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RESEARCH ARTICLE

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Development of a novel [^{18}F]fluorobenzyl derivative of the AT_1 receptor antagonist Candesartan

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Candesartan is a clinically approved angiotensin II type 1 receptor (AT_1R)-blocker that selectively binds AT_1Rs in high affinity. We report here the radiosynthesis and automation of the novel [^{18}F]fluorobenzyl derivative of Candesartan using the Sonogashira cross-coupling reaction. [^{18}F]Fluorobenzyl-Candesartan ([^{18}F]**7**) was developed from 4-[^{18}F]fluoroiodobenzene ([^{18}F]FIB) that was conjugated with alkyne-trityl-candesartan with the assistance of a Pd $(PPh_3)_4$/CuI catalyst followed by acid deprotection. The three-step two-reactor 2-HPLC purification process was automated resulting in >90% pure [^{18}F]**7** in a RCY of 4.6 ± 1.1% (decay corrected from EOB) and molar activities of 1,406-5,513 GBq/mmol. [^{18}F]FIB was reproducibly obtained by direct radiofluorination of the mono-iodinated triphenylsulfonium salt in the presence of K222/K_2CO_3 in an ~30% yield (decay-corrected). [^{18}F]**7** was stable (>97%) up to 4 h in solution and up to 1 h in rat plasma at 37°C. However, the use of Sonogashira cross-coupling reaction to produce [^{18}F]**7** in high yields and molar activities was found to be challenging for routine use in radiochemistry labs.

KEYWORDS
[^{18}]FIB prosthetic group, automated synthesis, Pd-catalyzed reaction, PET imaging, Sonogashira cross-coupling reaction

1 | INTRODUCTION

The renin-angiotensin system (RAS) is part of the regulatory system of cardiovascular and renal functions.[1] Alterations of RAS are characterized by potent antiatherosclerotic effects, which are mediated by their antihypertensive, anti-inflammatory, antiproliferative, and oxidative stress lowering properties.[2] Among other participants in RAS regulation, angiotensin (Ang) II plays a central role in the regulation of blood pressure. It acts mainly by stimulating the Ang II Type 1 receptors (AT_1Rs) that are expressed in the kidneys, adrenal glands, heart, brain, and vasculature.[3] Alterations of the expression levels of AT_1R have been linked to cardiac and renal diseases, such as cardiac and renal failures, hypertension, and some type of cancers.[4,5] Inhibitors of RAS, such as the angiotensin converting enzyme inhibitors and AT_1R blockers, are well-established treatments for hypertensive target organ damage, and progressive renal and cardiac diseases.[2]

Candesartan is an antagonist that binds selectively to AT_1Rs, giving a long-lasting blockage and suppression of Ang II response. It has high binding affinity (IC_{50} 0.6 nM) compared with the selective AT_1R antagonist Losartan (IC_{50} 20 nM,) and Valsartan (IC_{50} 2.7 nM).[6] Candesartan labeled with ^{125}I at the 22-position of the biphenyl moiety displayed binding to cardiac AT_1Rs with high heart-to-liver ratios, demonstrating its suitability to study AT_1R alterations in cardiovascular disorders.[7] Structure-activity relationship (SAR) studies demonstrated that large prosthetic molecules can be introduced at the 7-position of the benzimidazole ring of

Candesartan without changing its binding properties and pharmacological functions.[8] We have previously radiolabeled Candesartan with carbon-11 by [^{11}C]methylation of the carboxylic acid group, and this radioligand displayed potential for positron emission tomography (PET) imaging of AT_1Rs.[9] However, a high proportion of the microPET signal in the rat kidney corresponded to nonspecific binding from a ^{11}C-labeled hydrophobic metabolite. We hypothesized that an F-18 aromatic-substituted derivative at the same 7-position would provide a suitable radioactive derivative of the high affinity Candesartan for PET imaging. Furthermore, the longer half-life of a F-18 analog of Candesartan would offer some key advantages such as allowing multiple injections per formulation and shipping to sites without cyclotron or radiochemistry capability, as well as superior PET imaging resolution due to low positron energy.[10]

