A Traceless Approach to Amide and Peptide Construction from Thioacids and Dithiocarbamate-Terminal Amines

Wenteng Chen, [†, 1] Jiaan Shao, [†, 1] Miao Hu, [1] Wanwan Yu, [1] Marc A. Giulianotti, [2] Richard A. Houghten [*, 2] and Yongping Yu [*, 1, 2]

[1] Institute of Materia Medica, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, P. R. China
[2] Torrey Pines Institute for Molecular Studies, 11350 Village Parkway, Port St. Lucie, FL 34987, United States
*Phone: +86-571-88208452. Fax: +86-571-88208452. E-mail: yyu@zju.edu.cn
*Phone: +1-772-345-4590. Fax: +1-772-345-3649. Email: houghten@tpims.org
[†] These authors contributed equally.

Supporting Information

Table of Contents

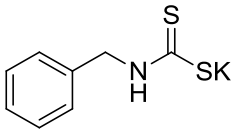
General Materials and Methods

Water was taken from a Mill-Q ultra-pure water purification system. Commercial reagents were purchased from Sigma-Aldrich and were used without further purification. Solvents were purchased from Sigma-Aldrich and were used directly. Resin, protected amino acids and EDC, PyBOP were purchased from Novabiochem. Deuterated solvents were purchased from Cambridge Isorope Laboratories Inc. LC-MS (APCI and ESI) were recorded on a Shimadzu LCMS-2010EVat LCQ mass spectrometer (ThermoQuest Corporation) using a Phenomenex Luna 5 μ C18, 100 Å, 150 × 4.60 mm 5 micron column at a flow rate of 0.5ml/min using liner gradients buffer B in A (B: CH_3CN containing 0.1 % formic acid, A: H_2O containing 0.1% formic acid). High resolution mass spectra were measured on an Agilent 1290 HPLC-6224 Time of Fight Mass Spectrometer. Preparative RP-HPLC was performed on a Shimadzu LC-8A preparative HPLC using a Phenomenex Luna 5 μ C18 (2) 100 Å column (75 × 21.2 mm, 5 micron).Chiral HPLC was measured on an Agilent 1200 using a Chiralcel OJ-RH column (4.6 mm × 150 mm, particle size: 5 μm) at a flow rate of 0.3 ml/min using 20 % B in buffer A (B: CH_3CN, A: H_2O containing 50 mM PBS buffer).

Characterization of the representative dithiocarbamate

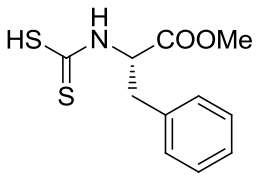
The preparation of dithiocarbamate salt is followed by Mishra, A.K. [1] and Zhang, J. [2]

Potassium of N-benzyl dithiocarbamate



Potassium hydroxide (1.0 mmol) was dissolved in MeOH: H_2O (10 ml: 1.0 ml), the solution cooled in an ice–salt bath and then benzyl amine was added (1.0 mmol) while stirring and carbon disulfide (5.0 mmol) then was added. The mixture was stirred for 2 h in an ice-salt bath. Most of the solvent was removed under reduced pressure, and the precipitate collected by filtration.
^{1}H NMR (500 MHz, DMSO) δ 7.94 (s, 1H), 7.32-7.24 (m, 5H), 4.69 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ 215.2, 140.7, 128.3, 127.9, 126.7, 58.2.

L-Phe-COOMe-derived dithiocarbamate



The *L*-Phe-COOMe (1.0 mmol) was dissolved in DCM (2.0 ml), the solution cooled in an ice–salt bath and then pyridine (0.1M) and carbon disulfide (0.1 M) were added while stirring. The

mixture was stirred for 1 h in an ice-salt bath. Most of the solvent was removed under reduced pressure, and the precipitate collected by filtration.
^{1}H NMR (500 MHz, DMSO) δ 7.82 (d, J = 7.5 Hz, 1H), 7.28-7.26 (m, 2H), 7.21-7.18 (m, 3H), 5.20 (dd, J_1 = 13.5 Hz, J_2 = 7.0 Hz, 1H), 3.55 (s, 3H), 3.04-3.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO) δ 216.2, 172.8, 138.1, 129.5, 128.7, 126.8, 60.4, 51.9, 37.5

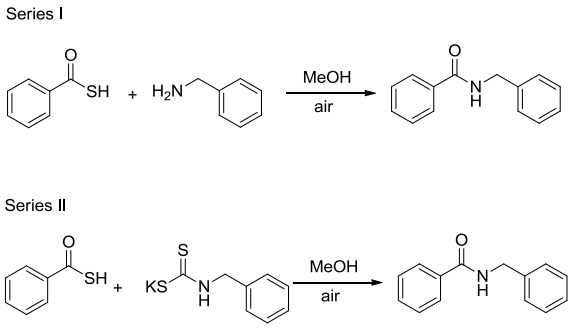
1. Mishra, A. K., Mishra, S. B., Manav, N., Saluja, D., Chandrac, R. & Kaushika, N. K. Synthesis, characterization, antibacterial and cytotoxic study of platinum (IV) complexes *Bioorg. Med. Chem.* **14**, 6333-6340, (2006).
2. Zhang, J., Lin, X., Ren, J., Liu, J. & Wang, X. Synthesis and biodistribution of a novel ^{99m}Tc nitride dithiocarbamate complex containing aromatic group for cerebral imaging. *Applied Radiation and Isotopes* **68** 101–104, (2010).

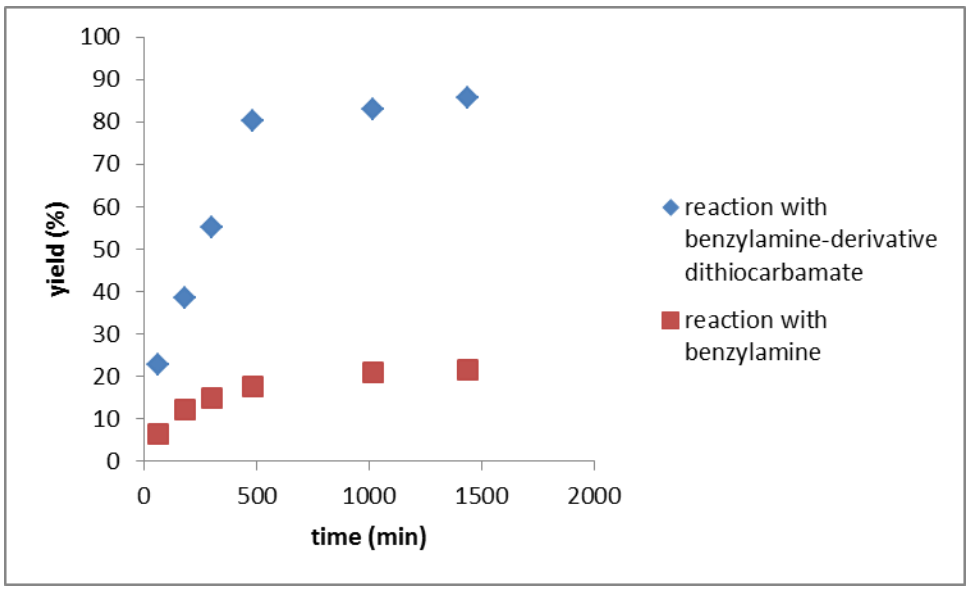
Studies of the Reaction Rate

In addition, to support the proposed mechanism, we designed the following reactions to prove dithiocarbamate was involved in this reaction. We compared the reaction rates of benzylamine derived dithocarbamate with thioacid and benzylamine with thioacid. (**Chart 1S**) The reaction rate of benzylamine derived dithocarbamate with thioacid is faster than benzylamine with thioacid. From the results, dithiocarbamate is indeed involved in this reaction.

General procedure:
The parallel reactions (x36) were proceeding in tubes. To a stirred solution of benzylamine or benzylamine derived dithiocarbamates (5.5 mmol) in methanol (30.0 ml) was added dropwise thiobenzoic acid (5.0 mmol) at room temperature. The reaction mixture was quenched with saturated $NaHCO_3$ solution at different time point. And the yield of the amide was determined based on isolated yields (the data are mean values of three independent experiments performed in duplicate).

Chart IS: reaction rate of benzylaimine-derived dithiocarbamate *vs* benzylamine





Studies of the Stability of Dithiocarbamates

1) For simple amine-derived dithiocarbamate

We test the decomposition of benzylamine-derived dithiocarbamate in MeOH and DCM on LC-MS. In fact, we cannot find the free amine peak. Instead, we found the thiourea peak. The decomposed amine may be reacted with dithiocarbamate to form thiourea (**Scheme 1S**). The decomposition rate is tested based on the formation of thiourea and consumption of dithiocarbamate. The result of decomposition rate is showed below (**Chart 2S**).

Scheme 1S

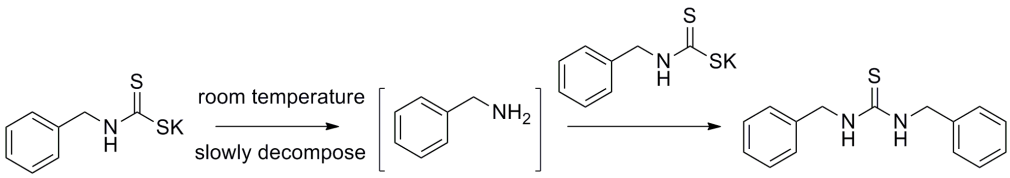
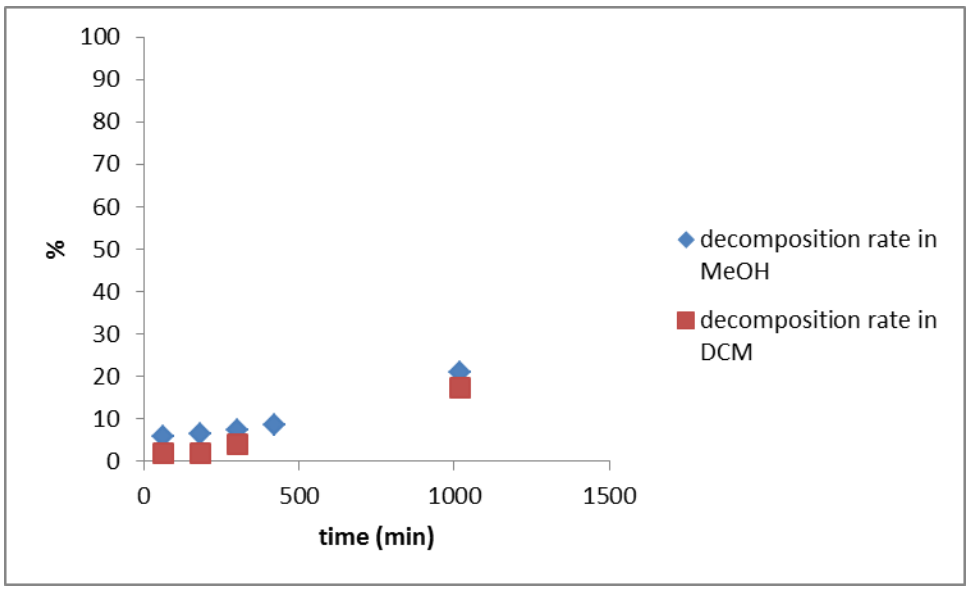


Chart 2S



2) For amino ester/amino acid derived dithiocarbamate

We probed by ^{1}HNMR. The results showed as follows (**Figure 1S and Figure 2S**).

Figure 1S. The ^{1}HNMR spectrum of ^{L}Phe-COOMe derived dithiocarbamate

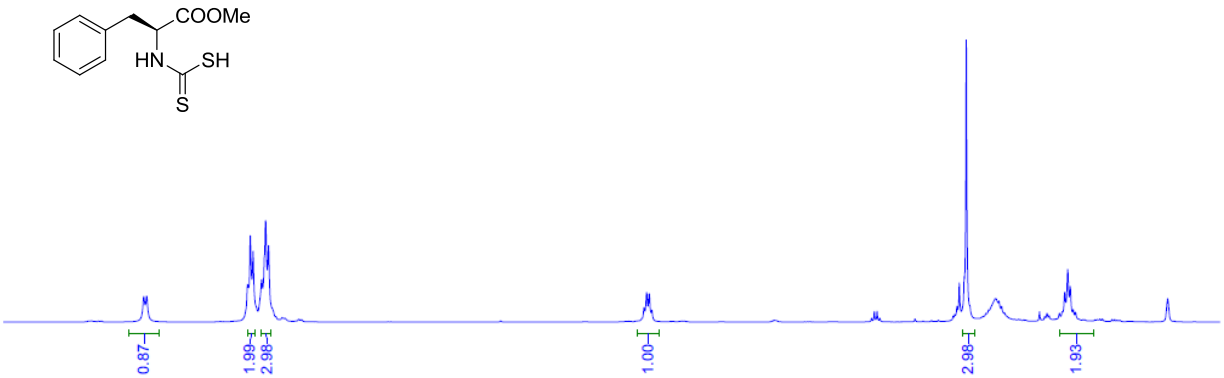
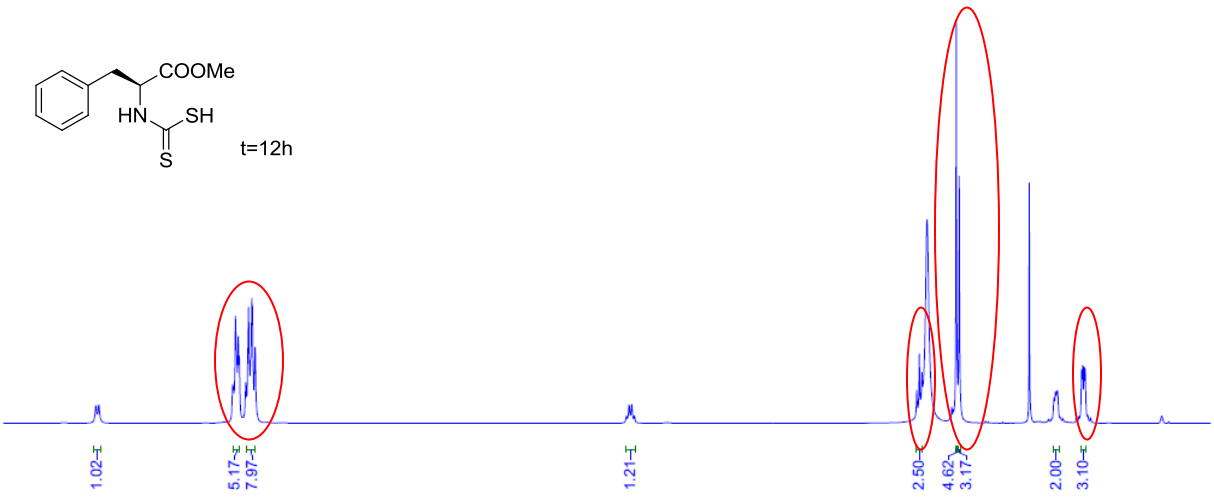
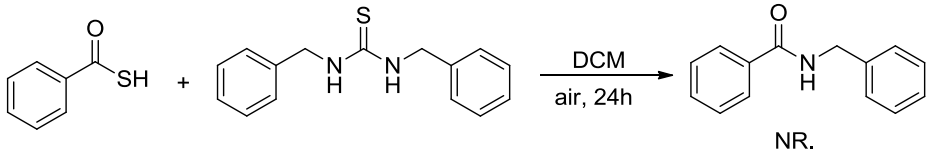


Figure 2S. The ^{1}HNMR spectrum of ^{L}Phe-COOMe derived dithiocarbamate after 12h at room temperature

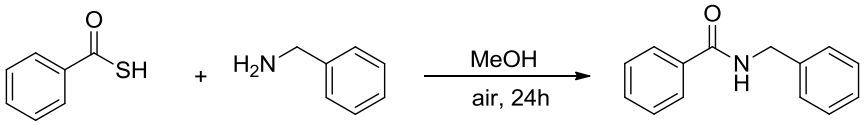


According to the ^{1}HNMR spectrum, the corresponding dithiocarbamate could decompose slowly (in red). Based on the results, we choose to prepare the amino ester / amino acid-derived dithiocarbamate *in situ* in the presence of excess CS_2, without further purification and directly used for next reactions.

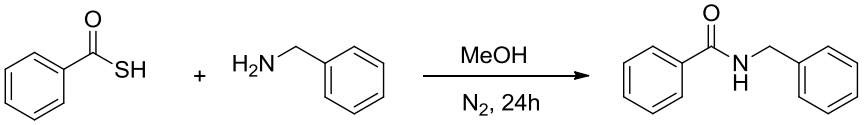
Control Experiments



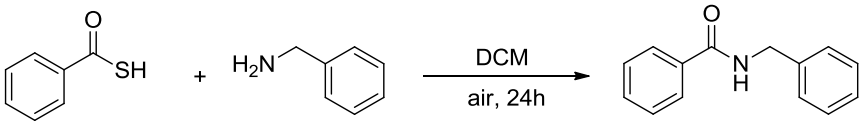
NR.



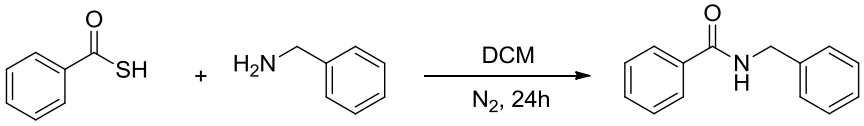
1.0 mmol 1.1 mmol 45.1mg(21.4%)



1.0 mmol 1.1 mmol 19.2mg(9.1%)



1.0 mmol 1.1 mmol 32.5 mg(15.4%)



1.0 mmol 1.1 mmol 11.2mg(5.3%)

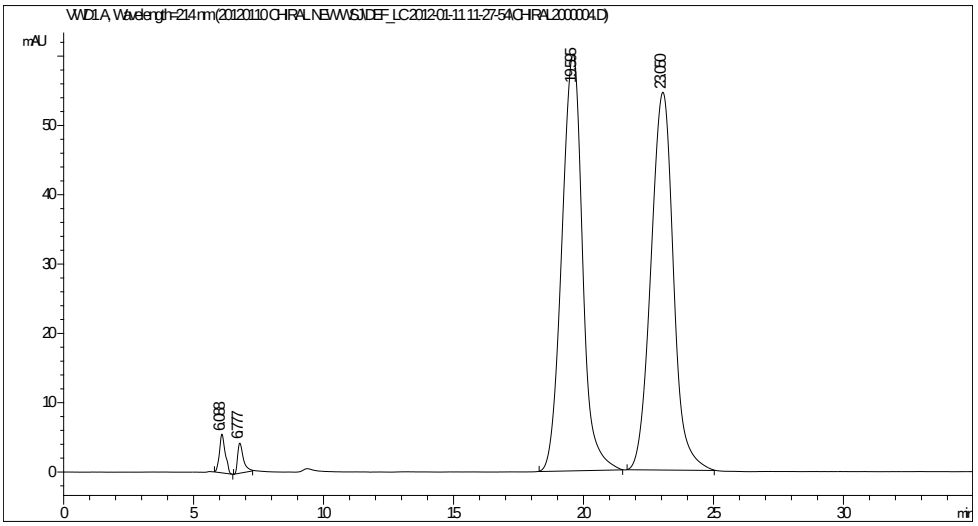
Epimerization Studies

1) Epimerization study of N-terminal

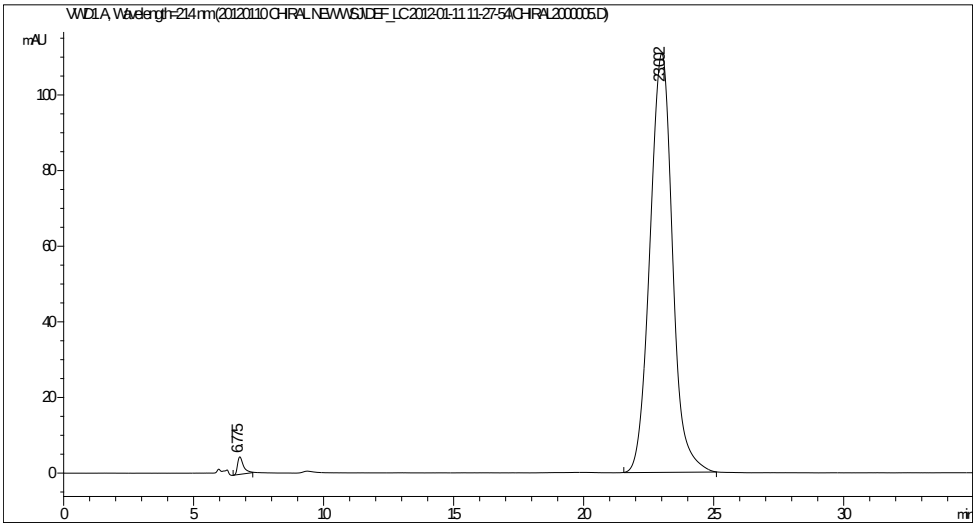
The reaction of AcSH and ^{L}Phe-OMe (^{D}Phe-OMe) derived dithiocarbamate is checked for epimerization, as also proved by the inspection of chiral HPLC spectrum listed in the following.

Chiral HPLC condition: 20 % B (MeCN) in A (H_2O with 50 mM PBS) over 35 min with a flow rate of 0.3 ml/ min and 214 UV detection.

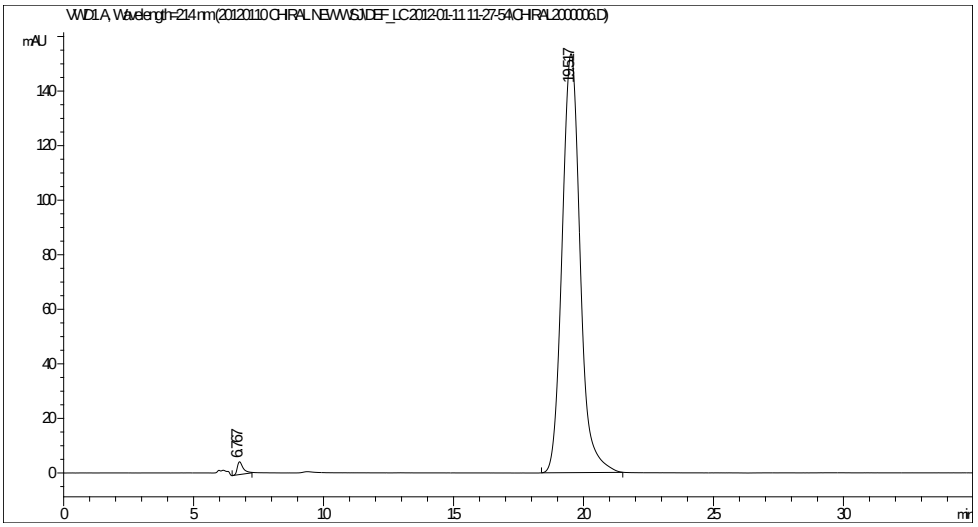
HPLC analysis of a mixture of Ac-^{L}Phe-COOMe and Ac-^{D}Phe-COOMe



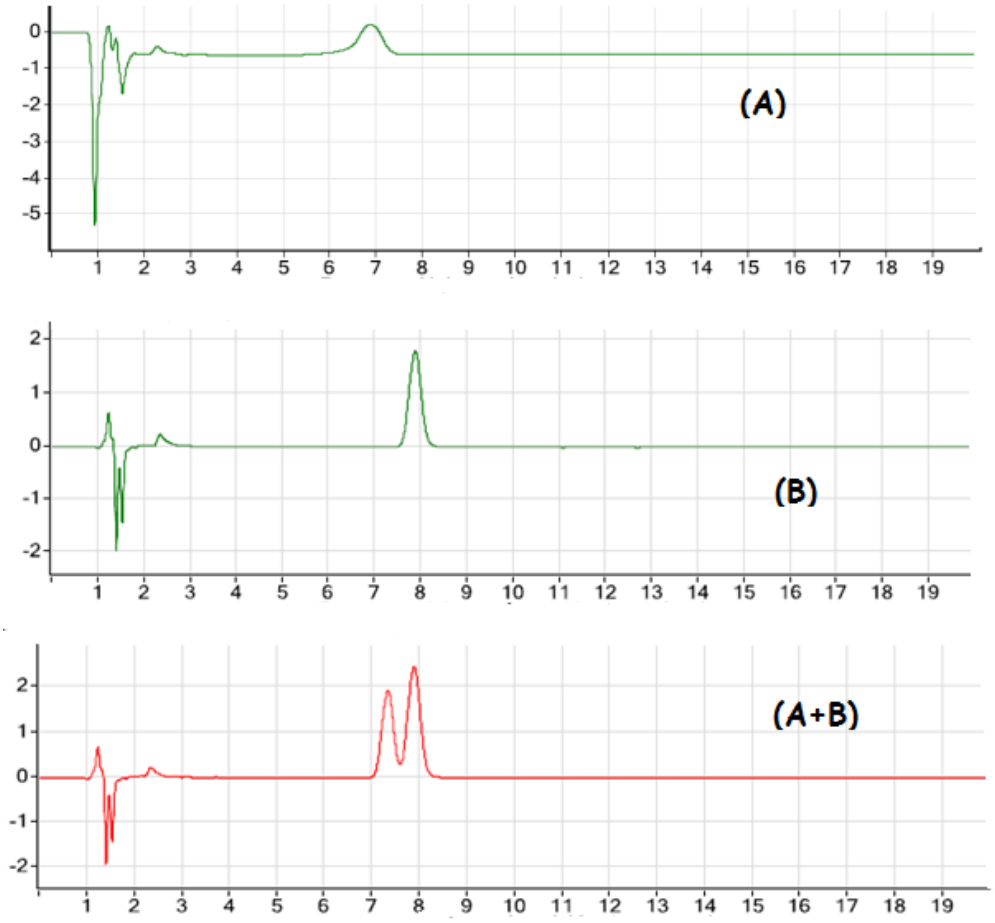
HPLC analysis of Ac-^{L}Phe-COOMe



HPLC analysis of Ac-^{D}Phe-COOMe

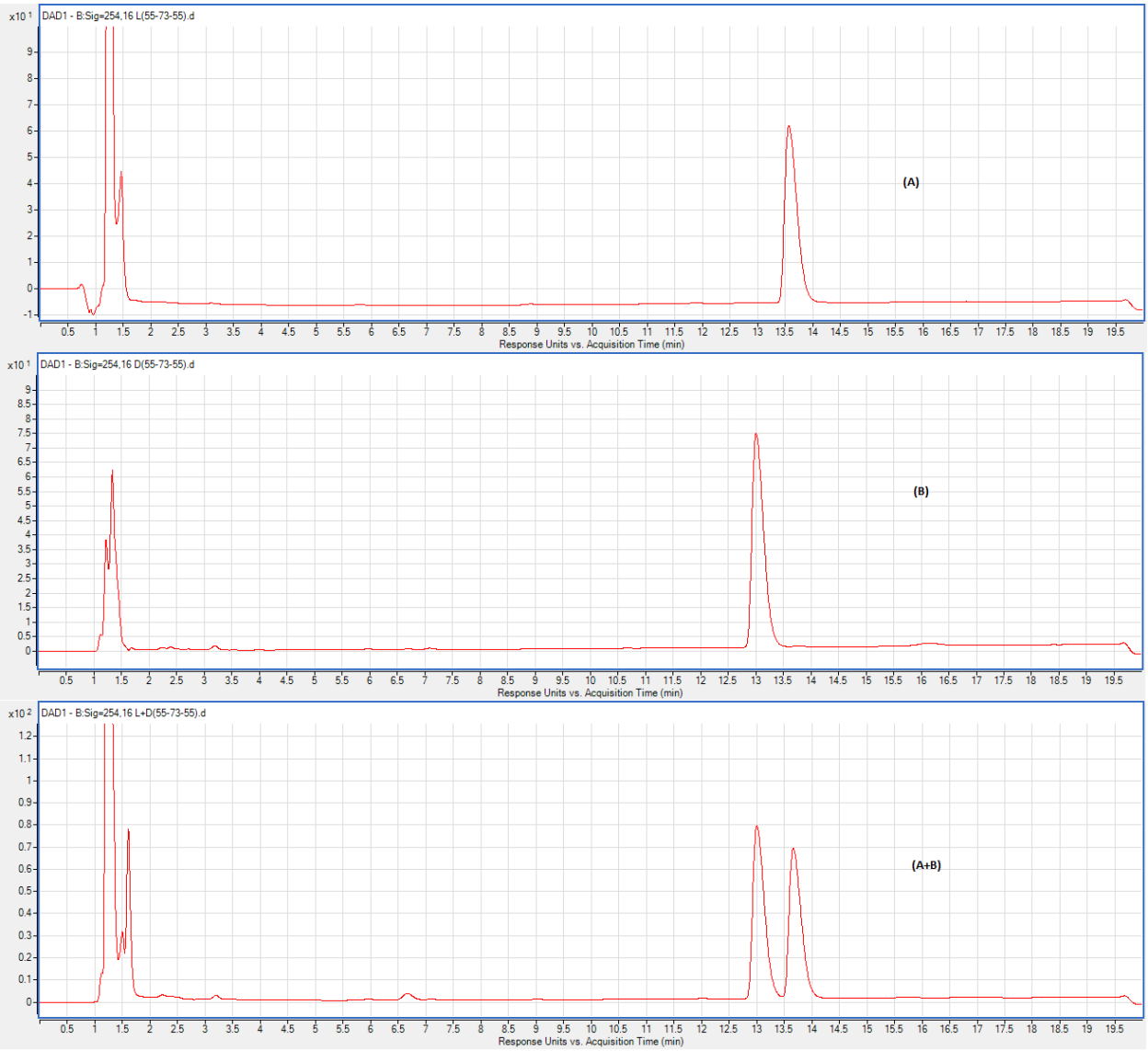


A = Boc-^{L}Phe-^{L}Phe-OMe, B = Boc-^{L}Phe-^{D}Phe-OMe A+B= Boc-^{L}Phe-(±)Phe-OMe
Eluent: 70 % CH_3CN (0.1 % formic acid) / 30 % H_2O (0.1 % formic acid) at 0.3 ml / min for a period of 20 mins.



2) Epimerization Study of C-terminal

A = Boc-^{L}Val-^{D}Phe-OMe, B = Boc-^{D}Val-^{D}Phe-OMe A+B= Boc-(±)Val-^{D}Phe-OMe
Eluent: 55-75 % CH_3OH (0.1 % formic acid) / 45-25 % H_2O (0.1 % formic acid) at 0.3 ml / min for a period of 18 mins, and then 75% CH_3OH (0.1 % formic acid) for a period of 2mins.

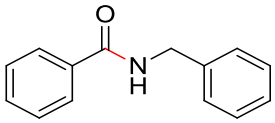


General Procedure A for Simple Amide Formation

To a stirred solution of the corresponding dithiocarbamate (1.0 mmol) in MeOH was added the thioacid (2.0 mmol). The reaction mixture was stirred for 8-17 h at room temperature then diluted with ethyl acetate and quenched with 1 M HCl solution. The organic layer was washed with saturated $NaHCO_3$ solution, brine, and then dried with anhydrous Na_2SO_4. After filtration and concentration under vacuum, the reaction residue was purified by chromatography to afford the desired amide.

Products from Table 1

N-benzylbenzamide (1-a)

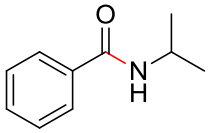


1-a

Prepared according to the general procedure A, stirred at room temperature for 8 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (194 mg, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 7.0 Hz, 2H), 7.42-7.25 (m, 8H), 6.66 (brs, 1H), 4.61 (d, J = 5.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO) δ 166.7, 140.2, 134.8, 131.7, 128.8, 128.7, 127.7, 127.6, 127.2, 43.1; LC-MS (ESI): *m/z* $([M+H])^+$: 212.3; HRMS (ESI): *m/z* calcd for $(C_{14}H_{13}NO+H)^+$: 212.1075; found: 212.1071.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 5.73 min.

N-isopropylbenzamide (1-b)

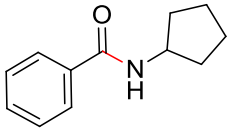


1-b

Prepared according to the general procedure A, stirred at room temperature for 8 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (139 mg, 85 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.48-7.45 (m, 1H), 7.41-7.38 (m, 2H), 6.16 (s, 1H), 4.31-4.24 (m, 1H), 1.26 (d, *J* = 1.5 Hz, 3H), 1.24 (d, *J* = 2.0 Hz, 3H); LC-MS (ESI): *m/z* $([M+H])^+$: 164.4; HRMS (ESI): *m/z* calcd for $(C_{10}H_{13}NO+H)^+$: 164.1075; found: 164.1075.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 4.67 min.

N-cyclopentylbenzamide (1-c)

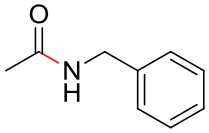


1-c

Prepared according to the general procedure A, stirred at room temperature for 8 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (172 mg, 91 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, *J*=7.5 Hz, 2H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 2H), 6.33 (s, 1H), 4.42-4.35 (m, 1H), 2.13-2.03 (m, 2H), 1.74-1.68 (m, 2H), 1.66-1.61 (m, 2H), 1.53-1.46 (m, 2H).LC-MS (ESI): *m/z* $([M+H])^+$: 190.4; HRMS (ESI): *m/z* calcd for $(C_{12}H_{15}NO+H)^+$: 190.1232; found: 190.1228.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 5.34 min.

N-benzylacetamide (1-d)

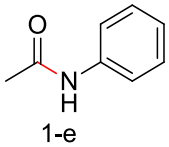


1-d

Prepared according to the general procedure A, stirred at room temperature for 8 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (128 mg, 86 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.27 (m, 5H), 6.04 (brs, 1H), 4.40 (d, *J* = 4.5 Hz, 2H), 2.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 138.3, 128.7, 127.8, 127.5, 43.7, 23.2; HRMS (ESI): *m/z* calcd for $(C_9H_{11}NO+H)^+$: 150.0919; found: 150.0915.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 3.81 min.

N-phenylacetamide (1-e)

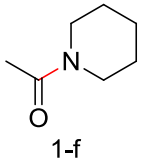


1-e

Prepared according to the general procedure A, stirred at room temperature for 12 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a yellow solid (81 mg, 60 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 2.16 (s, 3H). HRMS (ESI): *m/z* calcd for $(C_8H_9NO+H)^+$: 136.0762; found: 136.0761.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 4.02 min.

1-(piperidin-1-yl)ethanone (1-f)

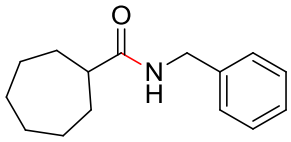


1-f

Prepared according to the general procedure A, stirred at room temperature for 12 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a yellow liquid (71 mg, 56 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.49- 3.46 (t, *J* = 5.5 Hz, 2H), 3.34-3.32 (t, *J* = 5.5 Hz, 2H), 2.02 (s, 3H), 1.59-1.55 (m, 2H), 1.51-1.48 (m, 2H), 1.48- 1.45 (m, 2H); HRMS (ESI): *m/z* calcd for $(C_7H_{13}NO+H)^+$: 128.1075; found: 128.1075.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 3.24 min.

N-benzylcycloheptanecarboxamide (1-g)

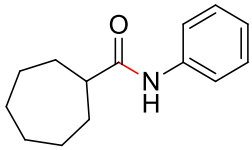


1-g

Prepared according to the general procedure A, stirred at room temperature for 8 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (155 mg, 67 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.32 (m, 2H), 7.29-7.26 (m, 3H), 5.67 (s, 1H), 4.43 (d, *J* = 5.5 Hz, 2H), 2.28-2.22(m, 1H), 1.94-1.89 (m, 2H), 1.80-1.42(m, 10H). HRMS (ESI): *m/z* calcd for $(C_{15}H_{21}NO+H)^+$: 232.1701; found: 232.1702.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 6.39 min.

N-phenylcycloheptanecarboxamide (1-h)

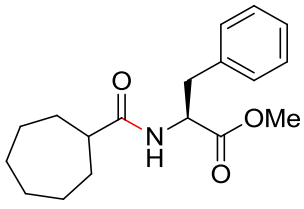


1-h

Prepared according to the general procedure A, stirred at room temperature for 12 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (115 mg, 53 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.19 (brs, 1H), 7.09 (t, *J* = 7.5Hz, 1H), 2.38-2.36 (m, 1H), 2.00-1.96 (m, 2H), 1.84-1.73 (m, 4H), 1.63-1.49 (m, 6H). HRMS (ESI): *m/z* calcd for $(C_{14}H_{19}NO+H)^+$: 218.1545; found: 218.1546.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 6.79 min.

(*S*)-methyl 2-(cycloheptanecarboxamido)-3-phenylpropanoate (1-i)



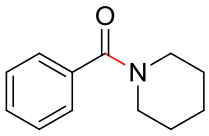
1-i

Prepared according to the general procedure A, stirred at room temperature for 8 h and purified

by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (209 mg, 69 % yield). ^{1}H NMR (500 MHz, DMSO) δ 8.12 (d, J = 7.5 Hz, 1H), 7.28-7.26 (m, 2H), 7.22-7.19 (m, 3H), 4.45-4.41 (m, 1H), 3.59 (s, 3H), 3.02 (dd, J_1 = 13.5 Hz, J_2=5.0 Hz, 1H), 2.88 (dd, J_1 = 14.0 Hz, J_2 = 10.0 Hz, 1H), 2.29-2.25 (m, 1H), 1.65-1.31 (m, 12H); HRMS (ESI): *m/z* calcd for $(C_{18}H_{25}NO_3+H)^+$: 304.1912; found: 304.1893.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 6.78 min.

phenyl(piperidin-1-yl)methanone (1-j)

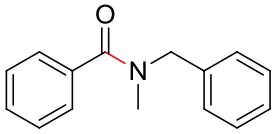


1-j

Prepared according to the general procedure A, stirred at room temperature for 17 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a pale white solid (109 mg, 58 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (s, 5H), 3.71 (s, 2H), 3.34 (s, 2H), 1.68 (s, 4H), 1.51 (s, 2H); HRMS (ESI): *m/z* calcd for $(C_{12}H_{15}NO+H)^+$: 190.1232; found: 190.1239.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 7.91 min.

N-benzyl-N-methylbenzamide (1-k)



1-k

Prepared according to the general procedure A, stirred at room temperature for 10 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a pale yellow oil (146 mg, 65 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.28 (m, 9H), 7.18 (brs, 1H), 4.76 (brs, 1H), 4.51 (brs, 1H), 3.03 (brs, 1.5H), 2.86 (brs, 1.5H); HRMS (ESI): *m/z* calcd for $(C_{15}H_{15}NO+H)^+$: 226.1232; found: 226.1237.

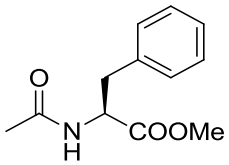
LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 8.27 min.

General Procedure B for Peptide Ester Formation

To a stirred solution of amino ester or peptide (0.2 mmol) and pyridine (0.1 M) in DCM (2.0 mL) was added CS_2 (0.1 M) at 0 ºC. After 1 h, the peptide thioacid (2.0 equiv.) was added and stirred in DCM for 24-48 h at room temperature then the reaction mixture was diluted with DCM and quenched with 1 M HCl solution. The organic layer was washed with saturated $NaHCO_3$ solution, brine, and then dried with anhydrous Na_2SO_4. After filtration and concentration under vacuum, the reaction residue was purified by chromatography to afford the desired peptide.

Products from Table 3

Ac-*L*-Phe-COOMe (3-a)

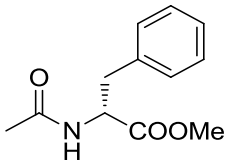


3-a

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 24 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (41 mg, 93 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31-7.25 (m, 3H), 7.09 (d, J = 7.0 Hz, 2H), 5.98 (brs, 1H), 4.91-4.87 (m, 1H), 3.73 (s, 3H), 3.15 (dd, J_1= 13.5 Hz, J_2 = 5.5 Hz, 1H), 3.11-3.08 (m, 1H), 1.99 (s, 3H). HRMS (ESI): *m/z* calcd for $(C_{12}H_{15}NO_3+H)^+$: 222.1130; found: 222.1125.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 5.84 min.

Ac-*D*-Phe-COOMe (3-b)

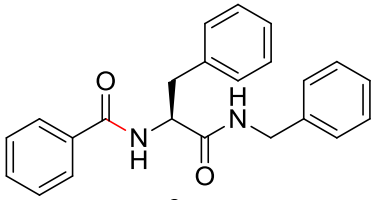


3-b

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 24 h and purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (42 mg, 94 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31-7.24 (m, 4H), 7.09 (d, J = 7.0 Hz, 2H), 5.91 (brs, 1H), 4.91-4.87 (m, 1H), 3.73 (s, 3H), 3.15 (dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz, 1H), 3.10 (dd, J_1 = 13.5 Hz, J_2 = 5.5 Hz, 1H), 1.99 (s, 3H). HRMS (ESI): *m/z* calcd for $(C_{12}H_{15}NO_3+H)^+$: 222.1130; found: 222.1125.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 5.84 min.

(*S*)-N-(1-(benzylamino)-1-oxo-3-phenylpropan-2-yl)benzamide (3-c)

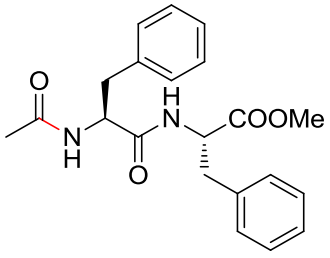


3-c

A solution of amine (1.0 mmol), pyridine (0.1 M) and CS_2 (0.1 M) in DCM (2.0 ml) was stirred at an ice-bath for 1h. Most of the solvent was removed under reduced pressure, and the precipitate collected by filtration. The resulting dithiocarbamate was used directly without purification. The thioacid (2.0 mmol) and the corresponding dithiocarbamate (1.0 mmol) were dissolved in DCM. The reaction mixture was stirred for 24 h at room temperature then diluted with DCM and quenched with 1 M HCl solution. The organic layer was washed with saturated $NaHCO_3$ solution, brine, and then dried with anhydrous Na_2SO_4. After filtration and concentration under vacuum, the reaction residue was purified by chromatography (30% ethyl acetate in hexanes) to give the product as a white solid (330 mg, 92 % yield). ^{1}H NMR (500 MHz, DMSO) δ 8.65-8.61 (m, 2H), 7.82 (d, J = 7.5 Hz, 2H), 7.51 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 7.35 (d, J = 7.5 Hz, 2H), 7.31 (t, J = 7.0 Hz, 2H), 7.27-7.22 (m, 5H), 7.17 (t, J = 7.0 Hz, 1H), 4.77-4.73 (m, 1H), 4.32 (d, J = 6.0 Hz, 2H), 3.15 (dd, J_1 = 14.0 Hz, J_2 = 4.5 Hz, 1H), 3.04 (dd, J_1 = 13.5, J_2 = 10.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO) δ 171.8, 166.8, 139.8, 138.9, 134.6, 131.7, 129.6, 128.7, 128.6, 128.5, 127.9, 127.6, 127.2, 126.7, 55.6, 42.6, 37.8; HRMS (ESI): *m/z* calcd for $(C_{23}H_{22}N_2O_2+H)^+$: 359.1759; found: 359.1747.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 6.21 min.

(S)-methyl 2-((S)-2-acetamido-3-phenylpropanamido)-3-phenylpropanoate (3-d)

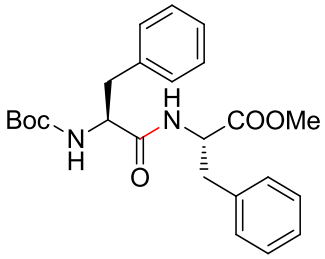


3-d

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (65 mg, 88 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.17 (m, 8H), 7.02-7.01 (m, 2H), 6.36 (d, J = 7.5 Hz, 1H), 6.18 (d, J = 8.0 Hz, 1H), 4.74 (dd, J_1 = 13.5 Hz, J_2 = 6.0 Hz, 1H), 4.65 (dd, J_1 = 14.5 Hz, J_2 = 7.0 Hz, 1H), 3.67 (s, 3H), 3.08 (dd, J_1 = 13.5 Hz, J_2 = 5.5 Hz, 1H), 3.02 (d, J = 7.0 Hz, 2H), 2.97 (dd, J_1 = 14.0 Hz, J_2 = 7.0 Hz, 1H), 1.92 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 170.6, 170.0, 136.4, 135.6, 129.3, 129.2, 128.6, 128.5, 127.1, 127.0, 54.3, 53.4, 52.3, 38.1, 37.9, 23.1.LC-MS (ESI): *m/z* $([M+H])^+$: 369.2; HRMS (ESI): *m/z* calcd for $(C_{21}H_{24}N_2O_4+H)^+$: 369.1814; found: 369.1811.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 5.36 min.

