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5-SUBSTITUTED PYRIMIDINE 1,5-ANHYDROHEXITOLS : CONFORMATIONAL ANALYSIS AND INTERACTION WITH VIRAL THYMIDINE KINASE.

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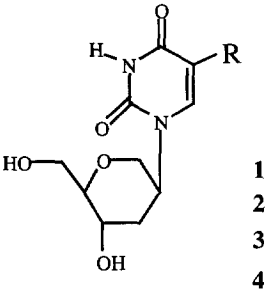
Abstract: Conformational analysis of anhydrohexitol nucleosides using a combination of experimental (X-ray crystallography) and computational methods indicates that those antiviral compounds occur in an equilibrium between two forms, one conformation being adopted in solid phase and in solution, the other found when the nucleosides are in complex with HSV-1 thymidine kinase. The conformational change induced by the enzyme has been investigated.

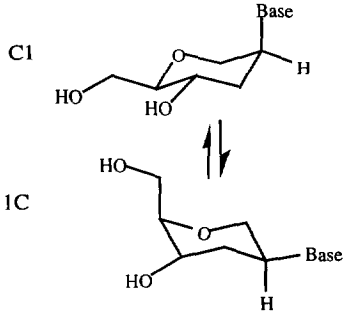
5-Substituted pyrimidines, bearing a 1,5-anhydro-2,3-dideoxy-D-arabino-hexitol moiety at N-1, demonstrate potent anti-HSV-1 activity. Among the compounds with highest selectivity index emerged the 5-iodouracil (**1**)[1], 5-ethyluracil (**2**)[2] and 5-trifluoromethyl analogue (**3**)[3]. These compounds are not active in the absence of viral thymidine kinase (TK) which points to the importance of intracellular phosphorylation as a biological activation process. Therefore, the binding mode of these nucleoside analogues to the viral thymidine kinase was investigated[3,4].



1 : R = I
2 : R = CH_2CH_3
3 : R = CF_3
4 : R = Cl
5 : R = CH_3

Compound **1** has been cocrystallized with the viral thymidine kinase and the structure of the complex was elucidated by X-ray crystallography[3,4]. The binding mode of the anhydrohexitol ring is very similar to that of the ribose moiety of deoxythymidine and of other modified pyrimidine nucleosides[4]. However, a striking difference has been observed between the conformation of the anhydrohexitol ring in the single nucleoside (as determined by X-ray diffraction and NMR) and in the co-crystallized complex (with the viral kinase).

The six-membered ring adopts a C1 conformation in the crystal structure of the small molecule alone[2], with the hydroxymethyl group oriented equatorially and the base moiety oriented axially. In the crystallized complex the hexitol ring adopts a 1C conformation with an axially oriented hydroxymethyl group and an equatorially oriented base moiety. This suggests that the viral thymidine kinase is able to induce conformational changes in the sugar moiety of anhydrohexitol nucleosides.



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Therefore we start to investigate the reason for this conformational change. Understanding this process may be important for the future design of novel anti-HSV-1 nucleosides. These investigations are initiated by a study of the conformation of three new anhydrohexitol nucleosides with high (**3**) and low (**4**, **5**) antiviral activity either alone (X-ray crystallography, geometry and optimized structure) or in complex with the viral protein (by docking of the inhibitor in the active site of the enzyme).

Results and Discussion

The structures of compounds **3** and **4** have been determined by crystallography[5]. Compound **3** cocrystallizes with two molecules of water. Compound **4** crystallizes with four molecules in the asymmetric unit and cocrystallizes with three molecules of water.

Both compounds adopt a C1 conformation (Fig. 1, Table 1). The hydroxymethyl group adopts different conformations, allowing optimal hydrogen bondings in the crystal packing. All other geometry parameters (bond lenghts and valence angles) are within experimental errors, and are consistent with values reported for similar structures[1,2,6,7]. The present structure investigation confirms the previous findings with related compounds[1-3, 6], and still no exception to this analysis is known.

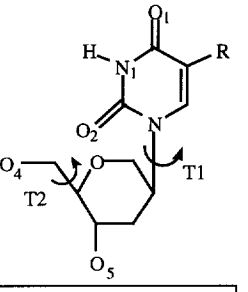


Table 1 Main structural features of compounds **3** and **4** (four molecules **I** to **IV**).

