Solid-State and Solution Conformation of 3'-Amino-3'-deoxythymidine, Precursor to a Noncompetitive Inhibitor of HIV-1 Reverse Transcriptase[1]

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The recent finding that 3'-amino-3'-deoxythymidine 5'-triphosphate is a noncompetitive inhibitor of the HIV-1 reverse transcriptase (Kedar, P. S.; et al. *Biochemistry* **1990**, *29*, 3603-3611), prompted an investigation of the conformation of 3'-amino-3'-deoxythymidine. An X-ray diffraction study has revealed that the glycosidic torsion angle of the nucleoside is in the less common syn region and this solid-state geometry is stabilized by a three-dimensional network of self-associated hydrogen-bonded molecules. On the other hand, the aqueous solution conformation, as determined by ^{1}H NMR, places the glycosidic torsion angle in the more usual anti region with the sugar in an equilibrium between C3'-endo and C2'-endo puckering. The energy barrier between the solid-state and solution conformation is relatively low as was demonstrated by the MM2 calculations.

Introduction

3'-Azido-3'-deoxythymidine (AZT, Zidovudine, Retrovir) has so far remained the only drug officially licensed for the treatment of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. (For a recent review, see ref 3.) AZT is activated by anabolic phosphorylation to its 5'-triphosphate (AZTTP), which inhibits the HIV-1 reverse transcriptase (RT).[4] This inhibition[5-10] is linear competitive with a K_i value of 20 nM against the natural substrate 2'-deoxythymidine 5'-triphosphate (dTTP) in the poly[r(A)]·oligo[d(T)] in vitro replication system.[11] The thymidine analogue, 3'-amino-3'-deoxythymidine 5'-triphosphate (NH_2dTTP), has also been found to be a strong inhibitor of HIV-1 RT with a K_i value of 42 nM, but the inhibition in this case is linear noncompetitive.[11] Unfortunately, the nucleoside 3'-amino-3'-deoxythymidine (NH_2dT) itself does not inhibit HIV replication at subcytotoxic concentrations in MT-4 cells, although it is inhibitory to the replication of Moloney murine sarcoma virus in C3H/3T3 cells (J. Balzarini, E. De Clercq, T. Kovács, and P. Torrence, unpublished observations). The nucleoside NH_2dT has been reported by Lin and Prusoff[12] and Fischer et al.[13] to be strongly cytotoxic to L1210 cells. Chen et al.[14] ascribed this cytotoxicity to an inhibition of DNA polymerase α by the corresponding 5'-triphosphate.

The observation that NH_2dTTP inhibited HIV-1 RT through a completely different mechanism than did AZTTP suggested the possibility of a completely different approach to inhibition of HIV RT as well as HIV itself. On the one hand, as proposed earlier, this inhibition could be the result of interaction of the NH_2dTTP with a completely different site of the RT than the site with which AZTTP interacts. Alternatively, the NH_2dTTP might bind to the normal deoxynucleotide triphosphate binding site, but may exert its effect on some enzyme form that occurs earlier or later than the incorporation step. Possible candidate forms have been presented in a proposed kinetic scheme.[15] Clearly, either of the above two modes of inhibition could be valuable if they could be exploited further since an agent that could inhibit HIV RT with a K_i similar to AZTTP may provide a synergistic combination or may help prevent resistance development. Moreover, since its toxicity spectrum would likely be different, it might be useful when AZT cannot be employed.

In this study, we have asked if there is any feature of the conformation associated with the parent nucleoside NH_2dT that might provide some clue to the potent and unusual mechanism of inhibition of HIV-1 RT by the corresponding triphosphate. In this report we describe the solid-state structure of NH_2dT as determined by X-ray diffraction and the solution conformation in water using proton NMR. The structural information of NH_2dT has been compared with similar data obtained previously on the conformation of AZT.

