Supporting Information

Head-to-tail cyclization for synthesis of naturally occuring cyclic peptides on organophosphorus small-molecular supports

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CONTENTS

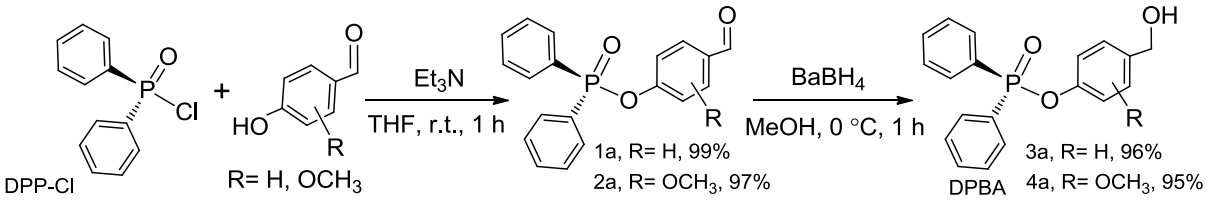
1. General considerations

All the involved Fmoc (or Boc) protected amino acids were purchased from G L Biochem (Shanghai) Ltd. All the chemical reagents and raw materials were purchased and used without further purification unless otherwise noted and remarked. All experiment procedures were carried out by standard experiment methods. The rotary evaporator (RE-52AA, Shanghai) and circulating water multi-purpose vacuum pump (SHB-III, Zhengzhou) were employed to concentrate the samples. Magnetic stirrers (ST 15 0SA, UK) were used to completed liquid phase stirring reactions. The four-use UV analyzer (ZF-8, Shanghai) and silica gel GF_{254} (0.15mm thick, Qingdao) plates were used for TLC analysis. The melting point (mp) test was implemented with the digital melting-point apparatus (WRS-1B, Shanghai). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and ^{31}P NMR (162 MHz) spectra were recorded on a Bruker NMR spectrometer (Bruker Avance 400 MHz, Germany). Spectra were obtained in DMSO-d_6 (δ_H 2.50 ppm, δ_C 39.52 ppm) or $CDCl_3$ (δ_H 7.26 ppm, δ_C 77.16 ppm,) or D_2O (δ_H 4.79 ppm). HRMS data were recorded on a Thermo Scientific LTQ Orbitrap XL using ESI ionization. HPLC analyses for the ultimate cyclopeptide products were performed with LC 3000 HPLC system with CXTH-3000 work station using analysis columns (Kromasil, NC-2546-06251151; 250 × 4.6 mm).

2. Abbreviations

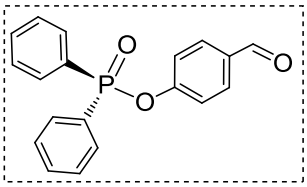
AA:	Amino acid
BDKO:	4,4’-bis(diphenylphosphinyloxyl) diphenyl ketoxime
DCM:	Dichloromethane
DEA:	Diethylamine
DIPEA:	*N, N’*-diisopropylethylamine
DPBA:	4-diphenyl phospholoxy benzyl alcohol
DMAP:	4-dimethylaminopyridine
DPP-Cl	Diphenylphosphinic chloride
EA:	Ethyl acetate
EDCl:	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
HOBt:	1-hydroxybenzotriazole
MeOH:	Methanol
MeCN:	Acetonitrile
PE:	Petroleum ether
PyBOP:	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
TFA:	Trifluoroacetic acid
SAP:	Support Aid Precipiation
GPS:	Greener Peptide Supports.

3. Synthesis of 4-diphenylphospholoxy benzyl alcohol DPBA supports

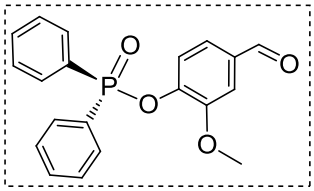


p-Hydroxy benzaldehyde (1.22 g, 10 mmol) was added to a solution of Et_3N (1.7 mL, 12 mmol) in THF (50 mL) at 0 °C and stirred for 20 min. The mixture was added with DPP-Cl (1.9 mL, 10 mmol) dropwise and stirred for 1 h. The mixture was quenched with 5 mL 0.1 mol/L H_2SO_4 and concentrated. The residue was then dissolved in 50 mL ethyl acetate and washed with H_2O and dried with $MgSO_4$. Concentration to afford **1a.** [**2a** according to the above procedure.]

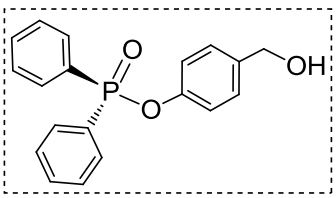
$NaBH_4$ (456 mg, 12 mmol) was added to a solution of **1a** (1.29 g, 4 mmol) in CH_3OH (25 mL) at 0 °C and the mixture was sealed and stirred for 1 h. The mixture was quenched by adding 10 mL saturated NH_4Cl and concentrated to remove the CH_3OH. The residue was dissolved in 30 mL EA and washed with H_2O, dried with $MgSO_4$. 5 mL EA was added to dissolve the sample after concentration and 30 mL of petroleum ether (V_{EA}/V_{PE} =1:6) was added dropwise and stirred. Precipitate appeared and filtered to afford the pure product **DPBA, 3a.** [**4a** according to the above procedure.]



1a, (3.18 g, 99% yield), white solid, R_f = 0.50, (*n*-hexane: EA= 4: 1), mp 93.1-94.3 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ 9.92 (s, 1H), 7.94-7.89 (dd, *J*= 8.0 Hz, 4H), 7.82-7.80 (d, *J*=8.0 Hz, 2H), 7.60-7.57 (m, 2H), 7.53-7.48 (m, 4H), 7.42-7.40 (d, *J*= 8.0 Hz, 2H) ppm; ^{13}C NMR (100MHz, $CDCl_3$), δ 190.8, 155.9, 132.8, 131.8, 131.6, 131.0, 129.6, 128.7, 121.2 ppm; ^{31}P NMR (162MHz, $CDCl_3$), δ 31.72 ppm; HRMS (ESI) m/z calcd for $C_{19}H_{16}O_3P^+$ $[M+H]^+$ 323.08316, found 323.08359.

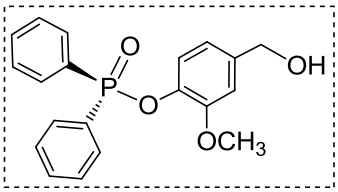


2a, (3.42 g, 97% yield), white solid, R_f = 0.30, (*n*-hexane: EA= 4: 1), mp 84.5-87.9 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ 9.85 (s, 1H), 7.97-7.92 (dd, *J*= 8.0 Hz, 4H), 7.62-7.60 (d, *J*= 8.0 Hz, 1H), 7.55-7.32 (m, 8H), 3.87 (s, 1H) ppm; ^{13}C NMR (100MHz, $CDCl_3$), δ 190.9, 151.4, 145.37, 145.3, 133.6, 132.7, 131.8, 131.3, 129.9, 128.5, 125.3, 122.0, 110.5, 55.9 ppm; ^{31}P NMR (162MHz, $CDCl_3$), δ 32.19 ppm; HRMS (ESI) m/z calcd for $C_{20}H_{18}O_4P^+$ $[M+H]^+$ 353.09372, found 353.09390.



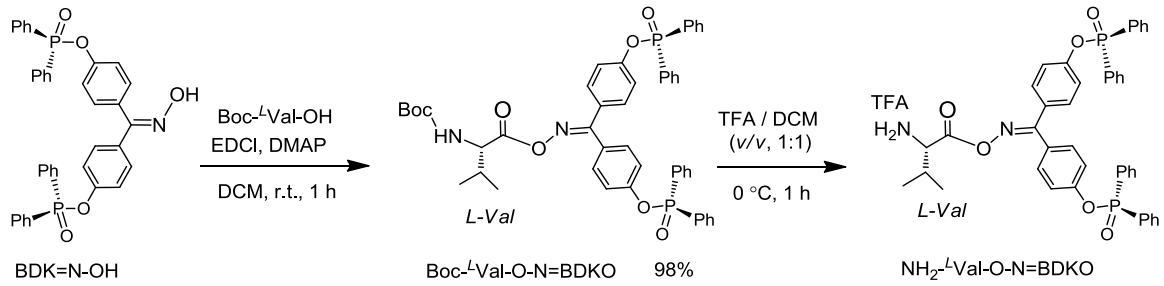
DPBA, 3a (1.23 g, 96% yield), white solid, R_f = 0.55 (*n*-hexane: EA= 2:5), mp 117.5-120.8 °C. ^{1}H NMR (400MHz, DMSO-d_6), δ 7.93-7.88 (m, 4H), 7.64-7.53 (m, 6H), 7.26-7.21 (t, 4H), 5.16-5.13 (t, 1H), 4.41-4.40 (d, *J*= 4.0 Hz, 2H) ppm; ^{13}C NMR (100MHz, DMSO-d_6), δ

149.8, 139.4, 133.2, 132.1, 130.6, 129.4, 128.3, 120.7, 62.7 ppm; ^{31}P NMR (162MHz, DMSO-d_6), δ 28.99 ppm. HRMS (ESI) m/z calcd for $C_{19}H_{18}O_3P^+$ $[M+H]^+$ 325.09881, found 325.09894.

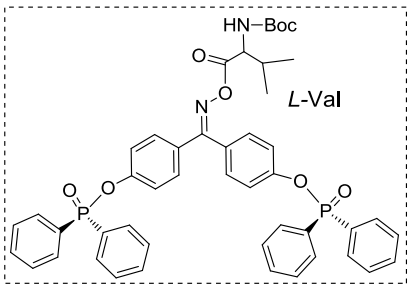


DPBA, 4a (1.35 g, 95% yield), white solid, R_f = 0.20，(*n*-hexane: EA= 1: 1), mp 92.9-96.1 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ 7.93-7.88 (dd, *J*= 8.0 HZ, 4H), 7.49-7.47 (m, 2H), 7.43-7.40 (m, 4H), 7.27-7.25 (d, *J*= 8.0 HZ, 1H), 6.88 (s, 1H), 6.71-6.69 (d, *J*= 8.0 HZ, 1H), 4.53 (s, 2H), 3.72 (s, 3H), 3.03 (s, 1H) ppm; ^{13}C NMR (100MHz, $CDCl_3$), δ 150.6, 138.9, 132.3, 131.9, 131.7, 130.4, 128.5, 121.7, 121.27, 118.9, 111.1, 64.5, 55.7 ppm; ^{31}P NMR (162MHz, $CDCl_3$), δ 31.30 ppm. HRMS (ESI) m/z calcd for $C_{20}H_{20}O_4P^+$ $[M+H]^+$ 355.10937, found 355.10992.

4. Synthesis of BDKO attached hexapeptide.

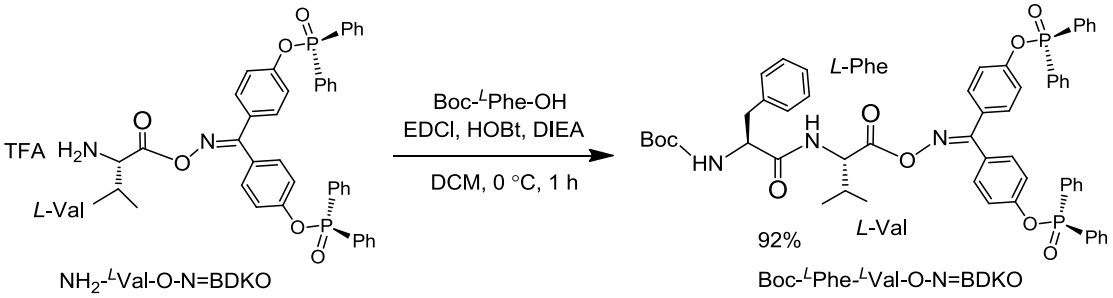


Synthesis of **Boc-^{L}Val-O-N=BDKO**: EDCl (458 mg, 2.4 mmol, 1.2 equiv) and DMAP (32 mg, 0.24 mmol, 0.12 equiv) were added to a solution of Boc-^{L}Val-OH (455 mg, 2.1 mmol, 1.05 equiv) in DCM (30 mL) at 0 °C and stirred for 10 min. The reaction mixture was then added with **BDKO** (1.26 g, 2.0 mmol, 1.0 equiv) and stirred at room temperature for 1 h. The mixture was then washed with saturated NH_4Cl and $NaHCO_3$ respectively and dried with anhydrous $MgSO_4$. 3.0 mL ethyl acetate was added to dissolve the sample after concentration, and 15 mL of petroleum ether (V_{EA}/V_{PE} =1:5) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the product **Boc-^{L}Val-O-N=BDKO** (1.62 g, 98% yield).



Boc-^{L}Val-O-N=BDKO. 1b-1, white solid, R_f = 0.45 (CH_2Cl_2: MeOH= 60:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95-7.86 (m, 8 H), 7.61-7.41 (m, 14 H), 7.31-7.29 (m, 2 H), 7.22-7.16 (m, 4 H), 5.01-4.98 (d, 1 H), 4.16-4.12 (m, 1 H), 1.92-1.88 (m, 1 H), 1.43 (s, 9 H), 0.85-0.83 (d, 3 H), 0.76-0.75 (d, 3 H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.77 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.8, 164.1, 155.5, 153.3, 152.0, 132.8, 131.8, 131.2, 130.7, 129.8, 128.8, 128.2, 120.7, 79.9, 57.7, 31.2, 28.3, 18.8, 17.6 ppm; HRMS (ESI) m/z calcd for $C_{47}H_{47}N_2O_8P_2^+$ $(M+H)^+$ 829.28022, found 829.28052.

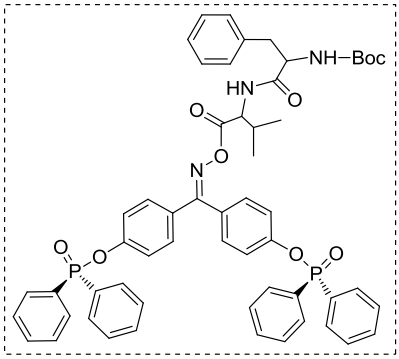
***de*-Boc** general procedure: The dry **Boc-^{L}Val-O-N=BDKO** (1.62 g, 1.95 mmol) was added to a dry rounded bottom flask with 3 mL dry DCM, and the solution was stirred at 0 °C for 10 min. Then 3 mL of dry TFA (99.9% purity) was added to the above solution and stirred for 1 h at 0 °C. Then removed most of the TFA by reduced pressure concentration at room temperature, and DCM was continuously added 3 times and concentrated to remove all the residual TFA to afford the *de*-Boc product: **TFA·NH$_2$-^{L}Val-O-N=BDKO** for next use.



NH$_2$-^{L}Val-O-N=BDKO

Boc-^{L}Phe-^{L}Val-O-N=BDKO

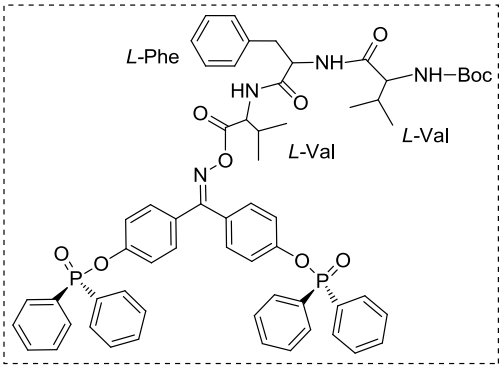
Synthesis of **Boc-^{L}Phe-^{L}Val-O-N=BDKO:** EDCl (460 mg, 2.4 mmol, 1.2 equiv), HOBt (285 mg, 2.1 mmol, 1.05 equiv) were added to a solution of Boc-^{L}Phe-OH (555 mg, 2.1 mmol, 1.05 equiv) in DCM (30 mL) at 0 °C and stirred for 0.5 h. Then TFA·NH$_2$-^{L}Phe-O-N=BDK (2.0 mmol, 1.0 equiv) was added to the mixture at 0 °C and stirred for 10 min. DIPEA (690 mL, 4.2 mmol, 2.1 equiv) was also added to the mixture subsequently and stirred for 1 h. The mixture was then washed with saturation $NaHCO_3$, dried with anhydrous $MgSO_4$. 7.0 mL ethyl acetate was added to dissolve the sample after concentration, and 50 mL of petroleum ether (V_{EA}/V_{PE} =1:7) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the product **Boc-^{L}Phe-^{L}Val-O-N=BDKO** (1.8 g, 92% yield).



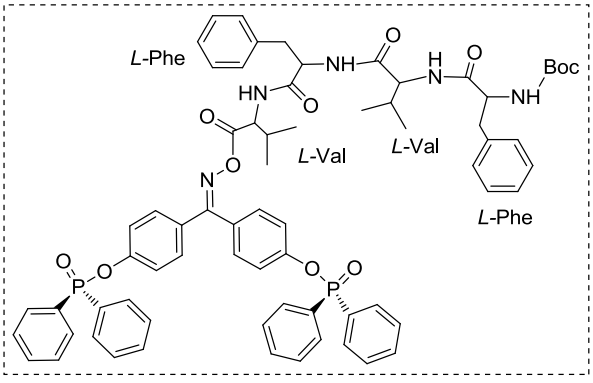
Boc-^{L}Phe-^{L}Val-O-N=BDKO. 1b-2, white solid, R_f= 0.35 (CH_2Cl_2: MeOH= 60:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94-7.86 (m, 8 H), 7.58-7.42 (m, 14 H), 7.31-7.20 (m, 9 H), 7.16-7.14 (m, 2 H), 6.51-6.49 (m, 1 H), 5.22-5.20 (m, 1 H), 4.40-4.30 (m, 2 H), 3.11-3.00 (m, 2 H), 1.95-1.88 (m, 1 H), 1.40 (s, 9 H), 0.78-0.76 (d, 3 H), 0.71-0.69 (d, 3 H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.93 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.2, 168.7, 164.2, 155.4, 153.4, 152.0, 136.7, 132.7, 131.7, 131.2, 130.5, 129.8, 129.4, 80.1, 56.4, 55.8, 37.9, 31.1, 20.3, 18.7, 17.7 ppm; HRMS (ESI) m/z calcd for $C_{56}H_{56}N_3O_9P_2^+$ $(M+H)^+$ 976.34863, found 976.34833.

Extension of BDKO attached peptide chains: According to the above synthesis procedures, and using the above coupling reagent system EDCl/HOBt/DIPEA (2.0 equiv) and the above *de*-Boc

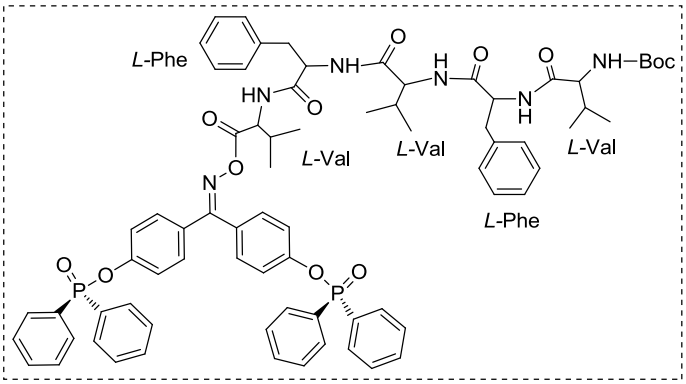
reagent system 50% TFA/DCM to extend the BDKO attached peptide chain. Therefore the intermediate peptide products were obtained as follows: [The intermediates of Boc-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO and Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO were precipitated by using the EA/PE solvent system with adjustable ratio, The intermediate peptides of Boc-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO and Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO were precipitated by using the EA/MeCN solvent system].



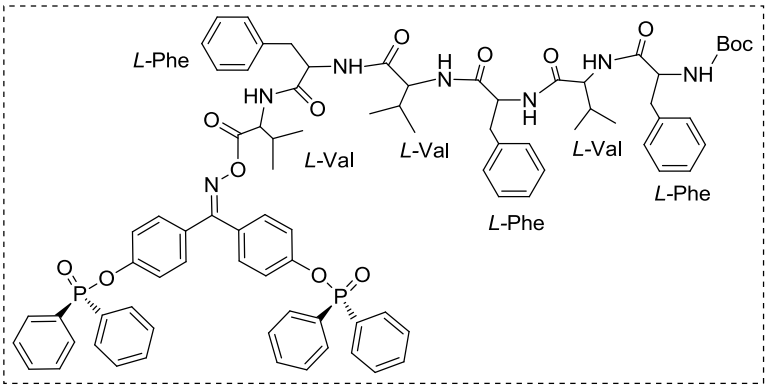
Boc-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO. 1b-3, 1.93 g, 90% yield, white solid, R_f = 0.42 (CH_2Cl_2: MeOH= 40:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95-7.87 (m, 8 H), 7.62-7.42 (m, 14 H), 7.29-7.20 (m, 9 H), 7.18-7.15 (m, 2 H), 6.86-6.84 (m, 1 H), 6.37-6.35 (m, 1 H), 5.08-5.06 (m, 1 H), 4.59-4.54 (m, 1 H), 4.35-4.32 (m, 1 H), 3.96-3.94 (m, 1 H), 3.10-2.98 (m, 2 H), 2.14-2.06 (m, 1 H), 1.99-1.91 (m, 1 H), 1.44 (s, 9 H), 0.90-0.88 (d, 3 H), 0.78-0.77 (d, 3 H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.99 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.9, 170.7, 168.7, 164.1, 155.8, 153.3, 151.9, 136.5, 132.7, 131.8, 131.3, 130.7, 129.8, 129.3, 128.7, 128.2, 127.0, 120.6, 79.8, 60.0, 56.6, 54.7, 37.8, 30.8, 20.3, 19.3, 18.8, 17.7, 17.5 ppm; HRMS (ESI) m/z calcd for $C_{61}H_{65}N_4O_{10}P_2^+$ $(M+H)^+$ 1075.41704, found 1075.41736.



Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO. 1b-4, 2.14 g, 88% yield, white solid, R_f = 0.40 (CH_2Cl_2: MeOH= 40:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95-7.87 (m, 8 H), 7.61-7.41 (m, 14 H), 7.30-7.15 (m, 16 H), 6.91-6.83 (m, 2 H), 6.66 (m, 1 H), 5.18-5.16 (m, 1 H), 4.68-4.66 (m, 1 H), 4.42-4.34 (m, 2 H), 4.28-4.24 (m, 1 H), 3.10-2.91 (m, 4 H), 2.13-2.08 (m, 1 H), 1.98-1.93 (m, 1 H), 1.39 (s, 9 H), 0.84-0.71 (m, 12 H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.75 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.6, 171.0, 170.7, 168.7, 164.1, 153.4, 152.0, 136.6, 132.7, 131.8, 131.2, 130.8, 129.8, 129.3, 128.6, 128.2, 126.9, 120.7, 80.3, 58.7, 56.7, 54.5, 37.6, 30.7, 29.7, 28.3, 19.2, 18.8, 17.8, 17.5 ppm; HRMS (ESI) m/z calcd for $C_{70}H_{74}N_5O_{11}P_2^+$ $(M+H)^+$ 1222.48546, found 1222.48596.



Boc-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO. 1b-5, 1.11 g, 84% yield, white solid, R_f = 0.45 (CH_2Cl_2: MeOH= 20:1). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.33-8.27 (m, 2 H), 8.06-8.04 (m, 1 H), 7.94-7.87 (m, 8 H), 7.85-7.83 (m, 1 H), 7.66-7.54 (m, 12 H), 7.37-7.36 (m, 5 H), 7.27-7.14 (m, 13 H), 6.63-6.60 (m, 1 H), 4.69-4.60 (m, 2 H), 4.18-4.14 (m, 1 H), 4.06-4.02 (m, 1 H), 3.72-3.61 (m, 1 H), 2.93-2.88 (m, 2 H), 2.76-2.69 (m, 2 H), 1.92-1.87 (m, 1 H), 1.82-1.76 (m, 2 H), 1.36 (s, 9 H), 0.76-0.64 (m, 18 H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 31.03 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.0, 171.5, 171.2, 171.0, 168.8, 164.1, 155.6, 153.4, 152.1, 138.1, 133.4, 132.0, 131.6, 130.8, 130.2, 129.5, 129.4, 128.4, 126.6, 121.3, 121.0, 79.7, 78.5, 60.4, 57.8, 57.0, 53.8, 31.3, 31.0, 30.1, 28.6, 19.6, 19.1, 18.5, 18.3 ppm; HRMS (ESI) m/z calcd for $C_{75}H_{83}N_6O_{12}P_2^+$ $(M+H)^+$ 1321.55387, found 1321.55359.

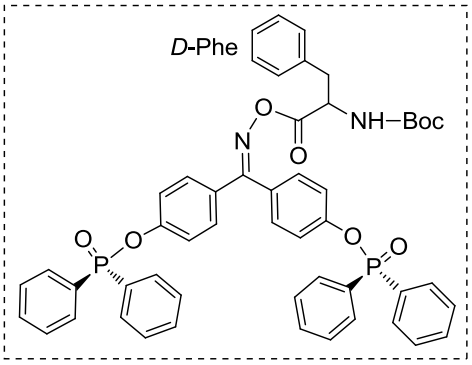


Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO. 1b-6, 1.27 g, 87% yield, white solid, R_f = 0.40 (CH_2Cl_2: MeOH= 20:1). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.32-8.26 (m, 2 H), 8.15-8.05 (m, 2 H), 7.94-7.89 (m, 8 H), 7.81-7.78 (m, 1 H), 7.65-7.56 (m, 12 H), 7.37-7.36 (m, 5 H), 7.26-7.15 (m, 18 H), 7.02-6.99 (m, 1 H), 4.67-4.58 (m, 2 H), 4.23-4.15 (m, 3 H), 4.05-4.02 (m, 1 H), 2.94-2.89 (m, 3 H), 2.78-2.67 (m, 3 H), 1.96-1.87 (m, 2 H), 1.82-1.77 (m, 1 H), 1.28 (s, 9 H), 0.79-0.66 (m, 18 H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 31.12 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.0, 171.8, 171.1, 171.0, 168.8, 164.1, 155.7, 153.4, 152.1, 138.8, 138.1, 133.4, 132.0, 131.6, 130.8, 130.2, 129.5, 128.4, 126.6, 121.3, 121.0, 79.7, 78.5, 57.5, 57.0, 56.2, 55.4, 54.0, 37.8, 37.7, 31.6, 31.4, 30.1, 28.6, 19.6, 19.5, 19.1, 18.5, 18.4, 18.2 ppm; HRMS (ESI) m/z calcd for $C_{84}H_{92}N_7O_{13}P_2^+$ $(M+H)^+$ 1468.62229, found 1468.62256.

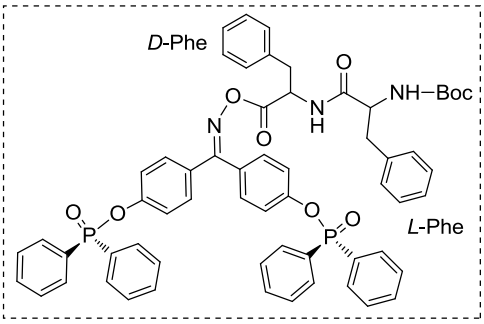
5. Synthesis of BDKO attached heptapeptides.

According to the above synthesis procedures, and using the above coupling reagent system such as EDCl/DMAP, EDCl/HOBt/DIPEA (2.0 equiv), and the above *de*-Boc reagent system (50% TFA/DCM) to extend the BDKO attached heptapeptide chains. The intermediates of

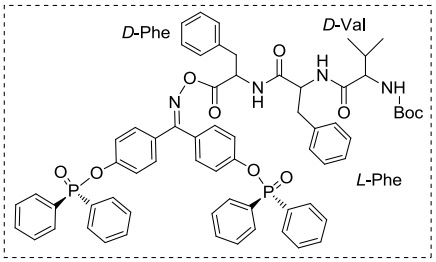
Boc-^{D}Phe-O-N=BDKO and Boc-^{L}Phe-^{D}Phe-O-N=BDKO were precipitated by using the EA/PE solvent system with an adjustable ratio. The intermediates of Boc-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO to intermediates heptapeptides Fmoc-^{D}Val-^{L}**Leu**-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO, Fmoc-^{D}Val-^{L}**Phe**-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO, Fmoc-^{D}Val-^{L}**Ile**-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO, Fmoc-^{D}Val-^{L}**Val**-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO, Fmoc-^{D}Val-^{L}**Pro**-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO were precipitated by using the EA/MeCN solvent system]



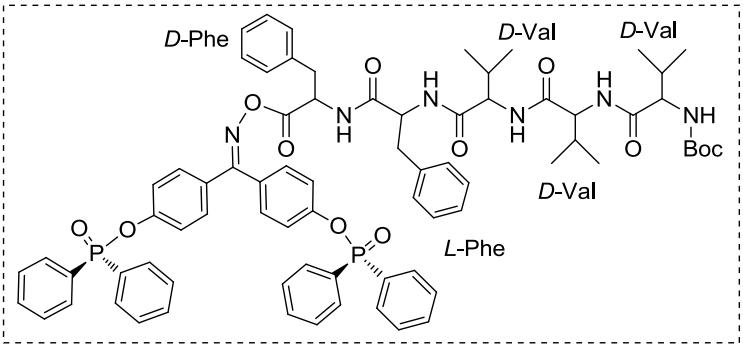
Boc-^{D}Phe-O-N=BDKO. 2b-1, 4.38 g, 99% yield, white solid, R_f = 0.42 (CH_2Cl_2: MeOH= 60:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.93-7.85 (m, 8H), 7.56-7.45 (m, 12H), 7.39-7.37 (d, J= 8.0 Hz, 2H), 7.22-7.18 (m, 7H), 7.04-7.01 (m, 2H), 6.95-6.93 (d, J= 8.0 Hz, 2H), 4.99-4.97 (d, J= 8.0 Hz, 1H), 4.49-4.46 (t, J= 12.0 Hz, 1H), 2.97-2.85 (m, 2H), 1.38 (s, 9H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.74 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.5, 163.9, 154.9, 153.3, 152.0, 135.5, 132.7, 131.7, 131.2, 130.8, 129.8, 129.3, 128.8, 127.8, 127.1, 120.5, 80.0, 53.7, 38.6, 28.3 ppm; HRMS (ESI) m/z calcd for $C_{51}H_{47}N_2O_8P_2^+$ $(M+H)^+$ 877.28022, found 877.28094.



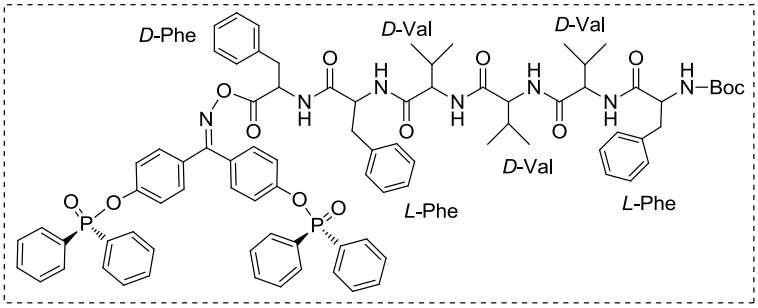
Boc-^{L}Phe-^{D}Phe-O-N=BDKO. 2b-2, 4.65 g, 95% yield, white solid, R_f = 0.40 (CH_2Cl_2: MeOH= 60:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94-7.84 (m, 8H), 7.56-7.43 (m, 12H), 7.37-7.35 (d, J= 8.0 Hz, 2H), 7.24-7.11 (m, 12H), 7.02-6.89 (m, 4H), 6.71 (m, 1H), 5.25-5.21 (m, 1H), 4.73-4.70 (m, 1H), 4.35 (m, 1H), 3.08-3.00 (m, 2H), 2.91-2.82 (m, 2H), 1.35 (s, 9H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.49 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.1, 168.8, 163.9, 153.4, 151.9, 136.7, 135.3, 132.8, 131.7, 130.8, 129.3, 128.7, 127.9, 127.2, 126.9, 120.8, 120.7, 120.6, 80.1, 52.5, 38.1, 28.3 ppm; HRMS (ESI) m/z calcd for $C_{60}H_{56}N_3O_9P_2^+$ $(M+H)^+$ 1024.34863, found 1024.34961.



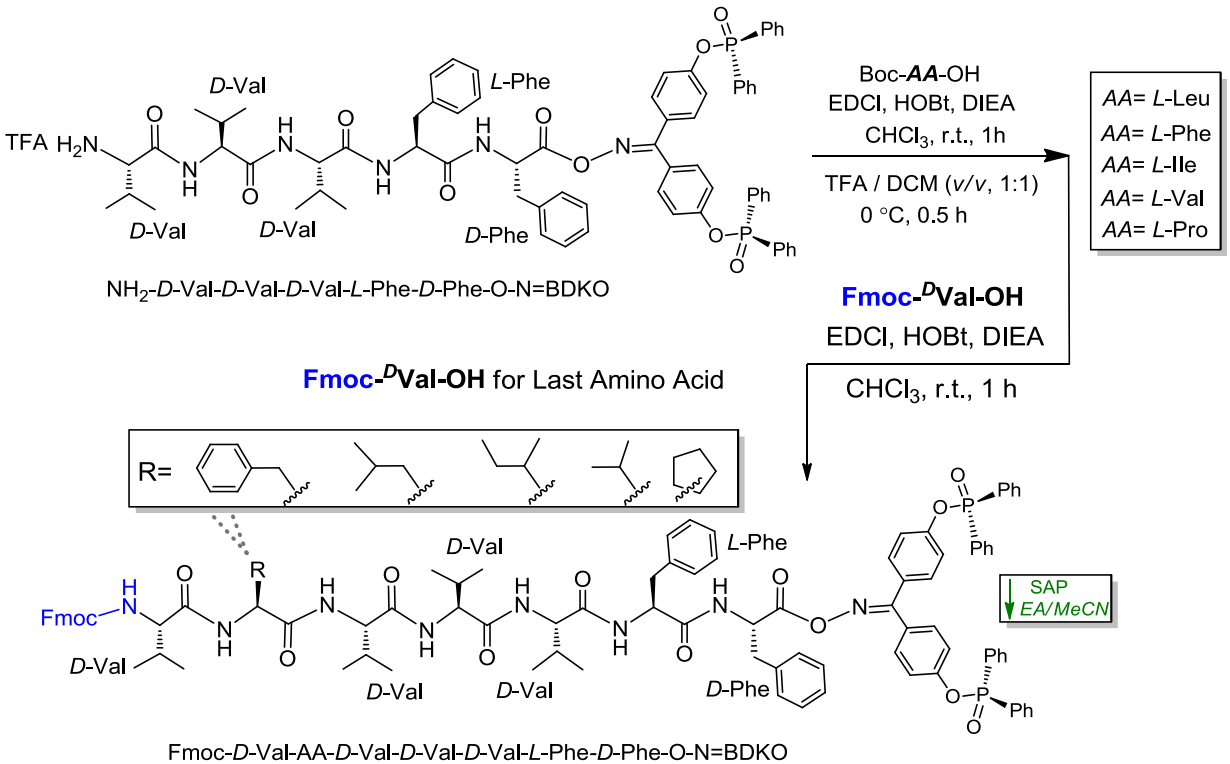
Boc-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO. 2b-3, 4.69 g, 82% yield, white solid, R_f= 0.42 (CH_2Cl_2: MeOH= 30:1). HRMS (ESI) m/z calcd for $C_{65}H_{64}N_4O_{10}P_2Na^+$ $(M+Na)^+$ 1145.39899, found 1145.39978.



Boc-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO. 2b-5, 83% yield, white solid, R_f= 0.40 (CH_2Cl_2: MeOH= 20:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 9.21 (m, 1H), 7.90-7.85 (m, 8H), 7.54-7.36 (m, 12H), 7.28-7.06 (m, 18H), 5.14-4.12 (m, 5H), 2.96 (m, 4H), 2.03-1.94 (m, 2H), 1.44 (s, 9H), 0.90-0.70 (dd, J= 8.0 Hz, 18H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.47 ppm;



Boc-Phe-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO. 2b-6, 4.90 g, yield 87%, white yield, R_f= 0.35 (CH_2Cl_2: MeOH= 20:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 9.33 (m, 3H), 7.92-7.79 (m, 8H), 7.56-7.40 (m, 12H), 7.22-6.95 (m, 23H), 5.34-4.21 (m, 6H), 3.45-2.85 (m, 6H), 2.29-1.94 (m, 3H), 1.26 (s, 9H), 0.90-0.68 (dd, J= 8.0 Hz, 18H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.39 ppm;



The use of 7th Fmoc protected Fmoc-^{D}Val-OH as the last amino acid residue to terminate peptide chain extension. The **BDKO** support attached heptapeptide intermediates:

Fmoc-^{D}Val-**Leu**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO, with 42% total coupling yield.

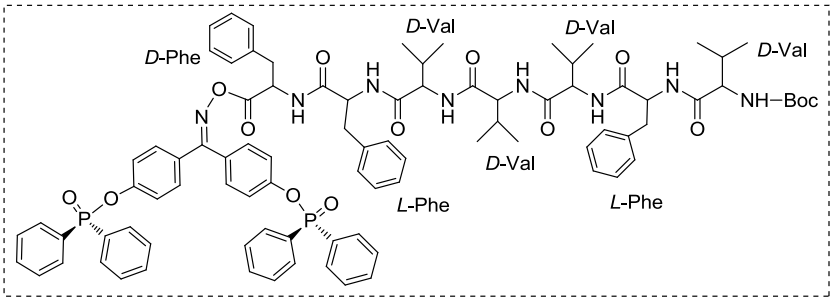
Fmoc-^{D}Val-**Phe**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO, with 41% total coupling yield.

Fmoc-^{D}Val-**Ile**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO, with 40% total coupling yield.

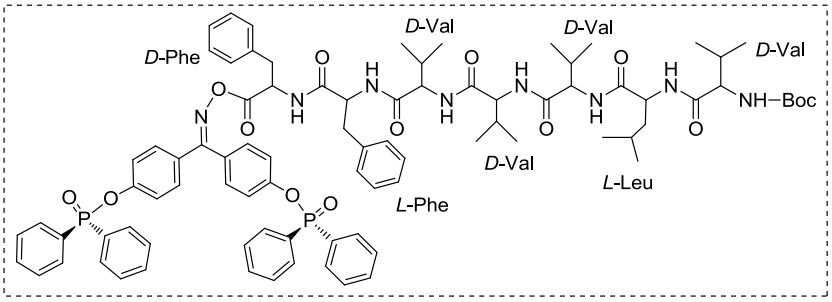
Fmoc-^{D}Val-**Val**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO, with 43% total coupling yield.

Fmoc-^{D}Val-**Pro**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO, with 41% total coupling yield.

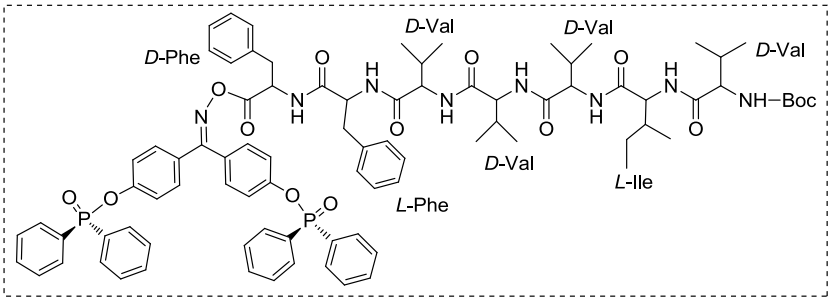
The heptapeptide products were precipitated by using the EA/MeCN solvent system with an adjustable ratio.



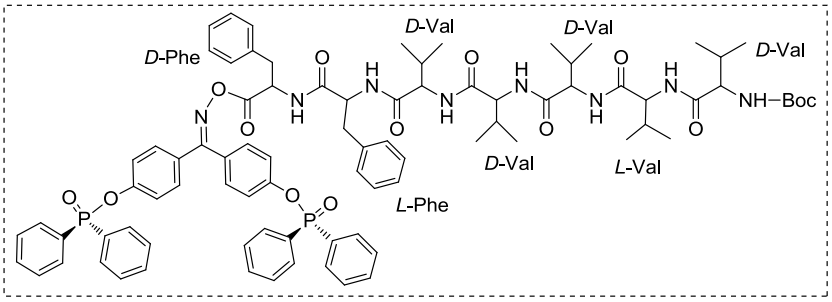
Fmoc-^{D}Val-Phe-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO. 2b-7, 1.45 g, 85% yield, white solid, R_f= 0.45 (CH_2Cl_2: MeOH= 10:1). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.65-8.60 (m, 1H), 8.26-8.24 (m, 6H), 7.93-7.83 (m, 11H), 7.73-7.53 (m, 15H), 7.43-7.04 (m, 25H), 4.60-3.91 (m, 10H), 3.01-2.76 (m, 6H), 1.94-1.89 (m, 3H), 1.75-1.72 (m, 1H), 0.82-0.75 (dd, J= 8.0 Hz, 18H), 0.53-0.51 (m, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.00 ppm; HRMS (ESI) m/z calcd for $C_{99}H_{103}N_8O_{14}P_2^+$ $(M+H)^+$ 1689.70635, found 1689.70520.



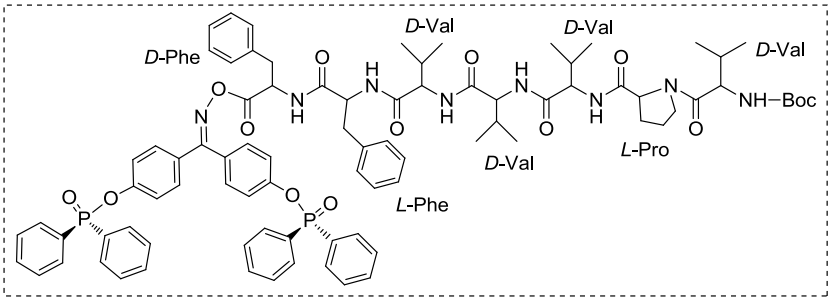
Fmoc-^{D}Val-Leu-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO. 2b-8, 1.46 g, 87% yield, white solid, R_f= 0.42 (CH_2Cl_2: MeOH=10:1). ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.16-8.59 (m, 4H), 8.32-7.07 (m, 46H), 4.59-3.89 (m, 10H), 3.20-2.76 (m, 4H), 2.11-1.45 (m, 10H), 0.81-0.74 (dd, J= 8.0 Hz, 24H), 0.53-0.50 (m, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.03 ppm;HRMS (ESI) m/z calcd for $C_{96}H_{104}N_8O_{14}P_2Na^+$ $(M+Na)^+$ 1677.70394, found 1677.70410.



Fmoc-^{D}Val-Ile-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO. 2b-9, 730 mg, 84% yield, white solid, R_f= 0.42 (CH_2Cl_2: MeOH= 10:1). HRMS (ESI) m/z calcd for $C_{96}H_{104}N_8O_{14}P_2Na^+$ $(M+Na)^+$ 1677.70394, found 1677.70581.



Fmoc-^{D}Val-^{L}Val-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO. 2b-10, 548 mg, 88% yield, white solid, R_f= 0.40 (CH_2Cl_2: MeOH= 10:1). HRMS (ESI) m/z calcd for $C_{95}H_{102}N_8O_{14}P_2Na^+$ $(M+Na)^+$ 1663.68829, found 1663.68982.



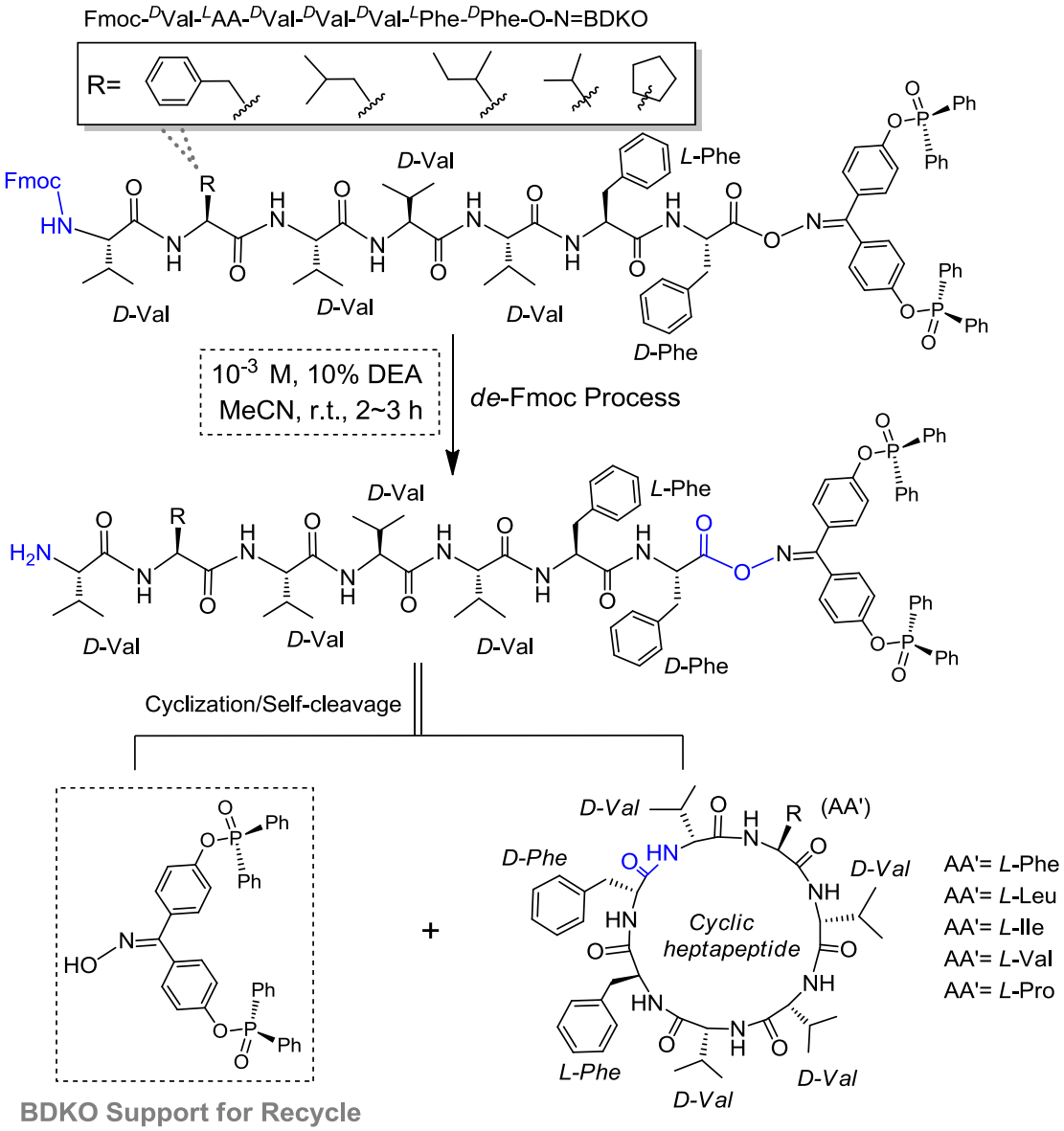
Fmoc-^{D}Val-^{L}Pro-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO. 2b-11, 710 mg, 86% yield, white solid, R_f= 0.50 (CH_2Cl_2: MeOH= 10:1). HRMS (ESI) m/z calcd for $C_{95}H_{100}N_8O_{14}P_2Na_2^{2+}$ $(M+2Na)^{2+}$ 831.33996, found 831.33923.

