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THE SYNTHESIS OF [^{18}F]-5-FLUOROURIDINE (F-18-5-FUR) AS A PROBE FOR MEASURING RNA SYNTHESIS AND TUMOR GROWTH RATES IN VIVO

Chyng-Yann Shiue, Joanna S. Fowler
Robert R. MacGregor, and Alfred P. Wolf

Chemistry Department
Brookhaven National Laboratory
Upton, New York 11973

ABSTRACT

A method for the rapid synthesis of high specific activity of [^{18}F]-5-fluorouridine is described. The ^{20}Ne(d,α)^{18}F nuclear reaction is used to produce high specific activity, anhydrous [^{18}F]-F_2 at the Brookhaven National Laboratory 60" cyclotron. Fluorination of 2',3',5'-tri-O-acetyluridine with [^{18}F]-F_2 in glacial acetic acid at room temperature followed by hydrolysis with sodium methoxide in methanol gives [^{18}F]-5-fluorouridine with a radiochemical yield of 5-7% in a synthesis time of 90 minutes from EOB. The compound is required for the study of RNA synthesis and tumor growth rates in vivo.

INTRODUCTION

Labeling of organic compounds with positron emitting nuclides where the labels are in non or in minimally bioactive positrons so as not to alter the functionality of the compound, has become especially attractive with the advent of the new positron emission tomographs. The localization of these compounds or more importantly their involvement in specific metabolic pathways can serve as a probe for metabolism in vivo. This principle of "metabolic trapping" has been used to design and synthesize the needed radiopharmaceutical. Of particular interest in this research is the preparation of a nucleotide or nucleoside labeled with a positron emitting nuclide and its use to study ribonucleic acid synthesis in vivo. The uracil analog, 5-fluorouracil (5-FU) has been used as an antineoplastic agent for the treatment of a variety of disseminated solid tumors, especially colorectal, gastrointestinal, and breast, with about 30% of the patients showing objective tumor remission (1-3). The mechanism of antitumor action of 5-FU is well documented (4). It is principally due to a competitive inhibition of thymidylate synthetase by its metabolite 5-fluoro-2'-deoxyuridine-5'-monophosphate. This produces thymidylic acid deficiency and thus blocks the synthesis of DNA. In mammalian and bacterial systems, 5-FU is converted to its ribonucleoside (5-FUR) by uridine phosphorylase. The ribonucleoside is then phosphorylated to its nucleotide, 5-fluorouridine-5'-monophosphate (5-FUMP) by uridine kinase. Formation of 5-FUMP may also result from direct conversion of 5-FU to 5-FUMP by uracil phosphoribosyltransferase. 5-FUMP is then readily incorporated into RNA (5). Therefore, if we label 5-FUMP or its precursors with a positron emitting nuclide, such as ^{18}F, it might be feasible to measure RNA synthesis in certain organs which might be applicable to tumor RNA synthesis measurements in vivo.

[^{18}F]-5-Fluorouridylate (^{18}F-5-FUMP) was recently synthesized enzymatically (6) from [^{18}F]-5-fluorouracil (^{18}F-5-FU)(7) and the tissue uptakes of these two compounds were studied. It was shown that the nucleotide was incorporated into most of the tissues studied to a greater extent than the free base (6). This was particularly evident in tissues with a known high rate of RNA synthesis such as the spleen and small intestine and agreed with the findings of Heidelberger that 5-fluorouridine was incorporated into RNA more efficiently than 5-fluorouracil (8) and that 5-fluorouridylate was dephosphorylated in vivo to give 5-fluorouridine (9). Therefore, it was of interest to synthesize ^{18}F-5-fluorouridine by chemical methods for use as a probe for measuring RNA synthesis and tumor growth rates in vivo.