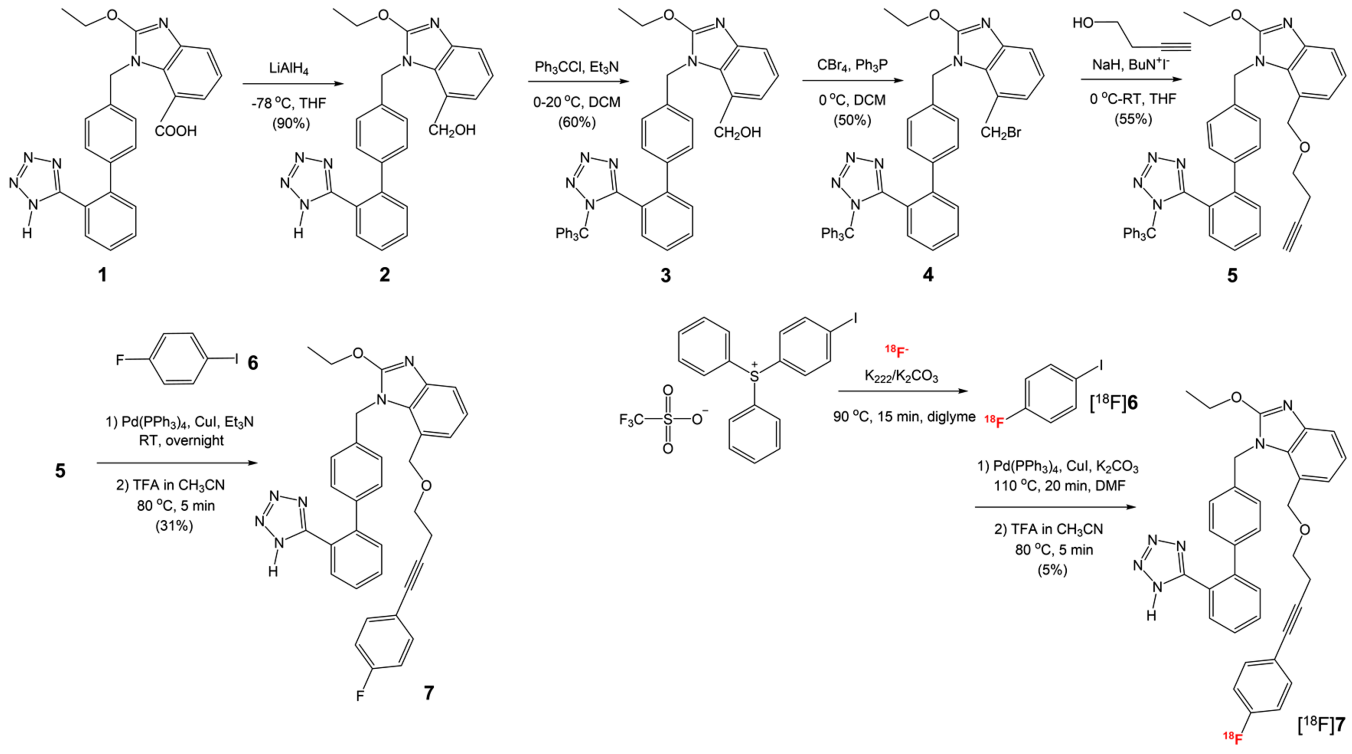
Incorporation of ^{18}F-labeled prosthetic groups onto biological active compound has been accomplished via acylation, amidation, imidation, alkylation, and photochemical conjugation.[11] Recently, our group developed the novel tracer [^{18}F]fluoropyridine-Candesartan labeled with the [^{18}F]FPyKYNE prosthetic via the copper-catalyzed azide-alkyne cycloaddition at the imidazole 7-position in 10% yield, high molar activity, and radiochemical purity (≥97%).[12] Biological studies revealed specific binding of [^{18}F]fluoropyridine-Candesartan in AT_1R-rich tissues such as the kidney cortex. Further studies to assess the full potential of [^{18}F]fluoropyridine-Candesartan are currently underway.

Several chemical approaches using the Pd-mediated cross-coupling reaction were previously investigated in radiochemistry with some favorable outcomes using the Stille,[13,14] Suzuki,[15] Sonogashira,[16,17] and Buchard *N*-arylation reactions.[18] Particularly, the Sonogashira cross-coupling reaction approach was successfully utilized to label alkyne-modified peptides with 4-[^{18}F]fluorohalobenzenes, such as 4-[^{18}F]bromofluorobenzene or 4-[^{18}F]fluoroiodobenzene ([^{18}F]FIB), in high yields and in vivo stability.[19] Recently, [^{18}F]FIB was produced from a commercially available precursor in high yields, relatively facile purification, fast conjugation, and versatile applications.[20] We report here the radiosynthesis and automation of the novel derivative [^{18}F]fluorobenzyl-Candesartan ([^{18}F]**7**) via the Sonogashira cross-coupling reaction with [^{18}F]FIB (Scheme 1) and in vitro plasma stability study to assess enzymatic breakdown.

