SUPPORTING INFORMATION

Synthesis and evaluation of [^{11}C]MCC950 for imaging NLRP3-mediated inflammation in atherosclerosis

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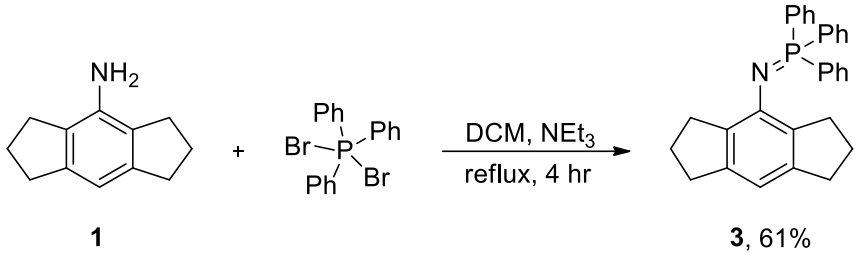
General information

All reagents and solvents purchased were not further purified unless stated otherwise. All reactions were carried out under inert (argon) atmosphere. All solvents used were anhydrous. Anhydrous 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was obtained by refluxing over CaH_2 followed by distillation under reduced pressured. Reaction products were confirmed using ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. ^{1}H-NMR spectra were obtained using a Bruker AVANCE 400 spectrometer. Spectral data are reported in ppm using solvent as a reference (^{1}H NMR $CHCl_3$ at 7.26 ppm). ^{1}H NMR data was reported as: multiplicity (ap = apparent, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constant(s) in Hz. Low resolution mass spectrometry was performed using a Waters Xevo TQD with an Acquity UPLC-H-Class Plus system. High resolution mass spectrometry was performed using a Kratos Concept – Magnetic Sector Electron Impact Mass Spectrometer. Radiochemical chromatograms were obtained using a Waters 2695 Alliance HPLC equipped with a Phenomenex Luna 10 µm C18(2) 100 Å column (250 × 4.6, 10 µmm), a Waters 996 photodiode array detector, and a Carroll & Ramsey Associates 105-S high-sensitivity radiation detector equipped with a 1 cm^3 CsI(Tl) scintillating crystal. Radiolabeled products were synthesized using a Synthra MeIplus Research module.

Sigma-Aldrich: triphenylphosphine dibromide

AstaTech: 4-(1-hydroxy-1-methylethyl)-2-furansulfonamide, 4-amino-1,2,3,5,6,7-hexahydro-*s*-indacene, MCC950 (sodium salt).

