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SHORT NOTE

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A simplified radiosynthesis of [^{18}F]MK-6240 for tau PET imaging

Robert Hopewell[1] | Karen Ross[1] | Alexey Kostikov[1] | Tharick A. Pascoal[1,2] | Thais Alberti[2] | Monica Lacatus-Samoila[1] | Jean-Paul Soucy[1] | Idriss Bennacef[3] | Eliane Kobayashi[1] | Min Su Kang[1,2] | Pedro Rosa-Neto[1,2] | Gassan Massarweh[1]

[1]Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

[2]Translational Neuroimaging Laboratory, The McGill University Research Centre for Studies in Aging, Montreal, Quebec, Canada

[3]Translational Biomarkers, Merck & Co, Inc, West Point, Pennsylvania, USA

Correspondence
Gassan Massarweh, Montreal Neurological Institute, 3801, University Street, Montreal, QC, Canada.
Email: gassan.massarweh@mcgill.ca

[^{18}F]MK-6240 (6-(fluoro)-3-(1*H*-pyrrolo[2,3-c]pyridin-1-yl)isoquinolin-5-amine) is a highly selective PET radiotracer for the in vivo imaging of neurofibrillary tangles (NFTs). [^{18}F]MK-6240 was synthesized in one step from its bis-Boc protected precursor *N*-[(*tert*-butoxy)carbonyl]-*N*-(6-nitro-3-[1*H*-pyrrolo[2,3-c]pyridin-1-yl]isoquinolin-5-yl) carbamate in DMSO using [^{18}F] fluoride with TEA HCO_3 with step-wise heating up to 150°C, resulting in an isolated radiochemical yield of 9.8% ± 1.8% (n = 3) calculated from the end of bombardment (5.2% ± 1.0% calculated from the end of synthesis). This new synthetic approach eliminates the acidic deprotection of the bis-Boc ^{18}F-labeled intermediate, which reduces the number of operations necessary for the synthesis as well as losses, which occur during deprotection and neutralization of the crude product mixture prior to the HPLC purification. The synthesis was performed automatically with a single-use cassette on an IBA Synthera+ synthesis module. This synthesis method affords the radioligand with a reliable radiochemical yield, high radiochemical purity, and a high molar activity. [^{18}F]MK-6240 synthesized with this method has been regularly (n > 60) used in our ongoing human and animal PET imaging studies.

KEYWORDS
Alzheimer disease, MK-6240, NFTs, tau

1 | INTRODUCTION

The development of a PET radiopharmaceutical with high affinity and selectivity for the in vivo quantification of neurofibrillary tangles (NFTs) is a critical challenge for AD imaging research. It is likely that an imaging agent able to accurately quantifying tau protein will allow a more precise AD staging and selection of participants with the highest probability of progression for clinical trials designed to test emerging therapeutic interventions.[1] In recent years, three classes of tau tracers have been investigated as candidates to measure NFTs in the living human brain. They are derivative of pyrido-indole (^{18}F-flortaucipir, AV-1451),[2,3] arylquinoline ([^{18}F]THK series),[4-6] and phenyl/pyridinyl-butadienyl-benzothiazoles/benzothiazolium ([^{11}C]PBB3).[7] These tracers have shown high affinity to NFTs; however, evidence from numerous studies has suggested that off-target binding significantly influences their signal. For instance, in a recent in vivo study, we have shown that the binding of [^{18}F]THK5351 within several cortical regions is strongly driven by MAO-B.[8] Similarly, in vitro evidence suggests that MAO-A may influence the signal of ^{18}F-

flortaucipir.[9] In addition, most of these tracers have shown high uptake in the striatum, which is not a region where histopathological studies indicate a high density of tangles in AD.[10] Therefore, an NFT imaging agent with low off-target uptake remains an important need to the AD research field.