2 | MATERIAL AND METHODS

2.1 | General

All chemicals, including FIB (**6**) and its triphenylsulfonium triflate precursor (**8**), are commercially available and used without further purification



SCHEME 1 Synthetic route of [^{18}F]**7**

unless stated. Flash chromatography is carried out with silica gel (40–60 μm). ^{1}H- and ^{13}C-NMR spectra are acquired with a Bruker 300 MHz spectrometer at ambient temperature. Spectral data are reported in parts per million (ppm) using residual solvent as a reference. High resolution and accurate mass measurements are acquired in positive mode by flow injection analysis into a Thermo Scientific Q-Exactive Plus Orbitrap Mass Spectrometer (San Jose, CA, USA) interfaced with a heated electrospray ion source. Sep-Pak C18 plus (360 mg, Waters) solid-phase extraction cartridges were preconditioned with 10-ml ethanol followed by 20-ml water. Analytical HPLC was performed on a Phenomenex Luna C18 (2) column (250 × 4.6 mm, 10 μm) with a Waters HPLC composed of 1,515 isocratic pump, 2,487 dual λ absorbance detector, and a Raytest Gabi Star radioactivity detector. Two analytical methods were utilized: Method 1 (2 ml/min, A: water and B: CH_3CN, linear gradient 50% B to 80% during 25 min) and Method 2 (2 ml/min, 55:45 acetonitrile/water 0.1% TFA). Radio-TLC chromatograms (solvent system, EtOAc/Hexanes/MeOH 80:20:5) were acquired with an AR-2000 radio-TLC imaging scanner (Eckert & Ziegler, Germany). Automated radiosyntheses were performed with the Synthra® RNPlus Research (Germany) synthesis module. Semi-preparative HPLC separations were carried out within the radiosynthesizer with a Phenomenex Luna C18 (2) HPLC column (250 × 10 mm, 10 μm) at a flow rate of 8 ml/min (first purification [^{18}F]**6**: 55:45 acetonitrile/water 0.1% TFA and second purification [^{18}F]**7**: 45:55 acetonitrile/water 0.1% TFA) with UV (254 nm) and radiation detection.

2.2 | Chemistry

Compounds (**2**), (**3**), and (**4**) were synthesized as reported previously[21] (see Data S1).

2.2.1 | Tetrazole-protected alkyne Candesartan (7-((but-3-yn-1-yloxy)methyl)-2-ethoxy-1-((2′-(1-trityl-1*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl)methyl)-1*H*-benzo[d]imidazole) (5)

But-3-yn-1-ol (12 mg, 0.18 mmol) was slowly added dropwise to NaH (9.5 mg, 0.39 mmol) and $Bu_4N^+I^-$ (13.2 mg, 0.04 mmol) in 1-ml anhydrous THF under nitrogen and stirred for 1 h at 0°C. Tetrazole-protected bromide Candesartan (**4**) (0.158 g, 0.20 mmol) in 2-ml anhydrous THF was added dropwise at 0°C and stirring continued overnight under N_2. The reaction was then quenched with cold saturated NaCl and extracted with ethyl acetate (3 × 20 ml), dried over $MgSO_4$, and the filtrated solution was partially evaporated under reduced pressure. The residue was purified by column chromatography using a solvent gradient EtOAc/Hexanes (15:85 to 30:70). The tetrazole-protected alkyne Candesartan (**5**) was obtained in 55% (yellowish solid, 84 mg). Purity of 98.2% (HPLC, 2 ml/min, AcN/0.1-M ammonium formate 80:20). Melting point: 94°C–98°C. ESI-MS: Calculated for $C_{47}H_{40}N_6O_2$: 720.3213 Found (MH^+): 721.3288. ^{1}H NMR ($CDCl_3$): δ: 7.95–6.84 (m, 26H); 5.45 (s, 2H); 4.60 (q, J = 6.96 Hz, 2H); 4.32 (s, 2H); 3.55–3.45 (m, 2H); 2.42 (td, J = 6.58, 2.63 Hz, 2H); 1.99 (m, 1H) 1.45 (t, J = 7.05 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.0, 157.6, 141.7, 141.3, 141.4, 140.3, 137.3, 132.5, 130.3, 130.2, 129.9, 129.8, 128.2, 127.5, 126.3, 124.9, 124.2, 121.2, 119.6, 118.4, 82.9, 81.6, 70.7, 69.4, 67.3, 66.4, 45.8, 19.8, 14.7.

2.2.2 | Fluorobenzyl-Candesartan (1-((2′-(1*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl)methyl)-2-ethoxy-7-(((4-(4-fluorophenyl)but-3-yn-1-yl)oxy)methyl)-1*H*-benzo[d]imidazole) (7)

