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Influence of Ribose 2′-*O*-Methylation on GpC Conformation by Classical Potential Energy Calculations

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Synopsis

Potential energy calculations were employed to examine the effect of ribose 2′-*O*-methylation on the conformation of GpC. Minimum energy conformations and allowed conformational regions were calculated for 2′MeGpC and Gp2′MeC. The two lowest energy conformations of 2′MeGpC and Gp2′MeC are similar to those of GpC itself. The helical RNA conformation (sugar pucker-C(3′)-*endo*, ω' and ω, g^-g^-, bases-anti) is the global minimum, and a helix-reversing conformation with ω', ω in the vicinity of 20°, 80° is next in energy. However, subtle differences between the three molecules are noted. When the substitution is on the 5′ ribose (Gp2′MeC), the energy of the helical conformation is less than that of GpC, due to favorable interactions of the added methyl group. When the substitution is at the 3′ ribose (2′MeGpC) these stabilizing interactions are outweighed by steric restrictions, and the helical conformation is of higher energy than for GpC. Furthermore, the statistical weight of the 2′MeGpC g^-g^- helical region is substantially less than the corresponding weight for Gp2′MeC. In addition, 2′MeGpC's methoxy group is conformationally restricted to a narrow range centered at 76°. This group has a broadly allowed region between 50 and 175° in Gp2′MeC. These differences occur because the appended methyl group in 2′MeGpC is located in the interior of the helix cylinder, as it would be in polynucleotide, while it hangs unimpeded in Gp2′MeC. These findings suggest that 2′-*O*-methylation has both stabilizing and destabilizing influences on the helical conformation of RNA. For 2′MeGpC the destabilizing steric hindrance imposed by the nature of the guanine base dominates.

INTRODUCTION

2′*O*-Methylated riboses are found in ribosomal and transfer RNAs,[1] and most recently have also been observed in viral RNA.[2] The tRNAs are of particular interest because of the availability of crystal structure data.[3,4] In the cloverleaf model of tRNAs, 2′-*O*-methylated nucleotides occur in regions which are usually not double stranded.[1] However, their function is not yet understood. One possibility is that the methyl group may act as a recognition device, either in a direct steric way, or indirectly by changing the conformation in the region surrounding the modified species.[5] In the monoclinic form of yeast phenylalanine tRNA,[4] it has been noted that the phosphodiester backbone of the anticodon loop actually kinks at

residues 32 and 34, which are 2′-*O*-methyl cytidine and 2′-*O*-methyl guanosine, respectively. An understanding of the influence of ribose methylation on conformation of nucleic acid subunits should help explain its function in natural RNAs.

The conformational influence of 2′-*O*-methylation on nucleosides has been previously examined by classical potential energy calculations. Stellman et al.[6] have made calculations for 2′-*O*-methyl cytidine, with particular attention to the effect of the added methyl group on the ribose pucker. These calculations produced a global minimum energy conformation, and a second conformation at 0.5 kcal/mole which were in very good agreement with the two conformers found in the crystal of 2′-*O*-methyl cytidine by Hingerty et al.[7] For this nucleoside, methylation caused the C(2′)-*endo*-pucker to be preferred (although low energy C(3′)-*endo* conformations were also calculated), while cytidine itself prefers C(3′)-*endo*. The other conformational angles were not perturbed from their usual ranges. Prusiner et al.[8] also found no unusual conformational angles in their calculation on 2′-*O*-methyl adenosine. Their calculations were restricted to the two sugar puckers actually found in the crystal of this molecule.

Potential energy calculations on guanylyl-3′,5′-cytidine (GpC)[9,10] have delineated two low energy conformations, both with ribose pucker C(3′)-*endo*. The global minimum is like A-RNA, and has ω', ω near 300°, 280°. A second low energy conformation is found with ω', ω-near 20°, 80°.

In the present work the influence of 2′-*O*-methylation on the low energy conformations of GpC was examined. Minimized potential energy calculations were made for 2′-*O*-methyl-GpC (2′MeGpC) and Gp-2′-*O*-methyl-C(Gp2′MeC), and statistical weights of the low energy regions were calculated from energy contour maps. In addition, the conformation space allowed to the methoxy angle m_1, C(2′)-O(2′), (which is unique to these rare nucleotides, Fig. 1) was investigated. While the two lowest energy conformations of GpC are similar in the 2′-*O*-methylated derivatives, a number of subtle but potentially significant differences are noted, especially for 2′MeGpC. The helical RNA conformation of 2′MeGpC has a lower statistical weight than for Gp2′MeC. Also the methyl group itself is more restricted in 2′MeGpC. The restrictions occur in 2′MeGpC because the appended methyl group is in the interior of the helix. Thus the flexibility of the molecule in this conformation is reduced. These steric constraints on the helical RNA conformation would occur in a (GC)-polymer. In Gp2′MeC the methyl hangs unimpeded on the outside of the helix, an effect which would occur only at the 3′ end of a polymer.

