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Structure of the anti-human immunodeficiency virus agent 3′-fluoro-3′-deoxythymidine and electronic charge calculations for 3′-deoxythymidines

(anti-AIDS agents/stereochemistry/drug design)

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ABSTRACT **The crystal and molecular structures of the anti-human immunodeficiency virus agent 3′-fluoro-3′-deoxythymidine have been determined by x-ray diffraction and stereochemical comparisons with thymidine have been made. Atomic charge distributions have been calculated by the complete neglect of differential overlap method for thymidine and antiretrovirally active and inactive C3′-substituted analogues. The structural and electronic results suggest that antiviral activity in these analogues may be correlated with the presence of an electronegative atom attached to C3′.**

Although 3′-azido-3′-deoxythymidine (AZT) is presently the only drug licensed in North America for use against acquired immunodeficiency syndrome (AIDS), other nucleoside analogues have also demonstrated anti-human immunodeficiency virus (HIV) activity to various degrees *in vitro*, and several are presently undergoing various phases of clinical trials for efficacy against AIDS. A considerable amount of activity is being devoted to preparing and testing substituted thymidines (or uridines), and rational stereochemical guidelines for synthetic endeavors would be very useful. 3′-Fluoro-3′-deoxythymidine (F-ddT) has been shown to be as potent an inhibitor of HIV replication as AZT in several cell lines [refs. 1 and 2; Polsky, B., Gold, J. W. M., Hardy, W. D., Jr., Baron, P. A., Zuckerman, E. E., Chou, T.-C., Levine, S. M., Flomenberg, N., Wang, L., Watanabe, K. A., Fox, J. J., & Armstrong, D. Twenty-seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, October 4–7, 1987, New York, p. P161 (abstr.)] although its selectivity appears to vary much more considerably than does AZT's from one cell line to another. Recent clinical results in Scandinavia, announced in the popular media, have indicated that F-ddT is as effective as AZT against AIDS *in vivo*, with somewhat less severe side effects. We have determined the crystal and molecular structures of F-ddT by x-ray diffraction and have calculated electronic charge densities in F-ddT and related compounds to identify stereochemical features which may play roles in anti-HIV activity in thymidine analogues.

MATERIALS AND METHODS

Colorless crystals of F-ddT were obtained from anhydrous methanol. The crystals are monoclinic, space group $P2_1$, with unit cell dimensions $a = 6.419(1)$ Å, $b = 18.731(2)$ Å, and $c = 9.336(2)$ Å and $\beta = 98.39(2)°$ (numbers in parentheses are standard deviation). The calculated density (1.461 g/cm^3) was reasonable, assuming two independent molecules in the asymmetric unit. Intensity data were collected on an automated diffractometer from a crystal measuring $0.76 \times 0.32 \times 0.27$ mm between Θ limits of 1° and 30°, using monochromated molybdenum Kα radiation and Θ–2Θ scans. A total of 3307 reflections were measured, of which 2574 had intensity $(I) > 2\sigma(I)$ [where $\sigma(I)$ is the estimated standard deviation in intensity obtained from counting statistics] and were considered observed. Psi scan data collected for two reflections showed absorption to be negligible.

The positions of all nonhydrogen atoms were obtained from direct-method procedures and were refined initially by full-matrix least-squares with isotropic temperature factors. Geometrically fixed hydrogen atom positions were calculated and methyl and hydroxyl hydrogens were located from difference electron density maps. All hydrogen coordinates were refined; their temperature factors were held fixed to the refined isotropic values of the atoms to which they are bonded. The final cycles of least-squares refinement, which were blocked with one molecule per block, $w = 1/\sigma^2(F)$ and anisotropic temperature factors for all nonhydrogen atoms, resulted in a final discrepancy factor $R = 0.043$ and $R_w = 0.041$. The quantity $\Sigma w(|F_o| - |F_c|)^2$ was minimized in the least-squares refinements.

