Supporting Information

In Vitro Metabolic Stability and *In Vivo* Biodistribution of 3-Methyl-4-furoxancarbaldehyde Using PET Imaging in Rats

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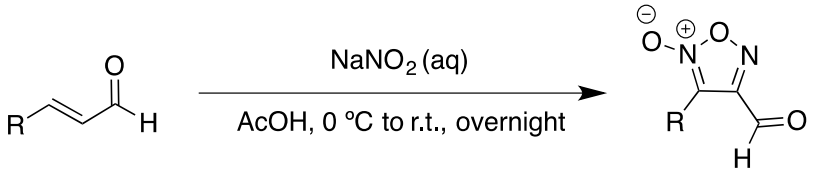
Experimental Section

General methods

All commercial reagents and anhydrous solvents were used without further purification unless otherwise specified; compounds **1** and **6** were synthesized following the published procedures[31, 35]. Crotonaldehyde was distilled prior to use. Analytical HPLC experiments were performed with a Waters Breeze HPLC system equipped with a Bioscan flow-count radioactivity detector and in line UV detectors set to monitor wavelengths 205 nm, 230 nm, and 254 nm (Waters Atlantis T3, Part No. 186003729, 3µm, 4.6 x 150 mm; mobile phase: deionized water; flow rate: 1.0 mL/min; T_R = 10 min). Anions were analyzed using a quaternary amine anion exchange column (Waters IC-Pak Anion, WAT007355, 10 µm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min). ^1H NMR (300 MHz) and ^{11}C NMR (75 MHz) spectra were recorded by means of a Mercury 300 plus.

Syntheses procedures



General procedure for the synthesis of 3-Alkyl-4-furoxancarbaldehydes[31]

To a stirring solution of the aldehyde (50 mmol) in acetic acid (10 ml, 175 mmol) under argon and cooled with an ice bath, was added saturated aqueous sodium nitrite solution (11 mL, 0.8 g/mL, 125 mmol). After 1 h, the ice bath was removed

and the reaction mixture was stirred overnight. The solution was diluted with H_2O (50 mL) and then extracted with dichloromethane (3 x 50 mL). The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtering, the solvent was removed under vacuum and the remaining residue was purified by silica gel flash chromatography (chloroform/ hexanes, gradient increasing to 1:1). 3-alkyl-4-furoxancarbaldehydes are an unstable source for mass spectrometry.

3-Methyl-4-furoxancarbaldehyde (1)[31]**:** (40%, white solid, mp: 40-42 lit.) ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.09 (s, 1H), 2.37 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.43, 154.36, 109.58, 8.38.

3-Ethyl-4-furoxancarbaldehyde (2): (45%, pale yellow oil) ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.08 (s, 1H), 2.80 (q, J = 7.5, 2H), 1.22 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.40, 154.17, 113.68, 16.59, 10.18, 16.59.

3-Propyl-4-furoxancarbaldehyde (3): (30%, clear oil) ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.08 (s, 1H), 2.75 (q, 2H), 1.66 (h, J = 7.5 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.35, 154.37, 112.57, 24.40, 19.36, 13.56.

3-isopropyl-4-furoxancarbaldehyde (4): (43%, pale yellow oil) ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.06 (s, 1H), 3.46 (hept, J = 7.1, 1H), 1.31 (d, J = 7.1, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.31, 153.95, 115.85, 23.90, 17.62.

Procedures for radiosyntheses

Radiosynthesis of [^{13}N] PRG150 (1) and derivatives (2-4)

No-carrier-added $^{13}NO_3^-$ was produced through a 20 min bombardment at 60 μA of a target containing water (sterile water for irrigation USP, B Braun Medical Inc.) by a Siemens 11 MeV RDS 111 cyclotron (Knoxville, TN, USA) at Emory University Center for Systems Imaging. The cyclotron delivered activity for 4 minutes at a rate of 5 mL/min (20 mL) into a reservoir column (Bio-Rad econo-column, Part No. 737-4716, 1.5 x 20 cm, max vol. 35 mL). The initial 10 mL of water contained no activity and was diverted with a 4-way valve (B. Braun Medical Inc., Discofix 4-way stopcock, Part No. 456020) before the cadmium reduction column. The remaining solution (10 mL) was eluted through a cadmium–copper reduction column (18 g Cd, 3 g sand, flow rate: 5 mL/min, Bio-Rad econo-column, Part No. 737-4716, 0.7 x 15 cm, max vol. 6 mL) prepared freshly and preconditioned as described in literature for the quantitative reduction of $^{13}NO_3^-$ to $^{13}NO_2^-$. Immediately after the column, was placed another 4-way valve (B. Braun Medical Inc., Discofix 4-way stopcock, Part No. 456020) connected directly to an alumina-basic Sep-Pak (Waters WAT020505, 1710 mg sorbent) followed by a small luer lock anion exchange cartridge (ORTG, 18F Trap & Release II, 500-102, 25 mg sorbent) attached to a 4-way valve (B. Braun Medical Inc., Discofix 4-way stopcock, Part No. 456020). Once the anion exchange cartridge reached maximum activity, it was dried with air for 10 s, and then the activity was eluted off the anion exchange cartridge with saline (0.15 mL, 23.4%) into a sealed conical vial (3 mL) containing solid sodium nitrite (22.5 mg, 3.3 mmol). A solution of aldehyde (20 μL, 2.4 mmol) in acetic acid (100 μL, 8.0 mmol) was

added to the vial via Hamilton syringe (250 µL). The reaction vessel was measured for activity (160 mCi) and then placed in a heating block (70 ºC) for 6 min. With the vessel still in the heating block, vacuum was applied for 30 s. The reaction mixture was diluted with water (0.5 mL), cooled in an ice bath for 10 s, and then measured for activity (89 mCi).

The crude reaction mixture was purified by pressure transferring it through two sequential t-C18 Sep-Paks (Waters, Part No. WAT036800, 900 mg sorbent, conditioned with 10 mL ethanol, then 10 mL H_2O) with argon (6 psi) into a 5 mL conical vial (A). The Sep-Paks were then washed with H_2O (1.5 mL). A 4-way valve (B. Braun Medical Inc., Discofix 4-way stopcock, Part No. 456020) was switched connecting the t-C18 Sep-Paks to four additional Sep-Paks in order: aminopropyl (WAT020535, 360 mg sorbent, conditioned with 10 mL H_2O), alumina-neutral (Waters, Part No. WAT020510, 1710 mg sorbent, conditioned with 30 mL H_2O), Accell QMA (Waters, Part No. WAT020545, 360 mg sorbent, conditioned with 1 M Na_2CO_3 10 mL, then 15 mL H_2O), Accell QMA (Waters, Part No. WAT020545, 360 mg sorbent, conditioned with 15 mL H_2O). The last Sep-Pak was connected to a 4-way valve (B. Braun Medical Inc., Discofix 4-way stopcock, Part No. 456020). Water (2.0 mL) was washed through the entire Sep-Pak train into a 5 mL conical vial (B). Once complete, the last 3-way valve was switched directing flow to a third 5 mL conical vial (C) and additional water (3.25 mL) was eluted through. That conical vial (C) was measured for activity (23.1 mCi) and then pressure transferred with argon (30 psi) through two- micron filters (1 µm and 0.2 µm) into a sterile vial. An aliquot (0.5 mL) was taken for analysis. The sterile vial was measured for activity (17.1 mCi, 2.5 mL)

and then sent for MicroPET imaging. The entire process from cyclotron delivery to MicroPET imaging was 22 min.

Radiosynthesis of [^{11}C] PRG150 (5)

No-carrier added [^{11}C] CO_2 was produced through the bombardment of $^{14}N_2$ gas containing 1% $^{16}O_2$ (single taret, 60 μA, 30 min) by a Siemens 11 MeV RDS 111 cyclotron (Knoxville, TN, USA) at Emory University Center for Systems Imaging through the ^{14}N [p,α] ^{11}C reaction. A GE TracerLab FX MeI system was employed for the conversion of [^{11}C] CO_2 to [^{11}C] CH_3I. $Pd_2(dba)_3$ (1.8 mg, 2.0 μmol), P(o-Tol)$_3$ (3.8 mg, 12.2 μmol), and NaOAc·$3H_2O$ (7.8 mg, 57 μmol) were placed in a 1-mL dry conical vial and then sealed. A solution of pinacolboron precursor **6** (3.3 mg, 13 μmol) in anhydrous THF (200 μL) was added to the vial, and then $^{11}CH_3I$ was bubbled through the solution by a stream of helium (10 mL/min) at room temperature. When the maximum activity of $^{11}CH_3I$ was reached, the vial was measured for activity (501 mCi) and then placed in a heating block (70 ºC) for 10 min, then cooled in an ice bath for 10 s and measured for activity (340 mCi). Acetic acid (100 μL, 8.0 mmol) was added, and then followed immediately by the addition of sat. $NaNO_2$ (0.05 ml, 0.58 mmol). The vial was placed back into the heating block (70 ºC) for an additional 10 min, then cooled 10 s, measured for activity (323 mCi), and then diluted with H_2O (0.5 mL).

The crude reaction mixture was purified by pressure transferring it through two sequential t-C18 Sep-Paks (Waters WAT036800, 900 mg sorbent, conditioned with 10 mL ethanol, then 10 mL H_2O) with argon (6 psi) into a 5 mL conical vial (A). The Sep Paks were then washed with H_2O (1.5 mL). A 3-way valve was switched

connecting the t-C18 Sep Paks to four additional Sep-Paks in order: aminopropyl (WAT020535, 360 mg sorbent, conditioned with 10 mL H_2O), alumina-neutral (WAT020510, 1710 mg sorbent, conditioned with 30 mL H_2O), Accell QMA (WAT020545, 360 mg sorbent, conditioned with 1 M Na_2CO_3 10 mL, then 15 mL H_2O), Accell QMA (WAT020545, 360 mg sorbent, conditioned with 15 mL H_2O). The last Sep-Pak was connected to a 3-way valve. Water (2.0 mL) was washed through the entire Sep-Pak train into a 5 mL conical vial (B). Once complete, the last 3-way valve was switched directing flow to a third 5 mL conical vial (C) and additional water (3.25 mL) was eluted through. That conical vial (C) was measured for activity (39 mCi) and then pressure transferred with argon (30 psi) through two- micron filters (1 μm and 0.2 μm) into a sterile vial. An aliquot (0.5 mL) was taken for analysis. The sterile vial was measured for activity (25 mCi, 2.5 mL) and then sent for MicroPET imaging. The entire process from cyclotron delivery to MicroPET imaging was 63 min.

