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SYNTHESIS OF HIGH SPECIFIC ACTIVITY ^{80m}Br AND ^{123}I LABELED 5-HALODEOXYURIDINES AND OTHER ^{80m}Br COMPOUNDS FOR THE STUDY OF AUGER ELECTRON TOXICITY.

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SUMMARY

Convenient preparations of ^{80m}Br- and ^{123}I-labeled 5-halodeoxyuridines, required for basic investigations of Auger electron radiotoxicity, are described. These radioactive thymidine analogs were synthesized from deoxyuridine, radiohalide and N-chlorosuccinimide in dilute sulfuric acid. Yields were 50-60% for ^{80m}Br and 60-70% for ^{123}I. Apparent specific radioactivities (based on UV absorption under the radioactive HPLC peaks) were 150-550 Ci/mmol and over 2000 Ci/mmol for ^{80m}Br and ^{123}I, respectively. 5-[^{80m}Br]bromouracil was produced in 89% yield when uracil was used. [^{80m}Br]Bromoantipyrine was produced in higher yield and specific activity using peroxyacetic acid, rather than N-chlorosuccinimide as oxidant (90% and 4,000 Ci/mmol *versus* 50% and 2000 Ci/mmol).

Key words: Auger electron, ^{80m}Br, ^{123}I, bromoantipyrine, 5-bromo-2'-deoxyuridine, 5-iodo-2'-deoxyuridine.

INTRODUCTION

It has been well established that the Auger electron emitters ^{77}Br and ^{125}I incorporated into DNA by incubation of cells with the thymidine analogs

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5-bromo and 5-iododeoxyuridine (BUdR, IUdR) exhibit radiotoxicity far in excess of that predicted by conventional dosimetric estimates (1-4). Radiotherapy based on incorporation of Auger electron emitting 5-halodeoxyuridines (XUdR) into tumors *in vivo* is an attractive concept since rapidly proliferating cancerous cells should take up more labeled pyrimidine than relatively quiescent normal cells. However, the utility of [^{125}I]IUdR is limited by its long half-life (60d) which limits the dose-rate achieveable by incorporation of a given amount of IUdR. The half-life of ^{77}Br is shorter (56h), but still long compared with expected rates of metabolism of BUdR. It is also less readily available.

Two very short half-lived Auger electron emitting halogens, ^{123}I and ^{80m}Br may also be considered for potential radiotherapy. ^{123}I is readily available commercially and has a convenient half-life (13.3h) as well as excellent imaging characteristics and a mean yield of 13 Auger electrons per decay (5). The radiotoxicity of ^{123}I has recently been established (6). Unlike ^{123}I, ^{80m}Br (half-life = 4.4 h) does not have suitable characteristics for medical imaging; however, it has potential for therapeutic use since its decay releases, on average, 7 Auger electrons (7,8). It is available in high specific activity from cyclotron targetry based on the ^{83}Kr(d, n,α) reaction (9); however, the ^{80}Se(p, n) reaction is also attractive because of its high yield and applicability to small medical cyclotrons (9,10). Our work with [^{80m}Br]BUdR has established that on average the decay of about 50 atoms/cell are required to kill Chinese hamster ovary (CHO) cells (11). This compares with [^{77}Br]-, [^{125}I]- and [^{123}I]XUdR, where the mean lethal number of decays in V79 cells are 340, 120 and 280, respectively (3,4,6).

Studies of the radiotoxicity of [^{80m}Br]BUdR also required labeled species which are not accumulated in the cell nucleus. In addition to ^{80m}Br$^-$, which is excluded from the cell, we prepared and used [^{80m}Br]bromoantipyrine (BAP). As an analog of iodoantipyrine (12), this is expected to freely diffuse throughout cellular water giving an indication of radiotoxicity not due to the extremely short-range Auger electron radiation. We also prepared 5-[^{80m}Br]bromouracil (5BU), the hydrolysis product of [^{80m}Br]BUdR.

