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Improved synthesis of 4-[^{18}F]fluoro-*m*-hydroxyphenethylguanidine using an iodonium ylide precursor

Running title: Improved synthesis of [^{18}F]4F-MHPG using an iodonium ylide precursor

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Total Word Count: 4224 (title page, abstract, text, references, tables, and figure legends).

Keywords: iodonium ylides, hypervalent iodine, radiofluorination, cardiac sympathetic innervation

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jlcr.3791

Abstract

Fluorine-18 labeled hydroxyphenethylguanidines were recently developed in our laboratory as a new class of PET radiopharmaceuticals for quantifying regional cardiac sympathetic nerve density in heart disease patients. Studies of 4-[^{18}F]fluoro-*m*-hydroxyphenethylguanidine ([^{18}F]4F-MHPG) and 3-[^{18}F]fluoro-*p*-hydroxyphenethylguanidine ([^{18}F]3F-PHPG) in human subjects have shown that these radiotracers can be used to generate high resolution maps of regional sympathetic nerve density using Patlak graphical analysis. Previously, these compounds were synthesized using iodonium salt precursors, which provided sufficient radiochemical yields for on-site clinical PET studies. However, we were interested in exploring new methods that could offer significantly higher radiochemical yields. Spirocyclic iodonium ylide precursors have recently been established as an attractive new approach to radiofluorination of electron-rich aromatic compounds, offering several advantages over iodonium salt precursors. The goal of this study was to prepare a spirocyclic iodonium ylide precursor for synthesizing [^{18}F]4F-MHPG and evaluate its efficacy in production of this radiopharmaceutical. Under optimized automated reaction conditions, the iodonium ylide precursor provided radiochemical yields averaging 7.8 ± 1.4% (n = 8, EOS, not decay corrected), around three-fold higher than those achieved previously using an iodonium salt precursor. With further optimization and scale-up, this approach could potentially support commercial distribution of [^{18}F]4F-MHPG to PET centers without on-site radiochemistry facilities.

1. Introduction

Noninvasive assessment of the extent and severity of regional cardiac denervation using radiopharmaceuticals that selectively localize in sympathetic nerve terminals is an emerging approach to assessing the risk of sudden cardiac death in patients with heart failure.[1] Our laboratory has recently developed ^{18}F-labeled hydroxyphenethylguanidines as a new class of radiopharmaceuticals for quantifying regional cardiac sympathetic nerve density using PET and tracer kinetic analysis. Our studies have focused on 4-[^{18}F]fluoro-*m*-hydroxyphenethylguanidine ([^{18}F]4F-MHPG) and its structural isomer 3-[^{18}F]fluoro-*p*-hydroxyphenethylguanidine ([^{18}F]3F-PHPG) (Figure 1). These radiotracers exhibit irreversible tissue kinetics through efficient intraneuronal retention in the norepinephrine storage vesicles localized in cardiac sympathetic nerve terminals. We previously described the use of iodonium salt precursors with the guanidine group completely protected (*tetrakis*-Boc) as an effective method for automated production of [^{18}F]4F-MHPG and [^{18}F]3F-PHPG.[2] For example, in production runs of [^{18}F]4F-MHPG (n = 15), this approach provided final product yields averaging 1.56 ± 0.68 GBq (range 0.61–2.84 GBq) at end of synthesis (EOS) with molar activities averaging 58 ± 25 GBq/μmol (range 21–105 GBq/μmol), which were adequate for on-site clinical studies. Under an exploratory Investigational New Drug clearance from the U.S. Food and Drug Administration, we recently conducted first-in-human studies of [^{18}F]4F-MHPG and [^{18}F]3F-PHPG in healthy control subjects.[3] Encouraged by the positive results of these studies, we were interested in evaluating alternative approaches to the production of [^{18}F]fluoro-hydroxyphenethylguanidines with the goal of achieving higher radiochemical yields for routine clinical production. Among several new methods developed for radiofluorination of electron-rich arenes, spirocyclic iodonium ylides offer several advantages, including excellent regioselectivity, high radiochemical yields, and mild, metal-free reaction conditions.[4-7] In practice, the spirocyclic iodonium ylide approach is quite

similar to our established method using iodonium salt precursors. The goal of this study was to prepare and evaluate the spirocyclic iodonium ylide analog of the iodonium salt precursor used previously for [^{18}F]4F-MHPG production to test the general utility of iodonium ylide method for radiofluorination of ^{18}F-labeled hydroxyphenethylguanidines. The results demonstrate that this new approach significantly increases the achievable radiochemical yields of [^{18}F]4F-MHPG, providing yet another example of the effectiveness of the use of spirocyclic ylide precursors for efficient radiofluorination of electron-rich aromatic compounds.

