Sonogashira diversification of unprotected halotryptophans, halotryptophan containing tripeptides; and generation of a new to nature bromo-natural product and its diversification in water

M. J. Corr,[a] S. V. Sharma,[a] C. Pubill-Ulldemolins,[a] R. T. Bown,[a] P. Poirot,[b] D. R. Smith,[a] C. Cartmell,[a] A. Abou Fayad, [c] R. J. M. Goss[a*]

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NMR spectra for small tryptophan, peptide derivatives and ^{19}F NMR analysis of cross-coupled cystargamide

General Chemical Experimental

All reagents were purchased from commercial suppliers and were used without further purification unless otherwise stated. Dry dichloromethane was dried and deoxygenated with an MBraun SPS-800 solvent purification system and the moisture content of the solvent was analysed using a Karl Fischer coulometer (Metler Toledo DL32).

Proton NMR (^{1}H), carbon NMR (^{13}C), phosphorus NMR (^{31}P) and fluorine NMR (^{19}F) were recorded on a Bruker Ascend 500 (500 MHz), Bruker 500 UltraShield (500 MHz), Bruker Ascend 700 (700 MHz), Bruker 400 UltraShield (400 MHz) or a Bruker UltraShield (300 MHz) spectrometer. Fluorine NMR were also recorded as proton decoupled (^{19}F{^{1}H}). Phosphorus NMR (^{31}P{^{1}H}) was recorded as proton decoupled. Using an HSQC experiment with multiplicity editing, the ^{13}C NMR signals were assigned to CH_3, CH_2, CH and C. The NMR experiments were carried out in deuterochloroform ($CDCl_3$) deuterated water (D_2O), deuterated DMSO (d_6-DMSO) or deuterated methanol (d_4-MeOH). The chemical shifts (δ) are quoted in parts per million (ppm). Multiplicities are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad for the ^{1}H NMR, ^{19}F NMR, ^{19}F{^{1}H} NMR and ^{13}C NMR spectra. Coupling constants are reported in Hertz (Hz).

Flash chromatography was performed using Davisil silica gel LC60A (40-63 micron). Thin layer chromatography (TLC) was performed using aluminium sheets of silica gel 60 F254 and was visualised under a Mineralight model UVGL-58 lamp (254 nm). The plates were developed with acidic methanolic vanillin solutions, ethanolic phosphomolybdic acid solutions or basic potassium permanganate solutions.

High and low resolution mass spectra were recorded at the University of St Andrews on a Waters Micromass LCT time of flight mass spectrometer coupled to a Waters 2975 HPLC system or on an Orbitrap ELOS pro. Optical rotation values were recorded in methanol on a Perkin Elmer Model 341 Polarimeter using a Na/Hal lamp (589 nm) at 20 °C in a 1 dm polarimeter cell and are given in 10^{-1} deg cm^2 g^{-1}. Freeze drying was carried out on a Scanvac CoolSafe^{TM} freeze dryer. Microwave reactions were carried out on a Biotage Initiator+.

5-Bromotryptophan **18**, 5-chlorotryptophan **20**, 6-bromotryptophan **21**, 7-bromotryptophan **22** and 7-iodotryptophan **23** were prepared as described previously.[1]

HPLC purification was carried out on an XBridge Prep Phenyl 5 μm column (10x250 mm) on a Gilson HPLC system (Gilson 322 pump, Gilson UV/Vis-151 detector, Gilson 402 syringe

pump). UPLC analysis was carried out on a Waters Acquity H-Class UPLC system fitted with an Acquity UPLC BEH C18 column (1.7 μm, 2.1x50 mm).

For purification of the cross coupling products from 5-bromotryptophan **18**, the following program was used: 10% acetonitrile/water for 5 min, 10% acetonitrile/water to 60% acetonitrile/water over 21 minutes, 60% acetonitrile/water to 90% acetonitrile/water over 5 minutes, 90% acetonitrile/water to 10% acetonitrile/water over 5 min, 10% acetonitrile/water for 4 min. Total run time 40 minutes. Flowrate 5 mL/min, detection wavelength 254 nm.

For purification of the cross coupling products from tripeptides **58** and **59**, the following program was used: 10% acetonitrile/water for 5 min, 10% acetonitrile/water to 60% acetonitrile/water over 30 minutes, 60% acetonitrile/water to 90% acetonitrile/water over 5 minutes, 90% acetonitrile/water to 10% acetonitrile/water over 5 min, 10% acetonitrile/water for 4 min. Total run time 50 minutes. Flowrate 5 mL/min, detection wavelength 330 nm.

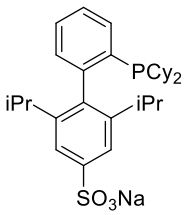
Purification of selected peptides was carried out on a Biotage Isolera Four using reverse-phase SNAP C18 12 g column cartridges. The purification was carried out using water/methanol on the following gradient: 12 mL/min elution, 2% methanol/water for 1.15 min, 2→15% methanol/water for 1.15 min, 15% methanol/water for 5.15 min, 15→95% methanol/water for 15 min, 95% methanol/water for 3.45 min. The collection wavelength was set a 254 nm.

UPLC analysis was carried out on a Waters Acquity H-Class UPLC system.

A 1:1 mixture of water:acetonitrile was freshly prepared before each set of cross coupling reactions. The solvent was degassed by bubbling nitrogen through the solutions for at least 15 minutes, then storing under nitrogen.

The IUPAC names of some compounds were obtained using ChemBioDraw Ultra (version 13.0.2.3012).

Sodium 2'-(Dicyclohexylphosphanyl)-2,6-diisopropyl-(1,1-biphenyl)-4-sulfonate (sXPhos) 12[2]



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Sulfuric acid (1.0 mL) and fuming sulfuric acid (3.0 mL) were added to a suspension of XPhos (433 mg, 0.9 mmol, 1.0 eq) in dry DCM (3 mL) at 0 °C under nitrogen. The reaction was allowed to warm to r.t. and stirred for 24 h. The reaction mixture was cooled to 0 °C and crushed ice (10 g) was added. The reaction mixture was neutralised to pH 7 using sodium hydroxide solution (6 N, ~25 mL). The reaction mixture was extracted with DCM (3 x 50 mL) and the solvent was removed *in vacuo*. The residue was dissolved in cold methanol (20 mL), filtered, the filtrate collected and the solvent removed *in vacuo* to give sodium 2'-(dicyclohexylphosphanyl)-2,6-diisopropyl-(1,1-biphenyl)-4-sulfonate **12** (0.49 g, 100%) as a tan solid; ^{1}H NMR (500 MHz, d_4-MeOH) δ = 0.98 (6H, d, J(H,H)= 6.7 Hz, CH_3), 1.08-1.31 (16H, m, CH_3, CH_2), 1.55-1.89 (12H, m, CH, CH_2), 2.45-2.50 (2H, m, CH), 7.11-7.13 (1H, m, ArH), 7.38-7.47 (2H, m, ArH), 7.65-7.69 (3H, m, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 23.1 (CH_3), 25.9 (CH_3), 27.5 (CH_2), 28.3 (d, J(C,P)= 8.9 Hz, CH_2), 28.5 (d, J(C,P)= 11.4 Hz, CH_2), 30.6 (d, J(C,P)= 12.0 Hz, CH_2), 31.9 (CH), 32.3 (d, J(C,P)= 16.1 Hz, CH_2), 35.6 (d, J(C,P)= 15.4 Hz, CH), 121.2 (CH), 128.0 (CH), 129.1 (CH), 132.3 (d, J(C,P)= 6.1 Hz, CH), 133.8 (CH), 137.2 (d, J(C,P)= 17.6 Hz, C), 142.7 (C), 145.5 (C), 147.5 (C), 147.7 (C), 148.3 (C); ^{31}P{^{1}H} NMR (162 MHz, d_4-MeOH) δ = -11.91 (1P, s); MS (ESI) 513 (100) $[M\text{-}Na]^-$; HRMS: m/z calcd for $C_{30}H_{42}O_3P_1S_1$ $[M\text{-}Na]^-$: 513.2598; found: 513.2596.

Standard Reaction Procedure of 5-bromoindole 15 under reflux:

5-Bromoindole **15** (20 mg, 0.1 mmol, 1.0 eq), *bis*(acetonitrile)dichloropalladium (II) (variable, see Table 1 in main text), sXPhos **12** (variable, see Table 1 in main text) and cesium carbonate (81 mg, 0.26 mmol, 2.5 eq) were added to a flask fitted with a reflux condenser under nitrogen. Degassed water/acetonitrile (1:1, 2 mL) was added to the flask, followed by the liquid alkyne substrate (variable, see Table 1 in main text). The reaction mixture was heated at 100 °C for 18 h. The reaction mixture was then cooled to r.t.

For analysis of the reaction conversions, 0.2 mL of reaction mixture was removed from the well-stirred reaction mixture and the solvent was removed *in vacuo*. The residue was dissolved in d_4-MeOH (0.7 mL) and analysed by ^{1}H NMR.

Microwave Reaction Procedure of 5-bromoindole 15:

5-Bromoindole **15** (20 mg, 0.1 mmol, 1.0 eq), *bis*(acetonitrile)dichloropalladium (II) (1.3 mg, 10 μmmol, 5 mol%), sXPhos **12** (8 mg, 30 μmmol, 15 mol%) and cesium carbonate (81 mg, 0.26 mmol, 2.5 eq) were added to a microwave tube, which was then fitted with a suba seal. The flask was evacuated and flushed with nitrogen three times. Degassed water/acetonitrile (1:1, 2 mL) and acetonitrile (1.0 mL) were added to the microwave tube, followed by the liquid alkyne substrate (33 μL, 0.3 mmol, 3.0 eq). The tube was sealed and heated at 100 °C for 2 h using a microwave.

For analysis of the reaction conversion, 0.2 mL of reaction mixture was removed from the well-stirred microwave tube and the solvent was removed *in vacuo*. The residue was dissolved in d_4-MeOH (0.7 mL) and analysed by ^{1}H NMR.

Standard Microwave Reaction Procedure for Tryptophans:

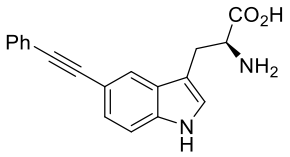
The halo-tryptophan obtained from our previously reported procedure[1] (28 mg, 0.1 mmol, 1.0 eq for bromotryptophans, 33 mg 0.1 mmol, 1.0 eq for 7-iodotryptophan), *bis*(acetonitrile)dichloropalladium (II) (1.3 mg, 10 μmol, 5 mol%), sXPhos **12** (8 mg, 30 μmol, 15 mol%), (in the reaction with 4-cyanophenylacetylene **25**, 3.0 eq of the solid alkyne was also added at this stage) and cesium carbonate (81 mg, 0.26 mmol, 2.5 eq) were added to a microwave tube, which was then fitted with a suba seal. The flask was evacuated and flushed with nitrogen three times. Degassed water:acetonitrile (1:1, 2.0 mL) were added to the

microwave tube, followed by the liquid alkyne substrate (0.3 mmol, 3.0 eq). The tube was sealed and heated at 100 °C for 2 h using a microwave.

For analysis of the reaction conversions, 0.2 mL of reaction mixture was removed from the well-stirred microwave tube and the solvent was removed *in vacuo*. The residue was dissolved in d_4-MeOH (0.7 mL) and analysed by ^{1}H NMR.

For purification of specified reactions, the reaction mixture was cooled to r.t. and diluted with water (6.0 mL). The reaction mixture was centrifuged (13,000 rpm, 16060 g, for 5 min). The ultrafiltrate was collected and purified by HPLC purification, as described above, in 1 mL injections. The appropriate HPLC fractions (generally eluted at 18-20 minutes) were collected and the solvent removed *in vacuo*. The residue was re-suspended in water (50-100 mL) and lyophilised to give the product.

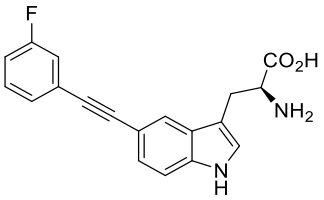
(*S*)-2-Amino-3-(5-(phenylethynyl)-1*H*-indol-3-yl)propanoic acid 19



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and phenylacetylene **16** (33 μL, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-(5-(phenylethynyl)-1*H*-indol-3-yl)propanoic acid **19** (29.4 mg, 97%) as a fluffy, white solid; $[\alpha]_D$ -41.1° (c 0.27, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 3.03 (1H, dd, *J*(H,H)= 14.1, 8.4 Hz, CH_AH$_B$), 3.37 (1H, d, *J*(H,H)= 14.4 Hz, CH$_A$$H_B$), 3.68 (1H, bs, CH), 7.22 (1H, s, ArH), 7.26 (1H, dd, *J*(H,H)= 8.4, 1.5 Hz, ArH), 7.29-7.37 (4H, m, ArH), 7.48-7.51 (2H, m, ArH), 7.96 (1H, d, *J*(H,H)= 0.7 Hz, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 31.0 (CH_2), 57.5 (CH), 87.4 (C), 92.5 (C), 111.9 (C), 112.5 (CH), 114.6 (C), 123.8 (CH), 125.6 (C), 125.96 (CH), 126.02 (CH), 128.7 (CH), 128.9 (C), 129.4 (CH), 132.2 (CH), 138.0 (C), 179.3 (CO); MS (ESI) 305 (100) $[M+H]^+$, 288 (80); HRMS: *m*/*z* calcd for $C_{19}H_{17}N_2O_2$ $[M+H]^+$: 305.1285; found: 305.1273.

The reaction was repeated to give 22.0 mg (73%) and 21.4 mg (70%) of the expected product **19** to give an average yield of 80% over three reactions.

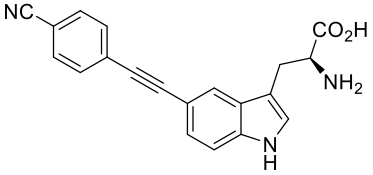
(*S*)-2-Amino-3-5-((3-fluorophenyl)ethynyl)-1*H*-indol-3-yl)propanoic acid 36



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and 3-fluorophenylacetylene **24** (34 μL, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-5-((3-fluorophenyl)ethynyl)-1*H*-indol-3-yl)propanoic acid **36** (32.2 mg, 100%) as a white solid; $[\alpha]_D$ -37.7° (c 0.26, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 3.14 (1H, dd, *J*(H,H)= 15.0, 8.9 Hz, CH_AH$_B$), 3.45-3.49 (1H, m, CH$_A$$H_B$), 3.81 (1H, dd, *J*(H,H)= 8.9, 3.8 Hz, CH), 7.07 (1H, ddd, *J*(H,H)= 9.0, 2.7 Hz, *J*(H,F)= 0.6 Hz, ArH), 7.22 (1H, ddd, *J*(H,H)= 2.7, 1.0 Hz, *J*(H,F)= 9.6 Hz, ArH), 7.25 (1H, s, ArH), 7.28-7.33 (2H, m, ArH), 7.35-7.40 (2H, m, ArH), 8.00 (1H, d, *J*(H,H)= 0.6 Hz, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 29.0 (CH$_2$), 56.9 (CH), 86.3 (C), 93.5 (C), 110.6 (C), 112.7 (CH), 114.3 (C), 115.8 (d, *J*(C,F)= 21.4 Hz, CH), 118.6 (d, *J*(C,F)= 22.9 Hz, CH), 123.8 (C), 126.2 (CH), 126.4 (CH), 127.6 (d, *J*(C,F)= 9.5 Hz, C), 128.3 (d, *J*(C,F)= 2.7 Hz, CH), 128.7 (C), 131.3 (d, *J*(C,F)= 8.8 Hz, C), 138.2 (C) 164.0 (d, *J*(C,F)= 244.8 Hz, CF), 175.7 (CO); ^{19}F{^{1}H} NMR (470 MHz, d_4-MeOH) δ= -115.34 (1F, s); ^{19}F NMR (376 MHz, d_4-MeOH) δ= -115.31 (1F, dddd, *J*(H,F)= 9.6, 8.5, 5.8, 0.6 Hz); MS (ESI) 323 (60) [M+H]$^+$, 306 (50), 196 (80), 118 (100); HRMS: *m*/*z* calcd for $C_{19}H_{16}F_1N_2O_2$ [M+H]$^+$: 323.1190; found: 323.1190.

The reaction was repeated to give 32.2 mg (100%) and 28.9 mg (90%) of the expected product **36** to give an average yield of 97% over three reactions.

(*S*)-2-Amino-3-5-((4-cyanophenyl)ethynyl)-1*H*-indol-3-yl)propanoic acid 39

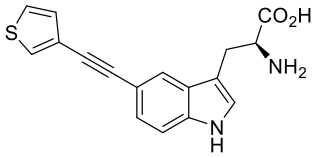


Using the standard procedure for tryptophans, 5-bromotryptophan **1**8 (28 mg, 0.1 mmol, 1.0 eq) and 4-cyanophenylacetylene **25** (38 mg, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-5-((3-cyanophenyl)ethynyl)-1*H*-indol-3-yl)propanoic acid **39** (26.1 mg, 79%) as a brown solid; $[\alpha]_D$ -37.7° (c 0.30, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 3.19 (1H, dd, *J*(H,H)= 15.2, 8.7 Hz, CH_AH$_B$), 3.50 (1H, dd, *J*(H,H)= 15.2, 4.0 Hz, CH$_A$$H_B$), 3.81 (1H, dd, *J*(H,H)= 8.7, 4.0 Hz, CH), 7.28 (1H, s, ArH), 7.32 (1H, dd, *J*(H,H)= 8.4, 1.3 Hz, ArH), 7.40 (1H, d, *J*(H,H)= 8.4 Hz,

ArH), 7.65-7.66 (2H, m, ArH), 7.72-7.74 (2H, m, ArH), 8.03 (1H, s, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 28.4 (CH_2), 56.7 (CH), 86.4 (C), 97.3 (C), 110.4 (C), 111.8 (C), 112.8 (CH), 113.8 (C), 119.5 (C), 124.2 (CH), 126.3 (CH), 126.7 (C), 128.6 (C), 130.6 (C), 132.9 (CH), 133.4 (CH), 138.5 (C), 174.6 (CO); MS (ESI) 330 (100) $[M+H]^+$, 313 (90), 196 (90), 118 (100); HRMS: *m*/*z* calcd for $C_{20}H_{16}N_3O_2$ $[M+H]^+$: 330.1237; found: 330.1237.

The reaction was repeated to give 25.9 mg (79%) and 26.0 mg (79%) of the expected product **39** to give an average yield of 79% over three reactions.

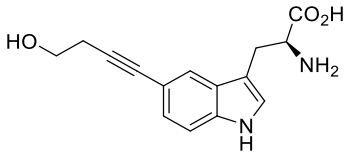
(*S*)-2-Amino-3-(5-(thiophen-3-ylethynyl)-1*H*-indol-3-yl)propanoic acid 42



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and 3-ethynylthiophene **26** (30 μL, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-(5-(thiophen-3-ylethynyl)-1*H*-indol-3-yl)propanoic acid **42** (28.1 mg, 91%) as a fluffy, white solid; $[\alpha]_D$ -47.8° (c 0.23, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 3.03 (1H, dd, *J*(H,H)= 12.4, 7.9 Hz, CH_AH$_B$), 3.37 (1H, d, *J*(H,H)= 14.3 Hz, CH$_A$$H_B$), 3.68 (1H, bs, CH), 7.18 (1H, dd, *J*(H,H)= 5.0, 1.1 Hz, ArH), 7.21 (1H, s, ArH), 7.23 (1H, dd, *J*(H,H)= 8.4, 1.3 Hz, ArH), 7.33 (1H, d, *J*(H,H)= 8.4 Hz, ArH), 7.42 (1H, dd, *J*(H,H)= 5.0, 3.0 Hz, ArH), 7.54 (1H, dd, *J*(H,H)= 3.0, 1.1 Hz, ArH), 7.94 (1H, s, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 30.9 (CH_2), 57.5 (CH), 82.6 (C), 91.7 (C), 111.8 (C), 112.5 (CH), 114.6 (C), 123.6 (CH), 124.5 (C), 125.9 (CH), 126.0 (CH), 1264 (CH), 128.54 (CH), 128.9 (C), 130.8 (CH), 137.9 (C), 179.5 (CO); MS (ESI) 311 (100) $[M+H]^+$, 294 (90); HRMS: *m*/*z* calcd for $C_{17}H_{15}N_2O_2S_1$ $[M+H]^+$: 311.0849; found: 311.0837.

The reaction was repeated to give 19.8 mg (64%) and 22.1 mg (71%) of the expected product **42** to give an average yield of 75% over three reactions.

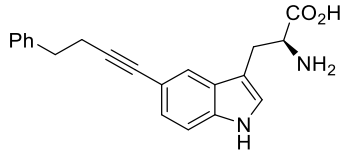
(*S*)-2-Amino-3-(5-(4-hydroxybut-1-yn-1-yl)-1*H*-indol-3-yl)propanoic acid 45



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and 3-butyn-1-ol **27** (23 μL, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-(5-(4-hydroxybut-1-yn-1-yl)-1*H*-indol-3-yl)propanoic acid **45** (21.4 mg, 79%) as a fluffy, white solid; $[\alpha]_D$ -30.0° (c 0.23, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 2.61 (2H, t, *J*(H,H)= 6.9 Hz, CH_2), 2.93 (1H, dd, *J*(H,H)= 14.3, 8.2 Hz, CH_AH$_B$), 3.26 (1H, dd, *J*(H,H)= 14.3, 4.3 Hz, CH$_A$$H_B$), 3.53-3.56 (1H, m, CH), 3.73 (2H, t, *J*(H,H)= 7.0 Hz, CH_2), 7.12 (1H, dd, *J*(H,H)= 8.4, 1.4 Hz, ArH), 7.16 (1H, s, ArH), 7.25 (1H, d, *J*(H,H)= 8.4 Hz, ArH), 7.80 (1H, s, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 24.4 (CH_2), 32.1 (CH_2), 57.9 (CH), 62.1 (CH_2), 84.2 (C), 84.3 (C), 112.2 (CH), 112.5 (C), 115.1 (C), 123.6 (CH), 125.6 (CH), 126.0 (CH), 128.9 (C), 137.6 (C), 181.3 (CO); MS (ESI) 273 (100) $[M+H]^+$, 256 (30), 198 (50), 196 (100), 158 (70); HRMS: *m*/*z* calcd for $C_{15}H_{17}N_2O_3$ $[M+H]^+$: 273.1234; found: 273.1233.

The reaction was repeated to give 17.3 mg (64%) and 27.0 mg (99%) of the expected product **45** to give an average yield of 81% over three reactions.

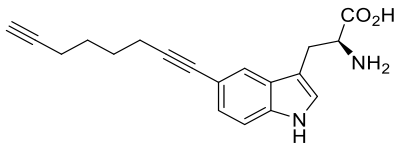
(*S*)-2-Amino-3-(5-(4-phenylbut-1-yn-1-yl)-1*H*-indol-3-yl)propanoic acid 48



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and 4-phenyl-1-butyne **28** (42 μL, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-(5-(4-phenylbut-1-yn-1-yl)-1*H*-indol-3-yl)propanoic acid **48** (8.9 mg, 27%) as a light brown solid; $[\alpha]_D$ -24.8° (c 0.27, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 2.67 (2H, t, *J*(H,H)= 7.5 Hz, CH_2), 2.90 (2H, t, *J*(H,H)= 7.5 Hz, CH_2), 3.00 (1H, dd, *J*(H,H)= 14.3, 8.6 Hz, CH_AH$_B$), 3.33-3.37 (1H, m, CH$_A$$H_B$), 3.67 (1H, bs, CH), 7.08 (1H, dd, *J*(H,H)= 8.3, 1.3 Hz, ArH), 7.17-7.21 (2H, m, ArH), 7.25-7.31 (5H, m, ArH), 7.75 (1H, s, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 23.1 (CH_2), 31.2 (CH_2), 37.1 (CH_2), 57.9 (CH), 84.4 (C), 87.4 (C), 112.0 (C), 112.7 (CH), 116.0 (CH), 123.7 (CH), 126.2 (CH), 126.6 (CH), 127.7 (CH), 129.2 (C), 129.8 (CH), 130.1 (CH), 138.1 (C) 142.9 (C), 179.5 (CO); MS (ESI) 333 (100) $[M+H]^+$, 316 (60); HRMS: *m*/*z* calcd for $C_{21}H_{21}N_2O_2$ $[M+H]^+$: 333.1598; found: 333.1591.

The reaction was repeated to give 8.3 mg (25%) and 8.7 mg (26%) of the expected product **48** to give an average yield of 26% over three reactions.

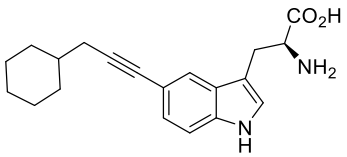
(*S*)-2-Amino-3-(5-(octa-1,7-diyn-1-yl)-1*H*-indol-3-yl)propanoic acid 51



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and 1,7-octadiyne **29** (40 μL, 0.3 mmol, 3.0 eq) gave (*S*)-2-amino-3-(5-(octa-1,7-diyn-1-yl)-1*H*-indol-3-yl)propanoic acid **51** (17.2 mg, 56%) as a tan solid; $[\alpha]_D$ -23.1° (c 0.29, MeOH); ^{1}H NMR (500 MHz, d_4-MeOH) δ = 1.68-1.75 (4H, m, CH_2), 2.22-2.27 (3H, CH, CH_2), 2.42-2.45 (2H, m, CH_2), 2.98 (1H, dd, *J*(H,H)= 14.6, 8.5 Hz, CH_AH$_B$), 3.33-3.35 (1H, m, CH$_A$$H_B$), 3.65 (1H, bs, CH), 7.11 (1H, dd, *J*(H,H)= 8.4, 1.4 Hz, ArH), 7.17 (1H, s, ArH), 7.26 (1H, d, *J*(H,H)= 8.4 Hz, ArH), 7.28 (1H, s, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 18.6 (CH_2), 19.6 (CH_2), 28.9 (CH_2), 29.2 (CH_2), 31.0 (CH_2), 57.5 (CH), 69.7 (CH), 83.5 (C), 84.8 (C), 86.9 (C), 111.6 (C), 112.2 (CH), 115.5 (CH), 123.2 (CH), 125.7 (CH), 126.1 (CH), 128.7 (C) 137.5 (C), 179.4 (CO); MS (ESI) 309 (100) $[M+H]^+$, 292 (70); HRMS: *m*/*z* calcd for $C_{19}H_{21}N_2O_2$ $[M+H]^+$: 309.1598; found: 309.1586.

The reaction was repeated to give 15.0 mg (49%) and 16.0 mg (52%) of the expected product **51** to give an average yield of 52% over three reactions.

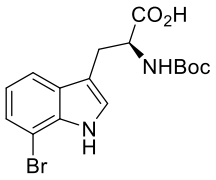
(*S*)-2-Amino-3-(3-cyclohexylprop-1-yn-1-yl)-1*H*-indol-3-yl)propanoic acid 54



Using the standard procedure for tryptophans, 5-bromotryptophan **18** (28 mg, 0.1 mmol, 1.0 eq) and 3-cyclohexyl-1-propyne **30** (43 μL, 0.3 mmol, 3.0 eq) at 100 °C for 8 h gave (*S*)-2-amino-3-(3-cyclohexylprop-1-yn-1-yl)propanoic acid **54** (8 mg, ~25%) as a fluffy, white solid that contained the sXPhos **12** ligand as an inseparable impurity; ^{1}H NMR (500 MHz, d_4-MeOH) δ = 1.10 (2H, dtt, *J*(H,H)= 12.5, 12.4, 3.1 Hz, CH_2), 1.17-1.43 (2H, m, CH_2), 1.50-1.58 (1H,

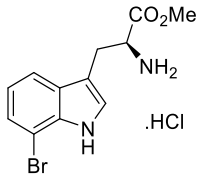
m, CH), 1.68-1.84 (2H, m, CH_2), 1.90-1.95 (2H, m, CH_2), 2.29 (2H, d, *J*(H,H)= 6.7 Hz, CH_2), 2.93 (1H, dd, *J*(H,H)= 14.2, 8.4 Hz, CH_AH_B), 3.328-3.30 (1H, m, CH_AH_B), 3.59 (1H, s, CH), 7.09 (1H, dd, *J*(H,H)= 8.4, 1.4 Hz, ArH), 7.16 (1H, s, ArH), 7.25 (1H, d, *J*(H,H)= 8.4 Hz, ArH), 7.76 (1H, s, ArH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 27.0 (CH_2), 27.4 (CH_2), 27.5 (CH_2), 31.7 (CH_2), 33.9 (CH_2), 39.2 (CH), 57.8 (CH), 84.2 (C), 86.1 (C), 112.1 (CH), 115.6 (C), 123.2 (CH), 125.6 (CH), 126.0 (CH), 128.8 (C), 137.4 (C), 147.9 (C), 180.7 (CO); MS (ESI) 325 (100) $[M+H]^+$, 308 (60); HRMS: *m*/*z* calcd for $C_{20}H_{25}N_2O_2$ $[M+H]^+$: 325.1911; found: 325.1890.

_N_-Boc-7-bromo-_S_-tryptophan 66



A suspension of 7-bromo-S-tryptophan **22** (100 mg, 0.35 mmol, 1.0 eq) and di-*t*-butyl dicarbonate (92 mg, 0.42 mmol, 1.2 eq) in 1,4-dioxane-water (1:1, 2 mL) was cooled to 0 °C. Aqueous KOH (1 M, 0.45 mL, 0.45 mmol, 1.25 eq) was added dropwise. The mixture was stirred for 4 h while warming to room temperature. The reaction was diluted with water (10 mL) and extracted with diethyl ether (2 x 10 mL). The aqueous layer was cooled in an ice-bath and the pH was adjusted to 2 using 1 M HCl. The resultant white suspension was extracted using ethyl acetate (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to give *N*-Boc-7-bromo-*S*-tryptophan **66** (120 mg, 89%) as a white, waxy solid that was used without further purification; ^{1}H NMR (300 MHz, d_4-MeOH) δ = 1.37 (9H, s, CH_3), 3.11 (1H, dd, *J*(H,H)= 14.6, 7.8 Hz, CH_AH_B), 3.31 (1H, dd, *J*(H,H)= 14.9, 5.1 Hz, CH_AH_B), 4.43 (1H, dd, *J*(H,H)= 7.8, 5.1 Hz, CH), 6.94 (1H, d, *J*(H,H)= 7.7 Hz, ArH), 7.17 (1H, s, ArH), 7.26 (1H, d, *J*(H,H)= 7.5 Hz, ArH), 7.56 (1H, dd, *J*(H,H)= 7.9, 0.7 Hz, ArH), 10.55 (1H, bs, NH); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 27.3 (CH_3), 24.7 (CH_2), 54.4 (CH), 79.2 (C), 104.1 (C), 111.18 (C), 111.23 (C), 117.5 (CH), 119.6 (CH), 123.5 (CH), 124.2 (CH), 124.3 (CH), 129.1 (C), 156.3 (CO), 174.3 (CO); MS (ESI) 407 (100) $[M(^{81}Br)+Na]^+$, 405 (100) $[M(^{79}Br)+Na]^+$; HRMS: *m*/*z* calcd for $C_{16}H_{19}Br_1N_2Na_1O_4$ $[M(^{79}Br)+Na]^+$: 405.0420; found: 405.0415.

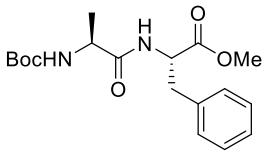
7-Bromo-_S_-tryptophan methyl ester hydrochloride 67



Thionyl chloride (290 µL, 4 mmol, 4.0 eq) was added to a flask containing dry methanol (10 mL) at 0 °C under argon. After stirring for 10 min, 7-bromo-*S*-tryptophan **22** (285 mg, 1 mmol, 1.0 eq) was added. The reaction was stirred overnight while allowing it to warm to r.t. The solvent was removed *in vacuo* to give 7-bromo-*S*-tryptophan methyl ester hydrochloride **67** (330 mg, 100%) as an off white hydrochloride salt that was used without further purification.

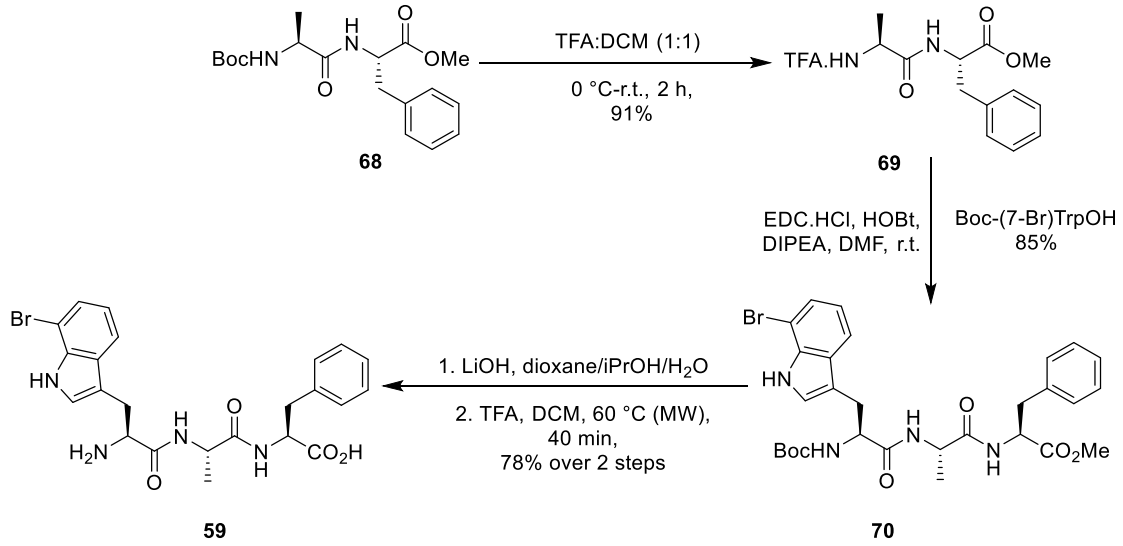
NMR on the HCl salt (d_6-DMSO) revealed broad signals. Hence, an analytical sample was obtained by desalting with dilute $NaHCO_3$ solution and extraction with ethyl acetate. Drying ($MgSO_4$) and evaporation of the solvent afforded the free base suitable for NMR in $CDCl_3$; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.08 (1H, dd, *J*(H,H)= 14.4, 7.4 Hz, CH_AH$_B$), 3.27 (1H, ddd, *J*(H,H)= 14.4, 5.0, 0.7 Hz, CH$_A$$H_B$), 3.73 (3H, s, CH_3), 3.85 (1H, dd, *J*(H,H)= 7.4, 5.0 Hz, CH), 7.03 (1H, d, *J*(H,H)= 7.8 Hz, ArH), 7.15 (1H, d, *J*(H,H)= 2.2 Hz, ArH), 7.37 (1H, dd, *J*(H,H)= 7.7, 0.7 Hz, ArH), 7.58 (1H, d, *J*(H,H)= 7.9 Hz, ArH), 8.37 (1H, bs, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 30.9 (CH_2), 52.2 (CH_3), 55.1 (CH), 105.0 (C), 112.7 (C), 118.2 (CH), 120.9 (CH), 123.6 (CH), 124.7 (CH), 128.9 (C), 135.1 (C), 175.8 (CO); MS (ESI) 299 (100) [M(^{81}Br)+H]$^+$, 297 (100) [M(^{79}Br)+H]$^+$, 282 (60); HRMS: *m*/*z* calcd for $C_{12}H_{14}Br_1N_2O_2$ [M(^{79}Br)+H]$^+$: 297.0233; found: 297.0228.

_N_-Boc-Ala-Phe-OMe 68

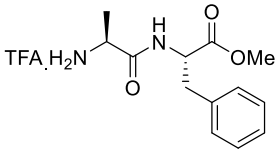


EDC.HCl (536 mg, 2.8 mmol, 1.2 eq) and HOBt (375mg, 2.8 mmol, 1.2 eq) were added to a microwave tube containing Boc-Ala-OH (440 mg, 2.3 mmol, 1.0 eq) in DMF (4.6 mL). The mixture was stirred for 5 min, then DIPEA (1 mL, 5.8 mmol, 2.5 eq) was added and the reaction mixture was stirred for 10 min. H-Phe-OMe.HCl (500 mg, 2.3 mmol, 1.0 eq) was added in one portion and reaction vial was sealed. The reaction was heated in a microwave reactor at 60 °C for 20 min. After cooling r.t., the reaction mixture was diluted with ethyl acetate (25 mL) and washed with water (3 x 10 mL), dilute sodium bicarbonate solution (10 mL) and brine (10 mL), dried over anhydrous $MgSO_4$ and the solvent removed *in vauco* to give *N*-Boc-Ala-Phe-OMe

68 (750 mg, 90%) as a white, waxy solid that was used without further purification; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.28 (3H, d, *J*(H,H)= 7.0 Hz, CH_3), 1.42 (9H, s, CH_3), 3.05 (1H, dd, *J*(H,H)= 13.8, 6.3 Hz, CH_AH_B), 3.13 (1H, dd, *J*(H,H)= 13.8, 5.9 Hz, CH_AH_B), 3.67 (3H, s, CH_3), 4.18 (1H, bs, CH), 4.83 (1H, q, *J*(H,H)= 6.2 Hz, CH), 5.27 (1H, bs, NH), 6.83 (1H, bs, NH), 7.03-7.15 (2H, m, ArH), 7.16-7.37 (3H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 18.4 (CH_3), 28.8 (CH_3), 37.9 (CH_2), 50.0 (CH), 52.3 (CH_3), 53.3 (CH), 79.9 (C), 127.0 (CH), 128.5 (CH), 129.3 (CH), 135.9 (C), 155.4 (CO), 171.8 (CO), 172.5 (CO).

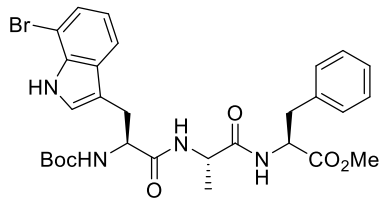


H-Ala-Phe-OMe.TFA 69



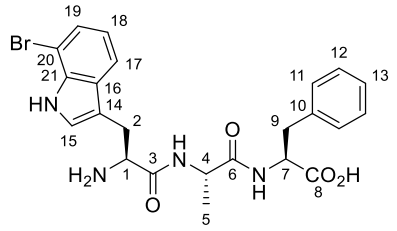
Water (0.2 mL) and TFA (2 mL) were added to a solution of *N*-Boc-Ala-Phe-OMe **68** (300 mg, 0.86 mmol, 1.0 eq) in DCM (3 mL) at 0 °C and stirred for 2 h, while warming to r.t. The solvent was removed *in vacuo*. The resulting pale, yellow oil was titurated with ice cold diethyl ether (3 x 5 mL) to give H-Ala-Phe-OMe.TFA **69** (310 mg, 91%) as an off white solid that was used without further purification; ^{1}H NMR (400 MHz, d_4-MeOH) δ = 1.50 (3H, d, *J*(H,H)= 7.1 Hz, CH_3), 3.02 (1H, dd, *J*(H,H)= 14.0, 9.2 Hz, CH_AH_B), 3.23 (1H, dd, *J*(H,H)= 14.0, 5.4 Hz, CH_AH_B), 3.73 (3H, s, CH_3), 3.88 (1H, q, *J*(H,H)= 7.1 Hz, CH), 4.73 (1H, dd, *J*(H,H)= 9.2, 5.4 Hz, CH), 7.24 (3H, dt, *J*(H,H)= 9.4, 3.2 Hz, ArH), 7.36-7.28 (2H, m, ArH).

***N*-Boc-Trp-(7-Br)-Ala-Phe-OMe 70**

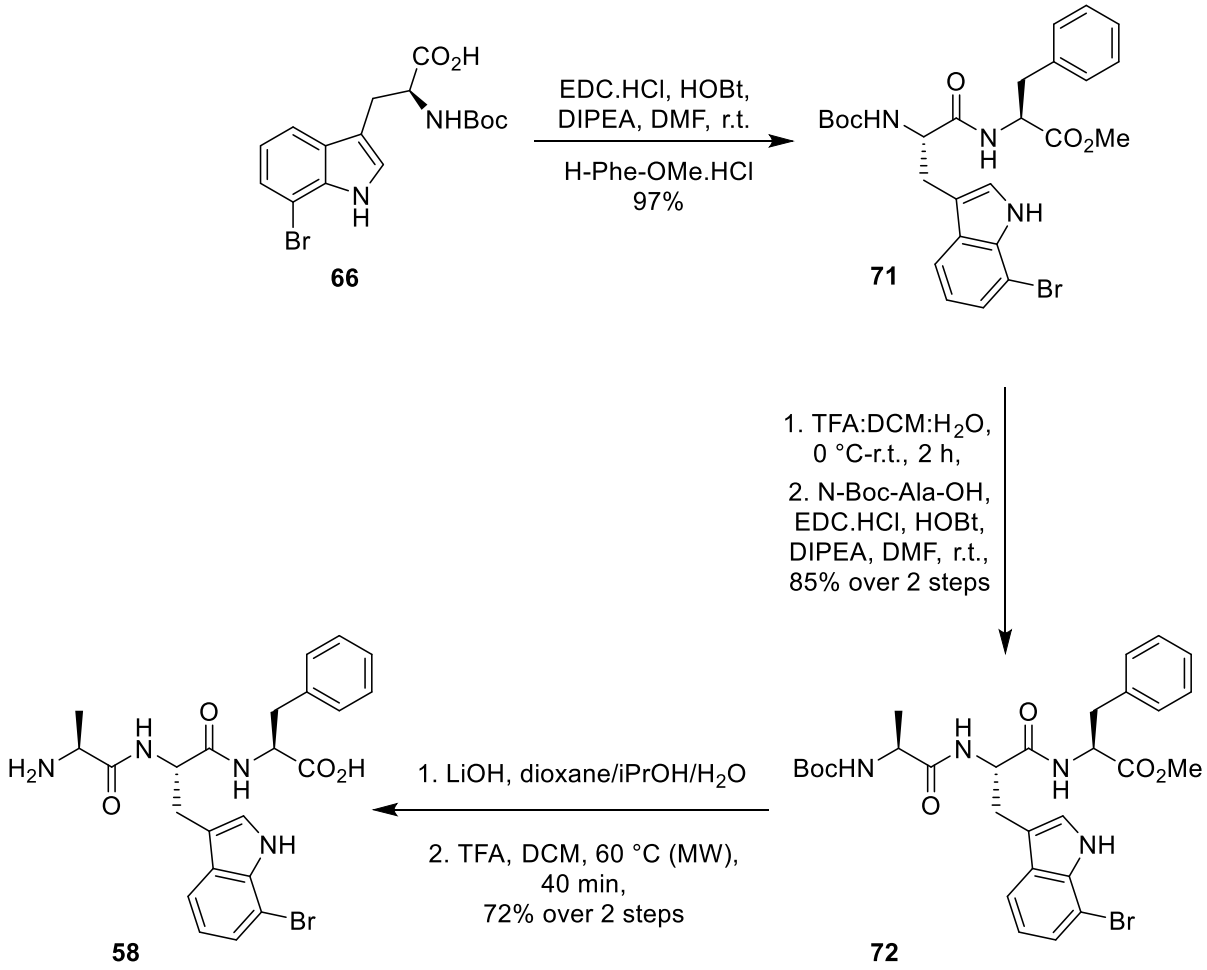


A solution of *N*-Boc-Trp-(7-Br)-OH **66** (220 mg, 0.57 mmol, 1.0 eq), H-Ala-Phe-OMe.TFA **69** (210 mg, 0.57 mmol, 1.0 eql) in DMF (4 mL) was cooled to 0 °C. Solid EDC.HCl (132 mg, 0.7 mmol, 1.2 eq) and HOBt (95 mg, 0.7 mmol, 1.2 eq) were added. The reaction mixture was stirred for 5 min, then DIPEA (0.28 mL, 1.6 mmol, 2.8 eq) was added dropwise. The reaction mixture was stirred for 20 h, while warming to r.t. The reaction mixture was diluted with ethyl acetate (20 mL) and washed successively with water (3 x 10 mL) and brine (10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. Purification by column chromatography using silica gel (ethyl acetate:hexanes, 10-90%) gave *N*-Boc-Trp-(7-Br)-Ala-Phe-OMe **70** (300 mg, 85%) as a waxy, white solid; ^{1}H NMR (300 MHz, d_4-MeOH) δ = 1.25 (3H, d, *J*(H,H)= 6.9 Hz, CH_3), 1.35 (9H, s, CH_3), 2.91-3.27 (4H, m, CH_2), 3.66 (3H, s, CH_3), 4.26-4.44 (2H, m, CH, CH), 4.61 (1H, d, *J*(H,H)= 6.0 Hz, CH), 6.92 (1H, t, *J*(H,H)= 7.7 Hz, ArH), 7.13-7.21 (4H, m, ArH), 7.22-7.28 (3H, m, ArH), 7.59 (1H, d, *J*(H,H)= 7.8 Hz, ArH); ^{13}C NMR (75 MHz, d_4-MeOH) δ= 18.4 (CH_3), 28.4 (CH_3), 28.5 (CH_2), 37.9 (CH_2), 49.0 (CH), 52.5 (CH_3), 53.6 (CH), 55.0 (CH), 80.2 (C), 104.9 (C), 111.8 (C), 118.2 (CH), 128.8 (CH), 124.0 (CH), 124.6 (CH), 127.2 (CH), 128.7 (CH), 128.8 (CH), 129.3 (CH), 134.9 (C), 136.0 (C), 155.6 (CO), 171.6 (CO), 171.8 (CO), 171.9 (CO); MS (ESI) 639 (100) [M(^{81}Br)+Na]$^+$, 637 (100) [M(^{79}Br)+Na]$^+$, 617 (15) [M(^{81}Br)+H]$^+$, 615 (15) [M(^{79}Br)+H]$^+$; HRMS: *m*/*z* calcd for $C_{29}H_{36}Br_1N_4O_6$ [M(^{79}Br)+H]$^+$: 615.1813; found: 615.1799.

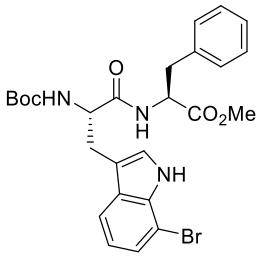
H-Trp-(7-Br)-Ala-Phe-OH 59



A solution of lithium hydroxide (14 mg, 0.6 mmol, 1.5 eq) in water (1 mL) was added to a solution of *N*-Boc-Trp-(7-Br)-Ala-Phe-OMe **70** (250 mg, 0.4 mmol, 10 eq) in 1,4-dioxane-iPrOH-water (1:1:0.5, 4 mL) at 0 °C. The reaction mixture was stirred vigorously until all the starting material has been consumed (as determined by TLC analysis). The reaction mixture was diluted with water (2 mL) and quenched by the addition of Amberlyst-120-H+ (to pH ~5-6). The resin was filtered and the solvent removed *in vacuo* to give the crude free acid (*N*-Boc-Ala-Phe-Trp-(7-Br)-OH). This intermediate was dissolved in DCM (3 mL) and transferred to a microwave vial. TFA (65 μL, 2 mmol, 5.0 eq) was added and reaction vial was sealed with an aluminium crimp cap. Reaction mixture was heated in a microwave reactor at 60 °C for 40 min. After cooling, the solvent was removed *in vacuo*. The residue was purified using gradient reversed phase chromatography (C-18, 12 g, water-MeOH, 5-95% gradient) to give H-Trp-(7-Br)-Ala-Phe-OH.TFA **59** (190 mg, 78% over 2 steps) as a white solid; ^{1}H NMR (500 MHz, d_4-MeOH) δ = 1.37 (3H, d, *J*(H,H)= 7.1 Hz, CH_3-C5), 3.00-3.03 (1H, dd, *J*(H,H)= 14.0, 8.1 Hz, CH_AH_B-C9), 3.12 (1H, dd, *J*(H,H)= 15.2, 9.0 Hz, CH_AH_B-C2), 3.21 (1H, dd, *J*(H,H)= 14.0, 5.2 Hz, CH_AH_B-C9), 3.36 (1H, dd, *J*(H,H)= 15.2, 5.2 Hz, CH_AH_B-C2), 4.11 (1H, dd, *J*(H,H)= 9.0, 5.2 Hz, CH-C1), 4.41 (1H, q, *J*(H,H)= 7.1 Hz, CH-C4), 4.63 (1H, dd, *J*(H,H)= 8.1, 5.2 Hz, CH-C7), 6.94 (1H, dd, *J*(H,H)= 7.8, 7.7 Hz, ArH-C18), 7.17 (1H, ddd, *J*(H,H)= 4.6, 4.1, 4.1 Hz, ArH-C15), 7.26-7.27 (5H, m, ArH-C11,C12,13), 7.30 (1H, d, *J*(H,H)= 7.5 Hz, ArH-C19), 7.66 (1H, d, *J*(H,H)= 7.9 Hz, ArH-C17); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 18.3 (CH_3, C5), 28.9 (CH_2, C2), 38.3 (CH_2, C9), 50.5 (CH, C4), 54.6 (CH, C1), 55.3 (CH, C7), 105.8 (C, C20), 109.4 (C, C14), 118.6 (C, C17), 121.5 (CH, C18), 125.5 (CH, C19), 126.9 (CH, C13), 127.8 (CH, C15), 129.5 (CH, C10), 129.9 (C, C16), 130.5 (C, C11), 136.8 (C, C21), 138.4 (C, C10), 169.6 (CO, C3), 174.1 (CO, C6), 174.6 (CO, C8); HRMS: *m*/*z* calcd for $C_{23}H_{26}Br_1N_4O_4$ $[M(^{79}Br)+H]^+$: 501.1132; found: 501.1132.



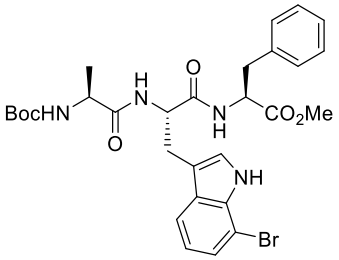
_N_-Boc-Trp-(7-Br)-Phe-OMe 71



A solution of *N*-Boc-Trp-(7-Br)-OH **66** (135 mg, 0.37 mmol, 1.0 eq), H-Phe-OMe.HCl (85 mg, 0.4 mmol, 1.1 eq) in DMF (2 mL) was cooled to 0 °C. Solid EDC.HCl (83 mg, 0.43 mmol, 1.2 eq) and HOBt (58 mg, 0.43 mmol, 1.2 eq) were added. The reaction mixture was stirred for 5 min, then DIPEA (0.19 mL, 1.1 mmol, 3.0 eq) was added dropwise. The reaction mixture was stirred for 16 h, while warming to r.t. The reaction mixture was diluted with ethyl acetate (20 mL) and washed successively with water (3 x 10 mL) and brine (10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. Purification by column chromatography using silica gel (MeOH:DCM, 0-10%) gave *N*-Boc-Trp-(7-Br)-

Phe-OMe **71** (194 mg, 97%) as a waxy, white solid; ^{1}H NMR (300 MHz, d_4-MeOH) δ= 1.36 (9H, s, CH_3), 2.86-3.06 (3H, m, CH, CH_AH$_B$), 3.15 (1H, dd, J(H,H)= 14.6, 5.9 Hz, CH$_A$$H_B$), 3.60 (3H, s, CH_3), 4.35 (1H, t, J(H,H)= 6.7 Hz, CH), 4.62 (1H, t, J(H,H)= 6.7 Hz, CH), 6.93 (1H, t, J(H,H)= 7.7 Hz, ArH), 7.06-7.17 (3H, m, ArH), 7.18-7.28 (4H, m, ArH), 7.57 (1H, d, J(H,H)= 7.8 Hz, ArH); ^{13}C NMR (75 MHz, d_4-MeOH) δ= 27.3 (CH_3), 27.9 (CH_2), 37.1 (CH_2), 51.3 (CH_3), 53.7 (CH), 55.2 (CH), 79.3 (C), 104.1 (C), 111.0 (C), 117.5 (CH), 119.6 (CH), 123.5 (C), 124.4 (CH), 126.5 (CH), 128.1 (CH), 128.9 (CH), 129.1 (CH), 134.9 (C), 136.4 (C), 156.0 (CO), 171.6 (CO), 172.8 (CO); MS (ESI) 568 (100) [M(^{81}Br)+Na]$^+$, 566 (100) [M(^{79}Br)+Na]$^+$, 546 (14), 544 (14); HRMS: m/z calcd for $C_{29}H_{36}Br_1N_4O_6$ [M(^{79}Br)+H]$^+$: 544.1442; found: 544.1431.

_N_-Boc-Ala-Trp-(7-Br)-Phe-OMe 72

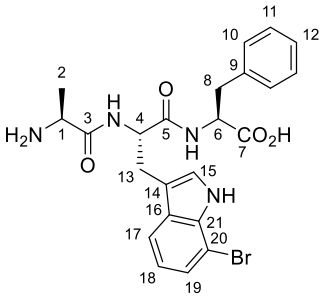


Water (0.1 mL) and TFA (1 mL) were added to a solution of *N*-Boc-Try-(7-Br)-Phe-OMe **71** (190 mg, 0.34 mmol, 1.0 eq) in DCM (2 mL) at 0 °C. The reaction mixture was stirred for 2 h, while warming to r.t. The solvent was removed *in vacuo*. The resultant pale yellow oil was triturated with cold diethyl ether (3 x 5 mL) to afford H-Trp-(7-Br)-Phe-OMe.TFA as a white foam.

A solution of H-Trp-(7-Br)-Phe-OMe.TFA (190 mg, 0.34 mmol, 1.0 eq) and *N*-Boc-Ala-OH (70 mg, 0.37 mmol, 1.1 eq) in DCM:DMF (1:1, 4 mL) was cooled to 0 °C. EDC.HCl (78 mg, 0.4 mmol, 1.2 eq) and HOBt (54 mg, 0.4 mmol, 1.2 eq) were added to the reaction mixture. DIPEA (0.2 mL, 1.2 mmol, 3.5 eq) was added dropwise and the reaction mixture was warmed to r.t. and stirred for 16 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed successively with water (3 x 10 mL) and brine (10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* to give *N*-Boc-Ala-Trp-(7-Br)-Phe-OMe **72** (190 mg, 85% over 2 steps) as a waxy, white solid that was used without further purification; ^{1}H NMR (500 MHz, d_6-DMSO) δ= 1.07 (3H, d, J(H,H)= 7.1 Hz, CH_3), 1.35 (9H, s, CH_3), 2.88-2.97 (2H, m, CH_AH$_B$, CH_AH$_B$), 3.00 (1H, dd, J(H,H)= 13.7, 6.1 Hz, CH$_A$$H_B$), 3.07 (1H, dd, J(H,H)= 14.7, 5.6 Hz, CH$_A$$H_B$), 3.56 (3H, s, CH_3), 3.86-4.00 (1H,

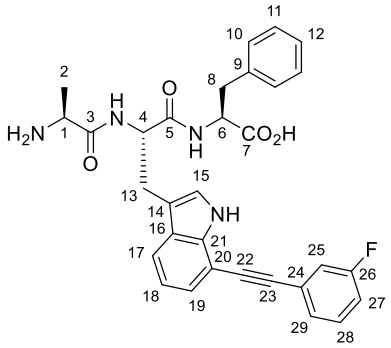
m, CH), 4.46 (1H, t, *J*(H,H)= 7.2 Hz, CH), 4.52-4.62 (1H, m, CH), 6.89 (1H, d, *J*(H,H)= 7.6 Hz, ArH), 6.93 (1H, t, *J*(H,H)= 7.7 Hz, ArH), 7.13-7.22 (4H, m, ArH), 7.23-7.31 (3H, m, ArH), 7.58 (1H, d, *J*(H,H)= 7.8 Hz, ArH); ^{13}C NMR (125 MHz, d_6-DMSO) δ = 18.3 (CH_3), 28.1 (CH_2), 28.5 (CH_3), 37.0 (CH_2), 50.0 (CH), 52.2 (CH_3), 53.0 (CH), 54.0 (CH), 78.7 (C), 104.3 (C), 111.3 (C), 118.4 (CH), 120.1 (CH), 123.8 (CH), 125.3 (CH), 127.0 (CH), 128.7 (C), 129.3 (CH), 129.5 (CH), 134.4 (C), 137.2 (C), 155.4 (CO), 171.5 (CO), 172.0 (CO), 172.8 (CO); MS (ESI) 639 (100) [M(^{81}Br)+Na]$^+$, 637 (100) [M(^{79}Br)+Na]$^+$, 617 (15) [M(^{81}Br)+H]$^+$, 615 (15) [M(^{79}Br)+H]$^+$; HRMS: *m*/*z* calcd for $C_{29}H_{36}Br_1N_4O_6$ [M(^{79}Br)+H]$^+$: 615.1813; found: 615.1800.

H-Ala-Trp-(7-Br)-Phe-OH 58



Following the procedure reported for **59**, *N*-Boc-Ala-Trp-(7-Br)-Phe-OMe **72** (170 mg, 0.27 mmol, 1.0 eq) gave H-Ala-Trp-(7-Br)-Phe-OH **58** (120 mg, 72% over 2 steps) as a white solid; ^{1}H NMR (500 MHz, d_4-MeOH) δ= 1.37 (3H, d, *J*(H,H)= 7.1 Hz, CH_3-C5), 3.00-3.03 (1H, dd, *J*(H,H)= 14.0, 8.1 Hz, CH_AH_B-C9), 3.12 (1H, dd, *J*(H,H)= 15.2, 9.0 Hz, CH_AH_B-C2), 3.21 (1H, dd, *J*(H,H)= 14.0, 5.2 Hz, CH_AH_B-C9), 3.36 (1H, dd, *J*(H,H)= 15.2, 5.2 Hz, CH_AH_B-C2), 4.11 (1H, dd, *J*(H,H)= 9.0, 5.2 Hz, CH-C1), 4.41 (1H, q, *J*(H,H)= 7.1 Hz, CH-C4), 4.63 (1H, dd, *J*(H,H)= 8.1, 5.2 Hz, CH-C7), 6.94 (1H, dd, *J*(H,H)= 7.8, 7.7 Hz, ArH-C18), 7.17 (1H, ddd, *J*(H,H)= 4.6, 4.1, 4.1 Hz, ArH-C15), 7.26-7.27 (5H, m, ArH-C11,C12,13), 7.30 (1H, d, *J*(H,H)= 7.5 Hz, ArH-C19), 7.66 (1H, d, *J*(H,H)= 7.9 Hz, ArH-C17); ^{13}C NMR (125 MHz, d_4-MeOH) δ= 18.3 (CH_3, C5), 28.9 (CH_2, C2), 38.3 (CH_2, C9), 50.5 (CH, C4), 54.6 (CH, C1), 55.3 (CH, C7), 105.8 (C, C20), 109.4 (C, C14), 118.6 (C, C17), 121.5 (CH, C18), 125.5 (CH, C19), 126.9 (CH, C13), 127.8 (CH, C15), 129.5 (CH, C10), 129.9 (C, C16), 130.5 (C, C11), 136.8 (C, C21), 138.4 (C, C10), 169.6 (CO, C3), 174.1 (CO, C6), 174.6 (CO, C8); HRMS: *m*/*z* calcd for $C_{23}H_{26}Br_1N_4O_4$ [M(^{79}Br)+H]$^+$: 501.1132; found: 501.1128.

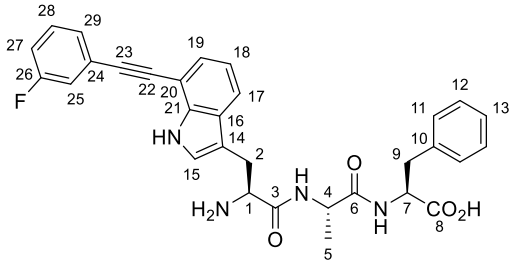
((*S*)-2-((*S*)-2-Aminopropanamido)-3-(7-((3-fluorophenyl)ethynyl)-1*H*-indol-3-yl)propanoyl)-L-phenylalanine 60



((*S*)-2-((*S*)-2-Aminopropanamido)-3-(7-bromo-1*H*-indol-3-yl)propanoyl)-L-phenylalanine **58** (4.0 mg, 8 μmol, 1.0 eq), sXPhos **12** (0.6 mg, 1.2 μmol, 15 mol%) and $CsCO_3$ (6.0 mg, 20 μmol, 2.5 eq) were added to a microwave tube and flushed with nitrogen. The microwave tube was sealed. A stock solution of *bis*(acetonitrile)dichloropalladium (II) (0.8 mg, 4 μmol, 0.5 eq) in degassed H_2O:CH_3CN (1:1, 1.0 mL) was prepared. The catalyst stock solution (0.1 mL, 0.4 μmol, 5 mol%) was injected into the microwave tube, followed by degassed H_2O:CH_3CN (1:1, 0.1 mL). 3-Fluorophenylacetylene **24** (10 μL, 80 μmol, 10.0 eq) was then injected into the microwave tube. The reaction mixture was well stirred, then heated at 100 °C for 2 h in a microwave. The reaction mixture was cooled to r.t. and diluted to 3 mL with H_2O:CH_3CN (9:1). The reaction mixture was centrifuged (13,000 rpm, 16060 g, 5 min) and the ultrafiltrate was collected and purified by HPLC purification as described above and lyophilised to give ((*S*)-2-((*S*)-2-aminopropanamido)-3-(7-((3-fluorophenyl)ethynyl)-1*H*-indol-3-yl)propanoyl)-L-phenylalanine **60** (2.0 mg, 47%) as a white solid; ^{1}H NMR (500 MHz, d_4-MeOH) δ = 1.24 (3H, d, *J*(H,H)= 6.9 Hz, CH_3-C2), 3.04 (1H, dd, *J*(H,H)= 13.5, 6.1 Hz, CH_AH$_B$-C8), 3.08 (1H, dd, *J*(H,H)= 14.6, 9.0 Hz, CH_AH$_B$-C13), 3.20 (1H, dd, *J*(H,H)= 13.5, 4.9 Hz, CH$_A$$H_B$-C8), 3.30-3.38 (1H, dd, *J*(H,H)= 14.6, 4.9 Hz, CH$_A$$H_B$-C13), 3.48 (1H, q, *J*(H,H)= 6.9 Hz, CH-C1), 4.45 (1H, dd, *J*(H,H)= 6.1, 4.9 Hz, CH-C6), 4.66 (1H, dd, *J*(H,H)= 9.0, 4.9 Hz, CH-C4), 7.06 (1H, dd, *J*(H,H)= 7.7, 7.6 Hz, ArH-C18), 7.10-7.21 (8H, m, ArH-C10,C11,C12,C15,C25), 7.30 (1H, dd, *J*(H,H)= 7.4, 0.7 Hz, ArH-C19), 7.38-7.44 (2H, m, ArH-C27,C28), 7.43-7.46 (1H, m, ArH-C29), 7.71 (1H, dd, *J*(H,H)= 8.0, 0.9 Hz, ArH-C17)- NOTE- the proton signal at 3.30-3.38 was obscured by the residual d_4-MeOH solvent peak, the position of the signal was determined by the 2D NMR experiments, with the coupling constants determined from the coupling constants of the protons on C4 and C13; ^{13}C NMR (125 MHz, d_4-MeOH) δ = 19.9 (CH_3, C2), 28.8 (CH_2, C13), 39.0 (CH_2, C8), 51.0 (CH, C1), 55.7 (CH, C4), 57.2 (CH, C6), 88.0 (C, C22), 92.4 (C, d, *J*(C,F)= 3.2 Hz, C23), 106.9 (C, C20), 112.1 (C, C14, C16), 116.3 (CH, d, *J*(C,F)= 21.8 Hz, C25), 119.0 (CH, d, *J*(C,F)= 23.1 Hz, C27), 121.0 (CH, C17), 125.5

(CH, C15), 126.3 (CH, C19), 127.0 (C, C24), 127.3 (CH, C12), 128.6 (CH, d, *J*(C,F)= 2.8 Hz, C29), 129.1 (CH, C10), 129.3 (C, C16), 130.8 (CH, C11), 131.4 (CH, d, *J*(C,F)= 8.8 Hz, C28), 138.2 (C, C21), 139.2 (C, C9), 164.0 (CF, d, *J*(C,F)= 244.4 Hz, C26), 172.6 (CO, C5), 176.7 (CO, C3), 177.3 (CO, C7); $^{19}F\{^{1}H\}$ NMR (376 MHz, d_4-MeOH) δ = -115.2 (1F, s); ^{19}F NMR (470 MHz, d_4-MeOH) δ = -115.2 (1F, ddd, *J*(H,F)= 9.5, 9.1, 5.6 Hz); MS (ESI) 541 (100) $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{31}H_{30}F_1N_4O_4$ $[M+H]^+$: 541.2246; found: 541.2221.

