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A Practical, Automated Synthesis of *meta*-[^{18}F]Fluorobenzylguanidine for Clinical Use

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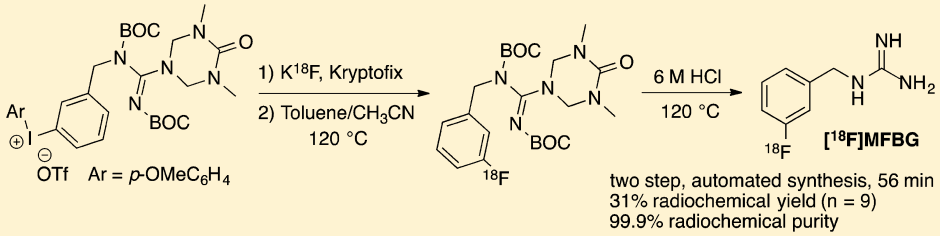
Supporting Information



ABSTRACT: Many neuroendocrine tumors, such as neuroblastoma (NB), arise from neural crest cells of the sympathetic nervous system. This nerve-like phenotype has been exploited for functional imaging using radioactive probes originally designed for neuronal and adrenal medullary applications. NB imaging with *meta*-[^{123}I]iodobenzylguanidine ([^{123}I]MIBG) is limited by the emissions of ^{123}I, which lead to poor image resolution and challenges in quantification of its accumulation in tumors. *meta*-[^{18}F]Fluorobenzylguanidine ([^{18}F]MFBG) is a promising alternative to [^{123}I]MIBG that could change the standard of practice for imaging neuroendocrine tumors, but interest in this PET radiotracer has suffered due to its complex and inefficient radiosynthesis. Here we report a two-step, automated method for the routine production of [^{18}F]MFBG by thermolysis of a diaryliodonium fluoride and subsequent acid deprotection. The synthesis was adapted for use on a commercially available synthesizer for routine production. Full characterization of [^{18}F]MFBG produced by this route demonstrated the tracer's suitability for human use. [^{18}F]MFBG was prepared in almost 3-fold higher yield than previously reported (31% corrected to end of bombardment, $n = 9$) in a synthesis time of 56 min with >99.9% radiochemical purity. Other than pH adjustment and dilution of the final product, no reformulation was necessary after purification. This method permits the automated production of multidose batches of clinical grade [^{18}F]MFBG. Moreover, if ongoing clinical imaging trials of [^{18}F]MFBG are successful, this methodology is suitable for rapid commercialization and can be easily adapted for use on most commercial automated radiosynthesis equipment.

KEYWORDS: *meta*-[^{18}F]*Fluorobenzylguanidine, [^{18}F]MFBG, positron emission tomography, diaryliodonium salts, fluorine-18*

Neuroblastoma (NB) is the third most common childhood cancer[1,2] with over 700 new cases diagnosed annually in the U.S.[3] Over 50% of patients have advanced stage disease at diagnosis, and sustained remissions of stage 4 NB are achieved in only ∼40% of cases.[4] Several new chemotherapeutic approaches for neuroblastoma (NB) are currently in development or in clinical trials,[5,6] but accurate assessment of these new therapies depends on precise and quantitative detection of metastatic disease and on the ability to measure response to therapy. Standard anatomical measurements of tumor volume by computed tomography (CT) or magnetic resonance imaging (MRI) are often delayed indicators of tumor response. Since tumor function is altered well in advance of anatomical changes,[7] functional imaging approaches are needed to assess the effects of new drugs and targeted therapies and to demonstrate early response to treatment.

Many neuroendocrine tumors such as NB and pheochromocytoma (PHEO) arise from neural crest cells of the sympathetic nervous system.[2,8] Most tumor cells of neural crest origin express many of the same transporter proteins and biosynthetic enzymes as do sympathetic neurons.[9] This nerve-like phenotype has been exploited for functional imaging using radioactive probes originally designed for neuronal and adrenal medullary applications.[10] Currently, evaluation of a variety of neuroendocrine tumors (particularly NB, PHEO and paraganglioma) depends on a combination of anatomical imaging using CT and functional imaging by single photon emission computed tomography (SPECT) using the radiotracer *meta*-

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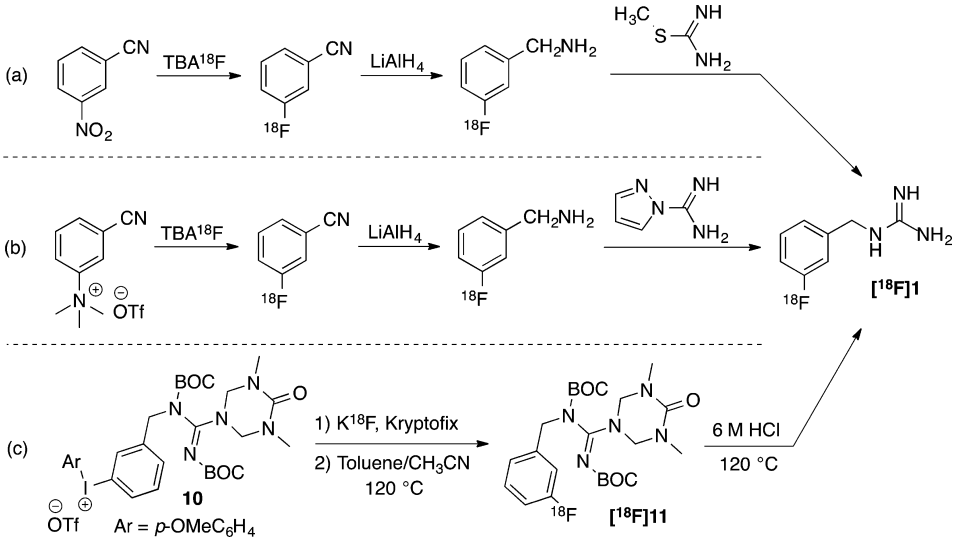

Figure 1. Comparison of the radiosynthesis of *meta*-[^{18}F]fluorobenzylguanidine ([^{18}F]MFBG) by (a) Garg et al.,[22] (b) Zhang et al.,[28] and (c) the method reported herein.

[^{123}I]iodobenzylguanidine ([^{123}I]MIBG).[8,10,11] Radioiodine ($^{123/125/131}$I) labeled MIBG was originally developed for imaging sympathetic innervation in the heart[12] and adrenal medulla[13,14] and is still popular for cardiac imaging to determine prognosis and assess response to therapy in several applications including heart failure,[15] as well as for staging of neuroendocrine tumors.[16,17] MIBG is taken into tumor cells via the selective norepinephrine transporter (NET) protein on the cell membrane. Over the nearly four intervening decades, [^{123}I]MIBG SPECT-CT has proven indispensible for diagnosis and staging of NB and has become essential for assessing response to treatment.[8,18]

Unfortunately, [^{123}I]MIBG is limited in sensitivity by the emissions of ^{123}I, which lead to poor image resolution, and quantification of accumulation is challenging and not routine. The inability to detect small metastatic lesions and the high incidence of false-positives due to significant background signal are barriers to efficient staging of disseminated disease. In addition, optimal clinical imaging of this tracer requires a 24-h uptake time, resulting in multiple visits to the clinic. MIBG has been radiolabeled with ^{124}I for use in positron emission tomography (PET);[19,20] however, only 25% of the radioactivity emitted by ^{124}I is from positron decay. The remaining 75% is by electron capture, adding to the PET image background and to the patient's radiation exposure; the estimated total body dose is at least 10 times higher in comparison to [^{123}I]MIBG.[21] The relatively long half-life of ^{124}I (4.2 days) also limits the injectable dose. These emission characteristics, paired with the limited availability of this radionuclide, high cost, and unfavorable dosimetry, suggest that [^{124}I]MIBG generally offers little advantage over the current standard of care.

