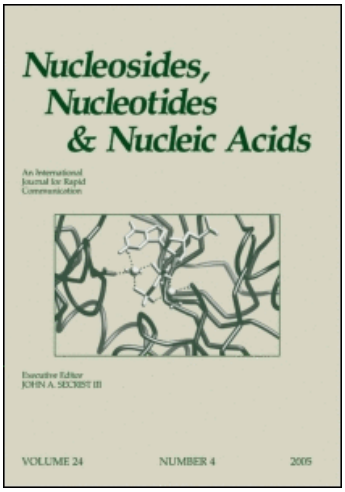

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CONFORMATIONS OF ACYCLONUCLEOSIDES: CRYSTAL STRUCTURE OF 9-(4-HYDROXYBUTYL)GUANINE, AN ANALOGUE OF ACYCLOVIR[1]

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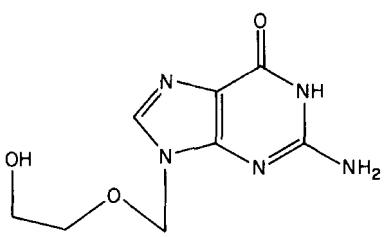
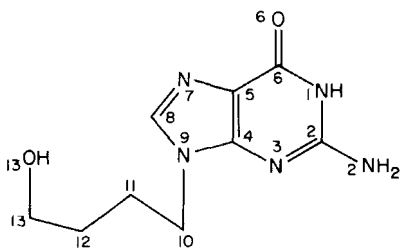
ABSTRACT. 9-(4-Hydroxybutyl)guanine is an analogue of acyclovir in which the ether oxygen is replaced by a methylene group. Crystals of the monohydrate belong to the monoclinic space group $P2_1/n$ with a = 4.350(1), b = 10.859(1), c = 23.684(4) A, β = 90.65(1)°. X-ray intensity data were measured with a diffractometer and the structure was determined by direct methods. Least-squares refinement converged at R = 0.055 for 1982 reflections. The side chain is fully extended and almost perpendicular to the guanine base.

In continuing investigations on the solid state and, in some instances, solution structures and conformations of acyclonucleosides with significant biological activities,[2-6] we report in this communication on the crystal structure of 9-(4-hydroxybutyl)guanine (HBG). As shown below, HBG is a structural analogue of the potent, and clinically licensed, antiherpes agent acyclovir (ACV, acycloguanosine), in which the ether oxygen of the acyclic chain of the latter has been replaced by a methylene group.



ACV



HBG

As is the case for acyclovir,[7] HBG is a substrate for HSV-1 and HSV-2 thymidine kinases,[7-9] but, like other analogues of acyclovir, it is not a substrate for the corresponding cellular kinase.[8,9] In in vitro systems (plaque reduction assays) it is known to inhibit replication of HSV-1 and HSV-2 with ID_{50} concentrations in the ranges 0.1-16 μM and 1.5-13 μM, respectively, depending on the virus strain and cell line employed.[9a] In analogy to acyclovir,[7] this activity is due to intracellular phosphorylation of HBG-phosphate by cellular kinases to the triphosphate,[8,9] which preferentially inhibits viral DNA polymerase as compared to cellular polymerases.[9b] In view of its in-vitro antiviral activity, we considered it worthwhile to determine the conformation of HBG by an X-ray analysis and to compare it to that of acyclovir.

EXPERIMENTAL

HBG, originally synthesized by Yamazaki,[10] was prepared by a condensation of 4-bromobutyl acetate with 2-amino-6-chloropurine, followed by acid hydrolysis. A more regioselective synthesis of HBG has recently been described.[11]

Crystals of HBG monohydrate, $C_9H_{13}N_5O_2.H_2O$ (f.w. = 241.25), obtained from aqueous ethanol, are thin plates. One of them, measuring 0.55 × 0.40 × 0.05 mm, was used for photography and data collection. Precession photographs showed systematic absences corresponding to the space group $P2_1/n$. The crystal was then mounted on a CAD-4 diffractometer, and the following data were obtained: Monoclinic, a = 4.350(1), b = 10.859(1), c = 23.684(4) Å, β = 90.65(1)°, V = 1118.5 Å^3, ρ_c = 1.43 g cm^{-3}, Z = 4 (20°C; CuKα$_1$, λ = 1.54056 Å); $F(000)$ = 512, μ(CuKα) = 8.8 cm^{-1}.

