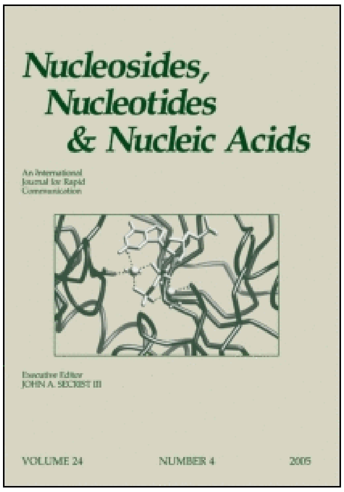

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SYNTHESIS, STRUCTURE, AND CONFORMATION OF ANTI-TUMOR AGENTS IN THE SOLID AND SOLUTION STATES: HYDROXYL DERIVATIVES OF FTORAFUR

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SYNTHESIS, STRUCTURE, AND CONFORMATION OF ANTI-TUMOR AGENTS IN THE SOLID AND SOLUTION STATES: HYDROXYL DERIVATIVES OF FTORAFUR

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ABSTRACT

The pyrimidine antimetabolite Ftorafur [FT; 5-fluoro-1-(tetrahydro-2-furyl)uracil] has shown significant antitumor activity in several adenocarcinomas with a spectrum of activity similar to, but less toxic than, 5-fluorouracil (5-FU). It is considered as a prodrug that acts as a depot form of 5-FU, and hence the two drugs exhibit a similar spectrum ofchemotherapeutic activity. Ftorafur is metabolized in animals and humans when hydroxyl groups are introduced into the tetrahydrofuran moiety. These metabolites are also thought to be as active as ftorafur but less toxic than 5-FU. Hydroxyl derivatives: 2′-hydroxyftorafur (III), 3′-hydroxyftorafur (IV) and 2′,3′-dihydroxyftorafur (II) were synthesized and X-ray and NMR studies of these hydroxyl derivatives were undertaken in our laboratories to study the structural and conformational features of Ftorafur and its metabolites in the solid and solution states. X-ray crystallographic investigations were carried out with data collected on a CAD-4 diffractometer. The structures were solved and refined using the SDP crystallographic

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package of Enraf-Nonius on PDP 11/34 and Microvax computers. All of the compounds studied had the base in the *anti* conformation. The glycosidic torsion angles varied from −20 to 60 degrees. There is an inverse correlation between the glycosyl bond distances and the χ angle. Molecules with a lower χ angle have a larger bond distance and vice versa. The sugar rings show a wide variation of conformations ranging from C2′-*endo* through C3′-*endo* to C4′-*exo*. The crystal structures are stabilized by hydrogen bonds involving the base nitrogen atom N3 and the hydroxyl oxygen atoms of the sugar rings as donors and the keto oxygens O2 and O4 of the base and the hydroxyl oxygen atoms O2′ and O3′ as acceptors. The NMR studies were carried out on Brüker 400 and 600 MHz instruments. Simulated proton spectra were obtained through Laocoon, and pseudorotational parameters were solved by Pseurot. Presence of *syn* or *anti* forms was demonstrated with the use of NOE experiments. The glycosyl conformations in solution vary more widely than in the solid state. The conformations of the sugar molecules are in agreement with the values obtained in the solid state. The studies of the structure and conformation in the solid and solution states give a model for the Ftorafur molecule that could be used in structure, function and biological activity correlation studies.

INTRODUCTION

The pyrimidine antimetabolite Ftorafur [FT; 5-fluoro-1-(tetrahydro-2-furyl)uracil] has shown antitumor activity in several adenocarcinomas including carcinomas of the breast and gastrointestinal tract. FT, thought to be a precursor of the much more toxic analog 5-fluorouracil, has nonetheless similar chemotherapeutic activity.[1–3] It was considered as a prodrug[4–6] that acts as a depot form of 5FU, and hence the two drugs exhibit similar spectra of chemotherapeutic activity. Studies by Lin and co-workers[7] and Sadee and his colleagues,[8–9] on the mechanism of activation of FT, showed the presence of hydroxylated FT in the urine of rats, rabbits and human cancer patients. They have isolated and identified the product as 4′-hydroxy- and 3′-hydroxy-ftorafurs. Recently Meyer and Lavennon[10] reported the synthesis of two hydroxylated Ftorafurs, namely 3′-hydroxyftorafur and 4′-hydroxyftorafur. The syntheses of these metabolites were also claimed by Lin and his co-worker.[7]

The hepatic drug metabolizing enzymes involving cytochrome P450 are probably involved in the conversion of FT to 5-FU in vivo.[11] This concept is in agreement with the rather low in vitro cytotoxic activity of FT in the absence of significant amount of cytochrome P450.[12] However, pharmacokinetic analysis of FT and 5-FU plasma levels following the administration of FT to several species indicate the presence of metabolites other than 5-FU that might be independently active or serve as intermediate(s) in the formation of 5-FU.[13–14] Several reports support the hypothesis that 5-FU formation may not be the only mechanism of FT activation. Smolyvan-Skaya and Tugarinov[6] have demonstrated the in vivo presence of microbiologically active metabolite formation of FT. Differences in FT and 5-FU toxicities in mice also supports an activation mechanism of FT other than or in addition to formation of 5-FU,[15] while therapeutic cross-resistance between FT and 5-FU suggests that the major part of therapeutic mechanism of action is common to FT and 5-FU.[16]

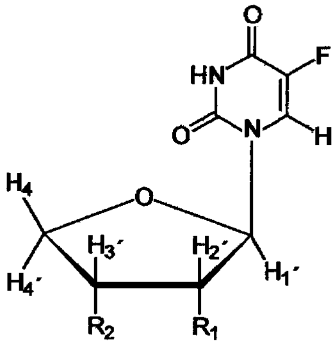
Hong has shown that FT-coupled to a thiolipid at the C4′ position shows little or no chemotherapeutic action against tumor cells. If one assumes that the delivery mechanism is preserved as that of Cytoros, then this reinforces the idea that FT must require the Cytochrome P450 to be modified to the 5-FU form in order to preserve activity.

In order to more effectively model the pharmacokinetic differences between derivatives and the parent compound, experimental data obtained from single crystal X-ray diffraction studies in combination with solution structures obtained with NMR is employed when possible. Correlation between structural differences and chemotherapeutic activity was investigated, along with possible reasons for differences between the solid and solution conformations. In this paper Ftorafur and three hydroylated derivatives were studied. The four compounds studied are: Ftorafur (FT, Compound I), 2′,3′-dihydroxyftorafur (2′,3′-di-OH FT, compound II), 2′-hydroxyftorafur (2′-OH FT, Compound III) and 3′-hydroxyftorafur (3′-OH FT, compound IV) (Fig. 1). Our attempts to synthesize the 1′-hydroxy and the 4′-hydroxy ftorafurs failed. We ran into problems of very low yields and difficulties in the purification of these products. So we are dropping these two products from including in this paper.

