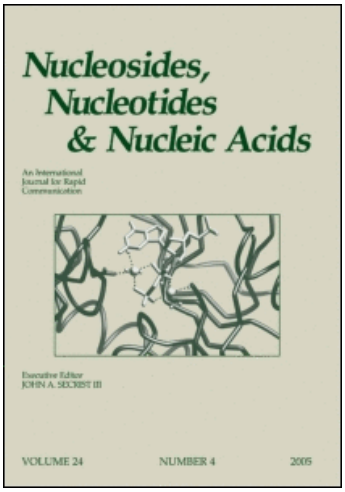

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Crystal Structure and Conformation of 5-Fluorouridine: Conformational Preferences for 5-Fluorinated Pyranosides

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ABSTRACT

Crystals of 5-fluorouridine (5FUrd) have unit cell dimensions a = 7.716(1), b = 5.861(2), c = 13.041(1)Å, $\alpha = \gamma = 90°$, $\beta = 96.70°$ (1), space group $P2_1$, Z = 2, ρ_{obs} = 1.56 gm/c.c and ρ_{calc} = 1574 gm/c.c The crystal structure was determined with diffractometric data and refined to a final reliability index of 0.042 for the observed 2205 reflections ($I \geq 3\sigma$). The nucleoside has the *anti* conformation [$\chi = 53.1(4)°$] with the furanose ring in the favorite C2′–*endo* conformation. The conformation across the sugar exocyclic bond is g^+, with values of 49.1(4) and $-69.3(4)°$ for Φ_{0c} and Φ_{∞} respectively. The pseudorotational amplitude τ_m is 34.5 (2) with a phase angle of 171.6(4)°. The crystal structure is stabilized by a network of N–H. . .O and O–H. . .O involving the N3 of the uracil base and the sugar O3′ and O2′ as donors and the O2 and O4 of the uracil base and O3′ oxygen as acceptors respectively. Fluorine is neither involved in the hydrogen bonding nor in the stacking interactions. Our studies of several 5-fluorinated nucleosides show the following preferred conformational features: 1) the most favored *anti* conformation for the nucleoside [χ varies from -20 to + 60°] 2) an inverse correlation between the glycosyl bond distance and the χ angle 3) a wide variation of conformations of the sugar ranging froni C2′–*endo* through

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C3′–*endo* to C4′-*exo* 4) the preferred g^+ across the exocyclic C4′–C5′ bond and 5) no role for the fluorine atom in the hydrogen bonding or base stacking interactions.

Key Words: Fluoropyrimidines; Conformational preferences; Structure-activity relationships.

INTRODUCTION

Certain purine and pyrimidine analogues readily replace the natural bases in nucleic acid if they are present during replication. Halogenated nucleic acids have been known for the past thirty years when for the first time it was found that 5-bromouracil and 5-iodouracil could be incorporated into the nucleic acids of *Streptococcus faecalis*.[1] It was also shown that 5-bromouracil inhibited the growth of *S. faecalis* although it was not clear from the preliminary experiments as to whether the analogue was present in the ribonucleic or deoxyribonucleic acid. Later in the same year it was shown by Zammenhof and Groboff[2] that when *E. coli* cells are grown in the presence of 5-bromouracil, the latter is incorporated into the DNA of *E. coli*. This provided the basis for the synthesis of 5-fluoro-nucleic acid bases and their use as a chemotherapeutic agent.

As early as 1954, it was observed that tumor cells utilized uracil to a greater extent than normal tissues.[3] The ability to incorporate halogenated pyrimidines into nucleic acids led Heidelberger and his coworkers[4] to develop a number of analogues, with replacement of hydrogen by fluorine. It was found that 5FUra exerts considerable anti-tumor activity against transplanted tumors in rats, mice and shows inhibitory activity against Gram-negative bacteria. 5FUracil is used in combination with other drugs to treat gastrointestinal malignancies,[5–8] stomach carcinoma,[9,10] ovarian cancer,[11] squamous cell cancer of head and neck,[12] malignant pleural effusions,[13] endocrine tumor of lung[14] and breast cancer.[15] Fluorinated analogues of uracil include the base, nucleoside, the nucleotide and the polynucleotide. Polymeric materials are widely used in the treatment of cancer and other diseases.[16–19] 5-Fluorodeoxyuridine (5FUdR) is often used in regional arterial chemotherapy for primary and metastatic malignancies. Poly (I.C) has been shown to inhibit the growth of a number of tumors like Ehrlich ascites tumor, fibrosarcoma, lymphoma ascites, leukemia and reticulum cell sarcoma.[20–22] We have synthesized a number of fluorinated polymers containing 5FU and have studied their structure by NMR and x-ray crystallography. This report describes the details of the crystal structure of 5FUrd and compares its structural details with other fluorinated nucleosides and nucleotides in the literature.