Boc-*L*-Phe-*L*-Phe-COOMe (3-e)

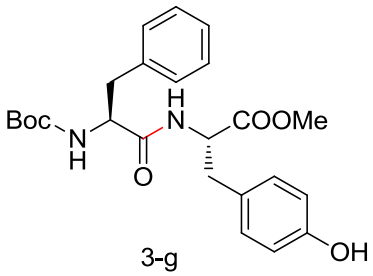


3-e

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (67 mg, 79 % yield); ^{1}H NMR (500 MHz, DMSO) δ 8.47 (d, *J* = 7.5 Hz, 1H), 7.29-7.22 (m, 10H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.52-4.48 (m, 1H), 4.21-4.16 (m, 1H), 3.58 (s, 3H), 3.07-2.97 (m, 2H), 2.90 (dd, J_1= 13.5Hz, J_2=4.0 Hz, 1H), 2.69 (dd, J_1= 13.5Hz, J_2=10.5 Hz, 1H), 1.28 (s, 9H); ^{13}C NMR (125 MHz, DMSO) δ 172.3, 172.2, 155.5, 138.5, 137.6, 129.7, 129.6, 128.7, 128.4, 127.0, 126.6, 78.5, 56.0, 54.1, 52.3, 37.9, 37.1, 28.6; HRMS (ESI): *m/z* calcd for $(C_{24}H_{30}N_2O_5+Na)^+$: 449.2053; found: 449.2043.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 7.12 min.

Boc-*L*-Phe-*L*-Tyr-COOMe (3-g)

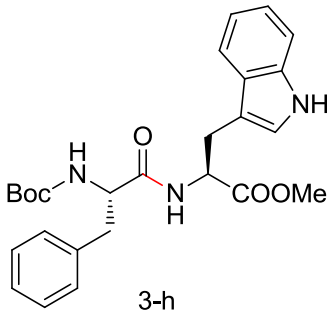


3-g

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (71 mg, 80 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.17 (m, 4H), 6.83-6.82 (m, 2H), 6.68 (m, 2H), 6.49 (m, 1H), 5.08 (m, 1H), 4.76 (m, 1H), 4.35 (m, 1H), 3.67 (s, 3H), 3.01-2.96 (m, 4H), 1.40 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.0, 154.3, 135.3, 129.3, 128.3, 127.6, 125.9, 114.5, 54.6, 52.5, 51.3, 37.3, 36.2, 28.7, 27.2; HRMS (ESI): *m/z* calcd for $(C_{24}H_{30}N_2O_6+Na)^+$: 465.2002; found: 465.1983.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 6.06 min.

Boc-*L*-Phe-*L*-Trp-COOMe (3-h)

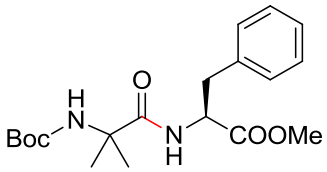


3-h

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (72 mg, 77 % yield). ^{1}H NMR (500 MHz, DMSO) δ 10.90 (s, 1H), 8.30 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.24-7.17 (m, 6H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.56-4.55 (m, 1H), 4.21-4.20 (m, 1H), 3.56 (s, 3H), 3.13-3.09 (m, 2H), 2.95-2.91 (m, 1H), 2.72-2.67 (m, 1H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, DMSO) δ 172.7, 1723, 155.6, 138.5, 136.6, 129.7, 128.4, 127.6, 126.6, 124.2, 121.5, 118.9, 118.5, 111.9, 109.6, 78.5, 56.0, 53.5, 52.3, 37.9, 28.6, 27.5; HRMS (ESI): *m/z* calcd for $(C_{26}H_{31}N_3O_5+Na)^+$: 488.2162; found: 488.2150.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 6.78 min.

Boc-Aib-*L*-Phe-COOMe (3-i)

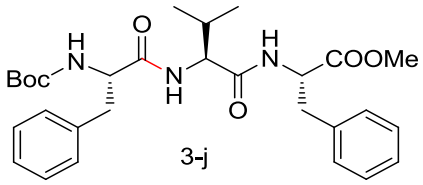


3-i

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (45 mg, 62 % yield); ^{1}H NMR (500 MHz, DMSO) δ 7.70 (d, *J* = 8.0 Hz, 1H), 7.27-7.24 (m, 2H), 7.21-7.18 (m, 3H), 6.80 (s, 1H), 4.52 (dd, J_1 = 13.5 Hz, J_2 = 8.0 Hz, 1H), 3.59 (s, 3H), 3.04-2.95 (m, 2H), 1.35 (s, 9H), 1.24-1.22 (m, 6H); ^{13}C NMR (125 MHz, DMSO) δ 174.3, 171.9, 154.1, 137.2, 129.1, 128.1, 126.4, 78.0, 55.6, 53.4, 51.8, 36.7, 28.1; LC-MS (ESI): *m/z* $([M+Na])^+$: 387.4; HRMS (ESI): *m/z* calcd for $(C_{19}H_{28}N_2O_5+Na)^+$: 387.1896; found: 387.1892.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 6.25 min.

Boc-*L*-Phe-*L*-Val-*L*-Phe-COOMe (3-j)



3-j

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a grey solid (75 mg, 71 % yield). ^{1}H NMR (500 MHz, DMSO) δ 8.50 (d, *J* = 7.0 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.27-7.18 (m, 10H), 7.03 (d, *J* = 8.5 Hz, 1H), 4.50-4.48 (m, 1H), 4.26 (m, 1H), 4.19 (m, 1H), 3.56 (s, 3H), 3.04 (dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz, 1H), 2.97-2.90 (m, 2H), 2.72 (t, *J* = 12.5 Hz, 1H), 1.96-1.93 (m, 1H), 1.29 (s, 9H), 0.84 (dd, J_1= 13.5 Hz, J_2 = 6.5 Hz, 6H).^{13}C NMR (125 MHz, DMSO) δ 172.2, 171.9, 171.4, 155.7, 138.7, 137.5, 129.6, 129.4, 128.7, 128.4, 127.0, 126.6, 78.6, 57.4, 56.2, 54.0, 52.2, 37.6, 37.0, 31.7, 28.6, 19.5, 18.3; HRMS (ESI): *m/z* calcd for $(C_{29}H_{39}N_3O_6+Na)^+$: 548.2737; found: 548.2731.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 7.02 min.

Boc-*L*-Phe-*L*-Val-*L*-Phe-CONHCH$_2$Ph (3-k)

Prepared according to the general procedure B, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (21 mg, 72 % yield). ^{1}HNMR (500 MHz, DMSO) δ 8.45 (s, 1H), 8.21 (d, *J* = 7.0 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.24-7.18 (m, 12H), 7.11 (d, *J* = 6.5 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 4.58 (d, *J* = 6.5 Hz, 1H), 4.24-4.18 (m, 4H), 2.98-2.83 (m, 3H), 2.74-2.69 (m, 1H), 1.99-1.94 (m, 1H), 1.29 (s, 9H), 0.79 (s, 6H). HRMS (ESI): *m/z* calcd for $(C_{35}H_{44}N_4O_5+Na)^+$: 623.3210; found: 623.3163.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 7.43 min.

Boc-*L*-Leu-*L*-Phe-*L*-Val-*L*-Trp-COOMe (3-l)

Prepared according to the general procedure B, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (17 mg, 51 % yield). ^{1}H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 8.43 (d, *J* = 6.8 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.17-7.12 (m, 6H), 7.07 (t, *J* = 7.2 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H),

6.92 (d, J = 8.4 Hz, 1H), 4.62-4.61 (m, 1H), 4.53 (dd, J_1 = 13.6, J_2 = 6.8 Hz, 1H), 4.28-4.24 (m, 1H), 3.89-3.88 (m, 1H), 3.53 (s, 3H), 3.18-3.05 (m, 2H), 2.96 (dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz, 1H), 2.79 (dd, J_1= 13.6 Hz, J_2 = 9.2 Hz, 1H), 1.99-1.91 (m, 1H), 1.51-1.43 (m, 1H), 1.35 (s, 9H), 0.85-0.77 (m, 13H). HRMS (ESI): *m/z* calcd for $(C_{37}H_{51}N_5O_7+H)^+$: 678.3866; found: 678.3850.

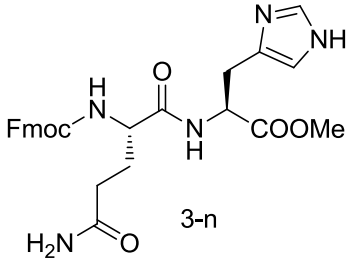
LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 7.28 min.

Boc-*L*-Leu-*L*-Phe-*L*-Val-*L*-Phe-*L*-Ile-COOMe (3-m)

Prepared according to the general procedure B, the reaction was performed at 0.2 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (83 mg, 55 % yield). HRMS (ESI): *m/z* calcd for $(C_{41}H_{61}N_5O_8+Na)^+$: 774.4418; found: 774.4345.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 7.97 min.

Fmoc-*L*-Gln-*L*-His-COOMe (3-n)

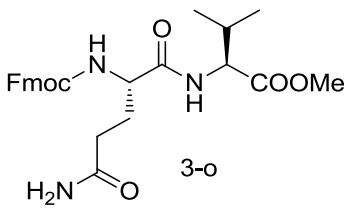


3-n

Prepared according to the general procedure B, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (16 mg, 60 % yield). LC-MS (ESI): *m/z* $([M+H])^+$: 520.5; HRMS (ESI): *m/z* calcd for $(C_{27}H_{29}N_5O_6+H)$+: 520.2196; found: 520.2180.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 3.64 min.

Fmoc-*L*-Gln-*L*-Val-COOMe (3-o)

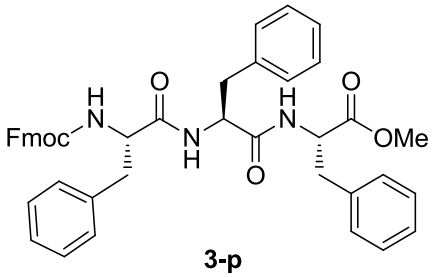


3-o

Prepared according to the general procedure B, the reaction was performed at 0.1 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (17 mg, 35 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.05-7.02 (m, 1H), 6.16 (m, 1H), 5.95 (m, 1H), 5.43 (m, 1H), 4.53-4.50 (m, 1H), 4.38 (d, *J* = 7.0 Hz, 2H), 4.28 (brs, 1H), 4.22 (t, *J* = 7.0Hz, 1H), 3.74 (s, 3H), 2.49 (m, 1H), 2.37 (m, 1H), 2.20-2.19 (m, 2H), 2.02 (m, 1H), 0.95 (d, *J* = 7.0 Hz, 3H), 0.90 (d, *J* = 7.0 Hz, 3H).LC-MS (ESI): *m/z* $([M+H])^+$: 482.4; HRMS (ESI): *m/z* calcd for $(C_{26}H_{31}N_3O_6+Na)^+$: 504.2111; found: 504.2104.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 5.85 min.

Fmoc-*L*-Phe-*L*-Phe-*L*-Phe-COOMe (3-p)

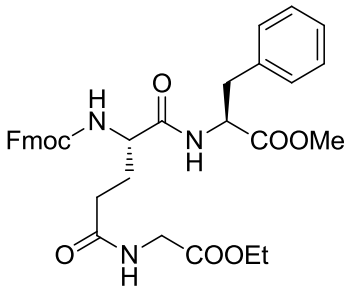


3-p

Prepared according to the general procedure B, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (14 mg, 40 % yield). LC-MS (ESI): *m/z* $([M+Na])^+$: 718.5; HRMS (ESI): *m/z* calcd for $(C_{43}H_{41}N_3O_6+Na)^+$: 718.2893; found: 718.2888.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 8.06 min.

Fmoc-*L*-Glu(Gly-COOEt)-*L*-Phe-COOMe (3-q)



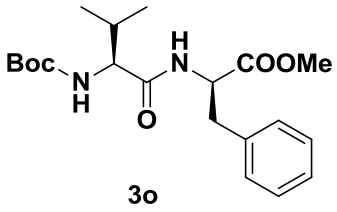
3-q

Prepared according to the general procedure B, the reaction was performed at 0.1 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the

product as a white solid (22 mg, 36 % yield). ^{1}H NMR (500 MHz, DMSO) δ 8.35 (d, *J* = 7.5 Hz, 1H), 8.25 (t, *J* = 6.0 Hz, 1H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 6.0 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.27-7.17 (m, 5H), 4.47 (dd, J_1 = 14.5 Hz, J_2 = 8.0 Hz, 1H), 4.28-4.19 (m, 3H), 4.09 (q, *J* = 7.0 Hz, 2H), 4.05-4.02 (m, 1H), 3.81 (d, *J* = 6.0 Hz, 2H), 3.58 (s, 3H), 3.02 (dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz, 1H), 2.95 (dd, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H), 2.25-2.14 (m, 2H), 1.92-1.85 (m, 1H), 1.78-1.71 (m, 1H), 1.19 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 172.1, 171.9, 171.8, 170.0, 155.9, 143.9, 143.8, 140.7, 137.0, 129.1, 128.3, 127.7, 127.1, 126.6, 125.4, 120.1, 65.7, 60.4, 54.0, 53.6, 51.9, 46.6, 40.7, 36.6, 31.5, 27.9, 14.1.LC-MS (ESI): *m/z* ([M+H])$^{+}$: 616.5; HRMS (ESI): *m/z* calcd for ($C_{34}H_{37}N_3O_8$+Na)$^{+}$: 638.2479; found: 638.2473.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 7.10 min.

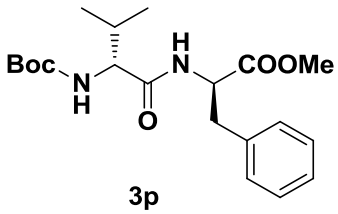
Boc-L-Val-D-Phe-COOMe (3o)



3o

Prepared according to the general procedure B, the reaction was performed at 1.0 mmol of the substrate, stirred at room temperature for 30 h and purified by chromatography to give the product as a white solid (295 mg, 78 % yield). HRMS (ESI): *m/z* calcd for ($C_{20}H_{30}N_2O_5$+H)$^{+}$: 379.2233; found: 379.2243.

Boc-D-Val-D-Phe-COOMe (3p)



3p

Prepared according to the general procedure B, the reaction was performed at 1.0 mmol of the substrate, stirred at room temperature for 30 h and purified by chromatography to give the product as a white solid (272 mg, 72 % yield). HRMS (ESI): *m/z* calcd for ($C_{20}H_{30}N_2O_5$+H)$^{+}$: 379.2233; found: 379.2245.

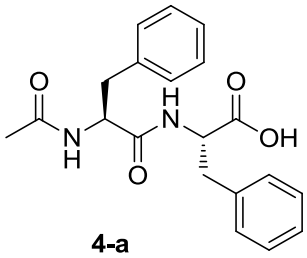
General Procedure C for Peptide Formation in Aqueous Media

Peptides or amino acid, pyridine (0.1 M) and CS_2 (0.1 M) were dissolved in 1.0 ml of ligation buffer [4:1 v/v *N*-methyl-2- pyrrolidinone (NMP): 1.0 M HEPES, pH = 7.0] [a]. This solution was transferred to a tube containing the thioacid peptide (approx. 2.0 equiv.). The ligation mixture was then stirred gently at room temperature every 12 h until the reaction was confirmed to be completed by LC-MS. The ligation reactions were quenched by the addition of 50 % HOAc in water (1.0 mL). The ligation residue was lyophilized and purified by semi-preparative HPLC.

[a] An aqueous buffer containing 1.0 M HEPES was prepared and adjusted to pH 7.0 using 25% aqueous sodium hydroxide solution. The resulting solution (1 mL) was diluted with NMP (4 mL) to produce the final buffer for use in the reactions.

Products from Table 4

(S)-2-((S)-2-acetamido-3-phenylpropanamido)-3-phenylpropanoic acid (4-a)



4-a

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (13 mg, 75 % yield). ^{1}H NMR (500 MHz, DMSO) δ 8.11 (d, J = 6.5 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.27-7.16 (m, 10H), 4.48 (m, 1H), 4.36 (m, 1H), 3.08 (dd, J_1= 13.5 Hz, J_2 = 5.0 Hz, 1H), 2.98 (dd, J_1 = 14.0 Hz, J_2 = 4.0 Hz, 1H), 2.93 (dd, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H), 2.66 (dd, J_1= 13.5 Hz, J_2 = 10.5 Hz, 1H), 1.71 (s, 3H). LC-MS (ESI): *m/z* $([M+H])^+$: 355.3; HRMS (ESI): *m/z* calcd for $(C_{20}H_{22}N_2O_4+H)^+$: 355.1658; found: 355.1646.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 5.07 min.

Boc-*L*-Phe-*L*-Val-*L*-Ala-*L*-Glu-*L*-Val-*L*-Tyr-$CONH_2$ (4-b)

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (15 mg, 37 % yield). LC-MS (ESI): *m/z* $([M+H])^+$: 826.6; HRMS (ESI): *m/z* calcd for $(C_{41}H_{59}N_7O_{11}+Na)^+$: 848.4171; found: 848.4148.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 5.20 min.

Boc-*L*-Phe-*L*-Val-*L*-Ala-*L*-Ile-*L*-Ser-*L*-Pro-*L*-Ala-COOH (4-c)

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (15 mg, 37 % yield). LC-MS (ESI): *m/z* $([M+H])^+$: 804.6; HRMS (ESI): *m/z* calcd for $(C_{39}H_{61}N_7O_{11}+Na)^+$: 826.4327; found: 826.4341.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 5.25 min.

Boc-*L*-Pro-*L*-Phe-*L*-Phe-*L*-Asn-*L*-Arg-Gly-*L*-Ala-COOH (4-d)

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (27 mg, 60 % yield). LC-MS (ESI): *m/z* $([M+H])^+$: 908.7; HRMS (ESI): *m/z* calcd for $(C_{43}H_{61}N_{11}O_{11}+H)^+$: 908.4630; found: 908.4620.

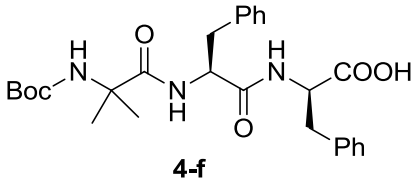
LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 4.25 min.

Boc-Aib-*L*-Ala-*L*-Glu-*L*-Ile-*L*-Gln-*L*-Thr-COOH (4-e)

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (15 mg, 40 % yield). LC-MS (ESI): *m/z* $([M+H])^+$: 746.3; HRMS (ESI): *m/z* calcd for $(C_{32}H_{55}N_7O_{13}+Na)^+$: 768.3756; found: 768.3753.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 4.10 min.

Boc-Aib-*L*-Phe-*L*-Phe-COOH (4-f)



4-f

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (14 mg, 55 % yield). LC-MS (ESI): *m/z* $([M+Na])^+$: 520.2; HRMS (ESI): *m/z* calcd for $(C_{27}H_{35}N_3O_6+H)^+$: 498.2604; found: 498.2602.

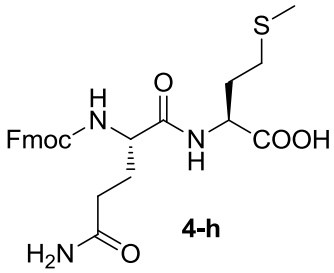
LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 6.13 min.

Boc-pGlu-*L*-Ala-*L*-Ile-*L*-Ser-*L*-Pro-*L*-Ala-COOH (4-g)

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (15 mg, 44 % yield). LC-MS (ESI): *m/z* $([M+Na])^+$: 691.3; HRMS (ESI): *m/z* calcd for $(C_{30}H_{48}N_6O_{11}+Na)^+$: 691.3279; found: 691.3271.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 3.68 min.

Fmoc-*L*-Gln-*L*-Met-COOH (4-h)

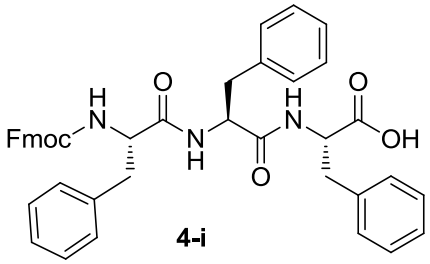


4-h

Prepared according to the general procedure C, the reaction was performed at 0.1 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (27 mg, 54 % yield). ^{1}H NMR (500 MHz, DMSO) δ 8.07 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.73 (t, *J* = 7.0 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 7.5Hz, 2H), 7.34 (t, *J* = 7.0 Hz, 2H), 7.29 (s, 1H), 6.78 (s, 1H), 4.26-4.22 (m, 4H), 4.02-3.97 (m, 1H), 2.46-2.40 (m, 2H), 2.21-2.11 (m, 2H), 2.01 (s, 3H), 1.97-1.82 (m, 3H), 1.77-1.69 (m, 1H). ^{13}C NMR (125 MHz, DMSO) δ 173.8, 173.3, 171.6, 155.9, 143.9, 143.8, 140.7, 127.7, 127.1, 125.4, 120.1, 65.7, 54.3, 51.4, 46.6, 31.6, 31.1, 29.6, 27.8, 14.6. LC-MS (ESI): *m/z* $([M+H])^+$: 500.4; HRMS (ESI): *m/z* calcd for $(C_{25}H_{29}N_3O_6S+H)^+$: 500.1855; found: 500.1852.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 214 nm UV detection, retention time = 5.45 min.

Fmoc-*L*-Phe-*L*-Phe-*L*-Phe-COOH (4-i)



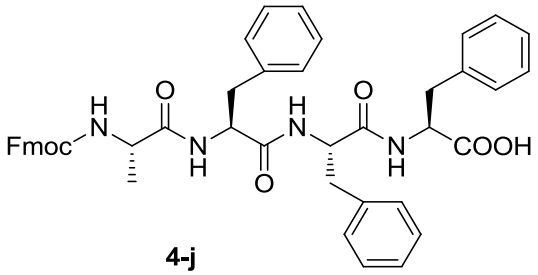
4-i

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the

product as a white solid (15 mg, 45 % yield). LC-MS (ESI): *m/z* $([M+Na])^+$: 704.5; HRMS (ESI): *m/z* calcd for $(C_{42}H_{39}N_3O_6+H)^+$: 682.2917; found: 682.2912.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 7.69 min.

Fmoc-*L*-Ala-*L*-Phe-*L*-Phe-L-Phe-COOH (4-j)

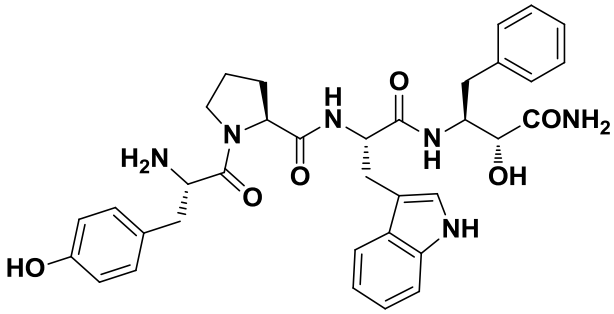


4-j

Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 36 h and purified by chromatography to give the product as a white solid (13 mg, 34 % yield). LC-MS (ESI): *m/z* $([M+H])^+$: 753.5; HRMS (ESI): *m/z* calcd for $(C_{45}H_{44}N_4O_7+H)^+$: 753.3288; found: 753.3281.

LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 7.42 min.

General Procedure for the Synthesis of Endomorphin Derivative 5a

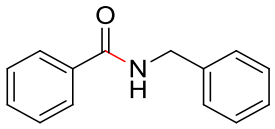


5a

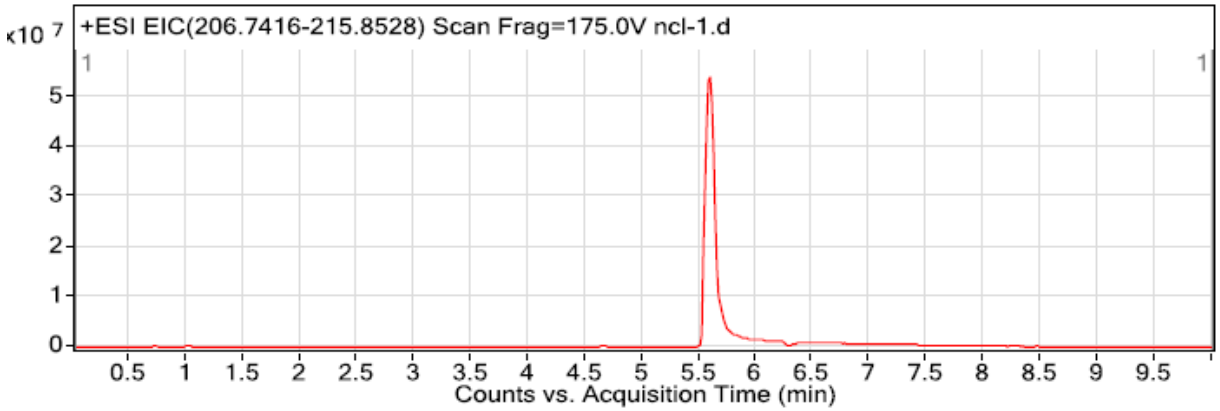
Prepared according to the general procedure C, the reaction was performed at 0.05 mmol of the substrate, stirred at room temperature for 24 h, deprotected with 10% piperidine/DMF and purified by chromatography to give the product as a white solid (13 mg, 20.5 % yield in two steps). ^{1}H NMR (400 MHz, DMSO) δ 10.75 (s, 1H), 8.05-7.74 (m, 3H), 7.53 (t, J = 8.4 Hz, 1H), 7.44-7.12 (m, 8H), 7.00 (ddd, J_1 = 23.5 Hz, J_2 =14.2 Hz, J_3 =7.0 Hz, 4H), 6.86 (d, J = 8.3 Hz, 1H), 6.67-6.60 (m, 2H), 4.59-4.21 (m, 3H), 3.90 (dd, J_1 = 5.0 Hz, J_2 =3.5 Hz, 1H), 3.53 (dd, J_1 = 8.1 Hz, J_2 = 2.5 Hz, 2H), 3.29 (dd, J_1 = 13.6 Hz, J_2 = 5.7 Hz, 1H), 3.20-3.11 (m, 1H), 3.04 (dd, J_1 = 14.9 Hz, J_2 = 5.1 Hz, 1H), 2.90 (dd, J_1 =15.4 Hz, J_2 = 8.0 Hz, 2H), 2.80-2.61 (m, 3H), 2.54 (d, J =

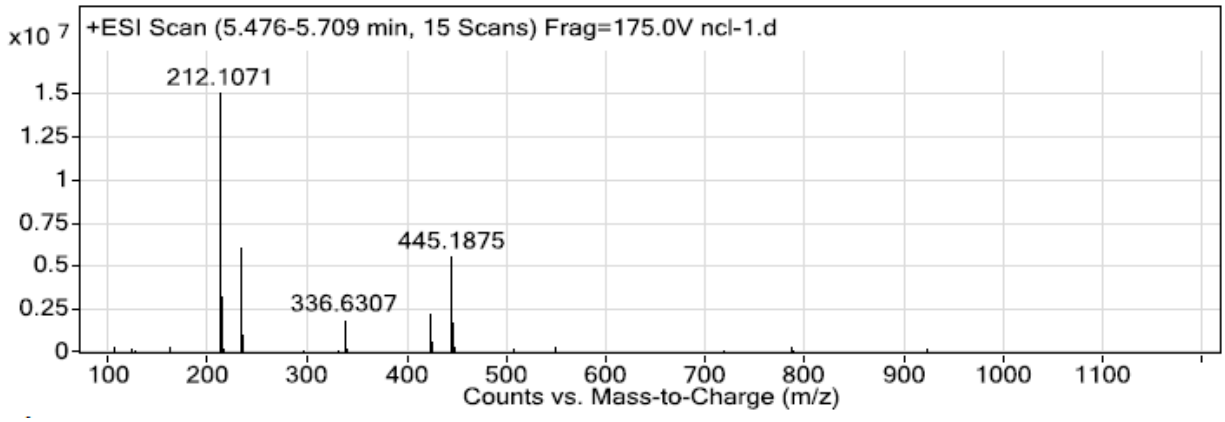
6.7 Hz, 1H), 1.99-1.66 (m, 2H), 1.35 (ddt, J_1 = 27.2 Hz, J_2 = 21.9 Hz, J_3 = 11.4 Hz, 3H). LC-MS (ESI): m/z ($[M+H]$)$^+$: 641.15;

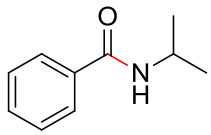
LCMS: 5-95% B over 6 min then 95% B over 1 min with a flow rate of 0.5 mL/min and 254 nm UV detection, retention time = 3.50 min.



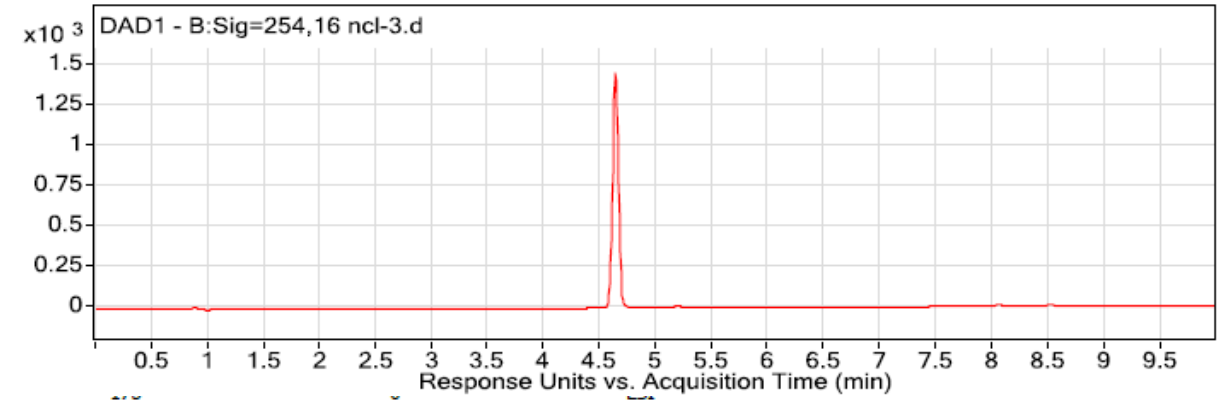
1-a

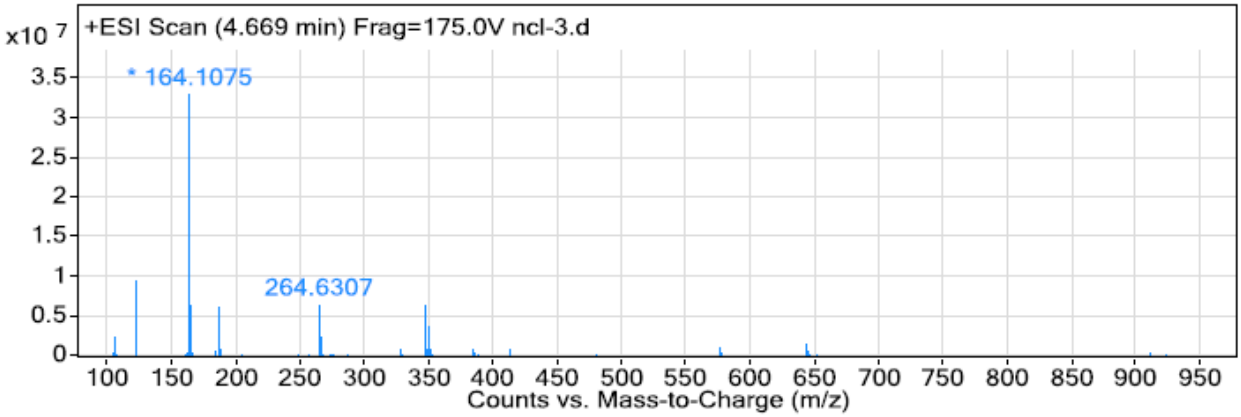


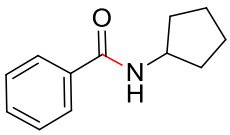




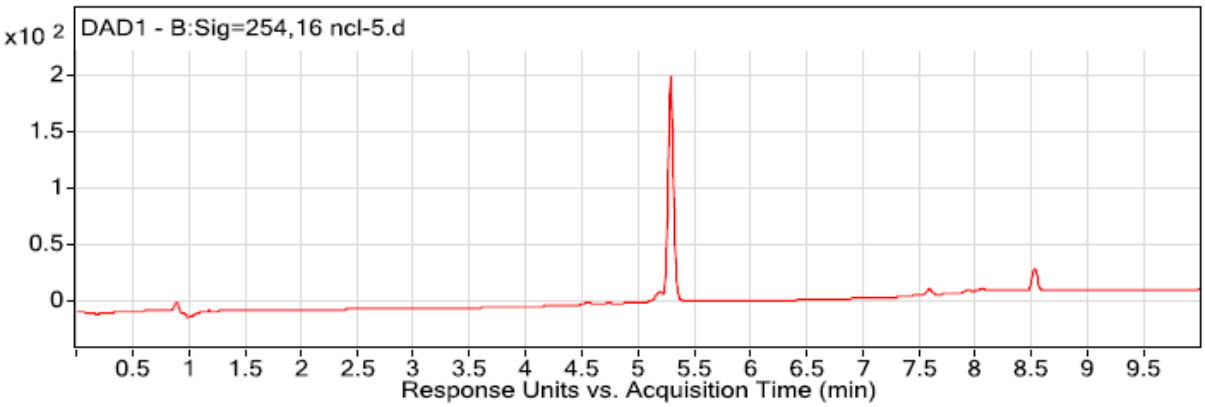
1-b

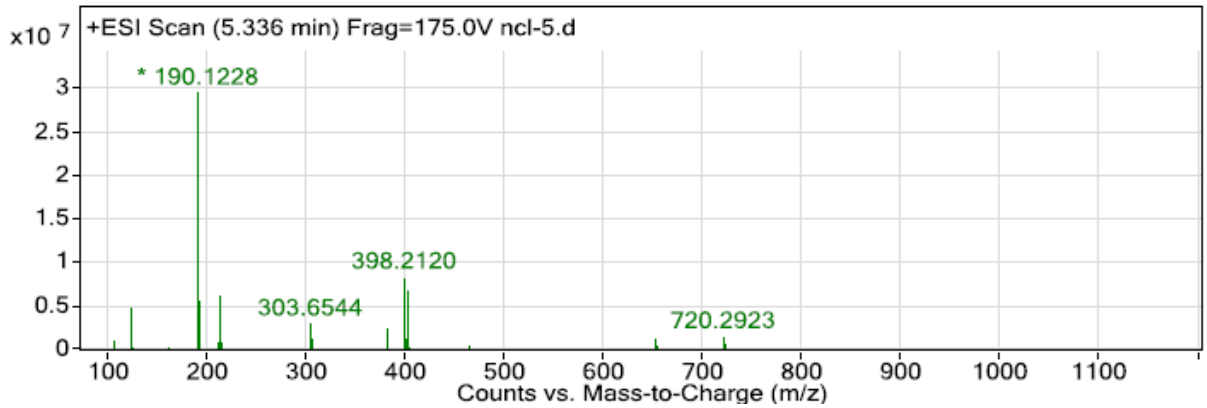


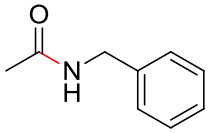




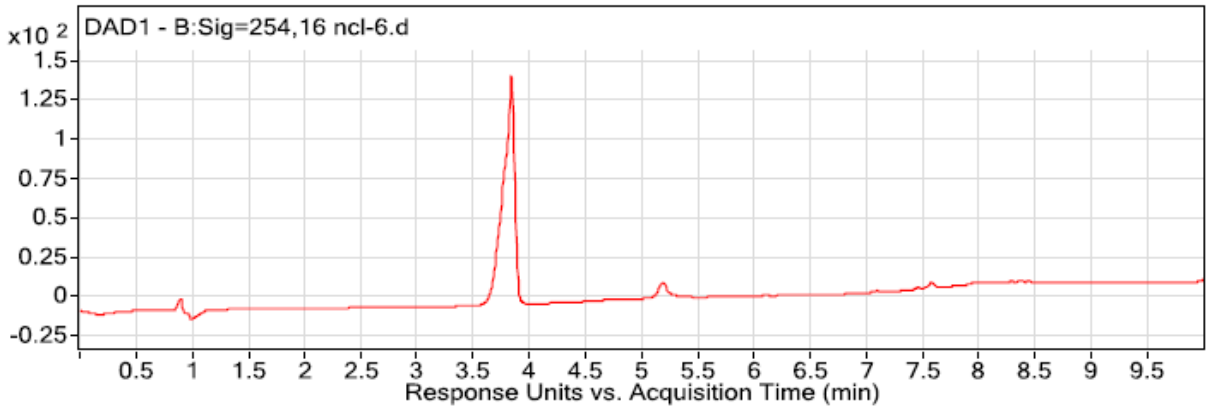
1-c

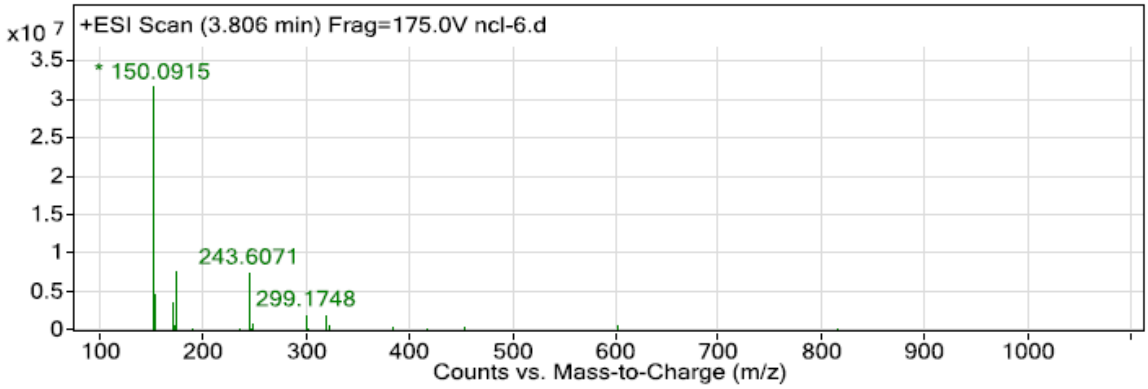


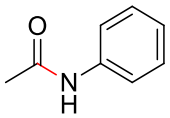




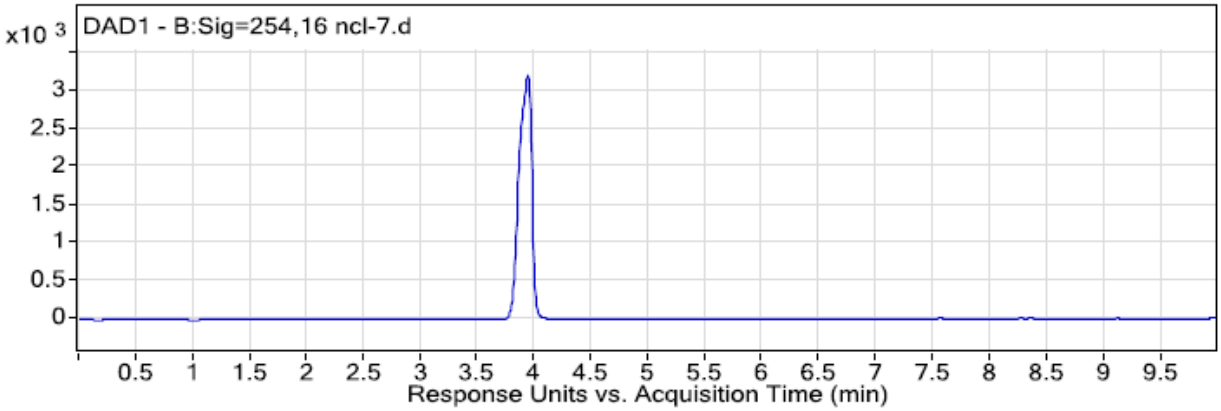
1-d

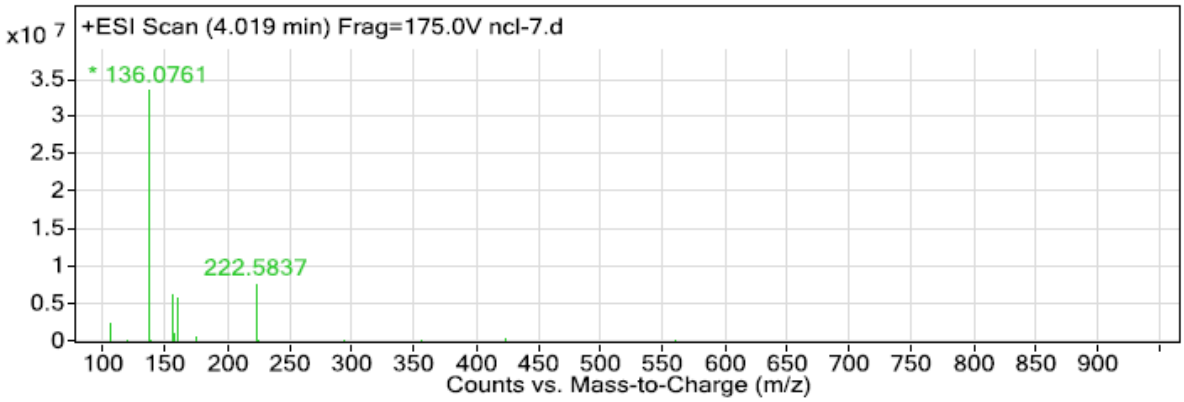


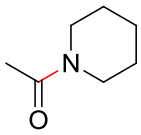




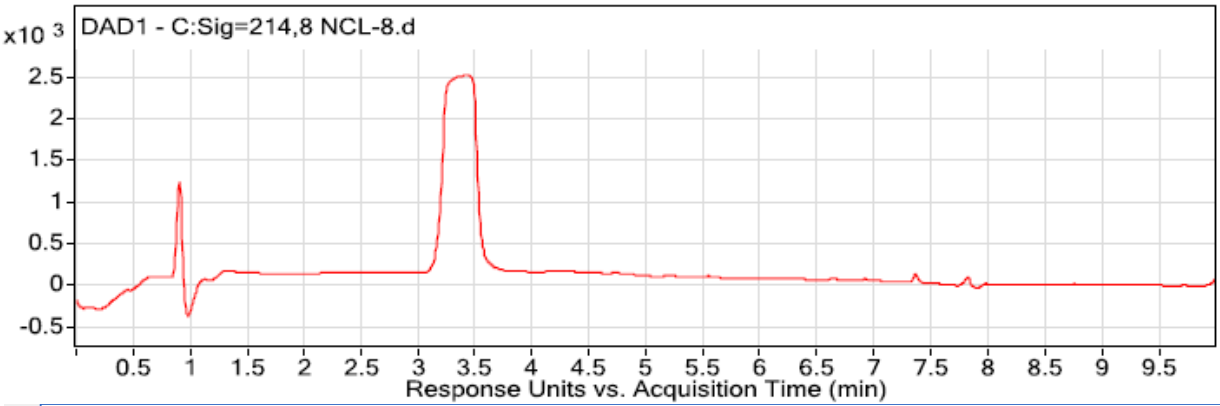
1-e

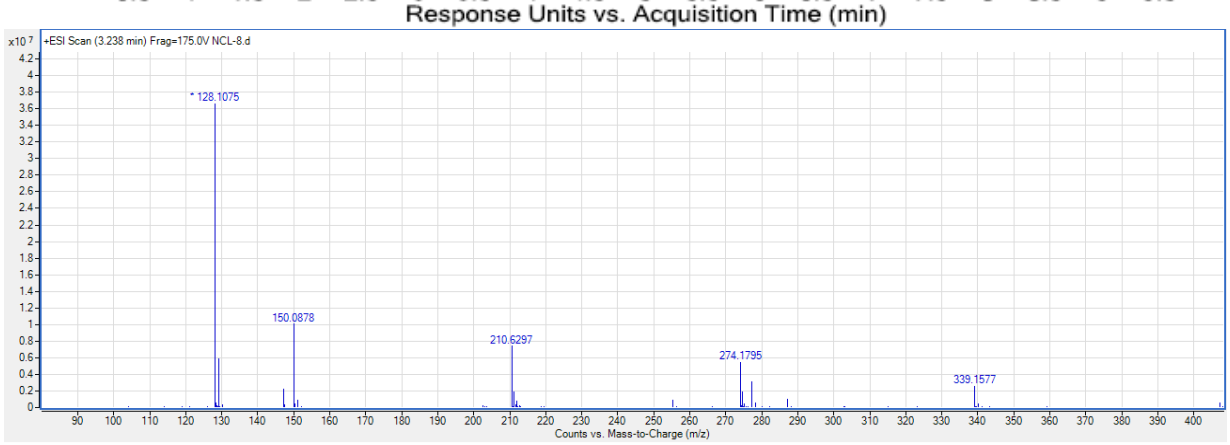


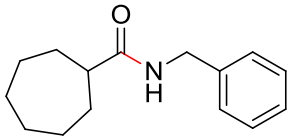



N
O

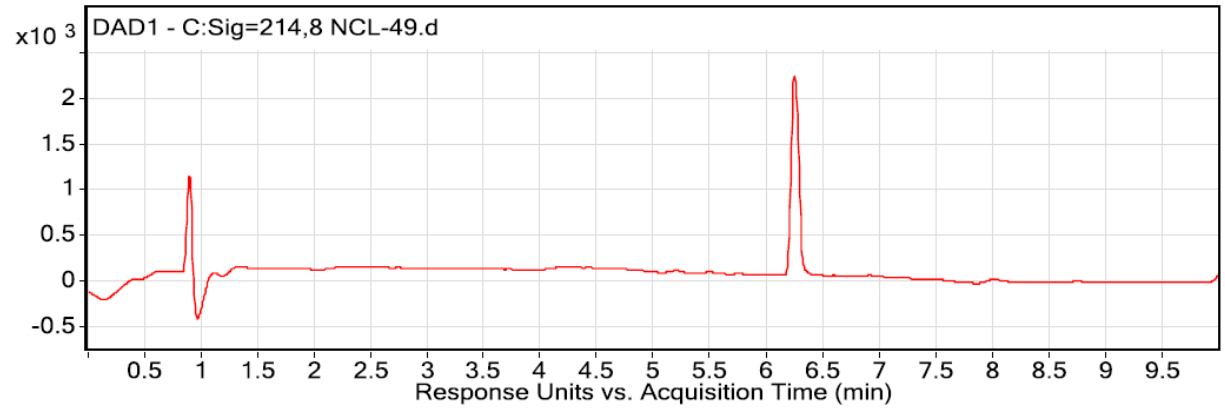
1-f

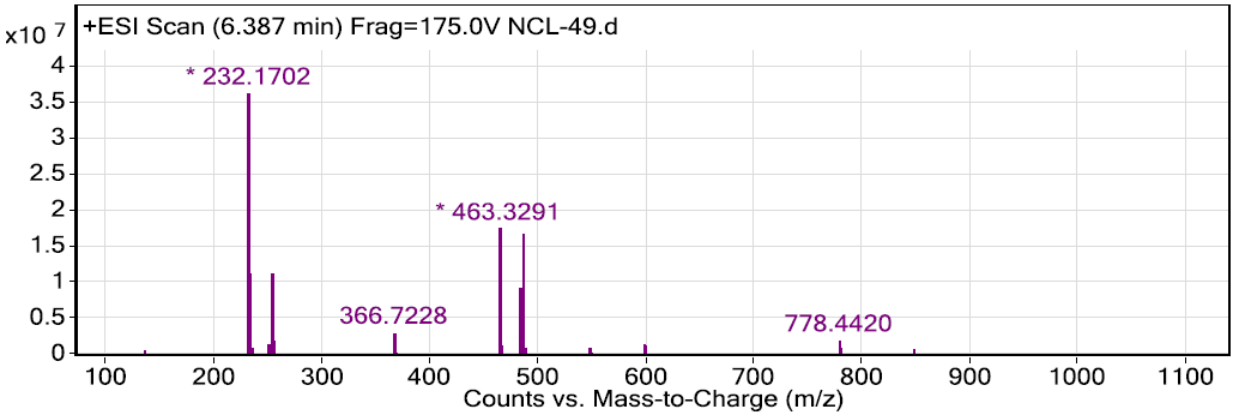

DAD1 - C:Sig=214,8 NCL-8.d
x10 3
Response Units vs. Acquisition Time (min)

