-78 °C, followed by the addition of methyl iodide (20 μL), and then the mixture was allowed to warm slowly to room temperature. The colorless solution was poured into water/ether, and the phases were separated. The aqueous phase was extracted with ether, and the combined extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed in vacuo. Flash column chromatography (10% ethyl acetate/hexane) afforded a mixture of the α- and β-epimers (30 mg, 78%). The epimers were separated by HPLC (4% ethyl acetate/hexane) to afford the individual isomers in a 74:26 (α:β) ratio.

**50**: $[\alpha]^{20}_D$ +51° (*c* 0.036, $CHCl_3$); UV $\lambda_{max}$ (EtOH) 316, 269, 225 nm ($\epsilon_{max}$ 2500, 8000, 23 200); IR ($CHCl_3$) 2950 (s), 1710 (s), 1580 (w), 1565 (w), 1460 (s), 1350 (m), 1335 (m), 1285 (s), 1235 (m), 1125 (s), 1000 (m), 910 (s) $cm^{-1}$; NMR (250 MHz, $CDCl_3$) δ 5.20 (br s, 1 H), 4.68 (t, *J* = 2 Hz, 1 H), 3.29 (dd, *J* = 15, 7 Hz, 1 H), 2.8-2.6 (m, 3 H), 2.48 (dd, *J* = 15, 4 Hz, 1 H), 2.23 (s, 3 H), 1.82 (m, 1 H), 1.58 (d, *J* = 9 Hz, 1 H), 1.27 (d, *J* = 7 Hz, 3 H), 1.23 (s, 3 H), 1.1-0.8 (m, 2 H), 1.0 (t, *J* = 7 Hz, 9 H), 0.85 (s superimposed upon 2d, 9 H); electron impact mass spectrum, *m/e* 410.2604 ($M^+$, calcd for $C_{26}H_{38}O_2Si$, 410.2641).

**51**: $[\alpha]^{20}_D$ +67.1° (*c* 0.098, $CHCl_3$); UV $\lambda_{max}$ (EtOH) 318, 270, 225 nm ($\epsilon_{max}$ 2050, 6300, 18 000); IR ($CHCl_3$) 2960 (s), 1710 (s), 1570 (m), 1450 (m), 1290 (s), 1030 (m) $cm^{-1}$; NMR (250 MHz, $CDCl_3$) δ 5.24 (br s, 1 H), 4.68 (br s, 1 H), 3.19 (dd, *J* = 15, 7 Hz, 1 H), 2.70-2.48 (m, 4 H), 2.24 (s, 3 H), 1.83 (m, 1 H), 1.59 (d, *J* = 9 Hz, 1 H), 1.28 (d, *J* = 7 Hz, 3 H), 1.23 (s, 3 H), 1.0 (m, 9 H), 1.0-0.8 (m, 2 H), 0.85 (s superimposed upon dq, 9 H); electron impact mass spectrum, *m/e* 410.2626 ($M^+$, calcd for $C_{26}H_{38}O_2Si$, 410.2641).

**Jatropholone B (2)**. To a stirred solution of silyl ether **51** (6 mg, 0.015 mmol) in dry THF (3 mL) at room temperature under argon was added tetra-*n*-butylammonium fluoride (10 μL, 1 M in THF). After 1 min, the reaction mixture was poured into water and extracted with ether, the combined extracts were washed with brine and dried ($MgSO_4$), and the solvent was removed in vacuo to afford, after flash column chromatography (33% ethyl acetate/hexane), 3.9 mg (88%) of jatropholone B: mp 226-228 °C [lit.[3] mp 228-230 °C]; $[\alpha]^{20}_D$ +77.0° (*c* 0.141, $CHCl_3$) [authentic sample[41] +80.3° (*c* 0.128, $CHCl_3$)].

**Jatropholone A (1)**. Using a procedure identical with that for jatropholone B (**2**), 7 mg of silyl ether **50** afforded 4.5 mg of jatropholone A: mp 215-218 °C [lit.[3] mp 218-220 °C]; $[\alpha]^{20}_D$ +102° (*c* 0.095) [authentic sample[41] +107.2° (*c* 0.070, $CHCl_3$)].

