

Dedicated to Professor David Shugar on the occasion of his 80th birthday

Crystal structures of some acyclonucleosides with antiviral activity and related compounds

Karin Bjåmer Birnbaum*

National Research Council of Canada, Ottawa, Canada, K1A 0R6

Key words: acyclonucleoside, acyclonucleotide, antiviral agent, antiviral activity, cyclic monophosphate, cyclic phosphate, X-ray diffraction

The crystal structure of the acyclonucleoside, 9-[4-hydroxy-2-(hydroxymethyl)-butyl]guanine (2HM-HBG), has been compared with related acyclonucleosides, and that of the acyclonucleotide, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine-3',5'-cyclic phosphate (DHPG-cMP), also with its parent second messenger cGMP and cyclic monophosphate nucleotides. There is considerable conformational flexibility in the acyclic chain of these compounds with several conformations coexisting in the solid state. This flexibility together with the narrow range of the glycosidic torsion angles (from 69° to 94°, with an average of 83(3)° for 11 molecules when the aglycon is guanine, and from 92° to 108° with an average of 103(3)° for 4 molecules when the aglycon is adenine) may be essential for the antiviral activity of these compounds and their ability to act as substrates. The mechanism of antiviral activity of, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine-3',5'-cyclic phosphate is different from that of the acyclonucleosides, and is discussed in the light of it being a close structural analogue to the second messenger cGMP.

The potent antiherpes activity of the clinically approved acyclovir stimulated the synthesis of a multitude of acyclonucleosides and nucleotides of purine and pyrimidine analogues. Many of these exhibit antiviral activity, and/or are inhibitors of specific enzymes which play key roles in chemotherapy [1]. The X-ray crystal structure determination of two of these compounds that was recently undertaken in collaboration with

Prof. Shugar and his coworkers at the University of Warsaw, will be reviewed below and compared with the structure of other related compounds: the first compound 2HM-HBG [2], is part of a group of acyclonucleosides that requires intracellular phosphorylation prior to exhibiting antiviral activity, while DHPG-cMP [3] is an acyclonucleotide that displays potent antiviral activity without prior "activation". DHPG-cMP may be active as such, i.e. as a

*NRCC No. 39095.

Abbreviations: ACV, acyclovir, 9-(2-hydroxyethoxymethyl)guanine; ganciclovir, DHPG, 2'-NDG, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine; DHPG-cMP, DHPG 3':5'-cyclic phosphate, 9[[[(2-hydroxy-1,3,2-dioxophosphorinan-5-yl)oxy]methyl]-P-oxide]guanine; DHPA, 9-(2',3'-dihydroxypropyl)adenine; DHP-Ade, 9-[(1,3-dihydroxy-2-propoxy)methyl]adenine; famciclovir, 9-[4-acetoxy-3-(acetoxymethyl)butyl]-2-aminopurine; HBG, 9-(4-hydroxybutyl)guanine; 2HM-HBG, 9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine; penciclovir, 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine; PMEAs, 9-(2'-phosphonomethoxyethyl)adenine; TK, thymidine kinase; HCMV, human cytomegalovirus; cIMP, inosine cyclic 3',5'-monophosphate; cUMPET₃-NH, triethylammonium salt of cyclic uridine-3',5'-phosphate; cTMP phenylthymidine phenyl cyclic 3',5'-monophosphate; cCMP, cytosine cyclic 3',5'-monophosphate.

structural analog of the second messenger cGMP.

STRUCTURE OF 2HM-HBG

Background

Several typical acyclonucleosides of guanine, including 2HM-HBG, the structure of which will be discussed below, are shown in Fig. 1. It should be noted that the acyclic chains may mimic the "upper" and/or "lower" portions of the sugar ring of natural nucleosides. The antiviral activities of these are dependant 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. 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 [4]. Ganciclovir (DHPG) does not code for TK, but is a clinically promising agent *vs* human cytomegalovirus (HCMV) and is phosphorylated by the HCMV UL97 gene product which shares regions of homology with protein kinases [5, 6]. This lends added interest to the structure of 2HM-

HBG, which like DHPG contains two primary hydroxyls in the acyclic chain.

Results

2HM-HBG crystallizes with two independent molecules A and B together with one water molecule in the asymmetric unit, and the three-dimensional structure and conformation is depicted in the stereoscopic view in Fig. 2.

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* [7].

The acyclic chain in molecule A is in the fully extended form with antiperiplanar orientation of the non-proton substituents on the chain about the bonds C1'-C2', C2'-C4', and C4'-C5' as can be seen from the torsion angles in Table 1. The conformation of C1' and O3' about C2'-C3' is *gauche* (+synclinal). The largest deviation from 180° is 23° and occurs in the C1'-C2'-C4'-C5' angle (157.1°). By comparison, the acyclic chain in molecule B is partially folded, a conformation that is stabilized by the formation of an intra-molecular hydrogen bond between O3'H and N3 of the guanine ring. This is achieved by a rotation of the hydroxy-methyl