In Vitro Methods

HPLC-UV Bioanalytical Method

The liquid chromatography system (Shimadzu Scientific Instruments (Oceania); Sydney, NSW) comprised a Shimadzu LC-20A solvent delivery module, a DGU-20A_3 degasser and a SIL-20AC HT autosampler. Chromatographic separation was achieved using a 150 mm × 4.60 mm × 5 μm Phenomenex (Torrance, CA) Luna C8 (2) 100A column and a 4 mm × 2 mm guard column (Phenomenex) placed in a CTO-20A column oven at 40°C. Analyte detection was achieved using a Shimadzu SPD-20A Prominence UV/Vis detector at 260 nm. The mobile phase comprised methanol (A) and sodium orthophosphate buffer (B, 5 mM, pH 7.4), delivered in isocratic mode for 12 min at a flow rate of 0.7 mL/min (A:B = 45:55 v/v). To prevent accumulation of endogenous microsomal matrix components in the HPLC column, stepwise gradient elution was used at a flow rate of 0.6 mL/min (A:B = 20:80 v/v) for 8 min before re-equilibration of the HPLC system with A:B (45:55) for 4 min. The acquisition and processing of data were performed using LabSolutions (Shimadzu Scientific Instruments (Oceania); Sydney, NSW, Australia,) software (Version 5.51).

Preparation of Calibration Standards

A stock solution of PRG150 (100 μg/ml) was prepared in methanol and stored at -20°C. Standard solutions were prepared by serial dilution with methanol: de-ionized water (40:60) and all solutions were kept on ice during preparation. A solution of the internal standard phenacetin (2000 ng/mL) was prepared in de-

ionized water. Calibration standards in the concentration range, 50 to 3200 ng/mL, were prepared by spiking aliquots (250 μL) of de-ionized water with aliquots (50 μL) of standard working solutions and aliquots (50 μL of the internal standard, phenacetin. Microsomal incubate samples were extracted using SPE cartridges. The concentration of PRG150 in the collected samples of rat liver microsomal incubate, were inverse predicted using linear regression of the peak area ratios of PRG150 to internal standard (phenacetin) for the calibration curves.

Solid Phase Extraction method

SPE cartridges (Oasis® HLB 1 cc 30 μm) were placed into a Speedisk® Pressure Processor (Avantor Performance Materials, Center Valley, PA, USA) and N_2 gas was used to draw fluids through the cartridges. In brief, SPE cartridges were conditioned with 1 mL aliquots of methanol followed by equilibration with 1 mL aliquots of de-ionized water. Individual incubate samples were loaded onto separate cartridges, washed with 1 mL aliquots of 5% methanol in water, and molecules of interest eluted with 350 μL aliquots of methanol. Eluents were diluted with water to have the 40:60 (water: methanol) ratio. Samples (10 μL) were then injected into the HPLC system.

Assessment of Metabolic Stability using Rat Liver Microsomes

Metabolic stability was assessed by incubation of rat liver microsomes (0.3, 0.6, 0.75, 0.9 and 1.2 mg protein/mL) with PRG150 at concentrations of 1, 10, 50 and 100 μM for 30 min. The percentage decrease from the initial PRG150

concentration during the 30 min incubation was calculated. Incubation conditions that resulted in an ~50% decrease in the concentration of PRG150 at 30 min was selected as optimal.

PRG150 (100 μM) was incubated with rat liver microsomes (1.2 mg protein/mL in EDTA-phosphate buffer; 100 mM, pH = 7.4); the final incubation volumes were 250 μL. Following pre-incubation of these microsomal mixtures for 5 min at 37°C on a shaking water bath to equilibrate, Phase 1 metabolism was initiated by addition of 50 μL aliquots of NADPH (5 mM) to produce a final concentration of 1 mM in each incubate. For control incubations, 50 μL aliquots of EDTA-phosphate buffer (100 mM, pH = 7.4) were added instead of NADPH. Incubate samples (250 μL in triplicate) were collected just prior to NADPH addition and then at 10, 15, 30 and 60 min after NADPH addition.[44] Metabolism was terminated immediately after incubate sample collection by the addition of 100 μL aliquots of ice-cold 1% formic acid followed by vigorous mixing of the samples placed on ice.

An aliquot (50 μL) of phenacetin solution (2000 ng/mL) was added to each sample. After vigorous mixing, precipitated proteins were separated by centrifugation (3000 x g, 5 min). Supernatants were then loaded onto SPE cartridges and eluted using 350 μL aliquots of methanol and analyzed alongside calibration standards for calculation of the amount of PRG150 remaining at each time of assessment. Eluants were diluted with water to have the 40:60 (water: methanol)

ratio. The evaporation and reconstitution steps were added to concentrate the samples and improve the lower limit of quantitation (LLOQ).

Peak area ratios of PRG150 to the internal standard for each sample were calculated using inverse prediction of the corresponding peak area ratios versus PRG150 concentrations of the calibration curves processed in parallel with each batch of incubate samples. The rate constant (k) for the decrease in PRG150 concentration in the microsomal matrix was estimated using linear regression of the log concentration versus time data as implemented in GraphPad® Prism (v 5.03). Other parameters derived from these data were metabolic half-life ($t_{1/2}$), and intrinsic clearance (CL_{int}), according to the following equations:

Elimination rate constant: k= -slope (from the linear regression)

Metabolic half-life: $t_{1/2}= 0.693/k$

Intrinsic Clearance: $(V \times 0.693)/t_{1/2}$

Animal experiments

The experiments were carried out with the approval of the Division of Animal Resources (Emory University, Atlanta, GA) in accordance with the Institutional Animal Care and Use Committee. Animal husbandry followed all the regulations of the Division of Animal Resources.

Anesthesia

Rats were anesthetized using isoflurane gas. Anesthesia was initiated 10 min ahead of imaging experiments by placing the animal in a cage ventilated with oxygen containing 1-2% isoflurane. Body temperature was held at 37 ºC using a temperature-controlled warm air convection system.

Injection of tracer

A catheter was placed in the tail vein by a certified veterinarian technician prior to imaging experiments. The catheter was filled with isotonic sodium chloride solution prior to injection of the tracer.

General biodistribution studies

Biodistribution studies were performed in male Sprague Dawley rats (body weight of 111 – 489 g). The rats were injected in the tail vein catheter with 500 – 1000 μCi of a high specific activity (> 0.1 Ci/μmol) of [^{13}N] and [^{11}C] furoxan compounds in 0.1-0.5 mL of isotonic saline (pH = 5-6).

PET Imaging

MicoPET data was acquired with a Siemens Inveon PET/CT system (Siemens Medical Solutions, Knoxville, TN, USA). After anesthesia and placement of the tail vein catheter, the animal was placed with its body located at the center of the field of view.

Radioactivity in the syringe was measured before and after the tracer was injected into the tail vein catheter using a Capintec CRC 15R (Capintec Inc, 6 Arrow Road Ramsey, NJ) dose calibrator.

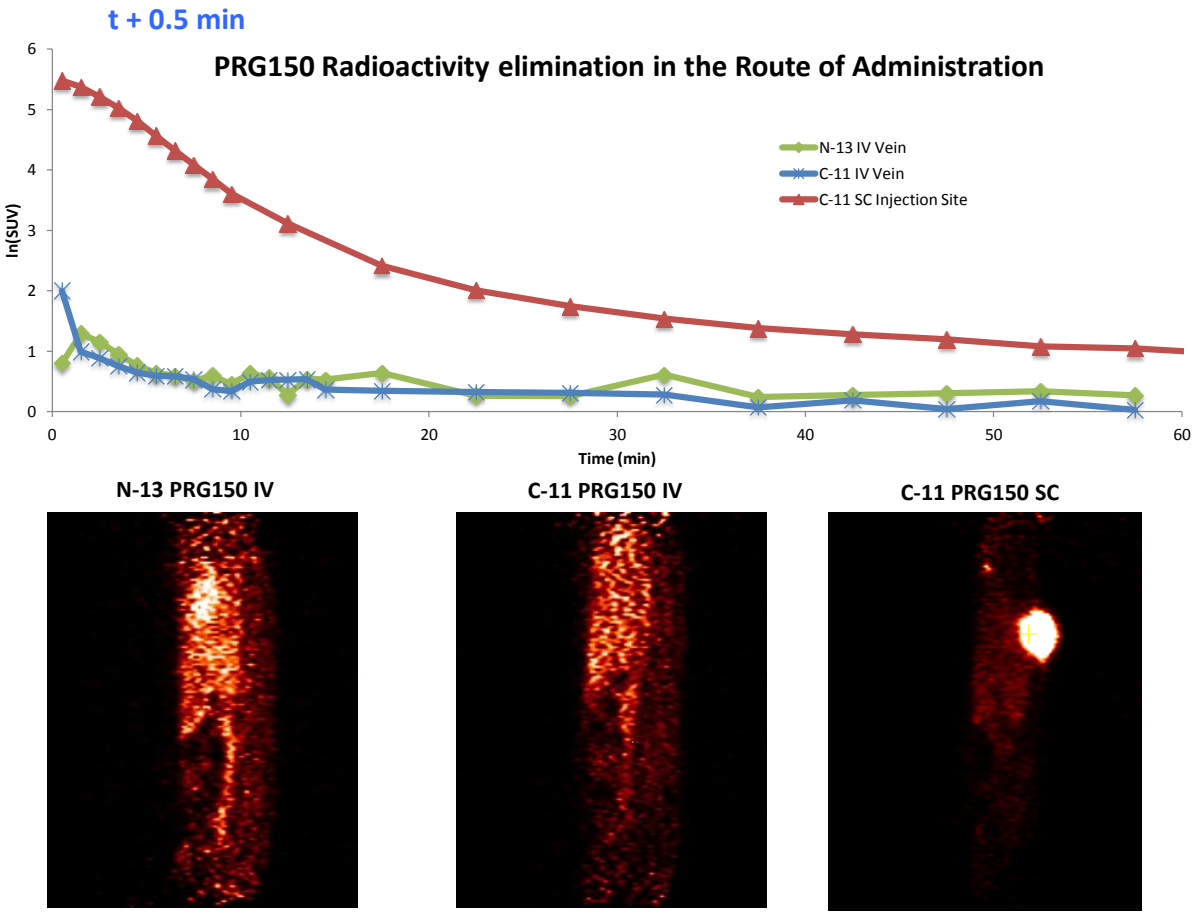
Data acquisition was performed for 60 min starting immediately following tracer injection. The emission data were normalized and corrected for decay and dead time. The images were reconstructed using an attenuation correction with a cobalt source into 15 x 1 min frames followed by 9 x 5 min frames. The image volume consisted of 128 x 128 x 159 voxels, each of a size of 0.78 x 0.78 x 0.80 mm.

After PET imaging, all animals underwent CT scan in the same position as the acquired PET data.

Data processing and co-registration

MicroPET data and CT data were co-registered using ASIPro. The CT template was used for definition of regions-of-interest (ROIs). The time-activity curves represent the mean activity in the regions-of-interest over time. The mean activity was normalized to standardized uptake values by multiplying by the animal weights (g) and dividing by the activity injected (nCi).

