Syntheses of 5'-Deoxy-5-[^{18}F]Fluorouridine and Related Compounds As Probes for Measuring Tissue Proliferation In Vivo

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SUMMARY

A rapid synthesis of 5'-deoxy-5-[^{18}F]fluorouridine (3a) from either [^{18}F]F_2 or $CH_3CO_2{}^{18}F$ is described. Fluorination of 2',3'-di-O-acetyl-5'-deoxyuridine (1a) with either [^{18}F]F_2 or $CH_3CO_2{}^{18}F$ in glacial acetic acid at room temperature followed by hydrolysis with sodium methoxide in methanol gives 5'-deoxy-5-[^{18}F]fluorouridine (3a) in radiochemical yields of 20-25% in a synthesis time of 80 min from EOB. The same method also has been used to synthesize 5-[^{18}F]fluorouridine (3b) and 2'-deoxy-5-[^{18}F]fluorouridine (3c) in similar radiochemical yields. Acetyl hypofluorite (CH_3CO_2F) is proposed as an intermediate for these reactions.

KEY WORDS: 5'-Deoxy-5-[^{18}F]fluorouridine; 5-[^{18}F]fluorouridine; 2'-Deoxy-5-[^{18}F]fluorouridine; [^{18}F]F_2; Acetyl hypofluorite

INTRODUCTION

The rapid development of positron emission tomography coupled with a rational choice of radiopharmaceuticals has made it possible to carry out the in vivo study of dynamic biochemical processes. For example, 2-deoxy-2-[^{18}F]fluoro-D-glucose is now widely used for the measurment of regional glucose metabolism in humans in normal and disease states (1). The principle of this technique could also apply to measuring tissue proliferation in vivo by using suitably labeled probes. Carbon-11 labeled thymidine has been used in tracer studies of DNA synthesis in mice and tumor bearing animals (2,3). The use of labeled uridine or uridine analogs could also provide an index of tissue proliferation via increased transport and increased intracellular phosphorylation and incorporation into RNA (4). Therefore, if uridine or its

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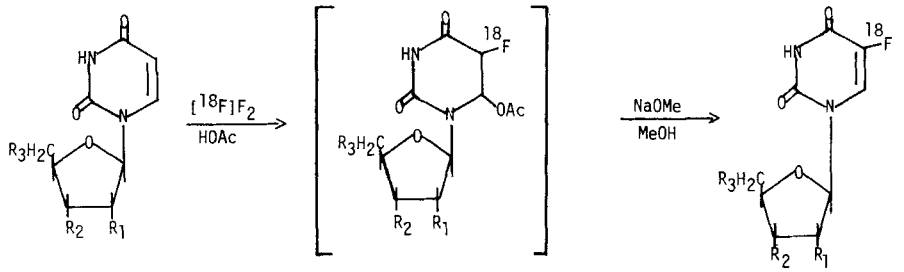
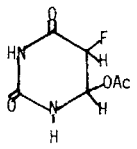


1 (a) R_1=R_2=OAc, R_3=H
(b) R_1=R_2=R_3=OAc
(c) R_1=H, R_2=R_3=OAc

2 (a) R_1=R_2=OAc, R_3=H
(b) R_1=R_2=R_3=OAc
(c) R_1=H, R_2=R_3=OAc

3 (a) R_1=R_2=OH, R_3=H
(b) R_1=R_2=R_3=OH
(c) R_1=H
R_2=R_3=OH



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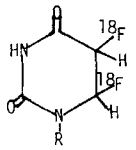

