ELECTRONIC SUPPLEMENTARY INFORMATION

Syntheses of [^{11}C]2- and [^{11}C]3-trifluoromethyl-4-aminopyridine: potential PET radioligands for demyelinating diseases

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Contents

Materials

Chemicals, including halopyridine precursors and reference trifluoromethyl pyridines, were obtained from Sigma Aldrich, Combi-blocks or Astatech. Sources of materials used to build the [^{11}C]fluoroform apparatus are given below.

General Methods

Safety and regulatory approval. All experiments involving nonhuman primates were performed in accordance with the U.S. Department of Agriculture (USDA) Animal Welfare Act and Animal Welfare Regulations (Animal Care Blue Book), Code of Federal Regulations (CFR), Title 9, Chapter 1, Subchapter A, Part 2, Subpart C, §2.31. 2017. Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Massachusetts General Hospital (MGH). Experiments involving radioactive materials were performed by trained personnel following all relevant regulations.

Radioactivity measurement. All values are decay corrected to time of HPLC injection for radiochemical conversions (RCCs) and for total synthesis time for yield in the production of PET radiotracer.

Statistics. Grouped data are reported as mean ± SE.

HPLC analysis of [^{11}C]fluoroform purity using acidic mobile phase. Analysis of the radiochemical purity of [^{11}C]fluoroform crude was performed using HPLC on an XBridge™ C-18 column (3.5 μm, 100 × 4.6 mm; Waters) eluted at 1 mL/min under acidic mobile phase (MeCN-0.1% TFA in water, 5:95, v/v). Under these conditions two radioactive peaks were observed with retention times *ca*. 3.42 and 4.68 min (**Figure S1-A**) with [^{11}C]fluoroform corresponding to the second peak.

HPLC analysis of [^{11}C]fluoroform crude using basic mobile phase. Because the analysis of the radiolabeling reactions was done using basic mobile phase (MeCN-10 mM NH_4HCO_3 in water), we analyzed the [^{11}C]fluoroform crude under these conditions on a Gemini® C-18 column (5 μm, 100 × 4.6 mm; Phenomenex). Under these conditions, three peaks were observed with retention times *ca*. 1.37, 2.59 and 3.90 min with [^{11}C]fluoroform corresponding to the third peak (**Figure S1-B**).

HPLC analysis of [^{11}C]fluoroform stability under basic conditions. To investigate the nature of the additional peak seen under basic HPLC conditions (t_R ~ 1.37 min), we tested the stability of purified [^{11}C]fluoroform under these conditions. [^{11}C]fluoroform was produced as described above, trapped in DMF (0.3 mL) and analyzed using HPLC with acidic mobile phase (**Figure S2-A**). A portion of the crude [^{11}C]fluoroform was purified via HPLC on an XBridge™ C-18 column (5 μm, 250 × 10 mm; Waters) eluted at 4 mL/min with MeCN-water (5:95, v/v). Pure [^{11}C]fluoroform was collected (t_R = 7 min) and analyzed via analytical HPLC on a Gemini® C-18 column (5 μm, 100 × 4.6 mm; Phenomenex) eluted at 1 mL/min with MeCN-0.1% TFA in water, 5:95 v/v (**Figure S2-B**). Stability of [^{11}C]fluoroform was examined by

addition of 100 µL of 100 mM NH_4HCO_3 to 900 µL of collected pure [^{11}C]fluoroform at RT (**Figure S2-C**) and 130 ºC (**Figure S2-D**). This experiment confirmed that the additional peak (t_R ~ 1.37 min) observed under basic conditions does not arise from the [^{11}C]fluoroform and therefore does not need to be accounted for when calculating radiolabeling yields.

Radiolabeling reactions and HPLC analysis. See manuscript text. Under the basic HPLC elution conditions, the [^{11}C]fluoroform shows an additional peak (t_R = 1.4 min, **Figure S1-B**) which results from interaction with the basic buffer.

Identification of [^{11}C]3-CF_3-4AP ([^{11}C]17) prepared from 7 and calculation of molar activity. After [^{11}C]fluoroform (4.24 GBq; 115 mCi) had been produced, a portion 548 MBq (14.8 mCi) was used to prepare [^{11}C]$CuCF_3$, as described above. A solution of precursor **7** (4.8 mg, 15 µmol) in DMF (0.1 mL) was then added to the reaction vial and the mixture heated to 130 ºC for 10 min. The Boc protecting group was removed by treatment with HCl (0.5 mL; 1 M) at 130 ºC for 10 min. The reaction vial was removed from heat and allowed to reach RT. NaOH solution (0.58 mL; 1 M) was then added to neutralize the reaction mixture and the crude product as analyzed with radio-HPLC on an XBridge™ C-18 column (5 µm; 250 × 10 mm; Waters) eluted at 3 mL/min with EtOH-10 mM NH_4HCO_3 (20:80 v/v). [^{11}C]**17** product was identified by co-injection with authentic **17**. Molar activity was determined by measuring injected radioactivity and calculating the mass of the product from the UV 254nm (**Figure S14**) using a previously determined calibration curve.

***In vivo* stability of [^{11}C]3-CF_3-4AP.** *Arterial blood sampling:* Arterial blood samples of 1 to 2 mL were drawn every 30 seconds immediately following radiotracer injection and decreased in frequency to every 30 minutes toward the end of the scan. [^{11}C]3-CF_3-4AP metabolism was measured from plasma samples following the centrifugation of whole blood samples acquired at 5, 10, 15, 30 and 60 minutes. *Arterial blood processing and radiometabolite analysis:* Radiometabolite analysis was performed using an automated column switching radioHPLC system[1,2]. Briefly, arterial plasma was injected onto the column switching radio-HPLC and initially trapped on a catch column (Waters Oasis HLB 30 µm) using mobile phase consisting of 99:1 10 mM ammonium bicarbonate pH 8 in water:MeCN at 1.8 mL/min (Waters 515 pump). After 4 minutes, the catch column was backflushed with 85:15 10 mM ammonium bicarbonate pH 8 in water:MeCN at 1 mL/min (second Waters 515 pump) and directed onto a Waters XBridge BEH C18 (130 Å, 3.5 µm, 4.6 mm x 100 mm) analytical column. Percent parent in plasma (%PP) was calculated as the area under the curve (AUC) of the parent peak over the AUC of all other peaks.