The tetrazole-protected alkyne Candesartan (**5**) (20 mg, 0.03 mmol) was added under argon to a solution containing Pd $(PPh_3)_4$ (0.70 mg, 0.6 μmol) and CuI (0.11 mg, 0.6 μmol) in 0.2-ml Et_3N at 0°C. After stirring for 10 min under argon, FIB (**6**) (7.4 mg, 0.33 mmol) previously dissolved in Et_3N (0.5 ml) was added dropwise and stirred at 0°C to room temperature for 20 h. After TLC testing (EtOAc/Hexanes 30:70, Rf = 0.4) for completion, the mixture was quenched with saturated aqueous solution of NH_4Cl, extracted with ether (2 × 10 ml) and washed with water (1 × 10 ml). A small aliquot was taken for MS. ESI-MS: Calculated for $C_{53}H_{43}FN_6O_2$: 814.3432 Found (MH^+): 815.3513. Subsequently, the product was hydrolyzed with 0.250 ml of TFA/AcN (1:1.5) at 60°C for 7 min. The final mixture was purified by semi-prep HPLC, and the collected fractions were lyophilized (FreeZone 12-84C, Labconco). The fluorobenzyl-Candesartan (**7**) was obtained in 31% (white solid, 5 mg) and a chemical purity of 98.9% (analytical HPLC using Method 2). Using Chem3D® (Perkin Elmer), the logP value was calculated to be 7.7. ESI-MS: Calculated for $C_{34}H_{29}FN_6O_2$: 572.2336 Found (MH^+): 573.2432. ^{1}H NMR ($CDCl_3$) δ 8.04 (d, J = 6.6 Hz, 1H), 7.68-7.54 (m, 2H), 7.32–7.28 (m, 5H), 7.06–6.78 (m, 8H), 5.53 (s, 2H), 4.24 (s, 2H), 4.05 (s, 1H), 3.49 (t, J = 6.5 Hz, 2H), 2.58 (t, J = 6.4 Hz, 2H), 0.92 (t, J = 6.05 Hz, 3H). ^{13}C NMR (151 MHz, CD_3CN): δ 162.98, 161.34, 157.35, 154.95, 141.29, 139.64, 138.79, 138.53, 138.19, 138.07, 133.47,

131.22, 130.70, 130.67, 130.65, 130.63, 129.65, 127.97, 125.44, 124.22, 123.32, 121.50, 115.60, 109.56, 87.69, 79.83, 79.80, 69.94, 67.67, 67.23, 46.34, 45.58, 44.19, 20.25, 13.84, 8.03.

2.3 | Radiochemistry

2.3.1 | Synthesis of [^{18}F]6

[^{18}F]FIB ([^{18}F]**6**) was synthesized by ^{18}F-radiofluorination of 4-(iodophenyl)diphenyl-sulfonium triflate (**8**) as reported previously.[20,22] Briefly, n.c.a. ^{18}F (740–5,550 MBq) was produced via an IBA Cyclone 18/9 cyclotron by the nuclear reaction ^{18}O(p,n)^{18}F. The ^{18}F was trapped into an anion exchange resin (QMA light, Waters), eluted with 1.5-ml Kryptofix2.2.2/K_2CO_3 (86:14), and then dried azeotropically with 0.4-ml CH_3CN. When the solution was completely dried, 7 mg of the triphenylsulfonium triflate precursor (**8**) in CH_3CN (0.25 ml) was added to the reactor 1 of the automated Synthra synthesis module. As part of the reaction optimization, various parameters such as the reaction solvent (CH_3CN, DMSO and diglyme), phase-transfer catalyst (K222, $TBAHCO_3$, and no catalyst), amount of precursor (7, 10 and 15 mg), and temperature (85, 90, and 120°C) were studied as presented in the Table 1. After reaction completion, the crude mixture was quenched with 20-ml water and purified either via a C18 Sep-Pak plus (Waters) or semi-prep HPLC. [^{18}F]**6** was eluted with 1 ml (either THF or DMF) into either a vial for manual optimization of the Sonogashira cross-coupling reaction (see Section 2.3.2) or into the second reactor of the Synthra module for the automated production of [^{18}F]**7** (see Section 2.3.3). The radiochemical purity of [^{18}F]**6** was analyzed by analytical HPLC (Method 1).

2.3.2 | Optimization of the synthesis of [^{18}F]7

Optimization of the Sonogashira cross-coupling conditions were initially evaluated with two different catalyst (Pd $(PPh_3)_4$ or Pd $(OAc)_2$) for producing other radioligands.[16,17] Such conditions (Table 2, entries 3 and 7) were utilized here for combining [^{18}F]FIB to the alkyne-Candesartan precursor. In general, in a conical vial (3 ml) under argon, the alkyne precursor (**5**), CuI, palladium catalyst, and the base were mixed with 29.6–148 MBq of [^{18}F]FIB [^{18}F]**6** (purified by Sep-Pak C18, entries 1–6 and 9 or semi-prep HPLC (Table 2, entries 7, 8, and 10). The vial was sealed and the reaction heated. After cooling, the deprotection of the tetrazole was carried out by means of 250 μl 40% TFA in CH_3CN at 70°C for 8 min to obtain [^{18}F]**7**. As part of the optimization process, the influence of various reaction parameter such as the amount of precursor (0.75–3 mg), temperature (60–110°C) and the base (TEA or K_2CO_3) were studied as presented in Table 2. Aliquots were taken for radio-HPLC (Method 2) or radio-TLC analysis.

