High Affinity Radiopharmaceuticals Based upon Lansoprazole for PET Imaging of Aggregated Tau in Alzheimer's Disease and Progressive Supranuclear Palsy: Synthesis, Pre-clinical Evaluation and Lead Selection

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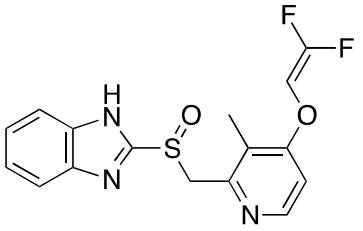
Electronic Supporting Information



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2-(((5-((2,2-difluorovinyl)oxy)-3-methylpyridin-2-yl)methyl)sulfinyl)-1*H*-benzo[*d*]imidazole (3)

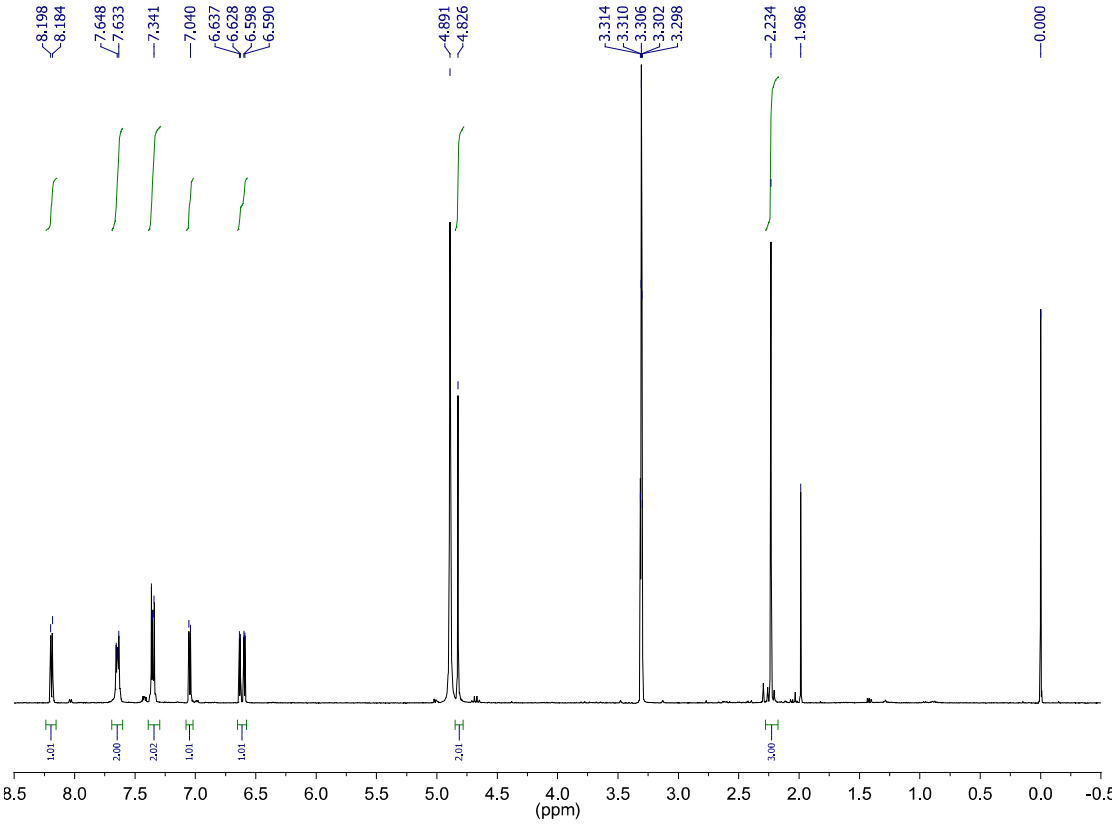


Figure S1: ^{1}H-NMR Spectrum of Precursor (**3**)

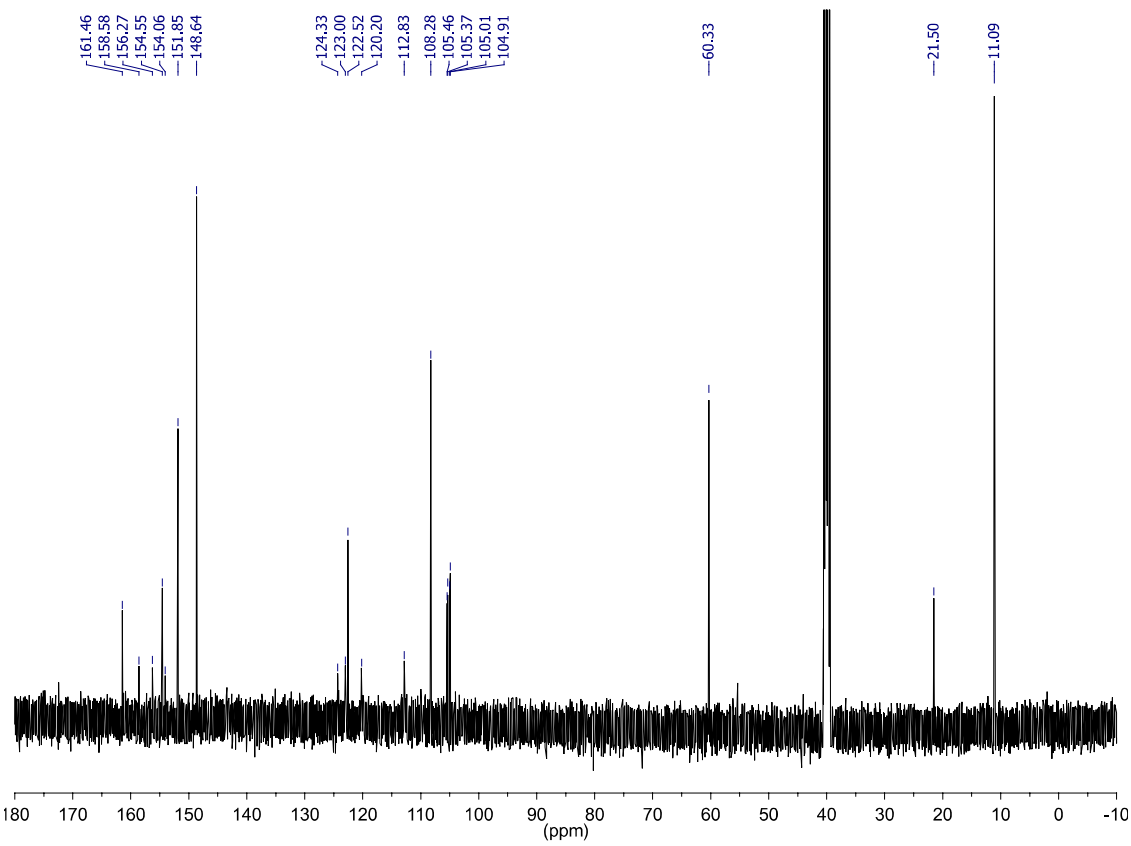


Figure S2: ^{13}C-NMR Spectrum of Precursor (**3**)

