DFT Energy Surfaces for Aminopurine Homodimers and Their Conjugate Acid Ions

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Dimers of free nucleobases with their conjugate acid ions can be assigned to either of two categories: protonated dimers or proton-bound dimers. In the former, the extra proton attaches to a lone pair of a neutral dimer. In the latter, the extra proton is situated between two lone pairs and participates in a proton bridge. In general, proton-bound dimers are found to be more tightly held together than protonated dimers. While neutral adenine and its isomer 8-aminopurine ($C_5H_5N_5$) are substantially more stable than their 7H tautomers, their conjugate acid ions and those of their respective 7H tautomers have nearly the same heats of formation. Correspondingly, the most stable $(C_5H_5N_5)_2H^+$ structures contain 7H tautomers as the neutral partner. Proton transit from one partner to the other within the most stable protonated dimer of 8-aminopurine has a low barrier (6 kJ mol^{-1}). The potential energy curve for the NH stretch in that case is better fitted as a double minimum rather than as a harmonic potential. Purine−purine mismatches have been observed in nucleic acids, to which calculated $(C_5H_5N_5)_2H^+$ dimer geometries appear nearly isosteric.

Nucleic acids contain nitrogen heterocycles ("nucleobases"), which recognize one another by specific patterns of hydrogen bonding. A number of additional interactions (such as base stacking) also influence the stability of base pairs within biological macromolecules. Considerable interest has recently arisen regarding mismatches (especially between two purines) and bases that have yet to be seen in nature. These variations come under the heading of non-canonical pairing. The NCIR database tabulates a number of non-canonical hydrogen-bonding motifs found in RNA. Figure 1 illustrates a pattern, Trans Hoogsteen/Hoogsteen pairing between two adenines, that represents one of the more common non-canonical motifs in ribosomal RNA.[1]

Neutral adenine (A) cannot form more than one hydrogen bond with itself in the orientation it adopts within the double helix of B-DNA (usually called the Watson−Crick orientation). However, as structure **1** illustrates, adenine can form multiple hydrogen bonds with itself in the Trans Hoogsteen/Hoogsteen orientation, as well as in the Hoogsteen (more properly, the Trans Watson−Crick/Hoogsteen[1]) or in the Reverse Watson−Crick orientations (which will be illustrated below). When one of the adenines is protonated, the Watson−Crick orientation can also form more than one hydrogen bond, as can the three aforementioned non-canonical motifs.

Given the widespread interest in nucleic acids as materials for construction of nanometer-scale structures,[2] there is increasing need for pH- or ligand-dependent noncanonical base pairs that allow the incorporation of controllable and well-defined structural transitions into molecular designs.[3] Specific adenine−adenine (A·A) base pairs could prove valuable for such applications. Structure **1** provides an example of an A·A base pair that exhibits pH dependence, because it forms a more stable duplex when the adenines are protonated at their N1 positions.[4] Additionally, we have recently demonstrated that the small molecule coralyne can be used to drive the assembly of homoadenine duplexes.[5] While the hydrogen-bonded structure of these coralyne-dependent A·A base pairs has yet to be determined, their reduced stability at pH <5 suggests that the A·A pairs in this structure are distinct from **1**. The versatility of adenine in forming base pairs with itself, with a diversity of pairing motifs, hydrogen bonds, and conditions for stability, has motivated us to explore the possibility that even more selective and energetically favored base pairs might be obtained between adenine and its analogues.

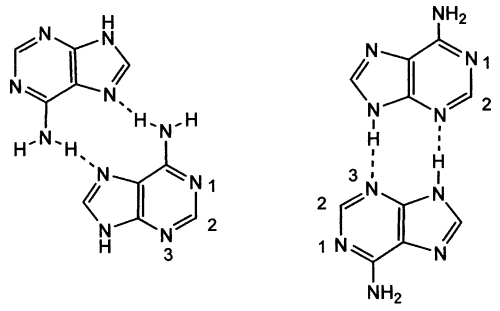

Figure 1. Two neutral dimers of adenine: **1**, which is accessible within nucleic acids, and **2**, which is not.

One way to isolate the contribution of hydrogen bonding in base pairing involves examining the interactions of free nucleobases in the gas phase. A potential drawback of this approach arises from the capacity of free nucleobases to interact in ways that are not possible for nucleosides, such as the "back-to-back" orientation of two adenines, **2**.

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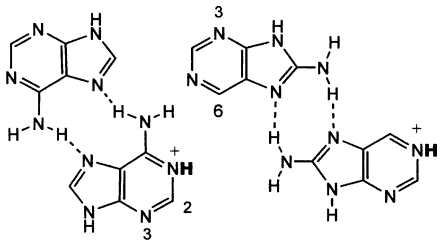


Protonated A·A Homodimer **3** | Protonated 8-Aminopurine Homodimer **4**

Figure 2. Protonated dimers of adenine and of 8-aminopurine in their Trans Hoogsteen/Hoogsteen orientations.

Another gas phase approach has been to look at the binding of nucleobases with their conjugate acid ions. In 1979 Mautner reported the enthalpies of dissociating singly protonated homodimers of the four DNA bases in the gas phase.[6] Many structures are possible, especially for the proton-bound dimer of adenine, $(C_5N_5H_5)_2H^+$. The present work surveys plausible $(C_5N_5H_5)_2H^+$ geometries, both for adenine and its isomer 8-aminopurine. Figure 2 illustrates protonated Trans Hoogsteen/Hoogsteen dimers of adenine (**3**) and of 8-aminopurine (**4**).

This study differentiates two categories of clusters between a protonated and a neutral molecule: protonated dimers (where the extra proton attaches to a lone pair of a neutral dimer) versus proton-bound dimers (in which the extra proton is situated between two lone pairs and participates in a proton bridge). The dimers shown in Figure 2 fall into the first category. Proton-bound dimers turn out to be considerably more stable. In some instances, proton-bound homodimers offer cases where transit of a proton from one heterocycle to the other has a low barrier.

Low-barrier hydrogen bonds have been extensively discussed, with particular reference to strong hydrogen bonds between an OH and a negatively charged oxygen.[7] Low-barrier proton bridges between two nitrogens have been less closely scrutinized.[8] Gas-phase ion chemists have looked at many simple cationic dimers that ought to exhibit this sort of bonding, but these have yet to prove amenable to examination of their vibrational structure. As one goal, the calculations described herein put forth molecular systems, which should be suitable for both gas phase and condensed phase investigations.

