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A Concise Radiosynthesis of the Tau Radiopharmaceutical, [¹⁸F]T807

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Abstract

Fluorine-18 labelled 7-(6-fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole ([¹⁸F]T807) is a potent and selective agent for imaging paired helical filaments of tau (PHF-tau) and is among the most promising PET radiopharmaceuticals for this target in early clinical trials. The present study reports a simplified one-step method for the synthesis of [¹⁸F]T807 that is broadly applicable for routine clinical production using a GE TracerlabTM FX_{FN} radiosynthesis module. Key facets of our optimized radiosynthesis include development and use of a more soluble protected precursor, *tert*-butyl 7-(6-nitropyridin-3-yl)-5H-pyrido[4,3-b]indole-5-carboxylate, as well as new HPLC separation conditions that enable a facile one-step synthesis. During the nucleophilic fluorinating reaction with potassium cryptand [¹⁸F]fluoride (K[¹⁸F]/K₂₂₂) in DMSO at 130 °C over 10 min, the precursor is concurrently deprotected. Formulated [¹⁸F]T807 was prepared in an uncorrected radiochemical yield of 14 ± 3%, with a specific activity of 216 ± 60 GBq/μmol (5837 ± 1621 mCi/μmol) at the end of synthesis (60 min; *n* = 3) and validated for human use. This methodology offers the advantage of faster synthesis in fewer steps, with simpler automation which we anticipate will facilitate widespread clinical use of [¹⁸F]T807.

Keywords

T807; Tau; PET; fluorine-18

1. Introduction

Development of positron emission tomography (PET) radiotracers specific for pathological tau accumulation in neurodegenerative diseases (tauopathies) represent one of the most active, yet most challenging, areas in neuroscience.¹ Pioneering developments for tau imaging in human subjects have been achieved with [¹⁸F]FDDNP,² which is effective at imaging hyperphosphorylated tau fibrillar aggregates but does not distinguish between

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amyloid- β plaques and tau. Development of a selective and specific imaging agent is essential for advancing our understanding of tauopathies, improving differential diagnostic accuracy, accelerating drug discovery and monitoring of therapeutics.¹ Recent preliminary efforts toward this goal have included exploration of [^{11}C]N-methyl lansoprazole³ and a [^{18}F]fluoroethyl astemizole derivative,⁴ which have shown promising properties *in vitro* for selective binding to tau but poor brain availability in preclinical studies *in vivo*. Okamura and co-workers have developed a series of tau radiotracer candidates based on benzoxazole, benzimidazole and quinoline scaffolds⁵ with [^{18}F]THK523 advancing to human imaging trials revealing mixed results.^{6–8} However, other derivatives based on these scaffolds including [^{18}F]THK5105 and [^{18}F]THK5117 appear to be more promising for imaging tauopathies in human subjects.⁹

Several ^{18}F -labelled benzimidazole pyrimidines have been discovered by Siemens Molecular Imaging Biomarker Research (recently acquired by Avid/Lilly). In particular, two compounds, [^{18}F]T807^{8,10} and [^{18}F]T808¹¹, were found to display excellent potency and selectivity *in vitro* for paired helical filaments of tau (PHF-tau) over A β -plaques. Both radiopharmaceuticals are currently in phase 0 clinical studies for Alzheimer's disease.⁸ Although both [^{18}F]T807^{8,10} and [^{18}F]T808¹¹ show promise as PHF-tau imaging agents, they were shown to have markedly different pharmacological profiles with regard to their biodistribution and metabolism. We focused our initial clinical tau imaging efforts on validation of [^{18}F]T807 because it revealed less defluorination *in vivo* than [^{18}F]T808.¹¹