Although ^{80m}Br has a lower yield of Auger electrons than ^{123}I, BUdR may be more stable *in vivo* since the carbon bromine bond is stronger than the carbon-iodine bond. Furthermore, unlike radioiodide, free radiobromide—produced by *in vivo* dehalogenation—does not accumulate in the thyroid (13).

MATERIALS AND METHODS

Uracil, 2'-deoxyuridine (UdR), BUdR, 5BU, N-chlorosuccinimide (NCS), and antipyrine (AP) were purchased from the Aldrich Chemical Company. ACS grade 30% H_2O_2, reagent grade glacial CH_3CO_2H, and reagent grade Br_2 were purchased from Fisher Scientific Company. Ultrex H_2SO_4 was purchased from J. T. Baker Chemical Company. ^{123}I dissolved in 0.1N NaOH at 30 mCi/mL was purchased from Nordion. Its specific activity was >>10,000 Ci/mmol (14). ^{80m}Br was prepared using the Argonne National Laboratory 60 inch cyclotron and was purified as previously reported (9,10,15). Reaction mixtures were injected directly into an HPLC; Columns, mobile phases and retention times are given below for each compound. Collected peaks were counted in a 3 x 3 in NaI gamma ray detector coupled to a multichannel analyzer. Radiochemical yields were determined by dividing the amount of radioactivity in the product by the total amount of radioactivity eluted from the HPLC column. Recoveries were >90%. The mass of the radioactive product, which was used in the specific activity determination, was calculated by comparing the area under its UV absorbance peak with that of known quantities of nonradioactive product. The authenticities of labeled products were checked by coinjection of labeled material with standards.

Bromoantipyrine was prepared from the dropwise addition of 0.81 mL (one equivalent) Br_2 to 3g AP dissolved in 100 mL CH_2Cl_2. After the addition was complete solvent was evaporated under vacuum. The residue was recrystallized from water, and dried under vacuum to give 2.2g (52%) white crystals. mp 110-112 °C (Lit. 115°) (16); nmr ($CDCl_3$) δ 7.48-7.26 (m, 5), 3.10 (s, 3), 2.31 (s, 3).

Chloroantipyrine was prepared by the addition of 2.3g (1.1 equivalent) NCS to 3g AP dissolved in 50 mL $CHCl_3$. This was heated at 50°C for 30

minutes. The $CHCl_3$ was evaporated under vacuum. The residue was recrystalized from water and dried under vacuum to give 1.5g (42%) white crystals. mp 128-130°C (Lit. 125-126°) (16); nmr ($CDCl_3$) δ 7.49-7.26 (m, 5), 3.07 (s, 3), 2.38 (s, 3).

Chlorouracil was prepared by the procedure of West and Barrett (17).

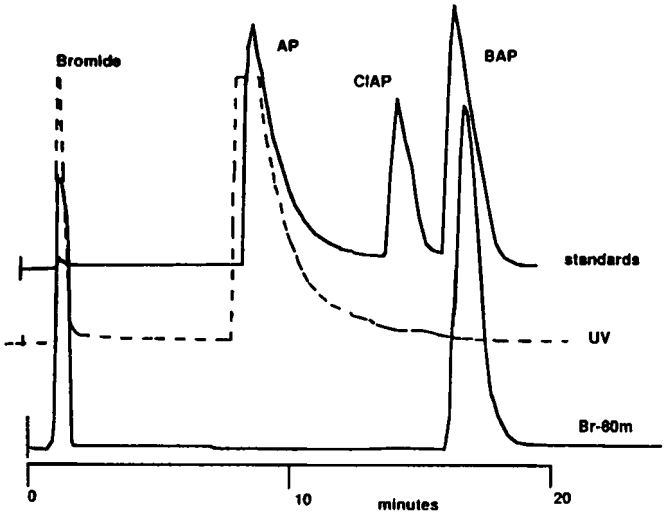
Radiobrominations were performed by placing 0.1-1 mL $^{80m}Br^-$ solution (3-5 mCi) into a 3.7 mL screw cap vial. Water was evaporated under a stream of nitrogen while the vial was heated at 100°C in an oil bath. After addition of reactants, the vial was sealed and shaken. After reaction the entire contents of the vial were then injected into the HPLC.