Garg and co-workers reported two ^{18}F-labeled benzylguanidines, *meta*- and *para*-[^{18}F]fluorobenzylguanidine ([^{18}F]MFBG (**1**, Figure 1a) and [^{18}F]PFBG), in 1994.[22] Preliminary results in mice indicated that [^{18}F]MFBG showed great potential as a PET analog of [^{123}I]MIBG. However, similar to other ^{18}F-labeled radiotracers with complex, low-yield, low specific activity (SA) radiochemistry such as 6-[^{18}F]fluoro-L-DOPA ([^{18}F]F-DOPA),[23,24] adoption of this radiotracer has been slow and limited.[25−27] In the case of [^{18}F]F-DOPA, while this radiotracer was used as a diagnostic tool for neuropsychiatric diseases, its potential to image peripheral malignancies was limited by the electrophilic, carrier-added synthesis that resulted in low specific activity (SA) product that was shown to cause pharmacological effects upon injection.[23] Recently, with the development of high SA nucleophilic syntheses and automation of this process, [^{18}F]F-DOPA has become broadly accessible as a radiotracer with utility outside of dopaminergic metabolism.[24]

The similar lack of interest in [^{18}F]MFBG is likely due to the complex, low-yield radiochemistry required for its synthesis and the general success and availability of [^{123}I]MIBG. With its more favorable imaging properties, the potential of [^{18}F]MFBG to replace [^{123}I]MIBG as the standard radiotracer for staging neuroendocrine tumors is only feasible with the development of a reliable, automated radiolabeling method.

More recently, Zhang et al. reported an improved synthesis of [^{18}F]MFBG (Figure 1b) with slightly better yields.[28] Their investigation of this radiotracer in NB cells expressing the NET showed that [^{18}F]MFBG had a 2−3-fold lower tumor cell uptake in vitro than [^{123}I]MIBG but was still specific and correlative with NET expression.[21,28] However, since [^{18}F]MFBG is more hydrophilic than MIBG, 70% of the injected dose remained unbound to plasma proteins. In contrast, only 12% of [^{123}I]MIBG remains unbound to plasma proteins. The consequence of diminished plasma protein binding is faster nontarget clearance, leading to high image contrast at only 4 h with [^{18}F]MFBG as opposed to 24−48 h for similar tumor to nontarget contrast with [^{123}I]MIBG. Dosimetry estimates in mice also demonstrated an overall reduction in absorbed radiation dose (140 μCi/kg vs <100 μCi/kg).[21]

These encouraging results have renewed interest in [^{18}F]MFBG for clinical investigation and an imaging efficacy trial in humans is currently underway.[29] Although the current best synthesis[28] is completed in only three formal chemical steps, it requires several manual extractions and evaporations, limiting its potential for automation. In addition, semi-preparative HPLC purification uses acetonitrile; thus, solvent evaporation and reformulation are required for patient dose preparation. Overall, this process requires approximately 3 h

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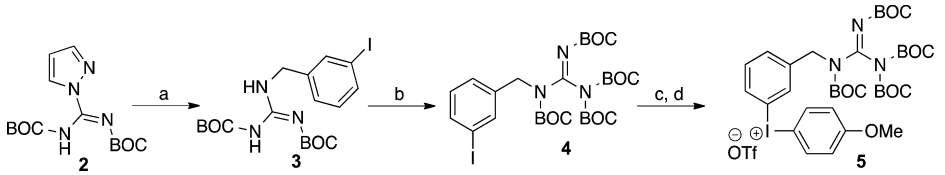
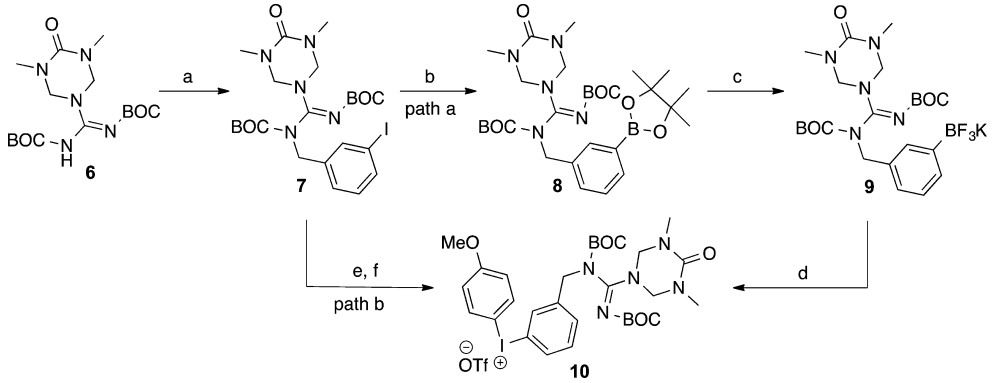
Scheme 1. Synthesis of *N,N′,N″,N‴*-Tetrakis-BOC-Protected Diaryliodonium Salt 5[a]



[a]Reagents and conditions: (a) *m*-iodobenzylamine HCl, Et_3N, $CHCl_3$, rt, 80%; (b) BOC_2O, cat. DMAP, Et_3N, THF, rt, 95%; (c) Selectfluor, TMSOAc, CH_3CN, rt; (d) potassium (4-methoxyphenyl)trifluoroborate, TMSOTf, CH_3CN, two steps, 68%.

Scheme 2. Synthesis of Diaryliodonium Salt 10[a]



[a]Reagents and conditions: (a) *m*-iodobenzyl bromide, DCM/H_2O, cat. Bu_4NI, KOH, 94%; (b) bis(pinacolato)diboron, cat. $PdCl_2(PhCN)_2$, cat. 1,1′-bis(diphenylphosphino)ferrocene (DPPF), KOAc, DMSO, 80 °C, overnight; (c) aq. KHF_2, methanol, two steps, 61%; (d) 4-methoxyphenyl-iodonium diacetate, TMSOTf, CH_3CN, 66%; (e) Selectfluor, TMSOAc, CH_3CN; (f) 4-methoxyphenyl-trifluoroborate, TMSOTf, CH_3CN, two steps, 60%.

and results in an overall decay-corrected radiochemical yield of 11% ± 2% (n = 12).[28]

If clinical trials of [^{18}F]MFBG are successful, this radiotracer has the potential to change the standard of care for detection and staging of NB and other neuroendocrine tumors. Therefore, we sought to develop a new, facile, automated method for the routine clinical production of [^{18}F]MFBG. Recently, we established robust methods for converting densely functionalized diaryliodonium salts into aryl fluorides with high regioselectivity and in good yield (Figure 1c).[30−32] We have also shown application of this strategy for rapid introduction of radiofluoride into other biologically active compounds for PET.[33−35] We demonstrate here the use of this chemistry as the key step in an automated, high specific activity (SA), high radiochemical yield synthesis of [^{18}F]MFBG that produces material suitable for human use. This new radiochemistry method is sufficiently superior to the existing method and because of these dramatic improvements is now being incorporated into the current [^{18}F]MFBG human trial (Jason S. Lewis, Memorial Sloan Kettering Cancer Center, personal communication).

RESULTS AND DISCUSSION

Syntheses of Diaryliodonium Salt Precursors 5 and 10. In the synthesis of diaryliodonium salt precursors for ^{18}F-radiochemistry, several key product characteristics must be addressed: (1) the purity of the final product must be high, and any impurities present must not compete with the trivalent iodine center for the trace amounts of ^{18}F-fluoride present in the labeling reaction mixture; (2) the compounds must be stable to long-term storage; (3) protection is required for potentially reducing or fluoride-binding functional groups; (4) these protective groups must be stable to the generally basic conditions used for radiosynthesis. In practice, the first two design criteria can be satisfied by syntheses that avoid heavy metal reagents and that result in crystalline diaryliodonium salt products. This need for crystallization of the final product naturally steers protective group strategy. For ease of synthesis, the *N,N′,N″,N‴*-tetrakis-BOC-protected MFBG precursor **5** (Scheme 1) was selected as the initial MFBG precursor.

