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8-Chloroguanosine: Solid-State and Solution Conformations and Their Biological Implications†

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ABSTRACT: The three-dimensional structure of 8-chloroguanosine dihydrate was determined by X-ray crystallography. The crystals belong to the orthorhombic space group $P2_12_12_1$, and the cell dimensions are a = 4.871 (1) Å, b = 12.040 (1) Å, and c = 24.506 (1) Å. The structure was determined by direct methods, and least-squares refinement, which included all hydrogen atoms, converged at R = 0.031 for 1599 observed reflections. The conformation about the glycosidic bond is syn with $\chi_{CN} = -131.1°$. The ribose ring has a C(2′)-endo/C(1′)-exo ($^2T_1$) pucker, and the gauche$^+$ conformation of the $-CH_2OH$ side chain is stabilized by an intramolecular O(5′)–H···N(3) hydrogen bond. Conformational analysis by means of ^{1}H NMR spectroscopy showed that, in dimethyl sulfoxide, the sugar ring exhibits a marked preference for the C(2′)-endo conformation (~70%) and a conformation about the glycosidic bond predominantly syn (~90%), hence similar to that in the solid state. However, the conformation of the exocyclic 5′-CH_2OH group exhibits only a moderate preference for the gauche$^+$ rotamer (~40%), presumably due to the inability to form the intramolecular hydrogen bond to N(3) in a polar medium. The conformational features are examined in relation to the behavior of 8-substituted purine nucleosides in several enzymatic systems, with due account taken of the steric bulk and electronegativities of the 8-substituents.

Following the demonstration, some years ago, that 8-bromoadenosine and 8-bromoguanosine are in the syn conformation about the glycosidic bond in the solid state (Tavale & Sobell, 1970) and in solution (Sarma et al., 1974), it was widely assumed that a bulky substituent at C(8) of a purine nucleoside or nucleotide restricts the conformation to syn in solution. For 8-bromoadenosine, this inference derived support from theoretical and hard-sphere calculations [see Birnbaum & Shugar (1978)], suggesting that the entire anti range was excluded by close contacts between the bromine and ribose atoms. Its validity was subsequently placed in doubt by the demonstration that 8-bromoadenosine diphosphate ribose, cocrystallized with alcohol dehydrogenase, shows the adenine moiety in the same anti conformation as in adenosine diphosphate ribose (ADP-ribose) and in NAD$^+$ (Abdallah et al., 1975). This led to the synthesis of purine nucleosides with C(8) substituents sufficiently bulky as to unequivocally exclude existence of the anti conformation (e.g., Birnbaum & Shugar, 1978). Concurrently it has been established that, while 8-

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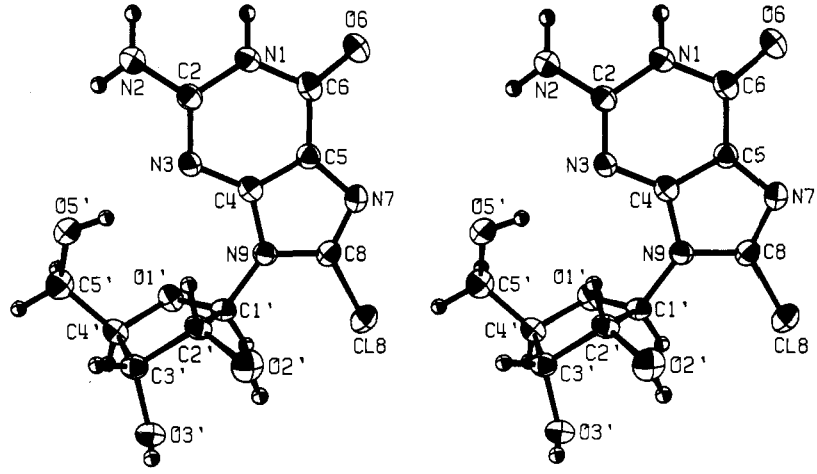


FIGURE 1: Stereoscopic view of 8-chloroguanosine. The thermal ellipsoids correspond to 50% probability.

bromo analogues of adenosine and guanosine are indeed predominantly in the syn conformation in solution, they are in dynamic equilibrium with a small proportion ($\lesssim$5%) of the anti conformers (Stolarski et al., 1980).

