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Solid-State and Solution Conformations of the Potent HIV Inhibitor, 4′-Azidothymidine[1]

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The three-dimensional structure of 4′-azidothymidine has been determined for the solid state and in solution. X-ray crystal analysis indicates the presence of two independent molecules (A and B) having the following conformational parameters: Phase angles, P_A = 13.7°, P_B = 12.6° (C3′-endo envelope); puckering amplitude Ψ_{m_A} = 32.4°, Ψ_{m_B} = 37.2°; glycosyl torsion angle χ_A = −88.2°, χ_B = −71.2°; 4′–5′ torsion angle γ_A = 58.5°, γ_B = 36.0°. The solution conformation was determined from NMR coupling constants in D_2O. Analysis using the computer programs PSEUROT and DAERM yielded phase angles (*P*) of 53.2° (C4′-exo envelope) (major conformer) and 63° (C4′-exo envelope), respectively, with corresponding puckering amplitudes (Ψ_m) of 34.9° and 45.8°. A gated ^{13}C NMR experiment was used to determined the ^1H–^{13}C vicinal coupling constants used to calculate the solution glycosyl torsion angle (χ) to be either −80° or −160° and a 4′–5′ torsion angle, γ, of ca. 180°. These studies show that 4′-azidothymidine is conformationally exceptional among the antiretroviral nucleosides both as a solid and in solution. The C3′-endo (northern) conformation determined by X-ray crystallography is rare among HIV-inhibitory nucleosides which usually exist in the solid state in a southern conformation. The solution structure is even more peculiar in that it exists in the extremely rare 4′-exo envelope conformation.

Over the past decade many modified nucleosides have been discovered that inhibit the replication of human immunodeficiency virus (HIV).[3] The majority of these analogues act via a similar mechanism in which they are first anabolized to the 5′-triphosphate derivatives which may then inhibit the viral reverse transcriptase or serve as a substrate, enabling the analogue to be incorporated into the viral DNA and cause chain termination.[4–6] Additionally, other enzymes may affect the activity of the nucleosides via modifications such as deamination, oxidation, or glycolysis. These inhibitors must, therefore, possess an exacting combination of enzymatic specificities in order to effectively achieve their therapeutic goal. It is no wonder then, that subtle differences in structure can lead to dramatic changes in activity.

The unique structural features which collaborate to produce a potent and selective inhibitor of HIV have been sought by many researchers.[7] However, the complexity of nucleoside metabolism and the relatively small number of active analogues conspire to limit structure–activity relationships (SAR) to only broad generalizations. The extrapolation of the structural features of active compounds to the design of new inhibitors can only be done with caution.

We have recently discovered a series of 4′-substituted nucleoside, HIV inhibitors that appear to run counter to many of the previous structural trends.[8] The most extensively studied example, 4′-azidothymidine, inhibits HIV (*in vitro* IC_{50} = 0.01 μM) via a mechanism similar to that of the other antiretroviral nucleosides (i.e. anabolic

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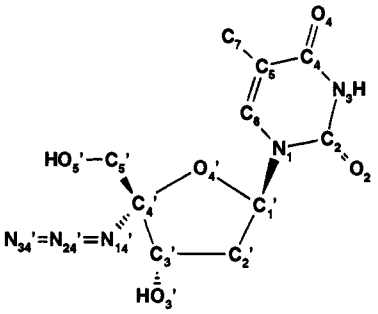

Figure 1. Molecular structure of 4′-azidothymidine with numbering scheme used in the X-ray analysis.

phosphorylation to its 5′-triphosphate which inhibits the HIV reverse transcriptase and/or incorporation into viral DNA leading to chain termination[9]). However, unlike all other nucleosides which are potent inhibitors of HIV, this series of 4′-substituted nucleosides retains a hydroxyl group at the 3′-position. In fact, if the 3′-hydroxyl group of 4-azidothymidine is removed, all anti-HIV activity is lost.[8] Our initial studies also revealed another structural difference from the majority of active nucleosides. Whereas X-ray crystallography has led to the suggestion that a 3′-endo conformation of the furanose ring is predictive of low anti-HIV activity,[7c,10] we have evidence to indicate that the active members of the 4′-substituted series strongly prefer a 3′-endo conformation.[8]

Because of these anomalies, we have undertaken more rigorous investigations into the conformation of 4′-azidothymidine. In this paper we describe the solid-state structure as determined by X-ray diffraction and structural studies of the solution conformation by NMR spectroscopy.

