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Radiosynthesis and in Vivo Evaluation of [¹¹C]A1070722, a High Affinity GSK-3 PET Tracer in Primate Brain

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Abstract

Dysfunction of glycogen synthase kinase 3 (GSK-3) is implicated in the etiology of Alzheimer's disease, Parkinson's disease, diabetes, pain, and cancer. A radiotracer for functional positron emission tomography (PET) imaging could be used to study the kinase in brain disorders and to facilitate the development of small molecule inhibitors of GSK-3 for treatment. At present, there is no target-specific or validated PET tracer available for the in vivo monitoring of GSK-3. We radiolabeled the small molecule inhibitor [¹¹C]1-(7-methoxy-quinolin-4-yl)-3-(6-(trifluoromethyl)pyridin-2-yl)urea ([¹¹C]A1070722) with high affinity to GSK-3 ($K_i = 0.6$ nM) in excellent radiochemical yield. PET imaging experiments in anesthetized vervet/African green monkey exhibited that [¹¹C]A1070722 penetrated the blood-brain barrier (BBB) and accumulated in brain regions, with highest radioactivity binding in frontal cortex followed by parietal cortex and anterior cingulate, and with the lowest bindings found in caudate, putamen, and thalamus, similarly to the known distribution of GSK-3 in human brain. Our studies suggest that

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Author Contributions

J.S.D.K. and J.P. conceived the idea and designed the experiments. K.K.S.S., J.P., and J.S.D.K. performed the experiments. F.Z. and H.R.-F. performed all analyses of the PET data. J.P., F.Z., K.K.S.S., H.R.-F., M.J.J., J.R.K., A.M., J.J.M., and J.S.D.K. analyzed and interpret the data. J.P. and J.S.D.K. drafted the manuscript. The manuscript was written through contributions of all authors and all authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

DEDICATION

This manuscript is dedicated to the memory of Professor Kenji Uneyama (1941–2017).

[¹¹C]A1070722 can be a potential PET radiotracer for the in vivo quantification of GSK-3 in brain.

Graphical Abstract

Keywords

GSK-3; radiotracer; PET; brain

INTRODUCTION

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase family of protein kinases, which is also known as tau phosphorylating kinase.^{1–3} Major functions of GSK-3 are regulating cell metabolism, cell survival, proliferation, neural development and neurotransmission. In humans, GSK-3 exists in three isoforms referred to as GSK-3 α , GSK-3 β , and GSK-3 γ . The GSK-3 α and GSK-3 β isoforms have 98% similarity, whereas GSK-3 γ is an alternative splice variant of GSK-3 β with additional 13 amino acids. Although the three isoforms are expressed widely in the brain and are structurally very similar, they perform nonidentical functions.^{1,4,5} Unlike other protein kinases, GSK-3 is constitutively active in resting conditions and is inhibited in response to upstream signals. It can be inhibited or upregulated by diverse post-transductional modifications such as phosphorylation in response to upstream signals. In addition, GSK-3 only phosphorylates a substrate, which is prephosphorylated by another protein kinase or signaling mediator. Human post-mortem studies using quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique, demonstrate that GSK-3 β is fairly well distributed in the adult human brain. High concentrations of GSK-3 β were found in cortical regions, locus coeruleus, hippocampus, and amygdala and the lowest in caudate and putamen.^{6–9}

Several studies have measured the density of GSK-3 in human and rodent brain in vitro. Postmortem studies of human brain using Western blot analyses showed 308 $\mu\text{U}/\mu\text{g}$ protein of GSK-3 in Brodmann area 9 (BA9) in the frontal cortex in control subjects, whereas a lower GSK-3 density was found in schizophrenic patients (251 $\mu\text{U}/\mu\text{g}$).⁴⁰ In different studies, GSK-3 β activity in the frontal cortex of control subjects, postmortem, was reported as 357 fmol/mg protein $\times$ min, while a 45% reduction in activity was found in schizophrenic patients.^{41,42} It was also found that GSK-3 density was 33% lower in the prefrontal cortex of prepubertally schizophrenia rat model (258 $\mu\text{U}/\mu\text{g}$ protein vs 386 $\mu\text{U}/\mu\text{g}$ protein) in comparison to control rats.⁴³ The above studies show the presence of a density of GSK-3 in brain that may be sufficient for visualizing this receptor in vivo using PET imaging.

