Modified Nucleosides and Conformation of Anticodon Loops: Crystal Structure of t^6A and g^6A†

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ABSTRACT: Accurate x-ray crystallographic investigations of the structure of t^6A, a hypermodified nucleoside that occurs on the 3′ side of anticodons in tRNAs, and g^6A, its glycine analogue, have been carried out. Crystals of t^6A and g^6A are monoclinic, space group $P2_1$ with cell dimensions, a = 10.447 (1), b = 16.894 (2), c = 5.001 (1) Å, β = 94.69 (5)° for t^6A and 7.572 (1), 21.015 (4), 4.909 (1) Å, 106.32 (2)°, respectively, for g^6A. The conformation about the glycosidic bond is anti for both the molecules with x_{CN} = 33.1° for t^6A and 4.0° for g^6A. The N^6 substituent is distal to the imidazole ring and the conformation of the amino acid side chain so arranges to form a bifurcated hydrogen bond from N(amino acid)–H to N(1) of adenine and to O^γ for t^6A, and O(carboxyl) for g^6A. This orientation of the threonine side chain of t^6A is also found in two other crystal forms with different electrostatic and hydrogen bonding environments. Using the known conformation of $tRNA^{Phe}$ and replacing Y by t^6A in its preferred conformation, it is found that the methyl group of t^6A will project toward the 3′ end of the anticodon loop and will limit the stacking of t^6A with the adjacent adenine. The carboxyl, projecting on the opposite side toward the third position of the anticodon base, has the potential to hydrogen bond to it or the backbone. These interactions arising from t^6A could lead to altered conformations for the anticodon loops that are different and selective to the codons being read.

The modified bases, nucleosides, and their analogues have, as components of tRNA and as "free agents," interesting biological and pharmacological properties. The modified nucleoside N^6-(*N*-threonylcarbonyl)adenosine[1], t^6A, occurs in several tRNAs (Chheda et al., 1969; Schweizer et al., 1969) which respond to the codons beginning with adenine (Takemura et al., 1969; Ishikura et al., 1969; see, however, Brambilla et al., 1976) and occupies a position adjacent to the 3′ end of the anticodons in the tRNAs mentioned above. The glycine analogue g^6A of t^6A has been isolated (Schweizer et al., 1970) from enzyme digests of unfractionated yeast tRNA, but it is not clear whether it occupies a position analogous to that of t^6A in tRNA (see also Cunningham and Gray, 1974; Elkins and Keller, 1974). This paper describes the results of our crystallographic studies on t^6A and g^6A and forms a part of our investigation relating the three-dimensional structure of modified components of tRNA to their biological activity.

Experimental Procedure

Synthetic samples of t^6A and g^6A (Chheda and Hong, 1971) were used for crystallization. After repeated attempts, *one* crystal of t^6A was obtained from water–ethanol solutions. This crystal had an irregular shape with a maximum thickness of about 0.3 mm. Crystals of g^6A were obtained readily from 50% water–methanol solutions.

Data Collection. The unit cell dimensions were refined (and their standard deviations estimated) by a least-square refinement of the 2θ values of more than 40 reflections at large 2θ angles, where the peaks from Cu $K\alpha_1$ and Cu $K\alpha_2$ could be distinguished. The relevant cell data for crystals of t^6A and g^6A are given in Table I. Complete three-dimensional intensity data were measured on a GE XRD 6 diffractometer by the stationary crystal–stationary counter technique (Furnas and Harker, 1955) using a 5° take-off angle. Balanced Ni–Co filters were used for monochromatization. The intensities were corrected for the Lorentz polarization and α_1–α_2 corrections. The difference in absorption as a function of ϕ, as measured for the axial reflections, was within the errors of observation; hence, no absorption correction was applied.

Phase Determination. The basis of the phase determining procedures is the multi-solution technique, using a modified version of the program MULTAN (Germain et al., 1971). The structure determination of t^6A turned out to be neither easy nor automatic. Three reflections 9 4 $\bar{2}$, 9 0 1, and 0 3 1 were used to fix the origin. Their $|E|$'s and assigned phases are, respectively, 3.15 and 45°, 3.01 and 0°, and 1.48 and 0°. The first reflection was also used for enantiomer discrimination. Three other reflections, 3 14 $\bar{1}$, 4 12 $\bar{2}$, and 9 6 1 were used to generate 64 sets of phases for 404 reflections of $|E|$'s > 1.25. These sets were sorted on their figures of merit; the first ten had their figures of merit ranging from 1.21 to 1.19. The corresponding E maps were calculated, but no structural solution could be obtained from these maps. An $(|E|^2 - 1)$ map was calculated from which the approximate orientation of the planar part of the molecule was derived. Using this orientation as a basis for searching the location of the molecule, the E maps were once again examined. The fourth set from the top with a figure of merit of 1.20 yielded a recognizable adenine ring with the ureido group. However, the peaks corresponding to this moiety ranged in peak heights from 300 to 800 (arbitrary units). Though there were several other peaks (ranging from 1800 to 3600) stronger than the ones that made chemical sense, it was felt that the adenine moiety might be useful for obtaining a better set of phases. Also, the peaks that made sense for the

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[1] The abbreviations used are: t^6A, N^6-(*N*-threonylcarbonyl)adenosine; g^6A, N^6-(*N*-glycylcarbonyl)adenosine; (tc^6Ade), N^6-(*N*-threonylcarbonyl)adenine; (gc^6Ade), N^6-(*N*-glycylcarbonyl)adenine; i^6A, N^6-(Δ^2-isopentenyl)adenosine; ms^2i^6A, N^6-(Δ^2-isopentenyl)-2-methylthioadenosine; $(tc^6Ade)^-K^+ \cdot 4H_2O$, crystal of the potassium salt of tc^6Ade tetrahydrate; $(gc^6Ade)^-K^+ \cdot H_2O$, crystal of the potassium salt of gc^6Ade monohydrate; $(tc^6Ade)^-Rb^+ \cdot 4H_2O$, crystal of the rubidium salt of tc^6Ade tetrahydrate; ac^6Ade, N^6-(*N*-alanylcarbonyl)adenine.

TABLE I: Crystallographic Data for t^6A and g^6A.

	t^6A	g^6A
Formula	$C_{15}H_{20}O_8N_6$	$C_{13}H_{16}O_7N_6$
a (Å)	10.477 (1)	7.572 (1)
b (Å)	16.894 (2)	21.015 (4)
c (Å)	5.001 (1)	4.909 (1)
β (deg)	94.69 (5)	106.32 (2)
Space group	$P2_1$	$P2_1$
Z	2	2
μ (cm^{-1})	11.09	11.72
D_{obsd} (g cm^{-3})	a	1.60
D_{calcd} (g cm^{-3})	1.56	1.63
Color	Transparent	Transparent
Mount, along ϕ axis	c^*	b^*
Dimensions of crystal (mm)	Irregular 0.1–0.3	0.44 × 0.23 × 0.16
No. of reflections (2θ = 164° for Cu Kα)	2035	1710
No. less than 2σ	93	57
$R = \frac{\Sigma\|\|F_o\| - \|F_c\|\|}{\Sigma\|F_o\|}$	0.041	0.046
	Temp = 22 ± 3 °C	
	Cu Kα = 1.54051 Å	

[a] Only one crystal was available; no density measurements were made for this compound.