2. Results and Discussion

The synthesis of the required *tetrakis*-Boc protected spirocyclic iodonium ylide precursor **2** is shown in Scheme 1. Compound **1 (***N,N′,N″,N″-tetrakis*(*tert*-butoxycarbonyl)-*N*-3-benzyloxy-4-iodophenethylguanidine), previously synthesized for our original approach to [^{18}F]4F-MHPG,[2] was first reacted with dimethyldioxirane (DMDO). The resulting intermediate was coupled with (1*r*,3*r*,5*r*,7*r*)-spiro[adamantan-2,2′-[1,3]-dioxane]-4′,6′-dione (SPIAd)[5] to give the spirocyclic iodonium ylide **2**.

For initial automated radiofluorination tests with **2**, we employed optimized reaction conditions reported by Rotstein *et al.*,[5] including the use of tetraethylammonium bicarbonate (TEAB; 4.0 mg) as the fluorine-18 counter ion, anhydrous *N*,*N*-dimethylformamide (DMF, 0.5 mL) as the reaction solvent, 5.0 – 6.0 mg of **2**, and reaction conditions of 120 °C for 10 min (Scheme 2). The subsequent steps for simultaneous deprotection of the benzyl ether and the *N,N′,N″,N″-tetrakis*-Boc groups using 3.0 N HBr followed by HPLC purification of [^{18}F]4F-MHPG were identical to those used in our original method.[2] Radiochemical yields of these pilot tests (Table 1, Runs 1–3) were dramatically higher than our original approach, averaging 9.2 ± 0.7% (range 8.4% – 9.7%) at EOS (not decay corrected), more than three-

fold higher than the average yield of [^{18}F]4F-MHPG achieved with our iodonium salt precursor approach .[2]

Encouraged by these trial studies, we sought to optimize reaction conditions through a series of manual reaction tests. First, we tested the effect of using different fluorine-18 counter ions, including TEA^+, K^+/Kryptofix 222 and Cs^+. The results showed that TEA^+ was the clear choice, as K^+/Kryptofix 222 and Cs^+ yielded only trace quantities of radiolabeled intermediate (data not shown). Next, we tested acetonitrile as the reaction solvent, based on a report suggesting it might provide better yields[8], but again low yields of product were obtained (data not shown). Finally, reaction temperatures and times were optimized using TEA^+ as the fluoride counter ion and DMF as the solvent. The effects of reaction temperature and reaction time using 4 mg of TEAB in 0.5 mL of DMF solvent are shown in Figure 2A. The best yields were obtained at 120 °C for reaction times between 3 – 10 min. Tests of three different concentrations of the fluoride counter ion showed that 4 mg of TEAB per 0.5 mL of DMF solvent gave the highest yields at a reaction temperature of 120 °C and reaction times of 5 – 10 min (Figure 2B). The general trend of decreasing radiochemical yields with longer reactions times is likely due to a combination of thermal decomposition of the spirocyclic iodonium ylide **2** and the radiolabeled intermediate [^{18}F]**3**. For example, melting point tests of **2** showed a broad melting point around 80 °C– 89 °C, followed by generation of bubbles in the open capillary tube at temperatures between 110 °C – 120 °C. We hypothesize that the bubbles were caused by generation of CO_2 during thermolysis of the *N,N′,N″,N‴-tetrakis*-Boc groups between 110 °C – 120 °C.[9,10]

The findings of the initial automated tests (Table 1, Runs 1–3) and the manual reaction tests were confirmed in some additional automated production runs of [^{18}F]4F-MHPG. Automated syntheses using Cs^+ and K^+/Kryptofix 222 as fluoride counter ions in DMF

solvent were again found to provide extremely low yields (Table 1, Runs 4–6). Similarly, yields were very low using TEA^+ as the fluoride counter ion in MeCN solvent (Table 1, Runs 7–8). Using TEA^+ in DMF, a short reaction time of 3 min at high temperature (150 °C) gave a moderate yield of 3.8% (Table 1, Run 9), while a higher yield of 6.2% was achieved for a 5 min reaction time at 120 °C (Table 1, Run 10). Finally, a few runs were performed using optimized conditions (TEA^+ in DMF for 10 min at 120 °C), and the final products were analyzed with the series of quality control (QC) tests currently used at our institution for clinical PET studies with this radiopharmaceutical (Table 1, Runs QC1-QC4). The time from end-of-beam to formulation was 90–95 min, including HPLC purification. An example of the HPLC data acquired during purification and collection of [^{18}F]4F-MHPG is shown in Figure 3. Good radiochemical yields were achieved (5.8% – 8.3%), consistent with the findings of the manual reaction tests. Radiochemical purities were high (>99%) and molar activities ranged from 105.8 to >225 GBq/mmol (Table 1). All other quality control tests (mass concentration, radiochemical identity, radionuclidic identity, pH, visual inspection for particulates and color, residual solvents tests, ethanol content, filter membrane integrity, and sterility) were passed by each batch of [^{18}F]4F-MHPG. Representative data of an HPLC study to confirm radiochemical identity of the product are shown in Figure 4. Together these results demonstrate the ability of the spirocyclic iodonium ylide method to reliably produce [^{18}F]4F-MHPG for clinical PET studies.