SYNTHESIS OF FTORAFUR DERIVATIVES

The syntheses of the two monohydroxylated ftorafurs were achieved by appropriate modification of their common precursor, 1-(β-D-erythrofuranosyl)-5-fluorouracil. The dihydroxy compound was synthesized via an extended Hilbert-Johnson procedure from 1,2,3-tri-*O*-acetyl-D-erythrofuranose and bis(trimethylsilyl)-5-fluorouracil using stannic chloride as a catalyst.

D-erythrose was prepared by lead tetraacetate oxidation of D-glucose according to Perlin.[17] However, the preparation of 1,2,3-tri-*O*-acetyl-D-erythrose was found to be problematic due to the self-condensation of D-erythrose to a dimer, a trimer, etc. Following the procedure described by Anderson and coworkers,[18] 1,2,3-tri-*O*-acetyl-D-erythrose was synthesized in variable yields and purified by column chromatography. Condensation of

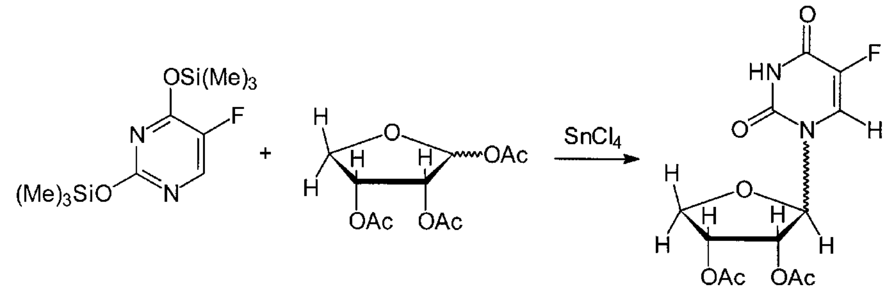


Compound*	R1	R2
I (Ftorafur)	H	H
II (2′, 3′-di-OH FT)	OH	OH
III (2′-OH FT)	OH	H
IV (3′-OH FT)	H	OH

*In this paper, we have used the nomenclature or numbering of hydroxyl derivatives of Ftorafur as that of the nucleoside derivatives. Several Workers[7–9] have used numbering of hydroxyl derivatives of Ftorafur as tetrahydrofuran derivatives.

Figure 1. A schematic diagram showing the structures of the different compounds under investigation in the study.

protected erythrose with silylated 5-FU in dry 1,2-dichloromethane with $SnCl_4$ catalyst gave a mixture of 1-(2,3-di-*O*-acetyl-D-erythrofuranosyl)-5-fluorouracil; α and β isomers were separated by column chromatography. The protected β isomer was then deblocked (de-*O*-acetylated) to give 5-fluoro-1-β-D-erythrofuranosyluracil [2′,3′-dihydroxyftorafur](II). Treatment of the β-nucleoside (II) with diphenylcarbonate in *N*,*N*-dimethylformamide (DMF) gave 5-fluoro-2,2′-*O*-anhydro-1-β-D-threofuranosyl)uracil (V).[19] Attempted synthesis of 5-fluoro-(2-bromo-2-deoxy-β-D-erythro-furanosyl)-uracil from compound (V) by treatment with HBr/AcOH and its catalytic reduction to give desired metabolite (IV) was not successful and gave a very

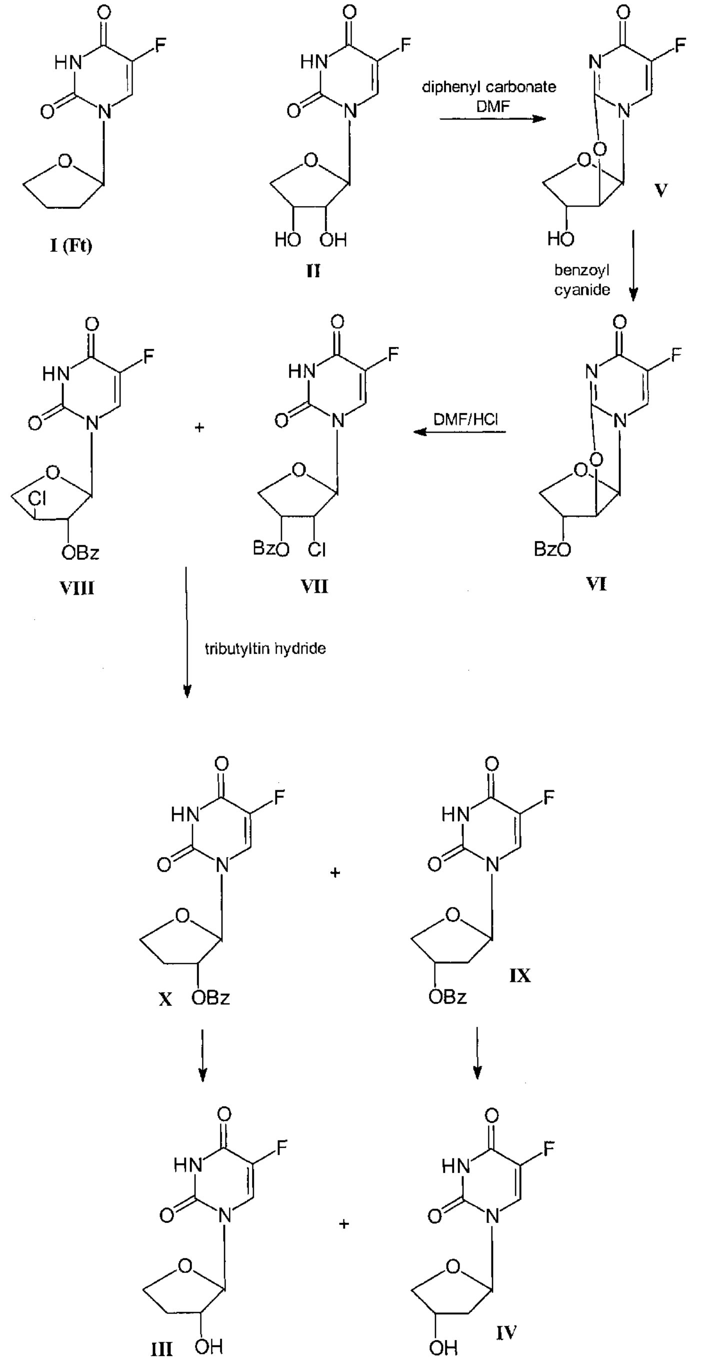


Scheme 1.