METHODS

The target, consisting of neon (Matheson Research Grade) containing 0.1% (60 μmol) of fluorine (F_2) carrier, was irradiated with deutrons at the Brookhaven National Laboratory 60" cyclotron (10). The ^{18}F-labeled fluorine was produced from the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction. The beam was degraded from 13.6 to 0 MeV in the target. Approximately 60-80% of the ^{18}F-F_2 produced was slowly purged through the target chamber (flow rate ~ 45 ml/min, total time 25-30 min) into the solution of 5.88 mg (15.9 μmol) of 2',3',5'-tri-O-acetyluridine (1) (Sigma) in 6 ml of glacial acetic acid in a reaction vessel (11) at room temperature. The reaction mixture was then transferred to a round-bottom flask, allowed to stand at room temperature for 10 min and evaporated in vacuo to give [^{18}F]-2',3',5'-tri-O-acetyl-5,6-dihydro-5,6,-difluorouridine (2). Compound 2 was dissolved in 10 ml of sodium methoxide-methanol solution and

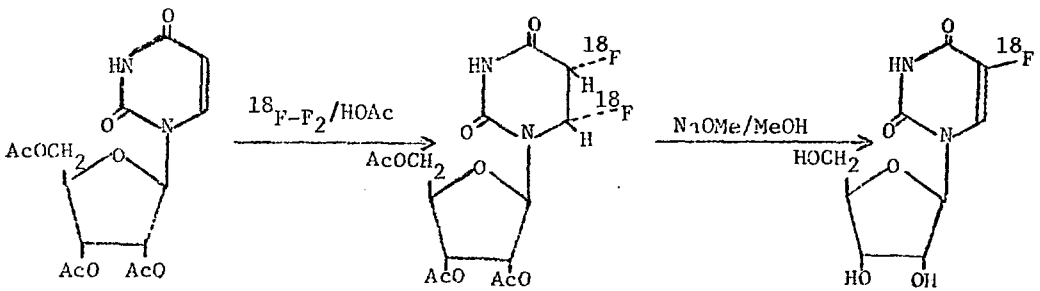
evaporated to dryness. The residue was dissolved in water for chromatographic separation of [^{18}F]-5-FUR.

Chromatographic separations were obtained on cation exchange column (AG 50 X-8, H^+ form), eluted with water. The eluate was evaporated *in vacuo* and the residue was extracted with ethyl acetate:acetone:water (v/v 70/40/5) and then passed through a silica gel column, eluted with same solvent system, and the eluate was evaporated *in vacuo* to give 1.11 mg of [^{18}F]-5-FUR (3)(53.5% chemical and 6.6% radiochemical yield).

RESULTS AND DISCUSSION

5-Fluorouracil, 5-fluorouridine, and 5-fluoro-2'-deoxyuridine have been synthesized by direct fluorination of uracil, 2',3',5'-tri-O-acetyluridine and 3',5'-di-O-acetyl-2'-deoxyuridine with trifluoromethyl hypofluorite (12). However, this method is not suitable for the synthesis of high specific activity of [^{18}F]-labeled fluoropyrimidines. Recently, these fluoropyrimidines were synthesized by fluorination of the pyrimidines with molecular fluorine (13,14). Since we are able to produce very high specific activity [^{18}F]-F_2, we have synthesized [^{18}F]-5-fluorouridine by direct fluorination of 2',3',5'-tri-O-acetyluridine (1) with [^{18}F]-F_2.

Fluorination of (1) with [^{18}F]-F_2 in glacial acetic acid gave a clean reaction product (2) as shown in Fig. 1. There are several reagents used to hydrolyze the acetyl groups, such as triethylamine (12,14) or ammonia (13). We, however, find that sodium methoxide in methanol is more efficient than tertiaryamines. Hydrolysis of 2 with NaOMe/MeOH followed by passage of the mixture in water through a cation exchange resin and silica gel column results in the production of [^{18}F]-5-FUR (3) in a radiochemical yield of 5-7% in a synthesis time of 90 minutes from EOB (equation 1). A silica gel column was used to separate [^{18}F]-fluoride and [^{18}F]-5-FUR, as shown in Fig. 2.



1 2 3

In order to achieve the maximum radiochemical yield of [^{18}F]-5-FUR (3) and without the contamination of uridine, the molar ratio of [^{18}F]-F_2 with substrate 1 should keep between 2.5-3.0. The purity of compound 3 was checked by tlc and UV.