Supplementary figures



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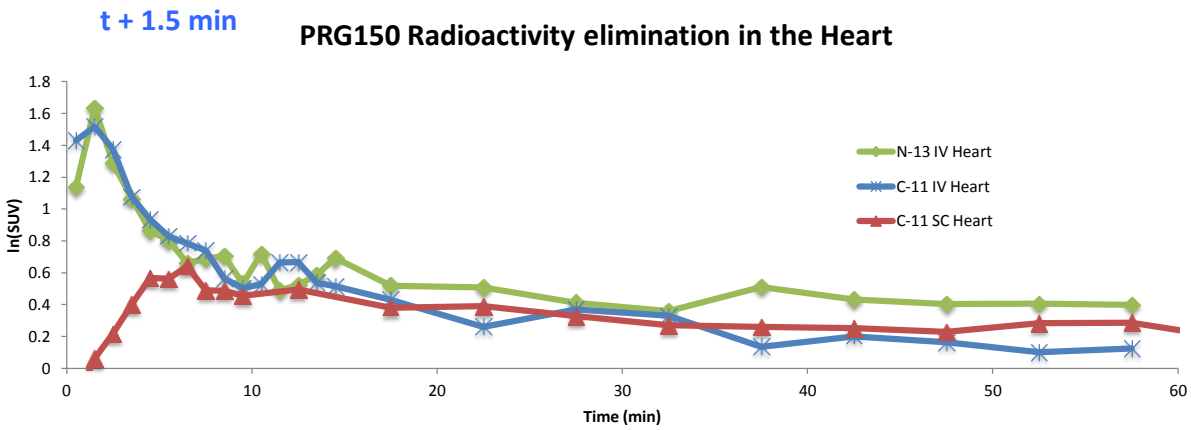

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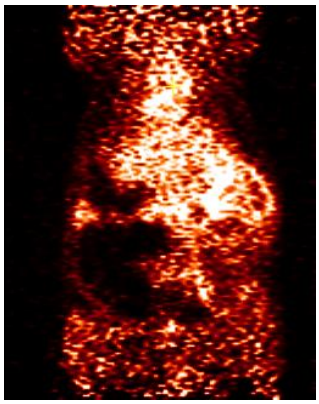

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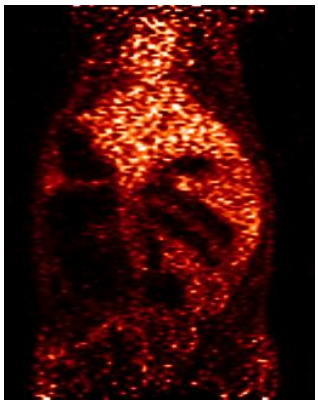

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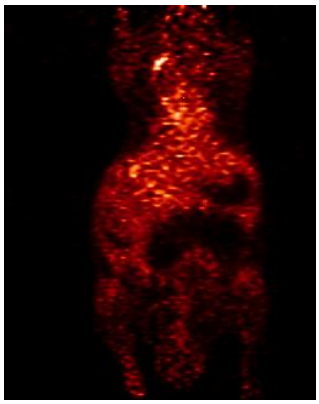

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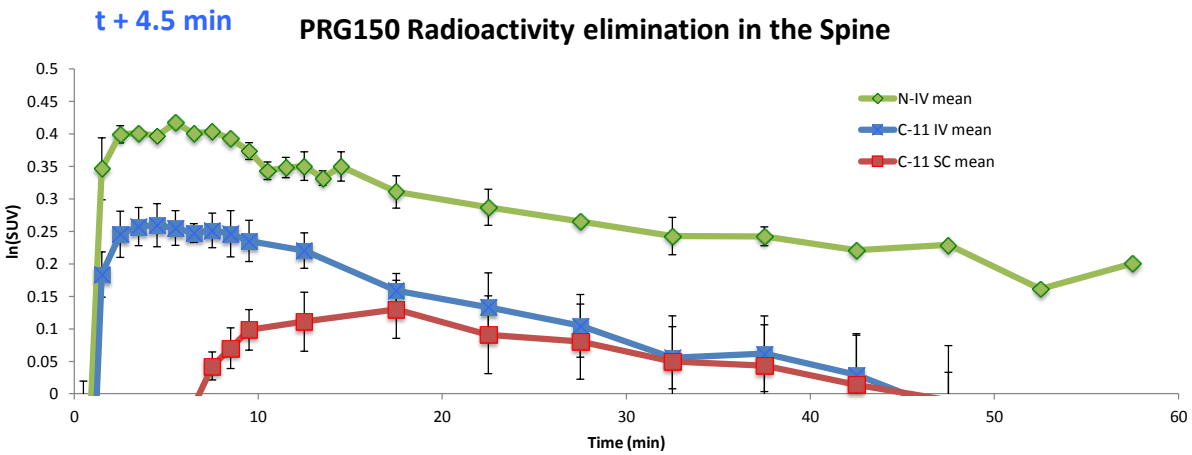

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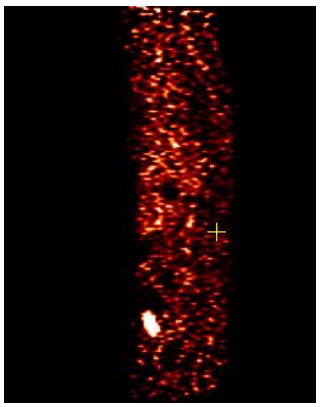

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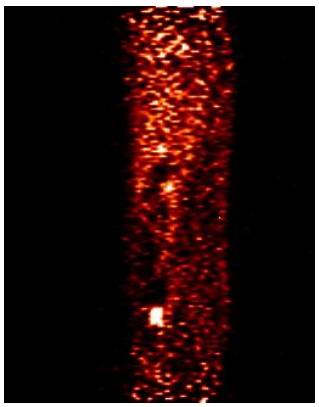

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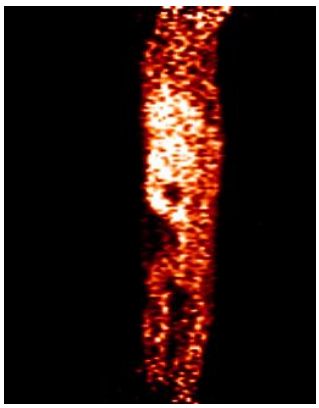

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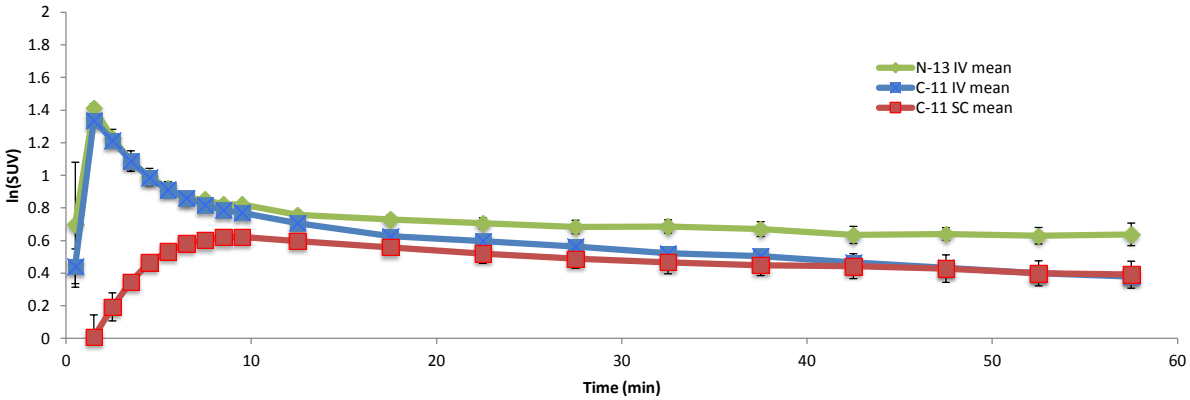
PRG150 Radioactivity elimination in the Liver



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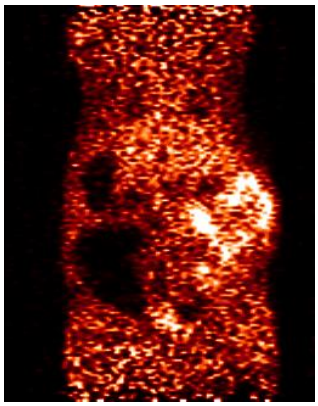

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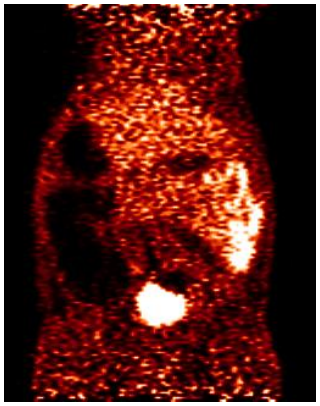

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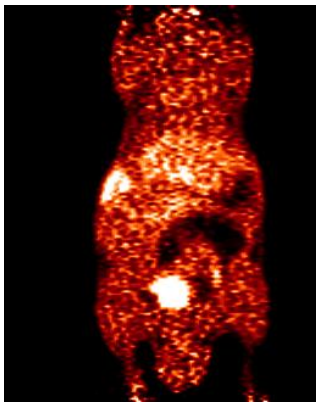

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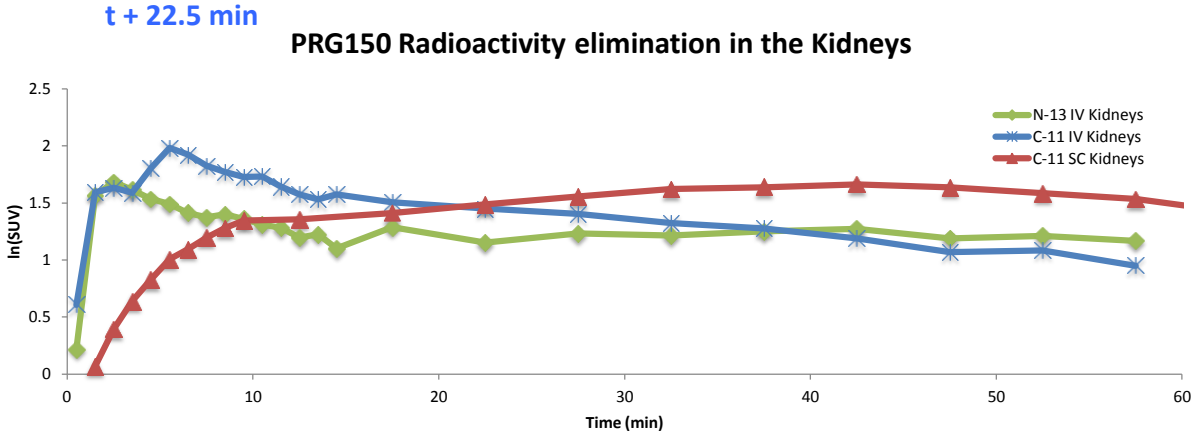

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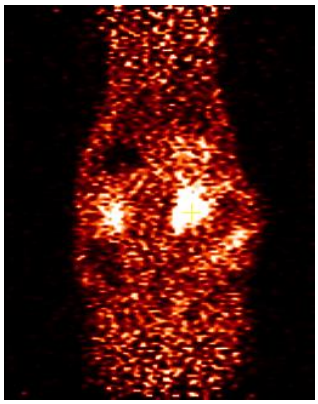

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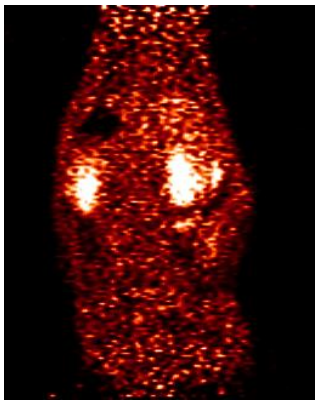