Biochemical, pharmacological, genetic, and rodent behavioral studies support the hypothesis that inhibition of GSK-3 represents a therapeutically relevant target for neuropsychiatric and neurodegenerative disorders.^{10–14} More recent studies suggest the modulation of GSK-3 in either the direct or downstream mechanism of action of mood stabilizers, antidepressants, and anti-inflammatory medications currently in use.^{15–18} Small molecule inhibitors of GSK-3 are currently under development for a broad range of central nervous system (CNS) disorders including bipolar disorder, depression, diabetes, schizophrenia, and Alzheimer's disease.^{19–22} Biological imaging of GSK-3 expression with radiolabeled small molecule inhibitors may offer a direct and more sensitive approach to monitor the clinical potential of targeted therapeutics and treatments. Noninvasive and in vivo detection of the changes in GSK-3 expression using PET can impact the choice of therapy as well as monitor the progress of treatment. Furthermore, accurate quantification of GSK-3 with radiolabeled inhibitors by PET could greatly accelerate drug discovery programs through occupancy measurement studies. At present there is no validated PET radiotracer available for the in vivo quantification of GSK-3.

Figure 1 summarizes the PET radiotracers that were reported for GSK-3.²² [¹¹C]AR-A014418 (IC_{50} = 170 nM), synthesized by labeling the molecule either at the methoxy or at the carbonyl position, showed a fast washout in brain despite the previously reported CNS effects of unlabeled AR-A014418.^{23–25} Autoradiography in rodent brain sections by phosphor imaging [¹¹C]PyrATP-1 (K_i = 4.9 nM) revealed significant nonspecific binding and moderate binding throughout the cortex and nucleus accumbens.²⁶ The radiotracer also exhibited poor brain uptake in rodent and primate brain with moderate increase in uptake during pretreatment with cyclosporine.^{26,27} [¹¹C]SB-216763 (K_i = 9 nM) penetrates the BBB in mice and baboon, but shows homogeneous distribution.^{28–30} In vivo evaluation of a series of [¹¹C]labeled oxindole analogues (IC_{50} = 66–35 nM) also was proven unsuccessful in normal and cold water stress (CWS)-induced tau hyperphosphorylation mice model.³¹ Although ¹¹C-radiolabeling of the nonselective CDK-GSK-3 tracer meisoindigotin has been reported, no in vivo data are available yet.³² The failure of these tracers for in vivo imaging of GSK-3 may be partially due to their inadequate lipophilicity ([¹¹C]SB-216763), low affinity to GSK-3 ([¹¹C]AR-A014418, [¹¹C]oxadiazoles), and poor BBB penetration associated with the affinity to p-GP ([¹¹C]PyrATP-1) (Figure 1). Recently reported GSK-3 radiotracer, [¹¹C]PF-367 (PF-04802367, K_i = 2.1 nM), crosses the BBB in rodent and nonhuman primates, but shows homogeneous distribution across brain regions, including cerebellum and white matter,³³ and significant uptake in skull, and was partially blocked by the administration of unlabeled PF-367.³³ This evidence suggests the need for exploring structurally different molecules in order to optimize a successful radiotracer to image GSK-3 in vivo in the brain. Herein we describe synthesis and in vivo evaluation of [¹¹C]A1070722 in anesthetized monkey by PET.

RESULT AND DISCUSSION

1-(7-Methoxyquinolin-4-yl)-3-(6-(trifluoromethyl)pyridin-2-yl)urea (A1070722, **1**) is a potent brain-penetrant GSK-3 inhibitor (ClogP = 3.2) with a K_i of 0.6 nM for both GSK-3 α and GSK-3 β .³⁴ A1070722 showed >50 fold selectivity for GSK-3 over a panel of other kinases.³⁴ A1070722 decreased tau phosphorylation, protected rat primary cortical neurons

