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3'-Intercalation of a *N*²-dG 1*R-trans-anti*-Benzo[*c*]phenanthrene DNA Adduct in an Iterated (CG)₃ Repeat

Yazhen Wang[†], Nathalie C. Schnetz-Boutaud[†], Heiko Kroth[‡], Haruhiko Yagi[‡], Jane M. Sayer[‡], Subodh Kumar[§], Donald M. Jerina[‡], and Michael P. Stone^{*,†}

[†]Department of Chemistry, Center in Molecular Toxicology, and the Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee 37235

[‡]Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, DHHS, Bethesda, Maryland 20892

[§]Environmental Toxicology and Chemistry Laboratory, Great Lakes Center, State University of New York College at Buffalo, Buffalo, New York 14222

Abstract

The conformation of the 1*R,2S,3R,4S*-benzo[*c*]phenanthrene-*N*²-dG adduct, arising from *trans* opening of the (+)-1*S,2R,3R,4S*-*anti*-benzo[*c*]phenanthrene diol epoxide, was examined in 5'-d(ATCGC^XCG-GCATG)-3'-5'-d(CATGCCG^CGCGAT)-3', where X = 1*R,2S,3R,4S*-B[*c*]P-*N*²-dG. This duplex, derived from the *hisD3052* frameshift tester strain of *Salmonella typhimurium*, contains a (CG)₃ iterated repeat, a hotspot for frameshift mutagenesis. NMR experiments showed a disconnection in sequential NOE connectivity between X⁴ and C⁵, and in the complementary strand, they showed another disconnection between G¹⁸ and C¹⁹. In the imino region of the ¹H NMR spectrum, a resonance was observed at the adducted base pair X⁴•C¹⁹. The X⁴ N1H and G¹⁸ N1H resonances shifted upfield as compared to the other guanine imino proton resonances. NOEs were observed between X⁴ N1H and C¹⁹ N⁴H and between C⁵ N⁴H and G¹⁸ N1H, indicating that base pairs X⁴•C¹⁹ and C⁵•G¹⁸ maintained Watson–Crick hydrogen bonding. No NOE connectivity was observed between X⁴ and G¹⁸ in the imino region of the spectrum. Chemical shift perturbations of greater than 0.1 ppm were localized at nucleotides X⁴ and C⁵ in the modified strand and G¹⁸ and C¹⁹ in the complementary strand. A total of 13 NOEs between the protons of the 1*R*-B[*c*]Ph moiety and the DNA were observed between B[*c*]Ph and major groove aromatic or amine protons at base pairs X⁴•C¹⁹ and 3'-neighbor C⁵•G¹⁸. Structural refinement was achieved using molecular dynamics calculations restrained by interproton distances and torsion angle restraints obtained from NMR data. The B[*c*]Ph moiety intercalated on the 3'-face of the X⁴•C¹⁹ base pair such that the terminal ring of 1*R*-B[*c*]Ph threaded the duplex and faced into the major groove. The torsion angle α' [X⁴]-N3-C2-N2-B[*c*]Ph-C1 was calculated to be -177°, maintaining an orientation in which the X⁴ exocyclic amine remained in plane with the purine. The torsion angle β' [X⁴]-C2-N2-[B[*c*]Ph]-C1-C2 was calculated to be 75°. This value governed the 3'-orientation of the B[*c*]Ph moiety with respect to X⁴. The helical rise between base pairs X⁴•C¹⁹ and C⁵•G¹⁸ increased and resulted in unwinding of the right-handed helix. The aromatic rings of the B[*c*]Ph moiety were below the Watson–Crick

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*To whom correspondence should be addressed. Tel: 615-322-2589. Fax: 615-322-7591. E-mail: michael.p.stone@vanderbilt.edu.

Supporting Information Available: Chemical shifts of nonexchangeable protons for the 1*R*-B[*c*]Ph-adducted *hisD3052* oligodeoxynucleotide (Table S1), chemical shifts of exchangeable protons for the 1*R*-B[*c*]Ph-adducted *hisD3052* oligodeoxynucleotide (Table S2), and experimental distance restraints used in the rMD calculations for the 1*R*-B[*c*]Ph-adducted *hisD3052* oligodeoxynucleotide (Table S3). Force field parameters for the B[*c*]Ph moiety (Figure S1), the rMD-simulated annealing protocol (Figure S2), and an overlay of the current structure with the previously determined structure of the 1*R*-B[*c*]Ph adduct in the 5'-CXC-3' sequence (30) (Figure S3). This material is available free of charge via the Internet at <http://pubs.acs.org>.

hydrogen-bonding face of the modified base pair X⁴•C¹⁹. The B[c]Ph moiety was stacked above nucleotide G¹⁸, in the complementary strand.

Introduction

Human exposure to polycyclic aromatic hydrocarbons (PAH)¹ comes from a combination of environmental, dietary, and lifestyle factors such as tobacco usage (1). As compared to the diol epoxide metabolites of the bay region PAHs, for example, benzo[*a*]pyrene (B[*a*]P), those of the fjord region PAHs, which include benzo[*c*]phenanthrene (B[*c*]Ph), are more tumorigenic (2–8) and thus, these PAHs have been of considerable interest. Fjord region PAHs undergo cytochrome P450-mediated (9,10) oxidation to diastereomeric *syn*- and *anti*-diol epoxides (11–13), including the mutagenic *anti*-B[*c*]Ph diol epoxides (14,15). Reaction of (+)- and (–)-*anti*-B[*c*]Ph diol epoxides with the exocyclic amines of purines forms the corresponding *cis*- and *trans-anti*-adducts (4,16,17) with *trans* addition being favored (Scheme 1) (18). The *anti*-B[*c*]Ph diol epoxide reacts more extensively with adenine than with guanine (16,19–22), although both adenine and guanine adducts are found in vivo (20,21) and modulate the biological processing of DNA. Site-specific mutagenesis in *Escherichia coli* revealed that the 1*R-trans-anti*-B[*c*]Ph-*N*²-dG adduct (Scheme 1) induced primarily G→A transitions (24). With *Vaccinia* topoisomerase I, DNA adducts in the purine sequence complementary to the 5'-C⁵C⁴C³T²T¹-3' recognition sequence immediately 5' to the position of cleavage at T¹ inhibit enzymatic activity. When placed opposite C⁴, the 1*R*-B[*c*]Ph dG adduct modestly inhibited enzymatic activity, and when opposite C³, it completely abolished enzymatic activity (25).

