# Striving to Understand the Properties of Universal Nucleobases: A Computational Study of Azole Carboxamides

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Density functional theory (DFT) is used to study the properties of a series of azole carboxamides in attempts to better understand why these molecules do not have an equal affinity for all natural DNA (RNA) nucleobases, which is an important criterion for universal bases. The thermodynamics and kinetics for bond rotations that afford four azole carboxamide conformers, which each bind to a different natural (DNA) base, are studied. It is concluded that a particular conformer of some azole carboxamides is favorably stabilized; therefore, these molecules will likely preferentially bind to a particular natural base. The geometries and binding energies are calculated for complexes formed between azole carboxamides and natural bases. Our calculations indicate that some complexes are highly distorted and therefore likely reduce the stability of duplexes. Our calculations also indicate that azole carboxamides bind to natural bases with varying affinities. Furthermore, the azole carboxamide binding interactions are generally significantly less than those in the corresponding natural base pair, with the exception of the thymine (or uracil) azole carboxamide complexes. Our calculations provide insight into interactions between azole carboxamides and the natural bases and allow suggestions to be made regarding why these compounds do not function as universal nucleobases.

## Introduction

There are many scientific techniques that rely on the selective pairing of DNA nucleobases. For example, the first step in the polymerase chain reaction (PCR) is the binding of a short oligonucleotide to the target DNA.[1] PCR amplifies the number of copies of DNA and is commonly used to produce enough DNA to identify a deceased person or a criminal suspect. Hybridization probes, which are used to detect DNA or to determine the amount of a particular base sequence in a sample, also use base-pairing properties to bind nucleotide strands to specific regions in DNA. Another application of base pairing is antigene (antisense) technology.[2] These techniques bind short nucleic acid segments to a sequence of nucleobases in a DNA (RNA) strand responsible for a disease. Binding of the synthetic oligonucleotide to DNA (RNA) leads to the formation a triple (double) helix, which prevents genetic disorders or diseases by stopping RNA (protein) synthesis.

A potential problem with these applications is that the exact sequence of nucleobases must be known in order to construct a complementary strand that will attach to a specific region in DNA (RNA). More specifically, the identity of one or more bases may not be known. For example, because of the degeneracy of the genetic code, the identity of all nucleobases is not always clear when a DNA coding sequence is predicted from a protein sequence. Furthermore, the power of these techniques could be extended by the development of a general hybridization probe or therapeutic that can bind to related yet distinct genes. In these instances, it would be useful to have a "universal nucleobase" that binds without discrimination to all natural bases and does not destabilize the resulting double (triple) helix or affect the biochemical utility of the modified oligonucleotide.[3]

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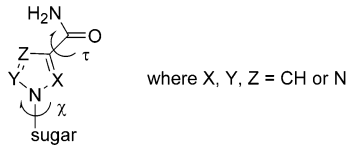
**SCHEME 1: Series of Azole Carboxamides Considered in the Present Study**




Although a universal nucleobase has many promising applications, it has proven difficult to design. Hypoxanthine (or its nucleotide form, 2′-deoxyinosine (**dI**)) was the first molecule to be identified as a potential universal nucleobase.[4] However, the applicability of **dI** as a universal nucleobase is limited.[5] To improve upon this molecule, some researchers emphasize the importance of strong stacking interactions, and others believe that stacking is not sufficient to stabilize double (triple) helices and molecules that also possess generic hydrogen bonding abilities must be considered. In particular, there could be a limit to the ability of non-hydrogen-bonded molecules to balance the loss of stability due to the presence of an unnatural base.[6] In general, improvements upon **dI** considered in the literature include molecules with neither strong hydrogen-bonding nor base-stacking preferences (e.g., abasic sites[7]), molecules with strong stacking interactions (e.g., nitroazoles,[8] nitropyrroles,[9] nitroindoles,[10] nitroindazoles,[11] benzimidazole[12]), and molecules with strong stacking and hydrogen-bonding interactions (e.g., azole carboxamides[6]). Other unnatural nucleobase analogues that solely rely on hydrophobic interactions,[13] as well as combinations of modified base and sugar residues,[11a,14] have also been discussed in the literature.

A promising design for a universal nucleobase is a family of azole carboxamides (Scheme 1),[6,15−23] which is derived from the structure of **dI**.[20] Azole carboxamides are attractive universal nucleobases because they can adopt four different conformations

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by rotation about the exocyclic carboxamide bond ($\tau$) and/or the glycosidic bond ($\chi$) (Scheme 1) and each conformation uniquely binds to one of the four natural DNA bases.[15,16,20] Binding is selective because of requirements such as the appropriate location of hydrogen bond acceptor and donor groups, the relative location of the sugar moieties, and the ability of the pair to fit adequately into a double (triple) helix without causing distortions. Although azole carboxamides have the potential to be good universal bases, experimental work has shown that these molecules preferentially bind to different natural nucleobases.[6,15−23]

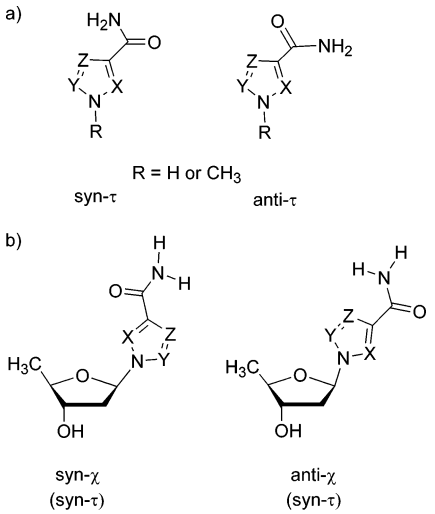
Before new universal nucleobases can be efficiently designed, unknowns surrounding interactions between molecules currently used for this application and the natural bases must be clarified. Because many properties of potential universal nucleobases, such as structure and hydrogen bond strengths with natural DNA bases, are difficult to obtain from experiment, computational chemistry is an ideal tool to use to investigate these systems. In the present work, computational chemistry is used to study a series of azole carboxamides (Scheme 1) to better understand the reason(s) that these molecules do not act as universal nucleobases. Properties to be investigated include the relative energies of conformers, the rotational barriers between conformers, and the hydrogen-bonding interactions with natural nucleobases. Although previous studies have used theory to consider select properties of individual azole carboxamides,[6,16,23] to our knowledge there has not been a systematic computational study of the series of azole carboxamides or their hydrogen-bonding properties. Attempts will be made to use our results to understand better the experimentally observed prejudice in the base-pairing properties of these molecules. It is hoped that this work will also provide general information that will aid the design of more efficient universal nucleobases in the future.

## Computational Details

Geometries of the azole carboxamides, as well as the rotational transition structures connecting different conformers, were optimized using Becke's three-parameter hybrid exchange functional (B3) in combination with Lee, Yang, and Parr's correlation functional (LYP) and Pople's 6-31G(d,p) basis set. Frequency calculations were performed at the same level of theory to characterize all structures. Intrinsic reaction coordinate (IRC) calculations were performed to verify that all transition structures connect the expected reactant and product. B3LYP/6-31G(d,p) zero-point energies were corrected using a scale factor of 0.9806. Single-point energy calculations were performed at the B3LYP/6-311G(2df,p) level.

Hydrogen-bonded pairs between the azole carboxamides and the natural DNA bases were optimized at the B3LYP/6-31G(d,p) level of theory. Zero-point energy corrections were calculated at the same level, and the appropriate scale factor was applied. Higher-level single-point calculations on hydrogen-bonded structures were performed using an extended basis set known to describe hydrogen bond energies (B3LYP/6-311+G(2df,p)) accurately. All binding energies were corrected by subtracting the basis set superposition error (BSSE) calculated according to the Boys and Bernardi counterpoise method.[24] The general importance of including this correction has been well documented in the literature.[25,26] We note that the reported binding energies represent the interaction energy between the nucleobase and the appropriate (syn-$\tau$ or anti-$\tau$) azole carboxamide conformer. Specifically, the energy difference between the syn-$\tau$ and anti-$\tau$ conformers is not included in the binding energies.

**SCHEME 2: Model Systems Used in the Present Study to Investigate the Properties of Azole Carboxamides**




In general, density functional theory (DFT) methods such as B3LYP are more appropriate for larger hydrogen-bonded systems compared with ab initio techniques such as MP2. Although some reservations have been expressed,[27] DFT has been successfully used to study hydrogen-bonded complexes.[28] Furthermore, although DFT has been shown to underestimate stabilization energies of stacked DNA base pairs,[29] it has been used successfully to predict relative hydrogen bond energies for a variety of systems. Additionally, DFT methods have been used to study, for example, radicals of DNA nucleobase pairs,[30] interactions between modified nucleobases,[31,32] and interactions between nucleobases and water.[33] It should also be emphasized that B3LYP hydrogen bond strengths are very appropriate for obtaining qualitative information about relative binding strengths, which is the primary focus of the present investigation.

