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Research Article

Radiosynthesis of 3-[^{18}F]fluoropropyl and 4-[^{18}F]fluorobenzyl triarylphosphonium ions

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Summary

3-[^{18}F]Fluoropropyl-, 4-[^{18}F]fluorobenzyl-triphenylphosphonium and 4-[^{18}F]fluorobenzyltris-4-dimethylaminophenylphosphonium cations were synthesized in multi-step reactions from no carrier added (nca) [^{18}F]fluoride. The time for synthesis, purification, and formulation was 56, 82, and 79 min with an average radiochemical yield of 12, 6 and 15%, respectively (not corrected for decay). The average specific radioactivity for the three radiolabeled compounds was 14.9 GB q/μmole (403 mCi/μmole) at end of synthesis (EOS).

Key Words: phosphonium; fluorine-18; positron emission tomography

Introduction

Recent studies using lipophilic cations including phosphonium cations indicate these cations accumulate in some cancer cells to a higher degree than in normal cells.[1,2] This phenomenon is attributed to the higher than normal electrochemical membrane potential of the mitochondria in cancer cells.[3,4] Furthermore, loss of mitochondrial membrane potential is an early event in cell death caused by pro-apoptotic agents.[5] Cumulative evidence suggests that mitochondria-controlled apoptosis underlies cell loss in heart failure.[6] Tumor and cardiac mitochondria membrane potential has been evaluated with tritium labeled phosphonium cations and fluorescent dyes, such as, the [^{3}H]tetraphenylphosphonium[1] and [^{3}H]methyltriphenylphosphonium[7–9] cations, and rhodamine 123.[10]

Positron emission tomographic (PET) animal brain tumor studies indicated the lipophilic cation radiotracer, [^{11}C]methyltriphenylphosphonium cation, had a tumor to normal brain uptake ratio of nearly 48:1 and a prolonged

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retention time.[11] Similarly, animal cardiac PET studies with the same radiotracer show heart to lung and heart to blood ratios of 14:1 and greater than 46:1, respectively, with a non-infarcted to infarcted myocardium ratio of 12:1 and prolonged retention.[12]

To provide a tracer similar to the [^{11}C]methyltriphenylphosphonium cation but with a longer half-life, the syntheses of ^{18}F labeled phosphonium cations were investigated. This paper describes the synthesis, purification, formulation, and characterization of 3-[^{18}F]fluoropropyltriphenylphosphonium, 4-[^{18}F]fluorobenzyltriphenylphosphonium and 4-[^{18}F]fluorobenzyltris-4-dimethylaminophenylphosphonium cations from [^{18}F]fluoride. Reported preliminary animal data on the 4-[^{18}F]fluorobenzyltriphenylphosphonium cation indicates a rapid blood clearance and myocardial accumulation with a heart to lung ratio of 15:1, prolonged retention and low non-specific binding in canines.[13,14] In a canine pacing model for heart failure, myocardium showed a global decrease of 4-[^{18}F]fluorobenzyltriphenylphosphonium cation uptake of 16% and regional apoptic site decreases of greater than 40%.[15] Also, mice bearing human breast carcinoma tumors showed a 45% reduced uptake of the 4-[^{18}F]fluorobenzyltriphenylphosphonium cation 48 h after administration of taxotere with no affect on heart, lung or liver uptake; and, low uptake in sites of inflammation.[16,17]

Results and discussion

The 3-[^{18}F]fluoropropyltriphenylphosphonium cation radiosynthesis (Figure 1) was performed in a single vessel. After drying the nca [^{18}F]fluoride in the presence of Kryptofix and potassium carbonate (K_2CO_3), propylene glycol ditosylate in acetonitrile was added and the solution heated for 4 min. Triphenylphosphine in toluene was subsequently added. After the solution was heated to boiling on a heat gun for 5 min, the toluene was evaporated, and high pressure liquid chromatography (HPLC) solvent added while cooling the mixture to room temperature. The mixture was filtered to remove the