The reported synthesis of [^{18}F]T807 involves a cumbersome, two-step reaction, that is semiautomated using a modified Siemens Explora radiosynthesis module.^{8,10} After [^{18}F]fluorination of 7-(6-nitropyridin-3-yl)-5H-pyrido[4,3-b]indole (**1a**), a second step is carried out with iron powder/formic acid in a separate vial off-line from the automated synthesis unit to reduce the nitro group on the remaining precursor to the respective 2-amino-pyridine derivative, thereby facilitating separation by HPLC. The semi-automated reduction step is not readily adaptable to commercial radiosynthesis platforms and poses a limitation for advancing large-scale multi-center trials and widespread use. Our goal was to develop a robust and facile method for production of this promising radiopharmaceutical to investigate a range of tauopathies¹ and traumatic brain injuries.^{12,13} Here we report an improved radiosynthesis of [^{18}F]T807 using a new *N*-tert-butoxycarbonyl (*t*-Boc) protected precursor, *tert*-butyl 7-(6-nitropyridin-3-yl)-5H-pyrido[4,3-b]indole-5-carboxylate (**1b**), in a one-step synthesis with a simple isocratic HPLC purification. The radiosynthesis of [^{18}F]T807 was carried out using the commonly used GE TRACERlabTM FX_{FN} module and its production was validated for routine human use.

2. Experimental

2.1 Chemicals and Reagents

Compounds **1a**, **1b**, and authentic standard, 7-(6-fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole (T807), were custom synthesized to GMP standards by Huayi Isotopes Company (Toronto, Canada). All other chemicals and reagents were obtained from commercial vendors and were used as received without further purification.

2.2 Automated Synthesis of [^{18}F]T807

A GE PETtrace 16/8.5 MeV cyclotron was used for [^{18}F]fluoride radionuclide production. A GE high yield niobium target containing >97% enriched O-18 water (Isotec, Taiyo Nippon Sanso or Rotem) was bombarded with protons at integrated currents up to 65 μA to produce [^{18}F]fluoride. Following completion of bombardment, the [^{18}F]fluoride was transferred to the TRACERlabTM FX_{FN} radiosynthesis module *via* helium gas overpressure.

A schematic diagram of the GE medical systems commercial TRACERlabTM FX_{FN} radiosynthesis module used for the synthesis of [^{18}F]T807 is shown in Figure 1. Automated synthesis involves: (1) azeotropic drying of [^{18}F]fluoride, (2) [^{18}F]fluorination of **1** (Scheme 1), (3) in-line solvent exchange and (4) HPLC purification, followed by solid-phase formulation of the final product. The synthesis module was operated in the following sequences with numerical references to Figure 1:

1. [^{18}F]Fluoride was produced by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module *via* line 10. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge 11 (Waters; activated with 6 mL of trace grade H_2O).
2. Automated synthesis began with the elution of resin-bound [^{18}F]fluoride (37 ± 0.37 GBq; 1.0 ± 0.1 Ci) using 1 mL of a solution (15 mg K_{222} , 1.5 mg K_2CO_3 , 0.6 mL trace grade H_2O , 0.6 mL acetonitrile), pre-loaded into vessel 1 and delivered to the reaction vial 12.
3. The reaction mixture in vial 12 was dried azeotropically by addition of 1 mL anhydrous CH_3CN , preloaded into vessel 4, at 85 °C under N_2 flow and vacuum over 8 min, then at 110 °C under N_2 flow and vacuum for 4 min.
4. After heating to 130 °C, the precursor (**1a** or **1b**; 1 mg in 1.5 mL DMSO) pre-loaded into vessel 3 was added to vial 12. The reactor was sealed *via* valve V24 and the reaction mixture was heated for 10 min.
5. The reaction mixture was then cooled to 50 °C, vented *via* valves V24 and V25, and diluted with 10 mL of H_2O , pre-loaded into vessel 5.
6. The contents of vial 12 were delivered onto an Oasis® HLB Light SPE cartridge 13 (Waters; pre-activated with 5 mL EtOH followed by 10 mL H_2O) and washed with 5 mL of water from vessel 6 to remove DMSO, unreacted ^{18}F -fluoride and other impurities. The crude reaction mixture was eluted from the cartridge with 1 mL of ethanol from vessel 2 into vessel 14 containing 1 mL of water. The contents of vessel 14 were transferred to the HPLC loop *via* N_2 pressure *via* a fluid detector, injected onto a semi-preparative column (X-Select HSS T3, 250 $\times$ 10.00 mm, 5 μ), and eluted with 18% EtOH/ H_2O by volume (pH 2, adjusted with HCl) at a flow rate of 5 mL/min. The eluent was monitored by UV ($\lambda = 254$ nm) and radiochemical detectors connected in series. The retention times of **1a** and T807 are 15 and 22 minutes, respectively (Figure 2). Compound **1b** does not elute under these

conditions and its absence from the formulated product was confirmed by analytical HPLC (see section 3.2).