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# Conformations of the 8-Methylated and Unmethylated Ribohexamer r(CGCGCG)$_2$

**Shashidhar N. Rao and Peter A. Kollman***

*Contribution from the Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143.* *Received February 4, 1985*

**Abstract:** Molecular mechanical calculations have been carried out on r(CGCGCG)$_2$, r(C-M$^8$G-C-m$^8$G-C-m$^8$G)$_2$, d(CGCGCG)$_2$, and d(C-m$^8$G-C-m$^8$G-C-m$^8$G)$_2$ in A, B, and $Z_I$ forms of polynucleotides. To our knowledge, this is the first atomic level molecular mechanical study of double-stranded RNA in the three polymorphic forms, and detailed structures are presented for the energy-refined models. The calculated energies when corrected for artifacts inherent in a model for RNA and DNA without inclusion of specific hydration are in general agreement with experimental results. Specifically, the B form is more stable than the A form in DNA, the reverse being true in RNA, and the counterion condensation promotes the B to Z transition in DNA and (with more difficulty) an A to Z transition in RNA. Further, 8-methylation of guanine bases potentiates an A to Z transition in RNA and, to a smaller extent, a B to Z transition in DNA. The effect of 8-methylation on promoting the A to Z transition in RNA can be attributed to an unfavorable steric interaction of the 8-methyl group with the backbone in the A structure, reducing favorable base-stacking interactions in this structure.

Oligoribonucleotides have been the targets of several recent studies by CD-ORD and NMR methods. Following the discovery of a novel left-handed Z structure for d(CGCGCG)$_2$ in the solid state,[1] and experimental data that suggested such a conformation in solution studies,[2-5] its ribo counterpart, r(CGCGCG)$_2$, has been analyzed spectroscopically to explore the possibilities of such unusual conformations in RNA structures. Two-dimensional NOE, CD, and ORD studies on this hexaribonucleotide reveal an A form, while it was concluded that the earlier predicted Z form was unlikely even at high salt concentrations.[6-8] No B to Z or A to Z transitions were observed. However, recent investigations of poly(G-C)·poly-(G-C) by NMR, CD, and absorbance techniques[9,10] have found a transition from the A form to the Z form at high salt (6 M $NaClO_4$) for this polyribonucleotide, CD-ORD and NMR studies on modified (8-substituted) oligoribonucleotides r(C-br$^8$G-C-br$^8$G) and r(C-m$^8$G-C-m$^8$G) have also found Z-like forms at both low and high salt concentrations.[11] These studies stimulated us to carry out molecular mechanics calculations on double-stranded RNA with both the normal and substituted guanines. To our knowledge, this is the first application of molecular mechanical methods to double-stranded RNAs in

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**Table I.** Molecular Mechanical Energies (kcal/mol) of the Hexanucleotides Investigated in the Present Study[a]

| hexamer | $E_\omega$ | $E_{cf}$ | $E_{2'\text{-OH}}$ | $E_{Cl}$ |
|---|---|---|---|---|
| RIA | -601.2 | -407.2 | -436.2 | -1251.6 |
| RIB | -594.4 | -413.1 | -419.4 | -1141.8 |
| RIZ | -588.2 | -421.4 | -408.6 | -1335.2 |
| RIIA | -585.9 | -394.3 | -419.5 | -1190.7 |
| RIIB | -623.2 | -429.2 | -404.5 | -1097.0 |
| RIIZ | -626.5 | -435.7 | -417.0 | -1315.8 |
| DIA | -529.5 | -378.3 | | -1202.1 |
| DIB | -538.5 | -384.1 | | -1087.7 |
| DIZ | -499.7 | -371.9 | | -1280.8 |
| DHA | -517.9 | -373.8 | | -1167.1 |
| DIIB | -525.4 | -378.4 | | -1079.9 |
| DIIZ | -492.9 | -369.5 | | -1246.6 |

[a] Key: $E_\omega$, total energy without counterions; $E_{cf}$, energy contributions from the central four base-paired nucleotides; $E_{2'\text{-OH}}$, energy of the central four base-paired nucleotides in ribohexanucleotides without theelectrostatic contribution due to the 2'-hydroxyl groups in ribose subars; $E_{Cl}$, total energy with counterions.

which all degrees of freedom have been energy refined. The results of our investigations not only are in qualitative agreement with the relative experimental energetics on these hexamers but also give mechanistic insight into the reason for the different behaviors of various polymers.

