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Synthesis and Tumor Uptake of 5-^{82}Br- and 5-^{131}I-Labeled 5-Halo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracils

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A synthesis of 5-bromo- and 5-iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (3 and 4) and their 5-^{82}Br and 5-^{131}I analogues has been developed. The tissue distribution of the radiolabeled compounds in BDF_1 mice bearing Lewis lung tumors has been investigated. After injection of the radiolabeled analogues of compounds 3 and 4 there was a rapid initial excretion of activity. Compound 3 was excreted unchanged in the urine. Residual activity in mice after 4 h showed a distribution characteristic of bromide (Br^-). Compound 4 was excreted mainly as unchanged starting material with increasing amounts of iodide (I^-) detected at later time periods, in addition to 5-iodouridine and unidentified metabolites at shorter time periods. Both 3 and 4 demonstrated a remarkable in vivo stability relative to related 5-substituted nucleosides that do not contain the 2′-fluoro group. The tumor uptake was minimal, with only the 5-bromo analogue demonstrating a slight elevation in tumor to blood ratios relative to other tissues. Compounds 3 and 4 were shown to compete with thymidine for the same binding site in the transport of nucleosides across the cell membrane in mouse erythrocytes. The inhibition constants (K_i) show that the compounds were weak competitors of thymidine binding to pyrimidine nucleoside transporter compared to physiological nucleosides. Other evidence indicates that compounds 3 and 4 are not substrates for mammalian kinase enzymes.

Pyrimidine nucleosides satisfy many of the requirements of a good tumor diagnostic agent. For example, naturally occurring pyrimidine nucleosides are used as building blocks for DNA and RNA, and many pyrimidine nucleoside analogues become trapped in neoplastic tissue by mimicking this behavior. Compounds such as 5-bromo-2′-deoxyuridine and 5-iodo-2′-deoxyuridine are incorporated into the DNA of animal and human tumor cells.[1,2] The radiolabeled deoxythymidine analogue [^{131}I]-5-iodo-2′-deoxyuridine has been shown to accumulate in a variety of animal tumors[3-6] and has recently been labeled with ^{123}I to scintigraphically image experimental tumors.[7] 5-Fluoro analogues such as 5-fluorouracil and 5-fluoro-2′-deoxyuridine have demonstrated selective accumulation in animal[8-10] and human tumors.[11,12] A number of ^{18}F-labeled pyrimidine nucleosides have also shown promise as imaging agents in studies with experimental in vivo tumor models.[13] The related arabino configuration nucleosides, 5-iodo- and 5-bromo-1-(2-deoxy-β-D-arabinofuranosyl)uracil, have also shown biological activity. These compounds, originally synthesized by Fox and co-workers,[14] were shown to have both antiherpetic activity against herpes simplex type 1 and type 2 infected cells and cytotoxicity toward normal lymphocytic cells in culture.

A series of radiolabeled pyrimidine nucleoside analogues with the structure 5-halo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil have been synthesized and investigated for their potential as scintigraphic radiotracers in diagnostic oncology. The presence of the 2′-fluoro ribo substituent has been shown to confer biochemical stability to other pyrimidine nucleosides by making them less susceptible to phosphorylytic cleavage catalyzed by the enzyme pyrimidine phosphorylase.[15,16] Two of the 5-halo compounds in this series, 5-chloro and 5-fluoro-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil, were recently prepared as ^{14}C-labeled analogues and were shown to have a high tumor to blood ratio in Lewis lung tumor bearing mice.[16] We now describe an original synthesis of the compounds 3 and 4 and report the biological distribution of their radiohalogenated derivatives in BDF_1 mice bearing a Lewis lung carcinoma. A structural diagram equivalent to 3 and 4 was presented in an earlier paper on a series of 5-substituted nucleosides.[17] However, this structure appears to be in