Unlike natural pyrimidine nucleosides, purine nucleosides in the solid state are found in both syn and anti conformations, the latter being the more common. At the polynucleotide level, in the Z form of DNA, guanosine residues are exclusively in the syn conformation (Rich et al., 1983), which is also involved in the hairpin twin-stranded helical structure of poly(U) (Rabczenko & Shugar, 1971) and in ordered helical structures of poly(8-bromoadenosine) and poly(8-methyladenosine) (Govil et al., 1981; Limn et al., 1983).

In view of the widespread continuing interest in the influence of C(8)-substituents on the glycosidic bond conformation of purine nucleosides and nucleotides and the associated biological effects (Lappi et al., 1980; Leng et al., 1980; Livneh et al., 1982; Huang et al., 1983; Birnbaum & Shugar, 1985), it appeared of interest to examine the role of a substituent slightly smaller than a bromine or a methyl. We have therefore profited from a new, improved procedure for the synthesis of 8-chloropurine nucleosides (Ryu & MacCoss, 1981) to prepare 8-chloroguanosine. We describe here the solid state and solution conformations of this compound, in which the van der Waals radius of the C(8)-substituent is smaller than that of bromine.

Experimental Procedures

8-Chloroguanosine was prepared by treatment of guanosine with anhydrous HCl in dimethylformamide, as described by Ryu & MacCoss (1981). Crystals of the dihydrate $C_{10}H_{12}N_5O_5Cl\cdot 2H_2O$, were obtained by very slow cooling of a seeded saturated aqueous solution from 70 °C to room temperature over a period of several days.

Precession photographs indicated the orthorhombic space group $P2_12_12_1$. A colorless crystal, measuring 0.15 × 0.15 × 0.40 mm, was mounted on a CAD-4 diffractometer. The unit cell dimensions were determined from angular settings of 22 reflections. The following data were obtained: a = 4.871 (1) Å, b = 12.040 (1) Å, c = 24.506 (1) Å, V = 1437.20 Å^3, D_x = 1.64 g cm^{-3}, Z = 4 (20 °C; Mo Kα_1, λ = 0.70930 Å), $F(000)$ = 736, and μ(Mo Kα) = 3.1 cm^{-1}.

Intensities were measured with Zr-filtered Mo Kα radiation up to 2θ = 55° by using the $\omega/(2\theta)$ scan technique with $\Delta\omega$ = 1.3 + 0.5 tan θ and a maximum scan time of 210 s per reflection. Three reflections, monitored every 100 min, showed intensity variations of <1%. Of the 1946 unique reflections, 1607 had $I > 2\sigma(I)$ and were considered observed. The intensities were corrected for Lorentz and polarization factors; absorption corrections were unnecessary.

The structure was determined by direct methods with the aid of the computer program MULTAN78 (Main et al., 1978). The atomic parameters were refined by the block-diagonal least-squares method with anisotropic temperature parameters. All hydrogen atoms were located on difference Fourier maps and refined with isotropic temperature parameters. The scattering factors were taken from the *International Tables for X-Ray Crystallography* (1974), and the chlorine and oxygen curves were corrected for anomalous dispersion. Throughout the refinement the function $\sum w(|F_o| - |F_c|)^2$ was minimized, and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages: $w = w_1w_2$, where $w_1 = 1$ for $|F_o| \leq 24$, $w_1 = 24/|F_o|$ for $|F_o| > 24$, $w_2 = \sin^2\theta/0.06$ for $\sin^2\theta < 0.06$, and $w_2 = 1$ for $\sin^2\theta \geq 0.06$. This scheme made the average values of $w(\Delta F^2)$ independent of $|F_o|$ and $\sin^2\theta$. After the final cycle the average parameter shift equaled 0.1σ and the largest 0.7σ. Eight strong, low-order reflections suffered from extinction effects and were given zero weights. The final conventional residual index R is 0.031, and the weighted index R_w is 0.036 for 1599 observed reflections. The coordinates are listed in Table I; anisotropic temperature parameters as well as a list of observed and calculated structure factors are provided as supplementary material (see paragraph at end of paper regarding supplementary material). The conformation of the molecule and the numbering of atoms are shown in Figure 1.

