Supporting Information for

Thioester-Isocyanide Reagents for the Synthesis of Cycle-Tail Peptides

Supplemental Information

Benjamin H. Rotstein,[a] David J. Winternheimer,[a] Lois M. Yin,[b] Charles M. Deber,[b] and Andrei K. Yudin*[a]

[a]*Davenport Research Laboratories, Department of Chemistry,*
University of Toronto, 80 St. George Street,
Toronto, Ontario, Canada, M5S 3H6
[b]*Division of Molecular Structure and Function, Research Institute,*
Hospital for Sick Children
Toronto, Ontario Canada, M5G 1X8

***General information*:** Anhydrous dichloromethane was obtained using the method described by Grubbs.[1] Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. 2,2,2-trifluoroethanol (TFE), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), and tris(2-carboxyethyl)phosphine hydrochloride were purchased from Oakwood Products (West Columbia, SC). All other solvents were of reagent grade quality. All other reagents were purchased from Sigma-Aldrich. Peptides were purchased from Sigma-Aldrich, synthesized by standard solution-phase chemistry, or synthesized on 2-chlorotrityl resin using an Aapptec Apex 396 automated peptide synthesizer.

Instrumentation: Infrared (IR) spectra were obtained on a Perkin-Elmer Spectrum 100 instrument equipped with a single-reflection diamond / ZnSe ATR accessory in the solid state. Spectral features are reported in wavenumber (cm^{-1}) and intensity (s-strong, m-medium, w-weak, br-broad).

Nuclear Magnetic Resonance Spectra: ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury 300 or 400 MHz spectrometers or a Bruker 400 MHz spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (ppm) relative to solvent (chloroform, 7.27 ppm; methanol, 3.31 ppm; dimethyl sulfoxide, 2.50 ppm). ^{13}C chemical shifts are reported in ppm, and referenced to solvent (chloroform, 77.0 ppm; methanol, 49.2 ppm; dimethyl sulfoxide, 39.5 ppm). Peak multiplicities are designated by the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dt, doublet of triplets; bt, broad triplets; br, broad; and *J*, coupling contant in Hz.

Mass Spectrometry: Low resolution mass spectra (ESI) were obtained at 60 eV and 100 eV on an Agilent Technologies 6130 Quadrupole LC / MS machine.

Chromatography: Flash column chromatography was performed using Silicycle 230-400 mesh silica gel. Analytical thin-layer chromatography was performed on pre-coated glass-backed plates and visualized using a UV lamp (254 nm), iodine and potassium permanganate stains. Analytical high-performance liquid chromatography (HPLC) was performed using an Agilent 1200 Series machine, and either an Agilent Poroshell 120 EC-C18 2.7 µm, 4.6 x 50 mm column or a Silicycle SiliaChrom XT C18 5 µm, 4.6 x 50 mm column. Mobile phases were acetonitrile (0.1 % formic acid) and water (0.1 % formic acid). Preparative separations were performed using a Waters Prep LC 4000 System machine, and a Silicycle, SiliaChrom XT C18 10 µm, 30 x 150 mm column. Mobile phases were acetonitrile (0.1 % TFA) and water (0.1 % TFA).

General procedures

***N*-Formylation of amino acids:**[2] An amino acid (1.0 equiv.) was dissolved in formic acid to a concentration of 1.0 M. The solution was warmed to 55 °C. Acetic anhydride (3.7 equiv.) was added dropwise to the warm solution. The reaction was then allowed to stir at room temperature for 2 hours, at which point it was diluted with water to a concentration of 0.3 M. The mixture was concentrated under reduced pressure with heating to give a white solid. This solid was suspended in diethyl ether, filtered and washed to remove residual acid.

Thioesterification of *N*-formyl amino acids

Method A: DCC coupling. Thiol (2.4 equiv.) and triethylamine (1.2 equiv.) were added to a 0.37 M solution of *N*-formyl amino acid (1.0 equiv.) in THF. The solution was cooled in an ice-water bath, and a 0.85 M solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (1.15 equiv.) in THF was added over a period of 20 minutes. The cooling bath was removed and the reaction was stirred at room temperature for 12 hours or until completion as judge by TLC. The flask was then cooled to -20 °C for 1 hour prior to filtration. The filtrate was concentrated and purified by flash chromatography (silica gel with ethyl acetate / hexanes) to give the thioester formamide.

Method B: Chloroformate coupling.[3] To a flame-dried flask equipped with a magnetic stir bar was added *N*-formyl amino acid (1.0 equiv.) and anhydrous DCM to prepare a 0.49 M solution. The flask was sealed, equipped with a nitrogen inlet, and cooled in an ice-water bath. Sequentially, isobutyl chloroformate (1.1 equiv.) and triethylamine (1.0 equiv.) were added to the flask dropwise. The flask was stirred for 10 minutes, after which thiol (2.2 equiv.) and triethylamine (1.0 equiv.) were added. The reaction was stirred for 20 minutes, after which it was diluted with diethyl ether to precipitate triethylammonium chloride. The mixture was filtered and washed with diethyl ether. The filtrate was concentrated and purified by flash chromatography (silica gel with ethyl acetate / hexanes) to give the thioester formamide.

Method C: Hydroxybenzotriazole coupling.[4] To a 0.23 M solution of *N*-formyl amino acid (1.0 equiv.) in anhydrous DCM were sequentially added 6-chloro-1-hydroxybenzotriazole (1.0 equiv.), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC HCl, 1.0 equiv.), and diisopropylethylamine (1.0 equiv.). The solution was stirred for 90 minutes before addition of thiol (2.4 equiv.). The solution was then stirred for 4 h at room temperature. The reaction mixture was then washed with 1 M $HCl_{(aq)}$, 1 % $NaHCO_{3(aq)}$, and water, dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography (silica gel with ethyl acetate / hexanes) to give the thioester formamide.

Synthesis of thioester isocyanides: Triethylamine (2.5 equiv.) was added to a 0.425 M solution of thioester formamide (1.0 equiv.) in anhydrous DCM. The solution was cooled in an ice-water bath and phosphorus oxychloride (1.0 equiv.) was added to the solution dropwise over 10 minutes. The ice-water bath was removed and the reaction was stirred for 1 hour at room temperature. Sodium carbonate (1.1 equiv.) was dissolved in water to give a 0.5 M solution that was added to the reaction mixture, which was then stirred for 30 minutes. The organic layer was separated and the aqueous layer was extracted 3 times with DCM. The combined organic layers were dried with anhydrous sodium sulfate, filtered, concentrated and purified by flash chromatography (silica gel with ethyl acetate / hexanes and triethylamine) to give the thioester isocyanide.

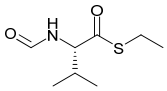
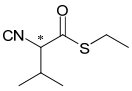
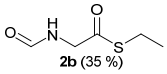
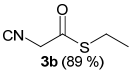
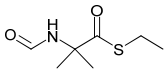
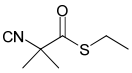
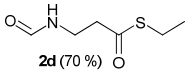
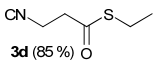
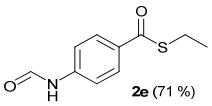
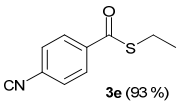
Macrocyclization of peptides using aziridine aldehyde and thioester isocyanide: Thioester isocyanide (1.1 equiv.) was added to a 50 mM solution of peptide (1.0 equiv.) with aziridine aldehyde dimer (0.6 equiv.) in 2,2,2-trifluoroethanol or 1,1,1,3,3,3-hexafluoro-2-propanol. The reaction was stirred for 3-6 hours or until >90 % consumption of starting peptide was determined by LCMS. The solvent was then evaporated via a stream of nitrogen or *in vacuo*. The residue was triturated with hexanes and diethyl ether before being purified by RP-HPLC.

Native chemical ligation: Thiophenol (10 equiv.) was added to a 4 mM solution of thioester peptide macrocycle (1.0 equiv.) with *N*-terminal cysteine peptide (2.0 equiv.), and tris(2-carboxyethyl)phosphine hydrochloride (20 equiv.) in Dulbecco's phosphate buffered saline. The pH was adjusted to ~7.5 using a 50 % aqueous solution of NaOH and 1 M aqueous hydrochloric acid. The reaction was stirred until full conversion, as measured by LCMS. The product was purified by RP-HPLC.

Aziridine ring opening using thiobenzoic acid: Thiobenzoic acid (4 equiv.) was added to a 0.1 M solution of aziridine containing macrocycle in chloroform with methanol present if necessary for solubility. The reaction was stirred for 1 h, at which point the reaction was judged by LCMS to be complete. The reaction was concentrated *in vacuo* and triturated with hexanes and diethyl ether.

Desulfurization: A slurry of Raney nickel (~1 mL / mmol peptide) was added to a 0.1 M solution of macrocyclic peptide in methanol. The reaction was stirred for 16 h, before filtration through a pad of Celite®, washing with methanol. The product was purified by RP-HPLC.

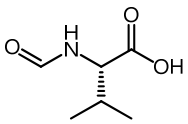
Table S1 Preparation of thioester formamides and thioester isocyanides.

Thioester formamide (**2**) (yield)[a]	Thioester isocyanide (**3**) (yield)[b]
 **2a** (68 %)	 **3a** (82 %)
 **2b** (35 %)	 **3b** (89 %)
 **2c** (91 %)	 **3c** (69 %)
 **2d** (70 %)	 **3d** (85 %)
 **2e** (71 %)	 **3e** (93 %)

[a] Isolated after two reactions from amino acids. [b] Isolated from dehydration of **2**. Conditions: $POCl_3$, NEt_3, DCM, 0 °C to rt, 1 h.

Characterization of isocyanides and precursors

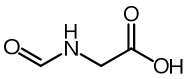
Note: Many of these compounds exhibit resolved rotamers in their proton and carbon spectra. Where possible, the chemical shifts have been assigned using the following notations: min. rot. (minor rotamer) and maj. rot. (major rotamer).



_N_-formyl valine (1a)

Yield: 95 % (9.5 mmol)

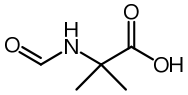
^{1}H NMR (400 MHz, DMSO) δ 12.69 (br s, 1H), 8.30 (d, *J* = 8 Hz, 1H), 8.07 (s, 1H), 4.21 (ddd, *J* = 12, 8, 2 Hz, 1H, maj. rot.), 3.38 (q, *J* = 8 Hz, 1H, min. rot.), 2.07 (m, 1H, maj. rot.), 1.09 (t, *J* = 8 Hz, 1H, min. rot.), 0.87 (app. t, 6H). ^{13}C NMR (100 MHz, DMSO) δ 173.3, 161.8, 56.2, 30.5, 19.8, 18.3.



_N_-formyl glycine (1b)

Yield: 87 % (87 mmol)

^{1}H NMR (400 MHz, DMSO) δ 8.30 (br s, 1H), 8.13 (s, 1H, maj. rot.), 8.07 (s, 1H, min. rot.), 3.84 (d, *J* = 8 Hz, 2H, min. rot.), 3.79 (d, *J* = 8 Hz, 2H, maj. rot.). ^{13}C NMR (100 MHz, DMSO) δ 170.9, 161.4, 39.2 (obscured by solvent).

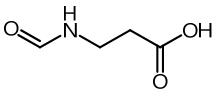


_N_-formyl 2-aminoisobutyric acid (1c)

Hexanes was substituted for diethyl ether during the workup of this product.

Yield: >99 % (50 mmol)

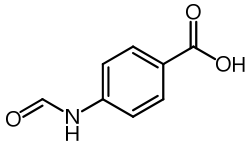
^{1}H NMR (300 MHz, DMSO) δ 8.25 (br s, 1H), 7.88 (s, 1H), 1.36 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 175.2, 160.4, 54.4, 24.8.



_N_-formyl β-alanine (1d)

Yield: 99 % (100 mmol)

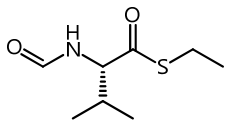
^{1}H NMR (400 MHz, DMSO) δ 8.07 (br s, 1H), 7.96 (s, 1H), 3.27 (apparent q, *J* = 8 Hz, 2H), 2.37 (t, *J* = 8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 172.9, 161.2, 33.8, 33.4.



_N_-formyl *para*-aminobenzoic acid (1e)

Yield: 95 % (20.8 mmol)

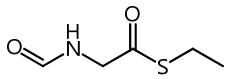
^{1}H NMR (300 MHz, DMSO) δ 10.45 (s, 1H, maj. rot.), 10.24 (s, 1H, min. rot.), 8.94 (d, *J* = 12 Hz, 1H, min. rot.) 8.33 (s, 1H, maj. rot.), 7.89 (apparent t, *J* = 9 Hz, 2H, both rot.), 7.68 (d, *J* = 9 Hz, 2 H, maj. rot.), 7.28 (d, *J* = 9 Hz, 2H, min. rot). ^{13}C NMR (75 MHz, DMSO) δ 166.9, 160.2, 142.2, 130.6, 125.6, 118.6.



_S_-ethyl _N_-formyl valine thioester (2a)

Method A. Yield: 72 % (5.42 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H, maj. rot.), 8.02 (d, 1H, min. rot.), 6.30 (br s, 1H), 4.74 (ddd, *J* = 8, 4, 1 Hz, 1H, maj. rot.), 3.94 (dd, *J* = 8, 4 Hz), 2.90 (m, 2H), 2.28 (m, 1H), 1.25 (t, *J* = 8 Hz, 3H), 0.99 (d, *J* = 8 Hz, 3H), 0.89 (d, *J* = 8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6, 161.2, 62.4, 31.7, 23.7, 19.6, 17.1, 14.7.



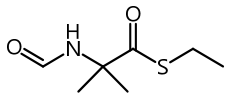
_S_-ethyl _N_-formyl glycine thioester (2b)

Method A. Yield: 22 % (1.05 mmol)

Method B. Yield: 40 % (7.79 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.24 (s, 1H, min. rot.), 6.16 (br s, 1H) 4.28 (d, *J* = 6 Hz, 2H), 2.97 (q, *J* = 9 Hz, 2H), 1.29 (t, *J* = 9 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.9, 160.8, 47.6, 23.3, 14.5.

R_f 0.12 (50 % ethyl acetate / hexanes)



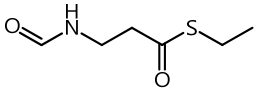
_S_-ethyl _N_-formyl 2-aminoisobutyrothioate (2c)

Method A. Yield: 44 % (2.25 mmol)

Method B. Yield: 91 % (63.9 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, min. rot.), 7.96 (s, 1H, maj. rot.), 2.73 (q, *J* = 8 Hz, 2H), 1.43 (s, 6H), 1.11 (t, *J* = 9 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 163.8 (min. rot.), 161.0 (maj. rot.), 62.4 (min. rot.), 61.93 (maj. rot.), 26.2 (min. rot.), 25.1 (maj. rot.) 23.0, 14.1.

R_f 0.24 (50 % ethyl acetate / hexanes)



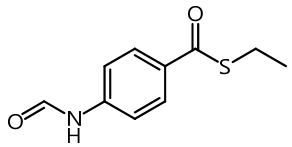
_S_-ethyl _N_-formyl β-alanine thioester (2d)

Method A. Yield: 68 % (32.9 mmol)

Method B. Yield: 70 % (69.7 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 6.25 (br s, 1H), 3.57 (apparent q, *J* = 8 Hz, 2H), 2.88 (apparent q, *J* = 8 Hz, 2H), 2.80 (t, *J* = 8 Hz, 2H), 1.24 (t, *J* = 8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.6, 161.1, 44.7 (min. rot.), 43.0 (maj. rot.), 37.4 (min. rot.), 33.8 (maj. rot.), 23.4, 14.6.

R_f 0.18 (50 % ethyl acetate / hexanes)

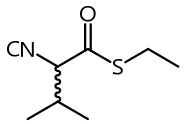


_S_-ethyl _N_-formyl _para_-aminobenzothioate (2e)

Method C. Yield: 75 % (2.28 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (br s, 1H, maj. rot.), 8.42 (m, 1H, maj. rot.), 8.25 (br s, 1H, min. rot.) 8.14 (br s, 1H, min. rot.), 7.94 (m, 2H, both rot.), 7.65 (m, 2 H, maj. rot.), 7.16 (d, *J* = 8 Hz, 2H, min. rot) 3.06 (m, 2H), 1.34 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1, 162.0 (min. rot.), 159.3 (maj. rot.), 141.4, 133.2, 128.4, 119.2 (maj. rot.), 117.2 (min. rot.), 23.4, 14.7.

R_f 0.38 (50 % ethyl acetate / hexanes)

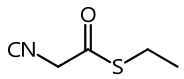


_S_-ethyl 2-isocyano-2-isopropylethanethioate (3a)

Yield: 82 % (0.19 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (d, *J* = 4 Hz, 1H), 2.96 (q, *J* = 8 Hz, 2H), 2.40 (br m, 1H) 1.30 (t, *J* = 8 Hz, 3H), 1.12 (d, *J* = 4 Hz, 3H), 1.00 (d, *J* = 4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.8, 161.3, 62.1, 31.7, 23.9, 19.5, 16.1, 14.2.

R_f 0.83 (50 % ethyl acetate / hexanes)

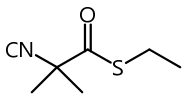


_S_-ethyl 2-isocyanoethanethioate (3b)

Yield: 89 % (3.02 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32 (s, 2H), 3.01 (q, *J* = 8 Hz, 2H), 1.32 (t, *J* = 8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 156.6, 50.6, 23.9, 14.3.

R_f 0.80 (50 % ethyl acetate / hexanes)

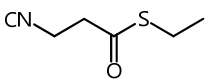


_S_-ethyl 2,2-dimethyl-2-isocyanoethanethioate (3c)

Yield: 69 % (13.8 mmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (q, *J* = 8 Hz, 2H), 1.63 (s, 6H), 1.28 (t, *J* = 8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 161.0, 65.9, 27.7, 24.0, 14.0.

R_f 0.88 (50 % ethyl acetate / hexanes)

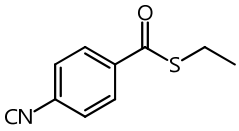


_S_-ethyl 3-isocyanopropanethioate (3d)

Yield: 85 % (17.1uu mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.69 (t, *J* = 6 Hz, 2H), 2.92 (m, 4H), 1.25 (t, *J* = 6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.3, 156.0, 42.8, 37.3, 23.8, 14.8.

