# SUPPORTING INFORMATION

## Hyperpolarized [6-$^{13}$C,$^{15}N_3$]-Arginine as a Probe for *in Vivo* Arginase Activity

Andrew Cho[1,2], Roozbeh Eskandari[3], Kristin L. Granlund[3], and Kayvan R. Keshari[1,3,4,*]

[1]Department of Biochemistry & Structural Biology, Weill Cornell Graduate School, New York, New York 10065, United States

[2]Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD-PhD Program, New York, New York 10065, United States

[3]Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, New York 10065, United States

[4]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, New York 10065, United States

## *In vivo* [6-$^{13}$C,$^{15}$N$_3$]-Arginine Tracing Experiment

**Method:**

3 female athymic nude mice, 10–11 weeks old, were used for this experiment. Each mouse was administered 250 μL 25 mM [6-$^{13}$C,$^{15}$N$_3$]-arginine (6250 nmol [6-$^{13}$C,$^{15}$N$_3$]-arginine) in 100 mM Tris, 1 mM EDTA, pH 7.4 *via* intravenous tail-vein injection over the course of 10s. The mouse was subsequently anesthetized with isoflurane and sacrificed *via* cervical dislocation 90s following the end of injection. Blood was collected *via* intracardiac puncture immediately following sacrifice, and the liver was subsequently collected and frozen in liquid nitrogen. Blood samples were set aside at room temperature for 30 mins to allow coagulation, after which they were centrifuged at 14000 RPM, 4°C for 10 min and the serum (supernatant) was collected for metabolite extraction.

In separate Eppendorf tubes, each liver or serum sample was treated with 4% perchloric acid in $H_2O$ (v/v) to precipitate proteins (200 μL perchloric acid solution was added to every 100 mg liver or 100 μL serum). The liver samples were blended with a handheld tissue homogenizer, and liver and serum samples were centrifuged at 14000 RPM, 4°C for 15 min. Every 1 mL of supernatant was subsequently mixed with 2 mL 78:22 chloroform:tri-n-octylamine (v/v), and the mixture was centrifuged at 4000 RPM, 4°C for 15 min. Afterwards, the aqueous layer was collected, frozen, and dried overnight on a lyophilizer. The resulting dried powder was resuspended in 700 μL $D_2O$ containing 1 mM Gd-DOTA and 250 μM [2-$^{13}$C]-glycine as a chemical shift reference and concentration standard. $^{13}$C-NMR spectra were acquired on a 14.1T Bruker NMR spectrometer. [$^{13}$C,$^{15}$N$_2$]-urea and [6-$^{13}$C,$^{15}$N$_3$]-arginine concentrations in each sample were calculated by comparing the corresponding peak integral to that of the glycine standard. [$^{13}$C,$^{15}$N$_2$]-urea and [6-$^{13}$C,$^{15}$N$_3$]-arginine concentration values for each mouse were

normalized to sample weight, averaged, and reported per 1 g liver or 1 mL serum, which is the approximate total liver mass and serum volume in these mice. Values are reported in nanomoles and as a percentage of the total injected moles of [6-$^{13}$C,$^{15}$N$_3$]-arginine.

**Results:**

[$^{13}$C,$^{15}$N$_2$]-urea per 1 g liver: 94.6 nmol ± 36.7 nmol, 1.51% ± 0.59% of injected dose

[6-$^{13}$C,$^{15}$N$_3$]-arginine per 1 g liver: Not Detected

[$^{13}$C,$^{15}$N$_2$]-urea per 1 mL serum: 78.7 nmol ± 8.8 nmol, 1.26% ± 0.14% of injected dose

[6-$^{13}$C,$^{15}$N$_3$]-arginine per 1 mL serum: 154.8 nmol ± 21.9 nmol, 2.48% ± 0.35% of injected dose

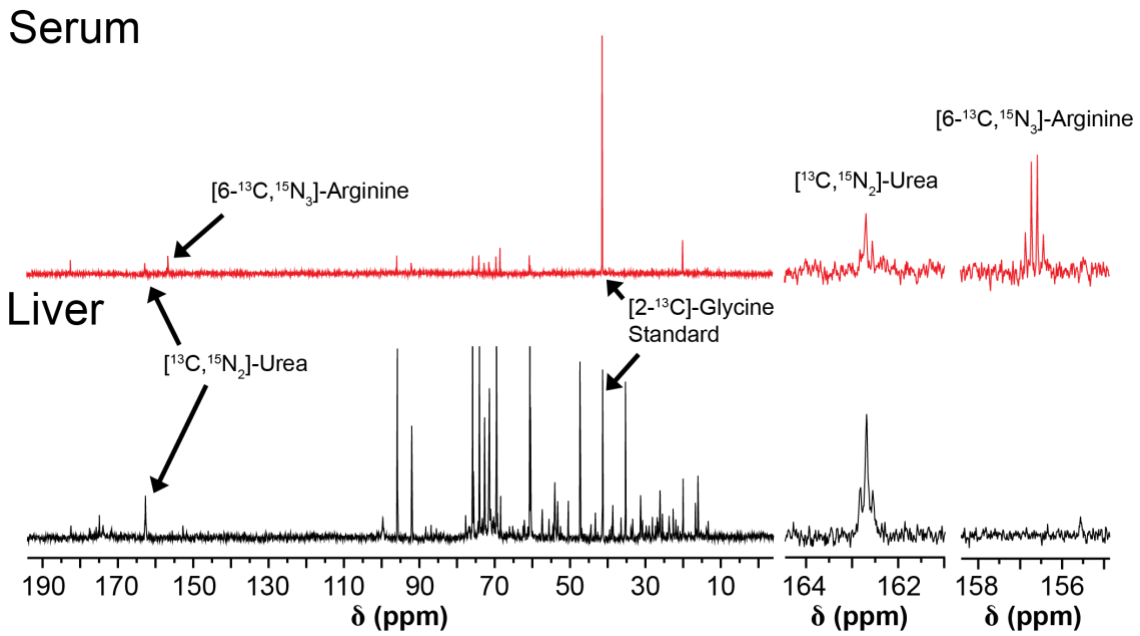



**Figure S1:** $^{13}$C-NMR of serum and liver metabolite extracts from Mouse 1 (left), with the carbon resonance of [$^{13}$C,$^{15}$N$_2$]-urea, carbon-6 resonance of [6-$^{13}$C,$^{15}$N$_3$]-arginine, and carbon-2 resonance of [2-$^{13}$C]-glycine indicated with arrows. The chemical shift range corresponding to urea (~163 ppm) and the carbon-6 resonance of arginine (~157 ppm) have been enlarged and are shown on the right.

## Synthesis of [6-$^{13}$C,$^{15}$N$_3$]-Arginine

**General Synthetic and Analytical Methods.** [$^{13}$C,$^{15}$N$_2$]-thiourea and potassium [$^{15}$N]-cyanide were purchased from Cambridge Isotope Laboratories, N-tert-Boc-L-aspartic acid tert-butyl ester (**compound 4**) was purchased from Carbosynth, and all other reagents were purchased from Sigma-Aldrich. All reagents were used without further purification. 230-400 mesh, grade 60 silica gel (Fisher Chemical) was used for column chromatography and MilliporeSigma 60 F254, 0.25 mm thickness, glass thin layer chromatography (TLC) plates (Thomas Scientific) were used for analytical TLC. TLC plates were visualized by fluorescence quenching under UV light or staining with vanillin or ninhydrin. $^1$H- and $^{13}$C-NMR for characterization of all intermediates and the final product were acquired in $CDCl_3$ or $D_2O$ (Cambridge Isotope Laboratories) at 25°C on a Bruker Avance III 600 MHz Spectrometer at the Memorial Sloan Kettering Nuclear Magnetic Resonance Core Facility. All chemical shift values are reported in parts per million ($\delta$). The residual proton signals of $CDCl_3$ or $D_2O$ and the carbon signals of $CDCl_3$ or MeOH were used as a chemical shift reference. Splitting patterns are labeled as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; ddd, doublet of doublets of doublets. The gray chemical shift value in the $^{13}$C-spectrum of **compounds 2** and **9** corresponds to the MeOH reference, while all other gray values correspond to unidentified impurities. Electrospray ionization high-resolution mass spectroscopy (ESI-HRMS) was performed on a Waters LCT Premier Time-of-Flight Mass Spectrometer at the Memorial Sloan Kettering Nuclear Magnetic Resonance Core Facility.

**Synthetic Methods**

**2-methylisothiouronium-$^{13}C$-$^{15}N_2$ iodide (2)**

In an oven-dried round bottom flask equipped with a magnetic stir bar, 2.5 g [$^{13}C$,$^{15}N_2$]-thiourea (31.6 mmol, 1 equiv.) was dissolved in 100 mL MeOH. To this, 5.15 g iodomethane (36.3 mmol, 1.15 equiv.) was added and this reaction mixture was stirred at reflux for 3 hours. The reaction mixture was cooled to room temperature and concentrated under vacuum to give pure **compound 2** as a white solid in 97% yield (6.7 g). $^1$H-NMR (600 MHz, $D_2O$): $\delta$ = 2.58 (d, 3H, $^3J_{H,C}$ = 4.8 Hz) ppm. $^{13}$C-NMR (151 MHz, $D_2O$, MeOH reference): $\delta$ = 173.3 (t, $^1J_{C,N}$ = 19.2 Hz), 13.6 ppm. ESI-HRMS: m/z calculated for $C^{13}CH_7{}^{15}N_2S$ $(M+H)^+$ 94.0304, found 94.0304.

