How Flexible Are Fleximer Nucleobases? A Computational Study

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Received: July 9, 2004; In Final Form: October 22, 2004

Density functional theory was used to study the potential energy surface for rotation about the carbon−carbon bonds in a variety of guanosine, adenosine, and inosine fleximers, which are modified purines with the imidazole and pyrimidine rings separated by a single carbon−carbon bond. Various connectivities between C4 or C5 of the imidazole ring and C5′ or C6′ of the pyrimidine ring were considered. Calculations on fleximer nucleobases in the absence of the ribose moiety suggest that a planar relative arrangement of the imidazole and pyrimidine rings is favored, and that all fleximers are indeed very flexible with regards to rotation about the carbon−carbon bond, where calculated barriers are generally less than 40 kJ mol^{-1}. Furthermore, calculated binding energies of fleximer−pyrimidine pairs indicate that the hydrogen-bonding properties of these modified nucleobases mimic those of the corresponding natural purine. Inclusion of the sugar moiety often leads to a favored nonplanar orientation of the two rings, and either a reduction in the rotational barrier height or small changes in the rotational surface depending on the connectivity and nucleobase considered. It is concluded that several connectivities may have favorable properties for biochemical applications where flexible nucleobases would be beneficial.

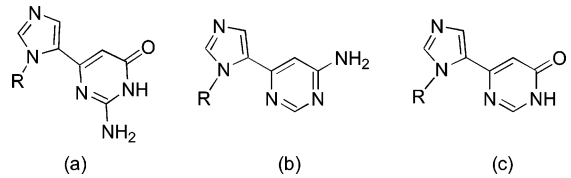
Introduction

Due to the distinct chemical structures and properties of nucleic acids, biochemical applications of modified DNA and RNA components have been explored over the past few decades.[1] A wide range of modifications to the sugar moiety, the phosphate backbone, and the nucleobases has been considered. Applications of modified nucleobases include therapeutics[2] and biochemical tools, such as polymerase chain reaction (PCR) primers[3] and bioprobes for drug design.[4−8]

Intriguing classes of modified nucleobases are derived by expanding the base structure. To the best of our knowledge, the first molecules based on extended nucleosides reported in the literature are the *lin*-, *prox*-, and *dist*-benzo nucleosides synthesized by Leonard and co-workers.[4−8] These analogues use benzene rings to separate the imidazole and pyrimidine components of the purines. Pyrimidine analogues, where a benzene ring is added between the base and sugar moieties, have also been considered recently.[9] Bases separated by other hydrocarbon spacers, such as benzocyclobutadiene or naphthalene,[5,6] or thienyl groups,[10] have also been examined, as well as biphenyl base surrogates.[11]

Modified DNA (RNA) components that contain different linkages between the sugar and nucleobase moieties are also of interest. For example, "split nucleosides" have been synthesized,[12−14] which possess a carbon bridge between the ribose moiety and the nucleobase such that two heterocyclic nucleobase rings are located on the methylene bridge. A methylene bridge between N1 or N9 of the heterocyclic base and C1′ of the pentofuranosyl ring has also been considered.[15] Other interesting extended nucleosides are the "double headed" nucleosides which contain a pyrimidine or purine base attached to both C1′ and C5′ of the sugar moiety.[16,17]

CHART 1: Guanosine (a), Adenosine (b), and Inosine (c) Fleximers Introduced by Seley et al.[18,19]



More recently, Seley et al. introduced an innovative group of modified purine nucleosides called "fleximers".[18,19] The molecules designed by Seley et al. split the rings of the guanosine, adenosine, and inosine bases such that the imidazole and pyrimidine components are connected by a single carbon−carbon bond (see Chart 1). The appeal of the fleximers designed by Seley and co-workers is that the molecules retain some of the chemical structure of the natural bases, such as the hydrogen-bonding scheme, but they are more flexible than the natural bases. Indeed, recent molecular docking studies have revealed an unexpected curved structure of the guanosine fleximer within the active site of *S*-adenosyl-L-homocysteine hydrolase (SAHase),[20] and NMR studies have considered the anti-syn and sugar conformational properties of the three fleximers.[21] One of the main appeals for increasing the flexibility of the nucleobase is the use of these molecules as bioprobes.[19]

Although evidence exists to support proposals of the flexible nature of these molecules, more information about the properties of fleximers can be obtained from computer calculations. Specifically, the geometries of minima and transition structures can be characterized and relative energy barriers between conformers determined. Select Hartree−Fock calculations have been performed to obtain optimized structures of the fleximers,[19] and to scan the potential energy surface of the guanosine fleximer with respect to rotation about the glycosidic and

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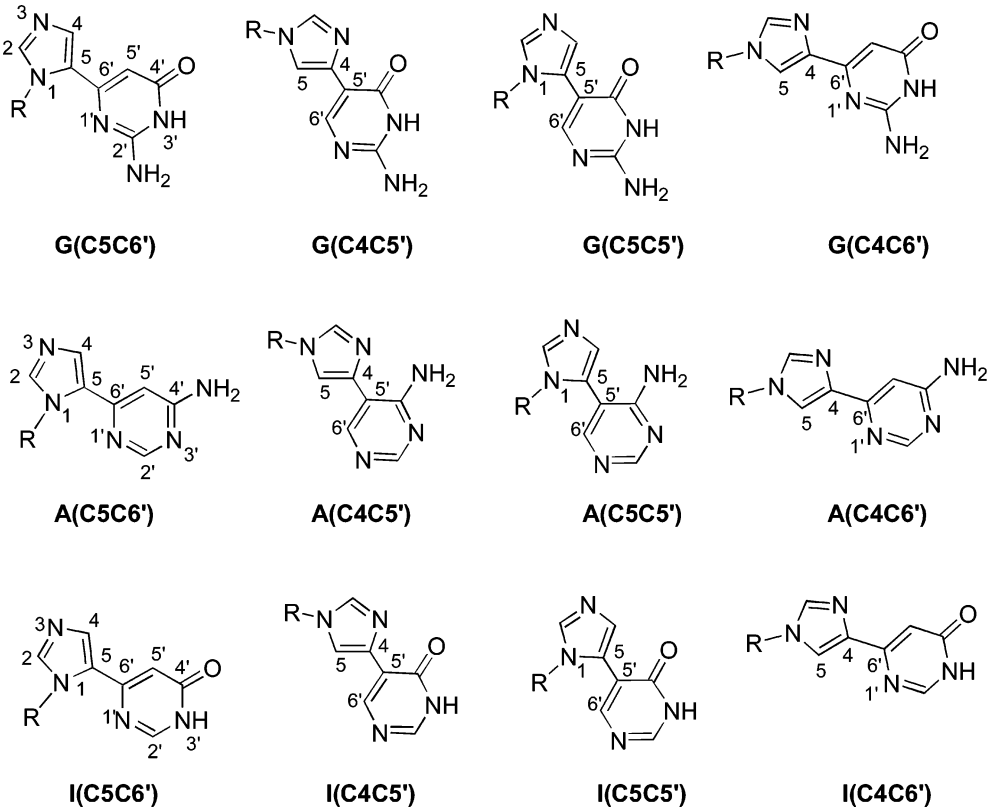
10.1021/jp046957w CCC: $30.25
Published on Web 12/18/2004

CHART 2: Guanosine, Adenosine, and Inosine Fleximers Considered in the Present Study, Where R = H or Ribose



fleximer bonds.[21] However, many questions related to the properties of the fleximer nucleobases remain unanswered.

The present work uses density functional theory to study the rotational path about the single carbon−carbon bond connecting the imidazole and pyrimidine rings in the guanosine, adenosine, and inosine fleximers. In addition to the fleximers introduced by Seley et al. that connect C5 of the imidazole ring to C6′ of the pyrimidine ring (Chart 1), we consider all combinations of connectivities involving C4 or C5 of the imidazole ring and C5′ or C6′ of the pyrimidine ring (see Chart 2 for structure, notation, and chemical numbering). Additionally, to understand the inherent flexibility within the nucleobase, models with and without a ribose moiety are considered. It is hoped that a greater understanding of the flexible properties of these interesting modified nucleobases will be obtained through the present work.

