Novel $^{18}$F-Labeled PET Imaging Agent FV45 Targeting the Renin–Angiotensin System

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ABSTRACT: Renin–angiotensin system (RAS) plays an important role in the regulation of blood pressure and hormonal balance. Using positron emission tomography (PET) technology, it is possible to monitor the physiological and pathological distribution of angiotensin II type 1 receptors (AT1), which reflects the functionality of RAS. A new $^{18}$F-labeled PET tracer derived from the clinically used AT1 antagonist valsartan showing the least possible chemical alteration from the valsartan structure has been designed and synthesized with several strategies, which can be applied for the syntheses of further derivatives. Radioligand binding study showed that the cold reference FV45 (Ki 14.6 nM) has almost equivalent binding affinity as its lead valsartan (Ki 11.8 nM) and angiotensin II (Ki 1.7 nM). Successful radiolabeling of FV45 in a one-pot radiofluorination followed by the deprotection procedure with 21.8 ± 8.5% radiochemical yield and >99% radiochemical purity (n = 5) enabled a distribution study in rats and opened a path to straightforward large-scale production. A fast and clear kidney uptake could be observed, and this renal uptake could be selectively blocked by pretreatment with AT1-selective antagonist valsartan. Overall, as the first $^{18}$F-labeled PET tracer based on a derivation from clinically used drug valsartan with almost identical chemical structure, $[^{18}$F]$^{}$FV45 will be a new tool for assessing the RAS function by visualizing AT1 receptor distributions and providing further information regarding cardiovascular system malfunction as well as possible applications in inflammation research and cancer diagnosis.

INTRODUCTION

The renin–angiotensin system (RAS) is a hormonal cascade that generates angiotensin peptides and is the main regulator of blood pressure as well as fluid and electrolyte balance.¹ The key mediator is the octapeptide angiotensin II, which stimulates mainly angiotensin II type 1 receptor (AT1), a G-protein coupled receptor, and thereby initiates further downstream effects and leads to vasoconstriction. AT1 receptors are mainly located in the heart, blood vessels, and kidneys. Therefore, it is currently one of the major therapeutic targets in the treatment of hypertension and heart failure (HF).² Furthermore, the angiotensin receptor/neprilysin inhibitor combination valsartan/sacubitril (LCZ696) has shown improved cardiovascular outcomes than ACE inhibitor enalapril as achieved in the prospective comparison of ARNI with ACEI to determine impact on global mortality and mobility in heart failure.³ Its approval (commercial name Entresto by Novartis) for the treatment of HF in both the United States and Europe is considered a further milestone for the application of AT1 antagonists.

In addition to the central role of RAS in the regulation of cardiovascular system mentioned above, it is also involved in a much broader range of functions in the body, including actions on growth factors, inflammation, mitosis, and cancer pathogenesis.¹ The majority of these functions are through the regulation of AT1 receptors. Several clinical investigations involving a large number of patients in recent years have been conducted to explore the possibility of using AT1 antagonists against several forms of cancer, e.g., prostate,⁴ lung,⁵ colon,⁶ and breast cancer.⁷ However, it should be mentioned that the results obtained remain controversial and the mechanism of these antitumor functions is not yet clarified. Studies have suggested that AT1 receptor level changes during disease progression, while AT1 blockade seems to be able to adjust the consequences of such alterations.⁸–¹² Therefore, AT1 level is considered a key reflector of RAS function. Molecular imaging
technique is ideal for monitoring in vivo AT₁ levels non-invasively. There is a high density of AT₁ receptors in the kidneys responsible for the regulation of blood pressure and furthermore functions. Thereby, in animal studies, the kidney represents the primary organ of RAS imaging to be investigated.