The guanylating agent *N,N′*-bis-BOC-1-guanylpyrazole **2** reacted readily with commercially available *m*-iodobenzylamine hydrochloride in the presence of triethylamine at room temperature to produce guanidine **3** in good yield.[36] Reaction of **3** with BOC-anhydride under alkaline conditions gave *N,N′,N″,N‴*-tetrakis-BOC-protected *m*-iodobenzylguanidine **4** in 95% yield. Diaryliodonium salt **5** was prepared from **4** using an exceptionally mild one-pot, two-step process in which an in situ Selectfluor oxidation was followed by treatment with potassium (4-methoxyphenyl)trifluoroborate.[37] Although **5** could be obtained in 68% yield with 95% purity, attempts to purify this material further by recrystallization failed, so **5** was abandoned as a potential radiochemical precursor.

With the aim of providing a more crystalline diaryliodonium salt product, the rigid and symmetrical triazone protecting group was selected. Triazones have been employed previously to protect both primary amines[38] and guanidine.[39] Two routes to the synthesis of the triazone protected diaryliodonium salt **10** are given in Scheme 2.

Reaction of **6** with *m*-iodobenzyl bromide in the presence of a phase-transfer catalyst and KOH afforded the triazone-protected MIBG **7** in 94% yield.[40] This alkylation also proceeded smoothly with NaH in DMF.[41,42] The subsequent palladium-catalyzed coupling reaction of the aryl iodide **7** and

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bis(pinacolato)diboron gave the desired arylboronic ester **8**,[43] which could be further converted into the trifluorborate derivative **9** by treating it with aqueous potassium bifluoride in methanol.[44] The synthesis of the key intermediate diaryliodonium salt **10** was achieved by coupling the organoborate intermediate **9** with activated (with TMSOTf) 4-methoxyphenyliodonium diacetate.[37] Because of our concern that trace heavy metal contaminants could affect the stability and ^{18}F-labeling of diaryliodonium salts, we performed a second, (umpoled) heavy-metal-free synthesis of **10**. Aryl iodide **7** was oxidized in situ with Selectfluor and treated with potassium (4-methoxyphenyl)trifluoroborate. The purified diaryliodonium salt **10** was obtained in 60% yield following ion-exchange and recrystallization by slow evaporation from CH_2Cl_2/hexane.

Radiochemistry. Our goal was to develop a facile, remote, high-yield method for the production of high purity [^{18}F]MFBG in quantities suitable for use in human subjects. Our approach builds on the diaryliodium salt chemistry that we have developed to synthesize 6-[^{18}F]fluorodopamine ([^{18}F]F-DA),[34,35,45] 6-[^{18}F]fluoro-L-dihydroxyphenylalanine ([^{18}F]F-DOPA),[34] and 4-[^{18}F]fluorophenylalanine.[33] Following a series of model studies that helped define the conditions required for the fluorination and deprotection steps (see Supporting Information), preliminary optimization of the radiochemical labeling reaction was carried out. While the radiochemistry reported here was performed using an IBA Synthera radiosynthesis instrument, our previous results[45] demonstrate the general compatibility of these methods to virtually any commercially available automated radiosynthesis platform. The selected reaction solvent (10% CH_3CN in toluene) reflects consideration of a dilemma in diaryliodonium salt radiochemistry: the ^{18}F-radiolabeling efficiency of diaryliodonium salts increases with decreasing solvent polarity, while fluoride solubility and availability increase with solvent polarity.

One consequence of the triazone protecting group and the highly crystalline nature of the diaryliodonium salt **10** is that its solubility in the reaction medium is limited (~10 mg/mL). In an attempt to elucidate the amount of **10** necessary for efficient radiolabeling, the full synthesis was conducted with varying amounts of precursor. Use of an amount of precursor (15 mg) that was greater than the room temperature solubility limit afforded a reduced yield of [^{18}F]MFBG at end of synthesis (EOS) (9.1% ± 2.1% radiochemical yield (RCY), n = 3) compared with similar experiments using 8 mg (21.7% ± 3.52% RCY, n = 9) or 5 mg (23.8% ± 5.19% RCY, n = 6).

While the conditions for radiofluorination of **10** were comparable to those used for the production of [^{18}F]F-DA and [^{18}F]F-DOPA,[34,35,46] some experimentation was required to optimize the deprotection conditions for the triazone group. Deprotection of [^{18}F]**11**, as determined by HPLC, was monitored as a function of time and temperature. Based upon the data summarized in Figure 2, deprotection with 6 M HCl for 10 min at 120 °C was selected as the best set of conditions because it afforded a high yield of [^{18}F]MFBG (82.1−89.4%) in a reasonable time without generating a significant overpressure in the reactor vial.

With these preliminary results in hand, a fully automated radiosynthesis of *meta*-[^{18}F]fluorobenzylguanidine was developed utilizing the IBA Synthera platform. The full synthesis was completed in 56 ± 2 min including HPLC purification. The desired purified product **1** was obtained in 21.7% ± 3.52% yield EOS (n = 9, 31% decay-corrected). Isocratic HPLC purification

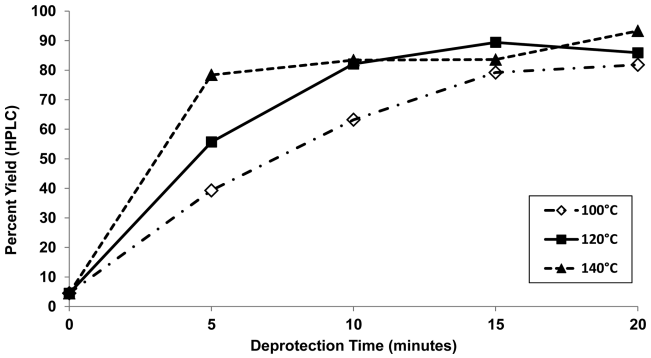

Figure 2. Deprotection of the fluorinated intermediate at various temperatures over time. Performed in 800 μL of 6 M HCl, reaction halted for sampling every 5 min; n = 1 for each temperature.

in 10% ethanol with 28 mM HCl, 20 mM ammonium acetate, and 0.04% ascorbic acid (all of which are normal components of human serum) allowed easy preparation for injection by dilution and pH adjustment to 4.5−6.5 with ammonium acetate (pH = 8.2) with no further reformulation necessary. This rapid, simple, automated synthesis provided [^{18}F]MFBG in significantly enhanced yields compared with previously published methods (Table 1).

Previously reported synthetic routes into [^{18}F]MFBG describe three-step methods in which *quaternary* ammonium salt or nitro leaving groups are displaced from 3-substituted benzonitriles to form 3-[^{18}F]fluorobenzonitrile. Subsequent $LiAlH_4$ reduction provides 3-[^{18}F]fluorobenzylamine, which is condensed with an amidino donor to provide the product (Figure 1). Each of the prior syntheses requires 16−17 manipulations, while the current synthesis requires only seven individual manipulations, beginning with elution of [^{18}F]-fluoride from a QMA resin and concluding with pH adjustment of the final product. This streamlined approach facilitates automation and remote synthesis.