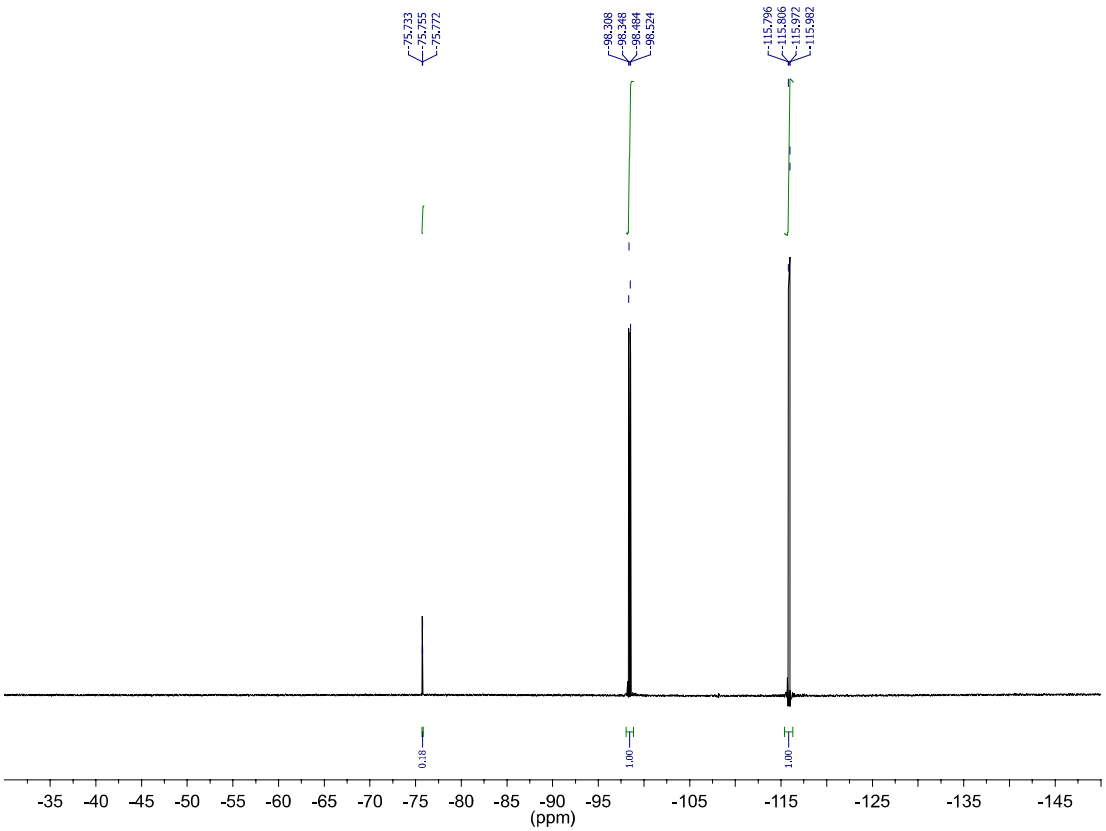


Figure S3: ^{19}F-NMR Spectrum of Precursor (**3**)

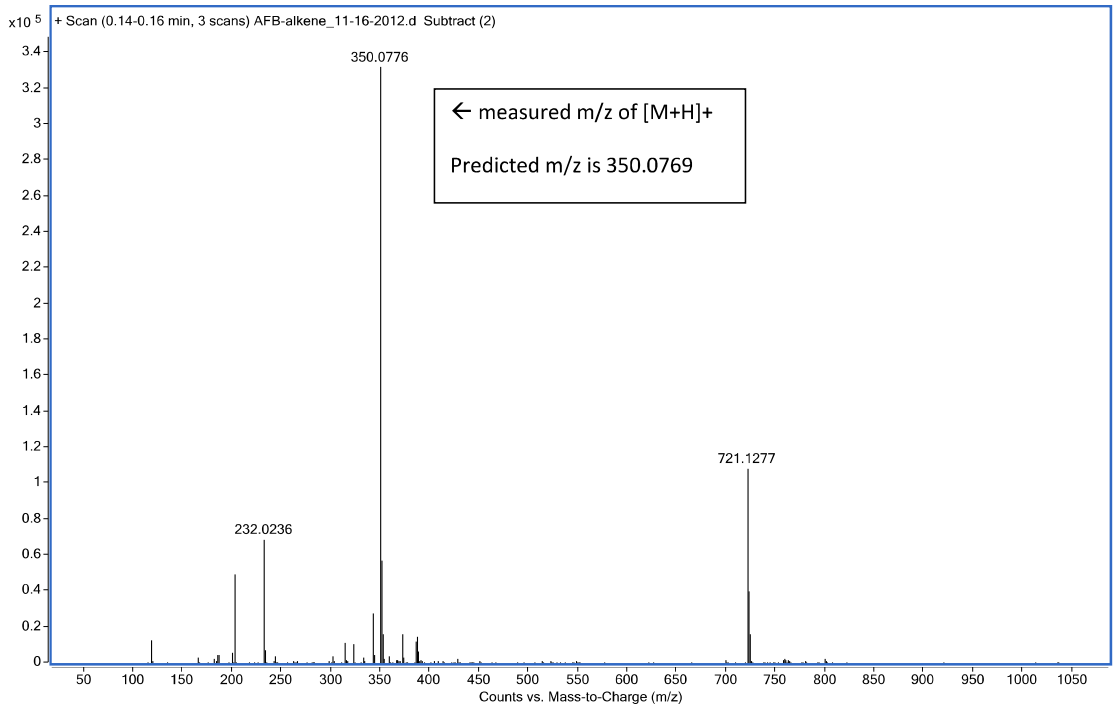
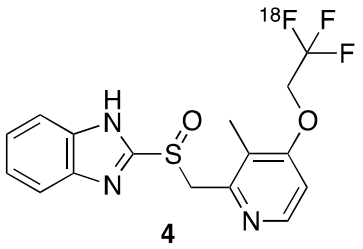


Figure S4: Mass Spectrum (ESI) of Precursor (**3**)



4

[^{18}F]Lansoprazole (4)

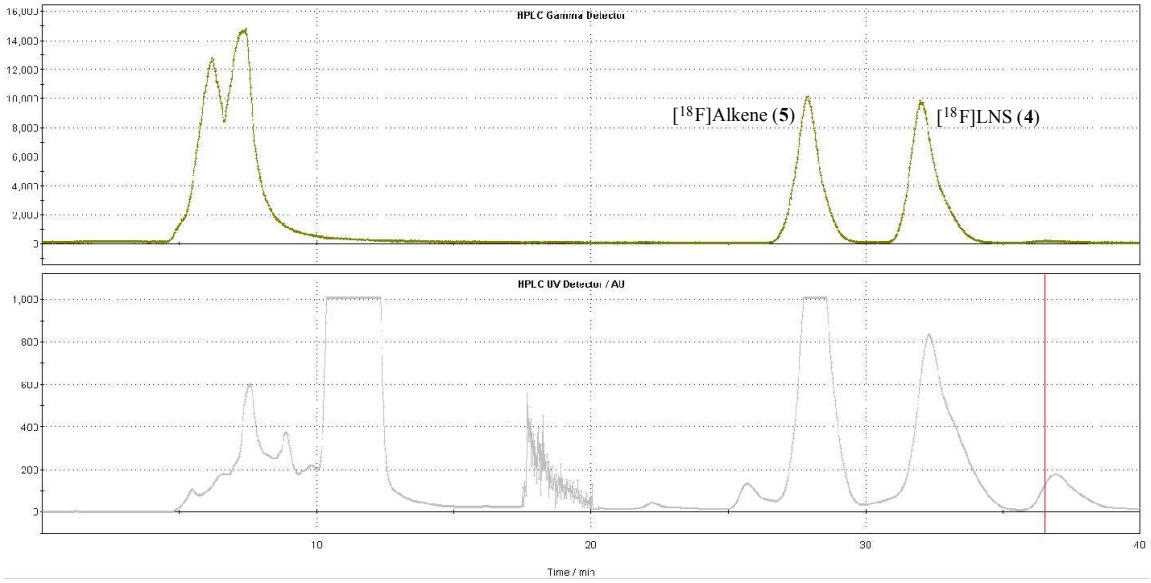
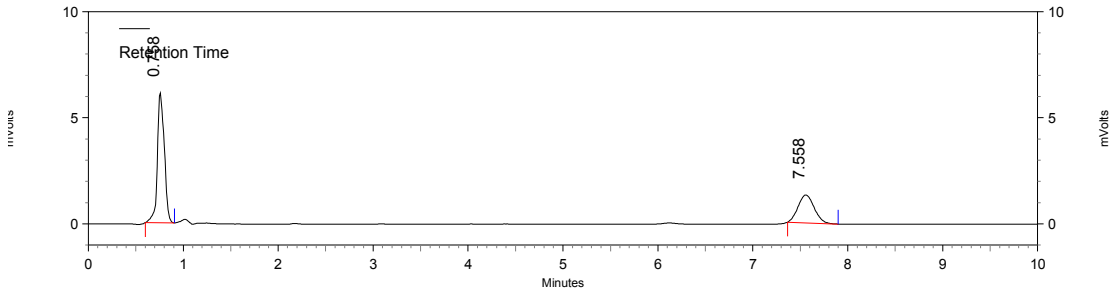