Nucleoside Geometry. The conformations of nucleosides and their analogues have been extensively reviewed by Sundaralingam,[16] Saenger,[17] and Birnbaum and Shu-

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Table I. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U(eq)[a]
O(2)	8459 (1)	9884 (3)	6530 (3)	46 (1)
O(4)	10271 (1)	13742 (3)	7857 (3)	44 (1)
O(4′)	9124 (1)	7180 (3)	4915 (2)	36 (1)
O(5′)	8422 (1)	5685 (3)	1238 (2)	41 (1)
N(1)	9707 (1)	9086 (3)	7278 (3)	32 (1)
N(3)	9390 (1)	11776 (3)	7285 (3)	31 (1)
N(3′)	8169 (1)	4272 (3)	6868 (3)	34 (1)
C(2)	9131 (1)	10214 (4)	6993 (3)	30 (1)
C(4)	10137 (1)	12287 (4)	7691 (3)	29 (1)
C(5)	10686 (1)	11074 (4)	7872 (3)	29 (1)
C(6)	10458 (1)	9549 (3)	7676 (3)	29 (1)
C(7)	11509 (1)	11517 (4)	8255 (5)	45 (1)
C(1′)	9518 (1)	7389 (3)	6922 (3)	29 (1)
C(2′)	9018 (1)	6654 (4)	7958 (3)	33 (1)
C(3′)	8360 (1)	5868 (4)	6319 (3)	26 (1)
C(4′)	8676 (1)	5763 (4)	4697 (3)	28 (1)
C(5′)	8091 (1)	5721 (4)	2682 (3)	35 (1)
H(3′A)	7658 (1)	4034 (4)	5774 (3)	50 (1)
H(3′B)	8129 (1)	4370 (4)	8117 (3)	50 (1)

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

Table II. Bond Lengths (Å)

O(2)-C(2)	1.213 (3)	O(4)-C(4)	1.243 (4)
O(4′)-C(1′)	1.416 (3)	O(4′)-C(4′)	1.431 (4)
O(5′)-C(5′)	1.423 (4)	N(1)-C(2)	1.392 (4)
N(1)-C(6)	1.385 (3)	N(1)-C(1′)	1.467 (4)
N(3)-C(2)	1.386 (4)	N(3)-C(4)	1.390 (3)
N(3′)-C(3′)	1.481 (4)	C(4)-C(5)	1.419 (4)
C(5)-C(6)	1.340 (4)	C(5)-C(7)	1.510 (4)
C(1′)-C(2′)	1.541 (4)	C(2′)-C(3′)	1.533 (3)
C(3′)-C(4′)	1.527 (4)	C(4′)-C(5′)	1.501 (3)

gar.[18] The three essential parameters are the geometry of the glycosyl bond, the rotation about the exocyclic C4′-C5′ bond, and the puckering of the sugar ring. The torsion angles χ(C2-N1-C1′-O4′) and γ(C3′-C4′-C5′-O5′) describe the orientations of the base and the 5′-hydroxyl group relative to the sugar ring. The puckering of the five-membered sugar ring is a continuous displacement of one (envelope) or two atoms (twist) out of the plane of the others. This displacement can be described by the degree of pucker ψ_m and the pseudorotational phase angle P, which is calculated from the endocyclic sugar torsion angles according to ref 19.

Crystal Structure. 3′-Amino-3′-deoxythymidine: $C_{10}H_{15}N_3O_4$, FW = 241.3. Crystal data: monoclinic, space group $C2$, a = 18.750 (5), b = 8.388 (2), and c = 7.435 (2) Å, β = 111.29 (2)°, V = 1089.6 (4) Å^3, ρ_c = 1.471 Mg/m^3, Z = 4, F(000) = 512, μ(Mo K$_\alpha$, λ = 0.710 73 Å) = 0.108 mm^{-1}.

The molecular conformation and the numbering are shown in Figure 1. Final atomic coordinates for the heavy atoms and the hydrogen atoms of the NH_2 group are listed in Table I. Their anisotropic temperature parameters and lists of hydrogen atom parameters and of observed and calculated structure factors (10 pages) are available on request.[20] The corresponding bonds lengths and bond angles are listed in Tables II and III, respectively. Designations used in describing the nucleoside torsion angles