Computational Details

Geometries were optimized by using the B3LYP functional in combination with the 6-31G(d,p) basis set. Frequency calculations were performed on all structures and scaled (0.9806) zero-point vibrational energies were added to all relative energies, which were obtained from B3LYP/6-311+G(2df,p) single-point calculations. Binding energies include basis set superposition error corrections calculated according to the Boys and Bernardi counterpoise method.[22] All calculations were performed with Gaussian 98 (revision A.11.3).[23] Some calculations were performed on a Linux cluster with Linda (version 7.1).[24]

The structure, notation, and chemical numbering of the fleximers considered in the present work are provided in Chart 2. It should be noted that atoms in the pyrimidine ring are denoted with primes. Two model fleximers were considered in the present study. One model contains a ribose moiety at N1 (R = ribose), while the other replaces the sugar with a hydrogen atom (R = H).

Although a very recent study carefully considered rotation about the base−sugar and fleximer bonds in the guanosine **G(C5C6′)** fleximer,[21] the present work focuses solely on rotation about the fleximer carbon−carbon bond connecting the imidazole and pyrimidine rings to gain a greater understanding of the intrinsic properties of these modified nucleobases. Since a large number of conformations of the ribose fleximers are possible, only select stationary points on the potential energy surface of these models are considered. First, only the anti orientation of the base with respect to ribose, which maximizes the distance between the hydrogens attached to C1′ of the sugar moiety and C8 of the natural purines, is considered. It should be noted that recent NMR studies indicate that substitution of purine nucleobases with the corresponding fleximer increases the population of the anti conformation and Hartree−Fock calculations on the guanosine C5C6′ fleximer indicate that the syn conformation has considerably higher energy.[21] Second, for consistency, only the C2′-endo (south) conformation of the sugar moiety, the favored conformation in RNA, is considered. The sugar was initially set to the C2′-endo conformation where an intramolecular (O2′−H···O3′) hydrogen bond is present between the C2′ hydroxyl hydrogen and the C3′ hydroxyl group. The C5′ hydroxyl group was directed away from the base at C1′ where the HO5′C5′C4′ and O5′C5′C4′H dihedral angles approximately equal 180°. Unless otherwise specified in the discussion, this sugar conformation was retained during the geometry optimization.

Results and Discussion

A. Relative Energies. It is of interest to compare the relative energies of guanosine, adenosine, and inosine fleximers with different connectivities, as well as the influence of the ribose sugar moiety on these energy differences. For this comparison, only the lowest energy minima found in the present study will be considered. A detailed study of all local minima, as well as

TABLE 1: Relative Energies (kJ mol^{-1}) of Purine Fleximers with Different Connectivities[a]

fleximer	R = H	R = ribose
G(C5C6′)	0.0	20.5
G(C4C5′)	8.4	6.6
G(C5C5′)	4.4	27.4
G(C4C6′)	4.3	0.0
A(C5C6′)	0.0	19.2
A(C4C5′)	10.3	4.5
A(C5C5′)	29.6	29.3
A(C4C6′)	4.5	0.0
I(C5C6′)	0.0	23.2
I(C4C5′)	5.7	5.8
I(C5C5′)	2.8	32.8
I(C4C6′)	1.8	0.0

[a] See Chart 2 for structure and notation.

transition structures for rotation about the CC fleximer bond, will be discussed in the following sections.

When the free fleximer bases are considered (R = H, Table 1), C5C6′ is the lowest energy connectivity for all nucleobases. Furthermore, the relative energy difference between various connectivities is less than 8, 30, and 6 kJ mol^{-1} for guanine, adenine, and hypoxanthine, respectively. It should be noted that a significantly larger relative energy occurs for adenine fleximers due to large repulsive interactions between the pyrimidine amino group and hydrogens in the imidazole ring in the C5C5′ connectivity.

Upon inclusion of the sugar moiety (R = ribose), C4C6′ becomes the lowest energy connectivity for all bases (Table 1). It should be noted that the imidazole and pyrimidine rings are planar with respect to one another in the lowest energy conformations of these connectivities. Other connectivities are up to 27, 29, and 33 kJ mol^{-1} higher in energy for guanine, adenine, and inosine, respectively. Thus, these results indicate that the presence of the sugar group likely has a significant effect on the properties of the fleximers. These effects will be examined in more detail in the following sections.

B. Binding Properties of Fleximers. As discussed in the Introduction, the primary reason for designing the purine fleximers was to increase the flexibility of the base, while maintaining the chemical structure and hydrogen-bonding pattern of the natural bases. In attempts to determine the effects of the change in structure on hydrogen-bonding properties, the binding strengths between the fleximers and the appropriate pyrimidine were calculated and compared with those of the corresponding natural base pairs (Table 2). It should be noted that only the lowest energy conformations of the models without the ribose moieties (R = H, Chart 2) were used to calculate the binding strengths.

The geometries of the fleximer−pyrimidine pairs are very similar to the corresponding natural base pair for all connectivities. It should be noted that the amino groups in the cytosine, adenine, and guanine fleximers are planar in all base pairs. All lowest energy arrangements of the fleximers (R = H) with various connectivities are planar, and remain planar in the corresponding pyrimidine pair with the exception of **A(C5C5′)**. The lowest energy conformer of the **A(C5C5′)** fleximer (R = H) has a ∠(N1C5C5′C6′) dihedral angle equal to 33.3° in the isolated base and 35.8° in the thymine−fleximer pair.

The average deviation in the hydrogen bond lengths among each set of pyrimidine−fleximer pairs considered in the present work is 0.02 Å, where the largest deviation is approximately 0.05 Å. Larger than average changes (up to 0.13 Å) occur for C−H···O hydrogen bond lengths. The hydrogen bond angles deviate by less than approximately 2°.

TABLE 2: Binding Strengths (kJ mol^{-1}) of the Pyrimidine Pairs with the Natural Purines and Purine Fleximers[a,b]

base pair	binding strength
G:C	96.1
G(C5C6′):C	99.4
G(C4C5′):C	91.8
G(C5C5′):C	94.8
G(C4C6′):C	95.6
A:T	44.0
A(C5C6′):T	44.8
A(C4C5′):T	39.3
A(C5C5′):T	42.4
A(C4C6′):T	44.7
I:C	71.9
I(C5C6′):C	75.1
I(C4C5′):C	66.7
I(C5C5′):C	70.1
I(C4C6′):C	71.1

[a] See Chart 2 for structure and notation. [b] In all structures, the sugar moiety was replaced by a hydrogen atom.

From Table 2, it can be seen that separating the imidazole and pyrimidine rings does not greatly affect the purine binding properties. Furthermore, the binding energies are not largely affected by the connectivity of the two rings. The C5C6′ fleximer has the largest binding energy for each purine, where the binding strengths are only 3.3, 0.8, and 3.2 kJ mol^{-1} larger than that of unmodified guanine, adenine, and hypoxanthine pairs, respectively. The C4C5′ fleximer has the smallest binding energy for each purine, where the binding strengths are 4.3, 4.7, and 5.2 kJ mol^{-1} below that of unmodified guanine, adenine, and hypoxanthine pairs, respectively.

In summary, the calculated binding energies of all pyrimidine−fleximer pairs are very similar to those of the corresponding natural base pairs. Thus, fleximer formation does not significantly affect the hydrogen-bonding properties of the (natural) purines.

C. Conformations and Rotational Barriers for Fleximers with R = H. We are mainly interested in the conformation about the CC fleximer bond in guanosine, adenosine, and inosine fleximers with varying connectivity. In the following sections, we discuss in detail the rotational surfaces for each fleximer studied with each model system (R = H and ribose, Chart 2), which is imperative for understanding the properties of these molecules. This discussion will be followed by a more general comparison of the rotational surfaces in the final section.