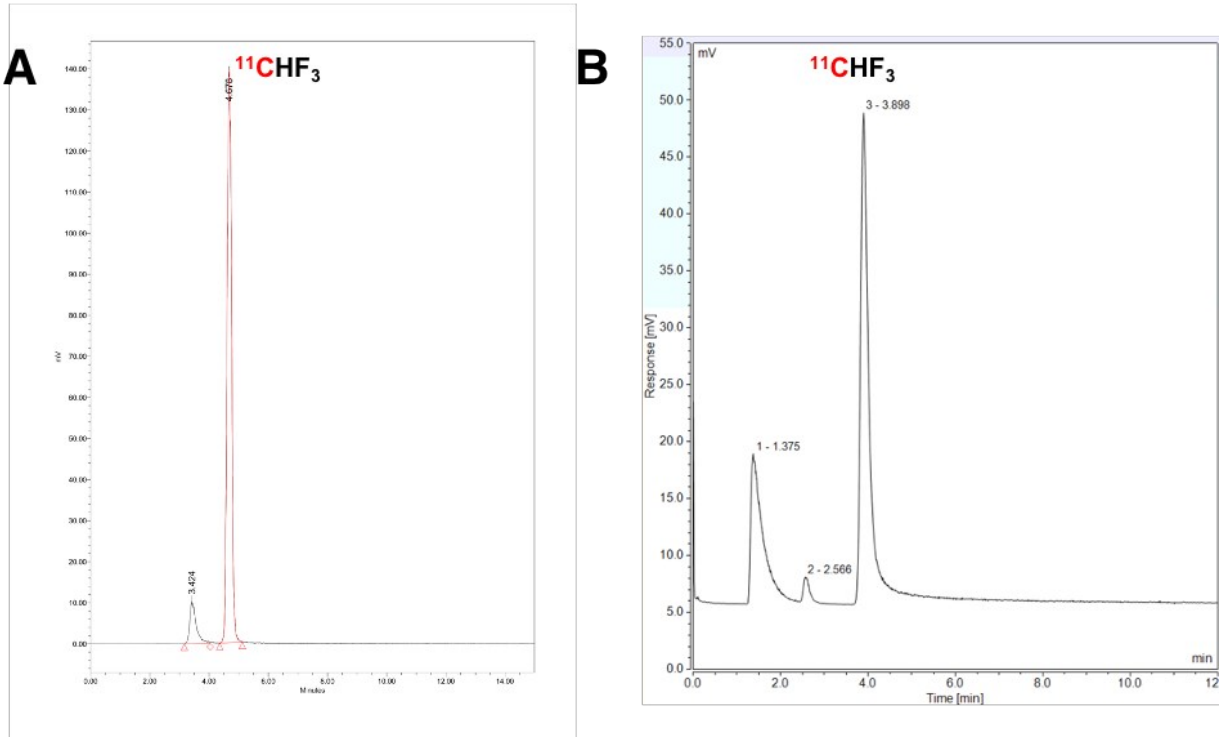


Figure S1. Radio-HPLC chromatograms from the analysis of crude [^{11}C]fluoroform under (A) acidic and (B) basic HPLC conditions, previous to radiolabeling reactions.

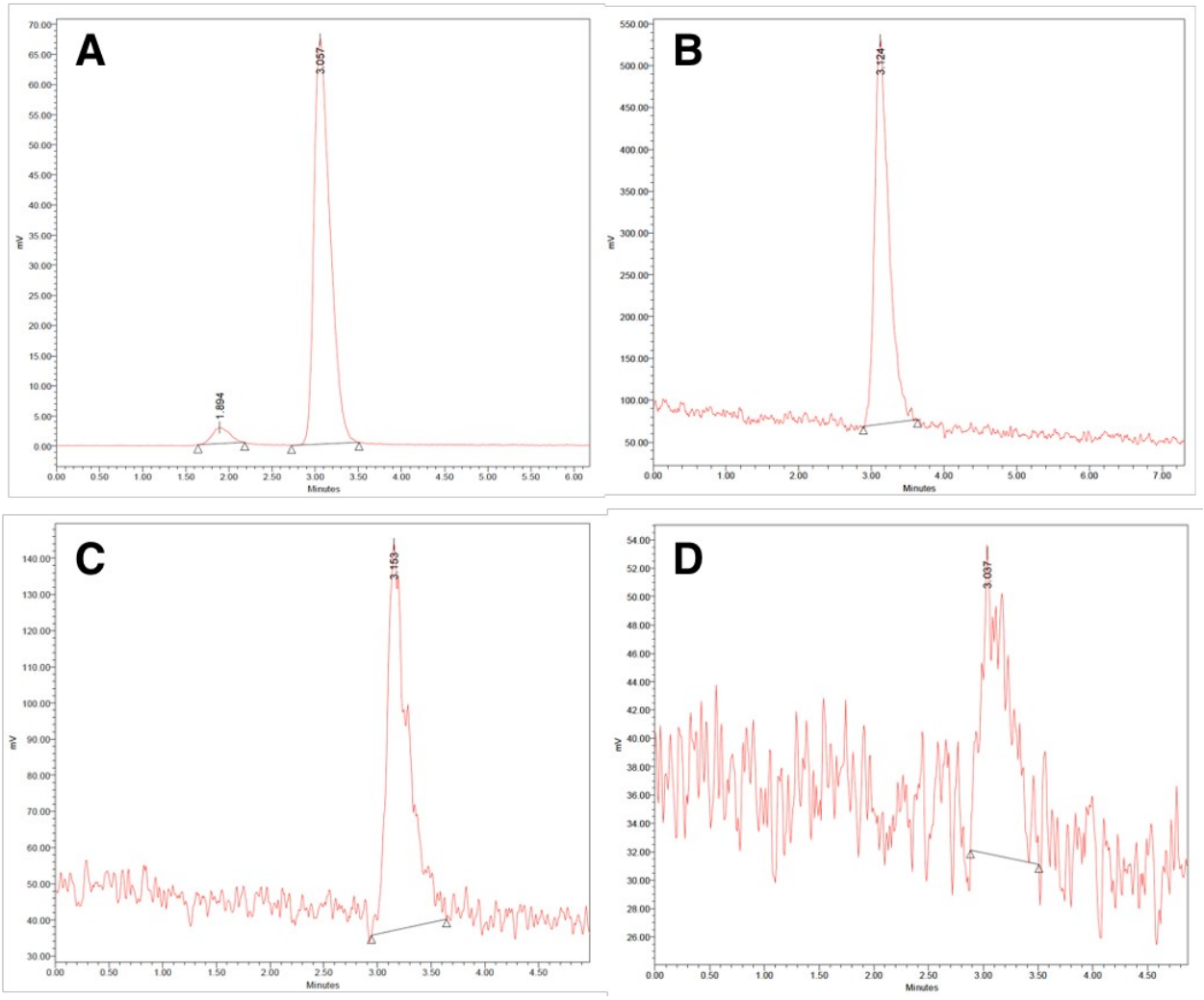
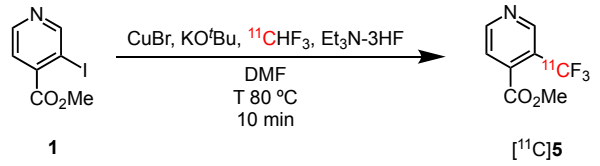