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Scheme 1

analog is labeled with a positron emitter, it might be feasible to measure tissue proliferation in vivo. Because of the favorable characteristics of ^{18}F($t_{1/2}$ = 110 min, 97% positron emission), ^{18}F-labeled compounds have been studied most extensively. The uridine analogs, 5-fluorouracil, 5-fluorouridine and 2'-deoxy-5-fluorouridine have been synthesized by direct fluorination of uracil, 2',3',5'-tri-O-acetyluridine and 3',5'-di-O-acetyl-2'-deoxyuridine either with trifluoromethyl hypofluorite or with molecular fluorine (5,6). The fluorine-18 labeled analog, 2'-deoxy-5-fluorouridine (3c) has been synthesized either by recoil ^{18}F labeling of 2'-deoxyuridine or by reaction of 2'-deoxyuridine with elemental $[^{18}F]F_2$ in glacial acetic acid in low yield (7). 5-[^{18}F]Fluorouracil, on the other hand, has been prepared in moderate yield by direct fluorination of uracil with $[^{18}F]F_2$ in either trifluoroacetic acid or in a mixture of glacial acetic acid and acetic anhydride (8,9).

Fluorine-18 labeled uridine and uridylate also have been synthesized chemically and enzymatically (10,11) and used as an index of tissue proliferation via increased transport and increased intracellular phosphorylation and incorporation into RNA (10,12). Recently, 5'-deoxy-5-fluorouridine has been synthesized and proven to have a superior therapeutic ratio than any other fluoropyrimidine tested (13). However, the method used to synthesize this compound is not suitable for the synthesis of the ^{18}F-labeled analog. We report here the syntheses of ^{18}F-labeled 5'-deoxy-5-fluorouridine (3a), 5-fluorouridine (3b) and 2'-deoxy-5-fluorouridine (3c) by direct fluorination of 2',3'-di-O-acetyl-5'-deoxyuridine (1a), 2',3',5'-tri-O-acetyluridine (1b) and 3',5'-di-O-acetyl-2'-deoxyuridine (1c) with either $[^{18}F]F_2$ or $CH_3CO_2{}^{18}F$ in glacial acetic acid. Preliminary reports of these syntheses have appeared (14,15).

EXPERIMENTAL

Materials. 2',3',5'-Tri-O-acetyluridine was purchased from Sigma Chemical Co., St. Louis, MO. 3',5'-Di-O-acetyl-2'-deoxyuridine was synthesized by acetylation of 2'-deoxyuridine with acetic anhydride in pyridine, mp 109-111°C (Lit (16) 107-110°C). 2',3'-Di-O-acetyl-5'-deoxyuridine and 5'-deoxy-5-fluorouridine were gifts from Dr. A. F. Cook of Hoffmann-LaRoche.

Thin-layer chromatographic analyses were performed on plastic-back TLC plates coated with silica gel 60F 0.2 mm thickness (Merck No. 5775). HPLC analyses were carried out with a Perkin-Elmer Series 3B liquid chromatograph equipped with a Berthold LB503 radioactivity monitor. An IBM C18 column (4.5 x 250 mm) was eluted with MeOH:H_2O (40:60; 1 ml/min). For the preparative separations an IBM C18 column (10 x 250 mm) was used with a flow rate of 4 ml/min.

Fluorine-18 labeled fluorine was produced from the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction as described previously (17). The target, consisting of neon containing 0.1% (40-45 μmole) of fluorine carrier, was irradiated with

deuterons at the Brookhaven National Laboratory 60 inch cyclotron. Approximately 60-80% of the $[^{18}F]F_2$ produced was slowly purged through the target chamber into the reaction solution.

Synthesis of $CH_3CO_2{}^{18}F$ from $[^{18}F]F_2$. Fluorine-18 labeled CH_3CO_2F was prepared as described previously (18). In a typical experiment, $[^{18}F]F_2$ was bubbled into a solution of aqueous ammonium hydroxide (58%) (50 µl) in 10 ml of glacial acetic acid over a period of 25 min to give a solution of $CH_3CO_2{}^{18}F$. The $CH_3CO_2{}^{18}F$ solution was used for the synthesis without further purification.

