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Enhanced copper-mediated ^{18}F-fluorination of aryl boronic esters provides eight radiotracers for PET applications†

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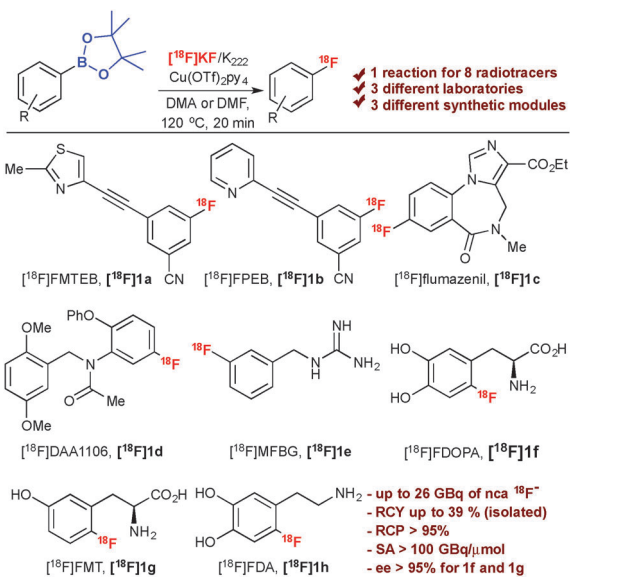
[^{18}F]FMTEB, [^{18}F]FPEB, [^{18}F]flumazenil, [^{18}F]DAA1106, [^{18}F]MFBG, [^{18}F]FDOPA, [^{18}F]FMT and [^{18}F]FDA are prepared from the corresponding arylboronic esters and [^{18}F]KF/K_{222} in the presence of $Cu(OTf)_2py_4$. The method was successfully applied using three radiosynthetic platforms, and up to 26 GBq of non-carrier added starting activity of ^{18}F-fluoride.

Positron emission tomography (PET) is a molecular imaging modality with wide-ranging applications in oncology, cardiology, neurology, as well as fundamental clinical research.[1] Despite the great success of PET imaging, the development of new radiotracers remains a formidable challenge. Among all PET radioisotopes, ^{18}F is a widely used clinically relevant radionuclide because of its advantageous properties;[2] specifically, the half-life of fluorine-18 (109 min) is long enough to allow remote-site application of radiopharmaceuticals as demonstrated worldwide by distribution of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG).[3,4] A recent upsurge in fluorination chemistry has revealed a number of novel ^{18}F-labeling methods,[5] including the preparation of ^{18}F-fluoroaromatics through aryl iodonium ylides,[6] aryl sulfonium salts,[7] preformed Pd^{IV} or Ni^{II} complexes,[8] and aryl boronic precursors.[9] These most recent advances could make an impact in the clinic if one progresses from proof of concept to the synthesis of radiotracers and radiopharmaceuticals, and ultimately apply these new methods for human use. Our group has demonstrated that arylboronates derived from pinacol are suitable substrates for Cu-mediated ^{18}F-labeling with ^{18}F-fluoride; one of the distinctive features of this reaction is its compatibility with arylboronates derived from electron rich, neutral and deficient arenes. In this report, we demonstrate that this transformation enables the preparation of eight radiotracers; [^{18}F]FMTEB, [^{18}F]FPEB, [^{18}F]flumazenil, [^{18}F]DAA1106, [^{18}F]MFBG, [^{18}F]FDOPA, [^{18}F]FMT, and [^{18}F]FDA (Scheme 1). To achieve this goal, our original reaction conditions were modified for radiotracers possessing an electron deficient fluoroarene. This study is significant as, for the first time, a range of radiotracers used in (pre)clinical studies, but difficult to prepare, is within reach applying a single reaction. Selected radiosyntheses were performed on automated platforms and in different laboratories, an advance indicating that the process is robust and amenable to broad use in PET radiochemistry facilities.



Scheme 1 Synthesis of eight radiotracers *via* Cu-mediated ^{18}F-fluorination of arylboronic esters. nca (non-carrier added), RCY (radiochemical yield), RCP (radiochemical purity), SA (specific activity).

