Synthesis of the Novel AT₁ Receptor Tracer [¹⁸F]Fluoropyridine—Candesartan via Click Chemistry

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1. INTRODUCTION

Cardiovascular diseases are among the leading causes of death in most countries worldwide. The octapeptide angiotensin II (AngII), the main hormone of the renin–angiotensin system (RAS), plays an important role in the regulation of blood pressure, sodium and water balance, aldosterone secretion, sympathetic nerve activity, and cardiovascular function. Its physiological effects are mainly mediated through AngII type-1 receptors (AT₁,R), which are expressed in the kidney (mainly cortex), adrenal glands, heart, brain, liver, gut, and vascular tissues. Several diseases including hypertension, heart and renal failure, and diabetes involve an alteration in the AT₁,R levels. Therapy with AT₁,R blockers (ARBs), for example, losartan and candesartan, was shown to reduce morbidity and mortality in patients with such diseases. The development of AT₁,R tracers offers the possibility to quantify AT₁,R during disease progression and follow-up therapy by positron emission tomography (PET) imaging.

Several compounds were labeled with carbon-11 and fluorine-18 for noninvasive in vivo PET imaging of AT₁,R receptors: [¹¹C]KR31173, [¹⁸F]FPY45 (valsartan derivative), and α-[¹⁸F]irbesartan exhibited binding specificity to renal cortex AT₁,R of multiple species. [¹¹C]Methyl-losartan, [¹⁸F]fluoropyridine—losartan ([¹⁸F]FPyKYNE-losartan), and [¹¹C]-methyl-candesartan derivatives, developed by our group, displayed specific binding for renal AT₁,R in rats and pigs. A superior in vivo kinetics and binding profile were obtained for [¹¹C]methyl-candesartan compared to [¹¹C]-methyl-losartan. Interestingly, high binding affinity, full antagonistic activity in the AngII pressor effect, high kidney-to-blood contrast, and minimal interference of the labeled metabolites on renal AT₁,R binding were observed with...
Fluoropyridine−losartan in rats. Labeling with $^{18}$F (half-life = 110 min and mean positron range = 0.6 mm) allows for multiple scans per tracer formulation, shipment to facilities without cyclotron, and high PET-image resolution compared to $^{11}$C (20.4 min, 1.2 mm). Based on previous structure−activity relationship studies, large prosthetic groups can be introduced at the 7-position of the benzimidazole ring of candesartan producing minimal changes both in binding properties and antagonist efficacy. Taking into account that candesartan has a higher affinity for AT$_1$R (IC$_{50}$ = 0.26 nM) compared to losartan (IC$_{50}$ = 34 nM), we present here the synthesis of the novel $^{18}$F-fluoropyridine−candesartan following the introduction of a (4-(3-((2-$^{18}$F$\text{)}$fluoropyridin-3-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)methyl group, using the same approach as for $^{18}$F-fluoropyridine−losartan, on the position 7 of candesartan via a Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) click reaction. In addition, we assessed the tracer specificity to renal AT$_1$R in rats using in vitro and ex vivo competitive assays.

2. RESULTS AND DISCUSSION

2.1. Chemistry. Fluoropyridine−candesartan standard was produced in a six-step route, as shown in Scheme 1. Compounds 2 and 2-fluoro-3-(pent-4-yn-1-yl)pyridine (FPyKYNE, 3) were successfully obtained in 70% and 67% yields, respectively. Commercially available candesartan (4) was reduced to its hydroxyl derivative (5) with lithium aluminum hydride (LiAlH$_4$) in 68% yield, and its tetrazole moiety was then protected with the trityl group to give 6 in 75% yield. The tritylcandesartan azide derivative (7) was synthesized following a general procedure reported previously to convert alcohols to azides using diphenylphosphoryl azide (DPPA). The hydroxyl group from the benzimidazole moiety on compound 6 was converted to the corresponding azide 7 in 71% yield under mild reaction conditions.