While the United States Pharmacopeia (USP) does not cite a release limit for tetraethylammonium ion (TEA^+), Institutional Review Boards may require QC testing of TEA^+ concentrations in the final [^{18}F]4F-MHPG formulation. For example, the European Pharmacopeia sets a release limit for the closely related tetrabutylammonium ion (TBA^+) of 2.6 mg/V (per patient dose), and QC tests have been developed to evaluate TBA^+ levels in PET radiopharmaceutical products.[11] Since our optimized iodonium ylide approach to

[^{18}F]4F-MHPG uses 4.0 mg of TEA^+, and the compound is purified using HPLC, it is unlikely that residual TEA^+ levels would preclude use of the final product in human subjects. Supporting this, a recent report on an improved synthesis of [^{18}F]fluorodopamine for human studies (which uses HPLC purification of the final product) described a QC testing method for TEA^+ concentrations, which averaged around 50 μg/mL when starting with up to 3.5 mg of tetraethylammonium bicarbonate per reaction.[12]

The radiochemical yields of [^{18}F]4F-MHPG achieved with the spirocyclic iodonium ylide **2** under optimized reaction conditions averaged 7.8 ± 1.4% (*n* = 8, range 5.8% – 9.7%, Table 1). In comparison, these are lower than the reported yield of 14% achieved with a closely related spirocyclic iodonium ylide precursor to prepare *meta*-[^{18}F]fluoro-benzylguanidine ([^{18}F]mFBG).[5] This is not surprising, as the protected *m*-hydroxyl group in precursor **2** is electron-rich, which would be expected to cause lower radiochemical yields for our compound. Nevertheless, the higher and more consistent radiochemical yields of [^{18}F]4F-MHPG achieved using **2** compared with our earlier results using an iodonium salt precursor represents a noteworthy improvement in the production of this radiopharmaceutical for clinical use.

3. Conclusions

The use of a spirocyclic iodonium ylide precursor for automated production of the cardiac sympathetic innervation radiotracer [^{18}F]4F-MHPG was evaluated and found to consistently provide higher radiochemical yields than the previous approach using an iodonium salt precursor. This improved method of [^{18}F]4F-MHPG production is well suited to on-site production for clinical PET studies, and could possibly be scaled up for distribution of the radiotracer to stand-alone PET centers without a cyclotron and radiochemistry facility.

4. Experimental

4.1 General

Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise noted. *N,N′,N″,N″-tetrakis*(*tert*-butoxycarbonyl)-*N*-3-benzyloxy-4-iodophenethylguanidine **1**, *N,N′,N″,N″-tetrakis*(*tert*-butoxycarbonyl)-*N*-3-benzyloxy-4-fluorophenethylguanidine **3** and 4-fluoro-*m*-hydroxyphenethylguanidine (4F-MHPG) were previously prepared in our laboratory.[2,13,14] Compound **1** was used to synthesize the spirocyclic iodonium ylide precursor **2**. Compound **3** and 4F-MHPG were used as reference standards for radio-HPLC and radio-TLC analysis. Dimethyldioxirane (DMDO)[15] and (1*r*,3*r*,5*r*,7*r*)-spiro[adamantan-2,2′-[1,3]-dioxane]-4′,6′-dione (SPIAd)[5] were prepared using previously reported methods.

NMR spectra were obtained on a Varian vnmrs 500 (500.10 MHz for ^{1}H; 125.70 MHz for ^{13}C) spectrometer. ^{1}H and ^{13}C NMR chemical shifts (δ) are reported in parts per million (ppm) relative to internal standard TMS and coupling constants (*J*) are in Hz. High-resolution mass spectra were obtained on a VG (Micromass) 70-250S spectrometer using electrospray ionization (ESI) in positive ion mode at 70 eV. Flash column chromatography was performed with E. Merck 230-400 mesh silica gel.

4.2 Precursor synthesis

4.2.1 (1*r*,3*r*,5*r*,7*r*)-spiro[adamantane-2,2′-[1,3]dioxane]-4′,6′-dion-[2-benzyloxy-4-{2′-(*N,N′,N″,N″-tetrakis*(*tert*-butoxycarbonyl)-guanidinyl)ethyl}phenyliodonium] ylide (2).

