Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 20 June 2014, Revised 23 September 2014, Accepted 24 September 2014 Published online 20 October 2014 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3241

# A microwave radiosynthesis of the 4-[18F]-fluorobenzyltriphenylphosphonium ion†

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**The 4-[$^{18}$F]-fluorobenzyltriphenylphosphonium cation was synthesized by a series of microwave reactions from no carrier added [$^{18}$F]-fluoride. The microwave procedure reduced the quantity of reagents used and synthesis time when compared with the original synthesis. In addition, problematic solid phase extraction, sodium borohydride reduction by column and inconsistent yields with excessive precipitate formation during the bromination step were eliminated. The 4-[$^{18}$F]-fluorobenzyltriphenylphosphonium cation was produced radiochemically pure in 8.3% yield with a specific radioactivity of 534.5 ± 371.4 GBq/μmole at end of synthesis.**

**Keywords:** phosphonium; fluorine-18; positron emission tomography; microwave

## Introduction

Recent studies using the lipophilic 4-[18F]-fluorobenzyltriphenyl phosphonium cation (18F-FBnTP) indicate that the radiotracer is useful for studying mitochondrial dysfunction in numerous diseases including cardiac and cancer.[1–4] $^{18}$F-FBnTP was initially synthesized by manual manipulation involving multiple steps including two solid phase extraction (SPE) procedures, a problematic on-column reduction, and a cumbersome bromination step resulting in inconsistent yields with excessive precipitate formation and significant radiation exposure.[5] All these factors were major deterrents to performing the radiosynthesis on a routine basis. To greatly reduce exposure, an initial automated and remote synthesis using a modified dual-run FDG synthesis module was developed (unpublished work). The dual-run synthesis module procedure following the original synthesis also proved problematic, frequently requiring regular manual intervention to avert a synthesis failure. In order to synthesize $^{18}$F-FBnTP for routine research studies, a modified reaction sequence that could be automated and would reduce the problems with the manual and module radiosynthesis was sought.

## Results and discussion

A microwave radiosynthesis[6] that greatly simplified the original production of $^{18}$F-FBnTP involving a similar four-step synthesis was developed (Figure 1). A hardware system (Figure 2) was built to accommodate specifically the $^{18}$F-FBnTP radiosynthesis but is adaptable to other fluorine-18 radiosyntheses. The custom hardware contains a heating block, custom microwave cavity, two Tecan Cavro syringe pumps, a multi-port cap constructed for standard 5 mL v-vials, and valved reagent addition vials. The synthesis module is controlled by a National Instruments Compact Fieldpoint module linked to a laptop computer running Labview Real-Time software (window examples are shown Figure 3). The entire hardware–software system allows for the complete control of every synthetic step from the collection of the [$^{18}$F]-fluoride to the injection of the reaction mixture onto the semi-preparative HPLC. The general radiosynthetic procedure for $^{18}$F-FBnTP is described in the succeeding texts.

The [$^{18}$F]-fluoride is unloaded from the target directly onto an ion exchange resin column. After trapping, the column is eluted with an aqueous acetonitrile (MeCN) solution of potassium carbonate ($K_2CO_3$) and Kryptofix into the 5 mL reaction vial. The solution is heated (in the heating block) to dryness with nitrogen flow. Two additional aliquots of MeCN are added with heating to ensure azoetropic removal of the water. The reaction vial is remotely transferred to the microwave cavity and cooled to room temperature before microwave irradiation. The precursor, 4-trimethylammoniumbenzaldehyde trifluoromethanesulfonate, in MeCN is added, and the solution is microwave irradiated in the sealed vial at 50 W for 30 s to produce 4-[$^{18}$F]fluorobenzaldehyde ($^{18}$F-FBnO). Aqueous sodium borohydride is added, and the mixture is microwave irradiated at 25 W for 30 s to produce 4-[$^{18}$F] fluorobenzyl alcohol ($^{18}$F-FBnOH). Next, aqueous hydrobromic acid (HBr) is added. The HBr solution is microwave irradiated at 25 W for 60 s. The 4-[$^{18}$F]fluorobenzyl bromide ($^{18}$F-FBnBr) solution is immediately eluted by nitrogen pressure through a Waters Oasis HLB Plus (Oasis SPE) cartridge. The reaction vial is rinsed with water and washed quickly through the SPE. The Oasis SPE cartridge is then eluted with an MeCN/acetic acid (HOAc) mixture into a second 5 mL reaction vessel containing triphenylphosphine (TP), and the solution is thermally heated. After cooling and adding water, a precipitate (triphenylphosphine oxide) forms. The mixture with precipitate is filtered and injected directly onto the semi-preparative HPLC column. The $^{18}$F-FBnTP product peak is collected into a reservoir containing HPLC water. The diluted product is loaded onto a Waters Oasis HLB Plus cartridge. An automated SPE reformulation

