the olefinic terminus which forms the strongest bond with the radical.

Regioselectivity is the net result of these two effects. When they join up regioselectivity will be large. When they oppose one another, regioselectivity will be smaller, and regioselectivity crossovers are expected. In most cases known to us, the spin-density effect wins over. The relative spin densities are found to parallel the relative HOMO coefficients of the olefin. Therefore the predictions of the spin-density effect an the HOMO-rule[6] will normally be identical. The ΔE_{dis} quantity is also usually a reliable regioselectivity index, and the smaller ΔE_{dis} usually coincides with the spin-rich site.

The role of "steric effects" cannot be ruled out. The way we define "steric effect" is that this is the specific contribution to the exchange repulsion between the electrons of the substituent and those of the radical. With this definition, "steric effect" is part of the ΔE_{rep} term (eq 2). As may be seen, ΔE_{rep} is usually, though not always, larger for the attack on the more substituted site.

Thus, in many cases all the regioselectivity indexes will coincide and reflect the total destabilization, ΔE_c, which the reactants have to undergo in order to achieve resonance with the excited states, Ψ^*_{RP}, in the diagram in Figure 1. It is then the integrated index ΔE_c ($\Delta E_c = \Delta E_{dis} + \Delta E_{rep}$) which will make the more successful predictions and is, itself, predictable by the balance of the spin density and bond strength effects.

Registry No. CH_2=CHX (X = Ph), 100-42-5; H_2C=CHF, 75-02-5; H_2C=CF_2, 75-38-7; FHC=CF_2, 359-11-5; H_2C=CHCl, 75-01-4; ClHC=CF_2, 359-10-4; H_2C=$CHCH_3$, 115-07-1; H_2C=$CHCF_3$, 677-21-4; H_2C=CHCN, 107-13-1; H_2C=CHCH=CH_2, 106-99-0; H_2C=$CHNH_2$, 593-67-9.

The Role of Intramolecular Hydrogen Bonding as a Determinant of the Conformational Profiles of cGMP and cAMP

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Abstract: Computational chemical studies using the AM1 semiempirical method and Hartree–Fock calculations with the STO-3G basis set using the AM1 structures have been performed on the cyclic nucleotides adenosine 3′,5′-cyclic monophosphate (cAMP), guanosine 3′,5′-cyclic monophosphate (cGMP), and model compounds. Consistent with earlier experimental and computational studies, cGMP is expected to prefer the syn conformation of the purine/sugar portions, while cAMP prefers the anti conformation. The present studies implicate an intramolecular hydrogen bond, between a hydrogen atom of the C2-amine of the purine and the axial oxygen atom of the cyclic phosphate, in increasing the relative stability of the syn conformation of cGMP. Analysis of the energetics and molecular electrostatic potentials of model compounds and model complexes reveals that the substituent at the C6 carbon atom and the phosphate ring conformation play critical roles in stabilizing this interaction. These conformational profiles along with tautomeric characteristics may help develop hypotheses to explain selectivity for binding to and activation of proteins by the cyclic nucleotides.

The cyclic nucleotides guanosine 3′,5′-cyclic monophosphate (cGMP) and adenosine 3′.5′-cyclic monophosphate (cAMP) (Figures 1 and 2) play central roles in many biochemical processes. Both serve as second messengers in diverse receptor mediated events. Insights into the mechanisms of interaction of these cyclic nucleotides as ligands for cGMP or cAMP dependent protein kinases (PKs) or as substrates for phosphodiesterases (PDEs) can be obtained through various routes. For instance, with use of the crystal structure of catabolite gene activator protein[1] (CAP) bound with cAMP together with sequence homologies between CAP and cAMP dependent protein kinase C (PKc), Weber et al.[2] have recently proposed a model for the structure of the cAMP binding site of PKc. In a similar fashion, modeling studies together with protein sequence and structural data have been synthesized to generate models for cGMP and cAMP dependent PDE.[3] Alternatively, Wells et al.[4] have developed a model for cGMP versus cAMP dependent PDEs based on an analysis of structure activity relationships of ligands for these enzymes. More recently, Erhardt and co-workers used structure activity relationships to develop a model for the cardiac cAMP PDE catalytic site.[5] Computational studies of the physicochemical properties of these isolated species have also been performed.[6,7] These physicochemical studies can be used to develop mechanistic models either independently or in conjunction with models developed through other means as described above. The present study represents the first of our computational studies on the physical chemical properties of cGMP and cAMP related compounds. In a separate paper,[8] we report the tautomeric properties of adenine and guanine. We focus herein on the conformational properties of cGMP and cAMP, and related models as these are salient features of existing models for their activity (e.g., ref 2 and 3).

Methods

The structures of the parent compounds cGMP, cAMP, and various analogues (defined herein) were fully optimized (unless otherwise indicated) by the use of the semiempirical AM1 method[9] as implemented in

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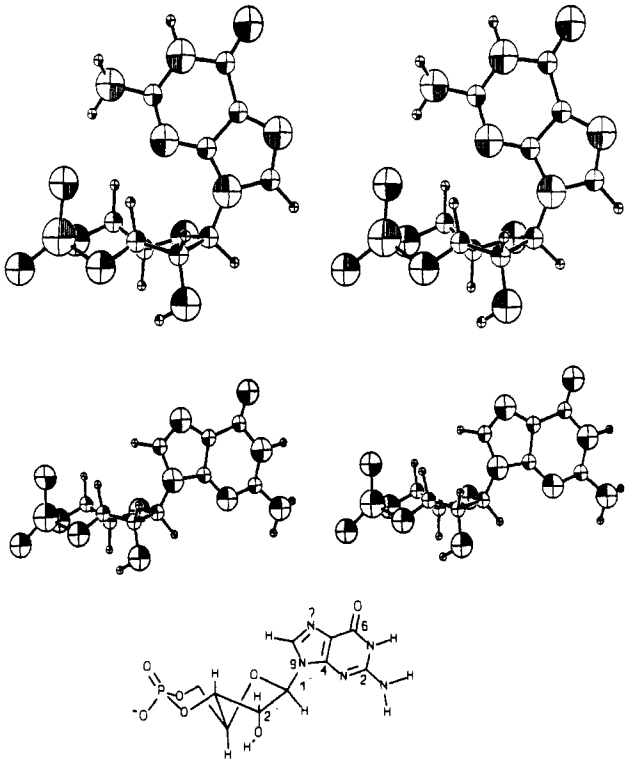

Figure 1. Stereodrawing of the AM1 optimized chair forms of the syn and anti conformations of cGMP.

