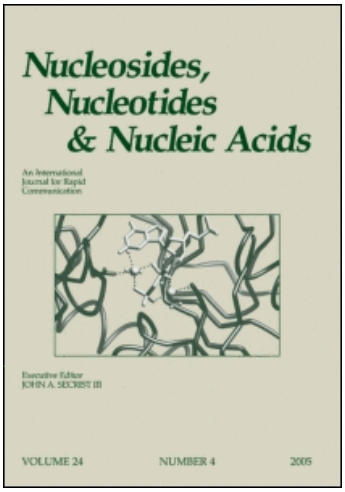

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Solid State and Solution Structure and Conformation of The Antiviral Acyclonucleoside 9-[4-Hydroxy-2-(hydroxymethyl)-butyl]guanine

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SOLID STATE AND SOLUTION STRUCTURE AND CONFORMATION OF THE ANTIVIRAL ACYCLONUCLEOSIDE 9-[4-HYDROXY-2-(HYDROXYMETHYL)-BUTYL]GUANINE

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ABSTRACT: The title compound, 9-[4-hydroxy-2-(hydroxymethyl)-butyl]guanine (2HM-HBG), crystallizes in the triclinic space group P1 with two independent molecules and one water molecule in the asymmetric unit. The acyclic chain of one molecule is in the fully extended form, and the other partially folded. The orientation of this chain with respect to the base corresponds to the conformation *syn* in natural nucleosides. The conformations of the two molecules were compared with the solution conformation from an analysis of the ^1H-^1H vicinal coupling constants. The comportment of some acyclonucleosides in several enzyme systems is examined in relation to their existence in folded or extended forms.

INTRODUCTION

The acyclonucleoside Acyclovir (ACV, 9-(hydroxyethoxy-methyl)guanine, see Fig. 1) is a potent inhibitor of HSV replication,[1] now widely applied clinically as an antiherpetic agent. This has prompted the synthesis of a multitude of acyclonucleosides of purine and pyrimidine analogues, extensively reviewed by Johansson.[2] Many of these exhibit antiviral activity, and/or are inhibitors of specific enzymes.[3] Several typical acyclonucleosides of guanine, including the title compound, 2HM-HBG are shown in Fig. 1. It will

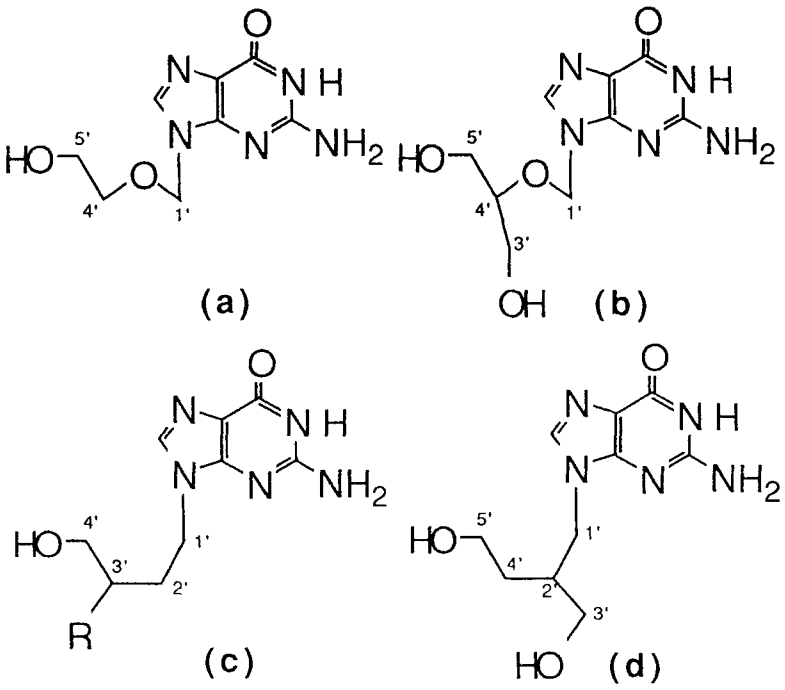

FIG. 1. Structures of acyclonucleosides: (a) Acyclovir; (b) Ganciclovir (DHPG); (c) HBG (R = H), Buciclovir (R = OH) and Penciclovir (R = CH_2OH); and (d) 2HM-HBG. Note that the carbons in the acyclic chains are numbered like the corresponding carbons of the pentose ring and that the oxygen atom in the acyclic chain in Acyclovir and Ganciclovir corresponds to the C2' methylene in HBG, Buciclovir, Penciclovir and 2HM-HBG.

be noted that the acyclic chains may mimic the "upper" and "lower" portions of the sugar ring of natural nucleosides.

The antiviral activities of ACV and the other acyclonucleosides shown in Fig. 1 are dependent on their prior intracellular phosphorylation in infected cells by a virus-coded, but not cellular, nucleoside kinase, and subsequent phosphorylation to the triphosphates, which are usually selective inhibitors of the viral, relative to cellular, DNA polymerases.

