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Theoretical Investigation of the Triphosphate Forms of Azidothymidine and Thymidine

Márcia Arissawa[†,]*, Judith Felcman[†] and Juan Omar Machucca Herrera[‡]

[†]Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro-Rua Marquês de São Vicente 225, Gávea, Rio de Janeiro, Brazil

[‡]Departamento de Química Inorgânica, Universidade Federal do Estado do Rio de Janeiro-Ilha do Fundão, Rio de Janeiro, Brazil

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In this paper we investigate (using AM1 semi-empirical as well as HF methods at the STO-3G, 3-21G, 6-31G, 6-31G* and 6-31+G level) the conformations, geometrical parameters, Mulliken charges, and solvation effects of the triphosphate form of AZT (AZTTP), as well as the thymidine nucleotide (dTTP) structure. Our calculated geometrical parameters and Mulliken charges, with and without solvation effects, are correlated with recent experimental results.**

Keywords: Azidothymidine triphosphate, Conformation, Ab initio, Solvent

Introduction

The nucleoside derivatives are the most active drugs against the HIV virus. Among them, an important compound, which the Food and Drug Administration (FDA) has approved, is 3'-azido-2',3'-dideoxythymidine (AZT). It inhibits the Reverse Transcriptase after phosphorylation to the triphosphate form. Several nucleoside derivatives have been synthesized to improve therapeutic treatment against AIDS. New drugs are being proposed. However, the mechanisms for more effective drugs still require more investigation.

The conformation analysis of several anti-HIV nucleoside analogues shows that the preferred sugar-ring orientation is based on C3'-exo conformations (Taylor *et al.*, 1990; Van Roey *et al.*, 1990) (Fig. 1). Some inactive compounds that have C3'-endo conformations (Fig. 1) were studied by Roey (Van Roey *et al.*, 1989). The C3'-exo and C3'-endo conformations place C5' in the axial and equatorial positions, respectively. This affects the location of the 5'-hydroxyl group in relation to the base conformation. The 5'-hydroxyl group is the phosphorylation site of the nucleoside.

Fisher *et al.* (1994) proposed 144 potentially bioactive conformations of AZT. They verified that the O2, O4' and O5' atoms are a potential pharmacophore for thymidine nucleoside analog inhibitors that are targeted at RT. The inclusion of triphosphate in the nucleoside results in modification of the geometry. As most of the analogues of nucleosides are variants of natural RT substrates that lack the 5'-hydroxyl group, their recognition by RT and subsequent incorporation into a growing DNA chain results in chain termination. As shown by several authors, chain terminators are potent inhibitors of viral replication, not because they compete very efficiently with their natural deoxynucleosides, but because of the high number of potential sites of chain termination in the viral genome(Van Roey *et al.*, 1992). The observation of the conformational preference in the active compounds after phosphorylation may be important for the development of new nucleosides, because this structure is the last step before the RT complexation (Balzarini *et al.*, 1986; McGuigan *et al.*, 1993; Marquez *et al.*, 1998).

After an *in vitro* analysis, Villahermosa *et al.* (1997) proposed that AZTTP significantly inhibited nucleotide incorporation at concentrations that are 2-3 orders of magnitude lower than the dTTP concentration. It has been demonstrated that the affinity of RT toward AZTTP and dTTP was nearly the same, and that dTTP was as efficient a substrate as AZTTP for the enzyme(Villahermosa *et al.*, 1997). Despite efforts for the crystallization of the AZTTP-RT complex with higher resolution, the real conformation of AZT remains obscure. In 1993, Painter *et al.* (1993) investigated the conformations of the 5'-triphosphates of zidovudine (AZT) and thymidine (dT) via Nuclear Overhauser experiments in order to determine the conformations of AZTTP and dTTP when bound to the HIV-1 Reverse Transcriptase. Subsequent studies(Painter *et al.*, 2000) used the Nuclear Overhauser effect to characterize the protein environment and

*To whom correspondence should be addressed.
Tel: 55-21-3882-9364; Fax: 55-21-2594-6147
E-mail: marissawa@bio.fiocruz.br

conformations of dTTP, dATP and AZTTP that are bound to HIV-RT in the ground state. These indicated that the binding sites for the nucleotides are not a complete coincident, and all of the bounded nucleotides assumed the same anti C4'-exo conformation.