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*†Additional supplementary material can be found in the online version of this article at the publisher's web-site.*

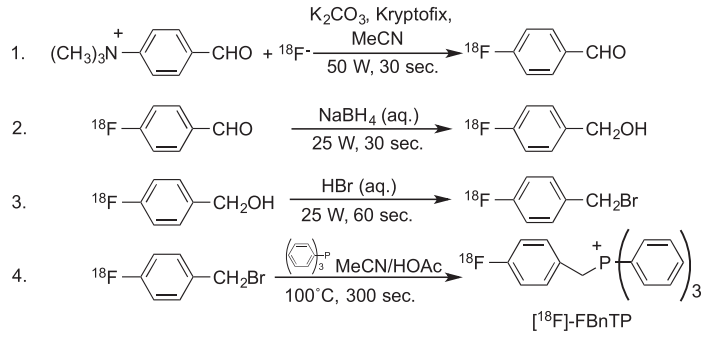


**Figure 1.** 4-[18F]-Fluorobenzyltriphenylphosphonium cation microwave radiosynthesis.

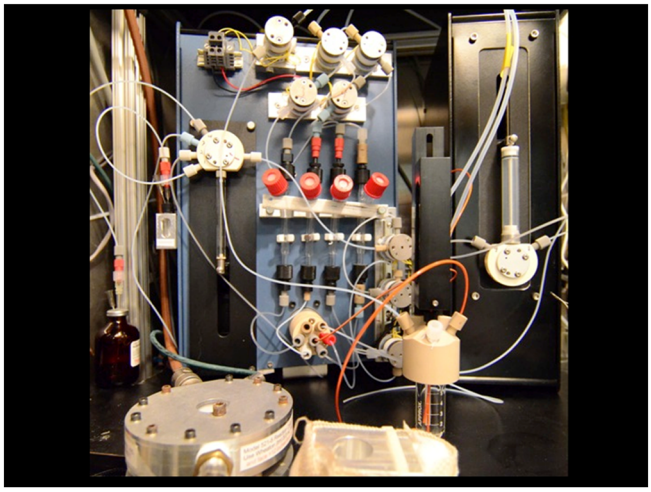

**Figure 2.** Custom automated microwave system.

includes a wash of the SPE with HPLC water, elution of the product with ethanol and sterile saline through a sterile filter to a final product vial. The preparative and final product chromatograms are presented in the Supporting Information.

The new microwave procedures allowed for the significant reduction in quantity and volume of precursor and other reagents.[6] Problems caused by larger volumes and precipitate solids were virtually eliminated. During the optimization of the microwave syntheses, yields of each radiosynthetic step ($n$=3) were determined by analytical HPLC. All chromatograms are displayed in the Supporting Information. The observed (isolated yields were not determined) HPLC yields were 57% for the fluorination reaction, 95% for the reduction reaction, 85% for the bromination reaction, and 95% for the alkylation reaction. These results are comparable to those observed in the manual and module radiosyntheses[5]; however, the microwave procedures reduced the total radiosynthesis time by 40 min with an increase in specific radioactivity and radiochemical yield.

