SYNTHESIS OF 2^1-[^{82}Br]-2^1-DEOXYURIDINE

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SUMMARY

Reaction of 2,2^1-anhydrouridine (2) with [^{82}Br]-ammonium bromide in the presence of one equivalent p-toluenesulfonic acid afforded 2^1-[^{82}Br]-2^1-deoxyuridine (3) in 73.4% chemical yield with a specific activity of 0.58 mCi mM^{-1}. Alternatively, direct activation of 2^1-bromo-2^1-deoxyuridine at a thermal neutron flux of 1 x 10^{12}n cm^{-2}s^{-1} gave 2^1-[^{82}Br]-2^1-deoxyuridine, with a specific activity of 0.6-0.7 mCi mM^{-1} for a 3 hr irradiation. 2^1-[^{82}Br]-2^1-deoxyuridine was rapidly purified by high pressure liquid chromatography with an elution time of 18 minutes, or by preparative thin layer chromatography.

Key Words: ^{82}Br-Labeled Radiopharmaceutical, 2^1-Bromo-2^1-deoxynucleoside, High Pressure Liquid Chromatography.

INTRODUCTION

It is well documented that the hydrogen or hydroxyl substituent on the 2^1-carbon of nucleic acids is the distinguishing feature between deoxyribonucleic (DNA) and ribonucleic acids (RNA). The differential distribution and tumor uptake of the radiohalogenated thymidine analogs 5-[^{18}F]-2^1-deoxyuridine (1), 5-[^{82}Br]-2^1-deoxyuridine (2,3) and 5-[^{131}I]-2^1-deoxyuridine (4) in mice have been reported. The results of these studies prompted investigation of nucleosides having a radiohalogen rather than a hydrogen or

0362-4803/80/0217-0269$01.00

Received October 6, 1978
Revised April 17, 1979

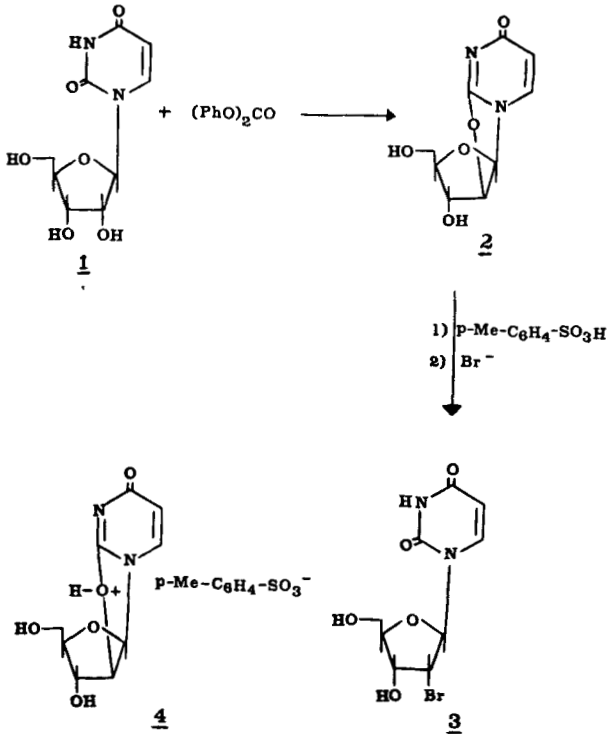
hydroxyl substituent at the 2^1-position of the sugar moiety. The ultimate goal of this study is to determine the potential of 2^1-radiohalogenated nucleosides for use in diagnostic oncology, specifically the non-invasive measurement of biochemical activity within a tumor before, during and after therapy. We now report (5) the synthesis of 2^1-[^{82}Br]-2^1-deoxyuridine (2^1-[^{82}Br]-2^1-UdR).

The rationale for selecting 2^1-[^{82}Br]-2^1-UdR for this study was: (i) Bromine-82 ($T_{½}$=35.4 hr) is readily available and is a suitable model for bromine-77 ($T_{½}$=56 hr) (6,7); (ii) Incorporation of bromine into the 2^1-position is less difficult than fluorine or iodine; (iii) the C-Br bond is 10-15 kcal/mol stronger than the corresponding C-I bond and hence the label is more stable (8).