METHODS

Figure 1 gives the structure and numbering scheme of 2′MeGpC and Gp2′MeC, and Table I defines the dihedral angles.

As in earlier work,[10] the Scott and Scheraga equation[11,12]

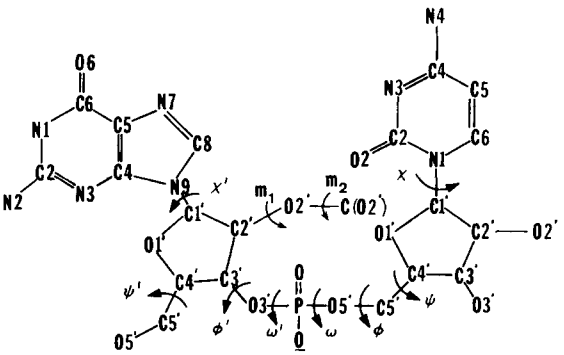


Fig. 1. Structure, numbering convention, and conformational angles of 2′MeGpC. For Gp2′MeC the methyl carbon is substituted at O(2′) of the cytidine ribose.

$$E = \sum_{i<j} \sum (a_{ij} r_{ij}^{-6} + b_{ij} r_{ij}^{-12}) + \sum_{i<j} \sum 332 q_i q_j r_{ij}^{-1} \epsilon^{-1} + \sum_{k=1}^{8} \frac{V_{0,k}}{2} (1 + \cos 3\theta_k) \quad (1)$$

was used to calculate the energy of the molecules. In this equation r_{ij} is the distance in angstroms between atoms i and j, q_i is the partial charge on atom i, ϵ is the dielectric constant, $V_{0,k}$ is the barrier to internal rotation for the kth dihedral angle, and θ_k is the value of that angle. Values for all parameters were taken from Refs. 13–15 (as before) except for the charges on the appended methyl group and the adjoining ribose. These are taken from Prusiner et al.[8] The rotational barrier height for the angle m_1 (see Table I) was assigned a value of 2.0 kcal/mole, as was done previously for 2′-*O*-methyl cytidine.[6] The geometrical parameters employed were the same as for the earlier work on GpC; they were taken from Arnott.[16] Standard bond lengths and angles were used for the methyl group.

Using a modification of the Powell algorithm,[17] the total energy was

TABLE I
Definition of Dihedral Angles for 2′MeGpC and Gp2′MeC[a]

Angle[a]	Bonds
χ'	O(1′)-C(1′)-N(9)-C(8)
ψ'	C(3′)-C(4′)-C(5′)-O(5′)
ϕ'	P-O(3′)-C(3′)-C(4′)
ω'	O(5′)-P-O(3′)-C(3′)
ω	C(5′)-O(5′)-P-O(3′)
ϕ	C(4′)-C(5′)-O(5′)-P
ψ	C(3′)-C(4′)-C(5′)-O(5′)
χ	C(6)-N(1)-C(1′)-O(1′)
m_1	C(1′)-C(2′)-O(2′)-C(02′)
m_2	C(2′)-O(2′)-C(02′)-H

[a] All angles A-B-C-D are measured by a clockwise rotation of D with respect to A when viewed along B-C. A eclipsing D is 0°.

minimized as a function of eight dihedral angles—the seven backbone angles and the methoxy angle m_1. ψ' and m_2 (Table I) were fixed at 60°. The sugar puckering was set in the C(3′)-*endo* or C(2′)-*endo* conformations. The C(2′)-*endo* and C(3′)-*endo* puckers are the only ones both calculated and observed for 2′-*O*-methyl cytidine.[6,7]

