Radiolabelling and positron emission tomography of PT70, a time-dependent inhibitor of InhA, the *Mycobacterium tuberculosis* enoyl-ACP reductase

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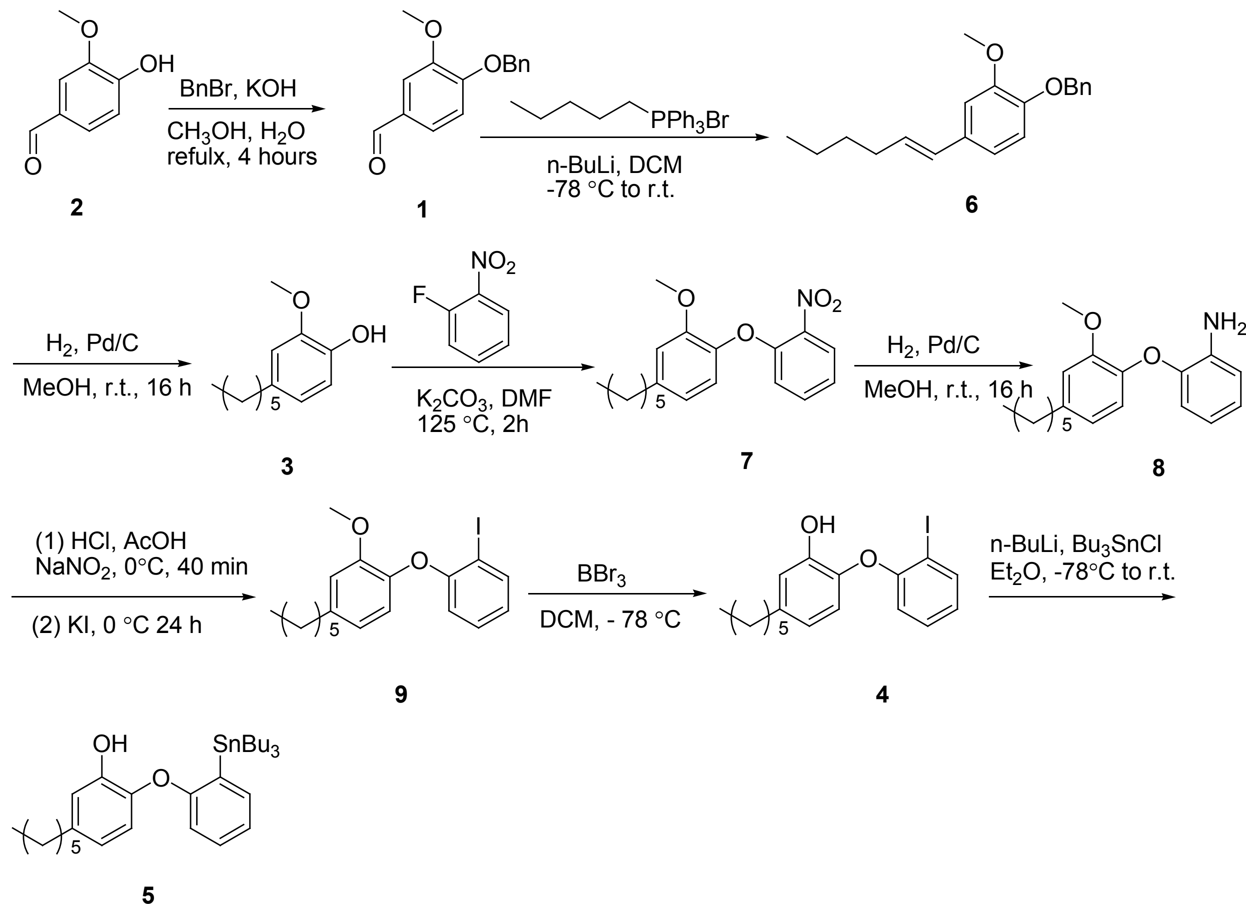
Supplementary Data

Organic Synthesis

The synthesis of the precursor for [^{11}C]PT70 has been described previously.[1] Compound **3** was prepared from commercially available vanillin (**2**) in three steps. Vanillin was protected with a benzyl group and coupled with a hexyl chain using a Wittig reaction to form compound **6**.[2] Reduction of compound **6** with hydrogen generated compound **3**. Compound **7** was synthesized by nucleophilic aromatic substitution of **3** with 1-fluoro-2-nitrobenzene, and was converted to compound **8** by reduction with H_2 catalyzed by palladium on charcoal. Compound **9** was prepared from **8** by utilizing the Sandmeyer reaction, which went through a diazonium salt intermediate prior to iodide anion attack. [3] The deprotection was carried out with tribromoborane in dichloromethane to form **4**, and compound **5** was prepared with additional treatment of **4** with tributylchlorostannane under strong basic conditions in diethyl ether (**Scheme S1**).