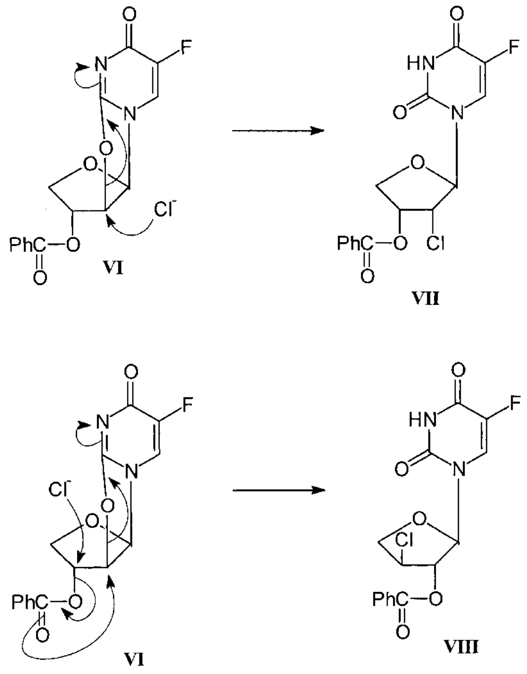
poor yield. An alternate and modified procedure described by Holy and coworkers[20] was then followed. 5-Fluoro-2, 2′-*O*-anhydro-1-(3-*O*-benzoyl-β-D-threofuranosyl)uracil (VI) was prepared from compound (V) by treatment with benzoyl cyanide (see Sch. 2). Treatment of compound VI with HCl/DMF gave a mixture of two chloro derivatives, namely 5-fluoro-1-(3-*O*-benzoyl-2-chloro-2-deoxy-β-D-erythrofuranosyl)uracil (VII) and presumably 5-fluoro-1-(2-*O*-benzoyl-3-chloro-3-deoxy-β-D-threofuranosyl)uracil (VIII). A possible mechanism for their formation is given in Sch. 3. Tributyltin hydride reduction of the mixture of VII and VIII gave a mixture of 5-fluoro-1-(3-*O*-benzoyl-2-deoxy-β-D-erythrofuranosy)uracil (IX) and 5-fluoro-1-(2-*O*-benzoyl-3-deoxy-β-D-erythrofuranosyl)uracil (X). Products (IX) and (X) were isolated and purified by chromatography. De-*O*-benzoylation of (IX) and (X) gave the desired metabolites: 5-fluoro-1-(2′-deoxy-β-D-erythrofuranosyl)uracil (IV) and 5-fluoro-1-(3′-deoxy-β-D-erythrofuranosyl)uracil (III) respectively. The sequence of the reactions is shown in sch. 2. The products were analyzed by elemental analysis, proton and ^{13}C NMR, U.V. and mass spectral data.

There is indication of formation of 5-fluoro-2,3′-anhydro-(β-D-threofuranosyl)uracil (XI) from compound (II) on treatment with diphenyl carbonate. This anhydro derivative (XI) is resistant to benzoylation by benzol cyanide. This phenomenon may be attributed to steric hinderance of the hydroxyl group at the 2′ position in (XI). No such steric hinderance was observed with compound (V). The possible structures are shown in Sch. 4.

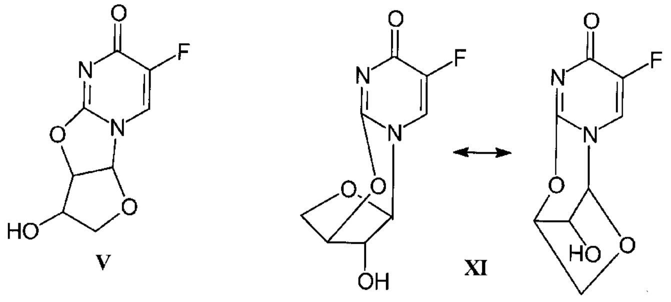
The isolated diol, 1-(β-D-erythrofuranosyl)-5-fluorouracil was shown to be the N-1 nucleoside (as opposed to the N-3 adduct) by the absence of the bathochromatic shift when the ultraviolet absorbance spectrum was determined in alkaline solution. Predominant production of the β-isomer was predicted by the "trans rule," and confirmation of the β configuration was provided by subsequent conversion of the isolated diol to the cyclonucleoside with diphenyl carbonate in DMF. The experimental details of the synthesis will be published at a later date.



Scheme 2. Synthetic scheme for the hydroxyl drerivatives of Ftorafur.



Scheme 3. Possible mechanism of formation of 2′- and 3′-chloro derivatives.



Scheme 4. Possible structure of 2,3′-anhydro-(XI) and 2,2′-(V) derivatives.

X-RAY CRYSTALLOGRAPHY

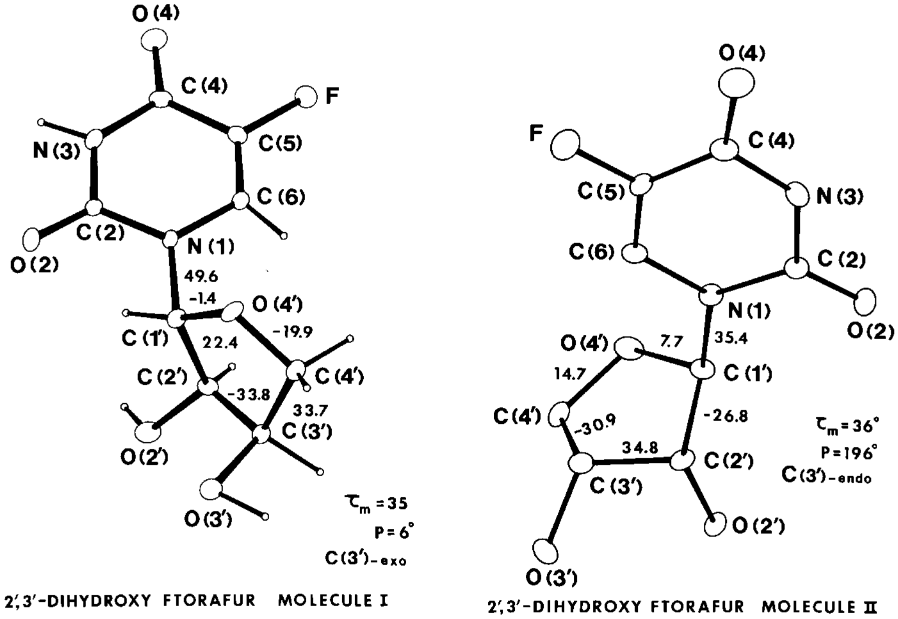
Ftorafur, 5-fluoro-1-(tetrahydro-2-furyl)uracil is a very potent antitumor agent which is used in the treatment of various cancers. Nakajima and his coworkers[21] identified the existence of two crystalline polymorphs with slightly different melting points and reported the crystal and molecular structure of one of these polymorphs. Table 1 gives the crystallographic and conformational parameters of ftorafur and its 2′-hydroxy, 3′-hydroxy and 2′,3′-dihydroxy derivatives.