TABLE II: Atomic Coordinates of Atoms in t^6A.[a]

	x	y	z
O(10)	8503(2)	−3537	−2319(6)
O(13a)	10943(3)	−4316(2)	1976(6)
O(13b)	12183(2)	−4460(2)	−1389(5)
O(14)	12544(2)	−2635(2)	2483(5)
O(1′)	4275(2)	−316(2)	8274(6)
O(2′)	6290(2)	1057(2)	5174(6)
O(3′)	4722(2)	1542(2)	8935(5)
O(5′)	2163(3)	−62(2)	4202(6)
N(1)	9101(3)	−1741(2)	3345(7)
N(3)	8140(3)	−733(2)	5985(7)
N(6)	7870(3)	−2656(2)	690(6)
N(7)	5558(3)	−1890(2)	2910(8)
N(9)	5798(3)	−837(2)	5642(7)
N(11)	10038(3)	−2882(2)	239(6)
C(2)	9128(3)	−1110(2)	5005(9)
C(4)	7016(3)	−1058(2)	5114(7)
C(5)	6846(3)	−1710(2)	3422(6)
C(6)	7959(3)	−2039(2)	2499(6)
C(8)	4966(3)	−1349(2)	4259(8)
C(10)	8838(3)	−3053(2)	−577(7)
C(12)	11111(3)	−3220(2)	−1046(6)
C(13)	11450(3)	−4066(2)	−185(6)
C(14)	12309(3)	−2698(2)	−362(7)
C(15)	12118(4)	−1864(2)	−1441(9)
C(1′)	5439(3)	−139(2)	7178(7)
C(2′)	5185(3)	594(2)	5407(6)
C(3′)	4172(3)	1040(2)	6852(7)
C(4′)	3452(3)	381(2)	8207(7)
C(5′)	2141(3)	168(2)	6923(8)

[a] Values × 10^4. Shifts related to y coordinates of O(10). Standard deviations given in parentheses relate to the last digit.

trial structure contained two sets of maxima close to each other; we ignored this doubling, and the appropriate peaks were chosen using stereochemical knowledge. The trial structure with 15 atoms gave an R of 0.49. From a series of least-squares refinement and electron-density difference maps, the entire structure was obtained (R = 0.20).

The crystal structure of g^6A was obtained readily using MULTAN. Thirty-two phase sets were generated using 380 $|E|$'s (>1.20); the set with the highest figure of merit of 1.0843 yielded the location of 18 atoms corresponding to the adenine ring, the ureido moiety and parts of the ribose. The R value for this trial structure was 0.39. The other 8 nonhydrogen atoms were obtained from successive electron-density difference maps (R = 0.17).

Refinement of the Structures. The positional and isotropic thermal parameters of the nonhydrogen atoms in t^6A and g^6A were subjected to several cycles of least-squares refinement (with block-diagonal approximation) until the discrepancy index R ($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$) dropped to 0.11 for t^6A and g^6A. Additional refinement with individual anisotropic thermal parameters reduced the R index to 0.063 and 0.067 for t^6A and g^6A, respectively. Electron-density difference maps at this stage revealed the locations of all the hydrogen atoms in the respective structures. Their positional and individual isotropic thermal parameters were included in further refinements, yielding final R values of 0.041 for t^6A and 0.046 for g^6A. Refinement was terminated at this point when the shifts of the atomic parameters were 0.1 times the estimated standard deviation of the corresponding parameters for nonhydrogen atoms and 0.3 times the estimated standard deviations for the hydrogen atoms. The differential synthesis weighting ($w = 1/f_c$ where f_c = scattering factor of carbon) was used for minimizing $\Sigma w(|F_o| - (1/k)|F_c|)^2$. For the stationary-crystal stationary counter data, this scheme yields a flat residual over the entire ($\sin\theta/\lambda$) range. The scattering factors used for the O, N, and C atoms are those given in International Tables (1968, 1974). For the hydrogens, the scattering factors given by Stewart et al. (1965) were used.

Results

Structure of t^6A. The final positional parameters for nonhydrogen atoms are given in Table II. The hydrogen atom parameters, the observed and calculated structure factors, and thermal parameters are provided in supplementary material (see paragraph at the end of paper regarding supplementary material). The bond lengths and angles (involving nonhydrogen atoms) are illustrated in Figures 1 and 2. Bond lengths and angles involving the hydrogen atoms fall in the usual range for x-ray determinations.

Bond Distances and Angles. The dimensions of the adenosine moiety are in general agreement with the values usually found in other adenosine structures. Substitution at N(6) results in a slight elongation of the C(6)–N(6) bond, as pointed out by us elsewhere (Parthasarathy et al., 1974a); however, the increase in the length of this bond in t^6A is considerably more than what has been observed previously. The C(8)–N(9) bond in t^6A is 0.027 Å longer than in $(tc^6Ade)^-K^+\cdot 4H_2O$ (Parthasarathy et al., 1974a); this difference, along with small changes in the ring angles in the imidazole moiety, indicates some electron delocalization and rearrangement in t^6A compared with $(tc^6Ade)^-K^+\cdot 4H_2O$.

The bond distances and angles in the threonine moiety agree well with the dimensions of amino acids obtained in other studies on compounds containing threonine moiety (Shoemaker et al., 1950; Mallikarjunan et al., 1969; Parthasarathy et al., 1974a). The carboxyl group is un-ionized, a result not wholly unexpected. A survey of the crystal structures of amino acids (Parthasarathy, unpublished) and linear peptides (Chen and Parthasarathy, 1977) shows that they all exhibit the zwitterionic form in the solid state (for an earlier survey, see Marsh and Donohue, 1967). In t^6A, N(11) is not very basic but N(1) is and, consequently, one may expect a zwitterionic form for t^6A in which the carboxyl group is ionized and the N(1) of adenine becomes $(\geqslant N(1)\text{–}H)^+$. In fact, AMP takes up such a zwitterionic form (Sundaralingam, 1966) and similar

