Available online at www.sciencedirect.com

SCIENCE DIRECT®

Nuclear Medicine and Biology 32 (2005) 811–819

NUCLEAR MEDICINE — AND — BIOLOGY

www.elsevier.com/locate/nucmedbio

Imaging herpes viral thymidine kinase-1 reporter gene expression with a new ^{18}F-labeled probe: 2′-fluoro-2′-deoxy-5-[^{18}F]fluoroethyl-1-β-D-arabinofuranosyl uracil

Julius A. Balatoni[a,c], Michael Doubrovin[b], Ludmila Ageyeva[b], Nagavarakishore Pillarsetty[b], Ronald D. Finn[a], Juri G. Gelovani[c], Ronald G. Blasberg[b,*]

[a]*Radiochemistry/Cyclotron Core Facility, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*
[b]*Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*
[c]*Department of Experimental Diagnostic Imaging, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA*

Received 25 May 2005; received in revised form 28 June 2005; accepted 7 July 2005

Abstract

The preparation and radiolabeling of 2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-(2-fluoroethyl)-uracil (FFEAU) with ^{18}F and its evaluation as a probe for imaging herpes simplex virus 1 thymidine kinase (HSV1-*tk*) gene expression are described. 2′-Fluoro-2′-deoxy-3′, 5′-di-*O*-benzoyl-1-β-D-arabinofuranosyl-3-*N*-benzoyl-5-(2-[^{18}F]fluoroethyl)-uracil **12** was prepared by nucleophilic substitution of the corresponding tosyl **8** or trifluoroethanesulfonyl **9** derivative with *n*-Bu$_4$N[^{18}F]F. Base hydrolysis was used to remove the benzoyl protecting groups, followed by HPLC purification, to afford [^{18}F]FFEAU **13**. The trifluoroethanesulfonyl substrate **9** appears to be the better labeling precursor. Carrier *n*-Bu$_4$NF was added to the labeling reaction, which resulted in specific activities of 40–70 Ci/mmol (estimated). Radiochemical purity averaged 94±4%. Although [^{18}F]FFEAU was obtained in low radiochemical yield with **9** and further optimization of the radiosynthesis will be required, sufficient product was available for a series of in vitro and in vivo studies. [^{18}F]FFEAU was directly compared with [^{3}H]TdR in a series of in vitro accumulation studies involving a HSV1-*tk* stably transduced cell line, RG2TK+ and a nontransduced, wild-type RG2 cells. The initial in vitro and in vivo imaging studies are promising; FFEAU has in vitro accumulation and sensitivity characteristics similar to that previously reported for FIAU, but greater selectivity than FIAU due to lower uptake and retention in nontransduced cells and tissues. The animal imaging experiment showed low levels of radioactivity in the lungs, with little or no radioactivity seen in the heart, liver, spleen and intestines.

Keywords: 2′-Fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-(2-fluoroethyl)-uracil; ^{18}F; HSV1-*tk*; PET; Reporter gene

1. Introduction

Several different radiolabeled probes have been developed to image HSV1 thymidine kinase gene (HSV1-*tk*) expression [1–12]. Pyrimidine nucleosides labeled with radioiodine, such as FIAU, appear to have several advantages over radiolabeled acycloguanosine probes such as FHBG and FHPG for imaging stably transduced cells [13,14], although FHBG may be a better probe for imaging the sr39 HSV1-tk variant (*HSV1-sr39tk*) [8]. The main advantage of using ^{18}F-labeled probes over radioiodine (^{123}I, ^{124}I or ^{131}I)-based probes is that the short half-life of ^{18}F allows repetitive and sequential imaging of the subjects.

We recently demonstrated that good images of HSV1-*tk* expression in transduced tissue can be obtained 1 to 2 h after intravenous administration of [^{124}I]FIAU [13]. Therefore, we decided to synthesize an ^{18}F-labeled analogue of FIAU that could be used for repetitive, sequential imaging studies of HSV1-*tk* expression and retain the imaging advantages of FIAU (greater sensitivity and dynamic range).

This report describes the synthesis and initial evaluation of ^{18}F-labeled 2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-(2-fluoroethyl)-uracil (FFEAU), a new probe for imaging HSV1-*tk* expression. FFEAU has similar sensitivity char-

* Corresponding author.

0969-8051/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.nucmedbio.2005.07.007

acteristics, but greater selectivity and dynamic range than FIAU, due to a marked reduction of uptake and retention in nontransduced cells and tissues.

