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# Structure of the antiviral stavudine using quantum chemical methods: Complete conformational space analysis, 3D potential energy surfaces and solid state simulations

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## HIGHLIGHTS

- The 25 optimum stable conformers were determined by potential energy surfaces (PES).
- The most stable conformer C1, has O4′-*endo* form, anti($\chi$)/g$^+$($\gamma$)/g$^+$($\beta$) is apparently amenable to phosphorilation on C5′—O5′bond.
- Rotations around C5′—O5′bond, $\beta$, is less restricted in D4T than in the natural nucleoside 3′-deoxythymidine dT.
- X-ray crystal unit cell state was accurate simulated by DFT methods taking into account the most stable conformers.

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## ABSTRACT

The molecular structure and energy of the anti-HIV, 2′,3′-didehydro-3′-deoxythymidine (D4T, stavudine or Zerit) nucleoside analogue was determined by using MP2, B3LYP and B971 quantum chemical methods. The global minimum was determined through 3D potential energy surfaces (PES). These surfaces were built by rotation of the exocyclic $\chi$, $\gamma$ and $\beta$ torsional angles, in steps of 20°, and full optimization of the remaining parameters. As consequence 5832 geometries were final optimized. The search located 25 local minimum, 4 of which are by MP2 within a 2 kcal/mol electronic energy range of the global minimum. The whole conformational parameters as well as *P*, $\nu_{max}$ were analyzed in all the stable conformers. The global minimum by MP2 corresponds to the calculated values of the exocyclic torsional angles: $\chi = -103.6°$, $\beta = 63.8°$ and $\gamma = 60.6°$. The results obtained are in accordance to those found in thymidine and in related anti-HIV nucleoside analogues. The effect of hydration on the two most stable conformers is analyzed by continuous and discrete models up to 20 water molecules. The solid state was also simulated. The dimer forms found in the crystal unit cell were accurately determined and they are in accordance to the X-ray data.



## 1. Introduction

HIV-1 RT reverse transcriptase has been a prominent target of anti-AIDS therapies due to its essential role in the viral life-cycle. Stavudine or D4T (2′,3′-didehydro-3′-deoxythymidine) is one of the most effective nucleoside reverse transcriptase inhibitors (NRTIs) that generally it is used in anti-HIV-1 combination therapy. This molecule is less cytotoxicity and less inhibitory to mitochondrial DNA replication than zidovudine or AZT [1]. Application of D4T as an agent alone is limited due to the development of drug resistances [2]. This drug acts as chain terminator following three phosphorylation steps at the substrate binding site of the reverse transcriptase (RT) [3].

2′,3′-didehydro-2′,3′-dideoxy nucleosides (ddNs) are the most important class of compounds active against HIV. Their structural features as compared to the natural nucleosides are the lack of hydroxyl group at the 3′ position and the planarity of ribose ring due to double bond. A higher degree of rigidity to the sugar moiety reduces the conformational possibilities. Differences in the ribose ring puckering lead to appreciable changes in positions of the thymine ring and the C5′—OH group [4]. Consequently, it appears differences in structural properties and intra- and inter-molecular hydrogen bonding possibilities. Other factors to be in account on molecular interactions of these molecules are the presence of tautomeric forms and the interaction with water environment into the cell. The importance of weak C—H···O hydrogen bonds in macromolecules is a well

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established phenomenon. Their significance has been described as supporting interactions of stronger N—H···O and O—H···O bonds in protein-ligand complexation [5].

In order to understand of the antiretroviral mechanism of this molecule, it appears necessary to study the complete molecular conformational flexibility considering all range for possible torsional angles values. Different authors have analyzed the conformers of several nucleosides and nucleotides analogues [6–10]. The interaction with enzyme inverse transcriptase (RT) has a complex mechanism and nucleosides analogues are expected to adopt different conformations in different phosphorilation and excision steps for binding to the active site of the enzyme.

In previous works, we have studied the effect of hydration on the two most stable conformers of D4T [11] and their tautomeric forms [12]. In this work we have analysed 3D and 2D conformational maps corresponding to potential energy surfaces (PES) by rotation the exocyclic $\chi$, $\gamma$, and $\beta$ torsional angles. The global minimum and 25 optimum stable conformers were determined. Another goal of present research is a simulation of the solid state structure. Several crystallographic studies have been reported on the polymorphism of D4T [13] yielding to triclinic [14], monoclinic [15], and orthorhombic [16,17] crystals. We have simulated well the dimer form found in these crystal unit cells.

## 2. Calculations

All the conformations were determined at several quantum chemical levels, including the MP2 method and the Density Functional methods (DFT), such as B3LYP and B971 hybrid functionals. These methods appear implemented in the GAUSSIAN 03 program package [18]. DFT methods provide adequate compromise between the desired chemical accuracy and the heavy demands put on computer time and power. Moreover, DFT methods have been used satisfactory in many studies of anti-HIV drugs [19]. Several basis sets were used starting from the 6-31G* to 6-311++G(3df,pd), but the 6-31G** represents a compromise between accuracy and computational cost, and thus it was the main base set selected for the calculations. With the MP2 method was used only the 6-31G** basis set. The B3LYP method was chosen because different studies have shown that the data obtained with this level of theory are in good agreement with those obtained by other more cost computational methods as MP2 calculations and it predicts vibrational wavenumbers of DNA bases better than the HF and MP2 methods [20–22].