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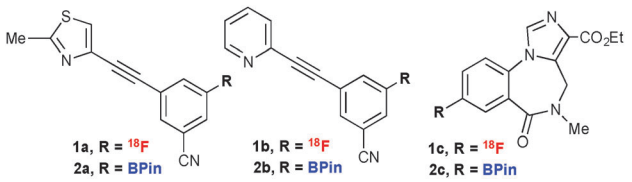
Our original protocol consists of treating the aryl boronic ester with [^{18}F]KF/K_{222} and $Cu(OTf)_2py_4$ in DMF at 110 °C. Under these reaction conditions, the copper-mediated ^{18}F-fluorination of aryl boronic esters is most effective for the ^{18}F-labeling of electron rich arenes. We therefore anticipated that the level of optimization required for the synthesis of [^{18}F]FMTEB **[^{18}F]1a**, [^{18}F]FPEB **[^{18}F]1b**, [^{18}F]flumazenil **[^{18}F]1c**, [^{18}F]DAA1106 **[^{18}F]1d**, [^{18}F]MFBG **[^{18}F]1e**, [^{18}F]FDOPA **[^{18}F]1f**, [^{18}F]FMT **[^{18}F]1g** and [^{18}F]FDA **[^{18}F]1h** from their respective arylboronate precursors **2a–h** would vary. This validation study was performed with a NanoTek platform applying a semi-automated protocol.[10] All radiochemical conversions (RCCs) reported in the present study are corrected for decay and radiochemical yields (RCYs) are non-decay corrected.

*Radiosynthesis of electron deficient fluoroarenes: [^{18}F]FMTEB **[^{18}F]1a**, [^{18}F]FPEB **[^{18}F]1b** and [^{18}F]flumazenil **[^{18}F]1c**.* 3-[^{18}F]Fluoro-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]benzonitrile ([^{18}F]FMTEB) and 3-[^{18}F]fluoro-5-[(pyridin-3-yl)ethynyl]benzo-nitrile ([^{18}F]FPEB) are radioligands developed for PET imaging of metabotropic glutamate receptor subtype 5 (mGlu5) in the central nervous system.[11,12] [^{18}F]Flumazenil is a suitable radioligand for PET assessment of central benzodiazepine receptors (BZR).[13] Applying the reaction conditions outlined in our original report for small scale reaction, [^{18}F]FMTEB was obtained from the corresponding arylboronic ester **2a** in 3% ± 1% RCC (n = 4). This reaction employed 20 MBq of [^{18}F]KF/K_{222}, 0.06 mmol of **2a** and 0.0053 mmol of $Cu(OTf)_2py_4$ in DMF (300 μL) at 110 °C for 20 minutes. This result was not unexpected as arenes with electron withdrawing substituents were among the most challenging precursors for ^{18}F-labeling using this methodology. Reevaluation of the reaction stoichiometry and the reaction solvent led to significant improvements. Reversing the substrate : Cu ratio from 10 : 1 to 1 : 1.5, and replacing DMF (*N*,*N*-dimethylformamide) with DMA (*N*,*N*-dimethylacetamide) gave [^{18}F]FMTEB in 71% RCC (n = 5). Under similar conditions (ratio **2b** : Cu = 1 : 1.3, ratio **2c** : Cu = 1 : 1.3), [^{18}F]FPEB **[^{18}F]1b** and [^{18}F]flumazenil **[^{18}F]1c** were obtained in 66% (n = 2) and 75% (n = 2) RCC, respectively. These encouraging results prompted further studies investigating how an increase of starting activity of non-carrier added (nca) ^{18}F-fluoride influences efficacy. The Oxford-based radiochemistry laboratory handles a maximum of 10 GBq of starting nca [^{18}F]fluoride, so the "scale-up" experiments were performed with 2.4–10 GBq of ^{18}F-fluoride. When using a full batch of [^{18}F]fluoride on a single reaction, rather than subdividing into multiple aliquots, the amount of K_2CO_3 employed to elute the [^{18}F]fluoride from the QMA cartridge became problematic for ^{18}F-labeling. A study investigating how the nature of the inorganic base affects efficacy and facilitates elution of trapped ^{18}F-fluoride from the QMA cartridge identified $K_2C_2O_4$ with minimal amounts of K_2CO_3 as the most suitable conditions.[14] With this modified protocol, [^{18}F]FMTEB **[^{18}F]1a**, [^{18}F]FPEB **[^{18}F]1b** and [^{18}F]flumazenil **[^{18}F]1c** were obtained in 29%, 13% and 35% RCY, respectively (Table 1, entries 1–3). When eluting with $Cu(OTf)_2py_4$ instead of $K_2C_2O_4$, a solution of 10% pyridine in DMF was used as the reaction solvent; this protocol gave 16% RCY of [^{18}F]flumazenil **[^{18}F]1c** (Table 1, entry 4). Finally, **[^{18}F]1c** is obtained in 17% RCY when $Cu(OTf)_2$ used in combination with pyridine is employed as an alternative to the preformed complex $Cu(OTf)_2py_4$ (Table 1, entry 5).