The dihedral angles of the ribose were set at the following values (in the notation of Altona and Sundaralingam[18]): for C(3′)-*endo*, $\theta_1 = -38°$, $\theta_2 = 25°$, $\theta_3 = -1°$, $\theta_4 = -24°$, $\theta_0 = 38°$, corresponding to a pseudorotation parameter **P** = 18°, and a puckering amplitude, θ_m, of 39.8°. Bond angles O1′-C1′-C2′, α_1, and O1′-C4′-C3′, α_2 were set at 106.5 and 103.5°, respectively. For C(2′)-*endo*, $\theta_1 = 20°$, $\theta_2 = 0°$, $\theta_3 = -21°$, $\theta_4 = 32°$, $\theta_0 = -31°$, corresponding to **P** = 162°, $\theta_m = 34.2°$. α_1 and α_2 were set at 106.7 and 103.1°. These values are not far from the energy minima calculated for unmethylated riboses by Sato.[6,19]

In the calculations of Sato and in those on 2′-*O*-methyl cytidine,[6] the ribose pucker was a variable, and a strain component was included with the terms considered in Eq. (1) for the ribose energy. In the present work the ribose pucker was not a variable. Therefore the total within-ring ribose energy is a constant for a given pucker and is excluded, as are all interactions that are invariant with changes in dihedral angles. Overall energies for C(2′)-*endo* and C(3′)-*endo* conformations may be validly compared because the calculated ribose energies for these two puckerings are virtually equal.[7,19] The methyl group has a small effect on the calculated charges of the ribose atoms.[8] It thus seems likely that the C(2′)-*endo* and C(3′)-*endo* methylated riboses have similar ring energies.

Starting conformations for the dihedral angles with C(3′)-*endo* sugar puckers were the global minimum and the next lowest energy conformation of GpC, as well as the g^-t conformation of ω',ω, which has been calculated to be of low energy for other ribo sequences.[21] The actual angles used are:

(1) $\chi' = 5°$ (anti); $\phi' = 203°$; $\omega',\omega = 298°, 279°$; $\phi = 182°$; $\psi = 57°$; $\chi = 27°$ (anti); $m_1 = 60°, 180°, 300°$ (staggered)

(2) $\chi' = 11°$ (anti): $\phi' = 181°$; $\omega',\omega = 16°, 83°$; $\phi = 192°$; $\psi = 62°$; $\chi = 39°$ (anti); $m_1 = 60°, 180°, 300°$ (staggered)

(3) $\chi' = 15°$ (anti); $\phi' = 200°$; $\omega',\omega = 290°, 180°$; $\phi = 180°$, $\psi = 60°$; $\chi = 15°$ (anti); $m_1 = 60°, 180°, 300°$ (staggered)

For C(2′)-*endo* pucker, starting conformations were:

(1) $\chi' = 55°$ (anti); $\phi' = 200°$; $\omega',\omega = 60°, 60°; 290°, 290°; 290°, 180°$; $\phi = 180°$; $\psi = 60°$; $\chi = 55°$ (anti); $m_1 = 60°, 180°, 300°$ (staggered)

H(O3′) of cytidine, whose position is uncertain, has been set such that C(2′) is *trans* to H(O3′), as this setting gives the deepest global minimum. However, the energy of the $\omega',\omega \sim 20°, 80°$ minimum for 2′MeGpC is lower when these atoms are eclipsed, and this setting was used for that case only.

The allowed regions of m_1 were examined for both 2′MeGpC and Gp2′

MeC by calculating the energy as a function of m_1 with the other angles fixed at either the global minimum or the next lowest energy conformation.

The ω',ω conformation space for the two molecules was investigated by calculating two dimensional energy contour maps. Energies were calculated at 18° intervals of each angle, for a total of 400 points. Two separate ω',ω maps were constructed for each molecule with ribose pucker C(3')-*endo;* one is for the g^-g^- helical RNA global minimum, and a second is for the $\omega',\omega \sim 20°, 80°$ minimum. For each map the angles other than ω',ω were fixed at the values they possess at the minimum. For a given molecule, we have compared the probabilities of the low energy conformations by estimating their statistical weights from the two energy maps. This was done by adopting the formula given by Olson[22] with a small modification.

$$w_M = \left[\sum_{m=1}^{2} \sum_{E=1}^{5} \exp(-E/RT) A_{M,m}(E) \right] / Z_{\omega',\omega} \qquad (2)$$

w_M is defined as the statistical weight for the minimum energy region M where M is specified by the ω',ω region, e.g., $\omega',\omega \sim 300°, 300°$. The m index sums over the two ω',ω maps. E is the energy in kcal/mole. The sum over E counts all conformational regions less than or equal to 5 kcal/mole. $A_{M,m}(E)$ is the area between the E and $E-1$ contours for the region M on the mth map. When $E = 1$ $A_{M,m}(1)$ is the area enclosed by the 1 kcal contour. $Z_{\omega',\omega}$, the partition function, is the sum of the area weighted Boltzmann factors from all energy regions less than 5 kcal/mole on both ω',ω maps. (There were only two regions per map.) Areas between contours were evaluated by graphical integration. In most cases the contours rose very steeply between 1 or 2 and 5 kcal/mole, and intermediate contours could not be distinguished. In that case, the area between contour intervals