CRYSTALLOGRAPHY

5FUrd was provided by *Hoffman La Roche Inc.* courtesy of Dr. W.E. Scott. Crystals of 5FUrd were grown by slow evaporation from a solution of water and methanol and the crystals were very stable at room temperature. A crystal of dimension of $0.2 \times 0.1 \times 0.1$ mm mounted on a glass fiber was used for data collection. Crystals are monoclinic with unit cell constants at (22 + 3°C) given in Table 1.

Systematic absences in 0k0, k odd established that the space group was $P2_1$. Accurate unit cell parameters were determined with Enraf-Nonius CAD-4 automatic

Table 1. Crystal data.

Empirical formula	$C_9N_2O_6H_{11}F$
F.W.	262.21
Crystal system	Monoclinic
Space group	$P2_l$
a	7.716 (1)Å
b	5.861 (2)
c	13.041 (2)
α	90°
β	96.7(1)°
γ	90°
Volume	544.7 A^3
Z	2
ρ_o	1.56 $g.cm^{-3}$ (flotation in bromoform/benzene)
ρ_c	1.574 $g.cm^{-3}$
μ	11.4 cm^{-1}
λ[CuKα]	1.5418 A
Total no. of reflections	2362 (2205 > 3σ)
Final R value	0.042

diffractometer using 25 reflections with $\theta > 51°$. Three dimensional data (to the limit $2\theta = 154°$ for CuKα radiation) were collected by the $\omega/2\theta$ scan; scan widths calculated using the expression $(0.5 + 0.15 \tan\theta)$, aperture widths using $(3.0 + 1.2 \tan\theta)$ mm. The maximum time spent on any reflection measurement was 100 seconds and the background count time was half the scan time. Faster scans were used for stronger reflections. The intensities of three reflections were monitored after every hour of x-ray exposure and the variation in intensities was less than 3% during the time of data collection. The orientation matrix was checked every 100 reflections. A total of 2362 unique reflections were measured of which 2205 had intensities greater than 3σ and these were used in structure determination and refinement. The intensity data were corrected for Lorentz-Polarization effects. The intensities of three reflections close to χ at 90° were measured for all values of φ from 0° to 360°, and the resultant curve of transmission as a function of φ was used to calculate the absorption corrections for all the reflections. The average transmission factor was 0.92.

The structure was solved by the multisolution technique with the help of the program MULTAN.[23] The E-map with the best figure of merit gave the positions for all the non-hydrogen atoms of the base and a part of sugar moiety. The positions of the remaining atoms were obtained from successive difference electron density maps. At the end of the isotropic refinement, the R factor was 0.070. The locations of all the hydrogen atoms were obtained from difference electron density maps. The structure was refined using the full matrix least-squares refinement with individual anisotropic temperature factors for the non-hydrogen atoms and isotopic temperature factors for hydrogen atoms. The final R value was 0.042 for the 2305 observed reflections ($I > 3\sigma$). The quantity minimized in full matrix refinement was $w\ (|F_O| - 1/k\ |F_C|)$ where $w = 4|F_O|^2/(\sigma(|F_O|^2)^2$, and $\sigma(|F_O|)^2 = [\sigma^2(I) + p^2\ I^2]^{1/2}/LP$ and p is an ''ignorance factor'' to reduce the weights of intense reflections ($p = 0.05$), $\sigma(I)$ is the standard

Table 2. Table of final fractional positional parameters and their estimated standard deviations in parentheses.