((S)-2-Amino-3-(7-((3-fluorophenyl)ethynyl)-1H-indol-3-yl)propanoyl)-L-alanyl-L-phenylalanine 61



Using the same procedure as **60**, ((*S*)-2-Amino-3-(7-bromo-1*H*-indol-3-yl)propanoyl)-L-alanyl-L-phenylalanine **59** (4 mg, 8 μmol, 1.0 eq) gave ((*S*)-2-amino-3-(7-((3-fluorophenyl)ethynyl)-1*H*-indol-3-yl)propanoyl)-L-alanyl-L-phenylalanine **61** (4.3 mg, 100%) as a white solid; ^{1}H NMR (500 MHz, d_4-MeOH) δ = 1.15 (3H, d, *J*(H,H)= 7.1 Hz, CH_3-C5), 3.00 (1H, dd, *J*(H,H)= 13.6, 6.8 Hz, CH_AH$_B$-C9), 3.02 (1H, dd, *J*(H,H)= 14.7, 7.3 Hz, CH_AH$_B$-C2), 3.17-3.22 (2H, m, CH$_A$$H_B$-C2,C9), 3.68 (1H, dd, *J*(H,H)= 7.3, 5.2 Hz, CH-C1), 4.29 (1H, q, *J*(H,H)= 7.1 Hz, CH-C4), 4.45 (1H, dd, *J*(H,H)= 6.8, 4.9 Hz, CH-C7), 7.04 (1H, dd, *J*(H,H)= 7.7, 7.5 Hz, ArH-C18), 7.09-7.13 (2H, m, ArH-C15,C25), 7.17-7.20 (5H, m, ArH-C11,C12,C13), 7.31 (1H, d, *J*(H,H)= 7.2 Hz, ArH-C19), 7.38-7.41 (2H, m, ArH-C27,28), 7.43-7.46 (1H, m, ArH-C29), 7.69 (1H, d, *J*(H,H)= 7.9 Hz, ArH-C17); ^{13}C NMR (125 MHz, d_4-MeOH) δ = 18.0 (CH_3, C5), 31.4 (CH_2, C2), 39.1 (CH_2, C9), 50.6 (CH, C4), 56.5 (CH, C1), 57.2 (CH, C7), 88.0 (C, C22), 92.4 (C, d, *J*(C,F)= 3.0 Hz, C23), 107.0 (C, C20), 111.8 (C, C14, C16), 116.3 (CH, d, *J*(C,F)= 21.5 Hz, C25), 119.0 (CH, d, *J*(C,F)= 23.1 Hz, C27), 119.9 (CH, C18), 121.1 (CH, C17), 125.9 (CH, C13), 126.3 (CH, C19), 127.0 (C, d, *J*(C,F)= 9.3 Hz, C24), 127.3 (CH, C15), 128.6 (CH, d, *J*(C,F)= 2.9 Hz, C29), 129.1 (CH, C11), 130.8 (CH, C12), 131.3 (CH, d, *J*(C,F)= 8.8 Hz, C28), 138.3 (C, C21), 139.3 (C, C10), 163.9 (CF, d, *J*(C,F)= 244.8 Hz, C26), 173.7 (CO, C6), 176.8 (CO, C3), 177.6 (CO, C8); $^{19}F\{^{1}H\}$ NMR (470 MHz, d_4-MeOH) δ = -115.2 (1F, s); ^{19}F NMR (470 MHz, d_4-MeOH) δ = -115.2 (1F, ddd, *J*(H,F)=

9.4, 9.1, 5.8 Hz); MS (ESI) 541 (100) $[M+H]^+$; HRMS: *m/z* calcd for $C_{31}H_{30}F_1N_4O_4$ $[M+H]^+$: 541.2246; found: 541.2224.

Enantiopurity analysis

The enantiopurity of the cross coupled products was analysed using Marfey's Reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, FDAA).

The tryptophan was dissolved in HCl solution (1 M, 10 mg/mL). An Eppendorf tube containing the tryptophan (50 μL), FDAA (1% *w/v* in actone, 100 μL) and $NaHCO_3$ solution (1M, 70 μL) was mixed thoroughly and incubated at 37 °C for 1 h. The reaction mixture was diluted (10 μL in 490 μL water) and centrifuged (13,000 rpm, 16060 g, 5 min) before UPLC analysis.

The UPLC analysis was carried out in acetonitrile/0.1% TFA in water at a gradient of 20% acetonitrile to 90% acetonitrile over 4.7 minutes. A 3:1 mixture of L-tryptophan to D-tryptophan was used as a standard. Two blank runs using acetone were conducted between each tryptophan sample. In all of the tryptophans analysed, only a single peak for the L-enantiomer was observed.

General Biological Experimental

Biological reagents and components for media, buffers and stock solutions were purchased from commercial suppliers, used without further purification and stored according to the supplier's instructions. Microorganisms were stored at -80 °C for long and short-term storage. Microorganisms were cultured under sterile conditions using a Faster BH-EN class II vertical laminar airflow cabinet and fermentation media was sterilised at 121 °C for 20 minutes at 1.3 bar in a Boxer Benchtop Denley autoclave prior to use. Alternatively, aqueous solutions of heat labile components were sterilised by passage through a 0.2 μm membrane. Culturing apparatus was sterilised by autoclaving as described above or alternatively, disposable pre-sterilised apparatus was used.

General apparatus: Pipetting of solutions and samples was done using LABNET Biopette autovclavable pipettes. Microbial cultures were incubated in a New Brunswick Scientific Innova 4300 incubator shaker, a New Brunswick Scientific I26 incubator shaker series, or Genlab incubator (static). pH measurements were taken using a Fisherbrand Hydrus 300 pH meter. Centrifugation was carried out using a Thermo Scientific IEC CL30R centrifuge.

***Kitasatospora cystargenia* culturing, and precursor directed biosynthesis of new to nature bromo-cystargamide**

Kitasatospora cystargenia NRRL-B16505 was obtained from the USDA agriculture research service culture collection. The strain was fermented in GY medium (10 g/L glucose, 10 g/L yeast extract) for 48 h at 28 °C (as previously reported by Kerr and coworkers)[3] and 200 rpm, in an incubator with a 2.54 cm throw. The mycelium from this culture was stored in 20% glycerol at -80 °C.

Small scale feeding experiments

In order to explore whether the halotryptophans could be incorporated too, we carried out small scale feeding experiments with 7, 6 and 5-chlorotryptophan and 7, 6 and5 -bromotryptophan at 0.25 and 1mM to both 4mL cultures in 24 deep well plates and 50mL cultures in 250mL Erlenmeyer flasks. The cultures were extracted and analysed by LCMSMS. Results demonstrated that all of the halotryptophans incorporated into cystargamide, generating new to nature halogenated analogues.

6Br-tryptophan 21 - Feeding at 0.25 mM scale

Detected Cystargamide Species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	5923338	10.0
Linear cystargamide[a]	972.4349	3036620	5.1
Cystargamide no hydroxylated[b]	938.4294	607443	1.0
(6BrW) Brominated cystargamide no hydroxylated[b]	1016.3400	122182	0.2
(6Brw) Brominated cystargamide	1032.3349	834562	1.4

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

<u>Ratios of the detected cystargamide species</u>:

Cyclic cystargamide species : Linear cystargamide species = **3:1**

Brominated cystargamide species : Brominated linear cystargamide species = **1:0**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **9:1**

Brominated cyclic cystargamide species : Brominated cystargamide no hydroxylated= **7:1**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	9574644	16.2
(6BrW) Brominated linear cystargamide[ALA] no hydroxylated[a],[b]	1048.3662	272892	0.5
(6BrW) Brominated linear cystargamide[ALA][a]	1064.3611	1312663	2.2

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

6Br-tryptophan 21 -Feeding at 1 mM scale

Detected Cystargamide species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	9135391	10.0
Linear cystargamide[a]	972.4349	4175252	4.6
Cystargamide no hydroxylated[b]	938.4294	54982	0.1
(6BrW) Brominated cystargamide no hydroxylated[b]	1016.3400	1035165	1.1
(6BrW) Brominated cystargamide	1032.3349	1928082	2.1

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

Ratios of the detected cystargamide species:

Cyclic cystargamide species : Linear cystargamide species = **3:1**

Brominated cystargamide species : Brominated linear cystargamide species = **1:0**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **10.4:1**

Brominated cyclic cystargamide species : Brominated cystargamide no hydroxylated= **2:1**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	15397915	16.9
Linear cystargamide[ALA] no hydroxylated[a],[b]	970.4557	60678	0.1
(6Br) Brominated linear cystargamide[ALA] no hydroxylated[a],[b]	1048.3662	1907165	2.1
(6Br) Brominated linear cystargamide[ALA][a]	1064.3611	2939735	3.2

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

5Br-tryptophan- Feeding at 1 mM scale

Detected Cystargamide species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	17929225	10.0
Linear cystargamide[a]	972.4349	600331	3.8
Cystargamide no hydroxylated[b]	938.4294	70645	0.3
(5BrW) Brominated cystargamide no hydroxylated[b]	1016.3400	312674	0.1

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

Ratios of the detected cystargamide species:

Cyclic cystargamide species : Linear cystargamide species = **3:1**

Brominated cystargamide species : Brominated linear cystargamide species = **1:0**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **21:1**

Brominated cyclic cystargamide species : Brominated cystargamide no hydroxylated= 0**:1**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	9536071	10.8
(5BrW) Brominated linear cystargamide[ALA] no hydroxylated[a],[b],[e]	1048.3662	266591	0.3

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10 [e] MS2 data was not recorded due to interfering masses

7Br-tryptophan-Feeding at 1 mM scale

Detected Cystargamide species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	3227118	10.0
Linear cystargamide[a]	972.4349	11589642	35.9
Cystargamide no hydroxylated[b]	938.4294	225555	0.7
(7BrW) Brominated cystargamide	1032.3349	348435	1.1

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

Ratios of the detected cystargamide species:

Cyclic cystargamide species : Linear cystargamide species = **0.3:1**

Brominated cystargamide species : Brominated linear cystargamide species = **1:0**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **16:1**

Brominated cyclic cystargamide species : Brominated cystargamide no hydroxylated= **1:0**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	26865004	23.2
Linear cystargamide[ALA] no hydroxylated[a],[b]	970.4557	1320235	1.1
(7BrW) Brominated linear cystargamide[ALA] no hydroxylated[a],[b],[e]	1048.3662	576202	0.5
(7BrW) Brominated linear cystargamide[ALA][a],[d]	1064.3611	2078672	1.8

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10 [e] MS2 data was not recorded due to interfering masses

5Cl-tryptophan-Feeding at 1 mM scale

Detected Cystargamide species	[M+H]+	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	1590311	10.0
Linear cystargamide[a]	972.4349	714633	4.5
Cystargamide no hydroxylated[b]	938.4294	155629	1.0
(5ClW) Chlorinated linear cystargamide no hydroxylated[a],[b]	990.401	191149	1.2

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

<u>Ratios of the detected cystargamide species</u>:

Cyclic cystargamide species : Linear cystargamide species = **1.93:1**

Chlorinated cystargamide species : Chlorinated linear cystargamide species = **0:1**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **10:1**

Chlorinated cyclic cystargamide species : Chlorinated cystargamide no hydroxylated= **0:1**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	2719153	17.1
Linear cystargamide[ALA] no hydroxylated[a],[b]	970.4557	13496	0.1
(5ClW) Chlorinated linear cystargamide[ALA] no hydroxylated[a],[b]	1004.4167	806781	5.1

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

6Cl-tryptophan-Feeding at 1 mM scale

Detected Cystargamide species	[M+H]+	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	699261	10.0
Linear cystargamide[a]	972.4349	1326172	19.0
Cystargamide no hydroxylated[b]	938.4294	23596	0.3
(6ClW) Chlorinated cystargamide no hydroxylated[b]	972.3905	1277854	18.3
(6ClW) Chlorinated linear cystargamide no hydroxylated[a],[b]	990.401	1440729	20.6
(6ClW) Chlorinated cystargamide	988.3854	4530292	64.8
(6ClW) Chlorinated linear cystargamide[a]	1006.3960	7364948	100.5

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

Ratios of the detected cystargamide species:

Cyclic cystargamide species : Linear cystargamide species = **0.6:1**

Chlorinated cystargamide species : Chlorinated linear cystargamide species = **0.1:1**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **4:1**

Chlorinated cyclic cystargamide species : Chlorinated cystargamide no hydroxylated= **4:1**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	4926993	70.5
Linear cystargamide[ALA] no hydroxylated[a],[b]	970.4557	102532	1.5
(6ClW) Chlorinated linear cystargamide[ALA] no hydroxylated[a],[b]	1004.4167	7641792	109.3
(6ClW) Chlorinated linear cystargamide[ALA][a]	1020.4116	24151097	345.4

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

7Cl-tryptophan-Feeding at 1 mM scale

Detected Cystargamide species	[M+H]+	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Cystargamide (WT)	954.4244	2106653	10.0
Linear cystargamide[a]	972.4349	1291560	6.1
Cystargamide no hydroxylated[b]	938.4294	300104	1.4
(7ClW) Chlorinated cystargamide no hydroxylated[b]	972.3905	668116	3.2
(7ClW) Chlorinated linear cystargamide no hydroxylated[a],[b]	990.401	94561	0.4
(7ClW) Chlorinated cystargamide[e]	988.3854	5313075	25.2
(7ClW) Chlorinated linear cystargamide[a]	1006.3960	2059653	9.8

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10 [e] MS2 data was not recorded due to interfering masses

Ratios of the detected cystargamide species:

Cyclic cystargamide species : Linear cystargamide species = **2:1**

Chlorinated cystargamide species : Chlorinated linear cystargamide species = **3:1**

Cyclic cystargamide species : cyclic cystargamide species no hydroxylated= **8:1**

Chlorinated cyclic cystargamide species : Chlorinated cystargamide no hydroxylated= **8:1**

Analysis of the extract by LC-MS also revealed quantities of analogues of cystargamide containing alanine [ALA] instead of glycine. In this case, only linear forms in which the ester bond appeared to have been hydrolysed were detected.

Detected Cystargamide[ALA] species	$[M+H]^+$	Absorbance peak area[c]	Ratio compared to Cystargamide (WT)[d]
Linear cystargamide[ALA][a]	986.4500	5187247	24.6
Linear cystargamide[ALA] no hydroxylated[a],[b]	970.4557	654399	3.1
(7ClW) Chlorinated linear cystargamide[ALA] no hydroxylated[a],[b]	1004.4167	956515	4.5
(7ClW) Chlorinated linear cystargamide[ALA][a]	1020.4116	7761149	36.8

[a] The ester bond appeared to have been hydrolysed [b] That lacked the 5-hydroxy on the tryptophan [c] Average area from the reaction performed in triplicate [d] Calculated as: (Absorbance peak area species/Absorbance peak area cystargamide wild type (WT))*10

Results demonstrated that all of the halotryptophans incorporated into cystargamide, generating new to nature halogenated analogues. As the concentration of tryptophan had no major impacted the amount of halo–cystargamide produced (see tables below) , we selected to scale up of the feeding of 6-bromotryptophan at 0.25 mM .

Scaled up feeding experiment with 6-bromotryptophan

A starter culture was prepared by inoculation of 50 mL GY medium in a 250 mL baffled conical flask with 2 mL glycerol mycelium stock, and fermented for 48 h at 28 °C and 200 rpm in an incubator with a 2.54 cm throw.
Fermentation cultures (8 L) were prepared by inoculating the production medium (0.4 g/L glucose, 0.8 g/L galactose, 0.8 g/L maltose, 1.6 g/L dextrin, 0.8 g/L soya peptone and 0.3 g/L ammonium sulfate, supplemented with 73 mg/L 6-bromotryptophan [0.26 mM] prepared as previously described[1] with starter culture (10% inoculum), and fermented for 72 h at 28 °C and 200 rpm.

The fermentation cultures were centrifuged (6000 rpm, 8980g, 30 min) and the supernatant was extracted with XAD7-HP resin overnight at 4 °C (20 mL resin / L supernatant). The resin was washed liberally with water, after which bound metabolites were eluted in 400 mL volumes each of 100% aqueous methanol. The presence of cystargamide and bromocystargamide was confirmed by UPLC and the extract was fractionated by gel permeation chromatography, using a 200 mL column of Sephadex LH-20 resin in methanol under gravity flow (25-30 cm/h). Cystargamide and bromocystargamide, both in their linear and cyclised forms, eluted between 120 – 180 mL. These fractions were further fractionated by RP-HPLC, using a Waters XBridge Prep Phenyl 10 x 250 mm 5 µm column with an isocratic gradient of 70% aqueous methanol and 0.1% formic acid and a flow rate of 6 mL/min, monitoring UV absorbance at 280 nm. Cystargamide was found to elute at 10.5 minutes, followed by bromocystargamide at 11 minutes. Solvent was removed from fractions using a Genevac centrifugal vacuum concentrator.

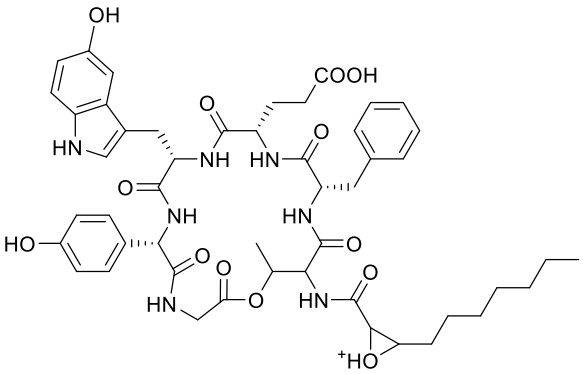
Throughout the purification, fractions were analysed by UPLC (95% acetonitrile and 0.1% trifluoroacetic acid with a flow rate of 0.6 ml/min, monitoring between 220 and 400 nm) or by LC-HRMS (XBridge C18 2.1 x 150 mm column, 5-95% 0.1% formic acid/water; acetonitrile, monitoring at 254 and 280 nm, HRMS: *m*/*z* calcd for $C_{49}H_{59}BrN_7O_{13}$ $[M+H]^+$: 1034.3349; found: 1032.3321.

The cystargamide and bromocystargamide were produced in linear and cyclic forms.

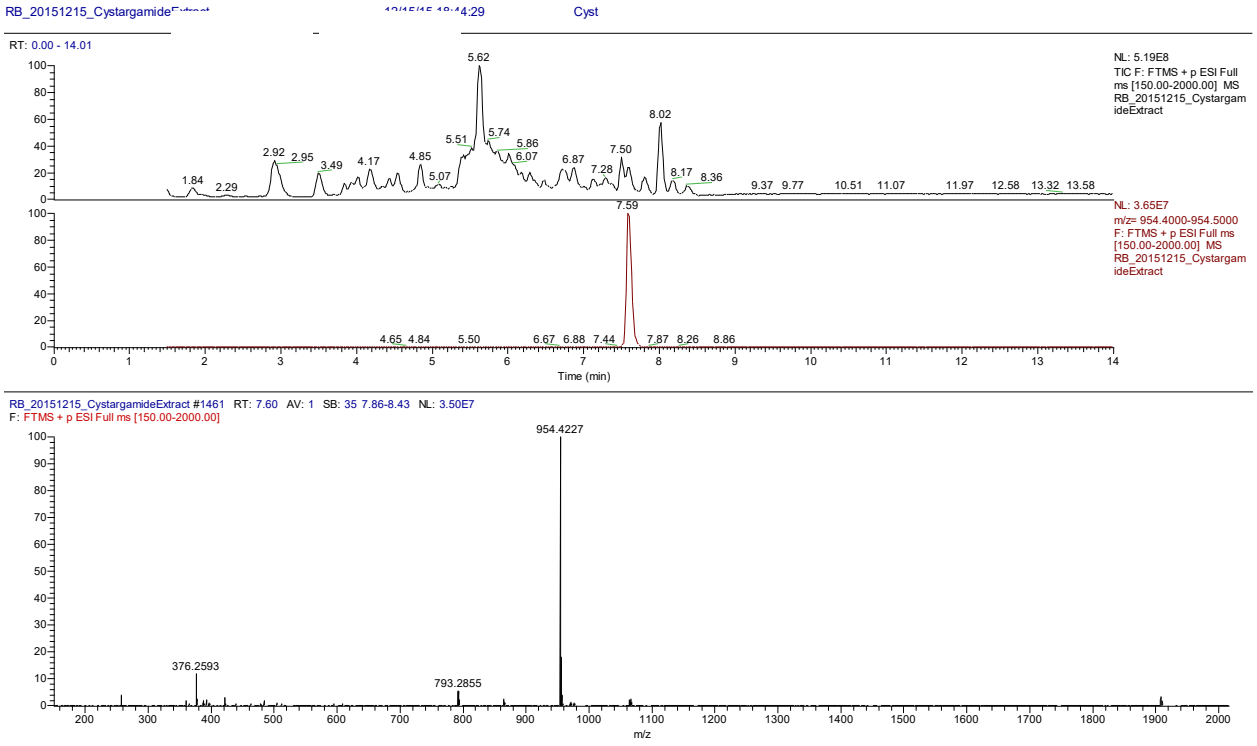
Extraction and purification resulted in a pure fraction containing 1.0 mg 6-bromocystargamide **9**. By comparison of the absorbance peak areas it could be estimated that the parent unhalogenated cystargamide **62**, a bromo-cystargamide analogue that lacked the 5-hydroxy on the tryptophan **63** and bromocystargamide **9**, were present in an approximate 10:0.2:1.4 ratio.

LC-MSMS data for cystargamide and its derivatives

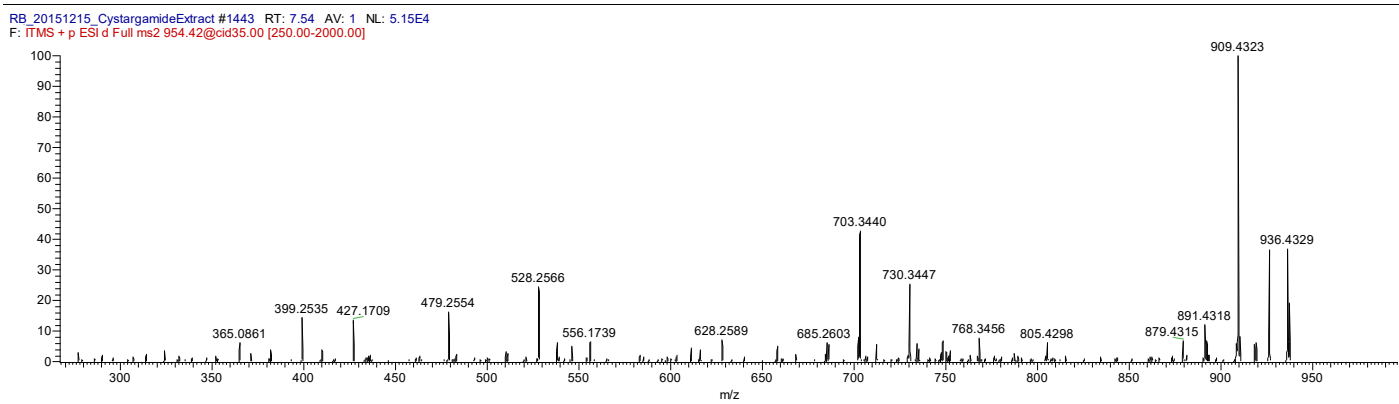
Natural Cyclic Cystargamide (62)

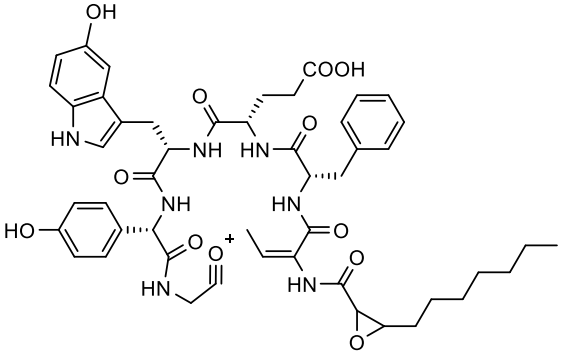


Chemical Formula: $C_{49}H_{60}N_7O_{13}^+$
Exact Mass: 954.4244

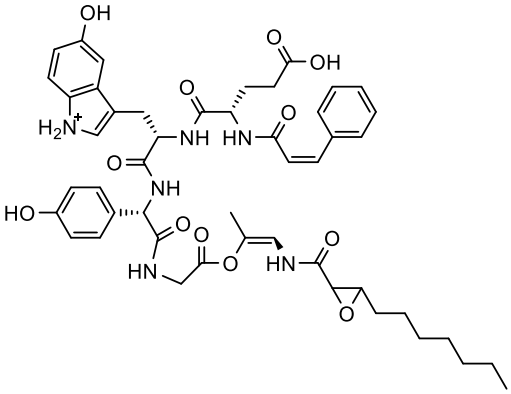


MS2 spectrum and fragment assignment

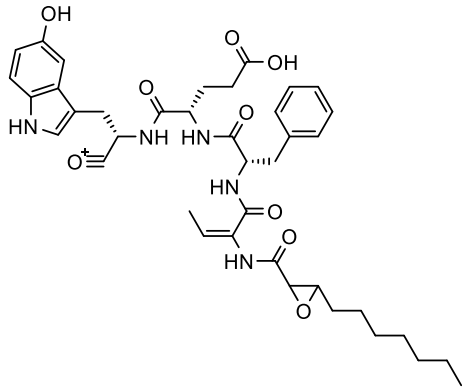




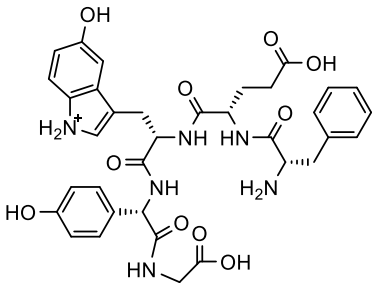
Chemical Formula: $C_{49}H_{58}N_7O_{12}^+$
Exact Mass: 936.4138



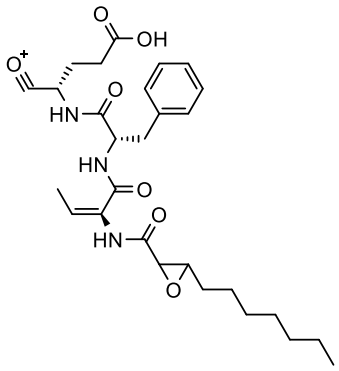
Chemical Formula: $C_{48}H_{57}N_6O_{12}^+$
Exact Mass: 909.4029



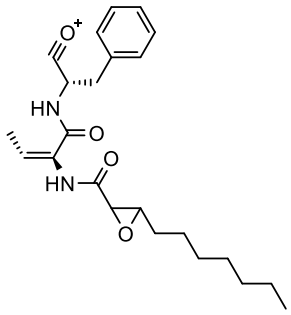
Chemical Formula: $C_{39}H_{48}N_5O_9^+$
Exact Mass: 730.3447



Chemical Formula: $C_{35}H_{39}N_6O_{10}^+$
Exact Mass: 703.2722

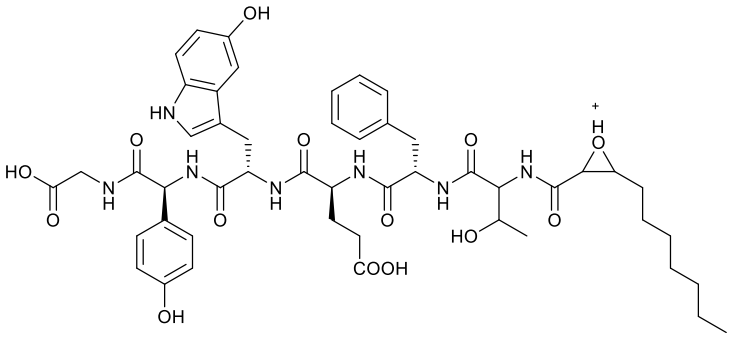


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

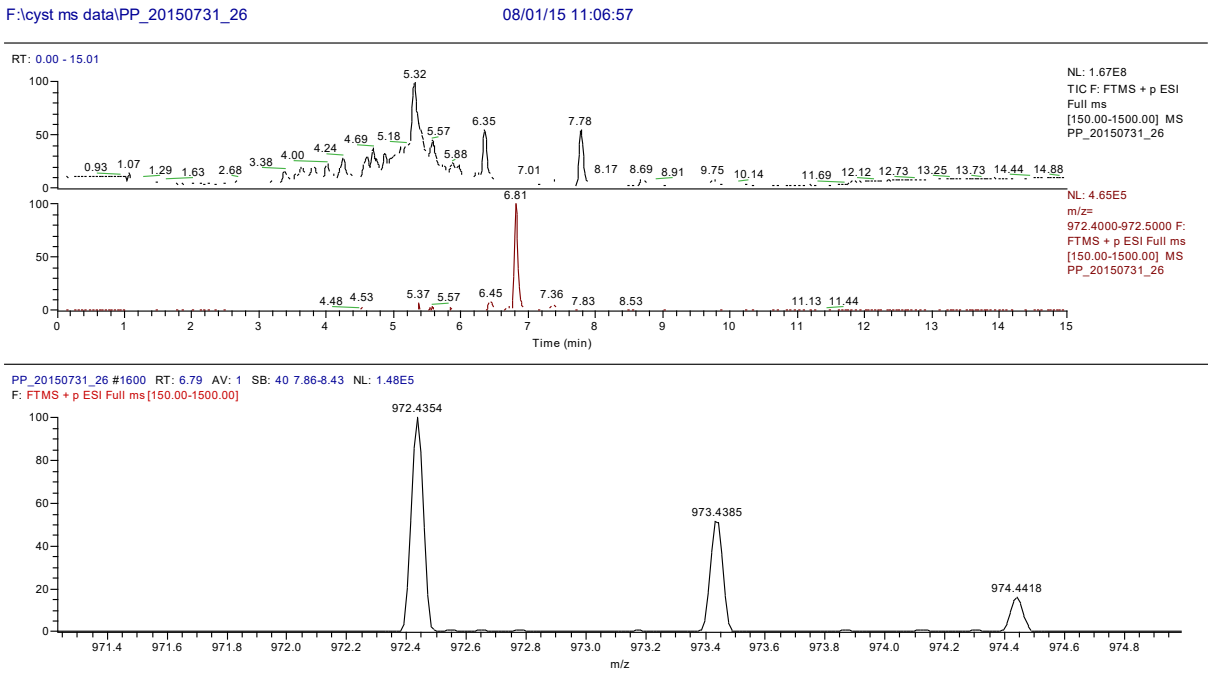


Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

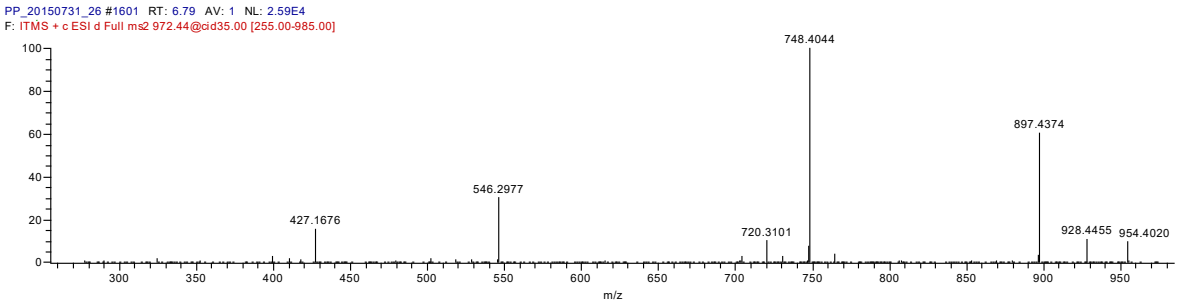
Linear cystargamide

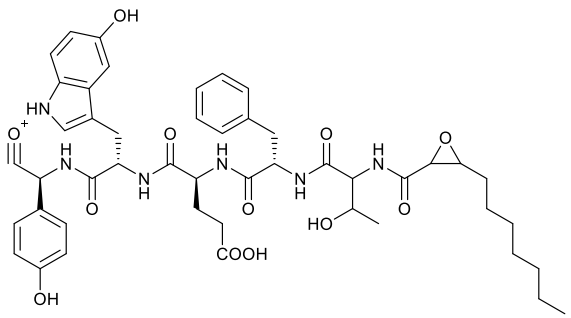


Chemical Formula: $C_{49}H_{62}N_7O_{14}^+$
Exact Mass: 972.4349

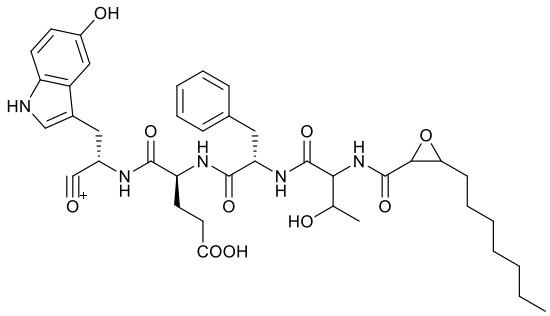


MS2 spectrum and fragment assignment

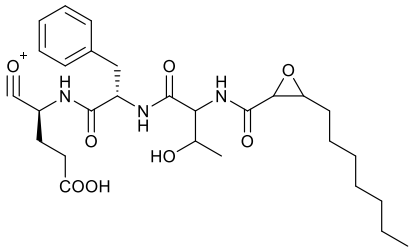




Chemical Formula: $C_{47}H_{57}N_6O_{12}^+$
Exact Mass: 897.4029

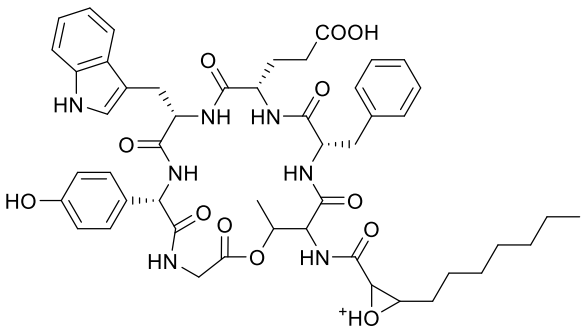


Chemical Formula: $C_{39}H_{50}N_5O_{10}^+$
Exact Mass: 748.3552

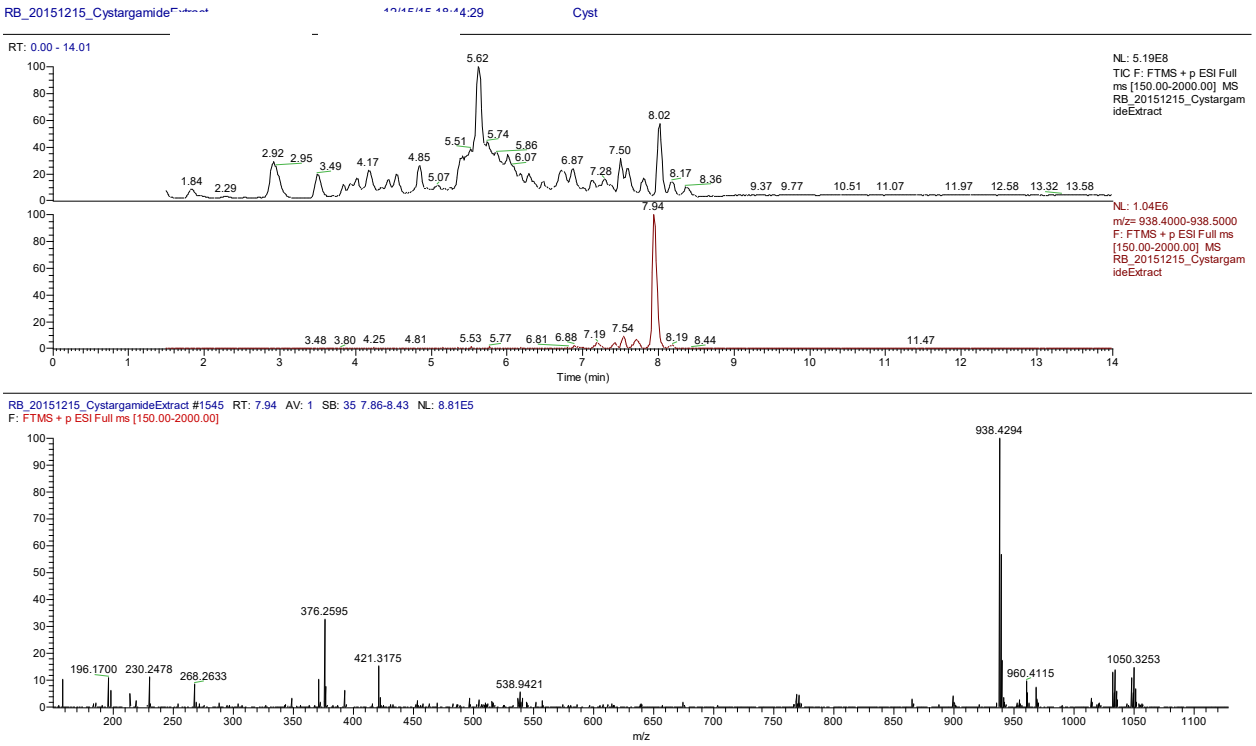


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

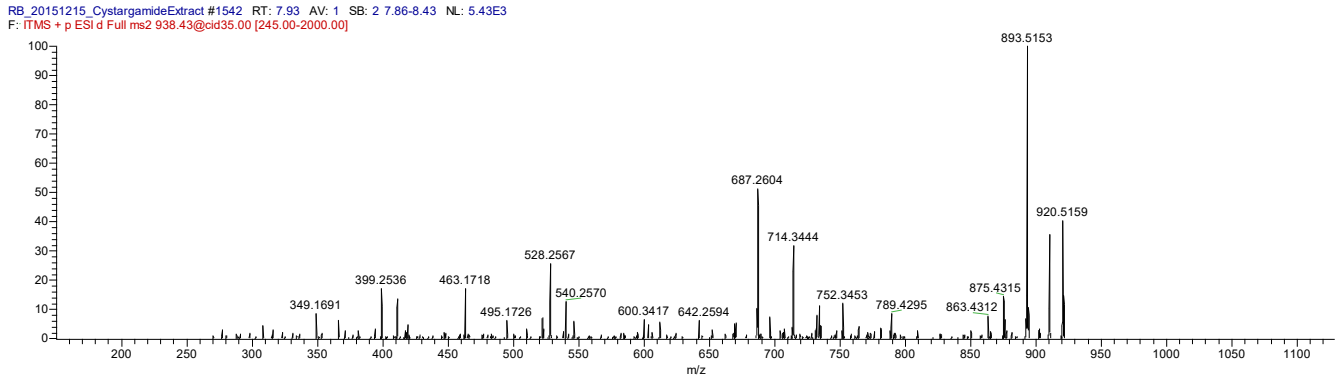
Natural cystargamide un-hydroxylated

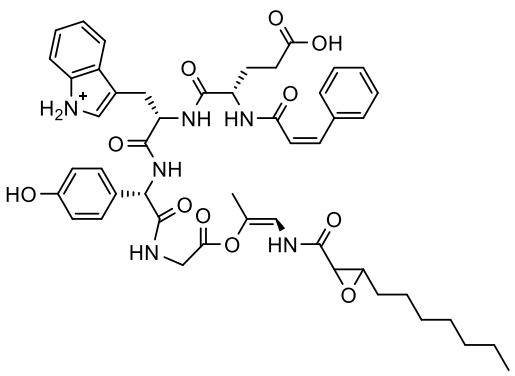


Chemical Formula: $C_{49}H_{60}N_7O_{12}^+$
Exact Mass: 938.4294

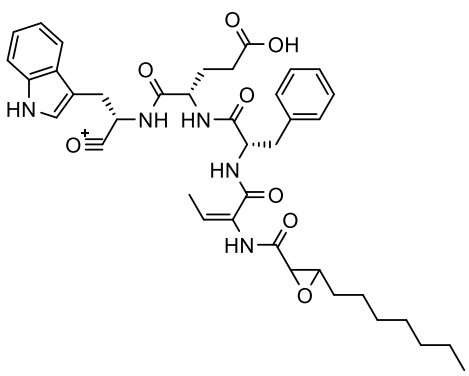


MS2 spectrum and fragment assignment

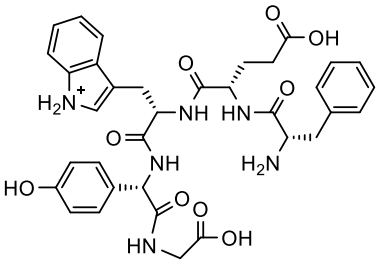




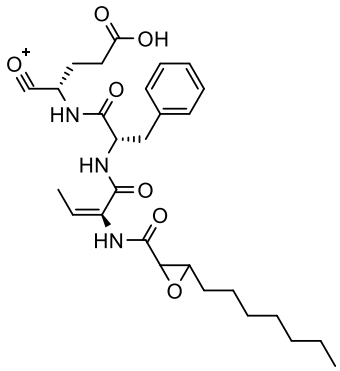
Chemical Formula: $C_{48}H_{57}N_6O_{11}^+$
Exact Mass: 893.4080



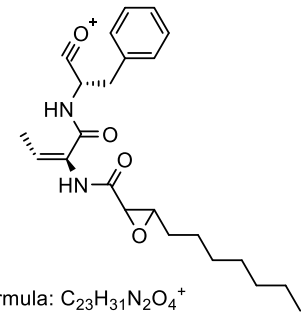
Chemical Formula: $C_{39}H_{48}N_5O_8^+$
Exact Mass: 714.3497



Chemical Formula: $C_{35}H_{39}N_6O_9^+$
Exact Mass: 687.2773

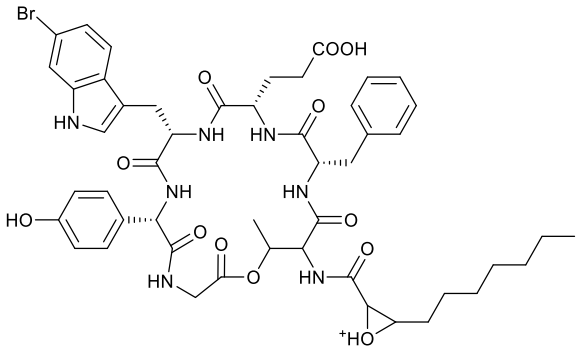


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

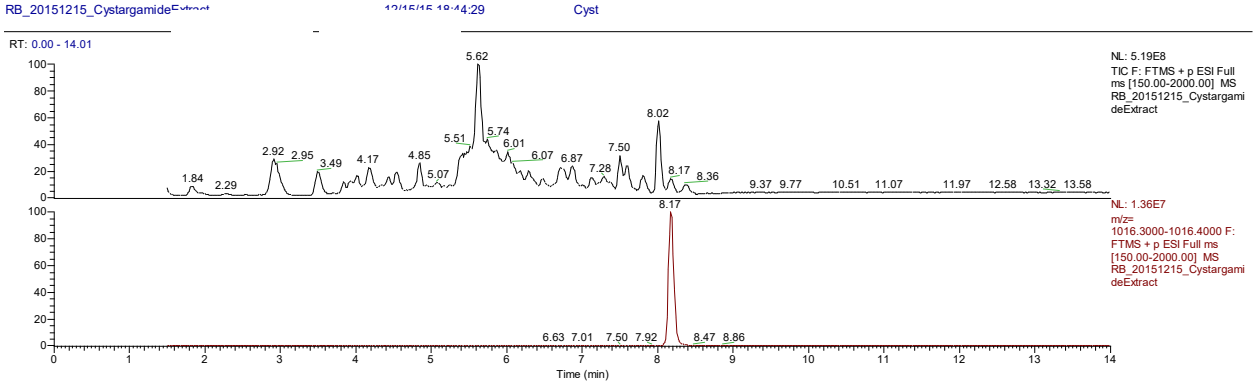


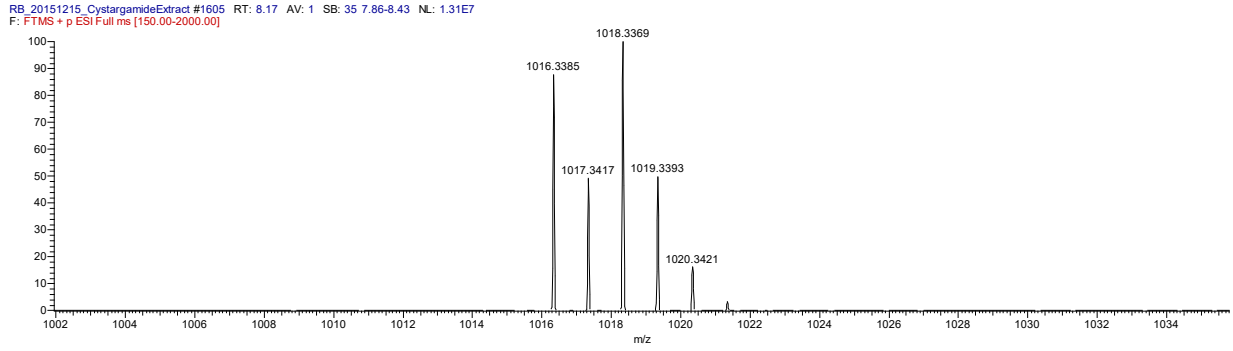
Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

Bromo-cystargamide un-hydroxylated (63)

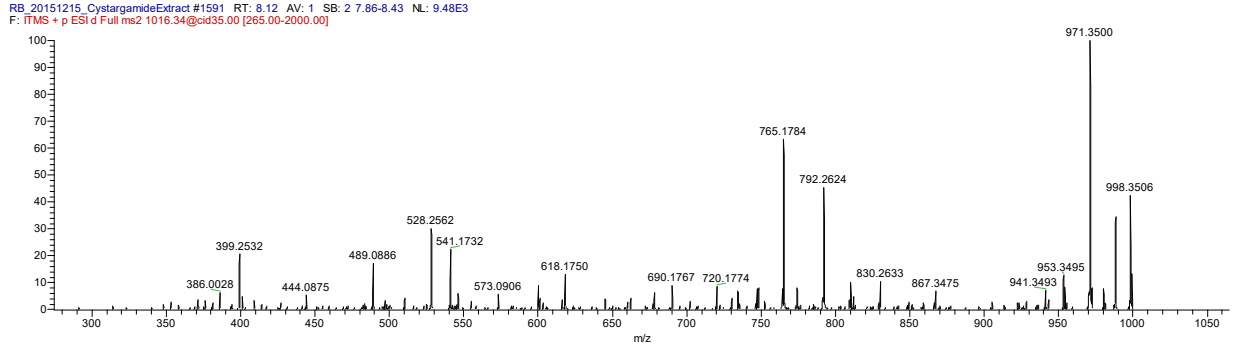


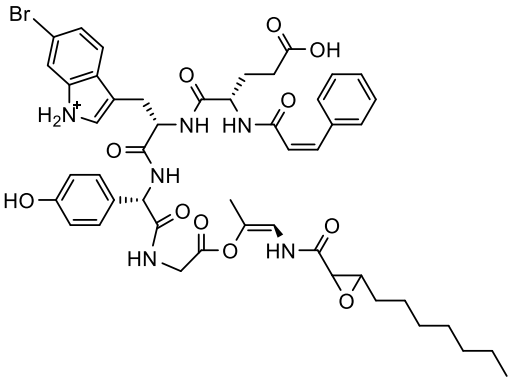
Chemical Formula: $C_{49}H_{59}BrN_7O_{12}^+$
Exact Mass: 1016.3400



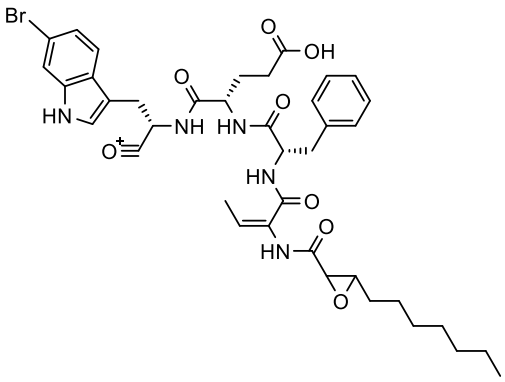


MS2 spectrum and fragment assignment

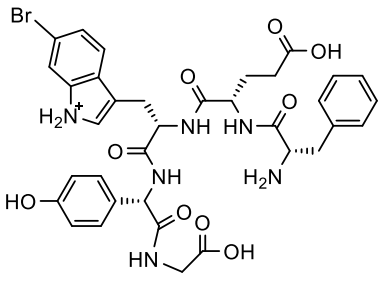




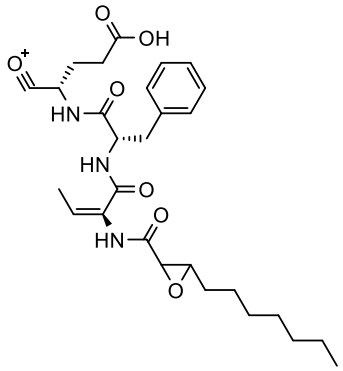
Chemical Formula: $C_{48}H_{56}BrN_6O_{11}^+$
Exact Mass: 971.3185



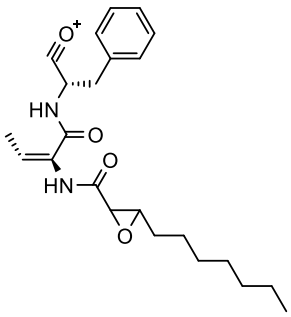
Chemical Formula: $C_{39}H_{47}BrN_5O_8^+$
Exact Mass: 792.2603



Chemical Formula: $C_{35}H_{38}BrN_6O_9^+$
Exact Mass: 765.1878

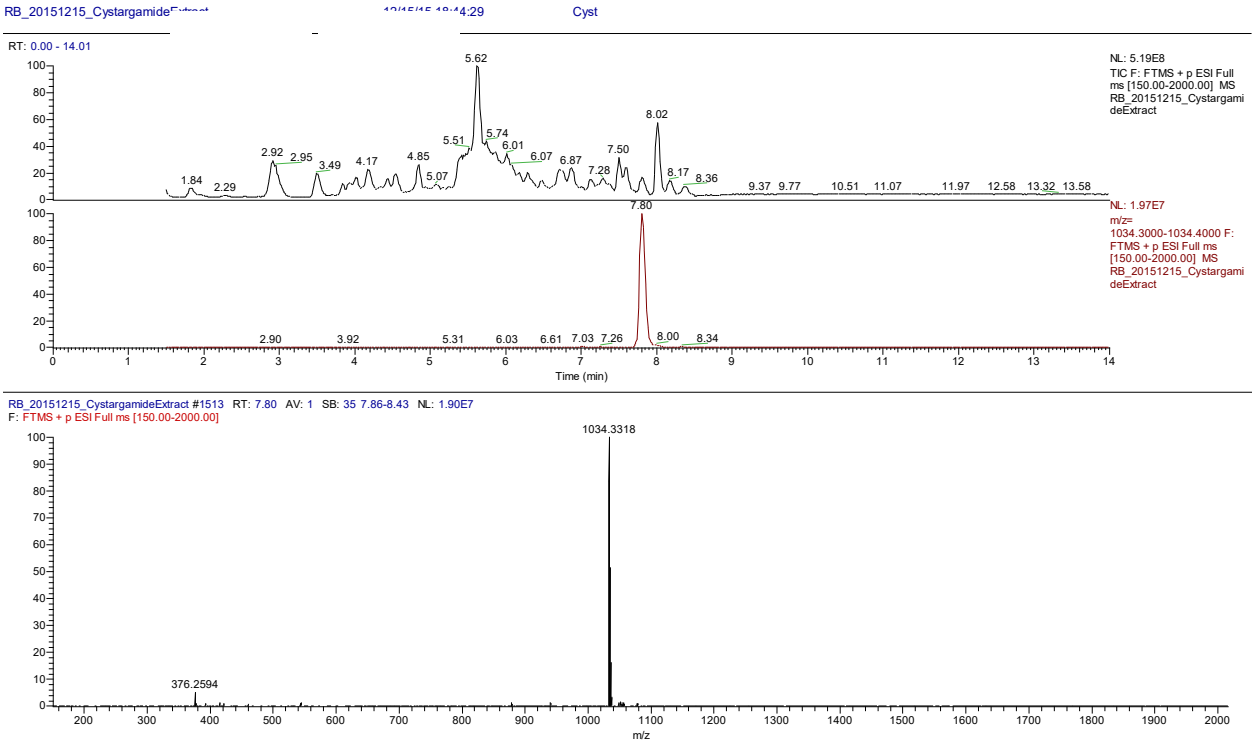


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

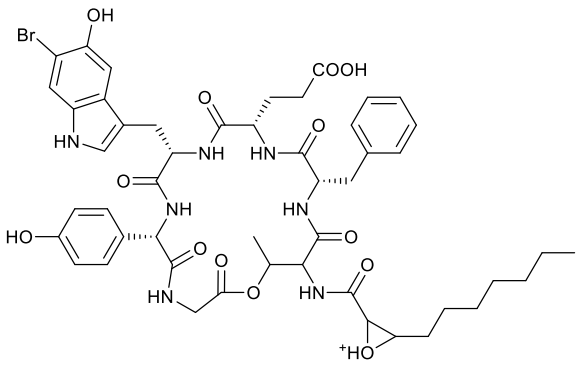


Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

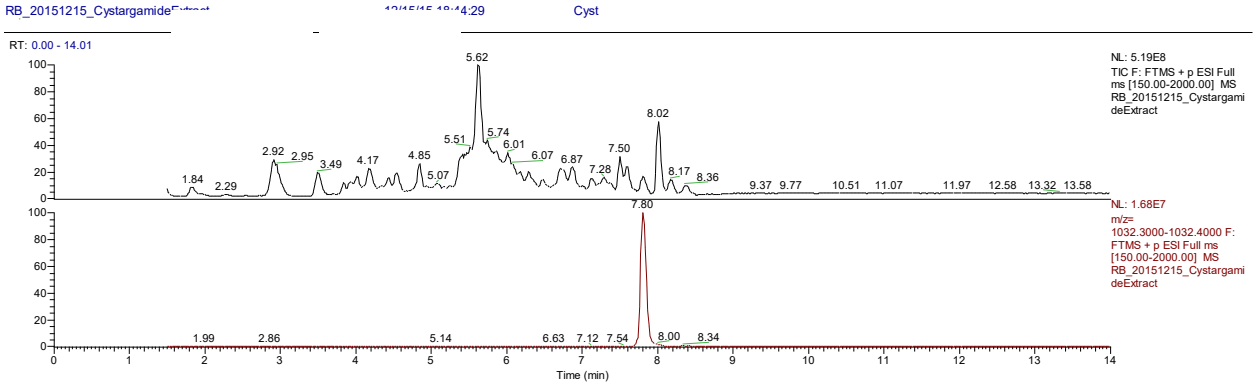
Brominated linear cystargamide un-hydroxylated

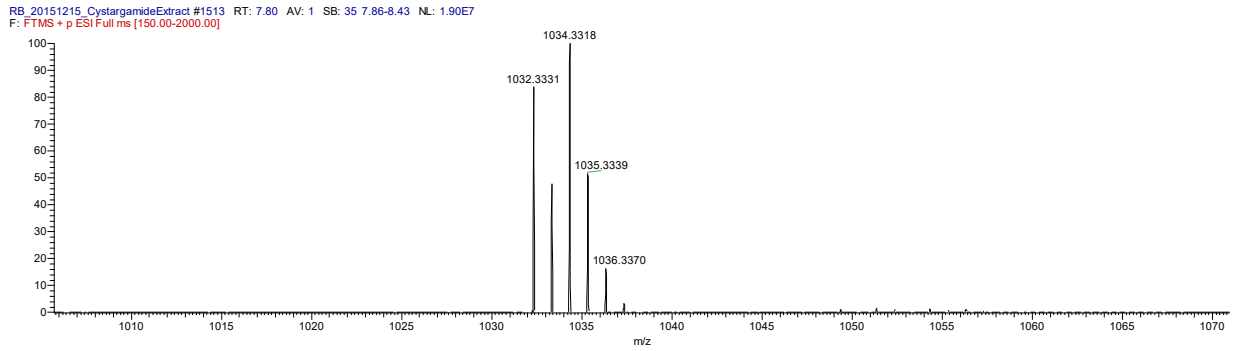


Brominated cystargamide (9)

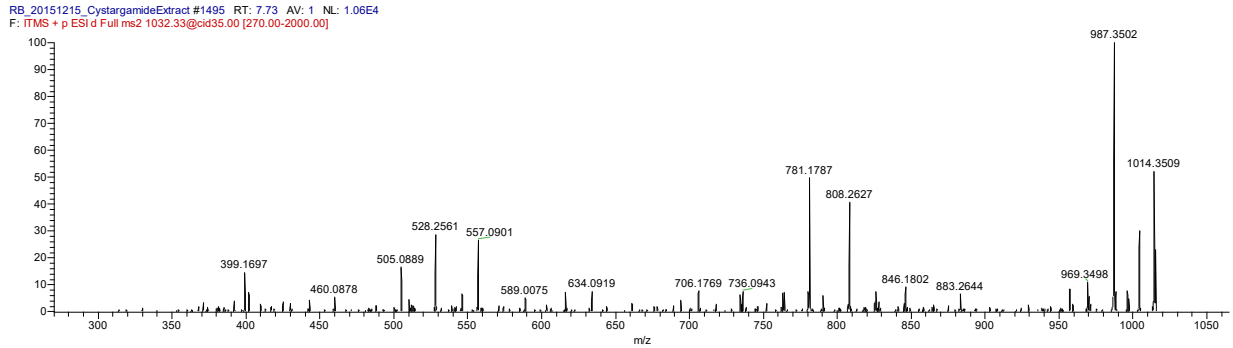


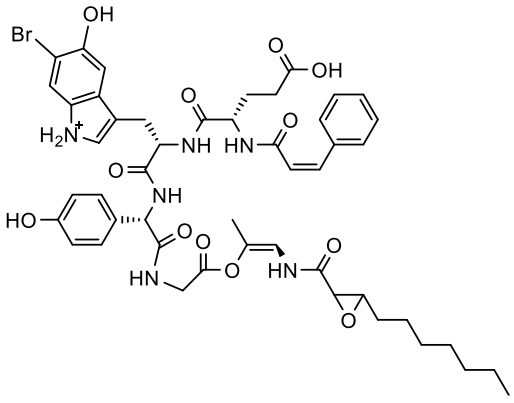
Chemical Formula: $C_{49}H_{59}BrN_7O_{13}^+$
Exact Mass: 1032.3349



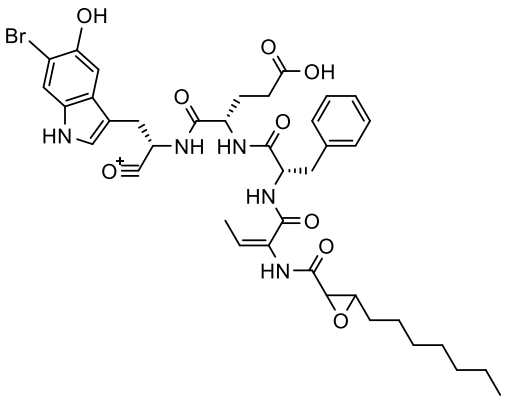


MS2 spectrum and fragment assignment

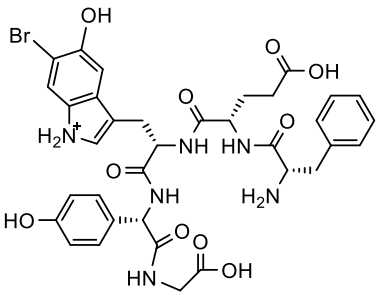




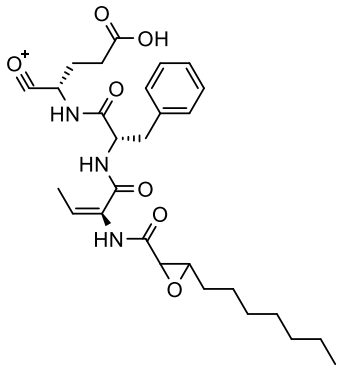
Chemical Formula: $C_{48}H_{56}BrN_6O_{12}^+$
Exact Mass: 987.3134



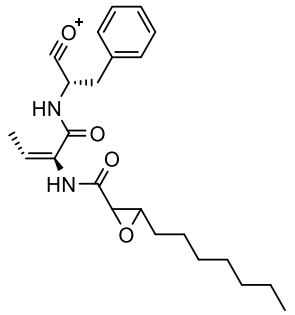
Chemical Formula: $C_{39}H_{47}BrN_5O_9^+$
Exact Mass: 808.2552