2.3.3 | Automation of the synthesis of [^{18}F]7

Automated synthesis of [^{18}F]**7** was carried out using the best conditions obtained in the optimization process for the production of [^{18}F]**6** (Table 1, entry 9) and coupling

TABLE 1 Optimization of [^{18}F]**6** reaction conditions

Entry	Phase-transfer catalyst	Precursor (mg)	Solvent	Time (min)	Temperature (°C)	RCY[b] (%)
1	K222	7	CH_3CN	15	85	13.1 ± 2.3
2	K222	7	DMSO	15	85	6.2 ± 1.4
3	K222	7	Diglyme	15	85	19.3 ± 2.8
4	$TBAHCO_3$	7	Diglyme	15	85	23.0 ± 1.5
5	None[a]	7	Diglyme	15	85	9.1 ± 1.8
6	K222	10	Diglyme	15	85	31.3 ± 3.8
7	K222	15	Diglyme	15	85	14.4 ± 1.5
8[c]	K222	10	Diglyme	15	85	26.4
9[c]	K222	10	Diglyme	15	90	29.8
10[c]	K222	10	Diglyme	15	120	<20

[a]Following Richarz et al.[23]
[b]Isolated radiochemical yield from start-of-synthesis ($n = 3$).
[c]Radiochemical conversion of [^{18}F]FIB compared with [^{18}F]fluorobenzene (by-product) for conditions described in entries 8, 9, and 10 are 93.1%, 99.8%, and 78.3%, respectively ($n = 1$).

TABLE 2 Optimization of Sonogashira cross-coupling on the synthesis of [^{18}F]**7**

Entry	Product 5 mg/μmol	CuI (mg/μmol)	Catalyst (mg/μmol)	Base	Solvent	Volume (ml)	Temp (°C)	Time (min)	RCC[a] (%)
1[d]	0.75/1	3/16	Pd $(PPh_3)_4$ 3/3	TEA	THF/TEA (1:1)	2	110	20	0.3[b]
2[d]	1.5/2	3/16	Pd $(PPh_3)_4$ 3/3	TEA	THF/TEA (1:1)	2	110	20	4.4[b]
3[d]	3/4	3/16	Pd $(PPh_3)_4$ 3/3	TEA	THF/TEA (1:1)	2	110	20	0-15[b]
4[d]	3/4	3/16	Pd $(PPh_3)_4$ 3/3	TEA	THF/TEA (1:1)	2	90	20	0[c]
5[d]	3/4	3/16	Pd $(PPh_3)_4$ 3/3	TEA	THF/TEA (1:1)	2	60	20	0[c]
6[d]	1.5/2	3/16	Pd $(OAc)_2$ 3/13	TEA	THF/TEA (40:1)	2	110	20	0[b]
7[e]	1/1	1/5	Pd $(OAc)_2$ 3/13	TEA	THF/TEA (40:1)	2	85	30	0[b]
8[e]	1.5/2	1/5	Pd $(PPh_3)_4$ 8/7	K_2CO_3	DMF	1	110	20	5.1[b]
9[d]	3/4	1/5	Pd $(PPh_3)_4$ 8/7	K_2CO_3	DMF	1	110	20	7.8[b]
10[e]	3/4	1/5	Pd $(PPh_3)_4$ 8/7	K_2CO_3	DMF	1	110	20	22[b]

[a]RCC was determined with $n = 1$ run, except for entry 3 ($n = 6$).
[b]Determined by analytical HPLC.
[c]Determined by radio-TLC.
[d][^{18}F]**6** purified by Sep-Pak C18, entries 1–6 and 9.
[e][^{18}F]**6** purified by semi-prep HPLC, entries 7, 8, and 10.

to the alkyne Candesartan precursor (**5**) (Table 2, entry 10) using the automated dual-reactor, two HPLC Synthra synthesis module. After elution from the QMA into reactor 1 with a solution of 86% K222/K_2CO_3 (1.5 ml), F-18 was dried azeotropically under vacuum in the presence of CH_3CN (0.4 ml). When completely dried, 10 mg of the precursor (**8**) in diglyme (0.25 ml) was added and heated at 90°C for 15 min. The reaction was quenched with water (5 ml) and transferred to a C18 Sep-Pak plus. The cartridge was washed with extra water (15 ml), and the product was eluted with THF (1.5 ml) into the V-Vial 1 (containing 7 ml of water). The mixture was purified by semi-prep C18 HPLC (t_R = 10.8 min) (see Data S3, Figure S3-1). The peak corresponding to [^{18}F]**6** was collected into the SP1 vessel (prefilled with 30 ml of water), then transferred and trapped on a C18 Sep-Pak plus. [^{18}F]**6** was eluted with DMF (1 ml) into the reactor 2 containing 3-mg K_2CO_3, 1-mg CuI, and 8-mg Pd $(PhP_3)_4$. After addition of the alkyne precursor (**5**) (3 mg in 0.2-ml DMF) to reactor 2 the reaction was heated at 110°C for 20 min. TFA (30%) in acetonitrile (0.25 ml) was added to the cooled reaction mixture for the tetrazol deprotection (60°C, 7 min).