There has been considerable interest to compare the natural nucleoside and their analogues, in order to search for similar characteristics that could probably explain the complexation of AZT into RT. One possible major contribution is the rotational angle χ (C1'-N1), illustrated in Fig. 1. It is often used in the stereochemical analysis of the nucleoside derivatives. Van Roey *et al*. (1989) proposed a dihedral angle that is related to the position of the OH group of the sugar ring γ (C4'-C5'). Anil Kumar and Mishra (1992) studied several molecules that exhibited varying degrees of anti-HIV activity. These include AZT conformers without triphosphorylation using the MNDO method. They indicated that the electric field region that is localized around C5, and an extended electric field region near C3' (around azido group), may be related to the anti-HIV activity of the AZT.

According to Marquez *et al*. (1998), the viral DNA incorporation of the nucleoside analogue should occur by substitution of the C3'-hydroxyl by the pyrophosphate group. The NMR study indicated that AZT 5'-triphosphate and thymidine-5'-triphosphate bind to RT with χ in the *anti* range and γ as the +*sc* rotamer (Painter *et al.*, 1993). Water pervades every portion of every cell and several interactions in biological macromolecules have a very significant influence on the three-dimensional structures of proteins, nucleic acids, polysaccharides and membrane lipids (Mulliken, 1955). This suggests the need to include solvation effects in our calculations

In this paper, we investigate the conformations, geometrical parameters, Mulliken charges (Mulliken, 1955) and solvation effects of the triphosphate form of AZT (AZTTP), as well as the thymidine nucleotide (dTTP) structure. For this investigation we use the AM1 semi-empirical method, as well as HF methods at the STO-3G, 3-21G, 6-31G, 6-31G* and 6-31+G** level. Our calculated geometrical parameters and Mulliken charges, with and without solvation effects, were correlated with recent experimental results.

Methodology

In this work, we used Gaussian 98(1998) on IBM RS6000 workstations for the semi-empirical (AM1) and ab initio calculations (STO-3G, 3-21G, 6-31G, 6-31G* and 6-31+G**) for both the gas and solvent phases of AZTTP and dTTP. The Onsager model (Wong *et al.*, 1991) was used to include the effects of the water solvent. We present relatively low-level calculations on several very large (from the computational perspective) molecules of substantial biomedical interest (AZTTP and TTP). These are very large molecules, however, on which to do calculations. Computational chemists expect to see methods that include electron correlation. In the present case, however, the number of basis functions that are needed to describe these molecules (about 500) is our justification for the technique choice that is used in this paper. Our calculations, using the Onsager model, were done with the gas phase optimization with solvent effects that were calculated afterwards.

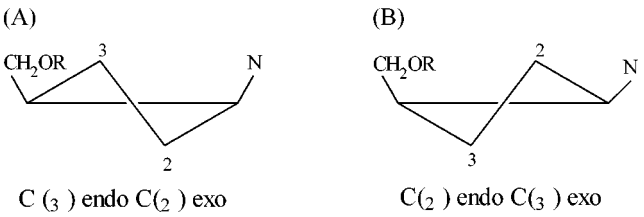

Fig. 1. Twist conformations of the furanose ring.

Results and Discussion

We made a full optimization of the 3'-azido-3'-deoxythymidine 5'-triphosphate (AZTTP) (Fig. 1A and 1B) using semi-empirical (AM1) as well as HF ab initio calculations with basis STO-3G and 3-21G sets in order to determine optimized geometries, Mulliken charges, and dipole moments, which could be compared with the experimental results. We investigated two conformations (A and B) for both dTTP and AZTTP (Tables 1-11). Table 1 gives the geometrical parameters that were obtained for AZTTP. Experimental values were given for comparison.