In contrast to anatomical techniques, molecular imaging techniques using radionuclide tracers focus more on subcellular down to molecular-level events. They provide a noninvasive method to examine functional changes in individual organs, with high sensitivity, specificity, and the possibility of quantifying alterations. Moreover, the rational design of radiotracers based on biological and chemical knowledge makes it feasible to bring forth valuable pathophysiological information in patients and further insight into in vivo conditions of specific targets. Currently, positron emission tomography (PET) is receiving more attention due to several advantages compared to single-photon emission computed tomography, namely, its higher count sensitivity, higher temporal and spatial resolution, as well as its ability to be used in dynamic and quantitative studies. In PET applications with the possibility of performing dynamic and quantitative studies, new fluorine-18-based radiotracers bring forth improved imaging characteristics over carbon-11-labeled counterparts. The use of fluorine-18 compensates the high cost for on-site radioisotope production due to its reasonably longer half-life (110 min) over carbon-11 (20 min), which has been one of the factors limiting the wide utilization of PET. This allows multiple scans each day with barely any in-patient cost. Besides, the application of fluorine-18 in PET may also provide higher flexibility in the design and syntheses of novel PET tracers together with an improvement of in vivo stability by reduced metabolism.

Figure 1. Chemical structures of currently reported AT₁ antagonist-derived radiotracers and their corresponding lead compounds.

Currently, positron emission tomography (PET) is receiving more attention due to several advantages compared to single-photon emission computed tomography, namely, its higher count sensitivity, higher temporal and spatial resolution, as well as its ability to be used in dynamic and quantitative studies. In PET applications with the possibility of performing dynamic and quantitative studies, new fluorine-18-based radiotracers bring forth improved imaging characteristics over carbon-11-labeled counterparts. The use of fluorine-18 compensates the high cost for on-site radioisotope production due to its reasonably longer half-life (110 min) over carbon-11 (20 min), which has been one of the factors limiting the wide utilization of PET. This allows multiple scans each day with barely any in-patient cost. Besides, the application of fluorine-18 in PET may also provide higher flexibility in the design and syntheses of novel PET tracers together with an improvement of in vivo stability by reduced metabolism.

Figure 2. Chemical structures of valsartan and the proposed ¹⁸F-labeled radiotracer [¹⁸F]FV45.
approach to achieve a corresponding precursor (on which the radioactive isotope is introduced to form the target radioactive tracer) would be chemically demanding. Since fluorine is smaller than hydrogen and has neutral properties, this modification should maintain its affinity and give access to a comparatively straightforward chemical synthetic scheme. In addition, fluorination on this position might block the formation of a potential metabolite, nonactive 4-hydroxyvalsartan, and consequently, alter the pharmacokinetics of this tracer, for instance, by decreasing liver uptake and increasing renal excretion.

**RESULTS**

**Chemistry.** To prepare the precursor for labeling, different synthetic approaches have been investigated for the preparation of both the precursor and the corresponding cold compound (nonradioactive fluorine derivate). Diphenyl tetrazole moiety was prepared according to the procedure described in the literature (Scheme 1). In the first attempt to build the precursor, 5-chlorovaleric acid was attached to the diphenyl valinate moiety, which was formed by reacting diphenyl tetrazole moiety with tert-butyl valinate. The chlorine atom in compound was replaced by iodine through the Finkelstein reaction. While trying to
introduce a tosylate moiety into compound 9 to serve as a leaving group for fluorination, the p-toluenesulfonic acid in silver tosylate leads to deprotection of the trityl group of the tetrazole and no product 10 was formed. The reaction could not take place either when adding 10% base in the attempt to neutralize excessive acid. Subsequently, a second attempt to achieve ω-hydroxyvalsartan 12 was performed by reacting the diphenyl valinate moiety 7 with δ-valerolactone 11 in different solvents, including chloroform, toluene, and xylene, at various temperatures. However, none of them gave desired products. While using aluminum chloride-catalyzed ammonolysis of compound 7 with lactone 11, the starting material decomposed in the reaction condition with no product formation. Using silver fluoride in an effort to replace either chloride of compound 8 or iodide of compound 9 also turned out to be unsuccessful and produced mainly ω-hydroxyvalsartan 12. Unfortunately, an attempt to react this byproduct compound 12 with tosyl chloride to get the precursor 10 failed, too. All unsuccessful attempts to achieve precursor and cold compound are illustrated in Scheme 2.