The purification of the crude [^{18}F]MFBG is also simplified in the current synthesis. Previously, purification of [^{18}F]MFBG by reversed-phase HPLC was performed with solvents that required reformulation of the final product (THF[22] and acetonitrile[28]); we sought a less cumbersome solvent system that would not require evaporation. Recently, Zhang et al. reported using a 0.1% trifluoroacetic acid (TFA)/acetonitrile gradient elution (2−100% CH_3CN) to elute [^{18}F]MFBG in a retention time of 11 min (during the transition from 10% to 20% CH_3CN).[28] We were able to achieve comparable separation efficiency by replacing acetonitrile with ethanol, a more physiologically compatible option. To simplify further, we performed an isocratic separation in 10% ethanol and replaced 0.1% TFA with 10 mM HCl, a less toxic acid. Ammonium acetate and ascorbic acid were added to the eluent, and the pH was maintained at 2, since this is essential for efficient elution of the desired product.

Quality Control for Human Use. The identity and radiochemical purity (RCP) of the final product was confirmed by HPLC in conjunction with an [^{19}F]MFBG standard sample (prepared as described in the Supporting Information). RCP was determined to be >99.9% after purification of the crude product (85−89% RCP, Figure 3) for all automated runs.

The presence of a UV peak corresponding to the retention time of the [^{19}F]MFBG standard was not observed in the final, formulated product. A serial dilution experiment using

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Table 1. Literature Comparison to Current Synthesis of [^{18}F]MFBG

	Garg et al.[22]	Zhang et al.[28]	our method
method	manual	manual	remote, automated
synthetic manipulations	17	16	7
average yield	10−15% (decay corrected)	11% (n = 12) (decay corrected)	31% (n = 9) (decay corrected) 21.7% ± 3.5%
radiochemical purity	94−98%	>98%	>99%
specific activity	*a*	505 mCi/μmol	>657 mCi/μmol
purification	HPLC, evaporation, dissolve in ethanol, evaporation, dissolve in buffer	HPLC + evaporation, dissolve in saline	HPLC + diluent, neutralization buffer
synthesis time	>62 min[b]	∼3 h	56 ± 2 min

[a]Not reported. [b]Reported synthesis time of 62 min = total time of reported heating steps and HPLC purification but does not include intermediate purifications, solvent evaporation, or final product reformulation.

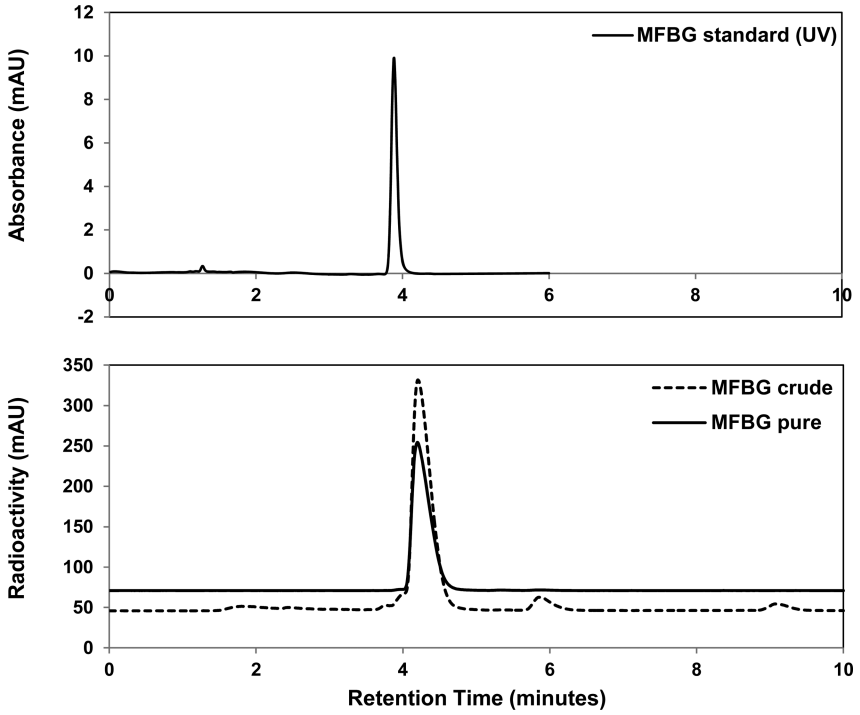

Figure 3. Analytical HPLC of [^{18}F]MFBG. Agilent Zorbax SB-Aq column (4.6 mm × 150 mm, 5 μm); 10% acetonitrile and 35 mM phosphoric acid, 25 mM monobasic sodium phosphate, pH 2; flow rate of 1 mL/min, monitoring at 220 nm. Note: a 0.3 min difference is expected between UV and radioactivity retention times due to the distance between the two detectors connected in series.

[^{19}F]MFBG established that the lowest detectable injected mass was 0.01 μg on our HPLC system. Using this detection threshold, the SA for a 450 mCi synthesis (97.9 mCi yield) of [^{18}F]MFBG produced by this method is greater than 657 mCi/μmol (>3285 mCi/μmol prior to dilution with sterile water), higher than the SA reported in other preparations.[28]

Ethanol has been established in USP Chapter <467> as a Class 3 volatile solvent, and is limited to 0.5% (or 50 mg/day);[48,49] however, the FDA has approved radiotracer formulations that contain up to 10% ethanol.[47] Because the main impetus for this work is use of the radiotracer in sensitive pediatric populations, we elected to use a maximum of 2% for ethanol for this soluble guanidinium salt. The final [^{18}F]MFBG product was augmented with 2 mL of 0.113 M ammonium acetate (pH 8.2) to adjust the pH to approximately 5 then diluted further (1:4) with sterile water to bring the ethanol concentration below 2%. Average ethanol concentration in the final prepared product (n = 9) was 1.57% ± 0.066%. A peak corresponding to acetonitrile was integrated in one of the nine samples resulting in a concentration of 0.002%. Ammonium acetate is also detected on GC analysis and was 0.042% ± 0.006%.

Residual Kryptofix [2.2.2] in the [^{18}F]MFBG final product was determined using a standard color spot test by visual comparison to standards spotted on silica pretreated with an acidic iodoplatinate solution.[50] No evidence of residual Kryptofix [2.2.2] was visualized in any of the final [^{18}F]MFBG solutions.

■ CONCLUSION

We describe here a rapid, remote, and automated method to produce quantities and quality of [^{18}F]MFBG suitable for human use. A crystalline diaryliodonium salt precursor is easily converted into the aryl fluoride with high regioselectivity and in good yield. Significant improvements in the synthesis of [^{18}F]MFBG included increases in yield and scale, automation, and a significant reduction in the number of synthetic manipulations. This method permits the automated production

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of multidose batches of clinical grade [^{18}F]MFBG for the first time. Moreover, this methodology is easily adapted for use on most commercial automated radiosynthesis equipment and because of these improvements is currently being implemented in an ongoing clinical trial.[51]

METHODS

General. All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), unless otherwise noted. Acetonitrile and acetonitrile-d_3 (Cambridge Isotope Laboratories) for precursor and standard syntheses were dried over P_2O_5, distilled into flame-dried storage tubes, and stored under dry nitrogen. Benzene and benzene-d_6 (Cambridge Isotope Laboratories) were dried over Na/benzenophenone and distilled into a flame-dried storage flask under dry nitrogen. All glassware, syringes, and NMR tubes were oven-dried (120 °C) for at least 12 h before they were transferred into a dry glovebox (N_2 atmosphere) for use. All NMR experiments were performed using a Bruker Avance 300, 400, 500, and 700 MHz NMR spectrometer in the NMR laboratory at the University of Nebraska—Lincoln. Glove box manipulations were performed in a MBraun Labmaster 150, equipped with a recirculating purifier. Yields from NMR scale reactions were determined by using the residual CHD_2CN solvent peak in CD_3CN.

All aqueous solutions were prepared using distilled, deionized water (Milli-Q Integral Water Purification System, Millipore Corp.; 18.2 MΩ·cm resistivity). Radioactive samples were analyzed in a CRC-15R dose calibrator (Capintec, Inc.) for determination of millicuries. HPLC was performed on an Agilent 1200 Series LC System (Agilent Technologies) using both diode array detection and a Bioscan Flow-Count radioisotope detector.