## Methods

We have considered the hexanucleotides r(CGCGCG)$_2$ (RI) and r-(C-m$^8$G-C-m$^8$G-C-m$^8$G)$_2$ (RII) in the standard A, B, and $Z_I$ forms of polynucleotids. In addition, the corresponding deoxyribohexanenucleotides have also been investigated in the above three polymorphic forms and have been referred to as DI and DII. The conformational analyses in the present investigations were carried out using the methods of molecular mechanics, wherein energy calculations were performed with the program AMBER-UCSF (assisted model building with energy refinement).[12] We have employed the force field parameters presented by Weiner et al.[13] The molecular mechanical energies were evaluated by using eq 1,

$$E_{total} = \sum_{bonds} K_r(r - r_{eq})^2 + \sum_{angles} K_\vartheta(\vartheta - \vartheta_{eq})^2 + \sum_{dihedrals} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] + \sum_{i<j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_iq_j}{\epsilon R_{ij}}\right] + \sum_{H\,bonds}\left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right] \quad (1)$$

In all the calculations, we have used a distance-dependent dielectric constant $\epsilon = R_{ij}$. The hexanucleotides were also energy refined in the presence of counterions (which had van der Waals parameters of R* = 1.6 Å and $\epsilon$ = 0.1) placed along the bisector of the angle between the pendant oxygens of the phosphate group at a distance of 5 Å from the phoshorus atoms. The hydrogen-bonding parameters used in the present investigations have been taken from ref 13 without any modifications. The two chains in all the hexanucleotides investigated have identical conformations, and hence the discussions on the conformational aspects of these structures have been restricted to only one of the chains. The residues in all the hexamers are referred to as CYT1, GUA2, CYT3, GUA4, CYT5, and GUA6. The total energies of these structures are listed in Table I.

## Results

**Conformations.** In the case of all the ribohexanucleotides, the energy-refined structures are not very different from the starting conformations. A few interesting deviations are detailed below. The A forms of both RI and RII are conformationally similar to the standard A RNA.[14] RIZ has similar backbone conformations as the standard deoxyribonucleotide Z form,[15] while RIIZ has ($g^+t$) phosphodiester conformations between GUA4 and CYT5 instead of the normal ($g^-t$) conformation. In RIB, the C3'–O3' and phosphodiester conformations vary over a larger range than in the standard B form.[16] For example, the C3'–O3' conformation between CYT3 and GUA4 and CYT5 and GUA6 are gauche$^-$ instead of the standard trans, with the corresponding phosphodiester conformations being ($tt$) instead of the ($g^-g^-$) normally found. It is likely that such unusual phosphodiester conformations may contribute to the instability of the ribohexamer in the B form. Furthermore, such conformations have been very rarely observed in the crystal structures of tRNA[17,18] and have been suggested[18] to be involved in structural features such as extension, and reversal of sugar–phosphate chains in tRNA.

**Table II.** Conformational Parameters of the Sugar Moieties in the A, B, and Z Forms of r(CGCGCG)$_2$ and d(CGCGCG)$_2$ in Terms of Phase ($W$) and Amplitude ($q$) of Pucker[33] Z Forms of d(CG*CG*CG*)$_2$ (DHZ) and r(CG*CG*CG*)$_2$ (RHZ), Where G* Is 8-Methylguanine

| | A form | | B form | | Z form | |
|---|---|---|---|---|---|---|
| residue | $q$ | $W$ | $q$ | $W$ | $a$ | $W$ |
| | | | (CGCGCG)$_2$ | | | |
| CYT1 | 0.38 | 25 | 0.48 | 165 | 0.35 | 164 |
| GUA2 | 0.38 | 33 | 0.41 | 168 | 0.33 | 110 |
| CYT3 | 0.35 | 8 | 0.49 | 145 | 0.42 | 144 |
| GUA4 | 0.33 | 3 | 0.40 | 183 | 0.33 | 100 |
| CYT5 | 0.37 | 1 | 0.46 | 144 | 0.44 | 149 |
| GUA6 | 0.33 | 0 | 0.32 | 185 | 0.35 | 19 |
| | | | d(CGCGCG)$_2$ | | | |
| CYT1 | 0.35 | 7 | 0.32 | 174 | 0.36 | 129 |
| GUA2 | 0.36 | 178 | 0.37 | 130 | 0.28 | 33 |
| CYT3 | 0.35 | 155 | 0.33 | 160 | 0.37 | 166 |
| GUA4 | 0.38 | 120 | 0.39 | 161 | 0.30 | 50 |
| CYT5 | 0.37 | 22 | 0.34 | 177 | 0.38 | 171 |
| GUA6 | 0.38 | 10 | 0.35 | 174 | 0.35 | 20 |

| | DHZ | | RHZ | |
|---|---|---|---|---|
| residue | $q$ | $W$ | $q$ | $W$ |
| CYT1 | 0.35 | 157 | 0.43 | 147 |
| GUA2 | 0.26 | 31 | 0.40 | 45 |
| CYT3 | 0.38 | 165 | 0.41 | 151 |
| GUA4 | 0.27 | 34 | 0.39 | 70 |
| CYT5 | 0.38 | 171 | 0.40 | 168 |
| GUA6 | 0.34 | 12 | 0.38 | 42 |