[***Remarked:*** The oxime ester formed by BDKO and peptide was partly hydrolyzed when the TFA contains water. Therefore, we should pay attention to several points when coupling and removing the Boc protection: (1) The process of removing the Boc protected group needs to be operated in a strict dry and anhydrous environment, the reaction solvent DCM or chloroform should be the newly opened, and the purity of trifluoroacetic acid should be controlled to 99.9%; (2) After the removal of Boc protection, when removed the DCM and TFA solvents by vacuum concentration, a

low-temperature environment should be maintained. The free TFA residue can be removed by vacuum drying. (3) In the coupling process, the addition order and amount of coupling reagents are also critical. First, Boc-AA-OH and the coupling reagent (EDCl/HOBt) form the activated ester at 0 °C. Then added the BDKO attached peptide products. The last step to add the base DIPEA.]

The coupling procedure of ketone oxime with amino acids can also refer to the methods in the reference.[1-4]

6. On-BDKO cyclization/ self-cleavage synthesis of cyclic heptapeptide.



General procedure for the head-to-tail cyclization /self-cleavage synthesis of cyclic heptapeptide: The linear Fmoc-heptapeptide-BDKO product Fmoc-^{D}Val-^{L}Phe-^{D}Val-^{D}Val-^{D}Val-^{L}Phe -^{D}Phe-O-N=BDKO (170 mg, 0.1 mmol) was added to a solution of 10% DEA/MeCM (55.5 mL,

V_{DEA}/V_{MeCN}= 1:9) and stirred at room temperature for 2 h. The solution was then concentrated under reduced pressure to afford the residue, Then cold diethyl ether was added to the above residue and assisted with ultrasonic treatment and grinding processing. Repeat the above operation 3 times to afford the white precipitate of crude cyclic heptapeptide: *Cyclo* (*D*Val-*L***Phe**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe). Then HPLC analysis was performed to test the cyclic heptapeptide. The diethyl ether phase was collected and concentrated under reduced pressure to afford the recyclable BDKO support with a 90% yield.

According to the above cyclization procedures, the corresponding cyclic heptapeptides were obtained as follows:

Cyclo(*D*Val-*L***Leu**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe) with 92% cyclization yield;

Cyclo(*D*Val-*L***Phe**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe) with 91% cyclization yield;

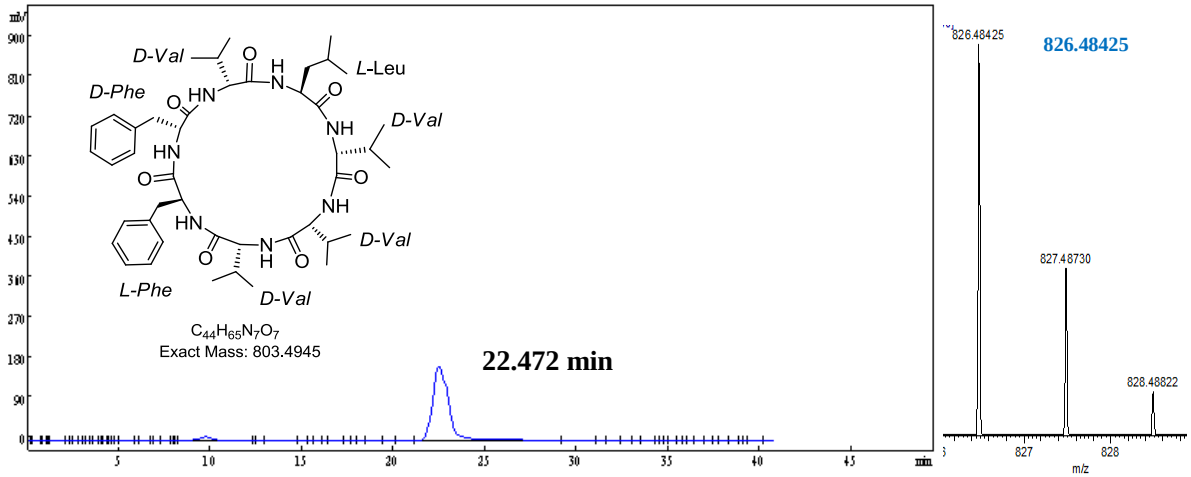
Cyclo(*D*Val-*L***Ile**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe) with 89% cyclization yield;

Cyclo(*D*Val-*L***Val**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe) with 85% cyclization yield;

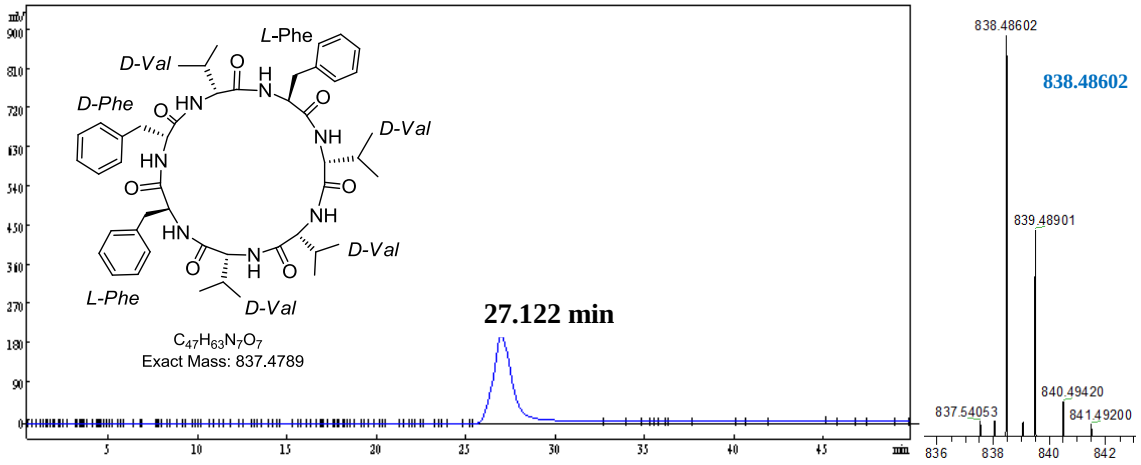
Cyclo(*D*Val-*L***Pro**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe) with 92% cyclization yield;

HPLC and HRMS (ESI) tests of the heptapeptide

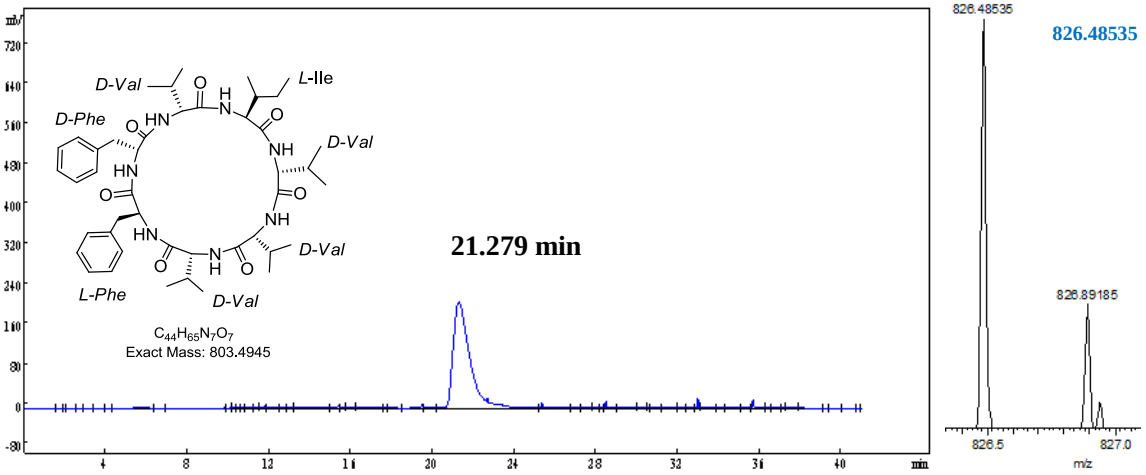
T *(min)*	Flow Rate *(mL/min)*	Elution		*UV* λ *(nm)*
		H_2O (0.1% TFA)	CH_3OH	
0.0	1.0	90	10	220
5.0	1.0	90	10	
40.0	1.0	10	90	
45.0	1.0	90	10	



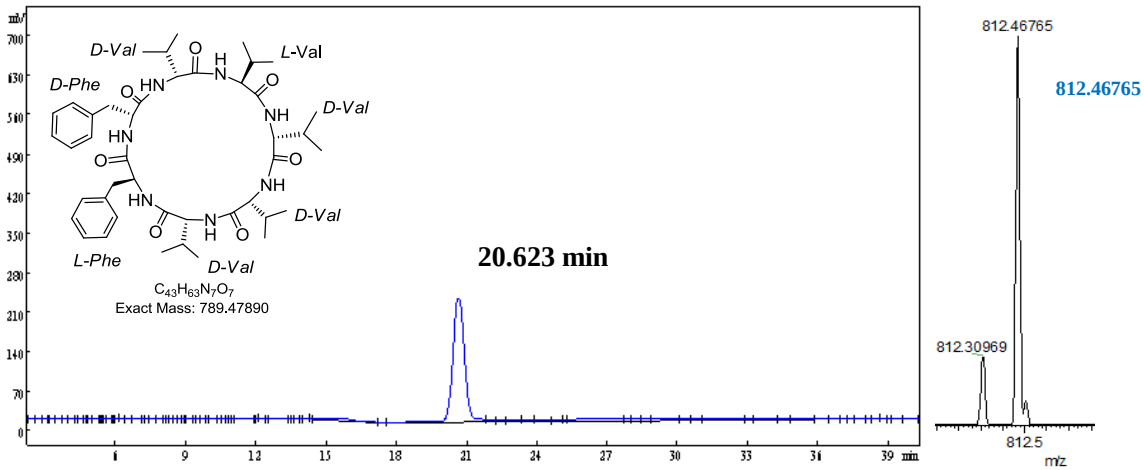
Cyclo (*D*Val-**Leu**-*D*Val-*D*Val-*D*Val-Phe-*D*Phe). Retention time *t*= 22.472 min, HRMS (ESI) m/z calcd for $C_{44}H_{65}N_7O_7Na^+$ $(M+Na)^+$ 826.48377, found 826.48425.



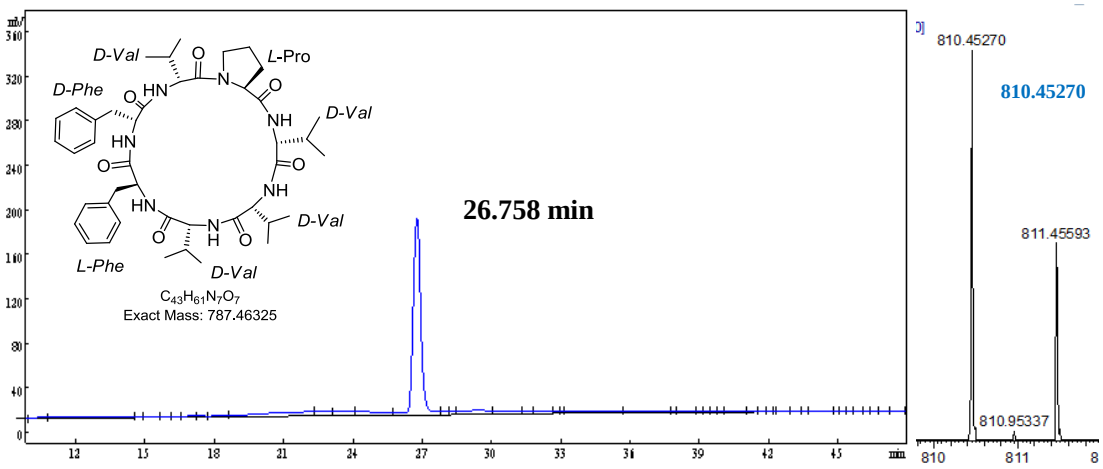
Cyclo (^{D}Val-**Phe**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe). Retention time *t*= 27.122 min, HRMS (ESI) m/z calcd for $C_{47}H_{64}N_7O_7^+$ $(M+H)^+$ 838.48617, found 838.48602.



Cyclo (^{D}Val-**Ile**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe). Retention time *t*= 21.279 min, HRMS (ESI) m/z calcd for $C_{44}H_{65}N_7O_7Na^+$ $(M+Na)^+$ 826.48377, found 826.48535.

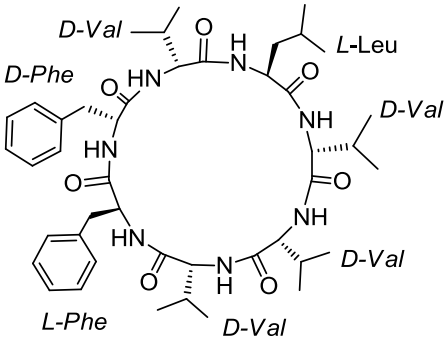


Cyclo (^{D}Val-**Val**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe). Retention time *t*= 20.623 min, HRMS (ESI) m/z calcd for $C_{43}H_{63}N_7O_7Na^+$ $(M+Na)^+$ 812.46812, found 812.46765.

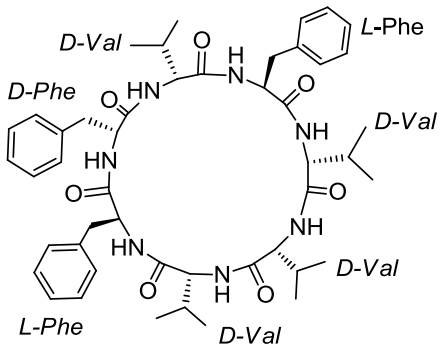


Cyclo (^{D}Val-**Pro**-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe). Retention time *t*= 26.758 min, HRMS (ESI) m/z calcd for $C_{43}H_{61}N_7O_7Na^+$ $(M+Na)^+$ 810.45247, found 810.45270.

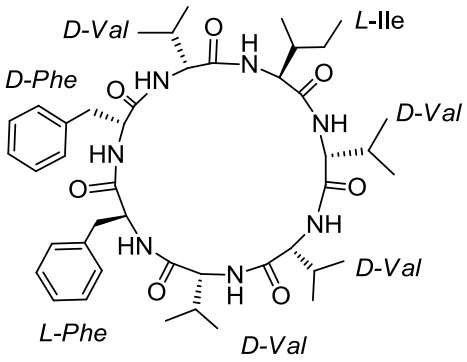
NMR and HRMS (ESI) Data of Cyclic heptapeptide



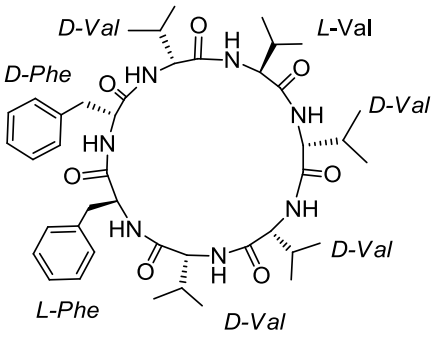
***Cyclo*(^D^Val-^L^Leu-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe). 2b-12,** (253 mg, 92% yield), white solid. ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.14-7.55 (m, 7H), 7.21-7.07 (m, 10H), 4.57-3.68 (m, 7H), 2.88-2.50 (m, 4H), 2.15-1.92 (m, 4H), 1.71-1.44 (m, 3H), 0.86-0.44 (dd, *J*= 8.0 Hz, 30H) ppm; HRMS (ESI) m/z calcd for $C_{44}H_{65}N_7O_7Na^+$ $(M+Na)^+$ 826.48377, found 826.48425.



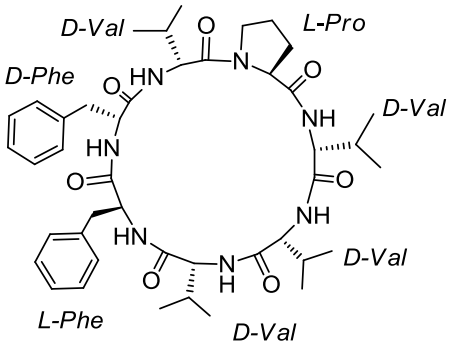
***Cyclo*(^D^Val-^L^Phe-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe). 2b-13,** (190 mg, 91%)，white solid. ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.70-7.65 (m, 4H), 7.24-7.13 (m, 15H), 4.99-4.12 (m, 4H), 3.58-2.70 (m, 10H), 1.93-1.72 (m, 4H), 1.09 (s, 1H), 0.79-0.44 (dd, *J*= 8.0 Hz, 24H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 173.2, 171.3, 170.7, 168.0, 138.3, 138.0, 137.8, 129.8, 129.6, 128.6, 128.3, 126.9, 126.6, 58.6, 57.9, 57.6, 54.1, 53.9, 31.5, 31.0, 30.3, 30.1, 19.7, 19.4, 19.0, 18.7, 18.2, 17.9, 16.9 ppm; HRMS (ESI) m/z calcd for $C_{47}H_{64}N_7O_7^+$ $(M+H)^+$ 838.48617, found 838.48602.



***Cyclo*(^D^Val-^L^Ile-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe). 2b-14,** (178 mg, 89%), white solid. ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.64-8.08 (m, 1H), 7.93-7.53 (m, 5H), 7.43-7.39 (m, 1H), 7.34-7.08 (m, 10H), 4.94-3.98 (m, 7H), 3.08-2.67 (m, 4H), 2.15-1.92 (m, 4H), 1.94-1.68 (m, 5H), 1.36-1.27 (m, 2H), 1.17-0.50 (m, 30H) ppm; HRMS (ESI) m/z calcd for $C_{44}H_{65}N_7O_7Na^+$ $(M+Na)^+$ 826.48377, found 826.48535.



***Cyclo* (^D^Val-^L^Val-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe). 2b-15,** (170 mg, 85% yield), white solid. HRMS (ESI) m/z calcd for $C_{43}H_{63}N_7O_7Na^+$ $(M+Na)^+$ 812.46812, found 812.46765.

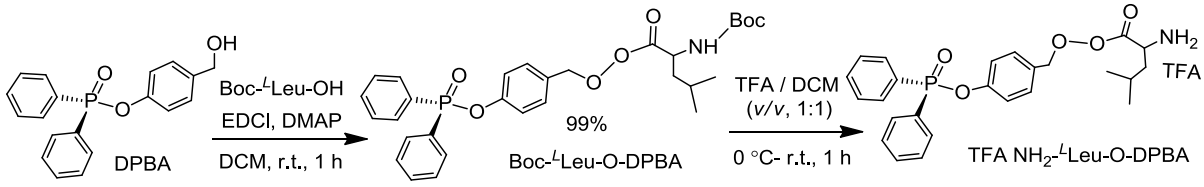


***Cyclo* (^D^Val-^L^Pro-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe). 2b-16,** (240 mg, 92% yield), white solid. ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.18-7.53 (m, 3H), 7.20-7.14 (m, 10H), 4.56-4.13 (m, 7H), 3.10-2.86 (m, 6H), 1.95-1.70 (m, 10H), 0.87-0.47 (dd, J= 8.0 Hz, 24H) ppm; HRMS (ESI) m/z calcd for $C_{43}H_{61}N_7O_7Na^+$ $(M+Na)^+$ 810.45247, found 810.45270.

On-BDKO cyclization/ self-cleavage of hexapeptide H-^L^Phe-^L^Val-^L^Phe-^L^Val-^L^Phe-^L^Val-O-N=BDKO

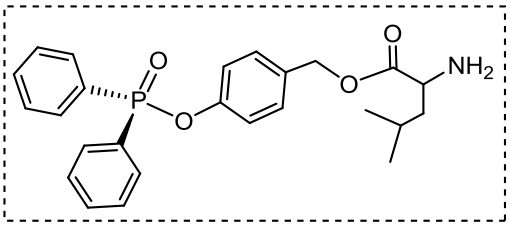
The linear hexapeptide product H-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO (0.1 mmol) was added to a solution of 10% DEA/MeCM (55.5 mL, V_{DEA}/V_{MeCN}= 1:9) and stirred at room temperature for 5 h. The mixture was concentrated under reduced pressure to afford the residue. There was no target cyclic hexapeptide product detected in the residue, only the mixtures containing the linear hexapeptide H-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-OH, uncleaved original product, and uncertain byproducts were detected.

7. Synthesis of DPBA-attached linear gramicidin S chain.

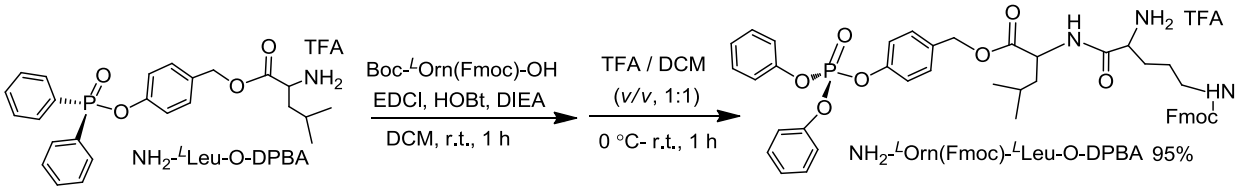


Synthesis of **Boc-Leu-O-DPBA**. EDCl (1.38 g, 7.2 mmol, 1.2 equiv) and DMAP (87.8 mg, 0.72 mmol, 0.12 equiv) were added to a solution of Boc-^{L}Leu-OH (1.46 g, 6.3 mmol, 1.05 equiv) in DCM (30 mL) at 0 °C and stirred for 10 min. The reaction mixture was added with **DPBA, 3a** (1.95 g, 6.0 mmol, 1.0 equiv) and stirred at room temperature for 1 h. The mixture was then washed with saturated NH_4Cl and $NaHCO_3$ respectively and dried with $MgSO_4$. 5.0 mL ethyl acetate was added to dissolve the sample after concentration, and 40 mL of petroleum ether (V_{EA}/V_{PE} =1:8) was added dropwise and stirred. Precipitate appeared, the precipitate was filtered, dried to afford the **Boc-Leu-O-DPBA** (3.3 g, 99% yield).

***de*-Boc** procedure: The **Boc-Leu-O-DPBA** was added to a dry flask with 4 mL dry DCM, and stirred at 0 °C for 15 min. Then 4 mL of TFA (99.9% purity) was added to the above solution and stirred for 1 h at r.t. Then removed the TFA by vacuum concentration at 25°C, and DCM was continuously added for 3 times and concentrated to remove TFA residue. The product was dissolved in DCM and washed with 0.1 M $NaHCO_3$, then concentrated to obtain the *de*-Boc product **H-Leu-O-DPBA.**

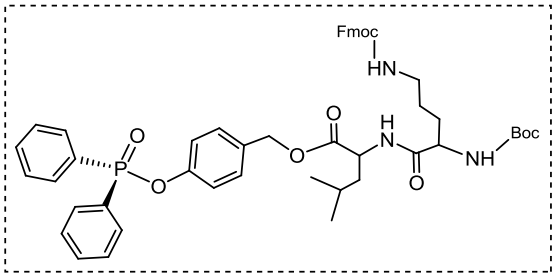


H-Leu-O-DPBA. 3a-1, 2.58 g, 99% yield, white solid. R_f= 0.45 (CH_2Cl_2: MeOH= 50:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.92-7.87 (m, 4H), 7.55-7.74 (m, 6H), 7.25-7.19 (m, 4H), 5.04 (s, 2H), 3.50-3.45 (m, 1H), 1.78-1.68 (m, 3H), 1.57-1.50 (m, 1H), 1.44-1.37 (m, 1H), 0.91-0.87 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.78 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 176.3, 150.9, 132.6, 132.1, 131.8, 131.5, 130.1, 129.8, 128.7, 120.9, 65.9, 52.9, 43.9, 24.7, 22.9, 21.8 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{29}NO_4P^+$ $(M+H)^+$ 438.18287, found 438.18219.



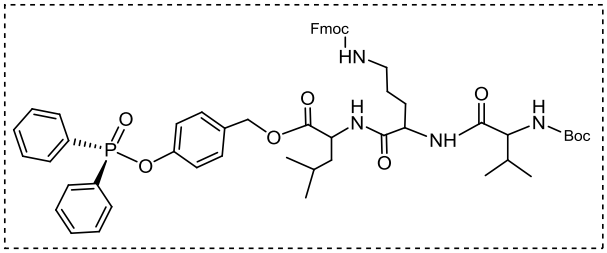
Synthesis of **Boc-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA.** EDCl (1.15 g, 6 mmol, 1.2 equiv) and HOBt (810 mg, 6 mmol, 1.2 equiv) were added to a solution of Boc-^{L}Orn(NHFmoc)-OH (2.38 g, 5.25

mmol, 1.05 equiv) in DCM (35 mL) at 0 °C and stirred for 0.5 h. **H-Leu-O-DPBA** (2.2 g, 5 mmol, 1.0 equiv) and DIEA (0.87 mL, 5.25 mmol, 1.05 equiv) were then added and stirred for 1 h. The mixture was then washed with H_2O, dried with $MgSO_4$. 7.0 mL ethyl acetate was added to dissolve the sample after concentration, and 55 mL of petroleum ether (V_{EA}/V_{PE} =1:8) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the product **Boc-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA**.

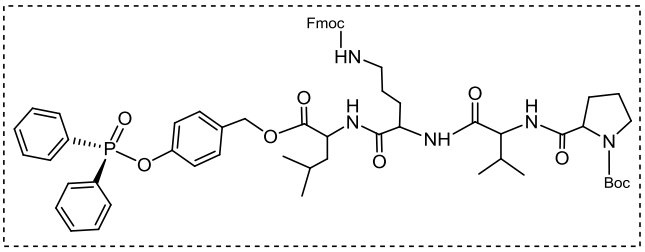


Boc-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-2, 4.15 g, 95% yield, white solid. R_f= 0.45 (CH_2Cl_2: MeOH= 40:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.91-7.86 (m, 4H), 7.74-7.72 (d, *J*= 8.0 Hz, 2H), 7.60-7.33 (m, 10H), 7.27-7.13 (m, 6H), 5.85-5.82 (m, 1H), 5.39-5.37 (m, 1H), 4.98-4.90 (m, 2H), 4.59-4.15 (m, 5H), 3.41-3.39 (m, 1H), 3.09-3.04 (m, 1H), 2.55 (s, 1H), 1.70-1.47 (m, 7H), 1.42 (s, 9 H), 0.86-0.85 (dd, *J*= 8.0, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.15 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.6, 172.5, 157.4, 155.8, 150.8, 144.0, 141.3, 132.7, 131.8, 131.4, 130.0, 128.6, 127.7, 127.1, 125.2, 120.9, 120.0, 79.6, 66.7, 66.3, 50.9, 47.2, 40.7, 30.3, 28.4, 26.0, 24.7, 22.9, 21.7 ppm; HRMS (ESI) m/z calcd for $C_{50}H_{56}N_3O_9PNa^+$ $(M+Na)^+$ 896.36464, found 896.36548.

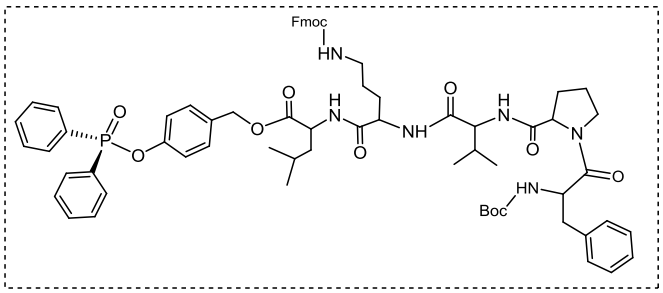
Extension of DPBA attached peptide chains: According to the above synthesis procedures, and using the above coupling reagent system such as EDCl/DMAP, EDCl/HOBt/DIPEA (2.0 equiv), and the above *de*-Boc reagent system (50% TFA/DCM) to extend the DBA-OH attached gramicidin S chain.



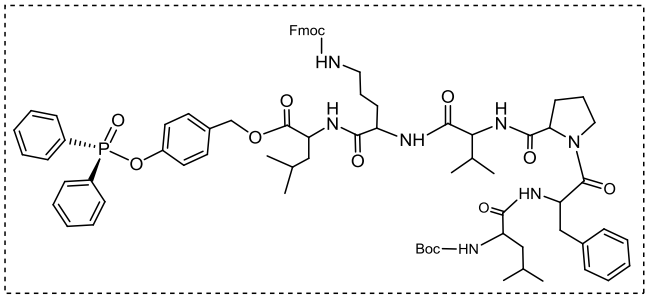
Boc-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-3, 3.65 g, 94% yield, white solid. R_f= 0.40 (CH_2Cl_2: MeOH= 40:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.91-7.86 (m, 4H), 7.75-7.73 (d, *J*= 8.0 Hz, 2H), 7.61-7.34 (m, 10H), 7.28-7.09 (m, 6H), 6.82-6.80 (m, 1H), 5.85-5.83 (m, 1H), 5.23-5.21 (m, 1H), 4.98-4.91 (m, 2H), 4.70-4.38 (m, 3H), 4.20-3.96 (m, 3H), 3.47-3.44 (m, 1H), 3.08-3.03 (m, 1H), 2.11-2.07 (m, 1H), 1.66-1.50 (m, 7H), 1.43 (s, 9 H), 0.94-0.83 (dd, *J*= 8.0, 12H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.22 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.3, 171.9, 171.8, 157.6, 155.9, 150.8, 150.7, 144.0, 143.9, 141.3, 132.7, 131.8, 131.5, 130.1, 128.8, 127.7, 127.1, 125.1, 120.9, 120.0, 79.8, 66.8, 66.3, 59.9, 51.0, 47.2, 40.6, 39.0, 31.1, 30.2, 28.3, 26.1, 24.7, 22.8, 21.6, 19.3, 17.8 ppm; HRMS (ESI) m/z calcd for $C_{55}H_{65}N_4O_{10}PNa^+$ $(M+Na)^+$ 995.43305, found 995.43433.



Boc-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-4, 3.89 g, 91% yield, white solid. R_f= 0.42 (CH_2Cl_2: MeOH= 30:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.91-7.86 (m, 4H), 7.75-7.73 (d, J= 8.0 Hz, 2H), 7.61-7.35 (m, 10H), 7.24-7.11 (m, 6H), 6.86 (m, 1H), 5.95-5.78 (m, 1H), 5.01-4.94 (m, 2H), 4.65-4.50 (m, 2H), 4.40-4.17 (m, 5H), 3.44-3.08 (m, 4H), 2.56 (m, 1H), 2.30-1.82 (m, 6H), 1.70-1.58 (m, 7H), 1.43 (s, 9 H), 0.93-0.84 (dd, J= 8.0, 12H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.00 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.7, 172.3, 171.8, 171.1, 157.2, 155.8, 150.7, 144.1, 141.2, 132.6, 131.8, 131.7, 131.5129.9, 128.6, 127.7, 127.1, 125.2, 120.8, 120.7, 119.9, 80.7, 66.8, 66.2, 60.4, 58.9, 51.6, 51.0, 47.2, 40.6, 30.0, 29.6, 28.6, 28.4, 26.1, 24.7, 22.9, 21.7, 19.4, 17.4 ppm; HRMS (ESI) m/z calcd for $C_{60}H_{72}N_5O_{11}PNa^+$ $(M+Na)^+$ 1092.48582, found 1092.48767.

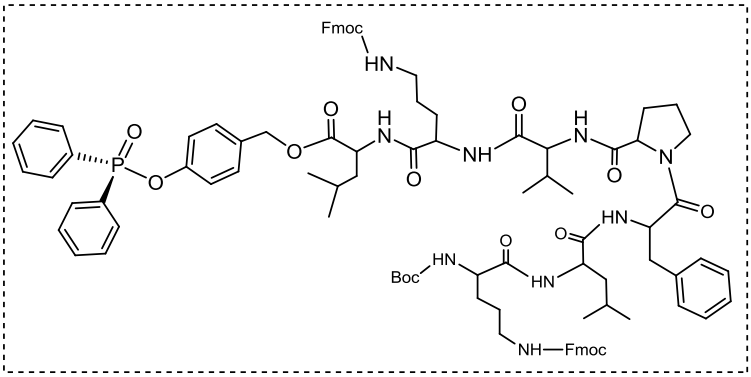


Boc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-5, 4.08 g, 96% yield, white solid. R_f= 0.35 (CH_2Cl_2: MeOH= 30:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.86 (m, 4H), 7.74-7.72 (d, J= 8.0 Hz, 2H), 7.61-7.34 (m, 10H), 7.25-7.08 (m, 11H), 6.94-6.92 (m, 1H), 6.75-6.74 (m, 1H), 6.28 (m, 1H), 6.04-6.00 (m, 1H), 5.07-4.98 (m, 2H), 4.59-4.15 (m, 8H), 3.73-3.64 (m, 1H), 3.45-2.92 (m, 5H), 2.57-2.26 (m, 2H), 2.04-1.93 (m, 2H), 1.78-1.49 (m, 8H), 1.41-1.34 (m, 10 H), 0.98-0.88 (dd, J= 8.0, 12H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.80 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 173.4, 173.0, 172.3, 172.0, 171.2, 157.5, 157.1, 155.8, 150.6, 143.9, 141.2, 135.8, 132.7, 131.4, 130.1, 129.7, 129.3, 128.7, 128.6, 127.7, 127.3, 127.1, 125.2, 120.7, 120.0, 80.1, 67.3, 66.8, 66.0, 61.6, 61.1, 59.3, 54.4, 53.5, 53.1, 51.0, 47.2, 40.5, 38.3, 29.1, 28.9, 28.7, 28.4, 26.9, 26.6, 25.0, 24.8, 24.5, 23.0, 21.7, 19.7, 18.7, 18.5 ppm; HRMS (ESI) m/z calcd for $C_{69}H_{81}N_6O_{12}PNa^+$ $(M+Na)^+$ 1239.55423, found 1239.55530.

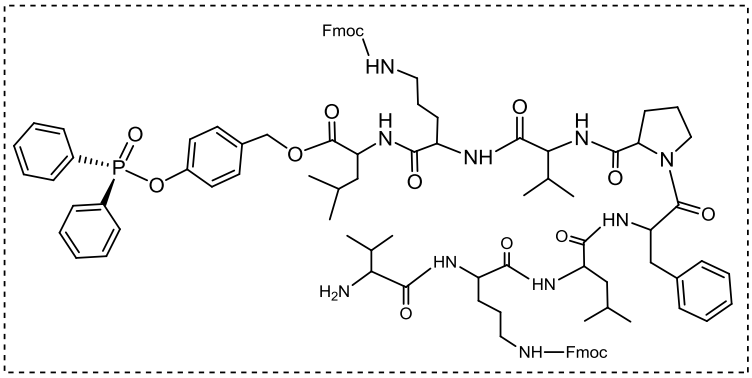


Boc-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-6, 4.42 g, 95% yield, white solid. R_f= 0.30 (CH_2Cl_2: MeOH= 30:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.71 (m, 6H), 7.61-7.31 (m, 10H), 7.27-7.00 (m, 11H), 6.93-6.89 (m, 1H), 6.72 (m, 1H), 6.20-5.80 (m, 1H), 5.34-5.31 (m, 1H), 5.10-5.00 (m, 2H), 4.57-4.01 (m, 9H), 3.83-3.46 (m, 2H), 3.27-2.88 (m, 4H), 2.64-1.98 (m, 3H), 1.85-1.20 (m, 23H), 1.01-0.85 (dd, J= 8.0, 18H) ppm; ^{31}P NMR (162 MHz,

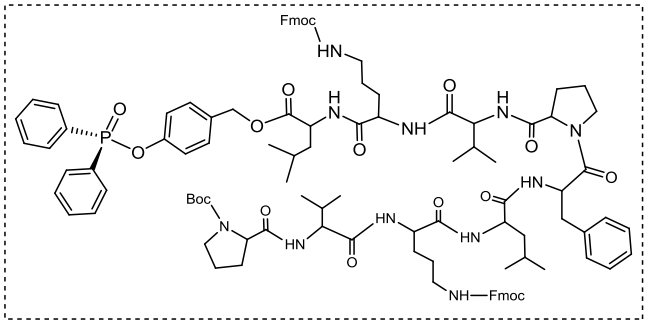
$CDCl_3$), δ 30.87 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.9, 173.8, 172.3, 172.3, 171.3, 170.7, 158.1, 156.0, 155.3, 150.6, 144.0, 141.2, 135.6, 132.6, 132.0, 131.8, 131.7, 131.5, 130.1, 129.8, 129.2, 128.6, 127.7, 127.1, 125.2, 120.7, 119.9, 79.9, 67.4, 66.9, 66.0, 61.4, 61.2, 59.9, 54.7, 54.0, 53.5, 52.9, 52.4, 51.0, 47.2, 43.0, 41.5, 41.2, 40.6, 40.3, 37.3, 36.9, 29.4, 29.0, 28.7, 28.4, 25.9, 24.8, 24.2, 24.0, 23.0, 21.9, 21.7, 21.5, 19.8, 19.6, 18.9 ppm; HRMS (ESI) m/z calcd for $C_{75}H_{92}N_7O_{13}PNa^+$ $(M+Na)^+$ 1352.63829, found 1352.63940.



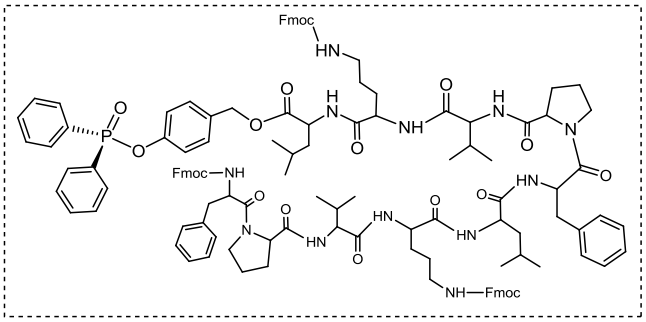
Boc-^{L}Orn(NHFmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-7, 4.70 g, 94% yield, white solid. R_f= 0.35 (CH_2Cl_2: MeOH= 20:1). ^1H NMR (400 MHz, $CDCl_3$), δ 8.56-8.54 (m, 1H), 8.24-8.05 (m, 1H), 7.93-7.88 (m, 4H), 7.76-7.56 (m, 10H), 7.51-7.32 (m, 10H), 7.27-7.14 (m, 11H), 6.97 (m, 1H), 6.35-6.27 (m, 1H), 5.94-5.38 (m, 1H), 5.10-4.95 (m, 2H), 4.84-4.03 (m, 14H), 3.44-3.39 (m, 1H), 3.22-3.01 (m, 6H), 2.36-2.06 (m, 3H), 1.90-1.36 (m, 28H), 0.96-0.85 (m, 18H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.98 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.7, 172.3, 172.1, 171.9, 171.8, 171.1, 170.9, 157.1, 156.9, 155.8, 150.8, 144.8, 144.2, 144.1, 143.9, 141.2, 135.5, 132.6, 132.0, 131.8, 131.4, 130.4, 130.0, 129.7, 129.2, 128.6, 127.6, 127.0, 125.2, 120.8, 119.9, 79.9, 67.1, 66.6, 66.1, 60.5, 59.5, 54.6, 53.5, 53.3, 52.7, 50.8, 50.3, 47.1, 46.5, 41.6, 41.1, 40.4, 39.8, 36.7, 32.3, 29.9, 29.3, 29.1, 28.4, 26.9, 25.9, 25.1, 24.8, 23.6, 23.1, 22.9, 22.8, 22.5, 21.2, 19.2 ppm; HRMS (ESI) m/z calcd for $C_{95}H_{112}N_9O_{16}PNa^+$ $(M+Na)^+$ 1688.78569, found 1688.78735.



H-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-8, 3.06 g, 92% yield, white solid. R_f= 0.35 (CH_2Cl_2: MeOH= 10:1). ^1H NMR (400 MHz, $CDCl_3$), δ 8.60-8.15 (m, 2H), 7.92-7.88 (m, 4H), 7.79-7.28 (m, 20H), 7.20-7.13 (m, 11H), 7.02-6.88 (m, 2H), 6.26-5.93 (m, 1H), 5.11-3.98 (m, 14H), 3.73-3.67 (m, 1H), 3.20-3.06 (m, 7H), 2.35-1.54 (m, 23H), 0.97-0.84 (dd, J= 8.0 Hz, 24H); ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.58 ppm; HRMS (ESI) m/z calcd for $C_{95}H_{113}N_{10}O_{15}PNa^+$ $(M+Na)^+$ 1687.80167, found 1687.80200.



Boc-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-9, 1.67 g, 90% yield, white solid. R_f= 0.40 (CH_2Cl_2: MeOH= 10:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.81 (m, 4H), 7.73-7.52 (m, 11H), 7.44-7.31 (m, 9H), 7.24-7.06 (m, 12H), 5.14-4.95 (m, 6H), 4.48-4.02 (m, 10H), 3.71-3.06 (m, 10H), 2.37-1.64 (m, 26H), 1.49-1.42 (m, 18H), 0.98-0.86 (dd, J= 8.0 Hz, 24H); ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.62 ppm; HRMS (ESI) m/z calcd for $C_{105}H_{128}N_{11}O_{18}PNa^+$ $(M+Na)^+$ 1884.90686, found 1884.90894.



Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA. 3a-10, 968 mg, 90% yield, white solid. R_f= 0.30 (CH_2Cl_2: MeOH= 10:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.93-7.88 (m, 4H), 7.79-7.69 (m, 6H), 7.65-7.59 (m, 6H), 7.49-7.31 (m, 16H), 7.26-7.09 (m, 16H), 6.90-6.83 (m, 2H), 6.20 (m, 1H), 5.79-5.70 (m, 1H), 5.12-5.05 (m, 2H), 4.65-3.92 (m, 19H), 3.77-3.54 (m, 4H), 3.35-3.04 (m, 8H), 2.71-2.39 (m, 3H), 2.04-1.46 (m, 16H), 1.31-1.25 (m, 1H), 1.08-0.79 (dd, J= 8.0 Hz, 24H); ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.67 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.2, 173.6, 173.4, 172.9, 172.3, 171.5, 158.0, 157.3, 157.1, 155.6, 150.6, 150.5, 144.3, 144.0, 143.8, 143.5, 143.3, 141.4, 141.2, 136.6, 135.7, 132.6, 132.4, 131.9, 131.5, 130.1, 129.7, 129.5, 129.2, 128.7, 128.6, 128.4, 127.9, 127.7, 127.1, 127.0, 126.9, 125.5, 125.2, 125.1, 125.0, 124.8, 124.6, 120.7, 119.9, 67.4, 67.1, 66.7, 65.9, 61.8, 61.5, 60.3, 56.0, 55.3, 54.5, 54.1, 53.5, 53.4, 52.3, 51.0, 47.6, 47.2, 47.0, 46.7, 41.1, 40.9, 40.6, 40.3, 39.7, 37.2, 36.6, 29.4, 29.2, 29.0, 28.7, 27.3, 25.8, 25.3, 24.8, 24.4, 23.3, 23.0, 21.8, 21.2, 20.8, 19.7, 19.5, 19.4, 19.2; HRMS (ESI) m/z calcd for $C_{124}H_{139}N_{12}O_{19}PNa^+$ $(M+Na)^+$ 2153.99093, found 2153.99512.

8. Head-to-tail cyclization synthesis of linear gramicidin S.

Method 1: General procedure for cyclization/ self-cleavage synthesis of cyclopeptide.

The linear product Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NHFmoc)-^{L}Leu-O-DPBA was added to a solution of 25% DEA/MeCM (v_{DEA}/v_{MeCN}= 1:3) and stirred at 35°C for 5 h and intermittently assisted with ultrasound treatment. The solution was then concentrated under reduced pressure to afford the residue. The results of TLC, HRMS (ESI), and NMR analysis

confirmed that the decapeptide Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn (Fmoc)-^{L}Leu-O-DPBA only completed the removal of the Fmoc protected group, and there was no head-to-tail cyclization/ self-cleavage product. Only the *de*-Fmoc product H-^{D}Phe-^{L}Pro-^{L}Val -^{L}Orn(NH_2)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH_2)-^{L}Leu-O-DPBA was obtained in this way.

Method 2: Conventional general procedure for synthesis of gramicidin S

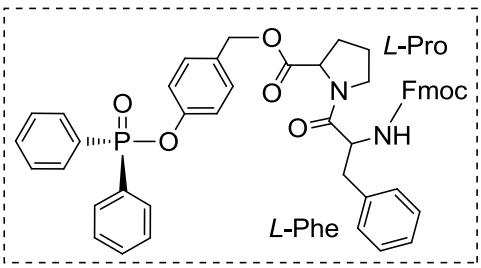
H-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH_2)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH_2)-^{L}Leu-O-DPBA (145 mg, 0.1 mmol) was added to a solution of LiOH/THF/H_2O (LiOH 3 equiv, *v/v*=10:1) cleavage reagent system and stirred for 3 h at room temperature. Then remove THF to derive the residue with water. The residue was neutralized with 0.1 M HCl and obtained the appeared precipitates. The precipitates were added with cold diethyl ether and assisted with grinding and ultrasonic treatment, the decapeptide precipitate was then obtained by centrifugation. Continue to add the diethyl ether and repeat the above operation 3 times to get the purified decapeptide precipitate product 95 mg in 82% yield.

PyBOP (35 mg, 0.06 mmol, 1.2 equiv) and DIPEA (17 μL, 0.1 mmol, 2.0 equiv) were added to a solution of linear gramicidin S (60 mg, 0.05 mmol, 1.0 equiv) in $CHCl_3$ (40 mL, 10^{-3} M). The mixture was stirred for 16 h at room temperature. The mixture was washed with saturated $NaHCO_3$ 2 times and concentrated to obtain the crude product residue. RP-HPLC was then performed to obtain the gramicidin S.

Cyclo [^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH_2)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH_2)-^{L}Leu], HRMS (ESI) m/z calcd for $C_{60}H_{93}N_{12}O_{10}^{+}$ $(M+H)^{+}$ 1141.71321, found 1141.71301.

9. On-DPBA support cyclization/ self-cleavage synthesis of cyclodipeptide

Extension of DPB-OH attached peptide chains: According to the above synthesis procedures, and using the above coupling reagent system such as EDCl/DMAP, EDCl/HOBt/DIEA (2.0 equiv), and the above *de*-Boc reagent system (50% TFA/DCM) to extend the DBA-OH attached dipeptide **Fmoc-^{L}Phe-^{L}Pro-O-DPBA**.