**Methyl *N,N'*-bis(*tert*-butoxycarbonyl)carbamimidothioate-$^{13}C$-$^{15}N_2$ (3)**

An oven dried round bottom flask was equipped with a magnetic stir bar and charged with 4.7 g **compound 2** (21.3 mmol, 1 equiv.), 75 mL DCM, and 71.5 mL saturated $NaHCO_3$ in $H_2O$. To this, 18.6 g di-tert-butyl dicarbonate (85.2 mmol, 4 equiv.) was added and the reaction mixture was stirred at ambient temperature over 5 days. The reaction mixture was diluted with 120 mL 1:1 DCM:$H_2O$ (v/v), the organic phase was collected, and the aqueous phase was extracted with DCM (2 x 100 mL). The pooled organic phase was washed with $H_2O$ (2 x 120 mL) and brine (120 mL), dried with $MgSO_4$, and concentrated under vacuum. Crude product was purified by flash chromatography (3% diethylether in hexanes, v/v) and dried to yield a white solid in 78.5% yield (4.9 g). $^1$H-NMR (600 MHz, $CDCl_3$): $\delta$ = 11.61 (d, 1H, $^1J_{H,N}$ = 90.6 Hz), 2.39 (d, 3H, $^3J_{H,C}$ = 4.8 Hz), 1.52 (s, 9H), 1.50 (s, 9H) ppm. $^{13}$C-NMR (151 MHz, $CDCl_3$): $\delta$ = 171.7–171.4 (m), 161.0–160.7 (m), 151.0–150.7 (m), 83.3, 81.1, 28.1, 14.5 (d, $^2J_{C,C}$ = 4.8 Hz) ppm. ESI-HRMS: m/z calculated for $C_{11}{}^{13}CH_{22}Na^{15}N_2O_4S$ $(M+Na)^+$ 316.1172, found 316.1195.

**(*S*)-4-(*tert*-butoxy)-3-((*tert*-butoxycarbonyl)amino)-4-oxobutanoic acid (5)**

An oven dried 2-neck round bottom flask was equipped with a magnetic stir bar and an argon inlet. The flask was charged with 5 g **compound 4** (17.3 mmol, 1 equiv.) and sealed under an argon atmosphere. 120 mL dry THF and 2.63 g triethylamine (26.0 mmol, 1.5 equiv.) were added to the flask, and the mixture was cooled in an ice-salt bath to between –5 and –10°C. To this, 2.82 g ethyl chloroformate (26.0 mmol, 1.5 equiv.) was added dropwise as the reaction mixture was stirred vigorously. While stirring, the mixture was subsequently removed from the ice bath and allowed to equilibrate to ambient temperature over 30 minutes, after which the reaction mixture was filtered and set aside. Meanwhile a second round bottom flask was equipped with a stir bar and charged with 1.37 g sodium borohydride (36.3 mmol, 2.1 equiv.) and 10 mL $H_2O$. This was cooled in an ice bath and the filtrate from the first reaction was added dropwise while stirring vigorously. After all the filtrate was added, the flask was removed from the ice bath and stirred at ambient temperature for 1 hour. The reaction mixture as washed with saturated $NaHCO_3$ (1 x 100 mL), the organic phase was collected, and the aqueous phase was extracted with EtOAc (3 x 100 mL). All organic fractions were pooled, dried with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography (35:75 EtOAc:Hexanes, v/v) and dried, affording a colorless oil in 83% yield (3.95 g). $^1$H-NMR (600 MHz, $CDCl_3$): $\delta$ = 5.35 (d, 1H, $^3J_{H,H}$ = 7.2 Hz), 4.39–4.29 (1H, m), 3.73–3.58 (2H, m), 2.17–2.08 (m, 1H), 1.57–1.47 (m, 1H), 1.45 (s, 9H), 1.43 (s, 9H) ppm. $^{13}$C-NMR (151 MHz, $CDCl_3$): $\delta$ = 172.1, 156.8, 82.4, 80.5, 58.3, 51.0, 36.7, 28.4, 28.1 ppm. ESI-HRMS: m/z calculated for $C_{13}H_{25}NaNO_5$ $(M+Na)^+$ 298.1629, found 298.1630.

***tert*-butyl *N*-(*tert*-butoxycarbonyl)-*O*-tosyl-*L*-homoserinate (6)**

To an oven dried round bottom flask equipped with a magnetic stir bar, 3.7 g **compound 5** (13.4 mmol, 1 equiv.) and 6.78 g triethylamine (67 mmol, 5 equiv.) were dissolved in 25 mL DCM and cooled in an ice bath. After cooling, 5.11 g p-toluenesulfonyl chloride (26.8 mmol, 2 equiv.) and 0.164 g DMAP (1.34 mmol, 0.1 equiv.) were added and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was subsequently diluted with 100 mL DCM and washed with $H_2O$ (4 x 60 mL), brine (1 x 60 mL), dried with $MgSO_4$, and concentrated under vacuum. Crude product was purified *via* flash chromatography (3:7 EtOAC:Hexanes, v/v) and dried under vacuum to yield a white tacky solid in 76% yield (4.4 g). $^1$H-NMR (600 MHz, $CDCl_3$): $\delta$ = 7.74 (d, 2H, $^3J_{H,H}$ = 7.8 Hz), 7.31 (d, 2H, $^3J_{H,H}$ = 7.8 Hz), 5.04 (d, 1H, $^3J_{H,H}$ = 7.8 Hz), 4.17–4.08 (m, 1H), 4.08–3.97 (m, 2H), 2.40 (s, 3H), 2.23–2.06 (m, 1H), 2.06–1.93 (m, 1H), 1.40 (s, 9H), 1.36 (s, 9H) ppm. $^{13}$C-NMR (151 MHz, $CDCl_3$): $\delta$ = 170.6, 155.2, 144.9, 132.7, 129.9, 128.0, 82.6, 79.8, 66.6, 51.0, 31.5, 28.3, 27.9, 21.6 ppm. ESI-HRMS: m/z calculated for $C_{20}H_{31}NaNO_7S$ $(M+Na)^+$ 452.1722, found 452.1719.

***tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-4-$^{15}$*N*-(cyano)butanoate (7)**

An oven-dried round bottom flask was equipped with a magnetic stir bar and charged with 4.4 g **compound 6** (10.2 mol, 1 equiv.), 3.06 g NaI (20.4 mmol, 2 equiv.), and 50 mL acetone. This mixture was stirred at reflux for 1 hour, cooled to room temperature, and diluted in 25 mL DCM. The reaction mixture was filtered, and the orange filtrate was dried under vacuum. The dry filtrate was diluted in an additional 90 mL DCM, filtered, and the filtrate was dried under vacuum in a round bottom flask. The flask containing the dry filtrate was equipped with a magnetic stir bar and charged with 50 mL DMSO and 0.809 g potassium [$^{15}$N]-cyanide (12.2 mmol, 1.2 equiv.). The

flask was placed in an oil bath set to 80°C and the reaction mixture was stirred under a reflux condenser for 18 hours. Afterwards, the magnetic stir bar was removed, the reaction mixture was frozen, and DMSO was removed under reduced pressure with a lyophilizer. The dried reaction mixture was diluted in 200 mL $H_2O$ and extracted with EtOAc (3 x 200 mL), after which the pooled organic phase was washed with $H_2O$ (2 x 300 mL) and brine (1 x 300 mL). The organic phase was dried under vacuum, and the crude product was purified with flash chromatography (2:8 EtOAc:Hexanes, v/v), yielding a white solid in 52% yield (1.5 g). $^1$H-NMR (600 MHz, $CDCl_3$): $\delta$ = 5.17 (d, 1H, $^3J_{H,H}$ = 6.0 Hz), 4.27–4.18 (m, 1H), 2.51–2.33 (m, 2H), 2.31–2.18 (m, 1H), 2.01–1.89 (m, 1H), 1.48 (s, 9H), 1.44 (s, 9H) ppm. $^{13}$C-NMR (151 MHz, $CDCl_3$): $\delta$ = 170.3, 155.5, 119.1 (d, $^1J_{C,N}$ = 16.9 Hz), 83.3, 80.4, 53.1, 29.3, 28.4, 28.1, 13.8 (d, $^2J_{C,N}$ = 3.0 Hz) ppm. ESI-HRMS: m/z calculated for $C_{14}H_{24}NaN^{15}NO_4$ $(M+Na)^+$ 308.1604, found 308.1597.

