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STRUCTURAL STUDIES OF THE ANTI-HIV AGENT 2',3'-DIDEHYDRO-2',3'-DIDEOXYTHYMIDINE (D4T)

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An x-ray crystallographic analysis of the potent anti-HIV agent D4T revealed two independent conformations (confomers a and b) with different glycosyl bonds and furanose geometries. Conformer a exhibits the unusual O4' exo configuration and χ (C2, N1, C1', O4') of -118 degrees. Conformer b exhibits a nearly planar furanose geometry and χ of -174 degrees. The reduced form of D4T, ddT, is poorly active against HIV and also exists in two independent conformations. χ of forms a and b (-129 and -170.9 degrees) are similar to that found with D4T. However, the furanoses exhibit the classical C2' endo and C3' endo geometries, respectively. These observed differences are not sufficient to account for the differing potencies of D4T versus ddT.

A number of structural studies have been performed on dideoxynucleoside analogues active against human immunodeficiency virus (HIV) in an attempt to gain insights into molecular features responsible for activity. Molecules evaluated by x-ray crystallography include 3'-azido-3'-deoxythymidine (AZT),[1] 2',3'-dideoxycytidine (ddC),[2] and 2',3'-didehydro-2',3'-dideoxycytidine (D4C).[3] The mechanism of action of these substances is complex and involves at least three enzymatic phosphorylations to a 5'-triphosphate. The 5' triphosphate exerts an antiviral effect by inhibition of the viral enzyme, reverse transcriptase (RT).[4] For selectivity, the triphosphate species should have a low affinity for host DNA-polymerases. Although some unusual structural features have been noted in the previous studies, no key determinants of activity have been proven. This is not surprising since activity depends upon activation by several enzymes about which only limited data is available.

In addition to AZT, another thymidine analogue, 2',3'-didehydro-2',3'-dideoxythymidine (D4T), has been selected for its unique biological properties[5] as a candidate for clinical development. D4T has apparent biochemical[6] and toxicity[7] advantages over AZT. A third thymidine analogue, 3'-deoxythymidine (ddT) is very similar in structure to D4T yet lacks exhibits greatly decreased activity against HIV in most systems.[8] Consequently, we initiated structural studies on D4T to determine if a basis for these advantages might be elucidated. We have also compared the structural features of D4T to those of ddT to determine if these features could account for the different biological properties.

EXPERIMENTAL SECTION

(a) General Procedures. Crystals of ddT and D4T were obtained by slow diffusion techniques. Both solid state structure determinations were performed under identical conditions unless specified. X-ray

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diffraction intensities were measured on an Enraf-Nonius CAD4 computer controlled kappa axis diffractometer using Ni-filtered Cu-Kα radiation (λ = 1.54184 Å). The data were collected at a temperature of 23(1)° using the ω–θ scan technique. Data were collected to a maximum 2θ of 120°. As a check on crystal and electronic stability 3 representative reflections were measured every 40 min. The data with $F>2\sigma$ were considered observed, where $\sigma^2(F) = (k/Lpl)[\sigma^2(I)+ (0.01I)^2]$. Lorentz and polarization corrections were applied to the data. The structures were determined by direct methods and refined in full-matrix least-squares where the function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = 1/\sigma^2(F)$ for the observed data and w = 0 for the unobserved data. Calculations were performed using SDP-PLUS. Pertinent crystallographic data is given in Table 1.

(b) D4T ($C_{10}H_{12}N_2O_4$). The sample was crystallized by toluene diffusion into a methanol solution of D4T. The crystals are monoclinic, belonging to space group $P2_1$. The cell constants are: a = 11.662 (1)Å, b = 5.422 (1)Å, c = 16.233 (3)Å, β= 92.64 (1)°, V = 1025.4 (5)Å^3, Z = 4, D_{calc} = 1.45 g/cc, μ = 9.2 cm^{-1}. 2770 unique reflections were used in the refinements. Final residuals were R = 0.036, R_w = 0.043. Highest peak in final difference map = 0.22 e/Å^3. All hydrogens were located in difference maps and refined isotropically.

(c) ddT ($C_{10}H_{14}N_2O_4$). The sample was crystallized by toluene diffusion into a methanol solution of ddT. The crystals are monoclinic, belonging to space group $P2_1$. The cell constants are: a = 11.040 (3)Å, b = 6.008 (5)Å, c = 15.892 (11)Å, β= 91.61 (5)°, V = 1054 (2)Å^3, Z = 4, D_{calc} = 1.42 g/cc, μ= 8.9 cm^{-1}. 1598 reflections were used in the refinements. Final residuals were R = 0.046. R_w = 0.045. Highest peak in final difference map = 0.27 e/Å^3. The 5'-CH_2OH group of molecule a exhibits disorder. The disorder is two-fold with model 1 at 67% and model 2 at 33% occupancy. All hydrogens were located in difference maps and refined isotropically.

