Synthesis of [^{11}C]Bexarotene by Cu-Mediated [^{11}C]Carbon Dioxide Fixation and Preliminary PET Imaging

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SUPPORTING INFORMATION

1. GENERAL INFORMATION

All solvents were of reagent or anhydrous grade quality and purchased from Sigma-Aldrich, Alfa Aesar, or Fisher Scientific. All reagents were purchased from Sigma-Aldrich, Alfa Aesar, or Fisher Scientific, except for bis(pinacolato)diboron, which was purchased from Frontier Scientific. *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TMEDA), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), *N*,*N*-dimethylformamide (DMF), and 1-methyl-2-pyrrolidinone (NMP) were distilled prior to use in radiochemical reactions, and stored over KOH at -20 °C. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker 300 MHz spectrometer, and resonances are given in parts per million (ppm) relative residual solvent. Peak multiplicities are designated by the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; br, broad; and *J*, coupling constant in Hz. Analytical thin-layer chromatography (TLC) was performed on pre-coated glass-backed plates (EMD TLC Silica gel 60 F_{254}) and visualized using a UV lamp (254 nm) and potassium permanganate stain. Flash column chromatography was performed using a Biotage Isolera One system and preloaded Biotage Zip silica gel columns.

Synthetic route: The syntheses of precursors **1a-c** were based on literature precedent.[1–8]