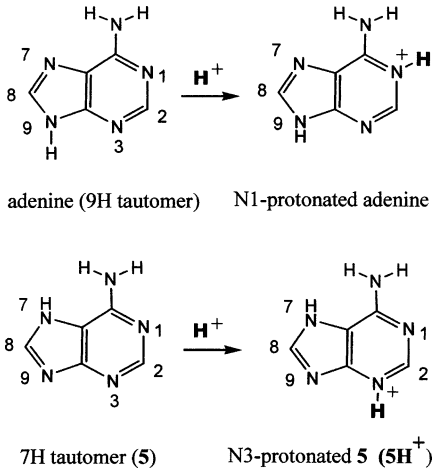
Computational Details

Monomer and dimer equilibrium geometries were optimized at the B3LYP/6-31G** level[9] using the GAUSSIAN98 and GAUSSIAN03 program suites. Stabilities of local minima were ascertained by performing harmonic normal modes calculations. Except where otherwise indicated, B3LYP/6-31G**//B3LYP/6-31G** electronic energies are reported. 0 K enthalpy differences include unscaled zero point energy corrections. Basis set superposition errors for dimer dissociations were estimated by the counterpoise method.[10] Single-point calculations were also performed at the B3LYP/6-31++G** level. Transition states for proton transit were determined by energy minimizations of geometries subject to twofold symmetry constraints and confirmed by normal modes calculations that give one negative force constant.

Results

This study differentiates between proton-bound dimers and protonated dimers. The first set of results surveys dimers of the nucleobase adenine with its conjugate acid ion. The second set of results deals with neutral homodimers of 8-aminopurine, an isomer of adenine. The third set of results examines dimers between 8-aminopurine and its conjugate acid ions.

SCHEME 1: Adenine (9H-6-aminopurine), Its 7H Tautomer, and Their Most Stable Conjugate Acids



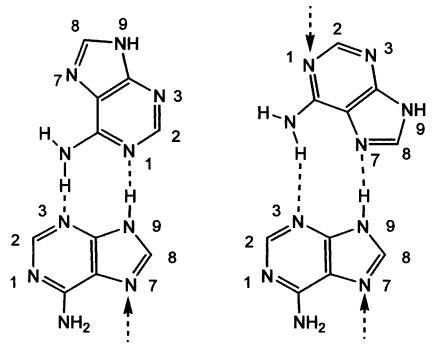
adenine (9H tautomer) | N1-protonated adenine

7H tautomer (**5**) | N3-protonated **5** (**5H**$^+$)

Adenine Dimers. In order to calibrate the computational method, the cationic dimers of adenine, $(C_5N_5H_5)_2H^+$, were examined and the results compared with reported experimental data. The free adenine nucleobase exists predominantly as the 9H tautomer in the gas phase. Small amounts of the 7H tautomer (**5**, drawn in Scheme 1) have been detected,[11] even though calculations place it approximately 30−35 kJ mol^{-1} higher in energy.[12]

Adenine's neutral homodimers have also been studied. High-level calculations have been reported for A·A dimers in the Reverse Watson−Crick, Hoogsteen, and Trans Hoogsteen/Hoogsteen geometries,[12] which give binding energies in the range of 45−55 kJ mol^{-1}. Hartree−Fock based calculations suggest that neutral homodimer **2**, with a back-to-back orientation (a pair of N9H···N3 hydrogen bonds), is more stable, but a R2PI study of jet-cooled, gaseous A·A homodimers is not consistent with any of those four geometries; the observed vibronic structure fits either the NH_2···N3; N9H···N1 (**6**) or the NH_2···N3; N9H···N7 (**7**) motifs illustrated in Figure 3.[11]

The binding energy of adenine to its conjugate acid ion in the gas phase was measured by Mautner in 1979 and is reported as $\Delta H°_{500} = -127 \pm 4$ kJ mol^{-1}.[6] The relative stabilities of the conjugate acid ions of the monomers play an important role in attempting to fit the experimental data: N1 is the most basic site of adenine, and the next most basic site is N3, which is



N1H···N9; NH_2···N3 **6** | N9H···N7; NH_2···N3 **7**

Figure 3. Neutral adenine homodimer structures that are consistent with reported R2PI spectra of jet-cooled gaseous samples.[11] Dashed arrows indicate the most basic nitrogens.

TABLE 1: Harmonic Zero-Point Energies and Relative Electronic Energies for B3LYP/6-31G Optimized Geometries of $C_5N_5H_5$ Isomers and Conjugate Acid Ions, as Well as Electronic Energies for CCSD/6-31G** Optimized Geometries**

	relative E^{el} (B3LYP/6-31G** geom)			E^{el} CCSD/6-31G** optimized geom
	6-31G**	6-31++G**	CCSD	
adenine−N1H$^+$	0	0	0	−466.400618
adenine + H$^+$	0.381143	0.371115	0.383318	−466.017329
20 + H$^+$	0.390597	0.381219	0.392435	−466.008254
21 + H$^+$	0.395439	0.386257	0.397957	−466.002740
7H−adenine−N3H$^+$ (**5H**$^+$)	0.000011	−0.000125	0.002243	−466.398920
7H−adenine−N1H$^+$	0.018809	0.017937	0.019700	−466.380872
N1 protonated **20** (**20H**$^+$)	0.003350	0.003042	0.007285	−466.393245
N7 protonated **20**	0.016313	0.016965	0.016573	−466.383873
N3 protonated **20**	0.023271	0.023222	0.027497	−466.373057
N3 protonated **21** (**21H**$^+$)	0.004260	0.004657	0.007428	−466.393062
N1 protonated **21**	0.006262	0.006254	0.010193	−466.390282

TABLE 2: Energies of Protonated Dimers of Adenine Based on Geometries Optimized at B3LYP/6-31G, with 0 K Enthalpies for Dissociation to Neutral Adenine Plus N1-Protonated Adenine, Corrected for BSSE Using Counterpoise and for ΔZPE (Unscaled)[a]**

