Supporting Information

Liquid-phase Total Synthesis of Plecanatide Aided by Diphenylphosphinyloxyl Diphenyl Ketone (DDK) Derivatives

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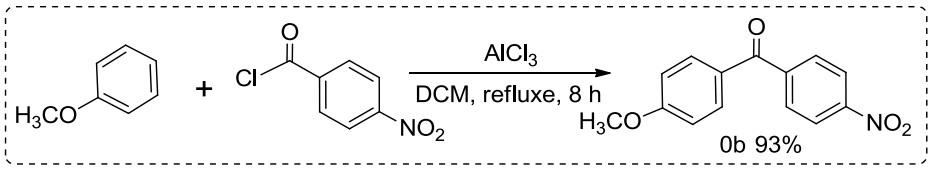
1. General considerations

All the Fmoc and Boc protected amino acids were obtained from G L Biochem (Shanghai) Ltd, all the reagents were purchased from commercial suppliers and used without further purification. All experiments were carried out using standard methods. The concentration of the samples was performed using the rotary evaporator (RE-52AA, Shanghai) and circulating water multi-purpose vacuum pump (SHB-III, Zhengzhou). The liquid phase stirring reactions were completed using the magnetic stirrer (ST 15 0SA, UK). TLC analysis was performed with the four-use UV analyzer (ZF-8, Shanghai) and silica gel GF_{254} (0.15mm thick, QingDao) plates. The melting point (mp) test was performed 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). Chemical shifts are expressed in ppm, and the coupling constant *J* values are given in Hz (TMS, δ 0.0 ppm). 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 peptides were performed with LC 3000 HPLC system with CXTH-3000 work station using analysis columns (Kromasil, NC-2546-06251151; 250 × 4.6 mm).

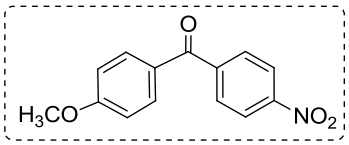
2. Abbreviations

AA: amino acid
Boc-Leu-OH: N-(tert-Butoxycarbonyl)-L-leucine
Boc-Val-OH: (S)-2-(tert-butoxycarbonylamino)-3-methylbutyric acid
DCM: dichloromethane
DDK: diphenylphosphinyloxyl diphenyl Ketone
DEA: diethylamine
DIEA: N, N-diisopropylethylamine
DMAP: 4-dimethylaminopyridine
EA: ethyl acetate
EDC·HCl: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDT: 1,2-ethanedithiol
Fmoc-Ala-OH: (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino) propanoic acid
Fmoc-Asp(tBu)-OH: (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-[(2-methylpropan-2-yl)oxy]-4-oxobutanoic acid
Fmoc-Asn(Trt)-OH: (S)-2-((((9h-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanoic acid
Fmoc-Cys(Acm)-OH: (R)-1-(9h-fluoren-9-yl)-3,10-dioxo-2-oxa-7-thia-4,9-diazaundecane-5-carboxylic acid
Fmco-Cys(Trt)-OH: 2-{[(9H-Fluoren-9-ylmethoxy)carbonyl]amino}-3-(tritylthio)propanoic acid
Fmoc-Glu(tBu)-OH: (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-5-[(2-methylpropan-2-yl)oxy]-5-oxopentanoic acid
Fmoc-Gly-OH: 2-(9H-fluoren-9-ylmethoxycarbonylamino) acetic acid
Fmoc-Leu-OH: (S)-2-((((9h-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid
Fmoc-Thr(tBu)-OH: (2S,3R)-2-(((9-Fluorenylmethoxy)carbonyl) amino)-3-(tert-butoxy) butanoicacid
Fmoc-Val-OH: (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methylbutanoic acid
HOBt: 1-hydroxybenzotriazole
MeCN: acetonitrile
MeOH: methanol
NCS: *N*-chlorosuccinimide
PE: petroleum ether
Py: pyridine
TEA: triethylamine
TFA: trifluoroacetic acid
THF: tetrahydrofuran
Tis: triisopropylsilane

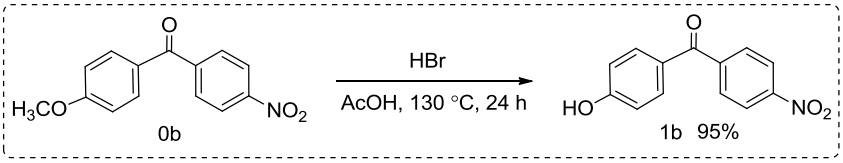
3. Synthesis of diphenylphosphinyloxyl diphenyl ketone (DDK) Derivatives



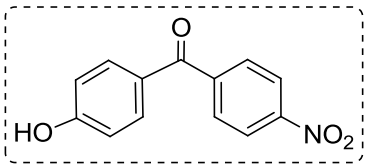
Synthesis of (**0b**, 4-methoxy-4'-nitrobenzophenone): 4-nitrobenzoyl chloride (1000 mg, 5.4 mmol) and anisole (0.60 mL, 5.5 mmol) were added to a solution of $AlCl_3$ (1670 mg, 12.5 mmol) in DCM (30 mL) at room temperature and the reaction mixture was refluxed for 8 h. Subsequently, 1.0 mol/L HCl was added to quench the reaction and to obtain the organic phase. The organic phase was washed by 1.0 mol/L NaOH for three times and dried with anhydrous $MgSO_4$. Then column chromatography was used to obtain the compound 4-methoxy-4'-nitrobenzophenone (**0b**) (1295 mg, 93% yield), pale yellow solid. R_f = 0.42 (CH_2Cl_2: MeOH= 60:1), mp 118.4- 121.2 °C.



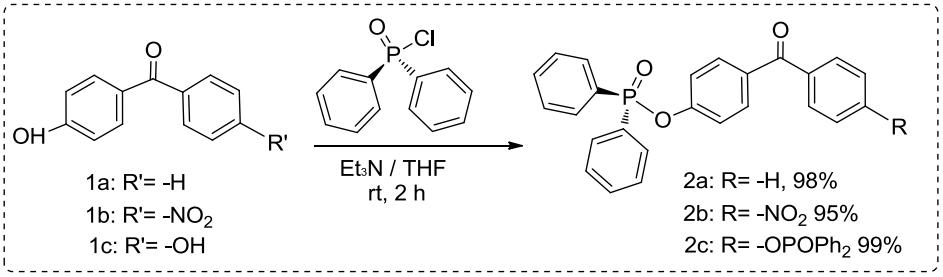
0b: ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.35-8.33 (d, J= 8.0 Hz, 2H), 7.90-7.81 (dd, J= 8.0 Hz, 4H), 7.02-7.00 (d, J= 8.0 Hz, 2H), 3.92 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 193.4, 164.0, 149.5, 143.8, 132.6, 130.3, 128.9, 123.5, 123.4, 114.0, 55.6 ppm; HRMS (ESI) m/z calcd for $C_{14}H_{12}NO_4^+$ $(M+H)^+$ 258.0760, found 258.0763.



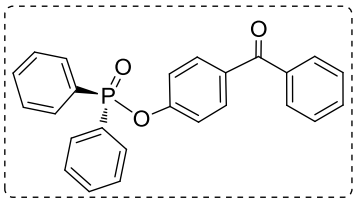
Synthesis of (**1b**, 4-hydroxy-4'-nitrobenzophenone): **0b** (1600 mg, 6.2 mmol) was added to a solution of 48% HBr (16 mL, 7.0 mmol) in AcOH (50 mL) at room temperature. The reaction mixture was stirred for 24 h at 130 °C and then quenched the reaction by adding ice water. Precipitation appeared after adding 1.0 mol/L NaOH to adjust the pH to 7.0~7.5. The precipitation was filtered to obtain the crude product. Then column chromatography was used to obtain the compound 4-hydroxy, 4'-nitrobenzophenone (**1b**) (1435 mg, 95% yield), pale yellow solid. R_f = 0.40 (CH_2Cl_2: MeOH= 40:1), mp 158.8- 162.0 °C.



1b: ^{1}H NMR (400 MHz, DMSO-d_6), δ 10.61 (s, 1H), 8.37-8.35 (d, J= 8.0 Hz, 2H), 7.90-7.88 (d, J= 8.0 Hz, 2H), 7.71-7.68 (d, J= 12.0 Hz, 2H), 6.94-6.92 (d, J= 8.0 Hz, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 193.3, 163.2, 149.4, 144.3, 133.2, 130.8, 130.6, 127.4, 124.1, 124.0, 116.0 ppm; HRMS (ESI) m/z calcd for $C_{13}H_{10}NO_4^+$ $(M+H)^+$ 244.0604, found 244.0603.

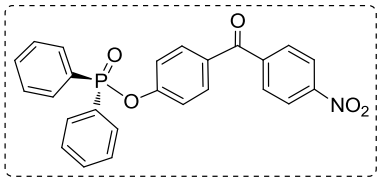


Synthesis of (**2a**, 4-diphenylphosphinyloxyl benzophenone): 4-Hydroxydiphenylketone (2380 mg, 12 mmol, 1.2 equiv) was added to a solution of Et_3N (1.7 mL, 12 mmol, 1.2 equiv) in THF (100 mL) at 0 °C and stirred at this temperature for 20 min. Subsequently, the reaction mixture was added with diphenylphosphinic chloride (1.9 mL, 10 mmol, 1.0 equiv) dropwise and stirred at room temperature for 2 h. The reaction mixture was quenched with 5 mL 0.1 mol/L H_2SO_4 and concentrated under reduced pressure. The dry residue was then dissolved in 50 mL ethyl acetate and washed with H_2O for three times and dried with $MgSO_4$. 5 mL ethyl acetate was added to dissolve the sample after concentrated, and 25 mL of petroleum ether (V_{EA}/V_{PE} =1:5) was added dropwise and stirred. Precipitate appeared, and the white precipitate was filtered and dried to afford the pure product **(2a)** (3910 mg, 98% yield), white solid. R_f = 0.48 (CH_2Cl_2: MeOH= 60:1), mp 140.4-141.5 °C.

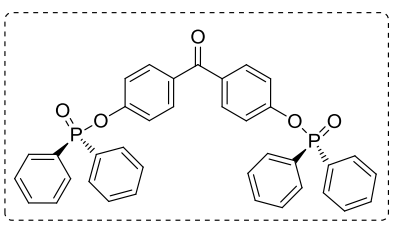


2a: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95-7.90 (m, 4H), 7.76-7.73 (m, 4H), 7.59-7.55 (m, 3H), 7.52-7.44 (m, 6H), 7.36-7.34 (d, J = 8.0 Hz, 2H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.51 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 195.4, 154.4, 137.5, 133.9, 132.8, 132.4, 132.1, 131.8, 131.7, 131.2, 129.9, 128.8, 128.7, 128.3, 120.5 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{20}O_3P^+$ $(M+H)^+$ 399.1144, found 399.1146.

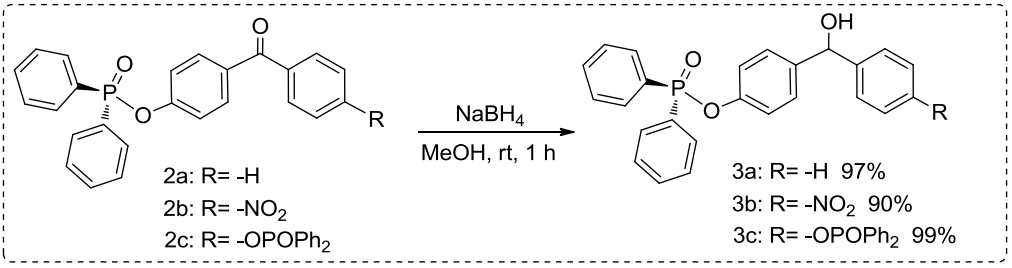
(2b) and **(2c)** were synthesized according to the above method of **(2a)**.



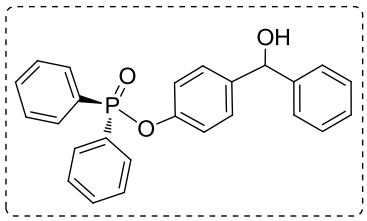
(**2b**, 4-diphenylphosphinyloxyl-4'-nitrobenzophenone)**:** (4215 mg, 95% yield), pale yellow solid, R_f = 0.45 (CH_2Cl_2: MeOH = 60:1), mp 151.9-154.8℃, (95% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.34-8.32 (d, J= 8.0 Hz, 1H), 7.95-7.89 (m, 2H), 7.82-7.72 (m, 5H), 7.59-7.49 (m, 4H), 7.41-7.38 (m, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 28.31 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 193.4, 155.3, 149.8, 142.8, 132.6, 132.2, 131.6, 131.2, 130.5, 128.9, 128.5, 123.6, 120.9 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{19}NO_5P^+$ $(M+H)^+$ 444.0995, found 444.0998.



(**2c**, 4,4'-bisdiphenylphosphinyloxyl benzophenone)**:** (6088 mg, 99% yield), white solid, R_f = 0.52 (CH_2Cl_2: MeOH = 60:1), mp 137.1-137.8℃, (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94-7.89 (m, 8H), 7.69-7.65 (m, 4H), 7.59-7.55 (m, 4H), 7.52-7.47 (m, 8H), 7.34-7.28 (m, 4H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.56 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 194.2, 154.4, 133.7, 132.8, 132.0, 131.8, 131.7, 131.1, 129.7, 128.9, 128.7, 120.5 ppm; HRMS (ESI) m/z calcd for $C_{37}H_{29}O_5P_2^+$ $(M+H)^+$ 615.1484, found 615.1484.

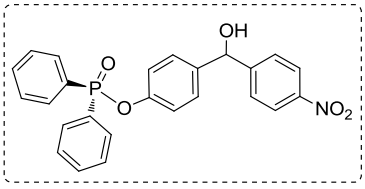


Synthesis of (**3a**, 4-diphenylphosphinyloxyl diphenylmethanol, **DDK-1**): $NaBH_4$ (456 mg, 12 mmol, 3.0 equiv) divided into three batches was added to a solution of **(2a)** (1600 mg, 4 mmol, 1.0 equiv) in CH_3OH (50 mL) at 0 ℃ and the reaction mixture was sealed and stirred at this temperature for 1 h. The reaction mixture was quenched by adding with 10 mL saturated NH_4Cl and concentrated to remove the CH_3OH, the residue was dissolved in 30 mL ethyl acetate and washed with H_2O for three times and dried with $MgSO_4$, 6 mL ethyl acetate was added to dissolve the sample after concentrated, and 42 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 pure product **(3a, DDK-1)** (1552 mg, 97% yield). White solid, R_f = 0.45 (CH_2Cl_2: MeOH= 40:1), mp 160.1-160.8 °C.

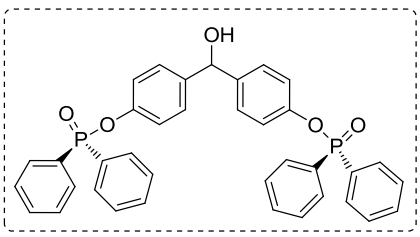


3a: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.84 (m, 4 H), 7.56-7.52 (m, 2H), 7.48-7.43 (m, 4H), 7.31-7.23 (m, 7H), 7.14-7.12 (d, *J*= 8.0 Hz, 2H), 5.74 (s, 1H), 2.99 (s, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.58 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 150.0, 143.9, 140.6, 132.5, 131.8, 131.7, 130.1, 128.7, 128.6, 128.4, 128.0, 127.4, 126.6, 120.6, 75.4 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{22}O_3P^+$ $(M+H)^+$ 401.1301, found 401.1298.

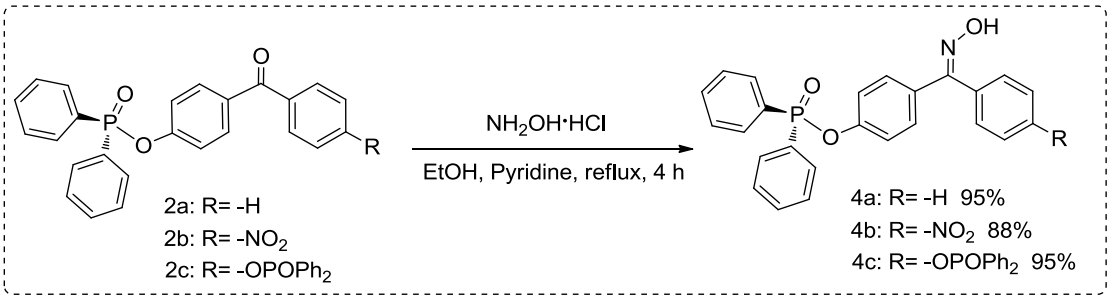
(3b) and **(3c)** were synthesized according to the above method of **(3a)**.



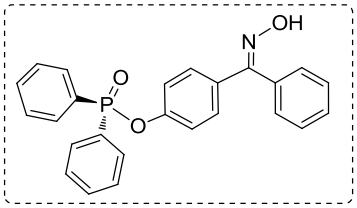
(**3b**, 4-diphenylphosphinyloxyl-4'-nitrodiphenylmethanol, **DDK-3):** (1605 mg, 90% yield), pale yellow solid, R_f = 0.40 (CH_2Cl_2: MeOH= 40:1), mp 182.2-184.1 °C, (90% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.17-8.15 (d, *J*= 8.0 Hz, 2H), 7.92-7.86 (dd, *J*= 8.0 Hz, 4H), 7.63-7.51 (m, 8H), 7.35-7.22 (dd, *J*= 8.0 Hz, 4H), 6.23-6.22 (d, *J*= 4.0 Hz, 1H), 5.81-5.80 (d, *J*= 4.0 Hz, 1H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.18 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 153.4, 150.2, 146.8, 141.4, 133.2, 132.0, 130.6, 129.4, 128.3, 127.6, 123.9, 120.9, 73.2 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{21}NO_5P^+$ $(M+H)^+$ 446.1152, found 446.1153.



(**3c**, 4,4'-bisdiphenylphosphinyloxyl diphenylmethanol, DDK-5)**:** (2442 mg, 99% yield), white solid, R_f = 0.50 (CH_2Cl_2: MeOH= 40:1), mp 187.2-189.3°C, (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.87-7.82 (m, 8H), 7.53-7.50 (m, 4H), 7.46-7.41 (m, 8H), 7.15-7.13 (d, *J*= 8.0 Hz, 4H), 7.07-7.05 (d, *J*= 8.0 Hz, 4H), 5.62 (s, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.54 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 140.5, 132.5, 131.8, 131.7, 131.5, 130.1, 128.7, 128.6, 128.0, 120.4, 74.5 ppm; HRMS (ESI) m/z calcd for $C_{37}H_{31}O_5P_2^+$ $(M+H)^+$ 617.1641, found 617.1641.

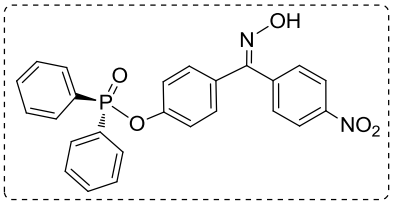


Synthesis of (**4a**, 4-diphenylphosphinyloxyl benzophenone oxime, **DDK-2**): $NH_2OH{\cdot}HCl$ (165 mg, 2.4 mmol, 1.2 equiv) was added to a solution of **(2a)** (800 mg, 2 mmol, 1.0 equiv) in pyridine and ethanol (33 mL, $V_{Ethanol:Pyridine}$ =10:1) and refluxed for 4 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in 30 mL ethyl acetate and washed with 0.1 mol/L HCl, dried with $MgSO_4$. Subsequently, 5 mL ethyl acetate was added to dissolve the sample after concentrated, and 30 mL of petroleum ether (V_{EA}/V_{PE} =1:6) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the pure product (**4a, DDK-2**) (780 mg, 95% yield). White solid, R_f = 0.47 (CH_2Cl_2: MeOH = 40:1), mp 168.1-169.2°C.

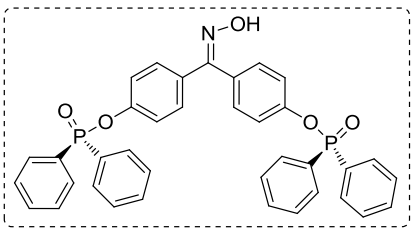


4a: ^{1}H NMR (400 MHz, $CDCl_3$), δ 9.50 (s, 1H), 7.99-7.90 (m, 4H), 7.59-7.28 (m, 14H), 7.22-7.20 (m, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.09 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 156.5, 151.3, 136.4, 132.7, 131.9, 131.3, 130.0, 129.8, 129.3, 129.0, 128.8, 128.6, 128.3, 128.0, 120.4 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{21}NO_3P^+$ $(M+H)^+$ 414.1253, found 414.1254.

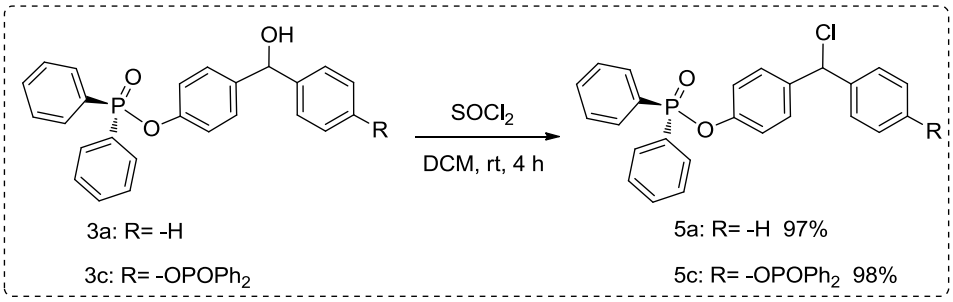
(4b) and **(4c)** were synthesized according to the above method of **(4a)**.



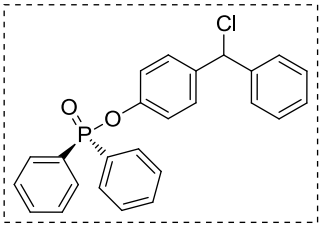
(**4b**, 4-diphenylphosphinyloxyl-4'-nitrobenzophenone oxime, **DDK-4**): (805 mg, 88% yield), pale yellow solid, R_f = 0.45 (CH_2Cl_2: MeOH= 40:1), mp 162.6-165.3℃, (88% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 11.98 (s, 1H), 8.30-8.20 (dd, J= 8.0 Hz, 2H), 7.98-7.90 (m, 4H), 7.65-7.53 (m, 8H), 7.41-7.31 (m, 4H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.76 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 153.5, 147.9, 143.3, 140.5, 133.3, 132.7, 132.0, 131.9, 131.7, 131.6, 131.3, 130.8, 130.6, 129.5, 129.4, 129.3, 129.2, 128.8, 128.5, 124.1, 120.9 ppm; HRMS (ESI) m/z calcd for $C_{25}H_{20}N_2O_5P^+$ $(M+H)^+$ 459.1104, found 459.1102.



(4c, 4,4'-bisdiphenylphosphinyloxyl benzophenone oxime, **DDK-6):** (1191 mg, 95% yield), white solid, R_f = 0.48 (CH_2Cl_2: MeOH= 40:1), mp 152.4-154.1℃, (95% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 10.68 (s, 1H), 7.96-7.87 (m, 8H), 7.57-7.43 (m, 12H), 7.32-7.30 (m, 6H), 7.21-7.19 (d, J= 8.0 Hz, 2H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.99 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 154.8, 151.4, 151.0, 133.5, 132.7, 131.9, 131.8, 131.3, 131.2, 131.0, 130.0, 129.6, 129.3, 129.1, 128.8, 128.7, 120.4, 120.2 ppm; HRMS (ESI) m/z calcd for $C_{37}H_{30}NO_5P_2^+$ $(M+H)^+$ 630.1593, found 630.1590.

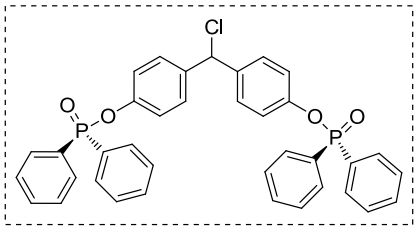


Synthesis of (**5a**, 4-diphenylphosphinyloxyl diphenylmethyl chloride, **DDK-7**): $SOCl_2$ (1 mL) was added dropwise to a solution of **(3a)** (400 mg, 1 mmol, 1 equiv) in dichloromethane (10 mL) at room temperature, then the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure at 40 ℃, then 3 mL ethyl acetate was added to the residue to dissolve the sample, and 18 mL of petroleum ether (V_{EA}/V_{PE} =1:6) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the pure product **(5a, DDK-7)** (405 mg, 97% yield), white solid, R_f = 0.40 (CH_2Cl_2: MeOH= 60:1), mp 78.3-79.6 °C.



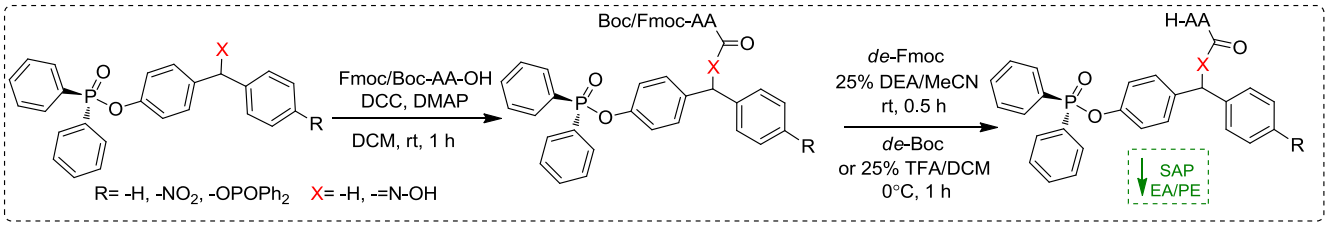
5a: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.85 (dd, *J*= 8.0 Hz, 4H), 7.48-7.39 (m, 6H), 7.32-7.16 (m, 9H), 6.02 (s, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.98 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 150.6, 140.8, 137.4, 132.7, 131.7, 129.3, 128.8, 128.6, 128.2, 127.8, 120.8, 63.6; HRMS (ESI) m/z calcd for $C_{25}H_{21}ClO_2P^+$ $(M+H)^+$ 419.0962, found 419.0960.

(5c, DDK-8) were synthesized according to the above method of **(5a)**.

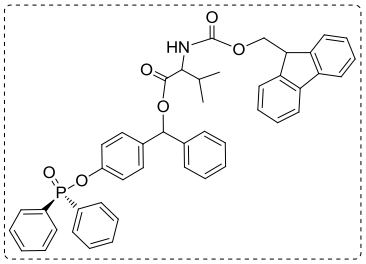


(**5c**, 4,4'-bisdiphenylphosphinyloxyl diphenylmethyl chloride, **DDK-8):** (620 mg, 98% yield), white solid, R_f = 0.45 (CH_2Cl_2: MeOH= 60:1), mp 153.5-156.1℃, (98% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.85 (dd, *J*= 8.0 Hz, 8H), 7.51-7.40 (m, 12H), 7.20-7.14 (m, 8H), 5.95 (s, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.00 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 150.6, 137.0, 132.7, 131.8, 131.4, 130.0, 129.2, 128.6, 120.8, 62.9; HRMS (ESI) m/z calcd for $C_{37}H_{30}ClO_4P_2^+$ $(M+H)^+$ 635.1302, found 635.1301.

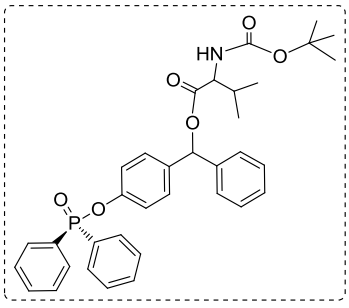
4. DDK derivatives coupled with Fmoc/Boc Amino acids



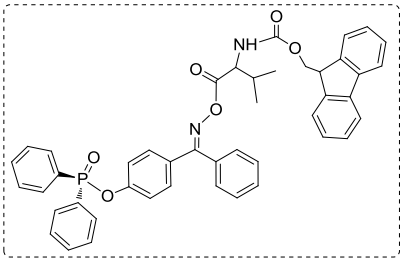
Synthesis of **Fmoc/Boc-AA-DDK-*n***: DCC (250 mg, 1.2 mmol, 1.2 equiv) and DMAP (15 mg, 0.12 mmol, 0.12 equiv) were added to a solution of Fmoc-Val-OH (or Boc-Val-OH) (1.2 mmol, 1.2 equiv) in dichloromethane (20 mL) at 0 °C and stirred at this temperature for 10 min. The reaction mixture was added with DDK-*n* (1.0 mmol, 1.0 equiv) and stirred at room temperature for 1 h. Subsequently, the insoluble was filtered out, and the solution was washed with saturated NH_4Cl for twice times and dried with anhydrous $MgSO_4$. 2 mL ethyl acetate was added to dissolve the sample after concentrated, and 18 mL of petroleum ether (V_{EA}/V_{PE}= 1:9) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the pure product as follows:



3a-1, Fmoc-Val-DDK-1: White solid, R_f = 0.45 (CH_2Cl_2: MeOH= 60:1), (730 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.84 (m, 4H), 7.76-7.74 (d, *J*= 8.0 Hz, 2H), 7.59-7.36 (m, 10H), 7.30-7.26 (m, 2H), 7.30-7.15 (m, 12H), 6.84 (s, 1H), 5.38-5.35 (d, *J*= 12.0 Hz 1H), 4.43-4.36 (m, 3H), 4.22-4.19 (m, 1H), 2.21-2.16 (m, 1H), 0.91-0.87 (dd, *J*= 8.0 Hz, 3H), 0.76-0.70 (dd, *J*= 8.0 Hz, 3H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.57 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.2, 156.3, 150.5, 143.8, 141.3, 139.1, 135.9, 132.6, 131.8, 130.1, 129.0, 128.6, 128.3, 127.7, 127.1, 125.2, 120.8, 120.0, 77.3, 67.1, 59.0, 47.2, 31.3, 19.1, 17.2 ppm; HRMS (ESI) m/z calcd for $C_{45}H_{40}NO_6PNa^+$ $(M+Na)^+$ 744.2486, found 744.2489.

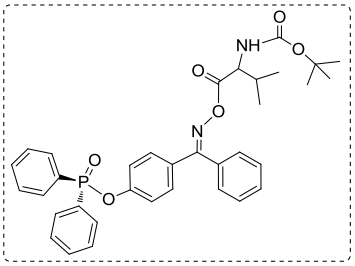


3a-2, Boc-Val-DDK-1: White solid, R_f = 0.43 (CH_2Cl_2: MeOH= 60:1), (615 mg, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.92-7.86 (m, 4H), 7.56-7.44 (m, 6H), 7.32-7.17 (m, 8H), 6.85 (s, 1H), 5.07-5.05 (d, *J*= 8.0 Hz, 1H), 4.35-4.33 (m, 1H), 2.18-2.14 (m, 1H), 1.44 (s, 9H), 0.91-0.88 (dd, *J*= 8.0 Hz, 3H), 0.75-0.69 (dd, *J*= 8.0 Hz, 3H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.64 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.5, 155.7, 150.6, 139.4, 136.1, 132.6, 131.8, 131.5, 130.1, 128.6, 128.2, 126.9, 120.8, 79.8, 77.0, 58.6, 31.2, 28.3, 19.1, 17.2 ppm; HRMS (ESI) m/z calcd for $C_{35}H_{38}NO_6PNa^+$ $(M+Na)^+$ 622.2329, found 622.2328.

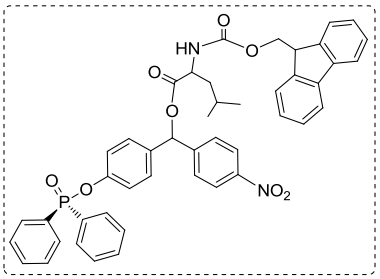


4a-1, Fmoc-Val-DDK-2: White solid, R_f = 0.42 (CH_2Cl_2: MeOH= 60:1), (712mg, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95-7.89 (m, 4H), 7.79-7.78 (d, *J*= 8.0 Hz, 2H), 7.62-7.25 (m, 21H), 5.36-5.31 (m, 1H), 4.43-4.39 (m, 2H), 4.29-4.23 (m, 2H), 2.01-1.99 (m, 1H), 0.98-0.80 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.56 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.6, 165.2, 156.1, 152.1, 143.7, 141.3, 134.1, 132.7, 131.8, 131.7, 131.3,

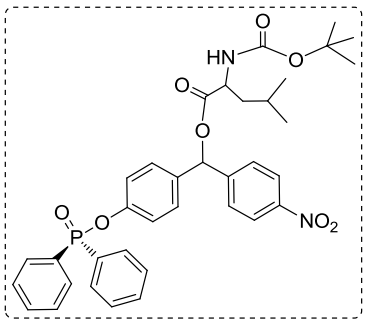
130.7, 130.6, 129.8, 129.2, 128.8, 128.7, 128.5, 128.3, 127.7, 127.1, 125.1, 120.7, 120.0, 67.1, 58.2, 47.2, 31.6, 22.7, 17.6 ppm; HRMS (ESI) m/z calcd for $C_{45}H_{40}N_2O_6P^+$ $(M+H)^+$ 735.2618, found 735.2617.



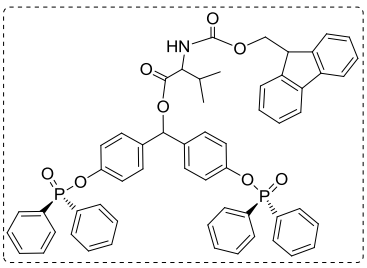
4a-2, Boc-Val-DDK-2: White solid, R_f = 0.43 (CH_2Cl_2: MeOH= 60:1), (600 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.97-7.87 (m, 4H), 7.61-7.21 (m, 15H), 5.04-5.02 (m, 1H), 4.20-4.17 (m, 1H), 1.95-1.90 (m, 1H), 1.45 (s, 9H), 0.88-0.77 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.66 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.9, 164.9, 155.5, 151.9, 134.2, 132.7, 132.1, 131.8, 131.7, 131.2, 130.6, 129.8, 129.1, 128.8, 128.7, 128.4, 128.3, 120.5, 79.9, 57.7, 31.3, 28.3, 18.8, 17.6 ppm; HRMS (ESI) m/z calcd for $C_{35}H_{38}N_2O_6P^+$ $(M+H)^+$ 613.2462, found 613.2461.



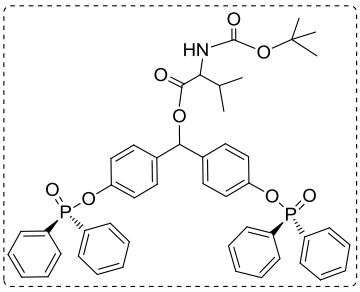
3b-1, Fmoc-Leu-DDK-3: White solid, R_f = 0.40 (CH_2Cl_2: MeOH= 60:1), (770mg, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.16-8.14 (d, *J*= 8.0 Hz, 2H), 7.92-7.78 (m, 6H), 7.59-7.39 (m, 12H), 7.32-7.19 (m, 6H), 6.87 (s, 1H), 5.19-5.16 (m, 1H), 4.50-4.41 (m, 3H), 4.23-4.19 (m, 1H), 1.67-1.53 (m, 3H), 0.94-0.89 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.18 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.8, 156.0, 151.0, 147.6, 146.4, 146.3, 143.7, 141.3, 134.4, 132.6, 131.8, 129.0, 128.8, 128.6, 127.8, 127.5, 127.1, 125.0, 123.9, 121.2, 120.0, 76.2, 67.1, 52.7, 47.1, 41.3, 24.8, 22.9, 21.8 ppm; HRMS (ESI) m/z calcd for $C_{46}H_{42}N_2O_8P^+$ $(M+H)^+$ 781.2673, found 781.2678.



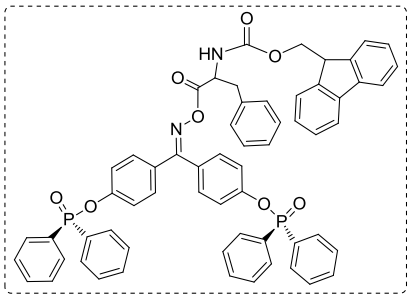
3b-2, Boc-Leu-DDK-3: White solid, R_f = 0.45 (CH_2Cl_2: MeOH= 60:1), (660mg, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.18-8.16 (m, 2H), 7.90-7.85 (m, 4H), 7.57-7.43 (m, 8H), 7.19 (m, 4H), 6.84 (s, 1H), 4.87-4.84 (m, 1H), 4.41-4.34 (m, 1H), 1.66-1.53 (m, 3H), 1.43 (s, 9H), 0.92-0.87 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 31.05 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.5, 155.4, 151.1, 147.6, 146.5, 134.6, 132.6, 131.8, 130.1, 128.7, 127.6, 123.8, 121.2, 88.1, 76.0, 52.3, 41.1, 28.3, 24.8, 22.8, 21.8 ppm; HRMS (ESI) m/z calcd for $C_{36}H_{39}N_2O_8PNa^+$ $(M+Na)^+$ 681.2336, found 681.2337.



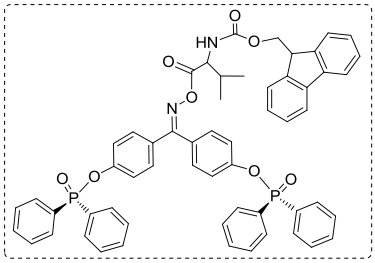
3c-1, Fmoc-Val-DDK-5: White solid, R_f = 0.45 (CH_2Cl_2: MeOH= 60:1), (920mg, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.83 (m, 8H), 7.76-7.74 (d, *J*= 8.0 Hz, 2H), 7.58-7.36 (m, 16H), 7.30-7.26 (m, 2H), 7.13 (m, 8H), 6.76 (s, 1H), 5.33-5.30 (d, *J*= 12.0 Hz 1H), 4.38-4.34 (m, 3H), 4.22-4.18 (m, 1H), 2.15-2.11 (m, 1H), 0.86-0.84 (dd, *J*= 8.0 Hz, 3H), 0.68-0.66 (dd, *J*= 8.0 Hz, 3H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.52 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.1, 156.3, 150.6, 143.9, 141.3, 135.6, 132.6, 131.8, 128.7, 128.5, 127.7, 127.1, 125.1, 120.8, 120.0, 76.6, 67.1, 58.9, 47.2, 31.3, 19.0, 17.2 ppm; HRMS (ESI) m/z calcd for $C_{49}H_{50}NO_8P_2Na^+$ $(M+Na)^+$ 960.2825, found 960.2820.



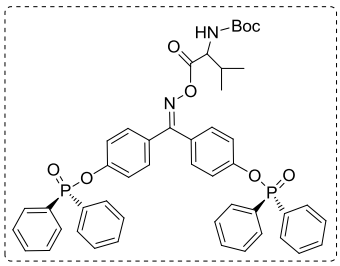
3c-2, Boc-Val-DDK-5: White solid, R_f = 0.47 (CH_2Cl_2: MeOH= 60:1), (805 mg, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.83 (m, 8H), 7.54-7.43 (m, 12H), 7.14 (m, 8H), 6.75 (s, 1H), 5.01-4.99 (d, *J*= 8.0 Hz, 1H), 4.27-4.24 (m, 1H), 2.11-2.06 (m, 1H), 1.41 (s, 9H), 0.84-0.83 (dd, *J*= 8.0 Hz, 3H), 0.65-0.63 (d, *J*= 8.0 Hz, 3H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.69 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.4, 155.7, 150.6, 135.7, 132.6, 131.8, 131.4, 130.1, 128.7, 120.7, 79.8, 76.4, 58.5, 31.1, 28.3, 19.0, 17.2 ppm; HRMS (ESI) m/z calcd for $C_{47}H_{48}NO_8P_2^+$ $(M+H)^+$ 816.2849, found 816.2845.



4c-1, Fmoc-Phe-DDK-6: White solid, R_f = 0.45 (CH_2Cl_2: MeOH= 60:1), (950 mg, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94-7.88 (m, 8H), 7.79-7.77 (d, *J*= 8.0 Hz, 2H), 7.59-7.54 (m, 6H), 7.52-7.45 (m, 8H), 7.43-7.39 (m, 4H), 7.33-7.22 (m, 9H), 7.04-6.98 (m, 4H), 5.33-5.30 (d, *J*= 12.0 Hz,1H), 4.62-4.57 (m, 1H), 4.43-4.32 (m, 2H), 4.23-4.19 (m, 1H), 3.04-2.91 (m, 2H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.74 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.1, 164.2, 155.4, 153.5, 152.1, 143.7, 141.3, 135.3, 132.7, 131.8, 131.2, 130.8, 130.6, 130.5, 129.8, 129.4, 128.8, 128.7, 127.7, 127.1, 125.1, 120.8, 120.0, 67.1, 54.2, 47.1, 38.5 ppm; HRMS (ESI) m/z calcd for $C_{61}H_{49}N_2O_8P_2^+$ $(M+H)^+$ 999.2958, found 999.2956.

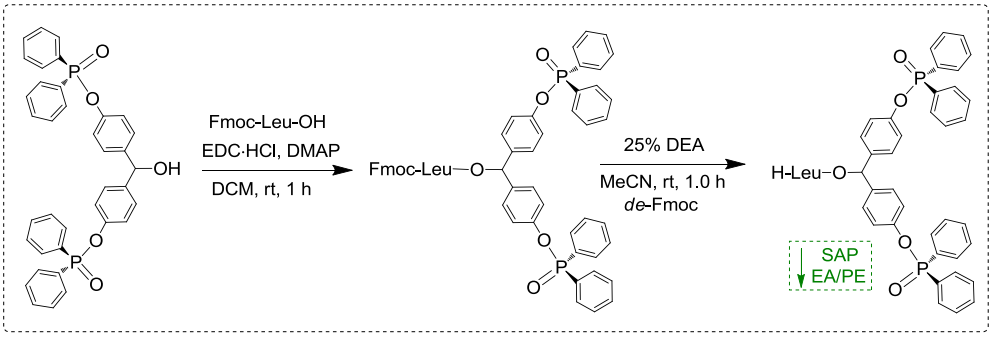


4c-2, Fmoc-Val-DDK-6: White solid, R_f = 0.47 (CH_2Cl_2: MeOH= 60:1), (920 mg, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.93-7.88 (m, 8H), 7.78-7.76 (d, *J*= 8.0 Hz, 2H), 7.62-7.55 (m, 6H), 7.51-7.39 (m, 12H), 7.34-7.29 (m, 4H), 7.24-7.16 (m, 4H), 5.34-5.31 (d, *J*= 12.0 Hz, 1H), 4.41-4.40 (d, *J*= 4.0 Hz, 2H), 4.25-4.21 (m, 2H), 2.01-1.93 (m, 1H), 0.87-0.85 (d, *J*= 8.0 Hz, 3H), 0.79-0.78 (d, *J*= 8.0 Hz, 3H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.79 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.5, 164.4, 156.1, 153.5, 152.1, 143.7, 141.3, 132.7, 131.8, 131.7, 131.2, 130.7, 130.5, 129.9, 129.8, 128.8, 128.7, 128.2, 127.7, 127.1, 125.1, 120.8, 120.7, 120.0, 67.1, 58.2, 47.2, 31.3, 18.8, 17.6 ppm; HRMS (ESI) m/z calcd for $C_{57}H_{49}N_2O_8P_2^+$ $(M+H)^+$ 951.2958, found 951.2960.

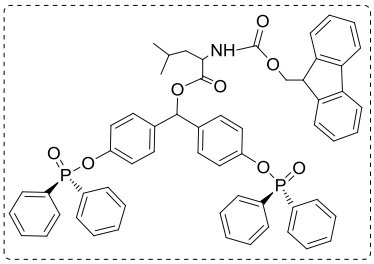


4c-3, Boc-Val-DDK-6: White solid, R_f = 0.48 (CH_2Cl_2: MeOH= 60:1), (780 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.95-7.86 (m, 8 H), 7.61-7.41 (m, 14 H), 7.31-7.29 (m, 2 H), 7.22-7.16 (m, 4 H), 5.01-4.98 (d, 1 H), 4.16-4.12 (m, 1 H), 1.92-1.88 (m, 1 H), 1.43 (s, 9 H), 0.85-0.83 (d, 3 H), 0.76-0.75 (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 $C_{47}H_{47}N_2O_8P_2^+$ $(M+H)^+$ 829.2802, found 829.2805.

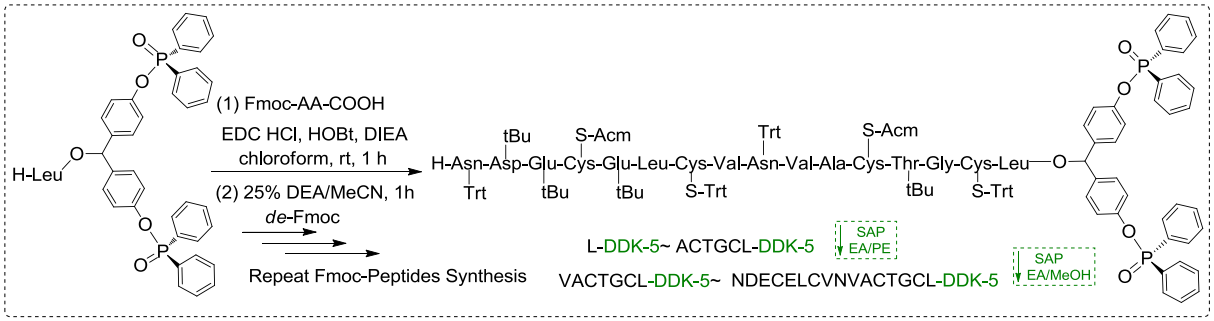
5. Synthesis procedure of Plecanatide



Synthesis of **Fmoc-Leu-DDK-5**: EDC·HCl (1146 mg, 6.0 mmol, 1.2 equiv) and DMAP (45 mg, 0.6 mmol, 0.12 equiv) were added to a solution of Fmoc-Leu-OH (2118 mg, 6.0 mmol, 1.2 equiv) in DCM (30 mL) at 0 °C and stirred for 10 min. The reaction mixture was added with (**3c**, **DDK-5**) (3085 mg, 5.0 mmol, 1.0 equiv) and stirred at room temperature for 1 h. The mixture was then washed with saturated NH_4Cl and Na_2CO_3 orderly and dried with $MgSO_4$. 4.0 mL ethyl acetate was added to dissolve the sample after concentrated, and 25 mL of petroleum ether (V_{EA}/V_{PE} =1:6) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the product **Fmoc-Leu-DDK-5** (4662 mg, 98% yield) Then the above **Fmoc-Leu-DDK-5** was added in 25% DEA/MeCN for 1 h to obtain the *de*-Fmoc product **H-Leu-DDK-5** for the next use.

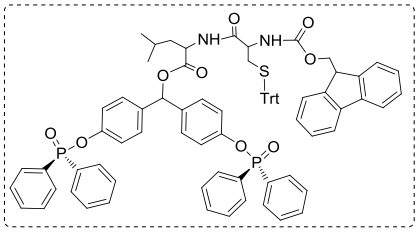


3c-3, Fmoc-Leu-DDK-5: White solid, R_f = 0.42 (CH_2Cl_2: MeOH= 60:1), (4662 mg, 4.9 mmol, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.88-7.83 (m, 8H), 7.75-7.74 (m, 2H), 7.58-7.50 (m, 6H), 7.39-7.35 (m, 10H), 7.29-7.25 (m, 2H), 7.11 (m, 8H), 6.74 (s, 1H), 5.22-5.20 (d, *J*= 8.0 Hz, 1H), 4.43-4.35 (m, 3H), 4.20-4.16 (m, 1H), 1.59-1.44 (m, 3H), 0.88-0.84 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 29.75 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.0, 155.9, 150.6, 143.7, 141.3, 135.5, 132.5, 131.8, 131.7, 130.2, 128.7, 128.6, 128.5, 127.7, 127.1, 125.1, 120.8, 120.0, 76.6, 67.0, 52.6, 47.2, 41.4, 24.7, 22.8, 21.8 ppm; HRMS (ESI) m/z calcd for $C_{58}H_{52}NO_8P_2^+$ $(M+H)^+$ 952.3162, found 952.3164.