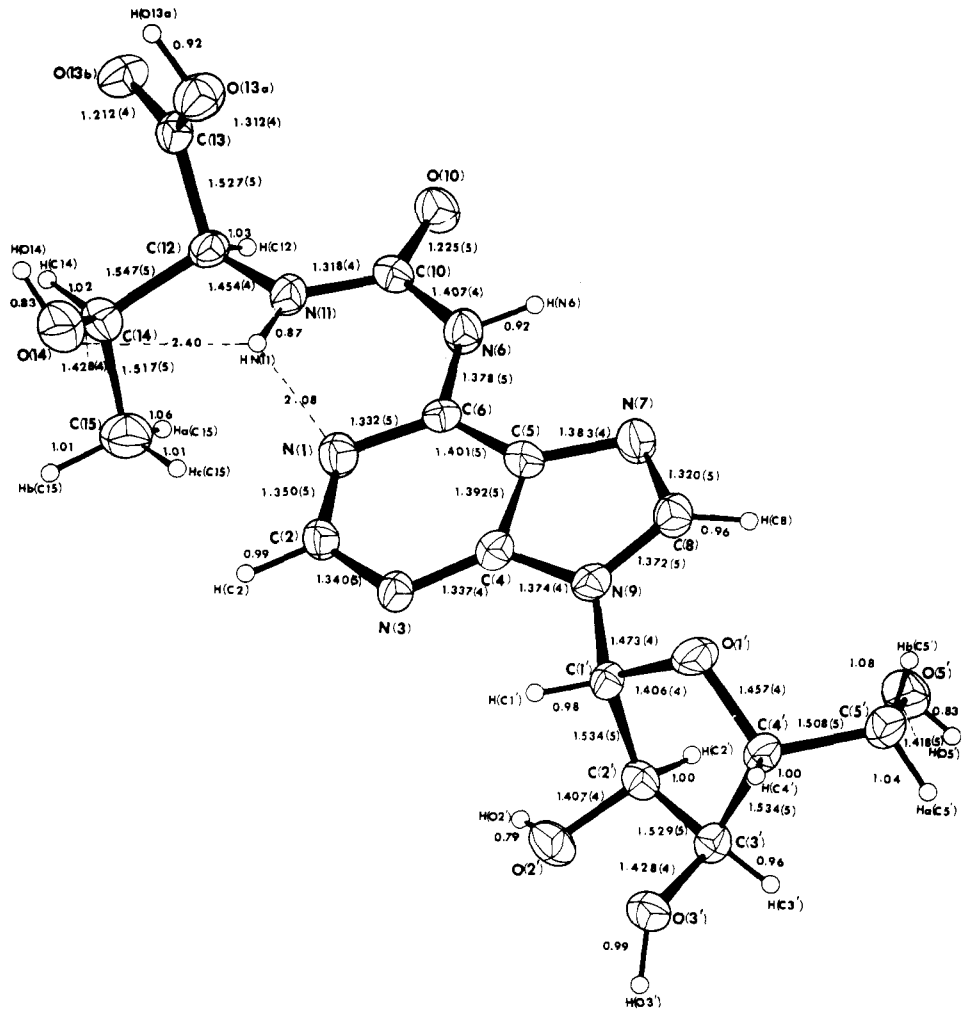


FIGURE 1: Bond distances (Å) in t^6A. The estimated standard deviations given in parentheses refer to the last digit. Note the bifurcated hydrogen bonding N(11)-H(N11)-O(14)[-N(1)].

results are seen in other nucleotides also (for a review, see Voet and Rich, 1970). The modified nucleoside t^6A cannot take up such a zwitterionic form since N(1) is involved in hydrogen bonding intramolecularly to HN(11) which is held rigidly in a particular orientation by a series of conjugated bonds (Parthasarathy et al., 1974a, 1976b).

The ureido group exhibits a remarkable degree of delocalization of electrons; this delocalization seems to depend on the nature of the substituents. In urea (Caron and Donohue, 1969), the C=O and C—N bonds are 1.270 (6) Å and 1.326 (1) Å long. Symmetrical substitution of urea with sp^2 atoms at the nitrogens as in carbohydrazide (Domiano et al., 1972) or with sp^3 atoms as in biotin (DeTitta et al., 1976) and dethiobiotin (Chen et al., 1976) increases the C—N bond lengths to 1.346–1.350 Å and decreases the C=O lengths to 1.242–1.244 Å. If the substitution of urea is asymmetric with one nitrogen carrying a sp^2 atom and another a sp^3 atom as in ureidopurines, the C=O bond and the C—N bond that carries the sp^3 substituent become slightly shorter than the urea bonds. The other C—N bond is considerably elongated to 1.407 Å in t^6A and 1.433 Å in N^1-(*N*-methylcarbomoyl)-N^3-methyl-5,6-dihydrouracil (Parthasarathy et al., 1973). In spite of this delocalization of electron density, the ureido group is planar to the precision of our analysis and is nearly in the plane of the adenine ring.

Conformation of t^6A. The N^6 substituent is "distal" (trans) to the imidazole ring. This orientation around the C(6)–N(6) bond leads to the formation of an intramolecular hydrogen bond from N(11)–H(N11) to N(1) giving rise to a planar six-membered ring. The importance of this conformation has been discussed by us elsewhere (Parthasarathy et al., 1974a,b, 1976a). The t^6A nucleoside has the anti conformation in the notation of Donohue and Trueblood (1960); the χ_{CN} angle (C(8)–N(9)–C(1′)–O(1′)) (Sundaralingam, 1969) is 33.1°. The ribose has the C(2′)-endo conformation. The best four atom least-squares plane is through C(3′), C(4′), O(1′), and C(1′); C(2′) and C(5′) deviate from this plane by 0.494 Å and 1.108 Å, respectively. The pseudo-rotation parameters (Altona and Sundaralingam, 1972) are $\tau_m = 31.8°$ and $P = 157.8°$. Since the torsion angle about the C(4′)–O(1′) bond is only 2.0°, the conformation of the sugar is ^2E (Altona and Sundaralingam, 1972). The torsion angles about the C(4′)–C(5′) bond denoted by ϕ_{OO} and ϕ_{CO} are −67.7° and 57.3° and show that the preferred gauche–gauche conformation across the C(4′)–C(5′) bond prevails in t^6A also.

Hydrogen Bonding. The hydrogen bonding and molecular packing are illustrated in Figures 3 and 4. The distances and angles involved in hydrogen bonding are deposited. There are seven polar hydrogens on t^6A that can take part in hydrogen bonding; all are found to do so. The hydrogen on C(1′) is involved in a weak C–H···O contact; this is not like the usual C(8)–H···O contacts in nucleosides since C(1′)–H bond is not