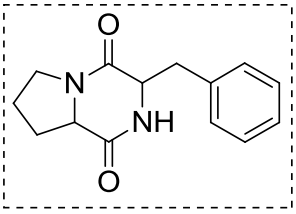


Fmoc-^{L}Phe-^{L}Pro-O-DPBA. 3a-11, 390 mg, 84% total yield. ^{1}H NMR (400MHz, $CDCl_3$), δ 7.92-7.87 (m, 5H), 7.75-7.74 (d, 2H), 7.57-7.36 (m, 12H), 7.28-7.16 (m, 9H), 5.14-4.99 (m, 2H), 4.77-4.71 (m, 1H), 4.55-4.52 (t, 2H), 4.37-4.32 (m, 1H), 4.26-4.22 (m, 1H), 3.64-3.58 (m, 1H), 3.23-3.08 (m, 2H), 2.99-2.93 (m, 1H), 2.12-2.09 (m, 1H), 1.87 (s, 3H) ppm; ^{13}C NMR (100MHz, $CDCl_3$), δ 171.54, 171.15, 170.47, 155.83, 150.91, 150.83, 143.86, 141.25, 136.22, 131.83, 131.73, 131.45, 128.75, 128.45, 127.70, 126.92, 125.27, 125.19, 120.87,

119.96,67.04, 66.11, 60.40, 59.08, 53.84, 47.09, 46.93, 38.86, 28.87, 24.88, 21.06 ppm; HRMS (ESI) m/z calcd for $C_{48}H_{44}N_2O_7P^+$ $[M+H]^+$ 791.28806, found 791.28796.

General procedure for on-DPBA cyclization/ self-cleavage synthesis of cyclodipeptide

The linear product **Fmoc-^{L}Phe-^{L}Pro-O-DPBA** (390mg, 0.5 mmol) was added to a solution of 25% DEA/MeCM (100 mL, v_{DEA}/v_{MeCN}= 1:3) and stirred at r.t for 2 h and intermittently assisted with ultrasound treatment. The solution was then concentrated under reduced pressure to afford the residue. Then petroleum ether was added to the above residue and assisted with ultrasonic treatment and grinding processing. Repeat the above operation 3 times to afford the white precipitate of DBA-OH and cyclic dipeptide. Then preparative TLC was performed to obtain the cyclodipeptide ***cyclo*(^{L}Phe-^{L}Pro)**.



***cyclo*(^{L}Phe-^{L}Pro). 3a-12,** 205 mg, 84% yield, ^{1}H NMR (400MHz, $CDCl_3$), δ 7.39-7.24 (m, 5H), 5.70 (s, 1H), 4.31-4.28 (d, 1H), 4.11-4.08 (t, 1H), 3.70-3.55 (m, 3H), 2.84-2.77 (d, 1H), 2.38-2.32 (m, 3H), 2.08-1.91 (m, 3H), 1.75 (s, 1H) ppm;^{13}C NMR (100MHz, $CDCl_3$), δ 169.40, 165.07, 135.95,129.28, 129.13, 127.56, 59.14, 56.19, 45.46, 36.79, 28.36, 22.55; HRMS (ESI) m/z calcd for $C_{14}H_{17}N_2O_2^+$ $[M+H]^+$ 245.12845, found 245.12816.

10. References

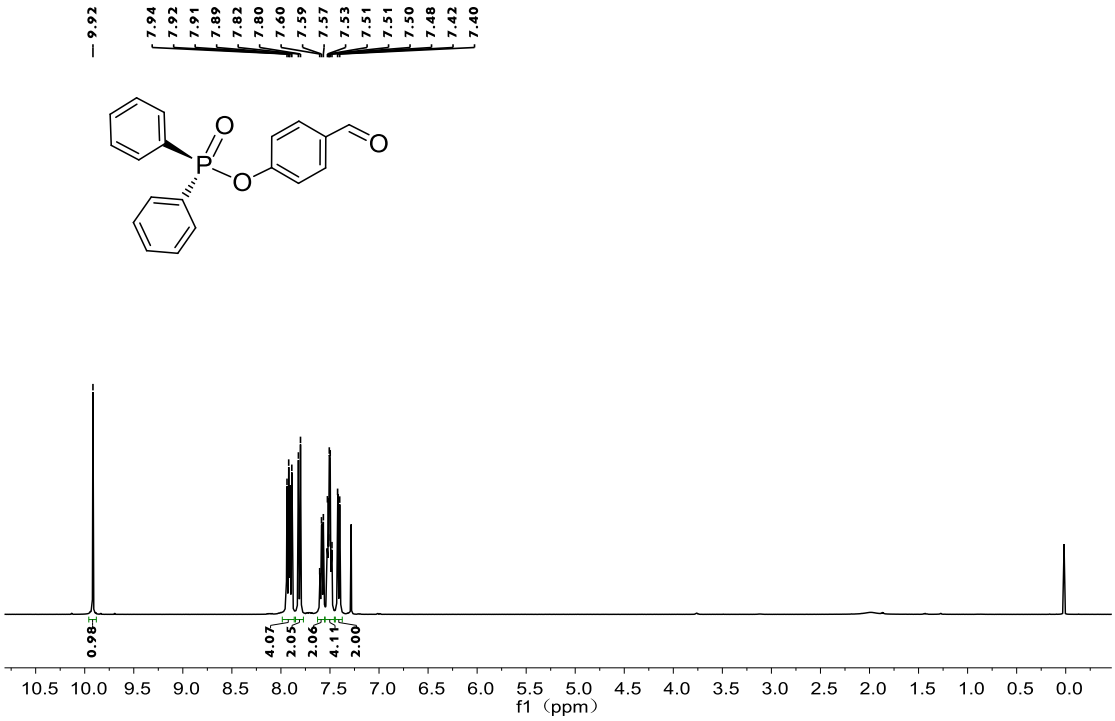
[1] C. Berube, D. Gagnon, A. Borgia, D. Richard and N. Voyer, Total synthesis and antimalarial activity of *mortiamides* A-D, *Chem. Commun.*, 2019, **55,** 7434-7437.

[2] C. Bérubé, A. Borgia and N. Voyer, A novel route towards cycle-tail peptides using oxime resin: teaching an old dog a new trick, *Org. Biomol. Chem.*, 2018, **16**, 9117-9123.

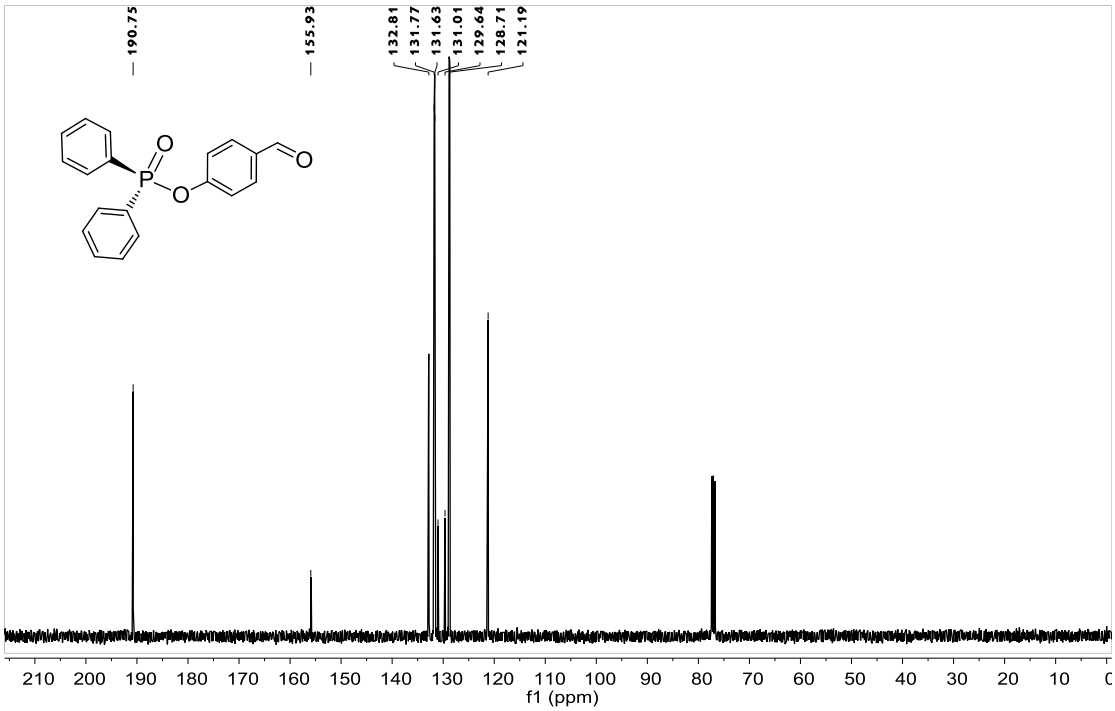
[3] C. Bérubé, A. Borgia and N. Voyer, Total synthesis of pseudacyclins A-E by an on-resin head-to-side chain concomitant cyclization-cleavage reaction, *Tetrahedron Lett.*, 2018, **59**, 4176-4179.

[4] T. Tremblay, G. Robert-Scott, C. Berube, A. Carpentier, N. Voyer and D. Giguere, Synthesis of C-terminal glycopeptides via oxime resin aminolysis, *Chem. Commun.*, 2019, **55**, 13741-13744.

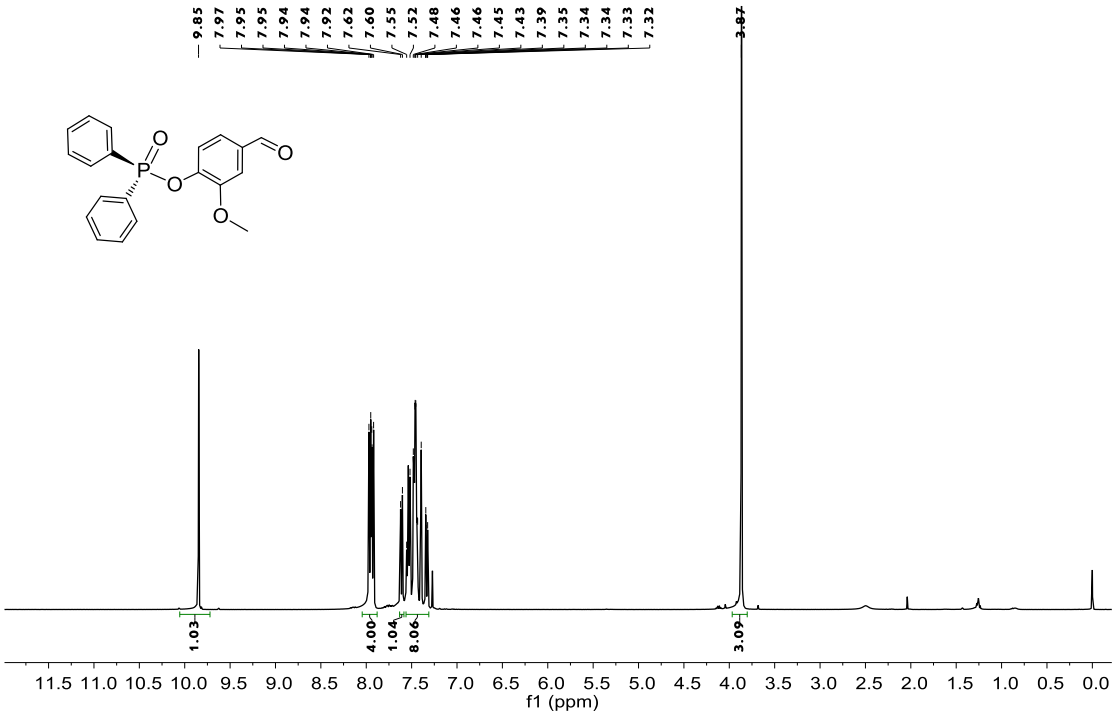
NMR Spectra and HRMS (ESI) Spectra



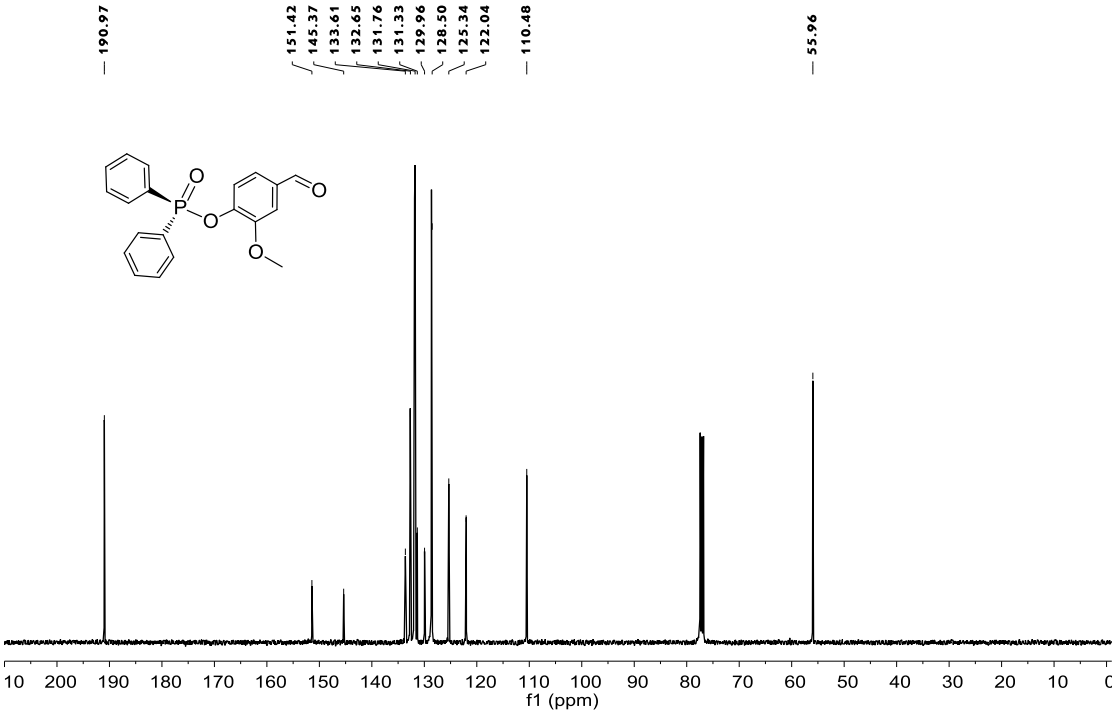
1a ^{1}H NMR (400 MHz, $CDCl_3$)



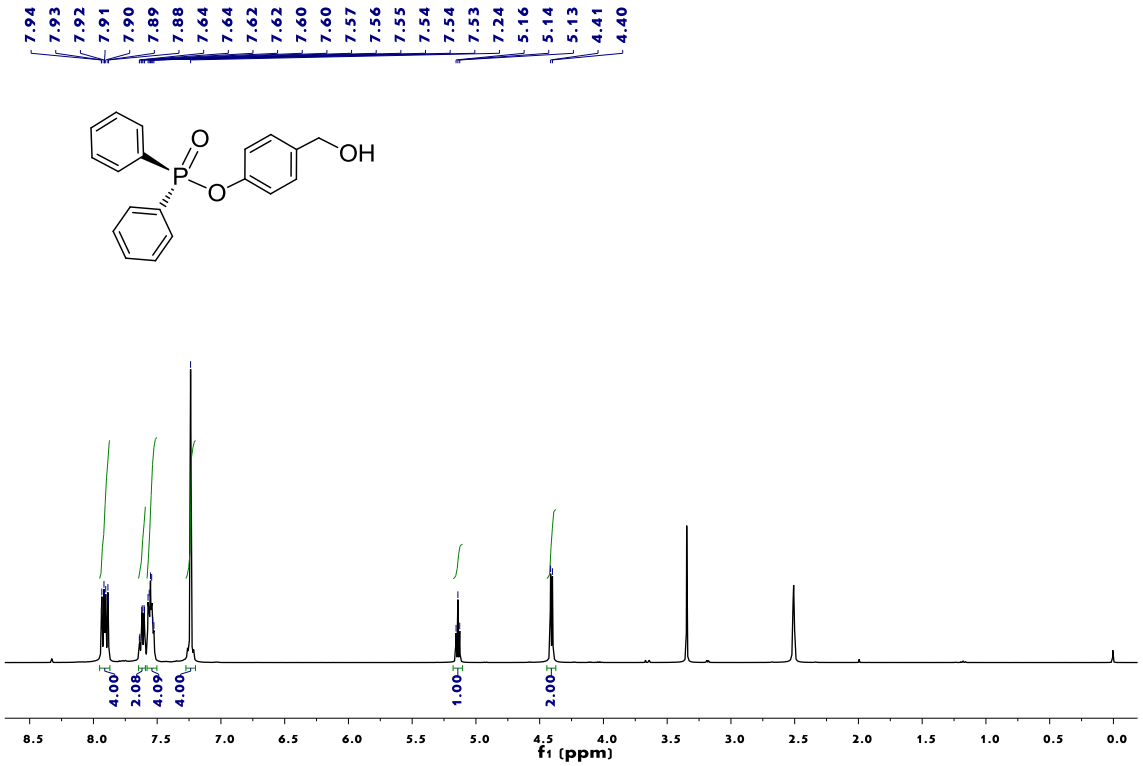
1a ^{13}C NMR (100 MHz, $CDCl_3$)



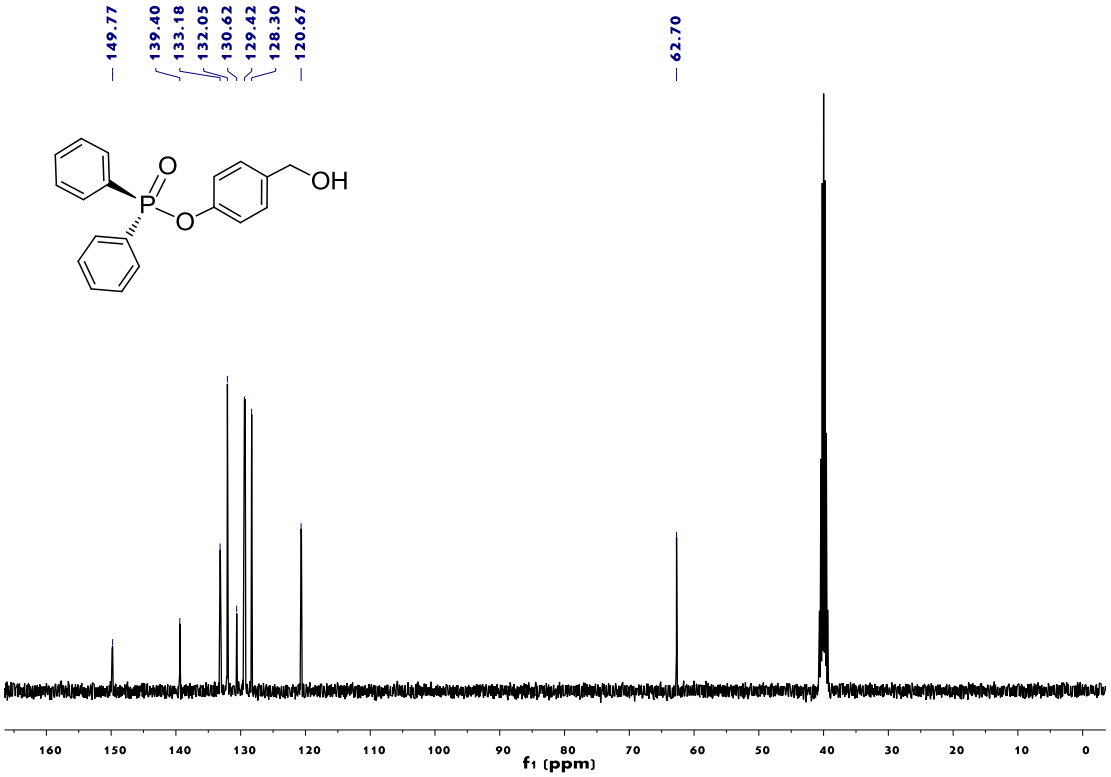
2a ^{1}H NMR (400 MHz, $CDCl_3$)



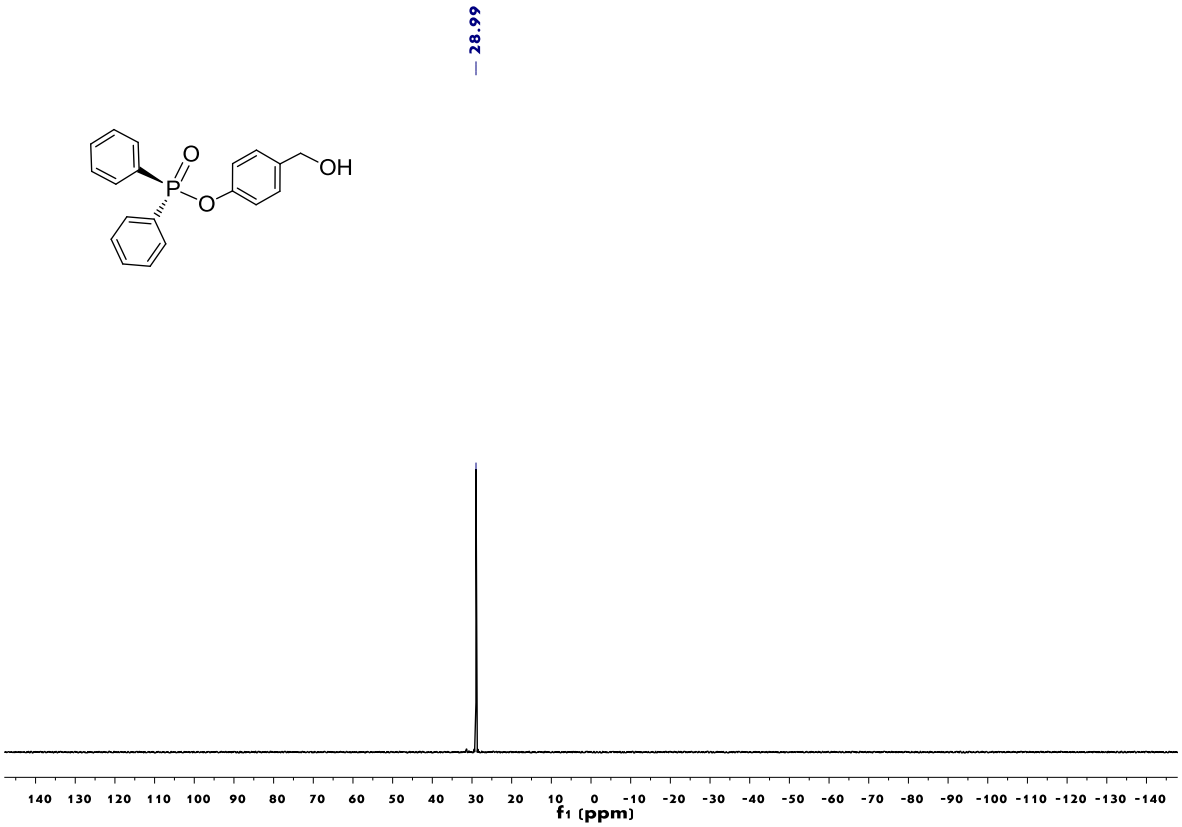
2a ^{13}C NMR (100 MHz, $CDCl_3$)



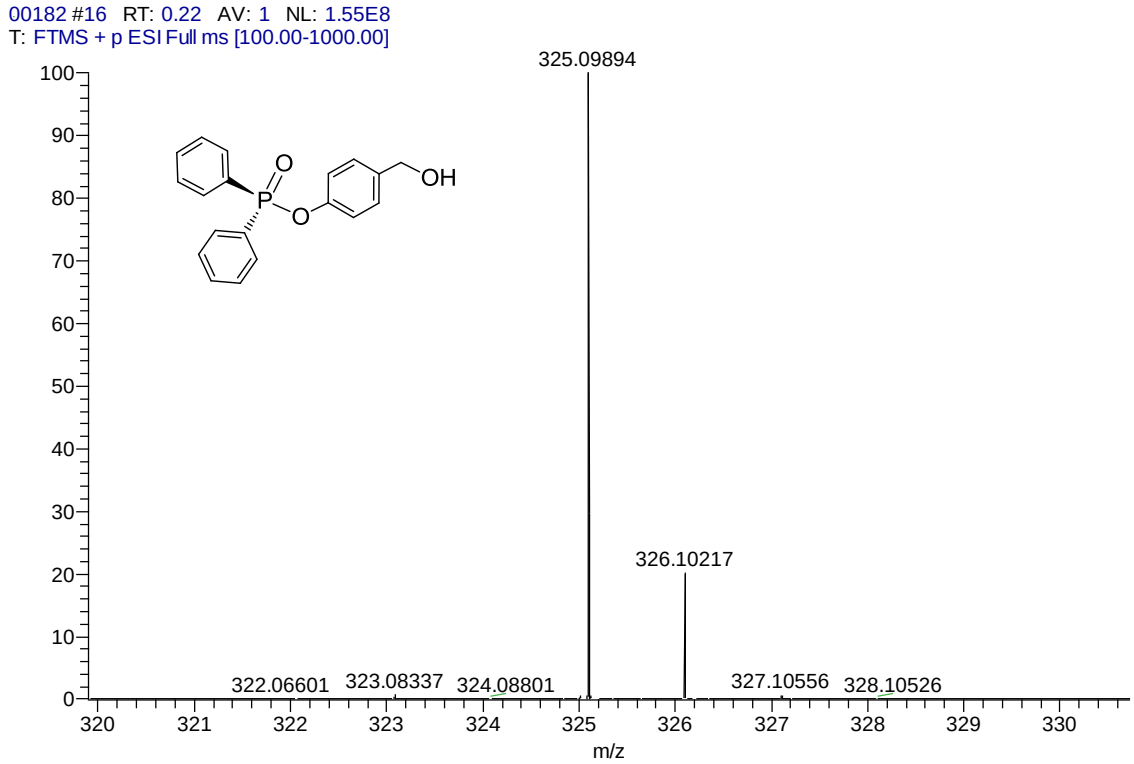
DPBA, 3a ^{1}H NMR (400 MHz, $CDCl_3$)



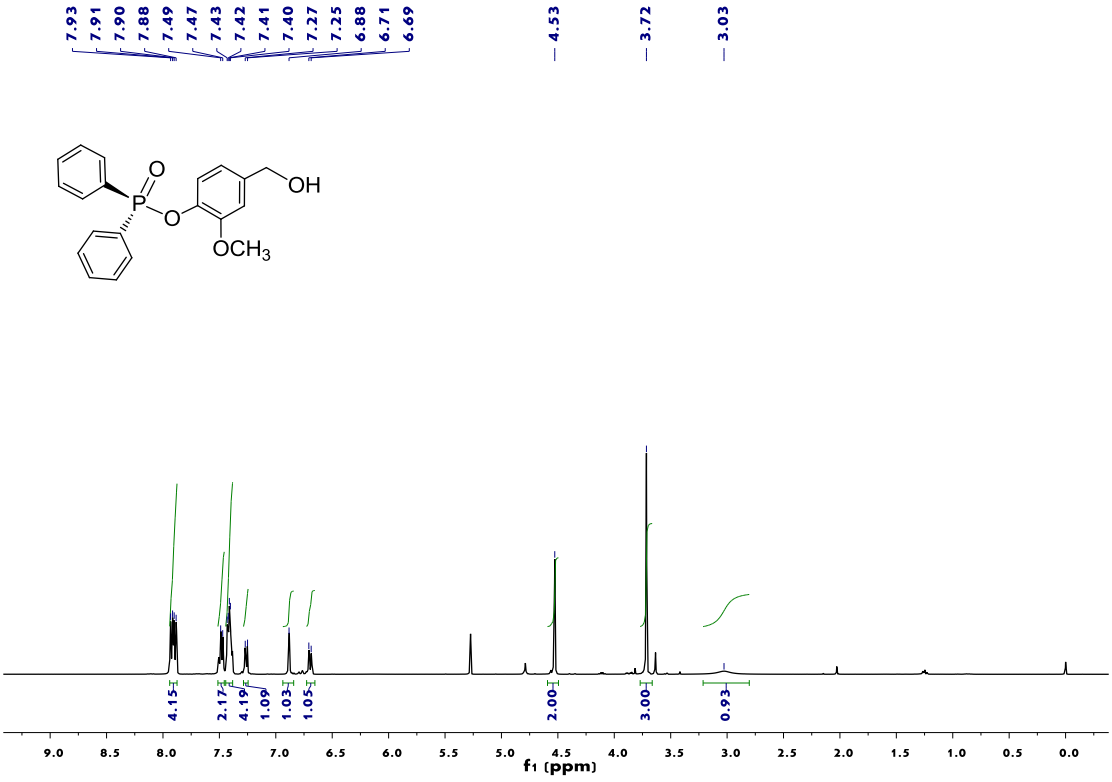
DPBA, 3a ^{13}C NMR (100 MHz, $CDCl_3$)



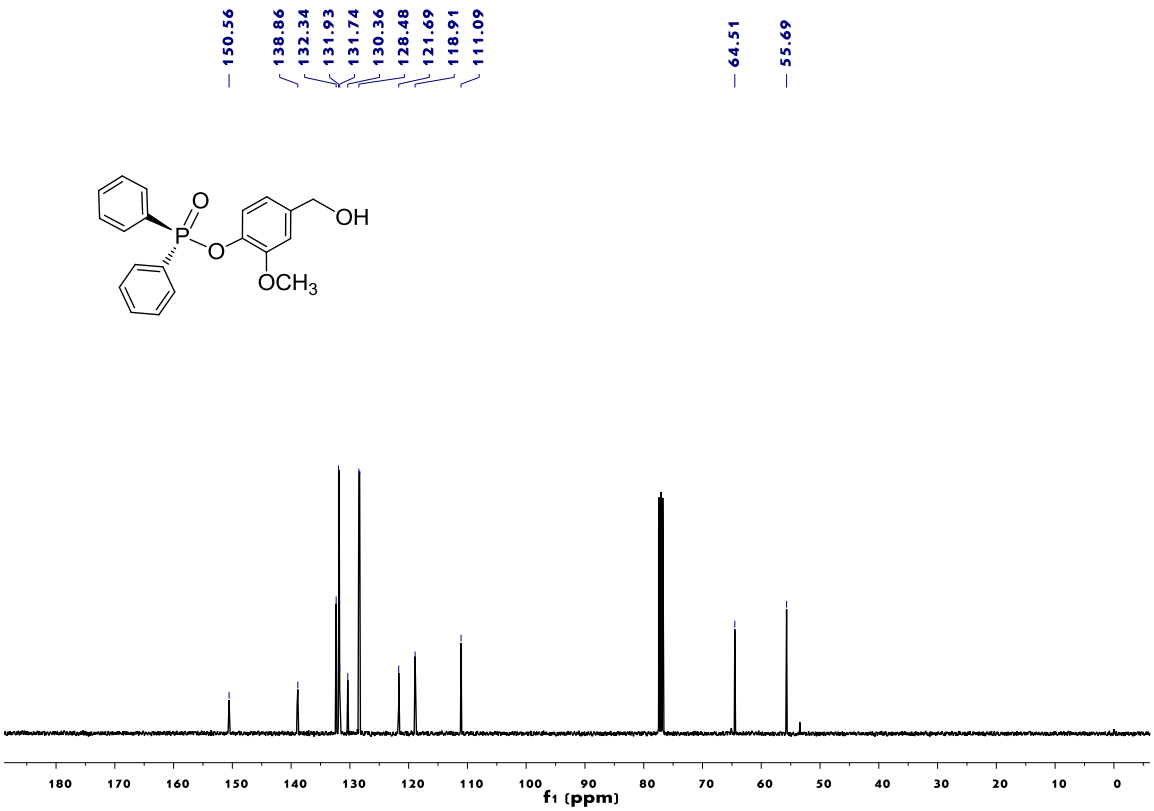
DPBA, 3a ^{31}P NMR (160 MHz, $CDCl_3$)



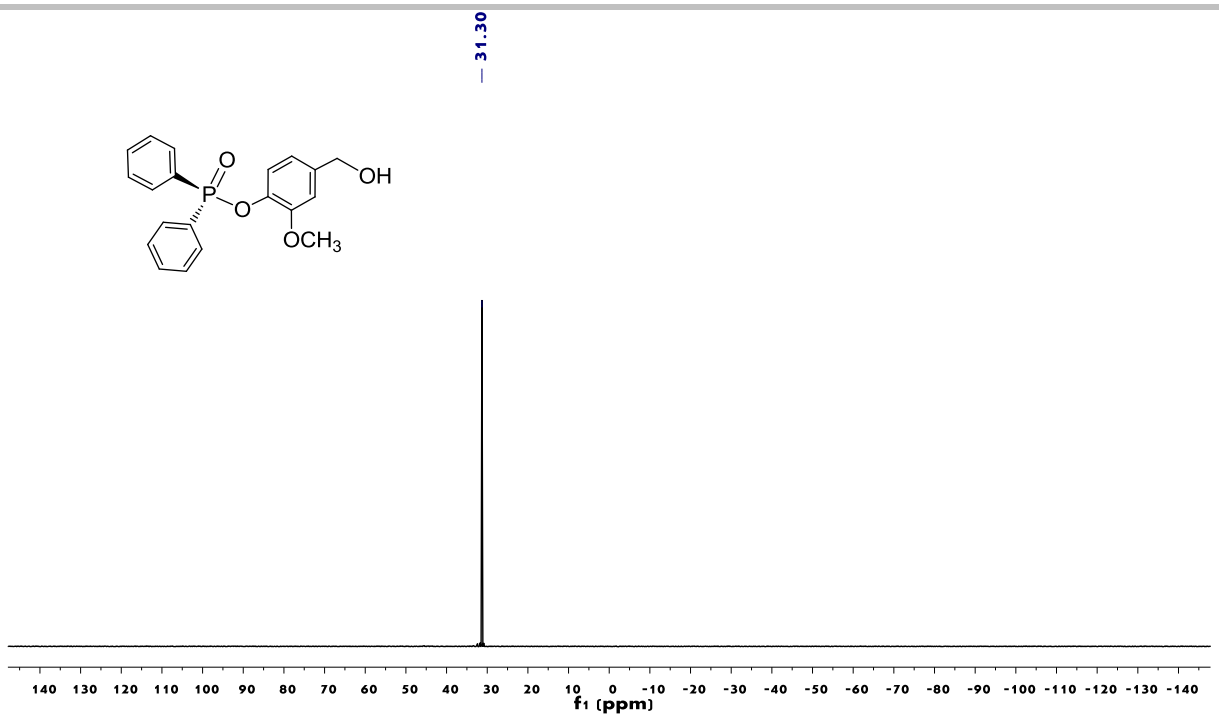
DPBA, 3a HRMS (ESI) m/z calcd for $C_{19}H_{18}O_3P^+$ $[M+H]^+$ 325.09881, found 325.09894.



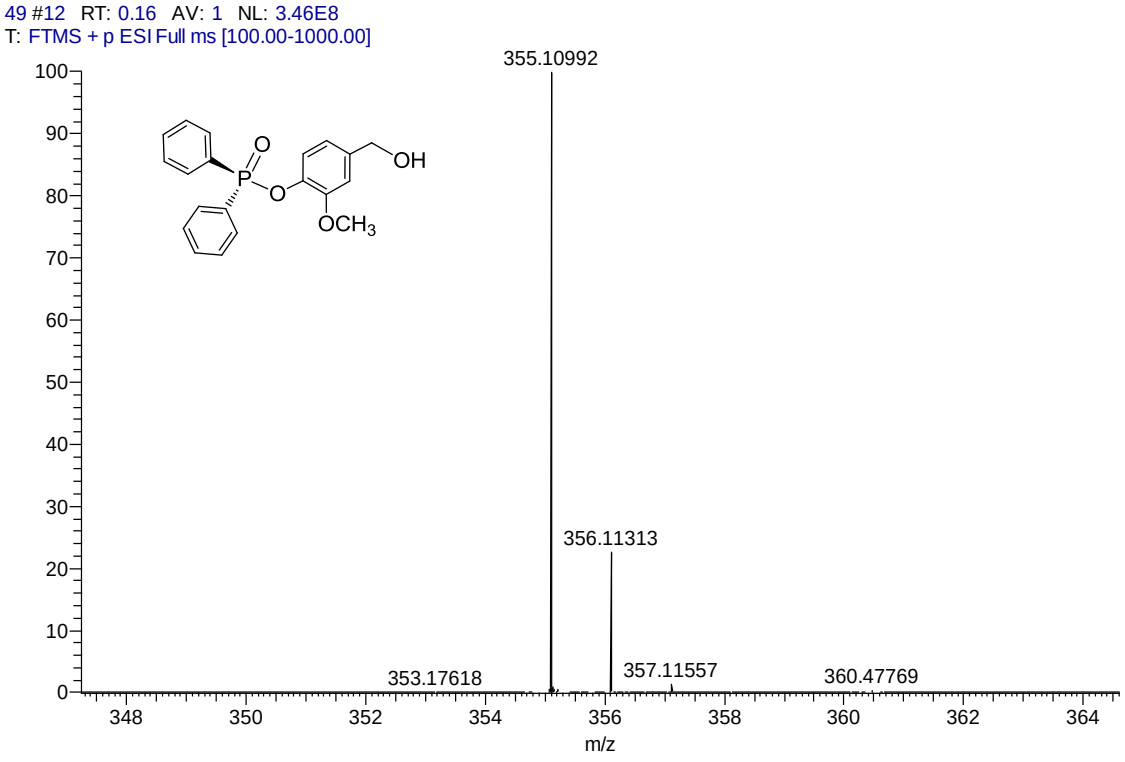
DPBA, 4a ^{1}H NMR (400 MHz, $CDCl_3$)



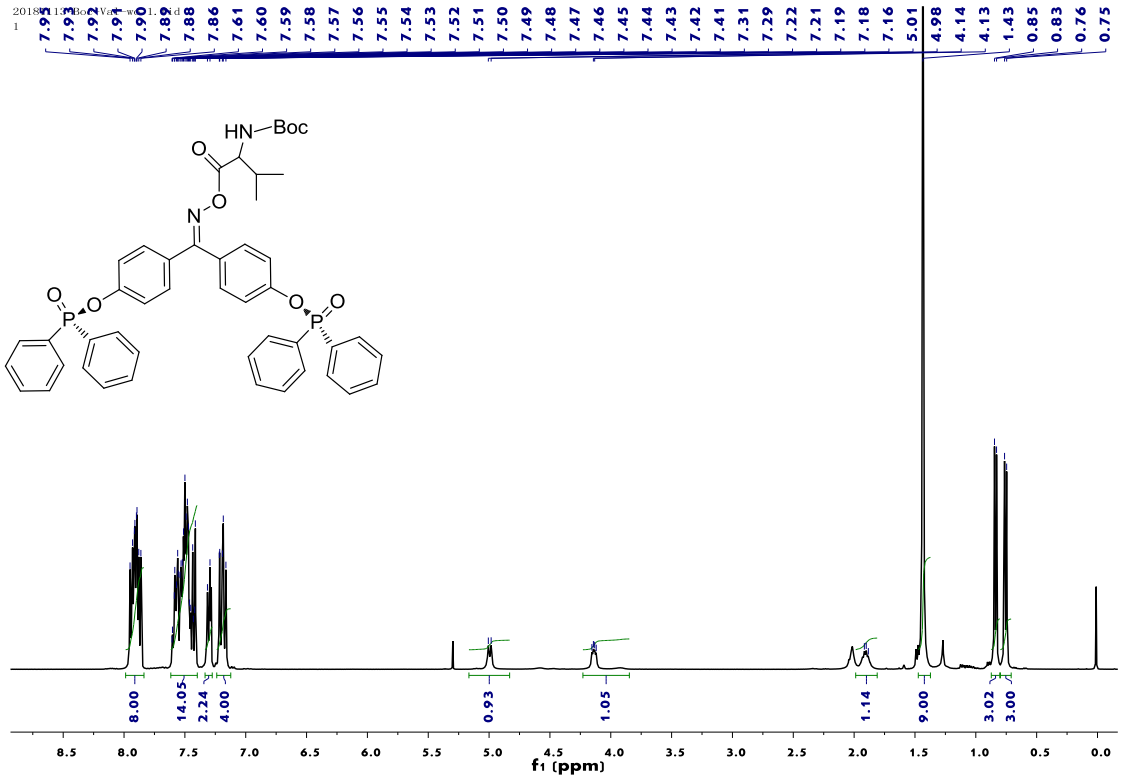
DPBA, 4a ^{13}C NMR (100 MHz, $CDCl_3$)



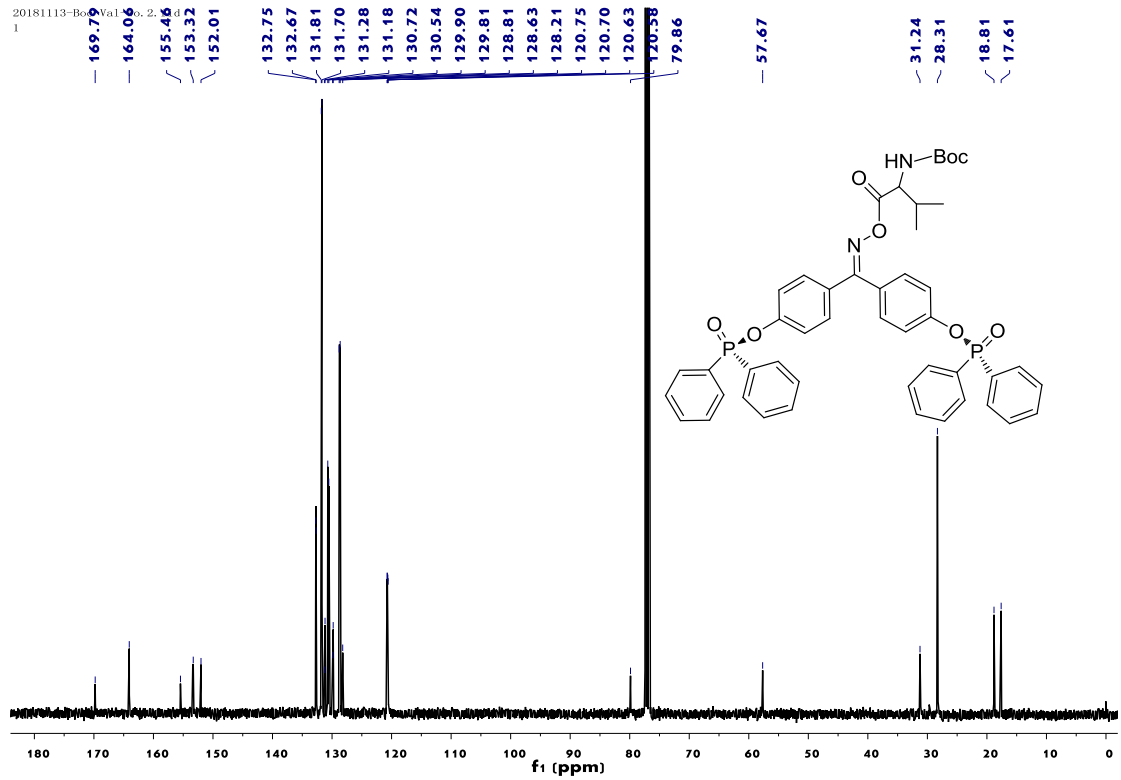
DPBA, 4a ^{13}C NMR (160 MHz, $CDCl_3$)



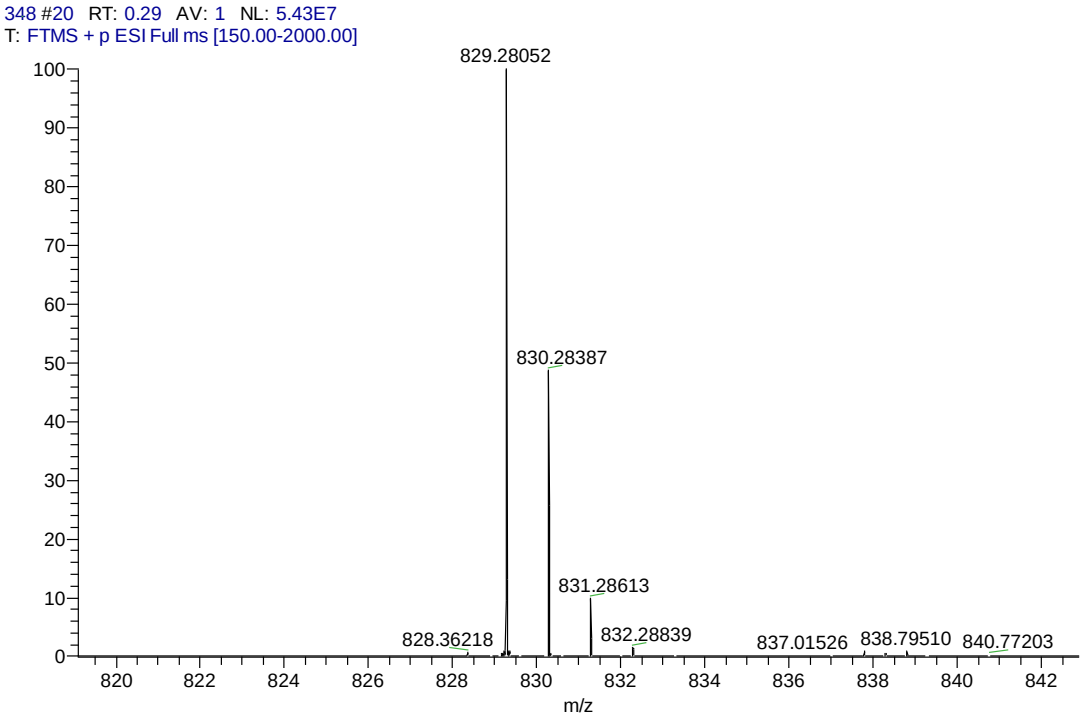
DPBA, 4a HRMS (ESI) m/z calcd for $C_{20}H_{20}O_4P^+$ $[M+H]^+$ 355.10937, found 355.10992.