Recent Nuclear Overhauser effect experiments were used to characterize the protein environment and conformation of AZTTP that bound to HIV-RT in the ground state (Painter *et al*., 1993; Painter *et al*., 2000). They indicated that the ligands that are exclusively *anti* in the presence of protein are limited to χ values between –95° to –155°. The γ torsion was fixed +synclinal (+*sc*) in accordance with other experimental data (Wong *et al.*, 1991). The glycosyl torsion angle c is noted. It determines the syn or anti disposition of the base relative to the sugar moiety (*syn)* when the C2 carbonyl of pyrimidines or N3 of purines lies over the sugar ring, and anti when these atoms are oriented in the opposite direction. The torsion angle γ determines the orientation of the 5'-OH with respect to C3', as represented by the three main rotamers, namely +*sc*, *ap*, -*sc*.

Our calculations in Table 1 that used full-optimized values with the basis sets (AM1, STO-3G, 3-21G) also gave yields for χ the *anti* orientation, which agreed with the recent Nuclear Overhauser experimental results (Van Roey *et al*., 1989; Dewar, 1992; Painter *et al*., 1993; Koch and Popelier, 1995; Painter *et al*., 2000; Fidanza *et al*., 2001). Our χ values were also between the expected experimental(Painter *et al.*, 1993; Painter *et al.*, 2000) values (–95° to –155°), i.e. 97.47°, –106.32° and –105.93° for AM1, STO-3G, 3-21G, respectively.

Table 2 gives the full-optimized geometries for 2'-deoxythymidine triphosphate (dTTP-Fig. 2) at the HF (AM1,

Table 1. Geometrical parameters (distances (Å) and angles (°)) of AZTTP (A and B conformers), full optimization at AM1 and HF (STO-3G and 3-21G) levels of theory

AZTTP Fig. 1B	Experimental Results (a)	Conformer A (b) AM1	Conformer A (b) HF/STO-3G	Conformer A (b) HF/3-21G	Conformer B (c)	Conformer B (c) HF/3-21G
P1=O3	1.4866	1.5352	1.5573	1.51667	1.4913	1.5446
P5=O6	1.4886	1.5380	1.5608	1.52389	1.4823	1.5319
P9=O10	1.4825	1.5490	1.6055	1.53417	1.5020	1.5337
C17-N18	1.4699	1.4573	1.5128	1.48881	1.4969	1.4589
N21-C20	1.4527	1.4519	1.4657	1.43986	1.489	1.4566
N18-N37-N46	134.036	169.006	169.744	172.928	171.381	169.406
C17-N18-N37-N46	160.874	-179.818	177.088	176.372	-172.145	179.924
C19-C17-N18-N37 (β)	157.978	60.358	75.289	89.933	157.978	177.075
C22-N21-C20-O16 (χ)	175.293	-97.472	-106.317	-105.934	-144.487	-97.787
O13-C14-C15-C17 (γ)	57.539	25.236	53.560	56.331	66.581	-57.640
P1-O13-C14-C15	-162.638	136.660	107.696	91.356	-162.641	109.932
C14-O13-P1-O4 (ϕ)	96.225	-139.542	-111.440	-122.647	-6.795	114.873
0O13-P1-O4-P5	-83.327	-36.834	-55.151	-53.546	-87.129	-177.526
P1-O4-P5-P8 (ε)	-116.723	-141.045	-117.316	-120.328	-131.176	-37.292
O4-P5-O8-P9	53.356	70.050	79.171	64.311	-72.634	-96.435
P5-O8-P9-O11 (τ)	175.698	-166.453	-179.555	-151.771	-68.101	54.228

(a) Protein databank
(b) Conformer A determine using input geometry from the literature (ref. 28)
(c) Conformer B using input geometry with modified torsion angles γ,ϕ,ε and τ.