TABLE II
Selected Minimum Energy Conformations of Gp2'MeC
Dihedral Angles, Degrees

χ'	ϕ'	ω'	ω	ϕ	ψ	χ	m_1	ΔE, [a] kcal/mole
			Sugar Pucker C(3')-*endo*					
5	204	297	279	181	57	266	71	0
6	203	297	279	182	56	267	155	1.0
6	203	298	279	182	56	267	289	8.6
19	183	10	92	189	64	21	72	2.7
18	271	323	169	196	54	23	72	7.2
			Sugar Pucker C(2')-*endo*					
−32	228	292	269	178	59	68	58	5.9
49	211	255	191	172	61	61	64	6.6
25	219	43	58	187	60	62	62	14.4

[a] ΔE is the difference in energy between the given conformation and the global minimum.

of 1 kcal/mole was approximated from the total area between the 2 distinguishable contours by distributing this total area uniformly among the 1 kcal/mole intervals.

RESULTS

Minimum Energy Conformations of Gp2′MeC and 2′MeGpC

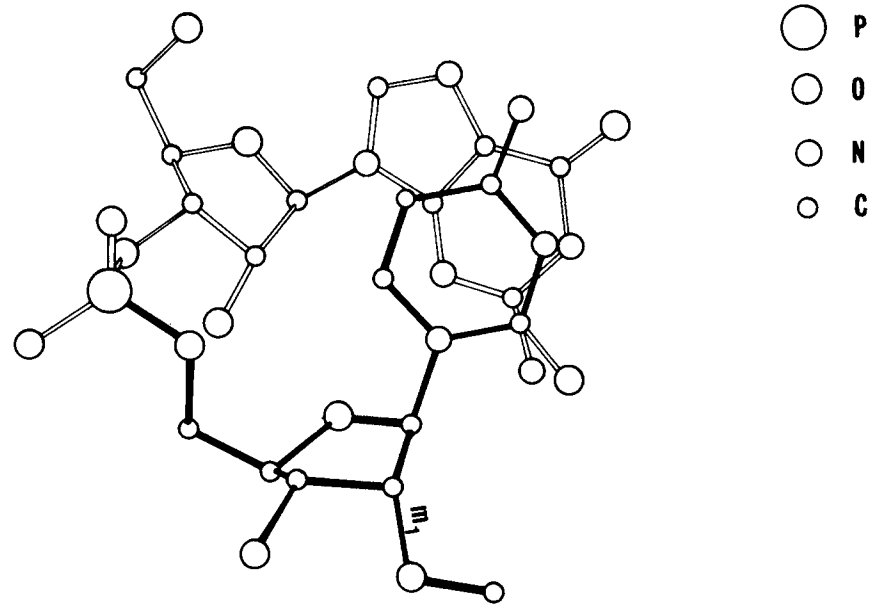
Tables II and III give results for Gp2′MeC and 2′MeGpC, respectively.

The global minimum for both molecules is like that of helical RNA. For Gp2′MeC, we find that the appended methyl group can be accommodated with virtually no changes in the backbone angles of the GpC helical conformation. The energy of Gp2′MeC at the angles of the GpC helical conformation is only 0.8 kcal/mole above the global minimum of Gp2′MeC and 1.5 kcal/mole below the GpC global minimum. Thus, the global minimum energy of Gp2′MeC is 2.3 kcal/mole lower than that of GpC. For 2′MeGpC more extensive changes in the dihedral angles of GpC are needed to accommodate the methyl in the helical conformation. The initial energy is 20.1 kcal/mole above the global minimum, but minimization produced the small necessary changes in the dihedral angles which reduced the energy. The change in χ' was responsible for most of the gain in energy. 2.4 kcal/mole was gained in the adjustment of m_1 from its starting value of 60°. A further indication that the helical conformation is less favored for 2′MeGpC than in Gp2′MeC is that the energy at the global minimum is 4.8 kcal/mole higher in 2′MeGpC. The methoxy angle m_1 prefers the neighborhood of 70° for both molecules, but for Gp2′MeC there is another minimum at 155° which is at only 1.0 kcal/mole, and no changes in backbone are needed. For 2′MeGpC, on the other hand, the energy of this second minimum is at 6.8