Table 1. Crystallographic and Conformational Parameters of Ftorafur and Its 2′-Hydroxy, 3′-Hydroxy and 2′,3′-Dihydroxyl Derivatives

Parameters Cell Dimensions	I	II	III	IV
a, Å	8.994 (8)Å	4.848 (2)Å	13.224 (1)Å	8.294 (1)Å
b	16.612 (9)	20.108 (6)	5.051 (1)	10.918 (1)
c	5.981 (5)	9.189 (2)	14.489 (1)	5.021 (1)
α( )	86.40 (6)	90.0	90.0	90.0
β	94.06 (15)	91.64 (2)	111.84 (2)	104.12 (2)
τ	80.29 (8)	90.0	90.0	90.0
V, Å^3	876	895.3	898.3	441
Z	4	4	4	2
Space group	P1	P2$_1$	P2$_1$	P2$_1$
D$_{obs}$ g·cm^{-3}	1.51	1.62	1.60	1.63
D$_{cal}$	1.51	1.618	1.598	1.62
Crystal size mm	0.6 × .37 × .25	.3 × .35 × .25	.45 × .25 × .15	.4 × .25 × .1
μ(CuKα) cm^1	11.52	12.93	11.97	12.19
CAD4-data	3363 (3125 > 3σ)	2028 (1273 > 3σ)	2121 (1178 > 3σ)	995 (606 > 3σ)
Final R factor	0.056	0.061	0.047	0.051
Goodness of fit	–	2.089	1.729	2.239
Glycosyl torsion	52.6, −17.3	49.6, 35.4	15, −2	56.6
Sugar pucker	C4′ exo C2′ exo C3′ endo	C3′ exo C3′ endo	C2′ exo C4′ endo	C2′ endo
τ_m	36, 41	36, 35	39, 38	38
P	38, 180	196, 6	351, 220	172

Crystals of 2′,3′-dihydroxyftorafur (II) ($C_8N_2O_5H_9F$), obtained from ethanol/methanol mixture, are monoclinic, space group P2$_1$ with unit cell dimensions (at 22 ± 3°C) a = 4.848(2), b = 20.180(6), c = 9.189(2)Å, β = 91.64(2)°, V = 895.3Å^3, Z = 4 (two independent molecules per asymmetric unit), and D$_c$ = 1.618 g.cm^{-3}. A single crystal of dimension 0.03 × 0.035 × 0.025 cm was used for data collection on a CAD4 diffractometer; 2028 unique reflections were measured, out of which 1273 reflections were significant ($I \geq 3\sigma$). The structure was solved by multisolution techniques and refined by full matrix least-squares method to a final R-value of 0.061 for the observed 1273 reflections. Both the molecules (Fig. 2) are in the *anti* conformation (χ = 49.6° and 35.4°), but their ring puckers are C3′-*exo* and C3′-*endo*. The crystal structure is stabilized by a number of hydrogen bonds from one molecule to the other involving the base and sugar, expect the fluorine which does not take part in any hydrogen bonding.

3′-Hydroxyftorafur (IV) was crystallized from 95% ethanol to give transparent needles, M.P. 212–213°C. Crystals of (III) ($C_8N_2O_4H_9F$) are monoclinic, space group P2$_1$ with the following unit cell dimensions



2',3'-DIHYDROXY FTORAFUR MOLECULE I

2',3'-DIHYDROXY FTORAFUR MOLECULE II

Figure 2. An ORTEP diagram showing the conformations in the molecules of 2',3'-dihydroxyftorafur.

(at 22 ± 3°C) a = 8.294(1), b = 10.918(1), c = 5.021(1)Å, $\beta = 104.12(2)°$, Z = 2, V = 441Å^3, $D_{calc} = 1.622\,g.cm^{-3}$. A small needle-like crystal of dimensions 0.04 × 0.025 × 0.01 cm was used for data collection on CAD-4 diffractometer by the $\omega/2\theta$ scan. 995 unique reflections were measured, out of which 606 were considered significant. The structure was solved by the application of multi solution techniques (22) and refined by full matrix least squares method to a final R value of 0.051 for the 606 reflections ($I \geq 3\sigma$). The molecule has *anti* conformation ($\chi = 56.6°$) with C2' *endo* (following nucleic acid nomenclature) for the ring pucker (Fig. 3). In the absence of the C5' carbon atom, N(1) of the base is used as the reference atom for describing the pucker of the ribose ring. The crystal structure does not have any base-pairing through hydrogen bonding, but is stabilized by a hydrogen bond between the base and the sugar. The fluorine does not take part in any hydrogen bonding interactions.

Crystals of 2'-hydroxyftorafur (III) ($C_8N_2O_4H_9F$), obtained from aqueous ethanol, are monoclinic, space group $P2_1$ with unit cell dimensions (at 22 ± 3°C) a = 13.224(1), b = 5.051(1), c = 14.489(1)Å, $\beta = 111.84(2)°$, V = 898.3Å^3, Z = 4 (two independent molecules per asymmetric unit), and $D_{calc} = 1.598\,g{\cdot}cm^{-3}$. A single crystal of dimension 0.045 × 0.025 × 0.015 cm was used for data collection on a CAD4 diffractometer. 2121 unique reflections were measured, out of which 1178 reflections were significant ($I \geq 3\sigma$). The structure was solved by multisolution techniques and refined by full

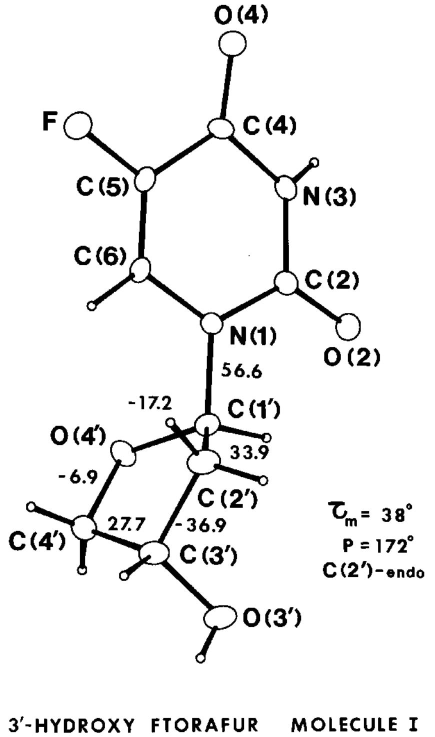


Figure 3. An ORTEP diagram of 3′-hydroxyftorafur showing the conformational angles in the ribose.

matrix least-squares method to a final R-value of 0.047 for the observed 1178 reflections. Both the molecules (Fig. 4) are in the *anti* conformation ($\chi =$ 15.0° and −2.0°), but their ring puckers are C2′-*exo* and C4′-*endo*. The crystal structure is stabilized by a number of hydrogen bonds from one molecule to the other involving the base and sugar, except the fluorine which does not take part in any hydrogen bonding.

