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Synthesis and Evaluation of Radiolabelled 5-Halo-1-(2'-Fluoro-2'-Deoxy-β-D-Ribofuranosyl)-Uracil Analogs As Non-Invasive Tumor Diagnostic Radiopharmaceuticals

by

John Robert Mercer

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Doctor of Philosophy

IN

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EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Synthesis and Evaluation of Radiolabelled 5-Halo-1-(2'-Fluoro-2'-Deoxy-β-D-Ribofuranosyl)Uracil Analogs As Non-Invasive Tumor Diagnostic Radiopharmaceuticals submitted by John Robert Mercer in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences.

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Supervisor

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External Examiner

Date April 17/1985

DEDICATION

For my parents who encouraged my early explorations into the wonders of science and who tolerated frogs in the bathtub, hydrogen sulfide in the kitchen and fires in the coal shed.

ABSTRACT

The pyrimidine nucleoside analogs 5-iodo-, 5-bromo-, 5-chloro- and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil and 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil were evaluated as non-invasive tumor imaging agents. Radiolabelled analogs of these compounds were synthesised for *in vivo* studies in animal models. [^{131}I]-5-Iodo- and [^{77}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil were synthesised by reacting 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil with [^{131}I]-I_2 and [^{77}Br]-BrCl respectively. [2-^{14}C]-5-Chloro- and [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil were synthesised by reacting [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil with Cl_2 and F_2 in acetic acid respectively. [^{123}I]-1-(3'-Iodo-3'-deoxy-β-D-arabinofuranosyl)uracil was synthesised by reacting 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil with aqueous [^{123}I]-HI.

The *in vivo* tissue distribution, excretion and metabolism of these compounds were evaluated in tumor bearing rodents. The 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracils were evaluated in BDF_1 mice bearing a Lewis lung tumor. 1-(3'-Iodo-3'-deoxy-β-D-arabinofuranosyl)uracil was evaluated in Wistar rats bearing a Walker 256 tumor. 5-Chloro- and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil demonstrated potential as non-invasive imaging radiopharmaceuticals by virtue of high tumor to blood ratios (4.2 and 10.3 at 4 hours respectively) and selective uptake in tumor tissue. All of the compounds examined demonstrated resistance to *in vivo* degradation.

The nuclear reaction ^{6}Li(n,α)^{3}H, ^{16}O(^{3}H,n)^{18}F was used to produce ^{18}F with the SLOWPOKE Nuclear Reactor Facility. ^{18}F was also produced with a Van de Graaff accelerator using the nuclear reaction ^{20}Ne(d,α)^{18}F. Targets and recovery systems were fabricated for use with the accelerator which allowed recovery of the ^{18}F activity as both [^{18}F]-HF and [^{18}F]-F_2. The ^{18}F was used to synthesise [2'-^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil and [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil in low yield.

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Many minds and many hands are represented in this work even though it bears my name alone as author. Although it is not possible to give credit here to all those who have assisted me in this project, I would like to recognize the special contributions of a number of people.

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I. INTRODUCTION

The development of radiopharmaceuticals for use in clinical nuclear medicine studies has been made possible by the co-operative efforts of researchers in a variety of disciplines. Biochemical research involving both endogenous biomolecules and xenobiotics has identified compounds which show specificity for the altered physical or biochemical situation present in diseased, injured or metabolically dysfunctioning tissues. Developments in radionuclide production and radiochemistry have permitted the preparation of radiolabelled analogs of a number of important biomolecules while the techniques of *in vivo* and *in vitro* screening in test systems have allowed rapid pre-clinical evaluation of the therapeutic potential of a particular compound.

The development of a new radiopharmaceutical must be based on criteria dictated by each of these disciplines. The compounds for investigation should have a recognized or a predicted biological function related to the diagnosis or treatment of a particular biological disorder. Synthesis of radiolabelled analogs of these compounds must be possible and must utilize radionuclides suitable for the particular counting or imaging technique to be used. In addition, a suitable *in vivo* or *in vitro* test system must be available for preliminary screening of the test compounds.

An additional criterion must be whether a demonstrated need exists for a particular class of radiopharmaceuticals. Such a need does appear to exist in the area of non-invasive diagnostic oncology. Berry and Ell have discussed the value of imaging agents in the pre-treatment assessment of cancer[1]. These agents are required for a number of reasons including the screening of populations for the early detection of cancer, assessing the extent of growth of a neoplasm, observing the response of a cancer to a particular treatment modality and early detection of the recurrence of a treated cancer.

Although many types of cancers are readily detected by existing radiopharmaceutical imaging techniques (for example primary hepatomas by $^{99}Tc^{m}$-sulfur colloid or malignant bone tumors such as osteogenic sarcomas by $^{99}Tc^{m}$-methylene diphosphonate) other tumors

are not easily or accurately detected. This is the case with many soft tissue cancers. Clinical tumor imaging has been performed with a variety of agents including ^{67}Ga-citrate, ^{169}Yb-citrate, ^{57}Co-bleomycin, ^{111}In-bleomycin and recently ^{111}In-labelled autologous white blood cells[2,3] and radioiodinated monoclonal antibodies[4,5]. ^{67}Ga-citrate is the most satisfactory of these agents and is the radiopharmaceutical of choice for tumor and abscess scanning[6]. This agent has however demonstrated a variety of problems including poor uptake in tumors arising from the alimentary and genito-urinary tract, concentration in normal tissues such as the liver, bowel, bone marrow and breast as well as accumulation in pyrogenic lesions[7]. Additional tumor localization agents specific for neoplastic tissue and without the complications encountered with ^{67}Ga-citrate would be of great value in clinical nuclear medicine.

From a clinical viewpoint an agent suitable for non-invasive localization of tumors would have the following characteristics:

1. The compound would be taken up in tumor tissue.
2. The compound would demonstrate selectivity of uptake so that the levels of radioactivity in body tissues and fluids would not interfere with the observation of the radioactivity in the tumor.
3. For non-invasive studies the compound would be labelled with a γ-ray or β^+-particle emitting radionuclide. The radionuclide would be readily available and would have a half-life suitable for the studies to be performed.
4. The compound should be non-toxic. This is a relative consideration since the biological mechanism of tumor localization often has toxic consequences. On the other hand the amounts of radiolabelled compounds used in an imaging study are usually many orders of magnitude below the toxic threshold.
5. The radiotoxicity of labelled compounds must be considered. Radionuclides with α or β^--particle emissions or those with long-half lives should not be used in human studies. All radionuclides, including those generally regarded as safe for human studies, cause some tissue damage and increase the risk of subsequent development of neoplasms. These

dangers must be weighed against the possible benefits derived from the nuclear medicine procedure.

6. The radiopharmaceuticals would be easily prepared from readily available compounds and radionuclides. The restriction of ready availability at one time permitted medical investigations to be carried out only with longer lived radionuclides (^{111}In, ^{67}Ga, ^{131}I, ^{125}I etc) or with generator produced radionuclides (^{68}Ga, $^{99}Tc^{m}$, $^{113}In^{m}$ etc). More recently, however, the development of in-house cyclotrons and reactors has permitted biomedical investigations of compounds labelled with short-lived radionuclides (^{18}F, ^{11}C, ^{15}O, ^{13}N etc).

Pyrimidine nucleosides satisfy many of the characteristics required of a good non-invasive tumor diagnostic agent. The naturally occuring pyrimidine nucleosides are utilized as building blocks for the synthesis of DNA and RNA within the cells. Many analogs of the natural pyrimidine nucleosides mimic this behaviour. In rapidly growing normal tissue and tumor tissue the concentration of these analogs will increase relative to that in body fluids and slower growing tissues. This behaviour has been observed for many pyrimidine nucleoside analogs. For example 5-fluorouracil and 5-fluoro-2'-deoxyuridine are taken up selectively by animal[9-10] and human[11,12] tumor cells. The deoxythymidine analog 5-iodo-2'-deoxyuridine is incorporated extensively into DNA in place of deoxythymidine[13] and the radiolabelled analog has been shown to accumulate in a variety of animal tumors[14-17]. The halogenated pyrimidine nucleosides are an important class of nucleoside analogs which are readily synthesised and often exhibit biological activity. In addition a variety of suitable halogen radioisotopes are available for tracer and imaging studies.

A series of pyrimidine nucleoside analogs 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil which have the structure 1 were examined in this study†. Compounds 1a and 1b were originally prepared and tested as antiherpes virus agents by Fox and co-workers[18].

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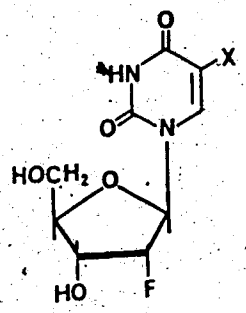
†Structural formulae are numbered sequentially in the text. In addition the more important structures are reproduced in Appendix 2 which may be folded out for quick reference.

These compounds were found to have both antiherpetic activity against *herpes simplex* virus type 1 (HSV-1) and *herpes simplex* virus type 2 (HSV-2) infected cells and cytotoxicity towards normal human lymphocytic cells in culture. The other two compounds in the series, 1c and 1d, have not been described previously. This series of compounds was chosen for testing as potential agents suitable for non-invasive diagnostic oncology.



1

a) X = I
b) X = Br
c) X = Cl
d) X = F

The Division of Cancer Treatment of The National Cancer Institute has defined a number of criteria for the selection of drugs for anticancer testing[19]. Since anticancer activity implies some selective uptake in or interaction with neoplastic tissue, these criteria also apply to compounds suitable as tumor-diagnostic agents.

Suitable compounds are those which are analogs of naturally occurring compounds, those which are prepared according to a rational structure-activity relationship, compounds which are analogs of known anticancer agents and those which have demonstrated biological activity in other test systems. The compounds with structure 1 satisfy all of these criteria. They are analogs of naturally occurring pyrimidine nucleosides such as deoxythymidine, uridine and 2'-deoxyuridine. Compounds with the structure 1 may, due to their structural similarity to natural nucleosides, cause perturbations in the normal cellular biochemistry as is observed with antimetabolites such as arabinosyl cytosine (ara-C) or 5-fluorouracil and its nucleosides. Compound 1d is an analog of 5-fluorouracil, a potent antitumor agent. The preliminary testing of compounds 1a and 1b as antiviral agents[11] revealed their toxicity toward normal lymphocytic cells. Although this observed cytotoxicity made these compounds unsuitable as antiviral agents

it did suggest that they had some involvement with the cellular biochemistry.

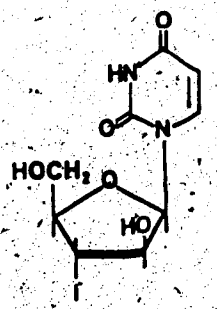
The presence of fluorine in compounds 1a-d also presented the potential for labelling them with the short-lived positron-emitting radionuclide ^{18}F. Compounds thus labelled would be ideal for imaging studies using positron emission tomography (PET), a technique capable of giving accurate, three dimentional, whole body imaging. ^{18}F is not used routinely in clinical nuclear medicine due to its short half-life ($T_{\frac{1}{2}}$ = 109.7 minutes†)[20] and difficult chemistry. This situation may change, however, since in-house cyclotron production facilities and improved chemical synthesis with fluorine have made a number of ^{18}F-labelled radiopharmaceuticals available for patient studies. Such has been the case, for example, with [^{18}F]-2-deoxy-2-fluoro-D-glucose which has been used for brain[21], tumor[22] and heart[21] imaging. Part of the present study was directed toward the production of ^{18}F and investigation of the synthesis of ^{18}F labelled compounds 1.

The *in vivo* test system employed in this study was the Lewis lung carcinoma. This carcinoma occurred spontaneously in 1951 in a C57BL/6 mouse[23]. Several factors have contributed to the popularity of this tumor model. The tumor spontaneously metastasises primarily to the lungs by 6 days after subcutaneous transplant[24]. The growth kinetics have been well characterized and can be fitted to a Gompertz equation[23-25]. Below 10^6 cells the growth kinetics after intramuscular injection of a cell suspension are best represented by a simple exponential curve[26]. The tumor doubling time has been reported as 1.02 days after intramuscular injection of Lewis lung carcinoma cells[26] or 1.18 days at a tumor volume of 1 / 1000 of the maximum tumor volume[25]. Subcutaneous implants gave tumor doubling times of 2.1 days at 5 days after implant increasing to 16.1 days at 30 days after implant[24]. The tumor has been used extensively as an indicator of the therapeutic potential of experimental anticancer agents. It was adopted as a test system for screening anticancer drugs by the Division of Cancer Treatment of The National Cancer Institute in 1971[19].

†The half-life values and other physical characteristics of the radionuclides quoted in this work unless otherwise specified are taken from "The Table of the Isotopes", 7th edition, C. M. Lederer and V. S. Shirley editors, Wiley-Interscience, New York (1978). Reference 20. See also Appendix 1 for data on selected radionuclides.

The widespread use of Lewis lung carcinoma as a test system for therapeutic anticancer agents has been criticized by Hewitt[27]. He presented evidence suggesting that over its long history of transplantation the tumor and host tissues may have developed histological incompatibilities. Consequently, in therapeutic studies the host's immunological response is in part responsible for the better than expected incidence of cures during anticancer drug treatment (reference 27 and references quoted therein). In addition, the tumors tend to grow beyond the ability of the vascularization to supply them with nutrients and older tumors develop necrotic centers.

In the present study we are concerned with diagnostic rather than therapeutic potential of our test agents and so the reservations noted in reference 27 are not as important. In addition, the distribution studies were carried out on "young" (10 to 20 day) tumors and extensive necrosis was not a problem. The tumor was transplanted into BDF_1 mice (C57BL/6 female cross with DBA/2 male) the strain most often employed for chemotherapeutic studies with Lewis lung tumors.



2

An additional study was carried out on the compound 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2). This compound is an analog of uridine or 2'-deoxyuridine which may have potential as a non-invasive diagnostic agent for tumor localization. It is postulated that the similarity of this compound to the natural nucleosides might permit its uptake into proliferating cells while the presence of a 3'-substituent (iodo in this case) would suppress the normal enzymatic degradation *via* pyrimidine nucleoside phosphorylases and allow metabolic

trapping of the compound. Synthetic studies were carried out with ^{123}I.

The *in vivo* test system employed for preliminary screening of this compound was the Walker 256 carcinosarcoma in male Wistar rats. This tumor arose spontaneously in rats of unspecified genetic constitution in 1928 and is generally used as an allotransplant (tumor tissue and host tissue not histologically identical) in Wistar rats[27]. Kinetic studies show the tumor to obey a Gomperz equation with a doubling time during the exponential growth phase as short as 0.5 days[25,28]. From 5 to 10 days after an intramuscular injection of Walker 256 carcinosarcoma the doubling time was shown to be 1.7 days, a rate of growth exceeding all normal rat tissues except that of the epithelium of the small intestine[29]. This readily transplantable, rapidly growing solid tumor has often been used for therapeutic anticancer studies and was adopted as an anticancer screening test by the European Organization for Research on Treatment of Cancer (EORTC)[30].

The primary aim of this study was to examine the potential this series of test compounds had as agents for non-invasive diagnostic oncology. This study will have a secondary value by increasing the information available concerning the structure-activity relationships of modified pyrimidine nucleosides.

II. LITERATURE REVIEW

A. The Production of ^{18}F

Introduction.

The radionuclide ^{18}F was artificially produced over 45 years ago. Within the space of several months this new isotope of fluorine was reported as a product of deuteron irradiation of neon gas[31], the proton bombardment of oxygen[32], the high energy neutron bombardment of fluorine[33] and the deuteron bombardment of oxygen[34]. The first tracer studies with ^{18}F, showing the distribution of the anion in the bones and teeth of experimental animals, were carried out several years later[35,36]. Despite its long history this radionuclide has until the last decade had a rather modest role in nuclear medicine. Reactor and cyclotron sources could produce ^{18}F only in its anionic form which localizes biologically in bones and teeth where it substitutes for hydroxyl and bicarbonate at the surface of the apatite crystals[37,38]. $^{99}Tc^{m}$-labelled phosphonates have proved superior to $^{18}F^-$ as bone scanning agents[39] although the recent developments in positron emission tomography (PET) have once again made ^{18}F an attractive choice[40]. An additional obstacle to the use of ^{18}F was the absence of synthetic methods for incorporating this radionuclide into organic molecules.

A number of factors have contributed to increased application of ^{18}F in addition to its limited role as a bone scanning agent. The development of positron emission tomography has enhanced the value of short-lived positron emitting radionuclides. In this class, which includes ^{18}F, ^{11}C, ^{13}N and ^{15}O, fluorine has the most desirable decay characteristics. Its half-life is 109.7 minutes[20] which is long enough to allow the syntheses of a number of organic radiopharmaceuticals. In addition the relatively long half-life permits the observation of slower metabolic processes in tracer studies. ^{18}F has a lower β^+-energy than ^{11}C, ^{13}N and ^{15}O and this gives the positron a shorter tissue penetration and correspondingly increased resolution in PET studies for compounds containing this radionuclide. In contrast to the other short-lived positron

emitters mentioned above fluorine is not a normal constitutent of biological molecules. Fluorine containing molecules can only be analogs of naturally occuring animal or human biomolecules.

Fluorine is strongly bonded to carbon and resembles in some respects both hydrogen (Van der Waals radii; H = 1.2 Å, F = 1.35 Å) and hydroxyl (electronegativity; OH = 3.5, F = 4.0). Substitution of hydrogen or hydroxyl by fluorine can give compounds that have similar or significantly altered pharmacological behaviour when compared to molecules for which they are analogs. For example 2-deoxy-2-fluoro-D-glucose (2-FDG) mimics deoxyglucose in its metabolism in tissue[41,42] while 5-fluorouracil (5-FUra) in contrast to uracil exhibits a profound toxicity to proliferating tissue[43].

The dual incentives provided by developments in PET and the recognition of biologically significant fluorine containing compounds have helped to remove the remaining obstacles to the production of ^{18}F-labelled pharmaceuticals. A variety of new synthetic methods have been developed which provide rapid, selective, high-yield syntheses of a variety of useful ^{18}F-labelled radiopharmaceuticals. These new synthetic methods will be discussed later. In addition to improved synthetic methods there have been improvements in the production methods for ^{18}F. Large quantities of this radionuclide are routinely produced by reactor and cyclotron methods. A complete survey of production methods for ^{18}F is beyond the scope of this review. A number of reviews of ^{18}F production have been published[44,45]. Various production methods will be discussed with emphasis on those methods which have yielded synthetically useful radionuclide.

Reactor production of ^{18}F.

There are several possibilities utilizing neutrons for the production of ^{18}F. The nuclear reaction ^{19}F(n,2n)^{18}F has been employed with fast, cyclotron-produced neutrons in recoil labelling[46] and for direct activation of fluorine containing organic compounds[47]. Direct activation led to extensive degradation such that greater than 80% of the radionuclide produced was no longer associated with the starting molecules[47]. This nuclear reaction is, however,

unsuitable for reactor production of ^{18}F due to the low energy of the reactor neutron population.

A reactor production method for ^{18}F giving higher yields and more versatility is the bombardment of ^{16}O, the naturally occuring isotope, with energetic ^{3}H. The ^{3}H with energy maximum of 2.73 MeV is produced by the reaction between reactor produced neutrons and ^{6}Li. The overall reaction is $^{6}Li(n,\alpha)^{3}H$, $^{16}O(^{3}H,n)^{18}F$. Compounds containing both lithium and oxygen are the logical choice for targets. Although other lithium targets have been used[48-50] Li_2CO_3 and $LiOH \cdot H_2O$ gave the best combination of relative yield and ease of purification[51-53]. The problems associated with reactor produced ^{18}F have been overcome to a great extent by improved methods of sample preparation and sample processing after irradiation. The yields of ^{18}F have been improved through the use of enriched ^{6}Li-targets (>90% ^{6}Li as compared to 7.42% ^{6}Li in natural abundance lithium compounds)[51,53-56]. In theory 9.25 gigabequerels (GBq) of ^{18}F per hour per gram of irradiated ^{6}Li as LiOH can be produced at a neutron flux of 3 x 10^{13} n cm^{-2} sec^{-1} [52]. In practice this value is greatly reduced[53,56]. One reason for the reduced activity is local flux depression in the reactor due to the very high capture cross-section of ^{6}Li for thermal neutrons[53].

The high capture cross section has required careful attention to the geometry of irradiated samples in order to obtain maximum yields. A 1 mm thick layer of $LiOH \cdot H_2O$ or Li_2CO_3 has been shown to effectively capture the majority of incident neutrons[53,57]. Consequently optimum yields resulted only when a thin layer of lithium salt was exposed evenly to the neutron flux. Thin aluminum pouches or plastic cylinders have been used[57] as well as quartz ampoules with spacers to reduce the sample thickness[53,55]. The salt has also been melted and applied as a thin film to the walls of the irradiation container[51]. The highest yields of reactor produced ^{18}F reported in the literature (2.8 GBq) were produced using an aluminum can with a graphite coating and a graphite spacer[56].

A variety of methods have been used to recover ^{18}F in a useable form from the reactor produced product. The ^{18}F has been washed from the insoluble salt (eg Li_2CO_3) with water and

then taken up on ion exchange resin[48,59,60]. In other recovery methods the ^{18}F has been precipitated as fluoride salt[57,61] or isolated as an appropriate synthetic precursor such as tetra-alkylammonium fluoride[53,62,63]. Alternately the lithium salt may be dissolved in concentrated sulfuric or perchloric acids and the ^{18}F removed by distillation under a flow of steam or inert gas[49,51-54,62]. A novel system was used for *in transit* recovery of ^{18}F from irradiated target material in which the ^{18}F was transformed to volatile trimethylsilyl fluoride and trapped in alkaline solution[64].

In addition to the separation problems, material destined for animal or human tracer studies must be made free of lithium and tritium residues. Tritium activities have been shown to be approximately one-half the activity of the ^{18}F produced[53]. This level of ^{3}H contamination requires careful purification of synthesised compounds and care to avoid contamination of working areas. The numerous problems associated with reactor production of ^{18}F in a form suitable for radiochemical synthesis make this the method of choice only when no accelerator or cyclotron production facility is available. Even so reactor produced ^{18}F has been used to produce a number of compounds suitable for tracer studies. These include fluoroalkanes[59,63], fatty acids[60], 5-fluoro-DOPA[55] and 3-fluorodeoxyglucose[62].

Accelerator methods for the production of ^{18}F.

The majority of ^{18}F-labelled radiopharmaceuticals are synthesised using accelerator produced radionuclide. Within the last few years improvements in targetry and a better understanding of fluorine chemistry have allowed the routine production and clinical utilization of a wide variety of fluorinated biologically active molecules. The accelerator production methods may be divided into those using water or solid target materials and those using gaseous target materials. The gaseous target materials may be further sub-divided into those producing [^{18}F]-anion and those producing [^{18}F]-molecular fluorine.

(a) water and solid targets

Early accelerator production of ^{18}F in quantities suitable for labelling involved almost exclusively the irradiation of water targets by either ^{3}He- or α-particles. The nuclear reactions employed are $^{16}O(^{3}He,p)^{18}F$ and $^{16}O(\alpha,pn)^{18}F$. The former reaction has a much higher activation cross section at a lower energy (374 millibarns maximum at 8 MeV for ^{3}He and 140 millibarns maximum at 36 MeV for α-particles)[66,67]. For particle beams of similar energies (about 25 MeV) the yield of ^{18}F is five times greater for ^{3}He[61].

The major production problems for water targets include the build up of heat and the development of pressure in enclosed systems. This latter effect is due to the radiolytic decomposition of target water. Early titanium target holders used catalytic recombination of evolved H_2 and O_2 gas[68-70], or a pressure resistant assembly[71] and cooling by an external water flow. Another solution to the heat and pressure build up in the targets was to allow the target water to circulate through an external circuit which contained a heat exchanger and a vent for the release of excess pressure[44,72,73].

Production methods of this type yielded a water solution of [^{18}F]-anion of an unidentified chemical composition[74]. However addition of carrier fluoride or exchange with fluoride forms of ion exchange resins or fluorinated compounds yielded precursor compounds suitable for the synthesis of biomolecules. For example fluoroborates[69,75], fluoride salts[76], fluoride resins[72,77] and tetraalkylammonium fluorides[70] have all been produced from water targets.

Some explorations of solid oxygen-containing target materials such as SiO_2 were undertaken[78]. Numerous problems such as the co-production of ^{32}P (from the nuclear reactions $^{29}Si(\alpha,p)^{32}P$ and $^{30}Si(\alpha,pn)^{32}P$), contamination of the solutions of ^{18}F with soluble silicon compounds and the necessity for carrier fluorine in solutions used to extract ^{18}F from target material, outweighed any advantages that might have resulted from the use of a solid target material. Enriched targets of [^{18}O]-water have also been used[79-81]. The $^{18}O(p,n)^{18}F$ reaction is particularly efficient with a high cross section maximum at a low proton energy (575 millibarns

at 5.3 MeV)[67,82]. Unfortunately the high cost of ^{18}O-enriched water is a significant deterrent to its widespread use, although small volume [^{18}O]-H_2O enriched targets which recover and recycle the ^{18}O have proven useful in the preparation of ^{18}F-fluoride[81].

(b) gas targets

At present most of the ^{18}F used for chemical synthesis makes use of gas targets composed of either $^{18}O_2$ for the $^{18}O(p,n)^{18}F$ nuclear reaction or ^{20}Ne for the $^{20}Ne(d,\alpha)^{18}F$ reaction. Although the latter reaction (225 millibarns at 6.3MeV)[66,67] is far less efficient than the former it has gained wide acceptance. It has the advantage of using natural abundance neon, a cheap, easily purified and chemically inert target material. Maximum recovery of ^{18}F in the expected chemical form has required very careful attention to the design and operation of targets and recovery systems and to the purity of the gas mixtures used as the target materials. Gas targets can be divided into those producing [^{18}F]-anion and those producing [^{18}F]-F_2.

(i) [^{18}F]-anion production

The standard [^{18}F]-anion radionuclide production system allows an energetic particle beam to pass through a thin foil and bombard the appropriate gas held under pressure in a target holder. The ^{18}F is produced as energetic atomic fluorine. In this state it is tremendously reactive and will form compounds immediately with constitutents of the target gas, impurities in the system or the target holder itself.

The high chemical reactivity of fluorine also requires special recovery procedures. A number of circulating gas targets have been used[40,83-86]. These allowed ^{18}F to be flushed from the target during the irradiation and recovered at a site external to the target holder. In deference to the known reactivity of fluorine and many of its compounds the target holders have been constructed of nickel which is known to resist chemical attack by fluorine[87]. Brass target holders with their surfaces passivated by previous reaction with molecular fluorine have also been used[40], and recently copper target holders have been successfully employed[86].

The nuclear reaction $^{20}Ne(^{3}He,\alpha n)^{18}Ne \rightarrow {}^{18}F$ has been utilized in a rapid gas flow system[15]. The rapid circulation of gas removed the initial product (^{18}Ne) from the target holder. The ^{18}Ne then decomposed ($T_{\frac{1}{2}}$ = 1.67 seconds)[20] to ^{18}F which reacted with H_2 in the target gas to produce [^{18}F]-HF which was then trapped from the circulating gas in a cooled section of teflon tubing.

Other flow systems have relied on the external trapping of [^{18}F]-HF formed from ^{18}F produced by the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction. Target gas containing the desired percentage of added H_2 and with a rapid gas flow have allowed trapping of [^{18}F]-HF in saline[40] or on CsOH coated silver wool[14]. It has been estimated that with 15% H_2 in the neon target gas the flow system removed 60% of the ^{18}F activity from the target holder of which 90% was trapped on the first of two silver wool plugs[14]. The [^{18}F]-CsF formed in this reaction has been successfully utilized as a precursor in the synthesis of a number of investigational radiopharmaceuticals[14,17,18]. The [^{18}F]-CsF has the dual advantages of being carrier free and anhydrous thereby providing the potential for high specific activity synthesis in situations where anhydrous reagents are required.

"Surface oxidized" silver wool, prepared electrochemically has been shown to quantitatively trap circulating [^{18}F]-HF[16]. This procedure has been used successfully to obtain 750 MBq quantities of [^{18}F]-HF for the synthesis of alkyl fluorides.

Glass target inserts have been used as an alternative to flow systems[44,89-92]. The ^{18}F trapped on the surface of the inserts during bombardment was subsequently removed by rinsing with water[44,90] or salt solutions[44,89,91] and utilized in radiochemical reactions or nuclear medicine procedures requiring ^{18}F-anion. A rotating quartz liner was used with ^{20}Ne as the target material in the routine production of ^{18}F. Sterile solutions of ^{18}F-anion were recovered from this target by flushing the insert with water or salt solution[44]. A stainless steel target holder using a niobium entrance foil and a silver surfaced pyrex liner has been used with $^{18}O_2$ as target material to obtain up to 5.5 GBq of ^{18}F per uA of beam current[92]. Most of the activity was trapped on the pyrex liner which was removed and used as a reaction vessel for subsequent

syntheses requiring ^{18}F-anion. Alternately the ^{18}F has been removed from the liner by boiling it in water[65]. The very expensive $^{18}O_2$ target material was cryogenically recovered after each irradiation without contamination by ^{18}F.

A prototype copper target holder has been developed which allows static irradiation of a neon target and subsequent recovery of up to 85% of the ^{18}F activity as carrier free [^{18}F]-HF[93,94]. The recovery procedure required heating of the target body to 300°C under a flow of 10% H_2 in He. This copper target system was regarded as superior to the fluorine passivated target holders which may contribute non-radioactive fluorine to recovered materials and thereby compromise the carrier free state of the ^{18}F[93]. Copper was selected for the target system after trial experiments with a number of metals. Absorbed ^{18}F was more readily released from copper upon heating than from any of the other metals tested. Copper has the added advantages of being easy to machine, inexpensive and readily available as a high purity metal.

Another static target system has been described for applications where a no-carrier-added product is not required[95]. The addition of 4.4% HF to the target neon gas has allowed for 100% recovery of the activity as [^{18}F]-HF after bombardment. Alternatively the problems associated with handling HF have been overcome by *in situ* radiolytic production of HF from CF_4 and H_2 mixed with the target gas. Although not carrier free the product [^{18}F]-HF was obtained in high yield and at high specific activity[95]. Recently a target system was described for the *in situ* production of [^{18}F]-HF by the deuteron irradiation of a neon / hydrogen gas mixture[341].

(ii) [^{18}F]-molecular fluorine production

Accelerator production can also yield ^{18}F as molecular fluorine. In this chemical form it may act as an electrophile and thereby permit the added synthetic potential for electrophilic additions and substitutions in suitable compounds. The first synthesis employing [^{18}F]-F_2 was reported in 1973[96] when Wolf and co-workers prepared ^{18}F-labelled 5-fluorouracil from the reaction of [^{18}F]-F_2 with uracil. The majority of work performed since this initial report with

[^{18}F]-F_2 is based on targetry developed and refined by Wolf and co-workers at the Brookhaven National Laboratory. Their passivated nickel target holder has been described in detail[97]. They have also reported refinements in the gas handling systems[98], purity requirements for the target gases[99] and the development of a shielded and remotely operated synthesis system[100]. The reader is referred to these papers for more details. This system has reliably produced [^{18}F]-F_2 which has been used by Wolf and co-workers for the synthesis of a variety of precursors and biologically important molecules[100-106]. Other workers have successfully used the Brookhaven targetry design for the production of [^{18}F]-F_2 for a variety of synthetic projects[107-112].

This target holder was constructed of high purity nickel polished inside to a mirror finish and isolated from the cyclotron or accelerator beam line by an aluminum backed nickel foil[98]. Gas delivery and recovery lines were constructed of monel metal as were all valves and other surfaces in contact with F_2. The system was passivated by heating at 300 °C for 48 hours under a pressure of 1 atmosphere of F_2. The NiF_2 coating formed by this procedure was inert to further attack and was reported not to exchange with F_2 in the gas phase[97] although there remains some uncertainty about this claim[96,113].

The target gas was typically a mixture of ultra pure neon and 0.1% to 1.0% F_2 as carrier. Impurities in the target gas must be rigorously excluded since the highly reactive ^{18}F has been shown to react with traces of N_2 and CO_2 as well as hydrocarbon impurities to form non-reactive species which affect the yield in subsequent syntheses requiring [^{18}F]-F_2[99]. Carrier free [^{18}F]-F_2 has not been recovered to date. Recovery of activity from the target holder is typically 50 to 60% of the total activity produced[100] and experience with the synthesis of [^{18}F]-fluorosugars has shown that specific activities of 0.7 GBq per milligram of product can be obtained[100,105].

A similar nickel target system has been used with a Van de Graaff Accelerator to produce 0.9 GBq of [^{18}F]-F_2[114]. The unpolished but passivated target holder used 1% or 2% F_2 in a neon target gas. This system suffered from the same loss of reactive [^{18}F]-F_2 due to the reaction between ^{18}F and impurities in the target gas and the target holder surfaces as described

previously.

The high yield nuclear reaction $^{18}O(p,n)^{18}F$ has also been used to produce $[^{18}F]$-F_2 in a nickel target system[113]. The procedure involved the proton irradiation of an $^{18}O_2$ enriched oxygen atmosphere. The ^{18}F produced adhered to the walls of the target chamber and allowed the $^{18}O_2$ to be cryogenically recovered. A second short irradiation of an inert gas / F_2 mixture allowed $[^{18}F]$-F_2 to be produced via exchange with the ^{18}F absorbed on the target walls and subsequently recovered from the target holder. The recovery was variable and low but the system seems to have potential for producing substantial quantities of $[^{18}F]$-F_2.

A recent inovation in ^{18}F-production was the development of multi-purpose target units which have permitted simultaneous or sequential production of ^{18}F in conjunction with other short lived radionuclides[81,115,116]. These systems have allowed semi-automated production of radionuclides without the necessity of manual switching of target units. These systems provide the advantages of rapid switching between the various isotope productions, economy in target construction, efficiency in the use of irradiation schedules and reduction in personnel radiation exposure.

Although $[^{18}F]$-F_2 has proved to be a valuable precursor for the preparation of $[^{18}F]$-labelled radiopharmaceuticals there remain a number of problems which limit its use. The production method requires a cyclotron capable of accelerating deuterons since the $^{20}Ne(d,\alpha)^{18}F$ nuclear is the only practical method of producing $[^{18}F]$-F_2 in quantities suitable for synthesis. The stringent requirements on gas purity, target fabrication and recovery methodology require dedicated resources and manpower in order to acheive "routine" production of this species. At present no-carrier-added $[^{18}F]$-F_2 is not available. Syntheses employing $[^{18}F]$-F_2 do not yield products with a sufficiently high specific activity to be used in some kinds of studies. For example the low uptake of ^{18}F-4-fluoroestradiol into the prostate of rats was attributed to the low specific activity of the fluorinated compound[117]. A final problem is the loss of at least 50% of the ^{18}F produced during synthesis due to the formation of non-reactive species or unwanted by-products. While $[^{18}F]$-anion is somewhat more accessable,

can be produced at no-carrier-added levels and can give theoretical yields of 100%, its use has been restricted somewhat by the absence of suitable synthetic procedures for its incorporation into organic molecules. A number of recent advances in radiofluorination methodology using [^{18}F]-anion have helped to improve the synthetic potential of this species.

B. Radiochemical Synthesis with ^{18}F

Introduction

The physical and chemical properties of fluorine make it a very difficult species with which to perform chemical synthesis. Fluorine is the most electronegative and reactive element producing fluorinated products on reaction with every other element except neon and helium. Reactions which proceed smoothly with other halogens often do not take place with fluorine or may lead to unexpected products. The fluorine anion has a high charge density and tends to be highly solvated in solution thereby greatly reducing its reactivity in nucleophilic substitution reactions. The chemical problems are compounded by the nature of the fluorine species that are available *via* the usual radionuclide production methods. Reactor produced ^{18}F requires solvent extraction or distillation of very minute amounts of fluorine (1 GBq of ^{18}F is 15.8×10^{-9} mmol). Fluorine is usually obtained from cyclotron targets as either [^{18}F]-HF or [^{18}F]-F_2. The former species reacts with glassware and the latter product requires dilution with substantial quantities of carrier F_2 before it can be removed from the target holder. The radiochemist also faces additional problems presented by the relatively short half-life of ^{18}F (109.7 minutes)[20] and the necessity for shielding from the γ-radiation produced by anihilation of the β^+-particle produced upon radioactive decay.

It is remarkable that despite the numerous problems inherent in synthesis with ^{18}F a great variety of organic radiopharmaceuticals have been prepared. Compounds such as [^{18}F]-2-deoxy-2-fluoro-D-glucose have reached the status of "routine" production[109,110], and a wide range of other biological tracers labelled with ^{18}F have been studied. The main synthetic

strategies for organic synthesis with ^{18}F involve either the enhancement of the anionic character of ^{18}F thereby making it a better nucleophile or preparation of a species in which ^{18}F is bonded to an element of similar electronegativity such as ^{18}F-F or ^{18}F-O-. In these compounds the bond to fluorine is considerably weaker than for instance the C-F bond and the fluorine acts as an electrophile for electrophilic addition or substitution reactions.

The following section deals briefly with the various ^{18}F precursor molecules that have been prepared and the types of compounds that can be synthesised employing these species.

Nucleophilic substitution reactions with ^{18}F-anion.

Despite the ease of synthesis using electrophilic ^{18}F-species, the fluorine anion remains a very attractive precursor for the preparation of ^{18}F-labelled compounds. It is readily accessable from reactor and cyclotron sources, it can be recovered in high yield from most target systems and can in theory produce high yields of no-carrier-added products. Large [redacted]F$^-$ are now available especially from the recently developed $^{18}O_2$ target[92] and [redacted]$^{18}O_2$ [118] target systems using the ^{18}O(p,n)^{18}F nuclear reaction. The major [redacted] the fluorine anion is that under most reaction conditions it tends to be a poor n[redacted]. The solvation shell surrounding the fluorine anion in polar protic solvents prevents flu[redacted]rom acting as an effective nucleophile. The salts tend to be poorly solvated in nor[redacted]r solvents and often long reaction times and vigorous reaction conditions are required to [redacted]eve acceptable yields. Water tends to be a better nucleophile in reactions with the flu[redacted]ne anion and therefore all reaction mixtures must be rigorously anhydrous. The drying of flu[redacted]de salts can be difficult and time consuming. Despite these problems a number of species have [redacted]en used to achieve acceptable yields in radiochemical synthesis with the ^{18}F-anion.

A number of fluoride salt / solvent combinations have been devised which seem to enhance the nucleophilic character of the fluoride anion. [^{18}F]-AgF was obtained by washing ^{18}F-production targets with carrier AgF in acetonitrile[91,119]. Nucleophilic substitutions with this salt gave [^{18}F]-fluorocholesterol from iodocholesterol in 20 minutes at room temperature[119].

Reaction of [^{18}F]-AgF with tetra-O-alkyl-α-D-glucosyl bromide gave the corresponding [^{18}F]-labelled sugar in 8.2% yield[91].

[^{18}F]-CsF has been produced by trapping circulating [^{18}F]-HF from a gas target on silver wool impregnated with CsOH[113,114] and by the reaction between $^{18}F^-$ and Cs_2CO_3[120]. This reagent has been used for a number of carrier-added[81,121] and no-carrier-added syntheses[21,87,120-122].

A typical procedure for synthesis with this reagent involves transfer of the silver wool [^{18}F]-CsF to a reaction vial containing the appropriate substrate in hexamethylphosphoric triamide and heating for an hour or more at 150 °C. A carrier-added synthesis of [^{18}F]-2-deoxy-2-fluoro-D-glucose was carried out using [^{18}F]-$CsHF_2$ in hexamethylphosphoric triamide at 130 °C for 25 minutes[14]. The cesium salt was prepared by trapping [^{18}F]-HF on CsF coated silver wool. [^{18}F]-Fluoroethanol, proposed as a microsphere analog for measuring blood flow, was prepared using carrier-free [^{18}F]-CsF to displace a tosylate or open a sulfite ring[17]. [^{18}F]-7-Fluoropalmitic acid has also been synthesised from [^{18}F]-CsF via nucleophilic displacement of sulfonic esters in dimethylsulfoxide[122]. Recently a series of no-carrier-added ^{18}F-labelled aryl fluorides has been prepared in yields up to 90% by the reaction between [^{18}F]-CsF and aryl compounds containing a trimethylammonium perchlorate leaving group[120].

The combination of the tetraethylammonium cation with the fluorine anion (tetraethylammonium fluoride) appeared to have a number of advantages over metal fluorides. The solutions of tetraethylammonium salts gave enhanced nucleophilicity and anionic character to the fluoride and in addition the tetraethylammonium salts were more readily freed of their hydration sphere than metal salts[62]. Mixing carrier tetraethylammonium fluoride with ^{18}F-anion in solution followed by careful drying gave the required reagent. If $^{18}F^-$ was added to tetraethylammonium hydroxide, carrier free [^{18}F]-tetraethylammonium fluoride could be obtained.

[^{18}F]-Tetraethylammonium fluoride has been used to prepare carrier free [^{18}F]-6-deoxy-6-fluoro-α-D-galactopyranose by tosyl group displacement[10,123] and carrier free

[^{18}F]-2-deoxy-2-fluoro-D-glucose by the opening of cyclic 2,3-sulfates[65]. In both cases the [^{18}F]-tetraethylammonium fluoride was prepared by adding tetraethylammonium hydroxide to aqueous solutions of no-carrier-added ^{18}F$^-$ and evaporating the solutions to dryness.

Gatley and co-workers have prepared a number of ^{18}F-labelled compounds using [^{18}F]-tetraethylammonium fluoride. Reactor irradiation of lithium salts was used to provide the necessary ^{18}F$^-$. They prepared fluoroalkanes by reaction of [^{18}F]-tetraethylammonium fluoride with the corresponding iodoalkanes[55,63] and [^{18}F]-3-deoxy-3-fluoro-D-glucose by nucleophilic displacement of the triflate group[53,62]. The [^{18}F]-tetraethylammonium fluoride was dried by an initial rotary evaporation to remove the bulk of the water followed by azeotropic distillation with acetonitrile to remove the final traces of water[53,59]. Despite these attempts to obtain anhydrous product it appears that truly anhydrous tetraethylammonium fluoride can not be prepared[124,125] and the fluoride remains strongly bonded to residual water[53,125]. In the F$^-$ for I$^-$ exchange reactions the use of silver (I) oxide as a catalyst gave a greatly improved yield of ^{18}F-labelled product[53,63].

The neuroleptic drugs [^{18}F]-spiroperidol[126] and [^{18}F]-haloperidol[127] have recently been prepared using no-carrier-added syntheses for use as markers for dopaminergic receptor sites. The reactions employed nucleophilic aromatic substitution of ^{18}F for NO_2 or Cl using no-carrier-added [^{18}F]-tetraethylammonium fluoride in dimethylsulfoxide. The [^{18}F]-spiroperidol was produced in <2% yield[126] and the [^{18}F]-haloperidol in 10% yield[127] by this procedure.

The nucleophilicity and basicity of fluoride anion is greatly enhanced in polar and non-polar aprotic solvents in the presence of crown ethers. The solubilized fluoride anion has been termed "naked" fluoride[128] and this species exhibits atypical behaviour and reactivity. The crown ether, 18-crown-6, has a strong affinity for potassium and complexes KF forming solutions in such solvents as dimethylformamide, acetonitrile, benzene and chloroform. These solutions have "naked" fluoride available for reaction. Solutions of [^{18}F]-KF dissolved in solvents containing 18-crown-6 were prepared from cyclotron produced ^{18}F$^-$ and used in the

synthesis of [^{18}F]-6-fluoro-9-β-D-ribofuranosyl purine by displacement of a trimethyl amino group[110]. The product was obtained in a 35% radiochemical yield in 30 minutes in a no-carrier-added synthesis and in 63.5% radiochemical yield when carrier was added. The same reagent was used to produce [^{18}F]-21-fluoroprogesterone from its mesyl precursor in a carrier-added synthesis[129,130]. No-carrier-added [^{18}F]-KF has been produced by evaporating to dryness a solution of $^{18}F^-$ and KOH. This reagent was used to synthesise [^{18}F]-21-fluor-progesterone in 12.1% radiochemical yield in a procedure similar to that described above[131]. The yield shows a strong dependance on the amount of carrier KOH. The KOH appears to suppress the deactivation of F^- by impurity cations in the reaction solutions[131]. The [^{18}F]-KF / 18-crown-6 complex has also been prepared from reactor produced ^{18}F by an ion exchange and distillation procedure[52].

Halogen-halogen exchange reactions.

Heterogenous nucleophilic exchange reactions have been used to synthesise ^{18}F-labelled materials from their corresponding bromo or chloro compounds using both reactor and cyclotron produced ^{18}F. Anion exchange resins labelled with ^{18}F have shown some potential in fluorine for halogen exchange reactions and compounds such as [^{18}F]-2-fluoroethanol[132], [^{18}F]-acid fluorides[133], [^{18}F]-benzyl fluoride[134] and a variety of [^{18}F]-fluoro fatty acid esters[60] have been synthesised. Reactor produced ^{18}F was isolated in two ion exchange steps[134] involving initial reaction of Li_2CO_3 with cationic resin followed by absorption of the $^{18}F^-$ on anionic resin. This form of resin once dried served as the $^{18}F^-$ source for exchange reactions in aceto-nitrile.

A labelled resin has also been used in a heterogenous exchange reaction with α-bromo fatty acid esters, the exchange taking place in a section of a gas chromatographic column[60]. This method had the advantage of providing a rapid work-up from reactor product to fluorinating species. The resin bound fluoride exhibited not only enhanced nucleophilicity but also strong basic properties as evidenced by the formation of some elimination products[134].

A gas chromatographic column was also used to carry out the synthesis and the separation of no-carrier-added [^{18}F]-alkyl fluorides by means of a heterogenous nucleophilic substitution reaction in the gas phase[16]. Yields as high as 60% of no-carrier-added products were obtained by passing alkyl iodides and bromides through a plug of "surface oxidized" silver wool with [^{18}F]-HF absorbed on its surface. The [^{18}F]-alkyl fluorides were then separated from the unreacted starting materials by passage through the gas chromatography column[16].

Nucleophilic ^{18}F for Br exchange with the methyl esters of 16-bromohexadecanoic acid and 17-bromoheptadecanoic acid has been effected using [^{18}F]-KF in an acetamide melt[76]. These compounds have potential for the measurement of myocardial extraction and elimination rates. The initial studies showed the reaction yield to have a strong dependance on the concentration of carrier KF with radiochemical yields approaching zero as no-carrier-added concentrations were approached[76]. Recently no-carrier-added radiochemical yields of 70% have been obtained for the synthesis of [^{18}F]-17-fluoroheptadecanoic acid by adding K_2CO_3 to the melt and carrying out the reaction in "glassy" carbon reaction vessels[135].

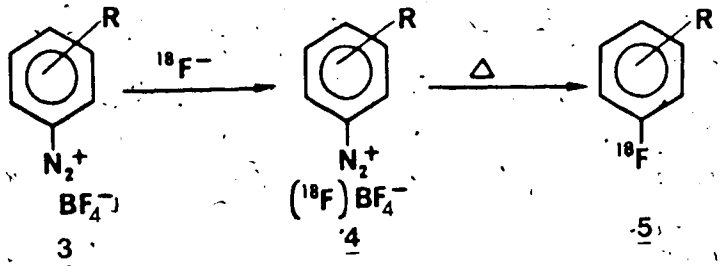
Homogeneous exchange reactions have been carried out on fluorinated aromatic compounds using [^{18}F]-RbF solutions in dimethyl sulfoxide[104]. Exchange yields as high as 90% and specific activities of products at greater than 100 GBq / mmol were obtained. This technique appears to have promise for reactions with suitably activated aromatic systems.

Balz-Schiemann reaction for the synthesis of [^{18}F]-aryl fluorides.

Diazonium tetrafluoroborate salts (3) undergo fluoride for ^{18}F exchange in solution to give the labelled salts(4). Decomposition of these ^{18}F-labelled salts by refluxing in high boiling solvents afforded [^{18}F]-aryl fluorides(5)[136] (Scheme II.1).

The Balz-Schiemann reaction suffered from a variety of problems. The exchange of ^{18}F$^-$ with the fluorine in the fluoroborate salt and the thermal decomposition reaction to form the [^{18}F]-aryl fluoride tended to be slow reactions. In addition sensitive molecules required the use of protecting groups which must be removed after the formation of the ^{18}F-labelled

compounds. These procedures can extend to several half-lives of ^{18}F. The maximum radiochemical yield is 25% since only one of the four fluorines of the fluoroborate salt becomes attached to the aromatic ring. In practice radiochemical yields were considerably lower than this[137]. The specific activities of the compounds produced were also very low.



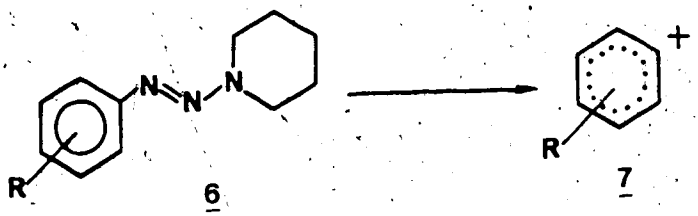
Scheme II.1 Balz-Schiemann reaction for the synthesis of ^{18}F-labelled aryl fluorides.

Some of the compounds produced by this method included [^{18}F]-p-fluorophenylalanine[138-140] and [^{18}F]-tryptophan[75] which were prepared as potential pancreas scanning agents, [^{18}F]-5-fluoro-DOPA[55] as a brain scanning agent and [^{18}F]-haloperidol[90] as a neuroleptic agent. The amino acids showed no advantage over ^{75}Se-L-selenomethionine in animal studies and the low specific activity of haloperidol and 5-fluoro-DOPA prevented their use in tracer studies. This method of producing ^{18}F-labelled aryl fluorides has limited utility.

Decomposition of aryl triazines.

It is likely that decomposition of aryl triazines (6) (Scheme II.2) gives rise to a transient and non-selective electron deficient aryl cation (7) which can react with a fluoride anion or other nucleophile or extract a hydride ion from solution[141]. The synthesis of haloperidol[141] and spiroperidol[142] in this way using [^{18}F]-CsF as the fluoride source resulted in a low product yield. This reaction does show some potential to produce carrier-free products

where receptor site specific radioactive tracers are required, provided the yields can be improved.



Scheme II.2. Decomposition of aryl triazines.

Diethylaminosulfur trifluoride (DAST).

The reagent diethylaminosulfur trifluoride (Et_2NSF_3, DAST) has shown some application as a fluorinating reagent in F for Cl substitutions in acid halides[143] and F for OH substitutions in a variety of alcohols[144] including the sugar 1,2,3,4-tetra-O-acetyl-β-D-gluco-pyranose[145], and a series of gluco- and talopyranoses[146]. This reagent has been used to prepare some simple [^{18}F]-alkanes and [^{18}F]-2-fluoroethanol[83].

Electrophilic fluorinating reagents.

Since the development of the Brookhaven targetry for the production of [^{18}F]-F_2[97,98], this reagent has been used to provide good yields (though not carrier-free) of a variety of useful molecules. Much attention has been given to the ^{18}F-labelled deoxy sugars [^{18}F]-2-deoxy-2-fluoro-D-glucose[100-102,108-110,114] and [^{18}F]-2-deoxy-2-fluoro-D-mannose[101,109]. These compounds have been used as probes in imaging studies of heart, brain and tumor[21,22,102,109] where they provide both structural and functional information. Elemental

fluorine when passed through a solution of 3,4,6-tri-O-acetyl-D-glucal in freon-11 ($CFCl_3$) followed by acid hydrolysis gave rise to the glucose and mannose sugars labelled at the 2-position with fluorine, the ratio of gluco to manno configuration being 4 to 1. The widespread use of these compounds has stimulated efforts to prepare the labelled product in a routine, reliable, and where possible, automated system[100,108].

^{18}F has been incorporated into 5-fluorouracil[107,112], 5-fluorouridine[112,147,148] and 5-fluoro-2'-deoxyuridine[112,148,149] using the reaction developed by Cech and co-workers for preparing fluorinated pyrimidines and their nucleosides[150]. The reaction procedure involves purging the target assembly containing $[^{18}F]$-F_2 through a solution of the pyrimidine base or nucleoside in acetic acid. $[^{18}F]$-5-fluorouracil was obtained in 28% radiochemical yield by this procedure[107]. $[^{18}F]$-4-Fluoroantipyrine, a potential cerebral blood flow tracer, has been prepared by the reaction of antipyrine with $[^{18}F]$-F_2 using acetic acid as the solvent[103]. The reagent $[^{18}F]$-XeF_2 has been used to prepare $[^{18}F]$-6-fluoro-DOPA in low yield[151]. Recently an improved synthesis of $[^{18}F]$-2-deoxy-2-fluoro-D-glucose has been reported which utilizes acetyl hypofluorite ($[^{18}F]$-CH_3COOF) as a fluorinating reagent[105]. This reagent seems to be milder and more selective than $[^{18}F]$-F_2[152,153] and has afforded higher yields of the ^{18}F-labelled fluoro sugar than previously reported.

Electrophilic aromatic substitution reactions have been perfotmed with $[^{18}F]$-CH_3COOF to yield the corresponding $[^{18}F]$-aryl fluorides. The reaction of arylmercury compounds with $[^{18}F]$-CH_3COOF in CH_3COOH at room temperature gave a 45 - 65% yield of the corresponding $[^{18}F]$-arylfluoride products[154]. ^{18}F-Labelled aryl fluorides have also been prepared using the electrophilic aromatic substitution reaction between either $[^{18}F]$-F_2 or $[^{18}F]$-CH_3COOF and aryltrimethylsilanes or arylpentafluorosilicates[106] and aryltributyltin compounds[155]. $[^{18}F]$-Perchlorylfluoride ($[^{18}F]$-$FClO_3$) has been synthesised recently from $[^{18}F]$-F_2 and $KClO_3$ and has been shown to react readily with functionalized aryl-lithium compounds to give the corresponding $[^{18}F]$-aryl fluorides in good radiochemical yield[111].

C. Synthesis of 5-Radiohalogenated Pyrimidine Bases and Nucleosides

Introduction.

The synthetic methods for preparing [^{18}F]-5-fluoropyrimidine bases and nucleosides were discussed in the previous section. The following section deals with the methods which have been employed for the incorporation of radioisotopes of iodine, bromine and chlorine into the C-5 position. The 5-halopyrimidines have received much attention since members of this class have exhibited antiviral and antitumor activity while compounds such as 5-iodo-2'-deoxyuridine and 5-bromo-2'-deoxyuridine are extensively incorporated into replicating DNA. The applications of radiolabelled compounds in this family include tracer studies, measurement of tumor response to therapy, metabolism studies and radiation induced destruction of tumor cells.

These compounds are relatively easy to synthesise. The uracil moiety is susceptible to attack by electrophilic species at the C-5 position, presumably due to the increased π-electron density at this position[156]. The reactions tend to proceed in moderate to high yield with a minimum of side products formed. A variety of synthetic methods have been devised for radiochemical synthesis. These methods have been investigated to improve some aspect of the existing syntheses by;

1. Improving overall radiochemical yield.
2. Synthesising compounds at higher specific activity or at no-carrier-added concentrations
3. Utilizing readily available forms of the isotopes
4. Simplifying the production techniques
5. Developing mild, selective radiohalogenation methods for sensitive molecules.

5-Radioiodine labelling of pyrimidines.

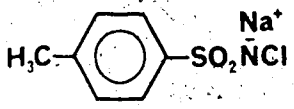
Iodopyrimidines were first synthesised in 1905. The iodination reagent, molecular iodine, was generated *in situ* by oxidation of KI using H_2SO_4 and KIO_3[157]. This technique was

used for the first preparation of radiolabelled [^{131}I]-5-iodouracil by Prusoff *etal* in 1953[158]. Prusoff also developed a reaction method using HNO_3 as the oxidizing species along with iodide or molecular iodine and various mixtures of organic solvents and water. This method was used for the first preparation of 5-iodo-2'-deoxyuridine[159] and for the synthesis of [^{131}I]-5-iodouridine[158]. The HNO_3 method was used for the small scale high specific activity synthesis of [^{125}I]- and [^{131}I]-labelled 5-iodo-2'-deoxyuridine, 5-iodouridine and 5-iodouracil for *in vivo* investigation of brain tumors in rats[160]. A simplified reaction utilizing HNO_3 was developed and used for the production of no-carrier-added [^{125}I]- and [^{131}I]-labelled 5-iodo-2'-deoxyuridine in yields up to 48%[161] and no-carrier-added [^{123}I]-labelled 5-iodo-2'-deoxyuridine in yields of 75 - 80%[162].