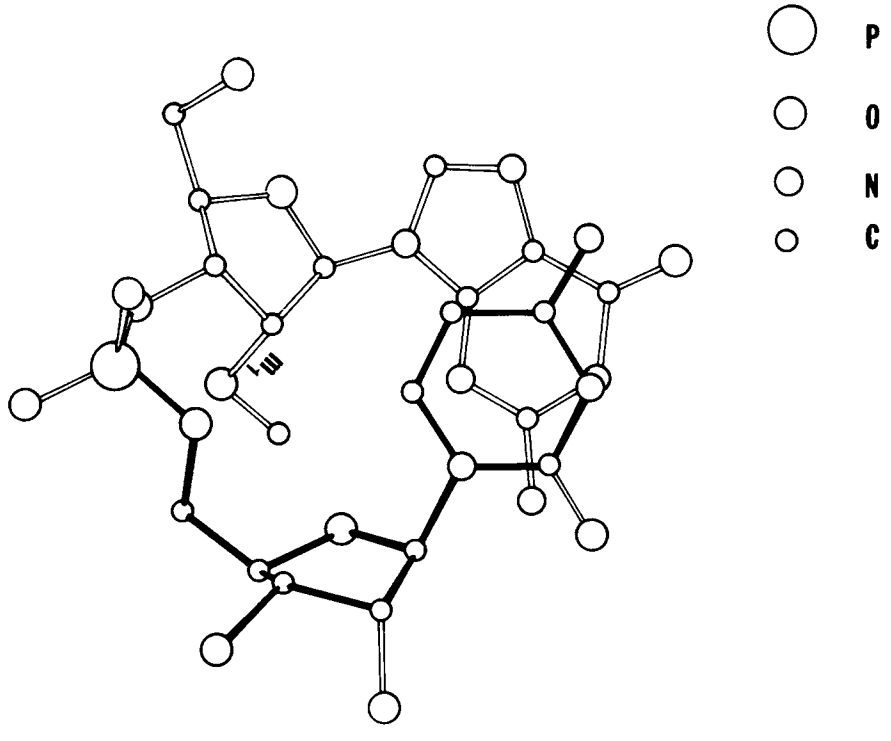
TABLE III
Selected Minimum Energy Conformations of 2′MeGpC Dihedral Angles, Degrees

χ'	ϕ'	ω'	ω	ϕ	ψ	χ	m_1	ΔE,[a] kcal/mole
			Sugar Pucker C(3′)-*endo*					
−4	204	301	271	193	54	28	76	0
−14	259	268	289	148	55	26	151	6.8
6	202	298	276	183	58	27	313	63.9
13	176	17	83	192	61	19	77	1.2
28	262	316	158	214	53	19	77	7.7
			Sugar Pucker C(2′)-*endo*					
49	198	254	194	173	61	58	67	4.5
38	276	280	289	169	56	65	176	7.4
39	268	59	89	172	61	40	175	10.5

[a] ΔE is the difference in energy between the given conformation and the global minimum.



(a)



(b)

Fig. 2. (a) Gp2′MeC in its helical RNA global minimum energy conformation (see Table II). (b) 2′MeGpC in its helical RNA global minimum energy conformation (see Table III).

kcal/mole, and rather substantial changes in backbone angles occur. The 300° region is totally inaccessible for this molecule (greater than 60 kcal/mole), but it lies at 8.6 kcal/mole for Gp2′MeC.

The next minimum has ω',ω near 20°, 80°. It occurs at 2.7 kcal/mole for Gp2′MeC, while it is at 1.2 kcal/mole for 2′MeGpC. The favored regions of m_1 follow the same pattern as the helical RNA conformation; results in Tables II and III are given only for the lowest energy of m_1.

The C(3′)-*endo* g^-t minimum energy conformers are above 7 kcal/mole and only the lowest energy of m_1 is shown.

As with GpC itself, the C(2′)-*endo* conformations are all of high energy; the B form conformation is at 5.9 kcal/mole for Gp2′MeC and 7.4 kcal/mole for 2′MeGpC. Results are again given only for the lowest energy of m_1.

