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Differential Solvation and Tautomer Stability of a Model Base Pair within the Minor and Major Grooves of DNA

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Abstract: 2-(2′-Hydroxyphenyl)benzoxazole (HBO) may be used as a model base pair to study solvation, duplex environment, and tautomerization within the major and minor groves of DNA duplexes. In its ground state, HBO possesses an enol moiety which may be oriented syn or anti relative to the imino nitrogen of the benzoxazole ring. In the absence of external hydrogen-bond donors and acceptors HBO exists as the internally hydrogen-bonded syn-enol, a mimic of the rare base pair tautomer found in DNA, which may be photoinduced to tautomerize and form the keto tautomer, a mimic of the dominant base pair tautomer. Previously, we demonstrated that when incorporated into DNA such that the enol moiety is positioned in the major groove, HBO is not solvated, exists exclusively as the internally hydrogen-bonded syn-enol which is efficiently photoinduced to tautomerize, and the corresponding keto tautomer is preferentially stabilized. In stark contrast, we now show that when HBO is incorporated in DNA such that the enol moiety is positioned in the minor groove, the enol tautomer is preferentially stabilized. Molecular dynamics simulations suggest that this results from the formation of a stable hydrogen-bond between the HBO enol and the O4′ atom of an adjacent nucleotide, an H-bond acceptor that is only available in the minor groove. The differential stabilization of the enol and keto tautomers in the major and minor grooves may reflect the functions for which these environments evolved, including duplex replication, stability, and recognition.

Introduction

DNA is virtually always discussed in terms of well-ordered, Watson−Crick hydrogen-bonded (H-bonded) base pairs between the keto−amine tautomers of the purine and pyrimidine nucleobases. However, each base pair may be converted to its minor, but often relatively stable, enol−imino tautomer by double proton transfer (prototropic tautomerism). Even transient tautomerization may impact duplex stability and dynamics, as well as compromise the integrity of the encoded sequence information.[1−3] While tautomerization has been extensively characterized computationally,[4−11] it has been more challenging to study experimentally due to the difficulties inherent to studying fast and reversible proton transfer as well as the polymeric nature of DNA which precludes the selective study of a single base pair. It would be useful for understanding DNA structure and dynamics if a model system was developed which allowed for the characterization of tautomer lifetime, stability, and solvation.

One model system used to study tautomerization is the dimer of 7-azaindole, where double proton transfer may be photoinitiated in different solvents.[12−16] For example, in hydrocarbon solvents, fluorescence upconversion experiments with this base pair model have shown that tautomerization occurs after excitation on a 1 ps time scale, while solvation dynamics evolve over a 12 ps time scale.[17] In contrast, in a more strongly H-bonding solvent, such as water or an alcohol, solvated 7-azaindole monomers are observed, and proton transfer is either blocked or occurs via solvent assistance.[18] These studies have elegantly elucidated the tautomerization and solvation dynamics

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of an isolated base pair in various solvents; however, they cannot address tautomerization within the DNA duplex environment.

While it is clear that the nucleobases within DNA generally adopt the keto−amino tautomers, environmental factors within the duplex, such as solvent and metal ion accessibility and local polarity, may facilitate tautomerization or may even stabilize the rare enol−imino tautomers.[1,19,20] Thus, in addition to being selectively recognized by proteins, other nucleic acid polymers, or small molecules,[21−25] the different environments of the major and minor grooves may contribute differently to base pair dynamics and solvation. The environments of the two grooves differ not only as a result of their structure but also due to differences in solvation and chemical functionalities. For example, while the majority of the nucleobase heteroatoms along the floor of both grooves are solvated, water molecules bound in the minor groove are more ordered than the those in the major groove.[26] In addition, while the walls of each groove are formed from the sugar−phosphate backbone as it spirals around the outer surface of the duplex, the ribose O4′ oxygen atoms are only accessible from the minor groove. These O4′ atoms are the only components of the minor groove, other than the nucleobase atoms, that are consistently found to interact with proteins and small molecules, suggesting that they may be an important component of the minor groove environment.[27−33]

