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# CONFORMATION OF $N^2$-METHYLGUANOSINE, A MODIFIED NUCLEOSIDE OF tRNA

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Summary: The crystal structure of $N^2$-methylguanosine ($m^2G$), a modified nucleoside of tRNA, has been determined from three-dimensional x-ray diffractometer data. The $N^2$-methyl substituent in the crystal structure is proximal to the imidazole ring, though in solution, it shows no restricted rotation across the C-N bond. From a comparison of the earlier crystal structure studies of G and $m_2^2G$ with $m^2G$, it is found that the methylation of the 2-amino group leads to altered stacking and conformation of the nucleoside. The methyl groups play a predominant role in the stacking of the bases. The molecules of $m^2G$ and $m_2^2G$ both have the syn conformation across the glycosidic bond despite the lack of $\overline{O(5')}$-H···N(3) hydrogen bond. Also, both the methylated molecules exhibit non-standard conformations across the C(4')-C(5') bond. It is suggested that such alterations in the stacking and conformation of $m^2G$, rather than destabilization of the Watson-Crick hydrogen bonding, is important in the biological role of $m^2G$ as a part of the DHU stem of tRNA.

Introduction: $N^2$-methylguanosine, $m^2G$, is a modified nucleoside that occurs at several specific locations in many tRNA's. From an examination of the available sequences of about 90 tRNA's (1), it is found that $m^2G$ occurs in 20 tRNA's at position 10, the beginning of the stem of the dihydrouridine loop. It also occurs at location 6 (in the CCA stem) in mammalian $tRNA^{Met}$, at location 9 in yeast (haploid) $tRNA^{Lys}$ and at location 26 in mammalian and yeast $tRNA^{Val}$ (1). It has been shown that $m^2G$ at position 10 can significantly affect the aminoacylation kinetics of a tRNA by synthetases and the possible role of this single methylation is

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to alter the specificity of the different reactions in which the tRNA is involved by altering their kinetic parameters (2). The introduction of alkyl groups on bases usually results in increased association (3,4). Monosubstitution of the amino groups in A, G and C results in two conformers, one capable of base-pairing in the Watson-Crick mode and the other not. The conformational preferences of these mono-substituted nucleosides have been examined at the monomeric level using x-ray diffraction (5,6) and n.m.r. (7,8) techniques. N.m.r. results for $m^6A$ and $m^4C$ indicated (8) restricted rotations across the C-N bond characterized by activation enthalpies of 11 to 18 K.cal/mol. and a 20:1 preference for distal conformation of the methyl group. This leads to preferential interference with Watson-Crick base-pairing. However, $m^2G$ and its dimethyl analog $m_2{}^2G$ showed no restricted rotation on the n.m.r. time scale (8). The present x-ray study was undertaken to find out the conformational preference of $m^2G$ in the solid state and to correlate these results with solution studies.

Methods: Crystals of the nucleoside $m^2G$ (from Sigma Chemical Co.) are triclinic, space group P1 with cell constants at (22±3)°C: $\underline{a}$ = 5.021(3), $\underline{b}$ = 10.072(2), $\underline{c}$ = 7.110(2)Å, $\alpha$ = 75.66(2), $\beta$ = 106.74(4), $\gamma$ = 101.09(3)°, Z = 1, $D_{obsd.}$ = 1.58, $D_{calc.}$ = 1.57 g $cm^{-3}$, formula of the asymmetric unit, $C_{11}H_{15}N_5O_5 \cdot H_2O$. Three dimensional intensity data (1645 reflections to the limit $2\theta$ = 165° for CuKα radiation) were collected using a GE XRD-6 diffractometer and Ross filters. The structure was solved by the rotation function method (9,10) using the program ROTRAN (11) and refined to an R of 0.043 using the least-squares method with the block-diagonal approximation. The locations of all the hydrogens except the three on the methyl group were obtained from electron-density difference

maps; their positional and individual isotropic thermal parameters were included in the refinement. Table 1 gives the positional coordinates and their estimated standard deviations.

Results and Discussion: Figure 1 illustrates the bond distances, bond angles and conformational angles of the molecule. The methyl group on N(2) is proximal to the imidazole ring, thusly enabling Watson-Crick base pairing with C. This molecule exhibits the syn conformation across the glycosidic bond with $\chi_{CN}$ =-114.6°. The sugar ring has the C(2')-endo pucker. The conformation across the C(4')-C(5') bond is not the preferred gauche$^+$ conformation, but the gauche$^-$ conformation with the torsion angles $\phi_{OO}$ = 165.6° and $\phi_{OC}$ =-76.9°. The pseudo-rotation parameters (12) are P = 167.8° and $\theta_{max}$ = 35.3°. Though there is no base association involving hydrogen bonds, extensive hydrogen bonding exists between the base and the sugar moiety (Figure 2). The bases are stacked with the methyl groups strongly participating in stacking; the planes of the bases are 3.4Å apart (Figure 3).