RESULTS AND DISCUSSION

SOLID-STATE CONFORMATIONAL PROPERTIES

The atomic coordinates and thermal parameters are listed in Table 2. Each crystal contains two independent molecules that are rotational conformers with respect to the N1-C1' bond. The observed C4'A- C5'A(2) bond of ddT is artificially long (1.7Å). This apparent bond elongation is due to the extension of disorder from the 5'-hydroxymethyl group to C4'A which results in two disordered C4' positions that are too close together to be resolved. The other bond lengths and angles fall within expected values. Figure 1 is a summary of the bond lengths and angles for the observed conformations of each molecule.

The ribose moiety of both ddT and D4T each exist in two geometries (a and b). One geometry of ddT has C2' of the ribose as endo, ddT(a), and has a χ angle of -129°. The other ddT geometry has C3'

TABLE 1. Crystallographic Data

	D4T	ddT
space group	$P2_1$	$P2_1$
a	11.662 (1)Å	11.030 (3)Å
b	5.422 (1)Å	6.008 (5)Å
c	16.233 (3)Å	15.892 (11)Å
β	92.64 (1)Å	91.61 (5)Å
z	4	4
V	1025 (5)Å^3	1054 (2)Å^3
D_{calc}	1.45g/cc	1.42g/cc
R	0.036	0.046
R_w	0.043	0.045
highest peak in final difference map	0.22e/Å^3	0.27e/Å^3