[^{18}F]MK-6240, a novel pyrrolopyridinyl isoquinoline amine derivative, has shown high affinity to NFTs, reduced off-target binding, fast brain penetration and kinetics, and absence of brain-permeable metabolites.[9] Therefore, [^{18}F]MK-6240 has emerged as a promising new generation tau-imaging agent for the quantification of NFTs load in the human brain. Two synthesis methods have been published recently for the preparation of [^{18}F]MK-6240 use either microwave-assisted heating[11] or standard thermal heating[12] with potassium cryptand [^{18}F] fluoride (K[^{18}F]F-K_{222} complex) as the fluorination agent followed by a Boc-deprotection step using TFA or other strong acids to yield the final product.

In the original description of the synthesis of [^{18}F]MK-6240, although the radiochemical yield was not reported, it was sufficient for the investigators to scan rhesus monkeys.[11] Using similar chemistry, Collier et al reported an automated synthesis compliant with current good manufacturing practices with a final radiochemical yield of 7.5 ± 1.9% (n = 3) and molar activity of 6.0 ± 1.8 Ci/μmol.[12] Using methodology equivalent to these syntheses, we were not able to achieve a radiochemical yield of 2.6 GBq or greater, which we require to perform scans of multiple (4-5) human subjects using [^{18}F]MK-6240 from a single production batch at our facility. Despite considerable effort to implement the synthesis at our facility using potassium cryptand [^{18}F] fluoride with an acid deprotection step, we were not able to reproduce the yield reported by Collier et al. Under these conditions, we observed significant [^{18}F]fluoride incorporation (10%-20% as monitored by r-TLC); however, the isolated yield we obtained was not satisfactory, suggesting that losses from the final deprotection and purification were significant. By utilizing thermal deprotection of the Boc protection groups with a different phase-transfer catalyst/base combination, we have been able to achieve final radiochemical yields of 3.0 to 4.4 GBq.

Herein, we report the cGMP compliant automated radiochemical synthesis of [^{18}F]MK-6240 with complete quality control specifications. This simplified synthesis uses di-Boc-protected nitro precursor with tetraethylammonium hydrogen carbonate (TEA HCO_3) as the fluorination complexing agent. Inspired by the concise radiosynthesis of ^{18}F-flortaucipir (AV1451, T807) described by Shoup et al,[13] we sought to similarly perform [^{18}F] fluoride incorporation concurrent to Boc deprotection by using an elevated reaction temperature under mildly basic conditions. By utilizing thermal deprotection, the final product was obtained in one step without the addition of a deprotection agent, streamlining the automation of the synthesis. This method offers a simpler alternative to the originally published method, that at our facility provided higher and more consistent yields of [^{18}F]MK-6240.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The standard compound 6-(fluoro)-3-(1*H*-pyrrolo[2,3-c]pyridin-1-yl)isoquinolin-5-amine ([^{19}F]MK-6240) and the precursor *N*-[(tert-butoxy)carbonyl]-*N*-(6-nitro-3-[1*H*-pyrrolo[2,3-c]pyridin-1-yl]isoquinolin-5-yl) carbamate (**1**) were prepared by Merck Research Laboratories. 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]MK-6240

[^{18}F] Fluoride was produced via the ^{18}O(p,n)^{18}F reaction in a Nitra XL liquid target in an IBA 18/9 Cyclone Cyclotron. The target containing ^{18}O-water was irradiated for 45 minutes with a 60 μA beam of 18 MeV protons, to produce up to 130 GBq of [^{18}F] fluoride. At the end of irradiation, the [^{18}F] fluoride was transferred with helium gas to an IBA Synthera+ synthesis module. The synthesis was performed automatically using the following operations sequentially with numerical references to the schematic diagram in Figure 1:

1. [^{18}F] fluoride was delivered through V05 to a QMA solid-phase extraction (SPE) ion exchange cartridge (Waters; conditioned with 10 mL of H_2O for irrigation), separating it from the target water that was collected in the recovery vial for recycling.
2. The [^{18}F] fluoride was eluted from the cartridge using 1.5 mL of a solution (15 mg TEA HCO_3, 150 μL H_2O for irrigation, and 1.35 mL acetonitrile), through V06 into the reaction vial.
3. The solution was then evaporated to dryness with acetonitrile at a temperature of 95°C, a stream of nitrogen, and reduced pressure provided by the vacuum.
4. After 15 minutes, the reactor was cooled, and a solution of 1 mg of MK-6240 precursor (**1**) in 1 mL DMSO was added to the reactor and was heated stepwise: 90°C, 110°C, and 120°C for 3 minutes each step and