The conformations of *N*²-dG *trans-anti*-B[*c*]Ph adducts depend upon an interplay of factors (26), including stereochemistry (27,28), PAH topology (29–31), DNA sequence (32), and whether the adducts are incorporated into model primer–template junctions (33,34) or interact with DNA polymerases (35,36). The need to accommodate the bulky fjord region PAH into the DNA helix, concomitant with maximizing aromatic stacking interactions of the PAH, provides a primary determinant of the conformations of *N*⁶-dA and *N*²-dG *trans-anti*-B[*c*]Ph adducts in DNA. Previously determined structures of *N*⁶-dA B[*c*]Ph adducts were intercalated, with the 5'- vs 3'-orientation of the intercalated adduct dependent upon stereochemistry at the benzylic carbon (37,38). In the 5'-d(C¹C²A³T⁴C⁵X⁶C⁷T⁸A⁹C¹⁰C¹¹)-3'-5'-d(G¹²G¹³T¹⁴A¹⁵G¹⁶C¹⁷G¹⁸A¹⁹T²⁰G²¹G²²)-3' oligodeoxy-nucleotide, where X = 1*R*,2*S*,3*R*,4*S*-B[*c*]P-*N*²-dG, the 1*R*-B[*c*]Ph *N*²-dG adduct intercalated into the DNA duplex in the 3'-direction, between base pairs X⁶•C¹⁷ and C⁷•G¹⁶ (30).

Iterated repeats, commonly encountered in microsatellite DNA, particularly in the absence of mismatch repair, are susceptible to frameshift mutations, which may lead to human disease (39). Iterated sequences occasionally undergo transient strand slippage during DNA replication, thus leading to deletion or addition mutations (40). DNA adducts located in iterated repeat sequences may be processed differently than adducts located in noniterated sequences (41). The iterated CG repeat sequence within the *S. typhimurium* *hisD3052* gene represents a hotspot for frameshift mutations, which has been examined in the context of exocyclic 1,*N*²-dG adducts arising endogenously in DNA as the result of oxidative damage (42–46).

¹Abbreviations: B[*c*]Ph, benzo[*c*]phenanthrene, B[*c*]Ph DE, benzo[*c*]phenanthrene diol epoxide, PAH, polycyclic aromatic hydrocarbon, EDTA, ethylenediaminetetraacetic acid, NOE, nuclear Overhauser enhancement, NOESY, two-dimensional NOE spectroscopy, TPPI, time proportional phase increment, α', the torsion angle [dG]N3–C2–N2–[B[*c*]Ph]–C1, β', the torsion angle [dG]C2–N2–[B[*c*]Ph]–C1–C2. A right superscript refers to numerical position in the sequence starting from the 5' terminus of chain A and proceeding to the 3' terminus of chain A and then from the 5' terminus of chain B to the 3' terminus of chain B. So as to keep the numbering scheme in register with previous studies on 5'-d(CGCXCG-GCATG)-3'-5'-d(CATGCCGCGCG)-3', the nucleotides have been numbered beginning with A^{–2}, T^{–1}, C¹, etc. C2, C5, C6, C8, C1', C2', C2'', etc. represent specific carbon nuclei. H2, H5, H6, H8, H1', H2', H2'', etc. represent protons attached to these carbons.

The present work describes the solution conformation of the *trans*-opened B[c]Ph N^2 -dG adduct arising from *trans* opening of the (+)-1*S*,2*R*,3*R*,4*S*-*anti*-benzo[c]phenanthrene diol epoxide (B[c]Ph DE), at position X⁴ in the 5'-d(A⁻²T⁻¹C¹G²C³X⁴C⁵G⁶G⁷C⁸A⁹T¹⁰G¹¹)-3'.5'-d(C¹²A¹³T¹⁴G¹⁵C¹⁶C¹⁷G¹⁸C¹⁹G²⁰C²¹G²²A²³T²⁴)-3', where X = 1*R*,2*S*,3*R*,4*S*-B[c]P- N^2 -dG, derived from the *hisD3052* frameshift tester strain of *S. typhimurium*, containing a (CG)₃ iterated repeat (Scheme 1). The (+)-1*S*,2*R*,3*R*,4*S*-*anti*-B[c]Ph DE is tumorigenic in mice (3) and mutagenic in the TA 98 and TA 100 strains of *S. typhimurium* (14). Structural refinement was achieved using molecular dynamics calculations restrained by interproton distances and torsion angle restraints obtained from NMR data. Similar to the isolated 5'-CXC-3' sequence (30), the B[c]Ph moiety intercalated on the 3'-face of the X⁴.C¹⁹ base pair such that the terminal ring of 1*R*-B[c]Ph threaded the duplex and faced into the major groove. The B[c]Ph moiety was stacked above nucleotide G¹⁸, in the complementary strand.

Materials and Methods

NMR Samples

The B[c]Ph diol epoxide-modified oligodeoxynucleotide was synthesized by a semiautomated method as described (47) using B[c]Ph-adducted *O*⁶-allyl-dG phosphoramidites as their mixed 1*R* and 1*S* diastereomers in the manual coupling step. Following removal of the allyl group with Pd(PPh₃)₄, the oligodeoxynucleotide was deprotected by heating in concentrated ammonium hydroxide and purified by reversephase HPLC on a Higgins Analytical DNA column (Thomson Instrument Co., Clear Brook, VA), 4.6 mm × 100 mm, eluted at 1.5 mL/min with a linear gradient that increased the percentage of solvent B in 0.1 M (NH₄)₂CO₃ buffer, pH 7.5, from 10 to 23% over 20 min followed by isocratic elution with 23% B, where B is a mixture of the above buffer with acetonitrile and methanol in a 45:45:10 ratio; *t*_R = 24.5 min. The diastereomeric identity was determined by enzymatic digestion to the nucleoside adduct (18,47). The circular dichroism spectrum of the oligodeoxynucleotide (H₂O, normalized to 1.0 A₂₆₀) exhibited θ_{obsd} [7.7 (289 nm), -19.0 (261 nm), and 8.9° (243 nm)]. These values were consistent with CD spectra (47) for several B[c]Ph dG-adducted oligodeoxynucleotides with different sequences, in which the 1*R* diastereomer exhibited a strong negative band at ~260 nm. Subsequently, the same oligodeoxynucleotide was synthesized from the 1*R*-phosphoramidite of known absolute configuration (48), an approach that greatly simplified product purification and characterization.