The fluoropyridine−candesartan standard (9) was produced from 7 via a Huisgen 1,3-dipolar CuAAC reaction with 3. The reactivity of tetrazoles (like azides) in Cu(I)-catalyzed click reactions has been reported because of the existence of the tetrazole/azole isomerism. Therefore, the triphenylmethyl (trityl)-protecting group was added to the tetrazole ring of the candesartan derivative 5 to give 6 in order to avoid the formation of an undesired adduct during the click reaction. The acid deprotection at 70 °C for 1 h was initially proposed for the last step of the synthesis (Scheme 1, product 8 to 9). Higher temperatures are not recommended because of the hydrolysis of the ethoxy group in the benzimidazole moiety of candesartan, as previously reported for methyl-candesartan.
which was converted to the cyclic urea derivative (TH4) in HCl (1 N) at 90 °C for 1.5 h. A first attempt to obtain the tetrazole-protected fluoropyridine−candesartan (8) from 7 was performed at 40 °C in the presence of a copper(II) sulfate/sodium ascorbate (NaAsc) catalytic system (1:2.4 CuSO4/NaAsc molar ratio) (adapted from ref 18). Unfortunately, it took more than 72 h to afford 8 in less than 15% yield, thus the deprotection reaction was not performed. Many CuAAC reactions are fast and efficient when NaAsc in 3- to 10-fold excess is used to generate Cu(I) in situ without the need for inert gas conditions. Nevertheless, it is recommended to minimize air exposure to prevent the oxidation of ascorbate by atmospheric oxygen. Using a 1:8 (CuSO4/NaAsc) molar ratio and increasing the temperature to 80 °C provided the fluoropyridine−candesartan standard (9) in 24 h, under a nitrogen (N2) atmosphere (yield 44%). During the reaction, almost complete deprotection of the precursor/product occurred; therefore, further addition of HCl was not necessary for the trityl deprotection after the click reaction to give 9 from 7. Very small amounts of impurities other than the deprotected precursor (candesartan azide, 10) were observed, suggesting good stability of the tetrazole group during the click reaction.

As reported previously, the stability of the tetrazole isomers depends on several factors, such as the nature of substituents, solvents, and temperature, which will determine the dominant form in the tetrazole/azide equilibrium. Our results confirm that the azide group at the benzimidazole moiety of 7 was more prone to react than the less favored azide open form of the tetrazole ring under the click reaction conditions used in this study.

2.2. Radiochemistry. Click chemistry reactions are widely utilized in the production of radiopharmaceuticals, especially for labeling biomolecules (e.g., peptides, proteins, and so on). Based on the previously reported [18F]-fluoropyridine−losartan synthesis, the CuAAC click reaction approach was used here to label the azido-modified candesartan with the [18F]FPyKYNE ([18F]3) prosthetic group.

[18F]3 was successfully synthesized in the first reactor by aromatic nucleophilic substitution, as reported previously. Reducing the amount of precursor from 30 to 10 mmol and the reaction time from 10 to 5 min (Scheme 2) did not affect the radiochemical yield (RCY). Pure [18F]3 was obtained in 40 ± 9% yield (n = 6) [decay-corrected from [18F] produced at the
end-of-beam (EOB).] Semi-preparative silica high-performance liquid chromatography (HPLC) allowed complete separation of \(^{18}F\)3 from its nitro precursor 2 (Figure 1A) preventing the formation of NO\(_2\)-pyridine-candesartan (11) in the second reactor (Scheme 2), which would decrease the apparent molar activity of the final product, as it is not possible to separate the fluorinated candesartan (9) from the nitro derivative (11) in the final semi-preparative C18 HPLC (Tables S1 and S2). The HPLC solvent was removed by transferring \(^{18}F\)3 to the second reactor via two consecutive silica cartridges. Elution with ether allowed a fast and efficient evaporation at low temperatures, avoiding the removal of the volatile \(^{18}F\)3. Less than 8% of the activity that reached the second reactor was lost in this step. \(^{18}F\)3 was then coupled with 7 at 80 °C for 10 min via the CuAAC click reaction, followed by acid deprotection at 60 °C for 7 min to produce \(^{18}F\)-fluoropyridine-candesartan (\(^{18}F\)9). The tracer was successfully purified from the deprotected precursor 10 by semi-preparative C18 HPLC (Figure 1B). A low-pH mobile phase (0.1% trifluoroacetic acid (TFA) in 33:67 methyl cyanide/water (CH\(_3\)CN/H\(_2\)O), pH 2) ensured optimal chemical purity and thus a high molar activity of the final product, following reformulation in 10% ethanol (EtOH)/saline (0.9%).

2.2.1. Optimization of the Click Reaction. Based on the synthesis of \(^{18}F\)fluoropyridine-losartan,\(^{16}\) a first experiment was performed using equimolar amounts of CuSO\(_4\) and NaAsc yielding \(^{18}F\)9 in very low RCY (Table 1, entry 1). This result was also possibly affected by the different click reaction conditions (80 °C, 10 min) compared to \(^{18}F\)fluoropyridine-losartan (95 °C, 30 min). However, the temperature was not increased to prevent the formation of the cyclic urea derivative.\(^{17}\) The conversion of \(^{18}F\)3 into \(^{18}F\)9 was calculated using the proportions of the peaks from the semi-preparative C18 HPLC chromatograms, decay-corrected to the retention time of \(^{18}F\)9. Before purification, the reaction mixture was passed through a C18 cartridge and washed with water to remove dimethyl sulphoxide (DMSO), hydrophilic compounds, and salts. The amount of activity removed to the waste in this step was negligible, hence it was not considered for calculations.