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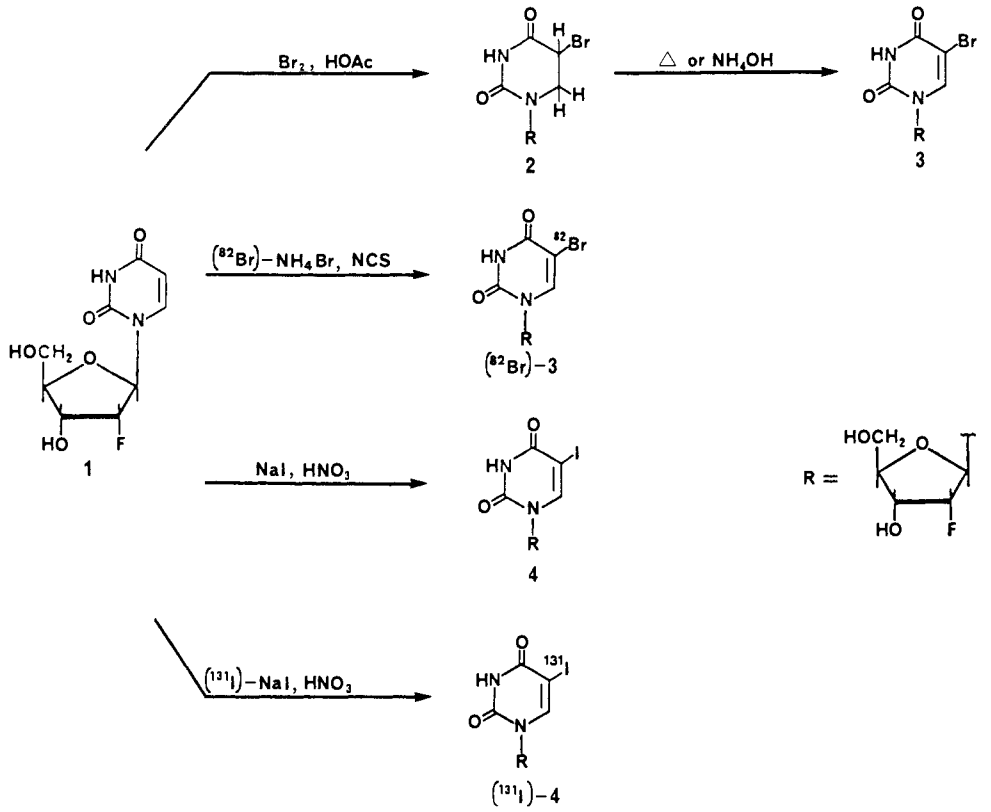
0022-2623/89/1832-1289$01.50/0

Scheme I



error since the accompanying text describes the synthesis and biological activity of the arabino configuration nucleosides.

Results and Discussion

Synthesis. The 5-bromo- and 5-iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracils (3 and 4) were prepared by electrophilic addition reactions as shown in Scheme I using 1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (1) as a precursor.

The classical reaction between electrophilic molecular bromine and the pyrimidine nucleoside[18,19] was used to synthesize the 5-bromo nucleoside 3 as shown in Scheme I. The stable dihydro intermediate 2 could be isolated as the major reaction product. This adduct was readily converted to the required nucleoside by heating or treatment with methanolic ammonia. Radiobromination was carried out with reactor-produced $NH_4{}^{82}Br$ with *N*-chlorosuccinimide as an oxidizing agent for in situ generation of electrophilic bromine. This procedure has been used for high-yield electrophilic radiobromination of aromatic rings. The reactive electrophilic species is believed to be $^{82}BrCl$.[20,21] The crude radiobrominated product was 80% pure. Purification of the crude material by preparative TLC gave a product of greater than 99% chemical and radiochemical purity with a specific activity of about 167 MBq/mmol at end of synthesis. Because of the relatively short half-life of ^{82}Br (35.3 h) the compound was used within 20–30 h after synthesis.

The 5-iodo compound 4 was synthesized in 89% yield according to an adaptation of the iodination method reported by Robins and Taylor.[7] In this reaction, I_2 produced in situ by oxidation of NaI with HNO_3 undergoes an electrophilic addition reaction with the electron-rich 5-position of the 2′-fluoro compound 1. After 16 h at 80 °C the brown color due to free iodine (I_2) had disappeared and the reaction was complete. At higher reaction temperatures (115 °C) the reaction time could be reduced to 90 min without a decrease in the yield. This reaction was also employed for the radiochemical synthesis of [^{131}I]-4. Chemical and radiochemical yields of greater than 60% in a "carrier-added" reaction and greater than 41% in a "no-carrier-added" synthesis were obtained. Purification of the carrier-added reaction product by TLC gave a compound of high chemical and radiochemical purity (>99%). The specific activity of the product depended on the amount of carrier iodine used in the synthesis and was generally in the range of 40–50 GBq/mmol at the time of its use in animal studies.

Tissue Distribution and Metabolism Studies. Animals were injected with doses of 47–130 μg of [^{82}Br]-5-bromo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil ([^{82}Br]-3) having ^{82}Br activities of 20–60 kBq. For the iodo compound [^{131}I]-4, doses of 0.5–1.8 μg with activities of 55–110 kBq were used. The data for the tissue distribution of these compounds are presented in Tables I and II as percent of injected dose per gram of tissue, and as organ to tumor ratios.