In RIIB, significant deviations are observed in the phosphodiester conformations at the 3' ends of GUA2 and GUA4 ($tg^-$) and the glycosidic orientations of the 8-methylated guanine residues. All the purines have high anti orientation ($\chi \sim 135$–$140°$) while all the pyrimidines have anti orientations ($\chi \sim 20$–$93°$). Thus, the largest structural changes due to methylation occur in the B form of the hexanucleotides. In contrast to the ribohexamers, the corresponding deoxy compounds show insignificant deviations in the phosphodiester, C3'–O3', C4'–C5', O5'–C5', and glycosidic conformations from those in the standard forms. Figure 1a–c shows stereopairs of the hexamers RIA, RIB, and RIZ, respectively.

The sugar-puckering profiles (Table II) in the case of RI structures are predominantly retained in their starting conformations in the A and B forms. However, in the Z form the sugars attached to the purines GUA2 and GUA4 tend to change their pucker to C2' endo rather retain the C3' endo geometries in the starting structures. In fact, in the energy-refined structure, these sugars have O1' endo pucker (Table II). The methylation of

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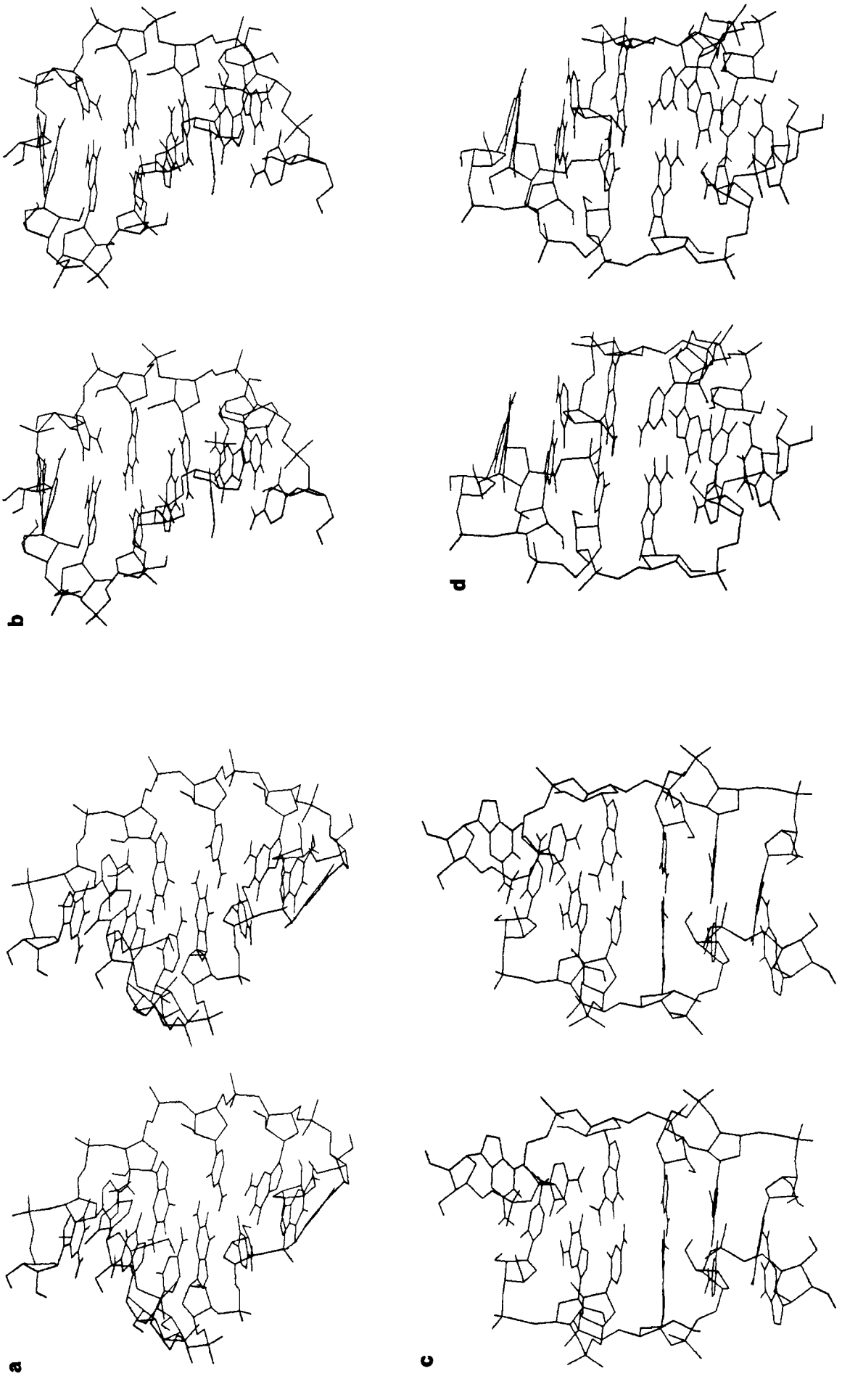