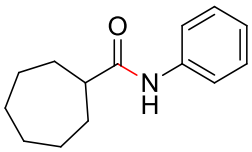

+ESI Scan (3.238 min) Frag=175.0V NCL-8.d
x10 7
* 128.1075
150.0878
210.6297
274.1795
339.1577
Counts vs. Mass-to-Charge (m/z)



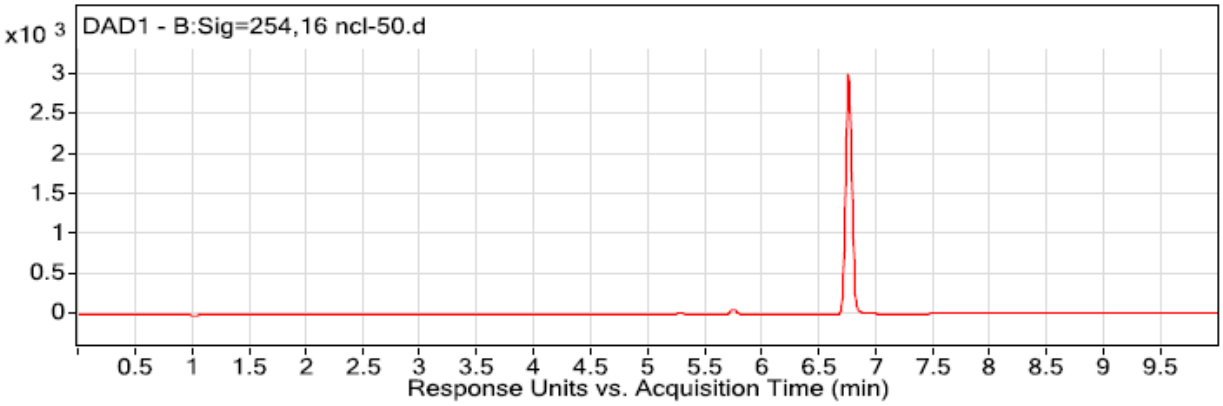
1-g

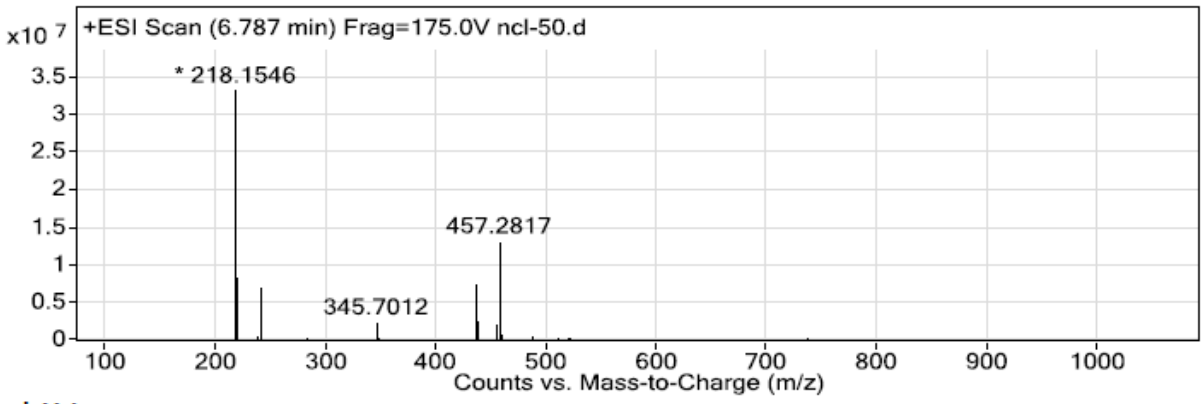


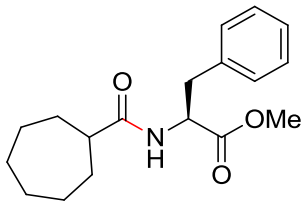



O
N
H

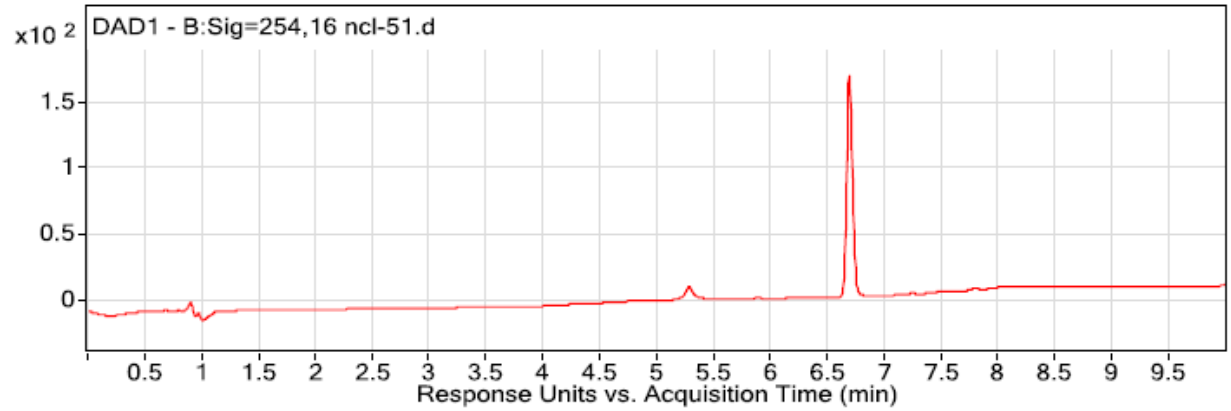
1-h

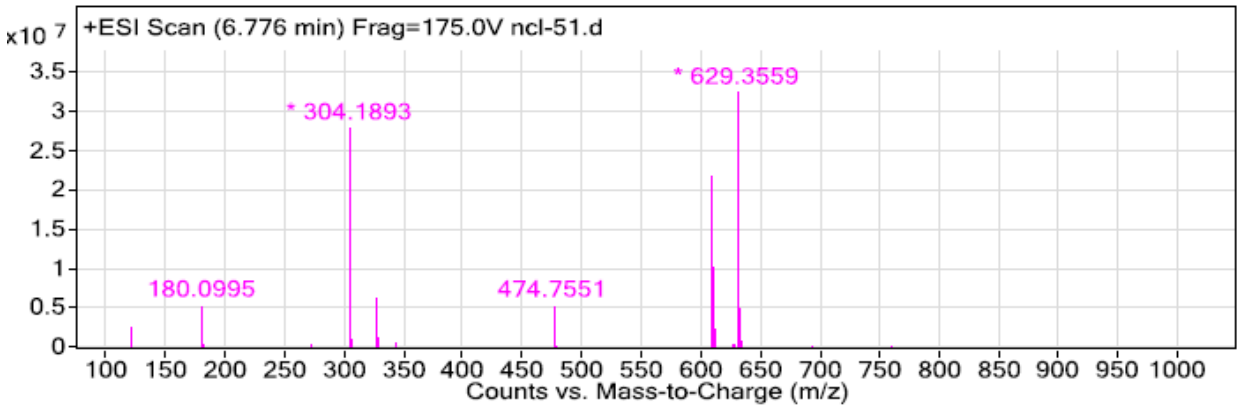

x10 3
DAD1 - B:Sig=254,16 ncl-50.d
3
2.5
2
1.5
1
0.5
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

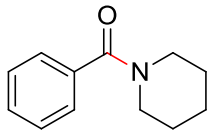

x10 7
+ESI Scan (6.787 min) Frag=175.0V ncl-50.d
* 218.1546
457.2817
345.7012
3.5
3
2.5
2
1.5
1
0.5
0
100 200 300 400 500 600 700 800 900 1000
Counts vs. Mass-to-Charge (m/z)



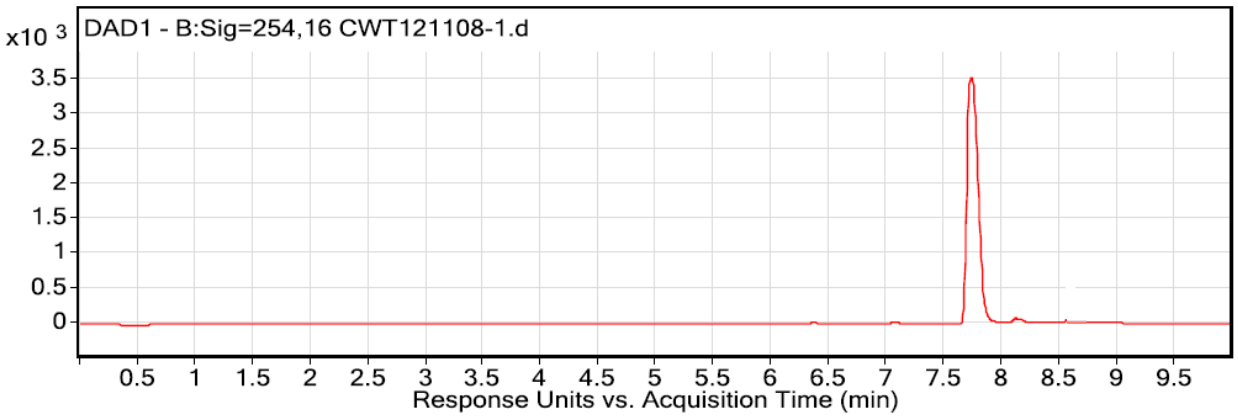
1-i

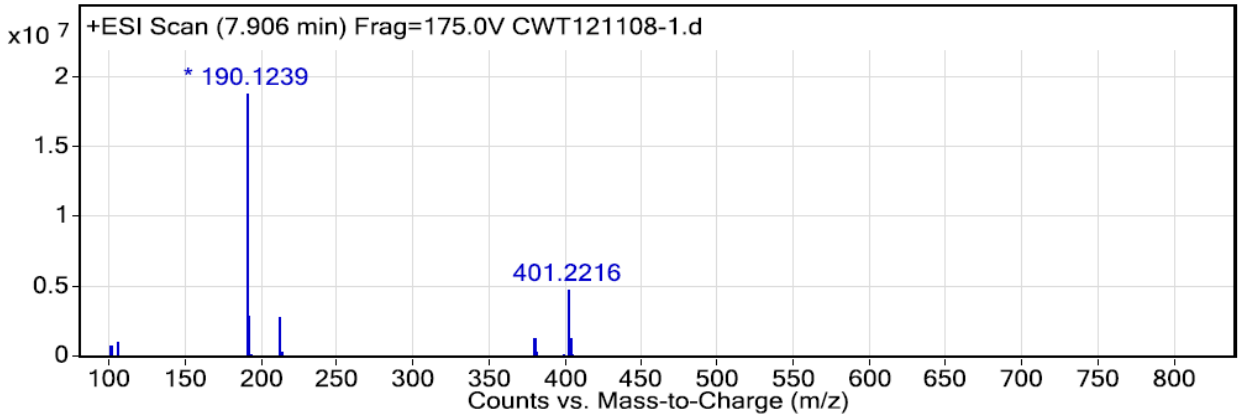


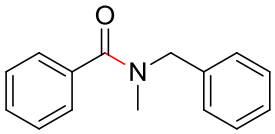



O
N

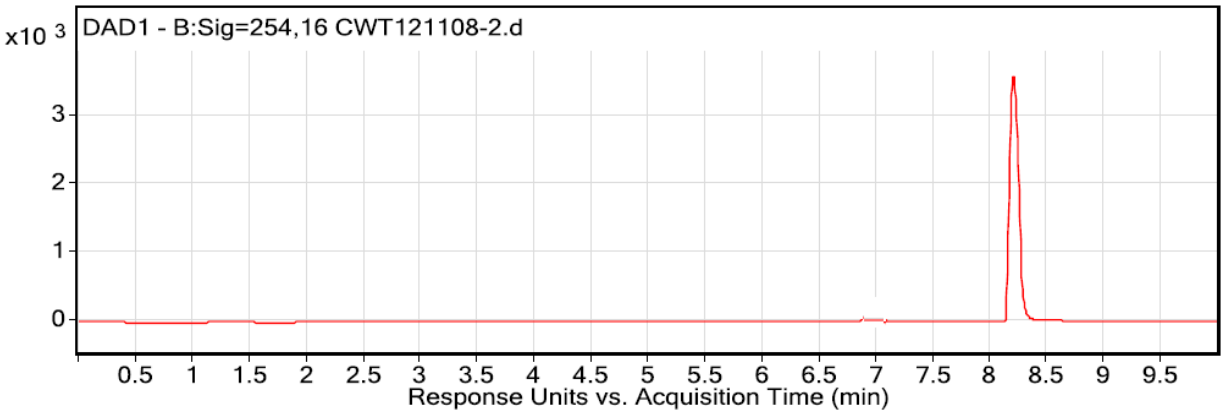
1-j

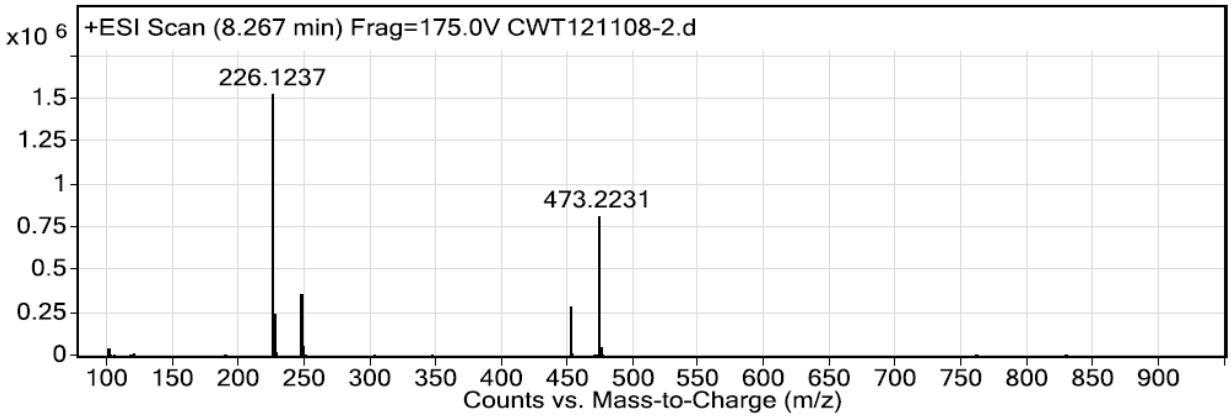

x10 3
DAD1 - B:Sig=254,16 CWT121108-1.d
3.5
3
2.5
2
1.5
1
0.5
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

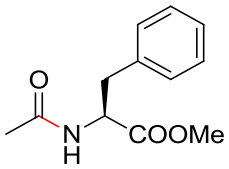

x10 7
+ESI Scan (7.906 min) Frag=175.0V CWT121108-1.d
* 190.1239
401.2216
2
1.5
1
0.5
0
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
Counts vs. Mass-to-Charge (m/z)



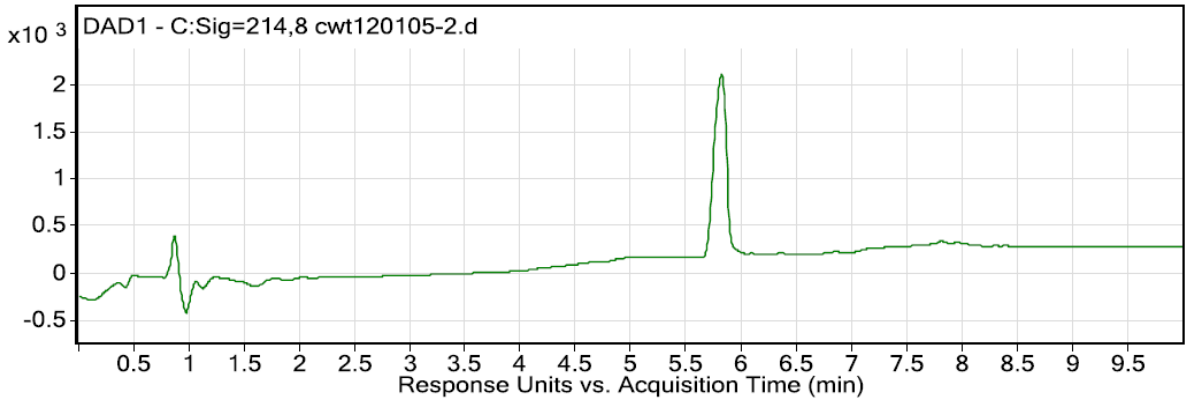
1-k

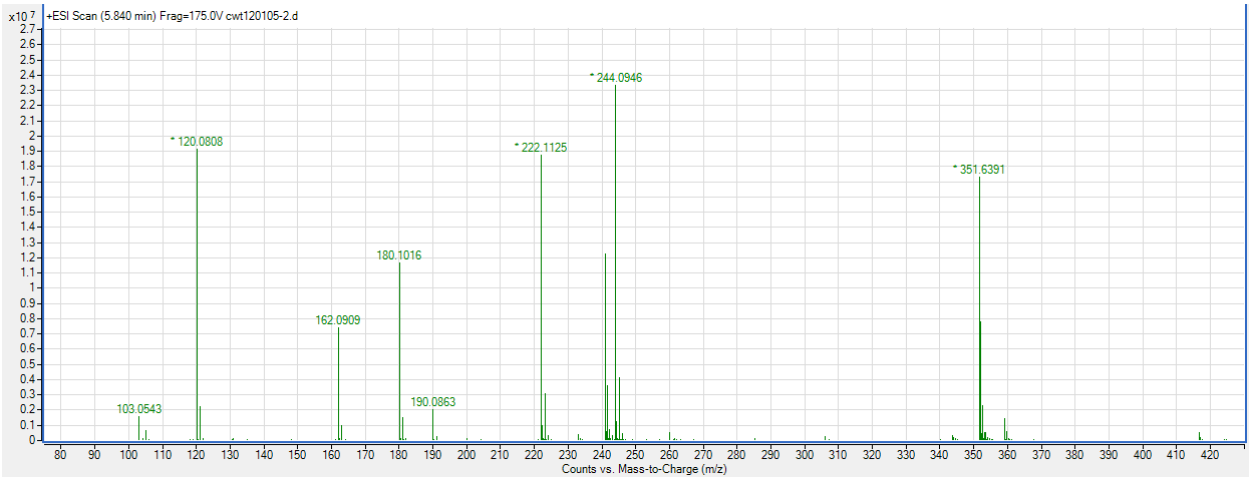


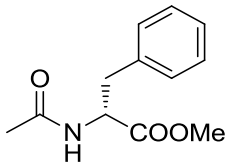



O
N
H
COOMe

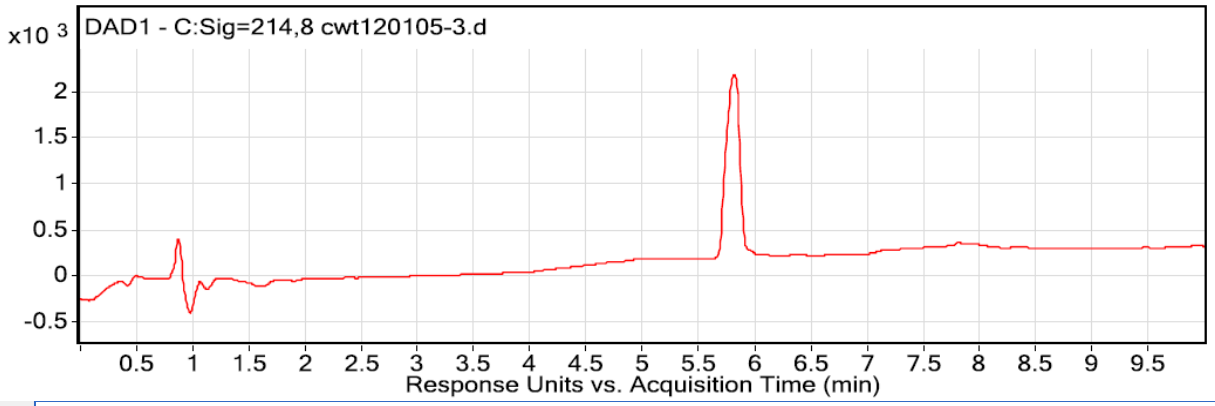
3-a

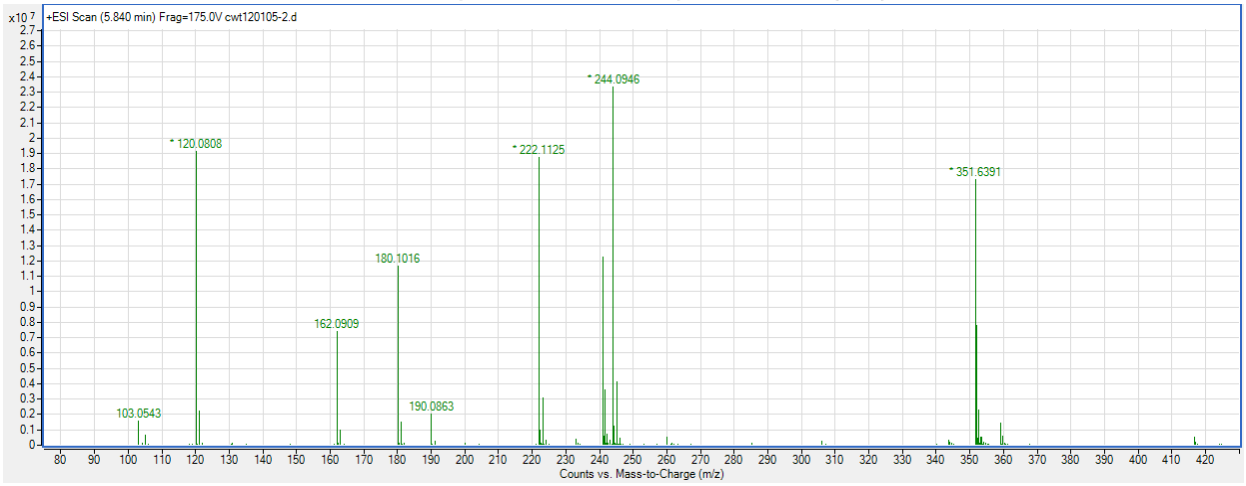

x10 3
DAD1 - C:Sig=214,8 cwt120105-2.d
2
1.5
1
0.5
0
-0.5
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

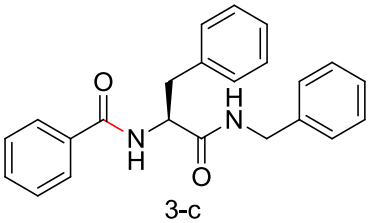

x10 7
+ESI Scan (5.840 min) Frag=175.0V cwt120105-2.d
* 244.0946
* 120.0808
* 222.1125
* 351.6391
180.1016
162.0909
190.0863
103.0543
Counts vs. Mass-to-Charge (m/z)


O
N
H
COOMe

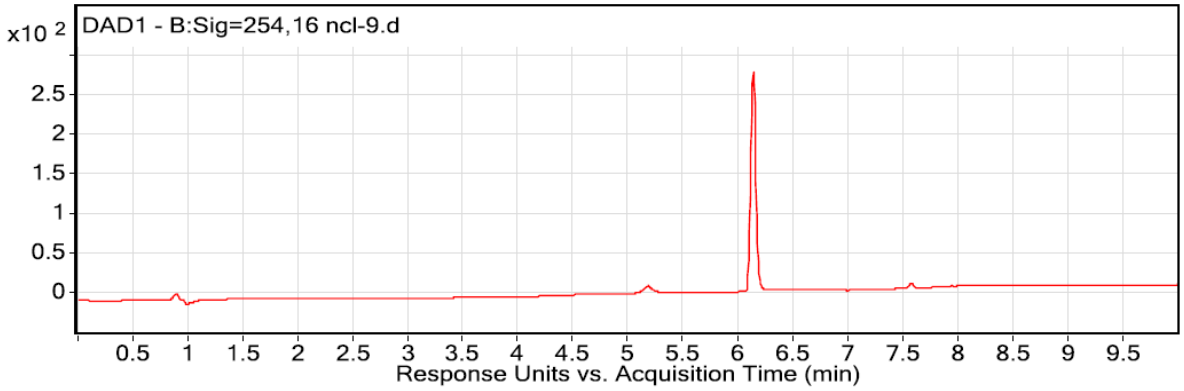
3-b

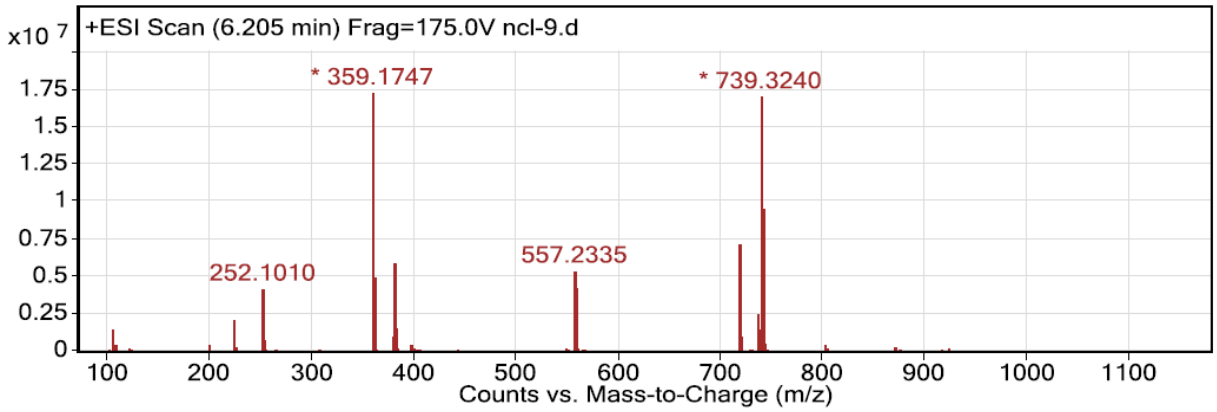

x10 3
DAD1 - C:Sig=214,8 cwt120105-3.d
Response Units vs. Acquisition Time (min)

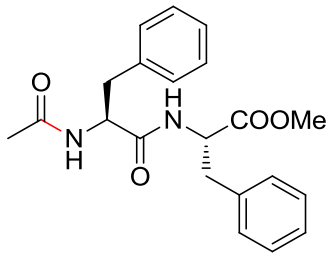

+ESI Scan (5.840 min) Frag=175.0V cwt120105-2.d
244.0946
120.0808
222.1125
351.6391
180.1016
162.0909
190.0863
103.0543
Counts vs. Mass-to-Charge (m/z)


O
N
H
H
N
O

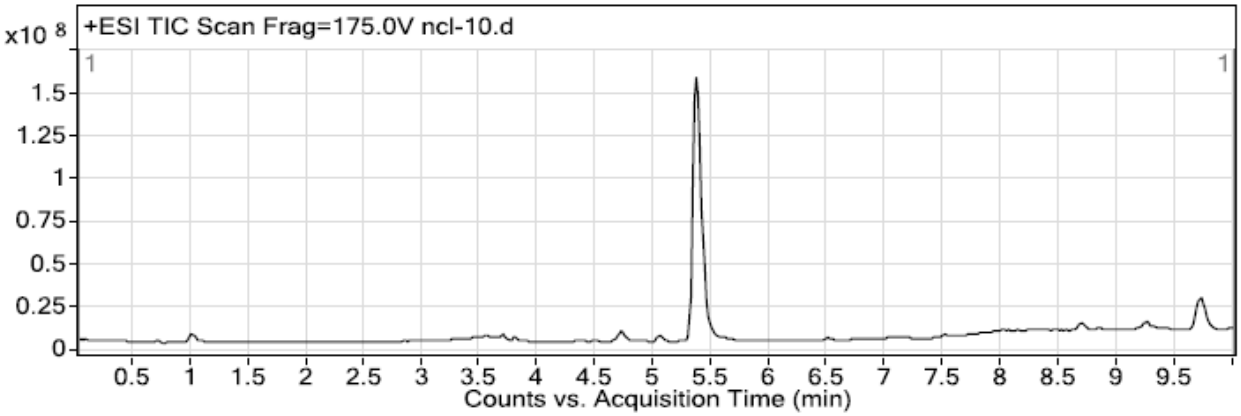
3-c

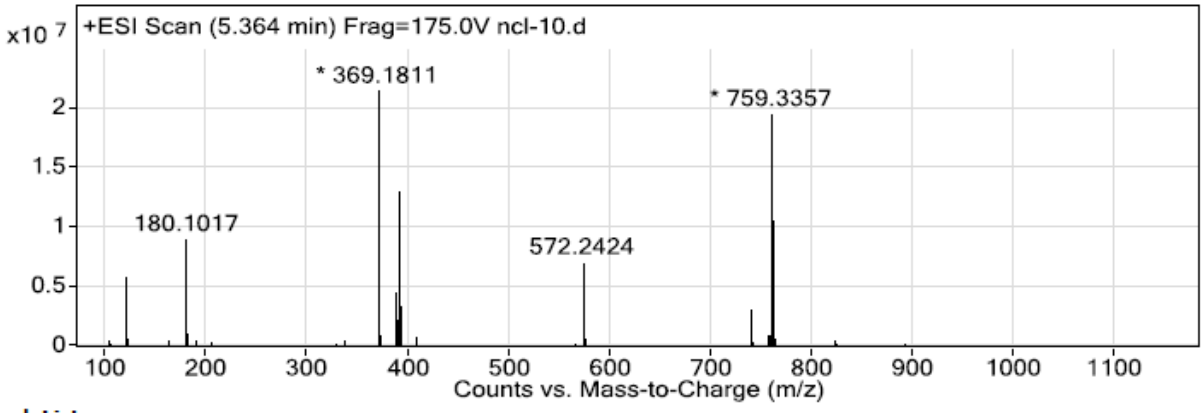

x10 2
DAD1 - B:Sig=254,16 ncl-9.d
2.5
2
1.5
1
0.5
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

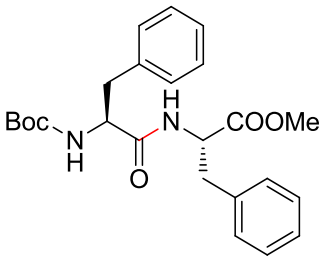

x10 7
+ESI Scan (6.205 min) Frag=175.0V ncl-9.d
* 359.1747
* 739.3240
252.1010
557.2335
1.75
1.5
1.25
1
0.75
0.5
0.25
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


O
N
H
H
N
O
COOMe

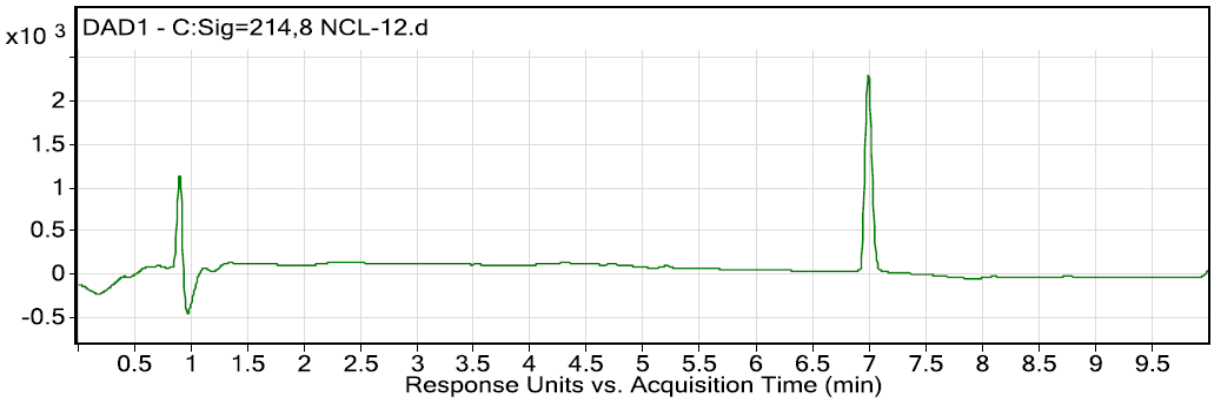
3-d

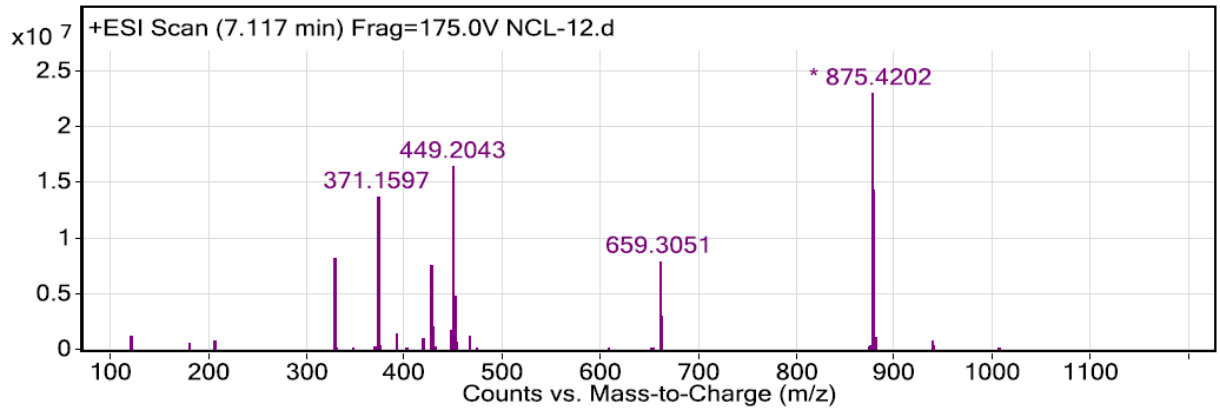

x10 8
+ESI TIC Scan Frag=175.0V ncl-10.d
1
1
1.5
1.25
1
0.75
0.5
0.25
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Counts vs. Acquisition Time (min)

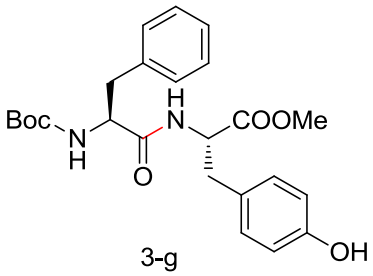

x10 7
+ESI Scan (5.364 min) Frag=175.0V ncl-10.d
* 369.1811
* 759.3357
180.1017
572.2424
2
1.5
1
0.5
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


Boc
N
H
H
N
O
COOMe

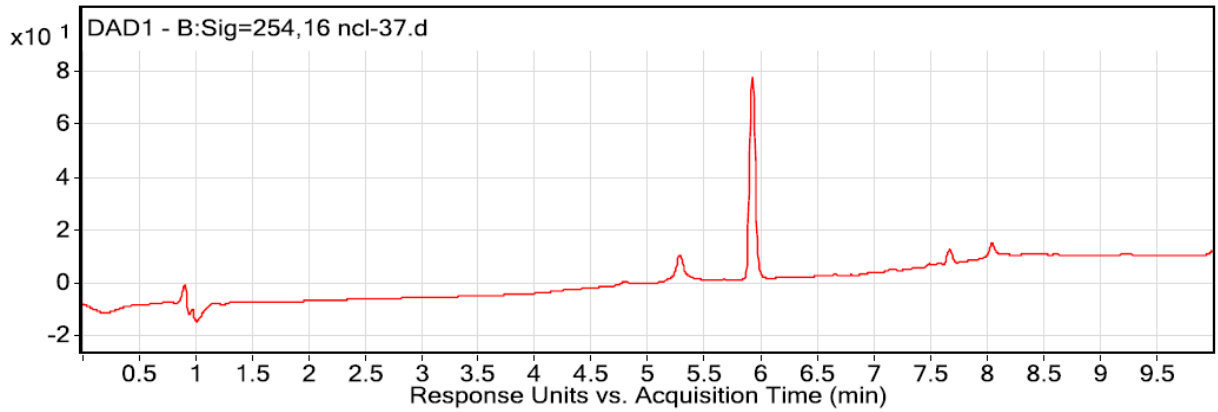
3-e

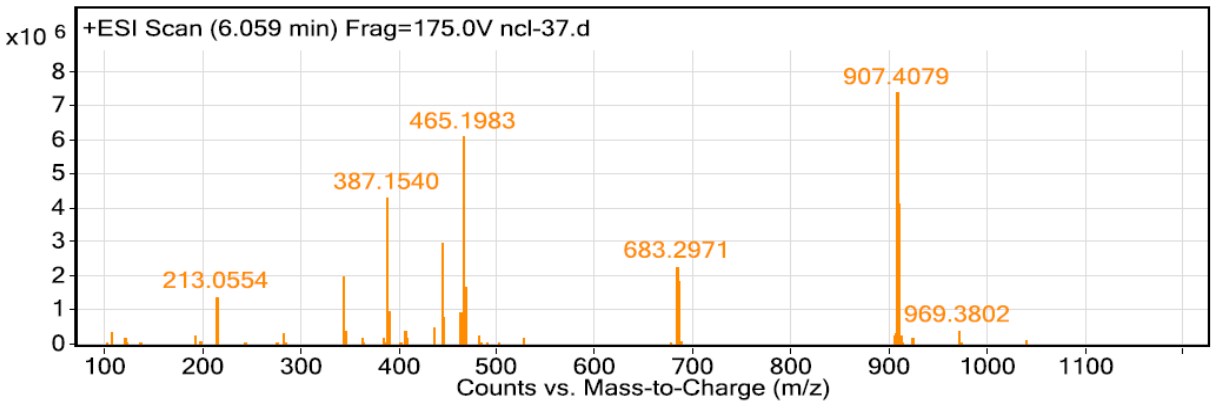

x10 3
DAD1 - C:Sig=214,8 NCL-12.d
2
1.5
1
0.5
0
-0.5
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

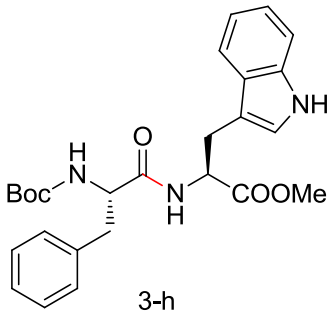

x10 7
+ESI Scan (7.117 min) Frag=175.0V NCL-12.d
2.5
2
1.5
1
0.5
0
* 875.4202
449.2043
371.1597
659.3051
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


Boc
N
H
H
N
O
COOMe
OH

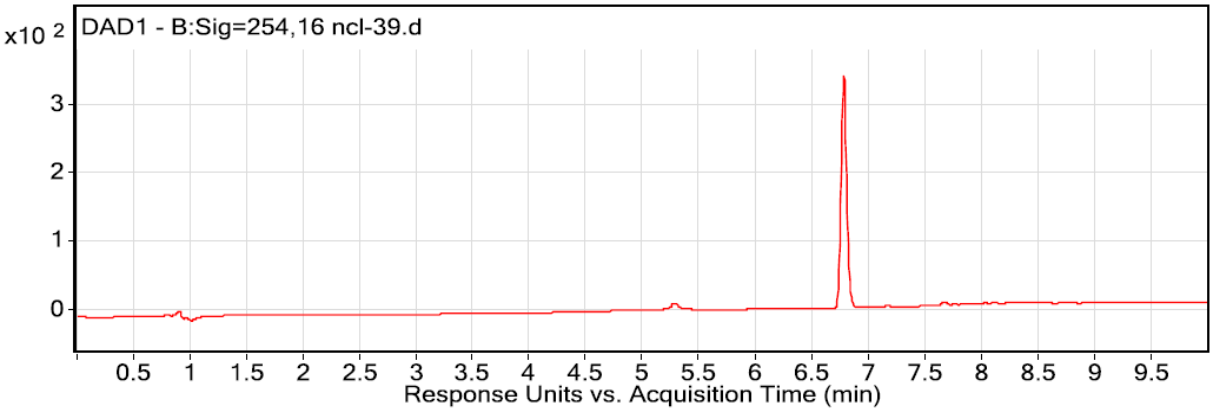
3-g

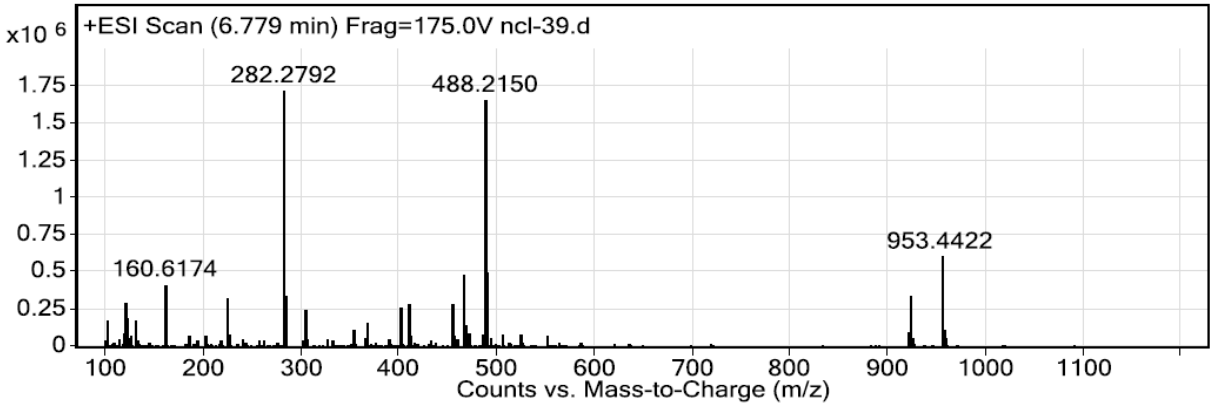

x10 1
DAD1 - B:Sig=254,16 ncl-37.d
8
6
4
2
0
-2
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

