Fluorination of uracil with acetylhypofluorite and fluorine in acetic acid: mechanistic investigation

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Fluorination of uracil with acetylhypofluorite and fluorine in acetic acid is described. The influence of the acetate ion on the products of the fluorination reaction was examined. Two stereoisomers (*cis* (**3**) and *trans* (**4**)) were found in the reaction mixture following fluorination in the absence of the acetate ion, but only one isomer (**4**) and 5-fluorouracil were found when the acetate ion was present during the fluorination reaction. ^2H nuclear magnetic resonance revealed that acetate from the solution containing acetate ion rather than from the residue from acetylhypofluorite binds to position 6 of uracil to form intermediates. The synthesis yields 5-fluorouracil isolated as pure compound in a chemical yield, relative to the fluorine introduced, of about 45%. The influence of inorganic cations on the yield of acetylhypofluorite was also evaluated.

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On décrit la fluoration de l'uracil à l'aide de l'hypofluorite d'acétyle et de fluor dans l'acide acétique. On a étudié l'influence de l'ion acétate sur la nature des produits de la réaction de fluoration. Lors des réactions effectuées en l'absence d'ions acétates, on a démontré que les mélanges réactionnels contiennent deux stéréoisomères, soit les produits *cis* (**3**) et *trans* (**4**); toutefois, les mélanges obtenus après des réactions effectuées en présence d'ions acétates ne contiennent que du fluoro-5 uracil et de l'isomère **4**. La rmn du ^2H permet de démontrer que ce sont les ions acétates de la solution plutôt que ceux de l'hypofluorite d'acétyle qui se lient à la position 6 de l'uracil pour former des intermédiares. La synthèse conduit au fluoro-5 uracil qui a été isolé à l'état pur, avec un rendement, basé sur la quantité de fluor utilisé, qui est d'environ 45%. On a ausi évalué l'influence des cations inorganiques sur le rendement de l'hypofluorite d'acétyle.

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Introduction

^{18}F-labelled 5-fluorouracil (5-[^{18}F]FU) has been suggested as a tracer for in vivo investigation of the pharmacokinetics of uracil (1). Since its metabolic fragment continues to carry a positron-emitting radioisotope, the ^{18}F label, it can be used to investigate, in vivo, by means of positron emission tomography (PET), the metabolism of 5-[^{18}F]fluorouracil (2), possible incorporation of the [^{18}F]-labelled fluorouracil into RNA (3) in tumors, and, with this, the rate of RNA synthesis. The rate of RNA synthesis might, in turn, provide some indication of the activity of a tumor, which could be useful in assessing tumor therapy (4). Recently, the tissue distribution of 5-[^{18}F]fluorouracil was extensively evaluated in tumor-bearing animals (5, 6) and in humans with cancer (4). The results of these studies suggest that 5-fluorouracil indeed accumulates in different animal (5, 6) and human tumors (4).

Although attempts have been made to explain the reaction mechanism of the fluorination of uracil (7), it has never been completely understood. The intermediate, 5-fluoro-6-acetoxy-5, 6-dihydrouracil isomers **3** or **4** (Fig. 1), was isolated in 1979, but details of the reaction mechanism and stereochemical structure remained unexplained (7).

Uracil was first fluorinated with trifluoromethylhypofluorite (CF_3OF) (8). The synthesis of 5-[^{18}F]fluorouracil (**5**) (5-[^{18}F]FU) was first reported in 1973 (9) and the fluorination of uracil with [^{18}F]F_2 in acetic acid was reported in 1979 along with isolation of an intermediate. Recently we also reported a convenient high-yield synthesis of 5-[^{18}F]FU (10). In the present paper the isolation of intermediates produced in the fluorination of uracil, as well as their nmr spectra, is reported. Although positive stereochemistry of these intermediates could not be established by nmr spectra or chemical reactivity, we can now suggest a structure for these intermediates.