5-Bromo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (3) was excreted rapidly and unchanged from the BDF_1 mice after its intravenous injection. The whole-body radioactivity data presented in Figure 1 show that more than 50% of the injected dose was excreted within 15 min and 75% of the dose was excreted in 30 min. The whole-body time–activity curve conforms to a two-component (biexponential) model with component half-lives of 0.26 and 33.5 h. Analysis of urinary metabolites by HPLC showed

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Table I. Tissue Distribution of Radioactivity in BDF_1 Female Mice Bearing a Subcutaneous Lewis Lung Carcinoma after Intravenous Injection of [^{82}Br]-5-Bromo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil ([^{82}Br]-**3**)

	time, h						
organ	0.25	0.50	1	2	4	8	24
blood	2.68 ± 0.35[a]	1.36 ± 0.22	0.78 ± 0.06	0.79 ± 0.04	0.60 ± 0.07	0.72 ± 0.07	0.51 ± 0.06
	1.9[b]	1.4	1.2	1.4	1.6	1.6	1.6
spleen	1.06 ± 0.17	0.59 ± 0.13	0.38 ± 0.03	0.35 ± 0.02	0.28 ± 0.03	0.34 ± 0.06	0.24 ± 0.03
	0.8	0.7	0.6	0.7	0.8	0.8	0.8
stomach	1.29 ± 0.29	0.79 ± 0.14	0.50 ± 0.03	0.52 ± 0.12	0.40 ± 0.02	0.53 ± 0.08	0.34 ± 0.03
	0.9	0.9	0.8	0.9	1.1	1.2	1.1
gut[c]	1.19 ± 0.29	0.72 ± 0.12	0.42 ± 0.02	0.41 ± 0.41	0.28 ± 0.28	0.33 ± 0.33	0.22 ± 0.22
	0.9	0.8	0.6	0.8	0.9	0.7	0.7
kidney	7.69 ± 0.18	2.68 ± 0.43	1.12 ± 0.22	0.71 ± 0.02	0.48 ± 0.07	0.53 ± 0.06	0.36 ± 0.02
	5.5	3.0	1.8	1.3	1.4	1.2	1.1
skin	1.39 ± 0.20	0.72 ± 0.16	0.45 ± 0.11	0.39 ± 0.07	0.31 ± 0.05	0.34 ± 0.01	0.22 ± 0.04
	1.0	0.8	0.7	0.7	0.9	0.8	0.7
muscle	0.89 ± 0.22	0.40 ± 0.03	0.21 ± 0.01	0.19 ± 0.04	0.12 ± 0.01	0.14 ± 0.01	0.09 ± 0.01
	0.6	0.4	0.3	0.3	0.3	0.3	0.3
bone	0.52 ± 0.10	0.30 ± 0.06	0.26 ± 0.07	0.26 ± 0.05	0.17 ± 0.04	0.23 ± 0.02	0.14 ± 0.03
	0.4	0.3	0.4	0.5	0.5	0.5	0.5
lung	1.70 ± 0.17	0.91 ± 0.21	0.52 ± 0.08	0.54 ± 0.03	0.38 ± 0.03	0.46 ± 0.06	0.35 ± 0.04
	1.2	1.0	0.8	1.0	1.1	1.0	1.1
heart	1.61 ± 0.21	0.76 ± 0.12	0.44 ± 0.02	0.48 ± 0.05	0.49 ± 0.01	0.45 ± 0.02	0.48 ± 0.01
	1.2	0.8	0.4	0.5	0.5	0.5	0.5
liver	1.45 ± 0.22	0.65 ± 0.14	0.34 ± 0.02	0.32 ± 0.04	0.23 ± 0.01	0.28 ± 0.02	0.18 ± 0.01
	1.0	0.7	0.5	0.6	0.6	0.6	0.6
tumor	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
	0.5[d]	0.7	0.9	0.7	0.6	0.7	0.6

[a] The numbers represent the mean ± standard deviation for percent of injected dose per gram of wet tissue for six animals. [b] Organ to tumor ratio. [c] Section of intestine. [d] Tumor to blood ratio.

Table II. Tissue Distribution of Radioactivity in BDF_1 Female Mice Bearing a Subcutaneous Lewis Lung Carcinoma after Intravenous Injection of [^{131}I]-5-Iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil ([^{131}I]-4)