Compounds **2**,[52,53] **6**,[39] and 4-diacetoxyiodoanisole[54] were prepared by previously reported methods. Synthetic details for the preparation of these known compounds and fluorination of **10** with [^{19}F]fluoride are provided in the Supporting Information.

*meta-Fluorobenzylguanidine (MFBG) Hydrochloride (**1**).* To a solution of *N*,*N*′-bis(*t*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (3.10 g, 10 mmol, 1.0 equiv) in 100 mL of $CHCl_3$ was added 12 mmol (1.50 g, 1.2 equiv) of 3-fluorobenzyl amine, and the resulting mixture was stirred at room temperature overnight. After completion of the reaction (as monitored by TLC), the solvent was evaporated in vacuo, and the residue was purified by silica gel chromatography (EtOAc/hexane, 1:10) to afford the *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-3-fluorobenzylguanidine intermediate as a colorless solid (3.21 g, 87%). This intermediate (1.10 g, 3 mmol) was treated with HCl in dioxane (10 mL, 4 M) and stirred at room temperature overnight. The precipitated, colorless solid was filtered, washed with hexane, and recrystallized from MeOH/methyl *tert*-butyl ether (MTBE) to produce *meta*-fluorobenzylguanidine hydrochloride (0.52 g, 85%). ^{1}H NMR (700 MHz, D_2O) δ 7.45 (td, J_1 = 7.4 Hz, J_2 = 6.0 Hz, 1 H), 7.19 (d, J = 7.7 Hz, 1 H), 7.14 (d, J_1 = 9.6 Hz, 1 H), 7.13 (dd, J_1 = 9.6 Hz, J_2 = 7.4 Hz, 1 H), 4.48 (s, 2 H). ^{13}C NMR (100 MHz, D_2O) δ 164.0, 161.6, 157.0, 138.8 (d, J = 7.5 Hz), 130.7 (d, J = 8.4 Hz), 122.5 (d, J = 2.6 Hz), 114.6 (d, J = 21.2 Hz), 113.5 (d, J = 22.3 Hz), 44.0. ^{19}F NMR (D_2O, 282 MHz): δ −113.4 (td, J_1 = 9.6 Hz, J_2 = 6.0 Hz, 1 F). HRMS (HREI) Calcd for $C_8H_{10}N_3F$ (M − HCl)$^+$: 167.0859. Found: 167.0856.

*N′,N″-Bis(tert-butoxycarbonyl)-N-3-iodobenzylguanidine (**3**).* To a solution of 3-iodobenzylamine hydrochloride (2.96 g, 11 mmol, 1.1 equiv) and Et_3N (1.7 mL, 12 mmol, 1.2 equiv) in 50 mL of chloroform was added *N*,*N*′-bis(*t*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (3.10 g, 10 mmol, 1.0 equiv) at room temperature. After the mixture was stirred for 4 h, it was treated with water, and the organic layer was separated. The water layer was extracted once with CH_2Cl_2, and the combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The obtained product **3** (colorless solid, 3.80 g, 80%) was sufficiently pure to be carried forward. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.53 (brs, 1 H), 8.59 (brs, 1 H), 7.67 (s, 1 H), 7.63 (d, J = 7.6 Hz, 1 H), 7.28 (d, J = 7.6 Hz, 1 H), 7.08 (t, J = 7.6 Hz, 1 H), 4.57 (d, J = 5.6 Hz, 1 H), 1.52 (s, 9 H), 1.49 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 154.5, 151.6, 138.2, 135.3, 135.1, 128.8, 125.5, 92.9, 81.7, 77.9, 42.5, 26.7, 26.5. HRMS (ESI) Calcd for $C_{18}H_{26}N_3O_4INa$ (M + Na)$^+$: 498.0866. Found: 498.0868.

*3-((1,2,3,3-Tetrakis(tert-butoxycarbonyl)guanidino)methyl)-iodobenzene (**4**).* DMAP (122 mg, 1.0 mmol, 0.1 equiv) was added to a solution of *N*′,*N*″-bis(*tert*-butoxycarbonyl)-*N*-3-iodobenzylguanidine (3.80 g, 8 mmol, 1.0 equiv) and Et_3N (4.2 mL, 30 mmol, 3.0 equiv) in THF (50 mL). A solution of di-*tert*-butyldicarbonate (4.36 g, 20 mmol, 2.0 equiv) in THF (40.0 mL) was added slowly (over approximately 5 h), and the solution was stirred overnight at room temperature. An additional aliquot of di-*tert*-butyldicarbonate (2.18 g, 10 mmol, 1.0 equiv) in THF (20.0 mL) was added to drive the reaction to completion. The mixture was concentrated in vacuo, and the residue was purified by silica gel chromatography (EtOAc/hexane, 1:10) to give **4** as colorless solid (5.12 g, 95% yield). ^{1}H NMR (400 MHz, CD_3CN) δ 7.75 (s, 1 H), 7.64 (d, J = 7.6 Hz, 1 H), 7.37 (d, J = 7.6 Hz, 1 H), 7.11 (t, J = 7.6 Hz, 1 H), 4.93 (s, 2 H), 1.45 (s, 9 H), 1.43 (s, 18 H), 1.39 (s, 9 H). ^{13}C NMR (700 MHz, $CDCl_3$) δ 157.4, 151.2, 147.2, 145.1, 140.2, 136.3, 136.2, 130.3, 126.9, 93.6, 84.1, 83.6, 81.8, 49.4, 27.2. HRMS (ESI) Calcd for $C_{28}H_{42}IN_3O_8Na$ (M + Na)$^+$: 698.1914. Found: 698.1901.

*(4-Methoxyphenyl)(3-((1,2,3,3-tetrakis(tert-butoxycarbonyl)-guanidino)methyl)phenyl)-iodonium Trifluoromethanesulfonate (**5**).* In a N_2 charged glovebox, a solution of TMSOAc (15.6 mmol, 2.06 g, 2.6 equiv) in 15 mL of dry CH_3CN was added dropwise to a solution of Selectfluor (7.8 mmol, 2.76 g, 1.3 equiv) in 15 mL of dry CH_3CN. The resulting colorless mixture was then added dropwise to a solution of 3-((1,2,3,3-tetrakis(*tert*-butoxycarbonyl)guanidino)-methyl)iodobenzene (6.0 mmol, 4.05 g, 1.0 equiv) in 20 mL of dry CH_3CN. The mixture was stirred at room temperature for 33 h before potassium 4-methoxyphenyltrifluoroborate (1.28 g, 6.0 mmol, 1.0 equiv) was added. Immediately thereafter, a solution of TMSOTf (1.20 g, 5.4 mmol, 0.90 equiv) in 10.0 mL of dry CH_3CN was added dropwise, and the mixture was allowed to stand at room temperature for 30 min. The CH_3CN was removed by rotary evaporation, 100 mL of deionized water was added, and the mixture was extracted (3 × 50 mL) with CH_2Cl_2. The combined organic extracts were washed with water (100 mL), and the aqueous layer was extracted (2 × 50 mL) with CH_2Cl_2 again. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was washed with hexane to give the diaryliodonium triflate. This compound was dissolved in 1 mL of CH_3CN/water (9:1 by volume) solution and slowly passed down an Amberlite IRA-400 ion exchange column (triflate counterion). After removal of the solvents under reduced pressure, the iodonium triflate product (3.80 g, 68% yield) was obtained as a colorless solid. ^{1}H NMR (400 MHz, CD_3CN) δ 8.09 (s, 1 H), 7.98 (d, J = 9.2 Hz, 2 H), 7.97 (d, J = 7.6 Hz, 1 H), 7.66 (d, J = 7.6 Hz, 1 H), 7.47 (t, J = 7.6 Hz, 1 H), 7.01 (d, J = 8.8 Hz, 1 H), 4.99 (s, 2 H), 3.81 (s, 3 H), 1.45 (s, 9 H), 1.41 (s, 18 H), 1.33 (s, 9 H). ^{13}C NMR (176 MHz, $CDCl_3$) δ 163,1, 157.2, 151.0, 147.4, 145.0, 142.2, 137.4, 133.9, 133.8, 132.0, 131.3, 118.0, 114.9, 102.7, 84.5, 83.9, 82.1, 55.7, 49.4, 27.3, 27.2, 27.2, 27.1, 26.2. HRMS (ESI) Calcd for $C_{35}H_{49}IN_3O_9$ (M − OTf)$^+$: 782.2514. Found: 782.2491.