Results and discussion

Reactions of trifluoroacetylhypofluorite with stilbene (11), acetylhypofluorite with triacetylglucal (12–15), and trifluoromethylhypofluorite with uracil (16) have been reported. In all these cases, 100% regiospecificity was reported. Stereoselectivity in the syntheses of these fluoro compounds was also very good, but not always 100% (11, 20). This stereoselectivity is apparently influenced by the hardness of the base involved in the reaction (11). A mixture of *threo* and *erythro* forms was produced in the stilbene reaction with acetylhypofluorite in the presence of sodium acetate (11, 17). An addition of acetylhypofluorite across the double bond in triacetylglucal also resulted in two stereoisomers (18–20). Fluorination of uracil with molecular fluorine in acetic acid has also been reported, but the details of the reaction mechanism have never been established (7, 9, 22, 23).

Our results show that two major stereoisomeric adducts, *cis*- and *trans*-5-fluoro-6-acetoxy-5, 6-dihydrouracil, **3** and **4**, are produced by fluorination of uracil with molecular fluorine in acetic acid (reagent grade), or with acetylhypofluorite in acetic acid; however, 5-fluorouracil and one isomer, **4**, are found in the crude reaction mixture under both reaction conditions when an acetate ion is present during the fluorination reaction (Table 1). The ^1H nmr and ^{19}F nmr spectra were measured on the

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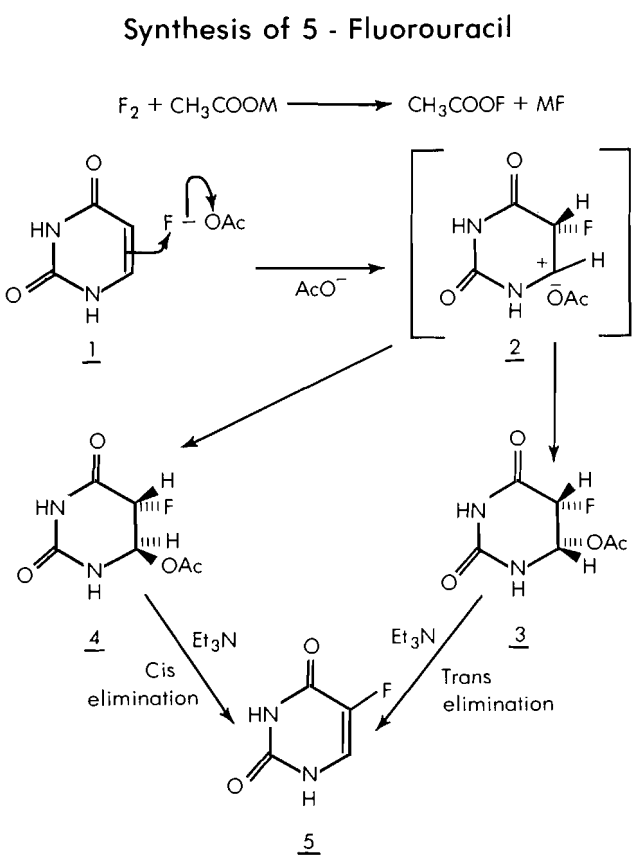

FIG. 1. Reaction scheme for the fluorination of uracil, **1**, with acethylhypofluorite.

relatively crude adducts prepared by fluorination of uracil (uracil present during bubbling) with F_2 in acetic acid (reagent grade). When acetate salt was not present during the reaction, the spectra revealed the presence of two geometric isomers (*trans* (**4**) and *cis* (**3**)) in a ratio of 1:2, but no 5-fluorouracil (Table 1). But when uracil was added (fluorination with CH_3COOF) after the end of bubbling, the ratio between *cis* (**3**) and *trans* (**4**) isomers was 4:1, and no 5-fluorouracil was found (Table 1). The presence of two geometric isomers indicates that the reaction does not have complete stereoselectivity. In contrast, in the presence of added acetate ion the spectra taken several hours after the end of bubbling showed the presence of a single isomer and 5-fluorouracil, suggesting that one of the isomers (Table 1) does not survive to the end of the reaction. We note that the reaction done in distilled acetic acid gave *trans* (**4**) and *cis* (**3**) isomers in a ratio of 2.5:1. This suggests that the removal of traces of impurities changes the mechanism of the reaction.