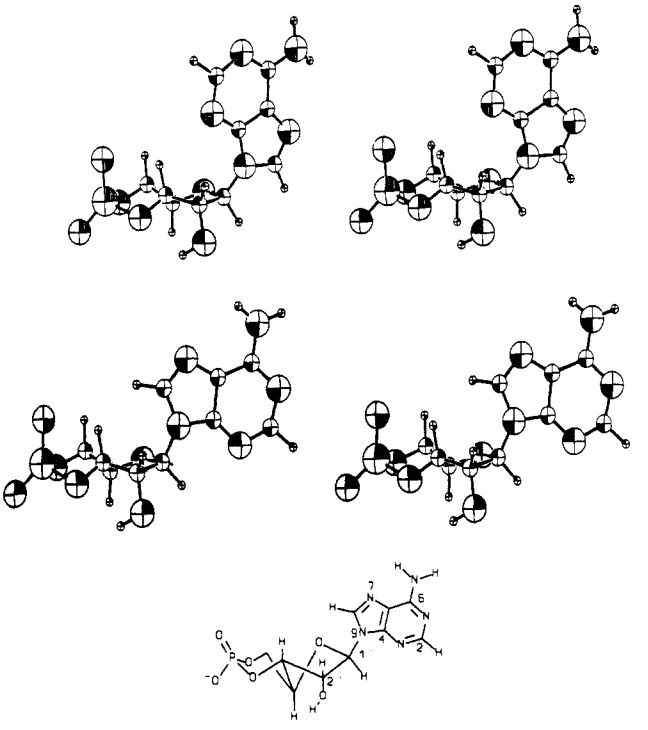

Figure 2. Stereodrawing of the AM1 optimized chair forms of the syn and anti conformation of cAMP.

Version 4.00 of MOPAC.[10] In some cases, as described, partial reoptimization was done. Energies of these structures were evaluated at the AM1 level (AM1//AM1) and Hartree–Fock level by using the STO-3G basis set[11] (STO-3G//AM1). Molecular electrostatic potential (MEP) maps of the base portions of cGMP and several of the various analogues studied herein were produced directly from the ab initio Hartree–Fock wave functions with the 3-21G basis set[12] obtained by using the GAUSSIAN 82 system of programs.[13] The MEPs were evaluated and displayed by using the CHEM-X system of programs.[14] The cGMP structures and energies were also determined by using the semiempirical PM3 method[15] as implemented in Version 5.00 of MOPAC.[16]

Results

I. Riboside Bond Conformational Minima. The greatest structural flexibility in cGMP and cAMP is associated with the riboside bond, i.e., the rotational angle $C_2'C_1'N_9C_4$ (see Figures 1 and 2). Full (360°) conformational scans have been done for both cGMP and cAMP at the semiempirical quantum chemical AM1 level including total optimization of all other structural parameters.[17] In addition, detailed semiempirical and ab initio analyses have been performed to establish that in the gas phase the purine ring tautomers depicted in Figures 1 and 2 are the lowest in energy.[8] While other tautomeric states may be significantly populated in the gas phase, those used here are nevertheless accepted as the relevant tautomers at physiological pH.

For the present report, we merely point out that both cGMP and cAMP are found to have two conformational minima (syn and anti). These energetic and structural results are summarized in Table I and depicted in Figures 1 and 2. Others have previously reported similar conformational studies by using molecular mechanics methods[6,7] in which the analogous local minima were obtained. Davis has recently summarized[6] the crystal structures of cGMP, cAMP, and other purine/ribose 3′,5′-cyclic phosphates. Consistent with the previous computational studies[6,7] and with its X-ray structure,[18-20] we find that the isolated cGMP has a lower energy in the syn conformation (Table I). Thus, at the AM1//AM1 level, while cAMP prefers the anti conformation by 3.61 kcal/mol, cGMP prefers the syn conformation by 1.36 kcal/mol, i.e., a difference of ca. 5 kcal/mol. This difference is even greater at the STO-3G//AM1 level, i.e., ca. 7 kcal/mol. Such a change in conformational preference would not easily be anticipated based on the interchange of the guanine with the adenine ring. Examining these structures (see Figures 1 and 2), we postulate that the source of this difference in conformational preference is the existence of an intramolecular hydrogen bond in the syn conformation of cGMP between the hydrogen atom of the guanine amine group and the axial phosphate oxygen atom. Surprisingly, a stabilization between the C2-amine group and the phosphate group has only briefly been alluded to in early theoretical studies.[7] The short distance between these two atoms (2.20 Å) clearly suggests the potential for hydrogen bonding. Because of the significance that this conformational feature may have in the various biochemical processes in which cGMP and cAMP are involved, we now examine this in greater detail.

II. Phosphate Ring Conformation. The phosphate ring of cGMP and cAMP can assume two conformations. We find the lower energy conformation is the chair conformation (Figures 1 and 2). This is consistent with X-ray structures for these and related compounds found in the Cambridge Crystal Data Base (see, e.g., ref 6 and 21). However, both cGMP and cAMP also

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Table I. The Geometry and Relative Stabilities of Syn and Anti Conformations of cGMP, cAMP, and Several of Their Derivatives

molecule synonym		complete AM1 geometry optimization[a] AM1//AM1 (kcal/mol)	STO-3G//AM1 (kcal/mol)	hydrogen bond distance (Å)	base–sugar dihedral angles (deg) $C_4N_9C_1'C_2'$	$N_9C_1'C_2'C_3'$
cGMP	syn			2.204	−62.28	98.56
(chair)	anti	−1.358	−4.310		132.34	105.61
cGMP	syn			5.713	−56.88	111.90
(twist-boat)	anti	+0.393	+0.074		133.39	106.05
6-CH_2 cGMP[b]	syn			2.245	−60.79	99.18
				(3.242)	(−59.15)	(107.68)
	anti	−0.206	−2.525		127.16	104.93
		(+0.203)	(−0.792)			
6-CN cGMP[c]	syn			3.689	−50.57	109.65
				(2.324)	(−63.20)	(98.82)
	anti	+0.865	[+0.151][d]		135.83	104.40
		(+1.460)	−0.972			
6-H cGMP	syn			3.845	−49.31	110.20
				(2.447)	(−56.01)	(101.54)
				{3.713}	{−59.10}	{111.05}
	anti	+1.434	+0.851		135.68	104.47
		(+2.081)	(+0.891)			
		{+2.146}	{+1.663}			
2-H cGMP	syn			no NH_2 group at	−49.20	113.01
(cIMP)	anti	+1.478	+0.877	the 2 position	−124.41	104.68
6-CH_3 cGMP[e]	syn			3.862	−49.55	110.40
				(3.736)	(−58.90)	(111.35)
	anti	+1.578	+0.986		135.74	104.33
		(+2.322)	(+1.712)			
6-NH_2 cGMP	syn			3.859	−49.13	110.27
(2-NH_2 cAMP)				(2.392)	(−55.37)	(101.32)
(AMcAMP)				{3.722}	{−60.06}	{111.19}
	anti	+1.676	+1.303		131.28	103.81
		(+2.319)	(+1.349)			
		{+2.619}	{+2.216}			
cAMP	syn	*f*		no NH_2 group at	−45.08	113.78
(chair)				the 2 position	(−47.37)	(114.73)
	anti	+3.608	+2.333		122.94	102.36
		(+3.826)	(+2.803)			
cAMP	syn			no NH_2 group at	−46.81	114.47
(twist-boat)	anti	+2.861	+1.655	the 2 position	124.80	102.81