	ΔE^{B3LYP} [b,c]		ΔZPE^{Harm}	0 K $\Delta H°_{Dissn}$	
structure	**6-31G****	**6-31++G***	**6-31G****	**6-31G****	ΔE^{DEF} [a]
		N3-Protonated			
17	129.6	114.6	1.5	117.6	56.1
19	128.0	113.1	2.0	115.5	55.2
		N1-Protonated			
N3H···N3;NH_2···N9 (**12**)	132.4	117.1	2.7	119.4	60.7
N1H···N9;NH_2···N3 (**13**)	129.9	114.5	3.5	116.1	45.4
Watson−Crick (**9**)	108.4	92.8	6.0	93.2	18.5
reverse Watson−Crick (**10**)	105.3	89.3	7.2	88.9	19.2
Hoogsteen (**11**)	102.5	85.6	6.4	85.5	21.7
N9H···N3;N9H···N3 (**8**)	87.7	74.6	−1.4	79.9	20.3
N9H···N7;NH_2···N3 (**14**)	88.9	74.1	1.0	78.0	28.5
trans Hoogsteen/Hoogsteen (**3**)	72.4	58.8	3.8	58.1	17.7
		N7-Protonated			
N9H···N7;NH_2···N3 (**15**)	91.7	77.2	3.8	77.9	82.5

[a] Deformation energies (ΔE^{DEF} in kJ mol^{-1}) computed as described in ref 15. All energies in kJ mol^{-1}. [b] Not corrected for BSSE. [c] Relative to N1-protonated adenine plus adenine.

reported to have a proton affinity 7 kJ mol^{-1} lower.[13] The third most basic site of adenine is N7, whose proton affinity has been calculated to be 34 kJ mol^{-1} less than that of N1.[13] Surprisingly, as Marian, Nolting, and Weinkauf have recently reported, N3-protonated 7H−adenine (**5H**$^+$ shown in Scheme 1) has a calculated heat of formation within 5 kJ mol^{-1} of that of N1-protonated adenine.[14] Table 1 summarizes computations at various levels, all of which agree with the previously published calculations. Since adenine is so much more stable than its 7H tautomer, these results mean that the 7H tautomer is approximately 30 kJ mol^{-1} more basic than adenine.

A variety of dimers can form between adenine and its N1-protonated conjugate acid ion, which possess two hydrogen bonds. Ion **5H**$^+$, though, has fewer options. Therefore, ten dimers that contain N1-protonated adenine have been considered (of which Table 2 lists eight), but only four that contain N3-protonated **5H**$^+$ (of which just two are stable).

The protonated A·A dimer drawn in Figure 2, **3**, corresponds to the Trans Hoogsteen/Hoogsteen homodimer with the proton on N1. Mautner suggested structures for two alternative possibilities: the Reverse Watson−Crick dimer with the proton on N1 (structure **10** in Figure 4) and the Trans Hoogsteen/Hoogsteen dimer with the proton on N7 (in which the NH_2 group of the unprotonated partner is twisted out of the plane of the ring and serves as a hydrogen bond acceptor). DFT calculations predict none of these to be the most stable geometry. A preferred geometry corresponds to placing a proton on N1 of the neutral homodimer with two N9H···N3 hydrogen bonds (the neutral geometry that is predicted to be the most stable[12]), species **8** depicted in Figure 4. However, all of the aforementioned structures have calculated binding energies far weaker than the experimental value, as Table 2 summarizes.

It turns out that the most favored $(C_5N_5H_5)_2H^+$ structure, **12**, corresponds to the proton-bound dimer of the neutral N7H tautomer of adenine with N1-protonated adenine, having hydrogen bonds between N1 and NH_2 of the cationic partner and N3 and N9 of the neutral partner, respectively. This geometry, which contains a C═N9 double bond, would not be accessible for nucleic acids, in which N9 is covalently bound to three atoms. A proton-bound dimer that is nearly as stable, **13**, is produced by placing H$^+$ on N7 of the NH_2···N3, N9H···N1 neutral dimer **6** (indicated by a dashed arrow), followed by a barrier-free shift of the bridging proton from N9 to the complementary N1.

Like **6**, neutral dimer **7** possesses four accessible, basic nitrogens. The dashed arrows in Figure 3 indicate the most basic ones. Placing H$^+$ on N1 or on N7 gives protonated dimer ions (**14** and **15**, respectively) that are close in energy (although they are among the least stable of those listed in Table 2). Although N7 is less basic than N1 or N3 in adenine itself, creation of a proton bridge in **15** gives N7 of **7**, a proton affinity virtually equal to that of the more basic N1 position.

Table 2 summarizes the 0 K enthalpies for dissociating various isomeric protonated A·A dimers to adenine plus its N1 conjugate acid ion. Ions **12** and **13** are the most stable dimers of N1-protonated adenine. The calculated 0 K dissociation enthalpy at B3LYP/6-31G** for the most stable isomer, **12**, is $\Delta H°_{Dissn}$ = 129.6 kJ mol^{-1} (without correction for BSSE); with

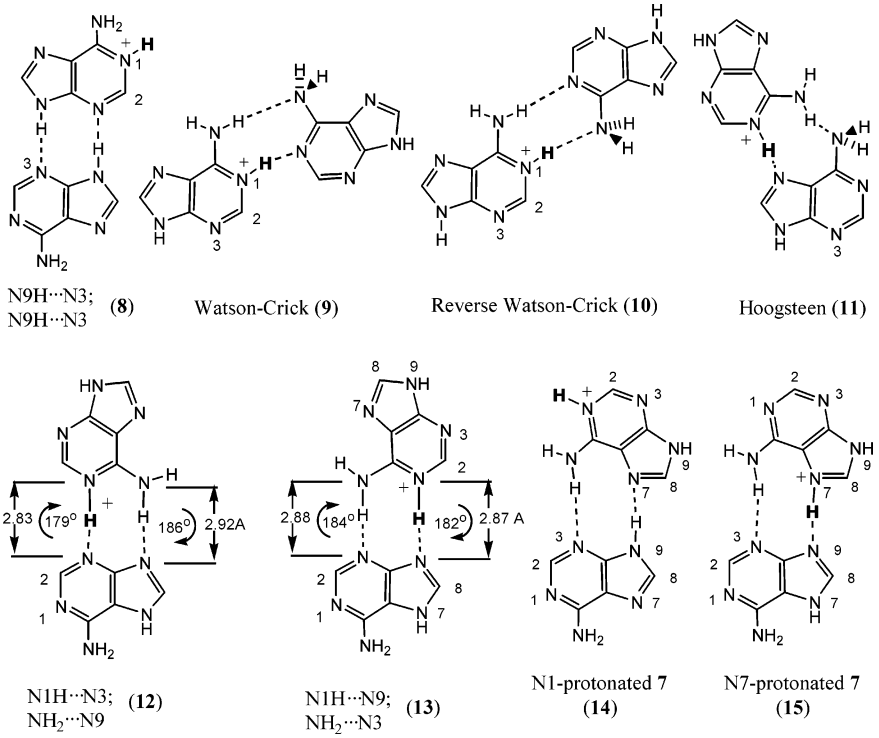


Figure 4. N1- and N7-protonated adenine homodimers (see Table 2).