TABLE 2. Positional Parameters and Their Standard Deviations

ATOM	x	y	z	B_{eq}/B
		D4T		
N1a	0.0408(2)	0.000	0.7095(1)	2.60(4)
C2a	0.1215(2)	0.1806(4)	0.7272(1)	2.56(5)
O2a	0.1536(1)	0.3237(3)	0.6752(1)	3.50(4)
N3a	0.1646(2)	0.1819(4)	0.8073(1)	2.79(4)
C4a	0.1356(2)	0.0255(5)	0.8708(1)	2.76(5)
O4a	0.1805(2)	0.0520(4)	0.9394(1)	3.88(4)
C5a	0.0526(2)	-0.1636(5)	0.8473(1)	2.61(5)
C6a	0.0101(2)	-0.1691(5)	0.7693(1)	2.72(5)
C7a	0.0204(2)	-0.3466(6)	0.9114(2)	3.85(6)
C1'a	-0.0145(2)	-0.0136(5)	0.6259(1)	2.72(5)
C2'a	-0.0029(2)	-0.2633(5)	0.5870(1)	3.07(5)
C3'a	-0.1056(2)	-0.3485(5)	0.5657(1)	3.29(5)
C4'a	-0.1957(2)	-0.1684(5)	0.5860(2)	3.29(5)
O4'a	-0.1340(1)	-0.0235(3)	0.6311(1)	3.33(4)
C5'a	-0.2920(2)	-0.2689(7)	0.6348(2)	5.00(8)
O5'a	-0.2551(2)	-0.3659(5)	0.7134(1)	5.90(5)
N1b	0.4302(2)	0.9259(4)	0.7931(1)	2.47(4)
C2b	0.3531(2)	0.7384(4)	0.7782(2)	2.60(5)
O2b	0.3220(1)	0.6000(3)	0.8326(1)	3.37(4)
N3b	0.3128(2)	0.7164(4)	0.6977(1)	2.97(4)
C4b	0.3408(2)	0.8670(5)	0.6319(2)	2.92(5)
O4b	0.2968(2)	0.8248(4)	0.5630(1)	4.32(4)
C5b	0.4205(2)	1.0602(5)	0.6520(2)	2.90(5)
C6b	0.4611(2)	1.0826(4)	0.7304(2)	2.68(5)
C7b	0.4548(2)	1.2333(6)	0.5857(2)	4.24(6)
C1'b	0.4717(2)	0.9648(5)	0.8811(1)	2.61(5)
C2'b	0.5480(2)	0.7604(5)	0.9129(2)	3.07(5)
C3'b	0.6489(2)	0.8488(5)	0.9351(2)	3.26(5)
C4'b	0.6531(2)	1.1204(5)	0.9220(1)	2.75(5)
O4'b	0.5400(1)	1.1771(3)	0.8862(1)	2.85(3)
C5'b	0.7460(2)	1.2140(6)	0.8683(2)	3.98(6)
O5'b	0.7400(2)	1.091(5)	0.7918(1)	5.48(5)
		ddT		
N1a	0.0205(3)	0.000	0.7189(2)	2.89(8)
C2a	0.0998(4)	0.1726(9)	0.7337(3)	2.8(1)
O2a	0.1183(3)	0.3218(7)	0.6840(2)	4.09(8)
N3a	0.1586(3)	0.1640(9)	0.8113(2)	3.13(8)
C4a	0.1440(4)	0.008(1)	0.8739(3)	2.9(1)
O4a	0.1999(3)	0.0243(8)	0.9416(2)	4.14(8)
C5a	0.0601(40	-0.172(1)	0.8524(3)	2.9(1)
C6a	0.0035(4)	-0.166(1)	0.7769(3)	3.1(1)
C7a	0.0390(5)	-0.350(1)	0.9161(3)	4.0(1)
C1'a	-0.0519(4)	0.002(1)	0.6392(3)	3.2(1)
C2'a	-0.0244(4)	0.186(1)	0.5799(3)	3.3(1)
C3'a	-0.1467(4)	-0.206(1)	0.5298(3)	3.6(1)
C4'a	-0.2392(4)	-0.164(1)	0.5973(3)	3.4(1)
O4'a	-0.1765(3)	-0.0307(8)	0.662(1)	4.12(8)
C5'a(1)	-0.2967(7)	-0.342(2)	0.6365(5)	3.9(2)*
C5'a(2)	-0.270(1)	-0.434(3)	0.6292(9)	3.0(3)*
05'a(1)	-0.2033(5)	-0.478(1)	0.6783(3)	4.5(1)*
05'a(2)	-0.351(1)	-0.418(3)	0.6942(8)	5.5(3)*
N1b	0.4274(3)	0.8443(8)	0.8008(2)	2.69(8)
C2b	0.3462(4)	0.677(1)	0.7817(3)	2.8(1)
02b	0.3180(3)	0.5364(7)	0.8344(2)	3.62(7)
N3b	0.3001(3)	0.6775(9)	0.7011(2)	3.31(9)
C4b	0.3291(4)	0.822(1)	0.6365(3)	3.3(1)
04b	0.2859(3)	0.7948(8)	0.5661(2)	4.71(9)
C5b	0.4110(4)	1.001(1)	0.6628(3)	3.1(1)
C6b	0.4552(4)	1.0032(9)	0.7415(3)	2.8(1)
C7b	0.4419(5)	1.173(1)	0.5990(4)	4.8(1)
C1'b	0.4801(4)	0.845(1)	0.8887(3)	2.9(1)
C2'b	0.5687(4)	0.654(1)	0.9036(3)	3.3(1)
C3'b	0.6914(4)	0.7626(9)	0.8822(3)	3.1(1)
C4'b	0.6744(4)	0.994(1)	0.9180(3)	2.8(1)
O4'b	0.5454(3)	1.0440(6)	0.9013(2)	2.90(7)
C5'b	0.7505(4)	1.174(1)	0.8799(3)	3.3(1)
O5'b	0.7265(3)	1.2090(7)	0.7922(2)	4.12(8)

* atoms were refined isotropically.