Bearing in mind the flexibility of the acyclic chains, these may adopt a variety of conformations.[4] In fact, the development of ACV

as an antiherpetic agent[1] was a direct outcome of the earlier observation that the adenine analogue of ACV, 9-(hydroxyethoxymethyl)adenine, is a mimic of adenosine and is deaminated by adenosine deaminase,[5] further discussed below. Numerous acyclonucleosides, and their phosphates and phosphonates, exhibit substrate/inhibitor properties in various enzyme systems,[3] e. g. the pyrophosphate of ACV is the most potent known *in vitro* inhibitor of purine nucleoside phosphorylase,[6].

The foregoing point to the utility of determining the preferred conformations of biologically active acyclonucleosides, a number of which have already been reported.[4,7] The significance of this is further underlined by the recent demonstration[8,9] that Ganciclovir (DHPG, Fig. 1b), a clinically promising agent *vs* human cytomegalovirus (HCMV), which does not code for a thymidine kinase, is phosphorylated by the HCMV UL97 gene product, which shares regions of homology with protein kinases, and suggests that DHPG may adopt a conformation resembling some local peptide region of a protein kinase substrate. This lends added interest to the structure and conformation of 2HM-HBG, which like DHPG, contains two primary hydroxyls on the acyclic chain, and is the subject of the present communication.

EXPERIMENTAL

Slow diffusion of acetone into an aqueous solution of the title compound, (R,S)-2HM-HBG,[2] at room temperature led to the formation of suitable crystals, in the form of platelets. A crystal, cut to size 0.20 x 0.20 x 0.20 mm was mounted and the cell dimensions determined from 32 reflections with 2Θ angles in the range 40 - 50° and their e.s.d.'s from a Θ least-squares. The crystal data are as follows: $O_3N_5C_{10}H_{15} \cdot 1/2H_2O$, mol wt. = 262.27, triclinic space group P1 with unit cell dimensions a = 4.657(2) Å, b = 11.030(2) Å, c = 12.059(3) Å, α = 87.43(2)°, β = 79.00(5)° and γ = 83.33(2)°, V = 603.7(3) $Å^3$, ρ_c = 1.443 Mg · m^{-3}, Z = 2, F(000) = 278.14, μ = 0.10 mm^{-1}, λ (Mo) = 0.70930 Å, 2Θ(max) = 49.8°. No absorption correction was considered necessary due to the low μ value.

The intensity data, collected at room temperature on a Nonius diffractometer, using the $\Theta/2\Theta$ scan mode, consist of 1981 unique

reflections that were considered observed, i.e. with intensities greater than 2.5σ. The structure was solved on the NRCVAX system, employing the symbolic addition method and refined with full-matrix least squares,[10] to: R = 0.029 (R' = 0.033) for the observed reflections, R = 0.032 (R' = 0.033) for all reflections, with 69 atoms, 459 parameters and 1981 observed out of 2113 reflections up to $2\Theta_{max}$ of 49.8°. Weights based on counting-statistics were used: $w = 1/(\sigma^2 F_o + k^2)$ with a k value of 0.0001. No shift was larger than 0.07σ after the last cycle of refinement. The last difference Fourier map has no significant features, with the deepest hole -0.15 e/Å^3 and the highest peak 0.13 e/Å^3.

^{1}H NMR spectra of 2HM-HBG, at a frequency of 500.13 MHz , were recorded on a Bruker 500 AM instrument at a concentration of 0.02 M in $(C^2H_3)_2SO$ at 30°C, with Me_4Si as internal standard.

RESULTS AND DISCUSSION

Solid state conformation of 2HM-HBG

The title molecule crystallized from aqueous/acetone medium, with two independent molecules A and B, and one water molecule, in the asymmetric unit. The final atomic coordinates, listed in Table 1, enable one to calculate the bond lengths and bond angles.* All the corresponding bond lengths in the two molecules agree with each other and with those in other related structures.[11] The O5'-HO5' bond in molecule B, 1.24(13) Å, seems long, but is still normal considering its large standard deviation - the hydrogen atom of O5' is not well defined due to high thermal vibration. There are slight variations between corresponding bond angles, however, which is to be expected due to the differences in conformation of the two molecules. The three-dimensional structure and conformation are depicted by the torsion angles (Table 2) and the stereoscopic view in Fig. 2.

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* Bond lengths and bond angles are available on request to the authors.

As can be seen from Planes 1 and 2 of the two independent molecules A and B (Table 3), the guanine residues are flat, with only a slight deviation (0.018 Å) of the endocyclic atom C6 in molecule A. Most of the exocyclic atoms, *viz.* N2, O6, C1' are slightly displaced from this plane, but the largest deviation is only 0.078 Å (for O6) and compares well with that in the 3':5'-cyclic phosphate of DHPG, i.e. DHPG-cMP.[12] The guanine planes of the two independent molecules are almost parallel, the torsion angle between them being only 6.2° (Table 3).