5-[^{80m}Br]Bromo-2'-deoxyuridine: To the dried $^{80m}Br^-$ was added 150 μL of 2M H_2SO_4 followed by 25 μL of NCS solution (freshly made in water at 33mM) and 20mg of UdR. It was heated at 80°C for 30 minutes, then purified with an Alltech Absorbosphere 10 micron C18 column (250 x 4.6 mm). The mobile phase was 3% water in CH_3CN at 1.5 mL/min. Retention times for uracil UdR, 5BU and BUdR were 3, 6, 8 and 23 minutes, respectively.

5-[^{80m}Br]Bromouracil: Method 1: To the dried $^{80m}Br^-$ was added 150 μL of a 0.01 M phosphate buffer pH = 7.0, 25 μL of NCS solution, and 5 mg uracil. It was allowed to stand at room temperature for 15 minutes, then purified by HPLC as above, except than 2% water was used at 1.0 mL/min. Retention times for uracil, 5-chlorouracil and 5BU were 4.5, 9 and 11 minutes. respectively. Method 2: The procedure for BUdR was followed, except that uracil was used in place of UdR.

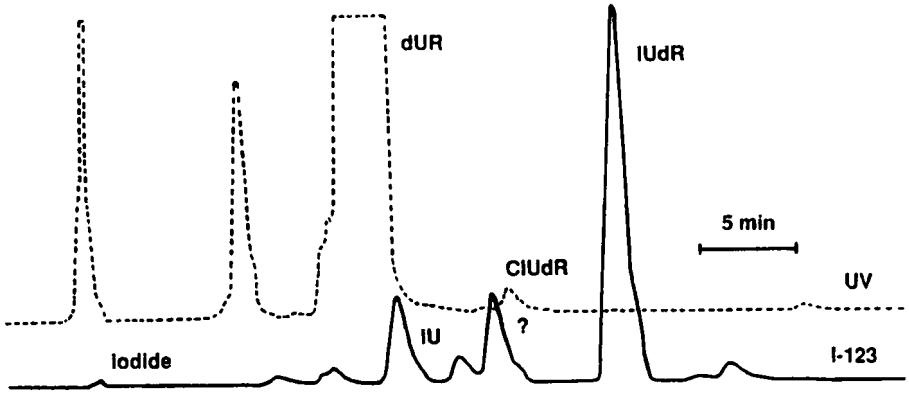
[^{80m}Br]Bromoantipyrine: Method 1: To the dried $^{80m}Br^-$ was added 50 μL water, 50 μL CH_3CN, 50 μL 1.0 x 10^{-2} M aqueous solution of AP, and 10 μL of NCS solution. Finally 3 μL of 1% H_2SO_4 was added to make the reaction mixture acidic (pH = 2.0). It was allowed to stand at room temperature 15 minutes, then purified by HPLC (Fig 1). Method 2: To the vial was added 50 μL of a 5% Na acetate in glacial CH_3CO_2H (pH = 4.5), 50 μL of a 2/1 30% H_2O_2/CH_3CO_2H solution, and 50 μL of a 1.0 x 10^{-2} aqueous solution of AP. It was allowed to stand at room temperature for 15 minutes, and purified as for Method 1.

FIGURE 1: HPLC purification of ^{80m}Br bromoantipyrene (BAP). An Alltech Absorbosphere 10 micron C_{18} column was used with mobile phase consisting of 85% water, 15% acetonitrile at a flow rate of 2.0 mL/min. Top trace shows standards of antipyrene (AP), chloroantipyrene (ClAP) and BAP. Middle trace shows UV and bottom trace shows radioactivity tracings in a typical synthesis.

