SUPPLEMENTARY INFORMATION

PET neuroimaging studies of [^{18}F]CABS13 in a double transgenic mouse model of Alzheimer's disease and non-human primates

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Experimental details

Animal husbandry

All animal experiments were conducted in compliance with Institutional Animal Care and Use Committee (IACUC) guidelines and the *Guide for the Care and Use of Laboratory Animals*. Female, wild-type B6C3F1/J mice (6 – 8 weeks old) and transgenic B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J (APP/PS1; Stock No.: 034829-JAX or MMRRC No. 034) mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were provided with food and water *ad libitum* and were allowed to acclimatize for 1 week prior to conducting PET/CT and biodistribution experiments.

Small-animal PET/CT imaging

Dynamic PET/CT imaging experiments were conducted on a dedicated small-animal PET/CT scanner (eXplore Vista-CT*,* Sedecal*,* Algete, Spain) equipped with VISTA-CT version 4.11 software. In separate studies (n=3), mice were administered formulations of [^{18}F]CABS13 (~4.0 – 16.0 MBq [~100 – 432 μCi], specific activity of > 2 Ci/μmol, in 200 μL sterile PBS, pH7.4, ≤5% v/v EtOH) *via* intravenous (i.v.) tail-vein injection using a catheter. Approximately 5 minutes prior to recording PET images, mice were anesthetized by inhalation of 3–4% isoflurane (Baxter Healthcare, Deerfield, IL)/oxygen gas mixture and, a catheter was inserted into the tail-vein, and then mice were transferred to the scanner bed and placed in the prone position. Anesthesia was maintained with 1-2% isoflurane/oxygen gas mixture (flow rate ~5 L/min). Co-registered dynamic PET/CT images were recorded for a total of 20 – 30 min. post-injection radiotracer injection (n≥3 mice/time point). List-mode data were acquired for 20 – 30 min. per scan using a γ-ray energy window of 250–700 keV. To ensure that the activity bolus was measured, Pet/CT data acquisition was initiated 20 s prior to injecting the radioactivity. Data were processed by 3-dimensional Fourier re-binning (3D-FORE), and images were reconstructed using 2-demensional ordered-subset expectation maximum (2D-OSEM) algorithm. Image data were normalized to correct for non-uniformity of response of the PET, dead-time count losses, positron branching ratio, and physical decay to the time of injection, but no attenuation, scatter, or partial-volume averaging correction was applied. An empirically determined system calibration factor (in units of Bq/cps) combined with the decay corrected administered activity and the animal weight were used to parameterize image activity in terms of the standardized uptake value (SUV). Manually drawn 2-dimensional regions-of-interest (ROIs) or 3-dimensional volumes-of-interest (VOIs) were used to determine the maximum and mean SUV radiotracer uptake in various tissues. Time-activity curves (TACs) were generated from ROI analysis on dynamic PET/CT data using 20 s frames. CT images were recorded using an X-ray current of 300 μA, 360 projections, and an image size of 63.8 mm × 63.8 mm × 46.0 mm. Data were acquired using the Vista CT 4.11 Build 701 software, and reconstructed images were analyzed by using VivoQuant® 1.23 (InviCRO, LLC, Boston, MA).

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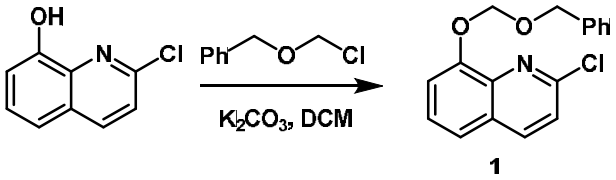
Data analysis and statistics

Data and statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA) and Microsoft Excel spreadsheets.