R_f 0.70 (50 % ethyl acetate / hexanes)



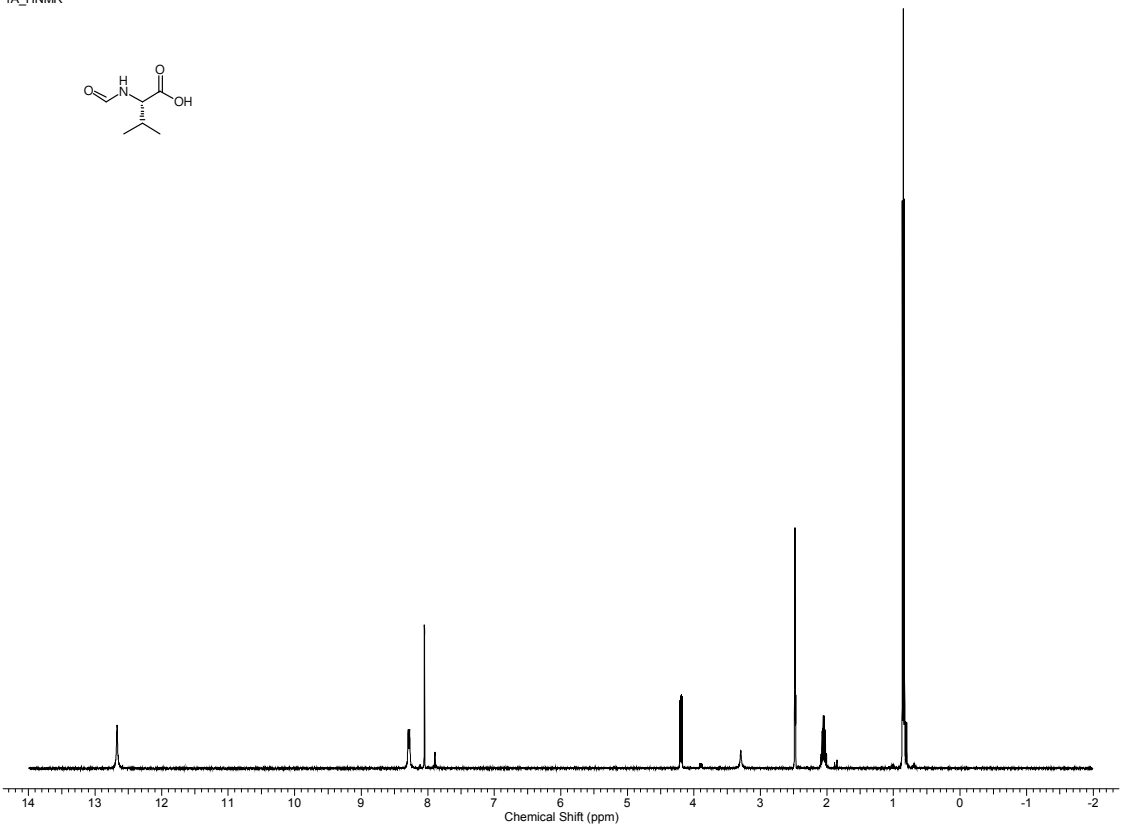
_S_-ethyl 4-isocyanobenzothioate (3e)

Yield: 93 % (2.12 mmol)

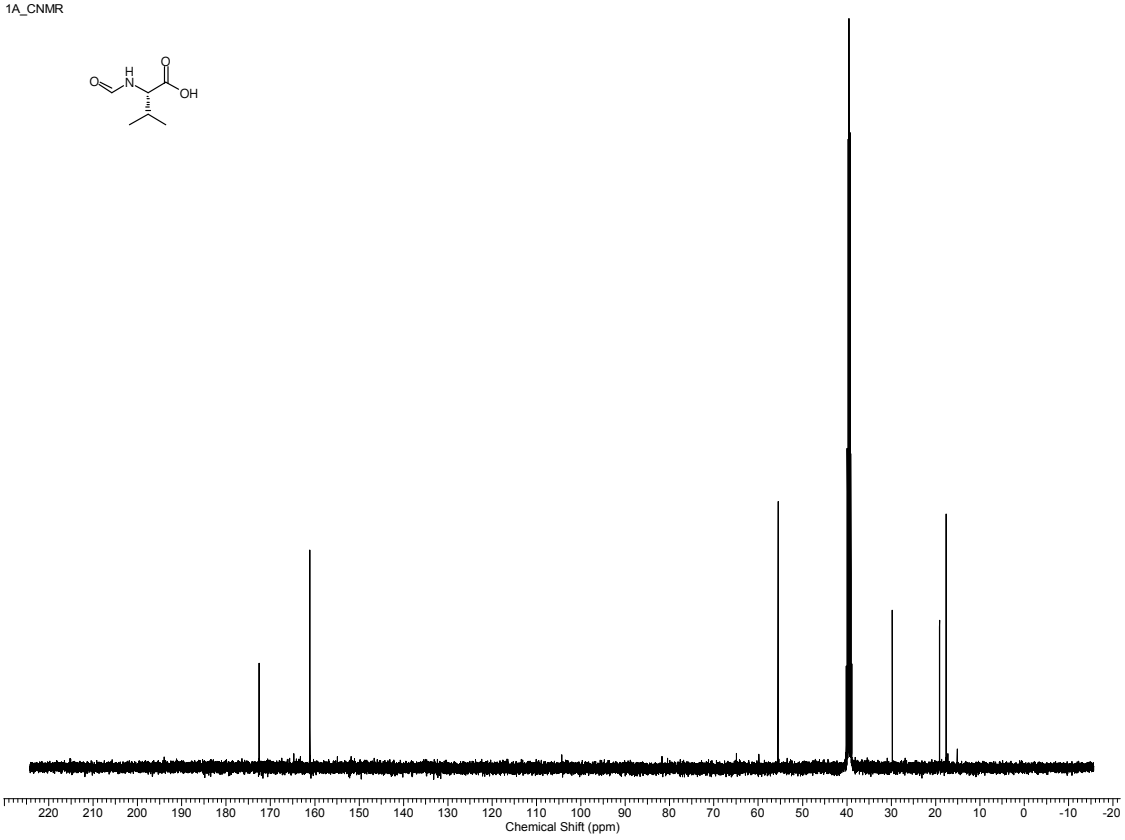
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8 Hz, 2H), 7.47 (d, *J* = 8 Hz, 2H), 3.11 (q, *J* = 8 Hz, 2H), 1.37 (t, *J* = 8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.5, 167.2, 149.4, 137.5, 128.3, 126.6, 23.8, 14.6.

R_f 0.84 (25 % ethyl acetate / hexanes)

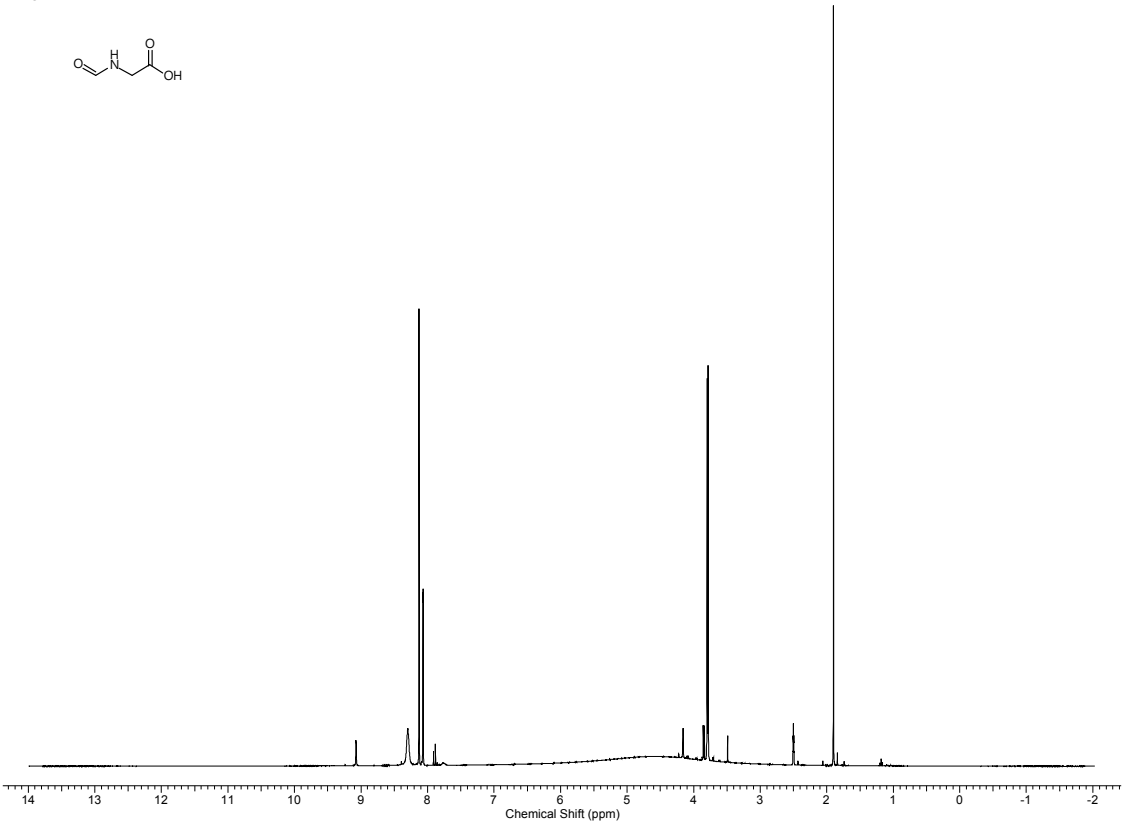
1A_HNMR



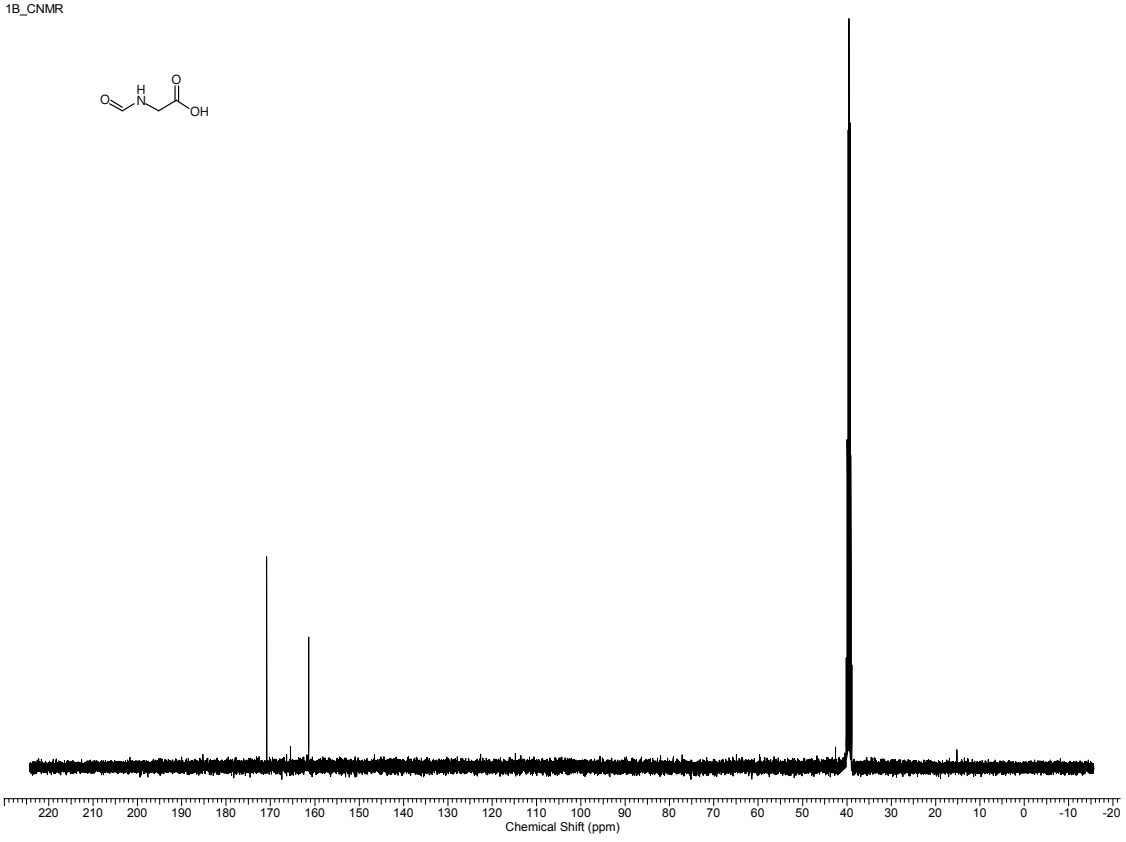
1A_CNMR

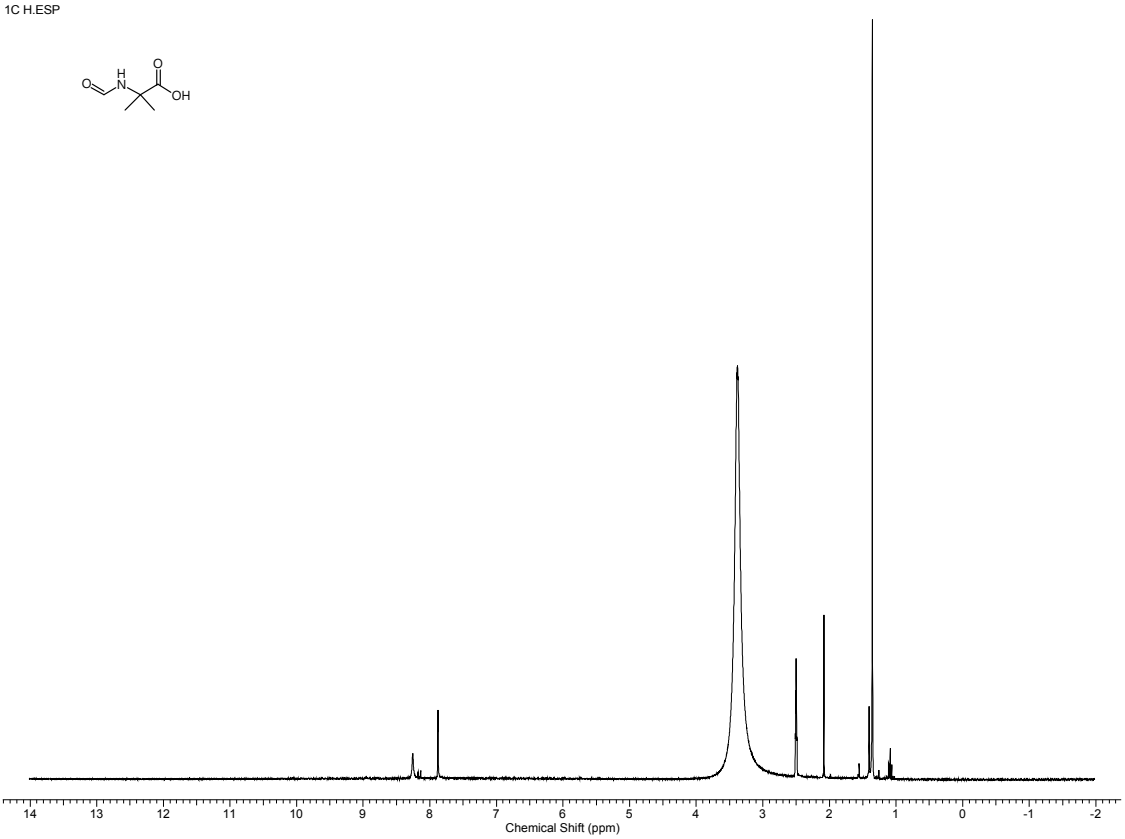


1B.ESP



1B_CNMR

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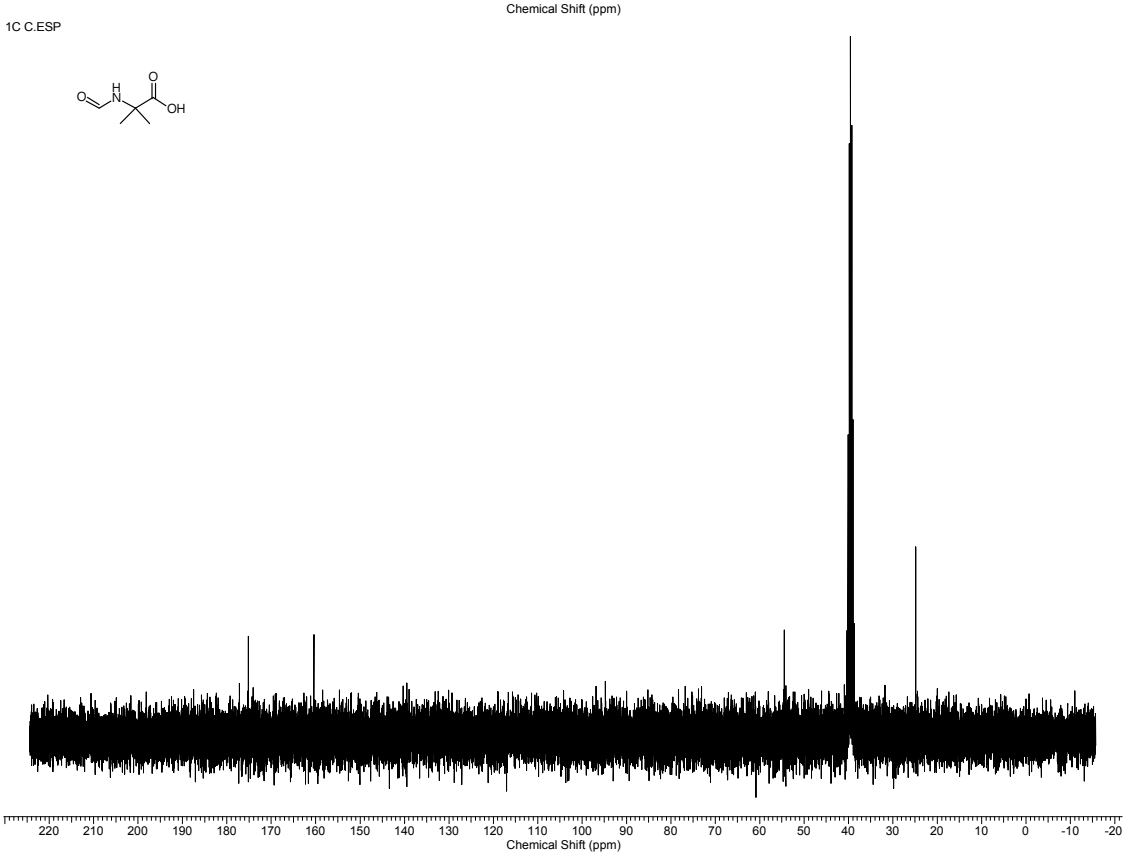
O
H
N
O
OH
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
Chemical Shift (ppm)


1C C.ESP
O
H
N
O
OH
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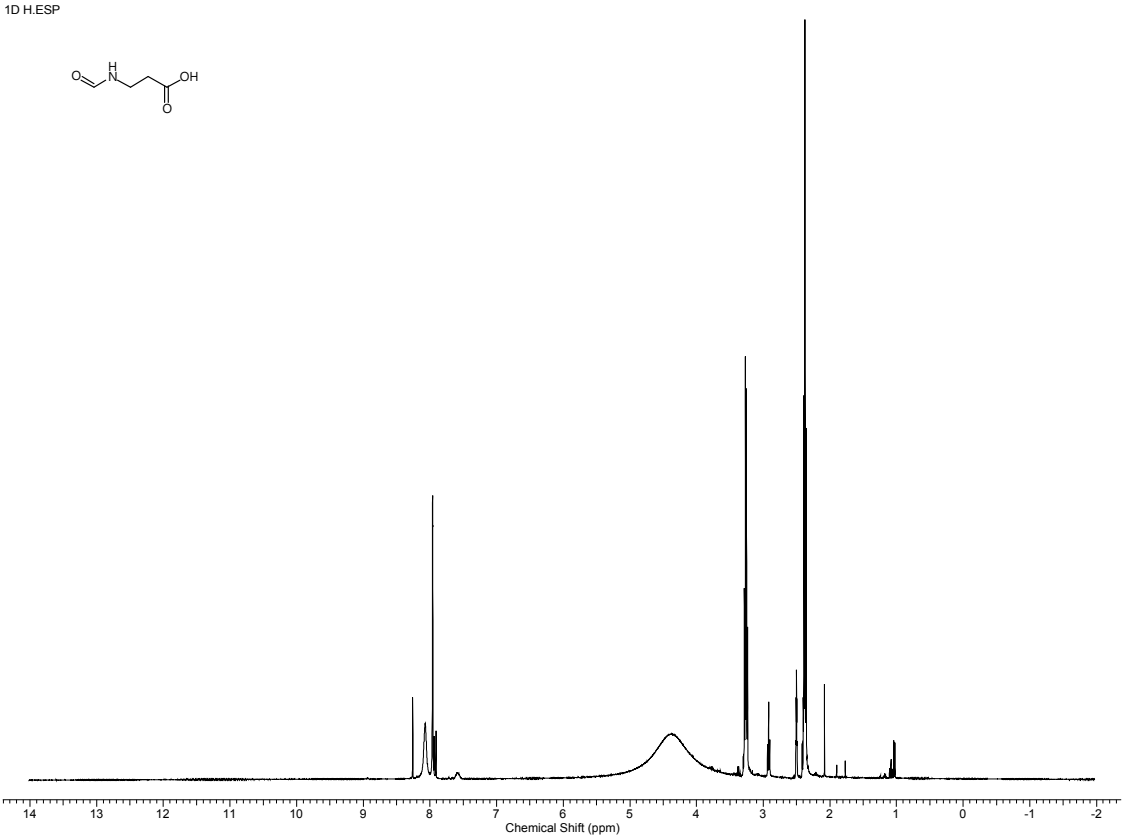
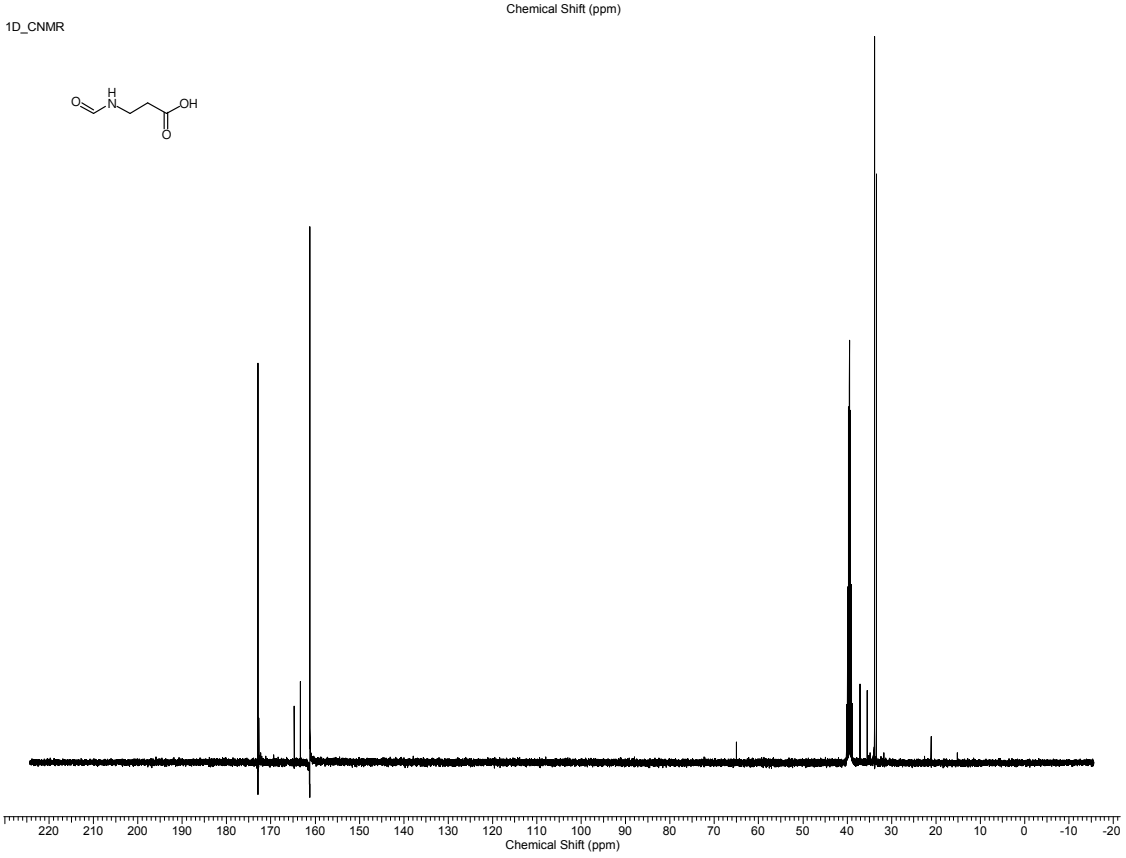
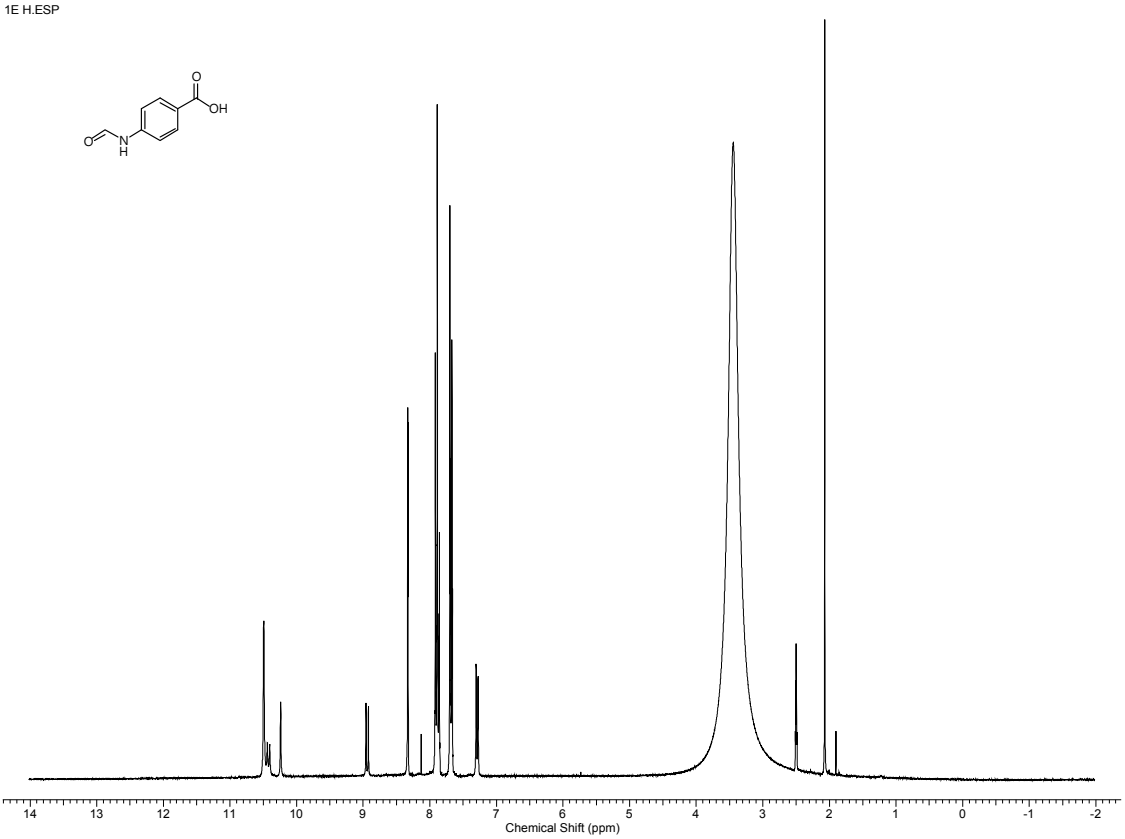
Chemical Shift (ppm)