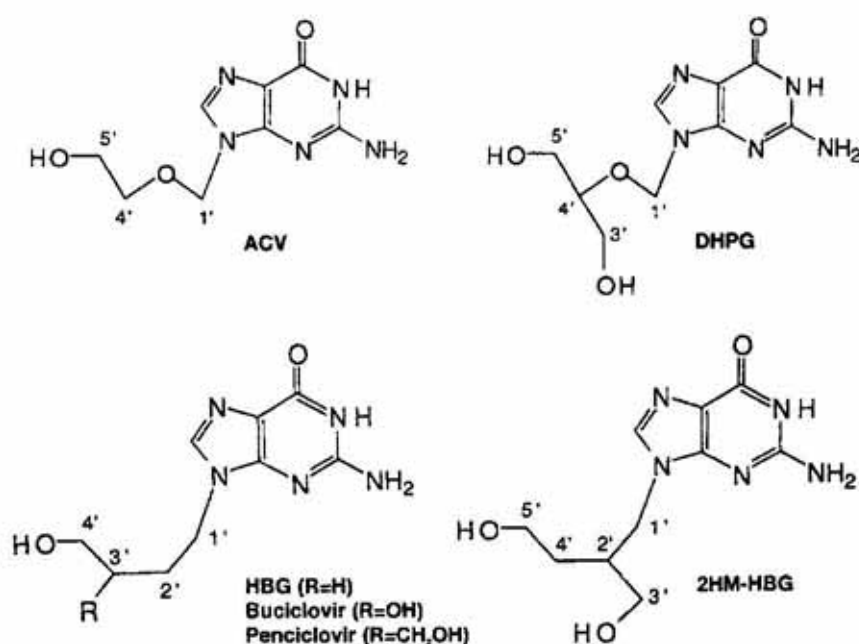


Fig. 1. Structures of acyclonucleosides: ACV; DHPG; HBG (R = H), Buciclovir (R = OH), Penciclovir (R = CH₂OH); 2HM-HBG. Note that the carbons in the acyclic chains are numbered like the corresponding carbons of the pentose ring.

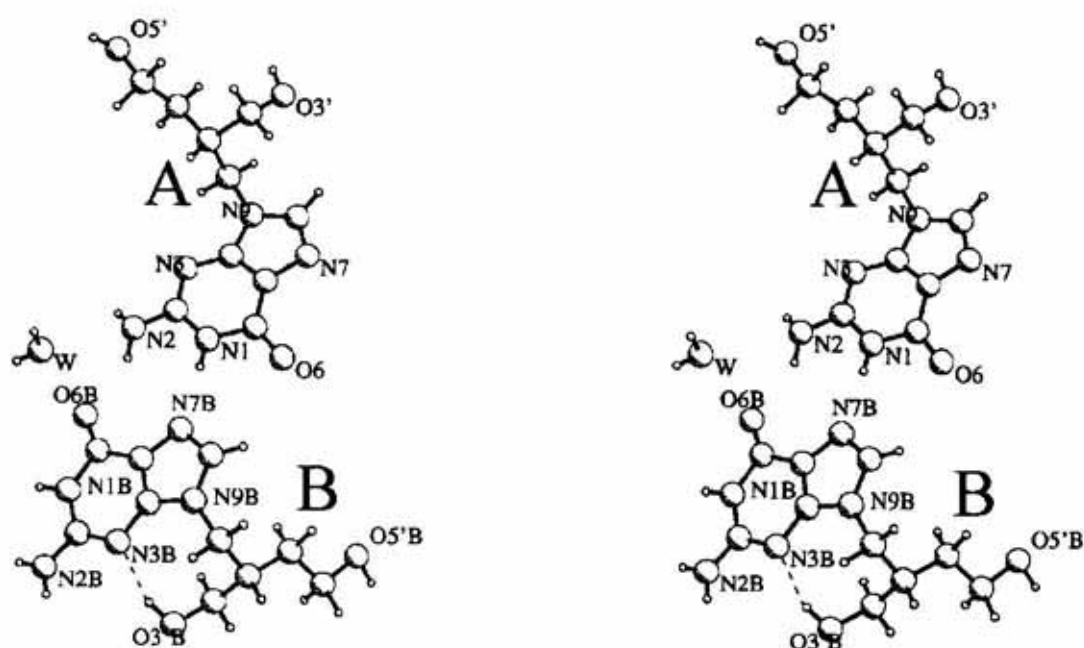


Fig. 2. Stereoscopic view of the solid state structure of 2HM-HBG, showing the two independent molecules A and B, and one water molecule, in the asymmetric unit.

group about the C1'-C2' bond, so that the atoms N9 and C4' are in the +*gauche* (+*sc*) orientation, 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 -*synclinal* conformation, manifested by the C4'-C2'-C3'-O3' angle of -179.5°, with the atoms C4' and O3' *trans* (-*ap*) to each other. This intramolecular hydrogen bond is creating 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) Å and 1.001(5) Å, and O3' and HO3' by -1.219(5) Å and -1.00(4) Å to the other side.