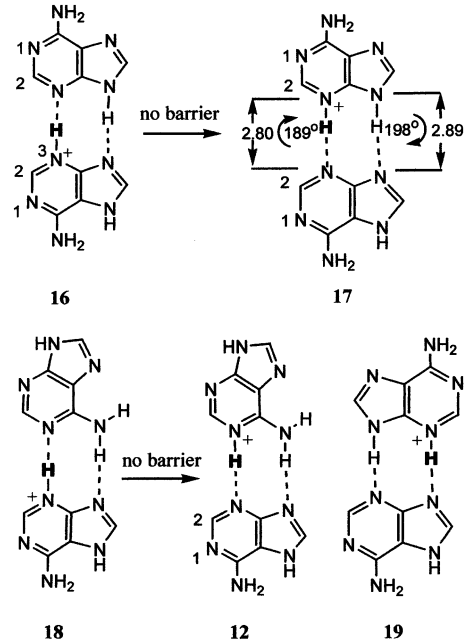
a counterpoise correction of 10.3 kJ mol^{-1}, the value in Table 2 compares well with the experimental $\Delta H°_{500}$ measurement. The calculated 0 K dissociation energy at B3LYP/6-31++G**, when corrected for BSSE with a counterpoise correction of 3.3 kJ mol^{-1}, is $\Delta H°_{\mathrm{Dissn}} = 110$ kJ mol^{-1}. The counterpoise correction to the B3LYP/6-31++G** dissociation. energy of **13** has the same value, which leads to a 0 K dissociation enthalpy of $\Delta H°_{\mathrm{Dissn}} = 108$ kJ mol^{-1} at that level. The inclusion of diffuse functions in the basis set does not improve agreement with experiment.

The geometrical comparison of **12** with **13** in Figure 4 gives an idea of the structural features that make for a favorable interaction in proton bound dimers having one hydrogen bond plus a proton bridge. Either N−H···N interaction can play the role of proton bridge, depending on which resonance structure is considered. Both interactions in the looser dimer, **13**, have nearly the same N−N distance and N−H···N angle. The tighter dimer, **12**, shortens one and brings it close to 180°, at the expense of lengthening the other and distorting it further from linearity. The geometrical features summarized for the dimer of the 7H tautomer with protonated adenine, **17** (discussed below), manifest the same effect.

As noted above, protonation of the 7H tautomer of adenine on N3 gives a $C_5H_6N^+$ cation, $\mathbf{5H}^+$, that is nearly as stable as protonated adenine. This result raises the question of the stability of proton-bound dimers containing that ion. Two orientations give a hydrogen bond plus a proton bridge, **16** and **18**. The angle at which the lone pair protrudes from N7 disfavors these dimers. Both represent labile geometries: **16** is unstable with respect to barrier-free transposition of a hydrogen (represented in boldface) to N3 of its partner, giving ion **17**, which is almost as stable as the most favorable proton-bound dimer of N1-protonated adenine; **18** transposes hydrogen without an intervening barrier to give **12**, as depicted in Scheme 2.

The neutral partner in **17** can be flipped 180° to give another stable proton-bound dimer, **19**, also drawn in Scheme 2, which is only slightly less stable. As Table 2 summarizes, the four

SCHEME 2: Adenine Homodimers Containining N3-Protonated Adenine or Its 7H Tautomer[a]

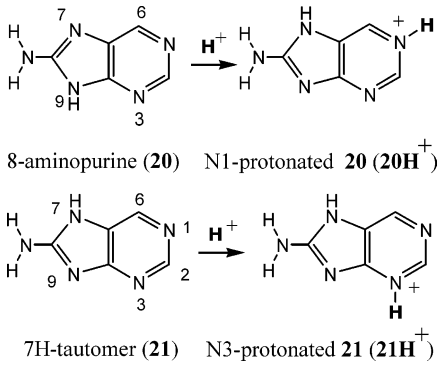


[a] Distances in Å.

most favorable $(C_5N_5H_5)_2H^+$ proton-bound dimers from adenine all contain the 7H tautomer as a neutral partner and have 0 K dissociation enthalpies within 4 kJ mol^{-1} of one another. At equilibrium, all should be present in substantial proportions at 500 K.

Counterpoise corrections can be incorporated into calculations of 0 K $\Delta H°_{\mathrm{Dissn}}$ values in two mathematically equivalent ways. Hobza and Šponer[15] advocate separating out the deformation energy, ΔE^{DEF}, which is the difference in electronic energy between the monomers in their geometries within the dimer and in their optimized geometries. As the last column illustrates, the deformation energy for the N1-protonated species in Table

SCHEME 3: 8-Aminopurine, Its 7H Tautomer, and Their Most Stable Conjugate Acids

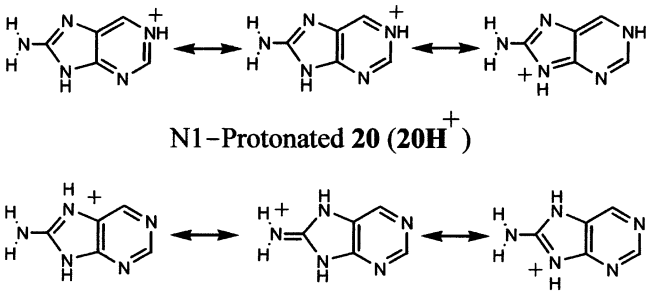


2 tends to get bigger as ΔE^{B3LYP} becomes more endothermic. The structures that deviate from this correlation are those that have an NH_2 group twisted out of the plane of the ring (the Watson−Crick, Reverse Watson−Crick, and Hoogsteen orientations **9**−**11**). The other dimer ions are nearly planar, with the exception of the Trans Hoogsteen/Hoogsteen motif (**3**), which has a propeller twist of 48°. A plot of ΔE^{DEF} versus ΔE^{B3LYP} for the N1- and N3-protonated conjugate acids that excludes **9**−**11** still shows considerable scatter and a poor linear correlation coefficient ($r^2 = 0.92$), but ΔE^{DEF} nevertheless exhibits a virtually monotone increase with ΔE^{B3LYP}.