It is interesting to note that two independent molecules in the crystal structure of guanosine (13) have the preferred anti conformation, inosine (13-17) without the exocyclic amino group exhibits both anti and syn conformations in different crystal forms, but both the substituted guanosines $m^2G$ and $m_2^2G$ (18) show a preference for the syn conformation. This is particularly significant since the syn conformation and the gauche$^+$ orientation of O(5') in purine nucleosides are usually stabilized by intramolecular hydrogen bonding from O(5')-H(O5')···N(3) (18), but such an intramolecular hydrogen bonding is not possible for $m^2G$ and $m_2^2G$ due to the blocking of N(3) by the methyl group. Both $m^2G$ and $m_2^2G$ exhibit the non-standard conformations, gauche$^-$ and trans, respectively, for the exocyclic oxygen O(5').

Table 1. Atomic coordinates and estimated standard deviations.

| Atom | x | y | z | Atom | x | y | z |
|---|---|---|---|---|---|---|---|
| O(6) | 0.4153(8) | -0.3094(3) | 0.6348(5) | C(2') | 0.9098(8) | 0.3535(4) | 0.4764(6) |
| O(W) | 1.1418(12) | -0.2444(4) | 0.0126(7) | C(3') | 0.9119(8) | 0.4887(4) | 0.3195(6) |
| O(1') | 0.4566(6) | 0.3572(3) | 0.2585(4) | C(4') | 0.6449(9) | 0.4617(4) | 0.1598(6) |
| O(2') | 1.0701(7) | 0.3626(3) | 0.6715(5) | C(5') | 0.6892(10) | 0.4031(4) | -0.0050(6) |
| O(3') | 0.8944(7) | 0.5997(3) | 0.4045(3) | H(O2') | 1.024(11) | 0.409(6) | 0.717(8) |
| O(5') | 0.8124(8) | 0.5082(3) | -0.1434(5) | H(O3') | 1.059(12) | 0.626(6) | 0.493(8) |
| N(1) | 0.6525(8) | -0.1774(4) | 0.4091(6) | H(O5') | 0.728(15) | 0.563(7) | -0.202(10) |
| N(2) | 0.8970(10) | -0.0637(4) | 0.1739(6) | H(N1) | 0.660(15) | -0.258(7) | 0.372(10) |
| N(3) | 0.7273(8) | 0.0682(3) | 0.3275(5) | H(N2) | 0.895(15) | -0.142(7) | 0.134(10) |
| N(7) | 0.3394(8) | -0.0302(4) | 0.7057(6) | H(C8) | 0.321(14) | 0.164(7) | 0.739(10) |
| N(9) | 0.5193(7) | 0.1646(3) | 0.5246(5) | H(C1') | 0.540(10) | 0.356(5) | 0.544(7) |
| C(2) | 0.7579(9) | -0.0543(4) | 0.3029(6) | H(C2') | 0.993(9) | 0.287(4) | 0.431(6) |
| C(4) | 0.5858(9) | 0.0563(4) | 0.4668(6) | H(C4') | 0.553(9) | 0.540(5) | 0.102(7) |
| C(5) | 0.4747(9) | -0.0621(4) | 0.5800(6) | H(C3') | 1.078(11) | 0.500(5) | 0.262(8) |
| C(6) | 0.5048(9) | -0.1911(4) | 0.5508(7) | H1(C5') | 0.835(12) | 0.333(6) | 0.064(9) |
| C(8) | 0.3710(9) | 0.1049(4) | 0.6680(6) | H2(C5') | 0.496(14) | 0.349(7) | -0.080(10) |
| C(10) | 1.0098(12) | 0.0581(5) | 0.0472(8) | H1(OW) | 1.187(17) | -0.197(8) | -0.044(12) |
| C(1') | 0.5965(8) | 0.3119(4) | 0.4616(6) | H2(OW) | 1.055(20) | -0.324(10) | -0.039(15) |

