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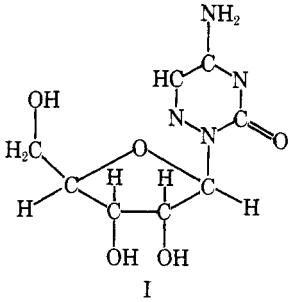
High-Anti Conformation in *o*-Azanucleosides. The Crystal and Molecular Structure of 6-Azacytidine†

Phirtu Singh and Derek J. Hodgson*

ABSTRACT: The crystal and molecular structure of 6-azacytidine has been determined from counter X-ray data. The nucleoside crystallizes in the space group $P2_12_12_1$ of the orthorhombic system with four molecules in a cell of dimensions $a = 7.623$ (6), $b = 6.993$ (7), and $c = 19.622$ (14) Å. Full matrix least-squares refinement using 1557 data has yielded a final value of the R factor (on F) of 0.031. The nucleoside adopts a glycosyl torsional angle, χ, of +99.1°, which is outside of the conventional syn and anti ranges and is in the "high-anti" region. The sugar pucker is the C-3′-endo (^3E) envelope conformation. The conformation about the extracyclic bond C-4′–C-5′ is the commonly occurring gauche–gauche. CNDO/2 molecular orbital calculations show that there is no residual charge on the aza atom N-6. The observed "high-anti" conformation may explain some of the known biochemical properties of 6-azacytidine.

6-Azacytidine (I) is an important carcinostatic agent



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(Sorm and Veseley, 1961) which interferes with the *de novo* synthesis of uridine through its inhibitory action on orotidylic acid decarboxylase. The inhibitory action of the nucleoside proceeds *via* two routes: (1) by the direct action of its 5′-nucleotide on the enzyme and (2) by first a deamination to 6-azauridine and then inhibition by the 5′-nucleotide of the latter (Skoda, 1963). Thus, 6-azacytidine and 6-azauridine are quite similar in their inhibition of orotidylic acid decarboxylase. Another similarity between the two nucleosides is seen in the ribosome binding experiments (Skoda, 1969). Thus, although GUU and GUC are the codon trip-

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lets for valine, GpUpazaU, GpazaUpU, and GpUpazaC do not stimulate the binding of [^{14}C]-Val-tRNA to ribosomes. It was, therefore, concluded from these experiments that the replacement of any pyrimidine ribonucleoside in the valine codon by either 6-azauridine or 6-azacytidine always results in a nonfunctional unit. An important difference, however, in the inhibitory action of the two azanucleosides is in the action of their 5′-diphosphates toward polynucleotide phosphorylase. Thus, while 6-azauridine 5′-diphosphate is an inhibitor, 6-azacytidine 5′-diphosphate is a substrate for polynucleotide phosphorylase (Skoda, 1969). A comparative study of the three-dimensional structures of the two nucleosides, namely, 6-azauridine and 6-azacytidine, would be of considerable value in trying to understand the structure–function relationship of 6-azapyrimidine nucleosides toward various enzymes of intermediary metabolism. The crystal and molecular structure of 6-azauridine and a molecular orbital calculation using the extended Hückel theory (EHT) has been reported by Schwalbe and Saenger (1973). In the present paper we report a precise determination of the crystal and molecular structure of 6-azacytidine. We also report molecular orbital calculations of the charge densities for both 6-azacytidine and 6-azauridine using the CNDO/2 approximation, which gives more reasonable values both qualitatively and quantitatively than the EHT procedure used by the previous authors for 6-azauridine. A preliminary report on the structure of 6-azacytidine has already appeared (Singh and Hodgson, 1974a).

Experimental Section

Data Collection. Large, colorless needles of 6-azacytidine were obtained from a concentrated aqueous solution of 6-azacytidine purchased from the Sigma Chemical Co., St. Louis, Mo. The unit-cell constants and the intensity data were measured using a small fragment of a crystal of approximate dimensions 0.40 × 0.35 × 0.25 mm on a Picker 4-Circle automatic diffractometer equipped with a Mo tube, a graphite monochromator, a scintillation counter, and a pulse-height analyzer. The intensities were collected at a tube take-off angle of 1.5° by the $\theta/2\theta$ scan technique at a scan rate of 0.5°/min with a stationary background count of 40 sec on each side of a peak which was scanned from 1° below the calculated α_1 peak position to 1° above the calculated α_2 peak position. The intensities of three standard reflections which were measured after every 50 measurements remained essentially constant throughout the duration of the data collection, indicating that there was no crystal decomposition or movement. The data collection was terminated at 2θ(Mo) = 60°, beyond which there was very little intensity observable above the background.

The data were processed by the method of Corfield *et al.* (1967) in a manner described elsewhere (Meyer *et al.*, 1972) with a local program which included a provision for Lorentz and polarization correction for a monochromator data set. No absorption corrections were applied since the linear absorption coefficient, μ, for Mo radiation and the crystal dimensions were quite small. The pertinent crystallographic data are presented in Table I.