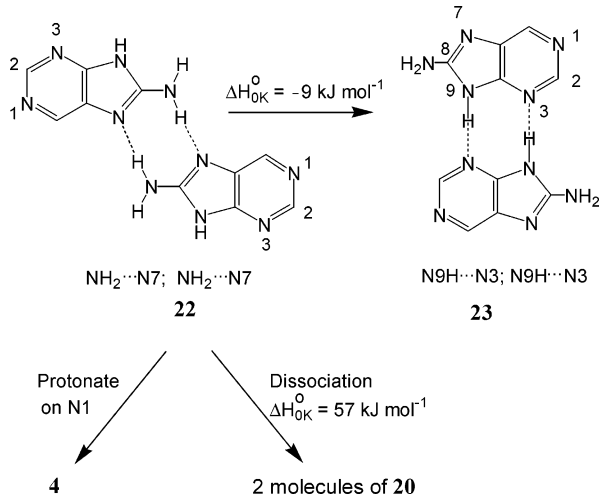
Converting the calculated 0 K enthalpies of dissociation to 500 K values (for comparison with experiment) introduces uncertainties because of errors from assuming harmonic vibrations. The calculated 500 K canonical energy of the most stable adenine dimer ion, **12**, turns out to be 8 kJ mol^{-1} greater than the sum of the canonical energies of adenine plus N1-protonated adenine. Taking into account the ΔPV term, the DFT value for comparison with experiment becomes $\Delta H°_{500} = 115.5$ kJ mol^{-1}. That result is consistent with previous reports that DFT methods tend to underestimate dimer dissociation energies by roughly 10 kJ mol^{-1}.[16] For purposes of comparing dimer structures, the degree of accuracy provided by B3LYP/6-31G** geometry optimizations can be considered adequate.

8-Aminopurine. Adenine and 8-aminopurine (**20**) both have the formula $C_5N_5H_5$. Adenine is an oligomer of HCN and can be produced in low but measurable yield simply by heating concentrated solutions of ammonium cyanide.[17] Under similar conditions, 8-aminopurine forms as a side product.[18] Hence, both $C_5N_5H_5$ isomers should have been present under prebiotic conditions, although natural selection has militated against including 8-aminopurine in nucleic acids. As in the case of adenine, 8-aminopurine has both 9H (**20**) and 7H (**21**) tautomers, drawn in Scheme 3. Like adenine, the 7H tautomer is calculated to be less stable. The different levels of computation in Table 1 give a calculated difference in 0 K heats of formation of 12.3 ± 0.3 kJ mol^{-1}.

SCHEME 4: Resonance Structures for N1-Protonated and N7-Protonated 8-Aminopurine



SCHEME 5: Neutral Homodimers of 8-Aminopurine Having Twofold Symmetry



N1 is the most basic site in adenine.[13] Enumeration of the resonance structures would suggest that N7 ought to be the most basic site in 8-aminopurine (**20**), as Scheme 4 summarizes. If every atom is constrained to have a complete octet, the N7 conjugate acid ion enjoys a half dozen resonance structures (the three shown in Scheme 4 plus three other Kekulé structures for the six-membered ring), while the N1 isomer has only three resonance structures. Surprisingly, computation predicts that N1 is the most basic site in 8-aminopurine, as Table 1 summarizes. CCSD/6-31G** reoptimizations confirm the ordering of basicities predicted by DFT.

As can be adduced from Table 1, the 0 K proton affinity of **20** is calculated to be 13.2 ± 6.5 kJ mol^{-1} greater than that of adenine, depending on the computational level. The 7H tautomer, **21**, has a calculated proton affinity 11.5 ± 2.5 kJ mol^{-1} greater than that of **20**. The basicity of **21** suggests that the 7H tautomer may serve as a better hydrogen bond acceptor than **20**. Despite the gap between stabilities of the tautomers of neutral 8-aminopurine, there is little or no preference for homodimers containing only **20** relative to mixed dimers that contain **20** and **21**.

Neutral Dimers of 8-Aminopurine. In terms of self-recognition, neutral 8-aminopurine enjoys two homodimer orientations having C_2 symmetry, **22** and **23**, as Scheme 5 portrays. The 7H tautomer, **21**, can also form a C_2 homodimer with the same sort of bonding as **22**, which lies 6.3 kJ mol^{-1} higher in energy. The corresponding mixed homodimer between **20** and **21** lies 3.6 kJ mol^{-1} higher in energy than **22**. As in the case of the neutral dimer of adenine,[11] the orientation with two N9H···N3 hydrogen bonds, **23**, is calculated to form the most stable neutral dimer. The 7H tautomer, **21**, cannot form a dimer with the same sort of bonding as **23**, nor can a mixed dimer of **20** plus **21**.

In spite of the fact that **21** is much less stable than **20**, **20**, and **21** can form a mixed dimer, **24**, which has a 0 K heat of formation only 1.3 kJ mol^{-1} higher than that of **23**. While the two purine rings in **24** are virtually coplanar, the length of the NH···N hydrogen bond between the two 5-membered rings, as Figure 5 portrays, permits only one other hydrogen bond to form. Dimer **24** leads to the most stable proton-bound dimer when protonated.

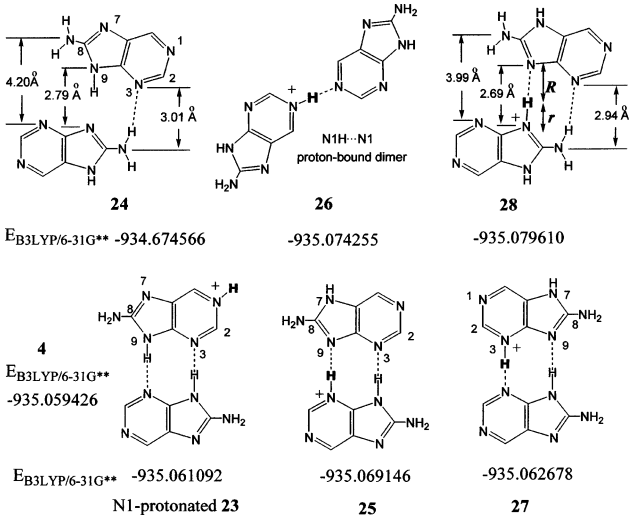


Figure 5. The most stable proton-bound dimer of 8-aminopurine (**28**) compared with its neutral conjugate base (**24**) and other cationic dimers containing **20** and **21**.