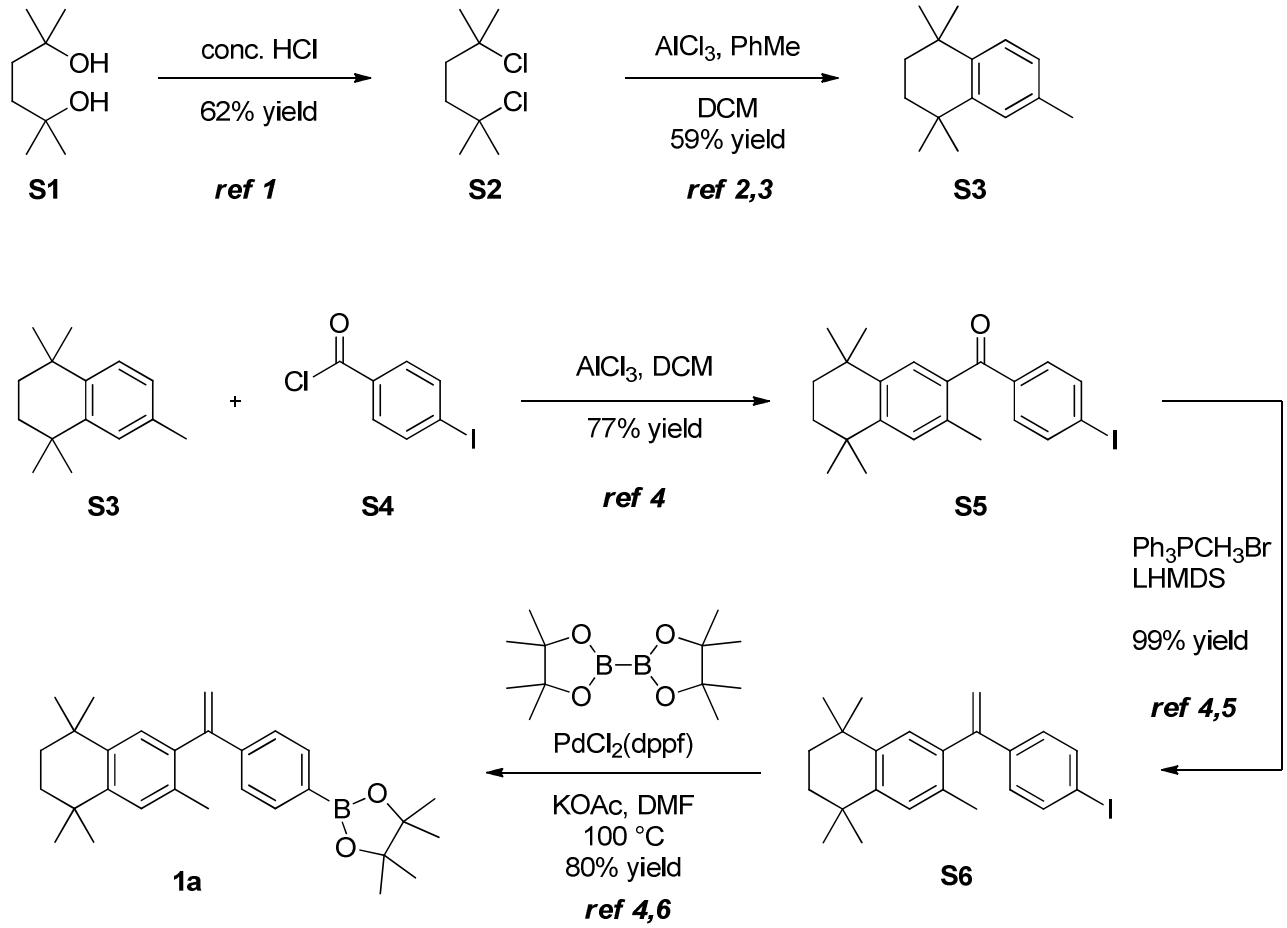


Figure S1: Synthesis of **1a**.

The syntheses of **S2**, **S3**, and **S5** were conducted as described in the cited references. The syntheses of **S6** and **1a** were conducted by adapted procedures to give products that matched those described in the literature.

Synthesis of 6-(1-(4-iodophenyl)vinyl)-1,1,4,4,7-pentamethyl-1,2,3,4-tetrahydronaphthalene (S6):[5]

A solution of lithium hexamethyldisilazane in toluene (1.0 M, 5.78 mL) was added over 5 min to an ice-water bath cooled stirred suspension of triphenylphosphine methyl bromide (2.07 g, 5.78 mmol) in anhydrous toluene (28.9 mL). The reaction was stirred for 15 min, and then cooled to -78 °C in an acetone-dry ice bath. A solution of **S5** (1.0 g, 2.31 mmol) in anhydrous toluene (23.1 mL) was added to the reaction mixture. The mixture was stirred until full conversion of **S5** was observed by TLC (~40 min, 10% EtOAc / hexanes). The reaction was quenched with saturated aqueous ammonium chloride, and extracted three times with diethyl ether. The combined organics were washed with brine, dried with $MgSO_4$, filtered and concentrated. The crude material was recrystallized from methanol to give a nearly quantitative yield of **S6**, which matched the literature description.[4]

Synthesis of 4,4,5,5-tetramethyl-2-(4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)phenyl)-1,3,2-dioxaborolane (1a):

An oven-dried reaction tube was cooled to room temperature under $Ar_{(g)}$ and charged with $PdCl_2$(dppf) (42.5 mg, 58 μmol), KOAc (171 mg, 1.74 mmol), and B_2pin_2 (443 mg, 1.74 mmol). A solution of **S6** (250 mg, 0.58 mmol) in anhydrous DMF (3.4 mL) was added, and the reaction was heated to 100 °C with stirring until full conversion of **S6** was observed by TLC (~90 min, hexanes). The reaction was diluted with toluene, and the organic layer was washed with water and brine

successively. The organic layer was dried with Na_2SO_4 and filtered through a short pad of silica gel. The filtrate was concentrated to a brown oil, which was purified by column chromatography (gradient, 0 – 10% ethyl acetate / hexanes). The product was recovered in 80% yield and matched literature description.[4] Prior to radiolabeling, this material was recrystallized 3 – 5 times from acetonitrile.