Results

Solid-State Structure. Suitable crystals for X-ray diffraction analyses were obtained from an acetonitrile solution. A colorless crystal of dimensions 0.30 × 0.35 × 0.45 mm was used to collect a room-temperature data set using Cu Kα (1.541 78 Å) radiation. 4′-Azidothymidine, $C_{10}H_{13}N_5O_5$, crystallizes in the monoclinic space group $P2_1$ with the following cell constants: a = 7.188(2), b = 22.804(7), and c = 8.042(2) Å and β = 109.91°. The unit cell contains two independent molecules (A and B) per asymmetric unit, for a total of four molecules. Figure 1 shows the molecular structure of 4′-azidothymidine with the numbering scheme used in the X-ray analysis. The crystal packing arrangement including the hydrogen bonding scheme is shown in Figure 2, and the fractional coordinates of the non-hydrogen atoms are listed in Table 1.

Molecules A and B are associated as dimers via two hydrogen bonds formed between the 5′-OH of one of the molecules to the O4 of the other, resembling an overall rectangular structure. The two pyrimidine rings are optimally stacked in an almost parallel orientation with a stacking distance of 3.5 Å, a distance which is commonly observed in B-DNA. The angle between the two idealized planes characterizing the pyrimidine rings is 2.8°. A Pluto representation of the asymmetric unit is shown in Figure 3. As shown in the figure, the pyrimidine rings are stacked in an offset position with a displacement of the center of the rings of 2.1 Å. The dipole moments of the pyrimidine rings, calculated from MOPAC derived charges, are at an angle of 60° to each other. The 5-methyl groups are in close contact providing a strong hydrophobic (van der Waals type) interaction. Such an arrangement is considered energetically highly favorable on theoretial and experimental grounds.[11]

The hydrogen bonds giving rise to the tight circular association of the two molecules in the asymmetric unit are part of extended systems as shown in Figure 2. There are two different systems, one spanning two, the other three asymmetric units. In the first case shown on the left in Figure 2, the hydrogen on N3 in molecule B forms a hydrogen bond to O5′ of a B molecule in the adjacent unit which donates its hydrogen to a hydrogen bond with O4 of molecule A in the same unit. In the second case, shown on the right in Figure 2, the hydrogen on N3 in molecule A of the first unit points to O3′ in molecule B in a second unit, which in turn has its hydrogen point to O3′ in a A molecule of a third unit. The hydrogen on O3′ of this third unit A molecule points back to O2 in the B molecule of the first unit. Table 2 list the hydrogen bond distances and angles identified in this analysis.

The two independent molecules (A and B) are very similar and differ mainly in their glycosyl torsion angles and the conformation of the 4′-azido group (Figure 4). They are essentially superimposable as far as the sugar conformation is concerned. Both are in a northern conformation with phase angles (P) of 12.7° and 14.8° and pucker amplitudes (Ψ_m) of 32.3° and 37.4°, respectively. The glycosyl torsion angle (χ) are in a close range, −88.2° and −71.2°, respectively. Both place the pyrimidine ring in the anti orientation (C6 above the sugar ring). The 4′-azido group is essentially linear in both cases, with bond angles of 173.5° and 175.1° at the central nitrogen atoms. Similar azido geometries have been observed in AZT in the solid state.[7h,12] The azido group is oriented either in a gauche (molecule A) or in an eclipsed (molecule B) conformation with respect to the C4′–O4′ bond. The latter orientation is probably a result of the crystal packing. Representative torsion angles for the two molecules are listed in Table 3 in comparison with the torsion angles derived from the NMR-based solution structure (see below).

Solution Conformation. Two methods were used to determine the nucleoside conformation from the NMR coupling constants shown in Table 4. Both are based on the generalized Karplus equations relating vicinal proton–proton coupling constants to their torsion angles. From the proton torsion angles, the furanose endocyclic torsion angles could be computed directly. Then using the relationships derived by Altona and Sundaralingam,[13] the phase angle of pseudorotation (P) and the degree of ring pucker (Ψ_m) were calculated. The first method, referred to as the "dihedral angle estimation by the ratio method" (DAERM)[14] requires the measurement of two $^3J_{H,H}$ couplings for each torsion angle. Therefore, one hydrogen must be vicinal to a pair of hydrogens in order to measure the vicinal hydrogen torsion angle. In the case of 4′-azidothymidine, this occurs between H1′ and H2′, H2″, and between H3′ and H2′, H2″. The advantage of this method is that the Karplus constants, which vary according to such factors as electronegativity and ring strain, and which can only be estimated or determined empirically, can be set up as a ratio. This ratio has been determined for general cases and found to remain essentially constant. A computer program is available[14] which calculates the possible angle sets for a given set of coupling constants.