Positively Charged Dimers of 8-Aminopurine. The N1-protonated conjugate acid of **23** is a protonated dimer (just as **3** is a protonated dimer of **1**), as drawin in Figure 5. By contrast, protonating **23** on N7 gives the proton-bound dimer **25**, which is substantially more stable. The initial placement of H^+ on N7 produces an unstable protonated dimer ion, which shifts a proton from N9 of the charged partner to N3 of the neutral, leading to **25**, which consists of N3-protonated **20** bound to **21**.

Protonation on N1 of the neutral Trans Hoogsteen/Hoogsteen dimer **22** gives protonated dimer **4**, which is even less stable than N1-protonated **23**. The N7 positions of **22** are not accessible, since their lone pairs are involved in the hydrogen bonds. Protonating N3 of **22** gives a conjugate acid that has an electronic energy 48 kJ mol^{-1} higher than that of **4**. None of the conjugate acid ions formed from the neutral C_2 dimers are as stable as **26**, a proton-bound dimer that has a proton bridge and no other hydrogen bonds.

Proton bound dimers **27** and **28** contain N3-protonated **21**. Both have a hydrogen bond plus a proton bridge, but **28** is the only dimer that is more stable than **26**. Ion **28**, which has the lowest heat of formation of all the charged dimers of 8-aminopurine, corresponds to **24** with a proton placed on the free N7. Ion **28** can be viewed as two molecules of **21** cemented together by a bridging proton, which can move back and forth between two nitrogens. Figure 5 summarizes the atom−atom distances that change most dramatically when **24** is protonated to give **28**.

Transit of the bridging H^+ from N9 of the charged partner to N9 of the neutral partner renders two molecules of **21** equivalent within **28**. From the vantage point of the bridging proton, this transit looks like an asymmetric stretch, at whose midpoint (which has C_2 symmetry) the central N−N distance shortens to 2.54 Å, while both of the flanking N−N distances become 3.27 Å. The B3LYP/6-31G** barrier to this transit is only 0.002285 a.u. (6.0 kJ mol^{-1}), as Figure 6 summarizes, suggesting that the proton bridge of **28** behaves as a low-barrier hydrogen bond.

Such a low barrier means that the harmonic normal-mode frequency calculated for that N−H stretch, $\nu = 2534$ cm^{-1}, may greatly overestimate its zero-point energy. Because a pronounced geometrical reorganization accompanies transit of H^+, whereby the long $NH_2\cdots$N3 distance and the short $NH_2\cdots$N3 distance change places within the plane of the dimer (a motion for which a harmonic normal modes calculation gives a frequency $\nu =$ 47 cm^{-1}), coupling between those motions makes it difficult to determine the correct spacing for the NH···N vibration.

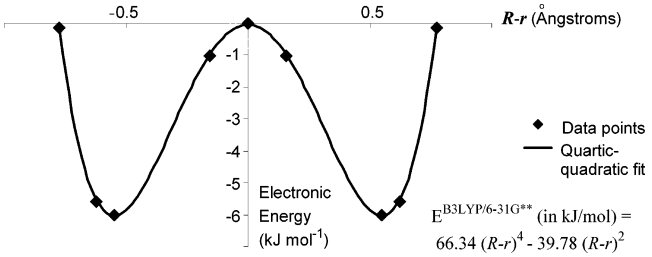


Figure 6. Electronic energy (B3LYP/6-31G**) as a function of the difference in N−H distances $R - r$ for the most stable proton-bound homodimer of 8-aminopurine, **28**.

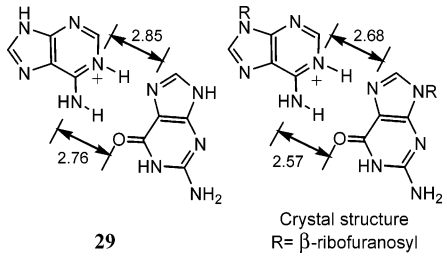


Figure 7. Comparison of the calculated gas-phase structure **29** with the X-ray structure[19] (distances in Å) for the Cis Watson−Crick/Hoogsteen dimer of N1-protonated adenine with guanine.

The 0 K dissociation enthalpy of **28** to **21H**$^+$ plus **20**, $\Delta H°$ = 130 kJ mol^{-1} (based on unscaled harmonic zero point energies), is not far from that of the proton-bound dimers of adenine with 7H−adenine (**12** or **13**). Those values may be compared with the binding of the Watson−Crick face of N1-protonated adenine to the Hoogsteen face of guanine in the *cis* orientation, drawn in Figure 7. That motif has been observed in double stranded RNA oligomers,[19] and its calculated 0 K dissociation enthalpy is $\Delta H° = 160$ kJ mol^{-1}.

Figure 7 compares the calculated (gas phase) geometry with that observed in the crystal structure. The two glucosidic carbons (the sp^3 carbons attached at the positions designated by R) in the X-ray structure are separated by 10.5 Å.[19] That separation is known as the C1′−C1′ distance. If sp^3 carbons were attached to the corresponding nitrogens of structure **29**, the C1′−C1′ distance would be 11.0 Å. These C1′−C1′ distances are sufficiently close to one another to justify making steric comparisons between DFT geometries and X-ray structures.

Discussion

In the gas phase, the conjugate acid dimer ions of nucleobases can be divided into two general classes: protonated dimers and proton-bound dimers. The former category comprises ions in which a proton affixes itself to a stable, hydrogen-bonded neutral dimer and does not produce a proton bridge. This class comprises the examples portrayed in Figure 1. The latter category embraces species in which appending an H^+ leads to a proton bridge. While the dichotomy between protonated dimers and proton-bound dimers is not always unambiguous, it is clear-cut in the examples studied here. In the present survey proton-bound dimers exhibit substantially greater dissociation enthalpies than do protonated dimers.