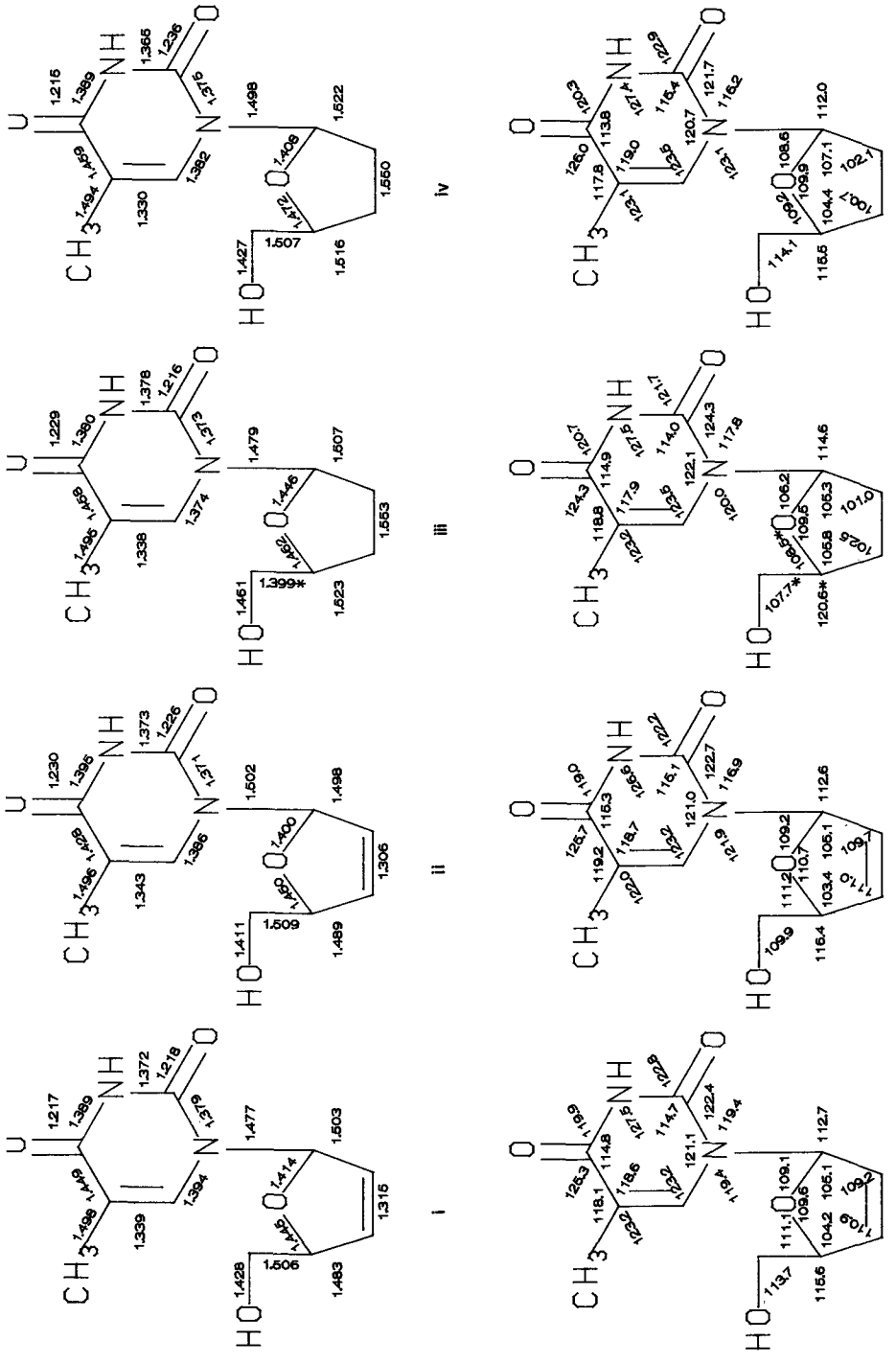

Figure 1. Summary of Bond Lengths and Angles.
lengths: i) D4T(a) ii) D4T(b) iii) ddT(a) iv) ddT(b) angles: v) D4T(a) vi) D4T(b) vii) ddT(a) viii) ddT(b)
* indicates disordered conformer where bond lengths and angles are reported for the model with 67% occupancy.

TABLE 3. Selected conformational parameters

MOLECULE	ddT(a)	ddT(b)	D4T(a)	D4T(b)
		Bond Lenghts(Å)		
N1-C1'	1.479	1.498	1.477	1.502
		Torsion Angles (deg)		
C2-N1-C1'-04'(χ)	-129.1	-170.9	-118.0	-174.0
N1-C1'-04'-C4'(ν)	-144.7	-117.2	-130.5	-123.1
C3'-C4'-C5'-05'(γ)	60.2	62.1	60.6	53.8
		Distance from Plance C1',C4',04' (Å)		
C2'	-0.561	0.099	0.237	-0.052
C3'	0.056	-0.529	0.242	-0.058

endo, ddT(b), and the χ angle is -171°. The correlation of the C2' endo conformer with the smaller χ angle and C3' endo with the larger χ angle is consistent with similar correlations observed by Van Rooey et al.[1a] and Saenger[9]. Conformer a of D4T has O4' exo by approximately 0.24Å while conformer b is nearly planer. The geometries for D4T are similar to the conformers seen in D4C[3]. The flattening of the furanose ring in the unsaturated series is presumably due to the presence of the double bond. Table 3 is a summary of selected conformational features, including the three characteristic torsional angles that describe the thymine-furanose geometry, χ, ν, and γ. Àpart from subtle differences shown in the Table, the conformational mimicry of ddT and D4T is complete. Figure 2 is a stereoview of the superimposed conformers observed in the asymmetric units of the crystal structures.