^{1}H NMR spectra were recorded on 0.2 M solutions in dimethyl sulfoxide-d_6, with Me_4Si as internal standard, on a Jeol-JNM-4H-100 cw instrument.

Apart from the MULTAN system, all crystallographic calculations were carried out with programs written by Ahmed et al. (1973). Figures 1 and 4 were drawn with the ORTEP program of Johnson (1970).

Results and Discussion

Purine Moiety. The bond lengths and bond angles in 8-chloroguanosine are shown in Figure 2. The effect of chloro substitution at C(8) can be assessed by comparison with recently published "standard" values (Taylor & Kennard, 1982). These values, based on only seven neutral guanine residues, can be improved by taking into account the results of the X-ray analysis of acycloguanosine (acyclovir) which crystallized with three independent molecules in the asymmetric unit (Birnbaum et al., 1981b). The N(7)–C(8)–N(9) bond angle is significantly larger, and the C(8)–N(9)–C(4) angle significantly smaller, than normal. A similar observation was made in the

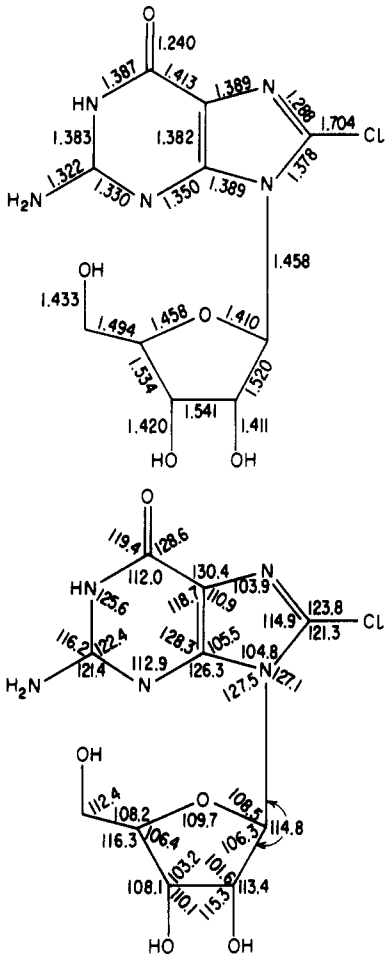


FIGURE 2: (Top) Bond distances (in angstroms): their estimated standard deviations (esd's) are 0.003–0.004 Å. (Bottom) Bond angles (in degrees): their esd's are 0.20–0.25°.

Table I: Final Atomic Parameters[a]

