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Synthesis and Evaluation of [¹⁸F]RAGER: A First Generation Small-Molecule PET Radioligand Targeting the Receptor for Advanced Glycation Endproducts

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Abstract

The receptor for advanced glycation endproducts (RAGE) is a 35kDa transmembrane receptor that belongs to the immunoglobulin superfamily of cell surface molecules. Its role in Alzheimer's disease (AD) is complex, but it is thought to mediate influx of circulating amyloid- β into the brain as well as amplify A β -induced pathogenic responses. RAGE is therefore of considerable interest as both a diagnostic and a therapeutic target in AD. Herein we report the synthesis and preliminary pre-clinical evaluation of [¹⁸F]RAGER, the first small molecule PET radiotracer for RAGE (K_d = 15 nM). Docking studies propose a likely binding interaction between RAGE and RAGER, [¹⁸F]RAGER autoradiography showed co-localization with RAGE identified by immunohistochemistry in AD brain samples, and [¹⁸F]RAGER microPET confirmed CNS penetration and increased uptake in areas of the brain known to express RAGE. This 1st generation radiotracer represents initial proof-of-concept and a promising first step towards quantifying CNS RAGE activity using PET. However, there were high levels of non-specific [¹⁸F]RAGER binding *in vitro*, likely due to its high logP (experimental logP = 3.5), and rapid metabolism of [¹⁸F]RAGER in rat liver microsome studies. Therefore development of 2nd generation ligands with improved imaging properties would be advantageous prior to anticipated translation into clinical PET imaging studies.

Graphical Abstract

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Supporting Information. Experimental procedures and spectra for all novel compounds synthesized; procedures for binding affinity experiments, docking experiments, microPET imaging, liver microsome metabolism studies, logP determination, autoradiography and immunohistochemistry. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

RAGE; radiotracer; positron emission tomography; fluorine-18

INTRODUCTION

Positron emission tomography (PET) is a powerful functional imaging technique for examining neurobiological parameters *in vivo*. Its use for clinical Alzheimer's disease (AD) research is extensive, focusing initially on the quantification of insoluble protein aggregates that accumulate in AD. Most efforts have concentrated on developing radiotracers to image beta-amyloid ($\text{A}\beta$) deposits, and notable probes in this category include AMYViD ($[^{18}\text{F}]\text{AV-45}$), Neuraceq, Vizamyl, and $[^{11}\text{C}]\text{Pittsburgh Compound B}$ ($[^{11}\text{C}]\text{PiB}$).^{1,2,3} More recently, focus has shifted to development of radiotracers that can quantify tau neurofibrillary tangles.⁴ The ability to quantify the pathology of amyloid and tau formation in AD will be critical in the development of disease modifying therapies for these targets. However, imaging these protein aggregates is only part of the story^{5,6} as they represent only two components of a complex and lengthy cascade. While the role of amyloid and tau in AD pathology is increasingly understood, the entire AD cascade that ultimately leads to severe cognitive decline remains ambiguous. When this gap in knowledge is considered along with the immense socioeconomic burden of AD,^{7,8} the urgent need for a series of PET radioligands to better understand the entire pathway of AD progression and support AD therapeutic development efforts is apparent (for recent perspectives on other potential imaging targets in AD, see: ^{9,10}). To this end, our lab and others have been working extensively to develop PET probes relevant to AD outside the scope of traditional $\text{A}\beta$ targeting, such as radioligands with affinity for metal- $\text{A}\beta$ species,^{11,12} glutaminyl-cyclase,¹³ and glycogen synthase kinase-3.¹⁴ Notably, we are increasingly interested in using PET to understand mechanisms underlying the increased accumulation of amyloid in the central nervous system (CNS) in AD. This includes potential upregulation of mediators responsible for moving amyloid across the blood-brain barrier into the CNS (e.g. Receptor for Advanced Glycation Endproducts (RAGE)), as well as down regulation of transporters responsible for clearing it back out again (e.g. P-glycoprotein (Pgp) and aquaporin-4). While there are PET radiotracers for Pgp¹⁵ and aquaporin-4,¹⁶ there are no small molecule radiotracers for quantifying CNS RAGE activity. Such a radiotracer could allow detection of AD prior to onset of amyloid accumulation and therefore, in an attempt to bolster the constellation of

alternative AD imaging agents, we examined the feasibility of RAGE as a target for AD imaging using PET.