The modified oligodeoxynucleotide was converted to the corresponding duplex by annealing in water with an excess of unmodified complementary strand, followed by HPLC on a TosoHaas HA-1000 hydroxyapatite HPLC column (5 μ m, 7.5 mm × 75 mm), eluted at 1.0 mL/min at room temperature with a linear gradient from 10 to 78 mM sodium phosphate buffer (pH 7.0) over 15 min; the duplex eluted at 13–14 min, and excess complement eluted at ~9 min. The duplex was desalted by passage through Sephadex G-25 (NAP, GE Healthcare) columns and lyophilized.

NMR Spectroscopy

NMR experiments were performed at a ¹H frequency of 800.13 MHz. The sample was prepared in 0.1 M NaCl, 10 mM NaH₂PO₄, and 50 μ M Na₂EDTA (ethylenediaminetetraacetic acid) at pH 7.0. The sample used for examining nonexchangeable protons was dissolved in 0.5 mL of 99.996% D₂O at 25 °C; that used for examining exchangeable protons was dissolved in 9:1 H₂O:D₂O at 10 °C. The concentration of the modified duplex was 2 mM. The watergate pulse sequence suppressed the water signal (49). Two-dimensional NOE spectroscopy (NOESY) spectra used for resonance assignment were recorded with a mixing time of 250 ms using time proportional phase increment (TPPI) quadrature detection. Distance restraints were derived from NOESY spectra recorded at mixing times of 150, 250, and 350 ms, respectively. There

were 512 real data in the t1 dimension and 2048 real data in the t2 dimension. The repetition rate was 1.9 s. The data in the t1 dimension were zero-filled to give a matrix of 2048 × 2048 real points. A skewed sinebell-square apodization function with a 90° phase shift was used in both dimensions. Double quantum-filtered ¹H correlation (DQF-COSY) (50,51) and exclusive COSY (E-COSY) (52) spectra were collected at 25 °C at 800.13 MHz and zero-filled to give a matrix of 1024 × 2048 real points. A skewed sine-bell square apodization function with a 90° phase shift and a skew factor of 1.0 was used in both dimensions. All NMR data were processed using FELIX (v. 97.0, Accelrys, Inc., San Diego, CA) on Silicon Graphics (Mountain View, CA) Octane workstations.

NMR Distance Restraints

Footprints were drawn around nuclear Overhauser enhancement (NOE) cross-peaks observed at a mixing time of 250 ms. The same set of footprints was applied to spectra measured at other mixing times. Cross-peak intensities were determined by volume integration of the areas under the footprints. The intensities were combined as necessary with intensities generated from complete relaxation matrix analysis of a starting DNA structure to generate a hybrid intensity matrix (53). The program MARDIGRAS (v. 3.0) (54,55) iteratively refined the hybrid matrix to optimize the agreement with experimental NOE intensities. The molecular motion was assumed isotropic. Calculations, generally requiring 2–5 cycles, were performed using DNA starting models generated by INSIGHT II (v. 97.0) (Accelrys, Inc.), the three mixing time NOE experiments, with four values of τ_c values (2, 3, 4, and 5 ns). The resulting sets of distances were analyzed to give the experimental NOE restraints used in restrained molecular dynamics calculations (56). For partially overlapped cross-peaks, lower or upper error bounds on the resulting distances were increased.

Restrained Molecular Dynamics

The B[c]Ph-adducted duplex was built starting with classical B-DNA (57) using INSIGHT II. Both minor groove and intercalated structures were constructed using the BUILDER module of INSIGHT II. The force field parameters used for the adduct were similar to those calculated by Wu et al. (58) The parametrization of the B[c]Ph moiety is provided as Figure S1 in the Supporting Information. These structures were energy-minimized using the steepest descents algorithm for 100 iterations, yielding the starting structures, named B[c]Ph-In (intercalated B [c]Ph moiety) and B[c]Ph-Mi (minor groove B[c]Ph moiety).

Calculations were performed using X-PLOR (v 2.4) (59) or AMBER (60). The X-PLOR force field was derived from CHARMM (61) and adapted for rMD calculations of nucleic acids (62). The van der Waals term was approximated using the Lennard–Jones potential function. The electrostatic term used the Coulomb function. The nonbonded pair list was updated if any atom moved more than 0.5 Å, and the cutoff radius for nonbonded interactions was 11 Å. Bond lengths involving hydrogen were fixed with the SHAKE algorithm (63). The effective energy function included terms describing distance and dihedral restraints, which were in the form of square well potentials (64). The distance restraints were divided into five classes based on the confidence factor obtained from MARDI-GRAS. Additional empirical restraints were included for deoxyribose pseudorotation. The backbone torsion angles ϵ and ζ were restrained to $165 \pm 30^\circ$ and $245 \pm 30^\circ$, consistent with B-like geometry. Additionally, empirical Watson–Crick hydrogen-bonding restraints between base pairs were used but not at the adduct site.

The rMD calculations utilizing a simulated annealing protocol were performed in vacuo without explicit counterions using X-PLOR. The simulated annealing procedure consisted of 28 ps of high temperature at 1100 K, 5 ps of cooling, and 15 ps of low temperature at 300 K, thus a total of 48 ps of rMD (Figure S2 in the Supporting Information). The rMD calculations were carried out for 28000 steps at 1100 K, then cooled to 300 K over 5000 steps, and continued

at 300 K for an additional 15000 steps. Sets of calculations were performed using both the B[c]Ph-In and the B[c]Ph-Mi starting structures. Random velocities were assigned, fitting a Maxwell–Boltzmann distribution. Calculations were initiated by coupling to a heating bath with a target temperature of 1100 K, with force constants of 50, 45, 40, 35, and 30 kcal mol⁻¹ Å⁻² for the five classes of NOE restraints. The force constants were maintained at the initial value for the first 10000 steps. During this first 10000 steps, no hydrogen bond restraints were applied to enable the B[c]Ph adduct to sample sufficient conformational space. Then, the force constants for the five classes of NOE restraints were scaled up to 200, 150, 100, 100, and 100 kcal mol⁻¹ Å⁻², and the hydrogen-bonding force constants were scaled up to 150 kcal mol⁻¹ Å⁻² over the next 5000 steps. The force constants were maintained for 20000 steps, then scaled back to 70 kcal mol⁻¹ Å⁻² for class 1 NOE restraints, to 50 kcal mol⁻¹ Å⁻² for classes 2–5 of NOE restraints and base pair restraints, respectively, over 3000 steps, and remained at these values for the final 10000 steps. The integration time step was 1 fs. Coordinate sets were archived every 0.1 ps, and structures from the last 6 ps were averaged and energy-minimized for 200 iterations using the conjugation gradients algorithm.