## Results and Discussion

**Geometries and Relative Energies of Azole Carboxamide Conformers.** For azole carboxamides to be universal nucleobases, there must be no preference for a particular conformer obtained through rotation about $\tau$ or $\chi$ (Scheme 1). Thermodynamically, there must be a small energy difference between all conformers. To investigate the relative stability of different azole carboxamide conformers, a series of model systems were investigated. The simplest models replace the sugar group required to add azole carboxamides to DNA (RNA) with a hydrogen atom or a methyl group (Scheme 2a). These models allow us to compare the anti-$\tau$ and syn-$\tau$ conformers. A larger model that better accounts for the sugar moiety was also considered (Scheme 2b). The sugar model replaces the DNA phosphate groups with a hydroxyl group at C3′ and a hydrogen atom at C5′. This model allows for differentiation between the anti-$\chi$ and syn-$\chi$ conformers and has been previously used to study the conformational properties of deoxyribose.[34]

In general, there is a good correlation between the geometries and relative energies for all model systems. Selected B3LYP/6-31G(d,p) geometrical parameters, which account for the most significant differences between the azole carboxamides, are presented in Table 1. We note that the model with a methyl group has been previously studied for some azole carboxamides, and in general, our results are in agreement with previous findings.[6,16,19,23,35,36]

The structure of the azole carboxamides depends on the functionality of X, Y, and Z. In particular, the largest variation

**TABLE 1: Selected Gas-Phase Geometrical Parameters (deg) and Relative Energies (kJ mol$^{-1}$) for Conformers of the Azole Carboxamides[a]**

| | | | | anti-$\tau$ | | | | | syn-$\tau$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| model[b] | X | Y | Z | ∠(NCCX) | ∠($H_1$NCC) | ∠($H_2$NCC) | $\chi^c$ | $\Delta E_\chi{}^d$ | ∠(OCCX) | ∠($H_1$NCC) | ∠($H_2$NCC) | $\chi^c$ | $\Delta E_\chi{}^d$ | $\Delta E_\tau{}^e$ |
| H | CH | CH | CH | 13.4 | 170.8 | 28.1 | | | 7.6 | 170.0 | 15.0 | | | 3.2 |
| $CH_3$ | CH | CH | CH | 12.6 | 170.7 | 28.1 | | | 7.6 | 170.4 | 21.9 | | | 4.3 |
| sugar (anti-$\chi$) | CH | CH | CH | 13.9 | 170.7 | 28.7 | 138.8 | | 8.9 | 170.3 | 22.8 | 143.8 | | 3.8 |
| sugar (syn-$\chi$) | CH | CH | CH | 13.9 | 170.5 | 28.7 | 151.0 | 0.0 | 9.6 | 170.3 | 23.0 | 140.6 | 0.4 | 4.2 |
| H | CH | CH | N | 22.7 | 170.9 | 30.8 | | | 0.0 | 180.0 | 0.0 | | | 33.7 |
| $CH_3$ | CH | CH | N | 22.4 | 172.6 | 30.9 | | | 0.0 | 180.0 | 0.0 | | | 34.2 |
| sugar (anti-$\chi$) | CH | CH | N | 23.6 | 172.5 | 31.2 | 159.9 | | 0.3 | 178.2 | 1.9 | 179.9 | | 33.9 |
| sugar (syn-$\chi$) | CH | CH | N | 21.6 | 172.2 | 30.1 | 171.5 | −1.1 | 0.5 | 179.0 | 1.2 | 116.3 | 2.6 | 37.7 |
| H | CH | N | CH | 12.6 | 171.4 | 26.2 | | | 6.6 | 172.1 | 19.1 | | | 5.1 |
| $CH_3$ | CH | N | CH | 11.7 | 171.1 | 26.7 | | | 6.2 | 171.5 | 19.7 | | | 5.3 |
| sugar (anti-$\chi$) | CH | N | CH | 12.9 | 171.1 | 26.2 | 90.8 | | 8.1 | 171.2 | 21.4 | 96.8 | | 3.1 |
| sugar (syn-$\chi$) | CH | N | CH | 10.3 | 170.8 | 24.9 | 169.0 | −3.0 | 9.1 | 171.1 | 22.1 | 172.2 | −3.5 | 2.6 |
| H | CH | N | N | 25.1 | 173.3 | 29.6 | | | 0.0 | 180.0 | 0.0 | | | 34.2 |
| $CH_3$ | CH | N | N | 24.1 | 173.0 | 29.7 | | | 0.0 | 180.0 | 0.0 | | | 34.7 |
| sugar (anti-$\chi$) | CH | N | N | 24.5 | 173.0 | 29.2 | 86.6 | | 0.0 | 179.4 | 0.5 | 90.4 | | 32.0 |
| sugar (syn-$\chi$) | CH | N | N | 22.7 | 172.8 | 28.4 | 161.5 | −2.1 | 0.3 | 179.1 | 0.9 | 163.5 | −2.3 | 31.8 |
| H | N | CH | CH | 0.0 | 180.0 | 0.0 | | | 20.0 | 170.0 | 10.0 | | | −28.9 |
| $CH_3$ | N | CH | CH | 0.0 | 180.0 | 0.0 | | | 20.2 | 171.9 | 25.4 | | | −24.8 |
| sugar (anti-$\chi$) | N | CH | CH | 0.7 | 174.7 | 6.2 | 171.5 | | 20.4 | 172.0 | 24.8 | 162.7 | | −22.6 |
| sugar (syn-$\chi$) | N | CH | CH | 0.1 | 175.2 | 5.9 | 98.0 | 2.2 | 19.5 | 172.1 | 24.4 | 92.8 | 4.7 | −20.1 |
| H | N | CH | N | 0.0 | 180.0 | 0.0 | | | 0.0 | 180.0 | 0.0 | | | 4.6 |
| $CH_3$ | N | CH | N | 0.0 | 180.0 | 0.0 | | | 0.0 | 180.0 | 0.0 | | | 5.5 |
| sugar (anti-$\chi$) | N | CH | N | 0.2 | 175.8 | 5.6 | 174.6 | | 0.9 | 179.3 | 0.6 | 169.8 | | 8.9 |
| sugar (syn-$\chi$) | N | CH | N | 0.7 | 175.7 | 5.9 | 75.2 | 1.9 | 0.1 | 179.4 | 0.6 | 95.2 | 4.5 | 11.4 |
| H | N | N | CH | 0.0 | 180.0 | 0.0 | | | 20.0 | 170.0 | 15.0 | | | −26.1 |
| $CH_3$ | N | N | CH | 0.0 | 180.0 | 0.0 | | | 19.5 | 173.2 | 24.8 | | | −23.8 |
| sugar (anti-$\chi$) | N | N | CH | 0.0 | 177.7 | 3.1 | 100.7 | | 22.3 | 173.0 | 25.8 | 108.3 | | −24.8 |
| sugar (syn-$\chi$) | N | N | CH | 0.6 | 176.3 | 5.2 | 108.4 | −3.4 | 19.4 | 173.2 | 23.8 | 104.5 | 2.8 | −18.5 |
| H | N | N | N | 0.0 | 180.0 | 0.0 | | | 0.0 | 180.0 | 0.0 | | | 6.4 |
| $CH_3$ | N | N | N | 0.0 | 180.0 | 0.0 | | | 0.0 | 180.0 | 0.0 | | | 7.0 |
| sugar (anti-$\chi$) | N | N | N | 1.1 | 177.9 | 3.1 | 97.4 | | 1.2 | 179.4 | 0.7 | 106.4 | | 5.0 |
| sugar (syn-$\chi$) | N | N | N | 1.8 | 176.7 | 5.3 | 112.2 | −1.8 | 0.6 | 179.6 | 0.4 | 106.2 | 4.8 | 11.6 |

[a] Geometries were optimized at the B3LYP/6-31G(d,p) level. Relative energies were obtained from B3LYP/6-311G(2df,p) single-point calculations and include scaled (0.9806) zero-point energy corrections. [b] See Scheme 2. [c] $\chi$ is the O1′C1′NX dihedral angle for the anti-$\tau$:anti-$\chi$ and syn-$\tau$:anti-$\chi$ conformers and the O1′C1′NY dihedral angle for the anti-$\tau$:syn-$\chi$ and syn-$\tau$:syn-$\chi$ conformers. [d] Relative energy calculated as $E$(anti-$\chi$) − $E$(syn-$\chi$). [e] Relative energy calculated as $E$(anti-$\tau$) − $E$(syn-$\tau$).