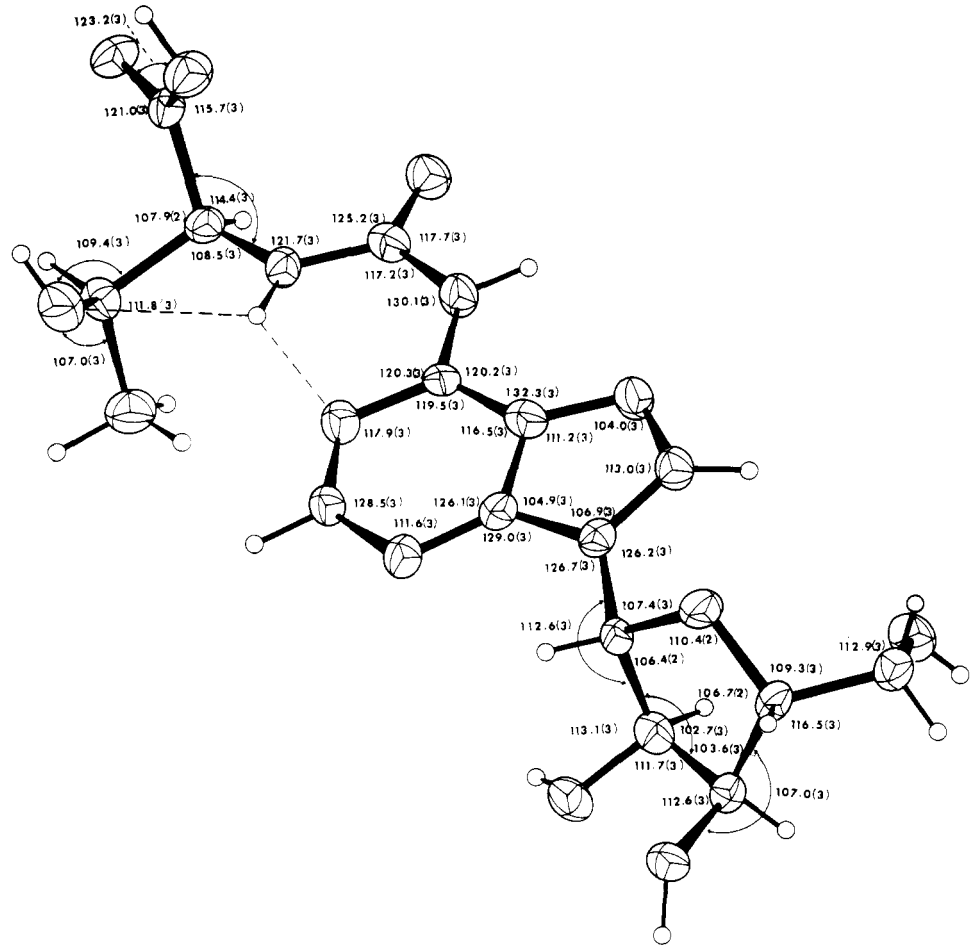


FIGURE 2: Bond angles (°) in t^6A.

TABLE III: Atomic Coordinates of Atoms in g^6A.[a]

	x	y	z
O(10)	3608(4)	7880	1665(7)
O(13a)	−3240(4)	8020(2)	263(8)
O(13b)	−1969(4)	7415(2)	4034(7)
O(1′)	8785(4)	5114(2)	15772(8)
O(2′)	5650(5)	4227(2)	15629(8)
O(3′)	7928(5)	3531(2)	13357(7)
O(5′)	11146(6)	4800(3)	12257(10)
N(1)	2338(5)	6525(2)	6943(9)
N(3)	3346(5)	5755(2)	10648(8)
N(6)	4332(5)	7112(2)	5002(8)
N(7)	7404(2)	6361(2)	9210(9)
N(9)	6668(5)	5640(2)	12086(8)
N(11)	1281(5)	7366(2)	2790(8)
C(2)	2075(6)	6088(2)	8787(11)
C(4)	5048(6)	5893(2)	10526(9)
C(5)	5520(6)	6342(2)	8737(10)
C(6)	4042(6)	6661(2)	6878(9)
C(8)	8027(6)	5947(2)	11222(10)
C(10)	3035(5)	7470(2)	3070(9)
C(12)	−164(6)	7745(2)	974(10)
C(13)	−1869(6)	7704(2)	1954(9)
C(1′)	6895(6)	5169(2)	14411(9)
C(2′)	6198(6)	4509(2)	13372(10)
C(3′)	7920(6)	4197(2)	12998(9)
C(4′)	9422(6)	4485(2)	15399(10)
C(5′)	11300(7)	4515(2)	14902(12)

[a] The entries in the table are values × 10^4. Standard deviations given in parentheses refer to the last digit. The shifts in y for all atoms are related to that of O(10).

expected to undergo such polarization. The hydrogen H(N11) is involved in an intramolecular bifurcated hydrogen bond to N(1) and O(14).

It is interesting to note that there is no adenine–adenine interaction, either by way of hydrogen bonding or stacking. All intermolecular contacts other than those already discussed are greater than the sum of the corresponding van der Waals' radii; the shortest of these contacts are O(13a)···O($2'^{ii}$), 3.180 Å, and O(10)–C($5'^{vii}$), 3.208 Å.

Structure of g^6A. The final positional parameters for nonhydrogen atoms are given in Table III. The hydrogen atom parameters, the observed and calculated structure factors and thermal parameters are provided in the supplementary material (see paragraph at the end of this paper regarding supplementary material). Figures 5 and 6 illustrate the bond lengths and angles of this molecule. Bond lengths and angles involving the hydrogen atoms fall in the usual range for x-ray determinations.

Bond Distances and Angles. The molecular structure and conformation of g^6A is very similar to that of t^6A. Consequently, most of the comments on features of t^6A apply equally well to the structure of g^6A.

Conformation of g^6A. The N^6 substituent is "distal" as in t^6A. The conformation of the glycine moiety is different from that found in $(gc^6Ade)^-K^+\cdot H_2O$ (Parthasarathy et al., 1974c, 1976b). The carboxyl group in $(gc^6Ade)^-K^+\cdot H_2O$ is nearly perpendicular to the ureido group whereas in g^6A, it is nearly in the plane of the ureido group. (For torsion angles, see supplementary material.) The approximate coplanarity of the