To study how the different environments within DNA affect tautomerization, we have developed 2-(2′-hydroxyphenyl)-benzoxazole (HBO) as a model base pair (Figure 1).[34−36] A nucleoside-bearing HBO may be synthesized such that when incorporated into DNA, the enol moiety of the model base pair is positioned in either the major or minor groove. In the ground state, HBO exists exclusively as the enol−imino tautomer, with the enol group oriented either syn or anti with respect to the imino nitrogen.[36] In aprotic solvents, the syn-enol dominates due to the stability of the internal H-bond, while in protic solvents, solvation disrupts the internal H-bond and HBO adopts a mixture of solvated syn- and anti-enols.[35] The internally H-bonded syn-enol, a mimic of the rare enol−imino tautomer in natural DNA, is efficiently photoinduced to tautomerize to the keto−amino tautomer (excited-state intramolecular proton transfer, ESIPT),[37−43] which is a mimic of the dominant tautomer in DNA. Because ESIPT results in a characteristic and experimentally observable red-shift of HBO fluorescence, and because the syn- and anti-enols have unique absorption wavelengths and excited-state lifetimes, the conformation and solvation state of HBO may be determined spectroscopically. Thus, by examining the photodynamics of HBO appropriately incorporated into DNA, the contributions of the major and minor groove environments to tautomer stability, solvation, and dynamics may be characterized.

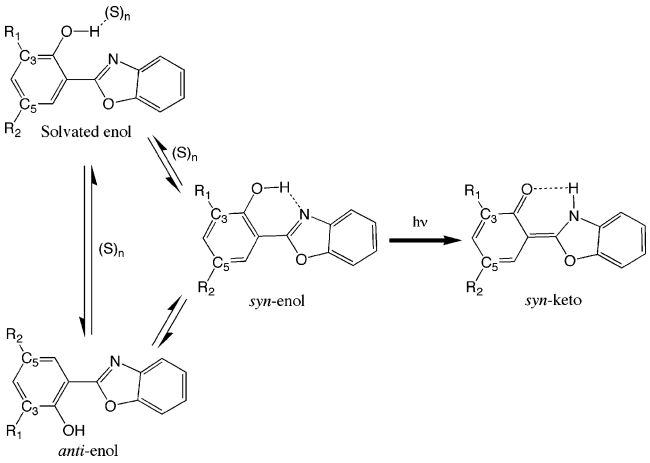

Figure 1. HBO ground-state equilibrium and photoinduced tautomerization. When R_1 = DNA and R_2 = H, the enol is positioned in the duplex minor groove, and when R_1 = H and R_2 = DNA, the enol is positioned in the major groove.

Using this model base pair, we previously characterized the major groove environment of a DNA duplex.[34,36] This was accomplished by synthesizing a C-nucleoside with HBO attached via a C5 aryl−glycosidic linkage (Figure 1), converting it to the corresponding phosphoramidite, and incorporating it into the oligonucleotide 5′-CGTTTC(HBO)TTCTC. The single-stranded DNA (ssDNA) was annealed to a complementary oligonucleotide containing an abasic site at the position opposite HBO. The circular dichroism (CD) and UV−vis spectra were both consistent with a well-packed duplex, and the duplex was virtually as stable to thermal denaturation as an analogous duplex containing a dA/dT base pair at the corresponding position (T_m = 38 and 39 °C, respectively).[36] The accommodation of the model base pair within a native-like duplex was also supported by molecular dynamics simulations, which indicated that HBO packs within the duplex with the phototautomerizable groups positioned in the major groove.[36]

Various spectroscopic techniques were used to characterize the conformation, solvation, and tautomeric dynamics within the major groove. In ssDNA, the model base pair populates both the syn- and anti-enol conformations[34,36,44] but is completely driven to the internally H-bonded syn-enol upon duplex forma-