Scheme S1. Synthesis of the precursor for [^{11}C]PT70

Radiosynthesis of [^{11}C]PT70

The synthesis of [^{11}C]PT70 was performed using organotin or stannanes **5** as the precursor.[4] The precursor (1.0 mg) was dissolved in 0.3 ml THF with 2 mg tetrakis(triphenylphosphine)-palladium(0). After [^{11}C]CH_3I was purged into the solution and trapped at 0 °C, the reaction vessel was sealed and heated at 100°C for 5 min in an oil bath. The reaction mixture was diluted with 1 ml of aqueous ammonium formate (0.1M) prior to loading onto a semi-preparative HPLC column. HPLC purification was performed using a reverse-phase PFP column (Phenomenex, Luna PFP 250×10, 5 µm), at a 5 ml/min flow rate with a mobile phase consisting of 68% MeCN/32% aqueous ammonium formate (0.1 M). The product was collected at the expected retention time (17 min) and the solvent was removed by rotary evaporation. After dilution with 4 ml saline and 1 ml sterile alcohol, the solution was filtered through an Acrodisc 13-mm Syringe Filter with 0.2 µm Supor membrane (Pall Corporation, Ann Arbor, MI) into a sterile vial for delivery. Radiochemical purity was determined by reverse-phase analytical HPLC using a Phenomenex, Luna PFP column (250×4.6 mm, 5 µm) operated at 1.0 ml/min flow rate using a mobile phase of 70% MeCN/30% water, which gave a retention time of 10 min. Subsequently, purity was verified using TLC (5% EA/95% HE, R_f = 0.45) by co-spotting the labeled product with a standard.

PET Imaging and Data Processing

All animal experiments performed in this study were approved by the Brookhaven Institutional Animal Care and Use Committee. Four baboons were included in this study. Ketamine hydrochloride (10 mg/kg) was administered intramuscularly as an anesthetic agent and anesthesia was further maintained with oxygen (800 ml/min), nitrous oxide (1500 ml/min) and isoflurane (Forane, 1-4%) during scanning. A catheter was placed in a radial arm vein for [^{11}C]-labeled drug injection. During the PET scanning, heart rate, respiration rate, body temperature and pO_2 were monitored. Siemens HR+ (Siemens high-resolution, whole-body PET scanner with 4.5×4.5×4.8 mm resolution at the center of field of view) was used to perform dynamic PET scans for a total of 90 min with the following time frames in 3D mode: 1×10, 12×5, 1×20, 1×30, 8×60, 4×300, 8×450 s. Correction of attenuation was obtained by a transmission scan of a ^{68}Ge rod source prior to each PET scan. Images were reconstructed by filtered back projection (FBP) and analyzed using AMIDE® software [5].

LogD and PPB Determination

LogD determination: A test tube containing 2.5 ml of octanol and 2.5 ml of phosphate buffer solution (pH 7.4) was mixed with ~50 µl aliquot of formulated [11C]-labeled drug by vortex for 2 min followed with centrifugation for 2 min to ensure full separation of the aqueous and organic phases. An aliquot from the octanol layer (0.1 ml) and aqueous layer (1 ml) were collected for radioactivity measurement. An additional 2.0 ml aliquot of the octanol layer was carefully transferred to a new test tube containing 0.5 ml octanol and 2.5 ml phosphate buffer (pH 7.4) and the previous procedure (vortex mixing, centrifugation, sampling, and transfer) was repeated for an additional five times to obtain six sets of samples. A well counter (Picker, Cleveland, OH) was used to measure radioactivity in each set of samples and the logD value of each sample was calculated by the following equation:

logD = log (decay-corrected radioactivity in octanol layer × 10/decay-corrected radioactivity in phosphate buffer layer).

PPB determination: A 10 µl aliquot of the formulated [11C]-labeled drug was mixed with a sample of baboon plasma (0.8 ml, collected from at least 4 different baboons and pooled) by gently inverting several times. The mixture was incubated for 10 min at room temperature and then a 20 µl aliquot was taken to determine the total radioactivity in the plasma sample (AT; AT=Abound+Aunbound). An additional 0.2 ml aliquot of plasma was placed in the upper level of a centrifree® tube (Amicon, Inc., Beverly, MA) and then the tube was centrifuged for 10 min. After discarding the upper part of the Centrifree tube, a 20 µl aliquot from the bottom part of the tube was taken to determine the amount of radioactivity that passed through the membrane (Aunbound). PPB was calculated by the following equation:

$$\% \text{ unbound} = \text{Aunbound} \times 100/\text{AT}$$

References

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