RESULTS AND DISCUSSION

Atomic coordinates are given in Table 1 and bond lengths and angles, in Fig. 1. The two crystallographically independent molecules have similar conformations and bear a striking conformational resemblance to thymidine in the highly hydrated dinucleotide 5′-phosphothymidylyl(3′-5′)thymidine [d(pTpT)] (3). The orientation of the thymine bases relative to the sugar rings is *anti* in both cases, with glycosyl torsion angles (O1′–C1′–N1–C6) of 46.6° and 26.5° for F-ddT A and B molecules, respectively, vs. 32° and 35° for d(pTpT). The thymine bases are in the usual keto form and are planar to within 0.02 Å. The sugar rings in both F-ddT molecules are best described as having C2′ *endo* envelope conformations with C2′ and C3′ distances from the C1′–O1′–C4′ plane of 0.50 and −0.04 Å for molecule A and 0.40 and −0.10 Å for molecule B. The phase angle of pseudorotation and amplitude of puckering (4) are $P = 164°$, $\tau_m = 36°$ and $P = 169°$, $\tau_m = 32°$ for molecules A and B, respectively. These conformations closely resemble the sugar conformations in d(pTpT), which are also C2′ *endo* envelopes with $P = 164°$, $\tau_m = 38°$ and $P = 173°$, $\tau_m = 29°$ in the two thymidine halves of the molecule. Thus the overall conformational characteristics of F-ddT and the thymidine moieties of d(pTpT) are similar in all respects; this is clearly illustrated in Fig. 2, in which atoms

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Abbreviations: HIV, human immunodeficiency virus; F-ddT, 3′-fluoro-3′-deoxythymidine; AZT, 3′-azido-3′-deoxythymidine; CN-ddT, 3′-cyano-3′-deoxythymidine; CNDO, complete neglect of differential overlap.

Table 1. Fractional atomic coordinates ($\times 10^4$) and equivalent isotropic temperature factors ($\times 10^3$) for F-ddT

	Molecule A				Molecule B			
Atom	x	y	z	U_{eq}	x	y	z	U_{eq}
N1	4246(3)	395(1)	2105(2)	43(1)	6256(3)	4824	2299(2)	44(1)
C2	5707(4)	905(1)	2598(3)	45(1)	4828(4)	4298(1)	1791(3)	42(1)
O2	7409(3)	765(1)	3319(2)	61(1)	3068(3)	4442(1)	1178(2)	54(1)
N3	5118(4)	1588(1)	2218(3)	48(1)	5513(4)	3617(1)	2071(3)	46(1)
C4	3215(4)	1812(1)	1451(3)	47(1)	7414(4)	3404(2)	2849(3)	46(1)
O4	2897(3)	2442(1)	1170(3)	70(1)	7802(3)	2764(1)	3082(3)	64(1)
C5	1703(4)	1246(1)	1040(3)	42(1)	8849(4)	3977(2)	3330(3)	45(1)
C6	2272(4)	583(1)	1381(3)	42(1)	8206(4)	4646(2)	3044(3)	46(1)
C7	−409(5)	1445(2)	254(4)	58(1)	10955(5)	3796(2)	4164(5)	62(1)
O1′	4009(3)	−761(1)	1186(2)	52(1)	7289(3)	6017(1)	2233(2)	53(1)
C1′	4706(4)	−360(1)	2449(3)	45(1)	5512(4)	5569(1)	2141(3)	47(1)
C2′	3533(5)	−661(2)	3604(3)	48(1)	4302(5)	5798(2)	3323(4)	53(1)
C3′	3326(5)	−1433(1)	3201(3)	49(1)	4805(4)	6581(2)	3489(3)	51(1)
C4′	3096(5)	−1430(2)	1564(3)	48(1)	7004(5)	6638(2)	3103(3)	49(1)
C5′	860(5)	−1491(2)	811(4)	60(1)	8718(5)	6670(2)	4385(4)	64(1)
O5′	−427(4)	−986(1)	1381(2)	70(1)	8498(4)	6085(2)	5301(3)	75(1)
F	5220(3)	−1779(1)	3757(2)	73(1)	3402(3)	6952(1)	2447(2)	76(1)

$U_{eq} = \frac{1}{3}\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$.

of the thymine base of each F-ddT have been superimposed with the thymine of the free 3′-OH thymidine of d(pTpT), and in Table 2, which lists values of selected torsion angles in F-ddT and d(pTpT).

