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A Synthetic Nucleoside Probe that Discerns a DNA Adduct from Unmodified DNA

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Biologically reactive chemicals alkylate DNA and induce structural modifications in the form of covalent adducts.[1] Certain bulky DNA adducts can persist, escape repair, and serve as templates for polymerase-mediated DNA synthesis, resulting in mutation and cancer.[2] Correlating chemical structures and quantitative levels of adducts with toxicity is central to understanding chemical mechanisms of carcinogenesis for specific agents. Major challenges include that DNA adducts are formed at exceedingly low levels, adduct mixtures are often formed, and minor lesions may have greater biological impact than more abundant products.[2] New molecular approaches for addressing specific low-abundance adducts are needed, and we describe here the first example of a synthetic nucleoside that may serve as the chemical basis for a probe of a bulky carcinogen−DNA adduct.

Dozens of thermodynamically stable synthetic base pairs have been reported[3] and continue to emerge as powerful tools in areas such as polymerase fidelity,[4] DNA helix stability,[5] nucleic acids with novel functionality,[6] and expanded genetic systems,[3] to cite selected examples. Recently, Hirao and co-workers successfully have amplified an entirely synthetic base pair.[7] Amplified in a polymerase-mediated process or used in hybridization-based strategies, synthetic nucleosides might act as probes of DNA damage, but to our knowledge, no examples of synthetic nucleosides that pair selectively with an adduct generated in a natural physiological system are known.

O^6-Benzyldeoxyguanosine (**1**, O^6-BnG; Figure 1) is a bulky DNA adduct chosen for analysis because of its prominent role in nucleic acid chemistry and biology and the high frequency of O^6-alkylguanine lesions.[8,9] This adduct results naturally from exposure to environmental carcinogens[8a,b] and is highly mutagenic, causing G to C and G to T transversion, and G to A transition mutations.[8c,d] O^6-Alkylguanine adducts have altered hydrogen-bonding capacity, increased size, and decreased hydrophilicity relative to G (Figure 1). On the basis of molecular modeling studies,[10] we anticipated that a diaminonaphthyl-derived nucleoside (**2**, dNap; Figure 1) would possess a hydrogen-bonding capacity complementary to O^6-BnG and favorable $\pi-\pi$ stacking and hydrophobic interactions between the benzyl moiety of O^6-BnG and the naphthyl moiety of dNap **2**.

To evaluate the O^6-BnG:dNap base pair in duplex DNA, we prepared a series of oligonucleotides containing selected combinations of DNA adduct, synthetic nucleoside, and/or natural bases. Nucleoside **2** was synthesized from diaminonaphthalene **3** (Scheme 1). Treatment of **3** with ethyl chloroformate produced perimidinone **4** (70% yield), which was coupled with bistoluoyl chloroglycoside to yield the β-isomer of **5** as the major product. Deprotection, 5′-tritylation, and conversion to the 3′-phosphoramidite **6**, required for oligonucleotide synthesis, were achieved in 50% yield overall.

Duplex DNA stability was determined by thermal denaturation of synthetic oligonucleotides. Melting temperatures (T_m) were measured for complementary sequences 5′-TTGTCGGTATAXC GG-3′ and 5′-CCGYTATACCGACAA-3′ with varying bases incorporated at positions X and Y. The results indicate that O^6-BnG:dNap is markedly stable (8.0 μM) with a T_m value one degree lower than

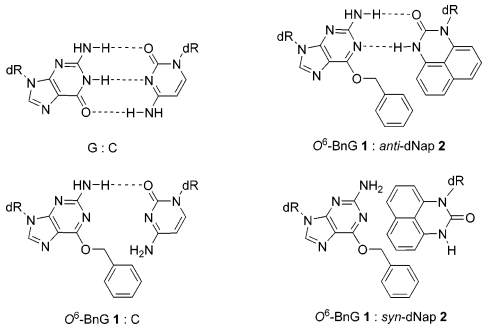
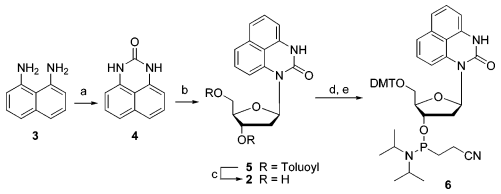


Figure 1. Schematic representation of base-pair interactions for a standard G:C pair, alkylation-damaged O^6-BnG **1**:C pair and proposed adduct:probe combination (dR = deoxyribose).

Scheme 1 [a]



[a] Reagents and conditions: (a) ethyl chloroformate, THF; (b) bistoluoyl chloroglycoside, NaH/THF; (c) NaOMe/methanol; (d) 4,4′-dimethoxytrityl chloride, pyridine; (e) *N*,*N*′-diisopropyl-2-*O*-cyanoethyl phosphoramidic chloride, Et_3N, CH_2Cl_2.

