Tuning the DNA Conformational Perturbations Induced by Cytotoxic Platinum−Acridine Bisintercalators: Effect of Metal Cis/Trans Isomerism and DNA Threading Groups

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Abstract: Four highly charged, water soluble platinum−acridine bisintercalating agents have been synthesized. Depending on the cis/trans isomerism of the metal and the nature of the acridine side chains, bisintercalation induces/stabilizes the classical Watson−Crick B-form or a non-B-form. Circular dichroism spectra and chemical footprinting experiments suggest that **4**, the most active derivative in HL-60 cells, produces a structurally severely perturbed DNA with features of a Hoogsteen base-paired biopolymer.

The design of genome-directed agents that target specific DNA structures and DNA−enzyme complexes has become a dominating strategy in anticancer drug development.[1] Since the discovery of echinomycin, a cytotoxic cyclic peptide quinoxaline natural product,[2] DNA bisintercalators have attracted considerable attention in the biomedical community. The unique DNA binding properties of these agents (high affinity, slow dissociation, efficient duplex unwinding, enhanced sequence specificity compared to analogous monointercalators) and their ability to interfere with DNA-processing enzymes, such as polymerases and topoisomerases, have inspired the development of several classes of synthetic bisintercalators.[3] Some promising recent developments include bifunctional acridines[4,5] and anthracyclines,[6] as well as mixed-chromophore agents.[7] In pursuing our interest in metal-containing pharmacophores that produce cancer cell kill via mechanisms other than DNA cross-linking, we have developed platinum–intercalator hybrid agents.[8] Recently, we reported on a new class of cytotoxic platinum–bis(acridin-9-ylthiourea) complexes that bind to DNA reversibly through bisintercalation with the metal residing in the minor groove.[9] Unlike classical platinum-containing drugs, which form coordinative bonds with nucleobase nitrogen, these compounds bind to DNA in a noncovalent fashion. This is a consequence of the lack of a suitable leaving group on the divalent metal center linking the two acridine chromophores.

Chart 1. Structures of Bisintercalators **1**−**4**

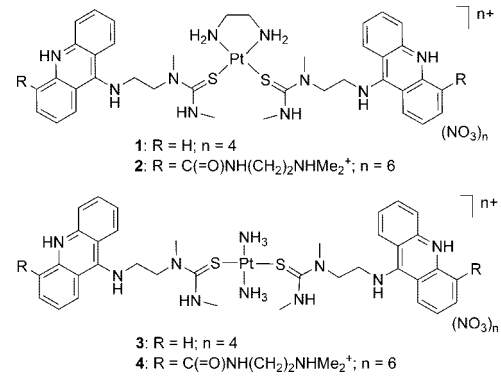

Table 1. Cytotoxicity of **1**−**4** in Human Leukemia[a]

compd	geometry/formal charge	$IC_{50} \pm SD$,[b] μM
1	cis/4+	28.18 ± 4.93
2	cis/6+	3.21 ± 0.46
3	trans/4+	4.06 ± 0.86
4	trans/6+	0.65 ± 0.12

[a] Determined in HL-60 cells using cell proliferation assays. [b] IC_{50} values (±standard deviations) are concentrations of drug required to kill 50% of the cells. Experiments were performed in triplicate.

To improve the biological activity of the prototypical agent,[9] PT-BIS(ACRAMTU[a]) (**1**, Chart 1), we have begun to make systematic changes to both the metal and intercalating moieties. Specifically, we studied the consequences of changes in the metal linker geometry and the effects caused by DNA threading acridines[10] containing charged substituents on C4 of the planar chromophores (Chart 1). In **2**, the acridines were modified with *N*-(2-(dimethylamino)ethyl)carboxamide groups,[10] while the *cis*-Pt(en) (en = ethane-1,2-diamine) linking group of the prototype, **1**, was retained. Conversely, complex **3** contains a *trans*-$Pt(NH_3)_2$ linker and unmodified ACRAMTU, and derivative **4** incorporates both the trans-bridging unit and carboxamide-modified acridine.