When the values of $J_{1',2'}$, $J_{1',2''}$, $J_{2',3'}$, $J_{2'',3'}$ were incor-

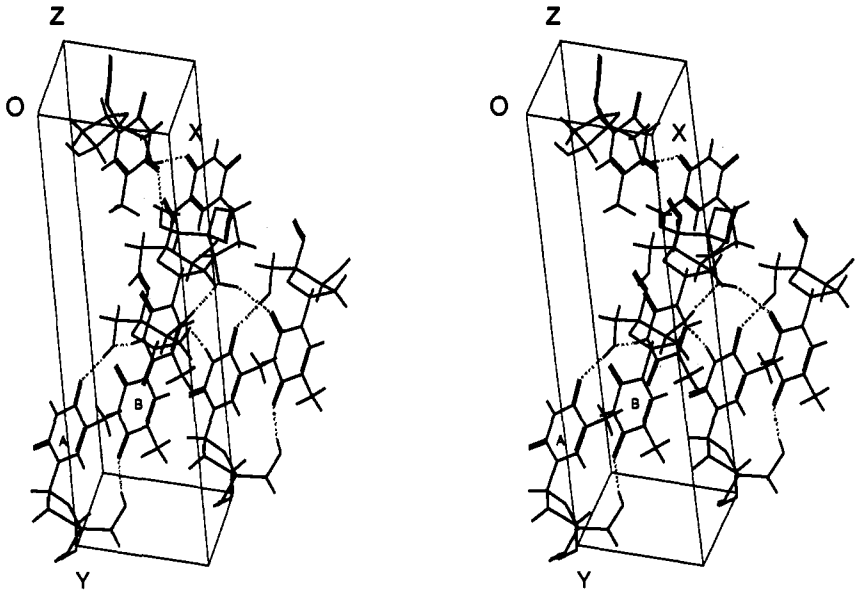

Figure 2. Relaxed stereoview of the unit cell packing arrangement of 4'-azidothymidine. The unit cell dimensions are indicated by thin solid lines. A minimum number of additional molecules, generated by the appropriate symmetry operations, are depicted outside the unit cell to illustrate all types of hydrogen bonds (broken lines) identified in the analysis. In the asymmetric unit at the lower left, the relative location of molecules A and B in that unit are indicated.

Table 1. Atomic Coordinates (× 10^4) and Equivalent Isotropic Displacement Coefficients (Å^2 × 10^3) of Molecules A and B in the Asymmetric Unit of 4'-Azidothymidine

	x	y	z	U(equiv)[a]
N(1A)	11014(4)	3122	9996(3)	32(1)
N(3A)	10890(4)	2113(2)	9517(3)	41(1)
N(14A)	10305(5)	4717(2)	13083(4)	45(1)
N(24A)	10311(5)	4518(2)	14511(4)	48(1)
N(34A)	10449(9)	4376(3)	15866(5)	92(2)
O(2A)	12287(4)	2478(2)	12302(3)	56(1)
O(4A)	9455(5)	1704(2)	6852(4)	57(1)
O(4'A)	10058(3)	3746(1)	11914(3)	36(1)
O(3'A)	10287(3)	5147(2)	9923(3)	41(1)
O(5'A)	6213(3)	3969(2)	9617(3)	46(1)
C(2A)	11456(5)	2559(2)	10720(4)	39(1)
C(4A)	9870(5)	2153(2)	7737(4)	37(1)
C(5A)	9355(5)	2745(2)	7067(4)	35(1)
C(6A)	9938(5)	3188(2)	8211(4)	35(1)
C(7A)	8287(6)	2829(2)	5109(5)	50(1)
C(1'A)	11619(4)	3620(2)	11230(4)	32(1)
C(2'A)	12011(4)	4187(2)	10415(4)	33(1)
C(3'A)	10107(4)	4533(2)	10044(4)	31(1)
C(4'A)	9379(4)	4329(2)	11537(4)	29(1)
C(5'A)	7157(5)	4330(2)	11087(4)	40(1)
N(1B)	4345(4)	1406(2)	6962(3)	33(1)
N(3B)	6043(4)	1787(2)	9731(4)	41(1)
O(2B)	6051(4)	799(2)	9216(3)	47(1)
O(3'B)	2494(3)	1019(2)	1009(3)	38(1)
O(4B)	6265(5)	2758(2)	10312(3)	61(1)
O(4'B)	5496(3)	670(2)	5507(3)	34(1)
O(5'B)	7521(3)	1398(1)	3418(3)	39(1)
C(2B)	5530(5)	1294(2)	8698(4)	35(1)
C(4B)	5657(5)	2360(2)	9214(4)	39(1)
C(6B)	3944(5)	1972(2)	6350(4)	35(1)
N(14B)	4562(5)	58(2)	2919(3)	43(1)
N(24B)	4656(4)	-321(2)	4008(4)	40(1)
N(34B)	4686(8)	-700(2)	4922(5)	79(2)
C(5B)	4534(5)	2448(2)	7361(4)	34(1)
C(7B)	4095(5)	3059(2)	6649(5)	43(1)
C(1'B)	3761(4)	883(2)	5831(4)	33(1)
C(2'B)	2232(5)	985(2)	4011(4)	39(1)
C(3'B)	3469(4)	1082(2)	2859(4)	30(1)
C(4'B)	5185(4)	655(2)	3674(4)	30(1)
C(5'B)	7136(5)	792(2)	3393(4)	37(1)