Figure S2. Analysis of the stability of [^{11}C]fluoroform under basic conditions. Radio-HPLC chromatograms of (A) crude [^{11}C]fluoroform, (B) pure [^{11}C]fluoroform, (C) pure [^{11}C]fluoroform with addition of base at room temperature and (D) pure [^{11}C]fluoroform with addition of base at 130 ºC.



1 [^{11}C]**5**

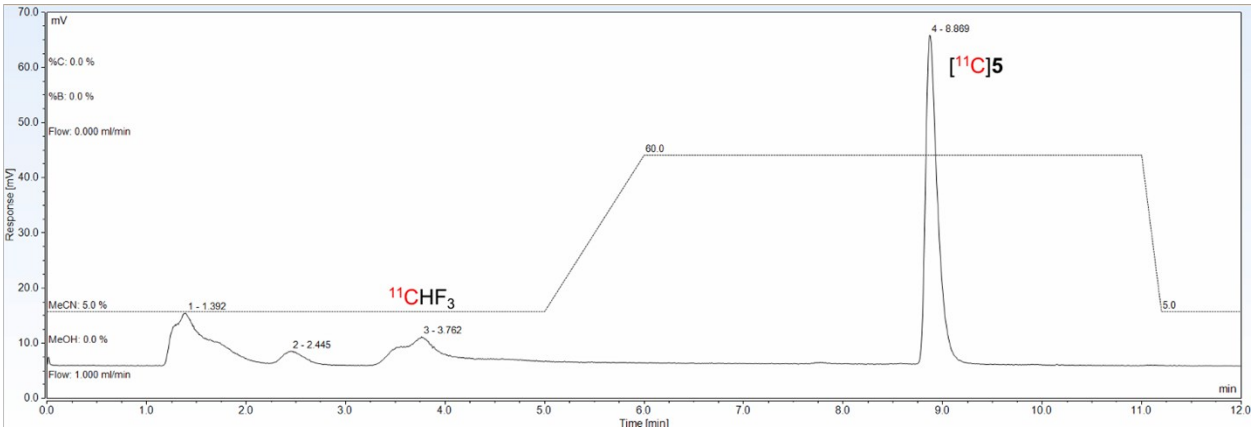
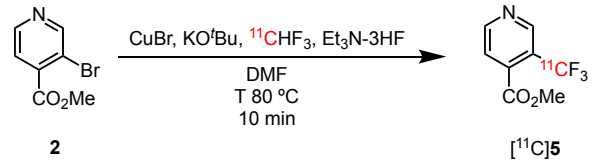


Figure S3. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of ester **1** with [^{11}C]$CuCF_3$ to produce [^{11}C]**5**.



2 [^{11}C]**5**

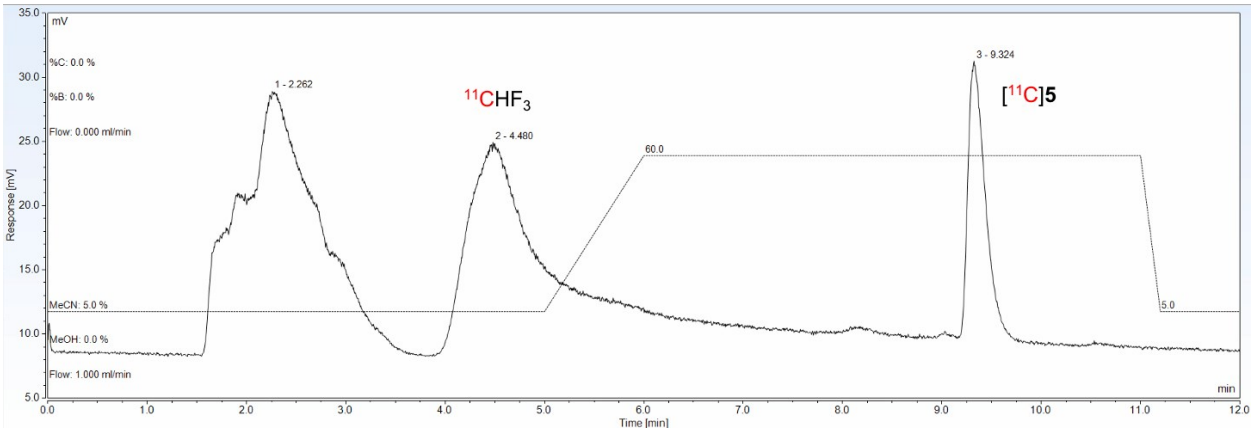
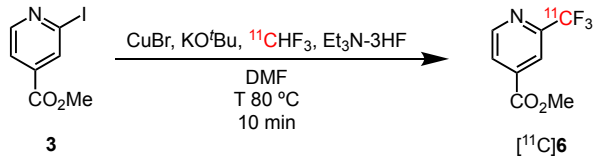


Figure S4. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of ester **2** with [^{11}C]$CuCF_3$ to produce [^{11}C]**5**.