Comparison of 2HM-HBG with related compounds

The crystal structure of 2HM-HBG may be compared with that of other acyclonucleosides with the same base and with similar characteristics of the side chain, *viz.* HBG [8], penciclovir [9], DHPG (see [10]), and acyclovir [11], the latter two with an oxygen instead of the C2' methylene in the side chain. Comparisons of torsion angles are shown in Table 1. The conformation of molecule A is closely related to other structures with fully extended chains, *viz.* HBG, penciclovir, DHPG, and molecule C of acyclovir, while that of molecule B resembles more those with folded chains, *viz.* molecules A and

B of acyclovir. In the light of the recent finding that DHPG is a clinically promising agent against human cytomegalovirus and may adopt a conformation resembling some local peptide region of a protein kinase substrate, it is particularly interesting to see the similarity of the fully extended molecule of 2HM-HBG to that of DHPG, even though the χ values differ by 23.8°.

Glycosidic torsion angle

The orientation of the aglycon, approximately perpendicular to the side chain, compares well with the values of the other compounds in Table 1. It should be noted that the χ value is somewhat less for the folded molecule (86.0°), *i.e.* part of the chain is closer to N3, than for the fully extended one (93.5°). A similar pattern exists for ACV, but with a larger spread between the values for the folded (76.5° and 74.4°) and extended (90.5°) chains.

The values range from 69° to 94°, with an average of 83(3)° for 8 molecules (Table 1) when the aglycon is guanine. With the aglycon being adenine rather than guanine, larger values were observed, ranging from 92° to 108° with an average of 102.6° (*high syn*) for 4 molecules (Table 2). (It should be noted that *high syn* is actually *anti*). In PME A [12] the side-chain is almost coplanar with the aglycon ($\chi = 177^\circ$).

Table 1

Torsion angles ($^{\circ}$)* of the 2HM-HBG side chain compared with those of HBG, penciclovir, DHPG and acyclovir

Side chain	2HM-HBG		HBG***	Penciclovir	DHPG**	ACV** , ***		
	A	B				A	B	C
C4 N9 C1' C2' [χ]	93.5(4)	86.0(4)	88.4	84.6	69.7	76.5	74.4	90.5
C8 N9 C1' C2'	-82.5(4)	-95.4(4)	-86.8	-97.9		-97.3	-104.3	-91.4
N9 C1' C2' C4'	-168.7(5)	65.9(3)	-179.5	<i>trans</i>	-152.1	76.9	66.3	173.3
C1' C2' C4' C5'	157.1(5)	171.3(5)	179.8	<i>trans</i>	157.0	-173.2	176.2	171.9
C2' C4' C5' O5'	175.3(6)	178.4(6)	178.3	<i>trans</i>	<i>trans</i>	-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)						

* When comparing these, it should be kept in mind that a torsion angle of, e.g. 173° , is the equivalent of -187° .

** In DHPG and acyclovir there is an oxygen (O4') instead of C2'.

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

This was assumed to be an example of a structure that most likely is an intermediate in the *syn-anti* conversion, and it was hence excluded from the average given above.

Acyclic chain

The conformation of the side chain of molecule A is similar to those of HBG, DHPG and molecule C of ACV, the largest deviations being about 23° , *viz.* in the χ angle relative to the one

in DHPG and in the C1'-C2'-C4'-C5' angle relative to the corresponding angle in HBG. There is much more variation between the individual torsion angles of the extended chain in molecule A of 2HM-HBG than in both HBG and molecule C of ACV, presumably due to the hydroxy-methyl substituent of the side chain in the former, while the latter two have no substituents. The conformation of the side chain of molecule B is similar to those of molecules A and B in ACV, with the chain folded about the C1'-C2' (or C1'-O4') bond. However, in acyclovir the oxygen atoms O4' and O5' are in the favoured *gauche* (*-sc*) position, while in 2HM-HBG where there is a carbon instead of O4', the conformation about the C4'-C5' is *trans* (*+ap*).

Table 2

Comparison of the glycosidic torsion angles χ ($^{\circ}$) when adenine is the aglycon (all listed for the same hand).

Acyclonucleoside	χ [O4' C1' N9 C4]
DHP-Ade (molecule A)	104.3 $^{\circ}$
DHP-Ade (molecule B)	107.5
S-DHPA	105.0
RS-DHPA	92.4
PMEA	177 $^{\circ}$,**
Average	102.6

*It should be noted that in order to adhere to the more recent convention used here, the torsion angle χ for PMEA is converted from the one given in the original paper [O4'-C1'-N9-C8] by subtracting 180° , and is thus accurate only to $\pm 3^{\circ}$.

**This value has been excluded from the average.

Concluding remarks for 2HM-HBG and other acyclic antiviral nucleosides

There is considerable conformational flexibility in the acyclic chain of these compounds in the solid state. The structure of 2HM-HBG is an example of two conformations coexisting in the asymmetric unit: one with the acyclic chain fully extended and the other with it partially folded and hence closer to that of the natural nucleoside. There are also two conformers in DHP-Ade and famciclovir [9], and even three conformers coexist for ACV.

However, in spite of the conformational flexibility of the acyclic chain, the glycosidic torsion angles for these antiviral agents are all in the *syn* or *high syn* conformation within a relatively narrow range, *viz.* from 69° to 94° , with an average of $83(3)^\circ$ for 8 molecules (Table 1) when the aglycon is guanine, and from 92° to 108° with an average of $103(3)^\circ$ for 4 molecules (Table 2) when the aglycon is adenine.