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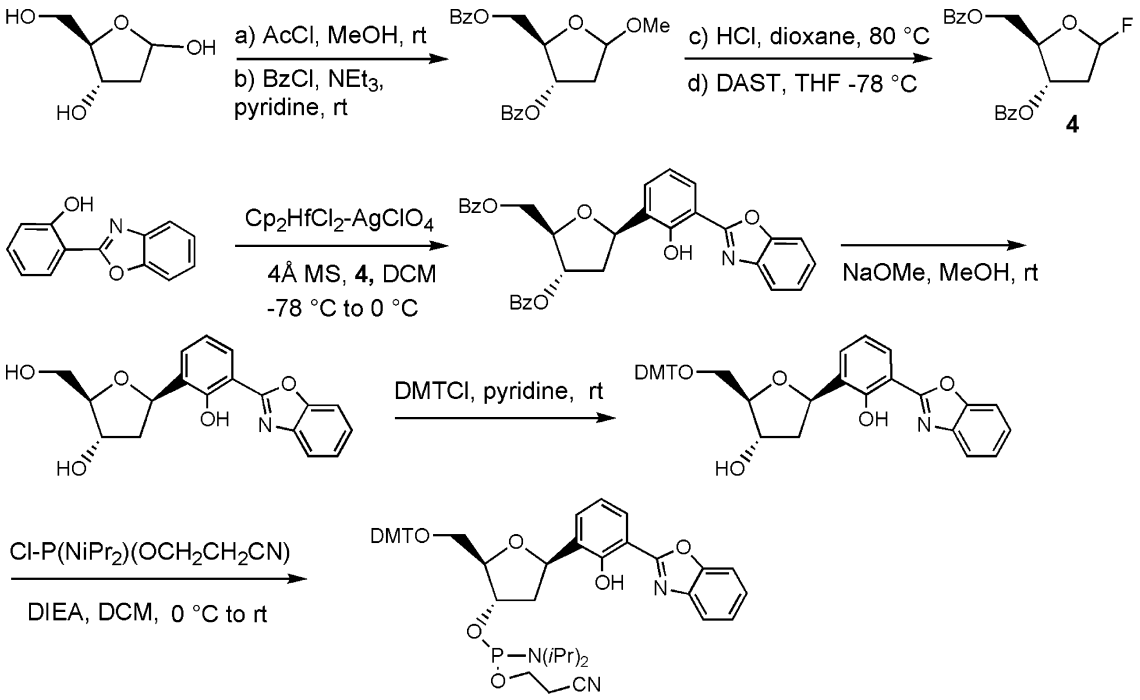
Scheme 1



tion. The major groove environment did not significantly affect the rate of proton transfer (which by fluorescence upconversion was found to be 150−170 fs for HBO in ssDNA, duplex DNA, hexane, methanol, and dimethyl sulfoxide[35,44]) but did increase the lifetime of the excited keto state by at least 17-fold relative to the other solvents examined.[34] Thus, the major groove environment appears to preclude solvation of the model base pair and stabilizes the keto−amino tautomer. To determine whether these properties are common to both the major and minor grooves, or unique to the major groove, we characterized the model base pair with its enol moiety positioned in the minor groove. We show that in contrast to the major groove, the minor groove environment stabilizes the enol−imino tautomer. Computational studies suggest that stabilization results from the formation of an internucleotide H-bond between the enol of the model base pair and the O4′ atom of an adjacent nucleotide. These observations may reflect fundamental differences in the environments provided by the major and minor grooves, which may have coevolved with the purine and pyrimidine base pairs to simultaneously ensure duplex stability, function, and recognition.

Results

The C-nucleoside, with HBO attached via C3, was synthesized as shown in Scheme 1 (see also the Supporting Information). The free aryl nucleoside was then converted to the corresponding phosphoramidite and incorporated into the DNA oligonucleotides 5′-CGTTTC(HBO)TTCTC, and 5′-GAGAA-(HBO)GAAACG using standard methods.[45] To form DNA duplexes containing the model base pair, each oligonucleotide was hybridized to a complementary oligonucleotide containing an abasic site at the position opposite HBO, resulting in duplexes **1** and **2**, respectively.

To characterize the stability of DNA containing the model base pair, the melting temperature, T_m, of each duplex was determined as described previously.[36] Both duplexes **1** and **2** showed simple two-state behavior with T_m values of 36.8 and 26.4 °C, respectively. The stability of **1** compares favorably with both that of the duplex containing a natural dA/dT, as well as with a duplex containing HBO with its enol−imino moieties disposed in the major groove (T_m = 38 and 39 °C, respectively). The greater stability of duplex **1**, relative to that of **2**, likely results from increased overlap of the benzoxazole moiety of HBO with flanking purines (dA and dG) of the opposite strand, as was observed in the same sequence context with HBO as a probe of the major groove.[36] CD measurements further supported the conclusion that duplexes **1** and **2** adopt native-like conformations. Both duplexes showed a negative band at ∼250 nm and a positive band at ∼280 nm, consistent with a B-form structure (see the Supporting Information).[46]

The steady-state emission spectra of the ssDNA as well as those of duplexes **1** and **2** are shown in Figure 2. The emission spectrum for HBO incorporated into DNA in the same sequence context as **1**, but with its enol group oriented in the major groove, is included for comparison and is referred to as duplex **3**.[36] In both ssDNA contexts, HBO fluorescence is only observed at ∼380 nm, in agreement with our previous results, suggesting that the model base pair is solvated or in its anti configuration.[36] As published previously,[36] upon formation of duplex **3**, emission from the model base pair is only detected at ∼400 nm, demonstrating the presence of an internal enol−imino H-bond, which efficiently tautomerizes upon excitation. In contrast, formation of duplex **1** or **2** does not result in any emission at longer wavelengths but rather results only in a small amount of fluorescence quenching. Thus, in contrast to the major groove, the minor groove environment appears to favor disruption of the internal HBO enol−imino H-bond.