Chemical Formula: $C_{35}H_{38}BrN_6O_{10}^+$
Exact Mass: 781.1827

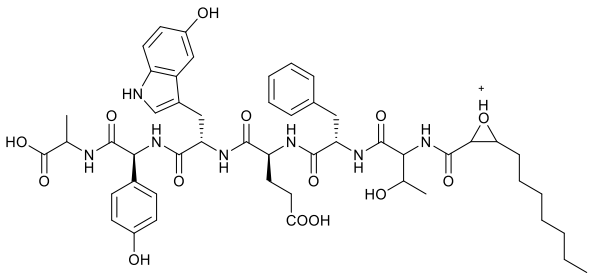


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

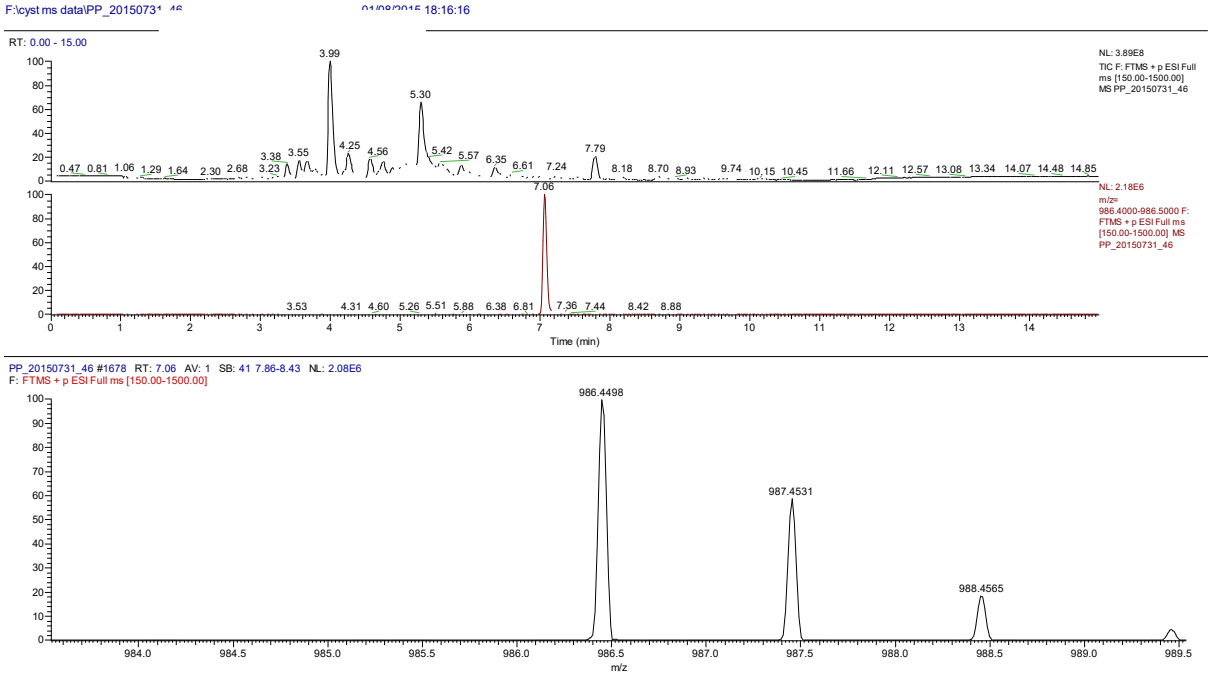


Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

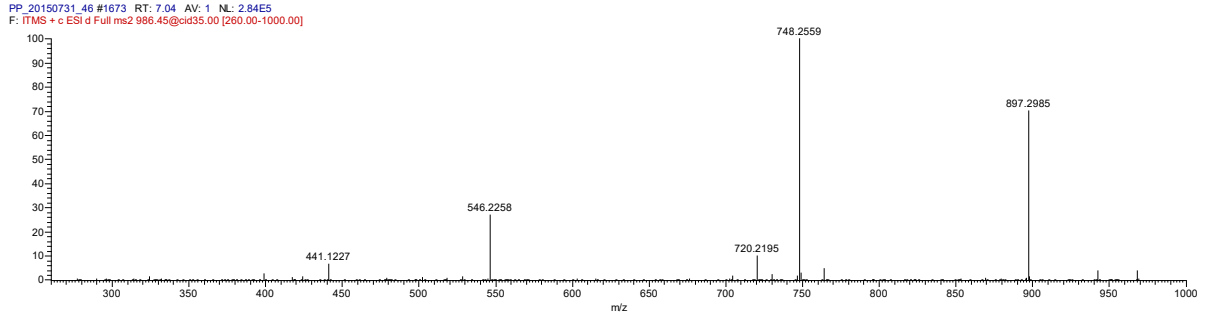
Linear cystargamide[ALA]

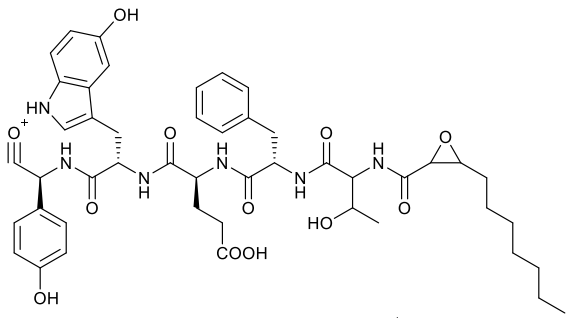


Chemical Formula: $C_{50}H_{64}N_7O_{14}^+$
Exact Mass: 986.4506

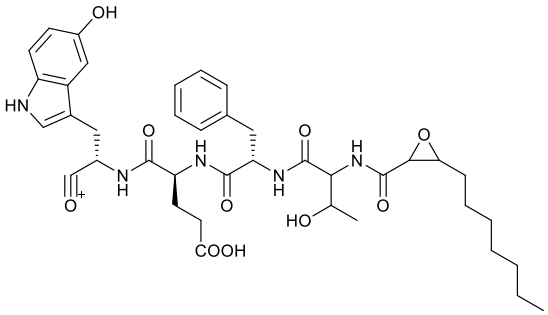


MS2 spectrum and fragment assignment

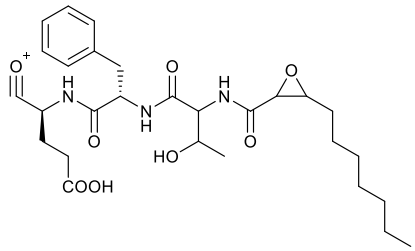




Chemical Formula: $C_{47}H_{57}N_6O_{12}^+$
Exact Mass: 897.4029

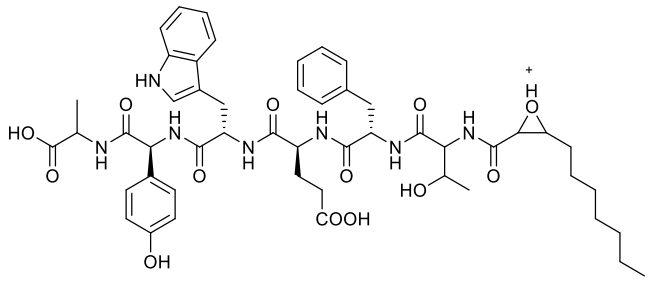


Chemical Formula: $C_{39}H_{50}N_5O_{10}^+$
Exact Mass: 748.3552

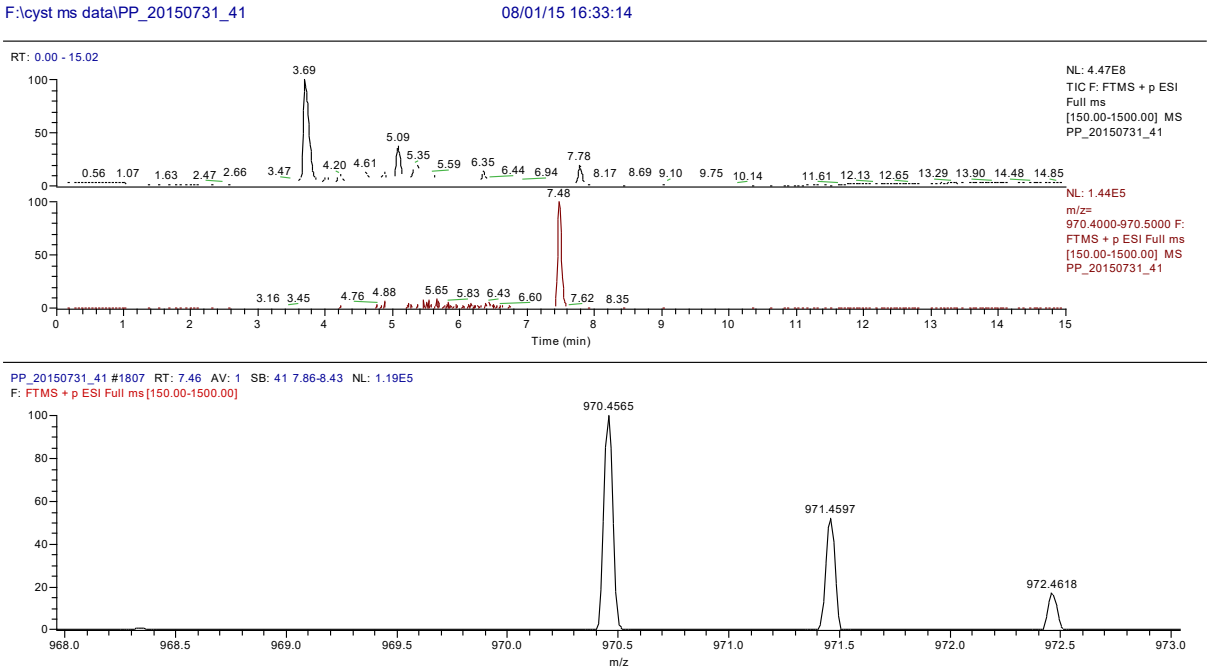


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

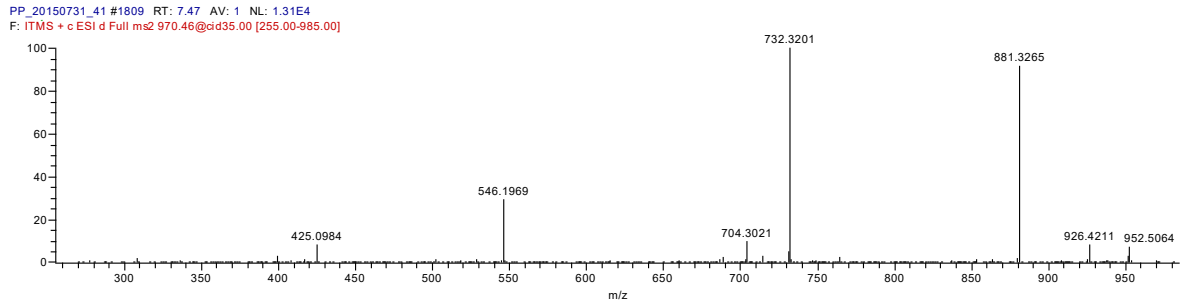
Linear cystargamide[ALA] un hydroxylated

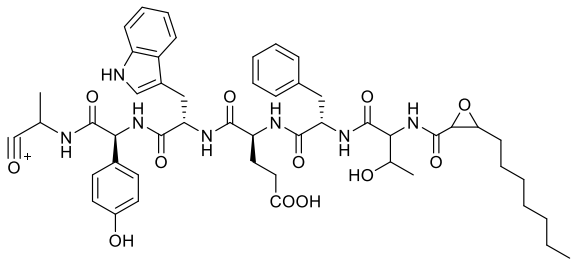


Chemical Formula: $C_{50}H_{64}N_7O_{13}^+$
Exact Mass: 970.4557

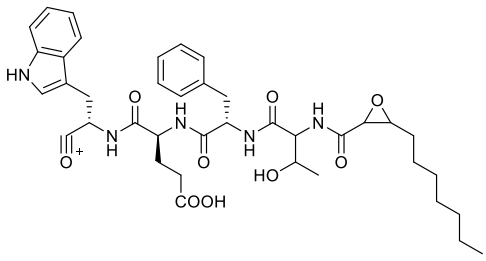


MS2 spectrum and fragment assignment

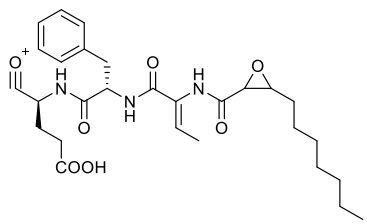




Chemical Formula: $C_{50}H_{62}N_7O_{12}^+$
Exact Mass: 952.4451

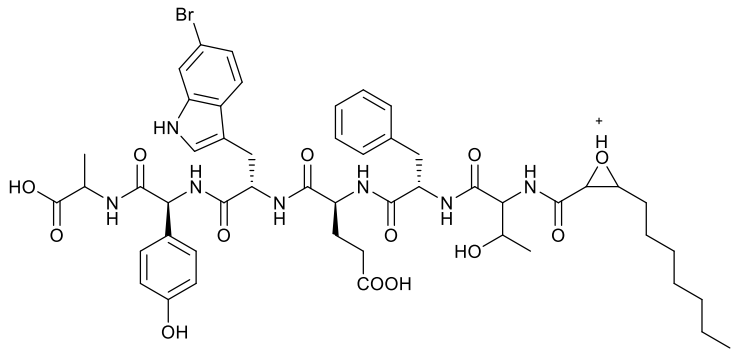


Chemical Formula: $C_{39}H_{50}N_5O_9^+$
Exact Mass: 732.3603

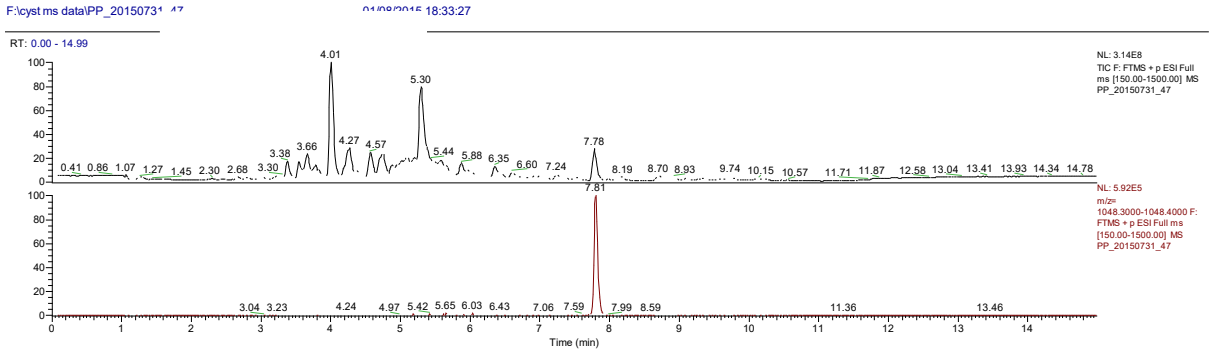


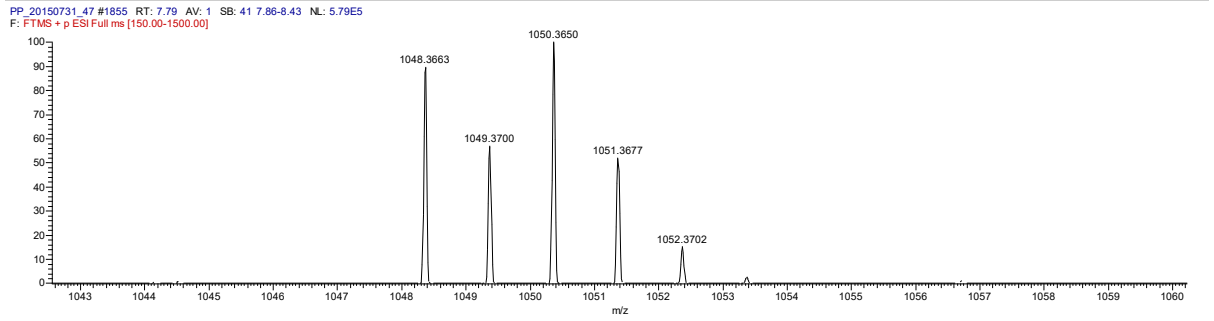
Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

Brominated linear cystargamide[ALA] un hydroxylated

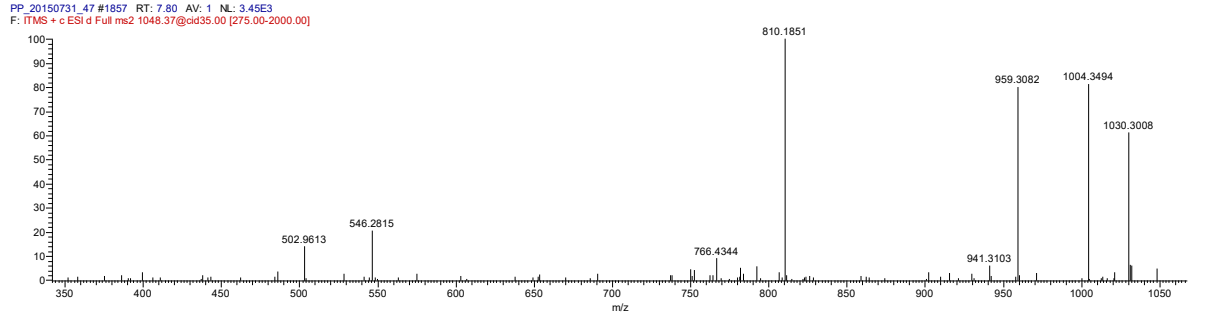


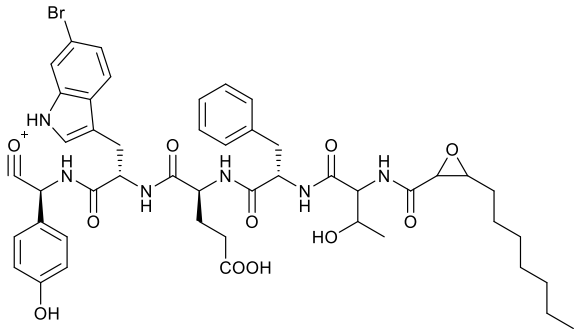
Chemical Formula: $C_{50}H_{63}BrN_7O_{13}^{+}$
Exact Mass: 1048.3662



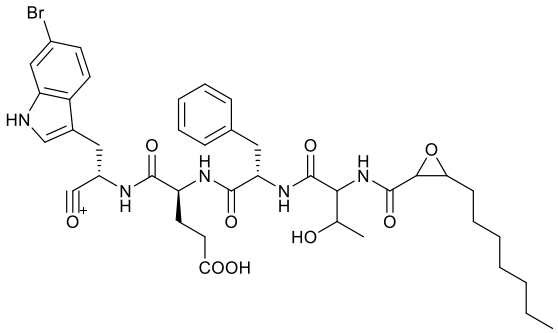


MS2 spectrum and fragment assignment

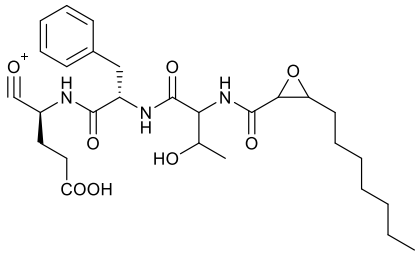




Chemical Formula: $C_{47}H_{56}BrN_6O_{11}^+$
Exact Mass: 959.3185

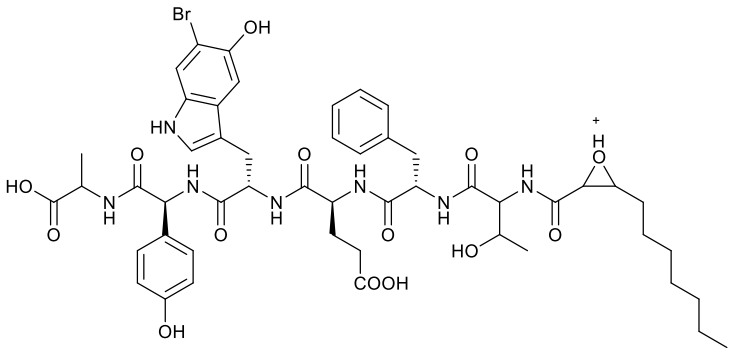


Chemical Formula: $C_{39}H_{49}BrN_5O_9^+$
Exact Mass: 810.2708

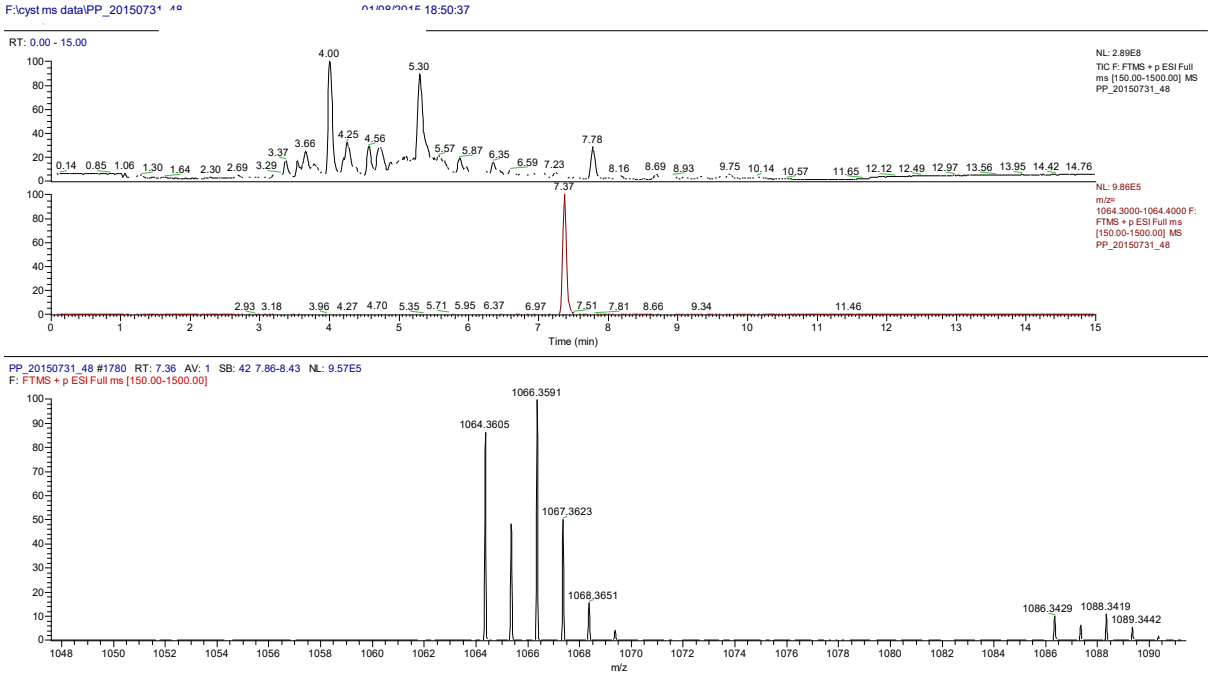


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

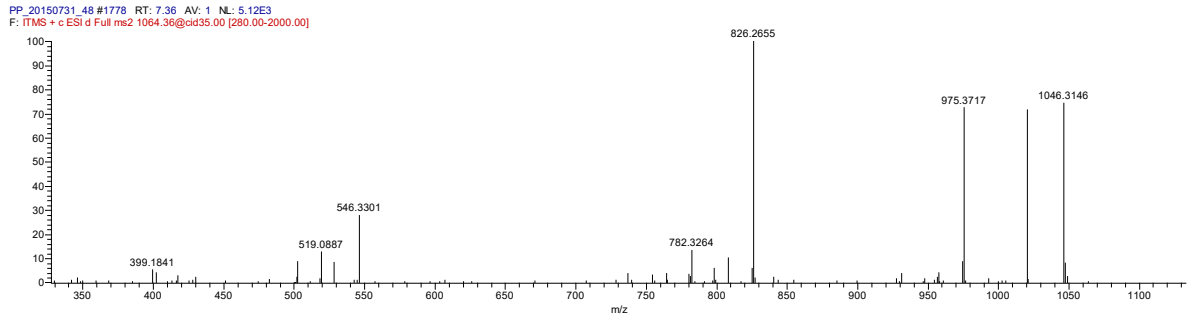
Brominated linear cystargamide[ALA]

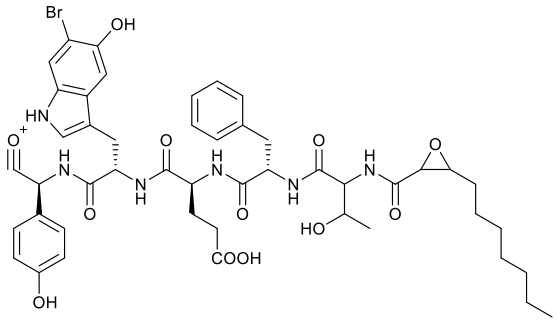


Chemical Formula: $C_{50}H_{63}BrN_7O_{14}^+$
Exact Mass: 1064.3611

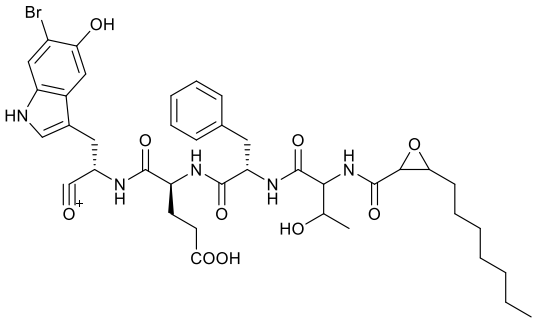


MS2 spectrum and fragment assignment

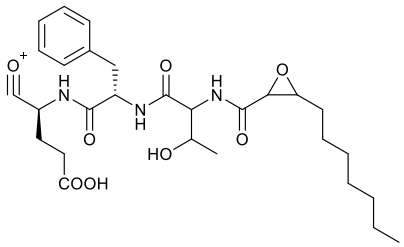




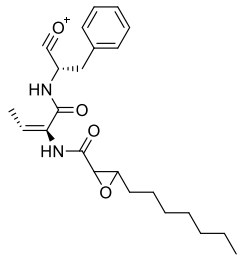
Chemical Formula: $C_{47}H_{56}BrN_6O_{12}^+$
Exact Mass: 975.3134



Chemical Formula: $C_{39}H_{49}BrN_5O_{10}^+$
Exact Mass: 826.2657

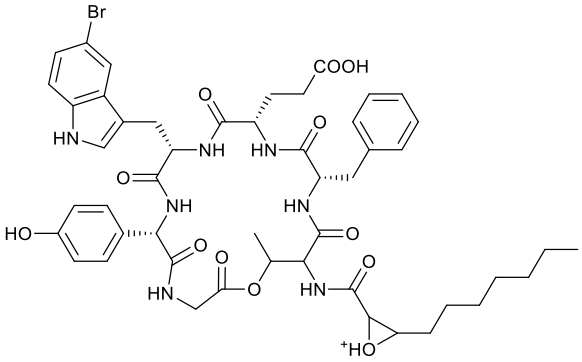


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

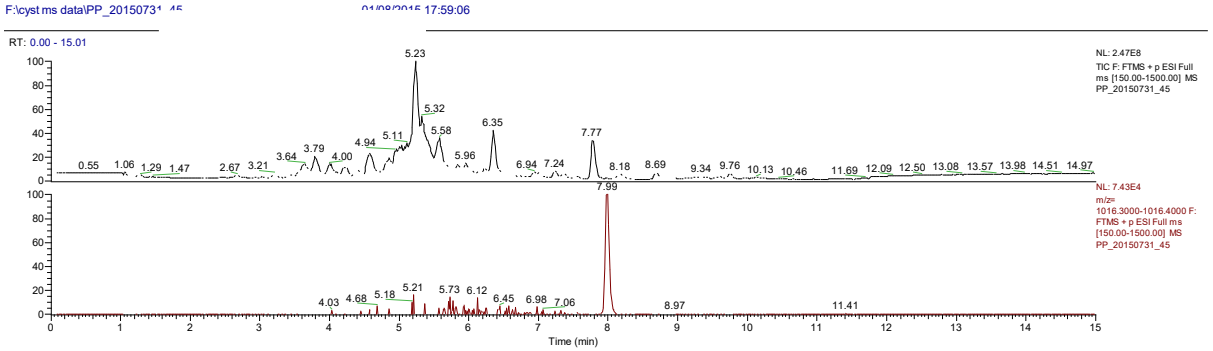


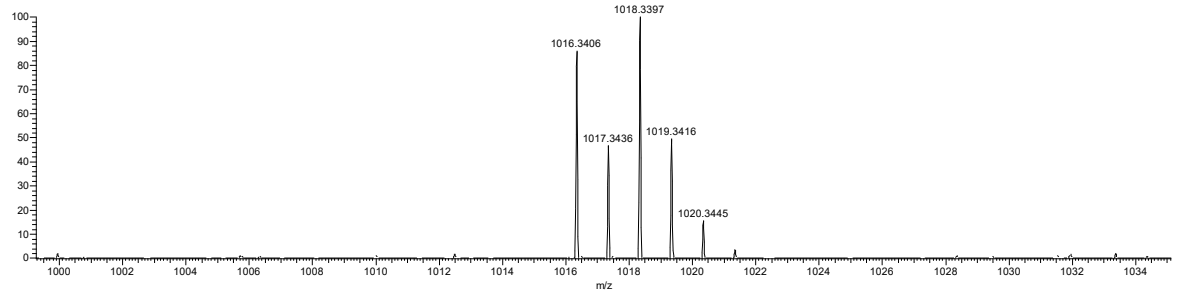
Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

(5BrW) Brominated cystargamide unhydroxylated

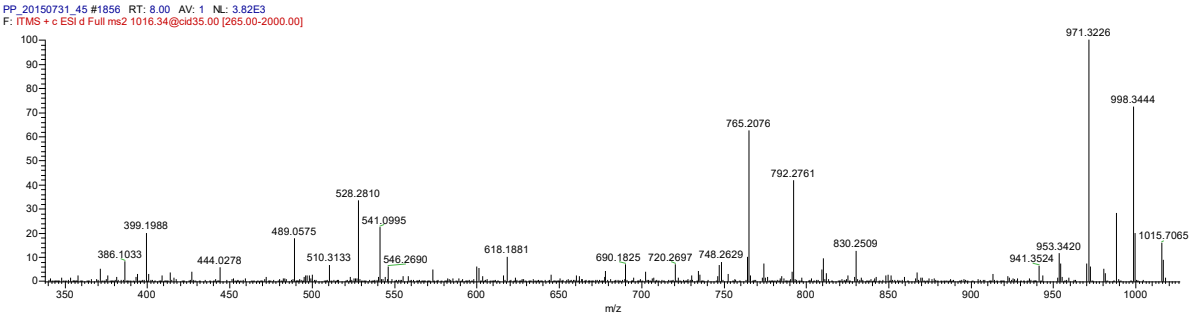


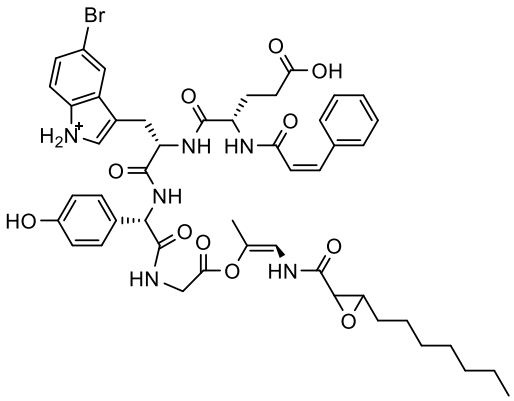
Chemical Formula: $C_{49}H_{59}BrN_7O_{12}^+$
Exact Mass: 1016.3400



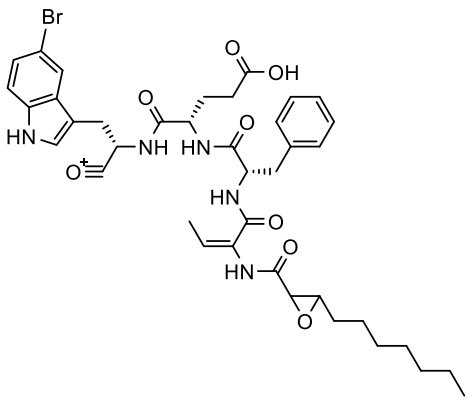


MS2 spectrum and fragment assignment

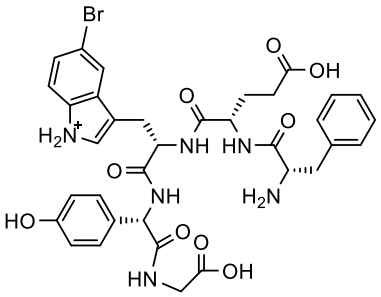




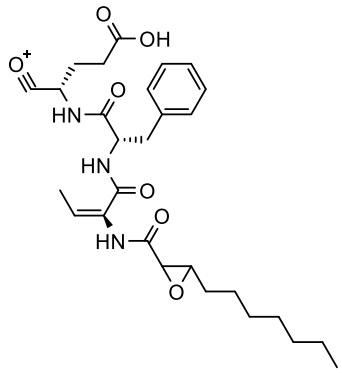
Chemical Formula: $C_{48}H_{56}BrN_6O_{11}^+$
Exact Mass: 971.3185



Chemical Formula: $C_{39}H_{47}BrN_5O_8^+$
Exact Mass: 792.2603

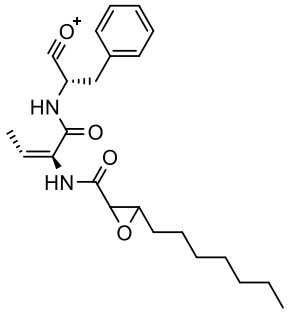


Chemical Formula: $C_{35}H_{38}BrN_6O_9^+$
Exact Mass: 765.1878

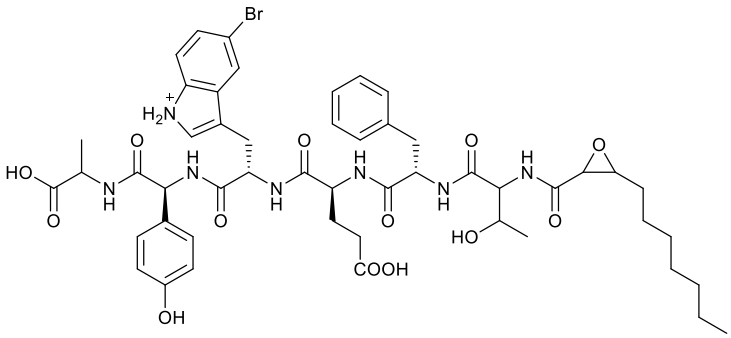


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

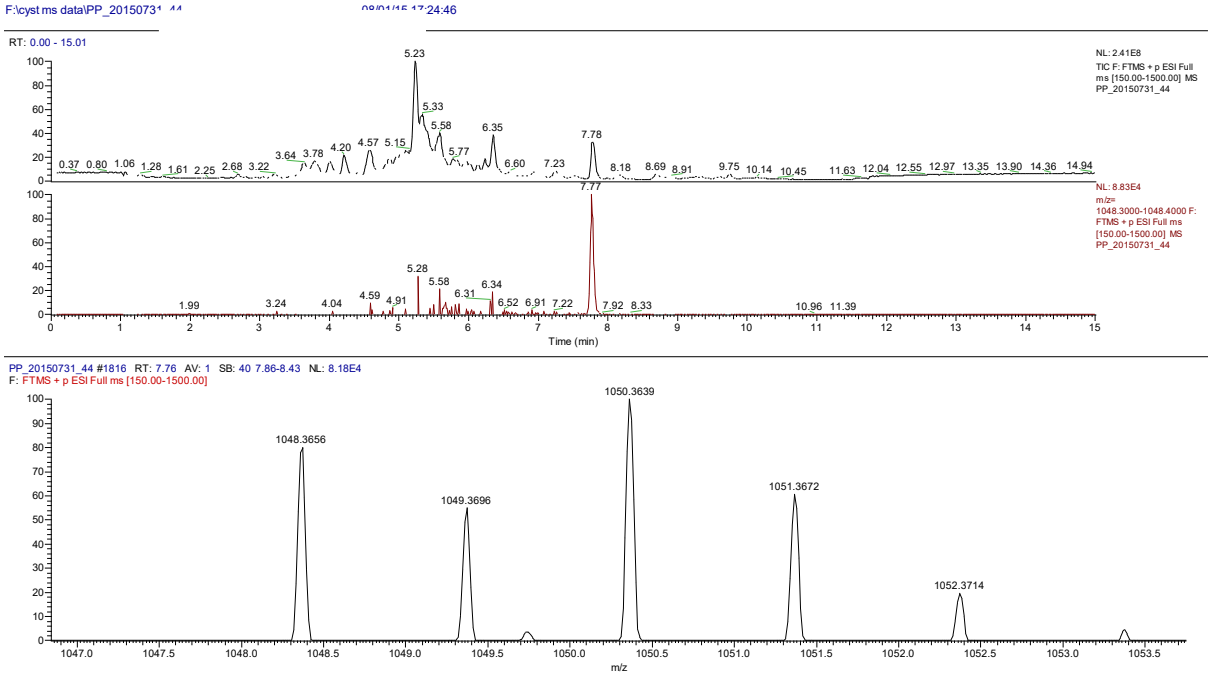
Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278



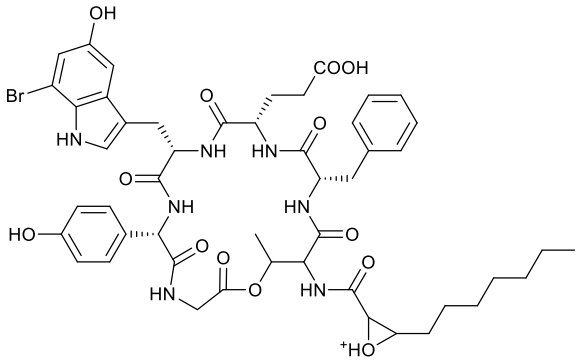
(5BrW) Brominated linear cystargamide[ALA] un hydroxylated



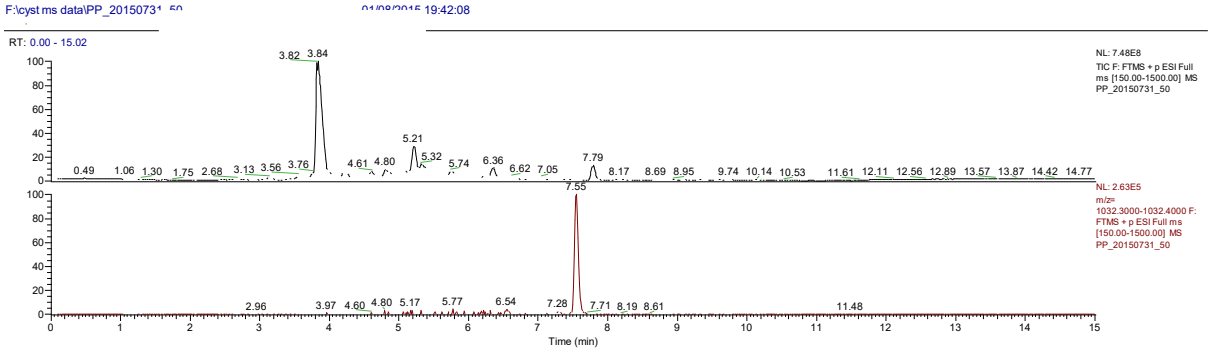
Chemical Formula: $C_{50}H_{63}BrN_7O_{13}^+$
Exact Mass: 1048.3662

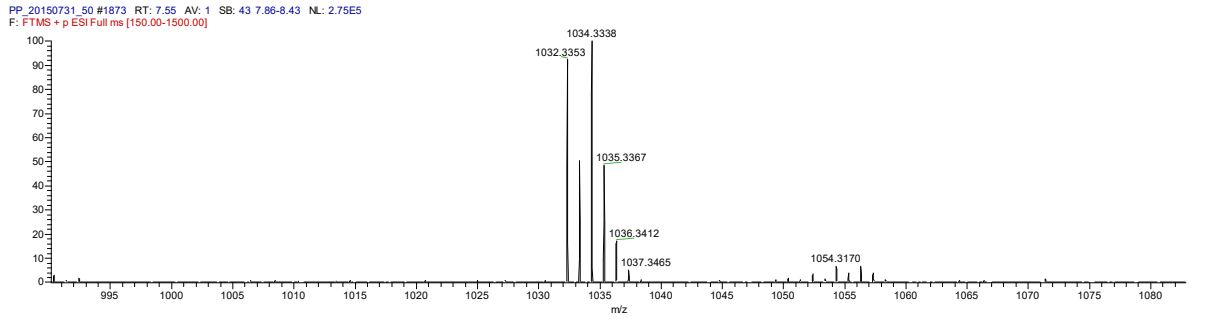


(7-BrW) Brominated cystargamide

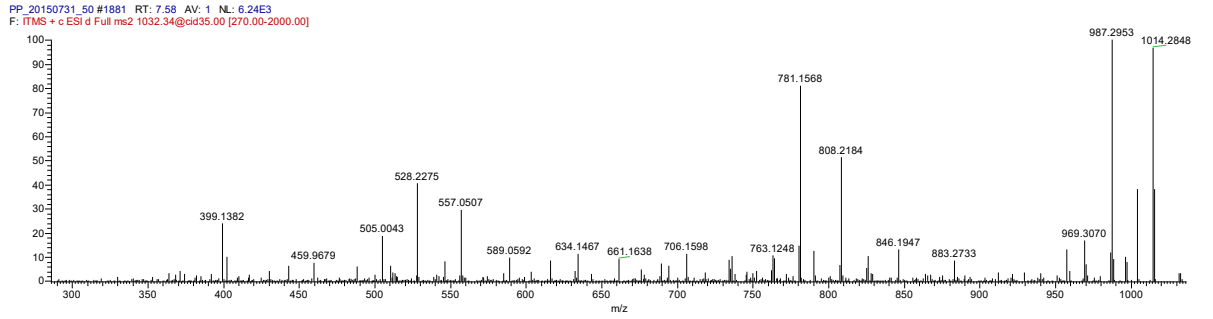


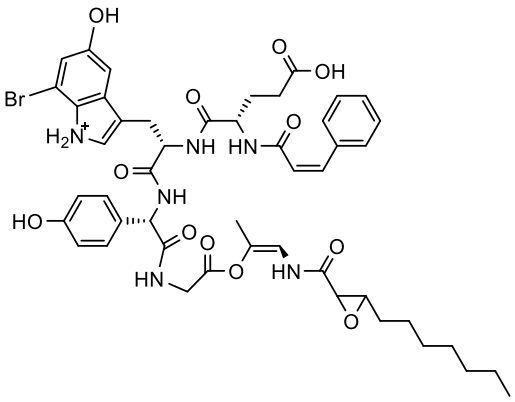
Chemical Formula: $C_{49}H_{59}BrN_7O_{13}^+$
Exact Mass: 1032.3349



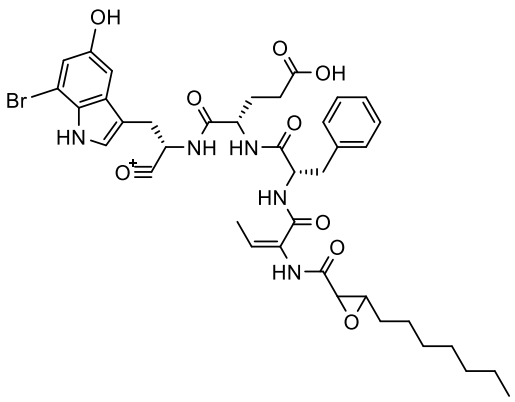


MS2 spectrum and fragment assignment

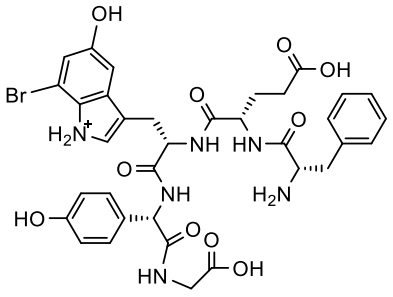




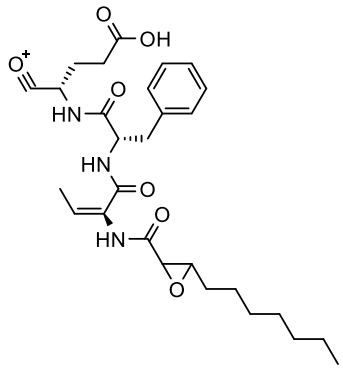
Chemical Formula: $C_{48}H_{56}BrN_6O_{12}^+$
Exact Mass: 987.3134



Chemical Formula: $C_{39}H_{47}BrN_5O_9^+$
Exact Mass: 808.2552

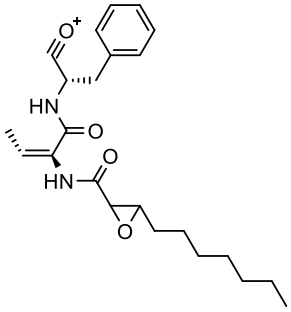


Chemical Formula: $C_{35}H_{38}BrN_6O_{10}^+$
Exact Mass: 781.1827

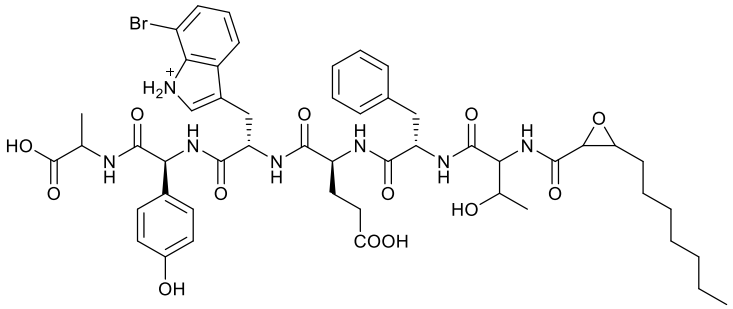


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

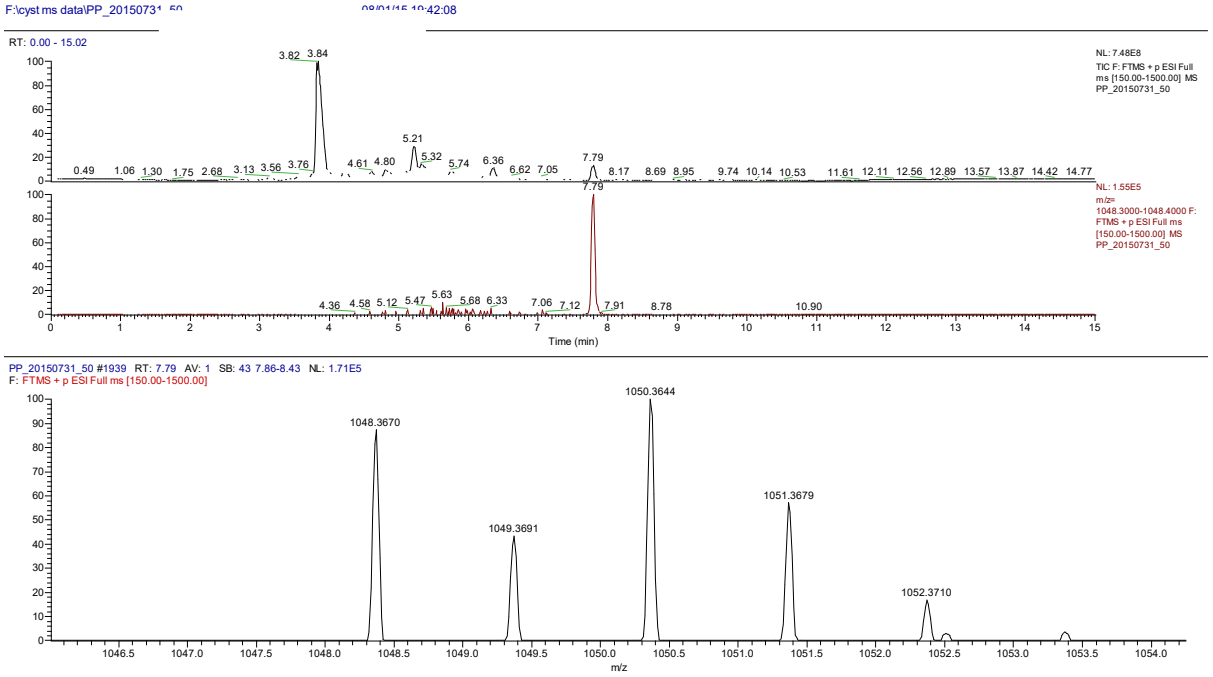
Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278



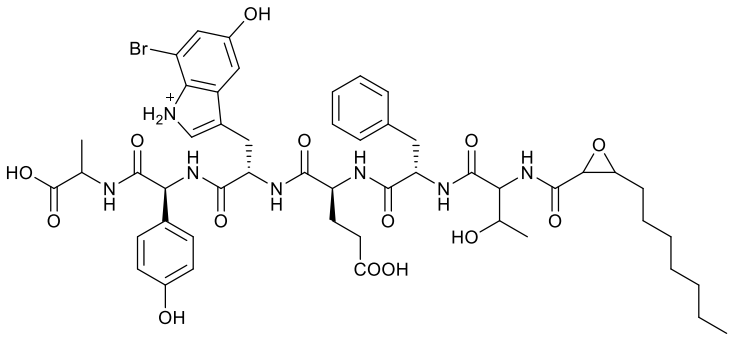
(7BrW) Brominated linear cystargamide[ALA] un hydroxylated



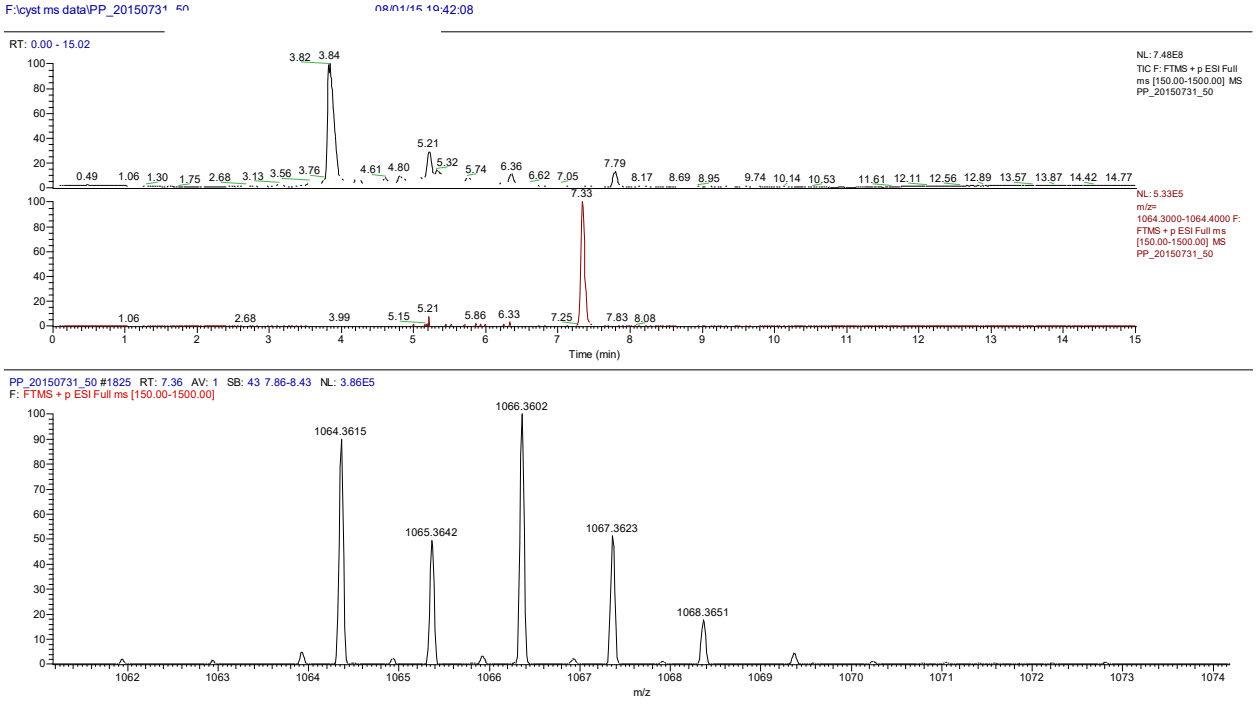
Chemical Formula: $C_{50}H_{63}BrN_7O_{13}^+$
Exact Mass: 1048.3662



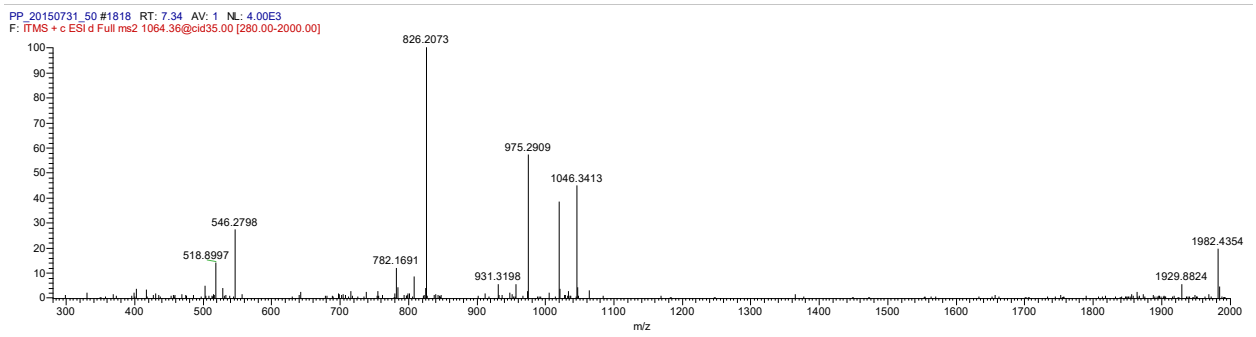
(6BrW) Brominated linear cystargamide[ALA]

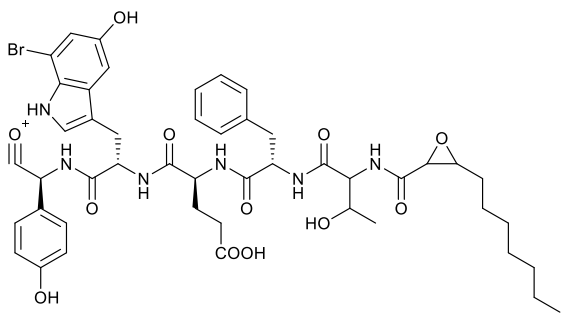


Chemical Formula: $C_{50}H_{63}BrN_7O_{14}^+$
Exact Mass: 1064.3611

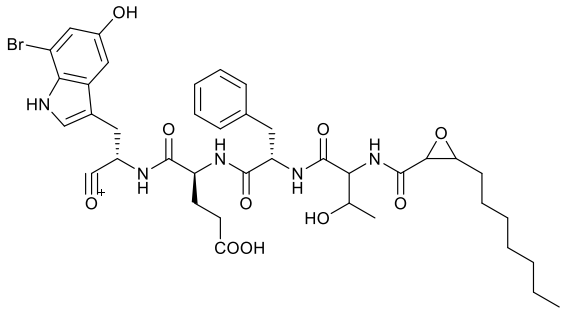


MS2 spectrum and fragment assignment

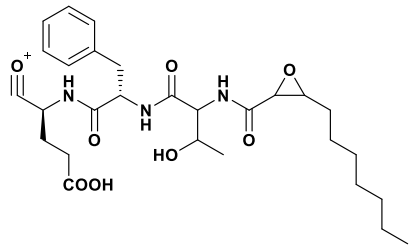




Chemical Formula: $C_{47}H_{56}BrN_6O_{12}^+$
Exact Mass: 975.3134

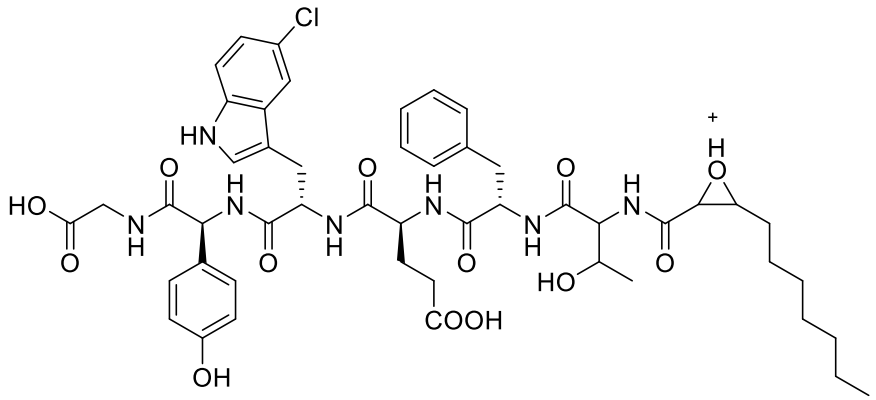


Chemical Formula: $C_{39}H_{49}BrN_5O_{10}^+$
Exact Mass: 826.2657

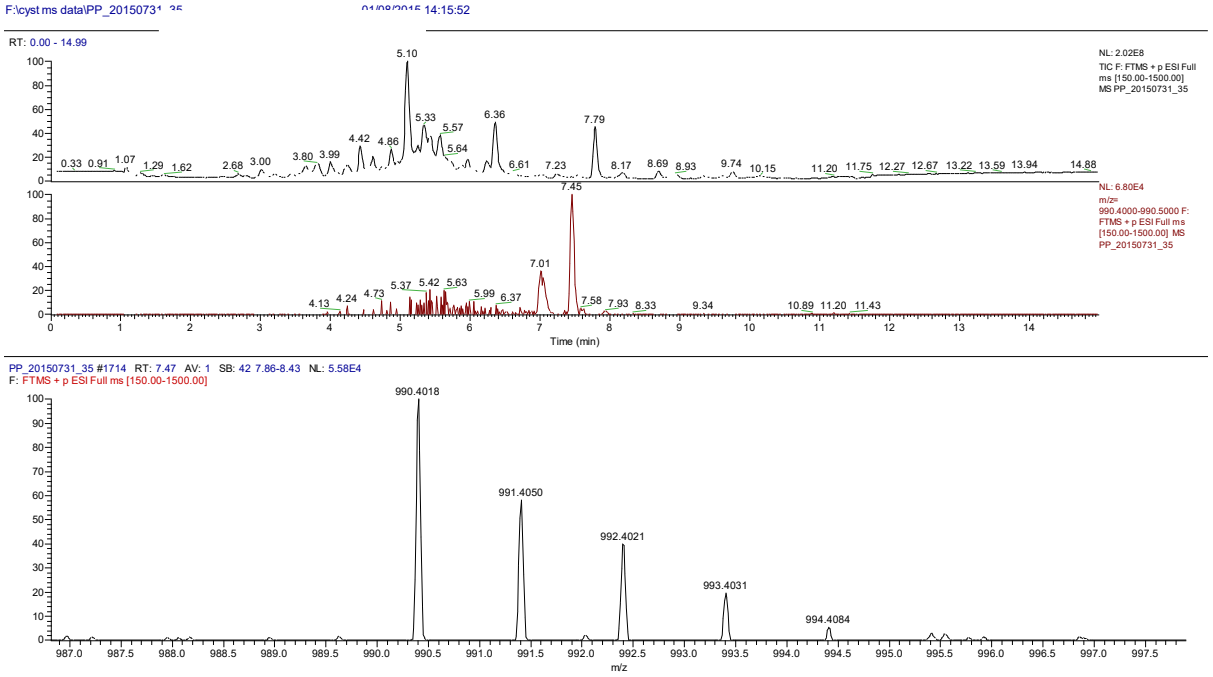


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

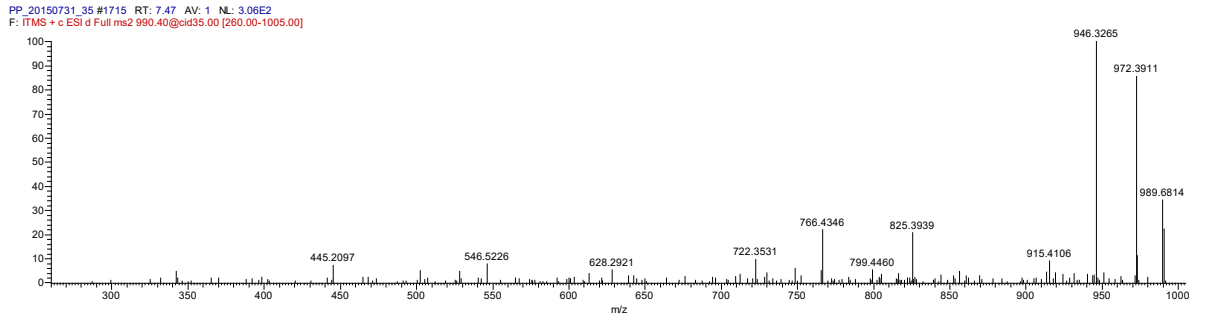
(5ClW) Chlorinated linear cystargamide no hydroxylated