STRUCTURE OF DHPG-cMP

Background

In contrast to the compounds discussed above (Fig. 1), the cyclic phosphate of ganciclovir (DHPG-cMP, see Fig. 3) displays potent broad-spectrum antiviral activity [13] without prior "activation" by intracellular phosphorylation by viral thymidine kinase, and is active against viruses which do not code for thymidine kinase [14]. Its mechanism of action, which still needs to be clarified, is thus independent of viral or cellular TK and it may be active as such, *i.e.* as a structural analogue of the second messenger cGMP. In fact, there are a number of reports to the effect that replication of viruses, including herpes simplex viruses and HIV, are modulated by intracellular levels of cAMP and cGMP [1, 15].

DHPG-cMP, which is readily taken up by cells, was the first nucleotide analogue described, and the first cyclic phosphate of an acyclonucleoside, with potent antiviral activity. In particular, it proved to be up to 20-fold more effective than its parental drug DHPG against a cytomegalovirus infection in guinea pigs [16].

A number of cyclic phosphates of acyclonucleosides have been examined for their substrate/inhibitor properties, *i.e.* their ability to recognize various specific phosphodiesterases and nucleases, in order to try to elucidate the mechanism of antiviral activity of DHPG-cMP [15]. This study indicates that the recognition by a nucleolytic enzyme of the acyclic chain is determined by the presence of the O4' ether oxygen in DHPG-cMP, since this atom cannot be substituted with a carbon without loss of activity.

Results

The three-dimensional structure and conformation of DHPG-cMP in its zwitterionic form with N7 of the guanine base protonated, can be seen from the stereoscopic view (Fig. 4). Its principal conformational features are as follows: the aglycon is in the *high syn* conformation about the glycosidic bond with the glycosidic torsion angle $[O4'-C1'-N9-C4]$ χ of 93.1° ; the acyclic chain is partially folded; the six-membered cyclic phosphate ring is in the chair form with P and C4' displaced in diametrically opposite directions from the plane through the other four atoms (C3', O3', C5' and O5') by $0.717(3)$ Å and $-0.628(5)$ Å, respectively; and O4' is attached to C4' in the axial position with respect to this ring. This structure is also consistent with NMR results that DHPG-cMP exhibits a single conformer in solution with O4' in the axial position [15].

Comparison of DHPG-cMP with related compounds

The structure of DHPG-cMP is comparable with those of cGMP sodium tetrahydrate [17]

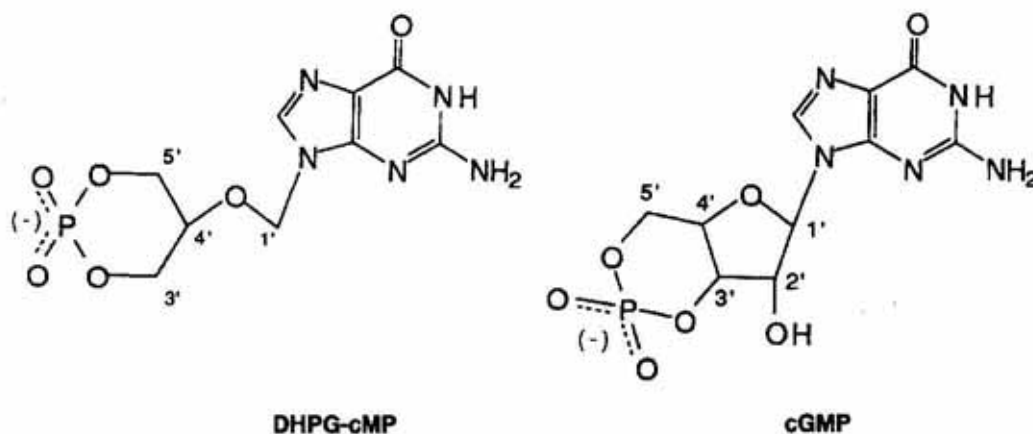


Fig. 3. Structures of DHPG-cMP and cGMP.

and the free acid of cGMP [18], both cyclic phosphates and structural analogues with identical conformations apart from small variations in bond angles and torsion angles. The conformation of the side chain is also compared to that of several acyclonucleosides, *viz.* the respective parent compound DHPG, DHP-Ade and acyclovir; all the appropriate torsion angles can be seen in Tables 3a, and 3b. In DHPG-cMP only the "upper" part of the ribose is present, which means that there is less rigidity in this structure than in that of cGMP and hence there is freedom of rotation not only around the N9-C1' bond, but also around the C1'-O4' and O4'-C4' bonds as in other acyclonucleosides. The preferred orientations around these bonds are described by their corresponding torsion angles.

Glycosidic torsion angle

The orientation of the aglycon relative to the sugar moiety (the glycosidic torsion angle [O4'-C1'-N9-C4] $\chi = 93.1^\circ$) is practically identical to that in the fully extended chains in 2HM-HBG (93.5°) and in acyclovir (90.5°), *i.e.* *high syn* (+ac) [7]. It lies in the range 69° – 94° for the guanine acyclonucleosides listed in Table 1, whose average is $83(3)^\circ$ (*syn*). The glycosidic torsion angles of the parent cGMP sodium tetrahydrate (78°) and cGMP free acid (82°) are very close to this average and hence in the typical *syn* ($\pm sc$) conformation.