In both the independent molecules the side chain is approximately perpendicular to the plane of the aglycon, the torsion angles C4-N9-C1'-C2' (equivalent to the glycosidic torsion angle χ in nucleosides) being 93.5° and 86.0°, respectively, and hence in the range characterized as *high syn* and *syn*.[13]

The acyclic chain in molecule A is in the fully extended form, with a transoidal, more accurately antiperiplanar,[13] orientation of the non-proton substituents on the chain about the bonds C1'-C2', C2'-C4' and C4'-C5', as shown below in Fig. 4. The conformation of C1' and O3' about C2'-C3' is *gauche* (+synclinical). The largest deviation from 180° is 23°, for the C1'-C2'-C4'-C5' torsion angle (157.1°). By comparison, the hydroxymethyl group in molecule B is rotated about the C1'-C2' bond, so that the atoms N9 and C4' are in the *gauche* (+synclinical) orientation (acyclic chain partially folded), with a torsion angle of 65.9(3)° for N9-C1'-C2'-C4'. There is also a rotation about the C2'-C3' bond to a -synclinical conformation, manifested by the C4'-C2'-C3'-O3' torsion angle of -179.5°, with the atoms C4' and O3' *trans* to each other, and the consequent formation of an intramolecular hydrogen bond between O3'H and N3 of the guanine ring. This intramolecular hydrogen bond thus creates an eight-membered ring where C1', C2', C3', and N3 are very close to being in one plane, C4 and N9 are displaced to one side by equal amounts, 0.997(5) and 1.001(5) Å, and O3' and HO3' by -1.219(5) and -1.00(4) Å to the other side.

As may be seen from the packing diagram (Fig. 3) and the hydrogen bond distances and angles listed in Table 4, the hydrogen bonding scheme is rather complex. All hydrogens of the OH, NH, and NH_2 groups are involved in hydrogen bonds, except for one

TABLE 1. Final atomic parameters x, y, z and Biso, the mean of the principal axes of the thermal ellipsoid. The e.s.d's refer to the last digit(s).

	x	y	z	Biso
N1A	0.9994(8)	0.7952(3)	0.3463(3)	2.78(11)
C2A	1.2206(9)	0.7577(3)	0.2583(3)	2.69(12)
N2A	1.2563(10)	0.6387(3)	0.2371(3)	3.84(13)
N3A	1.3910(7)	0.8321(3)	0.1952(3)	2.66(10)
C4A	1.3206(8)	0.9487(3)	0.2281(3)	2.36(11)
C5A	1.1042(9)	0.9952(3)	0.3141(3)	2.57(11)
C6A	0.9279(8)	0.9139(3)	0.3822(3)	2.72(12)
O6A	0.7321(8)	0.9375(3)	0.4663(3)	4.00(10)
N7A	1.1029(8)	1.1197(3)	0.3207(3)	3.35(12)
C8A	1.3184(9)	1.1466(4)	0.2398(3)	3.21(14)
N9A	1.4604(7)	1.0468(3)	0.1800(3)	2.63(10)
C1'A	1.6990(9)	1.0436(4)	0.0812(3)	2.78(12)
C2'A	1.5885(8)	1.0736(3)	-0.0287(3)	2.65(11)
C3'A	1.4509(9)	1.2057(3)	-0.0319(4)	3.17(13)
O3'A	1.64473	1.28906	-0.01253	3.88(11)
C4'A	1.8330(9)	1.0449(4)	-0.1313(3)	3.05(12)
C5'A	1.7127(10)	1.0250(5)	-0.2353(4)	4.73(19)
O5'A	1.9497(8)	1.0087(4)	-0.3295(3)	5.08(13)
N1B	0.6925(8)	0.3014(3)	0.4416(3)	2.98(10)
C2B	0.4518(9)	0.2801(3)	0.5215(3)	2.69(12)
N2B	0.4022(9)	0.1644(3)	0.5427(3)	3.39(13)
N3B	0.2760(8)	0.3686(3)	0.5785(3)	2.71(10)
C4B	0.3622(8)	0.4807(3)	0.5489(3)	2.39(11)
C5B	0.5977(9)	0.5103(3)	0.4703(3)	2.62(12)
C6B	0.7838(9)	0.4147(3)	0.4080(3)	2.92(12)
O6B	1.0000(8)	0.4235(3)	0.3330(3)	4.23(11)
N7B	0.6111(8)	0.6349(3)	0.4647(3)	2.79(10)
C8B	0.3853(9)	0.6776(3)	0.5398(3)	2.71(13)
N9B	0.2246(7)	0.5892(3)	0.5942(3)	2.50(9)
C1'B	-0.0424(8)	0.6073(4)	0.6810(3)	2.70(12)
C2'B	0.0096(8)	0.5935(3)	0.8019(3)	2.64(11)
C3'B	0.1525(9)	0.4669(3)	0.8279(4)	3.00(13)
O3'B	-0.0078(7)	0.3727(3)	0.8017(3)	3.83(10)