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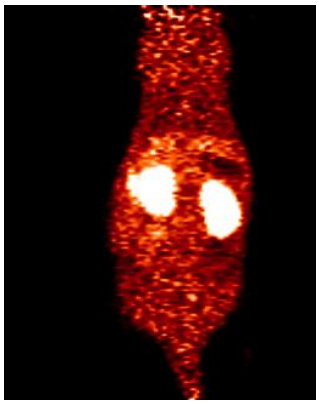

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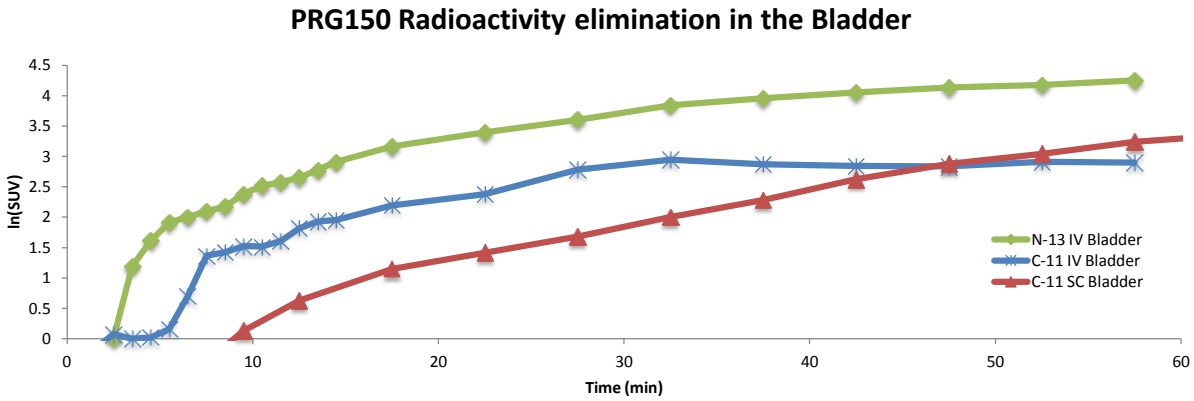

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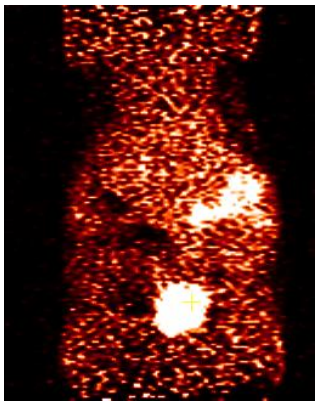

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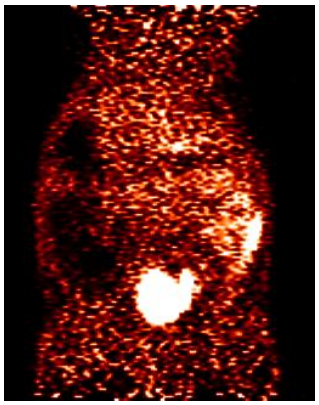

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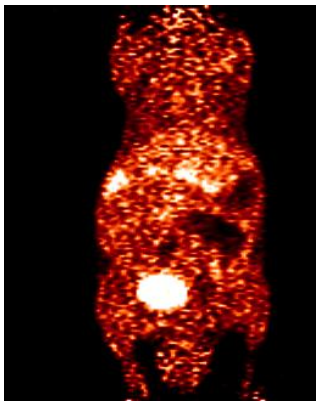
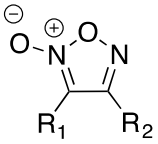


Description of HPLC systems including retention times



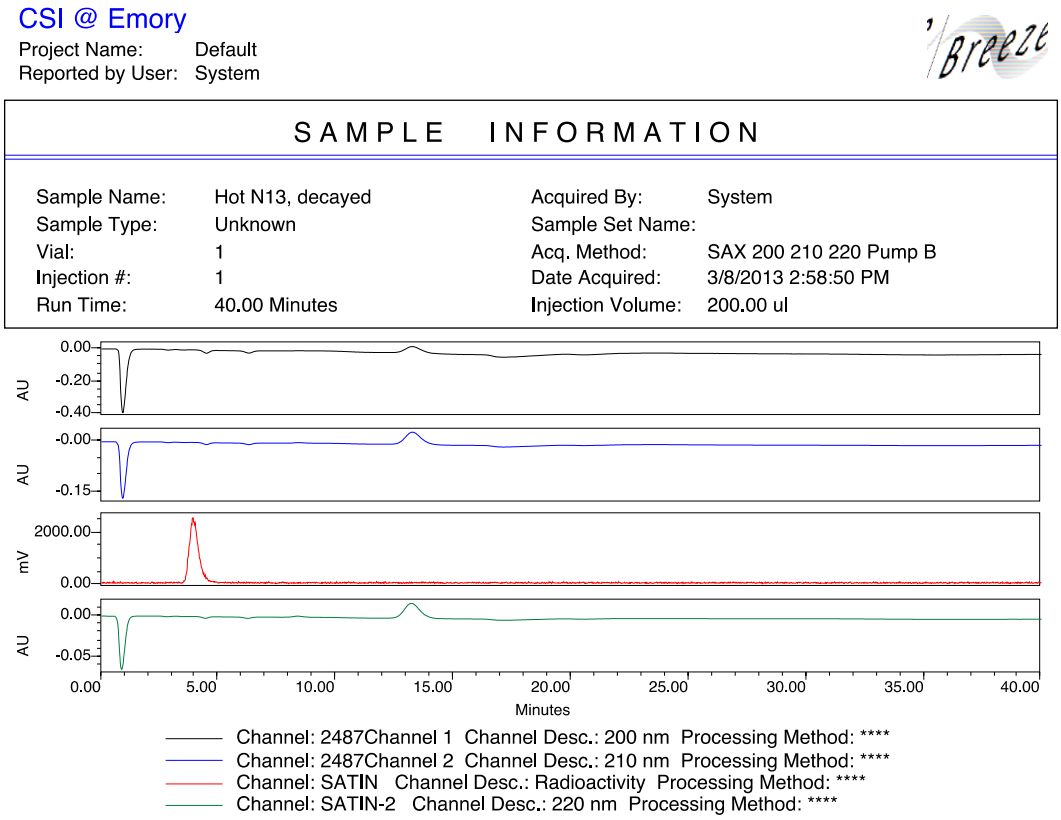
Compound	Isotope	R_1	R_2	HPLC
1	N-13	Me	CHO	5.5 min[a]
2	N-13	Et	CHO	9.8 min[a]
3	N-13	Pr	CHO	14.4 min[b]
4	N-13	iPr	CHO	13.3 min[b]
1	C-11	Me	CHO	9.8 min[c]

[a] HPLC system 1 was a Waters Nova-Pak C_{18} reverse phase column (Waters, WAT0011695, 4µm, 3.9 x 300 mm) eluted with water as the mobile phase. Flow rate was 1 ml/min

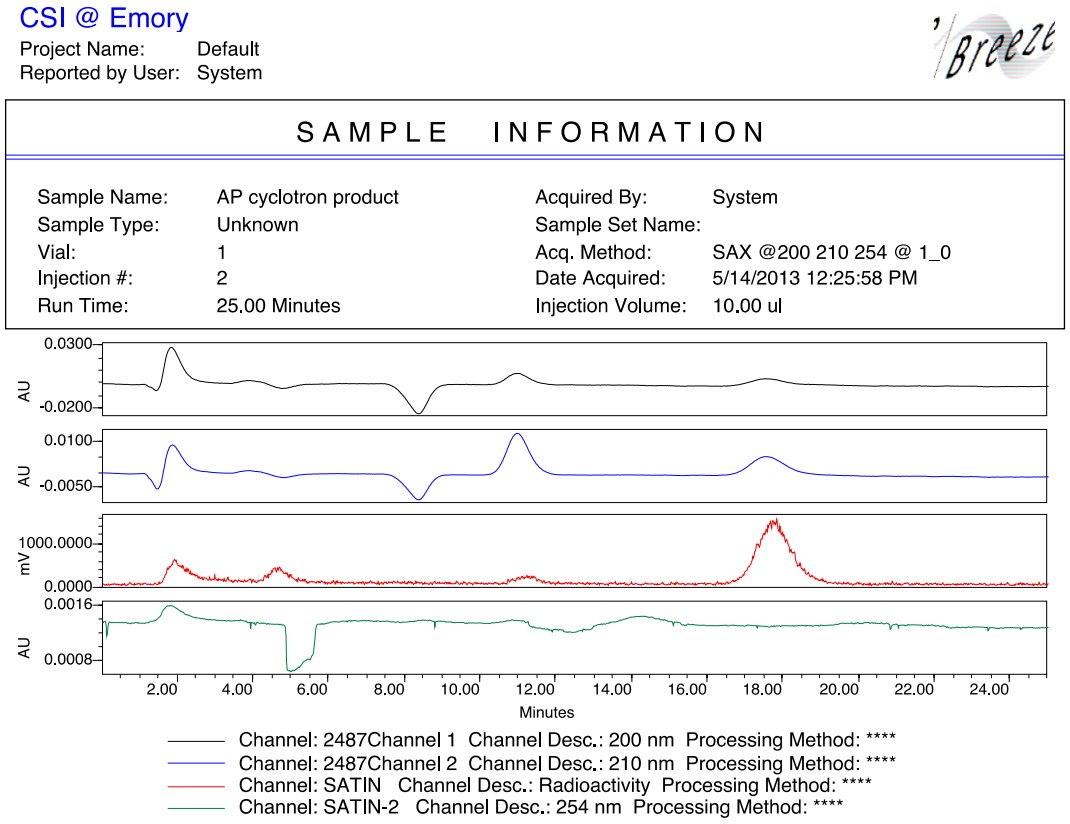
[b] HPLC system 2 was a Waters Nova-Pak C_{18} reverse phase column (Waters, WAT0011695, 4µm, 3.9 x 300 mm) eluted with water as the mobile phase. Flow rate was 1.5 ml/min

[c] HPLC system 3 was a Waters Atlantis T3 (Waters, 186003729, 3µm, 4.6 x 150 mm) eluted with water as the mobile phase. Flow rate was 1 ml/min

HPLC data



HPLC of the cyclotron product after a 30 min bombardment (60 μA) with Water containing ethanol (0.25% v/v) showing [$^{13}NH_4$] retention time of 5 min. (Waters IC-Pak Anion, WAT007355, 10 μm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min)