The results obtained from conformational searches about the fleximer carbon−carbon bond in models that replace the ribose moiety with a hydrogen atom are displayed in Tables 3−5 (R = H). In general, the calculated fleximer CC bonds are approximately 1.45−1.46 Å in minima and slightly longer (1.47−1.49 Å) in transition structures. It should be noted that some transition structures of the adenine fleximers (**A(C4C5′)** and **A(C5C5′)**) have shortened (1.45−1.46 Å) carbon−carbon bonds due to favorable interactions between the amino group of the pyrimidine ring and a hydrogen atom of the imidazole ring, which pull the two rings within closer proximity. These interesting geometrical features will be discussed in more detail below.

(*i*) *Guanine Fleximers.* The lowest energy conformer of **G(C5C6′)** has a ∠(N1C5C6′N1′) dihedral angle equal to 0.9° (Table 3 and Figure 1). A local minimum obtained by rotation about the C5C6′ bond by approximately 180° is 8.6 kJ mol^{-1} higher in energy. Both molecules are essentially planar, with the exception of a puckered amino group. The energy difference between the two conformers arises due to more favorable interactions between the N1′ lone pair and N1−H compared

TABLE 3: Selected Geometrical Parameters (deg, Å) and Relative Energies (kJ mol^{-1}) of Various Conformers in Guanine (R = H) and Guanosine (R = Ribose) Fleximers[a]

G(C5C6′)

R = H			R = ribose[b]			
∠(N1C5C6′N1′)	*R*(C5C6′)	Δ*E*	∠(N1C5C6′N1′)	∠(O1′C1′N1C5)[c]	*R*(C5C6′)	Δ*E*
0.9	1.454	0.0	25.8	223.3	1.462	0.0
93.3	1.483	36.5 (TS)	86.7	222.4	1.483	17.8 (TS)
179.9	1.454	8.6	154.3	235.8	1.460	3.1
267.7	1.483	36.8 (TS)	186.4	218.0	1.463	12.2 (TS)
			203.6	258.9	1.461	2.7
			265.8	237.4	1.483	18.0 (TS)
			333.7	240.2	1.460	3.1
			358.1	228.5	1.462	3.3 (TS)

G(C4C5′)

R = H			R = ribose[b]			
∠(C5C4C5′C6′)	*R*(C4C5′)	Δ*E*	∠(C5C4C5′C6′)	∠(O1′C1′N1C5)	*R*(C4C5′)	Δ*E*
180.7	1.459	0.00	180.2	234.6	1.459	0.0
272.0	1.478	29.2 (TS)	270.7	231.4	1.478	30.1 (TS)
314.7	1.468	28.4	316.3	232.2	1.468	28.6
0.5	1.469	38.4 (TS)	359.6	232.4	1.469	37.8 (TS)
44.9	1.468	28.8	44.0	230.2	1.468	28.6
88.1	1.478	29.6 (TS)	89.8	232.3	1.478	30.5 (TS)

G(C5C5′)

R = H			R = ribose[b]			
∠(N1C5C5′C6′)	*R*(C5C5′)	Δ*E*	∠(N1C5C5′C6′)	∠(O1′C1′N1C5)	*R*(C5C5′)	Δ*E*
179.2	1.455	0.0	134.7	238.0	1.465	0.0
280.3	1.472	32.8 (TS)	184.8	210.4	1.478	29.2 (TS)
359.0	1.453	19.7	225.6	216.1	1.465	4.7
79.8	1.472	32.6 (TS)	267.8	220.6	1.472	11.0 (TS)
			323.7	236.5	1.460	9.4
			10.6	203.6	1.465	27.9 (TS)
			34.4	261.3	1.460	9.3
			84.6	244.2	1.472	14.0 (TS)

G(C4C6′)

R = H			R = ribose[b]			
∠(C5C4C6′N1′)	*R*(C4C6′)	Δ*E*	∠(C5C4C6′N1′)	∠(O1′C1′N1C5)	*R*(C4C6′)	Δ*E*
359.1	1.462	0.0	0.7	233.1	1.460	0.0
88.4	1.488	32.0 (TS)	89.0	230.7	1.487	33.5 (TS)
168.1	1.471	17.4	182.9	232.4	1.469	17.8
175.0	1.470	17.3 (TS)	271.6	232.9	1.487	33.5 (TS)
195.6	1.470	17.3				
270.9	1.488	31.5 (TS)				

[a] See Chart 2 for structure and notation. [b] The ribose sugar is in the C2′-endo conformation. [c] The ∠(O1′C1′N1C4) dihedral angle in the (natural) guanosine ribonucleoside calculated at the same level of theory is 238.6°.

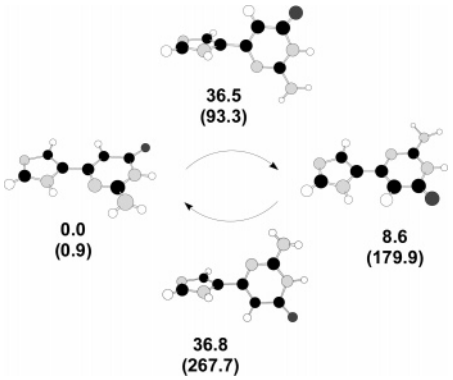


Figure 1. Relative energies (kJ mol^{-1}) and N1C5C6′N1′ dihedral angles (in parentheses, deg) for species involved in rotation about the C5C6′ bond in the guanine **G(C5C6′)** fleximer (R = H, Chart 2).

with C4−H. The two minima on the potential energy surface for rotation about the C5C6′ bond are connected via two transition structures that involve nearly perpendicular pyrimidine and imidazole rings (∠(N1C5C6′N1′) = 93.3 or 267.7°). The transition structures are 36.5−36.8 kJ mol^{-1} higher in energy than the global minimum.

The amino group is puckered in all **G(C4C5′)** structures. In the global minimum, ∠(C5C4C5′C6′) equals 180.7° (Figure 2). Two local minima are found via rotation about C4C5′, which contain dihedral angles of 44.9 and 314.7°. These minima are 28.8 and 28.4 kJ mol^{-1} higher in energy, respectively, than the global minimum, and are connected to the global minimum through transition structures (∠(C5C4C5′C6′) = 88.1 and 272.0°) that are approximately 30 kJ mol^{-1} above the global minimum. The transition structure connecting the two **G(C4C5′)** local minima has a ∠(C5C4C5′C6′) dihedral angle equal to 0.5° and is 38.4 kJ mol^{-1} above the global minimum. The high energy of this orientation likely arises due to repulsive interactions between the lone pairs of the carbonyl group in the pyrimidine ring and N3.

The potential energy surface for rotation about the C5C5′ bond in **G(C5C5′)** is very basic and similar to that for **G(C5C6′)**

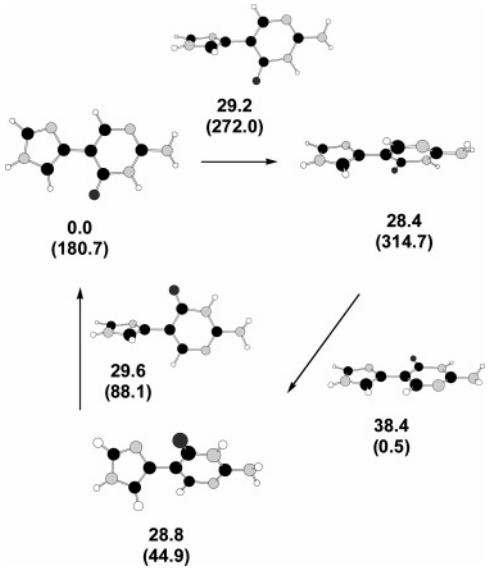


Figure 2. Relative energies (kJ mol^{-1}) and C5C4C5′C6′ dihedral angles (in parentheses, deg) for species involved in rotation about the C4C5′ bond in the guanine **G(C4C5′)** fleximer (R = H, Chart 2).

(Table 3). There is one global minimum ($\angle$(N1C5C5′C6′) = 179.2°) and one local minimum ($\angle$(N1C5C5′C6′) = 359.0°). Both structures are planar with the exception of a puckered amino group and are separated by 19.7 kJ mol^{-1} due to favored N1−H···O interactions with the pyrimidine carbonyl. The two minima are connected via approximately 33 kJ mol^{-1} barriers.