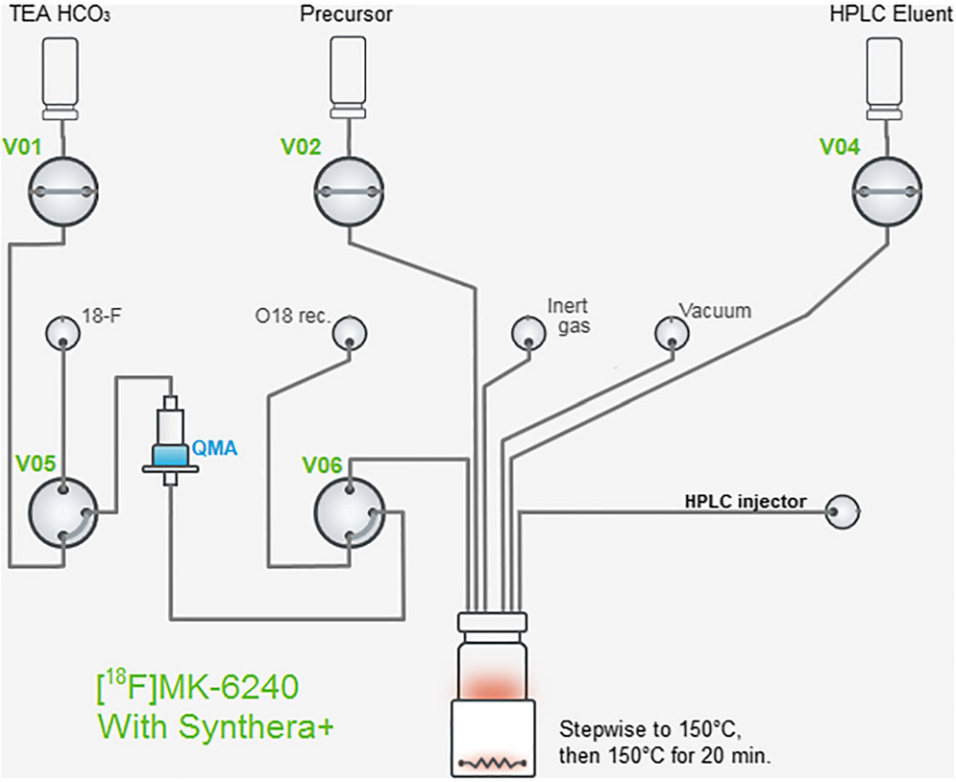
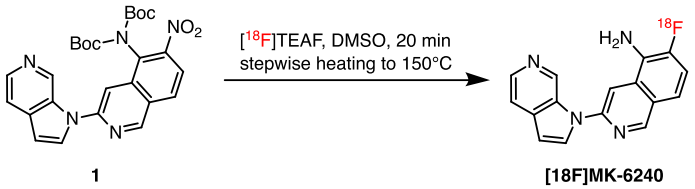


FIGURE 1 Schematic diagram of the automatic synthesis module for the synthesis of [^{18}F]MK-6240



SCHEME 1 Concerted [^{18}F]fluorination/deprotection radiosynthesis of [^{18}F]MK-6240

finally at 150°C for 20 minutes (Scheme 1). Both Boc groups are cleaved during heating, being fully removed after 20 minutes, providing the final product [^{18}F]MK-6240.