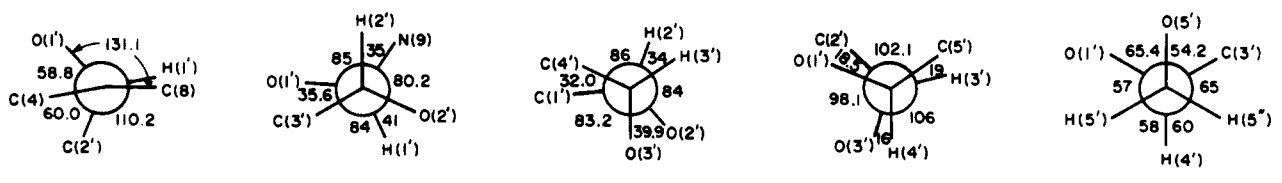
atom	x	y	z	U_{eq}/U_{iso}
N(1)	1365 (5)	31619 (18)	6586 (9)	279
C(2)	1909 (6)	34550 (22)	11935 (10)	267
N(2)	502 (6)	29189 (20)	15723 (9)	348
N(3)	3734 (5)	42322 (18)	13243 (8)	257
C(4)	4961 (6)	46880 (20)	8842 (9)	247
C(5)	4611 (6)	44169 (21)	3405 (10)	268
C(6)	2671 (6)	35911 (20)	2019 (10)	273
O(6)	2049 (4)	32352 (17)	−2566 (7)	336
N(7)	6290 (5)	50636 (20)	103 (9)	292
C(8)	7588 (6)	56898 (22)	3496 (11)	275
Cl(8)	10047 (2)	66214 (6)	1623 (3)	367
N(9)	6908 (5)	55313 (18)	8899 (9)	255
C(1′)	7748 (5)	62097 (21)	13535 (10)	249
O(1′)	8757 (4)	55016 (15)	17656 (7)	287
C(2′)	5459 (5)	68868 (20)	16125 (10)	249
O(2′)	4851 (5)	78729 (16)	13257 (8)	349
C(3′)	6562 (6)	70521 (21)	21964 (11)	257
O(3′)	8410 (4)	79634 (16)	22104 (8)	312
C(4′)	8210 (6)	59868 (22)	22998 (10)	268
C(5′)	6855 (6)	51248 (24)	26460 (11)	334
O(5′)	4198 (4)	48208 (16)	24449 (8)	328
O(W1)	3006 (7)	60789 (21)	36594 (10)	548
O(W2)	179 (6)	40567 (19)	39178 (9)	456
H(1)	16 (10)	266 (3)	58 (1)	23 (9)
H(1N2)	−56 (9)	238 (3)	148 (2)	25
H(2N2)	77 (9)	315 (3)	188 (2)	25
H(1′)	907 (9)	670 (3)	124 (2)	25
H(2′)	375 (9)	648 (3)	160 (2)	25
H(O2′)	628 (10)	817 (4)	128 (2)	39 (13)
H(3′)	511 (11)	715 (3)	246 (2)	25
H(O3′)	748 (8)	852 (3)	223 (1)	21 (9)
H(4′)	997 (11)	619 (3)	245 (2)	25
H(5′)	661 (9)	544 (3)	301 (2)	25
H(5″)	802 (9)	449 (3)	267 (2)	26 (10)
H(O5′)	435 (9)	471 (3)	211 (2)	27 (11)
H(1W1)	193 (12)	550 (4)	365 (2)	56 (16)
H(2W1)	335 (14)	615 (5)	401 (2)	67 (18)
H(1W2)	−41 (10)	437 (4)	422 (2)	34 (11)
H(2W2)	−124 (12)	381 (5)	377 (2)	59 (16)

[a] For nonhydrogen atoms x and U_{eq} were multiplied by 10^4 and y and z by 10^5. All hydrogen atom parameters were multiplied by 10^3.

structure of 8-bromoxylofuranosyladenine (Birnbaum et al., 1982). On the other hand, in 8-(α-hydroxyisopropyl)adenosine (Birnbaum & Shugar, 1978) and in 8-methyladenosines (Yasuniwa et al., 1979; Silverton et al., 1982) the N(7)–C(8)–N(9) angle is smaller than in unsubstituted adenosines. These changes presumably result from altered hybridization states of C(8) and depend on whether the substituent donates or withdraws electrons from the ring. The effect can also be seen in 5-substituted uracil residues. Thus, in 5-(hydroxymethyl)-2′-deoxyuridine the C(4)–C(5)–C(6) angle is 117.4° (Birnbaum et al., 1980) while in 5-chlorouridine it is 120.6° (Hawkinson & Coulter, 1971). As pointed out recently (Birnbaum & Gentry, 1983), substituent effects must be taken into consideration in the derivation of standard geometries of nucleic acid constituents.