RESULTS AND DISCUSSION

Reaction of uridine (1) with diphenylcarbonate using the procedure of Hampton and Nichol (9) gave rise to 2,2^1-anhydrouridine (2) in 58% yield. Treatment of 2 with bromide anion in the presence of one equivalent of p-toluenesulfonic acid in dimethylformamide at 100° for 4 hr afforded 2^1-bromo-2^1-deoxyuridine (3). The yield of 3 using ammonium, sodium and lithium bromide, as determined by quantitative preparative high pressure liquid chromatography (HPLC), was 62, 80 and 70% respectively. The reaction of 2 with alkalai metal bromides did not proceed in the absence of p-toluenesulfonic acid. The latter was likely required for protonation of the 2,2^1-anhydro ether linkage of 2 to yield the protonated intermediate 4 which subsequently reacts with bromide anion due to the increased

electrophilicity of the 2'-position (8). The 2'-bromo substituent of 3 was present in the ribo-configuration since 3 was identical (mp,ir,ms,nmr) to an authentic sample of known configuration (10) prepared from the reaction of 2 with anhydrous hydrogen bromide (8).



Reaction of 2,2'-anhydrouridine (2) with 11.5 equivalents [^{82}Br]-ammonium bromide in the presence of one equivalent p-toluenesulfonic acid at 100° for 6.5 hr gave 2'-[^{82}Br]-2'-deoxyuridine (3) in 73.4% chemical yield with a specific activity of 0.582 mCi mM^{-1} (6.3% radiochemical yield).

The direct irradiation of 3 in the University of Alberta Slowpoke reactor was also carried out using the ^{81}Br (n,γ) ^{82}Br reaction at a neutron flux of 1 x 10^{12}n $cm^{-2}s^{-1}$. The irradiated product 3 was allowed to stand for 24 hr to allow ^{80m}Br ($T_{½}$=4.4 hr) to decay since natural abundance ammonium bromide was used in the synthesis.

Quantitative high pressure liquid chromatographic (HPLC) separation and purification of the reaction mixture or the irradiated product 3 was carried out as illustrated in Figure 1. Preparative thin layer chromatography was equally effective for purifying and quantitating 3. Radiolytic decomposition due to reactor irradiation of 3 was substantial since 25% of the 2^{1}-Br-2^{1}-deoxyuridine was recovered 24 hr after the end of bombardment by HPLC or TLC purification. However TLC separation and counting indicated that only 19.3 ±2.4% of the total [^{82}Br]-radioactivity produced was present in 2^{1}-[^{82}Br]-2^{1}-deoxyuridine after irradiations of 3 at 1 x 10^{12}n $cm^{-2}s^{-1}$ for 1-3 hr. Excessive radiolysis was observed (sample discoloring) after longer irradiation periods. The specific activity of 2^{1}-[^{82}Br]-2^{1}-deoxyuridine for a 3 hr irradiation was 0.6-0.7 mCi mM^{-1} at the end of bombardment.

The reaction of 2,2^{1}-anhydrouridine (2) with alkalai metal bromides is a general and efficient reaction for the synthesis of 3 having radiobromine at the 2^{1}-position. Although the reaction of 2 with [^{77}Br]-NH_4Br has not been carried out it would provide ^{77}Br - labeled 3. It is expected that reaction of 2 with [^{81}Br]-NH_4Br (97% enriched; Oakridge National Laboratory) would give radiobrominated 3 with twice the specific activity after direct irradiation. Elimination of the decay period