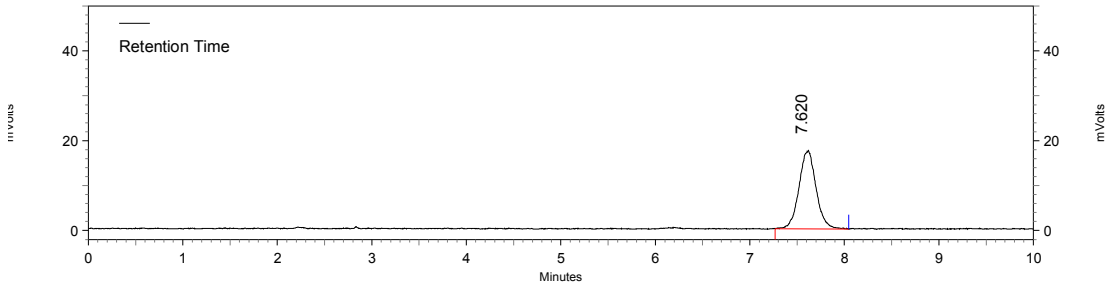
Figure S5: Semi-preparative HPLC Trace from a Representative Synthesis of [^{18}F]Lansoprazole (**4**)

UV



UV Detector Ch1-254nm Results Retention Time	Area	Area %	Height	Width	S/N (ASTM)
0.758	31074	67	6115	0.308	89.10
7.558	15537	33	1313	0.533	69.75

Gamma



RAD Results Retention Time	Area	Area %	Width
7.620	217385	100	0.78

Figure S6: Analytical HPLC Trace of [^{18}F]Lansoprazole (**4**)

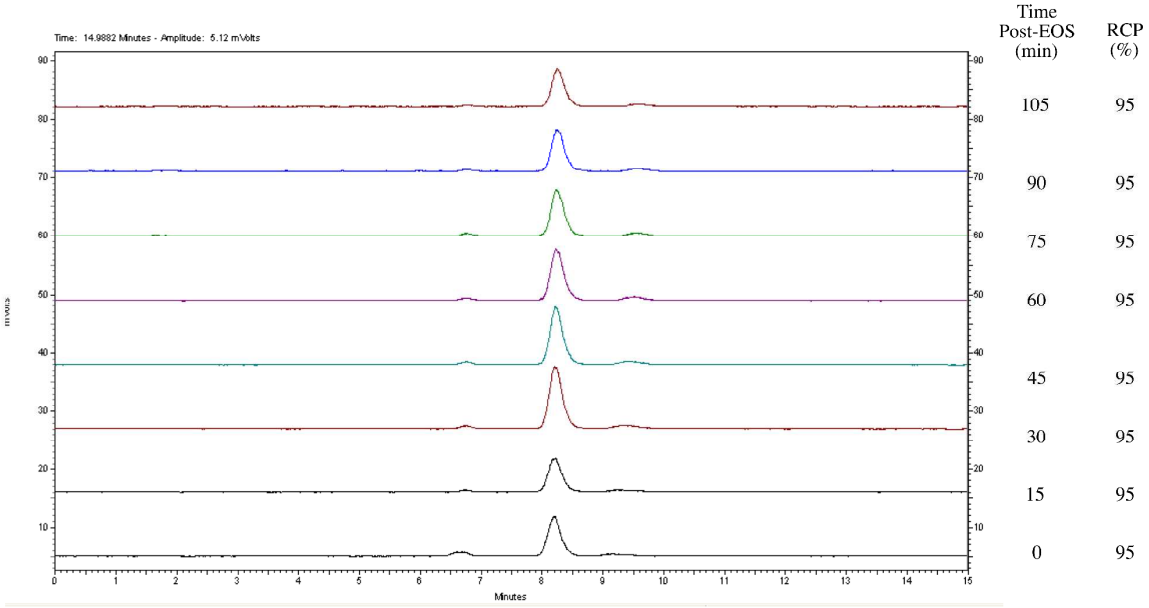
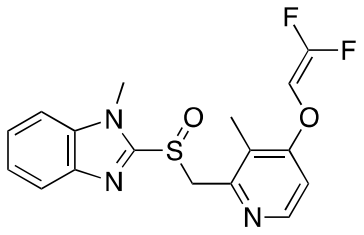


Figure S7: 2 h Stability Testing of [^{18}F]Lansoprazole (**4**)

(EOS = End of Synthesis; RCP = Radiochemical Purity)



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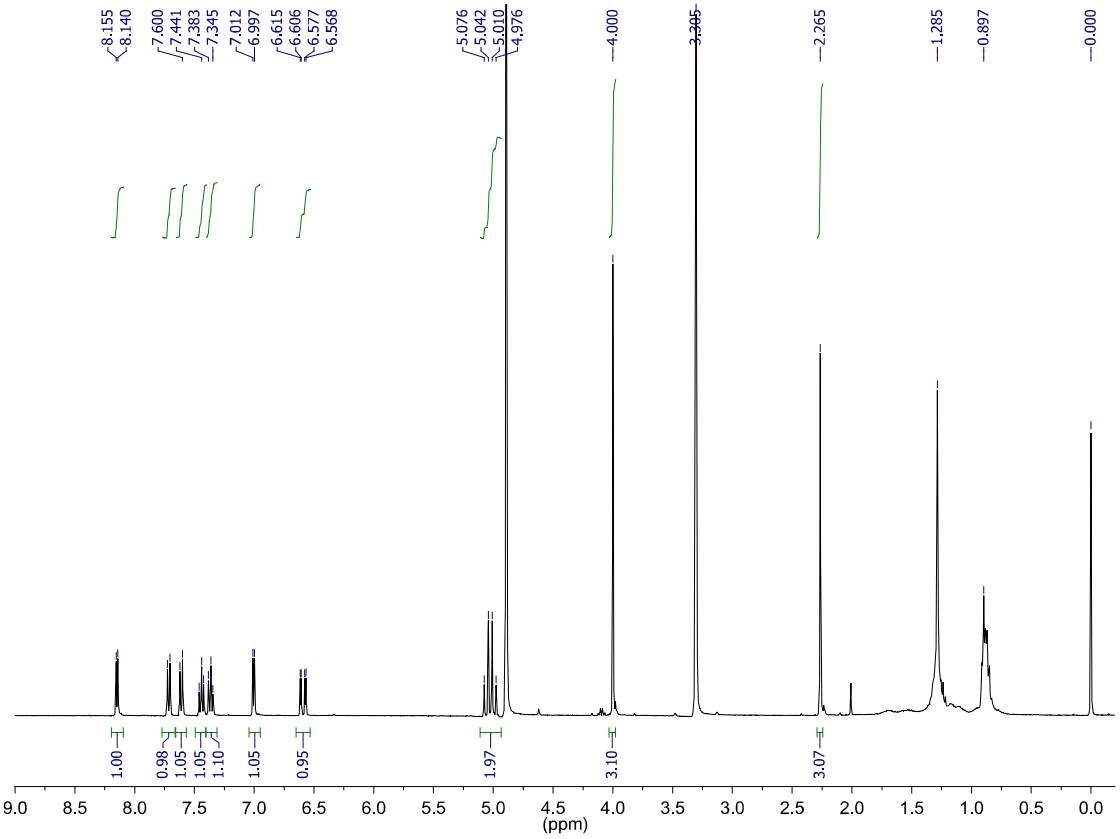