against β amyloid and glutamate challenge in vivo, thus supporting its brain permeability.³⁴ Hence we selected A1070722 as a candidate for GSK-3 radioligand development, based on its superior affinity, selectivity, ClogP, known BBB permeability, and availability of a potential labeling site with C-11. Compound **1**, the nonradioactive standard, was synthesized based on a reported procedure³⁵ and the radiolabeling precursor was synthesized as shown in Scheme 1. 7-methoxyquinolin-3-amine (**2**) was reacted with triphosgene in the presence of triethylamine in dichloromethane. The resulting mixture was coupled with 6-(trifluoromethyl)pyridin-2-amine (**3**) to afford reference standard **1** in 80% yield. Demethylation of **1** with BBr₃ followed by purification afforded radiolabeling precursor **4** in 90% yield (Scheme 1). The radiochemical synthesis of [¹¹C]A1070722 was optimized and automated on a GE-FX2MeI/FX2M radiochemistry module by alkylating the corresponding desmethyl-A1070722 (**4**) precursor [¹¹C]MeI in DMF using NaOH. [¹¹C]A1070722 was produced with a radiochemical yield of 40 ± 5%, (~1800–2146 MBq) with >97% radiochemical purity and a specific activity of 88.8 ± 15 GBq/μmol (*n* = 15, decay corrected to EOS based on [¹¹C]MeI, Scheme 1). We found the presence of an unidentified close running UV impurity with the radioproduct, which was difficult to remove below retention time 10 min. The optimal condition of semipreparative HPLC purification that resulted in maximum separation of the product and the impurity was using 60% ACN/40% 0.1 M AMF at 7 mL/min (λ = 254 nm) solvent system at which the retention time of the product is ~14 min. Our radiosynthesis proceeded with high yield and specific activity and therefore, this retention time did not affect the quality of the final product for further evaluation. With these acceptable and reproducible radiochemical yields and specific activities, the synthesis of [¹¹C]A1070722 can be easily translated to any automated radiochemistry modules.

PET study of [¹¹C]A1070722 was performed in fasted adult male vervet/African green monkeys (*Chlorocebus aethiops sabaues*) under anesthetic conditions. [¹¹C]A1070722 penetrated the BBB and showed retention in the brain (Figure 2) and a relatively heterogeneous pattern of binding. Time–activity curves (TACs) expressed in standard uptake value (SUV) indicated highest uptake of [¹¹C]A1070722 in frontal cortex, followed by parietal cortex and anterior cingulate. Low uptake was observed in caudate, putamen and thalamus (Figure 3). The radiotracer distribution in monkey brain is in agreement with the known distribution of GSK-3 in postmortem samples of human subjects.^{6–9} The radioactivity ratios of frontal cortex, parietal cortex, orbital cortex, anterior cingulate, cingulate cortex and amygdala to cerebellum were 1.5, 1.35, 1.3, 1.3, 1.2, 1.1, and 1, respectively, at 85 min. Average of the cortical gray matter region radioactivity of [¹¹C]A1070722 is similar to cerebellar regions. Radiotracer also shows significant binding to cortical and cerebellar white-matter regions (Figure 3). The global white matter uptake of radiotracer is overall similar to gray matter uptake. TACs also show that radiotracer did not reach equilibrium binding in some regions within 90 min scan duration (*N* = 2). One of our scans with 120 min duration also suggest that radiotracer did not reach equilibrium in some brain regions (data not presented).

The radioactivity levels in arterial blood and plasma peaked at 2 min, followed by a fast clearance (Figure 4B and C). HPLC analyses of the plasma samples indicated the evidence of polar metabolites and the percentage of unmetabolized [¹¹C]-A1070722 was found to be

92.5 ± 0.85% of total plasma radioactivity at 2 min, 89.0 ± 1% at 4 min, 51.9 ± 6.9% at 12 min, 45.1 ± 12.9% at 30 min, 35.6 ± 15% at 60 min, and 20.1 ± 9.5% at 90 min (Figure 4A). The extraction efficiency and column efficiency for metabolite analyses were >90%.

Regional values of [¹¹C]A1070722 total distribution volume (V_T) were calculated with a metabolite-corrected arterial input function using a 2-tissue compartment model³⁶ (2-TC) and graphical analysis (Logan plot)³⁷ (Figure 5). The two methods yielded comparable V_T values ($N = 2$). The highest V_T was found in the cortical regions such as frontal, parietal, anterior cingulate, and cerebellum. No significant differences in V_T 's were found for gray and white matters.