Synthetic procedures



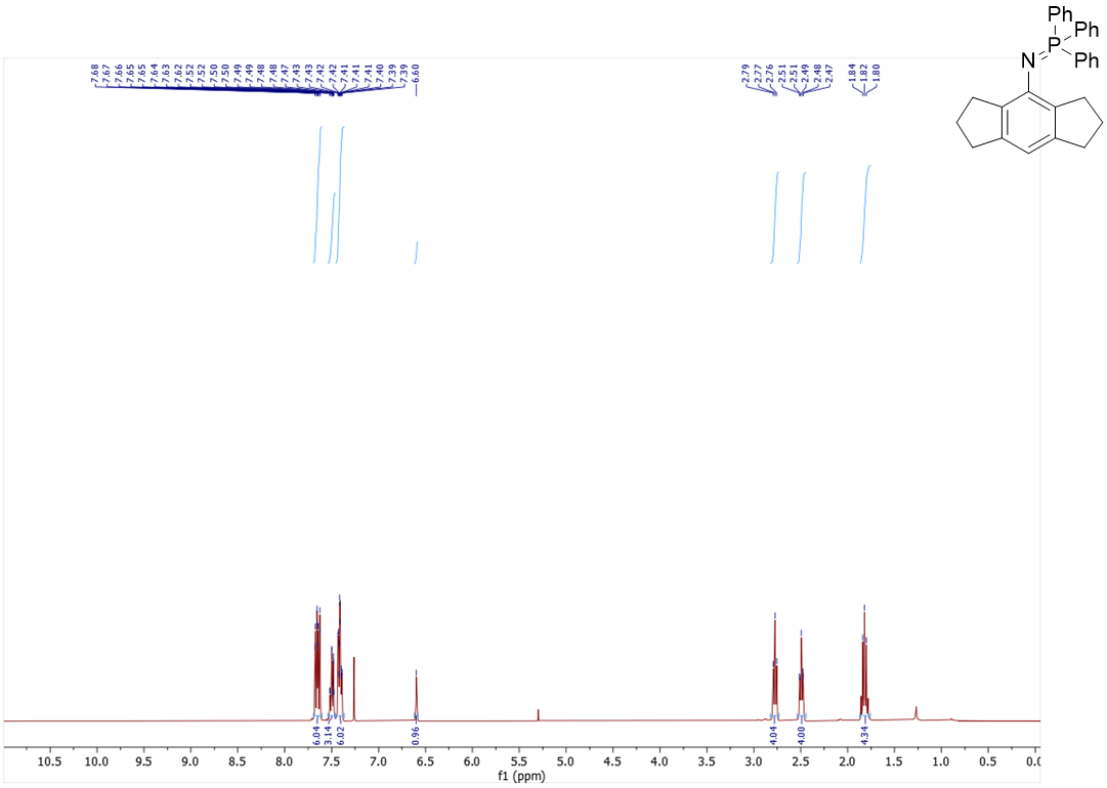
1 **3**, 61%

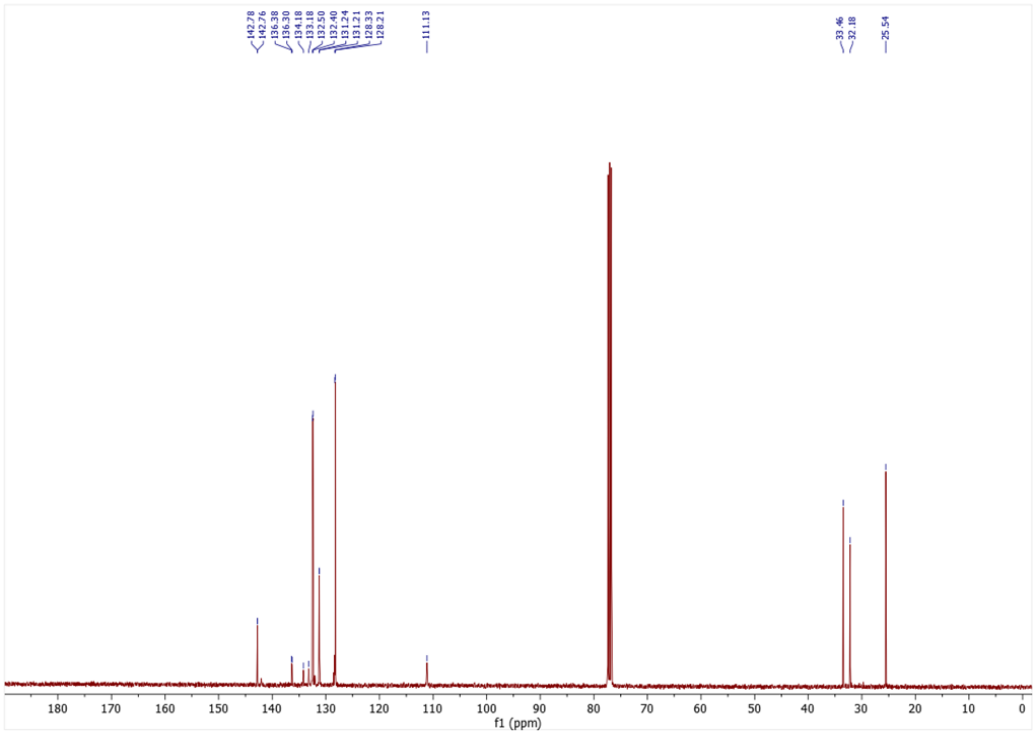
Scheme S1. Synthesis of iminophosphorane precursor