*tert-Butyl(((tert-butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)methyl)(3-iodobenzyl)carbamate (**7**).* A biphasic solution of *tert*-butyl (((*tert*-butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)methyl)carbamate (1.78 g, 4.80 mmol, 1.0 equiv), tetrabutylammonium iodide (0.19 g, 0.50 mmol, 0.10 equiv), and KOH (85%, 0.63 g, 9.6 mmol, 2.0 equiv) in a 1:1 mixture of CH_2Cl_2/H_2O (40.0 mL) was treated, dropwise, with 3-iodobenzyl bromide (1.69 g, 5.70 mmol, 1.2 equiv) in CH_2Cl_2 (8.0 mL) over 1.5 h. The mixture was stirred at room temperature for 12 h. At the completion of the reaction, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL × 2). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, EtOAc) to give the product (2.64 g, 94% yield) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1 H), 7.66 (d, J = 7.9 Hz, 1 H), 7.30 (d, J = 7.8 Hz, 1 H), 7.07 (t, J = 7.7 Hz, 1 H), 4.46 (brs, 6 H), 2.74 (brs, 6 H), 1.53 (s, 9 H), 1.47 (s,

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9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 156.2, 151.9, 150.2, 135.8, 135.4, 134.3, 132.1, 128.1, 83.8, 81.7, 79.3, 60.8, 50.9, 32.1, 27.4, 24.3. HRMS (ESI) Calcd for $C_{23}H_{34}N_5O_5INa$ $(M + Na)^+$: 610.1502. Found: 610.1532.

*N,N′-Bis(tert-butoxycarbonyl)-N-(3-(4′,4′,5′,5′-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-3,5-dimethyl-4-oxo-[1,3,5]triazinane-1-carboxamidine (**8**).* A flask charged with *tert*-butyl (((*tert*-butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)methyl)(3-iodobenzyl)carbamate (1.20 g, 2.0 mmol, 1.0 equiv), $PdCl_2(PhCN)_2$ (39 mg, 0.10 mmol, 0.05 equiv), bis-(diphenylphosphino)ferrocene (55 mg, 0.10 mmol, 0.05 equiv), bispinacolatodiboron (1.02 g, 4.0 mmol, 2.0 equiv), and KOAc (1.96 g, 20.0 mmol, 10.0 equiv) was flushed with nitrogen, and degassed DMSO (20 mL) was added. The mixture was heated at 80 °C and stirred for 12 h under N_2. After the mixture was allowed to cool to room temperature, it was extracted with ethyl acetate, washed with water, dried over anhydrous Na_2SO_4, and concentrated. The crude *tert*-butyl (((*tert*-butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)methyl)(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-benzyl)carbamate was obtained as colorless solid (1.05 g) after flash column chromatography (silica gel, EtOAc rapid flow rate to minimize exposure to silica gel) and was used without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.76 (s, 1 H), 7.70 (d, J = 7.4 Hz, 1 H), 7.51 (d, J = 7.5 Hz, 1 H), 7.38 (t, J = 7.5 Hz, 1 H), 4.50 (brs, 6 H), 2.66 (brs, 6 H), 1.52 (s, 9 H), 1.47 (s, 9 H), 1.33 (s, 12 H). ^{13}C NMR (100 MHz, acetone-d_6) δ 158.6, 157.0, 152.1, 150.6, 138.5, 138.1, 137.4, 130.6, 128.4, 94.5, 83.1, 80.7, 61.0, 50.6, 33.0, 28.2, 28.1. HRMS (ESI) Calcd for $C_{29}H_{46}BN_5O_7Na$ $(M + Na)^+$: 610.3388. Found: 610.3358.

*Potassium tert-Butyl (((tert-Butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl) methyl)(3-(trifluoroboryl)benzyl)-carbamate (**9**).* The above pinacol ester **8** (1.05 g) was dissolved in MeOH (20 mL) to which a solution of KHF_2(aq) (1.24 g, 15.9 mmol, 4.5 M solution) was added over the course of 1 h. This mixture was stirred for an additional hour before CH_2Cl_2 was added to generate a colorless precipitate. The precipitate was removed by filtration and washed with CH_2Cl_2. The mother liquor was concentrated to yield 1.22 g of crude product as colorless solid. This solid was washed with hexane to obtain the pure trifluoroborate salt **9** (0.69 g, two steps 61%). ^{1}H NMR (500 MHz, CD_3CN) δ 7.38 (d, J = 7.1 Hz, 1 H), 7.35 (s, 1 H), 7.14 (t, J = 7.5 Hz, 1 H), 7.07 (d, J = 7.4 Hz, 1 H), 4.91 (brs, 1 H), 4.46 (brs, 3 H), 4.01 (brs, 2 H), 2.60 (brs, 6 H), 1.94 (s, 9 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 156.6, 152.0, 151.1, 133.9, 132.3, 131.3, 126.9, 126.2, 81.7, 79.7, 60.6, 51.6, 32.1, 27.4, 24.3. HRMS (ESI) Calcd for $C_{23}H_{34}BF_3KN_5O_5Na$ $(M + Na)^+$: 590.2140. Found: 590.2166.

*(3-((N,N′-bis(tert-butoxycarbonyl)-3,5-dimethyl-4-oxo-1,3,5-triazinane-1-carboximidamido)methyl)phenyl)(4-methoxyphenyl)-iodonium Trifluoromethansulfonate (**10**).* In a N_2 charged glovebox, 1-(diacetoxyiodo)-4-methoxybenzene (224 mg, 0.64 mmol, 1.0 equiv) was dissolved in 7.0 mL of dry CH_3CN. The solution was combined with a solution of trifluoroborate salt **9** (397 mg, 0.70 mmol, 1.1 equiv) in 7.0 mL of dry CH_3CN. TMSOTf (155 mg, 0.70 mmol, 1.1 mmol) was added dropwise, and the mixture was allowed to stand at room temperature for 30 min. Saturated aqueous sodium acetate (20 mL) was added, and the mixture was extracted (3 × 20 mL) with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated. This compound was dissolved in a 1 mL of CH_3CN/water (9:1 by volume) solution and passed slowly down an Amberlite IRA-400 ion exchange column (triflate counterion). After removal of the solvents under reduced pressure, the purified diaryliodonium triflate product was obtained by washing the colorless residue with hexane to remove any organic impurities. The residue was recrystallized from CH_2Cl_2/hexane to give 350 mg (66%) of the title iodonium triflate. ^{1}H NMR (400 MHz, CD_3CN) δ 8.05 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 9.2 Hz, 2H), 8.01−7.99 (m, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.04 (d, J = 9.2 Hz, 2H), 4.40 (brs, 6H), 3.83 (s, 3H), 2.60 (brs, 6H), 1.48 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, CD_3CN) δ 163.2, 158.6, 156.3, 151.6, 150.4, 140.4, 137.5, 135.5, 134.9, 134.2, 132.7, 122.7, 119.5, 118.0, 114.1, 102.2, 82.7, 80.3, 60.5, 55.7, 50.1, 32.3, 27.3. ^{19}F NMR (CD_3CN, 376 MHz): δ −79.3 (s, 3F). HRMS (ESI) Calcd for $C_{30}H_{41}N_5O_6I$ $(M - OTf)^+$: 694.2102. Found: 694.2078.

