Supporting Information

Radiochemical Synthesis and Evaluation of ^{13}N-Labeled 5-Aminolevulinic Acid for PET Imaging of Glioma

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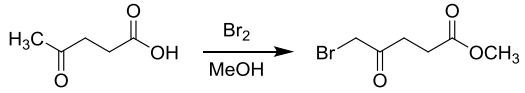
General Methods

All commercial reagents and anhydrous solvents were used without further purification unless otherwise specified. Levulinic acid and bromine were purchased from Sigma Aldrich. 5-Aminolevulinic acid hydrochloride was purchased from Oakwood Chemicals. Compounds **2** was synthesized following the published procedures[31, 35]. Analytical HPLC experiments were performed with a Waters Breeze HPLC system equipped with a Bioscan flow-count radioactivity detector and a in line UV detector set to monitor wavelengths 210 nm, 230 nm, and 254 nm (Waters Atlantis HILIC, Part No. 186002032, 5μm, 4.6 x 150 mm; mobile phase: acetonitrile/methanol/30 mM ammonium acetate (70:10:20); flow rate: 1.0 mL/min; T_R = 5.8 min). ^{1}H NMR (300 MHz) and ^{11}C NMR (75 MHz) spectra were recorded by means of a Mercury 300 plus. Radio thin layer chromatography (radioTLC) was scanned using a Rita Star (Raytest, Straubenhardt, Germany) and performed on silica gel (250 μM layer, aluminum backed, Whatman Ltd, Kent, England) using a mixture of acetonitrile/methanol/water/acetic acid (40:10:10:2) as a mobile phase.

Syntheses Procedures

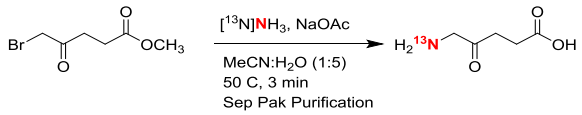
Synthesis of Methyl 5-bromolevulinate



Levulinic acid (5.8 g, 49.95 mmol) was placed in methanol (50 mL) and cooled with an ice-bath. Bromine (8.0 g, 50.0 mmol) was added dropwise over 20 minutes. Once the addition was completed, the ice-bath was removed and the reaction was stirred at ambient temperature for 2.5 hrs. The reaction was then refluxed for 1 hr. After cooling to room temperature, the solvent was removed via rotovap. Diethyl ether (50 mL) and water (10 mL) were added and the mixture was stirred until the oil was dissolved. Concentrated aqueous sodium bicarbonate (3 mL) was added to quench the remaining acid. The mixture was poured into a separatory funnel and the aqueous layer was removed. The ether layer was washed with concentrated aqueous sodium bicarbonate (10 mL) and then dried over sodium sulfate. After filtering away the solid, the ether layer was concentrated to give a yellow oil (7. 36 g). The oil was purified using silica gel column chromatography using a gradient of ethyl acetate/hexanes (5-20%) as an eluent to provide methyl 5-bromolevulinate as colorless oil.

Methyl 5-bromolevulinate (2) [31]**:** (30%, colorless oil, bp: 80 ºC lit.) ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 2 H), 3.68 (s, 1H), 2.96 (t, J = 6.4 Hz, 2 H), 2.65 (t, J = 6.4 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.9, 173.0, 52.2, 34.3, 28.3. HRMS (APCI+) m/z: $[M + H]^+$ calcd for $C_6H_9BrO_3$ 208.9808 Found 208.9808

Radiochemical Synthesis of [^{13}N] 5-ALA



No-carrier-added [^{13}N] ammonia was produced through a 30 minute bombardment at 60 μA of a target containing water/absolute ethanol (0.25% v/v, ABX) by a Siemens 11 MeV RDS 111 cyclotron (Knoxville, TN, USA). The cyclotron delivered the radioactivity for 4 minutes at a rate of 5 mL/min (20 mL) through a QMA Sep Pak (Waters, Accell QMA, Part No. WAT020545, 360 mg sorbent, chloride form) and a 3-way valve (B. Braun Medical Inc., Discofix 4-way stopcock, Part No. 456020) connecting a CM Sep Pak (Waters, Accell Plus CM, Part No. WAT020550, 360 mg sorbent, sodium form) into a 30 mL vial. Once the delivery was complete, the radioactivity was eluted off of the CM Sep Pak with sodium acetate (1.0 mL, 3 M) into a 3 mL v-vial (Kimble Chase, crimp-top, Part No. 60720-3) containing methyl 5-bromolevulinate (8 mg) in acetonitrile (250 μL) and the vial was measured for activity (typically ~250 mCi/9.25 GBq). The vial was placed in a heating block set to 50 ºC for 3 minutes. After heating, the vial was measured for activity (~210 mCi/7.7 GBq) and then purged with argon into a vent bag for 2 minutes to remove any unreacted [^{13}N] ammonia. The radiotracer was purified by vacuum transfer of the reaction mixture through 2 Sep Paks in tandem (tC18 Plus, Waters, Part No. WAT036800, 900 mg sorbent, and Oasis Plus HLB, Waters, Part No. 186000132) into a 3 mL v-vial. The Sep Paks were then washed with monosodium phosphate (3.0 mL, 0.2 M) into a 5 mL v-vial. The 5 mL vial was measured for activity (~100

mCi/3.7 GBq) and then pressure filtered through a 0.2 μm filter (Acrodisc CR 13 mm, Life Sciences) into a 10 mL sterile vial containing 6 mL of saline (0.9%). The sterile vial was measured for activity (~90 mCi/3.3 GBq) and then used for microPET imaging experiments.

