.7, which corresponded to the expected 5-mer, 5′-$G_7A_8T_9G_{10}G_{11}$-3′, containing a DB[*a*,*l*]P adduct ($[M - 2H]^{2-}$ = 946.2). This result thus confirmed the conclusion that the hydrocarbon was located on G_7 as deduced from the MS/MS data.

Characterization of Minor Adducted Product D. Given that the major product C was the result of migration of the hydrocarbon to G_7, it seemed likely that the minor product (peak D, Figure 1) resulted from migration to G_5 on the 5′-side of the complementary strand. Enzymatic hydrolysis to nucleosides with VPD and alkaline phosphatase gave the same cis 14*S* DB[*a*,*l*]P dG adducted nucleoside as obtained from C, as shown by its

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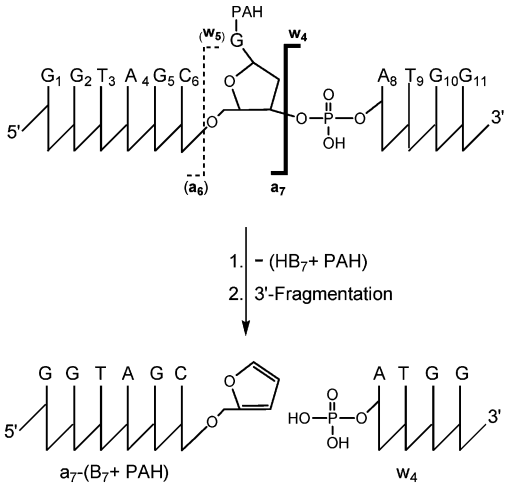

Fragment	m/z obsd (calcd)	Adducted	Fragment	m/z obsd (calcd)	Adducted
			w_{10}^{3-}	1178.6 (1178.6)	Yes
$(a_2\text{-}B_2)^-$	426.1 (426.1)	No	w_9^{2-}	1603.9 (1603.8)	Yes
$(a_3\text{-}B_3)^-$	—		w_8^{2-}	1451.8 (1451.8)	Yes
$(a_5\text{-}B_5)^-$	1372.0 (1372.2)	No	w_6^{2-}	1130.7 (1130.7)	Yes
$(a_6\text{-}B_6)^-$	—		w_5^{2-}	986.4 (986.2)	Yes
$(a_6\text{-}B_6)^{2-}$	850.3 (850.2)	No	w_5^-	—	
$(a_7\text{-}B_7)^{2-}$	994.7 (994.7)	No	w_4^-	1292.0 (1292.2)	No
$(a_8\text{-}B_8)^{2-}$	1335.4 (1335.3)	Yes	w_3^-	979.1 (979.2)	No

Figure 5. Assignment of MS/MS fragments observed from oligonucleotide C. Superscripts designate the charge of each fragment. The heavy line shows the cleavage that produces the diagnostic fragments (a_7–B_7) and w_4, whereas the dotted line shows the cleavage that produces (a_6–B_6) and w_5. *m*/*z* values above 2000 were outside the range of detection.

CD spectrum. Thus, this migration product also resulted from an inversion of configuration at C14. Insufficient amounts of oligonucleotide D were isolated to obtain satisfactory mass spectral results from either MS/MS or partial enzymatic hydrolysis experiments. In an alternative approach, the complementary strand oligonucleotide, 5′-GGTAG*$_5$C$_6$G$_7$ATGG-3′, was synthesized with a cis 14*S* DB[*a*,*l*]P adduct at the G*$_5$ position and found to be co-chromatographic with oligonucleotide D.

In addition, the synthesized oligonucleotide was subjected to partial enzyme digestion with SPD, and the resultant product was identified by MS as the 7-mer 5′-G*$_5$C$_6$G$_7$A$_8$T$_9$G$_{10}$G$_{11}$-3′ resulting from cleavage between A$_4$ and adducted G*$_5$. This partial digestion product was chromatographically identical to the corresponding partial digestion product obtained from oligonucleotide D. Thus, the most likely conclusion is that minor oligonucleotide D resulted from migration of the hydrocarbon to G$_5$.