	time, h						
organs	0.25	0.50	1	2	4	8	24
blood	19.0 ± 1.3[a]	16.0 ± 1.1	13.3 ± 0.6	8.90 ± 0.7	8.5 ± 0.5	4.1 ± 0.4	0.16 ± 0.7
	3.9[b]	4.5	3.2	2.3	2.7	2.2	2.1
spleen	3.2 ± 0.2	2.9 ± 0.2	2.5 ± 0.1	1.8 ± 0.3	1.8 ± 0.1	1.0 ± 0.1	0.5 ± 0.5
	0.7	0.8	0.6	0.5	0.6	0.6	0.7
stomach	3.7 ± 0.6	4.6 ± 0.8	4.5 ± 1.1	4.0 ± 1.0	5.7 ± 0.9	5.3 ± 1.1	0.21 ± 0.08
	0.7	1.3	1.1	1.1	1.8	2.8	2.8
gut[c]	3.1 ± 0.2	2.8 ± 0.1	2.5 ± 0.2	1.9 ± 0.3	2.1 ± 0.1	1.3 ± 0.2	0.07 ± 0.02
	0.6	0.8	0.6	0.5	0.6	0.7	1.0
kidney	21.1 ± 2.7	18.5 ± 2.7	12.0 ± 1.9	8.5 ± 1.1	7.3 ± 0.7	4.1 ± 0.6	0.14 ± 0.06
	4.3	5.1	2.9	2.2	2.3	2.2	1.8
skin	5.2 ± 0.6	3.8 ± 0.4	5.0 ± 1.5	3.2 ± 0.5	3.5 ± 0.6	2.1 ± 0.4	0.1 ± 0.05
	1.1	1.1	1.2	0.8	1.1	1.1	1.5
muscle	2.5 ± 0.2	2.0 ± 0.1	2.3 ± 0.4	1.3 ± 0.2	1.3 ± 0.2	0.7 ± 0.1	0.03 ± 0.01
	0.5	0.6	0.6	0.3	0.4	0.4	0.4
bone	2.4 ± 0.3	1.8 ± 0.3	1.5 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	0.7 ± 0.1	0.07 ± 0.09
	0.5	0.5	0.4	0.3	0.4	0.4	0.8
lung	7.9 ± 0.7	7.5 ± 0.8	7.0 ± 0.7	5.1 ± 0.9	4.5 ± 0.5	2.6 ± 0.5	0.1 ± 0.05
	1.6	2.1	1.7	1.3	1.4	1.4	1.4
heart	7.0 ± 1.1	6.4 ± 0.3	5.8 ± 0.2	5.7 ± 0.6	3.5 ± 0.1	1.6 ± 0.1	0.1 ± 0.1
	1.4	1.8	1.4	1.0	1.1	0.9	1.3
liver	5.3 ± 0.7	4.6 ± 0.6	3.6 ± 0.1	2.8 ± 0.3	2.6 ± 0.3	1.4 ± 0.2	0.1 ± 0.04
	1.1	1.3	0.9	0.7	0.8	0.8	1.3
tumor	4.9 ± 0.4	3.7 ± 0.9	4.1 ± 0.5	3.8 ± 0.3	3.3 ± 0.6	1.9 ± 0.4	0.08 ± 0.03
	0.3[d]	0.2	0.3	0.4	0.4	0.5	0.5

[a] The numbers represent the mean ± standard deviation for percent of injected dose per gram of wet tissue for six animals. [b] Organ to tumor ratio. [c] Section of intenstine. [d] Tumor to blood ratio.

that all measurable excreted activity was associated with unchanged starting compound. After 4 h the levels of excreted activity were below the detection level of the HPLC radioactivity detection system.

The ^{82}Br activity remaining in the mice after 4 h appeared to be mainly $^{82}Br^-$. Highest levels of activity were noted in the kidney, blood, stomach, and lungs, in agreement with the known tissue distribution of bromide anion in mice.[22] In addition, the measured excretion half-life of this activity was 33.5 h, which is in good agreement with previously published half-life determinations of 36[23] and 30 h[24] for Br^-.

Structurally related 5-bromopyrimidine nucleosides such as 5-bromouridine and 5-bromo-2′-deoxyuridine have been shown to be cytotoxic to bacterial and mammalian cells and to exhibit in vivo antiviral activity.[1] The accepted mode of cytotoxicity of these compounds is via incorpo-

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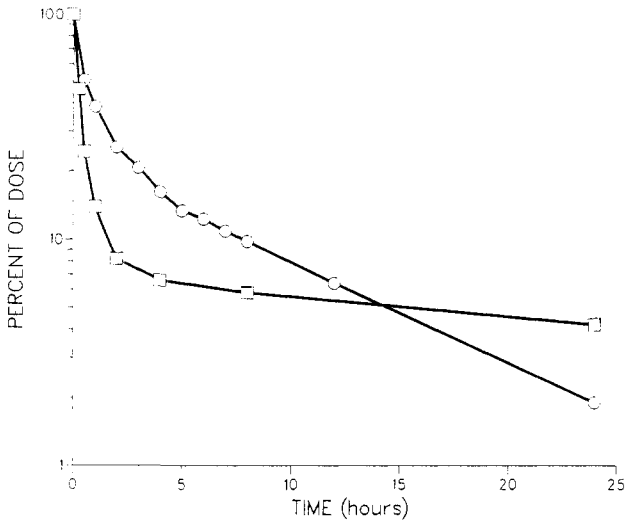

Figure 1. Whole-body activity in BDF_1 female mice after intraveneous injection of 5-halonucleosides: □ = [^{82}Br]-**3**; ○ = [^{131}I]-**4**.