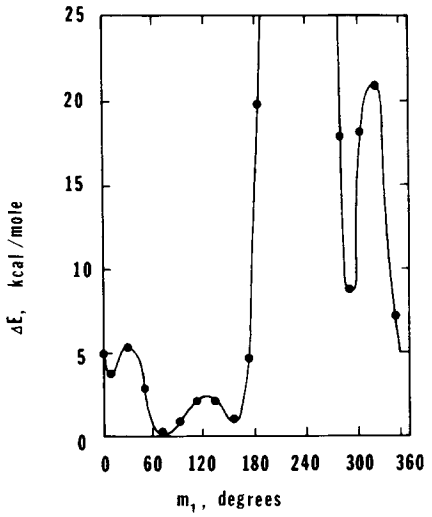
Figures 2a and b show Gp2′MeC and 2′MeGpC at their helical global minima. It is apparent that the methoxy is crowded into the inside of the helix in 2′MeGpC, as it would be in a polynucleotide, while it hangs on the outside of Gp2′MeC. This explains why delicate adjustment in the dihedral angles is needed for the group to be accommodated in the helical RNA conformation of 2′MeGpC. It causes the methoxy to be conformationally more restricted in 2′MeGpC than Gp2′MeC. The stacking of bases is slightly less for 2′MeGpC than for Gp2′MeC.

Energy Maps for Methoxy Angle, C(2′)-O(2′), m_1

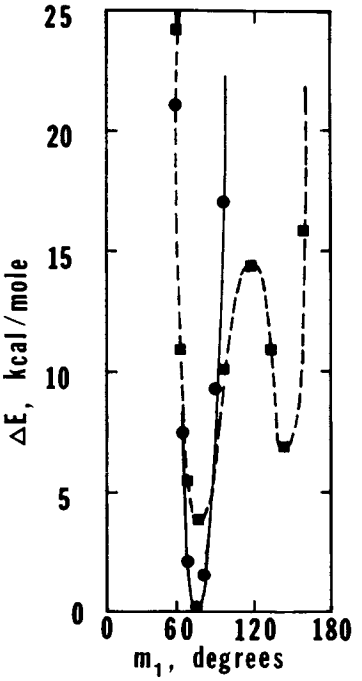
Figure 3a and b shows the energy of Gp2′MeC and 2′MeGpC, respectively, as a function of m_1. Other angles were fixed at the values they possess at their global minima. Results are similar for the 20°, 80° minima of ω',ω, and are not shown. For Gp2′MeC the entire region between 50 and 175° is of low energy, with the global minimum at 71°. The other minima are at 155 and 289°, the latter however being of high energy. With 2′MeGpC, when the other angles are fixed at the helical global minimum, only one narrow low energy region, centered at 75° is seen. When the backbone angles are fixed at the helical minimum corresponding to $m_1 = 151°$ (see Table III), a sharp, two pronged energy map is produced with a minimum at 75° and a second but considerably higher energy minimum at 151°. It is clear from the above that m_1 is much more conformationally restricted in 2′MeGpC than in Gp2′MeC. In the former case only one narrow conformation range is favored, while for Gp2′MeC a broad range encompassing two minima and more than 100° is found.

Energy Contour Maps for ω',ω

Figures 4 and 5 show low energy contours of ω' and ω for Gp2′MeC and 2′MeGpC, respectively. For 2′MeGpC it was not possible to choose one set of fixed values for the other angles that would accurately show the energy relationships between the two low energy regions. For this reason two

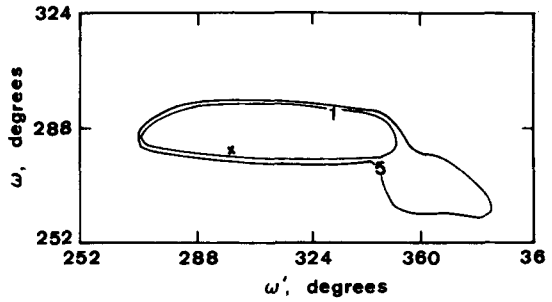


(a)



(b)

Fig. 3. (a). Energy of Gp2′MeC as a function of C(2′)-O(2′) angle m_1. Other angles set at global minimum (see Table II). (b) Energy of 2′meGpC as a function of C(2′)-O(2′) angle m_1. Other angles set at (—) helical RNA global minimum or (- - -) helical minimum for m_1 = 151° (see Table III).