Solution and Refinement of the Structure. The structure was solved by direct methods (Hauptman and Karle, 1953) using the multiple solution program MULTAN (Main *et al.*, 1971). The first solution tried from a set of 128 solutions revealed the locations of all the 19 nonhydrogen atoms, with only one spurious peak occurring among its highest 20 peaks. A total of 167 reflections with normalized structure factors, E, greater than 1.57 was used. It is interesting to note that four of the seven strongest E values were from the data set beyond the copper sphere ($2\theta > 55.5°$).

TABLE I: Crystallographic Data for 6-Azacytidine.

λ(Mo Kα_1) = 0.7093 Å
a = 7.623 (6), b = 6.993 (7), c = 19.622 (14) Å
Space group: $P2_12_12_1$ (orthorhombic) from the systematic absences $h00$ for h odd, $0k0$ for k odd, and $00l$ for l odd
Chemical formula: $C_8N_4O_5H_{12}$, mol wt 244.4
Specific gravity (measured by flotation in CCl_4–benzene) = 1.57 (2)
Specific gravity (calculated for Z = 4) = 1.55
Number of reflections observed above 3σ = 1557
R = 0.031 R_w = 0.040

The structure was refined by the full-matrix least-squares procedure of Busing *et al.* (1962). The program used was J. A. Ibers NUCLS. The atomic scattering factors for C, N, and O were taken from the International Tables for X-Ray Crystallography (1962) and those for H from Stewart *et al.* (1965). All the nonhydrogen atoms were refined with anisotropic temperature factors, and the hydrogen atoms, which were located from a difference Fourier, were refined with variable isotropic temperature factors. An examination of the data toward the end of the refinement revealed the presence of a significant amount of secondary extinction. Therefore, a correction for secondary extinction was applied in the manner described by Zachariasen (1968). The final agreement factors $R = \Sigma\|F_o| - |F_c\|/\Sigma|F_o|$ and $R_W = (\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2)^{1/2}$ were 0.031 and 0.040, respectively. No parameter shift was greater than 0.2 times its standard deviation in the final cycle of the least-squares refinement. The final difference Fourier was virtually featureless.

The positional and thermal parameters derived from the last cycle of least-squares refinement, along with their associated standard deviations as estimated from the inverse matrix, are presented in Table II. A table of observed and calculated structure factors is available (see paragraph at end of paper regarding supplementary material).

Molecular Orbital Calculations. The molecular orbital calculations of the total atomic charge density for 6-azacytidine were performed in the CNDO/2 approximation (Pople and Beveridge, 1970) with Quantum Chemistry Exchange Program No. 141. The geometry assumed for the molecule was that observed in the X-ray diffraction study.

Results and Discussion

The Base. The bond lengths and angles for 6-azacytidine not involving the hydrogen atoms are shown in Figure 1, and those involving the hydrogen atoms are presented in Table III (see paragraph at end of paper regarding supplementary material). A comparison of bond lengths in the pyrimidine portion of 6-azacytidine with those in the parent nucleoside cytidine (Furberg *et al.*, 1965) is given in Table IV. The substitution of N-6 for C-6–H has its most significant effect, as expected, on the C-5=C-6 double bond which, in 6-azacytidine, is a C=N bond and is 0.056 Å shorter than the equivalent C=C bond in cytidine. The C-4–C-5 bond is also affected, being 0.034 Å longer in 6-azacytidine than in cytidine. Also significant is the small difference (0.011 Å) between the N-1–C-6 bond in cytidine and

TABLE II: Positional and Thermal Parameters of Atoms in 6-Azacytidine.[a]