RAGE is a 35kDa transmembrane receptor that belongs to the immunoglobulin superfamily of cell surface molecules. RAGE binds a variety of ligands,¹⁷ and is heavily implicated in AD¹⁸ where it is thought to mediate the transport of peripheral A β across the BBB.¹⁸ Though the exact mechanism of transport is unknown, it has been shown in cell assays that A β binding to RAGE initiates an inflammatory response and alters the tight junctions through the Ca²⁺-Calcineurin pathway.¹⁹ Although in mice it has been suggested that the organic anion transporter polypeptide (Oatp1a4) is responsible for importing A β into the CNS, no comparable transporter has been identified in humans.²⁰ Therefore this loss of BBB integrity may simply lead to an increase in the influx of A β into the brain. Moreover, RAGE-dependent signaling mediates downregulation of exporters of A β ,²¹ upregulation of β -secretase,²² neuroinflammatory responses,²³ and stimulates tau phosphorylation.¹⁷ RAGE is upregulated and amplifies early A β neurotoxicity when A β levels are low.^{24,25} Low doses of Azeliragon (TTP-448), a RAGE antagonist, were associated with decreased cognitive decline relative to placebo in Phase 2 clinical trials for mild to moderate AD.²⁶

There are 22 documented isoforms of RAGE in humans, 12 of which are found in the brain.²⁷ These isoforms vary by alternative splicing, which includes soluble form in addition to the cell membrane bound receptors. A β binds to the V domain of RAGE,²⁸ which is varied in the different isoforms. In AD, there is an overexpression of RAGE and a lower amount of the soluble splice variant sRAGE, which is hypothesized to negatively regulate signal transduction of full length RAGE.²⁹ The relationship between RAGE and its various isoforms is not completely understood. PET radiotracers would aid in elucidating the complexity of this receptor and its dynamic relationship with A β in early stages of AD.

Prior efforts to produce radioligands for *in vivo* RAGE quantification have yielded a ^{99m}Tc monoclonal antibody³⁰ and ¹⁸F labeled S100 protein.³¹ While these probes have shown value imaging outside of the central nervous system,^{32,33} their preparation is relatively cumbersome and they are too large to efficiently cross the BBB. As macromolecules are largely precluded from neuroimaging, and as single photon emission computed tomography (SPECT) is of much lower sensitivity than PET,³⁴ this work commenced by investigating a promising small-molecule scaffold amenable to positron emitting isotopic labeling (ideally ¹¹C or ¹⁸F).

A number of small-molecule mediators of RAGE interactions have been reported.^{35,36,37,38} *N*-benzyl-4-chloro-*N*-cyclohexylbenzamide (FPS-ZM1, Figure 1) is a multimodal RAGE-specific inhibitor that reduced amyloid- β -mediated brain dysfunction in a mouse model of AD. Despite having high lipophilicity (CLogP = 5.25; ChemBioDraw), FPS-ZM1 appeared like a promising scaffold around which to develop a PET radiotracer because of its high affinity (25 nM K_i against RAGE-A β ₁₋₄₀), BBB permeability,³⁷ lack of toxicity in mice or cells, reported specificity, and amenability to nucleophilic aromatic radiofluorination. Moreover, in a mouse model of AD with confirmed A β pathology (aged *APP*^{sw/0} mice overexpressing human A β), FPS-ZM1 was found to bind exclusively to RAGE, inhibiting RAGE-mediated influx of circulating A β into the CNS, reduced microglia activation and

associated neuroinflammation.³⁷ In this work, we report the synthesis and preliminary preclinical evaluation of [¹⁸F]RAGER: a PET probe based on FPS-ZM1 and the first BBB-penetrative small molecule radiotracer for RAGE.