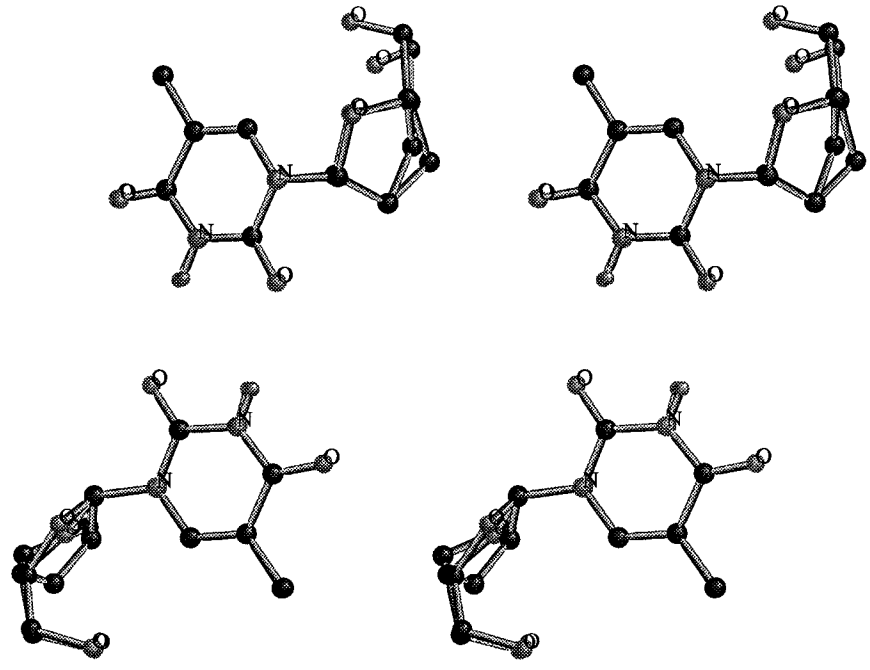

Figure 2. Stereoview of Superimposed Unit Cells.
Superposition of the asymmetric unit of ddT upon D4T. Conformers a displayed in top of figure, conformers b on the bottom.

TABLE 4. Intermolecular Hydrogen Bonding

bond:donor-acceptor	distance,(Å) D--A	angle,deg H(D)--A	D-H--A
ddT			
N3b-O2a (x,y,z)	2.939(2)	2.02(6)	160 (3)
N3a-O2b (x,y,z)	2.864(4)	1.99(7)	176 (4)
O5b'-O4a'(x-1,y-1,z)	2.762(5)	1.71(8)	157 (6)
O5a(1)'-O4a'(x,y-1,z)	3.34(4)		
D4T			
N3a-O2b (x,y,z)	2.934(3)	2.04(4)	170 (4)
N3b-O2a (x,y,z)	2.838(2)	1.83(5)	174 (3)
O5b'-O4a'(x,-1,y-1,z)	3.074(3)	2.58(8)	179 (5)
O5a'-O4a'(x,y-1,z)	3.862(5)	2.77(7)	140 (7)

HYDROGEN BONDING PATTERNS

The intermolecular bonding behavior of both D4T and ddT in the crystalline state are similar and involve two discrete sets of hydrogen bonds. The first set consists of base pairing which is a symmetrical interaction of O2 bonded to N3. The remaining hydrogen bonds involve the 5'hydroxyl and furanyl groups. The 5'-hydroxyl of conformer b hydrogen bonds to the furanyl oxygen of conformer a. The 5'-hydroxyl of conformer a hydrogen bonds to another confomer a furanyl oxygen in an adjacent unit cell. Table 4 is a summary of the hydrogen bonding data.

SUMMARY

The results presented in this paper show that the X-ray structures of ddT and D4T are similar in both their glycosyl geometries and hydrogen bonding behavior. The anti-HIV activities of these nucleosides, however, are markedly different. Since the triphosphates of ddT and D4T show similar affinity for RT,[8] the biological differences are presumably due to differences in the activation or pharmacokinetics of the two substances. This suggests that recognition of nucleosides by the relevant kinases is essential for biological activity. At the outset we thought that conformational differences might account for the recognition of the didehydro analogues by the relevant kinases. The results presented here suggest that these small changes do not, by themselves, account for the differences in biological activity. Only when the relevant interactions necessary for phosphorylation are elucidated will it be possible to fully study the effects of conformational change on RT efficacy. At present it seems more likely that it is the differences in conformational flexibility of these two molecules that contributes to the differences in biological efficacy and not the small differences in the low energy conformations.

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