During the development of the modified synthesis, some issues were noted with the system and procedure. Initial bromination reaction attempts used approximately 9 to 18-fold excess (880 to 1760 μmols HBr, 0.1 to 0.2 mL) of aqueous 48% HBr compared with a total of sodium borohydride and potassium carbonate (nearly 100 μmols as unreacted base) added to the reaction vial. Little to no reaction was observed. A larger excess, 0.8 mL (7070 μmols) of HBr, did provide an almost complete conversion of the $^{18}$F-FBnOH to $^{18}$F-FBnBr as observed by HPLC. Another confounding factor was that acetonitrile reacts with the aqueous HBr. A large excess of HBr reacted with both the acetonitrile and $^{18}$F-FBnOH. Attempts to remove the acetonitrile prior to bromination resulted in a substantial loss of $^{18}$F-FBnOH during the evaporation. Other reaction solvents tested proved incompatible with either the fluorination, reduction or bromination step.

A second issue was observed during the isolation of the $^{18}$F-FBnBr on the SPE. HPLC analysis of the isolated $^{18}$F-FBnBr from the Oasis SPE showed a large hydrophilic peak present (shown to be $^{18}$F-FBnOH). Analysis prior to the SPE indicated little $^{18}$F-FBnOH. The SPE water wash to remove the HBr apparently left a small amount of acidic water on the SPE. The quantity was sufficient to hydrolyze the $^{18}$F-FBnBr back to the $^{18}$F-FBnOH. The water wash could not be eliminated because the majority of the aqueous HBr needed to be removed to allow the reaction with a small quantity of triphenylphosphine (3 mg) with a small amount of precipitate. By performing the wash and elution of the SPE cartridge rapidly, the conversion back to $^{18}$F-FBnOH was significantly reduced (but not eliminated).

An HBr solution in acetic acid (HBr/HOAc) was examined in an effort to prevent the hydrolysis of the 18F-FBnBr. The HBr/HOAc reaction gave inconsistent results. As noted in the preceding texts, large amounts of the acid were needed to complete the reaction as a result of the acetonitrile. In addition, the 18F-FBnBr in the HBr/HOAc solution (even diluted with water) could not be retained on the SPE. Finally, the simple addition of TP to the 18F-FBnBr/HBr/HOAc reaction mixture required a large quantity of TP (21 mg) to complete the reaction. The excessive precipitate created was impossible to remove prior to injection onto the semi-preparative column.

A third issue (radiolysis) was noted when the final $^{18}$F-FBnTP tracer concentration was near or above 8 mCi/mL. Although not observed during initial quality control, a reduction in the radiochemical purity, below 80%, was noted during the 4-h stability check at the higher tracer concentrations. By reducing the starting [$^{18}$F]-fluoride quantity and thereby reducing amount of radiotracer synthesized and concentration in solution to less than 8 mCi/mL, the problem was resolved. The addition of an antioxidant to the final radiotracer solution might also reduce radiolysis.

## Experimental

[$^{18}$F]-Fluoride was produced by 18 MeV proton bombardment of a high pressure [$^{18}$O]-water target using a GE PETtrace cyclotron. All reagents were of ACS or HPLC purity. The precursor, 4-trimethylammoniumbenzaldehyde trifluoromethanesulfonate, was purchased from ABX GmbH. Authentic product, 4-fluorobenzyltriphenylphosphonium bromide, previously synthesized,[5] was used as the standard to determine the identity, purity and specific radioactivity of the final product. The semi-preparative chromatography system consisted of an Agilent 1260 Prep pump with a VICI injector, a Knauer 200 UV Detector (254 nm), a Bioscan Hot Cell interface with a diode radioactivity detector. The semi-preparative HPLC column was a Waters XBridge C18 10 μm 10 × 150 mm column eluted with a mixture

