General Information

All substrates, reagents, and solvents were purchased from commercial suppliers and used as received without any purification unless otherwise noted. Air- and moisture-sensitive manipulations were performed using oven-dried glassware under an atmosphere of argon or nitrogen. Air- and moisture-insensitive reactions were carried out under ambient atmosphere and monitored by thin-layer chromatography on silica gel (TLC-SG) or liquid chromatography-mass spectrometry (LC-MS). Microwave reactions were performed in a Biotage Initiator Classic microwave. Thin-layer chromatography was performed on pre-coated silica gel 60 F_{254} plates and visualized by fluorescence quenching under UV light. Flash chromatography purifications were performed using commercial normal-phase silica gel (40–63 µm particle size). Concentration under reduced pressure was performed by rotary evaporation at 23–40 °C at an appropriate pressure. Final products were purified by Grace Reveleris X2 Column chromatography using Grace Reveleris Silica cartridges (12g or 40g). Purified compounds were further dried under vacuum (10^{-6}–10^{-3} bar). Yields refer to purified and spectroscopically pure compounds.

Aqueous ^{18}F-fluoride used in this work was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction in an IBA (Louvain-la-Neuve, Belgium) Cyclone 18/9 cyclotron. Manual radiolabeling was performed in radiochemistry fume hoods at negative air pressure with respect to the laboratory. Radiolabeled products were monitored and identified by radio-TLC and radio-HPLC.

All animal procedures were carried out following the European Union directives for animal experiments (86/609/CEE, 2003/65/CE, and 2010/63/EU), and the protocols used (AVD105002016395 for mice works, and AVD10500201581 for guinea pig works) were previously approved by the Dutch National Committee on Animal Experiments and the Institutional Animal Care and User Committee of the University of Groningen.

8 male Dunkin Hartley guinea pigs (Envigo, Netherlands) weighing approximately 250 grams at the time of sensitization were used. The guinea pigs were housed conventionally in pairs, in ventilated cages in rooms maintained at a 12 hour light/dark cycle, and were provided *ad libitum* access to food and water.

32 immunocompromised male mice (6-8 weeks old BALB/c nude mice supplied by Envigo, Netherlands) were used. The animals were provided with sterilized chow and water *ad libitum*, and housed in individually ventilated cages equipped with a negative-pressure HEPA filtered air system. During tumor inoculation or PET scanning, the mice were anesthetized with isoflurane (5% for induction and 2% for maintenance).

Animals were scanned at least five days after the inoculation when the tumor reached a volume between 0.3 and 0.6 cm^3. Tumor diameters were measured 1 to 3 times per week with a caliper, and tumor volume was calculated using the following formula: $V_{tumor} = ab^2/2$, where a and b represent tumor length and width, respectively).

Spectroscopy and Instruments

High-resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI) mass spectra (MS) system from Waters Investigator Semi-prep 15 Super Critical Fluid Chromatography (SFC) with a 3100 MS-ESI detector using a solvent system of methanol (with ammonium hydroxide as an additive) and CO_2 on an ethyl pyridine 4.6x250 mm column or from the taken TLC-SG plate using an Advion plate express TLC-MS. Semi-preparative high-performance liquid chromatography (HPLC) was performed on a Waters system using a 1525 binary HPLC pump, a 2489 UV/visible detector and a Berthold Technologies Flowstar LB 513 radio flow detector. Analytical analysis of the synthesized radiotracers for assessment of final quality control (QC) was acquired using a Waters Acquity integrated system coupled to a Berthold Technologies Flowstar LB 513 radio flow detector. HPLC data were processed with Waters Empower 3 software. Radio-TLC's were scanned using a Perkin Elmer Packard Cyclone storage phosphor system and the acquired data analyzed with the OptiQuant 03.00 software. Gamma-counting was performed on a Perkin Elmer Wallac Wizard 1470 (Turku, Finland), with an open energy window (15-1000 keV) and 15 seconds of measuring time.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 500 spectrometer operating at 500 MHz and 126 MHz for ^{1}H and ^{13}C acquisitions, respectively, in deuterated solvents. For ^{1}H NMR, chemical shifts (δ) are reported in ppm, with the solvent residual peak as the internal standard, and coupling constants (*J*) in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Chemical shifts for ^{13}C NMR were reported in ppm relative to the solvent peak.

mRNA Isolation and PCR Analysis

Total mRNA was isolated using Trizol RNA extraction (TRI Reagent Solution, Applied Biosystems, Landsmeer, Netherlands), according to the manufacturer's instructions. cDNA was synthesized from equal amounts of RNA using Reverse Transcriptase System (Promega, Madison, WI, USA), and the following protocol: 10 min 25°C, 45 min 42°C, 5 min 99°C. rtPCR was performed with SYBR Green (Roche Diagnostics, Almere, Netherlands) and the following protocol including a final step to generate the melting curve: 2 min 95°C, 10 min 95 °C, 45× (30 s 95°C, 30 s 60°C, 30 s 72°C), 30 s 95°C, 30 s 55°C, 30 s 95°C. The rtPCR was performed in an Eco Illumina (Illumina, Eindhoven, Netherlands). For analysis, the LinReg software was used to calculate N0-values, which were normalized to N0 of the housekeeping genes HPRT1 and GAPDH as an internal control. Primer sets used to analyze gene expression are:

Gene:	Forward primer	Reverse primer:
HPRT1	AAGCCAGACTTTGTTGGATT	ACTGGCGATGTCAATAGGAC
GAPDH	CCAGCAAGAGCACAAGAGGA	GAGATTCAGTGTGGTGGGGG
ARG1	GGAGACCACAGTTTGGCAAT	CCACTTGTGGTTGTCAGTGG
ARG2	TGCATCCTTGAACTGTCAGC	ACAAGCTGCTGCTTTCCATT

Surface Plasmon Resonance

Binding kinetics of the inhibitors were determined by surface plasmon resonance using a Biacore T200 (GE Healthcare). Arg1 was immobilized on a Ni-nitrilotriacetic acid sensor chip by Ni-mediated affinity capturing and amine-coupling to a level of 4000 or 6000 resonance units using 60 μg/mL Arg1 in running buffer (50 mM Na_2HPO_4, pH 7.4,150 mM KCl, and 0.01% Tween-20). The arginase inhibitors were diluted in the same running buffer and were injected in an increasing concentration range of 0.1, 0.316, 1.0, 3.16, and 10 μM. Single-cycle kinetics were used for measuring compound binding with a flow rate of 30 μL/min, an association time of 100 s per injection, and a dissociation time of 1800 s. The compound response was subtracted with both the reference channel response and the blank injection. The Biacore Evaluation software was used to fit the data to the Langmuir 1:1 binding model, with x^2 values indicating minimal deviation between the fit and the experimental data. This minimal deviation was confirmed by determination of the reliability of the curve fits with standard Biacore checks. All combinations of the inhibitors and pH conditions were measured in at least two technical replicates to determine the kinetic constants k_a, k_d, and K_D. The target residence time (τ) was calculated from the k_d value using the formula $\tau=1/k_d$.

PET Acquisition, Image Reconstruction, and Biological Half-life Calculation

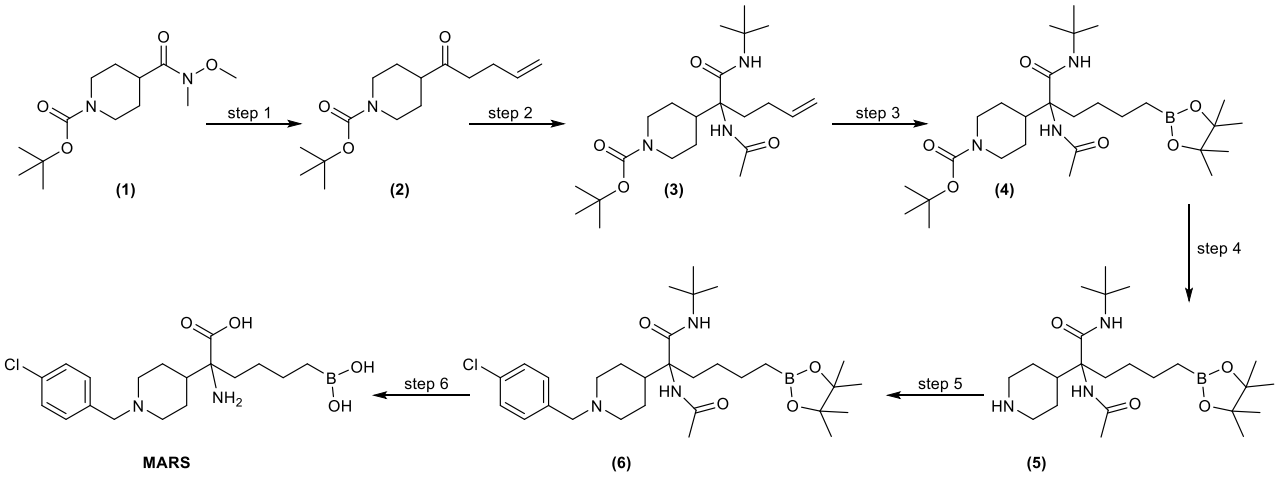
The anesthetized animals were placed in the micro-PET table in a prone position, on top of a heating pad at 38°C to keep constant body temperature, stretched out as much as possible to minimize organ superposition and with the tumor in the field of view. Subsequently, a 90 minutes emission scan was acquired with a Focus 220 rodent scanner (Siemens/Concorde). Between the injection time and the beginning of the scan, an average time of 5 minutes has passed. After completion of the PET scan, a 10 minutes transmission scan with a ^{57}Co point source was obtained for the correction of scatter and attenuation of 511 keV photons by tissue.