The final synthetic procedure used the acyl chain with either fluoride (for the cold reference) or tosylate (for the precursor) already connected, followed by coupling with the diphenyl valinate moiety 7. Accordingly, the successful approach started from 5-chlorovaleric acid 5, which was first esterified to protect the carboxyl group. The chlorine atom in compound 13 was
replaced by iodine through the Finkelstein reaction. This gave the possibility of further replacement by tosylate using silver tosylate, which was obtained by mixing equal molar quantities of silver oxide with 4-toluenesulfonic acid in acetonitrile in darkness. After removal of the benzyl ester selectively under mild hydrogenation conditions without affecting the tosylate, acid 17 reacted with oxalyl chloride in dry dichloromethane to form acid chloride 18. It was then reacted with diphenyl valinate moiety 7, and precursor 10 became available for labeling (Scheme 3). The fluoride atom of the cold compound was introduced by replacing the tosyl in intermediate 15. Fluorination conditions could also be evaluated as a reference for the radiolabeling procedure. Thereafter, FV45 was synthesized analogously as the precursor, with the only difference of using benzyl valinate 21 instead of tert-butyl ester (Scheme 3). The benzyl ester group is more stable during preparation, whereas tert-butyl ester in the precursor could be concomitantly removed after radiolabeling together with the trityl group under acidic condition.

**Radiochemistry.** As a good leaving group, a tosyl moiety could be substituted by fluorine-18 in the labeling procedure. Different solvents, reacting temperature, and sources of \(^{18}\text{F}\) were investigated. Dimethylformamide (DMF) provided high reaction temperature up to 140 °C. Acetonitrile gave mild reaction condition and temperature and is also easier to provide anhydrous condition by azeotropic distillation. In addition, Cs\(^{18}\text{F}\), K\(^{18}\text{F}\) and tetrabutylammonium \(^{18}\text{F}\) (\([^{18}\text{F}]\text{TBAF}\)) were used as sources of fluorine-18. Although all of these sources and conditions succeeded in radiolabeling, K\(^{18}\text{F}\) in acetonitrile with Kryptofix\(_{222}\) as phase transfer catalyst was selected as the most conventional and conveniently performed procedure. The last step, deprotection of both trityl on tetrazole and tert-butyl ester group, was first set for 10 min at 110 °C. During the purification via semi-preparative high-performance liquid chromatography (HPLC), the formation of a 30–50% of byproduct was observed. This byproduct was later identified as the tert-butyl ester of FV45 (Figure S2). As it had turned out that the tert-butyl ester is more stable than expected, the reaction time for deprotection was prolonged to 20 min. The intermediate was then also transformed to the target tracer, and the yield of \(^{18}\text{F}\)FV45 was greatly improved (Figure S3). Finally, a purification step using semi-preparative HPLC was still needed since the majority of the precursor decomposed during radiofluorination procedure and a couple of byproducts formed that could not be simply removed with a C18 Sep-Pak. In the end, with the improved labeling condition and procedure, the total synthesis of labeling takes approximately 120 min. The overall average radiochemical yield was 21.8 ± 8.5% (decay-corrected based on the starting radioactivity, calculated from 5 times of labeling records), and radiochemical purity was >99%. In summary, with one-pot reaction and straightforward labeling procedure, the rationale of the tracer design was successfully achieved. Additionally, such procedure would be easily scaled up for further preclinical or even clinical studies, with the possibility of using fully automated labeling system for tracer synthesis.

**Binding Studies on Human AT\(_1\) Receptors.** To evaluate the binding affinity of FV45 at AT\(_1\) receptors, it is necessary to compare the cold reference with both its lead valsartan and angiotensin II. By doing so, it would be able to confirm that such strategy of designing tracers from the corresponding clinically used drug is principally feasible. It would also provide further insight into the strategy, whether the introduction of fluorine at the terminal of the acyl chain would affect the binding affinity of competitive binding affinity of FV45, which retained in the same range as its lead valsartan (K\(_i\) value FV45 14.6 nM vs valsartan 11.8 nM), while slightly less compared to angiotensin II (1.7 nM). The almost identical affinity profile enables the application of our synthesis procedure to other sartans with similar structures to derive corresponding tracers or to develop further sartan derivatives for therapeutic purposes, for instance, to change bioavailability or pharmacokinetics.