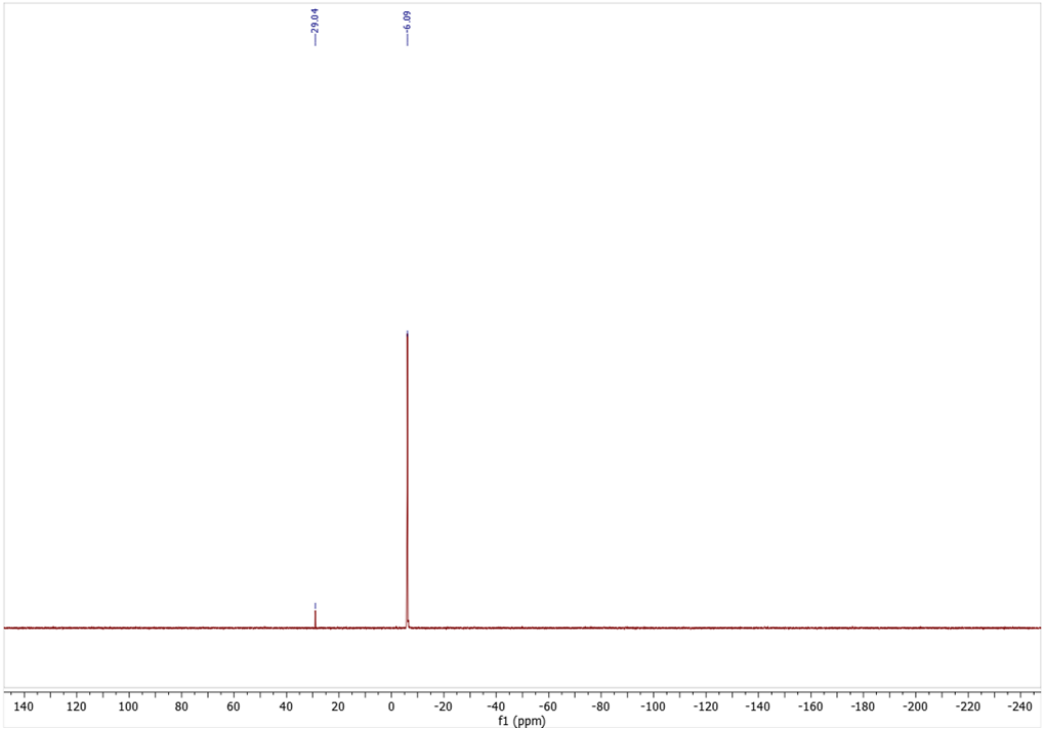
A flame dried flask was equipped with a magnetic stir bar and charged with triphenylphosphine dibromide (0.60 mmol) under inert atmosphere and dissolved in DCM (5.0 mL). The flask was placed in an ice bath, and a solution containing 4-amino-1,2,3,5,6,7-hexahydro-*s*-indacene (**1**, 0.57 mmol) and triethylamine (1.73 mmol) in DCM (5 mL) was added to the reactor in a dropwise manner over 10 minutes. The flask was placed under reflux for 4 hours. The solvent was removed under reduced pressure, and the product was purified by flash column chromatography on silica gel using a 0-25% hexane/ethyl acetate gradient to give **3** as an off-white solid (247 mg, 61%). ^{1}H-NMR (400 MHz $CDCl_3$): δ 7.68-7.62 (m, 6H), 7.52-7.47 (m, 3H), 7.43-7.39 (m, 6H), 6.60 (s, 1H), 2.79-2.76 (t, 4H), 2.51-2.47 (t, 4H), 1.85-1.78 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ 142.8 (d, J = 2 Hz), 136.4 (d, J = 8 Hz), 134.2, ,133.2, 132.5 (d, J = 10 Hz), 131.2 (d, J = 3 Hz), 128.2 (d, J = 12 Hz), 111.1, 33.5, 32.2, 25.5 ppm. ^{31}P-NMR (162 MHz, $CDCl_3$): 6.09 (s, 1P), 29.04 (s, 1P, TPPO) ppm. HRMS (ESI) calculated for $C_{30}H_{20}NP$ $[M + H]^+$ 434.2038. Found $[M + H]^+$ 434.2043.