An isotopic exchange reaction between 5-iodocytosine or 5-iodouracil and ^{131}I in solution at pH 7 was effected by heating the solution at 100 °C for 30 minutes. The yields in these reactions were low and the products contained carrier iodine[163]. These reaction conditions as well as those required for the HNO_3 reaction method are relatively harsh. A number of milder radioiodination methods have been developed and used for the preparation of radiolabelled 5-iodopyrimidines.

Iodine monochloride (ICl) has been exchange-labelled with carrier-free [^{131}I]-NaI and used for the synthesis of [^{131}I]-5-iodo-2'-deoxyuridine[164]. Although this particular reaction gave only moderate yields of radiolabelled product it appears that virtually quantitative yields could be obtained by reacting p-toluyl ester protected nucleosides with ICl in organic solvents[165]. ICl has also been obtained carrier-free by reacting ^{123}I with Cl_2 or NOCl[166,167]. No-carrier-added [^{123}I]-5-iodo-2'-deoxyuridine was obtained in 80 - 90% yield after a 3 hour reaction in ethanol at 90 °C using this reagent[167]. Chloramine-T (8, N-chloro-p-toluenesulfonamide sodium salt) has had widespread use in the iodination of proteins and has given excellent yields in the synthesis of no-carrier-added ^{125}I-labelled 5-iodouracil (95% radiochemical yield)[163], 5-iodouridine (75% radiochemical yield)[168] and ^{131}I-labelled N-1 substituted pyrimidines[169]. Chloramine-T acts as a mild oxidizing agent and slowly liberates hypochlorous

acid (HOCl) in aqueous solution which subsequently oxidizes radioiodide (I^-) to radioiodonium ion (I^+)[170]. This species then adds to the pyrimidine base at the electron rich 5-position to give the required radiolabelled products. Iodogen (9) has also been used as a mild oxidant for the preparation of a variety of N-1 substituted 5-[^{131}I]-iodopyrimidines[169].



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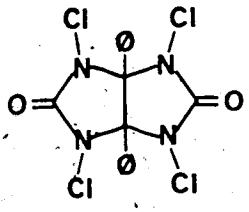

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The enzyme lactoperoxidase has been used to catalyse the 5-radioiodination of pyrimidines in the presence of H_2O_2[161]. Although this is a very mild iodination method, particularly when immobilized polymer bead supported enzyme is used, it suffers from a number of disadvantages. The radiochemical yields tended to be lower than other iodination methods[171] and the yields fell to zero as a no-carrier-added iodide concentration was approached[161].

The nuclear reaction $^{127}I(n,\gamma)^{128}I$ using reactor generated thermal neutrons was employed for the preparation of [^{128}I]-5-iodouracil and [^{128}I]-5-iodo-2'-deoxyuridine in 29% and 23% respectively using the Szilard-Chalmers reaction[172]. The reactant molecules, 5-iodouracil and 5-iodo-2'-deoxyuridine, were irradiated as dilute solutions frozen in an ice lattice. The "caging" of the iodopyrimidines in ice resulted in almost complete absence of the deiodination or decomposition of molecules that was noted when liquid solutions were similarly irradiated[172]. Although the specific activity of the products was relatively low (2.3 GBq mmol^{-1} for 5-iodouracil) and the ^{128}I isotope does not have suitable decay characteristics for biological work, this method may allow for rapid production of radiolabelled compounds not readily accessable by other methods.

The synthesis of C-5 mercurated pyrimidine nucleosides has been described[173] and this species has been shown to undergo mercury-halogen conversion at essentially quantitative levels at both the mononucleotide and polynucleotide levels[174]. The mononucleotide uridine triphosphate has also been iodinated with N-iodosuccinimide in dimethyl sulfoxide in the presence of N-butyl disulfide which appears to catalyze the reaction[175]. It was suggested that this method may be better than conventional methods for radioiodination of sensitive molecules.

5-Radiobromine labelling of pyrimidines.

5-Radiobrominated pyrimidines are readily formed by a variety of reactions. Bromine is somewhat more reactive than iodine and the bond strength of the C-Br bond is about 15 Kcal mol^{-1} stronger than the C-I bond making it less succeptible to *in vivo* dehalogenations. Simple treatment of uridine and 2'-deoxyuridine with solutions of Br_2 in water were used for the first syntheses of cold 5-bromouridine[176] and 5-bromo-2'-deoxyuridine[177].

Molecular radiobromine appears to be the reactive species in the excitation labelling experiments carried out with cyclotron produced ^{76}Kr and ^{77}Kr[178]. These radionuclides were allowed to decay to ^{76}Br and ^{77}Br respectively in the presence of $KBrO_3$ crystals. When the ^{76}Br / $KBrO_3$ reagent was reacted with uracil in HCl solution 5-bromouracil was obtained in 76% radiochemical yield. It was suggested that BrO_3^- acted as an oxidizing agent at low pH promoting the oxidation of Br^- to Br_2 which was the active radiobrominating species[178]. $KBrO_3$ crystals have been exposed to reactor produced $CF_3{}^{80}Br^m$ and this material was used similarly to give high-yield incorporation of radiobromine in Br for I exchange in biomolecules[179].

The reagent BrCl appears to be a particularly good radiobrominating intermediate. This was probably the reactive species formed when reactor irradiated CF_3Br was treated with Cl_2 gas[180]. The labelling yields improved from <1% (CF_3Br alone) to 27% (CF_3Br / Cl_2) in the synthesis of 5-bromo-2'-deoxyuridine. [^{82}Br]-5-bromo-2'-fluoro-2'-deoxyuridine has been synthesised in 75% radiochemical yield by *in situ* generation of [^{82}Br]-BrCl using reactor

produced $^{82}Br^-$ and N-chlorosuccinimide[181]. No-carrier-added [^{77}Br]-BrCl was prepared by reacting carrier-free $^{77}Br^-$ with Cl_2 in ethanol[167]. This reagent gave 80 - 90% yields of [^{77}Br]-5-bromo-2'-deoxyuridine after a 3 hour room temperature reaction. A rapid radiobromination method, which apparently involves the generation of gaseous BrCl, has recently been described[182] and used for the synthesis of a number of model compounds[183]. In this radiobromination method an aqueous solution of radiobromide was treated with $KMnO_4$ and HCl and the volatile radiobromine species was transferred in a stream of inert gas into a reaction vessel containing the compound to be brominated in solution in phosphate buffer. No-carrier-added [^{77}Br]-5-bromouracil[182] and [^{77}Br]-5-bromo-2'-deoxyuridine[183] were synthesised in 92% and 65% respectively using this method.

Chloramine-T has been investigated as an oxidizing agent for radiobromination in reactions analogous to those used successfully for radioiodinations[184]. While the radiobromination of uracil was successful giving an 83% radiochemical yield of [$^{80}Br^m$]-5-bromouracil, the same reaction failed to yield any product when uridine was the substrate.

A number of enzymatic methods have been investigated for radiobromination. Lactoperoxidase, the enzyme most commonly used to catalyze radioiodinations, does not catalyze radiobromination[185]. Chloroperoxidase has been used successfully for the preparation of [$^{80}Br^m$]-5-bromouracil[184] and a new brominating enzyme, bromoperoxidase, recently isolated from red algae appears to have some potential as a radiobrominating reagent as demonstrated by its success in radiobromine labelling of human serum albumin and canine fibrinogen[185].

In reactions analogous to those described previously for radioiodination, 5-bromopyrimidines have been prepared by reaction of 5-mercurated nucleotides and N-bromosuccinimide[174] and by the Szilard-Chalmers reaction on non-radioactive 5-bromopyrimidines held in an ice lattice[172].

5-Radiochlorine labelling of pyrimidines.

There has been little biological work carried out with radiochlorine labelled compounds since a suitable radioisotope of this halogen is not available. The majority of isotopes of chlorine are either short lived (^{32}Cl, ^{33}Cl, ^{34}Cl, $^{38}Cl^m$) or hard β^--ray emitters (^{38}Cl, ^{39}Cl, ^{40}Cl)[20]. The isotope ^{36}Cl is a moderate energy β^--emitter but has a very long half life (3.1×10^5 years)[20], can be obtained only in low specific activity and is unsuitable for imaging studies. The only useful isotope of chlorine suitable for imaging studies appears to be $^{34}Cl^m$ which decays by β^+-emission. $^{34}Cl^m$ has a half life of 32 minutes which places some restrictions on the types of synthetic and biological studies that can be carried out with $^{34}Cl^m$-labelled compounds. Although 5-chloropyrimidines are readily prepared in non-radioactive syntheses[165,186-189] it appears unlikely that 5-radiochlorinated pyrimidines would prove suitable for biological studies.

D. Synthes[redacted]enated Pyrimidine Nucleosides

Introductio[redacted]

Che[redacted]ification of pyrimidine nucleosides has provided compounds which ex[redacted]a wid[redacted]ctrum of biological activity. Compounds in this class have been used in diag[redacted]tic [redacted]herapeutic oncology and in antiviral therapy. Compounds of this type have also prov[redacted]rmation on various aspects of cellular biochemistry including pyrimidine nucleo[redacted]ransport, enzyme-substrate specificity and the mechanism of action of enzym[redacted]strate interactions. A number of different approaches have been used to synthesise these an[redacted]compounds.

A [redacted]mber of pyrimidine nucleoside analogs have been synthesised in multistep procedures, building the required skeleton from small carbon and nitrogen containing fragments. This approach has allowed for the incorporation of annular heteroatoms at various positions in[redacted]keleton and for the insertion of ^{14}C in both natural and analog pyrimidine nucleoside[redacted]s method has the disadvantage of requiring multiple synthetic steps and consequently may lead to overall low yields.

Many modified pyrimidine nucleosides have been prepared by the Hilbert-Johnson coupling reaction of pyrimidine bases and suitably blocked sugar moieties. This method has provided rapid and high yield syntheses of a variety of structures not readily available by other synthetic approaches. Coupling reactions can lead to mixtures of compounds due to lack of regiochemical (N-1 and N-3 coupling) and anomeric (α- and β-configuration) specificity. These mixtures may be difficult to separate. An additional problem with coupling reactions is that additional synthetic steps are required to prepare the blocked starting materials and to deprotect the coupled products.

The third approach used for the synthesis of modified pyrimidine nucleosides is *via* existing naturally occuring nucleosides or commercially available nucleoside analogs. This approach has the advantage of maintaining the β-configuration of the glycoside linkage and

keeping the pyrimidine and sugar rings intact while allowing alteration or addition of substituents at various positions. On the other hand there are limitations to the types of reactions which can be applied without compromising the integrity of the glycosidic linkage. In addition the presence of reactive substituents in the nucleoside often necessitates the use of blocking groups in synthetic procedures.

The following section reviews the literature concerning the synthesis of pyrimidine nucleosides modified by halogenation, with particular emphasis on the 2'- and 3'-positions of the sugar and the 5-position of the heterocycle. (See Figure II.1).

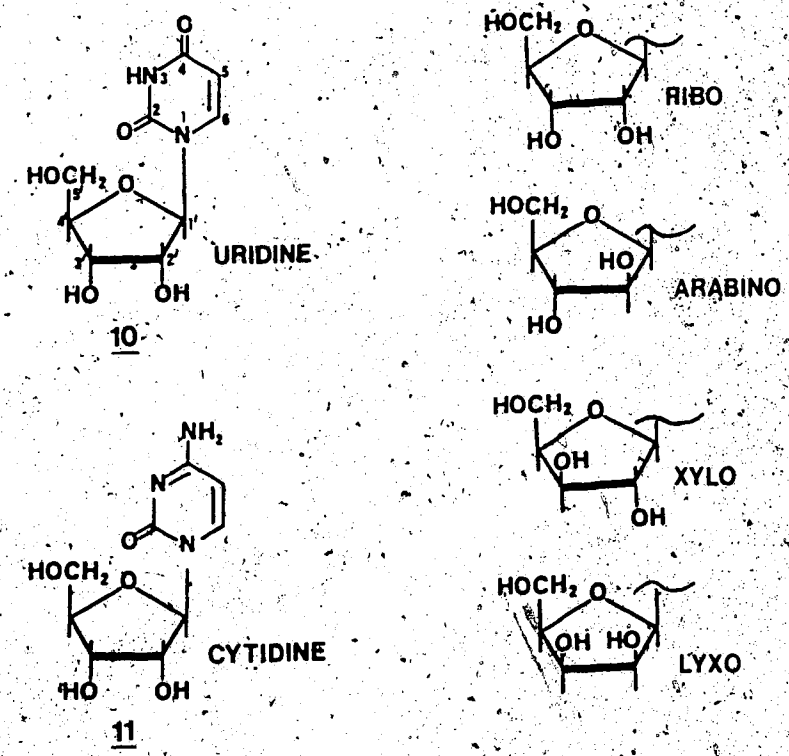
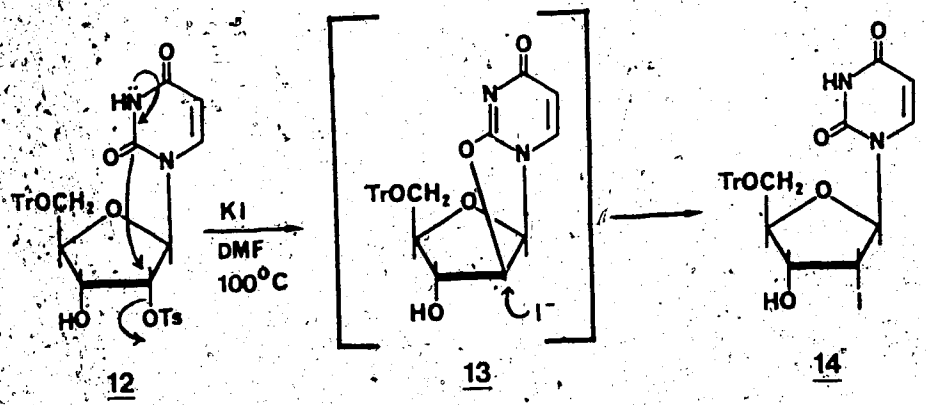


Figure II.1 The structure, numbering and configurational nomenclature of the pyrimidine nucleosides uridine and cytidine

Synthesis of 2'-halopyrimidine nucleoside analogs.

The best methods for the synthesis of 2'-halopyrimidine nucleosides are either a nucleophilic substitution reaction on the 2,2'-anhydro bond or the coupling of an appropriate

sugar and pyrimidine. The 2,2'-anhydro structure also appeared to act as an intermediate in some reactions in which simple nucleophilic substitution would be expected to take place. For example reaction of the 2'-O-tosyl ribo nucleoside 12 with iodide anion gave the 2'-iodo ribo nucleoside 14 [190,191]. The ribo configuration of the product (14) suggested that the 2,2'-intermediate 13 was formed before nucleophilic attack by the iodide occured. (Scheme II.3).



12 13 14

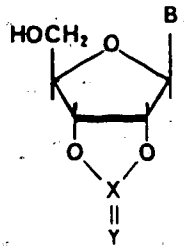
Scheme II.3 The reaction of 2'-O-tosyl-5'-O-trityluridine with iodide anion

A 2,2'-anhydro intermediate has also been proposed for the synthesis of 2'-chlorouridines by the reaction of acetylsalicyloyl chloride with 5'-O-acyluridines[192]. 2,2'-Anhydrouridine and -cytidine nucleosides are useful precursors for the synthesis of 2'-halo ribonucleosides. In addition acid or base hydrolysis of the appropriate 2,2'-anhydrocytosines gave rise to the potent antileukemic agent 1-β-D-arabinofuranosyl cytosine (ara C) and its analogs[193]. A variety or methods have been used to synthesise the 2,2'-anhydro intermediates. A review of early synthetic methods has been published[193].

Treatment of 2'-O-tosyl-5'-O-trityluridine with sodium benzoate in an acetamide melt gave 2,2'-anhydrouridine in 55% yield[194,195]. The mesyloxy salt of 2,2'-anhydro-3'-O-mesyl-cytidine was obtained in 70% yield when 5'-O-trityl-2',3'-di-O-mesylcytidine was refluxed in methanol-water[196]. These reactions are often not ideal since a good leaving group at the 2'-position and blocking groups at other positions are required. These are multistep syntheses

which gave low overall yields.

A variety of other reactions which afforded the 2,2'-anhydro nucleosides proceeded *via* 2',3'-cyclic intermediates 15.



15

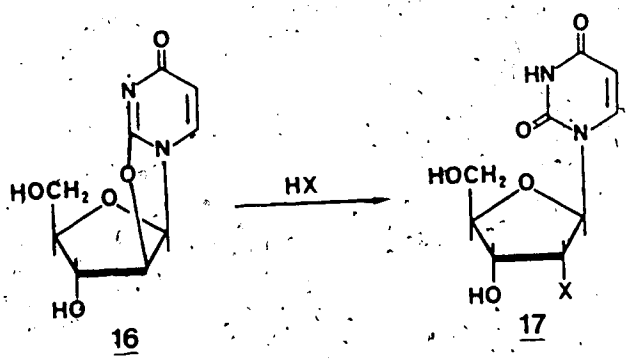
	B	X	Y	Ref.
a)	uracil	C	S	197
b)	uracil	C	O	198, 199
c)	uracil	S	O	200
d)	cytosine	S	O	200
e)	uracil	C	NH	201

The 2',3'-thiocarbonate (15a) was formed as a transient intermediate in the reaction of uridine with thiocarbonyldiimidazole in the synthesis of 2,2'-anhydrouridine[197]. Similarly the 2',3'-cyclic carbonate (15b) was a postulated intermediate when the reaction of uridine with diphenylcarbonate was used to prepare 2,2'-anhydrouridine[198,199] This postulate was supported by the isolation and characterization of stable 2',3'-cyclic carbonates when this reaction was carried out using purine ribonucleosides[191]. The 2',3'-O-sulfinates 15c and 15d were readily formed by the reaction of uridine or cytidine with thionyl chloride[200]. Acid hydrolysis of these stable intermediates subsequently afforded the 2,2'-anhydronucleosides in 73% and 47% yield respectively. The reaction of uridine with diiminosuccinonitrile appeared to proceed through the iminocarbonate 15e which reacted further to yield 2,2'-anhydrouridine and other products[201].

Reaction of N-4-substituted cytidines in ethyl acetate with partially hydrolyzed phosphorus oxychloride gave 2,2'-anhydrocytidines in 32 to 40% yield[202]. Phosphorus oxychloride and thionyl chloride reacted with dimethyl formamide to form the reactive species called Vilsmeier-Haack reagents. These in turn reacted readily with cytidine to yield, after work-up, 2,2'-anhydrocytidine hydrochloride (55% yield)[203]. Reaction of uridine with acetic anhydride and $BF_3{\cdot}Et_2O$, in acetonitrile, afforded 3',5'-di-O-acetyl-2,2'-anhydrouridine in 65%

yield[204]. 3',5'-Di-O-mesyl-2,2'-anhydrouridine has been prepared in high yield from the reaction of tri-O-mesyluridine with a small excess of 1,8-diazabicyclo(5-4-0)undec-7-ene[205].

2,2'-Anhydrouridine (16) underwent nucleophilic attack at the C-2'-carbon to give the 2'-halo ribonucleosides (17). The reaction appeared to proceed *via* initial protonation of the N-3 nitrogen when halogen acids were used followed by attack at C-2' by the anion[194].



16 17

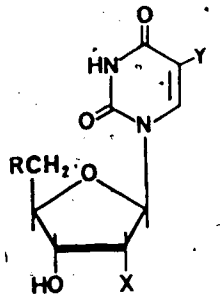
X	Ref.
a) Cl	192,194,195,206
b) Br	190, 194,195,207
c) F	194,195
d) I	190,208,209

The chloro and bromo compounds 17a and 17b were prepared in high yield from reaction of the appropriate halogen acid with 16 in dioxane (for 17a) or trifluoroacetic acid (for 17b). Preparation of the fluoro compound 17c required more vigorous conditions and gave lower yields[194,195]. Attempts to improve the yields using HF as the solvent afforded rearranged products[210]. The iodo compound 17d was obtained in low yield together with other unidentified iodo nucleosides when the hydroiodic acid was used as the iodine source. Water was excluded from these reactions since the hydrolytic reaction which gave arabinouridines was favoured even in the presence of a halide nucleophile[194].

Halide salts have also been used for the synthesis of the 2'-halouridines 17. The reaction of sodium chloride with 2,2'-anhydrouridine and trifluoroacetic acid in dimethyl formamide at 130 °C gave 17a in 53% yield[206]. The 2'-bromo compound was synthesised employing an analagous reaction using various bromide salts[207]. The 2'-iodo compound 17d was prepared by heating sodium iodide, 2,2'-anhydrouridine and trifluoroacetic acid in DMF under reflux[209]. The 2'-bromo and 2'-iodo nucleosides (17b and 17d) have also been prepared by

reaction of a 2,2'-anhydro intermediate, formed by an intramolecular reaction (Scheme II.3), and salts of iodine and bromine in dimethyl formamide at 100 °C[190].

The reaction 2,2'-anhydro compounds with nucleophiles has been used to synthesise a variety of other nucleoside analogs. These included analogs of thymidine (18a - c), nucleoside analogs of the potent antitumor agent 5-fluorouracil (18d) and a series of 5'-substituted nucleosides with potential carcinostatic activity (18e - g).

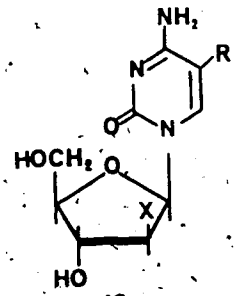


18

	R	X	Y	Ref.
a)	OH	Cl	CH_3	194
b)	OH	Br	CH_3	194
c)	OH	F	CH_3	194
d)	OH	F	F	194
e)	F	Br	H	211
f)	F	F	H	211
g)	F	F	I	211
h)	Cl	Cl	F	214
i)	Cl	Cl	Br	214
j)	Br	Br	F	214
k)	Br	Br	Br	214

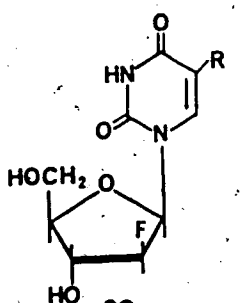
Reaction of 2,2'-anhydro-5-methyluridine with halogen acids in dioxane or trifluoroacetic acid yielded the 2'-halothymidine analogs (18a-c) in good yield[194]. 2'-Bromo--2',5'-dideoxy-5'-fluorouridine was prepared in 54% yield from its 2,2'-anhydro precursor upon reaction with NaBr in dimethyl formamide and p-toluenesulfonic acid as catalyst[211]. The 2'-fluoro compound 18f was obtained in 32% yield from the reaction of the 2,2'-anhydro precursor with 1% HF in dioxane at 170 °C in the presence of AlF_3[211]. A number of 2'-halouridines have been prepared as intermediates in the synthesis of 2'-deoxyuridines[212,213] since the 2'-halo group is readily displaced by hydride (tributyltin hydride). A number of 2',5'-dihalo--5-substituted nucleosides (18h-k) were synthesised by the reaction of 5-substituted uridines with the Vilsmeier reagent phosphorylhalide-dimethyl formamide. It has been proposed that the reaction proceeds *via* a 2,2'-anhydro intermediate[214].

Despite the ready availability of 2,2'-anhydrocytidine[200,202,203], reactions analogous to those described for the preparation of 2'-halouridines have not generally been successful for the preparation of 2'-halocytidines. The 2,2'-anhydro bond appeared to be unstable to basic reaction conditions and hydrolysis by traces of water in the reactants[215,216]. In addition 2,2'-anhydrocytidine appeared to be stable only in the salt form since attempts to isolate the neutral form by treatment of the salt with NaOH led to rapid conversion to arabinosyl cytosine[215]. 2'-Fluoro-2'-deoxycytidine was prepared by the reaction of 2,2'-anhydrocytidine hydrochloride with potassium fluoride and crown ether in rigorously dried dimethyl formamide[216]. The 2'-chloro- and 2'-fluorocytidines have also been prepared from the corresponding 2'-halouridines by amination[215] using the standard technique developed by Fox and co-workers[217]. The one step silylation-amination procedure developed by Vorbrugen appeared to be a significant improvement which had the potential for high yield synthesis of 2'-halo cytidine derivatives from the corresponding uridines[218].



19

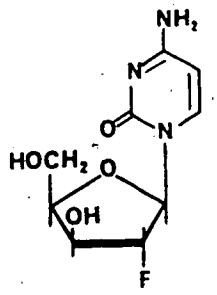
	R	X	Ref.
a)	H	OH	-
b)	H	F	220
c)	H	Cl	219
d)	F	F	221
e)	Cl	F	221
f)	Br	F	221
g)	I	F	221
h)	CH_3	F	221



20

	R	Ref.
a)	H	222
b)	F	221
c)	Cl	221
d)	Br	221
e)	I	221
f)	CH_3	221

The potent antileukemic activity of 1-β-D-arabinofuranosyl cytosine (ara C) (19a) suggested that the 2'-fluoro arabino substituent in these nucleosides may be a contributing factor to their biological activity. In fact both the 2'-arabinofluoro 19b and the 2'-arabinochloro 19c compounds show activity comparable to ara C against leukemias *in vitro*[219]. Compounds of structure 19 and 20 could not be synthesised from nucleoside precursors. Attempts to prepare the 2'-arabino compounds by nucleophilic displacement of a 2'-ribo substituent failed presumably due to intramolecular participation of the 2-carbonyl oxygen atom in the reaction[193]. Compounds 19 and 20 and the xylonucleoside 21[220] were prepared by condensation of the appropriate halogenated bromo sugars and bis-2,4-trimethylsilyl substituted pyrimidine bases[219-222].

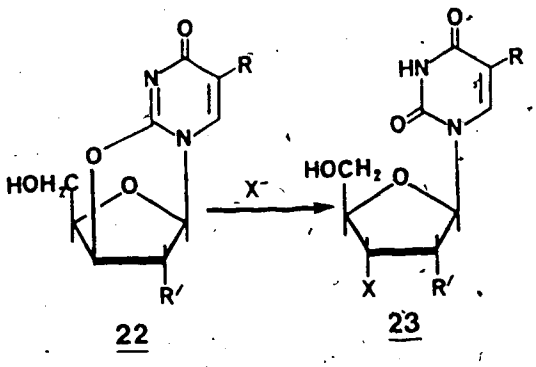


21

A variety of halo sugars have recently been prepared by displacement of a triflate substituent. The 2'-halo sugars obtained were subsequently used for the synthesis of 2'-halo arabinonucleosides using a coupling reaction[223].

Synthesis of 3'-halopyrimidine nucleoside analogs.

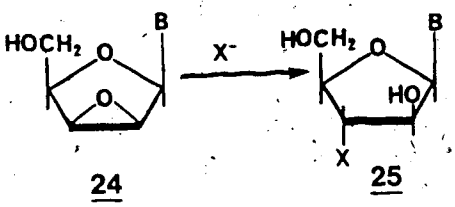
The majority of 3'-halopyrimidine nucleoside analogs have been prepared by reaction of 2,3'-anhydro compounds 22 or 2',3'-epoxy compounds 24 with nucleophiles or by coupling of halo sugars with suitably blocked pyrimidine bases. In addition to these methods there have been a number of other syntheses of 3'-halopyrimidine nucleosides reported.



22 23

	R	R'	
a)	H	OH	uridines
b)	H	H	deoxyuridines
c)	CH_3	H	deoxythymidines

X = halide

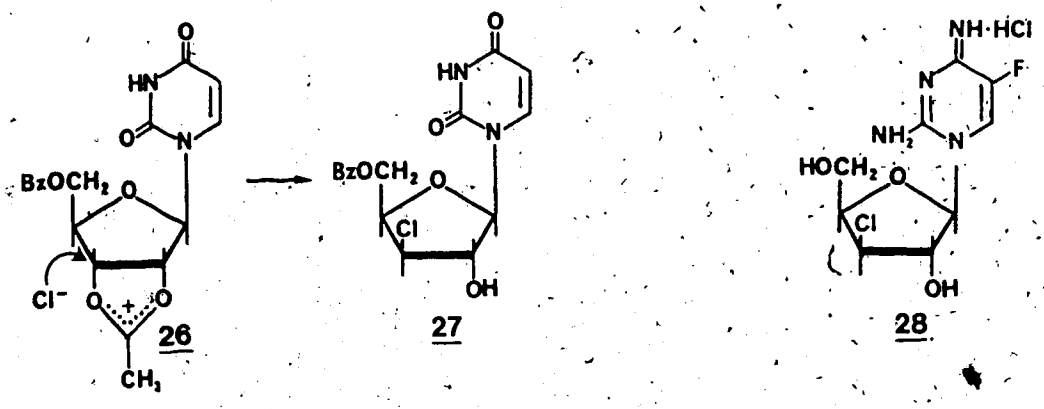


24 25

	B
a)	uracil
b)	cytidine

X = halide

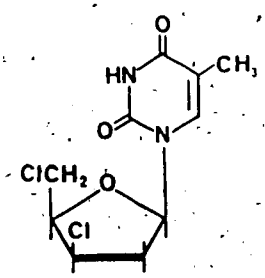
The 3'-chloro compound 27 has been identified as a product obtained from the reaction of 5'-O-benzoyluridine and acetylsalicyloyl chloride[192] and was presumed to arise from chloride anion attack on an intermediate acyloxonium ion (26). The acyloxonium ion has also been postulated as an intermediate in the synthesis of the 3'-chloro analog 28 which was obtained from reaction of the N-2-amino nucleoside and α-acetoxyisobutyryl chloride[224].



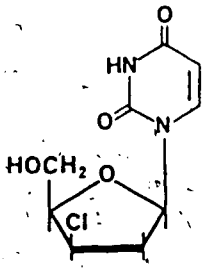
26 27 28

The reaction of thymidine and $SOCl_2$ in hexamethylphosphoric triamide yielded the 3',5'-dichlorothymidine analog having the *threo*-pentofuranosyl structure (29). This result indicated a simple nucleophilic substitution reaction with inversion of configuration at the

3'-position[225]. The reaction of 5'-O-trityl-2'-deoxyuridine with triphenylphosphine, dimethylacetamide and carbon tetrachloride gave the 3'-chloro-*threo*-pentofuranosyl compound 30 [226].

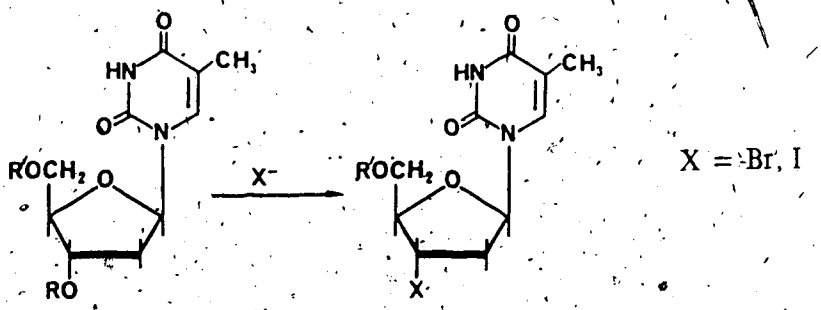


29



30

In other studies reaction of bromide or iodide anion with thymidine analogs 31 having a good leaving group at C-3' gave 3'-bromo- and 3'-iodothymidine nucleosides 32 with apparent retention of configuration[227-229]. It is resonable to postulate that these reactions proceeded *via* a 2,3'-anhydro intermediate formed by initial attack by the C-2 carbonyl oxygen atom at the 3'-position of the sugar (compare with Scheme II.3).



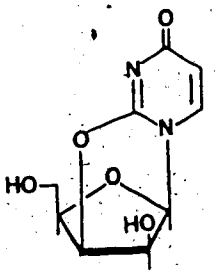
X = Br, I

31 32

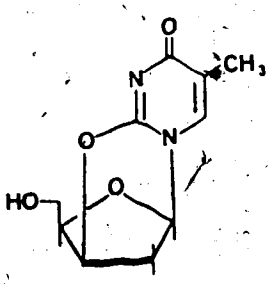
The 2,3'-anhydro compounds are readily prepared and a review of early work has been published[193]. Compounds 33, 22a and 22c were readily prepared by intramolecular C-2 carbonyl attack on a suitably blocked 3'-O-mesyl precursor[227,230,231].

Halogenation reactions analogous to those employed to prepare 2'-halo compounds from 2,2'-anhydrouridine have been used to synthesise 3'-halo analogs of 22c. Thus reaction of 22c with HF and AlF_3 in anhydrous dioxane gave the 3'-fluorothymidine analog (23c, X = F)[232,233]. The 3'-chlorothymidine analog (23c, X = Cl) was obtained from reaction of 22c with

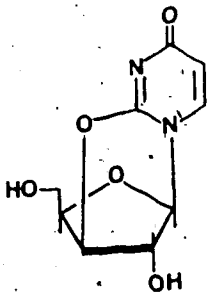
HCl in anhydrous dioxane[233]. Treatment of 5-fluoro-5'-iodo-2,3'-anhydrothymidine with dilute aqueous HI gave the 3',5'-diiodo compound in high yield[234].



33

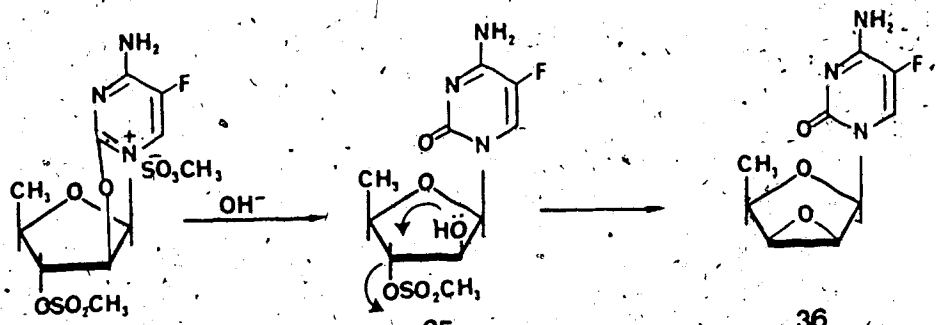


22c



22a

The 2',3'-epoxy nucleosides (24) were useful precursors for the synthesis of 3'-halo-arabino nucleosides. The 2',3'-epoxyuridine 25a was obtained from the reaction of 2',3',5'-tri-O-mesyluridine with three equivalents of base[193] or under more controlled reaction conditions by intramolecular attack of the 2'-arabino hydroxyl group at the 3'-carbon having an O-mesyl leaving group[235,236] upon treatment of the molecule with aqueous NaOH.



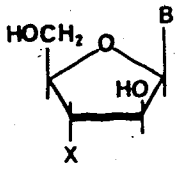
34 35 36

Scheme II.4 Proposed mechanism for the synthesis of 5-fluoro-1-(2',3'-epoxy-5'-deoxy-1-β-D-lyxofuranosyl)cytidine

The alkaline reaction conditions seemed to facilitate this reaction rather than a competitive reaction which involved attack by the C-2 carbonyl oxygen atom to give the 2,3'-anhydro

products. 2,2'-Anhydrouridines were obtained when 2',3',5'-tri-O-mesyluridines were refluxed in water[231]. Under alkaline reaction conditions 34 was similarly converted to 36 presumably *via* the 2'-hydroxyarabino cytidine intermediate 35 (Scheme II.4)[237].

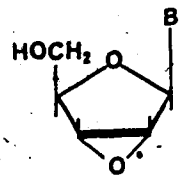
A series of 3'-halo nucleoside analogs 37a-e was synthesised by the reaction of the appropriate 2',3'-epoxide with ammonium halide salts in ethanol[238] or with aqueous acid[208,239,240].

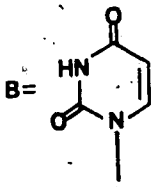


37

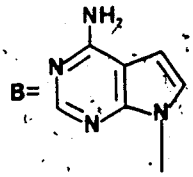
	B	X	Ref.
a)	cytosine	Br	238
b)	cytosine	I	238
c)	uracil	Cl	208,239
d)	uracil	Br	238,239
e)	uracil	I	208,240
f)	uracil	F	208,241

The 3'-fluoro compound 37f was obtained in low yield from reaction of 2',3'-epoxy nucleoside with HF in dioxane at high temperature under anhydrous reaction conditions[208,241]. Attack by halide occurred regioselectively at the 3'-position of the 2',3'-epoxides. This regioselectivity is presumably due to preferential attack by the incoming nucleophile at the less hindered 3'-position. No products resulting from attack at the C-2' position were isolated although they may have been produced as minor components in the reactions.





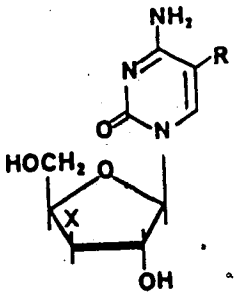
38a



38b

A number of purine nucleosides 38b having a ribo 2',3'-epoxide moiety have been synthesised[242-244]. Reaction of 38b with a variety of reagents gave 3'-bromo, 3'-chloro, 3'-iodo[244] and 3'-fluoro[245] lyxo nucleosides. A 2',3'-ribo epoxide has been postulated to be a transient intermediate during some pyrimidine nucleoside syntheses. The 2',3'-ribo epoxide intermediate is consistant with formation of 1-β-D-xylofuranosyluracil obtained after treatment of 2,2'-anhydrouridine with base[246]. A stable ribo 2',3'-epoxy nucleoside has never been isolated in the pyrimidine series. An attempted preparation of uridine 2',3'-ribo epoxide (38a) yielded only 2,2'-anhydrouridine (16) which presumably arises from the intramolecular attack by the C-2 carbonyl oxygen atom on a transient reactive 2',3'-epoxide[247].

However pyrimidine nucleosides having the xylo configuration can be synthesised using coupling reactions[220,248]. Thus condensation of silylated N-4-acetylcytosine or 5-fluoro cytosine with a variety of 3-halogenofuranoses gave a series of xylo 3'-halocytidines 39[248].



39

	R	X	Ref.
a)	H	F	220,248
b)	H	Cl	248
c)	H	Br	248
d)	H	I	248
e)	F	Cl	248
f)	F	Br	248
g)	F	I	248

Synthesis of 5-halopyrimidine nucleoside analogs.

The 5-halopyrimidine nucleoside analogs, as described previously, are readily synthesised *via* electrophilic attack by various halogenation reagents at the electron rich C-5 position. Within this series of compounds the 5-fluoronucleosides were the most difficult to synthesise. Fluoride salts could not be easily oxidized to yield electrophilic fluorine as was the case with the other halogens. Molecular fluorine (F_2) tended to be very reactive and non-selective for electrophilic fluorination reactions. Molecular fluorine and many related

electrophilic fluorination reagents are toxic and hazardous substances. Despite these problems a variety of methods have been developed to prepare 5-fluoropyrimidine nucleosides.

Nucleoside analogs of 5-fluorouracil and 5-fluorocytosine have been prepared using coupling reactions[221,248,249] and transnucleosidation[250]. Nucleosides have been reacted with a variety of novel electrophilic fluorinating reagents. The simplest electrophilic fluorinating reagent, a solution of F_2 in acetic acid, was used to prepare 5-fluorouridine in 92% yield from uridine[150]. This reaction has been shown to proceed through an unstable 5-fluoro-6-O-acetyl-5,6-dihydrouracil intermediate[212,251]. Somewhat lower yields were obtained when water was used as the solvent[150]. The lower yield is likely due to side reactions of the nucleoside and reactive species such as H_2O_2 and O_2 which are produced when F_2 is dissolved in water[252]. The reaction of trifluoromethyl hypofluorite (CF_3OF) with pyrimidine nucleosides formed a 6-alkoxy-5-fluoro-5,6-dihydropyrimidine adduct which underwent base catalyzed elimination to give 5-fluoropyrimidine nucleosides in high yield[253-255]. Several new fluorination reagents have shown promise for incorporation of fluorine at the C-5 position. A wide variety of additions to double bonds have been effected using XeF_2, including the synthesis of 5-fluorouracil from uracil[256,257]. The electrophilic fluorinating species acetyl hypofluorite (CH_3COOF) was prepared by bubbling nitrogen diluted elemental fluorine through acetic acid or acetic acid / freon solutions containing sodium salts[152,153]. The volatile acetyl hypofluorite could be removed from the solvent by a stream of inert gas and trapped in a second solvent, cooled to the appropriate temperature.

E. The Biochemistry of Pyrimidine Nucleosides

Introduction.

The role of pyrimidine nucleosides in cellular survival and reproduction has been recognized for a long time. Those interested in the design of new therapeutic and diagnostic nucleosides have tended to focus their attention toward the biochemical role nucleosides serve in

biosynthesis of DNA and RNA. It should however be recognized that nucleosides perform a variety of other functions in biological systems. Nucleosides are biosynthetic intermediates for the formation of glycoproteins, phosphoglycerides and glycogen as well as constituents of enzymes and metabolic regulators.

The cytotoxic behaviour of pyrimidine nucleoside analogs has been related to a number of effects including competition with natural pyrimidine nucleosides for enzymes, feedback inhibition of nucleoside synthesis and incorporation of modified nucleosides into DNA and RNA. The mechanism of action for a number of nucleoside analogs that have shown significant antitumor or antiviral effects has been examined in detail. The cytotoxic effect of nucleoside analogs has tended to be rather non-selective. Most chemotherapy using these compounds was based on their interference with constituents required for high metabolic activity in proliferating neoplasms. Consequently there is a concomitant hazard to rapidly growing normal tissues such as bone marrow and intestinal mucosa. Slowly growing tumors have tended to be refractory to such therapy.

Prusoff and Fischer[258] have defined a number of recognized or potential criteria for the selective antitumor and antiviral activities of nucleoside analogs. Recognition of altered transport, enzyme activity or specificity and chemical environment in tumor cells or virus infected cells may provide clues to the design of an effective therapy involving nucleoside analogs. Another aspect involves an understanding of the relationship between the structure of the analog and its biochemical behaviour. There has been a tendency to investigate in detail only those compounds which have demonstrated a substantial therapeutic effect. Consequently there are many unanswered questions pertaining to *in vivo* metabolism of nucleoside analogs and little predictive capacity in biochemical studies with, or therapeutic or diagnostic application of, novel nucleosides.

The review that follows examines the known biochemical behaviour of halogenated [redacted]osides with particular emphasis on the 2'- and 3'-positions of the sugar and [redacted]he base.

Biochemistry of naturally occuring pyrimidine nucleosides.

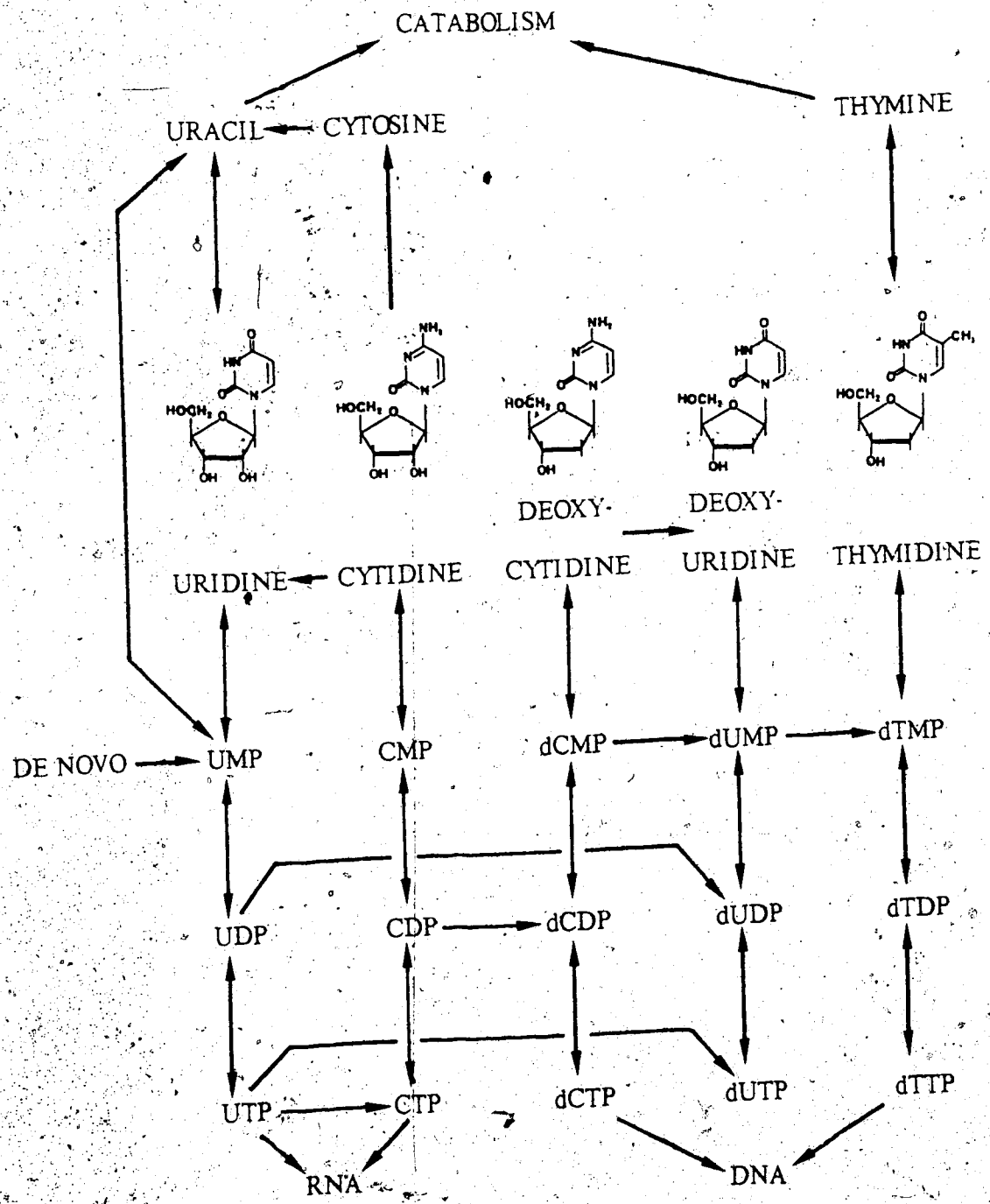
The biochemistry of naturally occuring nucleosides and nucleotides, their interconversion and incorporation into nucleic acids have been studied extensively and are well defined. The reader is referred to reviews of the subject for more detail[259,260]. The main pathways are shown in Scheme II.5.

Some features of this scheme are:

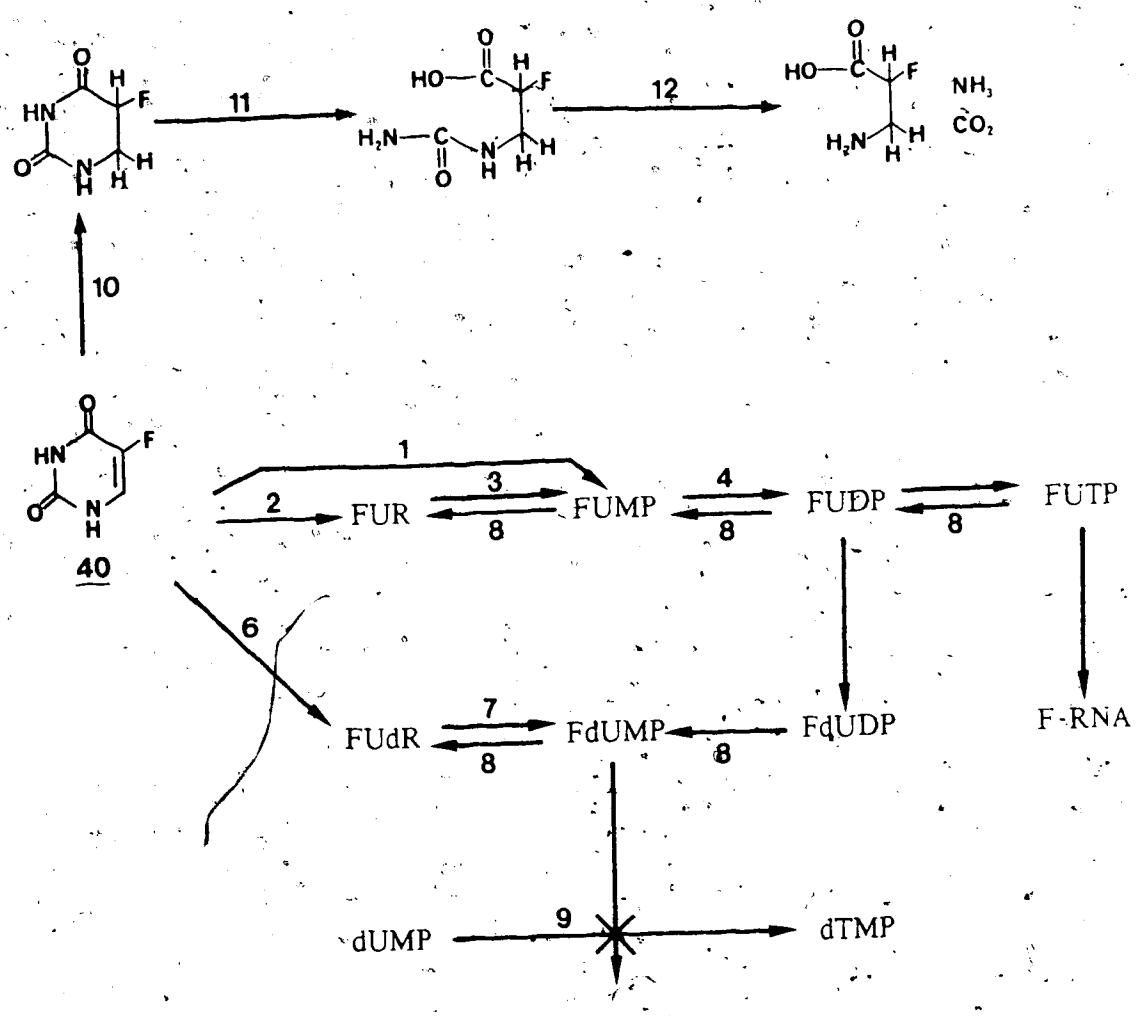
1. Other than salvage routes, all pyrimidine nucleosides arise *de novo* with uridine monophosphate as the progenitor of the other pyrimidine nucleosides.
2. Nucleotide triphosphates are the direct precursors for DNA and RNA. Kinase enzymes control the level of phosphorylation.
3. Cytidines arise from uridines at the triphosphate level with the reverse process occurring at the level of the nucleoside or free base or *via* nucleotide deoxycytidine monophosphate conversion to deoxyuridine monophosphate.
4. The deoxynucleotides are formed at the triphosphate level in mammalian systems and at the diphosphate level in *E. coli*.
5. Thymidine rather than deoxyuridine is incorporated *via* its triphosphate into DNA. Methylation of the deoxyuridine monophosphate gives the required thymidine.
6. Nucleoside phosphorylase enzymes can cleave the nucleosides to the corresponding ribose or deoxyribose sugar and pyrimidine heterocycle.

Biochemistry of 5-fluorouracil and its nucleosides.

Heidelberger and co-workers first reported the antitumor and antibacterial properties of 5-fluorouracil (40) in 1957[43]. Since this initial report a wide variety of analogs have been reported in the literature. A very limited number of these molecules possessed any advantage relative to 5-fluorouracil. The anabolic and catabolic pathways for 5-fluorouracil in mammalian systems are shown in Scheme II.6. 5-Fluorouracil mimics the behaviour of uracil with respect to both transformation to the active metabolite 5-fluoro-2'-deoxyuridine



Scheme II.5 Interconversions of naturally occurring nucleosides and nucleotides



ENZYMES

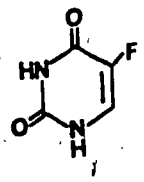
1. pyrimidine phosphoribosyl transferase
2. uridine phosphorylase
3. uridine kinase
4. uridylate kinase
5. ribonucleotide reductase
6. thymidine phosphorylase
7. thymidine kinase
8. phosphatase
9. thymidylate synthetase
10. uracil reductase
11. dihydropyrimidinase
12. β-ureidopropionase

Scheme II.6 *In vivo* metabolism of 5-fluorouracil in mammalian systems

monophosphate (FdUMP) and catabolism to α-fluoro-β-alanine. The metabolite FdUMP acts as an analog of deoxyuridine monophosphate (dUMP) except that it binds to the enzyme thymidylate synthetase in a virtually irreversable manner[262]. Thus the free enzyme is rapidly removed from tissues, thymidine pools are depleted by the absence of enzyme which effects the transformation of dUMP to deoxythymidine monophosphate and cell death is the consequence.

There have been a number of studies which correlated the concentration and clearance of FdUMP with antitumor effect in various tumor models[263,264]. It has also been suggested that incorporation of 5-fluorouridine triphosphate into RNA may be the major cytotoxic effect in many cell lines including human breast cancers[11,12,265]. The inhibitory action of 5-fluorouracil on ribosomal maturation has been well documented[266-268]. There may in fact be a spectrum of modes of action in various tumor lines between these two effects[265].

Cell lines which became resistant to 5-fluorouracil (40), 5-fluorouridine (41) and 5-fluoro-2'-deoxyuridine (42) have been shown to have reduced levels of the enzymes pyrimidine phosphoribosyl transferase, uridine kinase and thymidine kinase respectively[269]. A number of other mechanisms causing resistance have also been discovered[270].



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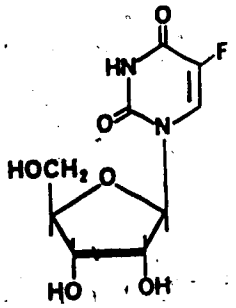

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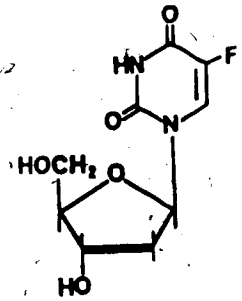
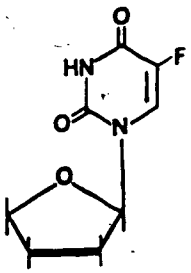


42

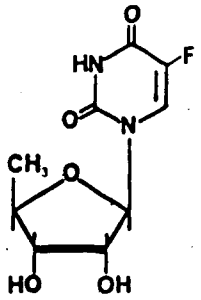
In some test systems the nucleosides 5-fluorouridine (41) and 5-fluoro-2'-deoxyuridine 42 have demonstrated enhanced or altered activity relative to 5-fluorouracil 40[1,271]. The 2'-deoxynucleoside 42 has shown greater tumor uptake and better tumor to blood ratios than 5-fluorouracil in some murine tumor test systems[9,10]. This may indicate a greater cellular uptake for the nucleoside than for the free base. The nucleosides have also been shown to exert

markedly different *in vitro* and *in vivo* activity against a murine lymphoma[272].

5-Fluorouracil has the disadvantage of being cytotoxic toward normal cells as well as tumor cells and has a relatively low therapeutic index. A number of nucleoside analogs of 5-fluorouracil have been prepared in an effort to enhance antitumor activity, to increase selectivity for tumor cells and to eliminate some of the potent cytotoxicity toward normal cells. Ftorafur (43) was shown to act as a slow release form of 5-fluorouracil. It had a median half-life of 18.6 hours in man compared with 0.5 hours for 5-fluorouracil[270,273,274]. The gradual formation of 5-fluorouracil from ftorafur (43) *via* activation by liver microsomes was regarded as the equivalent of a slow infusion of 5-fluorouracil but demonstrated no therapeutic improvement over this method[270].



43



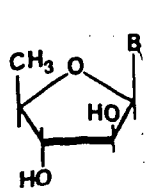
44

A recently synthesised antineoplastic agent, 5'-deoxy-5-fluorouridine (44), has shown therapeutic potential[275-277]. Its favourable therapeutic index and low host toxicity may represent preferential bioactivation in tumor cells[275]. Although the presence of novel metabolites could not be ruled out it appeared that compound 44 exhibited cytotoxicity after cleavage by pyrimidine nucleoside phosphorylase to 5-fluorouracil. The absence of an hydroxyl group at the 5'-position of the 5-fluoronucleoside 44 prevented direct phosphorylation. This compound also failed to exhibit *in vitro* cytotoxity, a result consistant with the proposed *in vivo* enzymatic activation[275]. The 5-fluoro-5'-deoxy analog (44) has shown relatively high rates of conversion to 5-fluorouracil in tumor tissue relative to normal tissue[277] and was not effective against a cell line which lacked nucleoside phosphorylase[277]. The "prodrug" mechanism was supported by the

absence of early inhibition of thymidylate synthetase when tissue concentrations of 44 were maximal[276].