This convenient method thus simplifies the production of [^{18}F]-5-FUR (3) and eliminates the possibility of macromolecular contamination introduced in the enzyme catalyzed synthetic sequence. It also can readily be used to provide

compound 3 with very high specific activity. Indeed, the specific activity of 3 depends on total irradiation dose (Table 1). [^{18}F]-5-FUR is currently being used as a probe for measuring RNA synthesis and tumor growth rates in vivo.

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TABLE 1. IRRADIATION DOSE DEPENDENCE OF THE SPECIFIC ACTIVITY OF [^{18}F]-5-FUR

Run No.	Compound 1 (μM)	[^{18}F]-F_2 Activity (mCi)	Specific Activity of [^{18}F]-5-FUR (μCi/μM)
5	13.8	16.4	94.7
6	13.5	8.6	50.1
7	13.9	8.5	58.8
10	17.5	48.5	424
11	18.1	16.9	128
12	15.9	102.9	642

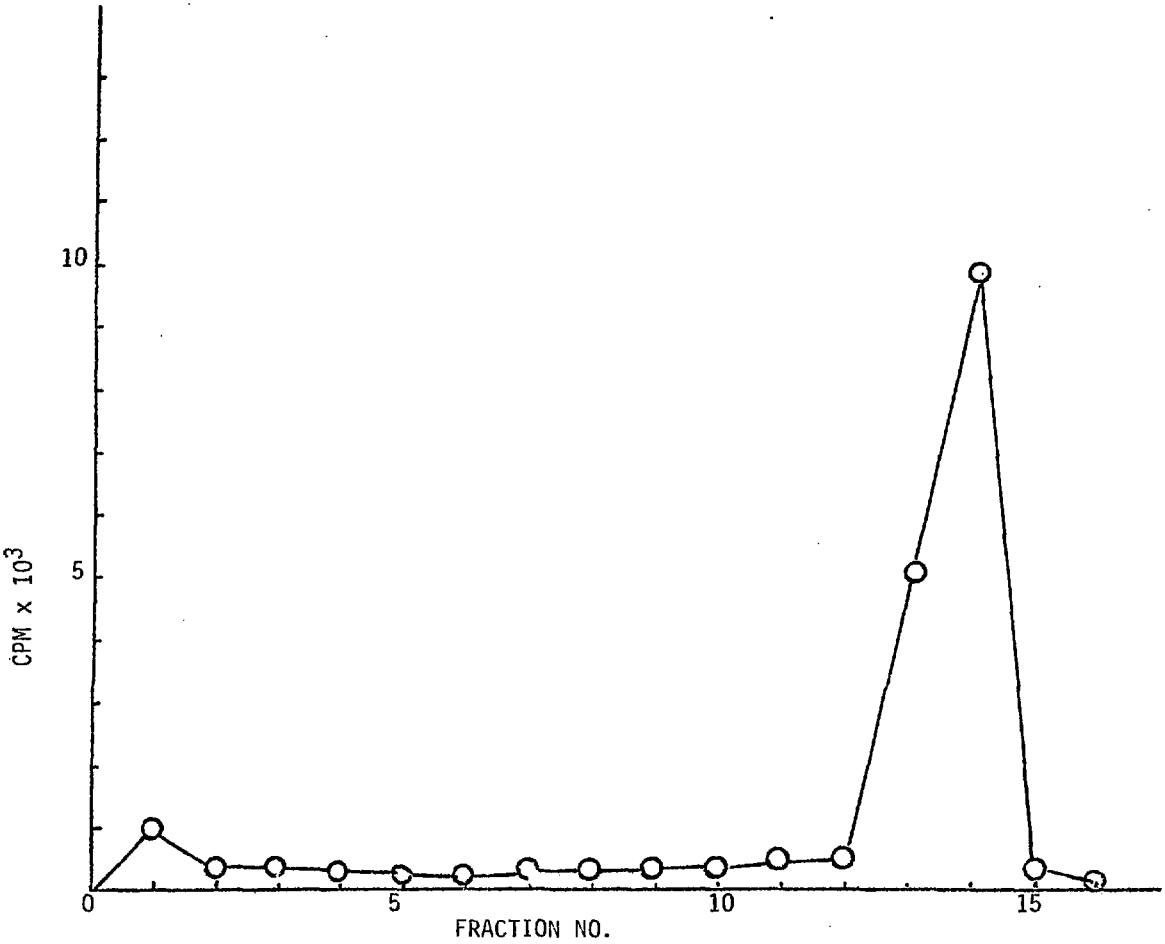

Fig.1. Radiochromatograms of the reaction mixture of 2',3',5'-tri-O-acetyluridine with ^{18}F-F_2