Intensities were measured with Ni-filtered CuKα radiation, using ω/2θ scans with variable scan ranges and speeds. There were 2352 unique reflections with 2θ ≤ 152°; of those, 1989 had $I \geq 3\sigma(I)$ and were considered observed. The intensities were corrected for Lorentz and polarization factors; absorption corrections were considered unnecessary.

The structure was determined by direct methods with the aid of the computer program MULTAN78.[12] Of the 8 starting sets subjected to tangent refinement, the solution with the highest combined figure of merit yielded an E map on which all atoms of the HBG molecule could be

located. The water oxygen atom was found on a difference Fourier map. The atomic parameters were refined by block-diagonal least squares with anisotropic temperature parameters. All hydrogen atoms, except one belonging to the water molecule, were found on difference Fourier maps and their coordinates and isotropic temperature parameters were refined. The scattering factors were taken from the International Tables for X-Ray Crystallography.[13] Throughout the refinement the function $\Sigma \underline{w}(|\underline{F}_o| - |\underline{F}_c|)^2$ was minimized and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages: $\underline{w} = \underline{w}_1 \cdot \underline{w}_2$, where $\underline{w}_1 = 1$ for $|\underline{F}_o| \leq 5$, $\underline{w}_1 = 5/|\underline{F}_o|$ for $|F_o| > 5$; and $\underline{w}_2 = \sin^2\theta/0.3$ for $\sin^2\theta < 0.3$, $\underline{w}_2 = 1$ for $\sin^2\theta \geq 0.3$. This scheme made the average values of $\underline{w}(\Delta \underline{F}^2)$ independent of $|\underline{F}_o|$ and $\sin^2\theta$. After the final cycle the average parameter shift equalled 0.05σ and the largest one, for the water hydrogen atom, 0.7σ. The conventional residual index $\underline{R}$ is 0.055 and the weighted index $\underline{R}'$ is 0.071 for 1982 observed reflections (seven low- angle reflections suffered from secondary extinction and were given zero weights). A final difference Fourier map showed no significant features. The atomic coordinates are listed in TABLE 1.

RESULTS AND DISCUSSION

The geometrical details of the HBG molecule are shown in FIG. 1. The side chain is almost perpendicular to the mean plane of the purine moiety, the C8-N9-C10-C11 torsion angle being 86.8°. This conformation is in accord with our previous observations and predictions.[2,3] The side chain is fully extended (all-*trans*), a conformation which is sterically the most stable one. A comparison of the side chain conformations in HBG and in acyclovir is presented in TABLE 2. Of the three molecules we found in the crystal structure of acyclovir,[2] only molecule C had a fully extended side chain. In the other two molecules, the two C-O bonds were found in the *gauche* conformation favored by the *gauche* effect. However, the *gauche* effect is not strong enough to preclude a *gauche* rotamer in HBG if it was required for binding to a receptor.

The bond lengths and bond angles in the guanine residue are in excellent agreement with those found in acyclovir and with previously published "standard" values.[14] The exocyclic bond angles at N9 are

TABLE 1. Final atomic parameters[a]

	x	y	z	U_{eq}/U_{iso}
N1	3880(4)	97668(14)	72276(6)	350
C2	5761(4)	100605(17)	67888(7)	354
N2	6206(5)	112593(16)	66903(8)	474
N3	7121(4)	92241(14)	64650(6)	358
C4	6433(4)	80600(16)	66212(7)	324
C5	4545(4)	76804(16)	70518(7)	337
C6	3139(4)	85810(16)	73938(7)	333
O6	1435(3)	84163(13)	78048(5)	415
N7	4444(4)	64072(14)	70804(6)	366
C8	6257(4)	60395(17)	66779(7)	378
N9	7537(3)	70007(14)	63842(6)	338
C10	9516(4)	69107(19)	58894(7)	381
C11	7672(4)	68473(18)	53457(7)	375
C12	9705(4)	67637(19)	48265(7)	387
C13	7846(5)	67067(25)	42881(8)	498
O13	9870(4)	65946(23)	38262(6)	627
OW	2471(21)	98122(38)	46826(17)	1954
H1	295(6)	1037(3)	742(1)	22(6)
H721	744(6)	1142(3)	643(1)	22(6)
H722	518(7)	1184(3)	689(1)	31(7)
H8	676(5)	521(2)	660(1)	14(5)
H101	1076(6)	618(3)	595(1)	21(6)
H102	1091(6)	759(3)	592(1)	18(6)
H111	629(7)	755(3)	531(1)	28(7)
H112	626(7)	615(3)	537(1)	31(7)
H121	1113(6)	602(3)	487(1)	21(6)
H122	1113(6)	745(3)	482(1)	20(6)
H131	637(7)	601(3)	428(1)	31(7)
H132	670(7)	745(3)	424(1)	29(7)
HO13	880(9)	658(4)	352(2)	44(9)
HW1	296(22)	1022(9)	429(4)	179(32)