TABLE 1. (continued)

C4'B	0.1817(9)	0.6939(4)	0.8286(3)	2.93(13)
C5'B	0.2054(10)	0.6997(4)	0.9511(3)	3.74(16)
O5'B	0.3791(9)	0.7957(3)	0.9598(3)	5.29(13)
OW	0.7615(12)	0.4784(5)	0.1182(4)	7.41(20)
H1A	0.895(8)	0.747(3)	0.384(3)	3.7(8)
H2A1	1.404(9)	0.616(4)	0.195(3)	4.0(9)
H2A2	1.141(10)	0.590(4)	0.283(4)	5.1(10)
H8A	1.382(7)	1.225(3)	0.223(3)	3.4(7)
H1'A1	1.808(7)	0.956(3)	0.081(3)	3.3(7)
H1'A2	1.832(7)	1.107(3)	0.096(3)	2.5(6)
H2'A	1.422(7)	1.021(3)	-0.027(3)	2.9(6)
H3'A1	1.267(9)	1.221(3)	0.031(3)	5.0(9)
H3'A2	1.399(6)	1.217(3)	-0.105(3)	2.8(6)
HO3'A	1.755(9)	1.303(4)	-0.073(3)	4.7(10)
H4'A1	1.971(7)	1.110(3)	-0.136(3)	3.5(7)
H4'A2	1.955(7)	0.972(3)	-0.114(3)	3.5(7)
H5'A1	1.578(9)	1.101(4)	-0.252(3)	5.6(10)
H5'A2	1.592(9)	0.944(4)	-0.226(4)	5.9(10)
HO5'A	1.879(12)	0.984(6)	-0.388(5)	8.9(16)
H1B	0.798(8)	0.235(4)	0.404(3)	3.7(8)
H2B1	0.252(8)	0.143(3)	0.589(3)	3.5(8)
H2B2	0.506(8)	0.107(4)	0.508(3)	4.2(9)
H8B	0.330(8)	0.757(3)	0.560(3)	3.5(7)
H1'B1	-0.148(8)	0.688(3)	0.668(3)	4.0(8)
H1'B2	-0.178(8)	0.546(3)	0.667(3)	4.0(7)
H2'B	-0.186(7)	0.604(3)	0.846(3)	2.8(6)
H3'B1	0.359(8)	0.457(3)	0.783(3)	3.4(7)
H3'B2	0.150(8)	0.453(3)	0.908(3)	4.6(8)
HO3'B	0.063(8)	0.354(3)	0.730(3)	4.0(8)
H4'B1	0.395(7)	0.685(3)	0.785(3)	3.3(7)
H4'B2	0.089(8)	0.781(4)	0.803(3)	4.4(8)
H5'B1	0.316(7)	0.623(3)	0.976(3)	3.3(7)
H5'B2	-0.019(8)	0.710(3)	1.006(3)	4.1(8)
HO5'B	0.452(25)	0.772(10)	1.053(11)	21.9(39)
HW1	0.630(10)	0.512(4)	0.066(4)	5.7(23)
HW2	0.741(16)	0.417(6)	0.106(6)	10.1(23)

TABLE 2. Torsion angles (°) of the side chains of molecules A and B of 2HM-HBG and comparison with those of HBG and molecules A, B and C of Acyclovir.

Side chain	2HM-HBG		HBG[a]	ACV[a,b]		
	A	B		A	B	C
C4-N9-C1'-C2'	93.5(4)	86.0(4)	88.4	76.5	74.4	90.5
C8-N9-C1'-C2'	-82.5(4)	-95.4(4)	-86.8	-97.3	-104.3	-91.4
N9-C1'-C2'-C4'	-168.7(5)	65.9(3)	-179.5	76.9	66.3	173.3
C1'-C2'-C4'-C5'	157.1(5)	171.3(5)	179.8	-173.2	176.2	171.9
C2'-C4'-C5'-O5'	175.3(6)	178.4(6)	178.3	-60.6	-73.5	174.4
N9-C1'-C2'-C3'	66.2(3)	-60.5(3)				
C3'-C2'-C4'-C5'	-78.2(4)	-62.1(3)				
C1'-C2'-C3'-O3'	54.4(3)	-53.7(3)				
C4'-C2'-C3'-O3'	-70.5(3)	-179.4(5)				

[a] For clarity, the values reported here for HBG and ACV are the ones corresponding to the same hand as for 2HM-HBG.

[b] In Acyclovir there is an oxygen atom instead of C2'.