For the micro-PET image analysis, all emission scans were iteratively reconstructed (OSEM2d, 4 iterations, 16 subsets) after being normalized and corrected for attenuation and radioactive decay. The list-mode data of the emission scans were separated into 24 frames (6x10s, 4x30s, 2x60s, 1x120s, 1x180s, 4x300s, and

6x600s). A three-dimensional volume of interest (VOI) was manually drawn by a single observer on the original data set, delineating the desired area on the summed PET images (0–90 min) using the PMOD software package (version 3.9; PMOD Technologies LLC). These VOIs were used to create the corresponding time-activity curves and to calculate standardized uptake values (SUV). A single exponential curve was fitted to the SUV time-activity curves (using values from 40 to 90 min) by an iterative nonlinear least-squares approach using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA) to calculate the biological half-life of the tracer.

Compound Synthesis and Characterization

2-amino-6-borono-2-(1-(4-chlorobenzyl)piperidin-4-yl)hexanoic acid (MARS)



SCHEME 1. Synthesis pathway for the production of MARS.

Step 1: Activated metallic Mg (8.07 mmol, 2.20 eq.) and anhydrous THF (10 mL) were kept under nitrogen atmosphere. A pinch of iodine was added to initiate the reaction and to keep track of it as a red color became visible. 4-Bromobutene (7.34 mmol, 2.00 eq.) was then added dropwise to the reaction mixture. After 30 minutes the red color vanishes, indicating that most Mg was consumed and the Grignard reagents were formed. At this point, the Weinreb amide **1** (CAS 139290-70-3, 3.67 mmol, 1.00 eq.) was diluted in anhydrous THF (8 mL) in a different round bottom flask, flushed with nitrogen and cooled down to 0°C. The Grignard reagents previously produced were then transferred dropwise to the Weinreb amide solution. This mixture was left to stir for at least 30 minutes and the reaction followed by TLC-SG (15 % EtAc:DCM). After the reaction was confirmed to be complete, a saturated ammonium chloride solution was added. The THF layer was extracted, and the ammonium chloride solution was washed with another portion of THF. The combined organic layers were washed with sodium bicarbonate and dried to yield product **2** (74 %).

Step 2: The previously produced ketone **2** (7.48 mmol, 1.00 eq.) was added together with ammonium acetate (29.92 mmol, 4.00 eq.), 2,2,2-Trifluoroethanol (1 mL) and *tert*-butyl-isocyanide (14.96 mmol, 2.00 eq.). This mixture was left to stir for 10 days and followed by TLC-SG (10 % EtAc in DCM, ninhydrin) until the reaction mixture showed more product formed than starting material. At this point, the organic layer was washed with water, then with brine, dried with $MgSO_4$, filtrated, and evaporated at reduced pressure to yield the Ugi product **3** (48 %).

Step 3: 1,2-Bis(diphenylphosphino)ethane (dppe, 0.22 mmol, 0.03 eq.) and $[Ir(cod)Cl]_2$ (0.07 mmol, 0.01 eq.) were transferred to an oven-dried round bottom flask, kept under nitrogen atmosphere and anhydrous DCM (10 mL) added. This mixture was left to stir until a homogenous mixture was formed. To this mixture was added the previously formed Ugi product **3** (7.32 mmol, 1.00 eq.) dissolved in anhydrous DCM (20 mL). After 15 minutes, 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (8.05 mmol, 1.10 eq.) was added with the round bottom

flask cooled down in a water bath (to prevent spontaneous heating). The reaction mixture was left to stir overnight at room temperature and followed by TLC-SG (50 % EtAc in PE, ninhydrin). The reaction mixture was slowly quenched with 3 mL of methanol and 30 mL of water. The aqueous layer was washed with DCM and the organic layer washed then with brine, dried with $MgSO_4$, and purified by flash chromatography to yield product **4** (59 %).

Step 4: Product **4** (4.28 mmol, 1.00 eq.) was dissolved in dioxane (10 mL). To this, 4N HCl in dioxane (17.12 mmol, 4.00 eq.) was added and the reaction mixture left to stir for 1 hour. The reaction was followed by TLC-SG (50 % EA in PE, ninhydrin). The mixture was then evaporated, dissolved in diethylether, and evaporated again to yield product **5** in its salt form (98 %).

Step 5: Salt **5** (0.42 mmol, 1.00 eq.) was dissolved in 1,2-dichloroethane (2 mL) and trimethylamine (0.42 mmol, 1.00 eq.) was added followed by 4-chlorobenzaldehyde (0.63 mmol, 1.50 eq.). The reaction mixture was left to stir for 1 hour and a first portion of sodium triacetoxyborohydride (0.53 mmol, 1.25 eq.) was added. This mixture was allowed to stir for 1 hour. Then a second portion of sodium triacetoxyborohydride (0.53 mmol, 1.25 eq.) was added and the mixture allowed to stir overnight. The reaction mixture was followed by TLC-SG (10 % MeOH in DCM, ninhydrin), washed with bicarbonate, and purified by flash chromatography to yield product **6** (70 %).

Step 6: Product **6** (0.18 mmol, 1.00 eq.) was dissolved in DCM (1 mL) and 4 mL of 6N HCl added. The mixture was refluxed overnight, and the aqueous layer was extracted and washed with DCM. The water was evaporated to yield the pure **MARS** product (98 %).

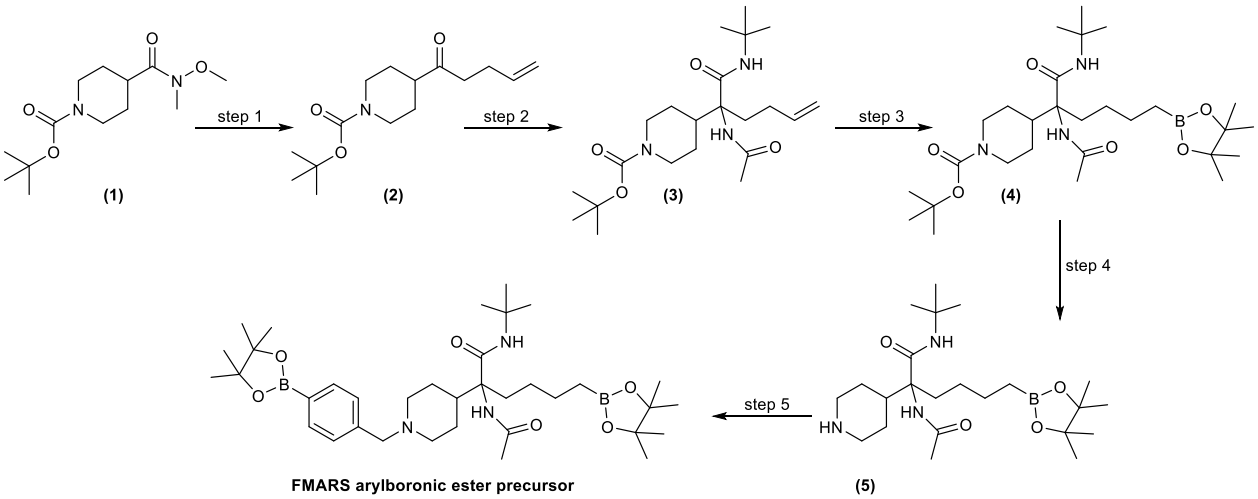
NMR spectroscopy of MARS

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (s, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 4.28 (s, 2H), 3.63 (s, 2H), 3.09 – 2.99 (m, 2H), 2.22 (t, *J* = 12.4 Hz, 1H), 2.13 (d, *J* = 14.2 Hz, 1H), 1.87 (d, *J* = 36.1 Hz, 3H), 1.59 – 1.48 (m, 1H), 1.42 – 1.39 (m, 1H), 1.33 (s, 8H), 1.17 (dq, *J* = 20.5, 7.3, 6.9 Hz, 1H), 0.76 (t, *J* = 7.6 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.07, 135.75, 132.89, 129.34, 127.02, 65.86, 62.65, 59.83, 52.08, 51.77, 38.53, 32.45, 26.75, 25.33, 23.98, 23.53, 13.85, 13.02.

HRMS-ESI: m/z [M-4+1H] (in presence of ammonium hydroxide) 379.10.

(5-acetamido-6-(*tert*-butylamino)-6-oxo-5-(1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl) piperidin-4-yl)hexyl)boronic acid (FMARS arylboronic ester precursor)



SCHEME 2. Synthesis pathway for the production of FMARS arylboronic ester precursor.

Step 1: Activated metallic Mg (8.07 mmol, 2.20 eq.) and anhydrous THF (10 mL) were kept under nitrogen atmosphere. A pinch of iodine was added to initiate the reaction and to keep track of it as a red color became visible. 4-Bromobutene (7.34 mmol, 2.00 eq.) was then added dropwise to the reaction mixture. After 30 minutes the red color vanishes, indicating that most Mg was consumed and the Grignard reagents were formed. At this point, the Weinreb amide **1** (CAS 139290-70-3, 3.67 mmol, 1.00 eq.) was diluted in anhydrous THF (8 mL) in a different round bottom flask, flushed with nitrogen and cooled down to 0°C. The Grignard reagents previously produced were then transferred dropwise to the Weinreb amide solution. This mixture was left to stir for at least 30 minutes and the reaction followed by TLC-SG (15 % EtAc:DCM). After the reaction was confirmed to be complete, a saturated ammonium chloride solution was added. The THF layer was extracted and the ammonium chloride solution washed with another portion of THF. The combined organic layers were washed with sodium bicarbonate and dried to yield product **2** (74 %).