Sugar moiety

The geometrical arrangement of the acyclic side-chain, *i.e.* the part comprised of the atoms

C1', O4', C4', and C3', is close to that of a sugar ring in the *C4'-endo* envelope (4E) conformation, but with the C2' atom missing. The two hydrogen atoms, H1'A and H3'A, attached to C1' and C3', are fairly close ($0.10(3)$ Å and $0.30(4)$ Å, respectively) to the plane through the three atoms C1', C3', and O4' while C4' is displaced by 0.780 Å to the opposite side. The *C4'-endo* envelope corresponds to a pseudorotation phase angle P of 237.7° based on v_o of 42.9° and v_{max} of -80.3° . With the sugar moiety being acyclic, there is much more puckering (v_{max} value as large as -80.3°) than in a fused system such as cGMP (Table 3a) where the conformation is twist ${}_4T^3$ [C4' *exo*, C3' *endo*] with P and v_{max} values of 42.6° and 44.2° , respectively. This is the usual conformation of nucleoside 3':5'-cyclicphosphates as discussed below (*cf.* Table 4).

The first part of the side chain is folded, the N9 and C4' atoms being in the *-gauche* (*-sc*) orientation, with a torsion angle of $-79.5(2)^\circ$ for N9-C1'-O4'-C4'. This is fairly close to the values in DHP-Ade (-68.0° and -70.0°), it is also *gauche* in molecules A and B of ACV (76.9° and 66.3°) but with opposite signs, and different from that in DHPG (-152.1°) and in molecule C of ACV (-173.3°) where it is *trans* and hence extended.

Another part of the side chain, *viz.* the atoms C1', O4', C4' and C5', form an almost extended chain, the C1'O4'C4'C5' torsion angle being $157.0(3)^\circ$. The deviation of 23.0° from an ideal extended chain is the same as that observed in DHPG, while the larger deviation of 44° observed in DHP-Ade shows that the latter is

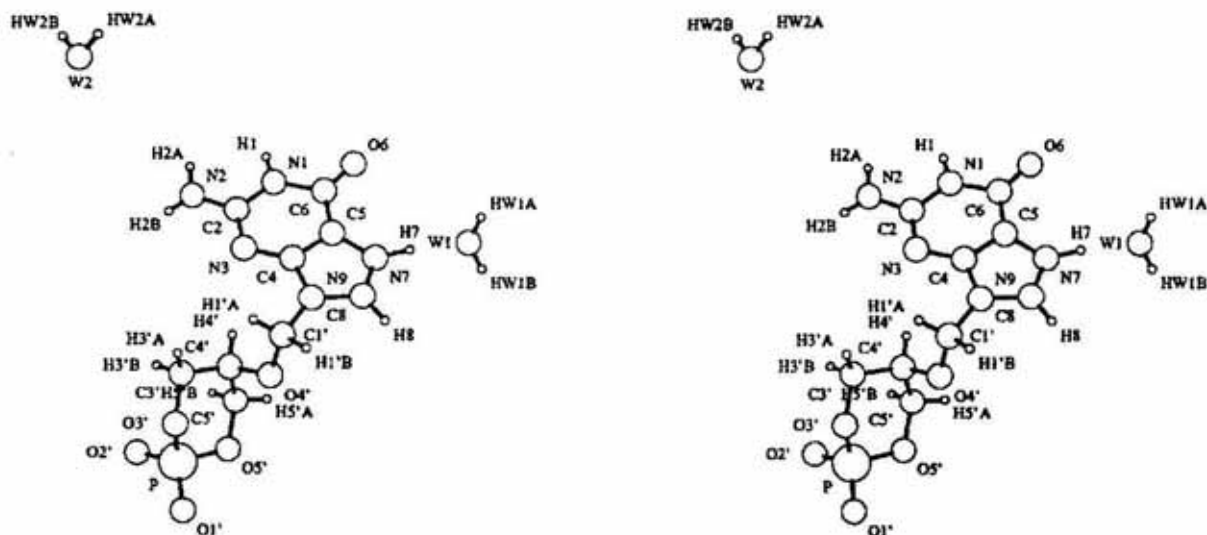


Fig. 4. Stereoscopic view of the solid state structure of DHPG-cMP and two molecules of water.

Table 3a
Comparison of torsion angles ($^{\circ}$) of DHPG-cMP sugar moiety and phosphate ring with those of cyclic GMP

Glycosyl angle and sugar moiety	DHPG-cMP	cGMPNa	cGMP, free acid
χ [O4' C1' N9 C4]*	93.1 (3)	78	82
ν_0 [here C4' O4' C1' H1' A]**	42.9 (17)	-18.4	
ν_1 [O4' C1' C2' C3']		-9.3	
ν_2 [C1' C2' C3' C4']		31.7	
ν_3 [here H3'A C3' C4' O4']**	54.8 (22)	-44.3	
ν_4 [C3' C4' O4' C1']	-80.3 (3)	38.7	
P	237.7	42.6	44
Puckering	\sim^4E	$4T^3$	$4T^3$
Phosphate ring conformation			
C3' O3' P O5'	54.2 (2)	44.2	
C4' C3' O3' P	-59.1 (2)	-59.8	
C5' C4' C3' O3'	55.5 (2)	68.5	
C3' C4' C5' O5'	-53.3 (2)	-60.5	
P O5' C5' C4'	54.8 (2)	53.6	
O3' P O5' C5'	-52.2 (2)	-44.3	

*It should be noted that in order to adhere to the more recent convention used here, the torsion angles χ for cGMP is converted from the ones given in the original papers [O4'-C1'-N9-C8] by subtracting 180° , and are thus accurate only to $\pm 3^{\circ}$.