Figure S8: ^{1}H-NMR Spectrum of Precursor (**6**)

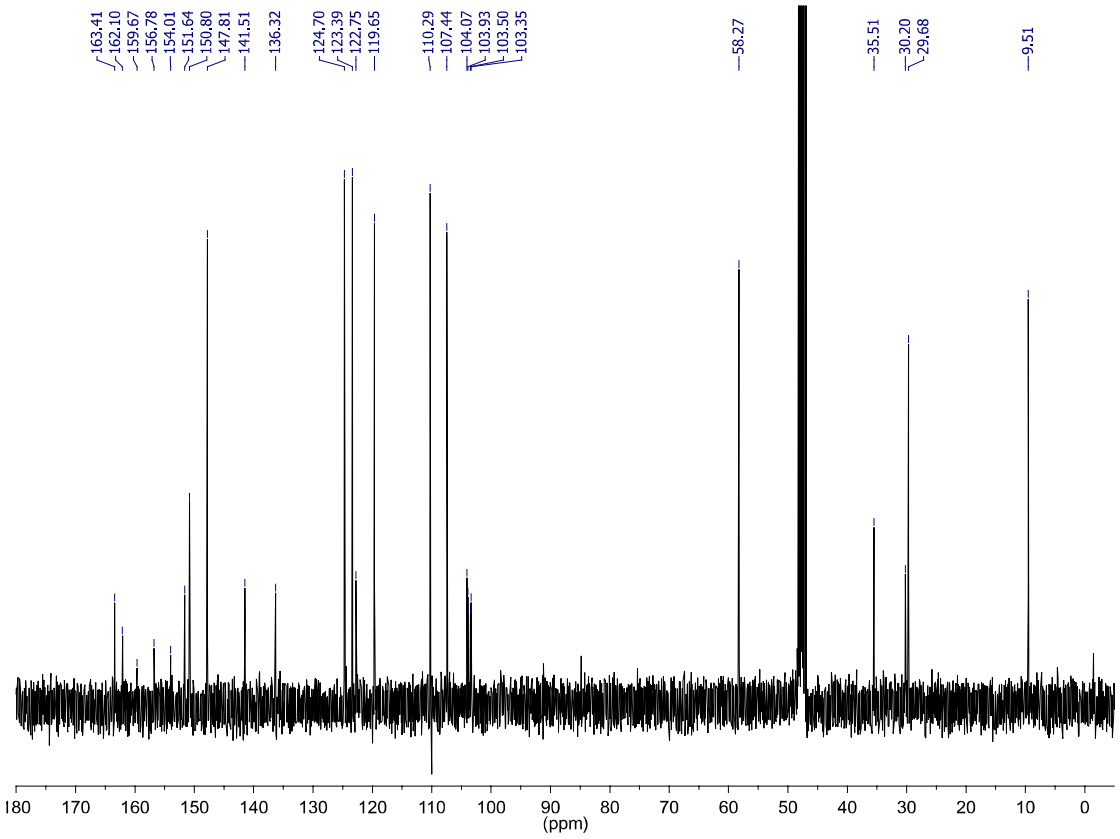


Figure S9: ^{13}C-NMR Spectrum of Precursor (**6**)

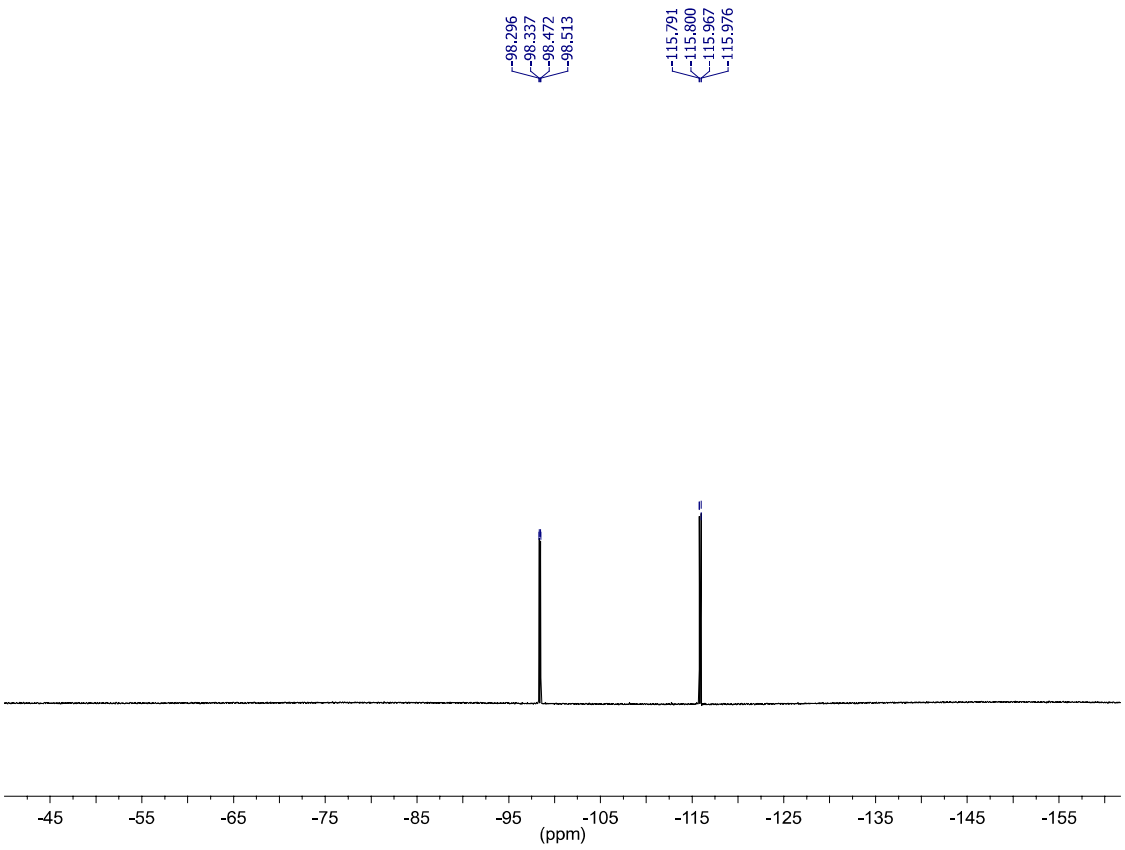


Figure S10: ^{19}F-NMR Spectrum of Precursor (**6**)