5. The reactor was then cooled to 70°C, and 1.5-mL HPLC eluent (20mM sodium phosphate/CH_3CN, 78/22) was added.
6. This mixture was transferred into the injector loop of the HPLC system and was purified on a Luna 10 μm C18 250 × 10 mm HPLC column (Phenomenex Inc) with a flow of 5 mL/min of the HPLC eluent (20mM sodium phosphate/CH_3CN, 78/22). The desired product eluted at a retention time of 22 to 24 minutes. Under these conditions, the precursor (**1**) has a retention time of 37 minutes, although none was detected. Unlabeled precursor that is deprotected has a retention time greater than 60 minutes and is eluted during postsynthesis column cleaning.
7. The product peak was collected in a 30-mL syringe containing 15 mL of water and 25 μL of ascorbic acid solution (25%; Sandoz). This solution was passed through a Sep-Pak Light C18 cartridge (Waters) and the [^{18}F]MK-6240 that was retained on the C18 cartridge was washed with additional 10 mL of water (Figure 2).
8. The product was then eluted from the cartridge through a 0.20-μm Millex - LG sterile filter (EMD Millipore) into the final product vial with 0.5 mL of USP ethanol followed by 9.5 mL of USP sodium chloride for injection (0.9%) containing 25 μL of ascorbic acid solution (25%; Sandoz) to yield [^{18}F]MK-6240 formulated for human injection.
9. This solution was subjected to quality control analysis to determine the radiochemical purity and identity, half-life, pH, chemical purity, residual solvents, and molar activity of the final tracer formulation (see Table 2).

The radiochemical identity and purity of the final [^{18}F]MK-6240 was determined using an Agilent 1200 series HPLC equipped with an in-line UV and radioactive detector (Raytest). The HPLC system consisted of an analytical HPLC column: Prodigy 5 μm ODS-3 100 Å

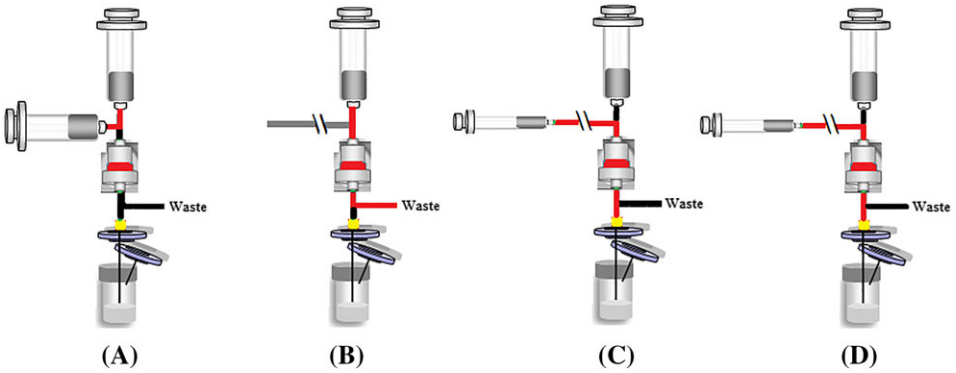


FIGURE 2 A, Thirty-milliliter syringe containing the diluted tracer HPLC fraction was connected to the side arm of the upper stopcock and aspirated by the syringe driver (Scintomics) at a rate of 100 mL/min. B, The upper stopcock was rotated so that the syringe driver dispenses the solution through the C18 cartridge to waste at a rate of 25 mL/min. C, A syringe containing 10 mL of water for injection was connected to the side arm of the upper stopcock using sterile extension tubing (B. Braun) and the upper stopcock was rotated back so that the C18 cartridge was washed with this syringe outside of the hotcell in order to reduce exposure to the operator. D, The lower stopcock was rotated towards the product vial, and 0.5 mL of EtOH was forced by syringe through the extension tubing, eluting the [^{18}F]MK-6240 through the sterile filter into the product vial; 9.5 mL of USP sodium chloride solution for injection (0.9%) containing 25 μL of ascorbic acid solution (25%, Sandoz) was then forced by syringe through the extension tubing and sterile filter, yielding the final formulated product