Protonating N3 of the monomeric 7H tautomers of $C_5N_5H_5$ gives ions (**5H**$^+$ and **21H**$^+$) that are nearly as stable as the N1-protonated 9H tautomers, as Table 1 summarizes. The electronic structures of 7H and 9H tautomers of adenine and guanine have

been the subject of recent theoretical scrutiny.[20] N1 is the most basic site in adenine,[13] and the present calculations show that protonation of 8-aminopurine on N1 (to give **20H**$^+$) also gives the most stable cation. The N3 positions of the 7H tautomers are substantially more basic. With regard to nucleoside chemistry, 7-(ribofuranosyl)purines should have greater proton affinities than the more conventional 9-(ribofuranosyl)purines, an effect that may be relevant to reported differences in their chemical ionization mass spectra.[21]

The greater basicity of the 7H tautomers has the consequence that the most stable dimeric cations from adenine—**7**, **13**, **17**, and **19**—contain the 7H tautomer **5**, as the neutral partner. The same holds true for 8-aminopurine: the most stable dimer ion, **28**, contains the 7H tautomer **21**. Despite the thermodynamic preference for 9H tautomers relative to 7H tautomers in neutral $C_5N_5H_5$, the present study finds that 7H tautomers represent more favorable hydrogen bonding acceptors in forming intramolecular proton bridges.

Under equilibrium conditions in the gas phase (such as described in ref 6), structures that prevail inside dimers rapidly interconvert with the most stable structures of the monomers. Thus, the fact that 7H tautomers are preferred within $(C_5N_5H_5)_2H^+$ does not require that the proton-bound dimer ions dissociate to give neutral 7H tautomers. Even a rigorously unimolecular dissociation can yield the most stable neutral if it passes through an ion-neutral complex in which proton exchange takes place between the partners.

The protonated dimers of 8-aminopurine show a comparatively small increase in binding relative to their neutral analogues, as distinct from the behavior of the proton-bound dimer ions. On the one hand, dissociation of neutral dimer **22** has a 0 K enthalpy of dissociation $\Delta H° = 57$ kJ mol^{-1}, while the value for its protonated analogue **4** is $\Delta H° = 61$ kJ mol^{-1}. On the other hand, dissociating the isomeric neutral dimer **24** to **20** plus **21** gives a 0 K dissociation enthalpy of $\Delta H° = 75$ kJ mol^{-1}, while dissociating its conjugate acid **28** to **21H**$^+$ plus **20** gives, as noted above, $\Delta H° = 130$ kJ mol^{-1}.

Protonated dimers are labile when they can convert to proton-bound structures by shifting the position of a hydrogen, as happens for the conjugate acid of the adenine dimer **6**, which converts to **13** upon protonation. Neutral dimer **24** from 8-aminopurine has two hydrogen bonds. Upon protonation, one of them becomes a proton bridge in **28**. Moving the bridging H$^+$ back and forth within this cationic dimer interconverts the roles of the two heterocyclic partners.

When a proton holds a pair of identical heterocycles together, placing H$^+$ equidistant between two nitrogens represents a local potential energy maximum. The stretching motion of the bridging proton is more accurately modeled as a double-well than as a harmonic potential. It is tempting to suppose that the normalized difference between the two N−H distances, $(R - r)/\sqrt{2}$, may serve as a reasonable approximation for the normal coordinate describing H$^+$ transit for the bridging hydrogen in **28**. If so, the net zero point energy change for dissociation of **28** to monomers (for which the DFT harmonic value for ΔZPE is close to zero) should become positive if the anharmonicity is taken into account.

Implications Regarding Possible Nucleic Acid Structures. Over the past few years there has been growing interest in the incorporation of nonstandard nucleotides within oligonucleotide duplexes, not only with respect to their biological implications, but also in regard to potential applications in nanotechnology.[22] The results presented here regarding the stability of proton-bound purine dimers and low barrier hydrogen bonds provides support for an experimental effort to use such bonds to create new, highly selective base pair interactions.

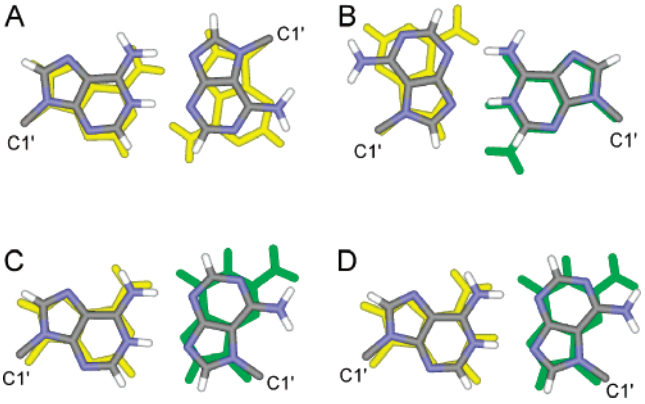


Figure 8. Steric comparisons of B3LYP/6-31G** geometries for proton-bound dimers of adenine superimposed upon known purine−purine pairings[1c] (underneath): (A) **12** superimposed over the Trans Watson−Crick/Hoogsteen A·A N7-amino base pair; (B) **13** superimposed over the G·A N7−N1 amino-carbonyl base pair; (C) **13** superimposed over the Cis Watson−Crick/Hoogsteen base pair of N1-protonated adenine with guanine (from the X-ray structure[19]); and (D) **13** superimposed over the B3LYP/6-31G** optimized geometry of **29**. For the known purine−purine pairs, A bases are shown in yellow and G bases in green.

A combined analysis of the relative stability and base pairing geometries for the purine−purine base pairs investigated here suggest two pairings of adenine that are especially well suited for incorporation into nucleic acid structures and could provide a means to stabilize particular strand geometries selectively as a function of pH. These two base pairs are structures **12** and **13**, proton-bound dimers of the neutral 7H tautomer of adenine with N1-protonated adenine. In both structures the 7H tautomer of adenine can be attached to a sugar without interfering with base pairing. The same is true for N1-protonated adenine. Thus, the analogous base pair structures ought to be possible within nucleic acids.

While the N7−β-ribofuranoside of adenine has not been investigated experimentally within oligonucleotides, the nucleoside has been synthesized and characterized by CD and NMR spectroscopy,[23] as well as by mass spectrometry.[21] As would be expected by the placement of the exocyclic amino group with respect to the glycoside bond, the glycosidic bond torsional angle (usually abbreviated as χ) of this N7−nucleoside is rigidly fixed in the *anti* conformation compared to adenosine.[23]

In exploring the possible existence of base pairs in RNA crystal structures that would be isosteric with structures **12** and **13**, the following aspects must be considered: distance between the C1′ atoms of the two ribose sugars in the base pairs, the orientations (i.e., in-plane angles) of the glycosidic bonds of the two paired nucleosides, and the glycosidic bond torsional angles (i.e., *anti* versus *syn*) for the nucleoside, which would correspond to the N7-ribofuranoside of adenine. Comparing these geometrical features with bone fide nucleic acid base pairs suggests promising isosteric base pairs for both structures **12** and **13**.