Experimental Data

3. *N*-(triphenylphosphoranylidene)-1,2,3,5,6,7-hexahydro-*s*-indacen-4-amine




142.78
142.76
135.38
135.30
134.18
133.18
132.50
132.40
131.74
131.21
128.33
128.21
111.13
33.46
32.18
25.54
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


29.04
6.09
140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200 -220 -240
f1 (ppm)

Radiosynthesis

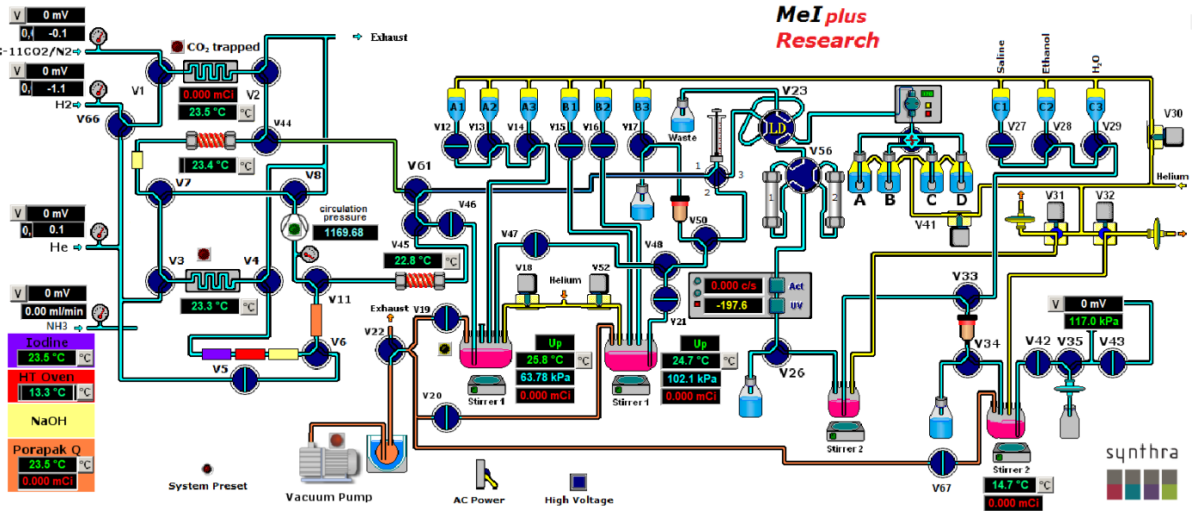


Figure S1. Syntha MeIplus Research Apparatus scheme.

Automated radiosynthesis of [^{11}C]MCC950

HPLC mobile phase (0.8 mL, 30% ACN/50 mM NH_4OAc) was loaded into V2. DMF and DBU were degassed using five freeze-thaw cycles prior to use. Precursor **3** (3.06 mg, 7.07 µmol) was loaded directly into the reactor. 4-(2-hydroxypropan-2-yl)furan-2-sulfonamide **2** (5.86 mg, 28.57 µmol) and KO^{t}Bu (3.14 mg, 27.9 µmol) were weighed in Teflon sealed vials and kept under inert atmosphere. Two minutes prior to the end-of-bombardment, **2** was dissolved in 100 µL of DMF and added to the vial containing KO^{t}Bu. After mixing for 30 seconds, the solution was loaded into the reactor, followed by the addition of DBU (7.46 µL of a 10 µL/mL solution in DMF, 9.97 µmol). The reaction vessel was tightly sealed, and a stream of helium was swept through the reactor after loading. Carbon-11 ([^{11}C]CO_2) was generated by the bombardment of a gas target filled with a pressurized N_2/O_2 mixture using a Siemens 11 MeV cyclotron, at 55 µA for 20 minutes, and was directed to a steel coil cooled at -180 ˚C. The coil was briefly flushed with $He_{(g)}$ prior to heating to 25 ˚C under a stream of helium at 3 mL/min to release [^{11}C]CO_2 into the reaction