All of the compounds were generated in their fully protonated 4+ and 6+ states, respectively. In physiological buffers, where the 9-aminoacridine and dimethylamino moieties can be expected to exist in their (at least partially) protonated forms, **1**−**4** prove to be highly soluble. In a colorimetric cell proliferation assay, an increase in activity of approximately 50-fold was noted for the doubly modified complex **4** compared to the prototype (Table 1). On the other hand, each modification alone resulted in a less pronounced cytotoxic enhancement, based on the inhibitory concentrations (IC_{50}) determined for complexes **2** and **3** (Table 1). It is also noteworthy that in each pair of geometric isomers (**1/2** and **3/4**) the highly charged derivative containing the 4-substituents showed significantly better activity. These structure−activity relationships prompted us to revisit the DNA interactions of **1**−**4**.

While all complexes show similar overall DNA affinity and no, or only marginal, preference for specific sequences or base content (Supporting Information), distinct differences exist in the structural perturbations induced by them in double-stranded DNA. We used circular dichroism (CD) spectropolarimetry to monitor the conformational changes in poly(dG-dC)$_2$ upon

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[a] Abbreviations: ACRAMTU, 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea; CD, circular dichroism; DMS, dimethyl sulfate.

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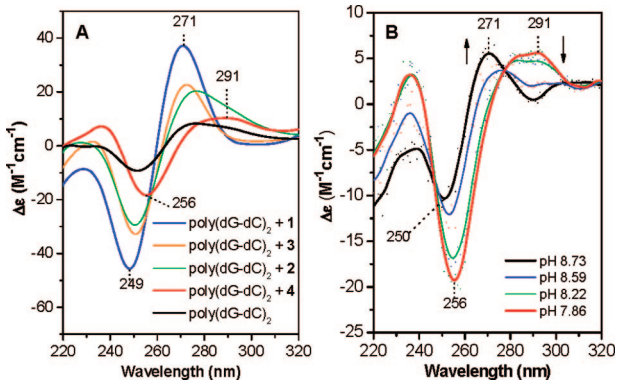

Figure 1. (A) CD spectra at 25 °C and pH 7.5 of poly(dG-dC)$_2$ modified with **1**−**4** at a drug-to-nucleotide ratio of 0.3. (B) Variable-pH CD spectra for poly(dG-dC)$_2$ modified with **4** recorded at 25 °C.

titration of aliquots of each of the conjugates **1**−**4** into a buffered solution of the alternating sequence (Figure 1A). Complex **1** produces a CD spectrum typical of B-form DNA with positive and negative Cotton effects at 271 and 249 nm, respectively, in accordance with previous biophysical studies of this compound.[9] In contrast, complex **4** turns the DNA into a non-B-type form. The CD is characterized by a pronounced hypochromic effect and bathochromic shifts of the positive and negative CD bands to 291 and 256 nm, respectively. A similar trend is observed in random-sequence DNA titrated with **4** (Supporting Information). Characteristically, **2** and **3** produce CD spectra that fall between these two extremes, indicating that only the cooperative action of the *trans*-platinum moiety and acridine side chains (R) in **4** is able to effect the conformational switch.

Variable-pH CD spectra recorded of drug-modified poly(dG-dC)$_2$ show that the structure induced by **4** is virtually unchanged between pH 7.5 and pH 3 (Supporting Information). Above physiological pH, however, a transition is observed from the non-B-form back to a B-form helix. The presence of isodichroic points at 246 and 277 nm is consistent with a proton-dependent transition between two distinct DNA conformations (Figure 1B).

One of the driving forces in the polymorphism of guanine-rich DNA is the high propensity of the purine base to undergo an *anti* → *syn* conformational switch at the glycosidic linkage. While the conformation found in Watson−Crick B-DNA is predominantly *anti*, other secondary structures are known to accommodate *syn*-G bases, including left-handed Z-form DNA and G-quadruplex structures.[11] A *syn* conformation is also found in the right-handed non-B-form structure adopted by alternating GC sequences under acidic conditions.[12,13] The *anti* → *syn* transition in this case is favored by protonation of cytosine-N3, which results in *syn*-GC$^+$ Hoogsteen base pairing.[13] Characteristically, the CD spectra[13] of this form of DNA show the same features as those observed for DNA modified with **4**. It is also noteworthy to mention that red-shifted UV and CD bands in the 290−300 nm region are one of the spectroscopic signatures of DNA structures containing G bases in the *syn* conformation.[14] On the basis of these observations, we hypothesize that **4** causes disruption of the classical internucleobase H-bonding pattern to induce a form of DNA containing Hoogsteen base pairs.