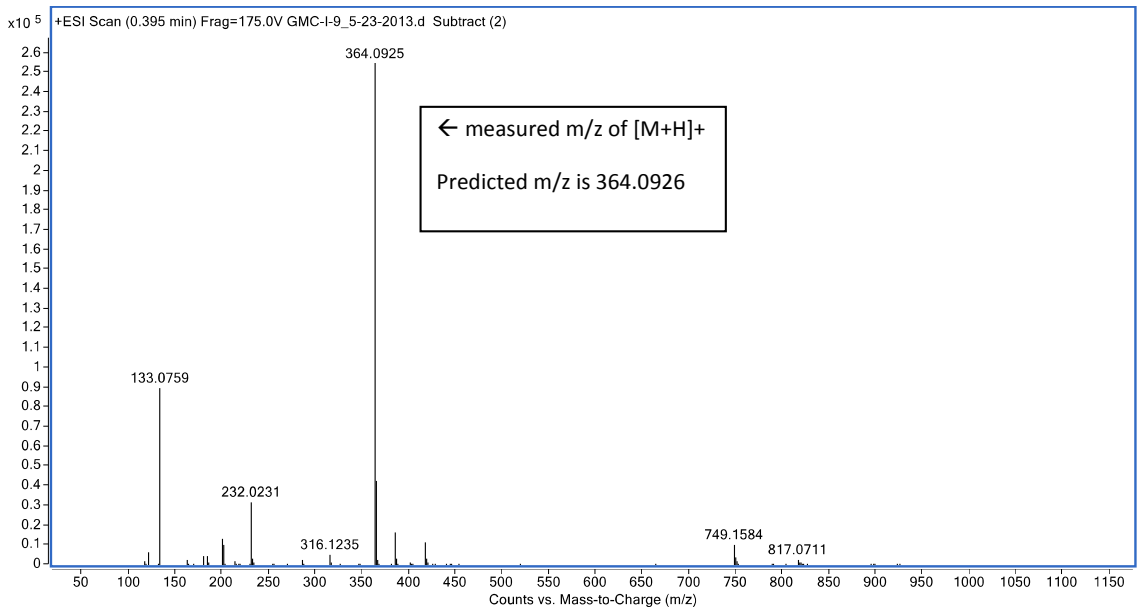
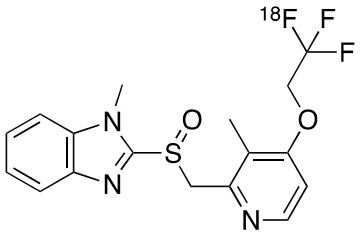


Figure S11: Mass Spectrum (ESI) of Precursor (**6**)



7

[^{18}F]*N*-Methyl Lansoprazole (7)

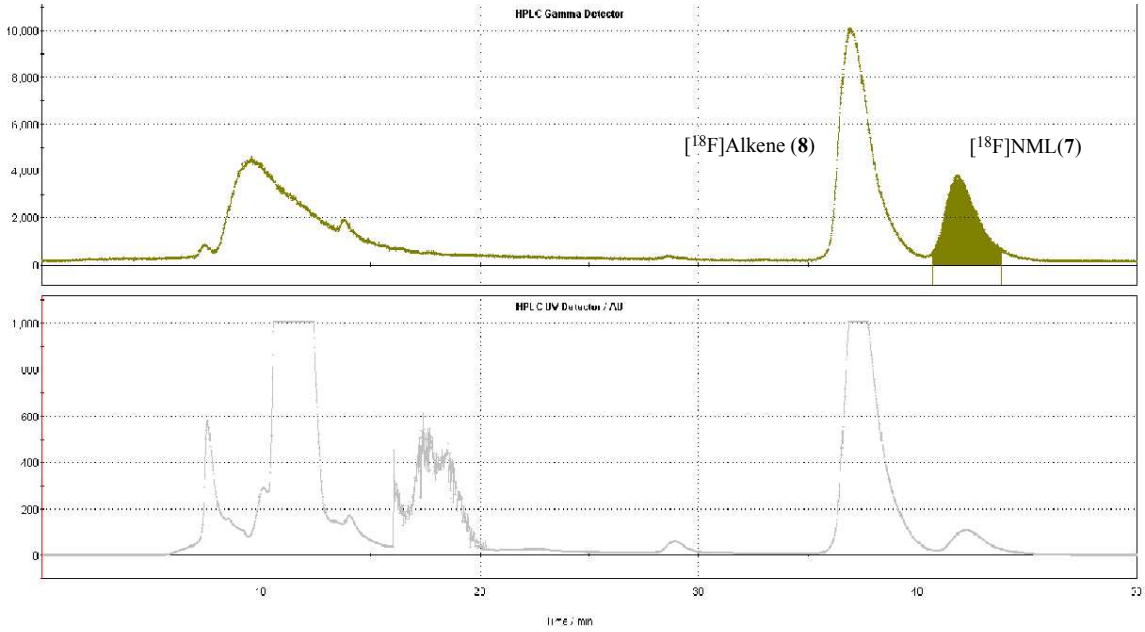
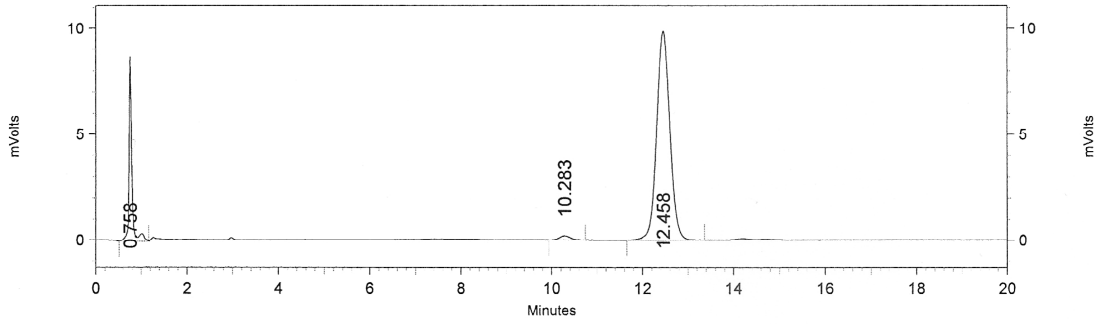


Figure S12: Semi-preparative HPLC Trace from a Representative Synthesis of [^{18}F]*N*-Methyl Lansoprazole (**7**)

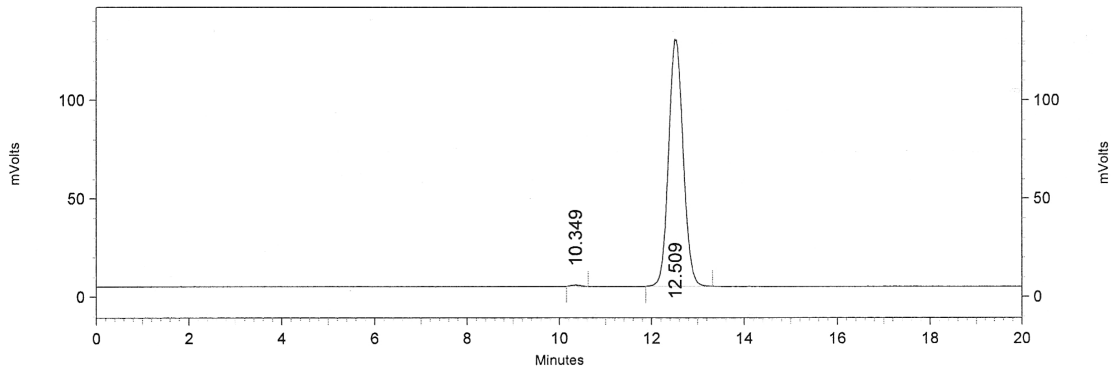
UV Detector Response



UV Detector
Ch1-254nm Results

Retention Time	Area	Area %
0.76	37352	15.62
10.28	3548	1.48
12.46	198291	82.90

Radioactivity Detector Response



RAD Results

Retention Time	Area	Area %
10.35	10893	0.39
12.51	2756186	99.61

Figure S13: Analytical HPLC Trace of [^{18}F]*N*-Methyl Lansoprazole (**7**)