Atom	X	Y	Z	B(A^2)†
N1	0.5853(3)	0.7941(6)	−0.2227(1)	3.44(4)
C2	0.7187(4)	0.9638(6)	−0.2091(2)	3.67(6)
O2	0.7359(3)	1.1138(5)	−0.2711(1)	5.30(5)
N3	0.8308(3)	0.9568(6)	−0.1149(2)	3.99(5)
C4	0.8225(3)	0.8001(7)	−0.0367(2)	3.62(5)
O4	0.9295(3)	0.8150(4)	0.0439(1)	4.74(5)
C5	0.6825(4)	0.6310(7)	−0.0607(2)	4.10(6)
F1	0.6641(3)	0.4732(5)	0.0127(1)	5.30(5)
C6	0.5686(4)	0.6295(7)	−0.1494(2)	3.76(5)
C1′	0.4481(3)	0.7996(6)	−0.3160(2)	3.26(4)
C2′	0.4583(3)	0.5915(6)	−0.3851(2)	3.23(5)
O2′	0.5909(3)	0.6310(6)	−0.4543(1)	4.31(4)
C3′	0.2532(3)	0.5703(6)	−0.4347(2)	3.59(5)
O3′	0.2178(3)	0.7216(5)	−0.5203(1)	4.58(5)
C4′	0.1428(3)	0.6551(8)	−0.3477(2)	4.20(6)
C5′	0.0786(5)	0.4670(1)	−0.2834(3)	6.05(8)
O5′	0.2302(4)	0.3161(7)	−0.2520(2)	6.35(6)
O4′	0.2687(2)	0.8041(5)	−0.2839(1)	4.04(4)
HN3	0.921(4)	1.072(7)	−0.099(2)	1.1(5)*
HC6	0.449(4)	0.535(7)	−0.157(2)	2.0(6)*
HC1′	0.475(4)	0.934(6)	−0.344(2)	1.1(5)*
HC2′	0.485(4)	0.444(7)	−0.337(2)	1.7(6)*
HC3′	0.214(3)	0.409(6)	−0.453(2)	0.5(5)*
HC4′	0.045(4)	0.749(7)	−0.372(2)	1.8(6)*
H1C5′	−0.015(6)	0.385(9)	−0.338(3)	5.0(1)*
H2C5′	0.027(4)	0.554(6)	−0.222(2)	0.6(5)*
HO2′	0.654(5)	0.480(1)	−0.464(3)	3.8(9)*
HO3′	0.250(4)	0.625(7)	−0.577(2)	1.8(6)*
HO5′	0.184(9)	0.170(1)	−0.229(4)	3.0(2)*

†Isotropic equivalent thermal parameter defined as: (4/3 [a^2B(1,1) + b^2 B(2,2) + c^2 B(3,3) + ab (cosγ) B(1,2) + ac (cosβ) B(1,3) + bc (cosα) B(2,3)]. Starred atoms were refined isotropically.

deviation in intensity I based on counting statistics and k is the scale factor. The atomic scattering factors are taken from International Tables for X-ray Crystallography.[24] Fourier and torsion angles programs written by Dr. S.T. Rao and ORTEP by Johnson were used (1965).[25] The final atomic coordinates are given in Table 2. Figure 1 gives an ORTEP diagram of the molecule.

RESULTS AND DISCUSSION

Bond Lengths and Angles

The bond length and angles for 5FUrd are given in Table 3. The average standard deviation in a bond length is 0.004 Å and in a bond angle 0.3°. The nucleoside has the

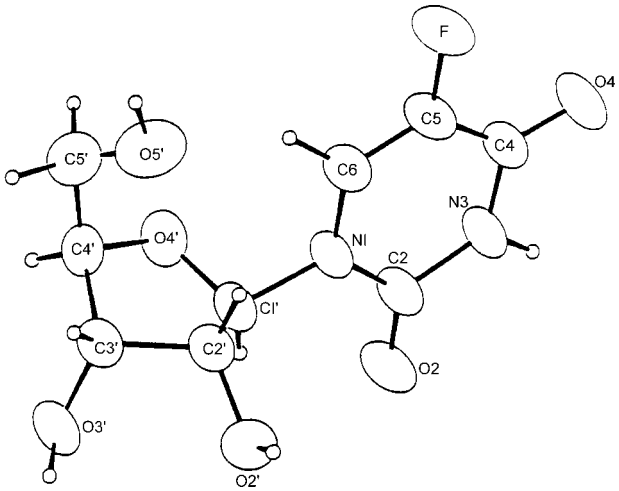

Figure 1. An ORTEP diagram of the molecule giving the numbering scheme in the molecule. Ellipsoids are drawn at 50% probability level. (From Ref. [25].)