Although our standard reaction conditions for observing migration were 2−6 days at ambient temperature, elevated temperature increased the rate of migration, and ∼70% conversion to migration products was observed after 4−5 days at 37 °C. No reverse migration from a synthesized bottom strand containing a cis 14*S* DB[*a*,*l*]P adduct at G$_7$ (major product C) to an unmodified top strand was detected after 2 days of incubating this duplex at 37 °C. These observations indicate qualitatively that the equilibrium for the transfer reaction strongly favors the observed migration products. This most likely reflects a stereochemical preference for hydrocarbon migration exhibited by the trans 14*R* adduct (rather than a sequence preference for migration to the bottom strand). Notably, we observed that this cis 14*S* adduct at position 6 of the top strand did not undergo significant migration (≤6%) even after 6 days at ambient temperature under our standard conditions (data not shown). We did, however, obtain evidence for a slow interstrand migration with the duplex containing the diastereomeric trans 14*S* adduct in the top strand. When a sample of this duplex was left at ambient temperature for 6 days, the HPLC trace of the mixture showed three new peaks, analogous to the DNA duplex containing the trans 14*R* isomer (Figure 6). However, the extent of reaction was much less than that observed with the 14*R* diastereomer in the same time period (cf., Figure 2). Notably, however, migration of the DB[*a*,*l*]P moiety of the cis 14*S* adduct (see above) is even slower than that observed (Figure 6) for the trans adduct with the same absolute configuration at C14. MALDI-TOF MS data for the small peaks C′ and D′

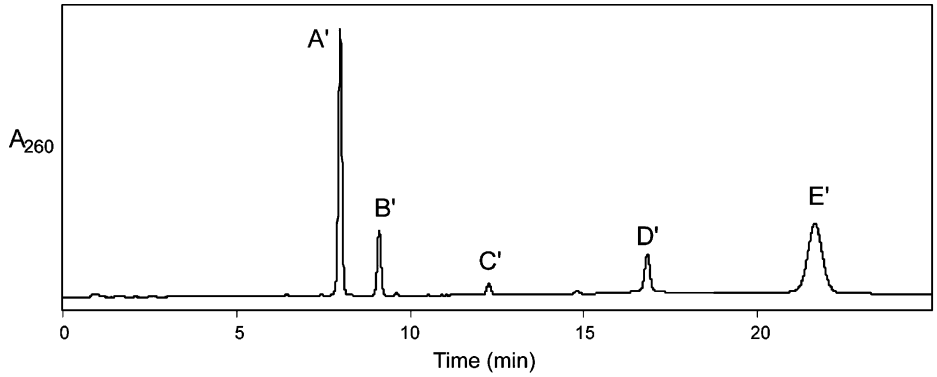

Figure 6. HPLC trace at 55 °C of the 11-mer duplex containing the trans 14*S* DB[*a*,*l*]P adduct after incubation for 6 days at pH 6.5. Peaks labeled C′ and D′ are newly formed products containing the hydrocarbon chromophore on the bottom strand. Note that reaction conditions and chromatographic conditions are the same as for Figure 2.

matched the calculated mass of the complementary strand containing a DB[*a*,*l*]P adduct, and the VPD digestion product from major peak D′ had a CD spectrum identical to that of an authentic sample of the cis 14*R* DB[*a*,*l*]P dG adduct, indicative of inversion of configuration at C14.

Discussion

The results presented here demonstrate a remarkable migration of a PAH moiety from a covalent dG adduct on one strand of a duplex oligonucleotide to a vicinal dG on its complement under mild experimental conditions that are typical for handling such duplexes. The reaction is only weakly pH-dependent near neutrality and is hence unlikely to involve a carbocation intermediate whose formation should be acid catalyzed. Furthermore, the inversion of configuration that occurs is consistent with S_N2 substitution at C14 of the adduct by the 2-amino group of the acceptor dG.

Hydrocarbon migration from one base to another within a modified DNA duplex has not previously been documented, and the existence of unambiguous NMR data[16,17] for duplexes containing benzo[*a*]pyrene dG adducts in the same sequence as the present one indicates that these duplexes do not undergo any migration under the NMR conditions of near-neutral pH and ambient temperatures. We initially speculated that the highly hindered fjord region might be responsible for the novel behavior of the present DB[*a*,*l*]P adducts. Benzo[*c*]phenanthrene (B[*c*]-Ph) is a prototype of a hydrocarbon that also contains a fjord region. Although migration of B[*c*]Ph appeared to be unlikely based on available NMR data for the trans 1*R* dG B[*c*]Ph adduct[18] in the same DNA duplex sequence as the present trans 14*R* DB[*a*,*l*]P adduct, we investigated this possibility and confirmed that such migration did not occur in this sequence. No new chromatographic peaks corresponding to migration were observed after incubation of this duplex at pH 6.5 for 2 days either at ambient temperature or at 37 °C. Although the lack of observable migration of the B[*c*]Ph moiety may be due in part to low chemical reactivity,[7] we speculate that differences in the relative orientations of the DB[*a*,*l*]P and B[*c*]Ph dG adducts may also contribute to the ability of the DB[*a*,*l*]P moiety to undergo this unique interstrand transfer. Examination of an NMR derived structure[18] of the 1*R* B[*c*]Ph−dG adducted duplex, which has the hydrocarbon intercalated to the 3′-side of the modified guanine base, suggests that this B[*c*]Ph adduct is poorly oriented for S_N2 substitution at C1 by either the G_5 or the G_7 of the complementary strand. Thus, for a putative migration to the exocyclic amino group of G_7 in the B[*c*]Ph adducted duplex (corresponding to the major migration product from the DB-[*a*,*l*]P adducted duplex), the C1−N^2 distance is 4.4 Å and the N−C−N angle between the entering and leaving nitrogen atoms is 59°. This angle is inconsistent with an S_N2 mechanism that would account for the inversion of configuration observed with the DB[*a*,*l*]P adduct. For formation of the postulated minor migration product at the complementary strand G_5, a more reasonable N−C−N angle of 124° for S_N2 substitution was measured, but the distance between C1 of the B[*c*]Ph adduct and the incoming N^2 of G_5 is large (5.7 Å). Detailed molecular conformations of the DB[*a*,*l*]P adducted oligonucleotide duplexes have yet to be determined.