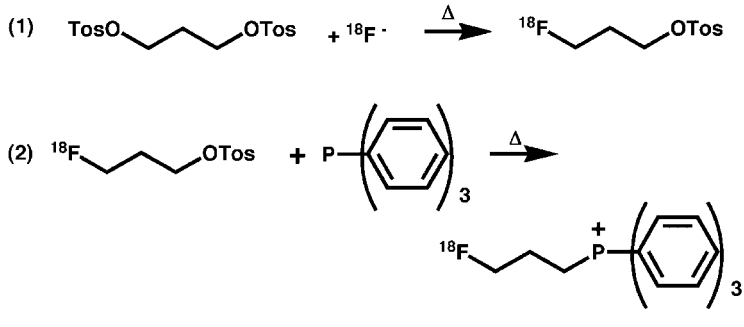

Figure 1. 3-[^{18}F]fluoropropyltriphenylphosphonium cation radiosynthesis

unreacted triphenylphosphine precipitate prior to injection on to the semi-preparative HPLC column. The radioproduct was collected, the solvent was evaporated, and residue was dissolved in normal saline to yield a radiochemically pure solution (>99%), as determined by analytical HPLC, in 12% yield (EOS) from starting [^{18}F]fluoride. The synthesis, purification and formulation were completed in 56 min ($n = 10$) at an average specific radioactivity of 15.5 GBq/μmole (420 mCi/μmole EOS). Authentic 3-fluoropropyltriphenylphosphonium bromide was synthesized and used to determine the identity, purity and specific radioactivity of the final product.

The radiosynthesis of the 4-[^{18}F]fluorobenzyltriphenylphosphonium cation involved the synthetic steps shown in Figure 2 and follows a reaction scheme previously investigated.[18,19] After drying the [^{18}F]fluoride in the presence of Kryptofix and K_2CO_3, the 4-trimethylammoniumbenzaldehyde trifluoromethanesulfonate in dimethylsulfoxide (DMSO) was added. The solution was heated in a sealed vial for 10 min. The cooled reaction mixture was diluted with water and passed through a reverse phase solid phase extraction (SPE) cartridge. After blowing with argon gas for 3–4 min, the SPE cartridge was eluted with ether through a column containing sodium borohydride and K_2CO_3 to yield the 4-[^{18}F]fluorobenzyl alcohol. The eluant was immediately added to a vial containing triphenylphosphine dibromide in methylene chloride (CH_2Cl_2). After 5 min the mixture containing 4-[^{18}F]fluorobenzyl