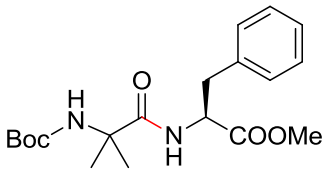

x10 6
+ESI Scan (6.059 min) Frag=175.0V ncl-37.d
907.4079
465.1983
387.1540
683.2971
213.0554
969.3802
8
7
6
5
4
3
2
1
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


NH
O
H
N
Boc
N
H
COOMe

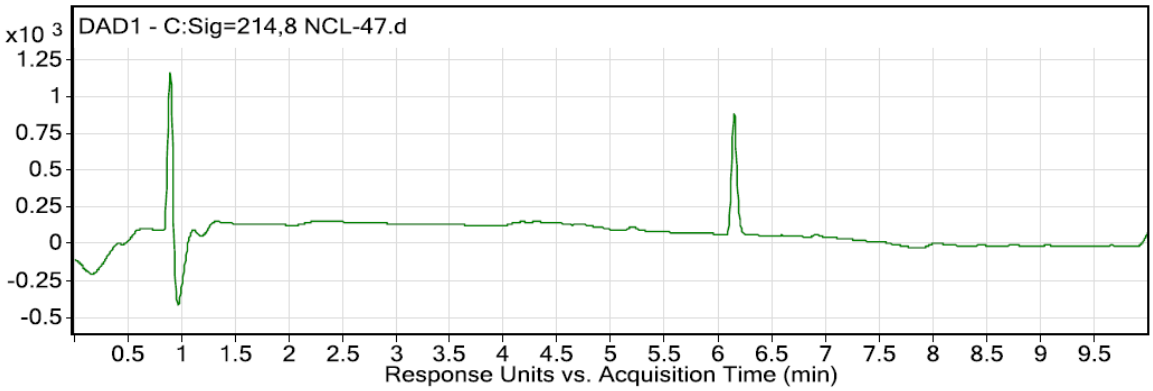
3-h

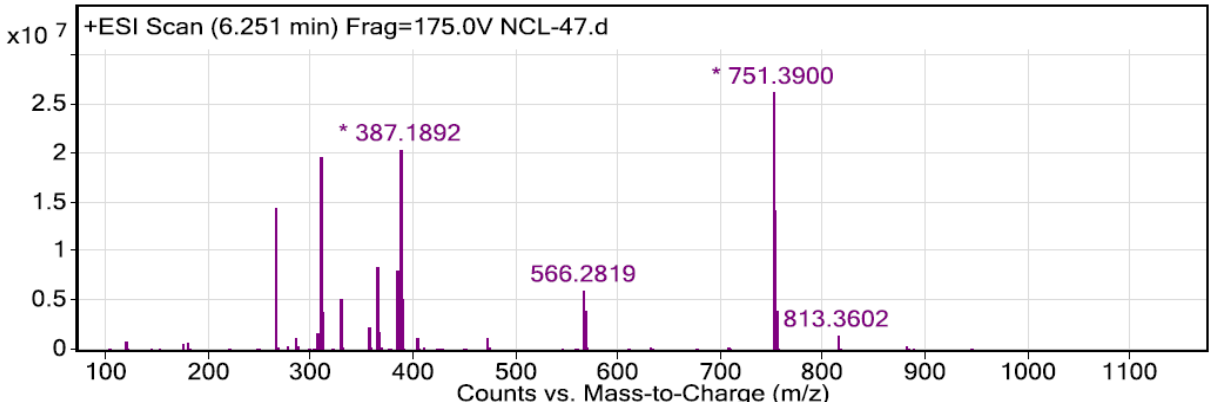

x10 2
DAD1 - B:Sig=254,16 ncl-39.d
3
2
1
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

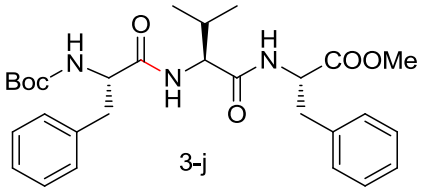

x10 6
+ESI Scan (6.779 min) Frag=175.0V ncl-39.d
282.2792
488.2150
953.4422
160.6174
1.75
1.5
1.25
1
0.75
0.5
0.25
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)



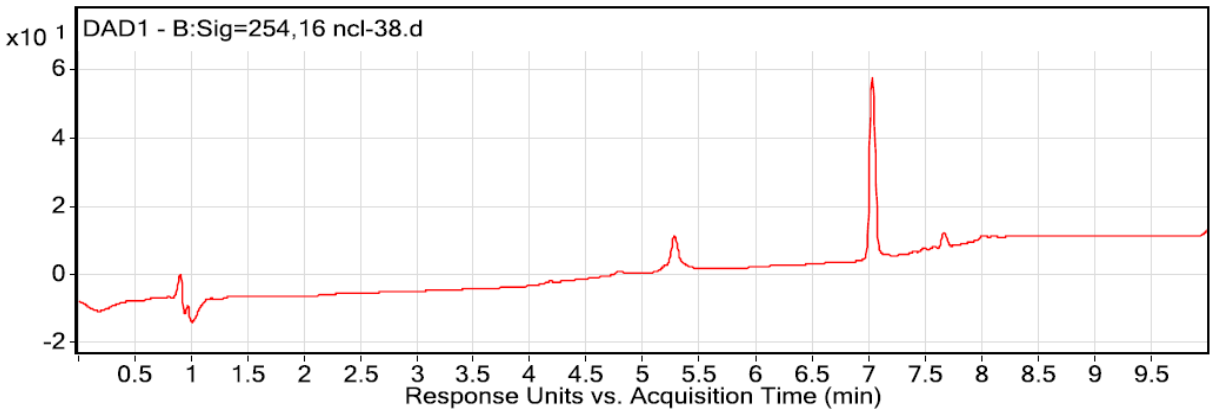
3-i

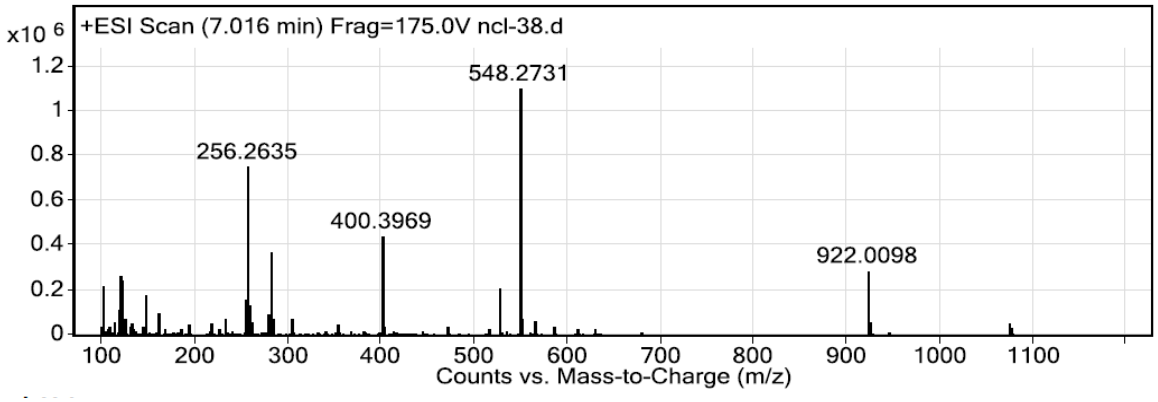


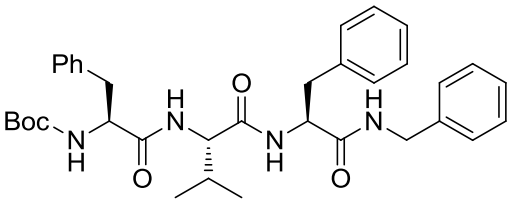




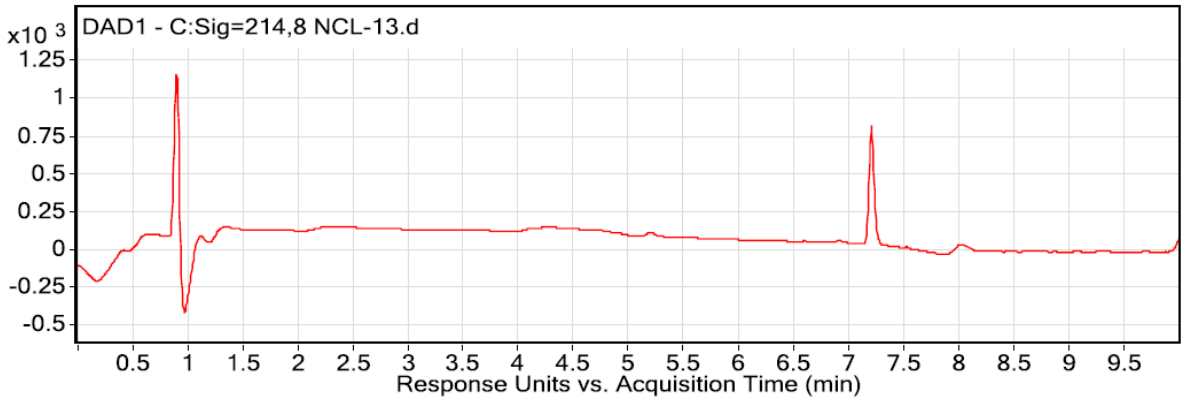
3-j

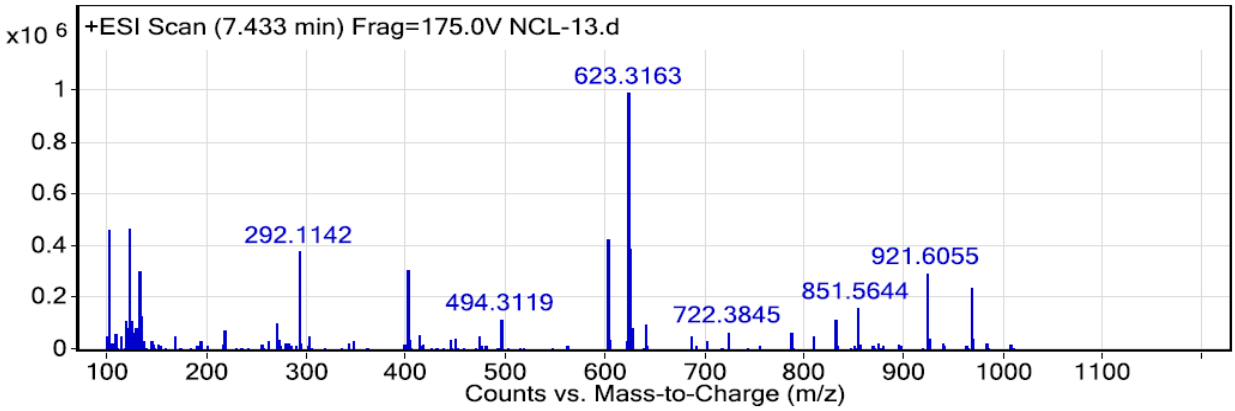


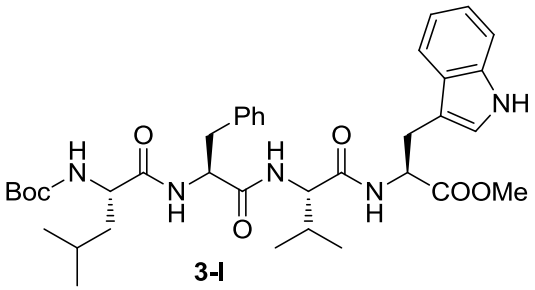



Ph
Boc
N
H
O
H
N
O
N
H
O
H
N

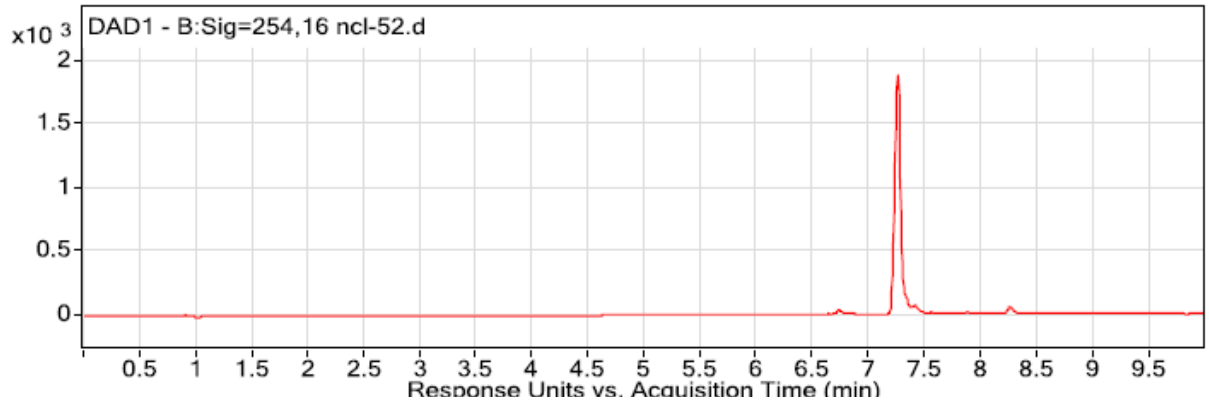
3-k

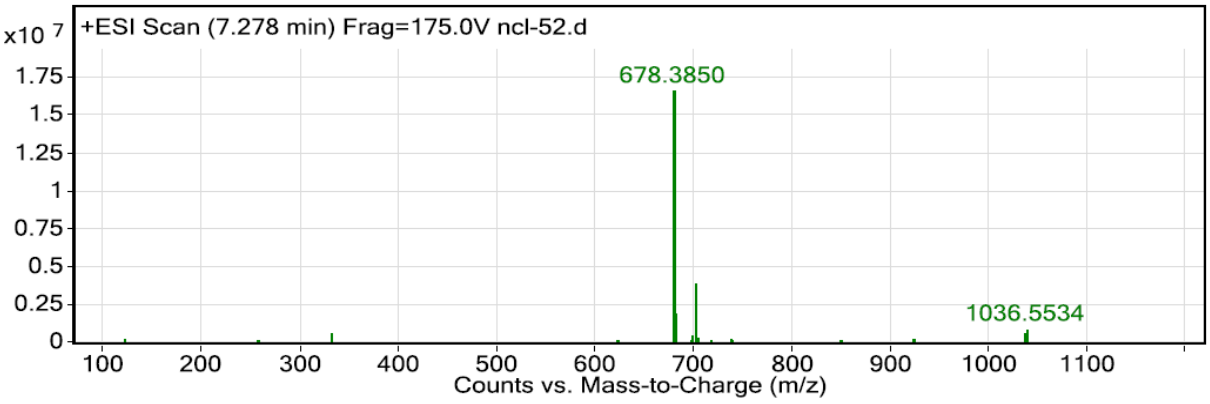

DAD1 - C:Sig=214,8 NCL-13.d
x10 3
1.25
1
0.75
0.5
0.25
0
-0.25
-0.5
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

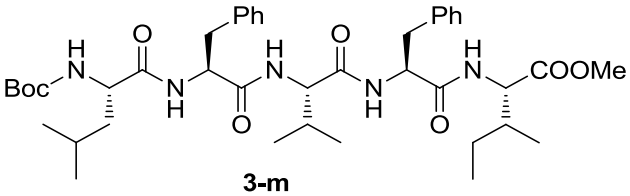

+ESI Scan (7.433 min) Frag=175.0V NCL-13.d
x10 6
1
0.8
0.6
0.4
0.2
0
623.3163
292.1142
494.3119
722.3845
851.5644
921.6055
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)



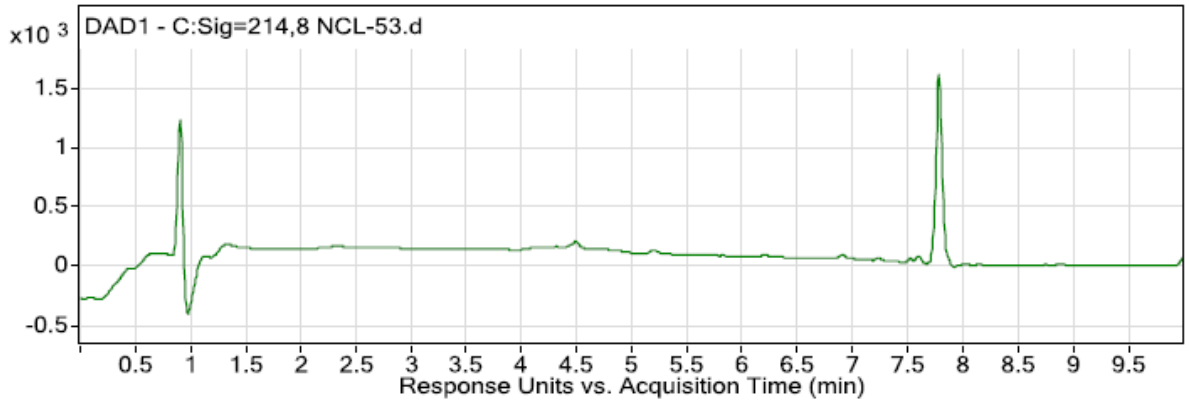
3-I

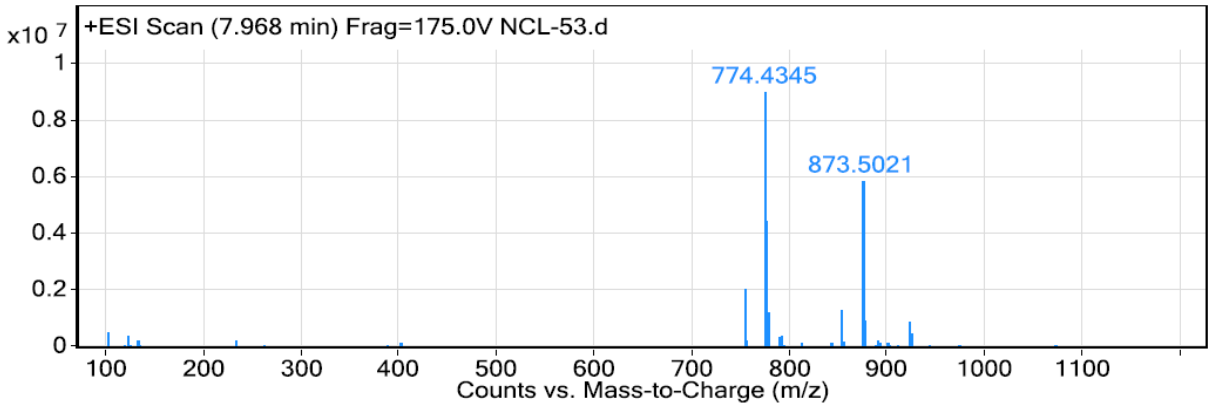


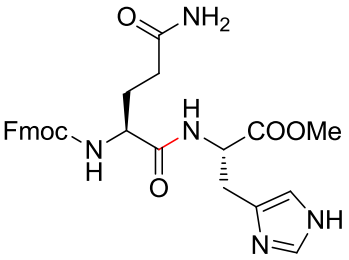



Boc
Ph
Ph
COOMe

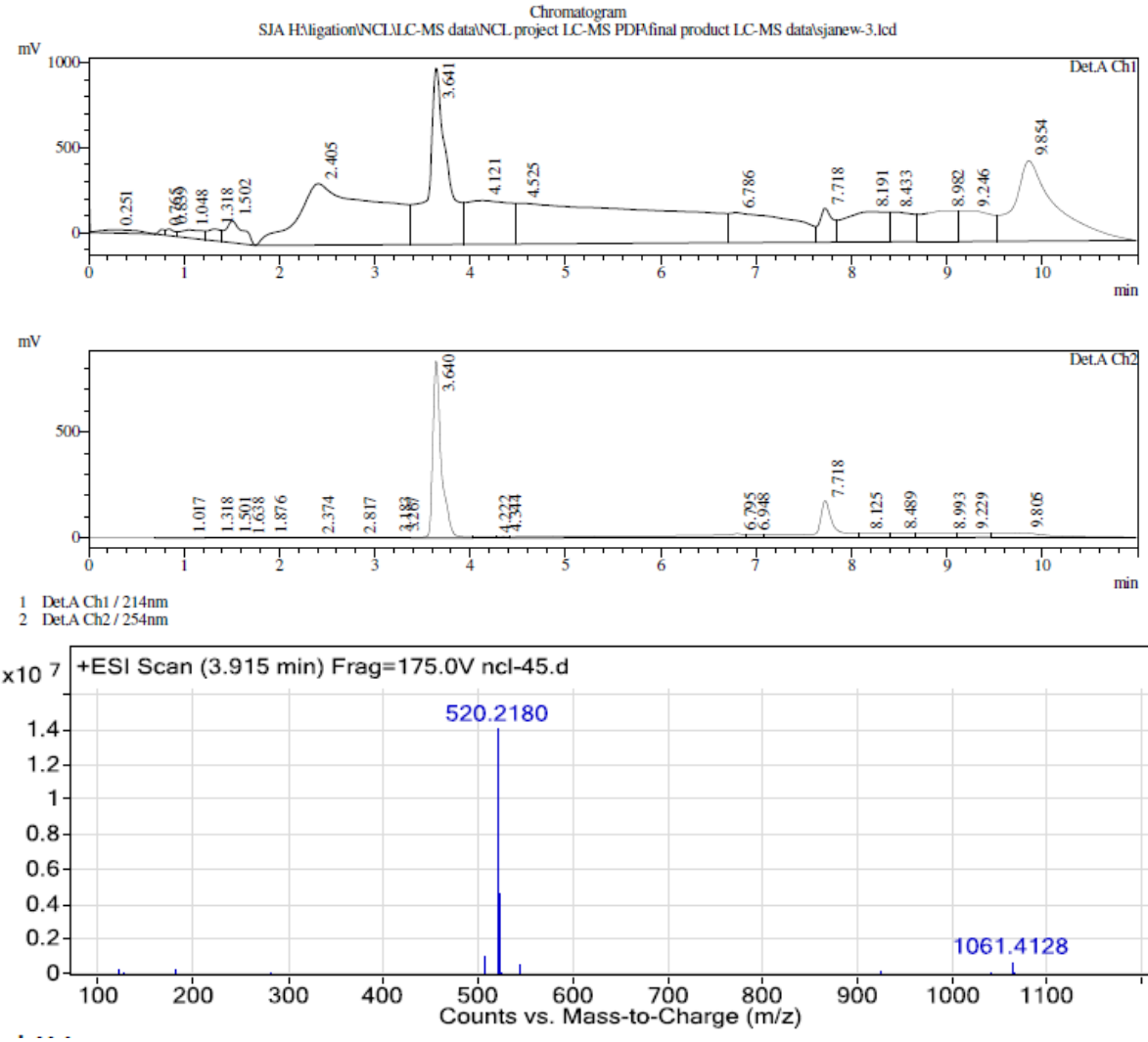
3-m

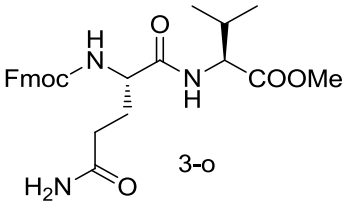

x10 3
DAD1 - C:Sig=214,8 NCL-53.d
Response Units vs. Acquisition Time (min)


x10 7
+ESI Scan (7.968 min) Frag=175.0V NCL-53.d
774.4345
873.5021
Counts vs. Mass-to-Charge (m/z)

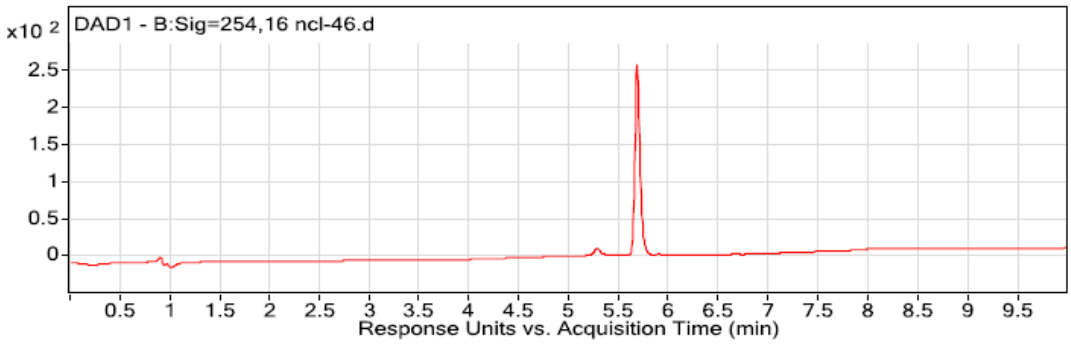

O
NH2
Fmoc
N
H
H
N
COOMe
O
NH
N

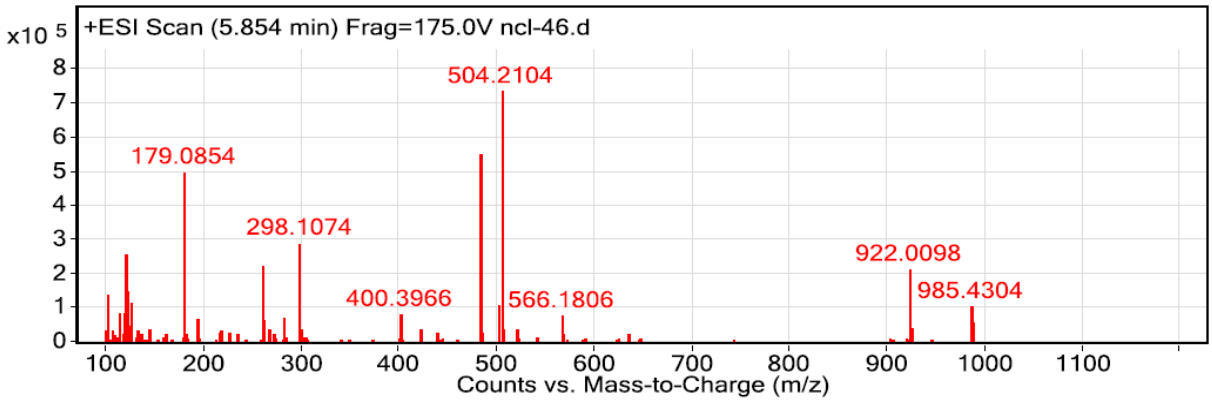
3-n

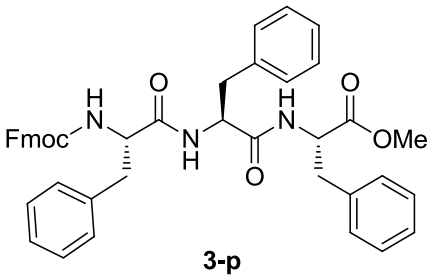

Chromatogram
SJA H\ligation\NCL\LC-MS data\NCL project LC-MS PDP\final product LC-MS data\sjanew-3.lcd
mV
DetA Ch1
0.251
1.048
1.318
1.502
2.405
3.641
4.121
4.525
6.786
7.718
8.191
8.433
8.982
9.246
9.854
min
mV
DetA Ch2
1.017
1.318
1.501
1.638
1.876
2.374
2.817
3.183
3.640
4.222
4.344
6.795
6.948
7.718
8.125
8.489
8.993
9.229
9.806
min
1 DetA Ch1 / 214nm
2 DetA Ch2 / 254nm
x10 7
+ESI Scan (3.915 min) Frag=175.0V ncl-45.d
520.2180
1061.4128
Counts vs. Mass-to-Charge (m/z)


Fmoc
H
N
O
N
H
COOMe
H_2N
O

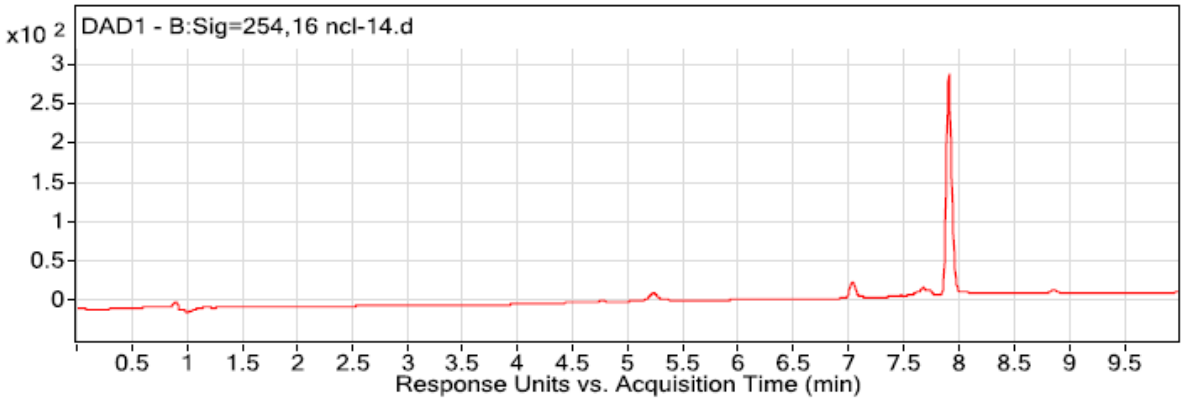
3-o

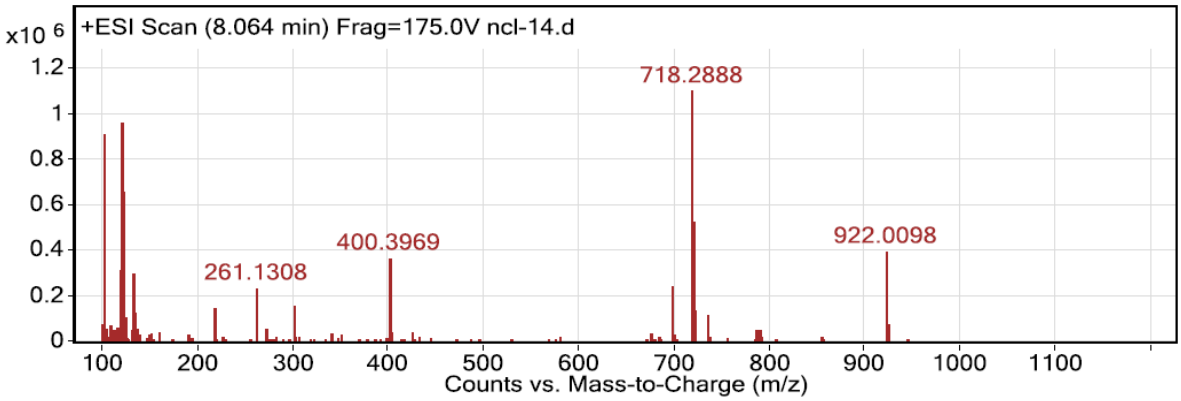

x10 2
DAD1 - B:Sig=254,16 ncl-46.d
2.5
2
1.5
1
0.5
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

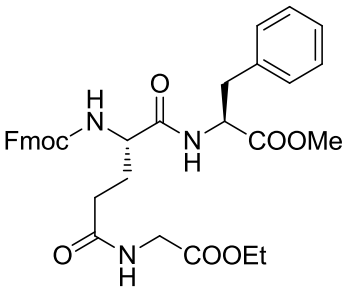

x10 5
+ESI Scan (5.854 min) Frag=175.0V ncl-46.d
504.2104
179.0854
298.1074
922.0098
985.4304
400.3966
566.1806
8
7
6
5
4
3
2
1
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


Fmoc
H
N
O
N
H
O
H
N
O
OMe

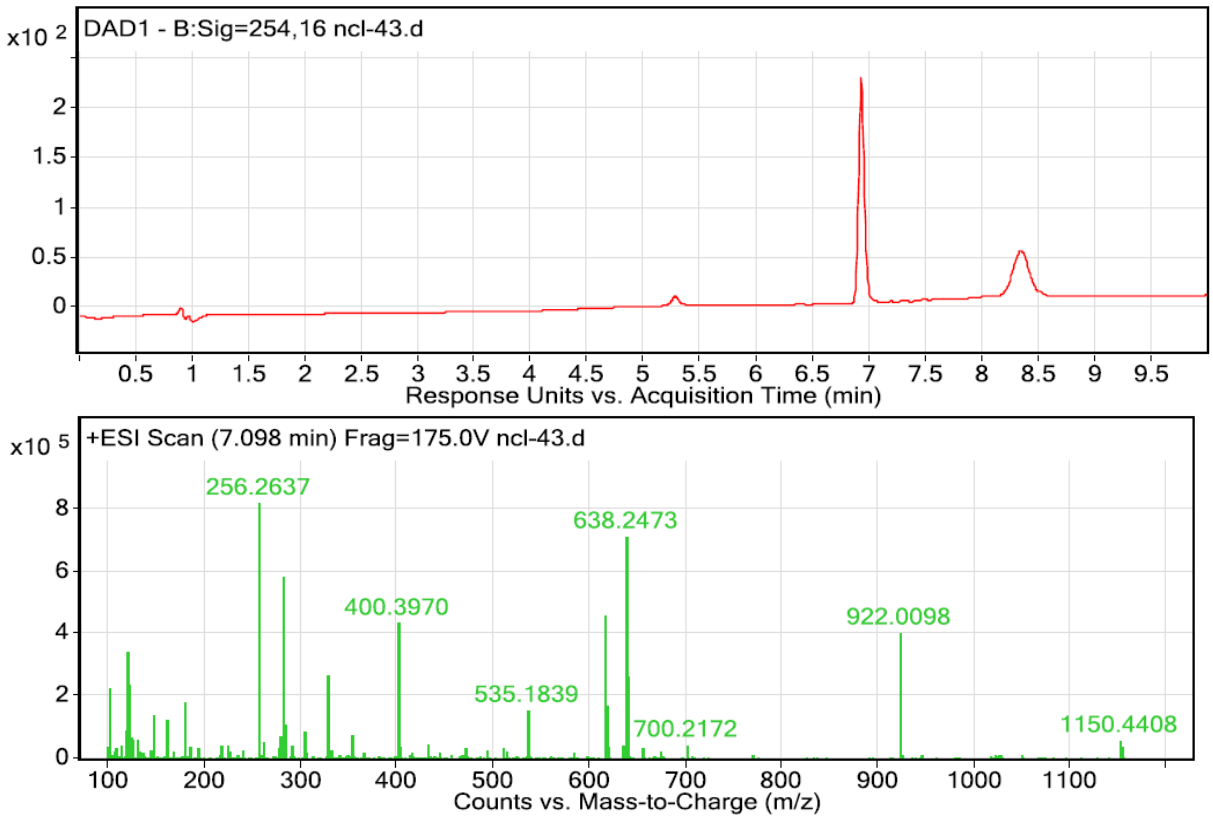
3-p

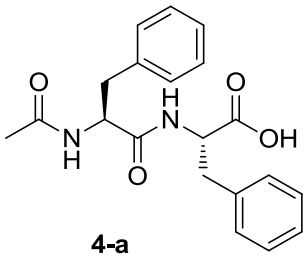

x10 2
DAD1 - B:Sig=254,16 ncl-14.d
3
2.5
2
1.5
1
0.5
0
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)


x10 6
+ESI Scan (8.064 min) Frag=175.0V ncl-14.d
1.2
1
0.8
0.6
0.4
0.2
0
718.2888
400.3969
922.0098
261.1308
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)

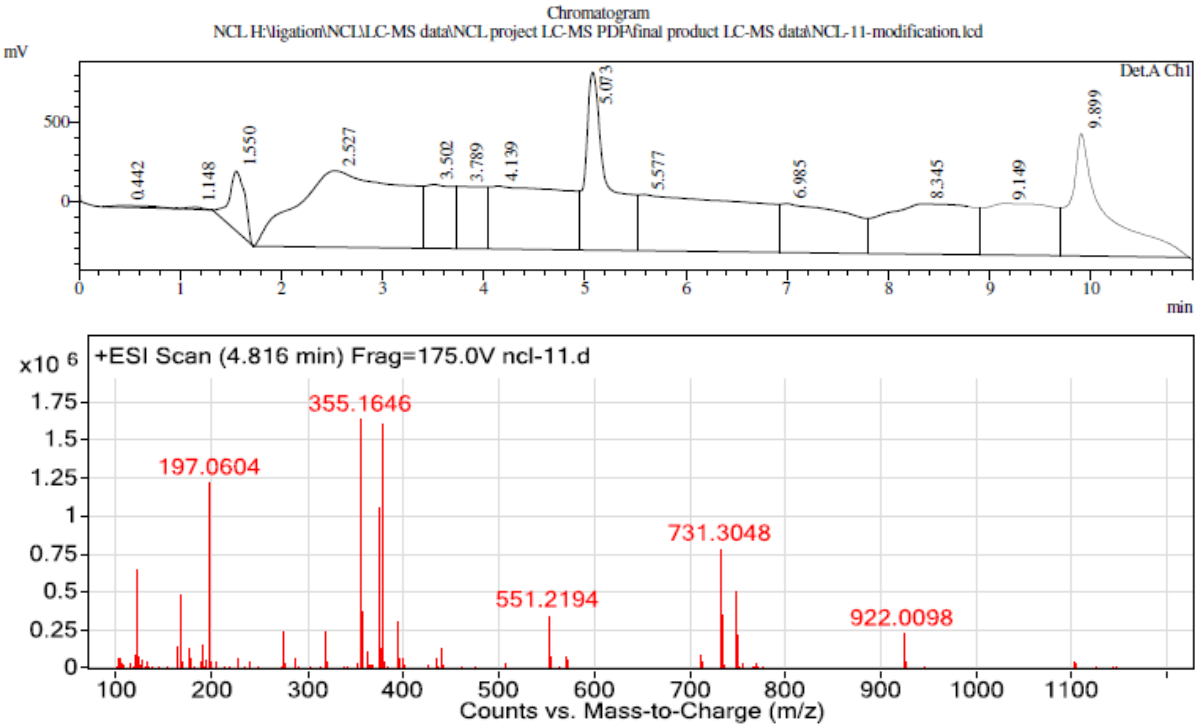


3-q

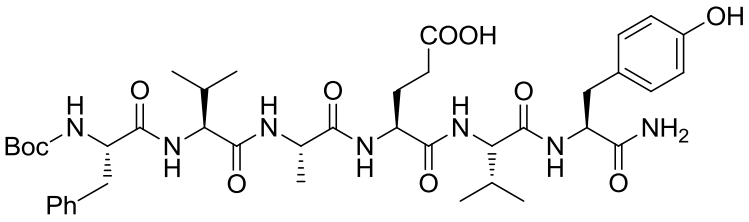



O
N
H
O
H
N
O
OH

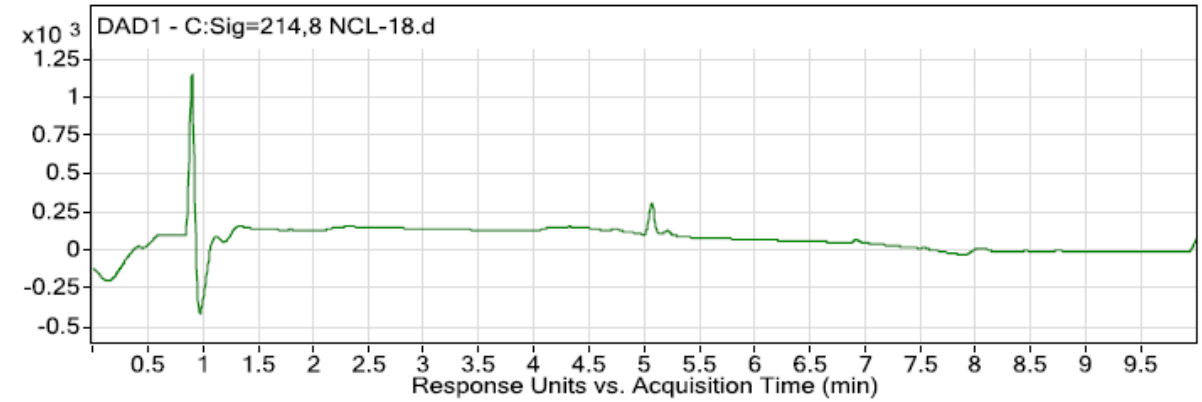
4-a

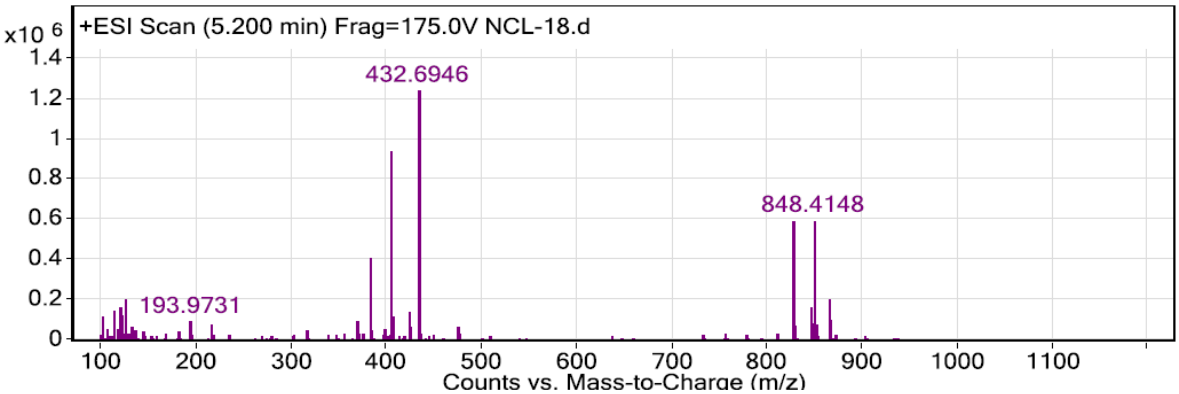

Chromatogram
NCL H:\ligation\NCL\LC-MS data\NCL project LC-MS PDF\final product LC-MS data\NCL-11-modification.lcd
mV
500
0
Det.A Ch1
0.442
1.148
1.550
2.527
3.502
3.789
4.139
5.073
5.577
6.985
8.345
9.149
9.899
0
1
2
3
4
5
6
7
8
9
10
min

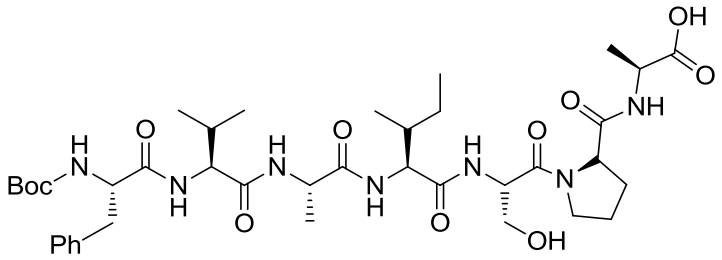
x10 6
+ESI Scan (4.816 min) Frag=175.0V ncl-11.d
1.75
1.5
1.25
1
0.75
0.5
0.25
0
355.1646
197.0604
731.3048
551.2194
922.0098
100
200
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)