[a] Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

porated in the DAERM relationships, torsion angles of H1'–C1'–C2'–H2' = 9.8°, H1'–C1'–C2'–H2'' = 129.8°, H2'–C2'–C3'–H3' = 27.5°, and H2''–C2'–C3'–H3' = 147.5° were obtained. Using the ALCHEMY III graphics program (Tripos Associates, Inc., 1992), a furanose ring was constructed from these hydrogen torsion angles. The endocyclic torsion angles were then measured from the computer graphic and incorporated into the calculation[13] to determine a phase angle (P) equal to 63°. From the phase angle and endocyclic torsion angles, a puckering amplitude (Ψ_m) of 46° was determined.[15]

We recognized two shortcomings of the DAERM methodology. First, there is only partial compensation for electronegative substituents. Small errors were likely introduced because of the presence of the azido group at C4' and the thymine ring at C1'. Second, nucleosides exist

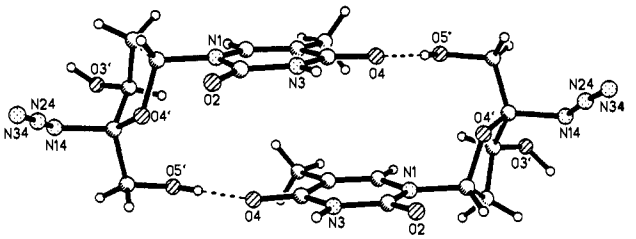

Figure 3. View along the pseudo 2-fold axis relating molecules A and B in the asymmetric unit, with molecule A on the right and molecule B on the left. The pyrimidine rings are offset by 2.1 Å and the dipole moments of them are rotated by 60° to each other. The stacking distance is 3.5 Å. Molecules A and B are connected by two hydrogen bonds from O5'H → O4 (broken lines).

Table 2. Hydrogen Bond Lengths and Angles

	distance (Å)		
atoms D–H → A[a]	D–A[a]	H–A[a]	angles (deg) D–H–A[a]
O5'BH → O4A[b]	2.82	1.98	169
O5'AH → O4B[b]	2.73	1.88	177
O3'AH → O2B[c]	2.89	2.03	148
O3'BH → O3'A[c]	2.74	1.89	172
N3AH → O3'B[c]	2.84	1.95	169
N3BH → O5'B[c]	2.93	2.06	160

[a] D = donor atom, A = acceptor atom. [b] Hydrogen bond between molecules within the same asymmetric unit. [c] Hydrogen bond between molecules in adjacent asymmetric units.

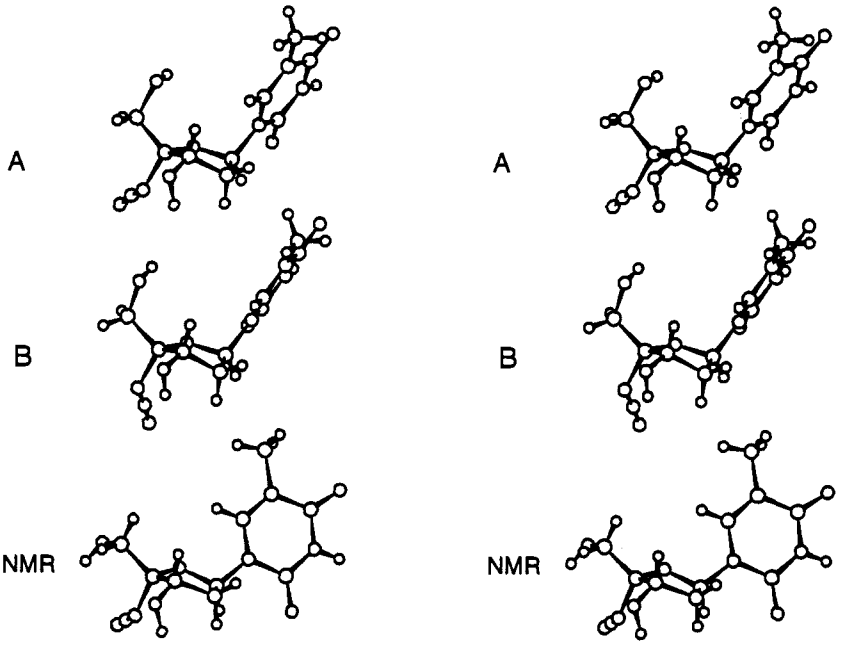

Figure 4. Relaxed stereoview of the aligned comformations of molecules A (top) and B (middle) from the X-ray analysis and the major conformer (bottom) derived from the NMR analysis of the solution structure.