Animal Experiments

All animal experiments were carried out under humane conditions and with the approval of the Division of Animal Resources (DAR) in accordance with the Institutional Animal Care and Use Committee (IUCAC) and Radiation Safety Committee (Emory University, Atlanta, GA). Animal husbandry followed all the regulations of the Division of Animal Resources.

Tumor Implantation

Rat 9L gliosarcoma cells were implanted into the brains of male Fischer rats (160-180g) as described previously.[1,2] Briefly, anesthetized rats placed in a stereotactic head holder were injected with a suspension of 4 X 10^4 rat 9L gliosarcoma cells (1 X 10^7 per mL) in a location 3 mm right of midline and 1 mm anterior to the bregma at a depth of 5 mm deep to the outer table. The injection was performed over the course of 2 minutes, and the needle was withdrawn slowly over the course of 1 minute to minimize the backflow of cells. The burr hole and scalp incision were closed, and the animals were returned to their original colony after recovering from the procedure. Intracranial tumors developed that produced weight loss, apathy and

hunched posture in the tumor-bearing rats, and the animals were used at 12-14 days after implantation.

Anesthesia

Rats were anesthetized using isoflurane gas. Anesthesia was initiated 10 minutes 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 the Radiotracer

The tracer was administered via a catheter placed in the tail vein prior to imaging experiments. The catheter was filled with isotonic sodium chloride solution prior to injection of the tracer. The radiotracer was diluted with saline to a final volume of 0.8 mL.

General Biodistribution Studies

Biodistribution studies were performed in male Fischer Rats (160-180 g) bearing 9L intracranial tumors. The rats were injected through the tail vein catheter with 300 – 400 μCi (0.0111 - 0.0148 GBq) of a high specific activity (> 1 Ci/μmol) of [^{13}N] 5-ALA in 0.8 mL of isotonic saline (pH = 6).

PET Imaging

MicroPET 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 minutes 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 fifteen 1 minute frames followed by nine 5 minute 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

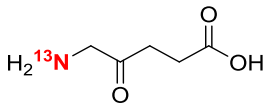
MicroPET data and CT data were co-registered using ASIPro. The CT template was used for definition of regions-of-interest (ROIs). The regions-of-interest were drawn around the tumor in the right hemisphere of the brain and compared to the symmetrical contralateral region in the left hemisphere. The time-activity 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).

Histology Staining and Optical Imaging

The rats were sacrificed right after the PET-CT scan. Whole brains were immediately collected and placed in an acrylic brain matrix mold (Stoelting, Part no. 51382) with 1 mm thickness before being snap-frozen with optimal cutting temperature (OCT) compound (Tissue-Teck®, VWR) in liquid nitrogen. The brain slices, including those bearing tumor, were cut with 1 mm thickness. The distribution of the tumor was determined by fluorescent imaging of the OCT-embedded slices using IVIS® Spectrum Series imaging system (PerkinElmer) with the excitation wavelength at 430 nm and emission wavelength at 620 nm. Afterwards, the OCT-embedded tissue sections were stained with hematoxylin and eosin (H&E) staining solutions to identify the tumor region. Briefly, the slices were sectioned to 7 μm thickness using microtome cryostat and fixed by acetone for five minutes on a glass microscope slide (Fisher Scientific). The slice was then rinsed by immersing into phosphate buffered saline (PBS) for five minutes. Hematoxylin 2 solution (400 μL, Richard-Allan Scientific, Cat No. 7231) was used to submerge the slice for two minutes to stain the cell nuclei. The glass slide was then immersed into PBS and 190 proof ethanol for 30 seconds, respectively, before the slice was counterstained by eosin Y solution (400 μL, Richard-Allan Scientific, Cat No. 7111) for 30 seconds. The staining was differentiated by dipping the slide in an acidic alcohol (1% (v/v) conc. HCl in 70% ethanol). Dehydration of H&E stained slice was carried out by dipping in ethanol (95%), ethanol (100%), and xylene (two times, two minutes each time), respectively.

HPLC data

Description of HPLC Systems



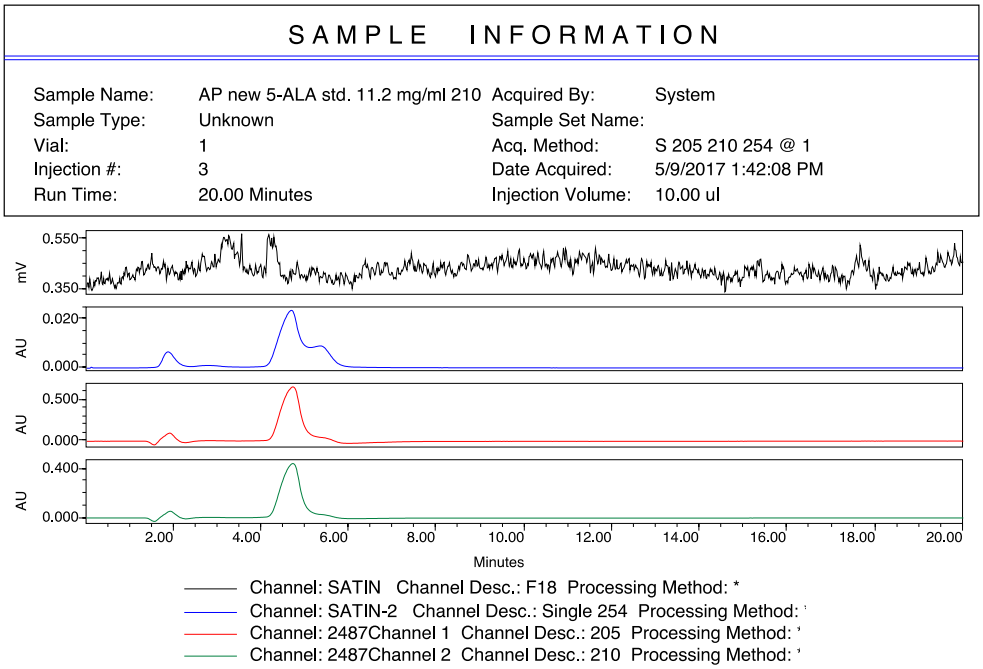
1
[^{13}N] 5-ALA

Compound	HPLC
1	5.3 min[a]
1	12.5 min[b]