The resulting structure was solvated, and explicit counterions were added. In all, 26 Na⁺ were added to neutralize the system using the Leap module in AMBER (65). The system was solvated with a rectangular box TIP3P waters extending ~10 Å from the DNA atoms in three dimensions. The equilibrium rMD calculations used the SANDER (66) module of AMBER and the Cornell et al. (67) force field, including the Parm99.dat parameter set (68). The Particle Mesh Ewald method (69) approximated nonbonded interactions. The solvated system was initially minimized with the positions of the DNA molecules fixed. Then, the entire system was minimized under constant volume conditions. The initial equilibration heated the system from 0 to 300 K under constant volume conditions and with weakly restrained DNA atoms. The equilibrium rMD calculations were run for 5.0 ns under constant pressure and temperature conditions. The NOE distance restraints were given a force constant of 10 kcal mol⁻¹. The sugar pucker and the torsion angle restraints were set to 1 kcal mol⁻¹ rad⁻¹. The SHAKE algorithm (63) constrained bonds involving protons to a tolerance of 0.0005 Å. A 1 fs time step was used. Coordinates were captured every 100 ps. Structures from the last 6 ps of equilibrium rMD calculations were averaged and energy-minimized for 200 iterations using the conjugation gradients algorithm. Back-calculation of theoretical NMR intensities from the emergent structures was performed using the program CORMA (v. 4.0) (53,70). Helicoidal analysis was performed using the program CURVES (71–73).

Results

NMR Spectroscopy. Resonance Assignments

The sequential assignments shown in Figure 1A,B were accomplished using standard protocols (56, 59). Disruptions in sequential NOE connectivity were observed in both the modified strand and the complementary strand. In the modified strand, there was a disconnection between X⁴ and C⁵, and in the complementary strand, another disconnection was between G¹⁸ and C¹⁹. The NOE cross-peaks between cytosine H5 and H6 at nucleotides C³ (5'-neighbor to the B[c]Ph adduct) and C¹⁹ (complementary to the adduct) were broadened as compared to the other seven cytosine H5–H6 cross-peaks in the NOESY spectrum. Also, the NOESY sequential connectivity cross-peaks involving cytosine nucleotides C⁵ in the modified strand, and C¹⁹ in the complementary strand, were broadened. The deoxyribose sugar proton resonances were assigned by utilizing a combination of DQF-COSY and NOESY spectra. With the exception of several of the H4' protons and the stereotopic assignments of the H5' and H5'' sugar protons, assignments were made unequivocally. In general, canonical B-DNA distances between the H4', H5', and H5'' protons were used to tentatively assign the H5' and H5'' deoxyribose protons.

The assignments of the nonexchangeable protons are provided in Table S1 in the Supporting Information.

The downfield region of the ^1H spectrum showing resonances from the base imino protons is shown in Figure 2. Nine resonances were observed between 11.2 and 13.5 ppm. There was spectral overlap between 12.3 and 12.6 ppm, corresponding to the guanine imino protons. A resonance was observed between the adducted base pair $\text{X}^4\cdot\text{C}^{19}$. X^4 N1H and G^{18} N1H were observed at higher field than the other guanine imino proton resonances. NOEs were observed between X^4 N1H and C^{19} N4H and between C^5 N4H and G^{18} N1H (74), indicating that both $\text{X}^4\cdot\text{C}^{19}$ and $\text{C}^5\cdot\text{G}^{18}$ maintained Watson–Crick hydrogen bonding. The NOE connectivity was disrupted between X^4 and G^{18} . At the 5' terminus of the adducted strand, imino proton resonances from base pairs $\text{T}^{-1}\cdot\text{A}^{23}$ and $\text{A}^{-2}\cdot\text{T}^{24}$ were not observed. At the 3' terminus of the adducted strand, the imino proton resonance from base pair $\text{G}^{11}\cdot\text{C}^{12}$ was not observed. This was presumably due to exchange broadening of the spectrum as a consequence of fraying of the duplex. The assignments of the exchangeable protons are provided in Table S2 of the Supporting Information.

The protons of the B[c]Ph moiety were identified by a combination of NOESY and COSY experiments. The aromatic protons were observed between 5.6 and 7.4 ppm (Figure 3). B[c]Ph H5 and H6 were furthest downfield, at 7.40 and 7.42 ppm. The B[c]Ph H6 proton showed an NOE to B[c]Ph H7 located at 6.57 ppm. The latter proton exhibited an NOE to B[c]Ph H8 located at 6.06 ppm. The B[c]Ph H8 proton showed an NOE to B[c]Ph H9 at 6.70 ppm. The B[c]Ph H9 proton showed an NOE to B[c]Ph H10 at 6.51 ppm, and B[c]Ph H10 had an NOE to B[c]Ph H11 at 5.57 ppm. The B[c]Ph H11 proton showed an NOE to B[c]Ph H12 at 7.18 ppm. The assignments of the B[c]Ph protons are provided in Table 1.

Chemical Shift Effects

Figure 4 shows ^1H chemical shift comparisons for DNA protons, between the 1R-B[c]Ph-adducted oligodeoxynucleotide and the unmodified *hisD3052* oligode-oxynucleotide. Chemical shift perturbations greater than 0.1 ppm were localized at positions X^4 and C^5 in the modified strand and G^{18} and C^{19} in the complementary strand. At the adducted base pair $\text{X}^4\cdot\text{C}^{19}$, an upfield shift of 0.48 ppm was observed for the X^4 H1' resonance. At the 3'-neighbor base pair $\text{C}^5\cdot\text{G}^{18}$, an upfield shift of 0.18 ppm was observed for C^5 H1', and a downfield shift of 0.28 ppm was observed for the G^{18} H1' resonance. At the modified base pair $\text{X}^4\cdot\text{C}^{19}$ and at the 3'-neighbor base pair $\text{C}^5\cdot\text{G}^{18}$, an upfield shift of 0.18 ppm was observed for C^{19} H5, and a downfield shift of 0.12 ppm was observed for C^5 H6. The latter protons were located in the major groove of the adducted duplex.

The B[c]Ph protons also exhibited significant upfield chemical shifts. Utilizing the reported chemical shifts of the B[c]Ph protons in the N^2 -[1R-(2S,3R,4S-triacetoxy-1,2,3,4-tetrahydrobenzo[c]phenanthryl)]- O^6 -allyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyguanosine adduct (47) as references, the B[c]Ph H5 proton shifted upfield by 0.19 ppm, the B[c]Ph H6 proton shifted upfield by 0.7 ppm, the B[c]Ph H7 proton shifted upfield by 1.3 ppm, the B[c]Ph H8 proton shifted upfield by 1.7 ppm, the B[c]Ph H9 proton shifted upfield by 1.3 ppm, the B[c]Ph H10 proton shifted upfield by 1 ppm, the B[c]Ph H11 proton shifted upfield by 1.7 ppm, and the B[c]Ph H12 proton shifted upfield by 1.5 ppm.

