SHORT NOTE

WILEY Journal of Labelled Compounds and Radiopharmaceuticals

Radiosynthesis of the norepinephrine transporter tracer [18F]NS12137 via copper-mediated 18F-labelling

Salla Lahdenpohja[1] (ID) | Thomas Keller[1] | Johan Rajander[2] | Anna K. Kirjavainen[1] (ID)

[1]Radiopharmaceutical Chemistry Laboratory, Turku PET Centre, University of Turku, Turku, Finland

[2]Accelerator Laboratory, Turku PET Centre, Åbo Akademi University, Turku, Finland

Correspondence
Anna K. Kirjavainen, Radiopharmaceutical Chemistry Laboratory, Turku PET Centre, University of Turku, Kiinamyllynkatu 4-8, FI-20520 Turku, Finland.
Email: anna.kirjavainen@utu.fi

[18F]NS12137 (*exo*-3-[(6-[18F]fluoro-2-pyridyl)oxy]8-azabicyclo[3.2.1]octane) is a highly selective norepinephrine transporter (NET) tracer. NETs are responsible for the reuptake of norepinephrine and dopamine and are linked to several neurodegenerative and neuropsychiatric disorders. The aim of this study was to develop a copper-mediated 18F-fluorination method for the production of [18F]NS12137 with straightforward synthesis conditions and high radiochemical yield and molar activity. [18F]NS12137 was produced in two steps. Radiofluorination of [18F]NS12137 was performed via a copper-mediated pathway starting with a stannane precursor and using [18F]F⁻ as the source of the fluorine-18 isotope. Deprotection was performed via acid hydrolysis. The radiofluorination reaction was nearly quantitative as was the deprotection based on HPLC analysis. The radiochemical yield of the synthesis was $15.1 \pm 0.5\%$. Molar activity of [18F]NS12137 was up to 300 GBq/μmol. The synthesis procedure is straightforward and can easily be automated and adapted for clinical production.

KEYWORDS
aryl stannanes, copper-mediated, fluorine-18, NET, NS12137, radiolabelling, radiofluorination

1 | INTRODUCTION

Fluorine-18 is the most common radionuclide for positron emission tomography (PET) due to its clean decay process (97% β⁺); its optimal positron energy (634 keV), which ensures high image resolution; and its adequate half-life (109.8 min), which allows multistep radiosynthesis and purification.[1] Norepinephrine transporters (NETs) are plasma membrane proteins located in the presynaptic noradrenergic neurons of the central nervous system. NETs are responsible for the reuptake of norepinephrine and dopamine, so disturbance of the NET system can lead to neurodegenerative and neuropsychiatric disorders.[2-4] [18F]NS12137[5-7] (*exo*-3-[(6-[18F] fluoro-2-pyridyl)oxy]8-azabicyclo[3.2.1]octane, [18F]**3**) is a highly selective NET tracer.

Radiofluorination of arenes remains a challenge for radiochemists. Previously, radiofluorination of many arenes was possible only using carrier-added electrophilic fluorination with [18F]F₂, which resulted in low molar activities.[1] Traditionally, high radiochemical yields (RCYs) and molar activities (A_ms) can be achieved with nucleophilic labelling, but the reactivity scope is limited. Several methods have recently been introduced to improve nucleophilic 18F-labelling reactions; for example, the use of iodonium[8] or sulfonium[9,10] salts enables 18F-labelling of nonactivated aromatic compounds. Recently, a new pathway, termed transition metal–mediated 18F-fluorination,

was introduced to produce ^{18}F-labelled arenes with high A_ms.[11-20] This transition metal–mediated synthesis pathway is based on the use of cyclotron-produced $[^{18}F]F^-$. In 2011, Lee et al.[11] reported the use of palladium complexes in ^{18}F-labelling reactions, but due to the moisture-sensitive nature of these complexes, a more convenient labelling method was still needed.[11,12] Nickel-mediated radiosynthesis followed the next year, but this still required the preparation and proper handling of highly complex precursor molecules, which was found to be cumbersome when designing clinical tracers that had to be synthetized according to good manufacturing practices (GMPs).[13-17] In 2013, Ye and Sanford[21] reported the mild copper-mediated fluorination of aryl stannanes and aryl trifluoroborates. Soon thereafter, Tredwell et al.[18] used this method for radiofluorination with $[^{18}F]$ KF starting from aryl boronic esters, and Mossine et al.[19] used it starting from boronic acids and Makaravage et al.[20] starting from aryl stannanes. Copper-mediated radiofluorination shows great promise as a suitable method for producing clinical tracers. We produced $[^{18}F]3$ starting from a stannylated precursor (exo-tert-butyl-3-[(6-trimethylstannyl-2-pyridyl)oxy]-8-azabicyclo-[3.2.1]octane-8-carboxylate, 1) (Figure 1).