The potential energy surface for rotation about the carbon−carbon bond in **G(C4C6′)** is similar to that for the corresponding bond in **G(C4C5′)**. The global minimum, which occurs when $\angle$(C5C4C6′N1′) = 359.1°, is 17.3−17.4 kJ mol^{-1} lower in energy than two local minima ($\angle$(C5C4C6′N1′) = 168.1° and 195.6°). The two local minima are connected via a transition structure with $\angle$(C5C4C6′N1′) = 175.0°, which upon inclusion of zero-point vibrational energy corrections is nearly energetically equivalent to the local minima. The high energy of the minima and the associated transition structure is due to unfavorable interactions between the N1′ and N3 lone pairs. The imidazole and pyrimidine rings are perpendicular with respect to one another in the transition structures connecting the **G(C4C6′)** global minimum and either local minima. As a result of this twisted configuration, the corresponding energy barriers with respect to the global minima are approximately 32 kJ mol^{-1}.

(*ii*) *Adenine Fleximers.* The external amino group on the pyrimidine ring of **A(C5C6′)** and **A(C4C6′)** fleximers is puckered in our gas-phase structures. The rotational surfaces for **A(C5C6′)** and **A(C4C6′)** (Table 4) are similar to those discussed for the analogous guanine fleximers (Table 3). **A-(C5C6′)** has a relatively simple C5C6′ bond rotational surface, which contains two planar minima that are connected by 40.6−40.7 kJ mol^{-1} barriers. Three minima are located on the **A-(C4C6′)** surface since the planar structure with $\angle$(C5C4C6′N1′) = 176.9° is a transition structure, which is nearly thermoneutral with respect to the local minima. The **A(C4C6′)** global and local minima are connected by approximately 36 kJ mol^{-1} barriers.

Unlike the fleximers discussed thus far, the amino group in the **A(C4C5′)** pyrimidine ring is puckered in different directions depending on the orientation about the C4C5′ bond (Figure 3). The change in puckering occurs due to close contacts between the pyrimidine amino group and the C5 hydrogen in the imidazole ring. Two **A(C4C5′)** transition structures, which are 25.1 kJ mol^{-1} above the global minimum, contain inverted amino groups with respect to each other and connect the global minimum to different local minima ($\angle$(C5C4C5′C6′) = 156.1 and 203.9°). The local minima, which are approximately 18 kJ mol^{-1} higher in energy than the global minimum, also contain inverted amino groups with respect to one another.

A change in puckering on the **A(C4C5′)** rotational surface is in part addressed by a planar amino group in the global minimum ($\angle$(C5C4C5′C6′) = 359.8°), which likely arises due to favorable interactions between the amino group and N3 in the imidazole ring. Additionally, a transition structure connecting the two **A(C4C5′)** local minima contains imidazole and pyrimidine rings that fall in the same plane ($\angle$(C5C4C5′C6′) = 180.0°) and an amino group that is significantly puckered and staggered with respect to the C4′N3′ bond. This arrangement avoids close contacts between an amino group hydrogen and C5−H. Intrinsic reaction coordinate (IRC) calculations verify the relationship between this transition structure and the local minima. Due to its unique geometry, this is a much higher energy transition structure (54.8 kJ mol^{-1} above the global minimum) compared with others discussed thus far.

The global minima on the potential energy surface for rotation about the C5C5′ bond in **A(C5C5′)** ($\angle$(N1C5C5′C6′) = 33.3°) is less than approximately 3 kJ mol^{-1} lower in energy than three additional minima on the surface (Table 4). Two transition structures have nearly perpendicular imidazole and pyrimidine rings ($\angle$(N1C5C5′C6′) = 93.2° and 266.2°) and fall 4.3 kJ mol^{-1} above the global minimum. The final two transition structures involve planar imidazole and pyrimidine rings and amino groups staggered with respect to the C4′N3′ bond as found for **A(C4C5′)** (Figure 3). The transition structures with $\angle$(N1C5C5′C6′) = 180.0° and 10.9° fall 29.7 and 50.3 kJ mol^{-1} above the global minimum, respectively, where the former barrier is smaller due to stronger N···H−N1 compared with N···H−C4 interactions. As discussed for **A(C4C5′)**, rotation of the amino group in these transition structures allows for differences in the puckering of the amino groups in the local minima and reduces repulsion due to close contacts between the amino group and N1−H or C4−H.

(*iii*) *Hypoxanthine Fleximers.* The rotational barriers about the CC fleximer bond in hypoxanthine fleximers are not complicated by the presence of an amino group and therefore closely mimic those for the corresponding guanine fleximer. Similar to **G(C5C6′)** and **G(C5C5′)** (Table 3), there are two planar minima on the **I(C5C6′)** and **I(C5C5′)** rotational surfaces that differ in energy by 10.3 and 19.6 kJ mol^{-1}, respectively (Table 5). The global minimum has favorable interactions between N1−H and N1′ (C5C6′) or the C4′ carbonyl (C5C5′). The minima are connected by 38−40 kJ mol^{-1} transition barriers that involve perpendicular imidazole and pyrimidine rings.

Both **I(C4C5′)** and **I(C4C6′)** rotational surfaces have a planar global minimum. The second planar arrangement of the imidazole and pyrimidine rings is a transition structure due to unfavorable interactions between the N3 lone pair and the pyrimidine carbonyl (**I(C4C5′)**) or N1′ (**I(C4C6′)**) This leads to two local minima on the surface which are separated from the global minimum through shallow barriers. The largest rotational barrier is 39.7 kJ mol^{-1} for **I(C4C5′)** and 34.7 kJ mol^{-1} for **I(C4C6′)**.

In summary, the potential energy surfaces for rotation about the carbon−carbon bond in various fleximers with R = H (Chart 2) are conceptually simple. The most basic surfaces involve two planar minima and two transition structures with perpendicular imidazole and pyrimidine rings. Depending on the fleximer

TABLE 4: Selected Geometrical Parameters (deg, Å) and Relative Energies (kJ mol^{-1}) of Various Conformers in Adenine (R = H) and Adenosine (R= Ribose) Fleximers[a]

A(C5C6′)

R = H			R = ribose[b]			
∠(N1C5C6′N1′)	*R*(C5C6′)	Δ*E*	∠(N1C5C6′N1′)	∠(O1′C1′N1C5)[c]	*R*(C5C6′)	Δ*E*
0.0	1.454	0.0	18.7	221.4	1.461	0.0
95.8	1.483	40.6 (TS)	90.0	223.4	1.484	20.2 (TS)
181.2	1.455	18.9	152.9	238.2	1.462	10.5
264.5	1.483	40.7 (TS)	186.6	217.8	1.465	21.9 (TS)
			205.1	260.5	1.463	10.3
			265.3	242.0	1.483	23.0 (TS)
			344.4	231.2	1.460	1.0
			357.3	227.0	1.461	1.0 (TS)

A(C4C5′)

R = H			R = ribose[b]			
∠(C5C4C5′C6′)	*R*(C4C5′)	Δ*E*	∠(C5C4C5′C6′)	∠(O1′C1′N1C5)	*R*(C4C5′)	Δ*E*
359.8	1.467	0.0	353.3	227.8	1.466	0.0
98.3	1.480	25.1 (TS)	96.6	230.7	1.480	24.3 (TS)
156.1	1.466	18.1	155.4	224.4	1.465	16.8
180.0	1.466	54.8 (TS)	180.4	232.1	1.464	55.5 (TS)
203.9	1.466	18.0	203.8	232.7	1.465	17.8
261.7	1.480	25.1 (TS)	262.1	230.5	1.480	25.2 (TS)

A(C5C5′)

R = H			R = ribose[b]			
∠(N1C5C5′C6′)	*R*(C5C5′)	Δ*E*	∠(N1C5C5′C6′)	∠(O1′C1′N1C5)	*R*(C5C5′)	Δ*E*
33.3	1.461	0.0	116.3	239.9	1.472	0.0
93.2	1.474	4.3 (TS)	237.3	255.2	1.469	1.3
143.4	1.461	3.0	256.4	222.0	1.473	1.3 (TS)
180.0	1.463	29.7 (TS)	311.6	233.6	1.468	1.2
216.6	1.461	3.1	50.0	244.0	1.468	1.0
266.2	1.474	4.3 (TS)	68.8	224.2	1.473	1.1 (TS)
326.7	1.461	0.1				
10.9	1.458	50.3 (TS)				