[a]The x coordinates and U_{eq} values of the non-hydrogen atoms were multiplied by 10^4, the y and z coordinates by 10^5. All hydrogen atom parameters were multiplied by 10^3.

identical. This is also the case in molecule C of acyclovir, the one with the fully extended side chain. In other acyclonucleosides C8-N9-C1' tends to be slightly larger (0-3°) than C4-N9-C1'. The geometry of the side chain is unexceptional and reflects, in part, increased thermal motion with increasing distance from the aglycon.

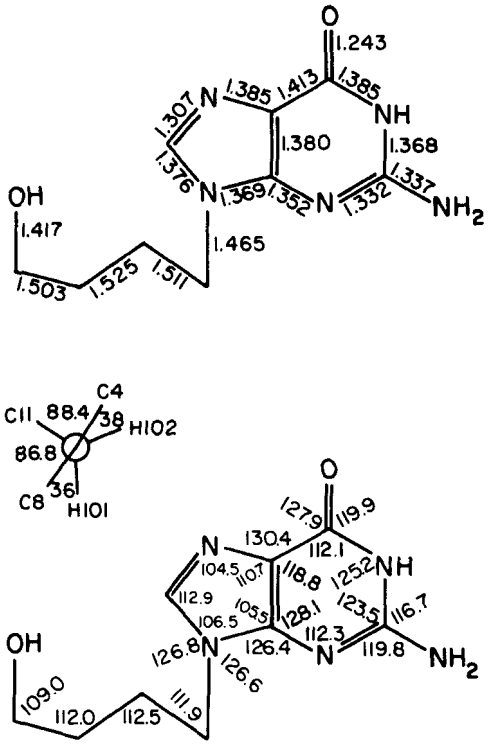
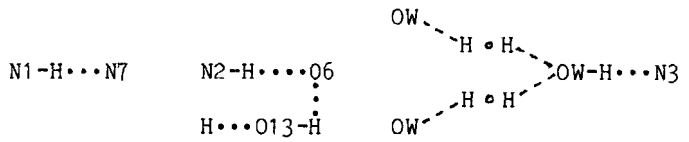


FIG. 1: Bond lengths (top) and bond angles (bottom) in HBG. Their estimated standard deviations are 0.002-0.003 Å and 0.15-0.18°, respectively. A Newman projection along N9-C10 is shown in the center.

TABLE 2. Torsion angles (deg) in HBG (left) and in acyclovir (right)

HBG		A	B	C	Acyclovir
C8-N9-C10-C11	86.8	97.3	104.3	91.4	C8-N9-C1'-O1'
N9-C10-C11-C12	179.5	-76.9	-66.3	-173.3	N9-C1'-O1'-C4'
C10-C11-C12-C13	-179.8	173.2	-176.2	-171.9	C1'-O1'-C4'-C5'
C11-C12-C13-O13	-178.3	60.6	73.5	-174.4	O1'-C4'-C5'-O5'

There are four protons in the HBG molecule which can participate in hydrogen bonds and the water molecule in the crystal lattice has two more. All of them do, in fact, take part in intermolecular hydrogen bonds; the interactions can be represented schematically as follows:



The geometry of the hydrogen bonds is given in TABLE 3. The covalent N-H and O-H bonds were normalized to their nominal values of 1.04 and 0.97 A, respectively, in order to assess the strengths of these interactions more accurately. Each water oxygen atom is in short contact with two others across centers of symmetry. Thus, the proton involved in this OW···OW interaction is too close to the center of symmetry to allow full occupancy of a symmetry-related proton.
It appears, therefore, that this proton is being donated alternately to the two other oxygen atoms which are related by a translation along a. We were unable to locate this proton on difference Fourier maps. The vibration parameters of the other two atoms of the water molecule are relatively high, reflecting the disorder of the water molecule. The interaction between N2 and O13 is rather weak but very likely an attractive one.