RESULTS AND DISCUSSION

CHEMISTRY

The first step when developing novel PET radiotracers is to synthesize both the precursor to be radiolabeled, and the corresponding unlabeled reference standard to confirm identity of the radiolabeled product by HPLC. Unlabeled reference standard **2** was produced in good yield (80% over 2-steps) via a reductive amination of benzaldehyde and cyclohexylamine to give **1**, followed by acylation with 4-fluorobenzoyl chloride. Trimethylammonium precursor **3** was prepared for radiolabeling as the charged precursor and uncharged product were expected to have markedly different retention times by reverse-phase HPLC. Precursor **3** was synthesized in the same manner as the reference standard, by acylation of intermediate **1** with *p*-(*N,N*-dimethylamino)benzoyl chloride; subsequent alkylation with methyl iodide provided **3** in 10% yield (over 3-steps).

With precursor and reference standard in hand, we moved to radiolabeling. Radiosynthesis of [¹⁸F]RAGER was automated using a commercial synthesis module (General Electric Tracerlab FX_{FN}) by reacting trimethylammonium precursor **3** with [¹⁸F]fluoride in the presence of kryptofix-222 (K2.2.2) in DMF at 130 °C for 30 minutes. Subsequent purification by semi-preparative HPLC and reformulation yielded [¹⁸F]RAGER (**4**) in satisfactory radiochemical yield (44 ± 10 mCi; 2.9% non-decay corrected based upon 1.5 Ci of [¹⁸F]fluoride), excellent radiochemical purity (RCP) (>99%), and high specific activity (3,740 ± 495 Ci/mmol); n = 6. Formulated [¹⁸F]RAGER was stable for at least 60 minutes post end-of-synthesis.

PRECLINICAL EVALUATION

In Vitro Immunohistochemistry/Autoradiography—As imaging of RAGE is of interest in the context of Alzheimer's disease, tissue samples from an Alzheimer's patient were obtained, along with samples from a healthy control, to evaluate [¹⁸F]RAGER. RAGE expression is known to be significantly elevated in the cortex,³⁹ and so cortical sections were selected from each subject for testing. Immunohistochemistry was performed with monoclonal anti-RAGE antibodies and HRP-conjugated secondary antibodies on each sample. For each AD patient, a 2.9-fold increase in RAGE expression was observed compared to normal control tissue (see Supporting Information for more details). This is consistent with the known increased expression of RAGE in AD from the literature.

Adjacent sections were then incubated with [¹⁸F]RAGER **4** (Figure 2). The images confirm co-localization of [¹⁸F]RAGER in grey matter areas with known RAGE expression, previously identified by immunohistochemistry. There was higher uptake in the AD samples than the normal control samples. Non-specific binding was investigated by repeating incubations in the presence of 1000-fold excess of unlabeled RAGER, which confirmed binding in the grey matter regions was displaceable to background levels. However, despite

the reported specificity of the parent compound,³⁷ we did observe significant non-specific binding in the white matter (the white matter signal in the non-specific binding experiments shown in Figure 2 actually appears higher than for the total binding experiments. The cause of this is unclear, but it is possible that all of the displaced radiotracer in the non-specific samples gets retained in the white matter leading to the higher signal). Although RAGER has lower lipophilicity (experimental LogP = 3.5; CLogP = 4.85, ChemBioDraw) than FPS-ZM1 (CLogP = 5.25, ChemBioDraw), it is still lipophilic and we believe this is the origin of the high white matter binding. Correcting for non-specific binding allowed estimation of K_d and B_{max} in both the AD tissue and normal control tissue (Table 1). Using both AD and control tissue samples, the K_d was found to be approximately 15 nM, similar to the reported K_i of 25 nM for FPS-ZM1.³⁷ The B_{max} in AD tissue was found to be 2.8-fold higher than the B_{max} in normal control tissue, which is also consistent with the 2.9-fold increase in RAGE expression confirmed by immunohistochemistry (*vide supra*). The binding potential (BP), estimated by the B_{max}/K_d value, is useful for predicting the suitability of a radiotracer for imaging a target binding site in human subjects. [¹⁸F]RAGER has a BP of 1.86 in AD tissue, suggesting potential suitability of this scaffold for future applications in clinical PET imaging and the feasibility of imaging RAGE with PET.

