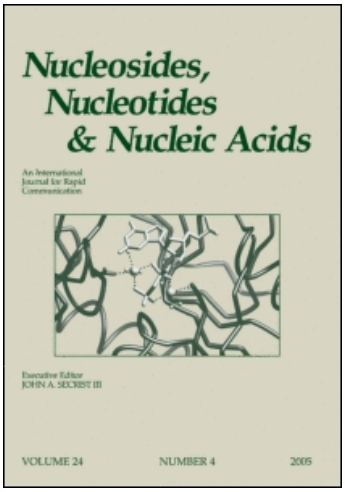

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CONFORMATIONAL ANALYSIS OF TWO ANTI-HIV NUCLEOSIDE ANALOGUES 2′,3′-DIDEOXY-3′-FLUOROCYTIDINE AND ITS N^4-DIMETHYLAMINOMETHYLENE PRODRUG DERIVATIVE#

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ABSTRACT. Crystal structure analysis of 2′,3′-dideoxy-3′-fluorocytidine (**1**) and its prodrug derivative, N^4-dimethylaminomethylene-2′,3′-dideoxy-3′-fluorocytidine (**2**), active anti-HIV nucleoside analogues, reveals that both structures adopt an anti conformation about the glycosyl bond. The furanose ring is C2′-endo for (**2**) and C2′-endo/C1′-exo and C2′-endo/C3′-exo for the two independent molecules of (**1**), respectively.

A prototype anti-HIV prodrug, N^4-dimethylaminomethylene-2′,3′-dideoxy-3′-fluorocytidine (NSC-614989), was designed and synthesized, as a representative of one of a rationally selected series[1,2]. The introduction of a proper substituent at the exocyclic NH_2 group of the parent deoxycytidine analogue (3′-F-ddC, $C_9H_{12}N_3O_3F$,**1**) lead to an increase in lipophilicity[3] of the resulting N^4-dimethylaminomethylene-2′,3′-dideoxy-3′-fluorocytidine (DDFC, $C_{13}H_{16}N4O2F$,**2**). The kinetics of the spontaneous hydrolysis of the side chain was determined and a feasible mechanism involving two distinct intermediates was established[3]. In the CEM cell line, complete protection against HIV was achieved by 6 μM **2**, without any cytotoxicity up to 200 μM, the highest concentration tested. Protection of CEM cells from HIV mirrored the lowering and elimination of p24 antigen levels[1-3]. Prodrug **2** and its congeners represent an active, non-cyctotoxic series having real therapeutic potential. Due to

#Dedicated to the memory of Prof. Tohru Ueda

TABLE 1. Crystal data for 2′,3′-dideoxy-3′-fluorocytidine (1) and N^4-dimethylaminomethylene-2′,3′-dideoxy-3′-fluorocytidine (2).

	(1)	(2)
Molecular Formula	$C_9H_{12}N_3O_3F$	$C_{13}H_{16}N4O2F$
Molecular Weight	229.22	279.30
Crystal System	Triclinic	Orthorhombic
Space group	P1	$P2_12_12_1$
Cell Dimensions		
a (Å)	6.973(1)	10.453(1)
b	7.315(1)	14.865(2)
c	10.5987(1)	8.6967(6)
α(°)	95.15(1)	90.0
β	107.84(1)	90.0
γ	104.38(1)	90.0
Z	2	4
V(Å^3)	490.3(1)	1351.29(2)
D_c (Mg/mm^3)	1.55	1.37
Crystal Size (mm)	0.2x0.4x0.5	0.3x0.5x0.6
Radiation (Å)	0.71069	0.71069
μ(cm^{-1})	1.23	0.98
T(K)	160	294
R (%)	3.1	5.6
wR	3.4	5.2
hkl range	-1<h<10,-10<k<10 -14<l<14	-1<h<15,-1<k<21 -1<l<13
reflections	2239	2020
Est. unit wt	0.367	0.669
Max Δ/σ	0.00	0.00
Δ/ρ_{max} (eÅ^{-3})	0.24	0.48
Δ/ρ_{min}(eÅ^{-3})	-.20	-.33

its novel structural features, this class of compounds may show a favorable pharmacokinetic profile, including efficient penetration into the central nervous system, warranting its further study and *in vivo* evaluation.