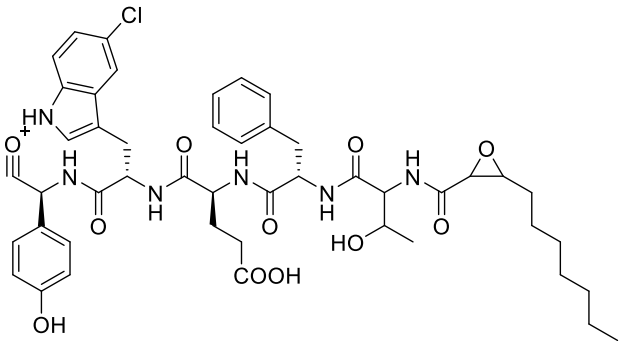


Chemical Formula: $C_{49}H_{61}ClN_7O_{13}^+$
Exact Mass: 990.4010

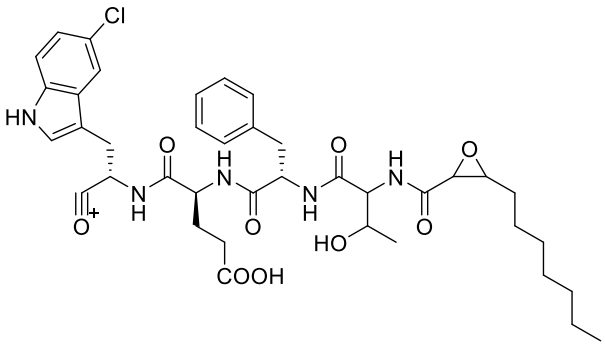


MS2 spectrum and fragment assignment

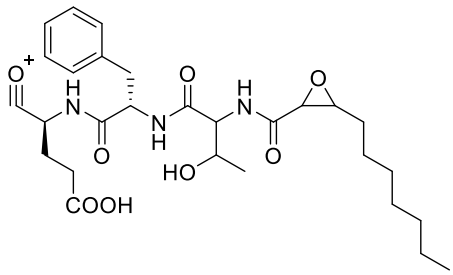




Chemical Formula: $C_{47}H_{56}ClN_6O_{11}^+$
Exact Mass: 915.3690

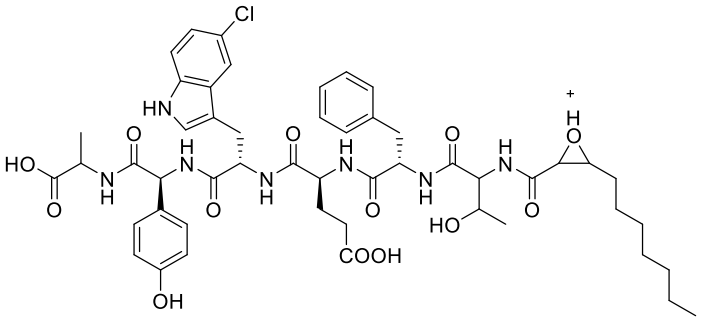


Chemical Formula: $C_{39}H_{49}ClN_5O_9^+$
Exact Mass: 766.3213

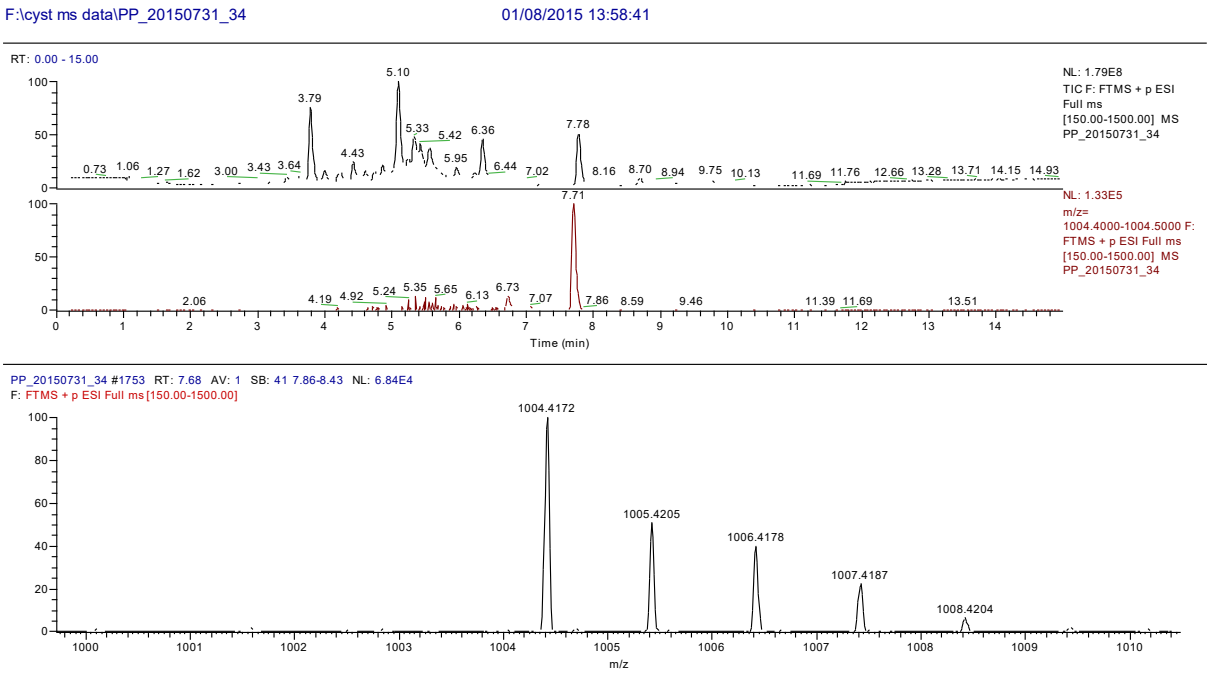


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

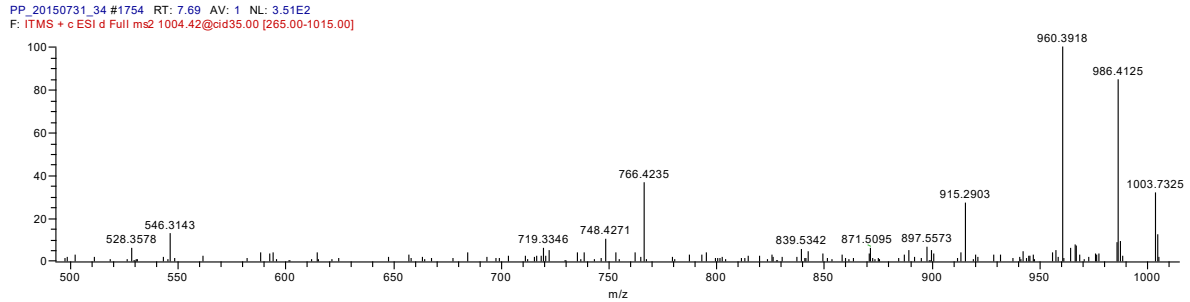
(5ClW) Chlorinated linear cystargamide[ALA] unhydroxylated



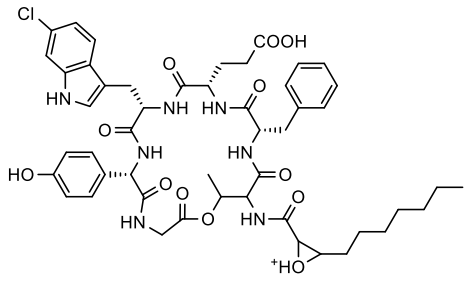
Chemical Formula: $C_{50}H_{63}ClN_7O_{13}^+$
Exact Mass: 1004.4167



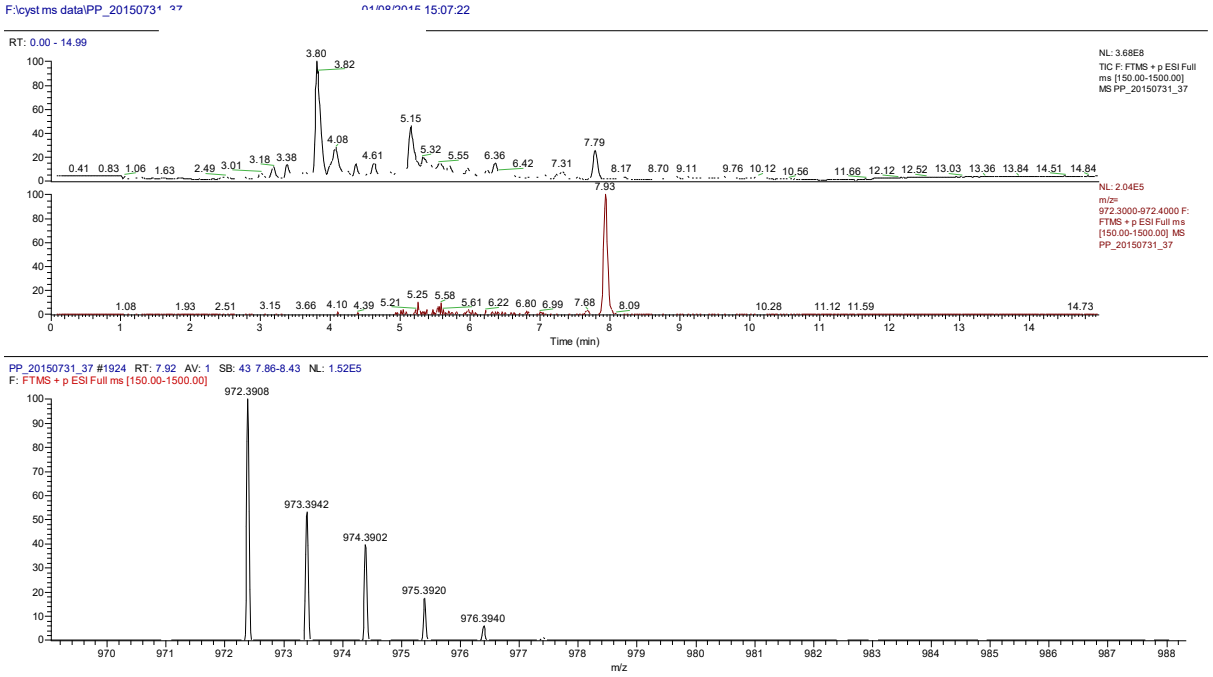
MS2 spectrum and fragment assignment



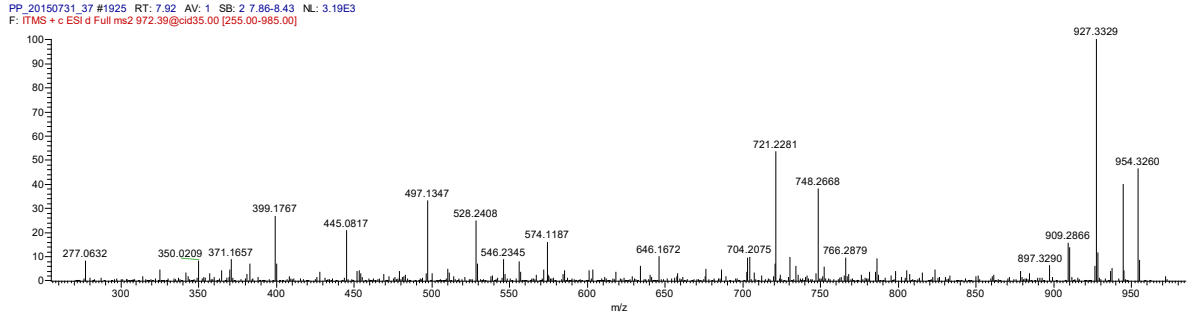
(6ClW) Chlorinated cystargamide un hydroxylated

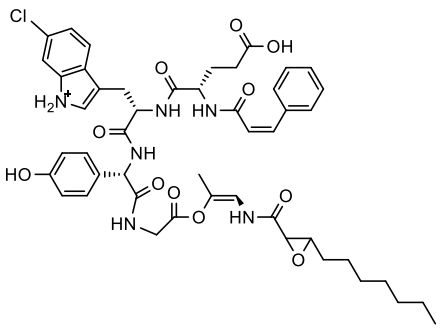


Chemical Formula: $C_{49}H_{59}ClN_7O_{12}^+$
Exact Mass: 972.3905

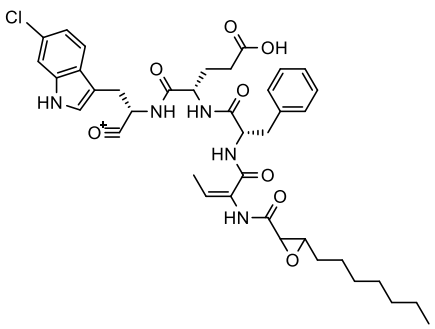


MS2 spectrum and fragment assignment

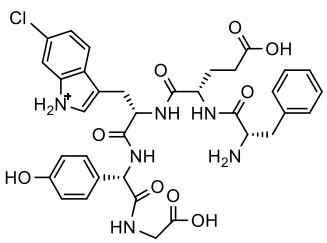




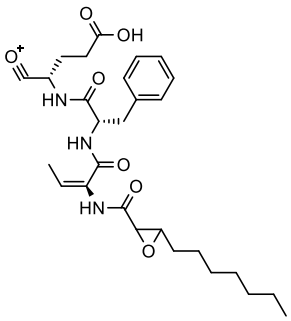
Chemical Formula: $C_{48}H_{56}ClN_6O_{11}^+$
Exact Mass: 927.3690



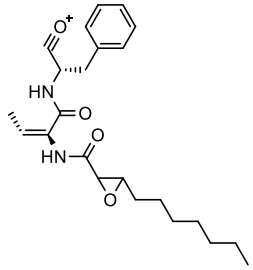
Chemical Formula: $C_{39}H_{47}ClN_5O_8^+$
Exact Mass: 748.3108



Chemical Formula: $C_{35}H_{38}ClN_6O_9^+$
Exact Mass: 721.2383

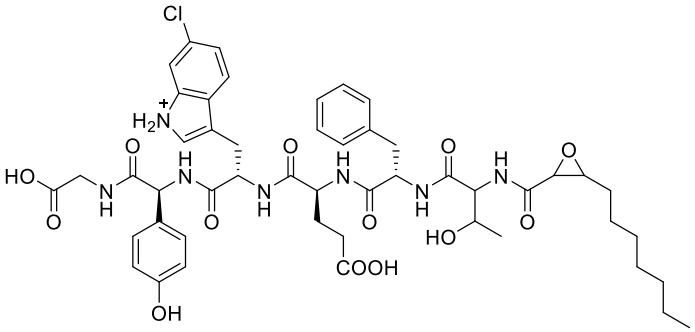


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

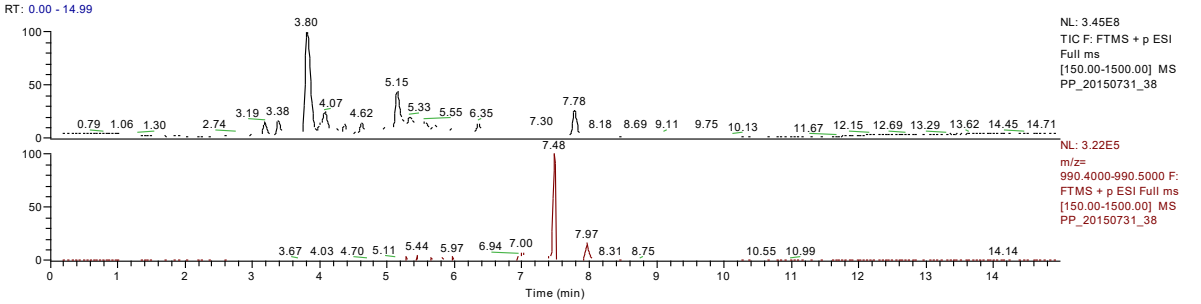


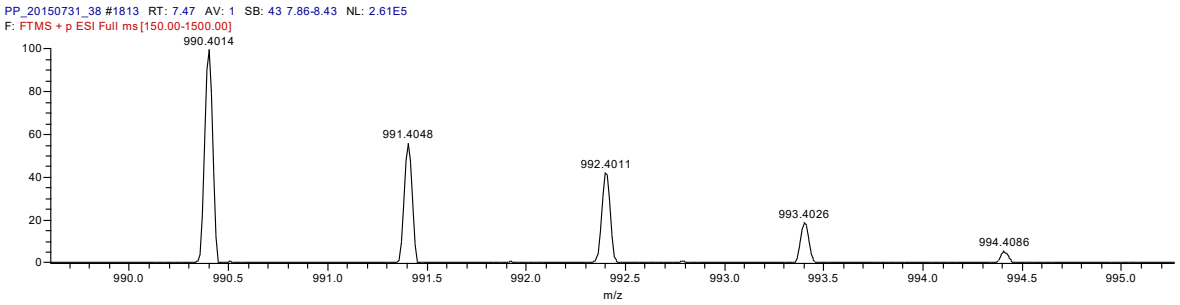
Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

(6ClW) Chlorinated linear cystargamide un hydroxylated

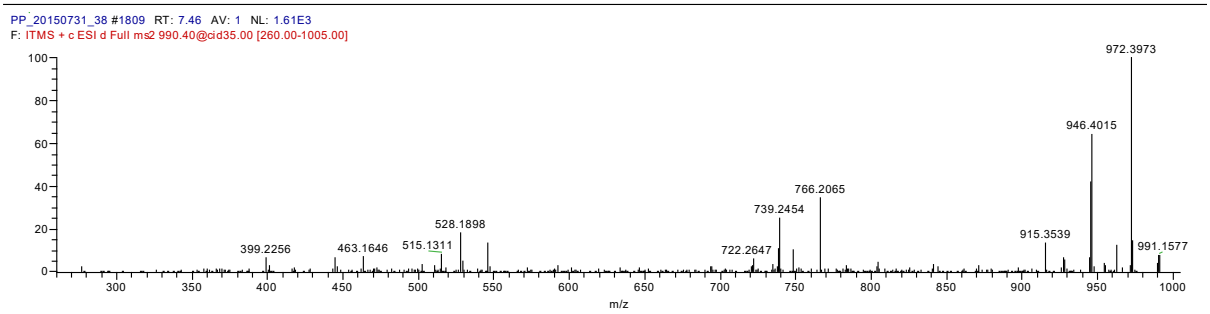


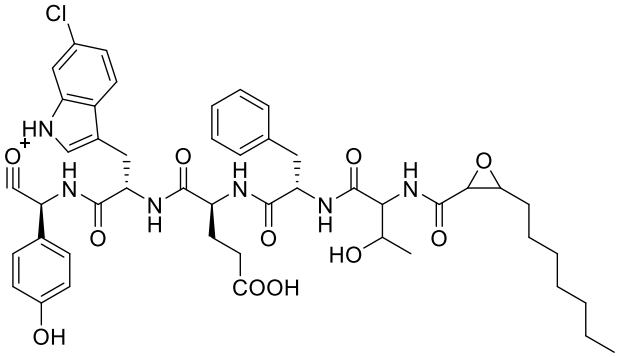
Chemical Formula: $C_{49}H_{61}ClN_7O_{13}^+$
Exact Mass: 990.4010



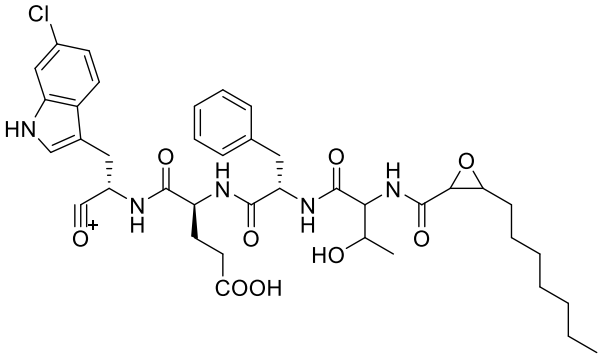


MS2 spectrum and fragment assignment

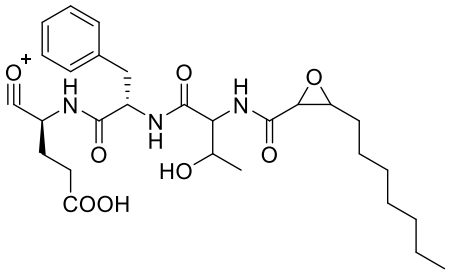




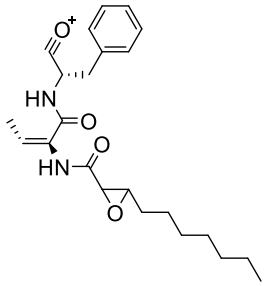
Chemical Formula: $C_{47}H_{56}ClN_6O_{11}^+$
Exact Mass: 915.3690



Chemical Formula: $C_{39}H_{49}ClN_5O_9^+$
Exact Mass: 766.3213

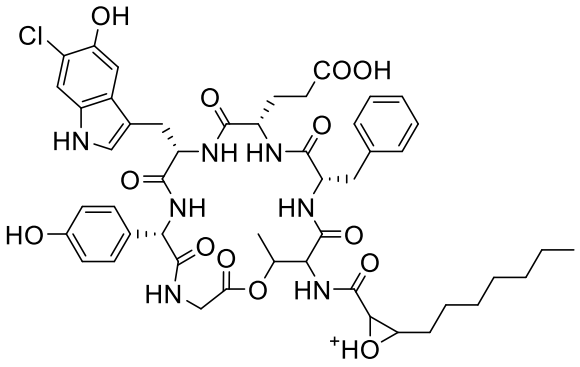


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

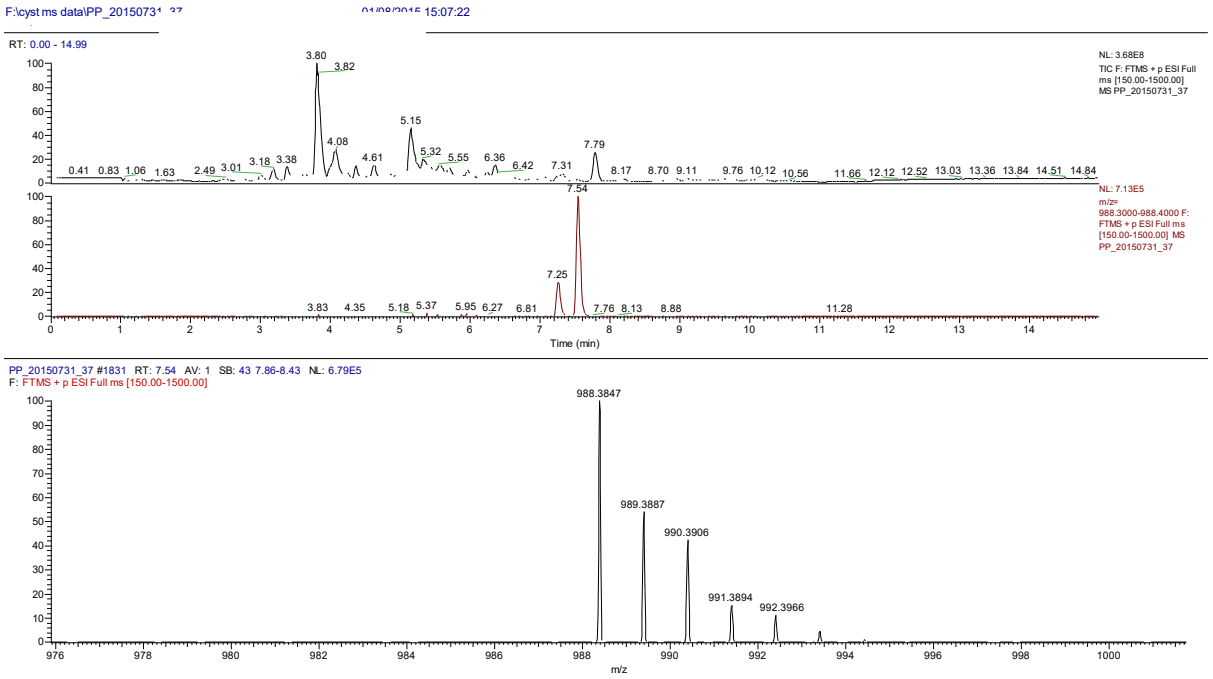


Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

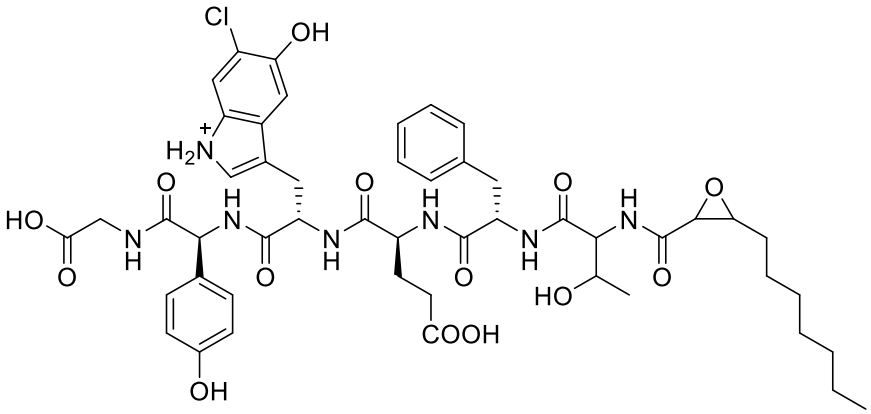
(6ClW) Chlorinated cystargamide



Chemical Formula: $C_{49}H_{59}ClN_7O_{13}^+$
Exact Mass: 988.3854

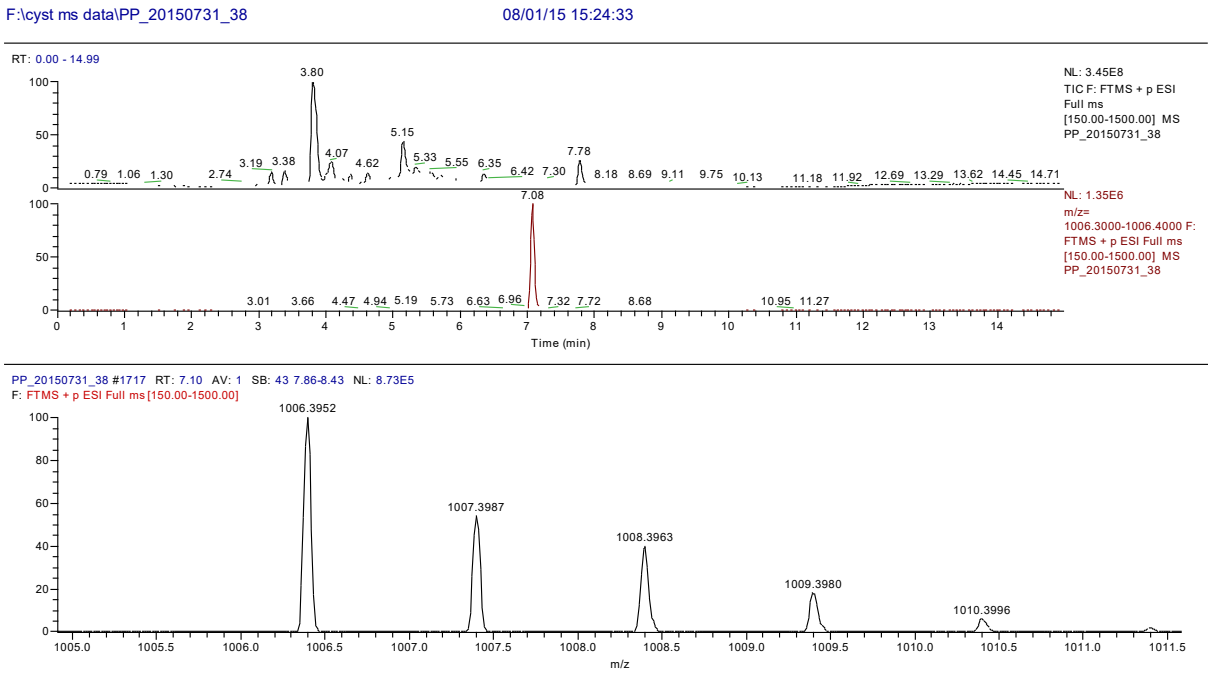


(6ClW) Chlorinated linear cystargamide

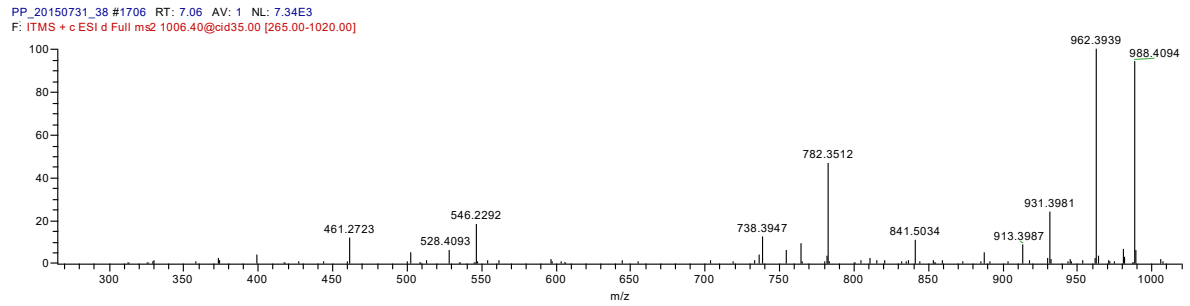


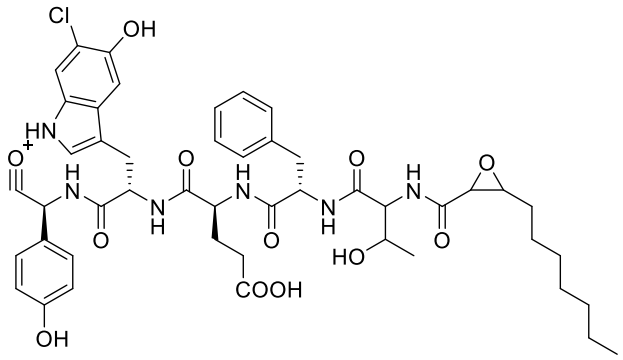
Chemical Formula: $C_{49}H_{61}ClN_7O_{14}^+$

Exact Mass: 1006.3960

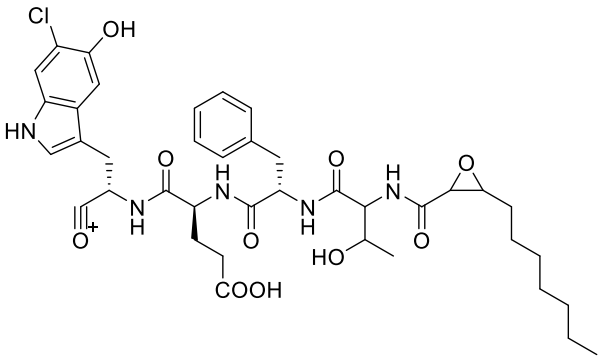


MS2 spectrum and fragment assignment

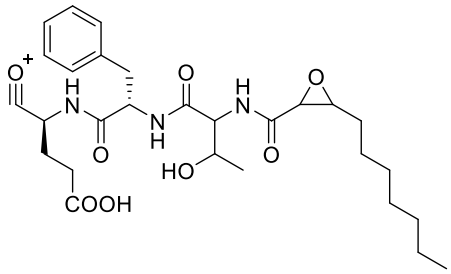




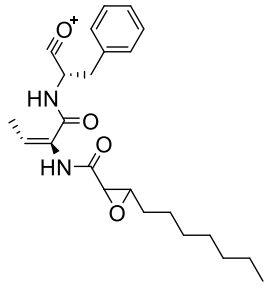
Chemical Formula: $C_{47}H_{56}ClN_6O_{12}^+$
Exact Mass: 931.3639



Chemical Formula: $C_{39}H_{49}ClN_5O_{10}^+$
Exact Mass: 782.3162

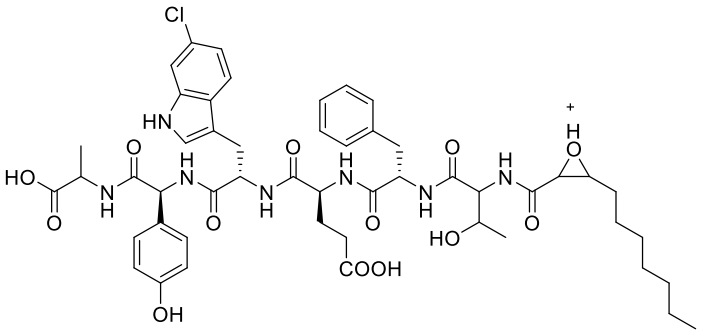


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

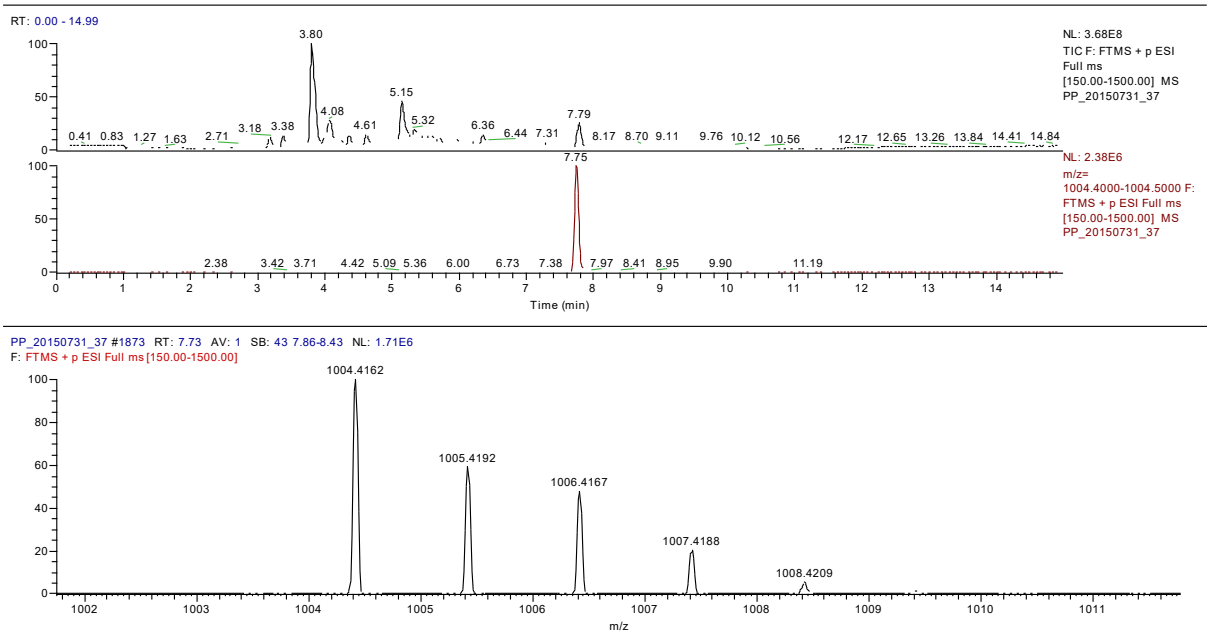


Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

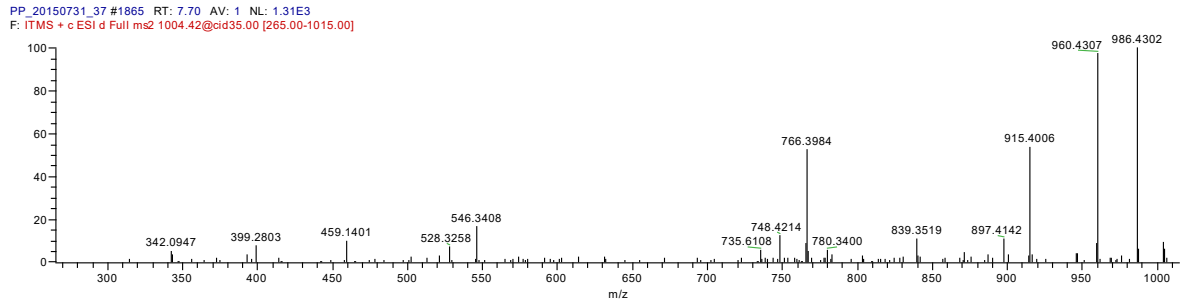
(6ClW) Chlorinated linear cystargamide[ALA] un hydroxylated

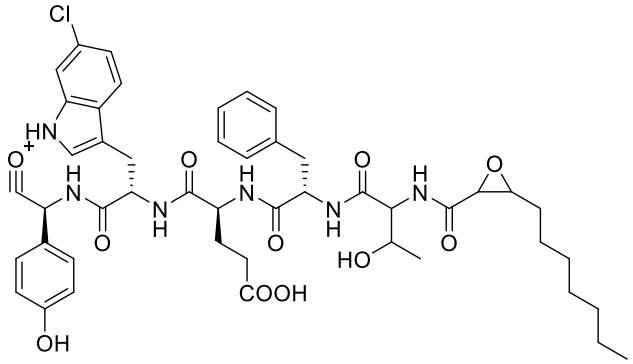


Chemical Formula: $C_{50}H_{63}ClN_7O_{13}^+$
Exact Mass: 1004.4167

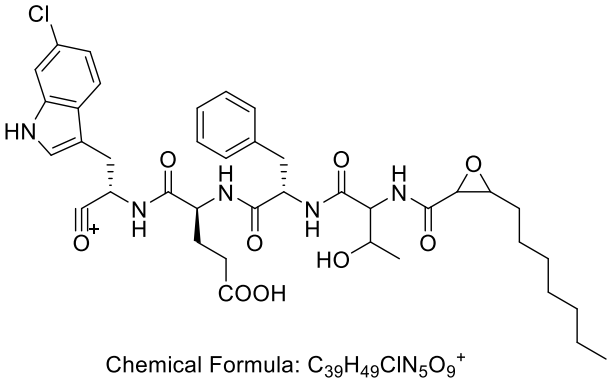


MS2 spectrum and fragment assignment

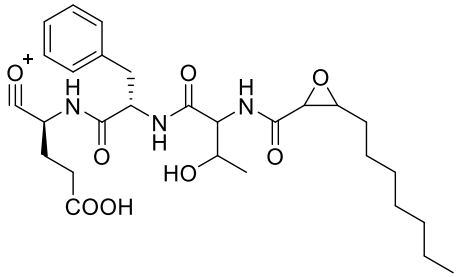




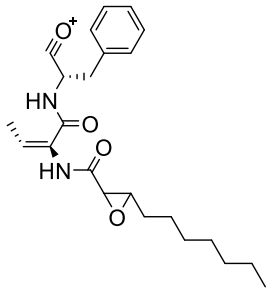
Chemical Formula: $C_{47}H_{56}ClN_6O_{11}^+$
Exact Mass: 915.3690



Chemical Formula: $C_{39}H_{49}ClN_5O_9^+$
Exact Mass: 766.3213

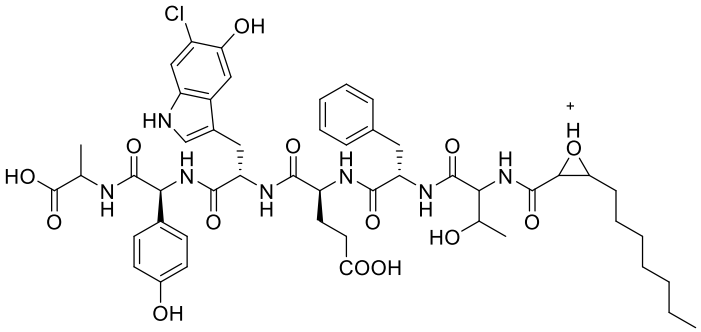


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810



Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

(6ClW)-Chlorinated linear cystargamide[ALA]



Chemical Formula: $C_{50}H_{63}ClN_7O_{14}^+$
Exact Mass: 1020.4116

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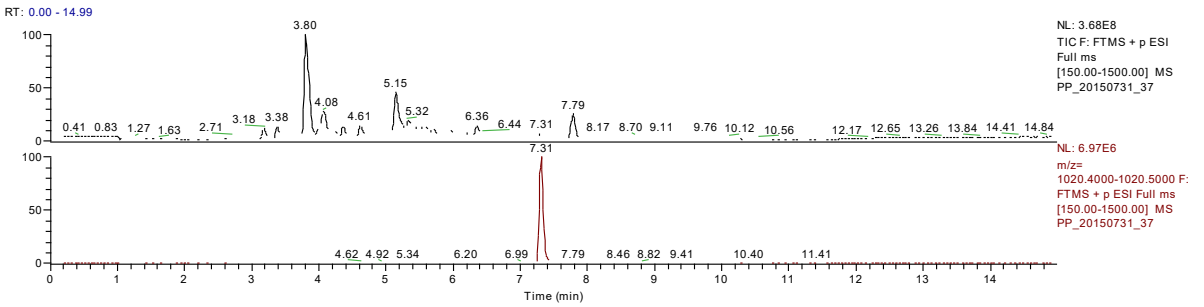
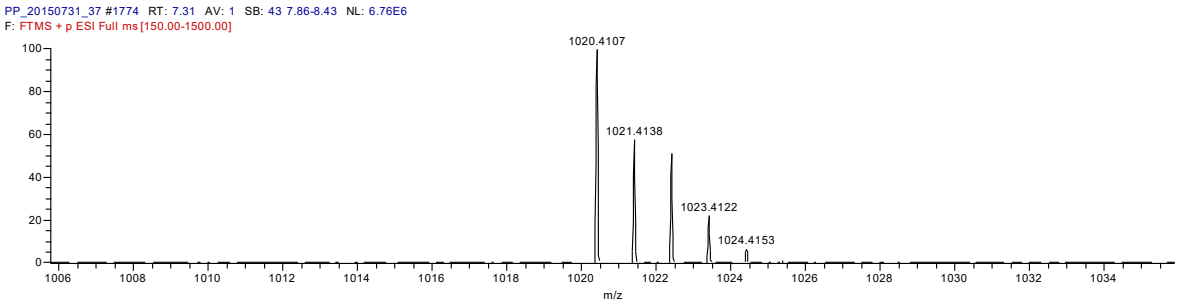
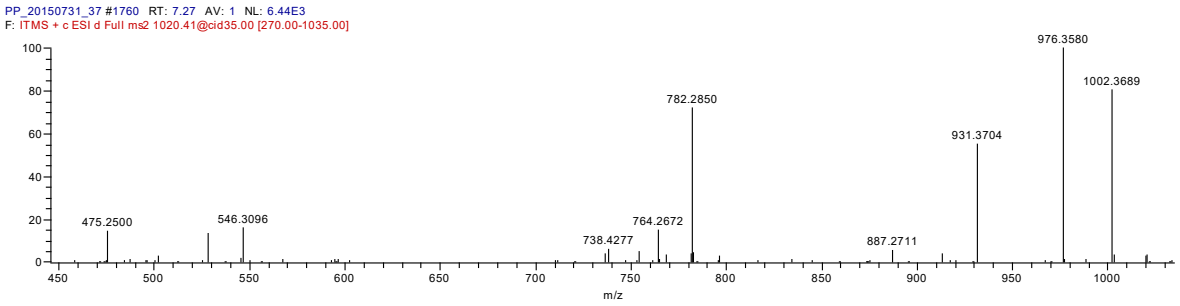
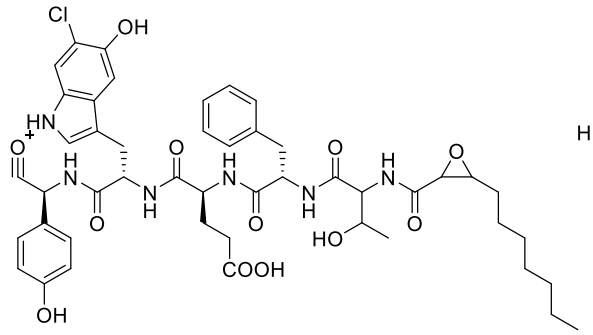
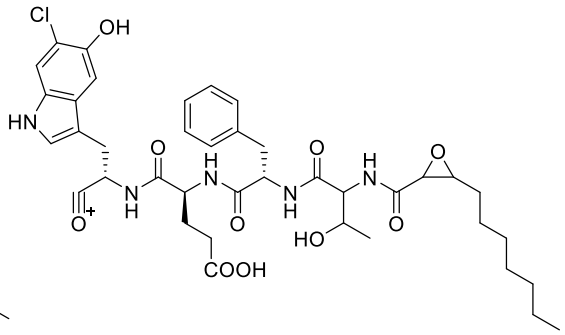




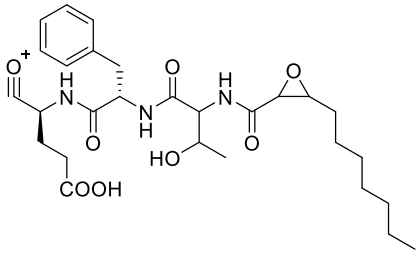




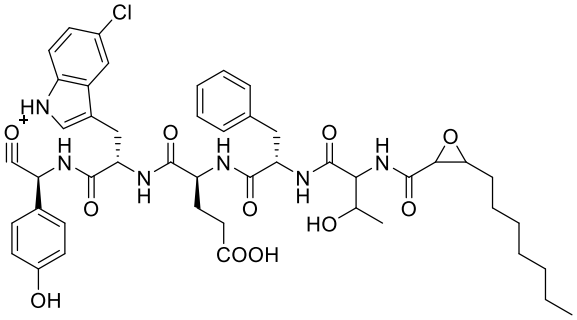
Chemical Formula: $C_{47}H_{56}ClN_6O_{12}^+$
Exact Mass: 931.3639



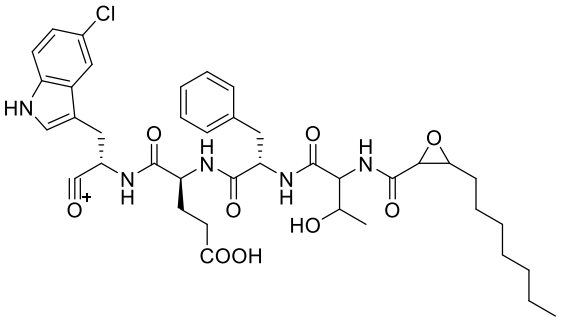
Chemical Formula: $C_{39}H_{49}ClN_5O_{10}^+$
Exact Mass: 782.3162



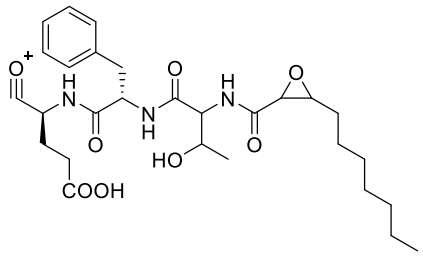
Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810



Chemical Formula: $C_{47}H_{56}ClN_6O_{11}^+$
Exact Mass: 915.3690

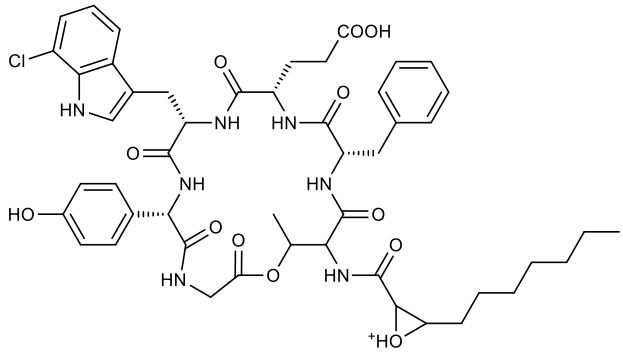


Chemical Formula: $C_{39}H_{49}ClN_5O_9^+$
Exact Mass: 766.3213

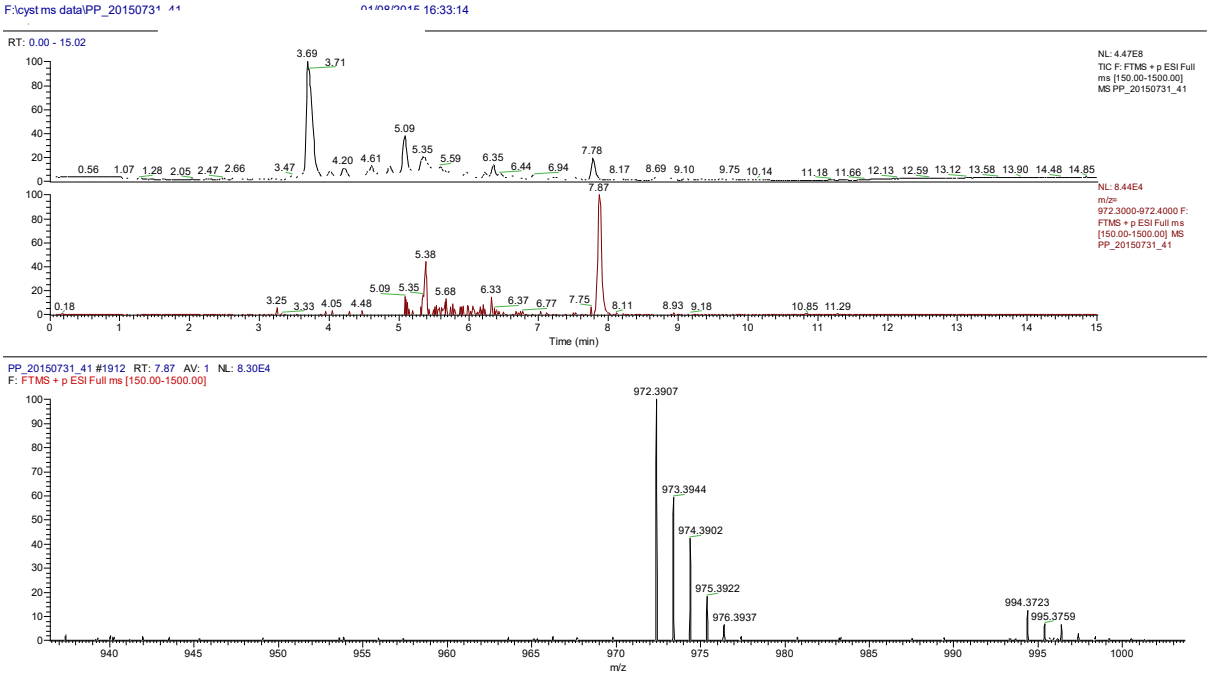


Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

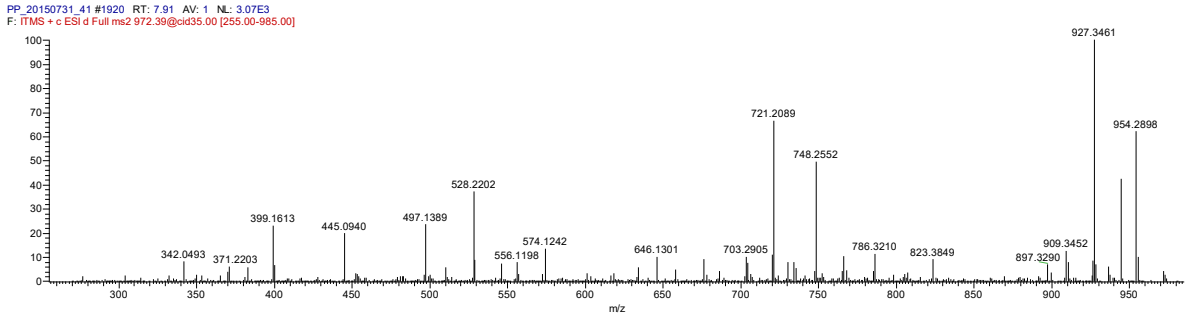
(7ClW) Chlorinated cystargamide unhydroxylated

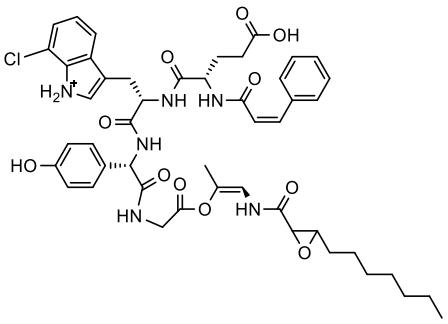


Chemical Formula: $C_{49}H_{59}ClN_7O_{12}^+$
Exact Mass: 972.3905

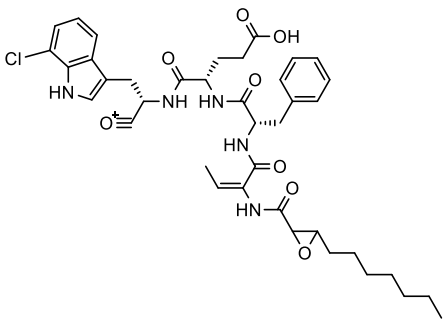


MS2 spectrum and fragment assignment

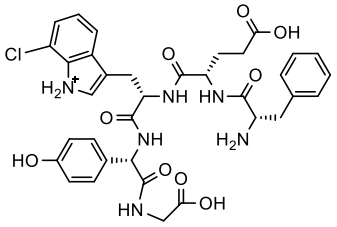




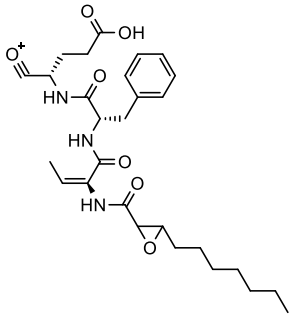
Chemical Formula: $C_{48}H_{56}ClN_6O_{11}^+$
Exact Mass: 927.3690



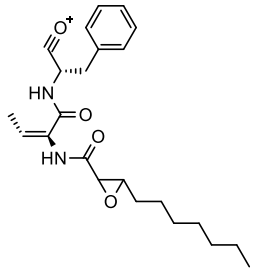
Chemical Formula: $C_{39}H_{47}ClN_5O_8^+$
Exact Mass: 748.3108



Chemical Formula: $C_{35}H_{38}ClN_6O_9^+$
Exact Mass: 721.2383

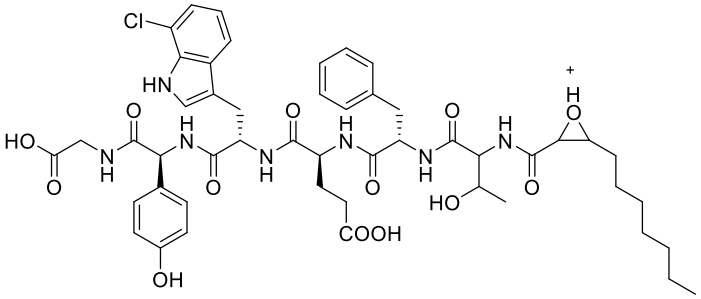


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

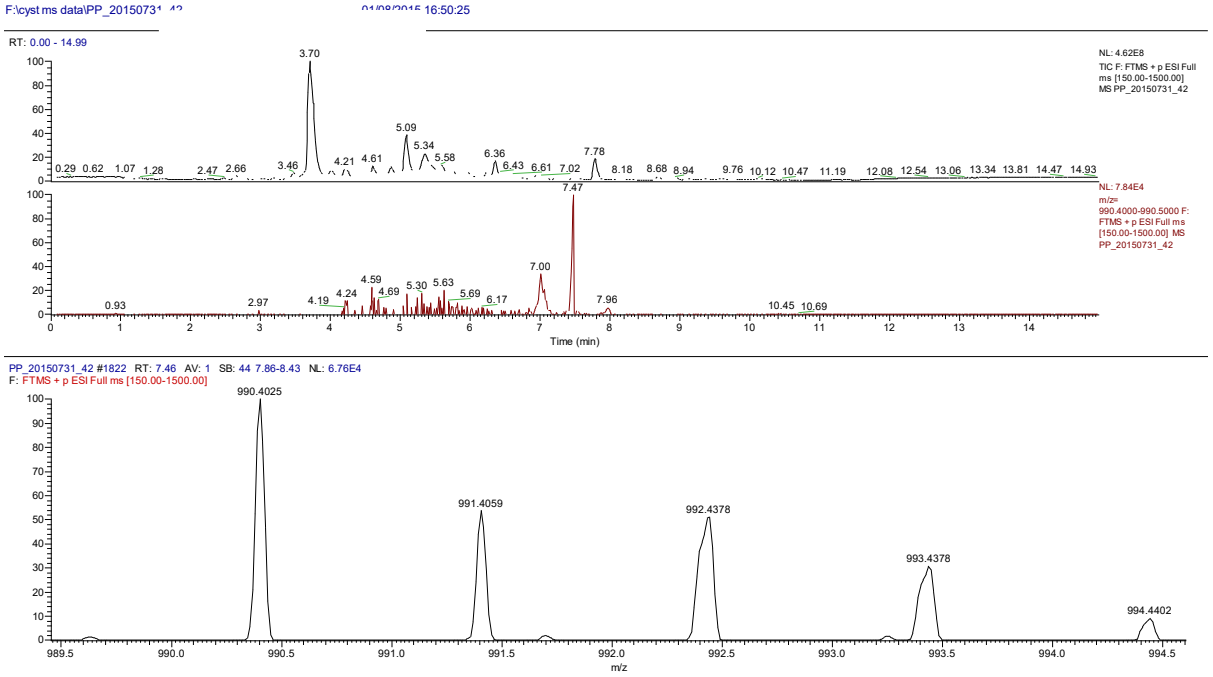


Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

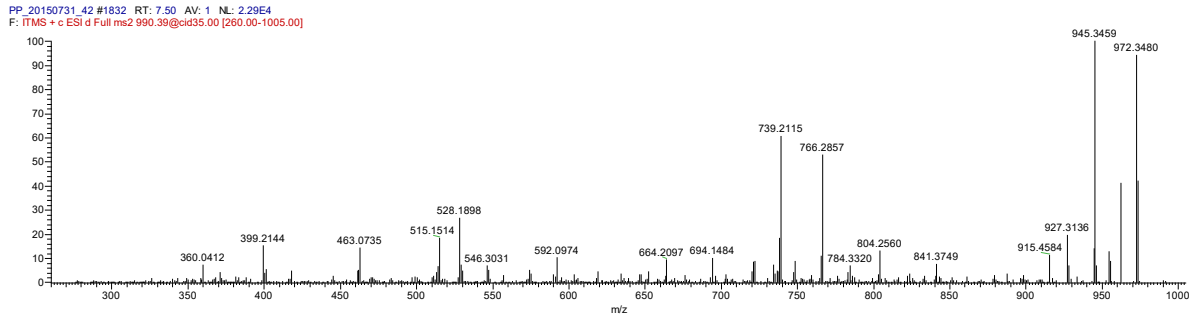
(7ClW) Chlorinated linear cystargamide un hydroxylated

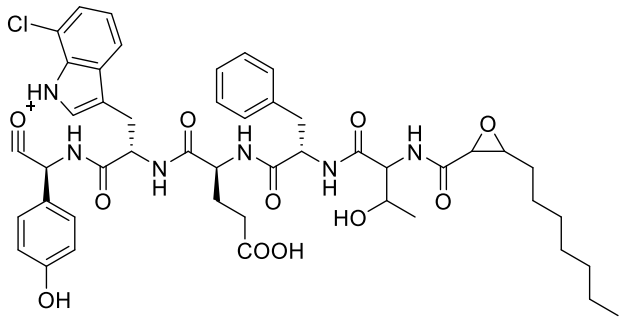


Chemical Formula: $C_{49}H_{61}ClN_7O_{13}^+$
Exact Mass: 990.4010

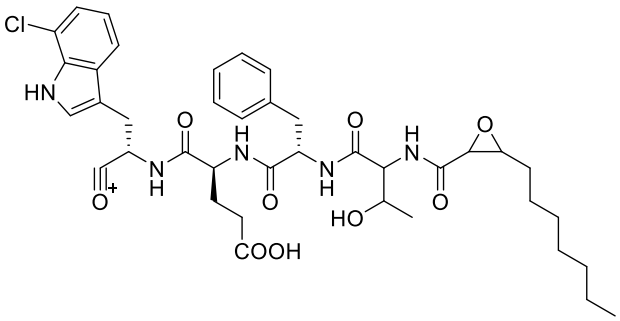


MS2 spectrum and fragment assignment

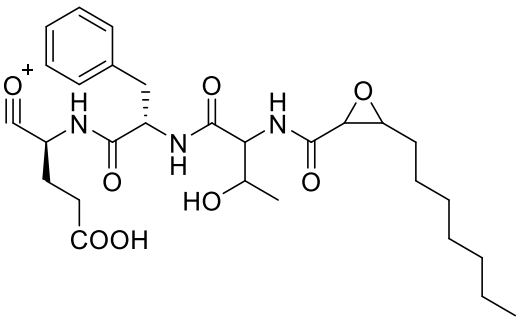




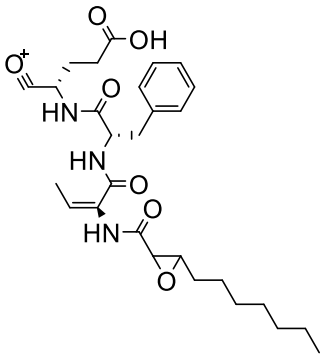
Chemical Formula: $C_{47}H_{56}ClN_6O_{11}^+$
Exact Mass: 915.3690



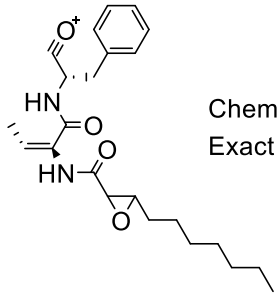
Chemical Formula: $C_{39}H_{49}ClN_5O_9^+$
Exact Mass: 766.3213



Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

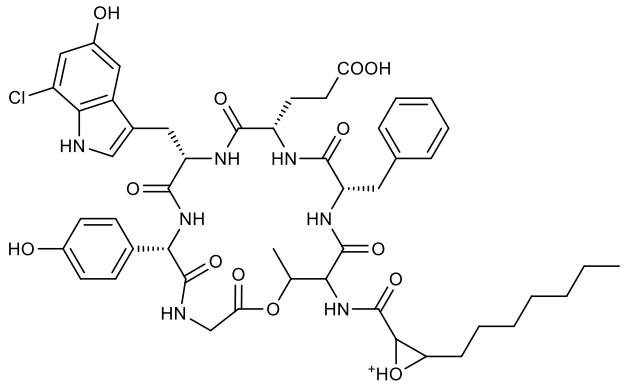


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704

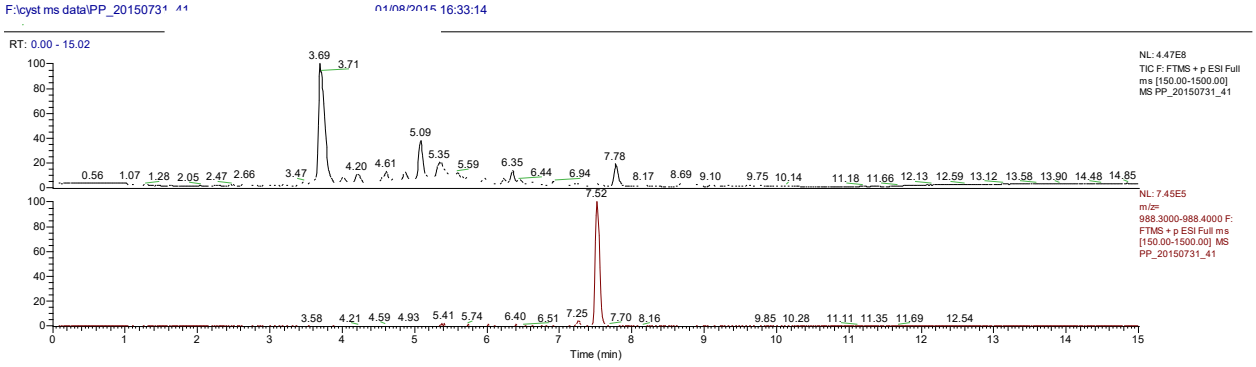


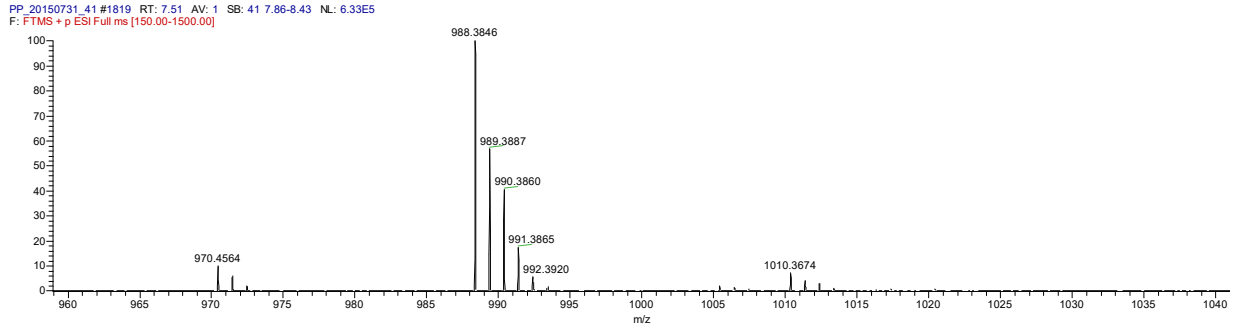
Chemical Formula: $C_{23}H_{31}N_2O_4^+$
Exact Mass: 399.2278