A(C4C6′)

R = H			R = ribose[b]			
∠(C5C4C6′N1′)	*R*(C4C6′)	Δ*E*	∠(C5C4C6′N1′)	∠(O1′C1′N1C5)	*R*(C4C6′)	Δ*E*
359.6	1.463	0.0	0.6	231.8	1.461	0.0
93.5	1.488	35.9 (TS)	93.2	229.2	1.487	36.4 (TS)
164.9	1.471	26.8	161.4	231.3	1.469	25.7
176.9	1.471	26.9 (TS)	266.9	232.4	1.487	37.6 (TS)
198.7	1.471	26.7				
266.3	1.487	35.8 (TS)				

[a] See Chart 2 for structure and notation. [b] The ribose sugar is in the C2′-endo conformation. [c] The ∠(O1′C1′N1C4) dihedral angle in the (natural) adenosine ribonucleoside calculated at the same level of theory is 238.5°.

connectivity, unfavorable interactions between the rings may occur, which lead to slightly more complicated surfaces. In these instances, either one or both of the structures with a planar relative arrangement of the rings are transition structures that connect two minima with slightly tilted rings (by up to 45°). The transition barriers are typically less than 40 kJ mol^{-1}, which suggests that the modified nucleobases have a significant degree of intrinsic flexibility.

The planar geometries found for the global minima of the C5C6′ fleximers in the present study are very different from those obtained by Seley et al. for models that include ribose moieties, where nonplanar arrangements of the two rings were found to predominate. Therefore, a closer examination of the dependence of the properties of the fleximer nucleobases on the presence of the ribose moiety is required.

D. Conformations and Rotational Barriers for Fleximers with R = Ribose. The results obtained from conformational searches conducted by rotating about the fleximer carbon−carbon bond in models that include a ribose moiety are displayed in Tables 3−5 (R = ribose). As mentioned in the Computational Details, the C2′-endo sugar conformations, which involve a O2′−H···O3′ hydrogen bond, and the anti orientation about the glycosidic bond are considered in the present study.

Experiments indicate that ∠(O1′C1′N9C4) dihedral angles in the anti conformations of the natural purine nucleosides typically range between 170° and 280°. The ∠(O1′C1′N9C4) dihedral angles in the (natural) guanine, adenine, and inosine nucleosides calculated with the same level of theory and sugar model implemented in the present work equal 238.6°, 238.5°, and 237.1°, respectively. For the purine fleximers, the ∠(O1′C1′N1C5) dihedral angle defines the conformation about the sugar−base bond. In some fleximers, this angle changes significantly with rotation about the fleximer carbon−carbon bond (Tables 3−5) and any significant changes will be discussed in the following sections.

The bond lengths of the fleximer CC bonds are also displayed in Tables 3−5. In general, the fleximer carbon−carbon bonds are approximately 1.46−1.47 Å in minima and 1.48−1.49 Å in transition structures, which are similar to those calculated for the R = H models.

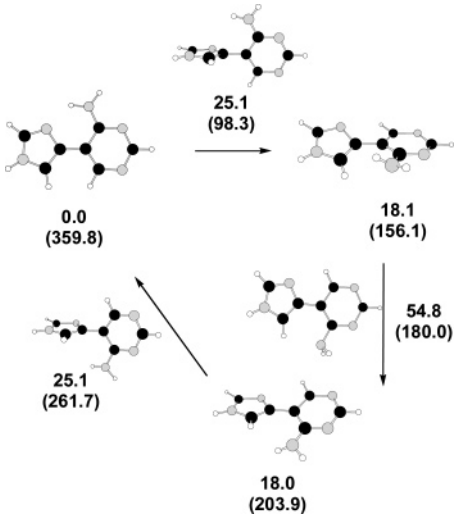


Figure 3. Relative energies (kJ mol^{-1}) and C5C4C5′C6′ dihedral angles (in parentheses, deg) for species involved in rotation about the C4C5′ bond in the adenine **A(C4C5′)** fleximer (R = H, Chart 2).

(*i*) *Guanosine Fleximers.* Close interatomic distances between the C1′ hydrogen in the sugar and the pyrimidine amino group or C5′ hydrogen are present in the guanosine **G(C5C6′)** fleximer (Figure 4). These interactions prevent structures with planar arrangements of the imidazole and pyrimidine rings from being minima on the potential energy surface. These interactions also lead to a large range (40.9°) in the ∠(O1′C1′N1C5) dihedral angle (Table 3), which deviates by up to 20° from the value in the guanosine ribonucleoside.

As a result of the close contact distances between the base and the sugar, four minima are present with respect to rotation about the C5C6′ bond (Table 3), which have ∠(N1C5C6′N1′) dihedral angles slightly greater or less than 0 or 180°.[25,26] All minima fall within 3.1 kJ mol^{-1}. The conformations with ∠(N1C5C6′N1′) approximately equal to 0 and 180° are transition structures, which fall 3.3 and 12.2 kJ mol^{-1} above the global minimum, respectively. Transition structures, which are approximately 18 kJ mol^{-1} above the global minimum, also occur when the imidazole and pyrimidine rings adopt a perpendicular arrangement.

The rotational surface for the guanosine **G(C4C5′)** fleximer is nearly identical with the surface discussed for the R = H model (Table 3 and Figure 2). The global minimum has a planar base, while two local minima (∠(C5C4C5′C6′) = 44.0° and 316.3°) fall 28.6 kJ mol^{-1} above the global minimum. Perpendicular arrangements of the rings lead to 30 kJ mol^{-1} barriers, while the planar nucleobase with ∠(C5C4C5′C6′) = 359.6° is a 37.8 kJ mol^{-1} transition structure.

The planar structure for the guanosine **G(C5C5′)** fleximer with ∠(N1C5C5′C6′) equal to 184.8° represents a (29.2 kJ mol^{-1}) transition structure due to close interatomic distances between the carbonyl group of the pyrimidine ring and C1′ hydrogen in the sugar (R(H···O) = 2.026 Å). It should also be noted that the C2′ hydroxyl group rotates to form a hydrogen bond with the pyrimidine carbonyl group (R(H···O) = 2.068 Å and ∠(O−H···O) = 135.4°). These interactions also lead to a relatively small ∠(O1′C1′N1C5) dihedral angle (210.4°).

The global minimum for **G(C5C5′)** (∠(N1C5C5′C6′) = 134.7°) also involves interactions between the C2′ hydroxyl group and the pyrimidine carbonyl group (R(H···O) = 1.905 Å and ∠(O−H···O) = 159.3°), which stabilize this conformer (Table 3). The remaining three local minima, which are up to 9.4 kJ mol^{-1} higher in energy than the global minimum, and three transition structures, which have barriers ranging from 11.0 to 29.2 kJ mol^{-1} with respect to the global minimum, do not have the aforementioned intramolecular hydrogen-bonding interactions. However, some conformations of **G(C5C5′)** involve small distances between the C1′ ribose hydrogen and the hydrogen at C6′ in the pyrimidine ring, which causes a change in the orientation of the base with respect to the sugar moiety (see ∠(O1′C1′N1C5), Table 3).

The guanosine **G(C4C6′)** fleximer has the simplest rotational surface among the guanosine fleximers where only two planar minima were characterized (Table 3), which differ in energy by 17.8 kJ mol^{-1}. Two transition structures, which involve perpendicular arrangements of the imidazole and pyrimidine rings, are 33.5 kJ mol^{-1} above the global minimum.

(*ii*) *Adenosine Fleximers.* As found for the R = H model, the adenosine **A(C5C6′)** and **A(C4C6′)** surfaces with R = ribose (Table 4) are very similar to the corresponding guanosine fleximer (R = ribose, Table 3). Four local minima exist on the **A(C5C6′)** surface since the planar arrangement of the imidazole and pyrimidine rings within the nucleobase is precluded due to close contact distance between the C1′ hydrogen atom in ribose and the pyrimidine carbonyl or C5′.[25] These close contacts also lead to a large range (42.7°) in the ∠(O1′C1′N1C5) dihedral angles. The **A(C4C6′)** surface is comparably simple since the two planar arrangements of the nucleobase rings are minima on the surface, which are separated by 37 kJ mol^{-1} barriers.