**In calculating ν_0 and ν_3 for DHPG-cMP the position of the hydrogen atoms H1'A and H3'A were used instead of the missing C2'.

Table 3b
Comparison of torsion angles ($^{\circ}$)* of the DHPG-cMP side chain of acyclonucleosides that mimic the "upper" portion of the sugar ring of natural nucleosides

Side chain	DHPG-cMP	DHPG	DHP-Ade		ACV**		
			A	B**	A	B	C
O4' C1' N9 C4 [χ]	93.1(3)	69.7	104.3	107.5	76.5	74.4	90.5
O4' C1' N9 C8	-89.7(3)				-97.3	-104.3	-91.4
N9 C1' O4' C4'	-79.5(2)	-152.1	-68.0	-70.0	76.9	66.3	173.3
C1' O4' C4' C5'	157.0 (3)	157.0	-135.5	-137.8	-173.2	176.2	171.9
O4' C4' C3' O3'	-64.3 (2)	<i>gauche</i>	61.4	52.6			
O4' C4' C5' O5'	67.8 (2)	<i>trans</i>	61.5	61.9	-60.6	-73.5	174.4
C5' C4' C3' O3'	55.5 (2)		-61.1	-62.3			
C3' C4' C5' O5'	-53.3 (2)		-174.2	178.8			

* When comparing these, it should be kept in mind that a torsion angle of, e.g. -173° , is the equivalent of 187° .

**For clarity, the values reported here for ACV and molecule B of DHP-Ade are the ones corresponding to the same hand as for DHPG-cMP.

more folded. In ACV, on the other hand, this part of the chain is fully extended with deviations being as small as 4° – 8° .

Cyclophosphate ring

The torsion angles of the phosphate ring are compared with those in cGMP (Table 3a). The conformation of the six-membered ring is the same in both compounds, but there is much more stress in cGMP due to the fusion with the ribose ring. This is manifested by an increased puckering in the C3', C4' half and a flattening in the P, O3', O5' half of the phosphate ring. There are hence much greater variations in the torsion angles in cGMP than in DHPG-cMP.

One significant difference between the structure of DHPG-cMP and cGMP is that O4' is attached to the cyclic phosphate ring in the axial position in the former, while in cGMP it is equatorial which is dictated by the ribose ring

being fused to the cyclic phosphate in the latter (Fig. 5).

The vicinal oxygen atoms all exhibit the favoured \pm *gauche* ($\pm 60^{\circ}$) orientations about the C-C bonds, the O4'-C4'-C3'-O3' and O4'-C4'-C5'-O5' being $-64.3(2)^{\circ}$ and $67.8(2)^{\circ}$, respectively. In DHP-Ade they are also both *gauche*, but both angles have the same sign and range in value from 52.6° to 61.9° . In cGMP, on the other hand, they are in the less favoured *trans* position to each other since O4' is in the equatorial position, and O4' and O5' are also *trans* to each other in DHPG.

Comparison of 3',5'-cyclic monophosphate nucleotides

In view of the reports that the biological specificity of cGMP is different from that of cAMP, and appears to have an opposing effect in cell proliferation and in other cellular events [19], and that the replication of viruses are modu-

Table 4
Comparison of glycosidic torsion angle χ ($^{\circ}$), sugar, and phosphate ring conformations of DHPG-cMP with those of 3,5'-cyclic monophosphate nucleotides

Compound	χ ($^{\circ}$)*	Sugar		Phosphate ring	O4'	Reference
		Conformation	P($^{\circ}$)			
DHPG-cMP	93.1 (<i>high syn</i>)	4E	237.7	chair	axial	[2]
cGMPNa	78 (<i>syn</i>)	${}^4T^3$	42.6	chair	equatorial	[17]
cGMP, free acid	82 (<i>syn</i>)	${}^4T^3$	44	chair	equatorial	[18]
cAMP (A)	-118 (<i>anti</i>)	${}^4T^3$	38	chair	equatorial	[30], see [25]
(B)	90 (<i>syn</i>)	${}^4T^3$	50	chair	equatorial	
5'-methylene-cAMP	54 (<i>syn</i>)	3T_4	37	chair	equatorial	[31]
8-[(2-aminoethyl)amino]-cAMP	73 (<i>syn</i>)	${}^4T^3$	51	chair	equatorial	[32]
8-Bromo-cAMP	109 (<i>anti</i>)**	3E	19	chair	equatorial	see [25]
cAMP, trigonal	-148 (<i>anti</i>)	3T_4	27	chair	equatorial	[25]
cAMPNa, monoclinic (A)	-149 (<i>anti</i>)	3T_4	33	chair	equatorial	[25]
(B)	-169 (<i>anti</i>)	3T_2	15	chair	equatorial	
cUMPEt ₃ NH (A)	-103 (<i>anti</i>)	${}^4T^3$	42	chair	equatorial	[23]
(B)	-122 (<i>anti</i>)	${}^4T^3$	48	chair	equatorial	
2'-acetyl-cUMP	71 (<i>syn</i>)	${}^4T^3$	49	chair	equatorial	[33]
cIMP	-162 (<i>anti</i>)	3T_4	28	chair	equatorial	[22]
cCMP	-168 (<i>anti</i>)	3T_2		chair	equatorial	see [25]
cTMPphenyl	-98 (<i>anti</i>)	3_4T		half chair		[24]