$[^{18}F]3$ was produced previously via an electrophilic pathway[6] and, most recently, via a nucleophilic pathway.[7] As expected, the electrophilic production of $[^{18}F]3$ was achieved with relatively low RCY and A_m.[6] Nucleophilic production of $[^{18}F]3$ was achieved with good RCY and A_m, but the reaction conditions were extreme.[7] Using no–carrier-added copper-mediated synthesis, our goal was to overcome the limits of electrophilic and nucleophilic radiofluorination and to produce $[^{18}F]3$ with a RCY and an A_m that was suitable for clinical use.

2 | EXPERIMENTAL

The precursor 1 and the reference compounds 2 (exo-tert-butyl-3-[(6-fluoro-2-pyridyl)oxy]-8-azabicyclo-[3.2.1] octane-8-carboxylate) and 3 were obtained from DanPET AB, Malmö, Sweden (for characterization, see the Supporting Information). All other reagents and solvents were used as received from commercial suppliers without further purification. Radiolabelling reactions were performed in hot cells with a remote-controlled synthesis device built in-house.

Semi-prepative HPLC purification of $[^{18}F]3$ was carried out as previously reported.[6] A Gemini C18 column (5 μm, 10 × 250 mm; Phenomenex, Milford, Massachusetts, USA) was used. The mobile phases were as follows: (A) 7mM KH_2PO_4 and (B) CH_3CN. The semi-preparative C18 column was eluted for the first 5 minutes with 100% A, which was the decreased to 92% A for 0.1 minute followed by 36 minutes of 92% A. The flow rate was 5.0 mL/min.

Analytical radio-HPLC was carried out with a VWR Hitachi L-2130 HPLC pump (VWR Hitachi, VWR International GmbH, Darmstadt, Germany) equipped with a VWR Hitachi L-2400 UV absorption detector (λ = 230 nm) and a 2 × 2-in NaI radioactivity detector. A Luna C5 column (5 μm, 4.6 × 150 mm; Phenomenex, Milford, Massachusetts) was used for analytical analysis of compound $[^{18}F]2$. The mobile phases were as follows: (A) 0.1% TFA in H_2O and (B) 0.1% TFA in CH_3CN. The analytical column was eluted for the first 3 minutes with 20% B, which was then increased to 60% B for 0.1 minute followed by 12 minutes of 60% B (analytical HPLC method 1). The flow rate was 1.5 mL/min. For compound $[^{18}F]3$, a Gemini C18 column (5 μm, 4.6 × 250 mm, Phenomenex) was used. The mobile phases were as follows: (A) 7mM KH_2PO_4 and (B) CH_3CN. The C18 column was eluted for the first 8 minutes with 100% A, which was then increased to 50% B for 4 minutes followed by 8 minutes of 50% B (analytical HPLC method 2). The flow rate was 1.5 mL/min.

All RCYs and A_ms are decay-corrected to the start of the synthesis. The radiochemical purities (RCPs) are based on the amount of overall radioactivity eluted from the HPLC column. The product fraction was collected in a separate vial. We determined that there was no leftover radioactivity in the injector or in the HPLC column after the analytical HPLC run. RCY and RCP values are expressed as means ± standard deviations. The A_m of compounds $[^{18}F]2$ and $[^{18}F]3$ was determined by comparing the UV
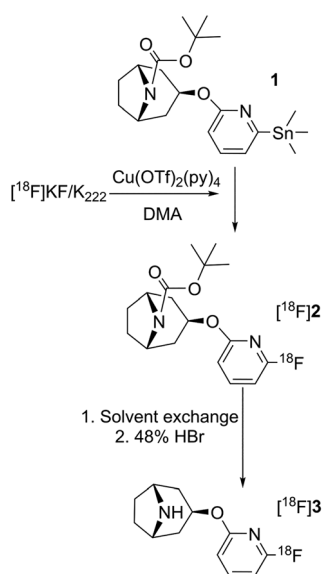

FIGURE 1 Reaction scheme for copper-mediated synthesis of $[^{18}F]$NS12137 ($[^{18}F]3$)

areas of the sample and a known concentration of the reference compound. The limit of detection (LOD; 0.1 µg/mL) of **3** was determined with the authentic reference compound and was used to determine the A_m of $[^{18}F]$**3**.