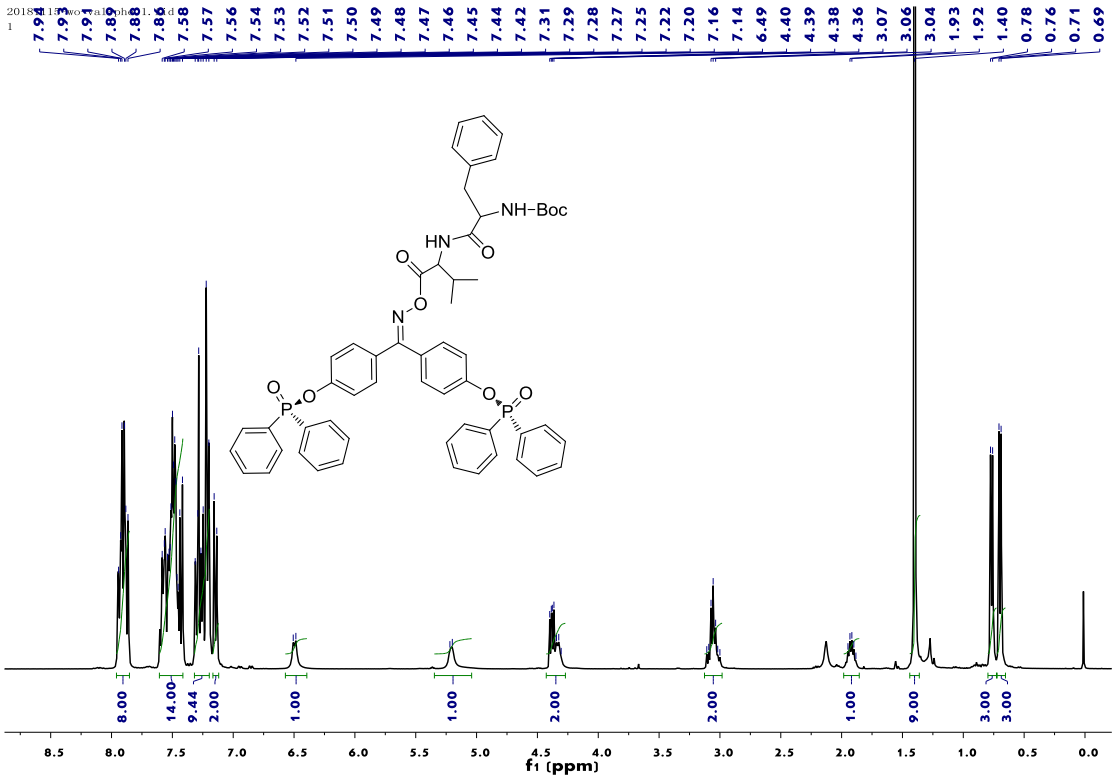
Boc-^{L}Val-O-N=BDKO ^{1}H NMR (400 MHz, $CDCl_3$)



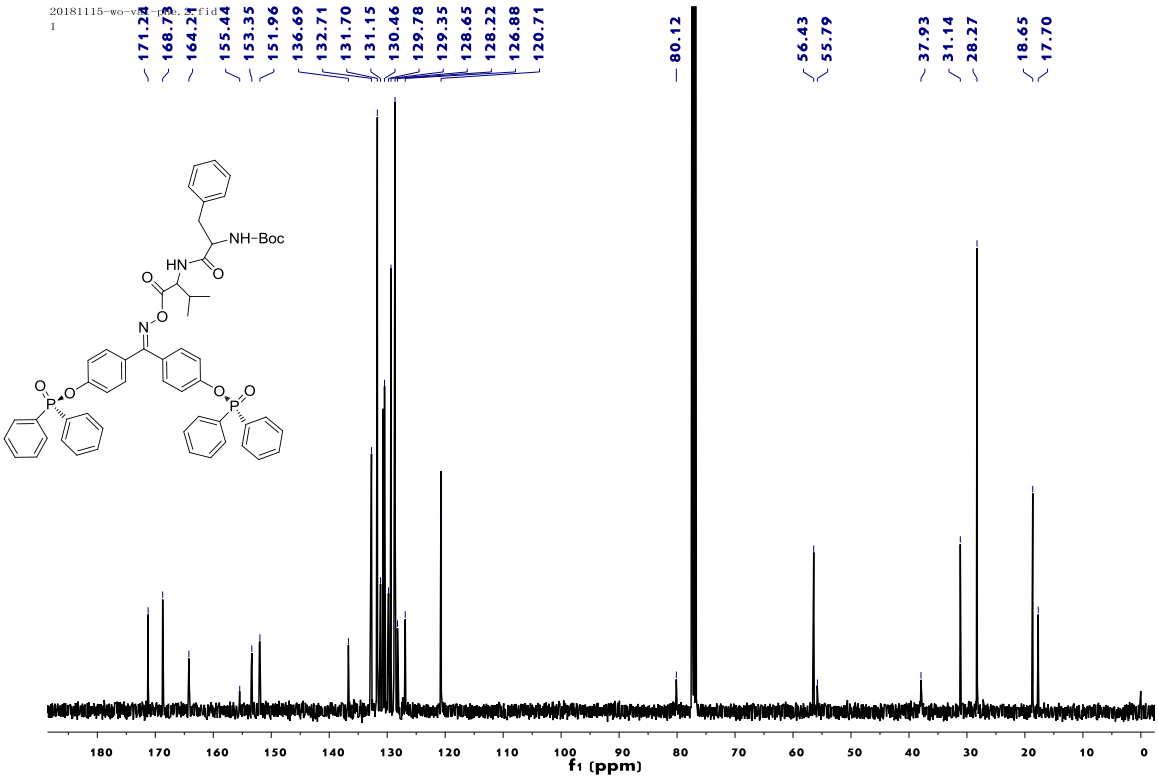
Boc-^{L}Val-O-N=BDKO ^{13}C NMR (100 MHz, $CDCl_3$)



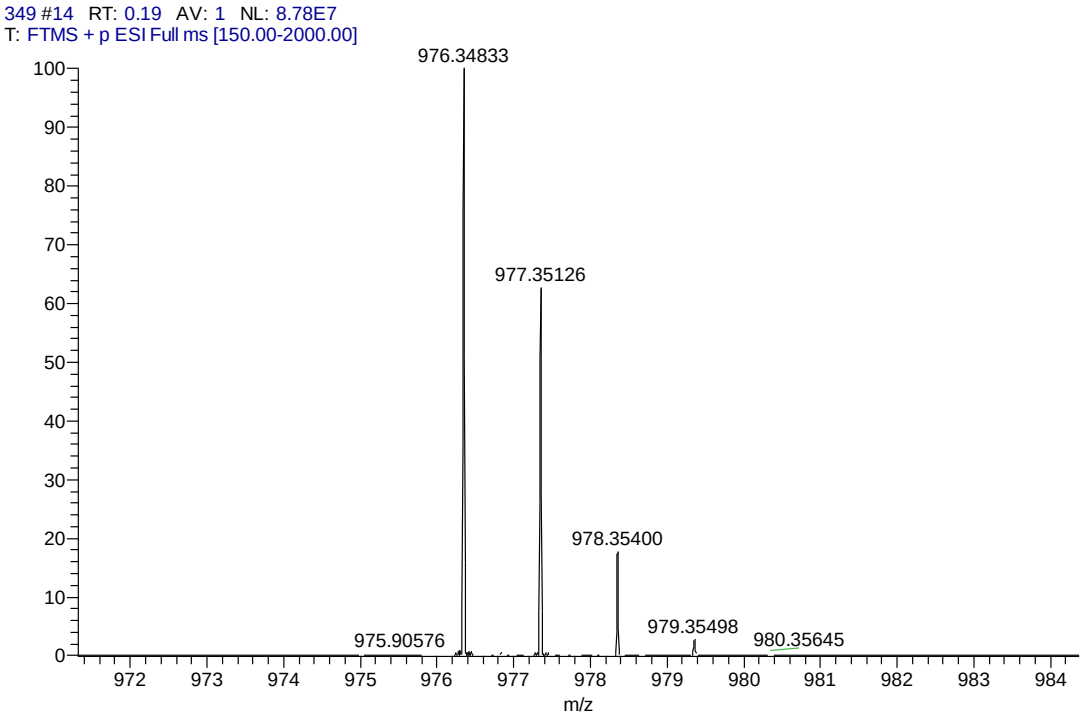
Boc-^{L}Val-O-N=BDKO HRMS (ESI) m/z calcd for $C_{47}H_{47}N_2O_8P_2^+$ $(M+H)^+$ 829.28022, found 829.28052.



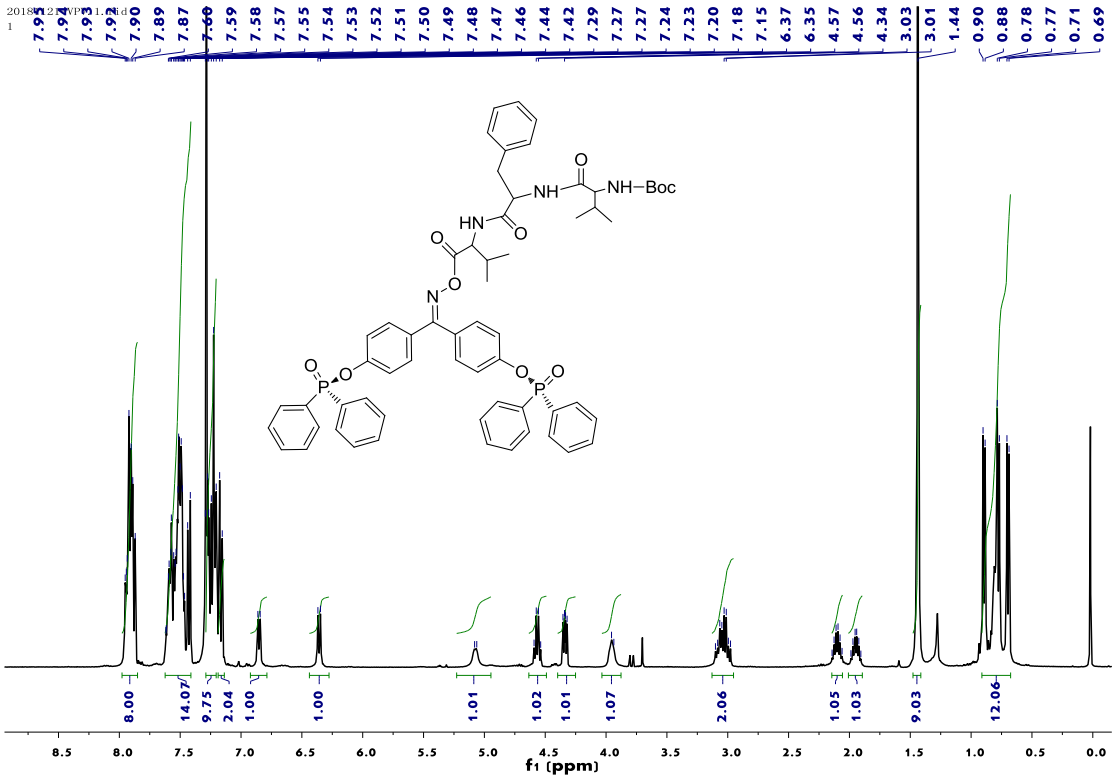
Boc-^{L}Phe-^{L}Val-O-N=BDKO ^{1}H NMR (400 MHz, $CDCl_3$)



Boc-^{L}Phe-^{L}Val-O-N=BDKO ^{13}C NMR (100 MHz, $CDCl_3$)



Boc-^{L}Phe-^{L}Val-O-N=BDKO HRMS (ESI) m/z calcd for $C_{56}H_{56}N_3O_9P_2^+$ $(M+H)^+$ 976.34863, found 976.34833.



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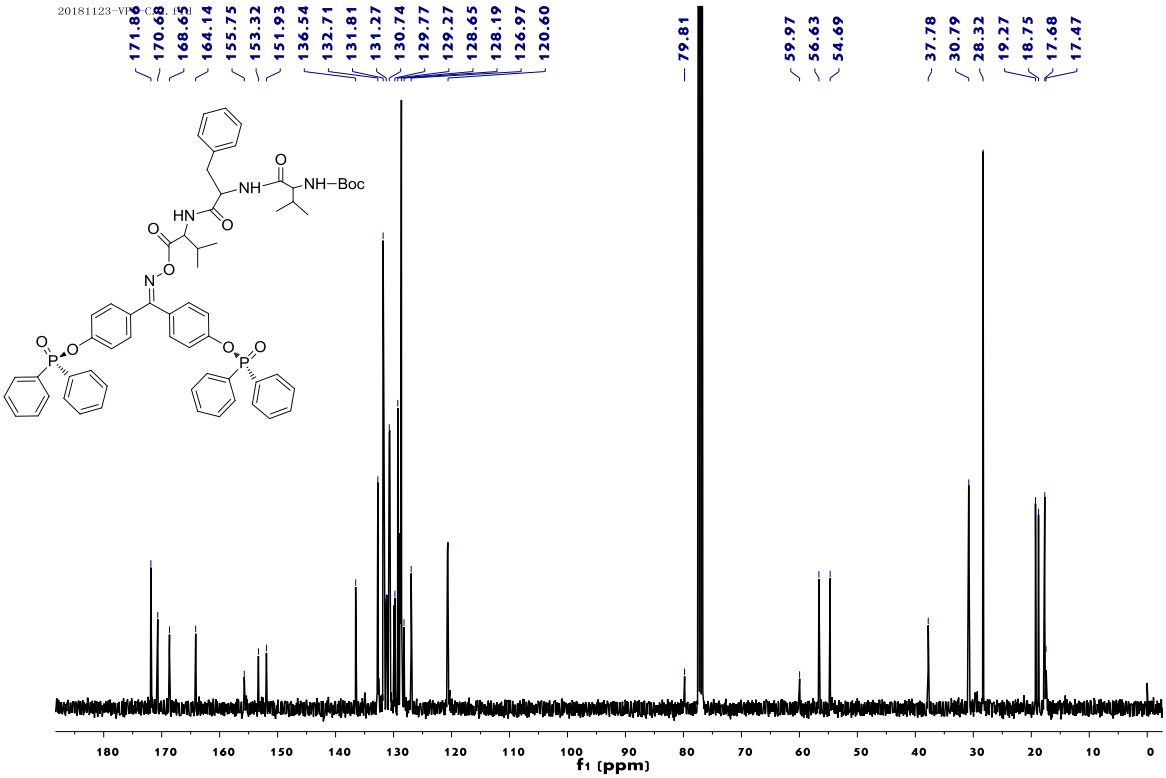
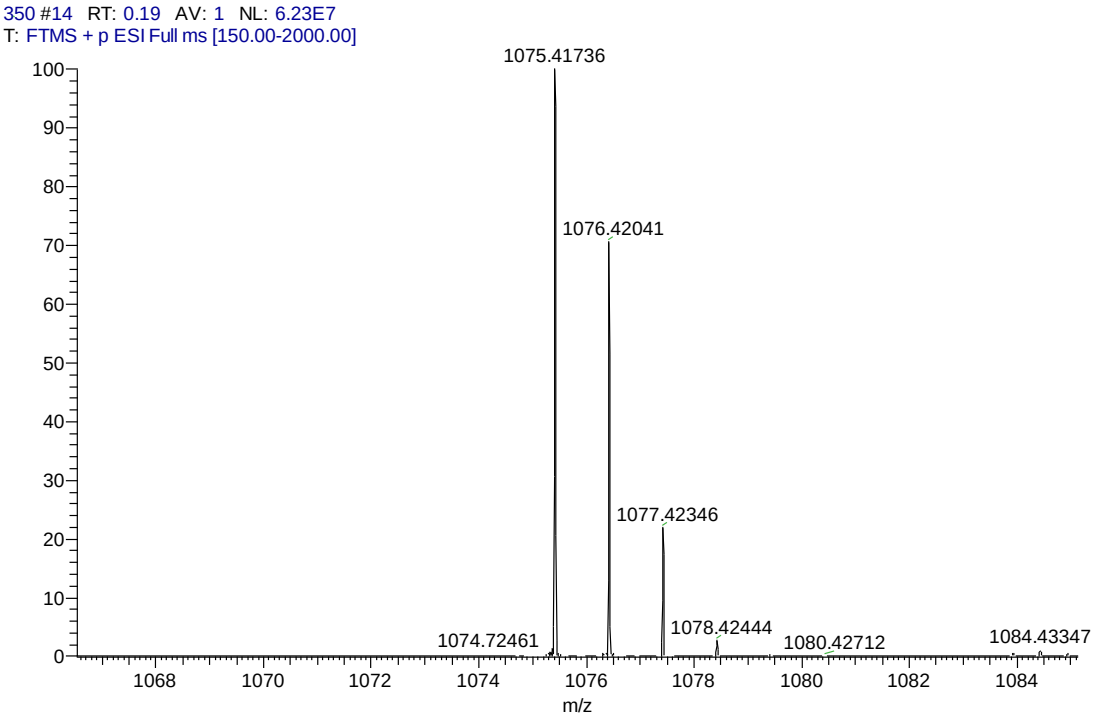
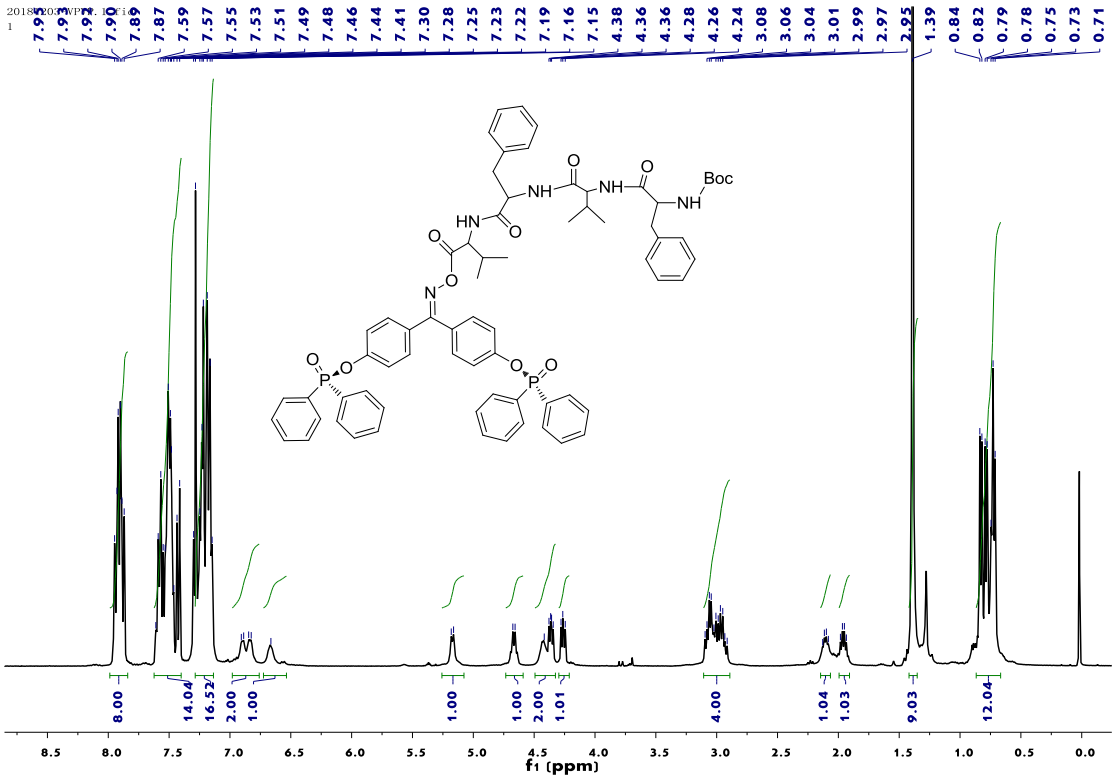


Boc-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO ^{13}C NMR (100 MHz, $CDCl_3$)



Boc-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO HRMS (ESI) m/z calcd for $C_{61}H_{65}N_4O_{10}P_2^+$ $(M+H)^+$ 1075.41704, found 1075.41736.



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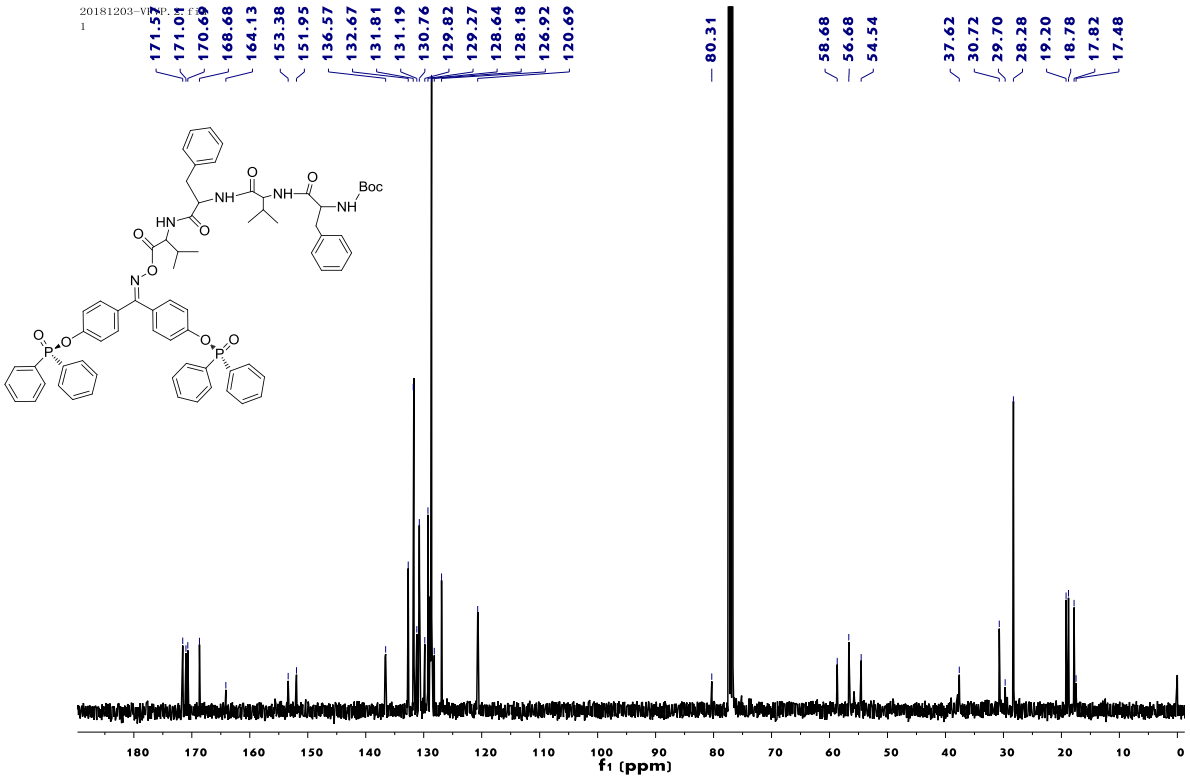

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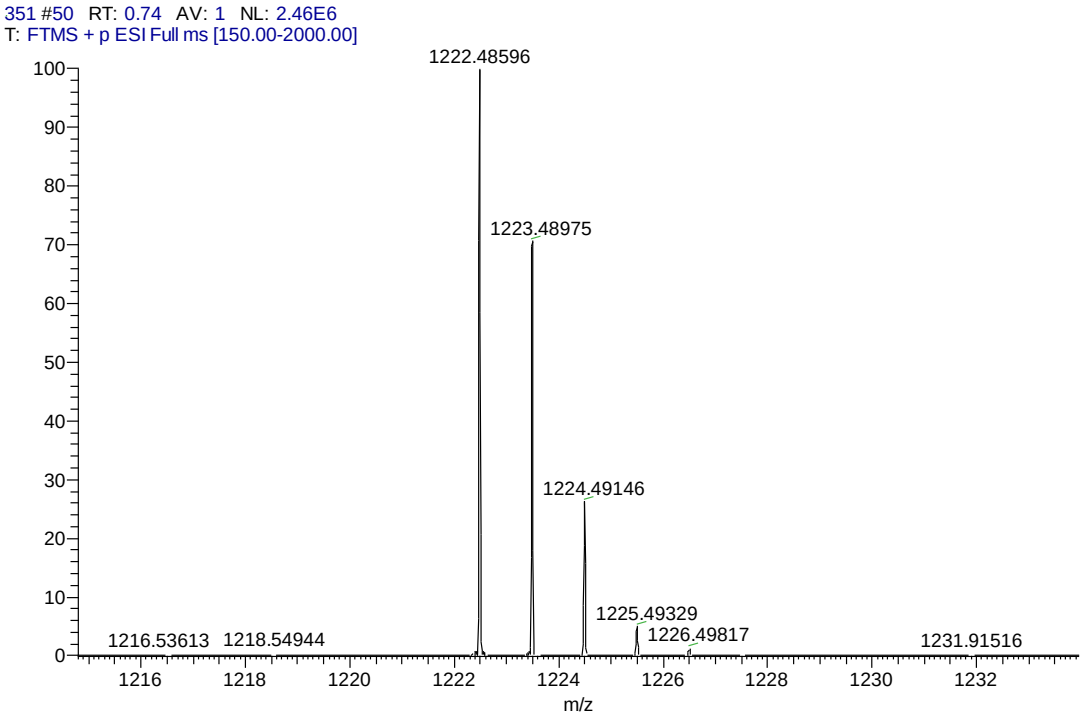

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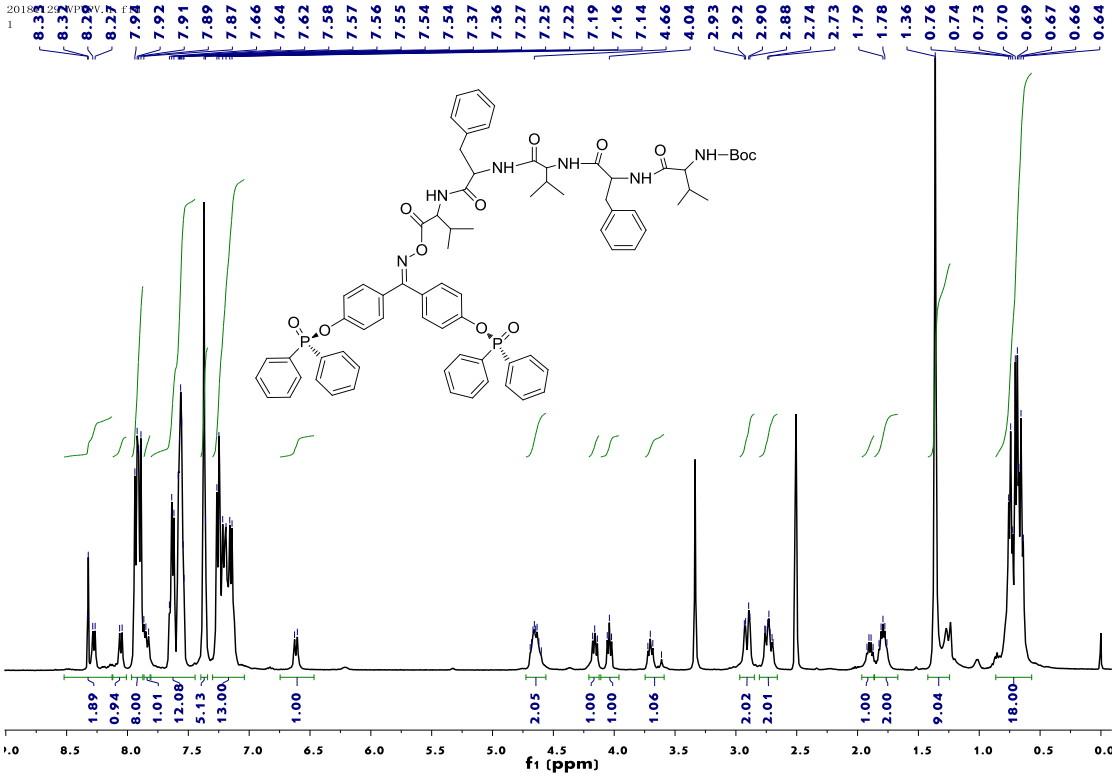

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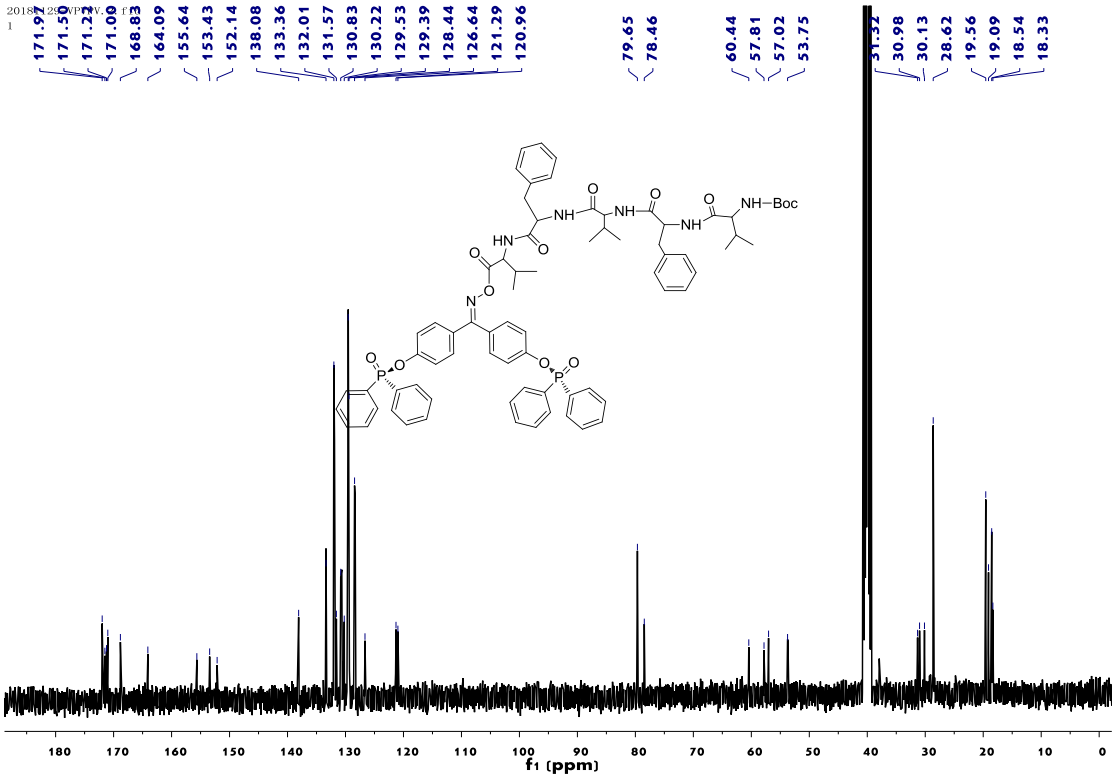
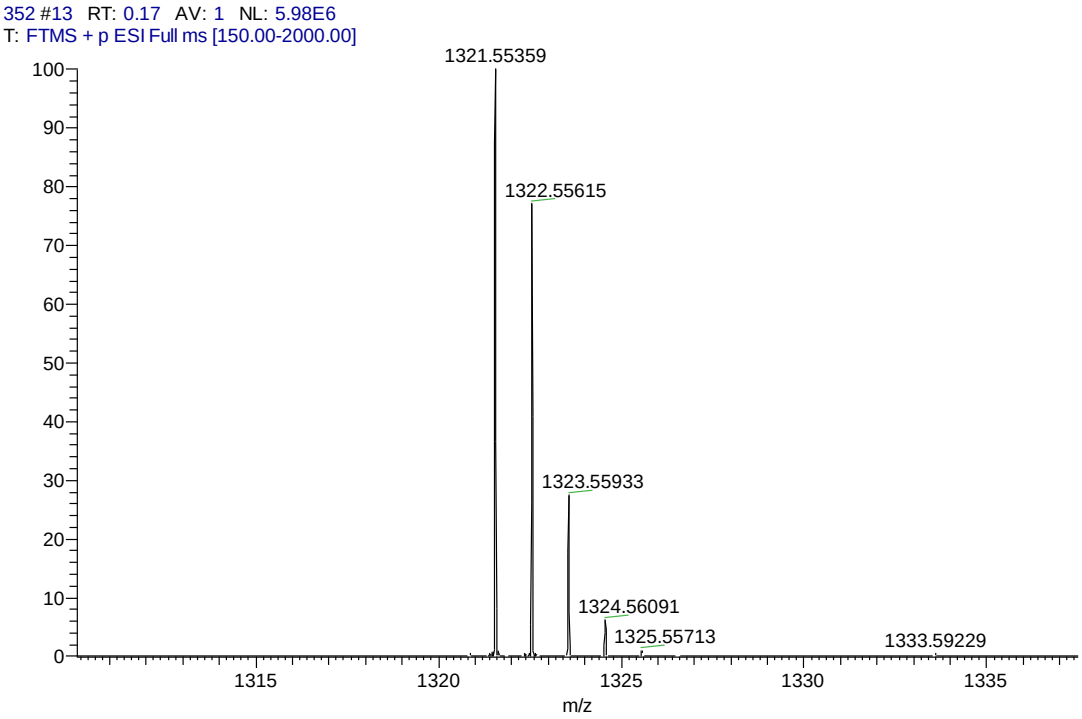
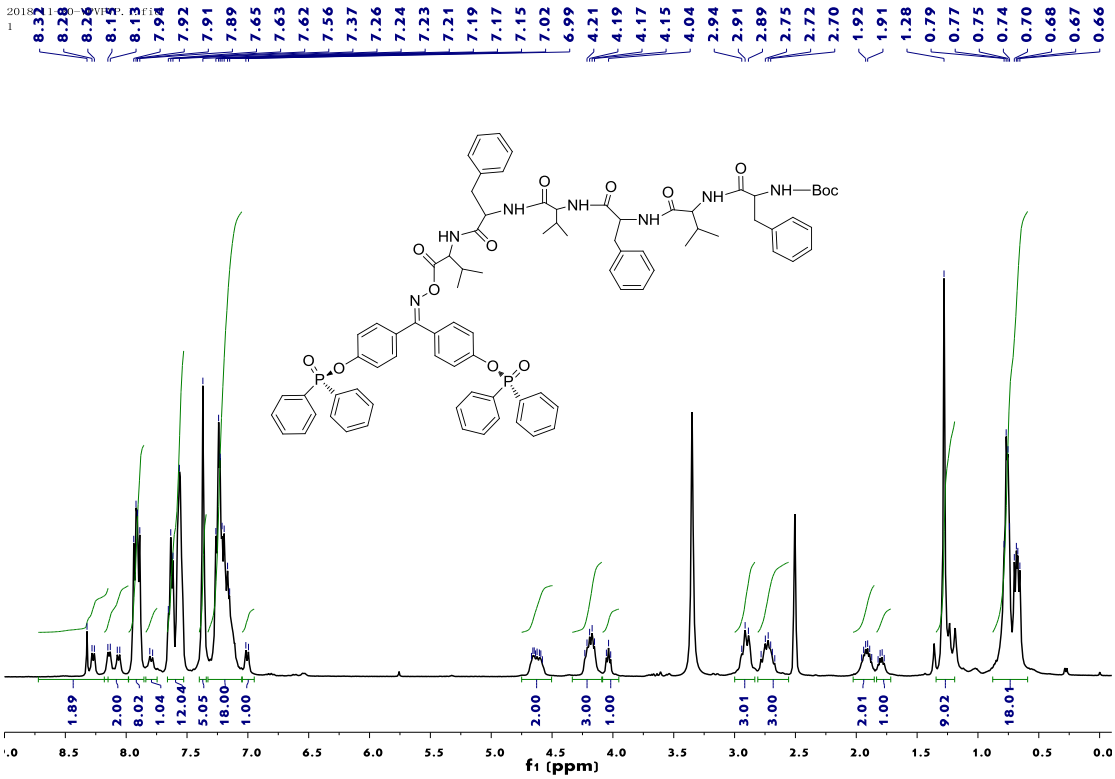


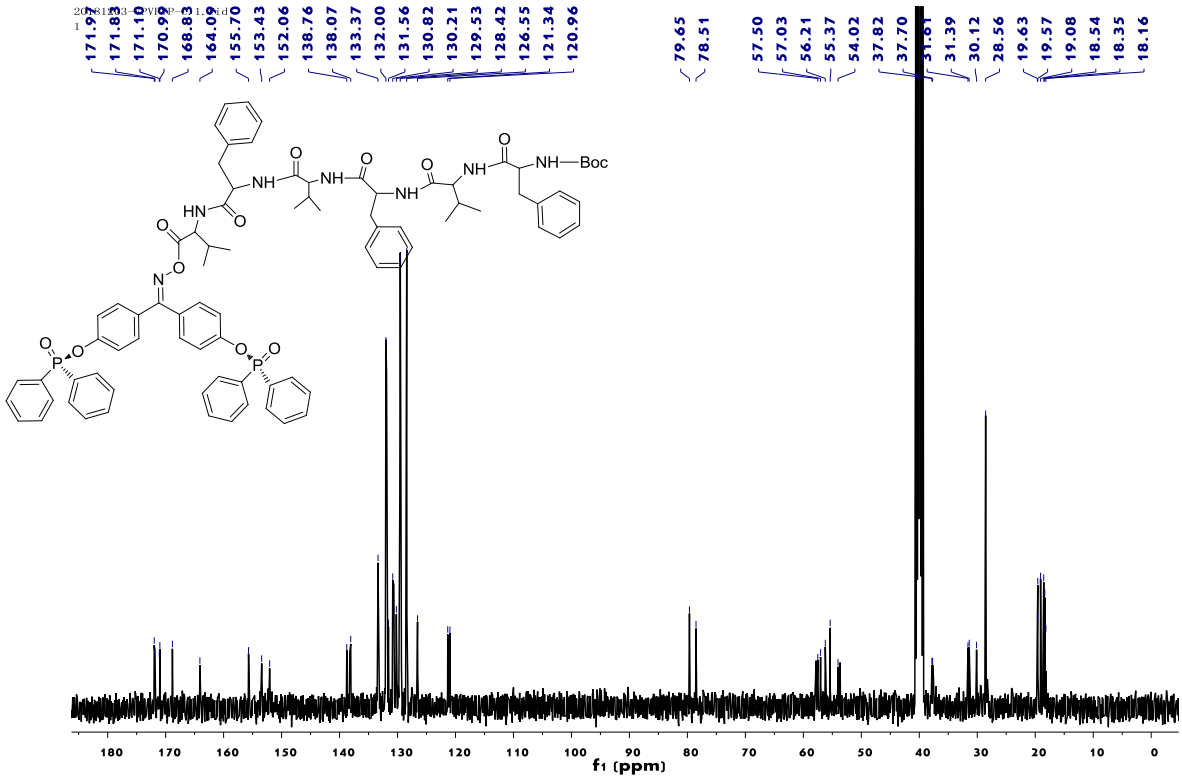
Boc-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO ^{13}C NMR (100 MHz, DMSO-d_6)



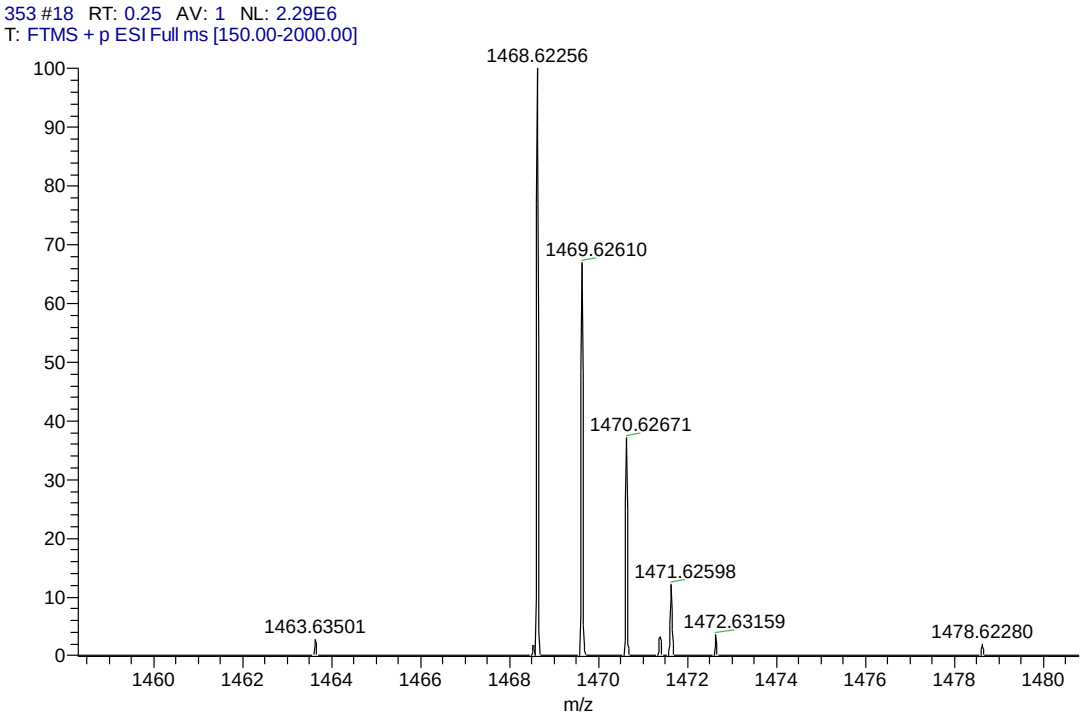
Boc-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO HRMS (ESI) m/z calcd for $C_{75}H_{83}N_6O_{12}P_2^+$ $(M+H)^+$ 1321.55387, found 1321.55359.



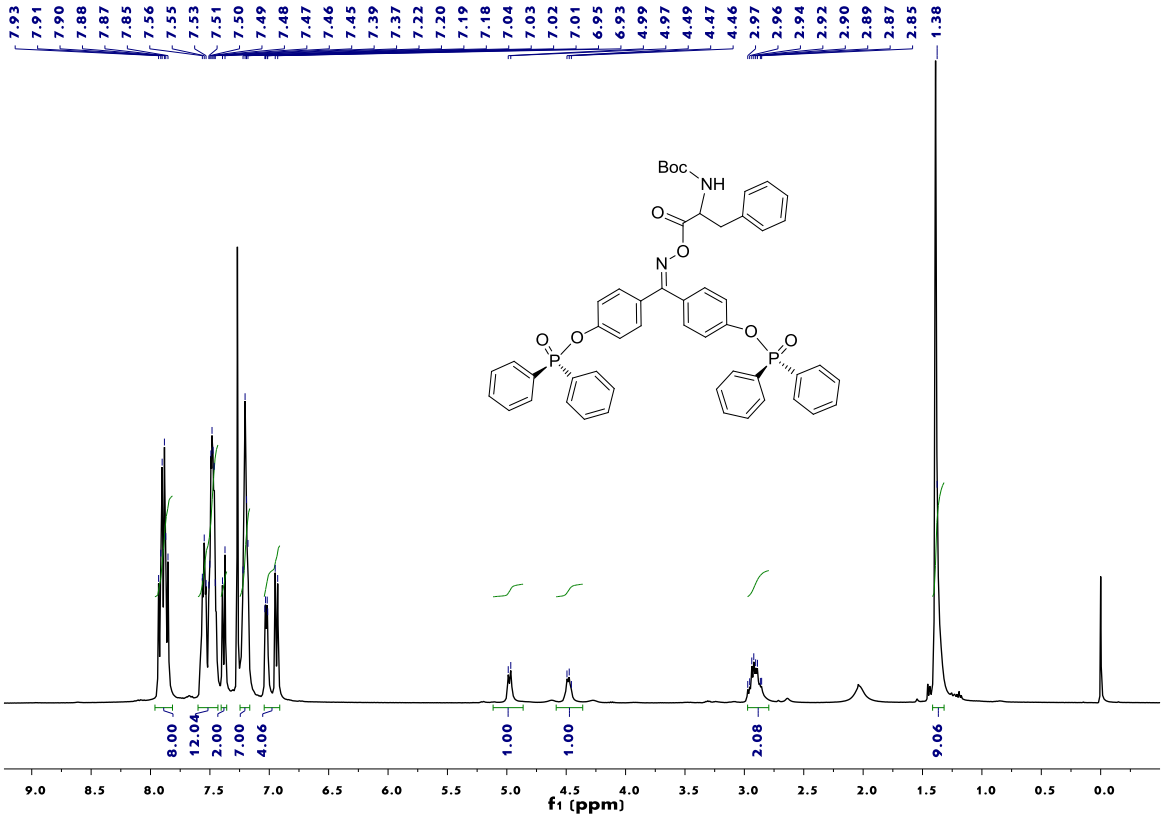
Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO ^{1}H NMR (400 MHz, DMSO-d_6)



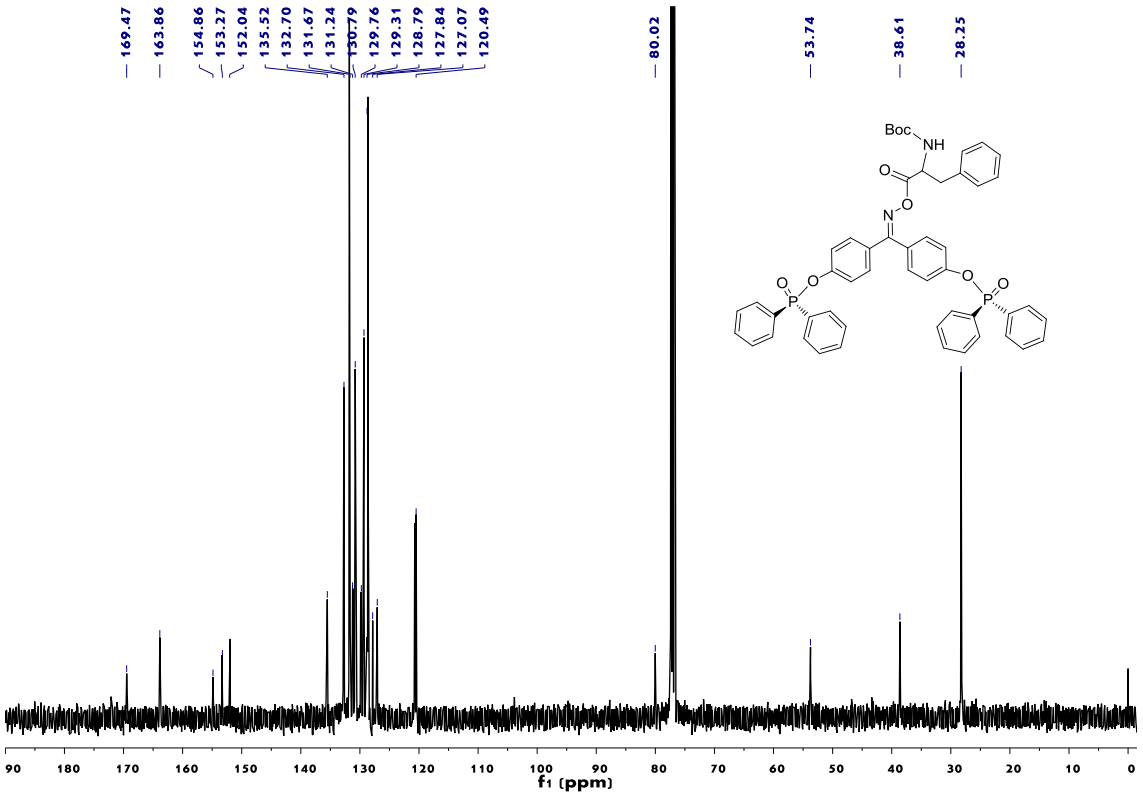
Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO ^{13}C NMR (100 MHz, DMSO-d_6)



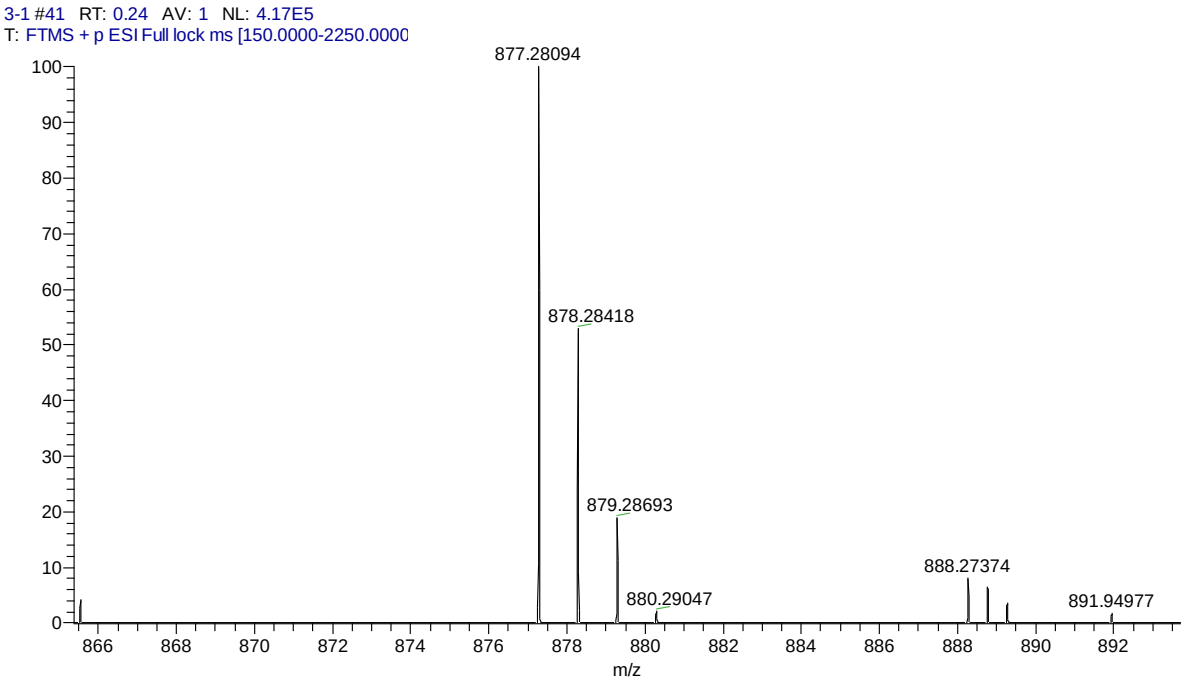
Boc-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-^{L}Phe-^{L}Val-O-N=BDKO HRMS (ESI) m/z calcd for $C_{84}H_{92}N_7O_{13}P_2^+(M+H)^+$ 1468.62229, found 1468.62256.