A solution of dimethyldioxirane (DMDO) in acetone was added dropwise to a cooled (0 °C) solution of *N,N′,N″,N″-tetrakis*(*tert*-butoxycarbonyl)-*N*-3-benzyloxy-4-iodophenethylguanidine **1** (200 mg, 0.25 mmol) in acetone and acetic acid (4:1, 2.5 mL) until the starting guanidine compound **1** had disappeared, as assessed by TLC analysis. The

mixture was stirred at 0 °C for 1 h and then warmed to room temperature. It was then concentrated under reduced pressure. The residue was diluted with ethanol (5 mL) and treated by addition of (1*r*,3*r*,5*r*,7*r*)-spiro[adamantan-2,2′-[1,3]-dioxane]-4′,6′-dione (SPIAd, 70 mg) in portions. The resulting solution was adjusted to pH 10 by addition of a 10% aqueous Na_2CO_3 solution, stirred for 2 h at room temperature, diluted with water (10 mL) and then extracted with ethyl acetate (3×50 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 30% – 40% ethyl acetate in hexane) to afford the product **2** (179 mg, 69%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44-7.39 (m, 5H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.04 (s, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 5.21 (s, 2H), 3.94 (t, *J* = 8.0 Hz, 2H), 2.98 (t, *J* = 8.0 Hz, 2H), 2.52 (br. s, 2H), 2.22 (br. s, 2H), 2.20 (br. s, 2H), 1.88 (br. s, 2H), 1.74 (br. s, 4H), 1.72 (br. s, 2H), 1.52-1.47(m, 36H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.80, 157.98, 154.82, 151.38, 147.80, 145.06, 144.12, 135.10, 129.26, 129.21, 129.14, 128.32, 125.59, 114.86, 107.90, 100.19, 84.32, 84.17, 82.53, 72.44, 48.79, 37.53, 36.95, 35.96, 34.08, 33.46, 28.34, 28.27, 28.25, 26.88; HRMS (ESI^+) *m/z* calculated for $C_{49}H_{64}IN_3O_{13}$ $[M+Na]^+$: 1052.3376, found 1052.3373.

4.3 Radiochemistry

4.3.1 General

Ethanol (200 proof, USP) was purchased from Decon Laboratories, Inc. USP grade sodium chloride 0.9%, and sterile water for injection, were sourced from Hospira. Millex GV (#SLGV013SL) and FG (#SLFG025LS) filters were purchased from Millipore. Sep-Pak® Light QMA cartridges (#WAT023525) were purchased from Waters Corporation. Light QMA cartridges were conditioned with sequential flushes of 10 mL of ethanol, followed by

10 mL of sterile water, 10 mL of 0.5M $NaHCO_3$ preconditioning solution and finally 10 mL of sterile water prior to use.

Using a GE Healthcare PETrace 880 cyclotron, a target containing water enriched with ^{18}O was irradiated with 16 MeV protons to yield [^{18}F]fluoride ([^{18}F]F^-) via the $^{18}O(p,n)^{18}F$ reaction. [^{18}F]F^- was isolated from the enriched water by trapping on a preconditioned Sep-Pak® Light QMA cartridge. The [^{18}F]fluoride was then used in manual reaction tests or for automated radiosyntheses of [^{18}F]4F-MHPG using a TRACERLab FX_{FN} synthesis module. Radiochemical reactions were analyzed by radio-TLC or HPLC. For manual tests, radio-TLC analysis was performed using a Bioscan AR 2000 Radio-TLC scanner with EMD Millipore TLC silica gel 60 plates (3.0 cm × 6.5 cm). For the automated syntheses, the radiochemical purity of [^{18}F]4F-MHPG was measured using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector under isocratic conditions (Jupiter C18 5µ column, 4.6 × 250 mm, 60 mM $NaHPO_4$ buffer, pH 5.4, with 10% ethanol, flow rate 1.1 mL/min, λ = 254 nm, 40 °C oven). A few batches of [^{18}F]4F-MHPG prepared under optimized automated reaction conditions were tested using the full quality control protocol developed for human PET studies, including molar activity determination and residual solvents analyses.