To test if in fact Hoogsteen H-bonding might be involved in the conformational switch produced by **4**, we introduced into a short model oligodeoxyribonucleotide the chemically modified nucleobase 7-deazaguanine (G′), which disrupts this type of base pairing.[15] The conformational changes produced by **4** were studied in the 13-mer duplexes d(CG′)$_6$C and d(CG)$_6$C. The

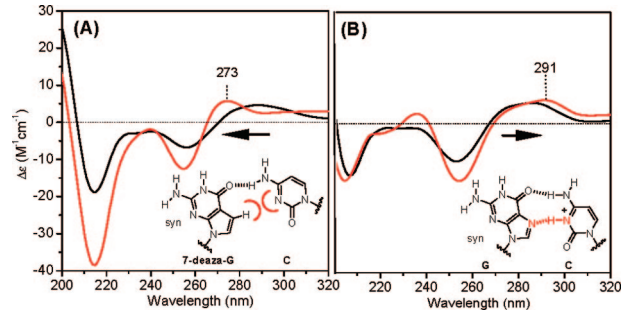

Figure 2. CD spectra at 25 °C and pH 7.5 recorded for d(CG′)$_6$C (A) and d(CG)$_6$C (B) modified with **4** at a drug-to-nucleotide ratio of 0.3. The insets illustrate the disruption (A) and formation (B) of Hoogsteen H-bonding. Arrows indicate CD band shifts resulting after titrating the unmodified sequences (black traces) with complex **4** (red traces).

CD spectra recorded of the drug-modified deaza sequence indeed confirm that **4** is unable to induce the non-B-form conformation but instead drives the equilibrium toward the classical Watson−Crick B-form (Figure 2A). In contrast, the complex efficiently generates the non-B-form structure in the analogous chemically unaltered sequence (Figure 2B). These results suggest that H-bonding involving the Hoogsteen face of guanine is a prerequisite for the observed conformational switch.

We also used chemical footprinting to shed light on the DNA binding of **4**. The alkylating agent dimethyl sulfate (DMS) is used routinely to detect Hoogsteen H-bonded guanine in DNA triplex and quadruplex secondary structures.[16,17] (Involvement of guanine-N7, the major target site of DMS alkylation, in Hoogsteen H-bonding protects the DNA from Maxam−Gilbert cleavage chemistry.) We have designed a 24-mer double-stranded DNA fragment, which contains several alternating purine/pyrimidine steps, the proposed target sequence of **4**. In this experiment, the sequence, whose top strand was 5′ end-labeled with ^{32}P, was titrated with varying concentrations of agent **4**, treated with DMS, and subjected to piperidine cleavage. The resulting fragments were analyzed on a denaturing polyacrylamide gel (Figure 3). Alkylation of guanine-N7 appears to be inhibited most efficiently at several TG steps, followed by CG, based on relative integrated band intensities. On the other hand, one guanine base, G8, becomes hyperreactive with DMS in the presence of **4**, while some G bases are virtually unaffected by drug binding.

Previous attempts to determine the binding sites of reversible DNA threading intercalators using Maxam−Gilbert DMS chemistry have proven unsuccessful because of the inability of the small footprinting agent to sense the presence of the DNA binder. Contrary to the expectation of steric protection of guanine-N7 from DMS in these cases, the conformational changes induced by the agents, most prominently unwinding, led to an *increase* in DMS cleavage activity.[18–20] These observations and the fact that **4** efficiently protects several G bases and causes opposite effects at the G7G8 step (⇵; see Figure 3) support the notion that DMS, in our experiment, indeed senses altered DNA structure rather than steric hindrance caused by the bisintercalators. Contributions to the cleavage inhibition from the latter mechanism, however, cannot be completely ruled out. The DMS cleavage pattern produced by **4** in the sequence TGTGG (residues 4−8), for instance, would be in agreement with a drug-induced change in DNA structure that protects guanine-N7 at TG steps but renders flanking G8 more susceptible to electrophilic attack. On the basis of the preliminary footprinting results, a plausible binding mode would involve nearest-neighbor excluded bisintercalation in which the acridine

