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Research Article

The sequential syntheses of [^{76}Br]FBAU 3′,5′-dibenzoate and [^{76}Br]FBAU

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Summary

Thymidine analogs labeled with positron emitting radionuclides are potential proliferation markers for positron emission tomography (PET). Bromine-76 ($T_{1/2} = 16.2$ h) is our choice of radionuclide, because it allows for maximal DNA incorporation of the tracer. Following the literature descriptions, ^{76}Br was produced using the ^{75}As (^{3}He, 2n) ^{76}Br reaction. We then recovered ^{76}Br from the target in the form of [^{76}Br]NH_4Br with a yield of 60 ± 12% ($n = 32$). Peracetic acid was used as the oxidant for electrophilic bromodestannylation to prepare [^{76}Br]FBAU 3′,5′-dibenzoate (71.2 ± 12.1%, RCY) and a basic hydrolysis of the dibenzoate then yielded [^{76}Br]FBAU. The yield of the hydrolysis reaction was 53.1 ± 9.2% when heated at 100°C for 15 min or quantitative (decay corrected) when left at room temperature overnight. The sequential synthesis of [^{76}Br]FBAU 3′,5′-dibenzoate and [^{76}Br]FBAU allowed us to perform a side-by-side comparison of their metabolic stabilities. While [^{76}Br]FBAU 3′,5′-dibenzoate was hydrolyzed to [^{76}Br]FBAU within 10 minutes by hepatocyte at 37°C, [^{76}Br]FBAU was stable and no [^{76}Br]Br^- was released from either radiopharmaceutical. Both compounds are potential proliferation markers for PET. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: bromine-76; thymidine; proliferation marker; PET; FBAU

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Introduction

Thymidine and its analogs have been labeled with various radioactive nuclides to assess proliferation potential using positron emission tomography (PET) or single photon emission computed tomography (SPECT). Ideally, a close relationship between the tracer uptake and rapidly proliferating cells would be established. The early attempts using [^{11}C]thymidine for PET studies lacked the long physical half-life to allow the observation of DNA incorporation and presented analytical difficulties.[1–5] Separately, the iodinated and brominated thymidine analogs, iodo- and bromo-deoxyuridine, were extensively used in the histological assessment of proliferation.[6,7] For imaging purposes, [^{77}Br]bromodeoxyuridine (BrdU), [^{123}I]iododeoxyuridine, [^{131}I]iodo-deoxyuridine and, most recently, [^{76}Br]BrdU have been developed as potential tracers.[8–11] These nuclides all provide half-lives long enough for extended observations. Unfortunately, these tracers suffer from rapid metabolism and thus have a very short biological half-life. A study using [^{82}Br]BrdU showed that 90% of plasma radioactivity was [^{82}Br]Br^- after only 10 min.[12] Bromide is poorly excreted with a half-life of 12 days in humans.[13,14] Once radioactive bromide is released, the background radiation becomes too great to perform high contrast imaging studies. In a study using [^{76}Br]BrdU, attempts to force excretion with diuretics and supplemental chloride replacement was not entirely successful in reducing background activity.[15] The rapid dissociation of [^{76}Br] as bromide *in* vivo not only impaired the imaging possibility, but also defeated the purpose of choosing ^{76}Br as the nuclide of interest for labeling. The main advantage of using ^{76}Br labeled compounds for PET imaging is to exploit its 16.2 h half-life for applications with slow kinetics.

Meanwhile, [^{124}I]5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-uracil (FIAU)[16] and [^{11}C]5-methyl-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) uracil (FMAU)[17] have been developed for imaging transgene expression with PET. These tracers were shown to be good substrates for the herpes simplex virus thymidine kinase gene (HSV1-tk) selected as a marker gene. The 2-fluoro substitution greatly reduced the metabolism of nucleosides compared to deoxyuridine-type analogs. This attribute will, in principle, eliminate much of the background radiation due to free 5-halouracil or dehalogenated uracil. Very recently, [^{76}Br]bromofluorodeoxyuridine ([^{76}Br]BFU) was developed.[18] The study in rats showed [^{76}Br]BFU was indeed much more stable than

[^{76}Br]BrdU *in vivo* and produced very limited amount of bromide. However, the low metabolism of the tracer did not increase its standardized uptake value, because 70% of injected activity was eliminated as intact compound through urinary excretion. Nevertheless, the study suggested that [^{76}Br]BFU was incorporated into DNA, and has potential as a proliferation marker for PET.