Experimental Distance and Torsion Angle Restraints

A total of 13 NOEs were observed between 1R-B[c]Ph protons and DNA (Table 1 and Figure 5). All of these NOEs involved the adducted nucleotide X^4 and, in the complementary strand, nucleotide G^{18} of the 3'-neighbor $\text{C}^5\cdot\text{G}^{18}$ base pair. NOEs were observed between B[c]Ph and major groove aromatic or amine protons at base pairs $\text{X}^4\cdot\text{C}^{19}$ and 3'-neighbor $\text{C}^5\cdot\text{G}^{18}$. The B

[c]Ph H6 and H7 protons showed medium intensity NOEs to G¹⁸ H1' (peaks f and h in Figure 5); the B[c]Ph H7 proton also showed a medium intensity NOE to G¹⁸ H2' (peak a in Figure 5). The B[c]Ph H8 and H9 protons showed weak NOEs to G¹⁸ H1' (peaks i and g in Figure 5). The B[c]Ph H9 and H10 protons showed strong and weak NOEs, respectively, to major groove proton G¹⁸ H8 (peaks d and e in Figure 5). The B[c]Ph H10 and H12 protons showed weak and medium NOEs to X⁴ N1H (not shown in Figure 5). The B[c]Ph H10 and H12 protons showed weak NOEs to G¹⁸ N1H (not shown in Figure 5). The B[c]Ph H11 proton showed a medium intensity NOE to X⁴ H2' and a weaker NOE to X⁴ H2'' (peaks b and c in Figure 5). Evaluation of DQF-COSY spectra indicated that the pseudorotation values for all deoxyribose sugars were in the C2'-endo sugar ring conformations (75). Inspection of ¹H NOESY data revealed that all glycosyl torsion angles were in the *anti* conformation.

Restrained Molecular Dynamics Calculations

Two starting structures were built from B-DNA using INSIGHTII (v. 97.0). In the first, the B[c]Ph moiety was intercalated between G¹⁸ and C¹⁹, while in the second the B[c]Ph moiety was oriented in the minor groove facing the 3'-direction. In both instances, the rMD calculations employed 579 distance restraints from NOESY experiments (Table 2). Of these, 401 were internucleotide restraints, and 177 were intranucleotide restraints, and nine were B[c]Ph-DNA restraints. No restraints were included for instances in which the H4', H5', and H5'' assignments were equivocal, including the H5' and H5'' protons at and adjacent to the adduct site. The NOE restraints used in the rMD calculations are listed in Table S3 of the Supporting Information. The force field parameters used for the 1R-B[c]Ph adduct are shown in Figure S1 of the Supporting Information.

The rMD calculations initiated from either starting structure converged to similar ensembles of structures (Figure 6). The refined structure was a right-handed duplex, in which the 1R-B[c]Ph moiety intercalated from the minor groove toward the 3'-side of the modified strand. Root mean square deviations are summarized in Table 3. Comparison of the individual sets of rMD-calculated structures showed rmsd values of 0.26 Å emergent from 1R-B[c]Ph-In and an rmsd of 0.21 Å emergent from 1R-B[c]Ph-Mi. The rmsd between the averaged structure emergent from 1R-B[c]Ph-In and the averaged structure emergent from 1R-B[c]Ph-Mi was 1.12 Å. The rmsd was 2.22 Å between the final structure and 1R-B[c]Ph-In and 2.88 Å between the final structure and 1R-B[c]Ph-Mi. Hence, the final structures were closer to 1R-B[c]Ph-In than to 1R-B[c]Ph-Mi.

The accuracy of the emergent structures was assessed by complete relaxation matrix calculations (53). The inverse six-root deviations were calculated with respect to the experiments measured at a mixing time of 250 ms using an isotropic molecular correlation time of 4 ns. The choice of 4 ns as the optimum value for the isotropic molecular correlation time was based upon calculations conducted over a range of correlation times from 3–5 ns and studies of the M₁dG adduct in this oligodeoxynucleotide duplex (76). The R_1^x values of the starting structures 1R-B[c]Ph-In and 1R-B[c]Ph-Mi indicated that the minor groove starting structure was less consistent with the NMR data than was the intercalated starting structure. Theoretical NOE intensities from each of the refined structures yielded R_1^x values of ~0.08 for intranucleotide NOEs and ~0.09 for internucleotide NOEs, irrespective of starting structures, which suggest that the refined structures were in improved agreement with the NOE data. A plot of the sixth root R factor R_1^x at all positions of this duplex is shown in Figure 7. At most positions, the R_1^x values were found to be between 5 and 10×10^{-2} . This suggested that the quality of the refinement was relatively consistent at all positions in the adducted duplex.

Conformation of the 1*R*-B[c]Ph Adduct

Figure 8 shows a view of the adducted base pair X⁴.C¹⁹, and the adjacent base pairs C³.G²⁰ and C⁵.G¹⁸, from the minor groove. The B[c]Ph moiety intercalated on the 3'-face of the X⁴.C¹⁹ base pair such that the phenanthrenyl ring intercalated into the helix and spanned between both minor and major grooves, with the terminal ring of 1*R*-B[c]Ph facing toward the major groove. The torsion angles α' and β' (Scheme 1) define the positioning of the B[c]Ph moiety with respect to the adducted base X⁴ (58). The α' torsion was calculated to be -177° (Table 3), maintaining an orientation in which the X⁴ exocyclic amine remained in plane with the purine, consistent with the anticipated partial double bond character of this bond. The β' torsion was calculated to be 75° . This value governed the 3'-orientation of the B[c]Ph moiety with respect to X⁴. The benzylic ring oriented in the minor groove in the half-chair conformation (58). This allowed the three hydroxyls on the benzylic ring to orient above the plane of the X⁴.C¹⁹ base pair. Intercalation of the B[c]Ph moiety increased the helical rise between base pairs X⁴.C¹⁹ and C⁵.G¹⁸ and resulted in unwinding of the right-handed helix. The adducted base pair X⁴.C¹⁹ and base pair C⁵.G¹⁸ remained Watson–Crick hydrogen bonded. Also evident was the twist of the B[c]Ph moiety characteristic of the fjord topology of the PAH (58,77). Figure 9 shows base-stacking interactions of the 1*R*-B[c]Ph adduct. The aromatic rings of the B[c]Ph moiety were directly below the Watson–Crick hydrogen-bonding face of the modified base pair X⁴.C¹⁹. The B[c]Ph moiety was stacked above nucleotide G¹⁸ in the complementary strand.