1D H.ESP
O
H
N
OH
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
Chemical Shift (ppm)


1D_CNMR
O
H
N
OH
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)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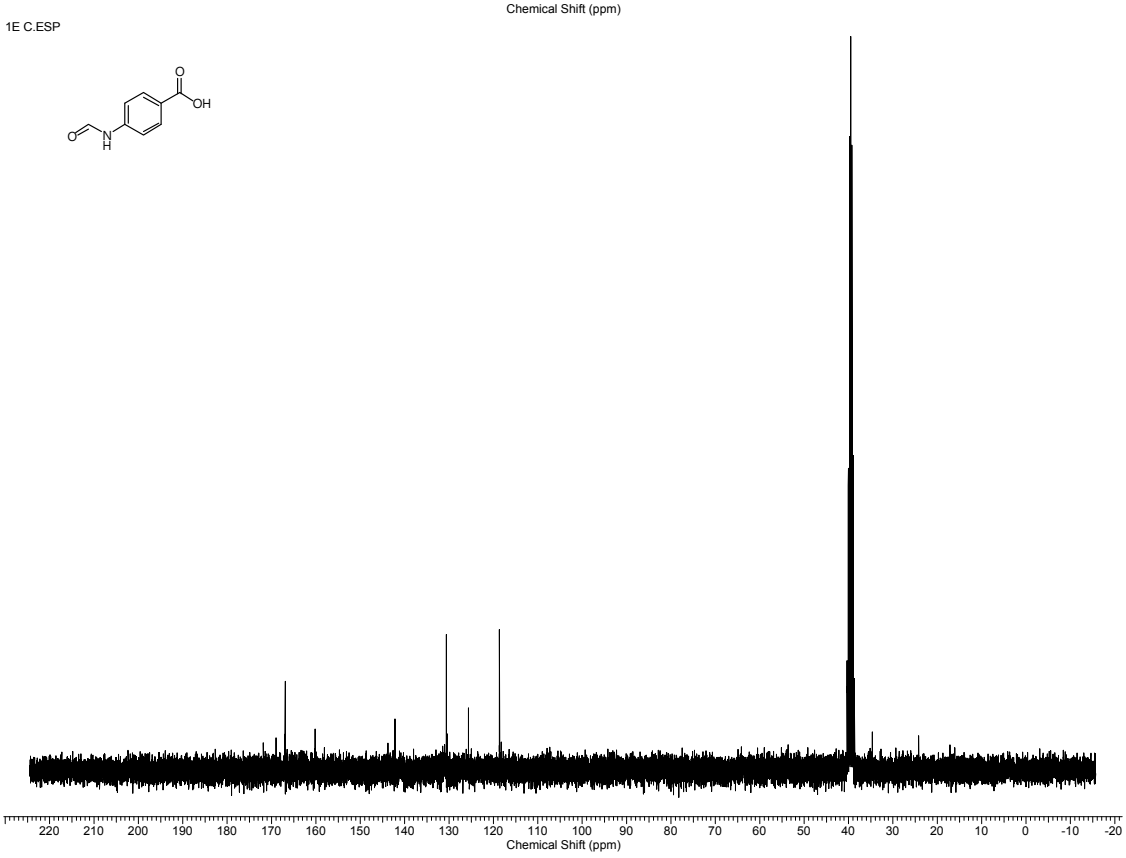
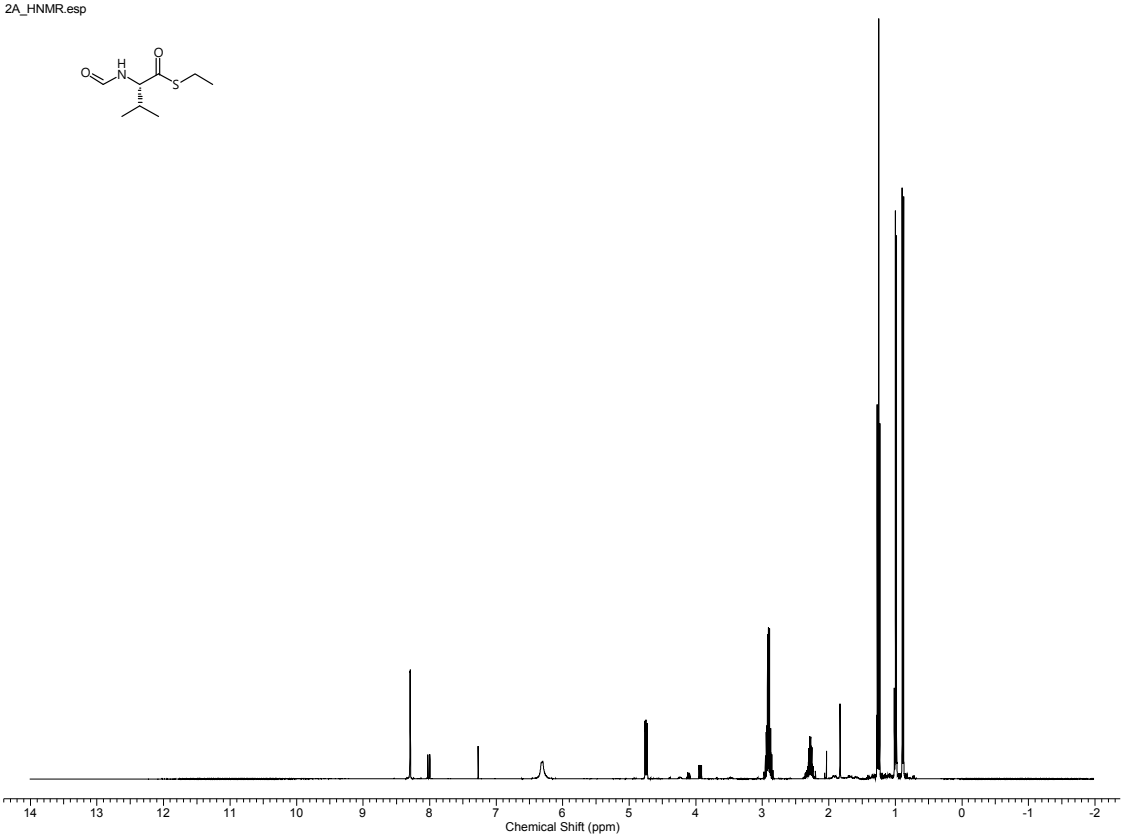
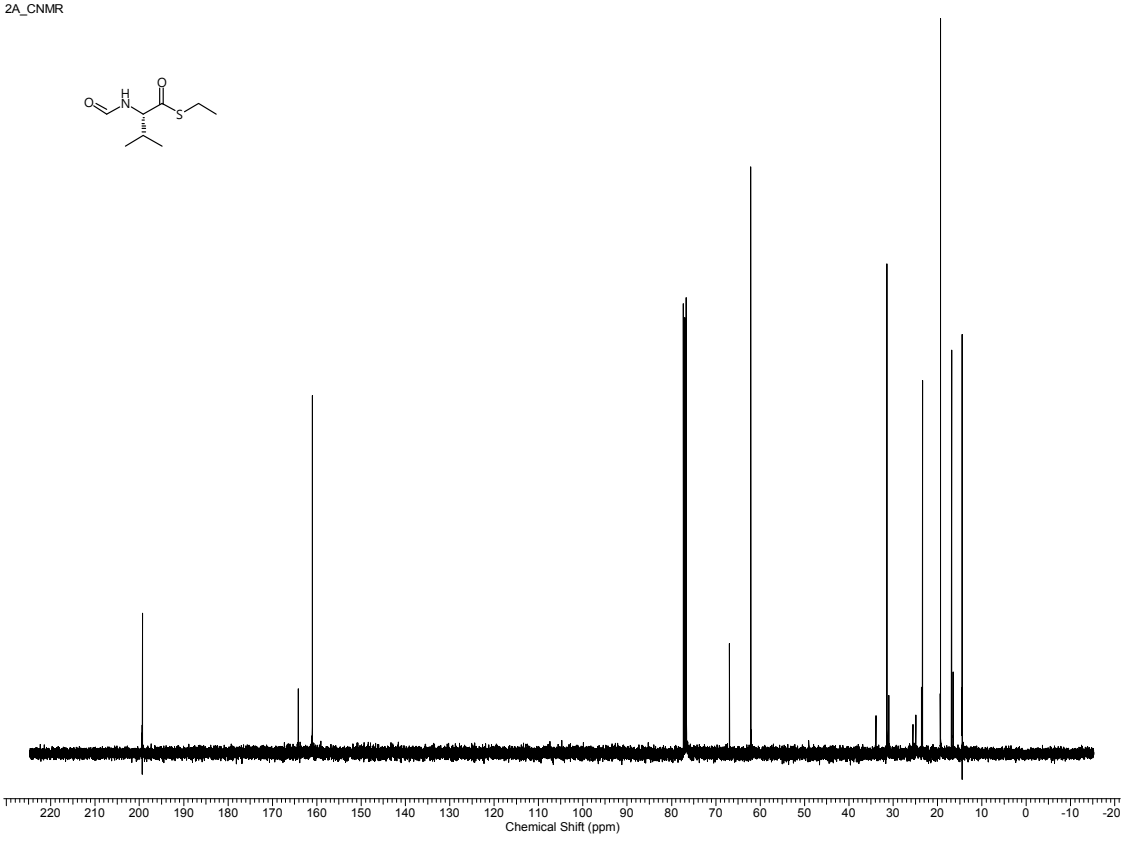
O
OH
O
N
H
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
Chemical Shift (ppm)