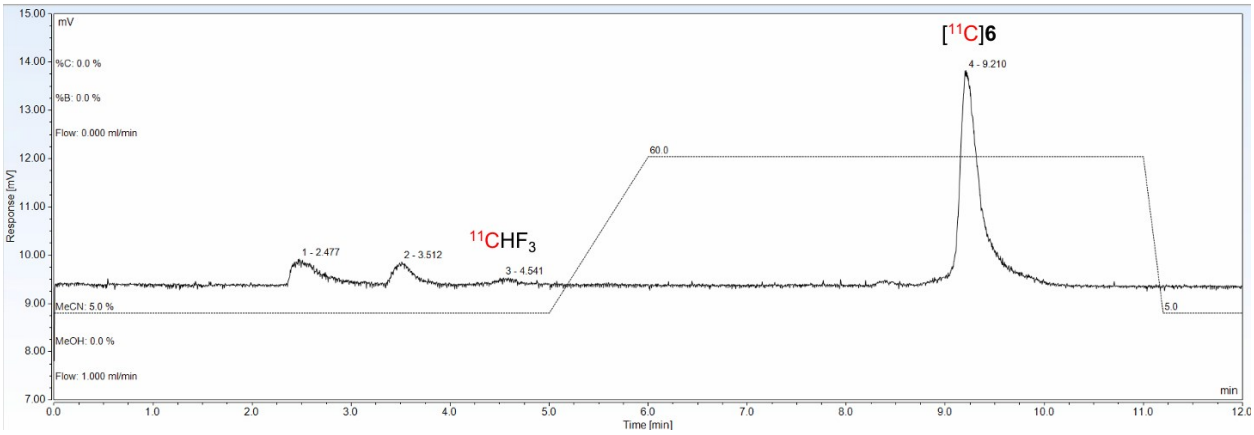
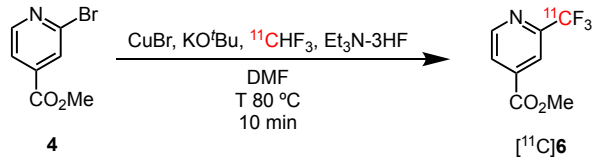


Figure S5. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of ester **3** with [^{11}C]$CuCF_3$ to produce [^{11}C]**6**.



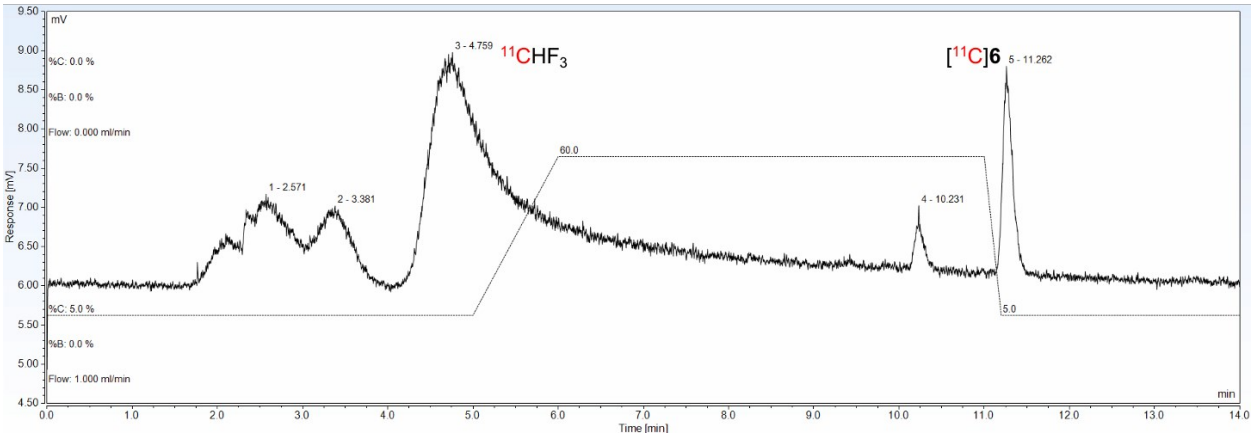
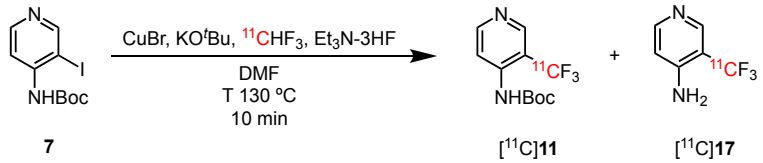


Figure S6. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of ester **3** with [^{11}C]CuCF$_3$ to produce [^{11}C]**6**.

Note: The integration on the [^{11}CHF$_3$] peak was calculated from 4.1 – 6.6 min, using a quasi-horizontal line corresponding to the baseline, which may partially underestimate the activity under the peak.



7 [^{11}C]**11** [^{11}C]**17**

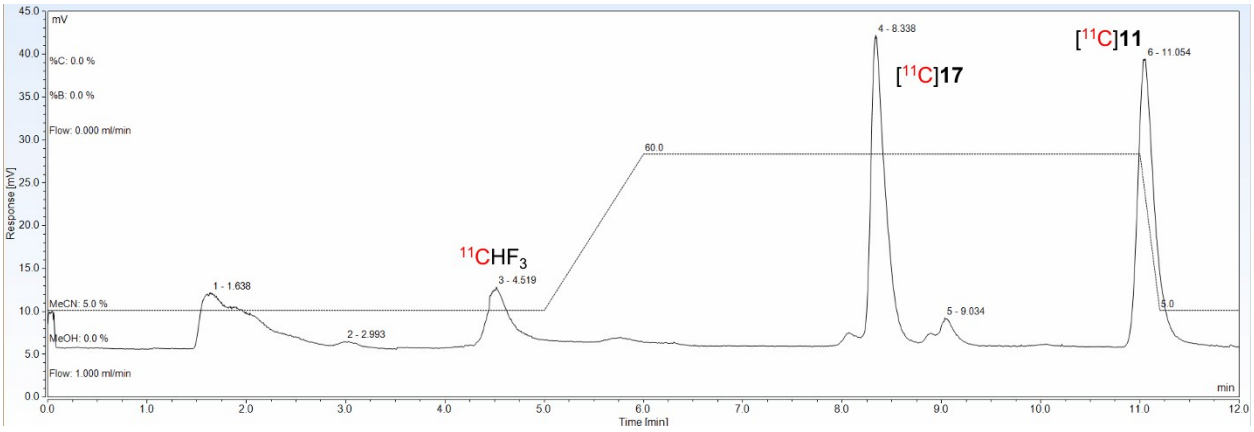
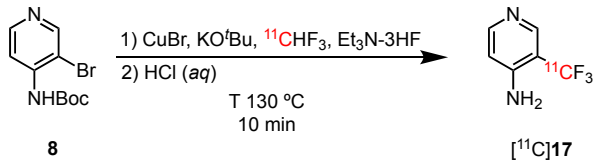


Figure S7. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of carbamate **7** with [^{11}C]$CuCF_3$ to produce [^{11}C]**11** plus [^{11}C]**17**.