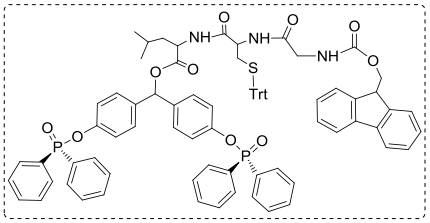
Synthesis of **Fmoc-Cys(Trt)-Leu-DDK-5:** EDC·HCl (1123 mg, 5.88 mmol, 1.2 equiv) and DIEA (1.0 mL, 5.88 mmol, 1.2 equiv) were added to a solution of H-Leu-DDK-5 (4662 mg, 4.9 mmol, 1.0 equiv), Fmoc-Cys(Trt)-OH (3439 mg, 5.88 mmol, 1.2 equiv), and HOBt (793 mg, 5.88 mmol, 1.2 equiv) in DCM (60 mL) at 0 °C and stirred for 1.0 h. The mixture was then washed with saturated Na_2CO_3, dried with $MgSO_4$. 5.0 mL ethyl acetate was added to dissolve the

sample after concentrated, and 30 mL of petroleum ether (V_{EA}/V_{PE} =1:6) was added dropwise and stirred. Precipitate appeared, and the precipitate was filtered and dried to afford the product **Fmoc-Cys(Trt)-Leu-DDK-5.**

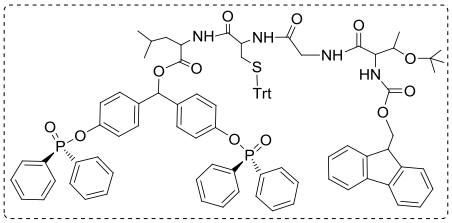


3c-4, Fmoc-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.42 (CH_2Cl_2: MeOH= 60:1), (6156 mg, 4.75 mmol, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.83 (m, 8H), 7.75-7.71 (m, 2H), 7.54-7.50 (m, 6H), 7.46-7.35 (m, 15H), 7.25-7.03 (m, 20H), 6.69 (m, 1H), 6.14-6.12 (d, *J*= 8.0 Hz, 1H), 5.00-4.98 (d, *J*= 8.0 Hz, 1H), 4.54-4.50 (m, 1H), 4.35-4.34 (d, *J*= 4.0 Hz, 2H), 4.18-4.15 (m, 1H), 3.72-3.70 (m, 1H), 2.72-2.51 (m, 2H), 1.53-1.36 (m, 3H), 0.78-0.74 (dd, *J*= 4.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 29.95 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.1, 169.8, 150.7, 144.3, 143.7, 141.3, 135.5, 132.6, 131.7, 130.2, 129.6, 128.7, 128.6, 128.1, 127.8, 126.9, 125.1, 120.8, 120.0, 76.5, 67.4, 67.1, 54.0, 50.9, 47.1, 41.0, 24.7, 22.7, 21.9 ppm; HRMS (ESI) m/z calcd for $C_{80}H_{71}N_2O_9P_2S^+$ $(M+H)^+$ 1297.4350, found 1297.4357.

Extension of DDK attached linear Plecanatide: Using the above coupling reagent system EDC•HCl/ HOBt/DIEA and the above *de*-Fmoc reagent system 25% DEA in MeCN to extend the DDK attached Plecanatide linear-chain and to obtain the intermediate peptide products as follows, [The intermediates of L-**DDK-5**~ACTGCL-**DDK-5** were precipitated by using the EA/PE solvent system, The DDK-5 attached intermediates of VACTGCL-**DDK-5~**NDECELCVNVACTGCL-**DDK-5** were precipitated by using the EA/ACN solvent system].

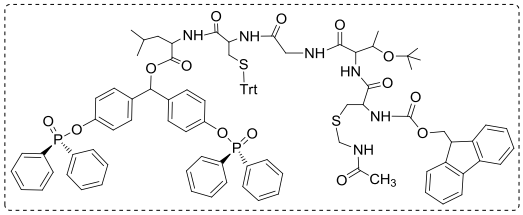


3c-5, Fmoc-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.40 (CH_2Cl_2: MeOH= 60:1), (6304 mg, 4.66 mmol, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.88-7.81 (m, 8H), 7.75-7.72 (m, 2H), 7.58-7.50 (m, 6H), 7.45-7.34 (m, 15H), 7.26-7.22 (m, 8H), 7.18-7.08 (m, 12H), 6.83-6.81 (d, *J*= 8.0 Hz, 1H), 6.66 (s, 1H), 6.50-6.47 (m, 1H), 6.34-6.32 (d, *J*= 8.0 Hz, 1H), 4.62-4.57 (m, 1H), 4.26-3.94 (m, 4 H), 3.65-3.64 (m, *J*= 4.0 Hz, 2H), 2.97-2.92 (m, 1 H), 2.44-2.40 (m, 1 H), 1.59-1.52 (m, 3H), 0.82-0.80 (m, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 29.85 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 170.9, 169.4, 168.9, 157.1, 150.5, 144.3, 143.8, 141.2, 135.7, 132.5, 131.6, 130.1, 129.5, 128.6, 128.1, 127.6, 126.9, 125.2, 120.7, 119.9, 76.3, 67.2, 52.1, 50.8, 46.9, 45.0, 40.4, 33.3, 24.6, 22.9, 21.7 ppm; HRMS (ESI) m/z calcd for $C_{82}H_{74}N_3O_{10}P_2S^+$ $(M+H)^+$ 1354.4564, found 1354.4563.

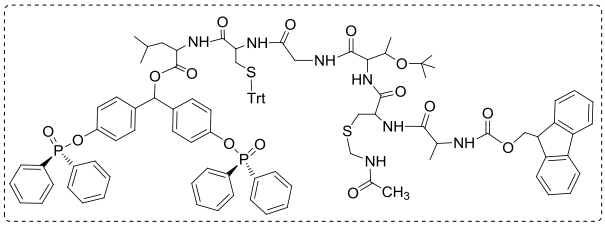


3c-6, Fmoc-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.40 (CH_2Cl_2: MeOH= 50:1), (6863 mg, 4.48 mmol, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.88-7.83 (m, 8H), 7.76-7.74 (d, *J*= 8.0 Hz, 2H), 7.60-7.50 (m, 6H), 7.46-7.36 (m, 15H), 7.30-7.17 (m, 12H), 7.10-7.07 (m, 9H), 6.69 (s, 1H), 6.42-6.37 (m, 2H), 5.92-5.91 (m, 1H), 4.50-4.37 (m, 3H), 4.22-4.13 (m, 3H), 3.90-3.73 (m, 2H), 3.65 (s, 1H), 2.85-2.80 (m, 1H), 2.50-2.45 (m, 1H), 1.54-1.41 (m, 3H), 1.24 (s, 9H), 1.02-1.01 (d, *J*= 4.0 Hz, 3H), 0.80-0.79 (m, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 29.99 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.1, 170.3, 169.4, 168.3, 156.1, 150.5, 144.4, 143.7, 141.3, 135.7. 132.6, 131.7, 130.2, 129.6, 128.7, 128.5, 128.1,

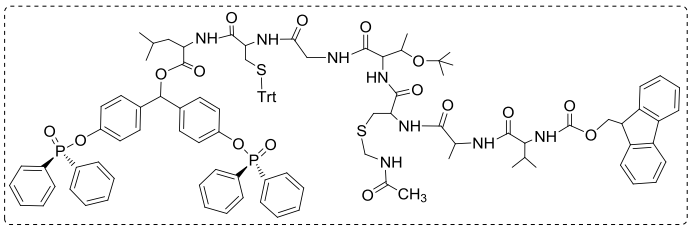
127.7, 126.9, 125.2, 120.7, 120.0, 77.3, 76.4, 75.6, 67.3, 64.5, 59.0, 52.4, 51.1, 47.2, 43.4, 40.9, 33.2, 28.2, 24.7, 22.8, 21.9, 17.5 ppm; HRMS (ESI) m/z calcd for $C_{90}H_{88}N_4O_{12}P_2SNa^+$ $(M+Na)^+$ 1533.5486, found 1533.5483.



3c-7, Fmoc-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.38 (CH_2Cl_2: MeOH= 50:1), (7330 mg, 4.35 mmol, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.88-7.83 (m, 8H), 7.76-7.74 (d, *J*= 8.0 Hz, 2H), 7.63-7.51 (m, 8H), 7.46-7.35 (m, 15H), 7.29-7.27 (m, 1H), 7.24-7.20 (m, 8H), 7.17-7.15 (m, 12H), 6.78-6.77 (m, 1H), 6.66 (s, 1H), 6.47-6.42 (m, 2H), 4.61-4.59 (m, 1H), 4.47-4.12 (m, 8H), 3.95-3.75 (m, 2H), 3.66 (s, 1H), 3.02-2.80 (m, 3H), 2.55-2.51 (m, 1H), 1.92 (s, 3H), 1.49-1.47 (m, 3H), 1.17 (s, 9H), 1.09-1.07 (d, *J*= 8.0 Hz, 3H), 0.80-0.77 (m, 6H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 29.49 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.5, 171.1, 170.6, 169.6, 168.6, 156.7, 150.5, 144.4, 143.8, 141.3, 135.7. 132.6, 131.8, 130.1, 129.6, 128.8, 128.1, 127.8, 127.1, 126.9, 125.3, 120.7, 120.0, 77.3, 76.3, 75.1, 67.2, 66.2, 64.6, 63.8, 59.1, 54.7, 52.8, 51.3, 47.1, 43.2, 40.6, 33.4, 28.2, 24.6, 23.1, 22.8, 21.8, 19.2 ppm; HRMS (ESI) m/z calcd for $C_{96}H_{99}N_6O_{14}P_2S_2^+$ $(M+H)^+$ 1685.6130, found 1685.6136.

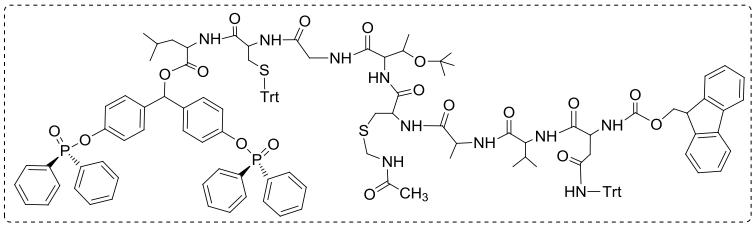


3c-8, Fmoc-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.45 (CH_2Cl_2: MeOH= 40:1), (7283 mg, 4.15 mmol, 95% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.53-8.50 (m, 1H), 8.38-8.37 (m, 1H), 8.32-8.26 (m, 4H), 7.90-7.84 (m, 10H), 7.76-7.72 (m, 2H), 7.63-7.53 (m, 13H), 7.43-7.40 (m, 2H), 7.35-7.10 (m, 25H), 6.64 (s, 1 H), 4.66-4.63 (m, 1H), 4.52-4.46 (m, 1H), 4.34-4.14 (m, 9H), 3.98-3.80 (m, 2H), 2.99-2.71 (m, 2H), 2.42-2.29 (m, 2H), 1.85 (s, 3H), 1.52-1.42 (m, 3H), 1.28-1.26 (m, *J*= 8.0 Hz, 3H), 1.10 (s, 9H), 1.00-0.99 (d, *J*= 4.0 Hz, 3H), 0.78-0.71 (dd, *J*= 8.0 Hz, 6H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.45 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 173.3, 171.1, 170.7, 170.3, 170.1, 169.7, 168.6, 156.1, 150.5, 144.7, 144.2, 141.2, 136.7, 133.3, 131.9, 130.6, 129.5, 128.5, 128.1, 127.5, 127.2, 125.8, 120.9, 120.6, 75.9, 74.3, 67.3, 66.3, 66.2, 65.5, 63.3, 58.0, 53.0, 51.7, 51.1, 50.4, 47.1, 28.4, 24.5, 23.1, 23.0, 21.8, 19.4, 18.9 ppm; HRMS (ESI) m/z calcd for $C_{99}H_{104}N_7O_{15}P_2S_2^+$ $(M+H)^+$ 1756.6501, found 1756.6502.

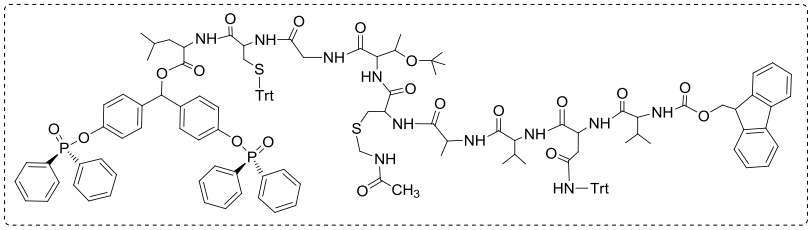


3c-9, Fmoc-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys (Trt)-Leu-DDK-5: White solid, R_f = 0.40 (CH_2Cl_2: MeOH= 40:1), (7382 mg, 3.98 mmol, 96% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.54-8.51 (m, 1H), 8.39-8.28 (m, 5H), 8.07-8.05 (m, 1H), 7.90-7.86 (m, 10H), 7.77-7.73 (m, 2H), 7.61-7.40 (m, 15H), 7.34-7.10 (m,25 H), 6.64 (s, 1 H), 4.68-4.62 (m, 1 H), 4.51-4.49 (m, 2 H), 4.43-4.20 (m, 8H), 3.98-3.80 (m, 3H), 2.99-2.69 (m, 2H), 2.42-2.30 (m, 2H), 2.04-1.99 (m, 1H), 1.86 (s, 3H), 1.52-1.42 (m, 3H), 1.26-1.25 (m, 3H), 1.10 (s, 9H), 1.01-0.99 (d, *J*= 4.0 Hz, 3H), 0.89-0.85 (m, 6H), 0.78-0.71 (m, 6H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.47 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.8, 171.2, 171.1, 170.6, 170.3, 170.1, 169.7, 168.6, 156.6, 150.5, 144.7, 141.2, 139.9, 137.9, 136.7, 133.2, 131.9, 130.6, 129.5, 128.5, 128.1, 127.7, 127.5, 127.2, 125.8, 121.8, 120.9, 120.5, 75.9, 74.3, 67.3, 66.3, 65.5, 63.3, 60.5, 58.0, 51.7, 51.1, 48.4, 47.2,

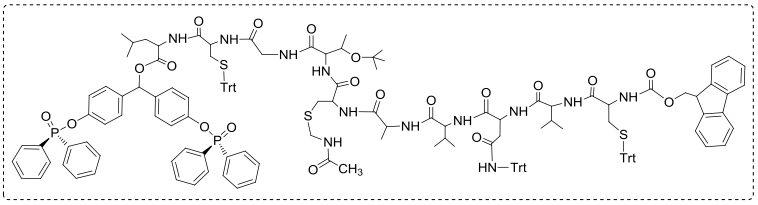
32.5, 30.9, 28.4, 24.5, 23.0, 21.8, 19.8, 19.4, 19.1, 18.6 ppm; HRMS (ESI) m/z calcd for $C_{104}H_{113}N_8O_{16}P_2S_2^+$ $(M+H)^+$ 1855.7185, found 1855.7175.



3c-10, Fmoc-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.42 (CH_2Cl_2: MeOH= 30:1), (8287 mg, 3.75 mmol, 94% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.57-8.51 (m, 2H), 8.39-8.15 (m, 7H), 7.91-7.86 (m, 11H), 7.74-7.72 (m, 3H), 7.61-7.53 (m, 13H), 7.42-7.41 (m, 2H), 7.26-7.16 (m, 38H), 6.64 (s, 1H), 4.65-4.64 (m, 1H), 4.48-4.24 (m, 11H), 3.97-3.81 (m, 3H), 2.96-2.60 (m, 4H), 2.39-2.32 (m, 2H), 2.00-1.98 (m, 1H), 1.85 (s, 3H), 1.48-1.44 (m, 3H), 1.26-1.21 (m, 3H), 1.10 (s, 9H), 1.00-0.99 (d, J= 4.0 Hz, 3H), 0.86-0.71 (m, 12H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.77 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.8, 171.6, 171.1, 170.8, 170.6, 170.3, 170.1, 169.7, 169.3, 168.6, 156.3, 150.5, 145.2, 144.7, 144.2, 141.2, 136.8, 133.2, 131.9, 131.8, 130.6, 129.5, 129.3, 129.0, 128.5, 128.1, 127.9, 127.6, 127.2, 126.8, 125.8, 120.9, 120.6, 75.9, 74.3, 69.9, 67.2, 66.3, 65.5, 63.3, 57.9, 57.7, 52.8, 51.7, 51.1, 48.5, 47.1, 31.3, 28.4, 24.5, 23.1, 21.8, 19.7, 19.4, 18.8, 18.3 ppm; HRMS (ESI) m/z calcd for $C_{127}H_{133}N_{10}O_{18}P_2S_2^+$ $(M+H)^+$ 2211.8710, found 2211.8728.

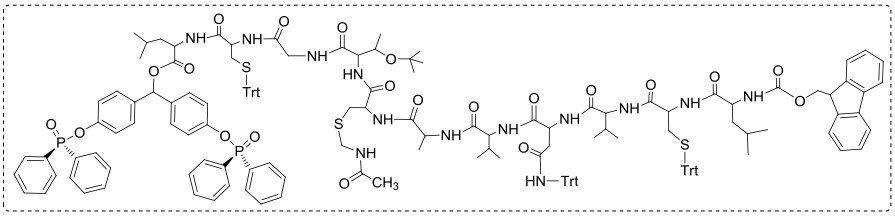


3c-11, Fmoc-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.40 (CH_2Cl_2: MeOH= 30:1), (8058 mg, 3.49 mmol, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.63-8.51 (m, 2H), 8.39-8.13 (m, 8H), 7.90-7.86 (m, 11H), 7.76-7.74 (m, 2H), 7.61-7.54 (m, 13H), 7.42-7.40 (m, 3H), 7.26-7.16 (m, 38H), 6.64 (s, 1H), 4.65-4.63 (m, 1H), 4.49-4.20 (m, 12H), 4.02-3.81 (m, 4H), 2.96-2.66 (m, 4H), 2.39-2.32 (m, 2H), 2.01-1.95 (m, 2H), 1.85 (s, 3H), 1.48-1.44 (m, 3H), 1.23-1.19 (m, 3H), 1.11 (s, 9H), 1.00-0.99 (d, J= 4.0 Hz, 3H), 0.90-0.71 (m, 18H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.97 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.8, 171.6, 171.1, 170.8, 170.6, 170.4, 170.3, 170.1, 169.7, 169.3, 168.6, 156.3, 150.5, 145.2, 144.7, 144.2, 141.2, 136.7, 133.2, 131.9, 131.8, 130.6, 129.5, 129.3, 129.0, 128.5, 128.1, 127.9, 127.5, 127.2, 126.7, 125.9, 120.9, 120.5, 75.9, 74.3, 69.9, 67.3, 66.3, 65.5, 63.3, 58.0, 57.6, 52.8, 51.7, 51.1, 48.5, 47.1, 31.3, 28.4, 24.5, 23.0, 21.8, 19.6, 19.4, 18.7, 18.5, 18.3 ppm; HRMS (ESI) m/z calcd for $C_{132}H_{142}N_{11}O_{19}P_2S_2^+$ $(M+H)^+$ 2310.9394, found 2310.9387.

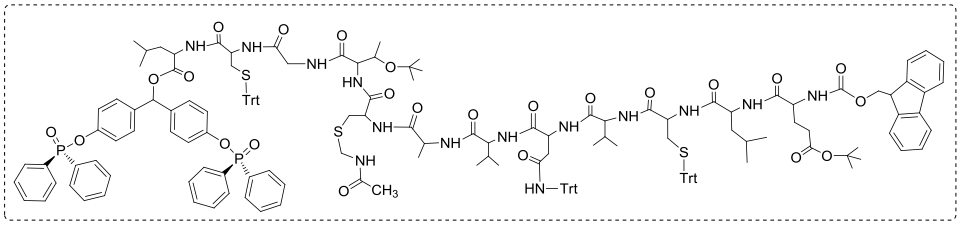


3c-12, Fmoc-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.38 (CH_2Cl_2: MeOH= 20:1), (9078 mg, 3.39 mmol, 97% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.64-8.53 (m, 2H), 8.43-8.12 (m, 8H), 7.90-7.86 (m, 11H), 7.73-7.71 (m, 2H), 7.61-7.52 (m, 13H), 7.41-7.10 (m, 57H), 6.64 (s, 1H), 4.67-4.47 (m, 3H), 4.34-4.16 (m, 10H), 3.98-3.80 (m, 4H), 2.98-2.93 (m, 1H), 2.74-2.54 (m, 3H), 2.46-2.30 (m, 3H), 2.00-

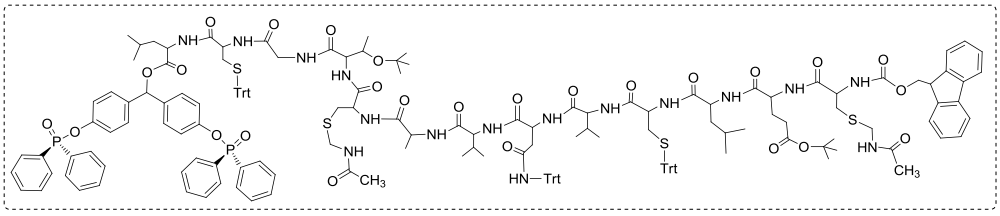
1.85 (m, 5H), 1.55-1.42 (m, 3H), 1.23-1.17 (m, 4H), 1.11 (s, 9H), 1.01-0.99 (d, *J*= 8.0 Hz, 3H), 0.83-0.69 (m, 18H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.91 ppm; ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.8, 171.1, 170.8, 170.7, 170.6, 170.3, 170.1, 169.9, 169.7, 169.2, 168.6, 156.2, 150.5, 145.1, 144.7, 144.3, 141.2, 136.8, 133.2, 131.9, 130.6, 129.5, 129.3, 129.0, 128.5, 128.1, 127.9, 127.5, 127.2, 126.7, 125.7, 120.9, 120.5, 75.9, 74.3, 69.8, 67.3, 66.5, 66.3, 58.0, 51.8, 47.1, 32.2, 31.3, 28.4, 24.5, 23.1, 21.8, 19.8, 19.6, 19.4, 18.8, 18.3, 17.6 ppm; HRMS (ESI) m/z calcd for $C_{154}H_{160}N_{12}O_{20}P_2S_3Na^+$ $(M+Na)^+$ 2678.0401, found 2678.0356.



3c-13, Fmoc-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.40 (CH_2Cl_2: MeOH= 20:1), (9070 mg, 3.25 mmol, 96% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.66-8.19 (m, 8H), 7.96-7.85 (m, 9H), 7.63-7.53 (m, 12H), 7.30-7.16 (m, 60H), 6.62 (s, 1H), 4.63-3.79 (m, 18H), 2.96-2.93 (m, 1H), 2.68 (m, 3H), 2.41-2.34 (m, 3H), 1.99-1.84 (m, 8H), 1.49-1.44 (m, 4H), 1.23-1.16 (m, 8H), 1.09 (s, 9H), 0.99-0.98 (m, 3H), 0.82-0.76 (m, 24H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.61 ppm; HRMS (ESI) m/z calcd for $C_{160}H_{171}N_{13}O_{21}P_2S_3Na^+$ $(M+Na)^+$ 2791.1242, found 2791.1145.

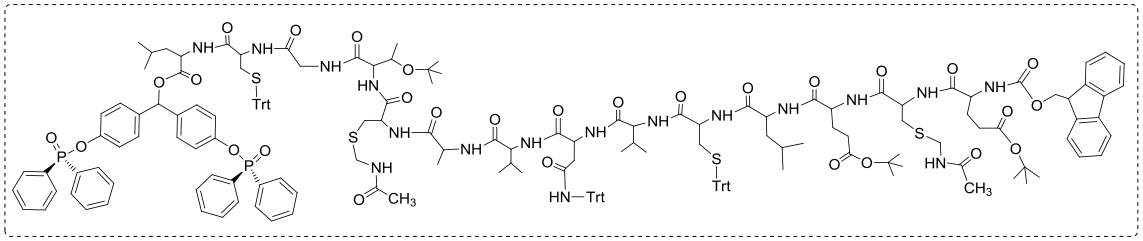


3c-14, Fmoc-Glu(*t*Bu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.50 (CH_2Cl_2: MeOH= 10:1), (9160 mg, 3.08 mmol, 95% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.63-8.60 (m, 2H), 8.44-8.16 (m, 8H), 7.96-7.85 (m, 13H), 7.73-7.71 (m, 2H), 7.63-7.52 (m, 14H), 7.42-7.09 (m, 56H), 6.62 (s, 1H), 4.63 (m, 2H), 4.47-3.79 (m, 17H), 2.96-2.89 (m, 2H), 2.73-2.64 (m, 3H), 2.41-2.24 (m, 5H), 2.00-1.84 (m, 7H), 1.67 (m, 2H), 1.52-1.35 (m, 8H), 1.23-1.16 (m, 8H), 1.09 (s, 9H), 0.99-0.98 (d, *J*= 4.0 Hz, 3H), 0.86-0.68 (m, 24H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.91 ppm; HRMS (ESI) m/z calcd for $C_{169}H_{186}N_{14}O_{24}P_2S_3Na^+$ $(M+Na)^+$ 2976.2294, found 2976.2203.

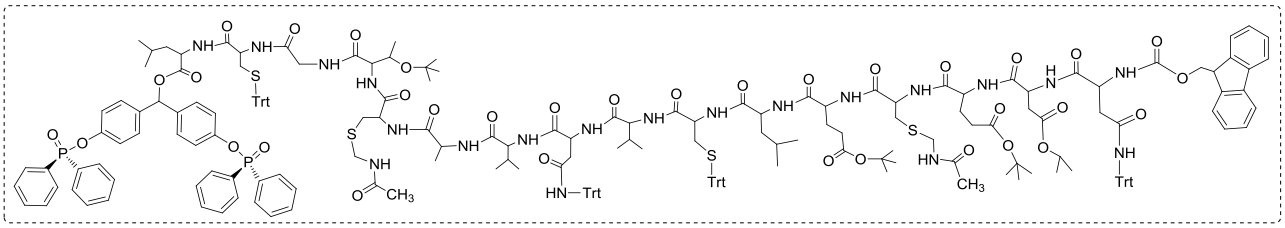


3c-15, Fmoc-Cys(Acm)-Glu(*t*Bu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.52 (CH_2Cl_2: MeOH= 10:1), (9135 mg, 2.90 mmol, 94% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.63-8.15 (m, 7H), 7.90-7.83 (m, 11H), 7.74-7.72 (m, 2H), 7.61-7.53 (m, 14H), 7.43-7.39 (m, 2H), 7.29-

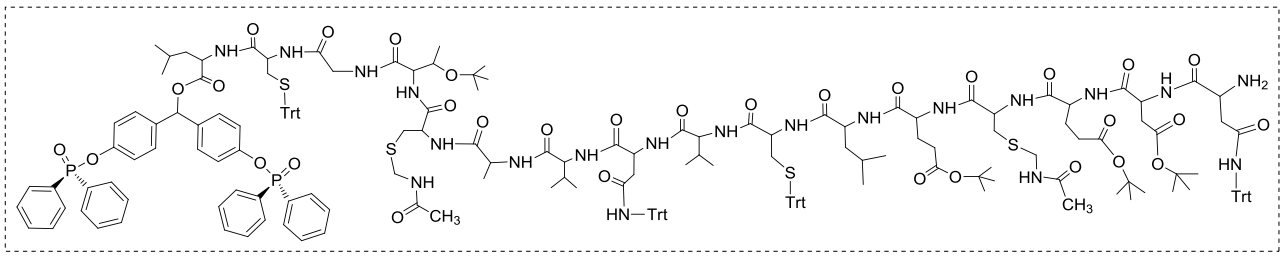
7.15 (m, 56H), 6.62 (s, 1H), 4.63-3.79 (m, 22H), 2.96-2.64 (m, 5H), 2.39-2.33 (m, 6H), 1.99-1.84 (m, 10H), 1.44-1.34 (m, 8H), 1.23-1.15 (m, 8H), 1.09 (s, 12H), 0.98 (d, *J*= 8.0 Hz, 3H), 0.82-0.70 (m, 24H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.76 ppm; HRMS (ESI) m/z calcd for $C_{175}H_{196}N_{16}O_{26}P_2S_4Na^+$ $(M+Na)^+$ 3150.2757, found 3150.2719.



3c-16, Fmoc-Glu(*t*Bu)-Cys(Acm)-Glu(*t*Bu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.50 (CH_2Cl_2: MeOH= 10:1), (9000 mg, 2.70 mmol, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.66-8.17 (m, 4H), 7.90-7.85 (m, 6H), 7.63-7.53 (m, 9H), 7.30-7.09 (m, 66H), 6.62 (s, 1H), 4.65-3.79 (m, 22H), 2.96-2.63 (m, 4H), 2.37-2.31 (m, 6H), 2.02-1.84 (m, 12H), 1.38-1.36 (m, 12H), 1.24-1.16 (m, 10H), 1.09 (s, 12H), 1.00-0.98 (m, 3H), 0.87-0.70 (m, 24H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.36 ppm; HRMS (ESI) m/z calcd for $C_{184}H_{211}N_{17}O_{29}P_2S_4Na^+$ $(M+Na)^+$ 3335.3809, found 3335.3854.



3c-17, Fmoc-Asn(Trt)-Asp(*t*Bu)-Glu(*t*Bu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.60 (CH_2Cl_2: MeOH= 10:1), (9890 mg, 2.57 mmol, 95% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.64-8.15 (m, 6H), 7.96-7.84 (m, 11H), 7.75-7.72 (m, 2H), 7.61-7.52 (m, 14H), 7.44-7.35 (m, 5H), 7.30-7.09 (m, 71H), 6.63 (s, 1H), 4.62 (m, 2H), 4.48-4.20 (m, 16H), 3.95-3.79 (m, 5H), 2.97-2.93 (m, 2H), 2.74-2.68 (m, 3H), 2.39-2.19 (m, 8H), 2.04-1.83 (m, 12H), 1.65 (m, 2H), 1.48-1.34 (m, 15H), 1.24-1.09 (m, 27H), 1.00-0.98 (m, 6H), 0.85-0.70 (m, 32H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.36 ppm; HRMS (ESI) m/z calcd for $C_{214}H_{242}N_{20}O_{34}P_2S_4Na^+$ $(M+Na)^+$ 3848.6076, found 3848.6069.



3c-18, (*de-Fmoc*) H-Asn(Trt)-Asp(*t*Bu)-Glu(*t*Bu)-Cys(Acm)-Glu(*t*Bu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(*t*Bu)-Gly-Cys(Trt)-Leu-DDK-5: White solid, R_f = 0.30 (CH_2Cl_2: MeOH= 10:1), (9250 mg, 2.54 mmol, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.63-8.15 (m, 15H), 7.89-7.73 (m, 6H), 7.61-7.54 (m, 8H), 7.30-7.10 (m, 78H), 6.64 (s, 1H), 4.66-4.51 (m, 3H), 4.35-4.08 (m, 16H), 3.97-3.82 (m, 5H), 3.19-2.94 (m, 2H), 2.70

(m, 4H), 2.40-2.09 (m, 7H), 1.97-1.86 (m, 12H), 1.58-1.38 (m, 11H), 1.21-1.01 (m, 30H), 0.84-0.71 (m, 36H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.40 ppm; HRMS (ESI) m/z calcd for $C_{200}H_{234}N_{20}O_{32}P_2S_4Na^+$ $(M+Na)^+$ 3640.5548, found 3640.5588.

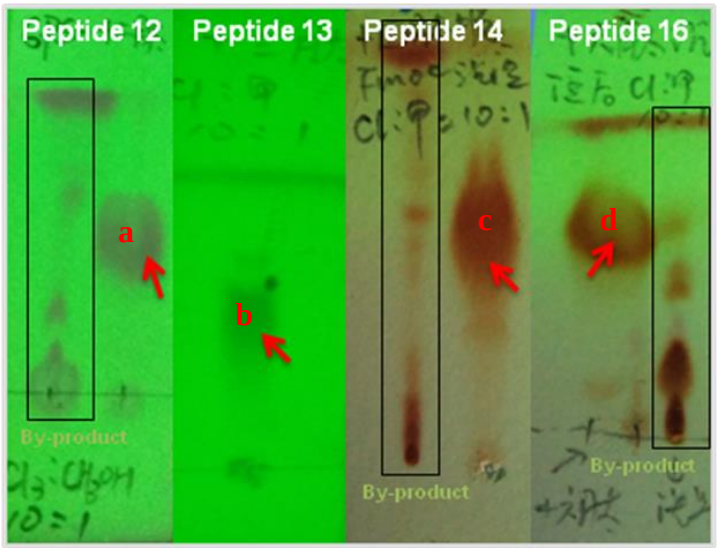


Figure S1 TLC analysis of DDK attached peptide chain ($CHCl_3$:CH_3OH=10:1).

a: Fmoc-ELCVNVACTGCL-DDK-5 TLC analysis;

b: Fmoc-CELCVNVACTGCL-DDK-5 TLC analysis;

c: Fmoc-ECELCVNVACTGCL-DDK-5 TLC analysis;

d: Fmoc-DNCELCVNVACTGCL-DDK-5 TLC analysis;

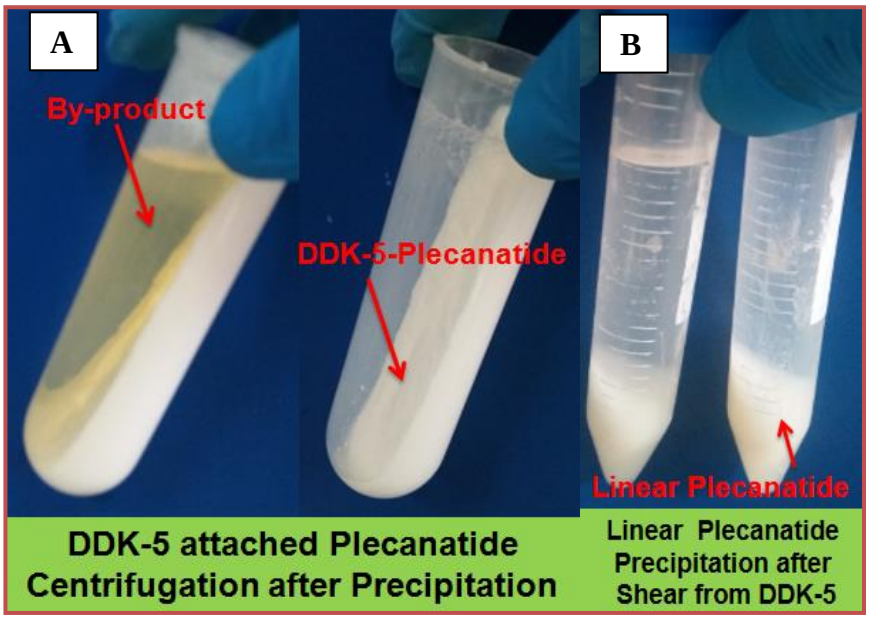


Figure S2 Optical photos of (**A**) DDK-attached Plecanatide precipitation and (**B**) linear Plecanatide precipitation by diethyl ether after shearing from the DDK support.

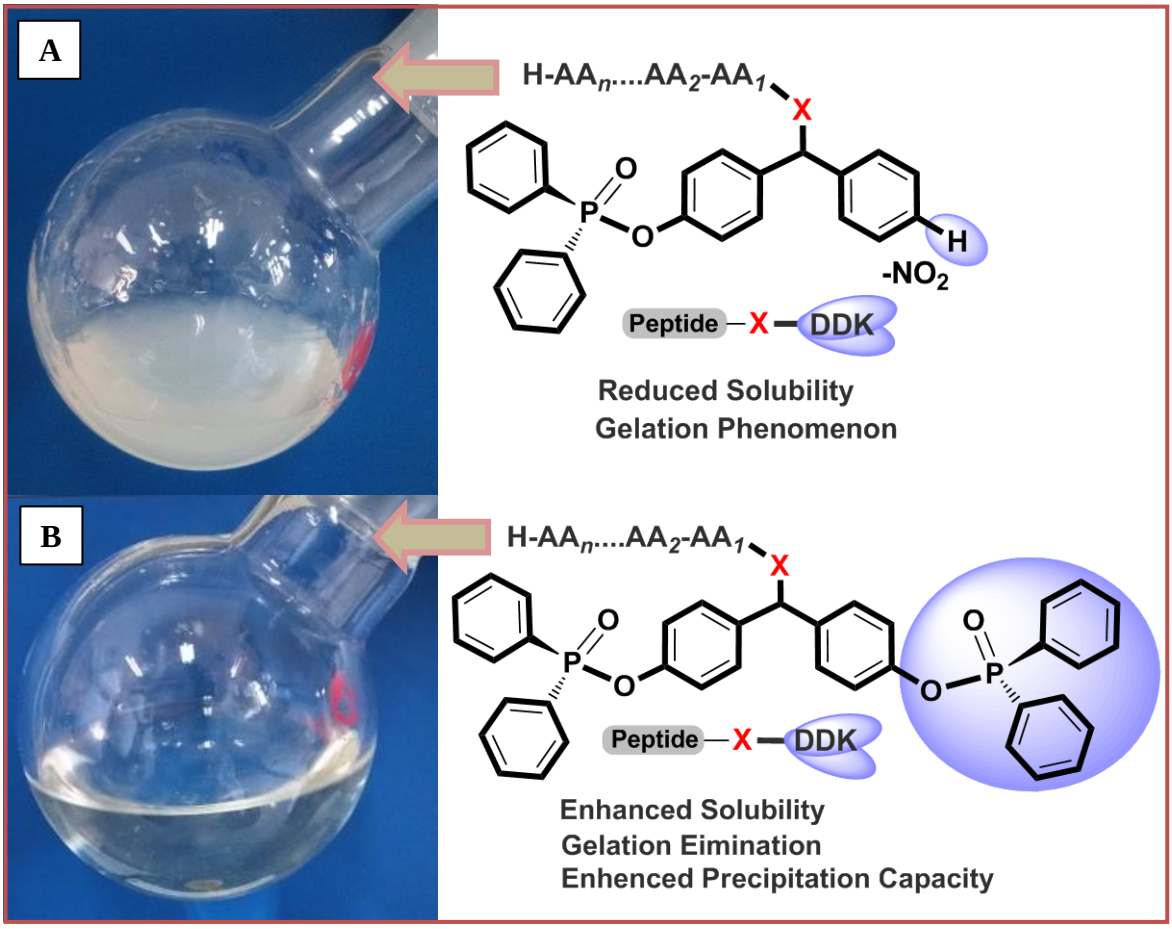
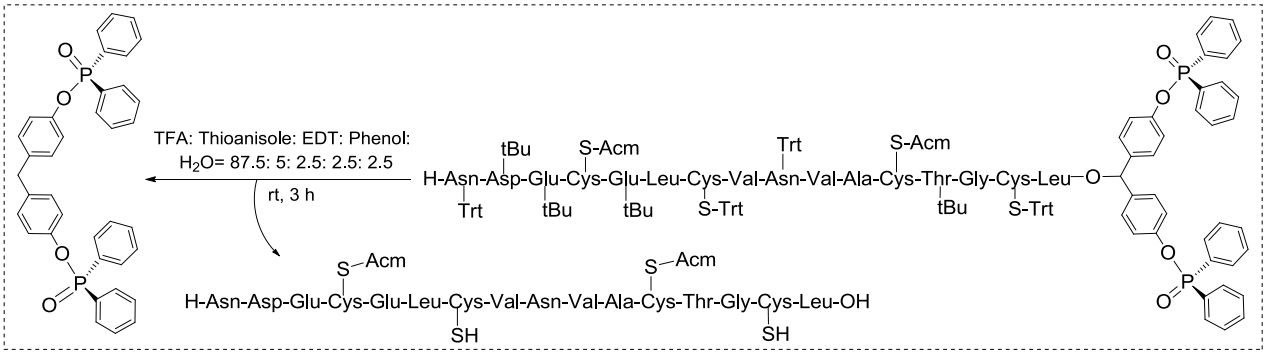
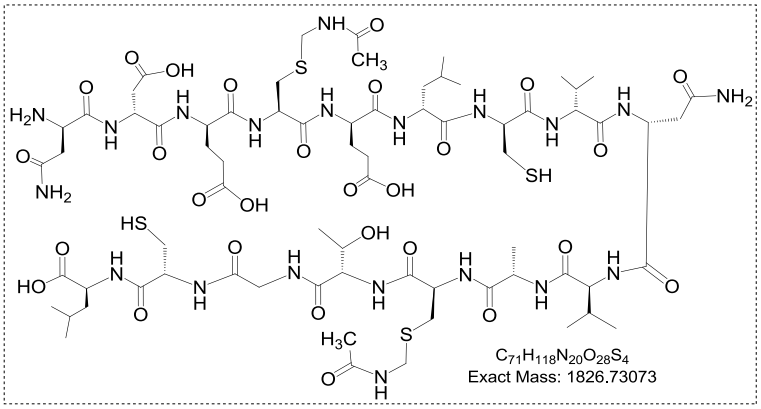


Figure S3 Optical photos of (**A**) DDK support attached peptide gelation and (**B**) Gelation phenomenon eimination after adjusting the group.

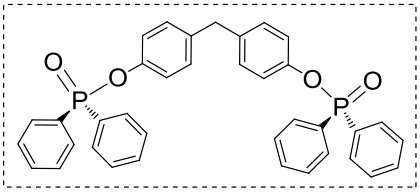


Shearing of DDK-5/Trt/*t*Bu group: The H-NDEC(Acm)ELCVNVAC(Acm)TGCL-**DDK-5** (730 mg, 0.2mmol) was added to the mixed solution of TFA/ Thioanisole/ EDT/ Phenol/ H_2O (3 mL, *v/v*, 87.5/5/2.5/2.5/2.5) at room temperature and stirred at this temperature for 3 h. The reaction mixture was then concentrated and added with cold diethyl ether (repeat 3 times) accompanied by ultrasound to afford the crude linear Plecanatide precipitate, and the precipitate was centrifuged to obtain the linear Plecanatide H-NDEC(Acm)ELCVNVAC(Acm)TGCL-OH (340 mg, 94% yield). The diethyl ether phase was collected to attain the DDK-5 residue (112 mg, 90 yield).

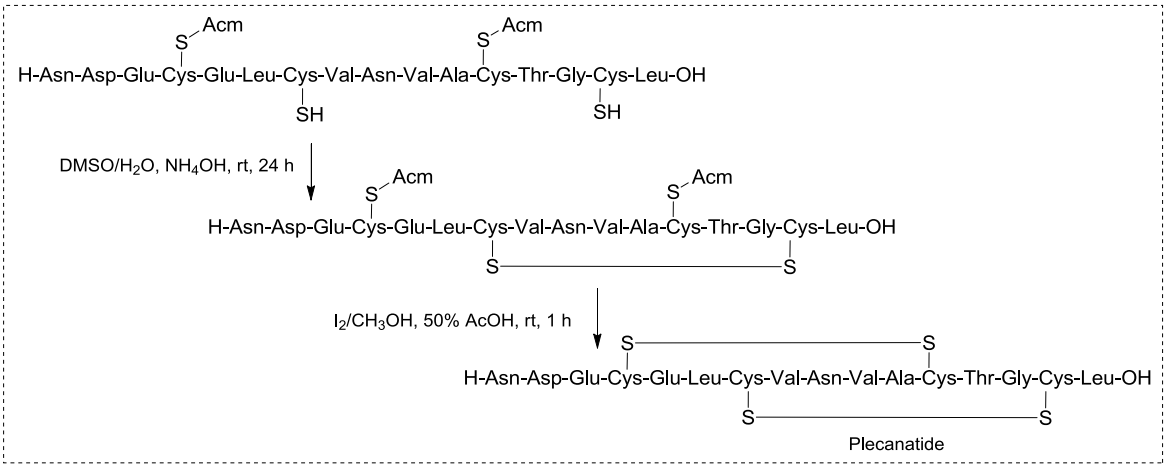
[Regeneration of **DDK**-5-residue: **DDK**-5 residue was added to a solution of NCS (or NBS) in chloroform and refluxed, then the reaction mixture was stirred at this condition for 5 h to obtained the regenerated DDK derivative.]



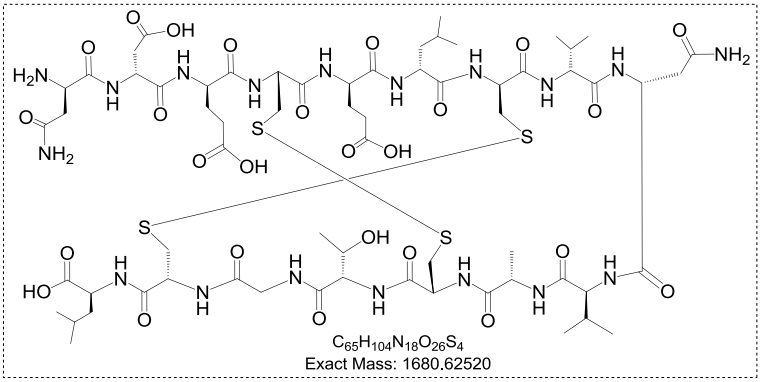
3c-19, Linear Plecanatide: H-Asn-Asp-Glu-Cys(Acm)-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys(Acm)-Thr-Gly-Cys-Leu-OH: ^{1}H NMR (400 MHz, DMSO-d_6), δ 12.46 (s, 4H), 8.54-7.73 (m, 19H), 7.43-7.33 (m, 5H), 6.97 (s, 1H), 5.02-3.80 (m, 22H), 3.38 (m, 5H), 2.93-2.71 (m, 10H), 2.28 (m, 5H), 1.98-1.87 (m, 8H), 1.65-1.46 (m, 6H), 1.24 (m, 6H), 1.05 (m, 3H), 0.89-0.84 (m, 24H) ppm; HRMS (ESI) m/z calcd for $C_{71}H_{119}N_{20}O_{28}S_4^+$ $(M+H)^+$ 1827.7380, found 1827.7375.



3c-0, DDK-5 Residue: White solid, R_f = 0.35 (CH_2Cl_2: MeOH= 60:1), mp 137.8-139.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ 7.91-7.86 (m, 8H), 7.59-7.50 (m, 12H), 7.17-7.06 (dd, *J*= 8.0 Hz, 8H), 3.75 (s, 2H) ppm; ^{31}P NMR (162 MHz, DMSO-d_6), δ 29.11 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 148.8, 137.4, 132.9, 131.9, 130.1, 128.8, 120.7, 40.2 ppm; HRMS (ESI) m/z calcd for $C_{37}H_{30}O_4P_2Na^+$ $(M+Na)^+$ 623.1511, found 623.1510.



Orthogonal oxidation formation of intramolecularly disulfide bonds: The linear Plecanatide peptide H-NDEC(Acm)ELCVNVAC(Acm)TGCL-OH (180 mg, 0.1 mmol) was dissolved in 10 mL DMSO/H_2O (V_{DMSO}:V_{H2O}= 0.05:0.95), and the pH was adjusted to 8.0 with dilute NH_3•H_2O. The reaction mixture was stirred for 24 h at room temperature and lyophilized for next use. Subsequently, the above lyophilized powder was dissolved in 20 ml 50% AcOH/H_2O solution, and then 1.5 mL of I_2/MeOH (0.1 mol/L) was added to the reaction mixture dropwise. The reaction mixture was stirred for 1 h and quenched by adding the ascorbic acid (1 mol/L, 1.5 mL). The reaction mixture was concentrated and directly subjected to RP-HPLC to obtain the target Plecanatide (96 mg, 53%).