In order to optimize the click reaction, several conditions were tested. Increasing the amount of NaAsc slightly improved the RCY of the process (Table 1, entry 2). The addition of Cu(I)-stabilizing ligands was demonstrated to accelerate the click reaction kinetics under mild conditions.\(^{39,36,39,40}\) In fact, in addition of tris[1-(benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), bathophenanthroline disulfonic acid disodium salt (BPDS), or tris[1-hydroxypropyl-1H-1,2,3-triazol-4-yl]-methyl]amine (THPTA) resulted in increased RCYs (Table 1, entries 3–7). The best results were obtained using THPTA that consistently provided conversions higher than 60% and RCYs higher than 10% (Table 1, entries 6–7). BPDS acted as an appropriate Cu(I) stabilizer at low \(^{18}F\) activities providing \(^{18}F\)9 in 9 ± 2% RCY (n = 3) (Table 1, entry 4), inducing a conversion of \(^{18}F\)3 into \(^{18}F\)9 similar to those with TBTA and THPTA (Table 1, entries 3 and 6). Unfortunately, starting with higher activities of \(^{18}F\)fluoride reduced the conversion by 1.7-fold leading to a lower RCY (Table 1, entry 5). A significant drawback of the Cu(I)–BPDS complex is its acute sensitivity to oxidation under a noninert atmosphere.\(^{41}\) The radiolysis of water during the click reaction could be enhanced at very high \(^{18}F\) activities, producing reactive oxygen species, especially hydroxyl radicals, that affect the stability of the Cu(I)–BPDS complex, consequently reducing the RCY. THPTA has interesting properties such as being soluble in water and stable under ambient conditions as opposed to TBTA. The Cu(I)–THPTA complex was shown to have a higher stability in the presence of oxidative byproducts,

### Table 1. Optimization of the Click Reaction Producing \(^{18}F\)9

| Entry | \[^{18}F\]F 3 Activity (GBq) | Precursor (mg) | Cu(I) Stabilizing Ligand | Molar Ratio\(^{b}\) P/Cu/NaAsc/L | Conversion of \[^{18}F\]3 into \[^{18}F\]9 (%) | RCY of \[^{18}F\]9 (%) |
|-------|-----------------------------|----------------|--------------------------|---------------------------------|---------------------------------|-----------------|
| 1 (n = 1) | 3.2 | 0.5 | None | 1:4:0 | 17 | 0.42 |
| 2 (n = 3) | 3.4–8.3 | 0.5–1 | None | 1:2:6:0, 1:4:12:0, 1:4:20:0 | 24 ± 5 | 2.4 ± 0.2 |
| 3 (n = 3) | 8.9–10.2 | 0.5 | TBTA | 1:4:20:5 | 55 ± 2 | 7 ± 3 |
| 4 (n = 3) | 3.5–8.2 | 0.5 | BPDS | 1:4:20:5 | 61 ± 7 | 9 ± 2 |
| 5 (n = 3) | 34–98 | 0.5 | BPDS | 1:4:20:5 | 34 ± 2 | 3.7 ± 0.2 |
| 6 (n = 3) | 1.1–96 | 0.5–1 | THPTA | 1:4:20:5 | 64 ± 6 | 11.0 ± 0.5 |
| 7 (n = 10) | 86–340 | 1 | THPTA | 1:2:10:2:5 | 70 ± 3 | 10 ± 2 |

\(^{a}\)The reaction conditions were set to 80 °C and 20 min, respectively, for all entries. \(^{b}\)P = precursor; L = Cu(I) stabilizing ligand. \(^{c}\)Conversion of \(^{18}F\)3 to \(^{18}F\)9 (mean ± SD), as calculated from C18 HPLC, decay-corrected to t\(_{EOB}\) for \(^{18}F\)9. \(^{d}\)RCY of \(^{18}F\)9 from \[^{18}F\]F (mean ± SD), decay-corrected to the EOB.
protecting Cu(1) and sensitive substrates from oxidation.41 Our results (Table 1, entry 7) confirm that THPTA is a strong Cu(1)-chelating ligand for click radiolabeling with high 18F activities. Considering that the click reaction using the deprotected precursor 10 with [18F]3 resulted in very low yields (RCY = 0.3–1.1%, n = 2) (Scheme 2), we selected THPTA, 1 mg of precursor 7, and 1:2:10:2.5 (precursor 7/Cu/NaAsc/THPTA) molar ratio (Table 1, entry 7) for scaling up the radiochemical procedure.

2.2.2. [18F]Fluoropyridine—Candesartan Stability. Starting from 86 to 111 GBq of [18F]fluoride, 0.40 ± 0.03 GBq/mL of [18F]9 was obtained with 97 ± 1% of radiochemical purity (RCP) (Figure 2A), 98.3 ± 0.5% chemical purity, and 203 ± 55 GBq/μmol molar activity at the end-of-synthesis (EOS) (n = 3). The identity of the tracer was confirmed by coelution of the product with the nonradioactive standard 9 on analytical HPLC (Figure 2B). The RCP decreased but remained over 90% for 6 h, as estimated for a radioactive concentration of 0.42 GBq/mL (n = 1) (Figure 3). The pH of the final formulation was 5.3.