in the geometries occurs for the ∠(NCCX) or ∠(OCCX) dihedral angles (Table 1), which represent a twisting of the external carboxamide group relative to the molecular plane of the azole ring. Combinations of X, Y, and Z that result in an eclipsed orientation of the external amino group with respect to a nitrogen atom in the azole ring (X = N for anti-$\tau$ conformers and Z = N for syn-$\tau$ conformers) lead to planar molecules (∠(NCCX) or ∠(OCCX) ≈ 0°). These planar structures likely arise from a favorable N···H−N intramolecular hydrogen bond. All other structures (X = CH for anti-$\tau$ and Z = CH for syn-$\tau$) display a twisting of the external carboxamide group relative to the azole ring, which is likely due to unfavorable steric interactions between the external amino group and CH in the azole ring. The degree of twisting depends on the interaction between the ring and the carbonyl oxygen. The smallest degree of distortion is observed in structures where a ring CH interacts with the carbonyl oxygen. (∠(NCCX) values range from 10.3 or 13.9° for anti-$\tau$ (X = Z = CH), and ∠(OCCX) values range from 6.2 to 9.6° for syn-$\tau$ (X = Z = CH).) A greater degree of distortion is observed when the external carbonyl group is a neighbor to nitrogen in the azole ring. (∠(NCCX) values range from 21.6 to 25.1° for anti-$\tau$ (X = CH,Z = N), and ∠(OCCX) values range from 19.4 to 22.3° for syn-$\tau$ (X = N,Z = CH).)

All structures with a twisted carboxamide group relative to the azole ring also exhibit a puckering of the amino group. The ∠($H_1$NCC) and ∠($H_2$NCC) dihedral angles range from 170 to 173.2° and 10.0 to 31.2°, respectively. There is no apparent correlation between the values of X, Y, and Z and the puckering of the amino group. The amino group puckering in structures where the external carboxamide and the azole ring are coplanar depends on the model, where the amino group is completely planar in models that replace the DNA sugar by a hydrogen atom or a methyl group and slightly puckered (by less than 6.2°) in the model that includes a (modified) sugar moiety.

The calculated relative energies of the anti-$\tau$ and syn-$\tau$ conformers are displayed in Table 1 ($\Delta E_\tau$), where a positive value indicates that the syn-$\tau$ conformer is more stable. As found for the geometries, the relative energies calculated for all model systems are very similar. For all but two combinations of X, Y, and Z, the syn-$\tau$ conformer is more stable than the anti-$\tau$ conformer. For the X = N,Y = Z = CH and X = Y = N,Z = CH combinations, the anti-$\tau$ conformer is more stable by approximately 20−30 kJ mol$^{-1}$. This likely arises from favorable N···H−N and C−H···O intramolecular interactions in this conformation. Similar interactions result in significant stabilization of the syn-$\tau$ conformer (by approximately 30−40 kJ mol$^{-1}$) for the X = Y = CH, Z = N and X = CH, Y = Z = N combinations. The remaining four combinations of X, Y, and Z exhibit very small (approximately 3−11 kJ mol$^{-1}$) energy differences between the two conformers. In all instances, X = Z; therefore, there are no preferable interactions when the carboxamide group is directed toward a particular side of the

**TABLE 2: Dihedral Angle (deg) in Optimized Transition Structures and Relative Energies (kJ mol$^{-1}$) for Interconversion between Anti-$\tau$ and Syn-$\tau$ Azole Carboxamide Conformers[a,b]**

| X | Y | Z | ∠(OCCX) | $\Delta E_{syn-\tau}$ | $\Delta E_{TS(syn-\tau\rightarrow anti-\tau)}$ | $\Delta E_{anti-\tau}$ | $\Delta E_{TS(anti-\tau\rightarrow syn-\tau)}$ |
|---|---|---|---|---|---|---|---|
| CH | CH | CH | 89.1 | 0.0 | 24.6 | 4.3 | 20.3 |
| CH | CH | N | 93.8 | 0.0 | 44.1 | 34.2 | 9.0 |
| CH | N | CH | 89.3 | 0.0 | 23.6 | 5.3 | 18.3 |
| CH | N | N | 96.3 | 0.0 | 41.3 | 34.7 | 6.6 |
| N | CH | CH | 83.6 | 0.0 | 11.0 | −24.8 | 35.8 |
| N | CH | N | 89.5 | 0.0 | 26.4 | 5.5 | 20.9 |
| N | N | CH | 83.8 | 0.0 | 9.2 | −23.8 | 33.0 |
| N | N | N | 91.0 | 0.0 | 24.7 | 7.0 | 17.7 |

[a] Calculations performed on models with R = $CH_3$ (Scheme 2a). [b] Geometries were optimized with B3LYP/6-31G(d,p). Relative energies were obtained from B3LYP/6-311G(2df,p) single-point calculations and include scaled (0.9806) zero-point energy corrections.

ring. Support for this statement comes from a comparison of the ∠(NCCX) and ∠(OCCX) dihedral angles (Table 1), which are very similar in instances where $\Delta E_\tau$ is small but deviate more significantly as $\Delta E_\tau$ increases.

In addition to a small energy difference between the syn-$\tau$ and anti-$\tau$ conformers, there must exist a small energy difference between the syn-$\chi$ and anti-$\chi$ conformers for azole carboxamides to function as universal nucleobases. The $\Delta E_\chi$ values displayed in Table 1 are small (less than 4.8 kJ mol$^{-1}$) regardless of the conformation about $\tau$. Thus, there is no particular preference for stabilization about $\chi$. We note that the magnitude of the dihedral angle that controls the relative orientation of the base with respect to the sugar ring ($\chi$, Table 1) ranges from approximately 90−180°.[37] Deviations of this dihedral angle from the corresponding value in natural DNA (RNA) nucleotides could lead to differences in interactions between azole carboxamides and natural bases in double helices because of disruptions in base pairing and stacking or distortions in the backbone.

Selected calculations on a larger sugar model that includes a hydroxyl group at C5′ indicate that the anti-$\chi$ conformer is favored when X = N and Y = CH and that the syn-$\chi$ conformer is favored when X = CH and Y = N. These preferences arise from favorable C−H···O intramolecular hydrogen bonding between CH in the ring and the hydroxyl group at C5′. It is not clear whether this preference will remain within a DNA (RNA) strand or whether this preference is lost when potentially stronger hydrogen bonds between the azole carboxamides and the natural bases are possible. Concerns that interactions between the external amino group in azole carboxamides and the phosphate backbone may stabilize one orientation have been previously expressed in the literature.[23] In contrast to these concerns, Klewer et al.[23] report evidence of a conformational exchange about $\chi$ when the X = Z = N, Y = CH azole carboxamide is paired with thymine. We note that an extensive computational investigation of the anti-$\chi$ and syn-$\chi$ conformers using a larger model is beyond the scope of the present work. These issues will be the subject of a more detailed study in the future.

In summary, the relative energies suggest that any azole carboxamide with X = Z may be a potential universal nucleobase if only the thermodynamic stability of the anti and syn conformers is taken into account. However, geometrical differences between conformers must also be considered. In particular, significant puckering and twisting of the carboxamide group relative to the azole molecular plane may disrupt and destabilize the double helix by resulting in poorer base stacking interactions. Thus, azole carboxamides with planar anti-$\tau$ and syn-$\tau$ conformers may be more desirable universal bases. We note that the anti-$\tau$ and syn-$\tau$ conformations of the azole carboxamides with X = Z = N and Y = CH or N are planar and thermodynamically equivalent.

**Rotational Barriers for Conversion Between Anti-$\tau$ and Syn-$\tau$ Azole Carboxamides.** Although a small energy difference between the anti and syn conformers for the series of azole carboxamides may provide some indication of the ability of these structures to act as universal nucleobases, kinetics may prohibit the interconversion between two conformers. Therefore, transition structures connecting the anti-$\tau$ and syn-$\tau$ conformers were located using our model that includes a methyl group (R = $CH_3$, Scheme 2a). Frequency calculations verify that all optimized geometries are transition structures with imaginary (negative) frequencies ranging from 75 to 96.3 cm$^{-1}$.

The geometries of the transition structures are very similar for each choice of X, Y, and Z. The carboxamide group is nearly perpendicular to the azole ring, where the ∠(OCCX) dihedral angles range from 83.6 to 96.3° (Table 2). The ∠(OCCX) dihedral angle is closest to 90° for structures with X = Z. Structures with X = N and Z = CH have the smallest dihedral angles (83.6 and 83.8°), and structures with X = CH and Z = N have the largest dihedral angles (93.8 and 96.3°). Deviations of ∠(OCCX) from 90° arise when X ≠ Z because the external amino group has more favorable interactions with a nitrogen atom in the azole ring compared with interactions with CH; therefore, the transition structure shows a slight preference for one side of the ring.