Analogs of 5'-deoxynucleosides 44 having a modified 5'-substituent retained *in vivo* activity but were essentially inactive *in vitro*[275]. These compounds also appeared to act *via* a "prodrug" mechanism. There was some restriction regarding the nature of the 5'-substituent if the compound was to remain a substrate for the enzyme nucleoside phosphorylase. For example the 5'-iodo-5'deoxy and the 2,3'-anhydro analogs of 44 showed no activity toward Sarcoma S-180 in mice[224].

Additional modifications to the 2'- and 3'-positions of the 5'-deoxynucleosides yielded compounds that were inactive against Sarcoma S-180, a tumor known to be sensitive to fluorinated pyrimidines. The compounds 45, 46[237], 47 and 48[234] did not appear to be substrates for nucleoside phosphorylase which converts them to 5-fluorouracil and its active metabolites.



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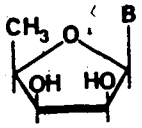

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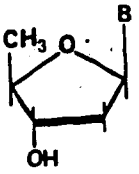

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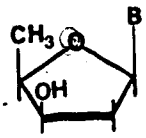

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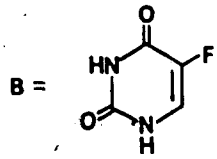
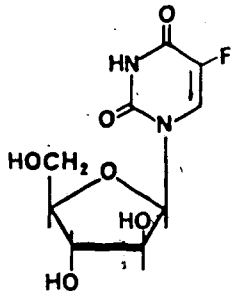


1-β-D-Arabinofuranosyl-5-fluorouracil (ara FU) (49) was shown to have a good chemotherapeutic index and a relatively high activity comparable to 5-fluoro-2'-deoxyuridine (42) against mouse leukemia S-82 [278]. Although it was originally proposed that ara FU (49) had a mode of action similar to 5-fluorouracil[278,279] recent studies revealed direct inhibition of the enzyme thymidylate synthetase *via* the monophosphate of the arabinonucleoside 49 [280].

2',5-Difluoro-1-arabinosyluracil 50 has shown moderate cytotoxicity toward tissue culture cells[221]. Recent experiments demonstrated that the cytotoxicity of this compound was due to the direct inhibition of the enzyme thymidylate synthetase by the nucleotide monophosphate[281]. In addition this compound was shown not to be a substrate for the

mammalian pyrimidine phosphorylases and hence might demonstrate a different spectrum of *in vivo* activity than 5-fluorouracil or 5-fluorouridine[211].



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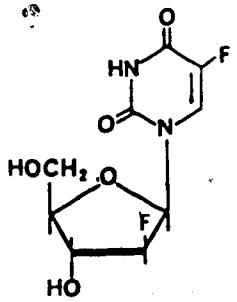

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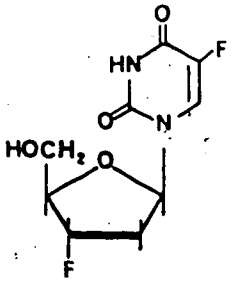
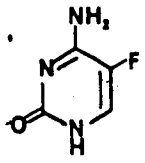


51

5-Fluoro-2',3'-dideoxy-3'-fluorouridine 51 was designed as an analog of 5-fluoro--2'-deoxyuridine (42) and was expected to be more resistant to phosphorylitic cleavage by the thymidine phosphorylase enzyme while maintaining the potential to interact with cellular thymidylate synthetase[212]. *In vitro* testing confirmed these expectations and 51 was shown to be moderately growth inhibitory to L 1210 mouse leukemia cells in culture[212].



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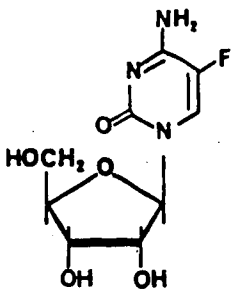

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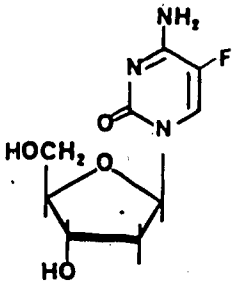


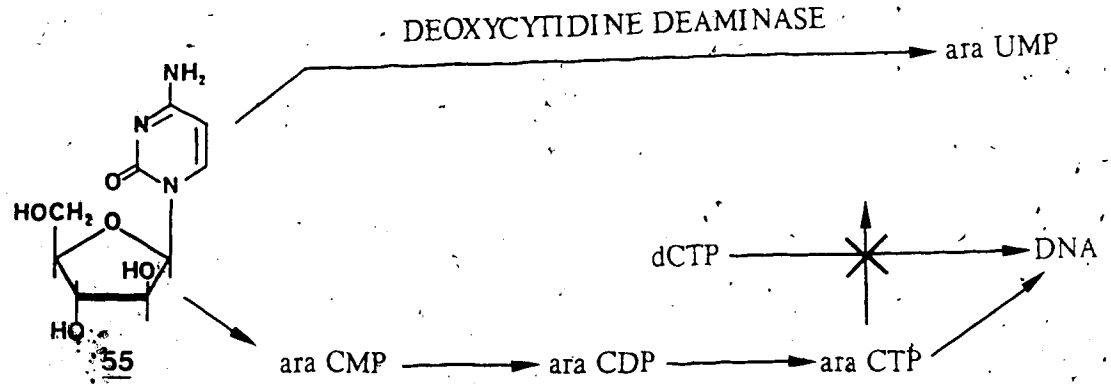
54

A number of 5-fluorocytidine analogs exhibited biological effects after conversion to the corresponding 5-fluorouridine analogs by deaminases *in vivo*. The pyrimidine base 5-fluorocytosine was not active in man due to the absence of cytosine deaminase or cytosine phosphoribosyl transferase enzyme activity[213]. This compound has shown antifungal activity due to selective activation by the deaminases present in fungal cells. 5-Fluorocytidine 53 and

5-fluoro-2'-deoxycytidine 54 are active against mouse leukemia. They are converted to the corresponding 5-fluoro nucleotides by deoxycytidylate deaminase thus providing inhibition of the enzyme thymidylate synthetase by the mechanism already discussed[214] (Scheme II,6).

Biochemistry of 1-β-D-arabinofuranosyl cytosine and its analogs.

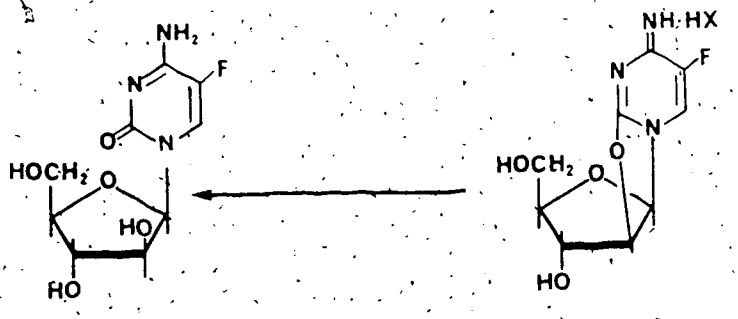
1-β-D-Arabinofuranosyl cytosine (ara C) (55) is the most important drug available for the treatment of acute myeloblastic leukemia. It is rapidly deactivated in mammalian systems by deoxycytidine deaminase in competition with its activation to ara C triphosphate by deoxycytidine kinases. It acts by inhibition of DNA polymerase, although incorporation into DNA may also play a role in its cytotoxicity[260] (Scheme II.7).



Scheme II.7 *In vivo* biochemical activation and deactivation of 1-β-D--arabinofuranosyl cytosine

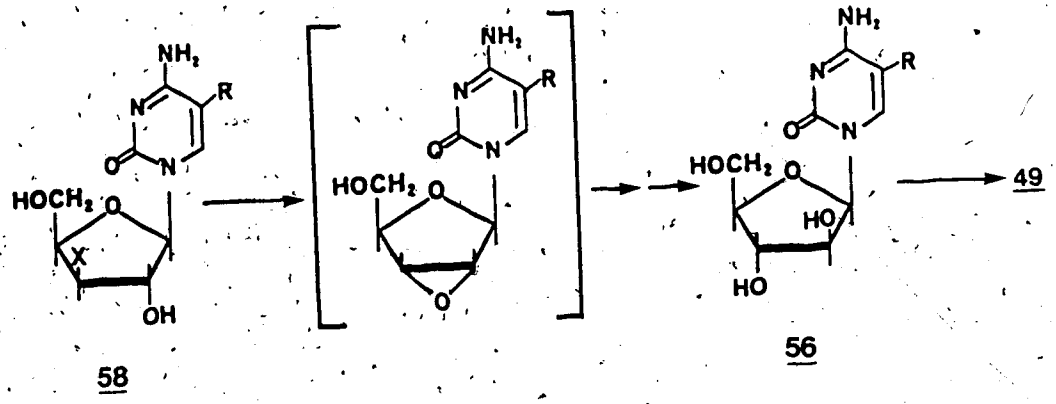
A number of halogen-containing analogs of ara C (55) were prepared with the hope of finding a compound which maintained the activity of the parent but was less schedule dependant and more resistant to deamination. 1-β-D-Arabinofuranosyl-5-fluorocytosine 56 was active against mouse leukemia and was more active than the parent compound ara C against a 5-fluorouracil resistant line of mouse leukemias[215]. The mode of action of this compound appeared to be the same as ara C except that deamination produced

1-β-D-arabinofuranosyl-5-fluorouracil (49), a compound which was also cytotoxic[271].



56 57

2,2'-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine 57 was very active against mouse leukemias[216] and was less schedule dependant than ara C. This compound was considered to be a "double barreled masked precursor" which gave rise to two active metabolites. Ara C (56) could result from cleavage of the anhydro linkage and the uridine analog 49 by subsequent deamination[279]. The anhydro compound 57 was active against both ara C sensitive and ara C resistant cell lines[241]. The activity in the latter case was reversed by the addition of thymidine, but not by deoxycytidine. This indicated that inhibition of thymidylate synthetase was responsible for cytotoxicity in this cell line.



58 56 49

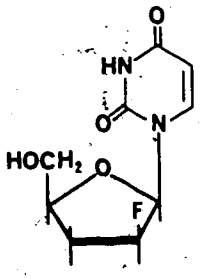
Scheme II.8 Conversion of "double barreled masked precursors" to the active metabolites 1-β-D-arabinofuranosyl-5-fluorocytosine(56) and 1-β-D--arabinofuranosyl-5-fluorouracil(49).

A number of 5-fluoro xylocytidines were designed as "double barreled masked precursors" of anticancer drugs[241]. The xylo compounds 58 where X was a good leaving group could give rise, via intramolecular conversion, to 1-β-D-arabinofuranosyl-5-fluorocytosine (56) and by subsequent deamination to 1-β-D-arabinofuranosyl-5-fluorouracil (49) (Scheme II.8). In ara C sensitive cell lines significant activity was noted with X = Br, I, O-mesyl, O-tosyl and to a lesser extent with Cl. The low activity when X = Cl and the absence of activity when X = F probably reflected the reduced ability of these halogens to act as leaving groups.

Significant cytotoxicity was also noted for the 5-fluoro compounds 58 against cell lines resistant to ara C. This effect was reversed by thymidine but not by deoxycytidine implicating thymidylate synthetase as the target enzyme[241].

The ribo cytidine analogs having 2'-chloro, 2'-fluoro and 2'-chloro-5-fluoro substituents were all active in cell culture[216]. A mechanism of activation was postulated in which active metabolites 55 or 56 were formed after initial formation of an anhydro intermediate by intramolecular attack of the C-2 carbonyl oxygen atom at the 2'-carbon with the halogen acting as a leaving group[216].

1-(2'-Fluoro-2'-deoxy-β-D-arabinofuranosyl)cytosine 59, 1-(2'-fluoro-2'-deoxy-β-D-xylofuranosyl)cytosine 60 and 1-(3'-fluoro-3'-deoxy-β-D-xylofuranosyl)cytosine 61 have been screened in L 1210 mouse suspension culture[220].



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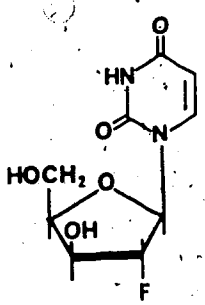

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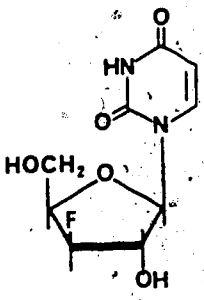


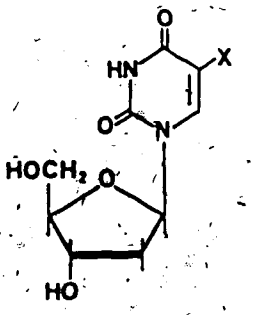
61

The 2'-arabinofluoro compound 59 exhibited an activity comparable to ara C (55) and the

5-fluoro analog 56 whereas the xylo compounds were considerably less active. Preliminary studies using the 2'-chloro analog of 59 showed it to be as active as ara C or 59 against mouse leukemia cells *in vivo*[219]. The lack of activity exhibited by 60 and 61 is probably due to a low affinity of these compounds for the cellular transport mechanisms and kinases required for conversion into active nucleotides.

Biochemistry of thymidine analogs.

Analogs of thymidine (62d) include compounds which retain the 5-methyl pyrimidine structure but have various modifications to the deoxyribose portion of the molecule. A second group of analogs are compounds in which the methyl group has been replaced by a substituent with an approximately equal steric influence. The latter group includes clinically useful compounds such as 5-iodo-2'-deoxyuridine (62a)[13], 5-bromo-2'-deoxyuridine (62b)[217] and 5-trifluoromethyl-2'-deoxyuridine (62c)[218].



62

	X
a)	I
b)	Br
c)	CF_3
d)	CH_3

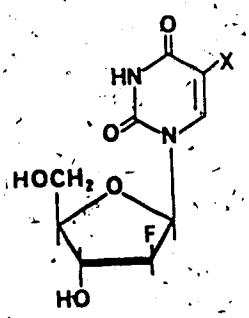
The accepted mechanism of action of compounds 62a-c involves their transport into cells and phosphorylation to the 5'-triphosphate nucleotides. At this level they inhibit viral induced DNA-polymerase or become incorporated into viral DNA[289,290]. These analogs also exhibited competitive and feedback inhibition of thymidine triphosphate synthesis.

The 5-iodo analog 62a has provided good results in the treatment of *herpes simplex* virus infection of the corneal epithelium in man with possible selective uptake of the compound into viral DNA being the cytotoxic effect[291]. A number of factors limited the usefulness of

these compounds as antiviral agents. These factors included their susceptibility to phosphorolytic cleavage[292] and their relatively small therapeutic index (eg 8 : 1 therapeutic to toxic ratio in a study of antiherpes virus activity with 62a[293].

Studies using the cytidine analogs of 62a and 62b in cell culture indicated they were equally effective in inhibiting replication of *herpes simplex* virus type 1 and type 2 but displayed a lower toxicity to uninfected cells than their corresponding uridine analogs[294,295]. This selectivity was due to the presence in infected cells of a virus-induced pyrimidine nucleoside kinase which enhanced the uptake of the cytidines relative to their uptake into non-infected normal cells[221].

Interesting results were obtained when various modifications to the sugar portion of 5-halo nucleosides were performed. Compounds 20 and 63 (X = F, Cl, Br, I) were shown to exhibit antiviral activity against *herpes simplex* virus type 1. Compounds 20b, d and e and 63d and e were the most potent[221].



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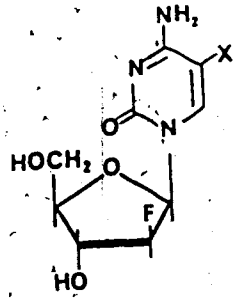


63

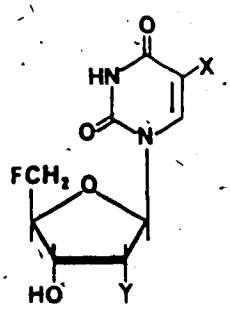
	X
a)	H
b)	F
c)	Cl
d)	Br
e)	I

The presence of a 2'-fluoro substituent in 20 and 63 in place of a 2'-H or 2'-OH enhanced their biological activity[121]. The 5-iodo-2'-arabinofluoro cytidine 63e was more active against *herpes simplex* virus type 1 and type 2 than either ara C (55) or 5-iodo-2'-deoxyuridine (62a)[297]. This compound appeared to be preferentially phosphorylated by the virus induced enzyme thymidine kinase thereby exhibiting a low toxicity toward uninfected cells[297]. The mechanism of cellular toxicity was predominantly inhibition of the enzymes thymidine kinase and / or thymidylate synthetase and the compound was cross resistant with ara C [298]. This

compound has recently undergone clinical trials in immunosuppressed cancer patients with herpes virus infections[299].

5-Iodo-2'-arabinofluoro uridine 20e exhibited potent antiviral and antileukemic activity[300]. It was highly active against leukemic lines which were resistant to ara C and it was recommended for clinical trials in patients with leukemias refractory to ara C therapy[300]. Compound 20e inhibited DNA polymerase and acted metabolically like thymidine and deoxycytidine[298].

The 5'-fluoro-5'-deoxy nucleosides 64a-d demonstrated a significant inhibitory effect on the enzyme thymidylate kinase and hence may possess antitumor or antiviral activity[211].



64

	X	Y
a)	Br	OH
b)	I	OH
c)	I	F
d)	I	H

3'-Deoxy-3'-fluorothymidine was shown to exhibit cytostatic behaviour[233]. This compound was found to be phosphorylated to the triphosphate level and its mechanism of action appeared to be uptake into DNA where it acted as a chain terminator due to the absence of a 3'-hydroxyl substituent in the molecule. This compound has also been shown to moderately inhibit the phosphorylation of thymidine to thymidylate[301].

III. EXPERIMENTAL

A. Cold Syntheses

Chemicals, solvents, gases and equipment.

Chemicals and solvents were reagent grade unless specified otherwise. Solvents were routinely dried and distilled prior to use and stored over 4 Å molecular sieves. Where "super-dry"[302] solvents were required the drying techniques described by Burfield *et al*[303,304] were employed. Gases were purchased from Matheson Gas Products, Canada and were generally of highest purity available from stock. Ultra-high-purity neon and research grade neon / fluorine mixtures required for the production of ^{18}F were specially prepared by the same supplier. A specialized gas handling system for studies involving the toxic and reactive fluorine gas was constructed of approved components purchased from Matheson Gas Products (Figure III.1).

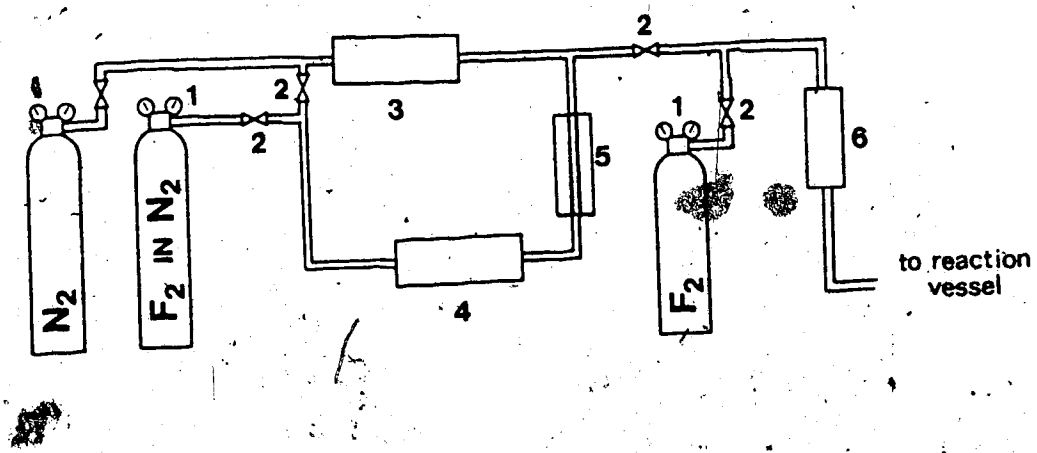


Figure III.1 Gas handling system for synthesis with F_2

This gas handling system consisted of B15F-679 regulators (1), 940-F valves (2), a model 8250 Mass Flow Controller (3), a model 8117 Mass Flow Meter (4), a model 7825

Flowmeter (5) and a model 68-1008 hydrogen fluoride trap (6).

Specialized glassware and teflon apparatus were fabricated by the University of Alberta Technical Services.

Instrumental analysis.

Melting points (mp) were determined with a Büchi capilliary apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were determined on a Varian EM-360A nmr spectrometer (60 MHz), a Bruker HFX 90 (90 MHz) or a Bruker AM-300 nmr spectrometer (300 MHz) using deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent and tetramethylsilane (tms) as an internal reference. Mass spectra (ms) were determined on a Hewlett-Packard Model 5995 A gas chromatograph / mass spectrometer or by an AEI MS-50 mass spectrometer. Exact mass measurements determined on the latter instrument were used in lieu of combustion analysis for determining the elemental composition.

Compounds synthesised as reference samples for radioactive compounds were purified by recrystallization or chromatography on columns (silica gel), thin layer chromatography plates (Whatman PLK5F or MK6F Microslides) or by high pressure liquid chromatography (hplc) on a Waters system (Model 860 Automated Gradient Controller, Models 510 and M-45 Solvent Pumps, Model U6K Injector and Model 480 LC ultraviolet (uv) detector) using a Whatman Partisil M9 10/25 ODS reverse phase column. Analytical hplc was carried out using C-18 radial compression columns supplied by Waters Scientific.

B. Radiochemical Syntheses

Radionuclides and specialized equipment.

The ^{14}C required for the synthesis of [^{14}C]-nucleosides was purchased from Atomic Energy of Canada Limited as $Ba^{14}CO_3$ (1.9 GBq/mmol). The ^{131}I was purchased from Merck-Frosst Laboratories as a no-carrier-added solution in NaOH. The ^{123}I was produced at

the German Cancer Research Center Cyclotron Facility, Heidelberg by the $^{124}Te(p,2n)^{123}I$ nuclear reaction (90.8% enriched in ^{124}Te)[305-307] or at the TRIUMF Cyclotron Facility at the University of British Columbia by the $^{127}I(p,5n)^{123}Xe \rightarrow {}^{123}I$ or the $^{133}Cs(p,2p9n)^{123}Xe \rightarrow {}^{123}I$ spallation reactions[301]. The ^{82}Br was obtained by neutron irradiation of natural abundance or 97.8% enriched (Oak Ridge National Laboratories) NH_4Br by the $^{81}Br(n,\gamma)^{82}Br$ nuclear reaction in the University of Alberta SLOWPOKE reactor facility at a neutron flux of 10^{12} n cm^{-2} sec^{-1}. The ^{18}F was obtained by neutron irradiation of enriched $^6Li_2CO_3$ (95% ^6Li (Oak Ridge National Laboratories)) at 10^{12} n cm^{-2} sec^{-1} in the University of Alberta SLOWPOKE reactor facility by the $^6Li(n,\alpha)^3H$, $^{16}O(^3H,n)^{18}F$ nuclear reaction. ^{18}F was also produced as both anion and molecular fluorine at the University of Alberta Nuclear Research Center using Van de Graaff accelerated deuterons employing the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction. The ^{14}C standards for calibration of the tissue oxidizer were obtained from Amersham.

Radiochemical reactions were generally carried out in small scale laboratory glassware. Pierce Reacti-Vials® and Tuf-Tainer® teflon vials were used for most syntheses with all-teflon systems used in reactions and manipulations of ^{18}F in gas and solution. Fluorine production targets were fabricated by the University of Alberta Technical Services Machine Shop. High purity nickel for one target was obtained from Atlas Alloys. Target foils were either 5 x 10^{-4} cm nickel or 7.6 x 10^{-4} cm molybdenum purchased from Hamilton Precision Metals, Lancaster, USA. Speciality gases were from Matheson Gas Products. Electrically operated teflon valves were purchased from Brunswick Technetics, New Jersey, USA, and automatic monel valves and controllers from Hoke Inc., New Jersey, USA. Manually operated stainless steel and brass valves were purchased from the Whitey Co. and miniature teflon valves from Mandel Scientific Co..

Measurement and analysis.

Radiochemical manipulations with γ-ray emitting compounds were monitored by a Victoreen "Frisker" or "Thyac III" GM-Detector. The activity of γ-ray emitting compounds

was measured with a Picker Isotope Calibrator Model 632507-1 or by γ-ray scintillation in a Beckman Gamma 8000. The activity of β^--emitting samples was determined with a Beckman LS 9000 or a Searle Mark III liquid scintillation counter with samples dissolved in Aquasol II (New England Nuclear) or a laboratory prepared toluene based fluor.

Tissue samples containing ^{14}C-labelled compounds were combusted in a H. J. Harvey Instrument Corporation Biological Oxidizer, Model OX 300, trapped as [^{14}C]-CO_2 in Harvey Carbon-14 Cocktail and counted by liquid scintillation.

Radiochromatograms developed on silica gel plates were analyzed on a Berthold LB 2832 Gas Flow Proportional Analyzer and a Canberra Series 40 Multichannel-Analyzer. Alternately the silica was divided into fractions, scraped from the plates and counted by liquid scintillation (lsc) using a Beckman LS 9000 and a Digital Integration Program. High pressure liquid radiochromatography was performed using the same hplc system described previously combined with a sodium iodide crystal detector and associated electronics to monitor the effluent for γ-radiation. β^--Radiation was detected by taking sequential samples of the effluent and counting by liquid scintillation counting.

C. ^{18}F Production and Precursor Formation

Reactor method for ^{18}F production.

The $^6Li(n,\alpha)^3H$, $^{16}O(^3H,n)^{18}F$ nuclear reaction was employed for reactor production of ^{18}F. The target material was Li_2CO_3 obtained as 95% ^6Li-enriched material from Oak Ridge National Laboratories. The irradiations were carried out in the University of Alberta SLOWPOKE Reactor Facility. The SLOWPOKE is a low energy reactor capable of producing a neutron flux of 1×10^{12} n cm^{-2} sec^{-1}. Activation yields of 390 to 880 KBq per milligram of ^6Li irradiated were obtained as ^{18}F after 2 hours of irradiation. Maximum yields per milligram of irradiated sample were obtained using smaller samples (larger surface to volume ratio) or by irradiating solutions of Li_2CO_3 in HNO_3.

All irradiations were carried out in heat sealed polyethylene irradiation vials supplied by the SLOWPOKE Facility. The irradiated Li_2CO_3 was then removed from the reactor and the activity of ^{18}F was measured in a dose calibrator. The samples were then transferred to a lead lined fume cupboard for further processing. Three methods were employed to obtain ^{18}F in a form suitable for radiochemical syntheses.

1. Distillation method.

The Li_2CO_3 powder was mixed with concentrated H_2SO_4 and this solution was heated at or near its boiling point. A slow stream of inert gas (N_2, He) or inert gas / hydrogen fluoride mixture was passed through the hot solution and the $H^{18}F$ was trapped by bubbling the gas through cool dry dioxane. The solution of $H^{18}F$ in dioxane thus obtained was employed for radiochemical synthesis.

2. Ion exchange method.

The irradiated Li_2CO_3 was added to Dowex 50W X4 ion exchange resin in H_2O and the mixture was stirred. The aqueous layer containing ^{18}F was passed through an anion exchange resin to trap the $^{18}F^-$. The resin was then washed with water, methanol and dry ether. The activity was eluted from the resin by a solution of carrier HF in dry dioxane and the radioactive eluate used for radiochemical synthesis.

3. Precipitation method.

The ^{18}F was separated from irradiated Li_2CO_3 (presumably as $Li^{18}F$) by triturating the solid with water. Carrier NaF was added to the aqueous solution and the fluoride was precipitated as CaF_2 by the addition of $Ca(NO_3)_2$ solution. The precipitate of [^{18}F]-CaF_2 was isolated by centrifugation and dried by washing with acetone and dry ether and removing traces of solvent with a flow of nitrogen. The isolated [^{18}F]-CaF_2 when heated with HF in dioxane in a stainless steel bomb underwent exchange of $^{18}F^-$ for F^-. The soluble ^{18}F activity was then used as a reagent for radiochemical synthesis.

Accelerator method for ^{18}F production.

The 7 MV Van de Graaff Accelerator at the University of Alberta was used for production of ^{18}F using the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction. The isotope production facility was developed in conjunction with an earlier study utilizing ^{11}C and ^{18}F[309]. The accelerator produced deuteron beams with an energy up to 7 MeV with a beam current up to 22 μA.

The target (Figure III.2) was a modification of a copper target used sucessfully to produce carrier free $H^{18}F$[93,94]. The target body (E) was machined from pure copper with an inside bore of 1.27 cm in diameter and 22.25 cm in length. The inside surface was polished to a mirror finish, washed and dried. This procedure was repeated each time the target foils were changed.

The target body was attached to the deuteron beam line by a series of plates (Figure III.2). Two stainless steel plates (B and D) allowed cold helium to be circulated over the beam line exit foil (F) and the target body entrance foil (G) during the irradiations. A central plate of Macor (Dow Corning®) ceramic material acted as both a thermal and electrical insulator between the target and the beam line. A final stainless steel plate allowed the target assembly to be attached to the beam line.

The foils (F and G) were circles of 7.6 x 10^{-4} cm thick molybdenum held in place by rubber or viton O-ring seals. The entrance foil (G) was further secured by silver epoxy to the copper target body. The edges of the bore in the stainless steel and copper components which came into contact with the foils were rounded slightly to avoid shearing forces which would weaken the foils. Inlet and outlet ports in the copper body were machined to accept 1/4 inch pipe thread fittings.

Figure III.3 shows the remaining components of the target system. The gas lines were copper and valves were monel or stainless steel except for valves 9 and 10 which were miniature teflon valves. A KOH trap prevented release of [^{18}F]-HF into the external (unshielded) gas lines and a drying tube prevented moisture from entering the recovery lines. An external control panel allowed operation of heaters and automatic valves and monitoring of temperatures of the

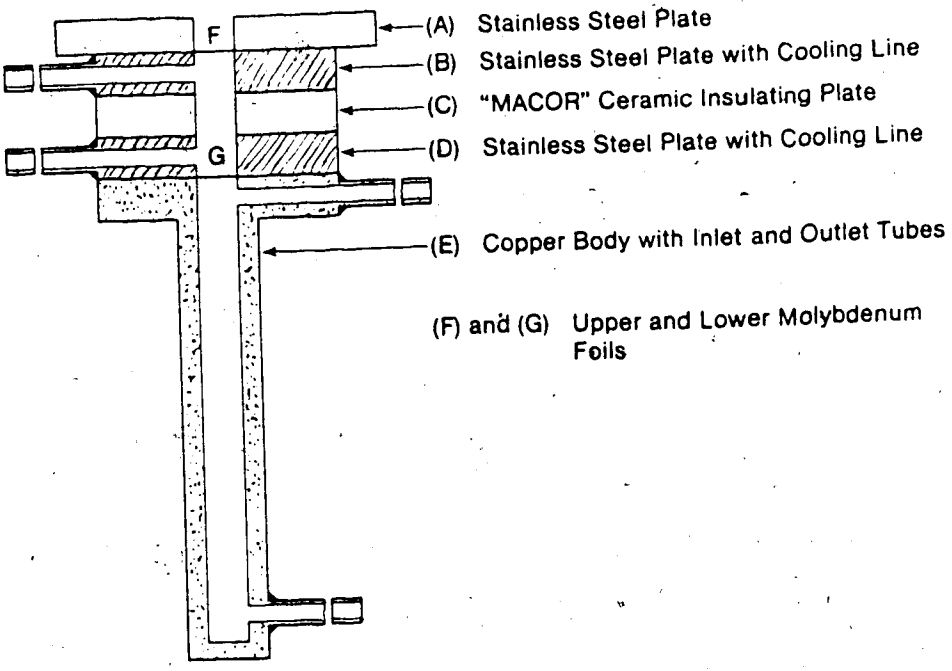


Figure III.2 Copper target for the production of [^{18}F]-HF

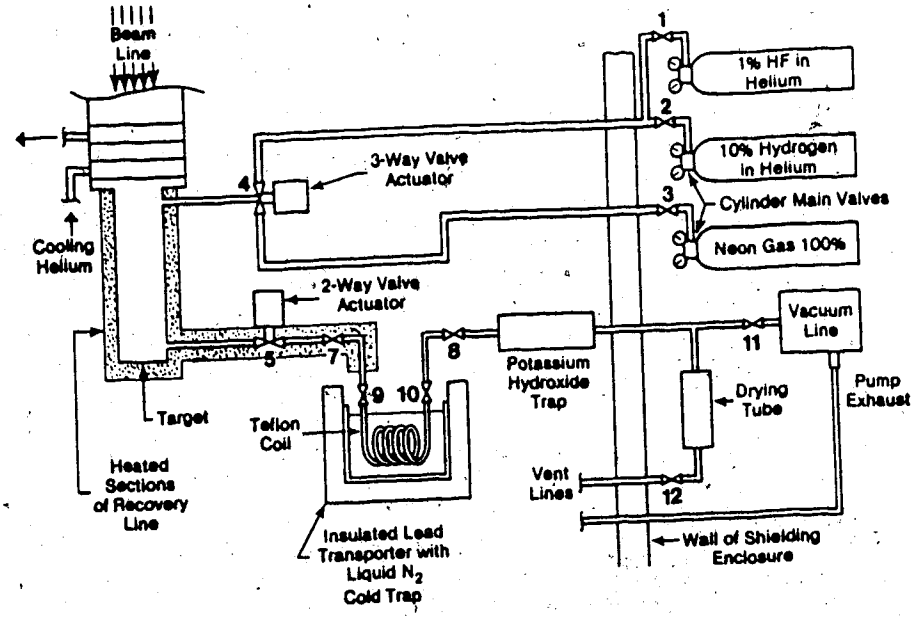


Figure III.3 Target system for the production and recovery of [^{18}F]-HF

target and recovery lines. After irradiation the radiation levels near the target were dangerously high. The system was therefore designed for remote operation of the recovery procedure in order to minimize personnel exposure to radiation.

A typical production run proceeded as follows;

The vacuum pump was used to evacuate the entire system to 1 x 10^{-4} torr. This also served as a leak test since this vacuum was not maintained if any air was entering the system. If the target or recovery lines had been exposed to atmosphere since the last run the system was degassed by heating these components (target 300°C, recovery lines 175°C). The heating was accomplished with heating tapes and carefully monitored by thermocouples. The system was allowed to cool and then filled with high purity neon gas at a pressure of 1 atmosphere.

The automatic valves (4 and 5 in Figure III.3) were closed to isolate the target from the rest of the system. All other valves were closed except 7, 8, 9 and 10. The cooling helium (cooled by liquid N_2) was allowed to flow over the foils and the neon gas in the target was irradiated for 2 hours with a beam of deuterons. A cool down time of 20 minutes was allowed after irradiation before the recovery procedure was performed.

The target body was heated to 300°C and the recovery line was heated to 175°C. Meanwhile the lead transporter containing the teflon coil was cooled with liquid nitrogen. Once the system had reached the required temperature the valve 1 was opened and the pressure was released from the system slowly through a needle valve 12. When the target had depressurized to atmospheric pressure valve 12 was closed and the target was evacuated with a roughing pump through valve 11.

The target was flushed using 10% H_2 in He. A second and third flush with He containing a trace of HF were usually employed to maximize the yield. The flushes were carried out with the target maintained at 300°C. The [^{18}F]-HF was transferred by this procedure to the cooled coil of teflon. The teflon coil was then isolated from the system by closing the miniature teflon valves 9 and 10. Teflon luer lock fittings allowed the coil assembly along with valves 9 and 10 to be rapidly released from the recovery line and sealed in the lead lined transporter. The

coil was then taken to the radiochemistry laboratory for subsequent manipulations.

Several procedures were investigated to recover ^{18}F-HF from the teflon coil in a form suitable for radiochemical synthesis. Water, saline, acid and base solutions all removed some or all of the activity. The most suitable procedure for our purpose used the apparatus shown in Figure III.4. The solution of HF in dioxane was slowly drawn through the valves and teflon coil by applying a vacuum to the teflon reaction vessel. The solution passing through the system carried the [^{18}F]-HF into the reaction vial. The activity was transferred virtually quantitatively by this procedure.

A modified target and recovery system was constructed for the production and isolation of ^{18}F as molecular fluorine [^{18}F]-F_2 (Figure III.5). A target similar in design to the one described previously (Figure III.2) was prepared from high purity nickel. The stainless steel plates B and D and the ceramic insulator C as described for the [^{18}F]-HF production target were replaced by a single teflon plate which served as an insulator and was machined to allow a flow of cooling helium to pass over the foils (5×10^{-4} cm nickel). The target gas for this system was a mixture of research grade neon and 1% v/v of high-purity F_2 specially prepared by Matheson Gas Products for [^{18}F]-F_2 production. The flush gas for this target was either the above mixture or ultra-high-purity neon gas. Remote actuated teflon solenoid valves were used inside the enclosure except for 9 and 10 which were manual miniature teflon valves. The gas delivery and recovery lines were 1/8 inch teflon tubing. The [^{18}F]-F_2 produced was trapped in a solution of NH_4OAc in glacial acetic acid.

A target passivation with F_2 has been recommended for optimum recovery of [^{18}F]-HF[97]. Our target was pressurized to 1 atm with 100% F_2 and heated at 180°C for 16 hours. The target was then flushed with N_2 and attached to the gas delivery and reco[illegible]

The procedure for production of [^{18}F]-F_2 was similar to that describ[illegible]y for [^{18}F]-HF. The system was presurized with 1% F_2 in Ne to 1.33 atmosphere after degassing the target and recovery lines under vacuum. The target was isolated from the system by closing valves 4 and 6 and irradiated with deuterons. After irradiation the excess pressure in the target

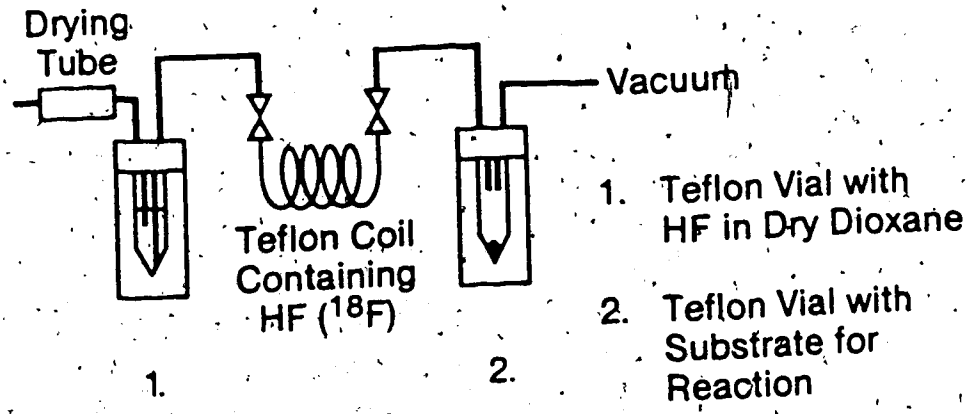


Figure III.4 Apparatus for recovery of [^{18}F]-HF as a solution in anhydrous dioxane

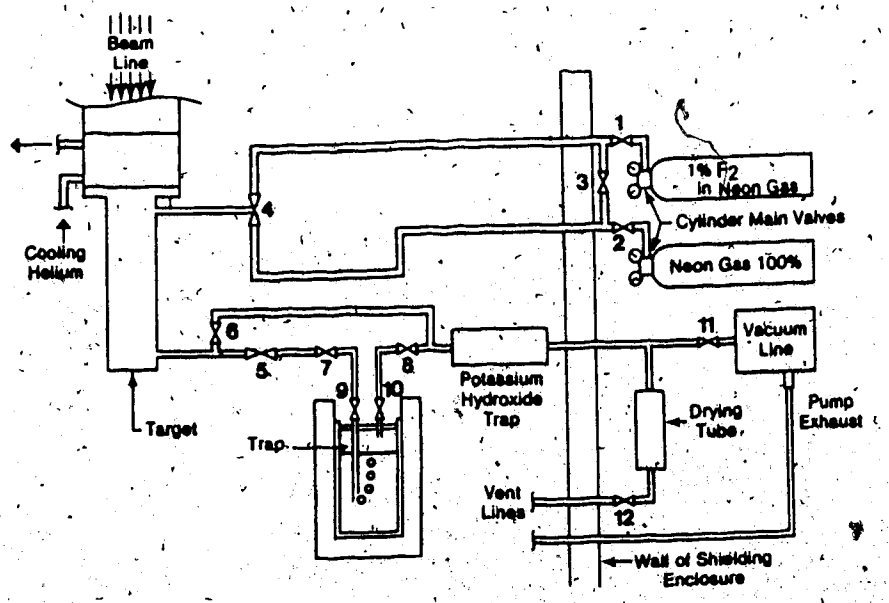


Figure III.5 Target system for the production and recovery of ^{18}F as [^{18}F]-F_2

was vented slowly through a needle valve 7 and the gas was allowed to bubble through the trapping solution. The target was flushed by re-pressurizing the system with the appropriate flush gas and bleeding off this pressure through the trapping solution. The trap was then isolated by closing the miniature teflon valves and disconnecting them from the recovery lines. The trap shielded in the lead lined transporter was then taken to the radiochemistry laboratory

D. Chemistry

5-Iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a).

A solution of NaI (65 mg, 0.43 mmol) in 1.5 mL of water was added to a solution of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (100 mg, 0.41 mmol) in 1.5 mL of 2 M HNO_3 in a 5 mL Reacti-Vial® *via* the septum. The sealed vial was heated at 80 °C overnight. The iodine color which developed on heating gradually disappeared to give a clear colorless solution. The reaction mixture was neutralized with NH_4OH and extracted with ethyl acetate (3 x 2 mL). The residue obtained after removal of solvent was chromatographed on four preparative tlc plates (Whatman PLK5F, 20 cm x 20 cm x 1 mm). The single major product visible under ultraviolet (uv) light after development with 15% v/v MeOH/CH_2Cl_2 was extracted from the silica with 20% v/v MeOH/CH_2Cl_2. The white powder obtained after removal of solvent (136 mg, 0.36 mmol, 88.6%) was crystallized from hot water to give the title compound (1a) as a microcrystalline solid: mp 216 -218 °C.

^{1}H nmr, (DMSO-d_6) δ; 11.56 [1H, s, N(3)-H], 8.56 [1H, s, C(6)-H], 5.88 [1H, d (J(1',F) = 16.5), C(1')-H], 5.63 [1H, d (J(OH,3') = 6.4), C(3')-OH], 5.42 [1H, t (J(OH,5') = 4.3, J(OH,5'') = 4.3), C(5')-OH], 5.05 [1H, dd (J(2',F) = 54, J(2',3') = 4.1), C(2')-H], 4.19 [1H, complex d (J(3',F) = 22.8) becomes ddd on D_2O exchange (J(3',F) = 22.8, J(3',4') = 8.2, J(3',2') = 4.1), C(3')-H], 3.91 [1H, broad d (J(4',3') = 8.2), C(4')-H], 3.83 and 3.67 [2H, complex d (J(geminal) = 12.0) becomes dd on D_2O exchange (J(gem) = 12.0, J(5',4') = 2.2), C(5')-H]. Signals at 11.56, 5.63 and 5.42 disappear after

exchange with D_2O.

Exact mass calc. for $C_9H_{10}N_2O_5FI$: 371.9617; measured (hrms), 371.9619; intensity = 2.9%.

5-Bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b):

The bromination reagent was prepared by dissolution of Br_2 (0.56 g, 3.50 mmol) in 26.28 g of glacial acetic acid. This solution contained 0.13 mmol Br_2 per mL. A solution of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (206 mg, 0.84 mmol) in 15 mL of glacial acetic acid was prepared in a 50 mL flask. This solution was heated at 50 °C and the bromination solution was added dropwise during 30 minutes until a distinct bromine color persisted in the solution (7.2 mL added, 0.94 mmol). The solution was heated at 100 °C for an additional 30 minutes. The solvent and unreacted Br_2 were removed at 50 °C on a rotary evaporator and the residue was dissolved in ethanol and reevaporated to give a pale yellow-brown foam. This foam was dissolved in a mixture of 5 mL of methanol and 0.5 mL of concentrated NH_4OH and stirred for 18 h. The crude product obtained after removal of solvent was purified by preparative hplc (Partisil ODS magnum column (Whatman), 90% H_2O, 10% MeOH, 3 mL/min) to give 116 mg of a colorless gum (0.36 mmol, 42%) This material was chromatographically pure as shown by tlc and hplc and exhibited a nmr spectrum consistant with that expected for the product. Attempts to crystallize this product from water, MeOH, EtOH and various mixed solvents were unsuccessful.

^1H nmr, (DMSO-d_6) δ; 11.8 [1H, s, N(3)-H], 8.55 [1H, s, C(6)-H], 5.87 [1H, d with fine structure (J(1',F) = 16.5, J(1',2') = small), C(1')-H], 5.63 [1H, broad s, C(3')-OH], 5.44 [1H, broad s, C(5')-OH], 5.05 [1H, dd (J(2',F) = 54, J(2',3') = 4.1), C(2')-H], 4.19 [1H, broad d (J(3',F) = 23.5, J(3',4'; 3',2'; 3',OH) = small) becomes ddd after D_2O exchange (J(3',F) = 23.5, J(3',4') = 8.5, J(3',2') = 4.1), C(3')-H], 3.90 [1H, broad d (J(4',3') = 8.5, J(4',5'; 4'5'') = small), C4'-H], 3.86 and 3.60 [2H, broad d (J(gem) = 12.5, unresolved fine structure) becomes dd after D_2O exchange (J(gem) = 12.5, J(5',4') =

2.1), C(5')-H]. Signals at 5.63, 5.44 and 11.80 disappear on D_2O exchange.

Exact mass calc. for $C_9H_{10}N_2O_5F^{81}Br$: 325.9736; measured (hrms), 325.9733; intensity = 0.55%. Exact mass calc. for $C_9H_9N_2O_5{}^{81}Br$ (M^+ - HF): 309.9674; measured (hrms), 309.9719; Exact mass calc. for $C_9H_9N_2O_5{}^{79}Br$ (M^+ - HF): 303.9694; measured (hrms), 303.9739.

5-Chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c).

A chlorination solution was prepared by bubbling Cl_2 gas into 30 mL of glacial acetic acid at room temperature. The molarity of the solution was determined by titrating the I_2 liberated after addition of excess KI with standard sodium thiosulfate solution.

The chlorination solution (1.5 mL, 0.85 M, 1.28 mmol) was added to 201 mg (0.82 mmol) of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) in 25 mL of glacial acetic acid. The reaction was stirred for 10 minutes at room temperature. The solution was evaporated to dryness on a rotary evaporator at 40 °C. The residue was dissolved twice in EtOH and the solvent removed along with traces of residual HOAc. This residue was treated with 0.5 mL of concentrated NH_4OH in 20 mL of MeOH with stirring for 18 hours.

The crude reaction mixture showed a major spot on tlc at Rf 0.50 (15% MeOH, 85% CH_2Cl_2, silica gel) in addition to several minor spots including polar material at the plate origin. The crude material was chromatographed on a column of silica gel (15 g, 1.6 cm x 16 cm, gradient elution from pure CH_2Cl_2 to 5% MeOH in CH_2Cl_2). Pure 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c) (120 mg, 0.43 mmol, 52%) was obtained as a colorless gum which resisted crystallization.

^1H nmr, (DMSO-d_6) δ; 11.90 [1H, s, N(3)-H], 8.52 [1H, s, C(6)-H], 5.92 [1H, d fine structure (J(1',F) = 16.5, J(1',2') = small), C(1')-H], 5.68 [1H, d (J(OH,3') = 6.5), C(3')-OH], 5.48 [1H, t (J(OH,5') = 4.5), C(5')-OH], 5.09 [1H, dd (J(2',F) = 54, J(1',2') = 4.1, fine structure J(2',1') = small), C(2')-H], 4.14-4.32 [1H, complex, becomes ddd on D_2O exchange J(3',F) = 23.5, J(3',4') = 8.5, J(3',2') = 4.1), C(3')-H], 3.96 [1H, broad d

(J(4',3') = 8.5, J(4',5'; 4',5'') = small), C(4')-H], 3.89 and 3.67 [2H, ddd (J(gem) = 12.5, J(5',OH) = 4.5, J(5',4') = 2.1) becomes dd on D_2O exchange (J(gem) = 12.5, J(5',4') = 2.1), C(5')-H]. Signals at 11.90, 5.68 and 5.48 disappear after D_2O exchange.

Exact mass calc. for $C_9H_{10}N_2O_5F^{37}Cl$: 282.0232; measured (hrms), 282.0230; intensity = 0.42%. Exact mass calc. for $C_9H_{10}N_2O_5F^{35}Cl$: 280.0262; measured (hrms), 280.0256; intensity = 1.27%. Exact mass calc. for $C_9H_9N_2O_5{}^{37}Cl$ (M^+ - HF); 262.0170, measured (hrms), 262.0173; intensity = 2.69%. Exact mass calc. for $C_9H_9N_2O_5{}^{35}Cl$ (M^+ - HF): 260.0200; measured (hrms), 260.0197.

5-Fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d).

A fluorination solution was prepared by bubbling nitrogen diluted F_2 (2.5% F_2) through 100 mL of glacial acetic acid (reagent grade) contained in a round bottom flask at room temperature. The fluorine gas handling system described earlier (Figure III.1) was used for this procedure. An aliquot of this solution was treated with excess KI and the liberated I_2 was titrated with standard thiosulfate solution.

The freshly prepared solution of F_2 in HOAc (60 mL, 0.0185 M; 1.11 mmol) was added to 162 mg (0.66 mmol) of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) in a 250 mL round bottom flask. The flask was stoppered and gently swirled at room temperature for 10 minutes. The glacial acetic acid was then removed on a rotary evaporator at 50 °C. The residue was redissolved twice in ethanol and the solvent removed along with residual HOAc. The yellow foam obtained contained 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) and a second major component believed to be 5-fluoro-6-O-acetyl-5,6-dihydro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (83). Treatment of this mixture with 1 mL of concentrated NH_4OH in 25 mL of MeOH at room temperature for 18 hours converted the dihydro adduct to 1d. Purification by column chromatography (1.5 cm x 15 cm, silica gel, 5% MeOH in CH_2Cl_2) gave a white foam (163 mg, 0.62 mmol, 94%) which resisted all attempts at crystallization. Hplc analysis (C-18 reverse phase radial compression column, 5% MeOH in

H_2O, 1.5 mL/min) showed this material to be >98% pure.

^1H nmr, (DMSO-d_6) δ; 11.70 [1H, broad s, N(3)-H], 8.38 [1H, d (J(6,F) = 8.4), C(6)-H], 5.90 [1H, d with fine structure (J(1',F) = 16.5, J(1',2') = small), C(1')-H], 5.68 [1H, d (J(OH,3) = 5.7 poorly resolved), C(3')-OH], 5.45 [1H, broad s coupled with C(5') but poorly resolved, C(5')-OH], 5.05 [1H, dd (J(2',F) =53, J(2',3') = 4.0, fine structure J(2',1') = small) C(2')-H], 4.20 [1H, complex d (J(3',F) =23.4) becomes ddd on D_2O exchange (J(3',F) = 23.4, J(3',4') = 8.5, J(3',2') = 4.0), C(3')-H], 3.92 [1H, broad d (J(4',3') = 8.5, J(4',5') = small), C(4')-H], 3.82 and 3.65 [2H, complex d (J(gem) =12.6, J(5',OH; 5',4') = unresolved) becomes dd after D_2O exchange (J(gem) = 12.6, J(5',4') = 2.0), C(5')-OH], Signals at 11.70, 5.68 and 5.45 disappear after D_2O exchange.

Exact mass calc. for $C_9H_{10}N_2O_5F_2$: 264.0557; measured (hrms), 264.0555; intensity = 9.2%. Exact mass calc. for $C_9H_9N_2O_5F$ (M^+ - HF): 244.0493; measured (hrms), 244.0494; intensity = 3.3%.

2,2'-Anhydro-1-(β-D-arabinofuranosyl)uracil (16).

Uridine (10) (2.50 g, 10.25 mmol), diphenyl carbonate (2.85 g, 13.32 mmol) and sodium bicarbonate (0.10 g, 1.19 mmol) were added to 6 mL of hexamethylphosphoric triamide in a 100 mL flask. The reaction mixture was heated to 130 °C at which point there was a vigorous evolution of gas. The temperature was gradually raised to 150 °C during 1 hour. The hot solution was poured into 500 mL of ice water, extracted three times with 100 mL portions of $CHCl_3$ and the aqueous phase evaporated to give 2.13 g of a white powder. Recrystallization from 95% EtOH gave the title compound (1.89 g, 8.36 mmol, 82%) in two crops, mp 238 - 239.5 °C; literature[191] 238 - 244 °C.

^1H nmr, (DMSO-d_6) δ; 7.89 [1H, d (J(6,5) = 7.5), C(6)-H], 6.34 [1H, d (J(1',2') = 5.9), C(1')-H], 5.92 [1H, d (J(OH,3') = 4.0), C(3')-H], 5.87 [1H, d (J(5,6) = 7.5), C(5)-H], 5.24 [1H, d (J(2',1') = 5.9), C(2')-H], 5.01 [1H, t (J(OH,5') = 5.4), C(5')-OH], 4.41 [1H, broad d (J(3',OH) = 4.0, J(3',4') = small) becomes broad s after D_2O exchange,

C(3')-H], 4.09 [1H, broad t (J(4',5') = 5.4, J(4',3') = small) C(4')-H], 3.31 and 3.21[2H, ddd (J(gem) = 12, J(5',4') = 5.4, J(5',OH) = 5,4) becomes dd after D_2O exchange (J(gem) = 12, J(5',4') = 5.0), C(5')-H]. Signals at 5.92 and 5.01 disappear after D_2O exchange.

Exact mass calc. for $C_9H_{10}N_2O_5$: 226.0589; measured (hrms), 226.0595; intensity = 45%.

1-(2'-Fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17).

Liquid HF (6.5 mL) was added to 750 mg of 2,2'-anhydro-1-(β-D-arabino-furanosyl)uracil (16, 3.32 mmol, dried at 100 °C over P_2O_5 *in vacuo*) suspended in 80 mL of dry dioxane in a stainless steel reaction bomb. The stainless steel reaction bomb was sealed and heated at 125 °C (oil bath temperature) for 18 hours. The reaction bomb was cooled in ice, opened and the contents poured into a polyethylene beaker containing 20 mL of water. This mixture was stirred during the addition of sufficient solid sodium carbonate to neutralize the excess HF. The suspended solid was removed by filtration and the liquid evaporated to dryness. This crude material was purified by column chromatography (1.5 cm x 35 cm, silica gel, gradient elution from 2% to 10% MeOH in CH_2Cl_2). The title compound was isolated as fluffy white crystals (337 mg, 1.37 mmol, 41%) by evaporation of the appropriate fractions; mp 149 - 150 °C; literature[195] 150 - 151 °C.

^{1}H nmr, (DMSO-d_6) δ; 11.44 [1H, s, N(3)-H], 7.95 [1H, d (J(6,5) = 8.4), C(6)-H], 5.92 [1H, dd (J(1',F) = 17.6, J(1',2') = 1.9), C(1')-H], 5.65 [1H, d (J(5,6) = 8.4), C(5)-H], 5.63 [1H, d (J(OH,3') = 6.5), C(3')-OH], 5.23 [1H, t (J(OH,5') = 4.8), C(5')-OH], 5.06 [1H, ddd (J(2',F) = 54, J(2',3') = 2.3, J(2',1') = 1.9), C(2')-H], 4.18 [1H, complex, becomes ddd after D_2O exchange (J(3',F) = 20.8, J(3',4') = 8.0, J(3',2') = 4.3), C(3')-H], 3.90 [1H, broad d (J(4',3') = 8.0, J(4',5') = small), C(4')-H], 3.79 and 3.61 [2H, complex d (J(gem) = 12.5, becomes dd after D_2O exchange J(gem) = 12.5, J(5',4') = 1.5, J(5'',4') = 3.0), C(5')-H]. Signals at 11.44, 5.63 and 5.23 disappear after D_2O exchange.

Exact mass calc. for $C_9H_{11}N_2O_5F$: 246.0652; measured (hrms), 246.0649; intensity = 2.7%. Exact mass calc. for $C_9H_{10}N_2O_5$ (M^+ - HF): 226.0589; measured (hrms), 226.0588; intensity = 16.7%.