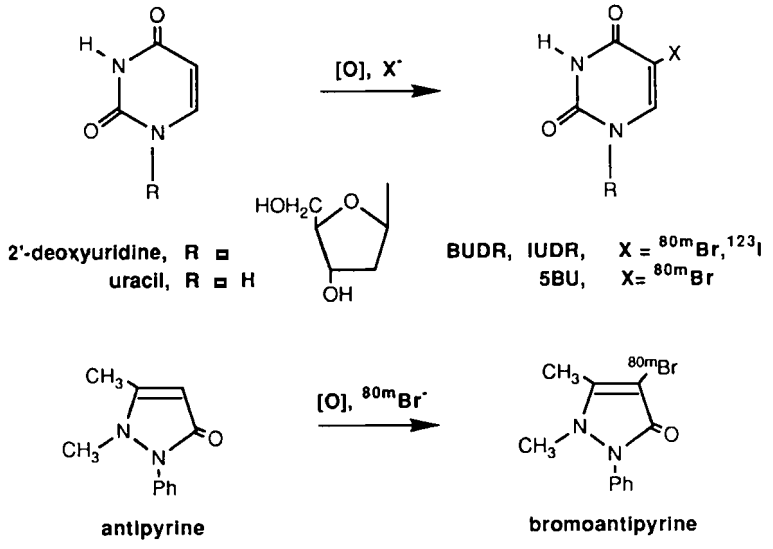


5-[^{123}I]Iododeoxyuridine: To 1 mL of 1N H_2SO_4 was added 50 µL of $^{123}I^-$ (5 mCi) and 10 mg of UdR. Reaction was initiated by addition of 25 µL of NCS solution. The mixture was held at room temperature for 15 min and then 80°C for a further 15 min before purification (Fig 2).

FIGURE 2: Purification of ^{123}I iododeoxyuridine (IUdR). An Alltech semi-preparative C_{18} column was used, with a gradient from H_2O to 10% C_2H_5OH over 30 minutes, followed by 10% C_2H_5OH for a further 20 minutes. The flow rate was 3.0 mL/min.



SCHEME I



RESULTS

Radiolabeling data are summarized in Table 1. Bromination of UdR was very slow at room temperature. Early experiments. in which 1 nmol of carrier bromide was added, gave radiochemical yields of [^{80m}Br]BUdR of 38, 57 and 38% at 65, 80 and 100°C respectively. At these temperatures labeled 5BU was also produced, in yields of 18, 22 and 41%. Subsequent no carrier added reactions at 80°C (Table 1) gave respectable yields of [^{80m}Br]BUdR. Several other labeled products appeared on HPLC, predominantly 5BU (15-20%). A co-eluting UV-active peak

Table 1. Labeling data.

Compound	Oxidant	Temp./Time	Yield	Apparent carrier
BUdR	NCS	80°/30min	50-60%	5-20 nmol
BAP	NCS	25°/15 min	50%	<1 nmol
BAP	CH_3CO_3H	25°/15 min	90%	<1 nmol
5BU	NCS (H_2SO_4)	80°/30 min	83%	<1 nmol
5BU	NCS (pH 7)	25°/15 min	89%	<1 nmol
IUdR	NCS	25°/15 min then 80°/15 min	60-70%	<1 nmol

corresponding to 5-20 nmol of BUdR was also present (see below). Other experiments in which peroxyacetic acid was used as oxidant failed to produce [^{80m}Br]BUdR (not shown). When uracil replaced deoxyuridine as substrate, a higher yield (83%) of desired product was obtained and HPLC indicated less than 1 nmol of product. Similarly, labeling of BAP was achieved at high specific radioactivity. Peroxyacetic acid gave a higher yield (90%) than NCS (50%). Fig 1 shows a sample HPLC tracing from an experiment where NCS was used. The general labeling conditions established for BUdR were also successful with ^{123}I. Radioiodide reacted with deoxyuridine at room temperature to give a labeled product which eluted prior to IUdR. However, this product disappeared and [^{123}I]IUdR appeared when the reaction mixture was heated at 80°C. Fig 2 shows a chromatogram in which the conversion was not complete.

DISCUSSION

The radiolabeling of nucleosides has received considerable attention (15). [^{77}Br]BUdR has been prepared by distilling an oxidized species of ^{77}Br into a solution of UdR (18-21). The specific activity of [^{77}Br]BUdR ranged from 90-125 Ci/mmole (18,22). [^{80m}Br]BUdR has also been prepared in poor yield (less than 30%) using [^{80m}Br]CF_3Br and various oxidizing agents (23).