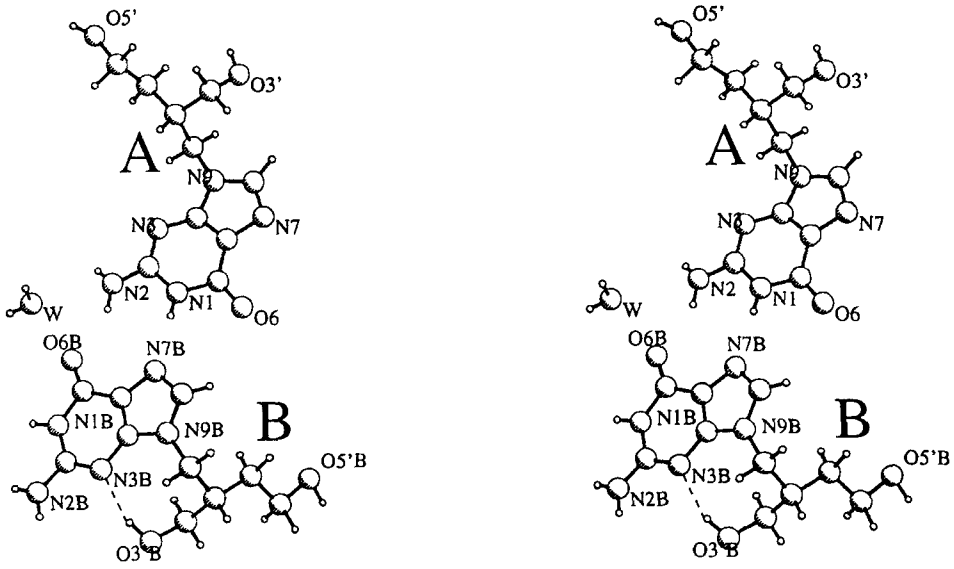

FIG 2. Stereoscopic view of the solid state structure of 2HM-HBG, showing the two independent molecules A and B, and one water molecule, in the asymmetric unit.

TABLE 3. Weighted least-squares planes. Atoms excluded from the calculations of the planes are denoted by an asterisk.

Guanine residues			8-membered ring	
	Plane 1 (Molecule A)	Plane 2 (Molecule B)		Plane 3 (Molecule B)
Atoms	Distance (Å)	Distance (Å)	Atoms	Distance(Å)
N1	-0.002(4)	0.009(5)	N3B	0.016(5)
C2	-0.001(5)	0.003(5)	C1'B	-0.042(5)
N3	-0.003(4)	-0.005(4)	C2'B	0.059(5)
C4	-0.005(4)	-0.002(4)	C3'B	-0.046(6)
C5	-0.012(4)	0.000(4)	C4B*	0.997(5)
C6	0.018(5)	-0.011(5)	N9B*	1.001(5)
N7	-0.011(5)	0.001(4)	O3'B*	-1.219(5)
C8	0.006(5)	0.002(5)	HO3'B*	-1.00(4)
N9	0.010(4)	0.002(4)		
N2*	-0.014(6)	0.038(5)		
O6*	0.078(5)	-0.038(5)		
C1'*	-0.040(6)	-0.020(6)		
H1*	-0.01(4)	-0.02(4)		
H21*	0.13(4)	0.00(4)		
H22*	0.01(5)	0.03(4)		
H8*	0.05(4)	0.03(4)		
χ^2	39.437	12.162	χ^2	295.290

Equations of the planes:

1. 3.574(4)X - 0.446(10)Y + 9.158(10)Z = 6.390(10) Å
2. 3.305(4)X + 0.097(10)Y + 9.903(9)Z = 6.682(5)
3. 4.166(5)X + 5.459(17)Y - 0.333(23)Z = 2.953(15)

Torsion angle between planes A and B:

A	B	Angle(°)
1	2	6.23(10)
2	3	62.76(14)