1-(β-D-Arabinofuranosyl)uracil (65).

To 130 mg (0.575 mmol) of 2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil (16) was added 50 mL of 0.1 M NaOH and the solution was warmed to 40 °C. After 10 minutes a tlc chromatogram showed complete conversion to a single product. The solution was neutralized with 0.10 M HCl, evaporated to dryness and the solid residue triturated with MeOH. The white powder obtained after removal of solvent (138 mg, 0.532 mmol, 93%) was recrystallized from 95% EtOH; mp 217 - 219 °C.

^{1}H nmr, (DMSO-d_6) δ: 11.30 [1H, s, N(3)-H], 7.64 [1H, d (J(6,5) = 8.0), C(6)-H], 5.99 [1H, d (J(1',2') = 4.5), C(1')-H], 5.62 [1H, unresolved d due to overlap with C(5)-H, C(2' or 3')-OH], 5.59 [1H, d (J(5,6) = 8.0), C(5)-H], 5.48 [1H, broad d (J(OH,3' or 2') = 3.5), C(3' or 2')-OH], 5.05 [1H, broad s, C(5')-OH], 4.01 [1H, complex, becomes dd after D_2O exchange (J(2',1') = 4.5, J(2',3') = 3.3), C(2' or 3')-H], 3.91 [1H, complex, obscured by HOD after D_2O exchange, C(3' or 2')-H], 3.74 [1H, complex, becomes ddd after D_2O exchange, (J(4',5') = 4.5, J(4' 5'') = 5.5, J(4',3') = 4.5), C(4')-H], 3.60 [2H, complex, becomes two overlapping dd after D_2O exchange at 3.66 (J(5',5'') = 12, J(5',4') = 4.5) and 3.63 (J(5'',5') = 12, J(5'',4') = 5.5), C(5')-H]. Signals at 11.30, 5.62, 5.48 and 5.05 disappear after D_2O exchange.

Exact mass calc. for $C_9H_{12}N_2O_6$: 244.0695; m[redacted]rms), 244.0690; intensity = 0.9%. Exact mass calc. for $C_8H_9N_2O_5$ (M^+ - CH_2OH): 213.0511, found (hrms), 213.0515; intensity = 15%.

1-(3'-Iodo-3'-deoxy-β-D-ribofuranosyl)uracil (2).

A 1.54 M aqueous solution of HI was prepared by bubbling HI gas through cool distilled water. To 200 μL (0.308 mmol) of this solution in a 1 mL Reacti-Vial® was added 63 mg (0.278 mmol) of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a). The vial was sealed, heated for 1 hour at 97 °C, cooled and filtered through a short column of cellulose impregnated with AgCl. The solvent was removed *in vacuo* and the residue was recrystallized from EtOH to give the title compound as a white microcrystalline solid (58 mg, 0.164 mmol, 59%); mp 204 - 205 °C; literature[201] 204 - 205 °C.

^{1}H nmr, (DMSO-d_6) δ: 11.23 [1H, s, N(3)-H], 7.75 [1H, d (J(6,5) = 8), C(6)-H], 6.14 [1H, d (J(OH,2') = 6), C(2')-OH], 6.06 [1H, d (J(1',2') = 7), C(1')-H], 5.55 [1H, dd (J(5,6) = 8, J(5,NH) = 2.5), becomes d after D_2O exchange (J(5,6) = 8), C(5)-H], 5.22 [1H, t (J(OH,5) = 5), C(5')-OH], 4.58 [1H, ddd (J(2',1') = 7, J(2',3') = 6, J(2',OH) = 6) becomes dd after D_2O exchange (J(2',1') = 7, J(2',3') = 6), C(2')-H], 3.9 - 4.2 [2H, complex multiplet, C(3')-H and C(4')-H], 3.58 - 3.78 [2H, broad unresolved, C(5')-H]. Signals at 11.23, 6.14 and 5.22 disappear after D_2O exchange.

Exact mass calc. for $C_8H_8N_2O_4I$ ($M^+ - CH_2OH$): 322.9528; measured (hrms): 322.9532; intensity = 1.8%.

5-[^{131}I]-Iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67):

a. Carrier added synthesis.

A mixture of of NaI (0.7 mg, 4.67 μmol) and 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (2.1 mg, 8.44 μmol) in a 1 mL Reacti-Vial® were treated with 0.289 GBq of carrier-free [^{131}I]-NaI as a solution in 75 μL of EtOH. The solvent was blown off with a stream of dry N_2 at room temperature and the residue was dissolved in 50 μL of 0.02 M NaOH. The vial was sealed with a septum cap and 50 μL of 2.0 M HNO_3 was added through the septum. The reaction mixture was heated at 115 °C for 90 minutes in a heating block at which time an initially formed brown coloration (I_2) had disappeared. Tlc indicated a single product (linear

analyzer) containing greater than 90% of the activity in the solution (tlc - lsc combined counting). The chemical and radiochemical yield calculated for NaI as the limiting reactant was greater than 90% for the crude product. The reaction mixture was treated with 80 μL of concentrated NH_4OH and the solvent removed under a stream of nitrogen. The residue was redissolved in EtOH and the solvent evaporated *in vacuo* at 40 °C. The sample was chromatographed on a 20 cm x 20 cm x 1 mm silica gel plate (Whatman PLK5F, 15% MeOH in CH_2Cl_2). The eluted product was chromatographically identical (tlc, hplc) with a sample of the authentic cold title compound (1a). The radiochemical purity (linear analyzer) was 99.3% and the chemical and radiochemical yields were 67%. The specific activity at end of synthesis was 45.9 GBq/mmol.

b. No-carrier-added synthesis.

An aqueous solution of no-carrier-added [^{131}I]-NaI (1 μL; 2.41 MBq) was added to 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (0.82 mg, 3.25 μmol) in 10 μL of 0.02 M NaOH in a 100 μL Reacti-Vial®. The vial was sealed and 10 μL of 2.0 M HNO_3 was added through the septum. The solution was heated at 112 °C for 90 minutes in a heating block, cooled and neutralized with several μL of concentrated NH_4OH. The solvent was removed *in vacuo*, the residue was dissolved in 20 μL of EtOH and the solvent evaporated. A tlc plate was spotted with authentic cold 1a, then spotted with the crude reaction product and developed with 15% MeOH in CH_2Cl_2. This plate was analyzed by combined tlc - hplc which showed that 57% of the total activity corresponded to the expected product 67. The product was purified on a 20 cm x 5 cm x .25 mm analytical tlc plate (Whatman K6F) after addition of 10 μg of carrier compound 1a. Removal and elution of the product peak gave 1.0 MBq (41.4% chemical and radiochemical yield) of title compound in greater than 95% radiochemical purity.

5-[^{82}Br]-Bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72).

[^{82}Br]-NH_4Br was produced by neutron activation as described previously. A suspension of [^{82}Br]-NH_4Br (13.2 MBq, 51.0 μmol) in 2 mL of glacial acetic acid was added to

1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (14 mg, 56.9 μmol) contained in a 3 mL teflon reaction vial . The vial was sealed with a teflon backed septum and 0.9 mL (9.0 mg, 67.4 μmol) of a 10 mg per mL solution of N-chlorosuccinimide in acetic acid was added through the septum. The initial brown coloration [^{82}Br]-BrCl rapidly disappeared at room temperature. The vial was heated at 45 °C for 1 hour. The cooled reaction mixture was transferred with several washes of EtOH to a 50 mL flask and evaporated to dryness *in vacuo* at 40 °C. The residue was reevaporated from 5 ml of EtOH and then treated with 10 drops of concentrated NH_4OH in 5 mL of EtOH at 50 °C for 10 minutes. Tlc (Whatman MK6F microslides, 20% MeOH in CH_2Cl_2) showed a single compound at Rf 0.68. This material accounted for 84% of the total activity detected on the plate. A further 12% of the activity (possibly [^{82}Br]-Br^-) occured as a broad area on the plate centered at Rf 0.58. The crude sample was purified by preparative tlc to give 72 (7.56 MBq, 57% chemical and radiochemical yield). This material had a radiochemical purity of 97% as determined by combined tlc-lsc and 99% as determined by hplc analysis. The end of synthesis specific activity was 0.167 GBq/mmol.

[^{14}C]-Cyanamide (74).

[^{14}C]-Barium carbonate (Atomic Energy of Canada Limited, 1.95 mmol, 1.92 GBq/mmol) held in a section of quartz tubing (1.2 cm od x 3 cm long) was placed into a quartz reaction tube (1.35 cm id x 40 cm long). A flow of anhydrous NH_3 was established through the tube (100 mL/minute) and this flow was maintained while the tube was heated at 850 °C for 2 hours in a Fisher Micro Combustion Electric Tube Furnace. The reaction product ([^{14}C]-barium cyanamide) was allowed to cool and then transferred to a centrifuge tube containing 5 mL of ice water. The solution was stirred at 0 °C for two hours and then treated with 1.95 mmol (191 mg) of concentrated H_2SO_4 added dropwise with stirring. The resulting suspension was stirred for 2 hours at 0 °C. The solid was compacted by centrifugation and the clear aqueous layer along with two 5 mL washes with water were transferred to a 100 mL round bottom flask. The pH was adjusted to 5.5 with 0.1 M NaOH and the aqueous solution was

extracted with of diethyl ether (7 x 3 mL). Evaporation of the ether gave 49 mg (1.11 mmol, 57%) of the title compound 74 as a fine crystalline coating on the flask; mp 38 - 40 °C; literature[310] 40 °C.

[2-^{14}C]-2-amino-β-D-arabinofurano-[1',2': 4,5]-2-oxazoline (76).

[^{14}C]-Cyanamide (74) (49 mg, 1.11 mmol) and D-arabinose (193 mg, 1.28 mmol) were dissolved in 4 ml of 1 M NH_4OH solution (1:1 water:MeOH v/v) and heated at 60 °C for 130 minutes. Evaporation of the solvent gave the title oxazoline (76) as a pale yellow oil which was used directly for the synthesis of [2-^{14}C]-2,2'-anhydro-(1-β-D-ribofuranosyl)uracil (77).

[2-^{14}C]-2,2'-Anhydro-(1-β-D-ribofuranosyl)uracil (77).

A solution of methyl propiolate (107 mg, 1.28 mmol) in 1 mL of tetrahydrofuran was added to the oxazoline 76 from the previous synthesis in a 30 mL teflon reaction vial. EtOH (3.5 mL) and H_2O (2.5 mL) and a magnetic stirring bar were added and the sealed vial was heated at 90 °C for 5 hours. Evaporation of the solvent at 50 °C *in vacuo* and reevaporation of two subsequent solutions in EtOH gave a yellow oil. Slow evaporation of a MeOH solution of this oil yielded 55.36 mg of the title compound (77) as white crystals (0.24 mmol, 12.3% from [^{14}C]-$BaCO_3$); mp 246.5 - 248 °C; literature (cold compound)[198] 246 - 248 °C.

This material was identical (tlc, hplc) with authentic cold compound (16). The mother-liquor contained a further 54.6 mg of 77 as determined by combined tlc - hplc counting. This product could be readily separated from the radioactive and non-radioactive impurities by hplc (5.8 min retention, 2.5% MeOH in water, Whatman C-18 Partisil column, 3 mL/minute). The overall chemical and radiochemical yield starting from barium carbonate was 24.6%. The product had a measured specific activity of 1.86 GBq/mmol.

[2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (78).

A sample of [2-^{14}C]-2,2'-anhydro-1(β-D-ribofuranosyl)uracil (77) (12.81 mg, 51.7 μmol, 96.2 MBq) in a 3 mL teflon reaction vial was dried for 18 hours *in vacuo* over P_2O_5. A solution of anhydrous HF in dioxane was prepared by condensing HF gas in a sealed teflon vial containing 3 mL of carefully dried and freshly distilled dioxane and fitted with a teflon backed septum. The resulting solution contained 250 mg/mL of HF. Hydrogen fluoride solution (1 mL, 250 mg HF, 12.5 mmol) was injected into the teflon vial containing 77 through a teflon backed septum. The sealed vial was heated at 115 °C (bath temperature) for 18 hours. The cooled solution and a 5 mL wash with water were transferred to a 30 mL teflon vial. This solution was treated with solid $CaCO_3$ until no further evolution of gas was noted. The aqueous layer was isolated from the solid by centrifugation, evaporated to dryness *in vacuo* at 45 °C, dissolved in EtOH and reevaporated. Analysis of this crude product by hplc showed the expected product 78 as the major component at a retention time of 13.77 minutes with starting material 77 at 3.88 minutes, uracil at 4.15 minutes as well as several minor components (Waters C-18 Radial Compression column, 2% MeOH in H_2O, 1 mL/minute). The identities of the hplc peaks were confirmed using authentic cold compounds as hplc standards. The crude material was purified by preparative hplc (3 mL/minute of 2% MeOH in H_2O, Whatman Partisil ODS reverse phase preparative column). The product after chromatography had a radiochemical and chemical purity of 98%. The total activity of the isolated product was 26.6 MBq (25.4%). The specific activity of of the product was 1.86 GBq/mmol.

[2-^{14}C]-5-Chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79).

A solution of Cl_2 in HOAc was prepared by bubbling Cl_2 gas through reagent grade HOAc until the solution retained a pale green color. Excess KI was added to an aliquot of this solution and the liberated I_2 was titrated with 0.1 M sodium thiosulfate. The solution was calculated to be 0.34 M in Cl_2. The freshly prepared solution of Cl_2 in HOAc (40 μL; 13.6 μmol, 2.4 equivalents) was added to [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil

(78) (1.39 mg, 5.60 μmol, 10.42 MBq) dissolved in 400 μL of HOAc in a 1 mL Reacti-Vial®. After 20 minutes at room temperature the solvent was blown off with dry N_2 and the residue treated with two drops of NH_4OH in 0.5 mL of EtOH at 50 °C for 10 minutes. The solvent was again blown off and the residue examined by tlc. The only product detected by uv and a combined tlc - lsc scan of the plate was 79 which indicated that greater than 90% of the activity corresponded to the product position. The crude product was purified by hplc (3 mL/minute of 2% MeOH in H_2O, Whatman C-18 reverse phase Partisil column). The isolated product was 99% pure and was identical (tlc, hplc) with the authentic cold compound 1c. The product had an activity of 8.03 MBq (77% yield) with a specific activity of 1.86 GBq/mmol.

[2-^{14}C]-5-Fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87).

A solution of F_2 in HOAc was prepared by bubbling 2.5% F_2 in N_2 through reagent grade HOAc at 100 mL/minute for 30 minutes. The solution was determined to be 9.4 μmol/mL in F_2 by titration of the I_2 liberated upon addition of KI to an aliquot of the solution with standard thiosulfate solution. The solution of fluorine in acetic acid (1 mL, 9.4 μmol F_2) was added to [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (78) (1.48 mg, 5.97 μmol, 11.1 MBq) in a 25 mL flask. After 20 minutes at room temperature the solvent was removed at 40 °C *in vacuo*. The residue was dissolved in EtOH and again evaporated to dryness. The pale yellow gum was treated with 100 μL of NH_4OH in 1 mL of MeOH for 10 minutes at 40 °C and again reduced to dryness *in vacuo*. A tlc chromatogram showed complete conversion to product (87) and combined tlc - lsc indicated 91.7% chemical and radiochemical yield of crude product. An aliquot of this product (7.10 MBq) was purified by hplc to give 6.44 MBq of 87 with a radiochemical purity of >99% (3 mL of 5% MeOH in H_2O, Whatman reverse phase Partisil column). This material was identical (tlc, hplc) with an authentic cold sample of (1d).

[^{123}I]-1-(3'-Iodo-3'-deoxy-β-D-ribofuranosyl)uracil (92).

1-(2',3'-Epoxy-β-D-lyxofuranosyl)uracil (24a) (1 mg, 4.42 μmol) was added to an aqueous solution of no-carrier-added [^{123}I]-I_2 (0.5 mL, 0.70 GBq). The solvent was removed at 50 °C with a stream of helium without loss of volatile iodine. The residue was transferred with two 20 μL water washes to a 100 μL Reacti-Vial® and treated with 4μL of freshly prepared HI solution (0.898 M, 3.6 μmol). The sealed vial was heated at 93 °C for 2.5 hours, cooled and the contents passed through a short column of DEAE-cellulose powder impregnated with AgCl. The column eluate contained the title compound (92) (0.64 GBq, 91%) and a trace of excess epoxide (24a). The radiochemical purity as determined by tlc was >99% and this material was chromatographically identical with authentic cold compound 2. The synthesis required 4 hours and gave an end of synthesis specific activity of 128 GBq/mmol. The unreacted epoxide (24a) present in this synthesis was eliminated in subsequent reactions by using a slight molar excess of HI.

[^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93).

Solutions of [^{18}F]-HF in anhydrous dioxane were prepared from reactor or accelerator produced ^{18}F. These solutions were reacted with 2,2'-anhydro-1-(β-D-ribofuronosyl)uracil (16) in stainless steel, teflon coated stainless steel and teflon reaction bombs at a variety of temperatures and reaction times. After reaction excess acid was neutralized with solid $CaCO_3$ and the filtered liquid was chromatographed by tlc. The title compound (93) was identified by comparison of retention times with that of an authentic cold reference compound. Yields were variable and very low (<1%).

E. Animal Studies

Preparation of animal model.

Lewis lung tumors were transplanted by trocar injection into young female BDF_1 mice weighing 18 to 20 grams using the following procedure. A donor BDF_1 mouse with a mature (2 cm diameter) subcutaneous Lewis lung tumor was sacrificed by cervical dislocation. The tumor was removed and viable sections were separated being careful to avoid soft or necrotic portions of the tumor mass. The portions of tumor were placed in sterile saline and sliced with a blade into small (8 mm^3) sections. Each section of tumor was drawn into a trocar (14 gauge, Custom Spinal, Popper and Sons Inc.) and injected subcutaneously into the host mouse. The mice were placed under light ether anesthesia and the tumor was injected into the left flank. The skin over the trocar was squeezed between the thumb and forefinger while the trocar was rotated and removed with the other hand. This insured that the tumor section was not removed with the needle. The tumors were generally palpable within 4 or 5 days and weighed 100 to 300 mg at 10 to 14 days after implant. The variability in tumor size reflected the differences in size and viability of the original transplant.

The Walker 256 carcinoma was injected as a cell suspension in saline subcutaneously into the right flank of male Wistar rats (210 to 230 g). The cell suspension was prepared by mincing a portion of tumor taken from the host rat and passing this material in saline through progressively smaller bore needles. After a final passage through a 26 gauge needle the cells were diluted with saline and 0.25 mL of this suspension was injected subcutaneously via a 23 gauge needle. The tumor was palpable after 2 days and ~2 cm in diameter after 6 days.

All animals were maintained *ad lib* with food pellets and tap water in standard plastic cages (6 mice per cage or 4 rats per cage).

Administration of the radiopharmaceuticals.

The radiolabelled compounds used in this study were all water soluble compounds. The compounds were administered intravenously via the tail vein as solutions in normal saline. The purified radioactive compounds were dissolved in ethanol and transferred to multidose vials. The solvent was then removed *in vacuo* and the compounds stored as a dry sterile residue at freezer temperature. The samples were reconstituted just prior to use with sterile saline. The appropriate dilutions to give the required activity to volume were determined using an isotope dose calibrator in the case of the γ-emitting nuclides. For ^{14}C-labelled compounds a partial dilution in saline was made, an aliquot was counted by lsc and further dilution was made to give the required concentration of radioactivity. The activity was adjusted so that 0.05 to 0.25 mL of solution was injected per animal.

The radiopharmaceuticals were generally administered to the Lewis lung bearing mice at approximately 14 days after tumor transplant. The Wistar rats containing the Walker 256 tumor were injected at 6 days after transplant.

Collection of biological samples.

The mice were sacrificed by asphyxiation with carbon dioxide. This was carried out in a 300 mL beaker containing dry ice over which a mesh covered watch glass was placed. The watch glass allowed collection of excreted urine. Once breathing had ceased the mice were removed from the beaker, the chest cavity was opened and the maximal quantity of blood was removed from the heart by cardiac puncture. Fifteen tissues were also examined in addition to the blood and urine. These included spleen, stomach, gastrointestinal tract (git), kidney, liver, muscle, bone, skin, tail, lungs, heart, thyroid, salivary glands, brain and tumor.

These tissues were handled differently depending on the radionuclide in use. When ^{82}Br- and ^{131}I-containing compounds were used, the entire organs were placed in plastic counting vials (Zinsser Polyvials®). A skin sample (~250 mg) containing fur was taken from the upper back. The muscle was taken from the upper right hind leg and the femur of this leg

was used as the bone sample. Stomach and intestine were emptied of contents before weighing. After removal of tissues the carcass was divided into two sections and placed in vials for counting. All tissues were weighed wet using a taring balance (Mettler AC 88). Samples not counted immediately were stored at -5°C.

When [^{14}C]-containing compounds were used, analysis of the activity required combustion of the samples and liquid scintillation counting of the ^{14}C-CO_2 thus produced. This necessity for sample combustion placed an upper weight limit of approximately 200 mg on each tissue sample and therefore tissue aliquots were used in some cases rather than the entire organ or tissue. This was the case for blood, intestine, kidney, liver, muscle, bone, skin and tumor. All tissues were weighed wet into paper combustion cups (Packard Instrument Co. Inc.) and dried under a heat lamp prior to combustion. The carcass was not counted in the mice injected with the ^{14}C-labelled compounds.

The Wistar rats were placed under ether anesthesia and sacrificed by exsanguination via cardiac puncture. The following tissues were collected and placed in 0.9 cm x 7 cm plastic counting tubes; blood (1 mL), heart, one lung, slices from edge and center of liver, spleen, one kidney, stomach, portion of intestine 10 cm long, one testicle, aliquot of tumor free from necrotic tissue, large muscle in hind leg contralateral to tumor, upper section of thigh bone, thyroid and surrounding tissues and contents of the bladder. The wet weights of the tissue aliquots as well as the whole organs were recorded. The tissues were stored at -5°C until required for counting.

Sample counting.

The tissue samples containing ^{82}Br and ^{131}I were counted using a Beckman Gamma 8000 gamma scintillation counter. The samples were generally counted for 1 minute with a window setting of 20 to 2000 KeV. This wide window maximized the counts. An aliquot of diluted injection solution was also counted as an activity standard. The count rate for this known activity allowed calculation of counting efficiency. The time at which the samples were counted

was recorded and decay corrections were made. The calculations for activity distribution were made using a computer program†.

The tissue samples containing ^{14}C were combusted at 900 °C in an oxygen atmosphere in an H. J. Harvey Instrument Corporation Biological Oxidizer The [^{14}C]-CO_2 produced by combustion was trapped in Harvey Carbon-14 Cocktail. The samples in standard liquid scintillation vials were counted using a Beskman LS 9000 or Searle Mark III liquid scintillation counter. The detection efficiency (product of trapping efficiency for ^{14}C-CO_2 and lsc counting efficiency) was determined by combusting and counting ^{14}C-standards of known activity. Lsc counting efficiency alone was determined by adding known volumes of standard [^{14}C]-n-hexadecane to the counted samples and recounting them.

Tissues from Wistar rats containing ^{123}I -labelled compound were counted using a Ge(Li) detector and a Canberra Series 80 Multichannel Analyzer equipped with an automatic sample changer.

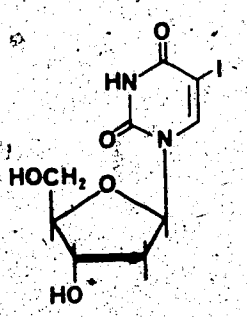
†A set of computer software has been prepared by R. Flanagan, Faculty of Pharmacy and Pharmaceutical Sciences at the University of Alberta for calculations of tissue distribution data and for comparison and graphical analysis of the results.

IV. RESULTS AND DISCUSSION

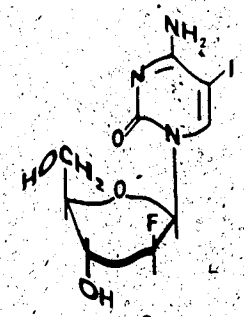
A, 5-Iodo-1-(2'-Fluoro-2'-Deoxy-β-D-Ribofuranosyl)-Uracil (1a)

Synthesis and purification.

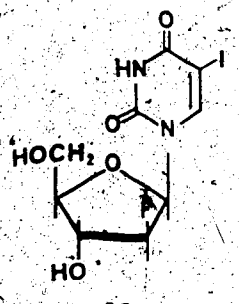
There has been considerable interest in 5-iodinated pyrimidine bases and nucleosides in medical and biochemical investigations. For example 5-iodo-2'-deoxyuridine (62a), first synthesised in 1959[159], was shown to have antiviral effects against *polyoma* virus[311] and *herpes simplex* virus[312] and was used clinically in the treatment of acute herpetic keratitis[313]. Another 5-iodopyrimidine nucleoside, 2'-fluoro-5-iodoarabinocytidine (63e), is a potent antiviral agent showing activity against *herpes simplex* virus type 1 and type 2 as well as a variety of other DNA viruses[221]. This compound has recently undergone Phase 1 Clinical Trials and has promoted healing of viral infections in immunosuppressed cancer patients[299]. This nucleoside and its uridine analog (20e) also show inhibitory effects against mouse and human leukemia and lymphoma lines[300].



62a



63e



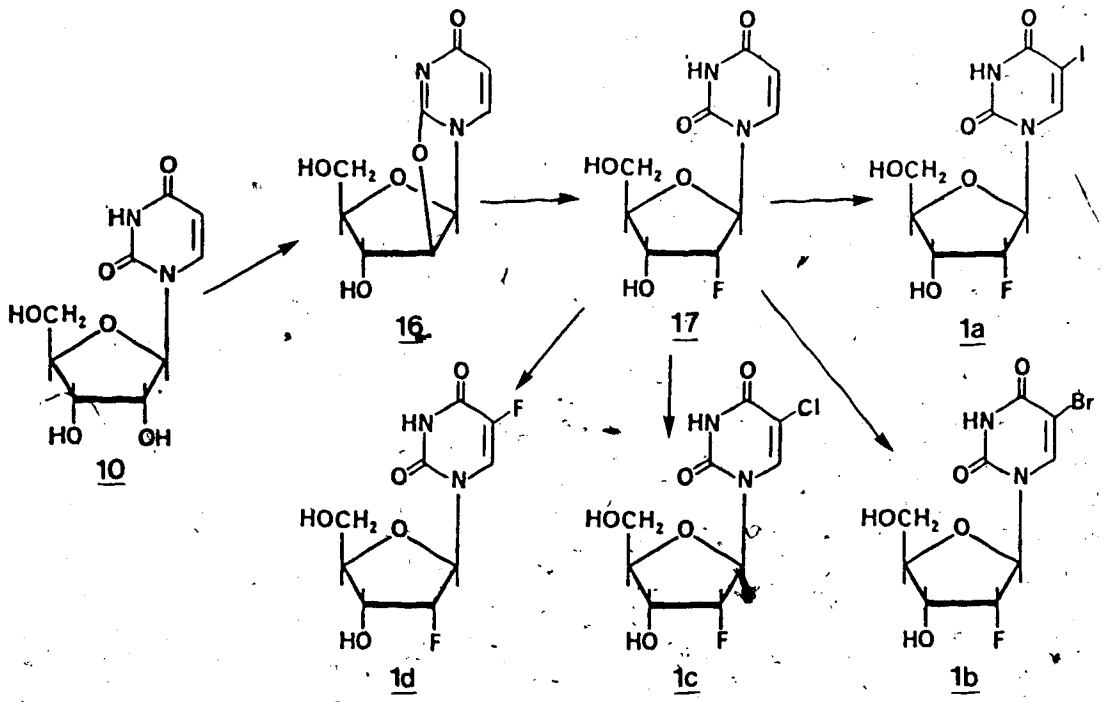
20e

Interest in these compounds and a variety of other 5-iodopyrimidines has resulted in the development of a number of procedures for the synthesis of both non-radiolabelled and radioiodinated compounds. These methods have been discussed in the literature survey. It was expected that one of these methods would prove suitable for the synthesis of

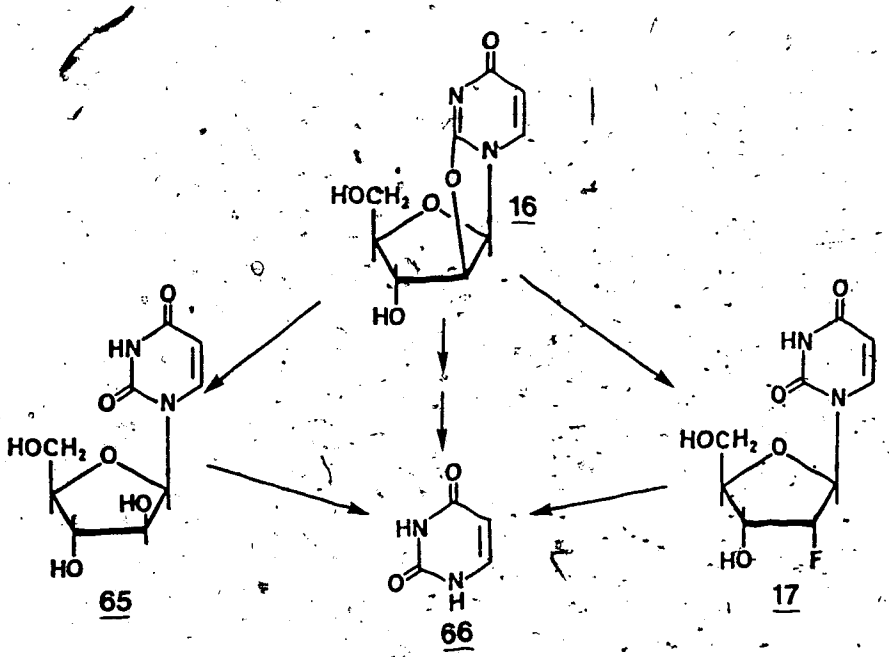
5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a).

The antiviral and cytotoxic effects of 5-iodo- and the 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a and 1b) were examined by Watanabe *et al*[1]. These two compounds were apparently synthesised by a method analogous to that used to prepare the closely related arabino configuration nucleosides. The methods employed[221,222] included condensation of *bis*-2,4-trimethylsilyl-5-halopyrimidine with the appropriate blocked sugar or direct iodination of 3'-O-acetyl-5'-O-benzoyl nucleosides with molecular iodine in fuming nitric acid. Both of these methods required further reaction steps after incorporation of the iodine and are therefore not as suitable for fadioiodinations as reactions in which iodination is the ultimate step.

The 5-iodo and 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils used in our study were synthesised from the precursor 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (17) as outlined in Scheme IV.1. The 2,2'-anhydronucleoside (16) was obtained in 82% yield from the reaction of uridine (10) and diphenyl carbonate in hexamethylphosphoric triamide at 150 °C[198,199]. Compound 17, the required precursor for the synthesis of the 5-halo-nucleosides, was prepared by reacting 16 with HF and dioxane at 115 - 120 °C in a stainless steel reaction bomb for 18 hours[195]. This procedure was subject to a number of complicating side reactions as outlined in Scheme IV.2. At temperatures above 120 °C the main product isolated after work-up was uracil (66) accompanied by a black tar due to degraded and polymerized sugar. At temperatures below 115 °C the reaction proceeded very slowly and even after 24 hours starting material comprised the bulk of the material in the reaction mixture. Traces of water in the solvents and reagents lead to isolation of 1-β-D-arabinofuranosyluracil (65). Since this latter reaction appeared to proceed more readily than the required 2'-fluorination reaction, water must be rigorously excluded from the reaction. We were able to synthesise 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) as outlined in Scheme IV.1 in 89% yield using an adaptation of the iodination method reported by Robins and Taylor[162] for the last step. In this reaction I_2, produced *in situ* by oxidation of NaI by HNO_3, undergoes an



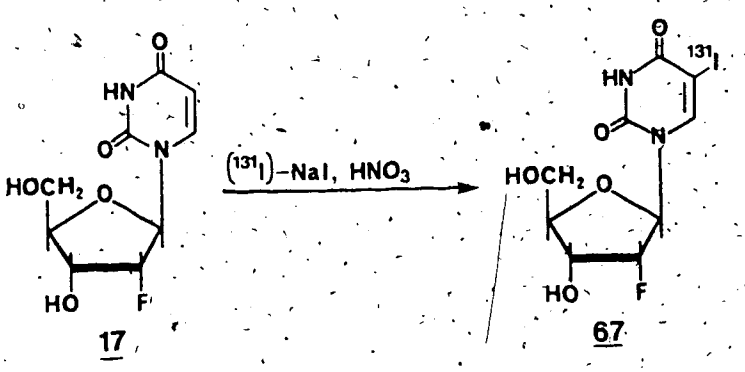
Scheme IV.1 Synthesis of 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d)



Scheme IV.2 Side reactions in the preparation of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (17)

electrophilic reaction with the electron-rich 5-position of the starting 2'-fluoro compound (17). The progress of the reaction was monitored by the disappearance of the free iodine color. The reaction was complete after 16 hours at 80 °C. At higher reaction temperatures (115 °C) the reaction time could be reduced to 90 minutes without reduction in yield.

This reaction was also used for the radiochemical synthesis of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) (Scheme IV.3).



Scheme IV.3 Synthesis of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67)

The reaction was rapid, gave high yields and, with NaI as the limiting reactant, resulted in all available iodine being added at the 5-position of the nucleoside. Chemical and radiochemical yields of greater than 60% after chromatographic (tlc) purification were obtained in the radioactive syntheses. The specific activity of the product (67) depended on the amount of carrier NaI added to the synthesis. End of synthesis specific activities of 40 to 50 GBq mmol^{-1} were obtained routinely when [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) was prepared for animal studies. In a no-carrier-added synthesis a 41.5% yield of 67 was obtained after purification of the product by tlc. Although carrier 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) was added to assist in the chromatographic

purification of this product it seems likely that purification by hplc would give a clean product at no-carrier-added levels.

Purification by thin layer chromatography gave material of high radiochemical purity (>99%) with no evidence of non-radioactive impurities. The purity was determined either by scanning developed tlc plates with a linear analyzer or by scraping the silica from the tlc plates in thin strips and counting by liquid scintillation using a Digital Integration program (Beckman). The chemical and radiochemical purity was confirmed by subsequent hplc analysis using ultraviolet and radioactivity detection. Only traces of radioactive and non-radioactive impurities were noted in the analysis (<1%).

Solutions of 67 in saline showed some degradation after 18 hours at room temperature. After 5 days at room temperature hplc analysis showed the presence of iodide (14%), 5-iodouracil (10%) and other unidentified degradation products (3%) as well as [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) (73%). Compound 67 used for animal studies was stored as a dry thin film in multidose vials at -5 °C. Samples were dissolved in saline just prior to use and unused portions were frozen if required for subsequent experiments. Reconstituted samples were discarded after 24 hours.

Tissue distribution.

The female BDF_1 mice bearing a Lewis lung carcinoma used in this experiment were injected with doses of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) ranging from 0.5 μg (1.3 pmol) to 1.8 μg (4.8 pmol) with injection activities from 55 kBq (15 minute tissue distribution) to 110 kBq (24 hour tissue distribution). The data is shown in Table IV.1 as percent dose per gram of tissue and as organ to tumor ratios and in Figure IV.1 and Figure IV.2 as organ to blood ratios.

[^{131}I]-5-Iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) demonstrated little uptake in tissues except for those which were related to the route of excretion (kidney) or those which had a high blood content (lung). At longer time periods (8 and 24 hours) the

	TIME(HOURS)						
ORGAN	0.25	0.50	1	2	4	8	24
BLOOD	19.0 (1.3)[1] 3.9[2]	16.0 (1.1) 4.5	13.3 (0.6) 3.2	8.90 (0.7) 2.3	8.5 (0.5) 2.7	4.1 (0.4) 2.2	0.16 (.07) 2.1
SPLEEN	3.2 (0.2) 0.7	2.9 (0.2) 0.8	2.5 (0.1) 0.6	1.8 (0.3) 0.5	1.8 (0.1) 0.6	1.0 (0.1) 0.6	0.5 (0.2) 0.7
STOMACH	3.7 (0.6) 0.7	4.6 (0.8) 1.3	4.5 (1.1) 1.1	4.0 (1.0) 1.1	5.7 (0.9) 1.8	5.3 (1.1) 2.8	.21 (.08) 2.8
GIT	3.1 (0.2) 0.6	2.8 (0.1) 0.8	2.5 (0.2) 0.6	1.9 (0.3) 0.5	2.1 (0.1) 0.6	1.3 (0.2) 0.7	.07 (.02) 1.0
KIDNEY	21.1 (2.7) 4.3	18.5 (2.7) 5.1	12.0 (1.9) 2.9	8.5 (1.1) 2.2	7.3 (0.7) 2.3	4.1 (0.6) 2.2	.14 (.06) 1.8
BRAIN	0.4 (.06) .08	0.4 (.03) 0.10	0.3 (.02) 0.08	0.2 (.03) 0.05	0.2 (.02) 0.07	0.1 (.01) 0.06	.01 (0.0) 0.14
SKIN	5.2 (0.6) 1.1	3.8 (0.4) 1.1	5.0 (1.5) 1.2	3.2 (0.5) 0.8	3.5 (0.6) 1.1	2.1 (0.4) 1:1	0.1 (.05) 1.5
MUSCLE	2.5 (0.2) 0.5	2.0 (0.1) 0.6	2.3 (0.4) 0.6	1.3 (0.2) 0.3	1.3 (0.2) 0.4	0.7 (0.1) 0.4	.03 (.01) 0.4
BONE	2.4 (0.3) 0.5	1.8 (0.3) 0.5	1.5 (0.3) 0.4	1.2 (0.2) 0.3	1.3 (0.2) 0.4	0.7 (0.1) 0.4	.07 (.09) 0.8
LUNG	7.9 (0.7) 1.6	7.5 (0.8) 2.1	7.0 (0.7) 1.7	5.1 (0.9) 1.3	4.5 (0.5) 1.4	2.6 (0.5) 1.4	0.1 (.05) 1.4
HEART	7.0 (1.1) 1.4	6.4 (0.3) 1.8	5.8 (0.2) 1.4	5.7 (0.6) 1.0	3.5 (0.1) 1.1	1.6 (0.1) 0.9	0.1 (0.1) 1.3
LIVER	5.3 (0.7) 1.1	4.6 (0.6) 1.3	3.6 (0.1) 0.9	2.8 (0.3) 0.7	2.6 (0.3) 0.8	1.4 (0.2) 0.8	0.1 (.04) 1.3
TUMOR	4.9 (0.4) 0.3[3]	3.7 (0.9) 0.2	4.1 (0.5) 0.3	3.8 (0.3) 0.4	3.3 (0.6) 0.4	1.9 (0.4) 0.5	.08 (.03) 0.5

[1] Percent of injected dose per gram of tissue (± standard deviation), (n = 6 animals).
[2] Organ to tumor ratio.
[3] Tumor to blood ratio.

Table IV:1 Tissue distribution in female BDF_1 mice bearing a Lewis lung tumor after intravenous injection of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) (n = 6 animals)

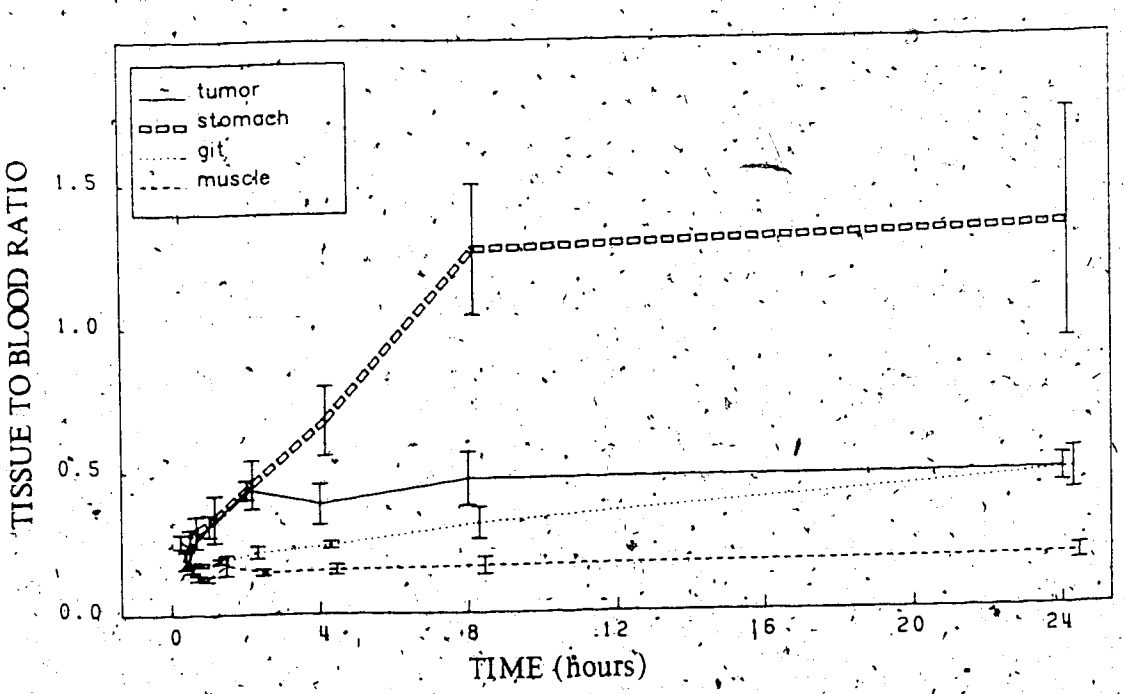


Figure IV.1 Organ to blood ratios for tumor, stomach, intestine and muscle after intravenous injection of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) into female BDF$_1$ mice bearing a Lewis lung tumor

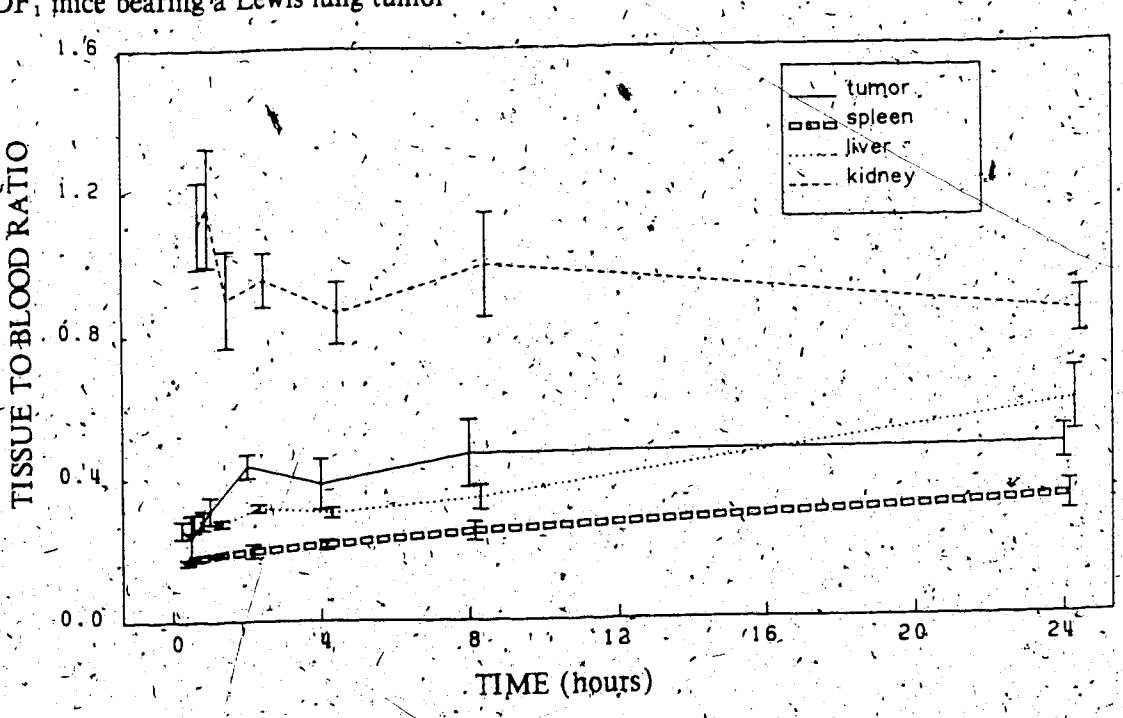


Figure IV.2 Organ to blood ratios for tumor, spleen, liver and kidney after intravenous injection of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) into female BDF$_1$ mice bearing a Lewis lung tumor

concentration of activity in the stomach exceeded that of the blood and the activity in the thyroid (data not shown in Table IV.1) increased significantly after 2 hours. At 4, 8 and 24 hours the percent of the injected dose in the thyroid was 1.5, 2.6 and 5.9 respectively. This distribution of activity at the longer time periods is characteristic of the metabolism of iodide anion. This suggests *in vivo* loss of radioiodine from [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67). This suspicion was supported by the detection of radioiodide in the urine as described later.

The tumor to blood ratios were plotted along with various other organ to blood ratios in Figure IV.1 and Figure IV.2. The tumor to blood ratios were always less than 0.5 and no tissues other than stomach and thyroid demonstrated accumulation of activity.

Whole body elimination and metabolism.

Three male BDF_1 mice each weighing approximately 22 grams were each injected *via* the tail vein with about 7.40 MBq of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) (52.2 GBq mmol^{-1}, 0.14 μmol, 52.8 μg) as a solution in 0.1 mL of normal saline. The whole body activity of the mice was measured at various times after injection using a Picker Isotope Dose Calibrator. During the measurements the mice were restrained in a defined geometry by the use of a plastic insert. Between measurements the animals were maintained *ad lib* with food and water and they were transferred to fresh cages at intervals to minimize the contamination of fur from bedding material. Urine was also collected at intervals by placing the mice in small metabolism cages. Additional urine was obtained at the time of sacrifice (24 hours). The data collected in this experiment are shown in Table IV.2 and in Figure IV.3. The compound was characterized by rapid initial excretion. Half of the injected dose was eliminated within 30 minutes and 75% of the dose by 2 hours. After 2 hours the excretion was slower. This data can be interpreted as a two component system (bi-exponential curve) with a longer lived component having a half-life of 6.79 hours and a shorter lived component having a half-life of 0.70 hours.

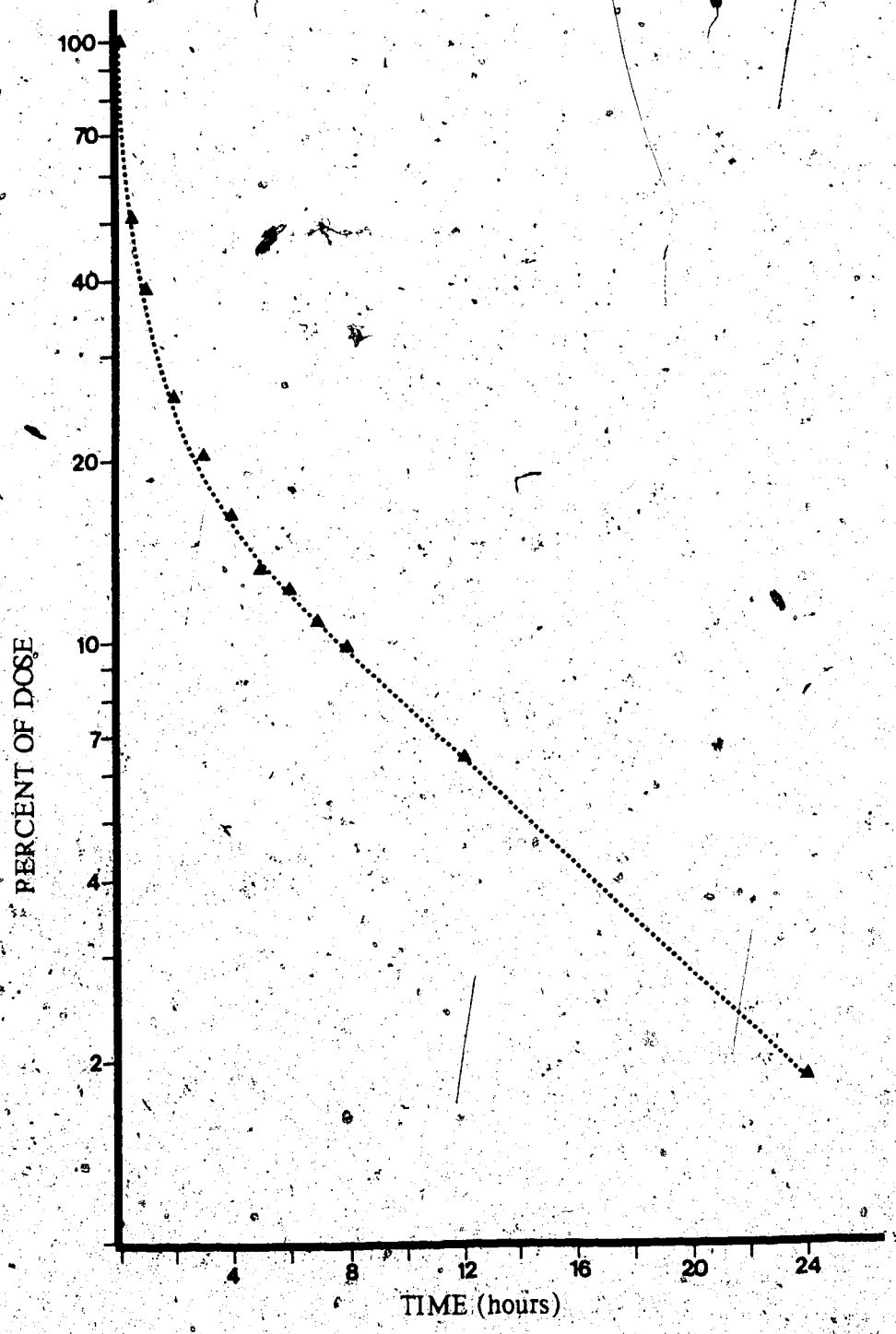


Figure IV.3 Plot of percent dose in whole body vs time after intravenous administration of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) into male BDF$_1$ mice

TIME (HOURS)	% DOSE IN WHOLE BODY 1	2	3	ave	(±sd)
0	100	100	100	100	0
.5	56.8	46.0	49.6	50.8	(5.5)
1	48.2	30.2	37.4	38.6	(9.1)
2	25.8	24.9	25.8	25.5	(5.2)
3	20.3	20.7	21.0	20.7	(0.4)
4	18.0	14.2	16.5	16.2	(1.9)
5	14.1	12.4	13.8	13.4	(0.9)
6	13.3	11.5	12.0	12.3	(0.9)
7	11.3	10.8	10.6	10.9	(0.4)
8	10.4	9.6	9.4	9.8	(0.5)
12	6.3	6.1	6.7	6.4	(0.3)
24	1.6	2.1	1.9	1.9	(0.2)

Table IV. 2 Percent of dose in whole body vs time after intravenous administration of approximately 7.40 MBq of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) into male BDF$_1$ mice

The chemical nature of the excreted activity in the urine was examined by hplc analysis of urine fractions collected during the intervals 0 - 30 minutes, 2 - 3 hours and 6 - 8 hours and at the time of sacrifice (24 hours). The analysis was carried out using a Waters C-18 reverse-phase radial compression column with 15% methanol in water as the mobile phase. The column effluent was analyzed first by a uv-detector and then by a sodium iodide crystal γ-detector. The main ^{131}I γ-ray photopeak at 364 KeV (82%) was detected using an energy window from 300 to 400 KeV.

Each of the stored frozen urine samples was thawed, mixed with a portion of the mobile phase and aliquots of 5 to 20 uL were injected directly onto the column. The identities of the radioactive components in the urine were determined by comparison of their retention times with authentic cold compounds. The series of cold compounds used as reference materials and their retention times were; 5-iodouracil (5.67 minutes), 5-iodouridine (6.78 minutes), 5-iodo-2'-deoxyuridine (62a) (8.75 minutes) and 5-iodo-2'-fluoro-2'-deoxyuridine (1a)

(12.12 minutes). These compounds were readily separated as shown in Figure IV.4.

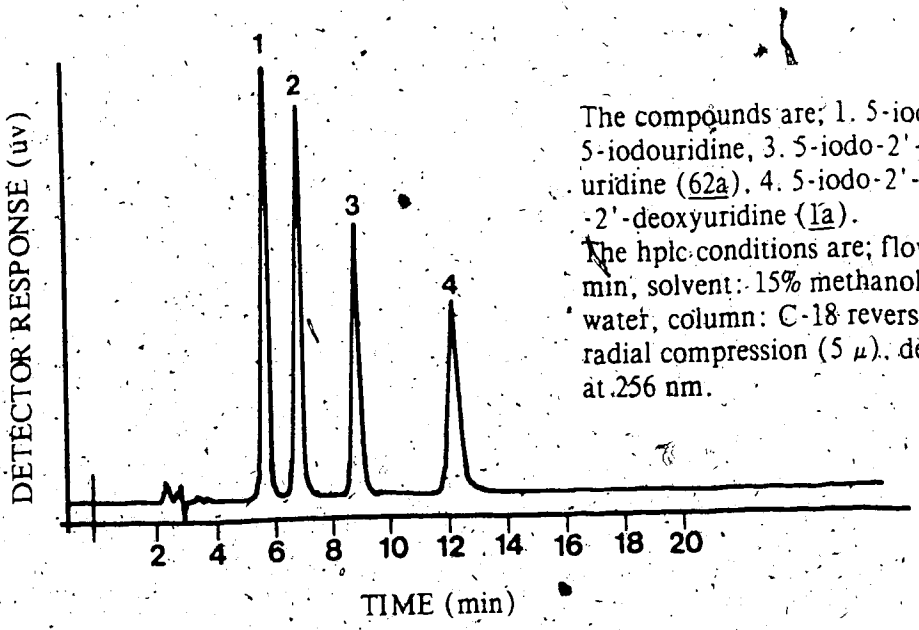


Figure IV.4 Retention times of 5-iodonucleosides and bases used as internal reference compounds for analysis of the urinary metabolites of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67)

INTERVAL	RADIOACTIVE CONSTITUENTS OF URINE (% of total)			
(hours)	iodide	5-I-2'-FUdR	5-IU	other
0 - 2	4.7	90.8	1.8	2.6
2 - 6	13.7	82.8	0	3.5
6 - 24	39.8	60.2	0	0
24	62.6	37.4	0	0

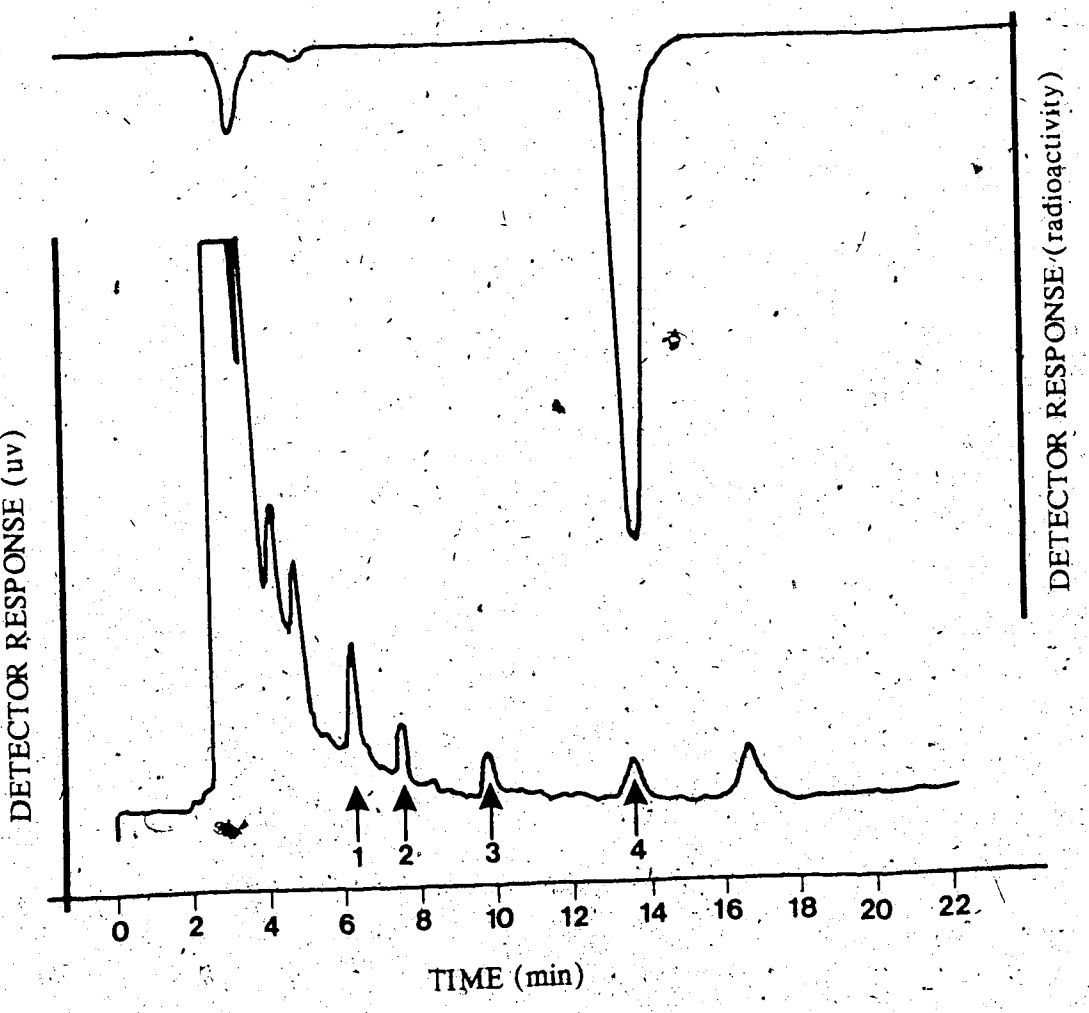
Table IV.3 Radioactive constituents present in urine at various time intervals after intravenous injection of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) into male BDF$_1$ mice

The urine was spiked with a mixture of the cold compounds shown in Figure IV.4 before injection. Superimposition of the uv and the radioactive traces allowed identification of the radioactive peaks. Figure IV.5 shows the 2 to 3 hour urine analyzed by this method and Table IV.3 illustrates the results for all the urine analyses. The column in Table IV. 3 labelled "iodide" refers to the activity detected at or near the solvent front using the reverse phase system. This very polar [^{131}I]-activity is probably [^{131}I]-I$^-$ although this was not proven in this experiment.