Levels of copper and tin in the final preparation were analyzed with inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, Elan DRC Plus). Commercial multielement standards were used for instrument calibration.

2.1 | Radiochemistry

The radiofluorination reaction was optimized by varying the following: the amount of $Cu(OTf)_2(py)_4$ and the base (K_2CO_3), the reaction solvent (MeCN or DMA), how the reaction solution was mixed (bubbling with air or helium), the reaction temperature, and the amount of precursor **1** (Table 1).

$[^{18}F]$Fluoride was produced as reported previously.[22] The initial amount of $[^{18}F]F^-$ used was 6.4 ± 2.5 GBq in the radiochemistry studies and 16.4 ± 1.5 GBq for A_m determinations. The $[^{18}F]KF$ solution was dried using azeotropic distillation with MeCN and Kryptofix K_{222} (15.5-16.5 µmol) at 120°C with helium flow to form the dry $[^{18}F]KF/K_{222}$ complex. The reaction vessel was allowed to cool for 1 minute, and $Cu(OTf)_2(py)_4$ (23.5-24.5 µmol, optimized amount) was added in MeCN (1 mL) or dimethylacetamide (DMA; 0.5 mL). Helium or air was bubbled through the solution for 10 seconds to 20 minutes. When $Cu(OTf)_2(py)_4$ was added in MeCN, the solvent was evaporated with a helium flow at 80°C to 120°C for 2 minutes. Compound **1** (7.5-8.5 µmol, optimized amount) was dissolved in DMA (0.5 mL) and added to the reaction vessel. The reaction solution was mixed by bubbling helium or air through the solution for 10 to 60 seconds and then heated at 70°C, 80°C, 100°C, or 120°C or kept at room temperature (RT) for 1 to 40 minutes. Samples (20 µL diluted in 200 µL CH_3CN) were collected during the reaction (at 1, 5, 15, and 40 min) and were analyzed using HPLC method 1.

A previously described method[7] was followed for deprotection of product $[^{18}F]$**2**. The reaction solvent was changed from DMA to tetrahydrofuran (THF) (1 mL) using a tC18 solid-phase extraction (SPE) cartridge (Waters Corporation, Milford, Massachusetts). THF was

TABLE 1 Optimization results of the synthesis of $[^{18}F]$**2**[a]

Entry	Equivalent Amount of Cu, Compared with Precursor 1, eq[b]	Cu and F⁻ Premixing	Premixing Time, min	Temperature, °C	Reaction Mixing	Reaction Time, min	RCP, %
1[c]	2	Air	10	85	-	30	0.5
2	2	Air	10	120	-	5	89[d]
3	3	He	10	120	-	1	85
4	3 (8 mg)	Air	10	120	-	5	94[d]
5	3 (5 mg)	Air	10	120	-	6	77
6	3	Air	10	120	-	1	76
7	3 (1 mg)	He	10	120	-	1	8
8	3	Air	10	RT	-	20	8[d]
9	3	Air	10	70	-	5	19
10	3	He	10	80	-	5	58
11	3	He	2	100	-	5	75
12	3	He	2	120	-	1	83
13[e]	3	He	2	120	He	2	88
14[e]	3	Air	0.2	120	Air	5	64

Abbreviations: DMA, dimethylacetamide; RCP, radiochemical purity.

[a]$Cu(OTf)_2(py)_4$ was premixed in MeCN, and the reaction solvent was DMA unless otherwise stated. Until entry 7, extra base was added to the reaction solution. After entry 7, no extra base was added.

[b]Three milligrams of precursor **1** unless stated otherwise.

[c]Reaction solvent MeCN.

[d]Based solely on the HPLC analysis of the crude product, no radioactivity was collected.

[e]Cu mixing done in DMA.

evaporated to dryness with helium flow at 80°C. Deprotection of the compound [^{18}F]**2** was carried out in 48% HBr (1 mL) by heating the mixture at 80°C for 5 minutes. The compound [^{18}F]**3** was analyzed using HPLC method 2.