	x/a	y/b	z/c	β_{11} or B (Å^2)	β_{22}	β_{33}	β_{12}	β_{13}	β_{13}
N-1	3646 (2)	−4080 (2)	4418 (1)	118 (2)	86 (3)	12 (0)	−6 (2)	0 (1)	−2 (1)
C-2	3519 (2)	−3651 (2)	5107 (1)	138 (3)	89 (3)	12 (0)	7 (3)	0 (1)	−4 (1)
N-3	3166 (2)	−5080 (2)	5555 (1)	183 (3)	101 (3)	13 (0)	5 (3)	7 (1)	−3 (1)
C-4	2956 (2)	−6833 (3)	5316 (1)	114 (3)	98 (3)	13 (0)	9 (3)	9 (1)	−1 (1)
C-5	2973 (3)	−7197 (3)	4586 (1)	152 (3)	87 (3)	14 (0)	−25 (3)	8 (1)	−7 (1)
N-6	3291 (2)	−5837 (2)	4160 (1)	152 (3)	98 (3)	14 (0)	−25 (3)	3 (1)	−8 (1)
O-2	3785 (2)	−1975 (2)	5296 (1)	260 (4)	89 (2)	14 (0)	−10 (3)	−2 (1)	−8 (1)
N-4	2699 (3)	−8282 (2)	5741 (1)	232 (4)	104 (3)	17 (0)	−6 (3)	18 (1)	3 (1)
C-1′	4114 (2)	−2563 (2)	3936 (1)	75 (2)	79 (3)	11 (0)	−7 (2)	−4 (1)	1 (1)
C-2′	4788 (2)	−3318 (2)	3249 (1)	60 (2)	90 (3)	12 (0)	3 (2)	0 (1)	3 (1)
C-2′	3121 (2)	−3222 (2)	2816 (1)	61 (2)	89 (3)	10 (0)	−4 (2)	0 (1)	−1 (1)
C-3′	2238 (2)	−1415 (2)	3080 (1)	73 (2)	88 (3)	12 (0)	8 (2)	0 (1)	3 (1)
C-4′	271 (2)	−1348 (3)	2986 (1)	74 (2)	165 (4)	15 (0)	32 (3)	−1 (1)	8 (1)
C-5′	2616 (2)	−1401 (2)	3799 (1)	99 (2)	111 (2)	12 (0)	30 (2)	−4 (1)	−8 (1)
O-1′	6040 (2)	−2011 (2)	2982 (1)	59 (2)	149 (3)	19 (0)	−18 (2)	2 (1)	13 (1)
O-2′	3385 (2)	−3097 (2)	2104 (1)	117 (2)	137 (3)	9 (0)	12 (2)	1 (1)	0 (1)
O-3′	−594 (1)	−2959 (2)	3281 (2)	70 (2)	179 (3)	15 (0)	−13 (2)	3 (1)	−6 (1)
O-5′	259 (4)	−840 (4)	440 (2)	4.5 (7)					
H-C-5	273 (4)	−799 (4)	618 (1)	4.6 (7)					
H-N-4	278 (4)	−946 (4)	555 (1)	3.6 (7)					
H-1-N-4	495 (4)	−173 (4)	415 (1)	3.9 (6)					
H-C-1′	529 (3)	−458 (3)	329 (1)	2.2 (4)					
H-C-3′	243 (2)	−431 (3)	295 (1)	0.8 (3)					
H-C-4′	277 (3)	−34 (3)	286 (1)	1.8 (4)					
H-C-5′	2 (4)	−138 (3)	251 (1)	2.7 (4)					
H-1-C-5′	−14 (4)	−17 (4)	319 (1)	3.0 (5)					
H-O-2′	696 (4)	−243 (4)	308 (1)	2.6 (6)					
H-O-3′	374 (3)	−426 (4)	199 (1)	3.5 (5)					
H-O-5′	−52 (3)	−284 (4)	370 (1)	3.5 (5)					

[a] Parameters of nonhydrogen atoms have been multiplied by 10^4 and those of hydrogen atoms, except for their B's, by 10^3. Form of the thermal ellipsoid is $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{33}kl)]$.

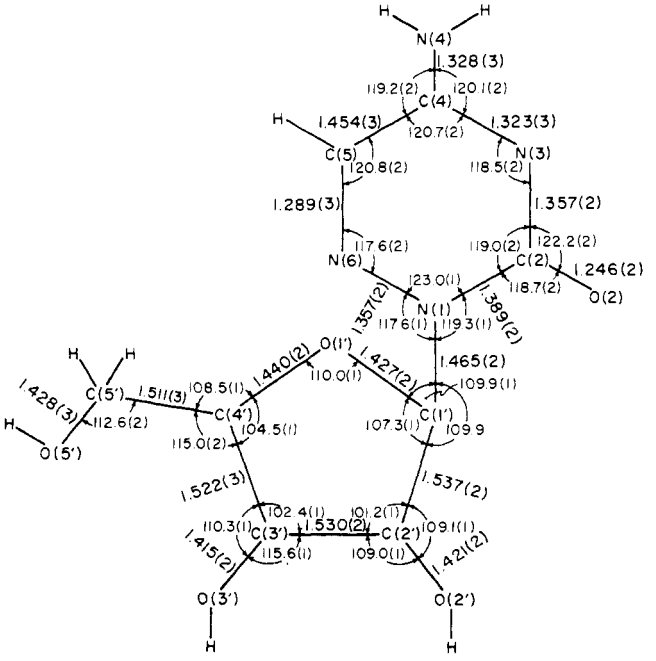

FIGURE 1: Bond lengths and bond angles, not involving the hydrogen atoms, in 6-azacytidine.

TABLE IV: Comparison of the Pyrimidine Bond Lengths in 6-Azacytidine and Cytidine.