Biological fate of injected [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67).

After its intravenous injection into female BDF$_1$ mice [^{131}I]-5-iodo-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (67) was rapidly excreted in the urine. The level of tissue uptake was low with little evidence of active uptake or accumulation in any tissue except the kidney during the early stages of the experiment (0 - 4 hours). The activity level in the tissues appeared to parallel the blood content of these tissues with organs such as the lung and heart being more active than less vascular tissues such as muscle and gastrointestinal tract. The distribution of activity at later time periods (4 - 24 hours) appeared to be due to a combination of the unchanged 67 and iodide anion. Evidence for the presence of iodide was provided by the detection of a very polar iodine species in the hplc analysis (Table IV.3) and from the high levels of activity measured in the thyroid and stomach at 8 and 24 hours after administration of the radiolabelled compound (67). The thyroid and stomach are known to extract and concentrate circulating iodide anion[314].

5-Iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) can be considered to be a structural analog of thymidine and is also related structurally to several compounds such as 5-iodo-2'-deoxyuridine (62a) and 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-arabinofuranosyl)uracil (20e) which have pronounced biological activity. The biochemical fate of 5-iodo-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (1a), however, appeared to differ from these related compounds. The failure of [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a)



The compounds are; 1. 5-iodouracil, 2. 5-iodouridine, 3. 5-iodo-2'-deoxyuridine (62a), 4. 5-iodo-2'-fluoro-2'-deoxyuridine (1a).
The hplc conditions are; flow: 1.mL / min, solvent: 15% methanol / 85% water, column: C-18 reverse phase radial compression (5 μ), detection: uv at 256 nm and radioactivity as γ-radiation between 300 and 400 KeV.

Figure IV.5 Hplc analysis by uv and radioactivity detection for the 2 to 3 hour urine sample collected from male BDF_1 mice after intravenous injection of [^{131}I]-5-iodo-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (67)

to accumulate in rapidly growing tissues such as intestine and tumor indicated that it was a poor substrate for the transport mechanisms required for cellular uptake and / or the kinase enzymes required to convert it to the triphosphate prior to incorporation into DNA.

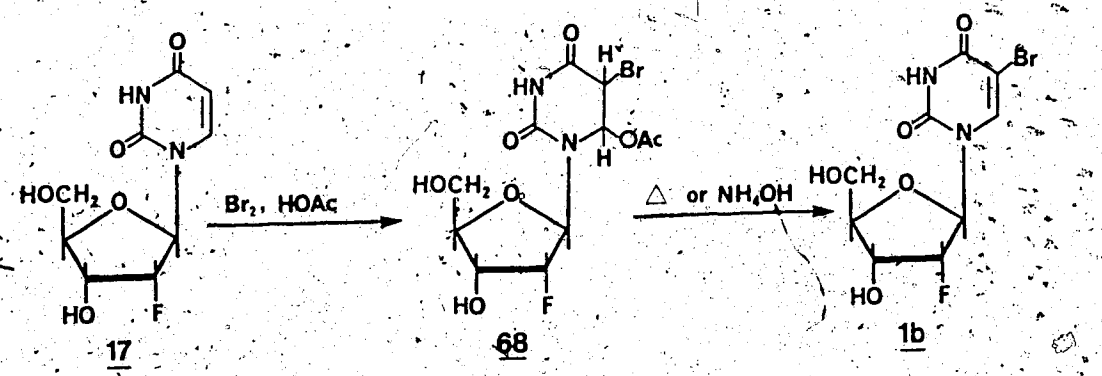
Unlike 5-iodo-2'-deoxyuridine (62a), 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) exhibited a remarkable *in vivo* stability. 5-Iodo-2'-deoxyuridine (62a) has been shown to be rapidly catabolized *via* phosphorolytic cleavage to iodouracil and deoxyribose catalyzed by the ubiquitous enzyme pyrimidine phosphorylase[315]. Iodine is rapidly lost from iodouracil after its enzymatic conversion to 5-iodo-5,6-dihydrouridine[316]. In addition thymidylate synthetase has been shown to catalyze the facile deiodination of 5-iodo-2'-deoxyuridylate[317]. These processes compete with uptake of 5-iodo-2'-deoxyuridine (62a) into DNA. In animal studies the half-life for uptake of 5-iodo-2'-deoxyuridine has been estimated at 5 minutes while the half-life of degradation has been measured at 3 minutes[15]. In the urine of rats injected with [^{131}I]-5-iodo-2'-deoxyuridine 99.9% of the radioactivity in the blood at 60 minutes was due to [^{131}I]-I$^-$ [14]. In contrast we observed that 5-iodo-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracil (1a) was very resistant to phosphorolytic cleavage and deiodination. Even after 24 hours unchanged (1a) comprised up to 37% of the activity present in the urine. The persistance of unchanged compound in the urine, despite the initial clearance half-life of 0.70 hours, was an indication that this material might have undergone reversible transport across the cellular membrane followed by a slow efflux from the cells back into the circulation.

The related compound 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-arabinofuranosyl)uracil (20e) has been shown to be a constituent of DNA and a major urinary metabolite in rats and mice dosed with 5-Iodo-1-(2'-fluoro-2'-deoxy-β-D-arabinofuranosyl)cytosine (63e)[318,319]. The uptake in DNA indicated that this compound must be a substrate for the kinases and polymerases present in cells. The observations of antiviral activity and cytotoxicity toward normal human lymphocytic cells in *in vitro* studies with 5-iodo-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracil (1a)[11] suggested that this compound must become involved in cellular metabolism although this supposition could not be confirmed in the present study.

B. 5-Bromo-1-(2'-Fluoro-2'-Deoxy-β-D-Ribofuranosyl)Uracil (1b)

Synthesis and purification.

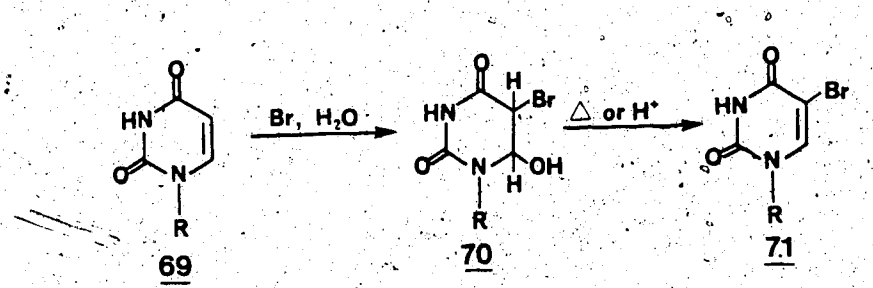
A variety of methods have been reported for the synthesis of 5-bromonucleosides as discussed previously. The classical reaction between electrophilic molecular bromine (Br_2) and an appropriate pyrimidine nucleoside was used for the first synthesis of 5-bromouridine[176] and 5-bromo-2'-deoxyuridine (62b)[177]. We employed this simple procedure for the synthesis of 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b) as shown in Scheme IV.4.



Scheme IV.4 Synthesis of 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b) *via* the stable intermediate 6-O-acetyl-5-bromo-5,6-dihydro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (68)

A solution of molecular bromine in glacial acetic acid reacted rapidly with 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) at 80 °C. When the product was isolated at this point by evaporation of the glacial acetic acid and excess bromine *in vacuo*, tlc analysis (15% MeOH in CH_2Cl_2, MK6F microslides) indicated complete consumption of starting material and formation of a complex reaction mixture containing a number of less polar materials in addition to the expected product (Rf .51). The major product was not visible when

the plates were viewed under a uv lamp but did become visible when the plates were sprayed with sulfuric acid and heated. It was also noted that heating the developed plates at 100 °C for 10 minutes caused the material at Rf .56 to become visible by the uv detection. If the heating was carried out after spotting the crude reaction mixture on the plate but prior to plate development; the product spot was much larger after development while the component at Rf .56 had disappeared. Treatment of the reaction mixture with NH_4OH also resulted in conversion of the mixture to a single major component at Rf .51. This observation suggested that the required bromonucleoside 1b arose *via* the stable intermediate 68 which upon heating or treatment with NH_4OH was converted to the required product as shown in Scheme IV.4. Stable intermediates (70) have been isolated when uracil (69, R = H) and N-1-substituted uracils (69, R = CH_3) were reacted with aqueous solutions of Br_2 as shown in Scheme IV.5[320,321].



Scheme IV.5 The reaction of N-1-substituted uracils with aqueous bromine solutions

These intermediates eliminated a molecule of water upon heating or treatment with acid to give 71. To insure formation of the required compound in our reactions, the crude reaction products were always treated with NH_4OH in MeOH until tlc indicated complete conversion to the required product.

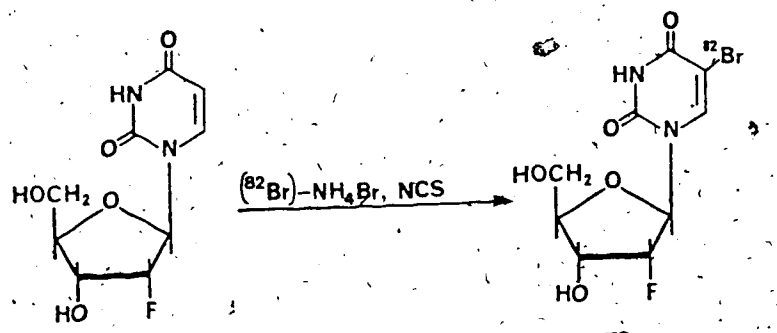
The crude reaction mixture was purified by preparative hplc but did not yield a crystalline product despite numerous crystallization attempts with different solvent systems. The clear gum or white foam obtained after evaporation of solvent was chromatographically pure (tlc, hplc) and showed no extra signals in the nmr spectrum.

Radiobromine (^{82}Br, $T_{\frac{1}{2}}$ = 35.3 hours) required for the synthesis of [^{82}Br]-5-bromo--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) was produced *via* the ^{81}Br(n,γ)^{82}Br nuclear reaction by neutron bombardment of 97.8% enriched [^{81}Br]-NH_4Br at a neutron flux of 1 x 10^{12} n cm^{-2} sec^{-1} for 4 hours. The calculated activity of ^{82}Br at end of bombardment of 5 mg of [^{81}Br]-NH_4Br was 13.2 MBq. The measured activities were very close to this calculated value. The irradiated samples were allowed to stand for 18 hours to permit decay of the co-produced and shorter-lived bromine isotopes (^{80}Br, $T_{\frac{1}{2}}$ = 17.6 minutes; ^{80}Br^{m}, $T_{\frac{1}{2}}$ = 4.38 hours; ^{82}Br^{m}, $T_{\frac{1}{2}}$ = 6.05 minutes)[320]. The bromine anion present in [^{82}Br]-NH_4Br was converted to electrophilic bromine by treatment with N-chlorosuccinimide. This procedure was adapted from a recent report on high-yield electrophilic radiobromination of aromatic rings using radiobromine anion with N-chlorosuccinimide as an oxidizing agent for *in situ* generation of electrophilic bromine[322]. The reactive species formed was probably [^{82}Br]-BrCl[322,323]. The reactions were carried out in Reacti-Vials® using a molar excess of N-chlorosuccinimide added through a teflon backed septum to a solution of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (17) and [^{82}Br]-NH_4Br in acetic acid. This procedure gave *in situ* formation of the reactive [^{82}Br]-BrCl species and provided high yields of the product (72) (Scheme IV.6).

The reaction proceeded readily at room temperature as indicated by the disappearance of the initial red-brown coloration ([^{82}Br]-BrCl) within a few minutes. Solutions were warmed at 40 °C for 1 hour to insure complete reaction. The crude reaction product contained >80% of the required product (72) (combined tlc-lsc). The recovered yields of 72 after ptlc were closer to 60% possibly indicating some sensitivity of the compound to the chromatographic system.

The specific activity of the produet was 167 MBq $mmol^{-1}$ at the end of the synthesis. The relatively short half-life of the ^{82}Br required that this material be used within 20 to 30

hours after the end of synthesis.



17 72

Scheme IV.6 Synthesis of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72)

Tissue distribution.

Within 30 hours after the completion of the synthesis of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) it was injected intravenously into female BDF$_1$ mice bearing a Lewis lung tumor. The distribution of activity was determined at selected time periods by sacrificing the animals and measuring the concentration of ^{82}Br-activity in the tissues. The injected doses ranged from 47 μg (0.14 μmol) to 130 μg (0.40 μmol) with activities of 20 kBq (15 minute tissue distribution) to 60 kBq (24 hour tissue distribution). The data are presented in Table IV.4 as percent dose per gram of tissue and organ to tumor ratios and in Figure IV.6 and IV.7 as organ to blood ratios.

[^{82}Br]-5-Bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) showed no significant uptake in any tissues. The compound was rapidly removed from the blood which contained only 3.7% of the injected dose at 15 minutes and 1.0% at 1 hour. This compound was rapidly and efficiently excreted *via* the urine. The kidneys did not accumulate significant levels of radioactivity. Other than the kidneys no tissues reached activity levels as high as that of the

ORGAN	TIME(HOURS) 0.25	0.50	1	2	4	8	24
BLOOD	2.68 (0.35)[1]	1.36 (.22)	0.78 (.06)	0.79 (.04)	0.60 (.07)	0.72 (.07)	0.51 (.06)
	1.92[2]	1.50	1.22	1.46	1.72	1.59	1.62
SPLEEN	1.06 (.17)	0.59 (.13)	0.38 (.03)	0.35 (.02)	0.28 (.03)	0.34 (.06)	0.24 (.03)
	0.76	0.65	0.58	0.65	0.79	0.76	0.75
STOMACH	1.29 (.29)	0.79 (.14)	0.50 (.03)	0.52 (.12)	0.40 (.02)	0.53 (.08)	0.34 (.03)
	0.93	0.86	0.76	0.94	1.14	1.17	1.09
GIT	1.19 (.29)	0.72 (.12)	0.42 (.02)	0.41 (.05)	0.28 (.03)	0.33 (.05)	0.22 (.02)
	0.86	0.80	0.64	0.76	0.79	0.73	0.69
KIDNEY	7.69 (.18)	2.68 (.42)	1.12 (.22)	0.71 (.02)	0.48 (.07)	0.53 (.06)	0.36 (.02)
	5.53	2.95	1.75	1.32	1.38	1.17	1.14
SKIN	1.39 (.20)	0.72 (.16)	0.45 (.11)	0.39 (.07)	0.31 (.05)	0.34 (.01)	0.22 (.04)
	1.00	0.80	0.71	0.74	0.88	0.77	0.69
MUSCLE	0.89 (.22)	0.40 (.03)	0.21 (.01)	0.19 (.04)	0.12 (.01)	0.14 (.01)	0.09 (.01)
	0.64	0.44	0.32	0.34	0.34	0.31	0.28
BONE	0.52 (.10)	0.30 (.06)	0.26 (.07)	0.26 (.05)	0.17 (.04)	0.23 (.02)	0.14 (.03)
	0.37	0.32	0.39	0.46	0.47	0.51	0.47
LUNG	1.70 (.17)	0.91 (.21)	0.52 (.08)	0.54 (.03)	0.38 (.03)	0.46 (.06)	0.35 (.04)
	1.22	1.00	0.81	1.00	1.08	1.02	1.10
HEART	1.61 (.21)	0.69 (.12)	0.29 (.02)	0.27 (.05)	0.17 (.01)	0.20 (.02)	0.15 (.01)
	1.16	0.76	0.44	0.48	0.49	0.45	0.48
LIVER	1.45 (.22)	0.65 (.14)	0.34 (.02)	0.32 (.04)	0.23 (.01)	0.28 (.02)	0.18 (.01)
	1.04	0.72	0.53	0.58	0.64	0.61	0.57
TUMOR	1.39 (.17)	0.95 (.19)	0.67 (.11)	0.57 (.16)	0.37 (.07)	0.46 (.06)	0.32 (.05)
	0.53[3]	0.71	0.86	0.73	0.63	0.65	0.63

[1] Percent of injected dose per gram of tissue (± standard deviation), (n = 6 animals)
[2] Organ to tumor ratio
[3] Tumor to blood ratios

Table IV.4 Tissue distribition in female BDF_1 mice bearing a Lewis lung tumor after intravenous injection of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) (n = 6 animals)

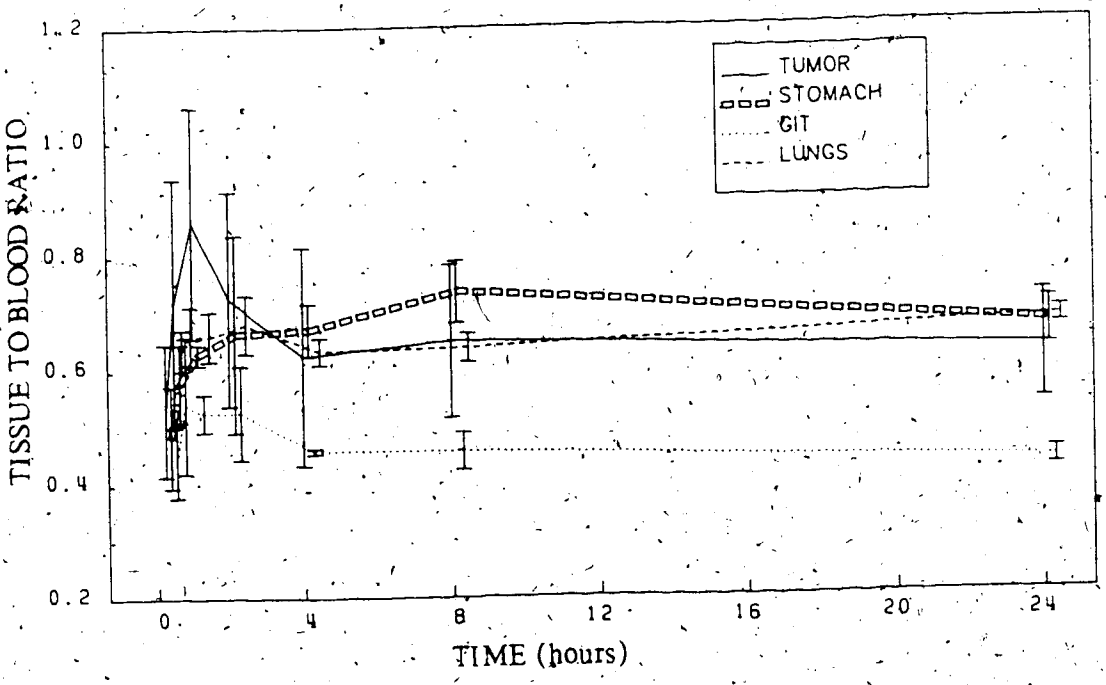


Figure IV.6 Organ to blood ratios for tumor, stomach, intestine and lung after intravenous injection of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) into female BDF$_1$ mice bearing a Lewis lung tumor

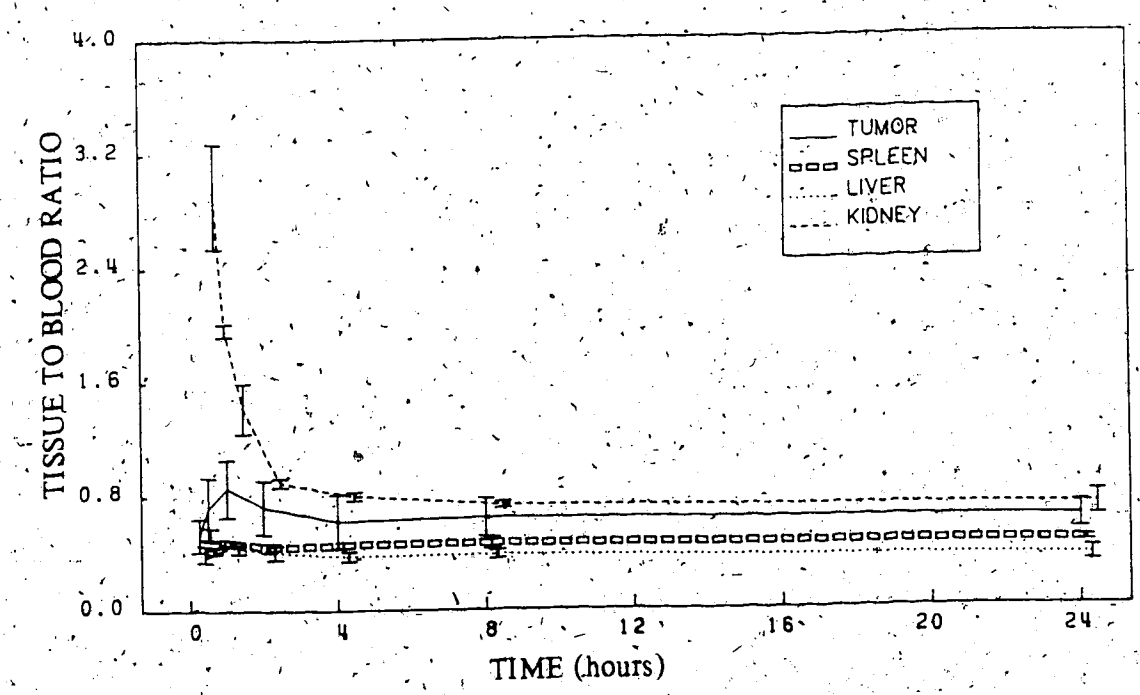


Figure IV.7 Organ to blood ratios for tumor, spleen, liver and kidney after intravenous injection of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) into female BDF$_1$ mice bearing a Lewis lung tumor

blood. The organ to blood ratios (Figure IV.6 and Figure IV.7) showed the highest concentrations of activity in the lungs, stomach and tumor. The lung activity was in part due to the high blood content of this tissue. The high concentration of activity in the stomach, lungs, blood and kidney at later time periods suggested that the radioactivity distribution was due to $^{82}Br^-$. This agrees with previous studies which describe the distribution of bromide anion in mice which reported stomach contents, blood, lungs, kidney and skin as the organs with the highest levels of radioactivity[324,325]. The radioactivity level in tumor suggested some selective uptake of radioactivity in this tissue. It is unlikely that this radioactivity was due to bromide since, except for the tissues noted above, bromide was restricted to extracellular distribution[314]. The vascular nature of the tumor tissue and hence its blood content may account for some of the noted radioactivity. This should not, however, be significant particularly since the animals were exsanguinated at the time of sacrifice. The tumor appeared to demonstrate a small selective uptake of activity as [^{82}Br]-5-bromo-1-(2'-fluoro-2''-deoxy-β-D-ribofuranosyl)-uracil (72) or one of its metabolites.

Whole body elimination and metabolism.

After removal of the required tissues for γ-ray counting the remaining carcass was divided between two counting vials and also counted. By summing the total activity in all the tissues and the carcass a value for whole body activity was obtained. These data are presented in Table IV.5 along with blood clearance data expressed as percent of dose in total blood. This latter value was determined by measuring the activity in a blood aliquot collected at the time of sacrifice and extrapolating this value to the total volume assuming blood to comprise 6.5% of the total body weight. Figure IV.8 shows the whole body activity plotted against the time after injection. The data conforms to a two component system (bi-exponential) with the half-life of the short lived component equal to 0.26 hours and the half-life of the long lived component equal to 33.5 hours. This data indicated a very rapid excretion of injected compound. Within 15 minutes 96.3% of the activity was cleared from the blood while the whole body activity reached

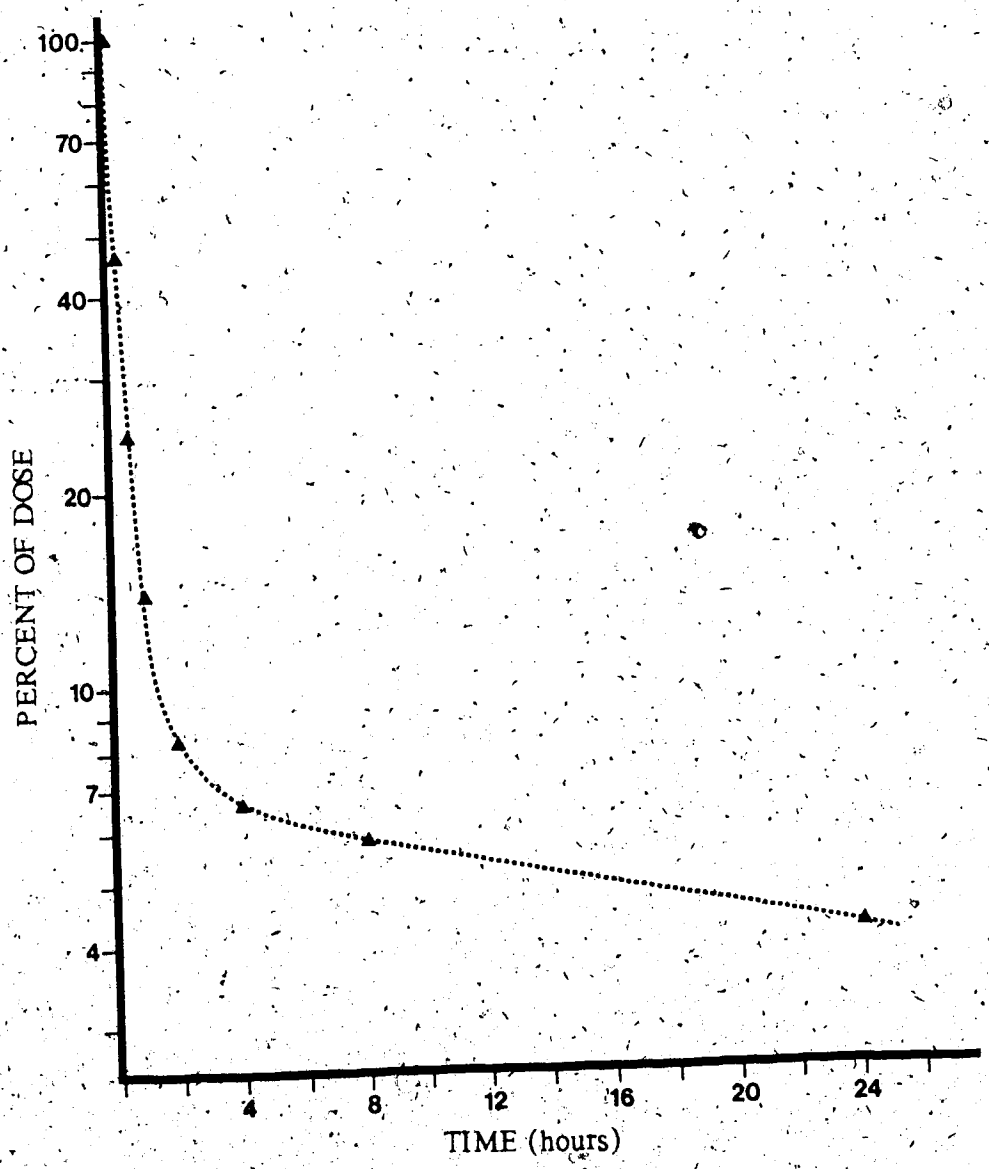


Figure IV.8 Plot of percent of dose in whole body vs time after intravenous administration of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) to female BDF$_1$ mice bearing a Lewis lung tumor

25% of injected dose after 30 minutes. The initial rapid excretion was probably due to efficient removal of unchanged bromonucleoside from the blood *via* the kidneys and bladder. Unchanged compound was the only species detected in the urine as described later. After this rapid excretion the radioactivity levels remaining in the mice dropped very slowly. This long biological half-life for the radioactivity was a further indication that the activity remaining in the blood and tissues at longer time periods was [^{82}Br]-Br$^-$.

TIME (hours)	% OF DOSE REMAINING	% OF DOSE IN BLOOD
0	100	100
.25	46.3	3.7
.5	24.4	1.8
1	13.9	1.0
2	11.2	1.0
4	6.6	0.8
8	5.8	0.9
24	4.2	0.7

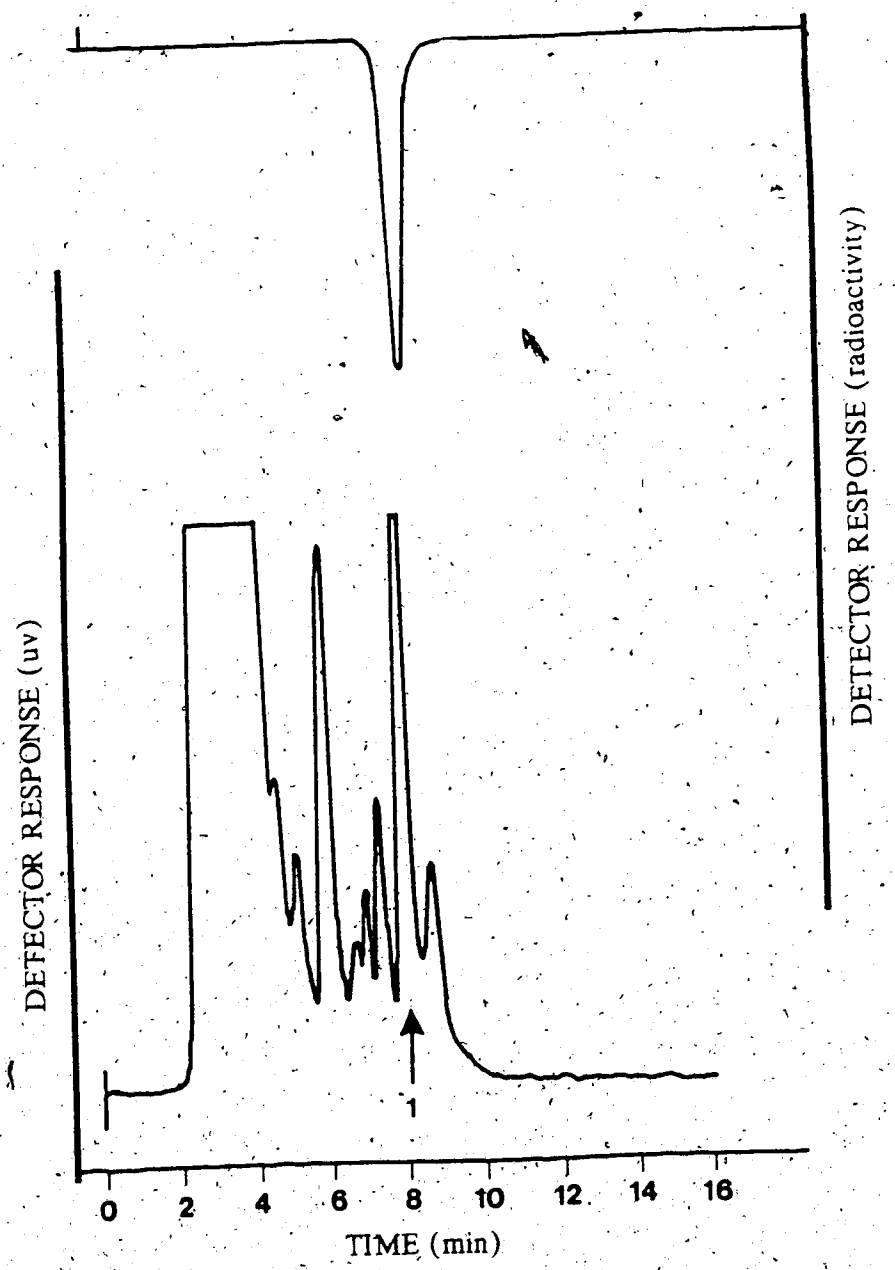
Table IV.5 Percent of dose in whole body and in blood after intravenous administration of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofurano-syl)uracil (72) to female BDF$_1$ mice containing a Lewis lung tumor

Samples of urine from the animals used for the tissue distribution study with [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72) were collected at the time of sacrifice and stored at -5 °C until analysis. The urine samples were allowed to warm to room temperature. These samples were then mixed with portions of hplc solvent, were spiked with non-radioactive reference 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b) and were analyzed directly using the Waters hplc system and the sodium iodide detector described previously. The mobile phase was 96% water and 4% MeOH. An energy window of from 200 KeV to 1.0 MeV was used. Figure IV.9 shows the results of a typical analysis carried

out on 2 hour urine. The only radioactive material detected was unchanged [^{82}Br]-5-bromo--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72). The retention times of the cold reference material and the urine activity corresponded exactly once a small correction was made to compensate for the delay between the flow of column effluent through the uv and radioactivity detectors. Analysis of urine samples collected from 15 minutes to 4 hours after injection of the radiobrominated nucleoside (72) showed only unchanged starting compound. Urine collected from animals sacrificed at 8 and 24 hours after injection of the radiopharmaceutical contained levels of radioactivity below the limit of detection. The urine samples were expected to contain [^{82}Br]-Br^- since this appeared to be the species present in mice at the longer time periods. However the low overall concentration of this species in the mice and its known long biological half life[326] combined to give concentrations in the urine below our limit of detection.

Biological fate of injected [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72)

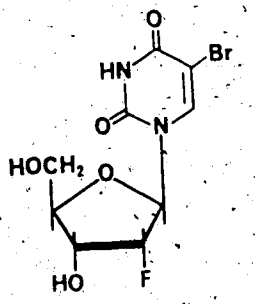
Intravenous injection of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (72) into female BDF_1 mice bearing a Lewis lung tumor was followed by very rapid excretion of unchanged compound into the urine. Unmetabolized [^{82}Br]-5-bromo-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (72) was detected at times up to 4 hours in the urine. It should be noted that the urinary bladder acts as a storage depot for urine radioactivity excreted by the kidneys and that radioactivity measured in urine collected at 4 hours may have been due to compound excreted much earlier. The activity remaining in mice after 4 hours appeared to be almost exclusively ^{82}Br-bromide. The highest levels of activity were noted in the kidney, blood, stomach and lungs in agreement with the known distribution of bromide anion in mice[324]. The radioactivity remaining at 4 hours was also excreted very slowly. If one assumes the long-lived component measured in the whole body excretion was due to Br^- then the measured half-life for this component of 33.5 hours was in good agreement with previous studies which showed half-lives of 36 hours[326] and 30 hours[325].



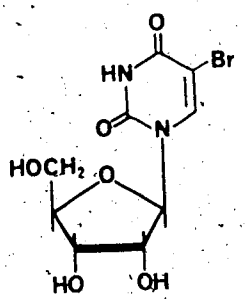
The reference compound (1) is; 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b).
The hplc conditions are; flow: 1 mL / min, solvent: 4% methanol / 96% water, column: C-18 reverse phase radial compression (5 μ), detection: uv at 256 nm and radioactivity as γ-radiation between 170 and 1000 Kev.

Figure IV.9 Hplc analysis by uv and radioactivity detection for the 2 hour urine sample collected from female BDF_1 mice after intravenous injection of [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72)

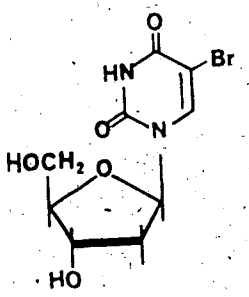
The related 5-bromopyrimidine nucleosides, 5-bromouridine (73) and 5-bromo-2'-deoxyuridine (62b) have been shown to be cytotoxic to bacterial and mammalian cells and exhibit *in vivo* antiviral activity[13]. The accepted mode of cytotoxicity of these compounds is *via* incorporation into the DNA of replicating cells in a manner analogous to the 5-iodonucleosides discussed previously. 5-Bromo-2'-deoxyuridine (62b) is incorporated very efficiently into the DNA of human and animal cells showing a level of replacement for thymidine of up to 43%[327]. In contrast compound (1b) prepared in this study showed no evidence of tissue uptake to the degree noted for 5-bromo-2'-deoxyuridine. Compound 1b demonstrated considerable *in vivo* stability since no radioactive species other than starting compound and a small level of bromide anion were detected.



1b



73



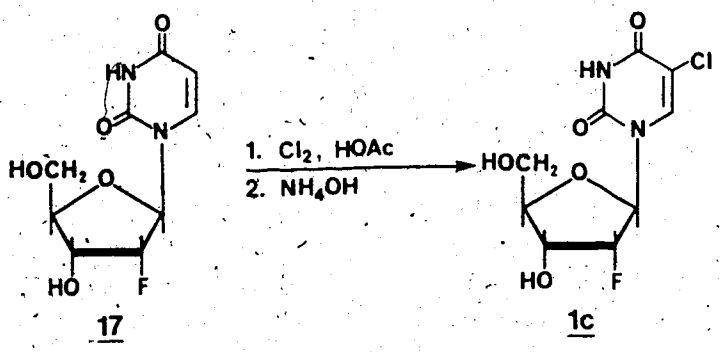
62b

An *in vitro* study with the 5-bromonucleoside (1b) showed it to exhibit anti-viral activity and growth inhibition toward normal human lymphocytic cells[18]. This indicated transport of the compound across the cell membrane and some involvement in the normal cellular biochemistry. This process was expected to be active in our *in vivo* system and this might account for the slightly elevated levels of activity noted in the tumor tissues. The *in vitro* studies involved long term exposure of cells to a constant concentration of substrate (0.1 μM for cytotoxicity toward *Herpes simplex* type 1 infected Vero cell monolayers and 0.9 μM for growth inhibition of human lymphocytic cells)[18]. In our *in vivo* experiments injections of up to 0.40 μmol of compound were used but the rapid excretion and *in vivo* debromination probably competed very effectively with any transport or biochemical involvement that might occur.

C. 5-Chloro-1-(2'-Fluoro-2'-Deoxy-β-D-Ribofuranosyl)Uracil (1c)

Synthesis and purification.

The non-radioactive reference compound 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c) was synthesised by the reaction of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) with a solution of Cl_2 in acetic acid as shown in Scheme IV.7. The required 5-chloronucleoside (1c) was obtained in 52% yield after purification by column chromatography. The colorless gum obtained after evaporation of the solvent resisted all attempts at crystallization but was shown to be a single compound (hplc, tlc, nmr and hrms).



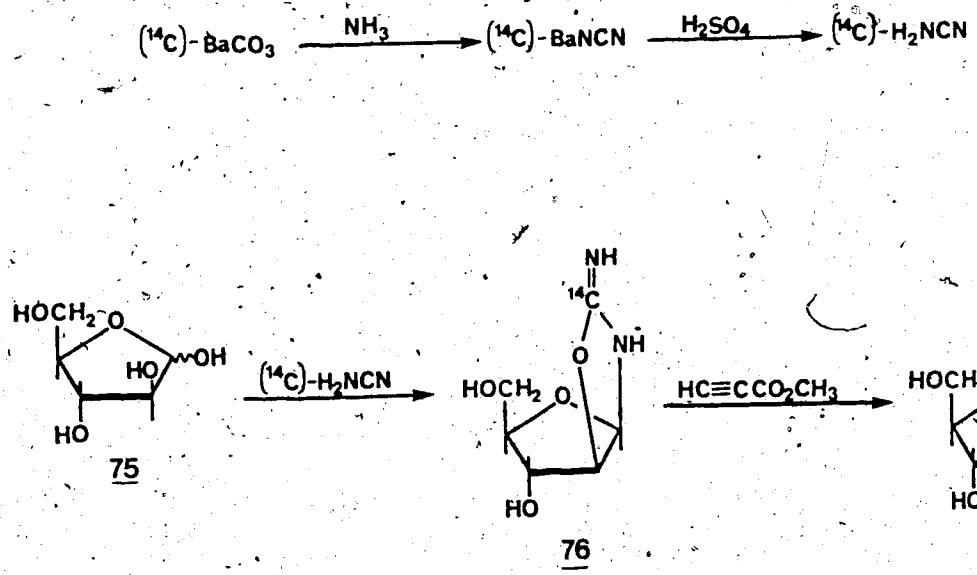
17 1c

Scheme IV.7 Synthesis of 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c)

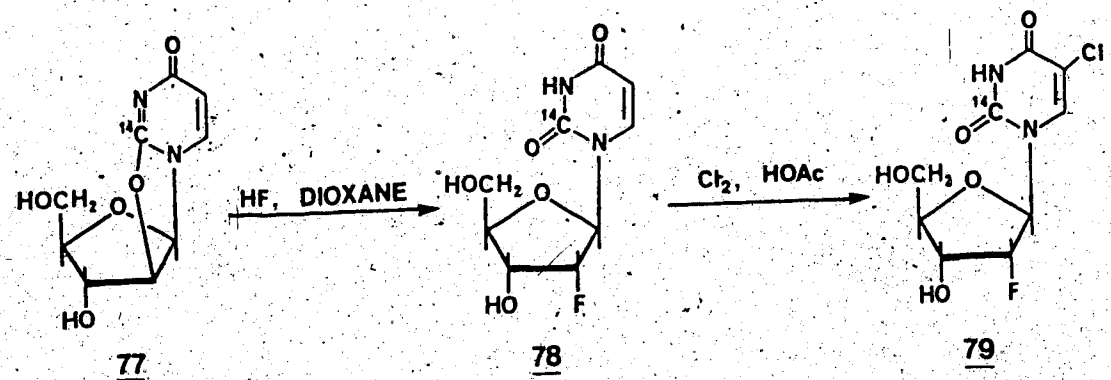
We were not able to adopt the direct radiohalogen labelling approach, which had proved successful for the synthesis of the [^{131}I]- and [^{82}Br]-labelled pyrimidine nucleosides 67 and 72 discussed previously, for the synthesis of the radiolabelled analog of 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c). The available radioisotopes of chlorine were either unsuitable for the synthetic or the biological requirements. The isotope ^{36}Cl was commercially

available but its low specific activity combined with its decay by low energy β^- emission made it unacceptable for biological studies. The cyclotron produced radionuclide $^{34}Cl^m$ is the most desirable isotope of chlorine for *in vivo* applications due to its relatively short half-life ($T\frac{1}{2}$ = 32 minutes) and its decay by positron emission (β^+) after isomeric transition to ^{34}Cl ($T\frac{1}{2}$ = 1.5 seconds)[20]. Production of this isotope however requires irradiation of suitable target materials with high energy particles generated by a cyclotron or accelerator[321-331] and its use is restricted to laboratories which have these facilities available. In addition the short half-life made it unsuitable for the present study in which long-term tissue distribution studies were undertaken. Some [^{38}Cl]-labelled pyrimidines have been prepared by the Szilard-Chalmers reaction using a nuclear reactor employing the nuclear reaction $^{37}Cl(n,\gamma)^{38}Cl$ [172] but this chlorine isotope has a short half-life ($T\frac{1}{2}$ = 37.2 minutes) and is a hard β^- emitter.

For our studies we decided to synthesise a ^{14}C-labelled analog of the 5-chloronucleoside (1c). A number of ^{14}C-labelled nucleosides suitable as precursors for the synthesis of the r[illegible]-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil are commercially available b[illegible] when larger quantities are required for animal studies. The cost of the co[illegible]ucts prompted us to synthesise the required ^{14}C-labelled nucleosides *via* the vers[illegible]ediate [2-^{14}C]-2,2'-anhydro-1-β-D-arabinofuranosyluracil (77) which was synth[illegible]as outlined in Scheme IV:8. This intermediate (77) has also been used for the synthe[illegible] [2-^{14}C]-labelled 5-ethynyl- and 5-ethyl-2'-deoxyuridine[332]. The commercial ^{14}C source[illegible] [^{14}C]-$BaCO_3$ having a reported specific activity of 1.92 GBq mmol^{-1}. [^{14}C]-$BaCO_3$ was c[illegible]erted to [^{14}C]-cyanamide (74) by reaction with NH_3 at high temperature followed by treatm[illegible] with sulfuric acid[310]. The intermediate [^{14}C]-2-amino-β-D-arabinofurano-[illegible]-[1',2'-[illegible]]-2-[illegible]xazoline (76) was readily prepared by reacting [^{14}C]-cyanamide (74) with D-arabinose (75) in 1 M NH_4OH solution. The oxazoline (76) was then reacted with a solution of methyl propiolate in tetrahydrofuran to give the required [^{14}C]-labelled 2,2'-anhydro-nucleoside (77) in 24,6% overall yield from [^{14}C]-$BaCO_3$ with a measured specific activity of 1.86 GBq mmol^{-1}.



Scheme IV.8 Synthesis of [2-^{14}C]-2,2'-anhydro-1-β-D-arabinofuranosyluracil (77) from [^{14}C]-$BaCO_3$



Scheme IV.9 Synthesis of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) from [2-^{14}C]-2,2'-anhydro-1-β-D-arabinofuranosyluracil (77)

[2-^{14}C]-5-Chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79), the compound required for the tissue distribution studies, was prepared in two steps from [2-^{14}C]--2,2'-anhydro-1-β-D-arabinofuranosyluracil (77) as shown in Scheme IV.9.

The ^{14}C-labelled 2'-fluoro compound (78) was synthesised using a procedure analogous to that used for the preparation of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17). The stainless steel cylinder used for the large scale reaction employing non-radioactive materials was replaced by a 5 mL teflon vial sealed with a teflon backed septum cap for the radioactive synthesis. The crude product appeared to contain both unreacted starting material (77) and [2-^{14}C]-1-β-D-arabinofuranosyluracil ([2-^{14}C]-65) in addition to the expected product 78 as indicated by hplc comparison with authentic cold reference compounds (see Scheme IV.2). The crude reaction product was purified by preparative hplc to yield [2-^{14}C]-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (78) in 25.4% radiochemical yield with a radiochemical purity of 98%.

Treatment of 78 with excess Cl_2 in HOAc followed by hplc purification of the crude reaction product gave [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) in 77% radiochemical yield with a radiochemical purity of 99% and a measured activity of 8.03 MBq. The specific activity of this product was 1.86 GBq / mmol. This ^{14}C-labelled product was divided between three multidose vials as a solution in methanol, evaporated to dryness and stored at -5 °C until required for the tissue distribution studies. The compound showed excellent chemical stability under these storage conditions. Samples were reconstituted with sterile normal saline just prior to use, stored frozen between animal studies and analyzed by hplc at the end of the animal studies to confirm the chemical integrity of the radiolabelled nucleoside.

Tissue distribution.

The female BDF_1 mice bearing a Lewis lung tumor used in this experiment were injected intravenously *via* the tail vein with [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) as a solution in normal saline. The quantity of ^{14}C-labelled nucleoside

injected varied from 22.6 kBq (.012 μmol, 3.37 μg) to 193 kBq (.103 μmol, 28.9 μg). After sacrifice tissue aliquots were weighed directly in paper combustion cups, the tissues were air dried under a heat lamp and then combusted using a Harvey Biological Oxidizer. The overall counting efficiency, which is the product of liquid scintillation counting efficiency and trapping efficiency of the biological oxidizer, was determined by combusting and counting of known ^{14}C-standards. The overall efficiency determined in this manner was 76.0% (±2.1%). Counting efficiency alone was determined by recounting the standards after addition of a known activity of [^{14}C]-n-hexadecane (Amersham, Reference Standard) The counting efficiency was 79.8% (±1.4%) indicating a trapping efficiency of 95.2% for the oxidizer. The tissue distribution of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) as determined by this experiment is presented in Table IV.6 as percent dose per gram of tissue and organ to tumor ratios and in Figure IV.10 and Figure IV.11 as tissue to blood ratios.

[2-^{14}C]-5-Chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) was rapidly removed from the blood after intravenous injection as demonstrated by blood levels of 3.54% per gram of tissue at 15 minutes and 0.58% at 1 hour. The compound appeared to be efficiently removed from the blood by the kidneys and was excreted *via* the urine. The kidneys showed a high concentration of activity during the first several hours and unchanged [2-^{14}C]-5-chloro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) was the major radioactive component in the urine as described later. No tissues demonstrated significant concentrations of activity and by 1 hour only the kidney had greater than 1% of the injected dose per gram of tissue. The activity level in all tissues continued to decline for the duration of the measurement period.

A number of tissues studied in this experiment demonstrated selective uptake of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79). Figures IV.10 and IV.11 indicated selective retention of activity in the spleen, tumor, stomach and intestine relative to the blood. The tumor to blood ratio was of particular interest since it reached a maximum value of 4.2 at 4 hours falling to 2.3 at 24 hours. The highest concentration of activity from about 6 hours onward was present in the spleen. The spleen displayed a maximum

	TIME(HOURS)						
ORGAN	0.25	0.50	1	2	4	8	24
BLOOD	3.54 (0.3)[1] 1.4[2]	1.82 (.07) 1.0	0.58 (.01) 1.0	0.18 (.02) 0.5	0.06 (.002) 0.2	0.03 (.002) 0.4	0.01 (.001) 0.4
SPLEEN	2.12 (.02) .83	1.08 (.02) .60	0.48 (.06) .83	0.34 (.03) .99	0.17 (.03) .74	0.15 (.02) 2.1	0.06 (.01) 2.0
STOMACH	1.41 (.14) .55	0.75 (.11) .41	0.27 (.02) .47	0.31 (.04) .90	0.07 (.03) .29	0.05 (.005) .69	0.01 (.003) .49
GIT	1.91 (.15) .75	1.02 (.18) .57	0.38 (.04) .67	0.17 (.02) .51	0.06 (.01) .26	0.04 (.006) .58	0.02 (.004) .72
KIDNEY	36.2 (2.2) 14.3	23.2 (1.7) 12.9	7.28 (.58) 12.9	1.60 (.14) 4.7	0.33 (.03) 1.5	0.09 (.02) 1.2	0.03 (.004) 1.2
SKIN	2.24 (.71) .85	1.24 (.31) .69	0.36 (.13) .63	0.13 (.07) .37	0.04 (.003) .15	0.02 (.008) .33	0.01 (.002) .32
MUSCLE	1.48 (.04) .58	0.78 (.08) .43	0.27 (.04) .49	0.15 (.02) .44	0.05 (.01) .19	0.03 (.001) .38	0.01 (.002) .42
BONE	0.77 (.11) .31	0.41 (.06) .23	0.17 (.03) .31	0.08 (.05) .25	0.04 (.02) .15	0.02 (.002) .30	0.01 (.002) .51
LUNG	2.73 (.26) 1.1	1.41 (.14) .78	0.43 (.07) .73	0.14 (.01) .42	0.06 (.008) .25	0.03 (.004) .46	0.01 (.003) .50
HEART	3.07 (.35) 1.2	1.56 (.16) .86	0.53 (.02) .94	0.17 (.02) .51	0.06 (.01) .26	0.03 (.004) .46	0.02 (.003) .54
LIVER	2.39 (.19) .93	1.43 (.17) .80	0.47 (.09) .86	0.15 (.02) .44	0.05 (.01) .23	0.03 (.005) .45	0.02 (.003) .54
TUMOR	2.62 (0.5) .74[3]	1.82 (0.2) 1.0	0.60 (.12) 1.0	0.34 (.04) 2.0	0.23 (.03) 4.2	0.07 (.01) 2.5	0.03 (.004) 2.3

[1] Percent of injected dose per gram of wet tissue ± standard deviation
[2] Organ to tumor ratios
[3] Tumor to blood ratios

Table IV.6 Tissue distribution in female BDF_1 mice after intravenous injection of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) (n = 6 animals)

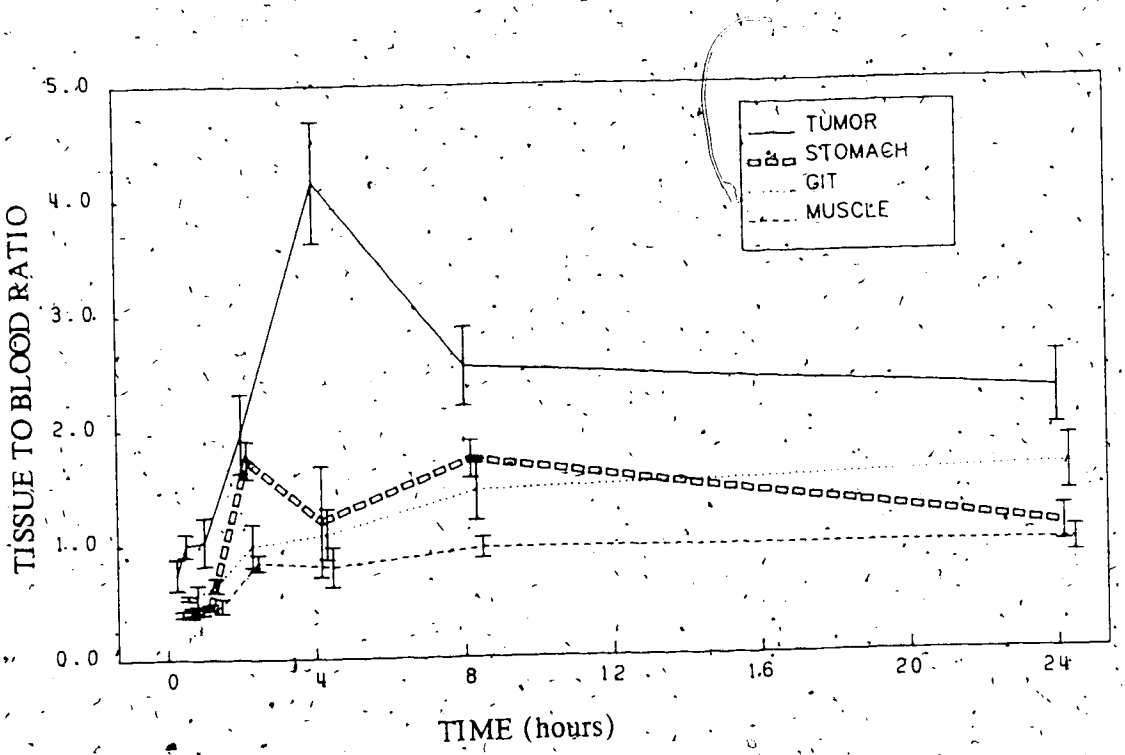


Figure IV.10 Organ to blood ratios for tumor, stomach, intestine and muscle after intravenous administration of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) into female BDF$_1$ mice bearing a Lewis lung tumor

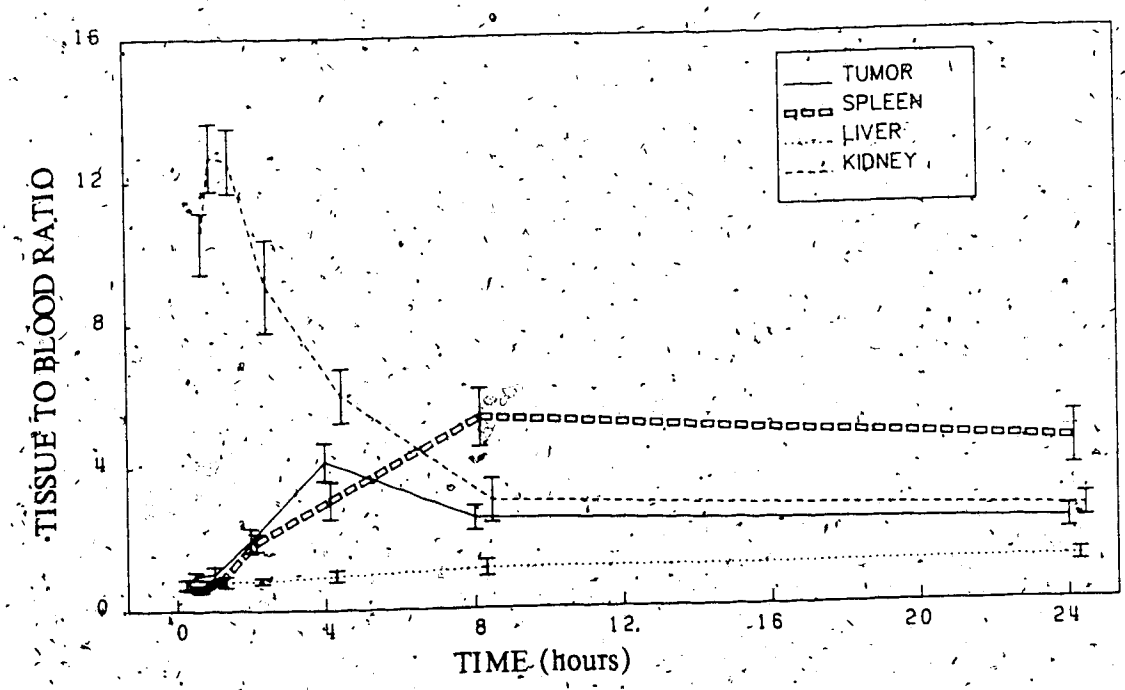


Figure IV.11 Organ to blood ratios for tumor, spleen, liver and kidney after intravenous administration of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) into female BDF$_1$ mice bearing a Lewis lung tumor

organ to blood ratio of 5.3 at 8 hours decreasing to 4.5 at 24 hours. The stomach and the intestine both contained higher concentrations of activity than the blood, reaching maximum organ to blood ratios of 1.7 at 8 hours and 1.6 at 24 hours respectively.