Step 2: The previously produced ketone **2** (7.48 mmol, 1.00 eq.) was added together with ammonium acetate (29.92 mmol, 4.00 eq.), 2,2,2-Trifluoroethanol (1 mL) and *tert*-butyl-isocyanide (14.96 mmol, 2.00 eq.). This mixture was left to stir for 10 days and followed by TLC-SG (10 % EtAc in DCM, ninhydrin) until the reaction mixture showed more product formed than starting material. At this point, the organic layer was washed with water, then with brine, dried with $MgSO_4$, filtrated, and evaporated at reduced pressure to yield the Ugi product **3** (48 %).

Step 3: 1,2-Bis(diphenylphosphino)ethane (dppe, 0.22 mmol, 0.03 eq.) and $[Ir(cod)Cl]_2$ (0.07 mmol, 0.01 eq.) were transferred to an oven-dried round bottom flask, kept under nitrogen atmosphere and anhydrous DCM (10 mL) added. This mixture was left to stir until a homogenous mixture was formed. To this mixture was added the previously formed Ugi product **3** (7.32 mmol, 1.00 eq.) dissolved in anhydrous DCM (20 mL). After 15 minutes, 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (8.05 mmol, 1.10 eq.) was added with the round bottom flask cooled down in a water bath (to prevent spontaneous heating). The reaction mixture was left to stir overnight at room temperature and followed by TLC-SG (50 % EtAc in PE, ninhydrin). The reaction mixture was slowly quenched with 3 mL of methanol and 30 mL of water. The aqueous layer was washed with DCM and the organic layer washed then with brine, dried with $MgSO_4$, and purified by flash chromatography to yield product **4** (59 %).

Step 4: Product **4** (4.28 mmol, 1.00 eq.) was dissolved in dioxane (10 mL). To this, 4N HCl in dioxane (17.2 mmol, 4.00 eq.) was added and the reaction mixture left to stir for 1 hour. The reaction was followed by TLC-SG (50 % EA in PE, ninhydrin). The mixture was then evaporated, dissolved in diethylether, and evaporated again to yield product **5** in its salt form (98 %).

Step 5: Salt **5** (0.42 mmol, 1.00 eq.) and K_2CO_3 (0.84 mmol, 2.00 eq.) were dissolved in DMF (2 mL). This mixture was left stirring for a few minutes to obtain the free base, which can be observed by a color change from grey to green. At this point, 4-bromomethylphenylboronic acid pinacol ester (0.46 mmol, 1.10 eq.) was added and left to stir for 2 hours. The reaction was followed by TLC-SG (5 % MeOH in DCM, ninhydrin). The reaction mixture was then poured on ice to induce precipitation and then filtered. The collected product was washed with water and dried to yield the **FMARS arylboronic ester precursor** as a white solid (68 %).

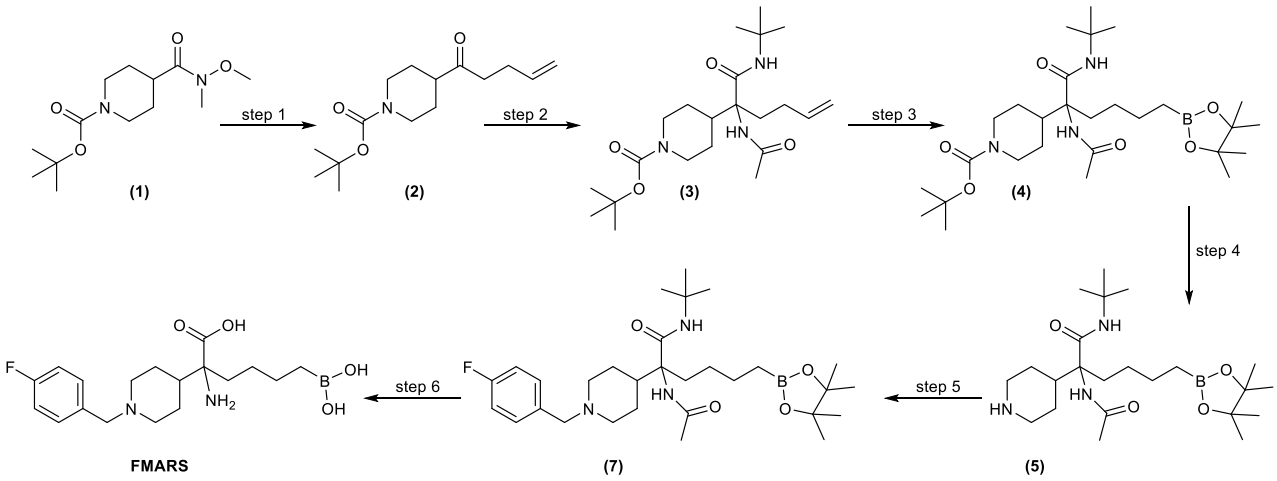
NMR spectroscopy of FMARS arylboronic ester precursor

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.8 Hz, 2H), 7.28 (d, *J* = 7.7 Hz, 2H), 6.93 (s, 1H), 5.53 (s, 1H), 3.56 – 3.39 (m, 2H), 2.87 (d, *J* = 5.6 Hz, 3H), 2.08 (t, *J* = 6.1 Hz, 1H), 1.97 (s, 3H), 1.95 – 1.82 (m, 2H), 1.71 (s, 4H), 1.58 (d, *J* = 27.2 Hz, 1H), 1.35 (d, *J* = 13.2 Hz, 25H), 1.22 (s, 11H), 1.08 – 0.95 (m, 1H), 0.73 (t, *J* = 7.9 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.02, 170.99, 169.17, 169.07, 141.70, 141.65, 134.73, 128.57, 83.80, 83.03, 66.67, 66.60, 63.11, 54.10, 53.81, 51.91, 42.82, 42.73, 32.11, 31.97, 28.85, 27.69, 27.45, 26.92, 26.33, 24.97, 24.93, 24.89, 24.74, 24.72, 24.28, 22.86, 14.13.

HRMS-ESI: m/z $[M+H]^+$ 654.48.

2-amino-6-borono-2-(1-(4-fluorobenzyl)piperidin-4-yl)hexanoic acid (FMARS)



SCHEME 3. Synthesis pathway for the production of FMARS.

Step 1: Activated metallic Mg (8.07 mmol, 2.20 eq.) and anhydrous THF (10 mL) were kept under nitrogen atmosphere. A pinch of iodine was added to initiate the reaction and to keep track of it as a red color became visible. 4-Bromobutene (7.34 mmol, 2.00 eq.) was then added dropwise to the reaction mixture. After 30 minutes the red color vanishes, indicating that most Mg was consumed and the Grignard reagents were formed. At this point, the Weinreb amide **1** (CAS 139290-70-3, 3.67 mmol, 1.00 eq.) was diluted in anhydrous THF (8 mL) in a different round bottom flask, flushed with nitrogen and cooled down to 0°C. The Grignard reagents previously produced were then transferred dropwise to the Weinreb amide solution. This mixture was left to stir for at least 30 minutes and the reaction followed by TLC-SG (15 % EtAc:DCM). After the reaction was confirmed to be complete, a saturated ammonium chloride solution was added. The THF layer was extracted and the ammonium chloride solution washed with another portion of THF. The combined organic layers were washed with sodium bicarbonate and dried to yield product **2** (74 %).

Step 2: The previously produced ketone **2** (7.48 mmol, 1.00 eq.) was added together with ammonium acetate (29.92 mmol, 4.00 eq.), 2,2,2-Trifluoroethanol (1 mL) and *tert*-butyl-isocyanide (14.96 mmol, 2.00 eq.). This mixture was left to stir for 10 days and followed by TLC-SG (10 % EtAc in DCM, ninhydrin) until the reaction mixture showed more product formed than starting material. At this point, the organic layer was washed with water, then with brine, dried with $MgSO_4$, filtrated, and evaporated at reduced pressure to yield the Ugi product **3** (48 %).

Step 3: 1,2-Bis(diphenylphosphino)ethane (dppe, 0.22 mmol, 0.03 eq.) and $[Ir(cod)Cl]_2$ (0.07 mmol, 0.01 eq.) were transferred to an oven-dried round bottom flask, kept under nitrogen atmosphere and anhydrous DCM (10 mL) added. This mixture was left to stir until a homogenous mixture was formed. To this mixture was added the previously formed Ugi product **3** (7.32 mmol, 1.00 eq.) dissolved in anhydrous DCM (20 mL). After 15 minutes, 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (8.05 mmol, 1.10 eq.) was added with the round bottom flask cooled down in a water bath (to prevent spontaneous heating). The reaction mixture was left to stir overnight at room temperature and followed by TLC-SG (50 % EtAc in PE, ninhydrin). The reaction mixture was slowly quenched with 3 mL of methanol and 30 mL of water. The aqueous layer was washed with DCM and the organic layer washed then with brine, dried with $MgSO_4$, and purified by flash chromatography to yield product **4** (59 %).