8 [^{11}C]**17**

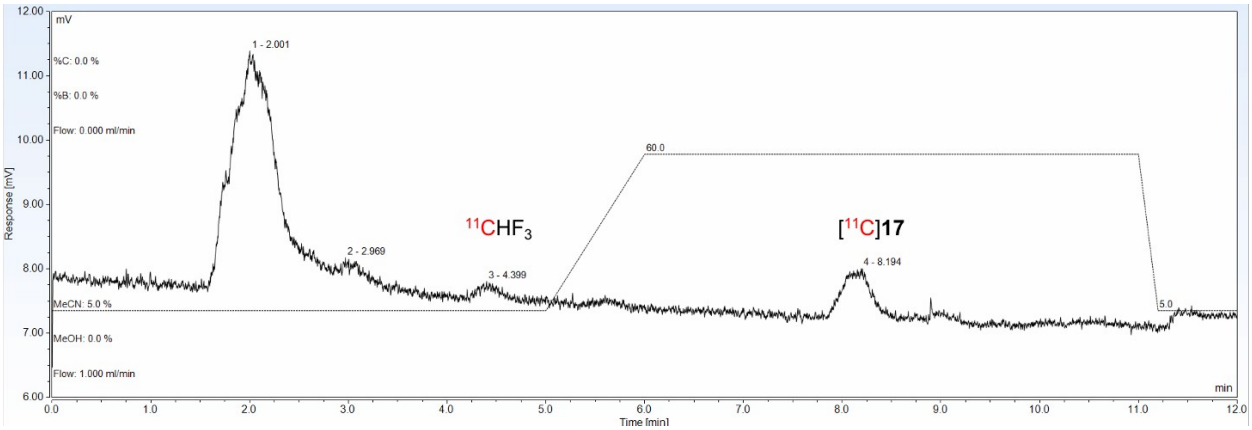
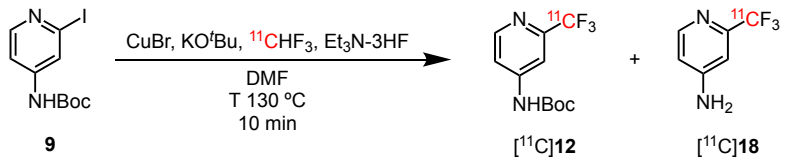


Figure S8. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of carbamate **8** with [^{11}C]$CuCF_3$ followed by acid hydrolysis to produce [^{11}C]**17**.



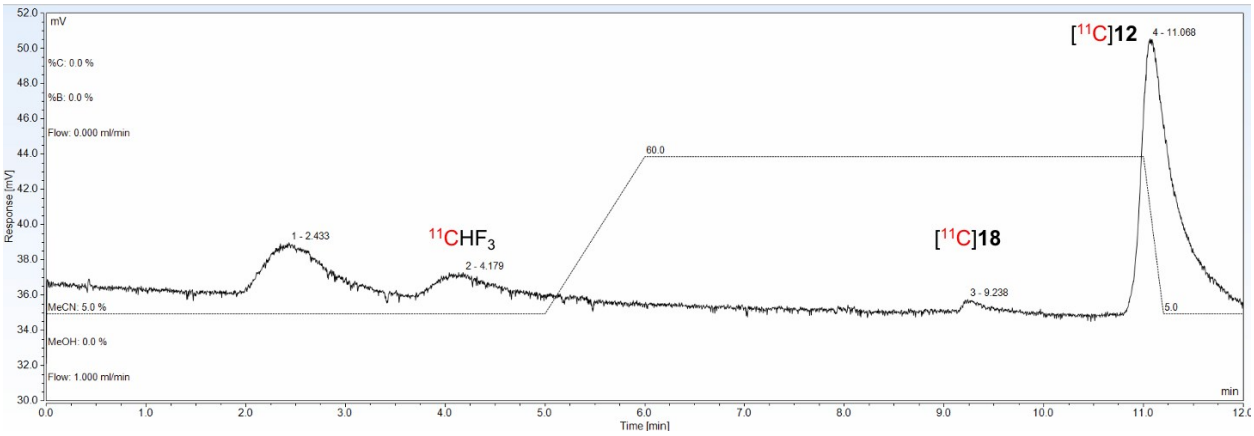
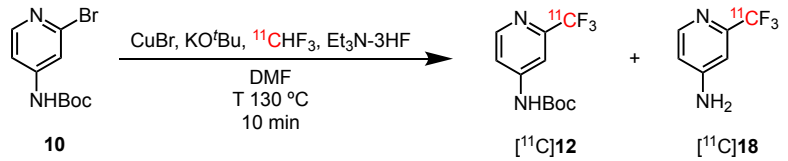


Figure S9. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of carbamate **9** with [^{11}C]$CuCF_3$ to produce [^{11}C]**12** plus [^{11}C]**18**.