Boc
Ph
COOH
OH
NH2

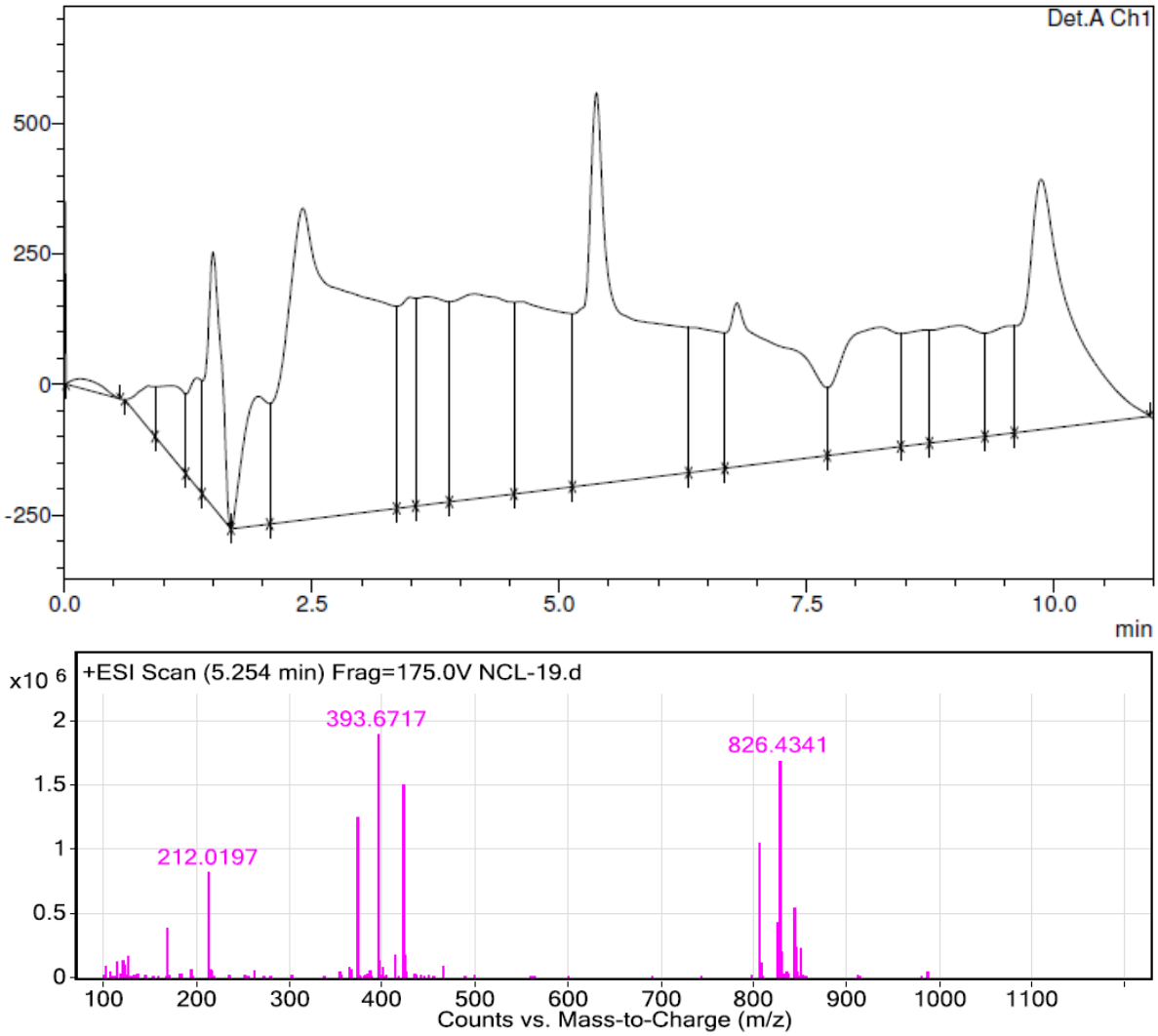
4-b

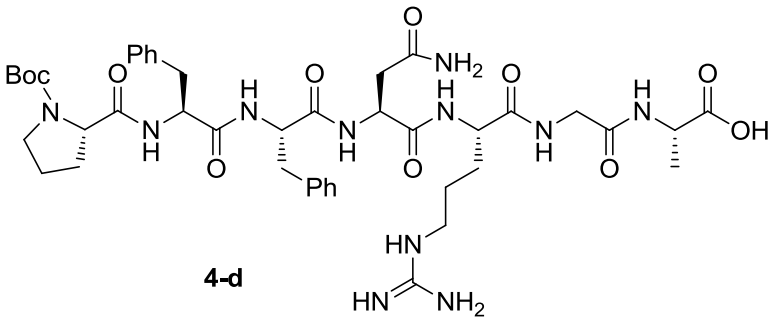

DAD1 - C:Sig=214,8 NCL-18.d
x10 3
Response Units vs. Acquisition Time (min)


+ESI Scan (5.200 min) Frag=175.0V NCL-18.d
x10 6
432.6946
848.4148
193.9731
Counts vs. Mass-to-Charge (m/z)

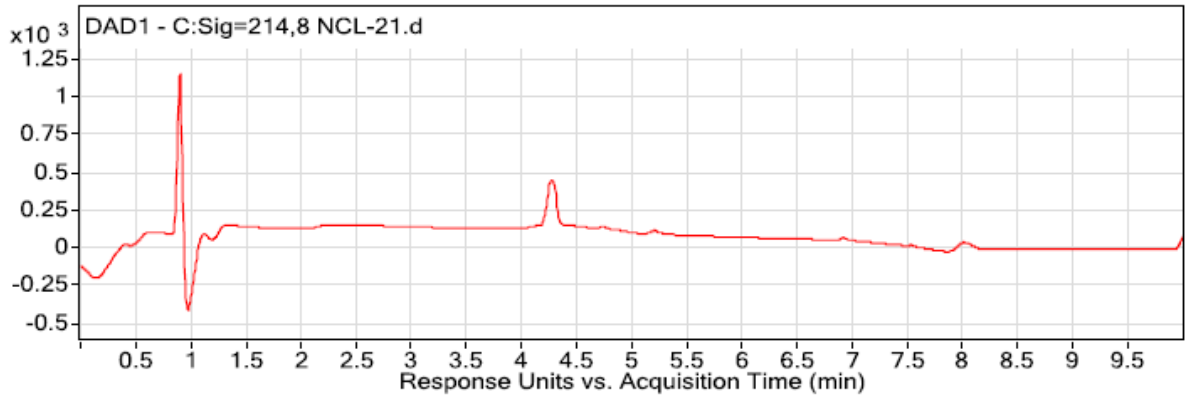

OH
O
O
NH
H
N
O
Boc
N
H
O
H
N
O
N
H
O
H
N
O
N
Ph
OH

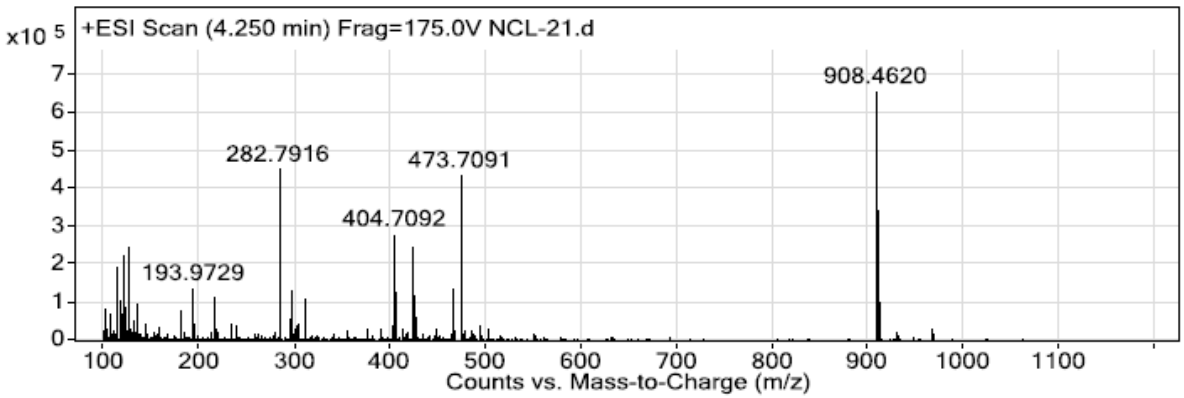
4-c

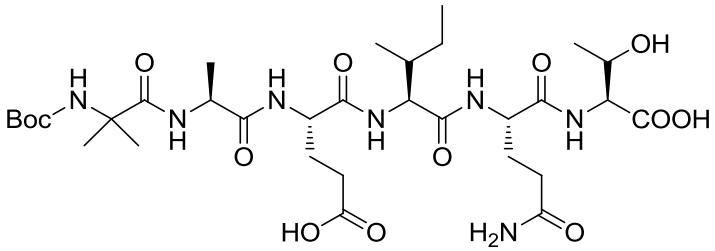

Det.A Ch1
500
250
0
-250
0.0
2.5
5.0
7.5
10.0
min
x10 6
+ESI Scan (5.254 min) Frag=175.0V NCL-19.d
393.6717
826.4341
212.0197
2
1.5
1
0.5
0
100
200
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)


Boc
Ph
NH2
OH
Ph
HN
HN
NH2

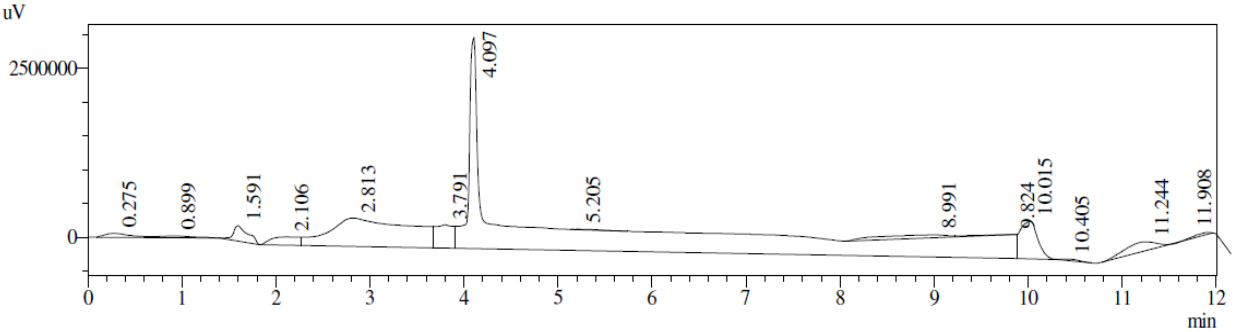
4-d

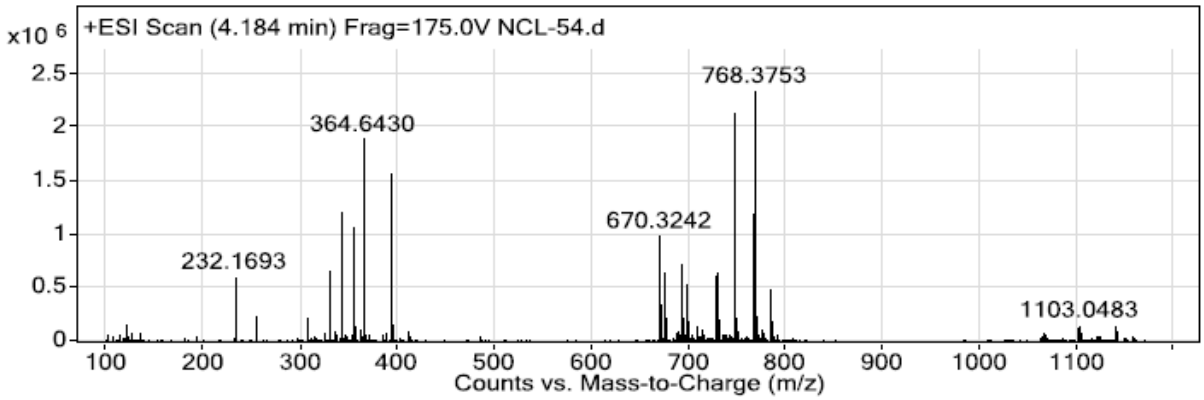

x10 3
DAD1 - C:Sig=214,8 NCL-21.d
1.25
1
0.75
0.5
0.25
0
-0.25
-0.5
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

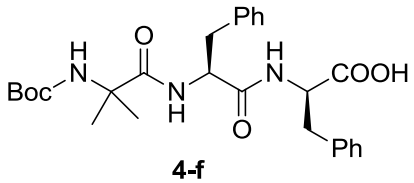

x10 5
+ESI Scan (4.250 min) Frag=175.0V NCL-21.d
908.4620
282.7916
473.7091
404.7092
193.9729
7
6
5
4
3
2
1
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


Boc
OH
COOH
HO
O
H2N
O

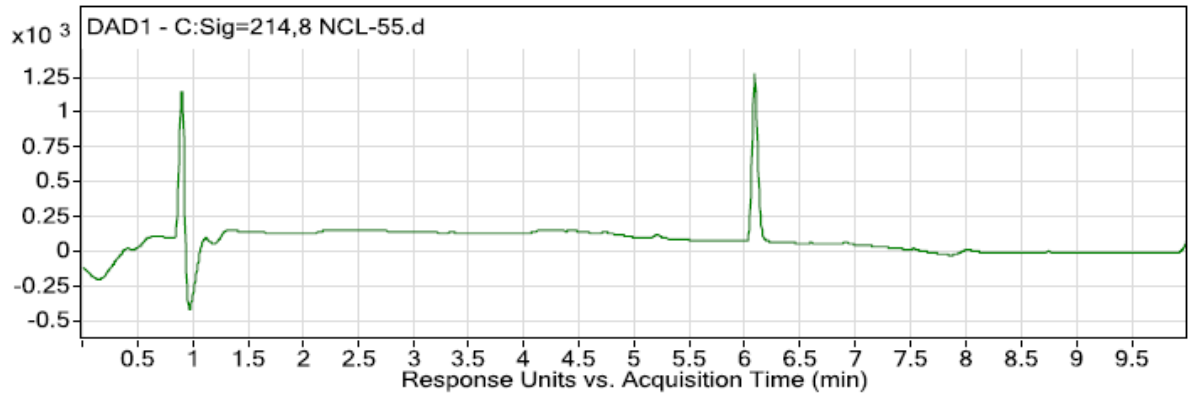
4-e

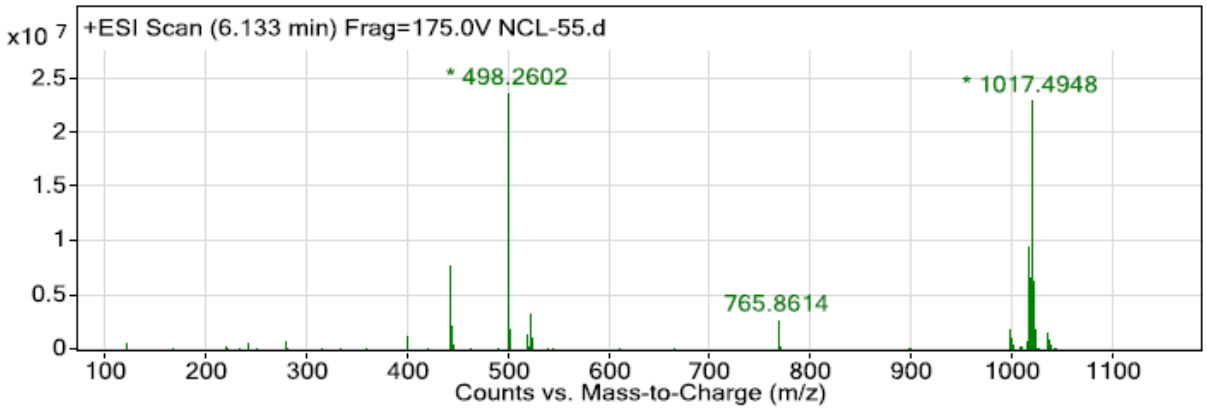

uV
2500000
0
0.275
0.899
1.591
2.106
2.813
3.791
4.097
5.205
8.991
9.824
10.015
10.405
11.244
11.908
0
1
2
3
4
5
6
7
8
9
10
11
12
min

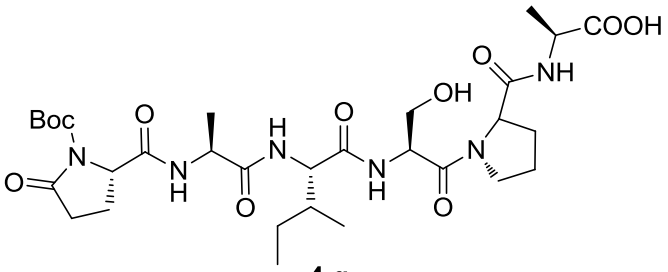

x10 6
+ESI Scan (4.184 min) Frag=175.0V NCL-54.d
2.5
2
1.5
1
0.5
0
768.3753
364.6430
670.3242
232.1693
1103.0483
100
200
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)


Ph
O
H
N
Boc
N
H
H
N
COOH
O
Ph

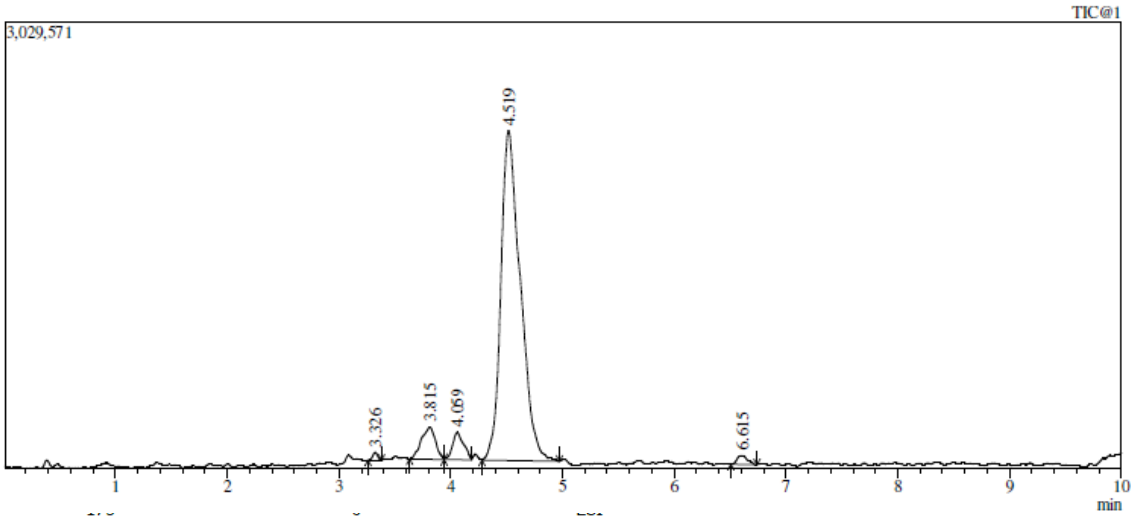
4-f

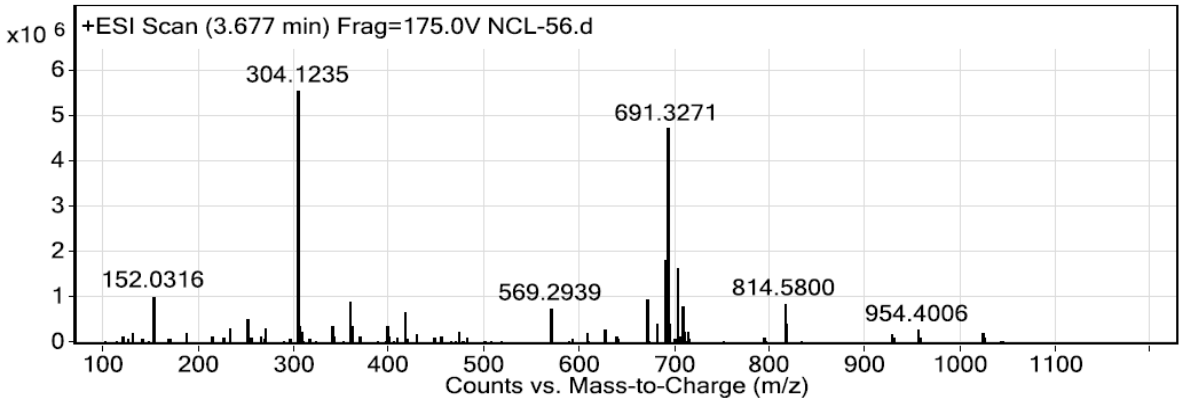

x10 3
DAD1 - C:Sig=214,8 NCL-55.d
1.25
1
0.75
0.5
0.25
0
-0.25
-0.5
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5
Response Units vs. Acquisition Time (min)

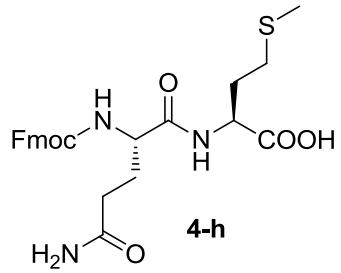

x10 7
+ESI Scan (6.133 min) Frag=175.0V NCL-55.d
* 498.2602
* 1017.4948
765.8614
2.5
2
1.5
1
0.5
0
100 200 300 400 500 600 700 800 900 1000 1100
Counts vs. Mass-to-Charge (m/z)


COOH
O
NH
OH
Boc
O
N
O
N
H
O
H
N
O
N
H
O
N
O

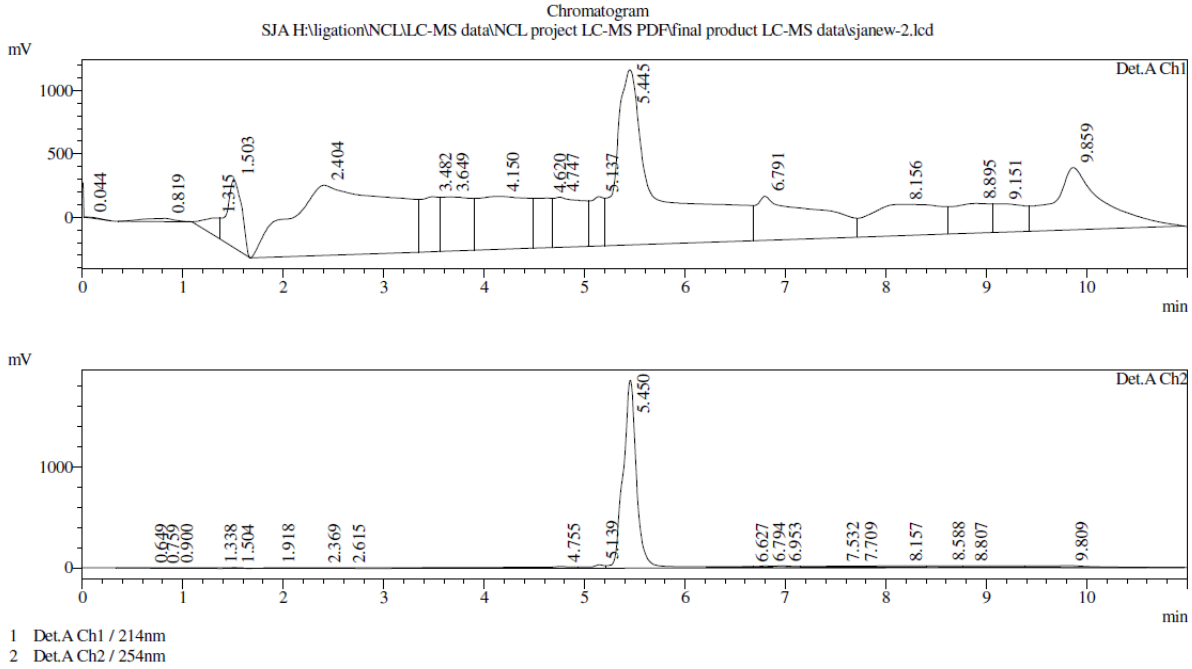
4-g

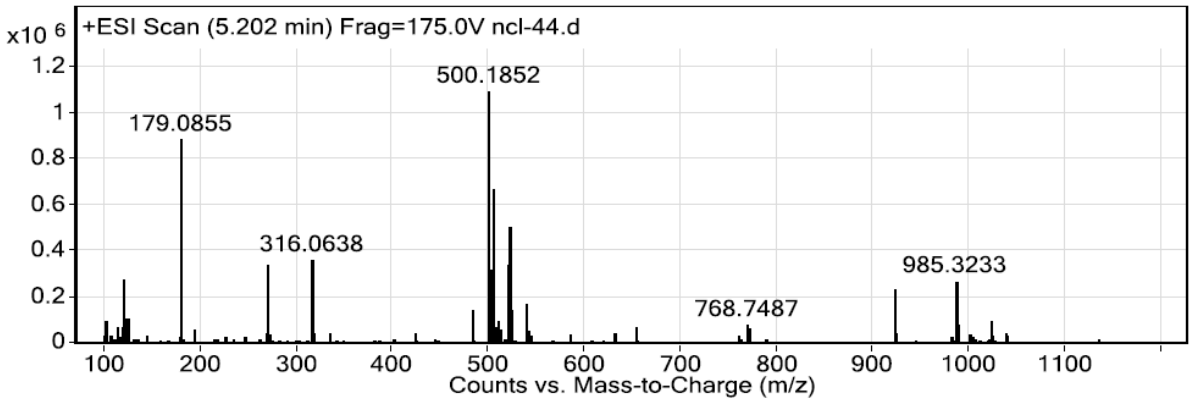

TIC@1
3,029,571
4.519
3.326
3.815
4.069
6.615
1
2
3
4
5
6
7
8
9
10
min

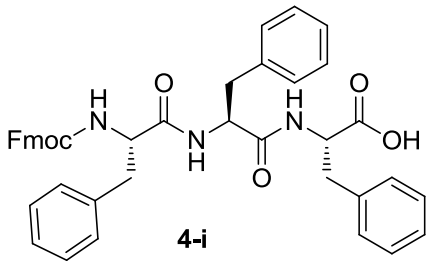

x10 6
+ESI Scan (3.677 min) Frag=175.0V NCL-56.d
304.1235
691.3271
152.0316
569.2939
814.5800
954.4006
0
1
2
3
4
5
6
100
200
300
400
500
600
700
800
900
1000
1100
Counts vs. Mass-to-Charge (m/z)


S
H
N
O
Fmoc
N
H
COOH
H2N
O

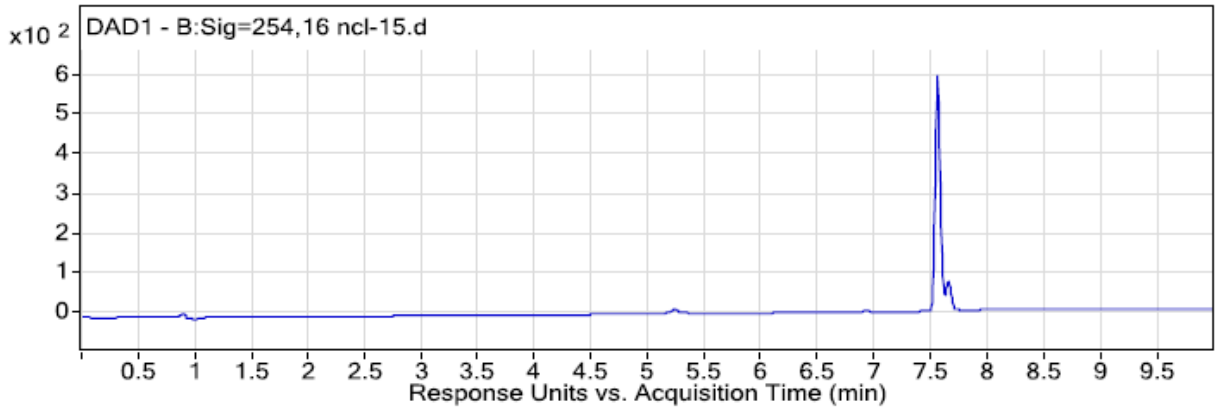
4-h

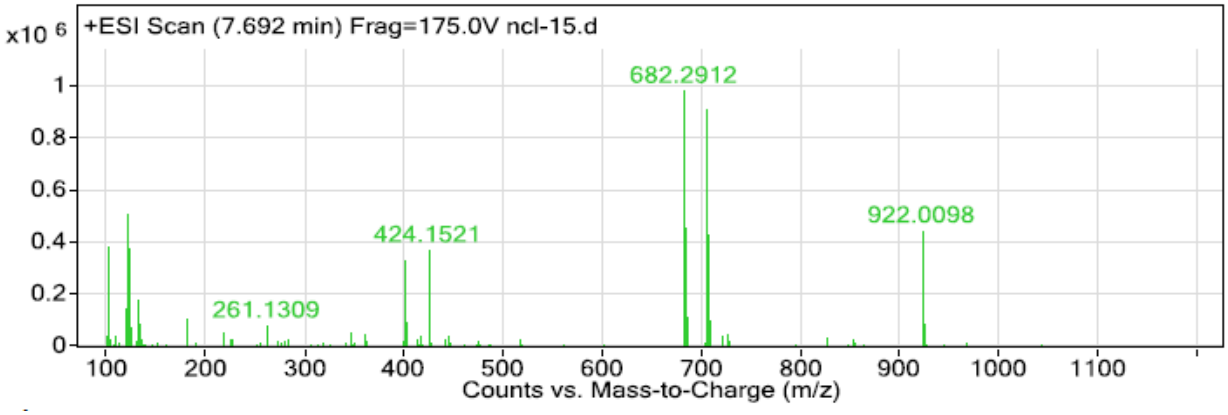

Chromatogram
SJA H:\ligation\NCL\LC-MS data\NCL project LC-MS PDF\final product LC-MS data\sjanew-2.lcd
mV
Det.A Ch1
0.044
0.819
1.315
1.503
2.404
3.482
3.649
4.150
4.620
4.747
5.137
5.445
6.791
8.156
8.895
9.151
9.859
min
mV
Det.A Ch2
0.649
0.759
0.900
1.338
1.504
1.918
2.369
2.615
4.755
5.139
5.450
6.627
6.794
6.953
7.532
7.709
8.157
8.588
8.807
9.809
min
1 Det.A Ch1 / 214nm
2 Det.A Ch2 / 254nm

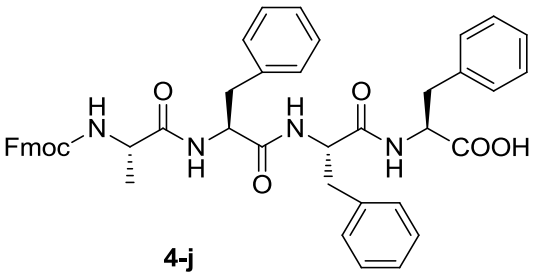

x10 6
+ESI Scan (5.202 min) Frag=175.0V ncl-44.d
500.1852
179.0855
316.0638
985.3233
768.7487
Counts vs. Mass-to-Charge (m/z)


Fmoc
H
N
O
N
H
H
N
O
O
OH

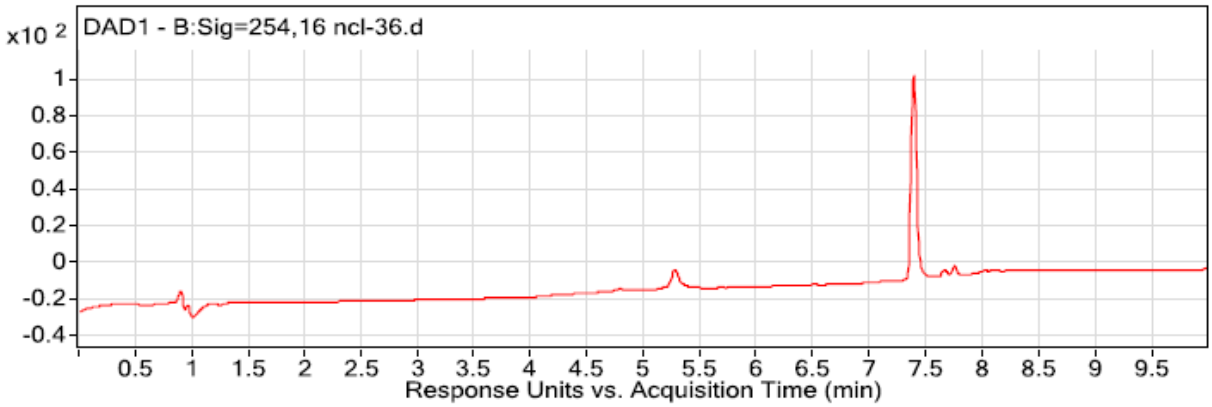
4-i

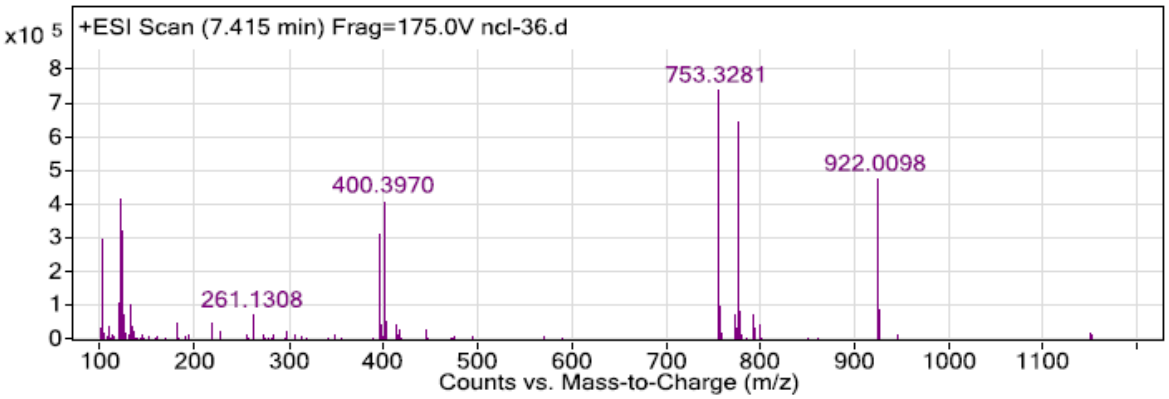

x10 2
DAD1 - B:Sig=254,16 ncl-15.d
Response Units vs. Acquisition Time (min)

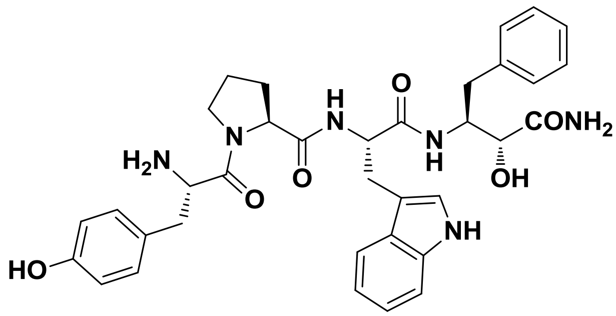

x10 6
+ESI Scan (7.692 min) Frag=175.0V ncl-15.d
682.2912
922.0098
424.1521
261.1309
Counts vs. Mass-to-Charge (m/z)



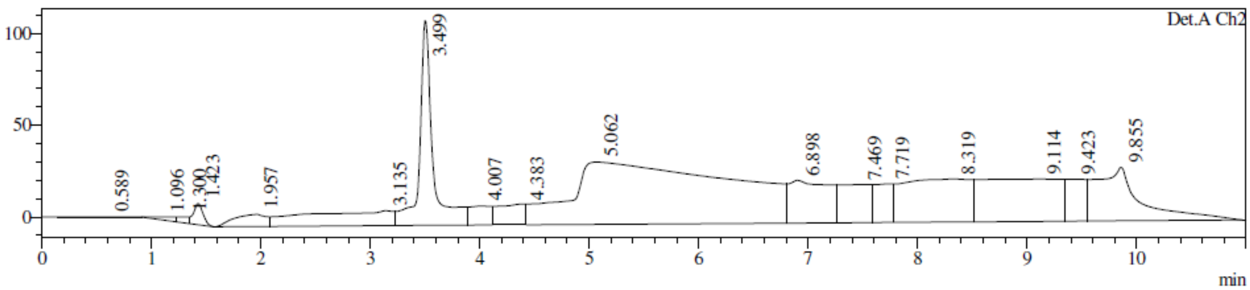
4-j

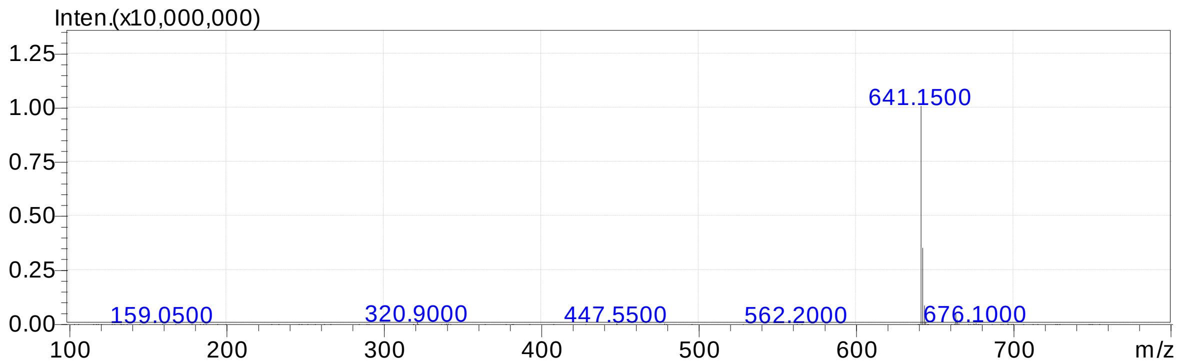


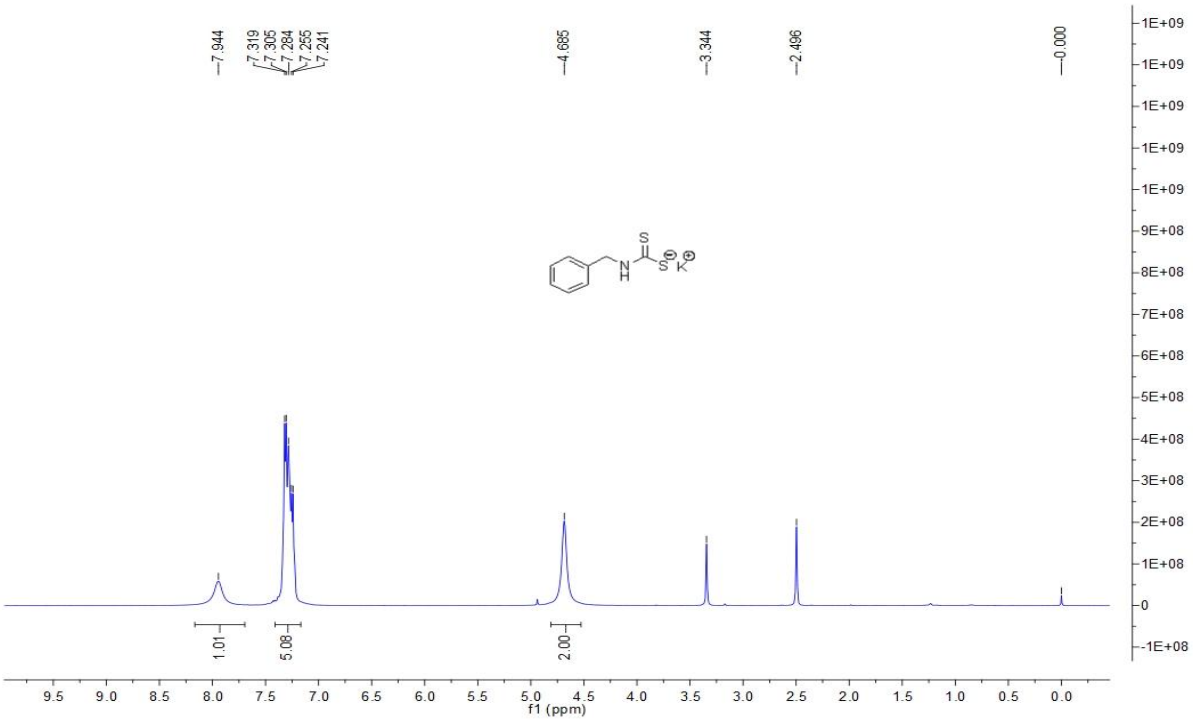


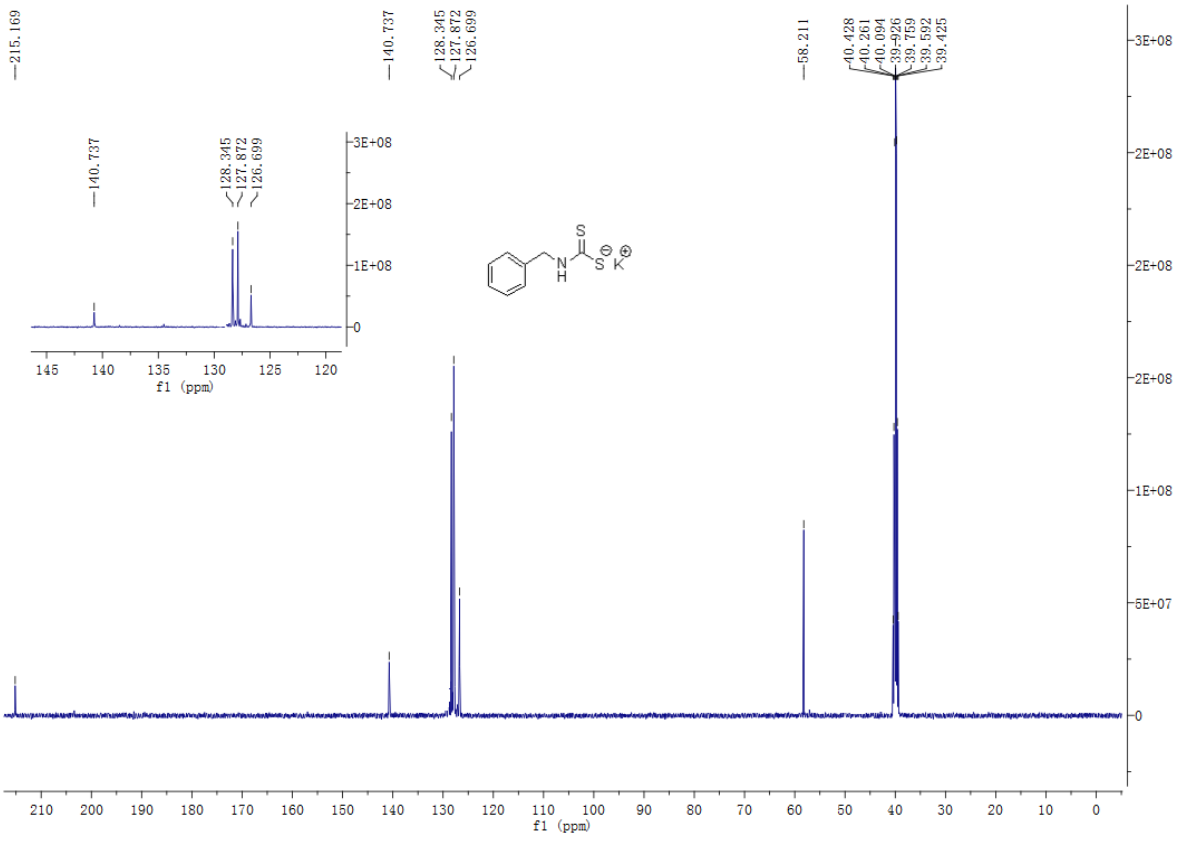

H
N
O
O
CONH$_2$
H_2N
N
O
N
H
OH
NH
HO

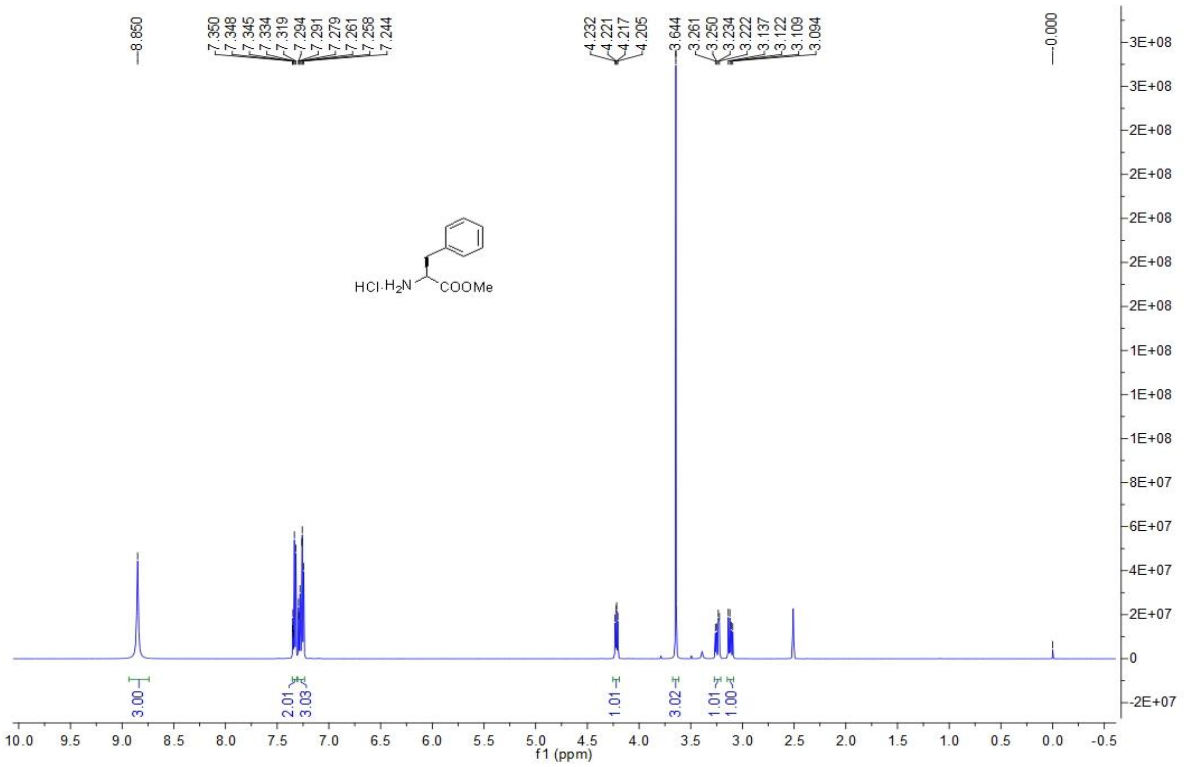
5a

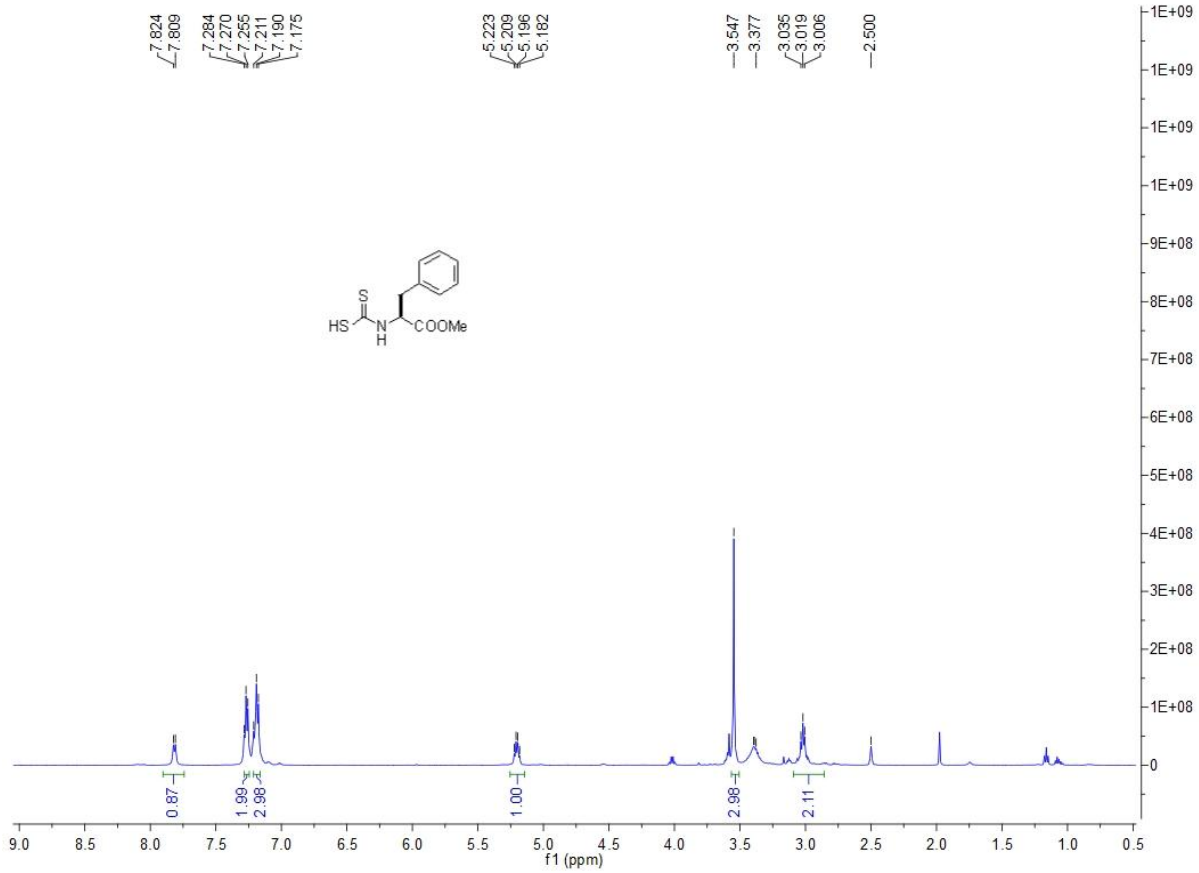

Det.A Ch2
100
50
0
0.589
1.096
1.300
1.423
1.957
3.135
3.499
4.007
4.383
5.062
6.898
7.469
7.719
8.319
9.114
9.423
9.855
0 1 2 3 4 5 6 7 8 9 10
min