Docking Studies—Docking studies were performed with Autodock Vina⁴⁰ using a 1.5 Å resolution crystal structure (PDB:3O3U⁴¹) to generate a feasible binding mode of the RAGER-RAGE complex (Figure 3; see Supporting Information for further details). As there is no known crystal structure of any small molecule RAGE antagonists with RAGE, Vina's search space was defined to encompass the interface residues for the vRAGE-S100P interaction as determined by the NMR-solved complex (PDB:2MJW).⁴² Yu *et al.* found FPS-ZM1 to be an inhibitor of the RAGE-S100P mediated cell proliferation induction,⁴³ and we reasoned, because of its structural similarities to FPS-ZM1, that RAGER would bind in the same pocket. This assumption is supported by the similar binding energies calculated for the lowest energy conformations of RAGER (−6.6 kcal/mol) and FPS-ZM1 (−6.5 kcal/mol). This, in combination with the apparent interaction the ligands with conserved residues (L49, W51) of the β3 strand, previously shown to be important for the RAGE-Aβ interaction,²⁸ suggest it is a reasonable docking configuration. The interaction appears to be largely dependent on hydrophobic interactions, although high-resolution structural information of a RAGE-inhibitor complex is needed to definitely confirm the binding mechanism of the scaffold. Nevertheless, these *in silico* studies do support our supposition that the chlorine/fluorine substitution of RAGER in relation to FPS-ZM1 had little effect on best bound-ligand conformation or energy.

Pre-Clinical Imaging—*In vivo* behavior of the radiotracers was investigated initially in rodents (n = 3). PET scans were conducted in Sprague Dawley rats and imaging was conducted for 90 min post-injection of the radiotracer. Following the scan, images were summed (Figure 4a) and a region-of-interest (ROI) was drawn over the whole brain on multiple planes. The volumetric ROIs were then applied to the full dynamic data set to generate a time- radioactivity curve (Figure 4b). The curve showed rapid uptake of [¹⁸F]RAGER (peak SUV ~2.5). Peak uptake occurred within 3 minutes post-injection, and was followed by virtually complete washout over the duration of the PET scan. Encouraged

by these results, we also evaluated the imaging properties of [^{18}F]RAGER in nonhuman primates. Similar imaging results were obtained in rhesus macaque (Figure 4c). A ROI was drawn initially for the whole brain and used to generate a time-radioactivity curve (Figure 4d), which revealed rapid uptake of [^{18}F]RAGER in the primate brain (peak SUV ~ 2.3). Peak uptake again occurred within 3 minutes post-injection, and was followed by washout over the duration of the PET scan. Washout was faster in rodents than primates, and we attribute this to increased tissue volumes and lower blood flow in the primates. The radiotracer provided reasonable levels of uptake in all grey matter regions (peak SUVs range from 2 – 4; see Figure 5 for regional time-radioactivity curves of [^{18}F]RAGER in nonhuman primate brain). For example, there was high uptake apparent in the cortex and cerebellum that agrees with the reported distribution of RAGE.³⁹ Notably, there was also extensive bilateral uptake and markedly slower washout associated with the hippocampal regions, which suggests specific binding. This is also consistent with the known expression of RAGE, where it has been shown that some RAGE expression is normal in the healthy hippocampus, but that elevated levels are present in the hippocampus of the Alzheimer's disease brain.^{39b-e} Very little white matter binding was apparent, in contrast to the high degrees of non-specific binding that complicated the *in vitro* studies described above, although there was extensive uptake in the brain stem and an unknown region outside of the brain (possibly tissue associated with the nasal cavity). The cause of differing non-specific binding between the *in vitro* and *in vivo* studies could simply be that there is less [^{18}F]RAGER available to bind in the brain during the *in vivo* studies because of uptake in the periphery (there is high RAGE expression in the lung⁴⁴), binding to circulating soluble RAGE and/or metabolism effects. To investigate the latter possibility, we next investigated metabolism of RAGER.

Metabolism—Metabolism of RAGER was examined by incubation with rat liver microsomes (see Supporting Information). The LC-MS/MS data analysis at microsome incubation time points out to 60 min demonstrated extremely fast metabolism of the radiotracer, with a half-life of 0.66 min and only $\sim 0.5\%$ of authentic RAGER remaining at 5 min. Although we have not confirmed this metabolism profile *in vivo*, the rodent time-radioactivity curves are consistent with rapid metabolism and do not suggest that any labeled metabolites enter the brain. Rapid metabolism would explain the difference between the high non-specific binding observed *in vitro* and the low levels apparent in the PET images. We have also not examined metabolism in primates, but would expect it to be slower than in rats. This could lead to higher non-specific binding in primates, which would be consistent with the slower washout apparent in the primate time-radioactivity curves (Figure 4). The rapid metabolism also suggests that brain uptake in known areas of RAGE expression is likely occurring before first pass metabolism, which is again consistent with the rapid peak uptake of the radiotracer in both rats and primates.