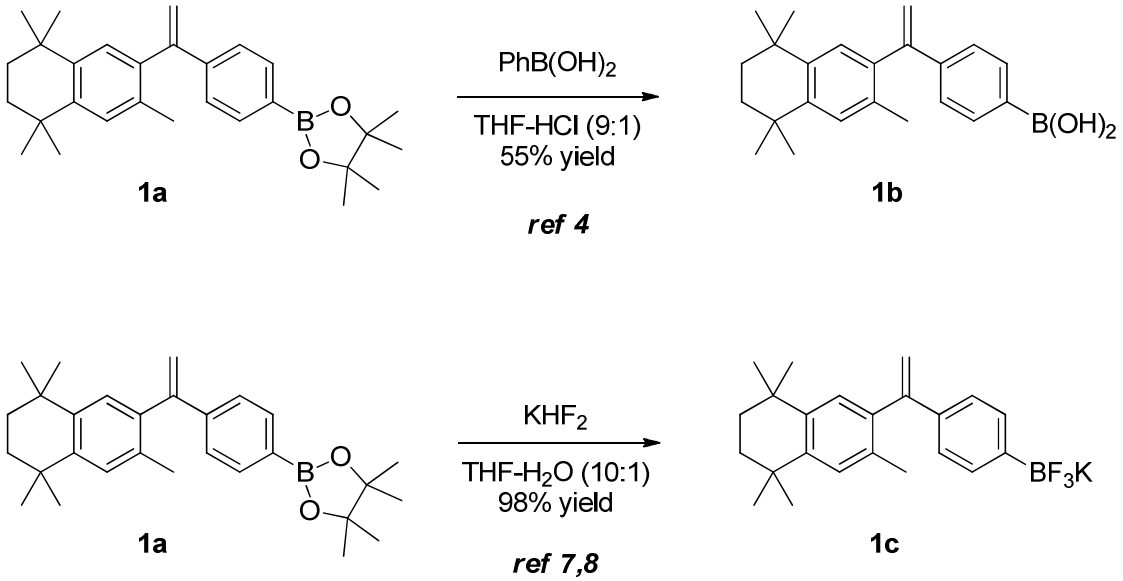


Figure S2: Syntheses of **1b** and **1c**.

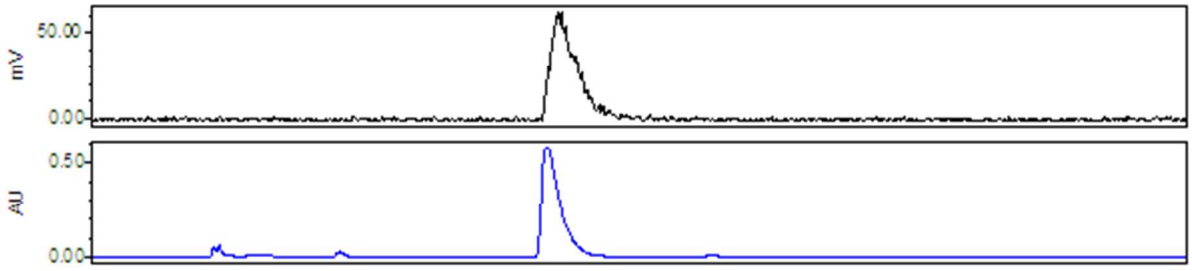
The synthesis of **1b** was conducted as described in the cited reference.[4]

Synthesis of potassium (4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl)phenyl)trifluoroborate (1c):

A solution of KHF_2 (63.5 mg, 0.81 mmol) in water (0.18 mL) was added to a solution of **1a** (100 mg, 0.23 mmol) in THF (1.8 mL) cooled to 0 °C in a round-bottom flask. The reaction mixture was stirred at 0 °C for 10 min, then warmed to room temperature and stirred for an additional 20 min. The reaction mixture was concentrated on a rotary evaporator to remove THF, and under high vacuum to remove water. The solid residue was extracted with acetone. The organics were concentrated and residual pinacol was removed under high vacuum with warming of the flask to 60 °C. Product **1c** was isolated as a white solid in 98% yield. ^{1}H NMR (300 MHz, d_6-AcMe) δ 7.41 (d, J = 8 Hz, 2H), 7.12 (s, 1H), 7.10 (s, 1H), 7.04 (d, J = 8 Hz), 5.64 (d, J = 2 Hz, 1H), 4.96 (d, J = 2 Hz, 1H, 1.95 (s, 3H), 1.70 (s, 4H), 1.28 (s, 6H), 1.26 (s, 6H) ppm. ^{19}F NMR (282 MHz, d_6-AcMe) δ -138.27 ppm. ^{13}C NMR (75 MHz, d_6-AcMe) 150.8, 142.8, 141.1, 139.3, 136.9, 134.2, 132.2, 131.1, 131.1, 127.1, 127.1, 125.2, 123.9, 110.9, 34.6 (2 nonsymmetrical C), 33.2, 33.0, 30.9, 30.8, 18.7 ppm.