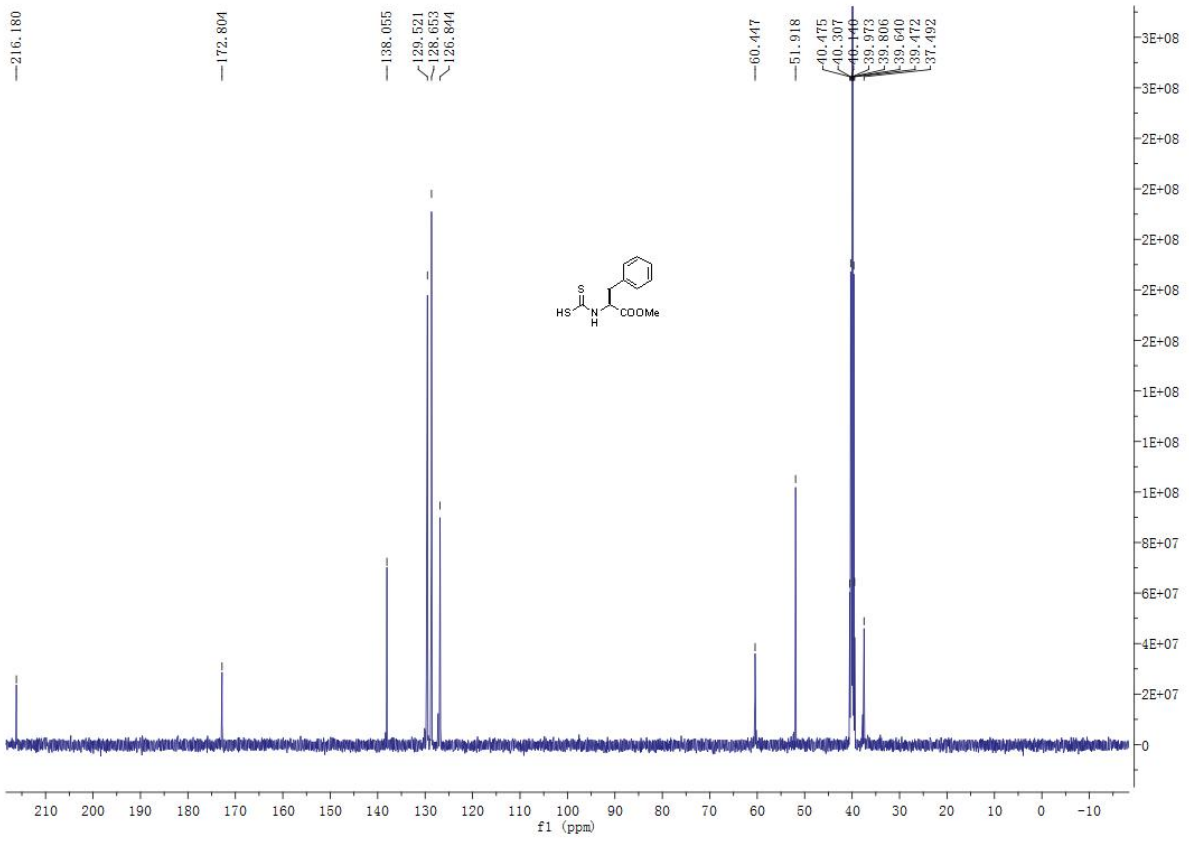

Inten.(x10,000,000)
1.25
1.00
0.75
0.50
0.25
0.00
641.1500
159.0500
320.9000
447.5500
562.2000
676.1000
100 200 300 400 500 600 700
m/z

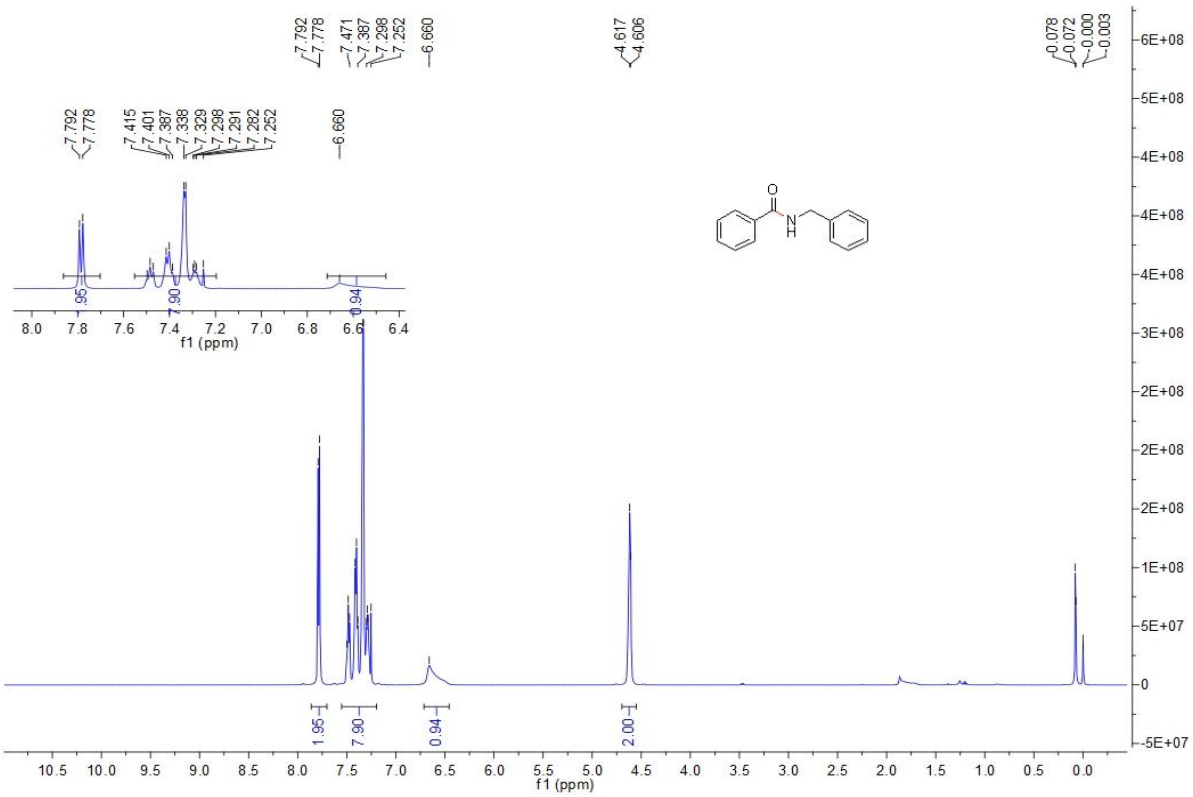

7.944
7.319
7.305
7.284
7.255
7.241
4.685
3.344
2.496
0.000
1.01
5.08
2.00
f1 (ppm)

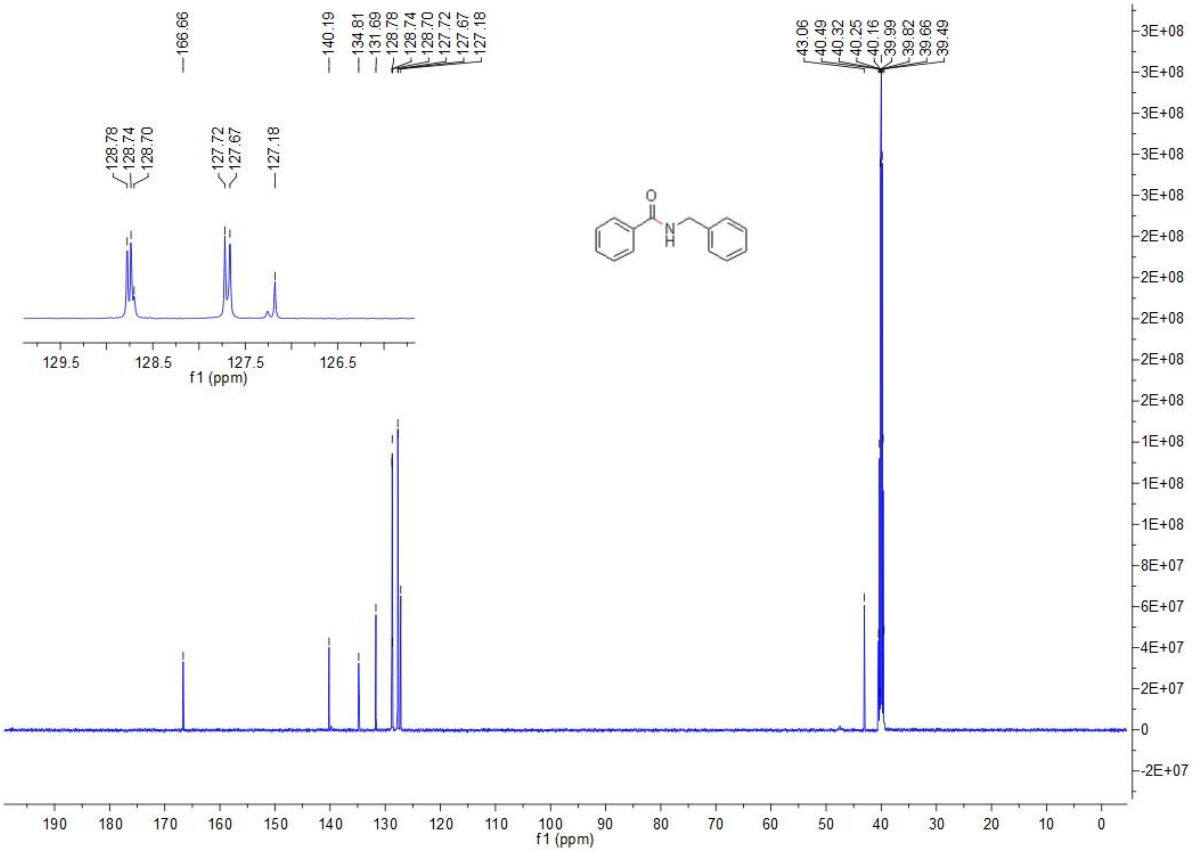

215.169
140.737
128.345
127.872
126.699
58.211
40.428
40.261
40.094
39.926
39.759
39.592
39.425
f1 (ppm)

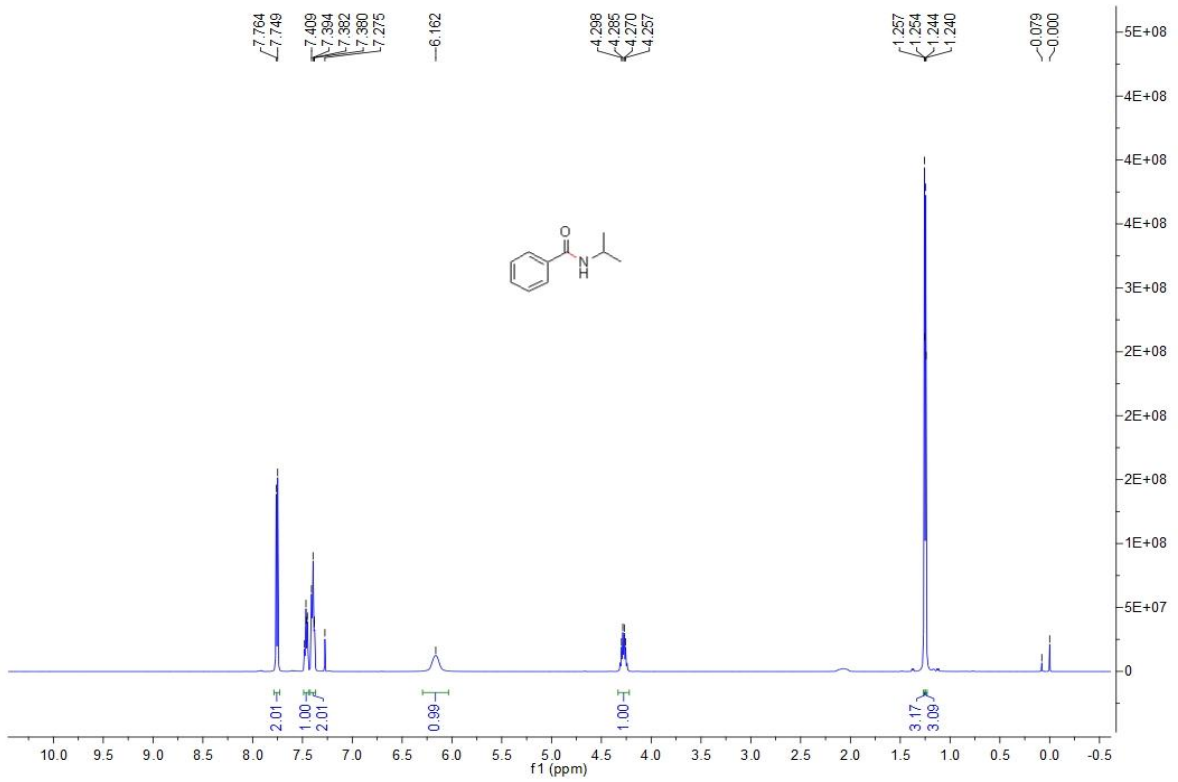

8.850
7.350
7.348
7.345
7.334
7.319
7.294
7.291
7.279
7.261
7.258
7.244
4.232
4.221
4.217
4.205
3.644
3.261
3.250
3.234
3.222
3.137
3.122
3.109
3.094
0.000
HCl·H2N
COOMe
3.00
2.01
3.03
1.01
3.02
1.01
1.00
f1 (ppm)

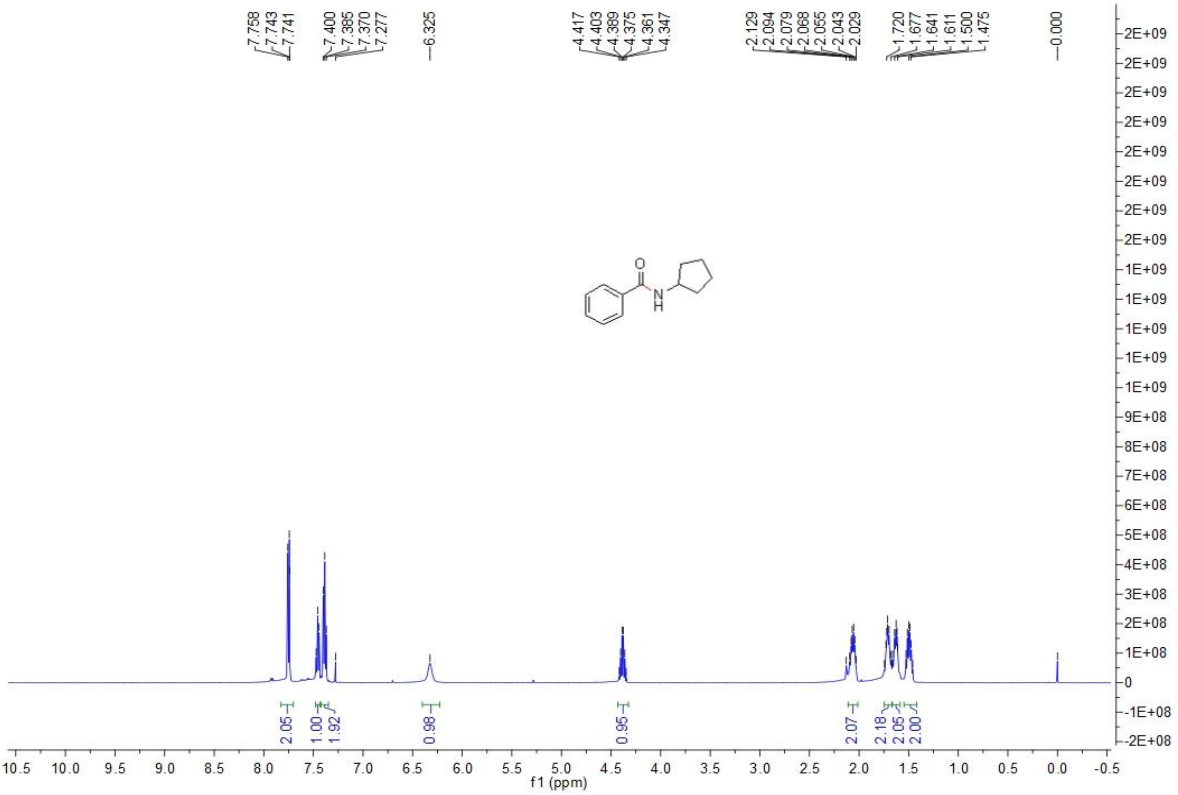

7.824
7.809
7.284
7.270
7.255
7.211
7.190
7.175
5.223
5.209
5.196
5.182
3.547
3.377
3.035
3.019
3.006
2.500
HS
N
H
COOMe
0.87
1.99
2.98
1.00
2.98
2.11
f1 (ppm)

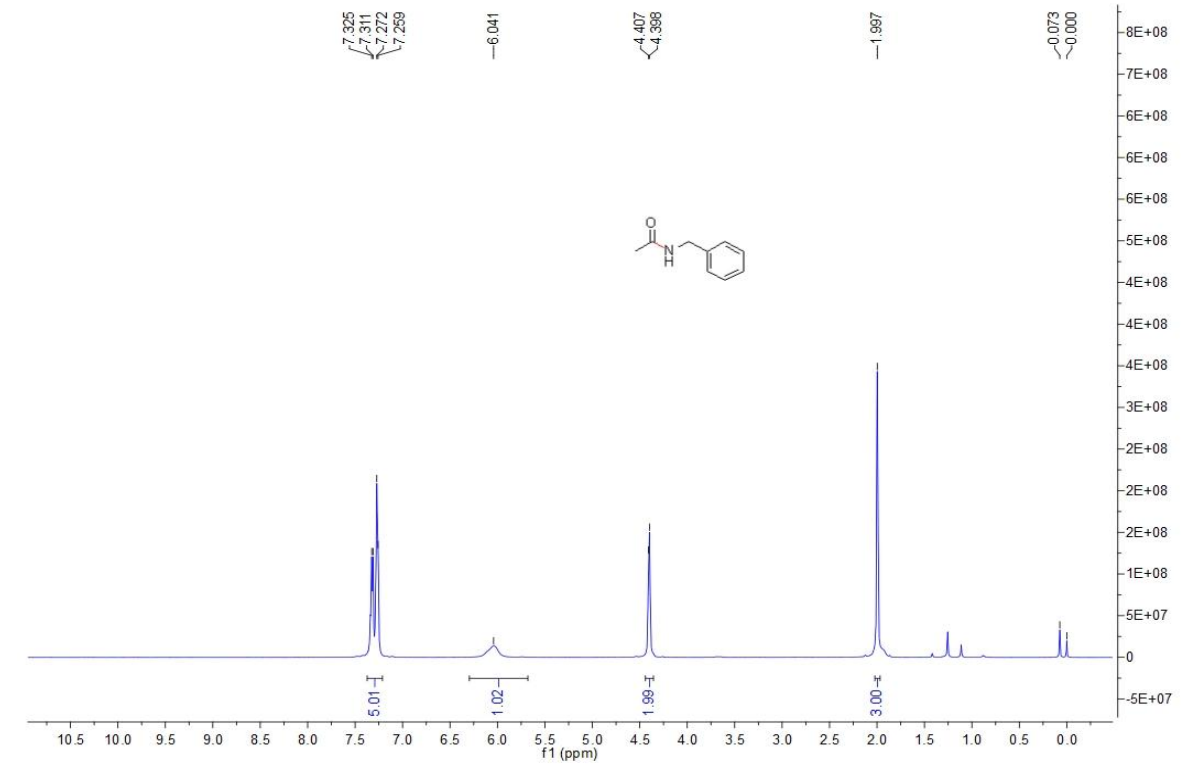


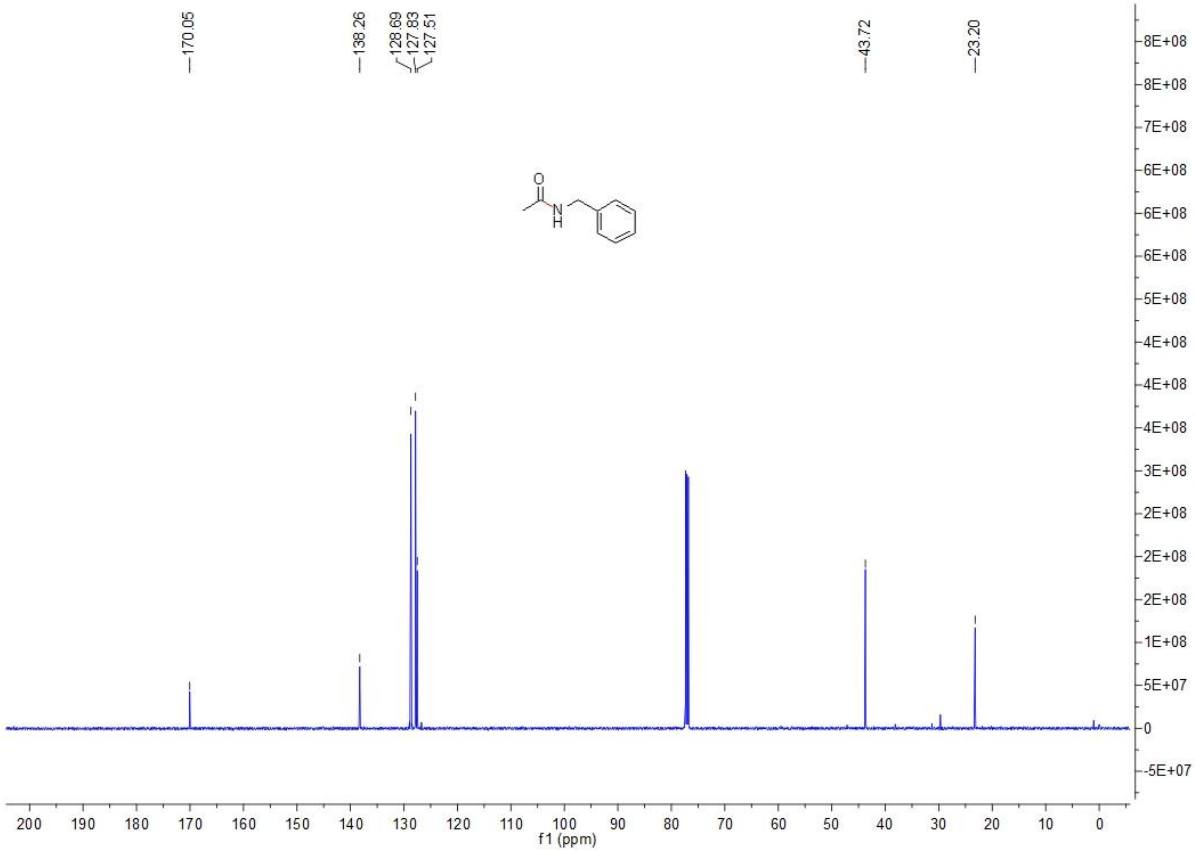

7.792
7.778
7.471
7.387
7.298
7.252
6.660
4.617
4.606
0.078
0.072
0.000
0.003
1.95
7.90
0.94
2.00
f1 (ppm)

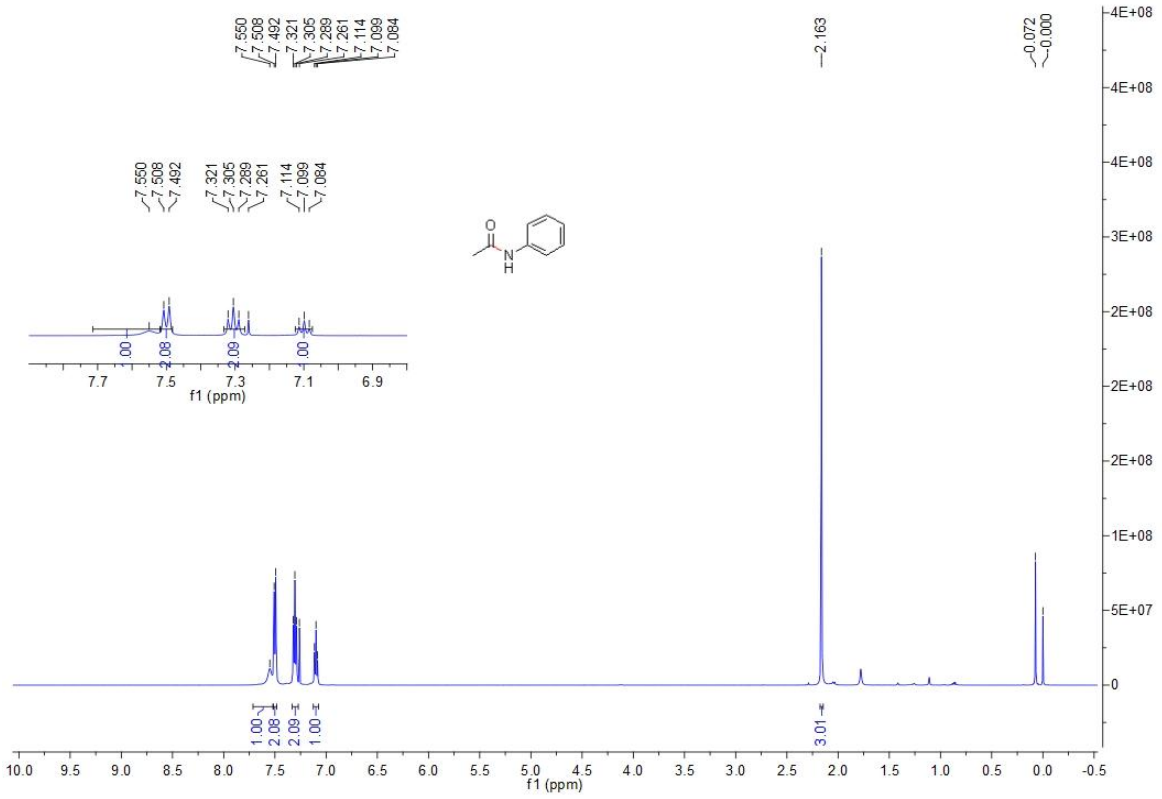

166.66
140.19
134.81
131.69
128.78
128.74
128.70
127.72
127.67
127.18
43.06
40.49
40.32
40.25
40.16
39.99
39.82
39.66
39.49
f1 (ppm)

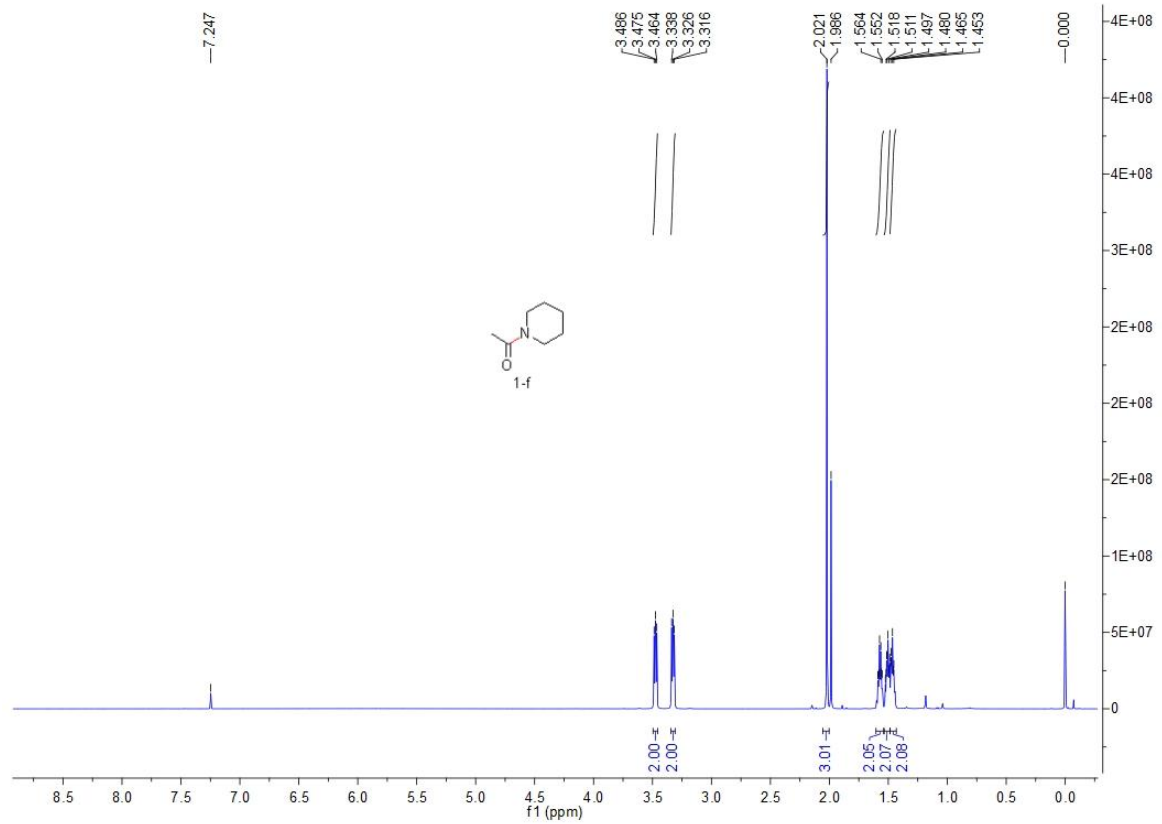

7.764
7.749
7.409
7.394
7.382
7.380
7.275
6.162
4.298
4.285
4.270
4.257
1.257
1.254
1.244
1.240
0.079
0.000
2.01
1.00
2.01
0.99
1.00
3.17
3.09
f1 (ppm)

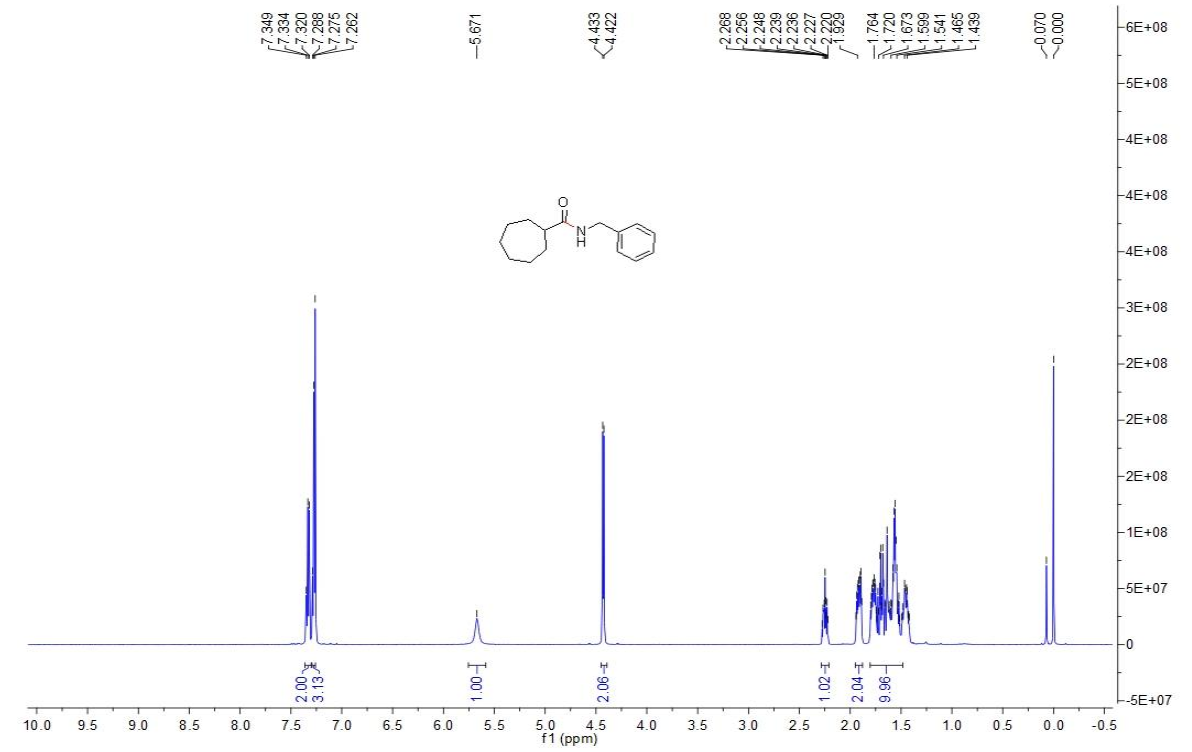

7.758
7.743
7.741
7.400
7.385
7.370
7.277
6.325
4.417
4.403
4.389
4.375
4.361
4.347
2.129
2.094
2.079
2.068
2.055
2.043
2.029
1.720
1.677
1.641
1.611
1.500
1.475
0.000
2.05
1.00
1.92
0.98
0.95
2.07
2.18
2.05
2.00
f1 (ppm)

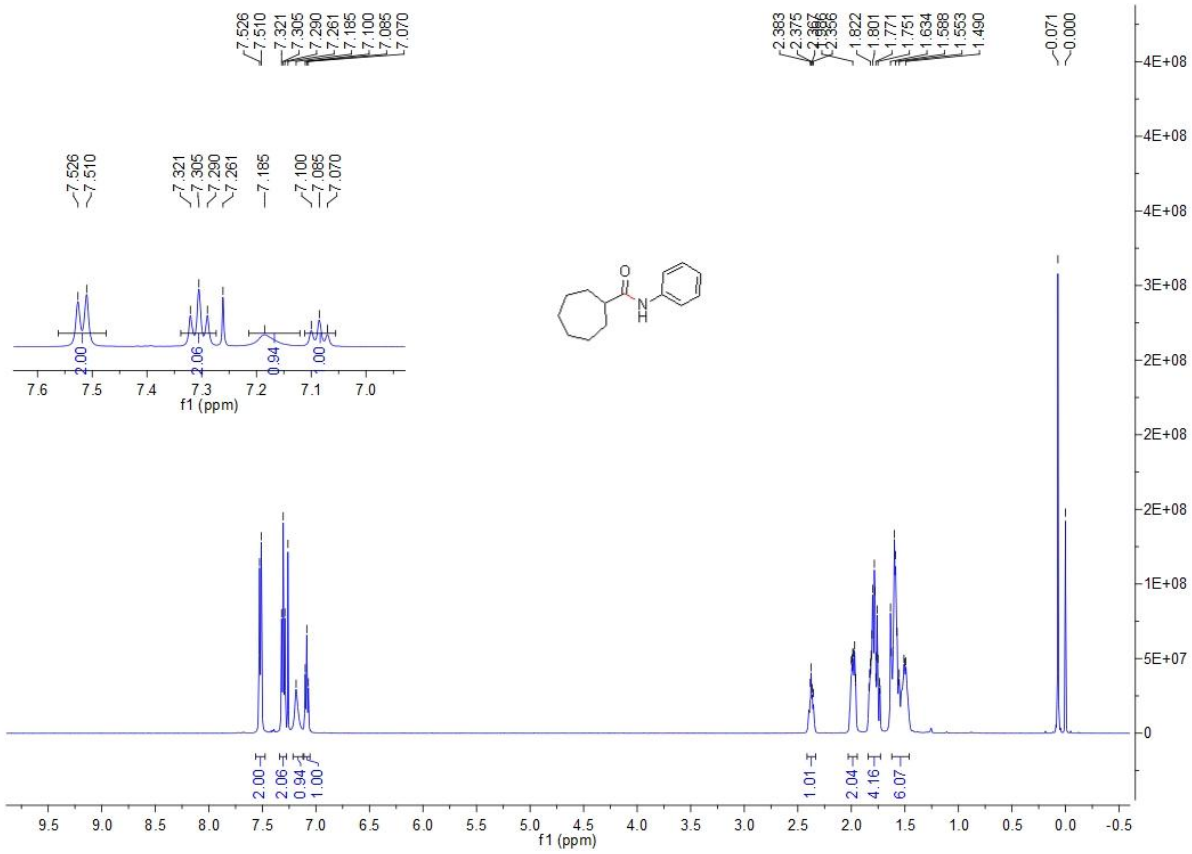

7.325
7.311
7.272
7.259
6.041
4.407
4.398
1.997
0.073
0.000
5.01
1.02
1.99
3.00
f1 (ppm)

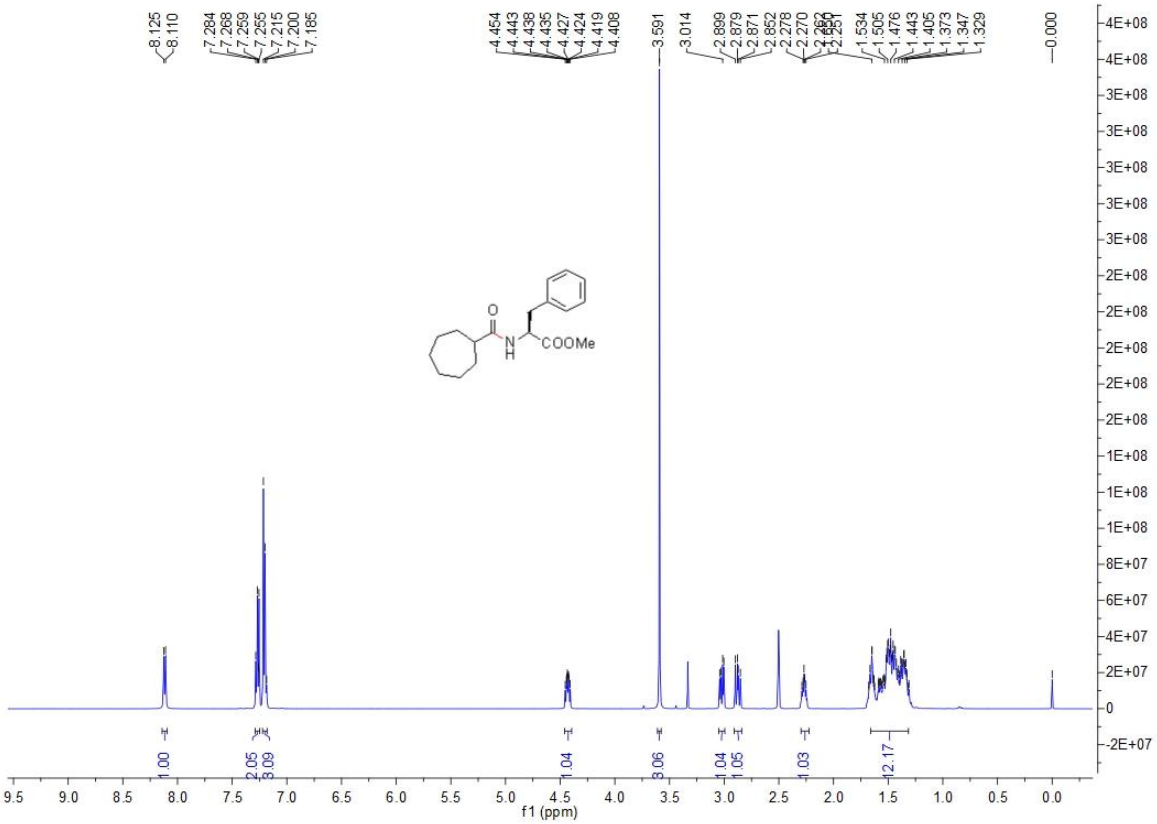

170.05
138.26
128.69
127.83
127.51
43.72
23.20
f1 (ppm)