The imidazole ring is perfectly planar, but Cl(8) and C(1′) deviate from the plane by 0.071 and 0.177 Å, respectively. The pyrimidine ring is not completely planar, the deviations of the six atoms ranging from 0.007 to 0.017 Å. The $-NH_2$ group is approximately coplanar with the ring. The dihedral angle between the mean planes of the two rings is 1.5°.

Glycosidic Angle. The conformation about the glycosidic bond is syn, the torsion angle χ_{CN} [C(8)–N(9)–C(1′)–O(1′)] being −131.1° (Figure 3). This value is fairly similar to those in other 8-substituted purine nucleosides, particularly those with an intramolecular O(5′)–H···N(3) hydrogen bond (Rao & Sundaralingam, 1970; Birnbaum & Shugar, 1978). Hence, although the van der Waals radius of chlorine is smaller than that of bromine (1.75 Å as compared to 1.85 Å), the chlorine atom is sufficiently bulky to force the syn–anti equilibrium toward the syn form, also reflected by the behavior in solution (see below). The same effect has been observed in numerous crystal structures of 8-iodo-, 8-bromo-, and 8-methylpurine nucleosides as well as in 8-thioxoadenosine (Mizuno et al., 1980). Thus far, only nitrogen substituents ($-N_3$, $-NHR$) have been shown to be small enough to favor the anti conformation (Ambady & Kartha, 1975; Neidle et al., 1979). In the latter case, the glycosidic conformation is stabilized by an intra-



C(3′)–C(4′)–O(1′)–C(1′) 3.7°

FIGURE 3: Newman projections, showing torsion angles along N(9)–C(1′), C(1′)–C(2′), C(2′)–C(3′), C(3′)–C(4′), and C(4′)–C(5′).

Table II: Distances and Angles for the Hydrogen Bonds

		distances (Å)			angles (deg)	
D–H···*A*	*A* at	*D*···*A*	H···*A*	H···A_{corr}	*D*–H···*A*	H–*D*···*A*
N(2)–H(1N2)···O(W1)	$(\bar{x}, {}^1/_2 + y, {}^1/_2 - z)$	2.855	2.00	1.82	174	4
N(2)–H(2N2)···O(3′)	$(1 - x, -{}^1/_2 + y, {}^1/_2 - z)$	3.030	2.26	2.07	152	19
O(3′)–H(O3′)···O(5′)	$(1 - x, {}^1/_2 + y, {}^1/_2 - z)$	2.707	1.94	1.79	157	15
O(5′)–H(O5′)···N(3)	(x, y, z)	2.845	2.03	1.90	163	11
O(W1)–H(1W1)···O(W2)	(x, y, z)	2.868	2.04	1.95	156	16
O(W1)–H(2W1)···O(6)	$({}^1/_2 - x, 1 - y, {}^1/_2 + z)$	2.782	1.95	1.87	155	17
N(1)–H(1)···O(6)	$(-{}^1/_2 + x, {}^1/_2 - y, z)$	2.867	2.02	1.85	164	10
O(W2)–H(1W2)···N(7)	$({}^1/_2 - x, 1 - y, {}^1/_2 + z)$	2.967	2.10	2.01	170	7
O(W2)–H(2W2)···O(2′)	$(\bar{x}, -{}^1/_2 + y, {}^1/_2 - z)$	2.897	2.10	1.97	158	15
O(2′)–H(O2′)···O(W2)	$(1 - x, {}^1/_2 + y, {}^1/_2 - z)$	2.872	2.09	1.91	172	5