Deposition of Structure Coordinates

The PDB entry is ZROU.

Discussion

This work provides a structure of the 1*R*-B[c]Ph *N*²-dG adduct, located site specifically within an iterated repeat frameshift-prone sequence in DNA, in this instance the iterated CG repeat sequence associated with the *S. typhimurium hisD3052* gene (78). The *hisD3052* strain of *S. typhimurium* arose from a ICR-191-induced deletion of a cytosine from the histidinol dehydrogenase gene (79,80) and has been commonly used in frameshift reversion assays. Iterated repeat sequences are prone to frameshift mutations due to their propensity to undergo transient strand slippage during replication (40). The common reversion in the *hisD3052* gene is a CG deletion in the iterated sequence (CG)₄ (81–84). Additions and deletions that restore the reading frame do not necessarily reverse the forward mutation (85).

Intercalation of the 1*R*-B[c]Ph Adduct

The intercalation of the 1*R*-B[c]Ph moiety in the 3'-direction within this iterated frameshift prone sequence is similar to the adduct conformation reported in an isolated 5'-CXC-3' sequence (30). The pattern of NOEs between the aromatic rings of B[c]Ph and DNA supports this conclusion. All of the NOEs observed between B[c]Ph protons and DNA protons are clustered at the adducted base pair X⁴.C¹⁹ and its 3'-neighbor, C⁵.G¹⁸ (Figure 5). The intercalation of the PAH moiety results in observation of several NOEs between B[c]Ph H9, H10, H11, and H12, located on the distal aromatic ring of B[c]Ph, and major groove protons of the DNA duplex. These are inconsistent with a 3'-minor groove orientation. Key NOEs are observed between both B[c]Ph H10 and H12 and the exocyclic amino protons of C¹⁹, located on the major groove face of the modified base pair X⁴.C¹⁹. In addition, B[c]Ph H11 shows NOEs to X⁴ H2' on the major groove side of the X⁴ deoxyribose moiety. Other NOEs diagnostic of intercalation involve B[c]Ph H6, H7, H9, and H10 and G¹⁸ H8, the latter proton also located in the major groove. The NOE between B[c]Ph H11 and X⁴ H2'' is attributed to spin diffusion.

Intercalation of the B[c]Ph moiety does not perturb Watson–Crick base pairing at X⁴·C¹⁹ or C⁵·G¹⁸, the base pairs flanking the intercalated PAH (Figure 2). Both X⁴ N1H and G¹⁸ N1H, the two imino protons flanking the intercalated B[c]Ph moiety, are observed in the ¹H spectrum. NOE connectivities are observed from X⁴ N1H to C¹⁹ N⁴H and from C⁵ N⁴H to G¹⁸ N1H, establishing Watson–Crick base pairing at both flanking base pairs. Intercalation of the B[c]Ph moiety results in approximately 1 ppm upfield shifts of both X⁴ N1H and G¹⁸ N1H, relative to the imino protons arising from other C·G base pairs in the *hisD3052* oligodeoxynucleotide. The base-stacking plots in Figure 9 show the emergence of the distal ring of B[c]Ph into the major groove of the DNA duplex. It is positioned below and above X⁴ N1H and G¹⁸ N1H, respectively, consistent with the upfield shifts observed for these protons. Simultaneously, the B[c]Ph protons exhibit upfield chemical shifts relative to the nucleoside adduct N²-[1R-(2S, 3R,4S-triacetoxy-1,2,3,4-tetrahydrobenzo[c]phenanthryl)]-O⁶-allyl-3',5'-di-O-(*tert*-butyldimethylsilyl)-2'-deoxyguanosine (47) that are attributed to stacking interactions with the neighboring DNA base pairs. These chemical shift perturbations are particularly notable for B[c]Ph H9, H10, H11, and H12. Notably, each of these protons resonates upfield of its resonance positions in the 1S B[c]Ph adduct (30). B[c]Ph H12, for example, exhibited an 0.6 ppm upfield chemical shift as compared to the 1S B[c]Ph adduct (30). Overall, DNA chemical shift perturbations are localized at base pairs X⁴·C¹⁹ and C⁵·G¹⁸, consistent with intercalation. The increased distance between these base pairs, a result of the intercalated B[c]Ph moiety, is reflected in the loss of sequential NOE connectivity between Watson–Crick hydrogen-bonded base pairs X⁴ and G¹⁸ (Figure 2). Likewise, there is a loss of sequential NOE connectivity observed in both the modified and the complementary strands of the duplex between X⁴ and C⁵ and between G¹⁸ and C¹⁹ (Figure 1). Propeller twisting of the fused B[c]Ph ring system (58) facilitates its intercalation on the 3'-face of the modified base pair X⁴·C¹⁹, similar to the 5'-CXC-3' sequence (30) (Figure 9).

The similarity of the present structure to that observed in the isolated 5'-CXC-3' sequence (30) suggests that for this adduct in duplex DNA, the identities of the nearest neighbor nucleotides, and not the isolated vs iterated repeat nature of the DNA sequence, provide the primary determinants of structure. Future studies designed to look at the processing of this adduct in iterated vs noniterated sequences by DNA damage processing enzymes will now be of considerable interest, and we would propose that the differential sequence contexts will become more evident in the context of the biological processing of this adduct. Additionally, it will be of considerable interest to examine the consequences of placing dA, T, or dG either 3' or 5' to the 1R B[c]Ph adduct.

Conformation of the PAH-Adducted N²-dG Amine

The orientation about torsion angle α' (Scheme 1) of the PAH-adducted N²-dG exocyclic amine facilitates intercalation of the fjord region PAH. In the *hisD3052* oligodeoxynucleotide, rMD calculations suggest that the N²-dG amine remains in plane with the modified dG and is positioned to form a Watson–Crick hydrogen bond with C¹⁹ O² (Figure 8). This conformation places the α' torsion angle in the 180° range, consistent with the partial double bond character of the exocyclic amine. It facilitates 3'-intercalation of the 1R-B[c]Ph adduct. The data suggest a rationale as to why 3'-intercalation would be favored as the lowest energy conformation for the 1R-B[c]Ph adduct. Intercalation of the 1R-B[c]Ph adduct exploits the favorable topology of the fjord region PAH to maximize stacking with the adducted base pair and its 3'-neighbor, thus shielding the PAH from solvent (30). Simultaneously, it accommodates the partial double bond character of the exocyclic amine and allows the modified base pair to maintain three Watson–Crick hydrogen bonds.