**Renal Imaging Studies in Rats.** Standard protocols and data analysis methods for noninvasive PET imaging of small animals have been well established in our working group. After \(^{18}\text{F}\)FV45 was successfully labeled as described above and radioactive purity was confirmed, the anesthetized rats of the control group were injected the tracer via the tail vein. To determine the specificity of \(^{18}\text{F}\)FV45, the rats were first treated with AT\(_1\) antagonist valsartan 10 min before the tracer was...
administered. A 60 min list-mode PET acquisition focusing on the kidney area was started shortly after the injection because there is high density of AT$_1$ receptors expressed in the kidney. The static images obtained demonstrated distinct $[^{18}F]$FV45 uptake in the kidney. Preadministration of AT$_1$ antagonist valsartan distinctly inhibits the kidney coronal uptake of this derived tracer $[^{18}F]$FV45.

**Figure 4.** Kidney uptake of $[^{18}F]$FV45 as control (left) and blockade by selective AT$_1$ antagonist valsartan (right). Preadministration of AT$_1$ antagonist valsartan distinctly inhibits the kidney coronal uptake of this derived tracer $[^{18}F]$FV45.

**Figure 5.** Dynamic coronal uptake of $[^{18}F]$FV45 as control (top) and blockade by selective AT$_1$ antagonist valsartan (bottom). Every 5 min is a time frame. Co-injection of AT$_1$ antagonist valsartan clearly reduced the kidney uptake of $[^{18}F]$FV45 that shows a selective AT$_1$ receptor-targeting mechanism.
accumulation in the kidneys, mainly renal cortex. Valsartan visibly inhibited kidney uptake of $[^{18}F]FV45$ (Figure 4), which indicates that the tracer uptake is specific to the AT$_1$ receptor. Further dynamic imaging of the tracer revealed that there was a fast and high uptake of $[^{18}F]FV45$ in the coronal section of the kidney 10 min after injection (Figure 5). This uptake could be blocked by pretreatment of valsartan, and no uptake in the kidney could be observed during the whole imaging time frame. In short, despite some unfavorable uptake into the liver (Figure 5, top), the selective uptake of $[^{18}F]FV45$ into renal cortex was clearly observed.

# DISCUSSION

The RAS plays a key role not only in renal and cardiovascular diseases but also in processes of inflammation and cancer cell proliferation and angiogenesis. Currently, there are only a couple of AT$_1$-targeting PET tracers reported in the literature. Most of them are connections of easily labeled chemical moieties and the parent sartan compounds, i.e., a simple connection without paying attention to SARs of these compounds, which may significantly change the binding properties at AT$_1$ receptor as well as pharmacokinetics and pharmacodynamics. These factors might be crucial to reflect the true status of RAS in the body under pathophysiological conditions. Therefore, it seems necessary to develop a novel class of $^{18}$F-labeled radiotracers targeting RAS through medicinal chemical techniques and applying rational drug design methodology, exemplified here with o-fluoro-valtsartan (FV45) as the first of its kind. By analyzing the SARs of sartan compounds, it was possible to design and develop compounds that retain or even improve the original ones' pharmacodynamic and pharmacokinetic properties.

Successful syntheses of the cold reference of FV45 and the corresponding precursor was achieved. The improved synthetic scheme is suitable to be scaled up, and the strategy could be applied to other sartans with similar structures, especially regarding the fluorine substitution and its position, as well as the chemical synthesis design. The introduction of fluorine would be ideal in any aliphatic moieties that bind to the same hydrophobic binding pocket of AT$_1$ receptor as illustrated in the SARs of losartan. For example, losartan, candesartan, irbesartan, and olmesartan all have such aliphatic tail in their structures, where the fluorine could be introduced to produce PET tracer following the same strategy. The radiolabeling of FV45 was carried out under no-carrier-added K$[^{18}F]$F/Kryptofix$_{2.2.2}$ condition using dry acetonitrile as solvent. Good yields, as well as high purity, could be achieved within the short-time labeling procedure, which might also serve as a standard labeling procedure for other sartan tracers derived from comparable principles due to their structural similarity.