Synthesis of 5'-Deoxy-5-[^{18}F]Fluorouridine (3a). Fluorine-18 labeled F_2 (35.33 mCi) produced as described previously (17) was slowly purged through the target chamber into the solution of 2',3'-di-O-acetyl-5'-deoxyuridine (1a, 11.03 mg, 35.32 µmole) in 15 ml of glacial acetic acid at room temperature over a period of ~ 20 min. The reaction mixture was then transferred to a round-bottom flask and evaporated *in vacuo* to dryness. The mixture was dissolved in 10 ml of NaOMe/MeOH solution (70 mmole) and evaporated to dryness. The residue was dissolved in H_2O, passed through a cation exchange column (AG50W-X8, H^+ form) and eluted with H_2O. The effluent was evaporated *in vacuo*, the residue was dissolve in ethyl acetate:acetone:water (v/v 70:40:5) and then loaded onto a silica gel column, eluted with the same solvent system and evaporated *in vacuo* to give 5'-deoxy-5-[^{18}F]fluorouridine (3a) (6.20 mCi at the end of synthesis, 30.54% radiochemical yield) in a synthesis time of 88 min from EOB. TLC showed compound 3a had R_f = 0.68 with ethyl acetate:acetone:water (v/v 70:40:5) as solvent. HPLC showed that compound 3 had a retention time of 4.54 min.

In an alternative method of synthesis, $[^{18}F]F_2$ (1.81 mCi) was slowly purged through the target chamber into a solution of aqueous ammonium hydroxide (58%) (50 µl) in 10 ml of glacial acetic acid over a period of 25 min. A solution of 2',3'-di-O-acetyl-5'-deoxyuridine (1a, 9.2 mg, 29.5 µmole) in 2 ml of glacial acetic acid was then added. The solution was allowed to

stand at room temperature for 10 min and then evaporated to dryness. The residue was extracted with chloroform:methanol (v/v 95:5) and passed through a silica gel column, eluted with the same solvent system and evaporated to dryness to give 0.39 mCi of reaction mixture. The radiochemical yield of this mixture was 21.6% (EOB). TLC showed this mixture had R_f = 0.45 with chloroform:methanol (v/v 95:5) as solvent. However, radio-HPLC showed this mixture had a major component with retention time of 9 min and an additional minor component with retention time of 13 min. The ratio of these two peaks was 75:25. The reaction mixture was dissolved in 10 ml of NaOMe/MeOH solution (78 mmole) and evaporated to dryness. Work-up as described above gave 5'-deoxy-5-[^{18}F]fluorouridine (3a) in a radiochemical yield of 16% (EOB).

Synthesis of 5-[^{18}F]Fluorouridine (3b). Compound 3b was synthesized by the same method as described for the synthesis of 5'-deoxy-5-[^{18}F]fluorouridine (3a). Typically, [^{18}F]F_2 (12.84 mCi at EOB) was bubbled into the solution of 2',3',5'-tri-O-acetyluridine (1b, 5.30 mg, 14.3 μmole) in glacial acetic acid and then work-up as described for 3a to give 5-[^{18}F]fluorouridine (3b) (2.14 mCi at EOB, 12.6% radiochemical yield) in a synthesis time of 88 min from EOB. TLC showed compound 3b had R_f = 0.60. HPLC showed compound 3b had a retention time of 4.07 min.

Synthesis of 2'-Deoxy-5-[^{18}F]Fluorouridine (3c). Compound 3c was synthesized by the same method as described above. Typically, [^{18}F]F_2 (5.81 mCi at EOB) was bubbled into the solution of 3',5'-di-O-acetyl-2'-deoxyuridine (1c, 10.17 mg, 33.5 μmole) in 10 ml of glacial acetic acid followed by work-up to give 3c in a radiochemical yield of 21.7% in a synthesis time of 80 min from EOB. TLC showed compound 3c had R_f = 0.6. HPLC showed compound 3c had a retention time of 4.06 min.