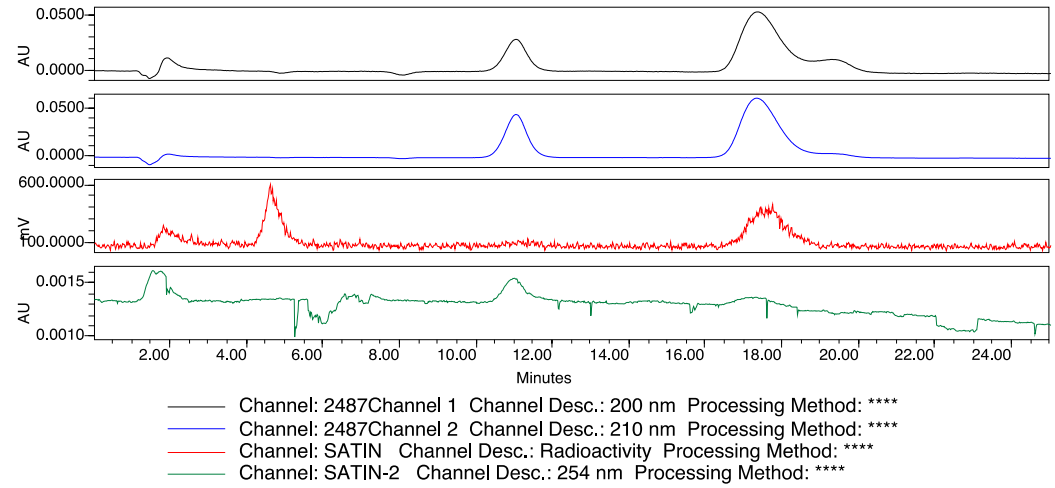
HPLC of the cyclotron product. ^{18}F, $^{13}NH_4$, $^{13}NO_2$, $^{13}NO_3$ (30 min bombardment at 60 μA). After 4 min delivery, 129 mCi was obtained. (Waters IC-Pak Anion, WAT007355, 10 μm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min)

CSI @ Emory

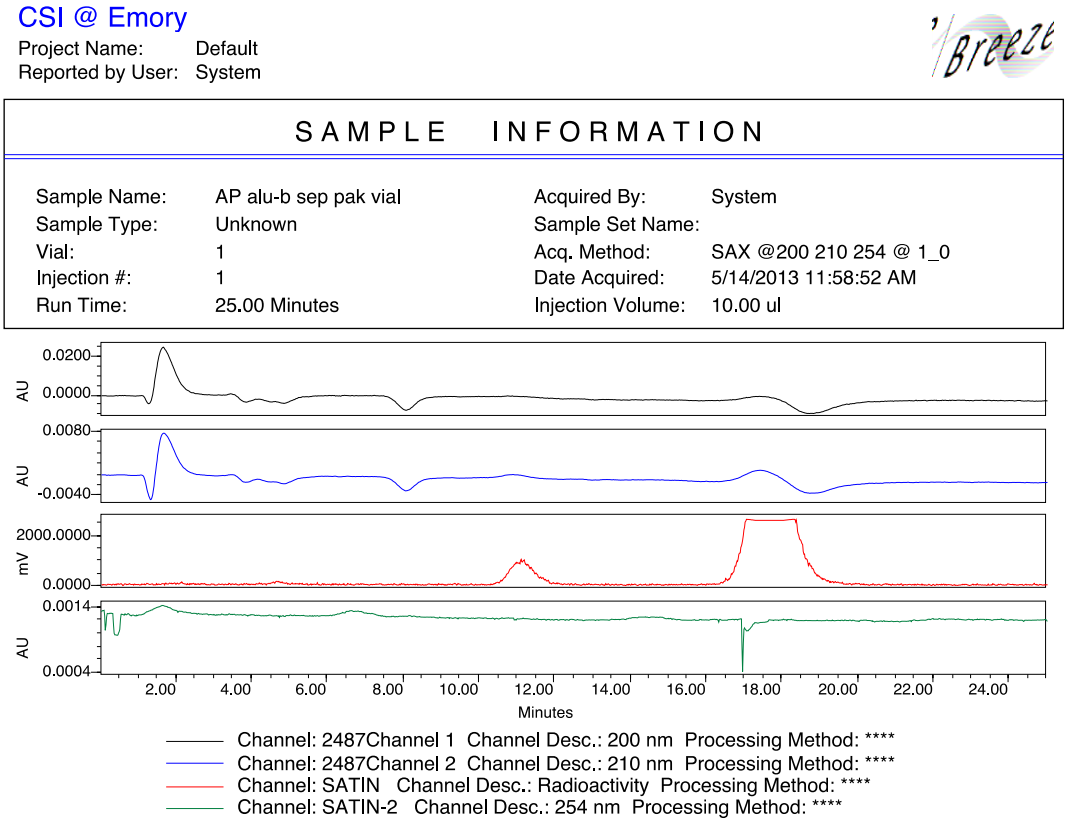
Project Name: Default
Reported by User: System

SAMPLE INFORMATION

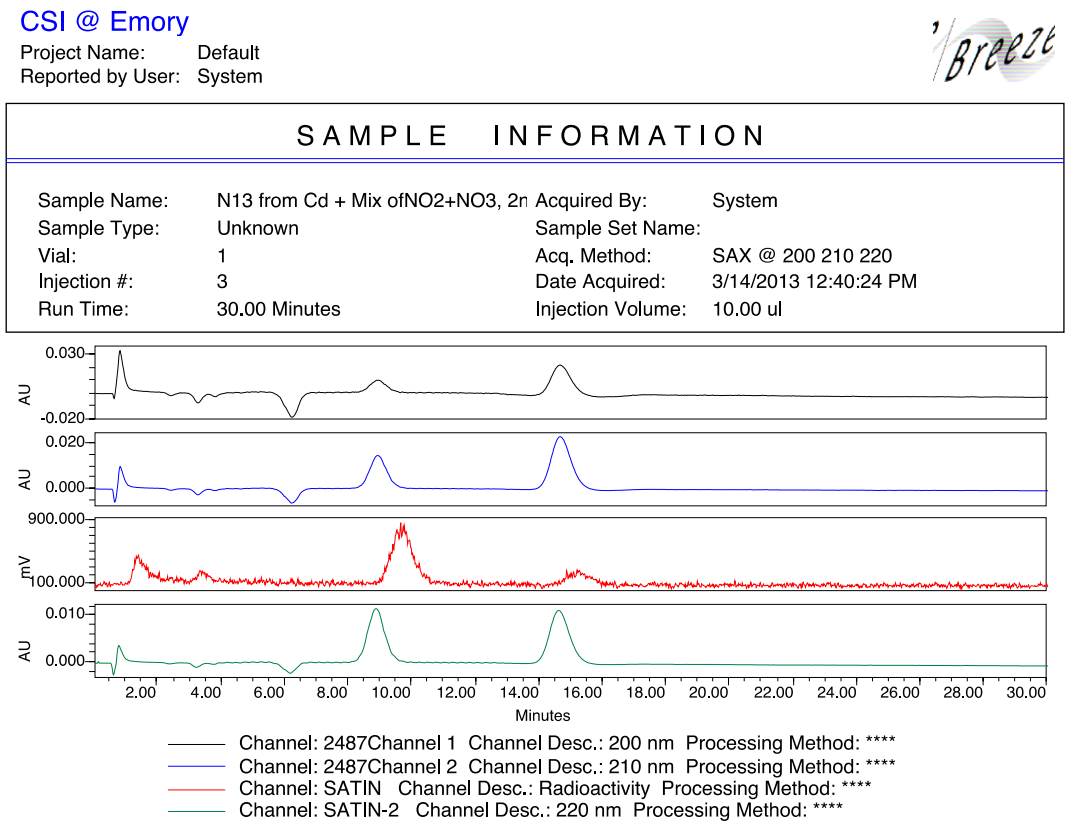
Sample Name:	AP cyclotron Cold NO2 NO3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method:	SAX @200 210 254 @ 1_0
Injection #:	3	Date Acquired:	5/14/2013 12:53:22 PM
Run Time:	25.00 Minutes	Injection Volume:	25.00 ul



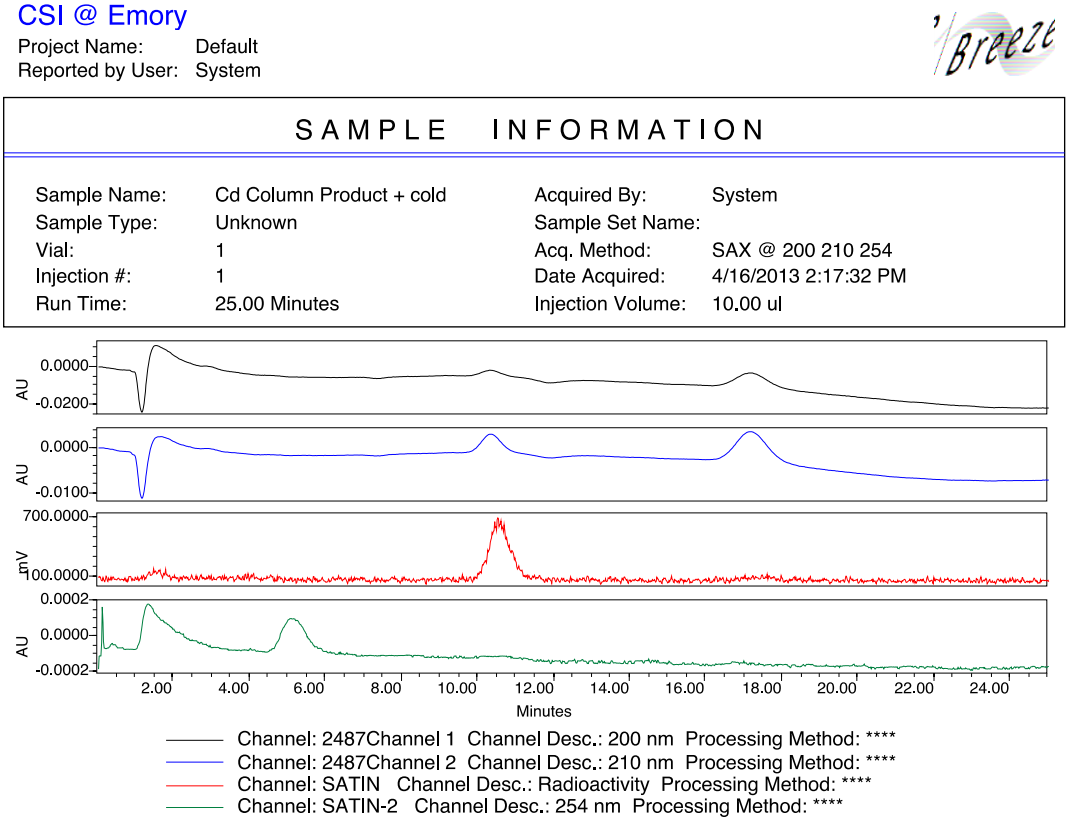
HPLC of the cyclotron product with $NaNO_2$ and $NaNO_3$ standard solutions (Waters IC-Pak Anion, WAT007355, 10 µm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min).