The ^1H and ^{19}F nmr spectra from positions 5 and 6 of the diastereomers were analyzed using iterative computer fitting, but positive identification of the stereochemistry at these positions could not be established on the basis of the coupling constants. Thus, $^3J_{H5-H6}$ is 3.9 and 4.3 Hz in **3** and **4**, respectively, while $^3J_{F5-H6}$ is 2.8 and 2.2 Hz in **3** and **4**, respectively. These values are too similar in magnitude to permit an unequivocal assignment of the configuration on the basis of the evidence of a coupling constant alone. Values of 4 and 2 Hz have also been reported for $^3J_{H5-H6}$ and $^3J_{F5-H6}$, respectively, for an isomer of 5-fluoro-6-acetyoxy-5,6-dihydrouracil prepared by fluorination of uracil in acetic anhydride – 5% acetic acid (23). Stereochemistry was not assigned (23).

TABLE 1. Ratios between *cis* (**3**) and *trans* (**4**) isomers formed under different fluorination conditions

Reaction medium	Ratio **3**:**4**	Acetate ion	Fluorinating reagent
AcOH (reagent)	Only **4***	Yes	F_2
AcOH (reagent)	2:1†	No	F_2
AcOH (distilled)	1:2.5†	No	F_2
AcOH (reagent)	4:1‡	No	CH_3COOF
AcOH (reagent)‡	8:1§	Yes	CH_3COOF

*Only one isomer (*trans* (**4**)) and 5-FUr found after evaporation of AcOH.
†Uracil present during bubbling.
‡Uracil added after the end of bubbling.
§Present in crude reaction mixture (before evaporation of AcOH), but after evaporation or about three hours after bubbling only *trans* (**4**) and 5-FUr were present.

Our results also suggest that the acetate ion is a strong enough base to facilitate acetic acid elimination in one of the isomers. This was confirmed in experiments where acetate ion was added to the crude reaction mixture containing the *cis*–*trans* mixture, ^{19}F nmr taken a few hours later showed that CH_3COOH was eliminated from the isomer assigned the *cis* configuration for that isomer (**3**), and the mixture contained only 5-fluorouracil and the *trans* isomer (**4**) in the same ratio in which the two isomers were present in the solution without acetate ion. The ease with which the *cis* isomer loses CH_3COOH supports our assignment, because the *cis* isomer (**3**) has H and CH_3COOH in the *trans* configuration favoured for the elimination of CH_3COOH.

Fluorination with fluorine

When fluorination with fluorine was carried out in the presence of an acetate ion, 60% of the fluorine was found in 5-fluorouracil (^{19}F nmr spectra), and the remainder (36–38%) in one stereoisomer (**4**), with a small admixture of 5,6-difluoro compound (2–4%). Fluorination of uracil with F_2 in CH_3COOH or CD_3COOD also yielded a minor product, which accounted for about 2–4% of the total F_2 approximately 1 h after introduction of F_2 was stopped. This compound was tentatively identified by its ^{19}F nmr spectrum as 5,6-difluoro-5,6-dihydrouracil. The $^{19}F\{^1H\}$ spectrum appeared as an AB quartet, $^3J_{F5-F6}=7.9$ Hz. When the fluorination reaction was carried out in CD_3COOD, the ^{19}F multiplet at δ −197.9 ppm showed loss of an H–F coupling (hydrogen on nitrogen-1). In consequence, this multiplet was assigned to F6, and the multiplet at −195.6 to F5. The geminal coupling constants, $^2J_{H5-F5}$ and $^2J_{H6-F6}$, were both 45.8 Hz.