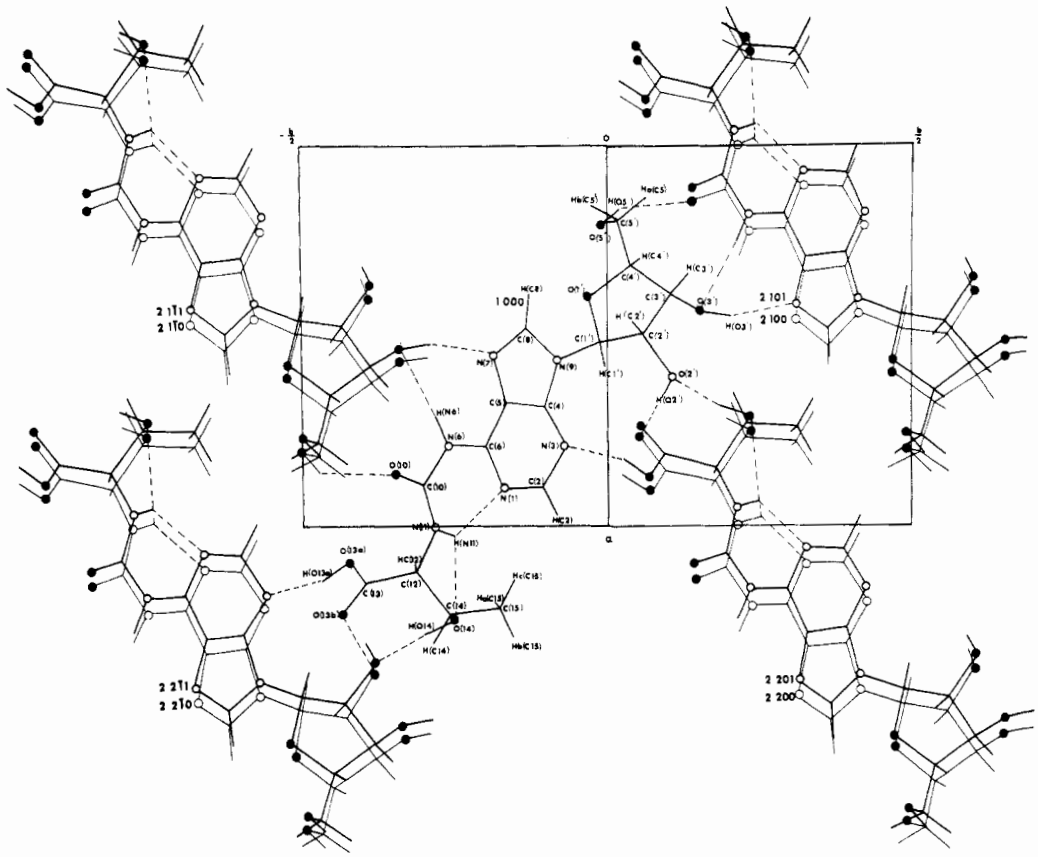


FIGURE 3: Hydrogen bonding, viewed down c axis. The open circles are nitrogen atoms and the filled ones are oxygen atoms. The numbers adjacent to the molecules (e.g., 2 $\bar{2}$11) denote the equivalent molecules related by symmetry and translations (see code for translations, supplementary material).

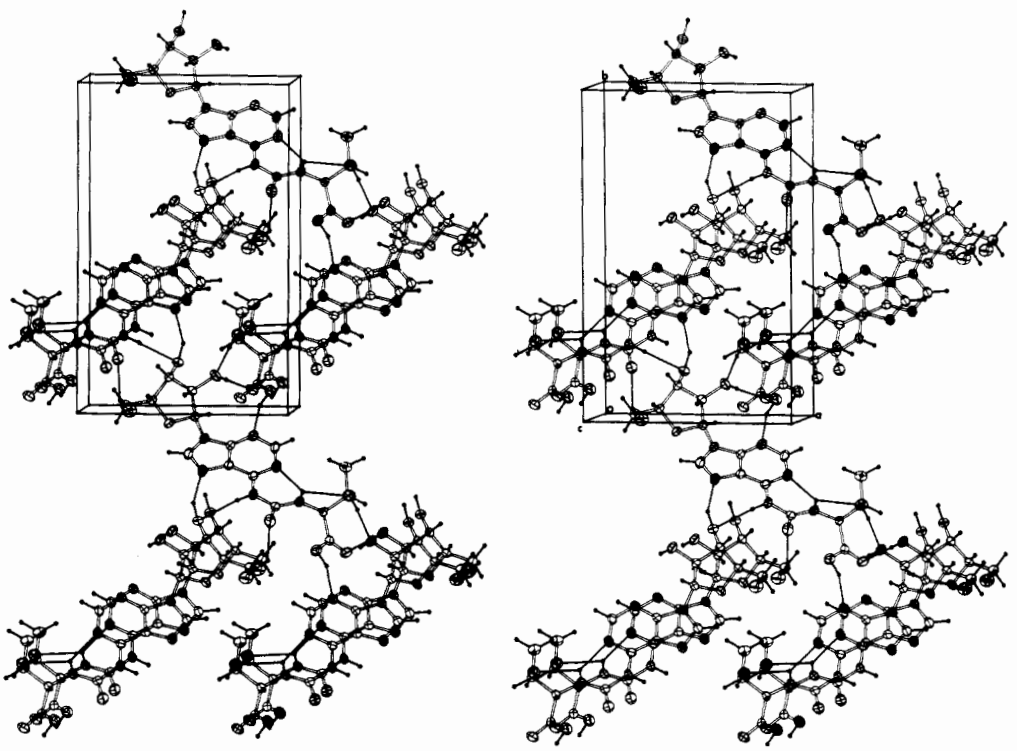


FIGURE 4: A stereo view of hydrogen bonding and packing.

carboxyl and ureido groups enable the hydrogen H(N11) to take part in a bifurcated hydrogen bond with N(1) and O(13b) as the two acceptors. Such a bifurcated bond with N(1) and O(13b) as two acceptors is clearly not possible in (gc^{6}Ade)-$^-$K$^+$·H_2O since the carboxyl group, which coordinates to K$^+$, is twisted away from the plane of the ureido group. Though the carboxyl in t^{6}A is not in a favorable position to form a bifurcated hydrogen bond with H(N11), a bifurcated bond is nevertheless formed, but with O(14) due to its favorable orientation.

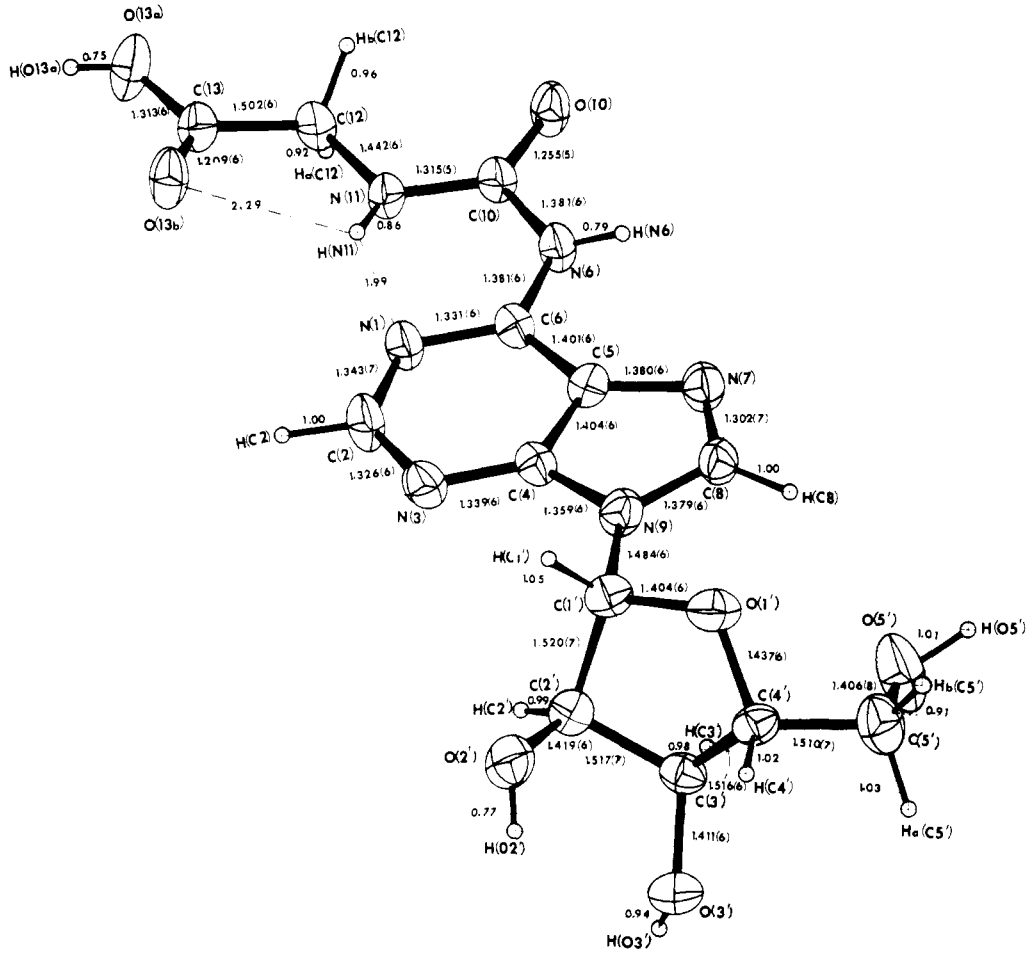
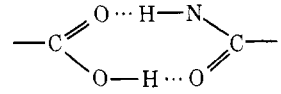