Complete three dimensional intensity data were collected on a CAD-4 diffractometer by the $\omega/2\theta$ technique. The intensities of all the reflections to the limit $2\theta < 150°$ for CuKα ($\lambda = 1.5418$ Å) were measured. Scan widths were calculated according to the relation ($A + B \tan\theta$) with values of 0.5 0.15° for A and B respectively. Aperture widths were calculated using the relation ($3.0 + 1.2 \tan\theta$) mm where θ is the Bragg angle. The maximum time spent on any reflection measured was 100 s and the background count time was half the scan time. A faster scan was used for strong reflections. The intensities for three reflections were monitored after every hour of X-ray exposure and the variation in intensities was less than 3% during the time of complete data collection. The orientation matrix was checked every 100 reflections. Lorentz and polarization corrections

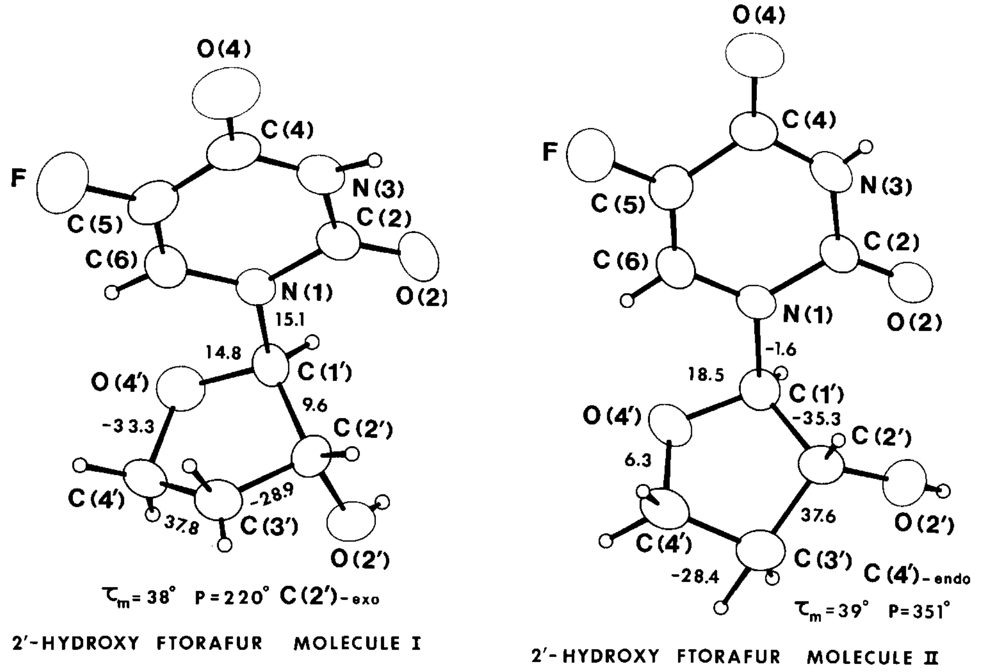


Figure 4. An ORTEP showing the conformation of the two molecules of 2′-hydroxyftorafur compounds.

were applied to all reflections. The intensities of three reflections close to χ of 90° were measured for all values of ϕ from 0 to 360° and the resultant curve of transmission was used to correct for absorption effects. The minimum and maximum transmission factors were 0.79 and 0.99 respectively with an average value of 0.92. The structures were solved by the application of the multisolution technique[22] and was refined by a full-matrix least squares procedure, initially with isotropic thermal parameters and later with anisotropic thermal parameters for the non-hydrogen atoms. A difference electron-density map was used to locate the positions of all the hydrogen atoms in the molecule. The structures were further refined with isotropic temperature factors for the hydrogen atoms and anisotropic factors for the other atoms. The function minimized was

$$w\left[|F_0| - \left(\frac{1}{k}\right)|F_c|\right]^2$$

where:

$$w = \frac{4|F_0|^2}{\sigma(|F_0|^2)^2}$$

Table 2. Table of Final Fractional Positional Parameters and Their Estimated Standard Deviations in Parentheses for 2′,3′-Dihydroxy Ftorafur

	X	Y	Z	B (Å^2)
Molecule A				
N1	−1.1542 (2)	−0.0748 (+)	1.2740 (2)	1.48 (4)
C2	−1.1817 (2)	−0.1173 (2)	1.1562 (2)	1.75 (4)
O2	−1.3436 (2)	−0.1627 (2)	1.1548 (2)	2.58 (4)
N3	−1.0085 (2)	−0.1051 (2)	1.0460 (2)	1.86 (4)
C4	−0.8088 (2)	−0.0561 (2)	1.0376 (2)	1.92 (4)
O4	−0.6561 (2)	−0.0516 (2)	0.9362 (2)	2.90 (4)
C5	−0.8126 (2)	−0.0117 (2)	1.1621 (2)	1.80 (4)
F1	−0.6329 (2)	0.0396 (2)	1.1601 (2)	2.95 (4)
C6	−0.9737 (2)	−0.0210 (2)	1.2751 (3)	1.77 (4)
C1′	−1.3013 (3)	−0.0886 (3)	1.4072 (3)	1.86 (4)
C2′	−1.1066 (3)	−0.1119 (3)	1.5339 (3)	1.79 (4)
C3′	−1.2335 (3)	−0.0794 (3)	1.6624 (3)	2.05 (4)
C4′	−1.3369 (3)	−0.0147 (3)	1.6008 (3)	2.20 (4)
O1′	−1.4303 (3)	−0.0303 (3)	1.4536 (3)	2.24 (4)
O2′	−1.0895 (3)	−0.1807 (3)	1.5483 (3)	2.59 (4)
O3′	−1.4496 (3)	−0.1210 (3)	1.7099 (3)	2.72 (4)
Molecule B				
N1	−1.3489 (2)	0.1874 (2)	0.8610 (2)	1.88 (4)
C2	−1.3157 (2)	0.2166 (3)	0.7271 (3)	1.72 (4)
O2	−1.1509 (2)	0.2595 (2)	0.7028 (2)	2.77 (4)
N3	−1.4982 (2)	0.1913 (2)	0.6170 (2)	2.23 (4)
C4	−1.6897 (2)	0.1426 (3)	0.6318 (3)	2.11 (4)
O4	−1.8338 (2)	0.1244 (3)	0.5221 (2)	3.60 (4)
C5	−1.7097 (3)	0.1178 (3)	0.7728 (3)	2.18 (4)
F1	−1.8993 (2)	0.0702 (2)	0.7961 (2)	3.03 (4)
C6	−1.5402 (3)	0.1382 (3)	0.8824 (3)	1.85 (4)
C1′	−1.1577 (3)	0.2080 (3)	0.9837 (3)	1.80 (4)
C2′	−1.2808 (3)	0.2572 (3)	1.0873 (3)	1.55 (4)
C3′	−1.4039 (3)	0.2130 (3)	1.2010 (3)	1.55 (4)
C4′	−1.1965 (3)	0.1573 (3)	1.2111 (3)	1.91 (4)
O1′	−1.0888 (2)	0.1507 (2)	1.0675 (2)	2.12 (4)
O2′	−1.0483 (2)	0.2925 (2)	1.1529 (2)	2.08 (4)
O3′	−1.4277 (2)	0.2439 (2)	1.3381 (2)	2.14 (4)