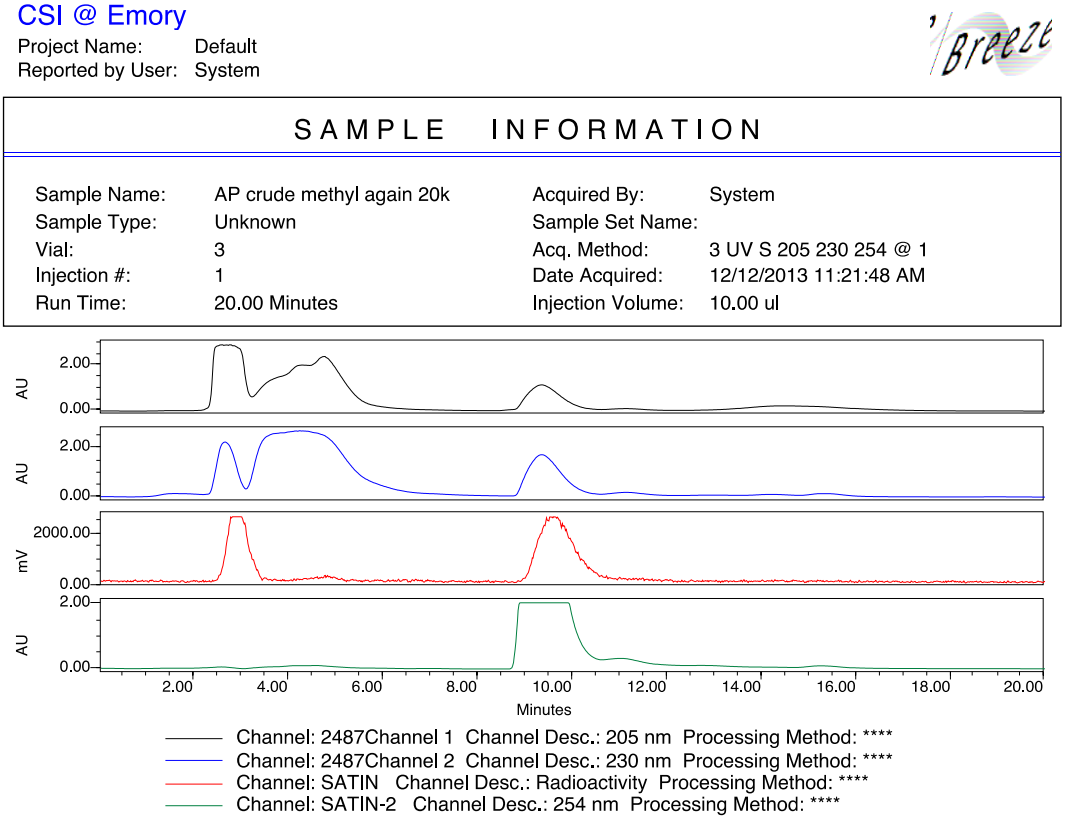
HPLC of the cyclotron product after elution through Alu-B Sep Pak. Sensitivity on the radioactivity channel was increased to show no ^{18}F at 2 min. (Waters IC-Pak Anion, WAT007355, 10 µm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min)



HPLC of the cyclotron product eluted through a Cd reduction column and cold standard $NaNO_2$ and $NaNO_3$, which shows the reduction of $^{13}NO_3$ to $^{13}NO_2$. (Waters IC-Pak Anion, WAT007355, 10 µm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min)



HPLC of the cyclotron product after elution through a cadmium reduction column with the addition of sand (18g cd: 3g sand), and cold standards $NaNO_2$ and $NaNO_3$, which shows increased reduction efficiency with sand. (Waters IC-Pak Anion, WAT007355, 10 µm, 4.6 x 50 mm; mobile phase: H_2O; flow rate: 1.0 mL/min)



HPLC of the ^{13}N crude reaction mixture before Sep Pak purification. (Waters Atlantis T3 3µm, 4.6 x 150 mm; mobile phase: deionized water; flow rate: 1.0 mL/min; T_R = 10 min).

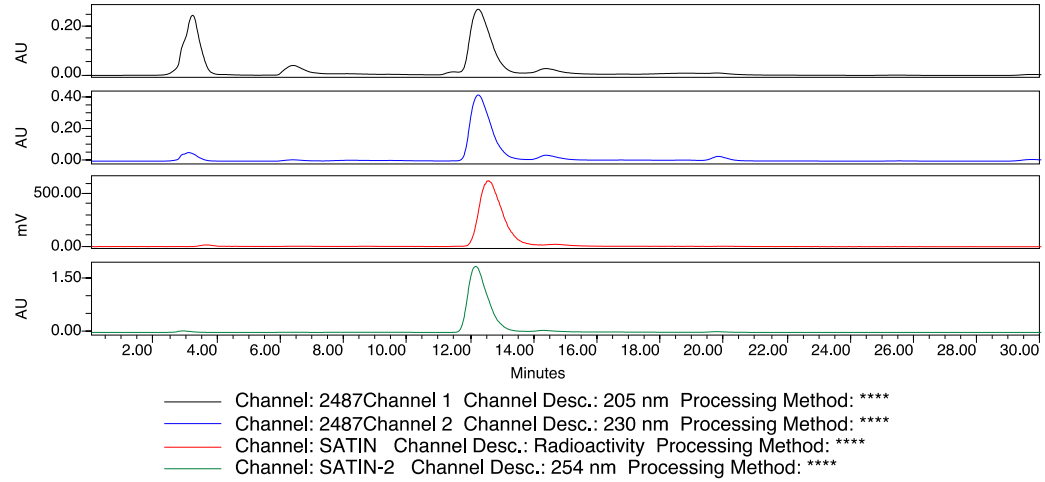
CSI @ Emory

Project Name: WW_248_2014

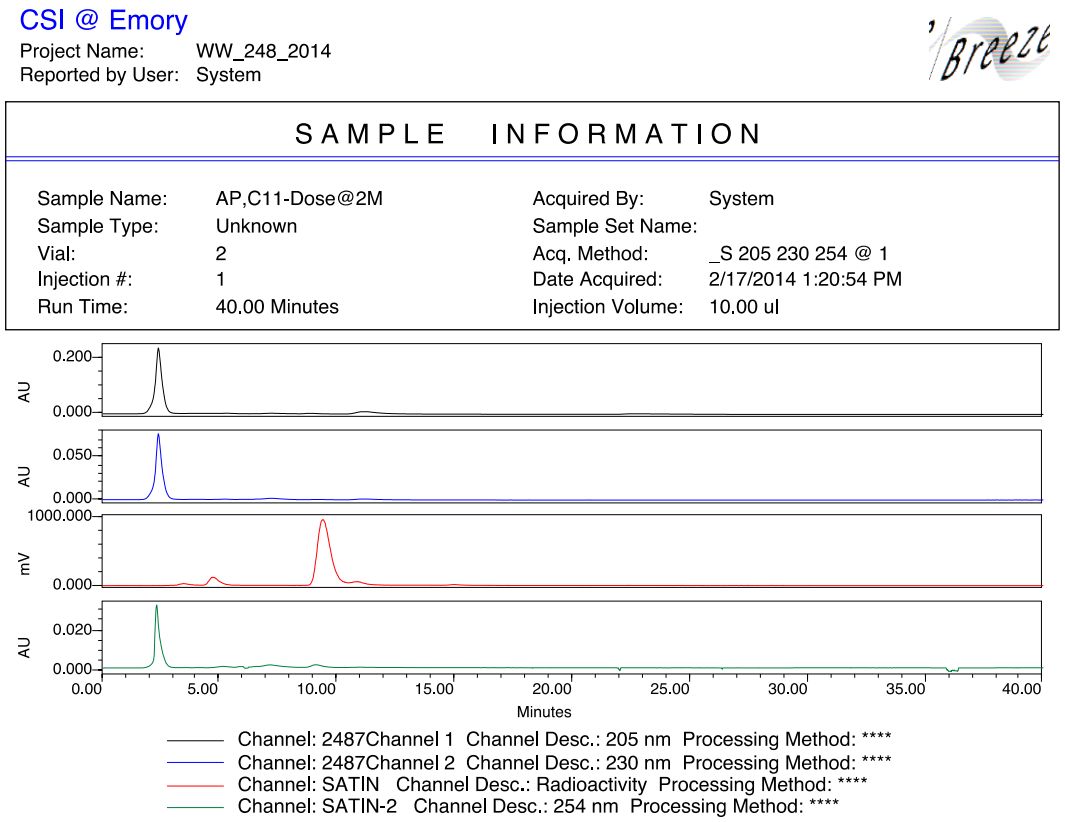
Reported by User: System

SAMPLE INFORMATION

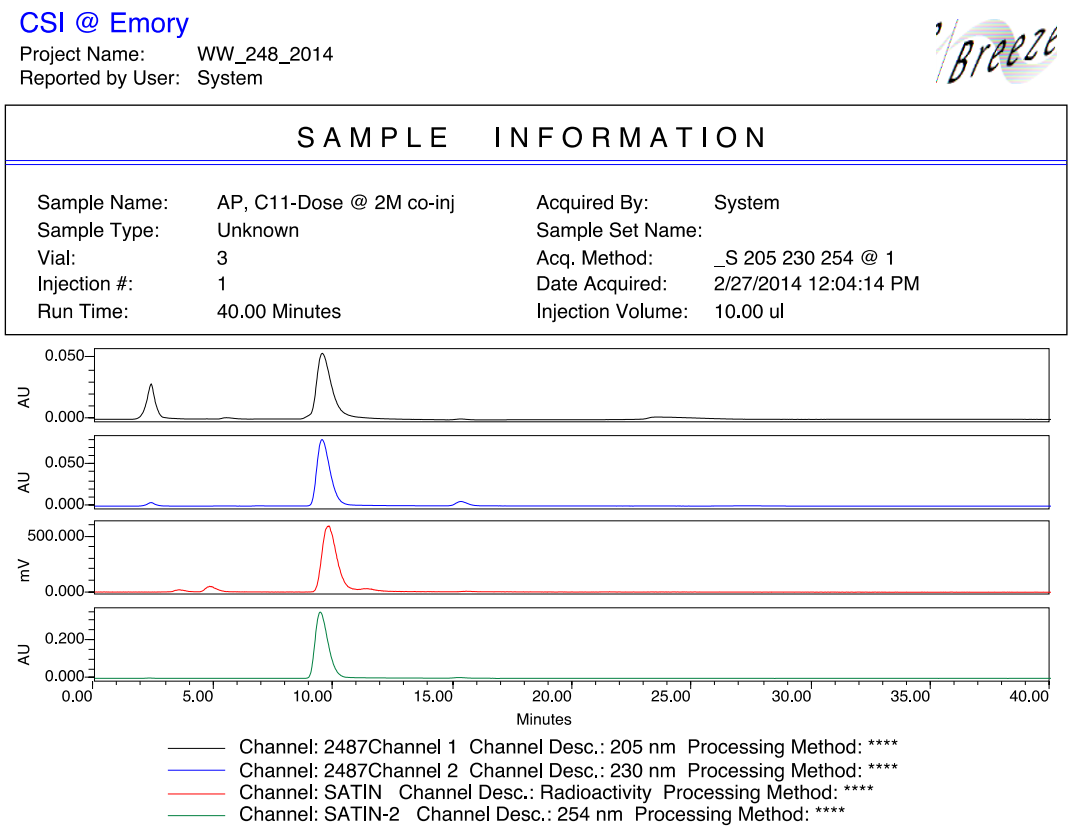
Sample Name:	AP, Dose @ 2M	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	3	Acq. Method:	_S 205 230 254 @ 1
Injection #:	1	Date Acquired:	2/5/2014 12:19:30 PM
Run Time:	30.00 Minutes	Injection Volume:	10.00 ul



HPLC of the carrier-added ^{13}N PRG150 purified product. (Waters Atlantis T3 3µm, 4.6 x 150 mm; mobile phase: deionized water; flow rate: 1.0 mL/min; T_R = 10 min).