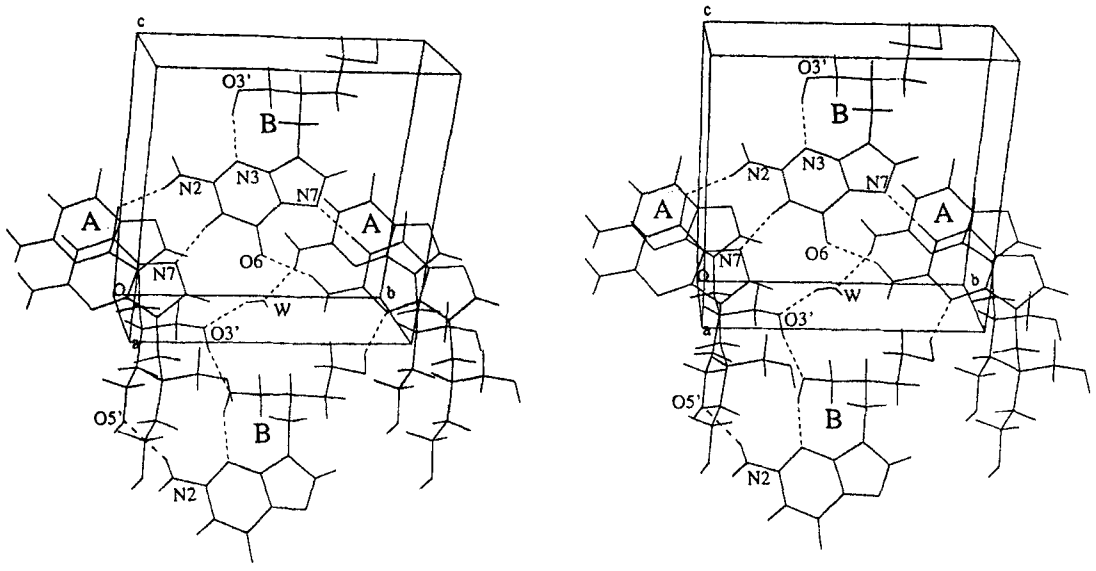

FIG. 3. Stereoscopic view of the packing and hydrogen bonding of 2HM-HBG in the solid state. One hydrogen bond is not shown, *viz.* the one from O5' to O6.

hydrogen atom of the water molecule. There are base-pairs of molecules A and B, linked *via* two pairs of hydrogen bonds: N1-H1...N7B and N2-H22...O6B, one pair from molecule A to molecule B and then the same pair also from B to A, thus forming parallel sheets in the direction of the aromatic planes. These sheets are then linked *via* the water molecule(s) to form an infinite spiral in the direction of the x-axis. However, the chain of hydrogen bonds within this spiral is finite, since the spiral also contains the side-chain of molecule B and a portion of molecule A. Infinite chains are energetically more favorable than finite ones due to the cooperative effect.[14] The molecules are also cross-linked by hydrogen bonds from N2 in molecule B to O6 and O5' in two different molecules of type A. The molecules do not pack to form π - π stacks of the hydrophobic guanine heterocyclic regions, since the shortest distance between atoms in two bases is as much as 4.66 Å, the length of the a-axis. However, the hydrophobic guanine heterocyclic regions still alternate with the hydrophilic side-chain regions, a common feature

TABLE 4. Hydrogen-bond distances (Å) and angles (°).

Donor atom	Acceptor atom	Position of acceptor atom	Distance D...A	Distance H...A	Angle	
OW	O3'A	x-1, y-1, z	2.833(5)	2.19(7)	OW-HW2...O3'A	148(7)
O3'A	O3'B	x+2, y+1, z-1	2.695(3)	1.88(4)	O3'A-HO3'A...O3'B	165(4)
O5'A	O6A	x+1, y, z-1	3.002(5)	2.10(7)	O5'A-HO5'A...O6A	175(5)
N2A	OW	x+1, y, z-1	2.948(6)	2.21(4)	N2A-H2A1...OW	154(4)
N2A	O6B	x, y, z	2.878(5)	2.05(5)	N2A-H2A2...O6B	152(4)
C8A	O6B	x, y+1, z	3.378(5)	2.86(3)	C8A-H8A...O6B	116(2)
O3'B	N3B	x, y, z	2.763(5)	1.91(4)	O3'B-HO3'B...N3B	158(3)
O5'B	N3A	x-1, y, z+1	2.896(5)	1.83(13)	O5'B-HO5'B...N3A	140(8)
N2B	O6A	x, y-1, z	2.871(5)	2.07(4)	N2B-H2B2...O6A	163(4)
N2B	O5'A	x-2, y-1, z+1	3.021(5)	2.23(4)	N2B-H2B1...O5'A	154(3)
N1A	N7B	x, y, z	2.822(5)	2.00(4)	N1A-H1A...N7B	173(3)
N1B	N7A	x, y-1, z	2.847(5)	1.95(4)	N1B-H1B...N7A	165(3)

in nucleoside crystal structures.[15] Both the side-chain and the guanine of molecule A are involved in hydrogen bonds to the water molecule, while molecule B is hydrogen-bonded only to molecule A. It is questionable whether there is a weak C-H hydrogen bond here, the C8...O6B and H8...O6B distances being 3.378(5) Å and 2.86(3) Å, respectively, with a C8-H8...O6B angle of 116(2)°. By using the van der Waals' potential minimum contact radii, r_H = 1.50, r_O = 1.65, r_C = 1.70,[16] the distances observed here are smaller than the sum of the respective radii, but longer than any of the C-H hydrogen bonds recorded, the longest of which is 2.651 Å.[17]

Comparison with HBG and Acyclovir

The crystal structure of 2HM-HBG may be compared with other acyclonucleosides with the same base and with similar characteristics of the side chains, viz. HBG [18] and Acyclovir [19], the latter with an oxygen instead of the C2' methylene in the side chain. Comparisons of torsion angles are shown in Table 2. When comparing these, it should be kept in mind that a torsion angle of, e.g. +173°, is the equivalent of -187°.

The conformation of molecule A is closely related to other structures with fully extended chains, *viz.* HBG and molecule C of ACV, while that of molecule B resembles more those with folded chains, viz. molecules A and B of Acyclovir.