The transition barriers for conversion from the syn-$\tau$ to the anti-$\tau$ conformer for the series of azole carboxamides range from 9.2 to 44.1 kJ mol$^{-1}$ ($\Delta E_{TS(syn-\tau\rightarrow anti-\tau)}$, Table 2). The smallest energy barriers (9.2−11.0 kJ mol$^{-1}$) occur for azole carboxamides with X = N and Z = CH, but conversion in the reverse direction (anti-$\tau$ → syn-$\tau$) for these combinations is associated with a much larger barrier (33.0−35.8 kJ mol$^{-1}$). Similarly, the largest barriers for conversion from the syn-$\tau$ to anti-$\tau$ conformer (41.3−44.1 kJ mol$^{-1}$) occur when X = CH and Z = N, but the reverse transition barriers are much smaller (6.6−9.0 kJ mol$^{-1}$). The large difference in the forward and reverse barriers for azole carboxamides with X ≠ Z arises from a thermodynamic preference for one conformer (Table 2). Alternatively, the transition barriers for conversion in either direction in azole carboxamides with X = Z, which have energetically equivalent syn-$\tau$ and anti-$\tau$ conformers, fall in a small range of 17.7−26.4 kJ mol$^{-1}$. There is no apparent correlation between the magnitude of the transition barriers and the relative stability of the syn-$\tau$ and anti-$\tau$ conformers among the four X = Z combinations. We note that the transition barriers calculated with density functional theory are larger than those estimated with AM1.[23]

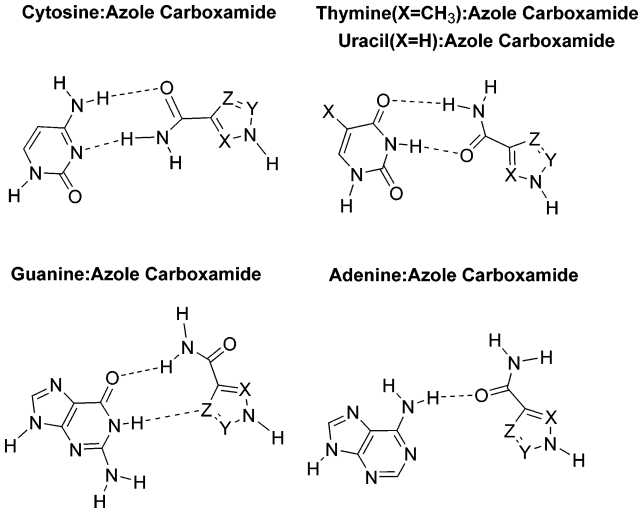
In summary, although the azole carboxamides with X ≠ Z have the lowest barriers for conversion from the syn-$\tau$ to the anti-$\tau$ conformer, the barriers in the reverse direction are much larger. This difference may lead to preferential stabilization of one conformer. Alternatively, azole carboxamides with X = Z

**TABLE 3: Calculated Binding Energies (kJ mol$^{-1}$) between Azole Carboxamides and Natural DNA (or RNA) Bases[a]**

| azole carboxamide[b] | | | | | | | |
|---|---|---|---|---|---|---|---|
| X | Y | Z | C:anti-$\tau$ | TL:syn-$\tau$ | U:syn-$\tau$ | G:syn-$\tau$ | A:anti-$\tau$ |
| CH | CH | CH | 56.1 | 43.9 | 45.7 | 24.4[c] | 23.6 |
| CH | CH | N | 57.3 | 40.5 | 41.1 | 28.7 | 16.9[c] |
| CH | N | CH | 58.0 | 44.3 | 44.1 | 32.9[c] | 22.7 |
| CH | N | N | 57.8 | 40.9 | 40.7 | 51.7 | 16.0[c] |
| N | CH | CH | 52.8 | 38.6 | 43.0 | 36.4[c] | 24.6 |
| N | CH | N | 54.1 | 40.2 | 41.8 | 25.7 | 16.7[c] |
| N | N | CH | 53.5 | 41.4 | 43.3 | 34.4[c] | 24.7 |
| N | N | N | 56.4 | 40.3 | 41.2 | 44.9 | 17.6[c] |

[a] Geometries were optimized at the B3LYP/6-31G(d,p) level. Relative energies were obtained from B3LYP/6-311+G(2df,p) single-point calculations and include scaled (0.9806) zero-point energy and BSSE corrections. [b] Calculations performed on models with R = H (Scheme 2a). [c] Highly distorted azole carboxamide pairs. See Figures 1 and 2 and the text for a discussion.

**SCHEME 3: Hydrogen-Bonding Interactions between Model Azole Carboxamides and Natural DNA (or RNA) Bases**




should relatively easily convert between anti-$\tau$ and syn-$\tau$ conformers. Therefore, these molecules are more suitable for applications as universal nucleobases. We note that the transition barrier for conversion between the anti-$\chi$ and syn-$\chi$ conformers is beyond the scope of the present study. These effects will be considered in more detail in a future study of the sugar moiety, which may also provide a greater understanding of the conformational exchange about $\chi$ reported experimentally.[23]

**Interactions Between Azole Carboxamides and Natural Nucleobases.** Although the relative energies of the syn and anti ($\tau$ or $\chi$) conformers, as well as the rotational barriers between conformers, are important considerations when contemplating the ability of azole carboxamides to act as universal bases, the binding energy of each species with the natural nucleobases is also an important factor and must be carefully considered. In particular, it is expected that the stability of a duplex containing an azole carboxamide depends at least in part on the stability of the pairs in the interior of the duplex. We discuss for the first time the calculated binding energies (Table 3) of complexes between the azole carboxamides and the natural bases using models that replace the DNA (RNA) sugar with a hydrogen atom (Scheme 3). Although only selected geometrical parameters are discussed in the text (Tables 4−7), full geometrical coordinates for all complexes are available in the Supporting Information. The interactions with each natural base will initially be discussed separately.

**Cytosine.** Cytosine can hydrogen bond with the anti-$\tau$ azole carboxamides.[38] All azole carboxamides that are planar in isolation (Table 1) are also planar when hydrogen bonded to cytosine (Table 4). Alternatively, azole carboxamides that exhibit twisting or puckering at the amino group in isolation (Table 1) generally show a decrease in twisting ($\angle$(NCCX) decreases by up to 6.7°) and less puckering ($\angle$($H_1$NCC) and $\angle$($H_2$NCC) angles decrease by up to 9.1°) when paired with cytosine. The most significant changes occur for the azole carboxamide with X = CH, Y = Z = N, which becomes planar upon binding with cytosine. Despite the fact that the distortion within the azole carboxamides decreases when interactions with cytosine are considered, distortion in three azole carboxamides (X = Y = Z = CH; X = Y = CH, Z = N; X = Z = CH, Y = N) leads to a slightly nonplanar cytosine pair.

The cytosine-azole carboxamide pairs contain two hydrogen-bonding interactions (Scheme 3). The N−H···O hydrogen bond lengths between the cytosine amino group and the external carbonyl oxygen of the azole carboxamide range from 1.789 to 1.843 Å. The N···H−N hydrogen bond length between cytosine and the external amino group of the azole carboxamide falls between 1.900 and 1.942 Å. Both of these hydrogen bonds are nearly linear, where hydrogen bond angles deviate from 180° by less than 10°. For comparison, the natural cytosine-guanine pair contains (linear) N−H···O, N···H−N, and O···H−N hydrogen-bonding interactions, which involve hydrogen bond distances (calculated at the same level of theory) of 1.750, 1.896, and 1.903 Å, respectively.

The calculated binding energies between cytosine and all anti-$\tau$ azole carboxamides fall within a 5.2 kJ mol$^{-1}$ range (Table 3). The smallest cytosine-azole carboxamide binding strength (52.8 kJ mol$^{-1}$) occurs for the X = N, Y = Z = CH combination, and the largest binding strength (58.0 kJ mol$^{-1}$) occurs for X = Z = CH, Y = N. Although the binding of each azole carboxamide to cytosine is very similar, the calculated binding energies are significantly smaller (by 38−44 kJ mol$^{-1}$) than that calculated for the cytosine-guanine base pair at the same level of theory (96.6 kJ mol$^{-1}$). This difference likely arises from differences in hydrogen bonding patterns, where the three hydrogen bonds present in the natural cytosine-guanine base pair are replaced by two hydrogen bonds in the cytosine-azole carboxamide pairs. Additionally, the hydrogen bonds in the cytosine azole-carboxamide pairs are significantly longer (by 0.01−0.09 Å) than corresponding bonds in the natural pair.