Step 4: Product **4** (4.28 mmol, 1.00 eq.) was dissolved in dioxane (10 mL). To this, 4N HCl in dioxane (17.12 mmol, 4.00 eq.) was added and the reaction mixture left to stir for 1 hour. The reaction was followed by TLC-SG (50 % EA in PE, ninhydrin). The mixture was then evaporated, dissolved in diethylether, and evaporated again to yield product **5** in its salt form (98 %).

Step 5: Salt **5** (0.42 mmol, 1.00 eq.) was dissolved in 1,2-dichloroethane (2 mL) and trimethylamine (0.42 mmol, 1.00 eq.) was added followed by 4-fluorobenzaldehyde (0.63 mmol, 1.50 eq.). The reaction mixture was left to stir for 1 hour and a first portion of sodium triacetoxyborohydride (0.53 mmol, 1.25 eq.) was added. This mixture was allowed to stir for 1 hour, and then a second portion of sodium triacetoxyborohydride (0.53 mmol, 1.25 eq.) was added and the mixture allowed to stir overnight. The reaction mixture was followed by TLC-SG (10 % MeOH in DCM, ninhydrin), washed with bicarbonate, and purified by flash chromatography to yield product **7** (70 %).

Step 6: Product **7** (0.18 mmol, 1.00 eq.) was dissolved in DCM (1 mL) and 4 mL of 6N HCl added. The mixture was refluxed overnight, and the aqueous layer was extracted and washed with DCM. The water was evaporated to yield the pure **FMARS** product (98 %).

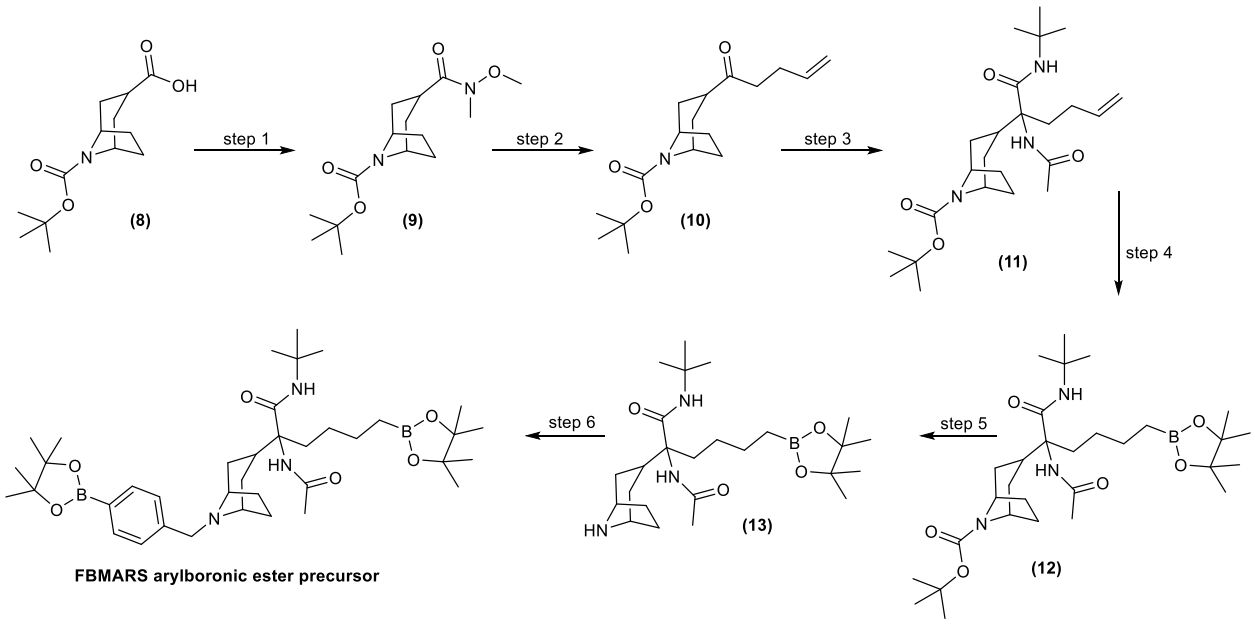
NMR spectroscopy of FMARS

^{1}H NMR (500 MHz, D_2O) δ 7.42 (dd, *J* = 8.5, 5.2 Hz, 2H), 7.14 (t, *J* = 8.6 Hz, 2H), 4.22 (s, 2H), 3.54 (d, *J* = 10.9 Hz, 1H), 2.98 (qd, *J* = 6.1, 2.9 Hz, 1H), 2.17 (tt, *J* = 12.7, 3.2 Hz, 1H), 2.07 (dt, *J* = 14.0, 2.9 Hz, 1H), 1.87 (d, *J* = 19.0 Hz, 2H), 1.82 – 1.70 (m, 1H), 1.48 (qd, *J* = 13.1, 3.9 Hz, 1H), 1.37 – 1.30 (m, 2H), 1.27 (s, 9H), 1.10 (dt, *J* = 25.5, 7.0 Hz, 1H), 0.69 (t, *J* = 7.6 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.93, 167.09, 165.12, 136.08, 136.01, 127.03, 118.80, 118.62, 68.59, 62.41, 54.26, 54.18, 41.17, 35.11, 29.24, 27.89, 26.59, 26.12.

HRMS-ESI: m/z [M-4+1H] (in presence of ammonium hydroxide) 363.26.

(5-acetamido-6-(*tert*-butylamino)-6-oxo-5-(3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-3-azabicyclo[3.2.1]octan-8-yl)hexyl)boronic acid (FBMARS arylboronic ester precursor)



SCHEME 4. Synthesis pathway for the production of FBMARS arylboronic ester precursor.

Step 1: Compound **8** (CAS 280762-00-7, 16.00 mmol, 1.00 eq.) and hydroxybenzotriazole (19.20 mmol, 1.20 eq.) were kept under nitrogen atmosphere in a round bottom flask. To this, it was added DCM (78 mL), trimethylamine (48.00 mmol, 3.00 eq.) and, after 5 minutes, N,O-hydroxyalamine hydrochloride (24.00 mmol, 1.50 eq.). The mixture was then left to stir at room temperature for 30 minutes, and then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (24.00 mmol, 1.50 eq.) was added. The reaction mixture was left to stir

overnight, and the reaction followed by TLC-SG (20 % EtAc in PE, ninhydrin). After the starting material completely vanishes from the TLC-SG profile, the reaction was quenched with 100 mL of water and 50 mL DCM was added. The organic layer was washed with 1N HCl and then with sodium bicarbonate. The product was then dried under high pressure and crystallized overnight to form the Weinreb amide **9** as a white solid (96 %).

Step 2: Activated metallic Mg (33.00 mmol, 2.20 eq.) and anhydrous THF (40 mL) were kept under nitrogen atmosphere. A pinch of iodine was added to initiate the reaction and to keep track of it as a red color became visible. 4-Bromobutene (10.50 mmol, 0.70 eq.) was then added dropwise to the reaction mixture. After 30 minutes a second portion of 4-bromobutene (21.00 mmol, 1.40 eq.) is added and left to stir for further 30 minutes until the red color vanishes, indicating that most Mg was consumed and the Grignard reagents were formed. At this point, the Weinreb amide **9** (15.00 mmol, 1.00 eq.) was diluted in anhydrous THF (40 mL) in a different round bottom flask, flushed with nitrogen and stirred overnight. The reaction was followed by TLC-SG (15 % EtAc in DCM, ninhydrin). After the reaction was confirmed to be complete, a saturated ammonium chloride solution was added. The THF layer was extracted and the ammonium chloride solution washed with another portion of THF. The combined organic layers were washed with sodium bicarbonate and dried to yield product **10** (99 %).

Step 3: The previously produced ketone **10** (3.30 mmol, 1.00 eq.) was added together with ammonium acetate (13.20 mmol, 4.00 eq.), 2,2,2-Trifluoroethanol (1 mL) and *tert*-butyl-isocyanide (6.60 mmol, 2.00 eq.). This mixture was left to stir for 3 weeks and followed by TLC-SG (10 % EtAc in DCM, ninhydrin) until the reaction mixture showed more product formed than starting material. At this point, the organic layer was washed with water, dried with $MgSO_4$, and purified by flash chromatography to yield the Ugi product **11** (57 %).

Step 4: 1,2-Bis(diphenylphosphino)ethane (dppe, 0.07 mmol, 0.03 eq.) and $[Ir(cod)Cl]_2$ (0.02 mmol, 0.01 eq.) were transferred to an oven-dried round bottom flask, kept under nitrogen atmosphere and anhydrous DCM (6.5 mL) added. This mixture was left to stir until a homogenous mixture was formed. To this mixture was added the previously formed Ugi product **11** (2.30 mmol, 1.00 eq.) dissolved in anhydrous DCM (6 mL). After 15 minutes, 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.50 mmol, 1.10 eq.) was added with the round bottom flask cooled down in a water bath (to prevent spontaneous heating). The reaction mixture was left to stir overnight at room temperature and followed by TLC-SG (50 % EtAc in PE, ninhydrin). The reaction mixture was slowly quenched with 0.5 mL of methanol and 10 mL of water. The aqueous layer was washed with DCM and the organic layer washed then with brine, dried with $MgSO_4$, and purified by flash chromatography to yield product **12** (73 %).

Step 5: Product **12** (2.70 mmol, 1.00 eq.) was dissolved in dioxane (5 mL). To this, 4N HCl in dioxane (10.80 mmol, 4.00 eq.) was added and the reaction mixture left to stir for 1 hour. The reaction was followed by TLC-SG (50 % EA in PE, ninhydrin). The mixture was then evaporated to yield **13** as a withe solid in its salt form (95 %).