Bond	6-Azacytidine (Å)	Cytidine[a] (Å)	Δ
N-1–C-2	1.389 (2)	1.379 (6)	+0.010 (6)
C-2–O-2	1.246 (2)	1.246 (5)	0 (5)
C-2–N-3	1.357 (2)	1.361 (6)	−0.004 (6)
N-3–C-4	1.323 (3)	1.335 (6)	−0.012 (7)
C-4–N-4	1.328 (3)	1.333 (7)	−0.005 (8)
C-4–C-5	1.454 (3)	1.420 (7)	+0.034 (8)
C-5–N-6[b]	1.289 (3)	1.345 (7)	−0.056 (8)
N-6[b]–N-1	1.357 (2)	1.368 (6)	−0.011 (6)

[a] Furberg *et al.* (1965). [b] C-6 in cytidine.

the N-1–N-6 bond in 6-azacytidine; in view of the much larger (approximately 0.04 Å) difference between the sizes of C and N, this suggests that there is more double bond character in N-1–C-6 of cytidine than in N-1–N-6 of 6-azacytidine. Similar effects were observed in comparisons between 6-azauridine and uridine (Schwalbe and Saenger, 1973) and 6-azauracil and uracil (Singh and Hodgson, 1974b).

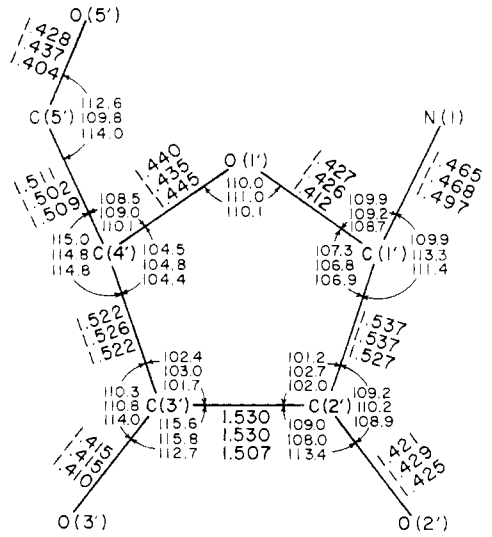

FIGURE 2: Bond lengths and angles in the ribose moieties of 6-azacytidine (top), 6-azauridine (middle), and cytidine (bottom).

The internal ring angle at the 6 position in 6-azacytidine has decreased by 2.5° and those at the neighboring atoms N-1 and C-5 have increased by 2.1 and 2.6°, respectively, from the corresponding angles in cytidine. The same trend has been observed in the 6-azauracil–uracil pair (Singh and Hodgson, 1974b; Stewart and Jensen, 1967) where the corresponding values are 4.5, 2.7, and 4.5°, respectively, and in the 6-azauridine–uridine pair (Schwalbe and Saenger, 1973; Voet and Rich, 1970) where the values are 2.6, 2.5, and 3.2°, respectively. A similar, but smaller, effect has also been observed in the imidazole ring of purines (Prusiner *et al.*, 1973; Voet and Rich, 1970). The values of the internal ring angles at both N-3 and N-6 are in general agreement with the observation of Singh (1965) and of Sundaralingam and Jensen (1965) that the internal ring angle at the nitrogen atom without an extraannular attachment should be smaller than 120°; those at C-2 and C-4 are consistent with the observation of Ringertz (1972) that the diminution in the internal ring angle is essentially proportional to the double bond character of the extraannular bond. The pyrimidine ring is approximately planar with a slight boat conformation, atoms N-1 and C-4 lying slightly off the plane on the same side and the substituent C-1′ and N-4 also off on the same side as N-1 and C-4 by 0.11 and 0.10 Å, respectively.

The Sugar. The bond lengths and angles in the ribose moiety of 6-azacytidine are compared with those of 6-azauridine and cytidine in Figure 2. The agreement between equivalent bond lengths in the two azanucleosides is excellent except for small differences involving the extracyclic bonds C-4′–C-5′ and C-5′–O-5′; it is noteworthy that the principal difference between the structures of these two compounds is in the conformation of the C-5′–O-5′ bond around the C-4′–C-5′ bond (*vide infra*). The agreement between equivalent bonds in 6-azacytidine and cytidine is also good, with the apparent exception of the C-2′–C-3′ and C-5′–O-5′ bonds.

As is commonly observed in nucleoside structures, the endocyclic C–O-1′ bond lengths are different, with C-1′–O-1′ significantly shorter than C-4′–O-1′. As in the other *o*-azanucleosides formycin (Prusiner *et al.*, 1973) and 6-azauridine (Schwalbe and Saenger, 1973), however, the C-1′–O-1′ bond in 6-azacytidine apparently contains less double bond character than that in cytidine. Conversely, the glycosyl N-1–C-1′ bonds in the 6-azapyrimidine nucleosides are significantly shorter than that in cytidine; a similar effect has been noted for the 8-azapurine nucleoside 8-azaadenosine (Singh and Hodgson, 1974c).