Vine *et al.* (7), who investigated the fluorination of uracil with molecular fluorine in acetic acid with the addition of 25% (by volume) of acetic anhydride, reported the ^1H nmr spectrum of a single isomer with δ 2.13 (CH_3), 5.48 (J_{H5-F5} = 47 Hz, $J_{H5-H6}=4.5$ Hz, H5), and 6.27 ($J_{H5-H6}=45$ Hz, $J_{H6-F5}=2.22$ Hz, H6) ppm in acetic acid-d_4. They assigned the *cis* (H5—H6) configuration to this isomer on the basis of the magnitudes of the coupling constants. However, our experimental data on the two isomers show that the coupling constants are too similar to permit an unequivocal stereochemical assignment on the basis of coupling constants alone. In our work, the isomer produced in the fluorination of uracil in the presence of inorganic acetate has a ^1H nmr spectrum very similar to that reported by Vine *et al.* (7). The only difference is the value of J_{H-F} (geminal), for which 47 Hz was reported, compared with our value of 45.14 Hz. When we repeated the procedure of Vine *et al.* (7) we

TABLE 2. Yield of acetylhypofluorite as a function of acetate/fluorine ratio and cation

Cation X	CH_3COOX/F_2 (mmol/mmol)	Yield (%)	Number of determinations
Na^+	0.096	53 ± 3	2
	0.29	53 ± 4	2
	1.92	69 ± 3	3
	0.29	83 ± 4	3
	4	75 ± 3	3
	8	75 ± 3	2
	12	80 ± 2	3
	160	68 ± 3	3
K^+	0.9	71 ± 2	2
	1.9	78 ± 3	3
	2.9	90 ± 4	3
NH_4^+	0.096	55 ± 3	2
	0.29	60 ± 3	3
	1.9	70 ± 2	3
	2.9	90 ± 3	3
H^+	Glacial acetic acid	30 ± 3	3

obtained an isomer with J_{H-F} (geminal) = 45 Hz, which agrees well with the value obtained for the compound prepared by other procedures. On the basis of the nmr evidence, we conclude that the compound prepared by Vine *et al.* (7) is identical to the one we synthesized in the presence of an acetate ion. Our work indicates that the stereochemical assignments of Vine *et al.* (7), made on the basis of coupling constants, may not be correct. We consider that the opposite configuration is more likely.

The *trans* configuration of the intermediate 5-fluoro-6-acetoxy-5,6-dihydrouracil, **4**, obtained in the reaction of uracil and the fluorinating agent in the presence of inorganic acetate, was assigned on the basis of the observation that acetate from solution, rather than residue from CH_3COOF, adds in the 6 position and that this isomer is relatively more stable towards elimination of CH_3COOH. (See the experiments with deuterated acetate, analyzed by ^2H nmr, and discussion of possible reaction mechanisms.) The *trans* configuration of the intermediate is in accordance with other reactions involving ionic mechanisms, in which a halogen in the presence of a nucleophile is added to a double bond, yielding a compound with halogen and nucleophile in a *trans* configuration (24).

Indirect evidence for the stereochemistry of the *cis* isomer, **3**, is obtained from ^{19}F chemical shifts. This isomer has a chemical shift (δ −211.0 ppm) similar to *cis*-5-fluoro-6-methoxy-5,6-dihydrouracil (δ −208 ppm), for which the structure was positively assigned by X-ray crystallography (16). In this case, the fluorination reaction with CF_3OF proceeds with complete stereoselectivity (16), with only *syn* addition, and there is conclusive evidence that the nucleophile (methoxy) which adds to position 6 comes from the solvent rather than from the trifluoromethoxy residue of the CF_3OF (16). Values of $^3J_{H5-H6}(cis)$ = 4.0 and $^3J_{F5-H6}(trans)$ = 2.2 Hz were reported for *cis*-5-fluoro-6-methoxy-5, 6-dihydrouracil (16), comparable to the values noted above for the *cis*-acetoxy analogs (22). The ease with which stereoisomer **3** loses CH_3COOH also supports the assignment of the structure.