anti conformation [$\chi = 53.1(4)^\circ$] with the furanose ring in the favorite C2′–*endo* conformation. The bond lengths and angles are very similar to the values found in 5-fluorodeoxyurdine,[26] 5-flurouracil[27] and uracil structures.[28] The bond lengths and angles are very similar to those found in nucleic acids with little change in secondary bonding patterns, in spite of the fluorine atom. When incorporated into RNA in place of uracil, 5FU behaves like uracil. Although hydrogen atom is replaced by a fluorine, they are quite similar sterically. When 5FUrd gets incorporated into DNA instead of thymidine, the fluorine and the methyl groups are sterically similar.

Torsion Angles

The torsion angles in the sugar ring are given in Table 4. Following the analysis of Altona and his coworkers[30,31] the conformation of the sugar ring is C2′–*endo* (^{2}E) with pseudorotation amplitude of 34.5(2)° and a phase angle of 171.6(4)°. The conformation across C(4′)–C(5′) is g^+ with values of 49.1(4)° and −69.3(4)° for Φ_{0c} and Φ_{∞} respectively.

Hydrogen Bonds and Molecular Packing

The packing of the molecules in the unit cell is shown in Fig. 2 which is a projection down the crystallographic a-axis. The hydrogen bond distances and angles are given in Table 5. The crystal structure is stabilized by a network of O–H...O and N–H...O hydrogen bonds. The fluorine atom is neither involved in the hydrogen bonding nor they are base paired. The hydrogen bonding scheme is different from that of uracil where one oxygen atom forms bifurcated hydrogen bonds to nitrogen atoms while the other forms no hydrogen bond at all. In 5FU, both the nitrogen atoms, N1 and N3 hydrogen bond with O2 and O4 respectively.

Table 3. Intramolecular bond distances (Å) and bond angles (°) in 5FUrd.

Bond	Distances (Å)	Bond angle	Angle (°)
N1–C2	1.387(4)	C2–N1–C6	122.2(2)
N1–C6	1.374(4)	N1–C2–N3	114.3(3)
N1–C1′	1.473(3)	C2–N3–C4	127.3(3)
C2–O2	1.210(4)	N3–C4–C5	113.1(2)
C2–N3	1.387(3)	C4–C5–C6	122.7(3)
N3–C4	1.378(4)	C5–C6–N1	120.3(3)
C4–O4	1.231(3)	N1–C2–O2	124.2(3)
C4–C5	1.420(5)	N3–C2–O2	121.3(3)
C5–C6	1.336(3)	N3–C4–O4	126.4(2)
C1′–C2′	1.524(4)	C5–C4–O4	116.6(2)
C2′–O2′	1.404(3)	F5–C5–C6	120.6(4)
C2′–C3′	1.542(3)	C6–N1–C1′	119.3(3)
C3′–O3′	1.425(4)	C2–N1–C1′	113.4(2)
C3′–C4′	1.540(4)	O4′–C1′C2′	107.5(3)
C4′–C5′	1.492(6)	C1′–C2′–C3′	101.7(3)
C4′–O4′	1.447(4)	C2′–C3′–C4′	102.3(3)
C5′–O5′	1.423(6)	C3′–C4′–O4′	106.2(2)
C1′–O4′	1.400(3)	C4′–O4′–O1′	110.6(3)
N3–HN3	0.94(3)	N1–O4′–O4′	107.7(2)
C6–HC6	1.01(3)	C1′–C2′–O2′	109.3(3)
C1′–HC1′	0.90(3)	O2′–C2′–C3′	115.4(2)
C2′–HC2′	1.07(4)	C2′–C3′–O3′	110.6(2)
C3′–HC3′	1.01(3)	O3′–C3′–C4′	108.4(2)
C4′–HC4′	0.92(3)	C3′–C4′–C5′	113.2(4)
C5′–H1C5′	1.04(3)	C4′–C5′–O5′	110.2(3)
C5′–H2C5′	1.05(3)	C5′–C4′–O4′	109.6(2)
O2′–HO2′	0.99(6)		
O3′–HO3′	0.98(3)		
O5′–HO5′	1.00(7)		

Table 4. Torsion angles (°) of 5-fluorocytidine.