In this study, we report the sequential preparation of [^{76}Br]1-(2-fluoro-2-deoxy-3,5-*O*-dibenzoyl-β-D-arabanofuranosyl)-5-bromouracil([^{76}Br]-FBAU3′,5′-dibenzoate) and [^{76}Br]1-(2-fluoro-2-deoxy-β-D-arabanofuranosyl)-5-bromouracil ([^{76}Br]FBAU). We also show the results of the hepatocyte metabolism studies of these two ^{76}Br compounds.

Results and discussion

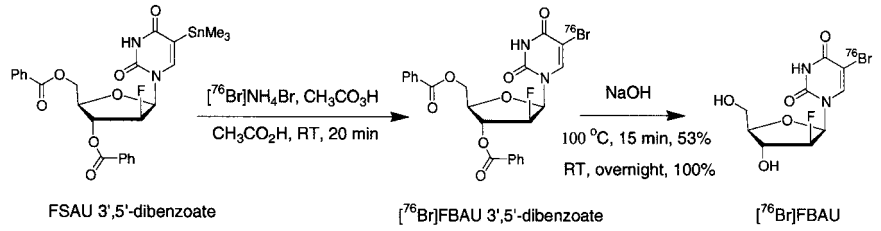
Production of ^{76}Br and preparation of [^{76}Br]NH_4Br

Bromine-76 is routinely produced on-site at the NIH Clinical Center utilizing the helium-3 capacity of the Cyclotron Corporation CS30 cyclotron. We use low cost natural arsenic metal as target to prepare ^{76}Br in the ^{75}As (^{3}He, 2*n*) ^{76}Br nuclear reaction.[19,20] A ^{76}Br production rate of 0.39 ± 0.09 mCi/μA h ($n = 32$) was obtained. Using 17 MeV incident ^{3}He particles, $0.4 \pm 0.1\%$ ^{75}Br (1.61 h) and $1.4 \pm 0.3\%$ ^{77}Br (57 h) were observed as radionuclidic impurities at 18 h post-EOB.

To isolate bromine activity from the target, we encountered problems obtaining [^{76}Br]NH_4Br using established methods.[21–23] Specifically, there was a substantial amount of ammonium sulfate contamination in the products, and when these products were used for labeling, radiochemical yields were usually very low. We then modified the distillation method of [^{76}Br]NH_4Br preparation, so that the ammonium sulfate contamination was negligible. The modified procedure also shortened the time of preparation to less than 1 h and the bromine activity was recovered from the target in $60 \pm 12\%$ ($n = 32$) decay corrected yield.

Syntheses of [^{76}Br]FBAU 3′,5′-dibenzoate and [^{76}Br]FBAU and the metabolic study

In this study, we synthesized [^{76}Br]FBAU 3′,5-dibenzoate and [^{76}Br]FBAU in sequence (Scheme 1) for the purpose of side-by-side



Scheme 1. Preparation of [^{76}Br]FBAU via its ester intermediate

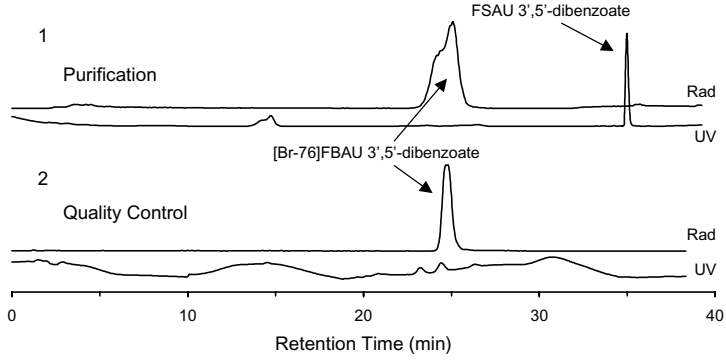


Figure 1. [^{76}Br]FBAU 3′,5′-dibenzoate (71.2%, RCY) was separated from the trimethylstannyl precursor easily. Quality control revealed good chemical and radiochemical purities, but a measurable amount of FBAU 3′,5′-dibenzoate (30 ng) lowered the specific activity