Synthesis of radiolabeling precursor, 1

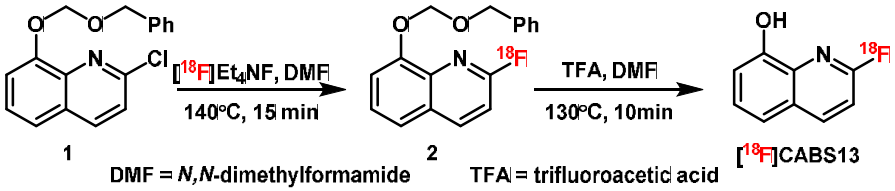


Supplementary Scheme 1. Synthetic scheme for the preparation of compound **1**

Procedure: To a 50 ml flask was added 2-chloro-8-hydroxyquinoline (200.0 mg, 1.12 mmol), $EtiPr_2N$ (584 µL, 3.36 mmol), benzyloxymethyl chloride (~60% pure by NMR, 522µL, 2.24 mmol) and CH_2Cl_2 (2 mL). The mixture was stirred at room temperature overnight, then concentrated in vacuo. The crude was purified by silica gel to yield 210 mg (63% yield) of desired product. ^{1}H NMR: (300.1 MHz, $CDCl_3$) δ 8.08 (d, J = 8.6 Hz, 1H), 7.57 - 7.45 (m, 3H), 7.41 (d, J = 8.5 Hz, 1H), 7.37 - 7.27 (m, 6H), 5.63 (s, 2H), 4.85 (s, 2H) ppm; ^{13}C NMR: (75.5 MHz, $CDCl_3$) δ 152.57, 150.04, 139.99, 138.89, 137.32, 128.42, 128.11, 127.99, 127.83, 127.74, 127.26, 122.93, 120.79, 114.32, 93.68, 70.54 ppm.

Radiochemistry

The fluorine-18 incorporation of **1** provided *ca.* 50-70% radiochemical conversions by radio-TLC (as detailed in Scheme 2). We next studied different deprotection conditions to remove the benzyloxy methyl and found that trifluoroacetic acid showed the optimal radiochemical conversions (>95%) among other common acids used in PET radiochemistry, including $HCl_{(aq.)}$, $HBr_{(aq.)}$, $TFA_{(aq.)}$ and $H_2SO_{4(aq.)}$. We also investigated the effects of temperature, reaction time and stoichiometry of trifluoroacetic acid, and determined that highest radiochemical conversions in deprotection reactions were achieved at 120°C for 10 min (Table 1).



Supplementary Scheme 2. Radiochemical synthesis of [^{18}F]CABS13

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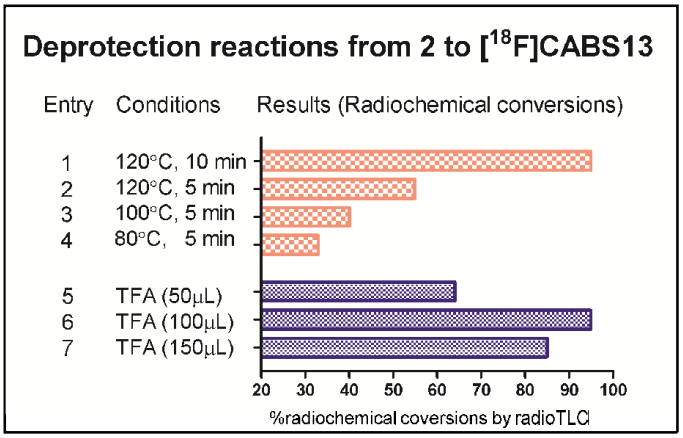

Supplementary Table 1. Optimization of temperature, time and amount of TFA for deprotection reactions