solution until peak activity. The reactor was then heated to 120 ˚C for 3 min prior to addition of 800 μL of mobile phase. The crude reaction mixture was purified by HPLC: Nucleodur C18 Pyramid 7 μm, 250 × 10 mm eluted with 30% ACN/50mM NH_4OAc at 5 mL/min (retention time 6–9 min). The product was collected in a bulk vessel loaded with 25 mL of H_2O and passed through a Sep-Pak C18 Plus Light cartridge. The cartridge was washed with 10 mL of H_2O and eluted with 1.5 mL of EtOH. 13.5 mL of saline was added to the vessel and the contents were passed through a 0.22 μm sterile filter. The identity was established by co-injection with the cold standard using a Waters 2695 Alliance HPLC equipped with a Phenomenex Luna 10 μm C18(2) (100 Å, 250 mm × 4.6 mm) column, a 996-photodiode array detector (Waters), and a Carroll & Ramsey Associates 105-S high-sensitivity radiation detector. Gradient: 80/20 H_2O/ACN for 2 min, linear gradient to 35/65 over 8 min, 35/65 for 2 min, linear gradient to 80/20 for 1 min, 80/20 for 7 min.

Radiochemical Identity and Isolation

Peaks indicated by solid arrows in chromatograms following co-injection of products with nonradioactive standard. The differences in elution times are due to UV-Vis and radiation detectors placed in series.