[a] With the exceptions of the cGMP and cAMP twist-boat forms, all geometry optimizations were performed with the CONVEX versions of MOPAC (v. 4.0) and GAUSSIAN86; the latter was used to determine the nature of the stationary points on the energy surface. The twist-boat forms of cGMP and cAMP were optimized with the VAX version of AMPAC 1.00. In addition to the lowest energy minima, higher energy local minima are reported in parentheses and brackets. The STO-3G//AM1 energies (in au and in the order of this table) of the structure in this table are cGMP (chair, syn) −1502.775 599 and (chair, anti) −1502.768 731; cGMP (twist-boat, syn) −1502.766 157 and (twist-boat, anti) −1502.766 274; 6-CH_2 cGMP (syn) −1467.470 132, −1467.466 107, and (anti) −1467.464 846; 6-CN cGMP (syn) −1519.484 143, −1519.485 933, and (anti) −1519.484 384; 6-H cGMP (syn) −1428.933 016, −1428.932 951, −1428.931 721, and (anti) −1428.934 371; 2-H cGMP (syn) −1448.440 399 and (anti) −1448.441 796; 6-CH_3 cGMP (syn) −1467.517 519, −1467.516 362, and (anti) −1467.519 090; 6-NH_2 cGMP (syn) −1483.259 797, −1483.259 724, −1483.258 343, and (anti) −1483.261 874; cAMP (chair, syn) −1428.930 246, −1428.929 497, and (anti) −1428.933 965; cAMP (twist-boat, syn) −1428.928 275 and (anti) −1428.930 911. The corresponding AM1//AM1 energies (in kcal/mol) are cGMP (chair, syn) −112 198.250 and (chair, anti) −112 196.892; cGMP (twist-boat, syn) −112 195.557 and (twist-boat, anti) −112 195.950; 6-CH_2 cGMP (syn) −108 377.627, 108 377.218, and (anti) −108 377.421; 6-CN cGMP (syn) −112 187.990, −112 187.395, and (anti) −112 188.855; 6-H cGMP (syn) −104 800.895, −104 800.248, −104 800.183, and (anti) −104 802.329; 2-H cGMP (syn) −107 105.736 and (anti) −107 107.214; 6-CH_3 cGMP (syn) −108 393.849, 108 393.105, and (anti) −108 395.427; 6-NH_2 cGMP (syn) −109 897.311, −109 896.668, 109 896.368, and (anti) −109 898.987; cAMP (chair, syn) −104 806.196, −104 805.978, and (anti) −104 809.804; cAMP (twist-boat, syn) −104 805.756 and (anti) −104 808.617. [b] This compound probably exists as 6-CH3 cGMP; compare the total molecular energies of 6-CH_2 cGMP and 6-CH3 cGMP. [c] For 6-CN cGMP, a saddle point was found (negative eigenvalue: −4.306) with an energy of −112 187.395 kcal/mol (i.e., the same as that of the syn minimum listed second) and with a geometry of 3.542 Å, −61.47°, 109.34° (i.e., similar to that of the syn minimum listed first). [d] Here, the conformation predicted to be the lower energy syn form at the AM1//AM1 level is not the lower energy syn structure at the STO-3G//AM1 level. [e] A hydrogen-bonding syn conformation of 6-CH_3 cGMP was not found. [f] The major difference in the geometries of the two cAMP syn conformations is in the orientation of the amino hydrogen atoms.

have a higher energy conformation, i.e., the twist-boat conformation (Figure 3). For example, full optimization of the structures of the ribose 3′,5′-cyclic phosphate above (i.e., without the adenine or guanine base portion) resulted in an energy difference of 1.03 kcal/mol between the chair and twist-boat conformations at the AM1//AM1 level.

To see what effect the phosphate ring conformation could have on the riboside bond conformation we have completely optimized the structures of cGMP and cAMP in the syn and anti conformations of the twist-boat structures of the phosphate ring (Figure 3 and Table I). In the anti conformation of cGMP, the changing of the phosphate ring from a chair to a twist-boat structure is not expected to have a significant effect as the base portion is far removed from the phosphate ring. We find that the twist-boat structure is 0.94 kcal/mol higher in energy than the chair conformer in the anti conformation of the riboside bond (Table I). Not surprisingly, this is strikingly close to the corresponding difference of 1.03 kcal/mol for ribose cyclic-3′,5′ phosphate. On the other hand, in the syn conformation of cGMP, the changing of the phosphate ring from a chair to a twist boat structure is accompanied by an increase in the base-to-sugar hydrogen bond length from 2.20 to 5.71 Å. This is due to rotation of the axial phosphate oxygen atom away from the guanine ring. As a result the twist-boat structure is 2.69 kcal/mol higher in energy at the

(21) *CAMBRIDGE Structural Database*; Cambridge Crystallographic Data Centre, University Chemical Laboratory: Lensfield Road, Cambridge CB2 1EW, England.