			5FCyd[29]	
Torsion angle	Symbol	5FUrd	Molecule A	Molecule B
C(6)–N(1)–C(1′)–O(4′)	χ	53.1	15.2	20.0
C(2′)–C(1′)–O(4′)–C(4′)	ζ_0	−15.9	−1.5	1.9
O(1′)–C(1′)–C(2′)–C(3′)	ζ_1	30.9	−23.0	−25.0
C(1′)–C(2′)–C(3′)–C(4′)	ζ_2	−33.0	37.6	36.9
C(2′)–C(3′)–C(4′)–O(4′)	ζ_3	−91.8	−39.4	−36.1
C(3′)–C(4′)–O(4′)–C(1′)	ζ_4	−6.3	25.9	21.8
O(5′)–C(5′)–C(4′)–C(3′)	Φ_{OC}	49.1	51.4	54.7
O(5′)–C(5′)–C(4′)–O(4′)	Φ_{OO}	−69.3	−64.5	−62.2

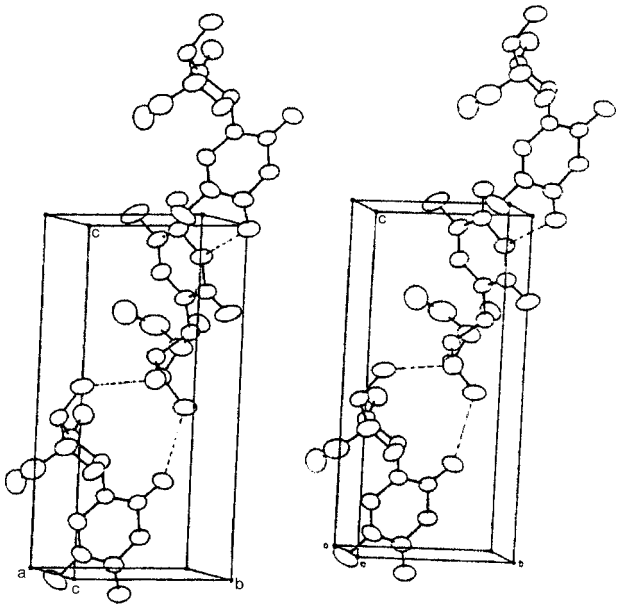

Figure 2. An ORTEP stereo diagram showing the packing of the molecules in the unit cell. Some of the intermolecular hydrogen bonds are shown by dashed lines. (From Ref. [25].)

In this structure O4 is the acceptor of a hydrogen bond from N3 whereas O2 takes part in a hydrogen bond with O3′. There is an O–H...O hydrogen with the exocyclic oxygen O5′ as the donor and the O4′ of the sugar ring as the acceptor. In addition to these two hydrogen bonds, we have additional C–H...O hydrogen bonding involving C5′ and O3′ exocyclic oxygen atom. The molecular packing and stacking is very much different compared with other halogenated nucleosides and nucleotides.[32–35] Crystal structures of 5-chloro, 5-bromo and 5-iodo compounds

Table 5. Geometry of the hydrogen bonds.

A	H	B	A...B	A–H	H...B	A–H...B
O3′	HO3′	O2	2.85^{I} Å	0.98 Å	1.99 Å	144.4°
O2′	HO2′	O3′	2.80^{II}	0.94	1.82	177
N3	HN3	O4	2.81^{I}	0.94	1.88	167
O5′	HO5′	O4′	3.05^{III}	1.00	2.34	144
C5′	H1C5′	O3′	3.44^{IV}	1.04	2.41	175

Subscript denote the following transformations

I	1–X	−1/2 + Y	1–Z
II	2–X	1/2 + Y	–Z
III	X	Y–1	Z
IV	–X	−1/2 + Y	1–Z

Table 6. Conformational details in 5-fluoropyranosides.