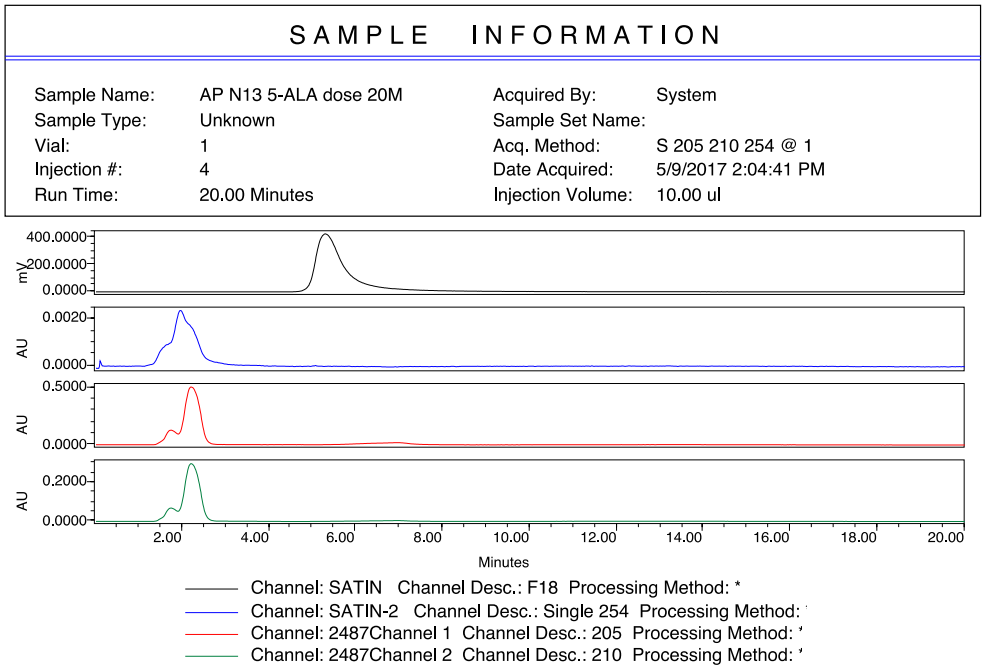
[a]HPLC system 1 was a Waters Atlantis HILIC (Waters, 186002032, 5µm, 4.6 x 150 mm) eluted with a mixture of acetonitrile/methanol/ammonium acetate (30 mM) (70:10:20) as the mobile phase. The flow rate was 1 ml/min.

[b]HPLC system 2 was a Waters Atlantis HILIC (Waters, 186002032, 5µm, 4.6 x 150 mm) eluted with a mixture of acetonitrile/ammonium acetate (30 mM) (80:20) as the mobile phase. The flow rate was 2 ml/min. This system gave better resolution but peak shape was poor and the retention time was inadequate for quality control of the short lived radiotracer **1**.