**Thymine and Uracil.** Because a universal nucleobase is advantageous for applications involving both DNA and RNA, it is of interest to consider the binding strength of the azole carboxamides to both thymine and uracil (Scheme 3). Thymine and uracil can hydrogen bond with the syn-$\tau$ azole carboxamides (Scheme 3). As for the cytosine pairs, the geometries of the azole carboxamides within the thymine and uracil pairs (Table 5) are relatively unchanged compared with the individually optimized structures (Table 1). More specifically, if the azole carboxamide is planar in isolation, then it remains planar upon pairing with thymine or uracil. However, if the monomer is distorted in isolation, then the distortion decreases upon binding. The most significant geometrical changes occur for azole carboxamides with X = Y = Z = CH or X = Z = CH, Y = N, which become planar upon base pairing. Thus, only the thymine (uracil)-azole carboxamide pairs with X = N and Z = CH are nonplanar, which arises from twisting within the azole carboxamide.

All thymine-azole carboxamide pairs possess very similar geometries, which contain two hydrogen bonds (Scheme 3). The

**TABLE 4: Selected Bond Lengths (Å) and Bond Angles (deg) for the Cytosine-Azole Carboxamide Pairs (Scheme 3)[a]**

| X | Y | Z | ∠(NCCX) | ∠($H_1$NCC) | ∠($H_2$NCC) | R(N−H···O) | R(N···H−N) | ∠(N−H···O) | ∠(N···H−N) |
|---|---|---|---|---|---|---|---|---|---|
| CH | CH | CH | 12.5 | 179.8 | 0.6 | 1.789 | 1.931 | 179.2 | 172.3 |
| CH | CH | N | 24.9 | 178.0 | 16.3 | 1.801 | 1.929 | 179.7 | 171.7 |
| CH | N | CH | 5.0 | 178.4 | 5.1 | 1.811 | 1.911 | 179.8 | 172.4 |
| CH | N | N | 0.0 | 180.0 | 0.0 | 1.825 | 1.902 | 179.0 | 171.9 |
| N | CH | CH | 0.0 | 180.0 | 0.0 | 1.807 | 1.942 | 179.3 | 170.0 |
| N | CH | N | 0.0 | 180.0 | 0.0 | 1.819 | 1.930 | 179.8 | 170.3 |
| N | N | CH | 0.0 | 180.0 | 0.0 | 1.828 | 1.917 | 179.6 | 170.4 |
| N | N | N | 0.0 | 180.0 | 0.0 | 1.843 | 1.900 | 178.6 | 170.7 |

[a] Geometries were optimized at the B3LYP/6-31G(d,p) level.

**TABLE 5: Selected Bond Lengths (Å) and Bond Angles (deg) for the Thymine-Azole Carboxamide and Uracil-Azole Carboxamide[a] Pairs (Scheme 3)[b]**

| X | Y | Z | ∠(OCCX) | ∠($H_1$NCC) | ∠($H_2$NCC) | R(O···H−N) | R(N−H···O) | ∠(O···H−N) | ∠(N−H···O) |
|---|---|---|---|---|---|---|---|---|---|
| CH | CH | CH | 0.0 | 180.0 | 0.0 | 1.849 | 1.838 | 172.1 | 177.6 |
| | | | (0.0) | (180.0) | (0.0) | (1.886) | (1.754) | (172.3) | (177.6) |
| CH | CH | N | 0.0 | 180.0 | 0.0 | 1.850 | 1.821 | 169.7 | 177.7 |
| | | | (0.0) | (180.0) | (0.0) | (1.894) | (1.766) | (169.8) | (177.5) |
| CH | N | CH | 0.0 | 180.0 | 0.0 | 1.871 | 1.809 | 172.4 | 176.9 |
| | | | (0.0) | (180.0) | (0.0) | (1.869) | (1.777) | (172.5) | (176.8) |
| CH | N | N | 0.0 | 180.0 | 0.0 | 1.870 | 1.799 | 170.3 | 176.7 |
| | | | (0.0) | (180.0) | (0.0) | (1.873) | (1.795) | (170.4) | (176.6) |
| N | CH | CH | 21.7 | 176.3 | 14.3 | 1.874 | 1.800 | 172.6 | 176.1 |
| | | | (21.7) | (176.5) | (14.0) | (1.869) | (1.794) | (172.6) | (176.1) |
| N | CH | N | 0.0 | 180.0 | 0.0 | 1.871 | 1.781 | 170.4 | 176.0 |
| | | | (0.0) | (180.0) | (0.0) | (1.870) | (1.804) | (170.4) | (176.0) |
| N | N | CH | 20.6 | 178.3 | 12.1 | 1.894 | 1.771 | 172.8 | 175.3 |
| | | | (20.5) | (177.6) | (12.7) | (1.850) | (1.816) | (173.0) | (175.5) |
| N | N | N | 0.0 | 180.0 | 0.0 | 1.887 | 1.758 | 171.0 | 175.2 |
| | | | (0.0) | (180.0) | (0.0) | (1.849) | (1.833) | (171.1) | (175.1) |

[a] In parentheses. [b] Geometries were optimized at the B3LYP/6-31G(d,p) level.

O···H−N hydrogen bond length between thymine and the amino group of the azole carboxamide varies from 1.849 to 1.894 Å. The N−H···O hydrogen bond interaction between thymine and the azole carboxamide carbonyl oxygen ranges from 1.758 to 1.838 Å. The corresponding hydrogen bond angles (approximately 169−179°) are nearly linear. The hydrogen bond geometries for uracil complexes are very close to the results for the corresponding thymine pair.

As reported for cytosine, the interaction energies between all azole carboxamides and thymine fall within a small (5.7 kJ $mol^{-1}$) range. The weakest binding (38.6 kJ $mol^{-1}$) occurs for the X = N, Y = Z = CH combination, and the strongest binding (44.3 kJ $mol^{-1}$) occurs for X = Z = CH, Y = N. It is interesting that the same combinations of X, Y, and Z result in the weakest and strongest binding to both cytosine and thymine. However, there is no further correlation between the relative strengths of the pairs within the two data sets. The binding strength of each azole carboxamide to uracil is within 4.4 kJ $mol^{-1}$ of the binding strength to thymine, where the largest differences occur for nonplanar pairs. In general, the binding strengths of azole carboxamides to uracil are slightly larger than those to thymine.

In contrast to the results for cytosine, the magnitude of the binding strengths between thymine and various azole carboxamides (38.6−44.3 kJ $mol^{-1}$) is similar to that of the natural thymine-adenine base pair calculated at the same level of theory (44.2 kJ $mol^{-1}$). A similar binding strength to the natural pair likely occurs because only a weak (2.805 Å) O···H−C hydrogen bond is absent in the thymine-azole carboxamide pairs compared with the thymine-adenine pair. Furthermore, the O···H−N hydrogen bond present in the thymine-azole carboxamide pairs is slightly shorter (Table 5) than that found in the natural pair (1.918 Å), and a (1.798 Å) N−H···N hydrogen bond in the thymine-adenine complex is replaced by a N−H···O hydrogen bond in the azole carboxamide pairs. The interaction energies between uracil and the azole carboxamides are also similar to that calculated for the natural uracil-adenine pair (45.4 kJ $mol^{-1}$).

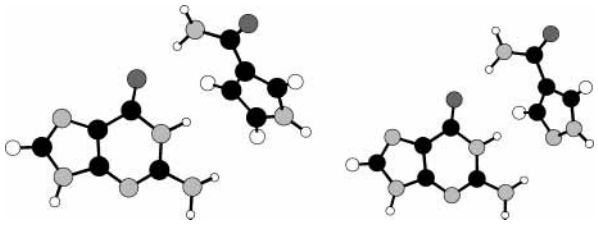


**Figure 1.** Examples of distorted guanine-azole carboxamide (X = Y = Z = CH and X = Z = CH, Y = N) pairs.

The nearly equal binding strength of thymine-azole carboxamide and thymine-adenine pairs reported in the present study contradicts experimental observations that the X = Y = Z = CH thymine-azole carboxamide pair is less stable than the analogous natural pair.[6] This suggests that other factors strongly influence duplex stability, such as poorer stacking interactions or negative changes in entropy upon duplex formation,[6] when these unnatural bases are incorporated into oligonucleotide strands.