Scaling up the process to 321 GBq of [18F]fluoride provided [18F]9 with 5-fold higher radioactive concentration (2.07 GBq/mL at EOS, n = 1). As expected, the molar activity was also increased (399.6 GBq/μmol), but the RCP rapidly decreased below 90% because of radiolytic decomposition of the tracer in the final formulation (Figure 3). However, the conversion of [18F]3 into [18F]9 and the RCP were not affected (68% and 15%, respectively). These results confirm that the tracer was stable up to the purification step, suggesting that the use of NaAsc as a reducing agent in the click reaction and the presence of TFA in the HPLC solvent favored the stability of the tracer. Previous studies reported the decomposition of 18F-labeled radiopharmaceuticals because of transient-reactive species such as hydroxyl radicals or hydrated electrons generated by the radiolysis of water during the purification and reformulation processes, especially for high molar activity formulations.42,43 The incorporation of antioxidant stabilizers such as ascorbic acid, sodium salts (ascorbate, nitrite, thiosulfate, iodide, formate, etc.), or EtOH into the HPLC solvent, collecting flask, washing solution, and/or final formulation was reported to inhibit the radiolytic decomposition of radiopharmaceuticals.42–44 Considering the stability of our product during the purification, we decided to use 0.1% TFA in water to dilute the collected fraction from the HPLC peak and wash the C18 cartridge, as the trifluoroacetate ion was reported as a scavenger of hydroxyl radicals and hydrated electrons.45 In comparison with no radiolytic inhibitors (0.42 GBq/mL or 2.07 GBq/mL), the radiolytic degradation of the tracer was successfully prevented with the presence of TFA in water (RCP >96% up to 8.5 h) for a radioactive concentration of 1.63 GBq/mL at EOS (n = 1) (Figure 3). Moreover, with the addition of NaAsc (10 mg/mL in saline) to the final formulation, the product (1.6 ± 0.2 GBq/mL at EOS, n = 3) was stable for up to 10 h with RCP >97% (Figure 3).

Injectable radiopharmaceuticals are formulated as isotonic solutions with a pH close to the one observed physiologically (pH 7.4); a range between 5 and 8 is acceptable because of the blood’s high buffer capacity. It has been found that pH values above 9 are related to tissue necrosis, whereas values lower than 3 can cause pain and phlebitis.46 Ascorbic acid was shown to be useful both as a radiolytic stabilizer and a buffer agent at pH 4–6.47 Addition of NaAsc (instead of ascorbic acid) to the [18F]9 formulation maintained the pH at 5.5–6, suitable for intravenous (IV) injection.

The whole process lasted about 2 h from the EOB and was reproducible (RCY = 10 ± 2%, n = 13) with high radiochemical and chemical purities (>97%) and very high molar activities (383 ± 27 GBq/μmol, n = 8) starting from 321 to 340 GBq of [18F]fluoride.

### 2.3. Ex Vivo Biodistribution Studies.

Previous biodistribution studies of [11C]methyl-candesartan (15 min post-injection)17 and [11C]methyl-losartan (10 min postinjection)19 confirmed AT1R-specific uptake in the kidney cortex and outer medulla, but very low retention in the adrenal gland, lungs, brain, kidney inner medulla, skeletal muscles, aorta, and heart. Taking into account that the PET time–activity curves of [11C]methyl-candesartan,17 [11C]methyl-losartan19 and [18F]-fluoropyridine–losartan18 exhibited high blood input and the highest accumulation in the kidney cortex at approximately 2–5 min postinjection, and a fast washout of the tracer from the kidney cortex reaching almost background values after 45 min, the 20 min time point was selected to assess [18F]-fluoropyridine–candesartan accumulation in the plasma, renal cortex, outer medulla, and heart (tissues of interest for future studies measuring AT1R expression in various disease states). A high radioactivity accumulation of the tracer was observed in the rat kidney cortex and outer medulla (Figure 4). These results are in accordance with the AT1R distribution in the rat kidney, associated with high densities of these receptors in the glomeruli and, in a more diffuse pattern, throughout the inner stripe of the outer medulla.3 As expected, cardiac uptake was negligible, as described for [11C]methyl-candesartan17 and [11C]methyl-losartan.19 It is important to note that under normal physiological conditions, the AT1R density in rat’s heart is very low,48 which results in receptor saturation with the unlabeled compound present in tracer formulation. However, AT1R expression in the heart was reported to increase in the infarct area in ischemia/reperfusion (1/R) rat and pig models, allowing the detection of cardiac AT1R by PET imaging.59,60 Specific binding to AT1R was demonstrated by pretreatment with saturating doses of candesartan (10 mg/kg, IV) or losartan (30 mg/kg, IV) 20 min before tracer injection, which
blocked the $[^{18}F]$fluoropyridine−candesartan retention in the kidney cortex by 82 and 91% ($p < 0.0001$), respectively, and in the outer medulla by 70 and 86% ($p = 0.0002$), respectively (Figure 4). The uptake reduction in the heart was not significant because of the low accumulation of the tracer in this tissue under normal conditions. $[^{18}F]$Fluoropyridine−candesartan exhibited a higher uptake (tissue-to-blood ratio $= 41 \pm 6, n = 4$) in the renal cortex at 20 min postinjection in comparison to $[^{11}C]$methyl-candesartan (tissue-to-blood ratio $\approx 18$ at 15 min postinjection)$^{17}$ and $[^{11}C]$methyl-losartan (tissue-to-blood ratio $\approx 10$ at 10 min postinjection).$^{19}$