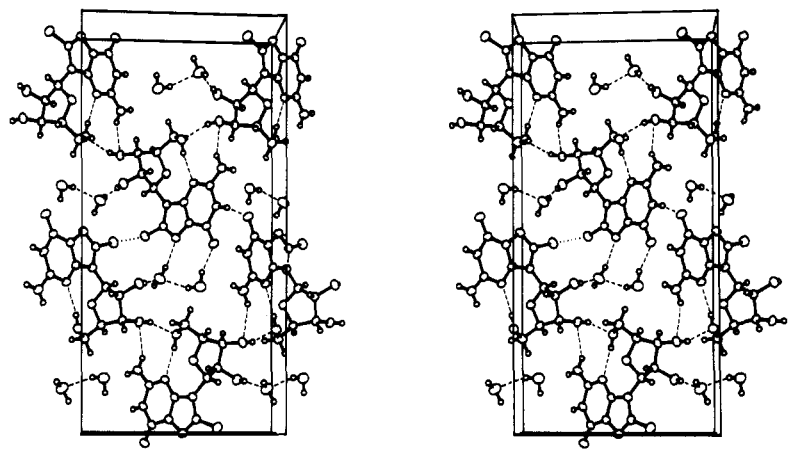
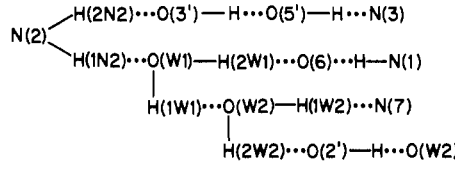


FIGURE 4: Stereoscopic view of the molecular packing in the crystal. Dashed lines indicate hydrogen bonds; the dotted line shows the short Cl···Cl contact.

molecular O(5′)–H···N(8) hydrogen bond.

Ribose Moiety. The conformation of the ribose is shown in Newman projections (Figure 3). The pucker of the furanose ring is C(2′)-endo/C(1′)-exo ($^2T_1$), as indicated by the phase angle of pseudorotation (P) of 155.0°; the maximum amplitude of puckering (τ_m) is 35.3° (Altona & Sundaralingam, 1972). The $-CH_2OH$ side chain occurs as the gauche$^+$ rotamer, the most common one in 8-substituted purine nucleosides (Birnbaum & Shugar, 1978), which is stabilized by an intramolecular hydrogen bond (see below). All bond lengths have normal values. The endocyclic angles are in excellent agreement (within 0.4°) with values calculated for P = 155.0 and τ_m = 35.3° (Birnbaum et al., 1981a).

Hydrogen Bonding and Packing. Each molecule has six protons capable of participating in hydrogen bonds. In addition, the crystal structure contains two water molecules. All 10 available protons are part of an intricate hydrogen bond network that can be represented schematically as follows:



The exact geometry of each hydrogen bond is given in Table II. As commonly observed in X-ray analyses, the O–H and N–H bonds appear shorter than their real values of 0.97 and 1.04 Å, respectively. By extending the covalent bond lengths to their nominal values, one obtains corrected H···*A* distances which reflect more accurately the strengths of these hydrogen bonds. Most bonds are seen to be of medium strength, but several of the bonds involving the water molecules are weaker than those between the nucleoside molecules. The only hydrogen bond between the guanine residues is N(1)–H···O(6), but as can be seen from the packing diagram (Figure 4), the bases are not at all coplanar.

Figure 4 also shows that base stacking, which is usually observed in halogenated pyrimidines and purines as well as in nucleosides (Bugg & Sternglanz, 1974; Birnbaum et al., 1979; Birnbaum et al., 1982), does not occur in the present structure. In guanosine, as well as in 8-bromoguanosine, the guanine residues are stacked, but the stacking mode is markedly different in the two crystal structures (Bugg & Thewalt, 1969). While 5-halogenated uracil and cytosine residues increase the stabilities of double- and triple-helical polynucleotides (Bugg & Sternglanz, 1974), the syn conformation of the residues in poly(8-bromoadenylate) prevents helix formation with anti-polynucleotides (Govil et al., 1981).

A notable feature of this crystal structure is the extremely short contact of 3.323 (1) Å between chlorine atoms in symmetry-related molecules. This distance is very significantly shorter than 3.50 Å, twice the value for the van der Waals radius of chlorine (Bondi, 1964; Taylor & Kennard, 1982). The C–Cl bonds in adjacent molecules are almost perpendicular to one another, the C–Cl···Cl and Cl···Cl–C angles being 88.2 and 177.3°, respectively. Munowitz et al. (1977) demonstrated that the Cl···Cl distance decreases as the sum of these two angles approaches 360°. The distance of 3.23 Å is considerably shorter than expected for $\theta_1 + \theta_2$ = 265.5°. The nature of Cl···Cl interactions has been a controversial subject (Yamasaki, 1962; Nyburg, 1964; Hsu & Williams, 1979; Nyburg & Wong-Ng, 1979). Recently, Price & Stone (1982) presented evidence in favor of a model that reflects the nonspherical shape of chlorine atoms and that involves the packing of the lone pairs on nearest-neighbor atoms.