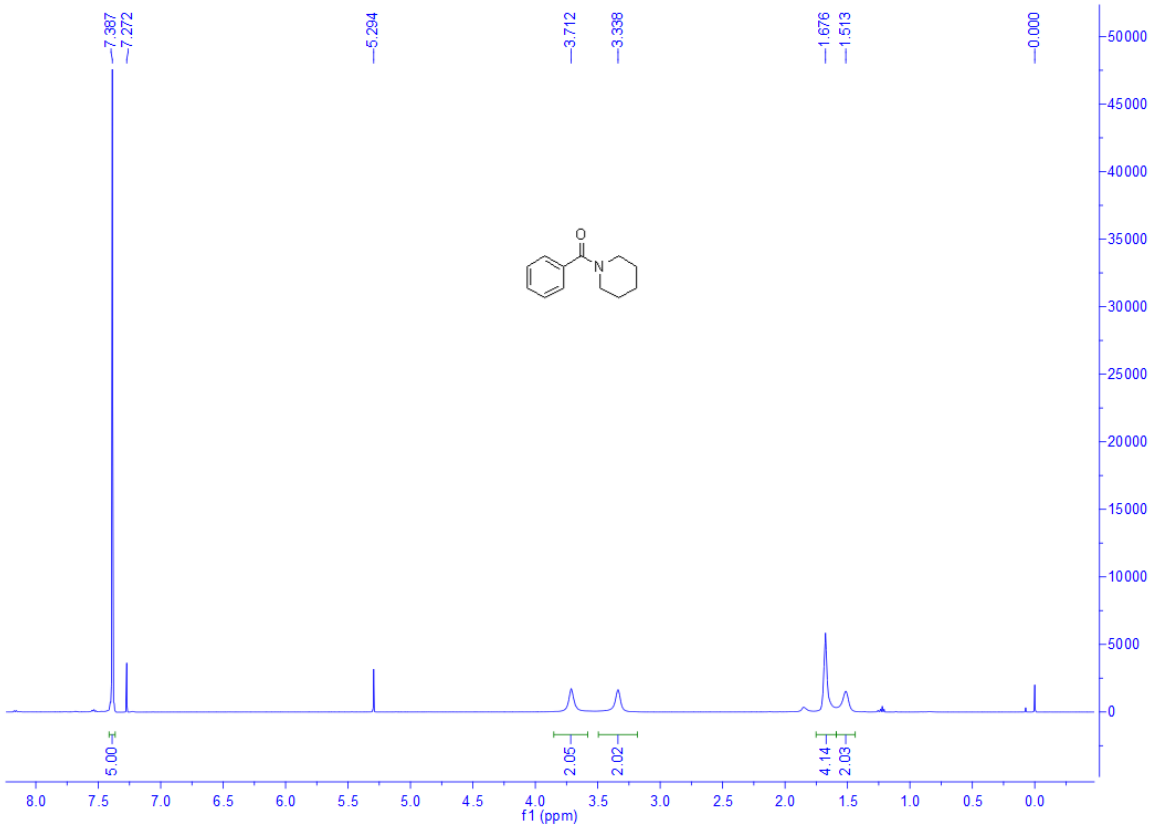

7.550
7.508
7.492
7.321
7.305
7.289
7.261
7.114
7.099
7.084
2.163
0.072
0.000
f1 (ppm)
1.00
2.08
2.09
1.00
3.01

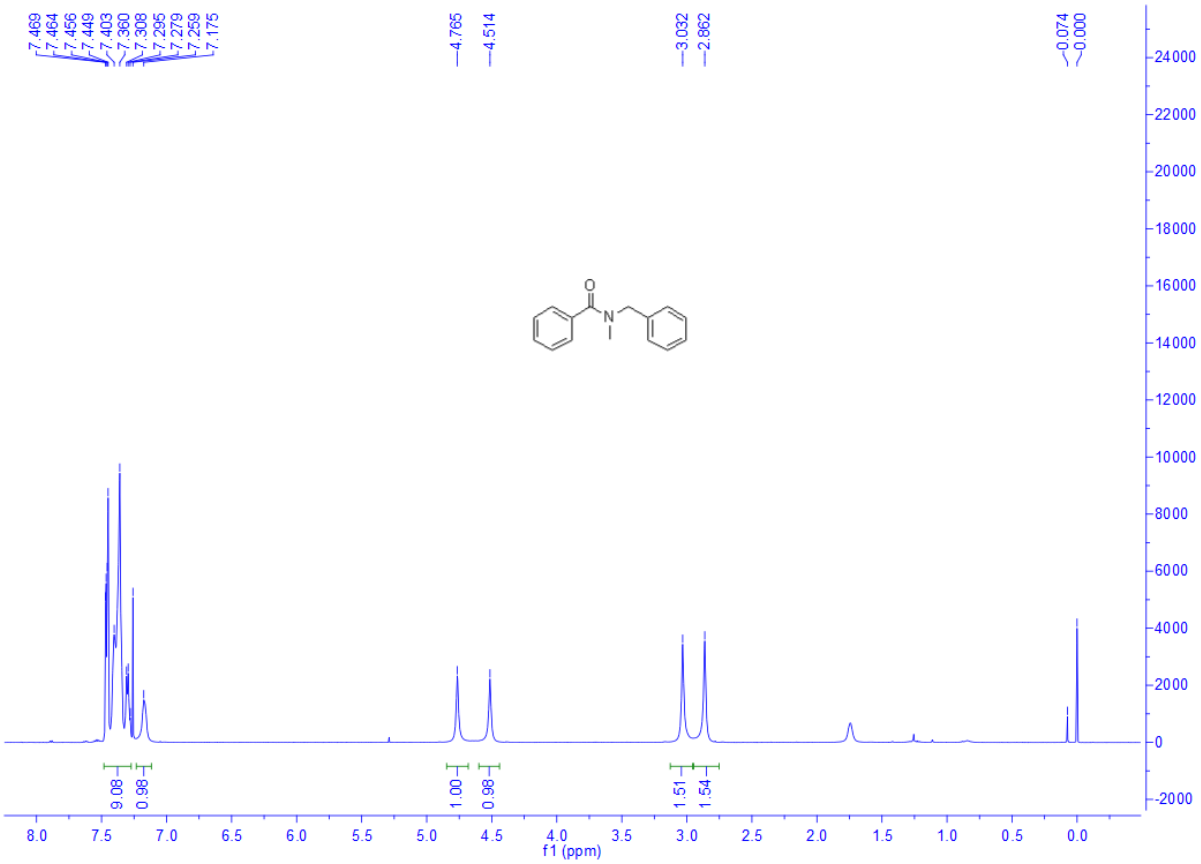

7.247
3.486
3.475
3.464
3.338
3.326
3.316
2.021
1.986
1.564
1.552
1.518
1.511
1.497
1.480
1.465
1.453
0.000
1-f
f1 (ppm)
2.00
2.00
3.01
2.05
2.07
2.08

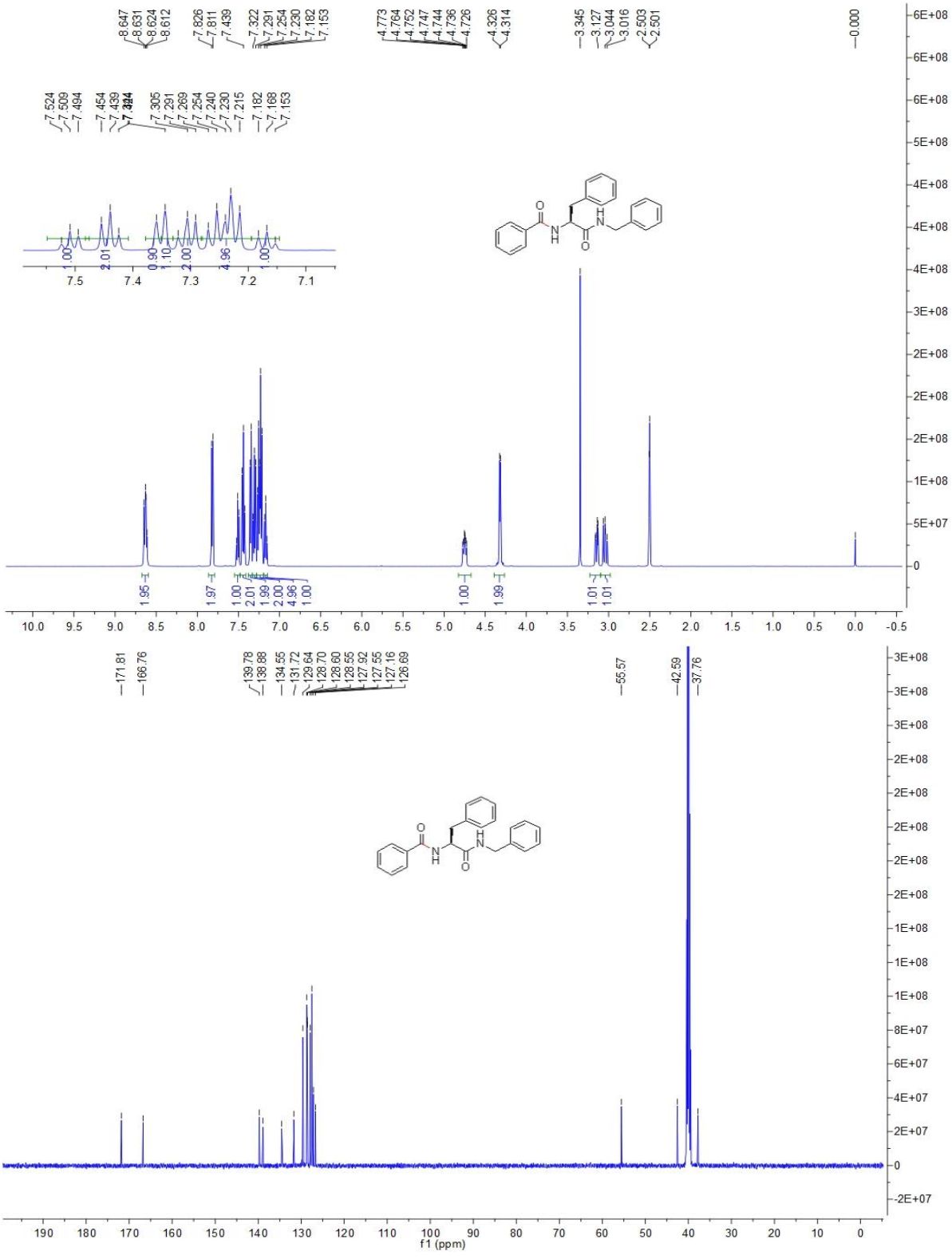

7.349
7.334
7.320
7.288
7.275
7.262
5.671
4.433
4.422
2.268
2.256
2.248
2.239
2.236
2.227
2.220
1.929
1.764
1.720
1.673
1.599
1.541
1.465
1.439
0.070
0.000
2.00
3.13
1.00
2.06
1.02
2.04
9.96
f1 (ppm)

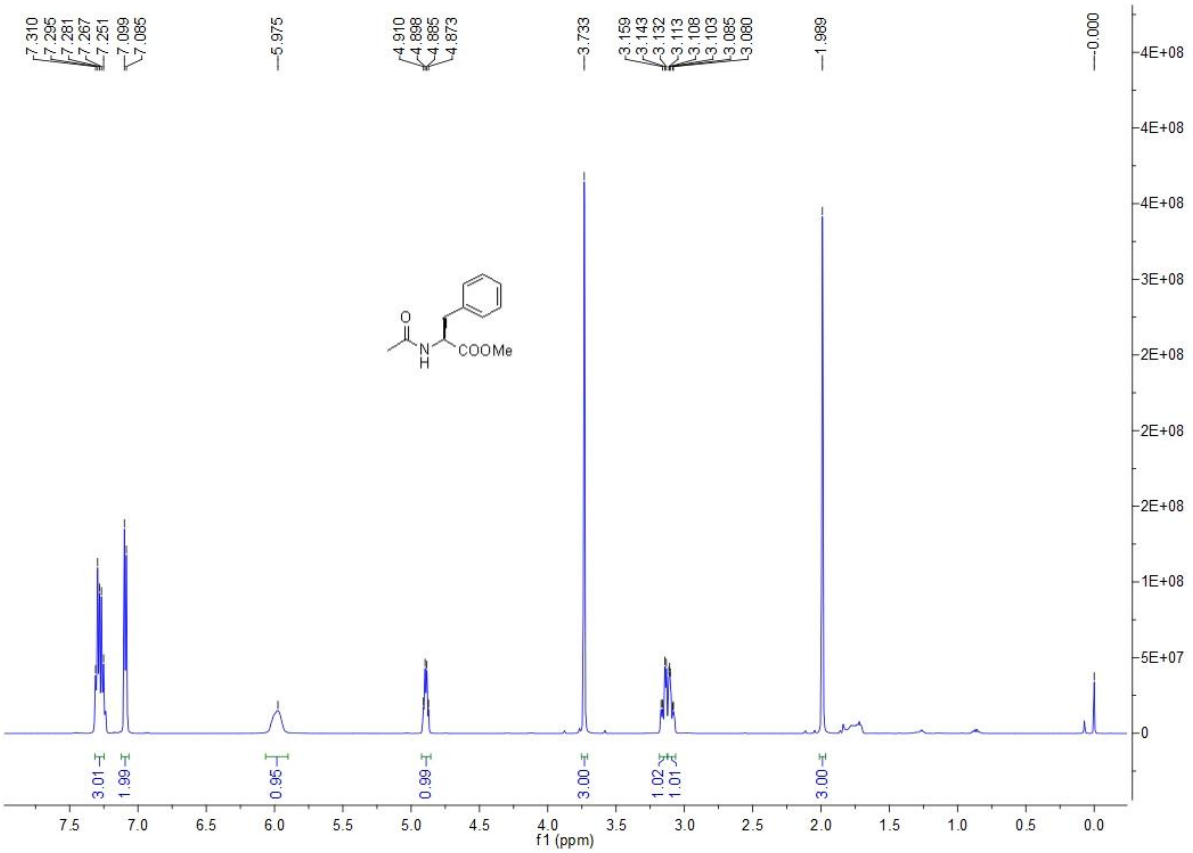

7.526
7.510
7.321
7.305
7.290
7.261
7.185
7.100
7.085
7.070
2.383
2.375
1.822
1.801
1.771
1.751
1.634
1.588
1.553
1.490
0.071
0.000
2.00
2.06
0.94
1.00
f1 (ppm)
2.00
2.06
0.94
1.00
1.01
2.04
4.16
6.07
f1 (ppm)

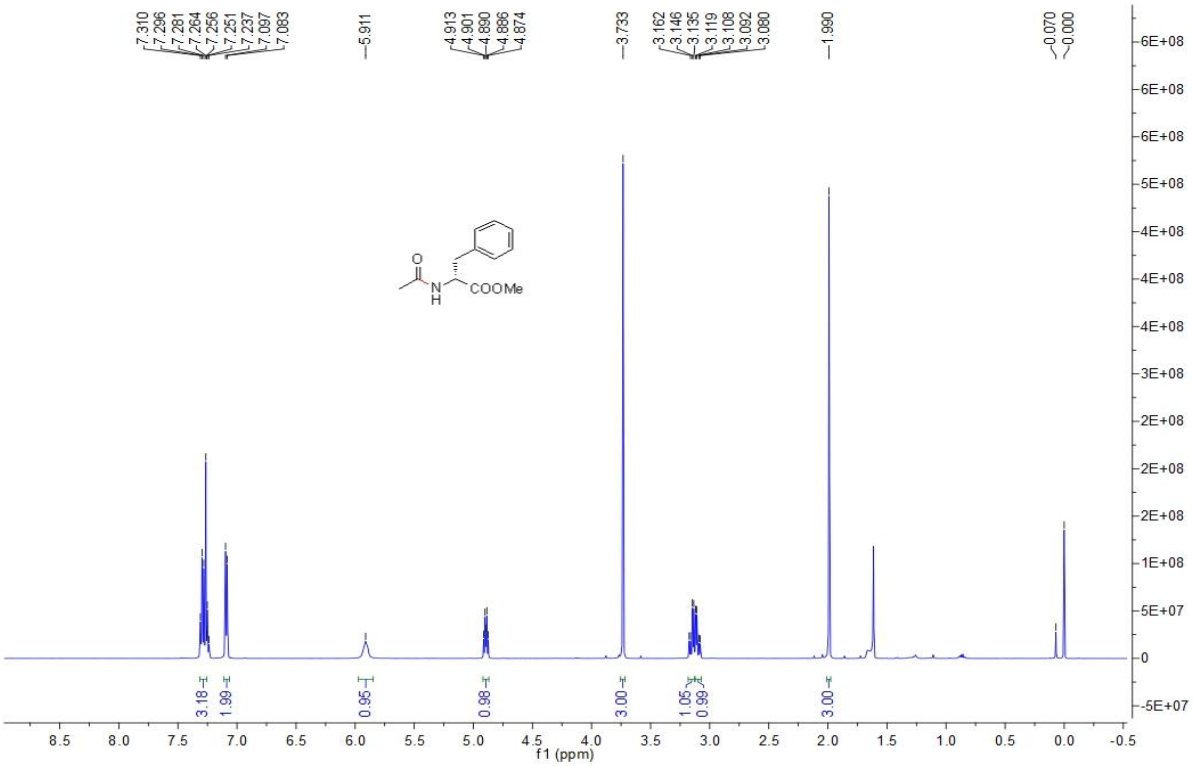

8.125
8.110
7.284
7.268
7.259
7.255
7.215
7.200
7.185
4.454
4.443
4.438
4.435
4.427
4.424
4.419
4.408
3.591
3.014
2.899
2.879
2.871
2.852
2.278
2.270
1.534
1.505
1.476
1.443
1.405
1.373
1.347
1.329
0.000
O
N
H
COOMe
1.00
2.05
3.09
1.04
3.06
1.04
1.05
1.03
2.17
f1 (ppm)

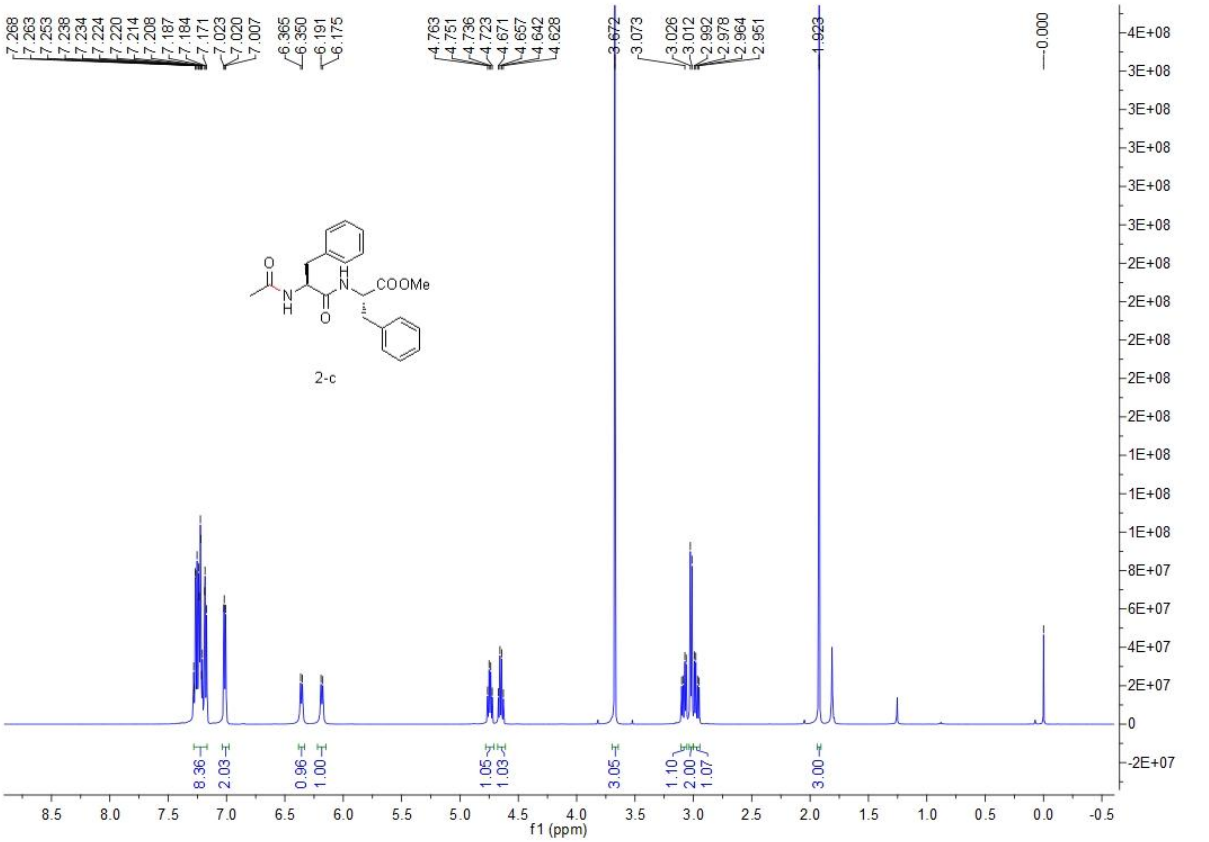

7.387
7.272
5.294
3.712
3.338
1.676
1.513
0.000
O
N
5.00
2.05
2.02
4.14
2.03
f1 (ppm)

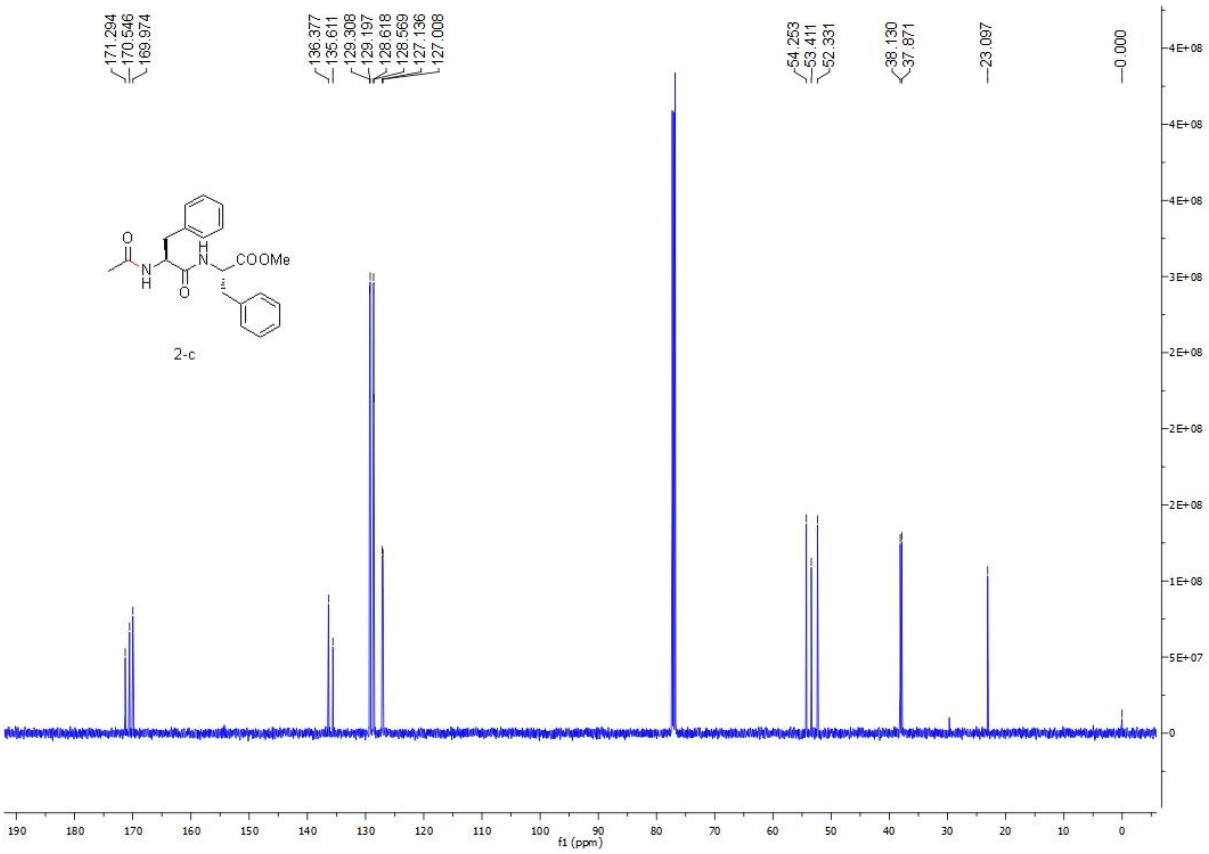

7.469
7.464
7.456
7.449
7.403
7.360
7.308
7.295
7.279
7.259
7.175
4.765
4.514
3.032
2.862
0.074
0.000
O
N
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
9.08
0.98
1.00
0.98
1.51
1.54
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

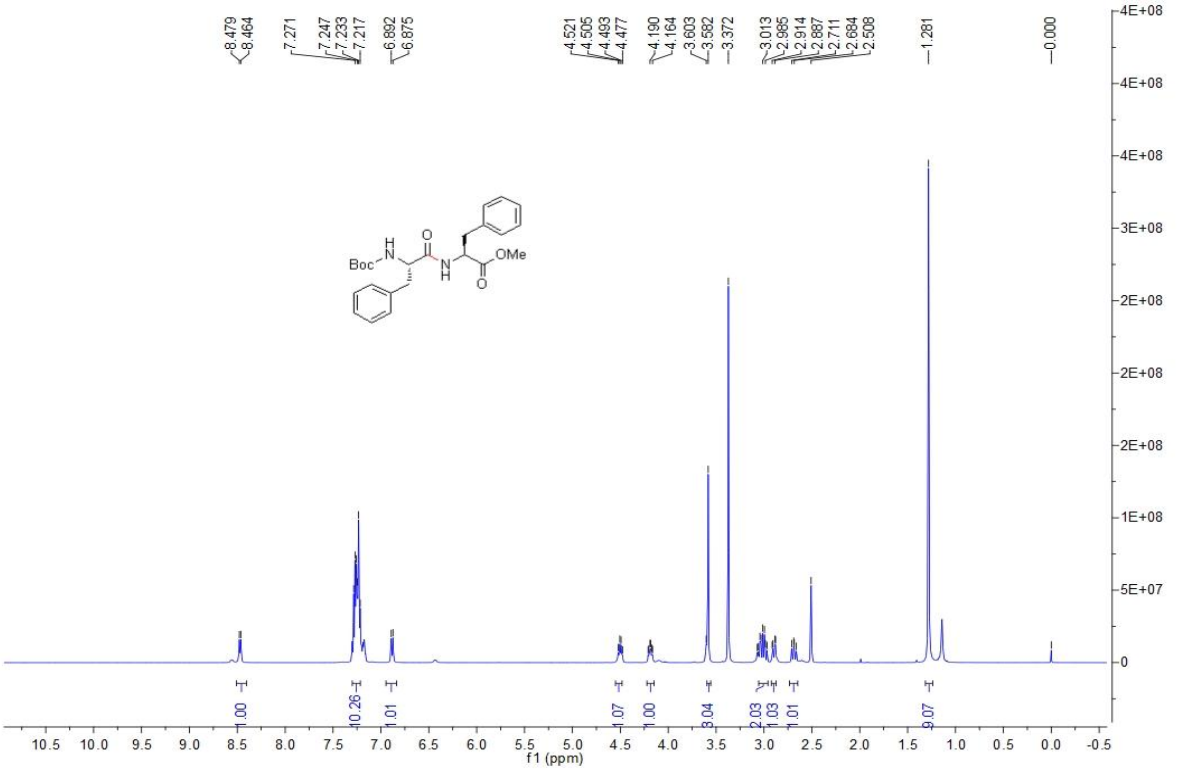

8.647
8.631
8.624
8.612
7.826
7.811
7.439
7.322
7.291
7.254
7.230
7.182
7.153
4.773
4.764
4.752
4.747
4.744
4.736
4.726
4.326
4.314
3.345
3.127
3.044
3.016
2.503
2.501
0.000
7.524
7.509
7.494
7.454
7.439
7.305
7.291
7.269
7.254
7.240
7.230
7.215
7.182
7.168
7.153
1.95
1.97
1.00
2.01
1.99
2.00
4.96
1.00
1.00
1.99
1.01
1.01
171.81
166.76
139.78
138.88
134.55
131.72
129.64
128.70
128.60
128.55
127.92
127.55
127.16
126.69
55.57
42.59
37.76
f1 (ppm)

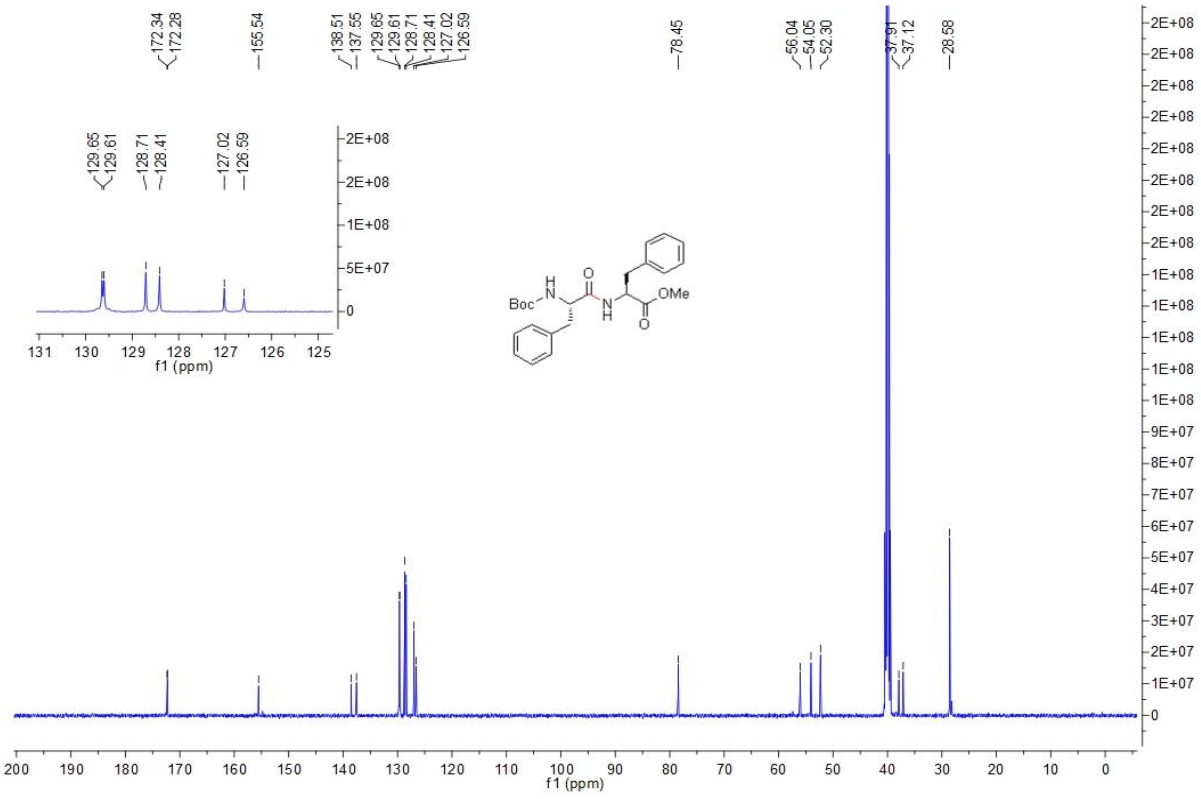

7.310
7.295
7.281
7.267
7.251
7.099
7.085
5.975
4.910
4.898
4.885
4.873
3.733
3.159
3.143
3.132
3.113
3.108
3.103
3.085
3.080
1.989
0.000
COOMe
3.01
1.99
0.95
0.99
3.00
1.02
1.01
3.00
f1 (ppm)

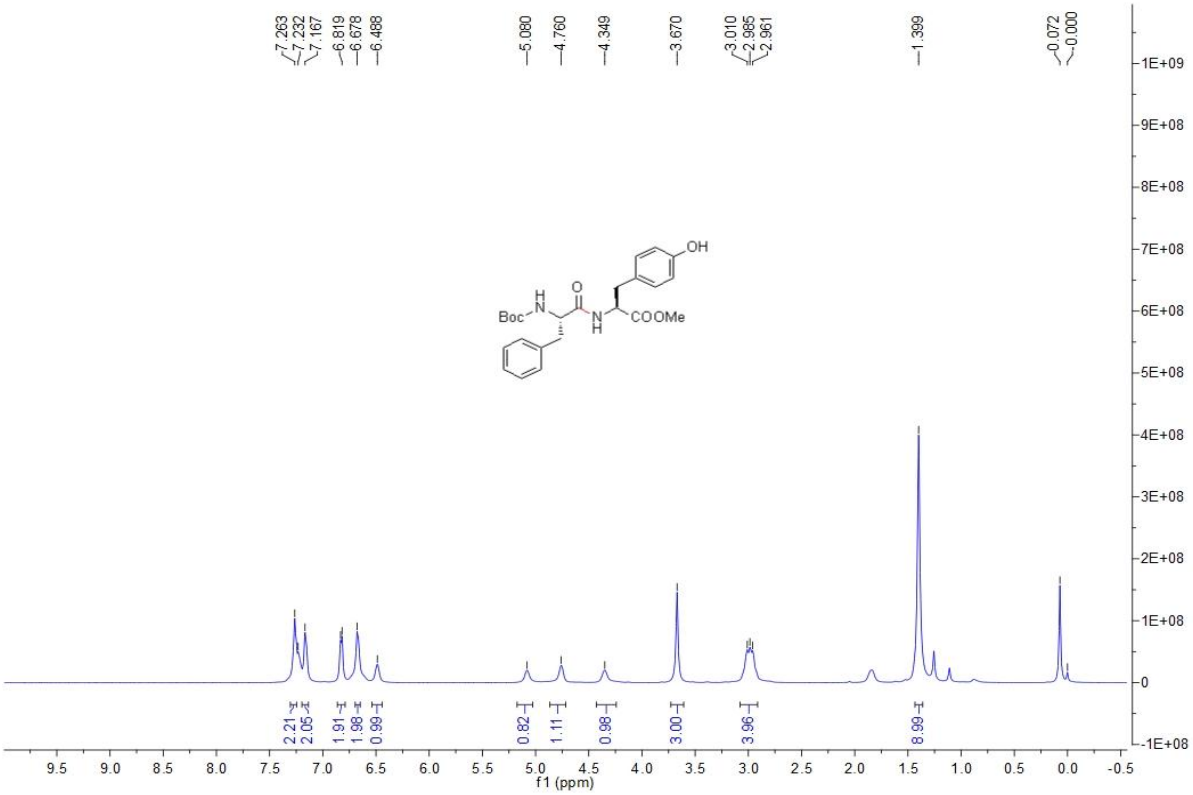

7.310
7.296
7.281
7.264
7.256
7.251
7.237
7.097
7.083
5.911
4.913
4.901
4.890
4.886
4.874
3.733
3.162
3.146
3.135
3.119
3.108
3.092
3.080
1.990
0.070
0.000
COOMe
3.18
1.99
0.95
0.98
3.00
1.05
0.99
3.00
f1 (ppm)

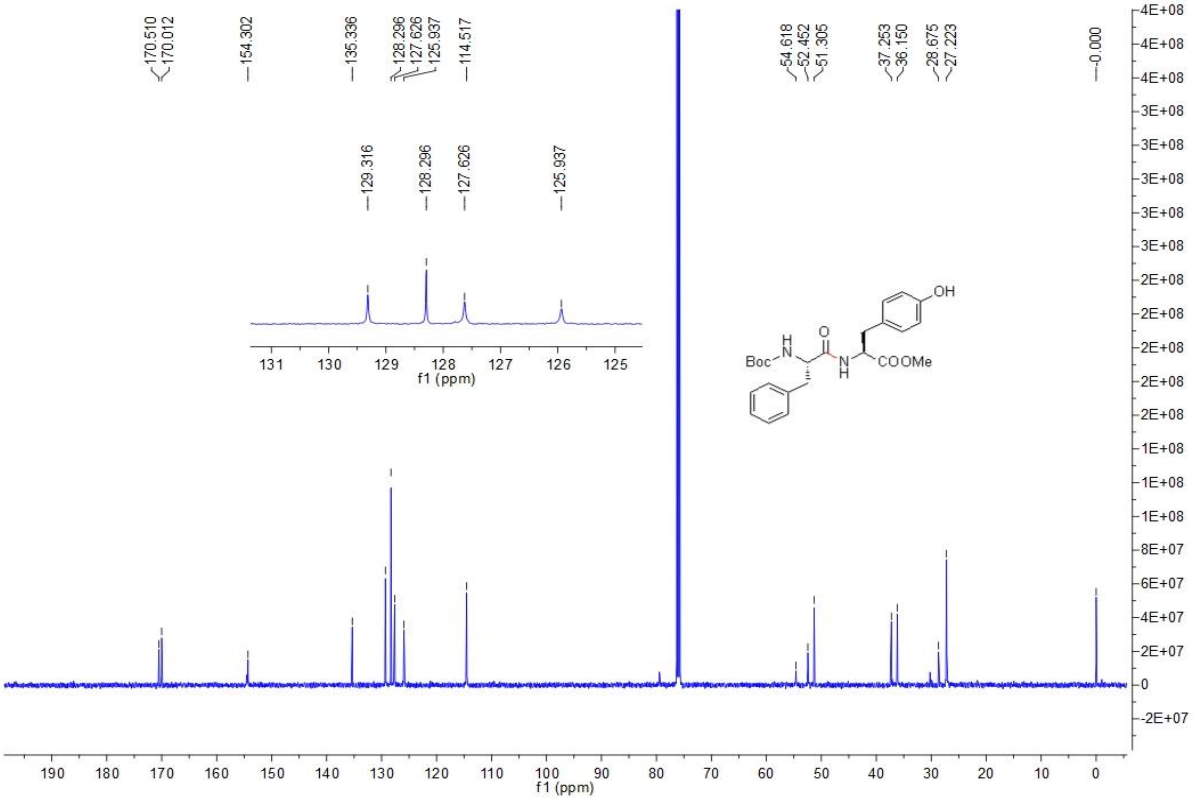

7.268
7.263
7.253
7.238
7.234
7.224
7.220
7.214
7.208
7.187
7.184
7.171
7.023
7.020
7.007
6.365
6.360
6.191
6.175
4.763
4.751
4.736
4.723
4.671
4.657
4.642
4.628
3.672
3.073
3.026
3.012
2.992
2.978
2.964
2.951
1.923
0.000
COOMe
2-c
8.36
2.03
0.96
1.00
1.05
1.03
3.05
1.10
2.00
1.07
3.00
f1 (ppm)

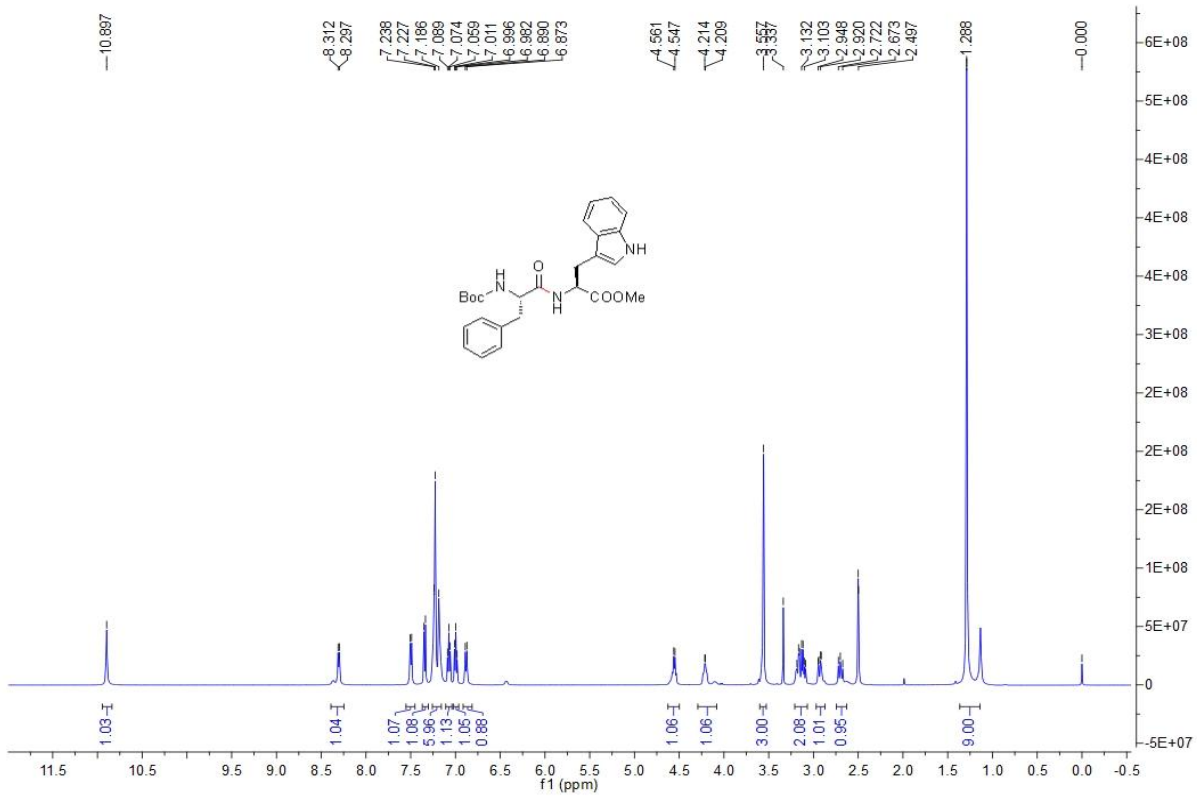

171.294
170.546
169.974
136.377
135.611
129.308
129.197
128.618
128.569
127.136
127.008
54.253
53.411
52.331
38.130
37.871
23.097
0.000
COOMe
2-c
f1 (ppm)

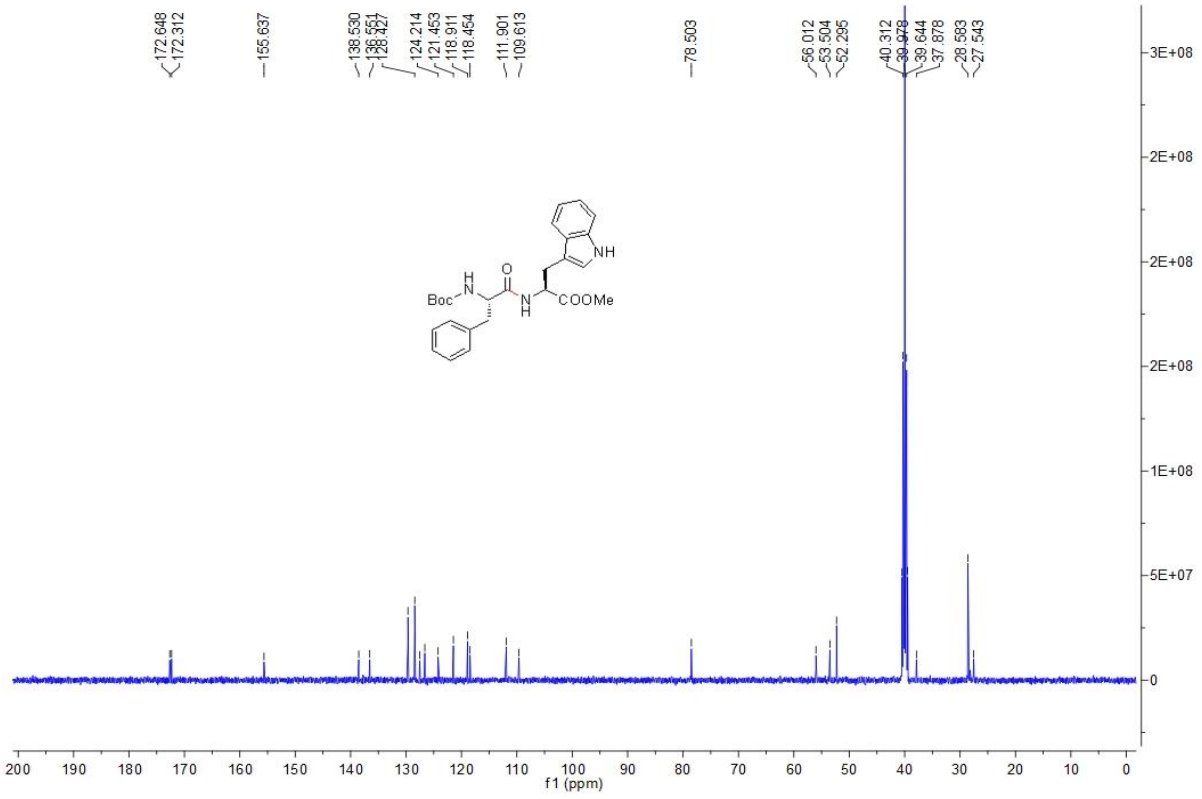

8.479
8.464
7.271
7.247
7.233
7.217
6.892
6.875
4.521
4.505
4.493
4.477
4.190
4.164
3.603
3.582
3.372
3.013
2.985
2.914
2.887
2.711
2.684
2.508
1.281
0.000
Boc
OMe
f1 (ppm)