3 | RESULTS AND DISCUSSION

[^{18}F]**3** was synthesized in two steps starting with the stannane precursor **1**. The first step was copper-mediated ^{18}F radiofluorination, and the subsequent step was deprotection by hydrolysis. The intermediate [^{18}F]**2** was produced with a maximum RCP of 89.2 ± 1.3% and up to 400 GBq/μmol of A_m (based on the HPLC analysis of the reaction solution) after 2-minute reaction at 120°C in DMA. After SPE, the RCP of [^{18}F]**2** was 98.2 ± 0.5%. Incorporation of the radiofluorination was 23.7 ± 2.2% according to the SPE. After hydrolysis, the RCP of the final product [^{18}F]**3** was 97.5 ± 0.5% (analyzed from the reaction solution). After semi-preparative HPLC purification, the radioactivity yield of the synthesis was 1.4 ± 0.2 GBq (n = 3, non–decay-corrected; starting activity 16.4 ± 1.5 GBq at the end of bombardment). Thus, the overall decay-corrected RCY of [^{18}F]**3** was 15.1 ± 0.5% (n = 3, based on the initial amount of [^{18}F]fluoride). A_m of purified [^{18}F]**3** was up to 300 GBq/μmol. The total synthesis time was 100 ± 11 minutes. According to the ICP-MS analysis, the amount of copper was less than 0.25 μg (Cu-63 residue, n = 3) and the amount of tin less than 0.25 μg (Sn-118 residue, n = 3) in the final product fraction. These amounts are low, well under the limits considered toxic.[23,24] This demonstrates that the relatively simple chromatographic methods can effectively remove copper and tin from the final product. Based on the used metal amounts, the purification factor is more than 6100 for copper and 3800 for tin.

The results of the optimization studies are presented in Table 1. In the preliminary labelling test, three equivalents of Cu(OTf)$_2$(py)$_4$ and one equivalent of precursor **1** was found to be optimal. Lowering the equivalent amount of Cu(OTf)$_2$(py)$_4$ decreased the RCP. The amount of base (K$_2$CO$_3$) did not affect the RCP. Notably, premixing the Cu(OTf)$_2$(py)$_4$ in MeCN or DMA, or mixing the reaction solution by bubbling helium or air through the solution, had no effect on the RCP. Changing the reaction solvent from MeCN to DMA before the addition of **1** resulted in a drastic increase in the RCP from 0.5% to 89% (Table 1, entries 1 and 2).

The amount of precursor **1** (Table 1, entries 4-7), and the time and temperature (Table 1, entries 8-12) of the radiofluorination reaction, substantially affected the RCP. The results of the reaction temperature and time

optimization experiments are presented in Figure 2. The reaction occurred even at RT (Table 1, entry 8), but the RCP at RT was less than 6% (n = 2) after a 20-minute reaction. Of the conditions tested, the optimal temperature and reaction time were 120°C for 2 minutes (n > 5) (Table 1, entries 12 and 13). Generally, increasing the reaction temperature resulted in exponential growth of the reaction rate (Figure 2B). It is also notable that a longer reaction time (>5 min) at a high temperature resulted in slightly lower RCP and increased the number of side products. This suggests that the product [^{18}F]**3** starts to slowly decompose at high temperatures. The amount of precursor **1** was varied between 1 and 8.5 mg (Table 1, entries 4-7). The lowest RCPs were observed when less than 3 mg of precursor **1** was used. With 1 mg of the precursor (Table 1, entry 7), the RCP was less than 10% (n = 1). The optimal amount of precursor was 3.5 mg,
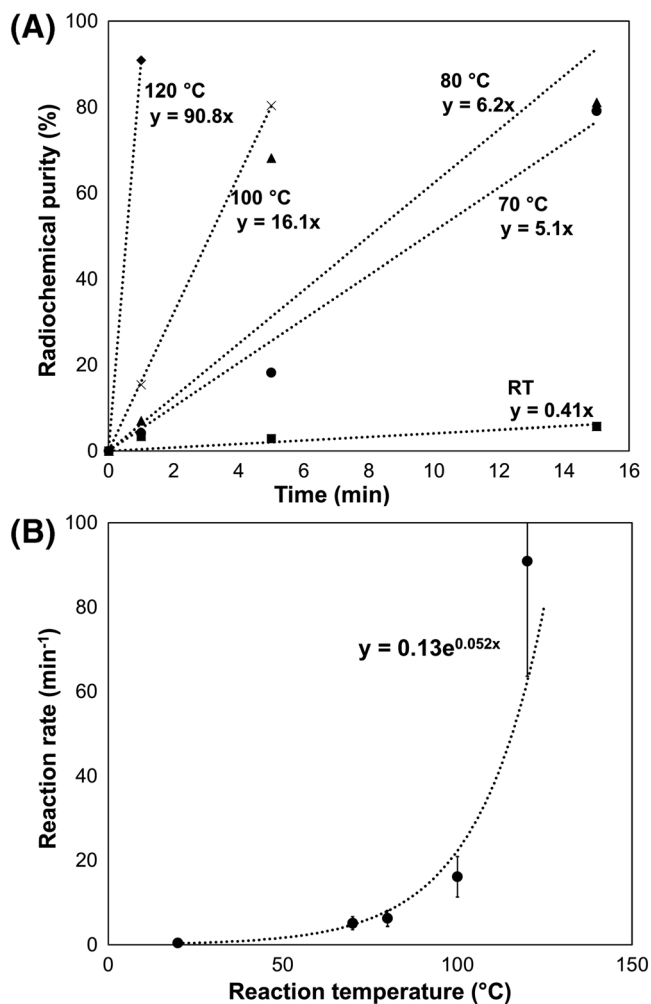