SUMMARY AND FUTURE OUTLOOK

In summary, we have demonstrated the synthesis and preliminary pre-clinical evaluation of [^{18}F]RAGER: the first small molecule BBB-permeable PET radioligand for the Receptor for Advanced Glycation End-products. We have confirmed upregulation of RAGE in AD post-

mortem cortical tissue with immunohistochemistry, and demonstrated that [^{18}F]RAGER co-localizes with areas of RAGE distribution. Using molecular docking, a reasonable binding site for RAGER was identified. MicroPET imaging in rodent and nonhuman primate confirmed brain uptake and extensive washout, and the latter indicated that the radiotracer accumulates in areas of known RAGE expression.

The binding potential (BP), estimated by the B_{max}/K_d value, is useful for predicting the suitability of a radiotracer for imaging a target binding site in human subjects. While the ideal binding potential is unknown, useful radiotracers have a binding potential that falls within a range of values, although 5 has recently been recommended for CNS radiotracers by the National Institutes of Health. [^{18}F]RAGER has a BP of 1.86 in AD tissue, suggesting potential suitability of this scaffold for future applications in clinical PET imaging of RAGE, but leaving scope for improvement. Second generation radiotracers with K_d 6 nM are desirable to achieve the target BP of 5 for anticipated successful *in vivo* clinical imaging of RAGE in the future. To address this, efforts to develop new radioligands based around the FPS-ZM1 scaffold that improve affinity, reduce lipophilicity to minimize non-specific binding, and address rapid metabolism of the tracer are ongoing and will be the subject of a future report. We expect PET radiotracers for RAGE to be useful in AD as well as other diseases that dysfunction of RAGE has been implicated in such as cancer, diabetes, and atherosclerosis.^{45,46}

EXPERIMENTAL SECTION

Chemistry

General Considerations—All the chemicals employed in the syntheses were sourced commercially, and used without further purification. ^1H -Nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz on Varian NMR spectrometer in CD_3OD or d_6 -DMSO solutions at room temperature with tetramethylsilane (TMS, $\delta = 0$) as an internal standard. ^{13}C -NMR spectra were obtained at 100 MHz and ^{19}F -NMR spectra were obtained at 376 MHz. Chemical shifts (δ) are reported in ppm and coupling constants are reported in Hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector. Mass spectra were performed on a Micromass VG 70-250-S Magnetic sector mass spectrometer, Micromass AutoSpec Ultima Magnetic sector mass spectrometer or Agilent Q-TOF HPLC-MS using the electrospray ionization (ESI) method or electron ionization (EI) method.

Preparation of N-benzyl-N-cyclohexyl-4-fluorobenzamide (2): Cyclohexylamine (250 mg, 2.52 mmol) and benzaldehyde (267 mg, 2.52 mmol) were dissolved in methanol (5 mL). Sodium sulfate was added, and the mixture was stirred for 18 h at 50 °C. The mixture was filtered and the filtrate was concentrated *in vacuo*. The white semi-solid was redissolved in methanol and cooled to 0 °C. Sodium borohydride (133 mg, 3.53 mmol) was slowly added and the reaction was stirred at rt for 4 h. The reaction was quenched with saturated ammonium chloride solution. The intermediate product was extracted with dichloromethane