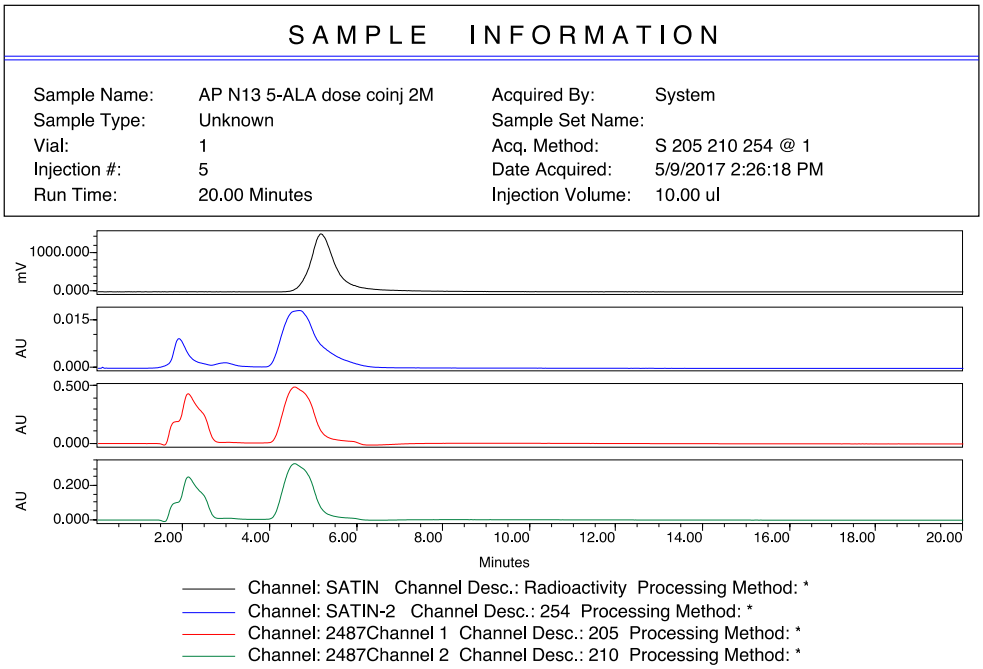
HPLC Chromatograms



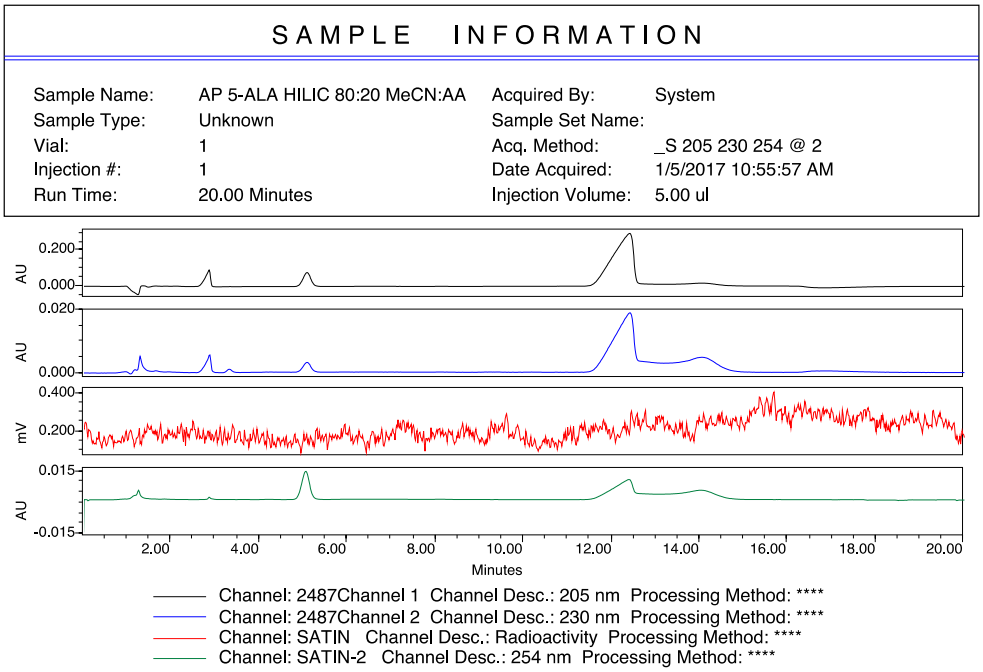
HPLC of 5-ALA HCl standard (11.2 mg/ml) (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ methanol/ ammonium acetate (30 mM) 70:10:20; flow rate: 1.0 mL/min). Order of chromatographs: 1 = radioactivity, 2 = 254 nm, 3 = 205 nm, 4 = 210 nm.



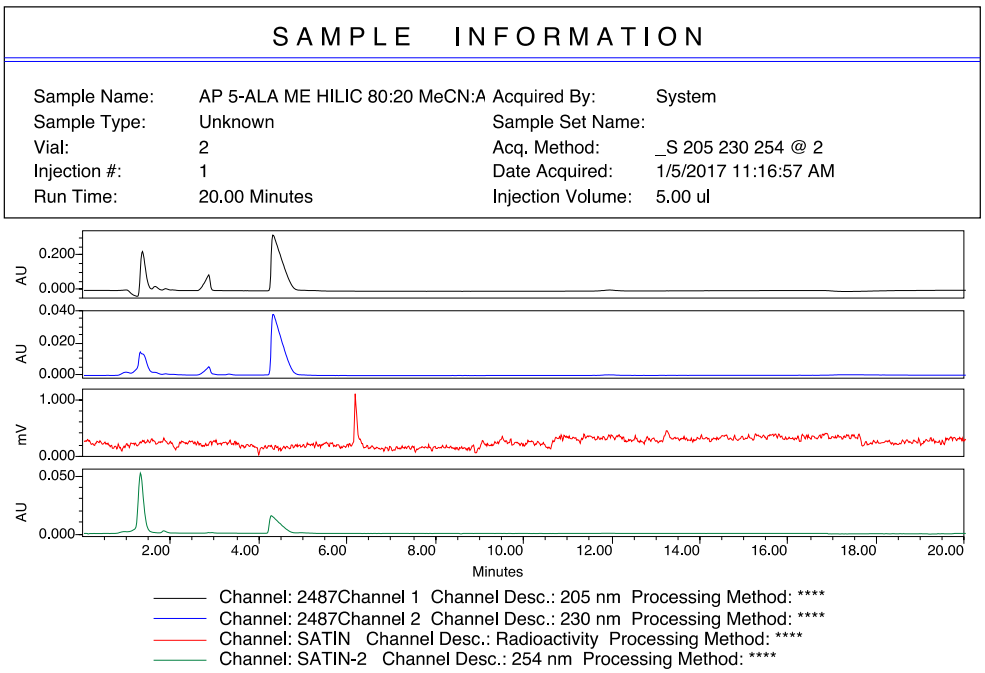
HPLC of [^{13}N] 5-ALA dose (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ methanol/ ammonium acetate (30 mM) 70:10:20; flow rate: 1.0 mL/min). Order of chromatographs: 1 = radioactivity, 2 = 254 nm, 3 = 205 nm, 4 = 210 nm.