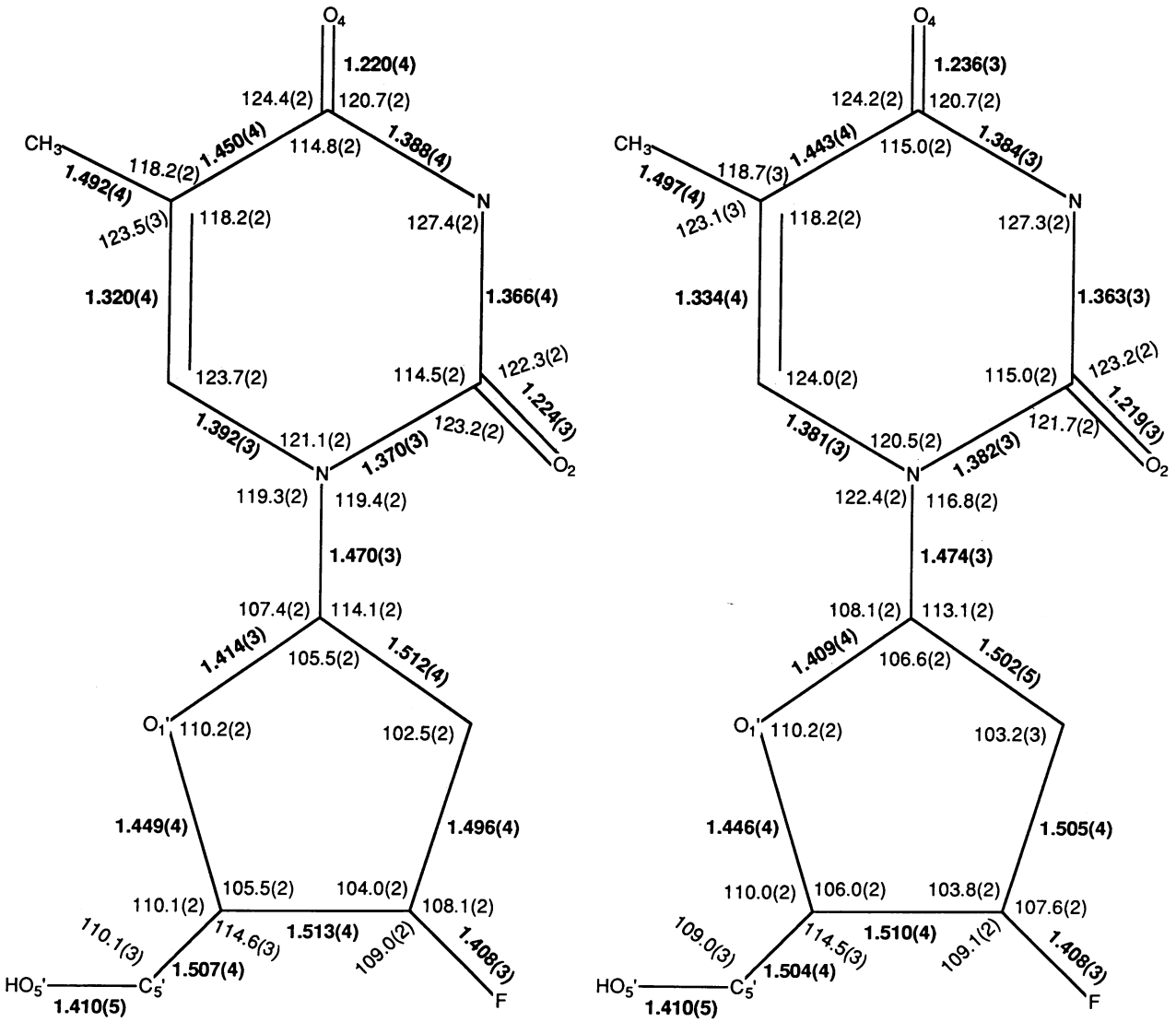

FIG. 1. Bond lengths (boldface; Å) and angles (lightface; °) in F-ddT. (*Left*) Molecule A. (*Right*) Molecule B.

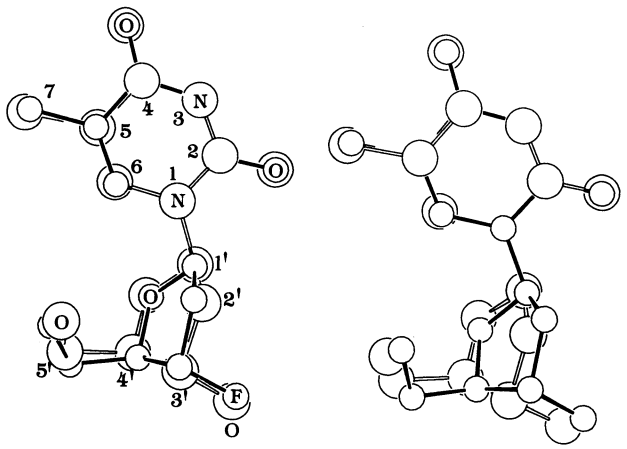

FIG. 2. Crystal structure conformations of the two independent molecules of F-ddT (dark bonds) shown with the thymine base of F-ddT superposed with thymine in the free 3'-OH thymidine moiety from hydrated 5'-phosphothymidylyl(3'-5')thymidine (3). (*Left*) Molecule A. (*Right*) Molecule B.