Full relaxation of all geometrical parameters were carried out. Berny optimization under the TIGHT convergence criterion was used. Atomic charges were determined with the Natural NBO [23,24] procedure.

The harmonic wavenumber computations were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to the nuclear displacement. Wavenumber calculations were performed in all the optimized conformers determined by DFT with the 6-31G** and 6-311++G(2d,p) basis set to asses that they correspond to real minimum. All the optimized structures only showed positive harmonic vibrations (true energy minimum). Relative energies were determined by including zero-point vibrational energies (ZPE). For the calculation of ZPE, the wavenumbers were retainted unscaled.

All quantum mechanical computations were performed on the alpha computer of the Computational Center from University Complutense of Madrid, as well as on a HP Integrity rx2600 server, with 2 Intel® Itanium® 2 processors at Computational Chemistry Laboratory from Universidad Nacional de Educación a Distancia (Lab-QC, UNED) [25].

**Table 1**
The exocyclic torsional angles and pseudorotational angle $P$ are in degrees, and the energy increments are in kcal mol$^{-1}$. The values correspond to the three most stable conformers in dT and D4T, calculated at the MP2/6-31G** level.

| | Conf. | $\chi$ | $\beta$ | $\gamma$ | $P$[a] | S[b] | $\nu_{max}$[c] | $\Delta E$ |
|---|---|---|---|---|---|---|---|---|
| dT | C1 | −128.9 | 176.1 | 50.1 | 163.3 | $^{2}$E | 37.0 | 0 |
| | C2 | −125.1 | 174.9 | 49.3 | 165.8 | $^{2}$E | 36.9 | 0.642 |
| | C3 | −134.1 | 67.7 | 64.5 | 24.7 | $_{3}^{4}$T | 35.4 | 0.971 |
| D4T | C1 | −103.6 | 63.8 | 60.6 | 77.2 | $^{0}$T$_{4}$ | 8.8 | 0 |
| | C2 | −134.4 | −63.8 | 49.3 | 95.0 | $^{0}$T$_{1}$ | 7.7 | 1.537 |
| | C3 | −122.3 | 165.0 | 43.7 | 83.3 | $^{0}$T$_{4}$ | 8.1 | 1.606 |

[a] $tgP = \frac{(\nu_4+\nu_1)-(\nu_3+\nu_0)}{2\nu_2(\sin(36)+\sin(72))}$.
[b] Notation used from Ref. [27].
[c] $\nu_{max} = \nu_2/\cos P$.

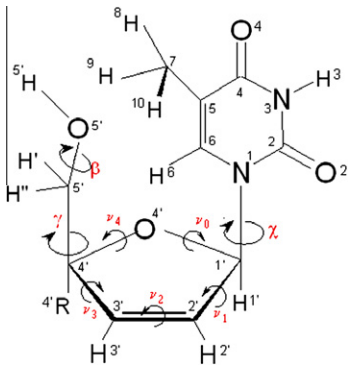
The Potential Energy Surface (PES) of this molecule was determined by rotation of the torsional angles $\chi$ (glycosidic bond), $\gamma$ (C4′—C5′ bond) and $\beta$ (C5′—O5′ bond). These dihedral angles simultaneously held fixed at values varying between 0° and 360° in steps of 60° in a first study (Table 1 is a resume with the global minimum) and of 20° in a more detailed second study (Tables S1 of ESI). All other geometrical parameters were relaxed during these optimizations. The optimized points were plotted by the SURFER program [26]. 216 optimized geometries were considered at the first step and 5832 in the second one.

## 3. Results and discussion

### 3.1. Definition of conformational angles

Following the Saenger's notation [27], the atomic description of this molecule as well as the most important exocyclic and endocyclic torsional angles are defined in Scheme 1. The different conformations in D4T can be characterized by the following four important structural parameters: (i) the glycosylic torsional angle, $\chi$ (O4′—C1′—N1—C2), which determines the two orientations of the base relative to the furanose ring, denoted as the *anti* and *syn* conformations. (ii) The exocyclic torsional angle $\gamma$ (C3′—C4′—C5′—O5′) which describes the orientation of the 5′-hydroxyl group relative to the furanose ring. This ring is twisted out-of-plane in order to minimize non-bonded interactions between their substituents. (iii) The exocyclic torsional angle $\beta$ (C4′—C5′—O5′—H5′) describes the orientation of the hydroxyl hydrogen H5′ relative to the furanose ring. (iv) The furanose pucker $P$ defined in the bottom of Table 1, which indicates north and south orientations.

An important structural characteristic of D4T is the presence of the C2′=C3′ double bond, a feature that renders the sugar ring nearly planar and imparts a high degree of rigidity to the sugar



**Scheme 1.** Molecular structure and definition of the exocyclic and endocyclic angles in D4T nucleoside.