The orientation of the base relative to the side chains in both molecules A and B of 2HM-HBG (χ = 93.5° and 86.0°, respectively) is similar to structures with extended side chains, *viz.* HBG (88.4°) and molecule C of ACV (90.5°), although the value in molecule B (86.0°) is somewhat closer to the smaller values 76.5° and 74.4° in molecules A and B of ACV, which have folded side chains; the latter values correspond more closely to the typical *syn* conformation

The conformation of the side chain of molecule A is similar to those of HBG and molecule C of ACV, the largest deviation being 23° in the C1'-C2'-C4'-C5' dihedral angle relative to the corresponding angle in HBG. Both in HBG and in molecule C of ACV, there is much less variation in the individual torsion angles of the extended chain than in molecule A of this structure, presumably due to the hydroxymethyl substituent of the side chain of the latter. The

conformation of the side chain of molecule B is similar to those of molecules A and B in ACV, except about the C4'-C5' bond, C2' and O5' being *trans* (+ap) in this structure but *gauche* (+sc) in Acyclovir.

Solution conformation of 2HM-HBG

The conformation of the acyclic chain was derived from the vicinal coupling constants ^1H-^1H which, with the aid of appropriate parametrization of the Karplus relationship, are related to the torsion angles in the ^1H-C-C-^1H molecular fragments.[20]

The measured NMR parameters (Table 5) clearly demonstrate the flexibility of the acyclic chain, with a dynamic equilibrium of all allowable classical conformers *trans* and *gauche* (ap, +sc, -sc, Fig. 4) with comparable populations (Table 5), and only a slight preference for the transoidal orientation, reflected in conformational flexibility in the solid state (see also below).

Concluding remarks and biological aspects.

We have previously compared the crystal structures of acyclonucleosides such as ACV,[19] DHPG and the analogous 9-[1,3-dihydroxy-2-propoxy)methyl]adenine (DHP-Ade)[11] with their corresponding nucleosides, and of DHPG-cMP with its parent second messenger cGMP.[12] These, together with the solid-state structures of HBG,[18] 2',3'-*seco*DRB ,[21] 2HM-HBG (this work), Penciclovir and Famciclovir,[22] the 2',3'-dihydroxypropyl derivatives of adenine,[23] as well as 1-(2',3'-O-isopropylidene-2',3'-dihydroxypropyl)uracil [24] and 9-(2'-phosphonomethoxyethyl)-adenine[25] permit some general conclusions.

A characteristic feature of the foregoing compounds is their appearance in the solid state as two (DHP-Ade, *seco*DRB, 2HM-HBG, Famciclovir), and even three (ACV) different conformers. In addition to one form with the acyclic chain fully extended, there is a partially folded form which mimics the pentose ring of the natural nucleoside. Analyses of solution conformations[26-28] lead to analogous conclusions regarding "conformational flexibility" of the acyclic chains, bearing in mind that the presence of an ether oxygen (as in the "upper" portion of the intact pentose ring) appears to confer somewhat more rigidity than a carbocyclic chain.

TABLE 5. Values of ^1H chemical shifts (ppm ±0.005 vs internal Me_4Si), ^1H-^1H coupling constants (±0.1 Hz) and conformer populations (%) for 2HM-HBG in $(C^2H_3)_2SO$ at 30°C.

H8	H1'	H1''	H2'	H3'	H3''	H4'	H4''	H5'	H5''	N1H	NH_2	HO3'	HO5'
7.618	3.944	3.887	2.029	3.28[a]	3.28[a]	1.295	1.430	3.441	3.382	9.407	6.419	4.415	4.663

J(^1H,^1H)														
1',2'	1'',2'	1',1''	2',3'	2',3''	3',3''	2',4'	2',4''	4',4''	4',5'	4'',5'	4',5''	4'',5''	5',5''	3',OH	5',OH
7.1	7.0	13.8	5.2	5.2	a	5.5	7.5	13.8	6.5	7.0	6.5	7.0	10.2	5.1	5.3

Populations												
C1' - C2'			C2' - C3'[a]			C2' - C4'			C4' - C5'		
+sc	ap	-sc	+sc	ap	-sc	+sc	ap	-sc	+sc	ap	-sc
13	44	43	33	34	33	26	48	26	33	34	33

[a] Values approximate due to coalescence of two proton signals.