**Figure 1.** Stereopairs of the energy-refined ribohexanucleotides r(CGCGCG)$_2$ in the (a) A form, (b) B form, and (c) Z form and (d) r(C-m$^8$G-C-m$^8$G-C-m$^8$G)$_2$ in the Z form.

guanine bases at C8 does not bring about any changes in the A and the B forms, while in the Z form the purines tend to retain the C3′ endo geometries of the starting structures.

In the case of the deoxyribohexamers, the sugars in the central region in the A form tend to pucker to C2′ endo geometries. This can be understood in light of the inherent preference for C2′ endo geometries over C3′ endo geometries in the case of deoxyribose sugars.[19] The above variation in sugar geometry profiles is also observed for the hexamer with 8-methylguanines. The sugar-puckering properties in the B and Z forms of this hexamer were quite similar to those in the standard structures, as was noted earlier.[20]

One of the most interesting conformational features of the ribohexanucleotides is the orientation of the O2′–HO2′ bond in the ribose sugars. For the sake of convenience, this orientation is described by the torsion angle C3′–C2′–O2′–HO2′ ($\sigma$), which in each structure corresponded to a trans orientation prior to refinement. In both RIA and RIIA, after refinement, this bond is mainly oriented toward the backbone atoms at the 3′ ends of the ribose sugars, contributing to the enhancement of the favorable electrostatic interactions between the HO2′s and negatively charged O5′ associated with the next nucleotide in the 3′–5′ direction and between O2′ and P. Typically, $\sigma$ lies in the gauche$^-$ range.

In RIB, the $\sigma$ angles in the GUA sugars are oriented as in RIA and RIIA, while those in the CYT sugars are oriented so as to enhance favorable hydrogen-bonding interactions with N7 atoms of the guanine bases at the 3′ ends. Here, $\sigma$ varies typically in the trans–gauche$^-$ ranges. In RIIB, on the other hand, this bond in the first four of the sugars in the 5′–3′ direction is pointed toward one of the pendant oxygens of the phosphate groups at the 3′ ends of the sugars and is locked in hydrogen-bonding interactions. In CYT5, the HO2′–O2′ bond is pointed away from the backbone and not specifically involved in hydrogen bonding with any group in the base. Such an arrangement may probably be attributed to the high $\omega'$ (P–O3′ torsion) value (305°) intermediate between CYT5 and GUA6 and very low values the glycosidic torsion ($\chi$ = 20°).

In both RIZ and RIIZ, the HO2′–O2′ bonds of the GUA sugars are oriented toward the backbone at the 3′ ends while those corresponding to CYT sugars are oriented so as to enhance favorable N–H···O hydrogen bonds involving the N2 atoms of the guanine bases at the 5′ ends. Typically, $\sigma$ lies in the gauche$^+$ and gauche$^-$ ranges for guanine and cytosine residues, respectively.

**Energetics.** There are five sets of experimental results to which we can attempt to relate our calculated energies: (1) It is known that normal double-stranded RNA prefers an "A" geometry to a "B" geometry. (2) Double-stranded DNA generally prefers a B geometry. (3) At high salt, appropriate sequences (mainly alternating C-G) can be induced to change their conformations to the Z form. (4) Adding an 8-methyl group on guanine causes RNA to prefer a Z to an A geometry. (5) Adding a bulky group to guanine C-8 apparently causes poly(dG-dC)·poly(dG-dC) to prefer a Z geometry.