Serial no. name	N1–C1′ (Å)	χ (°)	Sugar pucker	τ_m	P	Φ_{OO}	Φ_{OC}	Reference
1. Ftorafur†	1.504	52.6	C4′–*endo*	36.0	38.0	− +	− +	[46]
		−17.3	C2′–*exo*					
			C3′–*endo*	41.0	180.0			[47]
2. 2′,3′-Dihydroxy ftorafur†	1.516	49.6	C3′–*exo*	36.0	196.0	–	–	
	1.512	35.4	C3′–*endo*	35.0	6.0			[46]
3. 2′-Hydroxy ftorafur†	1.462	15.0	C2′–*exo*	15.0	351.0	–	–	
	1.496	2.0	C4′–*endo*	−2.0	220.0	–	–	[47]
4. 3′-Hydroxy ftorafur†	1.466	56.6	C2′–*endo*	38.0	172.0	–	–	[47]
5. 5-Flourouridine	1.473	53.1	C2′–*endo*	34.5	171.6	−69.3	49.1	This work
6. 5-Fluorodeoxyuridine	1.474	60.6	C2′–*endo*	35.6	172.8	−68.1	49.8	[48]
7. 5-Fluorocytidine	1.486	15.0	C3′–*endo*	40.4	20.5	−64.5	51.4	
	1.488	20.0	C3′–*endo*	39.4	14.4	62.2	54.7	[29]

†Ftorafur and its derivatives contain no exocyclic CH_2OH group, hence there are no Φ_{OO} and Φ_{OC} torsion angles.

show that the halogen stacks on the pyrimidine rings [5FUra > 5ClUra.5BrUra > 5IUra][33] but in this structure, no stacking of any form is observed.

CONFORMATIONAL PREFERENCES FOR 5-FLUORINATED PYRANOSIDES

5FU is a major chemotherapeutic agent that is widely used in the treatment of many tumors and its mechanism of action has been a subject of interest for many years. A systematic investigation of the biological, biophysical and photochemical properties of nucleic acids containing 5FU have been carried out in our department.[36] Chemical and enzymatic synthesis had been used to produce several dimers, trimers and oligomers containing 5FUrd and 5FCyd and their photochemistry and activity against L1210, Hela cells and HSV-1 cells have been published[37–39] from our institute. The pyrimidine antimetabolite Ftorafur (FT) has shown significant antitumor activity in several adenocarcinomas including carcinomas of the breast and gastrointestinal tract. FT is thought to be a precursor of 5FU and has chemotherapeutic activity very similar to 5FU.[40–42] It is considered as a prodrug that acts as a depot form of 5FU.[43–45] Several analogs of FT have been synthesized in our institute and their structure and conformation characterized by x-ray and NMR studies[46] and their chemotherapeutic activity evaluated and compared with 5FU. Table 6 gives the details of the conformations found in several 5-fluorinated pyranosides. An analysis of the conformational details found in several of the nucleosides enables us to deduce the following preferred conformational details in 5-fluorinated nucleosides: 1) they have the most

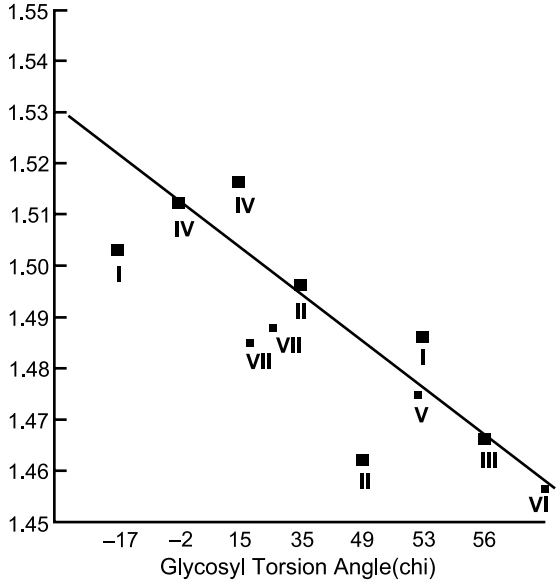

Figure 3. Graph showing the variation of the gylcosyl torsion angle (χ) with the glycosyl bond length [N1-C1′]. The numbering scheme followed is given in Table 6.

favored *anti* conformation for the nucleoside [χ varies from -20 to $+60°$] 2) an inverse correlation between the glycosyl bond distance and the χ angle (Fig. 3). 3) a wide variation of conformations of the sugar ranging from C2′–*endo* through C3′–*endo* to C4′–*exo* 4) the preferred g^+ across the exocyclic C4′–C5′ bond and 5) no role for the fluorine atom in the hydrogen bonding or base stacking interactions. The conformation of the sugar in the solution state shows more variability than that observed in the solid state. It appears that the presence of the fluorine atom at the C5 position of the base, has very little effect on the general conformation of the nucleoside or its base-stacking properties. We are currently trying to crystallize some of the di and trinucleotides containing the 5FU moiety. It is to be seen if these preferred conformations are conserved in the oligonucleotides.