Table 3. Conformational Parameters of the Solid-State and Solution Conformations of 4′-Azidothymidine

			torsion angles (deg)		
	phase angle (*P*) (deg)	pucker Ψ_m (deg)	glycosyl χ	γ^a	azido[b] orientation
		Solid State			
molecule A	13.7	32.4	-88.2	58.5	-161.1
molecule B	12.6	37.4	-71.2	36.0	-122.7
		Solution (D_2O)			
DAERM conformation	63	45.8			
PSEUROT					
major conformer (0.7 mol)	53.2	34.9			
minor conformer (0.3 mol)	-13.7	42.6			
calculated from ^3J ^{13}C–^1H couplings			-79.7° or -160.4°	180°	

[a] C3′–C4′–C5′–C5′O. [b] C3′–C4′–N14′–N24′.

Table 4. ^1H and ^{13}C Chemical Shifts (ppm) and Coupling Constants for 4′-Azidothymidine in D_2O

position	carbon[a]	hydrogen[b]	coupling constants[c] (Hz)
2	152.30		
4	167.26		
5	112.40		
6	138.50	7.59 q	$J_{6,5Me} = 1.24$
1′	85.54	6.48 dd	$J_{1',2'} = 3.79$, $J_{1',2''} = 8.17$
2′(β)	36.55	2.64 ddd	$J_{2',2''} = -14.22$, $J_{2',3'} = 8.13$
2″(α)		2.61 ddd	$J_{2'',3'} = 8.32$
3′	71.31	4.72 dd	
4′	99.85		
5′/5″	63.16	4.00 d	$J_{5',5''} = -12.57$
		3.94 d	
5-Me	12.29	1.97 d	

[a] Referenced to external dioxane in D_2O at 67.4 ppm. [b] Referenced to H_2O at 4.78 ppm. [c] Coupling constants for $J_{1'2'}$, $J_{1',2''}$, $J_{2',2''}$, $J_{2',3'}$, $J_{2'',3'}$ are the results of spectrum simulation.

in solution as a mixture of equilibrating conformations. By design, the DAERM calculation affords only an "apparent" conformation. That is to say, the measured proton couplings from which the conformation is derived are weighted, time-averaged values. The structure determined in this manner may not exist, but is the blend of contributing molecular forms as seen on the NMR time scale.

In an attempt to overcome these deficiencies, the NMR results were also analyzed using the computer program PSEUROT.[16] PSEUROT assumes an equilibrium mixture of two furanose conformations in order to find a best fit for the experimentally measured proton couplings. The electronegativities of substituents as well as Barfield transmission effects[17] are taken into account. The program calculates the phase angles (*P*), puckering amplitudes (Ψ_m), and mole fractions of the two conformers. To our disadvantage, only four hydrogen coupling constants are available, which is insufficient for the determination of the five parameters to be solved by PSEUROT. In these situations of underdeterminancy, one or more of the conformational parameters must be constrained.

As a starting point, the analysis was first carried out assuming that only a single conformer is present, that being the DAERM-derived conformation. However, under these constraints, a solution having a good fit between the observed and the calculated couplings could not be found. The solution had a phase angle of 40.3° and a pucker amplitude of 35.4° with an overall root mean square (rms) deviation in the coupling constants of 0.424 Hz.

Next, the analysis was performed assuming a two-state equilibrium in which alternately the furanose puckering amplitudes or the phase angles of the two conformers were constrained. Beginning with amplitudes set at 40°, which is close to the average for nucleosides,[15] a much better match was found between the calculated and the observed *J* values through this iterative process. After several cycles,

the overall rms dropped to 0.003 Hz, which is well below the experimental error. The calculated parameters were as follows: conformer 1, mole fraction = 0.7, P = 53.2°, Ψ_m = 34.9°; conformer 2, mole fraction 0.3, P = −13.7°, Ψ_m = 42.6°. Thus the best description of the solution conformation as determined by PSEUROT is an equilibrium mixture consisting of two northern conformations. It should be kept in mind that PSEUROT is designed to assume an equilibrium between two, low-energy furanose conformations. This appears to be a valid assumption for the majority of nucleosides examined. However, even though a reasonable correspondence to the observed hydrogen coupling of 4′-azidothymidine was found, this does not exclude the possibility that 4′-azidothymidine exists in solution as a more complex conformational mixture.