In summary, we synthesized a high affinity GSK-3 ligand, A1070722, and its radiolabeling precursor in high yield. The radiosynthesis of [¹¹C]A1070722 has been achieved by reacting [¹¹C]MeI and phenolate of the desmethyl-A1070722 in excellent yield and specific activity. PET study in anesthetized monkeys showed that [¹¹C]A1070722 penetrates the BBB and exhibits somehow heterogeneous binding across brain regions. The binding distribution of [¹¹C]A1070722 is in good agreement with the known distribution of GSK-3 in brain. Although the proof of concept of in vivo imaging using [¹¹C]A1070722 is demonstrated in monkey, the radiotracer showed low V_T values for GSK-3 in comparison to the values obtained with radiotracers for other well-established neuro-receptors. This may be partially attributed to the low concentration of GSK-3 in normative monkey brain. Therefore, PET studies in animal models with higher expression of GSK-3 may be required to establish the preclinical and potential clinical utility of this tracer. The radiotracer also did not reach equilibrium in some regions after 90 or 120 min of scan time (not shown), suggesting the need of a F-18 labeled radiotracer allows imaging over longer scan duration thereby facilitating quantitative kinetic studies and accurate modeling. By sharing these preliminary data, we intend to demonstrate the potential for A1080722-related structural skeleton as brain imaging agents for GSK-3, thereby facilitating the identification of an optimal small molecule radiotracer for in vivo PET imaging studies in brain.

METHODS

Synthesis of 1-(7-Methoxyquinolin-3-yl)-3-(6-(trifluoromethyl)pyridin-2-yl)urea (1)

Here 162 mg of 6-(trifluoromethyl)pyridin-2-amine (**3**, 1 mmol) and 0.275 mL of triethylamine (2 mmol) in 5 mL of dichloromethane was slowly added to a solution of 300 mg (0.5 mmol) triphosgene in 3 mL dichloromethane at 0 °C for 60 min. The reaction mixture was stirred for an additional 60 min at room temperature. Next, 175 mg of 7-methoxyquinolin-3-amine (**2**, 1 mmol) and 0.275 mL of triethylamine (2 mmol) in 5 mL dichloromethane were slowly added to the reaction mixture at room temperature. After stirring overnight, the reaction mixture was poured into ice water (100 mL) and stirred for additional 2h. The precipitation formed was filtered off, washed with ice-cold solution of hexane (2 × 20 mL) and dried in vacuum to afford 290 mg (80%) of **1** as yellow solid. The analytical data of **1** are in agreement with same compound reported in literature.³⁵

Synthesis of 1-(7-Hydroxyquinolin-3-yl)-3-(6-(trifluoromethyl)pyridin-2-yl)urea (4)

1-(7-Methoxyquinolin-4-yl)-3-(6-(trifluoromethyl)pyridin-2-yl)urea (**1**, 290 mg, 0.8 mmol) was taken in 8 mL of anhydrous dichloromethane and 8 mL of 1 M BBr₃ in dichloromethane at room temperature was added to it. The solution was stirred for 1 h at room temperature, followed by refluxing at 40 °C, for 12 h. After confirming the complete conversion of **1** by HPLC, the reaction mixture was quenched by adding methanol (2 mL) dropwise at 0 °C. The product mixture was basified by dropwise addition of ammonium hydroxide (pH = 8–9). Water (10 mL) was added to the crude mixture and resulting precipitate was filtered, washed with hexane (20 mL), followed by ice-cold solution of 1:1 hexane and ethyl acetate (20 mL). The precipitate was dried under vacuum to afford **4** in 240 mg, 90% yield as pale yellow solid.

4: ¹H NMR (300 MHz, CD₃OD) δ: 8.65 (d, 1H); 8.3 (d, 1H), 8.5 (d, 1H), 8 (m, 1H), 7.5 (m, 2H), 7.3 (m, 3H). HRMS (EI⁺) calculated for C₁₆H₁₁F₃N₄O₂: 348.0834. Found: 343.0853.