Solution Conformation. The conformational parameters of 8-chloroguanosine, and those of the parent guanosine, in dimethyl sulfoxide solution, were determined from the ^1H chemical shifts and the proton–proton vicinal coupling con-

Table III: ^1H Chemical Shifts (ppm vs. Internal Me_4Si) and Vicinal ^1H–^1H Coupling Constants (Hz) for Guanosine and 8-Chloroguanosine, Each at 0.2 M in Me_2SO-d_6[a]

	chemical shifts								coupling constants					
nucleoside	NH_2	H(8)	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)	1′,2′	2′,3′	3′,4′	4′,5′	4′,5″	5′,5″
guanosine	6.45	7.90	5.70	4.40	4.09	3.89	3.57[b]	3.57[b]	5.9	4.9	3.5	3.6[c]	3.6[c]	[c]
8-chloroguanosine	6.52		5.71	4.93	4.13	3.86	3.63	3.51	6.5	5.6	3.0	4.2	5.7	−12.0

[a] Chemical shifts to ±0.01 ppm; coupling constants to ±0.2 Hz. [b] Deceptively simple band center. [c] The deceptively simple character of the H(5′), H(5″) system permits the determination of only the mean J(4′,5′) and J(4′,5″) but not of J(5′,5″).

stants (Davies, 1978), which are shown in Table III.

The conformation of the sugar ring, deduced from the proton–proton vicinal coupling constants (Altona & Sundaralingam, 1973), corresponds to a C(2′)-endo population of about 70%, as compared to about 65% for guanosine.

It has been shown elsewhere, on the basis of an analysis of the chemical shifts of the sugar protons and carbons, that guanosine exhibits a moderate preference for the form anti, with a population of about 60% (Stolarski et al., 1984). The foregoing, and the presently observed deshielding of H(2′) in 8-chloroguanosine by 0.53 ppm relative to guanosine (Table III), points to an increase in the syn population to nearly 90%, as compared to ≳95% for 8-bromoguanosine (Stolarski et al., 1984).

The very marked increase in population of the syn conformer is accompanied by an appreciable decrease in the gauche$^+$ population of the exocyclic 5′-CH_2OH from 65% in guanosine to 30% in 8-chloroguanosine. This differs from the situation in the solid state, where the gauche$^+$ form is stabilized by hydrogen bonding to the ring N(3), as observed for a number of other nucleosides in the syn conformation in the solid state (Birnbaum & Shugar, 1978). The populations of the two remaining classical conformers of the exocyclic side chain in solution, also determined from the coupling constants between H(4′) and H(5′), H(5″) (Davies, 1978), were 40% for trans and 30% for gauche$^-$.

Biological Aspects. Considerable information has now been accumulated on the biological activities of purine nucleosides and nucleotides with C(8) substituents, including many with bulky substituents which impose the syn conformation about the glycosidic bond [for review, see Birnbaum & Shugar (1985)]. However, relatively few data are available on the behavior, in biological systems, of 8-chloropurines or their nucleosides, in part because the latter have not been readily accessible.