7. A typical semi-preparative HPLC chromatogram is shown in Figure 2. The fraction containing the major radiochemical product ($t_R = 21.5$ min) was collected, via valve 18, into a large dilution vessel 15 which was pre-loaded with 2 mL 8.4% sodium bicarbonate for injection (USP; Hospira) and 20 mL of USP sterile water for injection.
8. The product was loaded onto an Oasis® HLB Light SPE cartridge 16 (Waters; pre-activated with 5 mL EtOH followed by 10 mL H₂O).
9. The SPE cartridge was then washed to a waste container with 10 mL H₂O, pre-loaded into vessel 7, to remove traces of salts, CH₃CN, and [¹⁸F]fluoride.
10. The SPE cartridge was eluted with 1 mL USP EtOH, pre-loaded into vessel 8, into collection vial 17 followed by 10 mL 0.9% USP sodium chloride for injection pre-loaded into vessel 9, resulting in a solution of 10% EtOH (v/v) in 0.9% sodium chloride.
11. The solution was transferred, via line 18, and passed through a 0.22 µm PES vented sterilizing filter (B. Braun) connected to a 16 gauge hypodermic needle into a sterile 30 mL dose vial (Hospira) fitted with a sterile filtered venting needle (International Medical Industries)

Analyses of radioactive mixtures were performed by HPLC with an in-line UV ($\lambda = 254$ nm) detector in series with a CsI pin diode radioactivity detector. To determine the identity of [¹⁸F]T807, aliquots of the formulated product were injected onto an analytical HPLC system using a X-Select HSS T3, 150 × 4.6 mm, 3.5 µm and eluted with 20% EtOH/H₂O (pH 2 adjusted with HCl) with a flow rate of 1.2 mL/min, monitored at $\lambda = 254$ nm. The major radiochemical product was identified as [¹⁸F]T807 ($t_R = 8.8$ min; Figure 3). Further characterization and validation of [¹⁸F]T807 was carried out as described in sections 3.2 and 3.3.

3. Results and Discussion

3.1 Radiochemistry

The radiosynthesis of [¹⁸F]T807 was automated using a GE TRACERlab™ FX_{FN} radiosynthesis module, the most widely used apparatus that is specifically designed for nucleophilic [¹⁸F]fluorinations requiring HPLC purification. In order to make the radiosynthesis of [¹⁸F]T807 more easily amenable for routine automation we modified the previously published procedure.^{8,10} In the present work, a new precursor (**1b**) and optimized isocratic HPLC separation conditions enable a facile one-step synthesis of [¹⁸F]T807. Compound **1b** is concurrently deprotected during the nucleophilic fluorinating reaction with potassium cryptand [¹⁸F]fluoride (K[¹⁸F]/K₂₂₂) in DMSO at 130 °C, over 10 minutes. Although t-Boc protecting groups are commonly used in radiofluorinations and removed under acidic conditions, NH-heteroarenes, such as indoles, bearing electron-withdrawing groups can be deprotected under mildly basic conditions.¹⁴ The uncorrected RCY of [¹⁸F]T807 was 14 ± 3% ($n = 3$) and the total synthesis time was 60 minutes including

formulation. The product was prepared consistently with >95% radiochemical purity and the specific activity at the end of synthesis was 216 ± 60 GBq/ μ mol (5837 ± 1621 mCi/ μ mol).