(7ClW) Chlorinated cystargamide

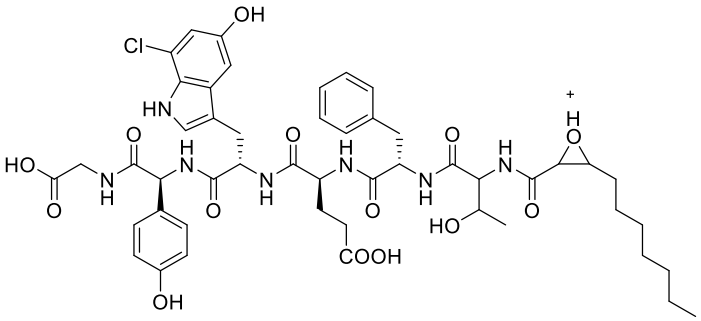


Chemical Formula: $C_{49}H_{59}ClN_7O_{13}^+$
Exact Mass: 988.3854

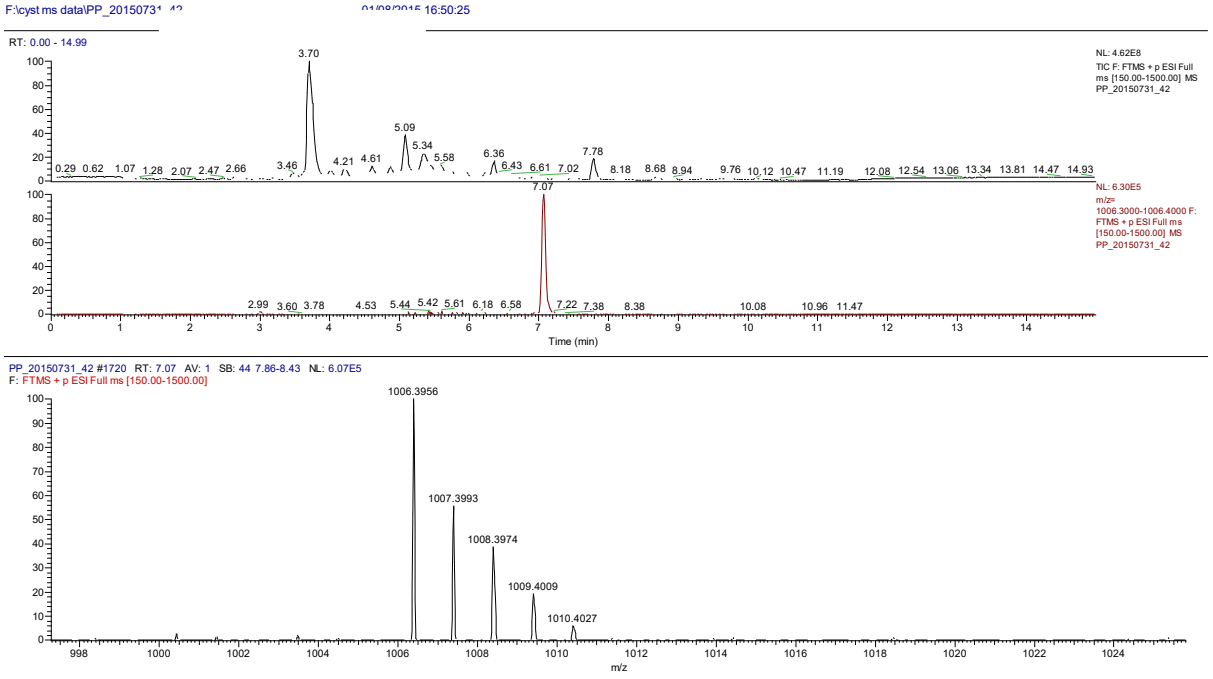




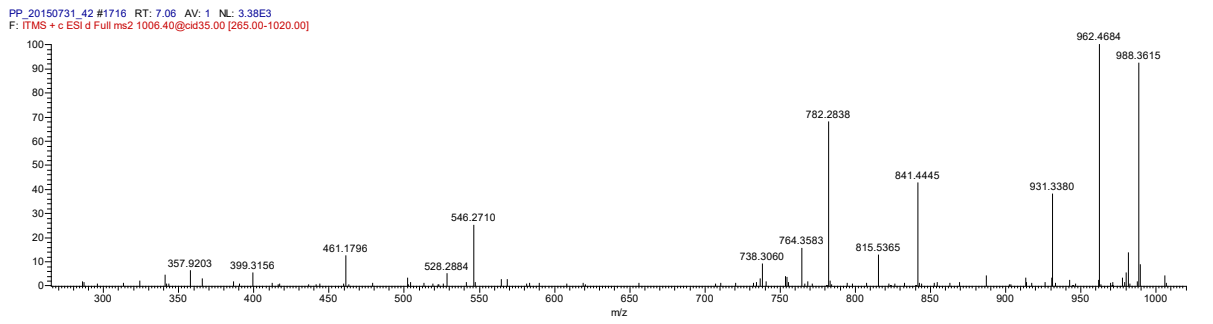
(7ClW) Chlorinated linear cystargamide

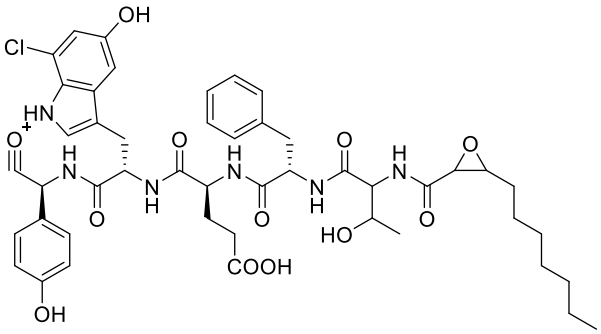


Chemical Formula: $C_{49}H_{61}ClN_7O_{14}^+$
Exact Mass: 1006.3960

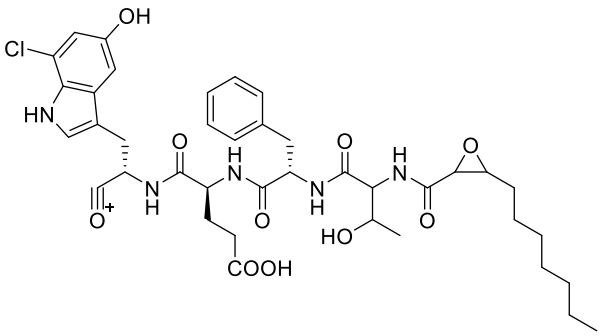


MS2 spectrum and fragment assignment

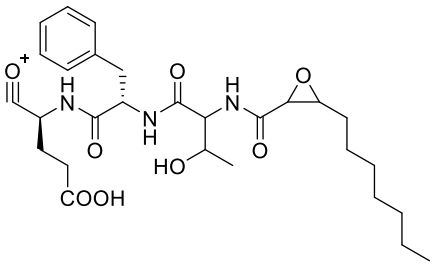




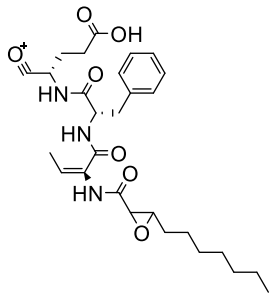
Chemical Formula: $C_{47}H_{56}ClN_6O_{12}^+$
Exact Mass: 931.3639



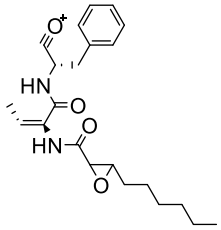
Chemical Formula: $C_{39}H_{49}ClN_5O_{10}^+$
Exact Mass: 782.3162



Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

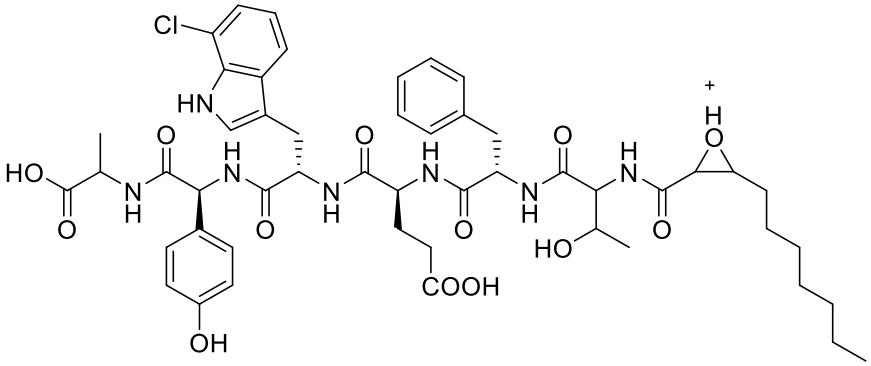


Chemical Formula: $C_{28}H_{38}N_3O_7^+$
Exact Mass: 528.2704



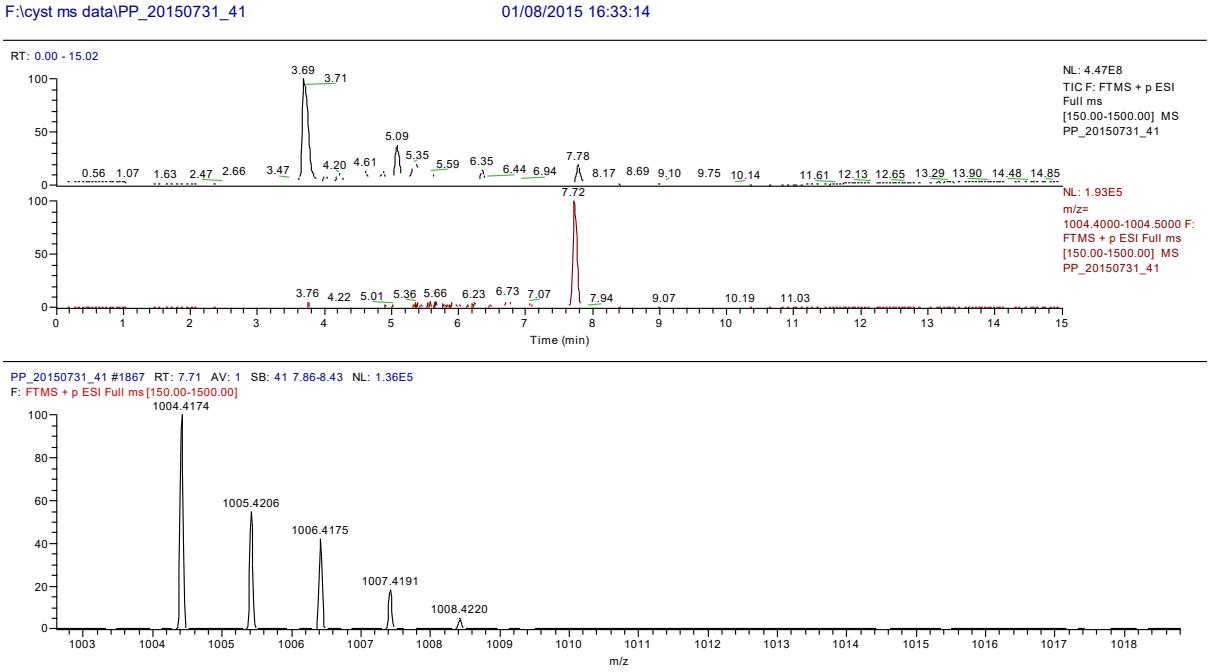
Chemical Formula: $C_{23}H_{33}N_2O_4^+$
Exact Mass: 401.2435

(7ClW) Chlorinated linear cystargamide[ALA] unhydroxylated

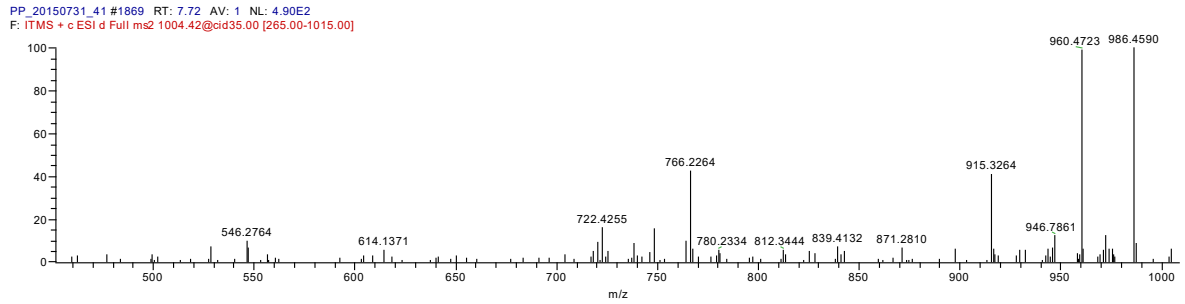


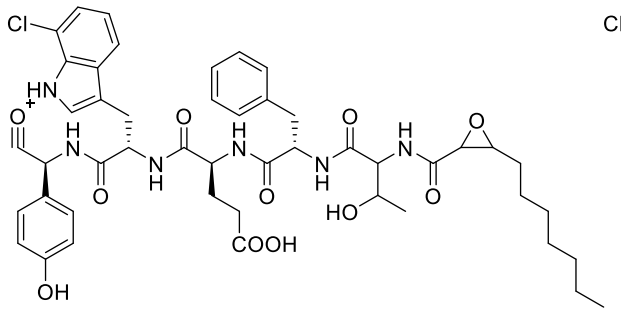
Chemical Formula: $C_{50}H_{63}ClN_7O_{13}^{+}$

Exact Mass: 1004.4167

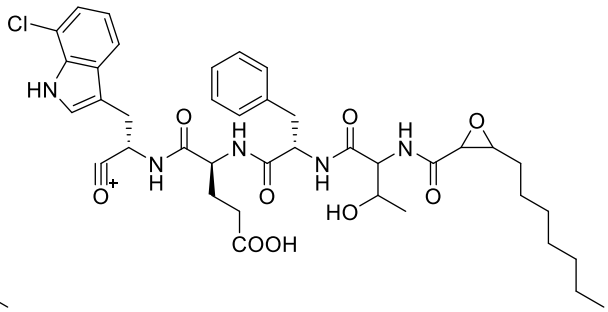


MS2 spectrum and fragment assignment

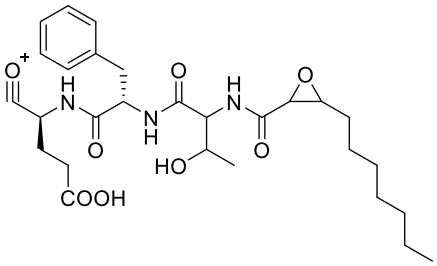




Chemical Formula: $C_{47}H_{56}ClN_6O_{11}^+$
Exact Mass: 915.3690

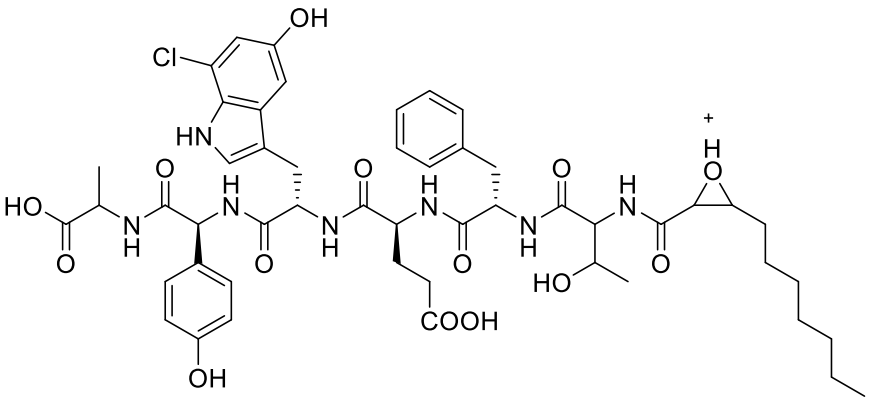


Chemical Formula: $C_{39}H_{49}ClN_5O_9^+$
Exact Mass: 766.3213



Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

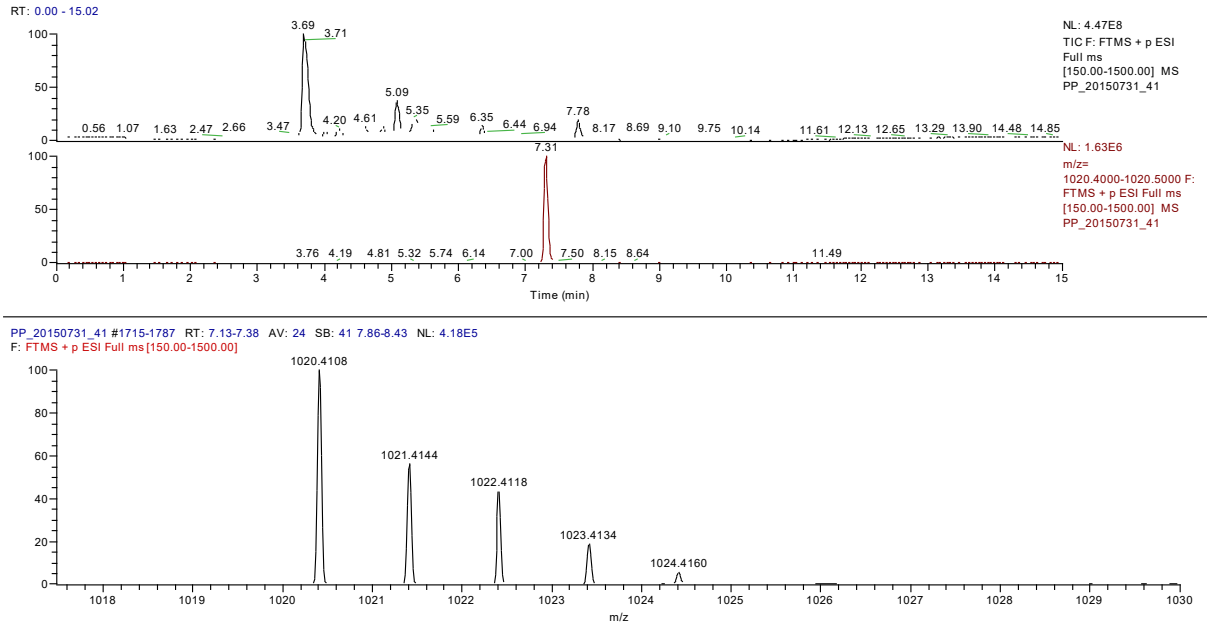
(7ClW) Chlorinated linear cystargamide[ALA]



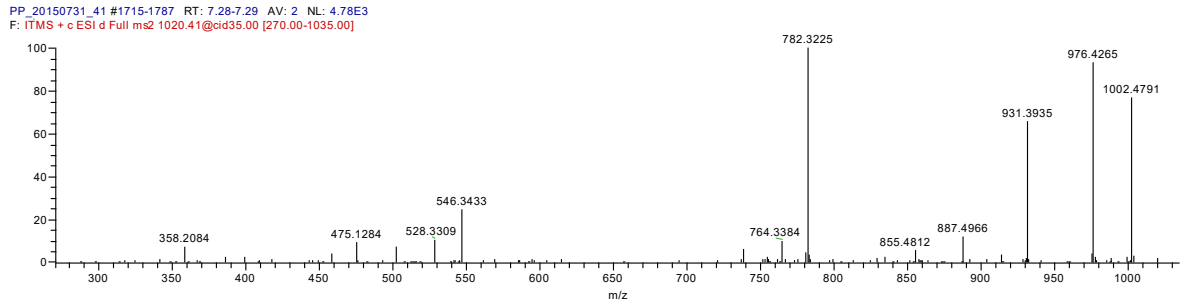
Chemical Formula: $C_{50}H_{63}ClN_7O_{14}^+$

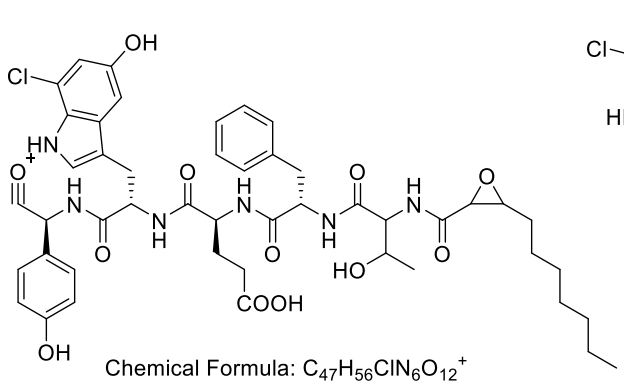
Exact Mass: 1020.4116

F:\cyst ms data\PP_20150731_41 01/08/2015 16:33:14

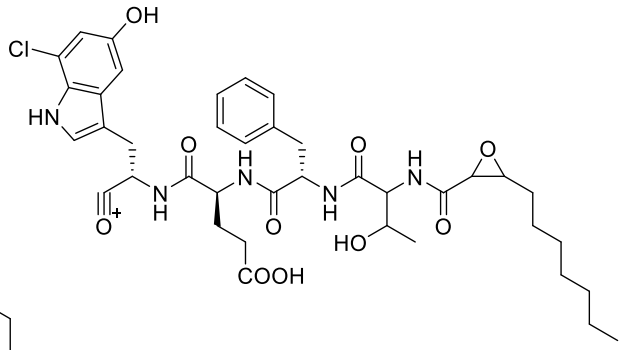


MS2 spectrum and fragment assignment

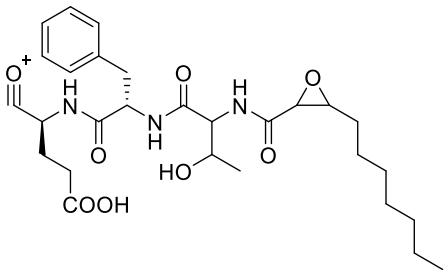




Chemical Formula: $C_{47}H_{56}ClN_6O_{12}^+$
Exact Mass: 931.3639



Chemical Formula: $C_{39}H_{49}ClN_5O_{10}^+$
Exact Mass: 782.3162



Chemical Formula: $C_{28}H_{40}N_3O_8^+$
Exact Mass: 546.2810

Cross coupling reaction on Br-Cystargamide

sXPhos **12** (0.6 mg, 1.2 μmol) and $CsCO_3$ (6.0 mg, 20 μmol) were added to a microwave tube and flushed with nitrogen. A stock solution of *bis*(acetonitrile)dichloropalladium (II) (0.8 mg, 4 μmol) in degassed H_2O:CH_3CN (1:1, 100 μL) was prepared. The catalyst stock solution (10 μL, 0.4 μmol) was added to the microwave tube, followed by a solution of the Br-Cystargamide **9** (1 mg) in H_2O:CH_3CN (1:1, 0.2 mL). 3-Fluorophenylacetylene **24** (10 μL) was then added. The microwave tube was flushed with nitrogen, then sealed. The reaction mixture was heated at 100 °C for 2 h. The reaction mixture was colled to r.t. A portion of the reaction mixture (0.1 mL) was removed and diluted with H_2O:CH_3CN (1:1, 0.2 mL). Polystyrene supported triphenylphosphine (5 mg) was added the sample was well shaken. The sample was filtered through a plug of cotton wool, then centrifuged (13,000 rpm, 16060 g, 5 min). The reaction mixture (30 μL) diluted with H_2O:CH_3CN (9:1, 970 µL) and analysed by LC-MS.

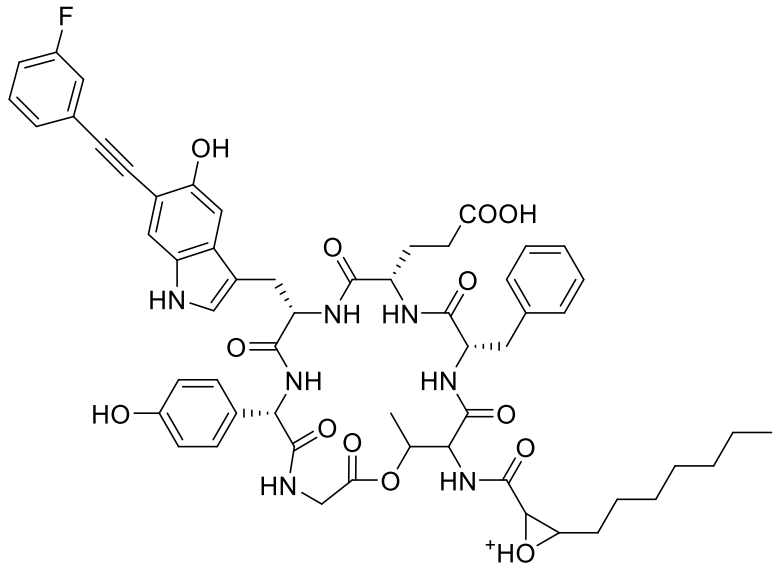
LC-MS analysis showed signals consistent with the product **64** at 1072 $[M+H]^+$, as well as the hydrolysed linear form of the peptide **65** at 1090 $[M+H]^+$.

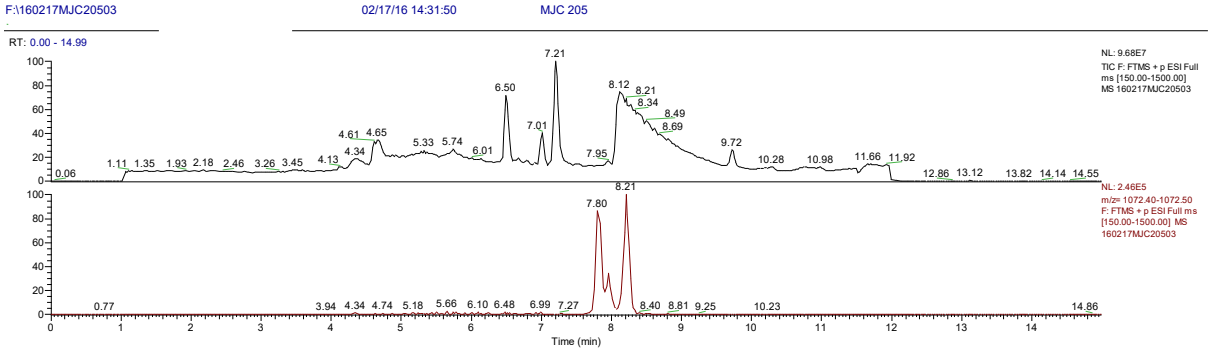
^{19}F NMR (470 MHz, D_2O) δ = -114.05 (1F, ddd, *J*(H,F)= 9.9, 9.3, 5.7 Hz), -114.43 (1F, ddd, *J*(H,F)= 9.7, 9.6, 6.3 Hz); Assignment of ^{19}F NMR signals to the cyclized and linear products respectively is tentative, and based on the observed ratios by LC-MS.

Compound **65** MS (ESI) 1090 (50) $[M+H]^+$, 1046 (40), 641 (40), 515 (100), 376 (50); HRMS: *m*/*z* calcd for $C_{57}H_{65}F_1N_7O_{14}$ $[M+H]^+$: 1090.4568; found: 1090.4562;

Compound **64** MS (ESI) 1072 (30) $[M+H]^+$, 1028 (20), 635 (40), 567 (20), 421 (60), 376 (100); HRMS: *m*/*z* calcd for $C_{57}H_{63}F_1N_7O_{13}$ $[M+H]^+$: 1072.4462; found: 1072.4464.

Cross Coupled Cyclised Cystargamide (64)

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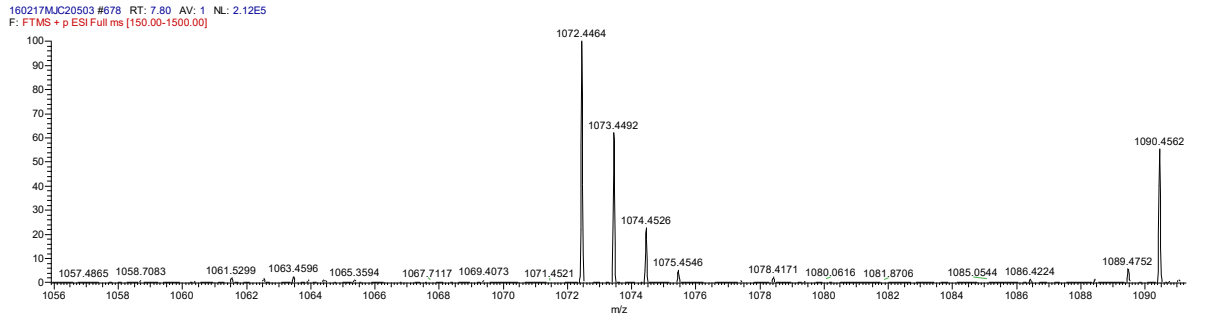
02/17/16 14:31:50
MJC 205
RT: 0.00 - 14.99
NL: 9.68E7
TIC F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
NL: 2.46E5
m/z= 1072.40-1072.50 F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
Time (min)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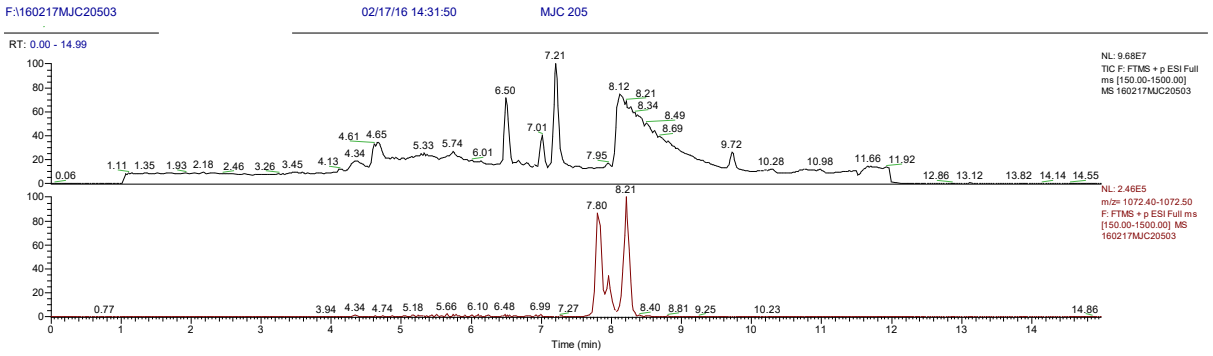
1090.4562
1074.4526
1075.4546
1089.4752
m/z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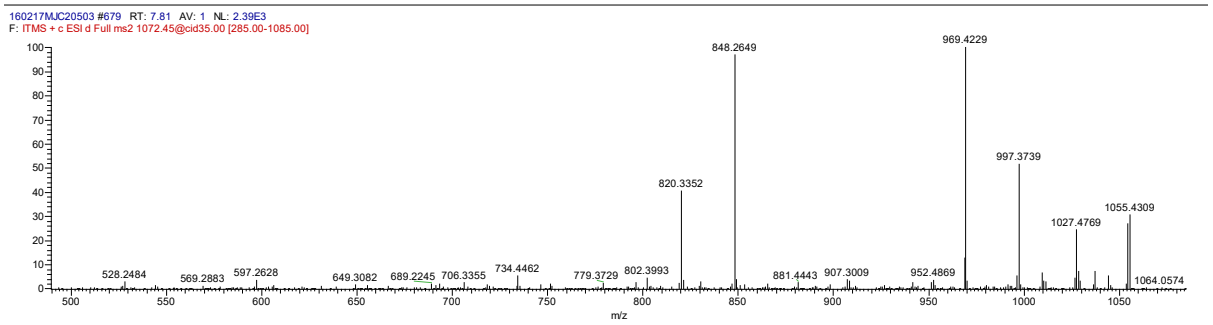
02/17/16 14:31:50
MJC 205
RT: 0.00 - 14.99
NL: 9.68E7
TIC F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
NL: 2.46E5
m/z= 1072.40-1072.50 F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
Time (min)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997.3739
820.3352
1055.4309
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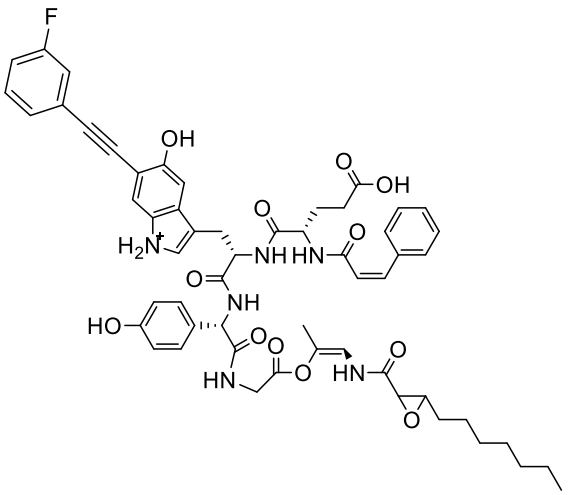
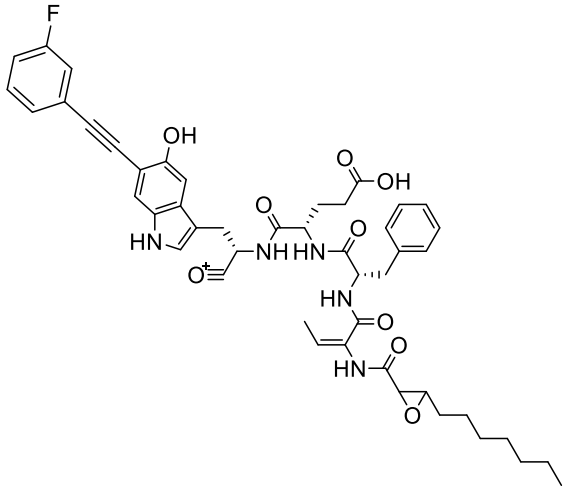
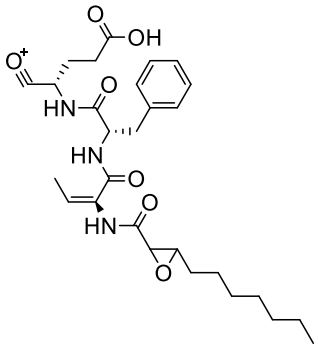
1064.0574
m/z



Exact Mass: 1027.4248

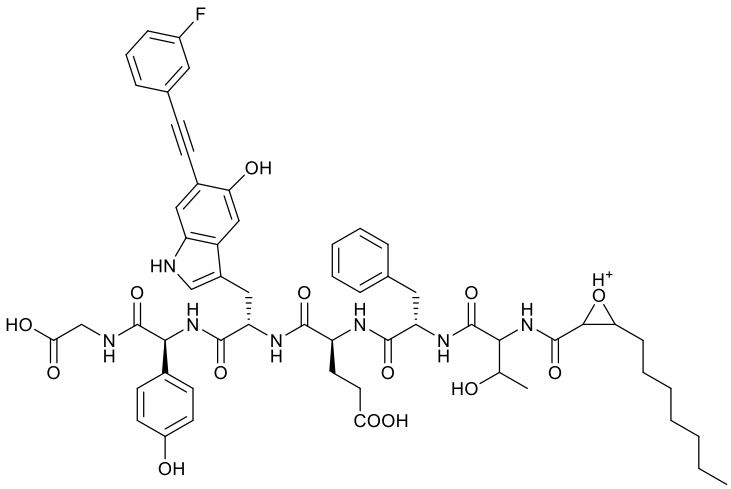


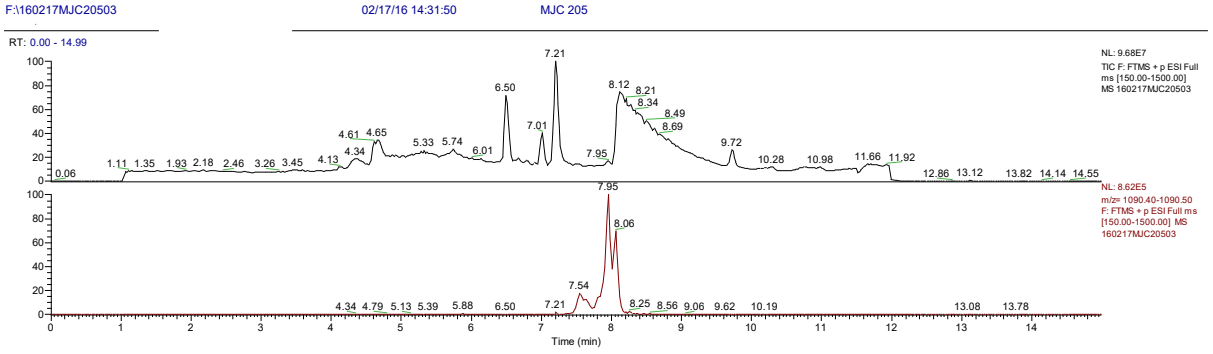
Exact Mass: 848.3665



Exact Mass: 528.2704

Cross Coupled Linear Cystargamide (65)

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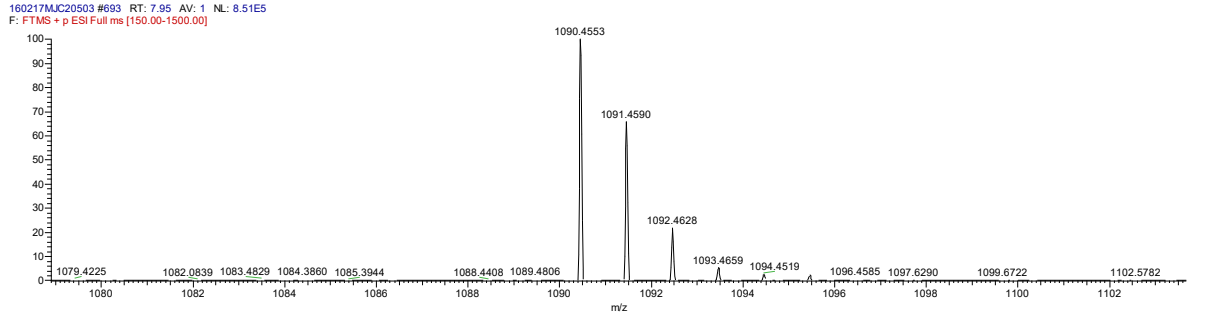
02/17/16 14:31:50
MJC 205
RT: 0.00 - 14.99
NL: 9.68E7
TIC F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
NL: 8.62E5
m/z= 1090.40-1090.50 F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
Time (min)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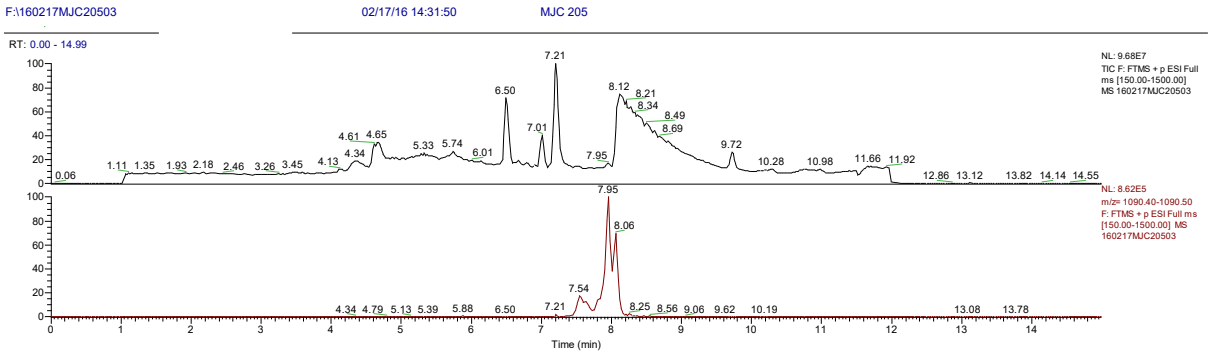
m/z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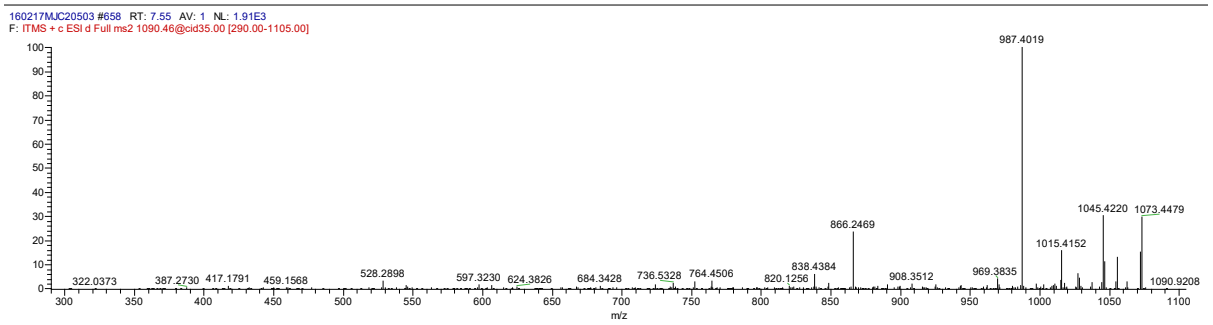
02/17/16 14:31:50
MJC 205
RT: 0.00 - 14.99
NL: 9.68E7
TIC F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
NL: 8.62E5
m/z= 1090.40-1090.50 F: FTMS + p ESI Full ms [150.00-1500.00] MS 160217MJC20503
Time (min)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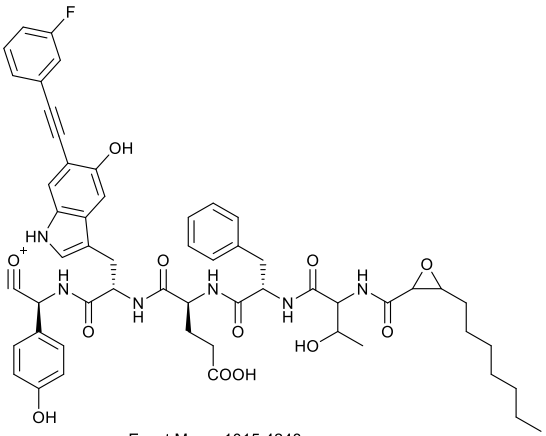
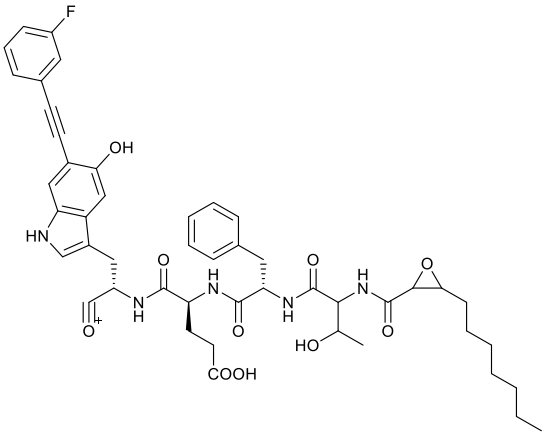
m/z



Exact Mass: 1015.4248



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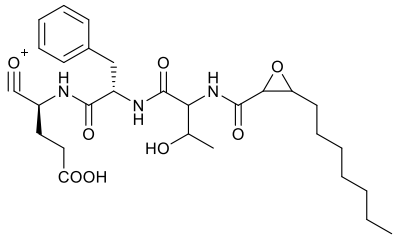
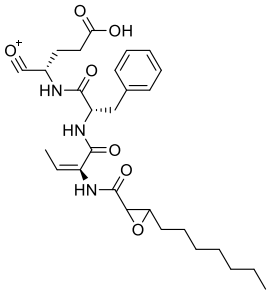
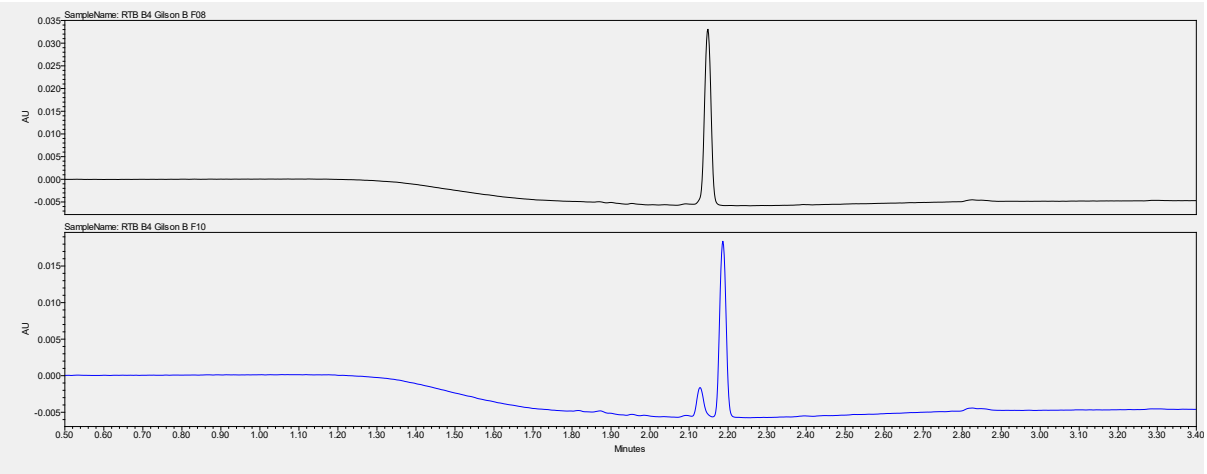


Exact Mass: 546.2810



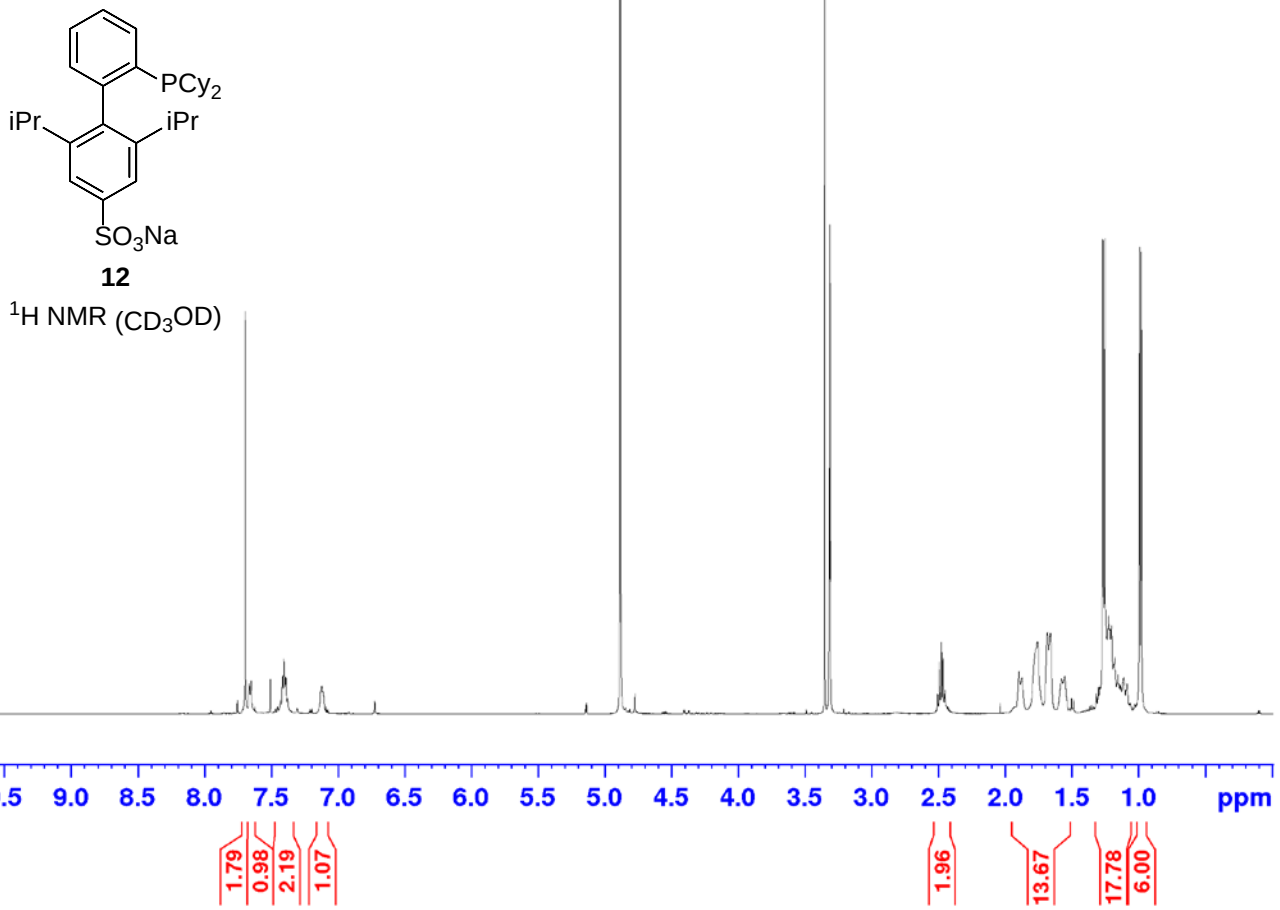
Exact Mass: 528.2704

UPLC chromatograms of purified cystargamide (top/black) and the best purified fraction of brominated cystargamide (bottom/blue) used for cross coupling experiment.



	Name	Retention Time	Area	% Area
1	Cystargamide (top chromatogram)	2.128	4619	14.53
2	Brominated cystargamide derivatives (bottom chromatogram)	2.187	27181	85.47

1. D. R. M. Smith, T. Willemse, D. S. Gkotsi, W. Schepens, B. U. W. Maes, S. Ballet and R. J. M. Goss, *Org. Lett*. 2014, **16**, 2622-2625.
2. K. W. Anderson and S. L. Buchwald, *Angew. Chem. Int. Ed.* 2005, **44**, 6173-6177.
3. K. A. Gill, F. Berrué, J. C. Arens and R. G. Kerr, *J. Nat. Prod*., 2014, **77**, 1372-1376.

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PROCNO                   1

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PULPROG                zg30
TD                    65536
SOLVENT                MeOD
NS                        8
DS                        2
SWH                7002.801 Hz
FIDRES            0.106854 Hz
AQ                4.6792703 sec
RG                     90.5
DW                   71.400 usec
DE                   11.40 usec
TE                   295.0 K
D1              1.00000000 sec
TD0                       1

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NUC1                     1H
P1                    10.35 usec
PLW1          24.00000000 W

F2 - Processing parameters
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WDW                      EM
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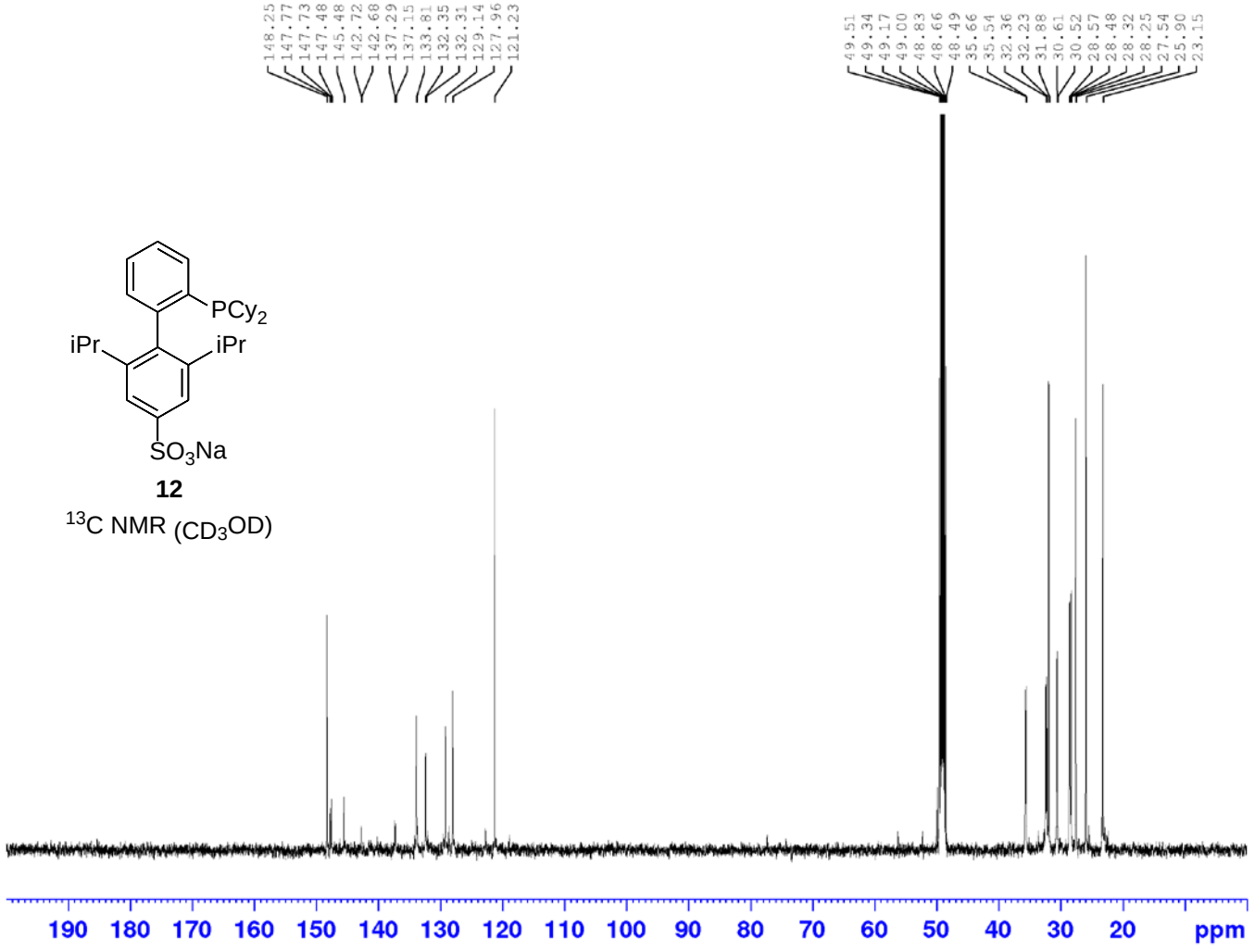


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PROCNO                   1

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PULPROG              udeft
TD                   21424
SOLVENT               MeOD
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DS                       0
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FIDRES            1.389185 Hz
AQ                0.3599232 sec
RG                     128
DW                  16.800 usec
DE                   7.96 usec
TE                  295.0 K
D1               3.00000000 sec
D11              0.03000000 sec
D12              0.00002000 sec
D20            200.00000000 sec
TD0                      1

======== CHANNEL f1 ========
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NUC1                    13C
P1                     8.90 usec
P13                 2000.00 usec
P26                  500.00 usec
PLW1          125.00000000 W
SPNAM[5]       Crp60comp.4
SPOAL5               0.500
SPOFFS5  0 Hz
SPW5           15.12800026 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8               0.500
SPOFFS8  0 Hz
SPW8           15.12800026 W

======== CHANNEL f2 ========
SFO2           500.1320005 MHz
NUC2                    1H
CPDPRG[2             waltz65
PCPD2                80.00 usec
PLW2          24.00000000 W
PLW12          0.39015001 W

F2 - Processing parameters
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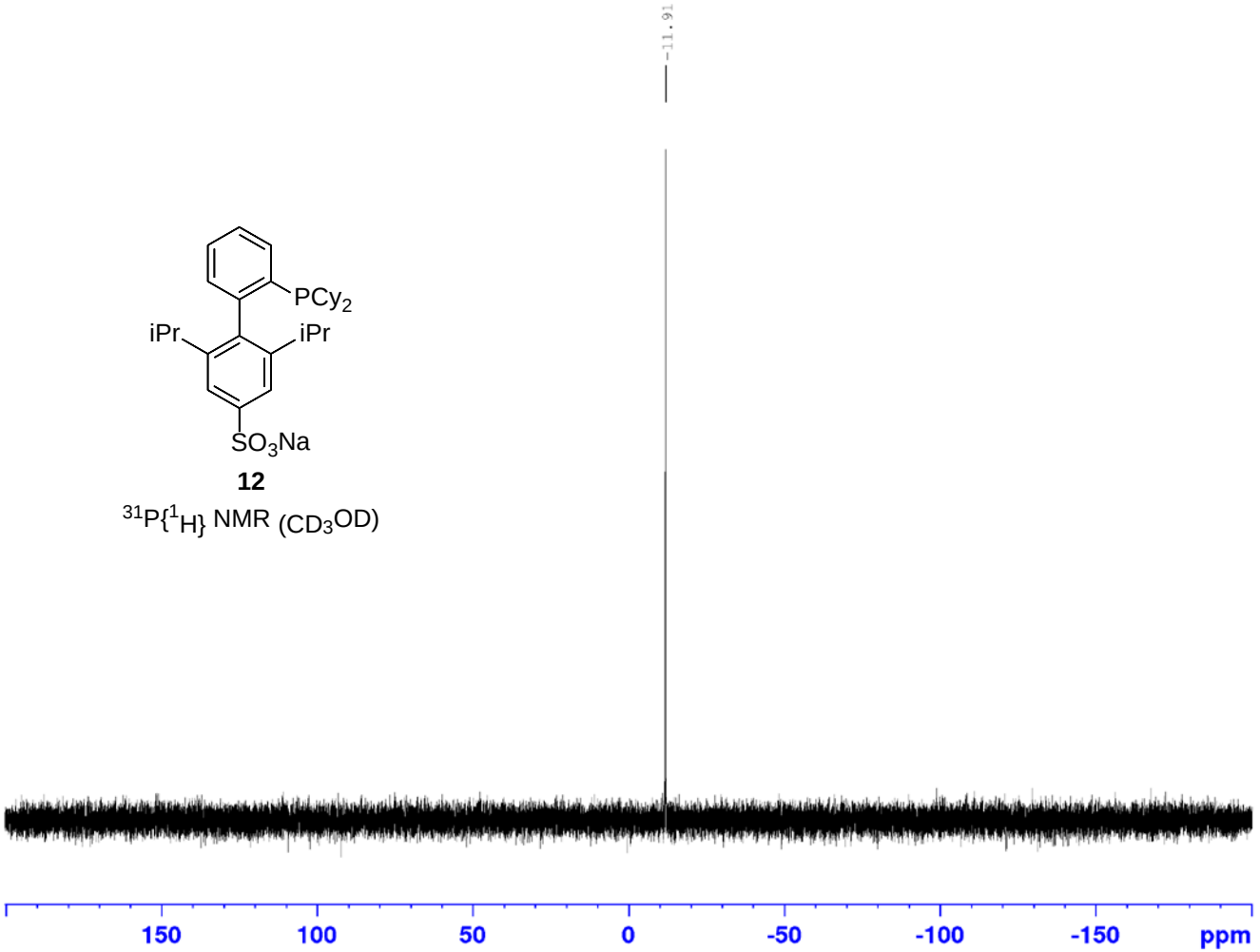
12

$^{31}P\{^{1}H\}$ NMR (CD_3OD)

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PROCNO                 1

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TD                65536
SOLVENT            MeOD
NS                  128
DS                    4
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FIDRES        0.990830 Hz
AQ            0.5046272 sec
RG                 2048
DW                7.700 usec
DE                6.50 usec
TE                297.7 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                31P
P1                15.00 usec
PL1                3.74 dB
PL1W        11.00252819 W
SFO1       162.0444100 MHz

======== CHANNEL f2 ========
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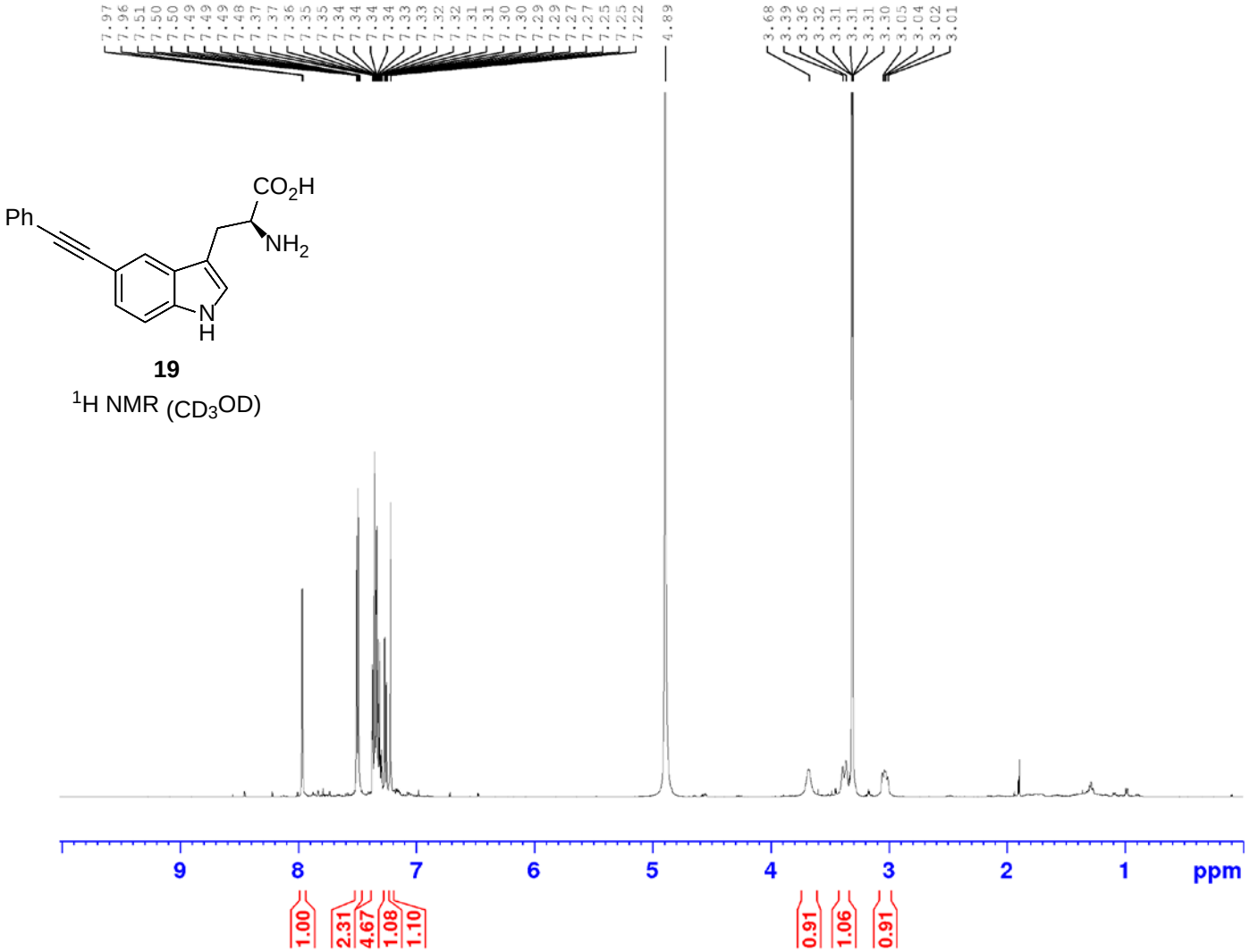
NUC2                1H
PCPD2             80.00 usec
PL2               -3.00 dB
PL12              14.00 dB
PL13              14.00 dB
PL2W        19.24057770 W
PL12W        0.38389999 W
PL13W        0.38389999 W
SFO2       400.3020015 MHz