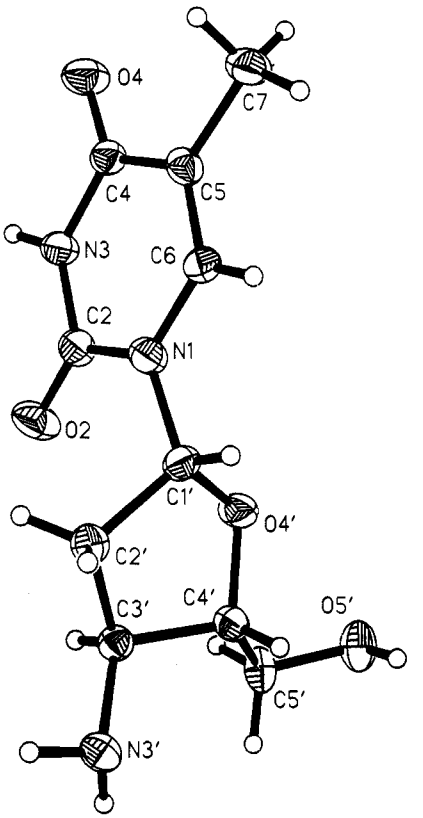

Figure 1. Molecular conformation and the numbering of NH_2dT. Thermal ellipsoids are drawn at the 50% probability level.

Table III. Bond Angles (deg)

C(1′)-O(4′)-C(4′)	106.4 (2)	C(2)-N(1)-C(6)	120.9 (3)
C(2)-N(1)-C(1′)	120.6 (2)	C(6)-N(1)-C(1′)	118.1 (2)
C(2)-N(3)-C(4)	126.4 (2)	O(2)-C(2)-N(1)	123.8 (3)
O(2)-C(2)-N(3)	121.9 (2)	N(1)-C(2)-N(3)	114.3 (2)
O(4)-C(4)-N(3)	118.4 (2)	O(4)-C(4)-C(5)	125.6 (2)
N(3)-C(4)-C(5)	116.1 (3)	C(4)-C(5)-C(6)	118.8 (2)
C(4)-C(5)-C(7)	119.9 (3)	C(6)-C(5)-C(7)	121.3 (3)
N(1)-C(6)-C(5)	123.4 (3)	O(4′)-C(1′)-N(1)	108.1 (2)
O(4′)-C(1′)-C(2′)	107.3 (2)	N(1)-C(1′)-C(2′)	116.7 (2)
C(1′)-C(2′)-C(3′)	103.6 (2)	N(3′)-C(3′)-C(2′)	112.0 (2)
N(3′)-C(3′)-C(4′)	111.5 (2)	C(2′)-C(3′)-C(4′)	102.8 (2)
O(4′)-C(4′)-C(3′)	104.0 (2)	O(4′)-C(4′)-C(5′)	108.6 (2)
C(3′)-C(4′)-C(5′)	115.9 (2)	O(5′)-C(5′)-C(4′)	113.1 (2)

are consistent with the rules of the IUPAC-IUB Commission on Biochemical Nomenclature.[21]

The glycosidic torsion angle was in the syn region with χ(C2-N1-C1′-O4′) = 67.5 (4)°. The sugar pucker was ^0_4T(O4′-endo/C4′-exo) with the pseudorotation phase angle P = 84.2° and the degree of pucker ψ_m = 40.2°. The side-chain conformation γ(O6′-C5′-C4′-C3′) was 177.7 (4)°, in the anti range. No lengthening of the N1-C1′ bond (1.467 (4) Å) was observed in NH_2dT.

Hydrogen Bonding. The solid-state structure of NH_2dT is a complex three-dimensional network held together by hydrogen bonding. A single molecule of the compound contains both donor (D) and acceptor (A) H-bonding sites that may be divided into three groups: (a) the two carbonyl groups of the thymine, C4-O4 and C2-O2, and the sugar oxygen O4′ are acceptors; (b) the amino group and the C6-H of the thymine act as H donors, and (c) the amino group and the alcohol group O5′-H are both donors and acceptors. Despite the range of potential H-bonding interactions, the actual structure is defined by