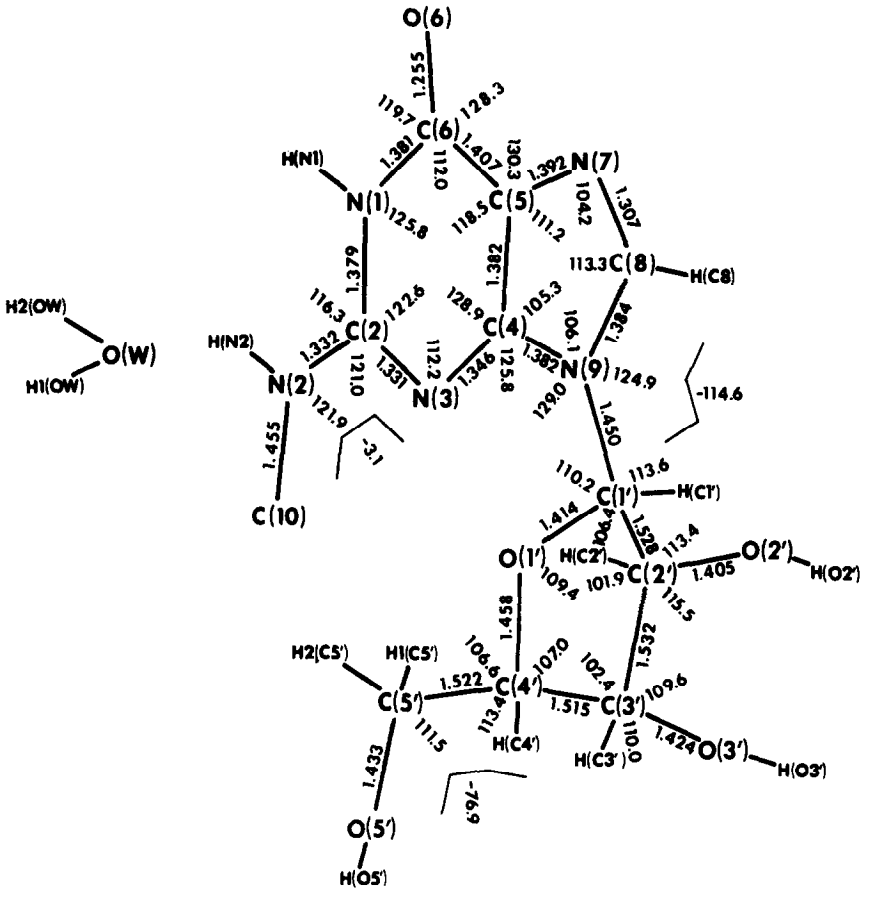


Figure 1. The bond distances, bond angles, and conformational angles of $m^2G$. The C(6)-O(6) bond is significantly longer than the usual value of 1.23 to 1.24Å found in other guanosine derivatives. The average e.s.d.'s of the bond distances and angles are, respectively, .006Å and 0.3° for non-hydrogen atoms.

The proximal conformation is seen for $m^2G$ in the solid state, but n.m.r. results (8) show no restricted rotation, quite unlike $m^6A$ and $m^4C$. The near equivalence of the geometry of the amide and the exocyclic C-N bond lengths of $m^2G$, $m_2^2G$ (18) and mono-substituted adenines (5) indicate the lack of such restricted rotations is not due to the lack of conjugation. In solution, $m^2G$ forms a stable complex with C and indicates no measurable destabilization to a hydrogen bonded complex due to methylation;

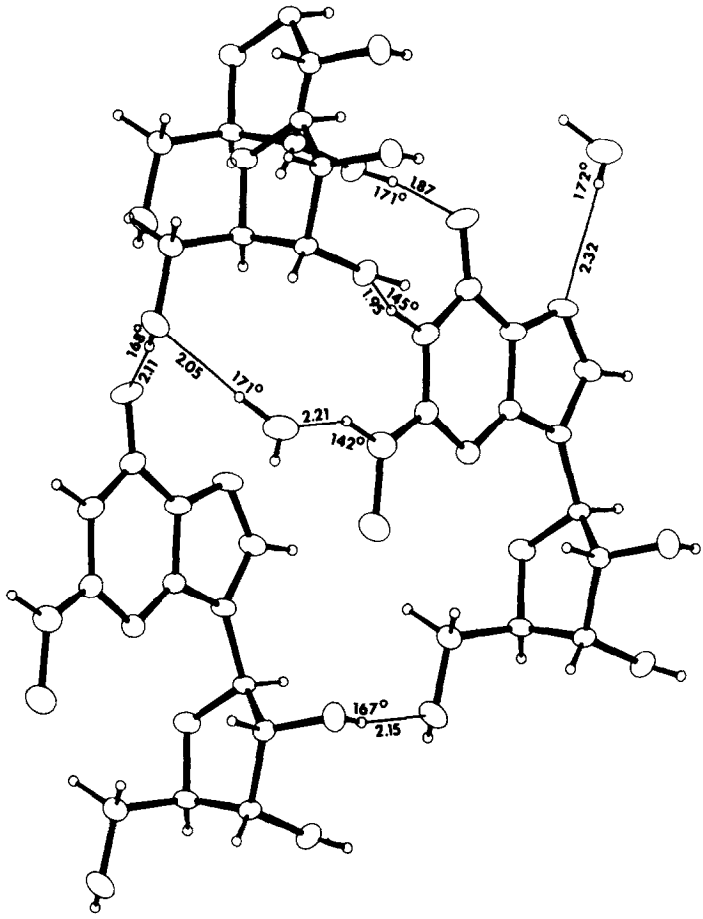


Figure 2. A view of the hydrogen bonding in the crystal structure of $m^2G$. The hydrogen to the acceptor distances (Å) and angles (°) at the hydrogen are illustrated in the figure.

the $m^2G \cdot C$ complex behaves nearly identically with G·C (8). Consequently at the monomer level, both in the solid and in solution the methylation does not seem to affect the Watson-Crick hydrogen bonding. It is also known that $m^2G$ does take part in tertiary hydrogen bonding in $tRNA^{Phe}$ (see, for example 19).