Step 6: Salt **13** (0.42 mmol, 1.00 eq.) and K_2CO_3 (0.84 mmol, 2.00 eq.) were dissolved in DMF (2 mL). This was left stirring for a few minutes to obtain the free base, which can be observed by a color change from grey to green. At this point, 4-bromomethylphenylboronic acid pinacol ester (0.46 mmol, 1.10 eq.) was added and left to stir overnight. The reaction was followed by TLC-SG (5 % MeOH in DCM, ninhydrin). The reaction mixture was then poured on ice to induce precipitation and then filtered. The collected product was washed with water and dried to yield the **FBMARS arylboronic ester** precursor as a white solid (71 %).

NMR spectroscopy of FBMARS arylboronic ester

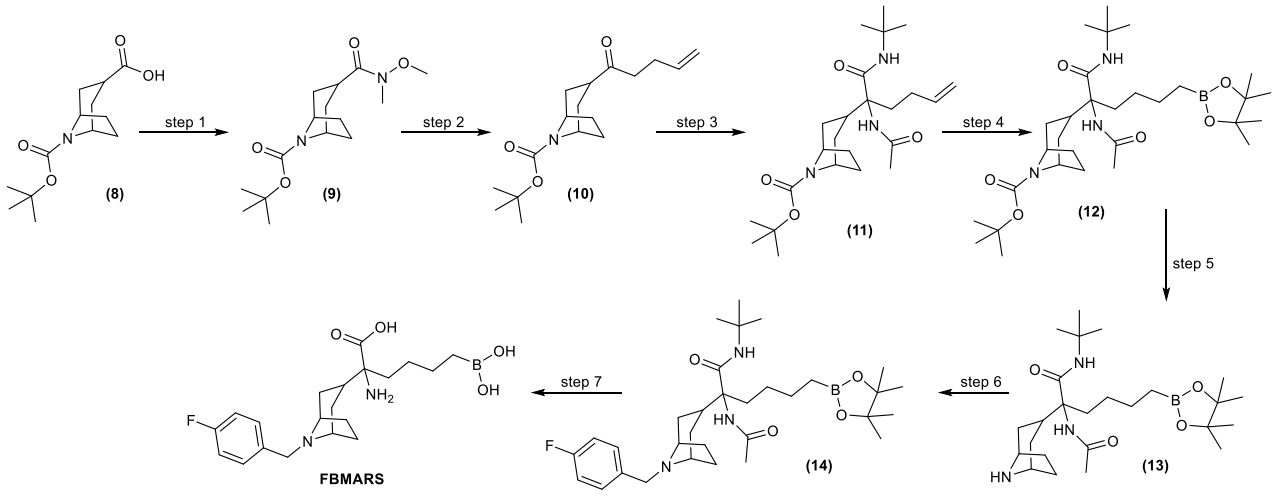
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.8 Hz, 2H), 7.35 (d, *J* = 7.7 Hz, 2H), 6.91 (s, 1H), 5.71 (s, 1H), 3.52 (s, 1H), 3.17 (s, 1H), 2.73 (t, *J* = 11.1 Hz, 1H), 2.64 (dt, *J* = 12.5, 6.9 Hz, 1H), 2.02 – 1.92 (m, 5H),

1.63 – 1.54 (m, 3H), 1.50 (d, *J* = 11.3 Hz, 2H), 1.40 (s, 12H), 1.34 (s, 16H), 1.22 (s, 13H), 0.73 (t, *J* = 7.8 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.27, 169.20, 143.77, 134.84, 134.70, 127.86, 83.79, 83.02, 66.84, 59.31, 58.95, 58.46, 56.32, 51.82, 36.59, 34.06, 32.83, 32.46, 32.23, 31.55, 28.98, 28.89, 26.95, 26.78, 26.54, 25.03, 24.99, 24.96, 24.93, 24.90, 24.87, 24.69, 24.63, 24.33.

HRMS-ESI: m/z $[M+H]^+$ 680.49.

2-amino-6-borono-2-(3-(4-fluorobenzyl)-3-azabicyclo[3.2.1]octan-8-yl)hexanoic acid (FBMARS)



SCHEME 5. Synthesis pathway for the production of FBMARS

Step 1: Compound **8** (CAS 280762-00-7, 16.00 mmol, 1.00 eq.) and hydroxybenzotriazole (19.20 mmol, 1.20 eq.) were kept under nitrogen atmosphere in a round bottom flask. To this, it was added DCM (78 mL), trimethylamine (48.00 mmol, 3.00 eq.) and, after 5 minutes, N,O-hydroxyalamine hydrochloride (24.00 mmol, 1.50 eq.). The mixture was then left to stir at room temperature for 30 minutes and then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (24.00 mmol, 1.50 eq.) was added. The reaction mixture was left to stir overnight, and the reaction followed by TLC-SG (20 % EtAc in PE, ninhydrin). After the starting material completely vanishes from the TLC-SG profile, the reaction was quenched with 100 mL of water and 50 mL DCM was added. The organic layer was washed with 1N HCl and then with sodium bicarbonate. The product was then dried under high pressure and crystallized overnight to form the Weinreb amide **9** as a white solid (96 %).

Step 2: Activated metallic Mg (33.00 mmol, 2.20 eq.) and anhydrous THF (40 mL) were kept under nitrogen atmosphere. A pinch of iodine was added to initiate the reaction and to keep track of it as a red color became visible. 4-Bromobutene (10.50 mmol, 0.70 eq.) was then added dropwise to the reaction mixture. After 30 minutes a second portion of 4-bromobutene (21.00 mmol, 1.40 eq.) is added and left to stir for further 30 minutes until the red color vanishes, indicating that most Mg was consumed and the Grignard reagents were formed. At this point, the Weinreb amide **9** (15.00 mmol, 1.00 eq.) was diluted in anhydrous THF (40 mL) in a different round bottom flask, flushed with nitrogen and stirred overnight. The reaction was followed by TLC-SG (15 % EtAc in DCM, ninhydrin). After the reaction was confirmed to be complete, a saturated ammonium chloride solution was added. The THF layer was extracted and the ammonium chloride solution washed with another portion of THF. The combined organic layers were washed with sodium bicarbonate and dried to yield product **10** (99 %).

Step 3: The previously produced ketone **10** (3.30 mmol, 1.00 eq.) was added together with ammonium acetate (13.20 mmol, 4.00 eq.), 2,2,2-Trifluoroethanol (1 mL) and *tert*-butyl-isocyanide (6.60 mmol, 2.00 eq.). This mixture was left to stir for 3 weeks and followed by TLC-SG (10 % EtAc in DCM, ninhydrin) until the

reaction mixture showed more product formed than starting material. At this point, the organic layer was washed with water, dried with $MgSO_4$, and purified by flash chromatography to yield the Ugi product **11** (57 %).

Step 4: 1,2-Bis(diphenylphosphino)ethane (dppe, 0.07 mmol, 0.03 eq.) and $[Ir(cod)Cl]_2$ (0.02 mmol, 0.01 eq.) were transferred to an oven-dried round bottom flask, kept under nitrogen atmosphere and anhydrous DCM (6.5 mL) added. This mixture was left to stir until a homogenous mixture was formed. To this mixture was added the previously formed Ugi product **11** (2.30 mmol, 1.00 eq.) dissolved in anhydrous DCM (6 mL). After 15 minutes, 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.50 mmol, 1.10 eq.) was added with the round bottom flask cooled down in a water bath (to prevent spontaneous heating). The reaction mixture was left to stir overnight at room temperature and followed by TLC-SG (50 % EtAc in PE, ninhydrin). The reaction mixture was slowly quenched with 0.5 mL of methanol and 10 mL of water. The aqueous layer was washed with DCM and the organic layer washed then with brine, dried with $MgSO_4$, and purified by flash chromatography to yield product **12** (73 %).

Step 5: Product **12** (2.70 mmol, 1.00 eq.) was dissolved in dioxane (5 mL). To this, 4N HCl in dioxane (10.80 mmol, 4.00 eq.) was added and the reaction mixture left to stir for 1 hour. The reaction was followed by TLC-SG (50 % EA in PE, ninhydrin). The mixture was then evaporated to yield **13** as a withe solid in its salt form (95 %).

Step 6: Salt **13** (0.43 mmol, 1.00 eq.) was dissolved in 1,2-dichloroethane (2 mL) and trimethylamine (0.43 mmol, 1.00 eq.) was added followed by 4-fluorobenzaldehyde (0.65 mmol, 1.50 eq.). The reaction mixture was left to stir for 1 hour and a first portion of sodium triacetoxyborohydride (0.54 mmol, 1.25 eq.) was added. This mixture was allowed to stir for 1 hour, and then a second portion of sodium triacetoxyborohydride (0.54 mmol, 1.25 eq.) was added and the mixture allowed to stir overnight. The reaction mixture was followed by TLC-SG (10 % MeOH in DCM, ninhydrin), washed with bicarbonate, and purified by flash chromatography to yield product **14** (54 %).

Step 7: Product **14** (0.21 mmol, 1.00 eq.) was dissolved in DCM (1 mL) and 4 mL of 6N HCl added. The mixture was refluxed overnight and the aqueous layer extracted and washed with DCM. The water was evaporated to yield the pure **FBMARS** product (95 %).