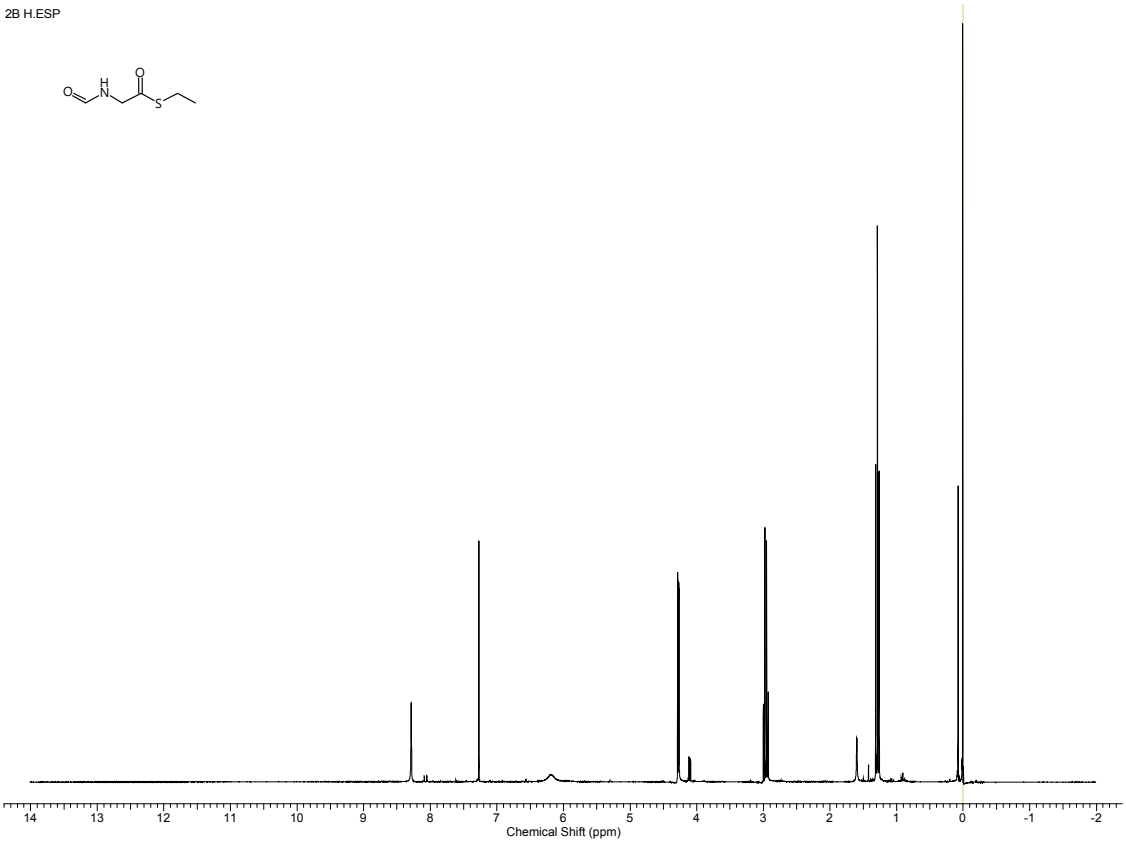

1E C.ESP
O
OH
O
N
H
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

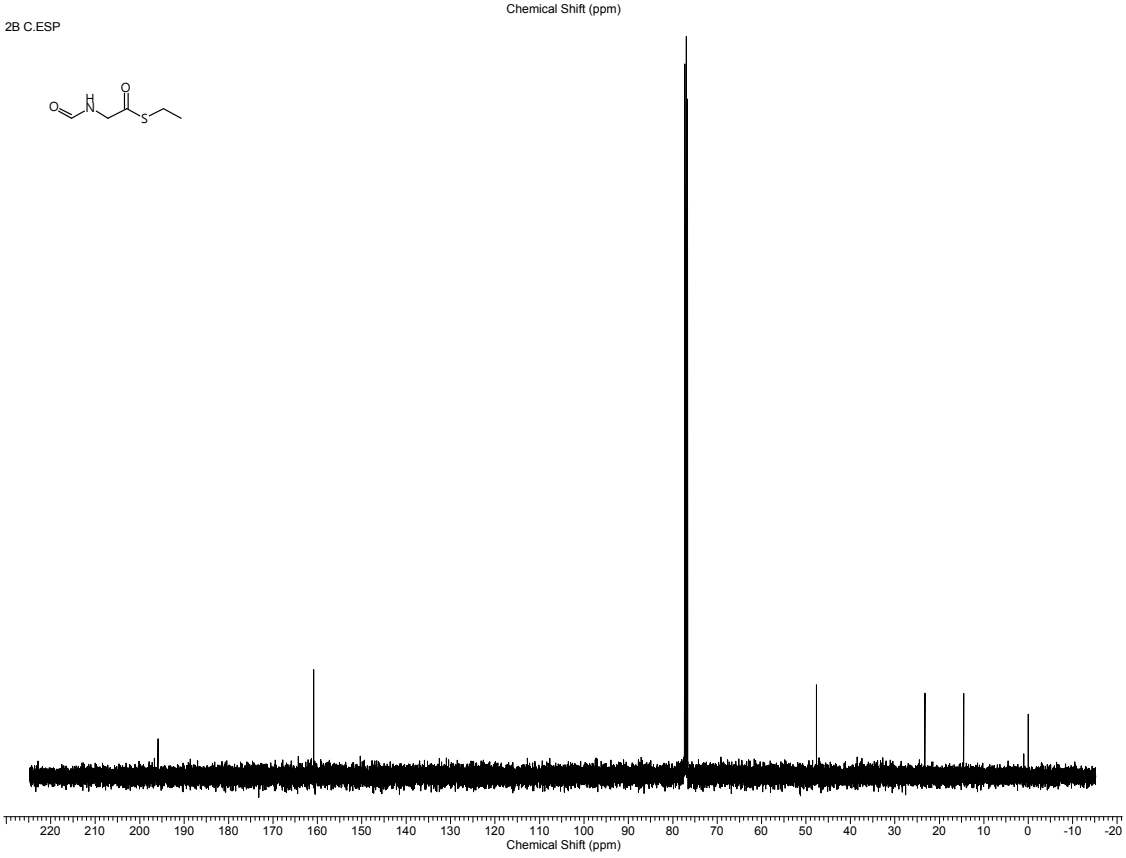
2A_HNMR.esp



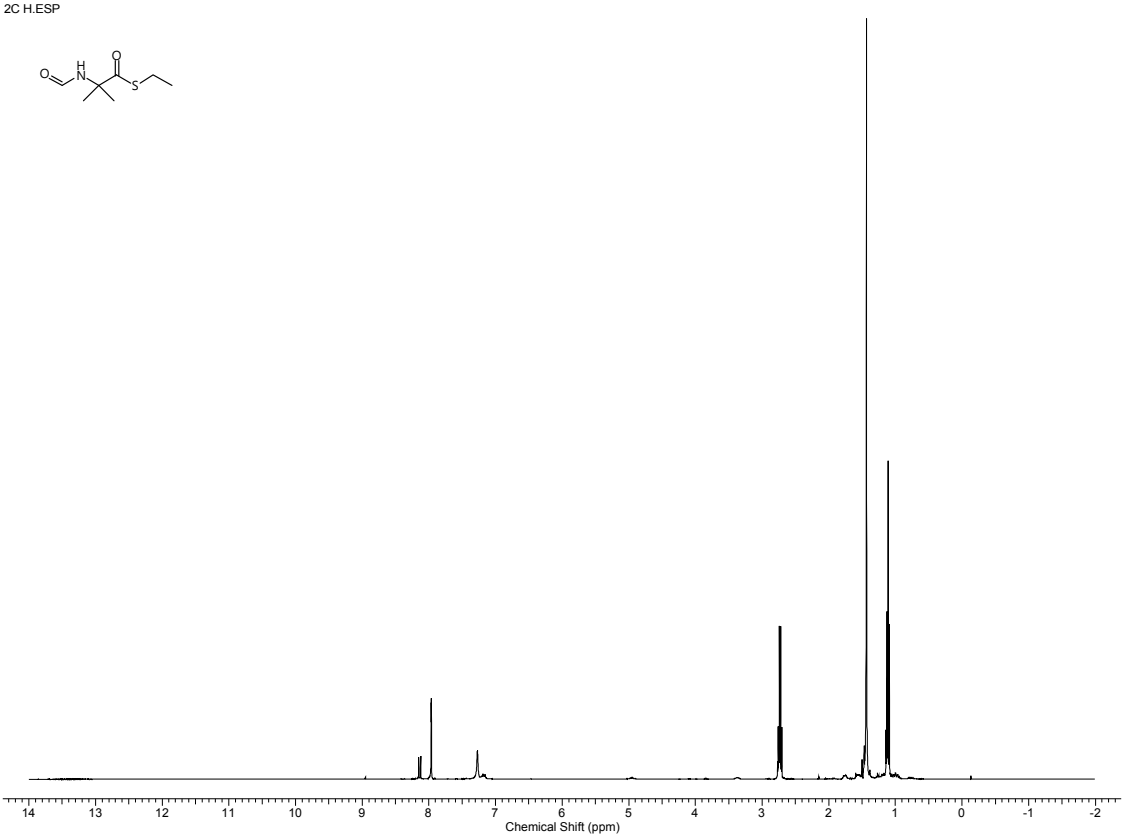
2A_CNMR



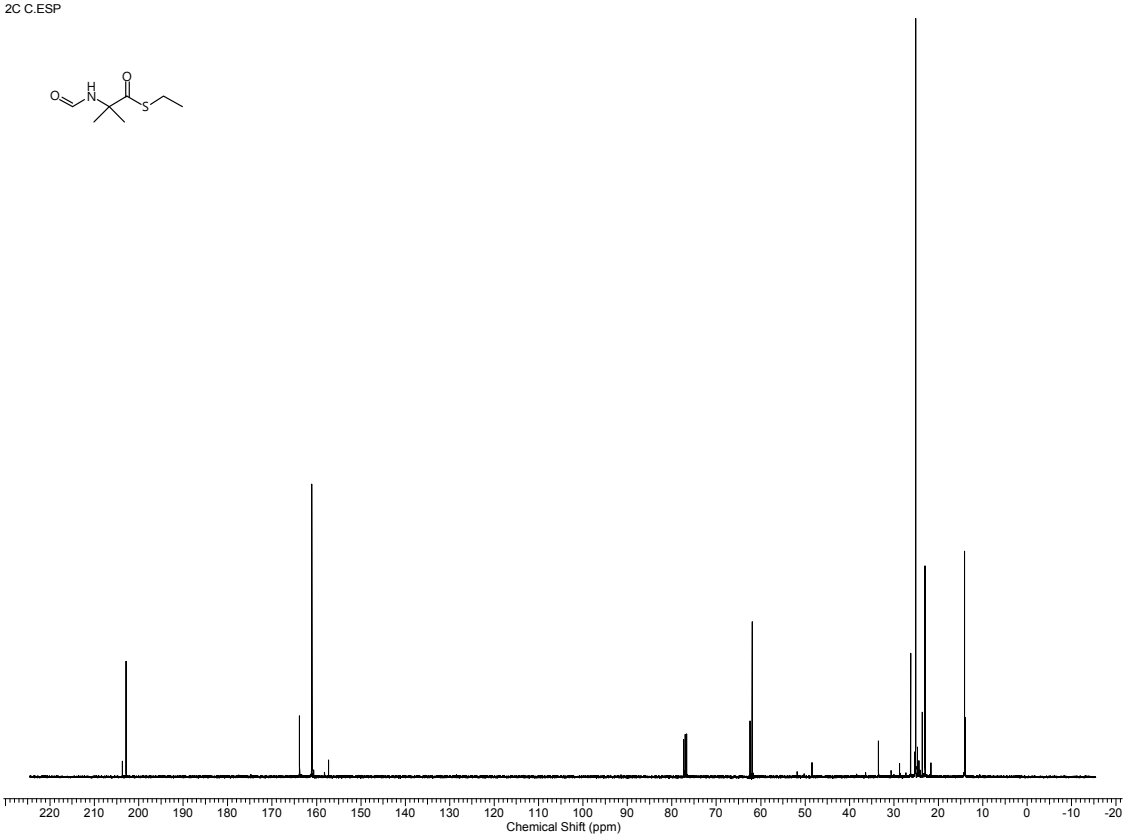

2B H.ESP
Chemical Shift (ppm)

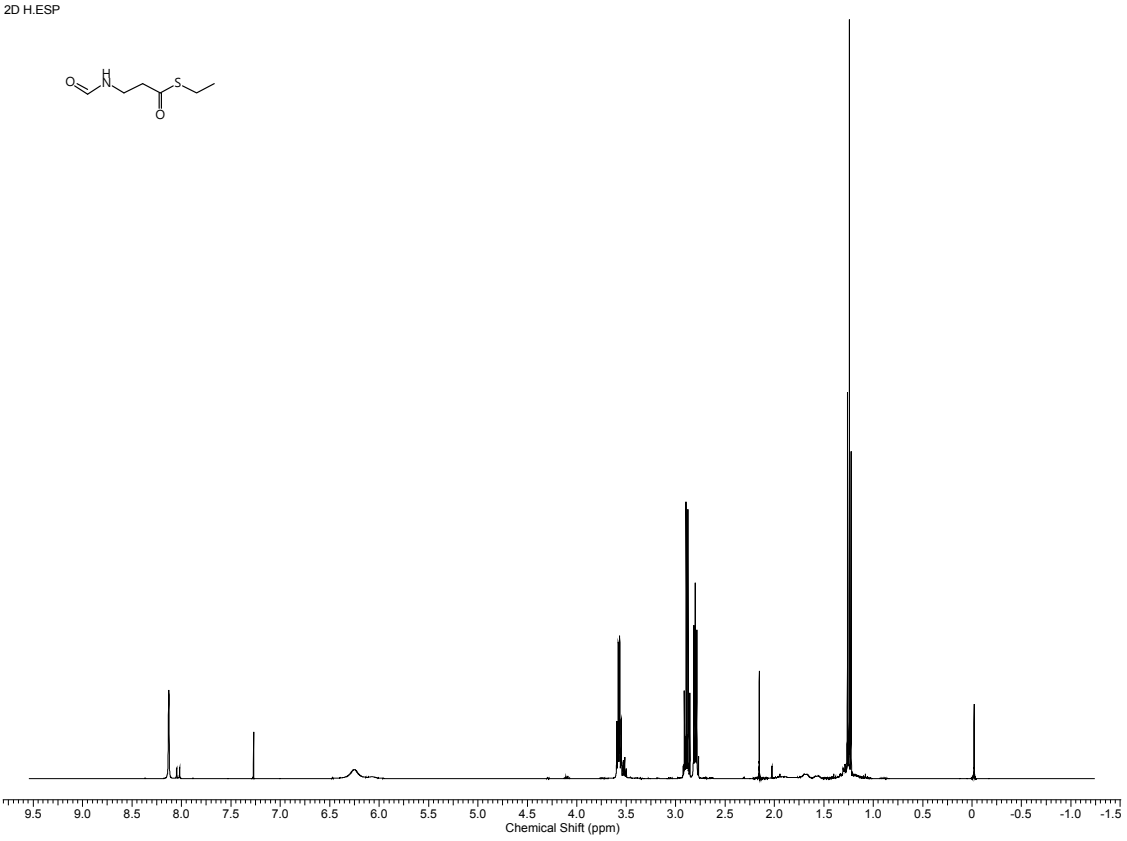

2B C.ESP
Chemical Shift (ppm)

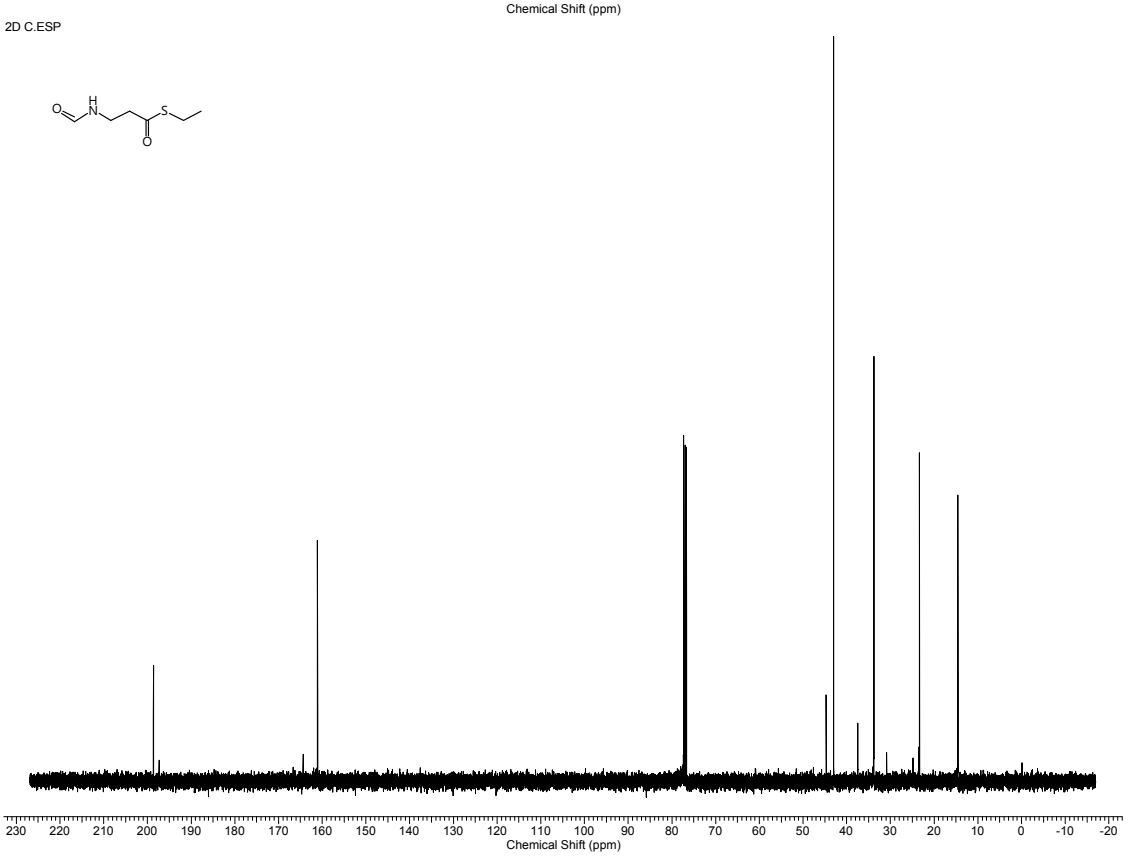
2C H.ESP

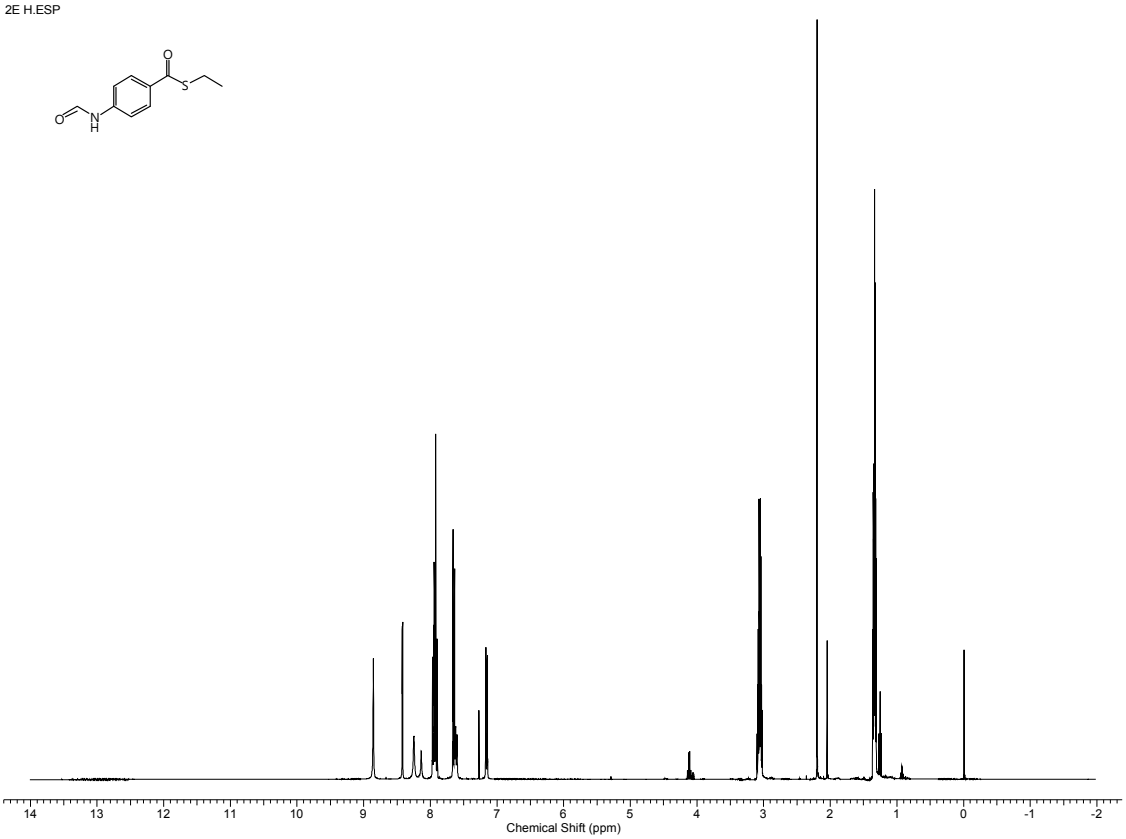


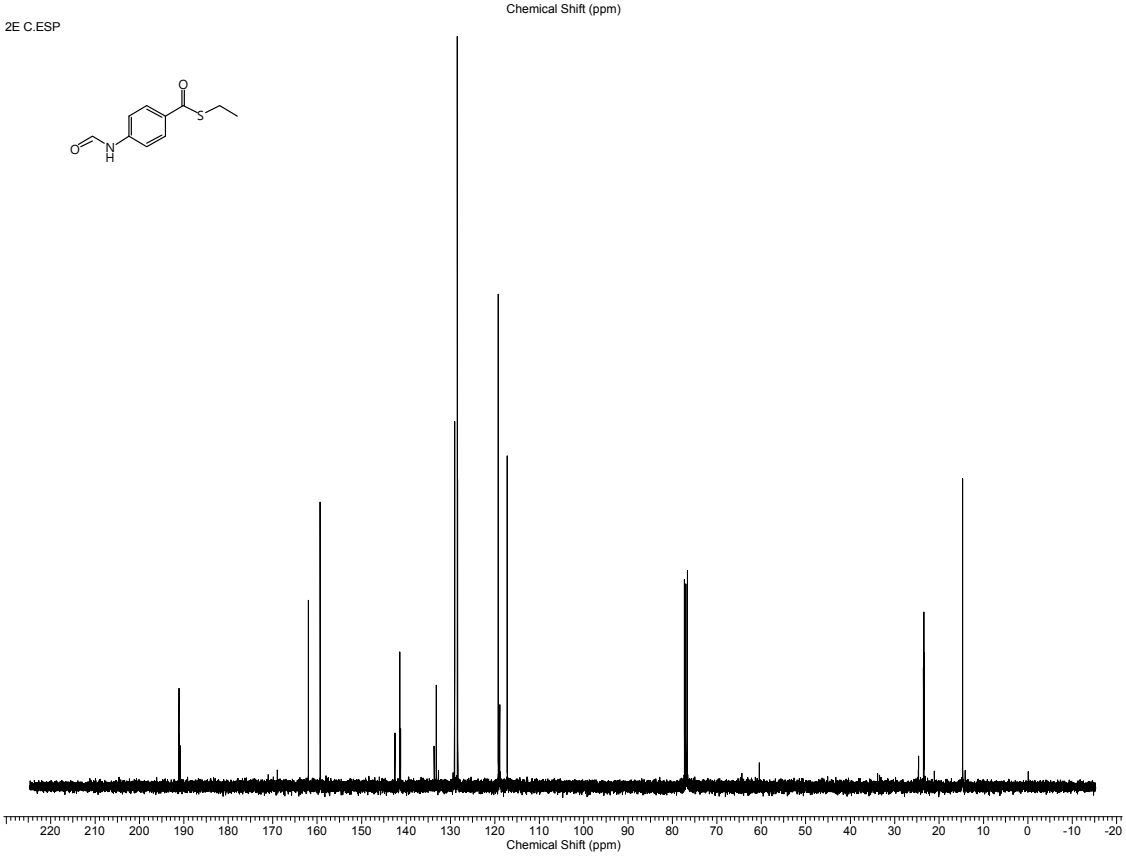
2C C.ESP

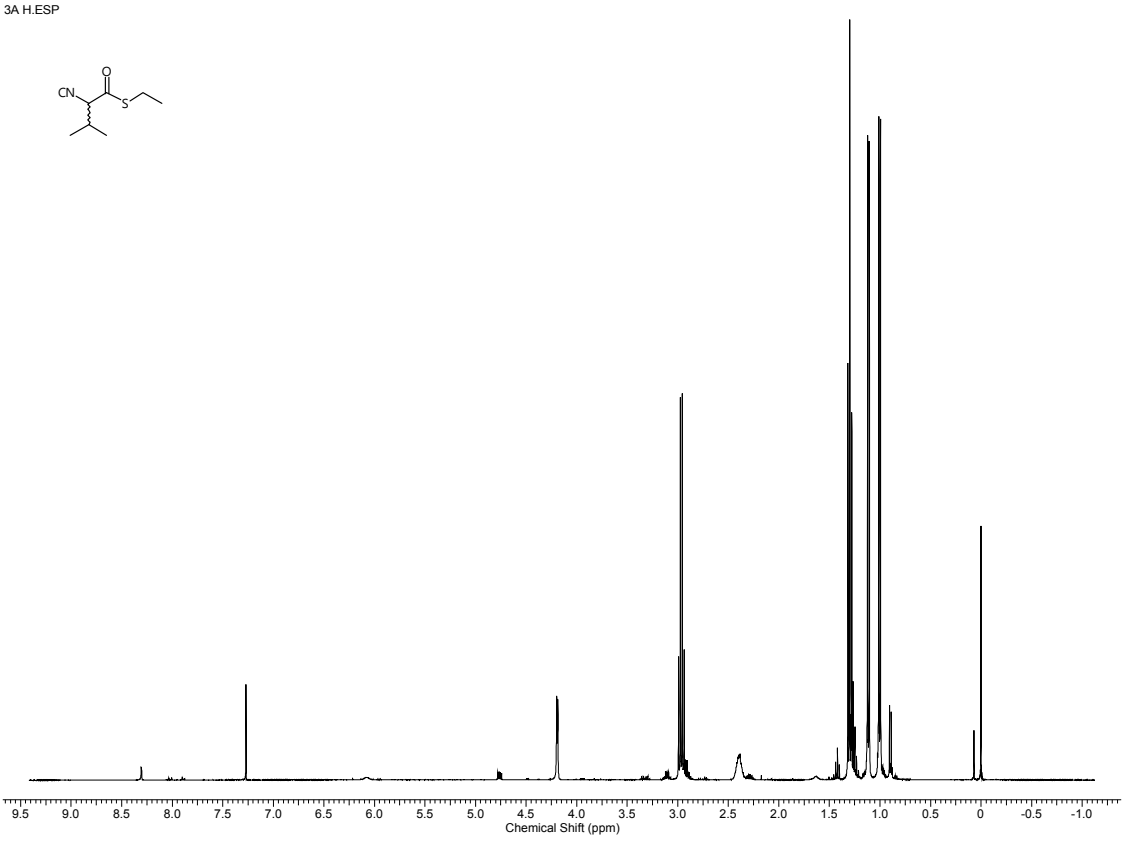


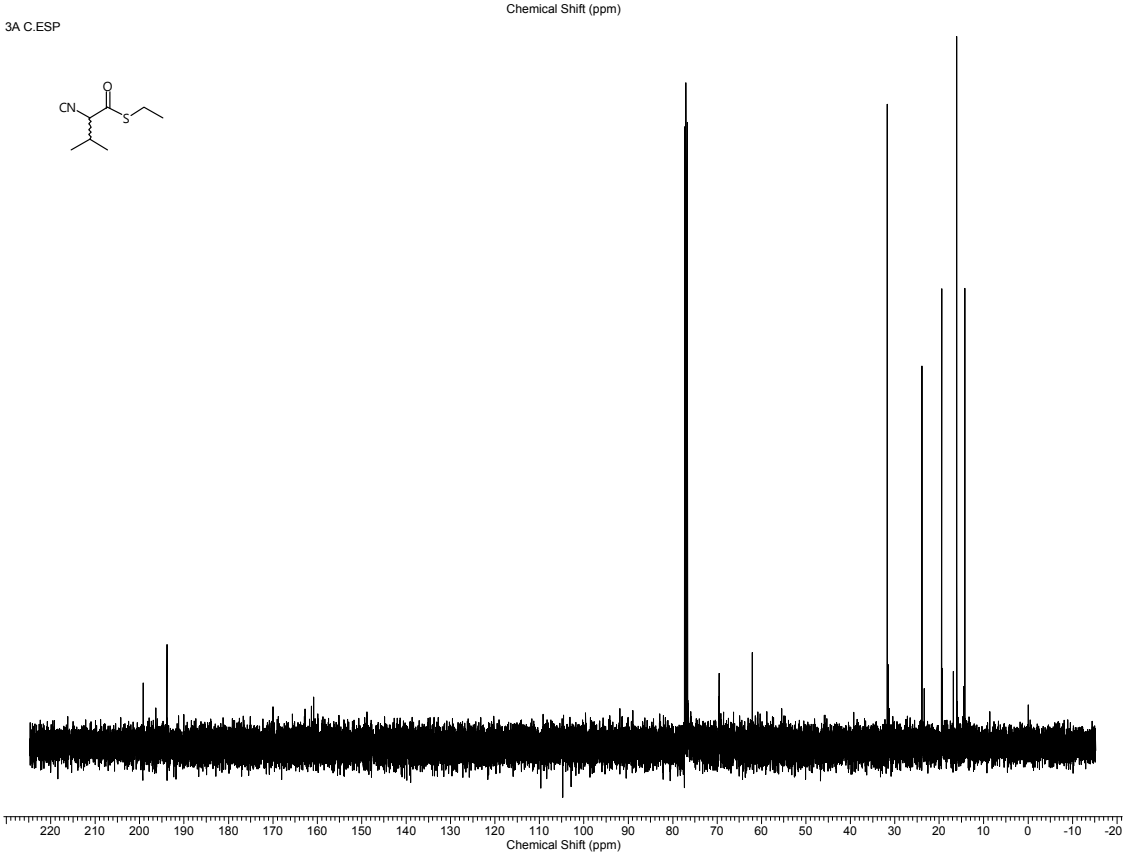

2D H.ESP
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5 -1.0 -1.5
Chemical Shift (ppm)