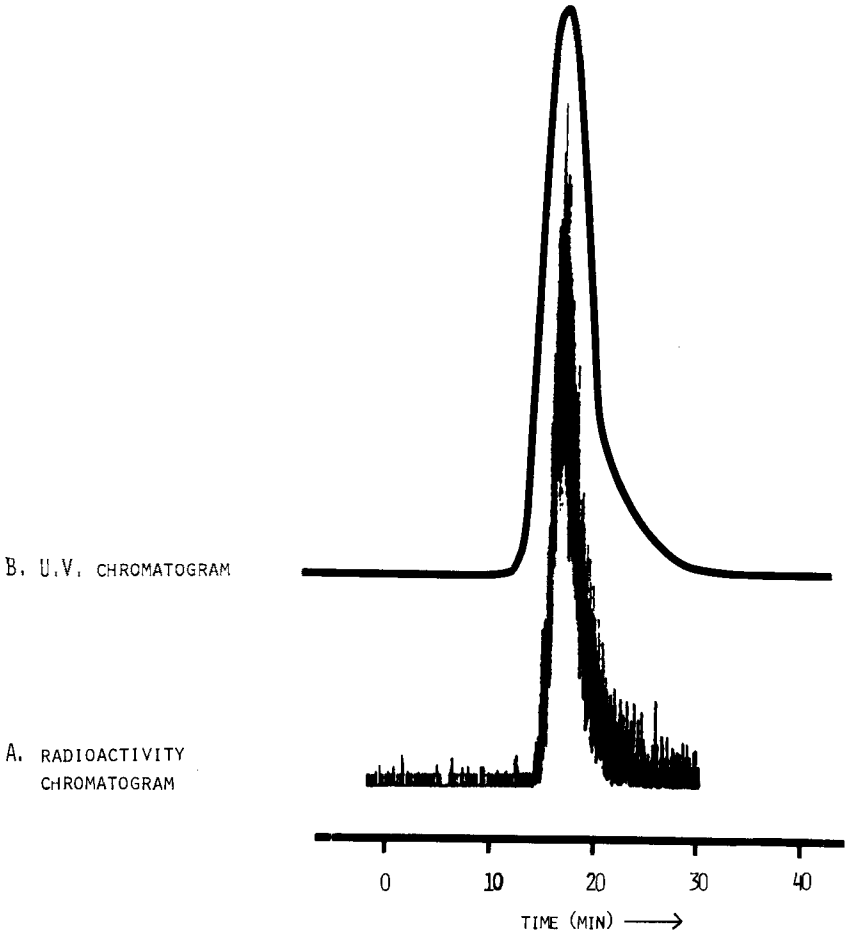


FIG. 1. RADIO HIGH PRESSURE LIQUID CHROMATOGRAM OF 2^1-[^{82}BR]-2^1-DEOXYURIDINE AFTER REACTOR IRRADIATION OF 3.

COLUMN: E. MERCK SILICA GEL 60 PREPACKED SIZE B; 2.5 x 26 CM

SOLVENT: MEOH:$CHCL_3$ (1:1 V/V)

FLOW RATE: 5 ML/MIN

U.V. DETECTION: 254 NM

RADIOACTIVITY DETECTOR: NAI (TL) DETECTOR

A. ^{82}BR-RADIOACTIVITY RECORDING OF COLUMN ELUANT

B. ULTRAVIOLET SPECTROMETRY RECORDING OF COLUMN ELUANT

required to allow decay of ^{80m}Br would then give an effective usable activity of almost 4 times that reported here for similar production procedures.

EXPERIMENTAL

2'-Bromo-2'-deoxyuridine. A solution of 2,2'-anhydrouridine (51.2mg, 0.227 mmol), ammonium bromide (50.6 mg, 0.516 mmol) and p-toluenesulfonic acid (39.1 mg, 0.227 mmol) in 5 ml dry dimethylformamide was heated at 100° for 4 hr. The solvent was removed in vacuo, 20 ml acetone was added to the residue and the insoluble material was removed by filtration. Removal of the acetone in vacuo gave a syrup-like liquid which was partitioned between 5 ml water and 10 ml benzene. Removal of the water in vacuo from the aqueous fraction gave 3 which was dissolved in 2 ml methanol. Quantitative preparative high pressure liquid chromatography using a E. Merck Silica gel 60 prepacked Size B column using methanol:chloroform (1:1 v/v) as solvent with a flow rate of 5 ml/min and U.V. detection at 254 nm gave 2'-bromo-2'-deoxyuridine (62%) with an elution time of 18 min. Removal of the solvent in vacuo gave 3 as a white solid which was identical (mp,ir,nmr,ms) with an authentic sample (8).