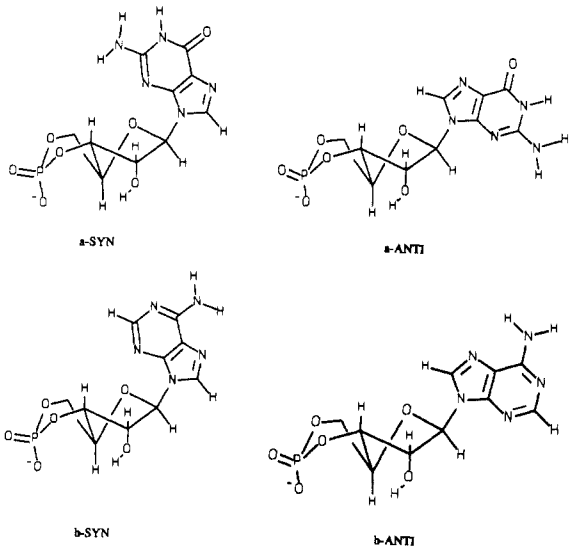

Figure 3. The twist-boat forms of the syn and anti conformations of (a) cGMP and (b) cAMP. See Figures 1 and 2 for labeling of the atoms.

AM1//AM1 level and 5.92 kcal/mol higher in energy at the STO-3G//AM1 level than the chair structure in the syn conformation as compared to 0.94 kcal/mol at the AM1//AM1 level and 1.54 kcal/mol at the STO-3G//AM1 level in the anti conformation.

For cAMP, on the other hand, the present analysis would suggest that the difference between the twist-boat and chair structures in the syn conformation is more nearly equal to the difference in the anti conformation. Indeed, we find that this energy difference for cAMP is 0.44 kcal/mol in the syn conformation and 1.19 kcal/mol in the anti conformation (Table I) at the AM1//AM1 level and 1.24 and 1.92 kcal/mol for the syn and anti conformations at the STO-3G//AM1 level, respectively. The slight lowering of the energy difference (for the twist-boat versus chair structure) in the syn conformation may be due to an increase in the energy of the chair structure of cAMP due to repulsion between the purine and phosphate groups which, for cAMP, do not benefit from the hydrogen bond described above. Nevertheless, for cAMP both energy differences are comparable to that of the isolated 3′,5′-cyclic monophosphate.

III. The C2-Amine Group. cGMP differs from cAMP by the presence of an amine in the 2 position of the purine ring and replacement of the amine group on C6 of the purine ring with an oxygen atom (i.e., conversion of C6 to a carbonyl group). To further explore the contribution of the C2-amine on the conformation of cGMP and cAMP we studied the model compounds 2,6-diaminopurine 3′,5′-cyclic monophosphate (aminocAMP; AMcAMP; see Figure 4a) and 6-oxopurine 3′,5′-cyclic monophosphate, the inosine analogue of cGMP in which the C2-amine group has been replaced by a hydrogen atom (cIMP; see Figure 4b). The relative energies of the fully optimized structures of these two compounds in the syn and anti conformational local minima are presented in Table I. As expected, elimination of the purported hydrogen bond by removal of the NH_2 group of cGMP (i.e., cIMP) results in changes of the relative preference of the anti over the syn conformation by 2.84 kcal/mol at the AM1//AM1 level and 5.19 kcal/mol at the STO-3G//AM1 level, so that the anti conformation is now more stable (by 1.48 kcal/mol at the AM1//AM1 level and 0.88 kcal/mol at the STO-3G//AM1 level). Analogously, introduction of a possible hydrogen bond by addition of an amine group at the 2 position of cAMP (AMcAMP) shifts the conformational preference by 1.93 kcal/mol toward the syn conformation relative to that of cAMP at the AM1//AM1 level and 1.03 kcal/mol at the STO-3G//AM1 level. Thus, qualitatively the results for both model compounds are in agreement in terms of the direction in which the amine shifts the conformational preference. We note, however, two differences which are seen through these model studies. First, introduction of the amine group into cAMP does not result in as great a

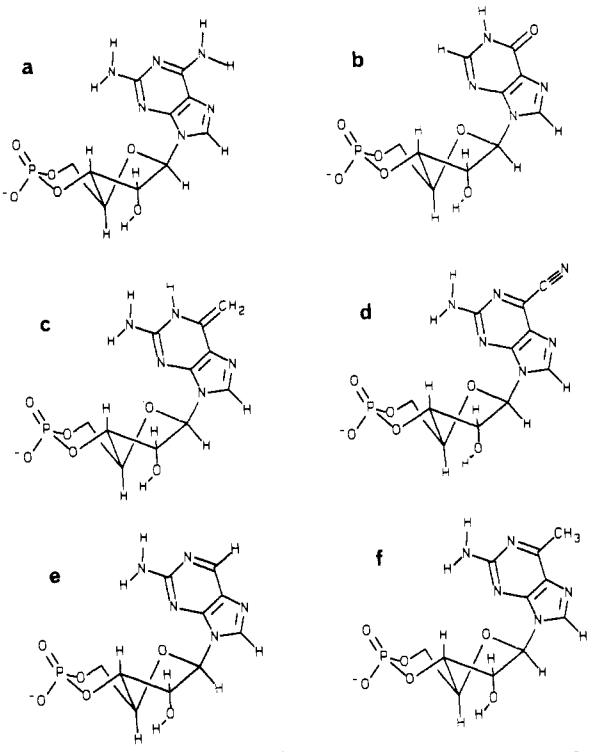

Figure 4. Model compounds: (a) AMcAMP, (b) cIMP, (c) 6-CH_2 cGMP, (d) 6-CN cGMP, (e) 6-H cGMP, and (f) 6-CH_3 cGMP.

difference in conformational preference (i.e., shifts in energy difference of 1.93 kcal/mol at the AM1//AM1 level and 1.03 kcal/mol at the STO-3G//AM1 level, respectively) as does elimination of the amine group of cGMP (2.84 kcal/mol at the AM1//AM1 level and 5.19 kcal/mol at the STO-3G level). Secondly, while the direction of the shift in the conformational preference which occurs when the amine group is added to cAMP is consistent with that for elimination of the amine group of cGMP, there is no reversal of the conformational preference in the former case, i.e., the anti conformation remains preferred for AMcAMP (albeit by less). Clearly the other difference between cGMP and cAMP, i.e., the carbonyl versus the amine group at the 6 position of the purine ring, must be considered. This is discussed below.