The endocyclic and exocyclic bond angles in the sugar moiety of 6-azacytidine are in good agreement with the values derived by Sundaralingam (1965) from an analysis of the results of several accurate structural studies of nucleosides. Sundaralingam (1965), however, has noted that the average internal C–C–C angle at the out-of-plane carbon atom in a large number of nucleosides is smaller than the average value at the in-plane carbon atom; hence, for a C-3′ endo sugar (*vide infra*) the endocyclic angle at C-3′ is normally smaller than that at C-2′. Saenger and Eckstein (1970), however, observe that the opposite effect is found for C-3′ endo sugars; examination of Figure 2 leads to the conclusion that for 6-azacytidine the values are in conformity with the latter observation.

From an examination of the deviations of the sugar atoms from the least-squares five-atom and best four-atom planes, it is evident that the atoms O-1′, C-1′, C-2′, and C-4′ are virtually coplanar, with C-3′ 0.58 Å from this plane on the same side as C-5′ and N-1. Hence, the puckering belongs to the C-3′ endo category, and has the envelope conformation ^3E; this conformation was also observed in 6-azauridine and in cytidine.

The principal difference between the structures of 6-azacytidine and 6-azauridine (Schwalbe and Saenger, 1973) is in the conformation around the extracyclic bond C-4′–C-5′. In 6-azauridine and its 5′-phosphate (Saenger and Suck, 1973) this conformation (Shefter and Trueblood, 1965; Sundaralingam, 1965) is the unusual gauche–trans (*gt*), but in 6-azacytidine it is the commonly occurring gauche–gauche (*gg*) with O-5′ located above the sugar ring. The angles ϕ_{OO} (*i.e.*, between the planes O-5′–C-5′–C-4′ and C-5′–C-4′–O-1′) and ϕ_{OC} are 61.2 and 55.4°, respectively. This solid state conformation, however, is in contrast to that in solution as deduced from nmr (Hruska *et al.*, 1971; Hruska, 1973), where considerable non-*gg* (*i.e.*, *gt* or *tg*) conformation is found. This observation leads us to conclude that the energy difference between the *gg* and *gt* forms may not be very great. The availability of the *gt* conformation in solution may make 6-azacytidine conformationally acceptable to orotidylic acid decarboxylase.

The observation of a *gg* conformation in 6-azacytidine is at variance with the extended Hückel calculations performed on 6-azauridine by Schwalbe and Saenger (1973). These workers calculate a residual charge of −0.47 e^- on N-6 in 6-azauridine, and note that this large negative charge would repel the negatively charged O-5′ and hence lead to the *gt* conformation. Our CNDO/2 calculations on 6-azacytidine and on 6-azauridine, which are compared in Table V with the EHT results for uridine and 6-azauridine, show a net residual charge on N-6 in both molecules of zero. Hence, there is probably very little coulombic interaction between N-6 and O-5′ in either system, which explains why the barrier to rotation around the C-4′–C-5′ bond may be small (*vide supra*). In uridine or cytidine, there is a positive attraction between O-5′ and C-6–H-6, which stabilizes the *gg* conformation (Yathindra and Sundaralingam, 1973).

Conformation around the Glycosyl Bond. The glycosyl torsion angle χ_{CN} describing the relative orientation of the sugar with respect to the base (Donohue and Trueblood,

TABLE V: Net Atomic Charge Densities on 6-Azacytidine (AC), Cytidine (C), and 6-Azauridine (AU) by the CNDO/2 Method and on 6-Azauridine and Uridine (U) by the Extended Hückel Theory (EHT).

	CNDO/2			EHT	
Atom	AC	C	AU	AU	U
N-1	−0.121	−0.165	−0.138	0.00	−0.207
C-2	0.410	0.409	0.444	1.38	1.371
N-3	−0.309	−0.340	−0.239	−0.31	−0.366
C-4	0.291	0.320	0.348	1.22	1.183
C-5	−0.052	−0.170	−0.036	0.24	−0.250
N-6, C-6	0.000	0.180	−0.007	−0.47	0.284
O-2	−0.406	−0.426	−0.368	−1.38	−1.415
N-4, O-4	−0.246	−0.246	−0.318	−1.38	−1.391
C-1′	0.219	0.228	0.220	0.68	0.768
C-2′	0.100	0.118	0.102	0.44	0.417
C-3′	0.131	0.125	0.123	0.42	0.497
C-4′	0.119	0.105	0.124	0.47	0.467
C-5′	0.117	0.133	0.126	0.29	0.322
O-1′	−0.246	−0.246	−0.243	−1.18	−1.201
O-2′	−0.281	−0.276	−0.268	−1.20	−1.155
O-3′	−0.262	−0.254	−0.280	−1.17	−1.183
O-5′	−0.281	−0.267	−0.253	−1.20	−1.194
H–N-3			0.144	0.33	0.307
H–C-5	0.023	0.027	0.035	0.14	0.121
H–N-4	0.149	0.132			
H-1–N-4	0.136		0.130		
H–C-1′	0.014	0.011	0.016	0.14	0.072
H–C-2′	−0.004	0.002	0.006	0.13	0.121
H–C-3′	−0.006	0.007	−0.010	0.14	0.084
H–C-4′	0.012	0.004	0.009	0.12	0.143
H–C-5′	0.011	−0.007	−0.012	0.29	0.128
H-1–C-5′	0.000	−0.006	0.008	0.15	0.125
H–O-2′	0.167	0.156	0.170	0.62	0.602
H–O-3′	0.149	0.174	0.138	0.61	0.617
H–O-5′	0.164	0.143	0.141	0.62	0.617