Table III. Inhibition Constants (K_i) for Nucleosides and Nucleoside Analogues for Influx Competition with Thymidine in Mouse Erythryocytes

nucleoside	K_i ± SD, mM
thymidine	0.08 ± 0.01
2′-deoxyuridine	0.13 ± 0.02
compound **3**	0.39 ± 0.00
compound **4**	0.41 ± 0.01
2′-fluoro-2′-deoxyuridine	0.12 ± 0.03
5-bromo-2′-deoxyuridine	0.14 ± 0.02
5-iodo-2′-deoxyuridine	0.21 ± 0.01

ration into the DNA of replicating cells as a structural analogue of deoxythymidine. For example, 5-bromo-2′-deoxyuridine is incorporated very efficiently into the DNA of human and mammalian cells, showing a level of replacement for thymidine of up to 43%.[2] The present study gave no evidence for this level of uptake with compound **3**. Compound **3** did demonstrate significant in vivo stability since no radioactive species other than the starting compound and bromine anion were detected. In our in vivo experiments injections of up to 40 μmol of 5-bromo nucleoside were used, but the rapid excretion and in vivo debromination appeared to compete very effectively with any transport or metabolism that might have occurred.

The transport behavior of the bromo nucleoside **3** was determined by using mouse erythrocytes and the methodology described previously.[25,26] Influx competition studies, in which the effect of **3** on the zero trans influx of [6-^{3}H]thymidine into the erythrocytes was measured, demonstrated competition of **3** with thymidine for the same binding sites on the nucleoside transporter. The value of the inhibition constant (K_i) for compound **3** was measured as 0.39 ± 0.00 mM, which is higher than those of physiological nucleosides such as thymidine and deoxyuridine (0.08 ± 0.01 mM and 0.13 ± 0.02 mM, respectively) (see Table III). This is interpreted to mean that compound **3** is a relatively poorer substrate for the nucleoside transporter than the physiological nucleosides.

Poor transport of the bromo nucleoside **3** may account in part for the absence of detectable incorporation of this

Table IV. Radioactive Constituents of Urine at Various Time Intervals after Injection of [^{131}I]-5-Iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil ([^{131}I]-**4**) into Female BDF_1 Mice Bearing a Lewis Lung Tumor

	radioactive constituents of the urine, % of total			
interval, h	iodide	5-I-2′-FUdR[a]	5-IU[b]	other[c]
0–0.5	4.7	90.8	1.8	2.6
2–3	13.7	82.8	0[d]	3.5
6–7	39.8	60.2	0	0
24	62.6	37.4	0	0

[a] [^{131}I]-5-Iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil. [b] [^{131}I]-5-Iodouracil. [c] Unidentified polar radioactive constituents of urine. [d] Below detection limit.

compound into the DNA of L1210 leukemia cells in an in vitro study.[27] Measurements of the incorporation of **3** into DNA were performed by using HPLC analysis of DNA hydrolysates of the L1210 leukemia cells. Incorporation of **3** was undetectable at the lower limit of 1 or 2% of base pair replacement, whereas the incorporation of 5-bromo-2′-deoxyuridine run in parallel approached 50%. The low upper limit for uptake of **3** into DNA, as demonstrated in this study, suggests that factors other than poor membrane transport impede incorporation of this compound into cellular macromolecules. It is likely that compounds **3** and **4** are poor substrates for the kinase enzymes necessary to convert them to the triphosphate precursors used in DNA synthesis. This behavior has been observed previously with the related compound 2′-fluoro-2′-deoxyuridine, which was well transported as indicated by a low inhibition constant (Table III) but was a poor kinase substrate.[28]

The ^{131}I-labeled 5-iodo nucleoside [^{131}I]-**4** was rapidly excreted after intravenous injection with little or no evidence of active uptake in any tissues except for the kidney. Whole-body radioactivity data (Figure 1) show that half of the injected dose was excreted in 30 min and 75% of the dose by 2 h. The curve in Figure 1 can be analyzed as a two-component system (biexponential curve) with component half-lives of 0.70 and 6.79 h. Tissue radioactivity levels for the early time periods (0–4 h) appeared to parallel blood content, with the vascular organs such as heart and lungs being more radioactive than less vascular tissues such as muscle and gastrointestinal tract. Later radioactivity distribution (8–24 h) indicated a contribution from unchanged starting nucleoside [^{131}I]-**4** as well as from ^{131}I$^-$. Evidence for the formation of ^{131}I$^-$ came from the detection of a polar radioiodine species in the HPLC analysis (Table IV) and from high levels of tissue activity in the stomach and thyroid. These tissues are known to extract and concentrate circulating iodide anion.[29]

Transporter binding studies with the iodo nucleoside **4** gave similar results to those reported previously for the bromo nucleoside **3** (Table III). Although **4** is competitive with thymidine for the nucleoside transporter, the value for the inhibition constant (K_i) indicates that this nucleoside analogue is a poorer substrate for the nucleoside transporter than the physiological nucleosides.