	T1 (°)	T2 (°)[a]	Hydrogen bondings : D..A (Å)
3	78.0(3)	62.3(3)	N1··O5$_i$: 2.84; O5··O3 : 2.82; O4··Ow1$_{ii}$: 2.73; O5··Ow2 : 2.85
4 I	81.3(5)	-65.6(6)	N1··O1^{III} : 2.79; O4··Ow1$_{iii}$: 2.87; O5··O3 : 2.87; O5··Ow2 : 2.77
II	77.4(5)	-54.1(7)	N1··O2$^{IV}_{iv}$: 2.86; O4··O2$_{iii}$: 2.90; O5··O3^{II} : 2.74; O5··Ow3 : 3.17
III	85.7(5)	49.1(11)	N1··O1^{I} : 2.86; O4··Ow2$_v$: 2.73; O5··O3I^{II} : 2.80; O5··Ow1$_{iv}$: 2.86
IV	71.6(5)	47.3(8)	N1··O2$^{II}_{vi}$: 2.77; O4··O2$^{III}_{vii}$: 2.89; O5··O4$^{I}_{viii}$: 2.77

[a] Only the main component of the disordered hydroxymethyl group was retained.
Symmetries : *i* = 1/2-x,-y,-1/2+z; *ii* = -x,-1/2+y,1/2-z; *iii* = x,y,1+z; *iv* = 1+x,y,1+z; *v* = 2-x,1/2+y,1-z; *vi* = -1+x,y,-1+z; *vii* = -1+x,y,z; *viii* = -1+x,y,1+z.

Conformational analysis (computational calculations) of the isolated molecules[8] (**3**, **4** and **5**) confirm the possibility of the existence of a C1 - 1C equilibrium with a ΔE of 0.29 kcal/mol, 2.70 kcal/mol, and 2.40 kcal/mol, respectively. It is interesting to note that the 5-substituent of the pyrimidine ring may influence the energy difference between both conformations. The chlorine group behaves as a perfect isosteric and isoelectronic substituent with respect to the CH_3 group. The geometries of the C1 conformation of **3** and **4** correspond to the crystallography structures. The energy barriers between both conformations are small enough to be overcome in a biological medium, as has been previously suggested[3]. Interestingly the most potent compound in this series (**3**) corresponds to the structure with the lowest energy barrier. The potential existence of a relationship between ΔE and biological activity will be further investigated.

In order to elucidate the influence of the protein on the conformation of anhydrohexitol nucleosides, and in particular on the C1 - 1C conformational shift of the inhibitor upon complexation with the protein, we have docked and energy minimized compound **5** in the active site of HSV-1 TK[9]. Our calculations indicate that the complex in which **5** adopts a 1C conformation is 10.25 kcal/mol more stable than the complex in which the ligand retains the conformation observed in the single molecule (C1 conformation) (Fig. 2). In the most stable geometry of the complex of **5** with HSV-1 TK, the hydroxymethyl group is placed axially and the base moiety is oriented equatorially.