The implications of facile interstrand migration for the biological activity of DB[*a*,*l*]P adducts are substantial, because an adduct formed on a specific base has now been shown to have the potential to migrate to neighboring base(s) on the complementary strand, thereby enabling mutations to occur at one or more sites that would not have been predicted based on the initial adduct position. The possibility of such migration obviously complicates the interpretation of site-specific mutagenesis studies. Furthermore, transcription coupled DNA repair, a process which may be initiated when transcription is blocked by bulky PAH adducts,[19] is specific for the transcribed strand. An adduct on the nontranscribed strand would escape one round of transcription-coupled repair; if the hydrocarbon subsequently migrates to the transcribed strand, this strand would need to undergo another round of repair. Because repair is not 100% efficient, migration makes possible a second chance for the adduct to compromise normal transcription. Although the present results are limited to migration of a DB[*a*,*l*]P adduct to a neighboring dG residue on the opposite strand, the scope of this reaction is at present unknown. Possible transfer to dC or dA residues in different sequence contexts as well as transfer from dA adducts remains to be evaluated. It is also not known to what extent the rest of the DNA sequence surrounding the

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donor and acceptor residues could influence the rate and extent of such transfer reactions.

Experimental Section

Oligonucleotides. Syntheses of the diastereomerically pure DB[*a*,*l*]P adducted nucleosides and phosphoramidites will be reported elsewhere. Unmodified oligonucleotides were obtained commercially (Oligos Etc., Wilsonville, OR). The single-stranded adducted 11-mers, 5′-CCA-TCG*CTACC-3′, with a DB[*a*,*l*]P adduct at the central G* were prepared by a semi-automated procedure as previously described[20] utilizing the diastereomerically pure trans 14*R* or 14*S* DB[*a*,*l*]P dG phosphoramidite. The 14*R* adducted oligonucleotide was purified by HPLC on a Waters Xterra MS C_{18} column (2.5 μm, 10 × 50 mm) eluted at 55 °C at 3 mL/min with a linear gradient, which increased the proportion of solvent B in solvent A from 5% to 20% in 15 min, where solvent A is 0.1 M $(NH_4)_2CO_3$, pH 7.5, and solvent B is a 1:1 mixture of solvent A with CH_3CN at the same pH (t_R 10.3 min). The 14*S* adducted oligonucleotide was purified by HPLC on the same column at 45 °C eluted with a linear gradient that increased the proportion of solvent B in solvent A from 15% to 28% in 12 min (t_R 8.7 min). Duplex stoichiometry was determined on a small scale as follows. An excess of each adducted strand was mixed with its complementary strand and annealed. The 14*R* duplex was isolated by HPLC at 8 °C on a TosoHaas HA-1000 hydroxyapatite column (7.5 × 75 mm), eluted at 1 mL/min with a linear gradient of 0.5 M sodium phosphate buffer (pH 7.0) in water that increased the buffer composition from 4% to 20% over 20 min. The 14*S* duplex was chromatographed on the hydroxyapatite column eluted at 4 °C with a phosphate buffer composition of 4% for 5 min followed by a linear gradient that increased the buffer composition to 13% over the next 8 min. In both cases, the duplex was significantly retained (6−12 min), whereas the excess adducted single strand eluted at ∼2.8 min. The isolated duplexes were desalted, lyophilized, and then separated into single strands by reverse phase HPLC at 55 °C, and the absorbance ratio of the single strands, collected separately, was determined at 260 nm. For larger scale duplex formation, the adducted and complementary strands were mixed according to the absorbance ratio of adducted to complementary strand thus obtained to obtain a 1:1 duplex: 1.0/0.9 A_{260} for 14*S* and 1.0/1.1 A_{260} for 14*R* adducted duplexes. Because it was noted that salts and divalent cations retard the rate of migration, DB[*a*,*l*]P-adducted and unadducted oligonucleotides were treated with Chelex 100 resin (BioRad) and lyophilized to dryness prior to use.