* The torsion angle χ [O4'-C1'-N9-C4] may have been converted from the original paper if it was given as [O4'-C1'-N9-C8].

**Listed in [25] as *syn*, but if the value is correct, it should be *high syn* which is actually *anti*.

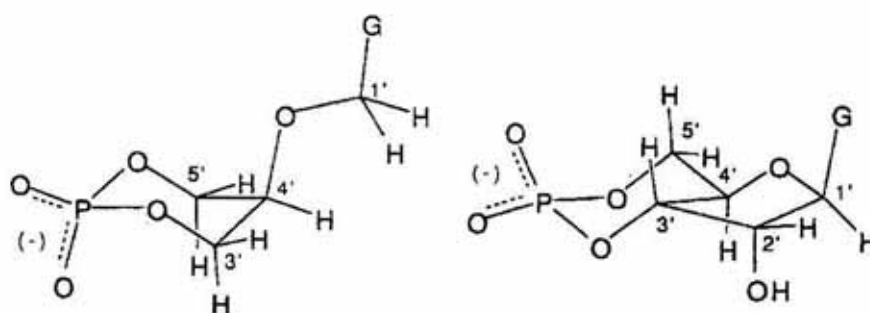


Fig. 5. Comparison of conformations of the cyclic phosphate rings of DHPG-cMP (left) and cGMP (right).

lated by intracellular levels of cAMP and cGMP [1, 15], a comparison of their structures is of interest. Another cyclic nucleotide of interest is cIMP, which was shown to be only slightly less potent than cAMP in stimulating cAMP-sensitive protein kinase in various tissues [20].

Some key structural features of several 3',5'-cyclic mononucleotides are summarized in Table 4. It shows that the fused cyclic phosphate and sugar rings have essentially the same conformations, while the relative orientations of the base and the sugar rings, as indicated by the glycosidic torsion angle χ , vary a great deal. However, only *syn* conformations have been observed when the base is guanine, and that of the acyclic analogue DHPG-cMP is close to that with a χ value in the *high syn* region. This is supported by the energy calculations on 3',5'-cyclic nucleotides [21] which show that cGMP prefers *syn* conformation while cAMP has a conformational preference for *anti* over *syn* in the ratio 7:3. The pyrimidine bases U, T, and C all have strong preferences for *anti* conformation, and cIMP ($\chi = -162^\circ$) [22], cUMP ($\chi = -103^\circ$ and -122°) [23], cTMP ($\chi = -98^\circ$) [24] and cCMP ($\chi = -168^\circ$) [25] all occur in the *anti* conformation.

Apart from cTMP, the cyclophosphate rings are all in a chair conformation that is flattened in the phosphate end and more puckered around the C3'-C4' bond due to the fusion with the pentose ring. This distortion of the ring is not observed in the nucleotide analogue DHPG-cMP where there is no such fusion. The latter compound is also the only one with the O4' oxygen in the axial position relative to the cyclophosphate ring. The half chair that is observed in thymidine phenyl cyclic 3',5'-monophosphate [24] is the intermediate conformation between a chair and the higher energy twist conformation.

Most of the pentose rings occur in the ${}^4T^3$ conformation [C4' *exo*, C3' *endo* with more puckering at C4'], but the similar conformations 3T [C4' *exo*, C3' *endo* with equal puckering at C3' and C4'], 3T_4 [C4' *exo*, C3' *endo* with less puckering at C4'] and 3E [C3' *endo* with no puckering at C4'] also occur. The pseudorotation phase angles (P) lie in a wider range (15° – 51°) than earlier thought, and include also the 3T_2 conformation at the low end of this scale. The value for DHPG-cMP is outside this range with the sugar moiety being approximately a C4' *endo* envelope (4E).

Concluding remarks for DHPG-cMP

The differences in the solid-state conformations of the sugar moieties between DHPG-cMP and cGMP, and how they are linked to the cyclic phosphate rings result in differing relative orientations of the planes of the cyclic phosphate rings and aglycon (Fig. 6). In view of this it is remarkable that the distance between the aglycon and the cyclic phosphate ring in DHPG-cMP of 5.893(2) Å (the N9-P distance) is only 0.05 Å greater than in cGMP (5.846(2) Å). This, together with the flexibility of the acyclic chain in DHPG-cMP, indicates possible reasonable overlap in the two structures despite the differences in conformations of the two compounds. The mechanism of antiviral activity of DHPG-cMP, which is different from that of the acyclonucleosides, may thus be linked to the fact that it is a close structural analogue to the second messenger cGMP.