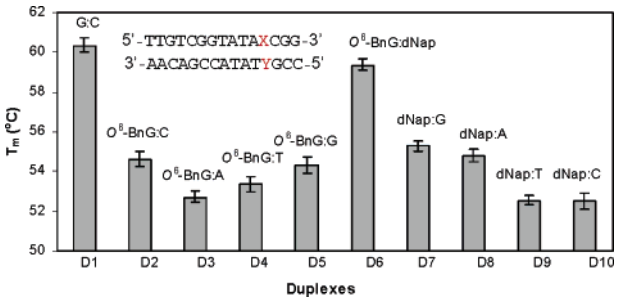


Figure 2. Thermal stabilities of natural, damaged, and dNap DNA.

that of the natural dG:dC pair (Figure 2, D1 T_m = 60.3 vs D6 T_m = 59.3). Sequence D5 represents a situation in which natural DNA is damaged, giving rise to the O^6-BnG adduct and a diminished thermal stability. Further, the adduct:probe pair T_m was compared to O^6-BnG paired with canonical bases. These combinations have diminished stabilities relative to the synthetic pair by 5.0, 6.6, 4.7, and 5.9 °C for dG, dA, dC, and dT, respectively (Figure 2). Similarly, for dNap paired opposite the natural bases, T_m diminished to 55.3, 54.8, 52.5, and 52.5 °C for dG, dA, dC, and dT, respectively. These data are comparable to optimized synthetic base pairs, in which approximate ranges of 4−9 °C in T_m depressions are considered highly stable and orthogonal systems.[3e] T_m values for point mutations in D1, which reflect the selectivity of natural base pairs, decrease by an estimated average of 9 °C.[11]

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Table 1. Thermodynamic Parameters for Duplex Formation

duplex	ΔH (kcal/mol)	ΔS (cal/K·mol)	ΔG_{298K} (kcal/mol)	$\Delta\Delta G_{298K}$ (kcal/mol)
D1	−85.9	−232	−17.7	
D2	−67.2	−195	−9.1	8.6
D6	−79.2	−218	−14.2	3.5

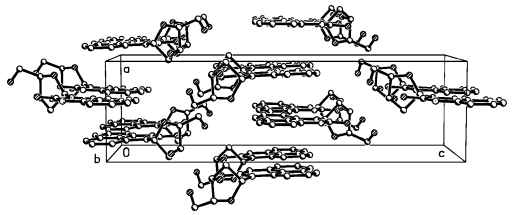

Figure 3. Stacking interactions in **2** indicated in X-ray crystal structure (unit cell dimensions: $a = 6.5$ Å; $b = 8.7$ Å; $c = 23.5$ Å).

A goal for potential biological applications is that dNap distinguish between isomeric adduct structures resulting from competing positions of base alkylation. We compared damaged oligonucleotides that contained O^6-BnG or the isomeric adduct N^2-benzyldeoxyguanosine (**7**, N^2-BnG). The N^2-BnG:dNap pair was less stable, but the difference was small (T_m of 57.4 °C, 1.9 °C lower than that of O^6-BnG:dNap). Many known synthetic nucleosides form stable self-pairs in duplex DNA.[3e−g] Similarly, the T_m for a duplex containing dNap:dNap is 60.3 °C, essentially as stable as dG:dC.

Thermodynamic relationships for key base pairs were evaluated further by a van't Hoff analysis (Table 1).[12] The relative free-energy changes parallel those observed for T_m values, with high T_m values associated with high free-energy changes upon duplex formation. Entropic contributions were similar for each example.

To verify whether the modified oligonucleotides formed strictly duplex structures, a titration study (Job plot) was performed by measuring UV absorbances at 260 nm for various molar ratios of the single strands. These data (Figure S3−S5, Supporting Information) clearly indicate 1:1 stoichiometric binding. To probe the helicity of the key duplexes in Figure 2, circular dichroism (CD) spectra of duplexes D1, D2, and D6 were obtained. The resulting CD spectra (Figure S6, Supporting Information) display positive signals at 271−274 nm and negative signals at 248−251 nm, indicating a B-form conformation of DNA duplexes. Similar patterns of CD spectra suggest that the synthetic base pair does not significantly perturb duplex conformation.

The relative stereochemistry of free dNap was assigned on the basis of NOESY correlations (obtained from **5**, Supporting Information) and confirmed by X-ray analysis of the free nucleoside **2** (Figure 3). Both indicate that the nucleoside favors a syn glycosidic torsion angle, contrasting the anti-conformation proposed to maximize H-bonding and π-stacking interactions (Figure 1). The energy barrier between syn and anti nucleoside conformations (Figure 1) is typically low,[13] and there may be structural differences among the same free nucleoside in solution or solid state, duplex DNA, or in the presence of other nucleosides.[14,15] The relationship of the dNap network in the crystal structure indicates a potential for $\pi-\pi$ interactions with an extensive array of face-to-face slipped $\pi-\pi$ stacking and hydrogen-bonding interactions of the naphthalene and deoxyribose moieties, respectively, with adjacent dNaps arranged in alternating orientations with about 3.2 Å between neighboring parallel naphthyl groups. Further studies to determine the nucleoside structure in the context of duplex DNA in the presence and absence of adduct are required to understand the origin of the experimentally observed stabilizing effect.

The novel naphthalene-derived nucleoside forms an orthogonal and thermodynamically stable base pair with the biologically significant DNA adduct O^6-BnG. This is the first report of a stable DNA base pair comprised of a biologically relevant bulky DNA adduct and a designed nucleoside partner. Synthetic nucleosides that base pair specifically with DNA adducts have diverse potential utility in the study of the impacts of chemical modification on DNA biology and chemistry. Continued studies are aimed at gaining a detailed understanding of the physical and structural origin of adduct:probe base-pair stability, the design of more selective analogues, and applications as structural probes.

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Supporting Information Available: Syntheses, NMR data, thermal denaturation studies, crystallographic analysis, Job plots, and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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