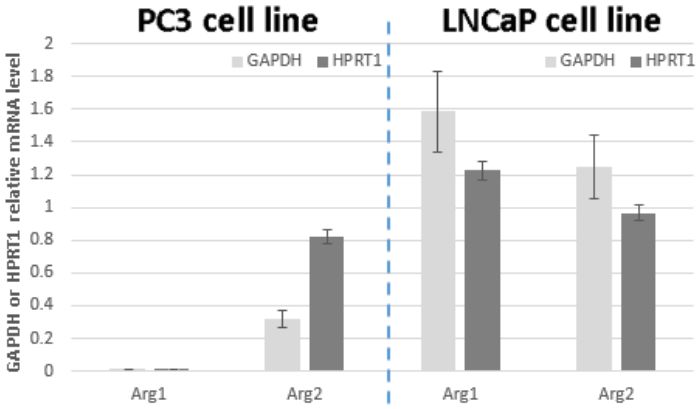
NMR spectroscopy of FBMARS

^{1}H NMR (500 MHz, CD_3OD) δ 7.73 – 7.56 (m, 2H), 7.23 (td, *J* = 8.6, 2.6 Hz, 2H), 4.21 (d, *J* = 11.5 Hz, 2H), 4.06 – 3.92 (m, 2H), 2.50 (d, *J* = 34.2 Hz, 3H), 2.33 – 2.18 (m, 1H), 2.09 (q, *J* = 10.5, 9.7 Hz, 3H), 2.03 – 1.74 (m, 4H), 1.44 (d, *J* = 12.5 Hz, 2H), 1.37 (d, *J* = 2.6 Hz, 7H), 1.24 (d, *J* = 12.6 Hz, 1H), 0.76 (s, 1H).

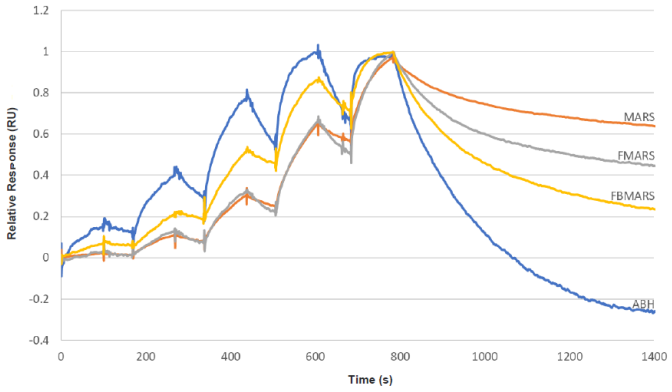
^{13}C NMR (126 MHz, D_2O) δ 172.44, 164.42, 162.45, 132.95, 125.14, 116.26, 65.92, 61.24, 60.97, 60.70, 54.13, 51.94, 48.83, 46.71, 32.37, 29.86, 26.59, 25.25, 23.43, 12.84.

HRMS-ESI: m/z [M-4+1H] (in presence of ammonium hydroxide) 389.3.

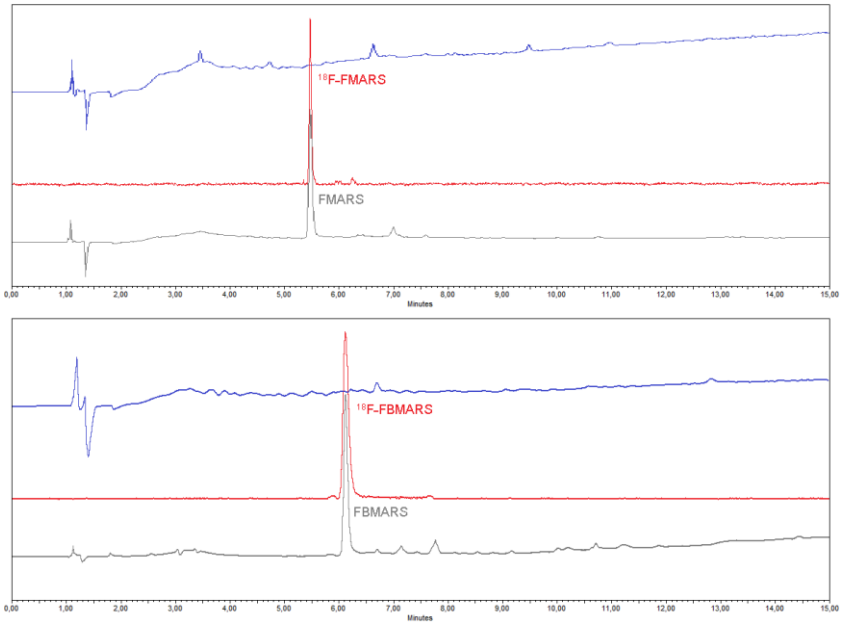
Supplementary Figures



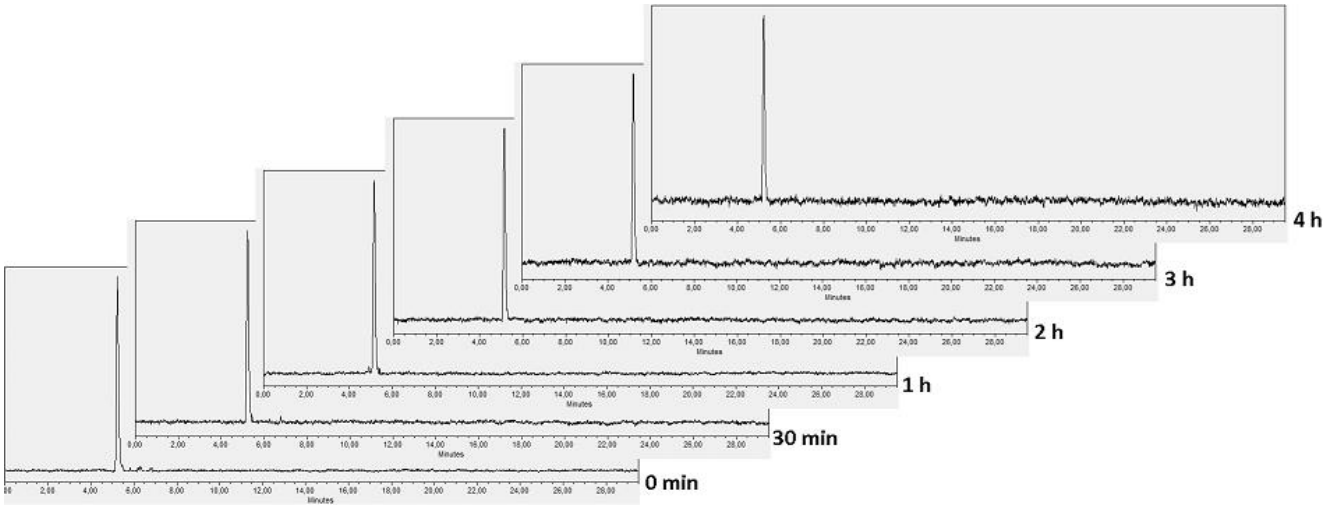
SUPPLEMENTAL FIGURE 1. Arg1 and Arg2 mRNA expression levels normalized with GAPDH and HPRT1 reference (housekeeping) genes in the used PC3 and LNCaP cell lines (*n*=3).



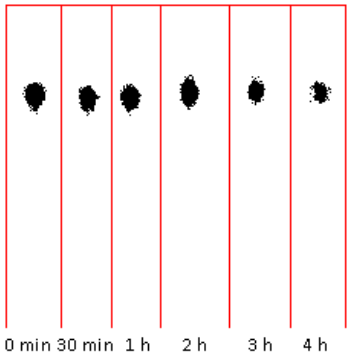
SUPPLEMENTAL FIGURE 2. Surface plasmon resonance sensorgrams (BiaCore T200) for Arg1 showing the binding of ABH, MARS, FMARS, and FBMARS (pH 7.4, inhibitor concentrations: 0.1-10 µM).



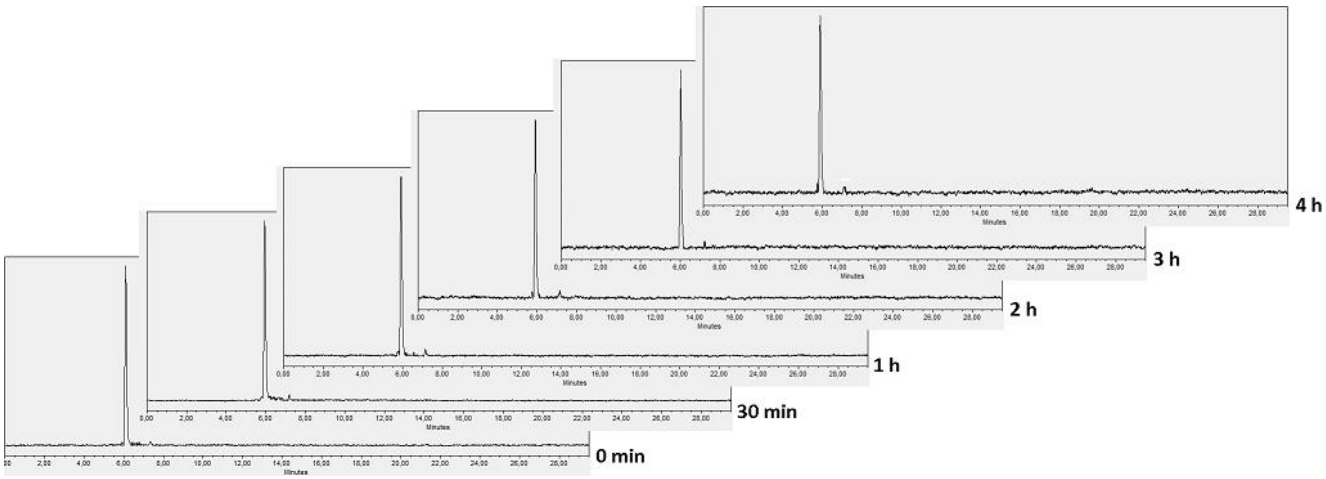
SUPPLEMENTAL FIGURE 3. Representative analytical HPLC profiles (blue, UV detector; red, γ detector) of ^{18}F-FMARS (top) and ^{18}F-FBMARS (bottom) with respective non-radioactive standards (gray UV signal).