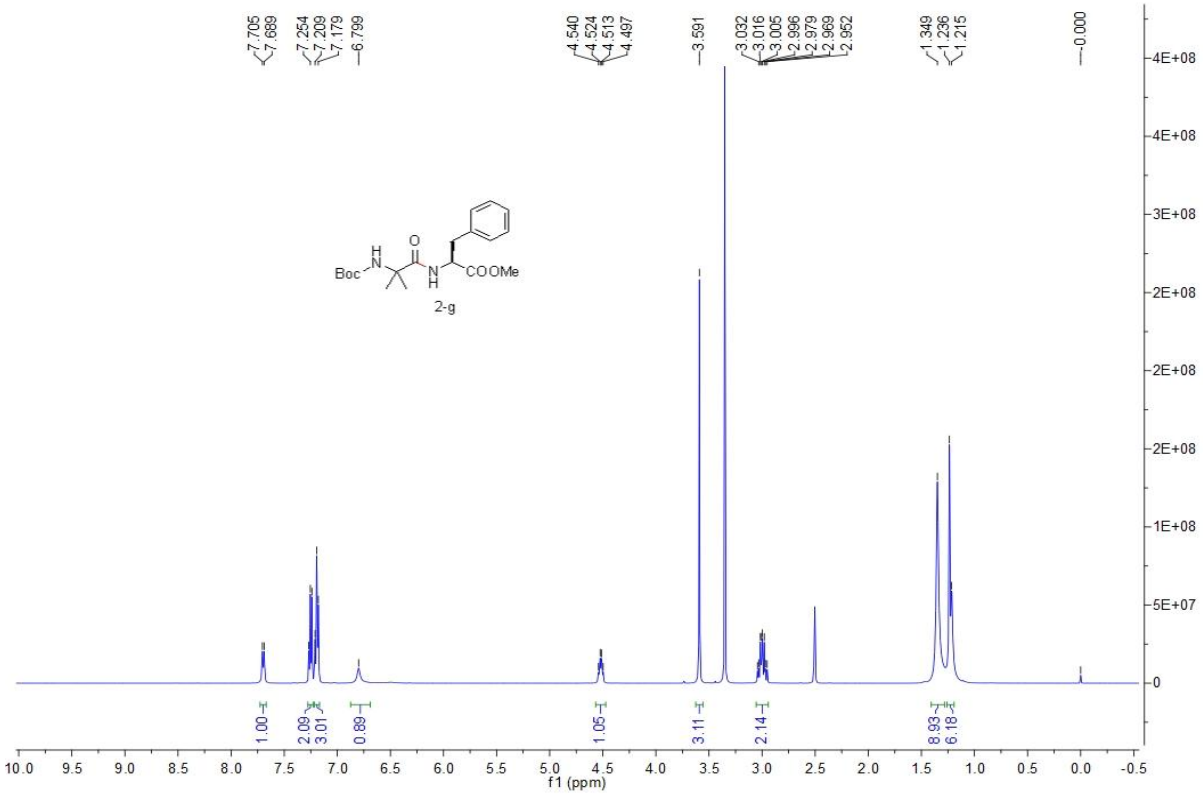

172.34
172.28
155.54
138.51
137.55
129.65
129.61
128.71
128.41
127.02
126.59
78.45
56.04
54.05
52.30
37.91
37.12
28.58
Boc
OMe
f1 (ppm)

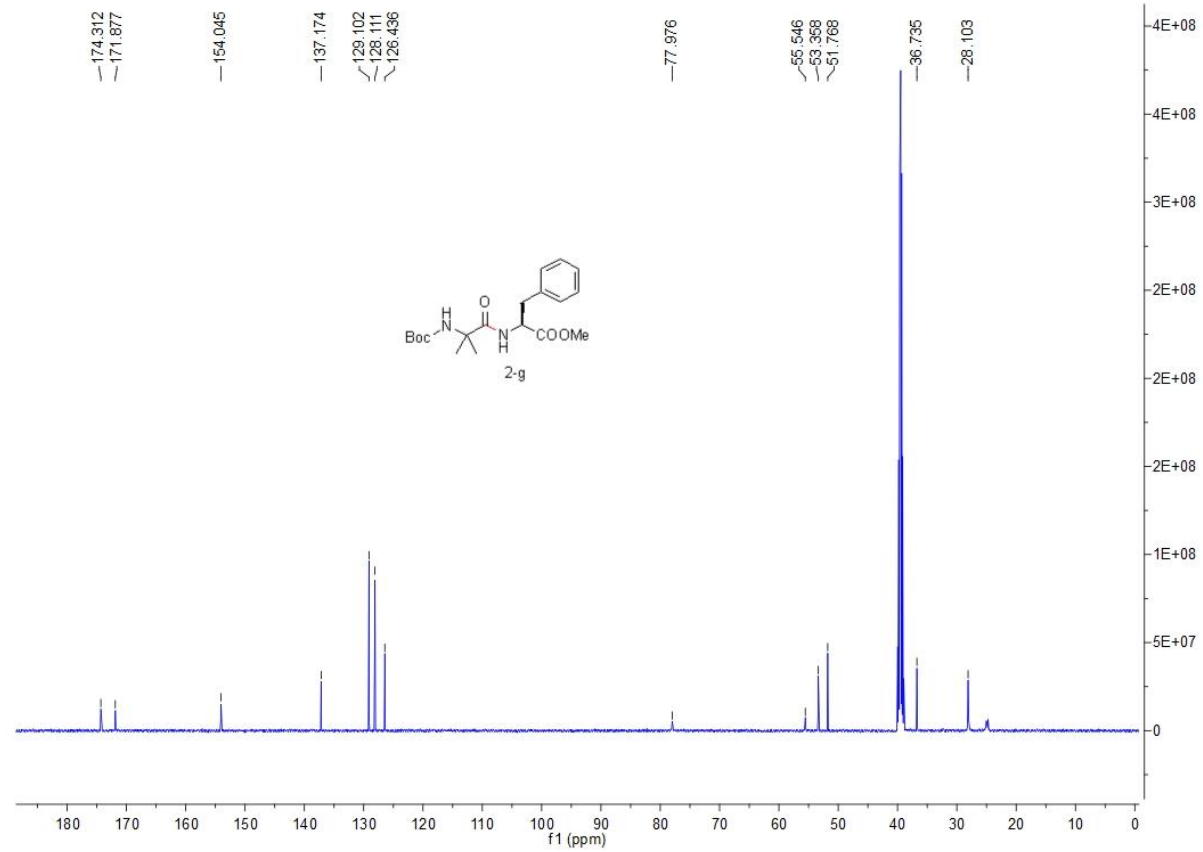

7.263
7.232
7.167
6.819
6.678
6.488
5.080
4.760
4.349
3.670
3.010
2.985
2.961
1.399
0.072
0.000
OH
Boc
N
H
O
N
H
COOMe
2.21
2.05
1.91
1.98
0.99
0.82
1.11
0.98
3.00
3.96
8.99
f1 (ppm)

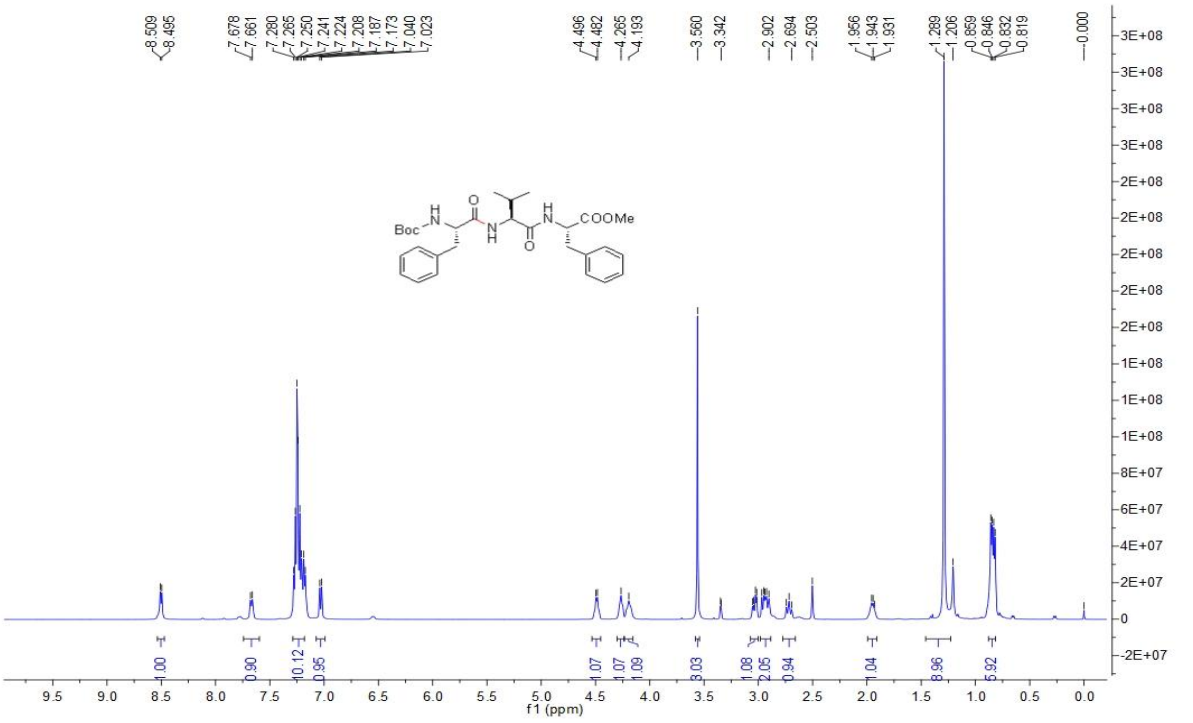

170.510
170.012
154.302
135.336
128.296
127.626
125.937
114.517
54.618
52.452
51.305
37.253
36.150
28.675
27.223
0.000
129.316
128.296
127.626
125.937
OH
Boc
N
H
O
N
H
COOMe
f1 (ppm)
f1 (ppm)

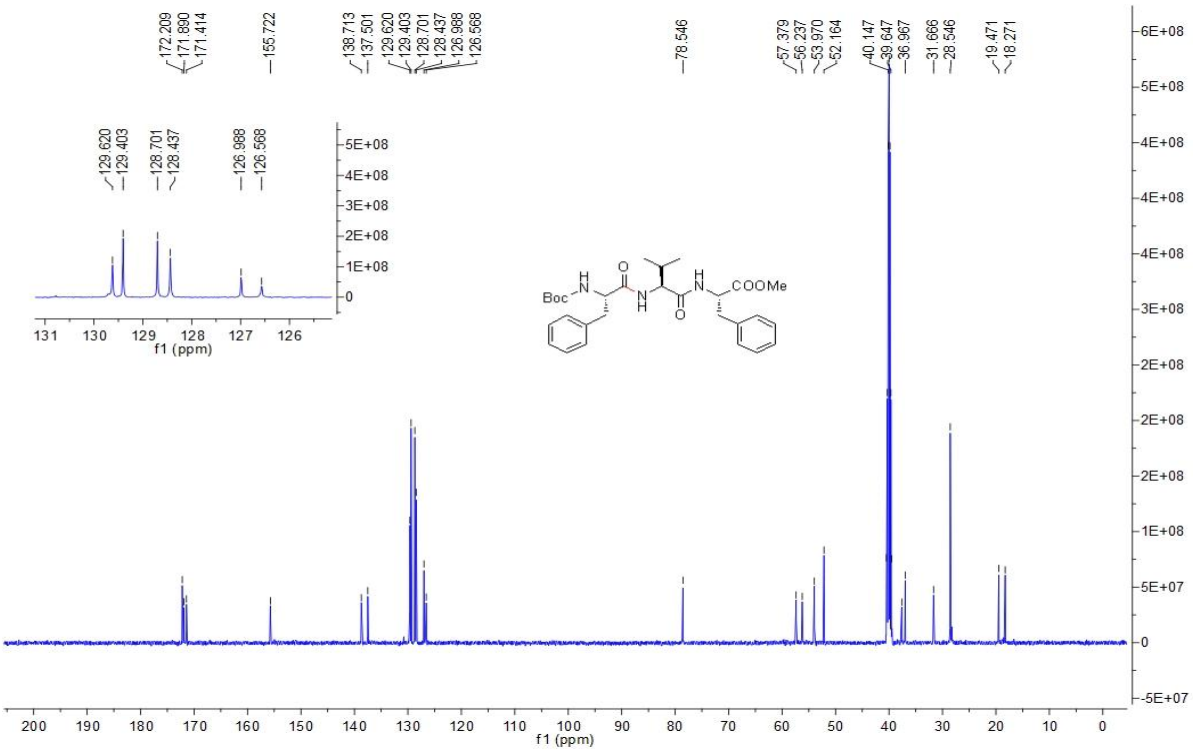

10.897
8.312
8.297
7.238
7.227
7.186
7.089
7.074
7.059
7.011
6.996
6.982
6.890
6.873
4.561
4.547
4.214
4.209
3.557
3.337
3.132
3.103
2.948
2.920
2.722
2.673
2.497
1.288
0.000
Boc
NH
COOMe
1.03
1.04
1.07
1.08
5.96
1.13
1.05
0.88
1.06
1.06
3.00
2.08
1.01
0.95
9.00
f1 (ppm)

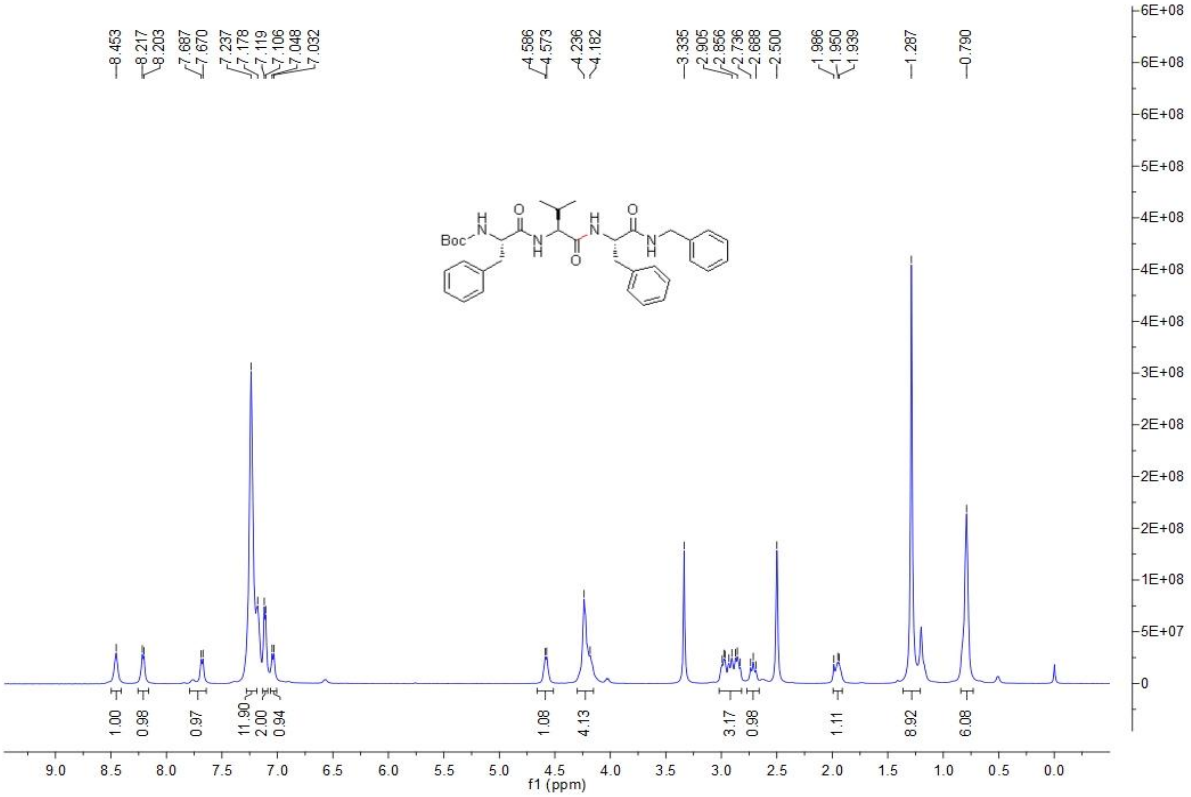

172.648
172.312
155.637
138.530
138.327
128.327
124.214
121.453
118.911
118.454
111.901
109.613
78.503
56.012
53.504
52.295
40.312
39.978
39.644
37.878
28.583
27.543
Boc
NH
COOMe
f1 (ppm)

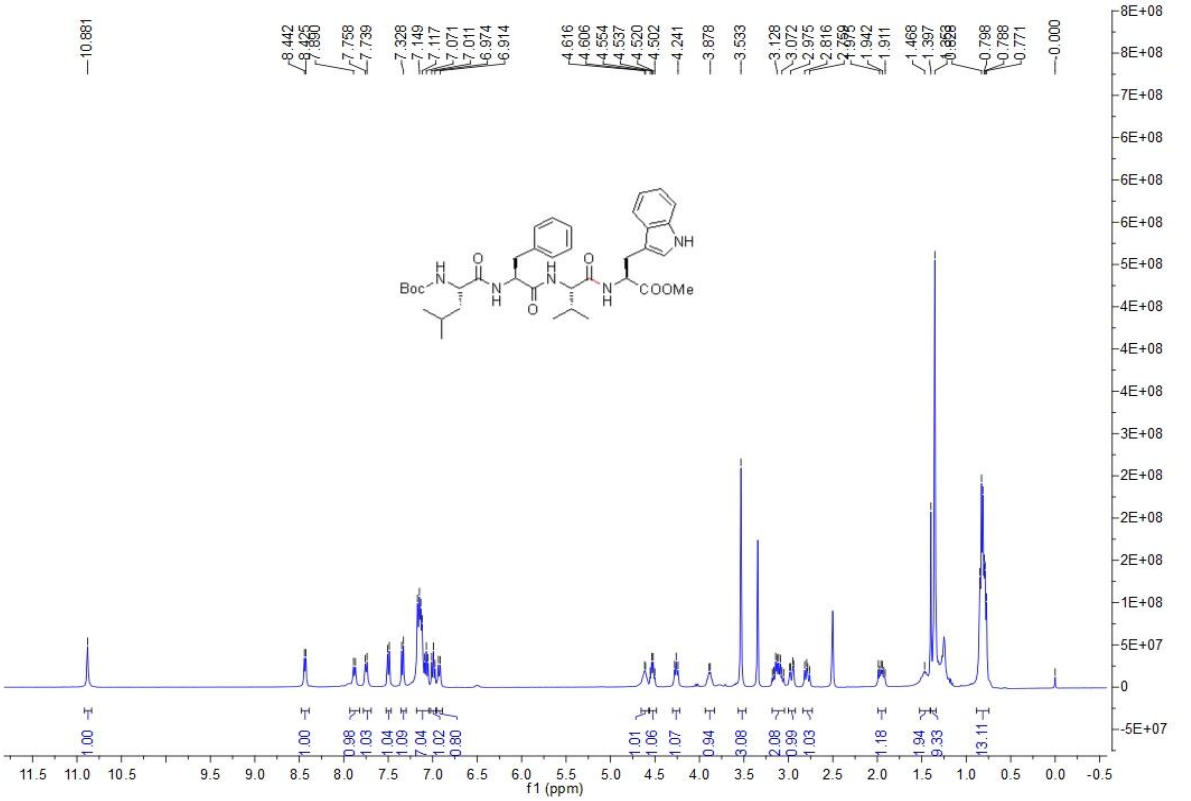

7.705
7.689
7.254
7.209
7.179
6.799
4.540
4.524
4.513
4.497
3.591
3.032
3.016
3.005
2.996
2.979
2.969
2.952
1.349
1.236
1.215
0.000
Boc
COOMe
2-g
1.00
2.09
3.01
0.89
1.05
3.11
2.14
8.93
6.18
f1 (ppm)

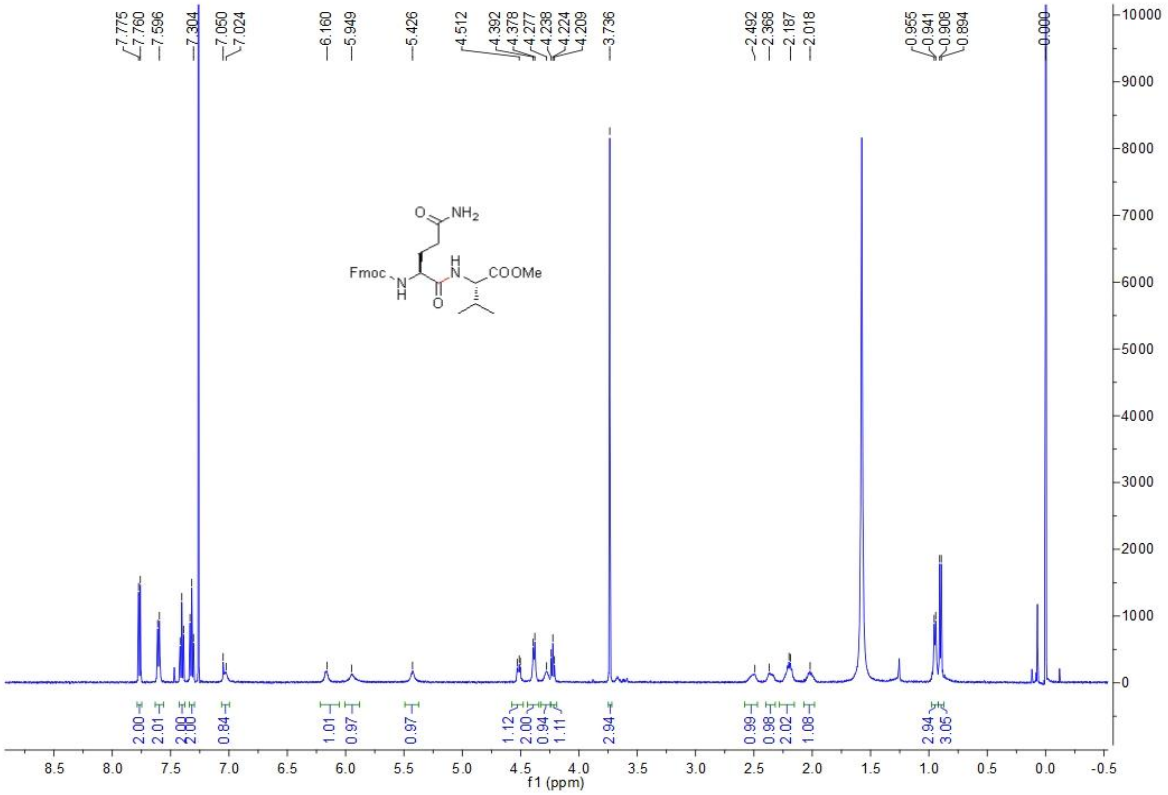

174.312
171.877
154.045
137.174
129.102
128.111
126.436
77.976
55.546
53.358
51.768
36.735
28.103
Boc
COOMe
2-g
f1 (ppm)

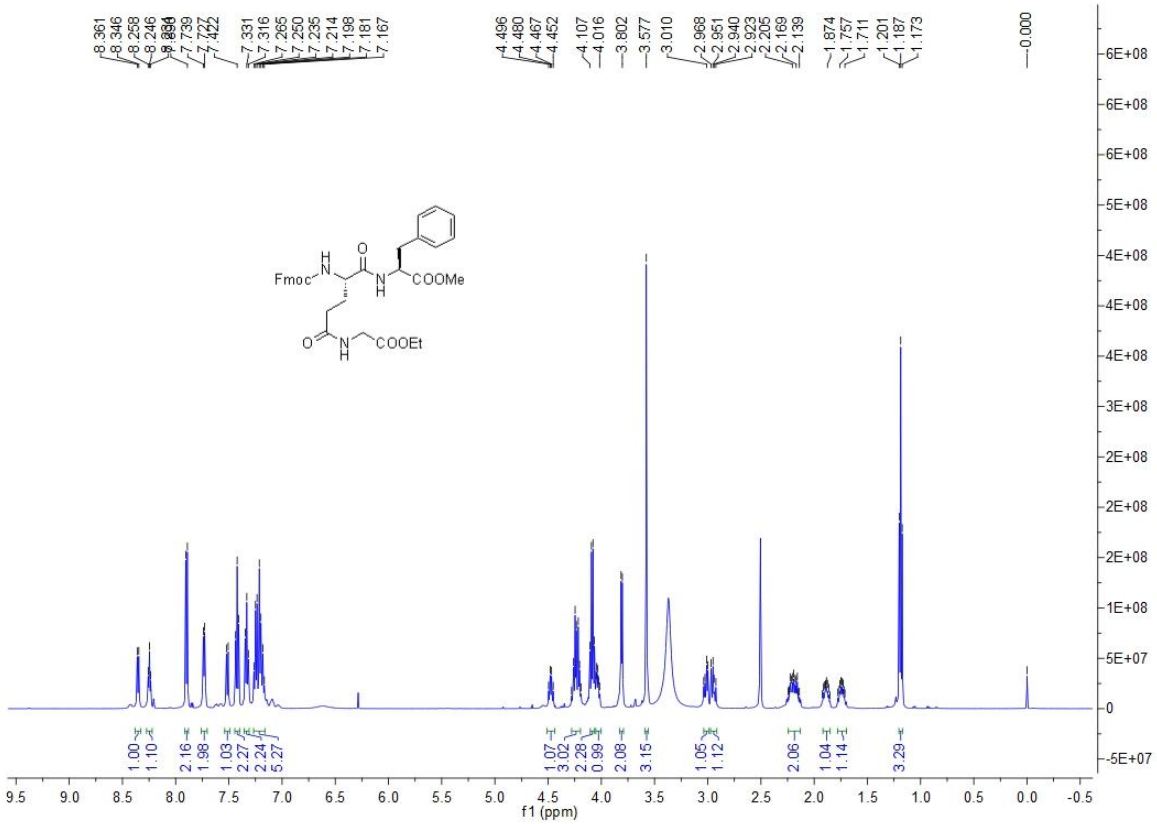

8.509
8.495
7.678
7.661
7.280
7.265
7.250
7.241
7.224
7.208
7.197
7.173
7.040
7.023
4.496
4.482
4.265
4.193
3.560
3.342
2.902
2.694
2.503
1.956
1.943
1.931
1.289
1.206
0.859
0.846
0.832
0.819
0.000
Boc
COOMe
1.00
0.90
10.12
0.95
1.07
1.07
1.09
3.03
1.08
2.05
0.94
1.04
8.96
5.92
f1 (ppm)

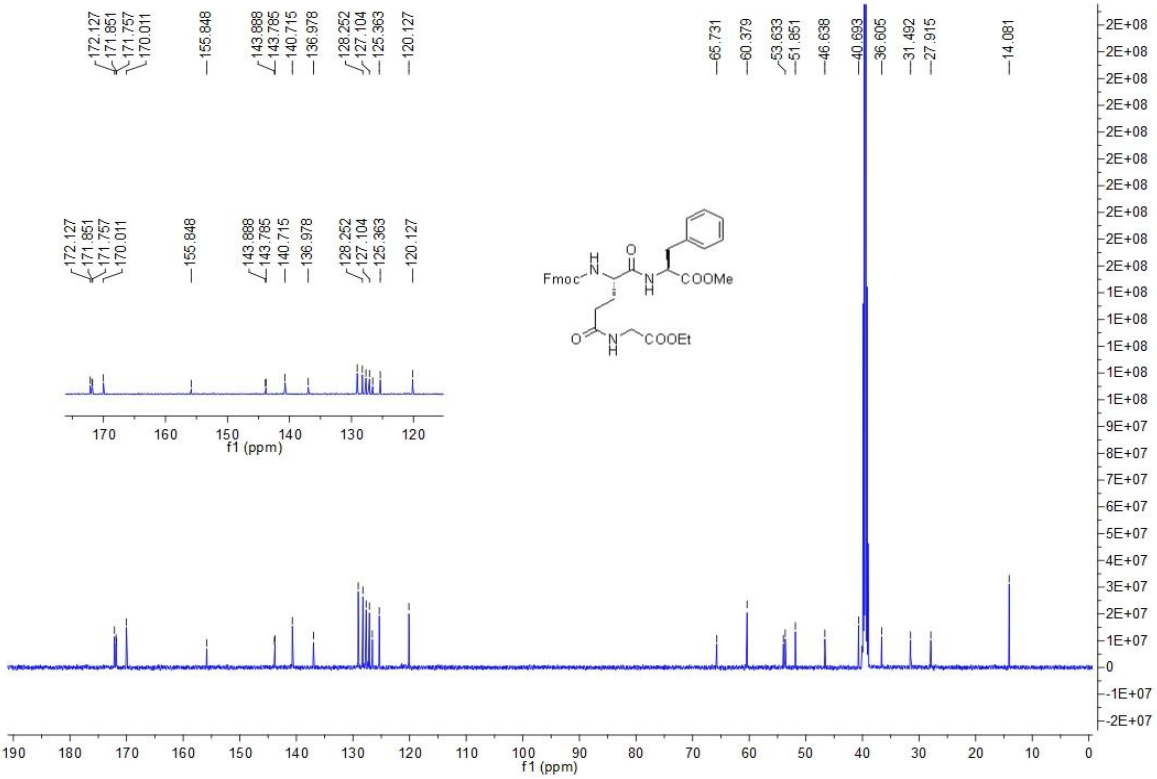

172.209
171.890
171.414
155.722
138.713
137.501
129.620
129.403
128.701
128.437
126.988
126.568
78.546
57.379
56.237
53.970
52.164
40.147
39.647
36.967
31.666
28.546
19.471
18.271
Boc
COOMe
f1 (ppm)

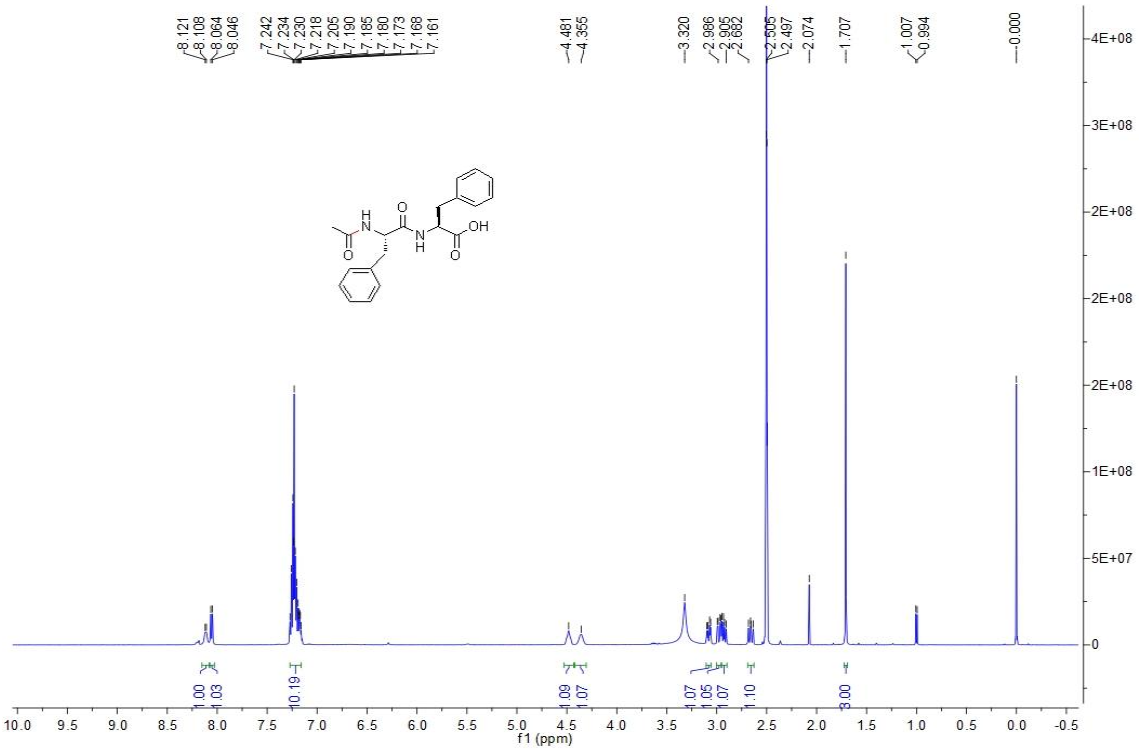

8.453
8.217
8.203
7.687
7.670
7.237
7.178
7.119
7.106
7.048
7.032
4.586
4.573
4.236
4.182
3.335
2.905
2.856
2.736
2.688
2.500
1.986
1.950
1.939
1.287
0.790
Boc
1.00
0.98
0.97
11.90
2.00
0.94
1.08
4.13
3.17
0.98
1.11
8.92
6.08
f1 (ppm)

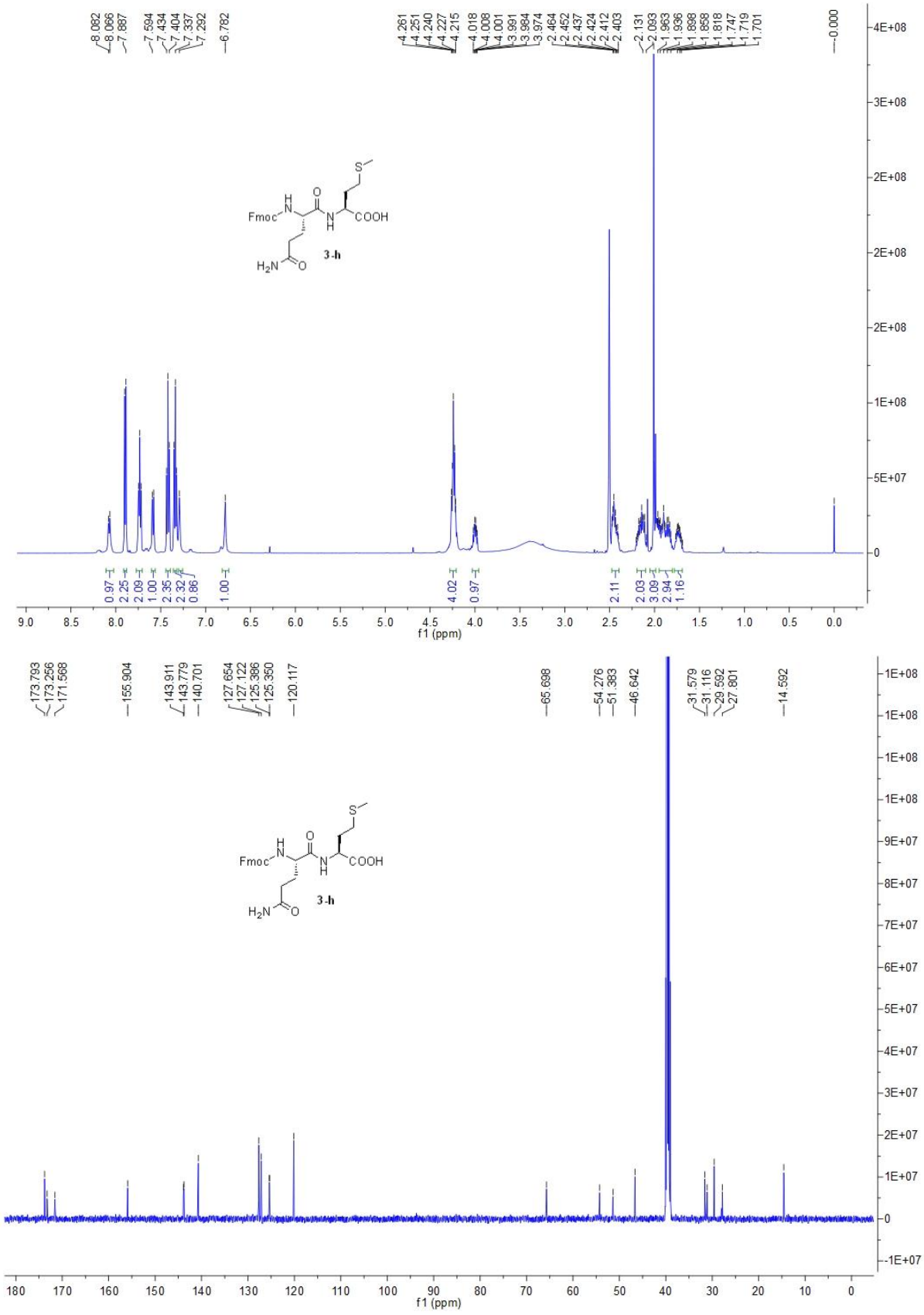

10.881
8.442
8.425
7.758
7.739
7.328
7.149
7.117
7.071
7.011
6.974
6.914
4.616
4.606
4.554
4.537
4.520
4.502
4.241
3.878
3.533
3.128
3.072
2.975
2.816
1.942
1.911
1.468
1.397
0.798
0.788
0.771
0.000
NH
Boc
COOMe
1.00
1.00
0.98
1.03
1.04
1.09
7.04
1.02
0.80
1.01
1.06
1.07
0.94
3.08
2.08
0.99
1.03
1.18
1.94
9.33
13.11
f1 (ppm)

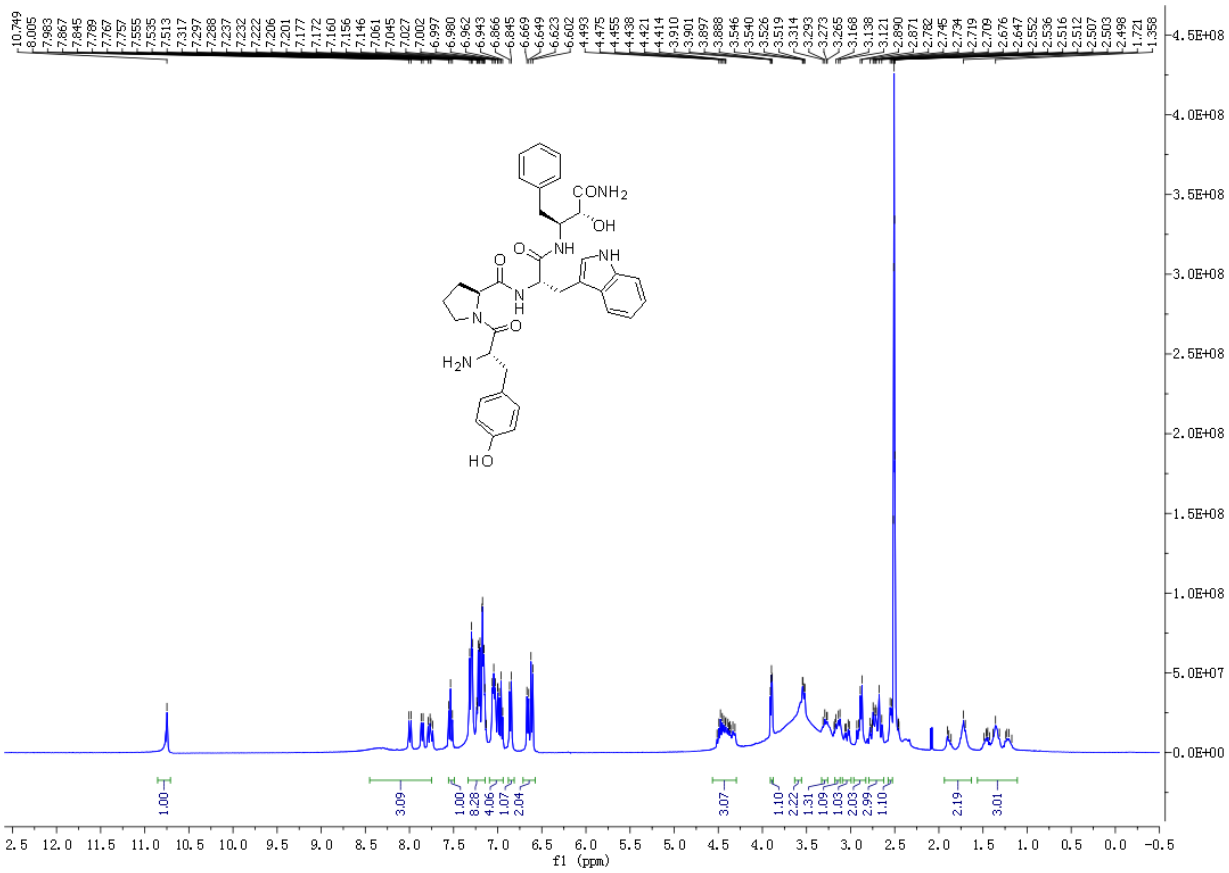

7.775
7.760
7.596
7.304
7.050
7.024
6.160
5.949
5.426
4.512
4.392
4.378
4.277
4.238
4.224
4.209
3.736
2.492
2.388
2.187
2.018
0.955
0.941
0.908
0.894
0.000
O
NH2
Fmoc
N
H
H
N
O
COOMe
2.00
2.01
2.88
0.84
1.01
0.97
0.97
1.12
2.00
0.94
1.11
2.94
0.99
0.98
2.02
1.08
2.94
3.05
f1 (ppm)


8.361
8.346
8.258
8.246
7.739
7.727
7.422
7.331
7.316
7.265
7.250
7.235
7.214
7.198
7.181
7.167
4.496
4.480
4.467
4.452
4.107
4.016
3.802
3.577
3.010
2.968
2.951
2.940
2.923
2.205
2.169
2.139
1.874
1.757
1.711
1.201
1.187
1.173
0.000
Fmoc
COOMe
COOEt
1.00
1.10
2.16
1.98
1.03
2.27
2.24
5.27
1.07
3.02
2.28
0.99
2.08
3.15
1.05
1.12
2.06
1.04
1.14
3.29
f1 (ppm)


172.127
171.851
171.757
170.011
155.848
143.888
143.785
140.715
136.978
128.252
127.104
125.363
120.127
65.731
60.379
53.633
51.851
46.638
40.693
36.605
31.492
27.915
14.081
Fmoc
COOMe
COOEt
f1 (ppm)


8.121
8.108
8.064
8.046
7.242
7.234
7.230
7.218
7.205
7.190
7.185
7.180
7.173
7.168
7.161
4.481
4.355
3.320
2.986
2.905
2.682
2.505
2.497
2.074
1.707
1.007
0.994
0.000
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
1.00
1.03
10.19
1.09
1.07
1.07
1.05
1.07
1.10
8.00
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)


8.082
8.066
7.887
7.594
7.434
7.404
7.337
7.292
6.782
4.261
4.251
4.240
4.227
4.215
4.018
4.008
4.001
3.991
3.984
3.974
2.464
2.452
2.437
2.424
2.412
2.403
2.131
2.093
1.963
1.936
1.898
1.858
1.818
1.747
1.719
1.701
0.000
Fmoc
COOH
3-h
0.97
2.25
2.09
1.00
2.35
2.32
0.86
1.00
4.02
0.97
2.11
2.03
3.09
2.94
1.16
f1 (ppm)
173.793
173.256
171.568
155.904
143.911
143.779
140.701
127.654
127.122
125.386
125.350
120.117
65.698
54.276
51.383
46.642
31.579
31.116
29.592
27.801
14.592
f1 (ppm)


CONH2
OH
NH
NH
O
N
H
N
O
H2N
HO
4.5E+08
4.0E+08
3.5E+08
3.0E+08
2.5E+08
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
f1 (ppm)