X-ray crystallographic analysis was carried out on DDFC **2** and its parent compound **1**. The results showed that the N^4-side chain of **2** is coplanar with the pyrimidine ring; the glycosyl bond is *anti*, as preferred for pyrimidine nucleosides, and the furanose ring is C2-endo/C1′(3′)-exo and C2′-endo for molecules **1** and **2**, respectively.

MATERIALS AND METHODS

Samples of **1**[4] were converted to **2** employing a previously published procedure[5] and recrystallized from aqueous ethanol. Crystal data for these compounds are listed in TABLE 1. Accurate cell dimensions were

TABLE 2. Atomic coordinates ($X10^4$) and isotropic thermal parameters ($Å^2X10^2$) for 2',3'-Dideoxy-3'-fluorocytidine (1).
Biso = $8/3\Pi^2\ \Sigma_i\Sigma_j\ U_{ij}\ a^*_i a^*_j\ a_i a_j$

ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	BISO*(σ)
N(1)	15272	20347	-3031	139(6)
C(2)	14804(5)	21798(5)	-3733(3)	147(7)
N(3)	16095(5)	22657(5)	-4363(3)	149(6)
C(4)	17830(5)	22129(5)	-4309(3)	134(7)
C(5)	18308(6)	20609(5)	-3630(4)	158(8)
C(6)	16989(5)	19744(5)	-3024(4)	156(8)
C(1')	13808(5)	19399(5)	-2[B8(4)	144(7)
C(2')	12308(6)	17482(5)	-3201(4)	171(7)
C(3')	11656(6)	16595(5)	-2113(4)	161(7)
O(1')	14982(5)	18971(4)	-1151(3)	160(5)
F(3')	10147(4)	17430(4)	-1869(3)	235(5)
C(4'1)	14862(6)	15828(5)	-559(4)	181(8)
O(4'2)	15616(5)	15317(5)	-1601(3)	238(7)
O(2)	13202(5)	22293(4)	-3732(3)	197(6)
N(4)	19077(5)	23052(5)	-4915(3)	163(7)
N(1*)	8964(5)	9073(4)	2874(3)	132(6)
C(2*)	9372(6)	7481(5)	3431(3)	130(7)
N(3*)	7930(5)	6384(5)	3875(3)	137(6)
C(4*)	6090(5)	6774(5)	3721(3)	130(7)
C(5*)	5562(5)	8265(5)	3032(3)	147(7)
C(6*)	7036(5)	9389(5)	2643(3)	145(7)
C(1'*)	10584(5)	10270(5)	2438(4)	147(7)
C(2'*)	10332(6)	9599(5)	972(4)	171(8)
C(3'*)	11361(6)	11459(5)	602(4)	185(8)
C(4'*)	10806(6)	12963(5)	1395(4)	153(7)
O(1'*)	10388(5)	12159(4)	2513(3)	166(6)
F(3'*)	13573(5)	11793(4)	1081(3)	304(7)
C(4'1*)	8853(6)	13443(5)	572(4)	184(8)
O(4'2*)	9444(5)	14508(5)	-381(3)	281(8)
O(2*)	11057(4)	7155(4)	3502(3)	185(6)
N(4*)	4760(5)	5722(5)	4223(3)	166(7)

*Molecule B

calculated from the least-squares analysis of 25 reflections for each crystal. Data were collected on a Nicolet P3V diffractometer using MoKα radiation. Both crystals were stable and showed no deterioration upon radiation. The data were corrected for Lorentz and polarization, but not for extinction or absorption effects. Both structures were solved by use of direct methods procedures using the programs MULTAN78[6] and NQEST[7]. Parameters were refined by full matrix least-squares techniques using anisotropic thermal parameters for the non-hydrogen atoms. Hydrogen atom positions were located in difference Fourier syntheses and refined isotropically. Atomic scattering factors were taken from the In-