3c-20, Plecanatide: H-Asn-Asp-Glu-Cys[4,12]-Glu-Leu-Cys[7,15]-Val-Asn-Val-Ala-Cys[4,12]-Thr-Gly-Cys[7,15]-Leu-OH: ^{1}H NMR (400 MHz, D_2O), δ 4.66-4.59 (dd, *J*= 28.0 Hz, 6H), 4.55-4.47 (m, 2H), 4.27-4.15 (s, 6H), 4.10-4.01 (m, 3H), 3.95-3.88 (m, 2H), 3.81-3.77 (d, *J*=16.0 Hz, 1H), 3.27-2.64 (m, 16H), 2.36-2.31 (m, 4H), 2.08-1.90 (m, 7H), 1.54-1.41 (m, 6H), 1.25-1.23 (d, *J*=8.0 Hz, 3H), 1.08-1.06 (d, *J*=8.0 Hz, 3H), 0.83-0.70 (m, 24H), ppm; ^{13}C NMR (100 MHz, D_2O), 177.6, 177.4, 177.2, 175.1, 174.4, 174.3, 173.9, 173.8, 173.1, 172.8, 172.6, 172.5, 172.2, 171.8, 171.7, 171.2, 168.8, 66.6, 60.2, 59.8, 54.3, 53.8, 53.4, 53.1, 52.7, 52.0, 50.8, 49.7, 42.6, 41.0, 39.9, 39.4, 38.9, 37.7, 36.4, 36.2, 34.8, 30.4, 30.1, 29.7, 25.8, 25.6, 24.4, 24.2, 22.3, 22.1, 20.7, 20.6, 18.7, 18.3, 17.6, 17.3, 15.8 ppm; HRMS (ESI) m/z calcd for HRMS (ESI) m/z calcd for $C_{65}H_{105}N_{18}O_{26}S_4^+$ $(M+H)^+$ 1681.6324, found 1681.6325.

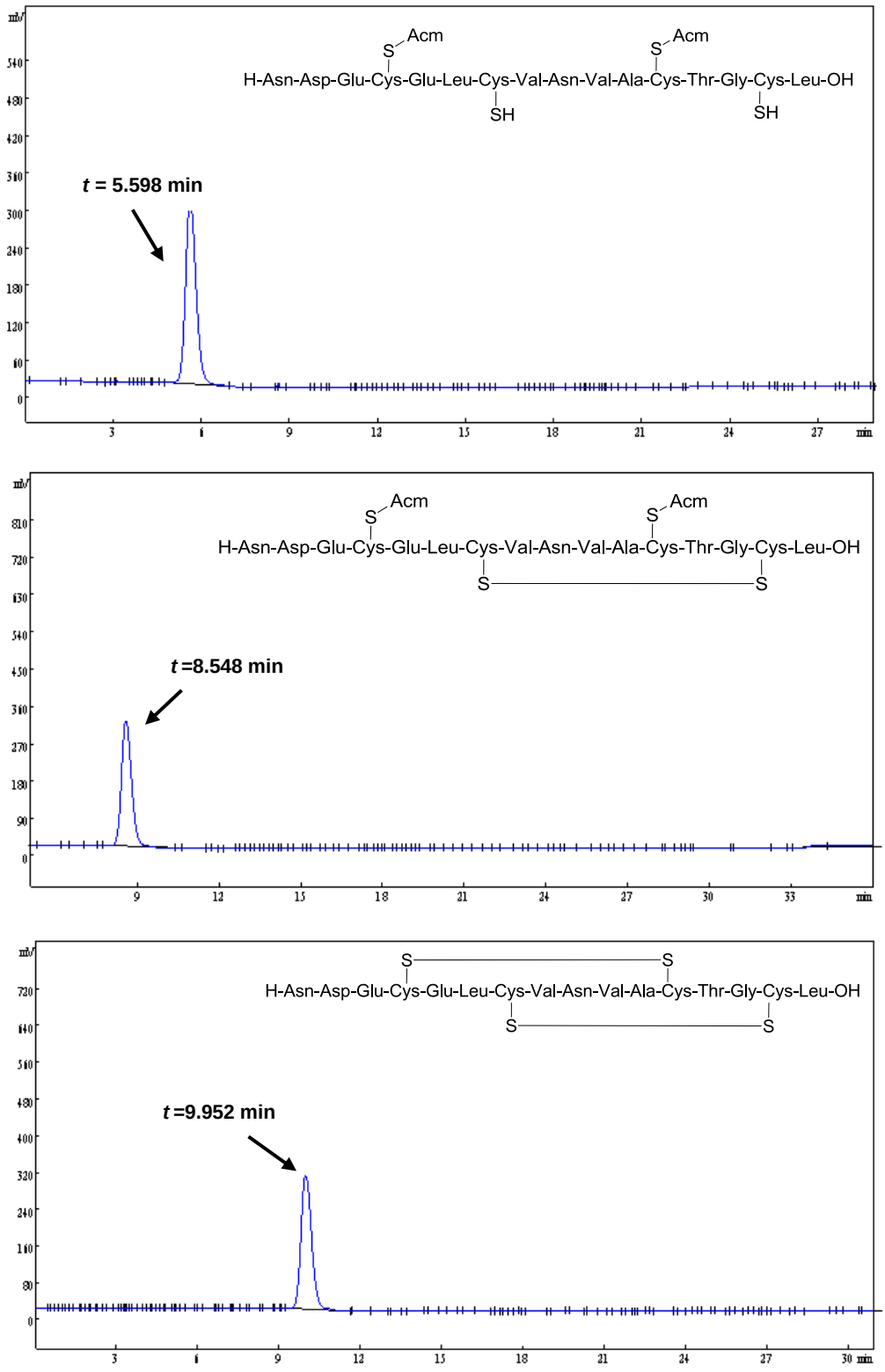


Figure S3 HPLC analysis of linear Plecanatide and Plecanatide. HPLC conditions: column, Kromasil, NC-2546-06251151; 250×4.6 mm; 25 °C.

T *(min)*	Flow Rate *(mL/min)*	Elution		*UV detection λ (nm)*
		H_2O (0.1% TFA)	CH_3OH	
0.0	1.0	95	5	220
5.0	1.0	90	10	
30.0	1.0	10	90	
40.0	1.0	95	5	

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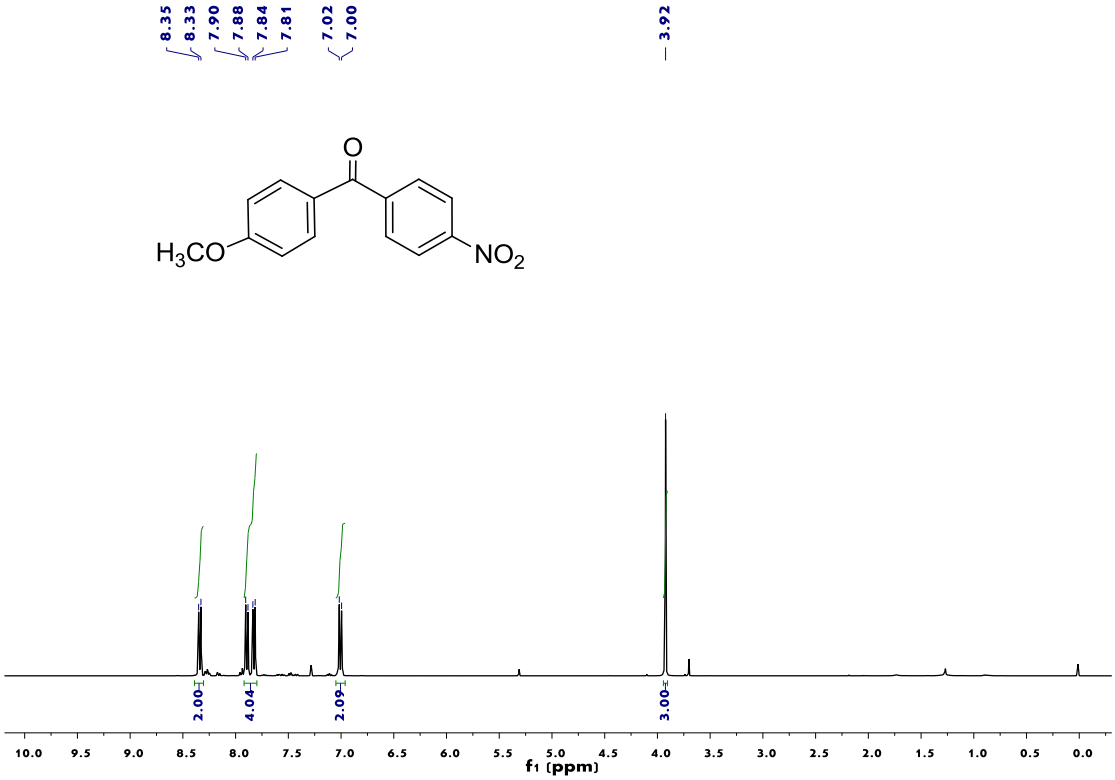
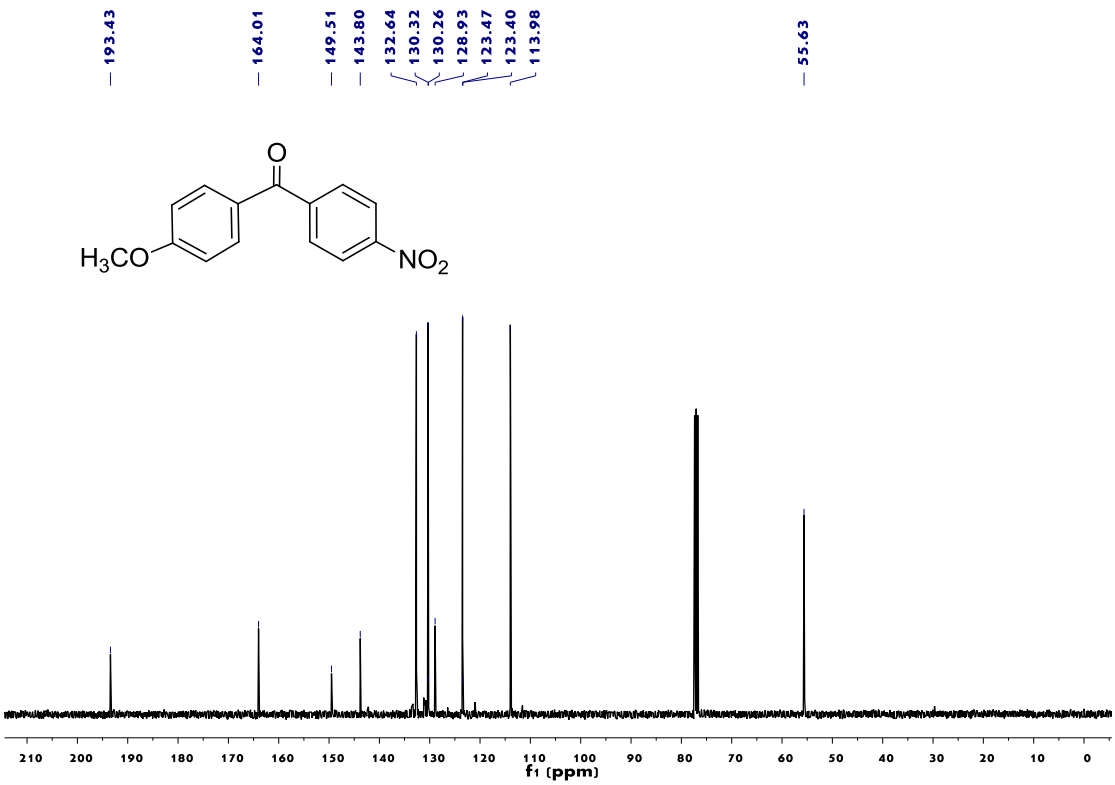
NMR Spectra and HRMS (ESI) Spectra

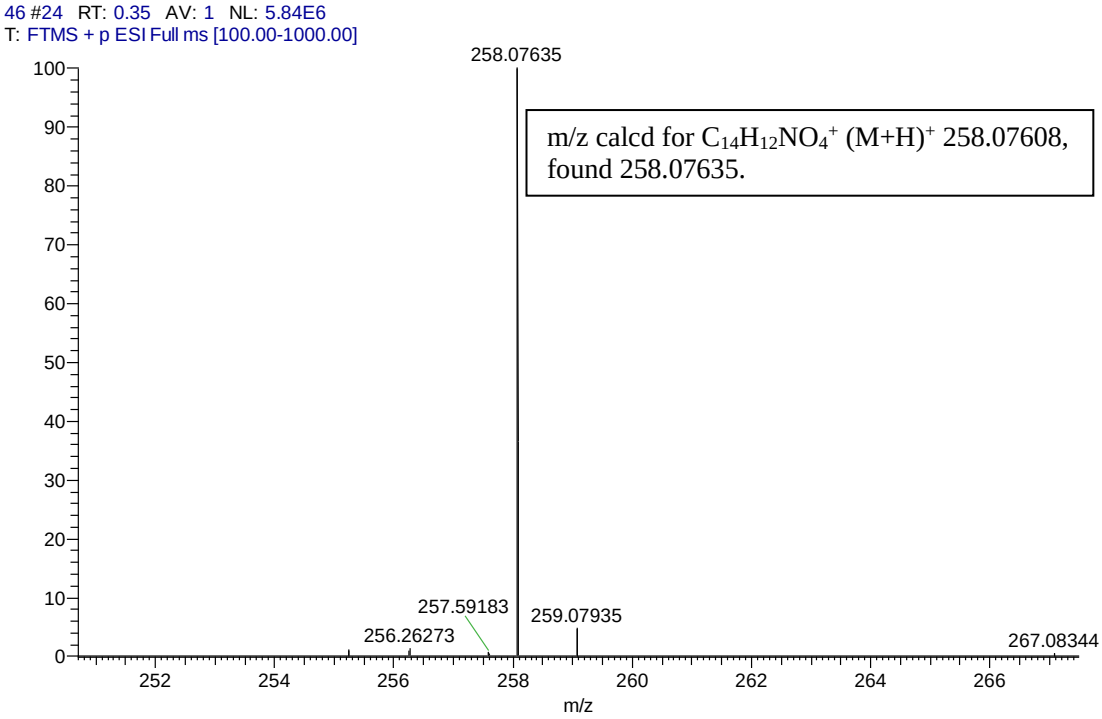
DDK derivatives Synthesis NMR and HRMS (ESI)



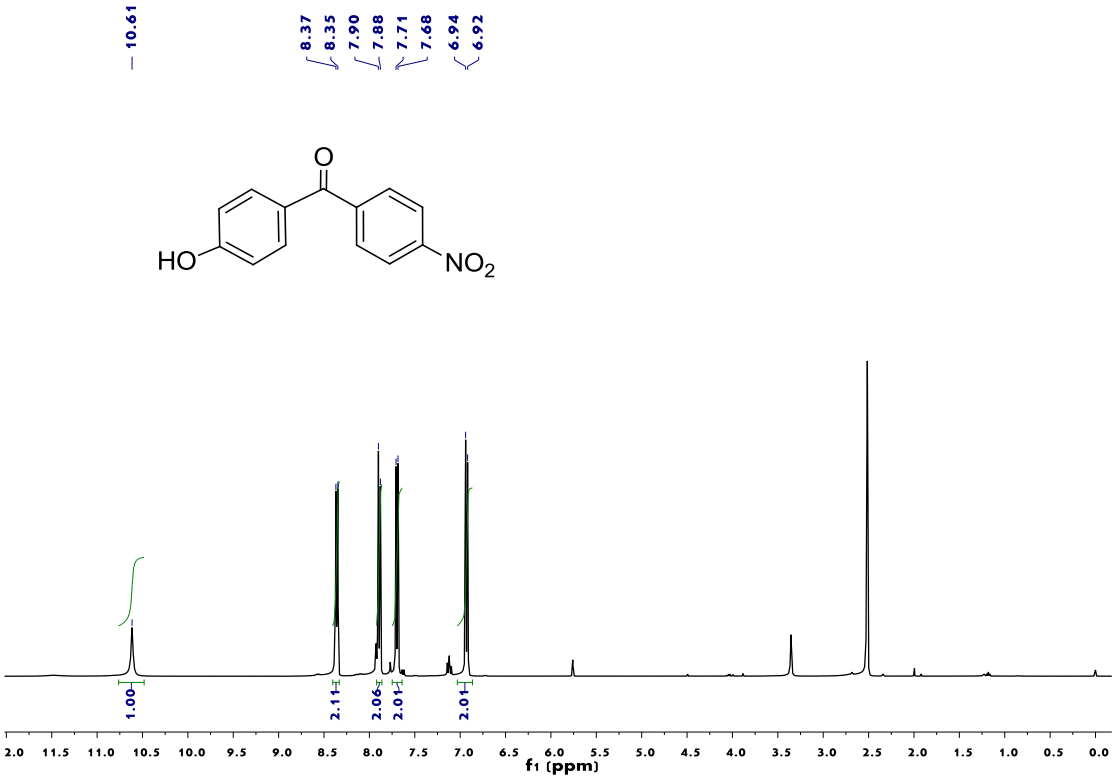
0b ^{1}H NMR (400 MHz, $CDCl_3$)



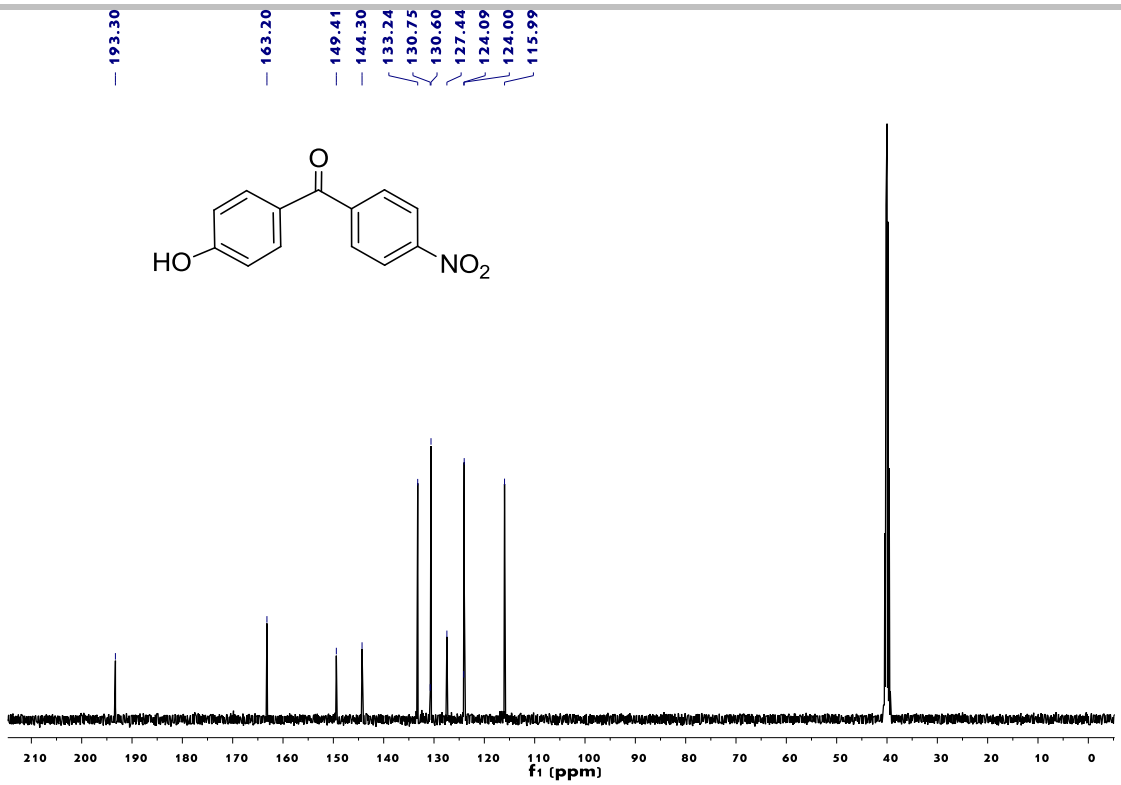
0b ^{13}C NMR (100 MHz, $CDCl_3$)



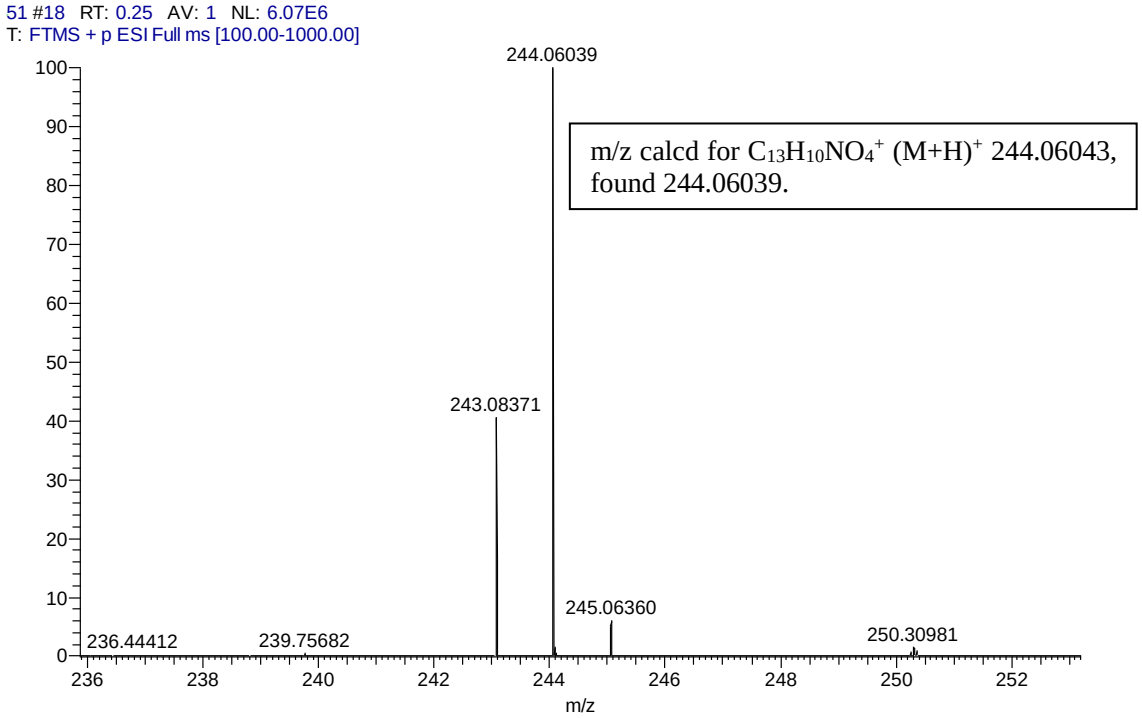
0b HRMS (ESI)



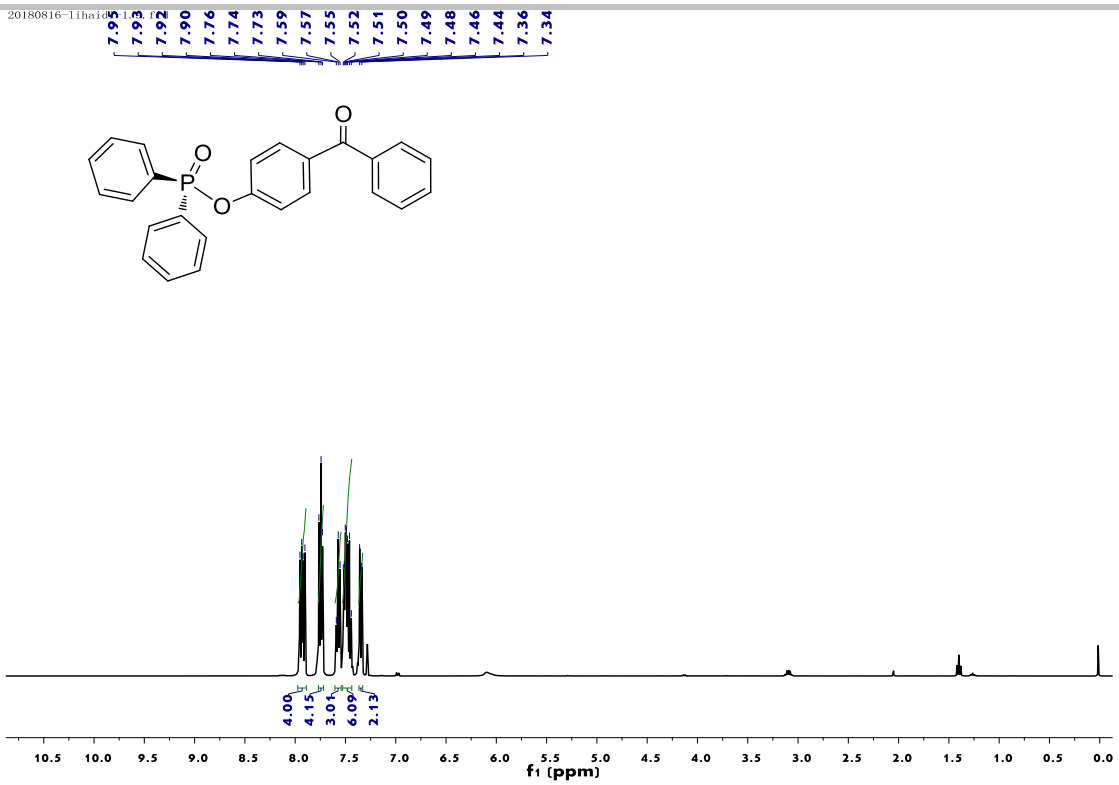
1b ^{1}H NMR (400 MHz, DMSO-d_6)



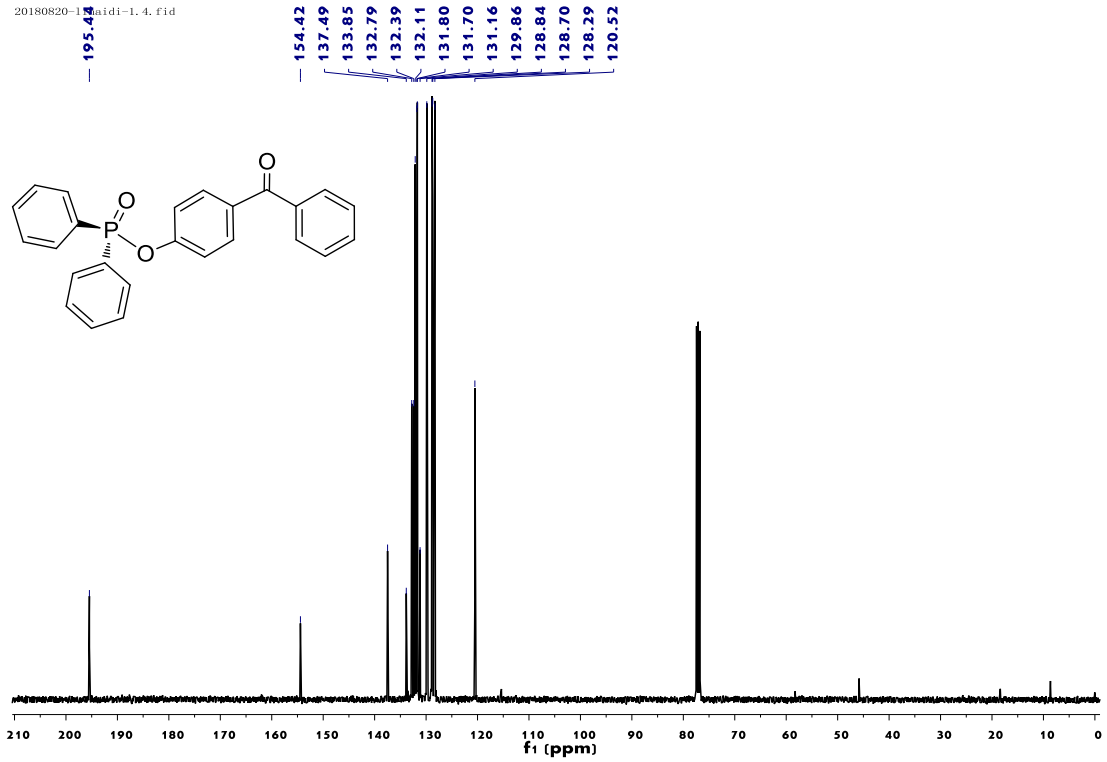
1b ^{13}C NMR (100 MHz, DMSO-d_6)



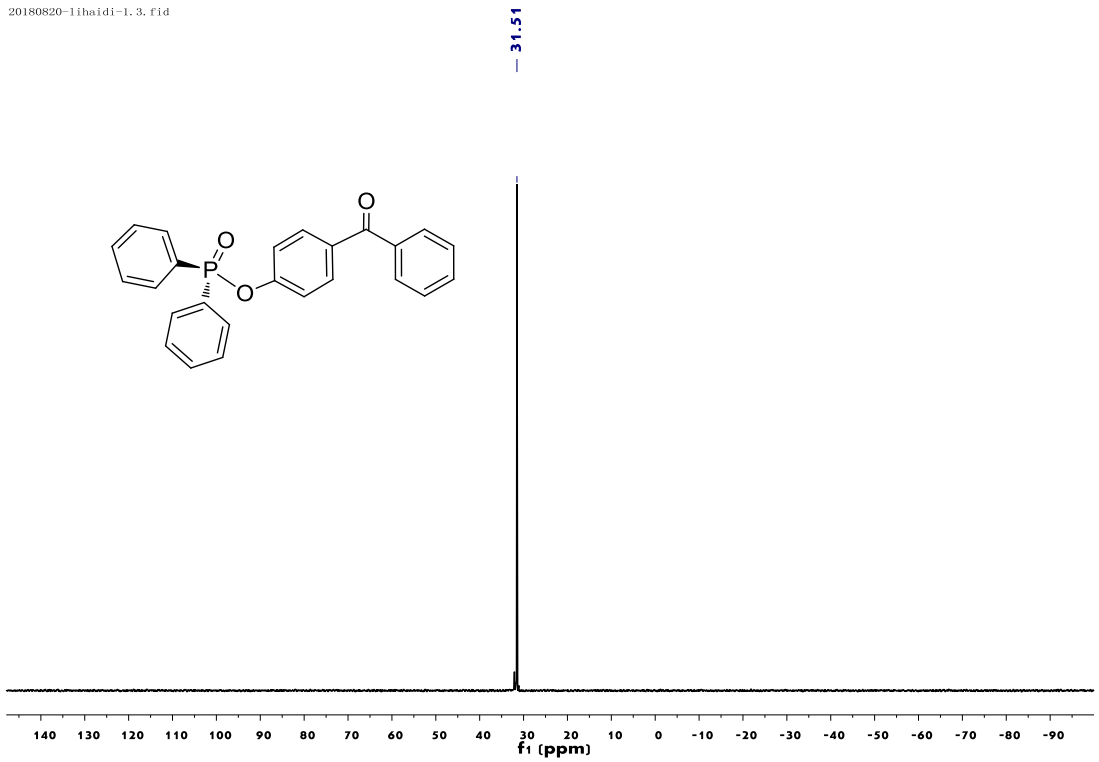
1b HRMS (ESI)



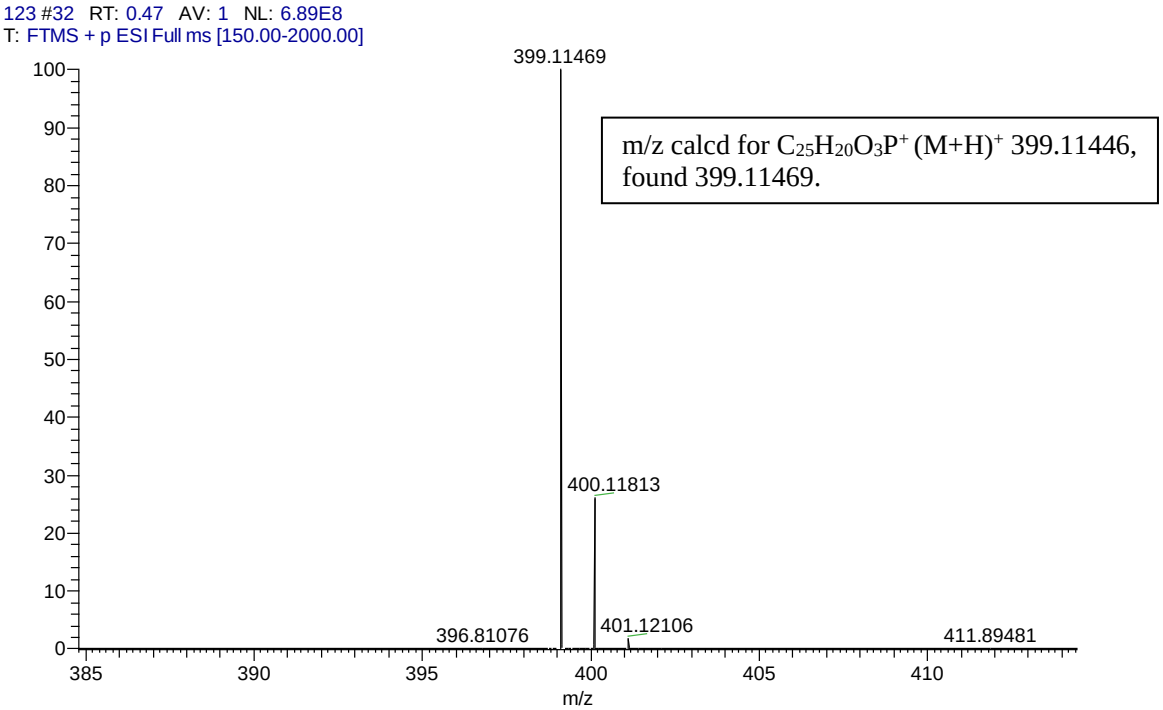
2a ^{1}H NMR (400 MHz, $CDCl_3$)



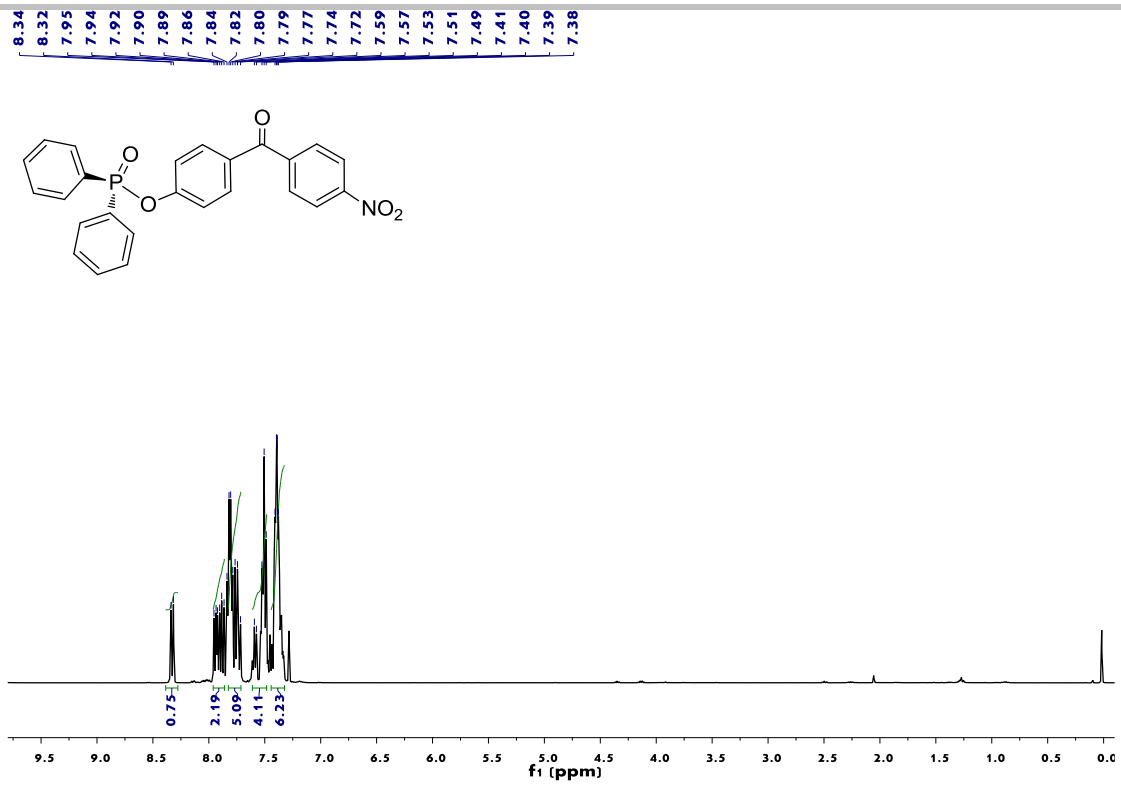
2a ^{13}C NMR (100 MHz, $CDCl_3$)



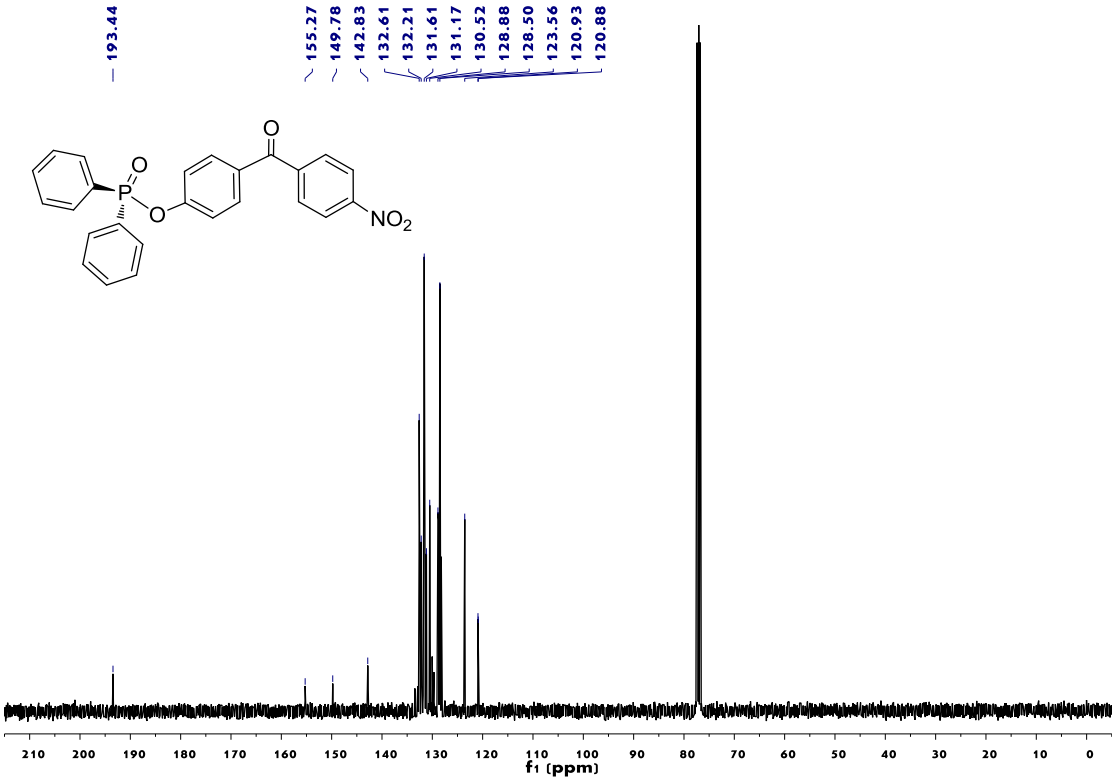
2a ^{31}P NMR (162 MHz, $CDCl_3$)



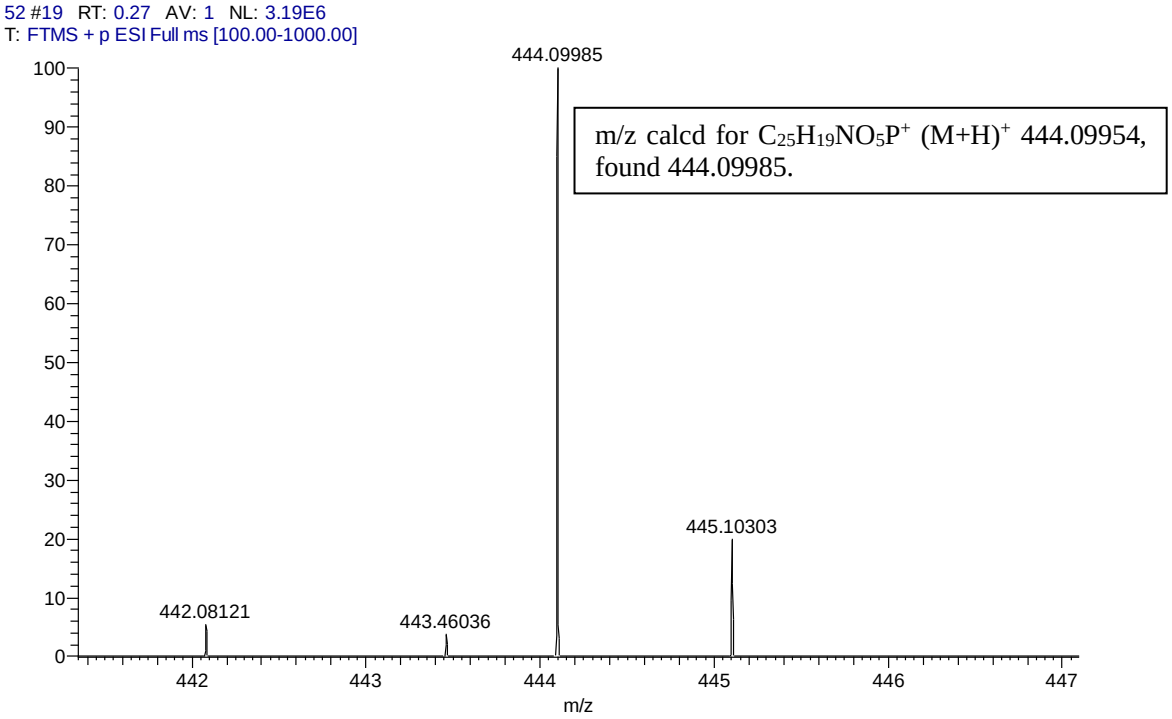
2a HRMS (ESI)



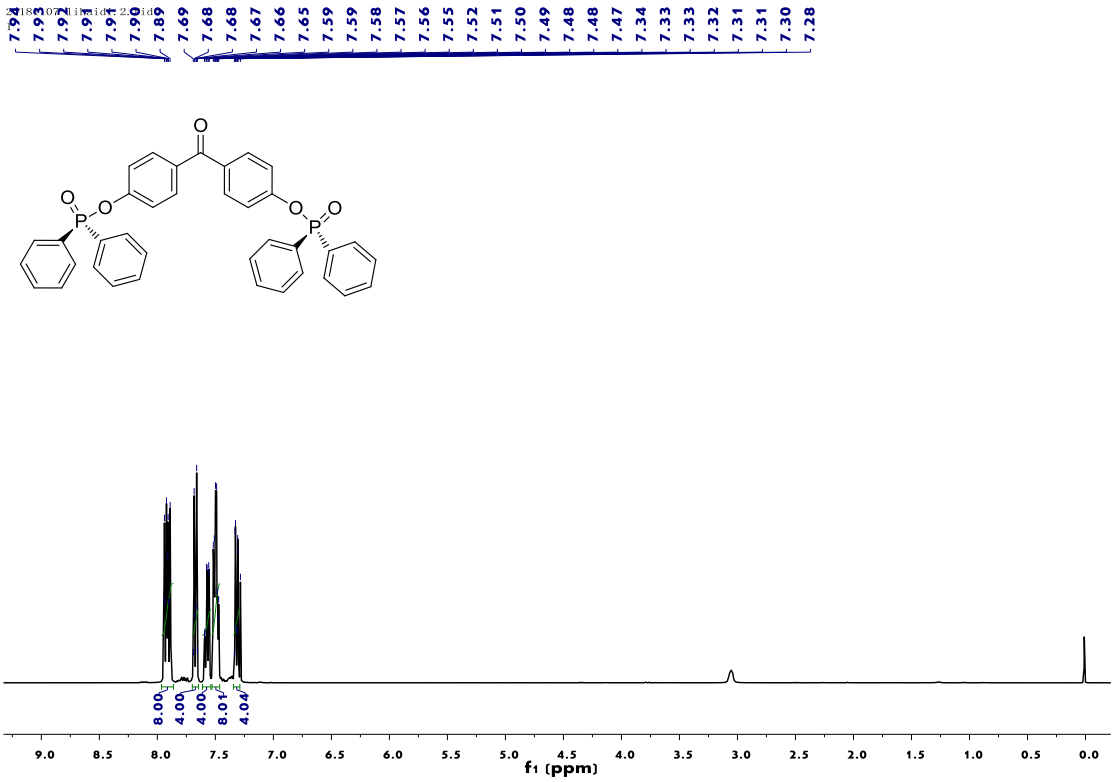
2b ^{1}H NMR (400 MHz, $CDCl_3$)



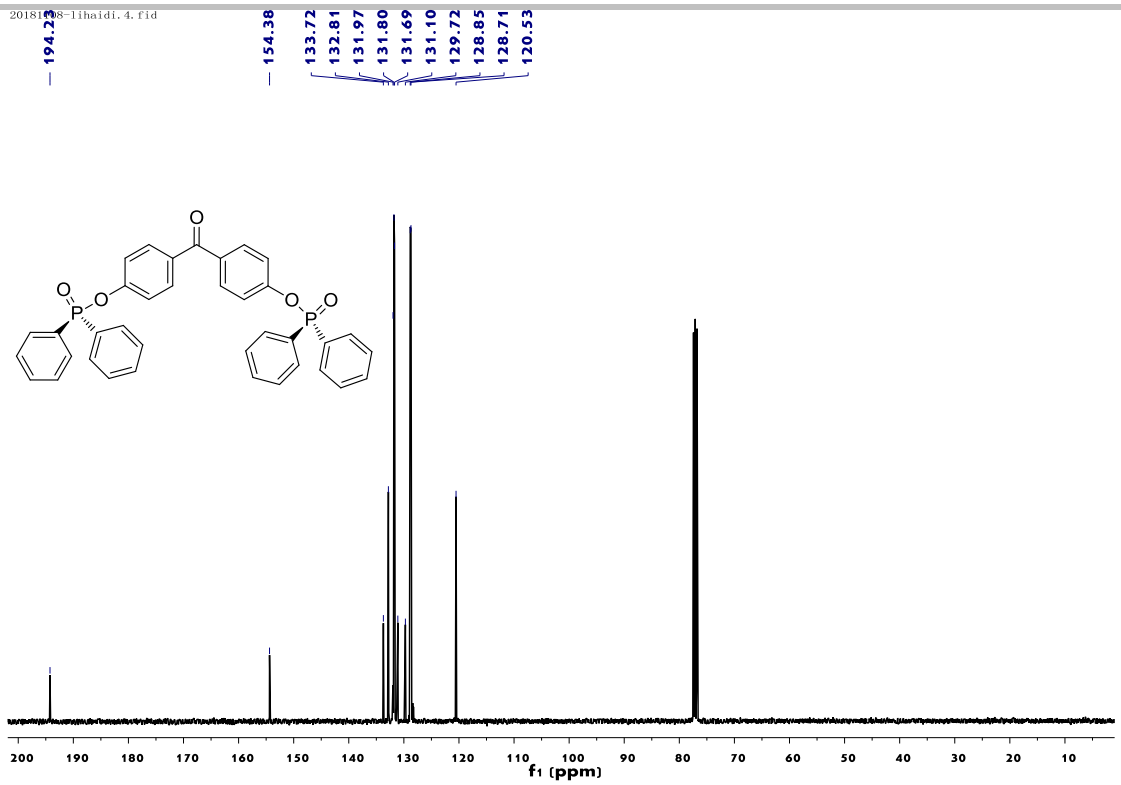
2b ^{13}C NMR (100 MHz, $CDCl_3$)