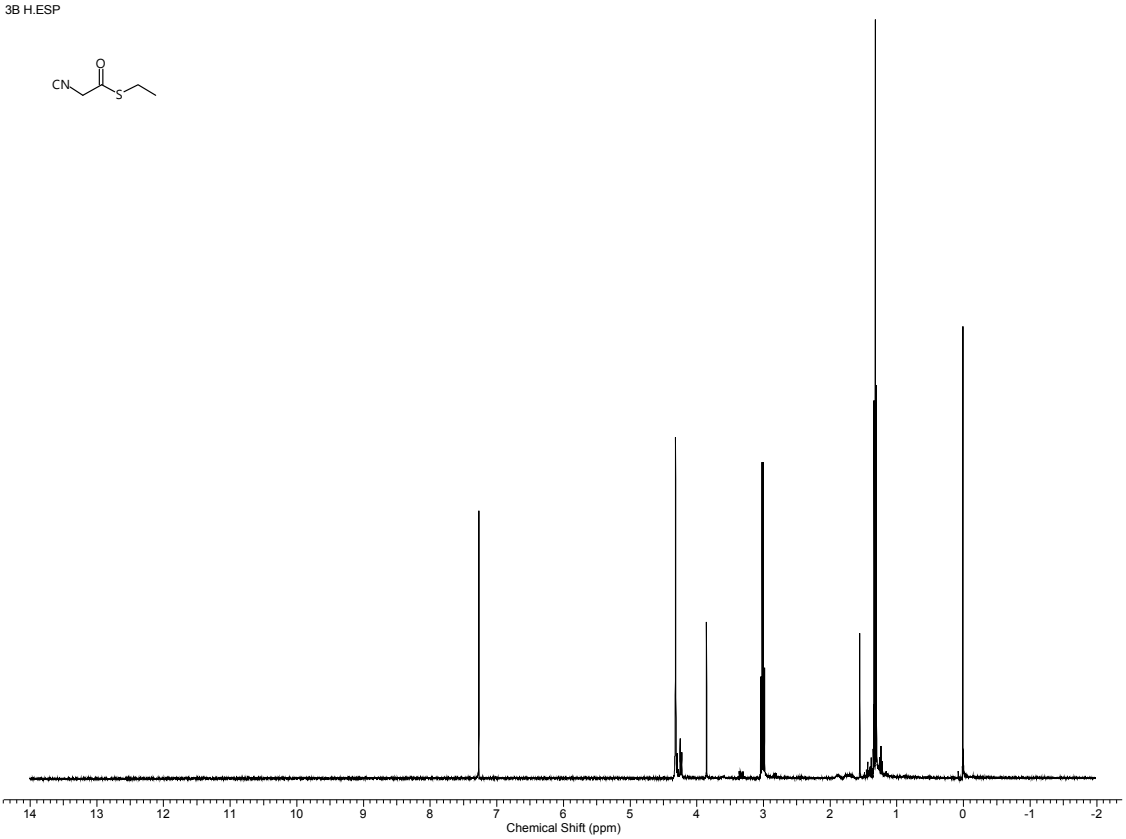

2D C.ESP
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

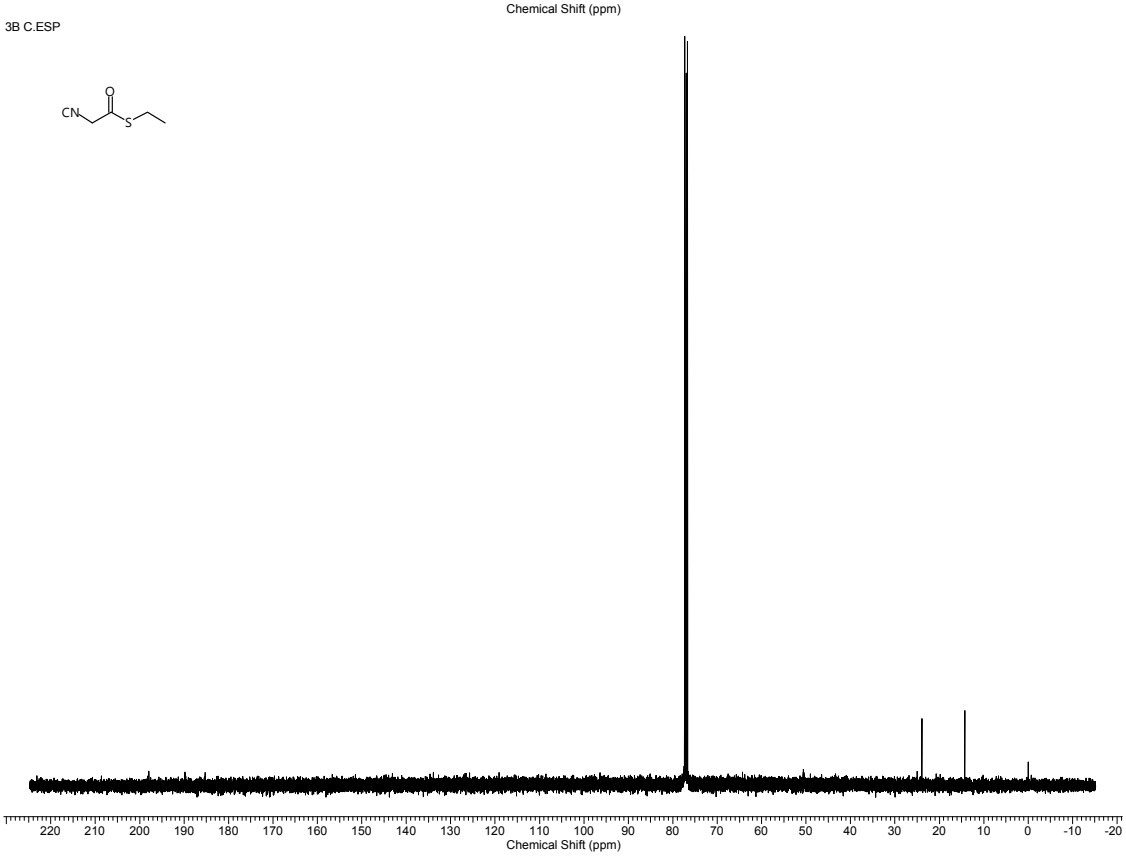

2E H.ESP
O
S
O
N
H
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
Chemical Shift (ppm)


2E C.ESP
O
S
O
N
H
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

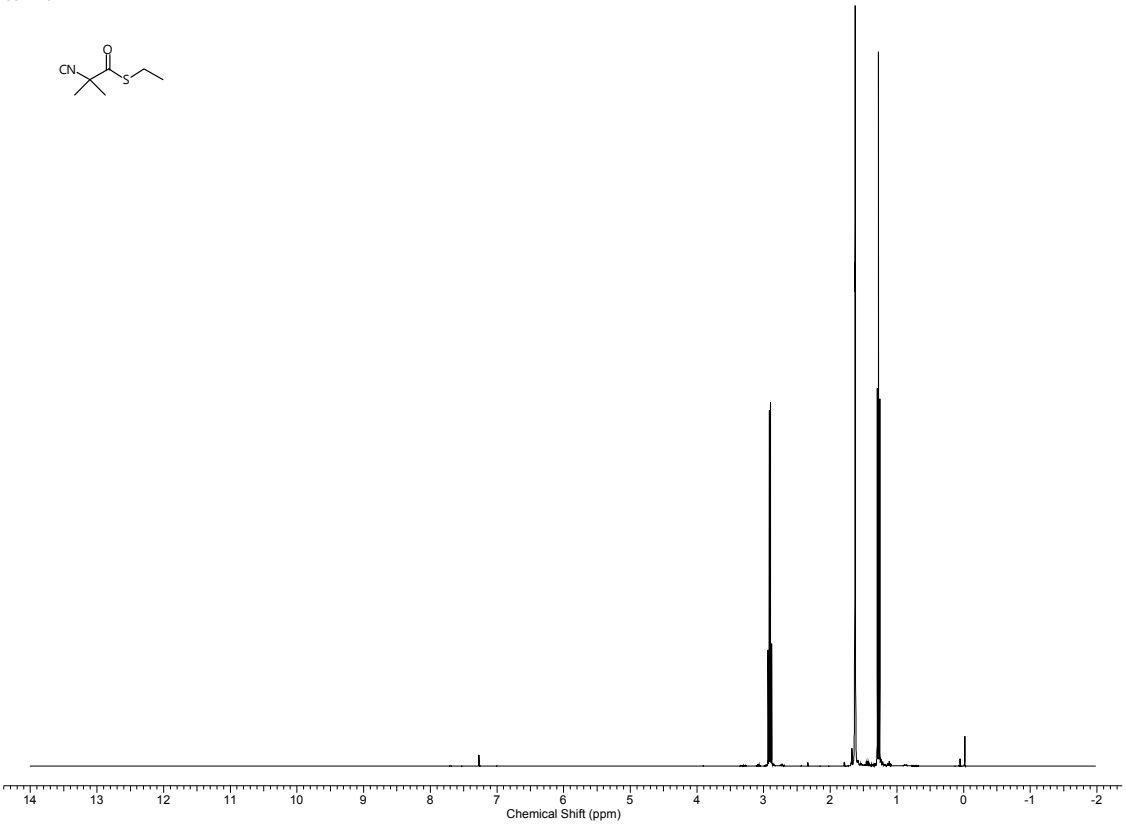

3A H.ESP
CN
O
S
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0 -0.5 -1.0
Chemical Shift (ppm)


3A C.ESP
CN
O
S
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

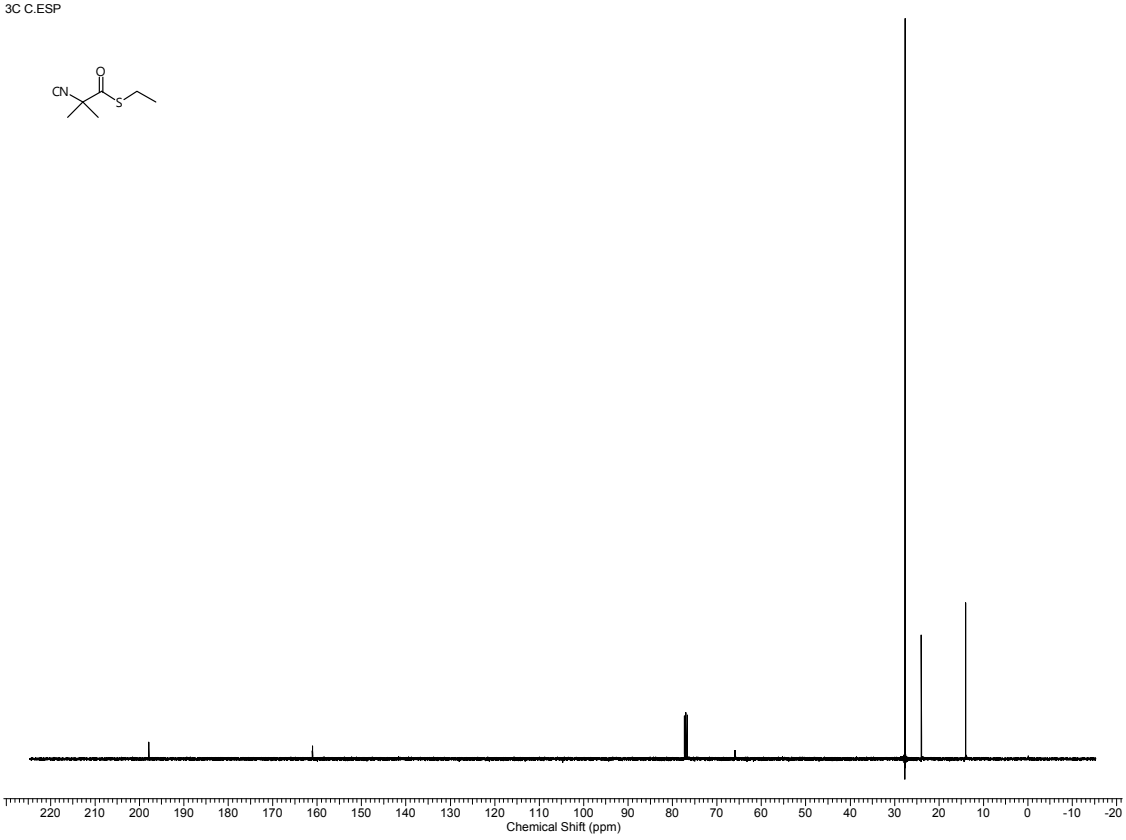

3B H.ESP
CN
O
S
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
Chemical Shift (ppm)

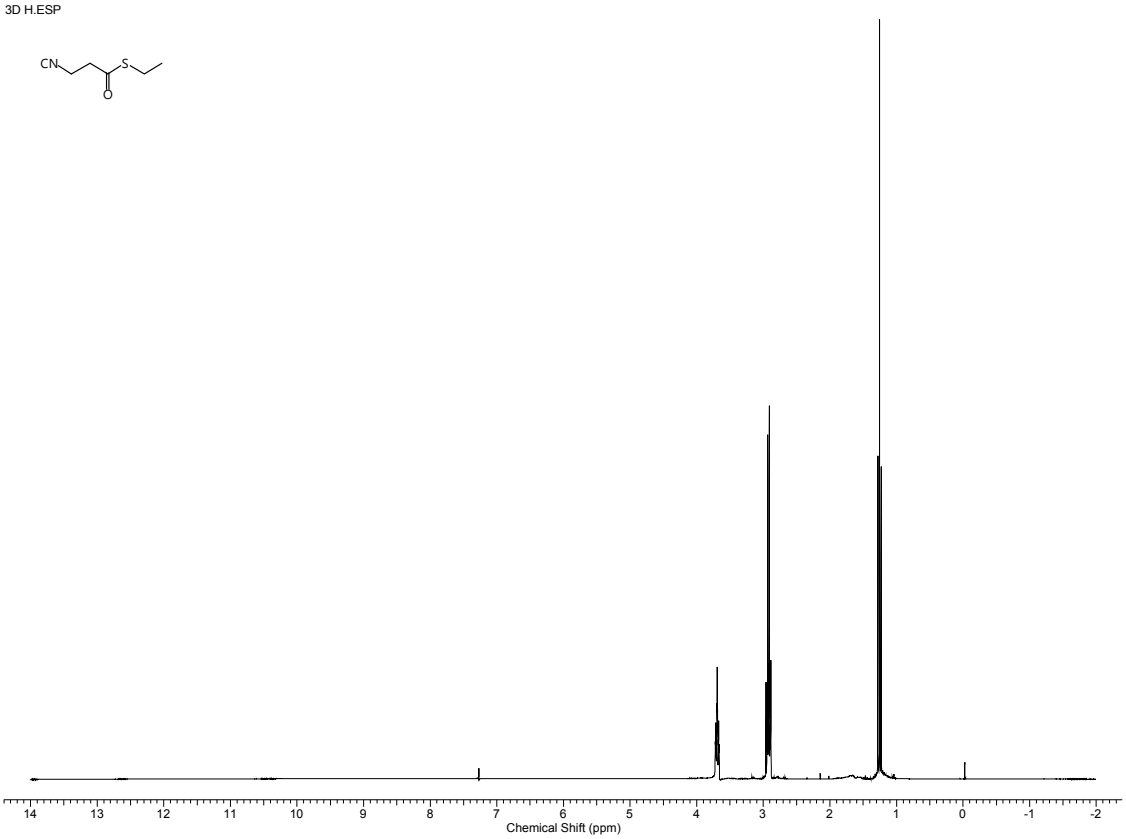

3B C.ESP
CN
O
S
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

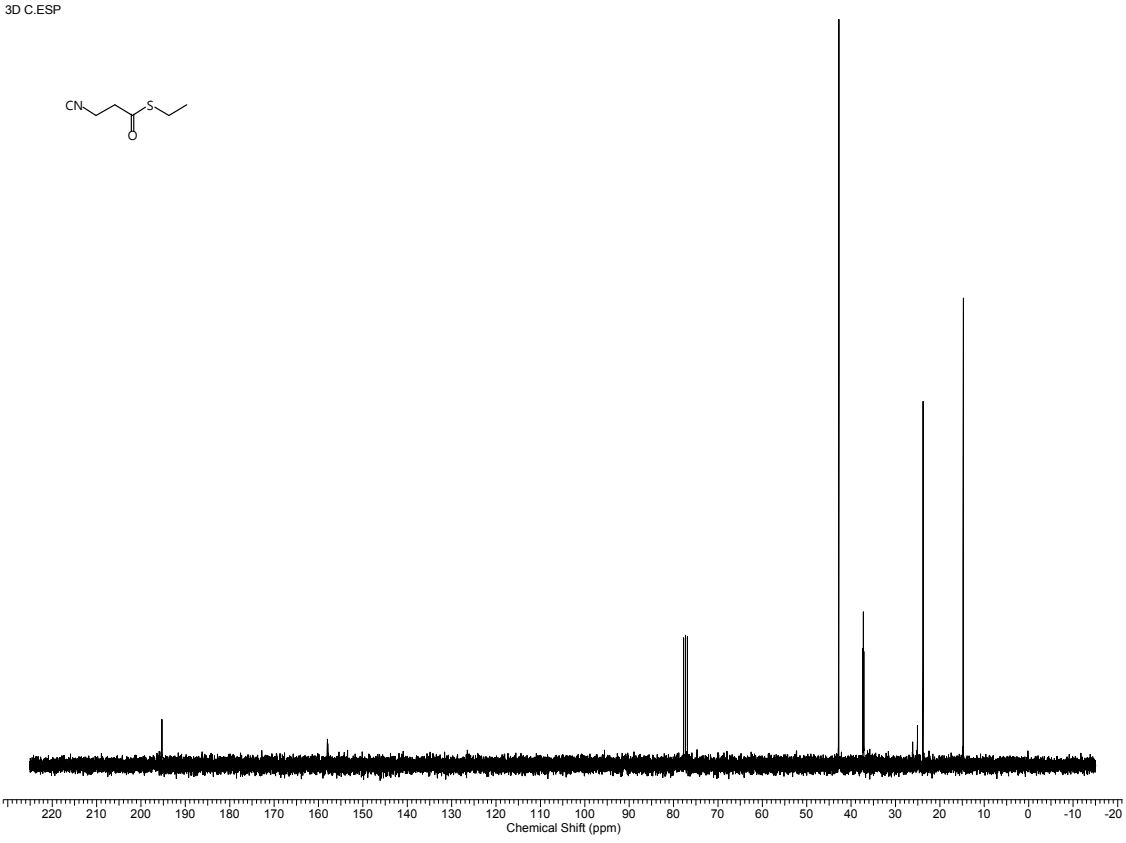
3C H.ESP

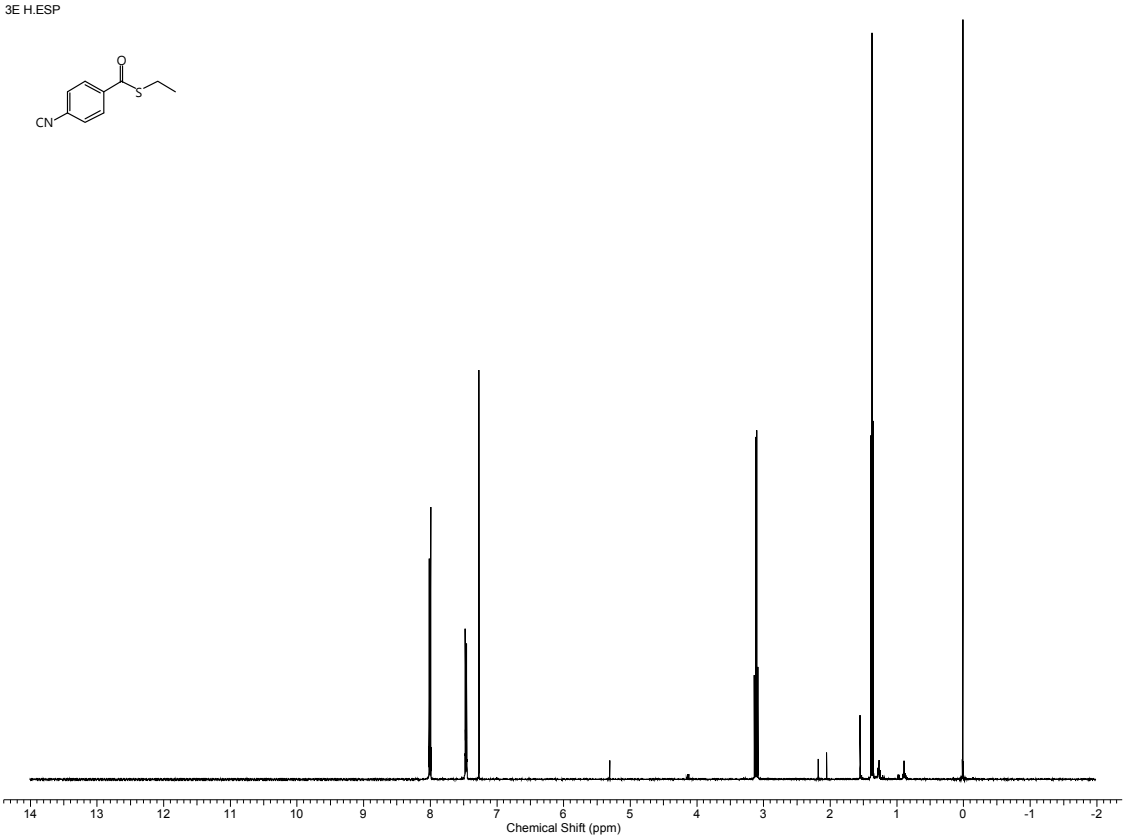


3C C.ESP




3D H.ESP
CN
S
O
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
Chemical Shift (ppm)