Radiosynthesis of [¹¹C] 1-(7-Methoxyquinolin-4-yl)-3-(6-(trifluoromethyl)pyridin-2-yl)urea ([¹¹C]1**)**

[¹¹C]MeI from FX2MeI module was bubbled to the reaction vial (15 mL/min) placed in FX2M module containing precursor **4** (0.5 mg) in anhydrous DMF (0.4 mL) and 5 N NaOH aqueous solution (5.0 μL) for ~5 min at room temperature. [¹¹C]CO₂ was produced in the Wake Forest PET Center Cyclotron Facility with a GE-PETtrace-800 cyclotron. GE-FX2MeI converts [¹¹C]CO₂ to [¹¹C]CH₄ using a nickel catalyst [shimalite-Ni (reduced)] at 360 °C. [¹¹C]CH₄ was then reacted with gaseous iodine at 760 °C to form [¹¹C]MeI. After the complete transfer of radioactivity, the sealed reaction vial was then heated at 80 °C for 5 min. The reaction mixture was quenched with HPLC mobile phase (0.7 mL) and injected onto a reverse-phase semipreparative Phenomenex ODS (250 × 10 mm, 10 μ) HPLC column to purify [¹¹C]A1070722. The isocratic HPLC mobile phase solution consisted of 40% acetonitrile, 60% 0.1 M aqueous ammonium formate solution (pH value 6.0–6.5) with UV wavelength at 254 nm and a flow rate of 7.0 mL/min. The product [¹¹C]A1070722 (*R*_t = 14.0–16.5 min) was collected and diluted with 100 mL of deionized water, and passed through C18 SepPak cartridge (WAT036800, Waters, Milford, MA) to trap the radiotracer [¹¹C]A1070722. Radioactive product was then eluted from the cartridge with absolute ethanol (1.0 mL) and formulated with saline (10% ethanol in saline). The final product [¹¹C]A1070722 was directly collected into a sterile vial through a sterile 0.22 μm pyrogen-free filter (Millipore Corp., Billerica, MA) for further animal studies and quality control analysis. [¹¹C]A1070722 purity was assessed using an analytical reverse phase Phenomenex ODS HPLC column (250 × 4.6 mm, 5 μ) and UV detection set at 254 nm. The mobile phase (1.5 mL/min) consisted of 60% acetonitrile and 40% 0.1 M aqueous ammonium formate pH 6.0–6.5 solution. [¹¹C]A1070722 showed a retention at 7.5 min, and authentication of the product was performed with coinjection of the nonradioactive standard A1070722, which demonstrated a similar retention times. The specific activities were determined at EOS based on the UV absorption and concentration standard A1070722 curves (λ = 254 nm).

PET Imaging of [^{11}C]A1070722 in Monkey

PET scans ($N = 3$) were performed in two vervet monkeys (~ 7.75 kg) using a GE 64-slice PET/CT Discovery VCT scanner (General Electric Medical Systems, Milwaukee, WI). In each scan, the fasted animal was immobilized with ketamine (10 mg/kg, i.m.) and anesthetized with 1.5–2.0% isoflurane via an endotracheal tube. Core temperature was kept constant at 37 °C with a heated water blanket. An intravenous infusion line with 0.9% NaCl was maintained during the experiment and used for hydration and radiotracer injection. An arterial line was placed for obtaining arterial blood samples for metabolite analysis and generation of an input function for full quantification of the brain PET data. The head was positioned at center of the field of view, and a 10 min transmission scan was performed before the tracer injection. [^{11}C]A1070722 radiotracer was injected (407 ± 80 MBq) as intravenous bolus over 30 s, and emission data were collected for 90 (in 2 scans) or 120 min (in 1 scan) in three-dimensional mode. Blood samples were collected manually at 1, 2, 4, 8, 12, 20, 30, 40, 60, 80, and 90 min after tracer injection, respectively. Of these, samples at 2, 4, 12, 30, 60, and 90 min after tracer injection were used for metabolite analysis. The supernatant liquid obtained after centrifugation of the blood sample at 3400 rpm for 10 min was transferred (0.5 mL) into a tube and mixed with acetonitrile (0.7 mL). The resulting mixture was vortexed for 10 s, and centrifuged at 14,000 rpm for 4 min. The supernatant liquid (1 mL) was removed and the radioactivity was measured in a well-counter (PerkinElmer 470 Wizard Gamma counter). All the acquired data were then subjected to correction for background radioactivity and physical decay to calculate the activity in the whole blood and plasma at different time points. Images were transferred into the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) for drawing and storing regions of interest. All PET images were coregistered within a dynamic study to the previous frame, and PET frames were then coregistered to the MRI. MRI scans were performed using a Siemens MAGNETOM Skyra 3T MRI Scanner with the following sequences: large fov localizer, small fov localizer 128 mm, MPRAGE Axial_ND and MPRAGE Axial (TE = 3.39 ms, TR = 2700 ms, TI = 880 ms, resolution = $0.5 \times 0.5 \times 0.5$ mm; FOV = 128 mm $\times$ 128 mm; matrix size = 256 $\times$ 256). Registrations from PET to MRI and MRI to standard space were performed with FMRIB Software Library (FSL) linear image registration tool (FLIRT).³⁸ Time activity curves (TACs) were then extracted as the mean PET radioactivity values within each region based on the INIA Primate atlas.³⁹ Radioactivity levels in the right and left regions were averaged. Regional total distribution volumes³⁸ (V_T) of [^{11}C]A1070722 were derived using the metabolite-corrected arterial input function and a 2-tissue compartment model³⁶ (2-TC) and graphical analysis (Logan plot).³⁷ V_T (mL $\cdot$ cm $^{-3}$) is defined as the ratio of the brain tissue tracer concentration in the region of interest to the plasma concentration of the tracer at equilibrium with metabolite correction. Image analysis was performed in MATLAB 2012b (The Mathworks, Natick, MA).