4.3.2 Manual reactions

Tetraethylammonium [^{18}F]fluoride (Et_4N[^{18}F]F) was prepared using a TRACERLab FX_{FN} synthesis module (General Electric, GE). [^{18}F]F^- was produced using a short beam time on the GE PETrace cyclotron (55 µA beam for 30 – 45 s) and trapped on a preconditioned Sep-Pak® Light QMA cartridge. The trapped [^{18}F]F^- was eluted into the reaction vessel with a mixture of tetraethylammonium bicarbonate (Et_4NHCO_3, 4.0 mg), H_2O (0.3 mL) and MeCN (0.7 mL). After additional MeCN (0.5 mL) was added to the reaction vessel, the resulting solution was azeotropically dried under vacuum at 90 °C for 4 min, followed by a nitrogen

stream and simultaneous vacuum draw at 70 °C for an additional 4 min. Anhydrous *N*,*N*-dimethylformamide (6 mL) was added into the reaction vessel, and the resulting solution was transferred to a sterile vial to provide ~ 1.5 GBq of anhydrous $Et_4N[^{18}F]F$ for manual radiolabeling tests.

Manual reaction tests for optimizing radiolabeling conditions using the spirocyclic iodonium ylide precursor **2** were carried out using different reaction temperatures, reaction times and tetraethylammonium bicarbonate concentrations. For most tests, a stock solution of Et_4NHCO_3 was prepared (72.0 mg of dried Et_4NHCO_3 in 9.0 mL of anhydrous DMF, 41.8 mM), and aliquots of this solution were used for manual reactions. A 0.5 mL aliquot of the Et_4NHCO_3 stock solution was added to a 4 mL amber glass vial containing precursor **2** (~ 4.0 mg, 3.8 μmol). The reaction vial was sealed with a PTFE/Silicone septum cap and the solution thoroughly mixed. Using a 100 μL glass syringe, a 50 μL aliquot of $Et_4N[^{18}F]F$ (7.5-15.0 MBq, prepared as described above) was added to the reaction vial. The vial was then heated in an aluminum block at one of four temperatures (105, 120, 135 or 150 °C). After reaction times of 3, 5, 10, 15 or 20 min, a 10 μL aliquot of the reaction mixture was withdrawn from the reaction vial and analyzed by radio-TLC (EtOAc/hexane, 1:3, v/v) to determine the radiochemical yield (%) of the radiofluorinated intermediate [^{18}F]**3** (see Scheme 2). Another series of tests evaluated the effect of different amounts of tetraethylammonium bicarbonate on radiochemical yields. Using a constant temperature of 120 °C, the radiochemical yields of [^{18}F]**3** achieved with 2.0, 4.0 and 6.0 mg of Et_4NHCO_3 dissolved in the 0.5 mL of DMF added to the reaction vial were measured at reaction times of 5, 10, 15 and 20 min.

4.3.3 Automated synthesis.

Our manual and automated reaction tests with the spirocyclic iodonium ylide precursor led to the following improved automated synthesis of [^{18}F]4F-MHPG (Scheme 2). For a 30 min target irradiation at 55 μA beam current, approximately 65 GBq of [^{18}F]F^- was produced and trapped on the preconditioned Sep-Pak Light QMA cartridge. [^{18}F]F^- was eluted from the cartridge into the reactor vessel of the TRACERlab FX_{FN} system with a solution of Et_4NHCO_3 (~4 mg in 0.4 mL of H_2O and 1.0 mL of MeCN). The water/acetonitrile mixture was evaporated under vacuum at 90 °C for 4 min, followed by a nitrogen stream and simultaneous vacuum draw at 70 °C for an additional 4 min to yield dried Et_4N[^{18}F]F. After cooling to 60 °C, a mixed solution of 0.5 mL of DMF containing 5.5−6.0 mg of the spirocyclic iodonium ylide precursor **2** was added to the reactor vessel containing Et_4N[^{18}F]F. The mixture was heated in the sealed reactor vessel at 120 °C for 10 min to produce 3-benzyloxy-4-[^{18}F]fluorophenethyl-*N,N′,N″,N″-tetrakis*-BOC-guanidine **[^{18}F]3** as intermediate. After cooling to 70 °C, a solution of 48% HBr (0.5 mL) and MeCN (0.5 mL) was added to the reaction mixture. The solution was heated at 120 °C for 15 min and then cooled to 50 °C. Next, a mixture of aqueous NaOH (1.0 mL, 4.0 M in H_2O) and buffer solution (1.5 mL, 5% EtOH in 40 mM NH_4OAc) was added into the reactor vessel. This mixture was injected onto a reverse phase HPLC column (Phenomenex Synergi 10 μm Hydro-RP 80Å, 250 × 10 mm, 5% EtOH in 40 mM NH_4OAc buffer, flow rate 4.0 mL/min, λ = 220 nm) and [^{18}F]4F-MHPG was collected at R_t = 27−32 min. The collected fraction was passed through a 0.22 μm sterilizing filter directly into a septum-sealed, sterile, pyrogen-free glass vial. An aliquot of the filtered product was analyzed with HPLC to confirm the radiochemical identity as [^{18}F]4F-MHPG.