2. Results and discussion

The preparation of ^{18}F-labeled FFEAU **13** was accomplished in two steps from either the tri-benzoyl protected tosyl **8** or trifluoroethanesulfonyl (tresyl) **9** derivatives as shown in Fig. 1. The labeling precursors **8** and **9** were synthesized as outlined in Fig. 2. Beginning with 5-(2-hydroxyethyl)-uracil **1** and 2-fluoro-2-deoxy-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose **2**, the initial 5-hydroxyethyl substituted nucleoside **4** was prepared in classical fashion by glycosylation of **1** with the 3,5-di-*O*-benzoyl-2-deoxy-α-D-arabinofuranosyl bromide 3. Compound **4** was obtained as a mixture of α- and β-anomers in a ratio (determined by ^{1}H NMR) of 9:91, respectively, in an overall yield of 82%. Previous attempts to prepare the 5-fluoroethyl substituted derivative from **4** using either diethylaminosulfur trifluoride (DAST) or conversion to the tosylate, followed by nucleophilic substitution with fluoride, have failed due to an undesirable ring closure between the 4-oxo group and the β-carbon of the ethyl side group [15]. Consequently, to prevent this ring closure from occurring, it was decided to protect the 3-*N* position as a benzoate, thus deactivating the nucleophilicity of the 4-oxo function. To add a benzoyl group to the 3-*N* position, the 5-hydroxyethyl moiety had to be first protected as a silyl ether. This was readily accomplished by treatment of **4** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in anhydrous DMF, which afforded the silyl-protected derivative **5** in good yield. Stirring **5** with a 10-fold excess of benzoyl chloride and 4-(dimethylamino)pyridine (DMAP) for a couple of days yielded the tribenzoate-protected product **6**. Overnight treatment of **6** with 80% glacial acetic acid in water cleanly provided the key tribenzoate intermediate **7**. Desilylation of **6** with tetrabutylammonium fluoride in THF led to partial decomposition and significantly reduced recovery of **7**.

Compound **7** was converted to the tosylate **8** via reaction with *p*-toluenesulfonyl chloride in the presence of DMAP in high (93%) yield. The tresylate **9** was prepared in analogous fashion using 2,2,2-trifluoroethanesulfonyl chloride and DMAP, but in moderate (39%) yield. In an effort to prepare the tosylate **8** in fewer synthetic steps, **4** was successfully tosylated, but then was very resistant to benzoylation, yielding only a minute amount of product and unchanged starting material. Therefore, the longer synthetic path as presented in Fig. 2 was followed. Attempts to prepare the triflate derivative from **7** failed. Additions of triflic anhydride to mixtures of **7** and pyridine in dichloromethane at −78°C (under nitrogen) resulted in significant decomposition of **7** and the recovery of unchanged starting material along with some unidentified byproducts. As a result, an alternative synthetic route is being investigated to obtain the triflate derivative.

Reaction of **7** with DAST in a mixture of dry diglyme (2-methoxyethyl ether) and dichloromethane, in the presence of pyridine, afforded the 5-fluoroethyl derivative **10** in low yield (12%). Due to the number of impurities still present after column chromatography, it was necessary to perform preparative HPLC to achieve a clean product.

2′-Fluoro-2′-deoxy-3′,5′-di-*O*-benzoyl-1-α-D-arabinofuranosyl-3-N-benzoyl-5-(2-[^{18}F]fluoroethyl)-uracil, [^{18}F]-FFEAU, 12 was produced by fluorination of **8** or **9** with *n*-Bu$_4$N[^{18}F]F in acetonitrile at 85°C for 30 min. The *n*-Bu$_4$N[^{18}F]F was prepared from aqueous [^{18}F]HF, ~0.5 μmol of *n*-Bu$_4$NOH and ~0.5 μmol of carrier *n*-Bu$_4$NF. Unfortunately, the % incorporation of ^{18}F was very low with both precursors, but the tresylate **9** gave better results. After Sep-Pak treatment, reaction mixtures were analyzed by radio-HPLC to estimate ^{18}F incorporation and found in the case of **9** that about 0.5% incorporation (n=6) was being obtained (relative to starting ^{18}F). Removal of benzoate groups was accomplished by treatment of the reaction mixture with sodium methoxide in anhydrous methanol at 70°C for 5–10 min. The final product, [^{18}F]FFEAU **13**, was isolated using preparative HPLC. Radiochemical yields averaged 0.2% (n=4; decay corrected). Radiochemical purity ranged from 91% to 98% (average value, 94%; n=4) with [^{18}F]fluoride present as impurity. Specific activity values were estimated to be in the order of 40–70 Ci/mmol, based on carrier *n*-Bu$_4$NF added and the radiochemical yields obtained. With the tosylate **8** as the precursor, a radiochemical yield of <0.1% (n=1; decay corrected) was obtained with a radiochemical purity of 95%.

In terms of radiolabeling yield, these early results are quite low, but this methodology still provided enough product to

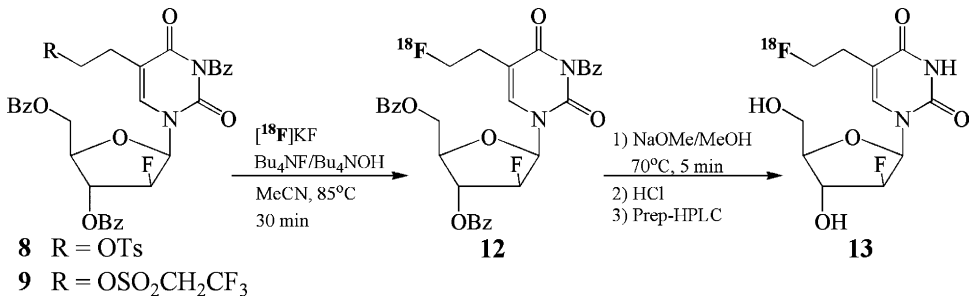

Fig. 1. The radiosynthesis of [^{18}F]FFEAU.