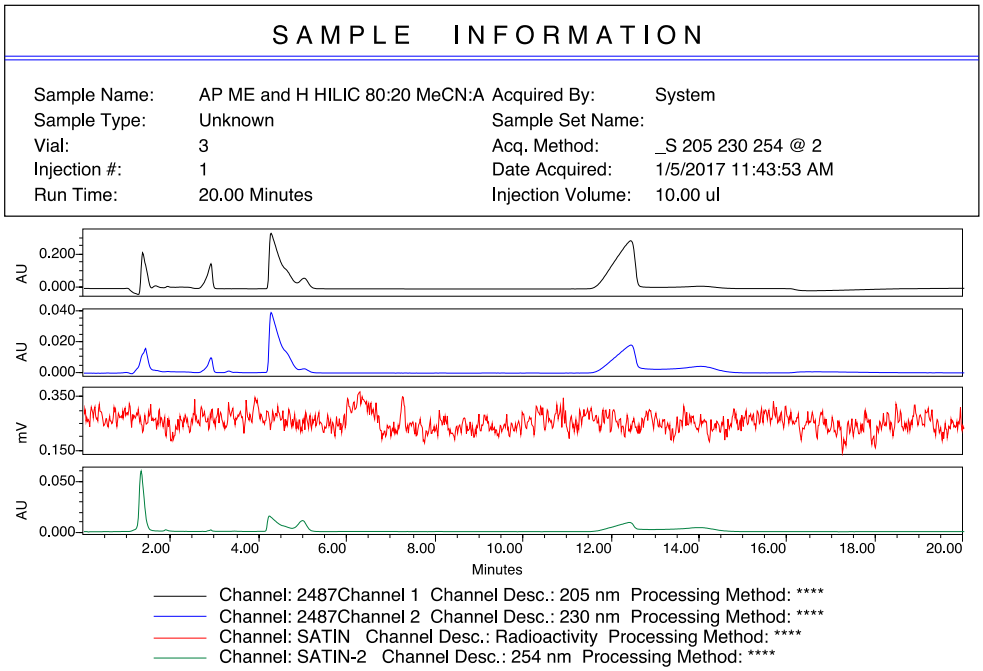
HPLC of [^{13}N] 5-ALA dose co-injected with 5-ALA HCl standard (11.2 mg/ml) (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ methanol/ ammonium acetate (30 mM) 70:10:20; flow rate: 1.0 mL/min). Order of chromatographs: 1 = radioactivity, 2 = 254 nm, 3 = 205 nm, 4 = 210 nm. The Bioscan radioactivity detector is ~30 s after UV detectors.



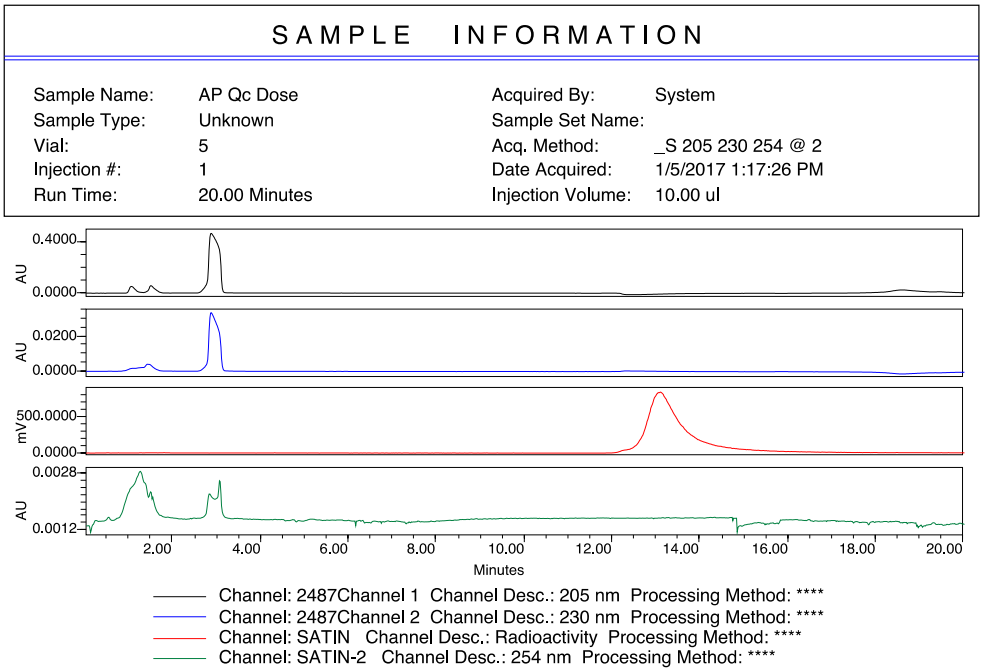
HPLC of 5-ALA HCl standard (10 mg/ml) (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ ammonium acetate (30 mM) 80:20; flow rate: 2.0 mL/min; T_R = 12.5 min). Order of chromatographs: 1 = 205 nm, 2 = 230 nm, 3 = radioactivity, 4 = 254 nm.