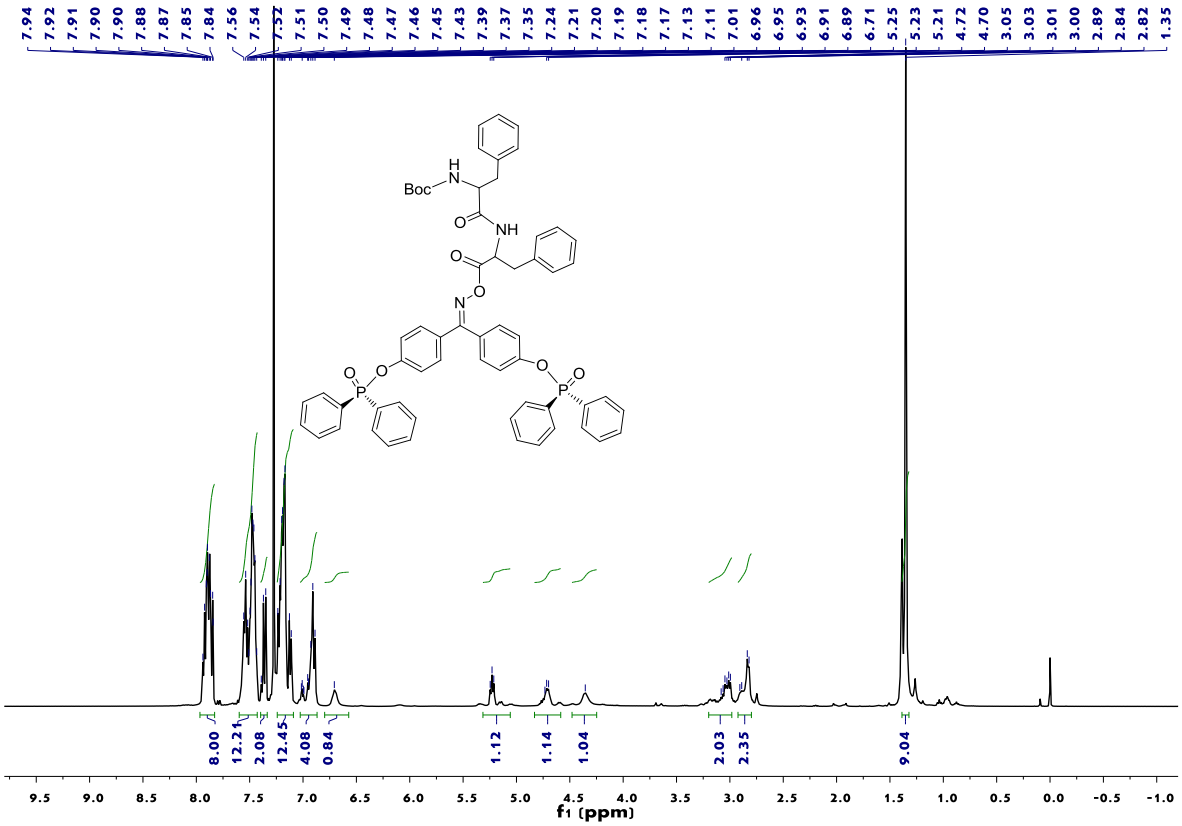
Boc-*D*Phe-O-N=BDKO ^{1}H NMR (400 Hz, $CDCl_3$)



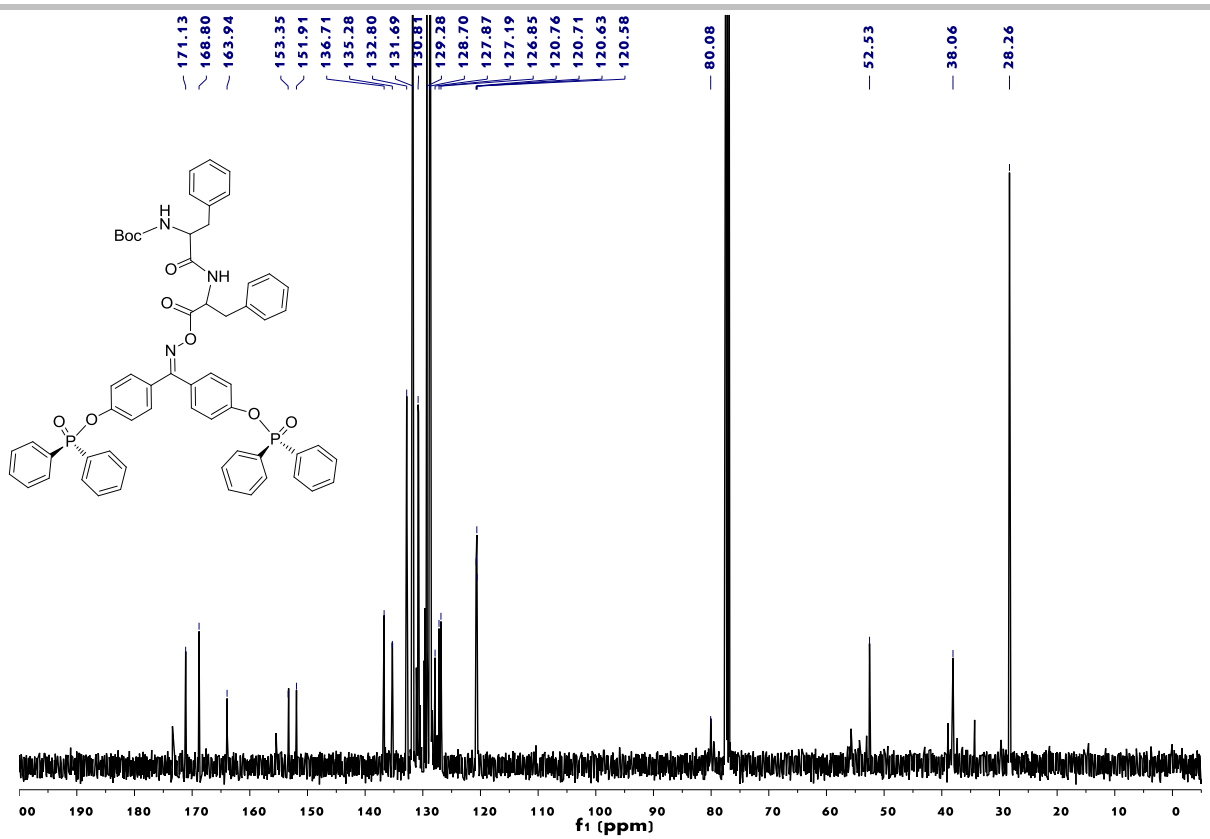
Boc-*D*Phe-O-N=BDKO ^{13}C NMR (100 Hz, $CDCl_3$)



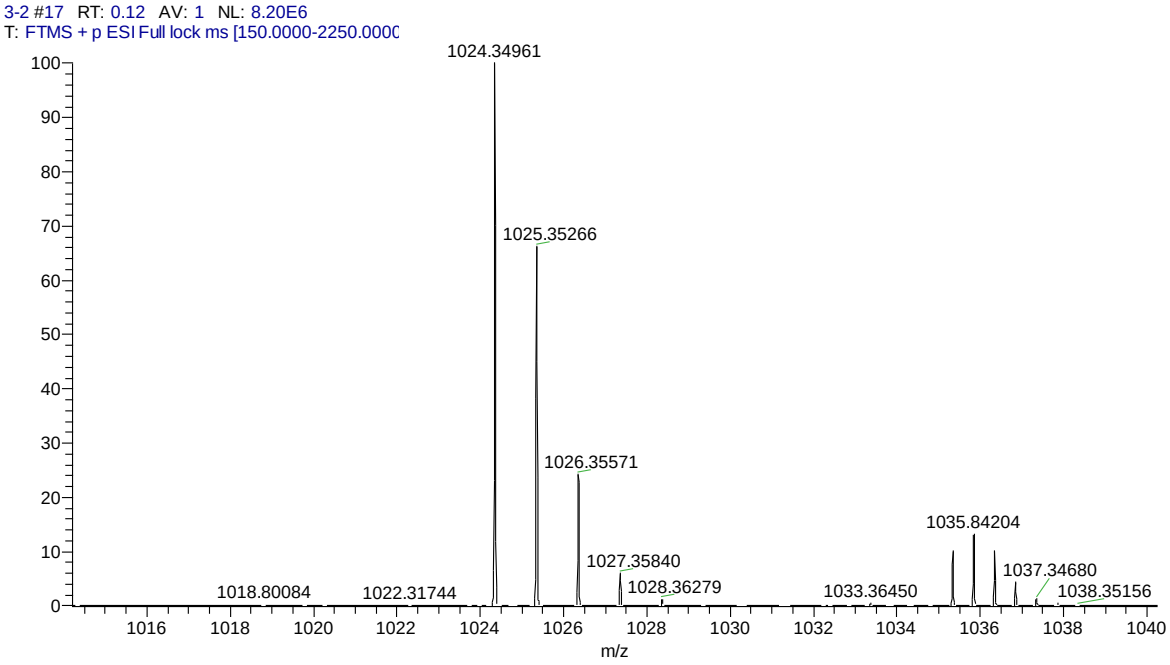
Boc-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{51}H_{47}N_2O_8P_2^+$ $(M+H)^+$ 877.28022, found 877.28094.



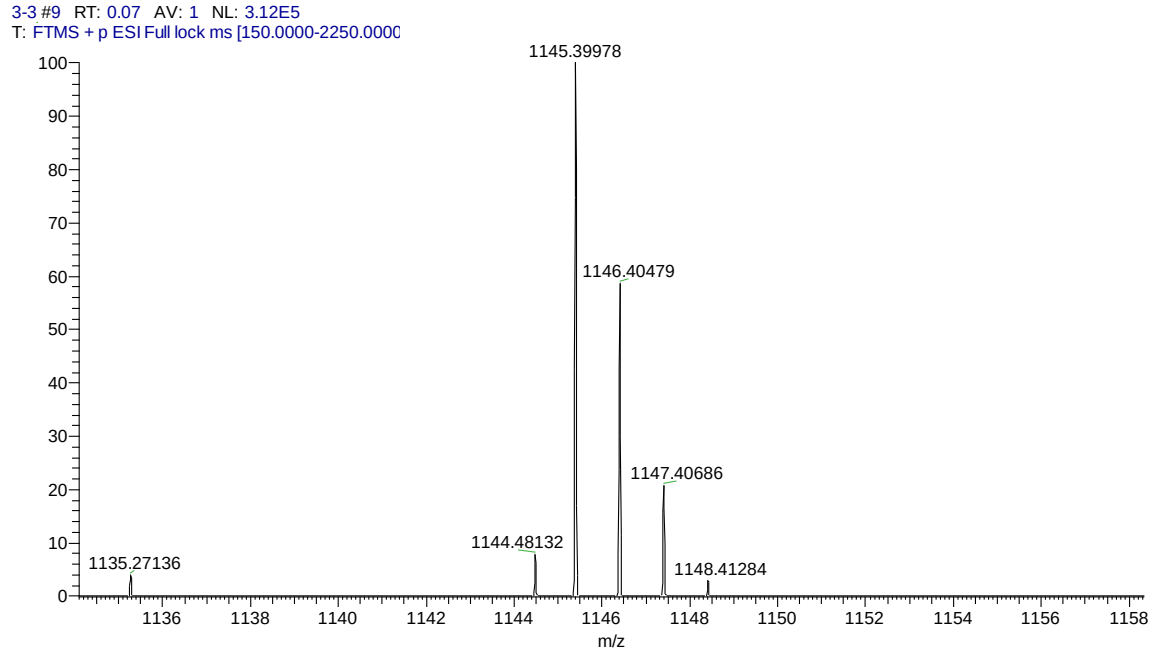
Boc-^{L}Phe-^{D}Phe-O-N=BDKO ^{1}H NMR (400 Hz, $CDCl_3$)



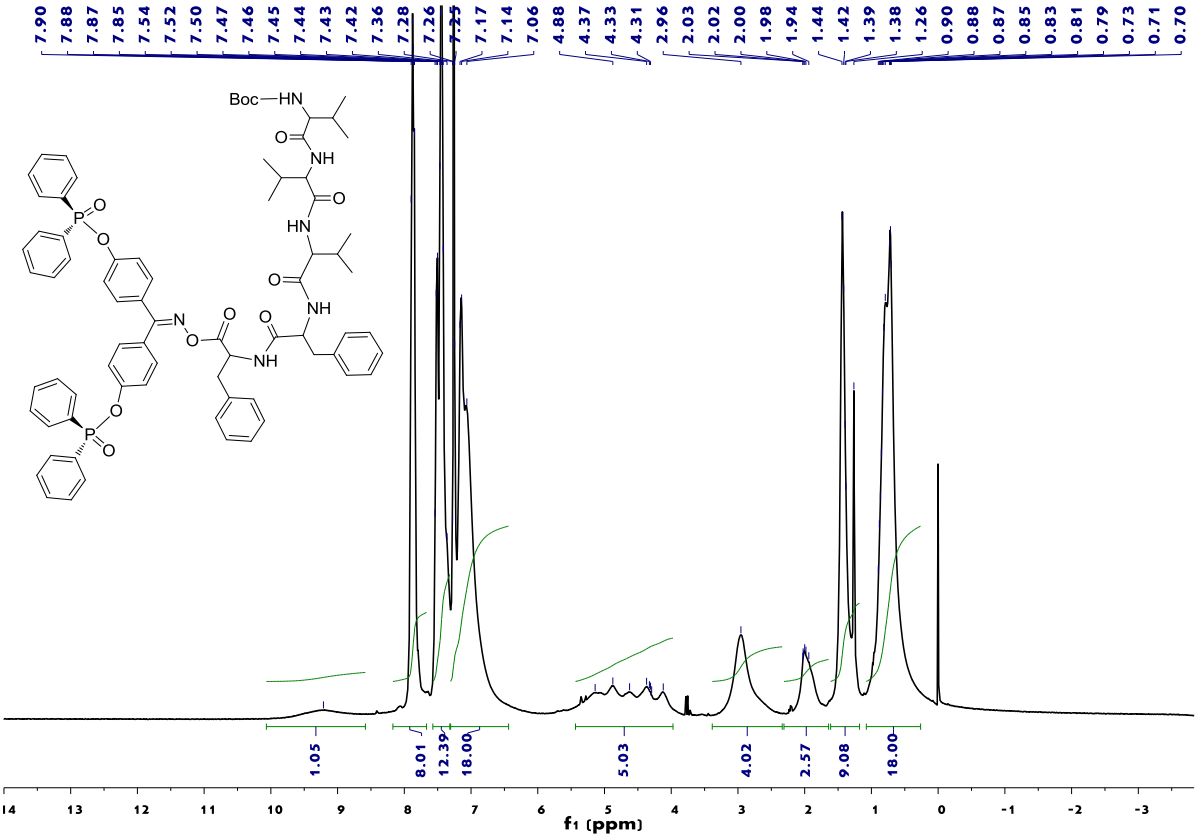
Boc-^{L}Phe-^{D}Phe-O-N=BDKO ^{13}C NMR (100 Hz, $CDCl_3$)



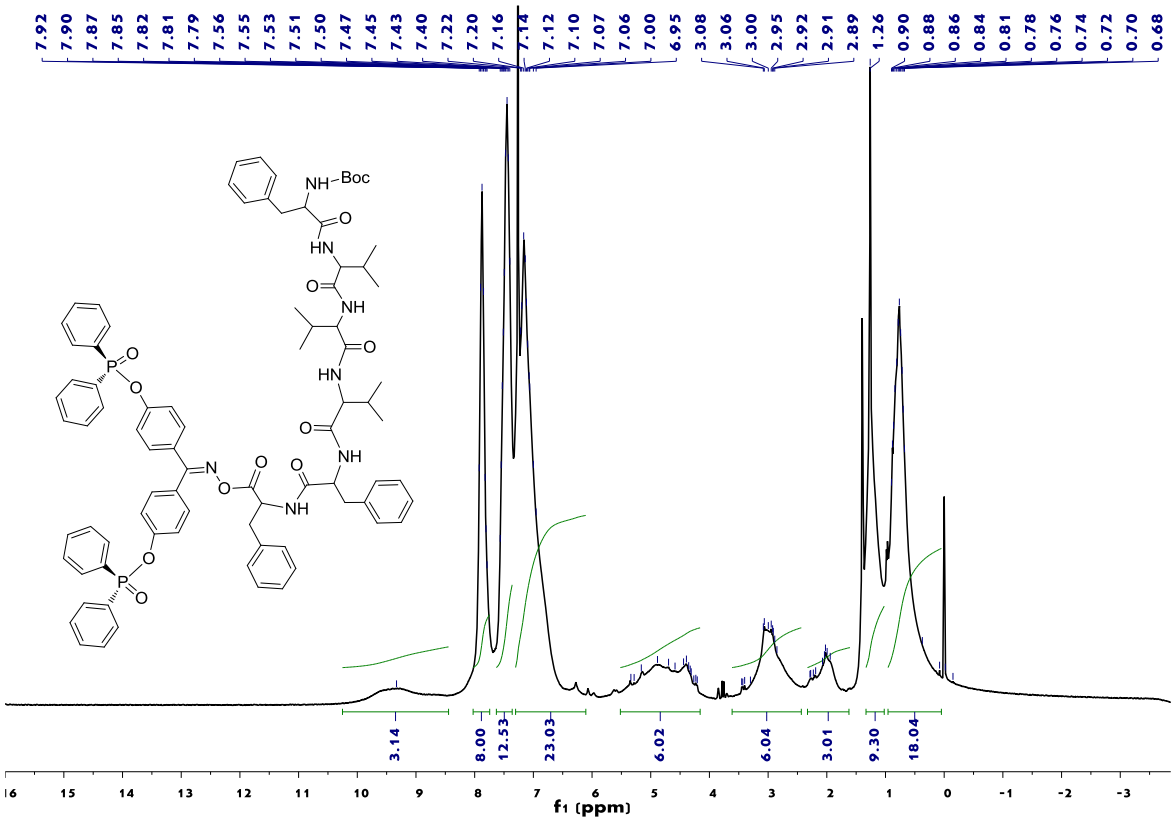
Boc-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{60}H_{56}N_3O_9P_2^+$ $(M+H)^+$ 1024.34863, found 1024.34961.



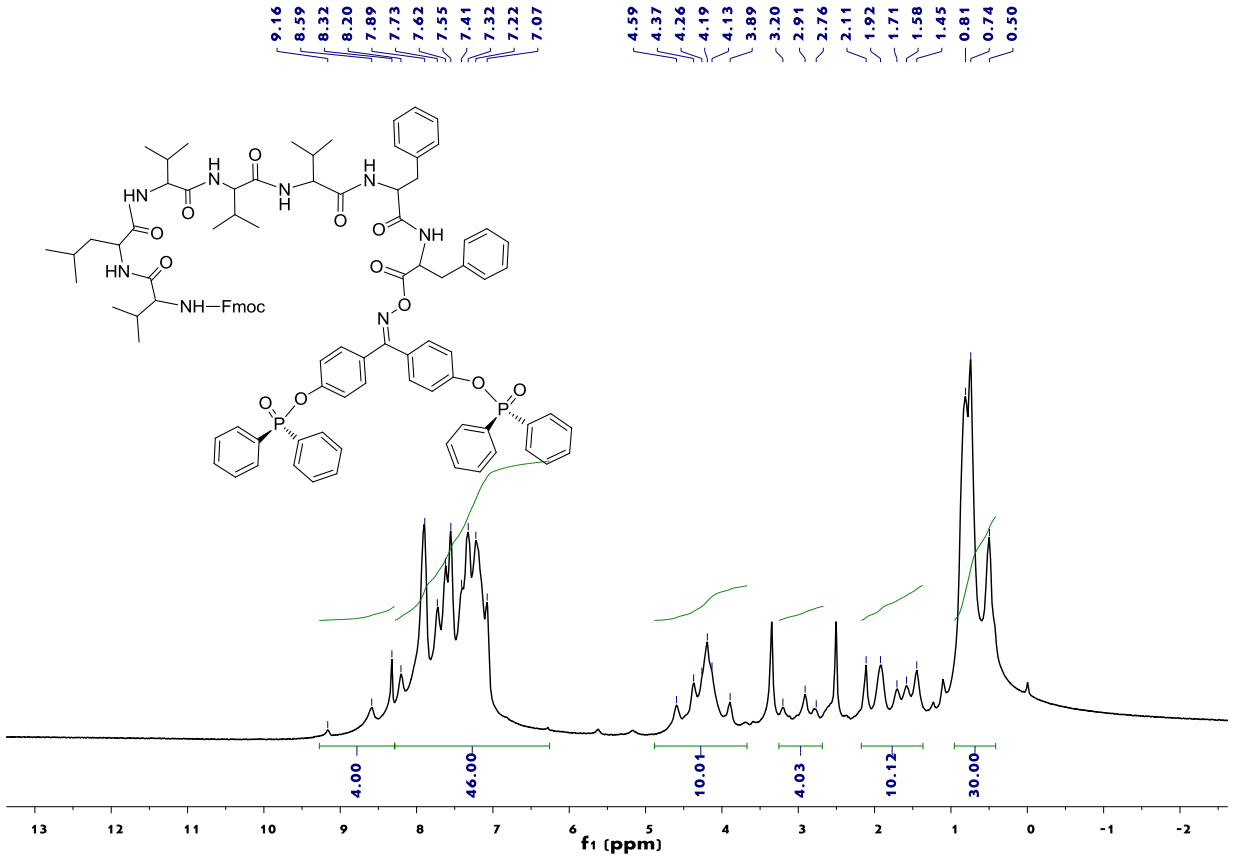
Boc-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{65}H_{64}N_4O_{10}P_2Na^+$ $(M+Na)^+$ 1145.39899, found 1145.39978.



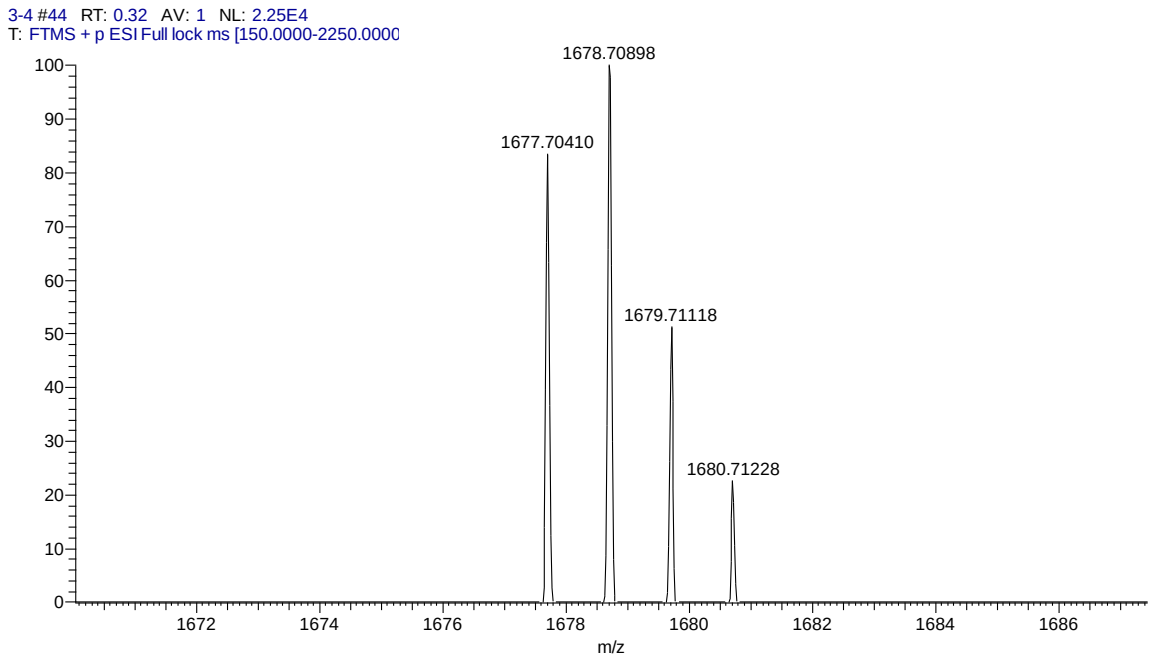
Boc-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO ^{1}H NMR (400 Hz, $CDCl_3$)



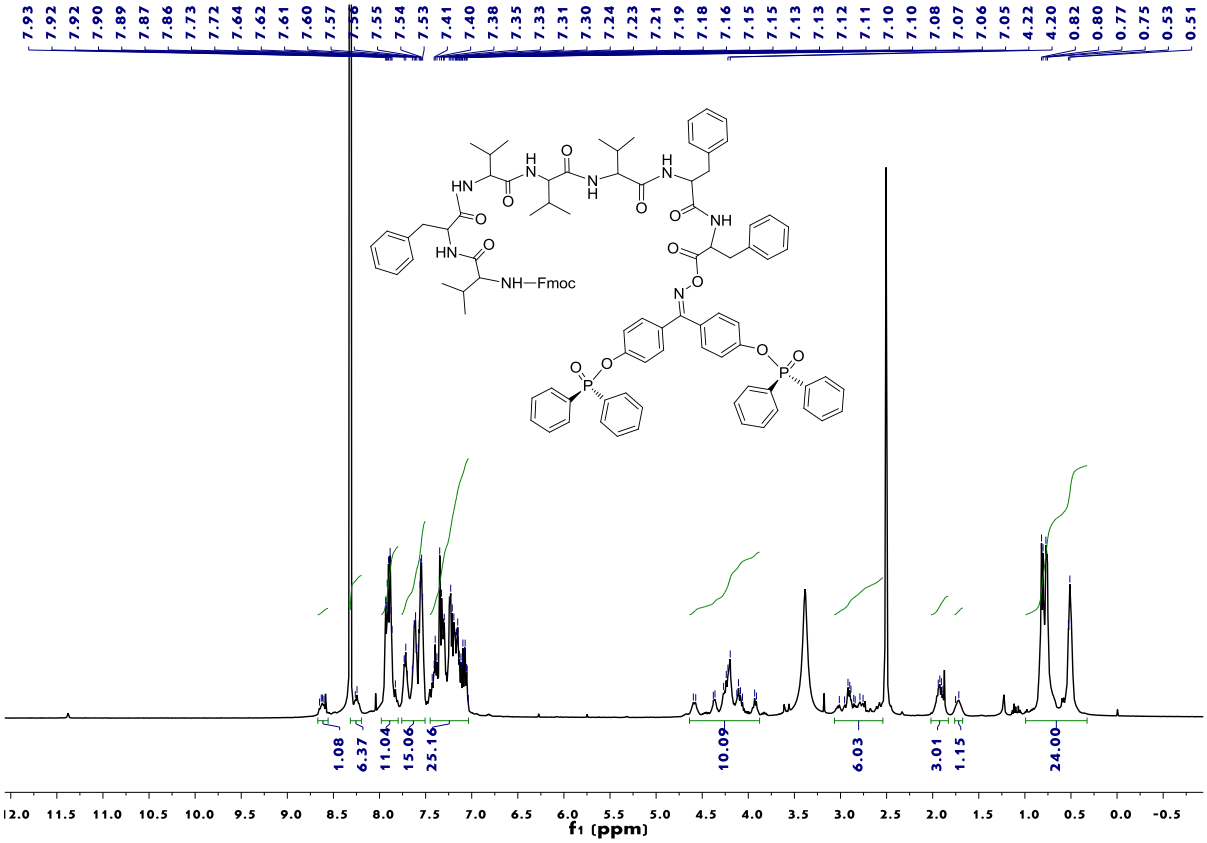
Boc-^{L}Phe-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO ^{1}H NMR (400 Hz, $CDCl_3$)



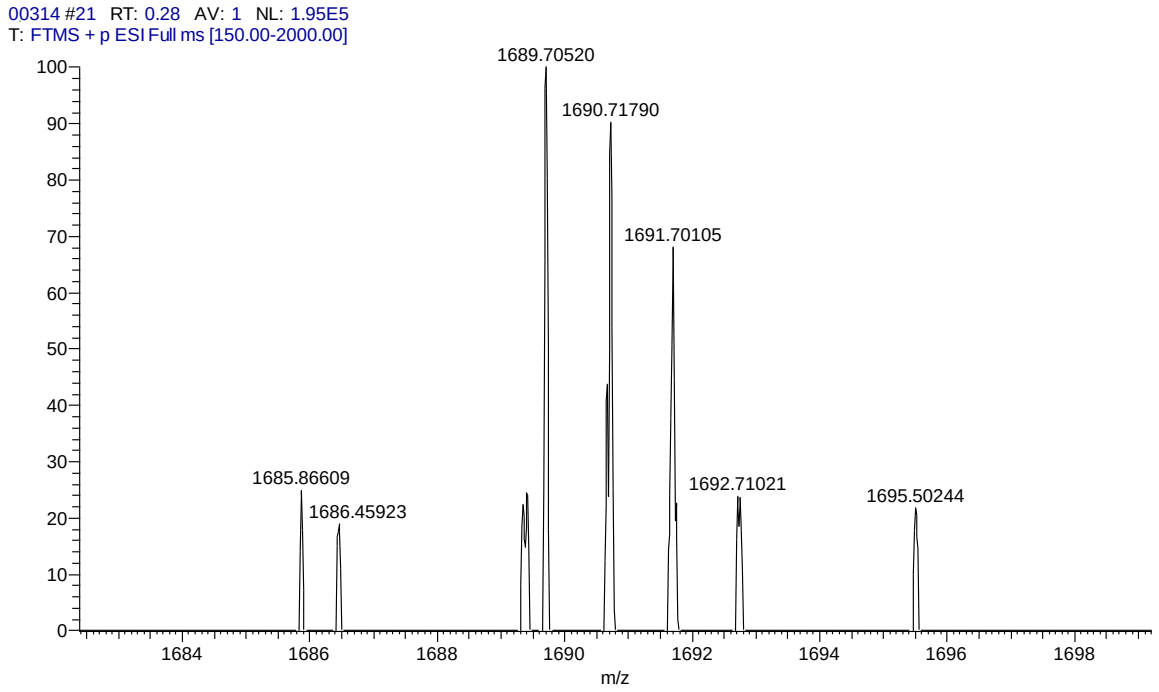
Fmoc-^{D}Val-^{L}Leu-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO ^{1}H NMR (400 Hz, DMSO-d_6)



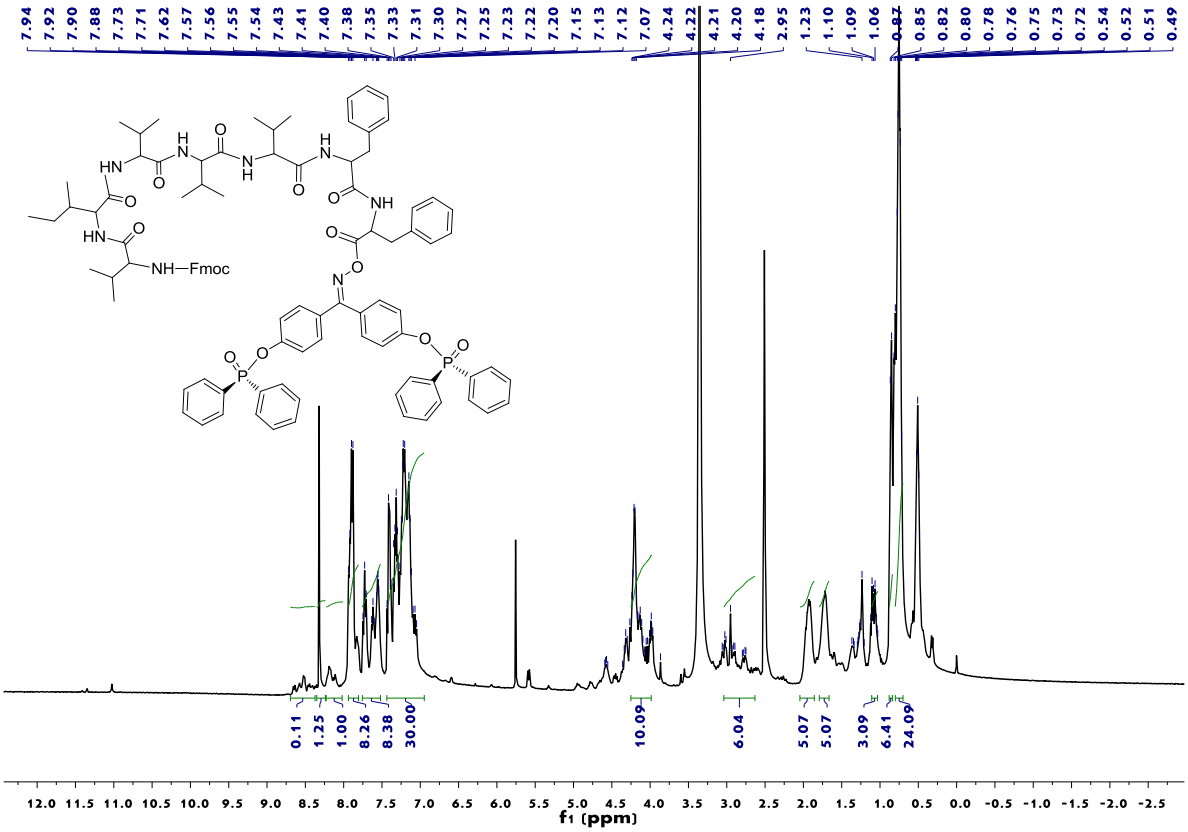
Fmoc-^{D}Val-^{L}Leu-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{96}H_{104}N_8O_{14}P_2Na^+$ $(M+Na)^+$ 1677.70394, found 1677.70410.



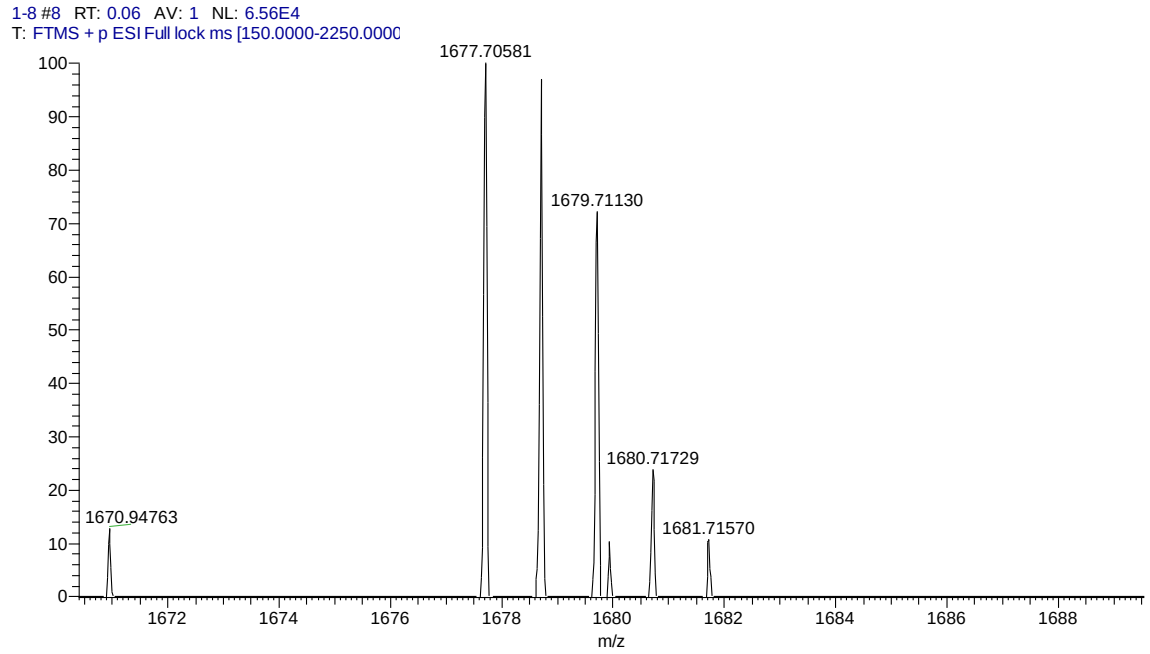
Fmoc-^{D}Val-^{L}Phe-^{D}Val-^{D}Val-^{D}Val-Phe-^{D}Phe-O-N=BDKO ^{1}H NMR (400 Hz, DMSO-d_6)



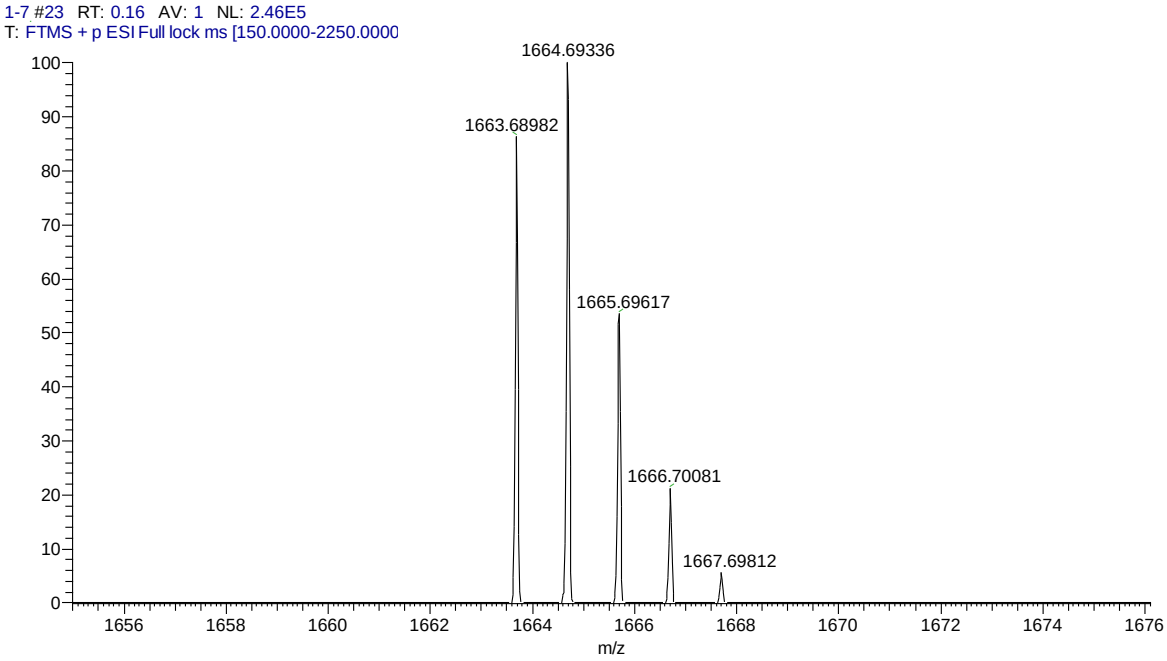
Fmoc-^{D}Val-^{L}Phe-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{99}H_{103}N_8O_{14}P_2^+$ $(M+H)^+$ 1689.70635, found 1689.70520.



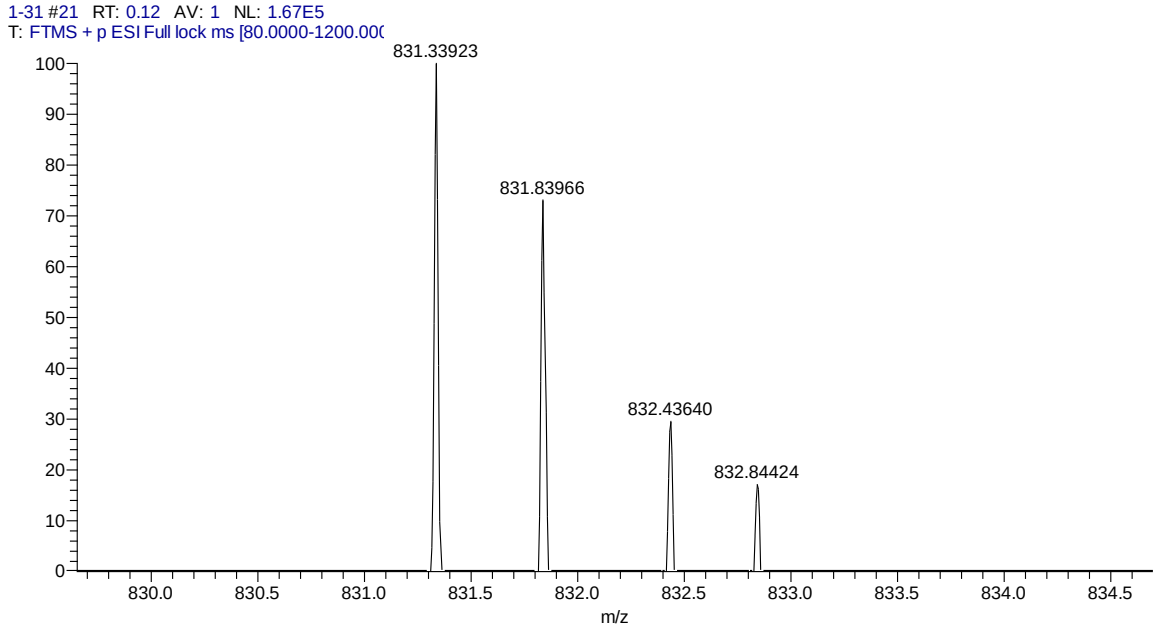
Fmoc-^{D}Val-^{L}Leu-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO ^{1}H NMR (400 Hz, DMSO-d_6)



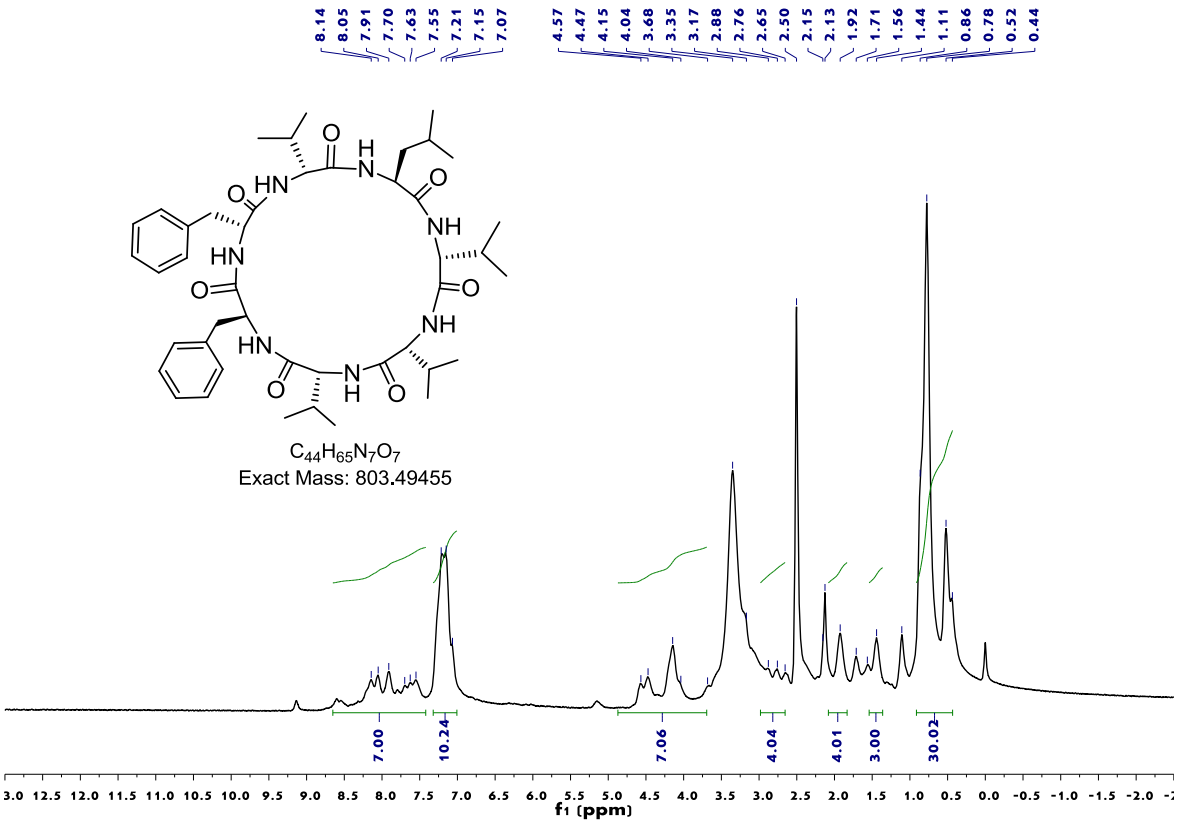
Fmoc-^{D}Val-^{L}Ile-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{96}H_{104}N_8O_{14}P_2Na^+$ $(M+Na)^+$ 1677.70394, found 1677.70581.