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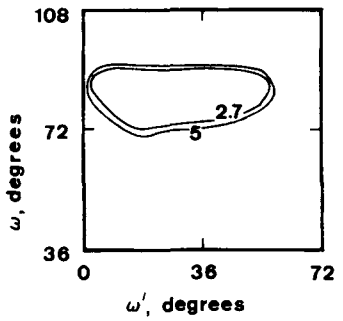

(b)

Fig. 4. Low energy contours of ω',ω for Gp2′MeC: (a) other angles fixed at helical RNA global minimum; (b) other angles fixed at second lowest energy minimum (see Table II).

separate ω',ω maps were drawn. Angles other than ω',ω were fixed at the minima of one of the two low energy regions (see Tables II and III). The same procedure was used for Gp2′MeC. Under these circumstances, the effective statistical weight of the 20°, 80° region in the map where the helical RNA angles were employed was less than 0.1%. The same is true of the statistical weight of the g^-g^- region in the map showing the conformation space near 20°, 80°. Contour energies are relative to the global minimum of each molecule. We note that the helical RNA conformation occupies a much smaller area within the 1 kcal/mole contour for 2′MeGpC than for Gp2′MeC. Statistical weights were calculated using Eq. (2), and are given in Table IV. The helical RNA-like conformation has a statistical weight of 0.96 for Gp2′MeC, while the statistical weight of this helical region is reduced to 0.59 in 2′MeGpC.

DISCUSSION

Comparison of Conformations of 2′MeGpC, Gp2′MeC, and GpC

The influence of 2′-*O*-methylation on the GpC conformation depends on whether this group is appended to the 3′ or the 5′ ribose. At first sight

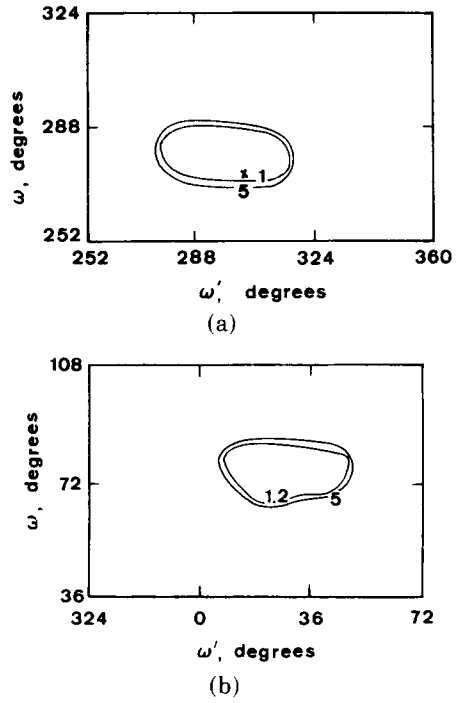

Fig. 5. Low energy contours of ω',ω for 2′MeGpC: (a) other angles fixed at helical RNA global minimum; (b) other angles fixed at second lowest energy minimum (see Table III).

it appears that the methyl substitution has little influence on GpC, since very similar minima are obtained for the methylated derivatives and GpC itself. On closer examination, however, differences emerge. The differences between GpC and Gp2′MeC are small. The most notable difference is that the energy at the global minimum is 2.3 kcal/mole lower in Gp2′MeC than in GpC. The Gp2′MeC helix is probably stabilized by attractive van der Waals interactions of the methyl with cytosine, and the adjoining ribose. In 2′MeGpC the stabilizing interactions of the methyl are outweighed by other effects. These are manifested in the following ways: (*1*) When 2′ MeGpC is set at the helical RNA global minimum of GpC, its energy prior to minimization is 20.1 kcal/mole while Gp2′MeC has an energy of only 0.8

TABLE IV
Statistical Weights for Low Energy Conformational Regions of 2′MeGpC and Gp2′MeC

Molecule	Conformational Region, ω', ω	Statistical Weight
2′MeGpC	301°, 271°	0.59
	17°, 83°	0.41
Gp2′MeC	297°, 279°	0.96
	10°, 92°	0.04

kcal/mole in the same conformation. Thus, adjustment in the dihedral angles from the GpC global minimum is needed to accommodate the methyl in the helical conformation of 2′MeGpC. A change in the glycosidic torsion angle χ' alleviates a steric repulsion between guanosine N3 and a methyl hydrogen. This accounts for a large part of the energy gain. With Gp2′MeC, on the other hand, only slight adjustment of the methoxy is needed. (2) The conformation space accessible to the methoxy angle m_1 is considerably more restricted in 2′MeGpC than in Gp2′MeC. (3) The statistical weight of the helical conformation is reduced from 0.96 in Gp2′MeC to 0.59 in 2′MeGpC. Stated in a possibly more significant way, the statistical weight of the "helix reversing" conformation[23] ($\omega'\omega \sim 20°, 80°$) is increased from 0.04 to 0.41. (4) The absolute energy of the helical RNA conformation of 2′MeGpC is 2.5 kcal/mole greater than that of GpC. These are all reflections of the crowding produced by the methyl when located in the interior of the 2′MeGpC helix.