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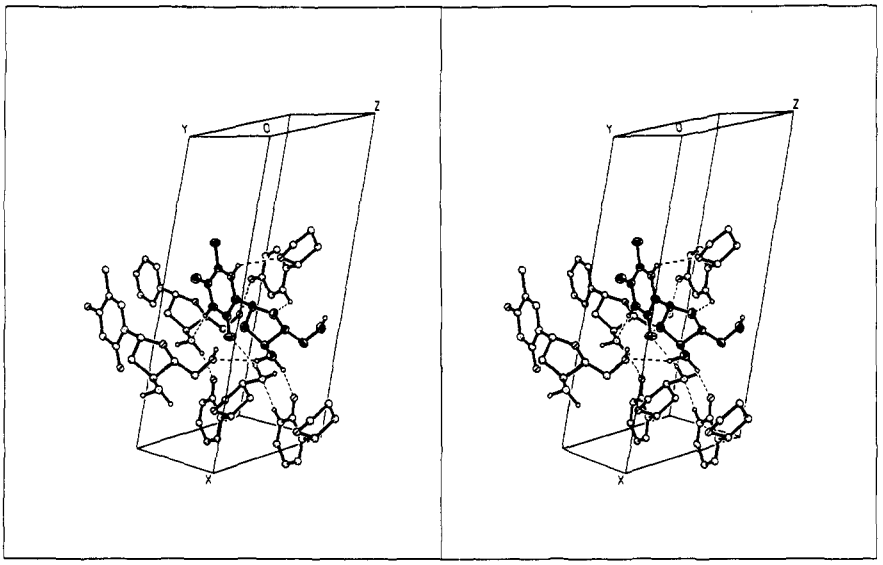

Figure 2. Stereoview showing the intramolecular hydrogen bonds.

Table IV. Hydrogen-Bond Lengths and Angles

	atoms D-H···A	distance (Å) D-A	distance (Å) H-A	angles (deg) D-A
I	N3'-H···O2	3.219 (5)	2.285 (5)	150 (0.5)
II	O5'-H···O4	2.814 (5)	1.936 (5)	161 (0.5)
III	C6-H···O4'	3.061 (5)	2.471 (5)	120 (0.5)
IV	N3-H···N3'	3.033 (5)	2.095 (5)	168 (0.5)
V	N3'-H···O5'	3.326 (5)	2.445 (5)	152 (0.5)

Symmetry Codes of Acceptor A
I: $(1 - x) + {}^1/_2, y - {}^1/_2, 1 - z$
II: $2 - x, y - 1, 1 - z$
III: $2 - x, y, 1 - z$
IV: $x, y + 1, z$
V: $x, y, 1 + z$

a very limited combination of the possibilities, and only five individual intermolecular H-bonds types are observed: four relatively strong (N3'-H···O2, O5'-H···O4, C6-H···O4', N3-H···N3') and a fifth relatively weak but important H-bond (N3-H···O5'). No intramolecular H-bonds are observed. The C6-H···O4' contact may be described as a hydrogen bond according to the interpretation of Taylor and Kennard.[22] A related non-classical H-bond, involving a pyrimidine C6-H to O5' of the 2'-deoxyribose moiety of a nucleoside, has been described recently.[23] The various bond lengths and angles involved in the H-bonds are presented in Table IV. Only H atoms involved in H-bonding are shown for the sake of clarity. A stereoview of the overall structure is presented in its thermal ellipsoid form in Figure 2. (A figure showing two major aspects of the H-bond network is available as supplementary material).

MM2 Calculations.[24] Molecular mechanics calculations were performed by using the standard MM2 force field. Contributions of the planar π-systems were also included in the energy. Calculations involved a VESCF[25] method for the planar π-electron system. Generation of the σ point charges were based on the concept of equalization of electronegativities.[26] A refinement starting from the X-ray coordinates resulted in the following structural parameters:

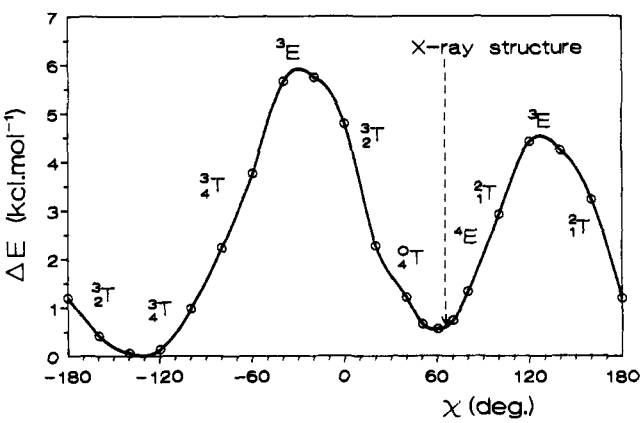

Figure 3. Energy function as a function of glycosidic torsion angle as calculated by MM2 force field. (See text for details.)