In the absence of added acetate ion, it is possible that fluorination (F_2 in CH_3COOH) of uracil proceeds through direct fluorination and *in situ* synthesis of acetylhypofluorite, which adds to the 5,6-double bond through a cation, **2**, (Fig. 1). The cation formed after the addition of fluorine (from F_2 or CH_3COOF) at C-5 will be more stable than fluorocarbon cations (16). This would allow the acetate ion to attack from either side and form two stereoisomers (equal probability would be expected only when the ion is planar and there is no hindrance of a nucleophilic attack).

The ratio between *trans* (**4**) and *cis* (**3**) isomers in the fluorination with F_2 in acetic acid differs from that observed in the fluorination with CH_3COOF (discussed later) (Table 1). This suggests that the reaction of molecular fluorine with uracil to form an intermediate occurs at a higher rate than that of fluorine with acetic acid to form acetylhypofluorite. The observation is not the same as that recently reported by Shiue *et al.* (21), who found no difference in the products in the fluorination of 2′,3′-di-O-acetyl-5′-deoxyuridine resulting from the two reactions.

Fluorination with acetylhypofluorite

The acetylhypofluorite yield is lower when it is synthesized in acetic acid without additional acetate ion (see Table 2 and ref. 13). As shown in Table 2, our results indicate that the yield of acetylhypofluorite is more a function of concentration than of the cation used. It is evident that the hypofluorite yield reaches a broad maximum when sodium acetate is used, while in other cases it increases with the concentration of acetate. A similar result was reported by Fowler *et al.* (13).

Fluorination of uracil with acetylhypofluorite (generated *in situ*) yielded intermediates **3** (*cis*) and **4** (*trans*) in different ratios (8:1) than fluorination with F_2, where it was 2:1 (Table 1). Fluorination done with acetylhypofluorite with acetate ion present during the reaction yielded mostly 5-fluorouracil (probably formed from *cis* isomer **3**) and a minor compound, *trans* isomer **4**. However, the fluorination of uracil with acetylhypofluorite when acetate ion was not present gave a ratio of 4:1 between the *cis* (**3**) and *trans* (**4**) isomers (Table 1).

That the incorporated acetate comes from the free acetate ion rather than from acetylhypofluorite was established by a labelling experiment (monitored by ^2H nmr) using deuterated acetate added to the reaction mixture after fluorine bubbling was stopped, but before uracil was added. No exchange between acetylhypofluorite and CD_3COO^- was observed. In this experiment, only one isomer was found, with deuterated acetate in position 6 of the intermediate. It was assigned the *anti*-configuration (*trans* (**4**)) as discussed above. The presence of deuterated, rather than regular, acetate in position 6, confirmed by ^2H nmr, supports the notion that the free acetate ion adds to position 6.

Fluorination of uracil with acetylhypofluorite also yields a small amount (2–4%) of the 5,5-difluoro adduct, 5,5-difluoro-6-acetoxy-5,6-dihydrouracil, identified by $^2J_{FF}$ = 284.2 Hz and the vicinal coupling constants $^3J_{FH}(cis)$ = 1.99 Hz and $^3J_{HF}(trans)$ = 6.73 Hz. The 6-methoxy analog of this compound has been reported by Robins *et al.* (16).

^{18}F-labelled 5-fluorouracil

The reaction scheme given in Fig. 1 for a non-radioactive synthesis was also applied when the ^{18}F-labelled compound was synthesized (10). ^{18}F-acetylhypofluorite was prepared first, and allowed to react *in situ* with uracil in the presence of acetate ion, yielding the intermediate, 5-[^{18}F]fluoro-6-acetoxy-5,6-dihydrouracil, **4**, which easily loses acetic acid, giving 5-[^{18}F]-fluorouracil (10).

Summary

On the basis of stability towards CH_3COOH, elimination of

one isomer, and the comparison of the ^{19}F chemical shifts with those of a related compound positively identified by X-ray crystallography (16), the stereochemistry of two intermediates formed in the fluorination reaction is suggested.