Ribose Pucker

2′-*O*-Methylation of cytidine causes the C(2′)-*endo* pucker to be preferred over C(3′)-*endo* in 2′-*O*-methyl cytidine.[7] However, in larger structures, such as GpC[20] and its methylated derivatives, C(2′)-*endo* is not of low energy. Solution studies of polymers are consistent with this observation. Nmr studies of poly(r2′Me-cytidilic acid)[24] indicate that this polymer is in the C(3′)-*endo* conformation in solution. Furthermore, Bobst et al.[25] have ruled out a major change in sugar conformation on 2′-*O*-methylation of both riboses in ApA, which is C(3′)-*endo* in solution. Sarma (private communication) has also come to this conclusion for 2′MeApA. Thus, the C(3′)-*endo* pucker preferred by common ribonucleotides is retained on 2′-*O*-methylation.

Conformations of Methoxy Angle m_1

Allowed conformations of the methoxy angle O(2′)-C(2′) calculated in this work (Fig. 3) are in good agreement with the calculations of Prusiner et al.[8] for the C(3′)-*endo* conformation of 2′-*O*-methyl adenosine 3′ phosphate and 2′-*O*-methyl adenosine 3′, 5′ diphosphate. For the 5′ nucleotide they calculate a permitted range of 80–160°, which may be compared with the minima at 71 and 154° calculated here for Gp2′MeC. The latter minimum is just 1.0 kcal/mole higher in energy than the former, and the two are separated by a barrier of only 1 to 2 kcal/mole (depending on direction). It is interesting to note that both these conformational regions occur in the crystal of 2′-*O*-methyl cytidine.[7] In the presence of the 3′ phosphate, Prusiner et al. find the methoxy to be more restricted, within the 90–130° range. We find a similar restriction for 2′MeGpC. The minima are at 76 and 151°, but the latter minimum is at 6.8 kcal/mole. Furthermore, con-

siderable adjustment in the backbone angles is needed to accommodate the higher energy conformation, and a barrier of 7–10 kcal/mole separates the two minima.

Comparison with Studies of Base Stacking in Solution

The influence of 2′-*O*-methylation on the extent of stacking and the stability of stacked structures in solution has been investigated by nmr, ORD, and CD spectroscopy, for dinucleoside phosphates (Refs. 26–28 and R. Sarma, personal communication) and for poly(r2′Me adenylic acid)[29] and poly(r2′Me cytidylic acid).[24] In these studies, stacking is implicitly equated with the helical RNA conformation. However, other stacked conformations are of low energy, notably the "helix-reversing" conformation, so that base stacking need not be correlated with the RNA helix.

In general, there is no profound difference between the spectra of the modified and the unmodified species for dinucleoside phosphates. This agrees with the similar helical global minima calculated here for GpC and its two methylated derivatives. Stacking appears to be enhanced or diminished, to a small extent, depending on sequence, with the substituted adenosines having a tendency to reduce stacking. The ORD of GpC and 2′MeGpC were compared by Singh and Hillier[27] at room temperature. They are of similar form, with a slightly diminished amplitude in the substituted derivative at pH 7. This suggested a small decrease in stacking, which also agrees with our findings. Warshaw and Cantor[26] found the CD spectra of 2′MeCpC and CpC to be almost identical, indicating a similar stacking geometry. However, the temperature dependence of the spectra showed a more stable structure for the methylated derivative. We find a less stable helical structure for methylation at the 3′ ribose. Possibly the smaller 3′ cytidine offers less steric hindrance, so that the favorable interactions of the methyl group overcome the steric restrictions which predominate in 2′MeGpC. Drake et al.[28] studied the CD spectra and their temperature dependence on 13 3′ substituted dinucleoside phosphates, as well as three that were substituted at both riboses. (They did not examine GpC derivatives.) They drew the general conclusion that stacking is enhanced by the modification, except for adenosines, but we believe their data can also be interpreted as showing that the stacked structure may be stabilized or destabilized by the substitution without appreciable change in the base overlap. Drake et al.[28] observed that substitution at the 5′ ribose has a smaller effect than at the 3′ ribose unit, which agrees with our results. In general, it appears that a competition exists between the stabilizing interactions of the methyl group and its destabilizing effect due to steric hindrance. The bases surrounding the methyl group may determine which prevails in a polymer.

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