5-Iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (**4**) can be considered to be a structural analogue of thymidine and is also structurally related to several other compounds

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such as 5-iodo-2'-deoxyuridine and 5-iodo-1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)uracil, which have pronounced biological activity.[4,14] The biochemical fate of compound 4, however, appeared to differ from these related compounds. Compound 4 did not accumulate in rapidly growing tissues such as intestine and tumor. This may be due in part to the fact that 4 is a poor substrate for the transport mechanisms required for cellular uptake. Table III shows that the inhibition constant for 4 is not greatly different than that of the related nucleoside 5-iodo-2'-deoxyuridine. This latter nucleoside analogue exhibits uptake into DNA of mammalian tissue, indicating that it is not only transported across the cell membrane but is also a substrate for the kinase enzymes. This suggests that the specificity of kinase enzymes rather than transport is the rate-limiting factor leading to the absence of measurable uptake of 4 into cellular metabolic products.

Compared to related 5-iodonucleosides, 5-iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (4) exhibited a remarkable in vivo stability. For example, 5-iodo-2'-deoxyuridine has been shown to be rapidly catabolized via phosphorylytic cleavage to iodouracil and deoxyribose by the ubiquitous enzyme pyrimidine phosphorylase.[30] Iodine is rapidly lost from iodouracil after its enzymatic conversion to 5-iodo-5,6-dihydrouracil.[31] In addition, thymidylate synthetase has been shown to catalyze the facile deiodination of 5-iodo-2'-deoxyuridylate.[32] These processes compete with the uptake of 5-iodo-2'-deoxyuridine into DNA. In animal studies the half-life for uptake of 5-iodo-2'-deoxyuridine has been estimated at 5 min while the half-life of degradation has been measured at 3 min.[4] In the urine of rats injected with [^{131}I]-5-iodo-2'-deoxyuridine, 99.9% of the radioactivity at 60 min was due to iodine anion.[3] In contrast, we observed that the 5-iodo nucleoside 4 was very resistant to phosphorylytic cleavage and deiodination. Even after 24 h, unchanged compound comprised up to 37% of the activity present in the urine. The persistence of unchanged compound in the urine, despite the initial clearance half-life of 0.70 h, 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.

It is apparent that the presence of a 2'-fluoro substituent in the ribo configuration is responsible for the high in vivo stability observed for compounds 3 and 4. Presumably, this substituent renders these compounds much poorer substrates for enzymatic degradation via glycosidic bond cleavage catalyzed by pyrimidine phosphorylase. The same feature has been observed in a variety of arabino configuration nucleosides such as 5-fluoro-1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)uracil[33] and 5-iodo-1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)cytosine.[34] This latter compound was observed to undergo rapid deamination in mice to the corresponding uridine analogue. The uridine analogue is resistant to further catabolism and is the major metabolite (75%) in pooled 24-h urine.

It is unfortunate that the same structural feature, a 2'-fluoro ribo substituent in 3 and 4, which confers biochemical stability on these compounds also appears to render them poor substrates for kinase enzymes. The absence of tumor selectivity and overall low uptake in tissues observed with these compounds make them unsuitable as imaging agents. It is possible that the compounds will exhibit some antiviral activity, as noted with the arabino configuration analogs,[14] since it is known that cells infected by herpes simplex virus, which encodes for thymidine kinase, will phosphorylate a broader range of nucleosides than uninfected cells.[35]

Experimental Section

Chemicals and solvents used were of reagent grade. Melting points were determined on a Büchi capillary apparatus and are uncorrected. NMR spectra were determined on a Brucker AM 300 spectrometer with deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent and tetramethylsilane [$(CH_3)_4Si$] as the internal reference. High-resolution mass spectra (HRMS), determined on an AEI MS 50 mass spectrometer, was used to determine the elemental composition. High-pressure liquid chromatographic (HPLC) analyses were carried out with a Waters system composed of a Model 860 automated gradient controller, Models 510 and M-45 solvent pumps, a Model U6K injector, and a Model 480LC ultraviolet detector with the wavelength set to 256 nm. Analytical HPLC was carried out on a Waters C-18 Radial-PAK reverse-phase column (column A) while preparative separations were performed with a Whatman Partisil M9 10/25 ODS reverse-phase column (column B). Reference compounds for HPLC analysis were obtained from commercial suppliers or were synthesized in our laboratory by established methods. Thin-layer chromatography (TLC) was performed on Whatman MK6F silica gel microslides. Preparative thin-layer chromatography was carried out on Whatman PLK5F plates. Radiochromatograms developed on silica gel plates were analyzed on a Berthold LB 2832 gas-flow proportional analyzer and a Canberra Series 40 multichannel analyzer (linear analyzer). 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 flux of 1×10^{12} n cm^{-2} s^{-1}. The ^{131}I was purchased from the Edmonton Radiopharmacy Center as a no-carrier-added solution of Na^{131}I in dilute NaOH. [6-^{3}H]Thymidine was obtained from New England Nuclear. Tissue samples were counted in a Beckman Gamma 8000 γ scintillation counter. Liquid scintillation counting was performed with a Searle Mark III counter.