and

$$\sigma(|F_0|)^2 = \frac{\sqrt{(\sigma^2(I) + p^2I^2)}}{LP}$$

where σ(I) is the standard deviation of intensity I based on the ignorance factor used to down weight intense reflections ($p = 0.05$). The final R factors for the significant reflections ($I \geq 3\sigma$) are given in Table 1 together with all the necessary crystallographic and structural data. The calculations were done on

Table 3. Table of Final Fractional Positional Parameters and Their Estimated Standard Deviations in Parentheses for 3′-Deoxy Ftorafur

	X	Y	Z	B (Å^2)
N1	−0.1788 (2)	0.3417 (2)	1.2783 (3)	2.37 (3)
C2	−0.1115 (3)	0.4536 (3)	1.3849 (4)	2.47 (3)
O2	−0.1661 (2)	0.5127 (3)	1.5467 (2)	3.35 (3)
N3	0.0234 (2)	0.4907 (3)	1.2928 (3)	2.35 (3)
C4	0.0931 (3)	0.4347 (3)	1.0973 (3)	2.17 (3)
O4	0.2051 (2)	0.4836 (2)	1.0206 (2)	2.75 (3)
C5	0.0157 (3)	0.3185 (3)	1.0122 (3)	2.29 (3)
F	0.0816 (2)	0.2564 (2)	0.8285 (2)	3.21 (3)
C6	−0.1085 (3)	0.2746 (3)	1.0949 (3)	2.15 (3)
C1′	−0.3239 (3)	0.2935 (3)	1.3617 (3)	2.28 (3)
C2′	−0.4745 (3)	0.2756 (3)	1.1270 (3)	3.27 (3)
C3′	−0.5655 (3)	0.1740 (3)	1.2333 (3)	2.89 (3)
C4′	−0.4256 (3)	0.0958 (3)	1.3868 (3)	2.85 (3)
O1′	−0.2832 (2)	0.1751 (2)	1.4791 (2)	2.67 (3)
O3′	−0.6522 (2)	0.2305 (2)	1.4160 (2)	3.26 (3)

a DEC Microvax II computer with the aid of the Enraf Nonius structure determination package.[23] Atomic scattering factors were taken from the "International Tables for X-ray Crystallography,"[24] Fourier and ORTEP, by Johnson (1965).[25]

The structure of the Ftorafur molecule is shown in Fig. 1. The final fractional atomic coordinates are given in Tables 2–4 for 2′,3′-dihydroxyftoraur, 3′-hydroxyftorafur and 2′-hydroxyftorafur respectively. Figure 5 is a composite diagram giving the final bond distances and angles in the three structures. The top two values are for the dihydroxy, followed by the values for the 3′-hydroxy and the bottom two values are for the 2′-hydroxyftorafur derivative respectively. Table 5 gives the hydrogen bond distances (Å) and angles (°) in Ftorafur and the derivatives. Figure 6 gives a plot of the variation of the glycosyl bond distance in Å with the glycosyl torsion analog (χ). This figure clearly demonstrates the *gauche* effect in the Ftorafur structures which states that the higher χ values have a lower glycosyl bond distance and vice versa.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The NMR spectra were acquired both at 400 and 600 MHz using Bruker AMX-400 and AMX-600 instruments, respectively. Chemical shifts and coupling constants listed in Table 6, obtained in D_2O, were refined using the interactive spin-simulation program NMR-LAOCN-4A (Quantum

Table 4. Table of Final Fractional Positional Parameters and Their Estimated Standard Deviations in Parentheses for 2′-Deoxy Ftorafur

	X	Y	Z	B (Å^2)
Molecule A				
N1	0.7229 (3)	1.8065 (+)	0.4777 (3)	2.41 (5)
C2	0.6537 (4)	1.6080 (1)	0.4225 (3)	2.54 (1)
O2	0.5641 (3)	1.5736 (1)	0.4248 (3)	3.55 (1)
N3	0.6944 (4)	1.4631 (3)	0.3546 (3)	3.07 (1)
C4	0.7944 (4)	1.4820 (1)	0.3564 (3)	2.96 (5)
O4	0.8236 (3)	1.3380 (1)	0.3040 (3)	5.91 (2)
C5	0.8585 (4)	1.6953 (1)	0.4156 (3)	3.47 (1)
F	0.9559 (2)	1.7419 (1)	0.4107 (2)	4.85 (4)
C6	0.8222 (3)	1.8468 (1)	0.4725 (4)	3.58 (1)
C1′	0.6867 (3)	1.9614 (1)	0.5488 (3)	2.71 (2)
C2′	0.6986 (3)	1.7903 (1)	0.6384 (3)	2.44 (3)
O2′	0.6379 (2)	1.9019 (1)	0.6931 (2)	3.05 (1)
C3′	0.8184 (4)	1.8284 (1)	0.6982 (4)	2.82 (2)
C4′	0.8372 (4)	2.1154 (1)	0.6806 (4)	2.91 (1)
O1′	0.7553 (3)	2.1763 (1)	0.5819 (3)	3.33 (2)
Molecule B				
N1	0.3036 (3)	0.6497 (1)	0.0703 (3)	2.60 (5)
C2	0.2940 (3)	0.8559 (1)	0.1301 (3)	2.56 (4)
O2	0.2078 (2)	0.9190 (1)	0.1325 (2)	3.40 (1)
N3	0.3907 (3)	0.9741 (1)	0.1845 (3)	2.97 (1)
C4	0.4927 (4)	0.9130 (1)	0.1901 (4)	2.78 (1)
O4	0.5744 (3)	1.0309 (1)	−0.0416 (3)	5.12 (4)
C5	0.4930 (4)	0.6939 (1)	0.1254 (4)	2.92 (1)
F	0.5895 (2)	0.6221 (1)	0.1226 (3)	4.95 (4)
C6	0.4022 (4)	0.5767 (1)	0.0701 (4)	2.79 (1)
C1′	0.1999 (3)	0.5150 (1)	−0.1586 (4)	2.97 (1)
C2′	0.1151 (4)	0.6719 (1)	−0.0637 (4)	3.15 (1)
O2′	0.0229 (2)	0.7318 (1)	−0.0416 (3)	4.47 (1)
C3′	0.0837 (4)	0.5150 (1)	−0.1586 (4)	3.44 (1)
C4′	0.1866 (4)	0.3620 (1)	−0.1434 (4)	3.64 (1)
O1′	0.2270 (3)	0.2947 (1)	−0.0374 (2)	3.35 (1)

Chemistry Program Exchange, program #458). NOESY and COSY spectra were obtained for each of the four compounds to verify coupling patterns and to suggest the position of the H6 proton of the base in relation to the furanose ring. Figure 7 gives the NOESY spectra for 3′-hydroxyftorafur. In order to determine the sugar pucker conformations, the coupling constants resulting from quantum mechanical spin simulations, were used as input values in PSEUROT (Quantum Chemistry Program Exchange, program #463).