$$\chi = 59.7° \quad P = 63.4 \quad \psi_m = 41.4° \ (^0_4T)$$

$$\text{N-C1'} = 1.47 \text{ Å} \quad \text{C1'-O4'} = 1.42 \text{ Å} \quad \gamma = -175.5°$$

The greatest change was approximately 10° in χ. The N-C1' bond varied by 0.01 Å during the calculations. The C1'-O4' bond was assumed to be the most sensitive to the conformational changes of the sugar ring. The rotation of a rigid sugar ring about the glycosidic bond (χ) resulted a very high barrier at the χ = -60–0° region. But a calculation in which the glycosidic torsion angle was frozen at 20° increments and the entire rest of the molecule was allowed to refine gave the energy function shown in Figure 3. The X-ray structure was near a local energy minimum, but the lowest energy obtained was in the anti range (-120–140°).

NMR Data of NH_2dT. Chemical shifts and coupling constants (Table V) were determined by first-order evaluation of the slightly resolution enhanced proton spectrum recorded in D_2O. Coupling constants for the sugar ring were further refined by spectrum simulation using NMR1 data processing software (New Methods Research, Inc., Syracuse, NY).

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Table V. Chemical Shifts (ppm) and Coupling Constants (Hz) for NH_2dT[a]

	coupling constants (J)								chemical shifts (δ/H_2O = 4.78)
	H-6	CH_3-5	H-5′/5″		H-4′	H-3′	H-2″	H-2′	H-1′	
H-1′	-	-	-	-	-	-	+7.26	+4.51	-	6.23
H-2′	-	-	-	-	-	+7.79	-14.09	-	-	2.41
H-2″	-	-	-	-	-	+7.43	-	-	-	2.29
H-3′	-	-	-	-	+7.09	-	-	-	-	3.57
H-4′	-	-	+2.94	+4.82	-	-	-	-	-	3.84
H-5′/5″	-	-	-12.65	-	-	-	-	-	-	3.78
H-5″/5′	-	-	-	-	-	-	-	-	-	3.90
CH_3-5	-/+1.7	-	-	-	-	-	-	-	-	1.89
H-6	-	-	-	-	-	-	-	-	-	7.67

[a] Coupling constant values are results of spectrum simulation, except that for H-6 and CH_3-5. Coupling constant values assumed to be zero were not iterated.

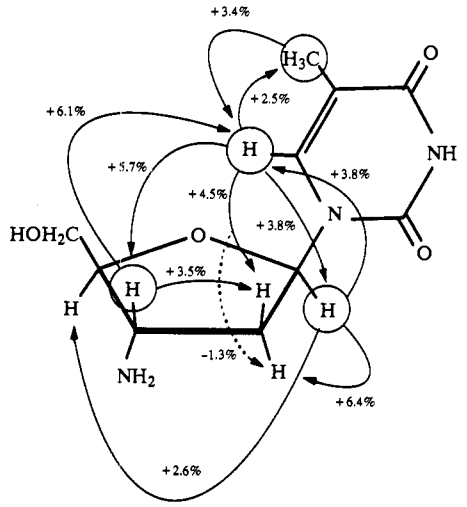

Figure 4. NOE enhancements.

The chemical shifts were in accordance with published data for similar structures.[27,28] Introduction of the polar and electron-rich NH_2 group at the 3′-position caused characteristic changes in chemical shifts and in some coupling constants as well, in comparison with other pyrimidine deoxyribonucleosides.[28] The effect of the amino group was expressed very well in the pH dependence of the chemical shifts primarily of the protons H-3′ and H-4′. (A figure showing chemical shifts as a function of pH is available as supplementary material.)