TABLE 3. Atomic coordinates ($X10^4$) and isotropic thermal parameters (A^2X10) for $\underline{N}^4$-Dimethylaminomethylene-2′,3′-dideoxy-3′-fluorocytidine (2)

$Biso = 8/3\Pi^2 \Sigma_i \Sigma_j U_{ij} a^*_i a^*_j a_i a_j$

ATOM	X/A(σ)	Y/B(σ)	Z/C(σ)	BISO(σ)
N(1)	-597(3)	7906(2)	-2068(3)	30(1)
C(2)	183(4)	8572(2)	-1463(4)	33(1)
N(3)	-39(3)	8878(2)	-26(3)	32(1)
C(4)	-949(3)	8494(2)	840(4)	28(1)
C(5)	-1707(4)	7777(2)	286(4)	35(1)
C(6)	-1514(4)	7507(2)	-1195(4)	34(1)
C(1′)	-367(3)	7620(2)	-3668(4)	31(1)
C(2′)	540(4)	6822(2)	-3781(4)	35(1)
C(3′)	49(3)	6327(3)	-5177(4)	37(1)
C(4′)	-1370(3)	6555(2)	-5250(4)	30(1)
O(1′)	-1543(2)	7346(2)	-4301(3)	34(1)
C(5′)	-2237(4)	5818(2)	-4694(4)	35(1)
O(5′)	-1945(3)	5563(2)	-3149(3)	41(1)
F(3′)	658(3)	6661(2)	-6491(3)	63(1)
O(2)	1077(3)	8869(2)	-2267(3)	50(1)
N(4)	-1230(3)	8815(2)	2283(3)	31(1)
C(41)	-438(3)	9423(2)	2827(4)	31(1)
N(42)	-681(3)	9867(2)	4116(3)	34(1)
C(421)	157(4)	10597(3)	4614(4)	42(1)
C(422)	-1859(4)	9706(3)	4976(5)	54(1)

ternational Tables for X-ray Crystallography[8]. All computations were performed on a VAX 8600 computer using the Nonius least-squares package. Positional parameters and isotropic thermal parameters for both structures[9] are listed in TABLES 2 and 3.

RESULTS AND DISCUSSION

The molecular conformation and geometry of **1** and **2** are illustrated in FIG. 1 and 2, respectively, and their conformational parameters are defined in TABLE 4. These data show that the conformation in the two independent molecules of **1**, and in **2**, adopt an anti conformation, as usually observed in other pyrimidine nucleoside structures. The pucker of the furanose ring is defined by the pseudorotation parameter P and the degree of ring flattening[13], ν_m. Although the values of ν_m normally cluster near 40°, that of **2** is significantly smaller, indicating a more flattened ring than in the two independent molecules of the parent nucleoside **1**. The furanose ring is twist, C2′-endo/C1′ or C3′-exo in **1** and C2′-endo in **2**. The pseudorotation parameter P in these three mole-