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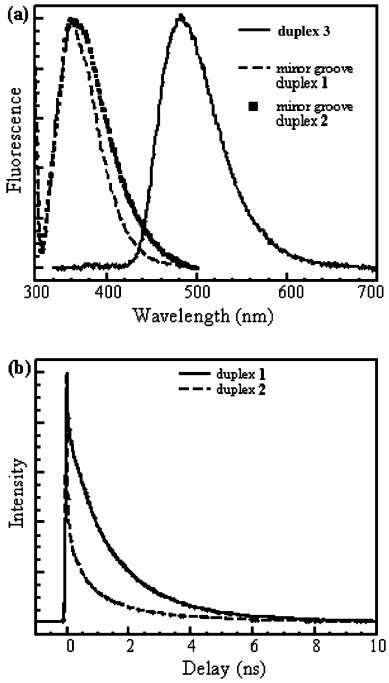

Figure 2. (a) Steady-state emission spectra of duplexes **1** and **2**. Also included is the spectrum of duplex **3** for comparison. (b) Fluorescence decay curves of **1** and **2**.

Table 1. Fluorescence Decay Rates

	A_1	k_1, (s^{-1})	A_2	k_2, (s^{-1})	A_3	k_3, (s^{-1})
duplex **1**	0.56	2.94×10^{10}	0.17	1.54×10^{9}	0.27	1.52×10^{8}
duplex **2**	0.87	4.17×10^{10}	0.08	2.56×10^{9}	0.05	5.56×10^{8}

Picosecond time-resolved fluorescence spectroscopy was employed to determine if the enol−imino H-bond is lost due to conformational dynamics or to disruption of the internal H-bond (for example, by solvation), as the syn- and anti-enols have characteristic lifetimes[35,44] (see the Supporting Information). With both duplexes **1** and **2**, three decay components were observed, a fast decay ($2.9-4.2 \times 10^{10}$ s^{-1}), an intermediate decay ($1.5-2.6 \times 10^{9}$ s^{-1}), and a slow decay ($1.5-5.6 \times 10^{8}$ s^{-1}) (Table 1). These time constants are in good agreement with those observed for HBO in different solvent environments that were previously assigned as vibrational relaxation (fast decay), the lifetime of the syn-enol (intermediate decay), and the lifetime of the anti-enol (slow decay).[35,44] The amplitudes of the intermediate and slow decays were approximately equal, and thus, we conclude that in this context HBO exists as an approximately 1:1 mixture of syn- and anti-enols. Thus, the loss of the internal enol−imino H-bond appears to result from both solvation, or other mechanism for disruption of the internal H-bond in the syn-enol, and rotational dynamics. Interestingly, the emission band for duplex **2** is significantly broader than that for **1**, which implies that duplex **2** has increased structural heterogeneity, consistent with the decreased stability of this duplex. The conformational, solvation, and tautomeric equilibria of HBO with its enol group oriented into the minor groove are distinctly different from that observed when the same hydroxyl group is positioned in the major groove.

To develop a molecular model that accounts for the different spectroscopic behavior of HBO with its enol moiety oriented in either the minor (duplex **1**) or major groove (duplex **3**), we conducted unrestrained molecular dynamics (MD) simulations

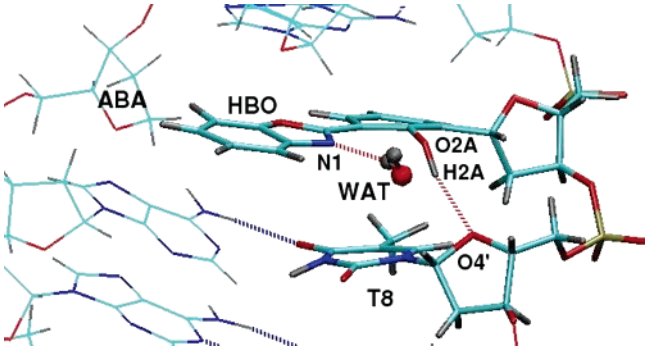

Figure 3. Average structure of duplex **1** predicted by MD simulations. The abasic site in the strand opposite HBO is labeled ABA, the enol−imino OH and N atoms are labeled O2A, H2A, and N1, respectively, and the bound water molecule is labeled WAT. HBO and the flanking dT nucleotide (labeled T8) are shown in bold.