**Guanine.** It has been proposed that guanine can hydrogen bond to the syn-$\tau$ azole carboxamides.[39] However, our calculations indicate that binding between guanine and the syn-$\tau$ conformers of some azole carboxamides is unfavorable because of crowding between substituents. In particular, azole carboxamides with Z = CH form greatly distorted guanine pairs, where the azole carboxamide is twisted such that it adopts a nearly perpendicular arrangement with respect to the molecular plane of guanine (Figure 1). This arrangement is likely due to crowding between the N(1) hydrogen in guanine and the Z = CH hydrogen in the azole carboxamides (Scheme 3). In these structures, only two weak hydrogen-bonding interactions are

**TABLE 6: Selected Bond Lengths (Å) and Bond Angles (deg) for the Guanine-Azole Carboxamide Pairs with Z = N (Scheme 3)[a]**

| X | Y | Z | ∠(OCCX) | ∠($H_1$NCC) | ∠($H_2$NCC) | R(O···H−N) | R(N−H···Z) | ∠(O···H−N) | ∠(N−H···Z) |
|---|---|---|---|---|---|---|---|---|---|
| CH | CH | N | 1.4 | 178.9 | 9.4 | 1.907 | 1.963 | 157.6 | 176.9 |
| CH | N | N | 2.6 | 179.2 | 3.5 | 1.910 | 1.913 | 159.9 | 172.6 |
| N | CH | N | 2.3 | 178.9 | 9.6 | 1.920 | 1.981 | 155.3 | 177.7 |
| N | N | N | 4.4 | 179.6 | 2.6 | 1.903 | 1.952 | 158.4 | 173.2 |

[a] Geometries were optimized at the B3LYP/6-31G(d,p) level.

possible. It is anticipated that the great distortion in these pairs may lead to unstable nucleotide strands.

In contrast, azole carboxamides with Z = N form nearly planar guanine pairs. The azole carboxamides within these pairs are slightly distorted (Table 6) even though they are planar in isolation (Table 1). The O···H−N hydrogen bond lengths between guanine and the external amino group of the azole carboxamides range from 1.903 to 1.920 Å (Table 6). The N−H···Z hydrogen bond lengths in these pairs show significant variation (1.913−1.981 Å) among different combinations of X, Y and Z. The N−H···Z hydrogen bonds are nearly linear, while the O···H−N interactions deviate from linearity by approximately 20−25°. A significant N−H···Y hydrogen bond is present when Y = N, but only a weak highly distorted hydrogen bond, which involves a twisted guanine amino group, is possible when Y = CH.

Unlike hydrogen bond strengths calculated for pyrimidine complexes, the guanine-azole carboxamide pairs show a range in binding energies (24.4−51.7 kJ mol$^{-1}$). The large range is in part due to significant deviations of some pairs from planarity (Figure 1). The distorted pairs have hydrogen bond strengths ranging from 24.4 kJ mol$^{-1}$ (X = Y = Z = CH) to 36.4 kJ mol$^{-1}$ (X = N, Y = Z = CH). The azole carboxamides with Y = Z = N form the strongest hydrogen bonds with guanine, where the binding is stronger when X = CH (51.7 kJ mol$^{-1}$) than with X = N (44.9 kJ mol$^{-1}$). As discussed, these pairs contain three hydrogen bonds (Scheme 3). However, the hydrogen bond between the guanine amino group and the Y position is much weaker in azole carboxamides with Y = CH and Z = N. The weakening of this hydrogen bond leads to a significant decrease in the interaction energy with guanine (by approximately 15−25 kJ mol$^{-1}$). As noted previously for the Y = Z = N combinations, the Y = CH, Z = N combination with X = CH (28.7 kJ mol$^{-1}$) shows stronger binding than that with X = N (25.7 kJ mol$^{-1}$).

It is interesting that even the strongest guanine-azole carboxamide hydrogen-bonded pair considered in the present work (X = CH,Y = Z = N) has a binding strength (51.7 kJ mol$^{-1}$) much smaller than that of the natural cytosine-guanine pair (96.6 kJ mol$^{-1}$). Indeed, the difference between the calculated binding strengths for the (planar) guanine-azole carboxamide pairs and the cytosine-guanine pair is 44.9−72.2 kJ mol$^{-1}$, which represents a decrease in the binding strength of the natural pair by on average 64%. Differences likely arise from the loss of a N−H···O interaction between the guanine amino group and cytosine and the distortion of a nearly linear O···H−N (178.0°) interaction between guanine and the cytosine amino group when guanine pairs with the azole carboxamides.

**Adenine.** Adenine has been proposed to pair with the anti-$\tau$ azole carboxamides (Scheme 3). However, our calculations show that interactions between these molecules and adenine are not always favorable.[38] We previously noted great distortion in guanine-azole carboxamide pairs with Z = CH. For adenine pairs, distortion occurs when Z = N. This distortion arises from unfavorable electronic interactions between N(1) in adenine and Z = N in the azole carboxamide (Scheme 3), which leads to a nearly perpendicular arrangement of the azole carboxamide with respect to the adenine molecular plane (Figure 2). Because of this arrangement, only one hydrogen bond (between the amino group in adenine and the external carbonyl oxygen of the azole carboxamide) is present in these complexes.

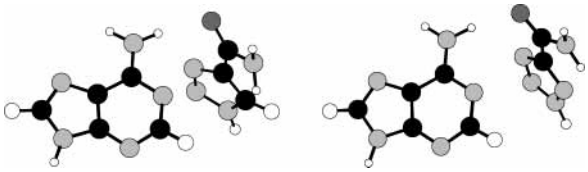


**Figure 2.** Examples of distorted adenine-azole carboxamide (X = CH, Y = Z = N and X = Y = Z = N) pairs.

Alternatively, azole carboxamides with Z = CH form reasonably planar adenine pairs. The adenine pairs with X = N, Z = CH are completely planar, but the pairs with X = CH, Z = CH exhibit a slight twisting within the azole carboxamide (Table 7). It should be noted that these azole carboxamides are also nonplanar in isolation (Table 1), but the degree of distortion decreases upon binding with adenine (Table 7). The adenine complexes include N−H···O hydrogen bonds (1.901−1.928 Å) to the carbonyl group of the azole carboxamide as well as N···Z(= CH) interactions (2.266−2.436 Å). The N···Z hydrogen bond angles are nearly linear (174.3−178.7°), but the N−H···O interactions deviate from 180° by up to 17°.

Similar to those of the pyrimidine complexes, the binding energies of the adenine-azole carboxamide pairs fall in a small range (8.6 kJ mol$^{-1}$). Because of the very similar geometry of the distorted adenine-azole carboxamide (Z = N) pairs, the binding strengths of these complexes fall within a 1.6 kJ mol$^{-1}$ range. The binding energies of the planar adenine-azole carboxamide pairs with Z = CH are slightly larger (by 8−9 kJ mol$^{-1}$) than those calculated for the distorted Z = N counterparts. The hydrogen bond strengths for the planar pairs are similar because all complexes contain two nearly equal hydrogen bonds.

The calculated binding strengths for the adenine-azole carboxamide complexes are nearly 50% less than the binding strengths calculated for the natural thymine-adenine (44.2 kJ mol$^{-1}$) and uracil-adenine (45.4 kJ mol$^{-1}$) base pairs. This decrease is likely due to the replacement of a N···H−N hydrogen bond in the natural pair with a N···H−C interaction in the azole carboxamide (Z = CH) pairs. Additionally, it should be noted that although the hydrogen bond distance between the adenine amino group and the azole carboxamide carbonyl group is similar to that calculated in the adenine-thymine pair (1.918 Å), the corresponding hydrogen bond angle deviates significantly from linearity in the azole carboxamide complexes. The weak C−H···O interaction in the adenine-thymine complex is lost upon pairing with the azole carboxamides.

It was suggested that the absence of an azole ring nitrogen in the X = Y = Z = CH azole carboxamide would decrease the likelihood that this molecule would form hydrogen bonds with the purines.[6] Although our calculations indicate that this

**TABLE 7: Selected Bond Lengths (Å) and Bond Angles (deg) for the Adenine-Azole Carboxamide Pairs with Z = CH (Scheme 3)[a]**

| X | Y | Z | ∠(NCCX) | ∠($H_1$NCC) | ∠($H_2$NCC) | R(N−H···O) | R(N···Z) | ∠(N−H···O) | ∠(N···Z) |
|---|---|---|---|---|---|---|---|---|---|
| CH | CH | CH | 8.8 | 171.5 | 24.4 | 1.901 | 2.436 | 163.5 | 174.3 |
| CH | N | CH | 7.5 | 172.4 | 21.9 | 1.923 | 2.345 | 166.5 | 174.9 |
| N | CH | CH | 0.1 | 179.8 | 0.2 | 1.905 | 2.347 | 163.9 | 178.7 |
| N | N | CH | 0.1 | 179.8 | 0.3 | 1.928 | 2.266 | 166.2 | 178.2 |

[a] Geometries were optimized at the B3LYP/6-31G(d,p) level.

azole carboxamide forms potentially unfavorable distorted pairs with guanine, we conclude that a planar hydrogen-bonded pair with adenine can be formed. Indeed, the calculated binding strength (23.6 kJ mol$^{-1}$) is similar to that of azole carboxamides with ring nitrogens (X = Z = CH, Y = N (22.7 kJ mol$^{-1}$); X = N, Y = Z = CH (24.6 kJ mol$^{-1}$); X = Y = N, Z = CH (24.7 kJ mol$^{-1}$)).