Automated synthesis of [^{18}F]CABS13

General description: A schematic diagram used for the synthesis of [^{18}F]CABS13 on GE TRACERlab™ FX_{FN} is shown in Supplementary Figure 1. [^{18}F]Fluoride was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge. Automated synthesis began with the elution of resin-bound [^{18}F]fluoride using a solution (0.075M, 0.8 mL) of tetraethylammonium bicarbonate, pre-loaded into vial 1 and delivered to the reaction vessel 12. The reaction mixture was dried azeotropically by addition of 1 mL anhydrous CH_3CN, pre-loaded into vial 5, at 85 °C under N_2 flow and vacuum over 8 min, then at 110 °C under N_2 flow and vacuum for 4 min. After heating to 140 °C, the precursor (**1**; 6 mg in 1.1 mL DMF) pre-loaded into vial 3 was added to 12. The reactor was sealed *via* the closure of valve V13, V20 and V24 and the reaction mixture was heated for 15 min. The reaction mixture was then cooled to 40 °C, vented *via* valves V24 and V25. The trifluoroacetic acid solution (100μL TFA in 300 μL DMF) pre-added into vial 4 was added to 12 and the reactor was sealed again and heated at 120°C for 10 min. The reaction mixture was then cooled to 40°C and diluted with 3M NaOAc (0.7 mL) and 30:70 CH_3CN / 0.1N ammonium formate (2.5 mL) pre-loaded into vial 6. The contents of reaction vessel were delivered into vessel 14, then to the HPLC loop *via* N_2 pressure *via* a fluid detector, injected onto a semi-preparative column (Luna C-18, 250 × 10.00 mm, 5μ), and eluted with 30:70 CH_3CN / 0.1 N ammonium formate by volume at a flow rate of 5 mL/min. The eluent was monitored by UV (λ = 254 nm) and radiochemical detectors connected in series. The retention time of [^{18}F]CABS13 is ca. 19 minutes (Supplementary Figure 2). The [^{18}F]CABS13 product peak was collected into the HPLC fraction collect vessel 15, where it was diluted with 23 mL of sterile water for injection, USP. The diluted HPLC fraction was then loaded on a tC18 SPE cartridge 16 for HPLC solvent removal. The tC18 SPE was washed with 10mL sterile water for injection, USP, preloaded into vial 7 and then the [^{18}F]CABS13 product was recovered in 1mL dehydrated alcohol for injection, USP (ethanol; vial 8) into vial 17 and 10 mL of 0.9% sodium chloride for injection, USP (vial 9). The solution was transferred and passed through a 0.22 μm Millipore GV sterilizing filter (EMD Millipore) into a vented sterile 30 mL dose vial.

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Step-by-step automation procedure: Automated synthesis involves the following: (1) azeotropic drying of [^{18}F]fluoride; (2) [^{18}F]fluorination; (3) HPLC purification, followed by solid-phase formulation of the final product. The synthesis module was operated in the following sequences with numerical references to Supplementary Figure 1.