A comparison of the NMR analysis of DAERM and PSEUROT indicates that both methods result in very similar solutions. The structure as derived by DAERM and the major conformer of the PSEUROT mixture both have similar phase angles, P = 63 and 53°, respectively. The phase angle of the minor contributory structure in PSEUROT was within 90° of this. Additionally, all the derived structures had reasonable puckering amplitudes.

In order to complete the solution conformational picture of 4′-azidothymidine, the glycosyl torsion angle (χ = O4′–C1′–N1–C2) and the position of the exocyclic 5′-OH (γ = C3′–C4′–C5′–O5′) were probed. The former angle was derived from the vicinal ^{13}C–^{1}H coupling constants $^{3}J_{H1'-C2}$ = 2.58 Hz and $^{3}J_{H1'-C6}$ = 5.28 Hz. Using the modified Karplus relation,[18] $^{3}J_{H-C} = 6.7 \cos \Phi^2 - 1.3 \cos \Phi$, the possible solutions for the angle Φ_1 = [C2–N1–C1′–H1′] were found to be equal to ±43.5° and ±122.1°, and for Φ_2 = [C6–N1–C1′–H1′] ±8.1° and ±142.8°. Only two of these solutions (Φ_1 = +43.5°, Φ_2 = −142.8° and Φ_1 = −43.5°, Φ_2 = +142.8°) placed C2 and C6 at approximately 180° angles from each other as required for a pyrimidine ring. These two solutions were translated to the glycosyl torsion angle (χ) by subtracting 120° from Φ_1 and 300° (120° + 180°) from Φ_2 to afford χ_1 = −76.5° and −163.5°; χ_2 = −82.8° and −157.2°. Taking the average, χ = −79.7° and −160.4°. The former result is essentially identical with both solid-state conformations, and both of the results place the thymine ring in an anti orientation which is normal for pyrimidines. Using the SYBYL graphics program (Tripos Associates, 1992), both of the solutions were constructed and analyzed. While in the case of the −160° glycosyl torsion angle, the distance between H6 and H2′ or H3′ was virtually identical (ca. 3.5 Å each); the −80° solution indicated a significant shorter distance from H6 to H2′ (ca. 2.0 Å) than to H3′ (ca. 3.5 Å). A quantitative NOE experiment in D_2O was undertaken to distinguish between the two cases. Due to interference in the form of spin diffusion observed between H2′ and H2″, no reliable quantitative measurement was possible in this experiment. Thus we were unable to experimentally determine the preference between the −80° and −160° glycosyl torsion angle solutions. A recent, comprehensive molecular modeling study of 2′-deoxynucleosides[19] suggests that the −160° solution may be energetically favored for phase angles (P) in the northern region. This study did not include substituents at the 4′-position and would have to be extended to include such cases to be of predictive value.

Traditionally, the position of the exocyclic C5′ hydroxymethyl group is determined via the ^{1}H NMR couplings, $J_{H4'-H5'}$ and $J_{H4'-H5''}$. Obviously, the absence of a 4′-hydrogen in 4′-azidothymidine precluded the use of this method. Alternatively the long-range ^{13}C–^{1}H ^{3}J coupling between C3′ and H5′ or H5″ can be used to determine the torsion angle for the C4′, C5′ bond. In the NMR experiment to determine the vicinal coupling constant $^{3}J_{H1'-C2}$ with a resolution of 0.48 Hz/point, no coupling was observed between C3′ and either hydrogen on C5′. This result indicates that both couplings are below 1 Hz, which is only consistent with an antiperiplanar orientation of O5′ relative to C3′.[20]

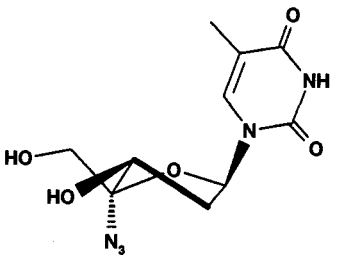

Figure 5. C3′-endo (N-type) conformation of 4′-azidothymidine.

Figure 4 is a depiction of 4′-azidothymidine combining the conformational features derived by the NMR solution studies, aligned with the two solid state conformations. For the NMR conformation, the glycosyl torsion angle (χ) is set to −160°, the phase angle (P) is 53°, and the puckering amplitude (Ψ_m) is 35°. The position of the 5′-hydroxy group has been set in the antiperiplanar orientation as predicted by NMR, with the orientation of the 4′-azido group arbitrarily set in an antiperiplanar orientation also. This is similar to the orientation in the A molecule of the solid state structure. However, we have no NMR evidence to support the azido orientation.