Results of the nuclear Overhauser effect difference spectroscopy (NOEDS) experiments are summarized in Figure 4. Resonances belonging to the circled spins were irradiated and the observed relative enhancements are noted. Slightly negative enhancement on the H-2″ while irradiating H-6 was due to the indirect NOE.[29]

The NOE data are very informative mainly from the point of view of the solution conformation around the glycosidic bond. In contrast to the results for the solid state, the solution conformation is definitely in the anti region, since the H-6 shows a strong NOE interaction to each of H-3′ and H-2′ as well as a moderate one to H-1′, respectively. Relatively strong NOE interaction between H-1′ and H-4′ (2.8%) as well as no observable NOE effect from H-1′ to H-2′ support the excess of *N*-type conformations with high population of ^3E(C3′-endo) sugar ring conformation. The partial contribution of some O4′-endo pseudorotamer also may be present.

According to the coupling constants H-4′/H-5′/5″ (2.94 and 4.82 Hz, respectively), the preferred conformation about C4′–C5′ bond is g^+ (about 60%).[27] Similarly, the coupling constants for H-1′/H-2′ (4.51 Hz) and H-1′/H-2″ (7.41 Hz) support the description of this structure in solution as a roughly 60:40 equilibrium of ^3E(C3′-endo): ^2E(C2′-endo) conformations.[28]

Discussion

Owing to the great biomedical importance of 3′-azido-3′-deoxythymidine (AZT), its solid-state structure has been investigated by a large number of groups.[30-35] AZT contains two independent molecules (A and B) in the asymmetric unit, and the following conformational parameters have been reported (molecule A values are given first): χ = -125.9 (5), -172.0 (5)° (anti); sugar puckers ^3_2T(C2′-endo/C3′-exo), P = 171°, ψ_m = 14° and ^3_4T(C4′-endo/C3′-exo), P = 213 (1), ψ_m = 11 (1)°; γ = 49.7 (5)° (+sc), 173.7(5)° (ap).

A study[36] of low-energy conformations of AZT by molecular mechanics techniques showed that it had a global energy minimum in the C3′-endo state with a rather low pseudorotational barrier, suggesting that in solution AZT would exist in both C3′-endo and C2′-endo state. Subsequent NMR investigation[27], revealed that in DMSO solution, AZT indeed had the sugar ring in equilibrium between C2′-endo and C3′-endo geometries, whereas γ was like that for the AZT molecule A in the solid state and χ was similar to that for AZT B geometry in the solid state. NMR results thus showed that the molecular geometry of AZT in solution was quite different from that obtained by X-ray studies and did not differ much from those geometries derived for several nucleosides by NMR.[17]

The anti-HIV activity of AZT was related to the conformational properties and preferred nucleoside conformers which would be expected to have relevance to the active, enzyme-bound phosphorylated structure and thus to the design of other reverse transcriptase inhibitors. On the basis of the rare occurrence of the higher potential energy associated conformational parameters of molecule

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B (extreme anti range of χ, ${}^3_4T$ sugar pucker), the conformation of molecule B of AZT in solid state was suggested to be the biologically active form.[31] An analysis of the solid-state conformations of 138 uridine analogues retrieved from the Cambridge Crystallographic Database[37] was used to correlate the preferred sugar ring conformation with activity of nucleoside analogues against HIV.[35,38] Futhermore, from a separate study of the crystal structure of the inactive 2',3'-dideoxyformycin A, it was concluded that the anti glycosidic conformation may be essential for anti-HIV activity.[39] Finally, very recently it has been demonstrated that 3'-fluoro-2',3'-dideoxy-5-chlorouridine is a potent and selective inhibitor of HIV-1 and HIV-2 replication, but its X-ray structure did not show a close conformational resemblance to AZT.[23]

The present X-ray diffraction study of 3'-amino-3'-deoxythymidine has revealed that the glycosidic torsion angle is in the less common syn region[17] and the sugar pucker is in the less-favored O4'-endo/C4'-exo conformations. Considered in terms of the pseudorotation concept,[19] this pucker lies between the commonly occurring C2'-endo and C3'-endo puckers.[16,17,40,41] According to the best of our knowledge, only two examples have been reported so far in the literature with this unusual sugar pucker: deoxyguanosine 5'-phosphate[42] and 2'-fluoro-2'-deoxyuridine[43,44] as determined by X-ray analysis. Some additional solid-state conformations having very similar O4'-endo sugar puckers also have been described.[45-50] Less-favored conformations, like those observed for 3'-amino-3'-deoxythymidine in the solid state, are considered to result from an equilibrium between intramolecular and intermolecular forces.[18,40,41,51] In the case of NH_2dT, the unusual conformations can be explained by the three-dimensional network of hydrogen-bonded molecules. This explanation is supported by the solution conformation of the nucleoside in which case the preferred conformations are very similar to those of standard 2'-deoxypyrimidines.[17] The energy barrier between the solid-state and the solution conformations is relatively low as was demonstrated by the MM2 calculations.