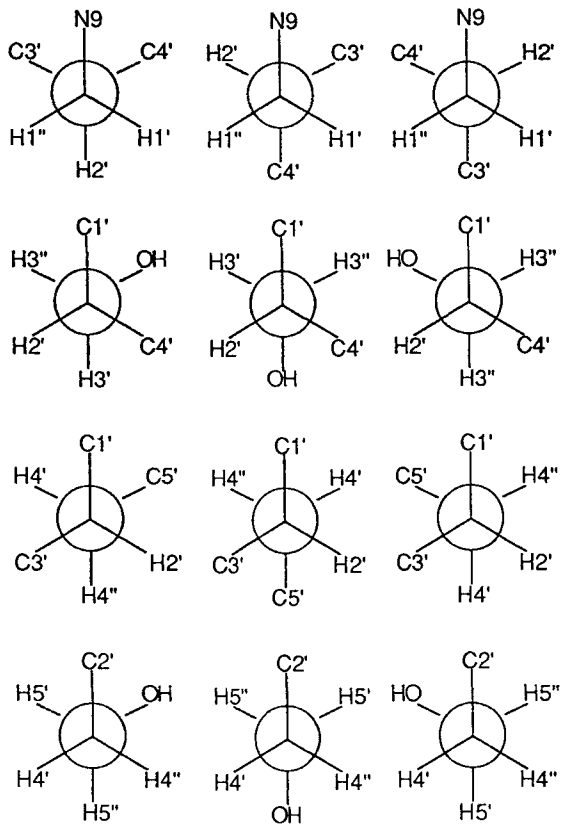

FIG. 4. Newman projections, from top to bottom, along C1'-C2', C2'-C3', C2'-C4' and C4'-C5', showing the three classical conformers, +sc (left), ap (middle) and -sc (right).

Numerous acyclonucleoside analogues, and their phosphates and phosphonates, exhibit substrate/inhibitor properties in various enzyme systems.[2,3] It was, in fact, the initial finding of Schaeffer et al.,[5] that 9-(2-hydroxyethoxymethyl)adenine, the adenine analogue of ACV, is a weak substrate of adenosine deaminase which led to the development of potent reversible inhibitors of this enzyme,[29] and susequently of ACV as a clinically approved antiherpetic drug.[1]

We have verified that the adenine analogue of ACV and (DHP-Ade) are both deaminated by adenosine deaminase at about 2% the rate for adenosine. Whereas the K_m for adenosine is 5 x 10^{-5} M, the

K_m values for the foregoing are 1.3×10^{-4} M for 9-(2-hydroxyethoxymethyl)adenine[29] and 1.3×10^{-3} M for DHP-Ade.[30] The higher K_m for the latter is most likely due to steric hindrance by its primary 3'-hydroxyl in place of the secondary 3'-hydroxyl in the parent adenosine. This effect is accentuated in 2',3'-*seco*adenosine, with an additional primary hydroxyl at C2'; it is not a substrate, but is a weak inhibitor, with $K_i = 1.7 \times 10^{-4}$ M.[31] In general 2',3'-*seco* nucleosides do not adopt conformations characteristic for such compounds as ACV and DHP-Ade, where the acyclic chain may mimic a portion of the intact pentose ring. As elsewhere shown, this is due to steric repulsion between the 2'-CH_2OH and 3'-CH_2OH.[21,26]

An excellent illustration of the constrainment of an acyclonucleoside to the fully extended form on interaction with an enzyme is ACV, the pyrophosphate of which is the most potent known inhibitor of human purine nucleoside phosphorylase (PNP), with $K_i = 9$ nM.[32] It is clearly a bi-substrate analogue inhibitor. The distal phosphate is located at the phosphate binding site of the enzyme. And the binding of the guanine base must be at the site for phosphorolysis, since replacement of the guanine by adenine virtually abolishes inhibitory activity, consistent with the fact that adenosine is not a substrate of the human enzyme. It is furthermore of interest that, whereas the (R) and (S) enantiomers of the pyrophosphate of DHPG are as active inhibitors as the pyrophosphate of ACV, the corresponding 2',3'-*seco*GDP is 3 orders of magnitude less effective. It was long ago demonstrated that, whereas ACV triphosphate readily substitutes for GTP in tubulin polymerization, 2',3'-*seco*GTP is quite ineffective[33].

The conformational features of 2HM-HBG should prove helpful in analyses of its biological properties. The racemate is a competitive inhibitor of PNP from calf thymus and human erythrocytes, with K_i values of 5 μM and 21 μM, respectively, reflecting the known differences between the two enzymes.[34] Preliminary measurements with the calf enzyme have shown that the (R)-enantiomer is 4-fold more effective than its (S)-counterpart (A. Bzowska, unpublished).

The racemate of 2HM-HBG is a substrate for the thymidine kinases (TK) of herpes simplex viruses types 1 and 2, but not the cellular enzyme, and is an *in vitro* inhibitor of replication of these

viruses.[35] With varicella-zoster virus the (R)-enantiomer is a potent inhibitor, whereas the (S) is inactive.[36] It would be of interest to determine whether one or both enantiomers are phosphorylated by the viral TK.

Surprisingly, both enantiomers are equally good inhibitors of human herpesvirus 6 (HHV-6), and more effective than DHPG.[37] Like HCMV (see above), HHV-6 appears not to code for a thymidine kinase.[38] And sequence data indicate that HHV-6 is closely related to HCMV.[37] It is, consequently, conceivable that, like HCMV,[8,9] HHV-6 may code for a protein kinase capable of phosphorylating one or both enantiomers of 2HM-HBG (and perhaps also DHPG). If so, the stereospecificity of this step, relevant to that for DHPG with the HCMV protein kinase,[39] would be of considerable interest.

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