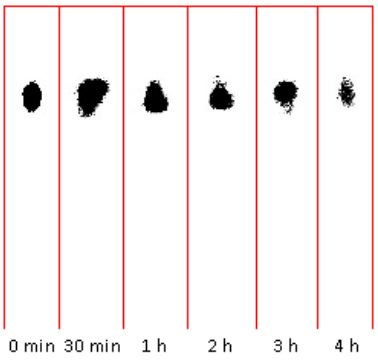
SUPPLEMENTAL FIGURE 4. Representative radio-HPLC of the in vitro stability tests performed for ^{18}F-FMARS by incubating the radiotracers with serum at 37°C for up to 4 hours.



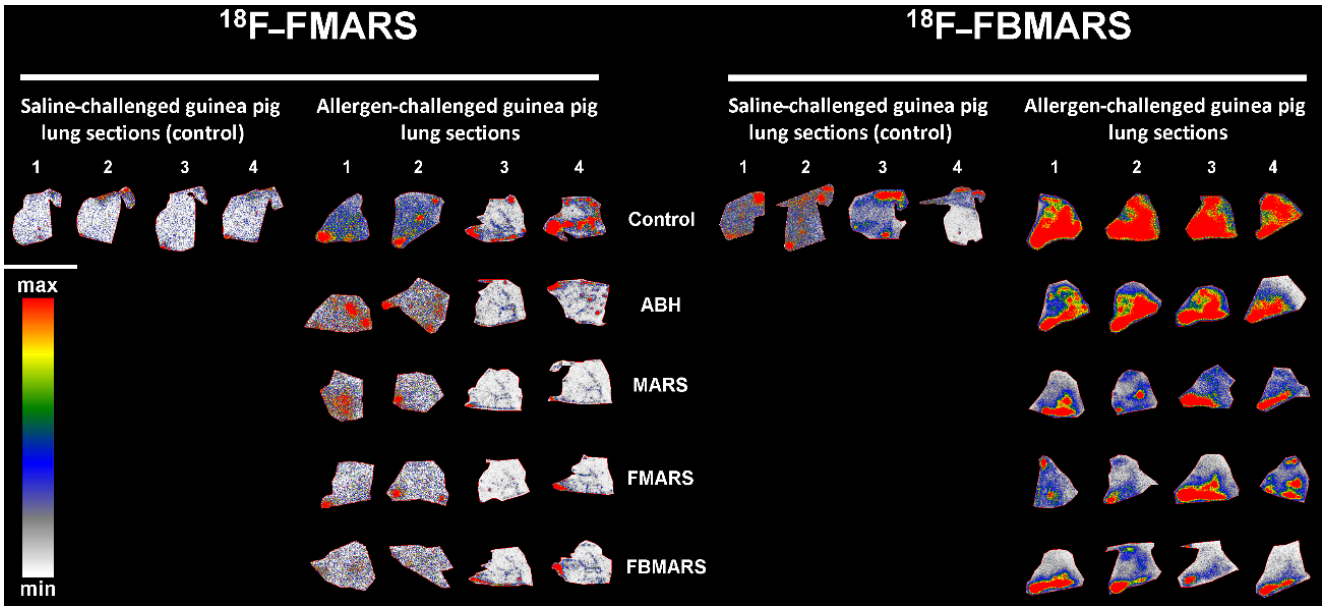
SUPPLEMENTAL FIGURE 5. Representative radio-TLC of the in vitro stability tests performed for ^{18}F-FMARS by incubating the radiotracers with serum at 37°C for up to 4 hours.



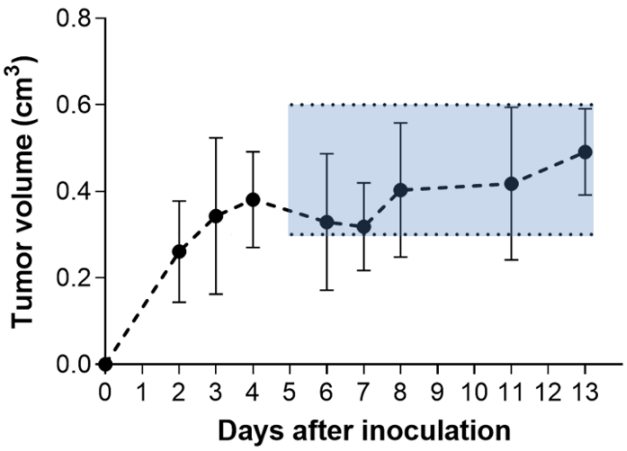
SUPPLEMENTAL FIGURE 6. Representative radio-HPLC of the in vitro stability tests performed for ^{18}F-FBMARS by incubating the radiotracers with serum at 37°C for up to 4 hours.



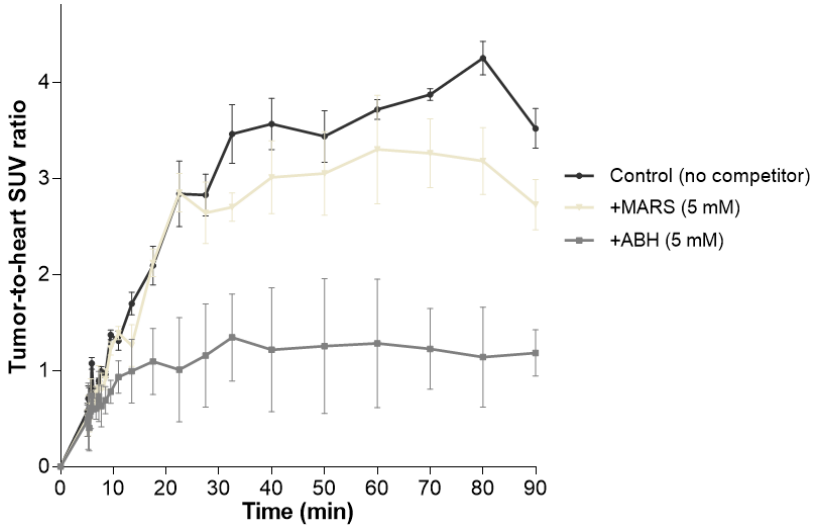
SUPPLEMENTAL FIGURE 7. Representative radio-TLC of the in vitro stability tests performed for ^{18}F-FBMARS by incubating the radiotracers with serum at 37°C for up to 4 hours.



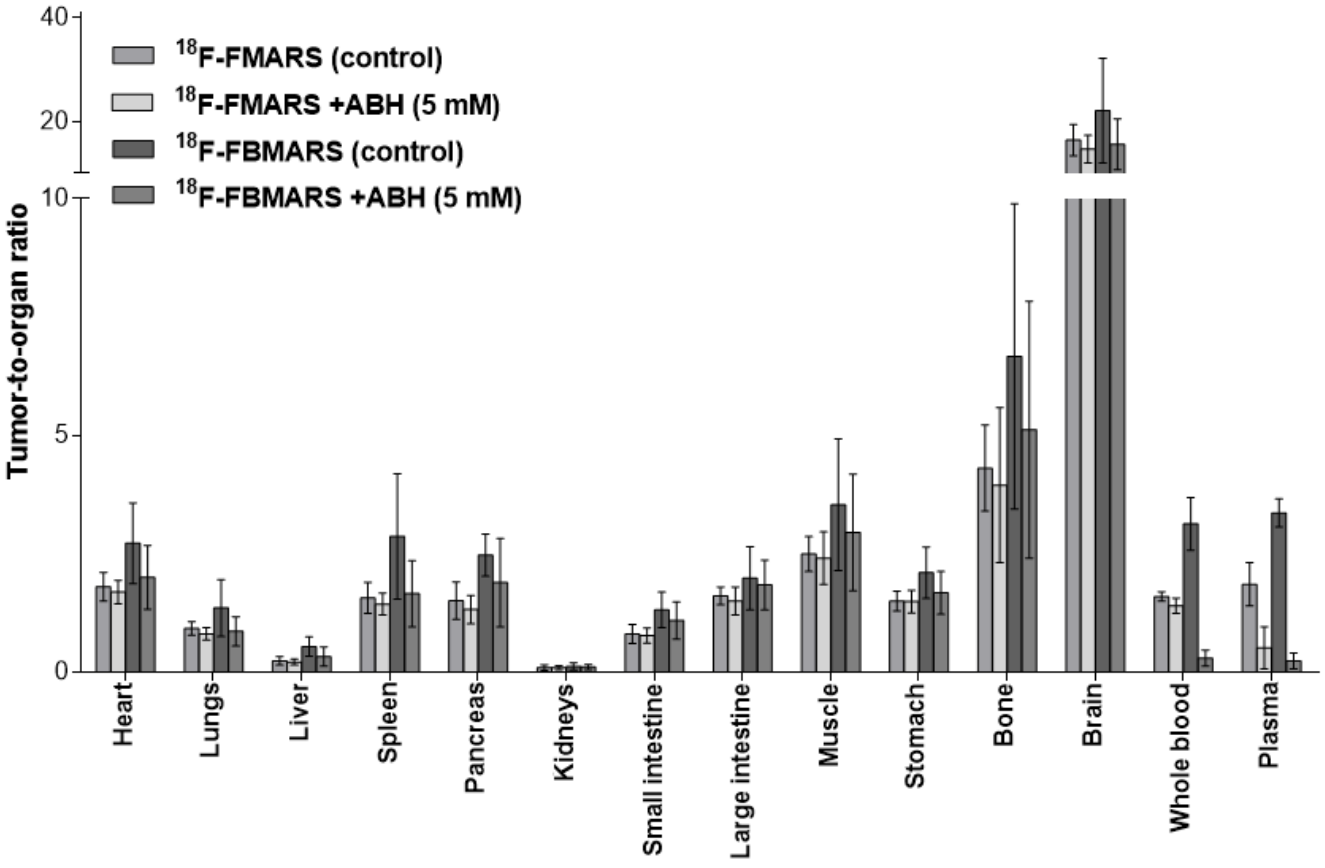
SUPPLEMENTAL FIGURE 8. Representative autoradiography images of saline- and allergen-challenged guinea pig lung sections with ^{18}F-FMARS and ^{18}F-FBMARS without (control) and with competitive arginase inhibition (*n*=4).