F2 - Processing parameters
SI                65536
SF         162.0444100 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



19

^{1}H NMR (CD_3OD)

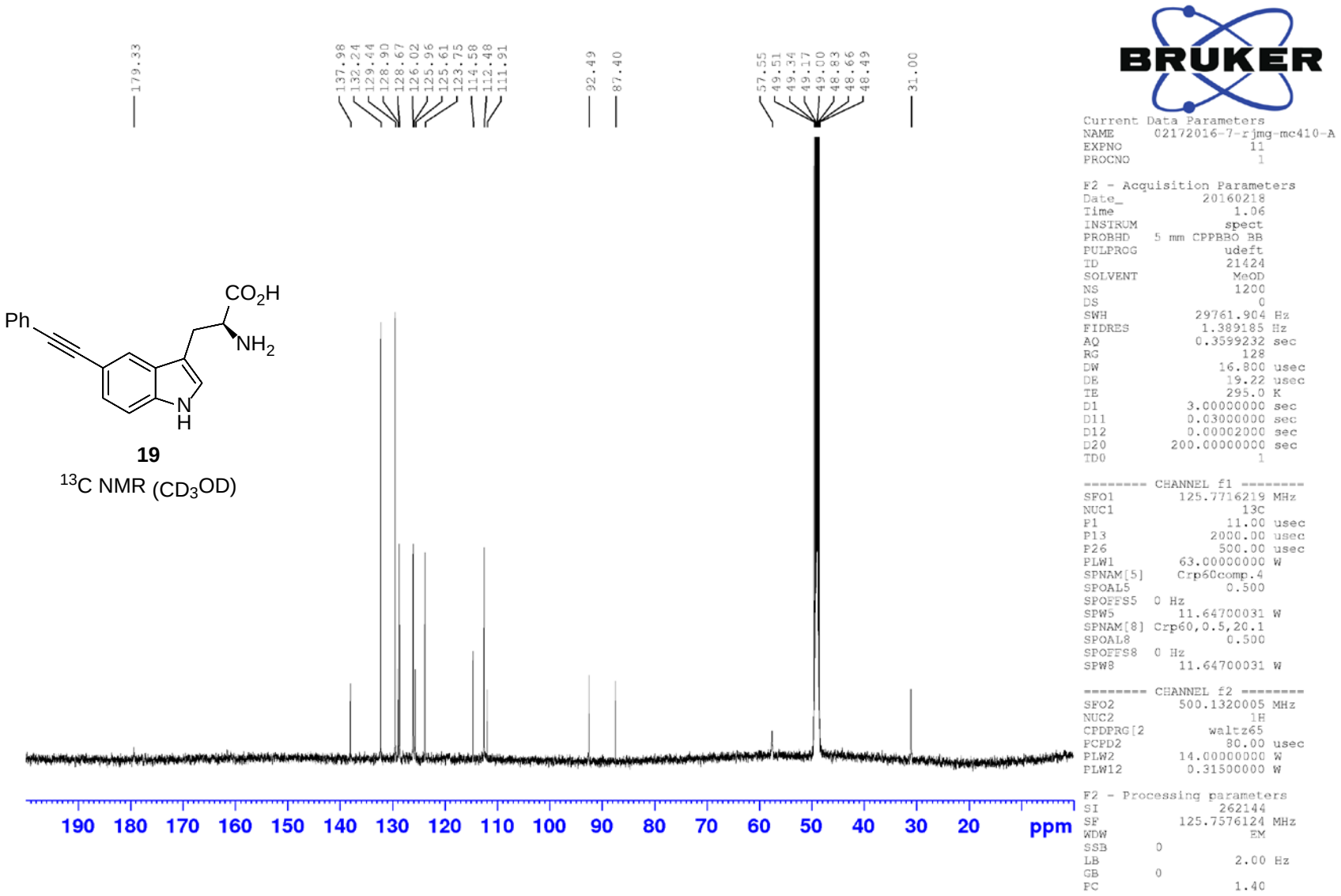
BRUKER

Current Data Parameters
NAME 02172016-7-rjmg-mc410-A
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160217
Time 23.47
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 64
DS 2
SWH 7002.801 Hz
FIDRES 0.106854 Hz
AQ 4.6792703 sec
RG 64
DW 71.400 usec
DE 10.95 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 500.1330008 MHz
NUC1 1H
P1 12.50 usec
PLW1 14.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300097 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



BRUKER

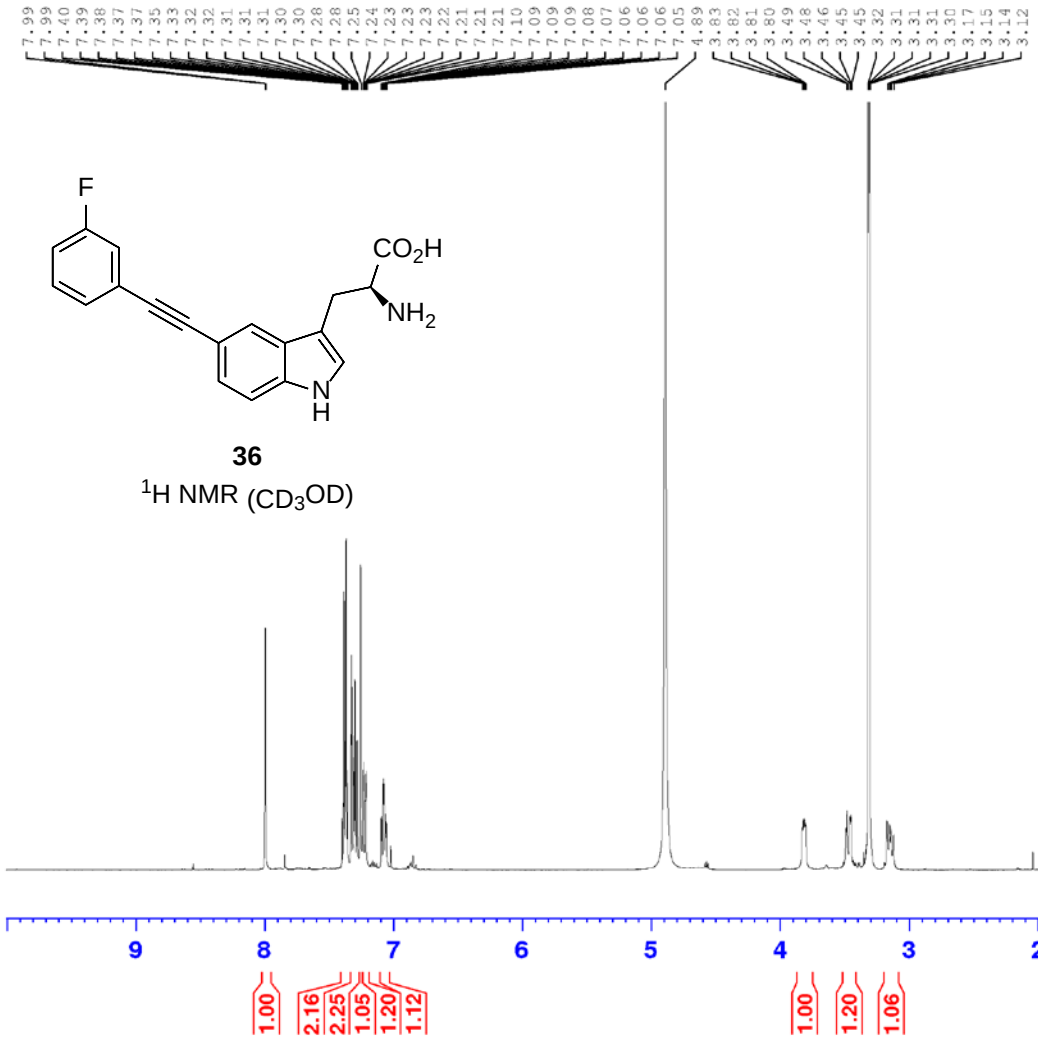
```
Current Data Parameters
NAME      02172016-7-rjmg-mc410-A
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160218
Time                1.06
INSTRUM            spect
PROBHD   5 mm CPPBBO BB
PULPROG             udeft
TD                  21424
SOLVENT              MeOD
NS                   1200
DS                      0
SWH           29761.904 Hz
FIDRES         1.389185 Hz
AQ             0.3599232 sec
RG                    128
DW               16.800 usec
DE               19.22 usec
TE                295.0 K
D1           3.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
D20        200.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.7716219 MHz
NUC1                 13C
P1                11.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1         63.00000000 W
SPNAM[5]     Crp60comp.4
SPOAL5             0.500
SPOFFS5  0 Hz
SPW5         11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8  0 Hz
SPW8         11.64700031 W

======== CHANNEL f2 ========
SFO2         500.1320005 MHz
NUC2                 1H
CPDPRG[2          waltz65
PCPD2             80.00 usec
PLW2         14.00000000 W
PLW12         0.31500000 W

F2 - Processing parameters
SI                 262144
SF           125.7576124 MHz
WDW                    EM
SSB       0
LB                2.00 Hz
GB        0
PC                1.40
```



36

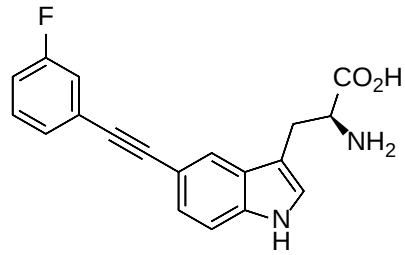
^{1}H NMR (CD_3OD)

```
Current Data Parameters
NAME      03112016-6-rjmg-mc410-A
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_              20160312
Time                  1.17
INSTRUM              spect
PROBHD   5 mm CPPBBO BB
PULPROG               zg30
TD                   65536
SOLVENT               MeOD
NS                      64
DS                       2
SWH                7002.801 Hz
FIDRES            0.106854 Hz
AQ                4.6792703 sec
RG                      64
DW                  71.400 usec
DE                  10.95 usec
TE                  295.0 K
D1             1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          500.1330008 MHz
NUC1                  1H
P1                  12.50 usec
PLW1          14.00000000 W

F2 - Processing parameters
SI                   65536
SF            500.1300097 MHz
WDW                    EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
```



36

$^{19}F\{^{1}H\}$ NMR (CD_3OD)

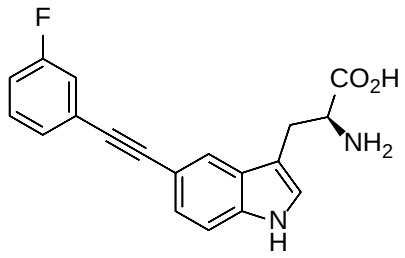

```
Current Data Parameters
NAME      01262016-1-rjmg-mc410-F
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_           20160126
Time              19.10
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG  zgfhigqn.3.and
TD              131072
SOLVENT             MeOD
NS                    64
DS                     4
SWH          125000.000 Hz
FIDRES         0.953674 Hz
AQ             0.5242880 sec
RG               121.22
DW                4.000 usec
DE                6.93 usec
TE               295.0 K
D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        470.3477405 MHz
NUC1                19F
P1                14.60 usec
PLW1        48.00000000 W

======== CHANNEL f2 ========
SFO2        499.9319997 MHz
NUC2                1H
CPDPRG[2         waltz65
PCPD2             80.00 usec
PLW2        16.00000000 W
PLW12        0.27563000 W

F2 - Processing parameters
SI                 65536
SF          470.4041892 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



36

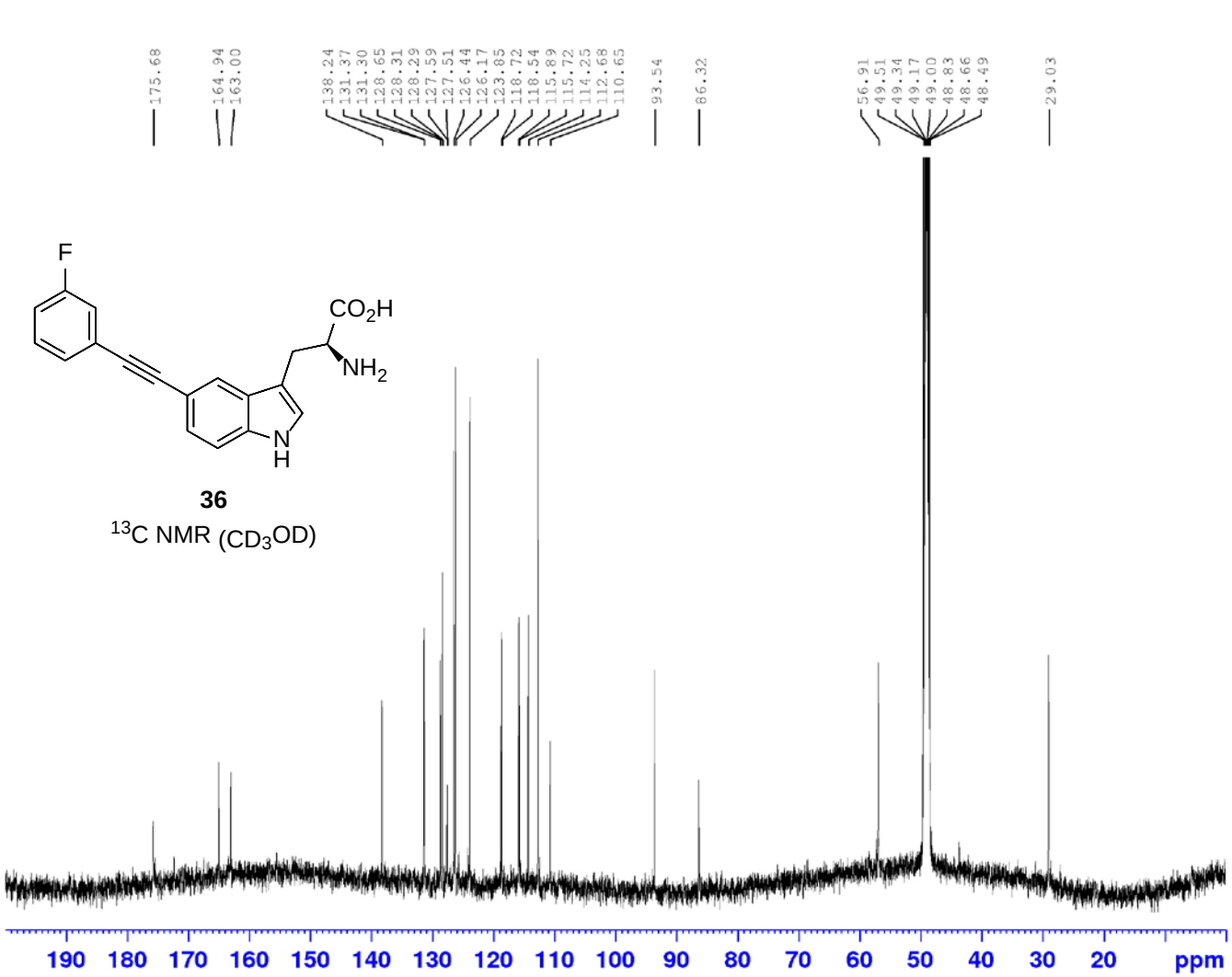
^{19}F NMR (CD_3OD)


```
Current Data Parameters
NAME      03162016-7-rjmg-mc410-M
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160316
Time              11.04
INSTRUM         AVII400
PROBHD   5 mm PABBO BB/
PULPROG          zgflqn
TD                65536
SOLVENT             MeOD
NS                   64
DS                    4
SWH           30000.000 Hz
FIDRES         0.457764 Hz
AQ             1.0922667 sec
RG                  128
DW                16.667 usec
DE                 8.37 usec
TE                294.8 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         376.4569514 MHz
NUC1                19F
P1                15.00 usec
PLW1         17.00000000 W

F2 - Processing parameters
SI                65536
SF           376.4983662 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



36

^{13}C NMR (CD_3OD)

BRUKER

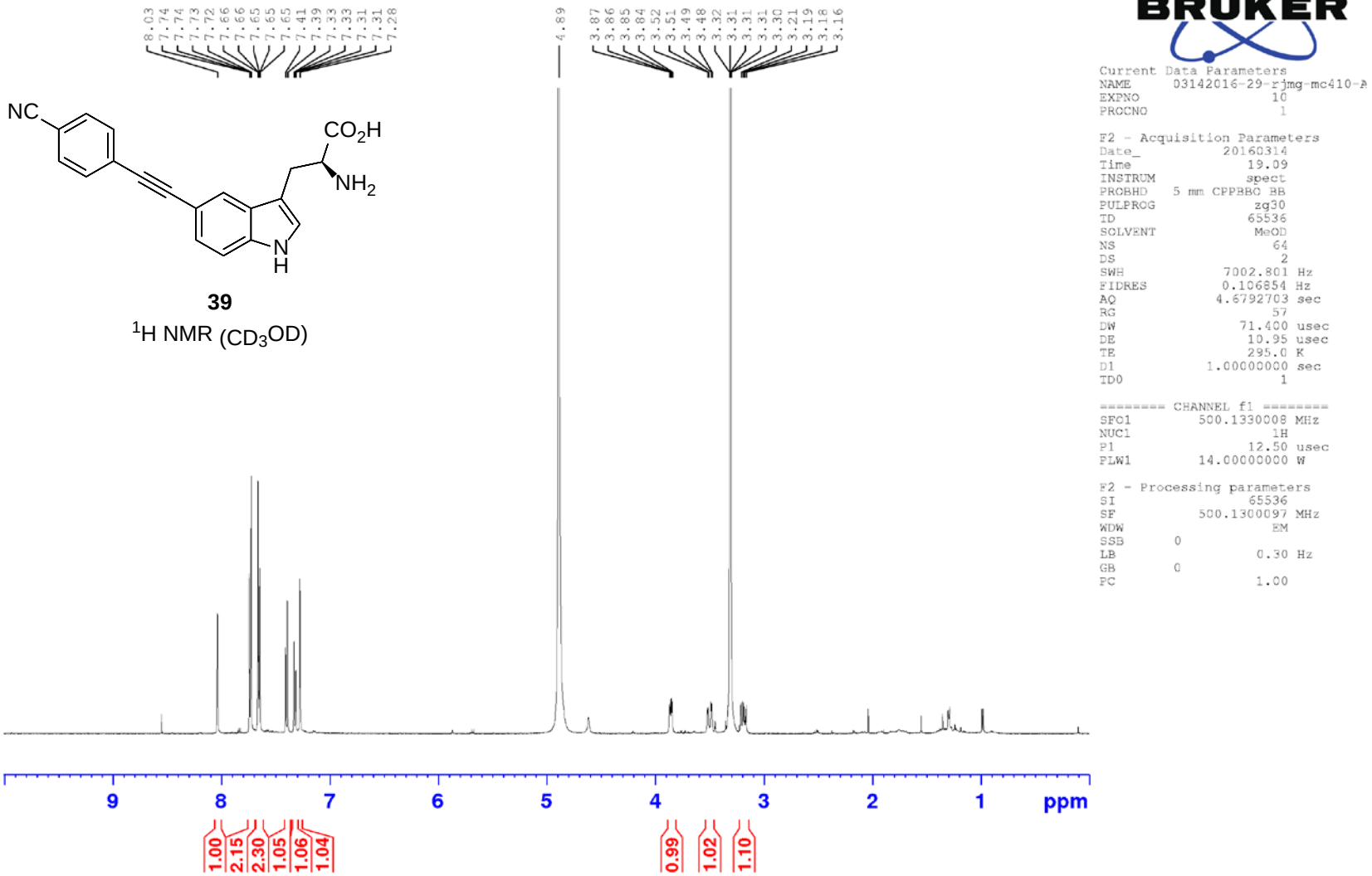
```
Current Data Parameters
NAME      03112016-6-rjmg-mc410-A
EXPNO                          11
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20160312
Time                         3.14
INSTRUM                     spect
PROBHD    5 mm CPPBBO BB
PULPROG                     udeft
TD                          21424
SOLVENT                      MeOD
NS                           1800
DS                              0
SWH                    29761.904 Hz
FIDRES                  1.389185 Hz
AQ                      0.3599232 sec
RG                            128
DW                         16.800 usec
DE                         19.22 usec
TE                         295.0 K
D1                    3.00000000 sec
D11                   0.03000000 sec
D12                   0.00002000 sec
D20                 200.00000000 sec
TD0                             1

======== CHANNEL f1 ========
SFO1                125.7716219 MHz
NUC1                         13C
P1                         11.00 usec
P13                      2000.00 usec
P26                       500.00 usec
PLW1                63.00000000 W
SPNAM[5]             Crp60comp.4
SPOAL5                     0.500
SPOFFS5  0 Hz
SPW5                11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8                     0.500
SPOFFS8  0 Hz
SPW8                11.64700031 W

======== CHANNEL f2 ========
SFO2                500.1320005 MHz
NUC2                         1H
CPDPRG[2                waltz65
PCPD2                      80.00 usec
PLW2                14.00000000 W
PLW12                0.31500000 W

F2 - Processing parameters
SI                          262144
SF                  125.7576124 MHz
WDW                             EM
SSB                0
LB                           2.00 Hz
GB                 0
PC                           1.40
```



39

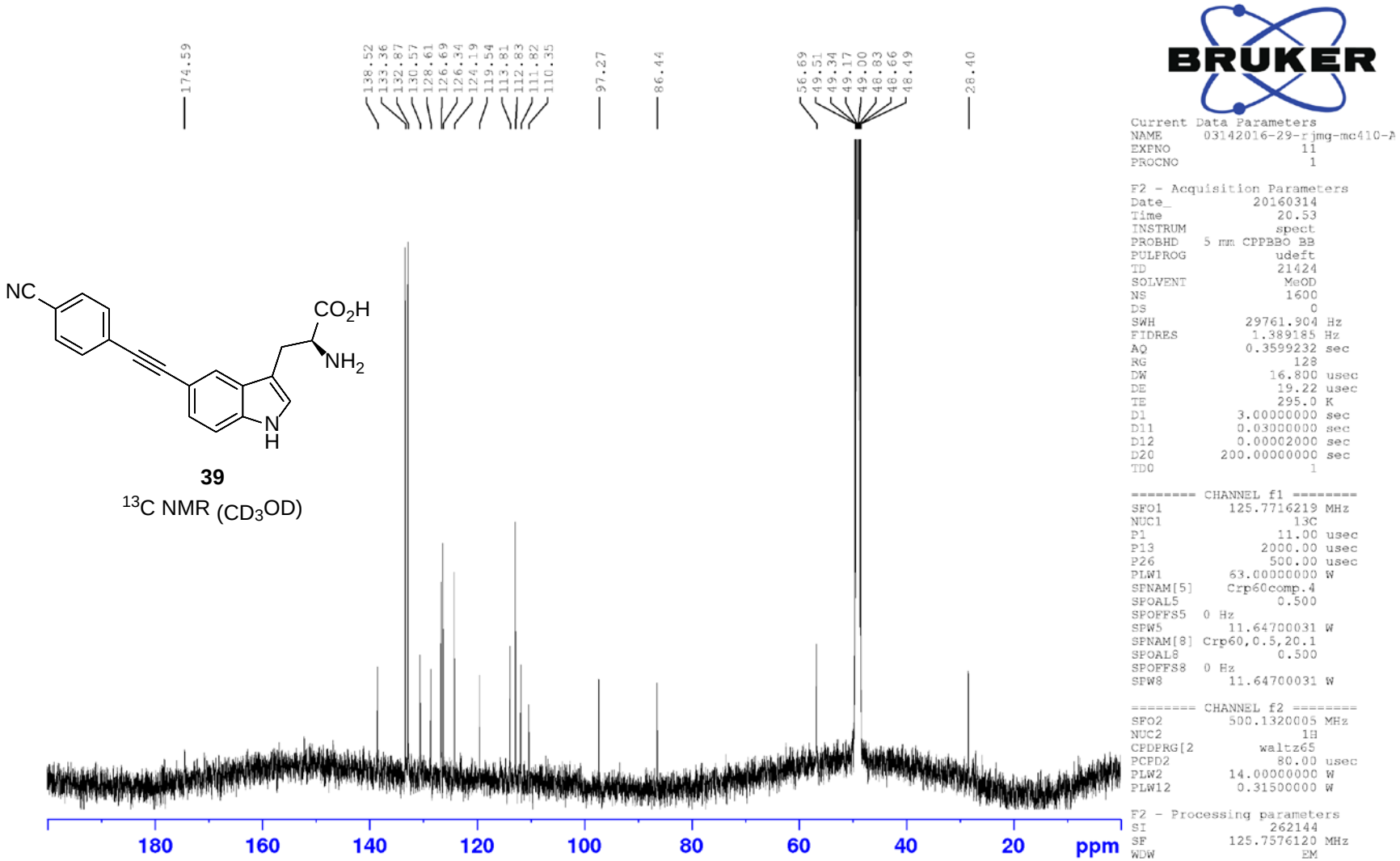
^{1}H NMR (CD_3OD)

```
Current Data Parameters
NAME      03142016-29-rjmg-mc410-A
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_              20160314
Time                 19.09
INSTRUM              spect
PROBHD   5 mm CPPBBO BB
PULPROG              zg30
TD                  65536
SOLVENT              MeOD
NS                     64
DS                      2
SWH               7002.801 Hz
FIDRES            0.106854 Hz
AQ                4.6792703 sec
RG                     57
DW                  71.400 usec
DE                  10.95 usec
TE                  295.0 K
D1              1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.1330008 MHz
NUC1                  1H
P1                  12.50 usec
PLW1          14.00000000 W

F2 - Processing parameters
SI                  65536
SF            500.1300097 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



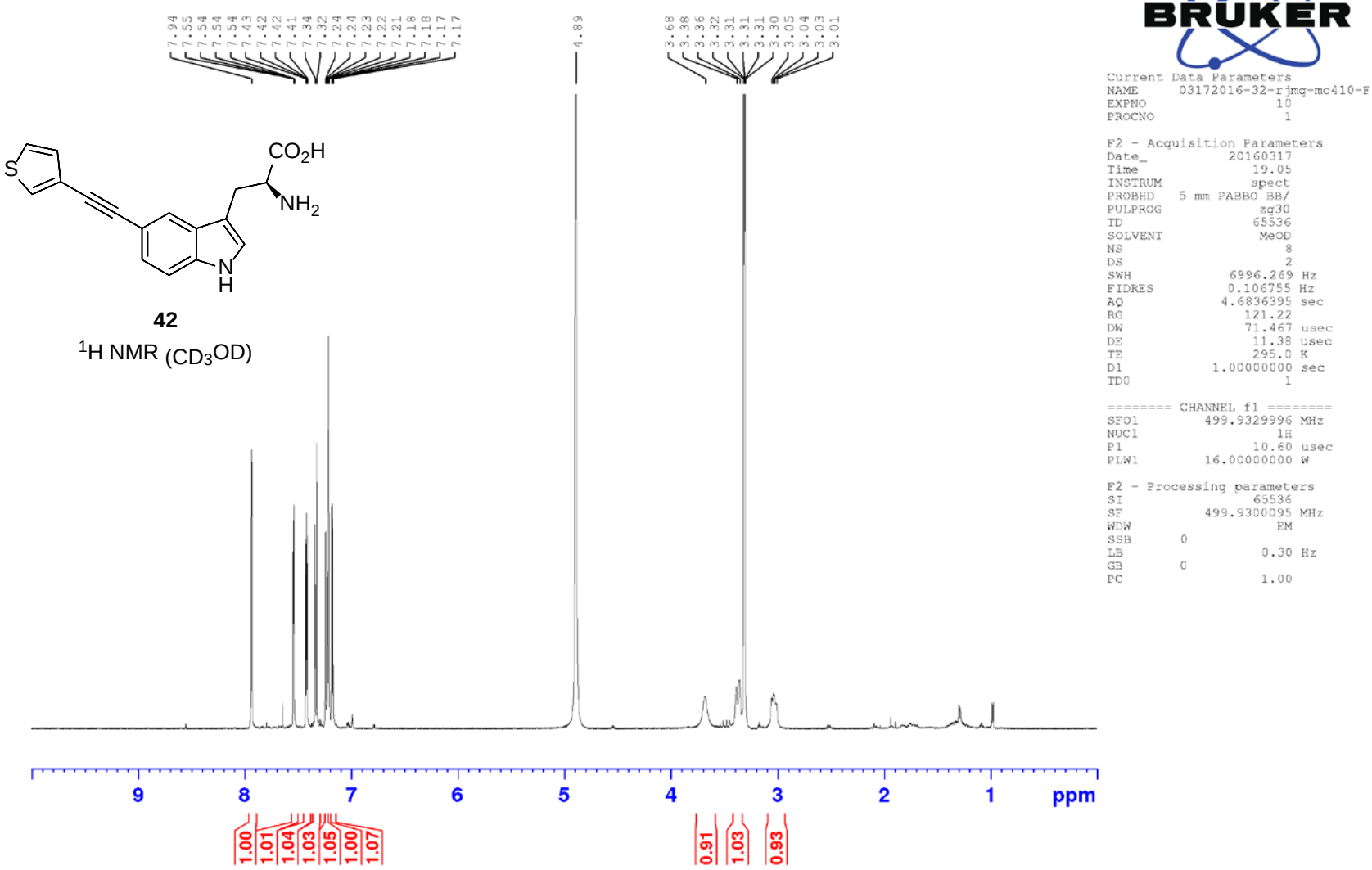

```
Current Data Parameters
NAME      03142016-29-rjmg-mc410-A
EXPNO                   11
PROCNO                   1

F2 - Acquisition Parameters
Date_           20160314
Time              20.53
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           udeft
TD                21424
SOLVENT            MeOD
NS                 1600
DS                    0
SWH           29761.904 Hz
FIDRES         1.389185 Hz
AQ            0.3599232 sec
RG                  128
DW               16.800 usec
DE               19.22 usec
TE                295.0 K
D1           3.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
D20        200.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.7716219 MHz
NUC1               13C
P1               11.00 usec
P13            2000.00 usec
P26             500.00 usec
PLW1         63.00000000 W
SPNAM[5]    Crp60comp.4
SPOAL5           0.500
SPOFFS5  0 Hz
SPW5         11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8           0.500
SPOFFS8  0 Hz
SPW8         11.64700031 W

======== CHANNEL f2 ========
SFO2        500.1320005 MHz
NUC2               1H
CPDPRG[2         waltz65
PCPD2             80.00 usec
PLW2         14.00000000 W
PLW12         0.31500000 W

F2 - Processing parameters
SI                262144
SF          125.7576120 MHz
WDW                 EM
SSB       0
LB                2.00 Hz
GB        0
PC                1.40
```



42

^{1}H NMR (CD_3OD)

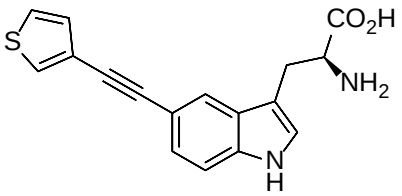
BRUKER

```
Current Data Parameters
NAME      03172016-32-rjmg-mc410-F
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160317
Time              19.05
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           zg30
TD               65536
SOLVENT           MeOD
NS                   8
DS                   2
SWH            6996.269 Hz
FIDRES        0.106755 Hz
AQ            4.6836395 sec
RG               121.22
DW               71.467 usec
DE                11.38 usec
TE                295.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         499.9329996 MHz
NUC1               1H
P1                10.60 usec
PLW1         16.00000000 W

F2 - Processing parameters
SI               65536
SF           499.9300095 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



42

^{13}C NMR (CD_3OD)

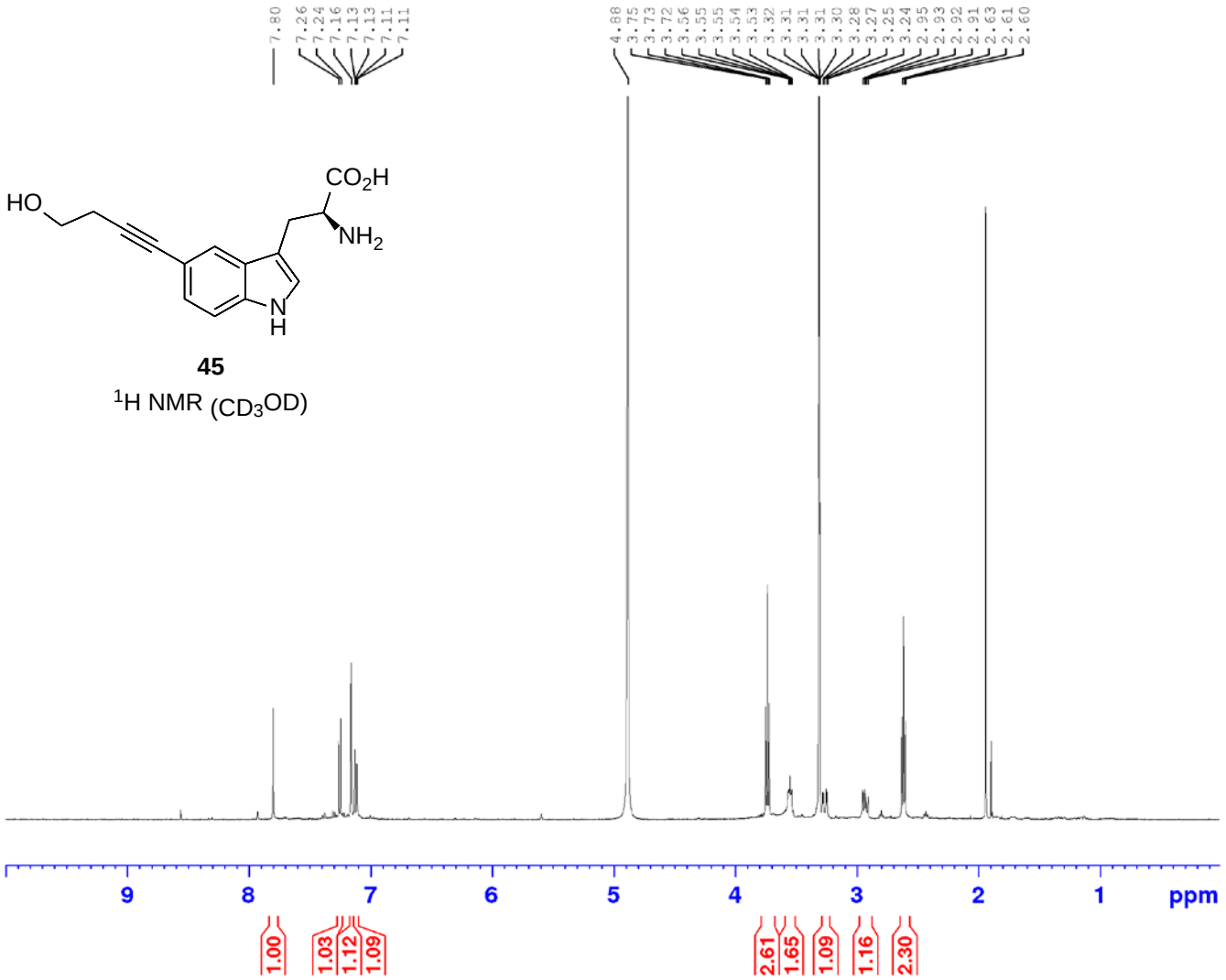

```
Current Data Parameters
NAME      03172016-32-rjmg-mc410-F
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160317
Time              20.49
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           udeft
TD                21424
SOLVENT            MeOD
NS                 1600
DS                    0
SWH           29761.904 Hz
FIDRES         1.389185 Hz
AQ            0.3599232 sec
RG               121.22
DW               16.800 usec
DE                9.36 usec
TE                295.0 K
D1           3.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
D20        200.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.7213263 MHz
NUC1               13C
P1               10.00 usec
P13            2000.00 usec
P26             500.00 usec
PLW1         85.00000000 W
SPNAM[5]    Crp60comp.4
SPOAL5           0.500
SPOFFS5  0 Hz
SPW5         12.98700047 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8           0.500
SPOFFS8  0 Hz
SFW8         12.98700047 W

======== CHANNEL f2 ========
SFO2        499.9319997 MHz
NUC2               1H
CPDPRG[2        waltz65
PCPD2            80.00 usec
PLW2         16.00000000 W
PLW12         0.27563000 W

F2 - Processing parameters
SI                262144
SF          125.7073222 MHz
WDW                 EM
SSB       0
LB                2.00 Hz
GB        0
PC                1.40
```



45

^{1}H NMR (CD_3OD)

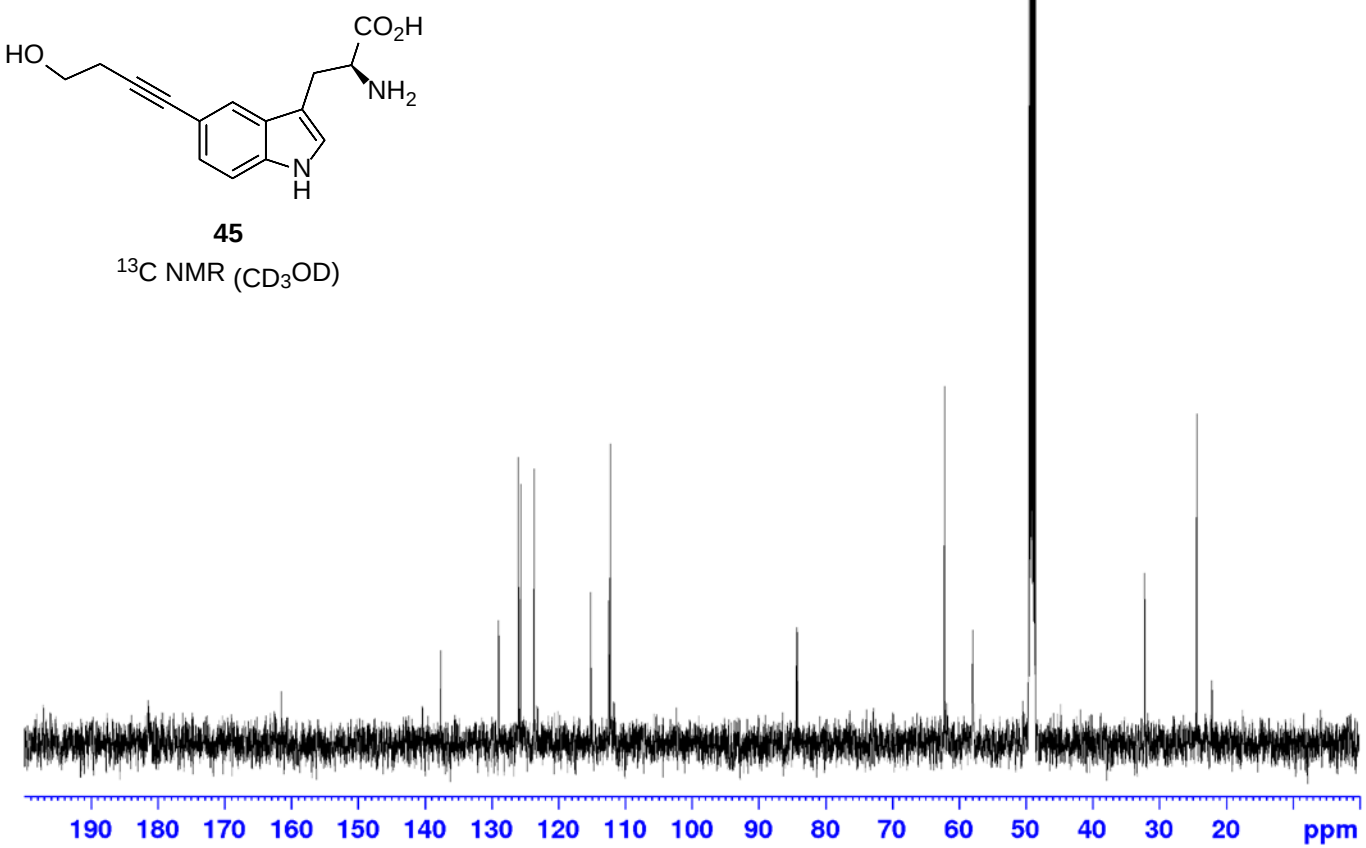
BRUKER

Current Data Parameters
NAME 02252015-18-rjmg-mc410-F
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150225
Time 22.59
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 8
DS 2
SWH 6996.269 Hz
FIDRES 0.106755 Hz
AQ 4.6836395 sec
RG 121.22
DW 71.467 usec
DE 11.38 usec
TE 295.8 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 499.9329996 MHz
NUC1 1H
P1 10.60 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 65536
SF 499.9300097 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



45

^{13}C NMR (CD_3OD)

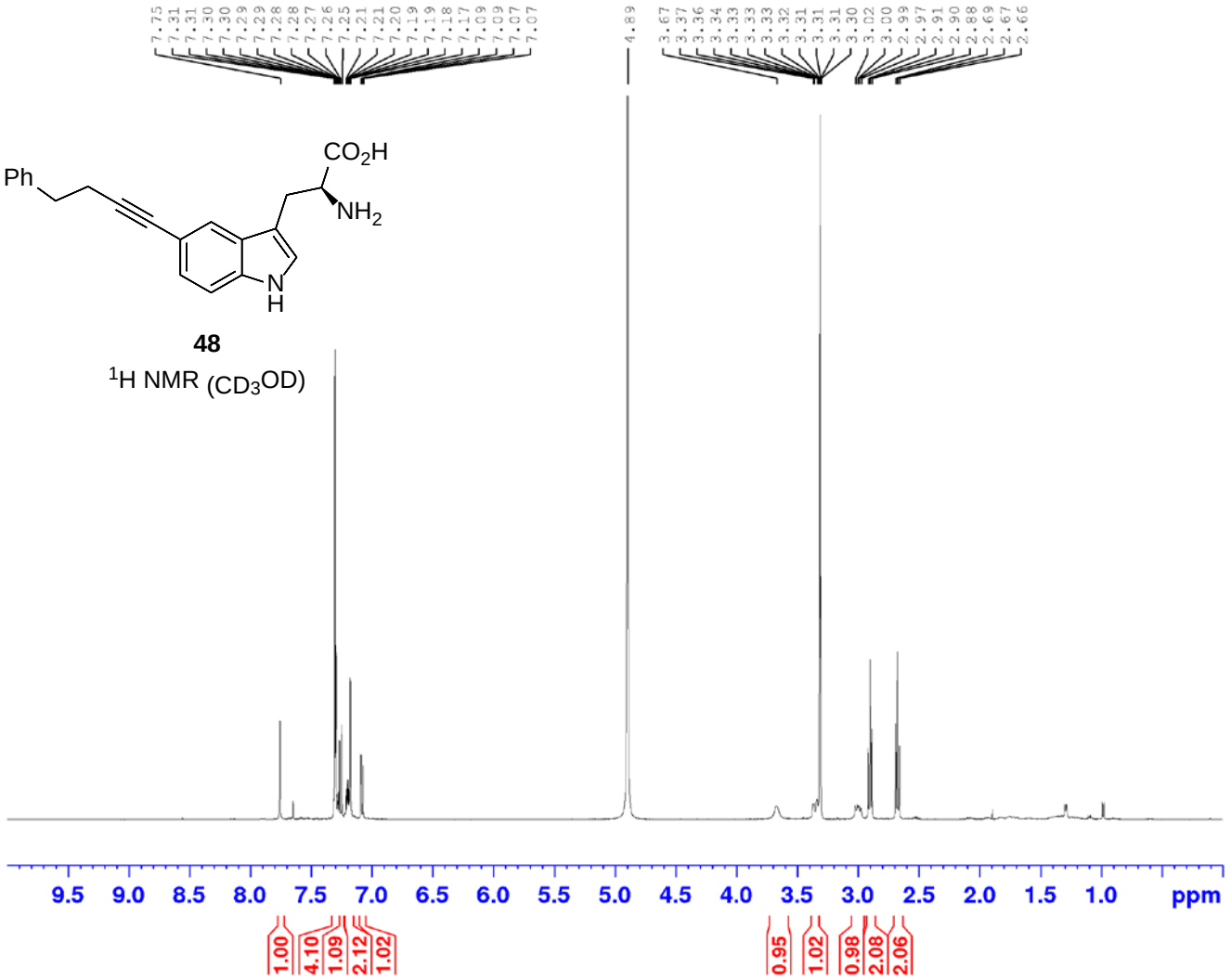
```
Current Data Parameters
NAME      02252015-18-rjmg-mc410-F
EXPNO                   11
PROCNO                   1

F2 - Acquisition Parameters
Date_           20150225
Time              23.54
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           udeft
TD                21424
SOLVENT            MeOD
NS                  800
DS                    0
SWH           29761.904 Hz
FIDRES         1.389185 Hz
AQ            0.3599232 sec
RG               121.22
DW               16.800 usec
DE                9.36 usec
TE               297.0 K
D1           3.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
D20        200.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.7213263 MHz
NUC1                13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1         85.00000000 W
SPNAM[5]    Crp60comp.4
SPOAL5             0.500
SPOFFS5  0 Hz
SPW5         12.98700047 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8  0 Hz
SPW8         12.98700047 W

======== CHANNEL f2 ========
SFO2         499.9319997 MHz
NUC2                1H
CPDPRG[2         waltz65
PCPD2             80.00 usec
PLW2         16.00000000 W
PLW12         0.27563000 W

F2 - Processing parameters
SI                262144
SF           125.7073225 MHz
WDW                 EM
SSB       0
LB                2.00 Hz
GB        0
PC                1.40
```



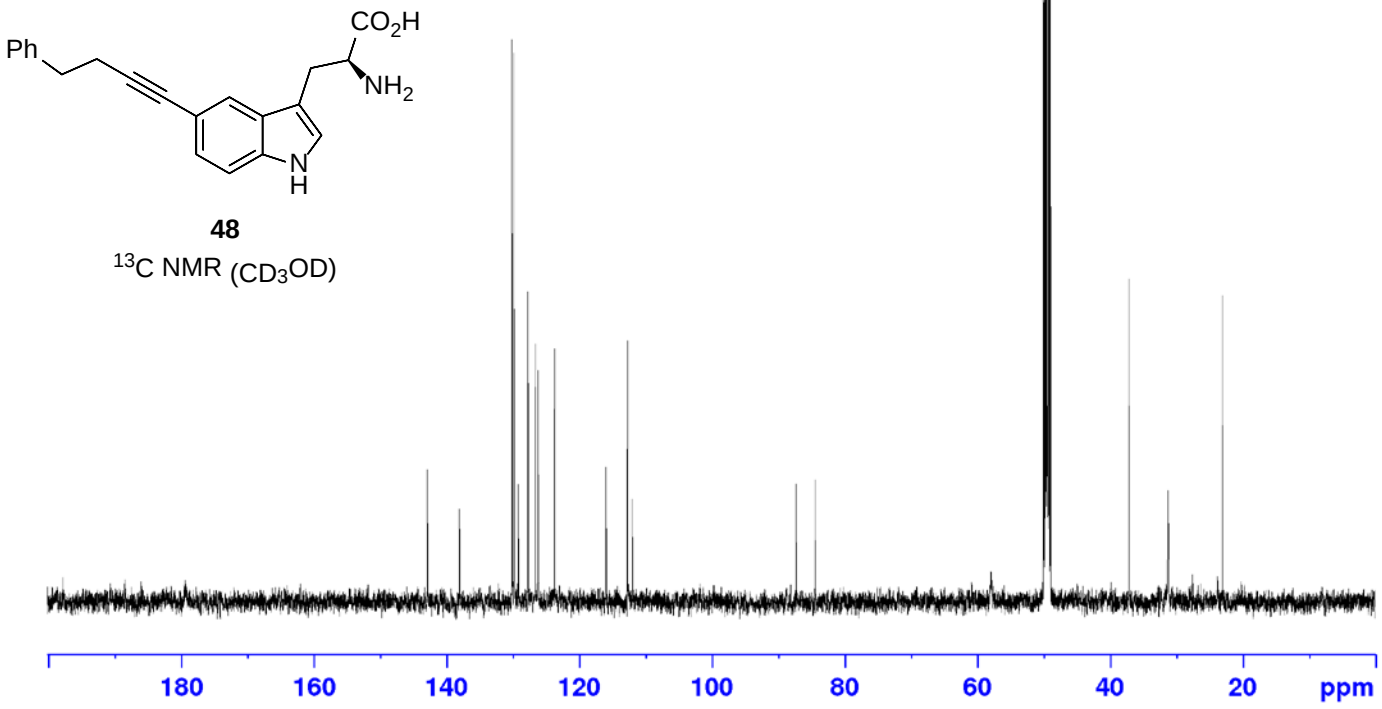
BRUKER

```
Current Data Parameters
NAME      01202016-5-rjmg-mc410-F
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160120
Time              19.04
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           zg30
TD               65536
SOLVENT            MeOD
NS                    8
DS                    2
SWH            6996.269 Hz
FIDRES        0.106755 Hz
AQ            4.6836395 sec
RG               121.22
DW               71.467 usec
DE                11.38 usec
TE                295.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         499.9329996 MHz
NUC1               1H
P1                10.60 usec
PLW1         16.00000000 W

F2 - Processing parameters
SI               65536
SF           499.9300096 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



48

^{13}C NMR (CD_3OD)

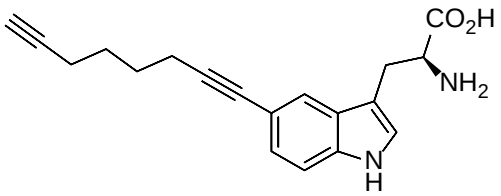

```
Current Data Parameters
NAME      01202016-5-rjmg-mc410-F
EXPNO                          11
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20160120
Time                        19.59
INSTRUM                     spect
PROBHD    5 mm PABBO BB/
PULPROG                     udeft
TD                          21424
SOLVENT                      MeOD
NS                            800
DS                              0
SWH                     29761.904 Hz
FIDRES                   1.389185 Hz
AQ                       0.3599232 sec
RG                          121.22
DW                          16.800 usec
DE                           9.36 usec
TE                          295.0 K
D1                    3.00000000 sec
D11                   0.03000000 sec
D12                   0.00002000 sec
D20                 200.00000000 sec
TD0                             1

======== CHANNEL f1 ========
SFO1                125.7213263 MHz
NUC1                        13C
P1                          10.00 usec
P13                       2000.00 usec
P26                        500.00 usec
PLW1                 85.00000000 W
SPNAM[5]             Crp60comp.4
SPOAL5                        0.500
SPOFFS5  0 Hz
SPW5                 12.98700047 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8                        0.500
SPOFFS8  0 Hz
SFW8                 12.98700047 W

======== CHANNEL f2 ========
SFO2                499.9319997 MHz
NUC2                        1H
CPDPRG[2                 waltz65
PCPD2                       80.00 usec
PLW2                 16.00000000 W
PLW12                 0.27563000 W

F2 - Processing parameters
SI                         262144
SF                  125.7072585 MHz
WDW                            EM
SSB       0
LB                           2.00 Hz
GB        0
PC                           1.40
```



51

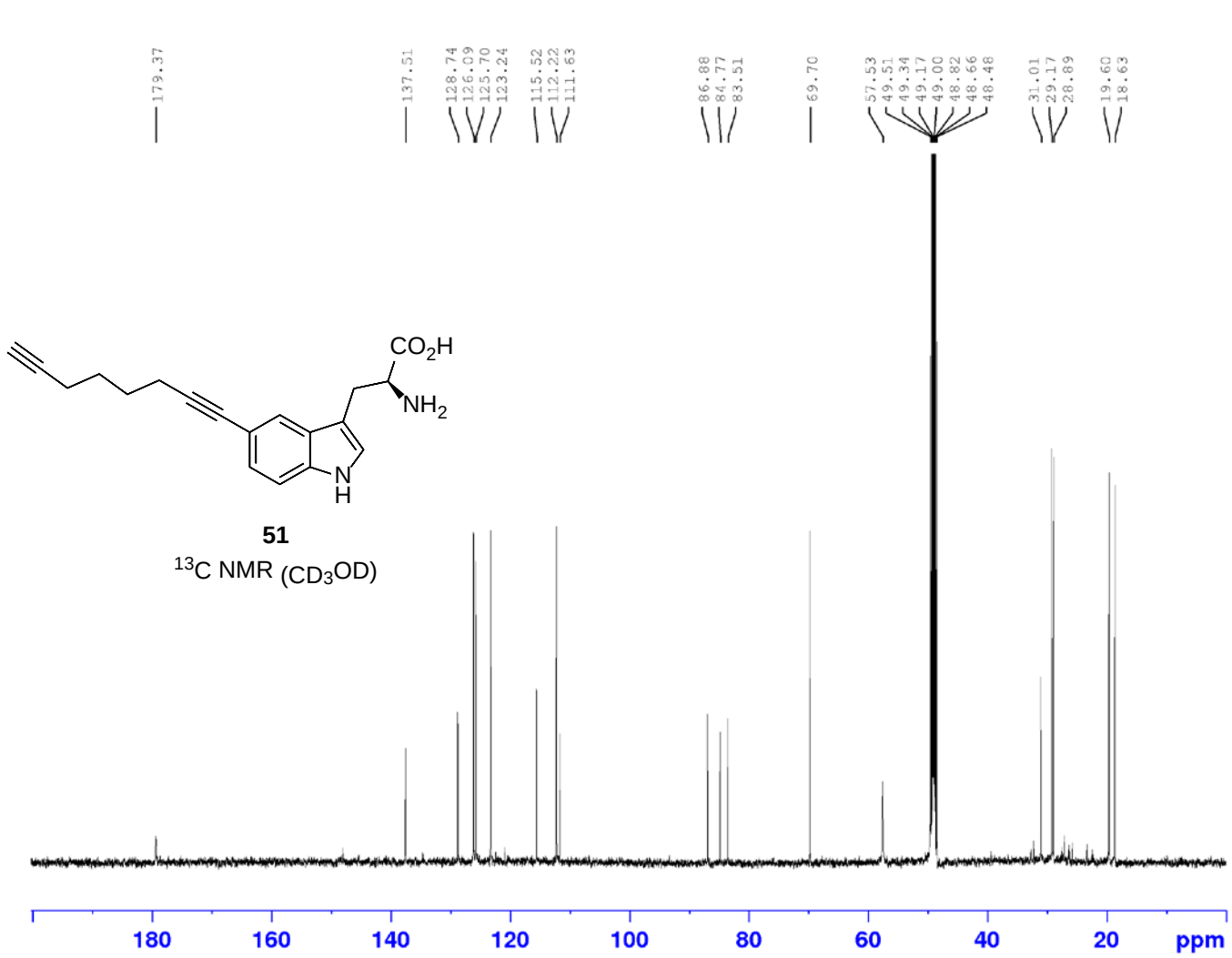
^{1}H NMR (CD_3OD)


```
Current Data Parameters
NAME      07142015-11-rjmg-mc410-M
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_            20150714
Time               22.42
INSTRUM          AVII400
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT             MeCD
NS                     8
DS                     2
SWH             5605.381 Hz
FIDRES          0.085531 Hz
AQ              5.8458114 sec
RG                   128
DW                89.200 usec
DE                13.44 usec
TE                294.9 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.1324008 MHz
NUC1                   1H
P1                  15.50 usec
PLW1        12.00000000 W

F2 - Processing parameters
SI                 65536
SF           400.1300079 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



51

^{13}C NMR (CD_3OD)

BRUKER

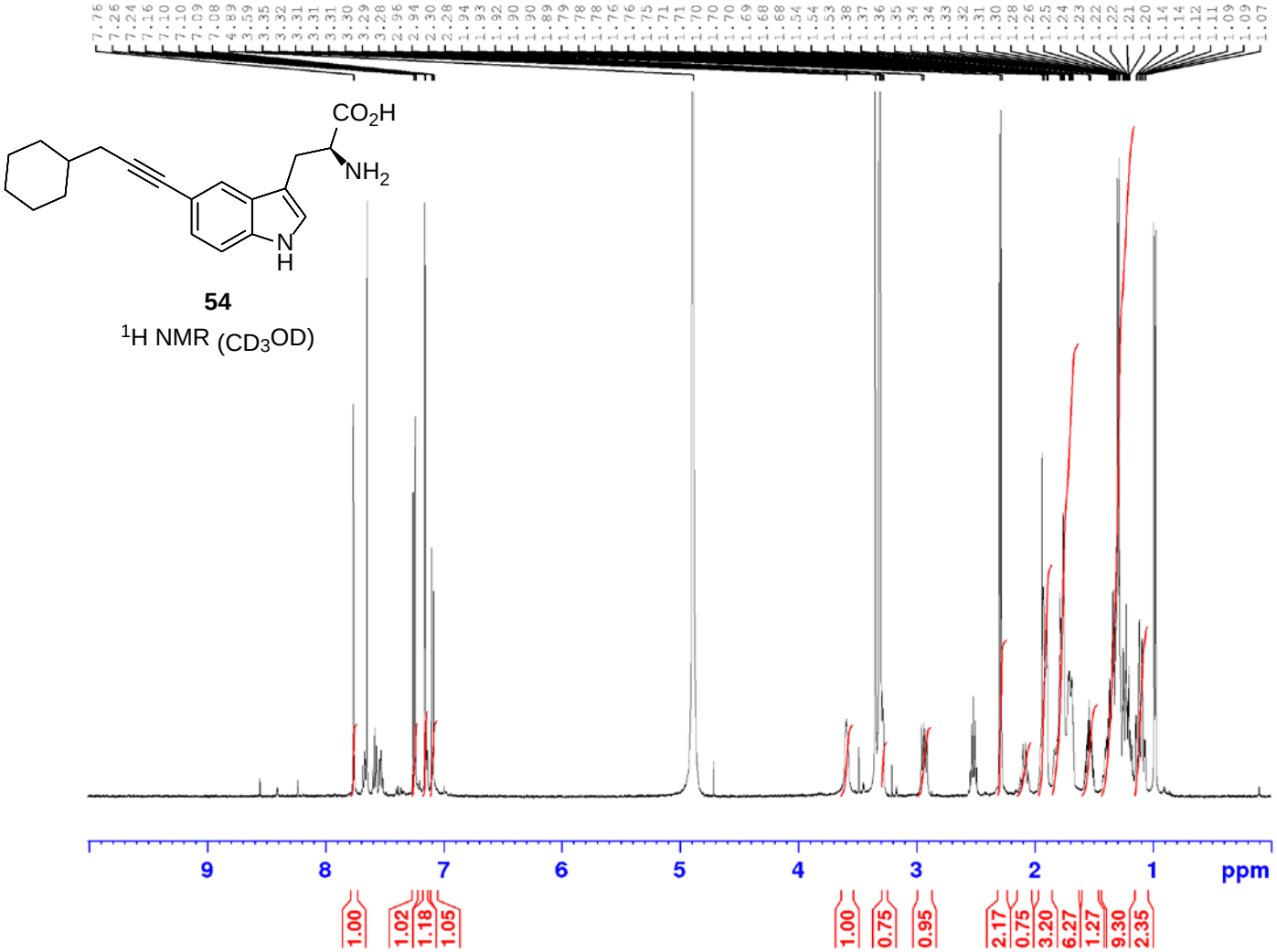
```
Current Data Parameters
NAME      07132015-11-rjmg-mc410-A
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_              20150713
Time                 21.04
INSTRUM              spect
PROBHD   5 mm CPPBBO BB
PULPROG              udeft
TD                   21424
SOLVENT               MeOD
NS                     400
DS                       0
SWH              29761.904 Hz
FIDRES            1.389185 Hz
AQ                0.3599232 sec
RG                     128
DW                  16.800 usec
DE                  19.86 usec
TE                  295.0 K
D1               3.00000000 sec
D11              0.03000000 sec
D12              0.00002000 sec
D20            200.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          125.7716219 MHz
NUC1                  13C
P1                  10.00 usec
P13               2000.00 usec
P26                500.00 usec
PLW1           68.00000000 W
SPNAM[5]      Crp60comp.4
SPOAL5               0.500
SPOFFS5  0 Hz
SPW5           10.39000034 W
SPNAM[8]  Crp60,0.5,20.1
SPOAL8               0.500
SPOFFS8  0 Hz
SPW8           10.39000034 W

======== CHANNEL f2 ========
SFO2          500.1320005 MHz
NUC2                  1H
CPDPRG[2          waltz65
PCPD2               80.00 usec
PLW2           15.00000000 W
PLW12           0.32633999 W

F2 - Processing parameters
SI                  262144
SF            125.7576133 MHz
WDW                   EM
SSB       0
LB                   2.00 Hz
GB        0
PC                   1.40
```