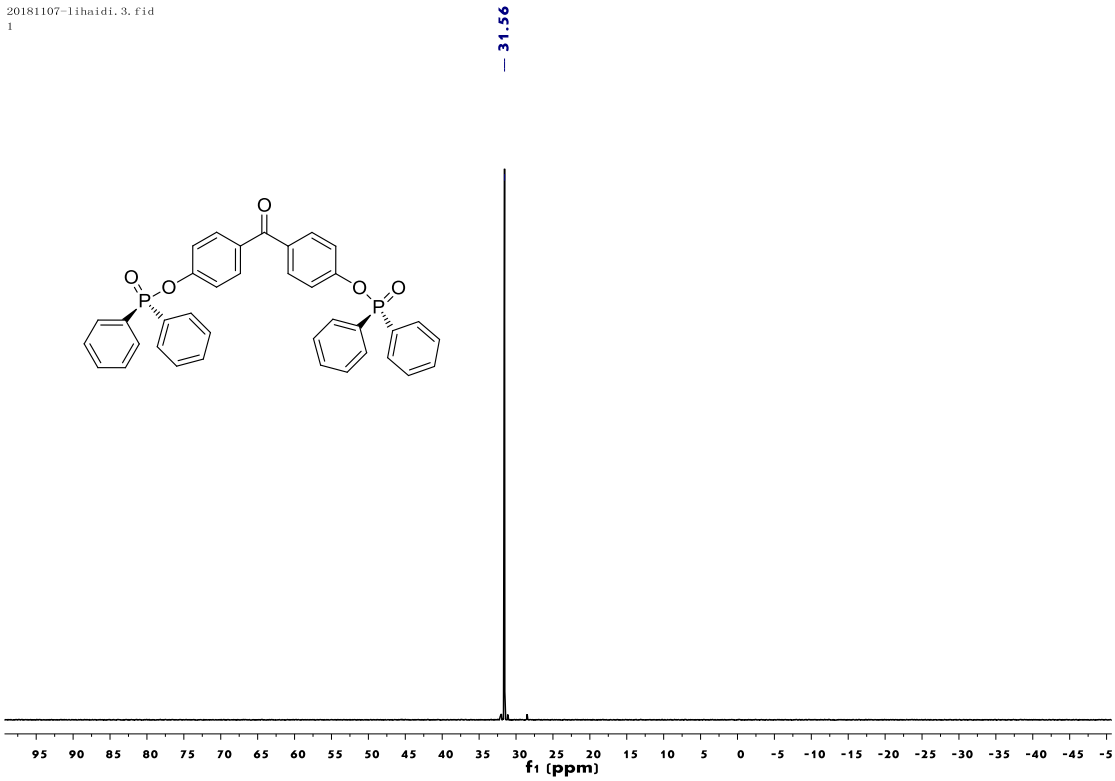
2b ^{31}P NMR (162 MHz, $CDCl_3$)



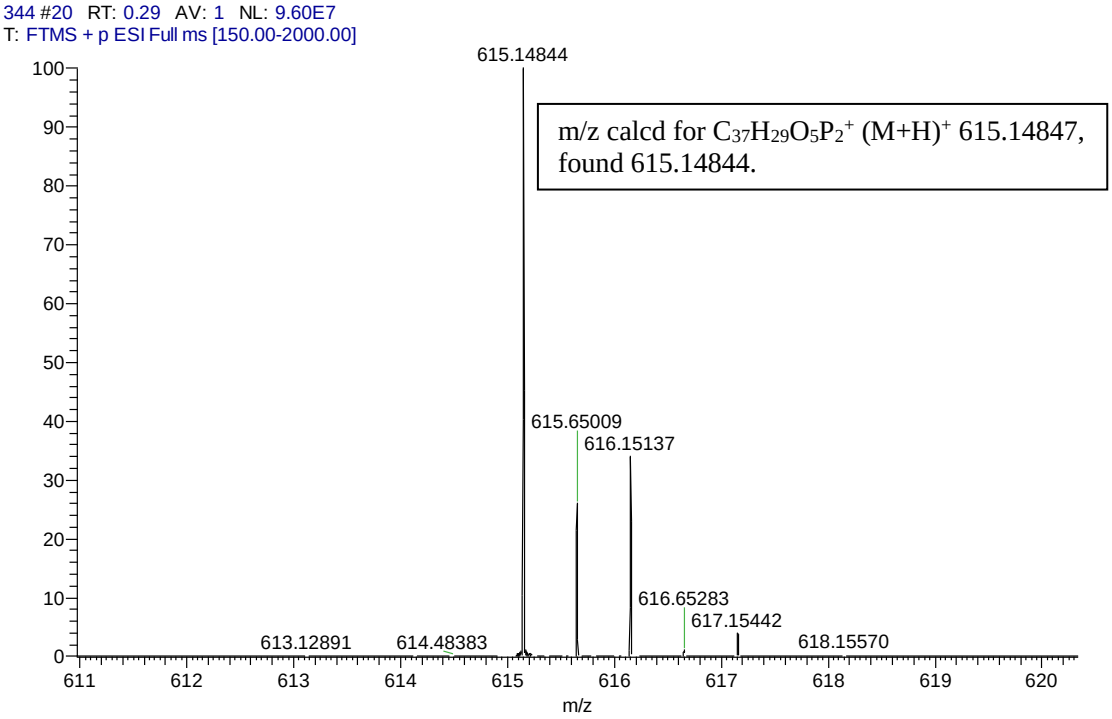
2c ^{1}H NMR (400 MHz, $CDCl_3$)



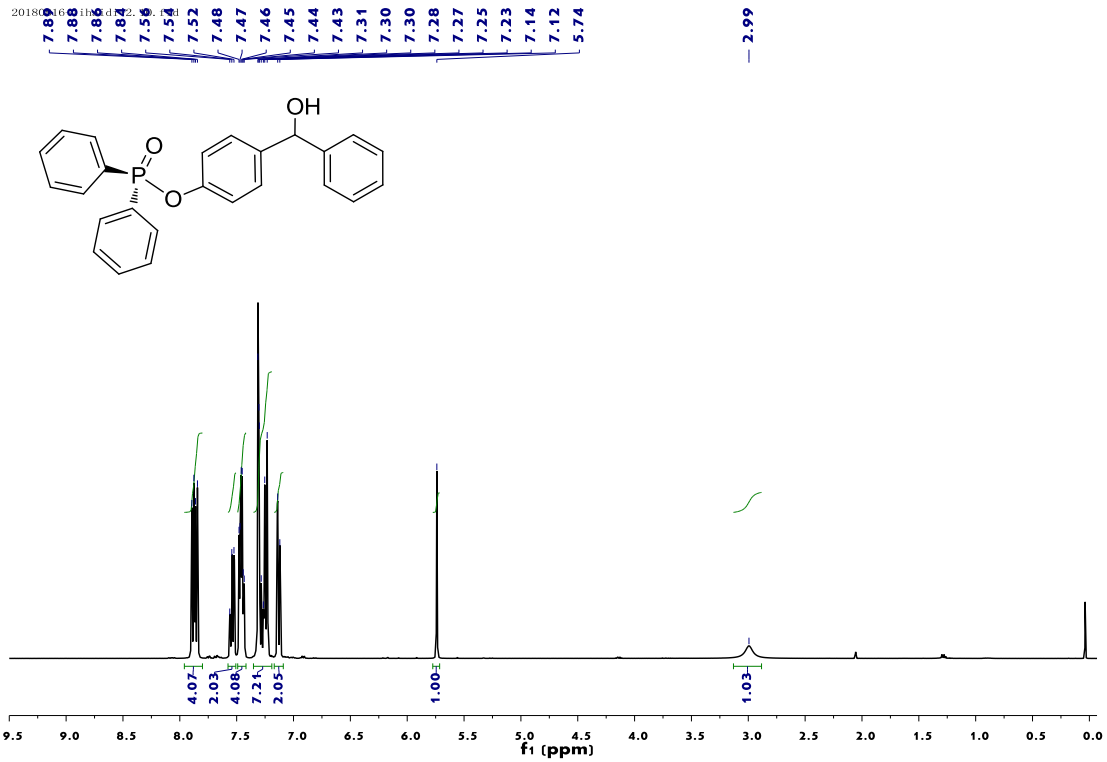
2c ^{13}C NMR (100 MHz, $CDCl_3$)



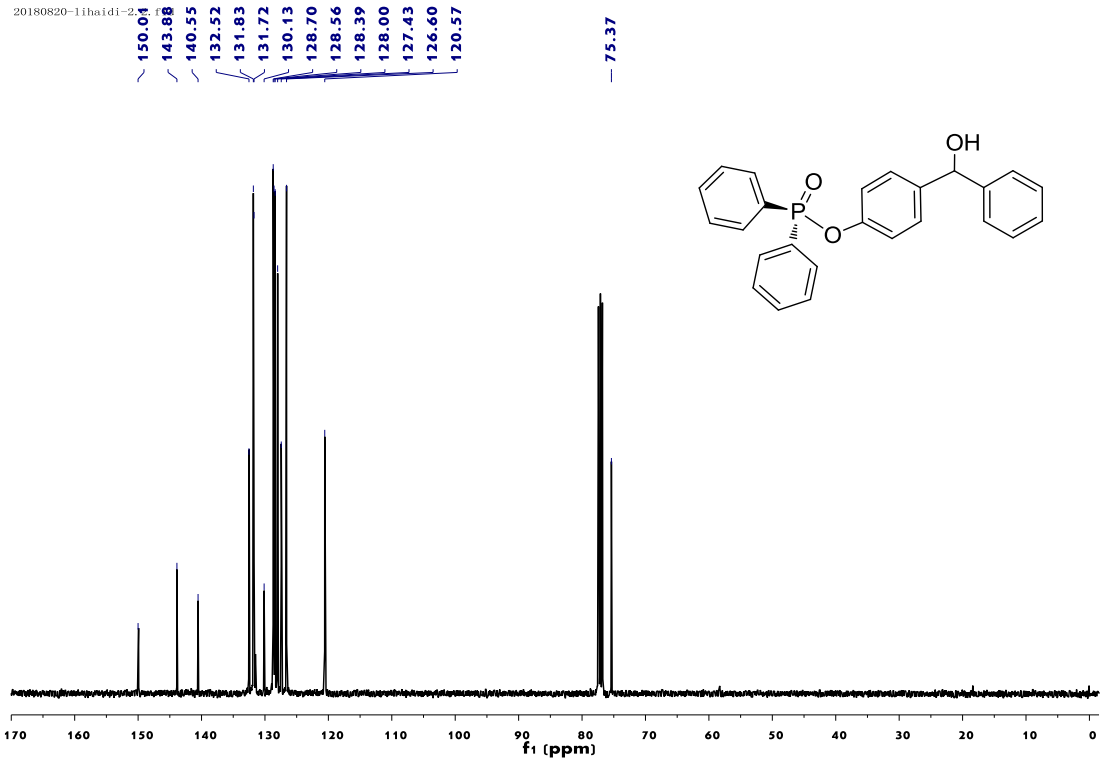
2c ^{31}P NMR (162 MHz, $CDCl_3$)



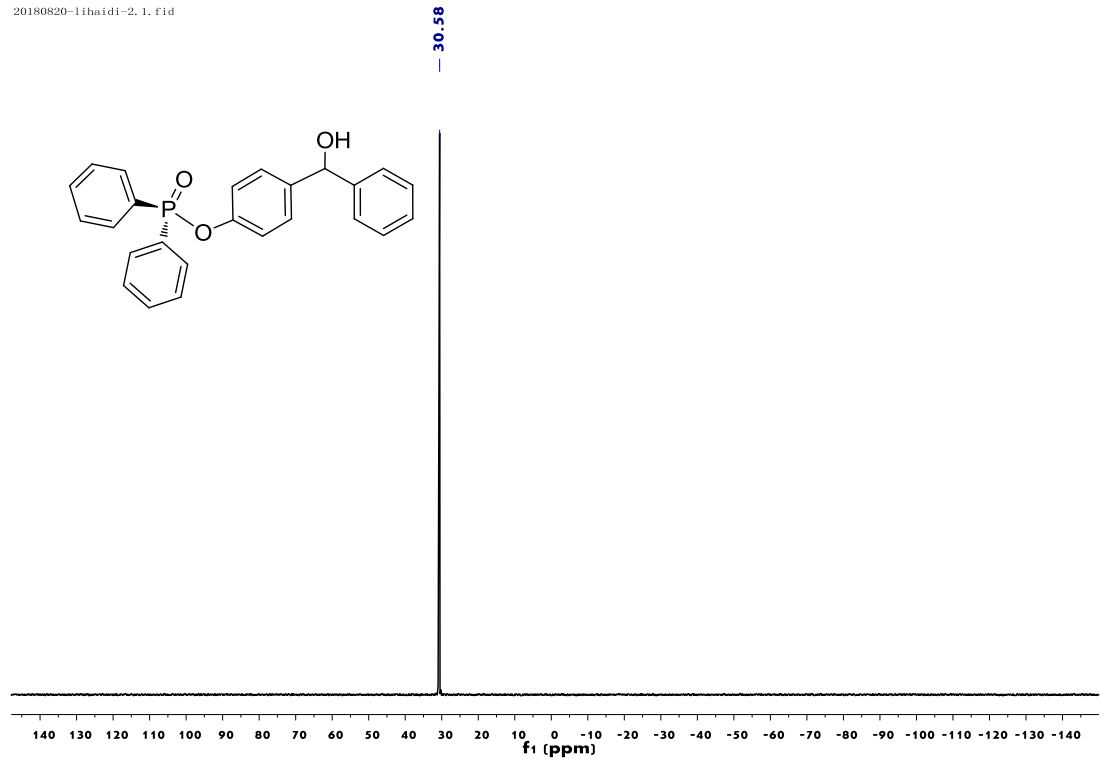
2c HRMS (ESI)



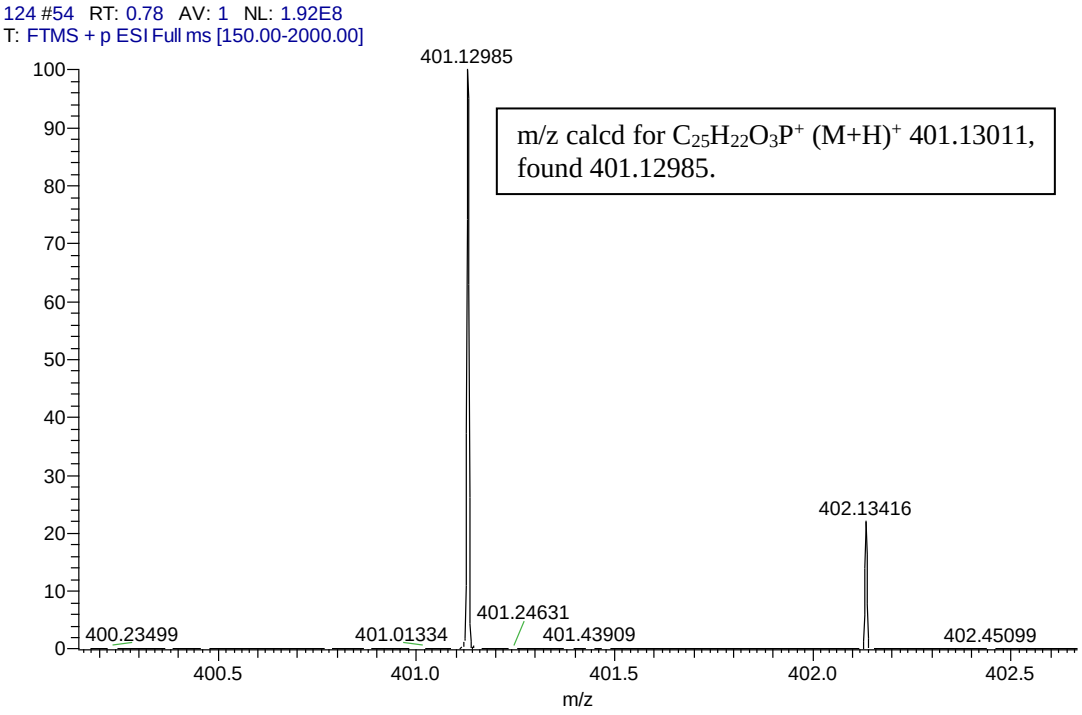
3a ^{1}H NMR (400 MHz, $CDCl_3$)



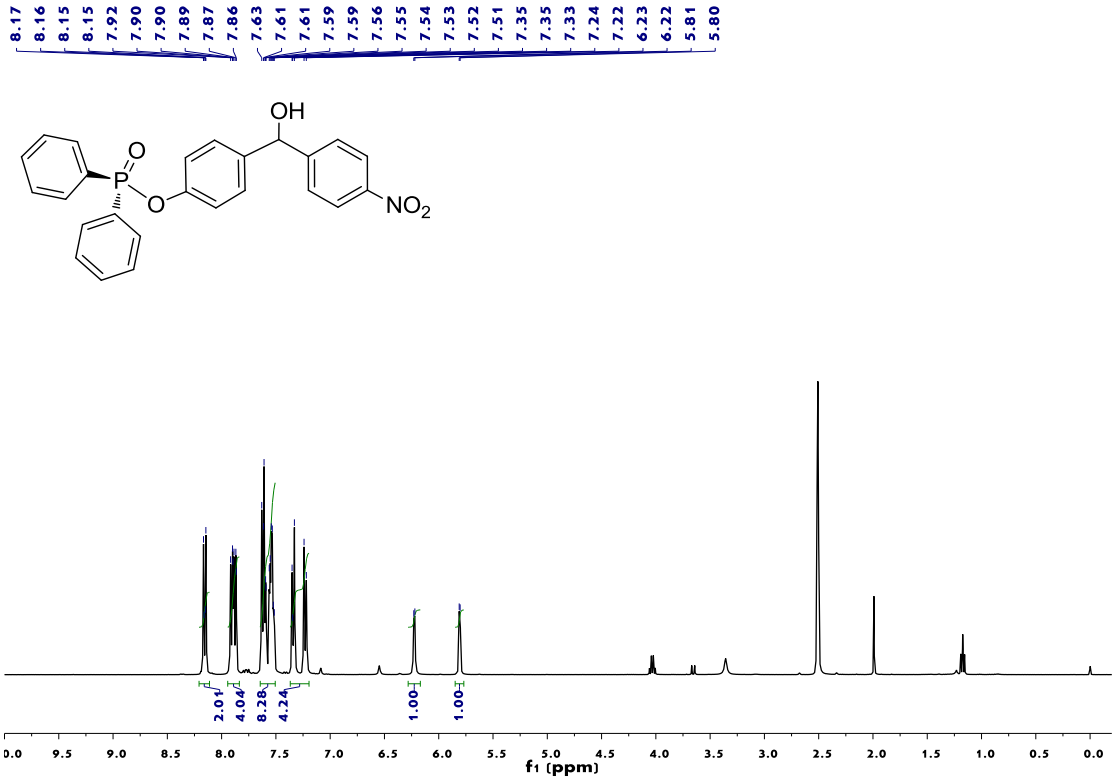
3a ^{13}C NMR (100 MHz, $CDCl_3$)



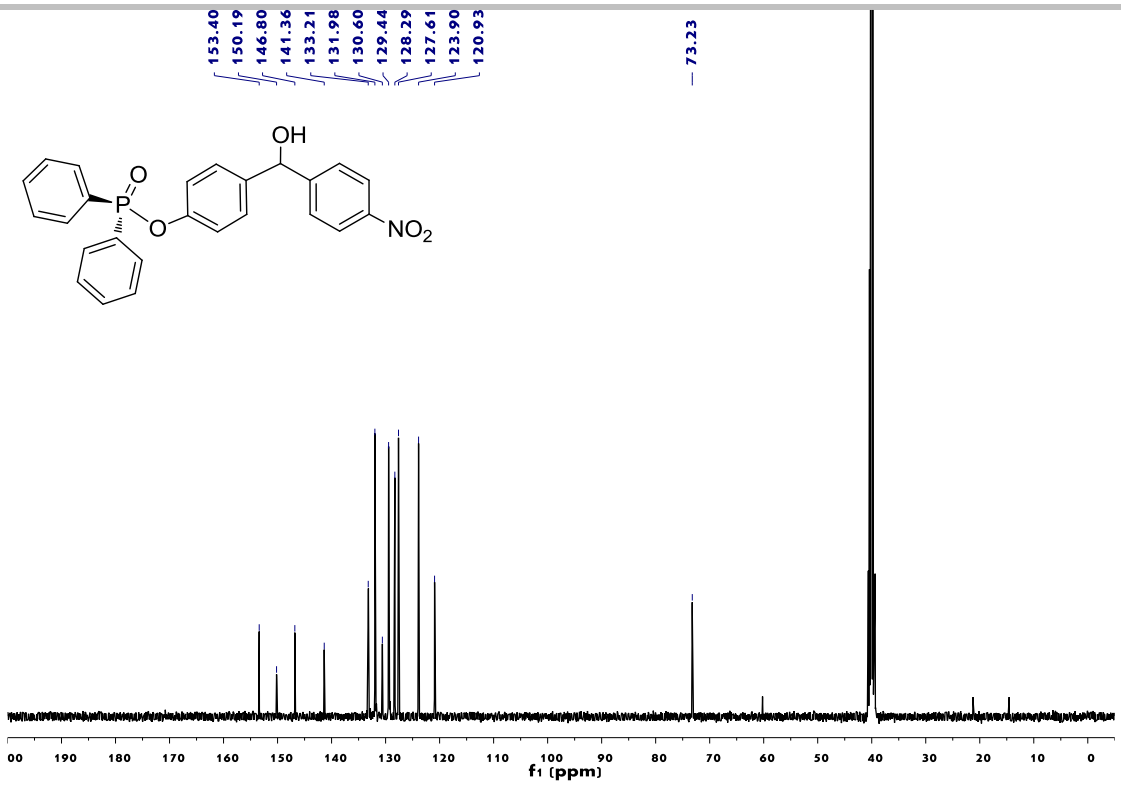
3a ^{31}P NMR (162 MHz, $CDCl_3$)



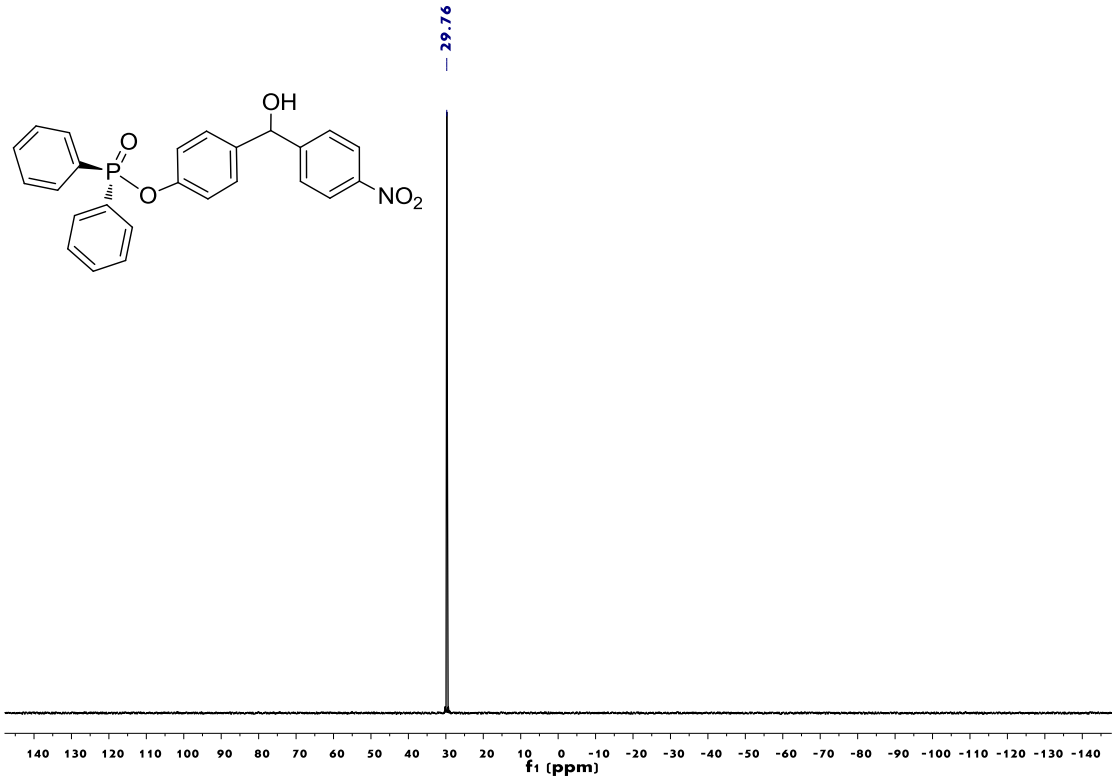
3a HRMS (ESI)



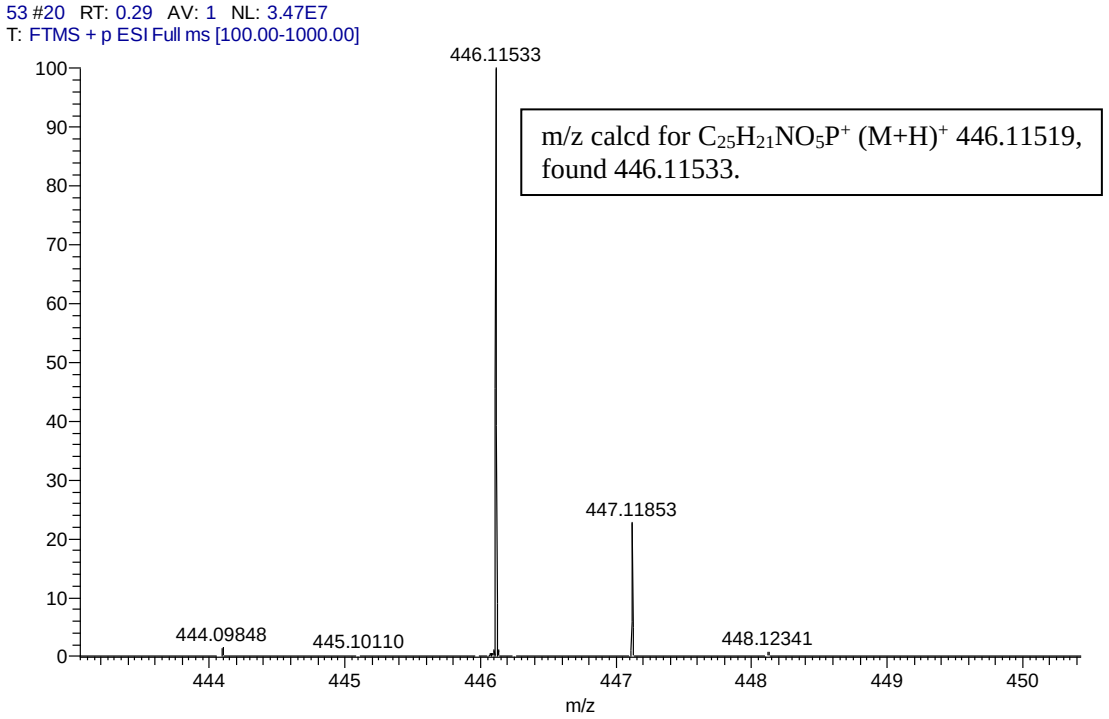
3b ^{1}H NMR (400 MHz, DMSO-d_6)



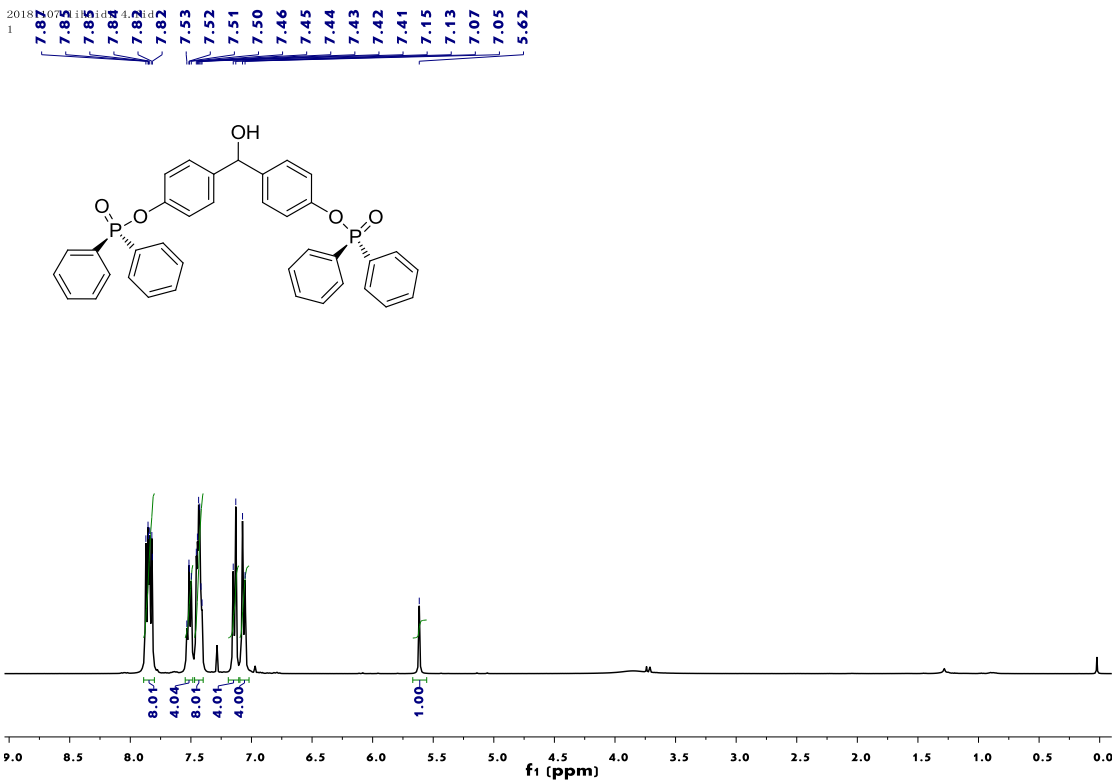
3b ^{13}C NMR (100 MHz, DMSO-d_6)



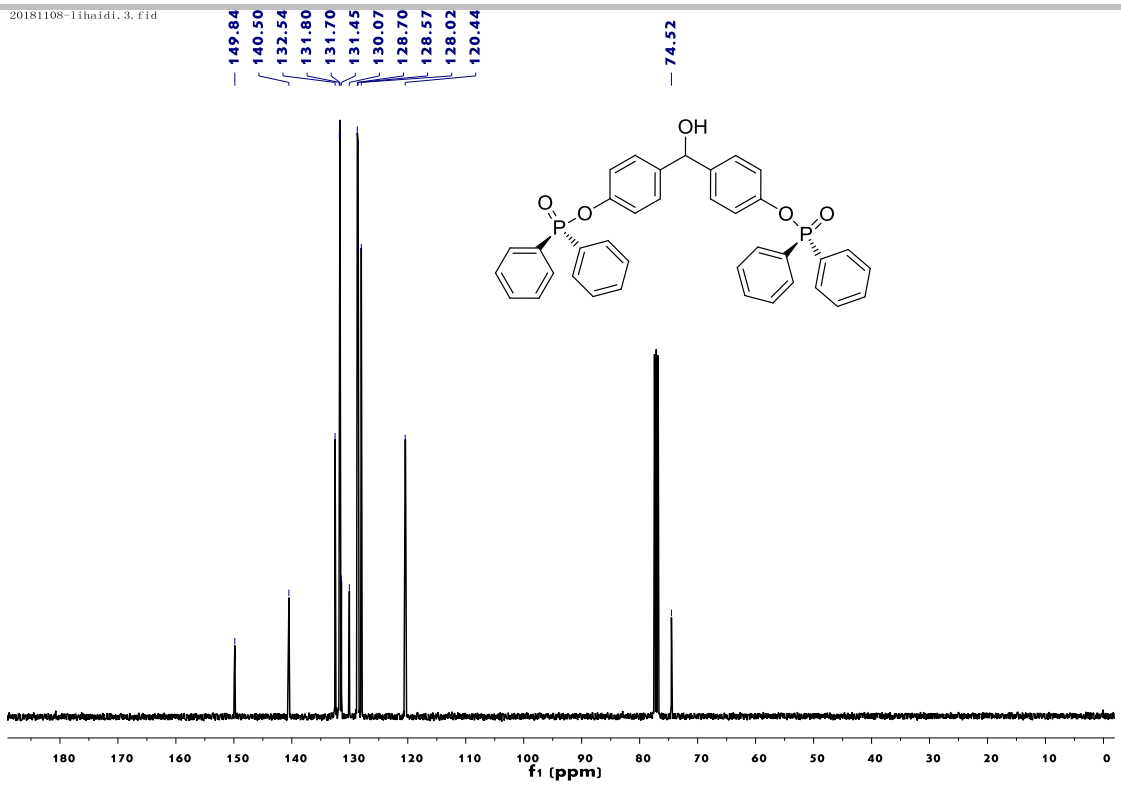
3b ^{31}P NMR (162 MHz, DMSO-d_6)



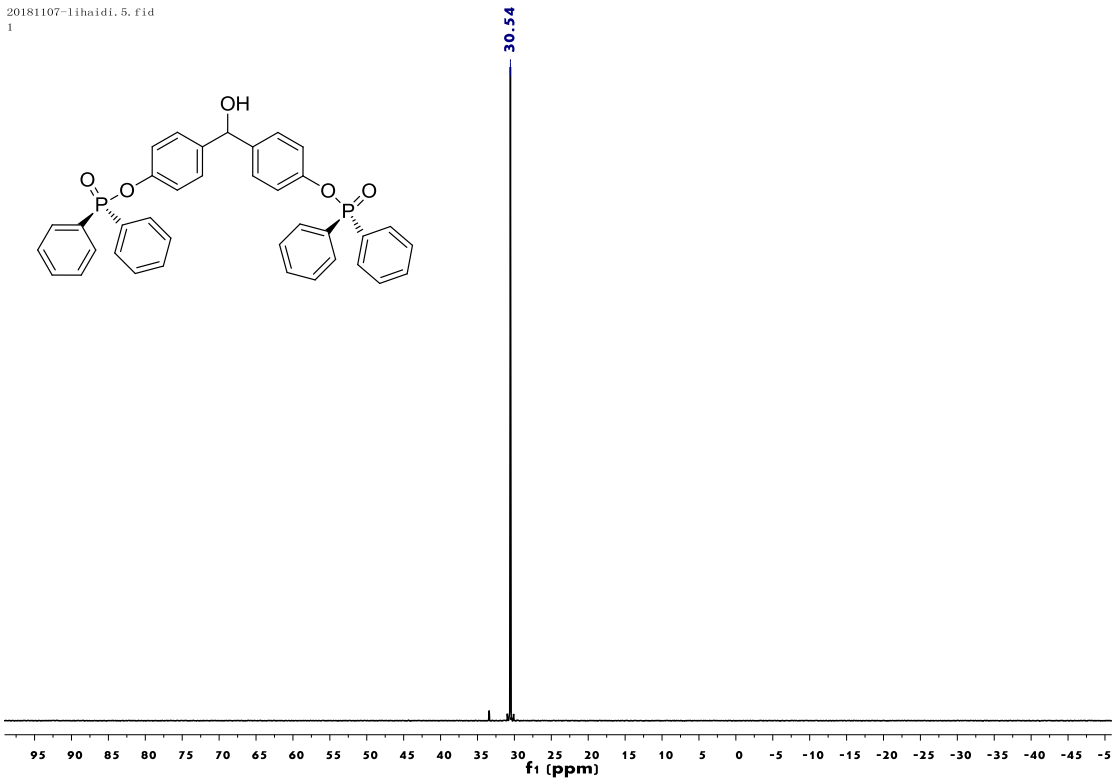
3b HRMS (ESI)



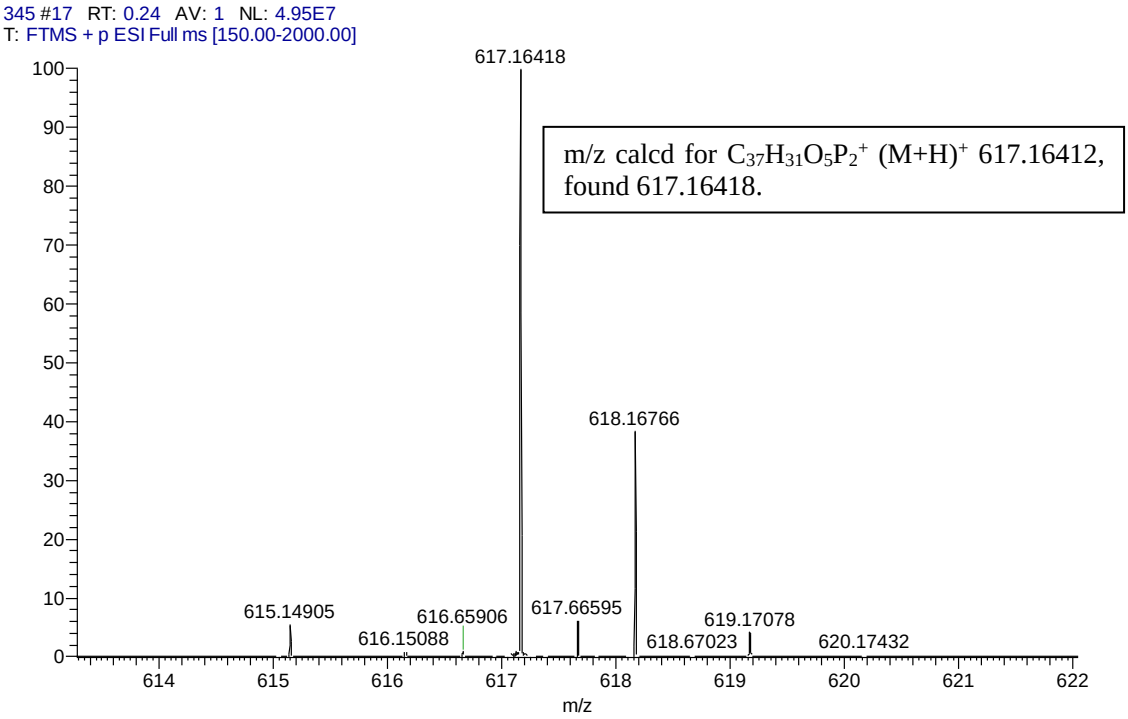
3c ^{1}H NMR (400 MHz, $CDCl_3$)



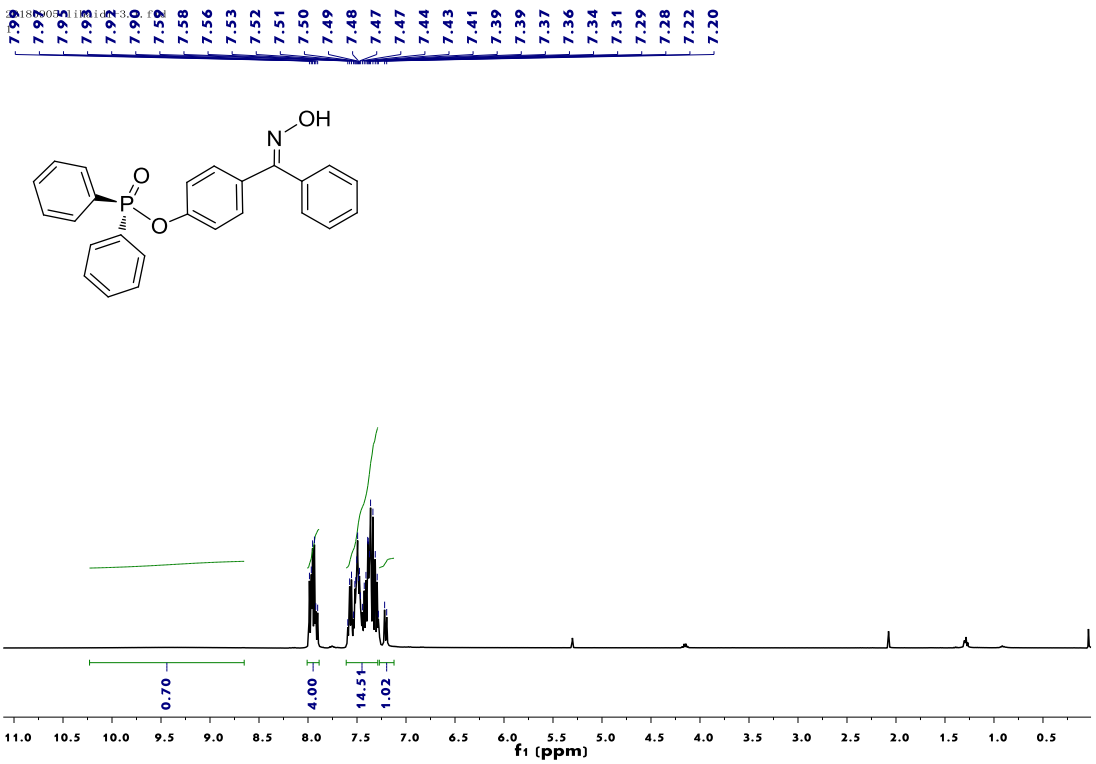
3c ^{13}C NMR (100 MHz, $CDCl_3$)



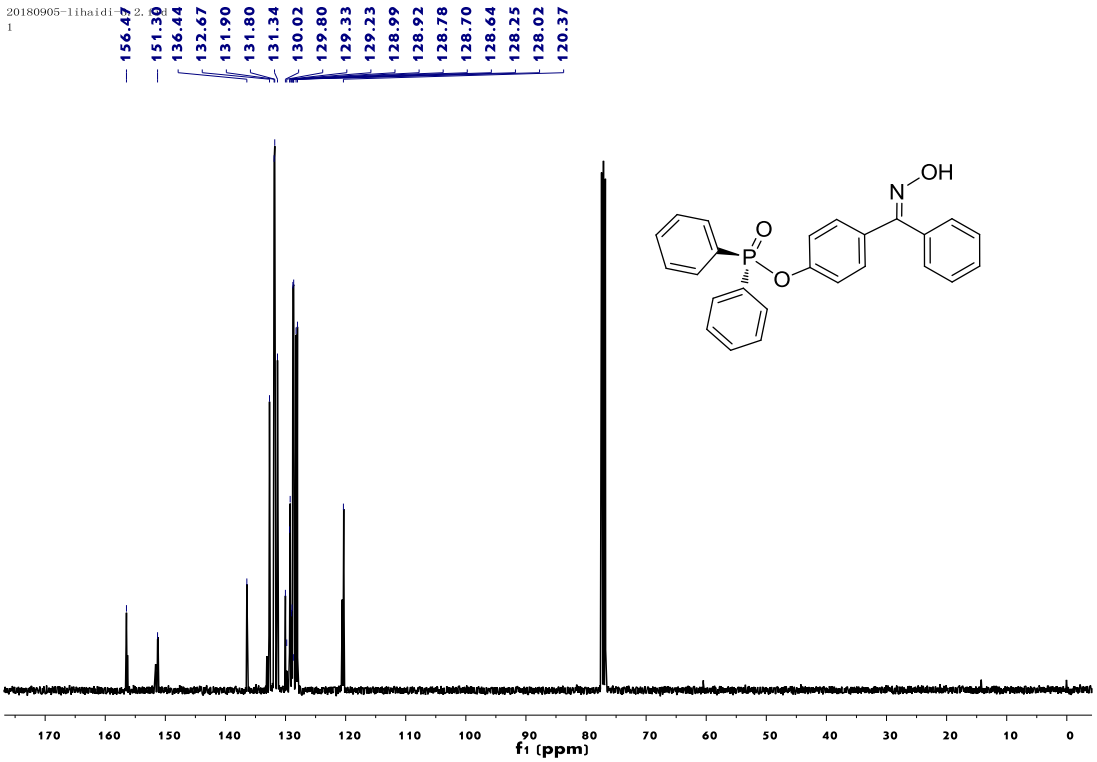
3c ^{31}P NMR (162 MHz, $CDCl_3$)



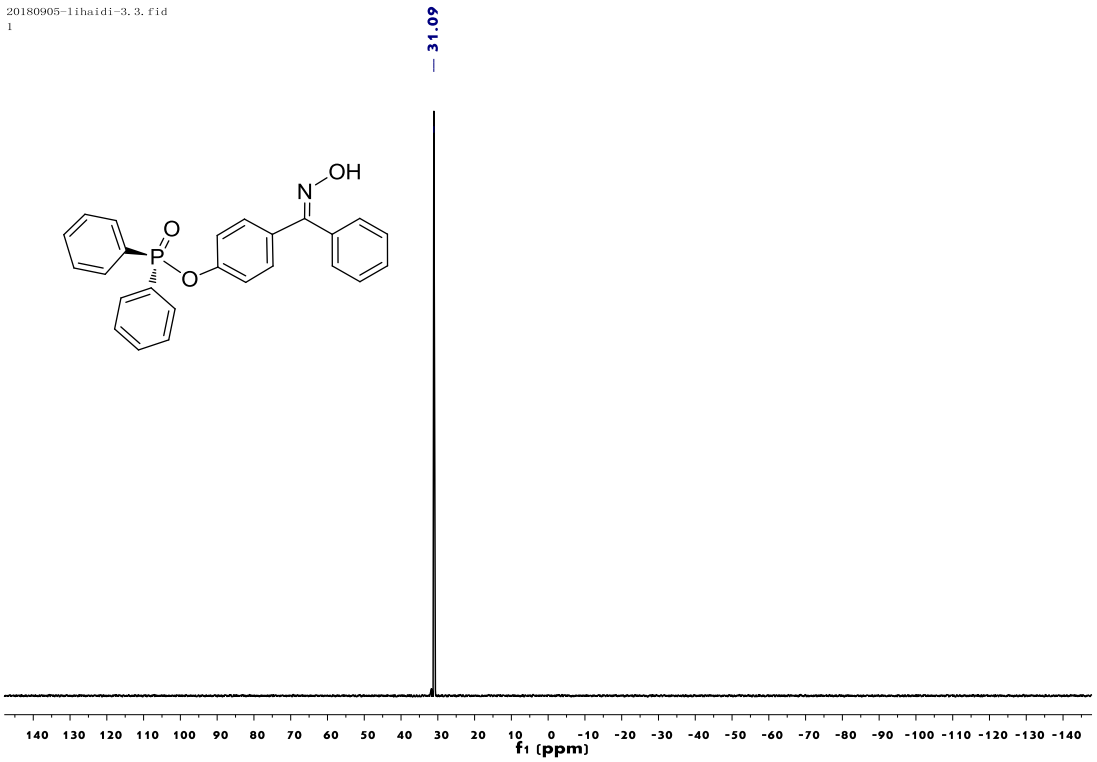
3c HRMS (ESI)



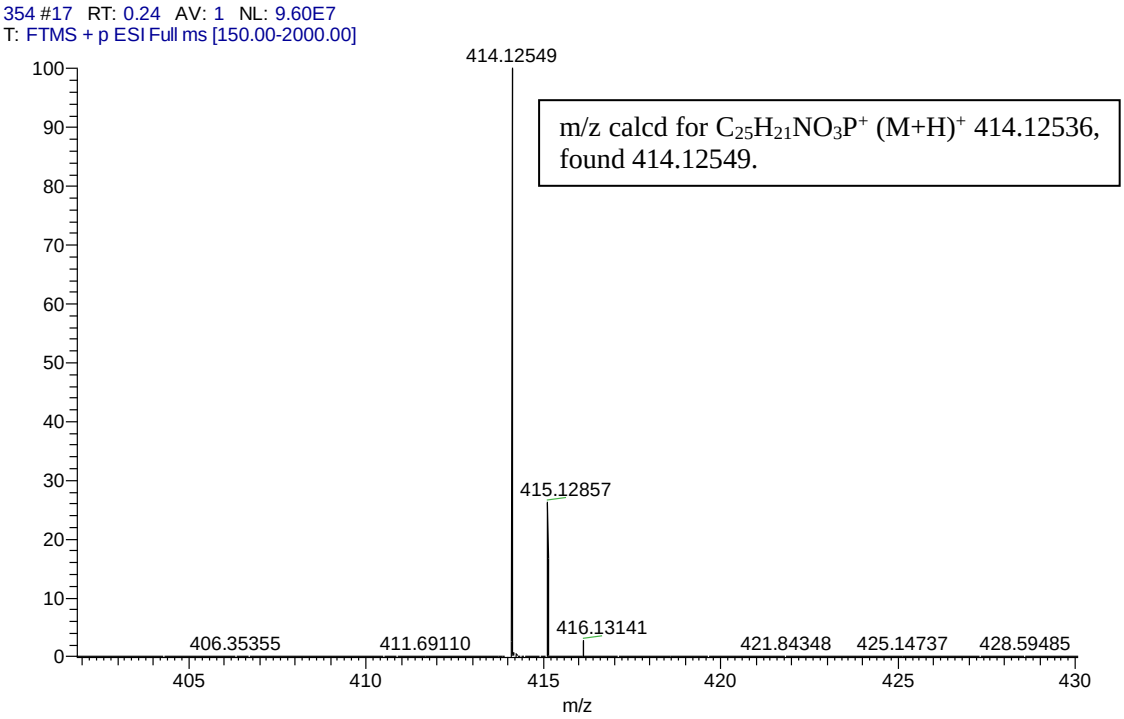
4a ^{1}H NMR (400 MHz, $CDCl_3$)



