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NOTE

A simplified protocol for the automated production of 2-[^{18}F]fluoro-3-[2-((S)-3-pyrrolinyl)methoxy]pyridine ([^{18}F]nifene) on an IBA Synthera® module

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The α4β2 nicotinic acetylcholine receptor (nAChR) ligand 2-[^{18}F]fluoro-3-[2-((S)-3-pyrrolinyl)methoxy]pyridine ([^{18}F]nifene) has been synthesized in 10% decay-corrected radiochemical yield using the IBA Synthera® platform (IBA Cyclotron Solutions, Louvain-la-Neuve, Belgium) with an integrated fluidic processor (IFP). Boc-nitronifene served as the precursor, and 20% trifluoroacetic acid (TFA) was used to deprotect the Boc-group after radiolabeling. By omitting the solvent extraction step after radiolabeling, the process was simplified to a single step with no manual intervention. [^{18}F]Nifene was obtained in decay-corrected radiochemical yields of 10 ± 2% ($n = 20$) and radiochemical purity >99%. Typical specific radioactivities of 2700–4865 mCi/μmole (100–180 GBq/μmol) were measured at the end of synthesis; total synthesis times were about 1 h 40 min.

KEYWORDS
IBA Synthera®, radiosynthesis, α4β2 nicotinic acetylcholine receptor (nAChR) ligand, [^{18}F]nifene

1 | INTRODUCTION

The α4β2 nicotinic acetylcholine receptor (nAChR) is a heteromeric neuronal-subtype receptor that has been correlated with a diverse array of neurological disorders that include Alzheimer's disease, Parkinson's disease, schizophrenia, and substance abuse.[1–5] Consequently, considerable effort has gone into the development and optimization of clinical imaging methods that map in vivo α4β2 nicotinic receptor expression, especially via positron emission tomography (PET) and single-photon emission computed tomography (SPECT).[6–8] To this end, a number of nAChR agonist-based imaging agents have been synthesized and evaluated, though systemic toxicity issues and slow binding kinetics have precluded most for clinical application.[8,9] Of the most efficiently targeted and best tolerated α4β2 nAChR agonists studied to date, two stand out: [^{18}F]2-FA85380 ([^{18}F]2-FA) and [^{123}I]5-IA85380 ([^{123}I]5-IA). Unfortunately, the kinetics of both radiotracers require prolonged imaging times for quantitation.[10] To reduce the imaging time needed for quantitation, and further characterize the binding kinetics and affinities of α4β2 nAChR agonists, we focused on the previously developed PET radiotracer [2-[^{18}F]fluoro-3-[2-((S)-3-pyrrolinyl)methoxy]pyridine ([^{18}F]nifene), an α4β2 nAChR agonist with more rapid

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kinetics than 2-FA and 5-IA, for potential nAChR α4β2 receptor imaging.[11] Nifene has previously been used to assess the efficacy of acetylcholinesterase inhibitors in small animal models because of its competition with the neurotransmitter acetylcholine in binding to nicotinic receptors.[12,13] Nifene studies with animal models of lung cancer have demonstrated upregulation of nicotinic receptors in the lung tumors.[14,15] Human studies with [^{18}F]nifene have shown it a promising nicotinic α4β2 receptor PET radiotracer for clinical research, with reliable distribution volume ratio test-retest reproducibility.[16] Human white matter thalamic radiations (tracts) have also shown good demarcation and quantification using [^{18}F]nifene.[17]

Our previously developed synthesis procedure of [^{18}F]nifene was tedious and involved manual intervention, with radiosynthesis conducted entirely in the CPCU box. After extensive drying of the [^{18}F]fluoride, the nitro precursor 2-nitro-3-[2-((S)-N-tert-butoxycarbonyl-3-pyrroline)methoxy]pyridine (dissolved in anhydrous dimethylsulfoxide–acetonitrile, 2:3 ratio) was added, and the mixture was heated at 126°C for 30 min. The contents of the reaction vessel were then extracted by the addition of methylene chloride (CH_2Cl_2). The CH_2Cl_2 extract was passed through neutral alumina to provide the intermediate N-Boc-[^{18}F]nifene. CH_2Cl_2 from the intermediate was then removed in vacuo, and the residue was dissolved in a mixture of CH_2Cl_2 and trifluoroacetic acid (TFA) (CF_3COOH) (5:1). The mixture was heated at 80°C for 30 min and dehydrated. The acidic residue was neutralized with 10% sodium bicarbonate ($NaHCO_3$) to pH 7.0 and added to the HPLC mobile-phase buffer. Reverse-phase HPLC purification was then conducted using an Alltech C18 column via mobile phase (60% acetonitrile, 40% 0.1 M ammonium formate) and vacuum dried to derive the product [^{18}F]nifene. The final formulation was obtained by diluting the collected fraction with 0.9% saline, followed by sterile filtration. The radiolabeled product [^{18}F]nifene (10–20 mCi) was thus obtained with >99% purity.