Additional evidence that the unusual conformation of the 3'-amino-3'-deoxythymidine is due to the intense hydrogen bonding through the free 3'-amino group derives from the earlier crystal structure determination of the hydrochloride salt of NH_2dT.[52] The conformational parameters given below indicate that the solid-state structure of the salt is close to the solution conformation of the free nucleoside itself: $\chi = -167.4°$ (anti), $P = 3.9°$, $\psi_m = 36.6°$; sugar pucker ${}^3T$(C3'-endo); and $\gamma = 57.0°$ (+sc).

It remains to be determined if the potent but unusual mode of inhibition of the HIV-1 RT by the 5'-triphosphate of NH_2dT may be in any manner related to the facile and intensified hydrogen-bonding capacity of this amino-nucleoside. The biological activity of a given nucleoside analogue is frequently determined by, among other factors, not only its conformation but also the changes of its conformation accompanying interaction with an enzyme. Crystallization of the HIV-1 RT has been achieved and studies are underway to determine its three-dimensional structure.[53,54] Studies of conformational changes resulting from interactions between the enzyme and its substrate and/or inhibitors such as NH_2dT may permit the rational design of inhibitors for HIV reverse transcriptase.

Experimental Section

The NH_2dT was synthesized from AZT by the method described in the literature.[12,55]

Crystal Structure Determination. Crystals were grown from a mixture of ethanol–acetone =1:1 (v/v). A colorless needle, measuring 0.15 × 0.18 × 0.33 mm, was cut off and mounted on a Nicolet R3m/V diffractometer equipped with a highly oriented graphite crystal monochromator. Unit cell parameters were obtained by least-square fit of 30 reflections in the $11° < 2\theta < 23°$ range. Data collection was performed at room temperature, using monochromated Mo K_α radiation in the 2θ range 3.5–45.0° using variable scan speed (1.5–20.0°/min.). Three check reflections were recorded after every 75 reflections and these showed no crystal decay. A total number of 2034 independent reflections were measured. The data were corrected for Lorentz and polarization effects. Because of the low absorption coefficient of the crystal, no absorption correction was applied. The structure was solved by using direct methods employing the SHELXTL PLUS software package on a Micro-VAX II computer and refined by full-matrix least squares. Hydrogen atomic positions were generated from assumed geometries except those of O5' and the amino group, which were located in a difference map. Contributions of the hydrogen atoms were included in structure factor calculations (riding model) but they were not refined. Isotropic temperature factors of the hydrogen atoms bound to the same atom were constrained to be equal. The final R indices are $R = 0.035$ ($R_w = 0.052$, weighting scheme $w^{-1} = \sigma^2(F) + 0.0046F^2$) for 1968 observed ($F > 3\sigma(F)$) and $R = 0.036$ for all data. Goodness-of-fit $S = 0.72$. The total number of variables refined was 153. The final cycle of refinement showed a largest shift/esd of 0.037 and a mean shift/esd of −0.001. The largest peak on the difference map was 0.79 e Å^{-3}.

NMR Measurement. ^{1}H NMR spectra of the 3'-amino-3'-deoxythymidine were recorded at 500.1 MHz (GN-500) in both D_2O (simple 1D and NOEDS acquisitions) and H_2O (pH dependence of the chemical shifts) solutions at 20 mM (D_2O) and