The possibility that the 1R B[c]Ph adduct undergoes a conformational change during error-prone translesion replication must be considered. When placed into a model primer–template

junction, the 10S N^2 -dG B[a]P adduct, arising from *trans* opening of the *anti*-B[a]P diol epoxide, reoriented from a 5'-minor groove conformation in duplex DNA to an intercalated conformation in which the modified dG was in the *syn* conformation about the glycosyl bond, prior to dNTP insertion opposite the damage site (33). This displaced the B[a]P adduct into the major groove. However, when dC was incorporated into the model primer-template junction opposite the lesion, the B[a]P moiety reverted to its minor groove orientation, with an *anti*-glycosyl torsion angle at the modified dG (34).

The structure of the 1S-B[c]Ph N^2 -dG adduct in a one nucleotide gapped complex with DNA polymerase β provides structural information for this adduct, in the context of a replication complex (86). In this structure, the B[c]Ph ring system stacked above the 3'-neighbor base pair, with respect to the adducted nucleotide, with the adducted deoxyguanosine base displaced and precluding formation of a catalytically competent complex. Structural analyses of these adducts in ternary complexes with Y-family polymerases will now be of considerable interest. The structure of the 10S-B[a]P adduct has also been determined in a complex with the *Bacillus stearothermophilus* DNA polymerase, placed opposite dC (87), and in three complexes with the *Sulfolobus solfataricus* P2 DNA polymerase IV (Dpo4) (88). In the first instance, the B[a]P adduct maintained a 5'-minor groove orientation and *anti*-glycosyl torsion angle as observed in duplex DNA (27), which may account for the fact that it creates a strong block to replication. With the Dpo4 polymerase, in the productive structures, the bulky adduct was flipped out of the DNA helix into a structural gap between the little finger and the core domains (88).

Structure–Activity Relationships

Site-specific mutagenesis studies of all possible B[a]P and B[c]Ph diol epoxide dG adducts (24,89) in two sequence contexts showed that that 10R- and 10S-B[a]P dG adducts, both of which are minor groove bound (27,90,91), give largely G→T or G→C transversions (purine misincorporation) and little or no G→A transitions (T misincorporation) regardless of the configuration at C10. In contrast, G→A transitions were favored by the 1R-B[c]Ph adducts (59–85% of total mutations), suggesting that the intercalated conformation of this adduct gives a fundamentally different mutagenic response as compared to the minor groove-oriented 10R- and 10S-B[a]P dG adducts (27,90,91). A similar effect is noted with *Vaccinia* topoisomerase I, for which DNA adducts opposite the 5'-C⁵C⁴C³T¹-3' recognition sequence immediately 5' to the position of cleavage at T¹ inhibit enzymatic activity. When placed opposite C⁴, the 10R-B[a]P N^2 -dG adduct, which orients in the minor groove toward C⁵ (27), had a 350-fold greater inhibitory effect on topoisomerase cleavage activity than did the 3'-intercalated 1R B[c]Ph dG adduct (25) that is intercalated in the same direction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Expanded plots from the aromatic–anomeric region of the NOESY spectrum for the 1*R*-B[c] Ph adduct in the *hisD3052* oligodeoxynucleotide. (A) Sequential NOE connectivities for the modified strand. (B) Sequential NOE connectivities for the complementary strand. The experiments were at 25 °C.

Figure 2.

Expanded plot showing sequential NOE connectivities for the base-paired imino protons of the 1R-B[c]Ph adduct. Crosspeaks: a, X⁴ N1H→C¹⁹ N⁴H2; b, X⁴ N1H→B[c]Ph H12; c, X⁴ N1H→C¹⁹ N⁴H1; d, X⁴ N1H→B[c]Ph H10; e, G¹⁸ N1H→C⁵ N⁴H2; f, G¹⁸ N1H→B[c]Ph H12; g, G¹⁸ N1H→C⁵ N⁴H1; and h, G¹⁸ N1H→B[c]Ph H10. The experiment was at 10 °C.

Figure 3.

Expanded plot showing the assignment of the B[c]Ph resonances in the 1R-adduct. The experiment was at 25 °C.

Figure 4.

(A) Chemical shift changes of nucleotide base protons in the modified strand between the 1*R*-B[c]Ph adduct relative to the unmodified *hisD3052* oligodeoxynucleotide. (B) Chemical shift changes of nucleotide base protons in the complementary strand between the 1*R*-B[c]Ph adduct relative to the unmodified *hisD3052* oligodeoxynucleotide. The nucleobase H6/H8 protons are in black. The cytosine H5 protons are in gray. The H1' protons are in white. In all instances, $\Delta\delta = \delta_{\text{modified oligodeoxynucleotide}} - \delta_{\text{unmodified oligodeoxynucleotide}}$ (ppm). (C) Plot of the B[c]Ph chemical shifts for the 1*R*-B[c]Ph-adducted duplex at 298 K.

Figure 5.

NOE cross-peaks between $1R$ -B[c]Ph protons and DNA protons. Crosspeaks: a, B[c]Ph H7 $\rightarrow$ G¹⁸ H2'; b, B[c]Ph H11 $\rightarrow$ X⁴ H2''; c, B[c]Ph H11 $\rightarrow$ X⁴ H2''; d, B[c]Ph H10 $\rightarrow$ G¹⁸ H8; e, B[c]Ph H9 $\rightarrow$ G¹⁸ H8; f, B[c]Ph H6 $\rightarrow$ G¹⁸ H1'; g, B[c]Ph H9 $\rightarrow$ G¹⁸ H1'; h, B[c]Ph H7 $\rightarrow$ G¹⁸ H1'; and i, B[c]Ph H8 $\rightarrow$ G¹⁸ H1'. The 800 MHz 250 ms mixing time NOESY experiment was at 25 °C.

Figure 6. Stereoview of the 1*R*-B[c]Ph-adducted duplex showing six superimposed structures obtained from rMD calculations.

Figure 7.

Comparison of six-root residual indices R_1^x for starting models and resulting rMD structures for the 1*R*-B[c]Ph-adducted *hisD3052* oligodeoxynucleotide. (A) The adducted strand. (B) The complementary strand. Only the inner 11 base pairs were used in the calculations, to exclude end effects. The mixing time was 250 ms. $R_1^x = \sum |(a_0)_i^{1/6} - (a_c)_i^{1/6}| / \sum |(a_0)_i^{1/6}|$, where a_0 and a_c are the intensities of observed nonzero and calculated NOE cross-peaks, respectively.