FIGURE 5: Bond distances (Å) in g^{6}A. Note the bifurcated hydrogen bonding N(11)-H(N11)-O(13b)[-N(1)].

The g^{6}A molecule has the anti conformation; the χ_{CN} angle is 4°. The "best" four atom least-squares plane is through C(1′), O(1′), C(2′), and C(4′) with a root-mean-square deviation of 0.031 Å; the next "best" plane is through C(1′), O(1′), C(3′), and C(4′) (root-mean-square deviation, 0.070 Å). The deviations of the atom C(3′) from the first plane and C(2′) from the second plane are, respectively, 0.539 and −0.508 Å; along with the torsion angles around the ring, they show that the sugar has the $^3T_2$ conformation (C(3′)-endo-C(2′)-exo). The torsional angles ϕ_{OO} and ϕ_{CO} (−67.8° and 51.0°) show that the sugar exhibits the preferred gauche-gauche conformation across the C(4′)-C(5′) bond.

Hydrogen Bonding and Packing. The hydrogen bonding (see supplementary material) and molecular packing are shown in Figures 7 and 8. The hydrogen bonding pattern of g^{6}A is quite different from that of t^{6}A. In g^{6}A, H(N11) participates in a bifurcated hydrogen bond with N(1) and O(13b) of the carboxyl group. In t^{6}A, the second acceptor is not O(13b) but the hydroxyl oxygen. The hydrogen H(O2′) is not involved in any hydrogen bonding, but makes two contacts, one intramolecular, with O(3′), and the other, intermolecular, with O(10) ($1 - x$, $-\frac{1}{2} + y$, $2 - z$). The distances and angles (H(O2′)···O(3′), 2.27 Å; H(O2′)···O(10), 2.55 Å; O(2′)-H(O2′)···O(3′), 119.2°; O(2′)-H(O2′)···O(10), 131.7°; O(10)···H(O2′)···O(3′), 85.8°) show that these contacts do not satisfy the linearity or planarity (Parthasarathy, 1969) conditions and hence are not interpretable as the usual hydrogen bonds nor as bifurcated hydrogen interactions. The hydrogen H(C8) has a weak intramolecular C-H···O contact with O(5′); similar C-H···O contacts have been observed in several nucleosides. There is an additional C(2)-H(C2)···O(1′^{iv}) (see supplementary material for the code for symmetry related atoms) contact (H···O, of 2.52 Å), but the angle C(2)-H(C2)···O(1′) is only 129.1°.

The mode of hydrogen bonding of the -COOH and -C(-N-H)=O group is interesting. They hydrogen bond to form a closed eight membered ring



linking one molecular to the next one related by a translation along the *a* axis, as in Figure 7. Such eight-membered hydrogen bonded rings are found in diverse structures such as biotin (DeTitta et al., 1976) and dethiobiotin (Chen et al., 1976). This pattern of hydrogen bonding between a COOH group and a ureido moiety seems to be a specific type of "recognition" between these two groups; this specific bonding suggests a model for activation of biotin which will be discussed elsewhere. There is no adenine-adenine interaction, either by way of hydrogen bonding or stacking. Intermolecular contacts other than those mentioned earlier are all larger than the sum of the corresponding van der Waals radii; the shortest of these contacts are: O(13′)···N(7^{iv}), 3.180 Å; C(13)···N(7^{iv}), 3.109 Å; N(11)···O(3′^{v}), 3.049 Å; C(10)···O(3′^{v}), 3.050 Å; O(10)···O(2′^{v}), 3.110 Å.

Discussion

Bifurcated hydrogen bonds and interactions, in the sense defined in Parthasarathy (1969) and Koetzle et al. (1972), are

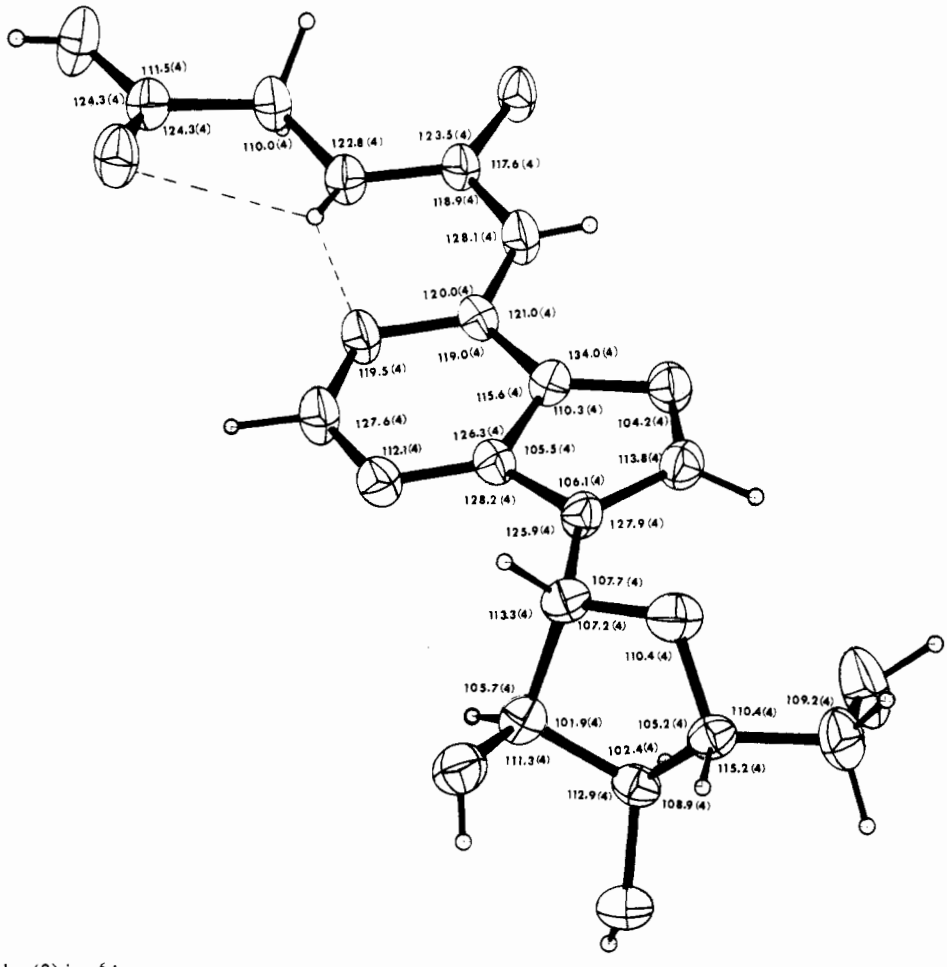


FIGURE 6: Bond angles (°) in g^{6}A.