Molecular dimensions in the two independent F-ddT molecules are closely similar. The only parameters which differ significantly in the two (Fig. 1) are the exocyclic angles around N1: the asymmetrical values in F-ddT B reflect the smaller glycosidic angle in that molecule and hence a closer approach to overlap of the N1–C6 bond with C1'–O1' of the sugar. The C3'—F bond length is 1.408 Å in both molecules; the other bond parameters are typical of thymidine nucleosides and require little comment.

Molecular packing is shown in Fig. 3. Molecules A and B form a base-paired hydrogen-bonded dimer involving the N3-H and O4 atoms of the thymine rings. Contacts between dimers are formed by hydrogen bonds from sugar O5'-H to thymine O2 atoms, forming an infinite sheet that lies approximately in the ($\bar{1}02$) crystallographic plane [angle between the plane of the bases and ($\bar{1}02$) is 9°]. This hydrogen-bonding pattern, where each molecule forms the same number and type of hydrogen bonds, allows the furanose rings in both independent F-ddT molecules to adopt the favored [as in d(pTpT)] C2'-*endo* conformation, as noted previously; this contrasts with the situation in the crystal structure of AZT (5), where different hydrogen-bonding patterns for the two molecules result in the sugar ring of one AZT molecule displaying a different conformation. There may also be electrostatic interactions between dimers in F-ddT, as there are short distances (3.1 Å) between antiparallel aligned polar C3'-F groups. Contacts between sheets occur primarily between the sugars of one independent type of molecule and the bases of the other in a head-to-tail arrangement, the closest approach (2.98 Å) being between sugar O1' A and electropositive (see Table 3) C2 B of thymine. There is no base stacking in the structure.

Table 2. Torsion angles (°) in F-ddT and 5'-phosphothymidylyl-(3'-5')thymidine [d(pTpT)]

Angle	F-ddT A	F-ddT B	d(pTpT) A	d(pTpT) B
O5'–C5'–C4'–C3'	50.3(4)	53.4(4)	44	42
C5'–C4'–C3'–O3'*	146.1(2)	146.2(3)	149	152
O1'–C1'–N1–C6	46.6(3)	26.5(3)	32	35
C4'–O1'–C1'–C2'	−19.9(3)	−16.2(3)	−21	−12
O1'–C1'–C2'–C3'	33.3(3)	29.5(3)	35	26
C1'–C2'–C3'–C4'	−33.6(3)	−31.0(3)	−36	−38
C2'–C3'–C4'–O1'	22.5(3)	22.1(3)	24	21
C3'–C4'–O1'–C1'	−1.6(3)	−3.9(3)	−1	−6
O1'–C4'–C5'–O5'	−68.4(3)	−65.8(3)	−68	−78

*F replaces O3' for F-ddT.

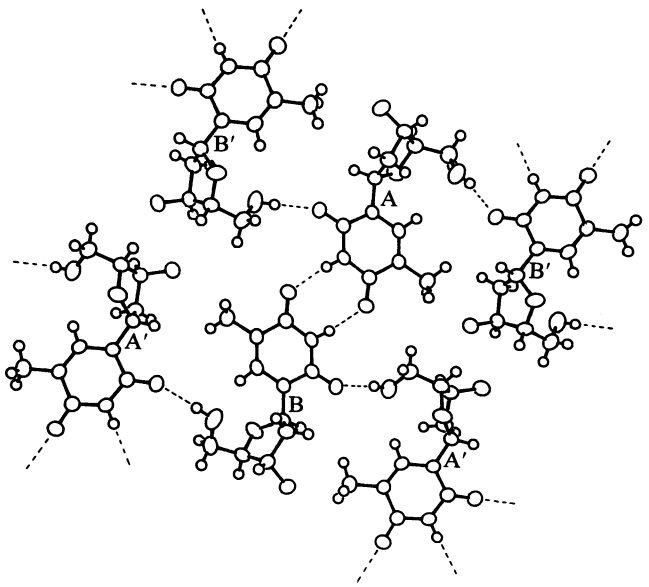

FIG. 3. Hydrogen bonding scheme in the crystal of F-ddT.