If we use the total energies (Table I, column 1) as criteria, these energies are qualitatively consistent with the above experimental results. For r(CGCGCG)$_2$, the A structure is lowest in energy, consistent with experimental results. However, a view of Figure 1a–c reveals that, only in the A form, this sequence has the terminal 5′-OH swung around, forming a hydrogen bond with the nearest phosphate group, an effect caused by the lack of explicit solvent in the calculations. To try to remove such "end effects", we have factored out the energies due only to the central four base-paired nucleotides, and these are listed in column 2 of Table I. As one can see, these parallel the relative total energies for the last three polymers r(C-m$^8$G-C-m$^8$G-C-m$^8$G)$_2$, d(CGCGCG)$_2$, and d(C-m$^2$G-C-m$^8$G-C-m$^8$G)$_2$, but for r(CGCGCG)$_2$, the A structure is now higher in total energy than B and Z. The question is why this occurs.

One of the difficulties in carrying out molecular mechanical calculations on double-stranded RNA (as opposed to DNA) in the absence of explicit counterions and solvation effects is the problem of treating the 2′-OH group, which does not exist in DNA. Not only does one know which direction to point the 2′-OH group (local minimum problem) but any intra-RNA hydrogen bonding is likely to be an artifact of lack of inclusion of explicit water molecules.

To deal with this problem as best we can with our simple model, we have evaluated the energies for this polymer and factored out the intra-RNA electrostatic and hydrogen-bond energies involving the 2′-OH groups and subtracted them from the energy contributions of the central four base-paired nucleotides ($E_{cf}$). These relative energies are reported in column 3 of Table I and "restore" the relative orders found in the total energies. A more detailed analysis of these energies ($E_{2'\text{-OH}}$), in which we break the energy into intra- and intergroup energies of bases, phosphates, and sugars, reveals, as has been noted earlier,[21] that the 2′-OH group has a less favorable van der Waals energy in B than in A due to short contacts with the atoms in the phosphate group at the 3′ end for C2′ endo sugars in the former. This causes more internal strain in the B form of the polymer, and that may be why RNA exists almost exclusively in an A rather than a B conformation, whereas DNA can be depending upon either salt or hydration conditions. It may also be noted that the A form was proposed for RNA[22-25] because of X-ray fiber diffraction studies, and not stereochemical criteria, while the earlier B model[26] (arrived because of the poor quality of the diffraction photographs) was rejected. Susequent X-ray fiber diffraction studies on DNA–RNA hybrids by Arnott and co-workers[27] suggested that while DNA could adopt both A and B forms, the presence of 2′-hydroxyl group in RNA would always force the latter to take up the A form.

The preference of DNA for a B rather than A conformational has been discussed in detail previously.[27] It has been pointed out that with such a preference comes the slightly better phosphate–base and inter- and intrastrand base-stacking interactions in the B form. Further, in the A form, on account of the sugars having C3′ endo geometries, the phosphate–phosphate distance is shorter and the phosphate–phosphate repulsions are larger than in the B form, which is characterized by C2′ endo sugars and a larger phosphate–phosphate distance.

Although we cannot deal with the salt effect in causing B to Z or A to Z transitions in a sophisticated way using molecular mechanics, column 4 of Table I reveals that the addition of $Na^+$ (1.6 Å) counterions complexed to the phosphates consistently changes the relative stability to Z > A > B in all the four hexamers. This trend parallels the phosphate–phosphate distances in the three structures. The phosphates are closer together, and the most effective cation–phosphate bridging occurs in the Z structure; the A structure is also better than B in this regard. This calculated order of stabilization can be related to the experimental fact that it requires much more drastic salt conditions to convert RNA,[9,10] which is in the A form in solution, to the Z form than is required for DNA, which is the B form in solution. Since salt stabilizes the A form more than the B form, A is a much more effective "competitor" with Z in determing the lowest energy