***tert*-butyl (*E*)-$N^2$,$N^{\omega}$,$N^{\omega'}$-tris(*tert*-butoxycarbonyl)-*L*-argininate-$C^{\omega}$-$^{13}C$-$N^{\delta}$,$N^{\omega}$,$N^{\omega'}$-$^{15}N$ (8)**

In an oven dried round bottom flask equipped with a magnetic stir bar, 1.7 g **compound 7** (5.96 mmol, 1 equiv.) was dissolved in 45 mL acetic acid. 1.26 g 10% Pd/C (w/w, dry basis, ~50% water, approx. 0.1 equiv. Pd) was subsequently added to the flask, and the flask was placed inside a Parr apparatus. The sealed Parr apparatus was depressurized with a vacuum line and subsequently charged with 70 PSI $H_2$. The reaction mixture was stirred at room temperature for 2 hours, after which $H_2$ gas was released from the vessel outlet. The reaction mixture was exposed to vacuum within the Parr apparatus for an additional 5 minutes to remove residual $H_2$ gas dissolved in the solvent. The Parr apparatus was subsequently opened, the magnetic stir bar was removed, and the mixture was filtered over a pad of celite. The filtrate was collected, frozen, and dried under reduced pressure in a lyophilizer. The resulting oil was the acetate salt of the reduced nitrile (confirmed by

NMR) and was used for the next step without purification. The oil was added to an oven dried round bottom flask equipped with a magnetic stir bar, which was combined with 50 mL DMSO, 1.92 g **compound 3** (6.56 mmol, 1.1 equiv.), and 3.02 g triethylamine (29.8 mmol, 5 equiv.). This reaction mixture was stirred at ambient temperature for 24 hours, after which the mixture was frozen and dried under reduced pressure in a lyophilizer to remove DMSO. The crude product was purified *via* flash chromatography (2:8 EtOAc:Hexanes, v/v) to yield a light yellow oil in 54% yield (1.73g). $^{1}$H-NMR (600 MHz, $CDCl_3$): $\delta$ = 11.47 (d, 1H, $^{1}J_{H,N}$ = 91.8 Hz), 8.46–8.23 (m, 1H), 5.16–4.99 (m, 1H), 4.23–4.12 (m, 1H), 3.45–3.35 (m, 2H), 1.88–1.73 (m, 1H), 1.68–1.59 (m, 2H), 1.59–1.50 (m, 1H), 1.47 (s, 9H), 1.46 (s, 9H), 1.44 (s, 9H), 1.42 (s, 9H) ppm. $^{13}$C-NMR (151 MHz, $CDCl_3$): $\delta$ = 171.7, 163.6–162.9 (m), 156.2 (ddd, $^{1}J_{C,N}$ = 8.4 Hz, $^{1}J_{C,N}$ = 14.4 Hz, $^{1}J_{C,N}$ = 24.6 Hz), 155.4, 153.3 (d, $^{1}J_{C,N}$ = 24.3 Hz), 83.3, 82.1, 79.8, 79.5, 53.6, 40.7–40.1 (m), 30.3, 28.4, 28.4, 28.1, 28.1, 24.9 ppm. ESI-HRMS: m/z calculated for $C_{24}{}^{13}CH_4N^{15}N_3O_8$ $(M+H)^+$ 535.3314, found 535.3338.

**[6-$^{13}$*C*,$^{15}$*N*$_3$]-L-Arginine-HCl (9)**

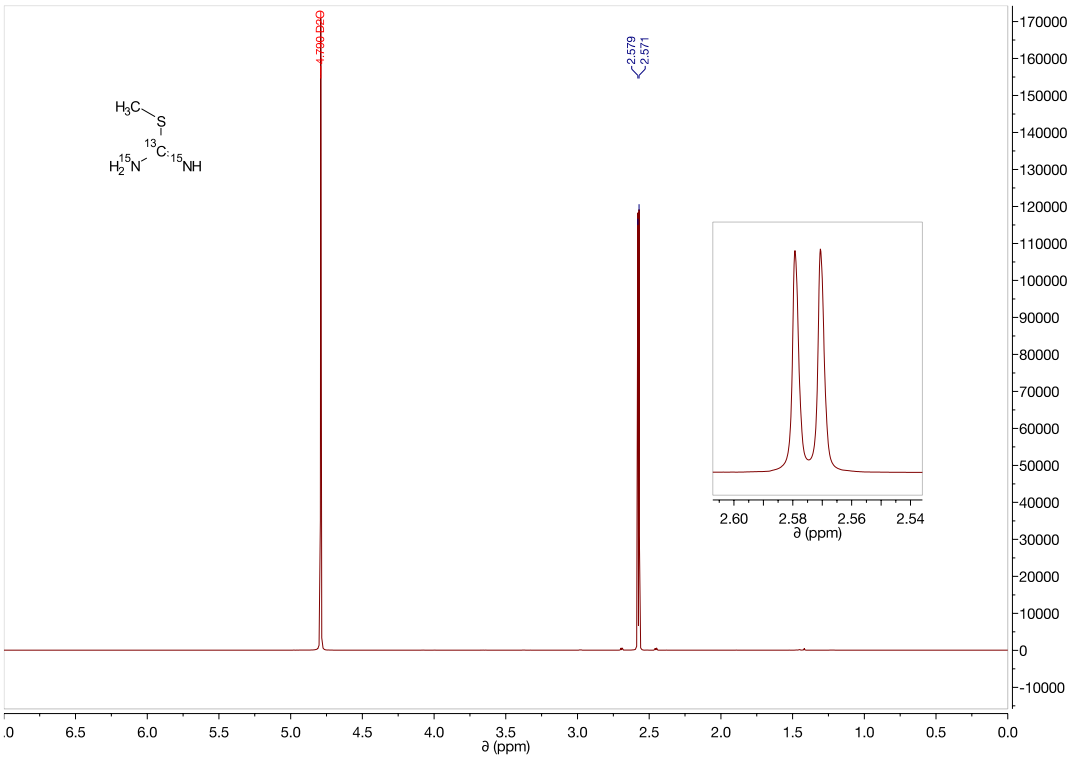
An oven dried round bottom flask was equipped with a magnetic stir bar and charged with 1.73 g **compound 8** (3.23 mmol). To this, 100 mL 1:9 trifluoroacetic acid:DCM (v/v) was added and the solution was stirred at ambient temperature for 18 hours. The mixture was dried under reduced pressure, after which 120 mL 1:1 DCM:$H_2O$ (v/v) was added to the flask and the solution was transferred to a separatory funnel. The aqueous layer was collected, and the organic phase was extracted with $H_2O$ (2 x 50 mL). Aqueous fractions were pooled, washed with 70 mL DCM, and dried under reduced pressure. The trifluoroacetate counter-ion was exchanged with a chloride counter-ion through 3 cycles of treatment with 100 mL 1M HCl and drying under reduced pressure.

Pure [6-$^{13}$C,$^{15}$N$_3$]-L-Arginine-HCl was crystallized from the remaining crude material using an adaptation of a previously reported method.[1] 640 mg of crude product was redissolved in 380 μL aniline (4.20 mmol, 1.3 equiv.) and 3 mL 1:9 $H_2O$:EtOH (v/v) at 50°C. This solution was left undisturbed at room temperature for 24 hours as [6-$^{13}$C,$^{15}$N$_3$]-L-Arginine-HCl crystallized out of solution. The resulting white solid was collected, washed with cold ethanol, and dried in an 80°C oven for 3 hours, yielding the pure monohydrochloride salt as a white powder in 53.2% yield (368 mg). $^1$H-NMR (600 MHz, $D_2O$): $\delta$ = 3.74 (t, 1H, $^2J_{C,N}$ = 6.0 Hz), 3.24–3.18 (m, 2H), 1.93–1.82 (m, 2H), 1.74–1.55 (m, 2H) ppm. $^{13}$C-NMR (151 MHz, $D_2O$, MeOH Reference): $\delta$ = 174.9 ppm, 157.4 (q, $^1J_{C,N}$ = 21.7 Hz), 54.9, 41.1 (d, $^2J_{C,N}$ = 10.0 Hz), 28.2, 24.5 ppm. ESI-HRMS: m/z calculated for $C_{12}{}^{13}CH_{15}N^{15}N_3O_2$ $(M+H)^+$ 179.1139, found 179.1140.