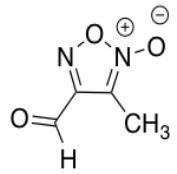
HPLC of the no-carrier-added ^{11}C PRG150 purified product. (Waters Atlantis T3 3µm, 4.6 x 150 mm; mobile phase: deionized water; flow rate: 1.0 mL/min; T_R = 10 min).

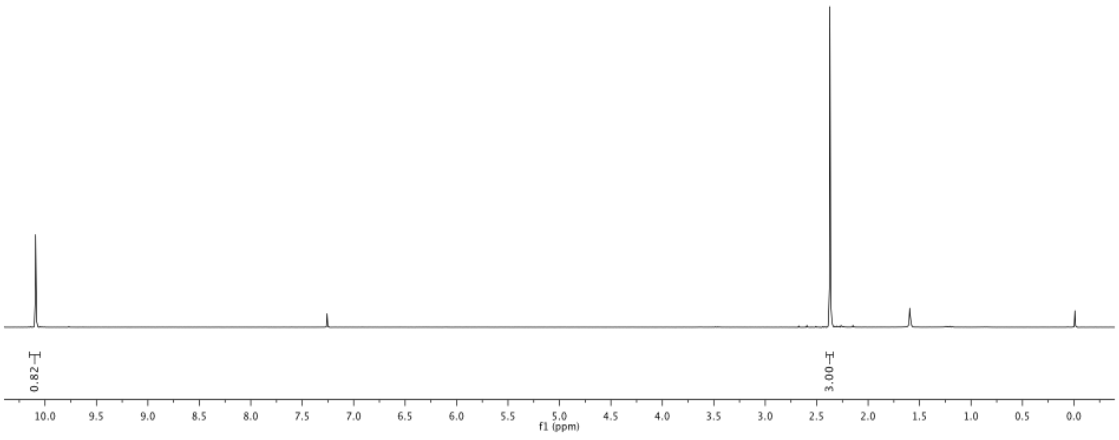


HPLC of the no-carrier-added ^{11}C PRG150 purified product spiked with cold standard PRG150. (Waters Atlantis T3 3µm, 4.6 x 150 mm; mobile phase: deionized water; flow rate: 1.0 mL/min; T_R = 10 min).

NMR Spectra

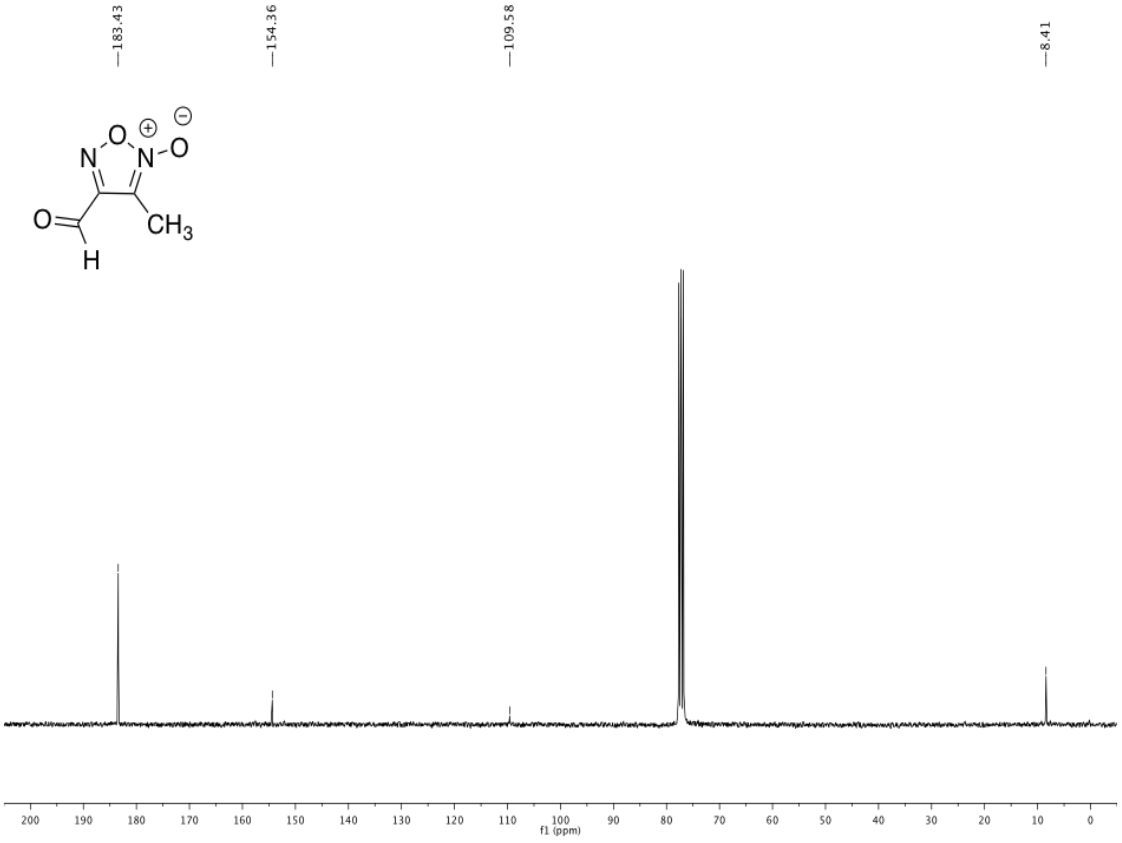
Compound **1**
^{1}H NMR, 300 MHz, $CDCl_3$ (with 0.03% v/v TMS)

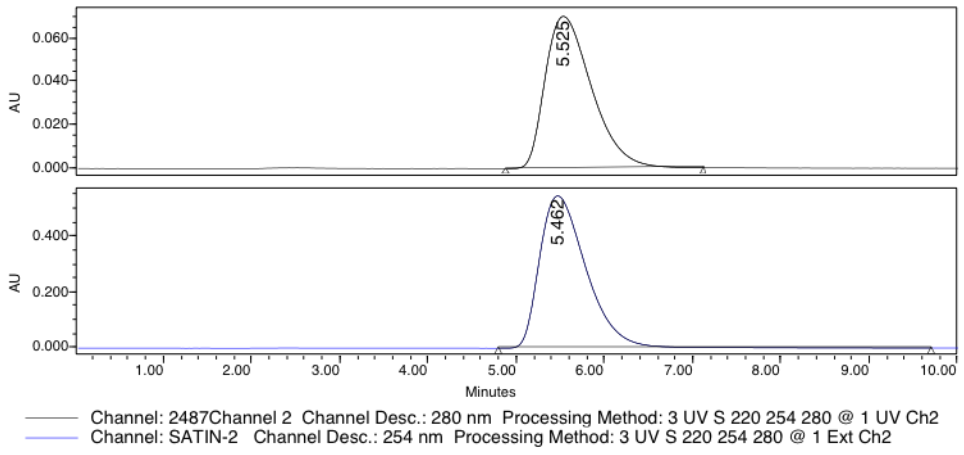




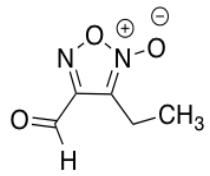
Compound **1**

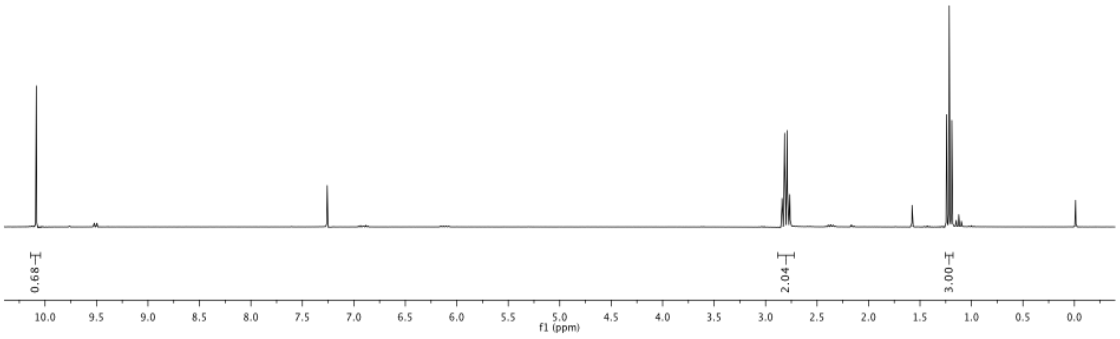
^{13}C NMR, 75 MHz, $CDCl_3$ (with 0.03% v/v TMS)





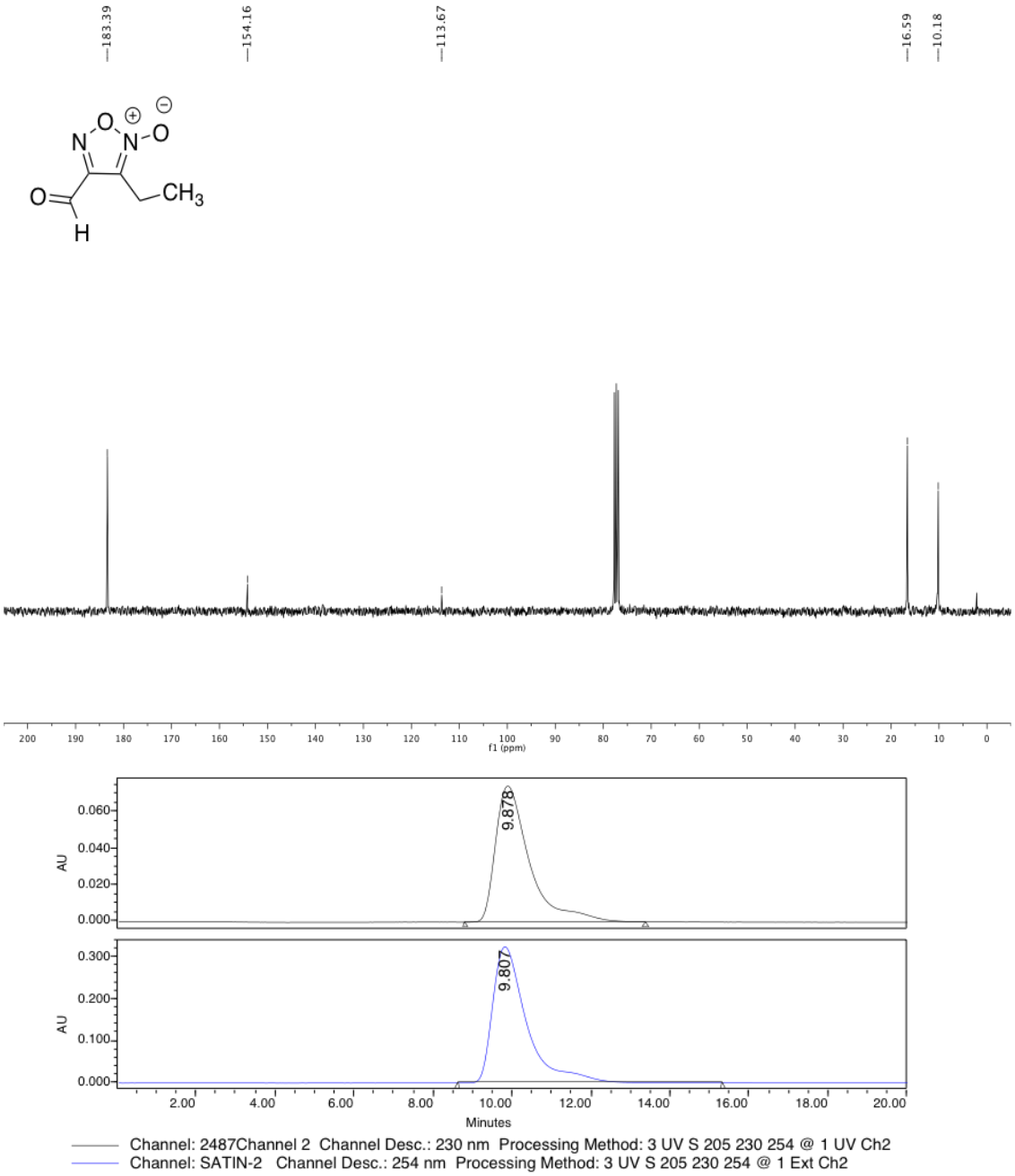
Compound **2**
^{1}H NMR, 300 MHz, $CDCl_3$ (with 0.03% v/v TMS)