Discussion

4′-Azidothymidine is an extremely potent and selective inhibitor of HIV. During its synthesis, evidence emerged to suggest that its conformation is substantially different from that of other nucleosides. After the 4′-position of thymidine had been substituted with an azido group, the ester protecting the 3′-hydroxyl readily migrated to 5′ when the 5′-carbon was activated toward nucleophiles.[8a] It was hypothesized that such a reaction between transdisposed substituents could only be accommodated if the furanose ring was in an extreme C3′-endo or C4′-exo conformation. This would situate the 3′-ester and the 5′-carbon pseudoequitorially on the ring in a synperiplanar configuration, thus achieving adequate proximity for the acyl migration (Figure 5).

Although such a conformation is rare among antiretroviral nucleosides, we rationalized its occurrence on the basis of the presence of the azido group in the 4′-position. Due to the anomeric effect,[21] an electronegative substituent, such as azido, would prefer a pseudoaxial orientation. The hydroxy group at 3′ would then be forced pseudoequatorially in order to avoid eclipsing the azido group, resulting in a C3′-endo/C4′-exo (northern) conformation of the furanose.

The present study now confirms that 4′-azidothymidine adopts a northern conformation both in the crystalline state and in solution. The two independent molecules in the crystal are in a C3′-endo conformation, while in solution the major contributing conformer is C4′-exo and the minor is C2′-exo. Thus, all structures position the azido group pseudoaxially with the 3′-hydroxyl group and 5′-hy-

droxymethyl group synperiplanar. This then supports the allegations provoked by the ester migration experiment and the theoretical, anomeric argument (*vide supra*).

In contrast to the unusual phase angle of the sugar ring, the other conformational parameters are in agreement with the majority of pyrimidine nucleosides. The thymine ring is in the common anti conformation, and the position of the 5′-hydroxyl group is in the expected + synclinal orientation[13] (determined in the solid state). The antiperiplanar orientation of the 5′-hydroxy group, as determined in solution, is more unusual but would be in agreement with that of many active anti-HIV compounds.[7c] The amplitude of nucleoside sugar puckering generally ranges from about 35° to 45°.[13] 4′-Azidothymidine is at the lower end of this range both when crystalline and when in solution.

The differences between the conformational parameters determined in the solid state and those determined in solution are not at all unexpected. In fact, there are examples where there is a substantial variance between the solid and solution conformations[22] or even where the crystal structure conformation is completely absent in solution.[23] In general, though, the two states usually correlate very well. In the case of 4′-azidothymidine, the differences between the solid and solution structure are relatively minor and are probably a consequence of the strong C3′-endo preference and the crystal packing forces. The C3′-endo conformation increases the distance between the pyrimidine base and the C5′-OH, allowing for an ideal stacking of the pyrimidine rings with concomitant hydrogen bonding of the 5′-OH to O4 of the other molecule in the asymmetric unit. Such an arrangement, resulting in a bimolecular large ring, is not possible in a pyrimidine nucleoside in a southern conformation, and to our knowledge has not been observed previously in the solid state.

Taken *in toto*, both X-ray and solution conformations are representative of a normal 2′-deoxypyrimidine nucleoside with the exception of the solution phase pseudorotational phase angle. Nucleosides generally prefer two narrow ranges of phase angles, those being a northern range of 3°–34° and a southern range of 145°–215°.[13] Most HIV inhibitory, 3′-substituted dideoxynucleosides fall in the southern range of 154°–215°.[7d] Although the phase angle of 4′-azidothymidine in the solid state (P = 14°) is in the common C3′-endo (northern) range, the major solution conformer (P = 53°) is in the C4′-exo (northern) envelope conformation. The C4′-exo envelope conformation, to our knowledge, has no precedent. On the basis of the combined arguments of chemical behavior (3′ → 5′ acyl migration), theoretical considerations (anomeric effect of the 4′-azido), X-ray diffraction, and NMR studies, it appears safe to say that 4′-azidothymidine has a strong preference for a northern conformation.

The significance of conformational preference is difficult to assess. Many researchers have observed that the majority of HIV-inhibitory nucleosides adopt a southern conformation in the crystalline state.[7d,10,24] Solution conformational studies of anti-HIV nucleosides have been infrequent, but often a nucleoside which crystallizes in a southern conformation has been found to favor a northern conformation or a north/south equilibrium in solution.[7f,25,26] Table 5 lists several anti-HIV nucleosides with their hemispherical preference in solution and as determined by X-ray crystallography. 4′-Azidothymidine is the only example found with a northern preference in both phases.

Table 5. Furanose Conformations[a] of HIV-Inhibitory Nucleosides

	method	
nucleoside	X-ray (solid state)	NMR (solution)
AZT	S	S/N
AxddU	S	S/N
FddT	S	S
ddA	S	N
ddC	S	N
ddI	S	N
ddG	S	N
4′-azidothymidine	N	N

[a] S = single conformer or major conformer in the southern region. N = single conformer or major conformer in the northern region. S/N = mixture of south and north conformers observed.