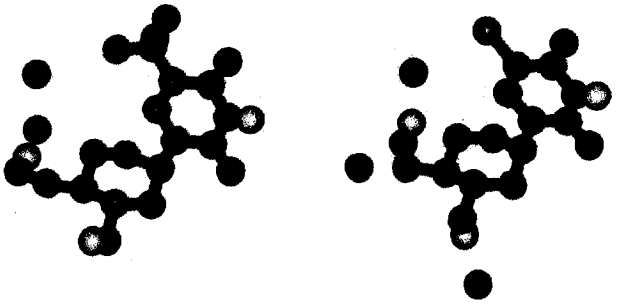
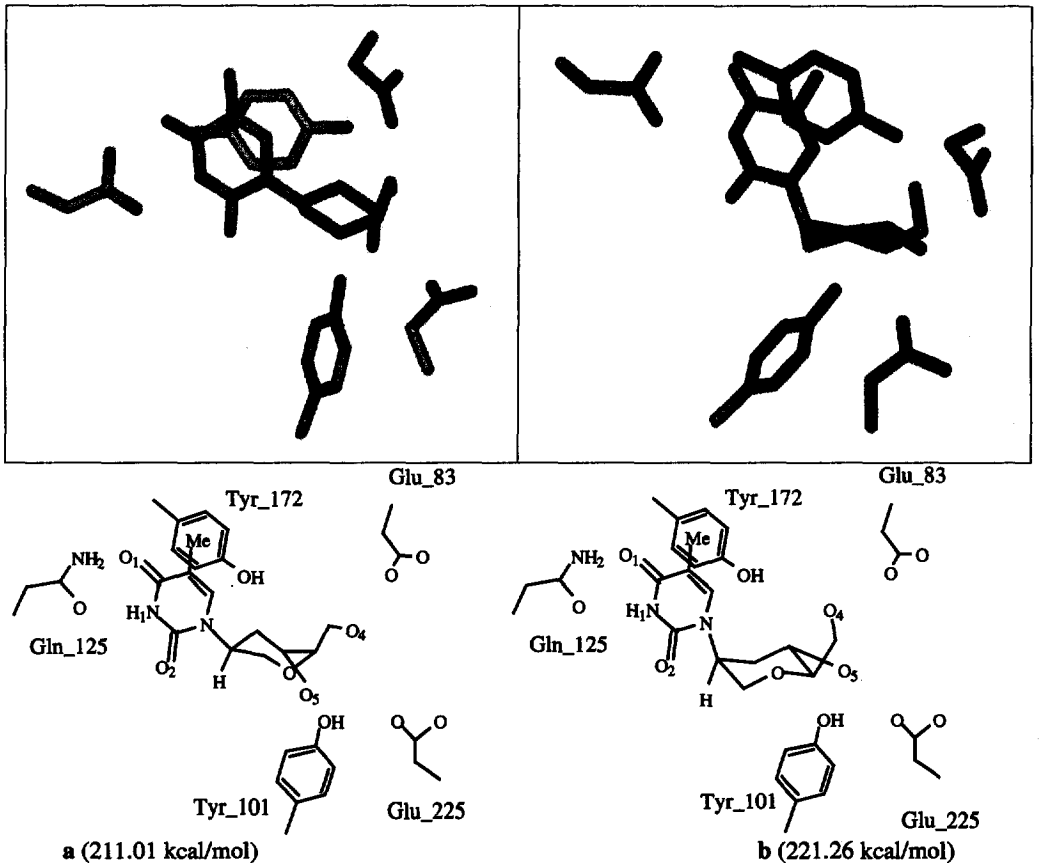


Fig 1. Conformations of compounds **3** and **4** deduced from X-ray crystallography. Note the different orientation of the hydroxymethyl group. Only one molecule of the asymmetric unit is shown for molecule **4**. Cocrystallization water molecules are involved in H bondings



Gln_125 $NE_2 \cdots O_1$ (2.77)	Gln_125 $NE_2 \cdots O_1$ (2.84)
$OE_1 \cdots N_1$ (2.94)	$OE_1 \cdots N_1$ (2.89)
Glu_225 $OE_1 \cdots O_5$ (2.49)	Glu_225 $OE_1 \cdots O_5$ (2.55)
Tyr_101 $OH \cdots O_5$ (2.55)	Tyr_101 no interaction (> 4)
Glu_83 $OE_2 \cdots O_4$ (2.60)	Glu_83 $OE_2 \cdots O_4$ (2.70)
$Ow_1 \cdots O_2$ (2.77)	$Ow_1 \cdots O_2$ (2.64)
$Ow_2 \cdots O_2$ (2.94)	$Ow_2 \cdots O_2$ (2.69)

Fig 2. Minimized structures of **5** in complex with HSV-1 TK for the ligand adopting either a 1C (a) or C1 (b) conformation. Selected intermolecular interactions are given (distances (Å) in parenthesis). Water molecules (Ow) are omitted from the figures.

This is in contrast to the orientation of both substituents in the crystal structure of the anhydrohexitol nucleosides and in the structure derived from the conformational analysis of the isolated compounds. These predictions are in agreement with experimental crystallographic structures of complexes between anhydrohexitol nucleosides and HSV-1 TK and allow a quantitative analysis of the conformational changes induced by the protein.