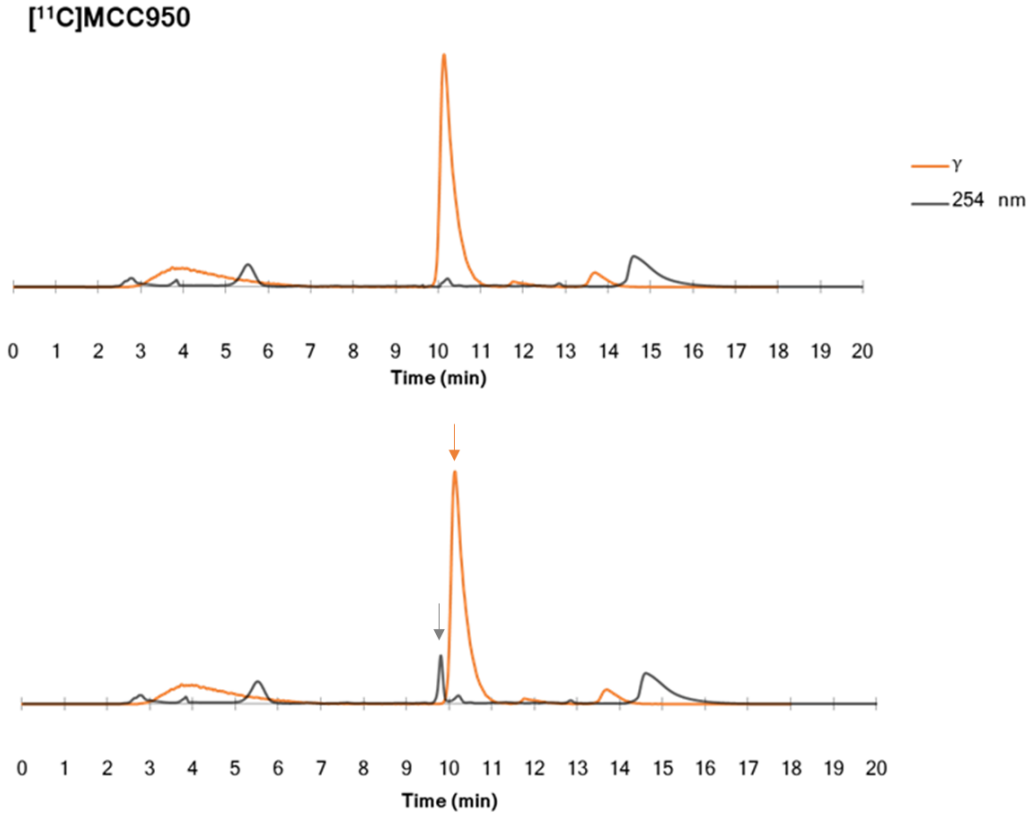


Figure S2. Chromatogram of crude [^{11}C]MCC950 reaction mixture. Crude reaction mixture with (bottom) and without (top) spiked reference standard.

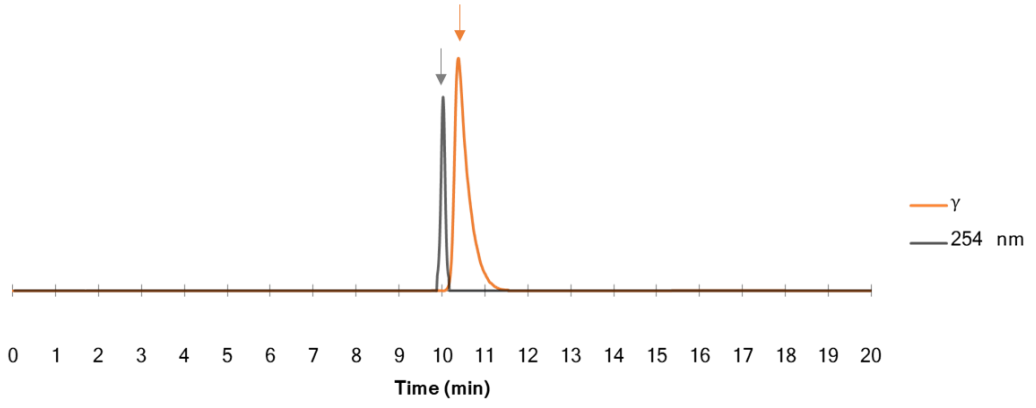


Figure S3. Chromatogram of isolated [^{11}C]MCC950 spiked with reference standard.