The direction of the amino group puckering in the adenosine **A(C4C5′)** fleximer changes upon rotation about the C4C5′ fleximer bond as discussed for the R = H model (Table 4 and Figure 3). This occurs due to a planar amino group in the global minimum (∠(C5C4C5′C6′) = 353.3°) and a twisted amino group in the transition structure at ∠(C5C4C5′C6′) = 180.4°. This transition structure, which connects the two local minima, has an associated 55.5 kJ mol^{-1} barrier, while two transition structures connecting the local minima to the global minimum have 24−25 kJ mol^{-1} barriers.

The rotational surface about the C5C5′ fleximer bond in **A(C5C5′)** is complicated by close contact distances between the ribose C1′ hydrogen and the pyrimidine amino group or C6′−H. Indeed, these interactions preclude the isolation of structures with ∠(N1C5C5′C6′) approximately equal to 0 or 180° that maintain the sugar geometry of interest in the present study. It is expected that these structures are very high-energy transition barriers and therefore these sections of the surface were not further investigated. It should be noted that in the present study we are interested in the anti conformation of the base and a select (C2′-endo) ribose conformation, and it is possible that examination of other constraints in future work will further the understanding of this system.

Other close contact distances between the sugar moiety and the fleximer amino group exist on the **A(C5C5′)** surface. The global minimum on the surface (∠(N1C5C5′C6′) = 116.3°) involves a O2′···H−N interaction (R(O2′···H) = 2.158 Å and ∠(O2′···H−N) = 143.9°), while a local minimum that falls 1.3 kJ mol^{-1} above the global minimum (∠(N1C5C5′C6′) = 237.7°) involves a O1′···H−N interaction (R(O1′···H) = 2.216 Å and ∠(O1′···H−N) = 146.5°). Two additional minima were characterized on the surface at ∠(N1C5C5′C6′) = 50.0 and 311.6°, where the latter contains weak O2′···H−C6′ interactions with the pyrimidine ring (R(O2′···H) = 2.443 Å and ∠(O2′···H−C6′) = 118.7°). Two transition structures with nearly perpendicular imidazole and pyrimidine rings were characterized on the surface. The relative energies for all characterized structures

TABLE 5: Selected Geometrical Parameters (deg, Å) and Relative Energies (kJ mol^{-1}) of Various Conformers in Hypoxanthine (R = H) and Inosine (R = Ribose) Fleximers[a]

I(C5C6′)

R = H			R = ribose[b]			
∠(N1C5C6′N1′)	*R*(C5C6′)	Δ*E*	∠(N1C5C6′N1′)	∠(O1′C1′N1C5)[c]	*R*(C5C6′)	Δ*E*
0.0	1.451	0.0	24.2	219.9	1.459	0.0
94.1	1.481	39.7 (TS)	88.2	222.2	1.481	19.8 (TS)
179.9	1.451	10.3	156.3	235.1	1.457	3.5
265.9	1.481	39.7 (TS)	187.4	216.3	1.459	11.6 (TS)
			201.6	263.6	1.458	3.1
			266.5	241.3	1.481	21.1 (TS)
			339.6	235.5	1.458	1.7
			358.1	226.8	1.459	2.3 (TS)

I(C4C5′)

R = H			R = ribose[b]			
∠(C5C4C5′C6′)	*R*(C4C5′)	Δ*E*	∠(C5C4C5′C6′)	∠(O1′C1′N1C5)	*R*(C4C5′)	Δ*E*
180.0	1.458	0.0	180.5	233.3	1.456	0.0
271.3	1.479	32.8 (TS)	270.4	231.1	1.479	33.7 (TS)
317.8	1.467	30.8	319.6	230.6	1.466	30.8
0.0	1.468	39.7 (TS)	0.0	230.9	1.466	38.8 (TS)
42.1	1.467	30.9	40.3	228.9	1.466	31.0
88.8	1.479	32.8 (TS)	90.0	231.3	1.479	34.3 (TS)

I(C5C5′)

R = H			R = ribose[b]			
∠(N1C5C5′C6′)	*R*(C5C5′)	Δ*E*	∠(N1C5C5′C6′)	∠(O1′C1′N1C5)	*R*(C5C5′)	Δ*E*
180.0	1.451	0.0	137.5	239.0	1.462	0.0
81.2	1.473	38.2 (TS)	183.6	209.4	1.473	26.0 (TS)
0.0	1.450	19.6	222.6	190.0	1.462	5.0
278.8	1.473	38.2 (TS)	269.1	220.1	1.474	11.3 (TS)
			328.1	236.1	1.456	5.7
			10.4	202.6	1.461	22.2 (TS)
			31.0	259.4	1.456	5.5
			87.2	243.7	1.474	14.9 (TS)

I(C4C6′)

R = H			R = ribose[b]			
∠(C5C4C6′N1′)	*R*(C4C6′)	Δ*E*	∠(C5C4C6′N1′)	∠(O1′C1′N1C5)	*R*(C4C6′)	Δ*E*
0.0	1.460	0.0	0.0	230.8	1.458	0.0
89.4	1.486	34.7 (TS)	89.4	227.6	1.485	36.6 (TS)
163.8	1.468	21.1	164.9	230.5	1.466	21.1
180.0	1.468	21.2 (TS)	179.8	230.8	1.466	21.2 (TS)
196.2	1.468	21.1	192.0	231.2	1.466	21.3
270.6	1.486	34.7 (TS)	270.3	233.3	1.485	37.1 (TS)

[a] See Chart 2 for structure and notation. [b] The ribose sugar is in the C2′-endo conformation. [c] The ∠(O1′C1′N1C4) dihedral angle in the (natural) inosine ribonucleoside calculated at the same level of theory is 237.1°.

on the adenosine **A(C5C5′)** surface indicate that the C5C5′ rotational surface is extremely flat (Table 4).

(*iii*) *Inosine Fleximers.* As discussed for the R = H model, the rotational barriers about the CC fleximer bond in inosine fleximers (R = ribose, Table 5) closely mimic those for the corresponding guanosine fleximer (Table 3). The **I(C5C6′)** and **I(C5C5′)** rotational surfaces contain four minima due to unfavorable interactions between the ribose moiety and the pyrimidine ring in structures with the appropriate ring dihedral angle equal to 0 or 180°.[27] These interactions lead to a large range in ∠(O1′C1′N1C5) for both fleximers. The rings are tilted by approximately 20° in **I(C5C6′)** and 30−40° in **I(C5C5′)** minima.[25] The largest rotational barrier is 21.1 and 26.0 kJ mol^{-1} for **I(C5C6′)** and **I(C5C5′)**, respectively.

The inosine **I(C4C5′)** fleximer has a planar nucleobase in the global minimum ∠(C5C4C5′C6′) = 180.5°, which is 38.8 kJ mol^{-1} lower in energy than a transition structure with ∠(C5C4C5′C6′) equal to zero due to repulsive interactions between the carbonyl group of the pyrimidine ring and the N3 lone pair. Two local minima are 30.8−31.0 kJ mol^{-1} higher in energy than the global minimum, where the local minima are connected to the global minimum by approximately 3 kJ mol^{-1} barriers with respect to the local minima.

The **I(C4C6′)** surface is slightly different from that discussed for **G(C4C6′)** with R = ribose due to the transition structure characterized at ∠(C5C4C6′N1′) = 179.8° for the inosine fleximer. However, it should be noted that this represents a very shallow barrier upon inclusion of zero-point vibrational energy corrections (Table 5). The structure with the ∠(C5C4C6′N1′) dihedral angle equal to 0 is the global minimum, which falls approximately 21 kJ mol^{-1} below two local minima (∠(C5C4C6′N1′) = 164.9 and 192.0°). A 37 kJ mol^{-1} barrier separates the local and global minima.