Poly($m^2G$), poly(2-methyl I) and poly(2-methylthio I) do not form a stable double helix with poly(C) (20-22), suggesting an inability of the methyl group to fit into the small groove of the

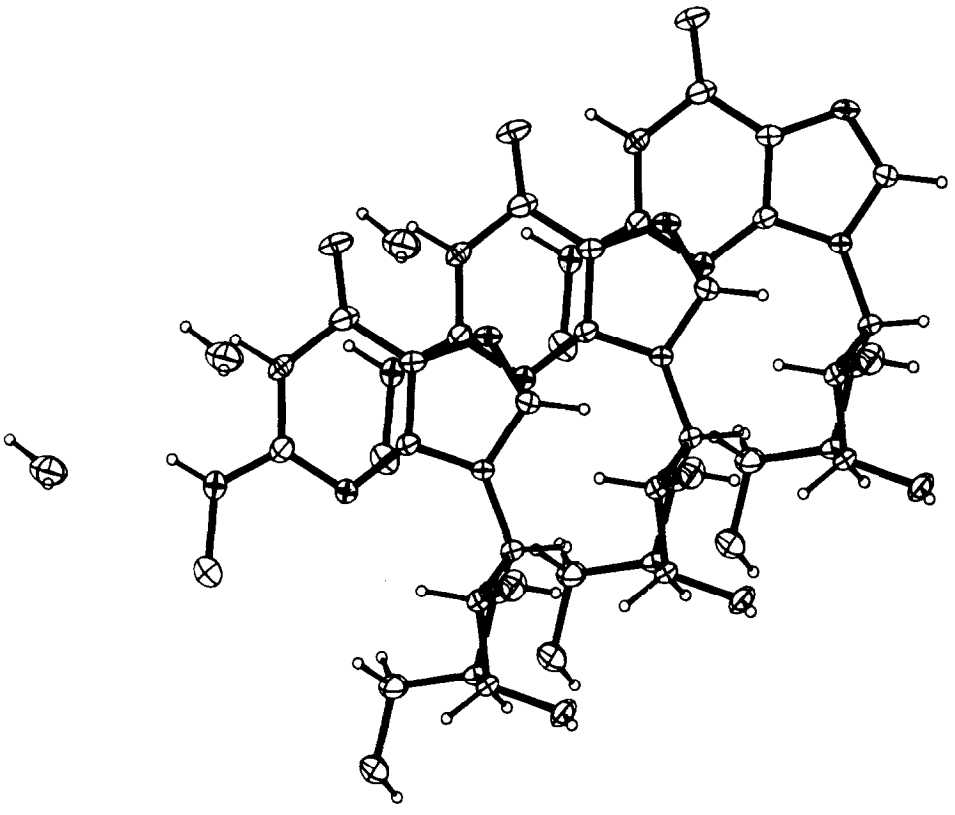

Figure 3. The base stacking of $m^2G$ molecules viewed normal to the guanine ring. The planes of the bases are 3.4Å apart.

double helix (8). Additional reason for this inability may be due to the altered stacking properties of $m^2G$ and $m_2^2G$ as compared to G. In the structure of guanosine dihydrate (13), there is extensive overlap between entire purine rings with little N(2) participation, but both in $m_2^2G$ (18) and $m^2G$, the N(2)-methyl groups play a predominant role in stacking interactions. A comparison the crystal structures of G (13), $m^2G$ and $m_2^2G$ (18) show that alkylation of the 2-amino groups lead to alterations in the stacking and conformation of the nucleosides. It is interesting to note that while $m^2G$ occurs instead of $m_2^2G$ at position 26 in some tRNA's, $m_2^2G$ which would hinder the Watson-Crick hydrogen bonding

has never been found at position 10, corresponding to the location of $m^2G$. These results suggest that such altered stacking and conformation, rather than destabilized Watson-Crick base pairing, might be important for the specific role played by $m^2G$ in the recognition of synthetases by some tRNA's (2).

During the preparation of the manuscript, we came across a recent abstract (23) containing a brief statement of another x-ray analysis of $m^2G$.

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