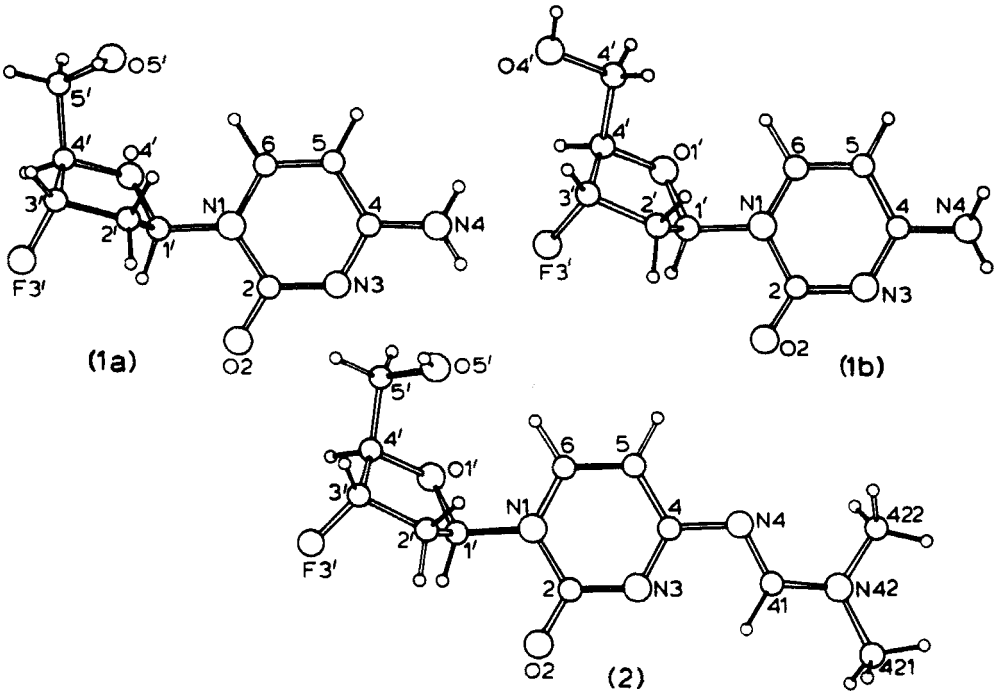

FIG 1. Molecular conformation of **1** and **2** with their numbering schemes.

cules is in general agreement with the other nucleosides with anti-HIV activity[14,15]. The conformation of the 5'-hydroxy oxygen is +sc with respect to the furanose ring in **1A** and **2**, and -sc in **1B**.

Comparison of these structures with other related nucleosides (TABLE 4) reveals their overall conformational similarity. The orientation of the $\underline{N}^4$-dimethylaminomethylene in **2** is cis to the cytosine N(3) as was also observed in the structure of $\underline{N}^4$-aminocytidine, another $\underline{N}^4$-substituted nucleoside (10). The conformation of ddC (11), which lacks 3'-F substitutent, is very different from those of **1** and **2**. While the conformations of **1** and **2** are similar to one of the AZT conformers (12), the other is significantly different.

The hydrogen bonding pattern of molecule **1** is described in TABLE 5 and shows that all functional groups participate in a two dimensional network of hydrogen bond interactions, typical of nucleosides. In mole-