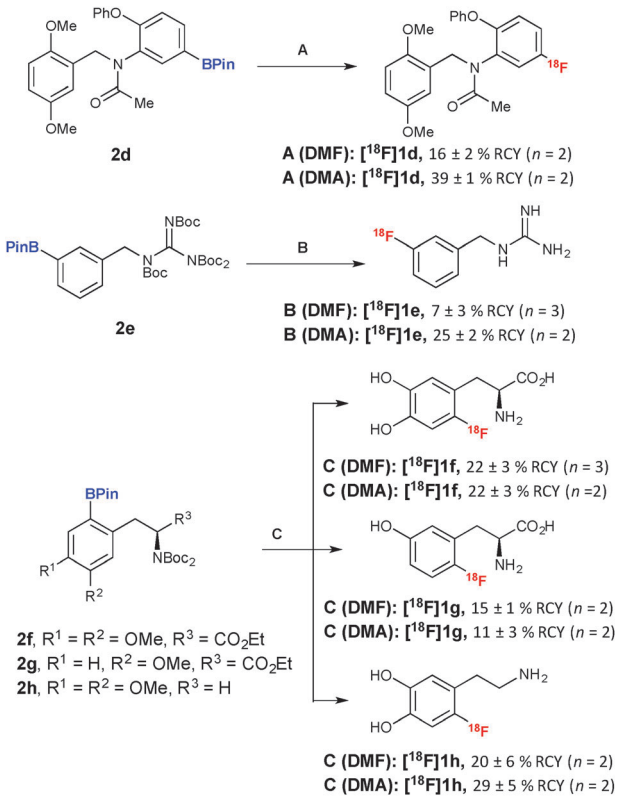
Table 1 Radiosynthesis of [^{18}F]FMTEB **[^{18}F]1a**, [^{18}F]FPEB **[^{18}F]1b** and [^{18}F]flumazenil **[^{18}F]1c** from **2a**, **2b** and **2c**, respectively, starting with 4.0–10.0 GBq of ^{18}F-fluoride



Entry	Radiotracer	Eluent	RCY[a] [%]
1	**[^{18}F]1a**	$K_2C_2O_4$	29 ± 6 (n = 2)
2	**[^{18}F]1b**	$K_2C_2O_4$	13 ± 5 (n = 2)
3	**[^{18}F]1c**	$K_2C_2O_4$	35 ± 7 (n = 3)
4[b]	**[^{18}F]1c**	$Cu(OTf)_2py_4$	16 (n = 1)
5[b,c]	**[^{18}F]1c**	$K_2C_2O_4$	17 (n = 1)

Reaction conditions: 0.03 mmol substrate, 0.04 mmol $Cu(OTf)_2py_4$, air, DMA 400 μL, 120 °C, 20 min. [a] RCY of isolated product. [b] DMF : pyridine 9 : 1 was used as reaction solvent. [c] $Cu(OTf)_2$ was used instead of $Cu(OTf)_2py_4$. BPin = boronic pinacol ester.