IV. The C6 Substituent: cAMP and cGMP Analogues. To evaluate the role of the substituent at the 6 position of the purine ring in cGMP on the riboside bond conformation we have performed the same computational studies as described above on analogues of cGMP and cAMP with other substitutions at the 6 position. The first of these, AMcAMP, has already been mentioned. Briefly, the replacement of the electron-withdrawing carbonyl oxygen atom of cGMP by an electron-donating (on an aromatic ring system) amine to form AMcAMP results in a large shift of the relative conformational preference from the syn to the anti form, e.g., 5.61 kcal/mol at the STO-3G//AM1 level (see Table I). To see whether the relative electron-withdrawing/donating effects are responsible for this shift in conformational preference we performed the same study with use of methyl, hydrogen, and cyano substituents at the C6 carbon atom (see Figure 4). As judged by the relative energy of the syn versus anti conformation, the relative interaction energies of the C2-amine with the axial phosphate oxygen atom are consistent with the relative electron-withdrawing effects anticipated for these compounds, i.e., $NH_2 < CH_3 < H < CN$. Interestingly, as opposed to the case of cGMP, none of these predict a greater stability for the syn conformation at the AM1//AM1 level. The C2-amine hydrogen atom to axial phosphate oxygen atom distance in these compounds is also longer than one would expect for a hydrogen bond (>3.6 Å). For the cyano analogue, for which this interaction is least repulsive, there is a second local minimum whose structure is consistent with a hydrogen bond, i.e., hydrogen bond length of 2.324 Å. While, as pointed out above, this local minimum is less

Table II. The Relative Stabilities of Syn and Anti Conformations of cGMP, cAMP, and Several of Their Derivatives in the AM1 Optimized Geometries of Syn and Anti cGMP

molecule (synonym)	partial AM1 geometry optimization[a] AM1//AM1 (kcal/mol)	STO-3G//AM1 (kcal/mol)
cGMP	−1.358	−4.310
6-CH_2 cGMP	−0.268	−3.604
6-CN cGMP	+0.869	−2.328
6-H cGMP	+2.014	−1.179
2-H cGMP (clMP)	+5.553	+5.232
6-CH_3 cGMP	+2.265	−0.869
6-NH_2 cGMP (2-NH_2 cAMP) (AMcAMP)	+2.652	−1.078
cAMP	+9.555	+8.008

[a] In CHEM-X, the AM1 optimized geometries of the syn and anti conformations of cGMP were converted to the structures in this table by the modification of the carbonyl oxygen atom at the 6 position and the removal (whenever appropriate) of the hydrogen atom at the 1 position. For 2-H cGMP and cAMP, the amino group of cGMP was replaced by a hydrogen atom. The resulting structures were used as starting guesses in very limited geometry optimizations in which only the substituent atom or group at the 6 position and, for 2-H cGMP and cAMP, the hydrogen atom at the 2 position (where the other structures have an amino group) were allowed to relax in a MOPAC (v 4.0) AM1 geometry optimization. The STO-3G//AM1 energies (in au and in the order of this table) are cGMP (chair, syn) −1502.775 599 and (chair, anti) −1502.768 731; 6-CH_2 cGMP (syn) −1467.469 626 and (anti) −1467.463 884; 6-CN cGMP (syn) −1519.472 175 and (anti) −1519.468 465; 6-H cGMP (syn) −1428.919 275 and (anti) −1428.917 396; 2-H cGMP (syn) −1448.430 484 and (anti) −1448.438 822; 6-CH_3 cGMP (syn) −1467.504 939 and (anti) −1467.503 555; 6-NH_2 cGMP (syn) −1483.246 160 and (anti) −1483.244 442; cAMP (chair, syn) −1428.905 658 and (chair, anti) −1428.918 420. The corresponding AM1//AM1 energies (in kcal/mol) are cGMP (chair, syn) −112 198.250 and (chair, anti) −112 196.892; 6-CH_2 cGMP (syn) −108 377.318 and (anti) −108 377.050; 6-CN cGMP (syn) −112 176.507 and (anti) −112 177.376; 6-H cGMP (syn) −104 787.726 and (anti) −104 789.740; 2-H cGMP (syn) −107 100.219 and (anti) −107 105.772; 6-CH_3 cGMP (syn) −108 382.665 and (anti) −108 384.930; 6-NH_2 cGMP (syn) −109 888.590 and (anti) −109 891.242; cAMP (chair, syn) −104 792.404 and (chair, anti) −104 801.959.

stable at the AM1//AM1 level, it is more stable than the anti form by 0.97 kcal/mol at the STO-3G//AM1 level. Nevertheless, while the rank order of the electron-withdrawing effects are as expected, even the cyano compound does not appear to induce an effect on the hydrogen bond which is comparable to that of the carbonyl oxygen atom of cGMP. This indicates that, in addition to the electron-withdrawing effects of the carbonyl oxygen atom of cGMP, other effects, such as the different ring conjugation resulting from the double bond of the carbonyl at C6 are important. Indeed, we see that if we examine the hypothetical methylene (rather than the above methyl) tautomer of the C6 substituent we increase the relative syn versus anti conformational energy by 1.78 and 3.51 kcal/mol at the AM1//AM1 and STO-3G//AM1 levels, respectively (as compared to the methyl tautomer). Thus, the syn conformation is now more stable by 0.21 and 2.53 kcal/mol at the AM1//AM1 and STO-3G//AM1 levels, respectively (see Table I). Also, the hydrogen bond length is 2.25 Å, i.e., only 0.04 Å greater than that for cGMP.

It is also worth noting that these effects can be predicted from an examination of the molecular electrostatic potentials (MEPs) of the respective base portions of these compounds. (Note that all base portions were optimized with a constraint of complete planarity, except for the methyl hydrogen atoms in 6CH_3-cGMP, with the AM1 method.) The relevant MEPs (produced with the ab initio Hartree–Fock wave functions with the 3-21G basis set) are presented in Figure 5. We see in comparing the positive region of the MEP of the lower (in Figure 5) C2-amine hydrogen atom, an increase in the positive nature of this region correlates with

Table III. Interaction Energy Differences (in kcal/mol) for Ammonia/Sugar Model Complexes

structure[a]	interaction energy AM1	STO-3G	STO-3G counterpoise-corrected
cGMP	−5.74	−7.04	−4.70
AMcAMP	−1.83	−1.77	−1.76

[a] Structural parameters for the complex shown in Figure 6 are derived from the parent compound indicated here. Geometries of the parent compounds are those obtained by full geometrical optimization at the AM1 level.

an increase in the relative stability of the syn conformation as described above. Indeed, a Mulliken population analysis, at the 3-21G//AM1 level, of the C2-amine hydrogen atom closer to the phosphate group for this series also supports this description (net charges on the hydrogen atoms are 0.364, 0.365, 0.367, 0.372, 0.384, and 0.388 e for NH_2, CH_3, H, CN, $=CH_2$, and =O, respectively).