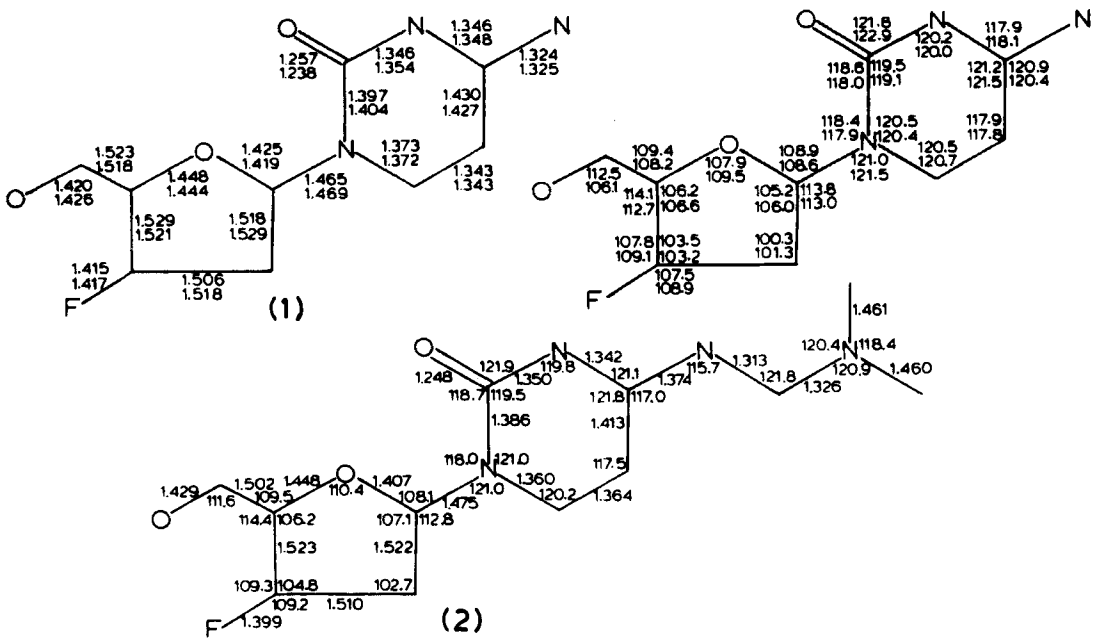

FIG. 2. Bond lengths and bond angles for 1 and 2, respectively. The two sets of values on 1 refer to molecule A and B respectively.

TABLE 4. Conformational comparison of some related pyrimidine nucleoside analogues.

MOLECULE	N1-C1'A	$\chi°$	$\gamma°$	$\nu_m°$	P°	Pucker	Ref
3'FddC 1A	1.465	-143.8	63.1	40	153	C2'-endo/C1'-exo	
1B	1.469	-153.1	-71.6	32	164	C2'-endo/C3'-exo	
DDFC (2)	1.475	-149.5	57.3	29	160	C2'-endo	
NH_2-Cyd A	1.490	-149.0	50.6	32	177	C2'-endo	(10)
B	1.477	-135.8	53.3	39	176	C2'-endo	(10)
ddC	1.485	-156.9	164.3	34	208	C3'-exo/C4'-endo	(11)
AZT(A)	1.460	-124.4	50.9	32	175	C2'-endo/C3'-exo	(12)
AZT(B)	1.502	-173.6	173.4	36	215	C3'-exo/C4'-endo	(12)

AZT-3'-azido-2',3'-dideoxythymidine; ddC, 2'-3'-dideoxycytidine; NH_2-Cyd, $\underline{N}^4$-aminocytidine.

TABLE 5. Hydrogen bonding in (1) and (2)

	D...A	D-H	H...A	<D-H...A
(1)				
N(4)-H1...N(3*)	3.021	0.89	2.15	170.0
-H2...O(2)	2.977	0.90	2.12	159.8
O(5')-H...O(2)	2.784	0.84	1.98	159.2
N(4*)-H1...N(3)	2.987	0.86	2.13	172.6
-H2...O(2*)	2.937	0.86	2.10	166.0
O(5'*)-H...O(5')	2.821	0.85	1.99	167.1
*Atoms in molecule B.				
(2)				
O(5')-H...O(2)	2.701Å	0.96Å	1.76Å	163.2°

cule 2 there is only one intermolecular hydrogen bond interaction between O(5') and O(2) which is much stronger than the corresponding hydrogen bond in structure 1 due to the electron donating conjugated side chain in 2 which makes O(2) a much better hydrogen bond acceptor. This resonance effect is also reflected by changes in the cytosine ring geometry (FIG. 2).

Recently, a report appeared[16] describing the crystal structure of 1. The data are in agreement with the results obtained in this independent work.

CONCLUSIONS

In both anti-HIV ddC derivatives 1 and 2, the 3'-F substituent alters significantly the overall conformation found in the unsubstituted ddC, but makes them resemble one of the two conformers of AZT, with similar sugar pucker and rotational angles. In prodrug 2, the presence of the conjugated $\underline{N}^4$-substituent and the resulting resonance effect enhances the electron density at O(2), making it a stronger hydrogen bond acceptor. The $\underline{N}^4$-side chain in 2 is coplanar with the pyrimidine ring and <u>cis</u> to N(3) [<u>syn</u> rotamer] due to steric interference by the H(5) in the opposite orientation.

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