*Radiosynthesis of electron rich fluoroarenes: [^{18}F]DAA1106 **[^{18}F]1d**, [^{18}F]MFBG **[^{18}F]1e**, [^{18}F]FDOPA **[^{18}F]1f**, [^{18}F]FMT **[^{18}F]1g** and [^{18}F]FDA **[^{18}F]1h**.* Initial studies employing 20 to 30 MBq of starting [^{18}F]fluoride and the model electron rich arylboronic ester, 2-(3,4-dimethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, indicated that the reaction solvent DMA was superior to DMF, and that the higher copper loading, found beneficial for the ^{18}F-labeling of electron deficient arene, was detrimental for reaction efficacy. Experiments with 2.4 to 7.4 GBq of starting [^{18}F]fluoride were conducted on precursors **2d–h** in either DMF and DMA (Scheme 2). Full batch isolation experiments using a 1 : 1 molar ratio of **2d** : Cu in DMF afforded [^{18}F]DAA1186 **[^{18}F]1d**, a radioligand for the translocator protein 18 kDa (TSPO), in 16 ± 2% RCY; significant improvement with a RCY of 39% was observed in DMA.[15] The radiotracer [^{18}F]MFBG **[^{18}F]1e** was obtained in a one pot two steps sequence from the tetraboc-protected aryl boronate **2e** in 7 ± 3% RCY using the same **2e** : Cu molar ratio of 1 : 1. For this radiosynthesis, the ^{18}F-fluorination step was followed by deprotection with 57% HI at 120 °C for 10 min. Similarly to [^{18}F]DAA1186, DMA proved to be a superior solvent allowing for **[^{18}F]1e** to be isolated in 25 ± 2% RCY. [^{18}F]MFBG is a promising agent for imaging NET-expressing neuroblastomas.[16] When the reaction was performed in DMF, 6-[^{18}F]fluoro-L-dopa ([^{18}F]FDOPA) **[^{18}F]1f**, 6-[^{18}F]fluoro-L-*m*-tyrosine ([^{18}F]FMT) **[^{18}F]1g** and 6-[^{18}F]-fluorodopamine ([^{18}F]FDA) **[^{18}F]1h** were isolated in 22%, 15% and 20% RCYs, respectively applying a one-pot ^{18}F-fluorination-deprotection sequence similar to the one applied for **[^{18}F]1e**. The use of DMA as an alternative solvent was beneficial only for the synthesis of **[^{18}F]1h**. Radiotracers **[^{18}F]1d–h** are used in the clinic for various applications. [^{18}F]FDOPA is used for human brain studies of the dopaminergic system, the evaluation of neuropsychiatric disorders, in studies of cognitive behaviour, and in oncology for the investigation of neuroendocrine tumors.[17] The detection of tumors such as pheochromocytomas and *para*-gangliomas has also been



Scheme 2 Radiosynthesis of [^{18}F]DAA1186 **[^{18}F]1d**, [^{18}F]MFBG **[^{18}F]1e**, [^{18}F]FDOPA **[^{18}F]1f**, [^{18}F]FMT **[^{18}F]1g** and [^{18}F]FDA **[^{18}F]1h** starting with 2.4–7.4 GBq of ^{18}F-fluoride. Conditions A: 4–7.4 GBq of [^{18}F]KF/K_{222}, **2d** (0.02 mmol), $Cu(OTf)_2py_4$ (0.02 mmol), air, DMF or DMA (400 μL), 120 °C, 20 min; conditions B: 4.9–6.3 GBq of [^{18}F]KF/K_{222}, **2e** (0.02 mmol), $Cu(OTf)_2py_4$ (0.02 mmol), air, DMF or DMA (400 μL), 120 °C, 20 min, then 57% HI (300 μL), 120 °C, 10 min; conditions C: 2.4–6.9 GBq of [^{18}F]KF/K_{222}, **2f–h** (0.02 mmol), $Cu(OTf)_2py_4$ (0.02 mmol), air, DMF or DMA (400 μL), 120 °C, 20 min, then 57% HI (400 μL), 150 °C, 10 min.