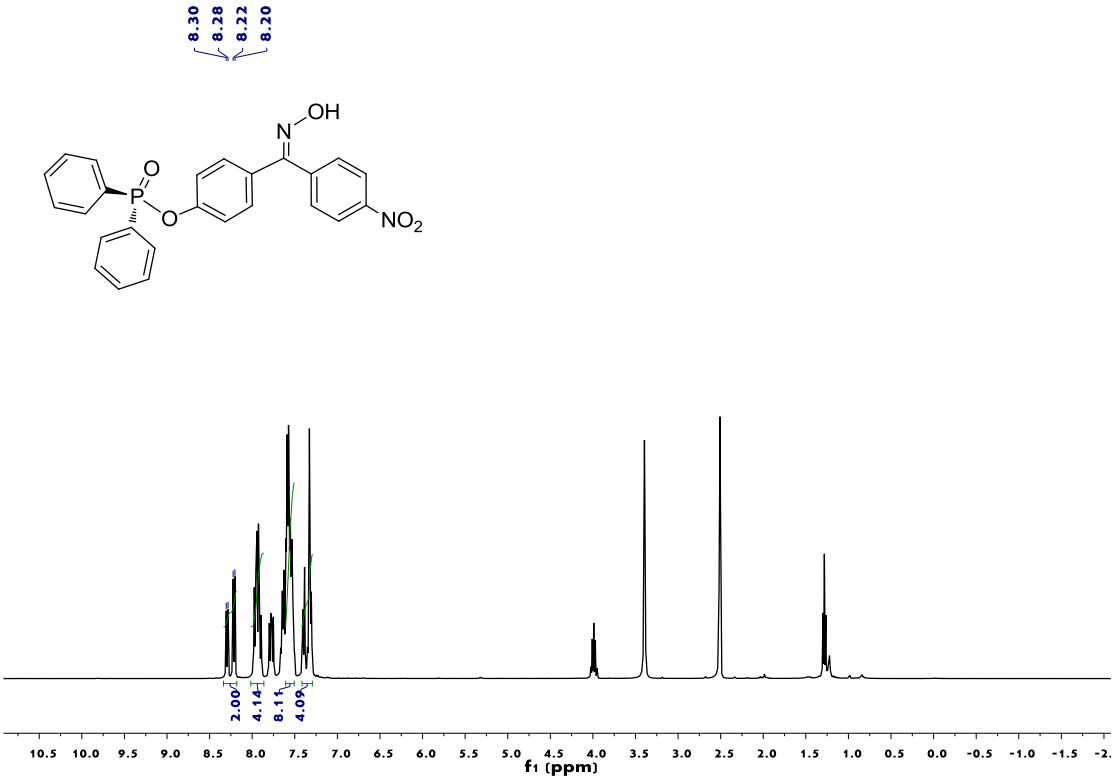
4a ^{13}C NMR (100 MHz, $CDCl_3$)



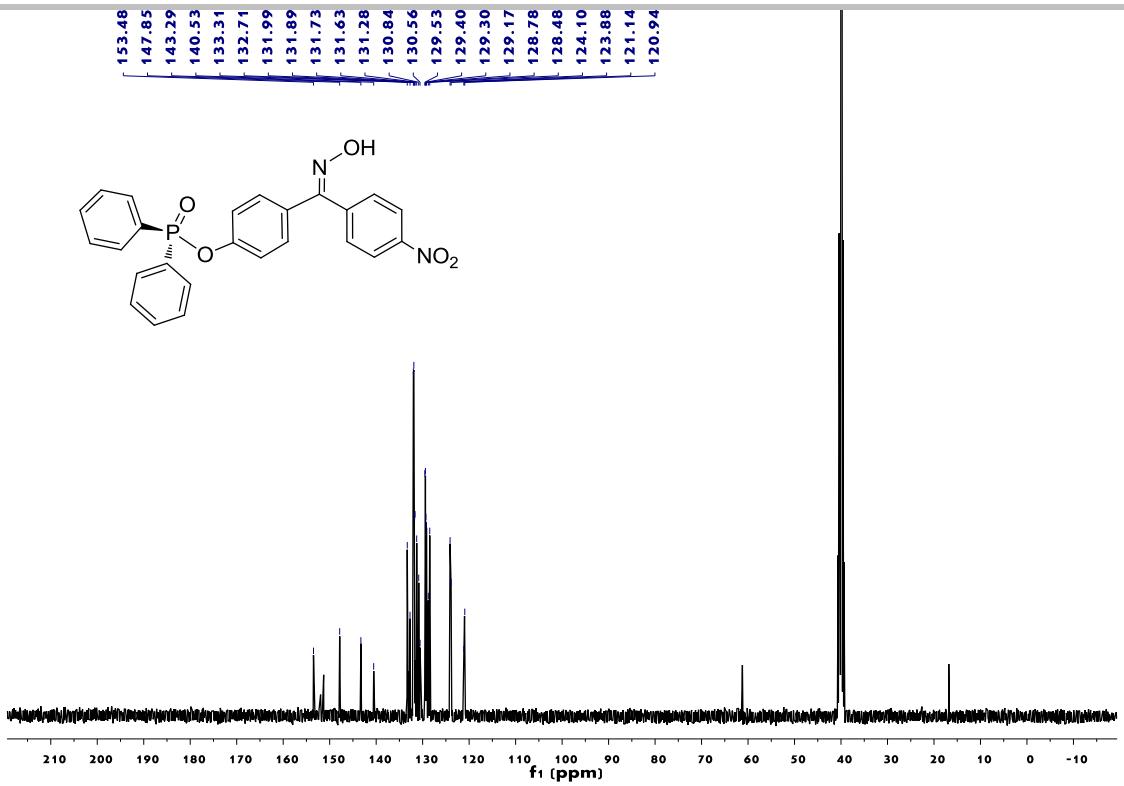
4a ^{31}P NMR (162 MHz, $CDCl_3$)



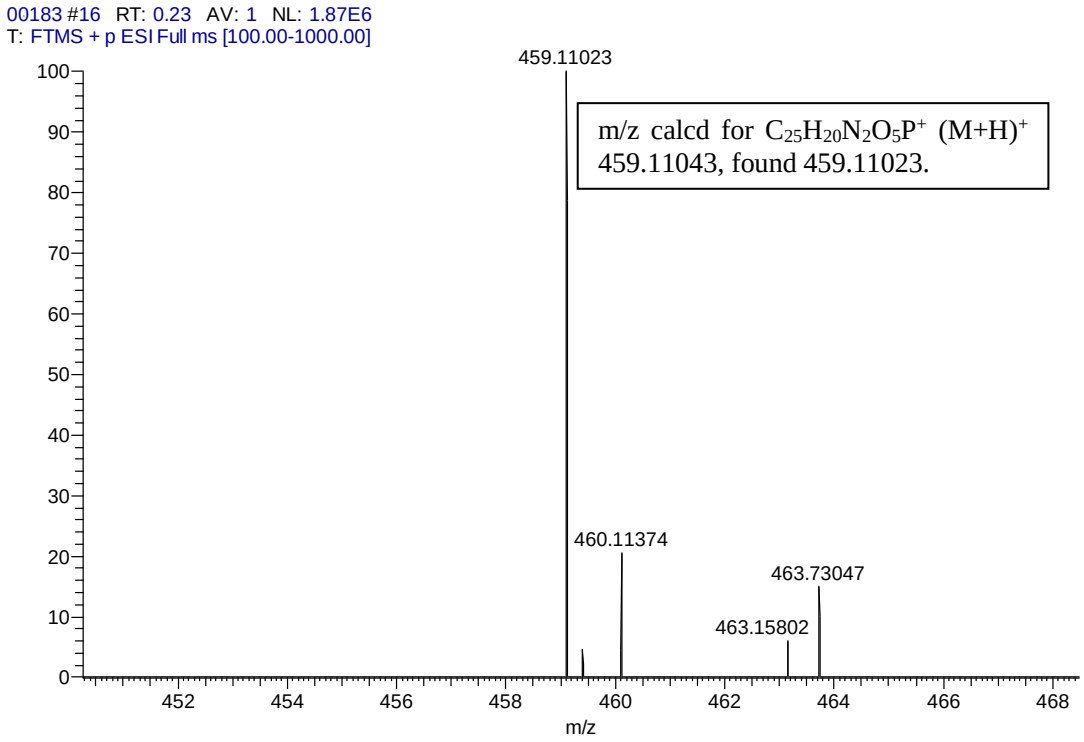
4a HRMS (ESI) m/z



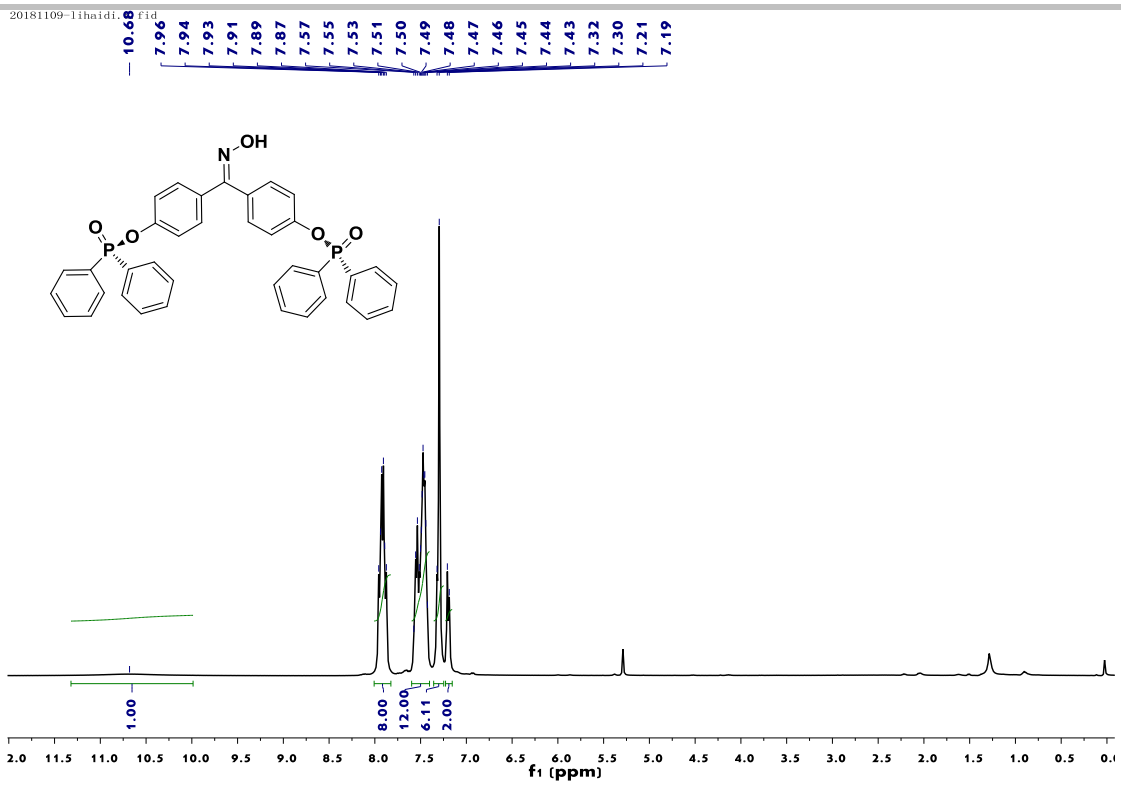
4b ^{1}H NMR (400 MHz, DMSO-d_6)



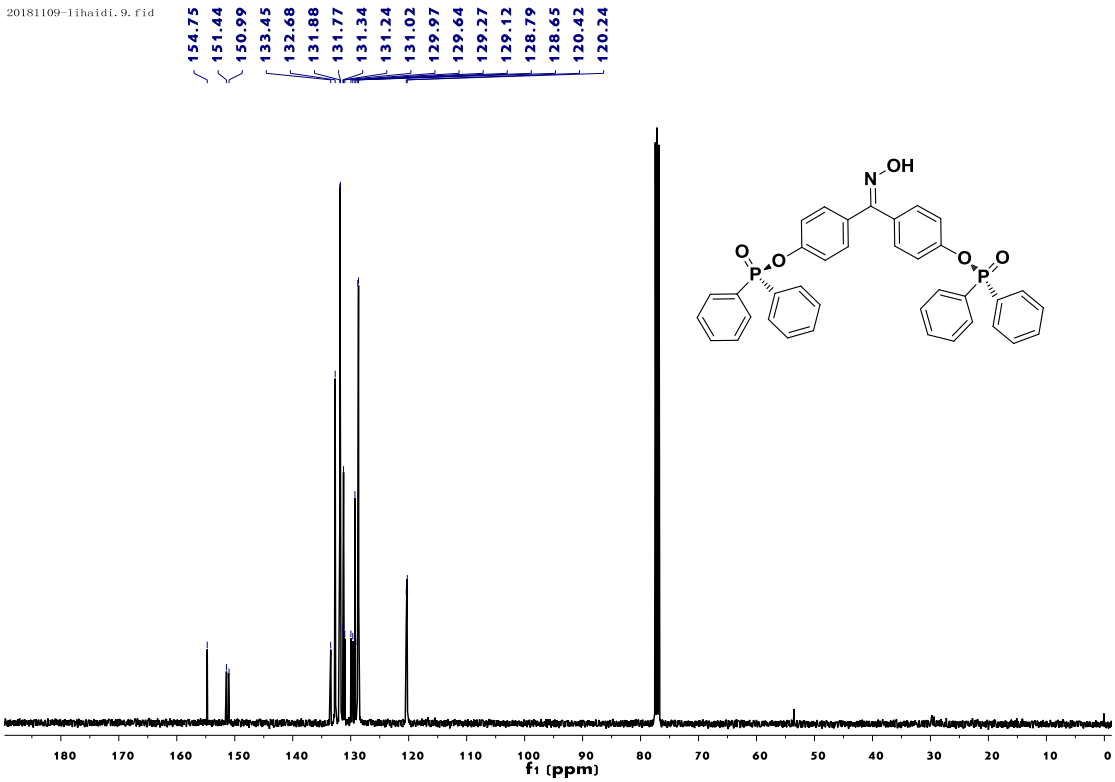
4b ^{13}C NMR (100 MHz, DMSO-d_6)



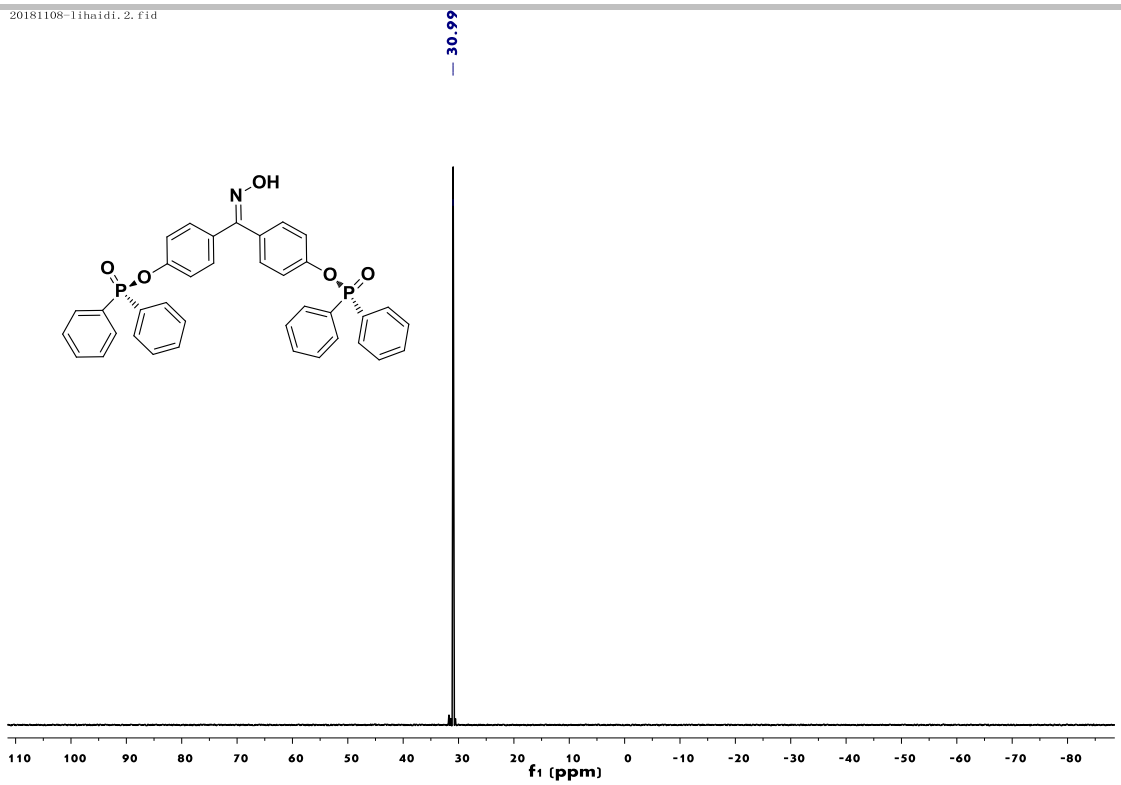
4b HRMS (ESI)



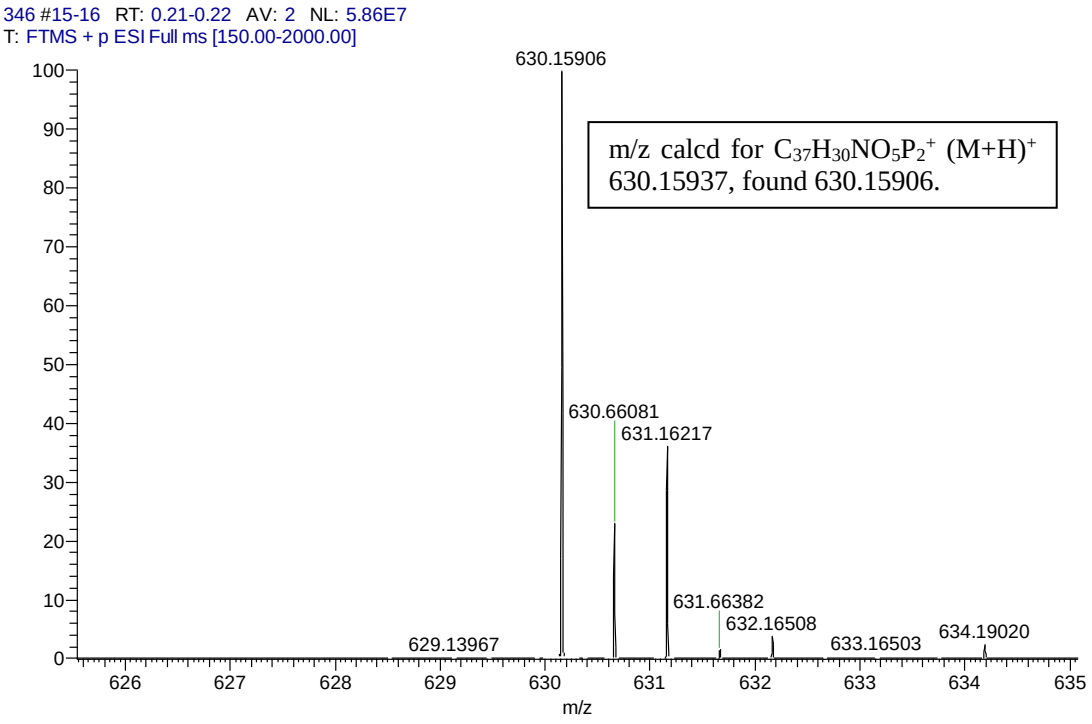
4c ^{1}H NMR (400 MHz, $CDCl_3$)



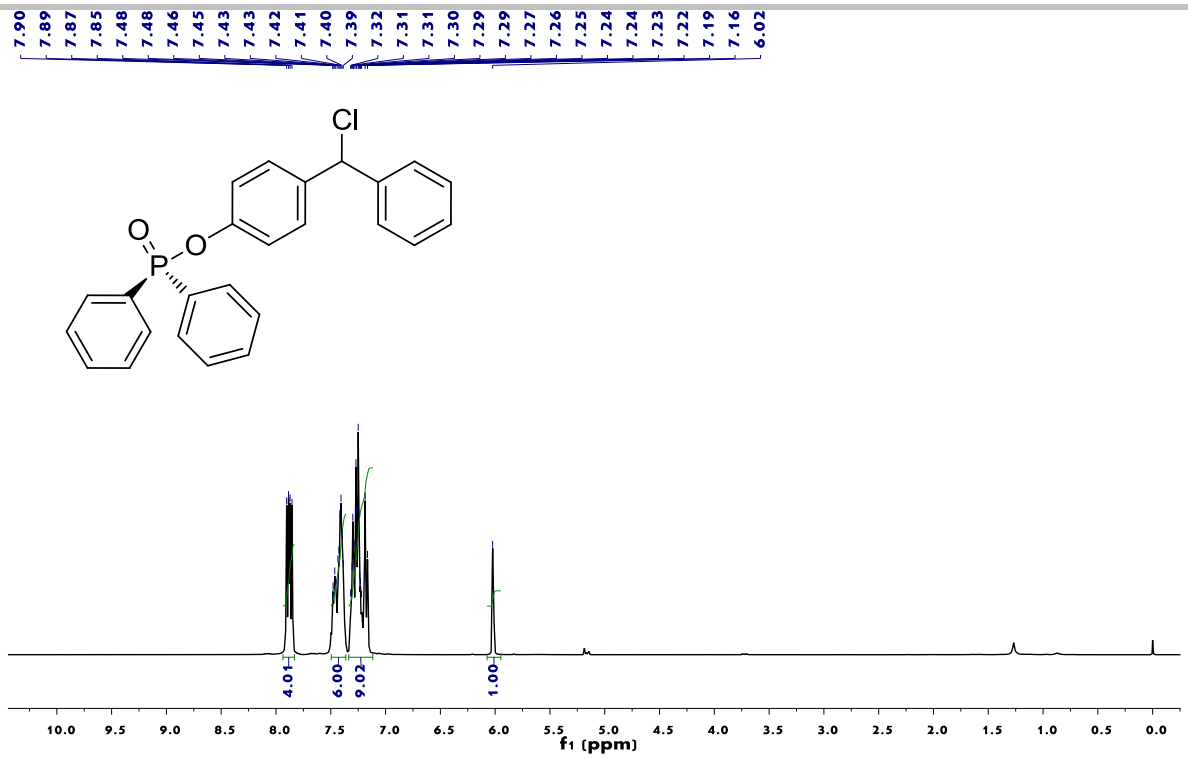
4c ^{13}C NMR (100 MHz, $CDCl_3$)



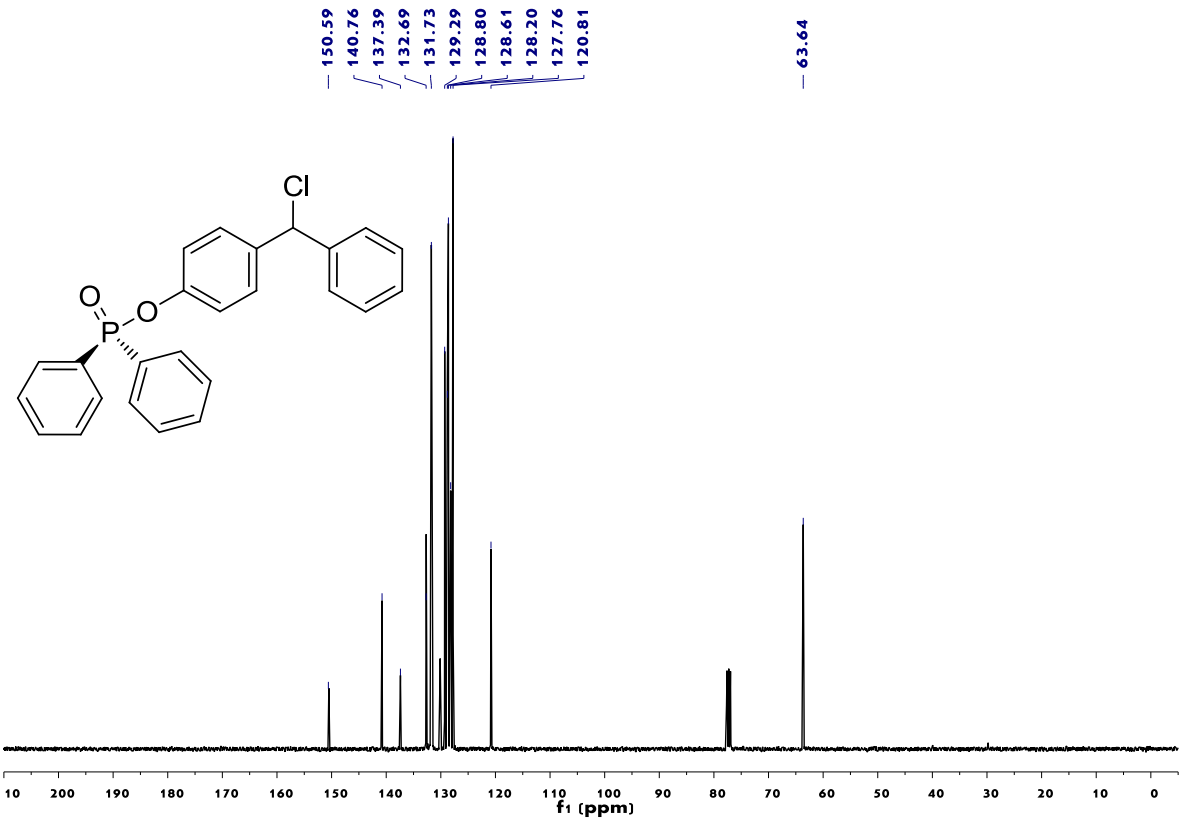
4c ^{31}P NMR (162 MHz, $CDCl_3$)



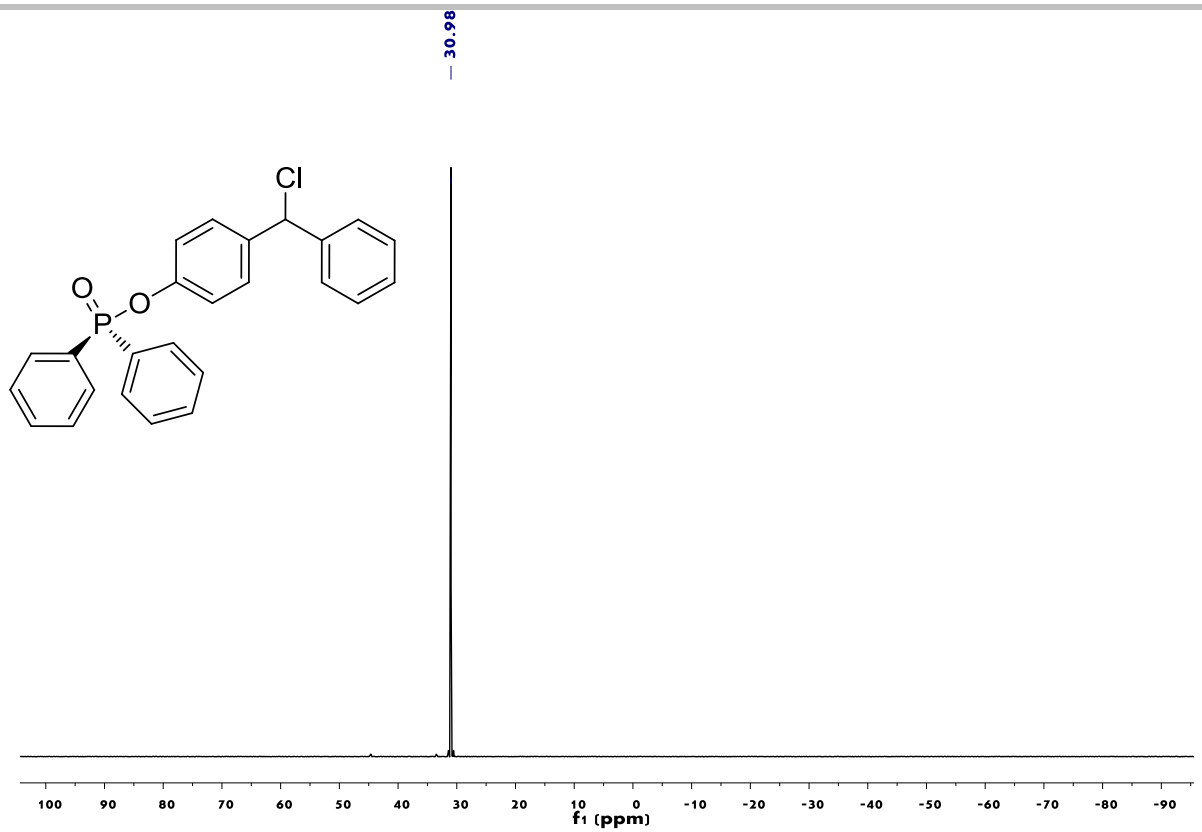
4c HRMS (ESI)



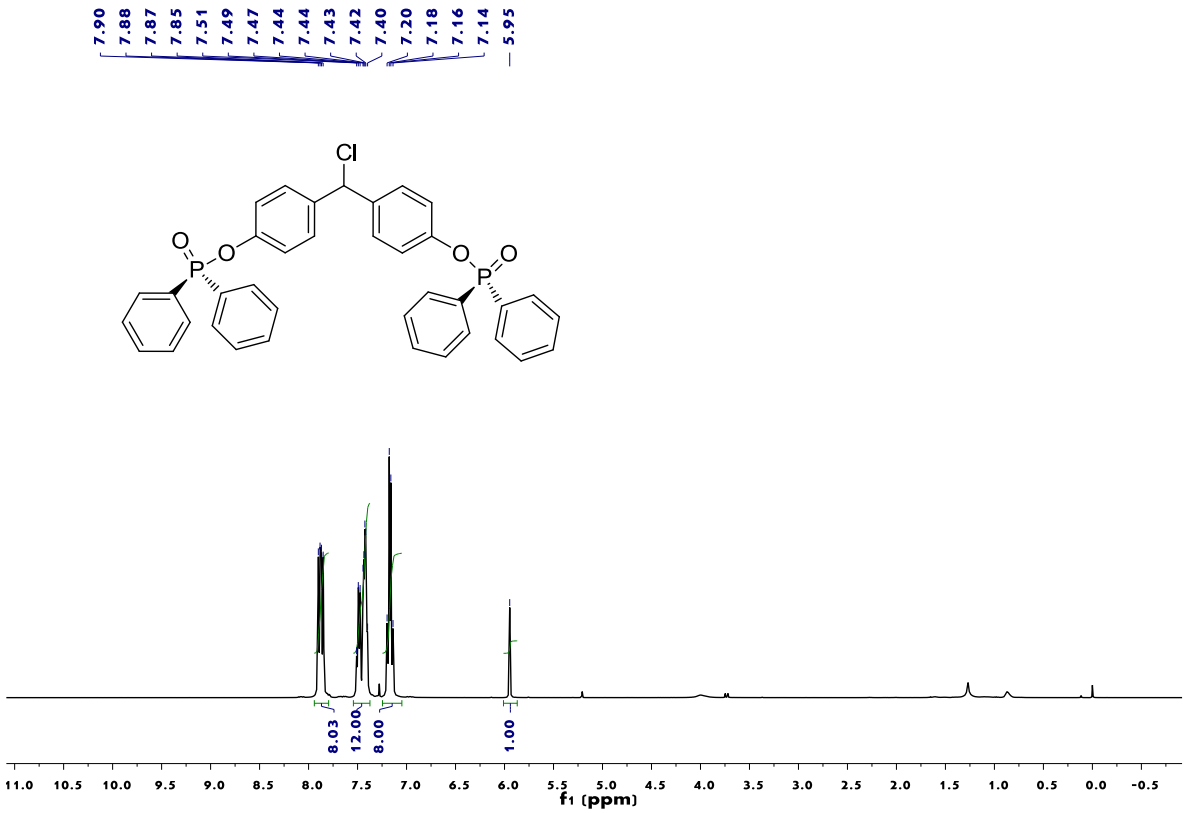
5a ^{1}H NMR (400 MHz, $CDCl_3$)



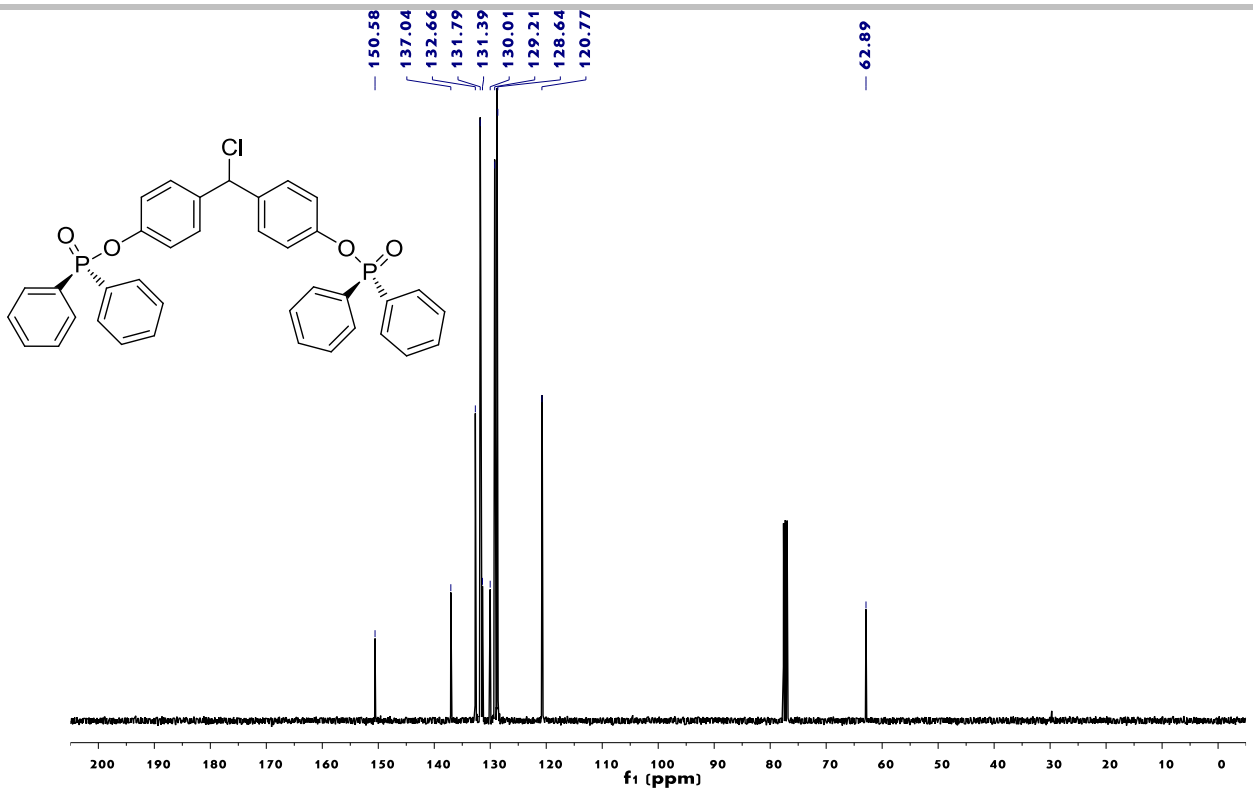
5a ^{13}C NMR (100 MHz, $CDCl_3$)



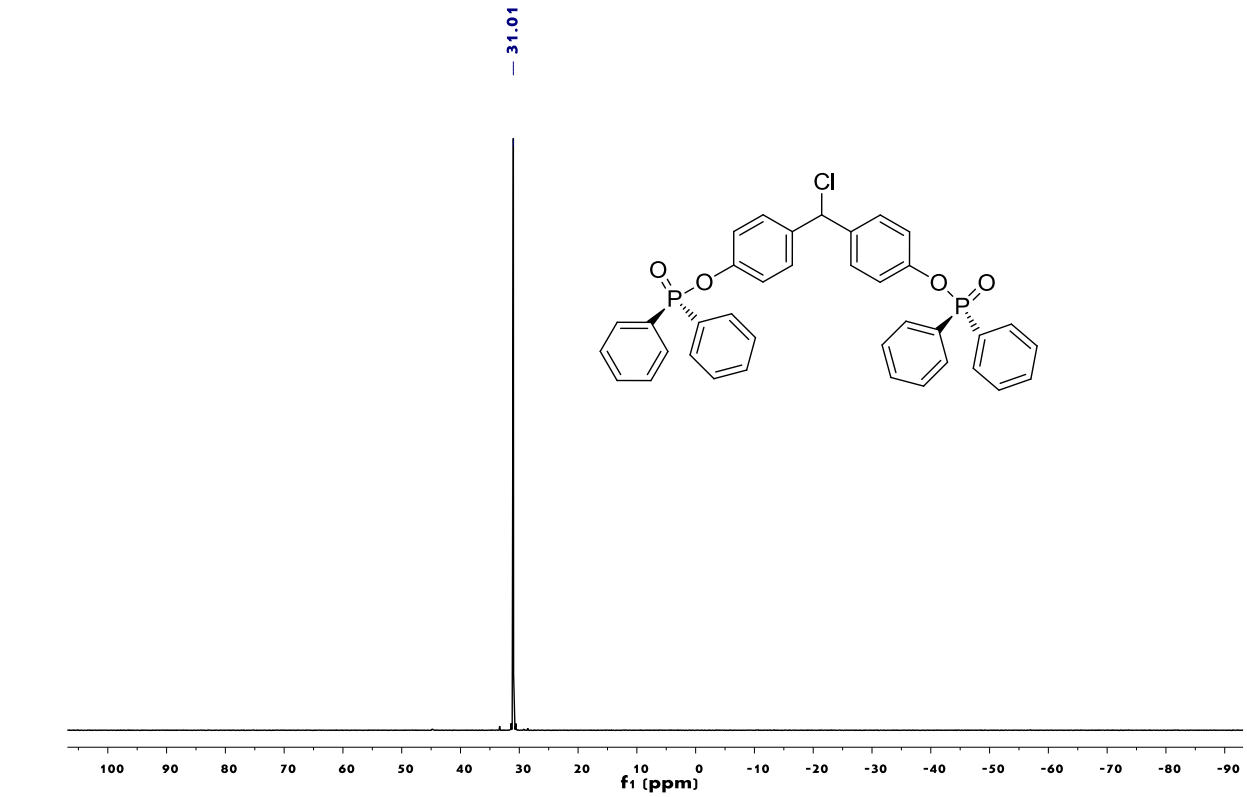
5a ^{31}P NMR (162 MHz, $CDCl_3$)



5c ^{1}H NMR (400 MHz, $CDCl_3$)

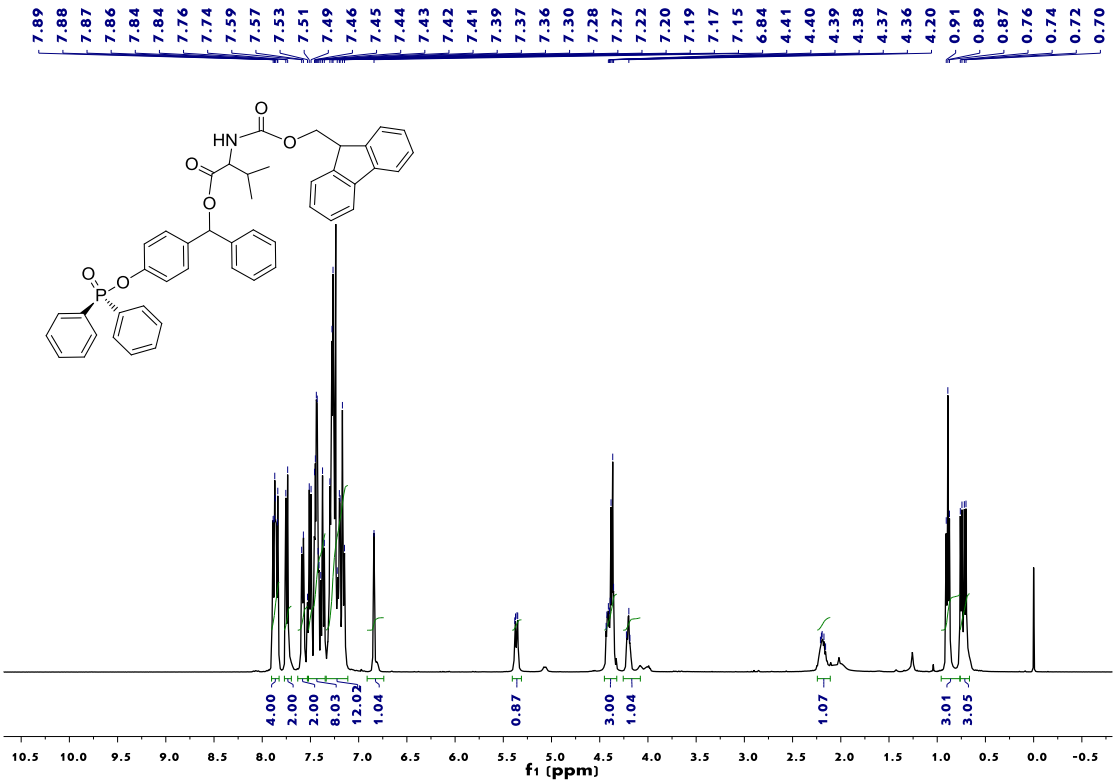


5c ^{13}C NMR (100 MHz, $CDCl_3$)

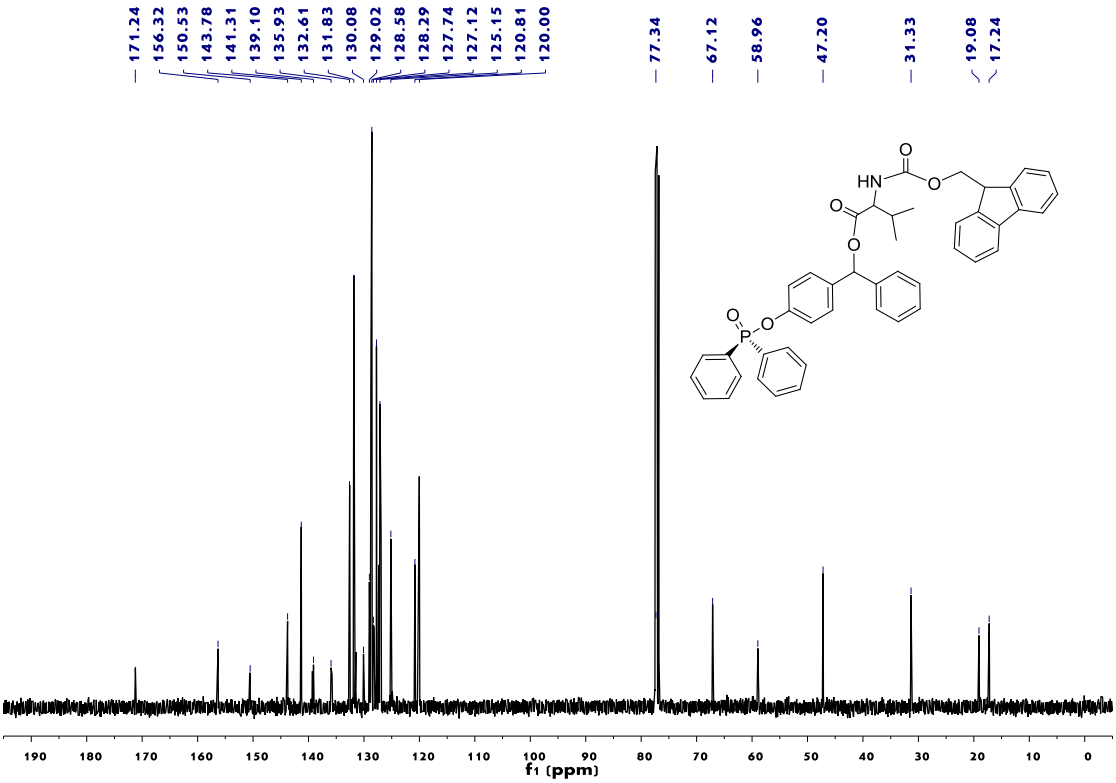


5c ^{31}P NMR (162MHz, $CDCl_3$)

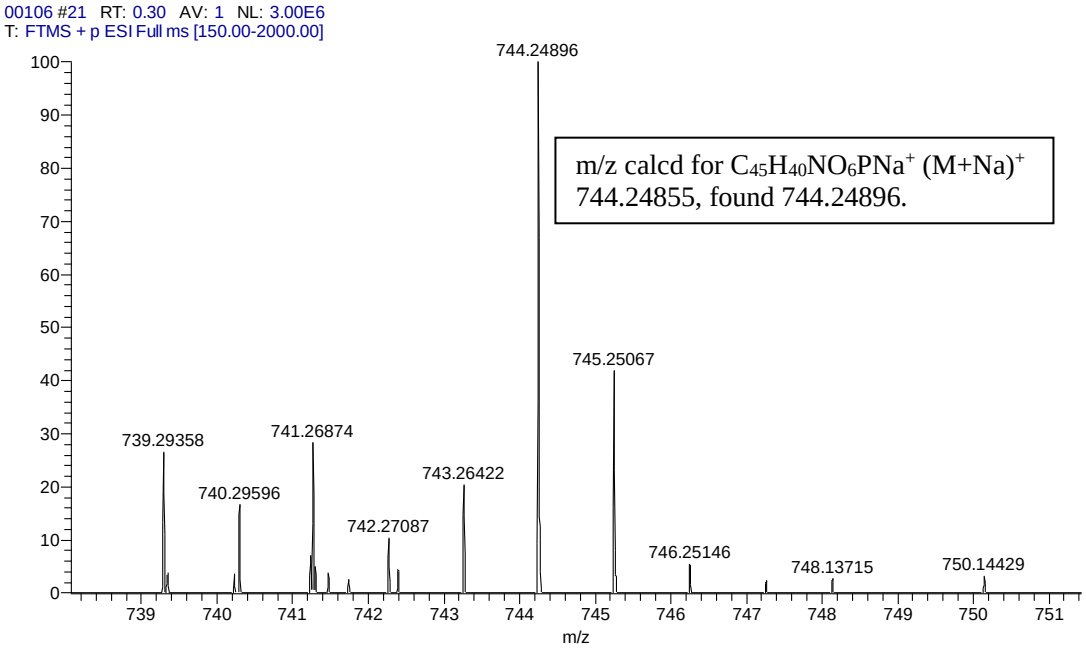
DDK derivates Coupling with Amino Acid NMR and HRMS (ESI)