(3x), dried over sodium sulfate, and solvent removed *in vacuo*. The intermediate product was dissolved in dichloromethane (6 mL), and the solution was cooled to 0 °C. Triethylamine (306 mg, 3.02 mmol) and 4-fluorobenzoyl chloride (439 mg, 2.77 mmol) were added and the solution was stirred for 18h under argon. The reaction was quenched with H₂O and the product was extracted with dichloromethane (3x) and purified by flash chromatography (SiO₂, 3:1=hexanes: ethyl acetate). This process yielded the product as white, crystalline solid (628 mg, 80%); R_f: 0.52 (SiO₂, 3:1=hexanes: ethyl acetate); ¹H NMR (400 MHz; DMSO-d₆)/δ (ppm) 0.97–1.63 (10H, m), 3.48 (1H, br), 4.63 (2H, s), 7.21–7.51 (9H, m); ¹³C NMR (100 MHz; DMSO-d₆)/δ (ppm): 25.03, 25.80, 31.45, 51.17, 59.20, 115.98, 126.85, 127.08, 128.76, 129.06, 134.14, 139.96, 163.84, 170.61; ¹⁹F NMR (376.3 MHz, DMSO-d₆)/δ (ppm): –111.71(1F, s); HPLC: 98%, retention time = 22 min, column: Phenomenex Gemini C18, 250×4.6 mm, mobile phase: 10 mM NH₄HCO₃ in 58% MeCN, plus 2 mL/L sat. NH₄OH solution, flow rate: 1.0 mL/min, wavelength: 254 nm; HRMS: calculated for [M+H]⁺(M = C₂₀H₂₂FNO), 312.1758, found 312.1756.

Preparation of 4-(benzyl(cyclohexyl)carbamoyl)-N,N,N-trimethylbenzenaminium

iodide (3): To a vial was added *N*-benzyl-*N*-cyclohexyl-4-(dimethylamino)benzamide (35.0 mg, 0.104 mmol, see Supporting Information for synthesis procedure) and methyl iodide (60.7 mg, 0.428 mmol). The vial was sealed, heated to 45°C, and stirred. Additional methyl iodide (60.7 mg, 0.428 mmol, 20 times) was added ever few hours. After 5 hours, an off-white, insoluble semi-solid was observed. After 12h, the solvent was evaporated under vacuum. The solid residue was washed with hexanes (3 × 2 mL), diethyl ether (1 × 2 mL), and dried under vacuum to yield the product as an off-white solid (35.6 mg, 0.0744 mmol, 72%); R_f: 0.53 (SiO₂, 3:1=dichloromethane:methanol); ¹H NMR (400 MHz; CD₃OD, 55°C)/δ (ppm): 1.09(3H, m), 1.59–1.73(7H, m), 3.70 (9H, s), 4.67(2H, s), 7.22–7.30(5, m), 7.66(2H, s), 7.98(2H, s); HRMS: calculated for [M]⁺(M = C₂₃H₃₁N₂O), 351.2431, found 351.2429.

Radiochemistry

General Considerations—Unless otherwise stated, reagents and solvents were commercially available and used without further purification: Sodium Chloride, 0.9% USP and Sterile Water for Injection, USP were purchased from Hospira; ethanol was purchased from American Regent; HPLC grade acetonitrile was purchased from Fisher Scientific. Other synthesis components were obtained as follows: sterile filters were obtained from Millipore; sterile product vials were purchased from Hollister-Stier; QMA-light and C18-light Sep-Paks[®] were purchased from Waters Corporation. Sep-Paks[®] were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use.

[¹⁸F]RAGER (4)

[¹⁸F]KF was prepared using a TRACERLab FX_{FN} automated radiochemistry synthesis module (General Electric, GE). [¹⁸F]Fluoride was produced via the ¹⁸O(p,n)¹⁸F nuclear reaction using a 16 MeV GE PETTrace cyclotron (40 μA beam for 30 min generated 1,500 mCi of [¹⁸F]fluoride). The [¹⁸F]fluoride was delivered to the synthesis module (in a 1.5 mL bolus of [¹⁸O]water) and trapped on a QMA-light Sep-Pak to remove [¹⁸O]water. [¹⁸F]Fluoride was eluted into the reaction vessel using K₂CO₃ (3.5 mg in 0.5 mL of water).