Bottom-strand oligonucleotide 11-mers, 5′- GGTAG*$_5$CG$_7$ATGG-3′ and 5′- GGTAG$_5$CG*$_7$ATGG-3′, with a cis 14*S* DB[*a*,*l*]P adduct at either of the starred positions, as well as a top-strand oligomer containing the cis 14*S* DB[*a*,*l*]P dG adduct at the central guanine, were synthesized as described[20] utilizing the cis 14*S* DB[*a*,*l*]P dG phosphoramidite in the manual coupling step. The bottom strand 11-mers were purified by HPLC as above on the Waters Xterra MS C_{18} column eluted at 55 °C with a linear gradient of solvent B in A (see above) that increased the percentage of B from 5% to 25% over 20 min; t_R for the G_5 adducted 11-mer, 16.6 min, and for the G_7 adducted 11-mer, 15.1 min; t_R for the top strand 11-mer with the cis 14*S* DB[*a*,*l*]P adduct at G_6, 18.7 min. Duplexes were formed by annealing each of these oligonucleotides with an unadducted complementary strand in a ratio of 1:1 based on A_{260}. The 11-mer sequence 5′-CCATCG*CTACC-3′ containing a trans 1*R* B[*c*]Ph dG adduct at the central position, a previously reported construct,[18] was similarly prepared utilizing the diastereomerically pure trans 1*R* B[*c*]Ph dG phosphoramidite[21] and converted to its duplex.

MS/MS Analysis of Oligonucleotide C. NanoElectrospray experiments were carried out using direct infusion (flow rate 1 μL/min) in negative ion mode. Full spectra were recorded over an *m*/*z* range from 510 to 2000. The precursor ion with *m*/*z* 1262 corresponding to the triply charged adducted oligonucleotide was selected manually, and a normalized collision energy of 45% was applied to form fragments in the ion trap. MS/MS data were processed manually.

Enzymatic Digestion to Nucleosides. Both migration products, C and D, from the 14*R* DB[*a*,*l*]P adduct as well as the major migration product, D′, from the 14*S* adduct were digested to the nucleoside level as follows. Each single-stranded oligonucleotide was digested at 37 °C for 18 h with a ratio of 10 units of VPD (Worthington) per 1 A_{260} of oligonucleotide in 500 mL of 40 mM Tris-HCl pH 9.0 buffer, containing 0.2% NaN_3 and 10 mM $MgCl_2$. The reaction was quenched by heating in boiling water for 2 min. *E. coli* alkaline phosphatase (Sigma-Aldrich) (2 units) was added, and the mixture was incubated for another 3 h at 37 °C. The reaction mixtures were separated by HPLC on a Beckman Ultrasphere C_{18} column, 4.2 × 250 mm, eluted at 1 mL/min with 50 mM sodium phosphate buffer, pH 7.0 (containing 5% MeOH), for 8 min, followed by a linear gradient of MeOH (containing 10% H_2O) that increased the percentage of the less-polar solvent to 10% over 7 min, followed by a linear gradient to 100% over the next 15 min. The hydrocarbon-containing products (absorbance at 344 nm) derived from digestion of both oligonucleotides C and D, as well as from D′, all of which elute at ∼31 min, were isolated and characterized by CD spectroscopy.

Partial Enzymatic Digestion. Oligonucleotide C from the trans 14*R* DB[*a*,*l*]P adducted duplex was partially digested by use of a ratio of 0.5 unit of SPD (Sigma Aldrich) per A_{260} of oligonucleotide in 0.5 mL of 150 mM NaOAc buffer (pH 6.0) at 37 °C for 5 min. Partial digestion of the minor oligonucleotide D, as well as the two synthesized oligonucleotides corresponding to the bottom strand with a cis 14*S* DB-[*a*,*l*]P adduct at G_5 or G_7, was carried out under the same conditions, but with a ratio of enzyme to oligonucleotide of 0.1 unit SPD per A_{260} and an incubation time of 15 min. Reactions were quenched by heating. HPLC separation utilized a Waters Xterra MS C_{18} column (4.6 × 50 mm) eluted at 1 mL/min with a linear gradient that increased the proportion of solvent B in solvent A from 10% to 30% in 20 min. Retention times (min) for the adducted short oligonucleotides resulting from partial digestion were as follows: product from oligonucleotide C (major migration product from trans 14*R* duplex), 19.8; product from oligonucleotide D (minor migration product from trans 14*R* duplex), 16.8; product from synthetic bottom strand with cis 14*S* adduct at G_7, 19.8; product from synthetic bottom strand with cis 14*S* adduct at G_5, 16.8.

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