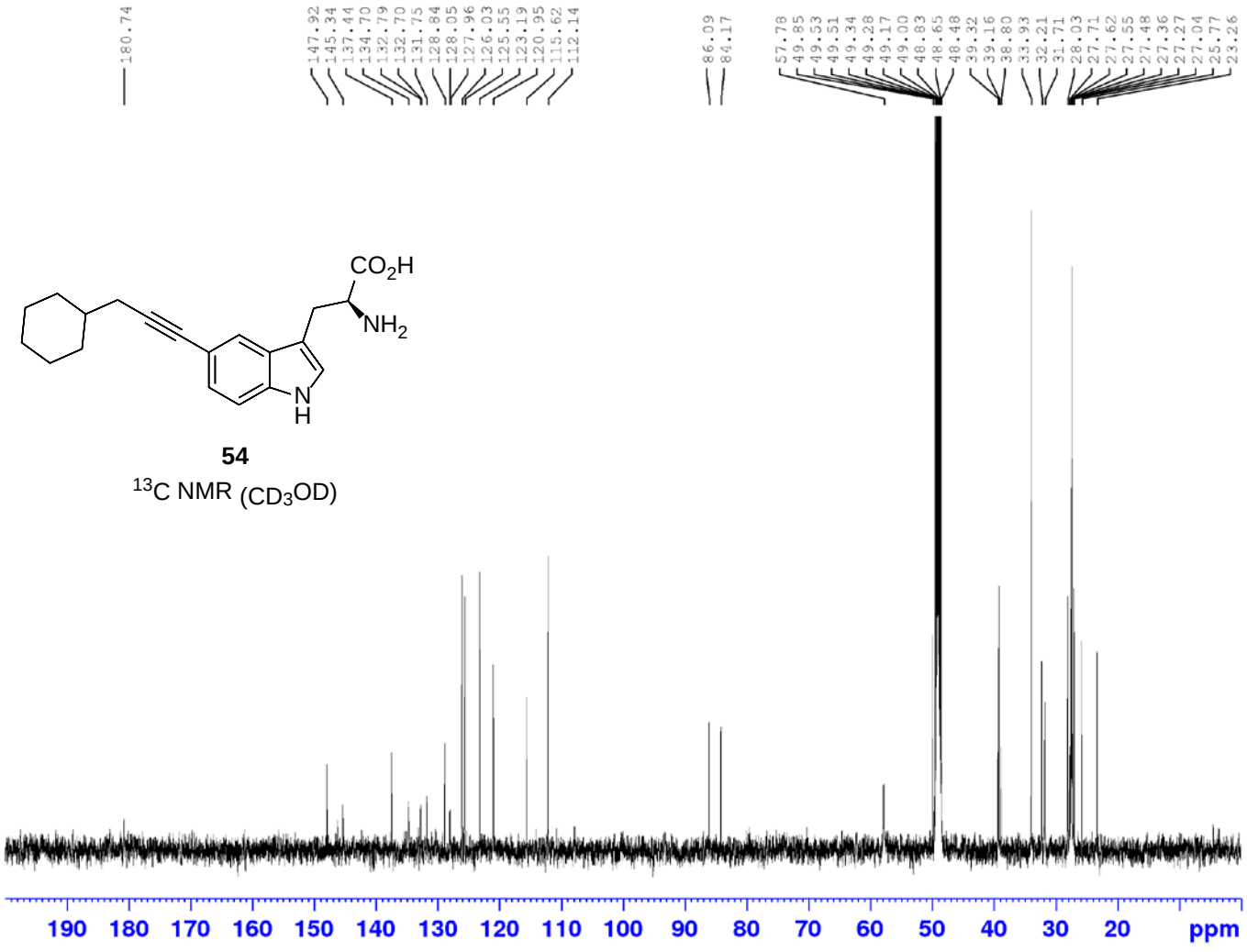
BRUKER

Current Data Parameters
NAME 03182016-14-rjmg-mc410-F
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160318
Time 21.11
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 8
DS 2
SWH 6996.269 Hz
FIDRES 0.106755 Hz
AQ 4.6836395 sec
RG 121.22
DW 71.467 usec
DE 11.38 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 499.9329996 MHz
NUC1 1H
P1 10.60 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 65536
SF 499.9300095 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



54

^{13}C NMR (CD_3OD)

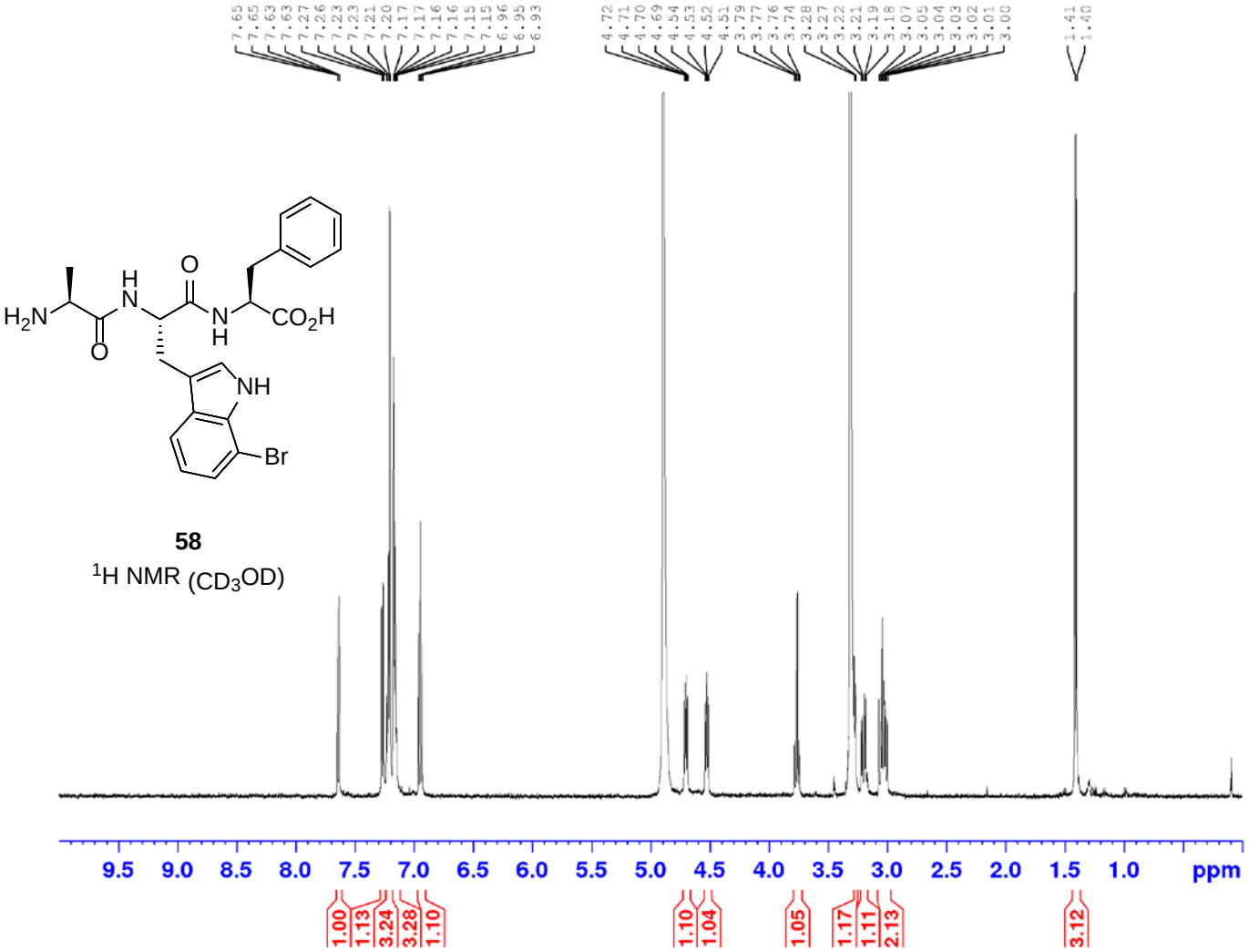
```
Current Data Parameters
NAME      03182016-14-rjmg-mc410-F
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160318
Time              23.20
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           udeft
TD                21424
SOLVENT             MeOD
NS                 2000
DS                    0
SWH           29761.904 Hz
FIDRES         1.389185 Hz
AQ             0.3599232 sec
RG               121.22
DW               16.800 usec
DE                9.36 usec
TE               295.0 K
D1            3.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
D20         200.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.7213263 MHz
NUC1                13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1          85.00000000 W
SPNAM[5]     Crp60comp.4
SPOAL5             0.500
SPOFFS5  0 Hz
SPW5          12.98700047 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8  0 Hz
SPW8          12.98700047 W

======== CHANNEL f2 ========
SFO2         499.9319997 MHz
NUC2                1H
CPDPRG[2          waltz65
PCPD2             80.00 usec
PLW2          16.00000000 W
PLW12          0.27563000 W

F2 - Processing parameters
SI                 262144
SF           125.7073227 MHz
WDW                  EM
SSB       0
LB                 2.00 Hz
GB        0
PC                 1.40
```



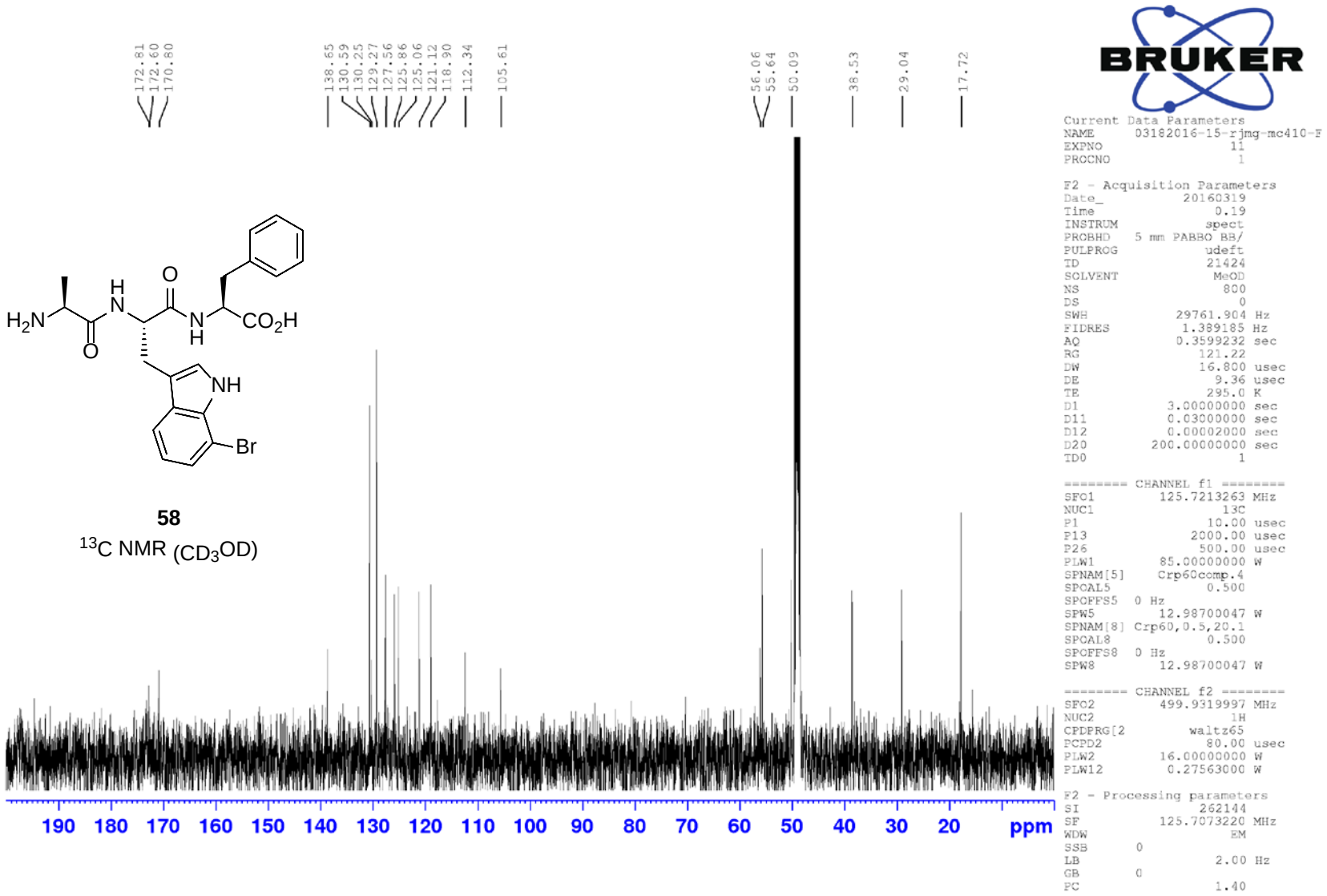
BRUKER

```
Current Data Parameters
NAME      03182016-15-rjmg-mc410-F
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160318
Time              23.25
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           zg30
TD               65536
SOLVENT           MeOD
NS                   8
DS                   2
SWH           6996.269 Hz
FIDRES        0.106755 Hz
AQ            4.6836395 sec
RG               121.22
DW               71.467 usec
DE                11.38 usec
TE                295.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         499.9329996 MHz
NUC1                 1H
P1                10.60 usec
PLW1         16.00000000 W

F2 - Processing parameters
SI                65536
SF           499.9300096 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



BRUKER

```
Current Data Parameters
NAME      03182016-15-rjmg-mc410-F
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160319
Time                0.19
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG            udeft
TD                 21424
SOLVENT             MeOD
NS                   800
DS                     0
SWH            29761.904 Hz
FIDRES          1.389185 Hz
AQ              0.3599232 sec
RG                 121.22
DW                 16.800 usec
DE                  9.36 usec
TE                 295.0 K
D1              3.00000000 sec
D11             0.03000000 sec
D12             0.00002000 sec
D20           200.00000000 sec
TD0                    1

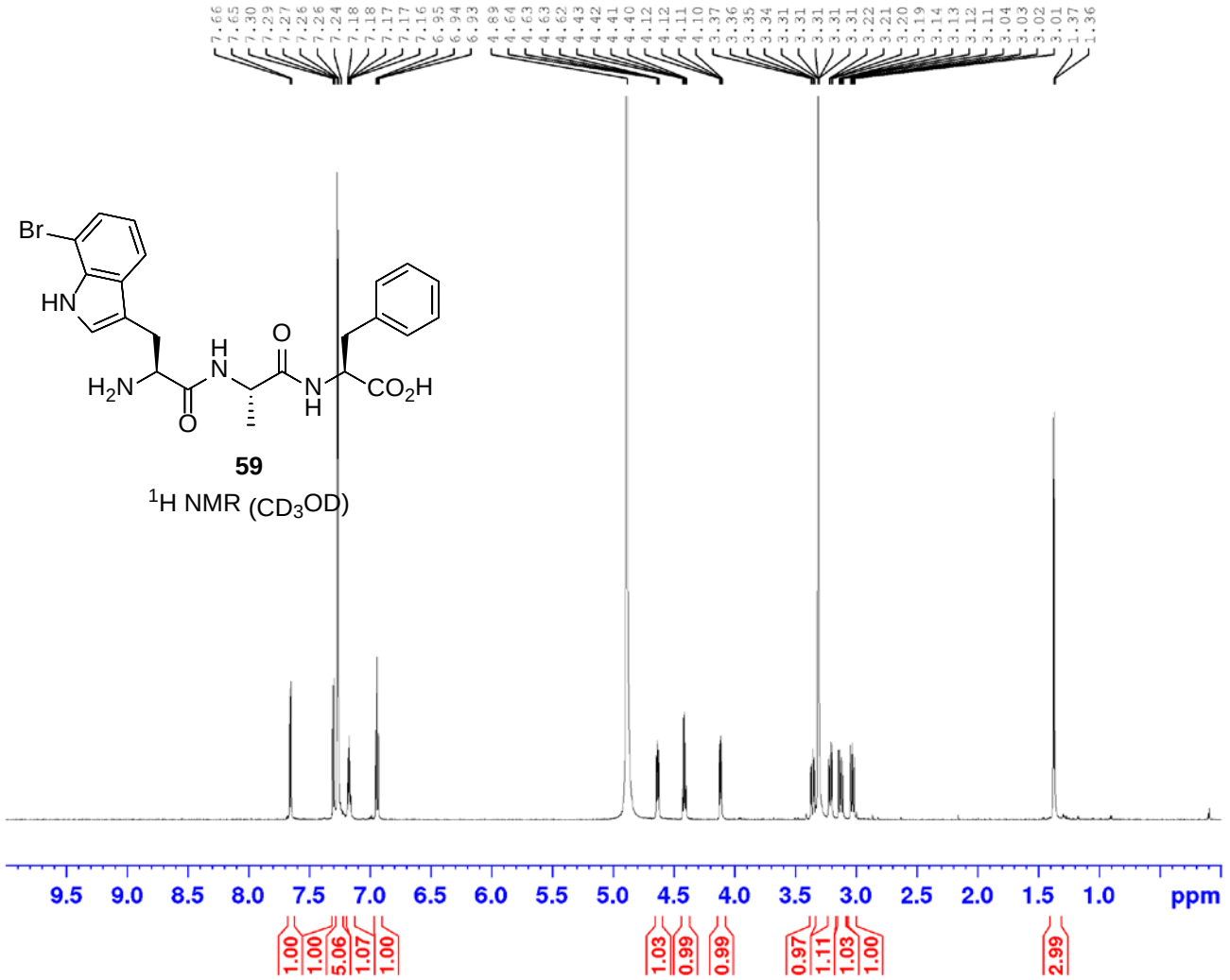
======== CHANNEL f1 ========
SFO1          125.7213263 MHz
NUC1                  13C
P1                  10.00 usec
P13               2000.00 usec
P26                500.00 usec
PLW1           85.00000000 W
SPNAM[5]     Crp60comp.4
SPOAL5               0.500
SPOFFS5  0 Hz
SPW5           12.98700047 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8               0.500
SPOFFS8  0 Hz
SPW8           12.98700047 W

======== CHANNEL f2 ========
SFO2          499.9319997 MHz
NUC2                  1H
CPDPRG[2           waltz65
PCPD2                80.00 usec
PLW2           16.00000000 W
PLW12           0.27563000 W

F2 - Processing parameters
SI                  262144
SF            125.7073220 MHz
WDW                    EM
SSB       0
LB                   2.00 Hz
GB        0
PC                   1.40
```

58

^{13}C NMR (CD_3OD)



59

^{1}H NMR (CD_3OD)

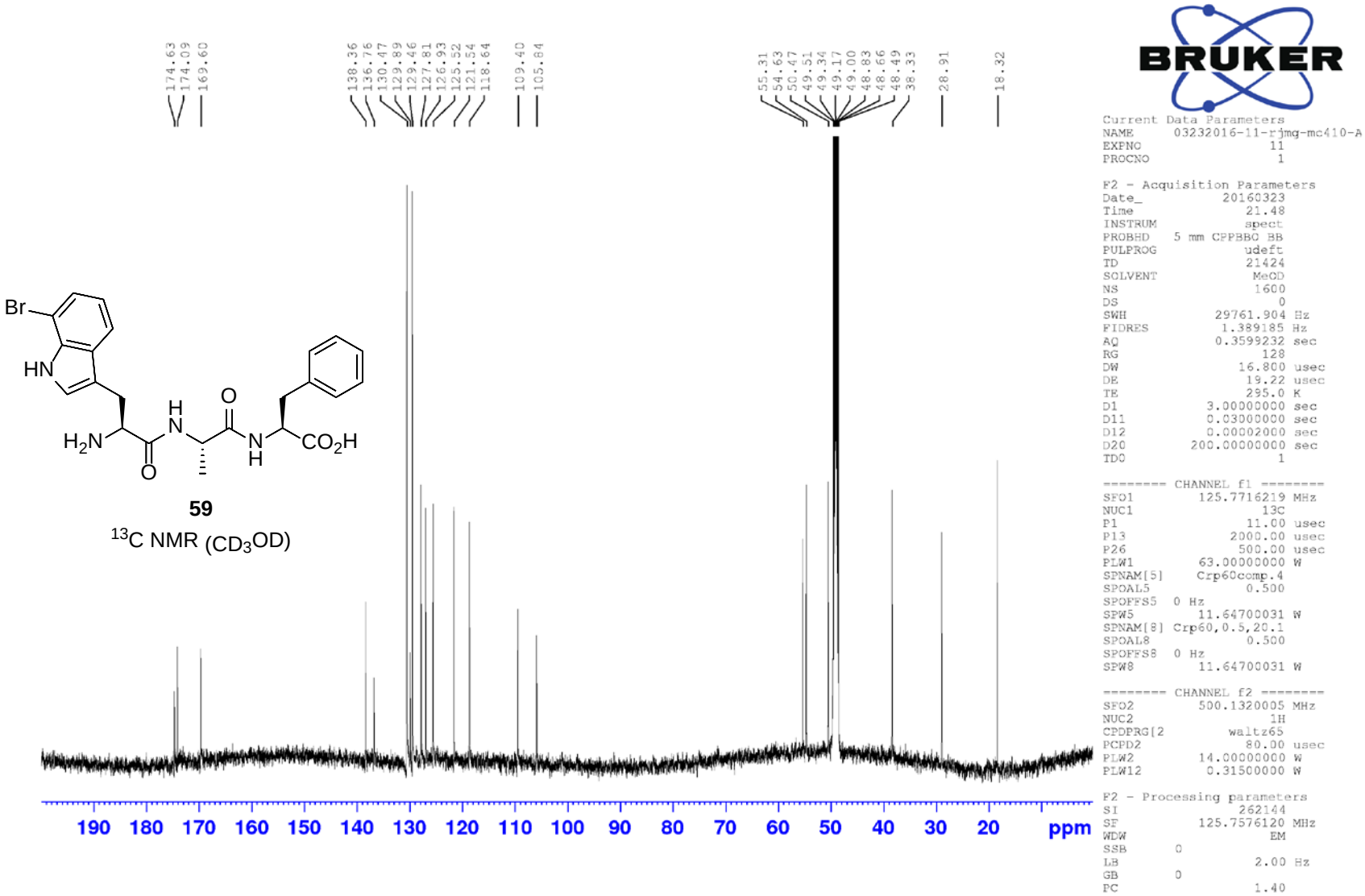
BRUKER

```
Current Data Parameters
NAME      03222016-11-rjmg-mc410-E
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160322
Time              16.49
INSTRUM           spect
PROBHD   5 mm CPPTCI 1H
PULPROG           zg30
TD               65536
SOLVENT           MeOD
NS                   4
DS                   2
SWH            9803.922 Hz
FIDRES        0.149596 Hz
AQ            3.3423359 sec
RG              111.35
DW              51.000 usec
DE              59.79 usec
TE              295.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1       700.1342008 MHz
NUC1               1H
P1                8.00 usec
PLW1       15.30000019 W

F2 - Processing parameters
SI               65536
SF         700.1300134 MHz
WDW                EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```



BRUKER

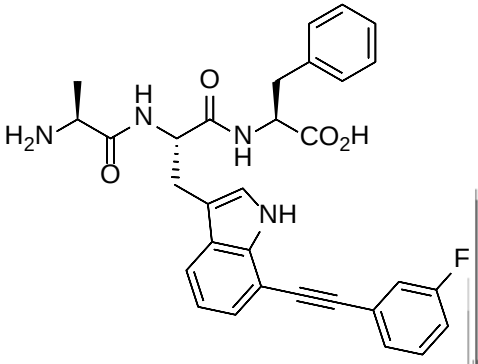
```
Current Data Parameters
NAME      03232016-11-rjmg-mc410-A
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_              20160323
Time                 21.48
INSTRUM              spect
PROBHD    5 mm CPPBBO BB
PULPROG              udeft
TD                   21424
SOLVENT               MeOD
NS                    1600
DS                       0
SWH              29761.904 Hz
FIDRES            1.389185 Hz
AQ                0.3599232 sec
RG                     128
DW                  16.800 usec
DE                  19.22 usec
TE                  295.0 K
D1               3.00000000 sec
D11              0.03000000 sec
D12              0.00002000 sec
D20            200.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1           125.7716219 MHz
NUC1                   13C
P1                  11.00 usec
P13               2000.00 usec
P26                500.00 usec
PLW1            63.00000000 W
SPNAM[5]      Crp60comp.4
SPOAL5               0.500
SPOFFS5   0 Hz
SPW5            11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8               0.500
SPOFFS8   0 Hz
SPW8            11.64700031 W

======== CHANNEL f2 ========
SFO2           500.1320005 MHz
NUC2                   1H
CPDPRG[2           waltz65
PCPD2                80.00 usec
PLW2            14.00000000 W
PLW12            0.31500000 W

F2 - Processing parameters
SI                   262144
SF             125.7576120 MHz
WDW                    EM
SSB       0
LB                   2.00 Hz
GB        0
PC                   1.40
```



60

^{1}H NMR (CD_3OD)

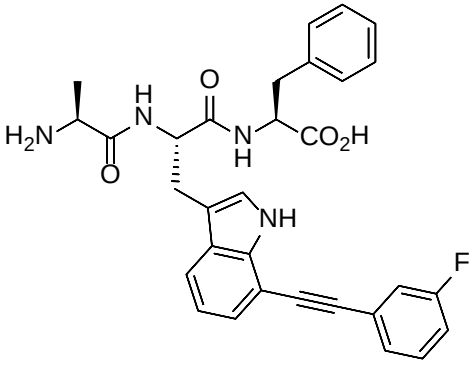
BRUKER

```
Current Data Parameters
NAME      03142016-30-rjmg-mc410-A
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160314
Time              21.02
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT           MeOD
NS                   64
DS                    2
SWH            7002.801 Hz
FIDRES        0.106854 Hz
AQ            4.6792703 sec
RG                   64
DW               71.400 usec
DE               10.95 usec
TE               295.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         500.1330008 MHz
NUC1               1H
P1               12.50 usec
PLW1         14.00000000 W

F2 - Processing parameters
SI               65536
SF           500.1300097 MHz
WDW                EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```



60

^{13}C NMR (CD_3OD)

BRUKER

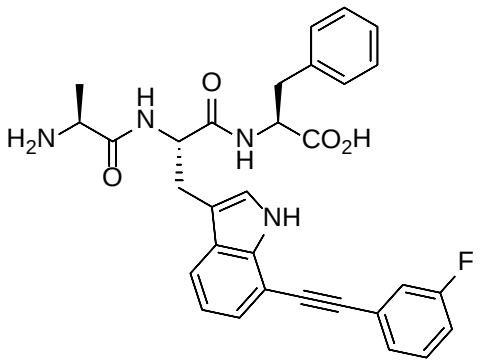
```
Current Data Parameters
NAME      03142016-30-rjmg-mc410-A
EXPNO                 12
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160314
Time              22.53
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           udeft
TD                21424
SOLVENT            MeOD
NS                 1600
DS                    0
SWH           29761.904 Hz
FIDRES         1.389185 Hz
AQ            0.3599232 sec
RG                  128
DW               16.800 usec
DE               19.22 usec
TE               295.0 K
D1            3.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
D20         200.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.7716219 MHz
NUC1                 13C
P1                11.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1          63.00000000 W
SPNAM[5]    Crp60comp.4
SPOAL5             0.500
SPOFFS5  0 Hz
SPW5          11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8  0 Hz
SPW8          11.64700031 W

======== CHANNEL f2 ========
SFO2         500.1320005 MHz
NUC2                 1H
CPDPRG[2          waltz65
PCPD2             80.00 usec
PLW2          14.00000000 W
PLW12          0.31500000 W

F2 - Processing parameters
SI                 262144
SF           125.7576119 MHz
WDW                  EM
SSB       0
LB                2.00 Hz
GB        0
PC                1.40
```



60

$^{19}F\{^{1}H\}$ NMR (CD_3OD)

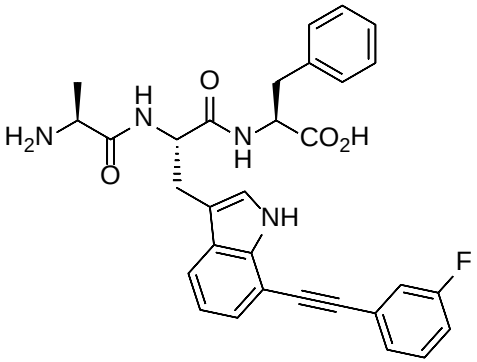

```
Current Data Parameters
NAME      03152016-2-rjmg-mc410-M
EXPNO                          10
PROCNO                          1

F2 - Acquisition Parameters
Date_                    20160315
Time                        16.50
INSTRUM                  AVII400
PROBHD    5 mm PABBO BB/
PULPROG   zgfhigqn.3.and
TD                           65536
SOLVENT                      MeOD
NS                             64
DS                              4
SWH                    30000.000 Hz
FIDRES                 0.457764 Hz
AQ                     1.0922667 sec
RG                           128
DW                        16.667 usec
DE                         8.37 usec
TE                        295.3 K
D1                     1.00000000 sec
D11                    0.03000000 sec
D12                    0.00002000 sec
TD0                             1

======== CHANNEL f1 ========
SFO1                376.4569514 MHz
NUC1                        19F
P1                        15.00 usec
PLW1                17.00000000 W

======== CHANNEL f2 ========
SFO2                400.1316005 MHz
NUC2                        1H
CPDPRG[2                waltz65
PCPD2                     90.00 usec
PLW2                16.00000000 W
PLW12                0.48688999 W

F2 - Processing parameters
SI                           65536
SF                  376.4983662 MHz
WDW                            EM
SSB                0
LB                         0.30 Hz
GB                 0
PC                         1.00
```



60

^{19}F NMR (CD_3OD)

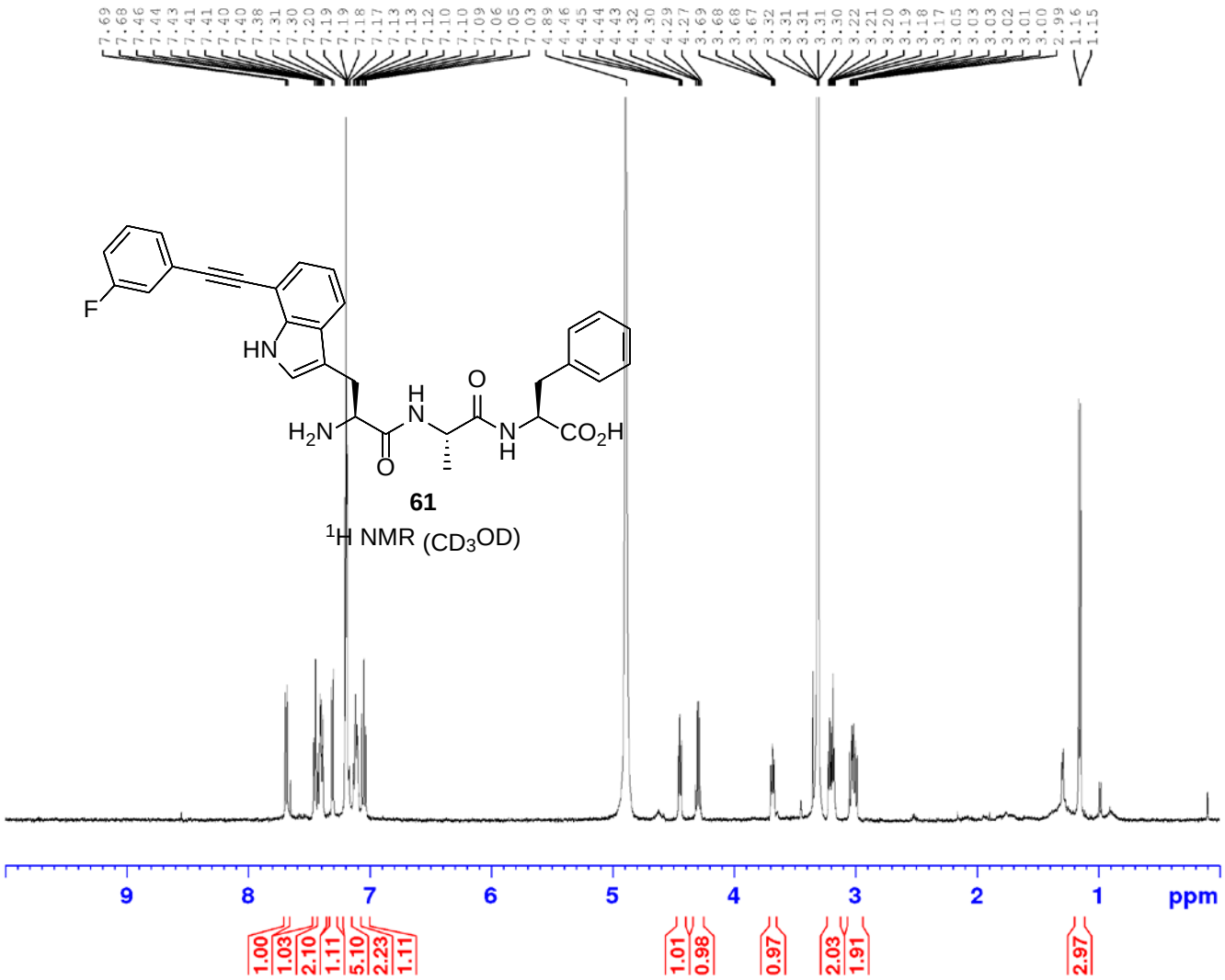
BRUKER

```
Current Data Parameters
NAME      03142016-30-rjmg-mc410-A
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160314
Time              21.06
INSTRUM           spect
PROBHD   5 mm CPFBBO BB
PULPROG               zg
TD                65536
SOLVENT            MeOD
NS                   32
DS                    4
SWH           37500.000 Hz
FIDRES        0.572205 Hz
AQ            0.8738133 sec
RG                 20.2
DW               13.333 usec
DE               18.16 usec
TE               295.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         470.5406119 MHz
NUC1                19F
P1                15.00 usec
PLW1         15.00000000 W

F2 - Processing parameters
SI                65536
SF           470.5923772 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



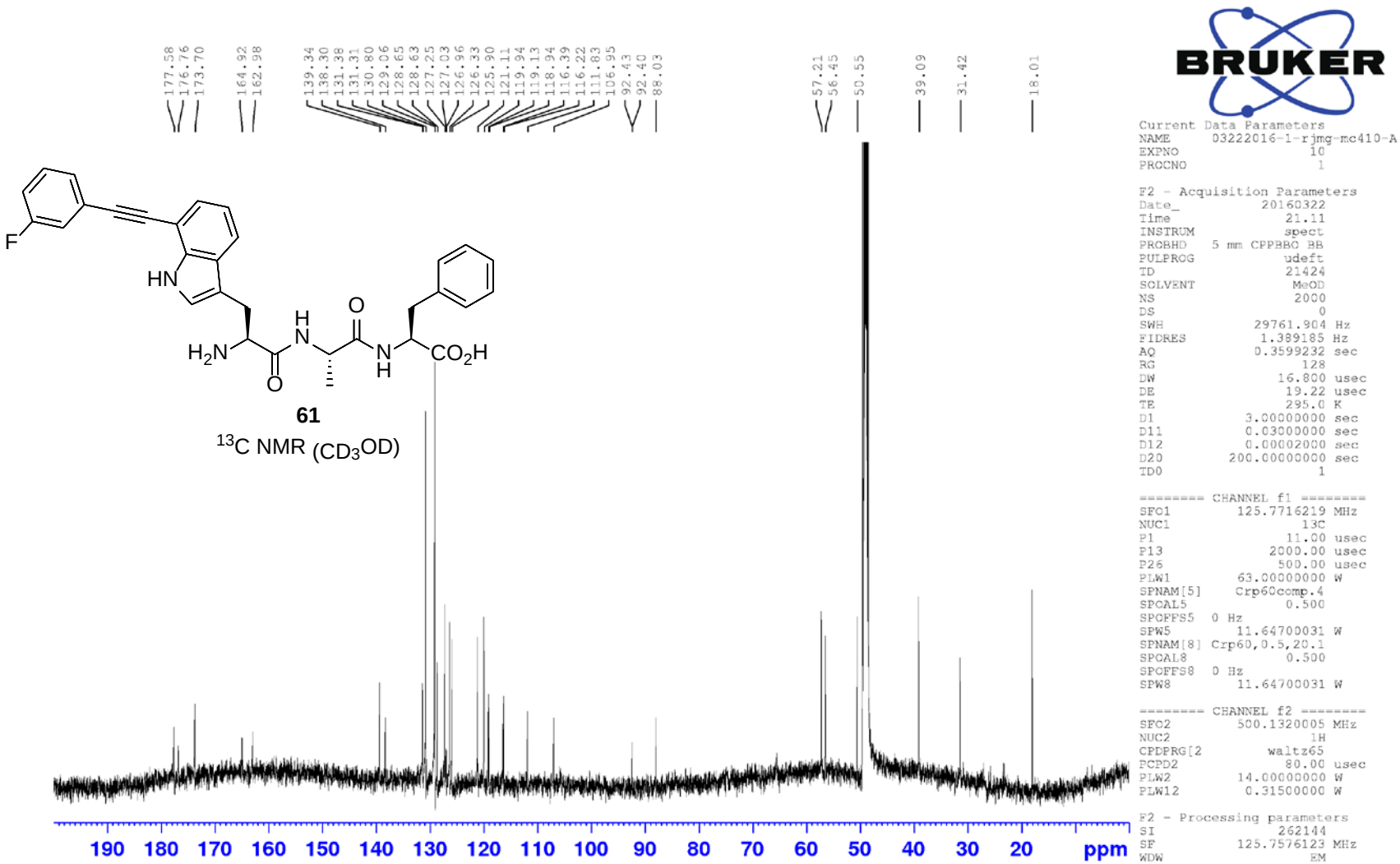
BRUKER

Current Data Parameters
NAME 03212016-17-rjmg-mc410-F
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160321
Time 16.28
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 8
DS 2
SWH 6996.269 Hz
FIDRES 0.106755 Hz
AQ 4.6836395 sec
RG 121.22
DW 71.467 usec
DE 11.38 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 499.9329996 MHz
NUC1 1H
P1 10.60 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 65536
SF 499.9300094 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



BRUKER

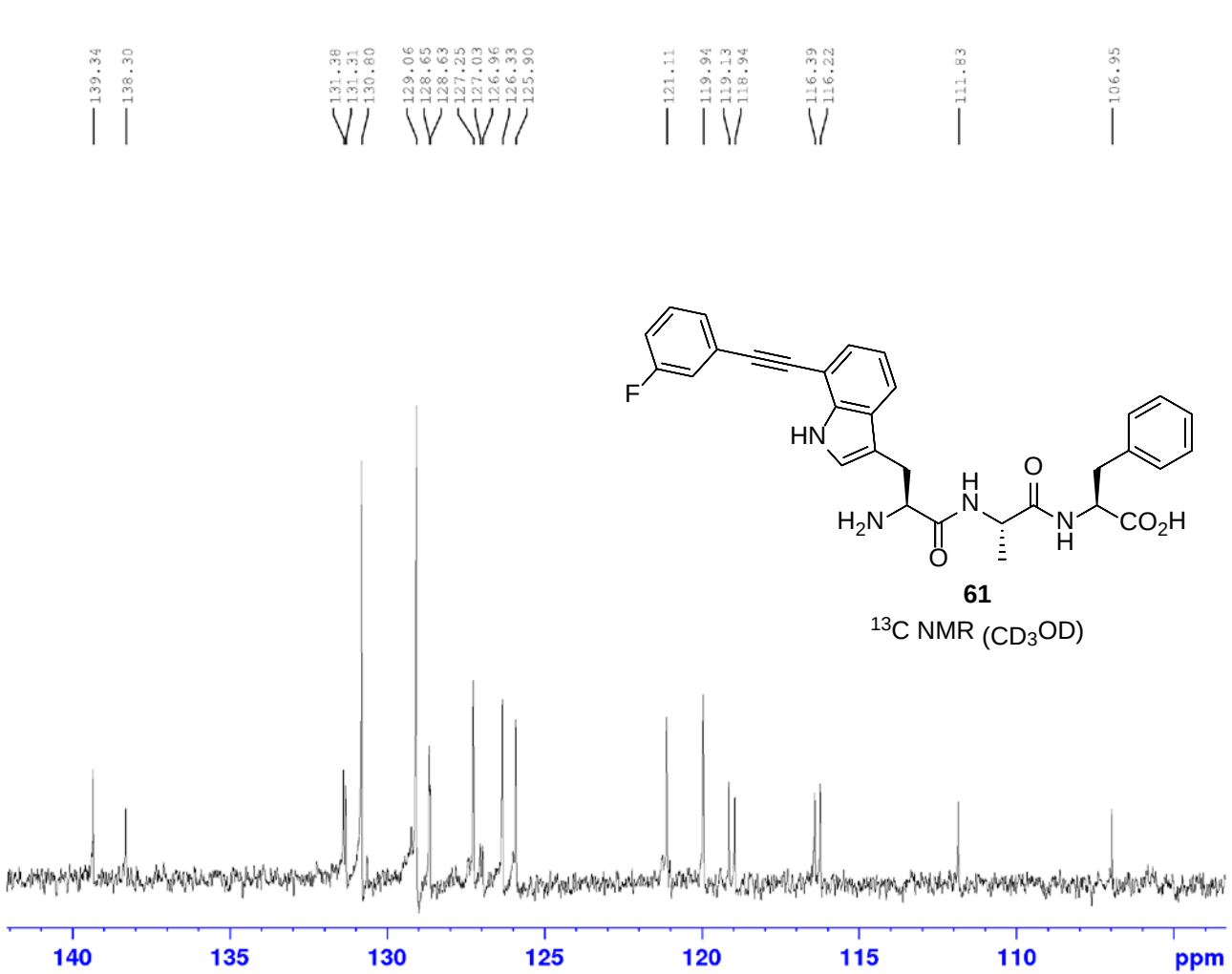
```
Current Data Parameters
NAME      03222016-1-rjmg-mc410-A
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_              20160322
Time                 21.11
INSTRUM              spect
PROBHD   5 mm CPPBBO BB
PULPROG              udeft
TD                   21424
SOLVENT               MeOD
NS                    2000
DS                       0
SWH              29761.904 Hz
FIDRES            1.389185 Hz
AQ                0.3599232 sec
RG                     128
DW                  16.800 usec
DE                  19.22 usec
TE                  295.0 K
D1               3.00000000 sec
D11              0.03000000 sec
D12              0.00002000 sec
D20            200.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1           125.7716219 MHz
NUC1                   13C
P1                  11.00 usec
P13               2000.00 usec
P26                500.00 usec
PLW1            63.00000000 W
SPNAM[5]     Crp60comp.4
SPOAL5               0.500
SPOFFS5  0 Hz
SPW5            11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8               0.500
SPOFFS8  0 Hz
SPW8            11.64700031 W

======== CHANNEL f2 ========
SFO2           500.1320005 MHz
NUC2                   1H
CPDPRG[2           waltz65
PCPD2                80.00 usec
PLW2            14.00000000 W
PLW12            0.31500000 W

F2 - Processing parameters
SI                   262144
SF             125.7576123 MHz
WDW                    EM
SSB       0
LB                   2.00 Hz
GB        0
PC                   1.40
```



61

^{13}C NMR (CD_3OD)

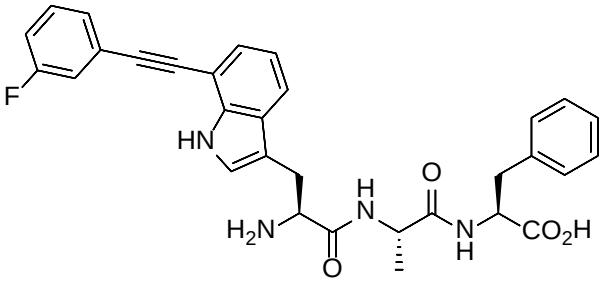
Current Data Parameters
NAME 03222016-1-rjmg-mc410-A
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160322
Time 21.11
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG udeft
TD 21424
SOLVENT MeOD
NS 2000
DS 0
SWH 29761.904 Hz
FIDRES 1.389185 Hz
AQ 0.3599232 sec
RG 128
DW 16.800 usec
DE 19.22 usec
TE 295.0 K
D1 3.00000000 sec
D11 0.03000000 sec
D12 0.00002000 sec
D20 200.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 125.7716219 MHz
NUC1 13C
P1 11.00 usec
P13 2000.00 usec
P26 500.00 usec
PLW1 63.00000000 W
SPNAM[5] Crp60comp.4
SPOAL5 0.500
SPOFFS5 0 Hz
SPW5 11.64700031 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8 0.500
SPOFFS8 0 Hz
SPW8 11.64700031 W

======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 80.00 usec
PLW2 14.00000000 W
PLW12 0.31500000 W

F2 - Processing parameters
SI 262144
SF 125.7576123 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.40



61

$^{19}F\{^{1}H\}$ NMR (CD_3OD)

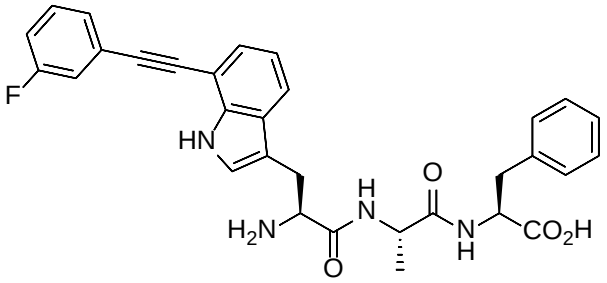

```
Current Data Parameters
NAME      03212016-17-rjmg-mc410-F
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160321
Time              16.31
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG  zgfhigqn.3.and
TD                65536
SOLVENT             MeOD
NS                    32
DS                     4
SWH           37500.000 Hz
FIDRES        0.572205 Hz
AQ            0.8738133 sec
RG                77.86
DW               13.333 usec
DE                6.75 usec
TE                295.0 K
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         470.3524446 MHz
NUC1                19F
P1                14.60 usec
PLW1         48.00000000 W

======== CHANNEL f2 ========
SFO2         499.9319997 MHz
NUC2                1H
CPDPRG[2          waltz65
PCPD2             80.00 usec
PLW2         16.00000000 W
PLW12         0.27563000 W

F2 - Processing parameters
SI                 65536
SF           470.4041892 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



61

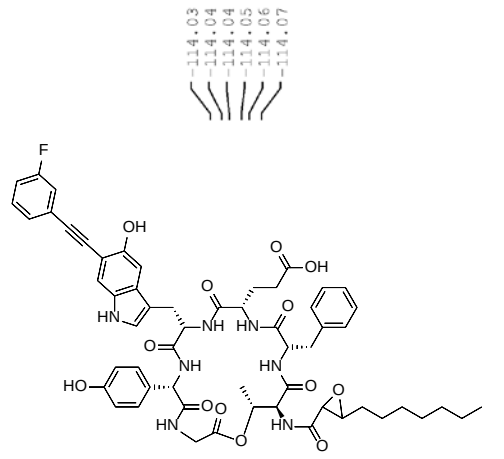
^{19}F NMR (CD_3OD)


```
Current Data Parameters
NAME      03232016-5-rjmg-mc410-F
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20160323
Time              14.45
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG         zgflqn
TD               65536
SOLVENT           MeOD
NS                  32
DS                   4
SWH           37500.000 Hz
FIDRES         0.572205 Hz
AQ             0.8738133 sec
RG               121.22
DW               13.333 usec
DE                6.75 usec
TE               295.0 K
D1            1.00000000 sec
TD0                  1

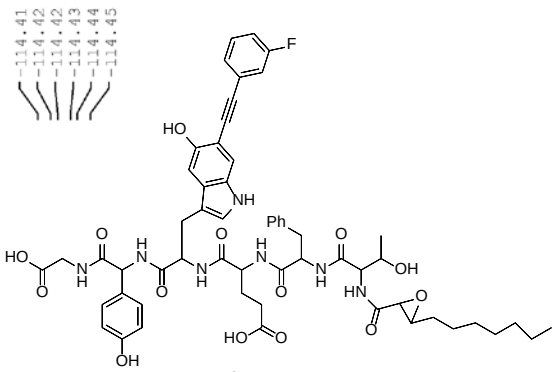
======== CHANNEL f1 ========
SFO1         470.3524446 MHz
NUC1               19F
P1               14.60 usec
PLW1         48.00000000 W

F2 - Processing parameters
SI                65536
SF           470.4041892 MHz
WDW                EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```



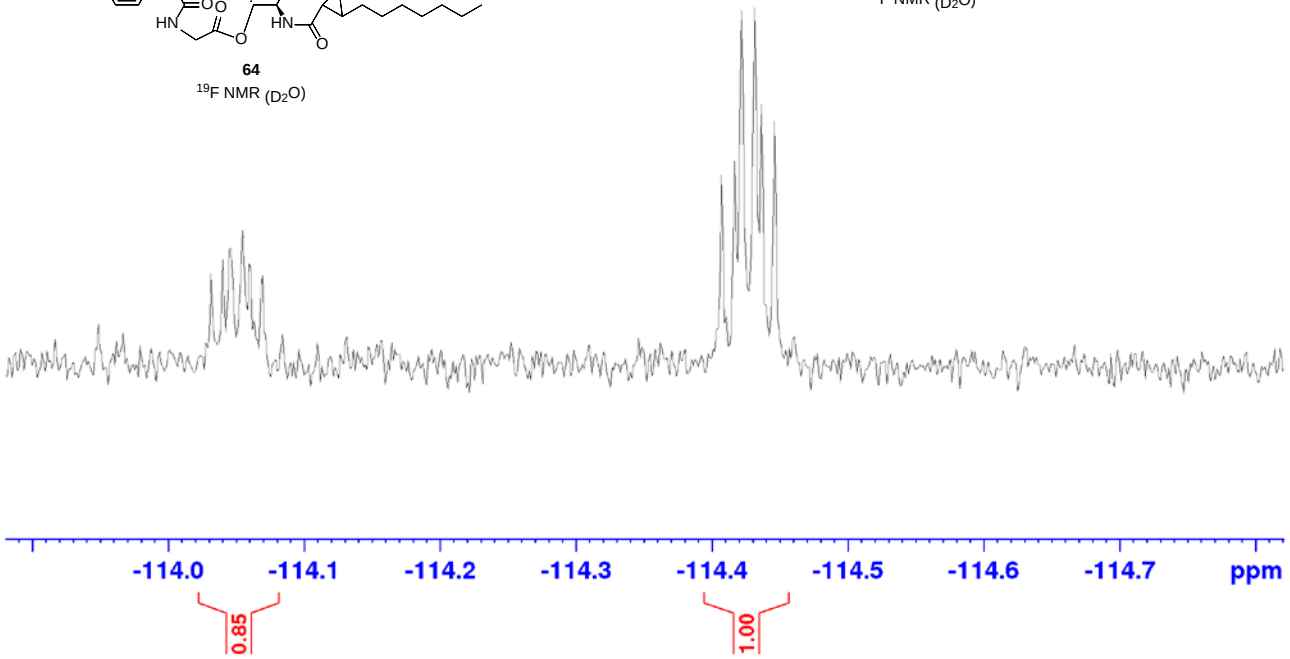
64

^{19}F NMR (D_2O)



65

^{19}F NMR (D_2O)