Table 2. Geometric parameters (distances (Å) and angles (°)) of the dTTP (A and B conformers), full optimization at AM1 and HF (STO-3G and 3-21G) levels of theory

DTTP Fig. 2	Experimental Results (a)	Conformer A (b) AM1	Conformer A (b) HF/STO-3G	Conformer A (b) HF/3-21G	Conformer B (c)	Conformer B (c) AM1
P1=03	1.4868	1.5353	1.5510	1.5206	1.4808	1.5363
P5=O6	1.4888	1.6781	1.6026	1.5229	1.4817	1.6734
P9=O10	1.4925	1.6775	1.5857	1.5291	1.4925	1.6781
C17-O18	1.4266	1.4169	1.4279	1.4492	1.4263	1.4093
O18-H37	0.9999	0.9693	1.0312	0.9722	0.9596	0.9662
N21-C20	1.4528	1.4565	1.4700	1.4669	1.4526	1.45252
C22-N21-C20-O16 (χ)	175.292	-114.456	-116.544	-157.888	175.292	-105.1583
O13-C14-C15-C17 (γ)	57.539	30.219	-35.766	47.352	57.539	43.508
P1-C13-C14-C15	-162.638	-150.886	-154.780	-132.418	-162.638	-177.579
C14-O13-P1-O4 (ϕ)	96.225	-13.355	174.430	67.336	-13.285	-58.574
O13-P1-O4-P5	-83.327	-21.430	-33.166	-70.039	-83.327	-110.109
P1-O4-P5-O8 (ε)	-116.723	-159.656	-130.973	-89.872	-116.723	-100.401
O4-P5-O8-P9	53.357	1.971	69.803	53.288	-58.614	-39.0481
P3-O4-P5-O12 (τ)	175.699	-71.876	-176.651	100.307	-60.511	-39.351
C19-C17-O18-H37 (β)	68.161	77.733	114.641	72.073	68.196	-54.076

(a) Protein DataBank[24]
(b) Conformer A determine using input geometry from the literature (ref. 24)
(c) Conformer B using input geometry with modified torsion angles γ,ϕ,ε and τ.

STO-3G, 3-21G). Experimental (Painter *et al.*, 1993; Painter *et al.*, 2000) values are given for comparison.

Our calculations in Table 2 for dTTP using full-optimized values with the basis sets (AM1, STO-3G, 3-21G) also yields χ (Fig. 1A), the anti orientation that agrees with the recent experimental (Painter *et al.*, 1993; Painter *et al.*, 2000) results. Our χ values (i.e. −114.46°, −116.54° and −157.89° for AM1, STO-3G, 3-21G, respectively) are also between the expected experimental values.

Our calculated value for dTTP, using the HF/3-21G level,

Table 3. Total energy and dipole moment (μ) of dTTPA

dTTPA	Total energy (a.u.)	μ
STO-3G	-2535.8081519	4.4892
3-21G	-2554.2067174	11.2693

Table 4. Total energy and dipole moment (μ) of AZTTPA

AZTTPA	Total energy (a.u.)	μ
STO-3G	-2622.4924043	6.0874
3-21G	-2641.4662789	6.7764

Table 5. Total energy and dipole moment (μ) for dTTP (conformer B)

dTTP	Total energy (a.u.)	μ (Debye)
HF/STO-3G	-2535.7174944	7.2500
HF/3-21G	-2554.06075599	10.2755
HF/6-31G	-2566.82419166	10.6897
HF/6-31G*	-2567.90764401	10.1942
HF/6-31+G**	-2568.0098347	10.2417

Table 6. Total energy and dipole moment (μ) for AZTTP (conformer B)

AZTTP	Total energy (a.u.)	μ (Debye)
HF/STO-3G	-2622.41126766	5.3065
HF/3-21G	-2641.35821613	7.5745
HF/6-31G	-2654.57902901	7.7222
HF/6-31G*	-2655.73836270	7.7430
HF/6-31+G**	-2655.8334501	8.0195

yields the important anti-HIV structural parameter γ (47.35°), which is close to the x-ray experimental result (Huang *et al.*,1998) (57.54°). Table 2 also gives our calculated HF/3-21G values of ϕ (67.34°), τ (100.31°), β (72.07°) and ε (–89.87°) compared to the experimental values ϕ (96.23°), τ (175.70°), β (68.16°) and ε (–116.723°) (Fig. 1A). We note that these parameters are sensitive to the dTTP conformation.