3D C.ESP
CN
S
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)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O
S
CN
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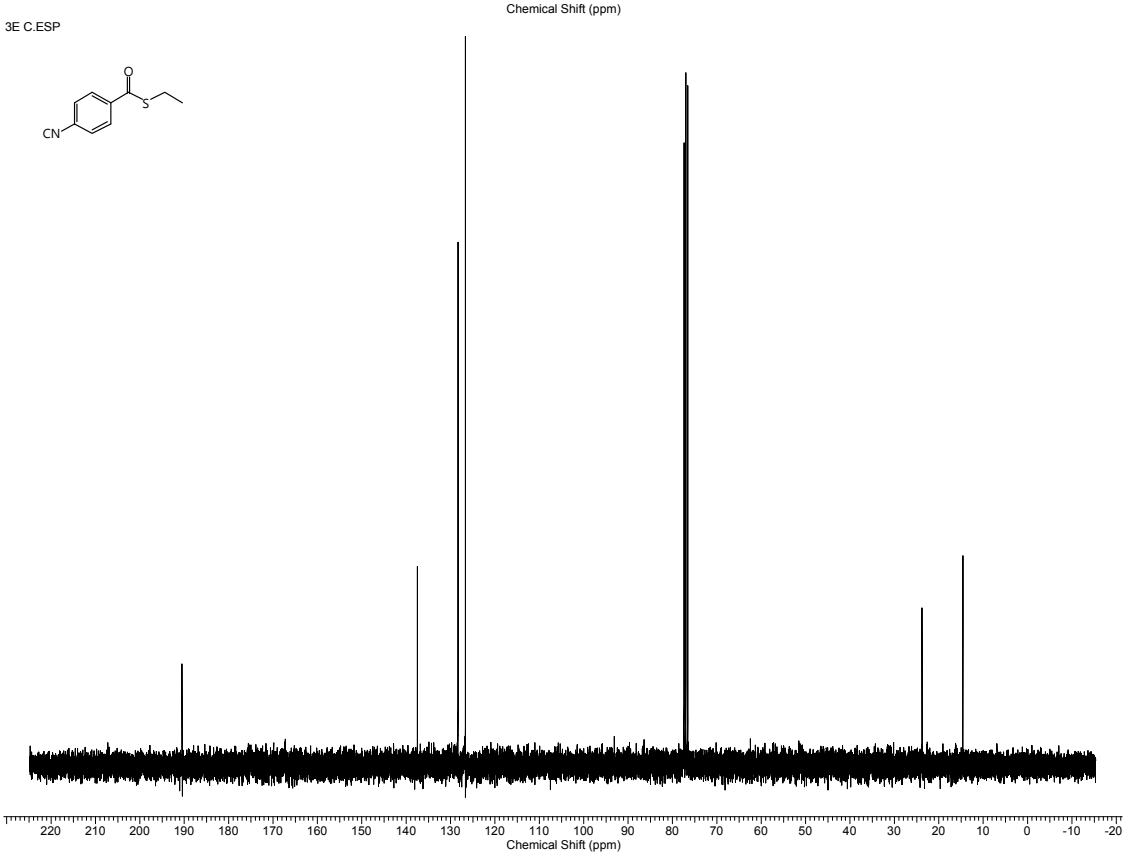
Chemical Shift (ppm)

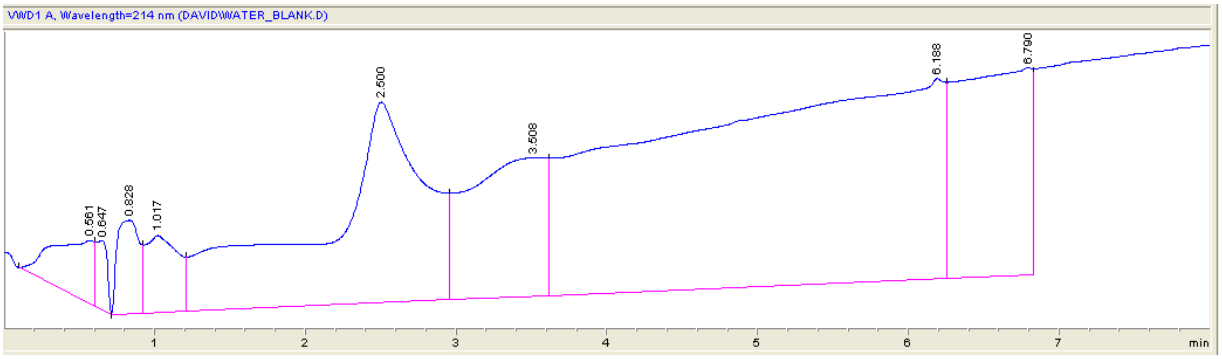

3E C.ESP
O
S
CN
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

Purification of peptide macrocycles

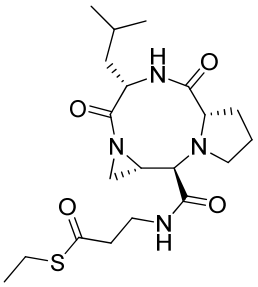
All peptides were purified by RP-HPLC using a C18 column with H_2O/MeCN gradient. Peptide purity was determined by LCMS analysis using UV integrations, and was in excess of 95% for most products. Peptide yields were determined by analysis of the crude reaction mixture using benzamide as an internal standard. Calibration curves were prepared using aliquots of 0.1 M solutions of purified peptide with a 0.1 M solution of benzamide and HFIP. These dilutions were then analyzed by LCMS and UV integrations used to construct curves for yield determination.

NOTE: During LCMS analysis of purified peptides both injection peaks (before 1 min) and solvent front peaks (between 2 and 3.5 min.) were observed. This was particularly prominent with dilute peptide solutions, and as such is not indicative of impurities.

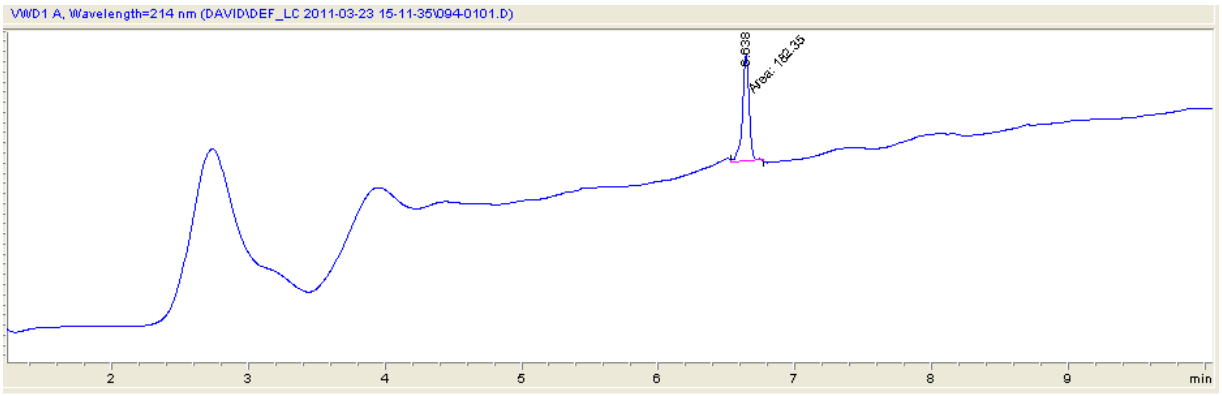
LCMS UV trace for water blank:

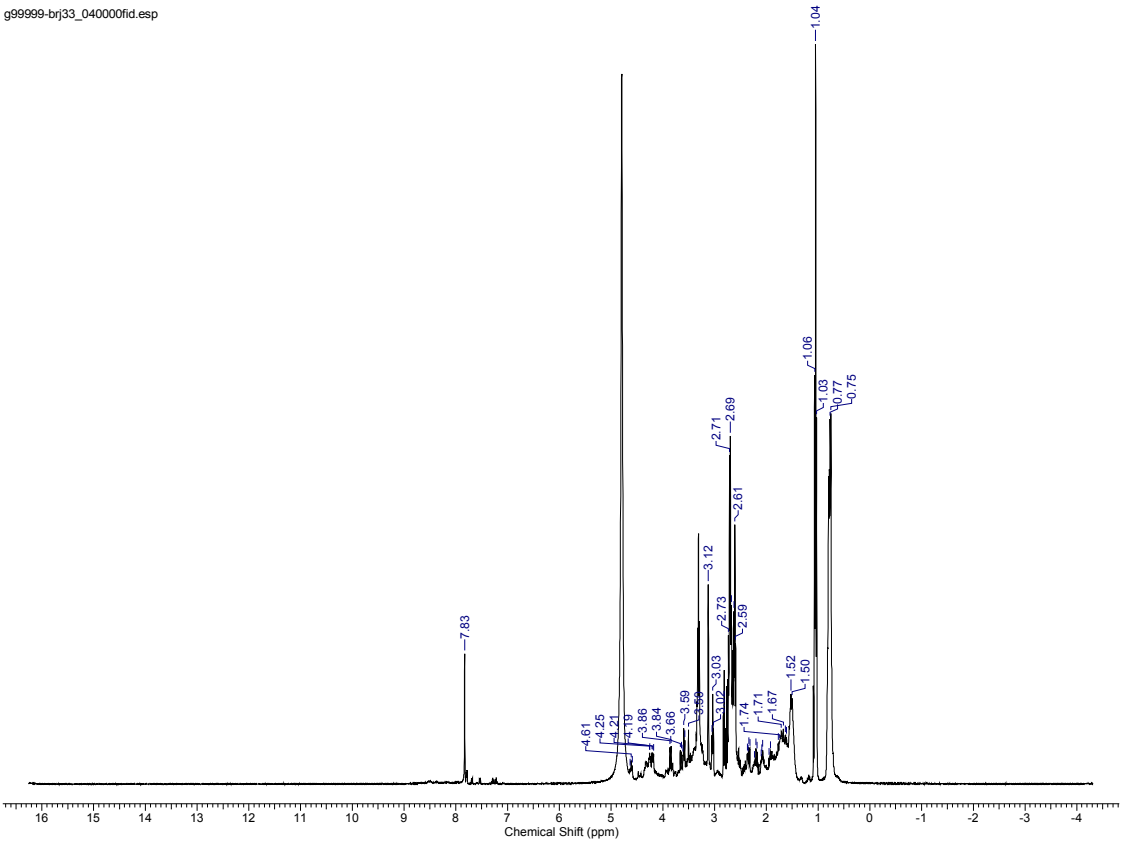


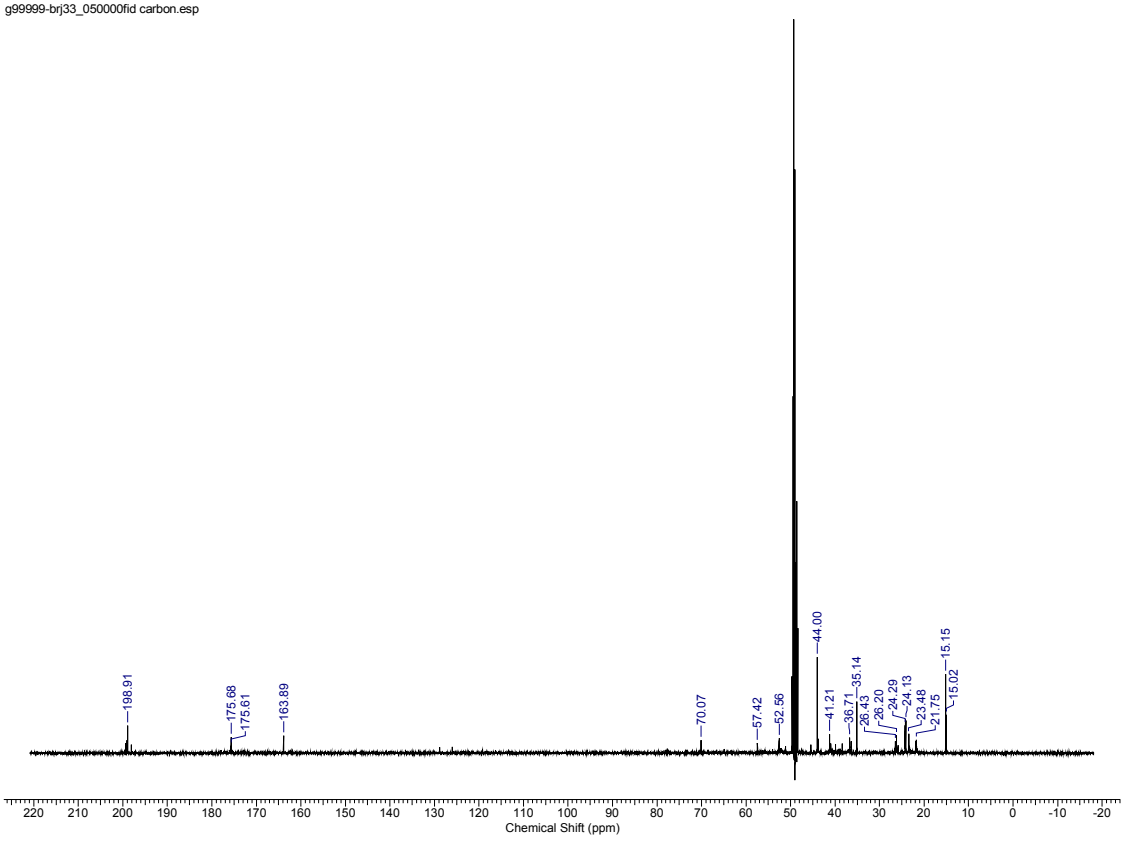
Characterization of peptide macrocycles

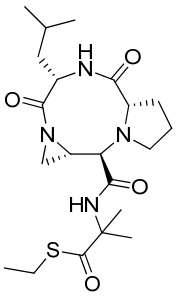


Compound 4a
Yield: 52 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{20}H_{33}N_4O_4S$ (MH^+) 425.2217 , found 425.2223
LCMS retention time on C18 (5% to 95% MeCN over 10 min.): 6.64 min.

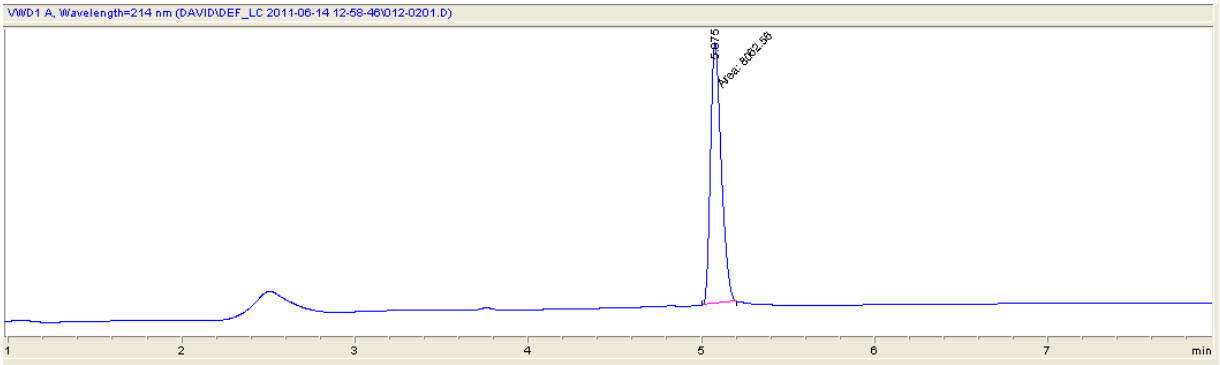


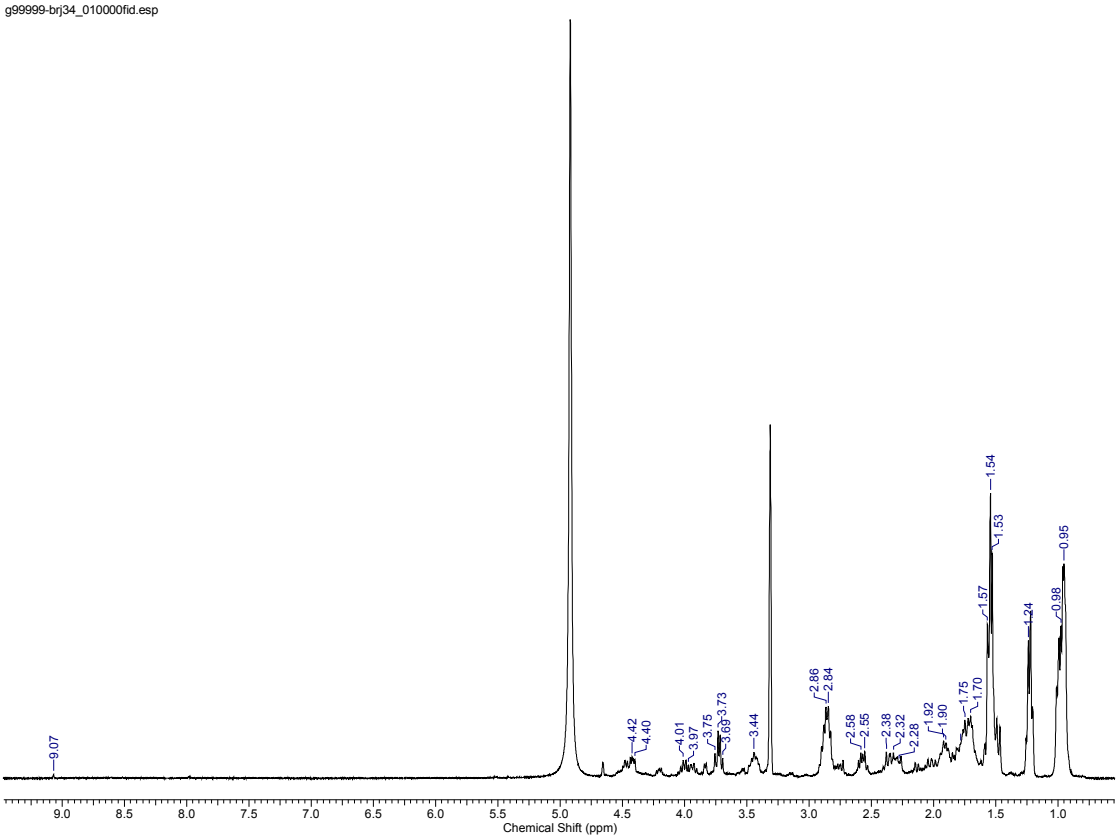

g99999-brj33_040000fid.esp
1.04
1.06
1.03
0.77
0.75
2.71
2.69
2.61
3.12
2.73
2.59
7.83
3.03
3.02
1.52
1.50
1.74
1.71
1.67
3.59
3.86
3.84
3.66
4.61
4.25
4.21
4.19
Chemical Shift (ppm)