possible with 6-[^{18}F]FDA.[18] 6-[^{18}F]FMT is reported to have improved imaging properties compared with the current clinical 'gold standard' 6-[^{18}F]FDOPA, but its use has been limited possibly due to the lack of a suitable manufacturing process.[19]

Today, the widespread application of **[^{18}F]1a–h** in the clinic is hampered by the paucity of effective production routes from [^{18}F]fluoride, a challenge that could be addressed if the methods described herein are amenable to automation on additional synthetic platforms. With these considerations in mind, we turned our attention to the Synthra and Neptis systems. The radiosynthesis of [^{18}F]flumazenil **[^{18}F]1c** was performed on a Synthra platform starting with 26 GBq of nca ^{18}F-fluoride. Applying the $K_2C_2O_4$ elution protocol with DMA as the reaction solvent, allowed isolation of 5.1 GBq of **[^{18}F]1c** (19% RCY), in >99% radiochemical purity (RCP) was >99%, and with a specific activity (SA) of 124 GBq μmol^{-1} (Table 2, entry 1). Flumazenil **[^{18}F]1c** was also prepared on the Neptis perform synthesizer. With this system, a solution of KH_2PO_4/K_2HPO_4 with K_{222} was used for elution. The ^{18}F-labeling was performed using 15 mg of flumazenil precursor **2c** and 30 mg of $Cu(OTf)_2py_4$ (molar ratio = 1 : 1.2) in DMA with 300 MBq of starting [^{18}F]fluoride.

Table 2 Radiosynthesis of [^{18}F]FPEB **[^{18}F]1b**, [^{18}F]flumazenil **[^{18}F]1c**, [^{18}F]FDOPA **[^{18}F]1f** and [^{18}F]FMT **[^{18}F]1g** on the SYNTHRA and NEPTIS platforms

Entry	Radiotracer	Synthra RCY [%] (n = 1)	NEPTIS RCY [%] (n = 10)
1	[^{18}F]flumazenil **[^{18}F]1c**	19[a]	16 ± 4[c]
2	[^{18}F]FPEB **[^{18}F]1b**	5[a]	—
3	[^{18}F]FDOPA **[^{18}F]1f**	9[b]	—
4	[^{18}F]FMT **[^{18}F]1g**	10[b]	—

Reaction conditions. [a] Radiosynthesis performed on the Synthra platform; 0.03 mmol substrate, 0.04 mmol $Cu(OTf)_2py_4$, air, DMA 400 μL, 120 °C, 20 min. [b] Radiosynthesis performed on the Synthra platform; 0.02 mmol substrate, 0.02 mmol $Cu(OTf)_2py_4$, air, DMF 400 μL, 120 °C, 20 min. [c] Radiosynthesis performed on Neptis perform synthesizer; 0.036 mmol substrate, 0.044 mmol $Cu(OTf)_2py_4$, air, DMA 1 mL, 130 °C, 10 min.

After HPLC purification, **[^{18}F]1c** was isolated in 16% ± 4% (n = 10) RCY (Table 2, entry 1). Three additional radiotracers were synthesized on the Synthra platform. Using DMA as the reaction solvent, [^{18}F]FPEB was obtained in 5% RCY with a SA of 120.8 GBq μmol^{-1} (Table 2, entry 2). The radiosyntheses of [^{18}F]FDOPA and [^{18}F]FMT performed in DMF gave ndc RCYs of 9% and 10%, respectively (Table 2, entries 3–4). Both radiotracers were produced as a single enantiomer (ee > 95%) with >99% RCP.

In summary, we have prepared eight clinically relevant radiotracers by applying our recently disclosed Cu-mediated non-carrier added nucleophilic ^{18}F-fluorination of arylboronic ester precursors. These precursors are easy to prepare and can be stored at room temperature under air. The reaction is reliable and reproducible, and employs commercially available $Cu(OTf)_2py_4$. The demonstration that eight radiotracers can be produced using a single reaction, some in different laboratories using different synthetic platforms, suggests that this radiochemistry could be broadly used in PET radiochemistry facilities. Current work focused on full automation for the production of radiopharmaceuticals that are most needed in the clinic.

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