Competitive binding assay proved that FV45 has similar binding affinity (17.4 nM) to the AT$_1$ receptor as angiotensin II (2.0 nM) and its lead valsartan (14.0 nM). In PET imaging studies in rats, clear and fast kidney uptake was observed after administration of $[^{18}F]FV45$. Renal imaging instead of cardiac imaging was performed because the distribution of AT$_1$ receptors in the kidney is highly conserved in all mammals, whereas in the rat heart, only low density of AT$_1$ receptors is expressed in the atrial and ventricular myocardium. Furthermore, the uptake in the kidney could be blocked by pretreatment of its parent compound—the selective AT$_1$ antagonist valsartan. It confirmed not only the specificity of FV45, but also our strategy to evolve AT$_1$ PET tracers from clinically used sartans with the minimum chemical modification. It is noteworthy that the tracer has a relatively short retention time in the kidneys, as can be seen from the dynamic scan. We do not consider this as a negative property for a novel designed tracer though: no obvious decomposition and/or metabolism happens in vivo, and no free fluorine-18 is released into the circulation system or long-term bone marrow storage (Figure 5). Approximately 15 min after tracer injection, $[^{18}F]FV45$ was washed out of the kidneys. This corresponds to one key point in our compound design that excretion from kidneys might be increased compared to the lead compound valsartan, or both sartan tracers mentioned above. The following two factors should be taken into account to give reasonable explanations for these changes: (a) metabolism and excretion in rodents are on average faster than in humans and (b) the relatively more polar structure of valsartan in comparison to other AT$_1$ antagonists makes it quickly excreted in the urine. This faster excretion might be influenced by the structural modification, i.e., the fluorination, since valsartan has an average half-life of around 6 h. The advantage of the fast excretion decreases the possibility of its accumulation in body and the consequent side effects. However, the strategy provided a basis for further tracer development, such as using alternative sartans with naturally longer half-life, which might be helpful to improve this property.

Furthermore, AT$_1$ antagonists also have the therapeutic potential, in particular, enhancing immunotherapy in cancer. As a consequence, a PET tracer, such as $[^{18}F]FV45$, might also be used in investigating RAS functions in oncology, e.g., in diagnosis of cancers. It could also be helpful in answering questions like whether AT$_1$ antagonists could interfere in the newly discovered ACE2/Ang-(1-7)/Mas axis of the RAS. In addition, provided that the iodine derivative of a sartan, such as $^{131}$I instead of $^{18}$F retains similar properties in vitro and in vivo, such compounds might hold potential for application in cancer therapy. Moreover, such modification derived from the clinically used sartans with the least structural changes might also provide novel directions in AT$_1$ antagonist development with regard to better pharmacokinetics and pharmacodynamics.

# CONCLUSIONS

The first of a new generation of PET tracers derived from clinically used AT$_1$ receptor antagonists targeting the RAS system with minimum structural modification has been designed, successfully synthesized, and evaluated both in vitro and in vivo. Fluorine-18 instead of carbon-11 was selected for radiolabeling due to its longer half-life and advantages in precursor design. The labeling procedure has been optimized with good yield and high radiochemical purity suitable for mass production. As bioisostere of hydrogen, fluorine could be introduced into the aliphatic tail of sartans without affecting the binding affinity. As a result, $[^{18}F]FV45$ showed almost identical AT$_1$ receptor binding affinity as its lead compound valsartan. An imaging study using Wistar rats showed fast and clear kidney uptake, which could be blocked selectively by pretreatment with the AT$_1$ antagonist valsartan. Overall, the strategy of evolving novel PET tracers from clinically used sartans with the least structural changes may point out a new direction in RAS imaging and even facilitate translational work to humans, exemplified here with valsartan derived tracer $[^{18}F]FV45$ that showed almost identical affinity in binding assay and good imaging properties of kidneys in rats. The work of this paper has been included in and filed as patent.
EXPERIMENTAL SECTION