NMR SPECTROSCOPY

A detailed determination of the biological, biophysical and photochemical properties of nucleic acids containing 5FU has been carried out in our laboratory.[36] Chemical and enzymatic synthesis has been used to produce several di- and trinucleotides containing 5-fluoronucleotides. These synthetic nucleotides were subjected to irradiation using UV-irradiation and the photoproducts were separated and analyzed using NMR and x-ray analyses. In addition, the polynucleotides were evaluated for their in vitro toxicity against L1210 (Leukemic mouse cells), HeLa (human cervical carcinoma cells) and HSV-1 (herpes simplex virus) cells. Henerson-Hasselbach titration curves were generated from H-1 and F-19 nmr data. This yields the relative pK_a values of N3–H ionization for 5FUrd base as a function of temperature ($60° < 50°$, $40°$, $30°$C). Figure 4 gives the nmr titration curve for 5FUrd. The H1′ chemical shifts are independent of pH over the temperature range of 30–60°C, whereas H6 shows a sigmaoidal behaviour. With increasing pH value, the H6 resonance shifts

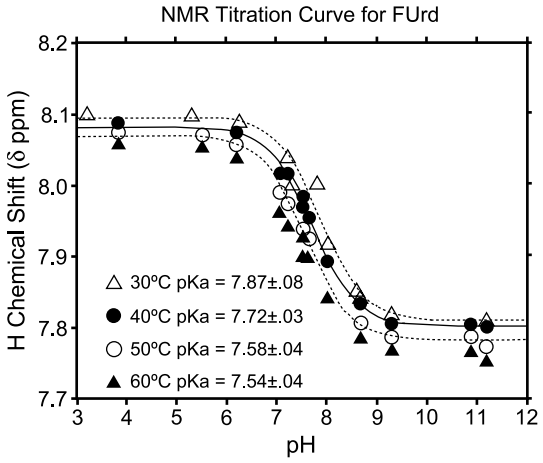

Figure 4. NMR titration curve for 5-fluorouridine.

Table 7. Antitumor effects against L1210.

Compound	Dosage (mg/kg)	% T/C
3′-OH-FT	100	96
	80	98
	60	108
5-FU	20	153
FT	80	102

upfield whereas the F5 resonance shifts downfield. The pK_a values show a significant temperature dependence and varies markedly as a function of the base sequence. In the case of 5FUrd, the pK_a is reduced by about 0.3 units on going from 30 to 60°C. Similar pK_a values were obtained from the F-19 nmr studies. In going from simple dinucleotide to the oligomers, the phosphate group contributes more negative charge thereby creating greater electrostatic potential. These results clearly demonstrate the ability of the neighboring bases to modulate the pK_a of the 5FU base, thereby providing the mechanistic base for a mutagenic event as a result of base mispairing.

ANTITUMOR STUDIES

Dunn and Smith[49] (1954) showed that 5-Bromouracil retards the growth of microorganisms and gets incorporated into DNA of *E. Coli* and its bacteriophages. Heidelberger[50] published the tumor inhibitory activity of 5-halopyrimidines. Since that time, several studies have been carried out on the action of 5FU and their derivatives on epithelial cells,[51,52] hepatoma cell lines[53] and other cell lines.[54,55] A systematic syntheses of several of these fluorinated purines and pyrimidines and their respective oligomers, their biological properties and their effects studied on L1210 and HeLa cell line have been reported in the doctoral dissertation of Dr. Soni.[36] These data are presented in Table 7 for Ftorafur and its derivatives and compared with 5FU. The effect of HeLa cell cycle traverse on these drugs are given in Table 8. These data confirm our

Table 8. Effect on HeLa cell cycle traverse.