FIGURE 2 A, Radiochemical purity of the intermediate [^{18}F]**2** as a function of time at the indicated temperatures. B, The reaction rate of the ^{18}F-radiofluorination reaction as a function of reaction temperature

which resulted in an RCP over 80% after a 1-minute reaction (n > 5).

In previous studies, nucleophilic ^{18}F-fluorination of [^{18}F]**3** with [^{18}F]F$^-$ was shown to be superior to electrophilic ^{18}F-fluorination with [^{18}F]F$_2$ or [^{18}F]Selectfluor bis(triflate).[6,7] Here, we showed that [^{18}F]**3** can be produced with similar RCY and A$_m$ with copper-mediated ^{18}F-fluorination as with traditional nucleophilic ^{18}F-fluorination.[7] Compared with the traditional nucleophilic synthesis route of [^{18}F]**3**, the copper-mediated method has two main advantages, namely, shorter synthesis time and lower reaction temperature. The procedure introduced here is simpler and therefore easier to automate than previously published methods. Future work will focus on further shortening the synthesis time by replacing azeotropic drying of the [^{18}F]F$^-$ with an SPE procedure.

4 | CONCLUSIONS

Here, we described the successful production of a NET tracer, [^{18}F]**3**, using no–carrier-added copper-mediated ^{18}F-labelling. The intermediate [^{18}F]**2** was produced with high RCP (>89%, based on the HPLC analysis of the reaction solution) and A$_m$ (up to 400 GBq/μmol) after a 2-minute reaction at 120°C. After hydrolysis, [^{18}F]**3** was achieved with high RCP (>97%). The overall RCY of [^{18}F]**3** was moderate (approx 15%) and the A$_m$ good (up to 300 GBq/μmol). The procedure can be automated in its present form and adapted for clinical GMP production. Our results show that copper-mediated ^{18}F-fluorination can be adapted to synthesize radiotracers of clinical interest, such as [^{18}F]**3**, and we aim to expand the scope further in the future.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Salla Lahdenpohja https://orcid.org/0000-0003-2456-5009
Anna K. Kirjavainen https://orcid.org/0000-0003-3981-2973

REFERENCES

1. Miller PW, Long NJ, Vilar R, Gee AD. Synthesis of ^{11}C, ^{18}F, ^{15}O, and ^{13}N radiolabels for positron emission tomography. *Angew Chem Int Ed.* 2008;47:8998-9033.

2. Tatsumi M, Groshan K, Blakely RD, Richelson E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol.* 1997;340:249-258.

3. Morón JA, Brockington A, Wise RA, Rocha BA, Hope BT. Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *J Neurosci.* 2002;22:389-395.

4. Torres GE, Caron MG, Gainetdinov RR. Plasma membrane monoamine transporters: structure, regulation and function. *Nat Rev Neurosci.* 2003;4:13-25.