It was also observed that the ratio of *trans* (**4**) to *cis* (**3**) is greatly influenced by the purity of acetic acid, giving a ratio of 2.5:1 when the fluorination with F_2 was done in distilled acetic acid rather than in reagent grade, where it was 1:2. The fluorination in the presence of an acetate also yielded two isomers, but one (*cis* (**3**)) did not survive the work-up. Chemical and radiochemical yields were similar in both synthetic routes.

Experimental

General

Research grade chemicals obtained from regular suppliers were used in the syntheses. Thin-layer chromatography (tlc) was done on silica gel hard layer plates with a fluorescence indicator (254 nm). Two solvents were used for development of tlc plates: A, THF–hexane (65:35) and B, ethyl acetate – H_2O–HCOOH (13:1:1). ^{18}F compounds were investigated by visual identification under 254-nm uv light and by thin-layer radiochromatography, using a scanner with a windowless gas-flow proportional detector with a 1-mm opening. The final product was purified by simple filtration through a Sep-Pak column, using THF–hexane (40:6) as an elution solvent. In "cold" reactions, identification of reaction products was done by ^{19}F nmr, ^1H nmr, ^2H nmr, mass spectrometry, melting point, and tlc on silica gel. Reactions were followed by tlc.

The ^1H nmr spectra were obtained at 200 MHz on a Varian XL-200 spectrometer using DMSO-d_6 or acetic acid-d_4 solutions, with TMS as an internal reference. The ^{19}F nmr spectra were obtained at 75.39 MHz on a Bruker WP-80SY spectrometer, on a reaction mixture in acetic acid, acetic acid-d_4, or DMSO-d_6, using trichlorotrifluoroethane (^{19}F chemical shift, δ −82.20 ppm) as an external reference. Iterative analysis of nmr spectra was carried out using the PANIC program on a Bruker Aspect 2000 computer.

An authentic sample of 5-fluorouracil, used as a reference, was isolated from the drug, fluorouracil (Roche), by lowering the pH to about 6.5 (mp (uncorrected) 281°C (lit. (**25**) mp. 282–283°C).

In the first set of experiments, uracil (60–80 mg, 0.5–0.7 mmol) was fluorinated with acetylhypofluorite (prepared *in situ*) in acetic acid (10 mL) and acetic acid-d_4 (10 mL), with and without inorganic acetate.

In the second set of reactions, uracil (80 mg, 0.7 mmol) was suspended in glacial acetic acid, acetic acid-d_4, or in those acids (10 mL) in the presence of an acetate (deuterated or regular). Fluorine (0.1–0.6 mmol) in nitrogen was then introduced. The work-up was carried out as described later under B. In some experiments, the intermediates were analyzed directly in the acid solutions by ^1H nmr (deuterated acids) and ^{19}F nmr (regular and deuterated acids). When the intermediates were isolated, ^1H and ^{19}F nmr spectra were taken in DMSO-d_6 solution. In experiments done in acetic acid to which sodium deuteroacetate had been added, ^2H nmr spectra were taken on a crude reaction mixture.

A. *Reaction of acetylhypofluorite with uracil*

Acetylhypofluorite was prepared by reacting a 0.5–5% mixture of fluorine (0.1–0.6 mmol of F_2) in nitrogen with acetic acid in the presence of added acetate (generally 0.15 *M*) or with acetic acid without an acetate ion present, as described earlier (11–15). The presence of an OF group was established in the reaction mixture (from which all F_2 had been removed) by the appearance of a signal at 161.9 ppm in the ^{19}F nmr spectrum (acetic acid or acetic acid-d_4). At room temperature (about 22°C) the half-life of acetylhypofluorite is 20 min, as measured by ^{19}F nmr.