Although many thymidine and uridine derivatives have been prepared as possible anti-HIV agents, and several have been or are currently being tested, the pursuit of such analogues would be improved by development of useful stereochemical guidelines. For example, 3'-cyano-3'-deoxythymidine (CN-ddT) was synthesized and evaluated as a potential anti-HIV drug (6) because the 3'-cyano group was reasoned to be electronically and sterically similar to hydroxy and azido groups, thus making the molecule similar overall to AZT; however, the compound is ineffective. Thus more sophisticated stereochemical analysis is required. We have previously (5) determined the crystal structure of AZT, compared it structurally with thymidine 3'-phosphate, and pointed out the similar spatial positionings of the two electronegative azide nitrogen atoms of AZT and two oxygens of thymidine 3'-phosphate. It is possible, therefore, that these stereochemical similarities of the 3' substituents are at least partially responsible for AZT's inhibition of HIV; that is, the azido group may increase binding to HIV reverse transcriptase or facilitate substrate-type binding to enzymes of the thymidine phosphorylation pathway. A comparable situation exists in the F-ddT structure (Fig. 2). Although only thymine ring atoms have been superposed in the F-ddT and thymidine structures, the fluorine and 3'-oxygen atoms occupy similar positions in space in the two molecules: distances between F and O3' are 0.46 Å and 0.97 Å for F-ddT molecules A and B, respectively, and only small changes in torsion angles would allow virtually exact superpositions.

To get a quantitative stereochemical electronic relationship among substituted deoxythymidines, we calculated charge distributions in thymidine and active (AZT, F-ddT) and inactive (CN-ddT) analogues by complete neglect of differential overlap (CNDO) approximations. The results are shown in Table 3. Most of the corresponding atoms in all four molecules have similar electronic charges (including the thymine atoms not shown); significant differences occur in the charge distributions at the C3' and C3'-substituent centers. For thymidine and the active azido and fluoro analogues the net atomic charges at the 3'-oxygen, 3'-nitrogen, and 3'-fluorine atoms, which we have shown to occupy similar positions in space in the superimposed structures, are similar (−0.295, −0.294, −0.230), while for the inactive CN-ddT, for which the carbon of the cyano group occupies the corresponding position, the electronic charge is very different (+0.079). The net charges at C3' for thymidine, AZT, and

Table 3. Calculated atomic net charges ($\bar{e}$) in 3′-substituted thymidines

Atom	3′-OH*	3′-F*	3′-N_3*	3′-CN
C2	0.43325	0.44336	0.44339	0.44949
N1	−0.16175	−0.19005	−0.18658	−0.17933
C1′	0.27236	0.26919	0.26030	0.24580
C2′	−0.06944	−0.07256	−0.04955	−0.04063
C3′	0.12906	0.20668	0.10252	0.00986
C4′	0.12818	0.11652	0.11642	0.15177
O1′	−0.25837	−0.25573	−0.25448	−0.24624
C5′	0.11596	0.12802	0.13033	0.11405
O5′	−0.27284	−0.28578	−0.30096	−0.28523
X(C3′)	−0.29467	−0.23010	−0.29393	0.07921
			0.38825	−0.16133
			−0.16082	
H(O3′)	0.15296			
H(C1′)	−0.00589	−0.00131	0.00049	0.00030
H(C2′)†	0.04103	0.04265	0.03028	0.02468
H(C3′)	0.01581	−0.00091	−0.01380	0.03463
H(C4′)	0.00373	0.01454	0.00047	−0.00290
H(C5′)†	−0.00812	−0.00045	−0.00360	0.00977
H(O5′)	0.16494	0.17102	0.17868	0.15562

*Averaged over the two crystallographically independent molecules.
†Averaged over two hydrogens in each of the two independent molecules.

F-ddT have values of +0.129, +0.103, and +0.207, while for CN-ddT the value is close to zero (+0.010). Interestingly, the slightly smaller negative charge on the fluorine in F-ddT relative to the hydroxy oxygen in thymidine and azido nitrogen in AZT and the higher positive charge on the F-ddT C3′ result in very similar C3′—X bond polarities [$\bar{e}$(C3′) − $\bar{e}$(X)] in all three compounds (+0.424, +0.396, +0.437); the bond polarity in CN-ddT, on the other hand, is −0.069.

The above results do not prove that an electronegative substituent at the C3′ position of thymidine (or deoxyuridine) analogues is required for anti-HIV activity, but they do indicate that in C3′-substituted analogues anti-HIV activity is consistent with the presence of a net negative charge on the C3′ substituent atom. Our structure determination and stereochemical comparisons have shown that C3′ substitution does not have a significant effect on molecular conformation in these derivatives, and therefore the nature of the substituent likely plays an important role in the antiretroviral properties of the compounds. That role appears to be mediated by negative net atomic charge on the C3′ substituent; this may strengthen molecular binding to reverse transcriptase (5), may facilitate binding to enzymes of the cellular thymidine phosphorylation system, or may even play a role in inhibiting degradative enzymes. Whatever the mechanism, it seems prudent to use these stereochemical results as a guideline in the design and synthesis of antiretroviral thymidine/deoxyuridine derivatives; specifically, intra- and exo-ring substitution at C3′ should maintain electronegative character of the attached atom and polarity of the bond.

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