5. Peters D, Eriksen BL, Nielsen EØ, Scheel-Krüger J, Olsen GM. Novel 8-azabicyclo[3.2.1]octane derivatives and their use as monoamine neurotransmitter re-uptake inhibitors. *WO.* 2004;2004(/113334):A1.

6. Kirjavainen AK, Forsback S, López-Picón FR, et al. ^{18}F-labeled norepinephrine transporter tracer [^{18}F]NS12137: radiosynthesis and preclinical evaluation. *Nucl Med Biol.* 2018;56:39-46.

7. López-Picón FR, Kirjavainen AK, Forsback S, et al. In vivo characterization of a novel norepinephrine transporter PET tracer [^{18}F]NS12137 in adult and immature Sprague-Dawley rats. *Theranostics.* 2019;9:11-19.

8. Pike VW, Aigbirhio FI. Reactions of cyclotron-produced [^{18}F] fluoride with diaryliodonium salts—a novel single-step route to no-carrier-added [^{18}F]fluoroarenes. *J Chem Soc Chem Commun.* 1995;1995:2215-2216.

9. Mu L, Fischer CR, Holland JP, et al. ^{18}F-Radiolabeling of aromatic compounds using triarylsulfonium salts. *Eur J Org Chem.* 2012;2012:889-892.

10. Gendron T, Sander K, Cybulska K, et al. Ring-closing synthesis of dibenzothiophene sulfonium salts and their use as leaving groups for aromatic ^{18}F-fluorination. *J Am Chem Soc.* 2018;140:11125-11132.

11. Lee E, Kamlet AS, Powers DC, et al. A fluoride-derived electrophilic late-stage fluorination reagent for PET imaging. *Science.* 2011;334:639-642.

12. Kamlet AS, Neumann CN, Lee E, et al. Application of palladium-mediated ^{18}F-fluorination to PET radiotracer development: overcoming hurdles to translation. *PLoS One.* 2013;8:1-10.

13. Lee E, Hooker JM, Ritter T. Nickel-mediated oxidative fluorination for PET with aqueous [^{18}F]fluoride. *J Am Chem Soc.* 2012;134:17456-17458.

14. Ren H, Wey H, Strebl M, Neelamegam R, Ritter T, Hooker JM. Synthesis and imaging validation of [^{18}F]MDL100907 enabled by Ni-mediated fluorination. *ACS Chem Neurosci.* 2014;5:611-615.

15. Zlatopolskiy BD, Zischler J, Urusova EA, et al. A Practical one-pot synthesis of positron emission tomography (PET) tracers via nickel-mediated radiofluorination. *ChemistryOpen.* 2015;4:457-462.

16. Sanford MS, Scott PJH. Moving metal-mediated ^{18}F-fluorination from concept to clinic. *ACS Cent Sci*. 2016;2:128-130.

17. Hoover A, Lazari M, Ren H, et al. A Transmetalation reaction enables the synthesis of [^{18}F]5-fluorouracil from [^{18}F]fluoride for human PET imaging. *Organometallics*. 2016;35:1008-1014.

18. Tredwell M, Preshlock SM, Taylor NJ, et al. A General copper-mediated nucleophilic ^{18}F-fluorination of arenes. *Angew Chem Int Ed*. 2014;53:7751-7755.

19. Mossine AV, Brooks AF, Makaravage KJ, et al. Synthesis of [^{18}F] arenes via the copper-mediated [^{18}F]fluorination of boronic acids. *Org Lett*. 2015;17:5780.

20. Makaravage KJ, Brooks AF, Mossine AV, Sanford MS, Scott PJH. Copper-mediated radiofluorination of arylstannanes with [^{18}F]KF. *Org Lett*. 2016;18:5440-5443.

21. Ye Y, Sanford MS. Mild copper-mediated fluorination of aryl stannanes and aryl trifluoroborates. *J Am Chem Soc*. 2013; 135:4648-4651.

22. Savisto N, Viljanen T, Kokkomäki E, Bergman J, Solin O. Automated production of [^{18}F]FTHA according GMP. *J Label Compd Radiopharm*. 2017;61:84-93.

23. 5.20. Metal catalyst or metal reagent residues. *Council of Europe. European Pharmacopoeia 9th Edition*. Strasbourg: Council of Europe; 2013.

24. Toxicological profile for tin and tin compounds, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. 2005.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.