2.4. In Vitro Binding Assay. High binding of $[^{18}F]$fluoropyridine−candesartan was observed in the AT$_1$R-rich rat kidney cortex with a dose-dependent increase in tracer binding between 0.74 and 1.3 nM (Figure 5). Coincubation with losartan (10 μM) reduced binding by 24% ($p = 0.0003$) and 29% ($p < 0.0001$), respectively, confirming binding specificity to AT$_1$R.

Further works on the full kinetic binding profile by microPET imaging and in vivo metabolism in plasma and kidney extracts are currently in progress.

3. CONCLUSIONS

Pure $[^{18}F]$fluoropyridine−candesartan was successfully produced in a three-step, two-pot, and two-step purification synthesis method using THPTA to stabilize Cu(I) species. The optimized process yielded $[^{18}F]$fluoropyridine−candesartan reproducibly in 10 ± 2% (from $[^{18}F]$fluoride) in molar activities higher than 380 GBq/μmol. The use of an acidic water solution during purification/reformulation and the presence of sodium ascorbate prevented tracer radioisolation in the final formulation. The significant blockade of $[^{18}F]$fluoropyridine−candesartan accumulation by pretreatment with the AT$_1$R antagonists candesartan and losartan demonstrates specific binding for AT$_1$R and supports further characterization studies as a PET imaging agent.

4. EXPERIMENTAL SECTION

4.1. General. Commercially available chemicals were used without further purification. HPLC and ACS grade solvents were purchased from Millipore, BDH, or Fisher; deuterated nuclear magnetic resonance (NMR) solvents from CIL; candesartan from AstaTech; losartan from LKT Laboratories; and optimal cutting temperature (OCT) compound (Sakura) from Cedarlane. EtOH (99.5%), sterile 0.9% sodium chloride (USP), anhydrous DMSO (99.8%), and all other reagents were purchased from Commercial Alcohols, Pfizer, Alfa Aesar, Fisher Scientific, VWR, Acros Organics, or Sigma-Aldrich. The reactions were monitored for completion by analytical thin-layer chromatography (TLC) on silica gel 60 F254-coated aluminum sheets (Millipore) or by HPLC using a Shimadzu LC-20AB system, with a Phenomenex Luna C18 (2) column (250 × 4.6 mm, 10 μm), at $\lambda = 254$ and 280 nm. Flash chromatography was carried out on FlashPure ID HP silica cartridges (24 g) using the RevelerisX2 system (Büchi) to purify the precursors and the standard. $^1$H NMR, $^{13}$C NMR, and $^{18}$F NMR spectra were acquired with a Bruker AVANCE III HD 300 MHz NMR 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 were performed in the positive mode by direct injection with electrospray ionization into the Q-Exactive Plus Orbitrap mass spectrometer (Thermo Scientific).