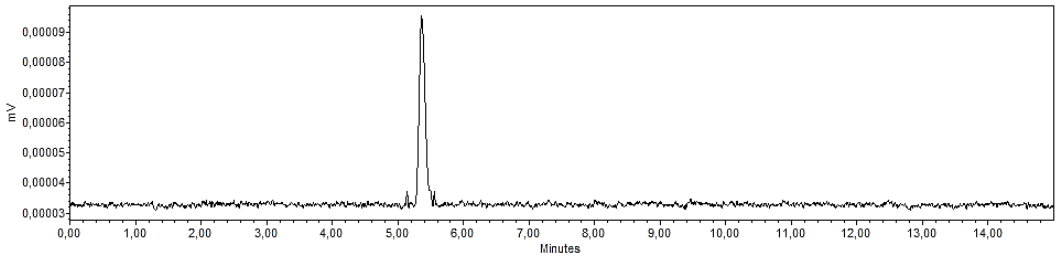
SUPPLEMENTAL FIGURE 9. PC3 solid mass growth after subcutaneous inoculation in mice. The blue area between grid lines represents the ideal tumor volume and moment to perform the PET scan (*n*=32).



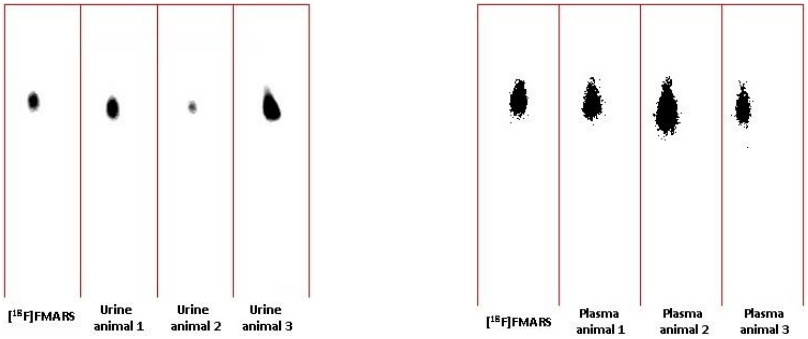
SUPPLEMENTAL FIGURE 10. Tumor-to-blood SUV ratio in the PC3 xenograft mouse model injected with ^{18}F-FBMARS without (control) and with co-injection of the competitive arginase inhibitors ABH and MARS (*n*=3).



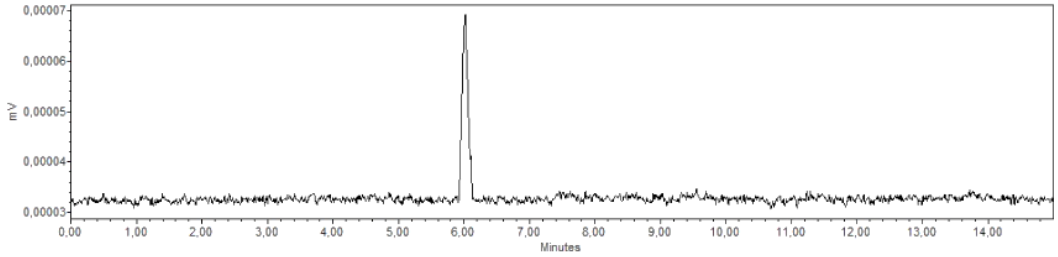
SUPPLEMENTAL FIGURE 11. Tumor-to-organ ratios of ^{18}F-FMARS (n=7) and ^{18}F-FBMARS (n=9), with and without ABH co-injection, approximately 2 hours after intravenous administration in PC3 xenograft mouse model.



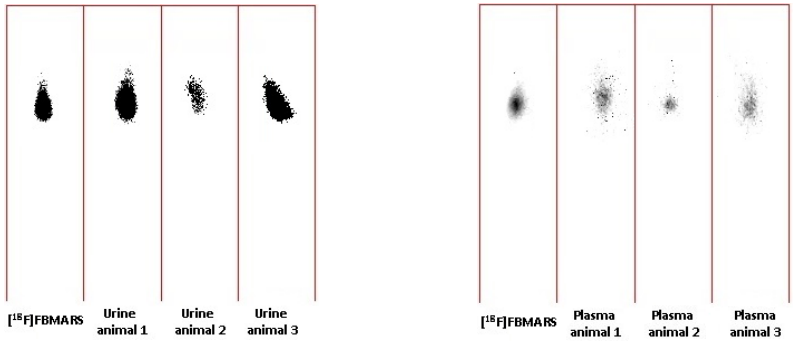
SUPPLEMENTAL FIGURE 12. Representative radio-HPLC of a sample of urine collected approximately 2 hours after ^{18}F-FMARS being intravenously injected in a PC3 xenograft mouse model.



SUPPLEMENTAL FIGURE 13. Representative radio-TLC of samples of urine (left) and plasma (right) collected approximately 2 hours after ^{18}F-FMARS being intravenously injected in a PC3 xenograft mouse model.



SUPPLEMENTAL FIGURE 14. Representative radio-HPLC of a sample of urine collected approximately 2 hours after ^{18}F-FBMARS being intravenously injected in a PC3 xenograft mouse model.



SUPPLEMENTAL FIGURE 15. Representative radio-TLC of samples of urine (left) and plasma (right) collected approximately 2 hours after ^{18}F-FBMARS being intravenously injected in a PC3 xenograft mouse model.

Supplementary tables

SUPPLEMENTAL TABLE 1

Ex vivo biodistribution of ^{18}F-FMARS and ^{18}F-FBMARS of PC3 xenografted immunocompromised male BALB/c nude mice approximately 2 hours after i.v. radiotracer administration (without/with ABH). Activity in each organ was measured and the percentage of the injected dose (%ID/g) was calculated.

Organs	**^{18}F-FMARS (%ID/g)**		**^{18}F-FBMARS (%ID/g)**	
	Control (*n*=7)	**+ABH (5 mM)** (*n*=7)	**Control** (*n*=9)	**+ABH (5 mM)** (*n*=9)
Heart	0.94 ± 0.48	0.55 ± 0.18	1.33 ± 0.66	0.47 ± 0.25
Lungs	1.82 ± 0.95	1.17 ± 0.41	2.89 ± 1.66	1.10 ± 0.55
Liver	7.15 ± 2.85	4.76 ± 2.22	6.78 ± 3.19	3.38 ± 2.27
Spleen	1.04 ± 0.48	0.64 ± 0.20	1.37 ± 0.71	0.58 ± 0.30
Pancreas	1.10 ± 0.49	0.72 ± 0.26	1.33 ± 0.43	0.53 ± 0.35
Kidneys	22.85 ± 14.2	11.02 ± 4.10	39.14 ± 25.02	11.40 ± 9.48
Small intestine	2.15 ± 1.25	1.21 ± 0.34	2.60 ± 0.89	0.88 ± 0.51
Large intestine	1.03 ± 0.55	0.62 ± 0.18	1.90 ± 1.01	0.48 ± 0.25
Muscle	0.67 ± 0.36	0.39 ± 0.13	1.07 ± 0.54	0.34 ± 0.18
Stomach	1.10 ± 0.55	0.61 ± 0.17	1.65 ± 0.75	0.57 ± 0.36
Bone	0.38 ± 0.19	0.26 ± 0.10	0.60 ± 0.32	0.21 ± 0.16
Brain	0.10 ± 0.05	0.06 ± 0.02	0.18 ± 0.11	0.06 ± 0.03
Tumor (PC3)	1.70 ± 1.00	0.92 ± 0.32	3.23 ± 1.05	0.92 ± 0.58
Whole blood	1.07 ± 0.36	1.91 ± 1.18	1.03 ± 0.61	3.19 ± 1.83
Plasma	0.92 ± 0.46	1.82 ± 1.38	0.96 ± 0.90	4.03 ± 2.54
Urine	250.02 ± 97.45	206.59 ±73.35	239.87 ± 54.75	168.20 ± 81.33