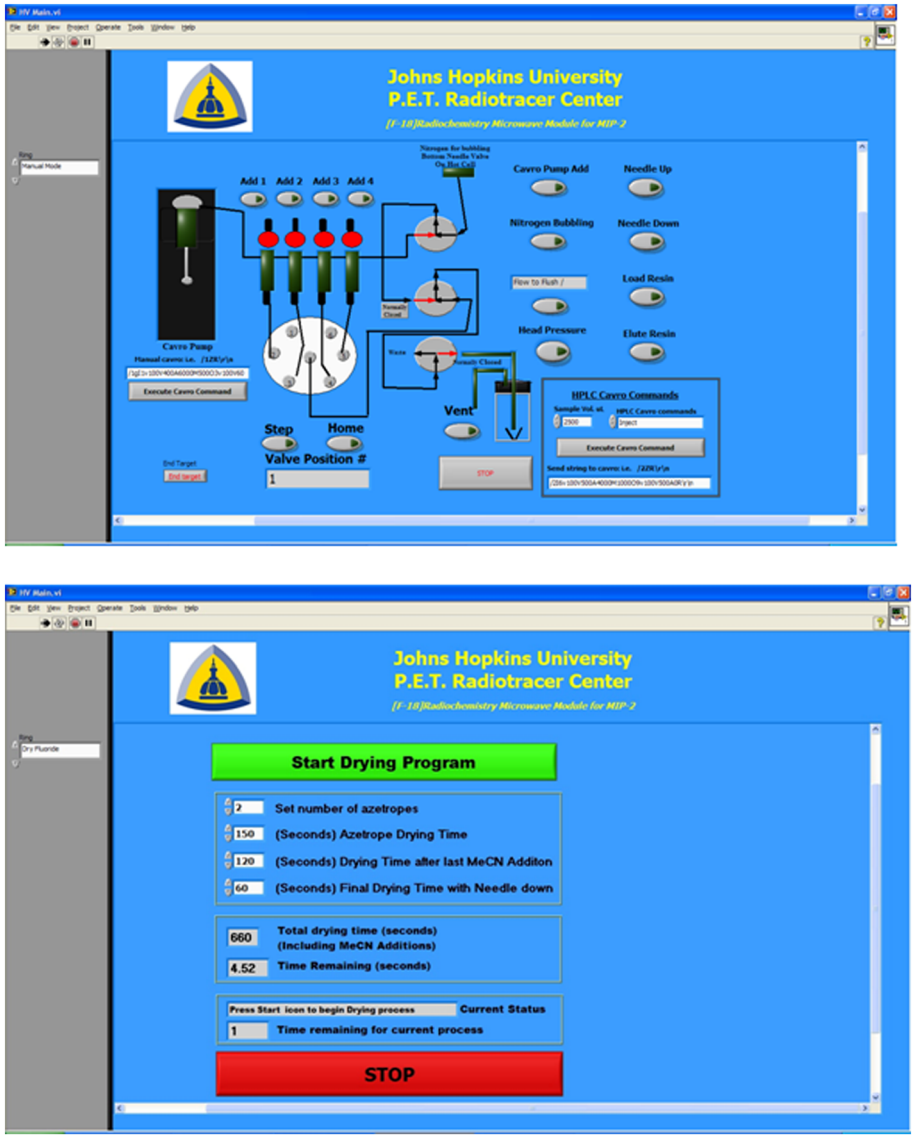


**Figure 3.** The software interface for the automated microwave system.

of 40:60 methanol : pH 3.2 triethylamine/phosphoric acid buffer at flow rate of 10 mL/min. The analytical chromatography system included an Agilent 1260 Infinity System incorporating a quaternary pump, HiP ALS autosampler, and DAD UV detector with a Max-Light flow cell set to 254 nm plus a Bioscan Flow-Count interface with a NaI radioactivity detector The analytical HPLC column was an Xbridge C18 3.5 μm 4.6 × 100 mm column eluted with a mixture of 35:65 acetonitrile : pH 3.2 triethylamine/phosphoric acid buffer at a flow rate of 2 mL/min. Chromatographic data were acquired and analyzed on an Agilent OpenLAB chromatography data system (Rev. A.04.02). Radioactivity measurements were made using a Capintec CRC-15R or Comcer model TALETE HC dose calibrator. Synthesis automation equipment included a custom synthesis module controlled by National Instruments LABView (2009 SP1) software and a National Instruments Compact Fieldpoint (CFP2110) system. The microwave system was a Resonance Instruments Model 521 fitted with a Model 521-5 reactor cavity for 5 mL v-vial modified for air cooling.