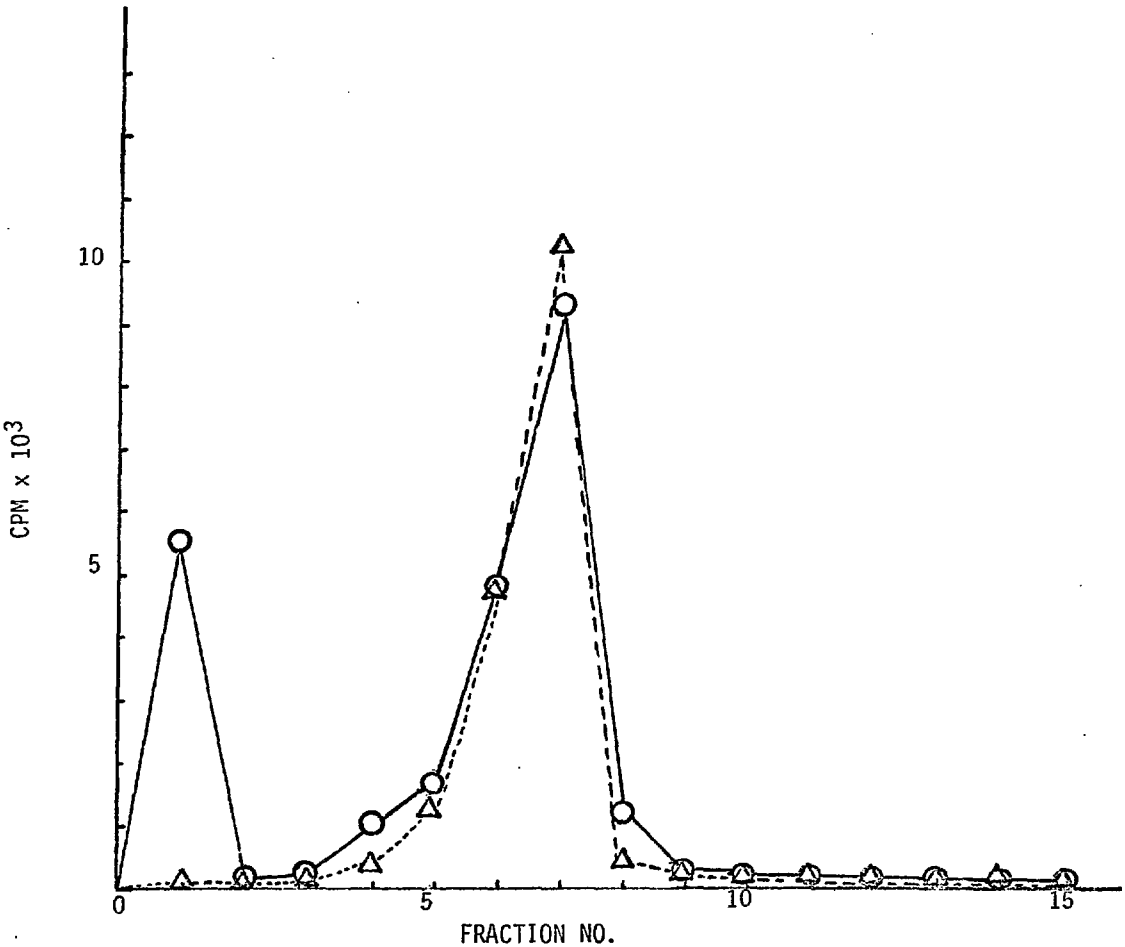

Fig.2. Radiochromatograms of ^{18}F-5-FUR (-O-O-O-) before silica gel column; (--Δ-Δ-Δ-) after silica gel column. Separation were achieved by eluting a silica gel column with ethyl acetate:acetone:water (70:40:5).