An automated radiosynthesizer Synthra RNplus Research, containing two reactors, two HPLC columns, ultraviolet (UV, $\lambda = 254$ nm) and gamma-ray detectors, was used for radiotracer synthesis, purification, and reformulation. Sep-Pak C18 Plus Short (360 mg, Waters) and Sep-Pak C18 1 cc Vac (100 mg, Waters) cartridges were preconditioned with EtOH (10 or 5 mL, respectively), followed by deionized water (20 or 10 mL, respectively). Sep-Pak Silica Plus Long cartridges (690 mg, Waters) were used as received. Sep-Pak Accell Plus QMA Plus Light cartridges (130 mg, Waters) were preconditioned with 5 mL of 8.4% sodium bicarbonate, followed by 10 mL of deionized water. The $^{18}$F eluent was prepared by dissolving 18.3 mg (0.13 mmol) of potassium carbonate (K$_2$CO$_3$) and 100 mg (0.26 mmol) of Kryptofix2.2.2 (K222) in 5 mL of 95%
DMSO was added, followed by a mixture of aqueous solutions, compound 3, MgSO4, and n-butanol. Fractions were combined, washed with brine, dried over MgSO4, and concentrated under reduced pressure. The crude residue was diluted in CH2Cl2 for purification by flash chromatography (ethyl acetate/EtOAc/hexane 10:90–30:70) and crystallized from hexane to provide a white powder (952 mg, 71%). HPLC (80:20 CH3CN/ammonium formate (AF 0.1 M), 2 mL/min): tR = 13.5 min. H NMR (300 MHz, CDCl3): δ 7.96–7.92 (m, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.54–7.43 (m, 2H), 7.34 (ddd, J = 8.4, 4.7, 1.6 Hz, 4H), 7.28 (d, J = 1.9 Hz, 3H), 7.24–7.16 (m, 3H), 7.11 (d, J = 8.2 Hz, 2H), 6.98–6.91 (m, 8H), 6.82 (d, J = 8.2 Hz, 2H), 5.38 (s, 2H), 4.72 (q, J = 7.0 Hz, 2H), 4.00 (s, 2H), 1.45 (t, J = 7.1 Hz, 3H). 13C NMR (75 MHz, CDCl3): δ 164.03, 147.17, 141.48, 141.25, 140.72, 140.72, 137.37, 137.19, 135.93, 130.62, 130.32, 130.15, 130.04, 129.98, 128.25, 127.94, 127.66, 126.34, 125.14, 124.20, 122.72, 122.11, 121.68, 120.79, 118.99, 116.44, 82.85, 68.31, 50.66, 45.83, 38.04, 28.40, 21.95, 14.63. \(^{19}F\) NMR (282 MHz, CDCl3): δ −84.06 (d, J = 9.9 Hz). HRMS (ESI): exact mass calc for C34H38F2O2 [M + H]\(^+\) = 547.2927, found, 547.2922.

4.2. Chemical Syntheses. 2-Nitro-3-(pent-4-yn-1-yl)-pyridine (NO2PyKNE, 2), 2-fluoro-3-(pent-4-yn-1-yl)-pyridine (FpyKNE, 3), (1-(2′-(2H-tetrazol-5-yl)-[1′,1′-biphenyl]-4-yl)methyl)-2-ethoxy-1H-benzoi[d]imidazole-7-yl)methanol (5), and (2-ethoxy-1-(2′-(2′-trityl-2H-tetrazol-5-yl)-[1′,1′-biphenyl]-4-yl)methyl)-1H-benzoi[d]imidazole-7-yl)methanol (6) were synthesized as previously reported. \(^{18,24}\) The characterization data were in agreement with the literature.

4.2.1. 7-(Azidomethyl)-2-ethoxy-1-(2′-(2H-tetrazol-5-yl)-[1′,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole (Tritylcandesartan Azide, 7). The protected hydroxyl derivative of candesartan 6 (1.3 g, 1.94 mmol) was dissolved in CH2Cl2 for purification by flash chromatography (ethyl acetate/EtOAc/hexane 10:90–30:70) and crystallized from hexane to provide a white powder (952 mg, 71%). HPLC (80:20 CH3CN/ammonium formate (AF 0.1 M), 2 mL/min): tR = 13.5 min. H NMR (300 MHz, CDCl3): δ 7.96–7.92 (m, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.54–7.43 (m, 2H), 7.34 (ddd, J = 8.4, 4.7, 1.6 Hz, 4H), 7.28 (d, J = 1.9 Hz, 3H), 7.24–7.16 (m, 3H), 7.11 (d, J = 8.2 Hz, 2H), 6.98–6.91 (m, 8H), 6.82 (d, J = 8.2 Hz, 2H), 5.38 (s, 2H), 4.72 (q, J = 7.0 Hz, 2H), 4.00 (s, 2H), 1.45 (t, J = 7.1 Hz, 3H).