1960) and defined by the dihedral angle O-1′C-1′–N-1–N-6 (Sundaralingam, 1969) has the unusual value of +99.1° which is outside the conventional anti and syn ranges (Donohue and Trueblood, 1960), and is shifted in the direction suggested by Rogers and Ulbricht (1971) to explain the unusual negative Cotton effect observed in optical rotatory dispersion studies in solution. The value of χ_{CN}, +99.1°, obtained by us in the solid state is almost exactly that predicted theoretically by Pullman and Berthod (1972). The values of χ_{CN} for all the 6-azapyrimidine and 8-azapurine nucleosides whose crystal structures we are aware of have been drawn in Figure 3. As we have noted elsewhere (Singh and Hodgson, 1974c), it is quite clear that, except for formycin hydrobromide, which has a proton on N-2, all the 6-azapyrimidine and 8-azapurine nucleosides lie outside the conventional syn and anti ranges and are grouped together in a rather narrow range with an average χ_{CN} of approximately +100°. It seems reasonable to conclude, therefore, that the favored conformation describing the relative orientation of the base and the sugar in ribonucleosides with a nitrogen atom next to the glycosyl bond would have χ_{CN} not far from +100°. This adoption of the "high-anti" conformation by both 6-azacytidine and 6-azauridine may be responsible for the failure of GpUpazaC and GpUpazaU to code for valine since, if the conformation is retained in the trinucleoside diphosphates containing them, the relative dis-

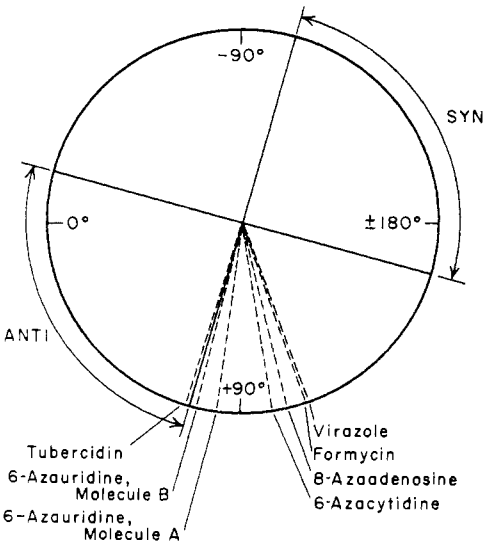

FIGURE 3: Glycosyl torsion angle, χ, for *o*-azanucleosides. The notation used is that of Sundaralingam (1969).

positions of hydrogen bonding and other interacting groups in these anomalous molecules would be different from those in the normal ones.