comparison in the subsequent metabolic study. [^{76}Br]FBAU 3′,5′-dibenzoate was prepared using an electrophilic substitution reaction from 1-(2-fluoro-2-deoxy-3,5-*O*-dibenzoyl-*β*-D-arabanofuranosyl)-5-trimethylstannyluracil (FSAU 3′,5′-dibenzoate)[24] and [^{76}Br]FBAU was prepared by basic hydrolysis of [^{76}Br]FBAU 3′,5′-dibenzoate.

The trimethylstannyl moiety of FSAU 3′,5′-dibenzoate is more lipophilic than the bromide of [^{76}Br]FBAU 3′,5′-dibenzoate and thus, the excess precursor is easily separated from the product by HPLC (Figure 1). The oxidation of [^{76}Br]Br^- was carried out by dilute peracetic acid (0.3%) and the electrophilic substitution was completed in acetic acid at room temperature in 20 min. We also found that when higher amounts of peracetic acid and hydrogen peroxide were used as oxidants in the presence of chloride, a large amount of chlorinated

analog was produced. The bromo and chloro analogs of the 3′,5′-dibenzoate compounds have nearly the same polarity and cannot be adequately separated by our HPLC system (experimental details not shown). Thus, the chloro analog could dilute the effective specific activity of [^{76}Br]FBAU 3′,5′-dibenzoate. We also avoided using chloramine-T and N-chlorosuccinamide as oxidants for the same reason.

When using FSAU 3′,5′-dibenzoate, one needs to be careful about the reaction conditions chosen, due to the propensity towards protodestannylation in the presence of strong acids. Once destannylated, there can be no regioselective radiobromination. It was determined that FSAU 3′,5′-dibenzoate was stable in acetic acid for at least 30 min but destannylated instantaneously if a trace of HCl was added. Both the destannylated product (retention time (RT): 14.7 min) and the trimethylstannyl precursor (RT: 35.0 min) can be easily separated from [^{76}Br]FBAU 3′,5′-dibenzoate (RT: 25.0 min) (Figure 1, chromatogram 1).

The reaction produced one major radioactive species, which was identified to be [^{76}Br]FBAU 3′,5′-dibenzoate by a co-injection with the authentic FBAU 3′,5′-dibenzoate (synthesized according to References.[24,25]). The rest of the activity eluted out at the polar region and there was also activity still retained on the HPLC column. The radiochemical yield based on the final purified product was $71.2 \pm 12.1\%$ ($n = 7$). Quality control showed that the product was radiochemically pure and had good chemical purity. (Figure 1, chromatogram 2) The specific activity was determined to be 0.6 Ci/μmol.

[^{76}Br]FBAU was prepared by hydrolysis of the benzoyl groups of [^{76}Br]FBAU 3′,5′-dibenzoate in base. After 15 min at 100°C, the hydrolysis was complete. However, only $53.1 \pm 9.2\%$ ($n = 5$) of the activity eluted as [^{76}Br]FBAU. (Figure 2, chromatogram 1) The remainder eluted earlier and was later determined to be [^{76}Br]Br^- by TLC. The hydrolysis was also performed at room temperature, and after an overnight reaction, a quantitative yield was afforded with decay correction. (Figure 2, chromatogram 2) The radiochemical purity was $>97\%$ after purification and was chemically pure. As this compound was a hydrolyzed product of the purified [^{76}Br]FBAU 3′,5′-dibenzoate, the specific activity of [^{76}Br]FBAU differed from that of the [^{76}Br]FBAU 3′,5′-dibenzoate only by the ^{76}Br decay over the time lapsed between the two preparations.