1-(2-Fluoro-2-deoxy-β-D-ribofuranosyl)uracil (1). The title compound was prepared by a literature procedure.[36,37] Purification by column chromatography (2%–10% gradient of MeOH in CH_2Cl_2, silica gel) yielded 41% of 1 as fine needles: mp 149–150 °C (lit.[38] mp 150–151 °C); exact mass calcd for $C_9H_{11}N_2O_5F$ 246.0652, found (HRMS) 246.0649 (M^+, 2.7%).

5-Bromo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (3). A solution of 1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (1) (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 a bromination solution (0.56 g of Br_2, 26.28 g of glacial acetic acid) was added dropwise over 30 min until a distinct brown color persisted in the solution (7.2 mL added, 0.94 mmol). The solution was then heated at 100 °C for 30 min. The solvent and unreacted bromine were removed at 50 °C on a rotary evaporator, and the residue was dissolved in ethanol and the solent evaporated to give a pale yellow-brown foam. This foam was dissolved in a mixture of 5 mL of ethanol and 0.5 mL of concentrated NH_4OH and stirred for 18 h. The crude product obtained after evaporation of solvent was purified by preparative HPLC (column B, 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 an NMR spectrum consistent with

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that expected for the product. Attempts to crystallize this compound from water, methanol, ethanol, and various mixed solvents were unsuccessful.

^{1}H NMR (DMSO-d_6) δ 11.8 [1 H, s, N(3)-H], 8.55 [1 H, s, C(6)-H], 5.87 [1 H, d (J(1′,F) = 16.5), C(1′)-H], 5.63 [1 H, br s, C(3′)-OH], 5.44 [1 H, br s, C(5′)-OH], 5.05 [1 H, dd (J(2′,F) = 54, J(2′,3′) = 4.1), C(2′)-H], 4.19 [1 H, br d (J(3′,F) = 23.5), becomes ddd after D_2O exchange (J(3′,F) = 23.5, J(3′,4′) = 8.5), C(4′)-H], 3.86 and 3.60 [2 H, br d (J(gem) = 12.5), becomes dd after D_2O exchange (J(gem) = 12.5, J(5′,4′) = 2.1), C(5′)-H]. Signals at δ 5.63, 5.44, and 11.8 disappear on D_2O exchange. Exact mass calcd for $C_9H_{10}N_2O_5F^{81}Br$: 325.9736; measured (HRMS), 325.9733; intensity = 0.55%. Exact mass calcd for $C_9H_9N_2O_5{}^{81}Br$ (M^+ – HF): 305.9674; measured (HRMS), 305.9719. Exact mass calcd for $C_9H_9N_2O_5{}^{79}Br$ (M^+ – HF): 303.9694; measured (HRMS), 303.9739.

5-Iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (4). A solution of NaI (65 mg, 0.43 mmol) in 4.5 mL of water was added to a solution of 1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (1) (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 × 2 mL). The residue obtained on removal of solvent was purified by preparative TLC (15% v/v MeOH/CH_2Cl_2). The single major product was recovered from the plates as a white powder (136 mg, 0.36 mmol, 88.6%), which gave pure title compound on recrystallization from hot water; mp 216–218 °C.

^{1}H NMR (DMSO-d_6) δ 11.6 [1 H, s, N(3)-H], 8.56 [1 H, s, C(6)-H], 5.88 [1 H, d (J(1′,F) = 16.5), C(1′)-H], 5.63 [1 H, d (J(OH,3′) = 6.4), C(3′)-OH], 5.42 [1 H, t (J(OH,5′) = 4.3), C-(5′)-OH], 5.05 [1 H, dd (J(2′,F) = 54, J(2′,3′) = 4.1), C(2′)-H], 4.19 [1 H, complex d (J(3′,F) = 22.8), becomes ddd after D_2O exchange (J(3′,F) = 22.8, J(3′,4′) = 8.2, J(3′,2′) = 4.1), C(3′)-H], 3.91 [1 H, br d (J(4′,3′) = 8.2), C(4′)-H], 3.83 and 3.67 [2 H, complex d (J(gem) = 12.0), becomes dd after D_2O exchange (J(gem) = 12.0, J(5′,4′) = 2.2), C(5′)-H]. Signals at δ 5.42, 5.63, and 11.6 disappear on D_2O exchange. Exact mass calcd for $C_9H_{10}N_2O_5I$: 371.9617; measured (HRMS), 371.9619; intensity = 2.9%.