V. Strain Energy Analysis. A. Partially Frozen Model Structures. In the analysis of the C6 substituted analogue structures described above, the relative stability of the syn conformation is consistent with expectations based on arguments of electron-withdrawing effects and is supported by the MEP results. With the completely optimized structures used above, the direct sugar-to-base interactions (as indicated in the MEPs) are coupled with the strain effects at the ribose bond. By using essentially equivalent structures for the sugar and base portions of all of the analogues presented above, one can maintain the same strain effects in all the compounds and thereby hopefully isolate the direct interactions. In Table II, we present the results of such a study in which the geometrical parameters of cGMP were used for all of the compounds with the exception of reoptimization of the C6 substituent, and, in the cases of cAMP and clMP, the C2 hydrogen atom. At the AM1//AM1 level, only the methylene derivative, for which the fully optimized results predict a hydrogen bond to exist, shows a (negligible) shift toward greater syn conformational preference from −0.21 to −0.27 kcal/mol when comparing the fully optimized results (Table I) to the partially optimized ones presented here. At the STO-3G//AM1 level, on the other hand, with the exception of cAMP and clMP, the conformational preferences of all of the compounds are shifted toward the syn conformation when these consistent (constant-strain) structures are used. In fact, all of the compounds with a C2-amino group now prefer the syn conformation, and, within this subset, the rank order of this relative preference is essentially consistent with those of the fully optimized structures. The only exception is the reversal, by only 0.20 kcal/mol, of the C6 methyl and amino compounds.

B. Model Complexes: The Ammonia/3′,5′-Cyclic Monophosphate Complex. While the studies described in the previous section attempt to analyze model systems with constant strain effects, it would also be interesting to conduct studies of models in which the strain effects do not exist. We therefore present results for such a model, i.e., the complex of NH_3 with 3′,5′-cyclic monophosphate. This complex represents a model for cGMP (or AMcAMP) in which not only the effects of strain but also the effect of the remainder of the purine portion (including the carbonyl of cGMP) on the interaction of the base with the sugar portion has been eliminated (see Figure 6). The interaction energy for this complex has been evaluated by using two sets of structural parameters which are taken from the optimized structures of the syn conformations of cGMP and AMcAMP described above, as representative of the two extremes of the above results. (Hydrogen atoms added to the complex were optimized, and then, in the calculation of the energy of the isolated fragments, all of the atoms were held fixed.) As summarized in Table III, the interaction energy for the cGMP model (−5.74 kcal/mol) is larger than that of the AMcAMP model (−1.83 kcal/mol) at the AM1//AM1 level. The corresponding results at the STO-3G//AM1 level are −7.04 kcal/mol and −1.77 kcal/mol, respectively. Clearly, in the present model system, this difference is due to the larger separation

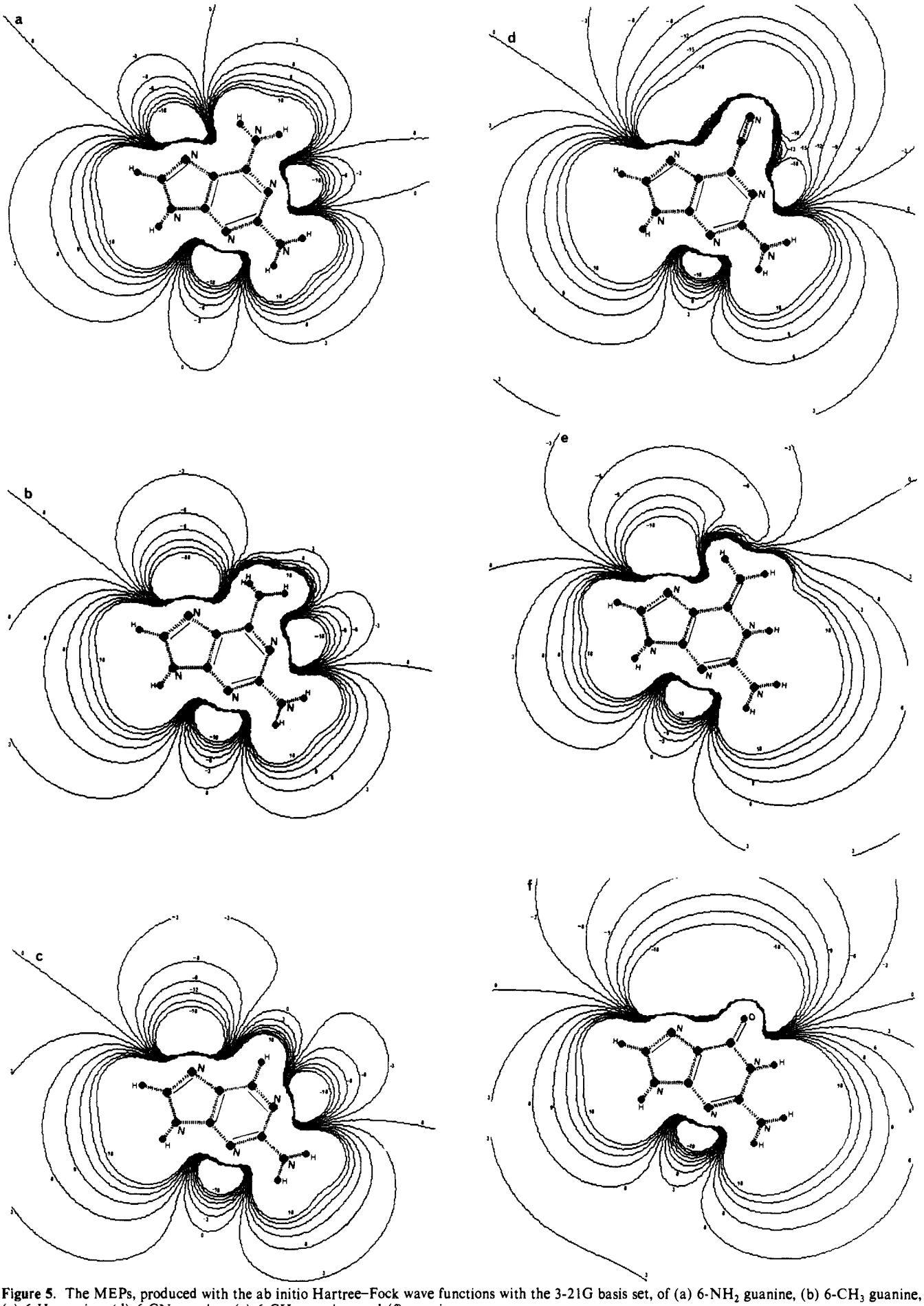

Figure 5. The MEPs, produced with the ab initio Hartree–Fock wave functions with the 3-21G basis set, of (a) 6-NH_2 guanine, (b) 6-CH_3 guanine, (c) 6-H guanine, (d) 6-CN guanine, (e) 6-CH_2 guanine, and (f) guanine.