13C NMR (75 MHz, CDCl3): δ 164.03, 147.17, 141.48, 141.25, 140.72, 140.72, 137.37, 137.19, 135.93, 130.62, 130.32, 130.15, 130.04, 129.98, 128.25, 127.94, 127.66, 126.34, 125.14, 124.20, 122.72, 122.11, 121.68, 120.79, 118.99, 116.44, 82.85, 68.31, 50.66, 45.83, 38.04, 28.40, 21.95, 14.63. \(^{19}F\) NMR (282 MHz, CDCl3): δ −84.06 (d, J = 9.9 Hz). HRMS (ESI): exact mass calc for C34H38F2O2 [M + H]\(^+\) = 547.2927, found, 547.2922. 1H NMR (300 MHz, CDCl3): δ 8.00 (dd, J = 7.5, 1.6 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.76–7.71 (m, 1H), 7.59–7.48 (m, 2H), 7.42 (d, J = 7.4, 1.6 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.24 (d, J = 6.9 Hz, 1H), 7.08–7.00 (m, 3H), 6.92 (d, J = 8.4 Hz, 2H), 6.61 (d, J = 8.1 Hz, 2H), 5.71 (s, 2H), 5.56 (s, 2H), 4.69 (q, J = 7.0 Hz, 2H), 3.81 (t, J = 5.9 Hz, 2H), 2.57 (t, J = 7.4 Hz, 2H), 1.93–1.81 (m, 2H), 1.44 (t, J = 7.1 Hz, 3H). 13C NMR (151 MHz, CDCl3): δ 163.10, 154.39, 152.81, 146.25, 141.95, 141.78, 139.96, 137.49, 137.40, 136.24, 131.68, 131.27, 131.24, 130.57, 130.41, 129.49, 129.30, 128.22, 128.10, 124.75, 124.30, 122.68, 121.79, 121.76, 111.46, 67.85, 50.72, 46.55, 40.22, 28.06, 21.65, 14.57. \(^{19}F\) NMR (282 MHz, CDCl3): δ −84.06 (d, J = 6.0 Hz). HRMS (ESI): exact mass calc for C34H38F2O2 [M + H]\(^+\) = 547.2927, found, 547.2922.
fluoropyridin-3-yl)oxy)propyl)-1H-1,2,3-triazol-1-yl)-methyl)-1H-benzo[d]imidazole ([18F]Fluoropyridine–Candesartan, [18F]9). [18F]Fluoride was produced in an IBA Cyclone 18/9 cyclotron via the 18O(p,n)18F nuclear reaction. It was transferred to the radiosynthesizer and trapped onto the QMA cartridge. It was then eluted with 1.5 mL of K2CO3/Kryptofix2.2.2 solution to the first reactor and brought to dryness following azeotropic drying of the solvent (with the addition of 0.4 mL CH3CN). [18F]PykYNE ([18F]3) was produced by radiofluorination of NO2PykYNE (2) (2 mg, 0.01 mmol in 200 μL DMSO) from ref 18). The reaction mixture diluted in deionized water (with or without 0.1% TFA). The reaction mixture diluted in deionized water (with or without 0.1% TFA) and trapped into a preconditioned Sep-Pak C18 Plus Short cartridge. The cartridge was washed with 15 mL of deionized water (2 min each, 4 × 2 min). Detritiation with TFA (100 μL) in CH3CN (150 μL) at 60 °C for 7 min provided [18F]9 (Scheme 2), which was purified by C18 HPLC after the removal of DMSO and aqueous species by a preconditioned Sep-Pak C18 Plus Short cartridge (HPLC: 0.1% TFA in 33:67 CH3CN/H2O, 10 mL/min, switched to 100% EtOH after tracer elution). The collected fraction was diluted in 45 mL of deionized water (with or without 0.1% TFA) and trapped into a preconditioned Sep-Pak C18 1 cc Vac cartridge. The cartridge was washed with 15 mL of deionized water (with or without 0.1% TFA). [18F]9 was then eluted with 0.8–1 mL of EtOH (USP) followed by 7.2–9 mL of sterile saline (0.9%) (with or without 10 mL NaAsc) to provide the final product [1/9 EtOH/saline (0.9%)]. The effects on the RCY of the amounts of NaAsc and tritylcandesartan azide (7), the starting [18F]F– activity, and the addition of TBTA, BPDS, or THPTA to stabilize the reaction. It was transferred to the radiosynthesizer and trapped into a preconditioned Sep-Pak C18 Plus Short cartridge before purification by silica gel HPLC in order to separate 2 from the prosthetic group [18F]3 (HPLC: 84:5:15:0.5 hexane/CH2Cl2/isopropanol, 9 mL/min, switched to 70:30 CH3Cl/isopropanol after peak collection). Pure [18F]3 was transferred to the second reactor via 2 Sep-Pak Silica Plus Long cartridges eluted with 4 mL of ether. After evaporation of the solvent at 40 °C under vacuum, it was conjugated with 0.5–1 mg (0.72–1.44 μmol) of tritylcandesartan azide (7) or 0.4–1 mg (0.88–2.21 μmol) of candesartan azide (10) in DMSO (400 μL) and the presence of aqueous CuSO4 (150 μL, 0.02–0.04 M) and NaAsc (200 μL, 0.015–0.15 M) with or without TBTA (1.9 mg, 3.58 μmol), BPDS (2.2 mg, 3.72 μmol), or THPTA (1.6–3.2 mg, 3.68–7.36 μmol) as Cu(I) stabilizing ligands (80 °C, 10 min). Detritiation with TFA (100 μL) in CH3CN (150 μL) at 60 °C for 7 min provided [18F]9 (Scheme 2), which was purified by C18 HPLC after the removal of DMSO and aqueous species by a preconditioned Sep-Pak C18 Plus Short cartridge (HPLC: 0.1% TFA in 33:67 CH3CN/H2O, 10 mL/min, switched to 100% EtOH after tracer elution). The collected fraction was diluted in 45 mL of deionized water (with or without 0.1% TFA) and trapped into a preconditioned Sep-Pak C18 1 cc Vac cartridge. The cartridge was washed with 15 mL of deionized water (with or without 0.1% TFA). [18F]9 was then eluted with 0.8–1 mL of EtOH (USP) followed by 7.2–9 mL of sterile saline (0.9%) (with or without 10 mL NaAsc) to provide the final product [1/9 EtOH/saline (0.9%)]. The effects on the RCY of the amounts of NaAsc and tritylcandesartan azide (7), the starting [18F]F– activity, and the addition of TBTA, BPDS, or THPTA to stabilize the Cu(I) species were assessed (see Table 1 for conditions).