*One-Pot, Two-Step Synthesis of (3-((N,N′-bis(tert-butoxycarbonyl)-3,5-dimethyl-4-oxo-1,3,5-triazinane-1-carboximidamido)-methyl)phenyl)(4-methoxyphenyl)iodonium Trifluoromethanesulfonate (**10**).* In a N_2 charged glovebox, a solution of TMSOAc (14.0 mmol, 1.85 g, 3.5 equiv) in 15 mL of dry CH_3CN was added dropwise to a solution of Selectfluor (6.8 mmol, 2.41 g, 1.7 equiv) in 15 mL of dry CH_3CN. The resulting colorless mixture was then added dropwise to a solution of *tert*-butyl (((*tert*-butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)methyl)(3-iodobenzyl)carbamate, **7** (3.45 mmol, 1. 50 g, 1.0 equiv), in 20 mL of dry CH_3CN. The mixture was stirred at room temperature for 2 days and at 40 °C for 3.5 h before potassium 4-methoxyphenyltrifluoroborate (0.86 g, 4.0 mmol, 1.0 equiv) was added. Immediately thereafter, a solution of TMSOTf (0.80 g, 3.6 mmol, 0.90 equiv) in 10.0 mL of dry CH_3CN was added dropwise, and the mixture was allowed to stand at room temperature for 30 min. The CH_3CN was removed by rotary evaporation, 100 mL of deionized water was added, and the mixture was extracted (3 × 50 mL) with CH_2Cl_2. The combined organic extracts were washed with water (100 mL), and the aqueous layer was extracted (2 × 50 mL) with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was recrystallized from dichloromethane/hexane to give the diaryliodonium triflate. This compound was dissolved in a 1 mL of CH_3CN/water (9:1 by volume) solution and slowly passed down an Amberlite IRA-400 ion exchange column (triflate counterion). After removal of the solvents under reduced pressure, the purified diaryliodonium triflate product (2.0 g, 60%) was obtained as a colorless, crystalline solid. The characterization data were identical with those reported above.

*N,N′-Bis(tert-butoxycarbonyl)-N″-(3-fluorobenzyl)-3,5-dimethyl-4-oxo-[1,3,5]triazinane-1-carboxamidine (**11**).* A biphasic solution of *tert*-butyl (((*tert*-butoxycarbonyl)imino)(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)methyl)carbamate (371 mg, 1.0 mmol, 1.0 equiv), tetrabutylammonium iodide (37 mg, 0.10 mmol, 0.10 equiv), and KOH (85%, 132 mg, 2.0 mmol, 2.0 equiv) in a 1:1 mixture of CH_2Cl_2/H_2O (10.0 mL) was added dropwise to 3-fluorobenzyl bromide (227 mg, 1.2 mmol, 1.2 equiv) in CH_2Cl_2 (4.0 mL) over 1 h. The reaction was stirred at room temperature for 12 h. At the completion of the reaction, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, EtOAc) to give the product (0.43 g, 90% yield) as a colorless solid. ^{1}H NMR (400 MHz, CD_3CN) δ 7.36 (td, J_1 = 7.9 Hz, J_2 = 6.0 Hz, 1 H), 7.17 (d, J = 7.6 Hz, 1 H), 7.12 (dt, J_1 = 9.8 Hz, J_2 = 2.4 Hz, J_3 = 1.6 Hz, 1 H), 7.07 (tdd, J_1 = 8.2 Hz, J_2 = 1.6 Hz, J_3 = 0.9 Hz, 1 H), 4.45 (brs, 6H), 2.66 (brs, 6H), 1.48 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 161.4, 158.8, 156.4, 152.0, 150.6, 138.9 (d, J = 7.3 Hz), 130.5 (d, J = 8.4 Hz), 125.3 (d, J = 2.8 Hz), 116.0 (d, J = 21.8 Hz), 114.9 (d, J = 21.2 Hz), 82.2, 79.9, 60.7, 50.4, 32.1, 27.3 (d, J = 7.2 Hz). ^{19}F NMR (CD_3CN, 376 MHz): δ −114.4 (td, J_1 = 9.8 Hz, J_2 = 6.0 Hz, 1 F). HRMS (ESI) Calcd for $C_{23}H_{34}N_5O_5FNa$ $(M + Na)^+$: 502.2442. Found: 502.2431.

*Hydrolysis of Protected MFBG Intermediate (**11**).* Compound **11** (0.25 mmol, 120 mg) was dissolved in 6 M HCl (5.0 mL) and heated to 100 °C in a sealed tube for 8 min (NMR scale showed 85% yield, which was determined by using an internal integration standard). The reaction mixture was washed with CH_2Cl_2 (2 × 10 mL), and the water layer was evaporated under reduced pressure. The resulting residue was dissolved in a minimal amount of acetone, precipitated with methyl *tert*-butyl ether (MTBE), filtered, and dried in vacuo to give the product MFBG as a colorless solid (37 mg, 72% isolated yield).

Radiochemistry. The purified precursor and organic solvents for radiosyntheses were stored in an anaerobic chamber (Coy Laboratory Products, Inc.) under argon to reduce exposure to water in the atmosphere. [^{18}F]MFBG was produced in a two-step synthesis by thermolysis and subsequent deprotection of a diaryliodonium fluoride

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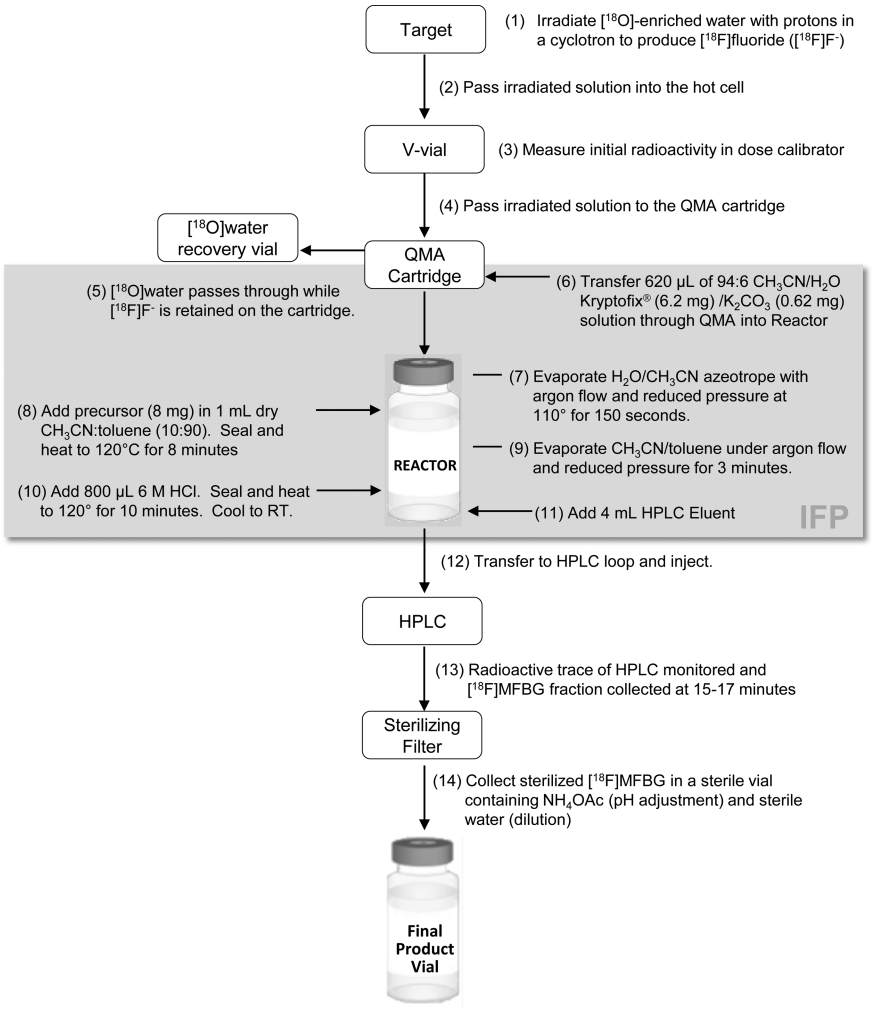

Figure 4. Stepwise process for synthesis of [^{18}F]MFBG.