As the procedure involved manual intervention, it mandated time-consuming manipulations as well as low initial activities to reduce radiation exposure to lower levels, a constraint that frequently resulted in low final product concentration and specific activity. To circumvent these issues and enhance [^{18}F]nifene radiosynthesis efficiency, we evaluated a new precursor, trimethylamino triflate (tmat) derivative, 2-(trimethylamino)-3-[2-((S)-N-tert-butoxycarbonyl-3-pyrrolinyl)methoxy]pyridine triflate 1 (tmat-Nifene), which afforded us the ability to exclude HPLC purification.[18] In the current work we report the fully automated synthesis of 2[^{18}F]fluoro-3-[2-((S)-3-pyrrolinyl)methoxy]pyridine ([^{18}F]nifene) without any manual intervention during synthesis, for use as a high affinity, α4β2 receptor binding contrast agent for PET.[19] The fully automated synthesis obviates all concerns of radiation exposure and readily enables the use of approximately 5.0 Ci starting activities to yield approximately 500 mCi in 10 mL; suitable for fractionation and injection.

2 | EXPERIMENTAL

2.1 | General

18 MeV protons from an IBA Cyclone® 18/9 cyclotron (Ion Beam Applications, Louvain-la-Neuve, Belgium) generated carrier-free [^{18}F]fluoride anions via ^{18}O(p, n)^{18}F nuclear reaction within a niobium target chamber bearing isotopically-enriched [^{18}O]H_2O (Medical Isotopes Inc.) at the University of Chicago's Cyclotron Facility. The target's entrance window was made of Havar and possessed an aluminum disk, not in contact with the enriched water, for use as an energy degrader—to slightly lower the beam's energy and better match the production cross-section of [^{18}F]. Typical irradiation parameters were 20–60 μA of protons for 30–120 min. Yields of [^{18}F] were roughly linear with beam current, up to >5 Ci for a 2-h irradiation. The [^{18}F]-fluoride, as hydrogen fluoride (HF), was transferred from the target to the designated synthesis box using helium gas (ultra high purity [UHP] grade, 99.999%).

A Synthera® V2 synthesis module with HPLC capabilities, also from IBA, was used for synthesis. The Synthera unit also possessed a Knaur Azura UVD 2.1 UV detector system. Nucleophilic integrated fluidic processor (IFP) kits were purchased from ABX (K-2751SYN; Advanced Biochemical Compounds, Germany) and used without any modification. A Waters preparative HPLC column Symmetry Prep C18, 7 μm, 19 × 300 mm, was used to purify the crude radiolabeled product. An in-house manufactured work-up frame was used to reconstitute the radiolabeled compound into injectable dose form using solid-phase extraction cartridges.

Solvents were purchased from Fisher Scientific and used as received. Reagents were purchased from ABX and Sigma-Aldrich and used without further purification. The Boc-Nitronifene precursor (2-Nitro-3-[2-((S)-N-tert-butoxycarbonyl-3-pyrroline)methoxy]pyridine) was purchased from the American Biochemicals (College Station, Texas, USA), a custom synthesis company for small molecule intermediates.

For quality control, an Agilent Infinity 1260 HPLC system, equipped with a vial sampler (G7129A, Agilent Infinity II series), 1260 VWD detector (G7114A, Agilent Infinity II series), and a 1260 Isocratic Pump (G7110B,

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Agilent Infinity II series), was used. The system was operated by Laura radiochromatography-HPLC software (LabLogic Systems, Ltd., UK). The stationary phase analytical column was a Phenomenex Luna C-18(2) 100 A (00G-4252-E0), 5 μm RP column, 250 × 4.6 mm. A mixture of 20% acetonitrile in 0.1 M ammonium formate was used as the mobile phase, and an isocratic elution technique was employed for analysis within 0–15 min of HPLC commencement. Specific radioactivity was calculated by HPLC using a standard mass curve of known concentrations of [^{19}F]nifene.