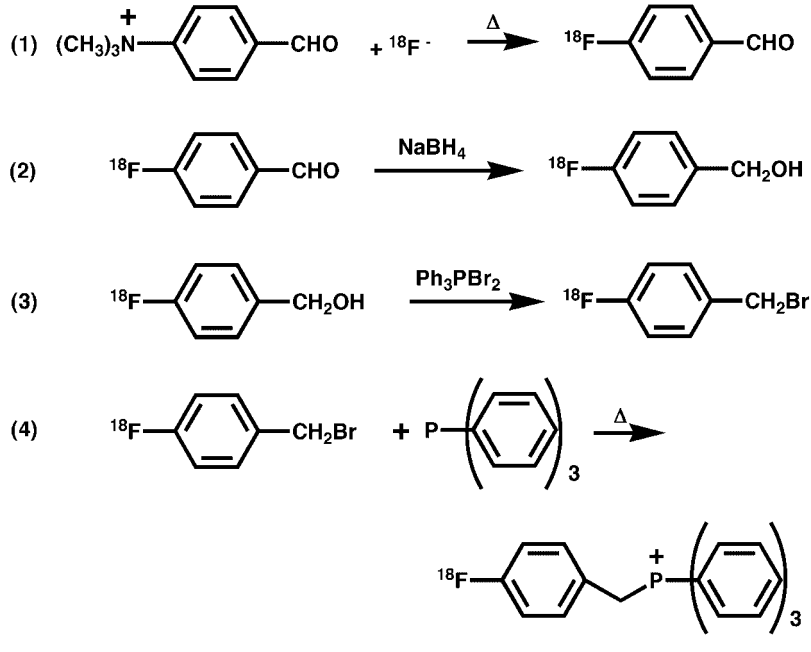

Figure 2. 4-[^{18}F]fluorobenzyltriphenylphosphonium cation radiosynthesis

bromide was eluted through a silica SPE cartridge into a vial containing triphenylphosphine in toluene. The solution was evaporated to a low volume, tightly capped and heated to boiling for 5 min. The workup of the toluene solution was the same as described in the 3-[^{18}F]fluoropropyltriphenylphosphonium synthesis above. A radiochemically pure product (>99%) was obtained in 6% yield (EOS) with an average synthesis time of 82 min ($n = 20$) and an average specific radioactivity of 16.7 GBq/μmole (451 mCi/μmole EOS). Authentic 4-[^{18}F]fluorobenzyltriphenylphosphonium bromide was synthesized and used to determine the identity, purity and specific radioactivity of the final product.

The radiosynthesis, purification and formulation of the 4-[^{18}F]fluorobenzyltris-4-dimethylaminophenylphosphonium cation was performed in the same manner as the 4-[^{18}F]fluorobenzyltriphenylphosphonium cation described above except tris-dimethylaminophosphine was substituted for triphenylphosphine in the last synthetic step (Figure 2). The radiochemically pure radioproduct (>99%) was obtained in 15% yield (EOS) with an average synthesis time of 80 min ($n = 6$) and an average specific radioactivity of 12.6 GBq/μmole (340 mCi/μmole EOS). Authentic *p*-fluorobenzyltris-4-dimethylaminotriphenylphosphonium bromide was synthesized and used to determine the identity, purity and specific radioactivity of the final product.

Experimental

[^{18}F]Fluoride was produced by 18 MeV proton bombardment of a high pressure ^{18}O-water target using a GE PETtrace biomedical cyclotron. All reagents were ACS or HPLC purity. The 4-trimethylammoniumbenzaldehyde trifluoromethanesulfonate was synthesized as previously described.[20] DMSO was distilled under vacuum from barium oxide prior to use. MALDI-Mass Spectra were performed by Anaspec, Inc. (San Jose, CA). Melting points were determined on a Mel-Temp (Thermolyne) melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab (Norcross, GA). NMR spectra were performed on a Varian 400 MHz instrument. Reverse phase HPLC analysis and purification were performed with two Waters 610 HPLC pumps, a Waters 441 fixed wavelength (254 nm) UV detector, and a Bioscan Flow Count PIN diode radioactivity detector. All HPLC chromatograms were recorded with a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax, version 1.4). Reverse phase HPLC semipreparative purification were performed on an Waters Novapak 6 μm C-18 column (7.8 × 300 mm) using the mobile phase described in the syntheses below at a flow rate of 7 ml/min. Chemical and radiochemical purity were determined using a Waters Novapak 4 μm C-18 HPLC column (3.9 × 150 mm) using the mobile phase described in the syntheses below at a

flow rate of 3 ml/min. A dose calibrator (Capintec 15R) was used for all radioactivity measurements.

Synthesis of 3-fluoropropyltriphenylphosphonium bromide

To 0.93 g (3.5 mmole) of triphenylphosphine (Aldrich) dissolved in 15 ml acetonitrile (CH_3CN) was added dropwise 0.32 ml (0.5 g, 3.5 mmole) 1-bromo-3-fluoropropane (Aldrich) in 15 ml of CH_3CN. The solution was refluxed 16 h. After rotary vacuum evaporation of the solvent, the white solid was recrystallized from methylene chloride (CH_2Cl_2). The solid was further purified on a short silica (EM Sciences, silica gel 60) column using 5% methanol in CH_2Cl_2 (1.21 g, 85%). mp: 232–235°C. ^{1}H NMR ($CDCl_3$): δ 1.81–2.17 (m, 2 H), 4.01–4.11 (m, 2 H), 4.72–4.75 (m, 1 H), 4.87–4.90 (m, 1 H), 7.69–7.88 (m, 15 H). MS: m/z 323.3 (C+). Elemental analysis calculated for $C_{21}H_{21}BrFP$: C, 62.55; H, 5.25; F, 4.71. Found: C, 62.26; H, 5.24; F, 4.47.