	Cells in			
Component (ug.ml)	Mitosis	Interphase	Total	Mitotic index %
5-FU (50)	95	205	300	31.7
5-FU (25)	78	141	218	35.8
5-FU (12.5)	137	164	300	45.7
FT (200)	108	192	300	36
FT (100)	181	119	300	60.3
3′-OH-FT (200)	104	196	300	34.7
3′-OH-FT (100)	192	108	300	64
Control, colcemid (.05)	194	106	300	64.7

earlier results[56] that fluorinated pyrimidines and their oligomers behave in a similar manner in their effects on L1210 and HeLa cell lines. Clinical evaluation of the antitumor activity of Ftorafur and its derivatives indicates that these agents are less toxic and more effective than 5FU, especially in the treatment of cancers of the breast and the gastrointestinal tract. Ftorafur is believed to be a repository form of 5FU. 5FUrd and poly (FU) behave in a very similar fashion with regard to their antiviral activity. A detailed comparison of the activities of the monomer and the polymeric fluorinated pyrimidines have been reported in the doctoral dissertation.[36] Our detailed studies demonstrate that the biological and biochemical studies show that fluorine substitution of the nucleic acid bases have varied effects. With fluorinated pyrmidine polynucleotides, cytotoxicity and anti-viral studies show that the polymer is more biologically active while the reverse effect is seen in purines. Our results are very encouraging and should be exploited further with an investigation of their biological activity in vivo for a proper assessment of their chemotherapeutic efficacy. These studies are in the preliminary stages of investigation and will be reported as soon as we can complete the analysis.

MECHANISM OF ACTION

Antimetabolites and agents that react with DNA comprise the largest number of compounds that have been approved by the FDA for treatment of cancer. Most of these drugs and/or their combinations are used in the treatment of human cancers. The drug combinations are used because of the differences, in general, of the mechanisms of action, as well as toxicity, between the two classes, although finally both interfere with either the synthesis or function of DNA. The development of 5FU by Heidelberger and his coworkers[50,57] is an important milestone in the history of chemotherapy. It represents one of the few instances when the design of the drug and synthesis of an anticancer agent were based on a biochemical rationale. 5FU and 5FdUR possess antitumor activity against various types of carcinomas, particularly of the breast and the gastrointestinal tract. Favorable results have also been obtained in topical treatment of premalignant keratoses of the skin and basal cell carcinomas with 5FU. 5FU is most commonly used with other drugs in combination for better efficacy and less toxicity. The mechanism of action of 5FU has been studied very extensively and several review[58–61] on this topic have been published. Multiple biochemical mechanisms appear to be responsible for the cytotoxicity of this drug. Primary mechanism by which 5FU exerts its cytotoxic effects are 1) inhibition of thymidylate synthase, following its conversion to 5-fluoro-2′-deoxyuridine monophosphate (FdUMP) 2) conversion to 5-fluorouridine triphosphate (FUTP) and its incorporation into RNA leading to impairment in the function and processing of RNA and 3) incorporation of 5FU residues into DNA, leading to the impairment of the integrity of DNA. The relative importance of each one of these mechanisms is not very clear and may differ from one cell line to the other. The three important metabolites of 5FU are FdUMP, FdUTP and FUTP.

Rapidly dividing cells are the target of 5FU and therefore, the primary toxic side effects associated with 5FU therapy are from its effect on bone marrow and intestinal

and oral mucosa. Because of the formation of active metabolite from the drug requires several enzymes, alteration of the properties of one of the key enzymes result in the development of resistance to 5FU. Several fluorinated pyrimidine derivatives can function as prodrug of 5FU or as a metabolic precursor of its metabolites. A large number of these prodrugs of 5FU have been prepared and found to possess useful antitumor properties. Most important among such derivatives is Ftorafur. This compound and its 1,3-bis(tetrahydro-2-furyl) analogues are active against a range of solid tumors.[62,63] In our institute, we have synthesized and tested the antitumor activities of the 2′-hydroxy, 3′-hydroxy and the 2′,3′-dihydroxy derivatives of ftorafur[47] and found them lobe very effective as a prodrug with 5FU. We have synthesized several oligomers of the fluorinated purines and pyrimidines and found them to have antitumor properties. Detailed investigation on some of these compounds as prodrugs awaits a complete evaluation of these and this will be reported in a future publication from our institute.

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