Acetylhypofluorite was allowed to react with uracil (80 mg, 0.7 mmol) by suspending it in a solution of hypofluorite in acetic acid, with (first set) and without (second set) added acetate ion. The reaction mixture from the first set was divided into two parts and a different work-up carried out on each. In the first part, acid was evaporated under reduced pressure (0.1 Torr; 1 Torr = 133.3 Pa) at a bath temperature of 60°C, the residue redissolved in DMSO-d_6 or acetic acid-d_4, and ^1H and ^{19}F nmr spectra taken. When the reaction was carried out in the presence of added acetate ion, *trans*-5-fluoro-6-acetoxy-5,6-dihydrouracil, **4**, was present, together with 5-fluorouracil. After evaporation of acetic acid, the residue proved to be over 80% final product, 5-fluorouracil, **5**, and only about 16–18% intermediate **4**. The second part was worked up by removing acid under reduced pressure and adding 0.7 mL of triethylamine to the residue. This was followed by a brief heating in a 60°C bath. The heating resulted in the complete elimination of CH_3COOH from the intermediate, giving pure 5-fluorouracil (**5**) in a quantitative yield.

In the second set (reaction done without addition of an acetate), the reaction mixture was divided and, on the first part, a similar work-up was done by evaporation under reduced pressure (0.1 Torr) in a 60°C bath. After evaporation, the residue consisted of about 88% of intermediate **3** and 12% of **4**, but no 5-fluorouracil in the first half. The second half of the reaction mixture was worked up with triethylamine as described above. Addition of 0.7 mL of triethylamine to the residue after evaporation, followed by brief heating in a 60°C bath, resulted in loss of CH_3COOH in both **3** and **4**, yielding 5-fluorouracil (**5**) or ^{18}F-labelled 5-fluorouracil. After evaporation of triethylamine, the residue was extracted with 1 mL of tetrahydrofuran–hexane (40:60) mixture, an the 5-fluorouracil or ^{18}F-labelled 5-FU was purified on a silica gel Sep-Pak column (10).

Purification of intermediate **3** was done only when working with non-radioactive materials. After fluorination in reagent grade acetic acid (no acetate added), isolation and purification were achieved using liquid chromatography (hplc) on silica gel with THF–hexane (35:75) as the solvent. It was not possible to determine the melting point because the intermediate was thermally unstable.

trans-*5-Fluoro-6-acetoxy-5,6-dihydrouracil (**4**)*

The ^1H nmr spectrum (acetic acid-d_4 δ : 2.07 (s, 3H, CH_3COO), 5.49 (d of d, 1H, $^2J_{H5-F5}$ = 45.1 Hz, $^3J_{H5-H6}$ = 4.27 Hz, H5), 6.31 (d, of d, 1H, $^3J_{H5-H6}$ = 4.38 Hz, $^3J_{H6-F5}$ = 2.26 Hz, H6); ^{19}F nmr spectrum (acetic acid-d_4) δ : −212.0 (d of d, 1F, $^2J_{F5-H5}$ = 45.24 Hz, $^3J_{F5-H6}$ = 2.19 Hz); (acetic acid/DMSO-d_6) δ : −212.0 (d of d, 1F, $^2J_{F5-H5}$ = 45.17 Hz, $^3J_{F5-H6}$ = 2.9 Hz). The ^{19}F nmr spectrum was best simulated by including a $^4J_{F5-H1}$ coupling of 2.2 Hz. The ^{19}F nmr spectra were analyzed by iterative computer fitting; TLC of the crude reaction mixture showed spots with R_f = 0.5 (5-FUr) and 0.81 in solvent A (*trans* (**4**) isomer). On heating, compound **4** was converted into 5-fluorouracil, mp 283°C, M^+ 130; tlc of the purified product showed R_f of 0.5 and 0.79, identical with an authentic sample of 5-fluorouracil, using, as developing solvent, solvent mixture A and solvent mixture B, respectively.

When the reaction was carried out in acetic acid in the presence of perdeuteroacetate, or in acetic acid-d_4 in the absence of acetate ion, the ^1H nmr spectrum of the reaction mixture showed no signal at 2.07 ppm, and it was unnecessary to use J_{F5-H1} in the simulation of the ^{19}F spectrum of the *trans* isomer.