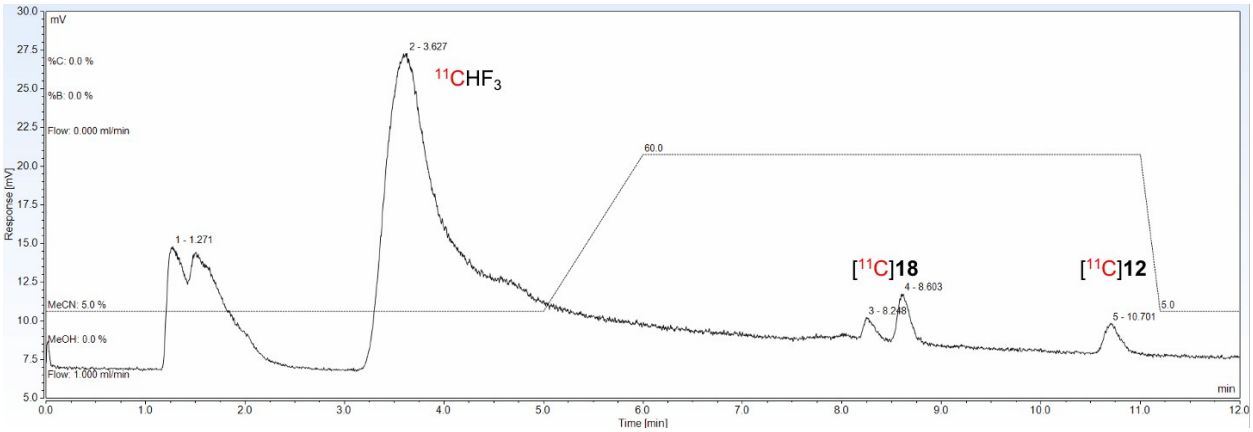
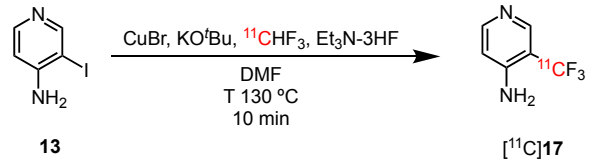


Figure S10. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of carbamate **10** with [^{11}C]$CuCF_3$ to produce [^{11}C]**12** plus [^{11}C]**18**.



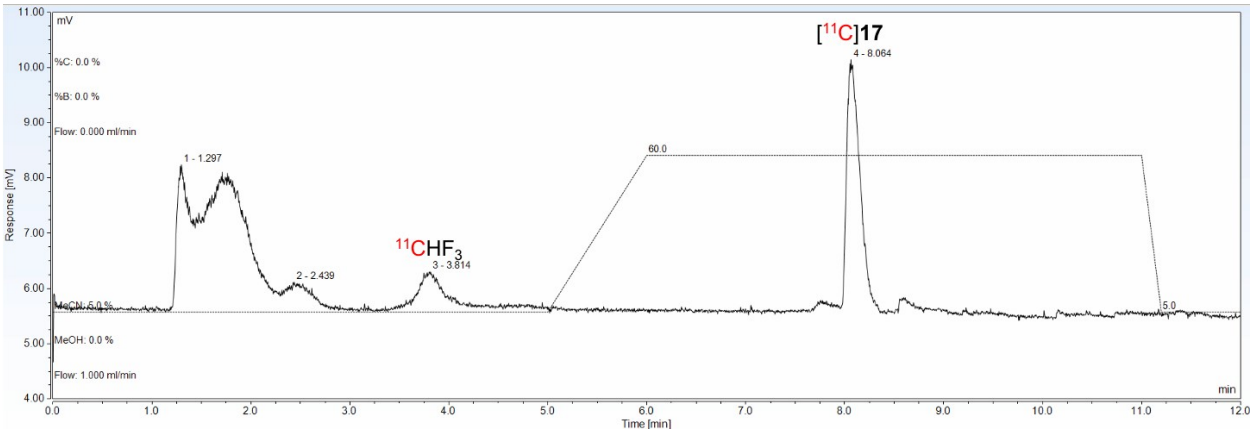
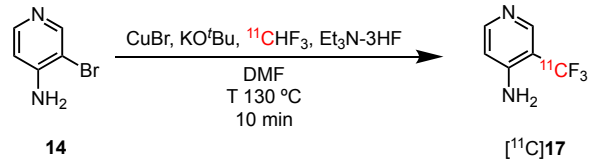


Figure S11. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of amine **13** with [^{11}C]$CuCF_3$ to produce [^{11}C**]17**.



14 [^{11}C]**17**

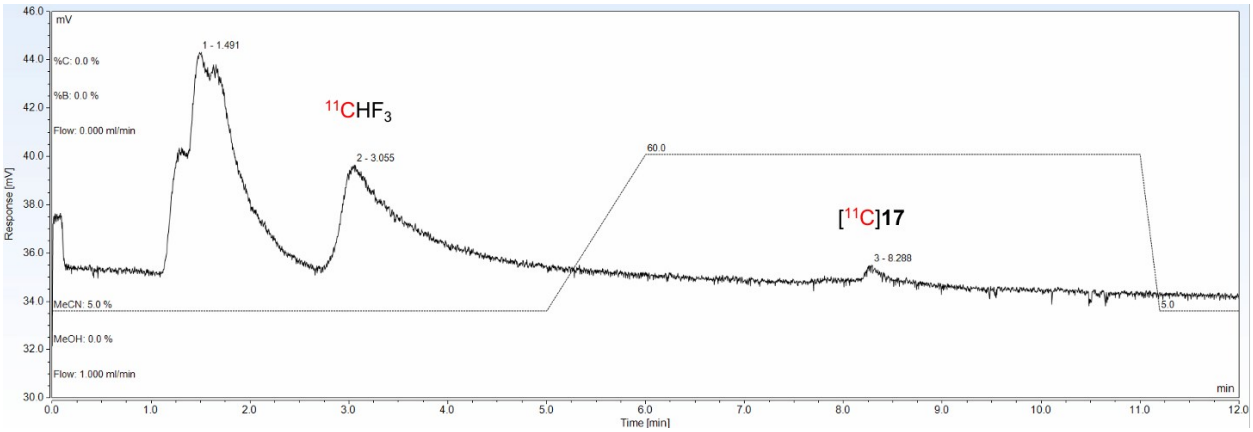
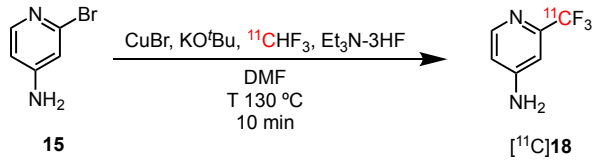


Figure S12. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of amine **14** with [^{11}C]$CuCF_3$ to produce [^{11}C**]17**.