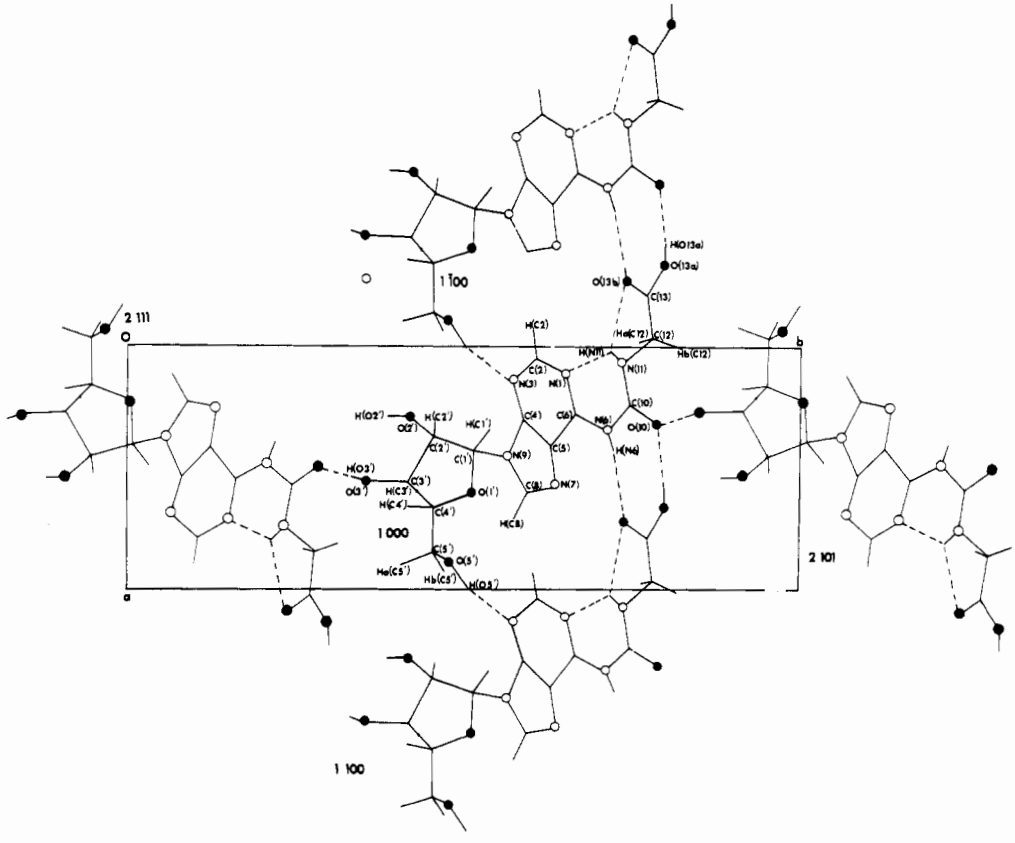


FIGURE 7: Hydrogen bonding, viewed down c axis (see code for translations, supplementary material).

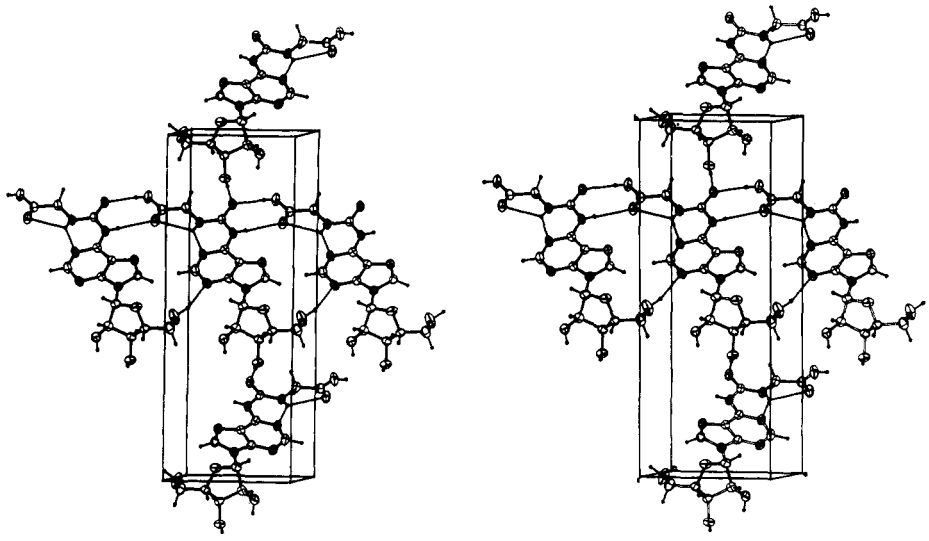


FIGURE 8: A stereo view of hydrogen bonding and packing.

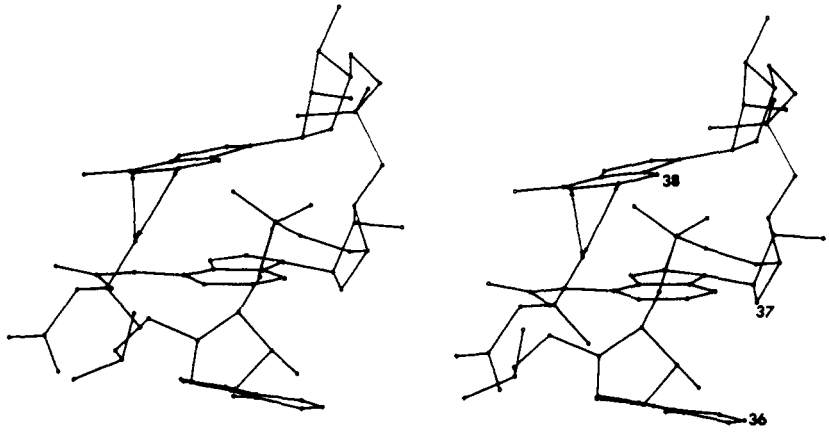
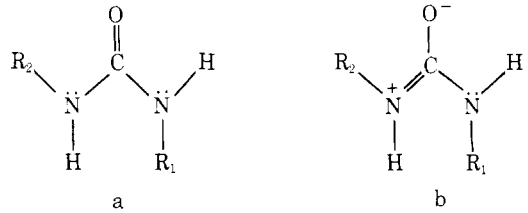
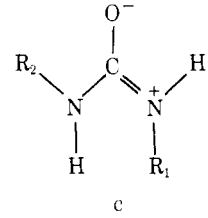