In our studies cyclotron produced ^{80m}Br in the form of Br^- was used. We therefore sought labeling procedures where the bromide anion is oxidized *in situ* in the presence of the labeling substrate. Such conditions have commonly been employed in radioiodinations using such oxidizing agents as chloramine-T, iodogen, and NCS (15,24). In particular, [^{131}I]IUdR was prepared at room temperature using chloramine-T (25). In this reaction the pH was kept below 4 to reduce the preferential labeling of uracil, UdR's hydrolysis product. The acidic reaction medium decreases the amount of the more reactive anionic form of uracil (26,27). Furthermore, Hadi (22) was unable to synthesize [^{80m}Br]BUdR using chloramine-T in a phosphate buffer at neutral pH, although [^{80m}Br]BU was prepared in 83% yield. Our attempts to prepare [^{80m}Br]BUdR

using UdR, NCS and $^{80m}Br^-$ in CH_3CO_2H or aqueous buffers from pH 4 to pH 12 were also unsuccessful, as was the use of H_2O_2 and CH_3CO_2H in lithium acetate. Bromination was successful when NCS in 2M H_2SO_4 was used.

Elevated temperatures (80°C) are necessary for the labeling of BUdR but at temperatures approaching 100°C hydrolysis of the UdR to uracil becomes pronounced. The production of labeled 5BU in radiobrominations of UdR have been previously reported, but attributed to a uracil impurity in the UdR (21). A combination of the labeling of a uracil impurity and hydrolysis of the product BUdR is probably involved.

The lower apparent specific activity of [^{80m}Br]BUdR is probably due to UV absorbing compounds other than BUdR, since 5BU and BAP were produced with <1 nmol carrier from the same source of ^{80m}Br. According to the manufacturer's specifications significant carrier Br^- was not present in the sulfuric acid. UV absorbing contaminants in [^{77}Br]BUdR have also been reported (18).

[^{82}Br]BAP has been prepared, in 88% radiochemical yield, using a Br-^{82}Br exchange reaction (28). However, this type of chemistry gives a low specific activity product less suitable for biological experiments Our method is similar to reported direct labeling with $^{125}I^-$ which has been achieved by incubation with NSC in the presence of AP (29), or by the reaction of $^{125}I^-$ and 1M HCl with AP in the presence of SiO_2 (30). Chloroantipyrine was produced in our reactions when NCS was the oxidant, but this could be separated from the labeled BAP by HPLC (Figure 1). Changing the oxidant to H_2O_2/CH_3CO_2H in an acetate buffer (31) greatly reduced the amount of chloroAP formed (since the only chlorine source was adventitious chloride in reagents), as well as improving the yield (Table 1).

It also proved possible to produce [^{123}I]IUdR in good yield and specific activity using NCS in dilute H_2SO_4. Our observations that the labeling process initially gives a labeling product more polar than IUdR (Figure 2) is consistent with Tee's demonstration that bromination of N1 substituted uracils in dilute

acid proceeded through a 5-bromo-6-hydroxy-5,6-dihydro derivative (26,27). Purification of [^{123}I]IUdR with gradient HPLC using water and aqueous ethanol is convenient because for many experiments the peak collected from the chromatograph can be used directly without an evaporation step.

While the present work was in progress, the synthesis of radioiodinated IUdR from 5-chloromercurideoxyuridine was reported (32). This method also produces material of high radiochemical purity and specific activity, and should also be applicable to the production of radiobrominated BUdR.

CONCLUSIONS

We have prepared in high yields ^{80m}Br labeled BUdR, 5BU and BAP and ^{123}I labeled IUdR. The labeling and purification procedures are fast and involve oxidation of radiohalide with either NCS or peroxyacetic acid in the presence of substrate, followed by HPLC separation. The labeled nucleosides are suitable for investigations of Auger electron toxicity (33) and for some purposes their short physical half-lives offer significant advantages over ^{77}Br- and ^{125}I -labeled XUdR.

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