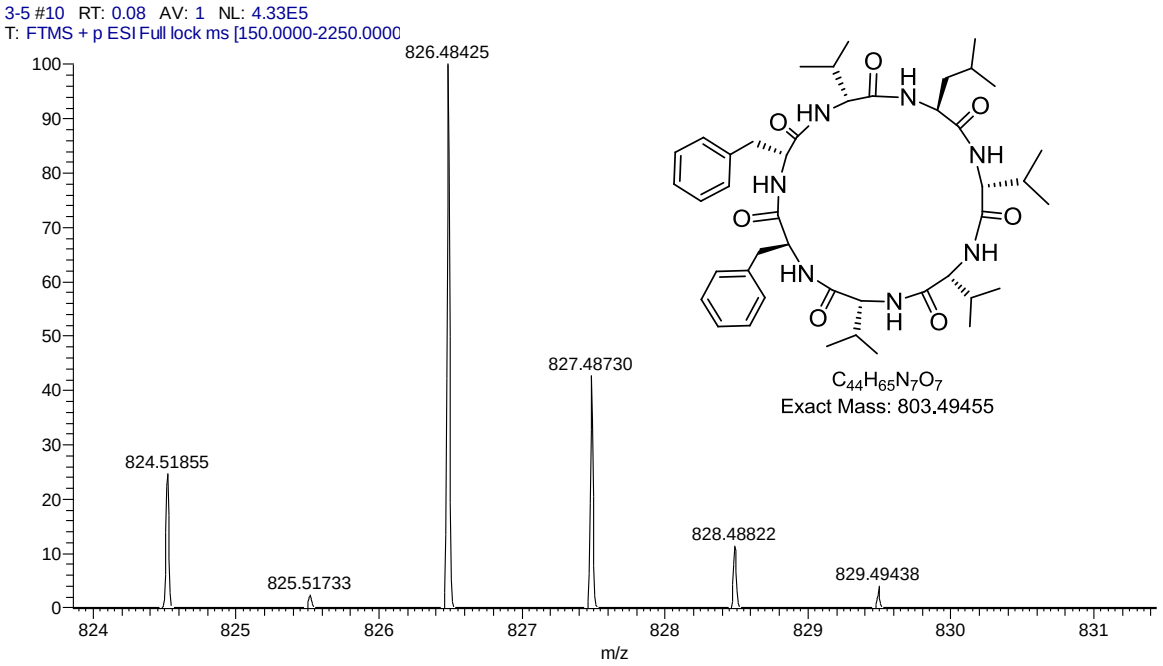
Fmoc-^{D}Val-^{L}Val-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{95}H_{102}N_8O_{14}P_2Na^+$ $(M+Na)^+$ 1663.68829, found 1663.68982.



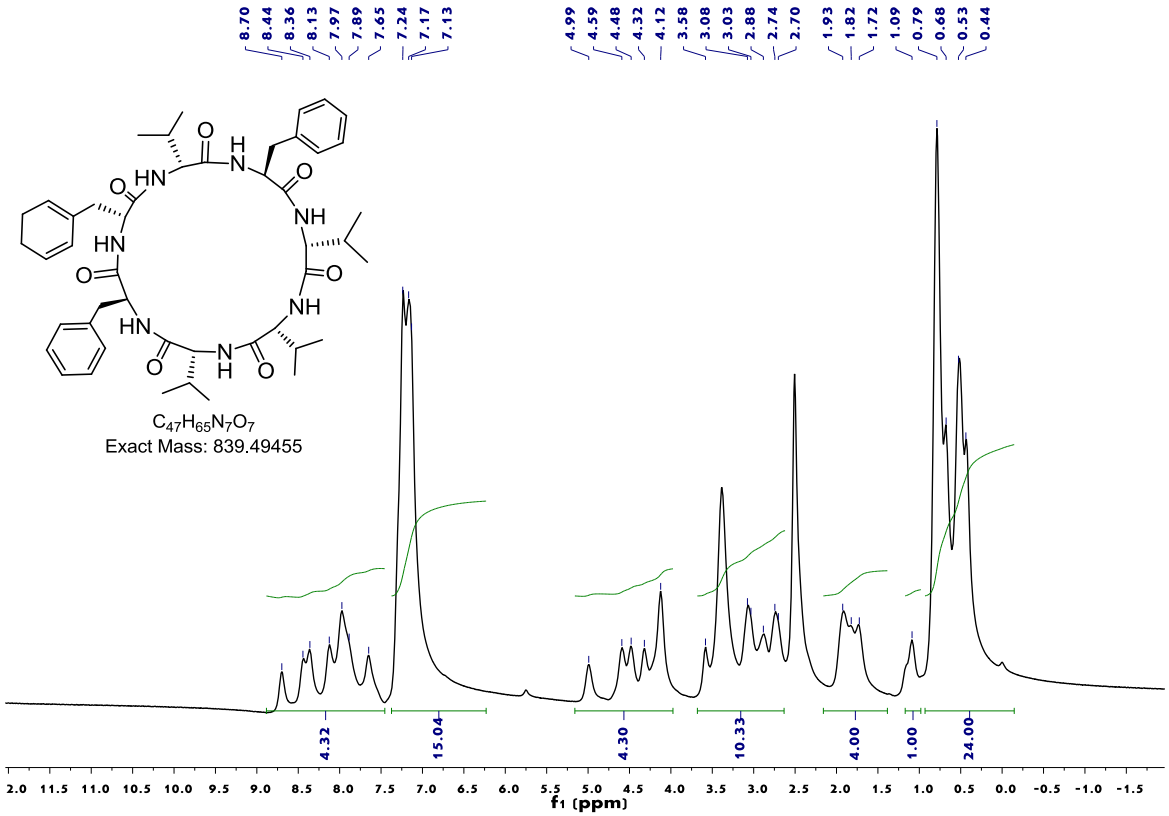
Fmoc-^{D}Val-^{L}Pro-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe-O-N=BDKO HRMS (ESI) m/z calcd for $C_{95}H_{100}N_8O_{14}P_2Na_2^{2+}$ $(M+2Na)^{2+}$ 831.33996, found 831.33923.



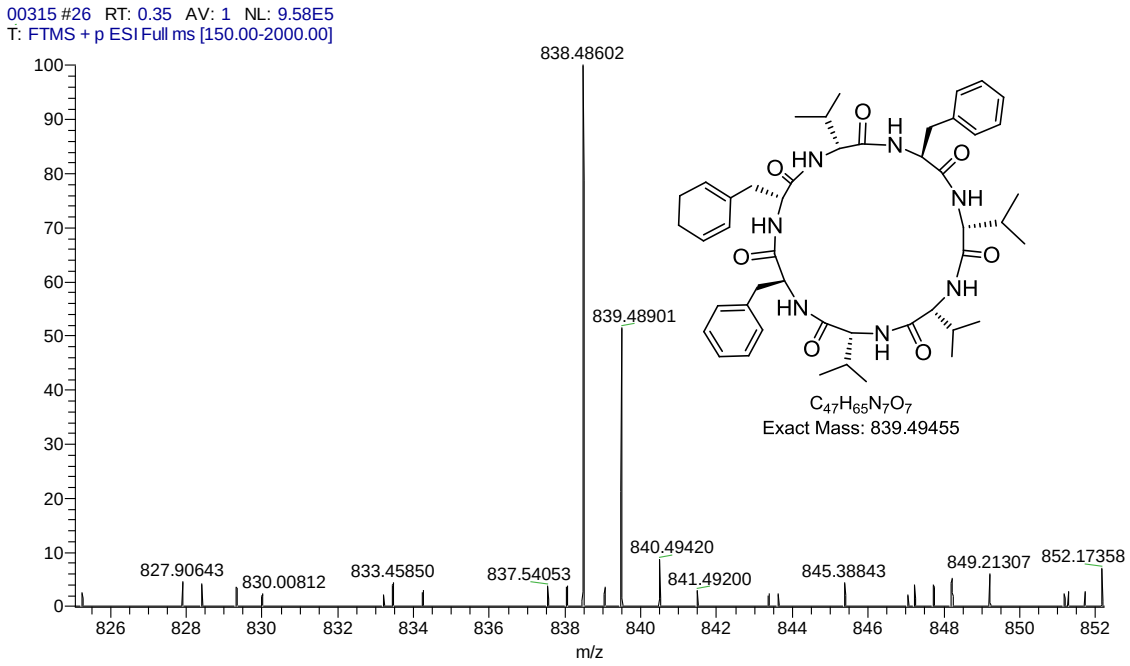
***Cyclo*(**^{D}Val-^{L}Leu-^{D}Val-^{D}Val-^{D}Val-^{L}Phe-^{D}Phe**)** ^{1}H NMR (400 Hz, DMSO-d_6)



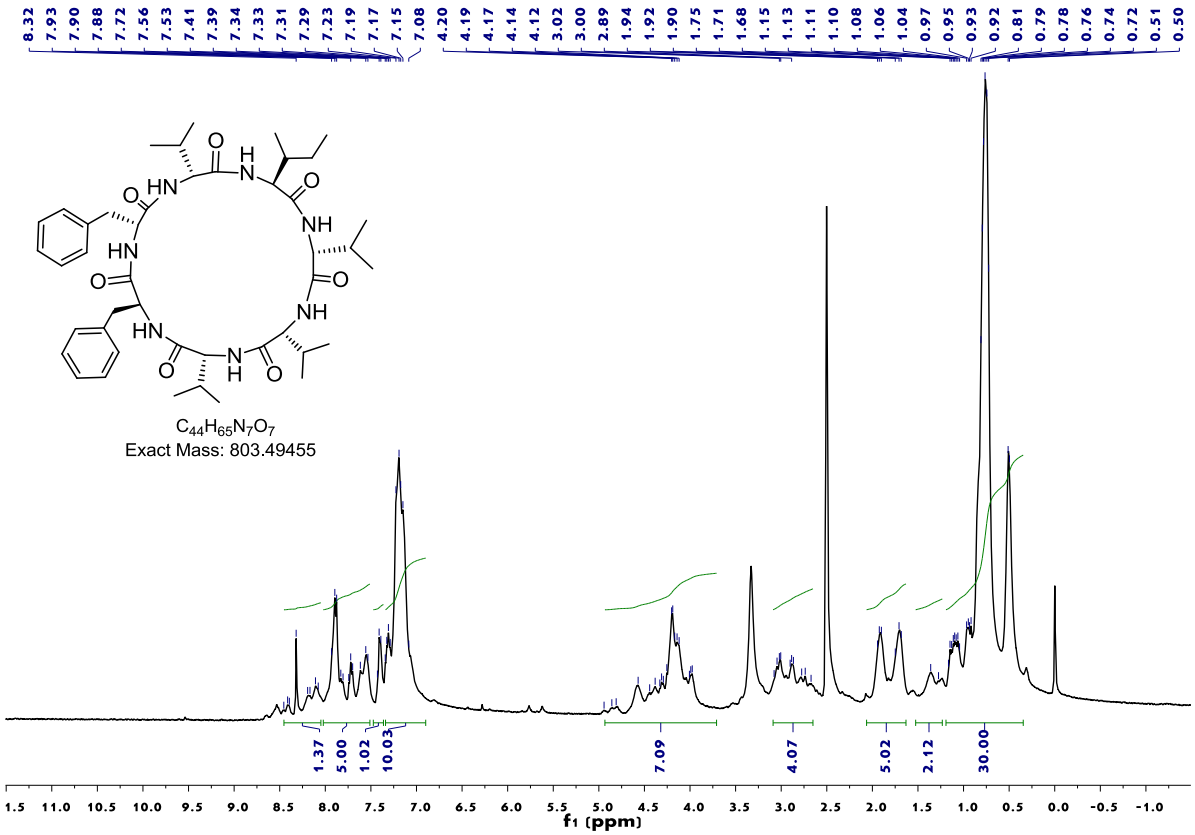
***Cyclo*(^D^Val-^L^Leu-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** HRMS (ESI) m/z calcd for $C_{44}H_{65}N_7O_7Na^+$ $(M+Na)^+$ 826.48377, found 826.48425.



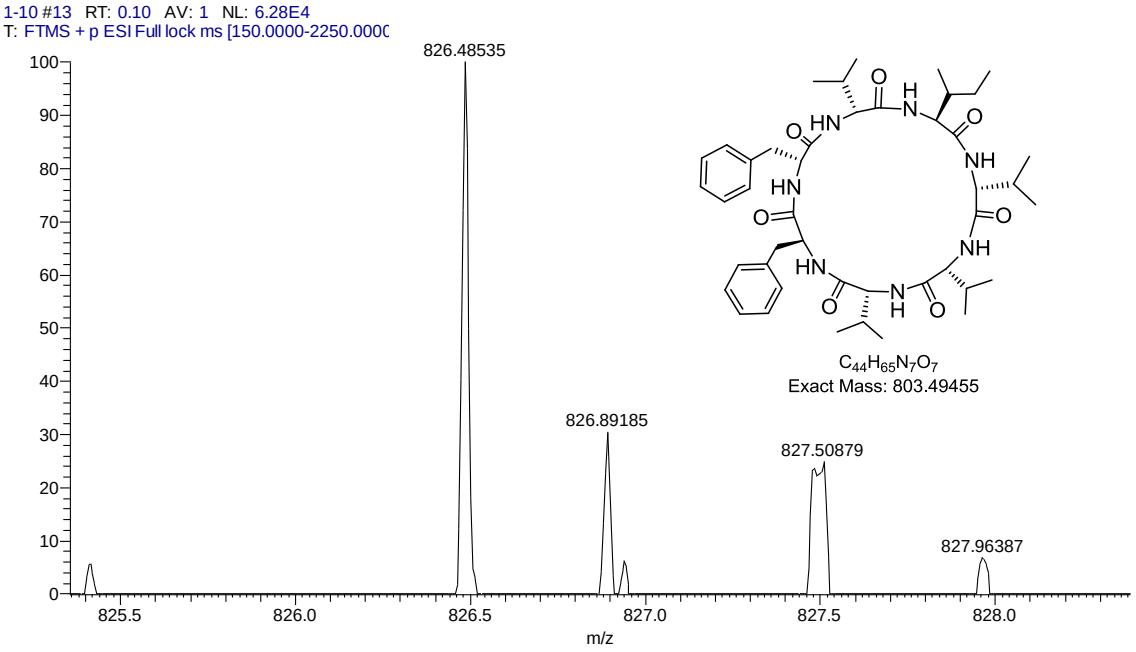
***Cyclo*(^D^Val-^L^Phe-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** ^{1}H NMR (400 Hz, DMSO-d_6)



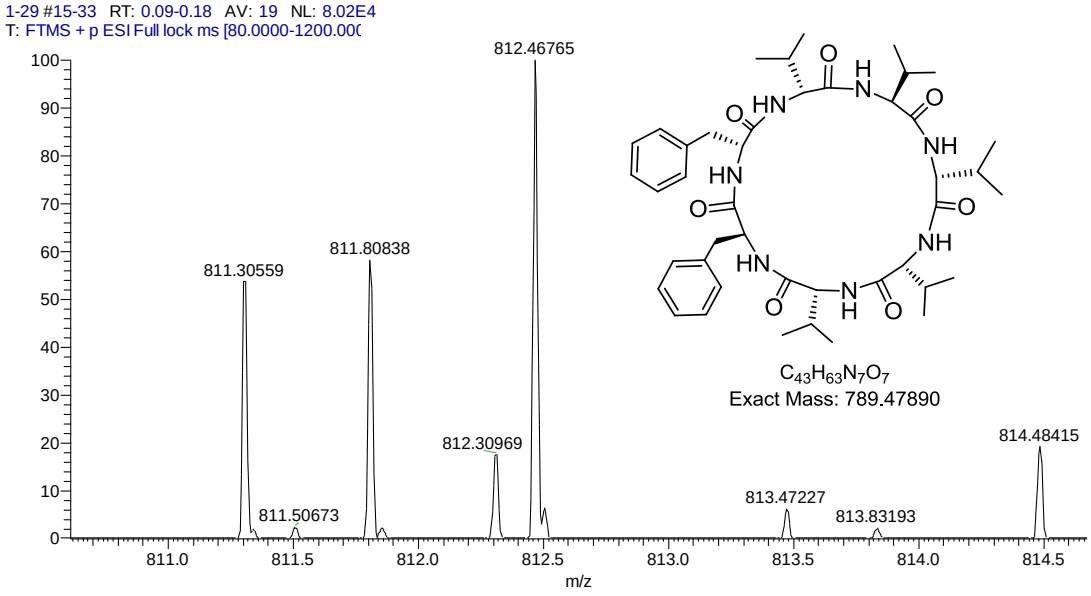
***Cyclo*(^D^Val-^L^Phe-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** HRMS (ESI) m/z calcd for $C_{47}H_{64}N_7O_7^+$ $(M+H)^+$ 838.48617, found 838.48602.



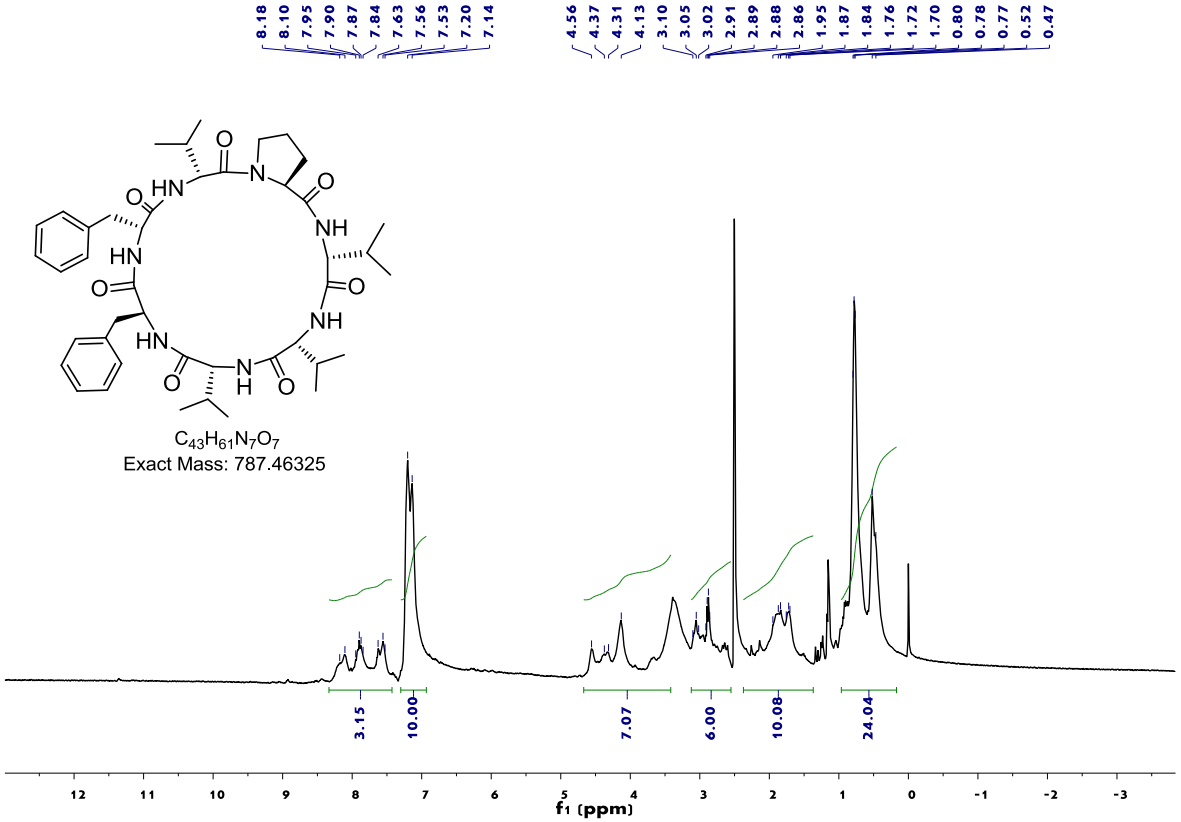
***Cyclo*(^D^Val-^L^IIe-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** ^{1}H NMR (400 Hz, DMSO-d_6)



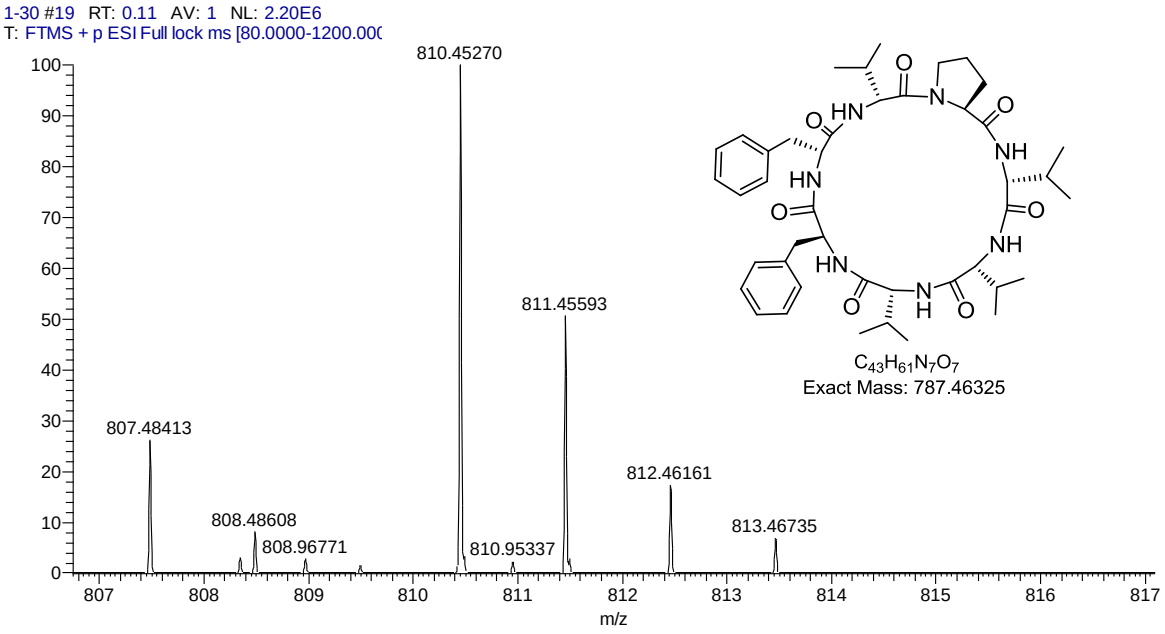
***Cyclo*(^D^Val-^L^IIe-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** HRMS (ESI) m/z calcd for $C_{44}H_{65}N_7O_7Na^+$ $(M+Na)^+$ 826.48377, found 826.48535.



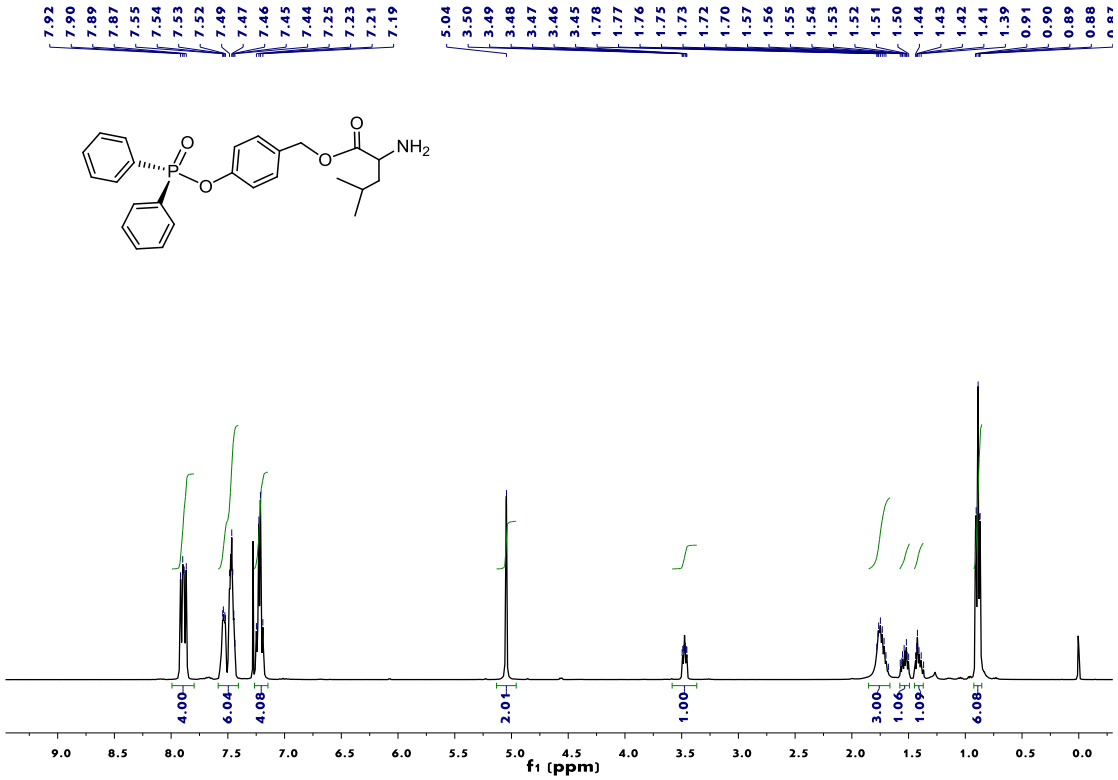
***Cyclo*(^D^Val-^L^Val-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** HRMS (ESI) m/z calcd for $C_{43}H_{63}N_7O_7Na^+$ $(M+Na)^+$ 812.46812, found 812.46765.



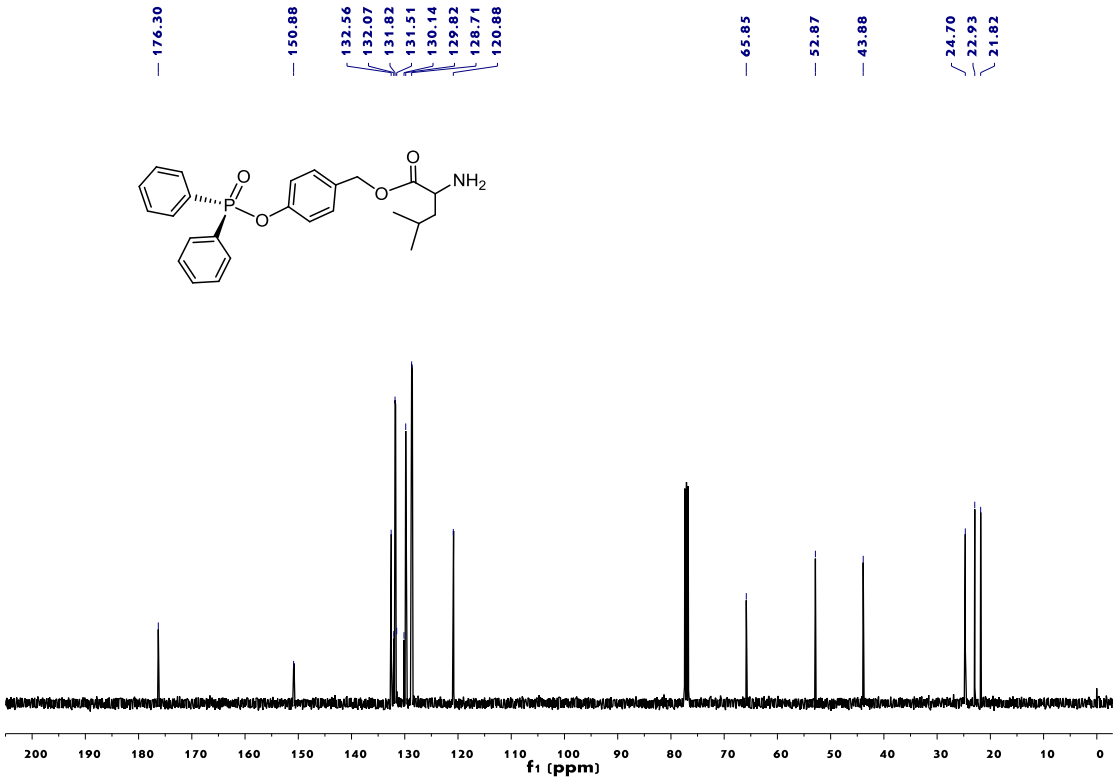
***Cyclo*(^D^Val-^L^Pro-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)** ^{1}H NMR (400 Hz, DMSO-d_6)



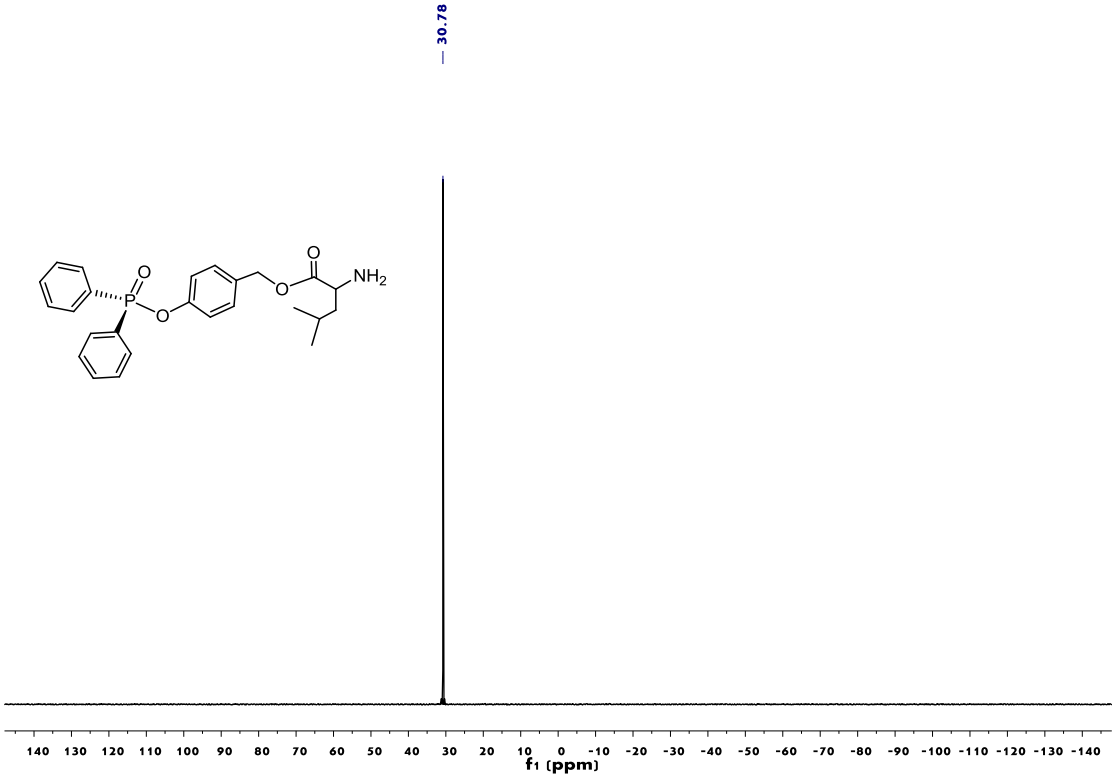
***Cyclo*(^D^Val-^L^Pro-^D^Val-^D^Val-^D^Val-^L^Phe-^D^Phe)**, HRMS (ESI) m/z calcd for $C_{43}H_{61}N_7O_7Na^+$ $(M+Na)^+$ 810.45247, found 810.45270.



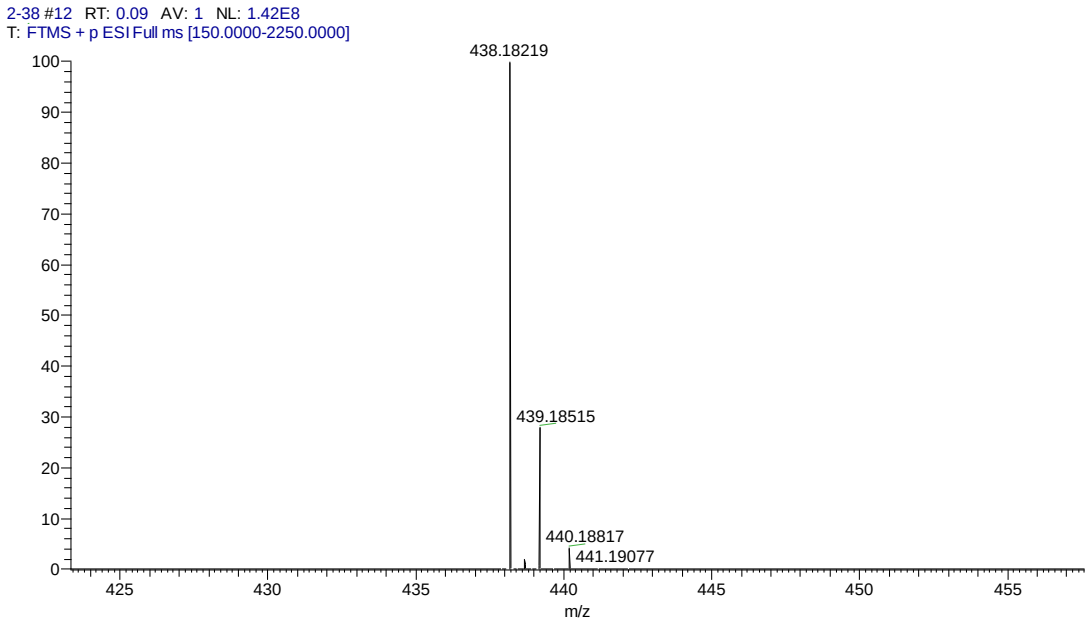
H-*L*Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



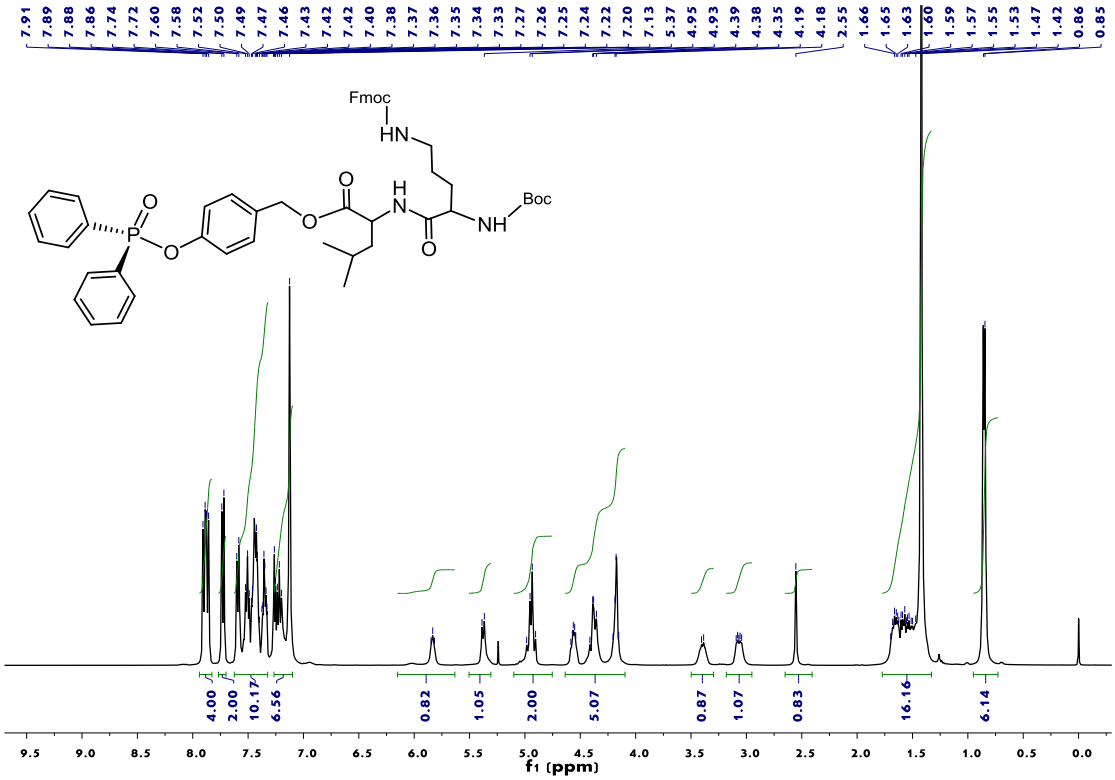
H-*L*Leu-O-DPBA ^{13}C NMR (100 Hz, $CDCl_3$)



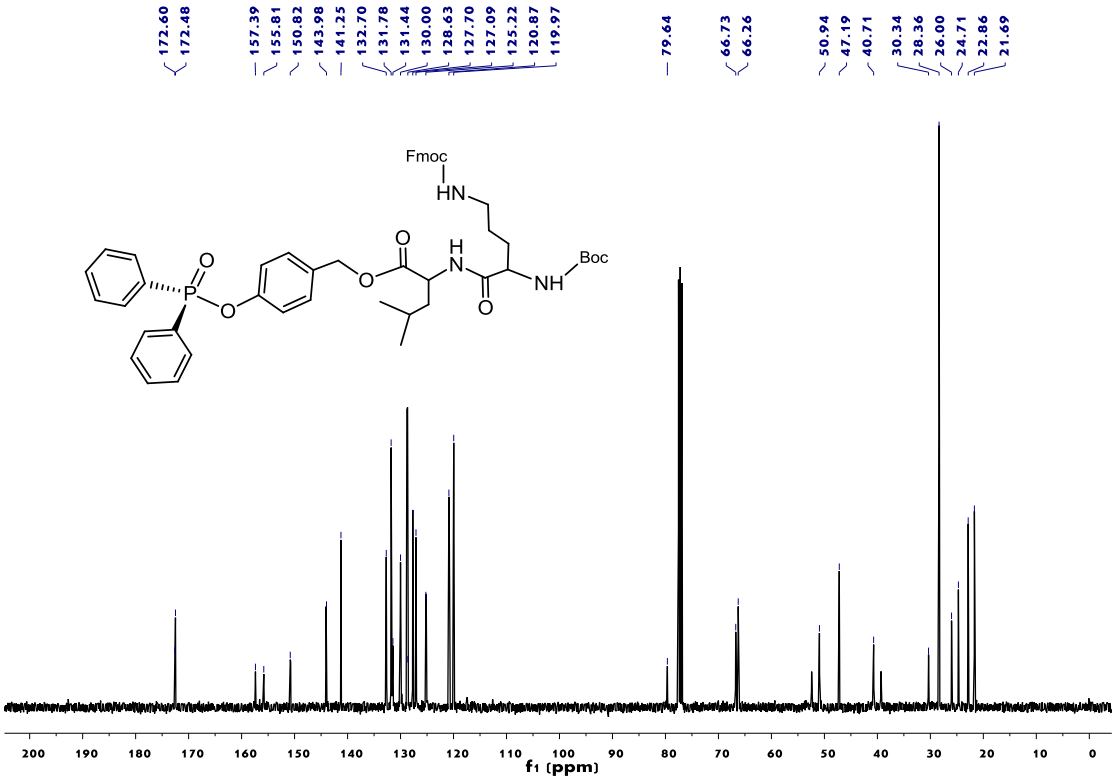
H-*L*Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



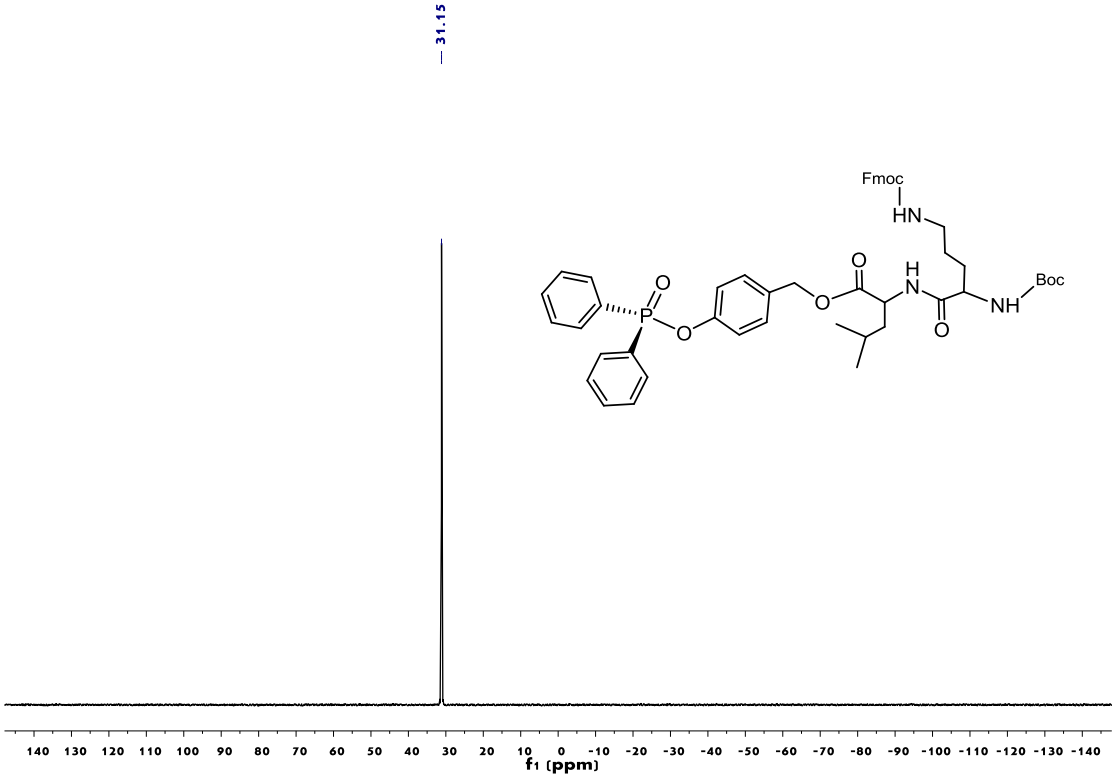
H-*L*Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$) HRMS (ESI) m/z calcd for $C_{25}H_{29}NO_4P^+$ $(M+H)^+$ 438.18287, found 438.18219.



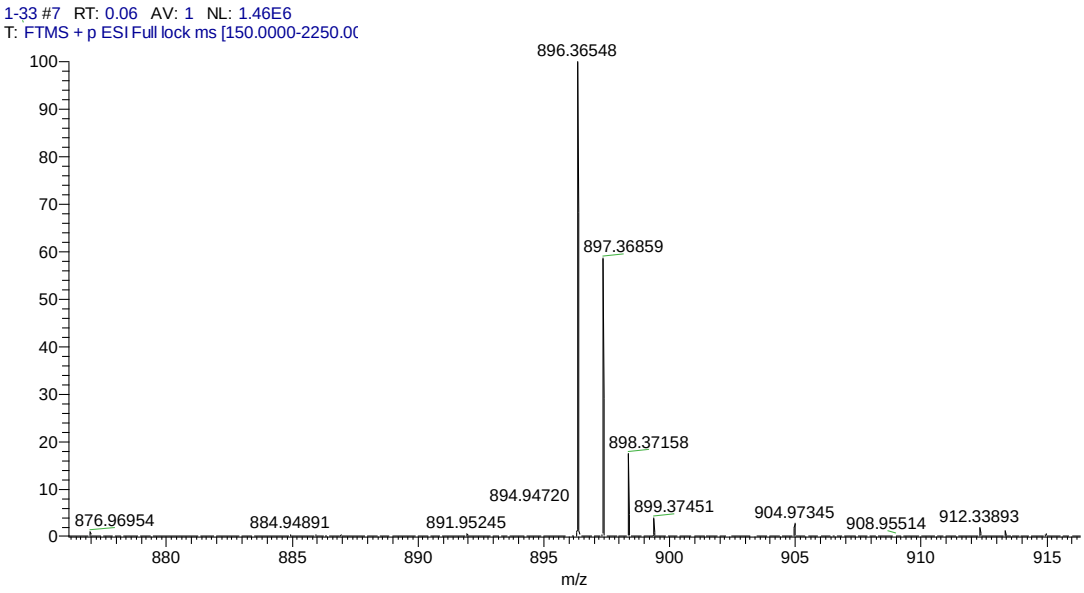
Boc-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



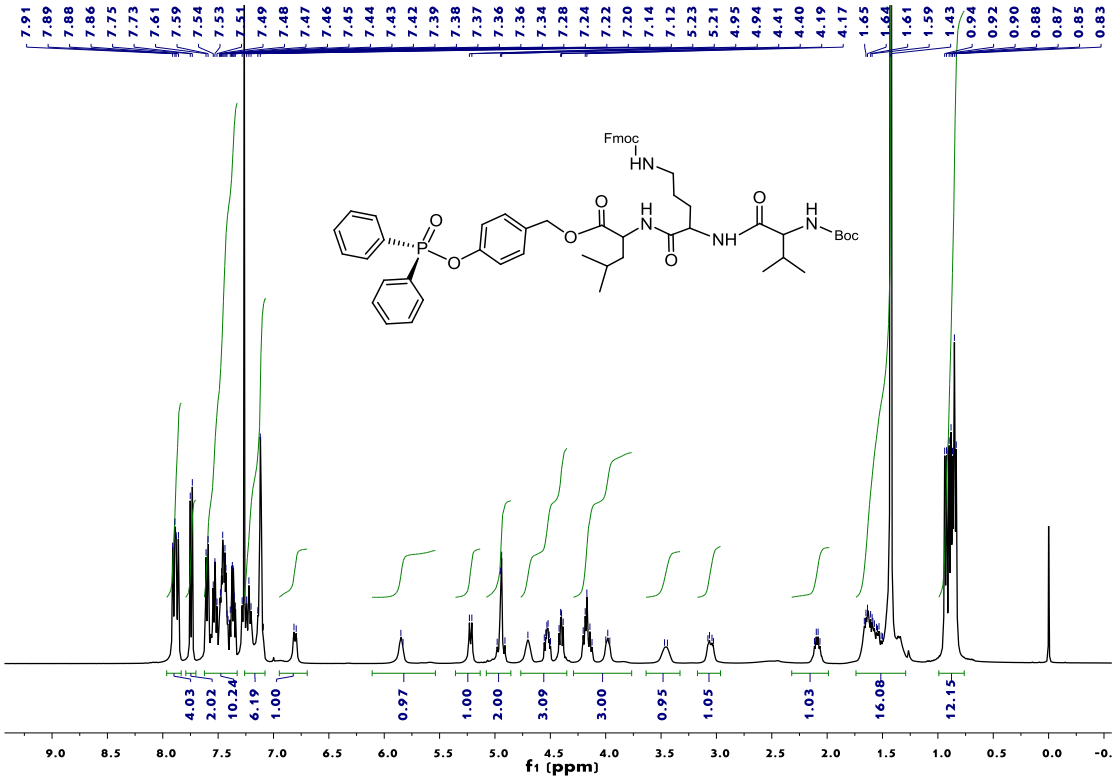
Boc-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100 Hz, $CDCl_3$)



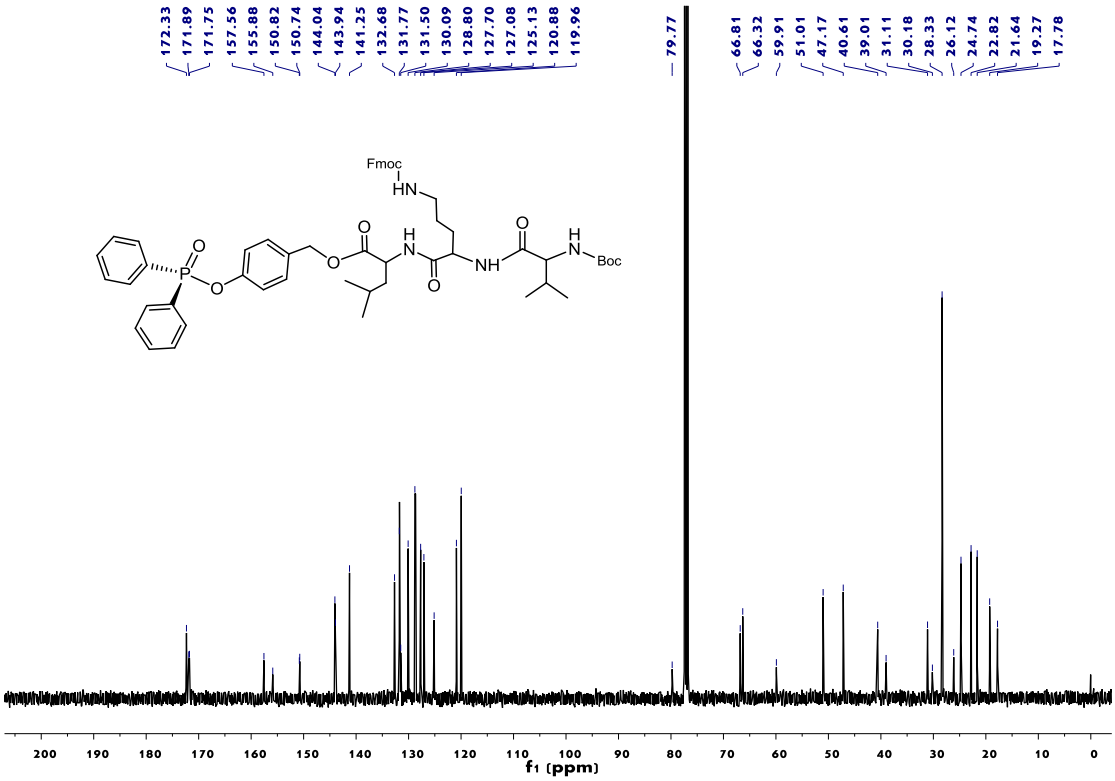
Boc-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



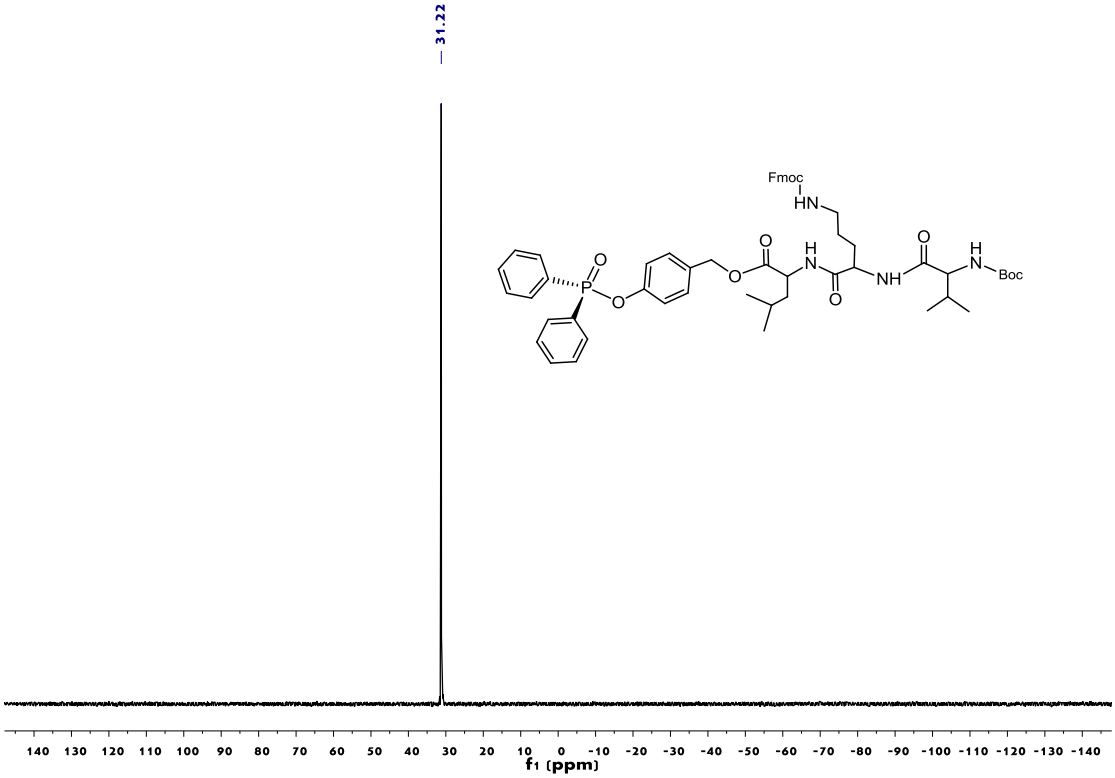
Boc-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{50}H_{56}N_3O_9PNa^+$ $(M+Na)^+$ 896.36464, found 896.36548.