Packing and Hydrogen Bonding. The packing of the 6-

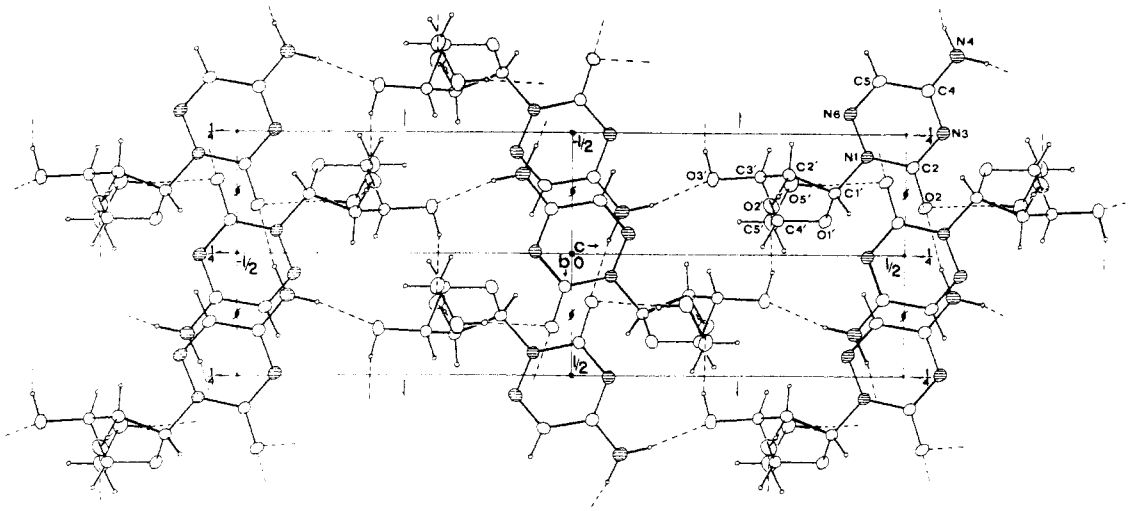

FIGURE 4: View of the structure along the *a* axis showing crystal packing and hydrogen bonding. Broken lines represent hydrogen bonds and the dotted lines represent possible C-5–H–C-5 · · · O-1′ interactions. Atoms belonging to the molecule whose coordinates are given in Table II are labeled.

TABLE VI: Lengths and Angles, Associated with the Hydrogen Bonds.

A–H · · · B	A–H (Å)	H · · · B (Å)	A · · · B (Å)	<A–H · · · B (deg)
N-4–H–N-4 · · · O-3′	0.89	2.14	2.96	152.0
N-4–H–N-4 · · · O-2	0.90	1.99	2.85	159.8
O-2′–H–O-2′ · · · O-5′	0.78	1.95	2.71	168.9
O-3′–H–O-3′ · · · O-2′	0.89	1.93	2.78	159.1
O-5′–H–O-5′ · · · O-2	0.83	2.05	2.83	158.0
C-5′–H–C-5′ · · · O-1′[a]	0.98	2.40	3.33	159.3

[a] Possibly a C–H · · · O type of hydrogen bond.

TABLE VII: Short Intramolecular Distances in the 6-Azacytidine Molecule.

	Distance (Å)	Sum of van der Waals Radii (Å)
N-6 · · · C-2′	2.76	3.30
N-6 · · · H–C-2′	2.46	2.80
N-6 · · · C-3′	3.21	3.30
N-6 · · · H–C-3′	2.69	2.80
C-2 · · · H–C-1′	2.55	2.90
C-2 · · · O-1′	3.09	3.10
O-2 · · · H–C-1′	2.41	2.60
O-5′ · · · C-3′	2.98	3.10

azacytidine molecules and their hydrogen bonding interactions in the crystal lattice are depicted in Figure 4, and the relevant distances and angles are presented in Table VI. The pyrimidine portion of the molecule is approximately perpendicular to the *a* axis. The packing is dominated by the hydrophobic interactions of the quasiaromatic pyrimidine rings, and hydrophilic interactions of the polar sugar moieties running in alternate channels parallel to the *b* axis, a phenomenon quite commonly observed in nucleosides. All the hydrogen atoms attached to an oxygen or a nitrogen atom participate in hydrogen bonding. Ring atoms N-3, N-6, O-1′ do not take part in any hydrogen bonding interactions. There is, however, a relatively short C-5–H · · · O-1′ intermolecular distance of 3.333 Å, with the H · · · O-1′ distance of 2.40 Å (0.20 Å less than the sum of the respective van der Waals radii) and the C-5–H · · · O-1′ angle of 159.3°. A similar C–H · · · O-1′ intermolecular separation has been observed in virazole,1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Sprang and Sundaralingam, 1973; Prusiner and Sundaralingam, 1973), with the C · · · O-1′ distance of 3.28 Å and the C–H · · · O-1′ angle of 176°. Significantly, virazole also has a nitrogen atom on the base next to the glycosyl bond similar to 6-azacytidine, and assumes an intermediate anti–syn conformation with a χ_{CN} = 110.0°. In nucleosides, O-1′ is known to accept a hydrogen bond in very few cases (Sundaralingam, 1968). It is, therefore, interesting that O-1′ does accept a hydrogen bond in formycin monohydrate (Prusiner *et al.*, 1973), 8-azaadenosine monohydrate (Singh and Hodgson, 1974c), and 6-azauridine (Schwalbe and Saenger, 1973), and that it has moderately close C–H · · · O-1′ contacts in virazole and 6-azacytidine. The net charge on atom O-1′, shown in Table V for 6-azacytidine, 6-azauridine, and cytidine does not indicate any electronic effects which might account for the participation of O-1′ in these interactions, since it is approximately the same for cytidine, which does not have this interaction, as it is for the aza compounds, which do. It has been suggested (Prusiner *et al.*, 1973) that the participation of O-1′ in hydrogen bonding in the crystal of formycin monohydrate is probably due to the balance of crystal packing and conformation energies; this also seems to be the most plausible explanation for the C–H · · · O-1′ interactions.