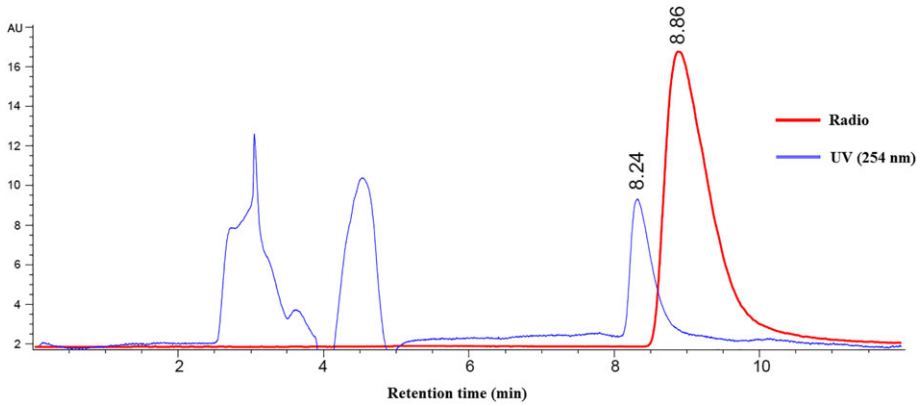


FIGURE 3 HPLC radio (red line) and UV (blue line) QC analysis of formulated [^{18}F]MK-6240 (0.6-min calibrated delay between UV detector and radioactivity detector). The radioactive [^{18}F]MK-6240 peak (RT = 8.86 min) corresponds to the retention time of MK-6240 standard (RT = 8.0-9.5 min). The absorbance peaks near the void volume are due to absorbance from the formulation matrix

TABLE 1 Labeling yields using thermal deprotection at 150°C for 20 minutes

Phase Transfer Catalyst/Base System	Quantity	% Isolated Yield Decay Corrected	% Isolated Yield End of Synthesis
K_{222}/K_2CO_3	10 mg/0.125M	0.9	0.5
$(CH_3CH_2CH_2CH_2)_4N(HCO_3)$	0.075M	1.9	1.0
$(CH_3CH_2)_4N(HCO_3)$	0.075M	9.8 ± 1.8 (n = 3)	5.2 ± 1.0 (n = 3)

column 250 × 4.6 mm (Phenomenex Inc), HPLC solvent: 40% acetonitrile, 60% aqueous 20mM sodium phosphate buffer, UV detector: wavelength 254 nm, and a flow rate of 0.7 mL/min. The retention time of [^{18}F]MK-6240 was between 8.0 to 9.5 minutes (Figure 3). A Perkin-Elmer GC 480 was used to determine the residual solvent content in the final product solution.

3 | RESULTS AND DISCUSSION

The previously described methods of the synthesis of [^{18}F]MK-6240 have used potassium carbonate with kryptofix to form [^{18}F] fluoride (K[^{18}F]/K_{222}) for ^{18}F incorporation followed by acid deprotection. Walji et al reported using microwave heating, which is not a

TABLE 2 Quality control acceptance criteria for [^{18}F]MK-6240

Test	Acceptance Criteria	[^{18}F]MK-6240 (n = 3)
Radiochemical yield	Report result at the end of synthesis	DC = 9.8% ± 1.8% EOS = 5.2% ± 1.0%
Product description	Clear, colorless, or slightly yellow solution. No foreign particulate matter	Pass
Radionuclidic identity	Half-life = 105-115 min	110.1 ± 1 min
Radiochemical purity	> 95%	99.1% ± 0.3%
Radiochemical identity	Retention time = 8-9.5 min	8.6 ± 0.4 min
pH	pH 4.5-7.5	6.5
Chemical purity	TEA ≤ 100 μg/mL	Pass
Residual solvents	Acetonitrile ≤ 400 ppm Ethanol ≤ 50 000 ppm DMSO ≤ 5000 ppm	Not detected 37 400 ± 400 ppm Not detected
Filter integrity test	≥ 50 psi	Pass
Molar activity	Report result	912 ± 322 GBq/μmol (24.7 ± 8.7 mCi/μmol)
Sterility	No growth after 14 d	Sterile
Bacterial endotoxins	≤17.5 EU/mL	<5 EU/mL

Abbreviations: DC, decay corrected; DMSO, dimethyl sulfoxide; EOS, end of synthesis; EU, endotoxin unit; ppm, parts per million; ppt, parts per thousand; psi, pounds per square inch; TEA, tetraethylammonium.