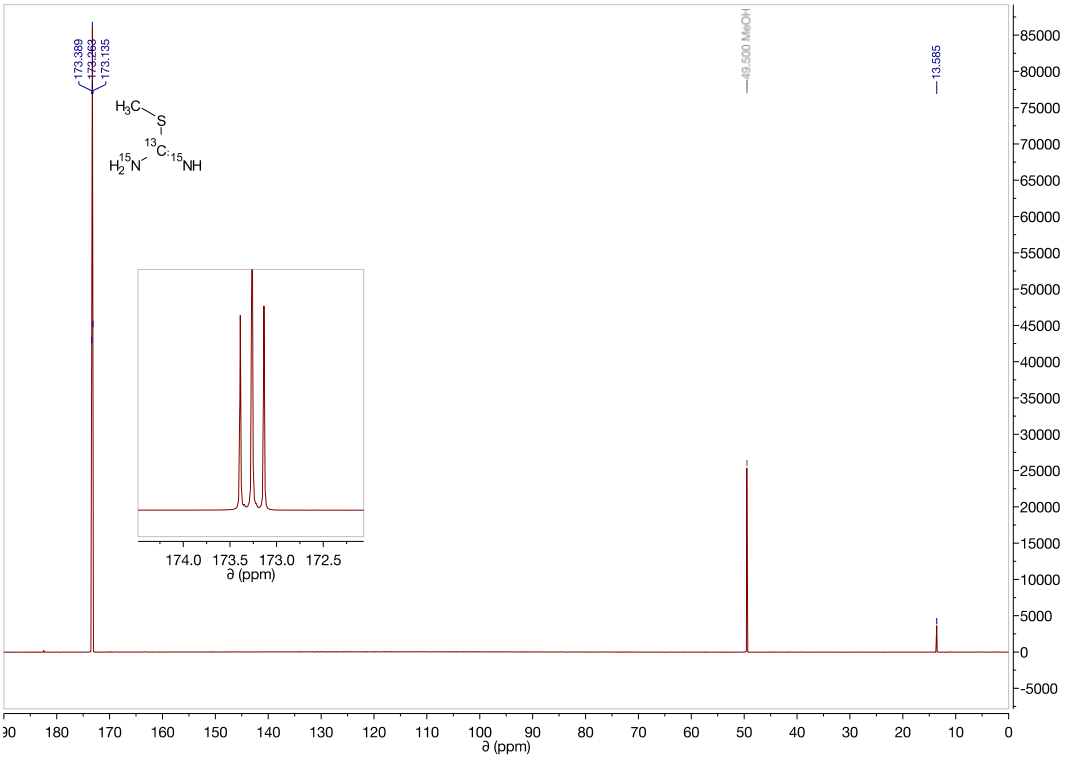
[1] Cox, J. *J. Biol. Chem.* **1928**, *78*, 475–479.