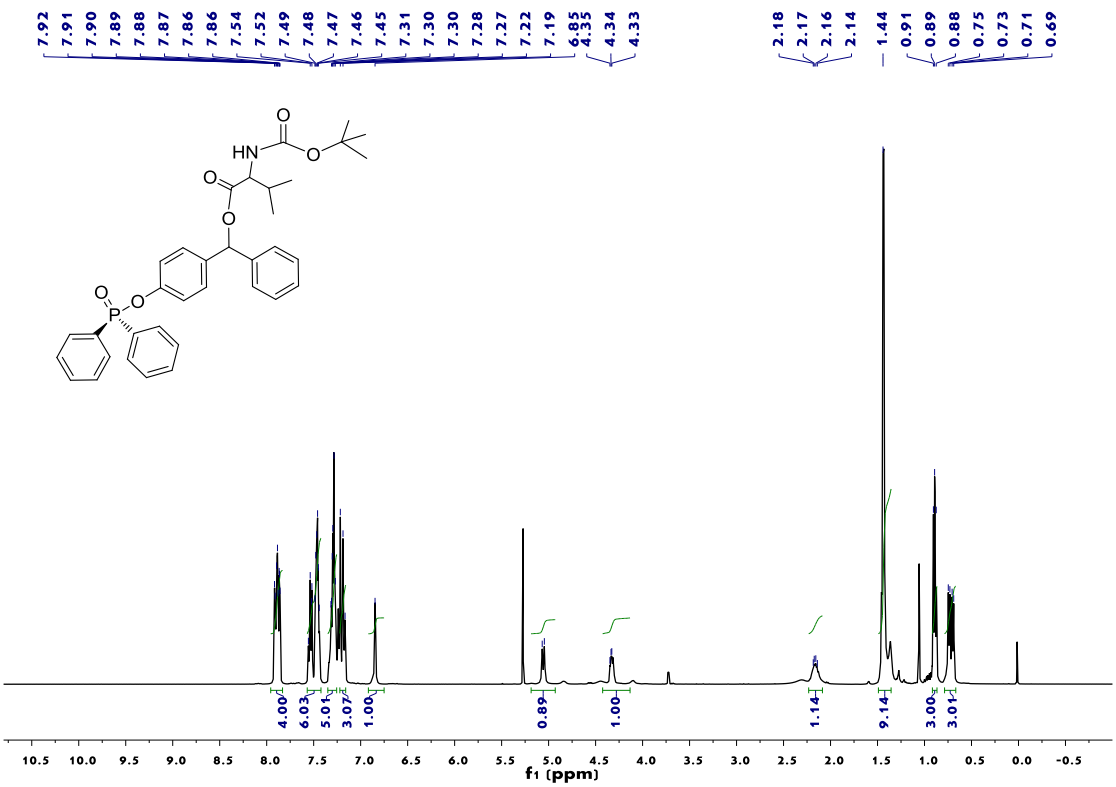
3a-1, Fmoc-Val-DDK-1 ^{1}H NMR (400 MHz, $CDCl_3$)



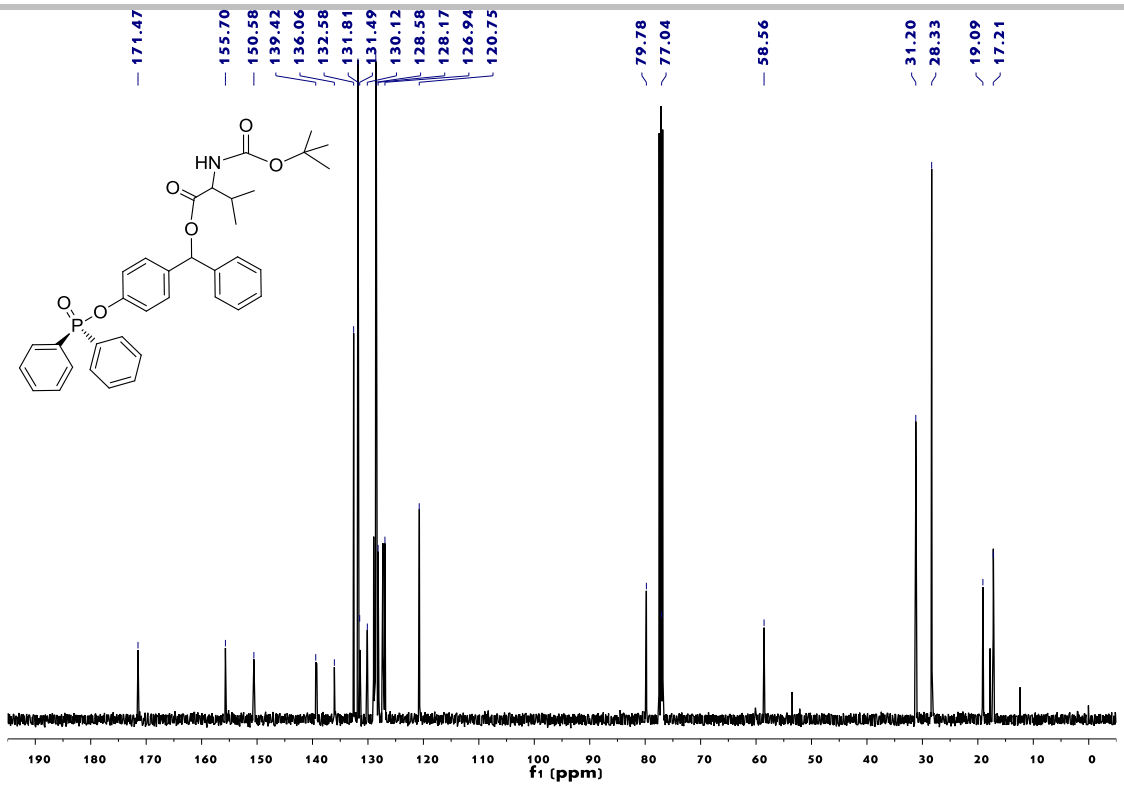
3a-1, Fmoc-Val-DDK-1 ^{13}C NMR (100 MHz, $CDCl_3$)



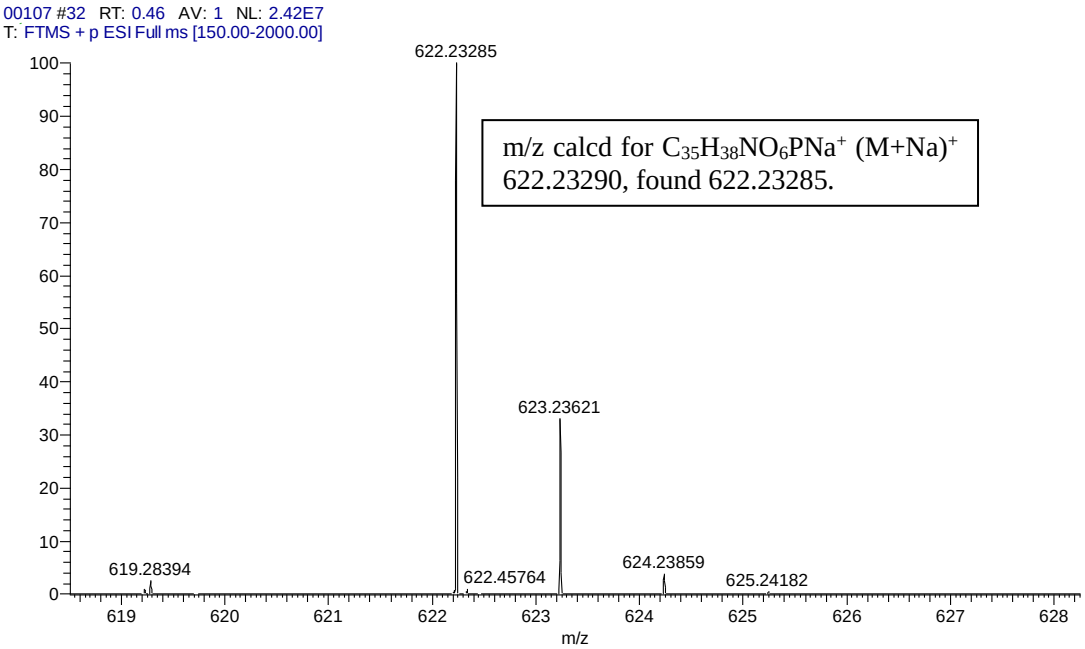
3a-1, Fmoc-Val-DDK-1 HRMS ESI



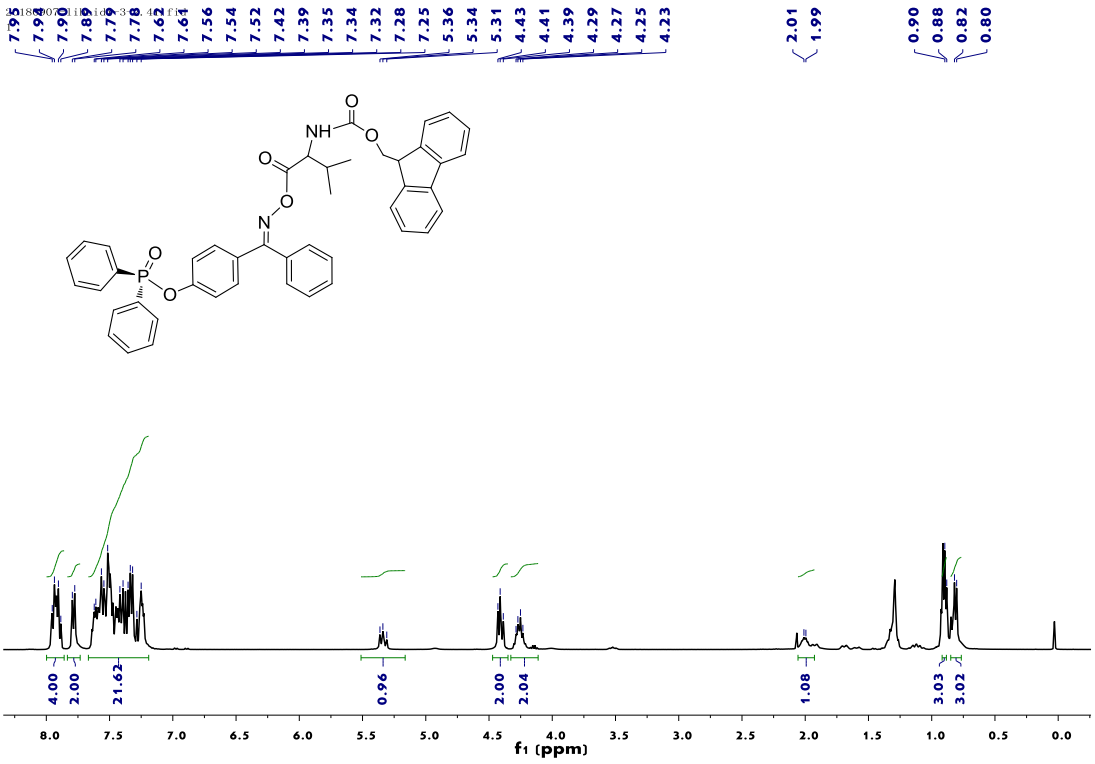
3a-2, Boc-Val-DDK-1 ^{1}H NMR (400 MHz, $CDCl_3$)



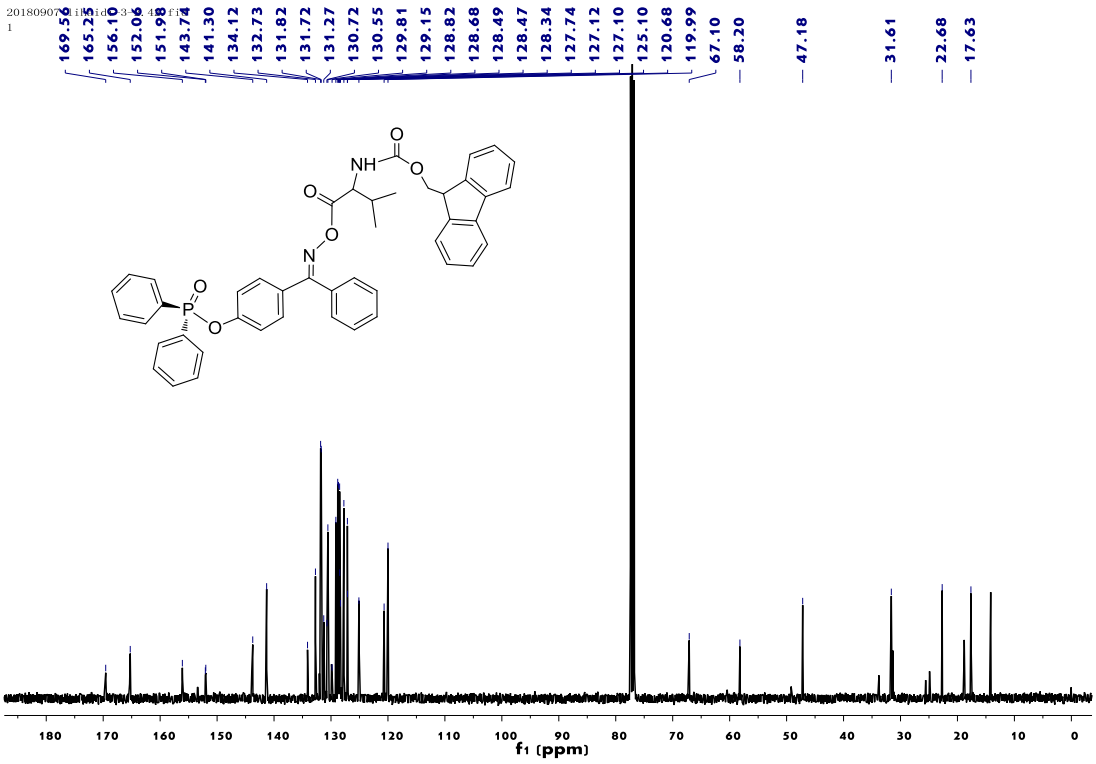
3a-2, Boc-Val-DDK-1 ^{13}C NMR (100 MHz, $CDCl_3$)



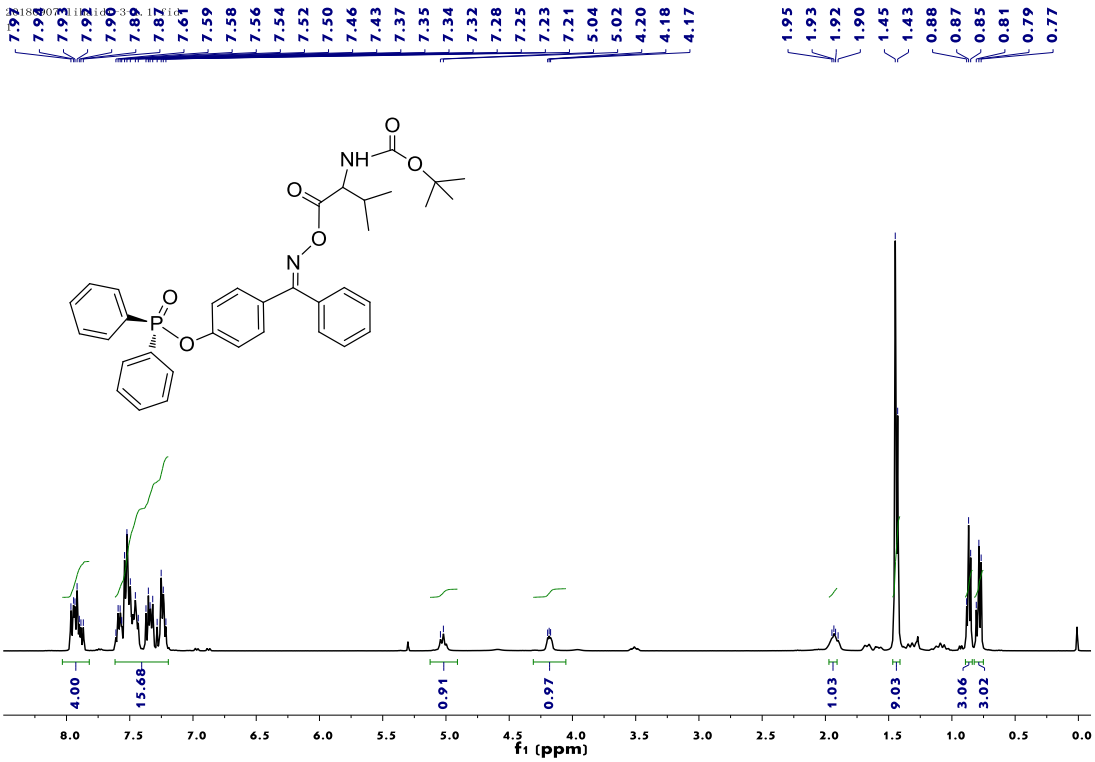
3a-2, Boc-Val-DDK-1 HRMS ESI



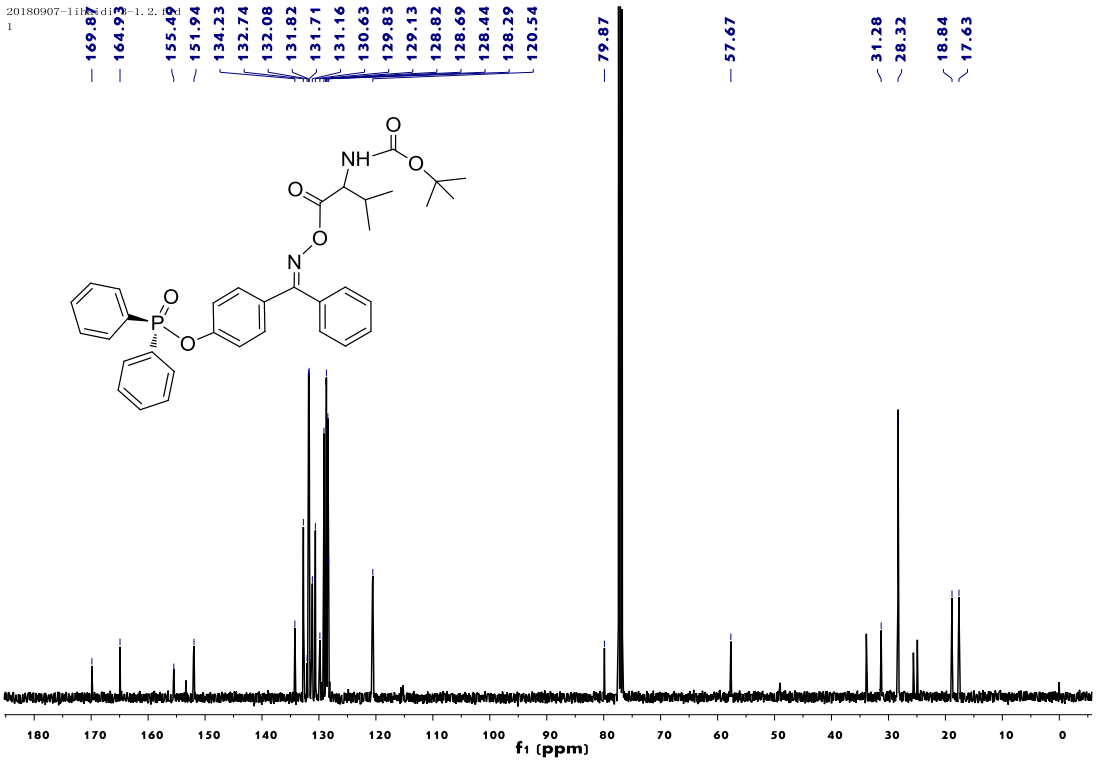
4a-1, Fmoc-Val-DDK-2 ^{1}H NMR (400 MHz, $CDCl_3$)



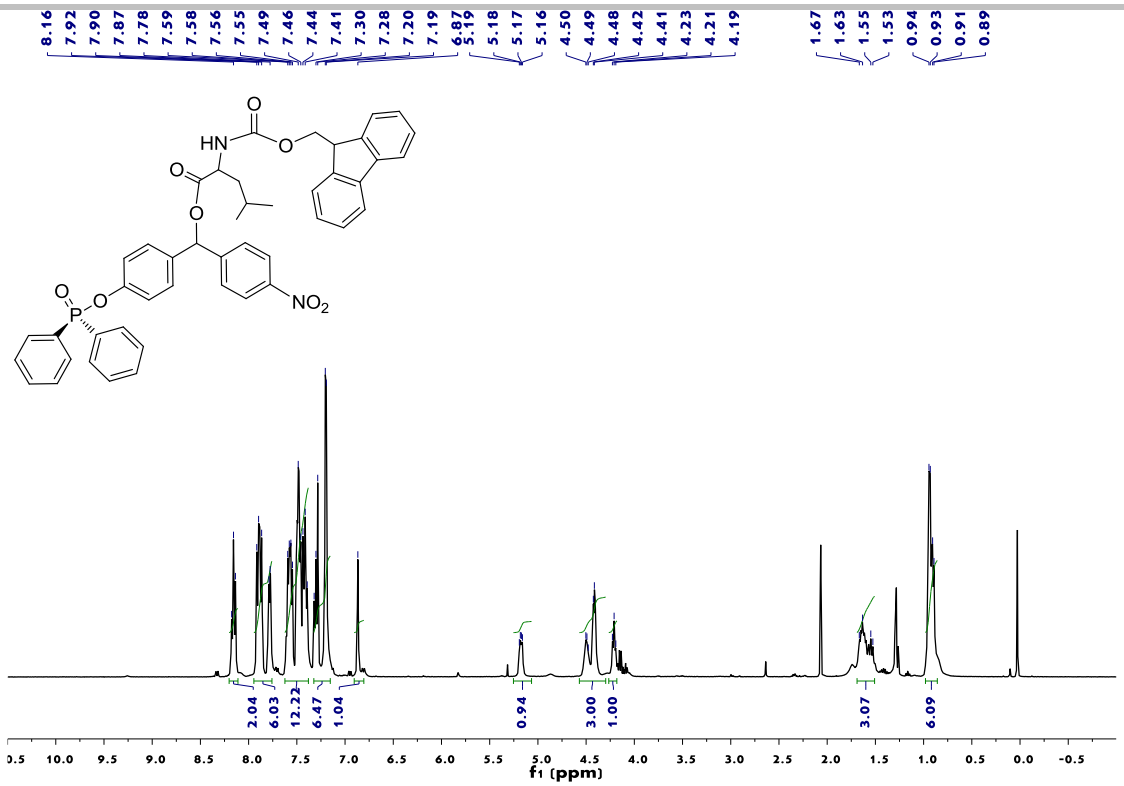
4a-1, Fmoc-Val-DDK-2 ^{13}C NMR (100 MHz, $CDCl_3$)



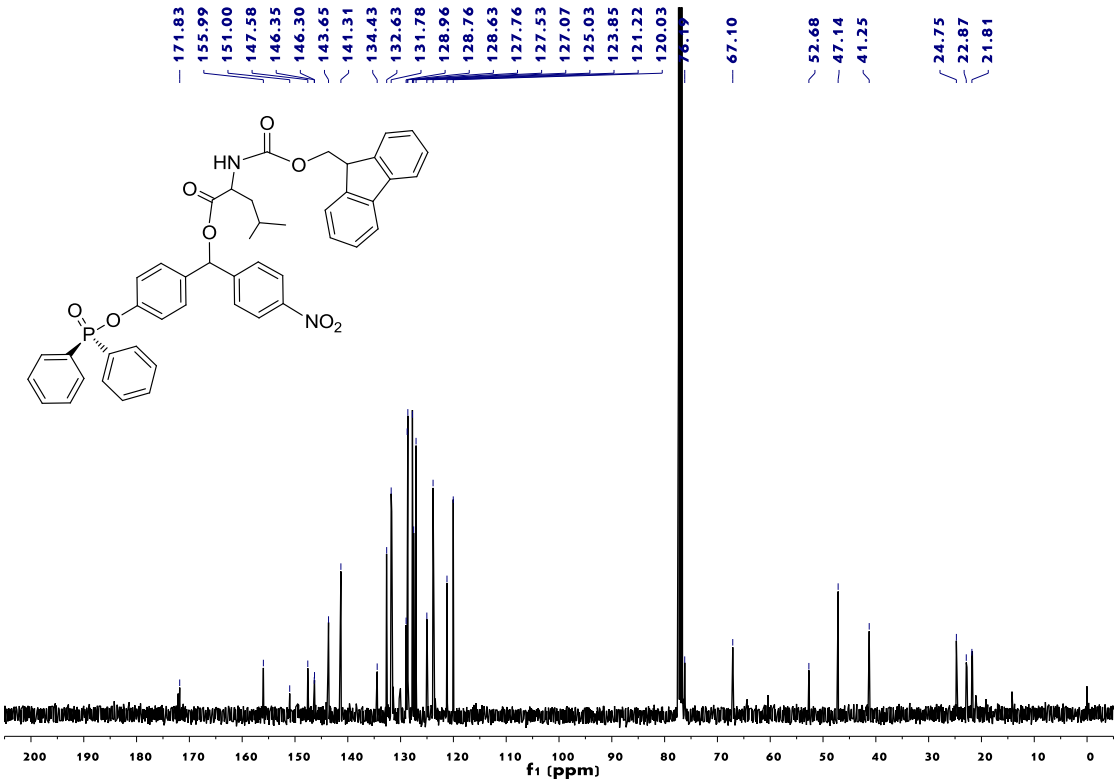
4a-2, Boc-Val-DDK-2 ^{1}H NMR (400 MHz, $CDCl_3$)



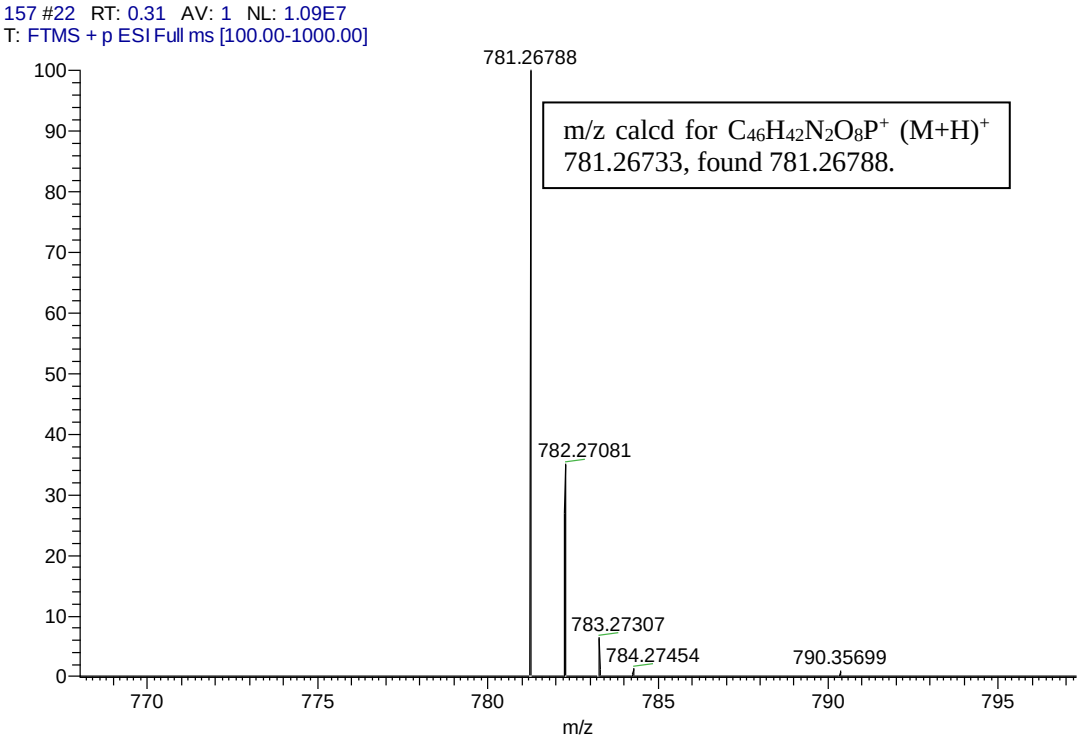
4a-2, Boc-Val-DDK-2 ^{13}C NMR (100 MHz, $CDCl_3$)



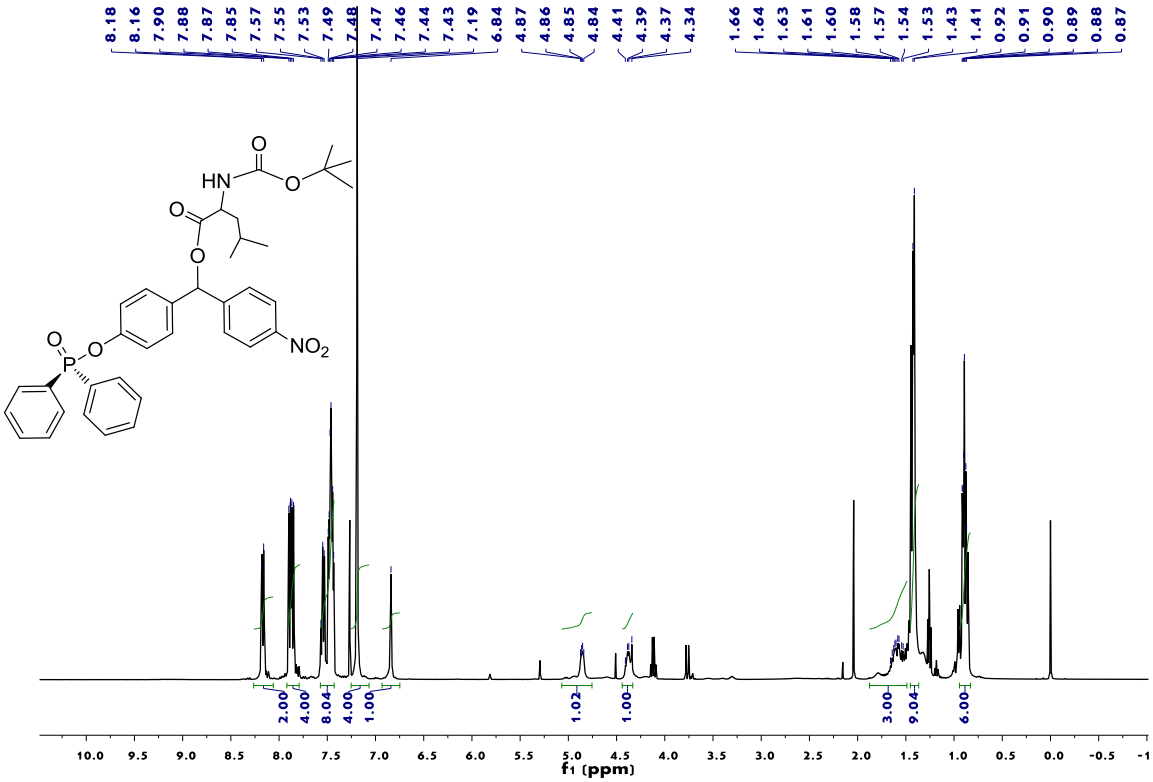
3b-1, Fmoc-Leu-DDK-3 ^{1}H NMR (400 MHz, $CDCl_3$)



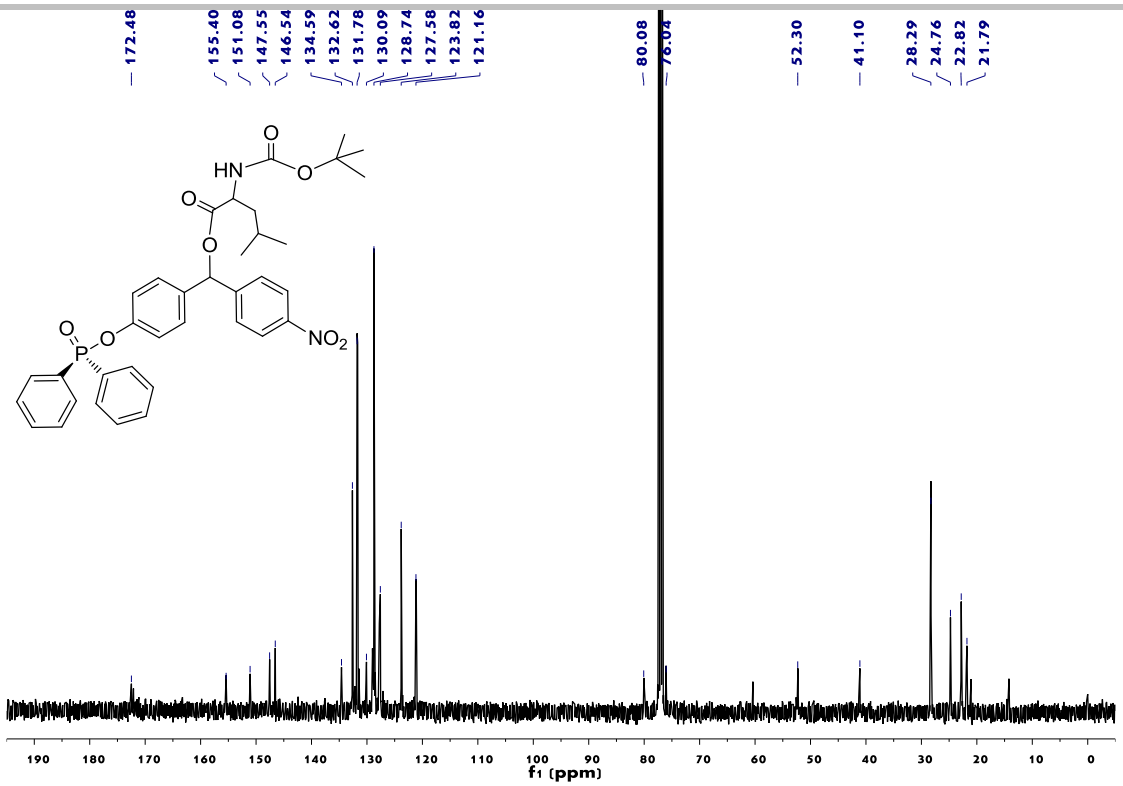
3b-1, Fmoc-Leu-DDK-3 ^{13}C NMR (100 MHz, $CDCl_3$)



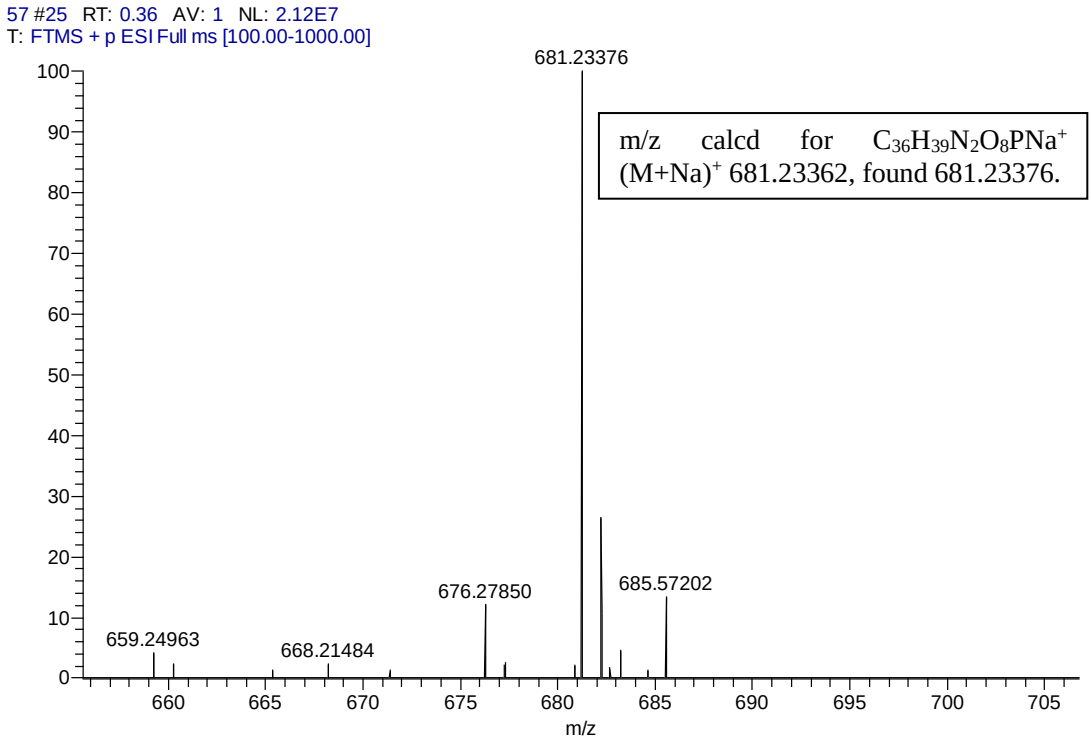
3b-1, Fmoc-Leu-DDK-3 HRMS (ESI)



3b-2, Boc-Leu-DDK-3 ^{1}H NMR (400 MHz, $CDCl_3$)

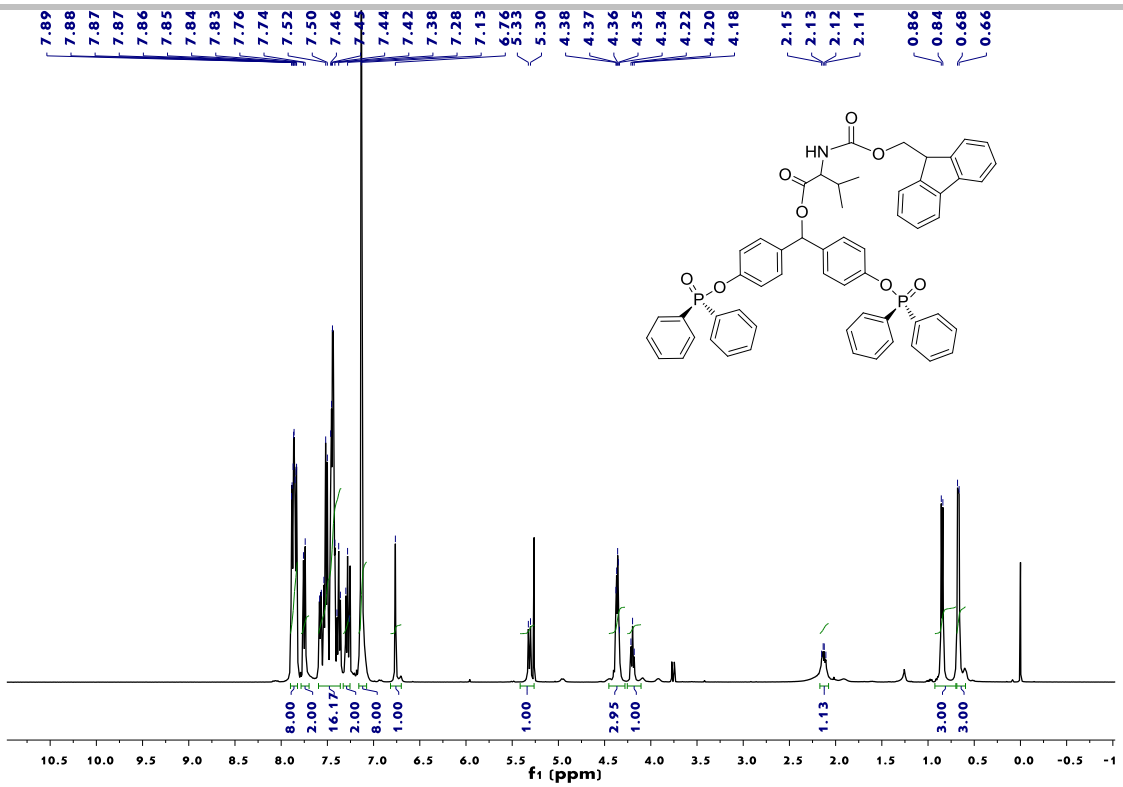


3b-2, Boc-Leu-DDK-3 ^{13}C NMR (100 MHz, $CDCl_3$)

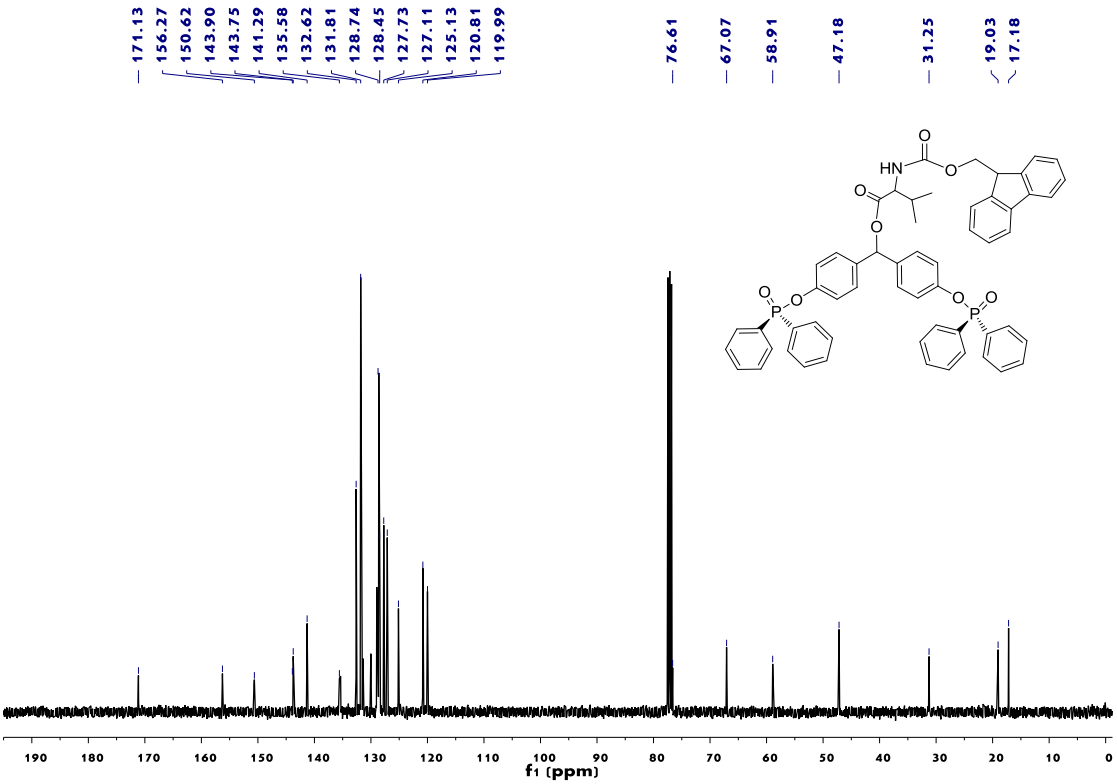


m/z calcd for $C_{36}H_{39}N_2O_8PNa^+$ $(M+Na)^+$ 681.23362, found 681.23376.