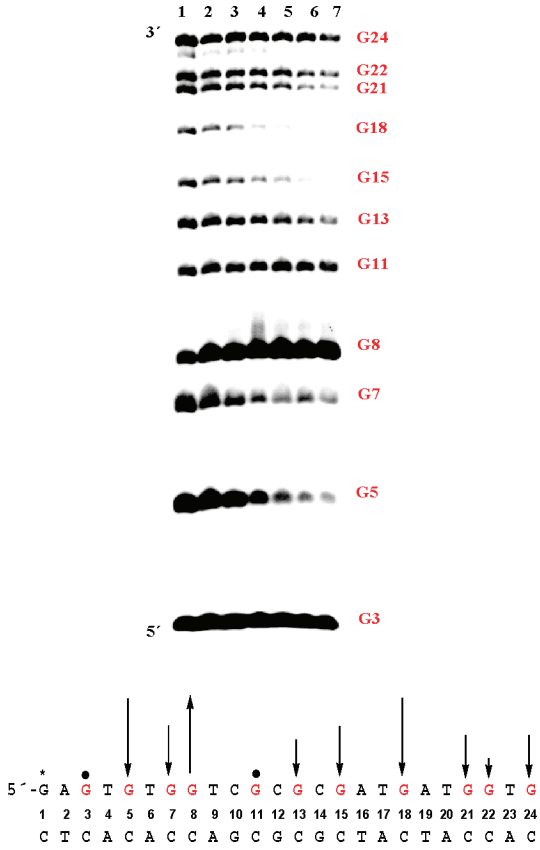

Figure 3. Footprinting analysis of a 24-mer DNA fragment modified with **4** using Maxam−Gilbert DMS/piperidine cleavage chemistry. Lane 1 on the denaturing polyacrylamide gel (top) shows the products resulting from treatment of the DNA with DMS in the absence of drug (control). Lanes 2−7 show the products resulting from the DMS reaction of drug-modified template at drug-to-nucleotide ratios of 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5, respectively. The asterisk in the sequence (bottom) indicates the radioactive label. Arrows show reduced and enhanced cleavage activity at a drug-to-nucleotide ratio of 0.3 relative to unmodified DNA. Arrow lengths are proportional to relative changes in integrated band intensities, and solid dots indicate unchanged cleavage activity.

chromophores bracket 5′-TG or 5′-CG steps. Molecular modeling results based on previous NMR solution structural studies of **1** in complex with a model oligodeoxyribonucleotide[9] suggest that this binding mode is geometrically feasible for **4** (Supporting Information). Unfortunately, attempts to extract structural information for complex **4** from 2D NMR experiments have been unsuccessful thus far because of severe line broadening caused by dynamic exchange phenomena on the NMR time scale in these model systems.

The present study suggests that it is possible to tune an intercalating agent to trigger the transition from Watson−Crick base pairs to thermodynamically less favorable Hoogsteen base pairs. Drug-induced Hoogsteen base pairs, *syn*-AT and *syn*-GC^+, have been reported previously for echinomycin and triostin A, which also bind to DNA through bisintercalation.[21,22] The stability and persistence in solution of this structural perturbation under physiological conditions and its biological relevance have been debated for many years.[2,23–25] Our data suggest that dramatic DNA conformational changes, such as those observed for **4**, may in fact contribute to the cytotoxic enhancement in this type of bisintercalator. Generation of a nonprossessible form of base pairing in genomic DNA can be expected to affect cellular viability. On the other hand, the targeting of transient DNA structures containing Hoogsteen base pairs and trapping of processes at the genome level associated with it may provide a novel mechanism of gene regulation and DNA-directed chemotherapeutic intervention. Several cases have been reported of Hoogsteen base pairing in (transient) DNA structures at the genome level. These include DNA in complex with TATA-binding protein[26] and DNA polymerase ι,[27] as well as DNA repeats in actively transcribed underwound regions of the genome,[28] suggesting a potential role of *syn*-GC^+ base pairs in gene expression and transcriptional control. While acidic conditions favor the formation of *syn*-GC^+ base pairs (the pK_a of free cytosine is <5[12]), evidence from crystal structural and solution studies exists for the persistence of this form of hydrogen bonding at and *above* neutral pH.[29–31] This shift in pK_a has been attributed to long-range electrostatic and hydrophobic effects in DNA secondary structures and DNA−ligand complexes.[27,29] The pH dependence of the conformational switch produced by **4** (Figure 2B) is in agreement with these observations.