## NMR Spectra

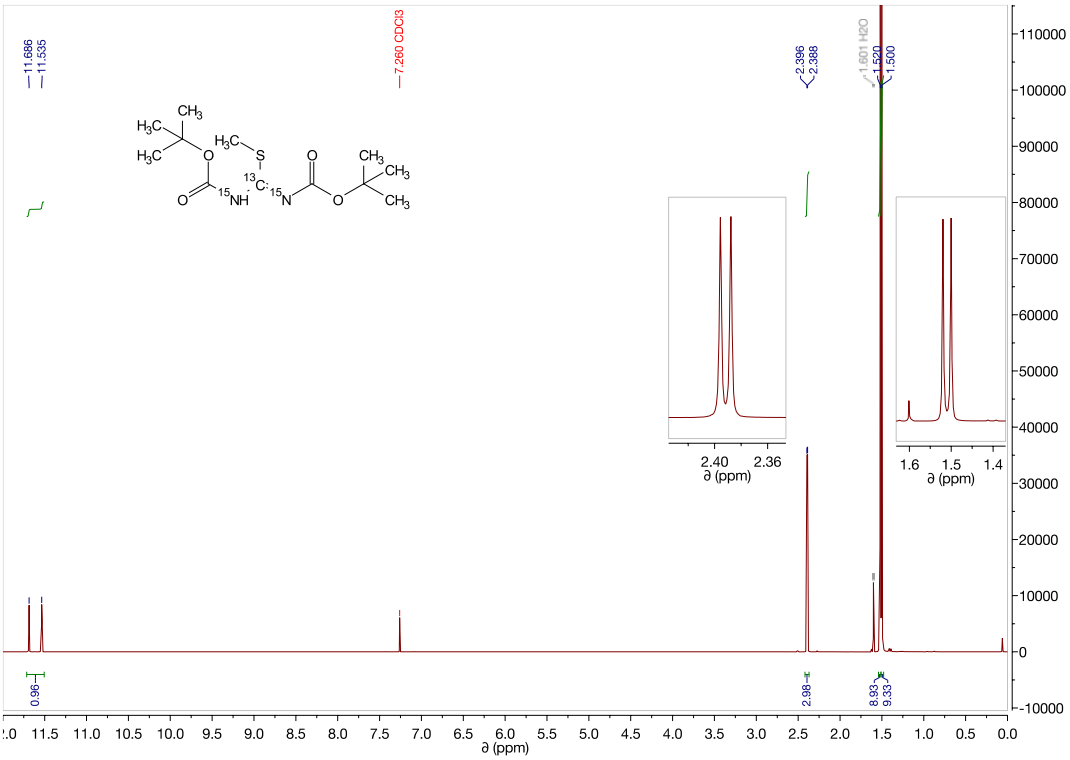
**Compound 2,** $^{1}$H-NMR, 14.1T



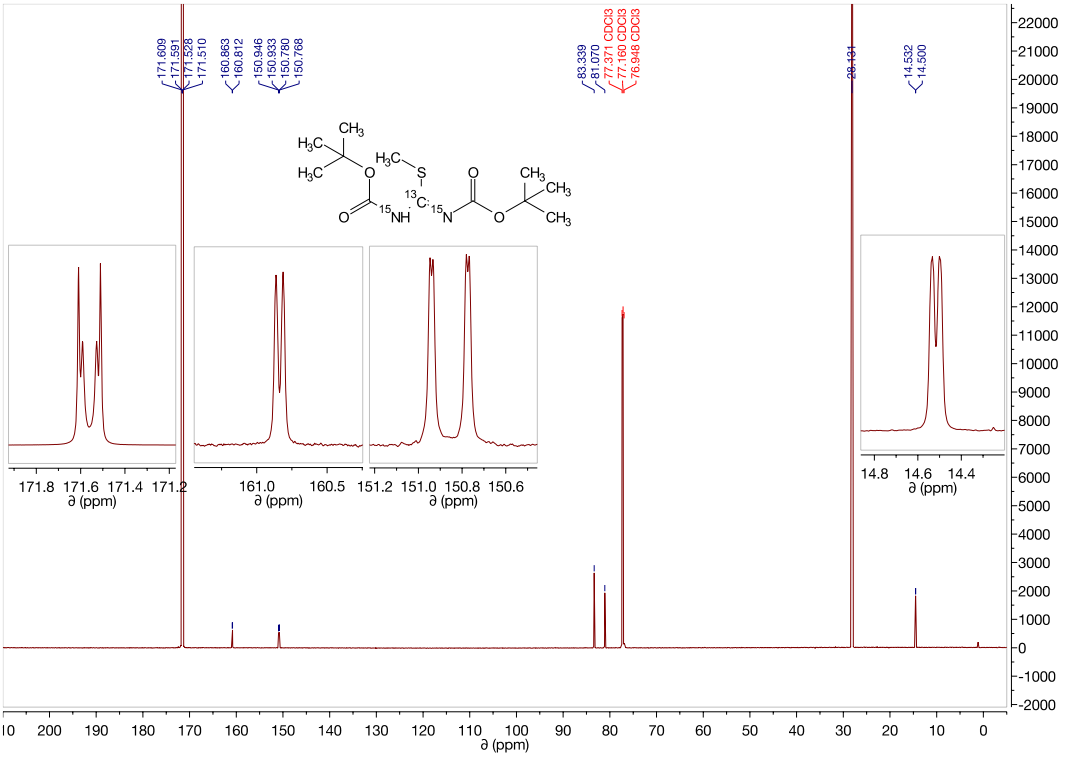

**Compound 2,** $^{13}$C-NMR, 14.1T



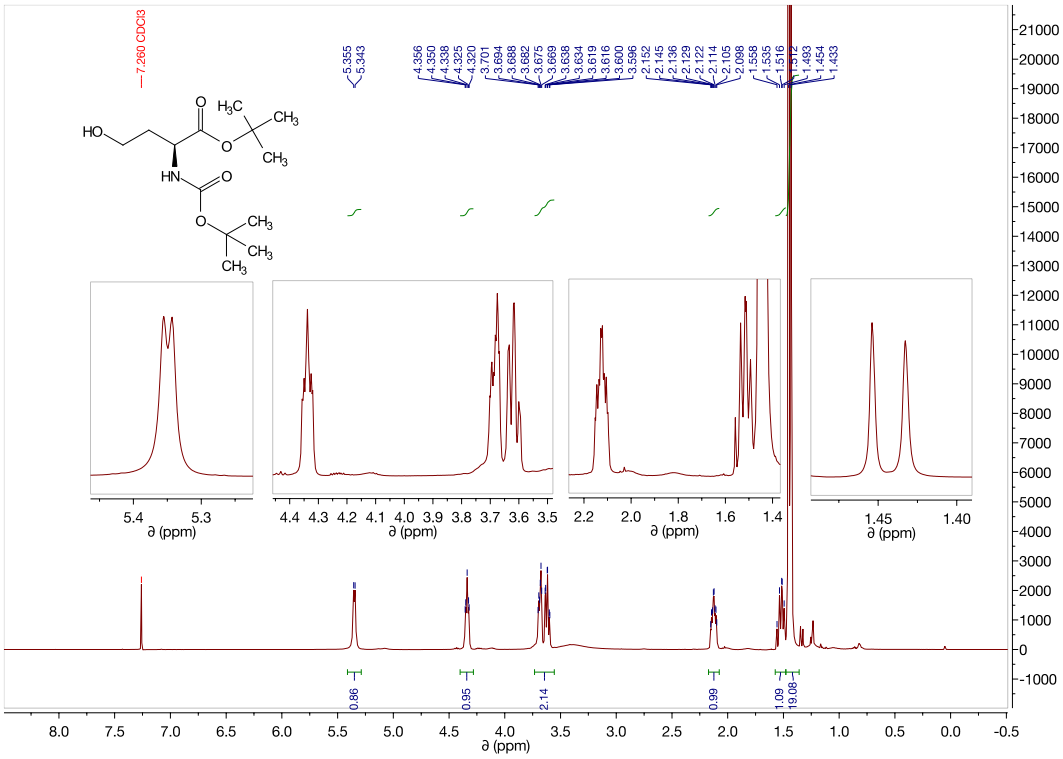
**Compound 3,** $^{1}$H-NMR, 14.1T



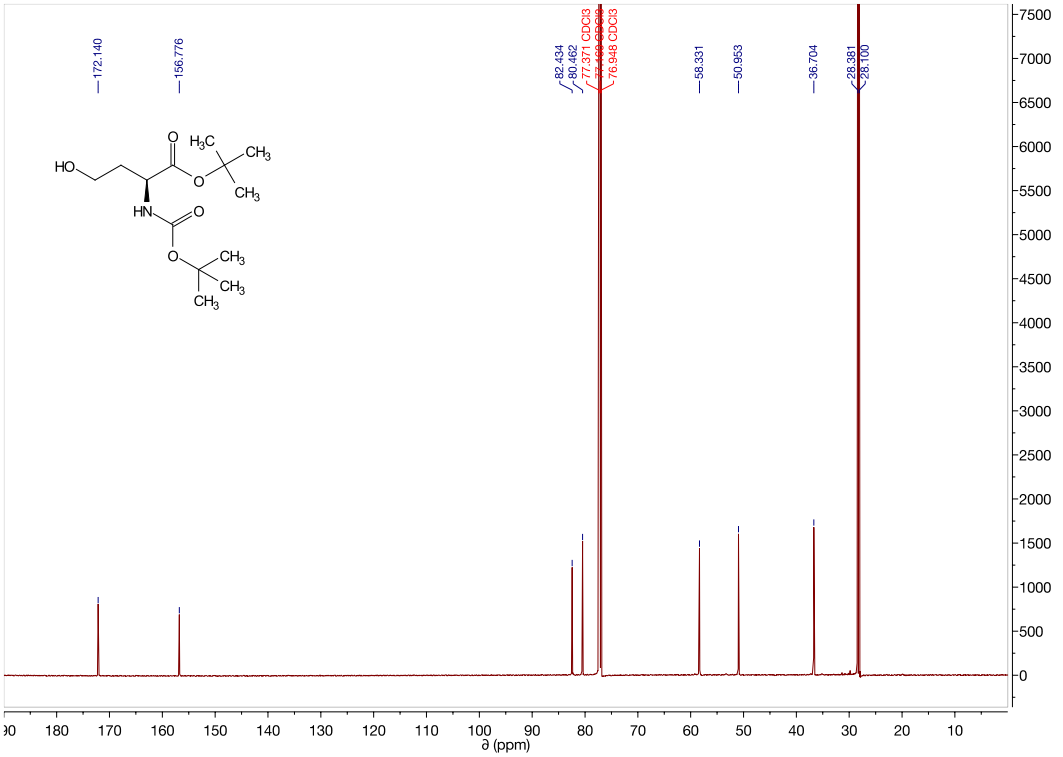

**Compound 3,** $^{13}$C-NMR, 14.1T



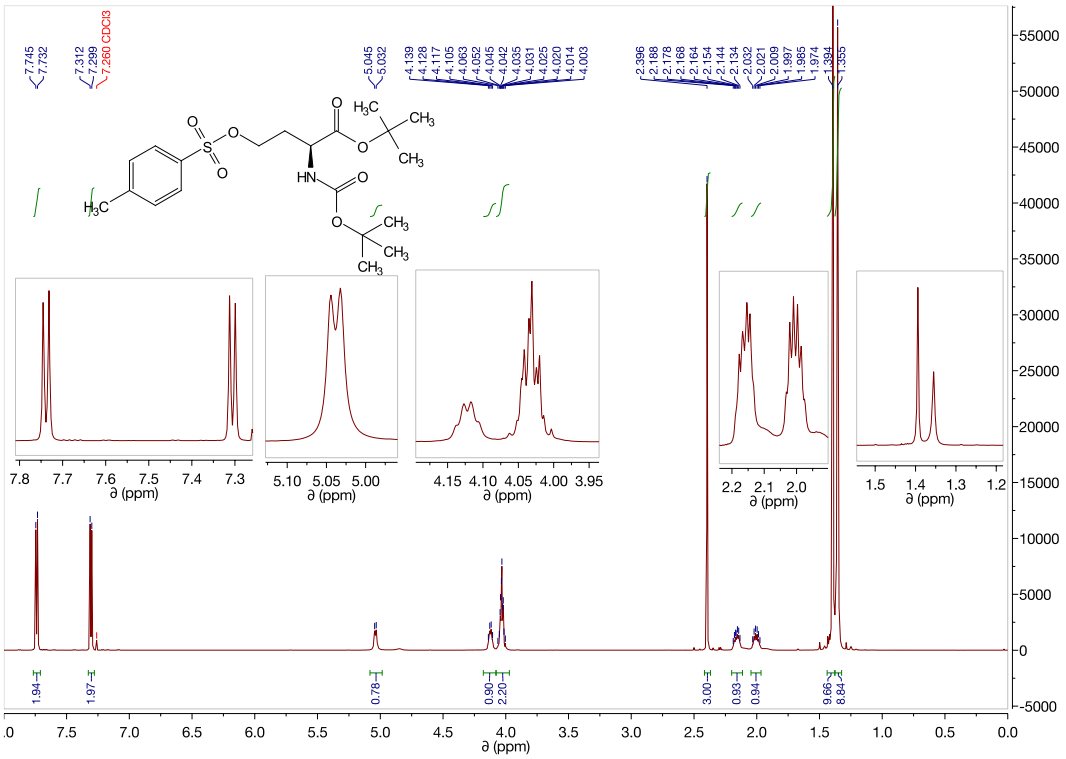
**Compound 5,** $^{1}$H-NMR, 14.1T



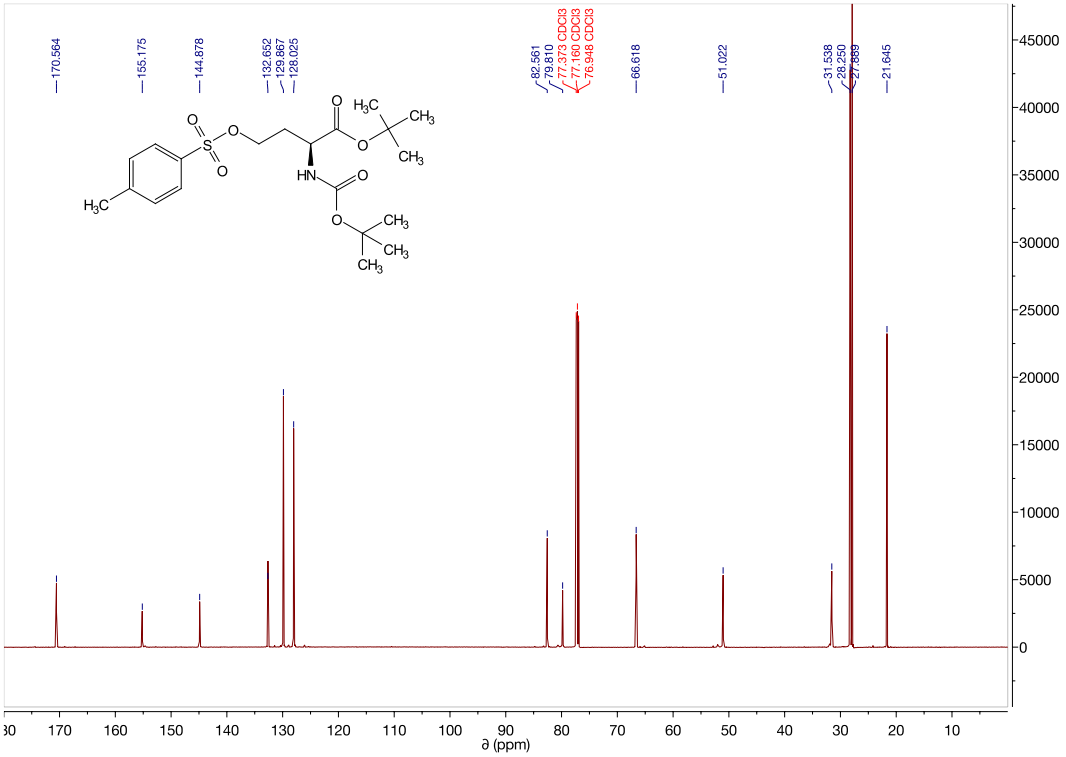

**Compound 5,** $^{13}$C-NMR, 14.1T



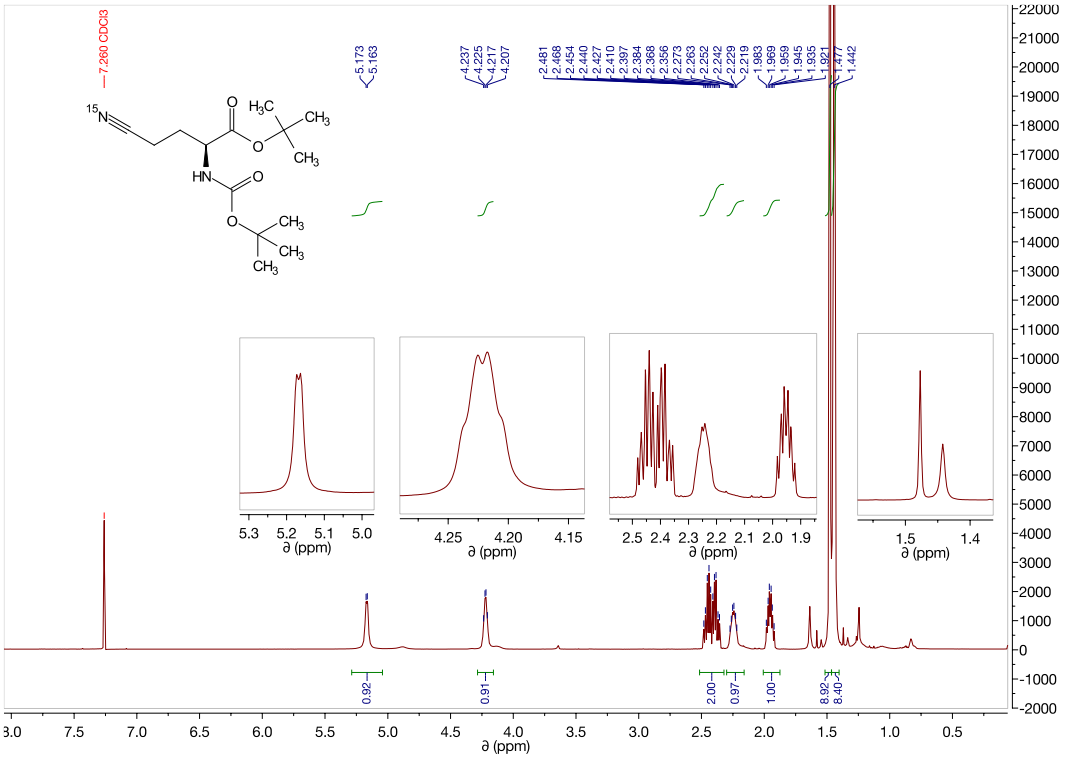
**Compound 6,** $^{1}$H-NMR, 14.1T



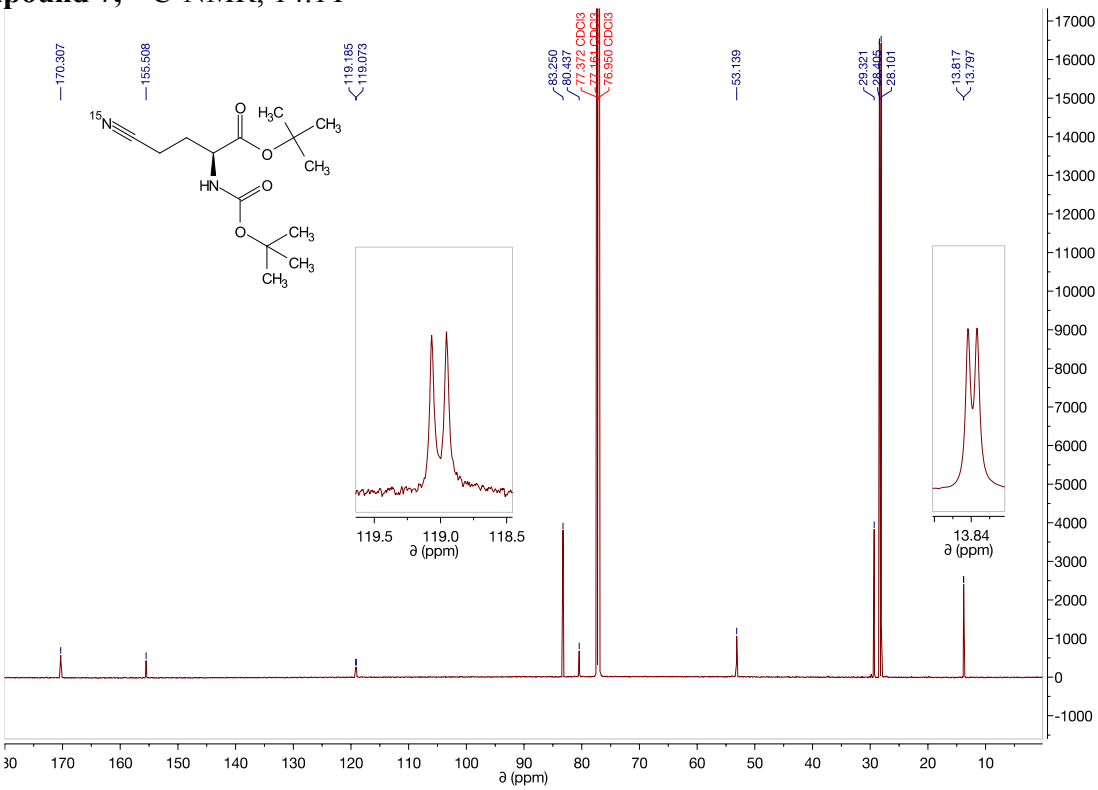
**Compound 6,** $^{13}$C-NMR, 14.1T



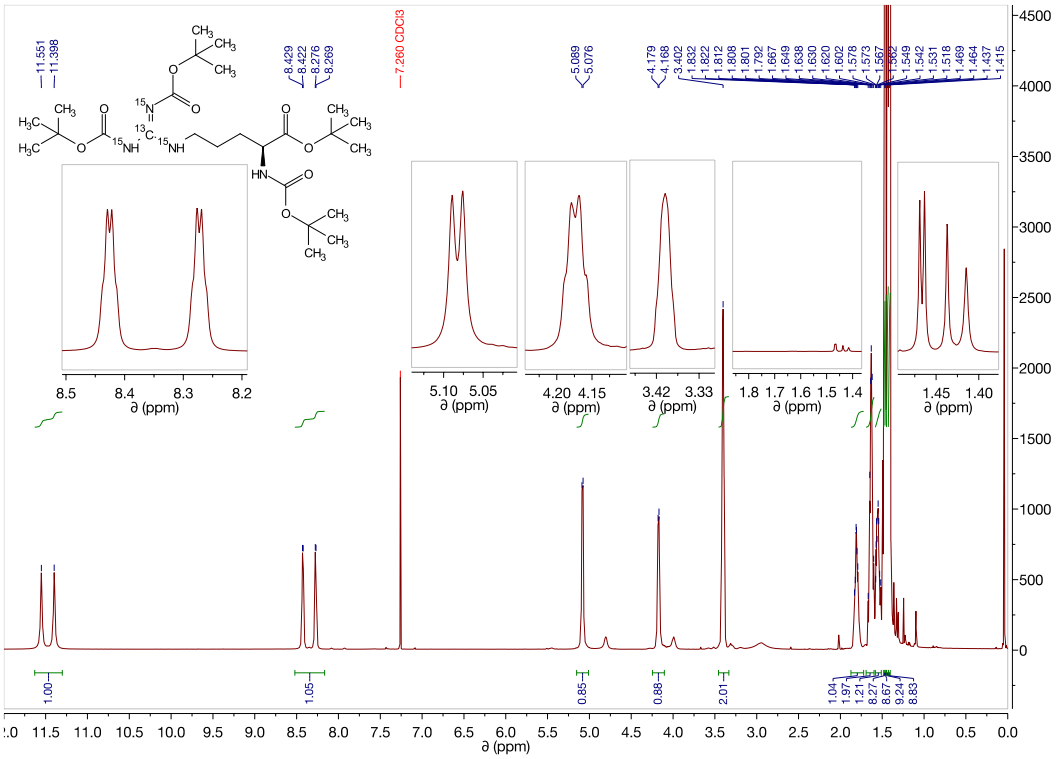
**Compound 7,** $^{1}$H-NMR, 14.1T



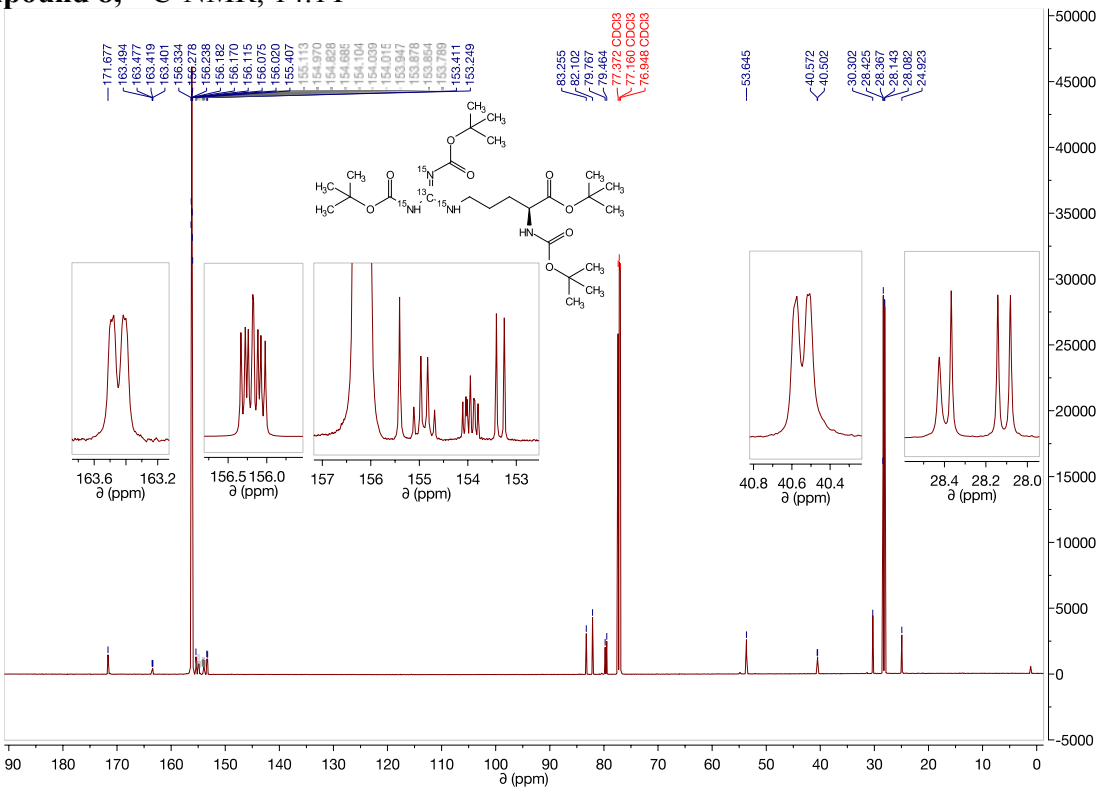

**Compound 7,** $^{13}$C-NMR, 14.1T



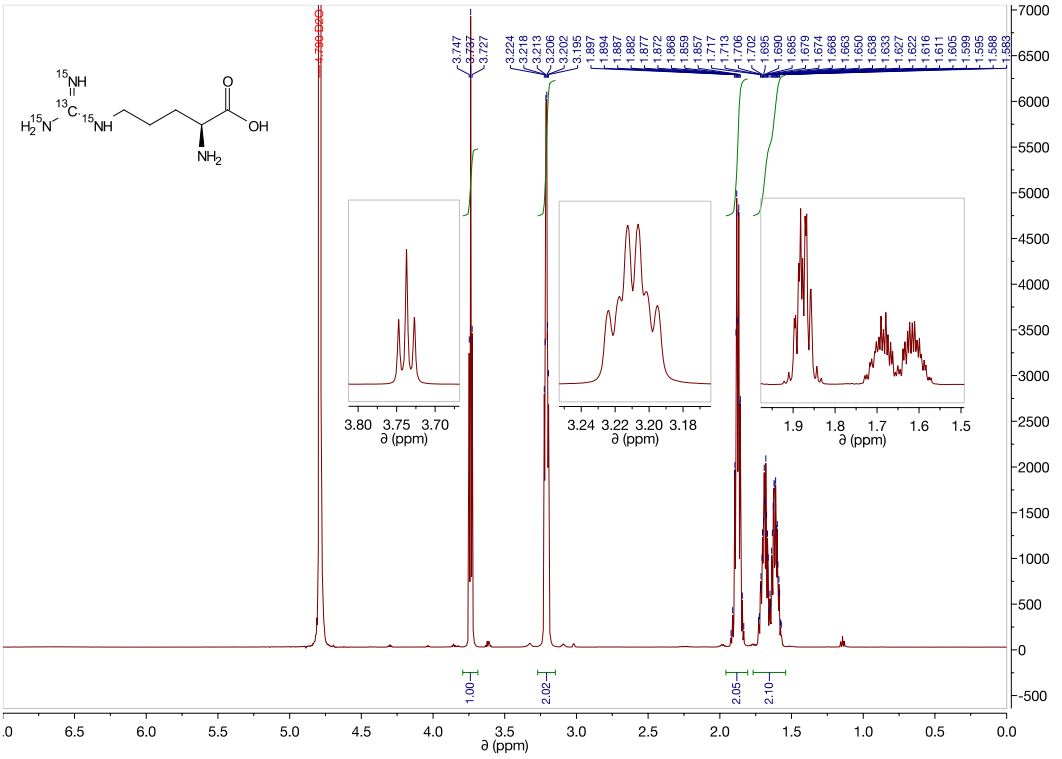
**Compound 8,** $^{1}$H-NMR, 14.1T



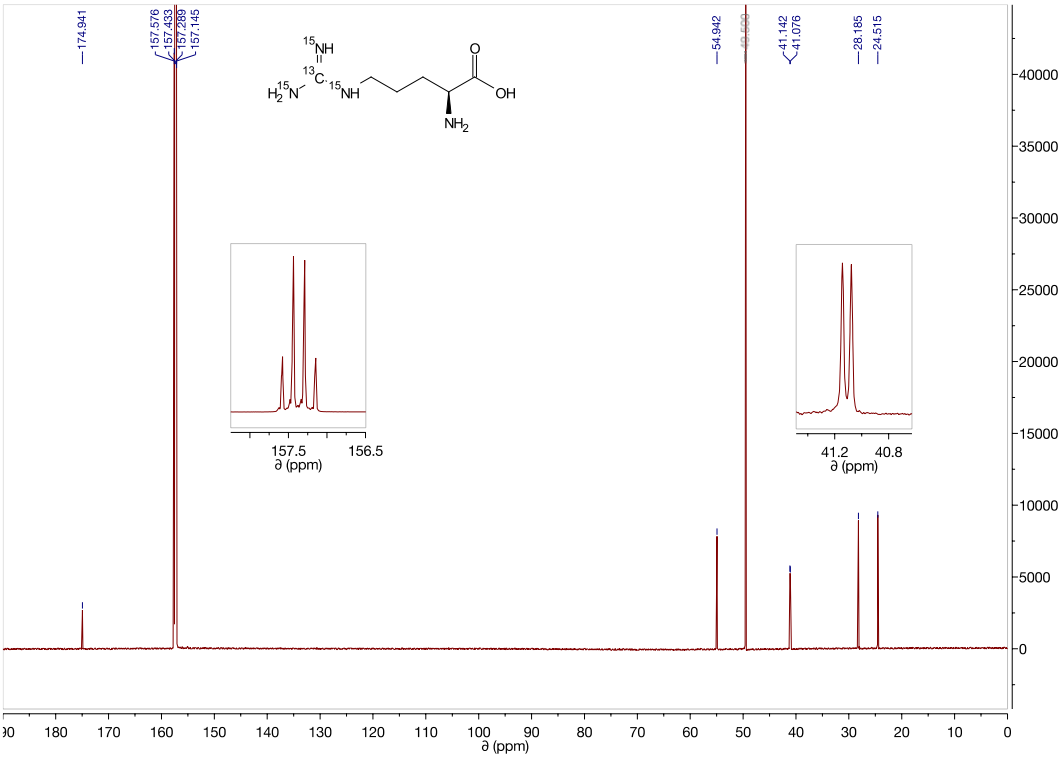
**Compound 8,** $^{13}$C-NMR, 14.1T



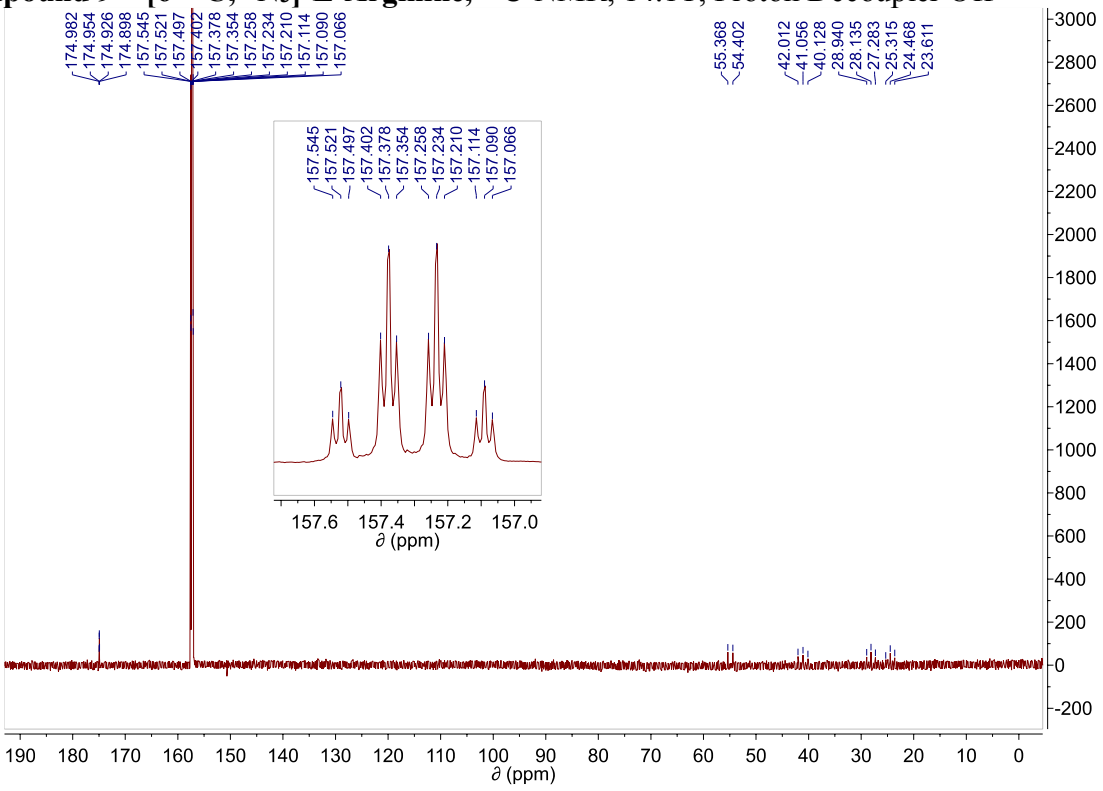