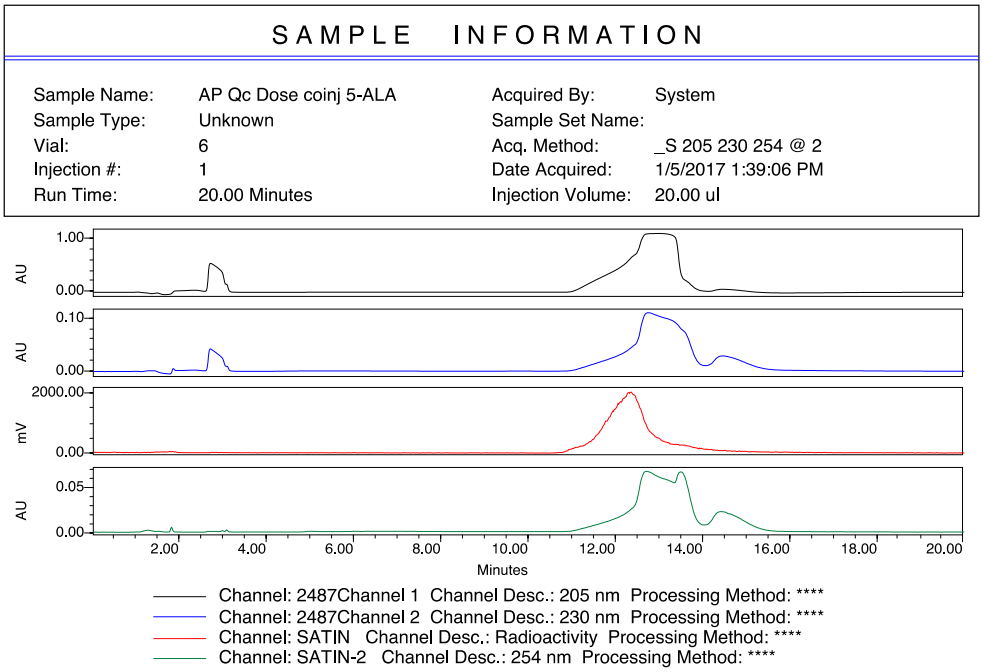
HPLC of 5-ALA methyl ester HCl standard (10 mg/ml) (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ ammonium acetate (30 mM) 80:20; flow rate: 2.0 mL/min; T_R = 4.5 min). Order of chromatographs: 1 = 205 nm, 2 = 230 nm, 3 = radioactivity, 4 = 254 nm.



HPLC of 5-ALA HCl standard (10 mg/ml) co-injected with 5-ALA methyl ester HCl standard (10 mg/ml) (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ ammonium acetate (80:20); flow rate: 2.0 mL/min; 5-ALA ME HCl T_R = 4.5 min 5-ALA HCl T_R = 12.5 min). Order of chromatographs: 1 = 205 nm, 2 = 230 nm, 3 = radioactivity, 4 = 254 nm.



HPLC of [^{13}N] 5-ALA dose (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ ammonium acetate (80:20); flow rate: 2.0 mL/min; T_R = 13 min). Order of chromatographs: 1 = 205 nm, 2 = 230 nm, 3 = radioactivity, 4 = 254 nm.

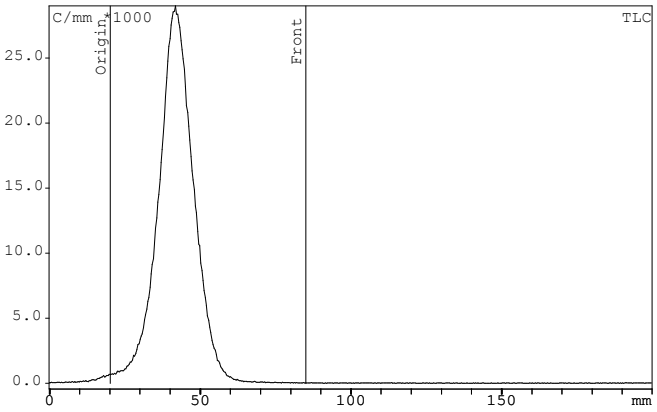


HPLC of [^{13}N] 5-ALA dose co-injected with 5-ALA HCl standard (Waters Atlantis HILIC, Part No. 186002032, 5µm, 4.6 x 150 mm; mobile phase: acetonitrile/ ammonium acetate (80:20); flow rate: 2.0 mL/min; T_R = 13 min). Order of chromatographs: 1 = 205 nm, 2 = 230 nm, 3 = radioactivity, 4 = 254 nm.
Note: Poor peak shape is likely due to the different counter anions, which highly affects peak shape and retention time when using a HILIC column. The 5-ALA standard is in hydrochloride form. The [^{13}N] 5-ALA is in acetate form. (See reference 3).

RadioTLC

[^{13}N] 5-ALA dose

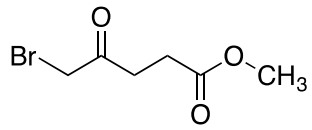
Solvent system: acetonitrile/methanol/water/acetic acid (40:10:10:2)