In the previous radiosynthesis method used for human validation of [^{18}F]T807,¹⁰ precursor **1a** was used and resulted in ~17% uncorrected RCY at the end of synthesis (EOS; 93 minutes) with specific activities > 1 Ci/ μ mol at the time of injection. Under our reaction conditions, we found that radiolabelling is facilitated using the *t*-Boc protected precursor (**1b**) and is attributed to increased solubility in DMSO compared with compound **1a**, which is only sparingly soluble in most organic solvents. Therefore, the precursor could be added remotely to the reaction vial with minimal volume and eliminates the possibility of the precursor precipitating out of solution before use. Moreover, the fluorination conditions employed simultaneously removes the *t*-Boc protecting group and eliminates the need for an additional deprotection step. Following ^{18}F -fluorination in the original procedures,^{8,10} an additional step was carried out to facilitate HPLC separation of [^{18}F]T807 from the precursor (**1a**). In this step, the reaction mixture was transferred to a separate vial containing iron powder/formic acid and to heated at 100 °C for 15 min to reduce the –NO₂ group on the unreacted precursor to the respective 2-amino-pyridine derivative. We found that it is possible to eliminate this step by developing suitable isocratic HPLC separation conditions for purification of [^{18}F]T807. A radiochemical reaction using the unprotected precursor (**1a**) was carried out using our optimized reaction conditions for **1b**, identified above. The uncorrected radiochemical yield was much lower (2.4%; $n = 1$) and specific activity at end of synthesis was 133 GBq/ μ mol (3615 mCi/ μ mol). The majority of the activity (55%) was isolated in the SPE waste (Figure 1; 13), and was identified as unreacted [^{18}F]fluoride by radio-TLC. In light of this comparatively low yielding result no further attempts were made to optimize the radiosynthesis with **1a** and validation of [^{18}F]T807 was carried out with the *t*-Boc protected precursor, **1b**. We speculate that the protecting group may also be responsible for the higher radiochemical yields by inhibiting side reactions with the –NH of the indole moiety.

3.2 Validation for Human Use

Three consecutive productions of [^{18}F]T807 were carried out to validate this radiopharmaceutical for human use. HPLC analysis of formulated product revealed high radiochemical (>95%) and chemical purities. Due to the increased lipophilicity of **1b**, an additional HPLC analysis was performed to confirm that this precursor was not present in the formulated product. This was achieved using an X-Select HSS T3 column (4.6×150 mm, 3.5 μ) and eluted with 50% CH₃OH/H₂O (pH 2 adjusted with HCl) with a flow rate of 1.2 mL/min, monitored at $\lambda = 254$ nm. Radio-TLC was performed to verify the radiochemical purity of [^{18}F]T807 using silica gel plates and 80:20 (v/v) CH₂Cl₂:CH₃OH as the mobile phase. Radio-TLC also confirmed high radiochemical purity (>97%). The integrity of the final filter was demonstrated by a bubble point filter test (>50 psi). Formulated [^{18}F]T807 maintained stability, as measured by HPLC and radio-TLC, as well as clarity and a pH of 5.5 over a period of 6 hours. The half-life was verified to be 109.7 minutes by a dose calibrator. No long lived isotopes were observed (5 days), as determined by analysis on a HPGE detector after ^{18}F -decay. The formulated product was free of pyrogens (Charles River Endosafe® PTS), sterile, and passed the Kryptofix® spot test (<50

µg/mL). Volatile organic compound analysis was carried out by GC-FID showing residual acetone, CH₃CN and DMSO below the lower limit of detection, thereby exceeding ICH requirements. Using this new methodology [¹⁸F]T807 is successfully validated for human PET studies meeting all FDA and USP requirements for a PET radiopharmaceutical.

4. Conclusion

An improved one-step synthesis of [¹⁸F]T807 followed by isocratic HPLC separation was achieved with a more soluble *t*-Boc protected precursor (**1b**). This radiosynthetic strategy which enables concurrent ¹⁸F-fluorination and deprotection should prove to be broadly applicable the preparation of ¹⁸F-labelled NH-heteroarenes bearing electron-withdrawing groups. [¹⁸F]T807 was validated for human use with a GE TRACERlab™ FX_{FN} radiosynthesis module offering a faster and simpler synthesis. The methodology described herein can facilitate multi-center trials and widespread use of this radiopharmaceutical for tauopathy imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Figure 2.

Figure 3.