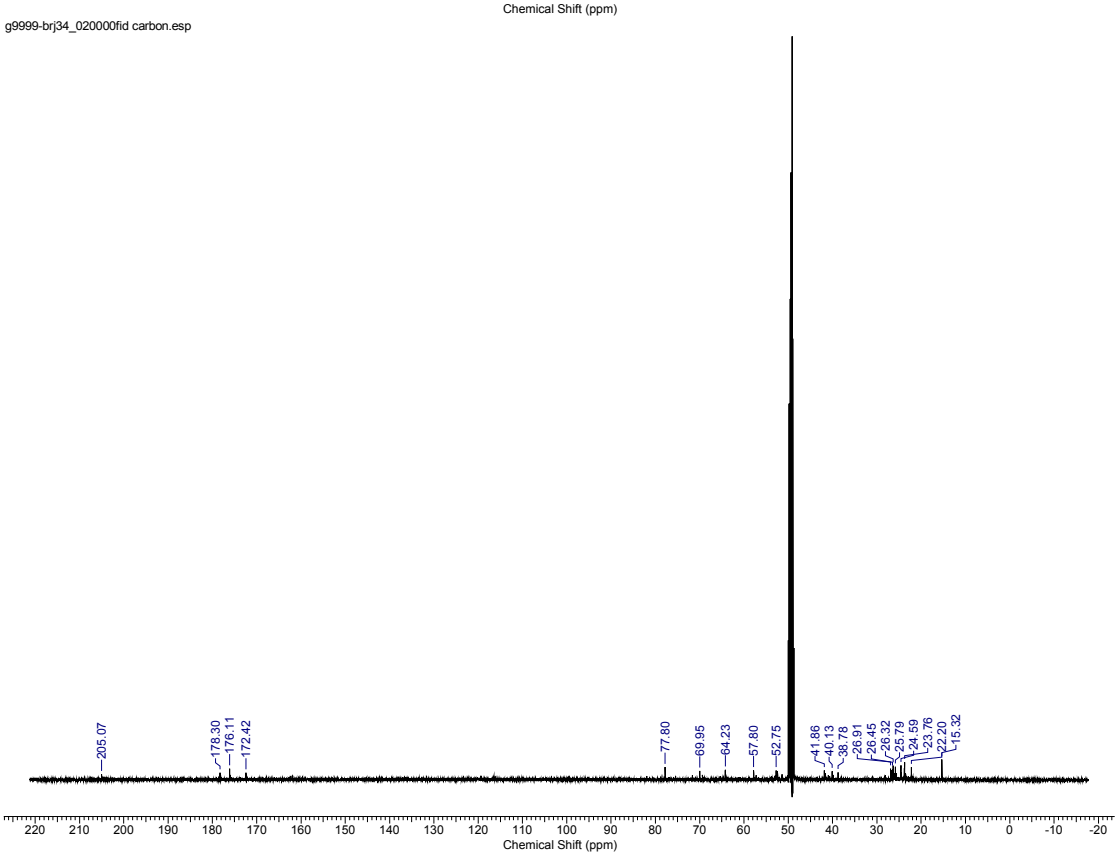

g99999-brj33_050000fid carbon.esp
198.91
175.68
175.61
163.89
70.07
57.42
52.56
44.00
41.21
36.71
35.14
26.43
26.20
24.29
24.13
23.48
21.75
15.15
15.02
Chemical Shift (ppm)

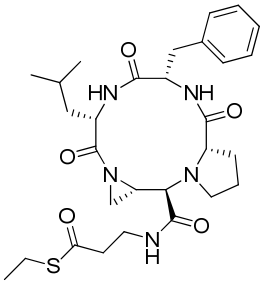


Compound 4b
Yield: 49 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{21}H_{35}N_4O_4S$ (MH^+) 439.2373, found 439.2370
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.08 min.

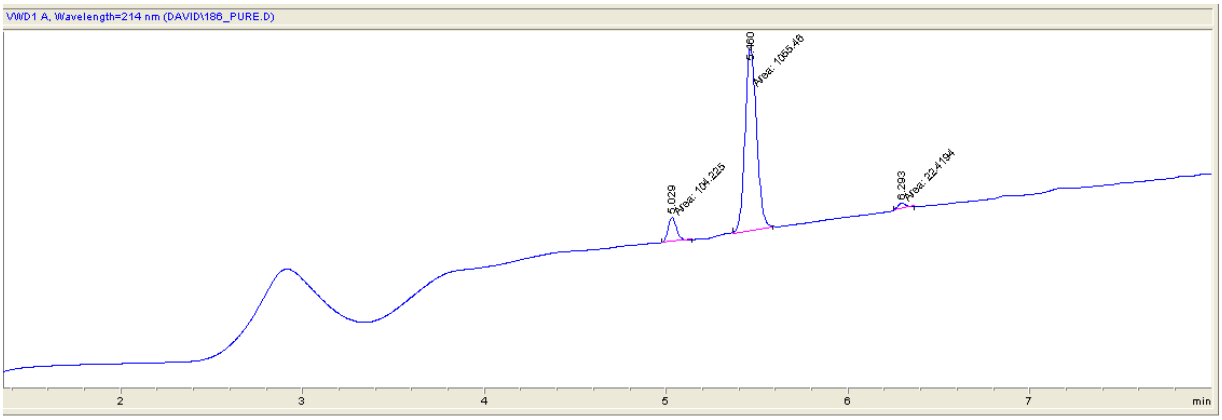


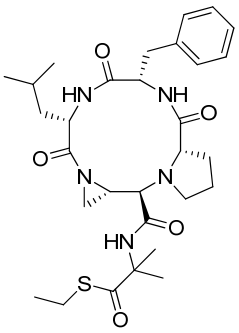

g99999-brj34_010000fid.esp
9.07
4.42
4.40
4.01
3.97
3.75
3.73
3.69
3.44
2.86
2.84
2.58
2.55
2.38
2.32
2.28
1.92
1.90
1.75
1.70
1.57
1.54
1.53
1.24
0.98
0.95
Chemical Shift (ppm)


g9999-brj34_020000fid carbon.esp
205.07
178.30
176.11
172.42
77.80
69.95
64.23
57.80
52.75
41.86
40.13
38.78
26.91
26.45
26.32
25.79
24.59
23.76
22.20
15.32
Chemical Shift (ppm)

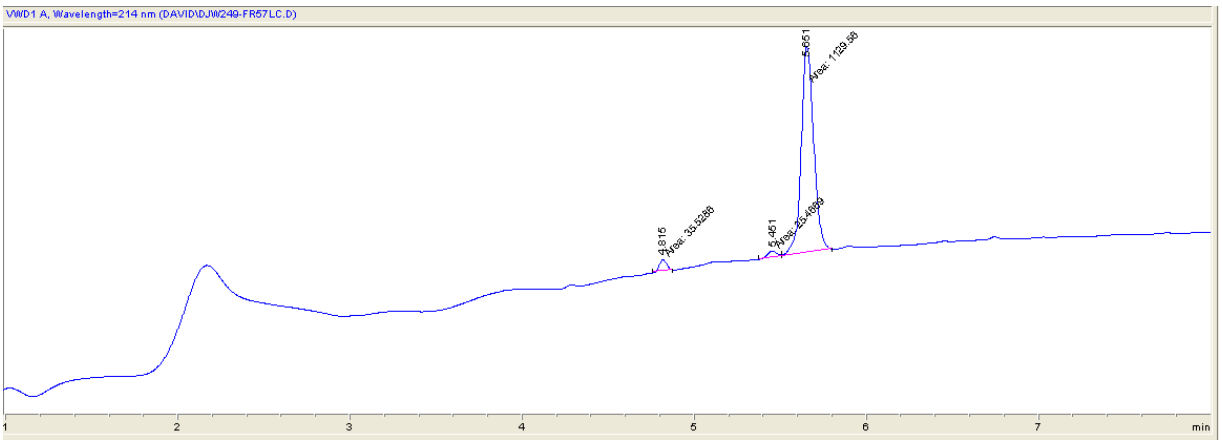


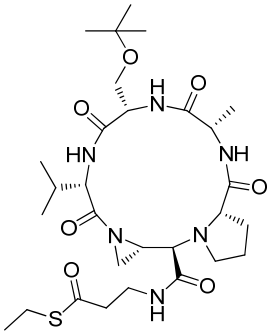
Compound 5a
Yield: 68 %
Purity: 90 %
HRMS (ESI) m/z calcd
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.46 min.





Compound 5b
Yield: 61 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{30}H_{44}N_5O_5S$ (MH^+) 586.3057, found 586.3057
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.65 min.





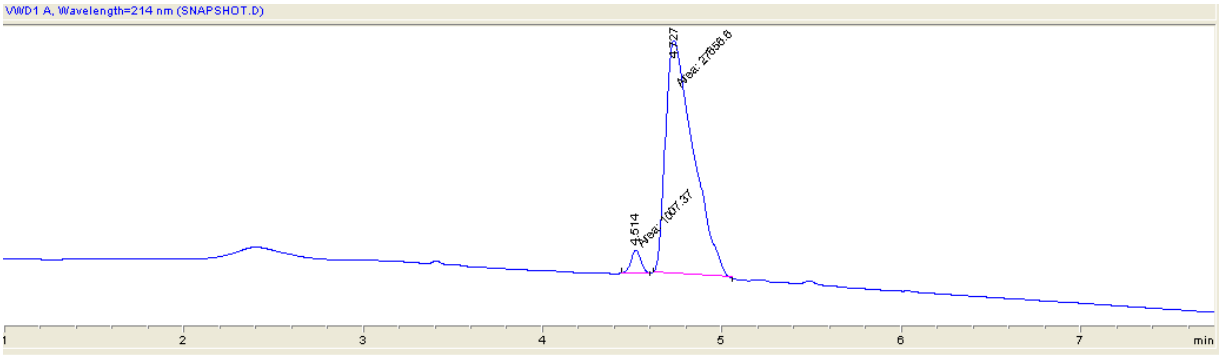
Compound 6a

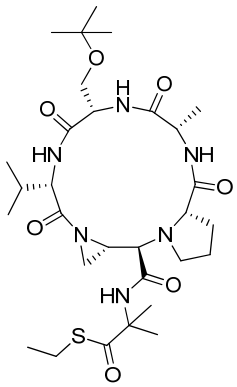
Yield: 31 %

Purity: >95 %

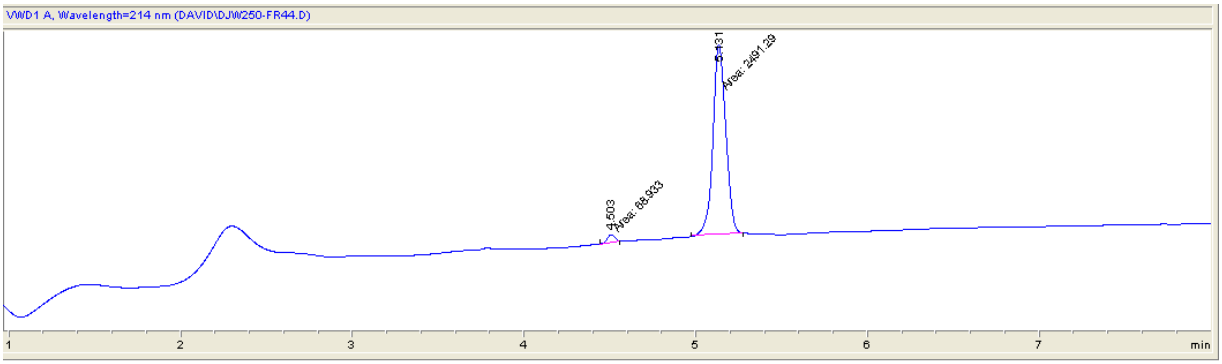
HRMS (ESI) m/z calcd for $C_{29}H_{49}N_6O_7S$ (MH^+) 625.3377, found 625.3367

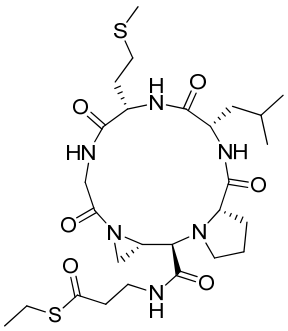
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 4.73 min.



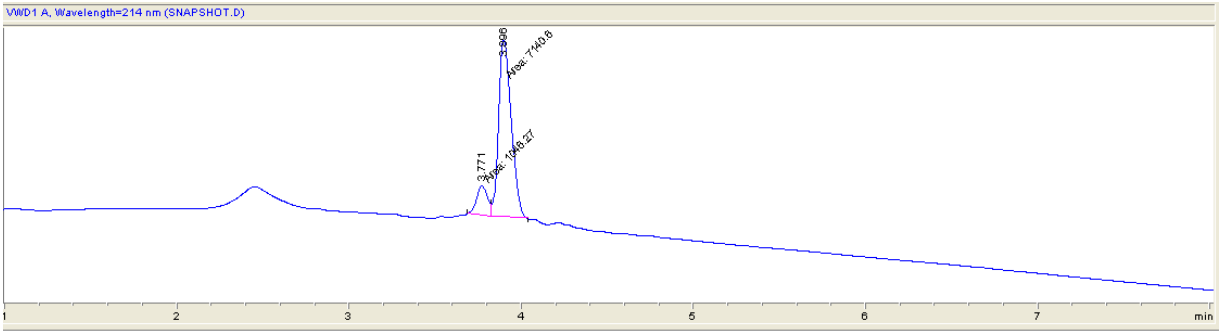


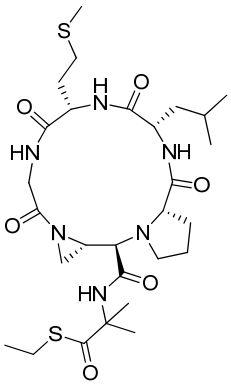
Compound 6b
Yield: 45 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{30}H_{51}N_6O_7S$ (MH^+) 639.3534, found 639.3547
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.08 min.



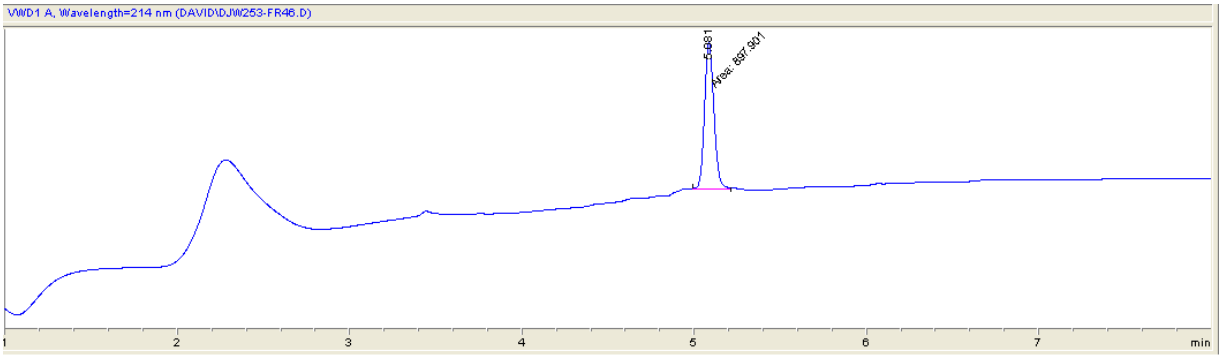


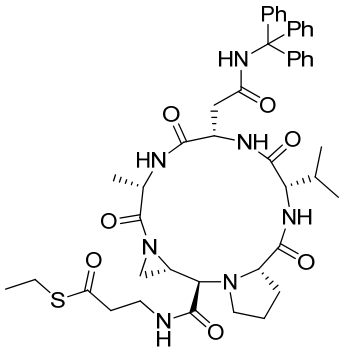
Compound 7a
Yield: 13 %
Purity: 87 %
HRMS (ESI) m/z calcd for $C_{27}H_{45}N_6O_6S_2$ (MH^+) 613.2843, found 613.2841
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 3.90 min.





Compound 7b
Yield: 34 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{30}H_{51}N_6O_7S$ (MH^+) 639.3534, found 639.3547
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.13 min.





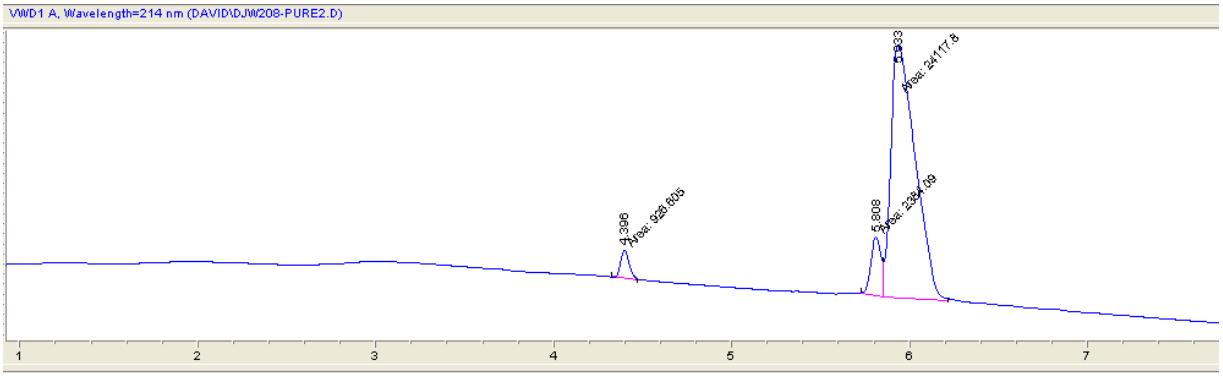
Compound 8a

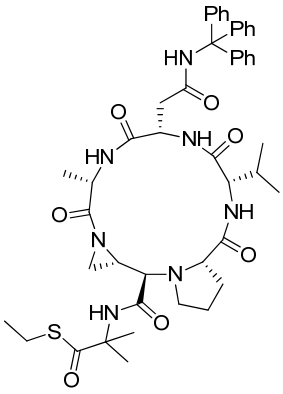
Yield: 51 %

Purity: 88 %

HRMS (ESI) m/z calcd for $C_{45}H_{56}N_7O_7S$ (MH^+) 838.3956, found 838.3994

LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.93 min.





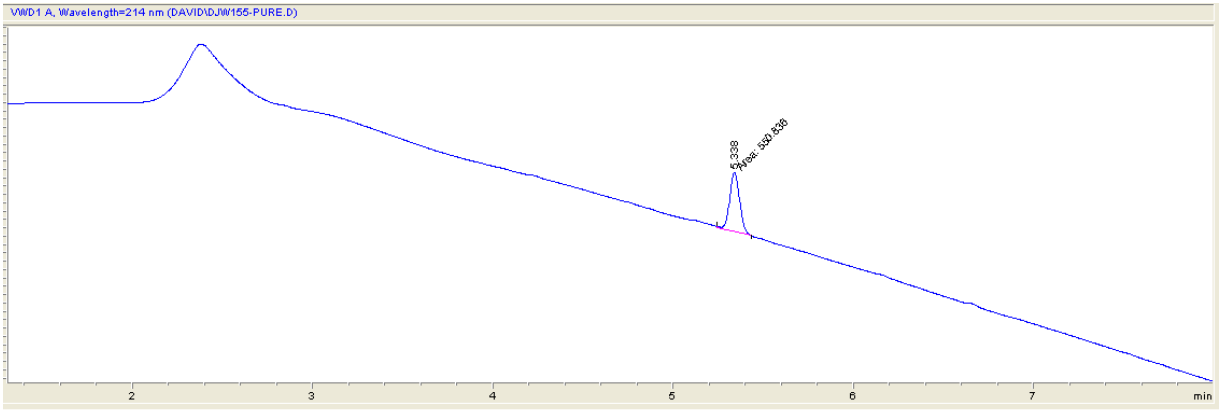
Compound 8b

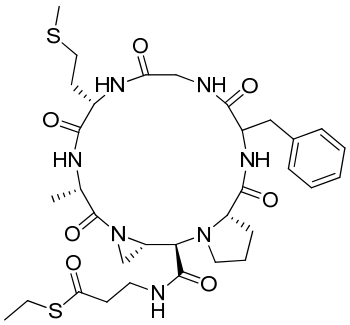
Yield: 51 %

Purity: >95 %

HRMS (ESI) m/z calcd for $C_{46}H_{58}N_7O_7S$ (MH^+) 852.4112, found 852.4141

LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.34 min.