Synthesis of 4-fluorobenzyltriphenylphosphonium bromide

To 1.0 g (4 mmole) of triphenylphosphine in 15 ml CH_3CN was added dropwise 0.5 ml (0.75 g, 4 mmole) 4-fluorobenzyl bromide (Aldrich) in 10 ml CH_3CN. The solution was stirred 2 days at room temperature. The white precipitate was collected and recrystallized from CH_3CN (1.44 g, 80%). mp: 313–315°C [lit: 280–282°C[21]]. ^{1}H NMR (D^6-DMSO): δ 5.17–5.21 (m, 2 H), 6.99–7.13 (m, 4 H), 7.67–7.94 (m, 15 H). MS: m/z 371.2 (C+). Elemental analysis calculated for $C_{25}H_{21}BrFP$: C, 66.53; H, 4.69; F, 4.21. Found: C, 66.55; H, 4.65; F, 4.15.

Synthesis of 4-fluorobenzyltris-4-dimethylaminophenylphosphonium bromide

To 1.6 g (4 mmole) tris(4-dimethylamino)phosphine (Organometallics, Inc.) dissolved in 160 ml of CH_3CN and 170 ml dimethylformamide (DMF) was added 0.5 ml (0.75 g, 4 mmole) 4-fluorobenzyl bromide in 10 ml DMF over 25 min. The solution was stirred 36 h. The solution was evaporated to a purple solid and recrystallized from CH_3CN to yield a white solid (1.88 g, 81%). mp: 302–304°C. ^{1}H NMR (CD_3OD): δ 3.08 (s, 18 H), 4.36, 4.40 (d, 2 H), 6.85–7.26 (m, 16 H). MS: m/z 500.3 (C+). Elemental analysis calculated for $C_{31}H_{36}BrFN_3P$: C, 64.14; H, 6.25; F, 3.27. Found: C, 64.18; H, 6.35; F, 3.11.

Radiosynthesis and purification of 3-[^{18}F]fluoropropyltriphenylphosphonium cation

Aqueous [^{18}F]fluoride (approx. 1 ml) was passed through a resin column [Trap and Release column (DW-TRC), D and W, Inc., Oakdale, TN, USA)]. The trapped [^{18}F]fluoride was eluted from the resin column with 2.3 mg (0.02 mmole) of K_2CO_3 (Aldrich, 99%) in 0.3 ml of water (Fluka) into a

5 ml crimp top v-vial containing Kryptofix (Aldrich, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8,8,8]-hexacosane) (13 mg, 0.03 mmole). The vented vial was heated to 120°C under argon flow and CH_3CN (1 ml) was added in aliquots to azeotropically remove water. After evaporation of water, the vial was heated under argon flow for 3 min. The flow was stopped and the vent was removed. Propylene glycol ditosylate (Aldrich) (15 mg, 0.036 mmole) in 0.2 ml CH_3CN was added to the vial and the solution heated at 80°C for 4 min. Triphenylphosphine (21 mg, 0.08 mmole) in 0.5 ml of toluene was added to the vial and the solution heated to boiling on a heat gun for 5 min. The vial was vented to remove the toluene and then cooled to room temperature. HPLC buffer [0.5 ml of 35:65 CH_3CN:H_2O (0.1 M ammonium formate (AF))] was added, the mixture filtered through a 0.45 μm teflon filter (Alltech, 13 mm) and injected onto the preparative HPLC column. After collection and evaporation to dryness, the product ($T_R = 5.3$ min, $k' = 3.1$) was dissolved in 3 ml of sterile normal saline and filtered through a sterile 0.2 μm filter (Gelman Acrodisc, 25 mm) into a sterile evacuated vial (Mallinckrodt Medical, 20 ml). An aliquot was removed aseptically to determine chemical and radiochemical purity of the final solution ($T_R = 1.7$ min, $k' = 1.8$) by analytical HPLC [40:60 CH_3CN:H_2O (0.1 M AF)].