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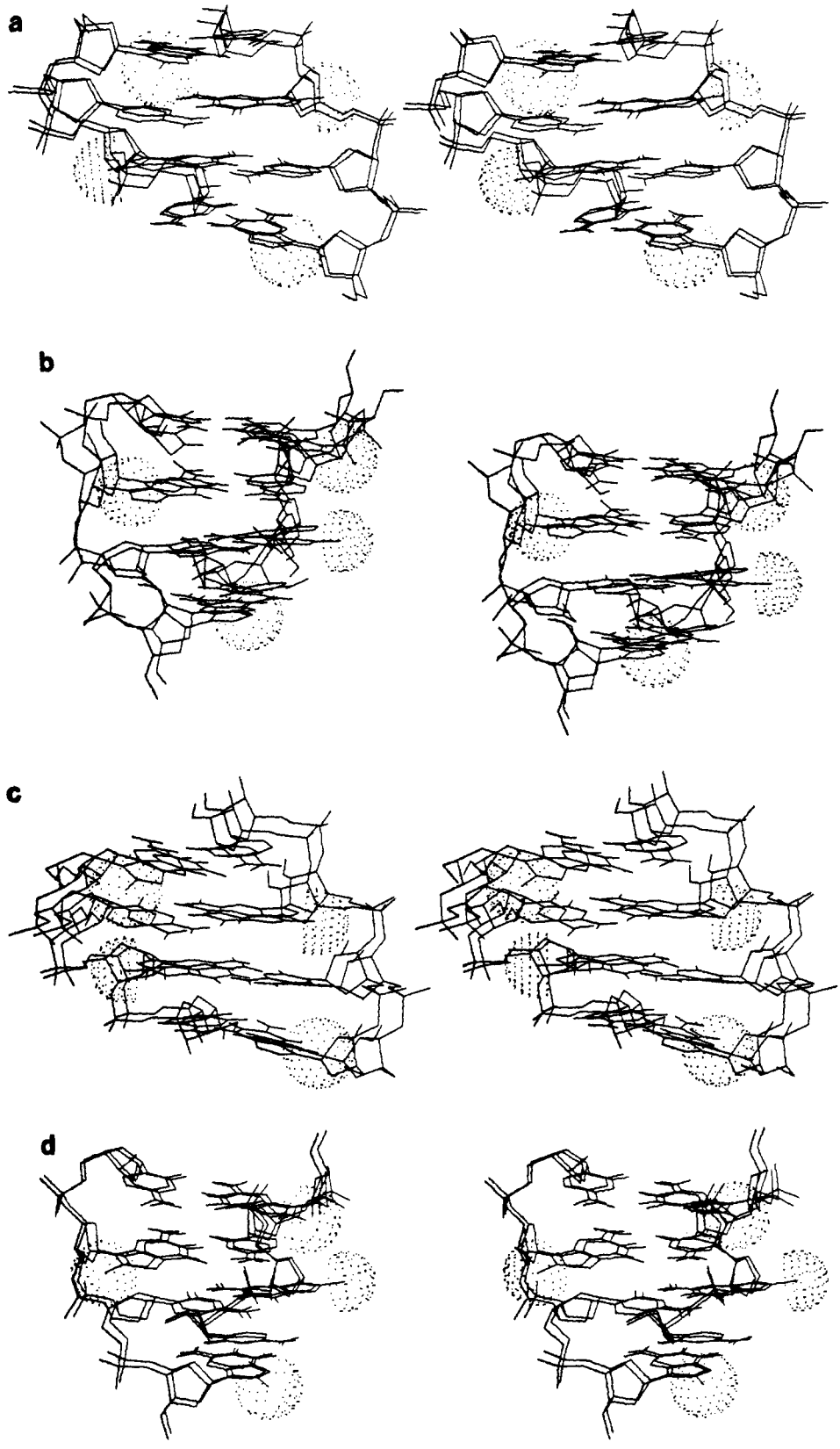


**Figure 2.** Stereopairs of the superpositions of the energy-refined hexanucleotides (a) RIA and RIIA, (b) RIZ and RIIZ, (c) DIB and DIIB, and (d) DIZ and DIIZ. Only the central four base-paired nucleotides have been shown along with the van der Waals surfaces around the 8-methyl groups of the guanines in the RII and DII structures.

structure under high-salt conditions.

The total energies and central four base-paired nucleotide energies reveal that adding an 8-methyl group to guanine changes the relative energies of RNA such that Z is now more stable than B or A, consistent with experimental results.[11] An analysis of the change in the energy components of $r(CGCGCG)_2$ upon methylation of guanine suggests that one can interpret this result as due to two factors:

The first factor is that in the A form, methylation causes destacking of CpG interactions by about 2.7 kcal/mol. This is mainly due to the fact that the accommodation of the methyl group in the major groove without causing unfavorable van der Waals

interactions with the 3′ end phosphates effectively pushes the guanine so as to cause destabilization of the stacking interactions with the cytosines at the 5′ end. This loss is in part compensated by cross base interactions between GUA4 and GUA10. In Figure 2a, we compare the structures of the methylated and unmethylated A forms of the hexamers in the central CG region, including a van der Waals surface for the 8-methyl group. As can be seen, the backbones are further apart and the base stacking is less favorable in the 8-methylated hexamer, reflecting the steric effect of the 8-methyl group. In the Z form, on the other hand, the base-stacking interaction energies are not significantly affected by methylation, and one can see why when one looks at Figure 2b, where the 8-methyl group sticks away from the structure. The methyl group at C8 in the RIIZ major groove is too far away from the 3′ end phosphate group to influence stacking with the cytosine at the 5′ end. It may also be noted that the differences in base–phosphate interactions upon methylation are almost equal in the A and the Z forms, suggesting that conformational changes occur to retain favorable nonbonded interactions between these two groups.