Short intramolecular contacts are given in Table VII and shown in Figure 5. All, except O-5′ · · · C-3′, are between an atom on the base and an atom on the ribose. There are four such contacts involving N-6 of the base with C-2′, H–C-2′, C-3′, and H–C-3′ of the ribose with distances of (van der Waals distances in parentheses) 2.76 (3.30), 2.46 (2.90), 3.21 (3.30), and 2.69 (2.90) Å, respectively. Similar short contacts were also observed in 6-azauridine (Schwalbe and Saenger, 1973). Interestingly, the N-8 atom in the two 8-

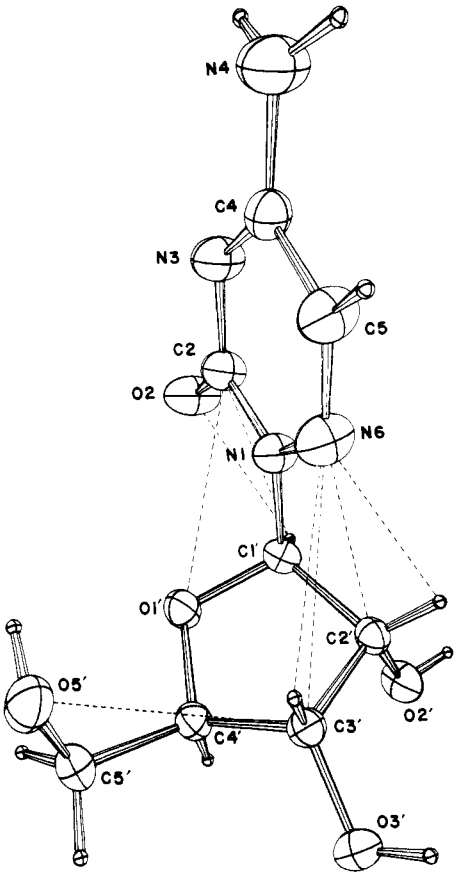

FIGURE 5: View of 6-azacytidine showing the atomic thermal ellipsoids (Johnson, 1965) and the atom numbering scheme for the nonhydrogen atoms. The hydrogen atoms are shown with artificially reduced thermal ellipsoids, and their numbering scheme follows from that of the atom to which they are attached.

azapurine nucleosides, 8-azaadenosine (Singh and Hodgson, 1974c) and formycin (Prusiner *et al.*, 1973), also has short contacts, but only with C-2′ and its associated hydrogen H-C-2′, the N-8 · · · C-2′ and N-8 · · · H-C-2′ distances being 2.84 and 2.28 Å, respectively, in 8-azaadenosine and 2.87 and 2.58 Å, respectively, in formycin. The short contacts of atoms N-6, C-2, and O-2 of the base with the ribose atoms are presumably a result of the adoption of the intermediate anti–syn conformation around the glycosyl bond by the azanucleoside. The contact between the intraribose atoms O-5′ and C-3′ is indicative of the gauche–gauche conformation adopted by the C-5′–O-5′ bond around the C-4′–C-5′ bond. There is, in addition to these intramolecular interactions, a short intermolecular contact between C-5′ and H–O-5′ of 2.65 Å between two molecules related by the *a*-axial translation (Figure 4).

Supplementary Material Available.

A listing of bond lengths and bond angles involving the hydrogen atoms and of observed and calculated structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number BIO-74-5445.

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CORRECTIONS

"Amino-Enzyme Intermediates in Pepsin-Catalyzed Reactions," by Marc S. Silver* and Mai Stoddard, Volume 11, Number 2, January 18, 1972, page 191.

The first sentence of the second new paragraph on page 197 should read "For Ac-Phe-Tyr, the data and equations of Denburg *et al.* (1968) predict k_c/K_m = 3600 M^{-1} hr^{-1} at pH 4.5, 35°, 3% methanol."

"Rhodopsin Content in the Outer Segment Membranes of Bovine and Frog Retinal Rods," by David S. Papermaster* and William J. Dreyer, Volume 13, Number 11, May 21, 1974, page 2438.

Page 2438, right-hand column, 7 lines from the bottom: 10 mM should be 1.0 mM; 6 lines from the bottom: 1 mM should be 0.1 mM. In the caption to Figure 3, line 8, 2 μg/ml should read 2 mg/ml.

"Affinity Labeling of Rabbit Muscle Myosin with a Cobalt(III)-Adenosine Triphosphate Complex," by M. M. Werber, A. Oplatka,* and A. Danchin, Volume 13, Number 13, June 18, 1974, page 2683.

Page 2685, line 20, should read k = 0.110 M^{-1} sec^{-1}, not k = 0.045 M^{-1} sec^{-1}.

"The Binding of Boronic Acids to Chymotrypsin," by J. David Rawn and Gustav E. Lienhard,* Volume 13, Number 15, July 16, 1974, page 3124.

Page 3128, right-hand column, paragraph 1, line 3, change 4 mM to 4×10^{-3}; same paragraph, line 6, change $0.025/55 = 4.7 \times 10^{-4}$ mM to $0.025/55{,}000 = 4.7 \times 10^{-7}$.