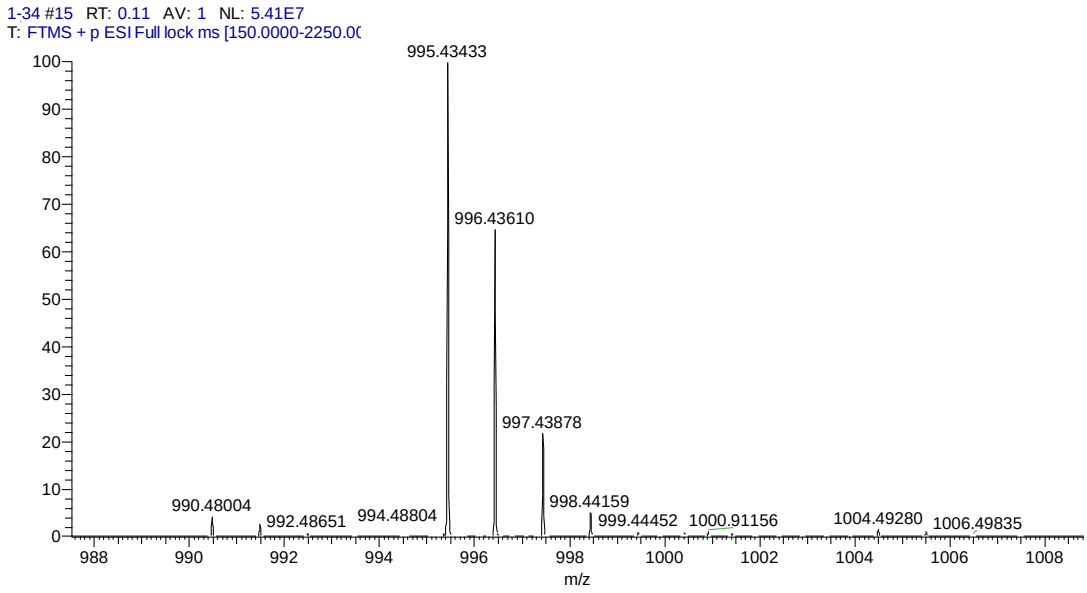
Boc-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



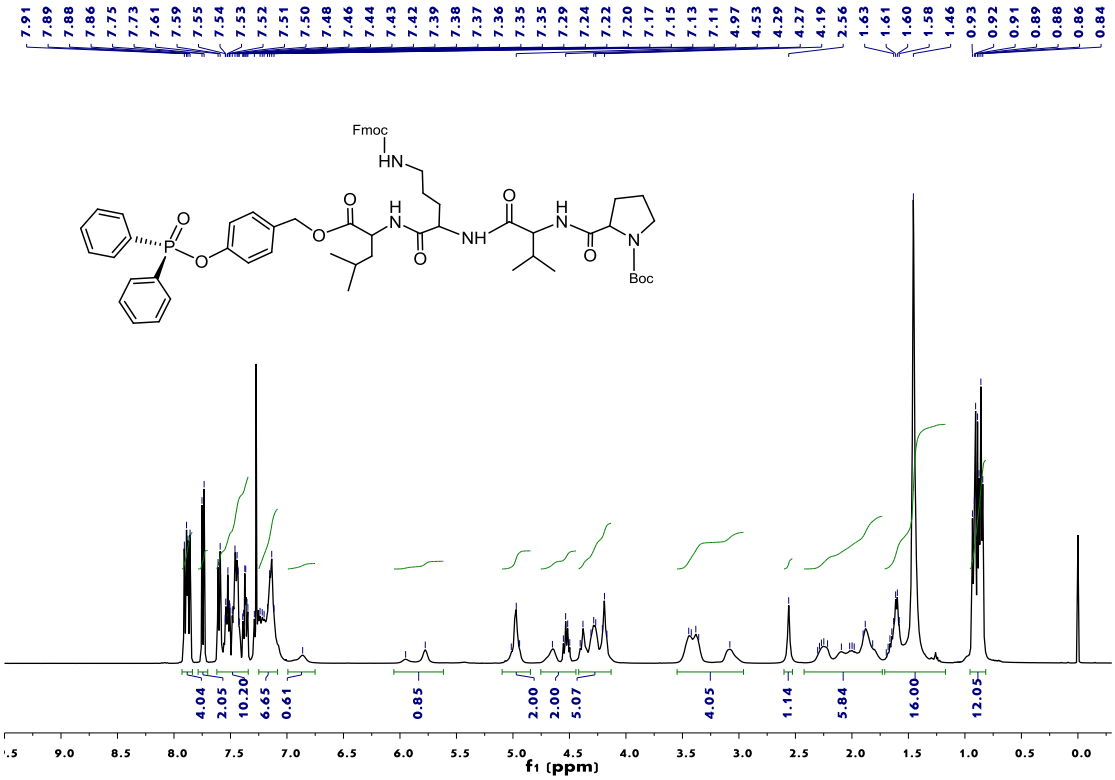
Boc-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100 Hz, $CDCl_3$)



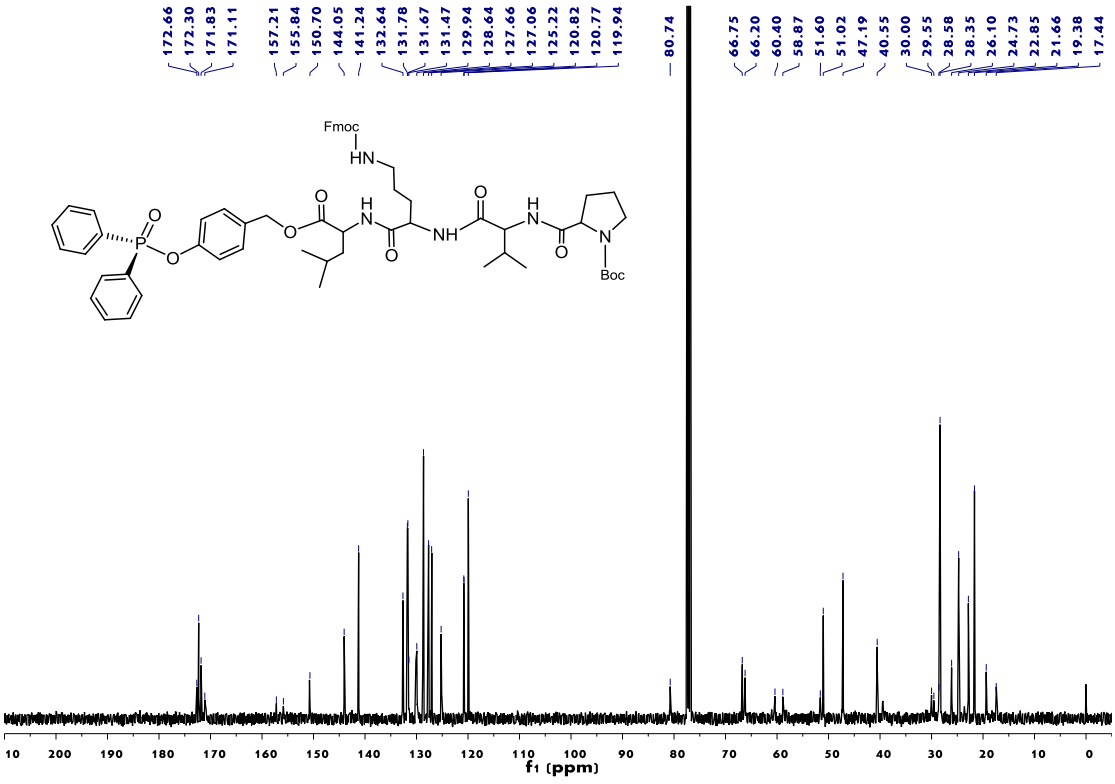
Boc-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



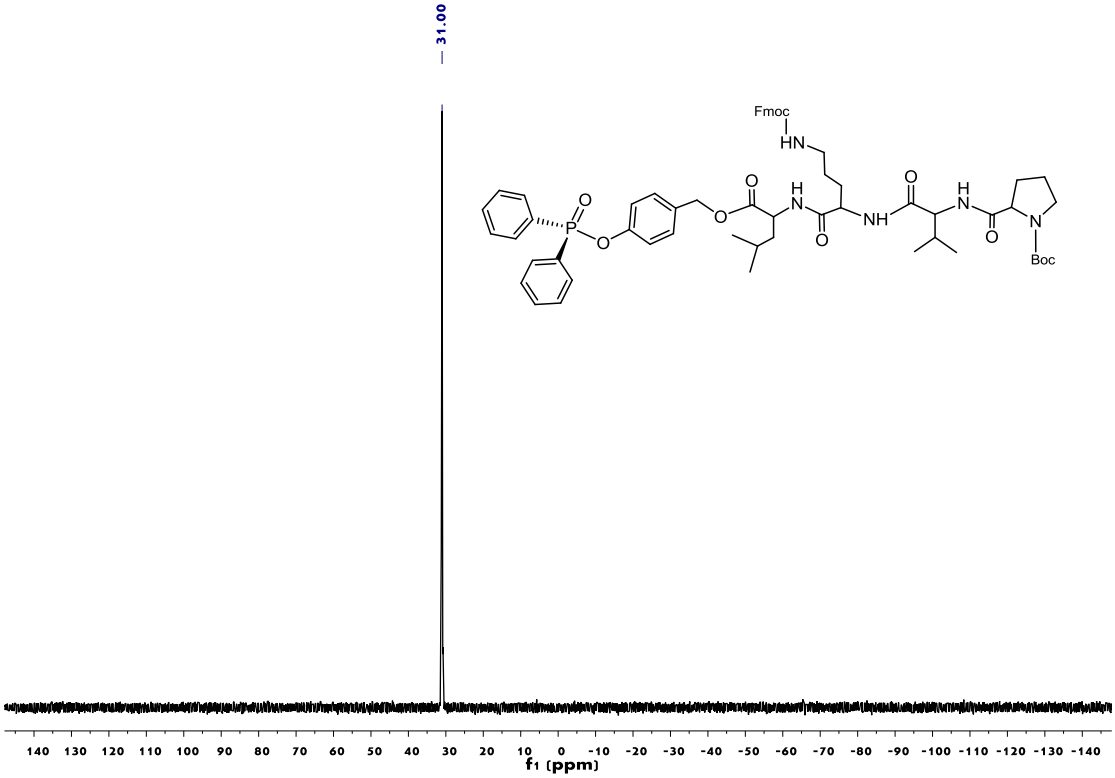
Boc-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{55}H_{65}N_4O_{10}PNa^+$ $(M+Na)^+$ 995.43305, found 995.43433.



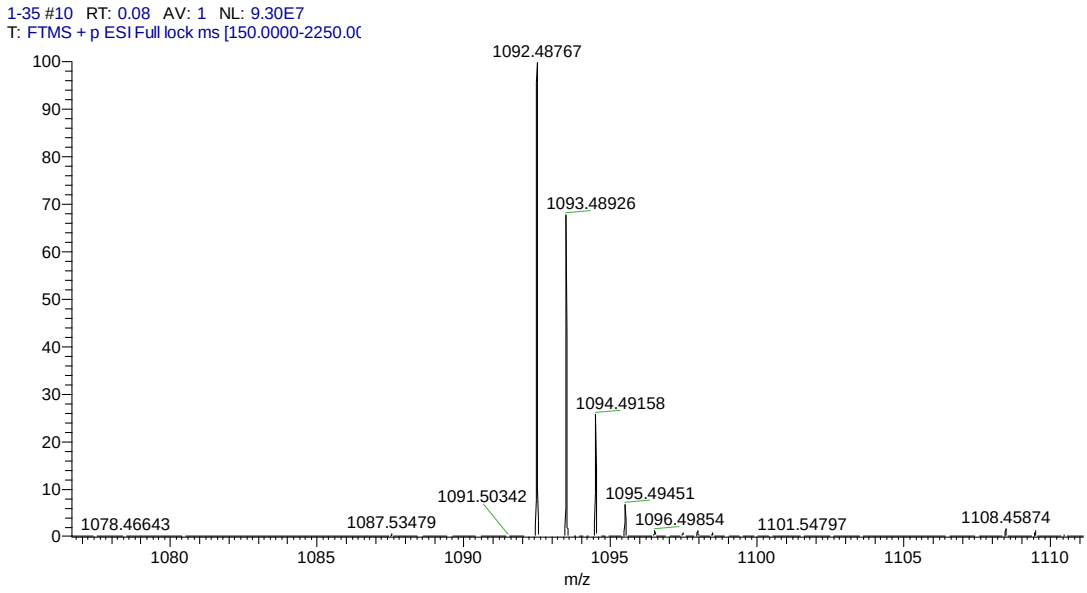
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



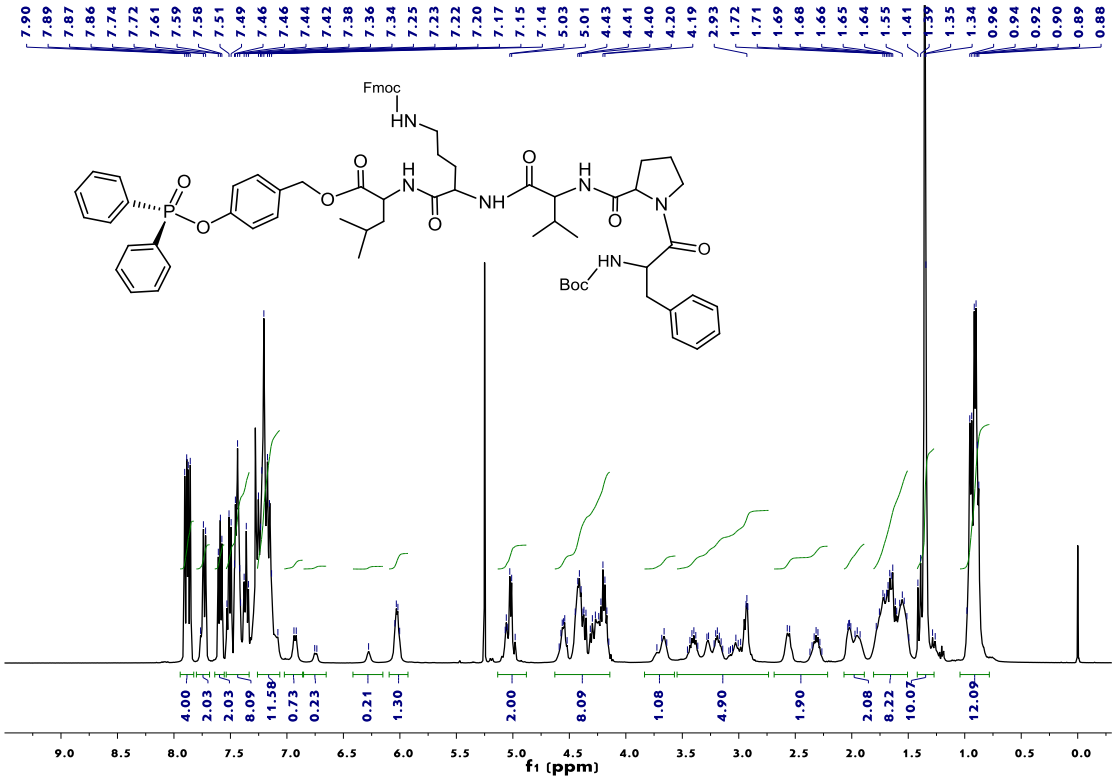
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100 Hz, $CDCl_3$)



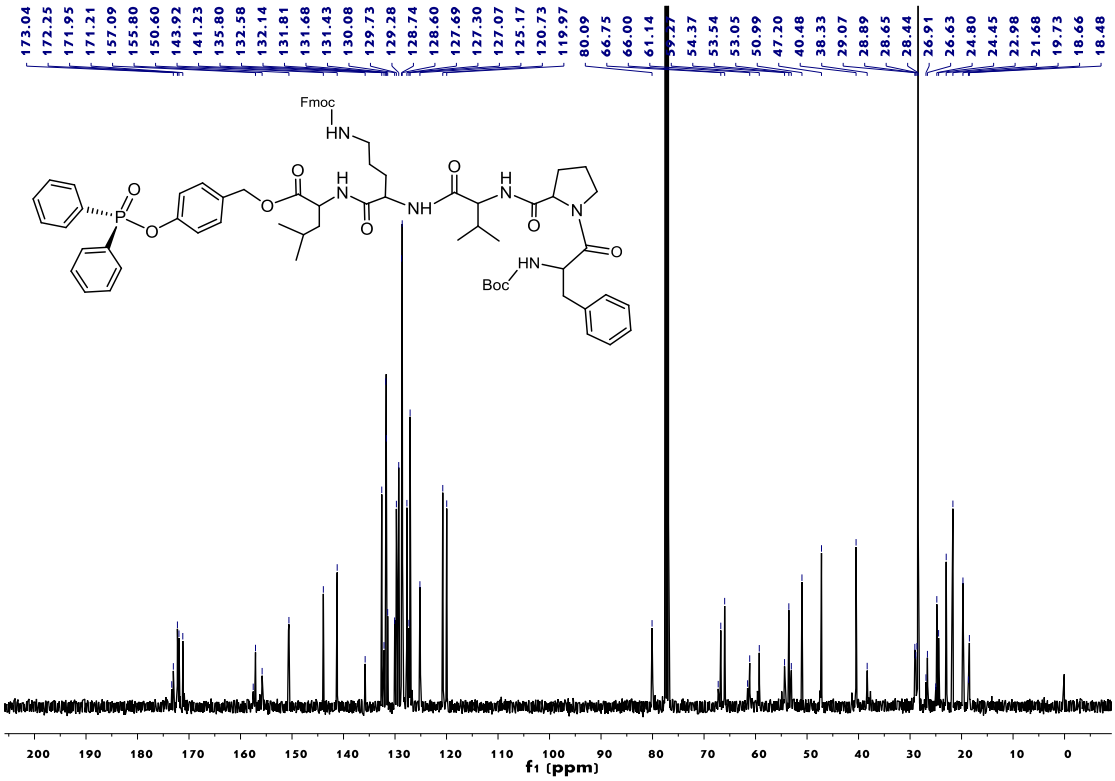
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163Hz, $CDCl_3$)



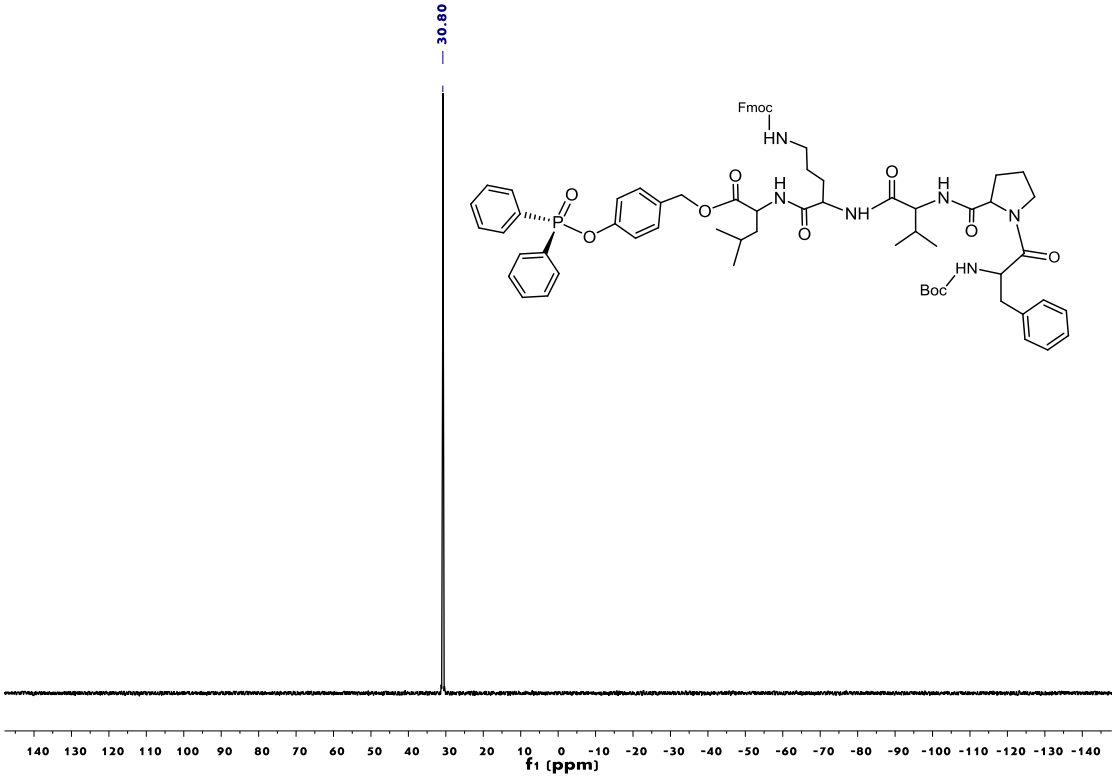
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{60}H_{72}N_5O_{11}PNa^+$ $(M+Na)^+$ 1092.48582, found 1092.48767.



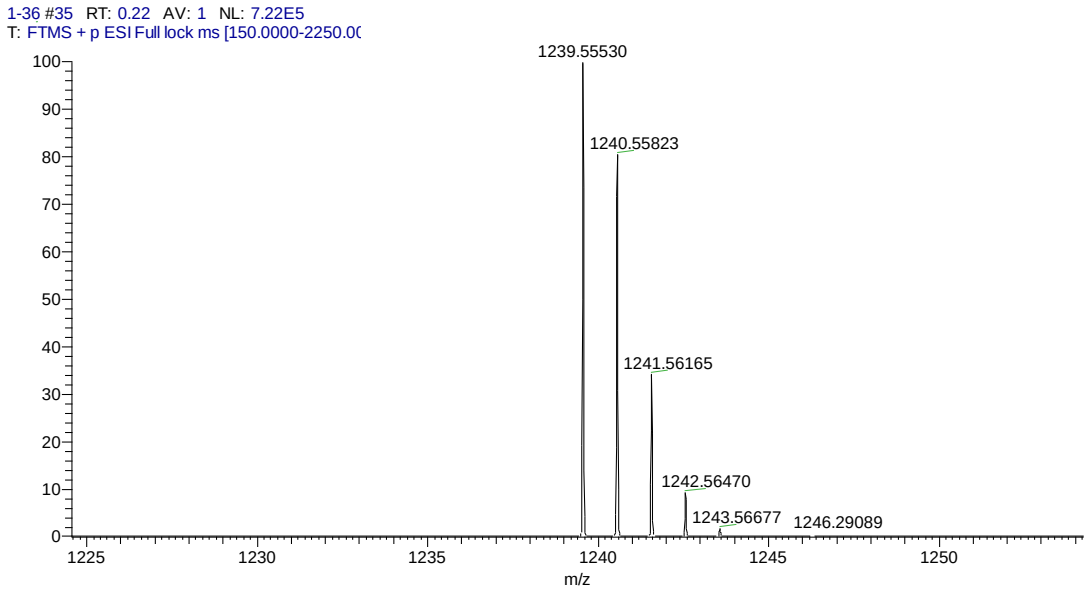
Boc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400Hz, $CDCl_3$)



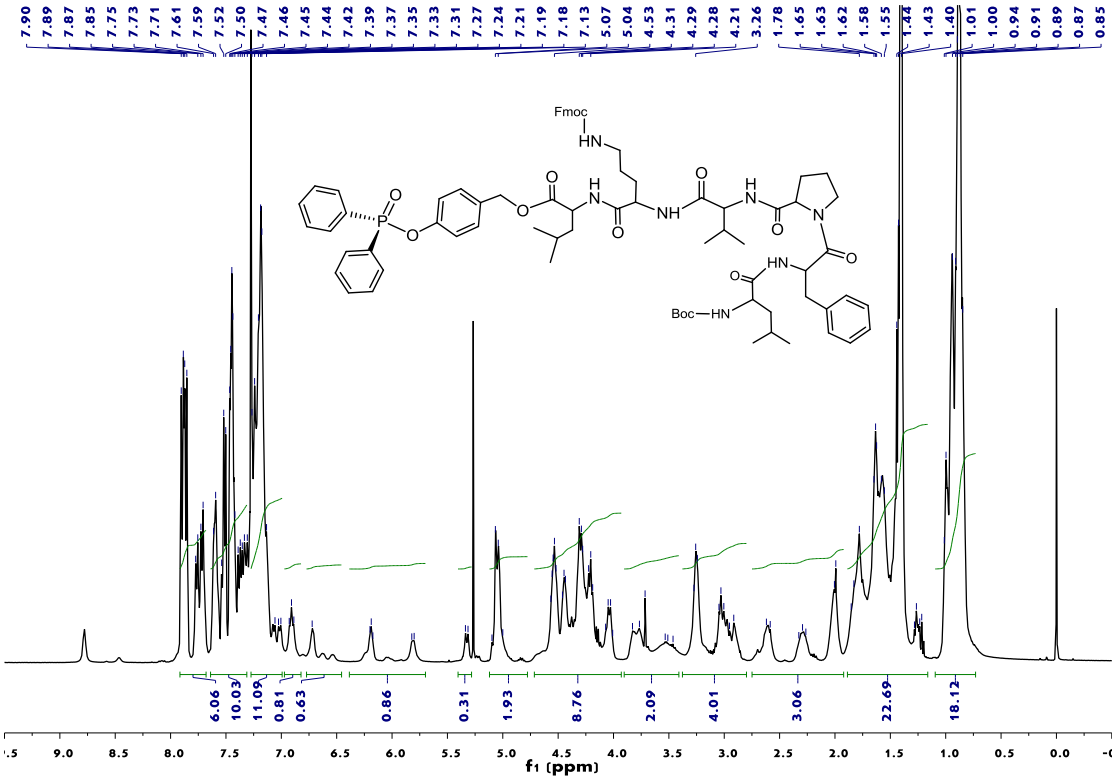
Boc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100Hz, $CDCl_3$)



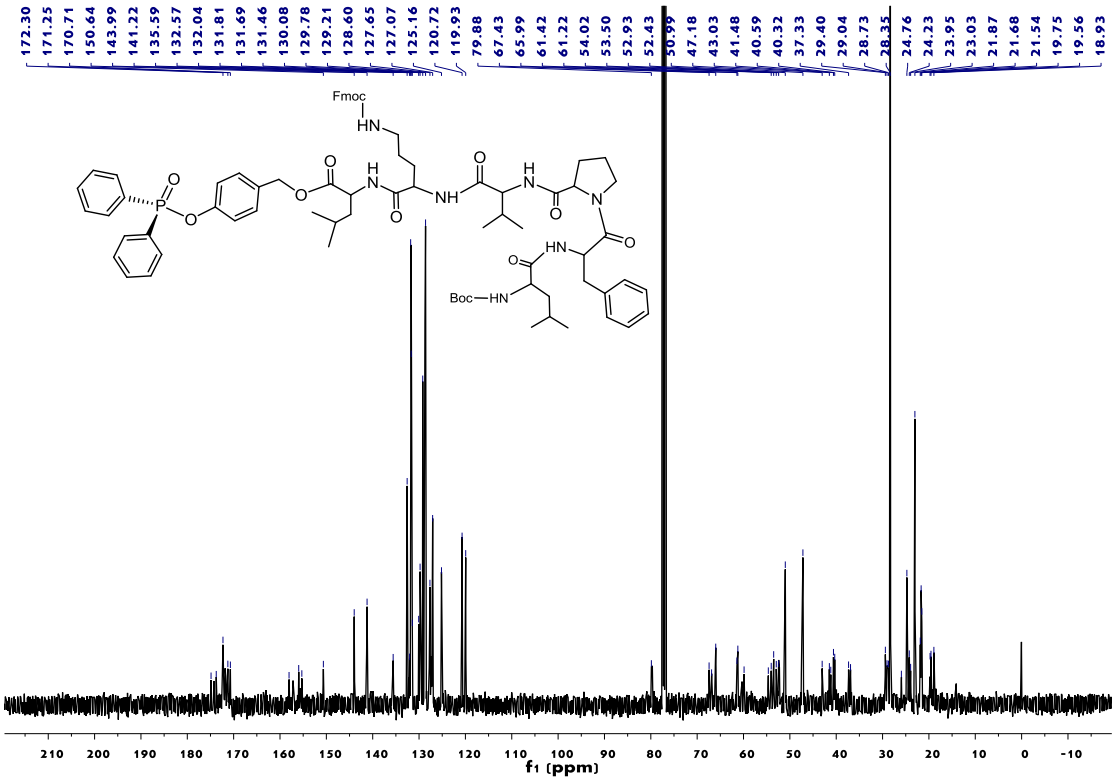
Boc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163Hz, $CDCl_3$)



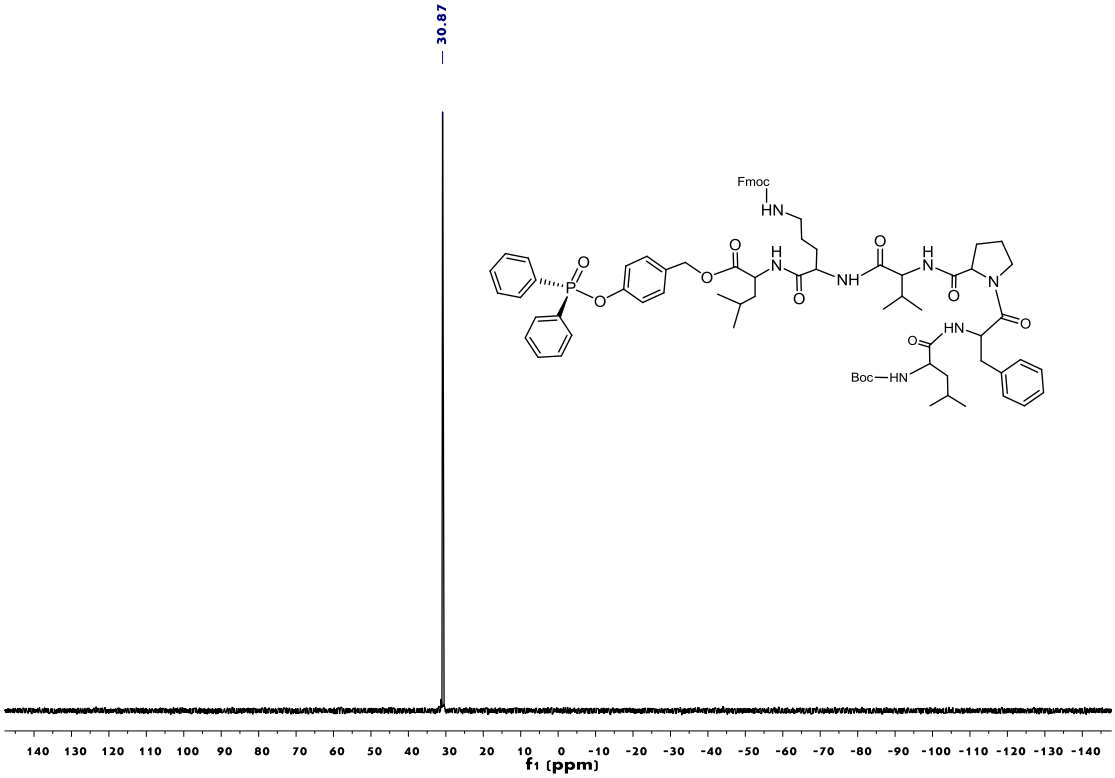
Boc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA. HRMS (ESI) m/z calcd for $C_{69}H_{81}N_6O_{12}PNa^+$ $(M+Na)^+$ 1239.55423, found 1239.55530.



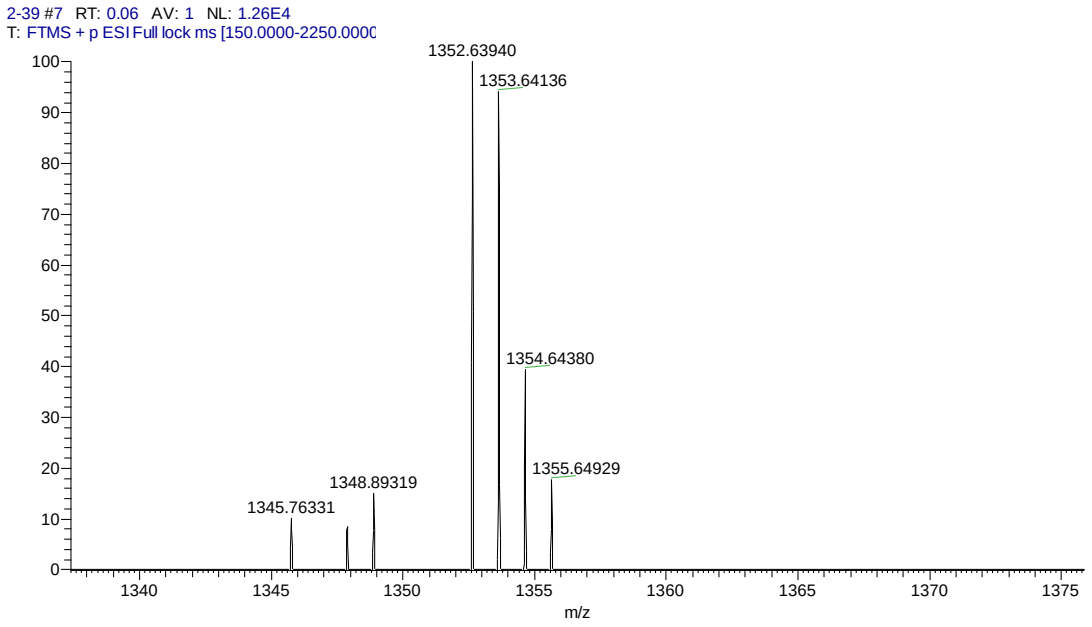
Boc-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400Hz, $CDCl_3$)



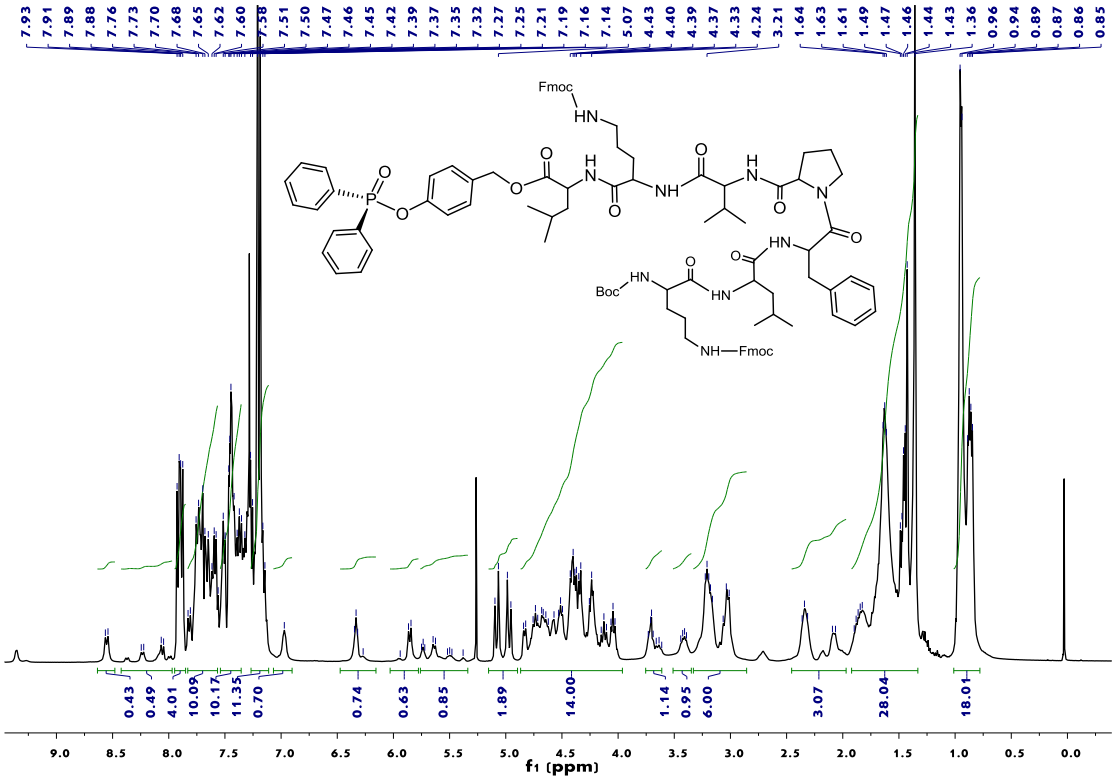
Boc-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100Hz, $CDCl_3$)



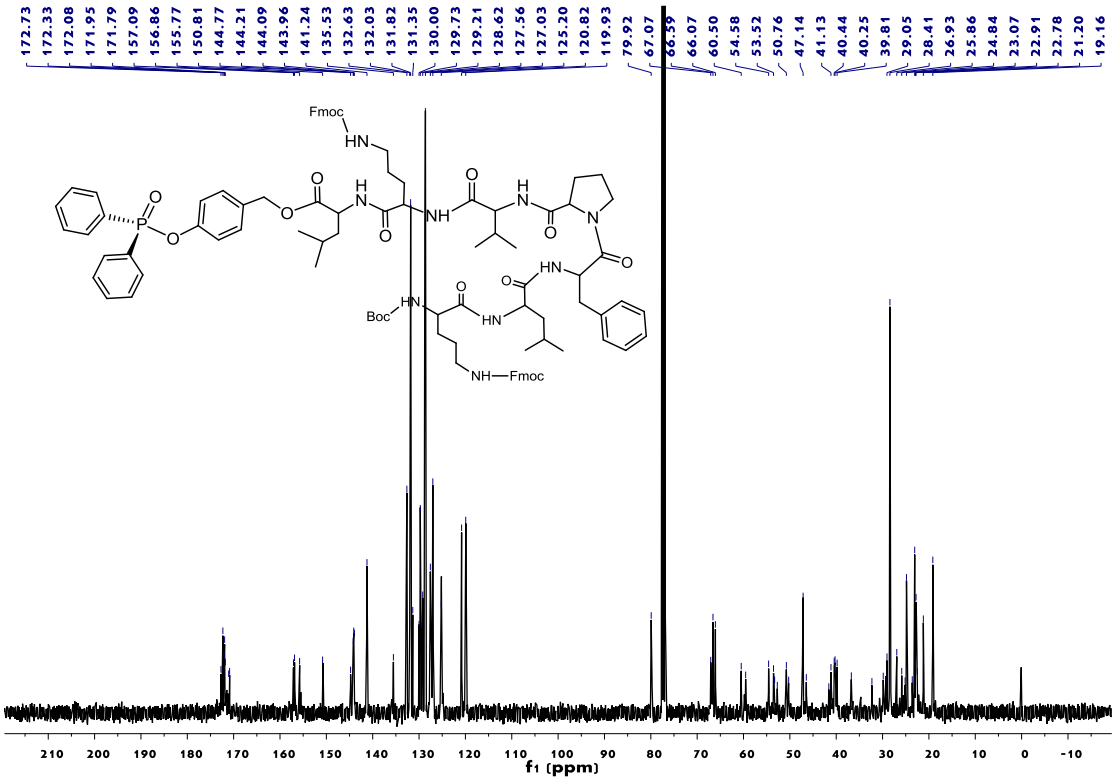
Boc-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



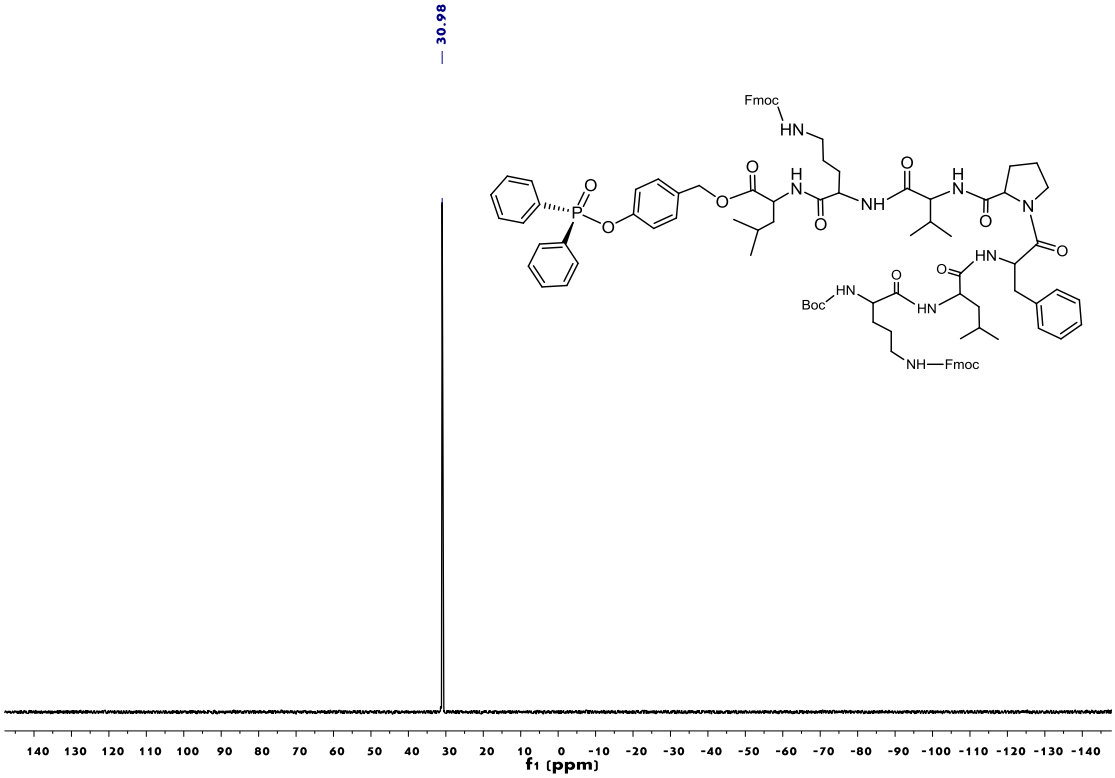
Boc-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{75}H_{92}N_7O_{13}PNa^+$ $(M+Na)^+$ 1352.63829, found 1352.63940.