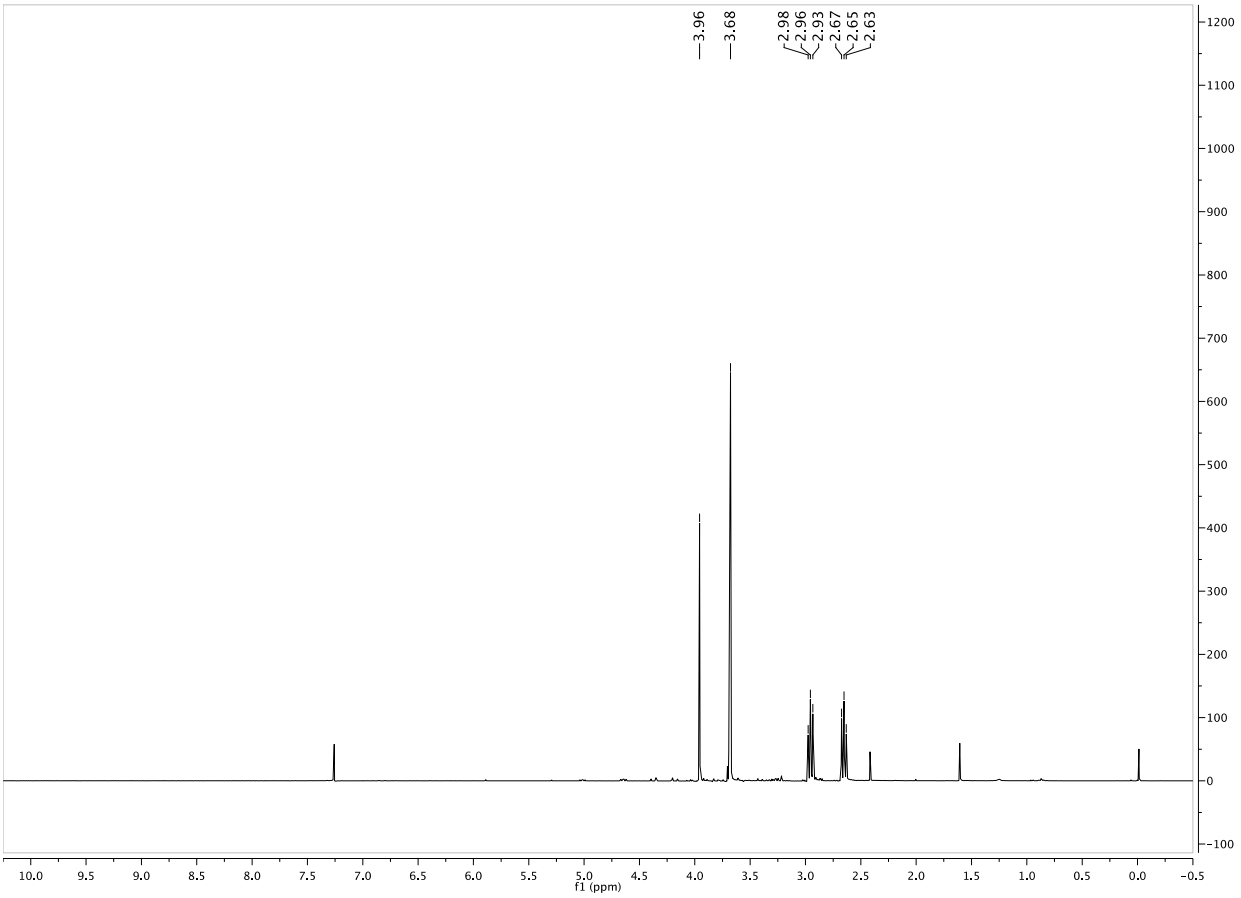


NMR Spectra

Compound **2**

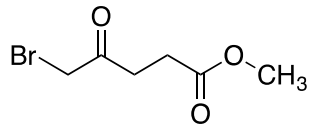
^{1}H NMR, 300 MHz, CDCl3 (with 0.03% v/v TMS)

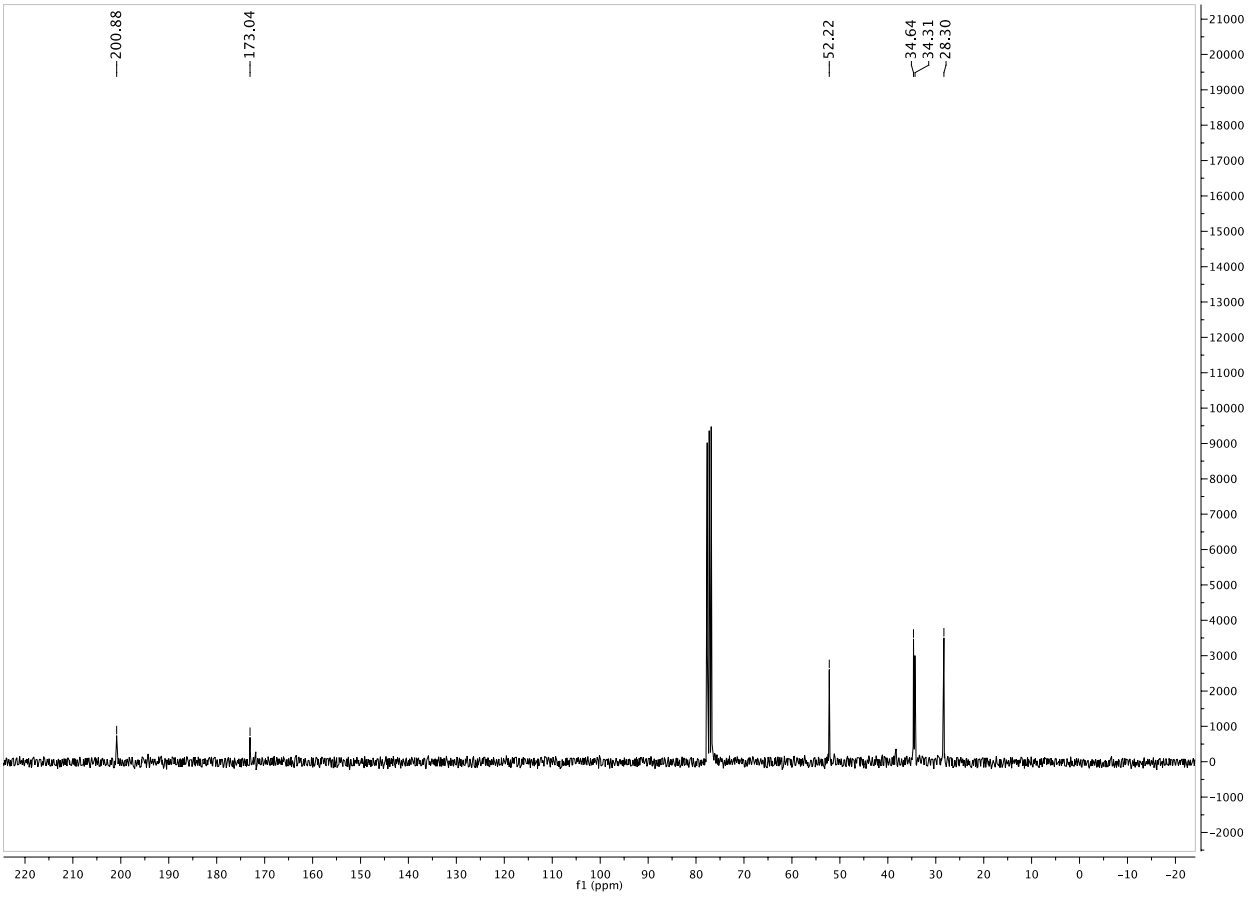




Compound **2**

^{13}C NMR, 75 MHz, CdCl3 (with 0.03% v/v TMS)