A packing diagram (FIG. 2) shows alternating regions of bases and aliphatic chains. As in acyclovir, the guanine bases are joined by N1-H···N7 and N2-H···O6 hydrogen bonds into infinite sheets. Translation of molecules along a results in base stacking and the distance between adjacent bases is 3.40(1) A.

The similarity in conformation of HBG to molecule C of acyclovir is of obvious interest in relation to the affinities of these molecules for the herpes virus thymidine kinases. The affinity for HSV-1 thymidine kinase of HBG (K_i = 2 μM) is much higher than that of acyclovir (K_i = 173 μM).[8] But the rate of phosphorylation of the former is only 10%, as compared to 27% for acyclovir, relative to the rate of phosphorylation of thymidine itself. The high affinity of HBG for HSV-1 thymidine kinase is further testified to by its effective inhibition of phosphorylation of acyclovir by this enzyme.[7]

TABLE 3. Distances and angles for hydrogen bonds

			Distances, Å		Angles, deg
D	A	A at	D•••A	H•••A_{corr}	D-H•••A
N1-H•••N7		1/2-x,1/2+y,3/2-z	2.829	1.81	168
N2-H1N2•••O13		2-x,$\bar{y}$,1-z	3.145	2.40	131
N2-N2N2•••O6		1/2-x,-3/2+y,3/2-z	2.875	1.86	164
O13-H•••O6		1/2+x,3/2-y,-1/2+z	2.829	1.86	178
OW-H1W•••N3		1-x,2-y,1-z	2.920	1.97	165
OW-H2W•••OW		$\bar{x}$,2-y,1-z	2.669		
OW-H2W'•••OW		1-x,2-y,1-z	2.682		

In the case of HSV-2 thymidine kinase, the K_i values for HBG and acyclovir, 30 μM and 140 μM, respectively, once again point to the higher affinity of the former for this enzyme. Furthermore, in this instance the rate of phosphorylation of HBG is 65%, and of acyclovir only 5%, relative to that for thymidine.[9] The triphosphates of HBG and ACV also differ in their inhibition of HSV DNA polymerase. The inhibition values (K_i) against DNA polymerases for both HSV-1 and HSV-2 are about 0.12 μM for HBGTP and 0.0015 μM for ACVTP.[9b]

Bearing in mind the flexibilities of the acyclic chains in both HBG and acyclovir, the differences in affinities for the viral thymidine kinases appear unlikely to be due to conformational factors. It appears more plausible that this is due to electronic effects resulting from replacement of the ether oxygen in acylcovir by a methylene group. During the course of a study, still in progress, on the inhibitory properties of various HBG and acyclovir analogues *vs* purine nucleoside phosphorylase from calf spleen, we have found that HBG is a markedly superior inhibitor (K_i ~40 μM) than acyclovir (K_i ~90 μM).