The second factor (related to the first) is that the methylation leads to electrostatic stabilization between the sugar in CYT3 and phosphate groups at its 3′ and 5′ ends by about 3.0 and 6.7 kcal/mol, respectively. Methylation also leads to lower energy sugar–phosphate interactions between (GUA2 and $P_{2-3}$), (CYT3 and $P_{2-3}$), (CYT3 and $P_{3-4}$), and (GUA4 and $P_{4-5}$) by about 5.0, 3.6, 7.3, and 8.5 kcal/mol, respectively. Since most of these changes are electrostatic in nature, they are more "tentative" than the first factor, given the uncertainty in treating electrostatic effects in solution. The first factor gives a simple and appealing explanation of why 8-methylated guanine stabilizes the Z form over the A form in RNA.

In DNA, the effect of 8-methyl substitution reduces the B–Z energy difference, as we have earlier found for 5-methyl substitution[20] in cytosine. So far, no experiments have been reported on the possibilities of Z DNA formation in polynucleotides containing 8-methylated guanines. However, given the consistency of the earlier calculations[20] with the experimental results[29] that suggest that the 5-methyl group in cytosines potentiate the B to Z transitions and the experimental results that suggest that 8-bromo substitutions in guanines potentiate the B to Z transition in DNA, the results of the calculations reported here would indicate the likelihood of potentiation to the Z form of alternating purine–pyrimidine sequences containing 8-methylated guanines. This is further supported by the fact that covalent adducts such as the bulky 2-(acetylamino)fluorene (AAF) to poly(dG-dC)·poly(dG-dC) apparently cause a B to Z transition even at low salt.[30-32]

Our calculations suggest that 8-methyl substitution would have a larger effect in potentiating the Z form of RNA than DNA. While this substitution brought about significant changes in the base-stacking interactions in the A form, it is found that in the B form the 8-methyl group can be accommodated without distortion in the base-stacking interactions, with the distances from the phosphates being similar to those found in the A form. Thus, the effects of methylation on the relative destabilization of the righ- and left-handed forms is larger in RNA than in DNA. The relative destabilization of the DNA B form compared to the Z form upon 8-methyl substitution is chiefly attributable to less favorable base–sugar interactions in the B form arising from the steric effect of the 8-methyl group. Methylation induces variations in the glycosidic torsions that cause each of the interactions between the guanines and sugars attached by the glycosidic bonds as well as those between guanines and sugars at their 5′ ends to be higher in energy by around 1 kcal/mol. Thus, as in the case of RNA, 8-methyl substitution on DNA potentiates a Z structure mainly by destabilizing the normal B form of the polymer.

## Discussion and Conclusions

Molecular mechanical simulations of CG ribohexamer and its derivative containing 8-methylguanine have been carried out in the three major polymorphic forms A, B, and Z. The results of our investigations are in general agreement with the recent experimental data on these compounds. It is found that methylation of guanine at C8 promotes transition to the Z form from the A form, which is the most stable structure for the unmethylated hexamer. This feature has its parallel in deoxyribonucleotides where methylation of cytosines at C5 and bromination of guanine at C8 lead to a predominantly Z form, even at low salt concentrations. Our calculations on the methylated deoxyribohexamer predict that the Z form is potentiated, but not as much as in RNA, by 8-methyl substitution.

The method of treating counterion and electrostatic effects in our calculations is very simple, and one cannot attach any great quantitative significance to the result. Nonetheless, the qualitative consistency with experimental energies and relative stabilities for ribohexamers is encouraging, particularly since we use the same approach and force field parameters previously reported by Weiner et al.[12] The most likely reason why methylation of C8 stabilizes the Z form of RNA over the A form compared to the unmethylated polymer is a steric effect between the 8-methyl group and the backbone in the A form, which is absent in the Z form where the 8-methyl group sticks out away from the backbone.

Our calculations lead to detailed models of r(CGCGCG)$_2$ and its derivative containing 8-methylguanine in both A and Z forms. Given that there is no precise structural data on these compounds in the literature, they can be viewed as a prediction, with the reasonable agreement between the calculated and observed DNA models lending support to the fact that such predictions may have basis in reality. Nonetheless, we stress that we have energy refined the structures starting with single but reasonable model-built structures and cannot rule out the possibility of other low-energy minima in these structures.

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