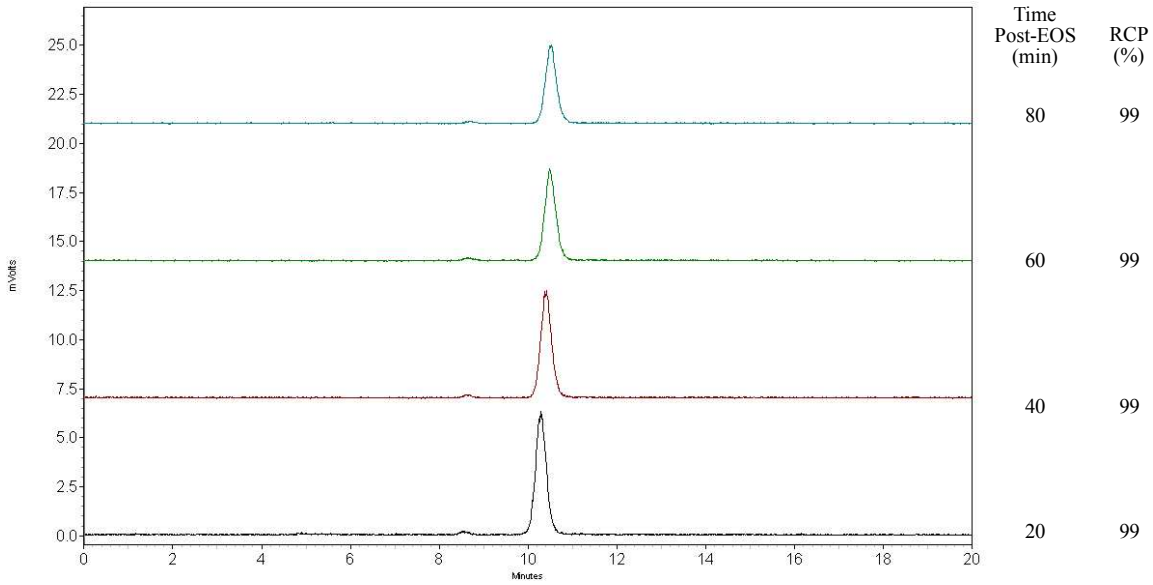


Figure S14: Stability Testing of [^{18}F]*N*-Methyl Lansoprazole (**7**)
(EOS = End of Synthesis; RCP = Radiochemical Purity)

Pre-clinical Evaluation

General Considerations. All animal studies were performed in accordance with the standards set by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan.

In Vitro Studies

Binding Affinity Studies

Preparation of Heparin Induced Tau and $A\beta_{1-42}$ Aggregates/ Congo Red Assay

Human Tau 441 (Sigma) was reconstituted with sterile water to a final concentration of 1 μg/μL solution (50 μg + 50 μL water). This was added to 2.5 mL of Hep-Lock (10 units/mL in 150 mM NaCl, pH 7.4) to produce a 1:1 molar ratio of each protein (heparin: 1 unit = 0.002 mg or 2 μg). The resulting mixture was incubated at 37ºC for six days, and then stored at -20 ºC until use.

$A\beta_{1-42}$ protein (Bachem) from a mouse was dissolved in PBS (pH 7.4) to produce a 36 ng/μL solution (25 μg + 700 μL buffer). The solution was then placed in the shaker-incubator at 37ºC and 100 rpm for 3 days. Optimal amyloid fibril formation was monitored using a Congo Red assay: 5 μL of 5 μM Congo Red was combined with 100 μL of 36 ng/μL amyloid solution in 96-well plate following by a 10 min incubation at room temperature; the absorbance of the solution was measured at 540 nm with Packard SpectraCount UV-Vis spectrophotometer. The maximal difference in absorbance

between Congo Red+protein and Congo Red+PBS was observed 3 days from the start of amyloid aggregation.

Tau and $A\beta_{1-42}$ Binding Affinity (K_d) Experiments

Tau: Whatman GF/C filters were pre-incubated in PBS/0.1% polyethylenimine (Acros) for 2 hours. Meanwhile, heparin-induced tau (25 μg) was added to PBS buffer (12.5 mL) to make the protein stock solution (2 μg/mL). [^{11}C]NML (or [^{18}F]LNS) was used to prepare 100 pM -100 nM stock dilutions (each in triplicate) in their respective tubes containing PBS buffer. Each reaction was initiated with addition of various concentrations of stock [^{11}C]NML (or [^{18}F]LNS) solution containing 0.5 μg (250 μL of 2 μg/mL) heparin-induced tau stock solution (500 μL total reaction volume). To investigate the non-specific binding, additional tubes were prepared containing 1000-fold excess of "cold" NML (or LNS) with respect to a particular [^{11}C]NML (or [^{18}F]LNS) concentration with 0.5 μg of heparin-induced tau. All tubes were incubated for 30 min at room temperature and then samples were run through the cell harvester (Brandel) to remove unbound [^{11}C]NML (or [^{18}F]LNS). This was followed by two washes with 2 mL PBS, pH 7.4. Filter circles were placed in plastic tubes and counted in the MINAXIγ Auto-Gamma 500 series gamma counter. Data was decay corrected and analyzed using a non-linear regression analysis (GraphPad Prism version 6.0b) to determine K_d and B_{max} values.

$A\beta_{1-42}$: The binding experiment and data analysis were performed as described above for tau with exception of using 0.5 μg of aggregated for 3 days $A\beta_{1-42}$ protein stock for each reaction instead of heparin-induced tau.

Autoradiography Studies.

Frozen blocks (1x1 inch) of inferior frontal cortex and anterior hippocampus from the postmortem brain of AD patients, globus pallidus from PSP patients and inferior frontal cortex from normal control patients (age range from 70 to 89) were used for the autoradiography binding studies. Tissue was obtained from the University of Michigan Alzheimer's Disease Center Brain Bank. Frozen blocks were sliced into 20 μm sections using a Hacker Instruments cryostat set to -15ºC. Tissue was thaw-mounted on the 1x3 inch polylysine-subbed glass slides. Sections used for autoradiography experiments were pre-conditioned for 5 min with phosphate buffer saline (PBS) – ethylenediaminetetraacetic acid (EDTA) buffer pH 7.4. To determine total binding, brain sections were transferred to a solution of 25 or 10 nM [^{11}C]NML, 10nM [^{18}F]NML, 10 nM [^{18}F]LNS or 0.85 nM [^{11}C]PiB in PBS-EDTA (pH 7.4), and incubated for 30 min. Similarly, the non-specific binding was determined by incubating the tissue sections (5 microns away) in the same concentration of "hot" [^{11}C]NML, [^{18}F]NML, [^{18}F]LNS or [^{11}C]PiB supplemented with 1000-fold excess of "cold" LNS or PiB in PBS-EDTA (pH 7.4) for 30 min at room temperature. Subsequently, all tissue sections were washed with ice-cold PBS-EDTA (pH 7.4) for 1 min (x2) and rinsed with ice-cold water for 5 sec to remove unbound radioactivity. Finally, all slides were dried under the continuous airflow for 5 min before being exposed to Kodak BioMax MR film (3 hours for C-11 and 2 hours for F-18 compounds). The exposed film was developed in Kodak D-19 solution for 4 min and then fixed in Kodak Rapid Fixer for 4 min. The autoradiography images were captured using a densiometer equipped with Sony XC-77 camera.