**Compound 9 – [6-$^{13}$C,$^{15}$N$_3$]-L-Arginine,** $^{1}$H-NMR, 14.1T




**Compound 9 – [6-$^{13}$C,$^{15}$N$_3$]-L-Arginine,** $^{13}$C-NMR, 14.1T



**Compound 9 – [6-$^{13}$C,$^{15}$N$_3$]-L-Arginine,** $^{13}$C-NMR, 14.1T, Proton Decoupler Off



# High Resolution Mass Spectrometry

## Compound 2

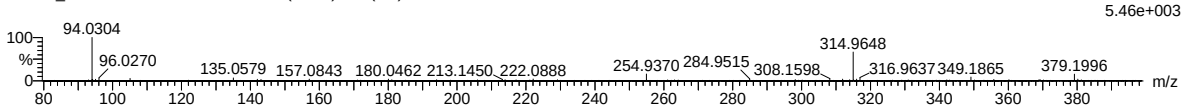
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
H: 0-8 14N: 0-1 15N: 0-2 S: 0-1 12C: 0-1 13C: 0-1 I: 0-1

C13CH6N2SHI
091516_13C15N-SMeThiomea-Exact 5 (0.132) Cm (3:6)

1: TOF MS ES+
5.46e+003




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 94.0304 | 94.0304 | 0.0 | 0.0 | 0.5 | 60.9 | H7 15N2 S 12C 13C |

## Compound 3

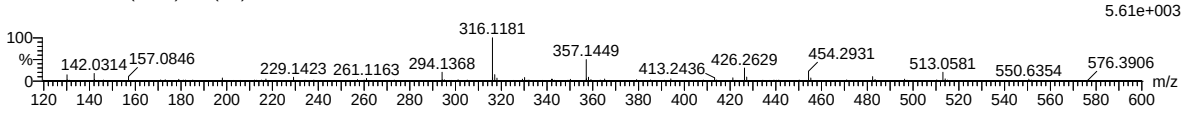
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
4177 formula(e) evaluated with 2 results within limits (up to 20 closest results for each mass)
Elements Used:
H: 0-25 O: 0-4 Na: 0-5 S: 0-1 12C: 0-11 13C: 0-1 15N: 0-2

C1113CH2215N2O4S
AC-BocSMeTU 2 (0.053) Cm (2:5)

1: TOF MS ES+
5.61e+003




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 316.1181 | 316.1172 | 0.9 | 2.8 | 2.5 | 374.6 | H22 O4 Na S 12C11 13C 15N2 |
| | 316.1195 | -1.4 | -4.4 | -0.5 | 568.6 | H22 O Na5 12C10 13C 15N2 |