FIGURE 9: A stereo view of the anticodon loop of tRNA. Here the modified nucleoside Y is replaced by t^6A. The arrows indicate short contacts. Note the orientation of the methyl and carboxyl groups of t^6A. The numbers 36, 37, and 38 denote the sequences of the bases in the corresponding $tRNA^{Phe}$.

important features of the molecular structure of ureidopurines (see also Parthasarathy et al., 1976a). A survey of over 100 crystal structures, several of which contain bifurcated hydrogen bonds, shows (Parthasarathy, 1977) that these bifurcated interactions occur for the following hydrogen donors: an $-NH_2$ or -NH bond when they are highly polarized (due to neighboring positive charges), charged cationic hetero nitrogen in nucleic acid bases, and the NH_3^+ group in amino acids. Without such a polarization due to neighboring charges, the NH groups, rarely if ever, take part in bifurcated hydrogen bonds or weaker interactions. When these bifurcated bonds are formed, the donor, the hydrogen and the two acceptors are nearly in a plane (Parthasarathy, 1969; Parthasarathy et al., 1976a). The hydrogen bonds involving N(11)-H(N11)-N(1)[-O(14)] in t^6A and N(11)-H(N11)-N(1)[-O(13b)] in g^6A satisfy the planarity condition (see supplementary material) as well as the electronic condition (see below) for bifurcated interactions. Typical resonance structures for the ureido moiety are



a b



c

The long C(10)-N(6) and the short C(10)-N(11) bonds observed in t^6A and g^6A show that the second resonance form (b) has a predominant contribution to the observed structure; consequently, N(11) is endowed with a positive charge enabling H(N11) to form bifurcated hydrogen bonds. The formation of intramolecular N(11)-H···N(1) hydrogen bond in all ureidopurines studied so far in the solid state and our nuclear magnetic resonance studies of the hydrogen bonding in ureidopurines and analogues as a function of temperature (Korytnyk et al., 1976) indicate that any conformational flexibility of ureidopurines rests in the rotations across N-C^α, C^α-C^β, and C^α-C′ bonds. The twist of the carboxyl group across C^α-C′, denoted by the angle ψ (IUPAC-IUB, 1970) is nearly 180° in all ureidopurines, remarkably similar to what is found in amino acids and peptides (Marsh and Donohue, 1967; Lakshminarayanan et al., 1967). The crystal structures of ureidopurines indicate that the preferred conformations of the amino acid side chains across these bonds are related to the formation of bifurcated hydrogen bonds either with the car-

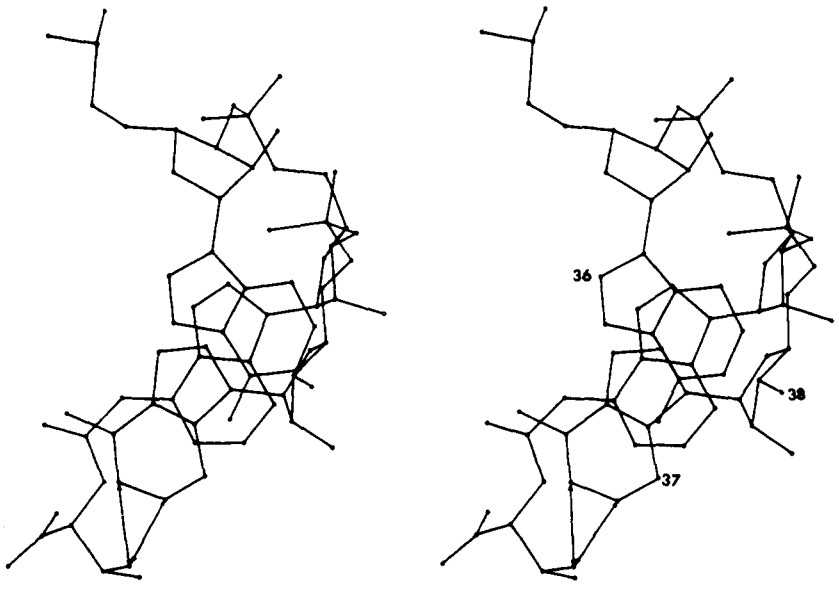


FIGURE 10: A stereo view of the stacking of the bases on the 3′ side of the anticodons.

boxyl group as in g^6A and ac^6Ade (Ohrt et al., 1977) or with O^γ of threonine in all the *three* threonine derivatives, namely, t^6A, $(tc^6Ade)^-K^+\cdot 4H_2O$, and $(tc^6Ade)^-Rb^+\cdot 4H_2O$ (Adamiak et al., 1975), studied so far. In view of the apparently preferred conformation of tc^6Ade that prevails in three crystal structures, we used this conformation in our model building studies (using Kendrew and CPK models) of anticodon loops of tRNA. For this study, we used the coordinates of yeast phenylalanine tRNA (Sussman and Kim, 1975; see also Ladner et al., 1975; Quigley et al., 1975; Stout et al., 1976), but replaced the base Y by tc^6Ade in such a way that the purine rings (especially atoms N(1), N(7), and N(9)) occupy the same locations. The model (Figures 9 and 10) indicates that the carboxyl group of tc^6Ade points toward the "third" residue (36) of the anticodon, whereas the methyl group will make short contacts of 2.7 Å with N(1) and C(2) of adenine (38), suggesting that the methyl and carboxyl groups have the potential to alter selectively the conformation of the anticodon loop, the stacking of the bases, and possibly hydrogen bonding around the "third" location of anticodon triplet. It might be possible to detect directly such selective alterations in conformations of the anticodon loops by a comparative study of anticodon loop with and without t^6A using physical tools such as nuclear magnetic resonance. Such selective alterations in the conformations of anticodon loops have been considered by several workers (Nishimura, 1972; Jukes, 1973; Engel and Von Hippel, 1974) and inferred from biochemical studies by others (Hogenauer et al., 1972; Miller et al., 1976; Schweizer et al., 1971; Kan et al., 1975; Grosjean et al., 1976); our studies indicate the details of one such alteration and suggest possible stereochemical factors to be considered in such studies. There is no doubt that such selective conformational changes in the anticodon loops are important in explaining the function of initiator $tRNA^{fMet}$ (Clark and Marcker, 1966; Ganem et al., 1973; Stewart et al., 1971) and other tRNAs that contain t^6A.

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Supplementary Material Available

Hydrogen atom parameters, the observed and calculated structure factors, torsion angles, hydrogen bonding parameters; and thermal parameters (38 pages). Ordering information is given on any current masthead page.

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