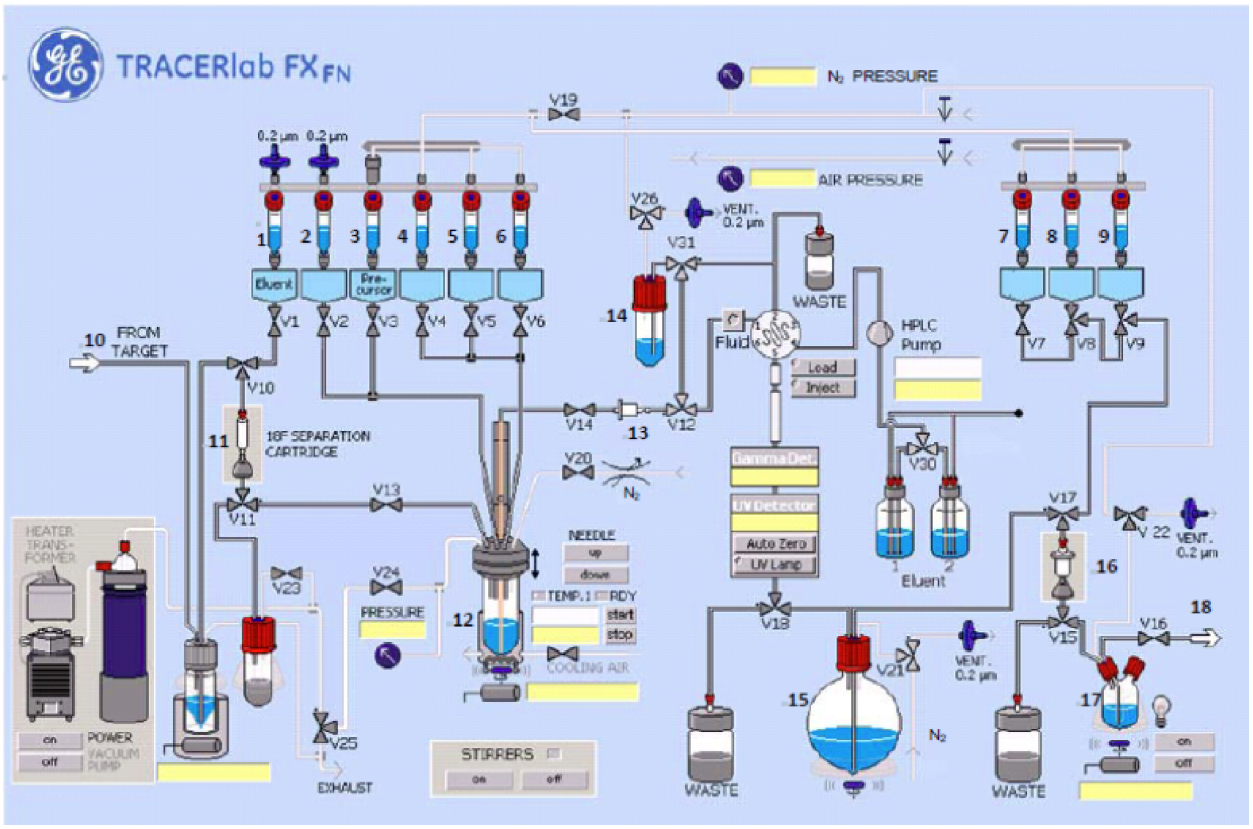
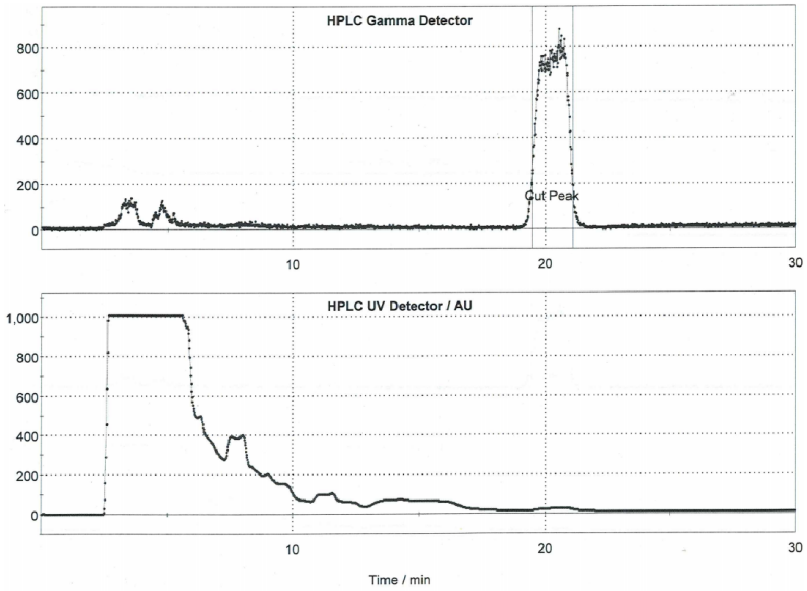


Figure 1: Schematic of the GE TRACERlab™ FX_{FN} radiosynthesis module automated synthesis manifold for [^{18}F]CABS13

1. [^{18}F]Fluoride was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module via 10. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge (Waters; activated with 6 mL of trace grade H_2O).
2. Automated synthesis began with the elution of resin-bound [^{18}F]fluoride using a solution (0.075M, 0.8 mL) of tetraethylammonium bicarbonate, pre-loaded into 1 and delivered to the reactor (12).
3. The reaction mixture (12) was dried azeotropically by addition of 1 mL anhydrous CH_3CN, pre-loaded into 5, at 85 °C under N_2 flow and vacuum over 8 min, then at 110 °C under N_2 flow and vacuum for 4 min.
4. After heating to 140 °C, the precursor (**1**; 6 mg in 1.1 mL DMF) pre-loaded into 3 was added to 12. The reactor was sealed *via* the closure of valve V13, V20 and V24 and the reaction mixture was heated for 15 min.
5. The reaction mixture was then cooled to 40 °C, vented *via* valves V24 and V25, and added with trifluoroacetic acid solution (100µL TFA in 300 µL DMF) pre-added into vial 4.