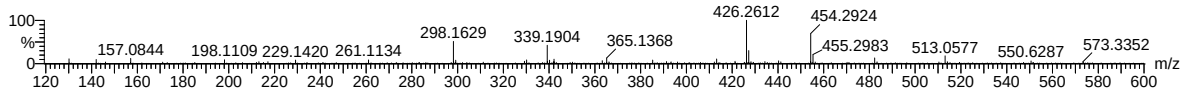
# Compound 5

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
92 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-13 H: 0-25 N: 0-1 O: 0-5 Na: 0-5

C13H25NO5
AC-BocHSOtbu 4 (0.088) Cm (3:6)




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 298.1629 | 298.1630 | -0.1 | -0.3 | 1.5 | 20.3 | C13 H25 N O5 Na |

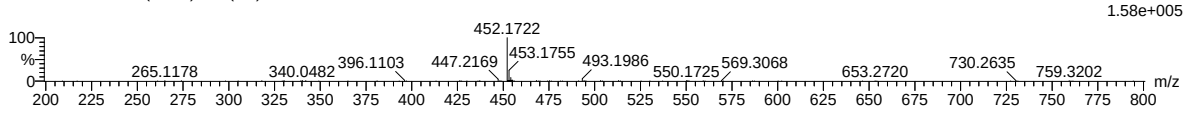
# Compound 6

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
62 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-20 H: 0-31 N: 0-1 O: 0-7 Na: 0-1 S: 0-1

C20H31NO5
AC-BocTOSOtbu 5 (0.127) Cm (2:5)




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 452.1722 | 452.1719 | 0.3 | 0.7 | 5.5 | 181.6 | C20 H31 N O7 Na S |

# Compound 7

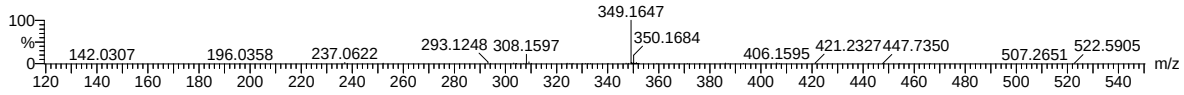
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
37 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-14 H: 0-26 14N: 0-1 15N: 0-1 O: 0-4 Na: 0-1

C14H24N15NO4
091516_15N-BocNitotBu-Exact-02 41 (0.949) Cm (41:44)

1: TOF MS ES+
1.76e+005