using the AMBER 8 program.[47] The adaptation of the Cornell et al. force field,[48] published by Cheatham et al., was used in all the simulations.[49] The RESP atomic charges of the abasic site and HBO nucleotide were derived following the strategy developed by the Kollman group.[50,51] Geometry optimizations and MEP computations were carried out at the HF/6-31G*// HF/6-31G* level of theory using the Gaussian 98 program[52] and the R.E.D. interface.[53] To better simulate potential H-bonding interactions, new dihedral force field parameters were developed for the HBO enol−imino moiety. These parameters better reproduce quantum mechanical structure and energy predictions in the presence of water molecules representing putative H-bond donors and acceptors (see the Supporting Information). The duplex was solvated in a box of explicit water with neutralizing sodium counterions and subject to 3 ns constant pressure productive MD after a 225 ps period of constant volume equilibration. The previously optimized structure of duplex **3** was used for comparison.[36]

During MD simulations of duplex **1**, the model base pair remained well packed by the flanking purines that are present of the opposite strand. As we observed with duplex **3**, the structure appears to be only slightly affected by the presence of the model base pair (Figure 3). The MD simulations predict that duplex **1** forms a B-DNA type conformation, and simulations starting with canonical B- and A-DNA initial structures led to similar average structures. This result is consistent with the general preference of DNA duplexes to adopt B-form structures in solution, as well as with the CD studies described above. While a slight widening of the minor groove at the site of the model base pair is apparent, the small perturbation does not appear to affect the H-bonding pattern of the flanking base pairs.

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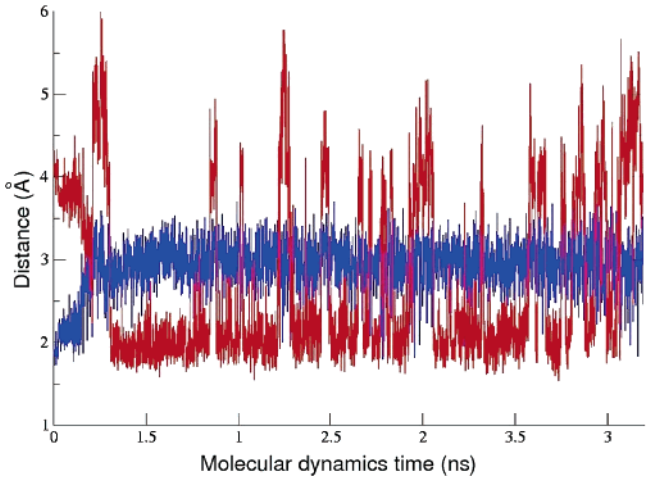

Figure 4. Time-dependent fluctuations of the internal enol−imino H-bond of HBO and the internucleotide H-bond between HBO and the O4′ atom of the flanking dT residue. The enol−imino H-bond distance is represented in blue, and the enol−O4′ H-bond distance is represented in red.

Interestingly, while neither duplex is distorted, the average structure predicted by the MD simulations is distinctly different for duplex **1** relative to that of duplex **3**. The average structure of duplex **1** predicts the disruption of the enol−imino H-bond that is present in the average structure of duplex **3**. Instead, two new H-bonds are observed. The imino nitrogen forms an H-bond with a water molecule within the minor groove, and the enol hydroxyl forms an H-bond with the O4′ endocyclic oxygen of the 3′ thymine nucleotide (Figure 3). This H-bond isomerization is clearly apparent upon examining the time-dependent fluctuations of the model base pair (Figure 4). Comparison of the δ and χ dihedral angle values of the HBO-7 and thymine-8 nucleotides measured during the MD in the presence of the H-bond (duplex **3**) or in its absence (duplex **1**) suggests that isomerization may occur without significantly perturbing the local structure of the duplex. Indeed, similar mean values and standard deviations were found for these dihedral angles in duplexes **1** and **3** (χ-7 = −132 ± 15 or −133 ± 15, χ-8 = −123 ± 20 or −125 ± 14, δ-7 = 119 ± 18 or 121 ± 17, δ-8 = 118 ± 19 or 118 ± 17, respectively). While all of the data suggest that duplex **1** adopts a B-type conformation, it should be noted that the two characteristic H-bonds predicted in the minor groove are also feasible in an A-form DNA conformation. Indeed, the distance between the HBO proton donor and the thymine-8 O4′ atoms measured for a canonical A-form DNA duplex is only 0.3 Å longer than the value found for the canonical B-form DNA duplex (3.83 and 3.53 Å, respectively). Therefore, the important structural differences between the major and minor grooves appear to be largely independent of whether the overall helix structure is of A- or B-form.