The reasonable agreement between our calculated geometrical parameters and experimental results (x-ray and NMR) may be useful to other workers. It is well known that experimental results may be affected by the use of solvents or the interaction with crystalline lattices.

The azidothymidine triphosphate (AZTTP) is a nucleotide analogue of 3'-deoxythymidine triphosphate (dTTP), whereas the geometrical parameters of the pyrimidinic and furanose rings of both complexes should in principle be retained. We note that in AZTTP (Fig. 2), selected bond lengths [N21-C29 (1.384 Å), C22 = O23 (1.215 Å), C29 = C27 (1.326 Å), N24-H45 (1.001 Å), C29-H46 (1.066 Å), C15-C17 (1.527 Å)] agree excellently with the geometrical parameters of dTTP (Fig. 2): [N21-C29 (1.387 Å), C22 = O23 (1.220 Å), C29 = C27 (1.327 Å), N24-H45 (1.001 Å), C29-H46 (1.065 Å), C15-C17 (1.529 Å)].

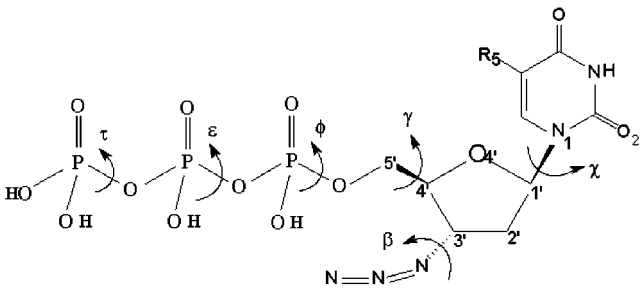

Fig. 2A. Azidothymidine triphosphate (AZTTP) indicating torsion angles.

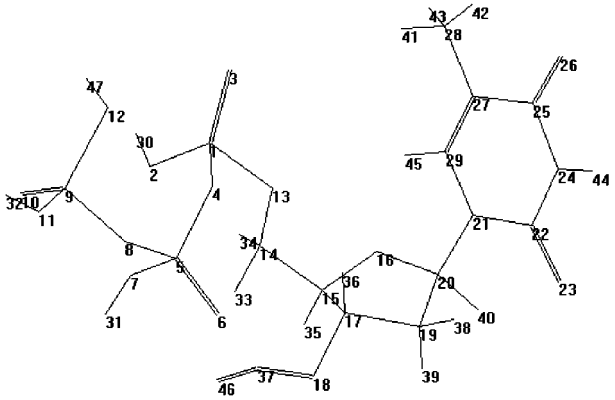

Fig. 2B. Azidothymidine triphosphate (AZTTP) indicating numbering of atoms used in tables.

We also calculated, using different basis sets (AM1, STO-3G, 3-21G, 6-31G, 6-31G* and 6-31+G**), the effects of solvent (water) on AZTTP and dTTP (Table 7 and 8). We observed that the solvent effects modify dipole moments and stabilize the total energy of both AZTTP and dTTP. A more detailed analysis should include the interaction of these molecules with the enzyme.

It may be useful to other authors and for future research to use our present experience in order to give some indication of the relative computational speed and best judgement on basis sets, level of calculation, as well as molecule sizes. Without generalizing, we suggest that molecule sizes that were investigated in our present work (the relatively fast and computationally inexpensive AM1) yielded reasonable qualitative results. However, when very close agreement is required for the experiment, and other effects (such as hydrogen bonding) must be accurately accounted for, then for future work we recommend the 6-31+G** basis set, using either the B3LYP or MP2 methods. If the molecules are very large (for example, when we must also consider the interaction between the drug and the receptor to better understand the mechanism of anti-HIV activity, discussed below), then we must again consider faster and cheaper computational methods, such as AM1 or even PM3.