2.2 | Radiosynthesis

The IFP, designed for the nucleophilic reactions on IBA Synthera® V2 modules, was prepared using the following vials:

1. Vial 1: Eluent (15 mg Kryptofix 2.2.2 (40 μmol) and 1.4 mg K_2CO_3 (10 μmol)) in 0.8 mL of acetonitrile plus 0.2 mL of water
2. Vial 2: 2 mg Boc-nitronifene (6.22 μmol) in 1 mL dry acetonitrile (MeCN)
3. Vial 3: 0.2 mL TFA (66 μmol) in 0.8 mL methylene chloride
4. Vial 4: 5 mL of 20% acetonitrile in 0.1 M ammonium formate.

The HPLC was primed and conditioned using the developed HPLC run sequence with 20% acetonitrile in 0.1 M ammonium formate at a flow rate of 6.5 mL/min. After loading the IFP, equipped with the requisite vials listed above, synthesis was performed using a programmed procedure on the Synthera® V2. [^{18}F]Fluoride from the target was trapped on a QMA ion exchange cartridge (Waters Corporation, Milford, MA, USA) and eluted using eluent from Vial 1. The Kryptofix complex was dried at 110°C for 5 min and 95°C for 3 min under vacuum and helium flow. After drying, the precursor was added from Vial 2 and labeling was achieved by heating the mixture to 130°C for 15 min. The reaction mixture was then evaporated to dryness at 80°C followed by the addition of the acid solution from Vial 3 and hydrolysis at 80°C for 5 min. The reaction was then diluted with 5 mL of HPLC mobile phase, for loading into the HPLC injection loop and injection into the preparative HPLC column for purification. A radioactive sample at peak elution time (11–12 min), was collected into the dilution vial of an in-house manufactured reformulation module, then diluted in 80 mL of HPLC-grade water basified with 3 mL of 0.5 M NaOH and subsequently trapped on a Chromafix PS-RP solid phase extraction cartridge (Macherey-Nagel GmbH &Co. KG, Duren, Germany). After washing the cartridge with 4 mL of HPLC-grade water and drying in nitrogen, [^{18}F]nifene was eluted with 1 mL of ethanol followed by 9 mL of 0.9% NaCl, into the final product vial. The product was then filter sterilized via a polyvinylidene fluoride (PVDF) membrane filter (0.22 mm). [^{18}F]Nifene was thereby obtained for decay-corrected radiochemical yields of 10 ± 2% ($n = 20$), and radiochemical purity of >99%. The specific radioactivity of [^{18}F]nifene was measured to be 2700–4865 mCi/μmole (100–180 GBq/μmol), with a total synthesis time of about 1 h 40 min.

3 | RESULTS AND DISCUSSION

The radiolabeling of [^{18}F]nifene was achieved by reacting the 2-nitro-3-[2-((S)-N-tert-butoxycarbonyl-3-pyrroline) methoxy]pyridine precursor with dried [^{18}F]KF kryptofix 2.2.2 complex in MeCN at 130°C for 15 min. After drying the solvents by heating at 80°C and simultaneous helium flow, 20% TFA in dichloromethane was added and the mixture was heated to 80°C for 5 min. This achieved deprotection of the tert-butoxycarbolyl group on the pyrroline nitrogen. The resulting crude reaction was diluted with HPLC mobile phase (20% MeCN in 0.1 M ammonium formate) and injected into the loop of the preparative HPLC column. The crude reaction mixture was purified by the HPLC system with a Waters Symmetry Prep C18, 7 μm, 19 × 300 mm column and a constant eluent flow at a speed of 6.5 mL/min. The [^{18}F]nifene peak was collected at approximately 11–14 min (Figure 1), transferred to the work-up frame where it was diluted in 77 mL of (Milli-Q) water plus 3 mL of 0.5 M NaOH and subsequently trapped on the preconditioned Chromafix PS-RP solid phase extraction cartridge and reconstituted in 10% EtOH in 0.9% saline for animal studies. Radio and UV-HPLC spiked with cold ^{19}Fnifene reference standard are shown in Figure 2. In a typical 1 h 40 min production run, the non-decay corrected yield was 210 mCi (4.5%) and the decay corrected yield of the entire process was 9.0% (decay corrected 423 mCi of [^{18}F] nifene from a 4700 mCi [^{18}F] starting activity), with >99% radiochemical purity (Table 1).