The organs involved in selective uptake of activity gave some indication of the mechanism by which this selective uptake occured. The stomach and intestine contain rapidly growing tissue, the spleen in mice contains active hematopoietic tissue and the Lewis lung tumor is a rapidly proliferating structure. The 5-chloronucleoside (79) appeared to be taken up into rapidly growing or metabolically active tissue *via* initial transport across the cell membrane and subsequent trapping of the compound by its involvement in the cellular metabolism.

Blood clearance and metabolism.

There were a number of disadvantages associated with the use of ^{14}C-labelled compounds for the animal studies. In order to measure tissue concentrations of activity extra manipulation of the tissue samples were required (aliquot separation, drying, combustion). In addition there was no practical method whereby the whole body activity could be measured. We were therefore restricted to qualitative descriptions for the rate of whole body elimination of the injected ^{14}C-labelled nucleosides. A second indicator of the elimination rate was obtained by plotting the blood clearance curves for these ^{14}C-labelled compound based on data collected during the tissue distribution study. The blood clearance data are shown in Table IV.7 and in Figure IV.12.

The blood clearance was extremely rapid. The intravenous injection placed 100% of the dose in the blood at the start of the experiment ($t = 0$). At 15 minutes after injection less than 5% of the dose remained in blood. The activity in the blood continued to drop very rapidly levelling off to a slower elimination rate after 4 hours at which time there was only 0.02% of the dose in the blood. None of the tissues examined except for the kidney showed significant levels of activity. These results indicated that the whole body activity drops very rapidly reaching a concentration of $<.05\%$ per gram of tissue (1% of the injected dose in a 20 g

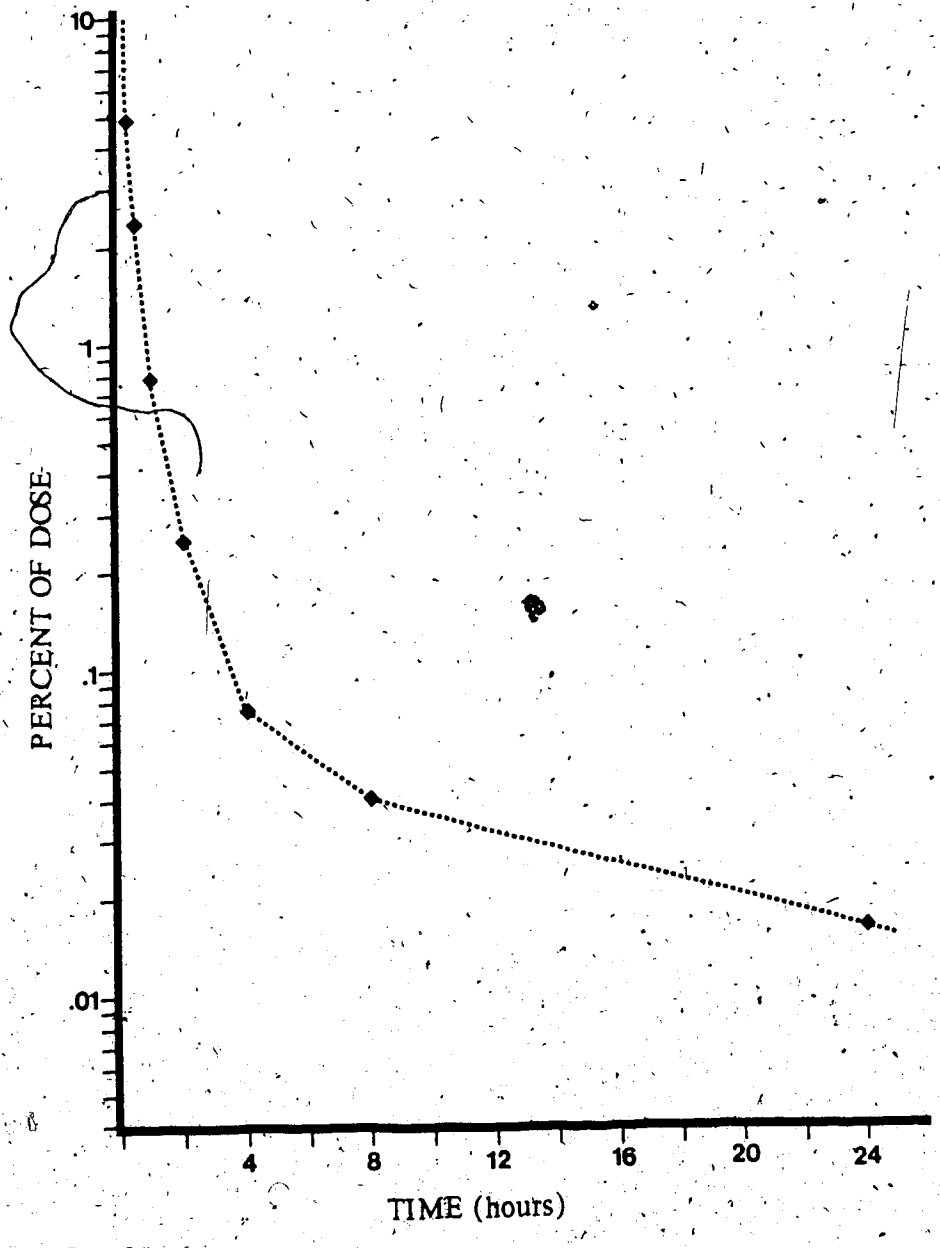


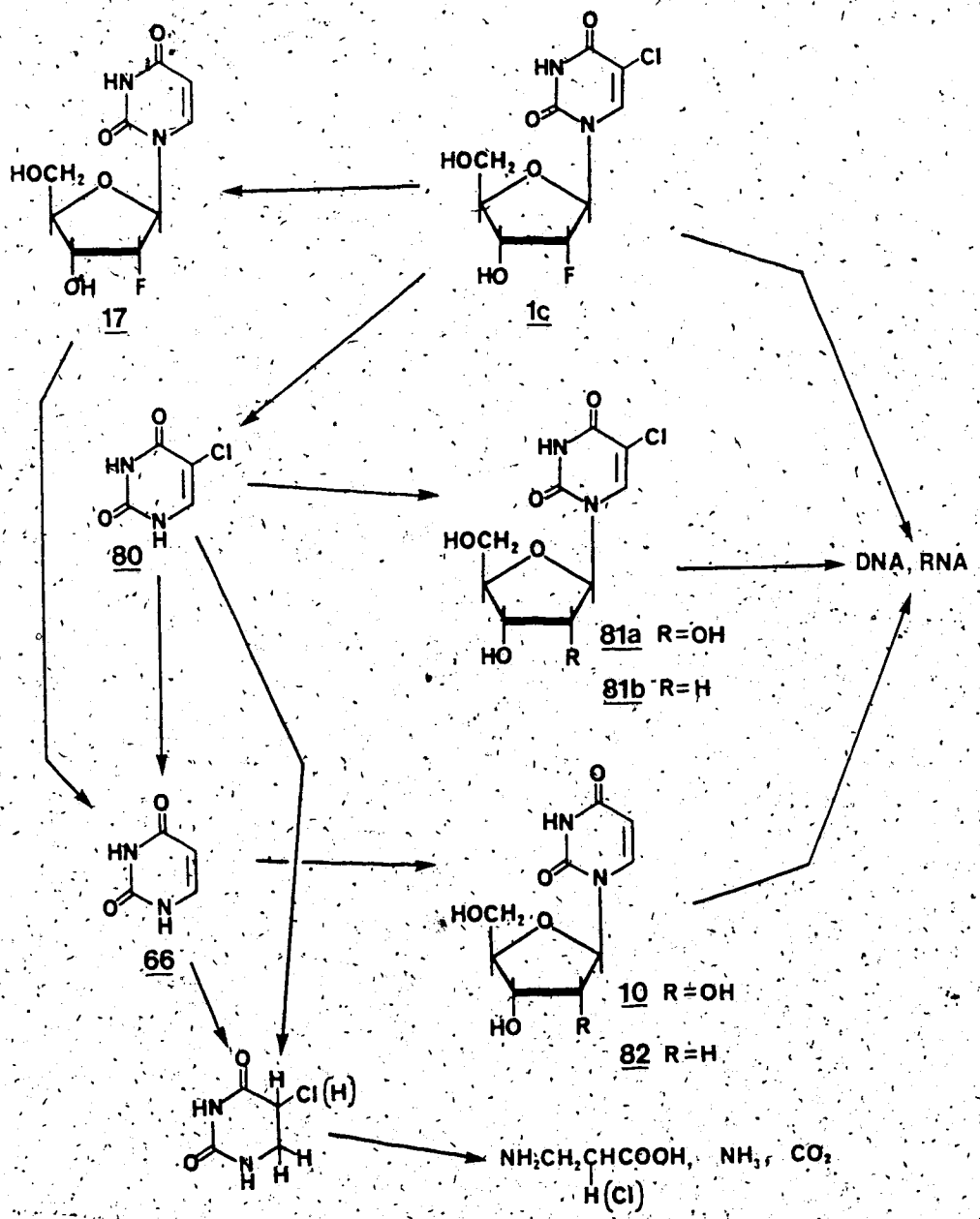
Figure IV.12 Plot of percent dose in blood vs time after intravenous injection of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) into female BDF$_1$ mice bearing a Lewis lung tumor

mouse) between 4 and 8 hours after injection. This estimate is exclusive of the urinary bladder contents.

TIME (hours)	% DOSE IN BLOOD
0	100
.25	4.80
.5	2.39
1	0.78
2	0.244
4	0.075
8	0.040
24	0.016

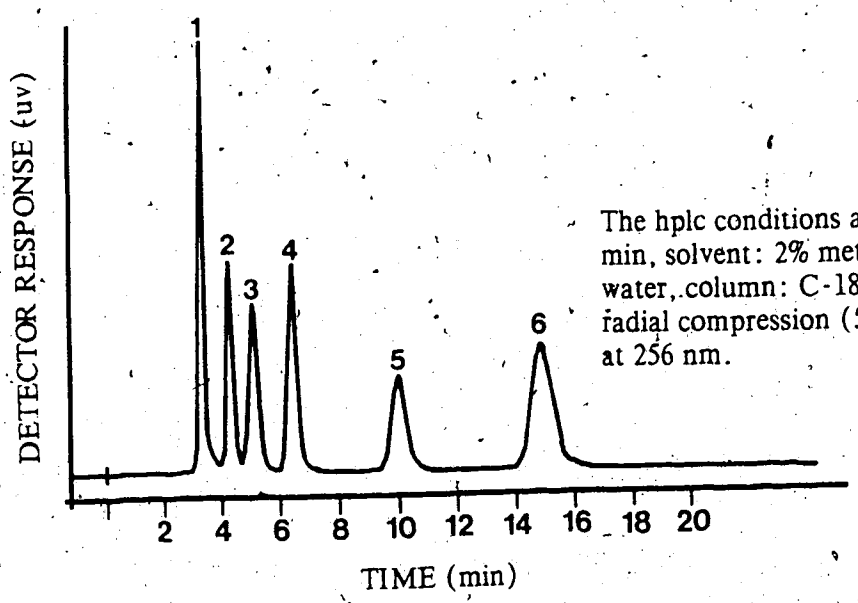
Table IV.7 Percent of dose in blood after intravenous administration of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) into female BDF$_1$ mice bearing a Lewis lung tumor

The identity of the radioactive compounds present in the urine was determined by hplc using the following procedure. A mixture of reference compounds was prepared and separated by hplc as shown in Figure IV.13. The reference compounds represented compounds which could potentially arise by simple metabolic or degradative biochemical steps from the starting 5-chloronucleoside (1c) as outlined in Scheme IV.10. Dehalogenation of 1c would produce 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) which could be converted *via* phosphorolysis to uracil (66) and hence to uridine (10) or 2'-deoxyuridine (82) by re-utilization of the free base. Dehalogenation is not usually observed with 5-halopyrimidine nucleosides. A more common catabolic route is via phosphorolysis, as reported for related nucleosides such as 5-chloro-2'-deoxyuridine (81d) [292], followed by dehalogenation of the resulting 5-halopyrimidine[316]. Dehalogenation of 5-halopyrimidine nucleosides has also been shown to take place *via* the monophosphate nucleotide catalyzed by thymidylate synthetase[317]. Phosphorolytic cleavage of 1c would give 5-chlorouracil (80) which could be converted to



Scheme IV.10 Possible primary *in vivo* metabolism of 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79)

5-chlorouridine (81a) or 5-chloro-2'-deoxyuridine (81b). Dehalogenation of 80 could lead to uridine (10) or deoxyuridine (82) via uracil (66).



The hplc conditions are; flow: 1 mL / min, solvent: 2% methanol / 98% water, column: C-18 reverse phase radial compression (5 μ), detection: uv at 256 nm.

The compounds are; (1)uracil (66) (3.19 min), (2)5-chlorouracil (80) (4.14 min), (3)2'-deoxyuridine (82) (4.97 min), (4)1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (6.33 min), (5)5-chloro-2'-deoxyuridine (81b) (9.87 min), (6)5-chloro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (1c) (14.76 min).

Figure IV.13 Retention times of nucleosides and bases used as internal reference compounds for the hplc analysis of the urinary metabolites of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) in female BDF$_1$ mice bearing a Lewis lung tumor

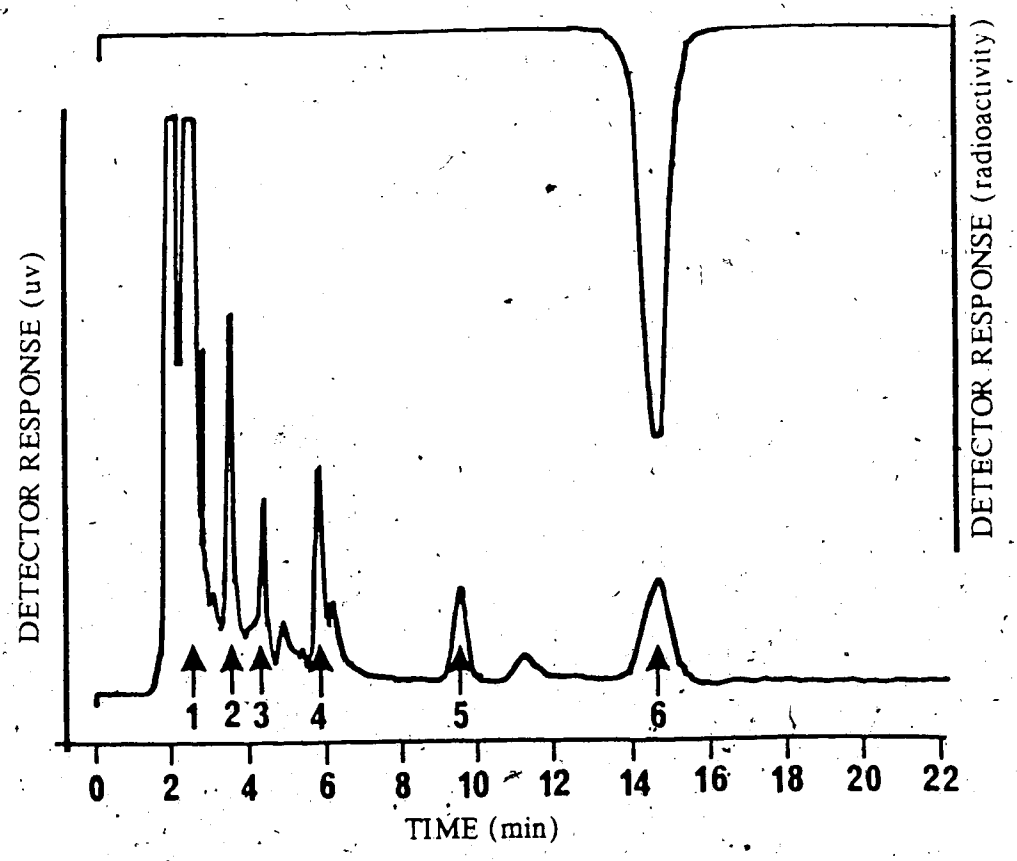
Urine was collected from the female BDF$_1$ mice at time of sacrifice during the tissue distribution study and stored frozen. The hplc analysis was performed by mixing 5 to 10 μL of urine with several microliters of the mixture of reference compounds described previously (Figure IV.13). This solution was injected directly onto a C-18 reverse phase radial compression column (Waters). The column effluent was monitored by uv and then collected as fractions (0.5 mL, 30 second intervals) in liquid scintillation vials. These fractions were mixed with Aquasol® fluor and counted by liquid scintillation. The counting results were analyzed

using a Digital Integration Program and this analysis was compared with the position of the reference compounds as determined by the uv analysis. The result of a typical analysis, in this case with 2 hour urine, is shown in Figure IV.14. This analysis method could not be used for the analysis of urine collected from the BDF_1 mice at 8 and 24 hours after injection due to the very low levels of activity in these samples.

The chromatogram (Figure IV.14) indicated that the major radioactive compound in the urine after intravenous injection of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) into female BDF_1 mice bearing a Lewis lung tumor was unchanged 5-chloro nucleoside (79). This was true for all samples of urine examined from animals sacrificed at times from 15 minutes to 4 hours. Quantitation of radioactivity by liquid scintillation showed that the unchanged 5-chloronucleoside (79) was responsible for >95% of the activity in the urine at all time periods. The 4 hour urine contained in addition to the unchanged nucleoside (95.6%) two other radioactive components comprising 2.0% and 2.3% of the overall activity and having the same retention time as uracil (66) and 5-chlorouracil (80) respectively.

Biological fate of injected [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil.

The pyrimidine nucleoside analog [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) was very rapidly eliminated from the blood following intravenous administration to female BDF_1 mice. The blood clearance was almost exclusively due to removal of the compound from the blood by the kidney followed by excretion in the urine. None of the tissues examined showed significant uptake or accumulation of activity. It was also apparent that the majority of ^{14}C-labelled nucleoside (79) was eliminated without biochemical modification of its structure. Analysis of the urine at times up to 4 hours after administration of the labelled nucleoside revealed that >95% of the ^{14}C-activity was due to unchanged (79). Small amounts of activity detected in 4 hour urine appeared to be present as [^{14}C]-uracil (2.0%) and [^{14}C]-5-chlorouracil (2.3%). The identification of these metabolites was based solely on



The compounds are; 1. uracil (66), 2. 5-chlorouracil (80), 3. 2'-deoxyuridine (82), 4. 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17), 5. 5-chloro-2'-deoxyuridine (81b), 6. 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c).
The hplc conditions are; flow: 1 mL / min, solvent: 2% methanol / 98% water, column: C-18 reverse phase radial compression (5 μ), detection: uv at 256 nm and radioactivity as β-radiation counted by lsc.

Figure IV.14 Hplc analysis by uv and radioactive detection for the 2 hour urine sample collected from female BDF_1 mice bearing a Lewis lung tumor after intravenous injection of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79)

their hplc retention times and is therefore open to question although the presence of these compounds is consistant with the expected route for catabolism of injected [2-^{14}C]-5-chloro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) (Scheme IV.10).

The tissue to blood ratios reached significant levels in a number of tissues. The relative levels of activity increased above the blood concentrations in spleen, tumor, stomach and intestine. The highest tissue to blood ratio was measured for the spleen. In mice this organ is known to be a site of hematopoeitic activity. The tumor contains rapidly proliferating cells and populations of cells in the stomach and intestine undergo rapid growth. The level of activity measured in a number of other tissues (lung, heart, liver) was in excess of that expected if the ^{14}C-activity was due solely to blood perfusion of these tissues. The results indicated that [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) was transported into the cells and became involved in the cellular metabolism.

The nature of the trapped activity within the cells was a matter for speculation since the experiments were not designed to determine the ultimate metabolic fate of the injected compounds. It is likely that [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) was the species transported into the cells. Metabolites of this compound were absent in the urine at times up to 2 hours after injection into mice and were minor components of the urine at 4 hours. It was unlikely that metabolites such as 5-chlorouracil (80) or 1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (17) were responsible for the observed tissue distribution since *in vivo* studies on these compounds have demonstrated different levels and distributions[333,334].

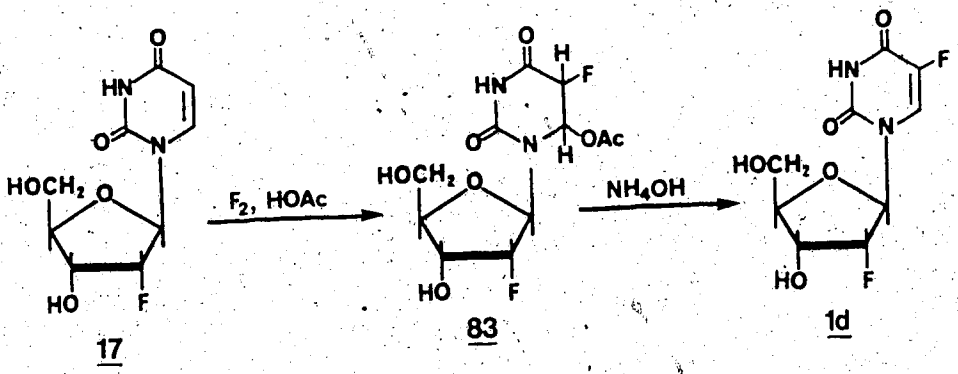
The *in vivo* stability of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (79) was high since it resisted degradation by pyrimidine nucleoside phosphorylases. On the other hand related 5-chloro-nucleosides such as 5-chloro-2'-deoxyuridine (81b)[292] underwent fairly rapid catabolism in the presence of this enzyme or intact blood platelets. This nucleoside (79) also resisted dehalogenation since we were unable to detect 1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (17) in the urine (Scheme IV.10). There does not appear

to be an enzym[illegible] dehalogenation operating at the nucleo*side* level although it has been demonstrated [illegible] and 5-bromonucleo*tides* are dehalogenated under catalysis by thymidylate s[illegible] ehalogenation also occurs rapidly following phosphorolysis[316] and may [illegible]unt, [illegible]t experiment, for the presence of uracil (66) in conjunction with 5-ch[illegible]racil [illegible]minor ^{14}C-labelled metabolites detected in the urine.

D. 5-Fluoro-1-(2'-Fluoro-2'-Deoxy-β-D-Ribofuranosyl)Uracil (1d)

Synthesis and purification.

The direct fluorination of the 5-position of pyrimidine nucleqsides occurred readily using a variety of electrophilic fluorinating reagents as discussed previously. The simplest of these reagents were solutions of elemental fluorine in water or acetic acid, both of which have been used for the synthesis of 5-fluorouridine[150] and a variety of other 5-fluorouracil nucleosides[335]. The use of glacial acetic acid as solvent tended to give the highest yields (92%) while water solutions gave some side products. This simple reagent proved to be very effective for the synthesis of 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) as shown in Scheme IV.11.

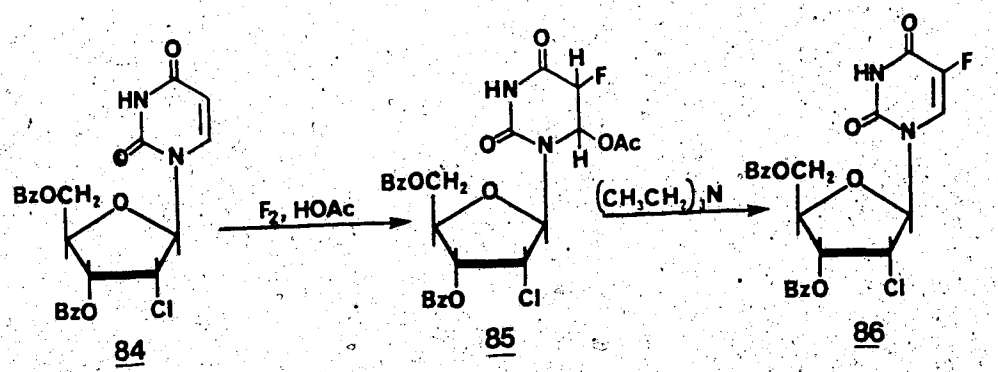


Scheme IV.11 Synthesis of 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d).

A solution of F_2 in HOAc was prepared by bubbling a dilute mixture of fluorine gas in nitrogen (1% F_2 in N_2) through HOAc at room temperature in a stirred round bottom flask. An excess molar quantity of the dilute solution of F_2 in HOAc prepared in this manner (generally 10 to 20 μM) was added to a solution of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) in HOAc at room temperature. The solvent was evaporated after 10 minutes and the residue

was treated with a methanol solution of NH_4OH. This procedure yielded the required 5-fluoro-nucleoside (1d) in virtually quantitative yield (94% yield after purification by column chromatography). The white foam obtained after evaporation of solvent could not be crystallized but was shown to be a single compound (hplc, tlc, nmr and hrms).

When the reaction product was worked up without treatment with NH_4OH two compounds were observed by tlc analysis. The less polar compound was not visible under uv light until the plates were heated but did become visible when sprayed with H_2SO_4. These observations suggested that 5-fluoro-6-O-acetyl-5,6-dihydro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (83) was formed in addition to the required 5-fluoronucleoside 1d (Scheme IV.11). Intermediates of this type have been identified in reactions of fluorine in glacial acetic acid with a variety of substituted uracils[107,212,251]. These intermediates were reported to be quite unstable[212] although 5-fluoro-6-O-acetyl-5,6-dihydrouracil has been isolated and characterized[107].



Scheme IV.12 Synthesis of stable 5-fluoro-6-O-acetyl-5,6-dihydro-1-(3',5'-di-O-benzoyl-2'-chloro-2'-deoxy-β-D-ribofuranosyl)uracil (85) and its conversion to 5-fluoro-1-(3',5'-di-O-benzoyl-2'-chloro-2'-deoxy-β-D-ribofuranosyl)uracil (86)

Of particular significance to the present study was the isolation and characterization of 5-fluoro-6-O-acetyl-5,6-dihydro-1-(3',5'-di-O-benzoyl-2'-chloro-2'-deoxy-β-D-ribofuranosyl)uracil (85) after reaction of 84 with F_2 in HOAc as shown in Scheme IV.12[212]. Compound 85 was stable since it required heating at reflux in the presence of triethylamine to convert it to the 5-fluoro compound 86. The stability of this intermediate was related to the presence of a 2'-halo group in the molecule since no such intermediate was isolated after the reaction of the 2'-deoxy or 2'-hydroxy analogs of 84 with F_2 in HOAc[212].

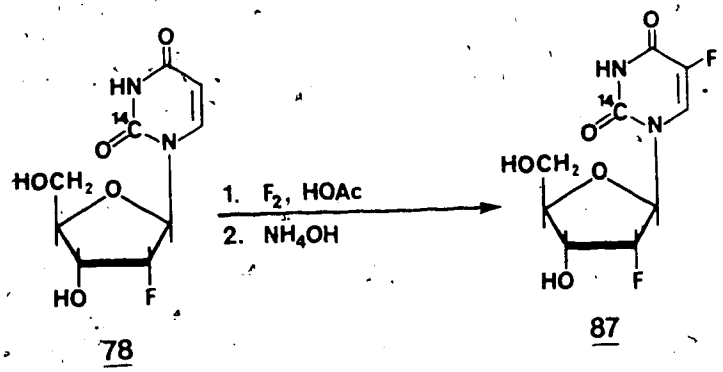
Conventional methods for conversion of the intermediate 5,6-dihydro adducts to the required 5-fluoronucleosides involved sublimation[107], rapid heating or reflux in triethylamine[212]. These methods proved to be less satisfactory in this study than treatment of the crude reaction product with a dilute solution of NH_4OH in MeOH.

In preliminary studies with this 5-fluorination reaction care was taken to dry and purify the glacial acetic acid by reflux with acetic anhydride followed by distillation. Commercial fluorine gas was purified by passage through an HF trap and in some cases 3',5'-di-O-acetyl nucleosides were used in place of the free hydroxy compounds. In addition the reaction of fluorine solutions and substrates were carried out under nitrogen with the exclusion of atmospheric moisture. These precautions proved unnecessary since virtually quantitative formation of the required 5-fluoronucleosides resulted using the simple procedure already described.

Two approaches were investigated for the preparation of a radiolabelled analog of 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d). One possibility was direct radiofluorination of a suitable precursor molecule. Of the 5 radioisotopes of fluorine only ^{18}F had a sufficiently long half life ($T_{\frac{1}{2}}$ = 109.7 minutes)[20] to be suitable for synthetic or *in vivo* studies. The features which make ^{18}F-labelled compounds virtually ideal for biological studies have been discussed previously. In addition 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) had the potential for ^{18}F-labelling at either the 5-position or the 2'-position of the molecule using existing synthetic techniques. Details of these synthetic approaches using

both reactor and cyclotron produced ^{18}F will be discussed later. This approach using ^{18}F production facilities at the University of Alberta was unlikely to produce sufficient radiolabelled material for the required animal studies and consequently the second approach involving synthesis of a ^{14}C-labelled analog was adopted.

The synthesis of the intermediate [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (78) has been described previously (Scheme IV.8 and Scheme IV.9). This compound reacted readily in a reaction analogous to that used to prepare the non-radioactive reference nucleoside 10 to yield [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) (Scheme IV.13).



78 87

Scheme IV.13 The synthesis of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracil (87) from [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracil (78).

The product (87) was obtained in 90.1% overall yield (6.44 MBq) with a radiochemical purity of >99% and a specific activity of 1.86 GBq mmol^{-1} after hplc purification. This purified material was transferred into multidose vials as a solution in ethanol, the ethanol was removed *in vacuo* and the dry residue was stored at -5 °C until required for the animal studies. Compound (87) showed excellent stability. Hplc analysis of the residue remaining in a

multidose vial (saline solution) after completion of the animal studies indicated 98% chemical purity and combined tlc - lsc showed that <1% of the ^{14}C-activity did not appear at the Rf of the product on the tlc plates.

Tissue distribution.

The tissue distribution of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) was determined in female BDF_1 mice using the methodology already described for [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79). The ^{14}C-labelled nucleoside (87) was reconstituted with normal saline just prior to the animal studies and injected intravenously *via* a tail vein. Doses ranged from 47.4 kBq (.025 μmol, 6.6 μg) to 158kBq (.085 μmol, 22.4 μg). The results obtained from combustion and counting of the dissected tissues at various time periods are presented in Table IV.8 as percent of dose per gram of wet tissue and in Figure IV.15 and Figure IV.16 as organ to blood ratios.

The ^{14}C-labelled 5-fluoronucleoside (87) was rapidly cleared from the blood. Blood contained only 3.5% of the injected dose per gram at 15 minutes after injection, 0.54% at 1 hour and less than 0.1% at 2 hours. The kidney showed very high concentrations of activity during the first hour indicating that excretion of activity *via* this organ was the main route for elimination. Unmetabolized [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil was the major radioactive component in the urine at times up to 8 hours, as discussed later, indicating efficient elimination of this compound from the blood by the kidneys. The activity level in all tissues continued to decline for the duration of the experiment. A number of tissues showed moderate concentrations of activity at 1 hour. The highest concentration of activity other than the kidney was measured in the tumor (2.24% of injected dose per gram of tissue) followed by the spleen (1.44%), intestine (0.71%) and the heart (0.58%).

The tissue to blood ratios reached significant levels in a number of tissues as shown in Figure IV.15 and Figure IV.16. The tumor to blood ratios (see also Table IV.8) were always greater than 1 and reached a maximum of 10.3 at 4 hours. After about 3 hours the only tissue

	TIME(HOURS)						
ORGANS	0.25	0.50	1	2	4	8	24
BLOOD	3.49 (0.2)[1] 0.7[2]	1.59 (.15) 0.4	0.54 (.08) 0.2	0.06 (.004) 0.1	0.02 (.001) 0.1	0.01 (.001) 0.1	0.01 (.001) 0.4
SPLEEN	8.72 (1.0) 1.8	3.77 (.13) 1.0	1.44 (.15) 0.7	0.29 (.03) 0.6	0.18 (.04) 1.1	0.09 (.01) 1.6	0.03 (.003) 2.0
STOMACH	2.18 (.08) 0.5	0.96 (.06) 0.3	0.38 (.04) 0.2	0.07 (.02) 0.1	0.02 (.007) 0.1	0.01 (.005) 0.2	.005 (.001) 0.3
GIT	3.35 (1.59) 0.7	1.59 (.18) 0.4	0.71 (.10) 0.3	0.11 (.03) 0.2	0.04 (.01) 0.3	0.02 (.004) 0.4	0.01 (.002) 0.8
KIDNEY	47.4 (6.3) 10.0	21.9 (2.6) 5.7	8.21 (1.5) 3.7	0.84 (.17) 1.8	0.08 (.009) 0.5	0.02 (.004) 0.5	0.01 (.003) 0.8
SKIN	3.30 (1.2) 0.7	1.18 (.12) 0.3	0.43 (.15) 0.2	0.06 (.01) 0.1	0.11 (.17) 0.6	0.01 (.002) 0.1	.004 (.001) 0.2
MUSCLE	2.48 (.18) 0.5	1.06 (.07) 0.3	0.37 (.04) 0.2	0.06 (.001) 0.1	0.02 (.003) 0.1	0.01 (.002) 0.3	.004 (.001) 0.3
BONE	1.69 (.27) 0.3	0.85 (.32) 0.2	0.35 (.06) 0.2	0.11 (.04) 0.3	0.07 (.03) 0.4	0.03 (.005) 0.6	0.02 (.002) 0.9
LUNG	3.37 (.32) 0.7	1.43 (.11) 0.4	0.51 (.07) 0.2	0.07 (.005) 0.2	0.02 (.001) 0.1	0.01 (.002) 0.2	.005 (.001) 0.3
HEART	3.80 (.19) 0.8	1.71 (.13) 0.4	0.58 (.06) 0.3	0.08 (.006) 0.2	0.02 (.002) 0.1	.009 (.002) 0.2	.005 (0) 0.3
LIVER	3.37 (.23) 0.7	1.54 (.20) 0.4	0.53 (.10) 0.2	0.08 (.01) 0.2	0.02 (.004) 0.2	0.01 (.003) 0.2	.005 (.001) 0.3
TUMOR	5.26 (1.5) 1.5[3]	3.88 (0.6) 2.4	2.24 (.37) 4.2	0.49 (.07) 7.7	0.16 (.02) 10.3	0.05 (.009) 7.6	0.02 (.002) 2.6

[1] Percent of injected dose per gram of wet tissue ±standard deviation
[2] Organ to tumor ratios
[3] Tumor to blood ratios

Table IV.8 Tissue distribution in female BDF_1 mice bearing a Lewis lung tumor after intravenous injection of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) (n = 6 animals)

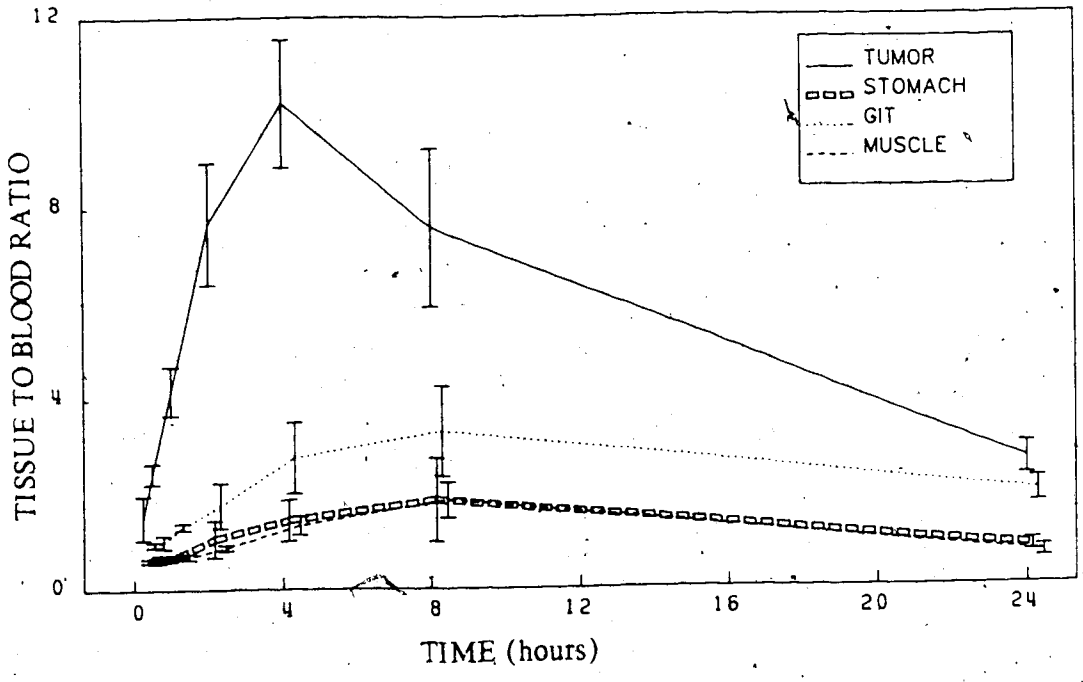


Figure IV.15 Organ to blood ratios for tumor, stomach, intestine and muscle after intravenous administration of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) into female BDF$_1$ mice bearing a Lewis lung tumor

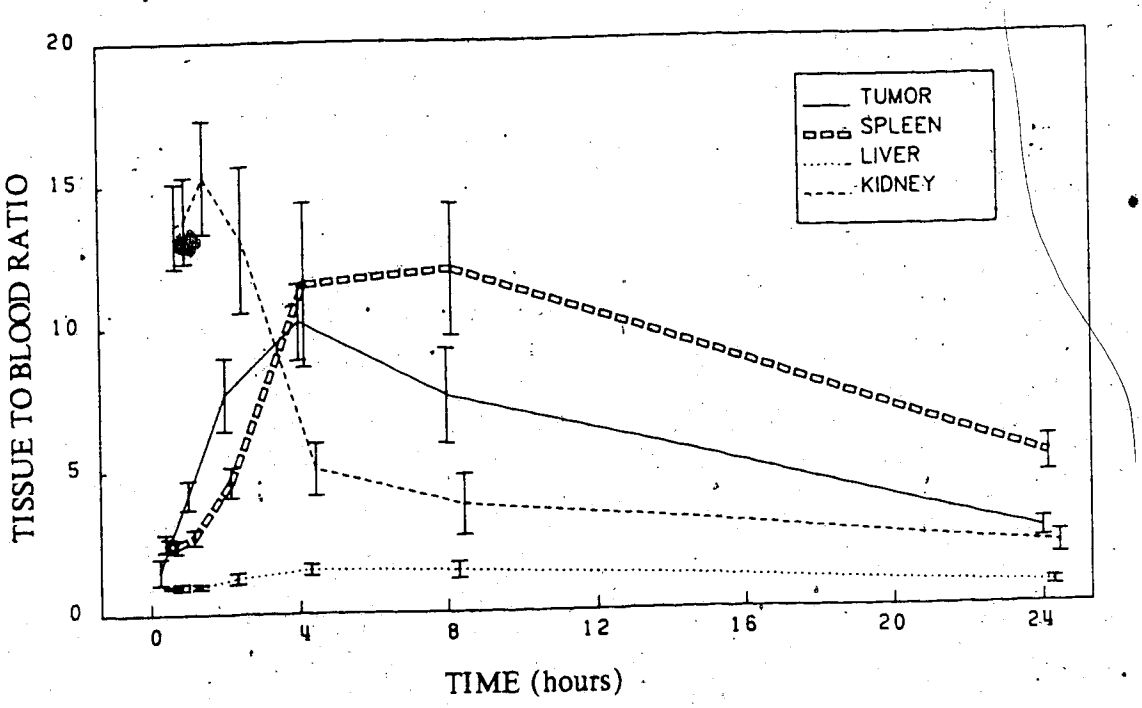


Figure IV.16 Organ to blood ratios for tumor, spleen, liver and kidney after intravenous administration of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) into female BDF$_1$ mice bearing a Lewis lung tumor

exhibiting a higher concentration of activity than the tumor was the spleen which reached a maximum value of 12.0 at 8 hours. In addition to these tissues, selective accumulation of activity relative to blood concentration was observed in the intestine and bone. The intestine showed a maximum tissue to blood ratio of 3.3 and the bone 4.2 at 8 hours after administration of the radiolabelled nucleoside. A number of other tissues such as liver, lung and heart showed levels of activity equivalent to blood levels over the first 8 hours.

These data suggested that [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) was transported freely into most tissues and the equilibrium between extracellular and intracellular concentrations of the nucleoside was rapidly achieved. A number of tissues having a population of cells with a high mitotic index such as intestine, spleen, tumor and bone appeared to accumulate activity against the concentration gradient and demonstrated a relative increase in activity when compared to blood and other organs. This mechanism of uptake was a reversible process since absolute levels of activity continued to decline rapidly even in the organs with the highest relative accumulation of activity.

Blood clearance and metabolism.

The use of ^{14}C-labelled nucleosides for the animal studies, as discussed previously, precluded the measurement of whole body activity and the construction of whole body elimination curves. It was possible however to obtain a qualitative description of the elimination rate for [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) from analysis of the tissue distribution data and further information gained from the blood clearance measurements.

[2-^{14}C]-5-Fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87), like the chloro analog (79), showed very rapid excretion as indicated by blood and tissue activity levels. The blood clearance (Table IV.9 and Figure IV.17) was identical within experimental error to that of the chloro compound (79) (Table IV.7 and Figure IV.12) during the first hour of the experiment. After one hour the 5-fluoronucleoside (87) showed a more rapid blood clearance

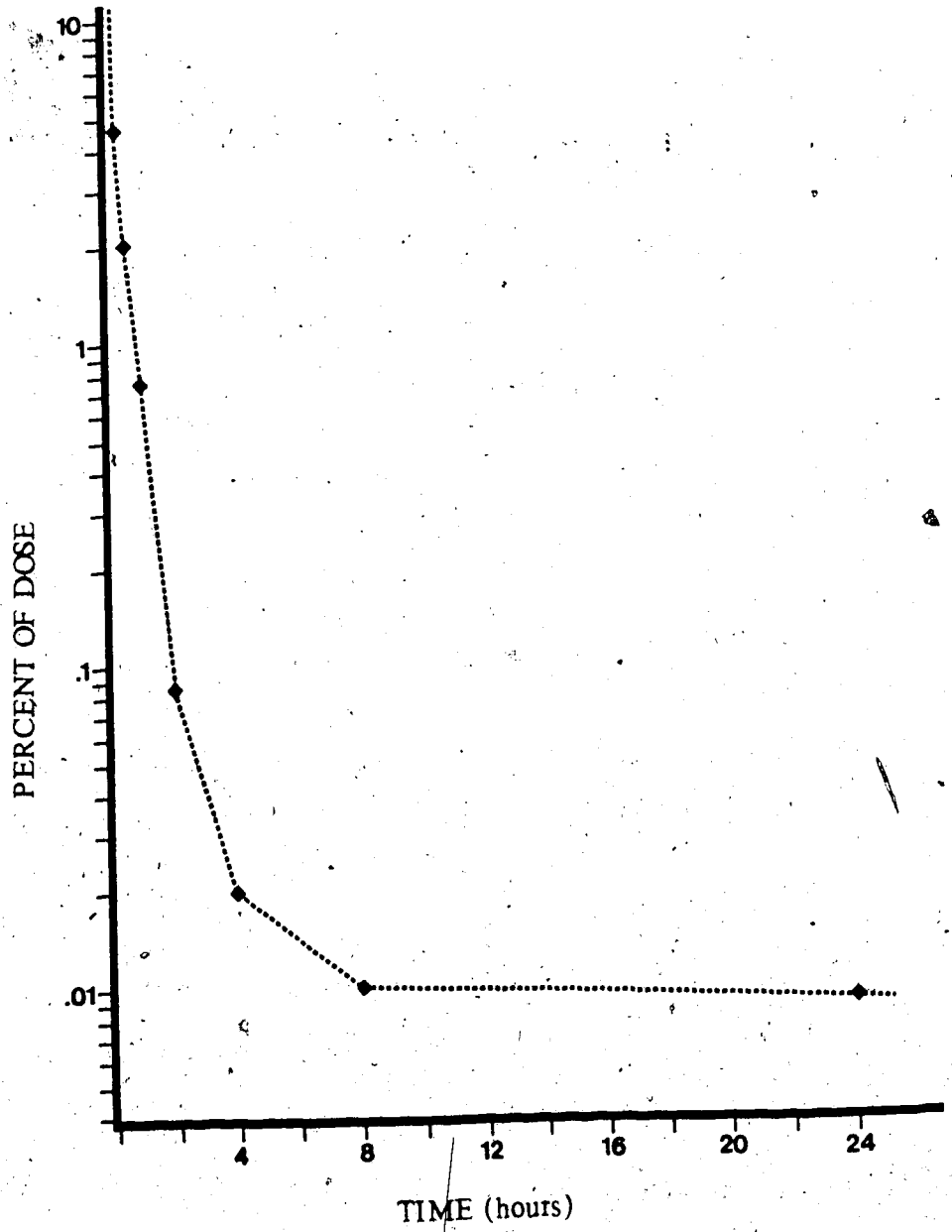


Figure IV.17 Plot of percent dose in blood vs time after intravenous injection of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) into BDF_1 mice bearing a Lewis lung tumor

than the 5-chloro analog (79) since at 4 hours the blood activity of the former was about one quarter that of the latter. After 8 hours a small but fairly stable level of activity persisted in the blood.

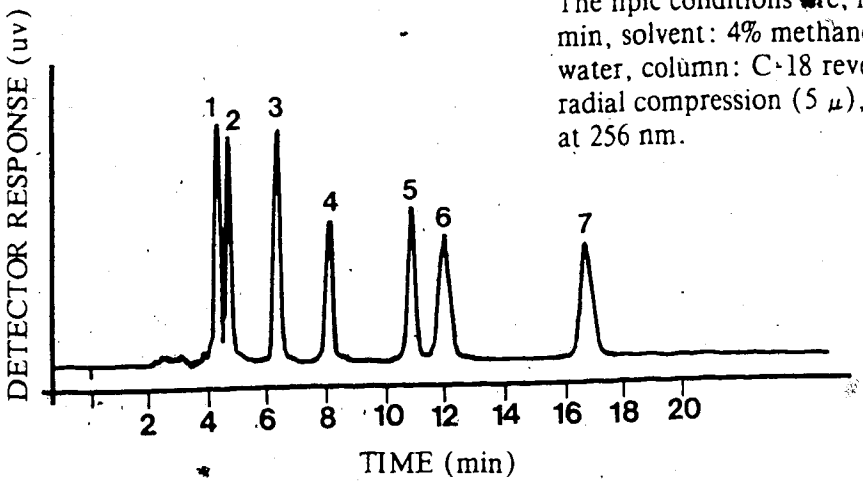
TIME (hours)	% DOSE IN BLOOD
0	100
.25	4.65
.5	2.05
1	0.752
2	0.085
4	0.020
8	0.010
24	0.009

Table IV.9 Percent of dose in blood after intravenous administration of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) into female BDF$_1$ mice bearing a Lewis lung tumor

While the blood concentration of the 5-chloro- and the 5-fluoronucleosides (79) and (87) were similar over the first hour the tissue levels were considerably higher for [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) and consequently the whole body activity would be higher for this compound. The level of ^{14}C activity in a number of tissues continued to parallel that of the blood. Therefore since the blood concentration of the 5-fluoronucleoside (87) dropped considerably below that of the 5-chloro analog (79) at later time periods the whole body activity would also be lower.

The identity of the radioactive constituents present in urine were determined by hplc comparison of their retention times with those of authentic reference compounds. A series of reference compounds was chosen on the basis of possible metabolites arising from chemical or enzymatic modification of the injected 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (1d) (Scheme IV.14). It should be noted that direct *in vivo* defluorination, here yielding

1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) or uracil (66), has not been observed in the catabolism of related 5-fluoropyrimidines and pyrimidine nucleosides[316,335]. These reference compounds and their hplc retention characteristics are shown in Figure IV.18. The components of the reference mixture were readily separated although uracil (66) and 5-fluorouracil (40) exhibited retention times quite close to each other.

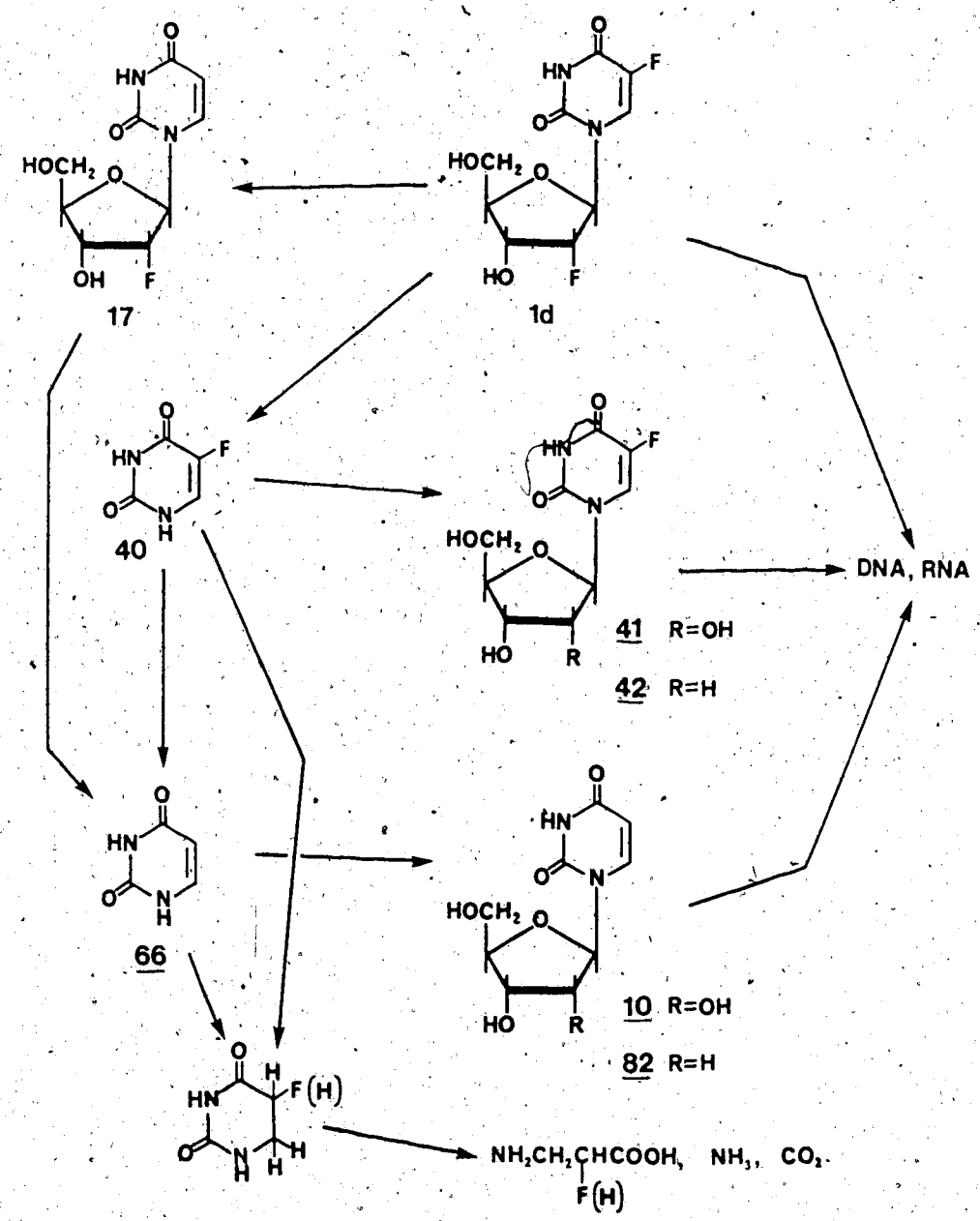


The hplc conditions were; flow: 1 mL / min, solvent: 4% methanol / 96% water, column: C-18 reverse phase radial compression (5 μ), detection: uv at 256 nm.

Compounds are; (1)uracil (66) (4.09 min), (2)5-fluorouracil (40) (4.47 min), (3)uridine (10) (6.12 min), (4)5-fluorouridine (41) (7.92 min) (5)5-fluoro-2'-deoxyuridine (42) (10.69 min), (6)1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (11.78 min) (7)5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) (16.54 min)

Figure IV.18 Retention times of nucleosides and bases used as internal reference compounds for the hplc analysis of the urinary metabolites of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) in female BDF$_1$ mice bearing a Lewis lung tumor

Urine was collected from female BDF$_1$ mice bearing a Lewis lung tumor at the time of sacrifice for tissue distribution studies. The urine was stored frozen until just before use. The urine was thawed, mixed with a portion of reference compound mixture in the hplc solvent and injected directly onto a C-18 reverse phase radial compression column. The column effluent



Scheme IV.14 Possible primary *in vivo* metabolism of 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d)

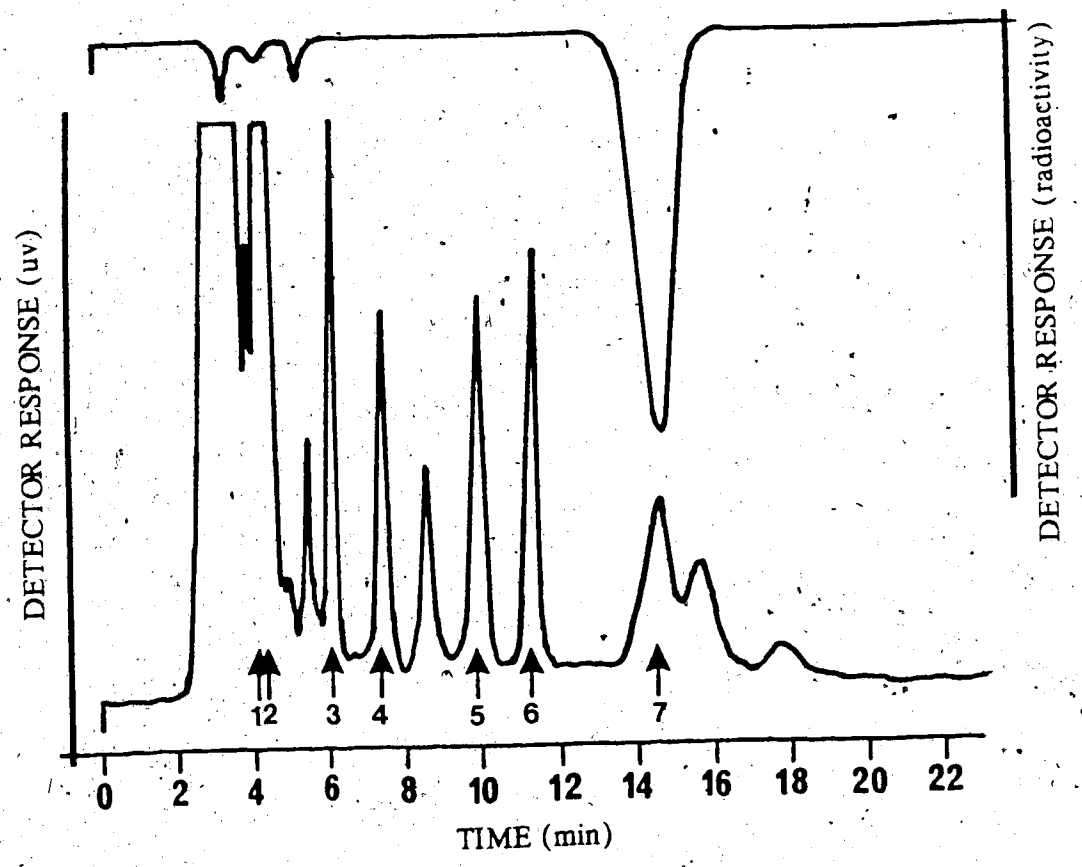
was analyzed by uv and by liquid scintillation counting of aliquots as described previously. The traces were superimposed and the identity of the radioactive components determined by the correspondence of the radioactive and the uv peaks. The hplc chromatogram for 2 hour urine is shown in Figure IV.19 and the results of analyses for time periods from 30 minutes to 8 hours are presented in Table IV.10.