In the nucleoside deoxyribosyltransferase system, it was reported by Holguin et al. (1975) that, with the enzyme from *Lactobacillus helveticus*, 8-substituted purines were inactive as acceptors, presumably because of steric hindrance; in particular, 8-chlorotheophylline was also inactive. A more extensive study by Huang et al. (1983) showed that the enzyme from *L. leichmanii* transfers deoxyribose from a donor to 8-substituted purines, including 8-chloroadenine, albeit at a considerably reduced rate relative to the parent purines. The surprising observation was made that the products included the N(3) deoxynucleosides. From a comparison of reaction rates, and yields of N(3) and N(9) nucleosides, with acceptors such as 8-methyl-, 8-bromo-, 8-chloro-, and 8-(trifluoromethyl)adenines, it was concluded that transfer to the deoxyribose to N(3), at the expense of N(9), was due more to electronegativity than to steric effects of the 8-substituents. This was based largely on the fact that, with the highly electron-withdrawing $-CF_3$ substituent, there was exclusively N(3) substitution, whereas the weak electron-donating $-CH_3$ group gave only N(9) substitution. However, the van der Waals radius of the $-CF_3$ group is 2.44 Å, as compared to 2.00 Å for $-CH_3$. Since an 8-bromopurine nucleoside (with a van der Waals radius of 1.85 Å for −Br) is already highly constrained (≳95%) to the syn conformation (Stolarski et al., 1984), the steric effects of $-CF_3$ must be considerably more pronounced. Furthermore, an 8-chloro substituent, which is more electronegative than an 8-bromo, was found to give a slightly higher yield of the N(9) nucleoside, hence apparently more in line with its smaller van der Waals radius. It would be desirable to examine the influence of other substituents, e.g., the highly electron-withdrawing 8-F with a van der Waals radius of 1.47 Å (Bondi, 1964), which would offer considerably less steric hindrance to adoption of the anti conformation [the synthesis of 8-fluoroadenosine has been reported by Secrist et al. (1982)]. This, in turn, might be compared with the behavior of an analogue such as the 8-thione (Mizuno et al., 1980), in which the =S substituent has a van der Waals radius of 1.75 Å (Bondi, 1964), similar to that of a chlorine atom, and an electronegativity slightly less than that of chlorine (Huheey, 1965). Apart from the foregoing, the enzyme system of Huang et al. (1983) provides a convenient procedure for the preparation of 8-substituted purine nucleosides, not easily accessible by chemical routes.

With bacterial purine-nucleoside phosphorylase, 8-bromoadenosine and 8-methylguanosine, both predominantly, but not exclusively, in the syn conformation (Stolarski et al., 1984), are neither substrates nor inhibitors (Doskocil & Holy, 1977). By contrast, 8-aminoadenosine and 8-aminoguanosine, which exhibit comparable populations of the syn and anti conformers (Evans & Kaplan, 1976), are both substrates, the former being also a good inhibitor. It is therefore possible that the anti conformation is required by the enzyme. However, examination of additional analogues is required, including the 8-chloro derivatives. Since purine-nucleoside phosphorylases are of importance because of the unusual metabolic abnormalities accompanying their deficiency (Stoeckler et al., 1982), extension of these studies to enzymes of mammalian origin would be desirable.

The foregoing enzymatic systems are of particular interest in the light of the demonstration that, with pyrimidine-nucleoside phosphorylases, e.g., uridine phosphorylase, the phosphorolysis reaction proceeds via an intermediate in the syn conformation (Krajewska & Shugar, 1982). Nonetheless, whereas 6-methyluridine, fixed in the syn conformation, is as readily phosphorylyzed as uridine, 6-methyluracil is not a substrate in the reverse, synthetic reaction.

Relatively little attention has been devoted to the antitumor or antiviral activities of 8-halogeno nucleosides. Kaneko et al. (1977) found that 8-substituted analogues of the antiviral ara-A, including the 8-bromo, were inactive against vaccinia and herpes simplex viruses and suggested that this is due to a requirement for the anti conformation. It would be desirable to examine these analogues, including the 8-chloro, with other viruses before arriving at a definitive conclusion.

Supplementary Material Available

Anisotropic temperature parameters and a list of observed

and calculated structure factors (9 pages). Ordering information is given on any current masthead page.

Registry No. 8-Chloroguanosine, 2104-68-9; 8-chloroguanosine dihydrate, 91294-62-1; guanosine, 118-00-3.

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