Radiosynthesis and purification of 4-[^{18}F]fluorobenzyltriphenylphosphonium cation

To the dried mixture of [^{18}F]fluoride ion, K_2CO_3 and Kryptofix prepared as described in the synthesis above was added 4-trimethylammoniumbenzaldehyde trifluoromethanesulfonate (7 mg, 0.02 mmole) in 0.2 ml of DMSO. The solution was heated at 120°C for 10 min. After cooling, 5 ml of HPLC water was added and the solution was passed through an activated C-18 solid phase extraction cartridge (SPE) (Waters Sep-Pak Plus). The C-18 SPE was washed with 10 ml of HPLC water and blown with argon flow for 3–4 min. Ether (2 ml) was passed through the C-18 SPE and a connected column containing 300 mg of sodium borohydride on alumina (Aldrich) above 300 mg K_2CO_3 into an open 5 ml v-vial containing triphenylphosphine dibromide (100 mg, 0.2 mmole) in 1 ml CH_2Cl_2. The vial was allowed to sit at room temperature for 5 min. The mixture was then passed through a silica SPE (Waters Sep-Pak Classic) followed by 1 ml CH_2Cl_2 into a vial containing triphenylphosphine (21 mg, 0.08 mmole) in 0.4 ml toluene. The volume was reduced to approximately 0.5 ml with argon and the vial was capped tightly. The solution was heated to boiling on a heat gun for 5 min. The vial was vented to remove the toluene and cooled to room temperature. HPLC buffer [0.5 ml of 50:50 CH_3CN:H_2O (0.1 M AF)] was added, the mixture filtered through a 0.45 μm teflon filter and injected onto the preparative HPLC column. After collection ($T_R = 3.9$ min, $k' = 3.2$) and evaporation to dryness,

the product was dissolved in 3 ml of sterile normal saline and filtered through a sterile 0.2 μ filter into a sterile evacuated vial. The chemical and radiochemical purity ($T_R = 3.9$ min, $k' = 5.5$) of the final solution were determined as described previously by analytical HPLC [40:60 CH_3CN:H_2O (0.1 M AF)].

Radiosynthesis and purification of 4-[^{18}F]fluorobenzyltris-4-dimethylaminophenyltriphenylphosphonium cation

The synthesis was performed following the procedure above for the 4-[^{18}F]fluorobenzyltriphenylphosphonium cation up to and including the bromination and silica SPE step. The eluant from the silica SPE was added to tris(4-dimethylaminophenyl)phosphine (21 mg, 0.05 mmole) in 0.4 ml toluene. After reducing volume and capping tightly, the solution was heated to boiling for 5 min on a heat gun. The mixture was vented, cooled, diluted and filtered as described above. After injection onto the preparative HPLC [70:30 CH_3CN:H_2O (0.1 M AF)] and collection, the product ($T_R = 4.3$ min, $k' = 2.3$) was dissolved in 3 ml of sterile normal saline and filtered through a sterile 0.2 μ filter into a sterile evacuated vial. The chemical and radiochemical purity ($T_R = 4.7$ min, $k' = 6.8$) of the final solution were determined as described previously by analytical HPLC [60:40 CH_3CN:H_2O (0.1 M AF)].

Conclusion

The radiosyntheses of the no carrier added 3-[^{18}F]fluoropropyl-, 4-[^{18}F]fluorobenzyltriphenylphosphonium and 4-[^{18}F]fluorobenzyltris-4-dimethylaminophenylphosphonium cations were achieved in modest radiochemical yields and specific radioactivities. Although the procedure has not been optimized and is being modified to a more remote and automated process for handling large amounts of radioactivity, the yields and specific radioactivities are sufficient for *in vivo* animal PET studies.

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