Compound (I) having a total of 7 ribose protons presented itself as the most difficult-to-simulate in Laocoon owing to the complex coupling patterns

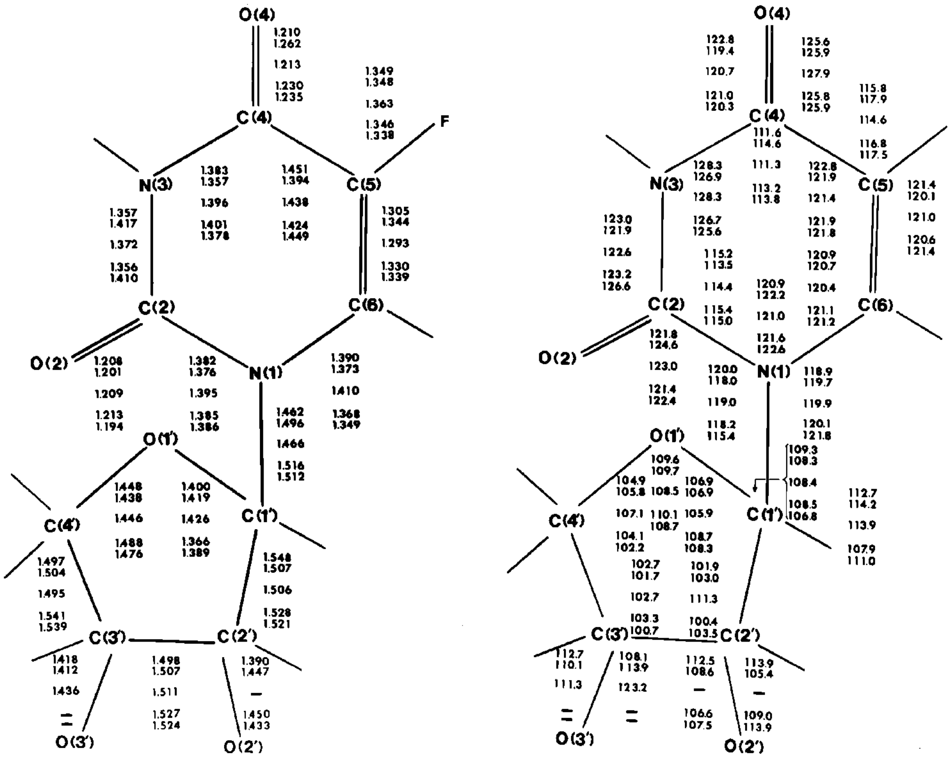


Figure 5.

arising from over 64 transitions per proton. With the use of a COSY experiment, assignments were made to each of the resonances.

Assignments for compound (II) were made from the 1D spectra by matching the coupling constants from each resonance. These assignments were also verified through a COSY experiment. Three of the five ribose resonances fell between 4.4 and 4.55 ppm, those resonances being the 2′,3′ and 4′ protons. Presumably the 4′ proton's chemical shift is further modified away from that of the 4″ through the influence of the 3′-OH residue. The final RMS value between the observed chemical shifts and coupling constants (94 total transitions) and the calculated values was 0.172, with the largest error being 0.047 Hz.

Compound (IV) has a total of 6 ribose protons, each of which was assigned first from the 1D spectra taken at 600 MHz, then again verified through a COSY spectrum. COSY crosspeaks identified couplings between 1′ and 2′, 2″, 3′ to 2′,2″, but lacked a long range coupling found in the 1D spectrum between the 2″ and the 4′ protons. An NOE experiment revealed the space couplings from the base H 6 proton to H1′, H4″ and H2′ protons. From the NOE experiment alone, the ring conformation consistent with

Table 5. Hydrogen Bond Distances (Å) and Angles (°) Observed in the Different Ftorafur Derivatives Studied

HYDROGEN BOND DISTANCES (IN Å) AND ANGLES (°)

			DISTANCES IN Å			ANGLE	
DONOR	HYDROGEN	ACCEPTOR	D-H	H...A	D...A	D-H...A	DATA SET
I FTORAFUR							
N(3)A	HN(3)A	O(2)B	0.98	1.91	2.884	170	-
N(3)B	HN(3)B	O(2)A	0.80	2.06	2.835	162	-
II 2',3'-DIHYDROXY FTORAFUR							
O(3')A	HO(3')A	O(4)A	1.06	1.75	2.719	149	(X-1, Y, 1+Z)
C(4')A	H2C(4')A	O(4)B	1.16	2.66	3.798	162	(1+X, Y, 1+Z)
N(3)B	HN(3)B	O(3')B	0.92	1.89	2.805	176	(X, Y, Z-1)
O(3')B	HO(3')B	O(4)B	1.02	2.58	3.544	159	(X, Y, 1+Z)
III 2'-HYDROXY FTORAFUR							
N(3)A	HN(3)A	O(4)B	0.86	2.07	2.870	156	(X, Y, Z)
O(2')A	HO(2')A	N(3)B	0.82	1.91	2.722	166	(1-X, ½+Y, 1-Z)
C(4')A	H2C(4')A	N(3)B	0.97	2.50	3.353	148	(1-X, +Y, 1-Z)
N(3)B	HN(3)B	O(2')A	0.62	2.29	2.908	168	(1-X, Y-½, 1-Z)
IV 3'-HYDROXY FTORAFUR							
C(2')A	H2C(2')A	O(4)	0.97	2.49	3.438	167	(X-1, Y, Z)

these cross peaks would place the 3′ OH group down or *exo* in the ring. Spin simulation of experimental transitions generated 792 transitions, each of which was assigned and fitted to experimental spectra with an RMS value of 0.075 Hz. The largest error was 0.013 Hz. PSEUROT yielded an overall RMS figure of .234 while allowing all variables to remain unconstrained; 87.9 percent of the molecule was in the 3′ *exo* form, with the remainder being in C3′ *endo* (phase amplitude of only 5).