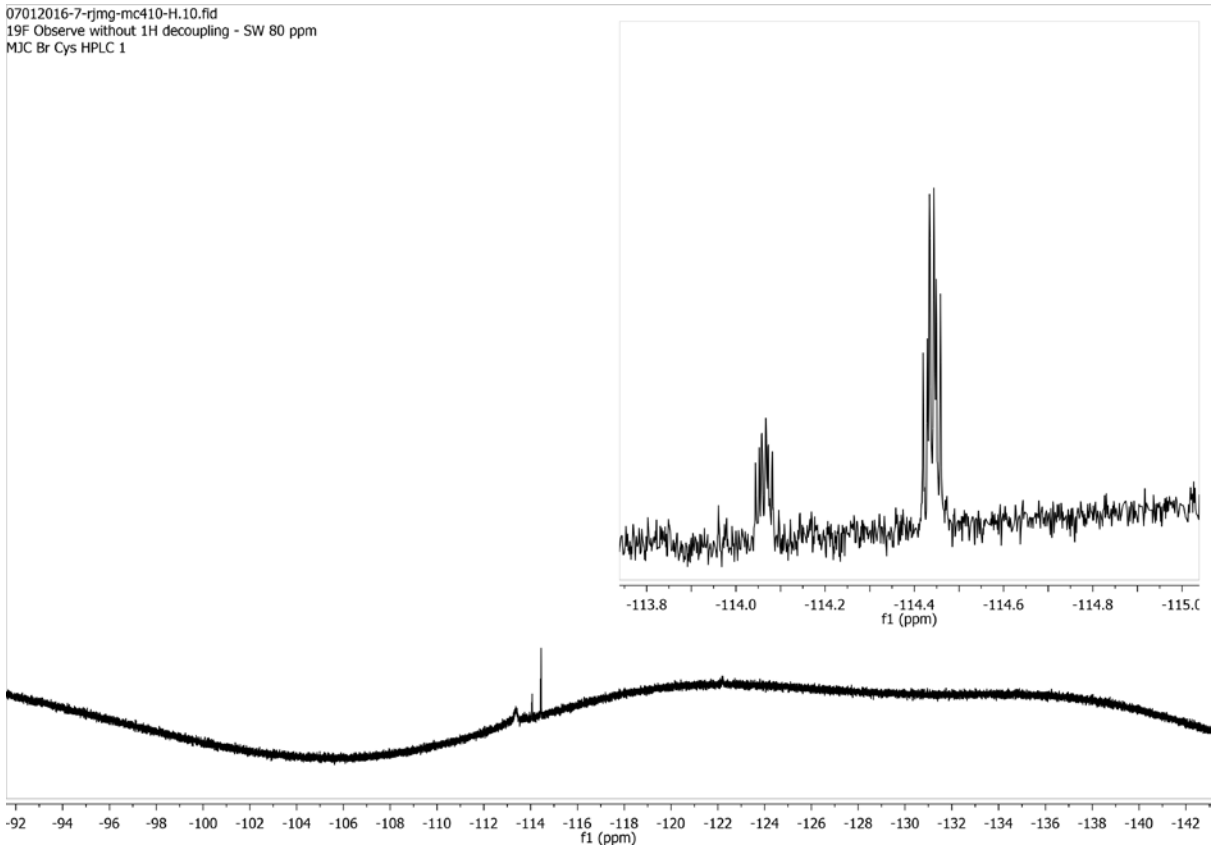



```
Current Data Parameters
NAME      07012016-7-rjmg-mc410-H
EXPNO                  10
PROCNO                  1

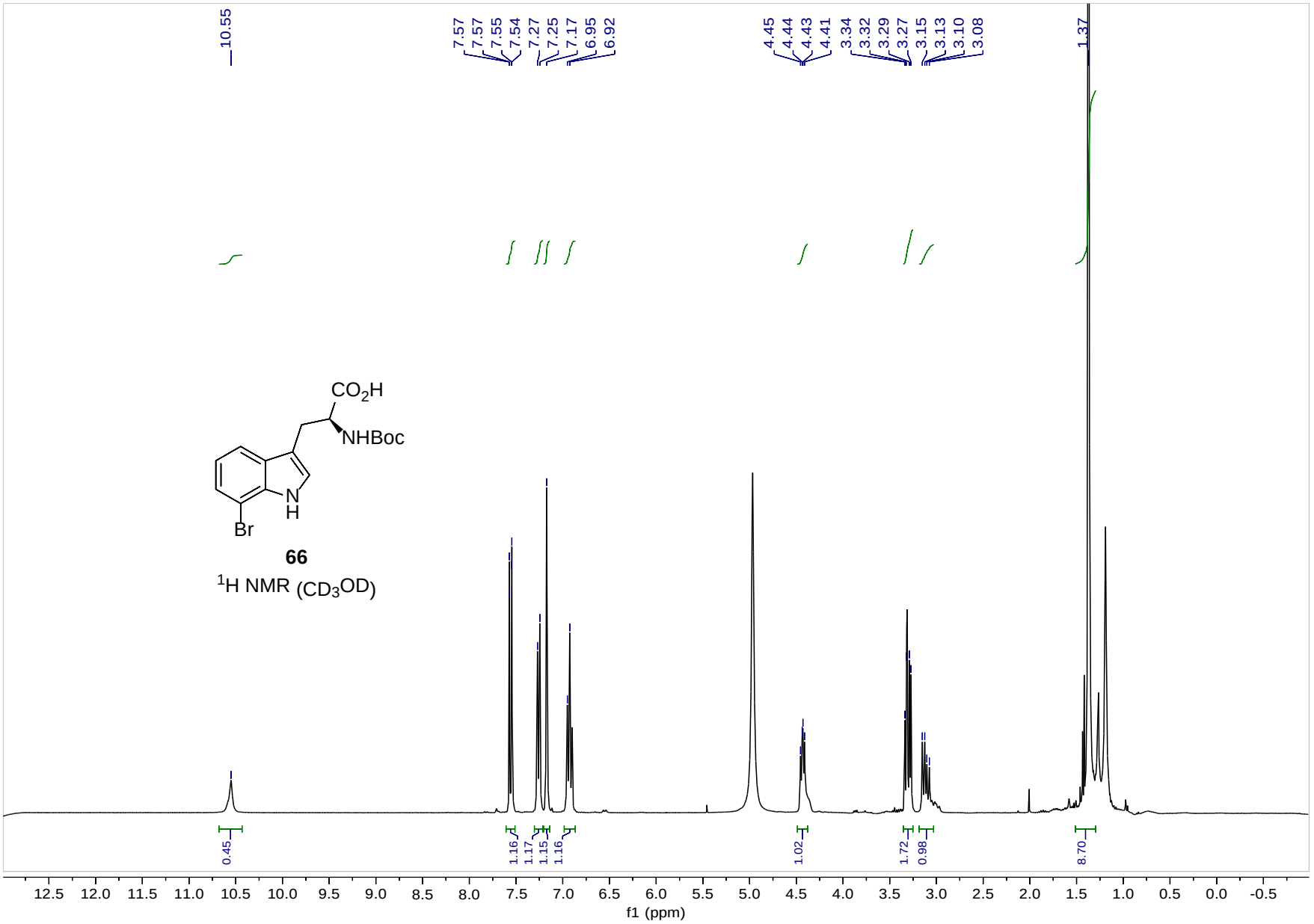
F2 - Acquisition Parameters
Date_            20160701
Time                15.34
INSTRUM             spect
PROBHD    5 mm CPPTCI 1H
PULPROG                 zg
TD                   65536
SOLVENT                D2O
NS                     256
DS                       4
SWH              52631.578 Hz
FIDRES            0.803094 Hz
AQ               0.6225920 sec
RG                   20.04
DW                 9.500 usec
DE                18.25 usec
TE                 295.0 K
D1              1.00000000 sec
TD0                      1

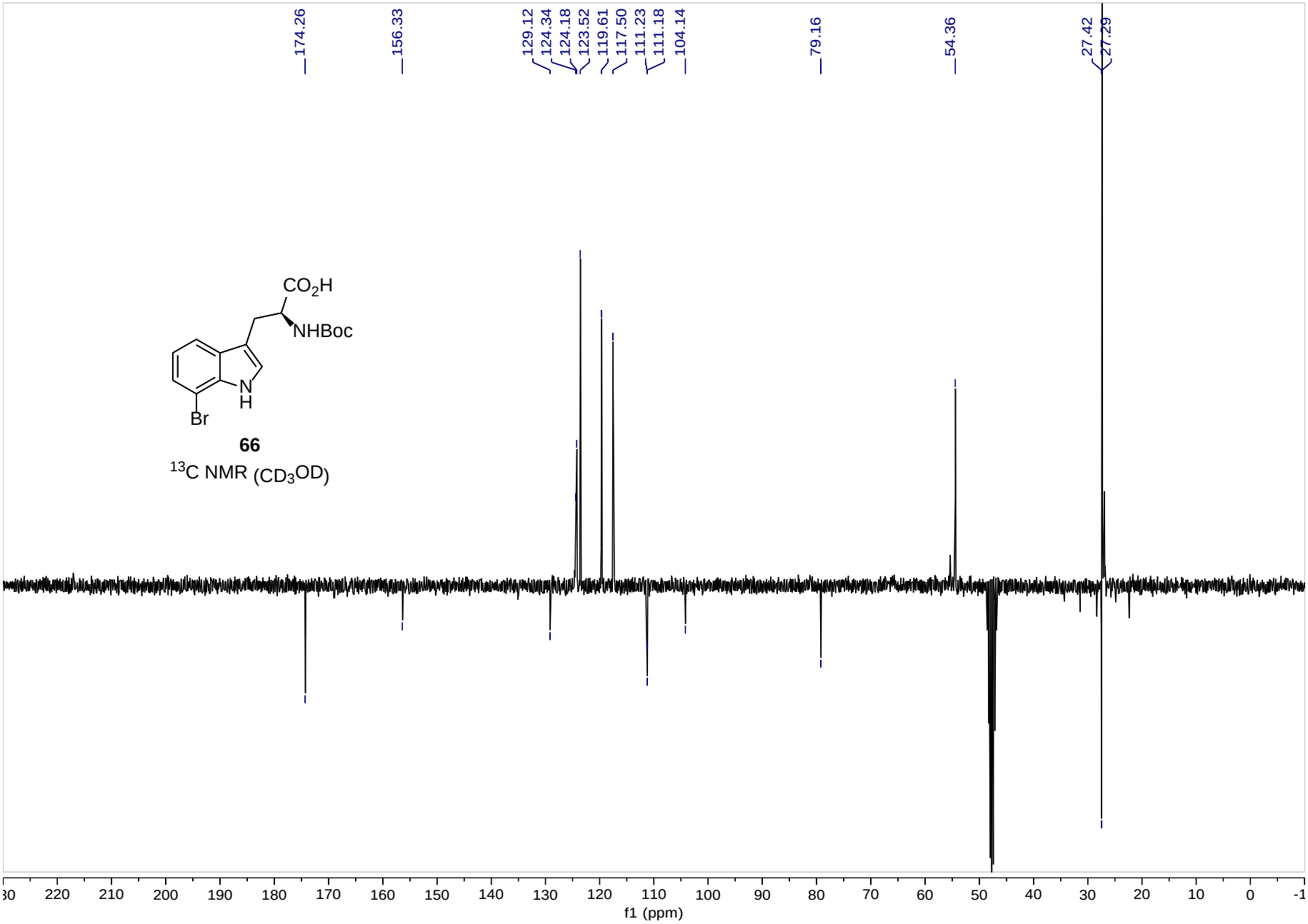
======== CHANNEL f1 ========
SFO1          658.7046395 MHz
NUC1                   19F
P1                  11.00 usec
PLW1          13.80000019 W

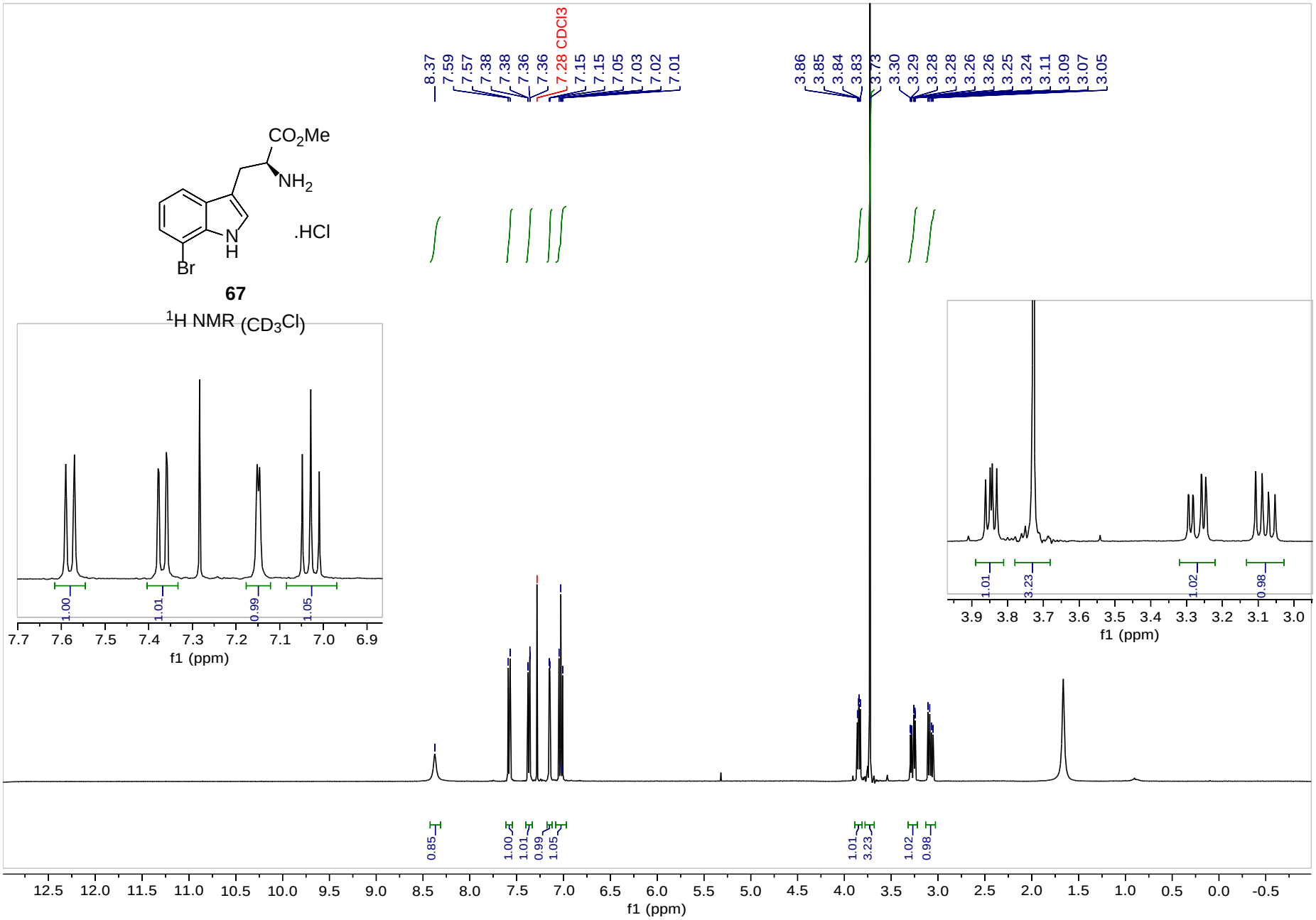
F2 - Processing parameters
SI                   65536
SF            658.7803992 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

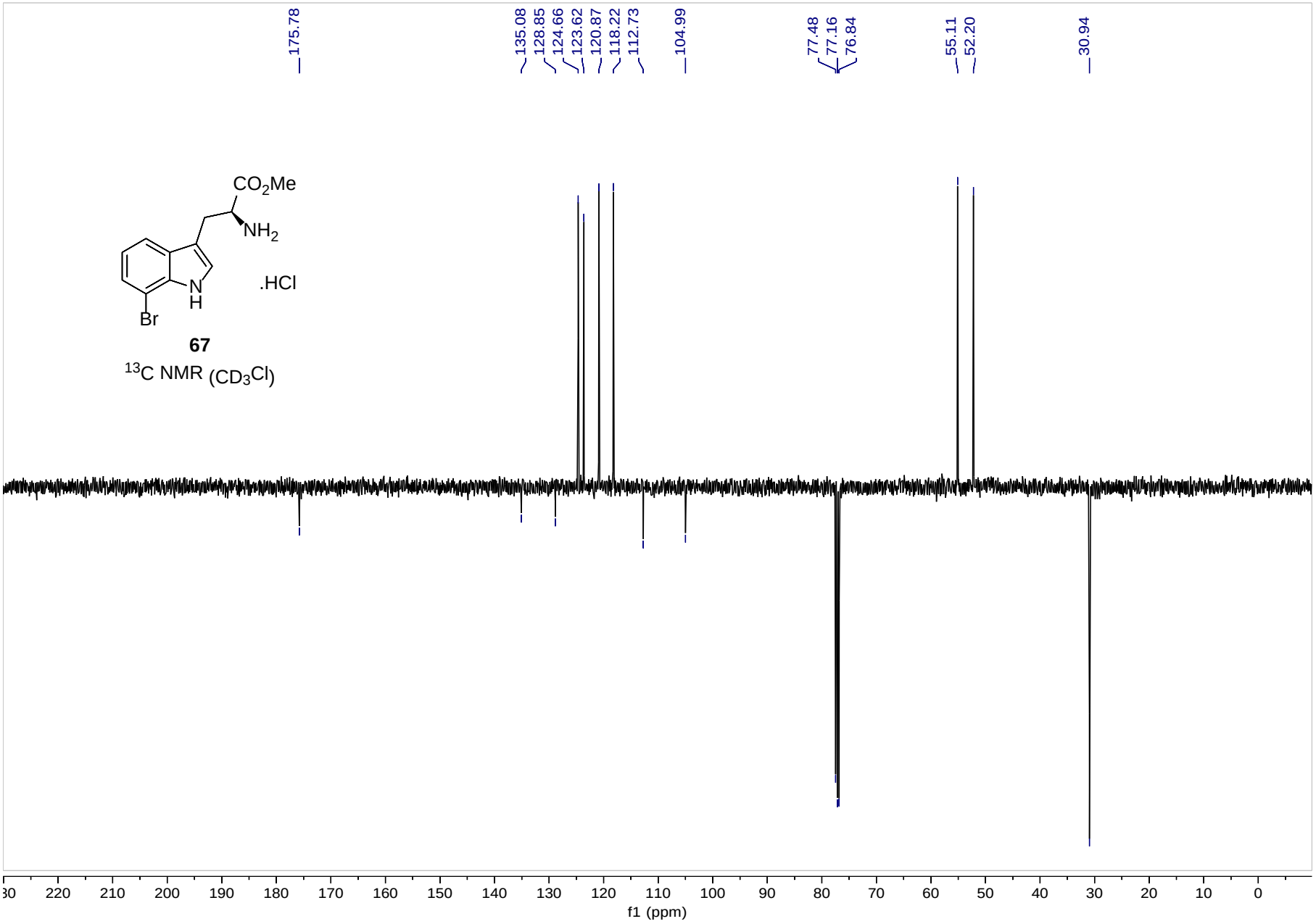


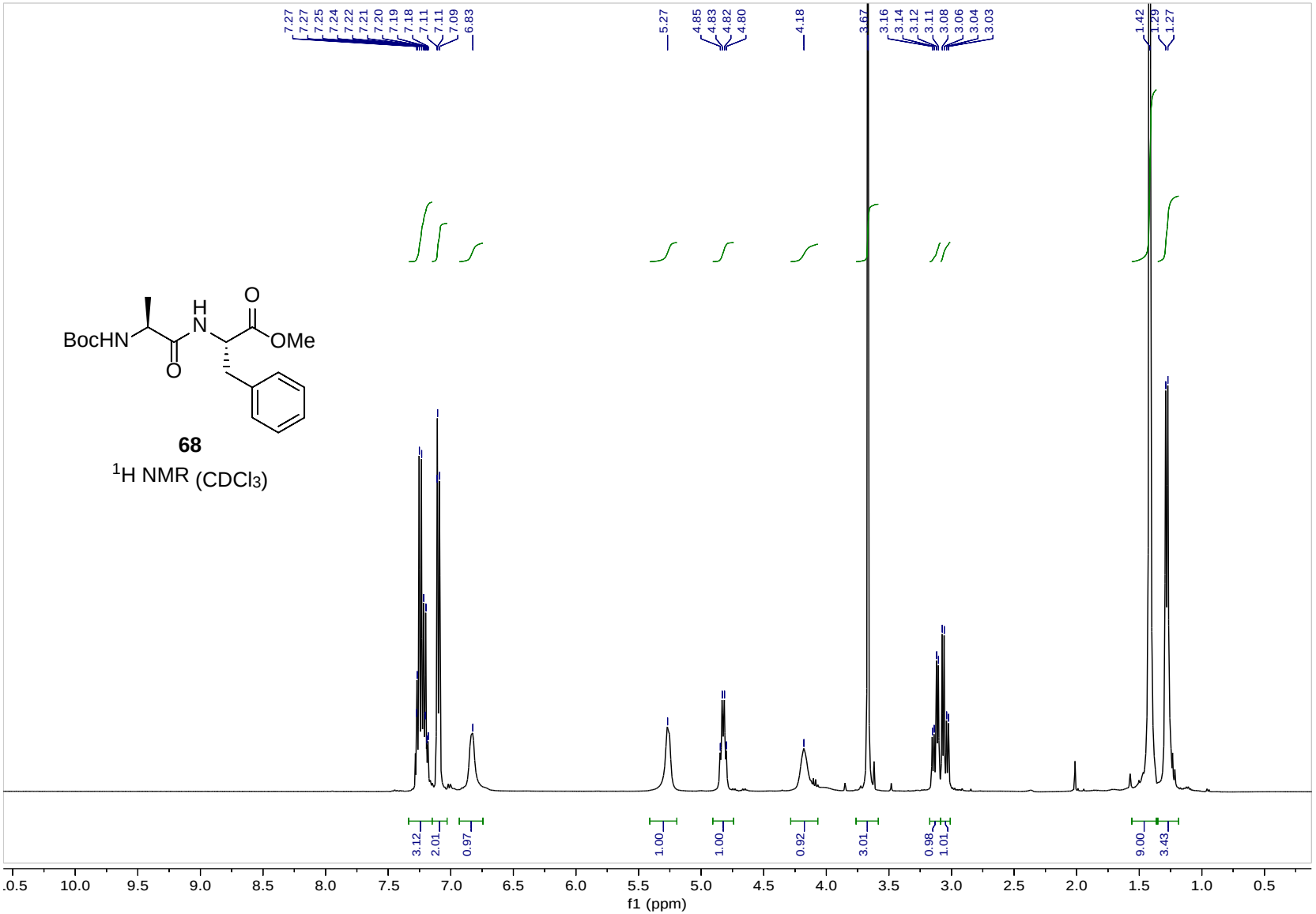
^{19}F NMR (D_2O) of **64** and **65**

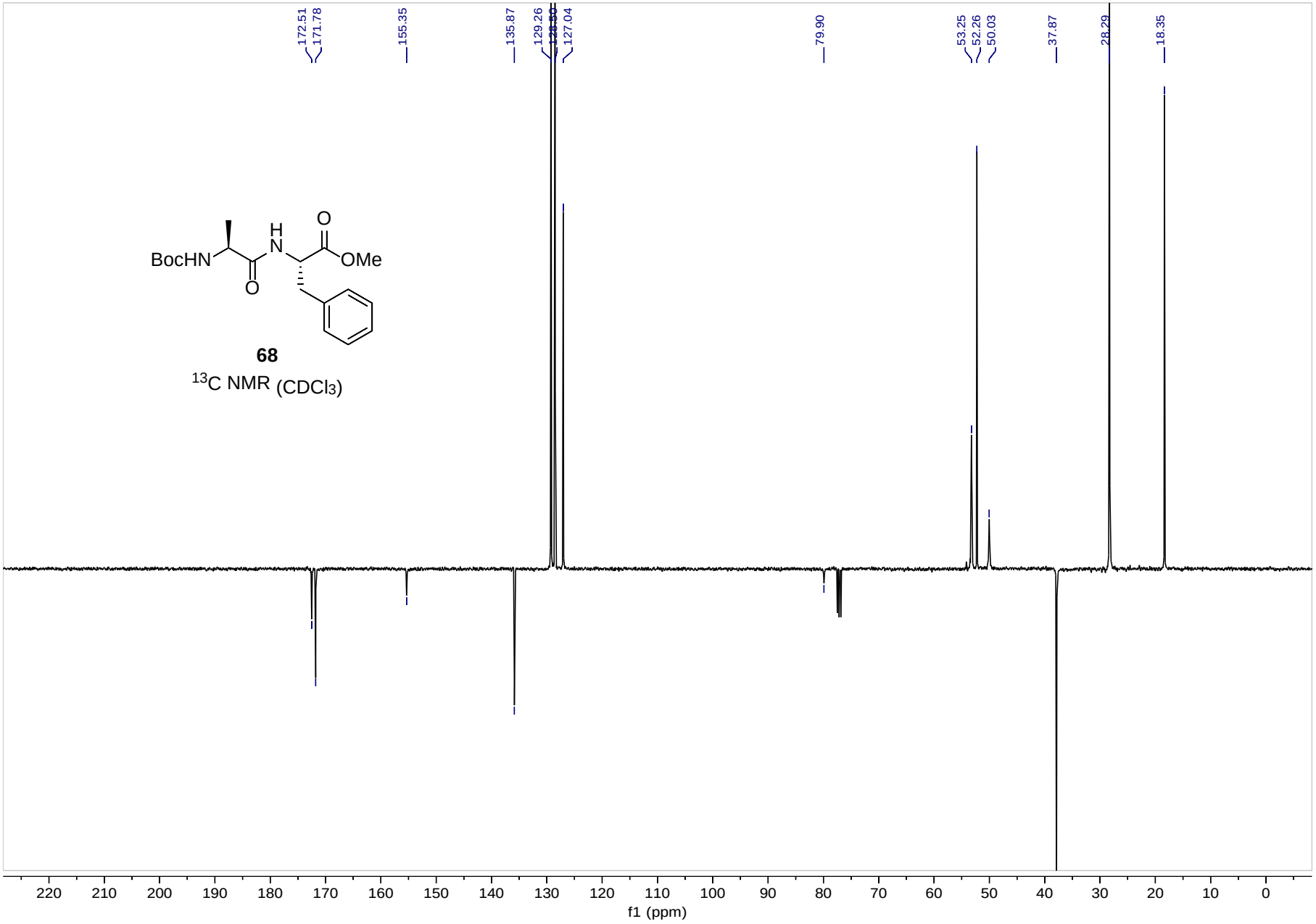

10.55
7.57
7.57
7.55
7.54
7.27
7.25
7.17
6.95
6.92
4.45
4.44
4.43
4.41
3.34
3.32
3.29
3.27
3.15
3.13
3.10
3.08
1.37
CO2H
NHBoc
N
H
Br
66
1H NMR (CD3OD)
0.45
1.16
1.17
1.15
1.16
1.02
1.72
0.98
8.70
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

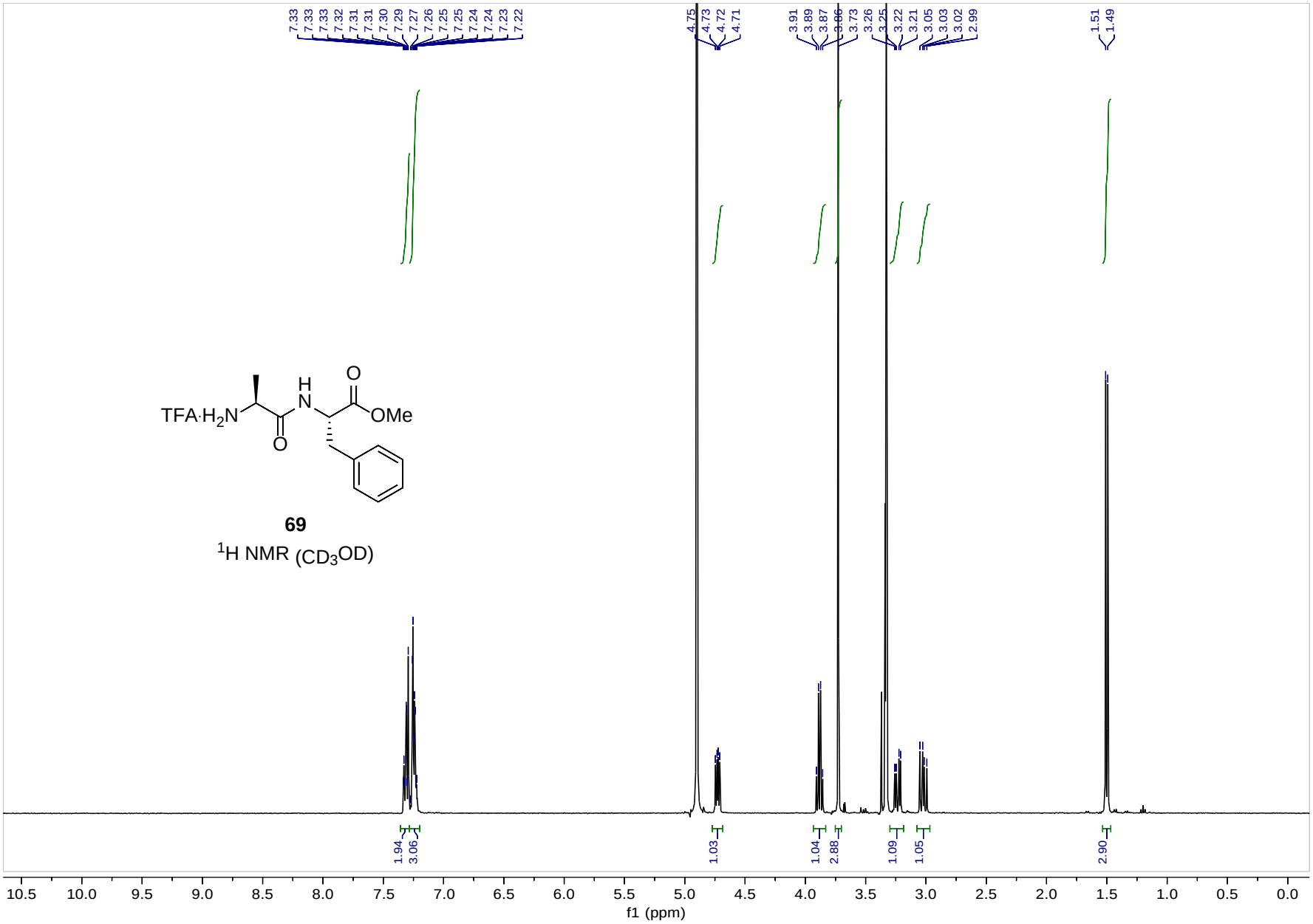

174.26
156.33
129.12
124.34
124.18
123.52
119.61
117.50
111.23
111.18
104.14
79.16
54.36
27.42
27.29
CO2H
NHBoc
N
H
Br
66
13C NMR (CD3OD)
f1 (ppm)

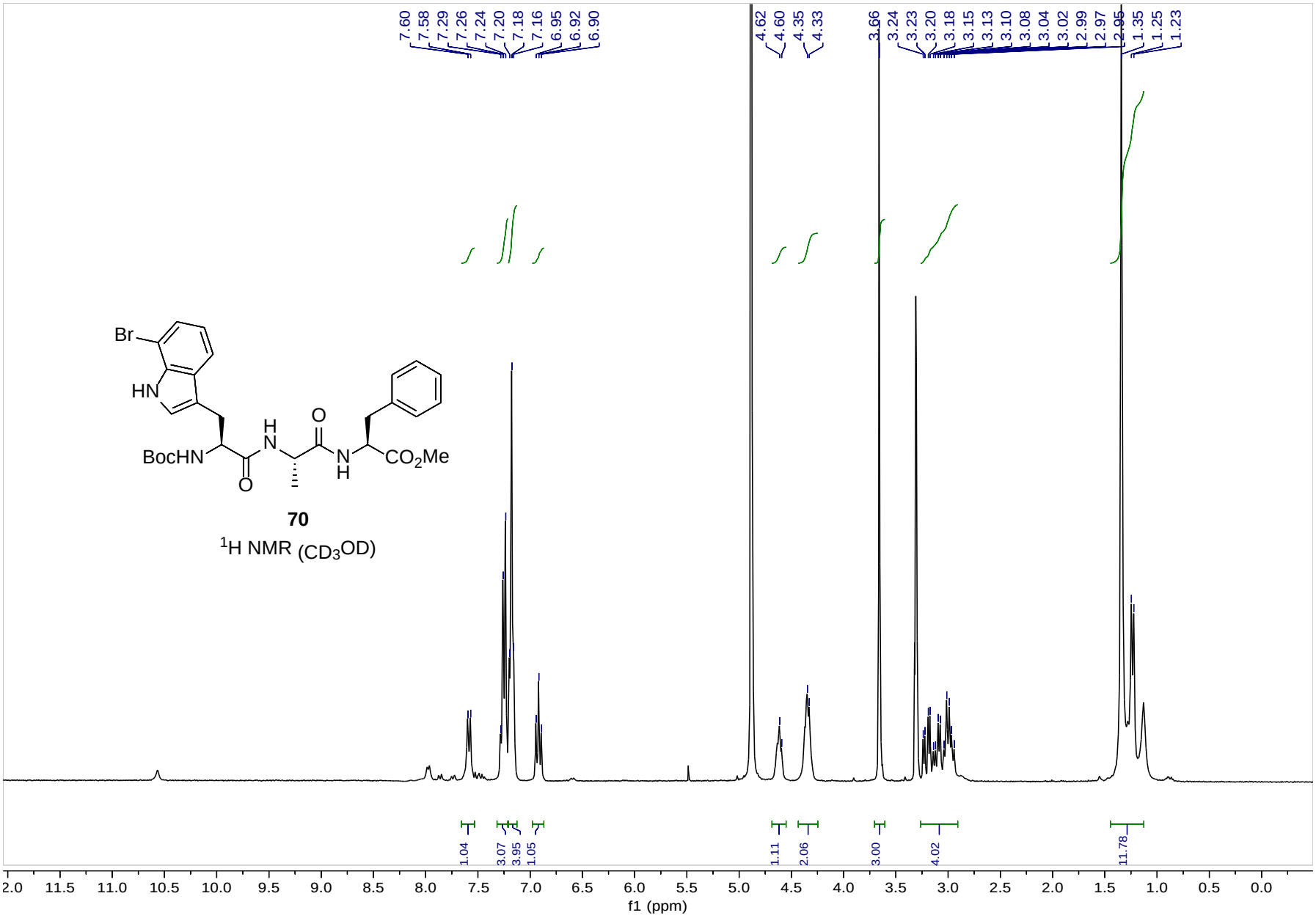

8.37
7.59
7.57
7.38
7.38
7.36
7.36
7.28 CDCl3
7.15
7.15
7.05
7.03
7.02
7.01
3.86
3.85
3.84
3.83
3.73
3.30
3.29
3.28
3.28
3.26
3.26
3.25
3.24
3.11
3.09
3.07
3.05
CO2Me
NH2
.HCl
N
H
Br
67
1H NMR (CD3Cl)
1.00
1.01
0.99
1.05
f1 (ppm)
1.01
3.23
1.02
0.98
f1 (ppm)
0.85
1.00
1.01
0.99
1.05
1.01
3.23
1.02
0.98
f1 (ppm)


175.78
135.08
128.85
124.66
123.62
120.87
118.22
112.73
104.99
77.48
77.16
76.84
55.11
52.20
30.94
CO2Me
NH2
.HCl
N
H
Br
67
13C NMR (CD3Cl)
f1 (ppm)


7.27
7.27
7.25
7.24
7.22
7.21
7.20
7.19
7.18
7.11
7.11
7.09
6.83
5.27
4.85
4.83
4.82
4.80
4.18
3.67
3.16
3.14
3.12
3.11
3.08
3.06
3.04
3.03
1.42
1.29
1.27
BocHN
H
N
O
O
OMe
68
1H NMR (CDCl3)
3.12
2.01
0.97
1.00
1.00
0.92
3.01
0.98
1.01
9.00
3.43
f1 (ppm)

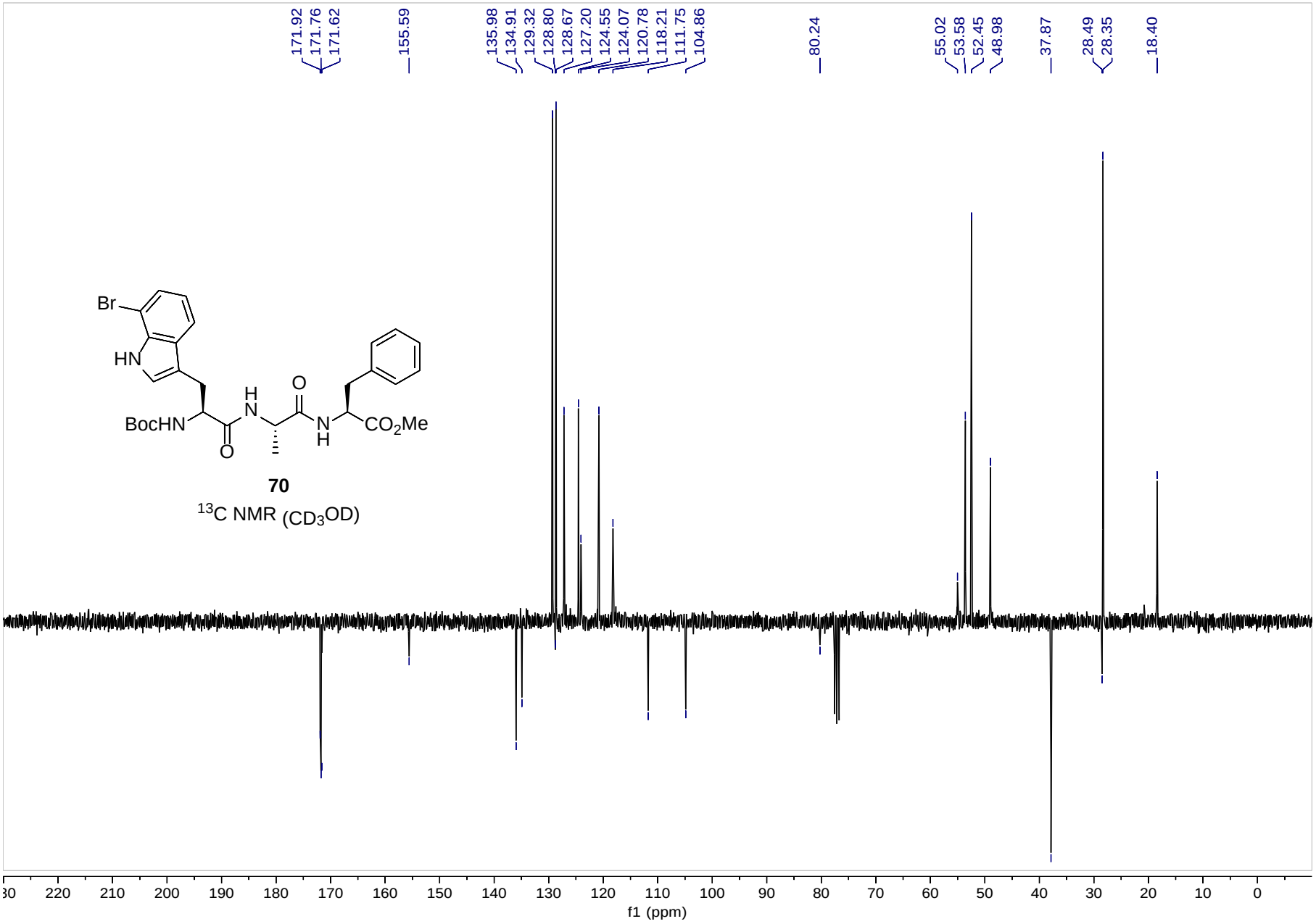

172.51
171.78
155.35
135.87
129.26
128.50
127.04
79.90
53.25
52.26
50.03
37.87
28.29
18.35
BocHN
H
N
O
O
OMe
68
13C NMR (CDCl3)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

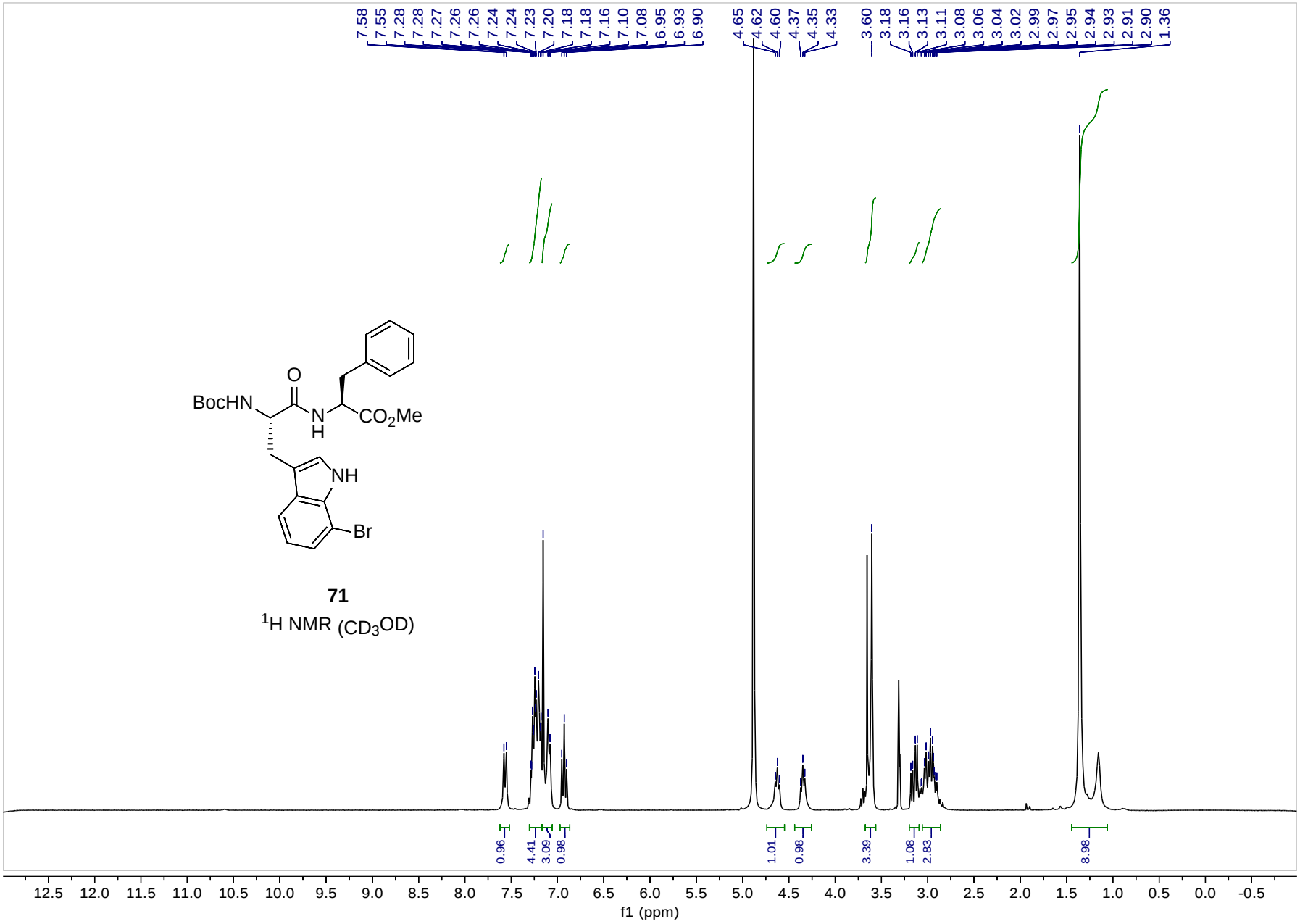

7.33
7.33
7.33
7.32
7.31
7.31
7.30
7.29
7.27
7.26
7.25
7.25
7.24
7.24
7.23
7.22
4.75
4.73
4.72
4.71
3.91
3.89
3.87
3.86
3.73
3.26
3.25
3.22
3.21
3.05
3.03
3.02
2.99
1.51
1.49
TFA·H2N
H
N
O
O
OMe
69
1H NMR (CD3OD)
1.94
3.06
1.03
1.04
2.88
1.09
1.05
2.90
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

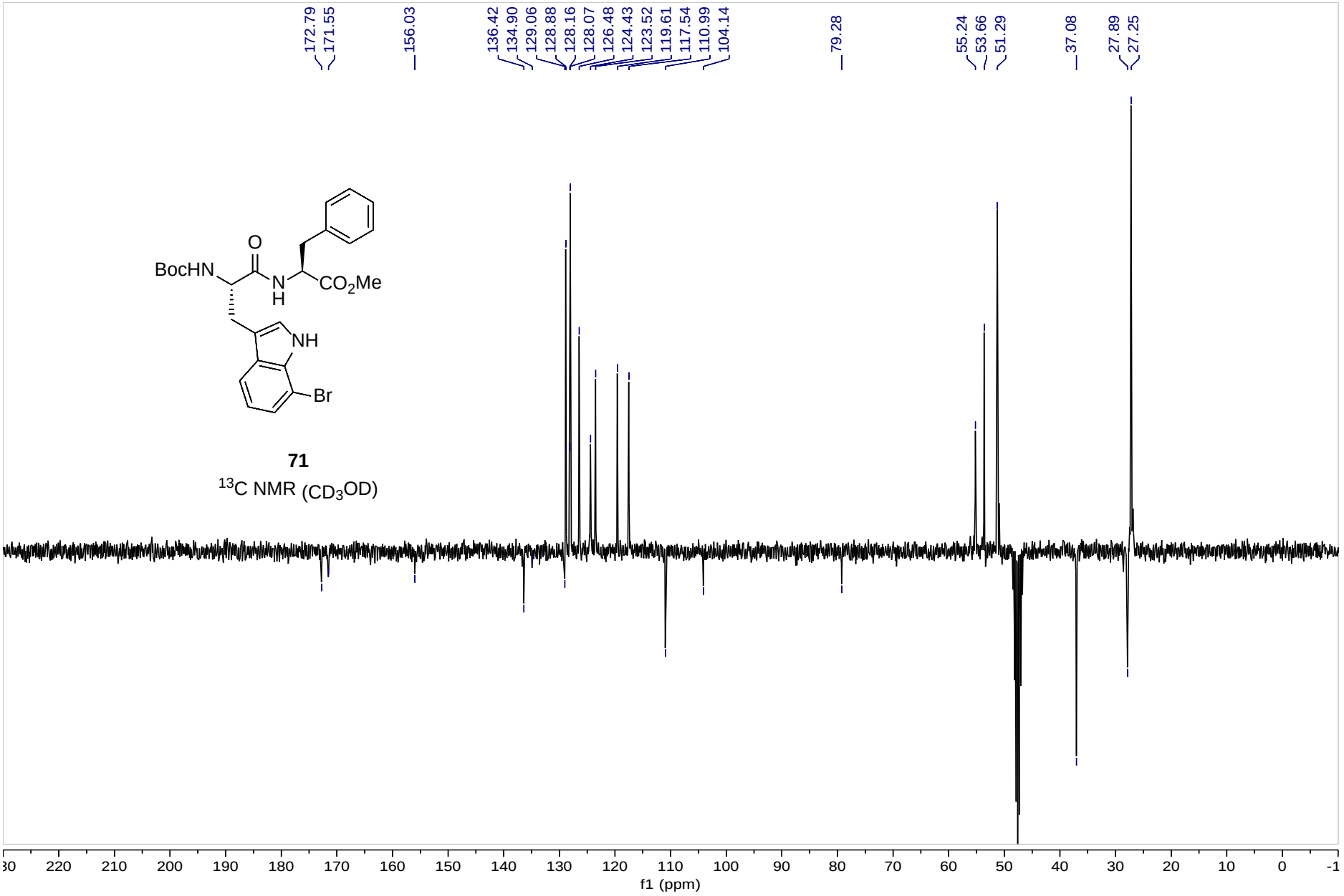


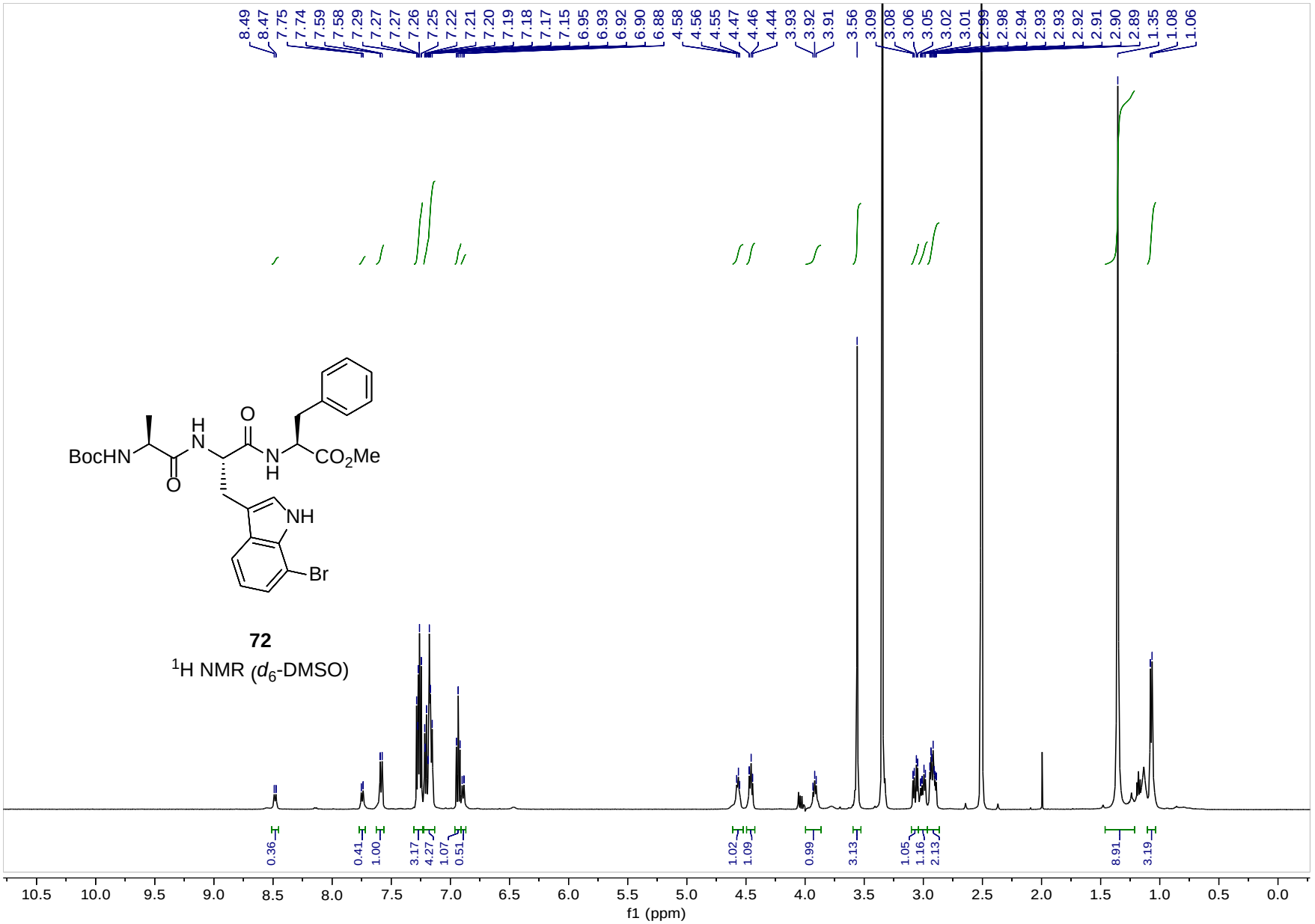
70

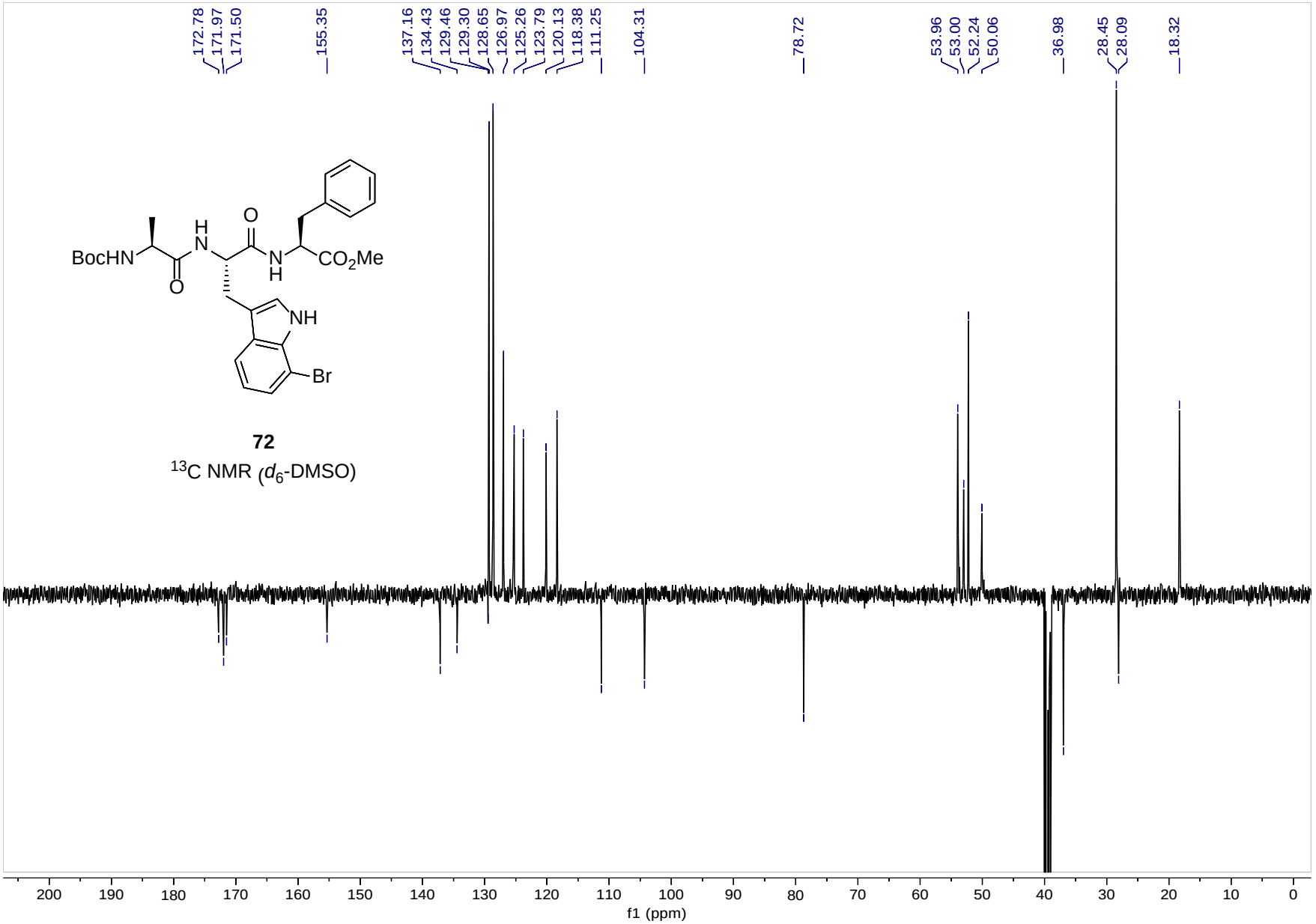
^{1}H NMR (CD_3OD)


171.92
171.76
171.62
155.59
135.98
134.91
129.32
128.80
128.67
127.20
124.55
124.07
120.78
118.21
111.75
104.86
80.24
55.02
53.58
52.45
48.98
37.87
28.49
28.35
18.40
Br
HN
BocHN
H
N
O
O
N
H
CO2Me
70
13C NMR (CD3OD)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

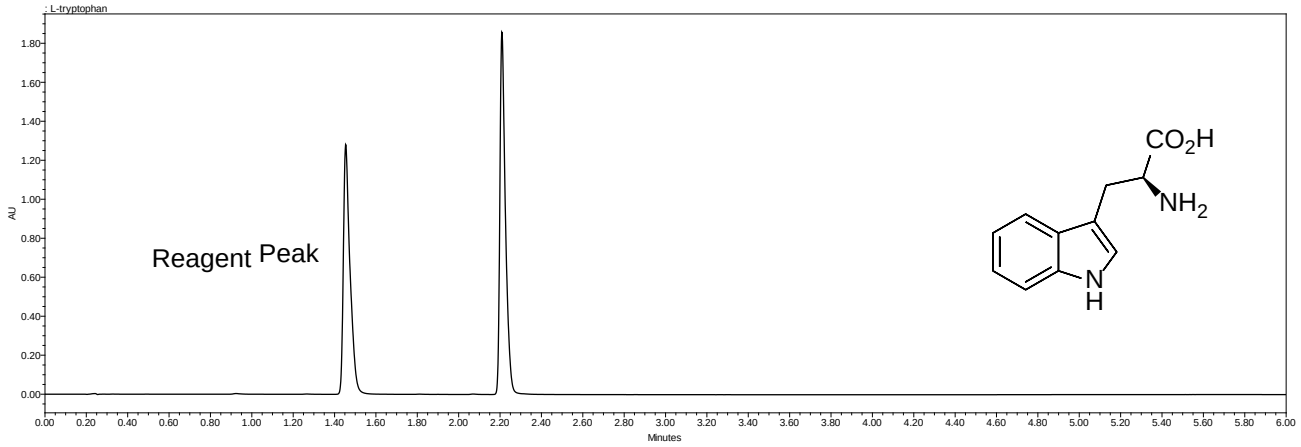

7.58
7.55
7.28
7.28
7.27
7.26
7.26
7.24
7.24
7.23
7.20
7.18
7.18
7.16
7.10
7.08
6.95
6.93
6.90
4.65
4.62
4.60
4.37
4.35
4.33
3.60
3.18
3.16
3.13
3.11
3.08
3.06
3.04
3.02
2.99
2.97
2.95
2.94
2.93
2.91
2.90
1.36
BocHN
O
N
H
CO2Me
NH
Br
71
1H NMR (CD3OD)
0.96
4.41
3.09
0.98
1.01
0.98
3.39
1.08
2.83
8.98
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

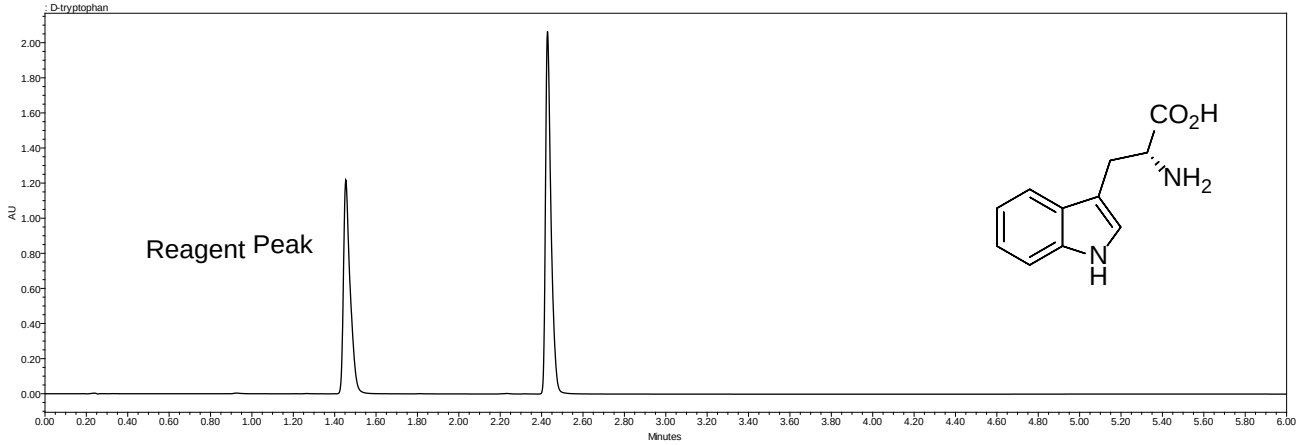

172.79
171.55
156.03
136.42
134.90
129.06
128.88
128.16
128.07
126.48
124.43
123.52
119.61
117.54
110.99
104.14
79.28
55.24
53.66
51.29
37.08
27.89
27.25
BocHN
O
N
H
CO2Me
NH
Br
71
13C NMR (CD3OD)
f1 (ppm)

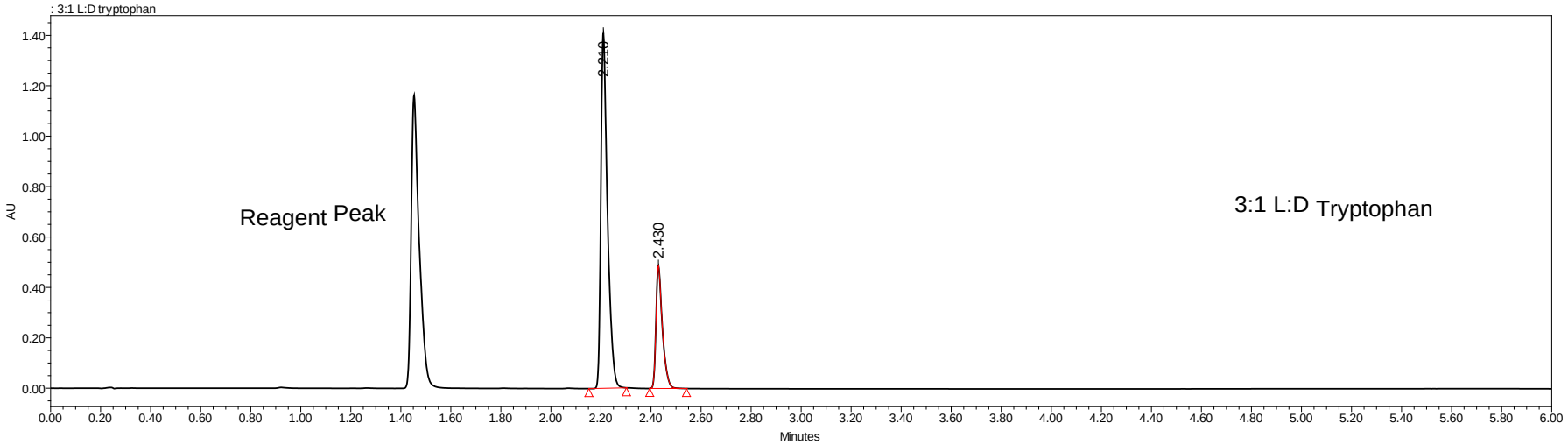

8.49
8.47
7.75
7.74
7.59
7.58
7.29
7.27
7.27
7.26
7.25
7.22
7.21
7.20
7.19
7.18
7.17
7.15
6.95
6.93
6.92
6.90
6.88
4.58
4.56
4.55
4.47
4.46
4.44
3.93
3.92
3.91
3.56
3.09
3.08
3.06
3.05
3.02
3.01
2.99
2.98
2.94
2.93
2.93
2.92
2.91
2.90
2.89
1.35
1.08
1.06
BocHN
O
H
N
O
N
H
CO2Me
NH
Br
72
1H NMR (d6-DMSO)
0.36
0.41
1.00
3.17
4.27
1.07
0.51
1.02
1.09
0.99
3.13
1.05
1.16
2.13
8.91
3.19
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


172.78
171.97
171.50
155.35
137.16
134.43
129.46
129.30
128.65
126.97
125.26
123.79
120.13
118.38
111.25
104.31
78.72
53.96
53.00
52.24
50.06
36.98
28.45
28.09
18.32
BocHN
H
N
O
O
N
H
CO2Me
NH
Br
72
13C NMR (d6-DMSO)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Enantiopurity analyses by UPLC using Marfey's Reagent

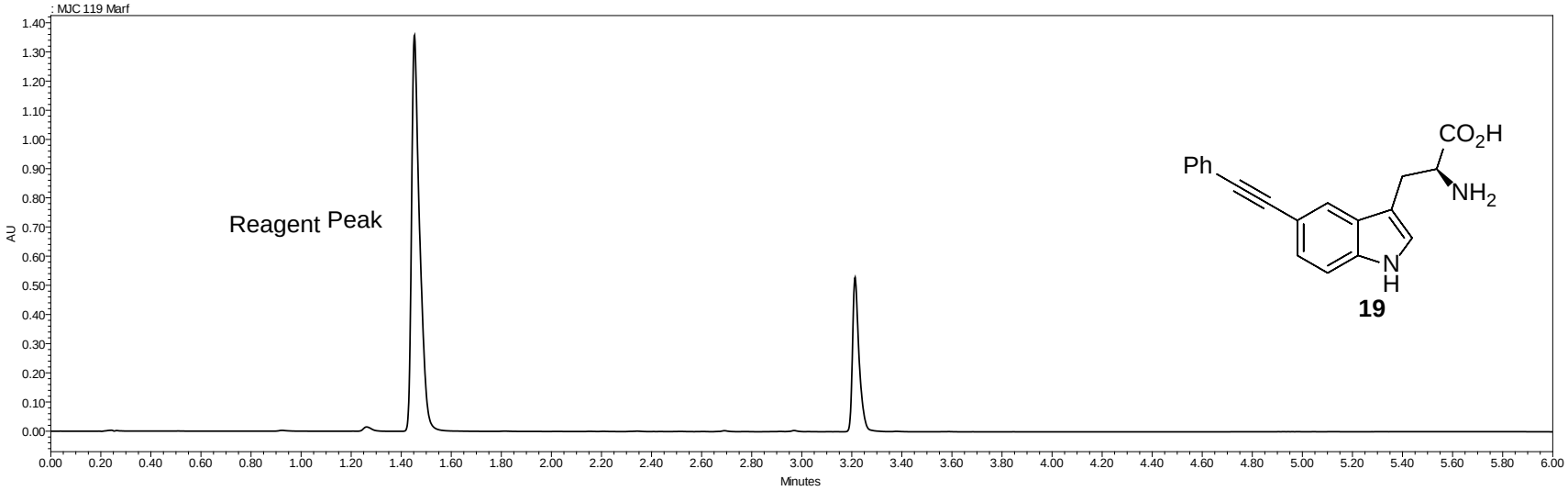


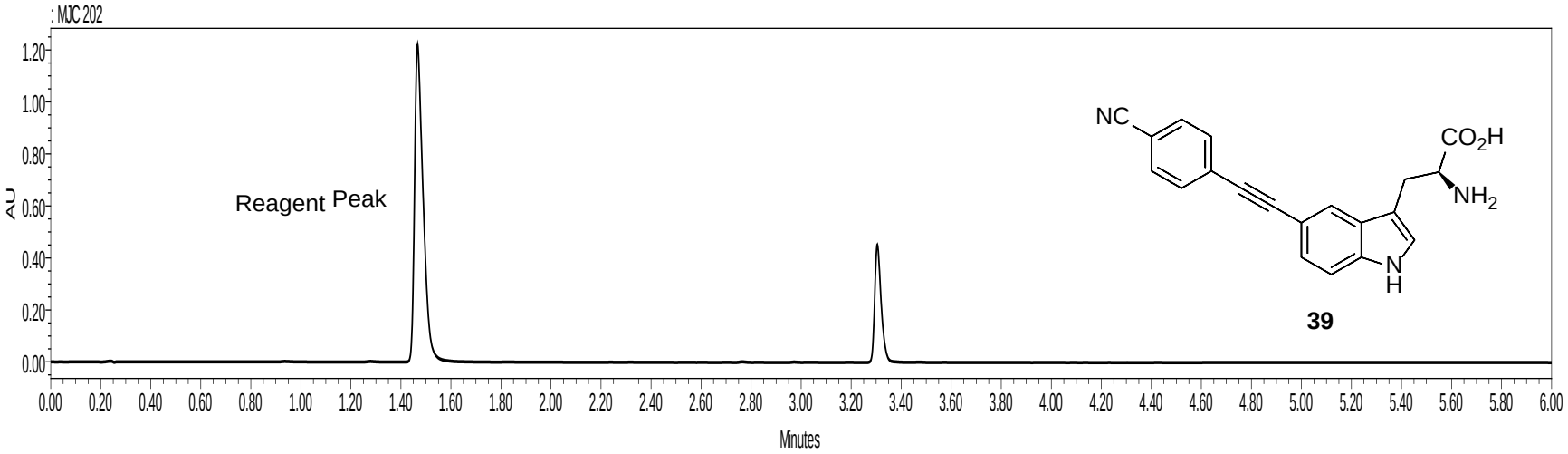


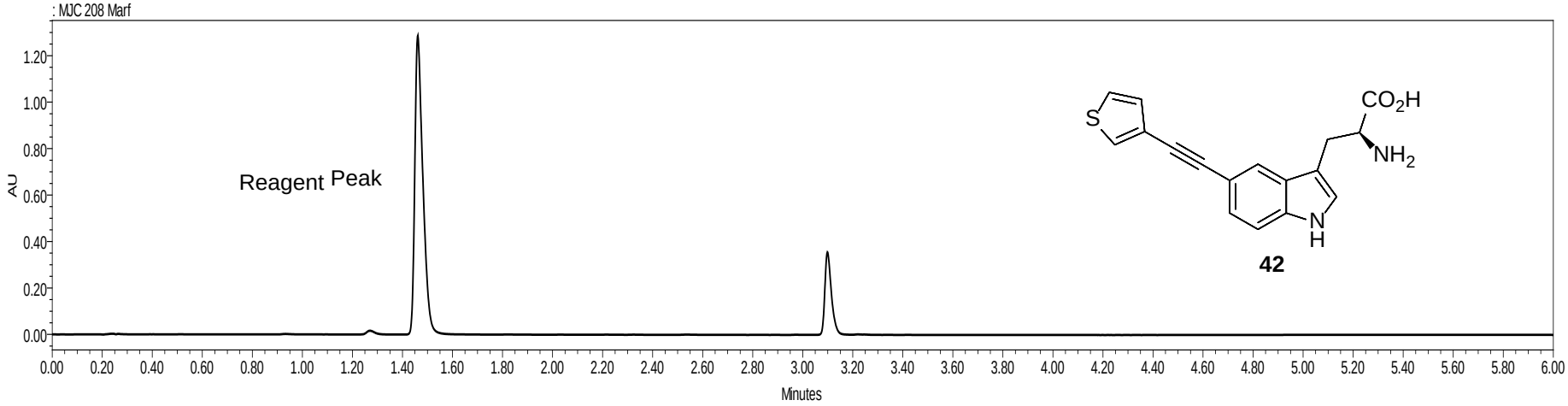


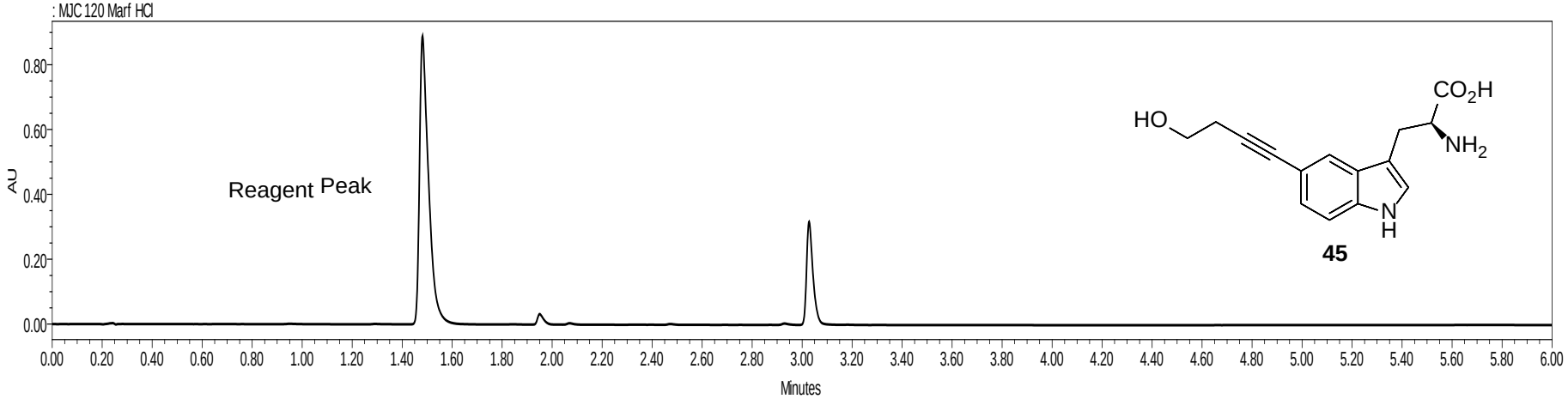
	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes	Match_Criteria	Purity
1		2.210	2588715	74.91	1409209	bb			Unknown			

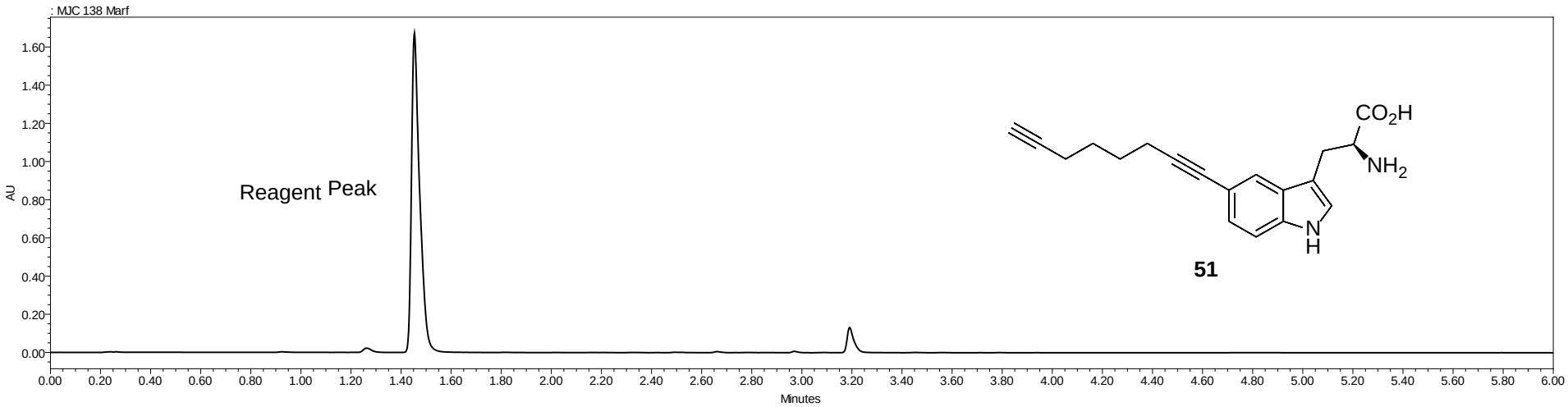
	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes	Match_Criteria	Purity
2		2.430	867178	25.09	486339	bb			Unknown			


: MJC 119 Marf
AU
Minutes
Reagent Peak
Ph
CO2H
NH2
N
H
19


: MJC 202
AU
Minutes
Reagent Peak
NC
CO2H
NH2
N
H
39


: MJC 208 Marf
AU
Reagent Peak
Minutes
42


: MJC 120 Marf HCl
AU
Reagent Peak
Minutes
45


: MJC 138 Marf
AU
Reagent Peak
Minutes
CO2H
NH2
N
H
51