Common reagents and solvents were obtained from commercial suppliers and were used without any further purification. Tetrahidrofuran (THF) was distilled from sodium/benzophenone under argon atmosphere. The reaction progress was monitored by using analytical thin-layer chromatography on precoated silica gel GF254 plates (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and spots were detected under UV light (λ = 254 nm) or by staining with iodine. NMR spectra were performed with a Bruker AV-400 NMR instrument (Bruker, Karlsruhe, Germany) in [D6]DMSO or CDCl3. Chemical shifts are expressed in parts per million relative to CHCl3/dimethyl sulfoxide (DMSO) (δ = 7.26/2.50 and 77.16/39.52 ppm for 1H- and 13C NMR spectroscopy, respectively). For purity and reaction analyzes, analytical HPLC analysis was performed with a system from Shimadzu equipped with a DGU-20A3R controller, LC20AB liquid chromatograph, and an SPD-20A UV/Vis detector. Stationary phase was a Synergy 4μm fusion-RP (150× 4.6 mm2) column (Phenomenex, Aschaffenburg, Germany). As mobile phase, H2O (phase A) and methanol (phase B) were used with 1 mL/min (conc. B: 5 µL to 90% from 0 to 8 min; 90% from 8 to 13 min; 90% to 5% from 13 to 15 min; 5% from 15 to 18 min). All target compounds were confirmed with purity over 95%. Electrospray ionization (ESI) mass spectral data were acquired with a Shimadzu LCMS-2020.

(S)-tert-Butyl 3-Methyl-2-(((2’-1-trityl-1H-tetrazol-5-yl)-[1,1’-biphenyl]-4-yl)-methylamino)butanoate (7). 2-Tetrazol(2-trityl)-4-methylbromide-biphenyl 4 (2.00 g, 3.59 mmol), and benzyl bromide (867 μmol) in toluene were stirred at room temperature (130.23, 130.20, 129.86, 129.15, 128.61, 128.43, 128.37, 128.30, 128.19, 127.70, 127.65, 127.61, 127.37, 126.42, 82.87, 66.96, 66.31, 52.27, 40.88, 31.70, 19.39, 18.61 ppm.

Benzyl 5-Chloro-1H-tetrazole (13). 5-Chlorotetrazolic acid 5 (1 g, 7.3 mmol) and benzyl bromide (867 μL, 7.3 mmol) were dissolved in acetonitrile. Sodium carbonate was added to the above solution. The mixture was heated to reflux under argon for 15 h. The reaction solution was cooled and concentrated under vacuum. The residue was diluted with diethyl ether (30 mL) and washed with water (10 mL) and then brine (10 mL). The organic phase was dried over sodium sulfate and concentrated under vacuum to afford the product as a colorless oil (1.65 g, 100%), which was used in the next step.

Benzyl 5-Iodovalerate (14). Benzyl 5-chlorovalerate 13 (3.17 g, 13.98 mmol) was dissolved in acetonitrile. Sodium iodide (2.60 g, 17.35 mmol) was added to the solution. This mixture was heated to reflux under argon for 5 h. The formed white solid was filtered off and the filtrate was removed under vacuum. The residue was diluted with diethyl ether (30 mL) and washed with water (10 mL) and then brine (10 mL). The organic phase was dried over sodium sulfate and concentrated under vacuum to afford the product as a colorless oil (3.51 g, 79%). 1H NMR (400 MHz, CDCl3) δ: 7.38–7.34 (m, 5H), 5.12 (s, 2H), 3.18 (t, 2H, J = 1.74 Hz), 2.39 (t, 2H, J = 2.39 Hz), 1.88–1.74 (m, 4H) ppm; 13C NMR (101 MHz, CDCl3) δ: 173.04, 136.07, 128.73, 128.41, 128.19, 128.02, 127.91, 127.90, 127.83, 127.67, 83.16, 67.85, 52.39, 32.04, 25.84, 19.67, 18.96 ppm.

5-(Toslyloxy)pentanamide (10). The solution of benzyl 5-(toslyloxy)pentanoate 15 (500 mg, 1.38 mmol) in ethanol (10 mL) was added palladium on charcoal (50 mg) under argon atmosphere. The flask was equipped with a hydrogen balloon. The gas in the flask was exchanged. After stirring vigorously for 3 h at room temperature, the catalyst was filtered off through celite and the filtrate was concentrated. The compound was obtained as a colorless oil (360 mg, 96%). 1H NMR (400 MHz, CDCl3) δ: 7.79–7.32 (m, 9H), 5.09 (s, 2H), 4.04–4.01 (t, 2H), 2.44 (s, 3H), 2.34–2.31 (t, 2H), 1.71–1.67 (m, 4H) ppm; 13C NMR (101 MHz, CDCl3) δ: 172.78, 69.88, 66.28, 33.37, 28.20, 21.63, 20.89 ppm.