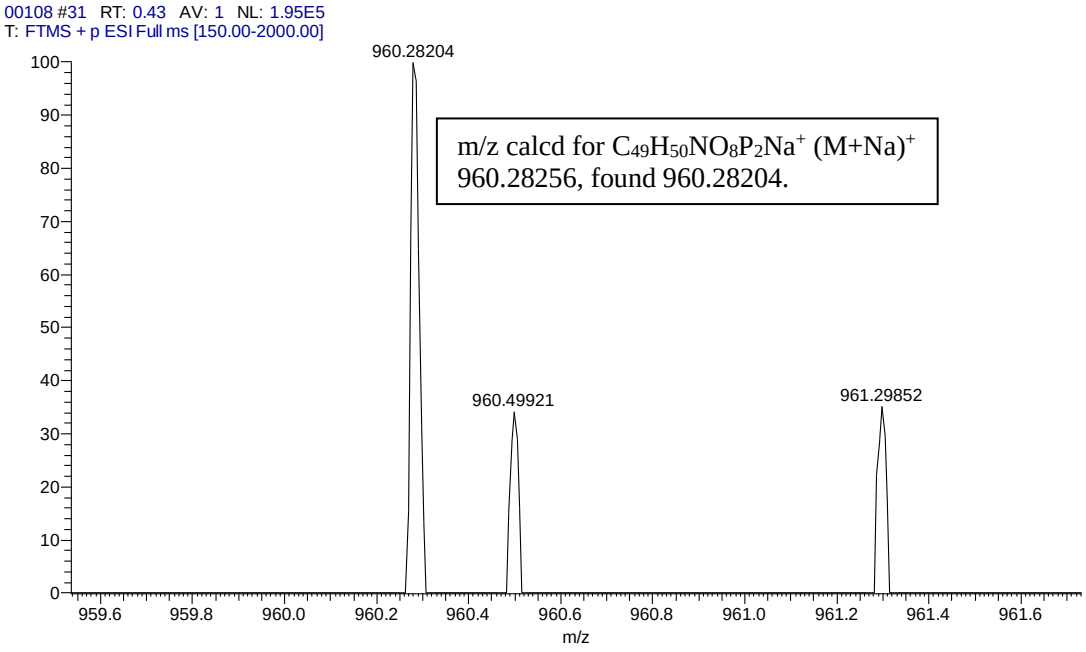
3b-2, Boc-Leu-DDK-3 HRMS (ESI)



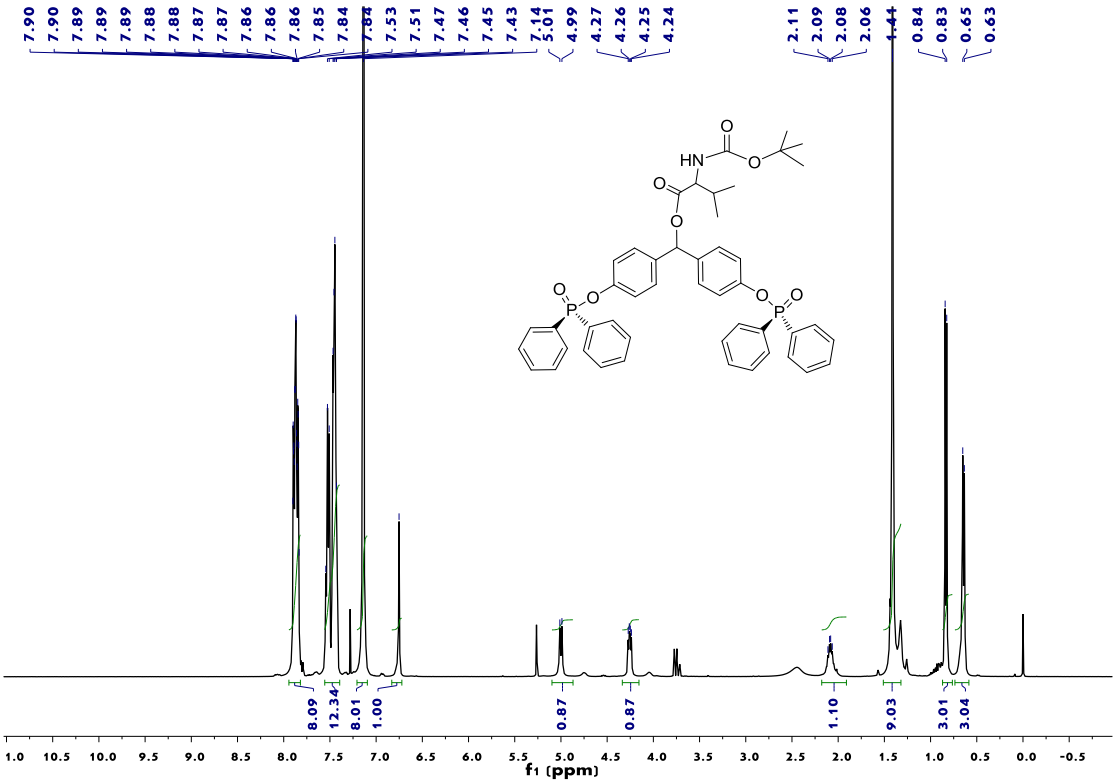
3c-1, Fmoc-Val-DDK-5 ^{1}H NMR (400 MHz, $CDCl_3$)



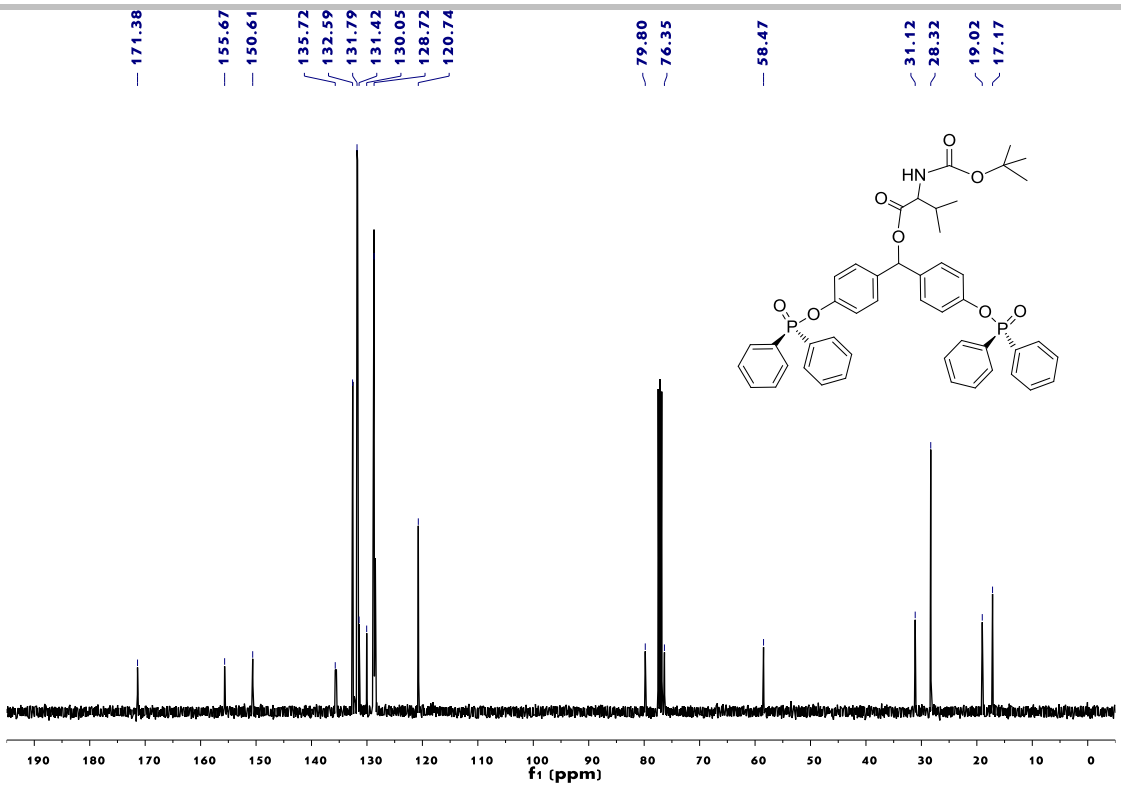
3c-1, Fmoc-Val-DDK-5 ^{13}C NMR (100 MHz, $CDCl_3$)



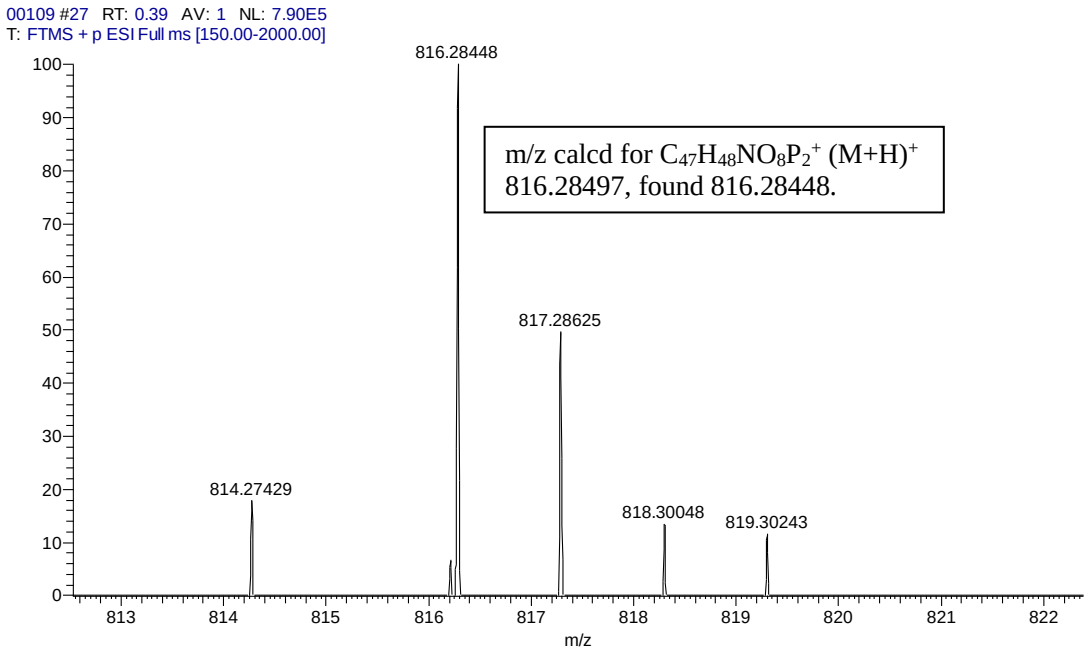
3c-1, Fmoc-Val-DDK-5 HRMS ESI



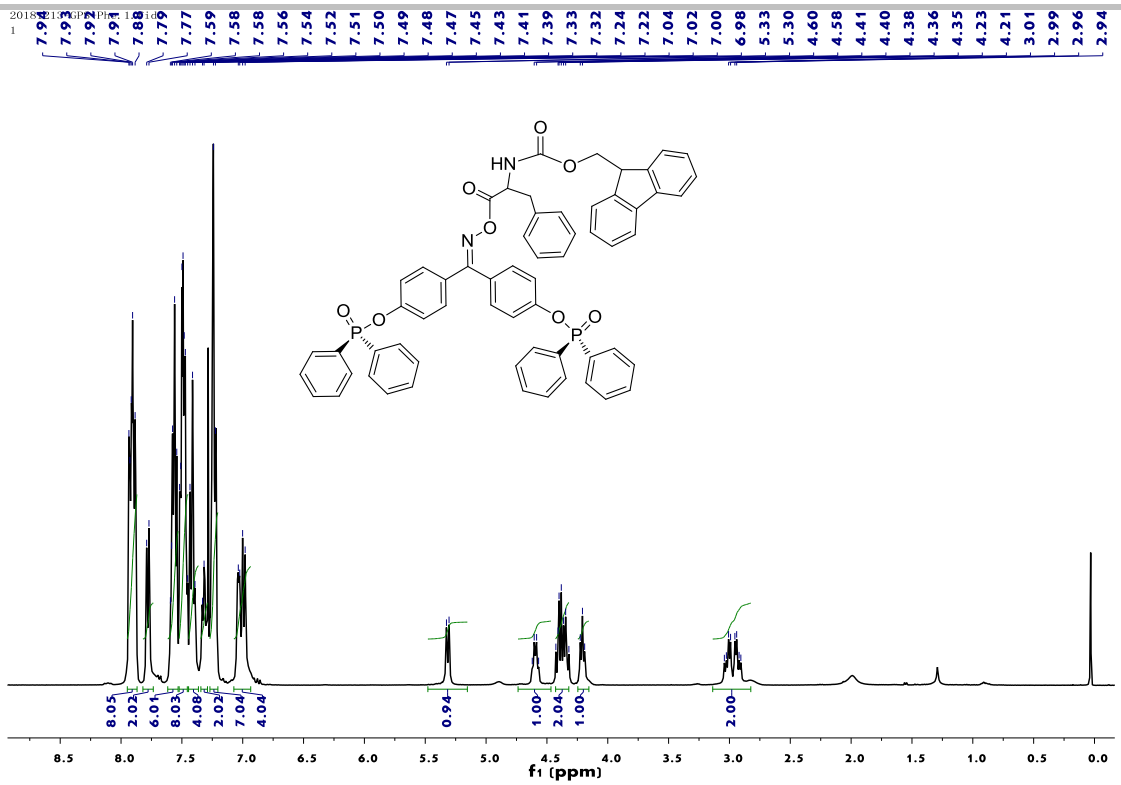
3c-2, Boc-Val-DDK-5 ^{1}H NMR (400 MHz, $CDCl_3$)



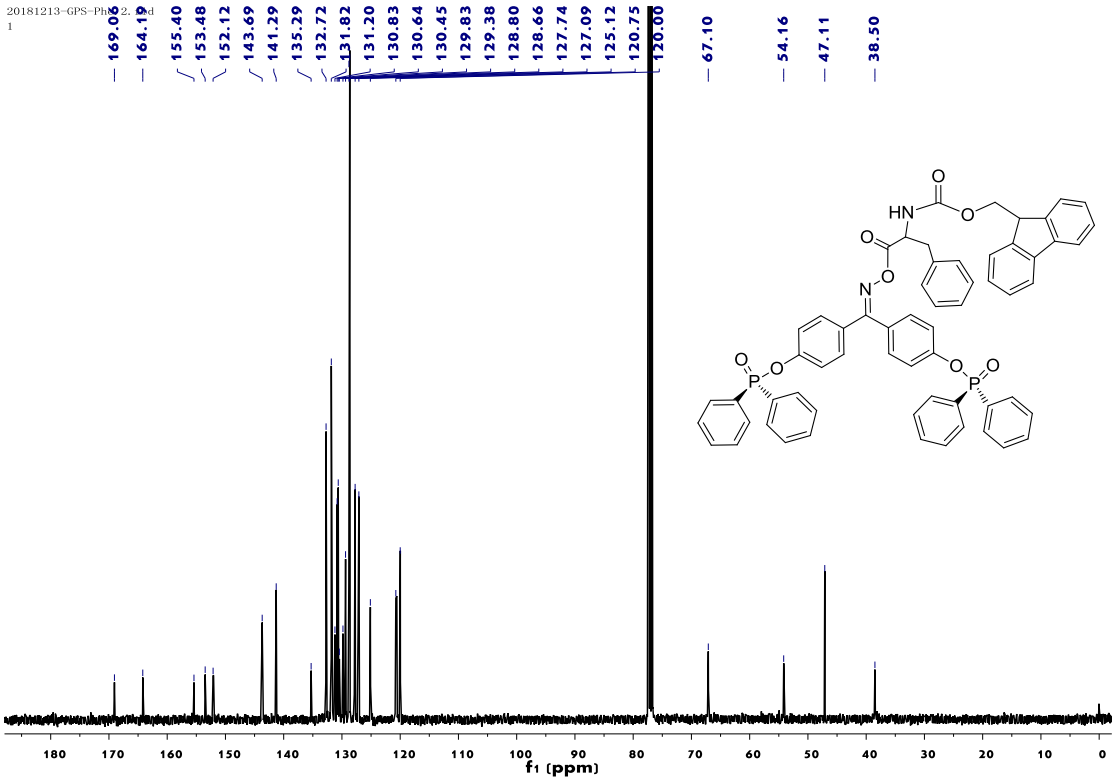
3c-2, Boc-Val-DDK-5 ^{13}C NMR (100 MHz, $CDCl_3$)



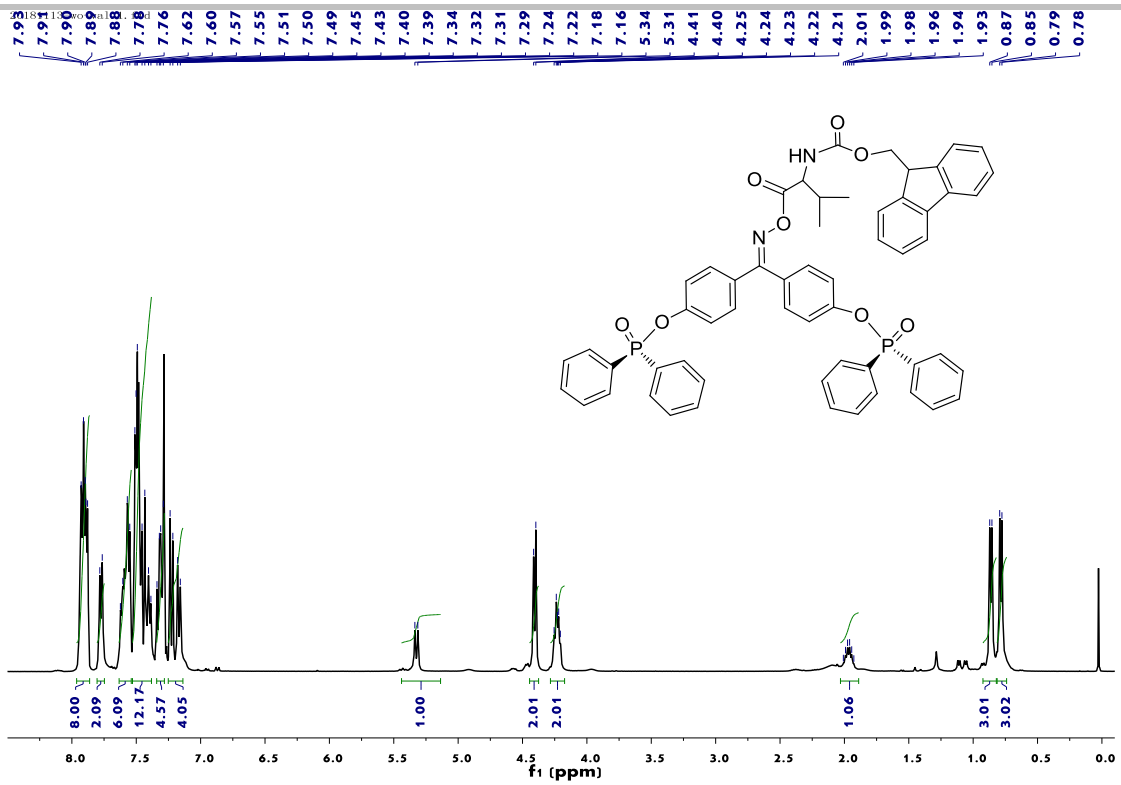
3c-2, Boc-Val-DDK-5 HRMS ESI



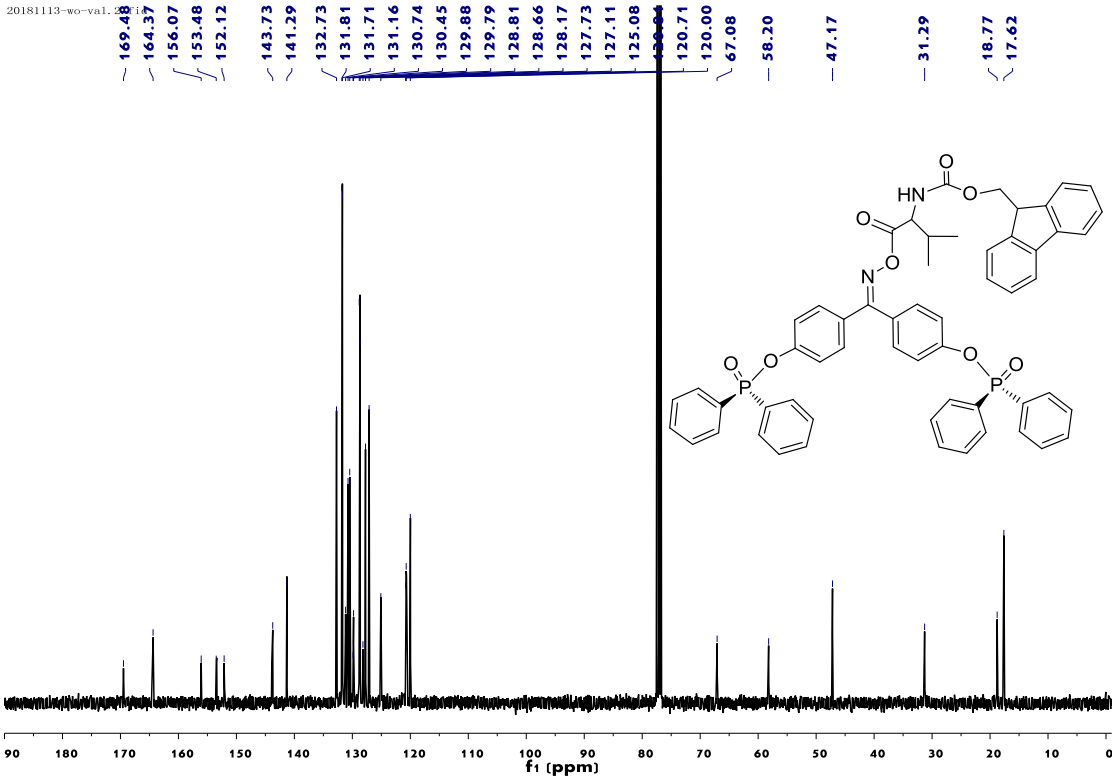
4c-1, Fmoc-Phe-DDK-6 ^{1}H NMR (400 MHz, $CDCl_3$)



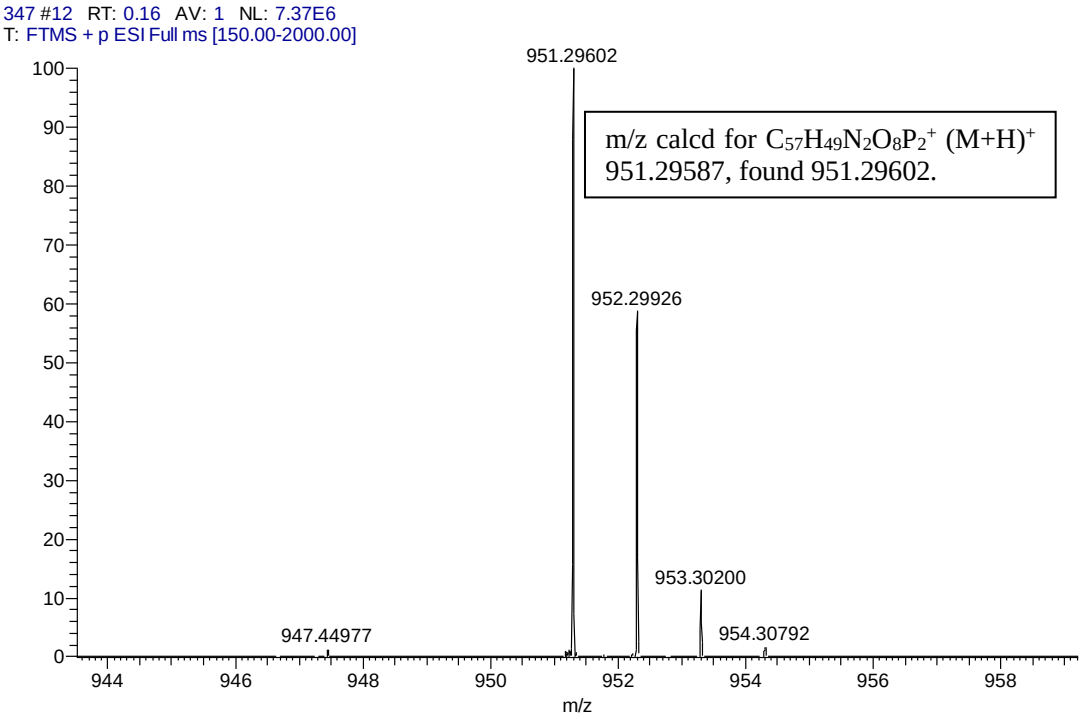
4c-1, Fmoc-Phe-DDK-6 ^{13}C NMR (100 MHz, $CDCl_3$)



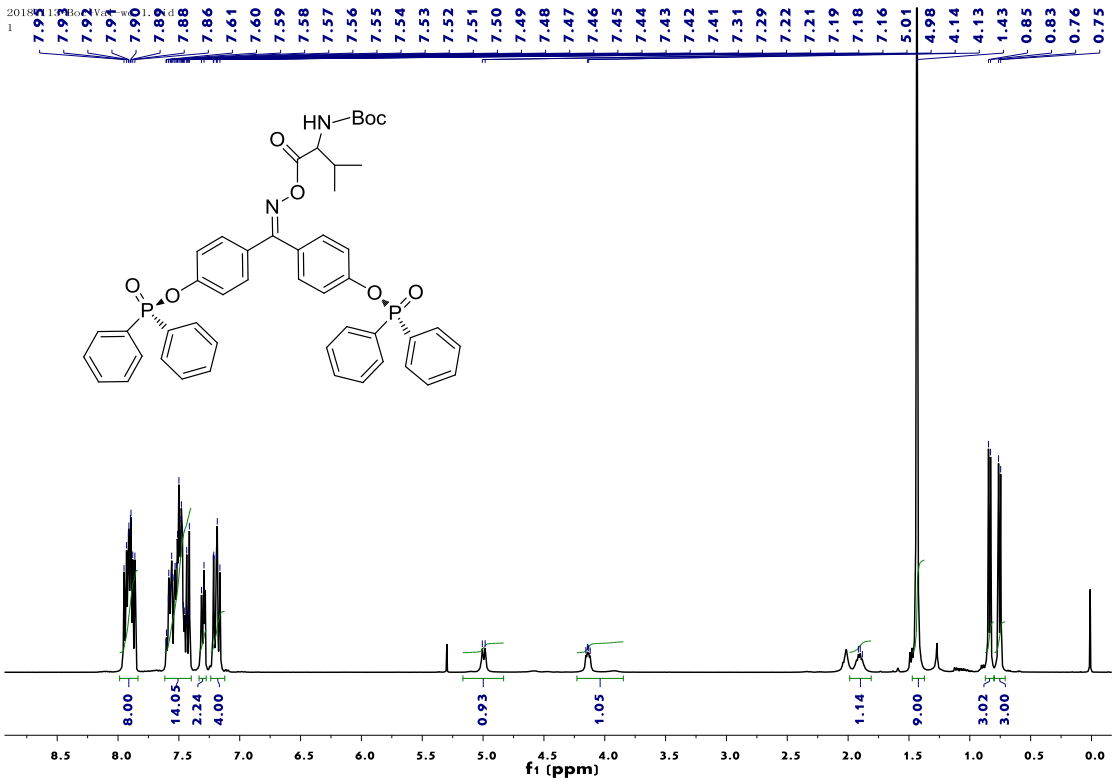
4c-2, Fmoc-Val-DDK-6 ^{1}H NMR (400 MHz, $CDCl_3$)



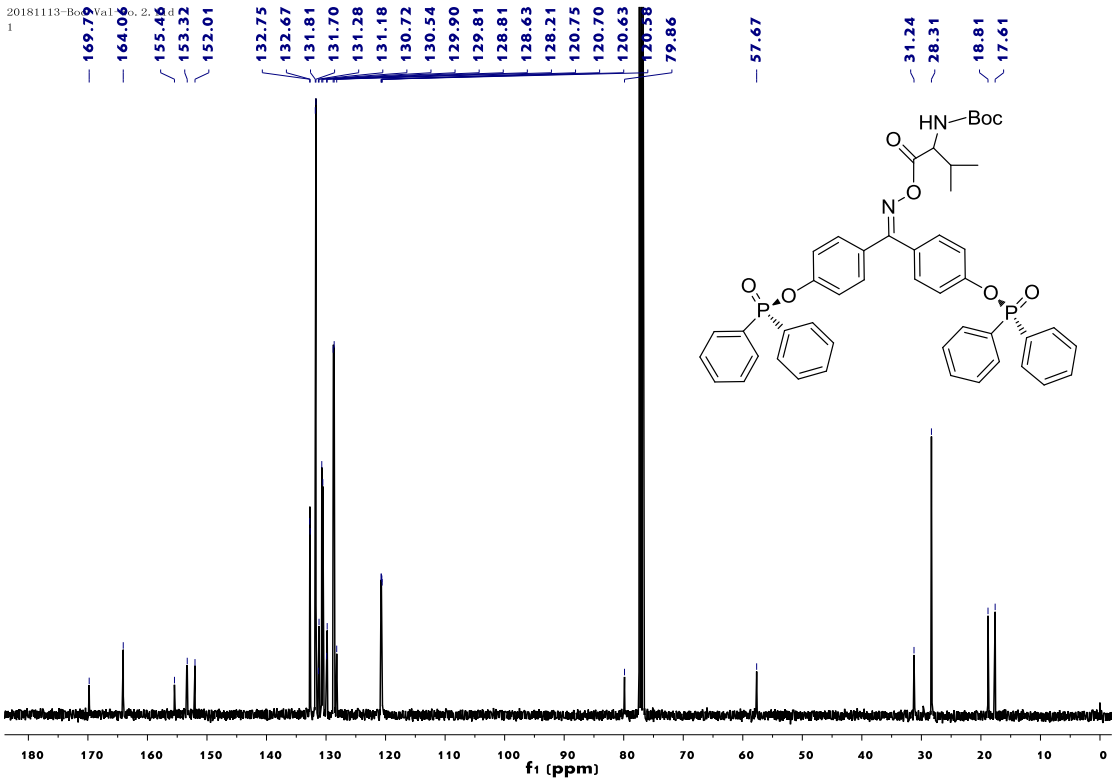
4c-2, Fmoc-Val-DDK-6 ^{13}C NMR (100 MHz, $CDCl_3$)



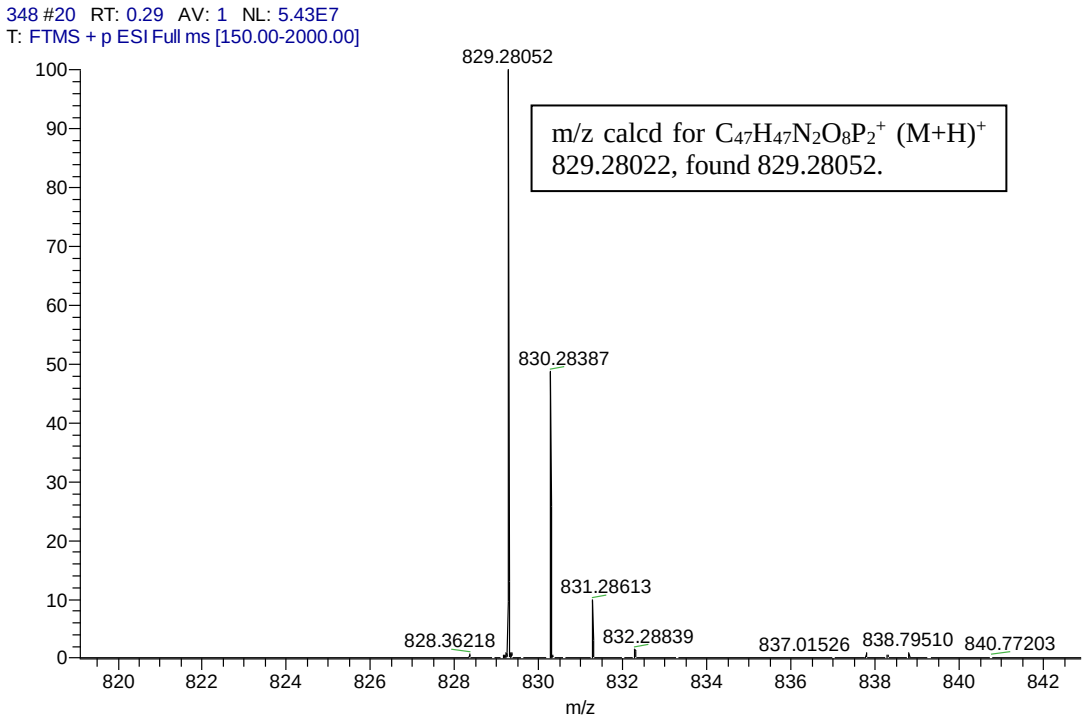
4c-2, Fmoc-Val-DDK-6 HRMS (ESI)



4c-3, Boc-Val-DDK-6 ^{1}H NMR (400 MHz, $CDCl_3$)

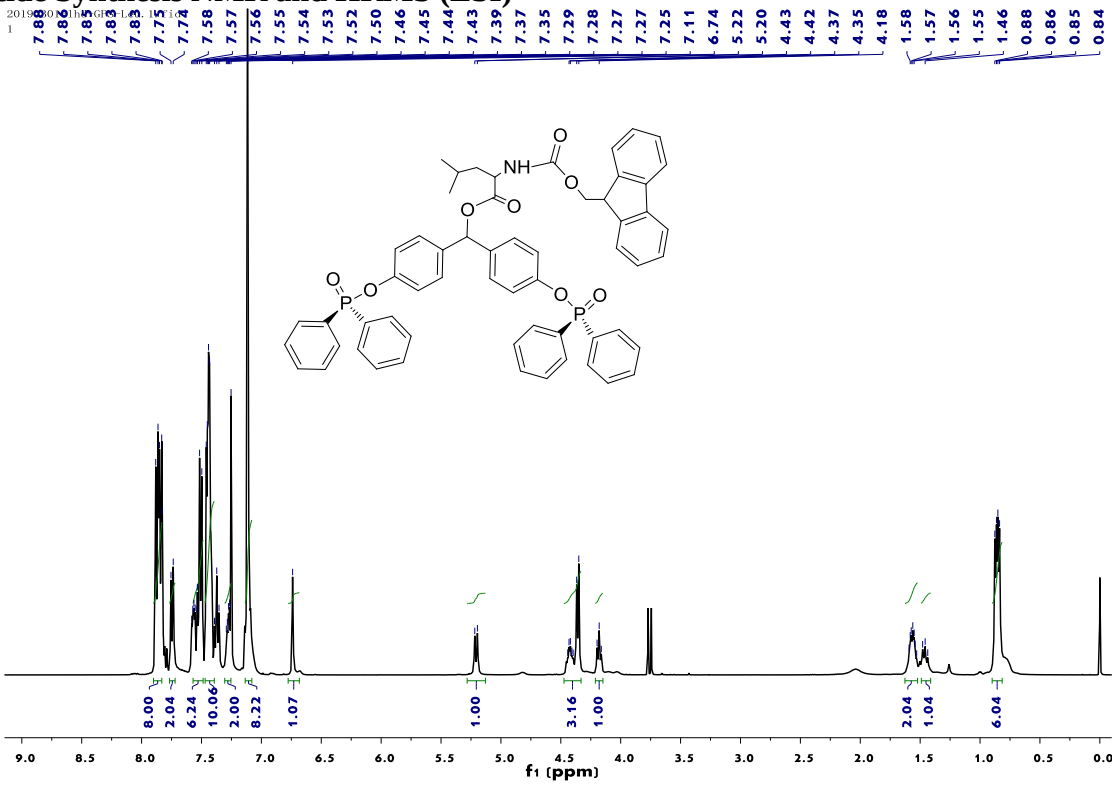


4c-3, Boc-Val-DDK-6 ^{13}C NMR (100 MHz, $CDCl_3$)

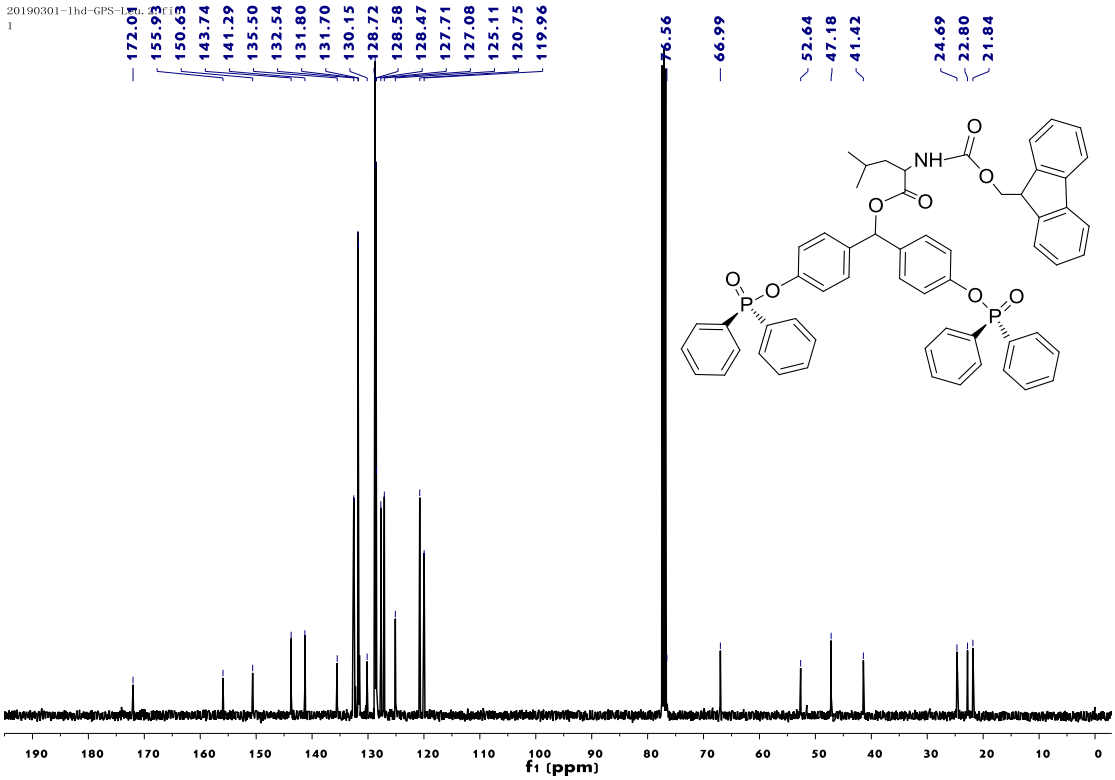


4c-3, Boc-Val-DDK-6 HRMS (ESI)

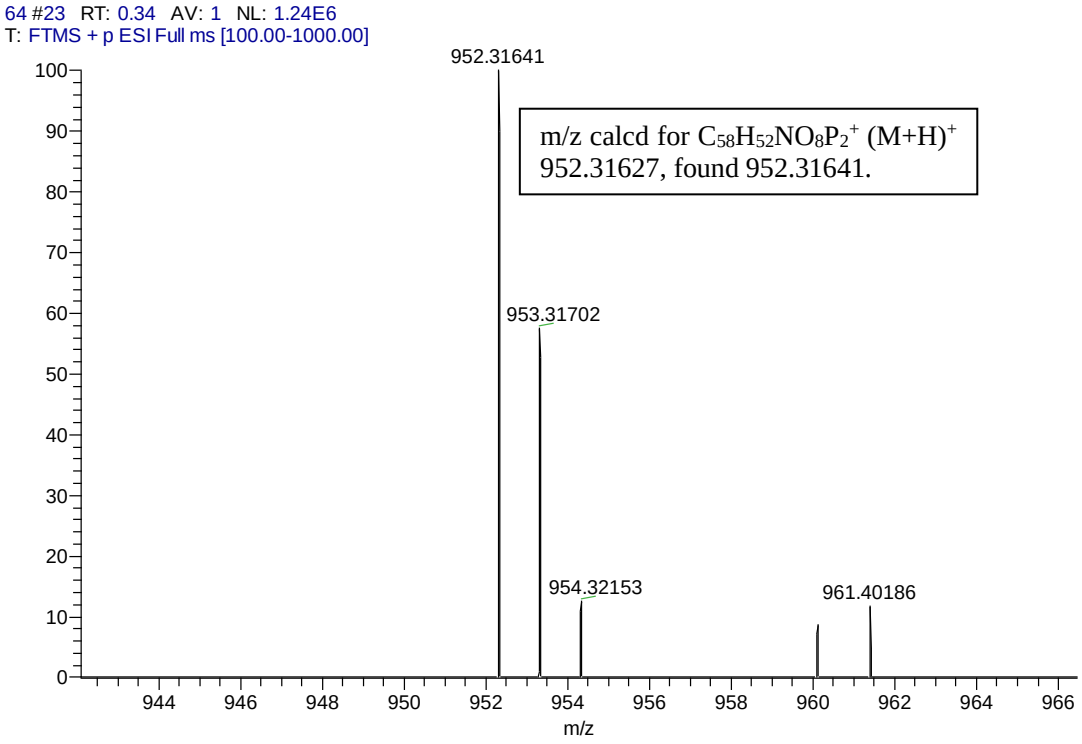
Plecanatide Synthesis NMR and HRMS (ESI)



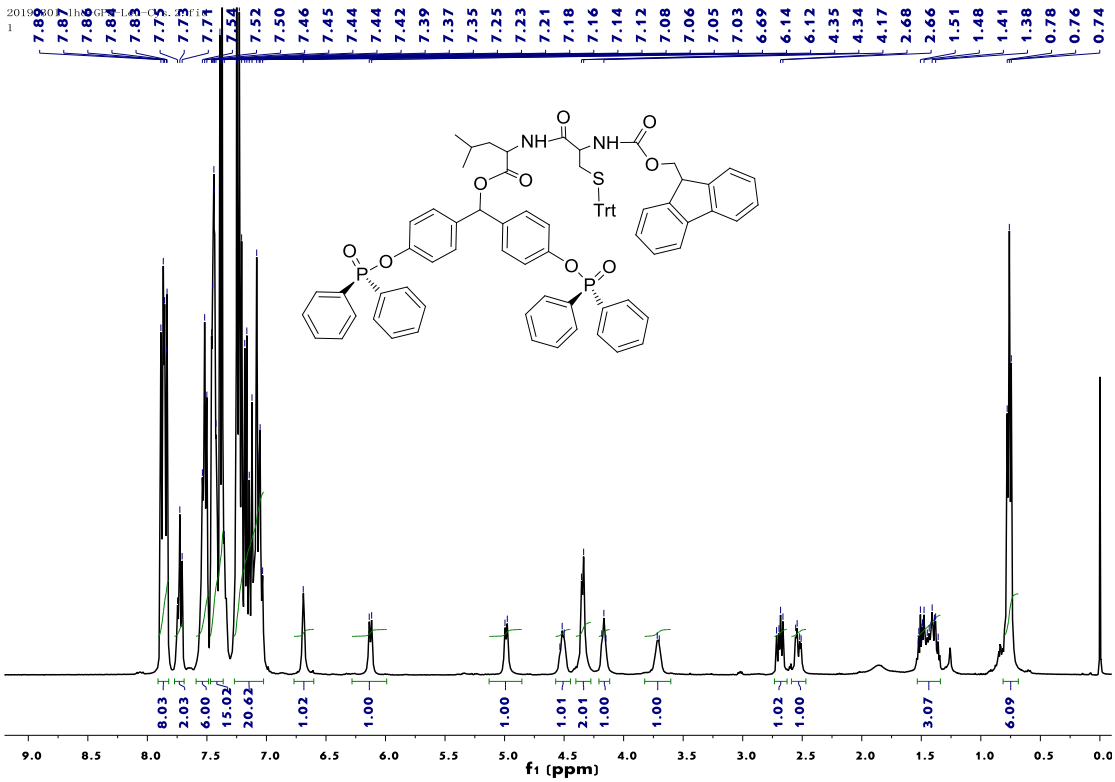
3c-3, Fmoc-Leu-DDK-5 ^{1}H NMR (400MHz, $CDCl_3$)



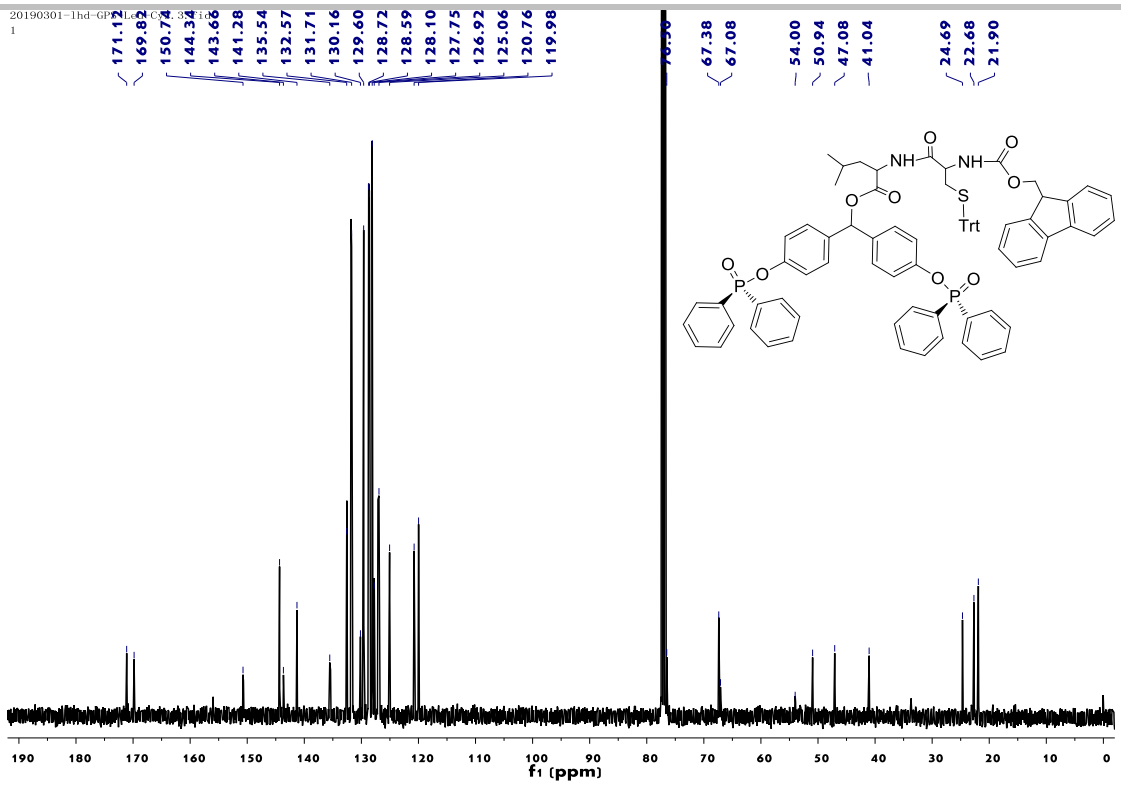
3c-3, Fmoc-Leu-DDK-5 ^{13}C NMR (100MHz, $CDCl_3$)



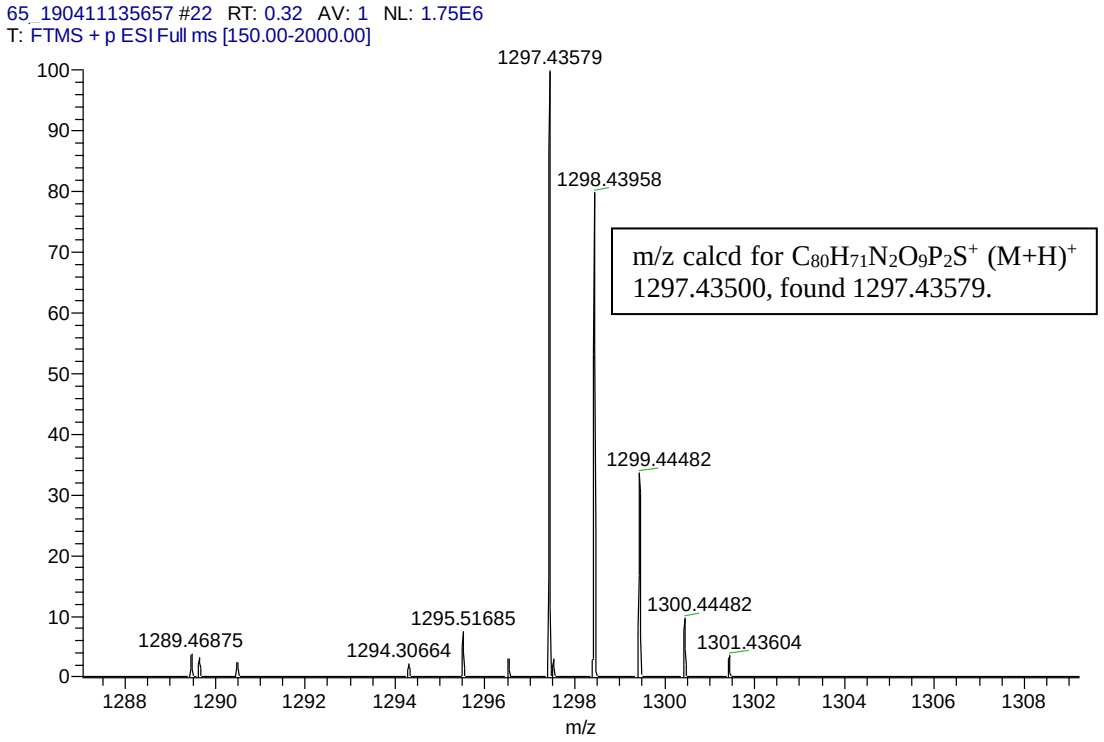
3c-3, Fmoc-Leu-DDK-5 HRMS (ESI)



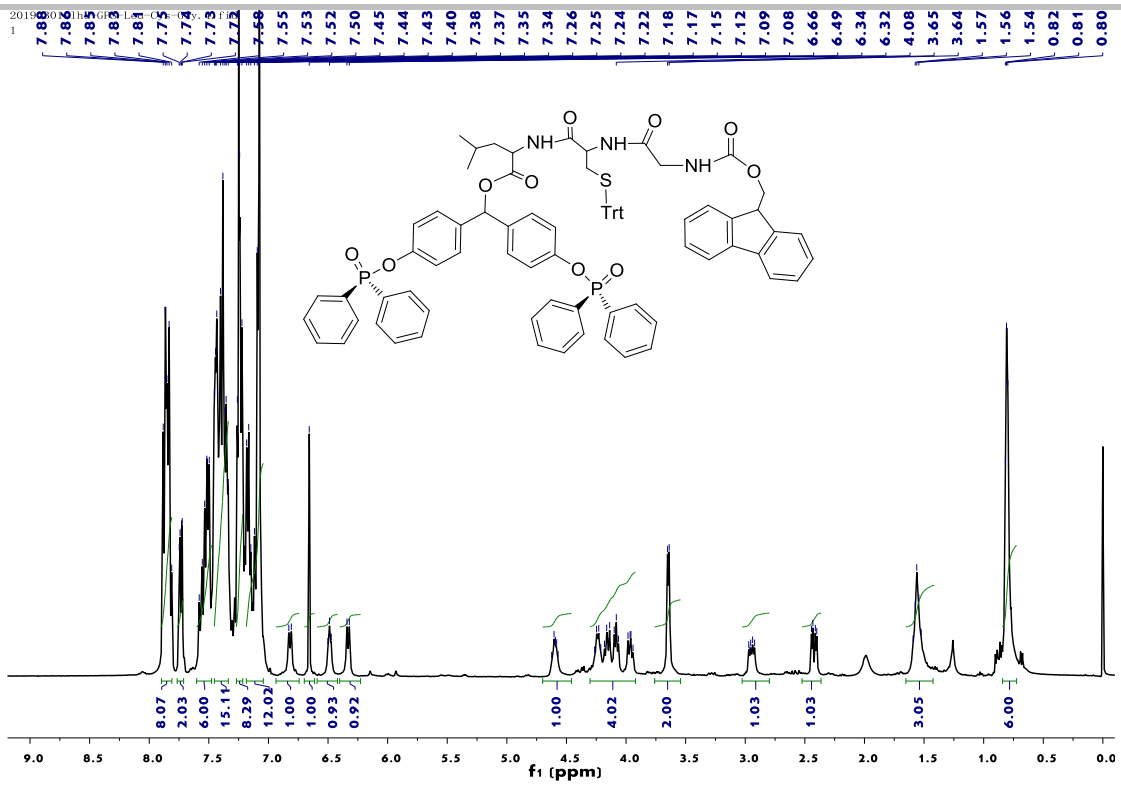
3c-4, Fmoc-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, $CDCl_3$)



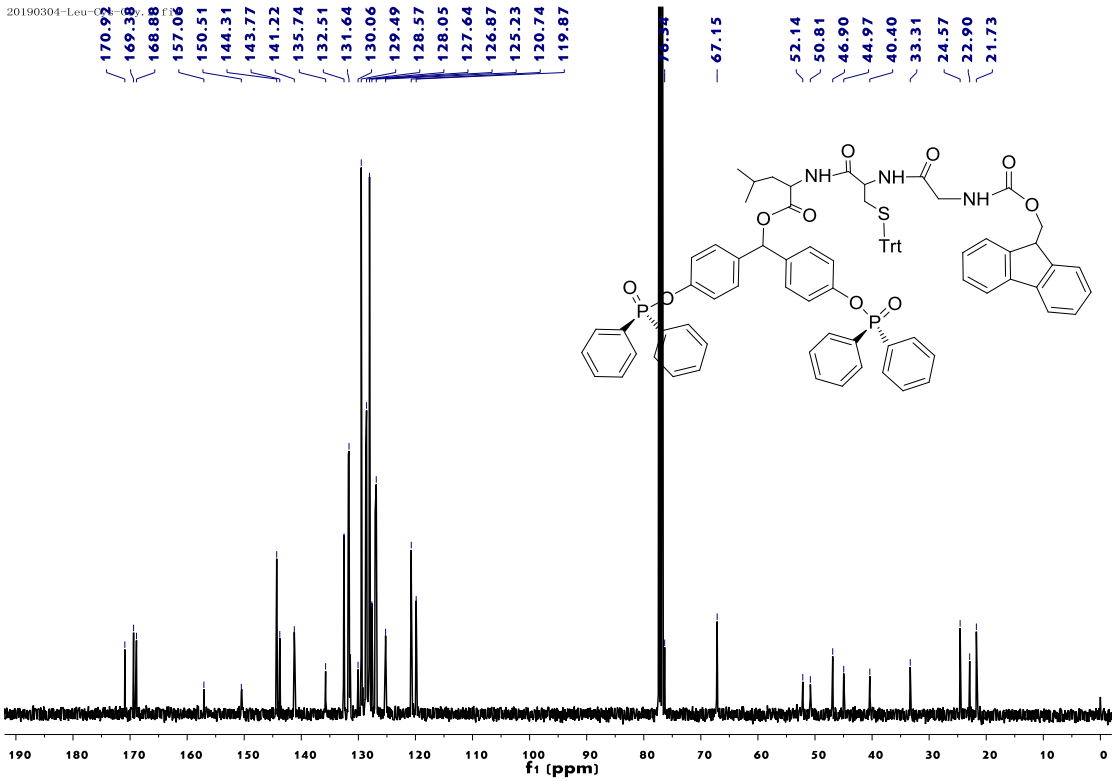
3c-4, Fmoc-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, $CDCl_3$)



