SHORT NOTE

Radiosynthesis of the norepinephrine transporter tracer \([^{18}\text{F}]\text{NS12137}\) via copper-mediated \(^{18}\text{F}\)-labelling

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[\(^{18}\text{F}]\text{NS12137}\) (exo-3-(6-[\(^{18}\text{F}\)]fluoro-2-pyridyloxy)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 \(^{18}\text{F}\)-fluorination method for the production of \([^{18}\text{F}]\text{NS12137}\) with straightforward synthesis conditions and high radiochemical yield and molar activity. \([^{18}\text{F}]\text{NS12137}\) was produced in two steps. Radiofluorination of \([^{18}\text{F}]\text{NS12137}\) was performed via a copper-mediated pathway starting with a stannane precursor and using [\(^{18}\text{F}\)]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 ± 0.5%. Molar activity of \([^{18}\text{F}]\text{NS12137}\) 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% \(\beta^+\)); 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.\(^2\)-\(^4\) \([^{18}\text{F}]\text{NS12137}\) (exo-3-(6-[\(^{18}\text{F}\)]fluoro-2-pyridyloxy)8-azabicyclo[3.2.1]octane, \([^{18}\text{F}]\text{3}\) 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 \([^{18}\text{F}\)]F\(_2\), which resulted in low molar activities.\(^1\) Traditionally, high radiochemical yields (RCYs) and molar activities (A\(_m\)) can be achieved with nucleophilic labelling, but the reactivity scope is limited. Several methods have recently been introduced to improve nucleophilic \(^{18}\text{F}\)-labelling reactions; for example, the use of iodonium\(^8\) or sulfonium\(^9\)-\(^10\) salts enables \(^{18}\text{F}\)-labelling of nonactivated aromatic compounds. Recently, a new pathway, termed transition metal–mediated \(^{18}\text{F}\)-fluorination,
was introduced to produce $^{18}$F-labelled arenes with high Am.$^{11-20}$ This transition metal–mediated synthesis pathway is based on the use of cyclotron-produced $[^{18}\text{F}]\text{F}^-$. 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 synthesized according to good manufacturing practices (GMPs).$^{13-17}$ In 2013, Ye and Sanford$^{21}$ reported to be synthetized according to good manufacturing practices (GMPs).$^{13-17}$ In 2013, Ye and Sanford$^{21}$ reported that, while copper-mediated fluorination follows the next year, but this was achieved with low RCY and Am.$^6$ Nucleophilic radiofluorination shows great promise as a suitable way.$^7$ As expected, the electrophilic production of $[^{18}\text{F}]$ was achieved with good RCY and Am, but the reaction conditions were extreme.$^7$ 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-preparative HPLC purification of $[^{18}\text{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) 7 mM KH$_2$PO$_4$ and (B) CH$_3$CN. 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}\text{F}]$2. The mobile phases were as follows: (A) 0.1% TFA in H$_2$O and (B) 0.1% TFA in CH$_3$CN. 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}\text{F}]$3, a Gemini C18 column (5 μm, 4.6 × 250 mm, Phenomenex) was used. The mobile phases were as follows: (A) 7 mM KH$_2$PO$_4$ and (B) CH$_3$CN. 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 Am's are decay-corrected to the start of the synthesis. The chemical 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 Am of compounds $[^{18}\text{F}]$2 and $[^{18}\text{F}]$3 was determined by comparing the UV

![FIGURE 1](image-url)  
**FIGURE 1** Reaction scheme for copper-mediated synthesis of $[^{18}\text{F}]$NS12137 ($[^{18}\text{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 Aₘ of [¹⁸F]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 (K₂CO₃), 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).

[¹⁸F]Fluoride was produced as reported previously.²² The initial amount of [¹⁸F]F⁻ used was 6.4 ± 2.5 GBq in the radiochemistry studies and 16.4 ± 1.5 GBq for Aₘ determinations. The [¹⁸F]KF solution was dried using azeotropic distillation with MeCN and Kryptofix K₂₂₂ (15.5-16.5 μmol) at 120°C with helium flow to form the dry [¹⁸F]KF/K₂₂₂ 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 2 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 CH₃CN) were collected during the reaction (at 1, 5, 15, and 40 min) and were analyzed using HPLC method 1.

A previously described method⁷ was followed for deprotection of product [¹⁸F]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

| Entry | Equivalent Amount of Cu, Compared with Precursor 1, eqb | Cu and F⁻ Premixing | Premixing Time, min | Temperature, °C | Reaction Mixing | Reaction Time, min | RCP, % |
|-------|-------------------------------------------------------|---------------------|---------------------|----------------|-----------------|-------------------|--------|
| 1c    | 2                                                     | Air                 | 10                  | 85             | -               | 30                | 0.5    |
| 2     | 2                                                     | Air                 | 10                  | 120            | -               | 5                 | 89d    |
| 3     | 3                                                     | He                  | 10                  | 120            | -               | 1                 | 85     |
| 4     | 3 (8 mg)                                              | Air                 | 10                  | 120            | -               | 5                 | 94d    |
| 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                | 8d     |
| 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     |
| 13e   | 3                                                     | He                  | 2                   | 120            | He              | 2                 | 88     |
| 14e   | 3                                                     | Air                 | 0.2                 | 120            | Air             | 5                 | 64     |

Abbreviations: DMA, dimethylacetamide; RCP, radiochemical purity.

*cCu(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.

*bThree milligrams of precursor 1 unless stated otherwise.

cReaction solvent MeCN.

dBased solely on the HPLC analysis of the crude product, no radioactivity was collected.

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

### 3 | RESULTS AND DISCUSSION

$[^{18}\text{F}]3$ was synthesized in two steps starting with the stannane precursor 1. The first step was copper-mediated $^{18}\text{F}$ radiofluorination, and the subsequent step was deprotection by hydrolysis. The intermediate $[^{18}\text{F}]2$ was produced with a maximum RCP of 89.2 ± 1.3% and up to 400 GBq/μmol of Am (based on the HPLC analysis of the reaction solution) after 2-minute reaction at 120°C in DMA. After SPE, the RCP of $[^{18}\text{F}]2$ 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 $[^{18}\text{F}]3$ 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 $[^{18}\text{F}]3$ was 15.1 ± 0.5% (n = 3, based on the initial amount of $[^{18}\text{F}]$fluoride). Am of purified $[^{18}\text{F}]3$ 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. 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 $[^{18}\text{F}]3$ 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,
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.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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