In conclusion, the preliminary biophysical and footprinting data presented in this Letter support a mechanism of bisintercalation that turns Watson−Crick base pairs into Hoogsteen base pairs. A relationship may exist between the cytotoxicity of the drugs and the severity of the DNA structural impact produced by them. To fully exploit the therapeutic potential of this type of bisintercalator, high-resolution structural studies (NMR, X-ray crystallography) are needed to establish the structural basis of the conformational switch produced by complex **4**. These studies are underway. Finally, the current study suggests that factors other than thermodynamic and kinetic DNA binding parameters may contribute to, or dominate, the biological activity of bisintercalators. In particular, the physiological and therapeutic implications of drug-induced non-Watson−Crick DNA should be revisited.

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Supporting Information Available: Details of complex synthesis and characterization, CD experiments, chemical footprinting, and cytotoxicity studies; DNA binding data for **1**−**4**; results of CD titrations for **1**−**4** and AMBER model of a duplex modified with **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hurley, L. H. DNA and its associated processes as targets for cancer therapy. *Nat. Rev. Cancer* **2002**, *2*, 188–200.
(2) Dawson, S.; Malkinson, J. P.; Paumier, D.; Searcey, M. Bisintercalator natural products with potential therapeutic applications: isolation, structure determination, synthetic and biological studies. *Nat. Prod. Rep.* **2007**, *24*, 109–126.
(3) Gago, F. Stacking interactions and intercalative DNA binding. *Methods* **1998**, *14*, 277–292.
(4) Wakelin, L. P.; Bu, X.; Eleftheriou, A.; Parmar, A.; Hayek, C.; Stewart, B. W. Bisintercalating threading diacridines: relationships between DNA binding, cytotoxicity, and cell cycle arrest. *J. Med. Chem.* **2003**, *46*, 5790–5802.
(5) Antonini, I.; Polucci, P.; Magnano, A.; Gatto, B.; Palumbo, M.; Menta, E.; Pescalli, N.; Martelli, S. Design, synthesis, and biological properties of new bis(acridine-4-carboxamides) as anticancer agents. *J. Med. Chem.* **2003**, *46*, 3109–3115.
(6) Portugal, J.; Cashman, D. J.; Trent, J. O.; Ferrer-Miralles, N.; Przewloka, T.; Fokt, I.; Priebe, W.; Chaires, J. B. A new bisintercalating anthracycline with picomolar DNA binding affinity. *J. Med. Chem.* **2005**, *48*, 8209–8219.
(7) Antonini, I.; Santoni, G.; Lucciarini, R.; Amantini, C.; Sparapani, S.; Magnano, A. Synthesis and biological evaluation of new asymmetrical bisintercalators as potential antitumor drugs. *J. Med. Chem.* **2006**, *49*, 7198–7207.
(8) Baruah, H.; Barry, C. G.; Bierbach, U. Platinum-intercalator conjugates: from DNA-targeted cisplatin derivatives to adenine binding complexes