**Comparison of Azole Carboxamide Interactions with Natural Nucleobases.** Among the natural nucleobases, the eight azole carboxamides bind most strongly with cytosine. For all but two azole carboxamides considered in the present study, the binding strength with the natural DNA bases decreases as C > T > G > A. On average, the binding with cytosine is approximately 15, 20, and 35 kJ mol$^{-1}$ greater than with thymine, guanine, and adenine, respectively. For the azole carboxamides with X = CH, Y = Z = N and X = Y = Z = N, the binding strength decreases as C > G > T > A. It should be noted that these combinations of X, Y, and Z lead to the strongest guanine-azole carboxamide pairs because of the formation of three hydrogen bonds. It should also be noted that uracil has binding interactions with the azole carboxamides very similar to those of thymine; therefore, uracil follows the same trend as thymine in the series.

If a nearly equal binding strength to all natural nucleobases is a criterion for a suitable universal base, then the differences in our calculated binding strengths suggest that no azole carboxamides are truly "universal". However, it could simply be unrealistic to expect a universal base to bind to each natural base with an equal affinity. In particular, the natural base pairs have different binding strengths, where the guanine-cytosine binding interactions (96.6 kJ mol$^{-1}$) are stronger than those in the adenine-thymine (uracil) pair (44.2 (45.4) kJ mol$^{-1}$). Furthermore, any mismatches that potentially disrupt these hydrogen bond interactions lead to duplex destabilization. Thus, a more realistic criterion may be that a molecule must form a complex that mimics the hydrogen bond pattern in, and hydrogen bond strength of, the corresponding natural (Watson−Crick) base pair.

Our calculations indicate that only azole carboxamide base pairs with thymine or uracil have binding strengths similar to that of the corresponding natural (thymine-adenine or uracil-adenine) base pair. On average, the binding strength between an azole carboxamide and thymine (uracil) is at most 13% (10%) smaller than that between adenine and thymine (uracil). The binding strengths of azole carboxamides to cytosine, guanine, and adenine are approximately 40, 61, and 54% smaller than the binding strengths of the related natural pair, respectively.

In general, the percent difference between the calculated binding strength in the azole carboxamide pair and the corresponding natural pair increases as T < C < A < G. Because of strong interactions between guanine and the X = CH, Y = Z = N and X = Y = Z = N combinations, the order for these azole carboxamides is T < C < G < A. Alternatively, because of the stabilization of the adenine pairs with X = Y = N, Z = CH and X = N, Y = Z = CH, the difference increases as T < A < C < G for these azole carboxamides.

The calculated hydrogen bond strengths may be useful in predicting the base that will preferentially bind with azole carboxamides in different applications. For example, if only relative hydrogen bond strengths need to be considered, then our calculations suggest that cytosine will be preferentially inserted opposite each azole carboxamide. Alternatively, if the binding strength between the azole carboxamides and a particular base must mimic the binding strength of the corresponding natural pair, then our calculations suggest that thymine will be preferentially inserted opposite all azole carboxamides. In summary, solely on the basis of the calculated binding energies and the criterion that a universal nucleobase should have equivalent binding strengths to all natural bases or should bind with a strength equal to that of the corresponding natural base pair, none of the azole carboxamides are suitable universal nucleobases.

## Discussion of Calculations and Experimental Data

As mentioned in the Introduction, the base-pairing properties of several azole carboxamides have been studied experimentally.[6,15−23] To act as a universal nucleobase, a molecule must satisfy several criteria,[10d] which include the ability to form a stable pair with all four natural bases and to direct the incorporation of all four natural bases by DNA polymerase. The primary experimental techniques used to assess these properties include DNA melt studies, which test the effects of modified bases on duplex stability, and enzyme-catalyzed DNA replication.[15−23] The latter technique incorporates a potential universal nucleobase into a DNA template (PCR primer) and monitors DNA strands generated by DNA polymerases to determine whether a particular natural base is preferentially inserted opposite the modified base. Factors in addition to base pair stability must be carefully considered when analyzing data from this method because enzyme recognition of modified bases may also play an important role.[20]

Our calculations predict that some azole carboxamides (X ≠ Z) prefer a particular conformation on both a thermodynamic and kinetic basis. For example, our calculations indicate that the syn-$\tau$ conformer for the X = Y = CH, Z = N azole carboxamide is thermodynamically more stable (by approximately 35 kJ mol$^{-1}$) than the anti-$\tau$ conformer (Table 1). Furthermore, our calculations predict a substantial barrier (44.1 kJ mol$^{-1}$) for conversion between the syn-$\tau$ and anti-$\tau$ conformers (Table 2). These results are consistent with calculations performed at a lower level of theory (AM1).[6,16,19,23,35] Because thymine and guanine form stable base pairs with the syn-$\tau$ conformer, calculations suggest that preferential binding to thymine and guanine should be observed. Indeed, studies by Sala et al.[18] indicate that preferential insertion of thymine opposite the X = Y = CH, Z = N azole carboxamide occurs during (*Taq*) DNA polymerase strand synthesis, and thermal melt studies by Johnson et al.[19] indicate that this azole carboxamide has a significantly higher affinity for thymine and guanine than for other natural bases. It should also be noted

that modeling studies suggest that structures of the thymine and guanine pairs with this azole carboxamide are spatially and geometrically similar to the natural base pairs.[19]

From the arguments above, one may conclude that calculations, in addition to simple thermodynamic and kinetic arguments, can explain experimental observations. However, discrepancies between calculations and experiment often arise. For example, preferential insertion of thymine and adenine opposite the X = Y = CH, Z = N azole carboxamide during PCR amplification of DNA has been reported.[20] The preference for adenine over guanine cannot be explained by our calculated relative thermodynamic stability or kinetic barriers.[40] Furthermore, our calculations indicate that the anti-$\tau$ conformer is particularly stable for the X = N, Y = Z = CH azole carboxamide (Table 1) and therefore favorable binding to cytosine and adenine is expected. However, both cytosine and thymine are preferentially inserted by DNA polymerase.[20] These examples strongly support suggestions that many factors must be considered when interpreting data from DNA polymerase studies[20] and indicate that thermodynamic and kinetic arguments are not always sufficient to explain the observed properties of azole carboxamides.

Our calculations predict that some azole carboxamides (X = Z) should not display a thermodynamic or kinetic preference for one conformation. However, all azole carboxamides show preferential binding with different natural bases.[6,15−23] Discrepancies may be addressed by comparing calculated hydrogen bond strengths in complexes between the azole carboxamides and natural bases.

Perhaps the most well studied (X = Z) azole carboxamide is the X = Y = Z = CH combination.[6,20] Although our calculations, in addition to those performed previously,[6,16,23] suggest that the energy difference between the syn-$\tau$ and anti-$\tau$ conformers is insignificant, both thermal melting studies[6] and experiments on PCR primers[20] indicate that this azole carboxamide preferentially pairs with thymine. Furthermore, experiments suggest that duplexes containing the X = Y = Z = CH cytosine-azole carboxamide pair are approximately 46 kJ mol$^{-1}$ less stable than those containing the analogous thymine pair.[6] It was suggested that the cytosine pair may be less stabilized by hydrogen-bonding interactions or that the anti-$\tau$ conformer (which binds with cytosine) may distort the backbone.[6]

Our calculations indicate that although the anti-$\tau$ conformer of the X = Y = Z = CH azole carboxamide is distorted in isolation (Table 1) the degree of distortion greatly decreases upon binding to cytosine (Table 4). Additionally, it is expected that the distortion will decrease further once stacking interactions are taken into account. Therefore, we do not believe that distortion leads to the observed energy difference. A consideration of our calculated hydrogen bond strengths indicates that interactions in the cytosine-azole carboxamide pair are larger than those of the thymine pair.[41] This contradicts the proposal that the cytosine pair is less stable.[6] However, we note that the calculated binding strength of the cytosine-azole carboxamide pair is much less than that of the natural cytosine-guanine base pair and the binding strength of the thymine pair is nearly equal to that of the natural thymine-adenine pair. Furthermore, the difference in the thymine and cytosine binding interactions with the azole carboxamides and adenine or guanine, respectively, (40.8 kJ mol$^{-1}$) is similar to the estimated difference in strand stability (approximately 46 kJ mol$^{-1}$). Thus, perhaps the difference in the binding strength with respect to the analogous natural base pair is at least in part responsible for the apparent discrepancy.