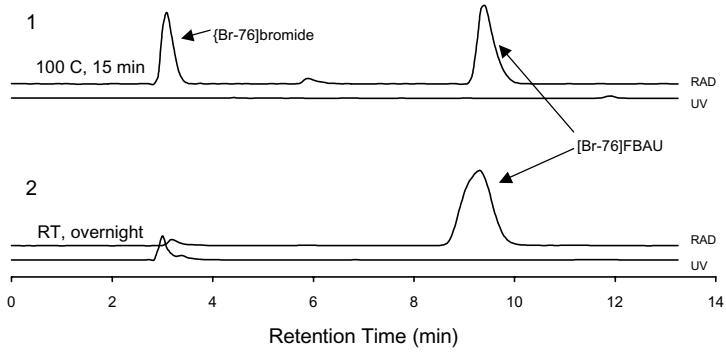


Figure 2. Hydrolysis of [^{76}Br]FBAU 3′,5′-dibenzoate under two different conditions produced [^{76}Br]FBAU with different yield

Our sequential syntheses produced a diester intermediate, [^{76}Br]FBAU 3′,5′-dibenzoate, which can be used as a lipophilic prodrug of [^{76}Br]FBAU for certain applications. In fact, incubation of [^{76}Br]FBAU 3′,5′-dibenzoate with either human or rat hepatocytes produced complete deesterification to [^{76}Br]FBAU in less than 10 min. Furthermore, [^{76}Br]FBAU remained intact, with no further degradation by hepatocytes for at least 4 h.

Experimental

Cyclotron production of ^{76}Br and preparation of [^{76}Br]NH_4Br

The cyclotron production of ^{76}Br was based on the established procedure.[19,20] In short, natural arsenic metal (200 mg) was pressed in a single-use aluminum external target cup equipped with a 0.38 mm aluminum degrader that reduced the ^{3}He incident beam energy to 17 MeV.Targets were irradiated at 20 μA for 1 to 3 h and processed after sufficient decay of ^{75}Br ($T_{1/2}$ =1.61 h).

To obtain [^{76}Br]NH_4Br for labeling, the arsenic target and $Na_2Cr_2O_7 \cdot H_2O$ (1.5 g) were mixed in a distillation flask before the aqueous sulfuric acid (50% v/v, 10 ml) was added. The mixture was heated at 125°C for 30 min. The activity carried over by nitrogen was trapped in 2 N NH_4OH. The NH_4OH solution containing [^{76}Br]NH_4Br was then evaporated to dryness before being reconstituted by water for labeling applications.

Synthesis of [^{76}Br] 1-(2-fluoro-2-deoxy-3,5-O-dibenzoyl-β-D-arabanofuranosyl)-5-bromouracil ([^{76}Br]FBAU 3′,5′-dibenzoate)

1-(2-Fluoro-2-deoxy-3,5-*O*-dibenzoyl-β-D-arabanofuranosyl)-5-trimethylstannylsuracil (synthesized based on[24]) (0.12 mg,. 0.20 μmol) was dissolved in peracetic acid (80 μl, 0.3% v/v in acetic acid). To this solution was added [^{76}Br]NH_4Br solution in water (10 μl, 1.86 mCi or 68 MBq). The mixture was allowed to stand at the room temperature for 30 min.Without quenching the reaction, the entire volume of the reaction was injected onto a HPLC for purification. The HPLC system used for purification comprised Gilson gradient HPLC pumps (model 305 and 306), adjustable wavelength UV detector (model 151), fraction collector (model 203b), and a Beckman radiation detector equipped with a NaI probe. Gilson UniPoint software controlled the system. The system was equipped with a Phenomenex, Luna 5μ C18(2) column (4.6 mm × 250 mm). An isocratic mobile phase of acetonitrile/water (50/50) running at 1 ml/min then eluted out the product at 25.0 min. At 26 min, the mobile phase was changed to 90% acetonitrile to elute out the remaining stannous precursor. The collected product (1.25 mCi or 46 MBq) was passed through a SepPak C18 Light cartridge and collected in 0.3 ml of EtOH. Without further formulation, the EtOH solution of [^{76}Br]FBAU 3′,5′-dibenzoate was used for the preparation of [^{76}Br]FBAU or for the *in vitro* metabolism study.