4.3.4 Quality Control Testing.

Molar activity was determined by analyzing an aliquot of [^{18}F]4F-MHPG with known activity A_0 (kBq) using a reverse-phase HPLC system with radiodetection. The area under the UV absorbance peak associated with the [^{18}F]4F-MHPG radioactivity peak was compared against a calibration curve to estimate the total mass of 4F-MHPG (μg) in the aliquot. The ratio of sample activity (A_0) to the measured total mass (converted from μg to μmol) gave the molar activity of the sample. Radiochemical purity of the product was measured as the fractional area under [^{18}F]4F-MHPG peak in the baseline-subtracted curve measured by the radiodetector. Additional quality control tests included radiochemical identity, radionuclidic identity, radionuclidic purity, pH, visual inspection for particulates and color, bacterial endotoxin test, and residual solvents testing.

Acknowledgements

We thank Dr. Allen Brooks for helpful discussions about the TracerLab FX_{FN} system and Bradley Henderson in the University of Michigan PET Radiopharmaceutical Production Program for performing quality control testing of several lots of [^{18}F]4F-MHPG.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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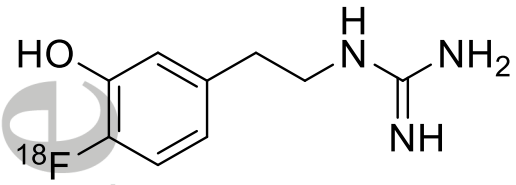
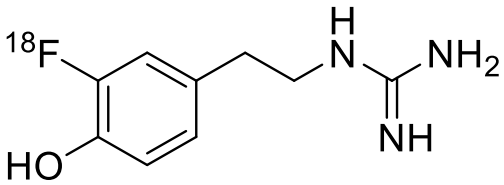
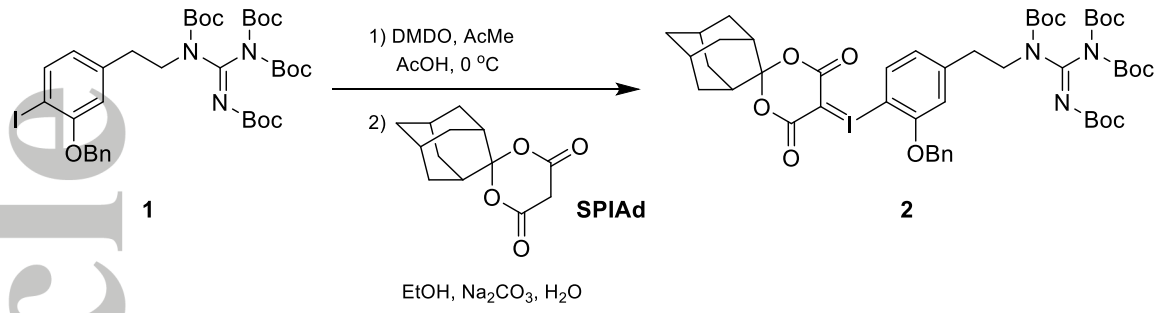