(Figure 1c) adapted for preparation on a Synthera automated synthesizer (IBA) using an integrated fluidic processor (ABX) and an automated script. Figure 4 outlines the necessary steps for synthesis of [^{18}F]MFBG using this method.

Aqueous [^{18}F]fluoride produced in an IBA Cyclone 18/9 cyclotron from 97% ^{18}O-enriched water was passed through a QMA cartridge (ORTG, Inc., pretreated with 2 mL of 1 M $NaHCO_3$ and rinsed with 5 mL of water) to trap the [^{18}F]fluoride while the [^{18}O]water passed through to waste. The [^{18}F]fluoride was eluted from the QMA resin with 620 μL of a 94:6 CH_3CN/H_2O solution containing K_2CO_3 (0.62 mg) and Kryptofix [2.2.2] (6.2 mg). The solvent was removed by azeotropic distillation under argon flow with reduced pressure and heating to 110 °C for 2.5 min. The diaryliodonium salt precursor (**11**), dissolved in 10% anhydrous CH_3CN and toluene (5−15 mg in 1 mL), was added to the reactor, and the mixture was heated to 120 °C for 8 min. The solvent was removed under reduced pressure with argon flow (approximately 3 min), and deprotection of the fluorinated complex was achieved by addition of 800 μL of 6 M HCl and heating to 120 °C for 10 min to afford the crude *meta*-[^{18}F]fluorobenzylguanidine product. The acidic solution was diluted by the addition of 4 mL of HPLC eluent and then passed through a 0.45 μm filter to a 5 mL HPLC loop. Purification was achieved by semipreparative HPLC utilizing a Hamilton PRP-1 column (10 mm × 250 mm, 10 μ) with an eluent of 10% ethanol and 28 mM HCl, 20 mM ammonium acetate, and 0.04% ascorbic acid (pH 2) at a flow rate of 3.5 mL/min. Detected by monitoring with a radioactivity detector, the desired product was collected in a 7−9 mL fraction at a retention time of approximately 15−17 min. Neutralization of the final product to pH 4.5−6.5 was achieved by the addition of 2 mL of 0.113 M ammonium acetate (pH 8.2). Sterile water was also added (1:4, v/v) to dilute the total ethanol concentration to below 2%. This method produced [^{18}F]MFBG (RCY = 21.7% ± 3.5% EOS, n = 9) in >99% radiochemical purity and a total synthesis time of 56 ± 2 min.

To confirm the ideal temperature and time for the deprotection step, a series of experiments was performed at various temperatures with sampling over time. The synthesis was initiated as described above and proceeded through the solvent evaporation following the thermolysis step. At this point, the acid deprotection was performed at one of three temperatures (100, 120, or 140 °C). After 5 min, the synthesis was paused, the reactor cooled, and the reactor pressure brought to atmospheric pressure. A small aliquot (~20 μL) was removed and diluted with 100 μL of analytical HPLC eluent. Heating was resumed to continue the deprotection at the desired temperature until the next time point. An additional minute was added to the heating time to account for the necessity of reheating the solution back to the hydrolysis temperature. Each sample of crude product was analyzed by analytical HPLC, and fractions of unreacted ^{18}F and side products were compared with the desired product to assess completion of the reaction.

Quality Control. Radiochemical purity was confirmed by analytical HPLC using an Agilent Zorbax SB-Aq column (4.6 mm × 150 mm, 5 μm) with an eluent of 10% acetonitrile and 35 mM phosphoric acid and 25 mM monobasic sodium phosphate, pH 2, at a flow rate of 1 mL/min with monitoring at 220 nm. If present, the area of the UV peak with a retention time corresponding to the standard solution of [^{19}F]MFBG of known concentration was compared with the area of that standard. From this, the total mass of MFBG in the final formulation was calculated. The radioactivity of a sample of the

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[^{18}F]MFBG was measured, and this value, along with the calculated total mass of the sample, was used to calculate the specific activity.

Residual Kryptofix [2.2.2] in the [^{18}F]MFBG final product was determined using a color spot test by visual comparison to standards spotted on silica pretreated with an acidic iodoplatinate solution.[50] Silica test strips were prepared in advance and spotted with 2 uL each of a 0, 20, and 50 μg/mL solution of Kryptofix [2.2.2] in the HPLC purification eluent (10% ethanol/28 mM HCl, 20 mM ammonium acetate, 0.04% ascorbic acid, pH 2). Standard spots were visually compared with 2 μL spots of each test sample of [^{18}F]MFBG final product.

To assess for any residual volatile organic compounds, a small aliquot (0.5 μL) of the [^{18}F]MFBG final formulation was analyzed by GC using an Agilent Technologies 7890A GC system, Carbowax column (J & W 122−7032; 30 m × 250 μm × 0.25 μm), inlet and detector temperatures of 250 and 300 °C, respectively, oven temperature of 80 °C, and flow of 1.5 mL/min. The GC peak retention times and areas were compared with standards of toluene (0.1% (v/v)), acetonitrile (0.1%), ethanol (0.1%), and ammonium acetate (0.15%, 20 mM). The amount of each volatile solvent is calculated based on the ratio of peak areas for the sample vs the standard.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00202.

A detailed description of experimental procedures and ^{1}H, ^{19}F, and ^{13}C NMR spectra of the compounds **1**−**11** (PDF)

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Notes

The authors declare the following competing financial interest(s): Kiel D. Neumann is a consultant for and a shareholder of Ground Fluor Pharmaceuticals, Inc., Lincoln, Nebraska. Stephen G. DiMagno holds a patent for the nucleophilic fluorination of aromatic ring systems, which includes the nucleophilic synthesis of meta-[18F]-fluorobenzylguanidine via a diaryliodonium salt precursor (US Patent 8,604,213 B2, Dec. 10, 2013) and is a consultant for and a shareholder in Ground Fluor Pharmaceuticals, Inc. Other authors declare no conflict of interest.

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■ ABBREVIATIONS

NB, neuroblastoma; MIBG, *meta*-iodobenzylguanidine; MFBG, *meta*-fluorobenzylguanidine; [^{18}F]F-DOPA, 6-[^{18}F]fluoro-L-dihydroxyphenylalanine; CT, computed tomography; MRI, magnetic resonance imaging; PHEO, pheochromocytoma; SPECT, single photon emission computed tomography; NET, norepinephrine transporter; PET, positron emission tomography; PFBG, *para*-fluorobenzylguanidine; SA, specific activity; TMSOAc, trimethylsilyl acetate; DPPF, 1,1′-bis-(diphenylphosphino)ferrocene; [^{18}F]F-DA, 6-[^{18}F]-fluorodopamine; EOS, end of synthesis; RCY, radiochemical yield; TFA, trifluoroacetic acid; RCP, radiochemical purity; MTBE, methyl *tert*-butyl ether

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