Quality control and specific activity determination were performed by the following procedures. The ethanol solution (15 μl) of the product (typically 30–80 μCi) was injected into the same HPLC system used for purification. Wavelength for UV detection was set at 276 nm, an absorption peak for the authentic FBAU 3′,5′-dibenzoate, and the sensitivity was set so that 0.01 μg gave rise to a distinguishable peak. The integration under the UV peak was compared to the standard curve of the absorption generated by FBAU 3′,5′-dibenzoate (synthesized based on[24,25]).

Synthesis of [^{76}Br] 1-(2-fluoro-2-deoxy-β-D-arabanofuranosyl)-5-bromouracil ([^{76}Br]FBAU)

To the ethanol solution of [^{76}Br]FBAU 3′,5′-dibenzoate (20 μl, 2.0 mCi. or 74 MBq) was added a solution of 3 N NaOH (20 μl). The reaction mixture was sealed in a 1 ml v-vial and heated at 100°C for 15 min.After cooling down, the solution was neutralized by 3 N HCl (20 μl). Alternatively, the reaction mixture was kept at room temperature overnight before being neutralized.

The entire solution was then injected into HPLC for purification. The HPLC system was the same one used in the preparation of [^{76}Br]FBAU 3′,5′-dibenzoate. The column used was a Supelco Supelcosil LC18S column (4.6 mm × 250 mm). Phosphate buffer (pH 5, 0.05 M) with 9% EtOH running at 1 ml/min eluted the product, and the fractions collected between 9.0 and 10.0 min were combined to give the product. The authentic FBAU used for co-injection was synthesized according to established methods.[26,27] The HPLC solvent was evaporated and reconstituted in EtOH for the *in vitro* metabolism studies. Quality control was performed by a procedure similar to that of the [^{76}Br]FBAU 3′,5′-dibenzoate.

Incubation of [^{76}Br]FBAU 3′,5′-dibenzoate and [^{76}Br]FBAU with hepatocytes

The cryo-preserved hepatocytes from both male Sprague–Dawley rats and male human liver tissue were thawed at 37°C in a water bath and gradually diluted with cell culture medium (RPMI Medium 164O media, Life Technologies, Rockville, MD). After washing the cells with the medium, the viable cell concentration was adjusted to 1.0 million/ml. The resulting cell suspension was then incubated at 37°C for 15 min before 20 µl EtOH solution of [^{76}Br]FBAU 3′,5′-dibenzoate (100 µCi, or 3.7 MBq) was added to the suspension. The suspension was maintained at 37°C, and at 10, 30, 60, 120 and 240 min, 100 µl of cell suspension was removed and mixed with 100 µl of EtOH. The mixture was centrifuged at 5000 rpm for 5 min, and the supernatant (20 µl) was injected onto an HPLC for analysis. The entire procedure was repeated for the incubation of [^{76}Br]FBAU.

Conclusion

Bromine-76 was produced on site based on the ^{75}As (^{3}He, 2*n*) ^{76}Br nuclear reaction and the ^{76}Br generated was recovered as [^{76}Br]NH_4Br for labeling. [^{76}Br]FBAU 3′,5′-dibenzoate and [^{76}Br]FBAU were successfully synthesized in sequence to provide both ^{76}Br compounds for side-by-side comparison of their metabolic stabilities. Incubations of both compounds with hepatocytes showed that [^{76}Br]FBAU 3′,5′-dibenzoate was quickly metabolized to [^{76}Br]FBAU, while [^{76}Br]FBAU was stable for up to 4 h. No [^{76}Br]Br^- was released from either

compound. Both compounds are good candidates for further studies to establish their potential as proliferation markers for PET.

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