In summary, as for the R = H models, the shape of the rotational surface of the fleximers with R = ribose depends on the connectivity between the imidazole and pyrimidine rings. However, the rotational surfaces about the carbon−carbon bond in various fleximers with R = ribose contain different and/or additional minima compared with R = H fleximers in many instances. Furthermore, the barriers for bond rotation change

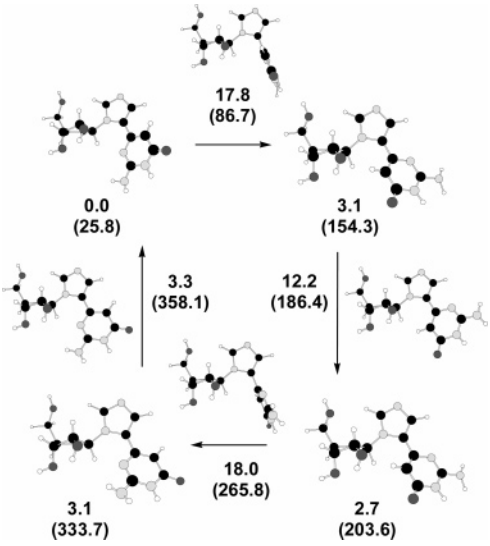


Figure 4. Relative energies (kJ mol^{-1}) and N1C5C6′N1′ dihedral angles (in parentheses, deg) for species involved in rotation about the C5C6′ bond in the guanosine **G(C5C6′)** fleximer (R = ribose, Chart 2).

in the presence of the sugar moiety. Trends in these effects will be summarized in the following section.

E. Comparison of Fleximer Properties. From the results presented in the previous sections, it is clear that the potential energy surface for rotation about the fleximer carbon−carbon bond is affected by the connectivity of the imidazole and pyrimidine rings, the nature of the (natural) nucleobase, and the presence of the ribose moiety. Table 6 summarizes the distinguishing features of the surfaces investigated in the present study and the global trends in the data are discussed below.

(*i*) *Effects of Connectivity.* Upon introduction of a single carbon−carbon bond to the purine nucleobases, it is energetically most favorable for the imidazole and pyrimidine rings to remain planar with respect to one another. This statement is supported by the fact that the reference dihedral angles for all global minima are approximately equal to 0 or 180° for R = H (Table 6). The only exception to this trend is **A(C5C5′)** where a nonplanar global minimum is characterized on the rotational surface due to close contact distances between the imidazole ring and the amino group of the pyrimidine ring.

For some fleximers, a local minimum with a planar arrangement of the imidazole and pyrimidine rings exists. However, depending on the connectivity of the fleximer, unfavorable intramolecular interactions, such as steric repulsion between hydrogen atoms or lone pairs, are sometimes present in the potential planar local minimum. In these instances, the planar arrangement becomes a transition structure on the rotational surface and two local minima are characterized, where the planes of the rings are typically tilted by 25−45°.[28] In general, the greater the tilt angle, the higher the energy of the local minimum compared with the planar global minimum.

When the properties of the nucleobase are considered in the absence of the sugar moiety (R = H), two minima are present for all C5C6′ and C5C5′ fleximers since there is no steric clashing between substituents on the imidazole and pyrimidine rings. Once again, the exception is the **A(C5C5′)** fleximer where four minima are characterized on the rotational surface due to the close contacts previously mentioned. Three minima are present for all C4C5′ fleximers, due to close contacts between N3 of the imidazole ring and the C4′ carbonyl in the guanine and hypoxanthine structures or C5−H of the imidazole ring and the amino group in the adenine fleximer. Three minima are also present on the C4C6′ fleximer surfaces due to unfavorable interactions between N3 and N1′.

In addition to the number of minima, the energy difference between minima varies with the connectivity of the imidazole and pyrimidine rings (ΔE_{min}, Table 6). The smallest energy differences occur for the guanine and hypoxanthine C5C6′ fleximers (8.6 and 10.3 kJ mol^{-1}, respectively), while the largest energy differences for these modified bases occur for the C4C5′ fleximers (28.8 and 30.9 kJ mol^{-1}, respectively), which also involve the most significantly twisted local minima. The C5C5′ and C4C6′ adenine fleximers have the smallest (3.1 kJ mol^{-1}) and largest (26.8 kJ mol^{-1}) energy differences between minima, respectively.

Although the number and relative energy of minima varies with the connectivity, the largest energy barriers are relatively constant among all fleximers with R = H. Guanine fleximer rotational barriers are within 6.4 kJ mol^{-1}, while the hypoxanthine barriers are within 5.0 kJ mol^{-1}. Transition barriers for the adenine fleximers span a greater (18.9 kJ mol^{-1}) range in part due to interactions between the pyrimidine amino group and the imidazole ring, which leads to high-energy (50−54 kJ mol^{-1}) transition structures for the **A(C4C5′)** and **A(C5C5′)** fleximers. It should be noted that a significant degree of flexibility can still be obtained in these adenine fleximers while avoiding this high-energy transition structure.

(*ii*) *Effects of Nucleobase.* The guanine and hypoxanthine potential energy surfaces for CC bond rotation are very similar due to the similarities in the nucleobase structure. In general, the magnitude of the relative energy between minima is slightly smaller (by 2−4 kJ mol^{-1}) for the guanine fleximers. The transition barriers are also slightly reduced for the guanine fleximers (by 1−5 kJ mol^{-1}).

The adenine rotational surfaces are different from the guanine and inosine surfaces due to the C4′ amino group, which leads to significant interactions between the separated rings. These interactions lead to alternate conformations of the fleximer base, which involve large changes in the orientation of the amino group. These changes lead to smaller relative energies for the C4C5′ and C5C5′ minima (by 10.7 and 16.6 kJ mol^{-1}) compared with the corresponding guanine fleximers (R = H). Despite the reduction in the relative energy of the minima, the largest transition barriers (50−54 kJ mol^{-1}) are much higher for these adenine fleximers, which have associated transition structures with a staggered amino group. It should be noted that if the high-energy barriers are not considered, the remaining transition barriers are less than 30 kJ mol^{-1}, which is smaller than those for the corresponding guanine and hypoxanthine fleximers.

The adenine C5C6′ and C4C6′ fleximers do not involve interactions between the amino group and the imidazole ring. In these instances, the magnitude of the relative energies significantly increases (by 10.3 and 9.4 kJ mol^{-1}) compared with that of the corresponding guanine fleximers, while the transition barriers only slightly increase (by approximately 4 kJ mol^{-1}). Although the intramolecular interactions appear to be similar in the adenine and guanine (hypoxanthine) fleximers, differences arise due to the electron-withdrawing properties of the guanine (hypoxanthine) carbonyl group.

(*iii*) *Effects of Ribose Moiety.* In addition to complications arising from intramolecular interactions within the nucleobases, different minima are found on the rotational surfaces of the R = ribose models due to interactions between the modified base and the sugar moiety. Although the sugar group has a significant effect on the CC bond rotational surface of some fleximers, the

TABLE 6: Summary of Distinguishing Features on the Potential Energy Surface for Rotation about Fleximer Carbon−Carbon Bonds[a,b]

	no. of minima		global minimum			ΔE_{min}		ΔE_{TS}	
	R = H	R = ribose		R = H	R = ribose	R = H	R = ribose	R = H	R = ribose
G(C5C6′)	2	4	∠(N1C5C6′N1′)	0.9	25.8	8.6	3.1	36.8	18.0
G(C4C5′)	3	3	∠(C5C4C5′C6′)	180.7	180.2	28.8	28.6	38.4	37.8
G(C5C5′)	2	4	∠(N1C5C5′C6′)	179.2	134.7	19.7	9.4	32.8	29.2
G(C4C6′)	3	2	∠(C5C4C6′N1′)	359.1	0.7	17.4	17.8	32.0	33.5
A(C5C6′)	2	4	∠(N1C5C6′N1′)	0.0	18.7	18.9	10.5	40.7	23.0
A(C4C5′)	3	3	∠(C5C4C5′C6′)	359.8	353.3	18.1	17.8	54.8	55.5
A(C5C5′)	4	4	∠(N1C5C5′C6′)	33.3	116.3	3.1	1.3	50.3	−[c]
A(C4C6′)	3	2	∠(C5C4C6′N1′)	359.6	0.6	26.8	25.7	35.9	37.6
I(C5C6′)	2	4	∠(N1C5C6′N1′)	0.0	24.2	10.3	3.5	39.7	21.1
I(C4C5′)	3	3	∠(C5C4C5′C6′)	180.0	180.5	30.9	31.0	39.7	38.1
I(C5C5′)	2	4	∠(N1C5C5′C6′)	180.0	137.5	19.6	5.7	38.2	26.0
I(C4C6′)	3	3	∠(C5C4C6′N1′)	0.0	0.0	21.1	21.3	34.7	37.1

[a] See Chart 2 for structure and notation. [b] The anti conformation of the base with respect to the ribose moiety was considered and the sugar maintains C2′-endo puckering. [c] The full rotational surface for the adenosine **A(C5C5′)** fleximer (R = ribose) was not characterized. See text for further details.

effect depends on the connectivity between the imidazole and pyrimidine rings and the nature of the (natural) nucleobase.