	PERCENT OF TOTAL URINE RADIOACTIVITY			
TIME (hours)	INJECTED COMPOUND	5-FLUORO-URACIL	URACIL	OTHER
.5	90.7	2.1	1.4	5.0
2	88.5	2.8	3.3	5.4
4	63.4	16.3	7.3	23.0
8	52.4	4.5	4.5	38.6

Table IV.10 Radioactive constituents in urine at various time intervals after injection of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (87) into female BDF$_1$ female mice bearing a Lewis lung tumor

These results should be interpreted with some caution since a number of factors contributed to a degree of uncertainty regarding the identity of the radioactive components of the urine. While the injected compound (87) was easily and unambiguously identified, 5-fluorouracil (40) and uracil (66) overlapped with constituents of the urine and exhibited some alteration of retention times depending on the quantity of urine injected. The values in Table IV.10 represented the measurement of the active components detected at the expected retention times for reference compounds but they should not be regarded as an absolute assignment.

The *in vivo* stability of the 5-fluoronucleoside was high as indicated by the persistance of substantial unmetabolized [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (87) at 8 hours after injection. The compound appeared to undergo phosphorolysis to



The compounds are; 1. uracil (66), 2. 5-fluorouracil (40), 3. uridine (10), 4. 5-fluorouridine (41), 5. 5-fluoro-2'-deoxyuridine (42), 6. 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17), 7. 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d).
The hplc conditions are; flow: 1 mL / min, solvent: 4% methanol / 96% water, column: C-18 reverse phase radial compression (5 μ), detection: uv at 256 nm and radioactivity as β-radiation counted by lsc.

[redacted] analysis by uv and radioactive detection for the 2 hour urine sample [redacted] BDF$_1$ mice bearing a Lewis lung tumor after intravenous injection of [redacted]'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87)

5-fluorouracil with subsequent dehalogenation of this species to yield uracil. Both of these compounds were detected in the urine. This is an unusual observation since the accepted mechanism of 5-fluorouracil catabolism in mammalian tissue is *via* degradation of the pyrimidine without defluorination[316]. A substantial portion of the radioactivity in the urine at 4 and 8 hours could not be assigned to any of the reference compounds other than the three compounds noted in Table IV.10. This suggested that the reference compounds were structures which had had only minor or transient involvement in the metabolism of 5-fluoronucleoside (87). The urinary activity at 24 hours was too low for hplc analysis.

Biological fate of injected [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87).

Following intravenous injection into female BDF_1 mice bearing a Lewis lung tumor the nucleoside analog 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) was rapidly eliminated from the blood by the kidneys and excreted in the urine. The compound appeared to permeate freely into tissues suggesting that it was a good substrate for the pyrimidine nucleoside transport mechanism. A number of tissues showed activity levels equivalent to blood indicating that the concentration gradient in itself was a sufficient driving force to move the compound across the cell membrane. It was also evident that unchanged nucleoside was the species transported since this was the major compound detected in the urine up to 8 hours after administration of the compound. A second group of tissues showed accumulation of activity in excess of the blood concentration. This suggested involvement of 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) in cellular metabolism, a supposition supported by the fact that the enhanced uptake of activity occured in tissues with a high mitotic index (spleen, tumor and intestine).

The actual biochemical involvement of the nucleoside within the cell appeared to be a reversible process. Although the relative concentration of the radioactivity within some tissues remained high the absolute level dropped very rapidly in response to rapidly decreasing blood concentrations of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87). For

example the spleen concentration at 1 hour after injection was 1.44% of the injected dose per gram of tissue, a value almost three times the blood concentration. This concentration was regarded as due to a combination of free permeability of the 5-fluoronucleoside across the cell membrane as a minor determinant and subsequent biochemical involvement as a major determinant. The concentration of activity in the spleen at 2 hours had dropped to 0.29%. This is still a high concentration relative to blood but a considerable decrease in absolute tissue concentration. Both the free and the metabolically involved forms of 5-fluoro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil must decrease in concentration within the cell in order to account for this rapid drop in observed ^{14}C activity between 1 and 2 hours. In addition the absence of significant concentrations of metabolic products in the urine at 2 hours suggested that the majority of the metabolically involved 5-fluoronucleoside had reappeared as unmetabolized extracellular [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87). These results were consistant with a model in which the nucleoside was reversably promoted *via* phosphorylation into the cellular nucleotide pool by the relatively non-selective uridine or thymidine kinases. The persistance of activity in some tissues (notably the spleen and tumor) and the increase in unidentified radioactive constituents in the urine at 4 and 8 hours suggested that 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil achieved a more intimate involvement in cellular metabolism possibly as a constituent of DNA or RNA pools.

E. Comparative Summary of Blood Levels, Tumor Levels and Tumor to Blood Ratios After Intravenous Injection of the 5-Halonucleosides (1a - d)

Tables IV.12 to IV.14 and Figures IV.20 to IV.22 compare some of the results obtained from the tissue distribution studies for radiolabelled analogs of 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d). The halogenated nucleoside analogs 1a - d differ from natural nucleosides (eg. uridine (10), deoxyuridine (82), thymidine (62d)) by substitution of a 2'-fluoro for a 2'-hydrogen or hydroxyl substituent and by the substitution of a 5-halo for a 5-hydrogen or methyl substituent. The different steric and electronic properties of the different halogens make compounds 1a - d different from each other. Some of the properties of the halogens and of the substituents that they have replaced are presented in Table IV.11.

SUBSTITUENT	VAN DER WAALS RADIUS(Å)[1]	ELECTRO-NEGATIVITY[1]	SINGLE BOND ENERGY(Kcal / mol)[2]
H	1.20	2.1	96 - 99
OH	1.40	3.5[3]	85 - 91
CH_3	2.00	2.4[4]	83 - 85
I	2.15	2.4	52
Br	1.95	2.8	66
Cl	1.80	3.0	79
F	1.35	4.0	116[5]

[1] Values taken from Pauling[336].
[2] Mean value when bonded to carbon for a series of compounds[337].
[3] Value for O.
[4] Group electronegativity[338].
[5] Value for C-F in CF_4[337].

Table IV.11 Comparison of some substituent characteristics for naturally occuring pyrimidine nucleosides and pyrimidine nucleoside analogs

Variation of the 5-halogen in the series of nucleoside analogs 1a - d alters the physicochemical properties of these compounds and results in altered behaviour in biological test systems. Some aspects of this altered behaviour are demonstrated in the data presented.

The blood activity levels shown in Table IV.12 and Figure IV.20 indicate a more rapid clearance of radiolabelled 5-fluoro- and 5-chloronucleosides 87 and 79 than for radiolabelled 5-bromo- and 5-iodonucleosides 72 and 67.

COMPOUND[1]	TIME (hours) 0.25	0.5	1	2	4	8	24
5-IODO (67)	24.19[2] (2.30)[3]	22.83 (1.12)	17.64 (0.82)	11.07 (1.45)	10.53 (0.83)	5.64 (0.44)	0.21 (0.09)
5-BROMO (72)	3.71 (0.55)	1.76 (0.19)	1.02 (0.14)	1.00 (0.14)	0.80 (0.12)	0.96 (0.16)	0.69 (0.15)
5-CHLORO (79)	4.80 (0.58)	2.39 (0.11)	0.78 (0.05)	0.24 (.027)	.075 (.008)	.040 (.003)	.016 (.002)
5-FLUORO (87)	4.65 (0.40)	2.05 (0.20)	0.75 (0.12)	.085 (.004)	.020 (.001)	.010 (.002)	.009 (.001)

[1] Compounds are; 67 [5-^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 72 [5-^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 79 [2-^{14}C]-5-chloro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil and 87 [2-^{14}C]-5-fluoro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil.
[2] Percent of the injected dose in the blood.
[3] ± Standard deviation.

Table IV.12 The percent of dose in blood at various time intervals after intravenous injection of radiolabelled 5-halo-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracils (1a - d) into female BDF$_1$ mice bearing a Lewis lung carcinoma.

The levels of blood activity between 1 and 8 hours were in the order 5-iodo > 5-bromo > 5-chloro > 5-fluoro. At 15 minutes and 30 minutes the activity levels for the 5-bromo, 5-chloro and 5-fluoro nucleosides were similar whereas the 5-iodo compound demonstrated a much higher activity level. These comparisons must be interpreted with some caution since two of the compounds, [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67) and [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72), have a radiohalogen

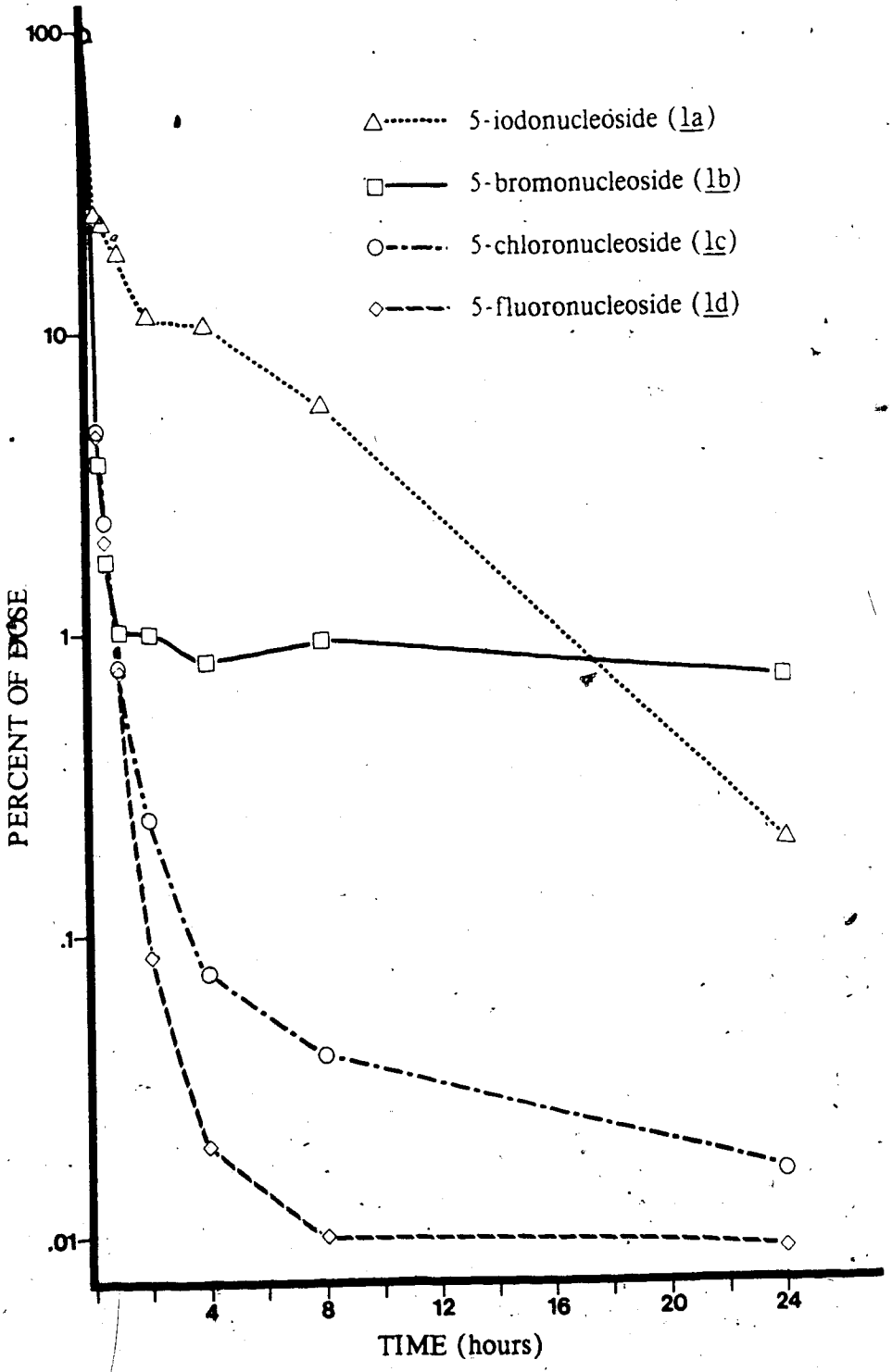


Figure IV.20 Plot of percent of dose in blood vs time after intravenous injection of radiolabelled 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d) into female BDF_1 mice bearing a Lewis lung carcinoma.

label while the other two, [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) and [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87), have a 2-^{14}C label. The final disposition of the radiolabel would differ when these two compounds undergo *in vivo* degradation. Analysis of the radioactive constituents excreted *via* the urine of BDF_1 mice after administration of each of the four radiolabelled 5-halonucleosides has shown unmetabolized starting material to be the major radioactive component at times up to 8 hours after injection. This suggested that the blood radioactivity levels, at least for the short time periods, represented intact nucleoside rather than metabolic products.

COMPOUND[1]	TIME (hours) 0.25	0.5	1	2	4	8	24
5-IODO (67)	4.90[2] (0.36)[3]	3.73 (0.95)	4.14 (0.52)	3.84 (0.26)	3.26 (0.55)	1.93 (0.38)	0.08 (.033)
5-BROMO (72)	1.39 (0.17)	0.95 (0.19)	0.67 (0.11)	0.57 (0.16)	0.37 (0.07)	0.46 (0.06)	0.32 (0.05)
5-CHLORO (79)	2.62 (0.50)	1.82 (0.23)	0.60 (0.12)	0.34 (.037)	0.23 (.028)	.072 (.007)	.028 (.004)
5-FLUORO (87)	5.26 (1.51)	3.88 (0.60)	2.24 (0.37)	0.49 (0.07)	.160 (.024)	.054 (.009)	.016 (.002)

[1] Compounds are; 67 [5-^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 72 [5-^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 79 [2-^{14}C]-5-chloro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil and 87 [2-^{14}C]-5-fluoro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil.
[2] Percent of the injected dose per gram of wet tissue.
[3] $\pm$ Standard deviation.

Table IV.13 The percent of dose per gram in tumor tissue at various time intervals after intravenous injection of radiolabelled 5-halo-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracils (1a - d) into female BDF_1 mice bearing a Lewis lung carcinoma.

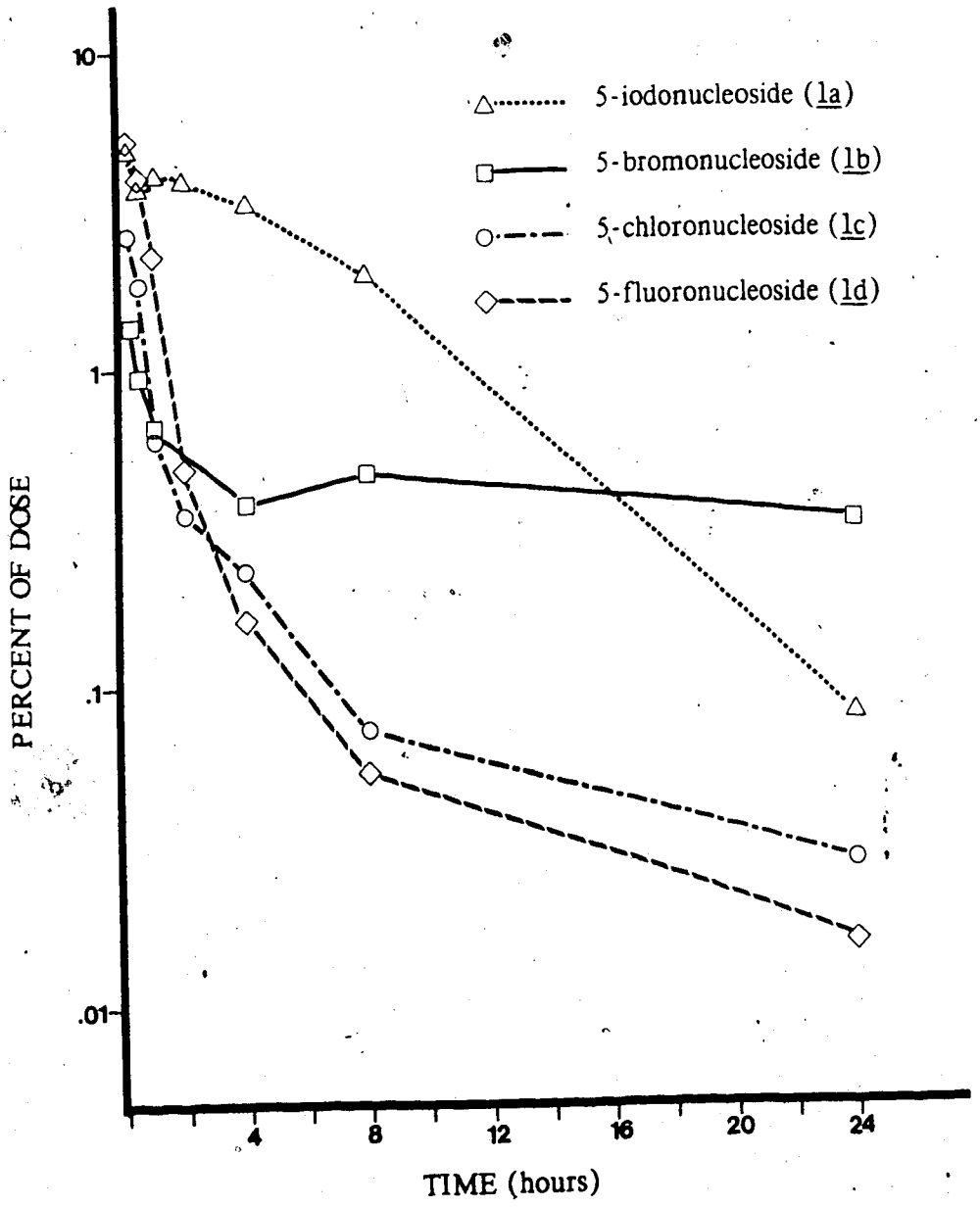


Figure IV.21 Plot of percent of dose per gram in tumor tissue vs time after intravenous injection of radiolabelled 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d) into female BDF_1 mice bearing a Lewis lung carcinoma.

The concentration of radioactivity in tumor tissue after injection of the radiolabelled 5 halonucleosides 67, 72, 79 and 87 into female BDF_1 mice bearing a Lewis lung tumor is shown in Table IV.13 and Figure IV.21. The levels of activity declined for all compounds up to the end of the measurement period (24 hours). None of the compounds were concentrated in the Lewis lung tumor tissue. At short time periods (15 minutes and 30 minutes) the tumor concentration of the 5-halonucleosides was in the order 5-fluoro > 5-iodo > 5-chloro > 5-bromo. This order changed at longer times (4 hours and 8 hours) to 5-iodo > 5-bromo > 5-chloro > 5-fluoro.

The tumor to blood ratios for the radiolabelled 5-halonucleosides 67, 72, 79 and 87 are given in Table IV.14 and Figure IV.22.

COMPOUND[1]	TIME (hours) 0.25	0.5	1	2	4	8	24
5-IODO (67)	0.26[2] (1.27)[3]	0.24 (0.89)	0.31 (1.28)	0.44 (1.69)	0.38 (1.23)	0.47 (0.91)	0.48 (0.04)
5-BROMO (72)	0.53 (0.73)	0.71 (0.67)	0.86 (0.58)	0.73 (0.42)	0.63 (0.23)	0.65 (0.30)	0.63 (0.20)
5-CHLORO (79)	0.74 (1.94)	1.00 (1.81)	1.03 (0.62)	1.97 (0.67)	4.16 (0.96)	2.54 (0.18)	2.28 (0.04)
5-FLUORO (87)	1.52 (7.99)	2.44 (9.46)	4.18 (9.36)	7.68 (3.76)	10.22 (1.63)	7.57 (0.41)	2.64 (0.04)

[1] Compounds are; 67 [5-^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 72 [5-^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 79 [2-^{14}C]-5-chloro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil and 87 [2-^{14}C]-5-fluoro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil.
[2] Tumor to blood ratio = percent of dose per gram of tumor divided by the percent of dose per gram of blood.
[3] Tumor index = percent dose per gram of tissue multiplied by the tumor to blood ratio.

Table IV.14 Tumor to blood ratio and tumor index at various time intervals after intravenous injection of radiolabelled 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d) into female BDF_1 mice bearing a Lewis lung carcinoma.

The 5-iodo- and 5-bromonucleosides 67 and 72 were not taken up selectively in Lewis lung tumor tissue. A small increase in the tumor to blood ratio was noted for the 5-bromonucleoside 67 at 30 minutes and 1 hour. This may have indicated a small uptake of activity into the tumor tissue when the blood concentration of the halonucleoside was maximal. In contrast, the tumor to blood levels for the ^{14}C-labelled 5-chloro- and 5-fluoronucleosides 79 and 87 showed selective uptake of activity in Lewis lung tissue. The maximum tumor to blood ratios (4.16 for 79 and 10.22 for 87) were recorded at 4 hours.

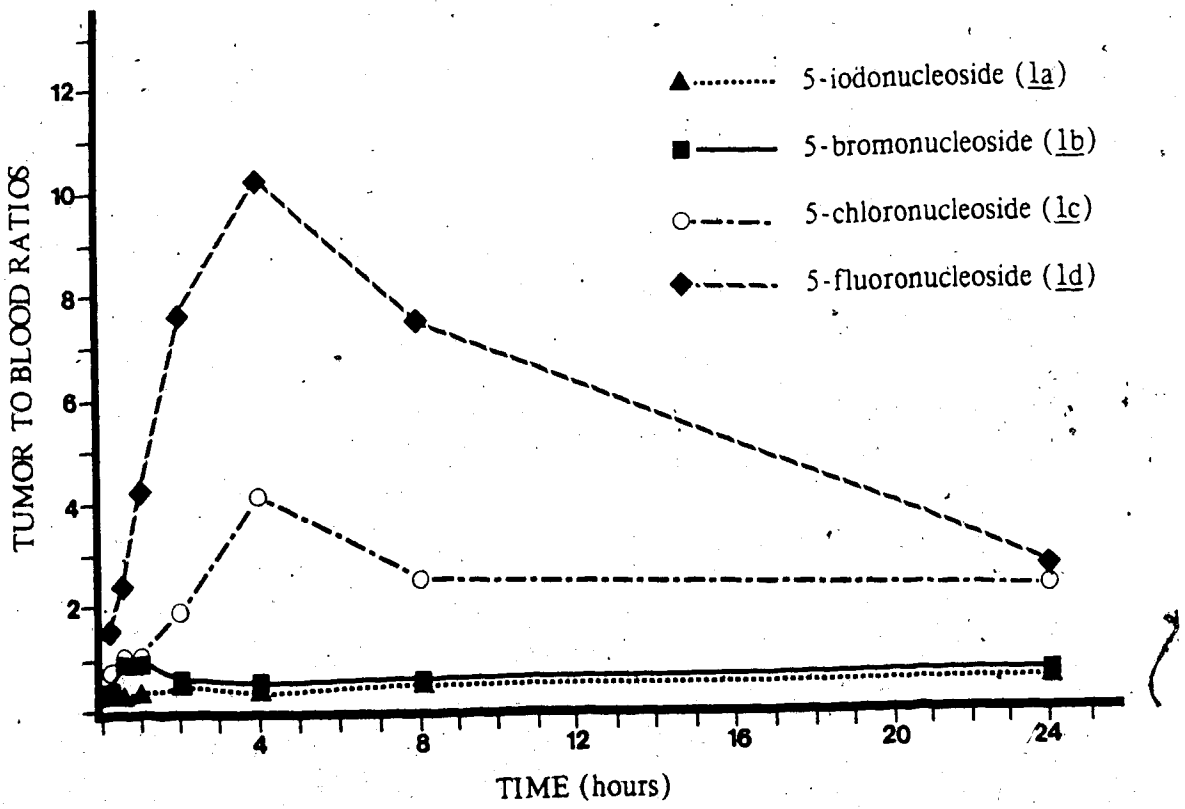


Figure IV.22 Plot of tumor to blood ratios vs time after intravenous injection of radiolabelled 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d) into female BDF_1 mice bearing a Lewis lung carcinoma.

Transport of nucleosides and nucleoside analogs into mammalian cells occurs *via* a rapid and non-concentrative mechanism which depends on the relative concentration of the

substrate on either side of the cell membrane[339] and on the affinity of the substrate for the transporter required to move it across the membrane. Accumulation of activity within the cell in excess of the activity in the blood (Figure IV.22) indicated further metabolic involvement of the ^{14}C-labelled compounds. The animal experiments carried out with the ^{14}C labelled nucleosides suggested that the transported nucleoside analogs ([2-^{14}C]-5-chloro-1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (79) and [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracil (87)) may be phosphorylated by kinase enzymes. This suggestion is consistent with the observed increase in the relative concentration of activity in the tumor tissue after injection of the ^{14}C-labelled nucleoside analogs. After phosphorylation, nucleosides have a very limited permeability through cell membranes[339]. Phosphorylation is also a reversible process and the formation of the free nucleoside analogs and their rapid efflux from the tumor cells would account for the steady decline in the absolute activity noted in tumor tissue (Figure IV.21)

The animal studies carried out in this work were designed to reveal the potential of the 5-halo compounds 1a - d as agents for non-invasive diagnostic oncology rather than to examine features of their *in vivo* metabolism. Consequently conclusions made concerning the metabolic fate of the 5-halo nucleoside analogs must be viewed with caution. It is possible however to make a number of general observations.

None of the 5-halonucleoside analogs 1a - d were concentrated in tissues of Lewis lung bearing BDF_1 mice. They were rapidly eliminated primarily as unmetabolized compound *via* the urine. The compounds were resistant to phosphorolysis catalysed by pyrimidine phosphorylase. This behaviour has been observed with a number of other pyrimidine nucleoside analogs with modified 2'- and 3'-positions[234,237,281,282].

The 5-iodo- and 5-bromonucleosides 1a and 1b were dehalogenated as indicated by the *in vivo* distribution of the radiolabel (^{131}I and ^{82}Br) observed 24 hours after injection of the 5-radiohalogenated analogs 67 and 72. Related 5-halopyrimidines are known to undergo rapid dehalogenation[315]. The established mechanism involved phosphorolytic cleavage followed by

loss of iodine from the resulting 5-halopyrimidine. Dehalogenation of 5-iodo-, 5-bromo- and 5-chlorouracil has been shown to proceed *via* a common mechanism[316]. It has also been noted that 5-iodo and 5-bromonucleotides undergo dehalogenation catalyzed by the enzyme thymidylate synthetase[317]. This process may also account in part for the observed radiohalide anion observed in the *in vivo* studies. There was also indirect evidence for dehalogenation of [2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) and [2-^{14}C]-5-fluoro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87). Both ^{14}C-labelled uridine and ^{14}C-labelled 5-halouridine were observed in the hplc analysis of the urinary metabolites after injection of these compounds into BDF_1 mice. Catabolism of 5-fluorouracil and its nucleosides is known to proceed *via* 5-fluoro-5,6-dihydrouracil, α-fluoro-β-ureidopropionic acid and α-fluoro-β-alanine as shown in Scheme III.6 [316]. However, the fluorine anion was detected in an *in vivo* study involving tumor uptake of 5-[^{18}F]-fluoro-2'-deoxyuridine. This observation suggested that an *in vivo* defluorination mechanism may exist[112].

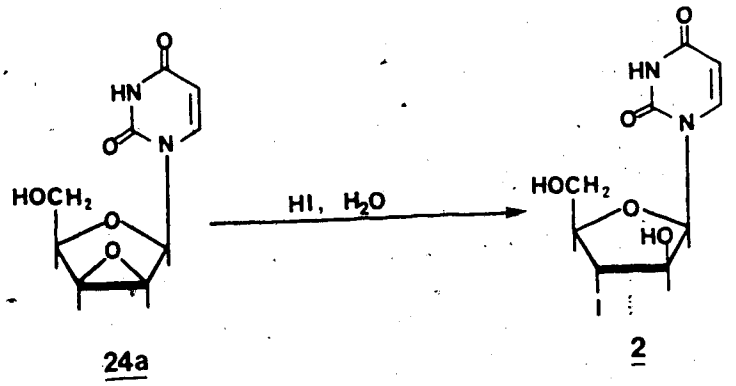
Among the four 5-halonucleosides tested, 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c) and in particular 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (1d) appeared to have potential as tumor imaging agents. The utility of a radiopharmaceutical for tumor scintigraphy is based on both selectivity for tumor tissue and extent of uptake in tumor tissue. Emrich and co-workers[340] suggested that the relative usefulness of radiopharmaceuticals could be determined by calculating a value called the "tumor index". This value was defined as the product of tumor uptake (percent of dose per gram of tissue) and tumor to blood ratio. The tumor index values for the 5-halonucleosides 67, 72, 79 and 87 are shown in Table IV.14. The values for [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) were particularly high with a maximum value of about 9.4 between 0.5 and 1 hour. At 1 hour 87 also demonstrated a good specificity for tumor tissue since the kidney and spleen were the only tissues showing comparable levels of activity. This compound has the added advantage of possessing fluorine at two positions as potential sites for labelling with ^{18}F. Literature results[194,195] and the synthetic trials reported earlier have indicated low yields for

incorporation of fluorine into the 2'-position of pyrimidine nucleosides. More favourable results have been obtained incorporating fluorine at the 5-position[150]. Recently a series of 5-[^{18}F]-fluoropyrimidines and pyrimidine nucleosides were synthesised in good yield[112,141] and positron emission tomographic images were obtained in tumor bearing rabbits[112].

F. 1-(3'-Iodo-3'-Deoxy-β-D-Arabinofuranosyl)Uracil (2)

Synthesis and purification.

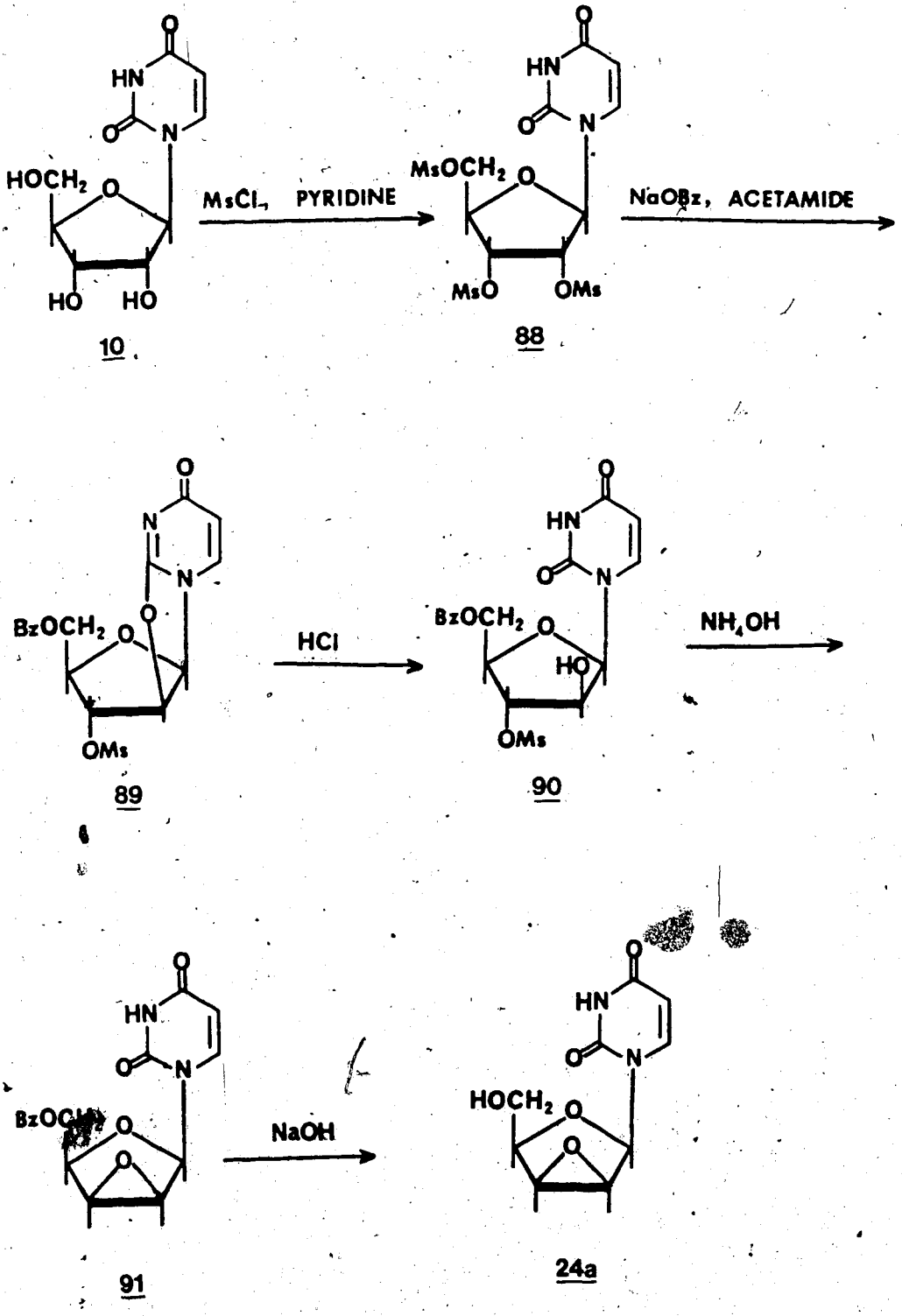
A series of 3'-halo-3'-deoxy nucleosides including the title compound (2) have been synthesised by nucleophilic attack of a halide anion on 1-(2',3'-epoxy-β-D-lyxofuranosyl)-uracil (24a)[201,231]. The epoxide (24a) was synthesised in five steps from uridine (10) using the procedure first reported by Codington and co-workers[235,246] (Scheme IV.16). Non-radioactive 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) was synthesised by reaction of hydroiodic acid with 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a) at 97 °C for 1 hour as shown in Scheme IV.15.



24a 2

Scheme IV.15 Synthesis of 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)-uracil (2) from 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a)

This reaction was superior to the literature procedure[201] where a similar reaction at 25 °C was reported to give a 54% yield of 2 after crystallization. In our hands this reaction was found to proceed very slowly at 25 °C and a substantial quantity of starting material was recovered. On the other hand, when the reaction mixture was heated at 97 °C a virtually quantitative conversion to the 3'-iodoarabinonucleoside 2 occured in 2 hours. There was only a



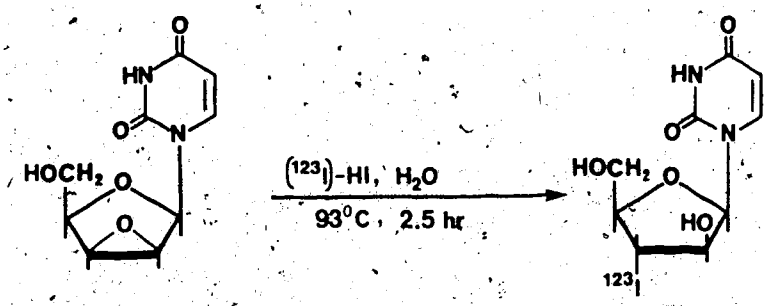
10 88 89 90 91 24a

Scheme IV.16 Synthesis of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a)

small loss of product during chromatography and recrystalization.

Radioiodinated 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) was synthesised using commercially available ^{131}I for some selected experiments but the majority of biological studies were performed using [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92). The ^{123}I was produced at the German Cancer Research Center Cyclotron Facility, Heidelberg by the $^{124}Te(p,2n)^{123}I$ nuclear reaction[305-307] using enriched ^{124}Te, or either the $^{127}I(p,5n)^{123}Xe \rightarrow {}^{123}I$ or the $^{133}Cs(p,2p9n)^{123}Xe \rightarrow {}^{123}I$ [308] spallation reactions at the TRIUMF Cyclotron Facility at the University of British Columbia, Vancouver. The ^{123}I produced by the $^{124}Te(p,2n)^{123}I$ nuclear reaction was recovered from the target by a dry distillation procedure which involved the passage of O_2 gas over molten $^{124}TeO_2$ and condensation of the liberated ^{123}I onto a cooled gold foil or a cold glass finger[305-307]. The molecular iodine was then washed from the trapping surface with water. The radioiodine was probably present as molecular iodine in solution. This assumption was consistant with the demonstrated loss of volatile radioactivity when the aqueous solutions were reduced in volume upon heating or under a gas stream.

Radioiodinated 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) was prepared as shown in Scheme IV.17.



24a 92

Scheme IV.17 Synthesis of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) from 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a)

The aqueous solutions of ^{123}I were mixed with 1 or 2 mg of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a) and the water was removed at 50 °C under a stream of helium. Addition of the epoxide stabilized the radioiodine since no activity was lost during evaporation of solvent. The residue was transfered to a 100 μL Reacti-Vial® and treated with a freshly prepared aqueous solution of HI. The reaction was found to be concentration dependant. Reaction mixtures in which the HI concentration was much lower than 0.1 M were slow to react and failed to go to completion. At HI concentrations of 0.1 M and greater the reaction was virtually quantitative going to completion within 2.5 hours. When equimolar quantities of [^{123}I]-HI and epoxide (24a) were used the crude reaction product showed the presence of small amounts of unreacted epoxide and [^{123}I]-iodide on tlc analysis. When a slight excess (1.1 equivalents) of [^{123}I]-HI was employed the reaction product contained no residual epoxide. The excess HI was removed by passing an aqueous or methanolic solution of the crude product through a short column of DEAE-cellulose powder impregnated with AgCl. This procedure generally yielded [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) in >99% radiochemical purity. No trace of starting epoxide or other non-radioactive impurities were present. End-of-synthesis specific activities >100 GBq mmol^{-1} were obtained routinely in this reaction.

The column purified [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) was dissolved in sterile normal saline and refrigerated at 5 °C prior to use. The biological studies were carried out within 36 hours after the end of the synthesis, a requirement dictated by the relatively short half-life of ^{123}I ($T_{\frac{1}{2}}$ = 13.3 hours)[20]. An analysis of the saline solution 6 days after preparation was possible due to the presence of long-lived radioiodine impurities present in the ^{123}I. The principal impurity was ^{124}I [306] ($T_{\frac{1}{2}}$ = 4.15 days). A tlc analysis indicated >99% of the radioactivity was still associated with 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) suggesting that the iodonucleoside was stable under these storage conditions.

Tissue distribution.

Preliminary biodistribution studies were carried out using [^{123}I]-1-(3'-iodo-3'-deoxy--β-D-arabinofuranosyl)uracil (92) injected intravenously into young male (~220 gram) Wistar rats bearing a 6 day old intramuscular Walker 256 Carcinoma. The injection doses were 70 μg (0.179 μmol) in 0.5 mL of normal saline. The injection doses measured 26.2 MBq at the time of their preparation and varied from 19.7 MBq to 13.7 MBq at time of injection. The tissue distribution data is presented in Table IV.15 as percent dose per gram of tissue and organ to tumor ratios and in Figure IV.23 and Figure IV.24 as tumor to blood ratios.

The information obtained from the tissue distribution study was supplemented by an imaging study . A female Wistar rat (237 g) was injected *via* the femoral vein with 0.48 mg (1.31 μmol, 72.5 MBq) of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) as a bolus dose in 0.3 mL of sterile saline. The rat was anesthetised and images were recorded at intervals up to 2 hours. The rat was then allowed to recover from the anesthetic and left *ad lib* with food and water. A final image was recorded at 24 hours at which time the rat was sacrificed and the activity remaining in selected tissues was measured. The animal was imaged using a Pho / Gamma III Counter (Nuclear Chicago) with a pin-hole collimator (50,000 counts accumulated).

Both studies indicated little early accumulation of activity in tissues except in organs related to the route of excretion (kidney and urinary bladder). The activity was excreted rapidly. At 15 minutes the blood and most tissues had about 0.5% of the injected dose per gram or about 11% of the total dose in a 220 gram rat. The kidneys contained 4% of the dose at 15 minutes. At 1 hour the concentration of activity in the stomach was greater than in all other tissues except the kidney At 4 hours the stomach and intestine showed the highest concentrations of activity. The kidney to blood ratio was initially very large but fell rapidly after 2 hours (Figure IV.24). The activity ratio in the stomach and intestine continued to increase with the stomach reaching a maximum value of 5.05 at 6 hours and the intestine 1.86 at 4 hours (Figure IV.23). Beyond these maxima the ratios in the stomach and intestine showed

	TIME(HOURS)							
ORGAN	0.25	0.50	1	2	4	6	8	24
BLOOD	0.41[1]	0.36	0.21	0.10	0.06	0.03	0.04	0.02
	0.7[2]	0.9	1.0	1.7	1.4	1.2	1.9	.81
SPLEEN	0.47	0.30	0.19	0.05	0.03	0.02[4]	-	-
	0.9	0.7	0.9	0.8	0.6	0.7	-	-
STOMACH	0.54	0.45	0.40	0.29	0.17	0.17	0.19	0.07
	1.0	1.1	2.0	4.8	5.3	6.3	8.3	-
GIT	0.62	0.44	0.33	0.13	0.12	0.06	0.07	-
	1.2	1.1	1.6	2.1	2.6	2.0	3.0	-
KIDNEY	2.20	2.12	0.98	0.53	0.05	0.03	0.02	-
	4.0	5.6	4.8	9.1	1.1	1.0	1.0	-
TESTES	0.14	0.11	0.10	0.07	0.06	0.03	0.03	-
	0.3	0.3	0.5	1.1	1.2	1.1	1.2	-
MUSCLE	0.53	0.39	0.24	0.05	-	-	-	-
	1.0	1.0	1.2	0.9	-	-	-	-
BONE	0.26	0.17	0.13	0.04	0.04	-	-	-
	0.5	0.4	0.6	0.7	0.8	-	-	-
LUNG	0.49	0.32	0.19	0.07	0.05	-	-	-
	0.9	0.8	0.9	1.1	1.1	-	-	-
HEART	0.48	0.31	0.16	0.04	0.03[4]	-	-	-
	0.9	0.8	0.8	0.7	0.6	-	-	-
LIVER	0.55	0.35	0.21	0.05	0.03	0.02	0.02[4]	-
	1.0	0.9	1.0	0.9	0.6	0.7	0.8	-
TUMOR	0.54	0.39	0.21	0.06	0.05[4]	0.03[4]	0.02	-
	1.34[3]	1.07	0.98	0.60	0.74	0.84	0.54	-

[1] Percent of injected dose per gram of wet tissue
[2] Organ to tumor ratios
[3] Tumor to blood ratios
[4] n = 1

Table IV.15 Tissue distribution in male Wistar rats bearing a Walker 256 carcinoma after intravenous injection of [^{123}I]-1-(3'-Iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92)

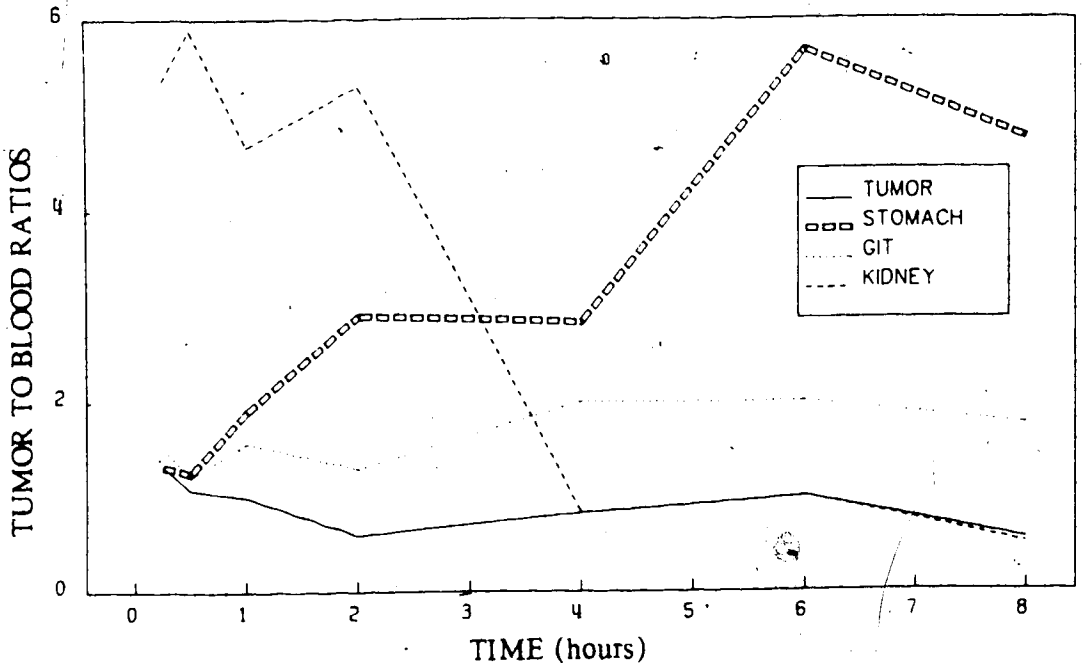


Figure IV.23 Organ to blood ratios for tumor, stomach, intestine and muscle after intravenous injection of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) into male Wistar rats bearing a Walker 256 tumor

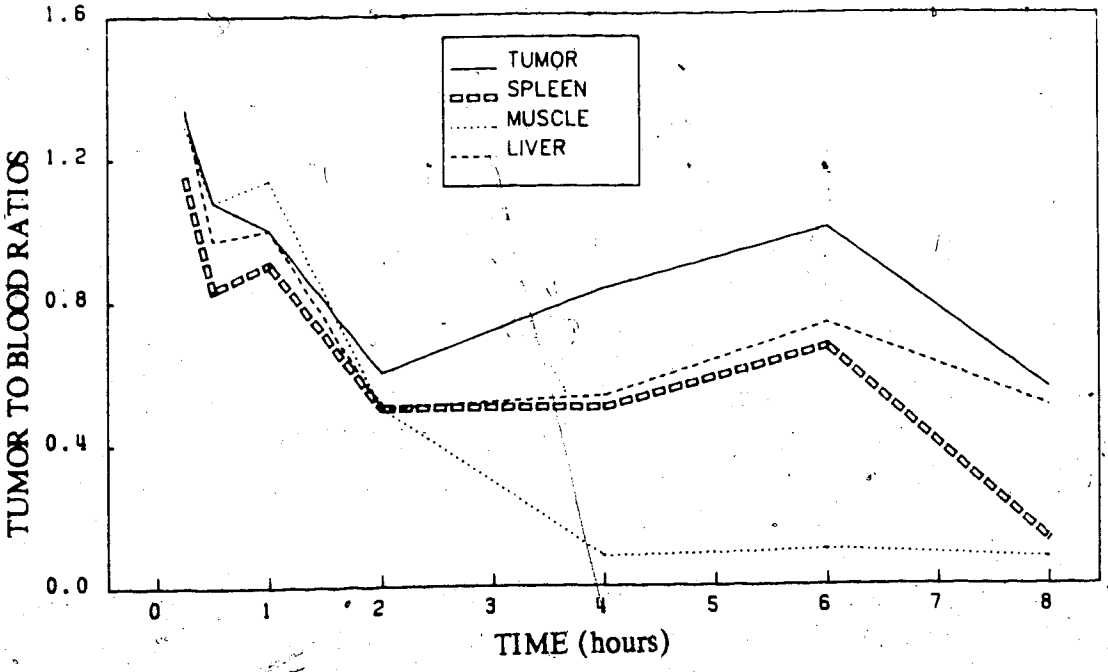


Figure IV.24 Organ to blood ratios for tumor, spleen, liver and kidney after intravenous injection of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) into male Wistar rats bearing a Walker 256 tumor

a gradual decline until the end of the measurement period. The tumor did not take up activity relative to other tissues. The levels of tumor activity paralleled tissues such as muscle indicating passive association with the radioactive compounds rather than active uptake.

The imaging study confirmed the data obtained in the tissue distribution study. An image recorded 7 minutes after injection of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) showed a general background activity due to blood perfusion and significant accumulation of activity in the kidney and bladder. At 1 hour activity was observed in the bladder and to a lesser extent in the kidney and stomach. The final image recorded at 24 hours showed that the stomach and thyroid were the only tissues having levels of radioactivity above the general background radiation. The thyroid and stomach were dissected and found to contain 0.16% and 0.15% of the injected dose or 12% and 11% of the residual dose respectively.

The data obtained in the tissue distribution study were consistent with rapid initial excretion of radioactivity, probably as unmetabolized starting compound. The majority of residual activity at 24 hours was iodide ($^{123}I^-$) as indicated by accumulations of activity in the stomach and thyroid. These organs are known to concentrate iodide anion[314].

Whole body elimination and metabolism.

A normal male Wistar rat was injected with 4.4 μg (11.8 pmol, 0.518 MBq) of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) in 0.25 mL of sterile normal saline. The rat was placed in a plastic tub and rotated on a turntable in a lead-shielded enclosure containing two sodium iodide crystal γ-ray detectors. Rotating the rat helped to correct for geometry effects due to the orientation of the rat within the plastic tub. A Canberra Multi-Channel Analyzer and associated electronics, with a window set to count the 159 keV γ-ray peak, were used to record the whole body activity of the rat. Three 1 minute determinations of activity were averaged at each time period. The data were collected for 20 hours. The collected data are shown in Table IV.16 along with percent of injected dose in the blood. The blood value was extrapolated from the tissue distribution studies with tumor bearing

rats as reported in Table IV.15 assuming blood comprises 6.5% of the rat total body weight.

TIME (hours)	% OF DOSE REMAINING	% OF DOSE IN BLOOD
0	100	100
.25	100	5.9
.5	99	5.1
1	56.0	3.0
2	26.0	1.4
3	17.6	-
4	13.0	0.86
6	9.6	0.43
8	8.2	0.57
10	6.8	-
20	4.7	-
24	-	0.29

Table IV.16 Percent of dose in whole body and in blood after intravenous administration of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) to normal male Wistar rats (whole body data) or male Wistar rats containing an intramuscular Walker 256 tumor (blood data)

The whole body elimination curve plotted from the data in Table IV.16 is shown in Figure IV.25. This curve is well fitted by a bi-exponential equation containing a short-lived component with a half-life of 0.97 hours for 90.1% of the dose and a long-lived component with a half-life of 18.6 hours for 9.9% of the activity. The initial rapid blood clearance and excretion was probably due to efficient removal of unmetabolized 3'-iodonucleoside from the blood *via* the kidneys and bladder. Analysis of the urine as described below showed the majority of urinary activity to be [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92). The slower excretion of activity at later times was due, at least in part, to [^{123}I]-iodide anion as demonstrated by the accumulation of activity in the stomach and thyroid.

The radioactive compounds excreted into the urine were examined after an intravenous injection of [^{131}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2, 3-^{131}I) in a male

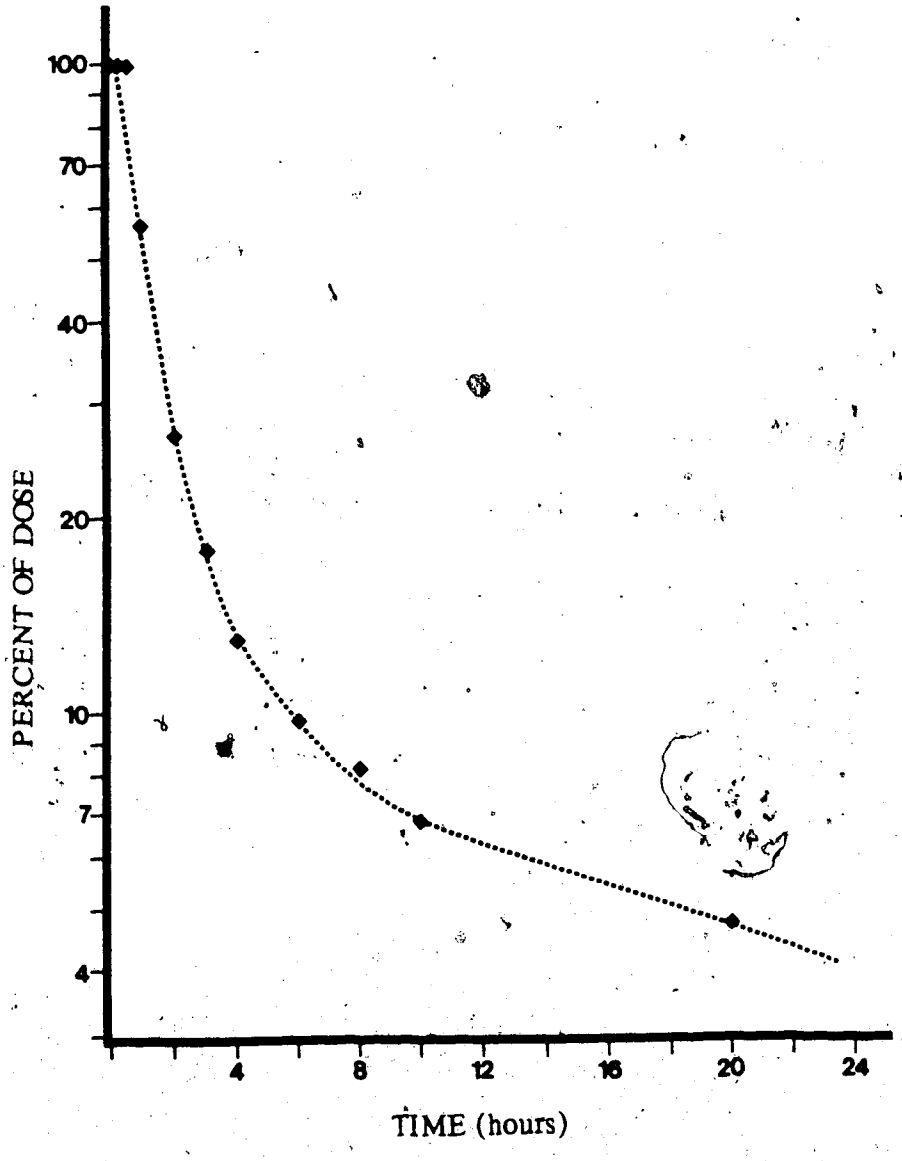


Figure IV.25 Plot of percent dose in whole body vs time after intravenous administration of [^{123}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (92) to male Wistar rats bearing an intramuscular Walker 256 carcinoma

Wistar rat weighing 234 grams. The animal was maintained under anesthesia during injection and for the duration of the experiment (2 hours) using a combination of Rompum and Ketamine. This anesthetic mixture relaxed the bladder sphincter. The rat excreted a slow flow of urine which was collected at intervals and measured by γ scintillation counting for both total activity and activity concentration. The data obtained in this experiment are shown in Table IV.17.

TIME INTERVAL (min)	TOTAL ACTIVITY IN URINE (MBq(% of dose))	CONCENTRATION OF ACTIVITY MBq / mL	PERCENT UNMETABOLIZED COMPOUND
0-5	-	-	-
5-10	0.135(1.08)	0.518	-
10-15	0.556(4.45)	1.465	-
15-20	0.910(7.27)	4.551	88.8
20-25	0.394(3.15)	2.812	-
25-70	0.081(0.65)	1.010	80.9
70-80	0.121(0.97)	0.932	87.5
80-90	0.272(2.23)	1.809	-
90-100	0.470(3.76)	1.236	86.5
100-110	0.457(3.65)	1.691	-
110-120	0.583(4.66)	1.458	88.0
TOTALS	3.986(31.8)	-	-

Table IV.17 Analysis of urinary activity in a normal male Wistar rat after intravenous injection of [^{131}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)-uracil (2, 3'-^{131}I)

Samples from various time periods were also analyzed by tlc. Urine (1μL) was spotted over a sample of authentic, non-radioactive, reference 1-(3'-iodo-3'-deoxy-β-D-arabino-furanosyl)uracil (2). The plates were developed with 15% MeOH in CH_2Cl_2, visualized by uv to locate the 3'-iodonucleoside (2) and the silica was scraped from the plates in 30 fractions and counted by lsc as described previously. A digital integration program was used to analyze the data from the liquid scintillation counting. In all cases the majority of the radioactivity

(>80%) detected on the plates corresponded with the Rf of 1-(3'-iodo-3'-deoxy-β-D--arabinofuranosyl)uracil (2) (Table IV.17). The remaining activity was located as a small peak at the plate origin and as a general low level activity between the plate origin and the product peak.