Standard Curve

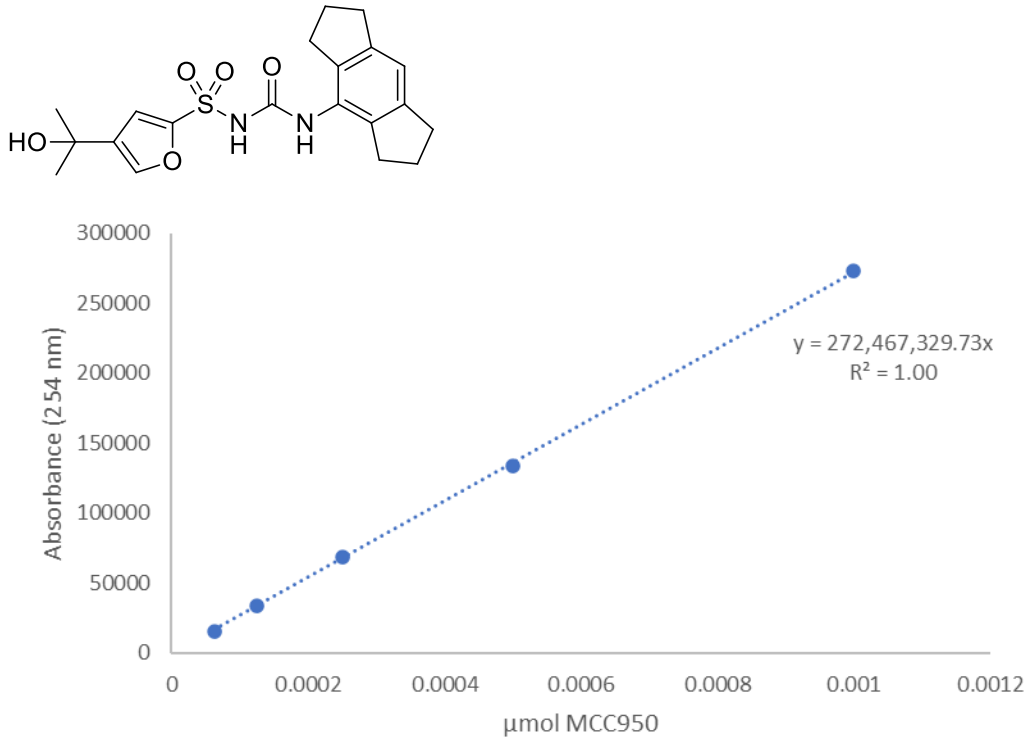


Figure S4. MCC950 standard curve

PET Imaging

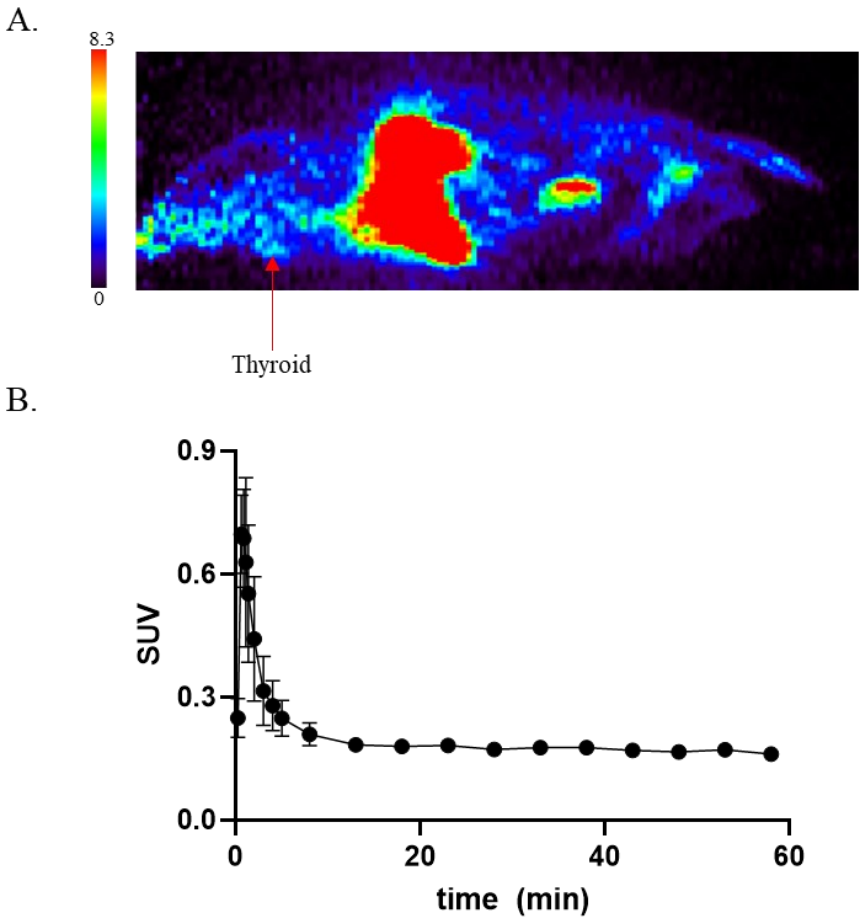


Figure S5. Representative summed (0-60 min) PET image of [^{11}C]MCC950 in C57BL/6 mice and corresponding time-activity curve. $n = 2$