Synthesis of [^{11}C-*carbonyl*]bexarotene ([^{11}C]4-[1-(3,5,5,8,8-pentamethyltetralin-2-yl)ethenyl]benzoic acid):

A GE PETtrace 16.5 MeV cyclotron was used for [^{11}C]CO_2 production by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a 50 μA proton beam current to irradiate $^{14}N_2$ containing 1% O_2. For the preclinical study, a Siemens Eclipse HP self-shielded 11 MeV cyclotron was used for isotope production. [^{11}C]CO_2 was dispensed in a stream of nitrogen (1 L/min) into a steel coil immersed in a liquid nitrogen bath. After delivery of [^{11}C]CO_2 was completed, the coil was flushed with helium (10 mL/min) to transfer activity through a nitrogen oxides trap[9] and a P_2O_5 column and into a 3 mL conical vial containing a solution of pinacol arylboronate (**1a**, 12.9 mg, 30 μmol), copper(I) thiophene-2-carboxylate (CuTc, 1.0 mg, 5 μmol), tetrabutylammonium difluorotriphenylsilicate (TBAT, 2.7 mg, 5 μmol), and *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TMEDA, 60 μL, 400 μmol) in 1-methyl-2-pyrrolidinone (NMP, 300 μL).When radioactivity in the vial reached a maximum (as measured by a proximal radioactivity detector), gas flow was ceased, the reactor was sealed and heated to 100 °C for 5 min. The reaction was subsequently quenched with HPLC mobile phase (1 mL, 80% CH_3CN, 20% 0.1 M $NH_4{\cdot}HCO_{2(aq)}$), loaded onto a 5 mL injection loop, and purified by semi-preparative reversed-phase HPLC (stationary phase: Phenomenex Luna C18, 250 x 10 mm, 5 μm; mobile phase as described above, 5 mL/min). The peak eluting at 7.6 min was collected and added to a solution of water (23 mL) and 8.4% $NaHCO_{3(aq)}$ (2 mL). The collection solution was then passed through a C18 SepPak cartridge (pre-activated by flushing with 5 mL EtOH, followed by 20 mL water). The cartridge was washed with water (10 mL), and then the product eluted with EtOH (1 mL). Saline (9 mL) was then passed through the cartridge and added to the ethanol solution containing [^{11}C]bexarotene. For animal studies, the product solution was sterile filtered prior to administration. Radiochemical purity and specific activity were determined by analytical reversed-phased HPLC (stationary phase: Phenomenex Luna C18, 250 x 4.6 mm, 5 μm; mobile phase: 80% CH_3CN, 20% 0.1 M $NH_4{\cdot}HCO_{2(aq)}$, 1 mL/min, t_R = 8.15 min). Product identity was confirmed by coinjection with bexarotene.



Determination of log$D_{7.4}$ of [^{11}C]bexarotene:[10]

Purified and reformulated [^{11}C]bexarotene (~50 μL) was added to a separatory funnel containing PBS buffer (pH 7.4) and 1-octanol (pre-saturated with PBS buffer) (~20 mL each). The octanol layer was collected and dispensed into 8 centrifuge tubes (2 mL each) containing PBS buffer (2 mL each). The tubes were mixed by vortex for 2 min and then centrifuged for 5 min. Approximately 0.5 mL of the octanol layer and 1.0 mL of the aqueous from each centrifuge tube was then transferred to pre-weighed test tubes (16 in total). The tubes were capped and assayed using an automated gamma counter. The tubes were then uncapped and weighed to determine the total volume of the liquid in each. The log$D_{7.4}$ of [^{11}C]bexarotene was determined to be 3.68.

All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Massachusetts General Hospital Institutional Animal Care and Use Facility.