Reaction of 2 (77.9 mg, 0.34 mmol), sodium bromide (0.191 g, 1.86 mmol) and p-toluenesulfonic acid (58.5 mg, 0.34 mmol) in 5 ml dry dimethylformamide followed by quantitative high pressure liquid chromatography as described above afforded 2'-bromo-2'-deoxyuridine (80%).

Similarly, reaction of 2 (45.6 mg, 0.2 mmol), lithium bromide (34.7 mg, 0.4 mmol) and p-toluenesulfonic acid (34.4 mg, 0.2 mmol) in 5 ml dry dimethylformamide followed by quantitative high pressure liquid chromatography as

described above gave 2'-bromo-2'-deoxyuridine (70%).

[^{82}Br]-Ammonium bromide. A solution of [^{81}Br]-NaBr (100 mg, 97.81% enriched in 81-bromine, Oak Ridge National Laboratory) in 1 ml deionized distilled water was added to a cation (ammonium) exchange column containing 50 g Amberlite IR-120 (Mallinckrodt Chemical Works). Elution with 25 ml deionized distilled water, removal of the solvent and drying in vacuo gave [^{81}Br]-NH_4Br. Titrimetric analysis of the product for ammonium and bromide indicated an ammonium:bromide ratio of 0.8:1 (11). Neutron activation analysis indicated the sodium line of 2754 keV to be less than 1% of the total activity. The [^{81}Br]-NH_4Br was used without further purification. Irradiation of [^{81}Br]-NH_4Br in the University of Alberta Slowpoke Reactor using the ^{81}Br (n,γ) ^{82}Br reaction at a neutron flux of 1 x 10^{12}n $cm^{-2}s^{-1}$ gave routine radiation yields of 9 uCi $mg^{-1}hr^{-1}$ of [^{82}Br]-NH_4Br. The [^{82}Br]-NH_4Br produced was used, after standing for 4 hr, without further purification.

2'-[^{82}Br]-2'-deoxyuridine

Method A: Reaction with [^{82}Br]-NH_4Br. A solution of 2,2'-anhydrouridine (3.6 mg, 0.016 mmol), [^{82}Br]-ammonium bromide (18 mg, 0.18 mmol, 2 hr irradiation with a neutron flux of 1 x 10^{12}n $cm^{-2}s^{-1}$) and p-toluenesulfonic acid (2.7 mg, 0.018 mmol) in 0.2 ml dry dimethylformamide was heated at 100° for 6.5 hr. After cooling in an ice bath the solution was applied to one 20 x 20 cm silica gel DSF-5 plate 1.0 mm in thickness which was developed using chloroform: 1,4-dioxane: methanol (8:6:3 v/v) as development solvent.

The Rf values for pure samples of [^{82}Br]-NH_4Br, 2,2^1-anhydrouridine, uridine and [^{82}Br]-2^1-deoxyuridine (3) were 0, 0.17, 0.41 and 0.60 respectively. Extraction of the fraction having Rf 0.60 with warm methanol (50 ml) and quantitation using ultraviolet adsorption spectrometry at 260 nm (MeOH) indicated a 73.4% chemical yield of 3 (3.6 mg) with a specific activity of 0.582 mCi mM^{-1} as determined by liquid scintillation counting (6.3% radiochemical yield).

Method B: Direct irradiation of 2^1-bromo-2^1-deoxyuridine. A 2 mg sample of 2^1-bromo-2^1-deoxyuridine (3) (containing natural abundance bromine) was irradiated in a double plastic vial using the $^{81}Br(n,\gamma)^{82}Br$ reaction in the University of Alberta Slowpoke Reactor at a neutron flux of 10^{12}n cm^{-2}s^{-1} for 3 hr. After standing for 24 hr the irradiated sample was dissolved in 1 ml methanol and was then purified using preparative quantitative high pressure liquid chromatography as described above (See Figure 1) to give 2^1-[^{82}Br]-2^1-deoxyuridine (25% chemical recovery). The specific activity was 0.6-0.7 mCi mM^{-1} as determined by liquid scintillation counting (Corrected to the end of bombardment).

ACKNOWLEDGEMENTS

We are grateful to the Medical Research Council of Canada (Grant MA-5965) for financial support of this work.

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