Procedures for Immunohistochemistry Studies.

Immunohistochemistry was performed according to Vectastain Elite ABC Kit instructions. Briefly, tissue sections used for autoradiography were formalin-fixed for 5 days and used directly for immunohistochemistry. Fixed sections were washed in 70% ethanol for 30 min followed by incubation with 20 μg/mL Proteinase K for 15 min at 37°C. Next, tissue sections were briefly washed with PBS pH 7.4 and then endogenous peroxidase activity was quenched with 0.3% H_2O_2 in 70% methanol for 15 min at room temperature. These sections were washed with PBS-T solution for 1 min (x3) and blocked with PBS-TBA for 30 min at room temperature. Then, the tissue slides were incubated for 24 hours at 4°C with primary antibody (Santa Cruz Biotechnology Inc. anti-tau (H-150) or Millipore anti-beta-amyloid 1-42) diluted 1:1000 or 1:500 accordingly in PBS-TBA. After 24 hours, these sections were washed with PBS-T for 5 min (x3) followed by the biotinylated secondary antibody application for 30 min at room temperature (diluted in PBS-T per Vectastain instructions). Once secondary antibody incubation was done, all sections were washed in PBS-T for 5 min (x3). The ABC solution (diluted in PBS-T per Vectastain instructions) was applied for 30 min at room temperature. Then, a 5 min rinse (x3) was performed before transferring sections to the DAB solution (tablet by Sigma) for 10 min at room temperature. Finally, the tissue sections were rinsed in water pH 4.0 and counterstained with Giemsa before coverslipping them with Permount.