standard feature on most synthesis modules.[11] Adapting this methodology in our laboratory, we were not able to produce a sufficient quantity of [^{18}F]MK-6240 to scan multiple human subjects (2.6 GBq or greater). Although Collier et al reported a RCY of 7.5 ± 1.9% (n = 3) with molar activity of 6.0 ± 1.8 Ci/μmol,[12] we were not able to achieve commensurate yields at our facility using potassium cryptand [^{18}F] fluoride with an acid deprotection step. Although [^{18}F] incorporation was fairly efficient (10%-20% as monitored by r-TLC), losses from hydrolysis and the need to neutralize the reaction to prevent HPLC column degradation considerably lowered the isolated yield of the final product. The basicity of these conditions, as well as inconsistent hydrolysis, may contribute to the inconsistency of these yields.

The radiosynthesis of [^{18}F]MK-6240 described herein was automated using an IBA Synthera+ synthesis module, and the product was obtained in one step, simplifying the automation of the synthesis by eliminating this final deprotection step. By utilizing TEA HCO_3 as the [^{18}F] fluoride eluent with a less basic bicarbonate counter-ion compared with carbonate, this methodology produced better and more consistent yields in our laboratory. Thermal deprotection of the product was achieved using K_{222}/K_2CO_3 without acid-catalyzed hydrolysis; however, the reaction afforded an insufficient isolated yield (Table 1). As we have previously described in our syntheses of [^{18}F] ABIDO[14] and [^{18}F] ODIBO,[15] the strongly basic carbonate anion apparently facilitates the decomposition of the labeling precursor and/or the ^{18}F-labeled product. Of the tetraalkylammonium cations we used with a bicarbonate counter anion, tetraethylammonium bicarbonate provided the highest yield (Table 1). Under these milder basic conditions, use of higher reaction temperatures permitted ^{18}F-fluorination concurrent to the removal of the bis *t*-Boc aniline protection, further improving the isolated yield. Without the addition of acid for deprotection or acetonitrile for azeotropic drying of the [^{18}F] fluoride, synthesis preparation for routine production is simplified, requiring less input vials for reagent additions.

3.1 | Quality control and validation for human use

The [^{18}F]MK-6240 was subjected to multiple quality control procedures as shown in Table 2 to confirm compliance with GMP specifications, which were established with guidance from the USP General Chapter <823>. After the final product sterile filtration, the final solution was examined visually to confirm it was a clear colorless liquid free of particulates. The radionuclidic purity was verified by measuring the half-life with a calibrated Capintec CRC-15R well counter. The formulated product was sterile, free of pyrogens, and pH was determined to be within range (4.5-7.5) using pH strips. TEA was

determined to be below the limit of 100 μg/mL by TLC developed in an iodine chamber in comparison with a TEA standard. The residual solvents were analyzed using a Perkin Elmer Clarus 480 gas chromatograph calibrated with external standards, using a Restek MTX-Wax column (30 m, ID 0.53 mm). The oven temperature was ramped from 35°C at 35°C/min to 130°C and held for 8 minutes. The radiochemical identity and purity were determined using an Agilent HPLC system equipped with UV and Raytest radioactivity detectors (Figure 3). The acceptance criteria for the final product solution are presented in Table 2.

4 | CONCLUSION

The methodology described herein uses di-Boc-protected nitro precursor with TEA HCO_3 as the fluorination complexing agent and performs [^{18}F] fluoride incorporation concurrent to Boc deprotection by using an elevated reaction temperature under mildly basic conditions. This simplified method is easily automated on most radiosynthesis modules, allowing for a streamlined cGMP compliant synthesis of the radiopharmaceutical [^{18}F]MK-6240 for the imaging of NFTs in humans.

CONFLICT OF INTEREST

The precursor and reference standard for [^{18}F]MK-6240 were made available to us without charge by Merck & Co, Inc (2000 Galloping Hill Road, Kenilworth, New Jersey 07033, USA).

ORCID

Robert Hopewell https://orcid.org/0000-0002-2478-5872
Alexey Kostikov https://orcid.org/0000-0003-1646-761X

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