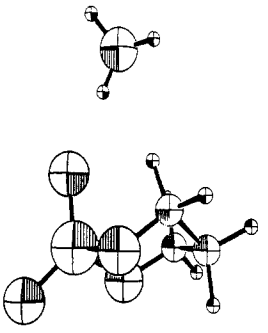

Figure 6. The ammonia/3',5'-cyclic monophosphate complex used as a model for the interactions of the base and sugar portions in cGMP.

between the NH_3 and the phosphate oxygen atom in the AM-cAMP model complex. This difference in separation is, in turn, due to the differential effects of the remainder of the purine group. Thus, while these substituents are not explicitly included in the present calculations we see that the interaction energies are significantly effected by the relative position of the C2-amine.

For the cGMP model complex, the interaction energy at the AM1//AM1 level (−5.74 kcal/mol) is significantly larger than the conformational energy difference between the syn and anti forms of cGMP (−1.36 kcal/mol). In part, the former represents the absence of the steric (base/sugar) strain energy for the syn conformation which is present in cGMP. To verify the insensitivity of these conclusions to the model selected, we have performed an analogous study on the cGMP model complex by using the purine and the corresponding pyrimidine fragments (where only the added hydrogen atoms were optimized) in place of the ammonia and, at the AM1//AM1 level, have found the interaction energies to be −6.31 and −4.71 kcal/mol, respectively.

C. A Bridging Water Molecule and the Crystal Structure. While the X-ray structure of cGMP has a syn conformation, the hydrogen bond discussed above is not usually implicated as responsible for this conformational preference. This is probably because the relevant hydrogen atom-to-oxygen atom distance is 4.37 Å (see Figure 7). Inspection of this structure, however, reveals a bridging water molecule which accommodates a hydrogen bond for the hydrogen and oxygen atoms discussed above. This would allow for a similar hydrogen bonding framework to exist while relieving the strain energy at the riboside bond in the syn conformation. Thus, in the crystal structure, the distances from the 2-amino hydrogen atom to the water oxygen atom and from one of the water hydrogen atoms to the axial phosphate oxygen atom are 2.130 Å and 2.098 Å, respectively, i.e., comparable to the 2-amino hydrogen to axial phosphate oxygen atom distance in cGMP. To assess the effects of such a water molecule, we have repeated the syn versus anti conformational studies of cGMP described above but with the inclusion of a (bridging) water molecule. We find that the syn versus anti conformational energy difference of cGMP is increased to 3.29 kcal/mol as compared to 1.36 kcal/mol in its absence at the AM1//AM1 level and 6.14 kcal/mol as compared to 4.31 kcal/mol at the STO-3G//AM1 level, when a bridging water molecule is included. Thus, both methods of calculation give a similar increase (ca. 2 kcal/mol) in the preference of the syn versus anti conformation of cGMP when a bridging water molecule is introduced. This difference (2 kcal/mol) in the conformational preference serves as another indication of the strain energy component, in the gas-phase model discussed above. Apparently, the aqueous conditions under which these crystals were grown show a preference for the use of such a bridging water molecule to achieve the hydrogen bond. On the other hand, it is possible that the binding site of a protein cannot accommodate a water molecule, and the gas-phase model implicitly used herein may be more appropriate in such instances. In either case (with or without the bridging water molecule), hydrogen bonding in the syn conformation of cGMP is strongly implicated as a key factor in determining the conformational profile.

VI. Accuracy Considerations. Much of this study focuses on an intramolecular hydrogen bond. Both the AM1 and ab initio

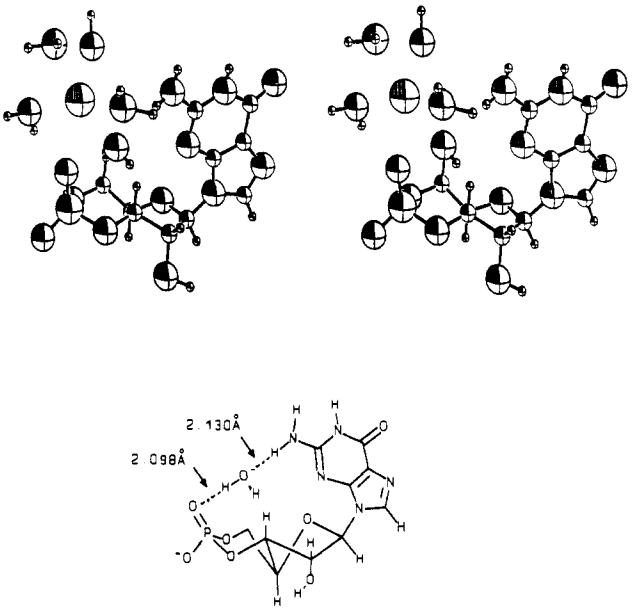

Figure 7. Stereodrawing of the X-ray structure of cGMP. One of the water molecules in the sodium/water cluster serves in hydrogen bonding as a bridge between the amino and phosphate groups.

STO-3G methods could be cause for concern in such systems.[22,23] Most importantly we note that the analysis presented herein is based on relative energy differences among model complexes and compounds. While the absolute values of the strength of the hydrogen bonds may be somewhat unreliable for these methods, these relative energies are less suspect. With respect to the AM1 results, we note that recent studies of water dimers compared with accurate extended basis set and correlation energy approximations suggest that the AM1 method gives reasonable results, particularly if care is taken to include all possible stationary states obtained with the AM1 method.[22] These additional, artificial stationary points are, however, of greater concern for more flexible systems, such as water dimers, where significantly different alternate structures are possible (e.g., linear, bifurcated, etc.). The intramolecular interaction considered herein is considerable more constrained. By comparison, the STO-3G results would be expected to overestimate the strength of hydrogen-bonding-like interactions due to the well-known basis set superposition error (BSSE). To help in assessing these issues as they relate to the present work, we have done two series of calculations as follows.