Following quenching with 3-ml HPLC solvent and filtration (0.45 μm Nylon, Whatman) into the V-vial 2, the filtered mixture was purified by semi-prep C18 HPLC (t_R = 7.2 min) (see Data S3, Figure S3-1). The peak corresponding to [^{18}F]**7** was collected into the SP2 vessel (prefilled with 45 ml 0.1% TFA water). [^{18}F]**7** was trapped onto a C18 Sep-Pak plus, eluted with 2-ml ethanol (95%). In order to have a more concentrated formulation, the ethanol was evaporated at 40°C under a gentle stream of nitrogen, and 0.9% saline (containing 10 mg/ml sodium ascorbate[24]) was added to reach a maximum of 10% ethanol in the final formulation.

2.3.4 | Analytical studies

The final formulation was analyzed by analytical HPLC (Method 2, t_R = 7.8 min, Figure 1) to determine the radiochemical purity and molar activity. The chemical identity of product [^{18}F]**7** was confirmed by coinjection with the nonradioactive standard (**7**). [^{18}F]**7** was assayed for stability by monitoring the radiochemical purity 4 h after EOS.

2.3.5 | In vitro plasma stability

In vitro tracer stability was evaluated in plasma samples from Sprague-Dawley rats. Plasma volumes of 0.5 ml were mixed with [^{18}F]**7** (~1.62 kBq) and then incubated

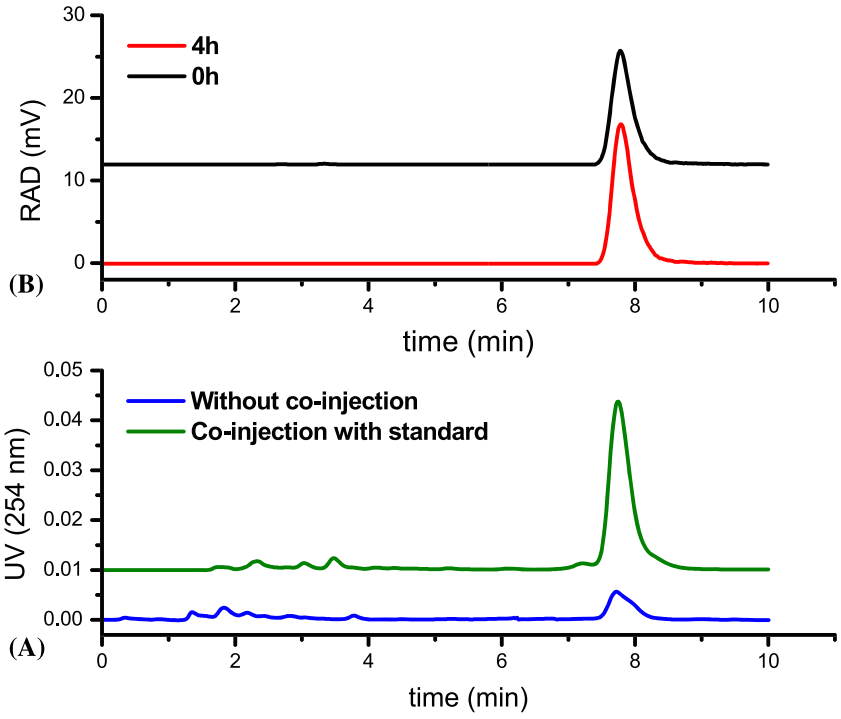


FIGURE 1 Representative analytical HPLC chromatograms of [^{18}F]**7**. Coinjection with authentic standard (**7**) in green (t_R = 7.78 min) (A). Radioactive signal at QC time (time 0) in red and 4 h post-synthesis in black (t_R = 7.80 min) (B)

at 37°C for 20, 40 and 60 min. Urea at final concentration of 0.35 g/ml was added to plasma samples to disrupt plasma protein binding. Prior to the experiments, samples of plasma directly spiked with authentic tracer before incubation at time zero (no enzymatic degradation) were processed as a control.

A previously described column-switch HPLC[25] consisted in a capture column (20 × 2 mm, hand packed with Oasis HLB), and an analytical column (Luna C18 10 µm 100 Å 250 × 4.60 mm, Phenomenex) was utilized to study the presence of plasma degradation products. Briefly, filtered samples injected in the system are trapped onto the capture column, and hydrophilic subproducts and/or macromolecules are eluted at 1 ml/min with 1:99 acetonitrile/water. Once the UV signal returned to baseline, solvent is switched to 55:45 acetonitrile/0.1% TFA to back-flushed the retained compounds (hydrophobic degradation products + intact tracer) off the capture column and over the analytical column at 2 ml/min. Signals were analyzed using PeakSimple 3.77 software and subsequently corrected for noise and radioactive decay. Integration of peaks was used to determine the proportion of hydrophilic/hydrophobic plasma degradation products over intact [^{18}F]**7**.