Following fluorination with acetylhypofluorite in acetic acid (produced in acetic acid but no acetate), the solvent was evaporated and the resaidue dissolved in DMSO-d_6. The nmr analysis showed 10% of the residue to consist of 5-fluorouracil, the bulk of the remaining material being **3** and **4** in a 4:1 ratio. However, the ^{19}F nmr of the reaction mixture before evaporation had only two isomers present. A small amount (2–4%) of 5,5-difluoro-6-acetoxy-5,6-dihydrouracil, identified by its ^{19}F spectrum (acetic acid or acetic acid-d_4), was always present. On evaporation of the solvent, the 5,5-difluoro adduct was transformed into **3**.

5,5-Difluoro-6-acetoxy-5,6-dihydrouracil

The ^{19}F spectrum (acetic or acetic acid-d_4 δ: −114.3 ($^2J_{F5-F5'}$ = 284.20, $^3J_{F5-F6}$ (*cis*) = 1.99 Hz, F5), −132.0 ($^3J_{F5'-H6}$(*trans*) = 6.73 Hz, F5'). On broadband ^1H decoupling, the ^{19}F spectrum consisted of an AB quartet.

B. *Fluorination of uracil in acetic acid with molecular fluorine*

When uracil (0.7 mmol) suspended in acetic acid (10 mL) was

allowed to react with molecular fluorine (~0.5 mmol) in the absence of added inorganic acetate, a mixture of 1 part *trans*- (**4**) and 2 parts *cis*-5-fluoro-6-acetoxy-5,6-dihydrouracil (**3**) was obtained (the stereochemical assignments are given under Results and discussion). After evaporation of acetic acid and addition and subsequent evaporation of triethylamine, both isomers yielded 5-fluorouracil, with properties (mass spectrum, ^{1}H and ^{19}F nmr spectra, and melting point) identical to an authentic sample.

cis-5-Fluoro-6-acetoxy-5,6-dihydrouracil (3)

^{1}H nmr spectrum (acetic acid-d_4) δ : 2.10 (s, 3H, CH_3COO), 5.30 (d of d, 1H, $^2J_{F5-H5}$ = 46.69 Hz, $^3J_{H5-H6}$ = 3.96 Hz, H5), 5.34 (d of d, 1H, $^3J_{F5-H6}$ = 2.29 Hz, $^3J_{H5-H6}$ = 3.02 Hz, H6); ^{19}F nmr spectrum (acetic acid and acetic acid-d_4 δ : −211.0 (d of d, 1F, $^2J_{H5-F5}$ = 46.11 Hz, $^3J_{H6-F5}$ = 2.76 Hz). The ^{19}F spectra were analyzed by computer fitting. The ^{19}F spectrum of the acetic acid solution was best computer-simulated by allowing for a J_{F5-H1} coupling of 2.2 Hz. The *trans* isomer (**4**) had chemical shifts and coupling constants as described above.

5-Fluorouracil was identified by mp (281–283°C), ^{1}H and ^{19}F nmr spectra, and mass spectrometry (M^+ 130). The ^{1}H nmr spectrum in DMSO-d_6 was identical to that of an authentic sample of 5-fluorouracil.

In distilled acetic acid, 5-fluorouracil and the *trans* isomer **4** were found in the reaction crude when fluorination was done in the presence of an acetate ion, but a mixture of *trans* (**4**) and *cis* (**3**) isomers (in a ratio of 2:1) was formed when the reaction was carried out without addition of acetate ion. About 4% of 5,6-difluoro-5,6-dihydrouracil was identified in the reaction mixture by its ^{19}F nmr spectrum.

5,6-Difluoro-5,6-dihydrouracil

The ^{19}F nmr spectrum (acetic acid) δ : −195.6 (d of d, 1F, $^2J_{F5-H5}$ = 45.78 Hz, $^3J_{F5-F6}$ = 7.90 Hz, F5), −197.9 (d of d, 1F, $^2J_{F6-H6}$ = 45.78 Hz, $^3J_{F5-F6}$ = 7.90 Hz, F6).

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