This result confirmed that the rapid excretion and blood clearance was due to efficient removal of 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) from the blood by the kidneys and excretion *via* the urine. The total activity excreted *via* the urine in 2 hours was 32% of the injected dose. In the whole-body excretion study 74% of the injected dose was excreted in the first 2 hours. The difference in these two values represented the slower physical and metabolic activity to be expected in the anesthetized animal. The detection of unmetabolized [^{131}I]-1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2, 3-^{131}I) as the major radioactive component in the urine at 2 hours after intravenous injection suggested that this compound was resistant to biochemical degradation.

Biological fate of injected 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2).

After its injection into male Wistar rats bearing a Walker 256 carcinoma unmetabolized radiolabelled 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil was rapidly cleared from the blood and excreted *via* the kidneys and urinary bladder. The level of activity in most tissues paralleled blood levels indicating that the measured activity was due to blood perfusion rather than tissue accumulation. Unmetabolized 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) represented >80% of the urinary activity at times up to 2 hours. The persistance of unchanged 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) *in vivo* suggested that this compound was a poor substrate for pyrimidine nucleoside phosphorylases and was fairly resistant to *in vivo* chemical deiodination. The gradual increase of activity in the stomach and intestine, and the detection of activity in the thyroid at 24 hours were indications that some of the residual activity in the rats was due to iodide anion. The distribution of activity at 24 hours was in agreement with the known biological distribution of iodide anion[314].

The 3'-halo compound 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) and the 3'-bromo, 3'-chloro and 3'-fluoro analogs were proposed as possible agents for non-invasive diagnostic oncology when synthesised with an appropriate radiolabel. These compounds are analogs of uridine or deoxyuridine and they may resemble the natural nucleosides sufficiently to become involved in the cellular metabolism of proliferating tissue. It was anticipated that the 3'-halo compounds would show some resistance to catabolism due to phosphorolytic cleavage catalyzed by the enzyme pyrimidine nucleoside phosphorylase. This enzyme system has shown a strong sensitivity to the nature of the 3'-substituent in a number of related compounds. For example 5-fluoro-2',3'-dideoxy-3'-fluorouridine was not a substrate for thymidine phosphorylase[282]. In contrast 5-fluoro-2'-deoxyuridine was rapidly degraded by this enzyme. The 3'-hydroxyl of thymidine is required for binding with thymidine phosphorylase[341] and compounds such as 3'-deoxythymidine[341] and 3'-fluoro-3'-deoxythymidine[301] are not degraded by pyrimidine phosphorylases. This study indicated that 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) undergoes a slow *in vivo* degradation. A similar result has been reported for the 3'-bromo and 3'-chloro analogs[342]. The observed persistance of unmetabolized 3'-haloarabinouridines in the urine was consistant with a postulate that these compounds were poor substrates for the ubiquitous pyrimidine nucleoside phosphorylases.

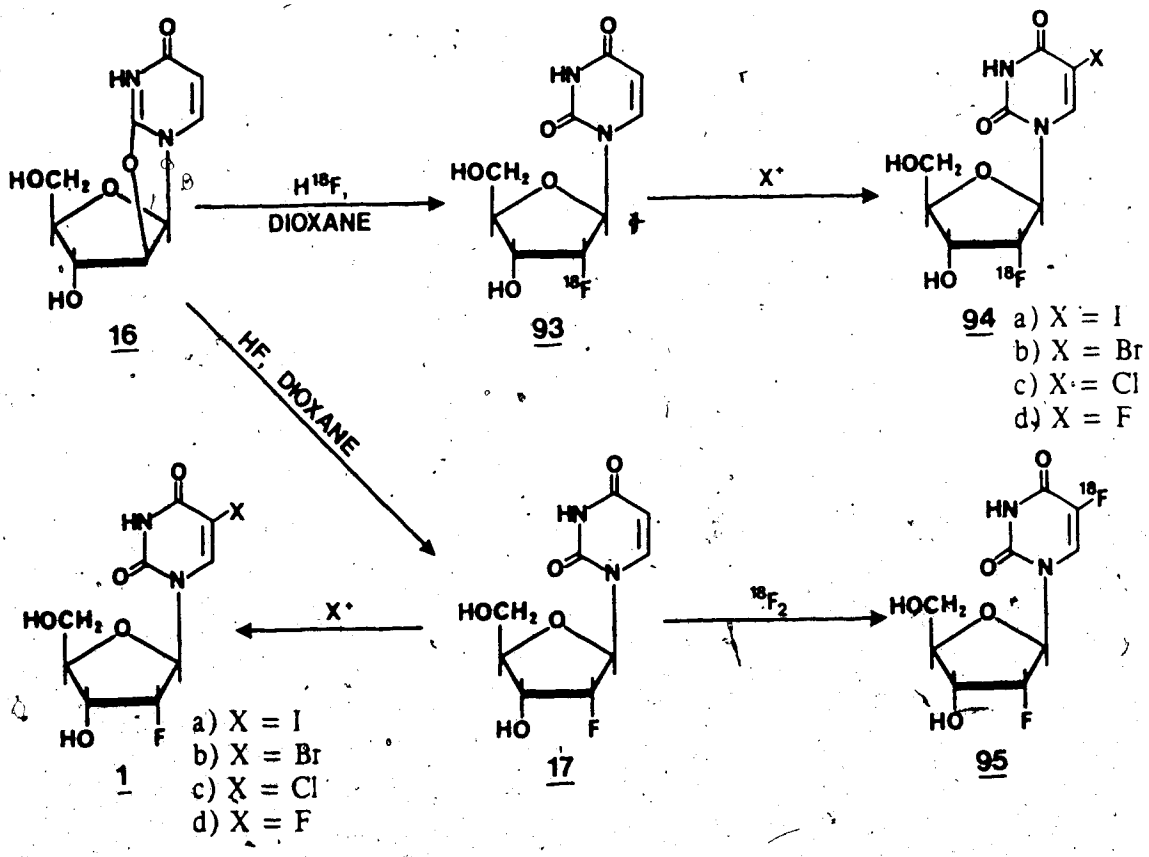
Despite its observed *in vivo* stability 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) did not demonstrate accumulation in tissues. Early activity in the kidney was due to urinary excretion and late accumulation of activity in the stomach, intestine and thyroid was attributed to iodide anion rather than intact nucleoside. It appeared that the 3'-iodo substituent affected the ability of this nucleoside to act as a substrate for the transport mechanism responsible for the movement of pyrimidine nucleosides into the cells and / or the kinase enzymes necessary for conversion of the nucleoside to the corresponding nucleotides. The 3'-hydroxyl group is not required for interaction of pyrimidine nucleosides with kinase enzymes since 5-fluoro-2',3'-dideoxy-3'-fluorouridine[282] and 3'-fluoro-3'-deoxythymidine[233] undergo *in vitro* phosphorylation. A recent study investigated the interaction of a variety of modified pyrimidine

nucleosides, including 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) and the 3'-bromo, 3'-chloro and 3'-fluoro analogs, with the mouse erythryocyte nucleoside transporter. These compounds were poor substrates for this transport mechanism[343].

G. The Production and Use of ^{18}F in Synthetic Studies

Introduction.

The syntheses and biological distribution of the 5-halo-2'-fluororibonucleosides 1a - d shown in Scheme IV.18 have been described previously (Chapter IV.A - IV.D).



Scheme IV.18 Proposed synthetic route to [2'-^{18}F]-5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (94) and [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil(95)

The biological distribution studies were carried out in female BDF-1 mice bearing a Lewis lung tumor using the radiolabelled analogs [^{131}I]-5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (67), [^{82}Br]-5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (72),

[2-^{14}C]-5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (79) and [2-^{14}C]-5-fluoro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87). These radiolabelled nucleosides were not however suitable for non-invasive imaging studies. The ^{14}C incorporated in the compounds 79 and 87 decays by a low energy β^--particle emission. ^{131}I and ^{82}Br have relatively long half-lives (8.05 days and 35.34 hours respectively), decay by β^--ray emission and have energetic γ-ray emissions. These factors contribute to a high patient radiation dose when these radionuclides are used for *in vivo* studies. The presence of a variety of γ radiations, including high energy γ-rays, in the emission spectra of these radionuclides makes them unsuitable for imaging techniques.

There are a number of halogen radioisotopes which would be suitable for imaging studies when incorporated as radiolabels into the 5-halonucleosides 1a - d. The relatively short-lived radionuclide ^{123}I ($T\frac{1}{2}$ = 13.3 hours) decays by emission of a single γ-ray with an energy of 159 keV. These favourable decay characteristics and the absence of β^--emissions make ^{123}I virtually ideal for single photon emission computerized tomography (SPECT). The useful bromine isotope ^{75}Br (β^+-ray decay, $T\frac{1}{2}$ = 95.5 minutes) has been suggested as a candidate for positron emission tomographic studies (PET) provided corrections can be made for the 286 keV γ-ray emission. The bromine isotope ^{77}Br ($T\frac{1}{2}$ = 57 hours) is one of the more useful medical isotopes of bromine decaying by electron capture (99%) and β^+-emission (1%). The 239 keV γ-ray is used for imaging. The majority of radioisotopes of chlorine are either short-lived or hard β^--emitters. The most suitable radioisotope is ^{34}Cl^m due to its short half-life ($T\frac{1}{2}$ = 32 minutes) and its decay by β^+-emission either directly from ^{34}Cl^m or after isomeric transition to ^{34}Cl ($T\frac{1}{2}$ = 1.53 seconds). Probably the most ideal radiohalogen with respect to imaging studies is ^{18}F which decays by β^+ (97%) and electron capture (3%), has a half-life of 109.7 minutes and has no β^- or γ-ray emissions.

In addition to its favourable physical characteristics, ^{18}F was regarded as a suitable radiolabel for compounds 1a - d for a number of practical reasons. The compounds under investigation all contained fluorine in the 2'-position of the molecule and in one case (1d), in

both the 2' and the 5 positions. In addition the synthetic sequences outlined in Scheme IV.18 for the preparation of the series of compounds [2'-^{18}F]-5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (94a - d) and [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (95) have been well established for non-radiolabelled syntheses. Finally, ^{18}F was locally available from both reactor and accelerator sources. The other radiohalogens suitable for imaging studies were not readily available.

The discussion which follows summarizes the production methods used to produce ^{18}F in a chemical form suitable for radiochemical synthesis and describes some preliminary synthetic studies.

Reactor production and recovery of ^{18}F.

The nuclear reaction ^{6}Li(n,^{3}H)^{4}He, ^{16}O(^{3}H,n)^{18}F was used to prepare ^{18}F. Thermal neutrons from a nuclear reactor interact with the ^{6}Li isotope of lithium to yield ^{4}He and ^{3}H. The ^{3}H with an energy maximum of 2.73 MeV then bombards ^{16}O also present in the target material to produce ^{18}F. The various types of target materials traditionally employed for this nuclear reaction and the methods used for ^{18}F recovery from the target materials have been outlined in the Literature Review (Chapter II.A). Li_2CO_3 was chosen as a target material since this material has demonstrated the best combination of ^{18}F yield and ease of processing after irradiation[51-53]. Target materials enriched in ^{6}Li (>90% as compared with 7.42% natural abundance) have been used by many workers to maximize ^{18}F production[51,53-56]. In the present study 96% ^{6}Li enriched Li_2CO_3 obtained from Oak Ridge National Laboratories was used. Table IV.18 outlines some of the irradiations that were performed during this study.

The very high cross section of ^{6}Li for thermal neutrons (953 barns†)[20] caused the majority of neutrons to be captured within the first millimeter of the sample surface in Li_2CO_3[53,57]. The best yields of ^{18}F were obtained when the surface area of the target was

†The **barn** is a measure of the effective area or cross section presented by a target nucleus to an impinging particle. The **barn** has no physical significance other than it frequently is the same order of magnitude as the actual nuclear cross section, 1 **barn** = 10^{-24} cm^2 = 10^{-28} m^2.

EXPERIMENT	^6Li SOURCE	WEIGHT OF ^6Li (mg)	RECOVERY METHOD[1]	ACTIVITY AT EOB[2] (MBq)	MBq PER mg OF ^6Li	% OF THEORY[3]
1	Li_2CO_3 solid	25.0	A	9.7	0.39	11.9
2	Li_2CO_3 solid	33.4	B	23.2	0.69	21.1
3	$LiNO_3$ solution	25.0	B	21.9	0.88	27.0
4	Li_2CO_3 solid	7.4	A	3.5	0.47	14.4
5	Li_2CO_3 solid	33.4	A	14.8	0.44	13.5
6	Li_2CO_3 solid	25.0	C	10.0	0.40	12.2
7	Li_2CO_3 solid	33.4	A	14.9	0.45	13.8
8	$LiNO_3$ solution	16.7	C	6.7	0.40	12.2
9	$LiNO_3$ solution	33.4	C	18.9	0.57	17.5

[1] Recovery methods are; A) distillation of HF from H_2SO_4 solutions, B)ion exchange and C) precipitation of ^{18}F as an insoluble salt.
[2] EOB = end of bombardment
[3] percent of theoretical maximum ^{18}F production based on the estimate in reference 52. (3.26 MBq / mg of ^6Li at 1 x 10^{12} n cm^{-2} sec^{-1}).

Table IV.18 Production of ^{18}F with reactor thermal neutrons at a flux of 1 x 10^{12} n cm^{-2} sec^{-1} for 2 hours in the SLOWPOKE reactor

increased as in experiment 2, Table IV.18, where four 50 mg samples of Li_2CO_3 were irradiated rather than a single 200 mg sample. Increased yields were also obtained by reducing the effective density of Li_2CO_3 by dissolving it in dilute HNO_3. In addition to the self-shielding effect caused by the high thermal neutron cross-section of ^6Li, local flux pulling effects were noted when samples of 200 mg or more were irradiated in the reactor. These effects limited the amount of Li_2CO_3 which could be irradiated in the reactor. The maximum production of ^{18}F in the SLOWPOKE Reactor at the University of Alberta was estimated to be 148 MBq [50]. Practical considerations of sample size and irradiation times reduced the production of ^{18}F to about 15% of this theoretical maximum.

A number of recovery techniques were used to obtain ^{18}F in a form suitable for radiochemical synthesis. [^{18}F]-HF in anhydrous dioxane was the required reagent for the proposed reaction sequence outlined in Scheme IV.18. Three different procedures for sample work-up were used in this study. These were, distillation of volatile [^{18}F]-HF from an H_2SO_4 solution of irradiated Li_2CO_3, ion exchange of [^{18}F]-F^- with various anion exchange resins or precipitation of [^{18}F]-F^- as an insoluble salt followed by $^{18}F^-$ for F^- exchange.

Distillation method.

After irradiation, Li_2CO_3 contained ^{18}F in an unspecified chemical form (probably as LiF) and a substantial quantity of ^3H as a radionuclidic impurity[53]. The irradiated samples of Li_2CO_3 were reacted with concentrated H_2SO_4 in a small flask. The resulting solutions were heated at about 300 °C and purged with an inert gas (N_2, He) or a mixture of inert gas and non-radioactive carrier HF. Volatile ^{18}F was then trapped by bubbling the inert gas through cooled anhydrous dioxane held in a teflon or stainless steel reaction vessel. This method resulted in a 10 or 15% transfer of the total ^{18}F radioactivity to the dioxane solution.

Substantial ^{18}F activity remained as a non-volatile residue in the reaction flask. Additional activity was present on the polyethylene or teflon tubing between the reaction flask and the trapping solution. In addition to the low overall transfer of activity to the dioxane solution this method was further complicated by ^3H contamination in the dioxane solution and

by the presence of a non-volatile acidic residue which remained after evaporation of the solvent. The acidic residue may be H_2SO_4 transfered from the reaction flask.

Ion exchange method.

Irradiated Li_2CO_3 was added to an aqueous slurry of Dowex 50W X4 ion exchange resin. The aqueous layer was decanted from the resin and the ^{18}F activity was trapped by passage of the aqueous solution through a short teflon column containing Amberlite IRA-400 anion exchange resin (F^- form). This procedure trapped 80% of the ^{18}F activity on the resin. Alternatively irradiated $LiNO_3$ solutions were slurried directly with Amberlite IRA-400 resin and the slurry transferred to a short teflon column. The resin was washed in succession with distilled water, methanol, dry diethyl ether and dry dioxane. The ^{18}F was eluted from the resin with a solution of HF in anhydrous dioxane. The resulting [^{18}F]-HF in dioxane was used in the synthetic studies. The ^{18}F activity adhered tightly to the resin and it was necessary to use 1 M HF in dioxane as the eluting solvent. The effluent contained 25 to 30% of the resin-bound activity in about 3 mL of 1 M HF in dioxane. This reagent was relatively free of ^3H contamination but a small amount of solid residue was obtained on evaporation of the solvent.

Precipitation method

The majority of ^{18}F activity could be removed from irradiated Li_2CO_3 by washing the solid with small volumes of cold water. Several milligrams of NaF were dissolved in the aqueous ^{18}F solutions thus obtained and the [^{18}F]-F^- was then precipitated as CaF_2 by the addition of excess $Ca(NO_3)_2$ solution. Alternatively, [^{18}F]-CaF_2 was precipitated directly from solutions of irradiated $^6LiNO_3$ by the addition of NaF and $Ca(NO_3)_2$. The precipitate was isolated by centrifugation, washed several times with small volumes of cold water to remove traces of ^3H and then washed sequentially with acetone and dry ether. About 60% of the ^{18}F activity originally in the irradiated Li_2CO_3 was present in the [^{18}F]-CaF_2. This precipitate was transferred to a stainless steel reaction bomb, mixed with 1 mL of 0.25 M HF in anhydrous dioxane, sealed and heated at 160 to 170 °C for 45 minutes. This procedure resulted in about 30% of the [^{18}F]-CaF_2 activity being transferred to the dioxane solution, presumably as

[^{18}F]-HF. The remaining activity was found in the precipitate of CaF_2 (6%) or adhering to the wall of the reaction vessel (64%).

The reactor production methods for ^{18}F available at the University of Alberta, although suitable for trial synthetic studies, did not seem likely to provide sufficient radionuclide to permit the synthesis of radiolabelled nucleosides for *in vivo* animal studies. A number of factors contributed to the unsuitability of this production method.

1. The processing time to convert irradiated Li_2CO_3 to the required ^{18}F precursor ranged from 1 to 2 half-lives of ^{18}F depending on which of the three methods described above was employed.
2. The overall conversion to the required precursor was moderate or low.
3. Additional processing of the target material was required to remove ^{3}H contamination and the [^{18}F]-HF in dioxane solution often contained ^{3}H as well as non-radioactive contaminants.
4. The neutron flux available in the SLOWPOKE nuclear reactor and practical considerations of irradiation times and sample sizes limited the quantities of ^{18}F that could be produced.
5. Substantial amounts of carrier fluorine were required for the processing steps. The resulting [^{18}F]-HF in dioxane solutions were of low specific activity.
6. The reagent required for the reaction outlined in Scheme IV.18 was [^{18}F]-HF in *anhydrous* dioxane but the processing steps used to recover the ^{18}F made it difficult to maintain the reagent in an anhydrous state.

In addition to these factors the overall yield in the synthetic sequence even under optimum conditions with non-radioactive reagents was only moderate (40 to 50%)[194,195].

Accelerator production and recovery of [^{18}F]-HF.

The Van de Graaff Accelerator at the University of Alberta was used to prepare ^{18}F for the synthesis outlined in Scheme IV.18. The Van de Graaff was capable of accelerating ^{4}He (α), ^{3}He, ^{2}H (deuterons, d) and ^{1}H (protons, p) through a potential difference of 7 million

volts (MV). There are a variety of nuclear reactions for producing ^{18}F using these particles, of which the most suitable for our purposes was the $^{20}Ne(d,\alpha)^{18}F$ reaction. This reaction possesses a number of advantages. It uses natural abundance neon which is a cheap, readily available, easily purified and chemically inert target material. Neon also has a relatively large cross section for interaction with ^{2}H at a fairly low particle energy (225 millibarns (mb) maximum at 6.3 MeV)[66,67] and at low particle energies (<9 MeV) no radionuclidic impurities are formed[66]. A more efficient production method for ^{18}F would be the nuclear reaction $^{18}O(p,n)^{18}F$ (575 mb maximum at 5.3 MeV)[67,82] which has been used with ^{18}O-enriched water[79-81] or $[^{18}O]$-O_2[92,113]. Unfortunately this is a very expensive target material which must be recovered from the target holder after each production run.

The Van de Graaff had the potential to produce substantial quantities of ^{18}F. Although it was not designed for radionuclide production this accelerator had been adapted for this purpose in a previous study involving the production of ^{18}F and ^{11}C [309]. The major problem was the requirement for adequate shielding of personnel from the prompt and delayed radiation produced in the radionuclide production. This problem was overcome by the use of a heavily shielded concrete bunker in the basement of the accelerator building. The details of this facility are described in reference 309.

The accelerator produced a beam of deuterons with an energy of 7 MeV and a beam current of 20 microamperes (μA). In actual production runs the available beam energy was somewhat lower (6.5 MeV). It was reduced further upon passage through the metal foils isolating the target gas from the beam line (see Appendix 3). With a deuteron beam of approximately 6 MeV entering the target, the expected production of ^{18}F was about 1.0 GBq / μA at saturation activity[66,97]. (Appendix 3 contains a sample calculation and a discussion of this value.) For an irradiation time of 2 hours which is just longer than the half-life of ^{18}F, the activity produced would be about 0.5 GBq / μA measured at the end of bombardment.

Two target assemblies were constructed during this study. A copper target was constructed for the preparation of $[^{18}F]$-HF. This target was based on a fluorine recovery

methodology developed by Dahl and co-workers[93,94]. Copper was shown to release ^{18}F as no-carrier-added [^{18}F]-HF more readily than a variety of other metals when heated in the presence of 10% H_2 in He[94]. Copper had the added advantages of being easy to machine and was relatively inexpensive. A second target to be used for the production of [^{18}F]-F_2 was constructed from nickel, based on the target design developed by Wolf and co-workers at Brookhaven[97]. This design and the ^{18}F recovery methodology have found wide application in the preparation of ^{18}F-labelled precursors and biologically important molecules[100-112].

The copper target and the system for the production and recovery of [^{18}F]-HF are shown in Figure III.2 and Figure III.3. The details of their construction and operation are given in the Experimental section. This target system was used for 11 successful production runs of [^{18}F]-HF. Recovered yields of ^{18}F in these reactions varied from 7.92 MBq to 1.69 GBq. The yields and production details are presented in Table IV.19.

The experiments 1 to 4 shown in Table IV.19 were used to make various refinements in the irradiation parameters, target preparation and recovery procedures. The ^{18}F produced was analyzed for radionuclidic impurities using a number of test systems. Aliquots of the recovered activity were analyzed by a half-life measurement using a Digital Equipment Corporation LSI-11-03 Microcomputer interfaced with an Ortec 490-B Amplifier and a Canberra 3 x 3 inch NaI crystal well detector. The 511 keV annihilation γ-ray was measured starting at 3 hours after the end of bombardment (EOB). The graph of time against log of the count rate was linear over 8 hours and gave a half-life value of 110 minutes (literature value for $T_{\frac{1}{2}}$ of ^{18}F = 109.7 minutes)[20]. The γ-ray spectrum was recorded at 3 hours after EOB using a Canberra Series 80 Multichannel Analyzer and a GeLi Detector. The only emission detected was the 511 keV γ-ray. Liquid scintillation counting of an aliquot of this sample at 4 days after EOB showed no trace of β-emitting radionuclides. Further confirmation that the activity produced was $^{18}F^-$ was obtained when an aliquot of the activity recovered from the trap in normal saline (1.0 MBq) was injected *via* the tail vein into a normal male Wistar rat. The rat was imaged 45 minutes after injection using the Pho / Gamma Camera equipped with a positron head. The

EXPERIMENT	CURRENT[1] (μA)	ENERGY[2] (MeV)	TIME hr	ACTIVITY (GBq) RECOVERED	ACTIVITY (GBq) PRODUCED	THEORY[3]	% OF THEORY
1	3	6.7-7.0	2	0.019	0.056	1.50	3.7
2	5	6.8-7.0	2	0.008	0.011	2.50	0.4
3	5	6.5	1	0.103	0.204	1.59	12.8
4	5	6.5	1	0.063	0.204	1.59	12.8
5	12-15	6.3-6.5	2	0.284	0.740	7.50	9.9
6	15-18	6.8-7.0	2	0.189	0.370	9.00	4.1
7	18-20	6.8-6.9	2	1.240	2.480	10.00	24.8
8	18-20	6.8	2	0.925	1.850	10.00	18.5
9	18-20	6.8-6.9	2	0.685	1.850	10.00	18.5
10	18-20	6.9	2	1.691	3.380	10.00	33.8
11	15-18	7.0	1.16	1.228	2.456	5.80	42.3

[1] Deuteron beam current measured as microamperes (μA)
[2] Deuteron beam energy
[3] Based on a saturation yield of 1 GBq / μA at 6 MeV beam current (see Appendix 3 for a sample calculation)

Table IV.19 Irradiation conditions and yields of ^{18}F produced by the deuteron bombardment of neon

positron scans indicated accumulation of activity in the bones, especially at the ends of the long bones, at the knees and shoulders as well as in the skull and spinal column. This result is in agreement with the recognized distribution pattern of the fluorine anion reported for *in vivo* studies with animals[36] and humans[38-40].

The remaining experiments listed in Table IV.19 were used to prepare anhydrous [^{18}F]-HF in dioxane as required for the synthesis of [2'-^{18}F]-5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil. The actual activity of ^{18}F produced was well below the theoretical expectation. A variety of factors contributed to this shortfall in activity. It was difficult to maintain both deuteron beam energy and beam current during the production runs and the 2 hour irradiation times often included periods during which one or the other or both of these parameters were reduced. In addition the geometry of the deuteron beam did not appear to be ideal and there was evidence for a sharp rather than a diffuse beam profile. Spreading of the beam inside the target and uncertainties in the measurements of the beam current and beam energy may also have accounted for the difference between the theoretical and the observed production of ^{18}F. The total activity produced was determined by measurement of the γ-field at a point on the target surface before and after the recovery procedure and then relating this difference in field intensity to the recovered activity. The recovered activity was accurately measured in a dose calibrator. This determination would also be subject to error.

Optimum recovery of ^{18}F was obtained by heating the target and recovery lines to 300 °C and 175 °C respectively and by using sequential flushes of the target with 10% H_2 in He and 1% HF in He. The resulting [^{18}F]-HF was trapped in a cooled section of 1/8 inch teflon or copper tubing positioned in a shielded and cooled lead transporter. The use of a 1% HF in He flush added about 25 μmol of cold carrier fluorine as HF to the ^{18}F. The recovery procedure and transportation of the ^{18}F to the chemistry lab required about 90 minutes. The specific activity of the [^{18}F]-HF at the time of its arrival in the radiochemistry lab varied from about 5.56 GBq / mmol to 39 GBq / mmol.

A variety of methods were used to recover [^{18}F]-HF from the trapping coils. The activity was washed from the coil with saline solution or dilute aqueous acid (HOAc) or base (NH_4OH). Anhydrous [^{18}F]-HF in dioxane was obtained by flushing the trapping coil with HF gas. The [^{18}F]-HF was then trapped in cooled anhydrous dioxane. Alternately the apparatus depicted in Figure III.4 was employed and the [^{18}F]-HF removed from the coil by passing a solution of HF in dioxane through the assembly. The teflon coil was found to release activity more readily to flushing gasses and liquids than copper and the all-teflon assembly shown in Figure III.4 was eventually adopted as the most suitable apparatus for [^{18}F]-HF recovery. More than 90% of the activity in the teflon coil was transferred to the reaction vial using 3 mL of 1 M HF in dioxane.

In an additional six experiments not listed in Table IV.19, ^{18}F was not produced or could not be recovered from the target due to perforation or rupture of the molybdenum foils at some point during the irradiation.

Accelerator production and recovery of [^{18}F]-F_2.

As indicated in Scheme IV.18, [^{18}F]-F_2 could be used to convert 1-(2'-fluoro--2'-deoxy-β-D-ribofuranosyl)uracil (17) to [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β--D-ribofuranosyl)uracil (95). This reaction was virtually quantitative for the analogous non-radioactive synthesis. A number of workers have prepared [^{18}F]-F_2 using the target methodology described by Wolf and co-workers[97-99] and a target system has also been described for use with a Van de Graaff accelerator[114]. The success of these targets has been reported to be dependent on the construction and inner surface preparation of the target gas holder[97,98] and on the composition and purity of the target gas[99].

Details of the nickel target used in the present study are given in the experimental section. The target system and gas delivery and recovery lines are shown in Figure III.5. The inside surface of the target was polished and degreased with distilled acetone and carbon tetrachloride and passivated by filling with F_2 at 1 atmosphere and heating at 180 °C for 16

hours. The resulting surface coating of NiF_2 provided a surface inert to further attack by fluorine but sensitive to traces of moisture[97]. The NiF_2 may also be responsible for exchange of non-radioactive fluorine for ^{18}F during the irradiation and recovery procedures[94,113]. The target gas was a mixture of ultra-high purity neon (99%) and high purity fluorine (1%) specially prepared by Matheson Gas Products. The target and gas delivery lines were degassed under high vacuum (1×10^{-4} torr) and flushed with ultra-high-purity neon before filling with the target gas (1% F_2 in Ne).

A number of trial irradiations were carried out with this target system. The ^{18}F activity was trapped in an acetic acid solution using the apparatus depicted in Figure III.5. This was accomplished by slowly venting the gas pressure through the solution followed by several flushes of the target with 1% F_2 in Ne. In addition to acetic acid the trapping solutions sometimes contained sodium acetate which has been shown to react with [^{18}F]-F_2 to form ^{18}F-labelled acetyl hypofluorite [^{18}F]-CH_3COOF[103,141]. These solutions were used in the radiochemical synthesis of [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (95) from 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) as described later.

Low overall production and recovery of ^{18}F from this target system was related in part to the factors already discussed with reference to the [^{18}F]-HF target. Problems encountered with the deuteron beam stability tended to limit the irradiation times and the beam energy and current that were available. Literature reports have demonstrated that 50% to 80% of the ^{18}F activity could be removed from the target by purging the target gas through a trapping solution[105,114,141]. In the trial reactions the majority of the activity could not be removed from the target despite several flushes of the target with 1% F_2 in neon. This may have indicated that the nickel surface passivation was incomplete and that ^{18}F was reacting with the nickel surface. The purged activity was not completely trapped by the acetic acid solutions and volatile ^{18}F activity was detected in the vacuum and venting lines. It was unlikely that this activity was [^{18}F]-F_2 which would be trapped in the acetic acid solution or absorbed by the KOH trap. It has been demonstrated that irradiation of F_2 / Ne gas mixtures containing gaseous impurities such

as N_2, CO_2 and CH_4 produces NF_3 and CF_4 with consequent loss of [^{18}F]-F_2 activity[99]. Species such as [^{18}F]-NF_3 or [^{18}F]-CF_4 may account for the volatile activity detected in these experiments.

Synthesis with ^{18}F.

The [^{18}F]-HF in anhydrous dioxane produced from reactor and accelerator sources was reacted with 2,2'-anhydrouridine (16) to form [2'-^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) as outlined in Scheme IV.18. The analogous reaction carried out on large scale with non-radioactive HF in dioxane gave a chemical yield of 40% but also demonstrated strong dependence on the reaction conditions. The presence of traces of water in the reactants gave rise to 1-β-D-arabinofuranosyluracil (65) rather than the required 2'-fluoronucleoside (17). Raising or lowering the reaction temperature resulted in the formation of decomposition products or unreacted starting material (see Scheme IV.2). Optimum yields for the preparation of non-radioactive 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) were obtained using a large excess of HF and an 18 hour reaction time[195]. These conditions were not suitable for radiochemical synthesis with the relatively short-lived ^{18}F ($T_{\frac{1}{2}}$ = 109.7 minutes). Yield optimization studies were undertaken in an attempt to maximize the yield with reactant concentrations and reaction times more suitable for synthesis with ^{18}F. The results of this investigation are presented in Table IV.20 taken from reference 206.

Examination of the results in Table IV.20 indicated acceptable yields of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) were obtained using 10 equivalents of HF for a reaction time of 1 hour and a temperature of 165 °C. These reaction conditions were more suitable for reactions with ^{18}F although the maximum yield of [2'-^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) would be about 3% relative to the [^{18}F]-HF used.

Trial reactions with reactor produced [^{18}F]-HF in anhydrous dioxane using the optimum conditions from Table IV.20 were disappointing. In these reactions only a trace of [^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) was detected in the product

mixtures. The poor yield (<<1%) was related to the low specific activity of ^{18}F in the [^{18}F]-HF solutions and to the presence of traces of moisture and other impurities in this reagent. Accelerator produced [^{18}F]-HF required fewer processing steps and provided a dioxane solution free of contaminants. The specific activity of this solution was however still fairly low. The reaction providing the best results in this series gave a 0.43% radiochemical yield of [^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) using a 77 fold excess of [^{18}F]-HF in anhydrous dioxane for a reaction time of 2 hours and a reaction temperature of 150 °C. At the end of the synthesis (4 hours after EOB) this product had an activity of 1.7 MBq and a specific activity of 0.32 GBq / mmol.

MOLAR RATIO[1]	CONCENTRATION OF HF (mM)	TIME (min)	TEMP (°C)	YIELD (%)
2	2	30	165	9
5	5	30	165	22[2]
5	5	60	165	27
10	10	30	165	29[2]
10	10	60	165	33
20	14.3	40	165-210	32

[1] Ratio of μmol of HF to μmol of 2,2'-anhydrouridine (16). All reactions were carried out using 1.0 μmol of 2,2'-anhydrouridine. The reaction vessel was a teflon-lined steel bomb. Product yields were determined by preparative hplc using a Merck B silica gel column.
[2] mean value of two trials.

Table IV.20 Yield optimization studies for the reaction of 2,2'-anhydro-uridine (16) and hydrogen fluoride in anhydrous dioxane[206]

The ^{18}F labelled 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) must be elaborated further to obtain [2'-^{18}F]-5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils 94a-d required for the *in vivo* studies. A further reduction in overall yield and further decay of the ^{18}F activity would take place during this final synthetic step. It seemed unlikely that sufficient ^{18}F labelled 94a-d would be obtained without substantial improvement in the

radiochemical yields for the synthesis of [2'-^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (93)

The synthesis of [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (95) was also investigated. Direct fluorination at the 5-position of pyrimidine nucleosides generally gave good yields of the 5-fluoronucleosides as discussed previously. For example the reaction of [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (78) and a solution of F_2 in HOAc gave a high yield (>90%) of [2-^{14}C]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (87) (see Scheme IV.13). Solutions of [^{18}F]-F_2 in HOAc, obtained from the Van de Graaff production and recovery procedure described earlier, were allowed to react with 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) and the reaction mixture was worked up as described for the non-radioactive synthesis. The residue was examined by tlc. The preliminary studies indicated a very low incorporation of ^{18}F into [5-^{18}F]-5-fluoro--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (95) (<<1%). The majority of ^{18}F in the acetic acid solutions appeared to be in the form of a volatile compound that was removed along with the solvent in the evaporation step. The ^{18}F activity was probably not [^{18}F]-F_2 since this reagent would be expected to react readily and rapidly with 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) as observed for the non-radioactive synthesis. It is more likely that the soluble activity represents an ^{18}F species such as [^{18}F]-CF_4 or other fluorinated hydrocarbons arising during the irradiation due to the presence of hydrocarbon impurities in the target system or target gas. This synthetic procedure does appear to have potential provided [^{18}F]-F_2 can be readily prepared. Recently Wolf and co-workers prepared [5-^{18}F]-5-fluoro-2'-deoxyuridine in 21.7% radiochemical yield using a similar reaction to the one described here[141]. In another study [5-^{18}F]-labelled pyrimidine nucleosides and pyrimidine bases were prepared in 10% to 20% radiochemical yield for use in imaging studies employing tumor bearing rats and rabbits[112].

V. CONCLUSIONS

The primary aim of this study was to evaluate the therapeutic potential of the nucleoside analogs 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a - d) and 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) as agents for scintigraphic evaluation of tumors. The results of this study can be summarized under 6 headings:

1. Non-radioactive syntheses
2. Radioactive syntheses for *in vivo* distribution studies
3. *In vivo* studies in murine tumors
4. ^{18}F Production
5. Radioactive syntheses for *in vivo* imaging studies
6. Potential of investigated compounds as non-invasive diagnostic imaging agents in oncology

1. Non-radioactive synthesis.

A series of 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (1a - d) were prepared in good to excellent yield from 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) (see Table V.1). Thus 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) was obtained in 89% yield, 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b) was obtained in 42% yield, 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c) was obtained in 52% yield and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) was obtained in 94% yield. The starting material for these reactions, 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17), was prepared in two steps from uridine in 34% overall yield. The nucleoside analog 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) was prepared in 59% overall yield from 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a). The chemical purity and identity of all compounds was confirmed by chromatographic and spectroscopic analysis.

COLD COMPOUND[1]	CHEMICAL YIELD(%)[2]	RADIO-LABELLED ANALOG	RADIO-CHEMICAL YIELD(%)	SPECIFIC ACTIVITY GBq/mmol[4]	MAXIMUM TUMOR TO BLOOD RATIO	MAXIMUM TUMOR INDEX[6]
5-iodo (1a)	89	5-^{123}I (67)	67	45.9[5]	0.5 (8 hr)	1.5 (2 hr)
5-bromo (1b)	42	5-^{77}Br (72)	41	0.17	0.86 (1 hr)	0.74 (15 min)
5-chloro (1c)	52	2-^{11}C (79)	77	1.86	4.2 (4 hr)	1.9 (15 min)
5-fluoro (1d)	94	2-^{11}C (87)	92	1.86	10.3 (4 hr)	9.4 (1 hr)
2'-fluoro (17)	41	2-^{11}C (78)	25	1.86	-	-
3'-iodo (2)	59	3'-^{123}I (92)	91	128	1.34 (15 min)	0.72 (15 min)
2',3'-epoxy (24a)	30[3]					

[1] The compounds are: 1a; 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 1b; 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 1c; 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 1d; 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 17; 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil, 2; 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil and 24a; 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil.
[2] Chemical yield (%) for the halogenation reaction (non radioactive synthesis).
[3] Overall yield in a 5-step synthesis from uridine (see reference 240).
[4] Specific activity measured at the end of synthesis and purification.
[5] Also prepared as a no-carrier-added product with a theoretical specific activity of 600 TBq / mmol.
[6] Tumor index is the product of percent of injected dose per gram of tissue and the tumor to blood ratio (see also reference 340).

Table V.1 Summary of data for the cold and radiolabelled synthesis and the tumor uptake of 5-iodo-, 5-bromo-, 5-chloro- and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a - d) and of 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2).

2. Radioactive syntheses for *in vivo* distribution studies.

Two approaches were used to prepare the radiolabelled analogs for a series of 5-halo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracils (see Table V.1). Radiolabelled analogs of 5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) and 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b) were prepared in 67% and 41% radiochemical yield respectively by the reaction of 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (17) and $^{131}I^{+}$ and $^{82}Br^{+}$ respectively. Radiolabelled analogs of 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c) and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) were prepared from [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (78) in 77% and 92% yield respectively. The ^{14}C labelled precursor, [2-^{14}C]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (78), was prepared in a multistep synthesis with [^{14}C]-barium carbonate as the radionuclide source. The overall yield of 78 from [^{14}C]-barium carbonate was about 6%. The radiolabelled analog of 1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2) was prepared in 91% radiochemical yield from 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (24a) and [^{123}I]-HI.

3. *In vivo* studies in murine tumors.

Two of the compounds examined, 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1c) and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d), were taken up selectively in tumor tissue in Lewis lung tumor bearing BDF$_1$ mice, reaching a maximum tumor to blood ratio of 4.2 and 10.3 respectively at 4 hours. The concentration of activity at this time exceeded that in all other tissues except the spleen. The other compounds examined showed no uptake into Lewis lung tumor tissue (5-iodo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1a) and 5-bromo-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1b)) or into Walker 256 tumor (1-(3'-iodo-3'-deoxy-β-D-arabinofuranosyl)uracil (2)). The 5-halonucleosides 1a - d were characterized by rapid overall excretion of activity and a high *in vivo* stability. These modified nucleosides were not good substrates for phosphorylase enzymes.

4. ^{18}F Production.

The SLOWPOKE Reactor facility was employed to prepare up to 23 MBq of ^{18}F by the nuclear reaction $^{6}Li(n,^{3}H)^{4}He$, $^{16}O(^{3}H,n)^{18}F$ using enriched [^{6}Li]-Li_2CO_3 or [^{6}Li]-$LiNO_3$ as target material. The Van de Graaff Accelerator was used to produce up to 3.38 GBq of ^{18}F with the nuclear reaction $^{20}Ne(d,\alpha)^{18}F$ using natural abundance neon as the target. The irradiated targets from both the reactor and accelerator production of ^{18}F were processed to yield [^{18}F]-HF which was used in subsequent synthetic procedures. Accelerator-produced [^{18}F]-HF was superior to reactor-produced product with respect to absolute quantity, recovery yield from targets, ease of preparation and chemical purity. Accelerator-produced ^{18}F was also recovered as [^{18}F]-F_2 although subsequent evidence suggested that this species was of low chemical purity.

5. Radioactive syntheses for *in vivo* imaging studies.

The reaction of reactor-produced [^{18}F]-HF in anhydrous dioxane and 2,2'-anhydro-uridine (16) yielded [2'-^{18}F]-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) in very low chemical yield. The same reaction using accelerator-produced [^{18}F]-HF gave [2'-^{18}F]--1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (93) in 0.43% radiochemical yield. The reaction of accelerator-produced [^{18}F]-F_2 and 1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil (17) gave [5-^{18}F]-5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (95) in <1% radiochemical yield.

6. Potential of investigated compounds as non-invasive diagnostic imaging agents in oncology.

Two of the compounds investigated, 5-chloro-1-(2'-fluoro-2'-deoxy-β-D-ribofurano-syl)uracil (1c) and 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)uracil (1d) demonstrated substantial relative accumulation in the tumor tissue of test animals. The Emrich tumor indicies (see Table V.1) were 1.9 (15 minutes) for 1c and 9.4 (1 hour) for 1d. Low absolute levels of activity in the tumor was due to rapid excretion of these compounds and

might be overcome by altering the rate or route of administration of the radiopharmaceutical. In particular the high relative uptake of 5-fluoro-1-(2'-fluoro-2'-deoxy-β-D-ribofuranosyl)-uracil 1d into Lewis lung tumor tissue commends this compound for further investigation.

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APPENDICES

A. Appendix 1. Characteristics of Selected Radionuclides

NUCLIDE	SYMBOL	HALF-LIFE	DECAY MODE[1]	MAJOR RADIATION[2] (MeV)
tritium	^{3}H	12.26 yr	β^-	β 0.019(100%)
carbon-11	^{11}C	20.34 min	β^+ EC	γ 0.511(200%)
carbon-14	^{14}C	5730 yr	β^-	β 0.156(100%)
nitrogen-13	^{13}N	9.96 min	β^+	γ 0.511(200%)
oxygen-15	^{15}O	123 sec	β^+ EC	γ 0.511(200%)
fluorine-18	^{18}F	109.7 min	β^+ EC	γ 0.511(194%)
neon-18	^{18}Ne	1.5 sec	β^+	γ 0.511(200%)
chlorine-34	^{34}Cl	1.56 sec	β^+	γ 0.511(200%)
chlorine-34*m*	$^{34}Cl^m$	31.99 min	β^+	γ 0.511(106%) γ 0.145(47%) γ 2.12(28%)
chlorine-36	^{36}Cl	3×10^5 yr	β^- EC	β 0.714(98.1%)
chlorine-38	^{38}Cl	37.29 min	β^-	β 4.91(53%) γ 1.60(38%) γ 2.170(47%)
chlorine-39	^{39}Cl	55.5 min	β^-	β 1.91(85%) β 2.18(8%) β 3.45(7%) γ 0.246(43%) γ 1.27(50%) γ 1.52(42%)

cobalt-57	^{57}Co	270 day	EC	γ 0.122(87%) γ 0.136(11%)
gallium-67	^{67}Ga	77.9 hr	EC	γ 0.093(40%) γ 0.184(24%) γ 0.296(22%)
gallium-68	^{68}Ga	68.3 min	β^+ EC	γ 0.511(176%)
bromine-75	^{75}Br	1.7 hr	β^+ EC	γ 0.511(180%)
bromine-76	^{76}Br	16.1 hr	β^+ EC	γ 0.511(133%) γ 0.559(63%) γ 0.650(19%) γ 1.21(13%) γ 1.86(11%)
bromine-77	^{77}Br	57 hr	EC β^+	γ 0.240(30%) γ 0.52(24%)
bromine-80	^{80}Br	17.6 min	β^- β^+ EC	β 2.00(92%)
bromine-80m	$^{80}Br^m$	4.38 hr	IT	γ 0.037(36%)
bromine-82	^{82}Br	35.34 hr	β^-	β 0.444(100%) γ 0.554(66%) γ 0.619(41%) γ 0.698(27%) γ 0.777(83%) γ 0.828(25%) γ 1.044(29%) γ 1.317(26%) γ 1.475(17%)
bromine-82m	$^{82}Br^m$	6.05 min	IT β^-	β 3.138(2.4%)
technetium-99m	$^{99}Tc^m$	6.05 hr	IT	γ 0.140(90%)
indium-111	^{111}In	2.81 day	EC	γ 0.173(89%) γ 0.247(94%)
indium-113m	$^{113}In^m$	99.8 min	IT	γ 0.393(64%)
iodine-123	^{123}I	13.3 hr	EC	γ 0.159(83%)

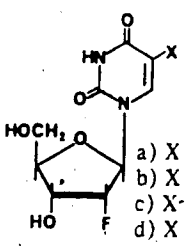
iodine-125	^{125}I	60.2 day	EC	γ 0.035(7%)
iodine-131	^{131}I	8.05 day	β^-	β 0.608(87%) γ 0.364(82%)
ytterbium-169	^{169}Yb	31.8 day	EC	γ 0.063(45%) γ 0.110(18%) γ 0.131(11%) γ 0.177(22%) γ 0.198(35%) γ 0.308(10%)

[1] Decay modes: β^- = beta particle decay, EC = electron capture, β^+ = positron decay, IT = internal transition

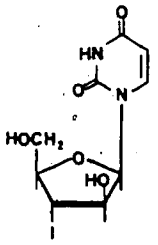
[2] Maximum energy (E-max) values given for β^--particle decay

Data for this table is taken from reference 20.

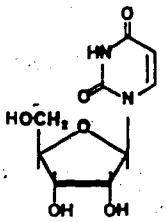
B. Appendix 2. Selected Structural Formulae


a) X = I
b) X = Br
c) X = Cl
d) X = F

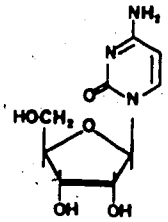
1



2



10



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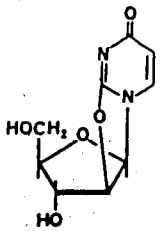

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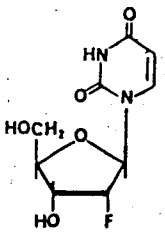

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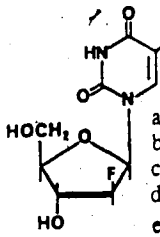
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c) X = Cl
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e) X = I
f) X = CH_3

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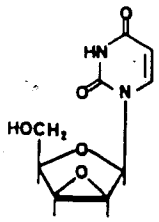

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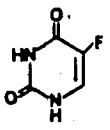

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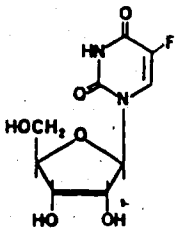

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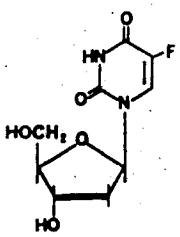

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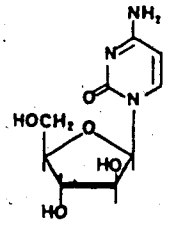

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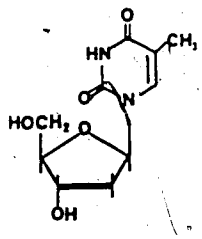

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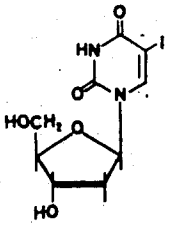

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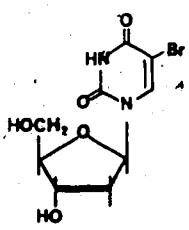

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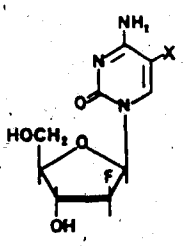
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c) X = Cl
d) X = Br
e) X = I

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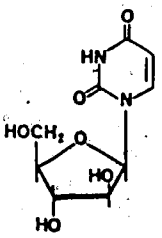

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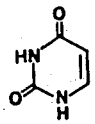

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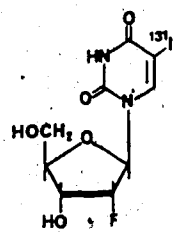

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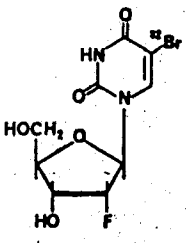

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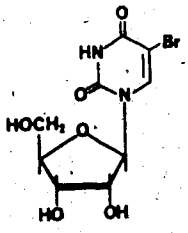

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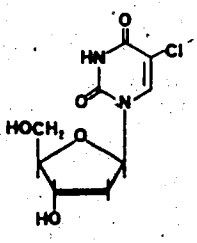

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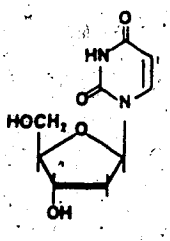

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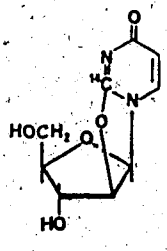

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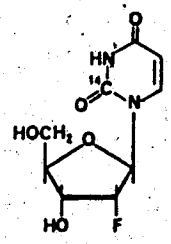

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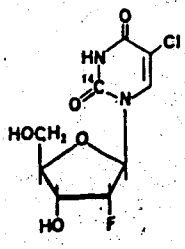

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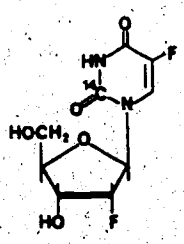

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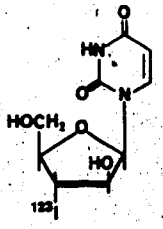

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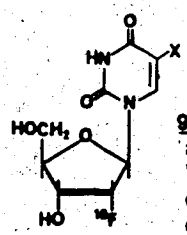
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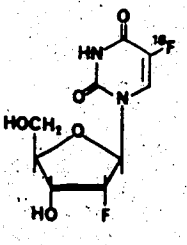
a) X = I
b) X = Br
c) X = Cl
d) X = F

94



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C. Appendix 3. Nuclear Physics Calculations

Yield calculation for a nuclear reaction.

The formula for the calculation of the yield in a nuclear reaction is given in (1) where A = the activity in nuclei per second, I = the number of bombarding particles per second or particle flux (particles / sec), x = the number of target nuclei per cm^2, δ = cross section in cm^2 and $(1 - \exp(-\lambda t))$ is a decay term to correct for the decay of the produced radionuclide during the production reaction. The decay term approaches unity as t becomes large.

$$\frac{dN}{dt} = A = I \times \delta (1 - \exp(-\lambda t)) \qquad (1)$$

The value for x is determined from the density of the target material and the thickness of the target material with a correction made for the isotopic abundance of the target nuclei if a mixture of isotopes is present. With our target system 22.25 cm in length the value for x is 3.6×10^{-2} gm cm^{-2}. This value is based on the assumption that the effective range of the incident particles is greater than 3.6×10^{-2} gm cm^{-2}. In fact the effective range for our particle energy (6 Mev → 2 Mev) is 3.2×10^{-2} gm cm^{-2} or 8.8×10^{20} nuclei cm^{-2} [345]. Our target may therefore be regarded as a thick target since the incident energy is degraded from 6 MeV to below the cross section threshold (2 MeV).

The cross section of interaction between deuterons at 6 MeV and ^{20}Ne has been measured as 215 millibarns (mb) = 215×10^{-27} cm^2 [66]. For 1 microampere (μA) of beam current the deuteron flux would be 6.3×10^{12} sec^{-1}. The activity produced under these conditions can be calculated from (1);

$$A = 6.3 \times 10^{12} \text{ sec}^{-1} \times 8.8 \times 10^{20} \text{ nuclei cm}^{-2} \times 215 \times 10^{-27} \text{ cm}^2 (1 - \exp(-\lambda t))$$

$$A = 1.19 \times 10^{9} \text{ nuclei sec}^{-1} (1 - \exp(-\lambda t))$$

As t becomes large the decay term $(1 - \exp(-\lambda t))$ approaches unity and the saturation activity is reached. The saturation activity would be 1.19×10^9 nuclei sec^{-1} or 1.19 GBq per μA

of current at 6 MeV. In the above calculation the assumption has been made that a uniform cross section of interaction exists as the particle beam is degraded from E_1 to 0 within the target. In fact equation (1) is valid only for thin targets where the energy of the beam entering and leaving the target is unchanged. An accurate thick target yield calculation would require an integration of the cross section over the energy range $E_1 \rightarrow 0$ and would be calculated from equation (2).

$$A = I \times \int \frac{\delta(E) \quad dE}{(dE/dx)} \qquad (2)$$

The term dE/dx is a measure of the energy change of the particle beam with respect to its penetration into the target. Equation (2) is the saturation activity formula for a thick target. For more information on application of this yield formula see reference 346.

In practice the term within the integral is difficult to determine with any accuracy due to the complex nature of the relationship between cross section and beam energy. Yield calculations are often carried out using equation (3) and average cross section values $\bar{\delta}$ as determined by numerical integration of experimentally determined cross section curves[61,347].

$$A = I \times \bar{\delta} (1-(\exp(-\lambda t)) \qquad (3)$$

In addition, experimental determination of thick target saturation yields have been carried out over various energy ranges allowing graphical interpolation of data. The reaction of deuterons on ^{20}Ne has been measured over the energy range 0 to 16 MeV[66,97]. These data indicate that the saturation activity of ^{18}F that would be produced in a thick target by 6 MeV deuterons would be about 1 X 10^9 decays per second (dps) = 1 GBq / μA. To obtain this value from equation (3) would require a $\bar{\delta}$ value of 179 mb over the range of particle energy 6 MeV $\rightarrow$ 2 MeV.

Calculation of the reduction in deuteron beam energy on passing through metal foils.

Charged particles lose some of their kinetic energy on passage through matter, predominantly by inelastic collision with orbital electrons. To calculate the loss of deuteron beam energy as it passes through the two foils separating the Van de Graaff Accelerator beam line from the target gas one can use equation (4) and the graphical plots of stopping power as compiled by Anderson and Ziegler[345].

$$E(A) = D\,S\,d \qquad (4)$$

E(A) = beam energy absorbed on passing through a material with density D (atoms cm^{-3}) with a stopping power of s (electron volts / atoms cm^{-2}) and a thickness of d (cm).

D for molybdenum = 6.407×10^{22}

s = 7.5 electron volts (ev) / 10^{15} atoms cm^{-2} at a beam energy of 7 MeV (Reference 345).

d = 10.16×10^{-4} cm

E(A) then becomes:

$$E(A) = 6.407 \times 10^{22} \times 7.5 \times 10^{-15} \times 10.16 \times 10^{-4}$$

$$E(A) = 0.488 \text{ MeV}$$

The energy reduction for a 7 MeV beam of deuterons on passage through two molybdenum foils in the target system used in this work would be 0.488 MeV.