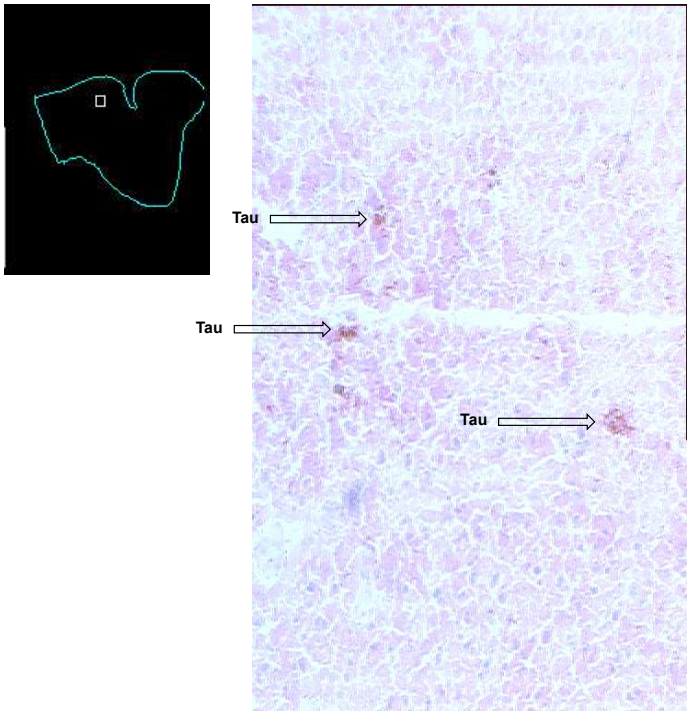


Figure S15: Tau Immunohisotchemistry of Subject #1649

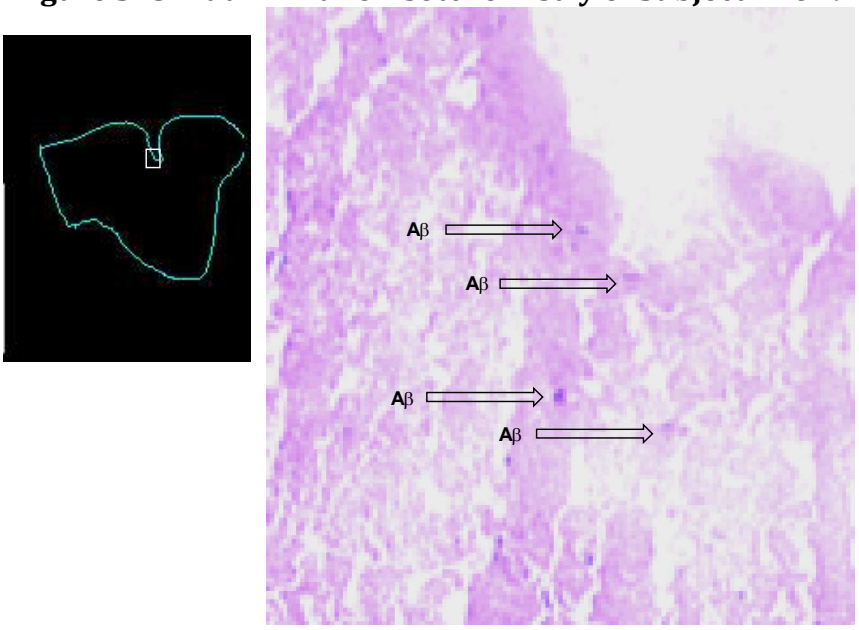


Figure S16: Amyloid Immunohisotchemistry of Subject #1649

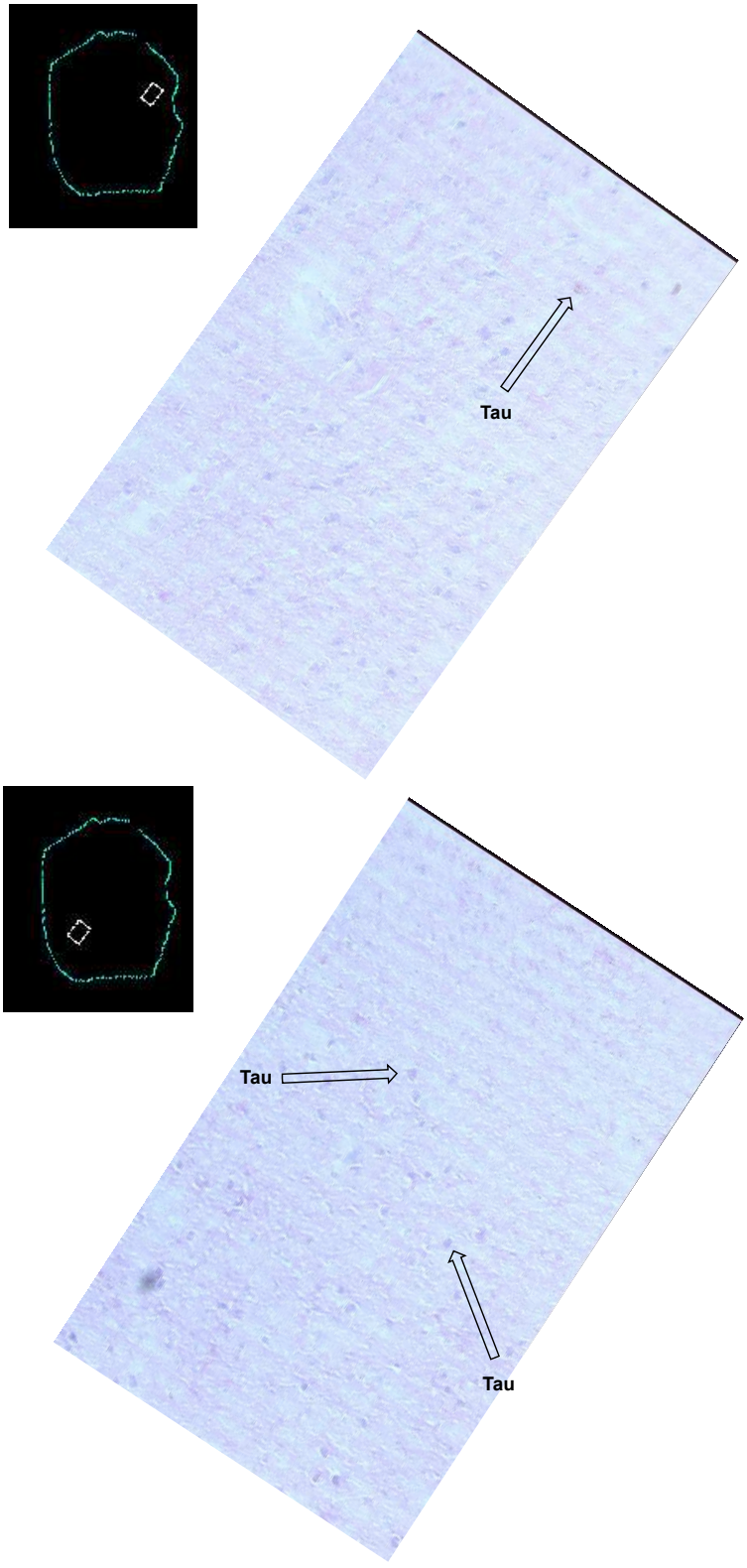


Figure S17: Tau Immunohisotchemistry of Subject #1657

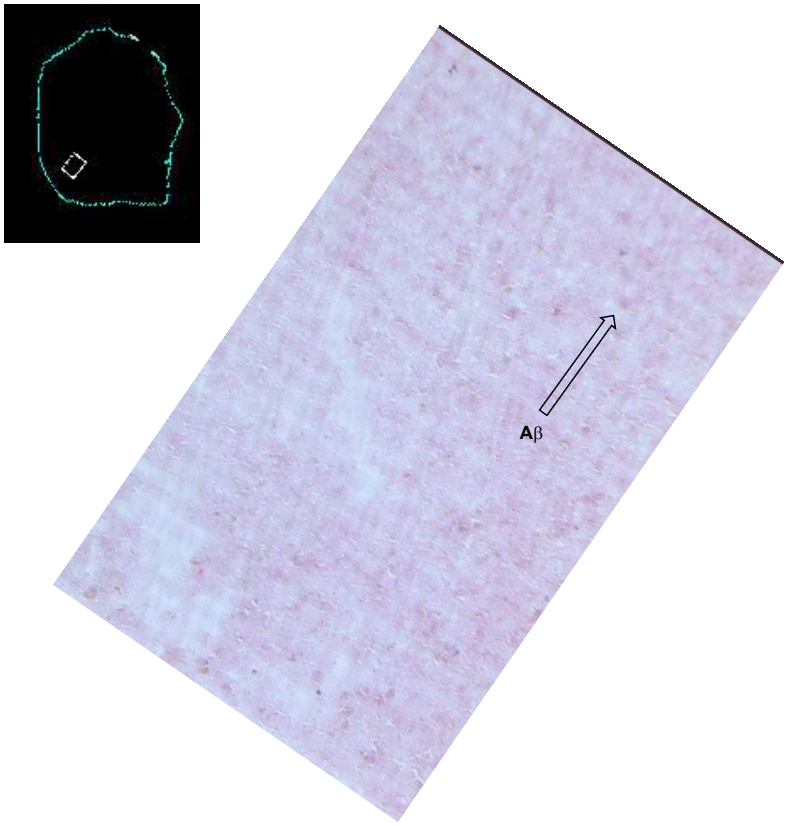


Figure S18: Amyloid Immunohisotchemistry of Subject #1657

In Vivo Imaging Studies

Rodent MicroPET Imaging. Rodent imaging studies were done using male Sprague Dawley rats. The animals were anesthetized (isoflurane), intubated, and positioned in a Concorde MicroPET R4 scanner. **[^{11}C]NML Scans** (n = 2, animal weights = 390 - 473 g): Following a transmission scan, the animals were injected i.v. (via tail vein catheter as a bolus over 1 min) with [^{11}C]NML (0.95 mCi), and the head imaged for 60 min (5 × 1 min frames – 2 x 2.5 min frames – 2 x 5 min frames – 4 x 10 min frames). **[^{18}F]LNS Scans** (n = 2, animal weights = 440 - 447 g): Following a transmission scan, the animal was injected i.v. (via tail vein catheter as a bolus over 1 min) with [^{18}F]LNS (0.5 mCi), and the head imaged for 90 min (18 x 5 min frames). In each case, emission data were corrected for attenuation and scatter, and reconstructed using the 3D maximum a priori (3D MAP) method (Figure 5A and 5B). By using a summed image, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves (Figure 6).

Rodent Pgp Blocking Studies. The imaging experiment described in the previous section was repeated using female Sprague Dawley rats (n = 2, animal weights = 288 – 325 g) pre-treated with cyclosporin A (manufactured for Bedford Labs, Bedford, OH. Lot # 1856175, each ml contains cyclosporin USP 50mg; polyoxyethyated castor oil of 650 mg; and absolute alcohol 33.2% (v/v)) per the procedure reported by Blanckaert *et al.*[1] Each rat was injected i.v. with 50mg/kg (approx. 0.3 mL of stock was diluted to 1.0 mL with saline) over a 3 minute infusion, one hr. prior to administration of the [^{11}C]*N*-methyl

lansoprazole tracer dose (0.95 mCi). The PET scan images of the rats show uptake of the tracer into the brain followed by rapid clearance. Emission data were corrected for attenuation and scatter, and reconstructed using the 3D maximum a priori (3D MAP) method (Figure 5C and 5D). By using a summed image, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves (Figure 6).

Primate MicroPET Imaging. Primate imaging studies were done using the same mature female rhesus monkey (weight = 5.7 kg without significant variation throughout the course of the studies). The monkey was anesthetized (isoflurane), intubated, a venous catheter inserted into one hindlimb and positioned in the bed of the Concorde MicroPET P4 gantry. Isoflurane anesthesia was continued throughout the study. **[^{11}C]NML Scans** (n = 2): Following a transmission scan, the animal was injected i.v. with [^{11}C]NML (5.4 mCi) as a bolus over 1 min, and the brain imaged for 60 min (5 x 2 min frames – 4 x 5 min frames – 3 x 10 min frames). **[^{18}F]NML Scans** (n = 2): Following a transmission scan, the animal was injected i.v. with [^{18}F]NML (4.5 mCi) as a bolus over 1 min, and the brain imaged for 90 min (18 x 5 min frames). **[^{18}F]LNS Scans** (n = 2): Following a transmission scan, the animal was injected i.v. with [^{18}F]LNS (4.6 mCi) as a bolus over 1 min, and the brain imaged for 60 min (12 x 5 min frames). In each case, emission data were corrected for attenuation and scatter, and reconstructed using the 3D maximum a priori (3D MAP) method (Figure 7). By using a summed image, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves (Figure 8).

References

1. Blanckaert, P., Burvenich, I., Staelens, S., De Bruyne, S., Moerman, L., Wyffels, L., and De Vos, F. (2009) Effect of cyclosporin A administration on the biodistribution and multipinhole μSPECT imaging of [^{123}I]R91150 in rodent brain., *Eur. J. Nucl. Med. Mol. Imaging 36*, 446-453.