[18F]4F-MHPG

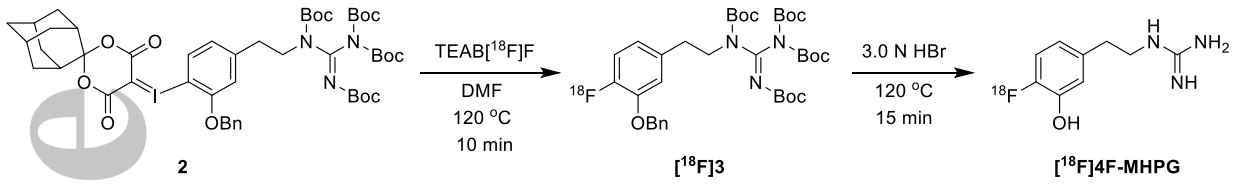


[18F]3F-PHPG

FIGURE 1. The structures of [18F]4F-MHPG and [18F]3F-PHPG



SCHEME 1. The synthesis of the spirocyclic iodonium ylide precursor **2**



SCHEME 2. The two-step reaction yielding [^{18}F]4F-MHPG from precursor **2**

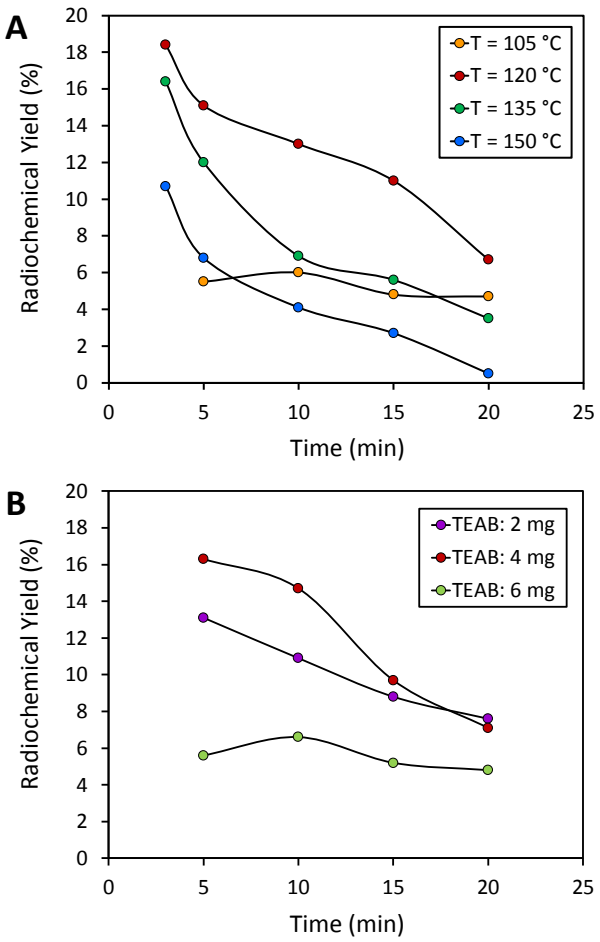


FIGURE 2 Radiochemical yields of the radiolabeled intermediate [^{18}F]**3** as a function of reaction time for: (A) different reaction temperatures, and (B) different amounts of tetraethylammonium bicarbonate (TEAB)

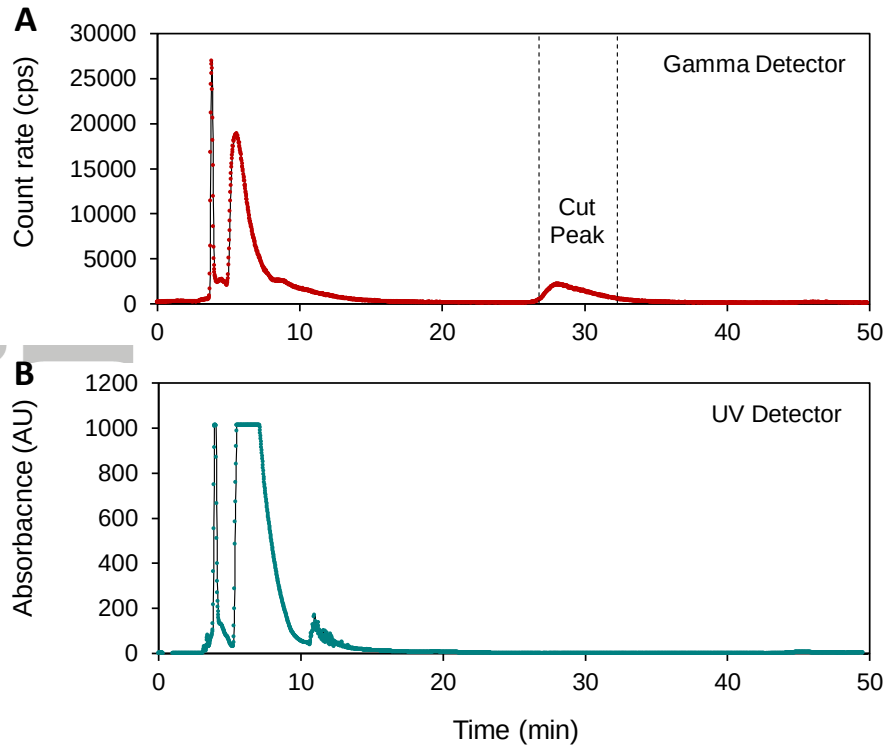


FIGURE 3 HPLC purification of [^{18}F]4F-MHPG; (A) radiation detector chromatogram, showing collection of [^{18}F]4F-MHPG from 27 to 32 min; and (B) the corresponding UV detector data at λ = 220 nm.