Table 7. Total Energies and dipole moments (μ) for dTTPB using SCRF-Onsager model of solvation at different levels of theory

	HF/STO-3G	HF/3-21G	HF/6-31G	HF/6-31G*	HF/6-31+G**
Total energy of solute	-2535.72381678	-2554.07419123	-2566.83898814	-2567.92112240	-2568.02378302
Total energy (include solvent)	-2535.72048881	-2554.06700332	-2566.83103173	-2567.91387174	-2568.01623764
Total energy (without reaction field)	-2535.71716083	-2554.05981540	-2566.82307531	-2567.90662108	-2568.00869225
Dipole moment (μ)	8.0583	11.8414	12.4640	11.8954	12.1577

Table 8. Total Energies and dipole moments (μ) for AZTTPB using SCRF-Onsager model of solvation at different levels of theory

	HF/STO-3G	HF/3-21G	HF/6-31G	HF/6-31G*	HF/6-31+G**
Total energy of solute	-2622.41453719	-2641.36531700	-2654.58656753	-2655.74632966	-2655.84226529
Total energy (include solvent)	-2622.41280895	-2641.36149780	-2654.58248789	-2655.74200054	-2655.83743192
Total energy (without reaction field)	-2622.41108071	-2641.35767861	-2654.57840825	-2655.73767142	-2655.83259855
Dipole moment (μ)	5.9265	8.8215	9.1181	9.2283	9.7653

Table 9. Mulliken charges from AZTTP (conformer B) using SCRF-Onsager model of solvation

	3-21G	3-21G (onsager)	6-31G*	6-31G* (onsager)
O6	-.708860	-.715597	-.632458	-.641051
O3	-.780041	-.788879	-.725450	-.735135
O10	-.745237	-.741227	-.667716	-.664185
O2	-.830975	-.830619	-.855110	-.854217
O7	-.839799	-.835064	-.849914	-.844525
O12	-.857303	-.856480	-.876007	-.874884
O11	-.801422	-.801805	-.801102	-.800936
C17	-.101259	-.097436	-.045439	-.041793
N18	-.318772	-.322096	-.455673	-.459443
N38	.036336	.034398	.385990	.384598
N46	.024943	.041673	-.175872	-.158316
H47	.457661	.462045	.512596	.516843
H30	.411508	.405751	.435067	.428776
H32	.453784	.451338	.522469	.520345
H31	.459543	.461710	.511831	.513218

In Table 9, 10 and 11 we give selected Mulliken charges using the conformers A and B of AZTTP and dTTP, and using HF at the 3-21G, 6-31+G** level with and without the effects of solvents. We note that with solvation and changes in conformations we may obtain larger modifications of the Mulliken charges, particularly for the non-bridging oxygen atoms of the triphosphate chain, as well as for N17 of the azido terminal group. Huang *et al.* (1998) prepared a HIV-1 RT complex with a DNA template:primer and a deoxynucleoside triphosphate (dNTP), and the crystal structure of the complex was determined. The presence of a dideoxynucleotide at the 3'-primer terminus allowed the capture of a state in which the substrates are poised for attack on the dNTP. Conformational changes that accompany the formation of the catalytic complex produce distinct clusters of the residues that are altered in viruses that are resistant to nucleoside analog drugs. The positioning of these residues in the dNTP neighborhood helps to resolve some long-standing puzzles about the molecular basis of resistance. The resistance mutations are likely to influence binding or reactivity of the inhibitors relative to normal dNTPs; the clustering of the mutations correlates with the chemical structure of the drug. The crystal of this catalytic complex contains bound dTTP in precisely the expected position for attack by the (missing) 3' OH. The base of the dTTP stacks on the terminus of the primer strand, almost as in a continuous DNA strand; the side chains of Arg^{72} and Gln^{151} pack against its outer surface. The triphosphate moiety is coordinated by Lys^{65}, Arg^{72}, main-chain -NH groups of residues 113 and 114, as well as two metal ions (Mg). In their model, the guanidinium group of Arg^{72}, which lies flat against the dNTP base, donates hydrogen bonds to the α-phosphate; the ε-amino group of Lys^{65} donates

Table 10. Mulliken charges from dTTP (conformer B) using SCRF-Onsager model of solvation