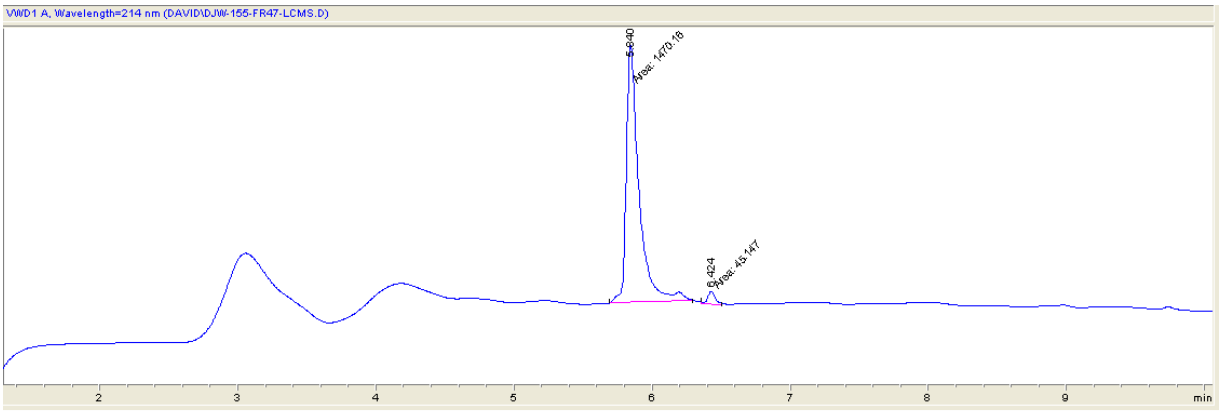
Compound 9a

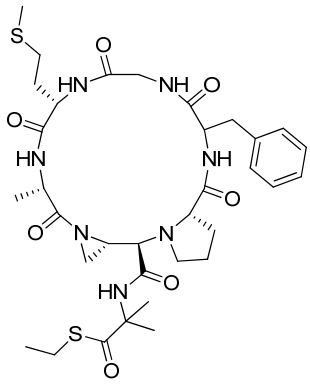
Yield: 20 %

Purity: >95 %

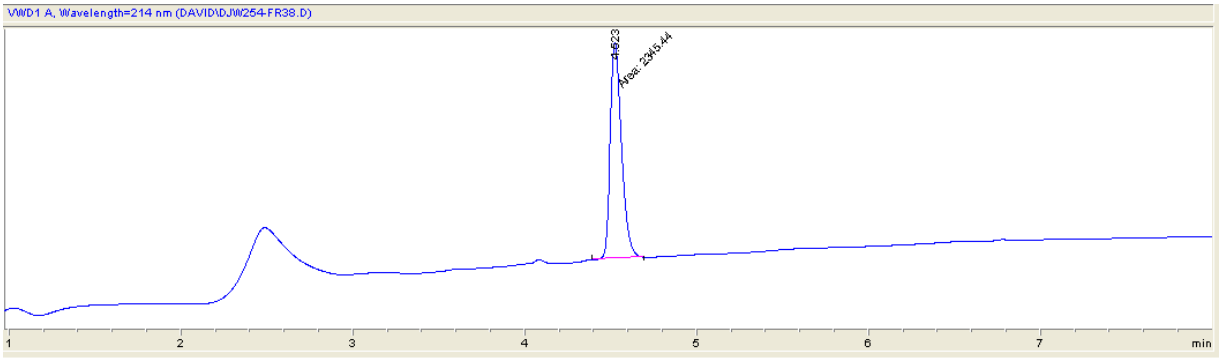
HRMS (ESI) m/z calcd for $C_{33}H_{48}N_7O_7S_2$ (MH^+) 718.3051, found 718.3048

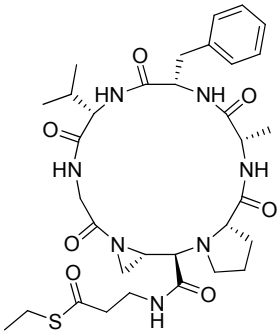
LCMS retention time on C18 (5% to 95% MeCN over 10 min.): 5.84 min.





Compound 9b
Yield: 20 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{34}H_{50}N_7O_7S$ (MH^+) 732.3207, found 732.3243
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 4.52 min.





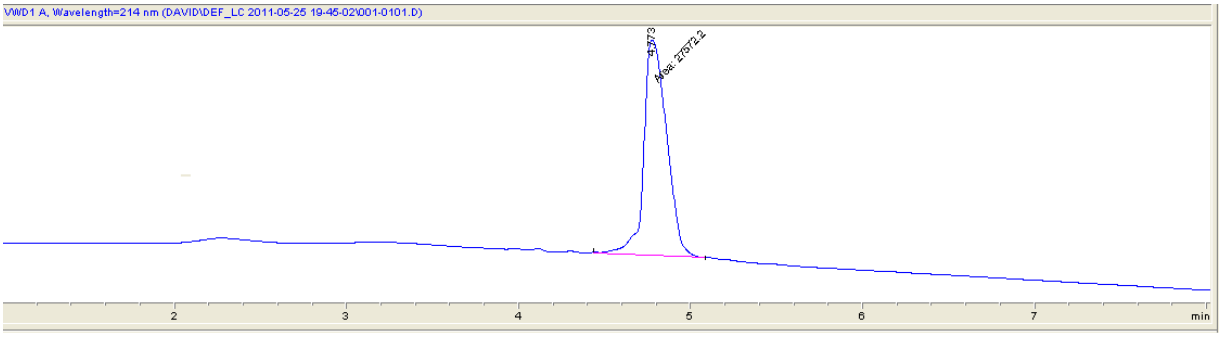
Compound 10a

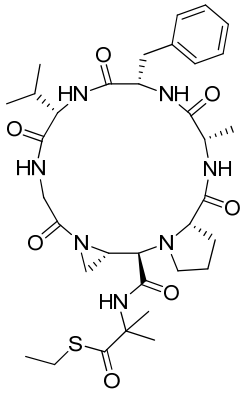
Yield: 13 %

Purity: >95 %

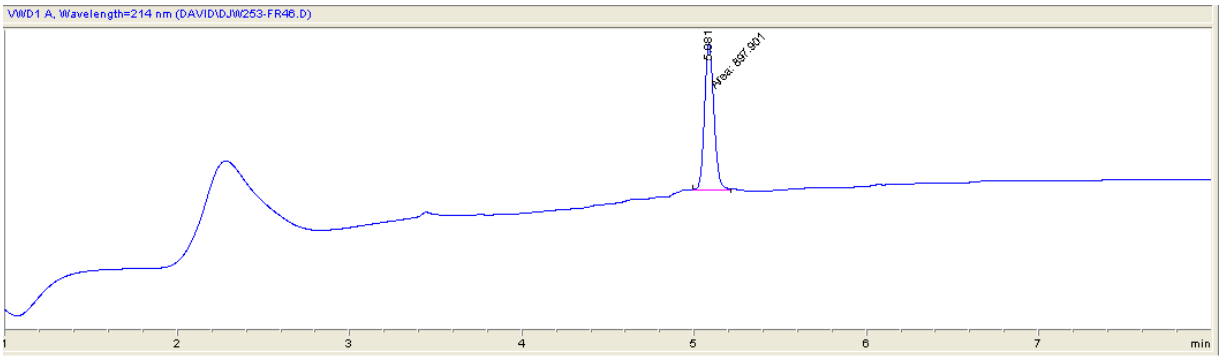
HRMS (ESI) m/z calcd for $C_{33}H_{48}N_7O_7S$ (MH^+) 686.3330, found 686.3356

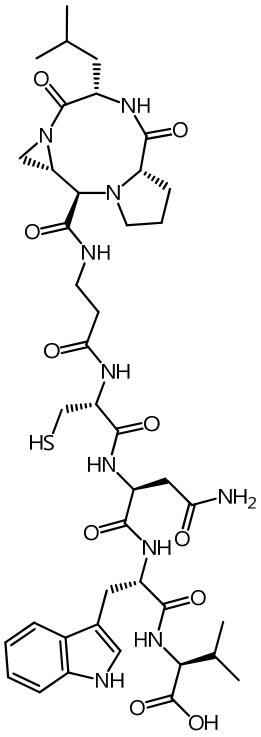
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 4.77 min.



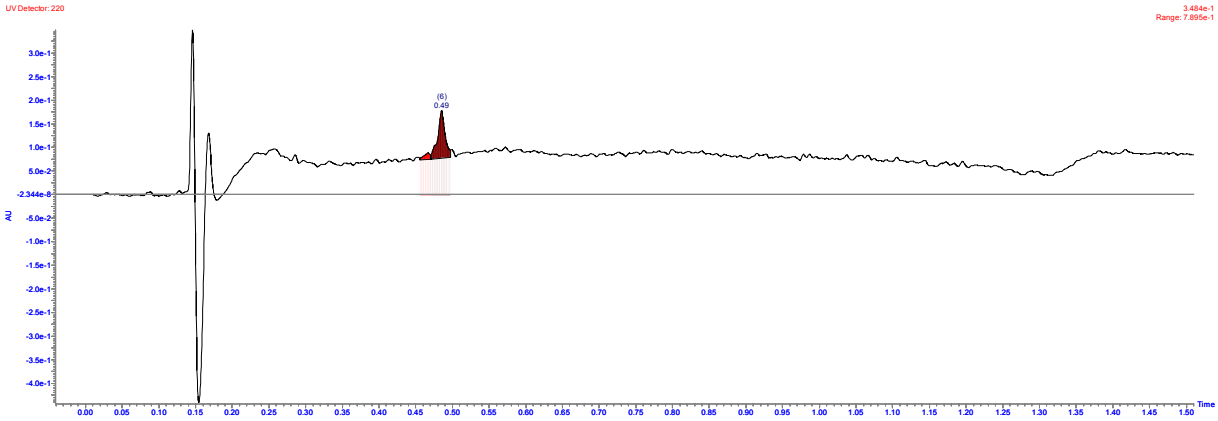


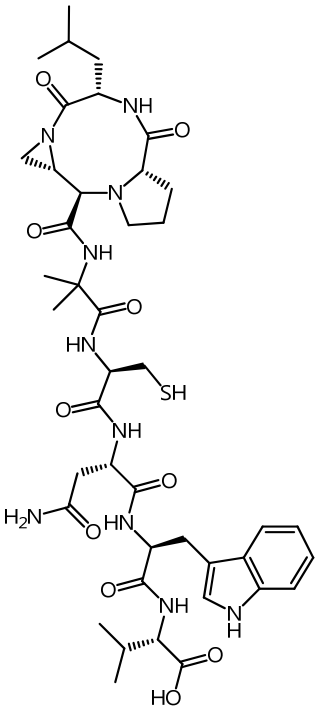
Compound 10b
Yield: 22 %
Purity: >95 %
HRMS (ESI) m/z calcd for $C_{28}H_{47}N_6O_6S$ (MH^+) 627.2993, found 627.3012
LCMS retention time on C18 (5% to 95% MeCN over 8 min.): 5.08 min.





Compound 11a
Yield: 73 %
Purity: 90 %
ESI m/z calcd for $C_{41}H_{58}N_{10}O_{10}S$ (MH^+) 883.41, found 883.55
LCMS retention time on C18 (5% to 95% MeCN over 3 min.): 0.49 min.





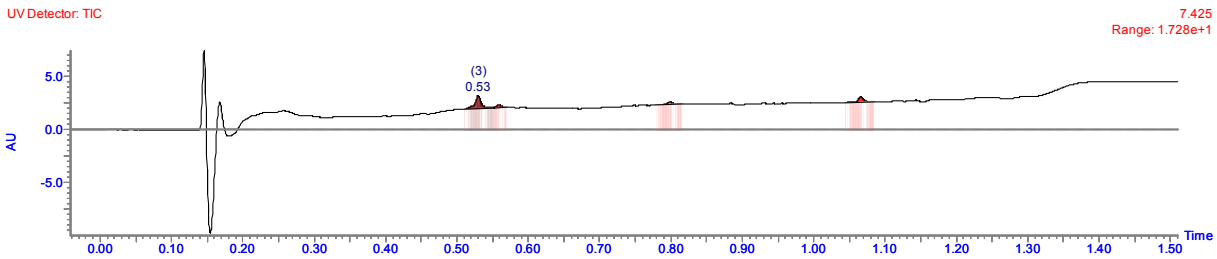
Compound 11b

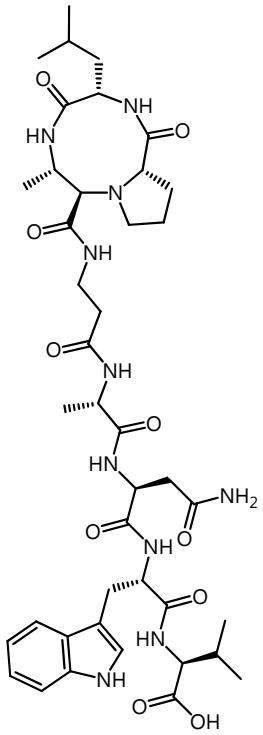
Yield: 43 %

Purity: 76 %

ESI m/z calcd for $C_{42}H_{60}N_{10}O_{10}S$ (MH^+) 897.43, found 897.49

LCMS retention time on C18 (5% to 95% MeCN over 3 min.): 0.54 min.





Compound 12
Yield: 71 %
Purity: 84 %
ESI m/z calcd for $C_{41}H_{60}N_{10}O_{10}$ (MH^+) 853.46, found 853.51
LCMS retention time on C18 (5% to 95% MeCN over 3 min.): 0.44 min.

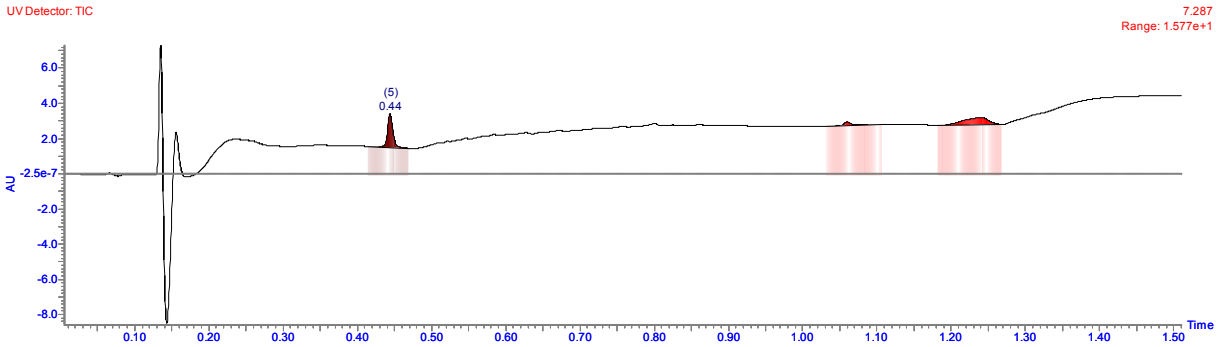
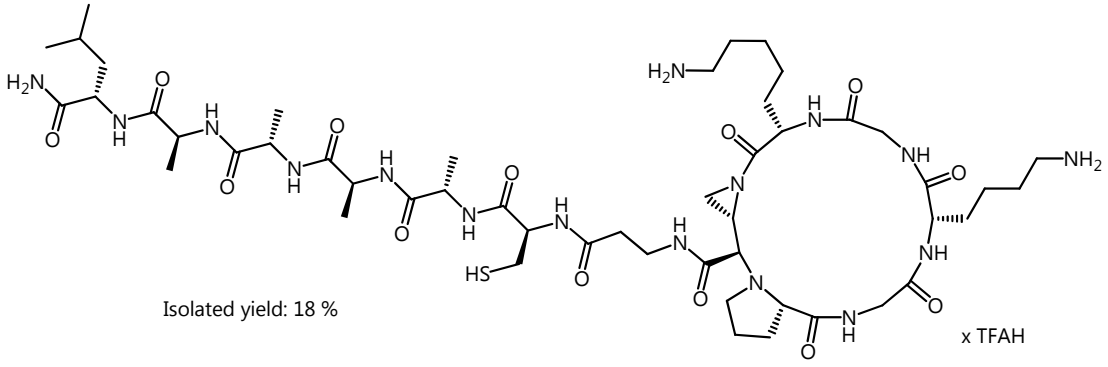
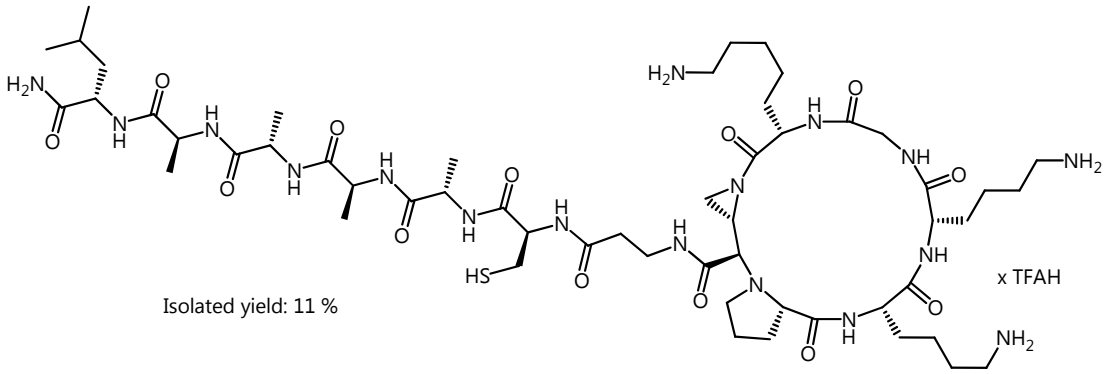


Figure S1 First generation of peptides synthesized for antimicrobial activity



Isolated yield: 18 %

x TFAH



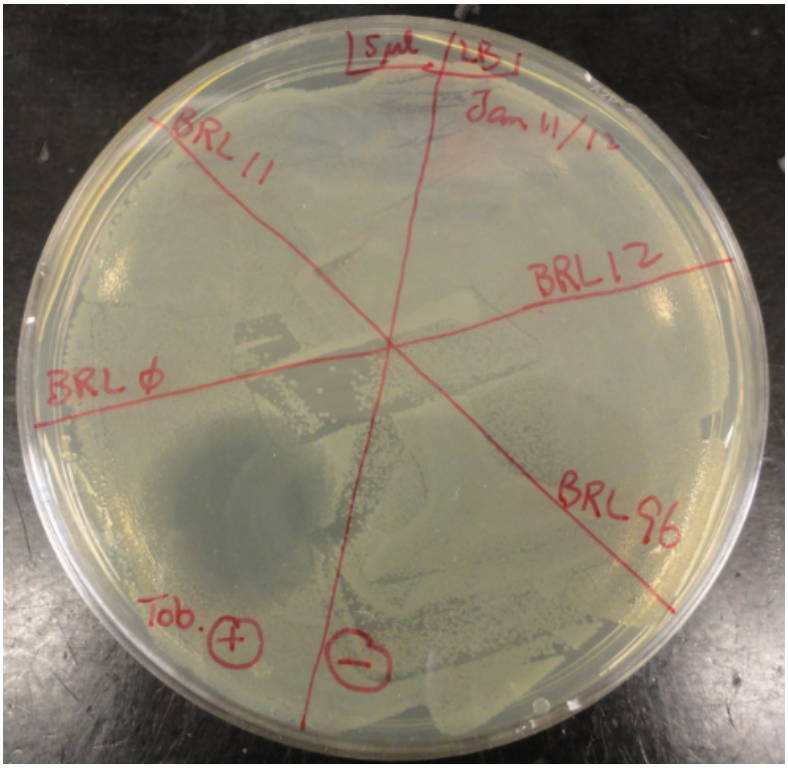
Isolated yield: 11 %

x TFAH

Antimicrobial Activity Screening

Zone-of-inhibition: Overnight E. coli (BL21 strain) culture was diluted to approximately 106 CFU/mL, plated on a LB (Bioshop) agar surface, and allowed to dry. 5 µL of each peptide dissolved in DMSO at 1 mg/mL was dropped on to the plate in a pre-labeled grid, and dried. Zones-of-inhibition were compared after incubating the plate at 37 ˚C overnight. Tobramycin (Sigma) at 1 mg/mL and DMSO without peptides were chosen to be the positive and negative controls.

Results: No clear zone of inhibition was detected for any of the tested peptides.



Minimal inhibitory concentration: The antimicrobial activity of each peptide was tested in sterile 96-well microtiter plates against *Escherichia coli* BL21 strain and *P. aeruginosa* PAO1 strain by following standard microtiter dilution protocols in Mueller Hinton Broth (MHB).[5] The bacterial strains were grown in MHB at 37 °C overnight, and diluted to a final concentration of 5 x 10^5 to 10^6 colony forming units as determined by UV spectrophotometer at optical density (OD) 600 nm. 10 µL of peptides of two-fold serial dilutions were added to 90 µL of diluted bacterial suspension. Plates were incubated at 37 °C overnight for 20 h, and the minimum inhibitory concentration (MIC) was taken as the concentration at which the bacterial growth was fully inhibited, detected at OD_{600} using a Genesys 5 microplate autoreader spectrophotometer.

Results: The peptides displayed no antimicrobial activity at tested concentrations up to 64 µg/mL against both bacterial strains.

References

[1] A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, *Organometallics*, 1996, **15**, 1518.
[2] J. C. Sheehan and D.-D. H. Yang, *J. Am. Chem. Soc.*, 1958, **80**, 1154.
[3] H. Tokuyama, S. Yokoshima, S.-C. Lin, L. Li and T. Fukuyama, *Synthesis*, 2002, 1121.
[4] J. A. Camarero, B. J. Hackel, J. J. de Yoreo and A. R. Mitchell, *J. Org. Chem.* 2004, **69**, 4145.
[5] I. Wiegand, K. Hilpert and R. E. Hancock, *Nat. Protoc.* 2008, **3,** 163.