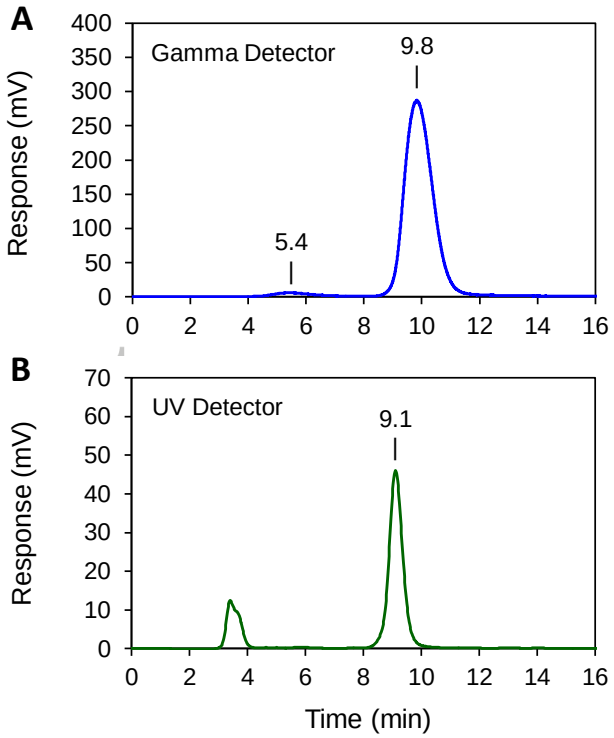


FIGURE 4. Coinjection study to confirm radiochemical identity. An aliquot of [^{18}F]4F-MHPG product was spiked with cold standard [^{19}F]4F-MHPG and analyzed using HPLC. The relative retention times of the peaks in the UV chromatogram (R_t = 9.1 min) and the radiation detector chromatogram (R_t = 9.8 min) is consistent with an established delay time of 0.7 min between the UV and radiation detectors. A small amount of free [^{18}F]F^- (~ 2% of the total activity) appears at R_t = 5.4 min in the radiation detector trace. HPLC conditions: Synergi 10µ Hydro-RP column, 4.6 × 250 mm, 60 mM sodium phosphate buffer, pH 5.4 with 10% ethanol, flow rate 1.0 mL/min, UV absorbance at 220 nm.

Table 1. Automated tests of [^{18}F]4F-MHPG production with spirocyclic iodonium ylide precursor **2**.

Run #	X^+ $[^{18}F]F^-$	Solvent	Temperature (°C)	Reaction Time (min)	Radiochemical Purity (%)	Radiochemical Yield (%)	Molar Activity (GBq/μmol)
1	TEA^+	DMF	120	10	> 98	8.4	—
2	TEA^+	DMF	120	10	> 98	9.4	—
3	TEA^+	DMF	120	10	> 99	9.7	—
4	Cs^+	DMF	120	10	—	—	—
5	K^+/K222	DMF	120	10	> 89	0.3	—
6	K^+/K222	DMF	120	10	> 96	1.1	—
7	TEA^+	MeCN	120	10	> 75	0.4	—
8	TEA^+	MeCN	120	10	> 95	1.0	—
9	TEA^+	DMF	150	3	> 99	3.8	—
10	TEA^+	DMF	120	5	> 96	6.2	—
11	TEA^+	DMF	120	10	> 98	8.3	—
QC1	TEA^+	DMF	120	10	> 99	6.9	130.5
QC2	TEA^+	DMF	120	10	> 99	5.8	105.8
QC3	TEA^+	DMF	120	10	> 99	6.7	137.9
QC4	TEA^+	DMF	120	10	> 99	7.0	>225

Reactions were performed in 0.5 mL of solvent using 5.0–6.6 mg precursor and 3.5–5.4 mg of fluoride counter ion.

Symbol '—' denotes that the value was not determined. K222 = Kryptofix 222.

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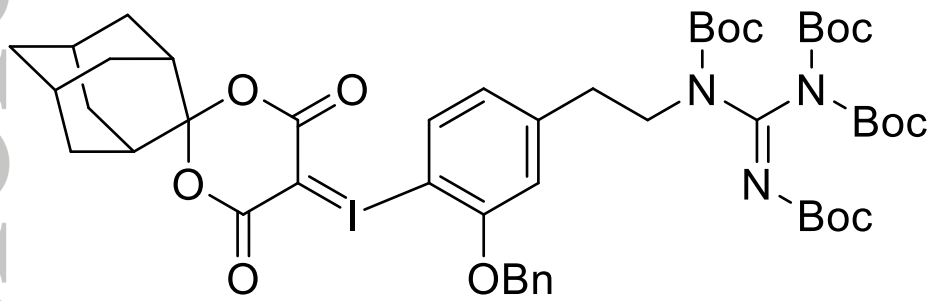

The use of a spirocyclic iodonium ylide precursor for preparation of the cardiac sympathetic innervation radiotracer 4-[^{18}F]fluoro-*m*-hydroxyphenethylguanidine has been shown to provide significantly higher radiochemical yields than were previously obtained with a structurally related iodonium salt precursor.