RESULTS AND DISCUSSION

Reaction of 2',3'-di-O-acetyl-5'-deoxyuridine (1a) with either $CH_3CO_2{}^{18}F$ or [^{18}F]F_2 in glacial acetic acid followed by hydrolysis and

purification gave 5'-deoxy-5-[^{18}F]fluorouridine (3a) in radiochemical yields of 20-25% (at EOB) in a synthesis time of ~ 80 min from EOB (Scheme 1). The same method also has been used to synthesize 5-[^{18}F]fluorouridine (3b) and 2'-deoxy-5-[^{18}F]fluorouridine (3c) in radiochemical yields of 15-20% (at EOB. The identities of compounds (3a-c) were verified by radio-TLC and radio-HPLC comparison with authentic samples.

The mechanism of this reaction is not fully understood. Giller et al have suggested that 5-fluoro-6-acetoxy-5,6-dihydrouracil (4) was the intermediate in the reaction of F_2 with uracil in acetic acid (19). This was later proven to be the case and its stereochemistry was determined on the basis of the NMR data (9). However, as the authors stated, the mechanism of this reaction is unclear and "we are still left without a satisfying mechanism of F_2 addition to uracil." In addition, in preparing 2'-deoxy-5-fluorouridine derivatives, Cech and Holy had also isolated 1-(3,5-di-O-benzoyl-2'-chloro-2'-deoxy-β-D-ribofuranosyl)-5-fluoro-6-acetoxy-5,6-dihydrouracil as an intermediate (6). They proposed an initial addition of fluorine to uracil and subsequent displacement of fluorine at C_6 by acetoxy. This, however, would give compound 4 with incorrect stereochemistry.

In our studies, the reaction of 2',3'-di-O-acetyl-5'-deoxyuridine (1a) with either ^{18}F-labeled acetyl hypofluorite ($CH_3CO_2{}^{18}F$) or [^{18}F]F_2 in glacial acetic acid gave the same mixture. Radio-HPLC analysis of this mixture showed a major peak with R_T = 9 min and a minor peak with R_T = 13 min with a ratio of 75:25. Hydrolysis of this mixture with NaOMe/MeOH followed by purification with silica gel column gave 5'-deoxy-5-[^{18}F]fluorouridine (3a) with 25% of the radioactivity originally present being held on the silica gel column. In a separate experiment, the reaction mixture of compound 1a with $CH_3CO_2{}^{18}F$ was separated by a preparative C18 column and was hydrolyzed with NaOMe/MeOH, separately. When the peak with R_T = 9 min was hydrolyzed, less than 10% of the radioactivity originally present was held on the silica gel

column. On the other hand, when the peak with R_T = 13 min was hydrolyzed, greater than 80% of the radioactivity originally present was held on the silica gel column. This would suggest that the radioactivity originally present in the reaction mixture being held on the silica gel column (25%) originated from the peak with R_T = 13 min and demonstrated that the intermediate of this reaction is probably 2',3'-di-O-acetyl-5'-deoxy-5-[^{18}F]fluoro-6-acetoxy-5,6-dihydrouridine (2), not 2',3'-di-O-acetyl-5'-deoxy-5,6-[^{18}F]difluoro-5,6-dihydrouridine (5). If the intermediate was 5, it should have lost 50% of the radioactivity after hydrolysis with NaOMe/MeOH solution. Therefore, acetyl hypofluorite (CH_3CO_2F) seems to be the key intermediate in the fluorination of pyrimidines with F_2 in glacial acetic acid. In the case of uracil, CH_3CO_2F adds across the C_5 and C_6 double bond of uracil to give cis-5-fluoro-6-acetoxy-5,6-dihydrouracil (4) as an intermediate which is in agreement with the stereospecificity (syn addition) and regiospecificity of its addition to olefins (18,20).

This synthetic method thus provides a general method for the production of ^{18}F-labeled 5-fluoropyrimidines. It is also important for it removes the possibility of allergic reaction due to the presence of antigenic and pyrogenic protein contaminants, an inherent disadvantage of using enzymes in the biosynthetic preparation of these ^{18}F-labeled fluoronucleosides.

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