From this data it appears that directly relating activity to furanose conformation should only be done with trepidation. Nevertheless, it is worth noting that in a recent study by Painter et al.[27] it was shown that dTTP and AZTTP, when bound to HIV-1 reverse transcriptase, assume a peculiar furanose phase angle. The angles were 55° for dTTP and 60° for AZTTP. This is virtually identical to the furnose conformation of 4′-azidothymidine in solution. Therefore, the strong preference that 4′-azidothymidine has for a 50–60° phase angle may facilitate the binding of its 5′-triphosphate ester to reverse transcriptase.

It is becoming increasingly obvious that because of the complexity of antiretroviral nucleoside metabolism, activity is dependent in very subtle ways on many structural features. The interplay of these features is also important. For instance, the furanose ring pucker influences the distance between the 5′-hydroxyl group and the base which could affect the binding to a kinase.[7c,24] At the same time, the orientation of 3′ is determined by the pucker which could be important to the mechanism of 3′-elongation of a DNA strand after the nucleoside is incorporated.[8b] Electronegative substituents on the sugar can dictate both the C4–C5′ rotational angle and the furanose pucker via gauche effects.[7c,7g,28] Modifications to the base can change the pK_a's of NH and OH groups which, in turn, affect base pairing and enzyme binding as well as influencing the glycosyl torsion angle. Thus small changes can cause a cascade of effects greatly complicating the derivation of SAR's. 4′-Azidothymidine is a unique example of a potent inhibitor of HIV which runs counter to the previous SAR of nucleosides. It retains a 3′-hydroxyl group, it is substituted at the 4′-position, and it prefers a northern furanose conformation. Why this unusual combination of features is so effective in preventing viral replication remains a subject for speculation and further study.

Experimental Section

NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Bruker AMX500 NMR spectrometer in D_2O solution. Accurate J couplings for the glycosidic resonances and the chemical shifts of the H2′ and the H2″ were simulted using the Bruker microprogram PANIC (a minicomputer version of the LAOCOON type simulation program). A NOE difference experiment in acetone-d_6, where the entire ^{1}H NMR spectrum of 4′-azidothymidine is first order, was used to verify the β and α assignment of H2′ and H2″. Varying the sample temperature from 280 K to 353 K showed only small changes in the J couplings as was also observed in AZT and other similar nucleosides.[7f,29] The ^{1}H–^{13}C vicinal couplings $^3J_{H1'-C2}$ = 2.58 Hz and $^3J_{H1'-C6}$ = 5.28 Hz used to calculate the glycosyl torsion angle were measured in D_2O using a gated ^{13}C experiment with a 0.48 Hz/point resolution. Conformations were estimated based on the couplings constants

using (1) a Fortran version running under VMS of the program DAERM[14] and (2) a PC (DOS) version of the program PSEUROT (QCPE program No. 463).[16b]

Crystal Structure. Colorless, block-like, single crystals of 4'-azidothymidine were obtained after recrystallization from acetonitrile. A crystal of dimensions 0.30 × 0.35 × 0.40 mm was selected to collect a room-temperature (23 °C) X-ray crystallographic data set to a maximum $2\theta = 116°$, using Cu Kα (1.541 78 Å) radiation. The axial photos revealed monoclinic symmetry ($P2_1$) with unit cell dimensions $a = 7.188(2)$, $b = 22.804(7)$, and $c = 8.042(2)$ Å and $\beta = 109.91°$. Two independent copies of $C_{10}H_{13}N_5O_5$ form the asymmetric unit. A total of 1747 unique reflections were collected using θ–2θ scan technique, with 1° ω scans at a variable ω scan speed of 2.00 to 29.30 deg/min, using a Siemens R3M diffractometer. Of these, 1731 (91%) were considered observed [$|F_o| \geq 3.0\sigma(F_o)$]. Data were corrected for Lorentz-polarization effects, but no absorption correction was applied. The structure was solved via *direct methods*, using the SHELXTL PLUS Siemens software library. Full-matrix least-squares techniques were employed for the positional and thermal anisotropic refinements for all non-hydrogen atoms. All atoms were located in the difference Fourier maps and refined with isotropic thermal parameters. The refinements lead to a final discrepancy residual factor $R = 3.49\%$ and a weighted $R_w = 4.67\%$, where $w = 1/(\sigma^2(F)\text{'s} + 0.001(F)^2)$. The final difference Fourier maps were featureless with a largest difference peak of 0.17 e Å^3.

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Supplementary Material Available: Crystal data, data collection and refinement details, bond lengths, bond angles, anisotropic displacement coefficients, and H atom coordinates (8 pages). Ordering information is given on any current masthead page.

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