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separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and dried over anhydrous sodium sulfate. The compound was obtained after concentration and purification via column chromatography (petroleum ether/ethyl acetate, 3:1) as a yellow oil (100 mg, 36%). ESI-MS: 926.4 m/z [M + Na]⁺.

\(^1\)H NMR (400 MHz, CDCl₃) δ: 7.80−7.62 (m, 3H), 7.44−7.36 (m, 3H), 7.22−7.19 (m, 1H), 7.05−6.86 (m, 7H), 4.74−4.30 (m, 3H), 3.99 and 3.82 (dt, 2H, J = 3.82 Hz, 2.42−1.97 (m, 3H), 2.34 (s, 3H), 1.68−1.67 (m, 2H), 1.44−1.41 (m, 2H), 1.22−1.20 (d, 9H), 0.91−0.75 (m, 6H) ppm; \(^13\)C NMR (101 MHz, CDCl₃) δ: 171.16, 164.15, 146.90, 141.32, 133.11, 130.79, 130.21, 129.81, 129.53, 129.27, 128.29, 128.22, 128.08, 127.94, 127.90, 127.67, 127.26, 127.23, 125.73, 82.88, 81.59, 70.29, 60.40, 32.79, 27.82, 27.71, 23.85, 21.61, 21.04, 14.20 ppm.

Benzyl 5-Fluorovalerate (16). The mixture of benzyl 5-(tosloyloxy)pentanoate (15) (500 mg, 1.38 mmol), potassium fluoride (80 mg, 1.38 mmol), and 18-crown-6 (364 mg, 1.38 mmol) in dry dichloromethane (5 mL) was added oxalyl chloride (51 mg, 0.5 mmol) in the presence of palladium on charcoal (6 mg) in 0.7 mL of dry acetonitrile. Azeotropic drying of the solution containing various concentrations of test compounds (radioactivity of \(^18\)O was isolated by trapping on Sep-Pak Light QMA cartridge, followed by washing with 3 mL of water. Fluoride was eluted with a solution of K₂CO₃ in 0.3 mL of water (50.6 mM) into a sealed glass vial containing a solution of Krypto-10470·(2′-(1H-Tetrazol-5-yl)-[1,1′-biphenyl]-4-yl)-methyl-5-fluoropentanamido)-3-methylbutanoic Acid (FV45). To the solution of compound 21 in methanol was added palladium charcoal. The resulting mixture was stirred vigorously under hydrogen atmosphere overnight. The catalyst was filtered and the filtrate was concentrated under vacuum. The residue was dissolved in dry dichloromethane followed by formic acid. The solution was stirred at room temperature for 2 h. The reaction solution was concentrated under vacuum. The residue was purified via column chromatography using petroleum ether/ethyl acetate/formic acid (1:1:0.05) as the eluent system. The target cold compound was obtained as a colorless oil (48 mg, 45%). ESI-MS: 454.3 m/z [M + H]⁺. \(^1\)H NMR (400 MHz, CDCl₃) δ: 7.98−7.96 (m, 1H), 7.60−7.44 (m, 3H), 7.17−7.11 (m, 3H), 6.97−6.95 (m, 1H), 4.90 and 4.31 (dd, 2H); 4.53 and 4.41 (dt, 2H); 4.02 and 3.68 (dd, 1H); 2.63−2.39 (m, 3H); 1.87−1.73 (m, 4H); 0.98 (d, 3H); 0.94 (d, 3H) ppm; \(^13\)C NMR (101 MHz, CDCl₃) δ: 176.33, 172.74, 154.60, 140.31, 139.25, 135.19, 131.31, 131.09, 131.30, 129.21, 129.81, 129.53, 129.27, 128.29, 128.22, 128.08, 127.94, 127.90, 127.67, 127.26, 127.23, 125.73, 82.88, 81.59, 70.29, 60.40, 32.79, 27.82, 27.71, 23.85, 21.61, 21.04, 14.20 ppm.