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

In order to prepare the hydroxy derivative of Candesartan (**2**), the carboxylic acid moiety was reduced by means of lithium aluminum hydride in high yields (Scheme 1). The alkyne tetrazole-protected Candesartan (**5**) was prepared from (**2**) with trityl chloride in the presence of TEA and dichloromethane. To produce the alkyne Candesartan precursor (**5**), two synthetic strategies were evaluated. The first one was to form the alkoxide of the hydroxy derivative of Candesartan (**2**) by means of NaH and then proceed to the ether formation reaction with 4-chlorobut-1-yne. The second strategy was to use the Mitsunobu reaction between of hydroxy derivative of Candesartan (**2**) and 3-butyn-1-ol, but unfortunately, both approaches did not yield the desired compound (**5**). Interestingly, the reaction to produce (**5**) proceeded in high yields by inverting the conditions of the first strategy, where the alkoxide of 3-butyn-1-ol reacted with the trityl bromine Candesartan (**4**) following a modified version of Williamson ether synthesis.

3.2 | Radiochemistry

3.2.1 | Synthesis of [^{18}F]6

After several attempts following the conditions reported in Way and Wuest,[20] the highest RCY of [^{18}F]FIB was of 13.1 ± 2.3% (Table 1, entry 1). Further experiments were designed to find optimal conditions (in our hands) and to increase the RCY. As presented in Table 1, the use of diglyme (entry 3) yielded ~19% compared with ~6% with anhydrous DMSO (entry 2). Therefore, diglyme was used in all subsequent conditions (entries 4–10). No difference

was found with K222 or $TBAHCO_3$ (Table 1, entries 3 and 4), whereas using no catalyst produced [^{18}F]**6** in lower yields (Table 1, entry 5). Richarz et al[23] published a minimalist method to produce [^{18}F]FIB in 66% radioconversion (RCC) without the need of catalyst and/or azeotropic drying. In their study, F-18 was trapped in a Chromafix cartridge, rinsed extensively with anhydrous methanol, and then eluted into the reactor using the same sulfonium salt precursor as here. Using their approach, [^{18}F]FIB production was lower compared with the use of K222 or $TBAHCO_3$. Furthermore, approximately 30% of the trapped F-18 was not completely eluted when 10 mg of sulfonium salt precursor was utilized. Therefore, we selected the classical $K222/K_2CO_3$ approach for producing [^{18}F]FIB in high RCY.

After evaluating the amount of precursor (Table 1, entries 3, 6, and 7), the highest RCY for producing [^{18}F]**6** (at 93% radiochemical purity) was obtained with 10 mg of precursor. As previously reported,[20] we also found a relationship between the temperature and the radiochemical purity of [^{18}F]**6** (Table 1, entries 8, 9, and 10). The formation of the by-product [^{18}F]fluorobenzene was practically prevented with reactions at 90°C, indicating that at this temperature (see Data S2, Figure S2-1), the mechanism favored the electron-poor iodinated system leading to the desired product [^{18}F]**6**.[22] Finally, the optimal condition (Table 1, entry 9) produced [^{18}F]**6** in a similar RCY (31.3 ± 3.8, $n = 8$) as previously reported by Mu et al.[22]

3.2.2 | Optimization of the synthesis of [^{18}F]7

Since the reaction proposal by Kenkichi Sonogashira in the 1970s,[26] the Sonogashira cross-coupling reaction has not been extensively used in radiochemistry. The first report came in 2003 when Wüst and Kniess[16] reported the radiolabeling of an estradiol derivative with [^{18}F]FIB. Twelve years later, the same group extended [^{18}F]FIB application to the radiolabeling of alkyne-modified peptides under mild conditions with Pd $(OAc)_2$ in high RCYs.[19] Their reported Sonogashira conditions were investigated here for radiolabeling the protected alkyne-Candesartan derivative (**5**) with [^{18}]FIB (Table 2, entries 3 and 7). As observed in entry 7, addition of Pd $(OAc)_2$ to the reaction mixture did not produce the final compound [^{18}F]**7** from [^{18}F]**6** either at 85°C or 110°C (Table 2, entries 7 and 6, respectively). Therefore, we decided to use the Pd $(PPh_3)_4$ catalyst in all subsequent experiments.

While performing optimization experiments with 3 mg of precursor and TEA as the base, random conversion yields where obtained varying from no reaction to 22% RCC. The analysis of HPLC chromatograms (Table 2, entry 3) revealed the formation of more radioactive impurities and the presence of the unprotected final product. To overcome these problems, we hypothesized that using less precursor would favorize the formation of [^{18}F]**7** over the [^{18}F]by-products (Table 2, entries 1 and 2). Contrary to our hypothesis, the formation yield was also reduced to 0.3% with 0.75 mg of precursor (Table 2, entry 1). Reducing the reaction temperature to 60°C or 90°C (Table 2, entries 5 or 4, respectively) was not beneficial with no reaction, demonstrating that 110°C is required for completion of the cross-coupling reaction in higher RCCs.