With respect to the AM1 calculations, the new semiempirical method PM3, which has recently been presented,[15] has been designed as an improvement over the AM1 method. In the water dimer case, the PM3 results[15] do not have the discrepancies with ab initio results described above. We have repeated the AM1 studies of the syn versus anti conformational energies of cGMP and cAMP by using the PM3 method (including full geometry optimization at the PM3 level) and find that the relative energy differences are −0.533 kcal/mol and 4.354 kcal/mol, respectively. These results are in good qualitative agreement with the AM1//AM1 results of −1.358 and 3.608 kcal/mol. Indeed, the *difference between the relative preference* for the syn conformation in cGMP versus cAMP is remarkably close for the AM1 and PM3 results, i.e., 4.966 and 4.887 kcal/mol, respectively (<0.1 kcal/mol!).

With regard to possible errors due to the BSSE effect on the intramolecular interaction discussed herein, we call attention to the following. While the BSSE effect has been widely discussed, it is understood in *inter*molecular terms. It seem intuitively obvious that the *intra*molecular interactions discussed here could be subject to similar errors. However, a proper identification and treatment

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of such effects (if it can be shown to exist and be separable from other intramolecular basis set limitations) is beyond the scope of this paper. Nevertheless, to the extent that the complex discussed herein (between NH_3 and 3′,5′-cyclic monophosphate) is a suitable model for the key interactions, we can attempt some evaluation of the BSSE effect by determining its value in this model by means of the counterpoise correction approximation.[24] Thus, we find that the counterpoise corrected interaction energies for these complexes at the cGMP and AMcAMP model geometries are −4.70 and −1.76 kcal/mol, respectively (Table III). These results compare reasonably well with the uncorrected values of −7.04 and −1.77 kcal/mol, respectively. As expected, the greater BSSE error occurs for the closer proximity of the two molecules. The difference between the counterpoise corrected interaction energies is in closer agreement with the semiempirical result than the uncorrected ab initio value (Table III).

The numerical results presented herein may change somewhat if more extensive basis sets are used or approximate corrections for correlation energies are included. Such calculations are beyond the scope of the present study. It is likely that these changes will be uniform for the systems studied here and that the rank order will not be effected. It is also possible that more extensive methods would predict that, e.g., in the gas phase other tautomers of the purine moieties may be energetically accessible or even more stable.[8,25] The enol tautomer of cGMP would be expected to have differences in conformational energy from the keto tautomer which are analogous to those shown for the C2-methyl versus methylene tautomers. Provided that there is not enough excess stability of the enol tautomer over the keto tautomer to overcome the greater stability expected for the hydrogen bond of the keto tautomer in the syn conformation, the numerical results presented here are likely to be the pertinent ones for this system. Preliminary studies[26] seem to confirm this. The present study is aimed at modeling events at physiological pH where the keto tautomer is more likely to be the dominant form. In any event, it is unlikely that our conclusion that the nature of the C6 substituent plays an important role in its effects on the intramolecular interactions described herein will be changed.

Discussion

Our study supports the previous computational studies identifying two conformational minima for both cGMP and cAMP. Our results identify the source of the relative stabilization of the syn conformation of cGMP as a hydrogen bond between the C2-amine group of the purine and the nearby phosphate oxygen atom. (This possibility does not exist for cAMP.) This is consistent with studies of the crystal structure of cGMP which reveals a water molecule as a hydrogen-bonded bridge between one of the 2-amino hydrogen atoms and the axial phosphate oxygen atom. Our analyses also reveal the role of the various components of cGMP in establishing this hydrogen bond. The bridging water molecule provides a means for maintaining the intramolecular hydrogen bond framework while somewhat relieving the strain energy of the syn conformation.

Converting the 3′,5′-cyclic monophosphate from the lower energy chair structure to the twist-boat structure increases the hydrogen bond length significantly and is accompanied by a shift (small reversal) in the relative stability of the syn versus anti conformation.

Introduction of an amine group at the C2-carbon atom of cAMP shifts the relative stability of the syn versus anti conformation toward the syn conformation for the compounds studied here. However, introduction of the amine at the 2 position alone does not result in the same degree of conformational preference of the syn versus anti structures that exists for cGMP. On the basis of the models studied herein, the strongest base-to-sugar interaction energy (and therefore relative preference for the syn conformation) will occur when there is an oxygen atom at the 6 position of the purine. The relative stability of the syn versus anti conformation varies with the substituent at the C6 position in a manner consistent with the expected electron-withdrawing effects on the aromatic purine ring system. Thus, the C6 substituent plays an important role in stabilizing the syn conformation of these analogues. Furthermore, the effect on the conjugation in the purine ring, imparted by the carbonyl group, is important in stabilizing this hydrogen bond as evidenced also by the methylene analogue.

It is interesting to note that the relative energy of the syn versus anti conformation of the C2-amino cAMP analogue (AMcAMP), which is intermediate between that of cAMP and cGMP, correlates with its observed activities on cAMP and cGMP dependent protein kinases. Thus, the activation of cAMP dependent protein kinase by AMcAMP is less than that by cAMP but more than that by cGMP, while the reverse is true for these activities on cGMP dependent protein kinases.[22-24] Similarly, cIMP shows a reduced activity at cAMP and cGMP dependent protein kinase receptors.[20,25] On the other hand, the relative energies of the syn versus anti conformations of cGMP, cIMP, and AMcAMP do not correlate with the inhibition of cAMP dependent phosphodiesterases.[26]

It is worth emphasizing that the energetic analyses reported here are obtained by using the AM1//AM1 and STO-3G//AM1 methods. Certainly, many quantitative aspects of the present results are therefore subject to change if reevaluated with ab initio methods which use extensive basis sets and include approximations of correlation effects. We do not, however, expect any significant changes in the qualitative results presented herein (see discussion in previous section).

Conclusions

The conformational profiles of cAMP, cGMP, and their analogues are to a large extent determined by their ability to form an intramolecular hydrogen bond between the hydrogen atom of the C2-amine group and the axial oxygen atom of the ring phosphate. Thus, the presence of the C2-amine group is important in stabilizing the syn conformation of these cyclic nucleotides and their analogues. The strength of this interaction energy is highly sensitive to the substituent at the 6 position of the purine. This preference of syn conformation for cGMP and the relatively low-energy anti conformation may have important implications for recognition and binding of cGMP and cAMP at various receptor and enzyme sites and, thus, may be useful in designing potential analogues for therapeutic use.

Registry No. cGMP, 7665-99-8; cAMP, 60-92-4; 6-CH_2 cGMP, 124419-30-3; 6-CN cGMP, 124419-31-4; 6-H cGMP, 42467-66-3; cIMP, 3545-76-4; 6-CH_3 cGMP, 124419-32-5; H_2O, 7732-18-5.

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