For all fleximers with C5C6′ connectivity, the presence of the sugar moiety increases the number of minima and transition structures for rotation about the fleximer carbon−carbon bond from two to four. Although the C5C6′ global minima for R = H are planar, the global minima for R = ribose contain slightly tilted orientations of the two rings as reported by Seley et al.[19,25] The inclusion of the sugar moiety significantly decreases (by 5.5−8.4 kJ mol^{-1}) the relative energy between the C5C6′ global and local minima. Additionally, the rotational barriers in these fleximers are reduced by approximately 18 kJ mol^{-1} upon inclusion of the sugar moiety.

Similarly, upon inclusion of the sugar moiety in the guanine and inosine C5C5′ fleximers, the number of minima increases from two to four and the global minima are no longer planar. It is interesting to note that the guanosine and inosine C5C5′ fleximers have significantly reduced relative energies (by 10−14 kJ mol^{-1}) upon inclusion of the sugar moiety. Although the transition barriers decrease by a smaller extent (3−12 kJ mol^{-1}), the guanosine and inosine C5C5′ barriers are the second smallest among connectivities with R = ribose. On the other hand, although the relative energies between minima are small, the flexibility in **A(C5C5′)** is complicated by close contacts between the amino group and the ribose moiety.

Although the C5C6′ and C5C5′ surfaces change considerably upon inclusion of ribose, the global minima for all C4C5′ and C4C6′ fleximers remain planar upon inclusion of the sugar moiety. Furthermore, all C4C5′ fleximers, as well as **I(C4C6′)**, have the same number of minima and transition structures (three), the relative energy differences between minima are within 1 kJ mol^{-1}, and the transition barriers are within 2 kJ mol^{-1} upon inclusion of the sugar. Although the surfaces for the **G(C4C6′)** and **A(C4C6′)** fleximers appear to be simplified upon increasing the model size from R = H to ribose, the apparent simplification arises due to the loss of a very low energy transition structure, and therefore the R = H and ribose surfaces are very similar.

The transition barriers for the R = ribose models vary more significantly with connectivity than those discussed for R = H due to intramolecular interactions between the nucleobase and the sugar moiety. The guanosine, adenosine, and inosine fleximer transition barriers vary by 19.8, 32.5, and 17.0 kJ mol^{-1} with ring connectivity, respectively. These larger differences in the transition barriers make some structures significantly more flexible than others with respect to rotation about the carbon−carbon fleximer bond.

Among the fleximers studied in the present work, **G(C5C6′)** has the lowest barrier for rotation about the fleximer CC bond and the smallest relative energy between minima on the surface. **I(C5C6′)** has very similar properties to **G(C5C6′)**. Although **A(C5C6′)** is the most flexible adenosine fleximer, it has a significantly larger energy difference between minima and a slightly larger transition barrier than **G(C5C6′)**. The **G(C5C5′)** and **I(C5C5′)** fleximers also possess small relative energies and transition barriers. Although other structural factors must be examined in future work, such as alternate sugar conformations and syn orientations about the glycosidic bond, our data suggest that these molecules may be appropriate targets for future synthesis.

Conclusions

Modified nucleobases are used in a variety of applications including therapeutics and bioprobes. The present study examined the properties of a class of modified nucleobases called fleximers, which are purine derivatives with the imidazole and pyrimidine rings separated by a single carbon−carbon bond. A variety of guanosine, adenosine, and inosine fleximers with different connectivities between C4 or C5 of the imidazole ring and C5′ or C6′ of the pyrimidine ring were considered. Two model systems were investigated in the present study, where the first replaces the sugar with a hydrogen atom and the second contains a ribose moiety. The investigation of two model systems allows us to determine properties that are intrinsic to the modified nucleobase and those due to interactions between the nucleobase and the sugar moiety.

While studying the properties of the nucleobase in the absence of the ribose moiety, we found that separating the imidazole and pyrimidine rings with a single carbon−carbon bond leads to highly flexible molecules with respect to rotation about the fleximer bond, while maintaining the integrity of the hydrogen-bonding patterns and properties of the natural purines. All global minima (without ribose) contain planar relative arrangements of the imidazole and pyrimidine rings with the exception of that for the adenine **A(C5C5′)** fleximer. One, two, or three local minima are characterized on the surface depending on the connectivity and (natural) nucleobase structure. The variation in the number of minima arises due to unfavorable intramolecular interactions between the pyrimidine and imidazole rings

in some structures. Despite these differences, the minima on the surfaces are separated by less than 40 kJ mol^{-1} for most fleximers.

We find that the effects of the sugar moiety on the carbon−carbon bond rotational surface depend on the connectivity of the fleximer. In some instances, additional minima and transition structures are isolated on the rotational surface, which is generally accompanied by a nonplanar global minimum where the imidazole and pyrimidine rings are tilted by approximately 25−40° and a reduction in the rotational barriers. These features generally arise due to interactions between the sugar and nucleobase. In other fleximers, the rotational surfaces are not significantly affected by the presence of the sugar moiety.

In summary, the present study is the first systematic, high-level computational study on an intriguing class of modified nucleobases. A greater understanding of the dependence of fleximer properties on the connectivity, the nature of the nucleobase, and the ribose moiety has been obtained. Our calculations show that the C5C6′ fleximers introduced by Seley et al.[18,19] are indeed very flexible with respect to rotation about the carbon−carbon bond, although the favored nucleobase conformation is influenced by the ribose moiety. Additionally, the guanosine and inosine C5C5′ fleximers considered for the first time in the present work were found to have similar barrier heights to the C5C6′ fleximers. Although additional geometrical degrees of freedom related to the sugar moiety must be considered in future work, these structures may be good targets for future synthesis and biochemical tools.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation (CFI), the New Brunswick Innovation Foundation (NBIF), and the New Brunswick Department of Training and Employment Development for financial support. We also thank the Mount Allison Cluster for Advanced Research (TORCH) for generous allocations of computer resources.

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(25) It should be noted that Seley et al. report average ∠(N1C5C6′N1′) dihedral angles of 333.4°, 347.1°, and 340.9° for the guanosine, adenosine, and inosine C5C6′ fleximers, respectively (see ref 19). Although we find these structures to be local minima on the rotational surfaces, different global minima were characterized. However, in our study, the O5′C5′C4′H dihedral angle is approximately equal to 180°, while an alternate arrangement of the C5′ hydroxyl group may have been considered by Seley et al.

(26) Polak et al. suggest that a hydrogen bond between O2′ in the ribose moiety and the pyrimidine amino group stabilizes the C5C6′ fleximer that adopts a C3′-endo puckering (see ref 21). We do not find evidence of this hydrogen bond when C2′-endo sugar puckering is considered.

(27) The global minimum on the **I(C5C5′)** surface contains a hydrogen bond between the C2′ hydroxyl group in the sugar moiety and the pyrimidine carbonyl group (R(H···O) = 1.941 Å and ∠(O−H···O) = 153.6°).

(28) It should be noted that a smaller tilt (12−20°) is observed for the C4C6′ fleximers, where the associated transition barriers are extremely flat.