as potential modulators of gene regulation. *Curr. Top. Med. Chem.* **2004**, *4*, 1537–1549.
(9) Choudhury, J. R.; Bierbach, U. Characterization of the bisintercalative DNA binding mode of a bifunctional platinum−acridine agent. *Nucleic Acids Res.* **2005**, *33*, 5622–5632.
(10) Guddneppanavar, R.; Saluta, G.; Kucera, G. L.; Bierbach, U. Synthesis, biological activity, and DNA-damage profile of platinum-threading intercalator conjugates designed to target adenine. *J. Med. Chem.* **2006**, *49*, 3204–3214.
(11) Neidle, S. *Nucleic Acid Structure and Recognition*; Oxford University Press: New York, 2002; pp 62−88.
(12) Airoldi, M.; Boicelli, C. A.; Gennaro, G.; Giomini, M.; Giuliani, A. M.; Giustini, M. Acid titrations of poly(dG-dC) ·poly(dG-dC) in aqueous solution and in a w/o microemulsion. *J. Biomol. Struct. Dyn.* **2006**, *23*, 465–478.
(13) Segers-Nolten, G. M.; Sijtsema, N. M.; Otto, C. Evidence for Hoogsteen GC base pairs in the proton-induced transition from right-handed to left-handed poly(dG-dC) ·poly(dG-dC). *Biochemistry* **1997**, *36*, 13241–13247.
(14) Rodriguez, R.; Pantos, G. D.; Goncalves, D. P.; Sanders, J. K.; Balasubramanian, S. Ligand-driven G-quadruplex conformational switching by using an unusual mode of interaction. *Angew. Chem., Int. Ed.* **2007**, *46*, 5405–5407.
(15) Seela, F.; Driller, H. Alternating d(G-C)3 and d(C-G)3 hexanucleotides containing 7-deaza-2′-deoxyguanosine or 8-aza-7-deaza-2′-deoxyguanosine in place of dG. *Nucleic Acids Res.* **1989**, *17*, 901–910.
(16) Guo, K.; Pourpak, A.; Beetz-Rogers, K.; Gokhale, V.; Sun, D.; Hurley, L. H. Formation of pseudosymmetrical G-quadruplex and i-motif structures in the proximal promoter region of the RET oncogene. *J. Am. Chem. Soc.* **2007**, *129*, 10220–10228.
(17) Wells, R. D.; Collier, D. A.; Hanvey, J. C.; Shimizu, M.; Wohlrab, F. The chemistry and biology of unusual DNA structures adopted by oligopurine·oligopyrimidine sequences. *FASEB J.* **1988**, *2*, 2939–2949.
(18) Lokey, R. S.; Kwok, Y.; Guelev, V.; Pursell, C. J.; Hurley, L. H.; Iverson, B. L. A new class of polyintercalating molecules. *J. Am. Chem. Soc.* **1997**, *119*, 7202–7210.
(19) Shelton, C. J.; Harding, M. M.; Prakash, A. S. Enzymatic and chemical footprinting of anthracycline antitumor antibiotics and related saccharide side chains. *Biochemistry* **1996**, *35*, 7974–7982.
(20) Stankus, A.; Goodisman, J.; Dabrowiak, J. C. Quantitative footprinting analysis of the chromomycin A3−DNA interaction. *Biochemistry* **1992**, *31*, 9310–9318.
(21) Cuesta-Seijo, J. A.; Sheldrick, G. M. Structures of complexes between echinomycin and duplex DNA. *Acta. Crystallogr., Sect. D.: Biol. Crystallogr.* **2005**, *61*, 442–448.
(22) Ughetto, G.; Wang, A. H.; Quigley, G. J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. A comparison of the structure of echinomycin and triostin A complexed to a DNA fragment. *Nucleic Acids Res.* **1985**, *13*, 2305–2323.
(23) Gilbert, D. E.; van der Marel, G. A.; van Boom, J. H.; Feigon, J. Unstable Hoogsteen base pairs adjacent to echinomycin binding sites within a DNA duplex. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3006–3010.
(24) McLean, M. J.; Seela, F.; Waring, M. J. Echinomycin-induced hypersensitivity to osmium tetroxide of DNA fragments incapable of forming Hoogsteen base pairs. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9687–9691.
(25) Mendel, D.; Dervan, P. B. Hoogsteen base pairs proximal and distal to echinomycin binding sites on DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 910–914.
(26) Patikoglou, G. A.; Kim, J. L.; Sun, L.; Yang, S. H.; Kodadek, T.; Burley, S. K. TATA element recognition by the TATA box-binding protein has been conserved throughout evolution. *Genes Dev.* **1999**, *13*, 3217–3230.
(27) Nair, D. T.; Johnson, R. E.; Prakash, L.; Prakash, S.; Aggarwal, A. K. Hoogsteen base pair formation promotes synthesis opposite the 1,*N*6-ethenodeoxyadenosine lesion by human DNA polymerase iota. *Nat. Struct. Mol. Biol.* **2006**, *13*, 619–625.
(28) Johnston, B. H. The S1-sensitive form of d(C-T)n·d(A-G)n: chemical evidence for a three-stranded structure in plasmids. *Science* **1988**, *241*, 1800–1804.
(29) Plum, G. E.; Breslauer, K. J. Thermodynamics of an intramolecular DNA triple helix: a calorimetric and spectroscopic study of the pH and salt dependence of thermally induced structural transitions. *J. Mol. Biol.* **1995**, *248*, 679–695.
(30) Nunn, C. M.; Trent, J. O.; Neidle, S. A model for the [C+-G×C]n triple helix derived from observation of the C+-G×C base triplet in a crystal structure. *FEBS Lett.* **1997**, *416*, 86–89.
(31) Betts, L.; Josey, J. A.; Veal, J. M.; Jordan, S. R. A nucleic acid triple helix formed by a peptide nucleic acid−DNA complex. *Science* **1995**, *270*, 1838–1841.

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