Radiochemistry. \(^{[18]}\)F⁻ produced via proton bombardment of H₂¹⁸O was isolated by trapping on Sep-Pak Light QMA cartridge, followed by washing with 3 mL of water. Fluoride was eluted with a solution of K₂CO₃ in 0.3 mL of water (0.56 mM) into a sealed glass vial containing a solution of Krypto-10470·(2′-(1H-Tetrazol-5-yl)-[1,1′-biphenyl]-4-yl)-methyl-5-fluoropentanamido)-3-methylbutanoic Acid (FV45). To the solution of compound 21 in methanol was added palladium charcoal. The resulting mixture was stirred vigorously under hydrogen atmosphere overnight. The catalyst was filtered and the filtrate was concentrated under vacuum. The residue was dissolved in dry dichloromethane followed by formic acid. The solution was stirred at room temperature for 2 h. The reaction solution was concentrated under vacuum. The residue was purified via column chromatography using petroleum ether/ethyl acetate/formic acid (1:1:0.05) as the eluent system. The target cold compound was obtained as a colorless oil (48 mg, 45%). ESI-MS: 454.3 m/z [M + H]⁺. \(^1\)H NMR (400 MHz, CDCl₃) δ: 7.98−7.96 (m, 1H), 7.60−7.44 (m, 3H), 7.17−7.11 (m, 3H), 6.97−6.95 (m, 1H), 4.90 and 4.31 (dd, 2H); 4.53 and 4.41 (dt, 2H); 4.02 and 3.68 (dd, 1H); 2.63−2.39 (m, 3H); 1.87−1.73 (m, 4H); 0.98 (d, 3H); 0.94 (d, 3H) ppm; \(^13\)C NMR (101 MHz, CDCl₃) δ: 176.33, 172.74, 154.60, 140.31, 139.25, 135.19, 131.31, 131.09, 131.30, 129.21, 129.81, 129.53, 129.27, 128.29, 128.22, 128.08, 127.94, 127.90, 127.67, 127.26, 127.23, 125.73, 82.88, 81.59, 70.29, 60.40, 32.79, 27.82, 27.71, 23.85, 21.61, 21.04, 14.20 ppm.

**Competitive Binding Study.** The radioligand binding assay was performed by using cell membrane preparations from CHO-K1 cells expressing human AT₁ receptors (Membrane Target Systems; PerkinElmer, Waltham, MA). Human AT₁ receptor (0.6 µg of membrane protein/well) was incubated with assay buffer (50 mM Tris-HCl, 5 mM MgCl₂, pH 7.4) containing various concentrations of test compounds (radiotracer) and 125I-Sar₁-Ile₆-AngII (final concentration, 0.3 nM) in 200 µL total volume in 96-well plate at room temperature. After 1 h, the plate was washed nine times with 250 µL/well of wash buffer (50 mM Tris-HCl, pH 7.4) to remove unbound tracer.
Membrane-bound radioactivity was counted using a γ-counter (FH 412; Frieseke & Höpfner, Erlangen, Germany). Non-specific binding of $^{125}$I-Sar$^1$-Ile$^8$-AngII was estimated in the presence of 10 M unlabeled AngII. Specific binding is defined as total binding minus non-specific binding. $K_i$ values are calculated from the $IC_{50}$ values using the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + [L]/K_d)$, where the final concentration of radioligand is 0.03 nM and $K_d$ of $^{125}$I-Sar$^1$-Ile$^8$-AngII is 0.16 nM.

**Animal Imaging Study.** Healthy male Wistar rats (weighing 200–250 g) were used. The animal protocols were approved by the local institutional animal care and use committee and were conducted strictly according to the Guide for the Care and Use of Laboratory Animals. The rats were maintained under anesthesia by 2% isoflurane during the whole experiment. $[^{18}F]$FV45 (20–25 MBq) was administered via the tail vein as two different medications: intravenous injection of the tracer ($n = 3$); a pretreatment of 30 mg/kg valsartan orally 3 h before the study and 10 min intravenous before tracer delivery. Imaging was performed using a dedicated small-animal PET system (Inveon, Siemens Healthcare). A 60 min list-mode PET acquisition was started shortly after injection. The reconstructed PET images were analyzed using an imaging-processing application (AMIDE-bin, version 1.0.2).

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