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10 mM (H_2O) concentrations, respectively, and 298 K temperature. For the D_2O sample the pH was not regulated; however, in H_2O the pH of the original solution was measured to be 8.5. For all the experiments the water peak was used as internal reference ($\delta = 4.78$ ppm). For the D_2O solution no saturation of the residual HDO peak was necessary, while in H_2O the very intense solvent peak was eliminated from the spectrum by using the jump-return-echo sequence.[56] The pH was adjusted by using small amounts of concentrated HCl and NaOH solutions, respectively. Selective irradiation NOE difference spectra were recorded by using low power CW preirradiation of ca. 40 Hz strength for 5 s. Approximate measurement of T_1 have shown values between 0.8 and 2.5 s. An additional relaxation delay was introduced extra to the preirradiation time to avoid saturation resulting in 10-s overall recycling time. Assignments of resonances were straightforward in most cases, except for the protons 2',2'' and 5',5''. For the former pair unique assignments were possible according to the NOE effects, while the 5',5'' signals are still not specifically assigned.

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Supplementary Material Available: Figures of the two major aspects of the H-bond network in NH_2dT and chemical shifts as a function of pH NH_2dT and tables of anisotropic and isotropic displacement coefficients (5 pages); observed and calculated structure factors for 3'-amino-3'-deoxythymidine (8 pages). Ordering information is given on any current masthead page.

Targeting 5'-Deoxy-5'-(methylthio)adenosine Phosphorylase by 5'-Haloalkyl Analogues of 5'-Deoxy-5'-(methylthio)adenosine

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A series of 5'-haloalkyl-modified analogues of 5'-deoxy-5'-(methylthio)adenosine (MTA), a nucleoside byproduct of polyamine biosynthesis, has been synthesized: 5'-deoxy-5'-[(2-monofluoroethyl)thio]adenosine (**10**), 5'-deoxy-5'-[(2-chloroethyl)thio]adenosine (**4**), 5'-deoxy-5'-[(2-bromoethyl)thio]adenosine (**5**), and 5'-deoxy-5'-[(3-monofluoropropyl)thio]adenosine (**13**). On the basis of their abilities to serve as substrates of MTA phosphorylase prepared from mouse liver, several of these analogues were characterized for their growth inhibitory effects in MTA phosphorylase-containing (murine L5178Y and human MOLT-4) and MTA phosphorylase-deficient (murine L1210 and human CCRF-CEM) leukemia cell lines. The MTA phosphorylase-containing tumor cell lines, especially of human origin, were found to be more sensitive to treatment by these analogues. Of the analogue series, **10** was the most potent inhibitor of growth in each of the cell lines tested. The analogues, especially compound **10**, displayed a reduced capacity to alter polyamine pools relative to MTA, mechanistically indicating a decreased potential for interactions at sites other than MTA phosphorylase. The results indicate that of the analogues tested, compound **10** displayed the best inhibitor/substrate interaction with MTA phosphorylase, which, in turn, correlated with more potent growth inhibition in tumor cell lines containing MTA phosphorylase. Overall, this supports the concept that MTA phosphorylase plays a role in the activation of such analogues.

5'-Deoxy-5'-(methylthio)adenosine (MTA) is a nucleoside metabolite of *S*-adenosylmethionine (AdoMet) produced stoichiometrically during biosynthesis of the polyamines spermidine (SPD) and spermine (SPM). MTA, itself, has well-documented growth inhibitory activity.[1] Although it serves as a substrate of MTA phosphorylase and is known to interact with several other cellular enzyme targets, including SPD synthase, SPM synthase, *S*-adenosylhomocysteine hydrolase, cyclic AMP phosphodiesterase, and adenosine kinase, the exact mechanism of its growth inhibition remains obscure. Numerous analogues of MTA have been synthesized and their chemotherapeutic potential extensively explored.[2,3] Some impetus has come from the observations that certain tumor cell lines[4] and clinically obtained leukemia and solid tumor samples[5,6] have been found to be devoid of MTA phosphorylase activity. However, strategies which successfully exploit this tumor-specific enzyme deficiency have proved elusive.

A critical component of the cellular biochemistry of MTA is MTA phosphorylase, the enzyme which catalyzes the degradation of MTA to adenine and 5-(methylthio)-ribose 1-phosphate (MTRP). Adenine is then recycled via purine salvage pathways, and MTRP is converted via a multistep pathway to methionine. The degradation of MTA by MTA phosphorylase maintains extremely low cellular concentrations of MTA and thereby protects the cell from the growth inhibitory effects of MTA.

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