## Microwave radiosynthesis and purification of 4-[$^{18}$F]-fluorobenzyltriphenylphosphonium cation

Aqueous [$^{18}$F]-fluoride was trapped on a Chromafix 30-PS-HCO3 cartridge (ABX). The cartridge was eluted with 0.15 mL of a $K_2CO_3$ : Kryptofix solution (9.5 mg $K_2CO_3$ : 48 mg Kryptofix in 0.6 mL of 50% aqueous MeCN) into a 5 mL vial with a multi-port cap. After rinsing the cartridge with 0.25 mL MeCN, the solution was dried at 110°C with controlled nitrogen flow (325 mL/min) for 150 s. Two separate additions of 0.25 mL of MeCN were heated for 150 and 180 s respectively. The vial was transferred remotely to the microwave cavity, cooled for 2 min using compressed air (flow approx. 6 L per minute). 4-Trimethylammoniumbenzaldehyde trifluoromethanesulfonate (12.8 μmols, 4 mg in 0.4 mL MeCN) was added from a valved reservoir attached through the multi-port cap. The solution was microwave irradiated at 50 W for 30 s. Next, sodium borohydride (31.7 μmols, 1.2 mg in 0.2 mL HPLC water) was added from a valved reservoir, and microwave irradiation applied at 25 W for 30 s. Through a third valved reservoir, 0.8 mL of aqueous HBr (48%) was added and the solution was microwave irradiated at 25 W for 60 s. The hot solution was rapidly eluted through a Waters Oasis HLB Plus LP cartridge using positive pressure of nitrogen gas followed by 15 s of drying at approx. 125 mL/min. The vessel was rinsed with 1 mL of water, and the solution eluted and dried in the same manner. The Oasis cartridge was eluted with a mixture of MeCN (0.9 mL), and glacial acetic acid (0.1 mL) into a new 5 mL reaction v-vial containing triphenylphosphine (11.4 μmols, 3 mg). The vessel was heated to 100°C for 5 min followed by cooling for 2 min. HPLC water (1 mL) was added to the vial, and the solution was injected onto the semi-preparative column through a 0.45 μm Teflon filter (17 mm diameter). The product, 18F-FBnTP (RT = 10.3 min, k′ = 6.2), was collected in 50 mL HPLC water for SPE reformulation. The water

  

solution was eluted through a Waters Oasis HLB Plus LP cartridge, and the cartridge was washed with 10 mL saline. The cartridge was eluted with 1 mL absolute ethanol followed by 10 mL sterile saline through a 0.2 μm sterile Millipore FG filter (25 mm) into a sterile vial containing 4 mL sterile saline. An aliquot was removed for quality control analysis. Analytical HPLC was performed to determine radiochemical identity (RT = 4.4 min, k′ = 7.8), purity, and specific activity.

## Conclusion

The microwave radiosynthesis of $^{18}$F-FBnTP provided an automated method for the radiotracer production that simplified and eliminated steps of the original synthesis that were burdensome. The new radiosynthesis allowed the radiofluorination, reduction and bromination to occur in one pot, reduced the need for large quantities of reagents and changed an unreliable bromination step into a reliable and consistent procedure. A radiochemically pure product (>99%) was obtained in 8.3 ± 2.4% yield (end of synthesis) with an average synthesis time of 52.4 ± 14 min ($n$ = 27) and an average specific radioactivity of 534.5 ± 371.4 GBq/μmole (14,446 ± 10,039 mCi/μmole end of synthesis).

## Conflict of Interest

The authors did not report any conflict of interest.

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## Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.