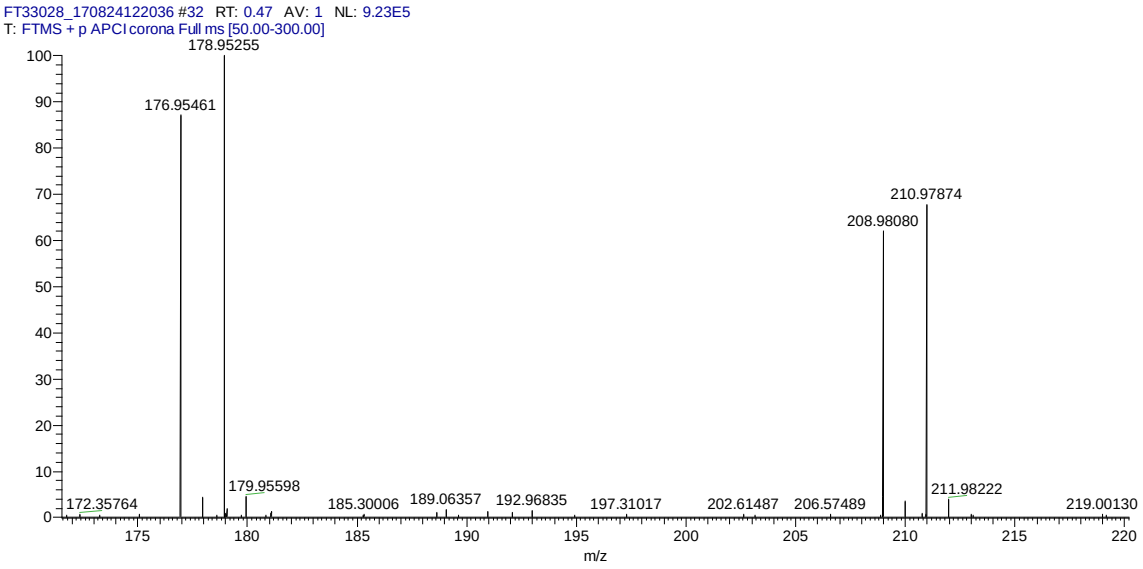


High Resolution Mass Spectrometry

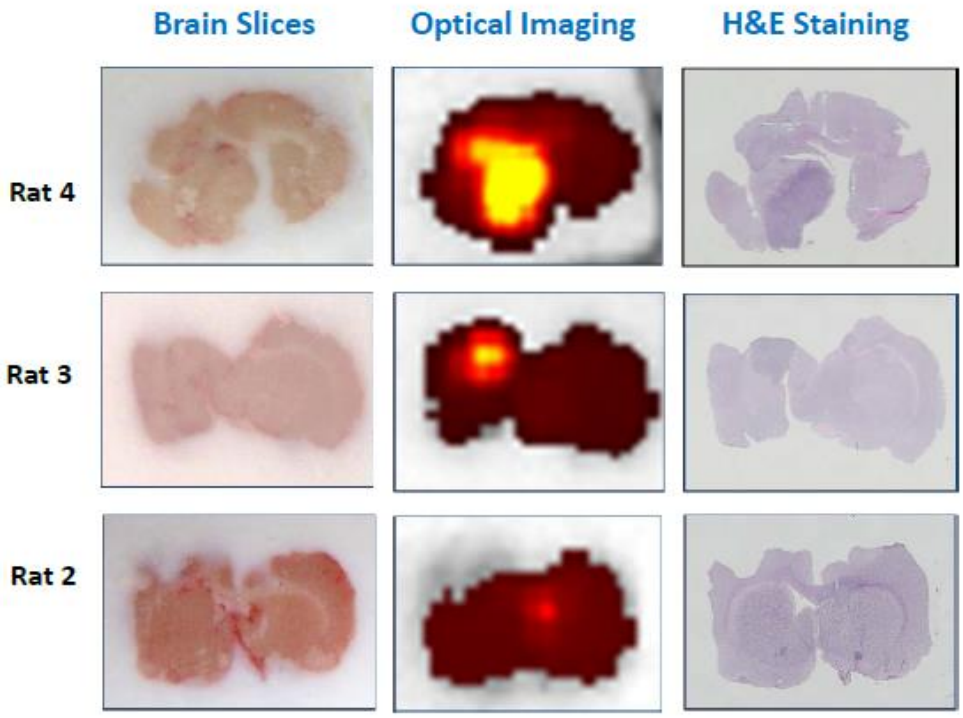
Elemental composition search on mass 208.98080

m/z= 203.98080-213.98080

m/z	Theo. Mass	Delta (mmu)	RDB equiv.	Composition
208.98080	208.98078	0.02	1.5	$C_6H_{10}O_3Br$



Supplemental Images



References

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