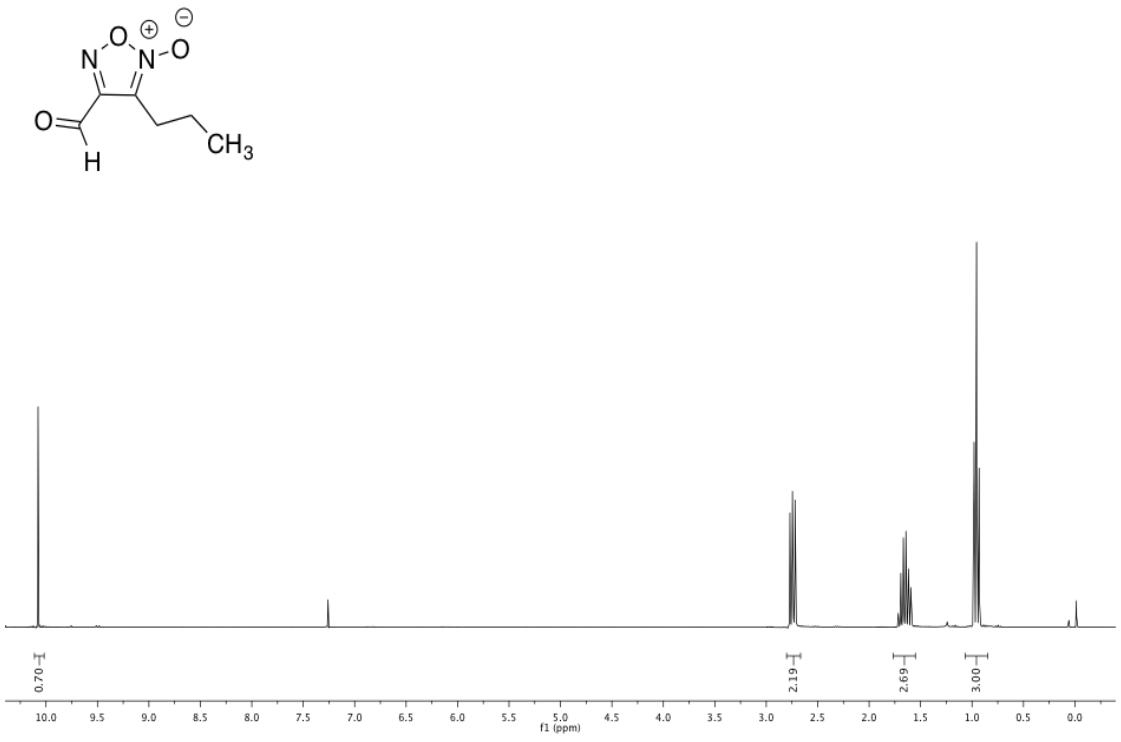


Compound **2**

^{13}C NMR, 75 MHz, $CDCl_3$ (with 0.03% v/v TMS)

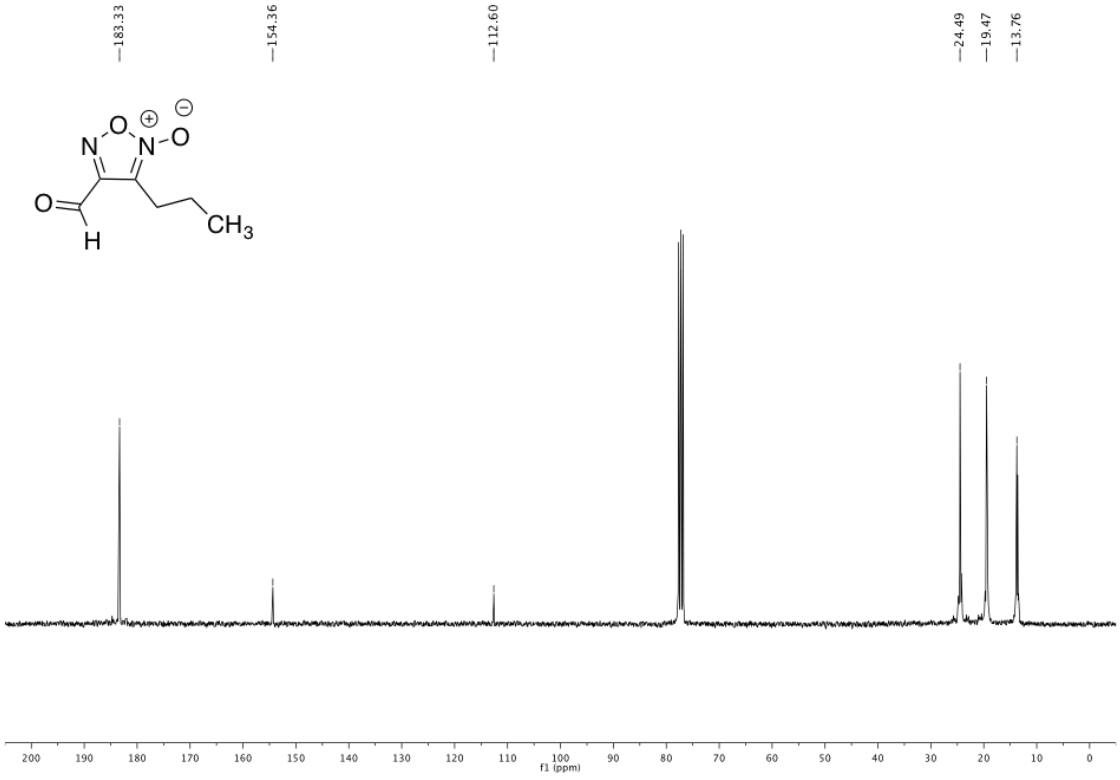


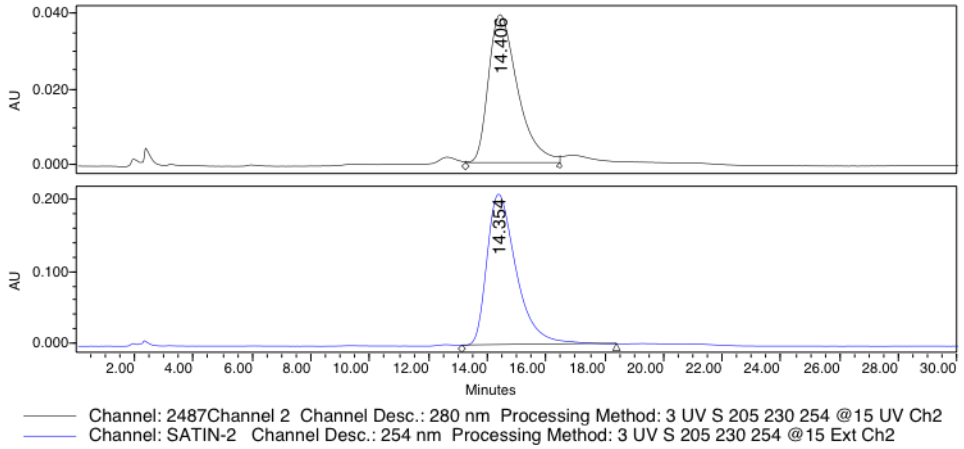
Compound **3**
^{1}H NMR, 300 MHz, $CDCl_3$ (with 0.03% v/v TMS)



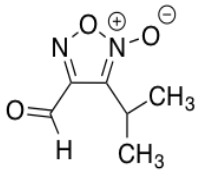
Compound **3**

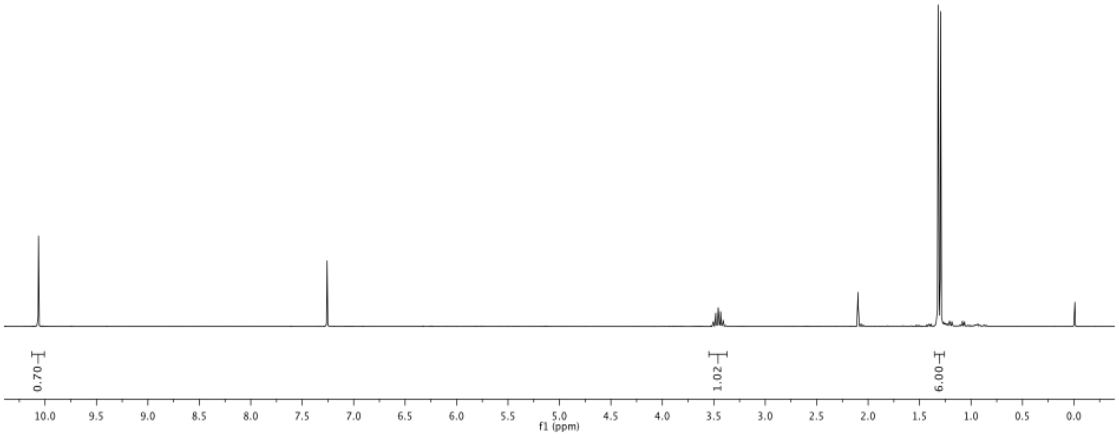
^{13}C NMR, 75 MHz, $CDCl_3$ (with 0.03% v/v TMS)





Compound **4**
^{1}H NMR, 300 MHz, $CDCl_3$ (with 0.03% v/v TMS)





Compound **4**

^{13}C NMR, 75 MHz, $CDCl_3$ (with 0.03% v/v TMS)