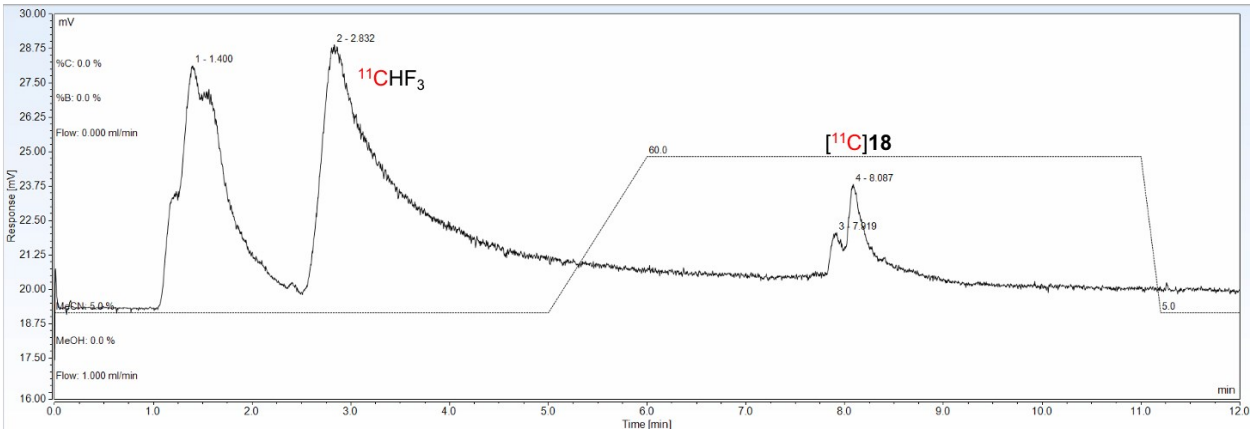


Figure S13. Radio-HPLC chromatogram from the analysis of the reaction mixture from the treatment of amine **15** with [^{11}C]CuCF$_3$ to produce [^{11}C]**18**.

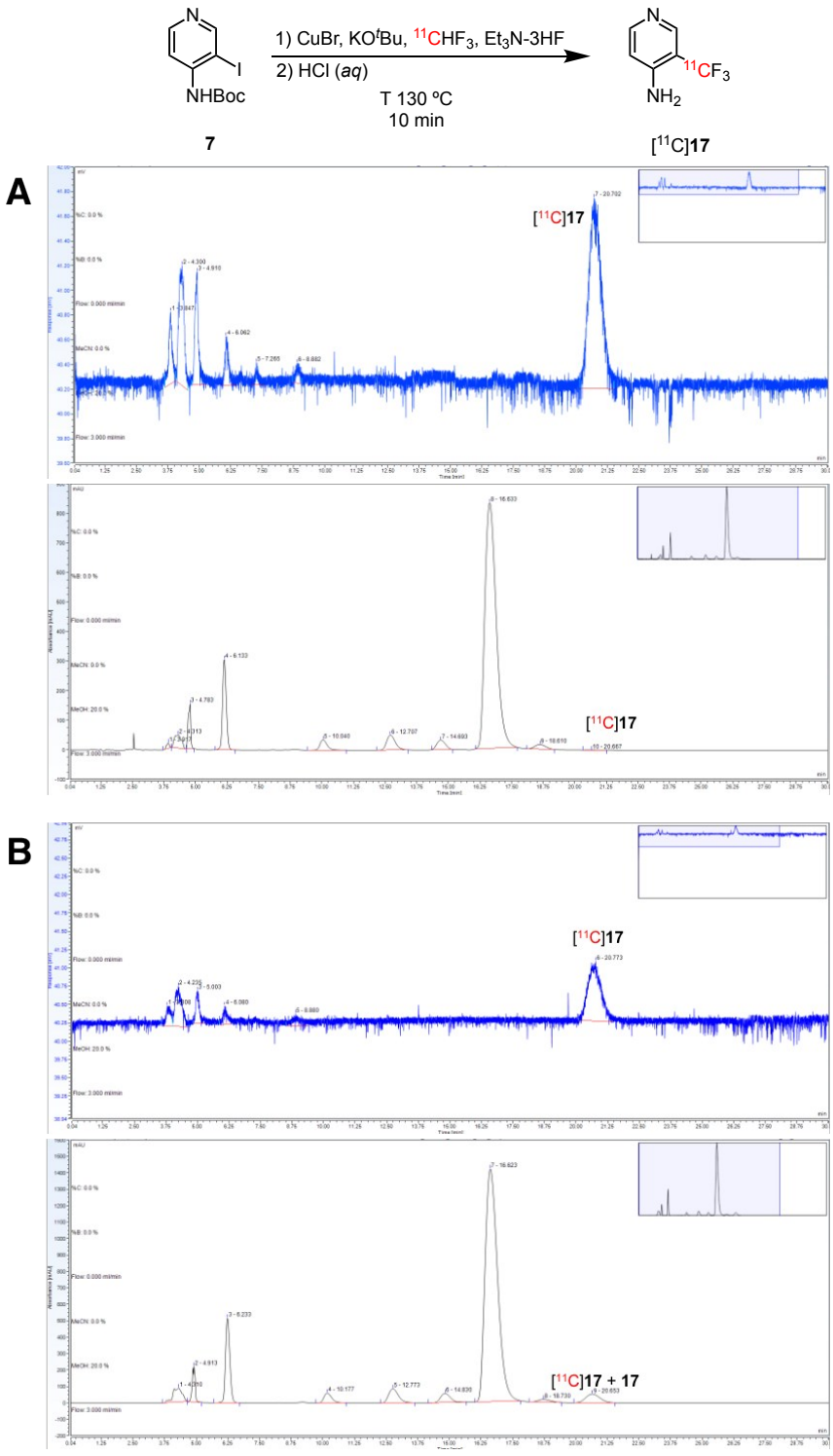


Figure S14. Identification of [^{11}C]3-CF_3-4AP ([^{11}C]17) prepared from 7. A. Radio- (top panel) and UV absorbance (bottom panel) HPLC chromatograms for the crude reaction mixture from the radiosynthesis of **[^{11}C]3-CF_3-4AP** ([^{11}C]**17**) from precursor **7**. **B.** Radio- (top panel) and UV absorbance (bottom panel) HPLC chromatograms of the crude reaction mixture spiked with reference **17** to identify radioactive product.