The acid hydrolysis of the trityl-protected [^{18}F]**7** was not reproducible due to the excess of TEA. It was reported that using concentrated acids (e.g. TFA or HCl) led to the formation of the des-ethylated Candesartan analog for trityl deprotection.[9] To circumvent this problem, alternative bases such K_2CO_3 and Cs_2CO_3 were explored as previously reported as a modified version of the classic Sonogashira reaction.[27] Adapting this reaction condition to our context, 3 eq of K_2CO_3 in DMF produced [^{18}F]**7** in 5% yield with almost no trityl-protected (**5**) left (Table 2, entry 8). In addition, increasing the amount of precursor to 3 mg (Table 2, entry 9) increased the RCC up to 8%. Repeating the entry 9 with HPLC pure [^{18}F]**6** (Table 2, entry 10) led to the production of [^{18}F]**7** in in higher RCC (22%). Such observation was previously discussed by Wüst and Kniess.[16] Based in their findings, iodinated impurities produced during the thermal decomposition of product (**8**)[28,29] are not separated from [^{18F}]FIB following C18 SepPak purification, causing competitive cross-coupling reactions reducing the RCC yields. Whereas such competition was not observed with [^{18}F]**6** after HPLC purification. The conditions described in entry 10 were thus selected for automatized radiosynthesis.

3.3 | Automation of the synthesis of [^{18}F]7

Automation of the radiosynthesis (see Data S3, Figure S3-1) was initially performed with 1 mg of precursor giving [^{18}F]**7** in an overall RCY of 0.19 ± 0.03% ($n = 3$, decay corrected from EOB). Increasing the amount of precursor to 3 mg of the protected alkyne-Candesartan (**5**) gave radiochemically pure (99.8%) [^{18}F]**7** in 4.6 ± 1.1% ($n = 4$, decay corrected from EOB) (Figure 1A). Its identity was confirmed by coinjection with the authentic (**7**) (Figure 1B). Fawdry[24] reported that 10 mg/ml of sodium ascorbate in concentrated formulations of FDG (11 GBq/ml) led to a stability of 99%

up to 14 h. After ethanol evaporation and reformulation in saline-containing sodium ascorbate, the final formulation at 1.48 GBq/ml was stable for 4 h after EOS at room temperature (Figure 1B). The molar activity of the final formulation was low (1,406–5,513 GBq/mmol) due to the formation of structurally close impurities during the coupling and acid hydrolysis as detected by semi-prep HPLC (see Data S3, Figure S3-2). Attempts to further improve the isolated radiochemical yield and molar activities turned out to be challenging. However, new strategies for reducing the side-products formation by exploring new Pd catalyst, less aggressive acid hydrolysis of tetrazole group and new semi-prep HPLC system are currently under way.

3.4 | In vitro plasma stability

Hydrolysis of compounds by plasmatic enzymes is among a wide variety of mechanisms utilized for clearing drugs from circulation. This process reduces the lifetime of pharmaceutical compounds in circulation, affecting the availability of the parent product to produce pharmacological effect.[30] [^{18}F]**7** was found to be stable in plasma with the presence of less than 3% of radioactive by-products after 60 min of incubation at 37°C. Previous reports suggested that compounds containing functional groups such as esters, amides, lactones, lactams, carbamides, sulphonamides, and peptic mimetics are more susceptible to hydrolysis in plasma.[31] Taking our results into account, we have found the carbon–carbon bond between a terminal alkyne and an aryl halide to be highly stable to enzymatic break-down, suggesting metabolic stability of [^{18}F]fluorobenzyl-Candesartan. In support to this finding, the propargylglycine derivative [^{18}F]FPhPA labeled with [^{18}F]FIB using a similar Sonogashira conditions was also found to be a stable in vivo. PET imaging of this tracer in MT-6 tumor-bearing BALB/c mice revealed high tumor uptake and no abnormal biodistribution pattern as compared with the amino acid transporter [^{18}F]FET, indicating the stability of the alkyne-benzene (sp–sp^2 carbon–carbon) planar arrangement.[17]

4 | CONCLUSIONS

A fully automated synthesis of the novel [^{18}F] fluorobenzyl-Candesartan has been developed in a three-steps two-reactor two-HPLC purification process using [^{18}F]FIB as prosthetic group. The tracer was obtained in high radiochemical purity and remained stable up to 4 h in solution and up to 1 h in plasma at 37°C. Nonetheless, the use of Sonogashira cross-coupling reaction to produce [^{18}F]**7** in high yields and molar activities was found to be challenging for routine use in radiochemistry labs.

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