Apart from minor accommodation for the binding of the two conformations, the overall structure of the active site is conserved in both complexes. The binding mode (Fig. 2) of the two conformers of **5** are particular different with respect to the influence of O5 on the stability of the complex. For both conformers, the pyrimidine ring is stabilized by hydrogen bonds (O1 and N1 interact with Gln_125, O2 binds two water molecules) and van der Waals interactions (Tyr_172 stabilizes the ring *via* stacking (π–π) interactions). In both complexes, the hydroxymethyl group (O4) of **5** forms a hydrogen bond with Glu_225. The secondary hydroxyl group (O5) is hydrogen binding to Glu_225 in conformation C1. In contrast, in the 1C conformation, this hydroxyl group O5 is stabilized by both Gln_225 and Tyr_101. These intermolecular interactions are in agreement with experimental structure of complexes[3,4]. The extra stabilization of the anhydrohexitol moiety in the 1C conformation explains the increased stability of the complex. In particular, the additional interaction with Tyr_101 is one of the driving forces explaining the induced fit imposed by HSV-1 TK on the conformation of **5**. This information will be used further for the design and synthesis of new anti-HSV-1 compounds.

References and Notes

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3. Ostrowski, T. et al. *J. Med. Chem.* **1998**, *41*, 4343-4353.
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5. Compound **3** : $C_{11}H_{13}N_2O_5F_3$. 2 H_2O, orthorhombic, $P2_12_12_1$, a = 6.620(2) Å, b = 10.442(2) Å, c = 20.659(4) Å, V = 1428.1(7) Å^3, Z = 4, μ = 1.39 mm^{-1}, D_x = 1.611 g cm^{-3}, λ *(Cu Kα)* = 1.54178 Å, *F(000)* = 720, T = 290 K, 2080 unique reflections (R_{int} = 0.022), $R1$ = 0.0376 for 1993 $F_O > 4\sigma(F_O)$ and $wR2$ = 0.1327, *GOF* = S= 1.098. Absorption effects were corrected using psi-scan methods T_{max} = 0.998 and T_{min} = 0.802. Full matrix least-squares on F^2 using the program Shelxl97 (Sheldrick, G. Univ Goettingen, Germany).
   Compound **4** : $(C_{10}H_{13}N_2O_5Cl)_4$. 3.5 H_2O, monoclinic, $P2_1$, a = 6.999(2) Å, b = 29.945(4) Å, c = 12.381(3) Å, V = 2540.8(7) Å^3, Z = 4, μ = 2.92 mm^{-1}, D_x = 1.517 g cm^{-3}, λ *(Cu Kα)* = 1.54178 Å, *F(000)* = 1204, T = 290 K, 6898 unique reflections (R_{int} = 0.017), $R1$ = 0.0526 for 6043 $F_O > 4\sigma(F_O)$ and $wR2$ = 0.1586, *GOF* = S= 1.082. Absorption effects were corrected using psi-scan methods T_{max} = 0.998 and T_{min} = 0.701. Full matrix least-squares on F^2.
   Lists of atomic coordinates, displacement parameters, structure factors and complete geometry have been deposited with the IUCr.
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8. Geometries corresponding to the C1 or 1C conformations of **3**, **4**, and **5**, were constructed with the *Builder* program (*InsightII* package, MSI, San Diego) and energy minimized with the *Discover* program using the cvff ForceField.
9. Energy calculations on enzyme-inhibitor (**5**) complexes were performed with the *Discover* program (Amber ForceField) using the geometry of the active site observed in the crystallographic structure **2vtk** (PDB code). The ADP and a structural water were retained in the calculations and their positions fixed during the minimization protocol. All residues further than 8Å from the ligand were fixed. The solvent effect was approached by using a distance dependent dielectric constant (1*r) and by retaining individual water molecules from the crystal structure, in the active site (free to move). After the inhibitor (**5**) had been docked into the active site (either in a C1 or 1C conformation), the complexes were energy minimized until the energy gradient dropped below 0.01 kcal/mol.