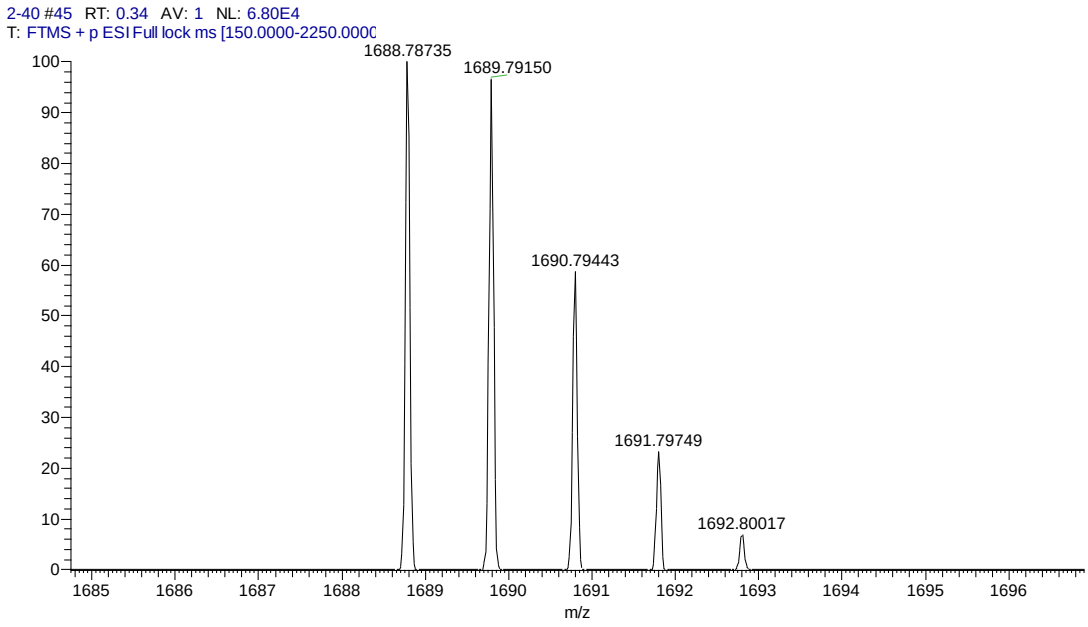
Boc-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400Hz, $CDCl_3$)



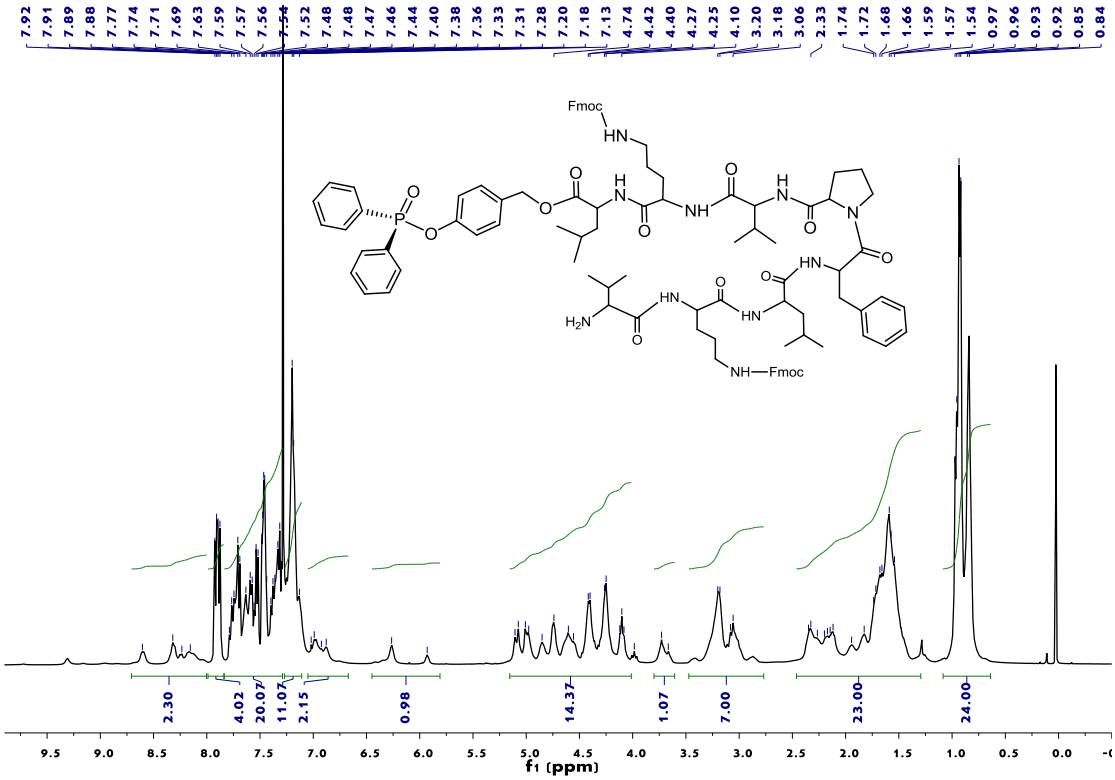
Boc-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100Hz, $CDCl_3$)



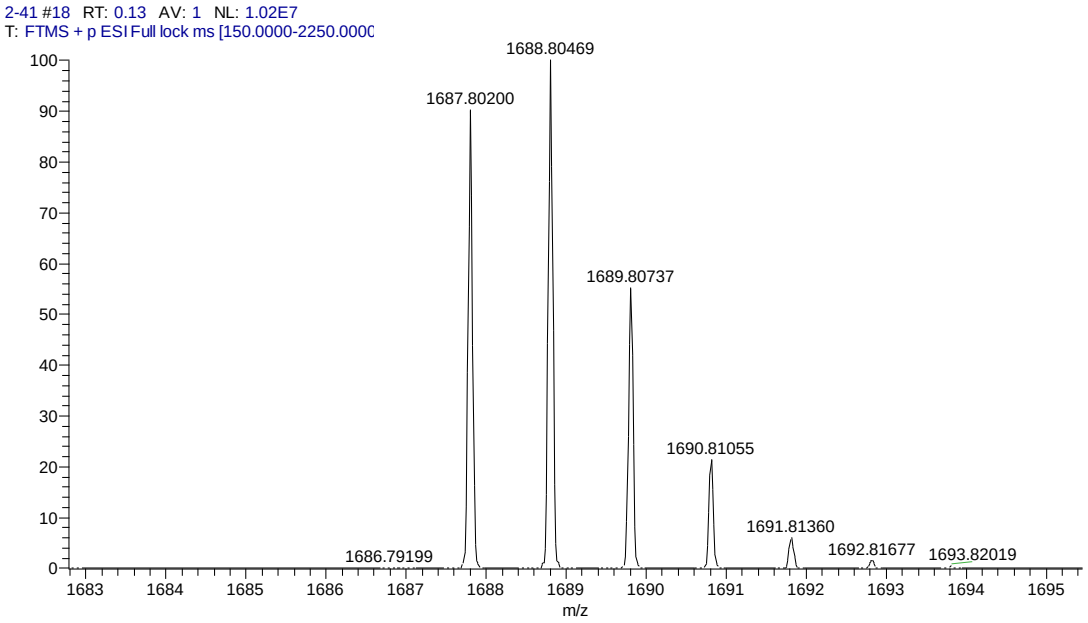
Boc-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



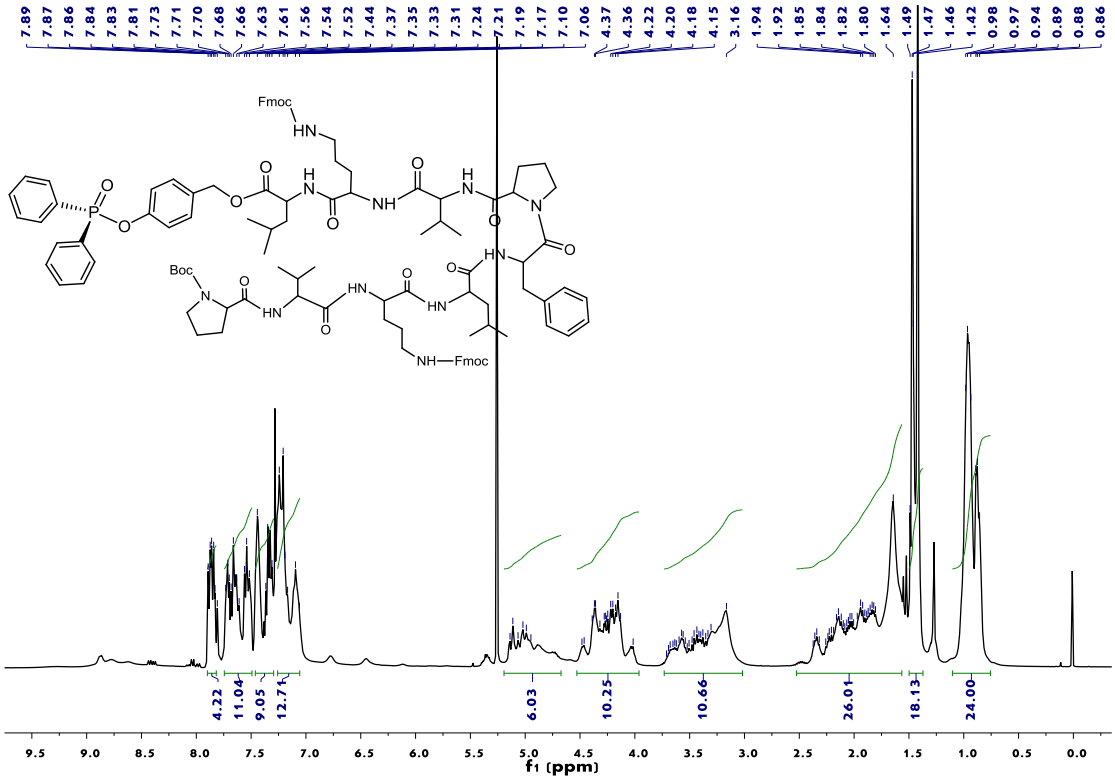
Boc-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{95}H_{112}N_9O_{16}PNa^+$ $(M+Na)^+$ 1688.78569, found 1688.78735.



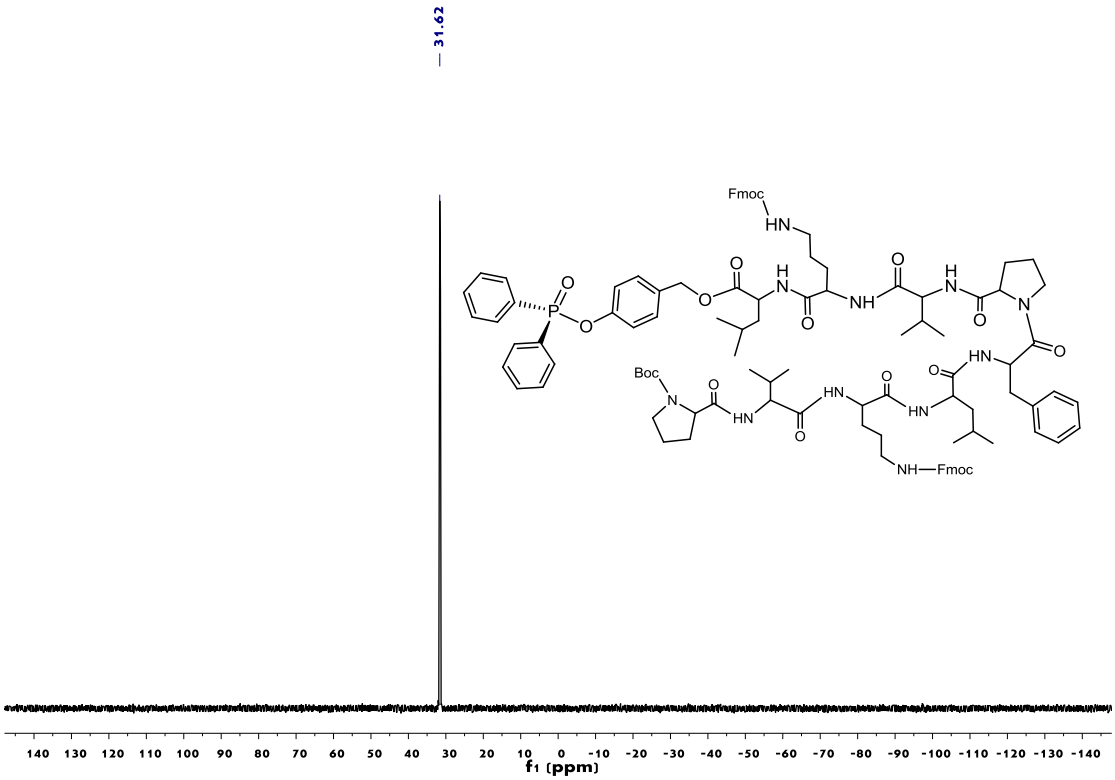
H-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



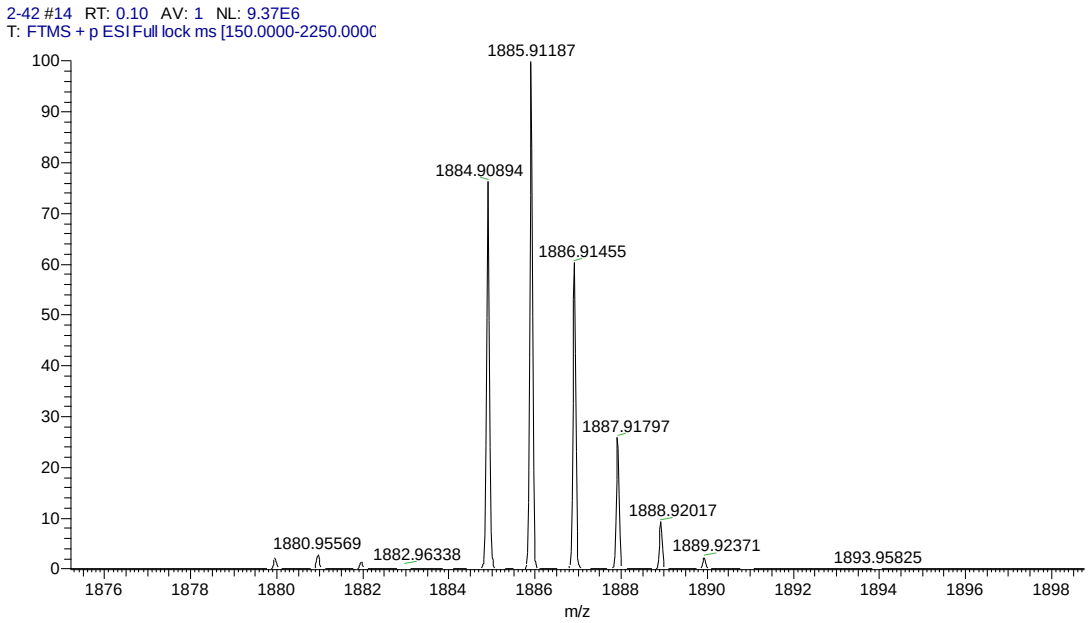
H-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{95}H_{113}N_{10}O_{15}PNa^{+}$ $(M+Na)^{+}$ 1687.80167, found 1687.80200.



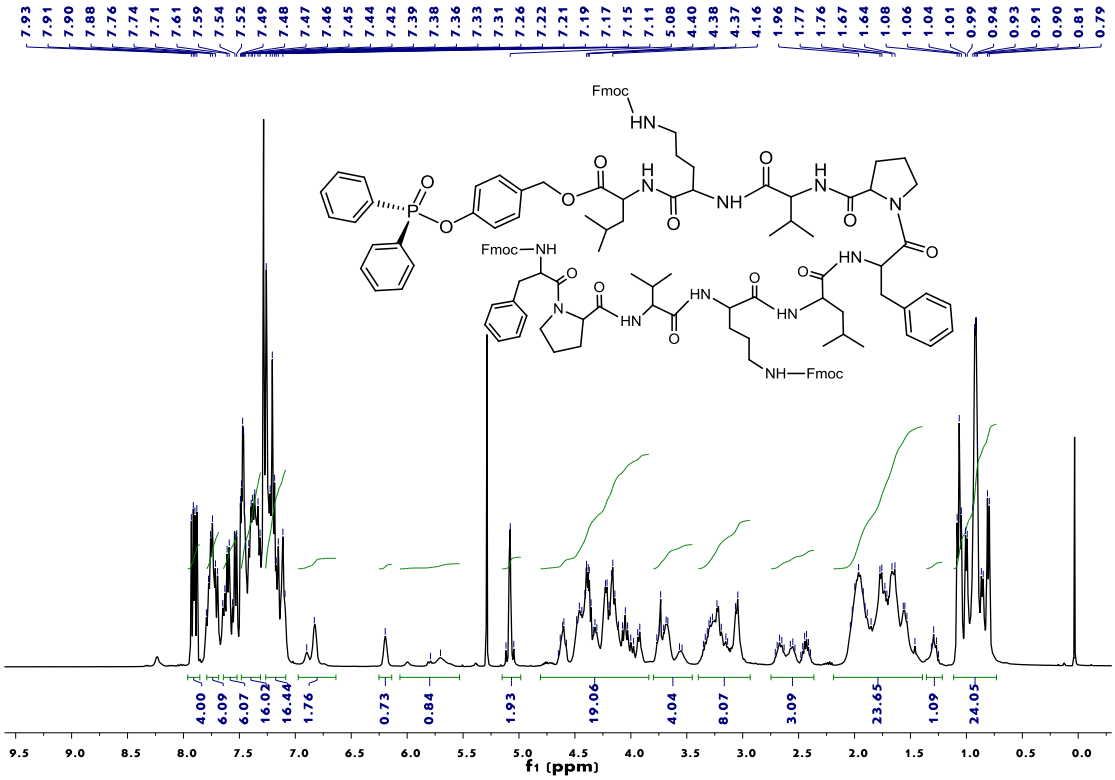
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



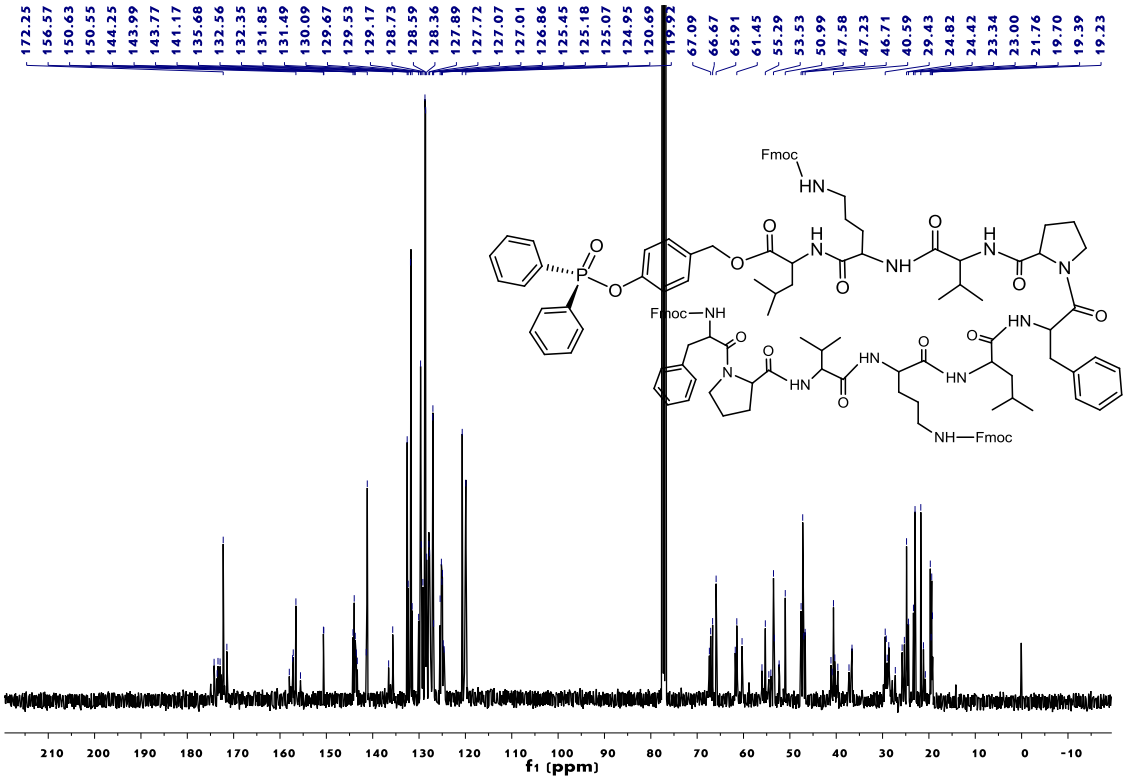
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



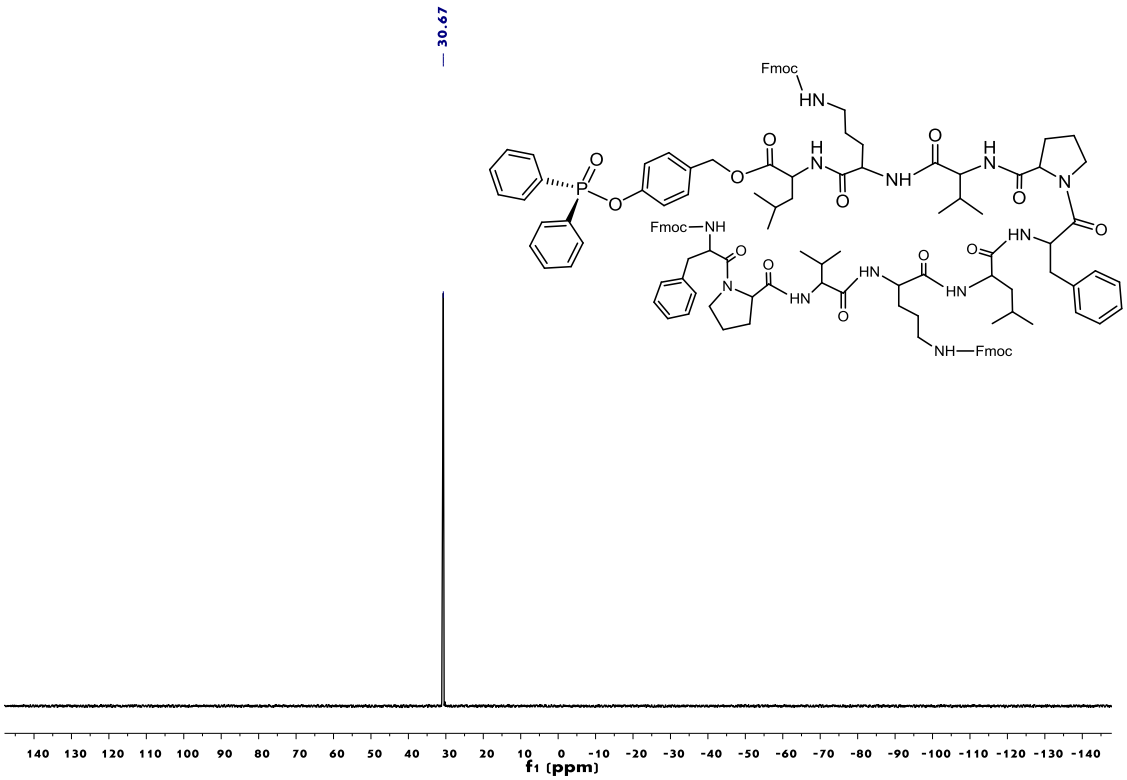
Boc-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{105}H_{128}N_{11}O_{18}PNa^{+}$ $(M+Na)^{+}$ 1884.90686, found 1884.90894.



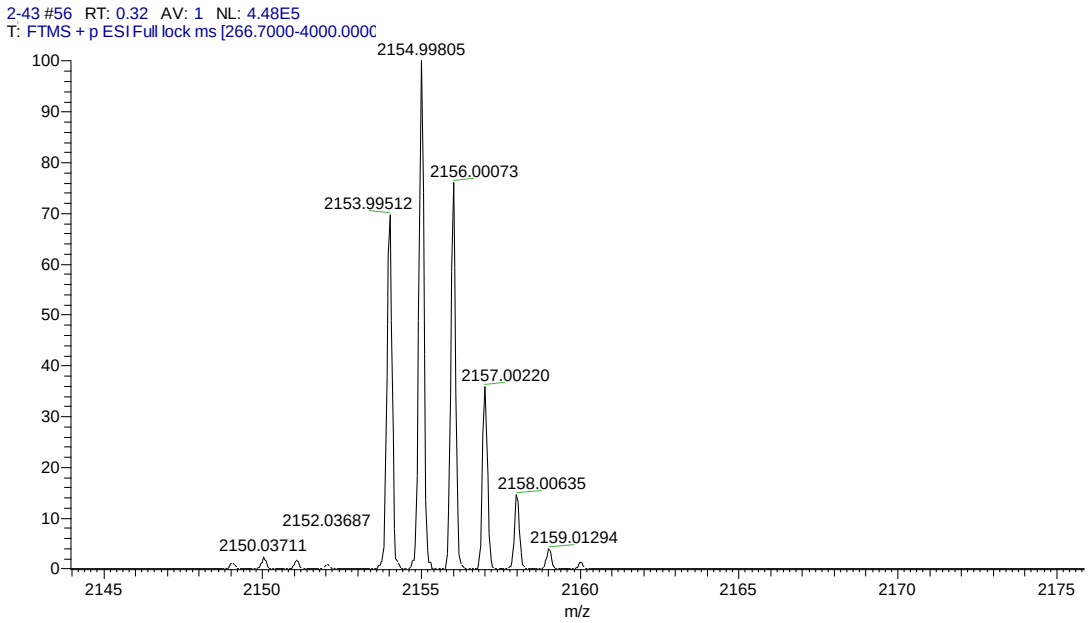
Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{1}H NMR (400 Hz, $CDCl_3$)



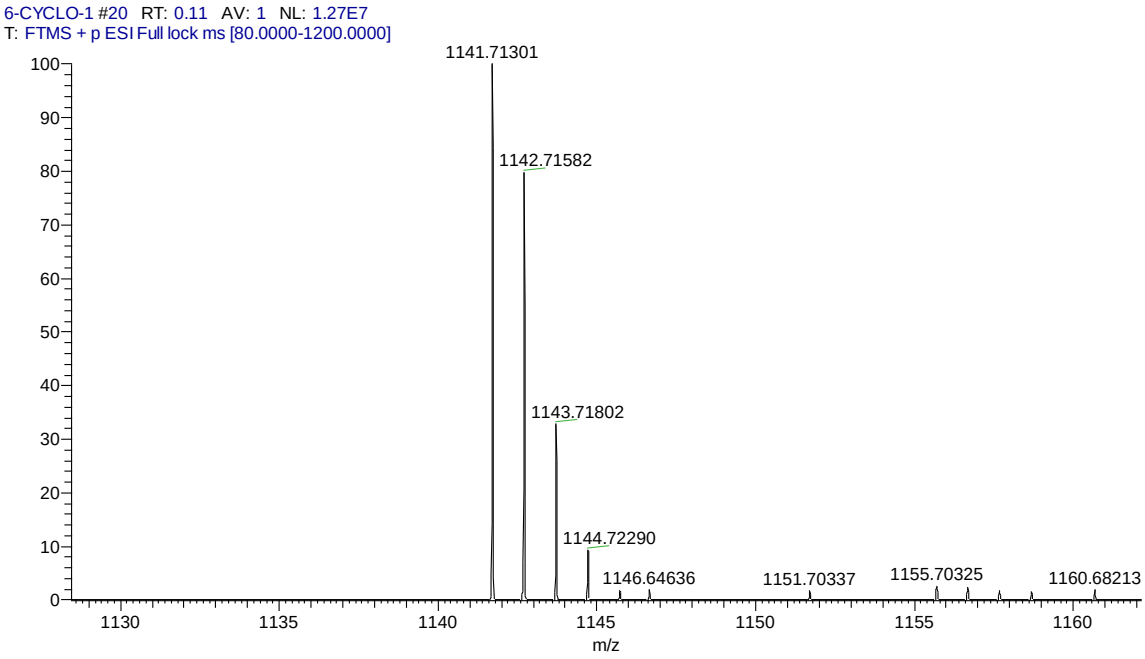
Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{13}C NMR (100 Hz, $CDCl_3$)



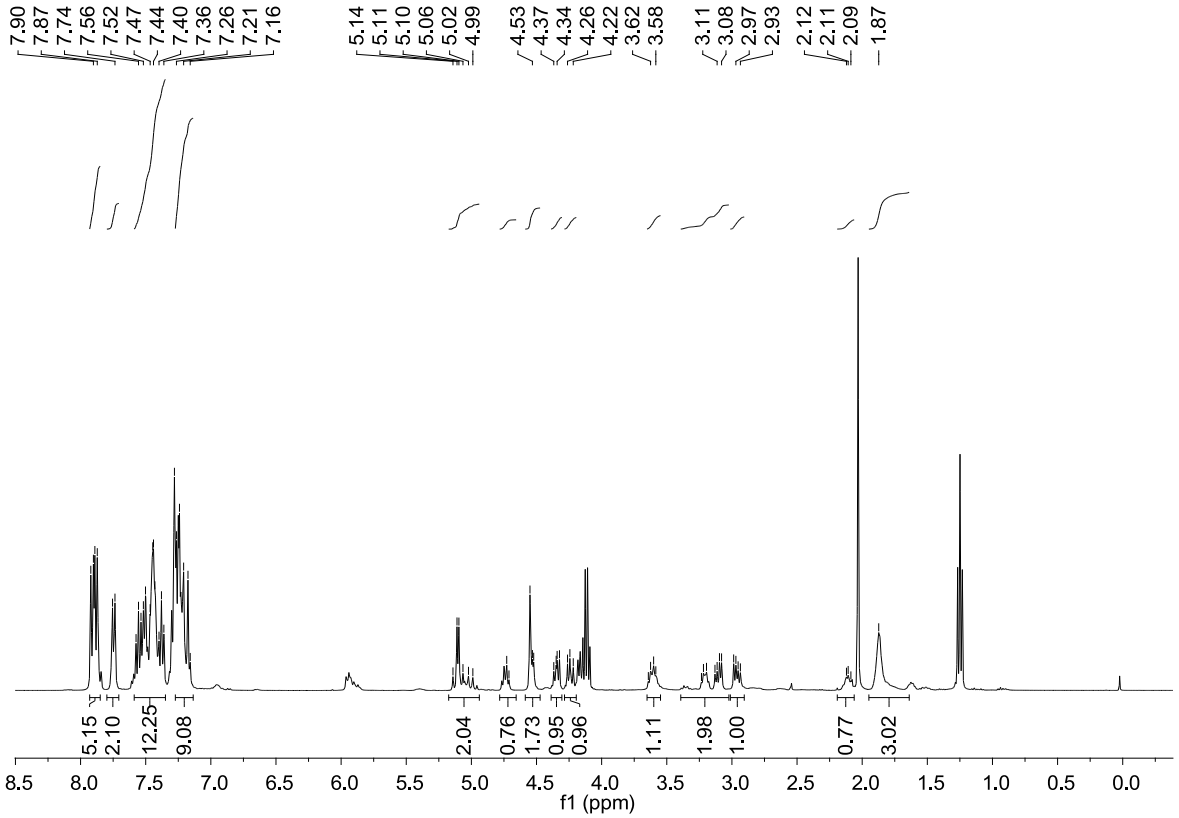
Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA ^{31}P NMR (163 Hz, $CDCl_3$)



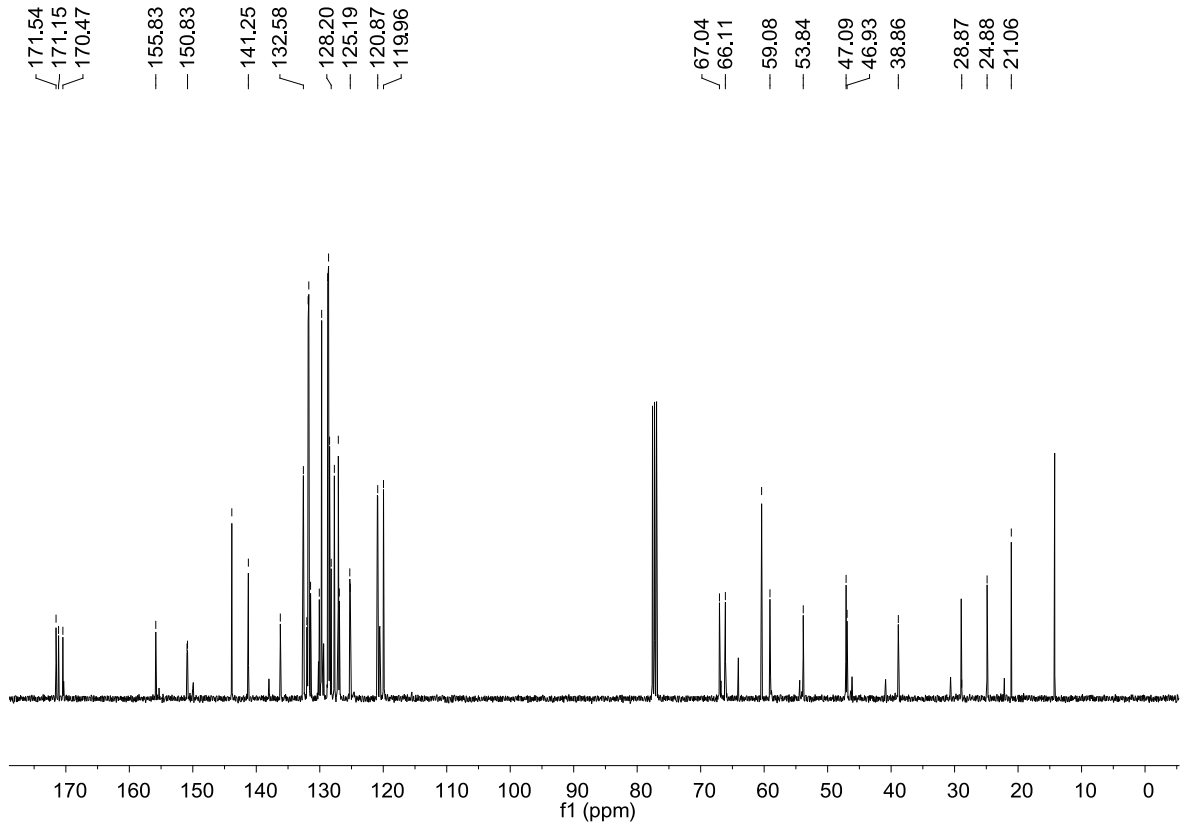
Fmoc-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(Fmoc)-^{L}Leu-O-DPBA HRMS (ESI) m/z calcd for $C_{124}H_{139}N_{12}O_{19}PNa^{+}$ $(M+Na)^{+}$ 2153.99093, found 2153.99512.



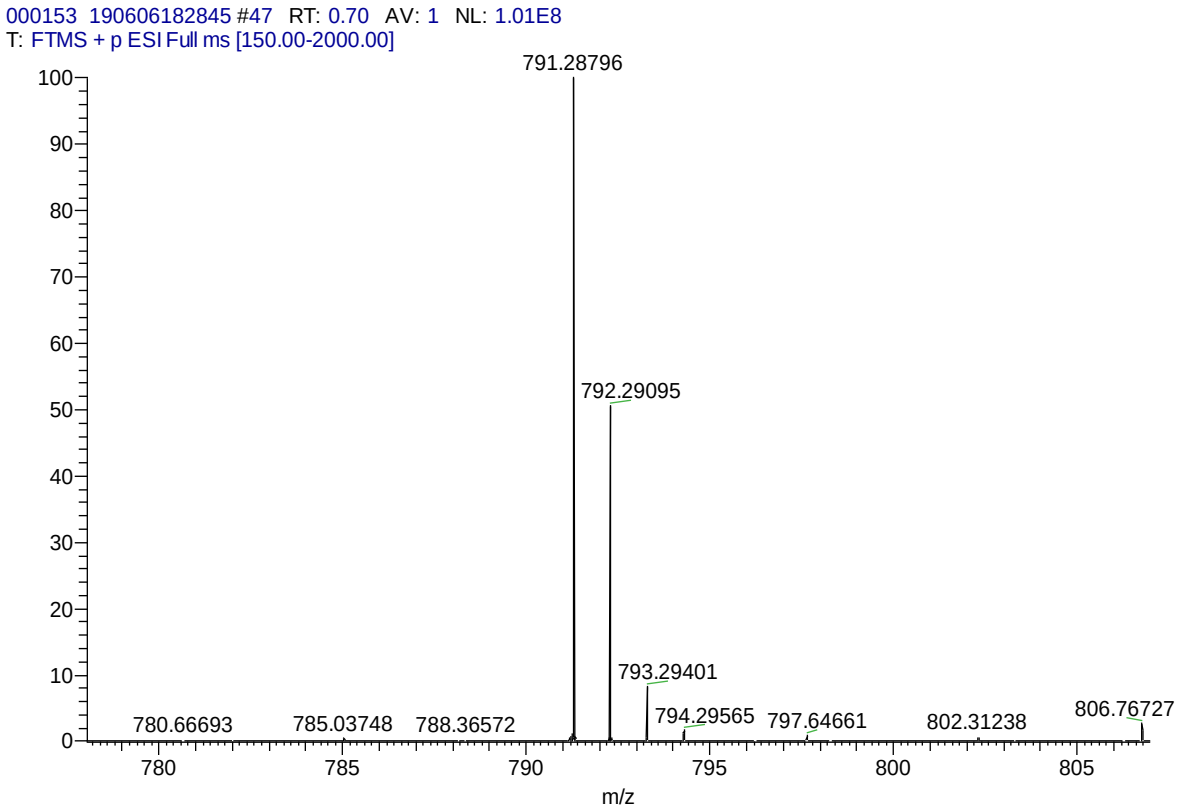
Gramicidin S *Cyclo*[^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH$_2$)-^{L}Leu-^{D}Phe-^{L}Pro-^{L}Val-^{L}Orn(NH$_2$)-^{L}Leu] HRMS (ESI) m/z calcd for $C_{60}H_{93}N_{12}O_{10}^{+}$ $(M+H)^{+}$ 1141.71321, found 1141.71301.



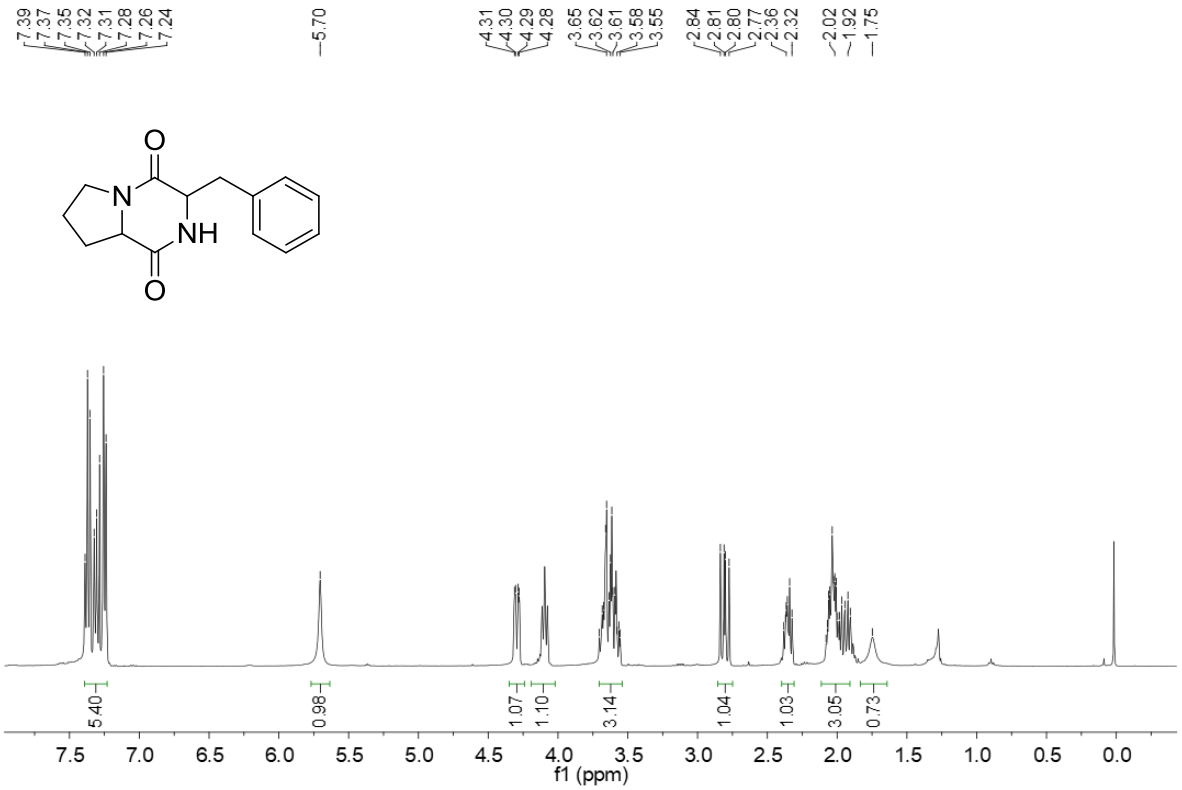
Fmoc-^{L}Phe-^{L}Pro-O-DPBA ^{1}H NMR (400MHz, $CDCl_3$)



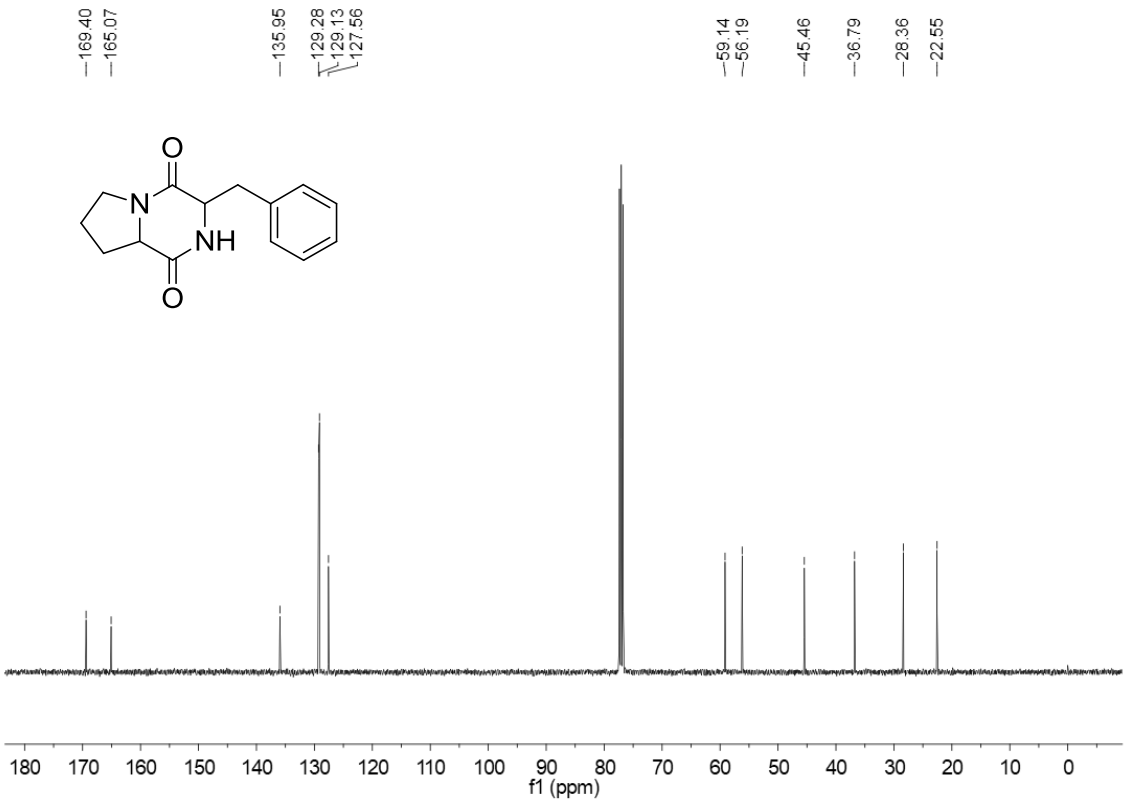
Fmoc-^{L}Phe-^{L}Pro-O-DPBA ^{13}C NMR (100MHz, $CDCl_3$)



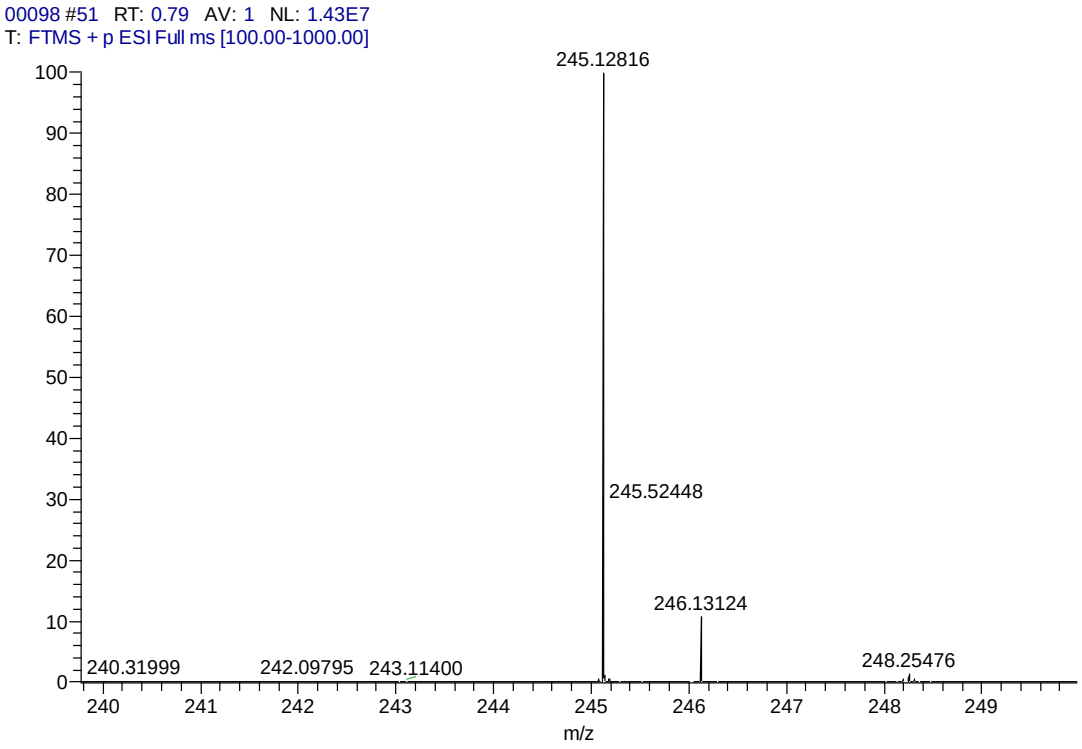
Fmoc-^{L}Phe-^{L}Pro-O-DPBA HRMS (ESI) m/z calcd for $C_{48}H_{44}N_2O_7P^+$ $[M+H]^+$ 791.28806, found 791.28796.



***Cyclo* (**^{L}**Phe-**^{L}**Pro)** ^{1}H NMR (400MHz, $CDCl_3$)



***Cyclo* (^LPhe-^LPro)** ^{13}C NMR (100MHz, $CDCl_3$)



***Cyclo* (^LPhe-^LPro)** HRMS (ESI) m/z calcd for $C_{14}H_{17}N_2O_2^+$ $[M+H]^+$ 245.12845, found 245.12816.