Metabolite Analyses of [^{11}C]A1070722 in Monkey Plasma

The percentage of radioactivity unchanged [^{11}C]A1070722 in plasma was determined by reverse phase HPLC method.⁴⁴ Plasma samples (~ 0.5 mL) obtained at 2, 4, 12, 30, 60, and 90 min after radiotracer injection was transferred into a tube and mixed with acetonitrile (0.7 mL). The resulting mixture was vortexed for 10 s, and centrifuged at 14 000 rpm for 4 min. The supernatant liquid (1 mL) was removed, the radioactivity was measured in a well-

counter, and the majority extract (0.8 mL) was subsequently injected onto the HPLC column (column: Phenomenex, Prodigy ODS (3) 4.6 × 250 mm, 5 μ m; mobile phase: acetonitrile: 0.1 M ammonium formate (60:40), flow rate 1.5 mL/min, retention time 7–8 min) equipped with a radioactivity detector. The metabolite and parent radiotracer fractions were collected using a PerkinElmer 470 Wizard Gamma counter. All the acquired data were then subjected to correction for background radioactivity and physical decay to calculate the percentage of the parent compound in the plasma at different time points. To reaffirm that the retention time of the parent had not shifted during the course of the metabolite analysis, a quality control sample of [^{11}C]A1070722 was injected at the beginning and at the end of the study. The metabolite fractions per time point are determined by dividing the fractions correspond to radioactivity counts of parent radiotracer with total HPLC fraction counts.

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Figure 1.
Attempted PET radioligands for GSK-3.

Figure 2. MRI coregistered PET images (sum of radioactivity 0–90 min after injection) of a representative [^{11}C]A1070722 brain scan in vervet monkey.

Figure 3.

TACs of $[^{11}\text{C}]\text{A1070722}$ in representative vervet monkey brain (ACN, anterior cingulate; AMY, amygdala; CAU, caudate; CER, cerebellum; FRT, frontal cortex; GM, gray matter; HIP, hippocampus; OCC, occipital cortex; ORB, orbital cortex; PAR, parietal cortex; PUT, putamen; THA, thalamus; WM, white matter).

Figure 4. (A) Unmetabolized parent fraction of $[^{11}\text{C}]\text{A1070722}$ in vervet monkey plasma. (B) Blood radioactivity of $[^{11}\text{C}]\text{A1070722}$ in vervet monkey. (C) Plasma radioactivity of $[^{11}\text{C}]\text{A1070722}$ in vervet monkey. The data are means $\pm$ SD of two independent experiments on same monkey.

Figure 5.

Average of the metabolite corrected volume of distribution of [^{11}C]1070722 in monkey brain regions ($N=2$) (ACN, anterior cingulate; AMY, amygdala; CAU, caudate; CER, cerebellum; FRT, frontal cortex; GM, cerebral gray matter; HIP, hippocampus; OCC, occipital cortex; PAR, parietal cortex; PUT, putamen; THA, thalamus; WM, cerebral white matter).

Scheme 1. Synthesis of A1070722 and Radiosynthesis of [¹¹C]A1070722