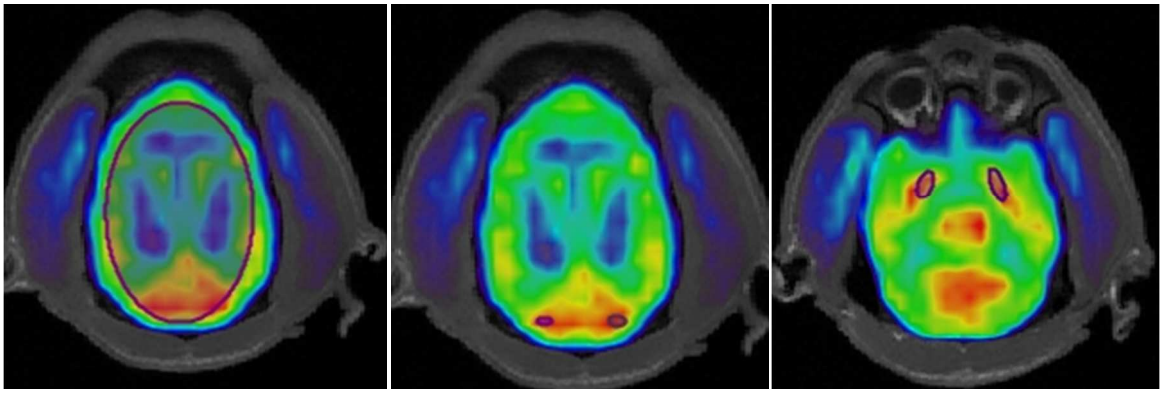
A female *Papio anubis* baboon, deprived of food for 12 h prior to the study, was administered intramuscular ketamine (10 mg/kg) and intubated. For maintenance of anesthesia throughout the study, the baboon was provided 1 – 4% isoflurane (Forane) in a mixture of medical oxygen and nitrogen. The baboon was catheterized antecubitally for radiotracer injection. PET-MR images were acquired in a Biograph mMR scanner (Siemens, Munich, Germany), with a PET resolution of 5 mm and field-of-view of 59.4 and 25.8 cm (transaxial and axial, respectively). Dynamic PET image acquisition was initiated followed by administration of the radiotracer in a homogenous solution of 10% ethanol and 90% isotonic saline. An MEMPRAGE sequence was initiated for anatomic coregistration. Static PET and MR image acquisition of thoracic and abdominal regions were initiated 60 min after radiotracer administration, for durations of 20 min, each.

Dynamic data from the PET scans were recorded in list mode and corrected for attenuation. Baboon data were reconstructed using a 3D-OSEM method resulting in a full width at half-maximum resolution of 4 mm. Reconstructed images were exported from the scanner in DICOM format along with an anatomic MRI for baboon scans. These files were imported to AMIDE and manually coregistered using six degrees of freedom. Volumes of interest (VOIs) were drawn manually as ellipsoids in brain regions guided by a simultaneously acquired high resolution MPRAGE scan for structural images and summed PET data, with a radius no less than double that of the PET voxel size to minimize partial volume effects (4 mm). Time–activity curves (TACs) were exported in terms of decay-corrected activity per unit volume at specified time points with gradually increasing intervals.

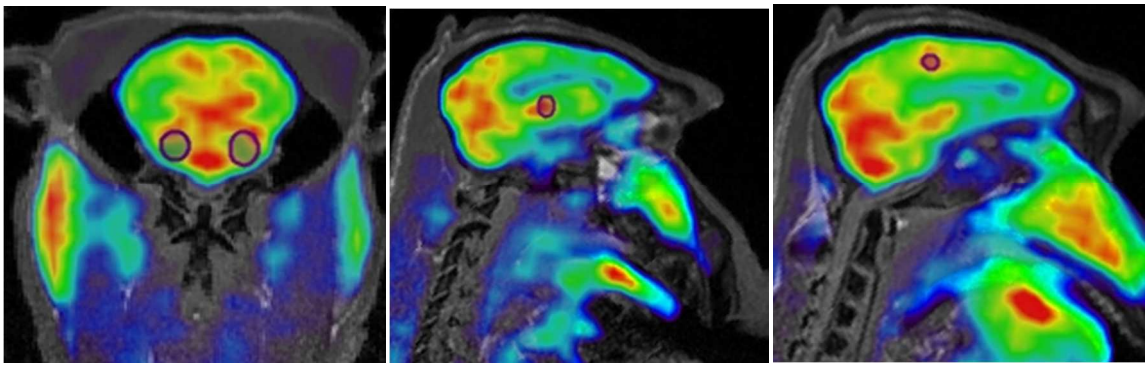
ROI Placement

ROIs were drawn on summed PET data manually coregistered with a simultaneously acquired MPRAGE scan (high resolution MRI), and were subsequently confirmed by close examination of the MRI structural images.

Whole brain (WB) Occipital Cortex (Occ Ctx) Putamen (Pu)



Cerebral Cortex (Crb Ctx) Thalamus (Th) Cingulate Sulcus (CGS)



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