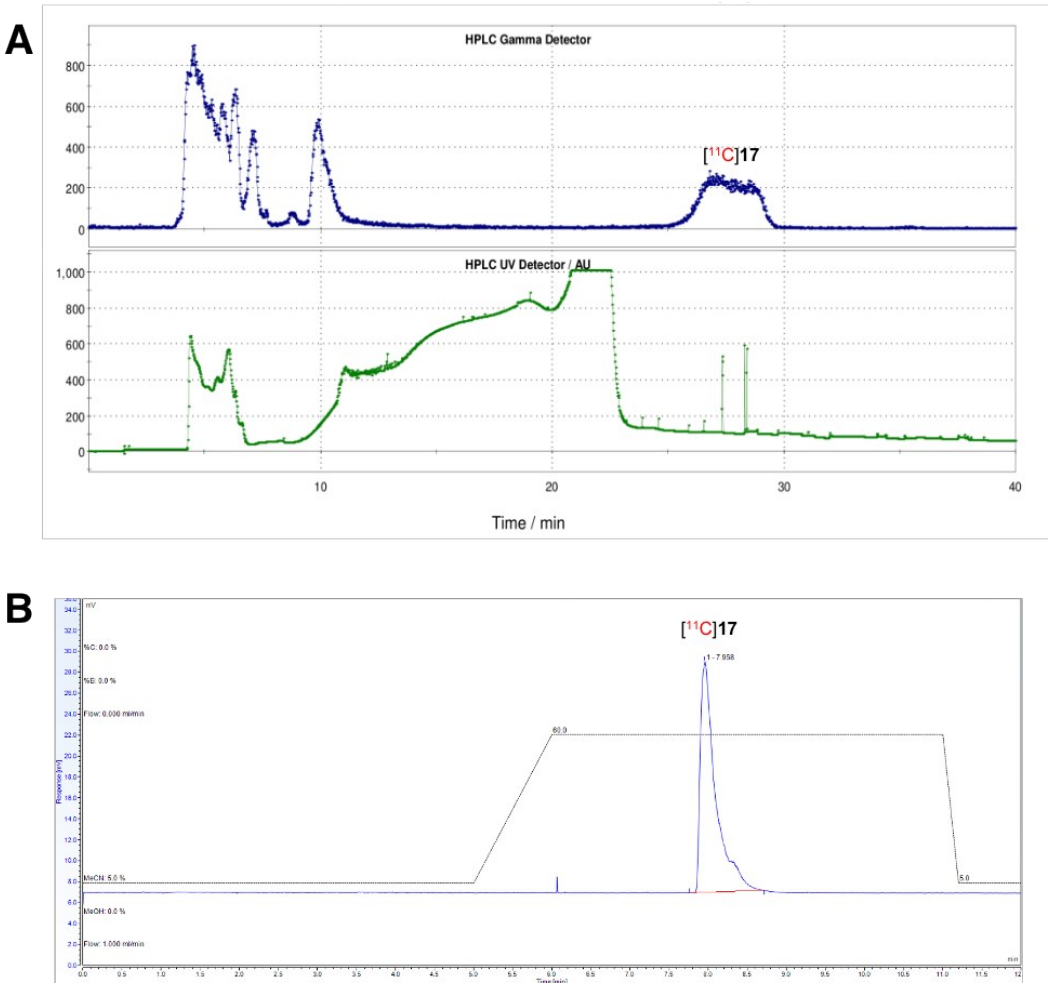


Figure S15. Radiochromatograms from the full-scale synthesis and analysis of [^{11}C]3-CF_3-4AP from 13. A. Radio- (top panel) and UV absorbance (bottom panel) HPLC chromatograms from the HPLC separation of **[^{11}C]3-CF_3-4AP** ([^{11}C]**17**) prepared from precursor **13**. **B.** Radiochromatogram of purified **[^{11}C]3-CF_3-4AP** ([^{11}C]**17**).

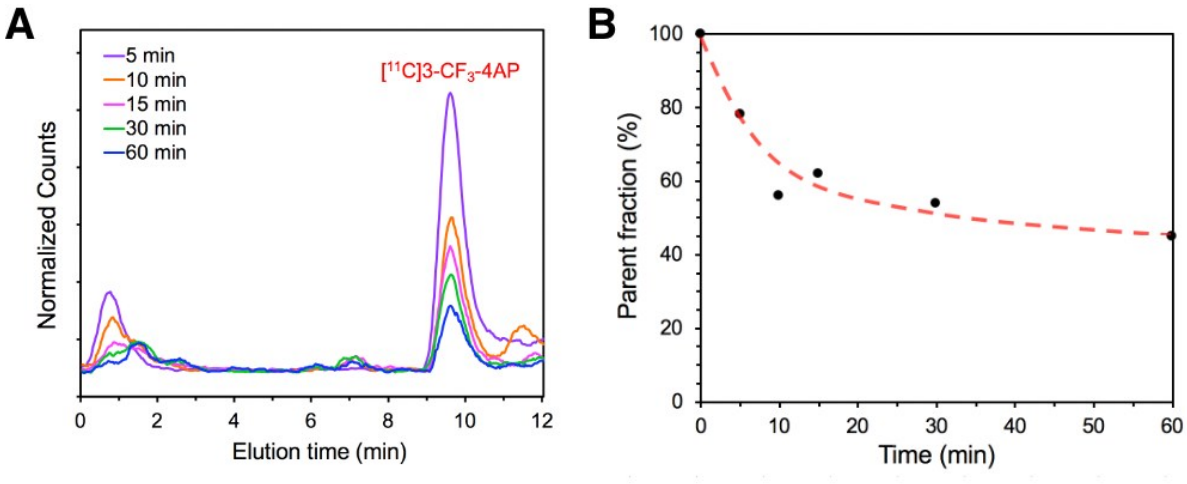


Figure S16. ***In vivo*** **stability of [^{11}C]3-CF$_3$-4AP.** (A) RadioHPLC chromatogram of plasma samples. (B) Time course of remaining parent compound in plasma.

Supplementary References

1. Collier T, Normandin M, El Fakhri G, Vasdev N. Automation of column-switching HPLC for analysis of radiopharmaceuticals and their metabolites in plasma. *Journal of Nuclear Medicine Annual Meeting Abstracts,* 2013, **54**,1133.
2. Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF. Column-switching HPLC for the analysis of plasma in PET imaging studies. *Nucl Med Biol.*, 2000, **27**, 627-630.