The shorter synthesis time was achieved because this method does not require solvent extraction of the intermediate reaction mixture before the removal of the Boc protection by TFA. From our experience with modified nucleophilic synthesizers, we believe that hydrolysis takes place at the end of the acetonitrile evaporation where the amount of acetonitrile is <5%. It is also important to note that with the exception of the [^{18}F]KF Kryptofix 2.2.2 complex, evaporation to dryness is not

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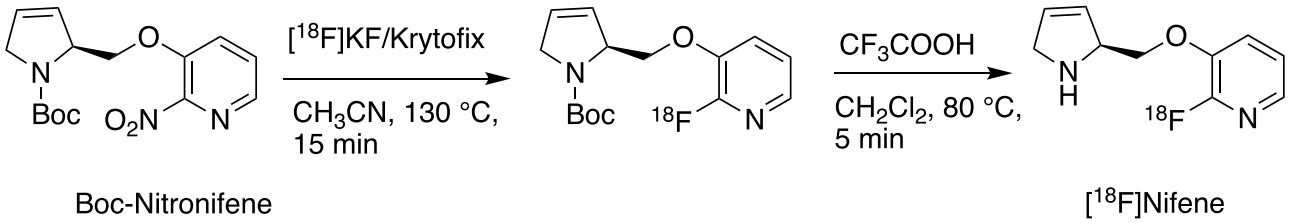

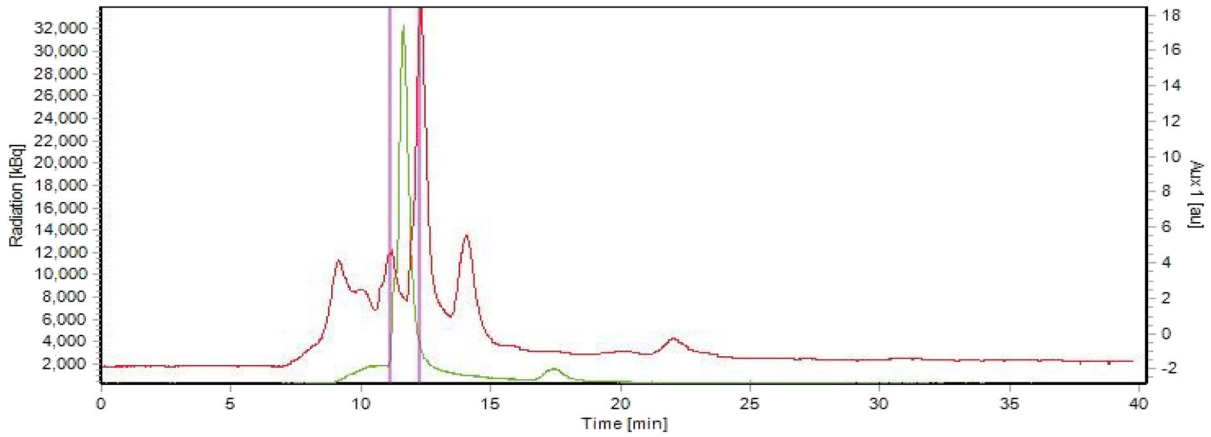

FIGURE 1 Synthesis scheme (top) and Synthera® preparative HPLC purification profile (bottom) of [^{18}F]nifene. The chromatogram in green is radioactive and the red is UV. The purple lines indicate the desired peak collection times of the [^{18}F]nifene product.