COMMON FEATURES

Base

The guanine residues are usually fairly flat, which is the case for both 2HM-HBG and

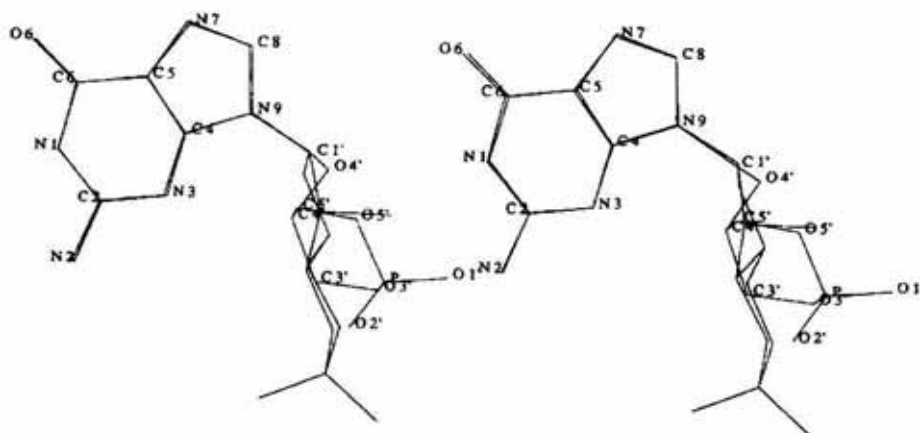


Fig. 6. Stereoscopic view of the superposition of the solid-state structures of DHPG-cMP and cGMP. Atoms in the DHPG-cMP molecule are numbered.

DHPG-cMP. Most of the exocyclic atoms, *viz.* N2, O6, and C1' are displaced from this plane by a small amount, but in both compounds the maximum deviation is no more than about -0.08 \AA (for C1' and O6, respectively) which is much less than in cGMP where it is as large as 0.184 \AA (for C1').

Hydrogen bonding and packing

Many of these compounds crystallize with water molecules, which play an important part in the hydrogen bonding scheme. This is the case for both 2HM-HBG and DHPG-cMP, as well as for ACV and cGMP sodium tetrahydrate. The molecules usually pack in such a way that the hydrophilic water regions are being alternated with hydrophobic ones, consisting of the guanine heterocycles, often forming π - π stacks, such as in DHPG-cMP where the distances between atoms in the overlapping bases range from 3.157 \AA (C6 \cdots C6) to 3.367 \AA (N3 \cdots O6). This type of packing is a common feature in nucleoside and nucleotide crystal structures [26]. In 2HM-HBG where the guanine heterocycles do not form π - π stacks, the hydrophobic heterocyclic regions still alternate with the hydrophilic regions.

CONCLUSION

It is clearly of interest to determine the role of the structure and conformation of a given analogue relative to those of the natural substrate. The comparison here of DHPG-cMP with its parent second messenger cGMP, previous comparisons of the crystal structures of acyclonu-

cleosides such as ACV [11], DHPG and the analogous DHP-Ade [10] with their corresponding nucleosides, together with the solid state structures of HBG [8], 2HM-HBG, penciclovir and famciclovir [9] the 2',3'-dihydroxypropyl derivatives of adenine [27] as well as 1-(2',3'-*O*-isopropylidene-2',3'-dihydroxypropyl)uracil [28] and 9-(2'-phosphonomethoxyethyl)adenine (PMEA) [12] lead to some general conclusions.

A characteristic feature of the above mentioned compounds is that not only is there "conformational flexibility" in solution of the acyclic chain, but also the co-existence of two (2HM-HBG, DHP-Ade, famciclovir), and even three (ACV) different conformers in the solid state. Sometimes two of the conformers have essentially the same conformation, but with some variation in dihedral angles, such as the molecules A and B of ACV and famciclovir. In addition to a form with the acyclic chain fully extended, there is often also a partially folded form, generally folded about the C1'-C2' (or C1'-O4') bond, which mimics the pentose ring of the natural nucleoside. In the case of 2HM-HBG the partially folded chain is stabilized by an intra-molecular hydrogen bond.

In spite of the conformational flexibility of the acyclic chain, the glycosidic torsion angles for these antiviral agents, both the acyclonucleoside and the acyclonucleotide DHPG-cMP, are all in the *syn* or *high syn* conformation within a relatively narrow range, *viz.* from 69° to 94° , with an average of $83(3)^\circ$ for 11 molecules (Tables 1 and 3a) when the aglycon is guanine, and from 92° to 108° with an average of $103(3)^\circ$ for

4 molecules (Table 2) when the aglycon is adenine.

Hence, these experimental results support the suggestion based on theoretical calculations for ribavirin [29] that a glycosidic torsion angle within these ranges is essential to the antiviral activity. The flexibility of the acyclic chain, and its ability to adopt a number of conformations, including folded ones that mimic the pentose ring of the natural nucleoside or nucleotide, may also be essential for the ability of these compounds to act as substrates for nucleoside kinases, phosphodiesterases, nucleases, or protein kinases.

The work reported here was carried out in collaboration with Professor David Shugar and Dr Ryszard Stolarski — my sincere thanks go to both of them.

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