	3-21G	3-21G (Onsager)	6-31G*	6-31g* (Onsager)
O10	-.877610	-.875203	-.889848	-.887266
O12	-.721253	-.733516	-.640773	-.652809
O9	-.810472	-.813418	-.807709	-.810481
O3	-.838505	-.831522	-.854394	-.847392
O7	-.744140	-.753380	-.666743	-.676454
O11	-.740238	-.740765	-.654776	-.655969
O6	-.833231	-.830608	-.833120	-.830588
H32	.453976	.457807	.514867	.518658
H30	.448066	.453324	.508504	.513726
H31	.458097	.459576	.516985	.518075
H46	.410273	.405434	.434077	.429396
C17	.077338	.080495	.144715	.147419
O18	-.672250	-.666535	-.743231	-.737359
H37	.272185	.268985	.222883	.219191

Table 11. Mulliken charges on principal active sites of dTTPA and AZTTPA (Onsager solvation model)

dTTPA Fig. 2	dTTPA 3-21G	dTTPA 6-31+G** (Onsager)	AZTTPA Fig. 1B	AZTTPA 3-21G	AZTTPA 6-31+G** (Onsager)
O10	-.847630	-1.099783	O10	-.838271	-1.065728
O6	-.794238	-.885357	O6	-.774879	-.785047
O3	-.769596	-.835513	O3	-.928291	-.767644
O2	-.944609	-1.013338	O2	-.943080	-1.045208
O7	-.914327	-1.003848	O7	-.928291	-1.038301
O11	-.847630	-.896699	O11	-.838271	-.884950
O12	-.914327	-1.003848	O12	-.756875	-1.038301
H32	.476824	.443155	H32	.489018	.441800
H30	.520581	.580090	H30	.507215	.556240
H31	.486850	.510284	H31	.489018	.486737
H46	.477955	.447659	H47	.479516	.440658
C17	.031708	-.258136	C17	-.089924	-.338663
O18	-.733458	-.760050	N18	-.324428	-.504524
H37	.434071	.448116	N46	.043342	-.172696

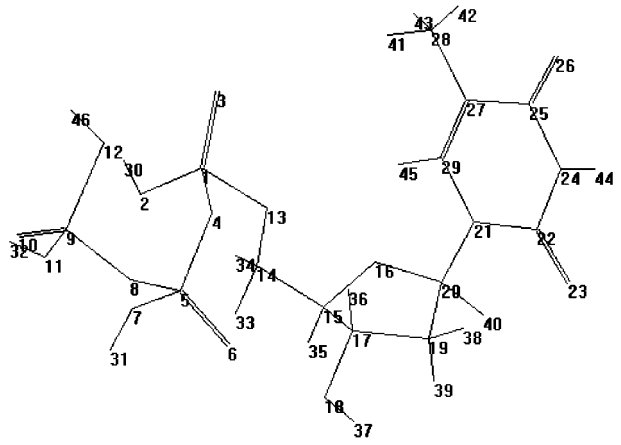

Fig. 3. Deoxythymidine triphosphate (dTTP) indicating numbering of atoms used in tables.

hydrogen bonds to the γ-phosphate. The triphosphate wraps around one of the Mg ions. A non-bridging oxygen from each of the phosphates contributes to the octahedral coordination of this metal.

The theoretical model of Huang *et al.* (1998) agrees with our present calculations. Three non-bridging oxygens, which should participate in the octahedral coordination with Mg, have large negative charges in the gas phase that is further increased in the presence of the solvent. Conformation changes suggest that these negative oxygens ions can effectively interact with the strongly positive two Mg ions.

Because most of the nucleoside analog drugs have modifications at the 3'-position, this "3'-pocket" is of particular significance for understanding structure-activity relations and analyzing resistance mutations. The pocket can probably accommodate two or three water molecules in

addition to the 3'-OH; it clearly has room for the azido group of AZTTP. We note, however, that there is still a need for models to elucidate clearly the mechanisms for resistant mutations of HIV-1. Our calculations indicate that the terminal nitrogen charge of the azido chain at 3' (N18-Fig. 2) may be modified in the presence of solvents. This effect is interesting since it is well known that nitrogen atoms with appropriate charges may participate in Hydrogen bonding. There is, therefore, the possibility that the azido group may not be able to always function as a terminal group, leading to one of the possible mechanisms for resistance mutations.

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