Compound (III) was chronologically the first compound to be studied and was the first to exhibit the long range coupling between the 2′ and the 4″ protons. Long range coupling is possible when the conformation between the protons assumes a zigzag pattern, thus maximizing the backlobe orbitals optimal influence upon one another. A total of 223 calculated transitions were assigned experimental peaks, yielding a final RMS value of 0.160 Hz, with a maximum error of 0.30 Hz. Fitted chemical shifts and coupling constants were then used to calculate the pseudo-rotational parameters.

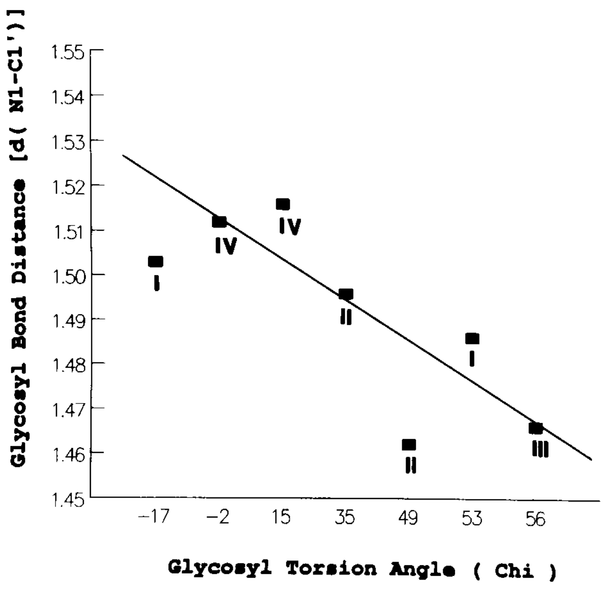


Figure 6. Graph showing the variation of the glycosyl torion angle (χ) with the glycosyl bond length d[N1-C1′].

COMPARISON OF X-RAY AND NMR

The structural differences are given in Table 7. Despite the definite angle given for χ in the solution states, it was apparent in most cases that at least two positions *syn* and *anti* were populated in Ftorafur and in two of its derivatives. P in Table 7 is the pseudo-rotation phase angle and MF indicates the mole fraction present. The number of hydrogen bonds available to

Table 6. Table of Chemical Shifts (in PPM)

Compound (ppm):	H6	H1′	H2′	H2′	H3′	H3″	H4′	H4″
I	7.88	6.05	2.16	2.42	2.03	2.10	4.02	4.31
II	7.91	5.85	4.77	—	4.71	—	4.32	4.73
III	7.9	6.28	2.26	2.62	4.75	—	4.13	4.35
IV	7.80	5.76	4.54	—	2.15	2.07	4.26	4.56

Table of Coupling Constants (in Hertz)

Compound J(hz)	H6,F	H6,1′	1′,2′	1′,2″	2′,3′	2′,3″	2″,3′	2″,3″	3′,4′	3′,4″	3″,4′	3″,4″
I	6.43	1.45	6.60	4.64	NA	NA	NA	NA	NA	NA	NA	NA
II	6.31	1.34	5.81	—	4.45	—	—	—	4.08	2.11	—	—
III	6.47	1.46	7.08	6.47	5.25	—	1.54	—	1.54	3.66	—	—
IV	6.42	1.24	1.24	—	4.92	2.27	—	—	6.84	8.49	6.84	3.29

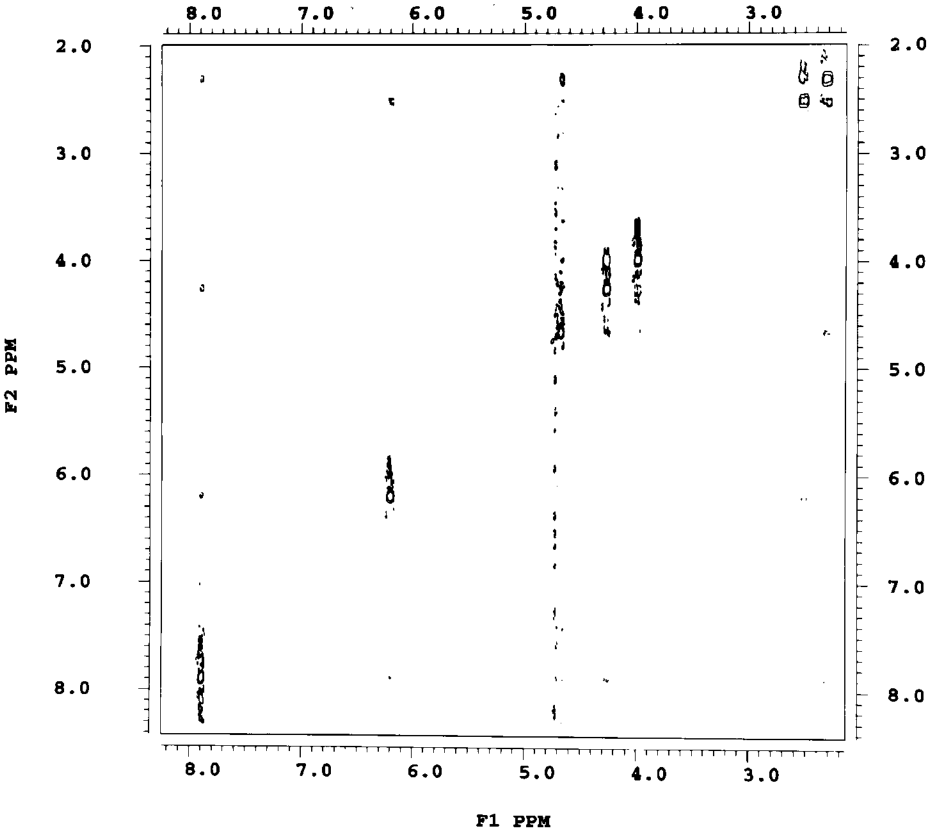


Figure 7. A NOESY spectrum of 3′-hydroxyftorafur.

Table 7. Parameters of Crystal and Solutions Structures

Compound	I	II	III	IV
X-ray				
R_1	H	OH	OH	H
R_2	H	OH	H	OH
χ_{CN}	53°	50°	57°	15°
	− 17°	35°	—	− 2°
τ_m	36°	36°	38°	38°
	41°	35°	—	− 39°
P	38°	196°	176°	351°
	180°	6°	—	220°
NMR				
τ_m	NA	45	37	36.4
P_1/MF*	NA	201/.79	207/.88	7/.88
P_2/MF*	NA	56/.21	33/.12	229/.12
RMS	NA	.32	.23	.6

compounds in the crystal (solid) state combined with the packing forces restrict any significant χ movement. In aqueous solution however, χ movement is seemingly unrestricted, as are the puckering motions of the ribose ring puckers. Despite the solution-state's increased mobility, much of the X-ray conformations are preserved at least in part (% mol fraction, and X-ray conformer and % phase change) in the solution phase.

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