Figure 8.

View from the minor groove of the 1R-B[c]Ph-adducted oligodeoxynucleotide, showing base pairs C³·G²⁰, X⁴·C¹⁹, and C⁵·G¹⁸. The adducted base pair X⁴·C¹⁹ is shown in yellow. The protons of 1R-B[c]Ph moiety are shown in gray. The carbon atoms of 1R-B[c]Ph are shown in black. The oxygen atoms of 1R-B[c]Ph are shown in red.

Figure 9.

(A) Stacking of C³·G²⁰ above X⁴·C¹⁹ in the 1R-B[c]Ph adduct. (B) Stacking of X⁴·C¹⁹ above the 1R-B[c]Ph adduct. (C) Stacking of the 1R-B[c]Ph adduct above C⁵·G¹⁸. (D) Stacking of C³·G²⁰ and G⁴·C¹⁹ in the unmodified *hisD3052* oligodeoxynucleotide. (E) Stacking of G⁴·C¹⁹ and C⁵·G¹⁸ in the unmodified *hisD3052* oligodeoxynucleotide. The adducted base pair X⁴·C¹⁹ and the unmodified G⁴·C¹⁹ are in yellow. Base pairs C⁵·G¹⁸ and C³·G²⁰ are shown in blue.

**A. Formation of *trans*-opened
1*R*,2*S*,3*R*,4*S*-Benzo[*c*]phenanthrenyl-*N*²-dG Adduct in DNA**

B. The *Salmonella typhimurium hisD3052* Oligodeoxynucleotide

Scheme 1.

(A) Formation of the *trans*-Opened 1*R*, 2*S*, 3*R*, 4*S*-Benzo[*c*]phenanthrenyl-*N*²-dG Adduct in DNA from the (+)-1*S*, 2*R*, 3*R*, 4*S*-anti-B[*c*]Ph DE and (B) Numbering Scheme for the *Salmonella typhimurium hisD3052* Oligodeoxynucleotide^a

^a So as to keep the numbering scheme in register with previous studies on 5'-d(CGCXCGGCATG)-3'-5'-d(CATGCCGCGCG)-3', the nucleotides have been numbered beginning with A⁻², T⁻¹, C¹, etc.

Table 1
NOEs Involving the 1*R*-B[c]Ph Adduct in the *hisD3052* Duplex

proton	chemical shift (ppm)	NOEs ^a	distance measured in final rMD structure (Å)
H1	5.73	H2 (m), H12 (s)	
H2	4.24	H1 (m)	
H3	4.08	H4 (m)	
H4	4.82	H3 (m)	
H5	7.40	H6 (s)	
H6	7.42	H5 (s), H7 (s), H8 (w), G ¹⁸ H1' (m)	G ¹⁸ H1' (3.76)
H7	6.57	H6 (s), H8 (s), H9 (w), G ¹⁸ H1' (m), G ¹⁸ H2 (m)	G ¹⁸ H1' (3.54), G ¹⁸ H2' (4.03)
H8	6.06	H6 (w), H7 (s), H10 (w), G ¹⁸ H1' (w)	G ¹⁸ H1' (3.95)
H9	6.70	H7 (w), H8 (s), H10 (s), H11 (w), G ¹⁸ H1' (w), G ¹⁸ H8 (s)	G ¹⁸ H1' (4.89), G ¹⁸ H8 (2.89)
H10	6.51	H9 (s), H8 (w), H11 (s), H12 (w), G ¹⁸ H8 (w), G ¹⁸ N1H (w), X ⁴ N1H (w)	G ¹⁸ H8 (4.51), G ¹⁸ N1H (5.66), X ⁴ N1H (5.32)
H11	5.57	H12 (s), H10 (s), H9 (w), X ⁴ H2'' (w), X ⁴ H2' (m)	X ⁴ H2'' (4.81), X ⁴ H2' (4.41)
H12	7.18	H11 (s) H10 (w), H1 (s), G ¹⁸ N1H (w), X ⁴ N1H (m)	G ¹⁸ N1H (4.79), X ⁴ N1H (3.75)

^a s represents strong NOE, m represents medium NOE, and w represents weak NOE, determined by integration of cross-peak volumes at 150 and 250 ms NOE mixing times.

Table 2
Root Mean Square Deviations (rmsd), Excluding the End Base Pairs, between Various Initial Structures and Intermediate Structures of the 1*R*-B[c]Ph Adduct in the *hisD3052* Oligodeoxynucleotide^a

atomic rmsd (Å)	
NMR restraints	
total no. of distance restraints	579
interresidue distance restraints	401
intraresidue distance restraints	177
DNA-1 <i>R</i> distance restraints	9
deoxyribose pseudorotation restraints	110
backbone torsion angle restraints	127
hydrogen-bonding restraints	61
initial structures	
1 <i>R</i> -B[c]PhG-In vs 1 <i>R</i> -B[c]PhG-Mi	2.74
rms shifts	
1 <i>R</i> -B[c]PhG-In vs <rMD-In>	2.22 ± 0.10
1 <i>R</i> -B[c]PhG-Mi vs <rMD-Mi>	2.88 ± 0.14
rms shifts distributions	
<rMD-In> vs <rMD-In>	0.26 ± 0.10
<rMD-Mi> vs <rMD-Mi>	0.21 ± 0.10
<rMD-In> vs <rMD-Mi>	1.12 ± 0.10
<rMD-In> vs <rMD>	0.52 ± 0.04
<rMD-Mi> vs <rMD>	0.57 ± 0.04

^a 1*R*-B[c]PhG-In is the initial structure with the 1*R* adduct intercalated into the duplex, 1*R*-B[c]PhG-Mi is the initial structure with the 1*R* adduct residing in the minor groove of the duplex, <rMD-In> is the average structure emergent from rMD calculations initiated from the initial structure 1*R*-B[c]PhG-In, <rMD-Mi> is the average structure emergent from rMD calculations initiated from the initial structure 1*R*-B[c]PhG-Mi, and <rMD> is the average structure derived from from <rMD-In> and from <rMD-Mi>.

Table 3Torsion Angle Measurements for the B[c]Ph Moiety in the Refined Structure^a

torsion angle	1 <i>R</i> -B[c]Ph <i>N</i> ² -dG adduct (deg)
α'	− 177
β''	75
γ'_1	− 70
γ'_2	48
δ'	18

^aThe torsion angles are as defined by Wu et al. (58).