[^{82}Br]-5-Bromo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)-uracil ([^{82}Br]-3). A suspension of $NH_4{}^{82}Br$ (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 (1) (14 mg, 56.9 μmol) contained in a 3-mL Teflon reaction vial. The vial was sealed, and 0.9 mL (9 mg, 67.4 μmol) of a 10% w/v solution of *N*-chlorosuccinimide in acetic acid was added through the septum. The initial brown coloration (^{82}BrCl) rapidly disappeared at room temperature. The vial was heated at 45 °C for 1 h. The cooled reaction mixture and several washes with EtOH were transferred to a 50-mL flask and evaporated to dryness in vacuo at 40 °C. The residue was dissolved in 5 mL of EtOH evaporated to dryness and then treated with 10 drops of concentrated NH_4OH in 5 mL of EtOH at 50 °C for 10 min. TLC (20% MeOH in CH_2Cl_2) showed a single compound at R_f 0.68. This material accounted for 84% of the total activity on the plate. A further 12% of the activity (possibly $^{82}Br^-$) occurred as a broad area on the plate centered at R_f 0.58. The crude sample was purified by preparative TLC to give the title compound in 57% chemical and radiochemical yield. This material had a radiochemical purity of 99% as determined by HPLC analysis. The end of synthesis specific activity was 0.167 GBq/mmol.

[^{131}I]-5-Iodo-1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil ([^{131}I]-4). Carrier-Added Synthesis. A mixture of NaI (0.7 mg, 4.67 μmol) and 1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (1) (2.1 mg, 8.44 μmol) in a 1-mL Reacti-Vial was treated with 0.289 GBq of carrier-free $Na^{131}I$ as a solution in 75 μL of EtOH. The solvent was removed with a stream of dry nitrogen 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 min in a heating block at which time an initially formed brown coloration (I_2) had disappeared. TLC indicated a single product containing 90% of the total plate activity. 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 crude product was purified by preparative TLC (15% MeOH in CH_2Cl_2) to give a product that was identical (HPLC, TLC) to an authentic unlabeled sample of 1. The radiochemical purity (linear analyzer) was 99.3%, and the chemical and radiochemical yields were 67%. The product specific activity at the end of the synthesis was 45.9 GBq/mmol.

No-Carrier-Added Synthesis. An aqueous solution of no-carrier-added $Na^{131}I$ (1 μL, 2.41 MBq) was added to 1-(2-fluoro-2-deoxy-β-D-ribofuranosyl)uracil (1) (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 min in a heating block, cooled, and neutralized with several microliters of concentrated NH_4OH. The solvent was removed in vacuo, and the residue was dissolved in 20 μL of EtOH and the solvent evaporated. This crude material was treated with 10 μg of nonradioactive carrier compound and then analyzed and purified by TLC. The product was obtained in 41% radiochemical yield with a radiochemical purity of greater than 95%.

Tissue Distribution Studies. Radiolabeled compounds were dissolved in normal saline and injected via the tail vein into female BDF_1 mice (18–22 g) bearing a subcutaneous Lewis lung carcinoma. Details of the transplantation and maintenance of this tumor model were reported previously.[39] Animals were sacrificed by cardiac puncture exsanguination after asphyxiation with CO_2. Tissues were weighed wet and transferred to plastic vials for γ counting. The carcass was also placed into two vials and counted, and the total activity in all tissues was used as the whole-body activity at the time of sacrifice. Whole-body elimination was also determined by injecting BDF_1 mice with the radioactive compounds and then measuring the whole-body in vivo activity at various time intervals with a Picker isotope dose calibrator. During measurements the mice were maintained in a defined geometry by the use of a plastic insert. Urine was collected at intervals by placing the mice in small metabolism cages.

Transport Studies. Transport of compounds **3** and 4 and the other nucleosides listed in Table III was determined by using freshly harvested mouse erythrocytes exposed to various concentrations of intracellular test nucleoside and [6-^{3}H]thymidine. The concentration of [6-^{3}H]thymidine in the extracellular fluid at various times after this exposure was determined by counting aliquots by liquid scintillation counting. These procedures and the method of interpreting the results have been described in detail elsewhere.[25,26]

HPLC Analysis of Urinary Metabolites. Urine was collected during the whole-body activity measurements and at the time of sacrifice and stored frozen until the analysis was performed. Each of the stored frozen urine samples was thawed, mixed with a portion of the HPLC solvent to be used, and filtered through a 0.45-μm filter, and aliquots of 5–20 μL were injected directly onto HPLC columns. The column effluent was analyzed first by a UV detector and then by a sodium iodide crystal γ-scintillation detector. For ^{131}I the major photopeak at 364 keV (82%) was detected in a window from 300 to 400 keV while for ^{82}Br a window from 200 to 1000 keV was used.

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