Discussion

As characterized by the HBO model base pair, the minor and major grooves provide distinctly different environments. In the major groove, we previously demonstrated that the keto−amino tautomer is significantly stabilized relative to the enol−imino tautomer. The major groove environment may be optimized to stabilize these tautomers to ensure the high fidelity storage and retrieval of genetic information. This is in stark contrast to the minor groove environment characterized in this study. In the minor groove, the HBO enol−imino ground-state tautomer is favored by formation of an internucleotide H-bond with a neighboring O4′ oxygen H-bond acceptor and an imino−water H-bond. The fact that HBO also populates the anti conformation when the enol is positioned in the minor groove, where the benzoxazole nitrogen atom is rotated into the major groove, suggests that minor groove solvation is not the dominant driving force behind the disruption of the internal H-bond. Rather, the major factor favoring disruption of the enol−imino H-bond appears to be the availability of the O4′ sugar atoms along the wall of the minor groove, which serve as H-bond acceptors for the HBO enol proton. In the major groove, this H-bond acceptor is not available to the enol (∼7.5 Å distant), and thus the enol forms an internal H-bond with the imino nitrogen.

At first glance, the data suggest that the minor groove O4′ H-bond acceptors might jeopardize duplex stability of function by stabilizing rare tautomers within DNA. However, neither a dG/dC nor a dA/dT base pair may maintain a Watson−Crick geometry and tautomerize its minor groove moieties without concomitantly tautomerizing its major groove moieties. In contrast, both base pairs may tautomerize their major groove moieties without concomitant tautomerization in the minor groove. Thus, sufficient stabilization of the keto−amine moieties in the major groove maybe sufficient to control base pair tautomerization.

Because the major groove environment strongly stabilizes the keto−amine tautomers important for sequence-specific recognition, the minor groove environment is free to possess functionality important for duplex stability or nonspecific recognition. An important component of stability and nonspecific recognition is a network of H-bonding and packing interactions in the minor groove involving nucleobase heteroatoms along the floor of the groove and the O4′ sugar atoms along the wall.[19] In fact, these moieties of adjacent nucleotides commonly collaborate to bind molecules within the minor groove. For example, DNA polymerases typically engage primer-template DNA, especially at the primer terminus, via interactions with the O4′ sugar atoms.[33] In addition, protein side chains and small molecules bound within the minor grove are commonly observed to bridge the O4′ atom of one nucleotide and the nucleobase heteroatom of an adjacent nucleotide.[27−32,54] In addition, water molecules are commonly observed to bridge the O4′ H-bond acceptor and the O2 of neighboring pyrimidines, the N2 of neighboring guanines, or the N3 of neighboring adenines and are thought to contribute to duplex stability.[55−58] These bridging water molecules may also be of functional significance as they have been suggested to narrow the minor groove, which is thought to be important for recognition by different proteins and drugs.[59] In contrast to DNA, the C2′ hydroxyl group of RNA provides a minor groove H-bond donor, and interestingly it forms an H-bond with the O4′ atom that stabilizes the A-form duplex.[55,56] Thus, within the minor groove, H-bond donors and acceptors along the floor and wall appear to interact directly, or via bridging molecules, to mediate duplex stability and recognition. When positioned in the minor groove, the enol H-bond acceptor of HBO is

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positioned to participate in this network of interactions. Thus, the stabilization of the HBO enol tautomer in the minor groove appears to have a very different origin than the stabilization of the keto tautomer observed in the major groove, the former being structural and the latter being electrostatic.

While arguments about why the major and minor grooves may have evolved to be different are heuristic by nature, the effects of the different environments on the HBO model base pair are nonetheless striking. It seems likely that such differences would be manifest in unique properties of the natural base pair functionalities within the major and minor grooves and thus contribute substantially to DNA dynamics and probably to function, as well. Further studies directed at the effects of metal ion and protein binding are currently underway and are expected to further define the differences between the major and minor grooves and how they impact the storage and retrieval of genetic information.

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Supporting Information Available: Synthetic procedures, CD spectra, T_m data, force field parametrization, details of the molecular dynamics simulations, and complete citations for refs 47 and 52. This material is available free of charge via the Internet at http://pubs.acs.org.

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