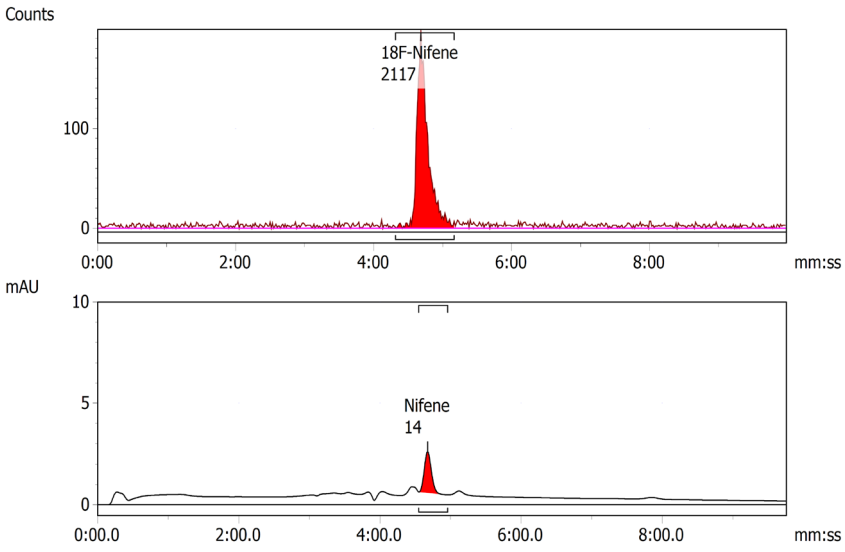

FIGURE 2 Radio and UV-HPLC spiked with cold [^{19}F]nifene reference standard.

achieved at any point of the previous synthesis protocol because of the presence of high boiling DMSO. So, we replaced DMSO with extra dry acetonitrile. Because of the protocol modifications described here, it has been possible to perform the [^{18}F]nifene synthesis using an unmodified nucleophilic IFP (typically used for fluorothymidine production) with only four reagent vials. The nucleophilic IFP is a disposable kit that clips onto the Synthera module and can be used to perform nucleophilic substitution reactions followed by base or acid hydrolysis. This kit is commonly used for the synthesis of [^{18}F]FLT, [^{18}F]FAZA, or [^{18}F]FMISO. The IFP can be ejected at the end of synthesis because all radioactivity is contained within the fluidic processor. The HPLC purification time was reduced by approximately 10 min using the optimized mobile phase and flowrate.

In summary, the two-step (radiolabeling and deprotection) reaction process was simplified to one step, which significantly reduced the time of synthesis and radiation exposure. The synthesis process needed only

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TABLE 1 Summary of key findings from the technique-development runs.

Run #	Starting activity (mCi)	Yield (mCi)	Synthesis time (min)	Decay-corrected yield (mCi)	Decay-corrected yield (%)	Radiochemical purity (%)	Specific activity (mCi/μmole)
1	3920	97.0	100	182	4.65	>99	2678
2	4300	58.0	100	109	2.54	>99	4003
3	4400	124	100	233	5.30	>99	1597
4	3500	78.0	112	158	4.52	>99	2319
5	3800	165	112	335	8.81	>99	3065
6	4600	137	102	261	5.67	>99	18908
7	4900	117	98	217	4.43	>99	5797
8	4100	100	125	220	5.37	>99	1111
9	4900	355	100	668	13.6	>99	4397
10	5200	300	106	586	11.3	>99	3295
11	3900	112	120	239	6.12	>99	4327
12	1500	34.6	98	64.3	4.28	>99	667.6
13	4700	104	118	219	4.66	>99	802.8
14	4700	210	112	426	9.07	>99	13524
Ave	4173	142	107	280	6.45	>99	4749
SD	911	90.0	9.10	172	3.10	0	5189

four reagent vials including the [^{18}F]-eluant and precursor. Dose formulation was done remotely in series and there was no manual intervention in the entire synthesis process. With this fully automated procedure, it is possible to produce high concentration doses with the desired specific activity. The overall synthesis plus dose preparation time is reduced from the previous 2 h 30 min to 1 h 40 min. Though the yield percent is relatively lower than our previous method, completely remote operation in the highly shielded radiosynthesis hot cells can afford us to use high starting activity (~5 Ci) and end up producing activity with the required concentration (~50 mCi/mL) and specific activity for in-vivo imaging studies. Based upon these improvements in synthesis, lower starting activities can also be used, making this automated procedure especially useful when access to an on-site cyclotron is not available.

4 | CONCLUSION

Using the IBA Synthera® modules as well as a modified synthesis procedure, the entire synthesis was carried out inside the Comecer® hot cell with no manual intervention. The decay-corrected radiochemical yields of [^{18}F]nifene have been 10 ± 2%; however, the synthesis time has been shortened greatly and radiation exposure minimized considerably. This setup is now routinely used in our laboratory for the [^{18}F]nifene production. This simplified protocol can potentially be implemented in other automated synthesis modules.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data associated with the findings of this study are all included in the article.

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