It should be noted that the majority of azole carboxamides studied experimentally generally show a preference for hydrogen bonding with thymine.[6,19,20] However, we calculate the largest hydrogen bond strengths for the cytosine-azole carboxamide pairs. Therefore, if pairing with azole carboxamides is dictated by hydrogen bond strength, then our calculations suggest that azole carboxamides will preferentially pair with cytosine. We note that for X = Y = CH, Z = N and X = CH, Y = Z = N the anti-$\tau$ conformer is much higher in energy than the syn-$\tau$ conformer. If this energy differences is taken into account, then binding to thymine is more favorable for these azole carboxamides. Nevertheless, our calculations indicate that only thymine (or uracil) azole carboxamide pairs have binding energies equal to that of the analogous natural pair. The binding strength of all other natural bases to the azole carboxamides is much smaller than that in the corresponding natural pair. Thus, the difference between the binding strength of the azole carboxamide pair and the corresponding natural pair may be an important criterion for predicting preferential pairing of the azole carboxamides with nucleobases.

Calculations of interactions between azole carboxamides and the natural bases cannot always explain experimental observations. For example, the thermodynamic stability of base pairs decreases as T > G > A > C for the X = Y = Z = CH azole carboxamide[6] and as G > T ≈ A > C for the X = Z = N, Y = CH azole carboxamide.[21] These results do not match the calculated properties of the azole carboxamide conformers, the relative binding strengths, the difference in the hydrogen-bonding strength of the azole carboxamide pair and that of the corresponding natural base pair, or the distorted structures calculated for the purine pairs.[42]

We note that our calculations of the thermodynamics, kinetics, and hydrogen-bonding properties of the azole carboxamides cannot fully explain experimental observations. Therefore, other issues must be considered in future studies. A major approximation employed in the present study is gas-phase calculations. Environmental effects, such as the polarity inside the double helix or stacking interactions, may change the results. We note that although a dielectric constant ($\epsilon$ = 40) that mimics the base-pairing region of the DNA helix only slightly changes the relative gas-phase (anti-$\tau$ and syn-$\tau$) energies,[6] solvation effects could change the geometries and binding energies of base pairs. In particular, the large deviations from planarity found for some base pairs may not be possible within the DNA double helix.

Another complication when comparing calculations to experiment is that gas-phase binding energies were calculated but experiment typically yields information about the change in the Gibbs energy for duplex formation. $\Delta G$ also accounts for changes in entropy upon hybridization and temperature effects, and is not solely a measure of the difference in the interaction energy of one pair in the oligonucleotide. Therefore, a direct comparison of our theoretical results and experimental data is difficult. It should also be noted that discrepancies sometimes arise in experimental results depending on whether base insertion by DNA polymerases or DNA melts is considered. More research is required to understand better the function of DNA polymerases, including their interactions with the azole carboxamides, to interpret data obtained from studies involving these enzymes.

## Conclusions

Our calculations on a series of eight azole carboxamides (Scheme 1) reveal some key characteristics that may help

explain why these molecules are not efficient universal nucleobases. In particular, the stabilization of one conformer of an azole carboxamide decreases the likelihood that this molecule will act as a universal nucleobase because each natural base pairs with a different conformer. Our calculations show that one conformer of some (isolated) azole carboxamides is preferentially stabilized on both a thermodynamic and kinetic basis because of intramolecular hydrogen bonding. Initial model studies also suggest that a more complete investigation of intramolecular interactions between azole carboxamides and the sugar moiety is necessary in order to understand whether stabilization about the glycosidic bond occurs in some derivatives.

For the first time, calculated geometries and hydrogen bond strengths of complexes between azole carboxamides and natural nucleobases are presented. The calculated geometries indicate that although some azole carboxamides are nonplanar in isolation, the degree of distortion generally decreases when hydrogen-bonding interactions with natural bases are taken into account. It is also likely that other environmental effects, such as stacking interactions, will further reduce the distortion. Thus, distortion within the azole carboxamide is expected to be limited in duplexes and therefore not lead to significant differences in strand stabilities.

Optimized guanine-azole carboxamide pairs with Z = CH and adenine-azole carboxamide pairs with Z = N are highly distorted. These structures will likely cause great instability within double helices. Because each azole carboxamide forms a distorted pair with either guanine or adenine, none of the azole carboxamides will experience equal binding with all natural (DNA or RNA) bases. We note that our calculations were made in the gas phase and the degree of distortion may decrease once the pair is incorporated into a DNA strand because of other (stacking) interactions. Future studies will consider the effects of the environment on these distorted geometries.

Our calculations indicate that the azole carboxamides bind to each natural nucleobase with varying affinities. The binding strength of azole carboxamides to the natural base pairs decreases as C > T > G > A, with the exception of that for the X = CH, Y = Z = N and X = Y = Z = N combinations, which decreases as C > G > T > A. Our calculations also indicate that the binding energies of the azole carboxamide complexes can be very different from those of the natural base pairs. Indeed, only the binding strengths of thymine (or uracil) azole carboxamide pairs are equal to that of the analogous natural (thymine (uracil)-adenine) pair. The azole carboxamides bind to all other natural bases with a much smaller affinity than the corresponding natural pair. The similar binding energies of the thymine-azole carboxamide pairs and the thymine-adenine pair may help explain why many azole carboxamides show preferential binding to thymine.

In summary, calculations are useful in providing insight into the relative stability of different conformations of azole carboxamides and their relative binding strength to the natural bases. Although calculations cannot fully explain all experimental observations, our calculations indicate that none of the azole carboxamides pair equally with all bases or have binding energies with all bases similar to those of the corresponding natural pairs. Therefore, our calculations offer a possible explanation for the bias in the base-pairing properties of these molecules and shed light on why azole carboxamides do not function as universal nucleobases. Further areas of study have been identified to understand better the mechanism by which the azole carboxamides operate.

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**Supporting Information Available:** Geometrical coordinates for the azole carboxamides, the transition structures for rotation about $\tau$, and the complexes with natural bases. This material is available free of charge via the Internet at http://pubs.acs.org.

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(35) We note that the conformation of the X = Y = C, Z = N azole carboxamide that Johnson et al.[21] refer to as anti corresponds to our syn-$\tau$ conformer. For consistency, we use the same nomenclature for all azole carboxamides (Scheme 2).

(36) We note that Klewer et al.[25] report the anti conformer of the X = Z = N, Y = CH azole carboxamide to be more stable than the syn conformer. However, the relative energies reported by the authors ($E_{SCF(rel)}$, Table 2, ref 25) indicate that the syn conformer is more stable. Our calculations indicate that the syn-$\tau$ conformer is more stable for this azole carboxamide (Table 1).

(37) The glycosyl torsion angle ($\chi$) is defined as $\angle$(O1′C1′N1C2) in pyrimidine and $\angle$(O1′C1′N9C4) in purine nucleotides. This dihedral angle ranges from 90 to 270° for the anti conformation typically found in A-DNA and B-DNA in right-handed and left-handed nucleic acid helices. $\chi$ varies between +90 and −90° for the syn orientation often found in left-handed (Z-form) helices of DNA and RNA. (See Saenger, W. *Principles of Nucleic Acid Structure*; Cantor, C. R., Ed.; Springer-Verlag: New York, 1984.)

(38) We note that Klewer et al.[25] report that molecular modeling of the X = Z = N, Y = CH azole carboxamide contained within a DNA duplex reveals that this molecule cannot adequately pair with adenine or cytosine.

(39) It should be noted that Klewer et al.[25] report a conformational exchange about $\tau$ when the X = Z = N, Y = CH azole carboxamide is paired opposite guanine.

(40) It should also be noted that calculations predict that this azole carboxamide forms a distorted pair with adenine; therefore, binding to adenine is expected to be unfavorable.

(41) We note that cytosine interacts with the anti-$\tau$ conformer, which is slightly less stable than the syn-$\tau$ conformer that interacts with thymine. However, even if this energy difference is taken into account, the binding strength of the cytosine pair is still greater than that of the corresponding thymine pair.

(42) It should be noted that Klewer et al.[25] suggested that differences in stabilities observed experimentally for the X = Z = N, Y = CH azole carboxamide may actually be due to differences in intrastrand sequences introduced by changing the pairing sequence.[25,40]