3.3.1. HPLC Analyses and tracer Stability. Radiochemical and chemical purities of [18F]9 were determined by analytical HPLC. The identity of the tracer was confirmed by coinjection with the nonradioactive standard (9). The molar activity was calculated by comparing the UV responses (λ = 254 nm) of the tracer and the standard on separate injections. The stability of the tracer was monitored over 10 h as a function of the RCP. The use of 0.1% TFA/water in the reformulation step and the addition of NaAsc to the final formulation were evaluated to prevent the radiolysis of the product.

4.4. Animal Studies. Male Sprague–Dawley rats (356.58 ± 40.84 g, n = 12, Charles River Laboratories, Senneville, QC, Canada) were housed in a temperature-controlled facility, with 12 h day/night cycle, and fed with standard rat chow and water ad libitum. All animal experiments were conducted in accordance with the Canadian Council on Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee of the CRCHUM.

4.4.1. Ex Vivo biodistribution Studies. Biodistribution studies were performed to evaluate tissue uptake and binding specificity of [18F]9 using previously published methods (adapted from refs 19 and 20). Briefly, animals were anesthetized with isoflurane (4% for induction, 2% for maintaining) and injected by the IV route via the lateral tail vein with the tracer. Control animals received 8.2–70.8 MBq of tracer alone (0.02–0.5 μg, n = 4), whereas the other two groups received saturating doses of AT1R antagonists losartan (30 mg/kg, n = 4) or candesartan (10 mg/kg, n = 4). IV, 20 min prior to the injection of 44–136 MBq (0.15–1.6 μg) and 23–94 MBq (0.2–0.9 μg) of tracer, respectively. Rats were sacrificed by decapitation at 20 min postinjection. Trunk blood was collected in heparinized tubes, and plasma was obtained by centrifugation for 15 min (1500g, 4 °C). Samples of blood, plasma, dissected kidney cortex, and medulla were transferred into preweighed polypropylene tubes with caps and measured in a gamma counter Wizard 3470 (PerkinElmer Inc), along with a tracer solution as a 1% volumetric standard. The percentages of the injected dose (decay-corrected) per gram of tissue (% ID/g) were calculated. The uptake is expressed as a tissue-to-blood ratio.

4.4.2. In Vitro Binding Assay. In vitro autoradiography of [18F]9 was carried out using the methods published previously (adapted from refs 20 and 51), to investigate its binding to AT1R using rat kidney slices, as they exhibit high AT1R density. Following rat decapitation, dissected kidneys were immersed in OCT compound, frozen on dry ice, and stored at −80 °C. Briefly, 20 μm thick kidney sections were thaw-mounted onto glass slides and stored at −80 °C. On the day of the experiment, the slides were preincubated in assay buffer (150 mM NaCl, 50 mM Na2HPO4, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM bacitracin, and 0.1% bovine serum albumin, pH 7.4) for 20 min at room temperature, in the presence or absence of the AT1R antagonist losartan (10 μM). The slides were then incubated with 0.74 or 1.302 nM [18F]9 for 90 min at room temperature, with or without losartan (10 μM) (n = 6 each). Following incubation, the slides were washed sequentially in deionized water, buffer, and deionized water (2 × 2 min each, 4 °C) and then dried. Sections were exposed along with a set of different concentrations of [18F]9 as radioactivity standards to an imaging phosphor screen (Amersham Bioscience) for 2 h. The screen was imaged at 50 μm resolution with a molecular imager Typhoon Trio (Amersham) and analyzed using ImageJ 1.52a software (NIH, USA). Quantification of the samples was performed by manually tracing the kidney cortex; the corresponding tracer binding (fmol/cm2) was calculated.

4.5. Statistical Analyses. All results are expressed as mean ± standard deviation (SD), as indicated. Biodistribution data were analyzed using one-way ANOVA, followed by Tukey’s post hoc test. Two-way ANOVA followed by Bonferroni’s post hoc test was used to determine the source of variability and difference between groups in autoradiography studies. Differences were considered statistically significant when p < 0.05. All statistical analyses were performed using Prism (GraphPad Software; version 8.4.1).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02310.
Comparison of HPLC retention times of fluoropyridine–candesartan (9) and nitropyridine–candesartan (11) and characterization data of novel compounds (HRMS and NMR spectra) (PDF)

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Notes
The authors declare no competing financial interest.

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