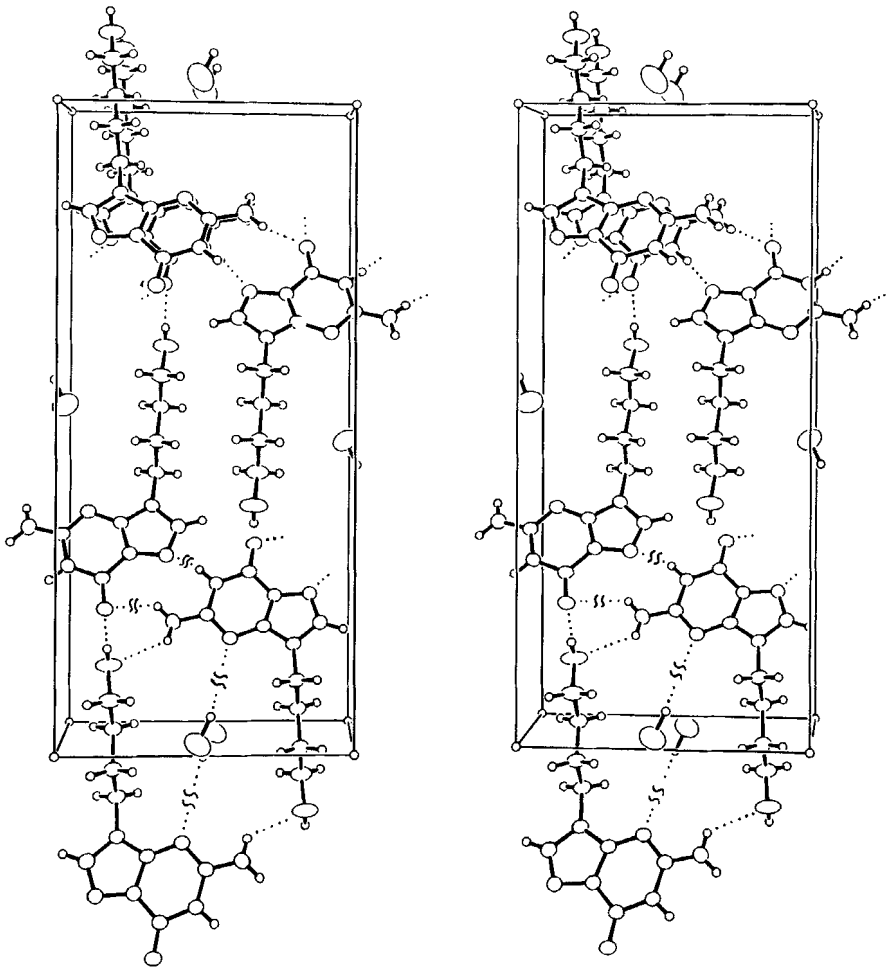


FIG 2: Stereoscopic view of molecular packing in the crystal. The directions of the axes are x ⊙, y →, z ↑. A molecule translated by a (top left) shows base stacking.

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REFERENCES AND FOOTNOTES

(1) Issued as NRCC No. 26358.

(2) Birnbaum, G.I.; Cygler, M.; Shugar, D. Can. J. Chem. 1984, 62, 2646.

(3) Birnbaum, G.I.; Stolarski, R.; Kazimierczuk, Z.; Shugar, D. Can. J. Chem. 1985, 63, 1215.

(4) Birnbaum, G.I.; De Clercq, E.; Hatfield, P.W.; Robins, M.J. Heterocycles, 1986, 25, in press.

(5) Birnbaum, G.I.; Brisson, J.-R.; Chu, S.H.; Chen, Z.H.; Rowe, E.C. Can. J. Chem. 1986, 64, in press.

(6) For a review see Birnbaum, G.I.; Shugar, D. In "Topics in Nucleic Acid Structure", Part 3; Neidle, S., Ed.; Macmillan: London, in press.

(7) Keller, P.M.; Fyfe, J.A.; Beauchamp, L.; Lubbers, C.M.; Furman, P.A.; Schaeffer, H.J.; Elion, G.B. Biochem. Pharmacol. 1981, 30, 3071.

(8) Larsson, A.; Alenius, S.; Johansson, N.-G.; Oberg, B. Antiviral Res. 1983, 3, 77.

(9) (a) Ericson, A.-C.; Larsson, A.; Aoki, F.Y.; Yisak, W.-A.; Johansson, N.G.; Oberg, B.; Datema, R. Antimicrob. Agents Chemother. 1985, 27, 753. (b) Larsson, A.; Sundquist, A.; Parnerud, A.-M. ibid. 1986, in press.

(10) Yamazaki, A. Chem. Pharm. Bull. 1969, 17, 1268.

(11) Kjellberg, J.; Liljenberg, M.; Johansson, N.G. Tetrahedron Lett. 1986, 27, 877.

(12) Main, P.; Hull, S.E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M.M. MULTAN78, University of York, England, and University of Louvain, Belgium, 1978.

(13) "International Tables for X-ray Crystallography"; Ibers, J.A.; Hamilton, W.C., Eds.; Kynoch Press: Birmingham, England, 1978.

(14) Taylor, R.; Kennard, O. J. Am. Chem. Soc. 1982, 104, 3209.

(15) Ahmed, F.R.; Hall, S.R.; Pippy, M.E.; Huber, C.P. J. Appl. Crystallogr. 1973, 6, 309.

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