Figure 8A shows structure **12** (bases modeled as nucleosides) superimposed upon the uncharged Trans Watson−Crick/Hoogsteen A·A N7−amino base pair, a well-documented motif, which has been observed in tRNA, the ribosome, and a synthetic duplex with tandem mismatched bases.[24] These two structures are sufficiently close as to suggest that the bases of structure **12** could be accommodated within nucleic acid duplexes that contain an A·A N7−amino base pair. The C1′−C1′ distance

for nucleosides that would form structure **12** is calculated to be 12.3 Å, compared to 12.2 Å for the A·A N7−amino base pair. The angles between the C1′−N7/C1′−N9 glycosidic bonds in the plane of the base pair for these two structures are also similar, with deviations between the two bases of structure **12** and those of an A·A N7-amino base pair being 7° and 13°.

For structure **13** a close match was found with the uncharged G·A N7−N1, amino-carbonyl base pair (Figure 8B). The C1′−C1′ for structure **13** is calculated to be 11.4 Å, which is comparable to the 11.1 Å C1′−C1′ distance measured for the G·A N7−N1, amino-carbonyl base pair. The C1′−N7/C1′−N9 bond angles in the plane of the base pair are also very close to those of the G·A N7−N1, amino-carbonyl base pair, with only one base in structure **13** being rotated by 7° from the corresponding base of the G·A base pair.

The glycosidic bond torsional angles are within the *anti* conformation for all four of the nucleosides in the known uncharged base pairs that are compared with structures **12** and **13** above. However, the orientations of the oligonucleotide strands in which these nucleosides are found to differ between the two known structures. The A·A N7-amino base pair is found within an antiparallel strand orientation (analogous to the Watson−Crick double helix). By contrast, the G·A N7−N1 amino−carbonyl base pair occurs in a parallel strand orientation. Thus, it is expected that structure **12** can be accommodated into antiparallel structures, such as simple duplexes,[24] while structure **13** may be restricted to structures associated with less regular nucleic acid folds.

The incorporation of either structure **12** or **13** will, of course require protonation at N1 of the adenosine nucleoside. At low pH, free N7−(β-ribofuranosyl)adenine is somewhat labile, hydrolyzing nearly 30 times faster than free adenosine.[21] Incorporating it into a nucleic acid may provide considerable stabilization if it forms a proton bridge with an N1-protonated adenosine residue.

In addition to the two uncharged base pairs discussed above, a charged base pair, **29**, represents another isostere for **13**. Both dimers possess proton bridges formed from the Watson−Crick face of N1-protonated adenine. Substituting **13** for **29** replaces guanine with the N7-β-ribofuranoside of adenine. Parts C and D of Figure 8 portray their degree of similarity. To date, the principal example of RNA that contains **29** orients the guanine *syn*.[19] Nevertheless, preparation of oligomeric nucleic acids in which **12** or **13** might form warrants exploration.

This discussion has limited itself to the possible incorporation of proton-bound dimers involving the nucleobase adenine. As noted above, base pairs might also form, which possess low barrier hydrogen bonds, such as **28**. The potential well for N−H stretching vibrations in proton-bound dimers like **12** or **13** must deviate substantially from a harmonic potential, but the asymmetry of that well probably does not affect the zero point level dramatically. The double well potential illustrated in Figure 6, on the other hand, has a sufficiently low central barrier that its zero point level should lie much lower than a normal modes analysis would predict.

Lowering of the zero point level for proton transit can markedly increase the stability of a base pair. The example of **28**, which contains two 8-aminopurines, suffers from the fact that large geometry changes accompany proton transit. Nor do base pairs incorporating two 8-aminopurines present obvious isosteric analogues to conventional nucleobase pairs. Low barrier hydrogen bound pairings are possible, though, which exhibit much smaller motions of the heavy atoms during proton transit. These promise to provide a very selective mode of base−base recognition. Exploration of this possibility is currently underway in our laboratories.

Conclusions

Adenine is known to be more stable than its 7H tautomer (**5**), but the conjugate acid ion of the 7H tautomer **5H**$^+$ has a heat of formation that lies within 5 kJ mol^{-1} of protonated adenine. Likewise, the 9H tautomer of 8-aminopurine (**20**) is more stable than the 7H tautomer (**21**), but their conjugate acids ions have virtually the same heats of formation. The most basic site in adenine and 8-aminopurine is the nitrogen at position 1, while the most basic site in their 7H tautomers is the nitrogen at position 3.

The 7H tautomers of purine bases have never been found in natural nucleic acids. Because the unnatural 7H tautomer of adenine is more basic than adenine itself, proton-bound dimers of the free nucleobase that contain the 7H tautomer (e.g., **12**) as the neutral partner turn out to have the lowest calculated heats of formation. The same holds true for 8-aminopurine.

Charged pairs that can be described as proton-bound dimers have greater stability than their isomeric protonated dimers. Dimer ions in which proton transit renders the two bases equivalent exhibit low barriers for transferring H^+ from one nitrogen to the other. This has recently been reported for the proton-bound dimer of cytosine,[25] for which the reported barrier height corresponds to an electronic energy difference of 16−19 kJ mol^{-1}. In the present study, the most stable proton-bound dimer of 8-aminopurine, **28**, has an even lower barrier, with an electronic energy difference of only 6 kJ mol^{-1}. A study of the vibrational spectroscopy of such species would provide insight into the nature of low barrier hydrogen bonds.

Some $(C_5N_5H_5)_2H^+$ proton-bound dimers are isosteric with purine−purine mismatches that have been observed in RNA. Of the four most stable proton-bound dimer structures predicted for free adenine, at least two resemble base pairings seen in nucleic acid duplexes. The systems described herein are amenable to rational synthesis for experimental studies. Detailed investigations of functionalized 8-aminopurines are in progress.

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Supporting Information Available: Electronic energies and Cartesian coordinates for dimers. This material is available free of charge via the Internet at http://pubs.acs.org.

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