6. The reactor (12) was sealed again and heated at 130°C for 10 min. The reaction mixture was then cooled to 40°C and diluted with 3M NaOAc (0.7 mL) and 30:70 CH_3CN / 0.1N ammonium formate (2.5 mL) pre-loaded into vial 6.
7. The contents of reaction vessel were delivered into vessel 14, then to the HPLC loop *via* N_2 pressure *via* a fluid detector, injected onto a semi-preparative column (Luna C-18, 250 × 10.00 mm, 5μ), and eluted with 30:70 CH_3CN / 0.1 N ammonium formate by volume at a flow rate of 5 mL/min. The eluent was monitored by UV (λ = 254 nm) and radiochemical detectors connected in series.
8. A typical semi-preparative HPLC chromatogram is shown in Supplementary Figure 2. The fraction containing the major radiochemical product (t_R = 19 min) was collected, via valve 18, into a large dilution vessel (15), which was preloaded with 23 mL of sterile water for injection (United States Pharmacopeia (USP); Hospira).



Supplementary Figure 2: Semi-preparative HPLC trace of a typical radiosynthesis of [^{18}F]CABS13

9. The diluted HPLC fraction was then loaded onto a tC18 SPE cartridge (16) (Waters; pre-activated with 5 mL EtOH followed by 10 mL H_2O).
10. The content of 16 was washed with 10 mL sterile water for injection, USP, preloaded into 7, to remove traces of salts, CH_3CN, and [^{18}F]fluoride.
11. The content of 16 was eluted with 1 mL dehydrated alcohol for injection, USP (Ethanol) preloaded into 8, into collection vial 17 followed by 10 mL 0.9% sodium chloride for injection, USP preloaded into 9.
12. The solution was transferred and passed through a 0.22 μm Millipore GV sterilizing filter (EMD Millipore) into a vented sterile 30 mL dose vial (Hospira).

Analyses of radioactive mixtures were performed by HPLC with an in-line UV ((λ = 254 nm) detector in series with a CsI PIN diode radioactivity detector. To determine the identity of [^{18}F]CABS13,

aliquots of the formulated product were injected onto an analytical HPLC system using an analytical Luna C-18 (150 × 4.6 mm, 5 μm) and eluted with mobile phase (CH_3CN / 0.1N ammonium formate, 70:30 v/v) with a flow rate of 1 mL/min, monitored at λ = 254 nm. The major radiochemical product was identified as [^{18}F]CABS13 (t_R = ~5.8 min).

Quality control for [^{18}F]CABS13

Visual Inspection: The [^{18}F]CABS13 dose was clear, colorless, and free of particulate matter.

Radiochemical Identity, Radiochemical Purity, Chemical Purity and Specific Activity: To determine the identity of [^{18}F]CABS13, aliquots of the formulated product were injected onto an analytical HPLC system using an analytical Luna C-18 (150 × 4.6 mm, 5 μm) and eluted with mobile phase (CH_3CN / 0.1N ammonium formate, 70:30 v/v) with a flow rate of 1 mL/min., monitored at λ = 254 nm. After completion of the chromatograph, peaks on UV and radioactivity detector were integrated and the radiochemical and chemical purity were determined by the area of integration.

The major radiochemical product was identified as [^{18}F]CABS13 (t_R = ~ 5.8 min.), followed by co-injection with the reference standard CABS13. The retention time of [^{18}F]CABS13 was compared to that of the standard [^{19}F]CABS13 and was within ±10% error. The radiochemical purity was ≥99% and chemical purify was ≥95%. Specific activity was determined using authentic CABS13 to generate a calibration curve.