A solution of K-2.2.2 (15 mg in 1 mL of acetonitrile) was added to the reaction vessel, and the resulting solution was azeotropically dried by heating the reaction vessel to 100 °C and drawing vacuum for 4 min. After this time, the reaction vessel was subjected to an argon stream and simultaneous vacuum draw for an additional 4 min. RAGER precursor **3** (1.5 mg dissolved in 0.5 mL DMF) was delivered to the reaction vessel, and the reaction was heated to 130 °C and stirred for 30 min. After this time, the reactor was cooled to 55 °C and 2 mL semi-preparative HPLC solvent was added to the crude reaction mixture. This mixture was injected onto a semi-preparative HPLC column (column: Phenomenex Gemini C18, 250×10 mm, mobile phase: 10 mM NH₄HCO₃ in 58% pH 9.0 adjusted with 2mL/L sat. NH₄OH, flow rate: 2.5 mL/min, see Supporting Information Figure S1 for a representative HPLC trace). The product peak (~36 – 37 min retention time) was collected and diluted into a round-bottom flask containing 50 mL water. The solution was then passed through a C-18 extraction disk to remove organic solvent. The disk was washed with 5 mL sterile water. The product was eluted with 0.5 mL of ethanol followed by 4.5 mL of normal saline. The final formulation was passed through a 0.2 µm needle filter into a sterile dose vial. Using Nitro-precursor: (15.3 ± 6.4 mCi (566 ± 237 MBq), 1.0% uncorrected yield, >99% RCP, pH = 5–5.5 *n* = 4). Using trimethyl-ammonium precursor: (44.6 ± 10 mCi (1650 ± 385 MBq), 3.0% uncorrected yield, >99% RCP, 3740 ± 495 Ci/mmol (138 ± 18 GBq/mmol), pH = 5.5 *n* = 6).

Quality Control—Quality control of radiopharmaceutical doses was conducted using the following tests:

Visual inspection: doses were examined visually to confirm that they were clear, colorless and free of particulate matter.

Dose pH: the pH of the doses was analyzed by applying a small amount of the dose to pH-indicator strips and determined by visual comparison with the provided scale.

HPLC analysis: Radiochemical purity of [¹⁸F]RAGER was assessed using Shimadzu LC-2010A HT system equipped with the UV and Rad detectors (column: Phenomenex Gemini C18, 250×4.6 mm; mobile phase: 10 mM NH₄HCO₃ in 58% MeCN, pH 9 adjusted with 2mL/L sat. NH₄OH solution; flow rate: 1.0 mL/min; wavelength: 254 nm; room temperature; product peak: ~22.5 min; see Supporting Information Figure S2 for a representative HPLC trace).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

Aβ	amyloid beta
AD	Alzheimer's disease
BBB	blood-brain barrier
CNS	central nervous system
HPLC	high-performance liquid chromatography
IPA	isopropanol
K2.2.2	kryptofix-2.2.2
MAP	maximum a posteriori
MS	mass spectrometry
NMR	nuclear magnetic resonance
PET	positron emission tomography
Pgp	P-Glycoprotein Transporter
PiB	Pittsburgh Compound B
QMA	quaternary methylammonium
RAGE	Receptor for Advanced Glycation End-products
RCP	radiochemical purity
RCY	radiochemical yield
ROI	region-of-interest
SA	specific activity
TACs	time-radioactivity curves

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Figure 1.
FPS-ZM1 and [¹⁸F]RAGER

Figure 2.
Autoradiographic images of [^{18}F]RAGER in AD and normal control frontal cortex samples.

Figure 3. Possible configuration of RAGER bound to vRAGE. (A) The blue color indicates interface residues of RAGE-S100P, and the bright red is β -strand important for the RAGE-A β interaction.²⁸ (B) Close up of possible binding with labeled residues.

Figure 4.

Representative microPET imaging data for rodent (**A**, summed images 0 – 90 minutes post i.v. injection of the radiotracer (○; = brain); **B**, rodent whole brain time-radioactivity curves) and non-human primate (**C**, summed images 0 – 90 minutes post i.v. injection of the radiotracer; **D**, non-human primate whole brain time-radioactivity curves) imaged with [^{18}F]RAGER

Figure 5.
 $[^{18}\text{F}]\text{RAGER}$ regional time-radioactivity curves in the non-human primate brain

Scheme 1.

Radiosynthesis of HPLC reference standard (**2**), precursor (**3**) and [¹⁸F]RAGER (**4**).

Table 1Binding Affinity Data for [¹⁸F]RAGER

	K_d	B_{max}	BP (B_{max}/K_d)
AD Tissue	15.5 nM	28 nM	1.86
Normal Control Tissue	15.3 nM	10 nM	0.65