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 308.1597 | 308.1604 | -0.7 | -2.3 | 3.5 | 67.0 | C14 H24 14N 15N O4 Na |

# Compound 8

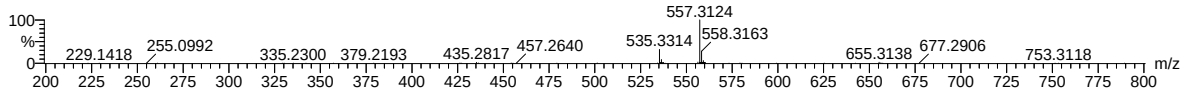
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
3600 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
12C: 0-24 13C: 0-1 H: 0-47 14N: 0-1 15N: 0-3 O: 0-8

C2413CH46N15N3O8
AC-Prot-Arg 25 (0.580) Cm (19:28)

1: TOF MS ES+
4.90e+005




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 535.3314 | 535.3338 | -2.4 | -4.5 | 4.5 | 22317.2 | 12C24 13C H47 14N 15N3 O8 |

## Compound 9 – [6-$^{13}C$,$^{15}N_3$]-L-Arginine

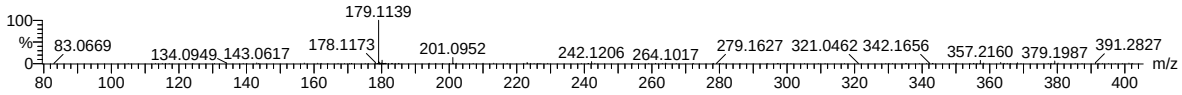
**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
383 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
12C: 0-5 13C: 0-1 H: 0-15 14N: 0-1 15N: 0-3 O: 0-3

C513CH14N15N3O2
Label-Arg 7 (0.161) Cm (6:7)

1: TOF MS ES+
4.07e+004




| | | | | | | |
|---|---|---|---|---|---|---|
| Minimum: | | | | -1.5 | | |
| Maximum: | | 5.0 | 5.0 | 110.0 | | |
| Mass | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
| 179.1139 | 179.1140 | -0.1 | -0.6 | 1.5 | 1547.7 | 12C5 13C H15 14N 15N3 O2 |