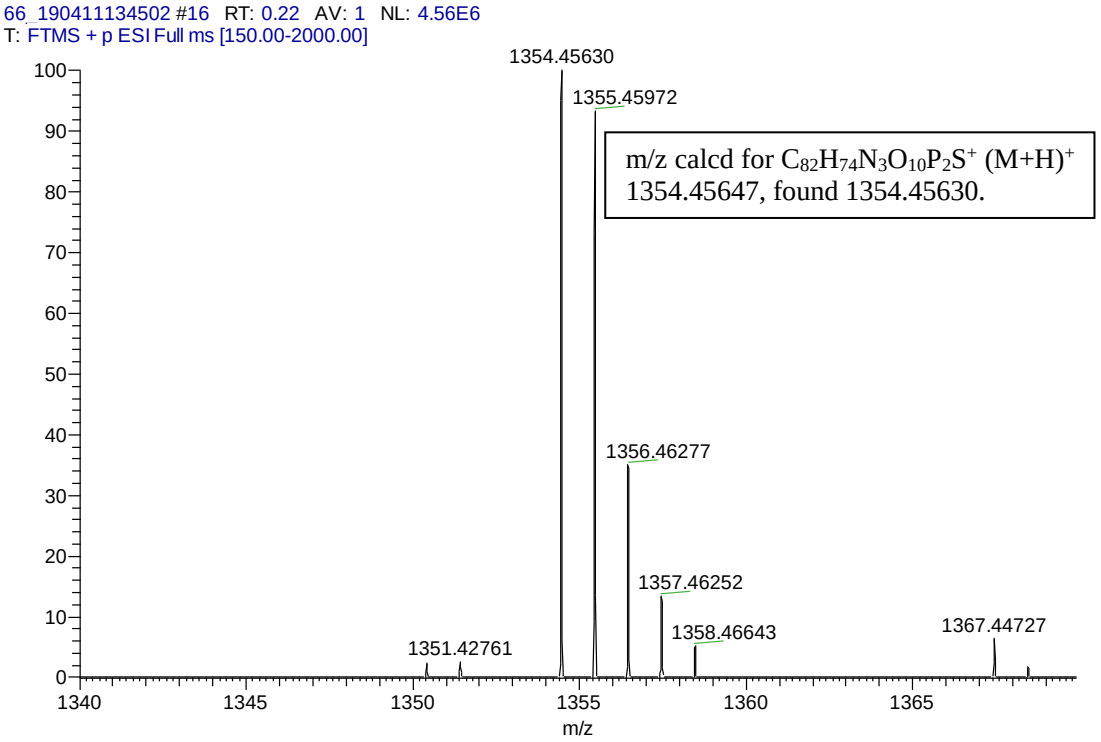
3c-4, Fmoc-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



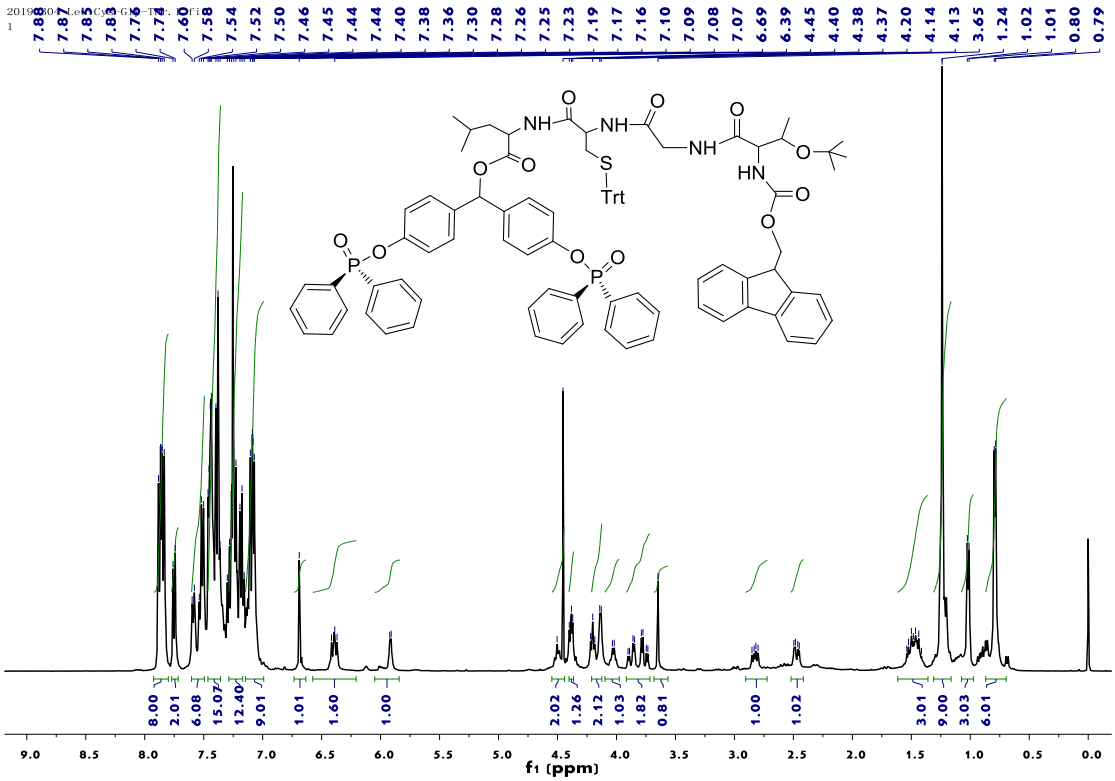
3c-5, Fmoc-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, $CDCl_3$)



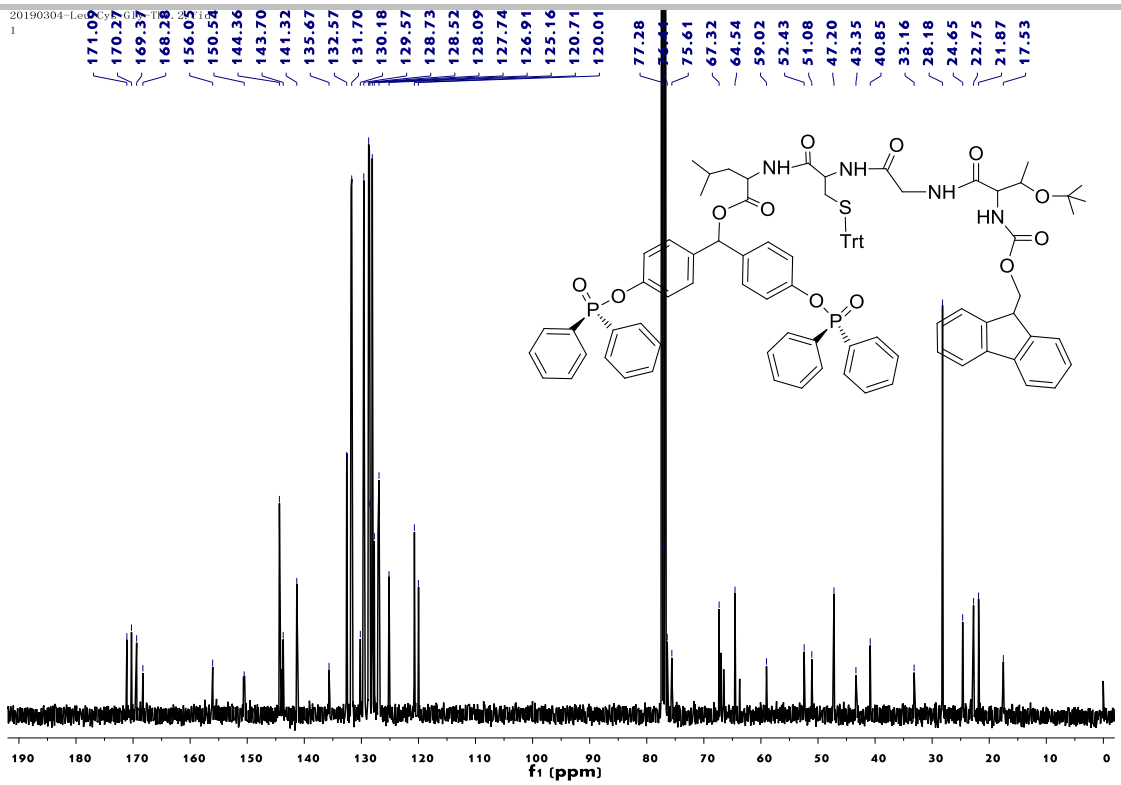
3c-5, Fmoc-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, $CDCl_3$)



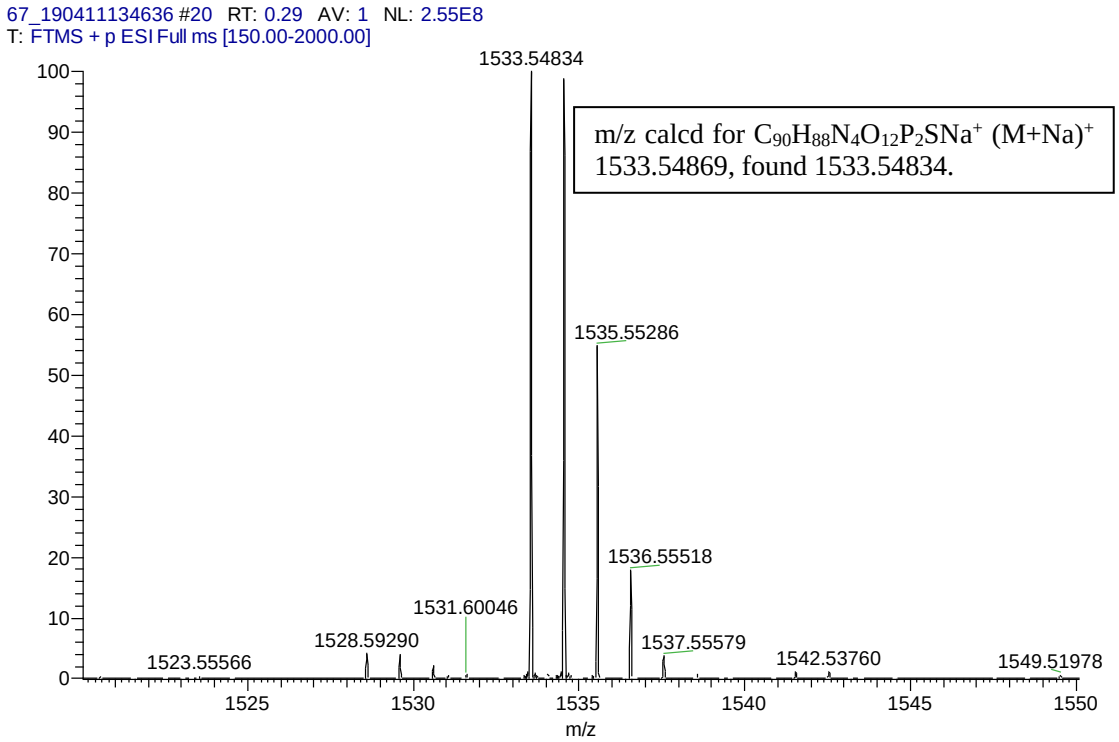
3c-5, Fmoc-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



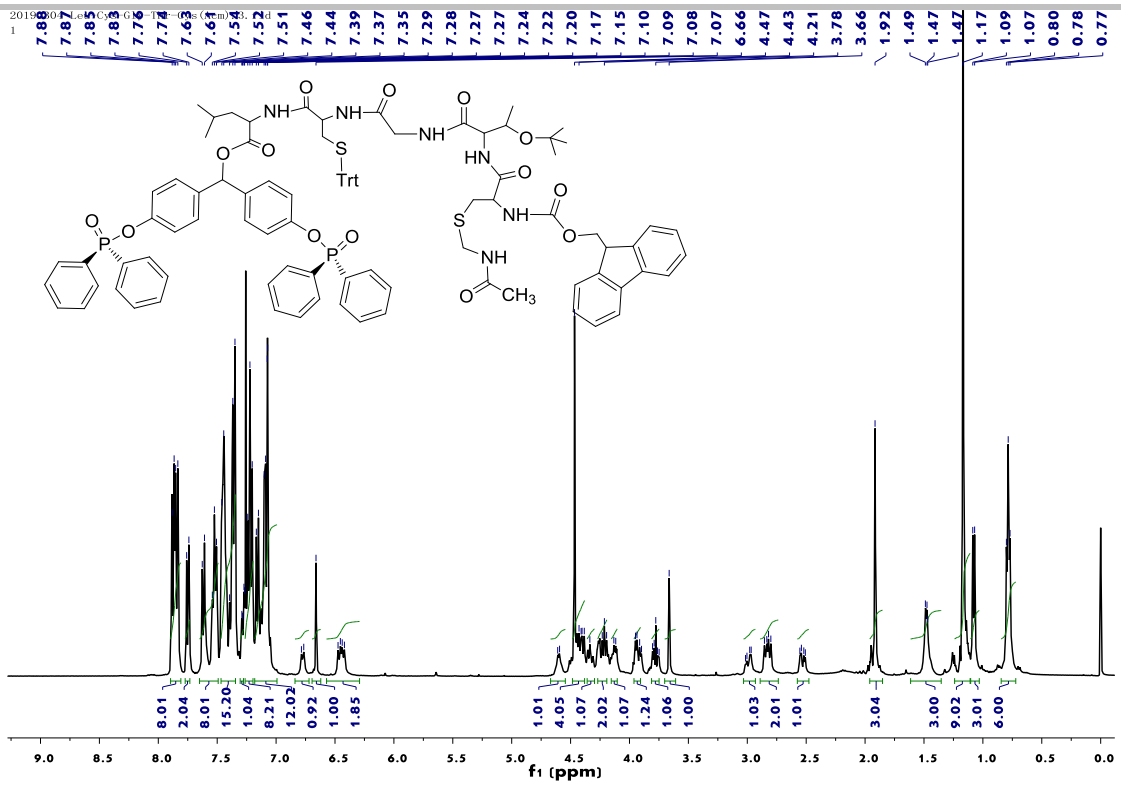
3c-6, Fmoc-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, $CDCl_3$)



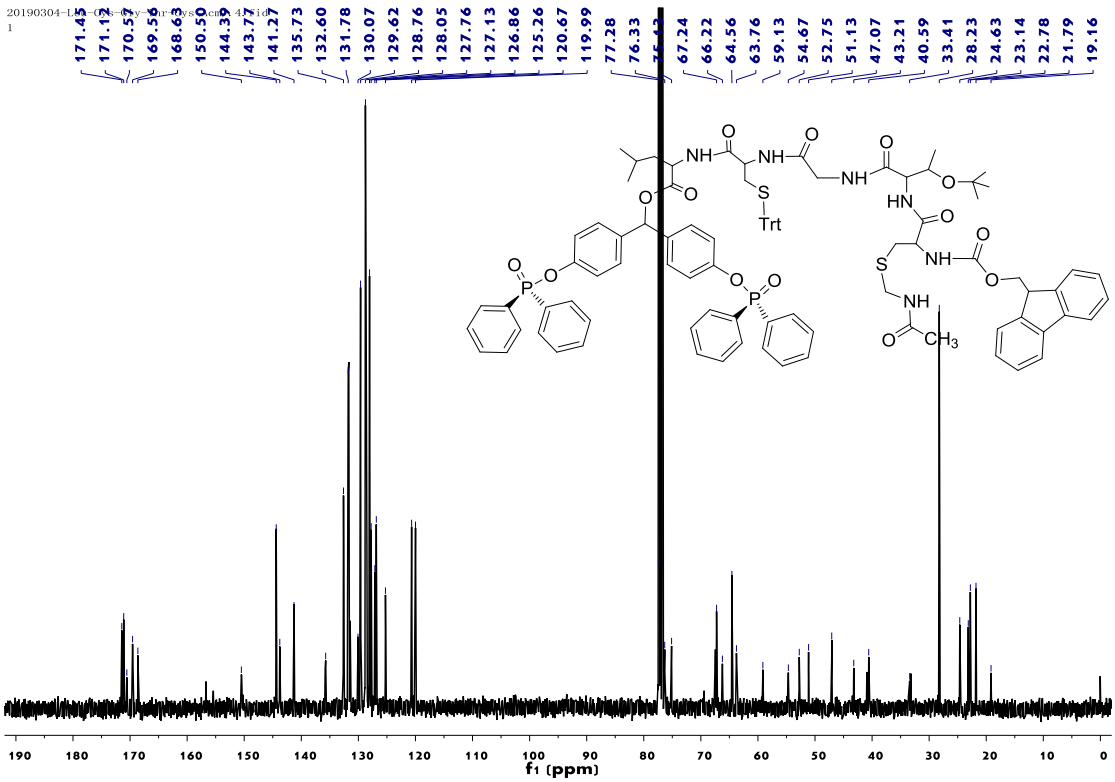
3c-6, Fmoc-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, $CDCl_3$)



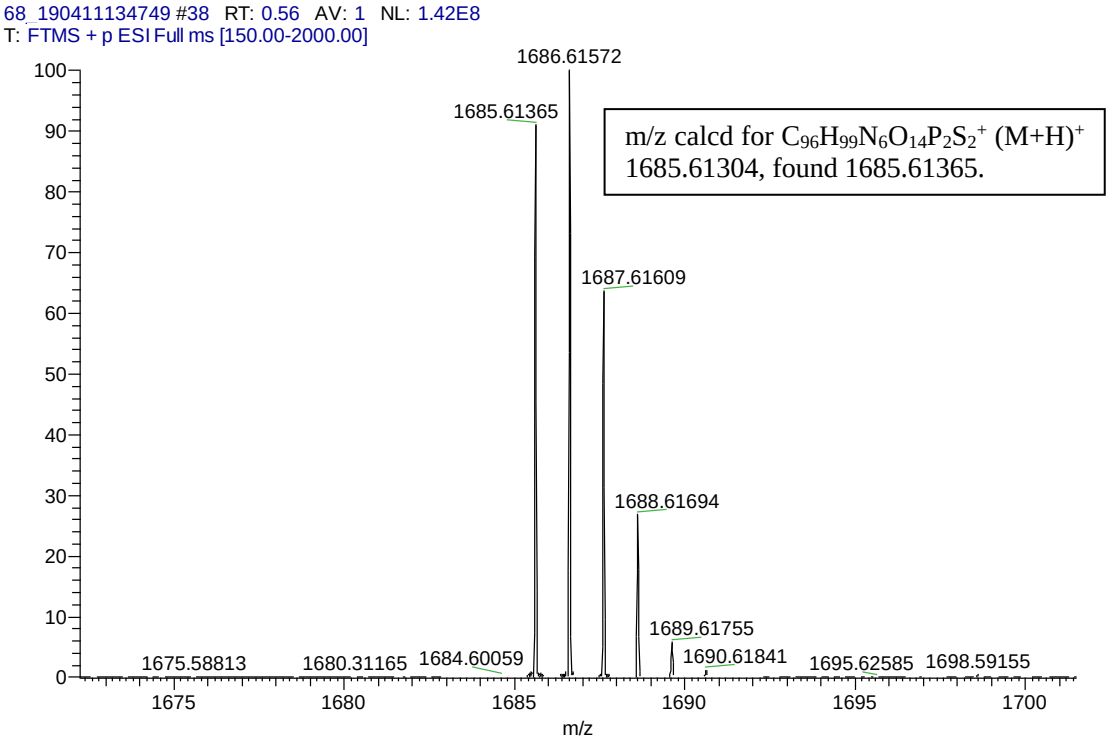
3c-6, Fmoc-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



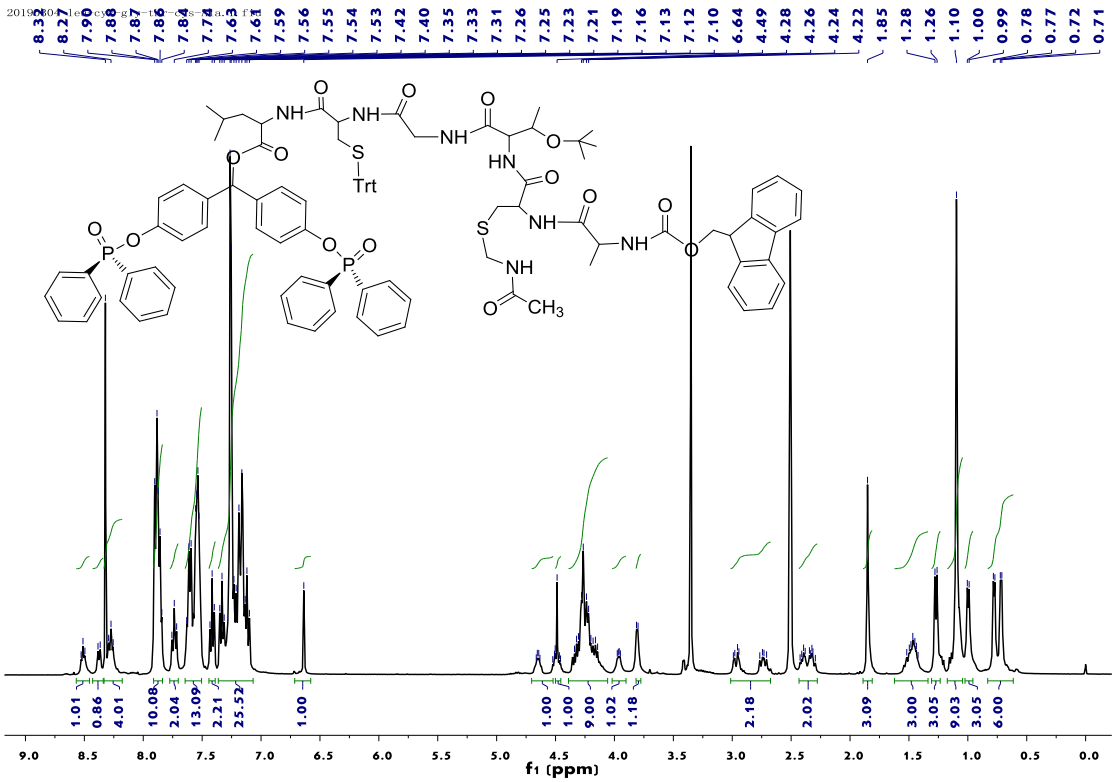
3c-7, Fmoc-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, $CDCl_3$)



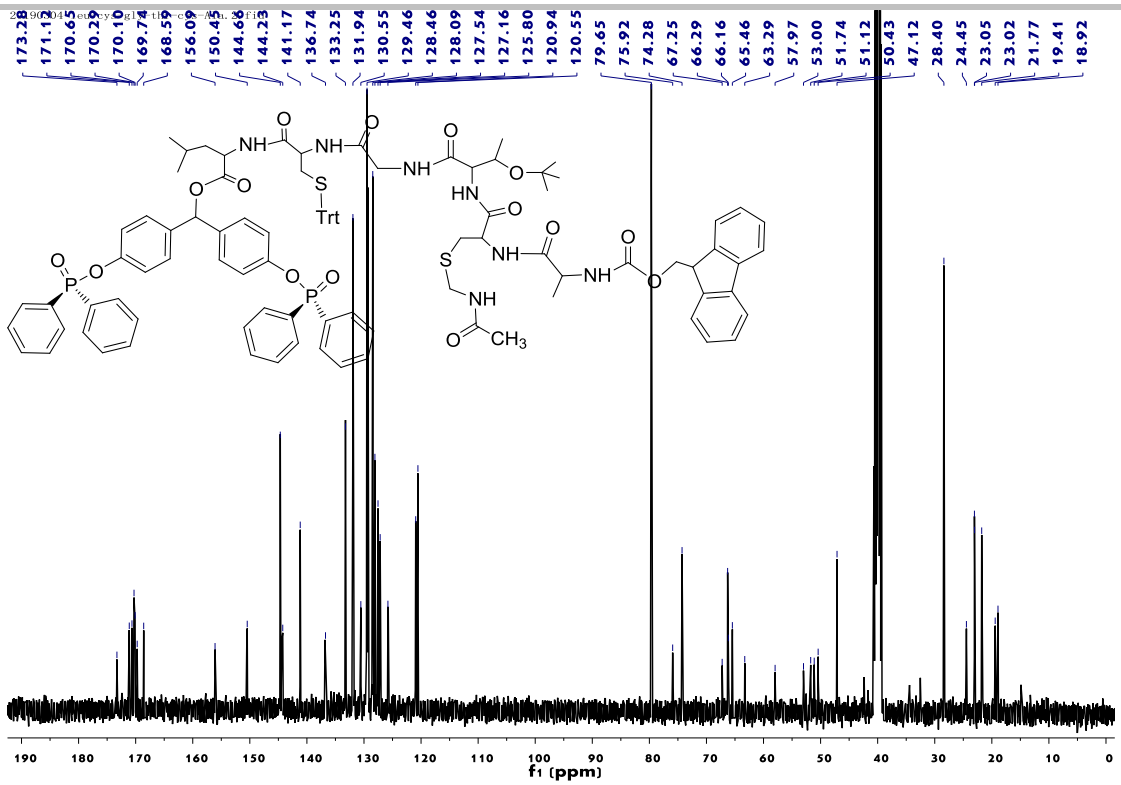
3c-7, Fmoc-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, $CDCl_3$)



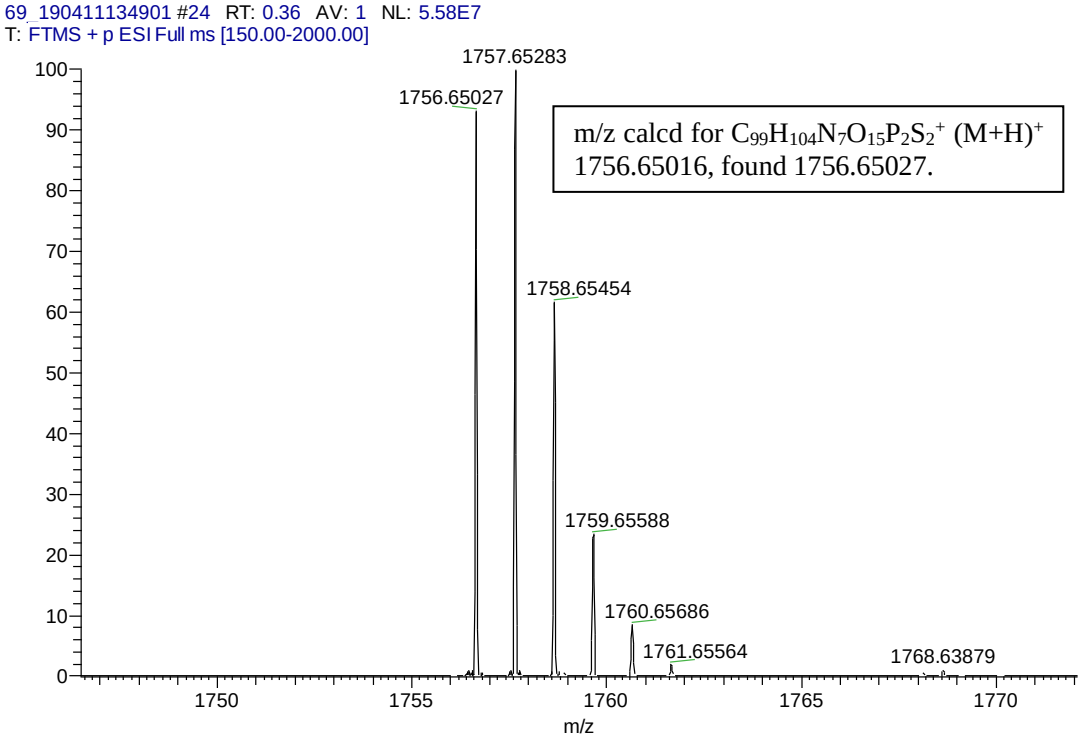
3c-7, Fmoc-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



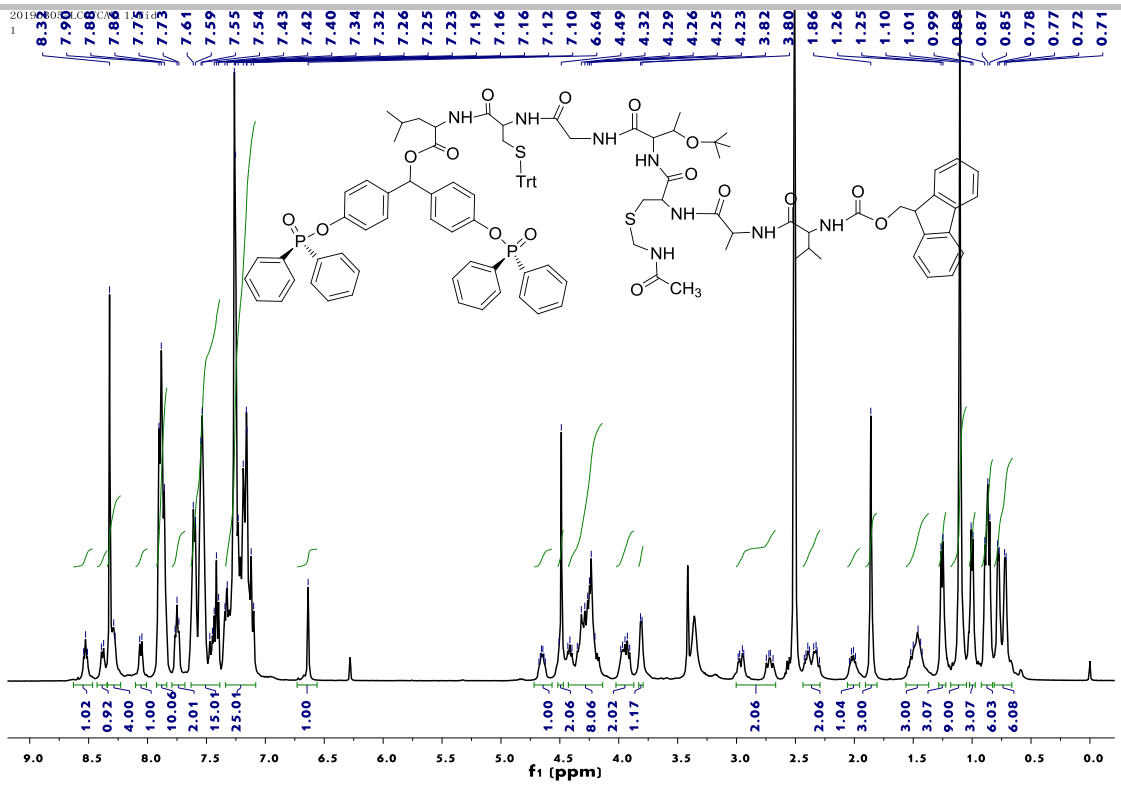
3c-8, Fmoc-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



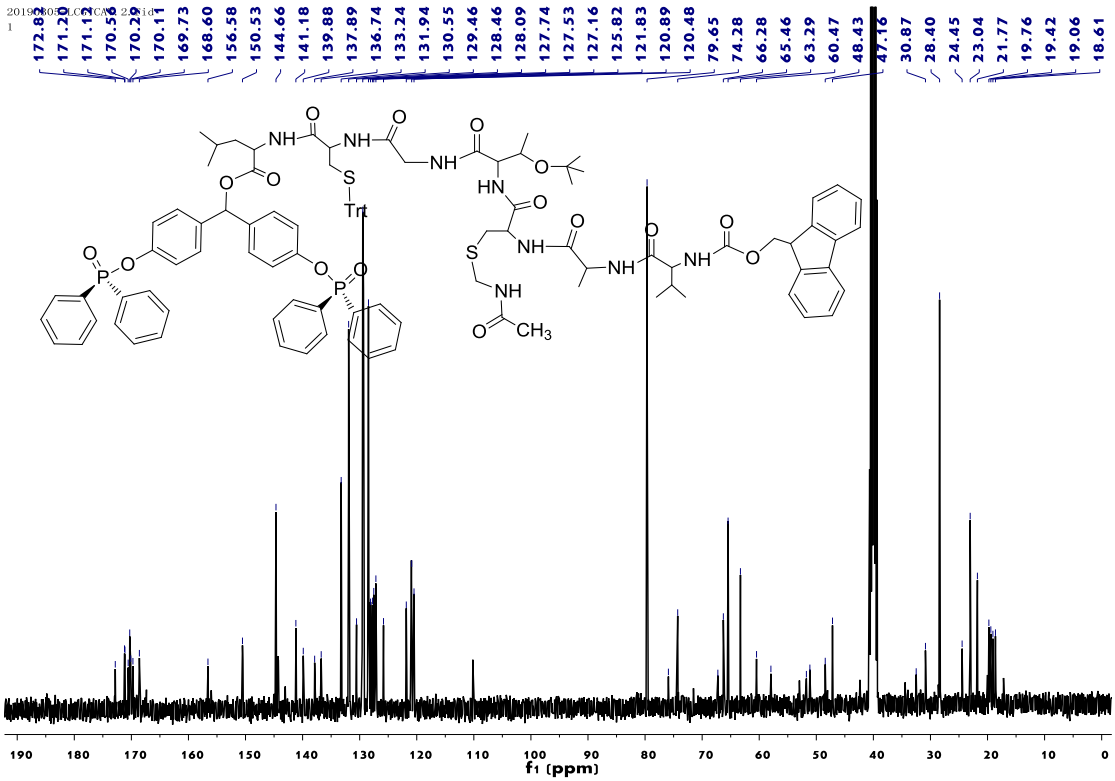
3c-8, Fmoc-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, DMSO-d_6)



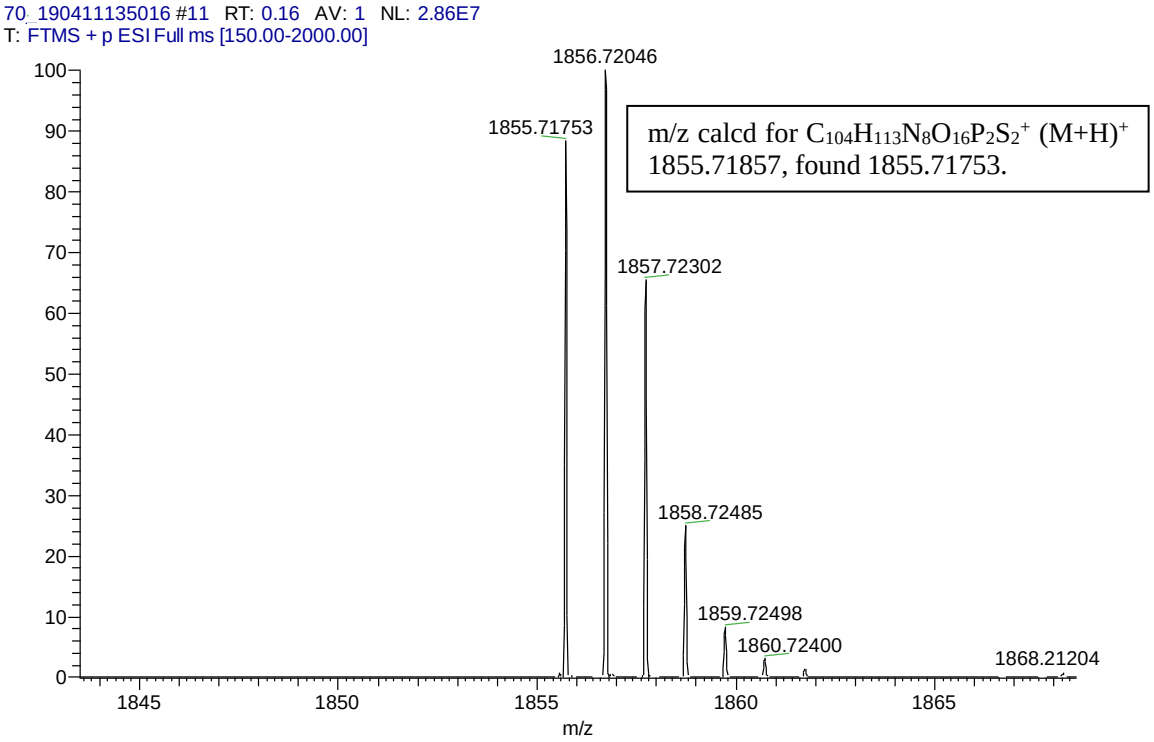
3c-8, Fmoc-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



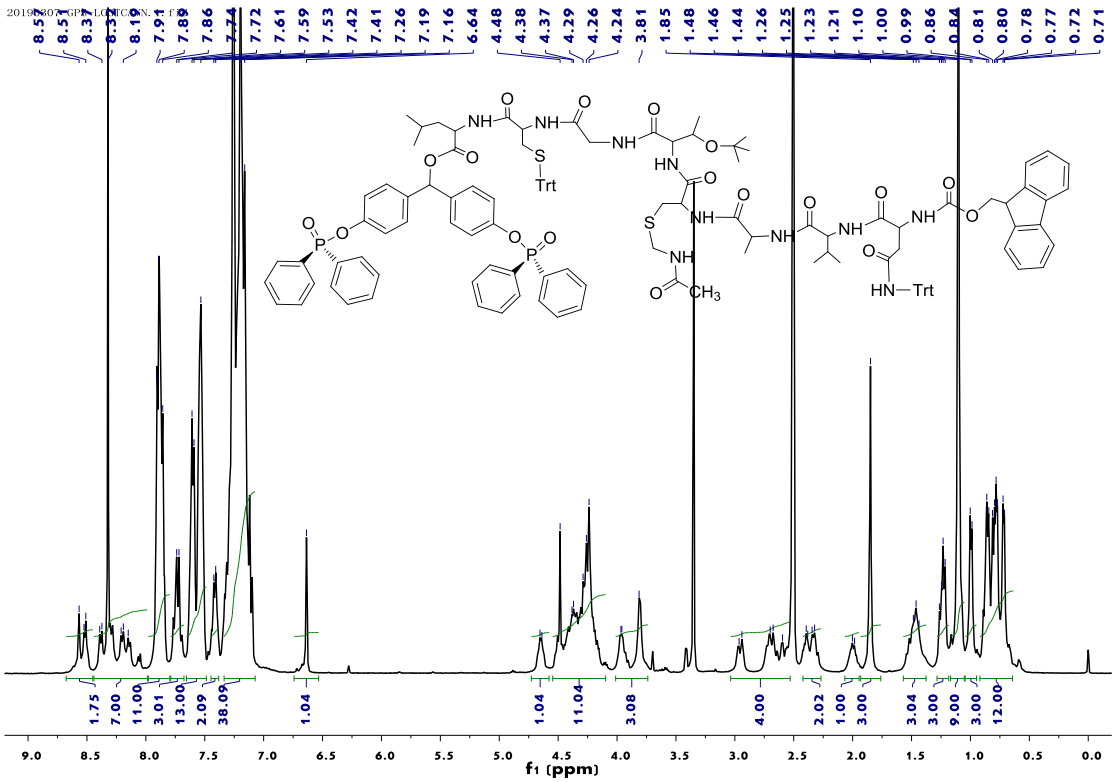
3c-9, Fmoc-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



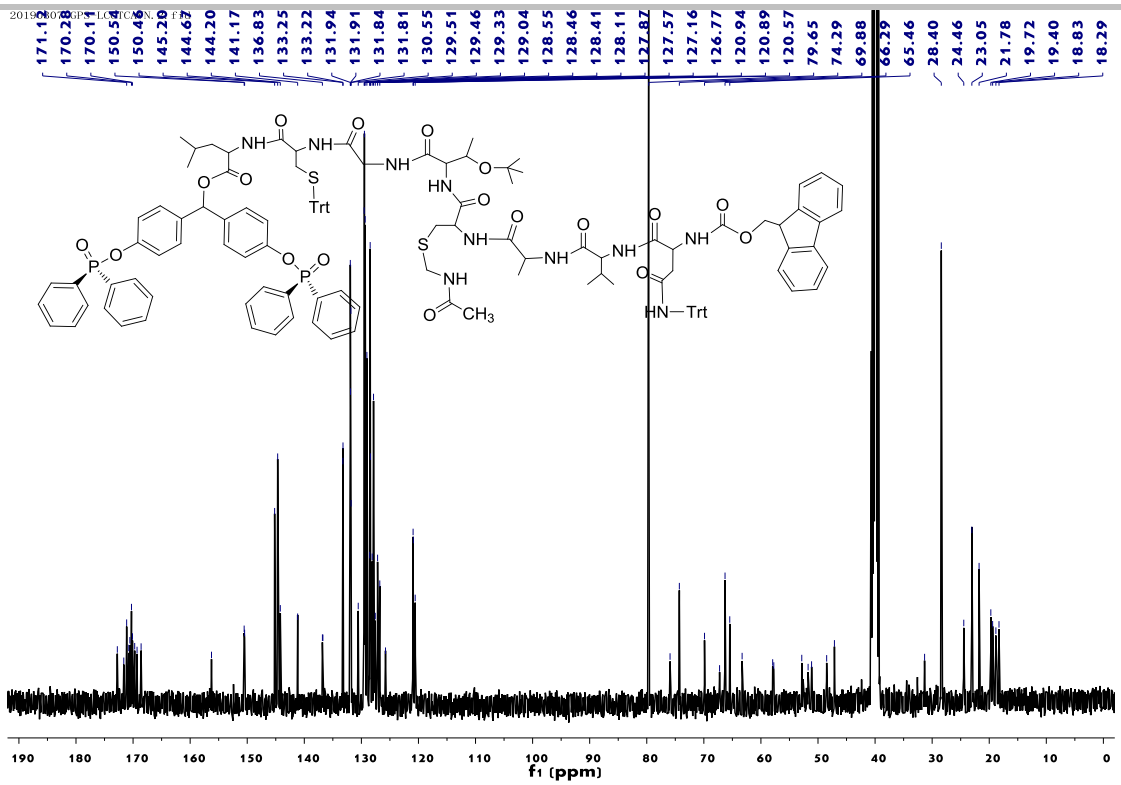
3c-9, Fmoc-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, DMSO-d_6)



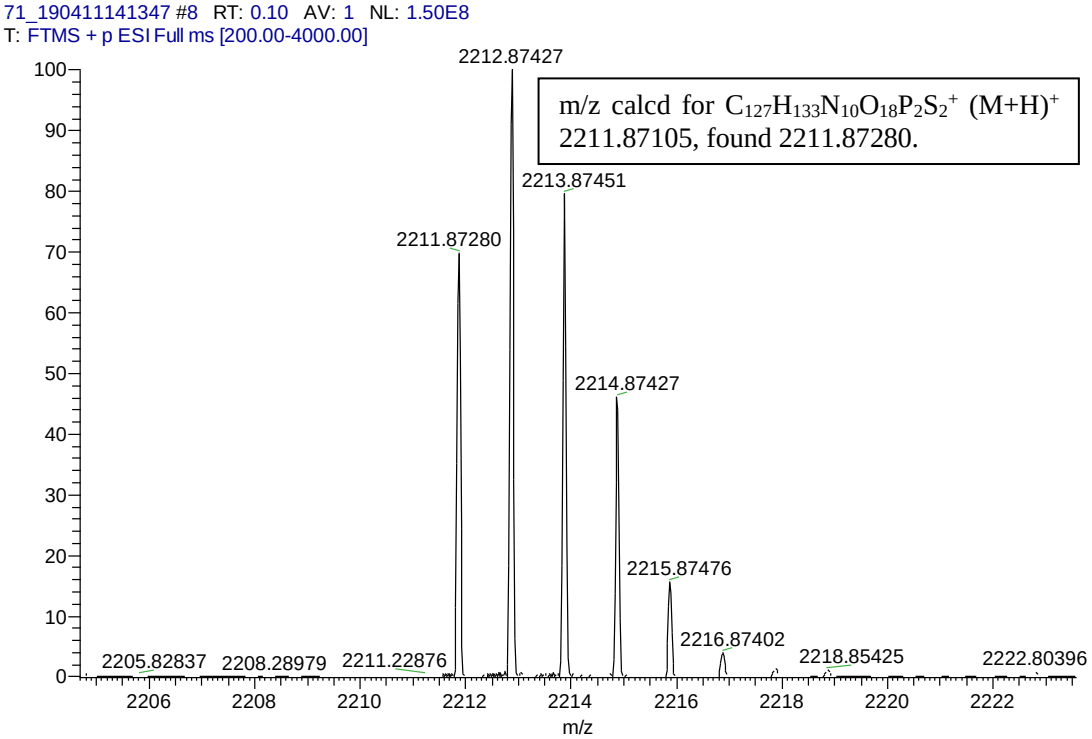
3c-9, Fmoc-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



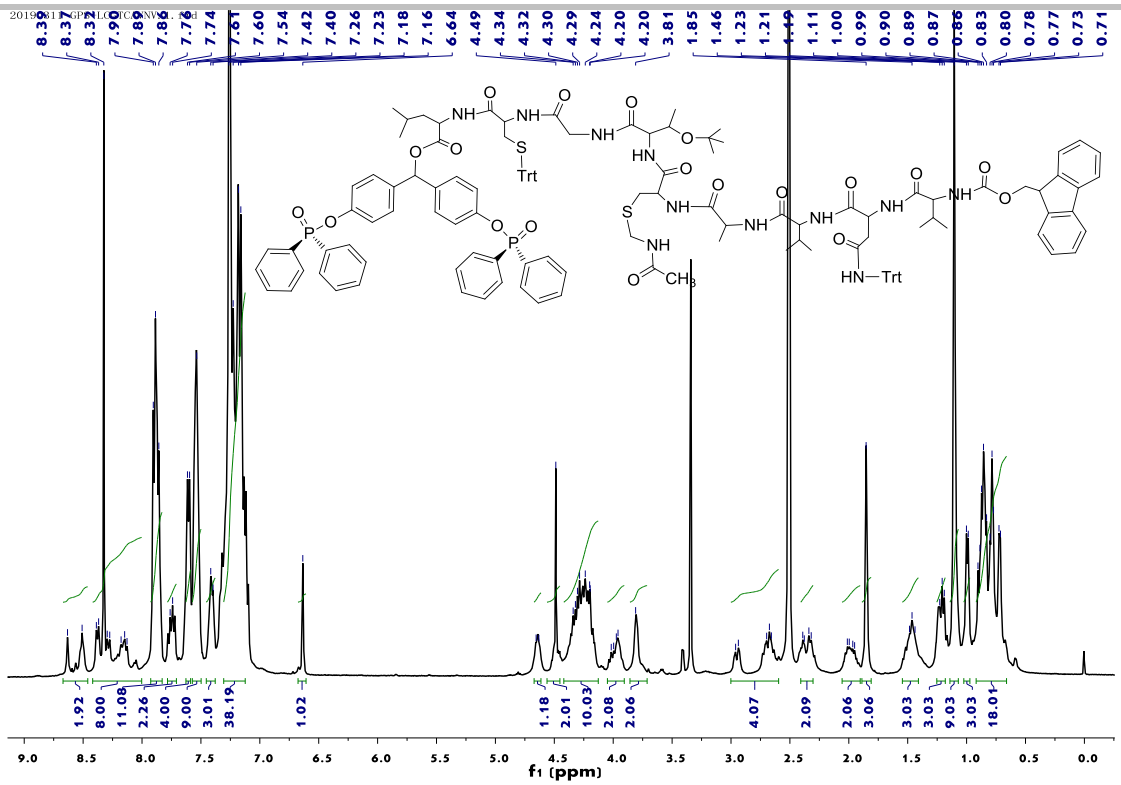
3c-10, Fmoc-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



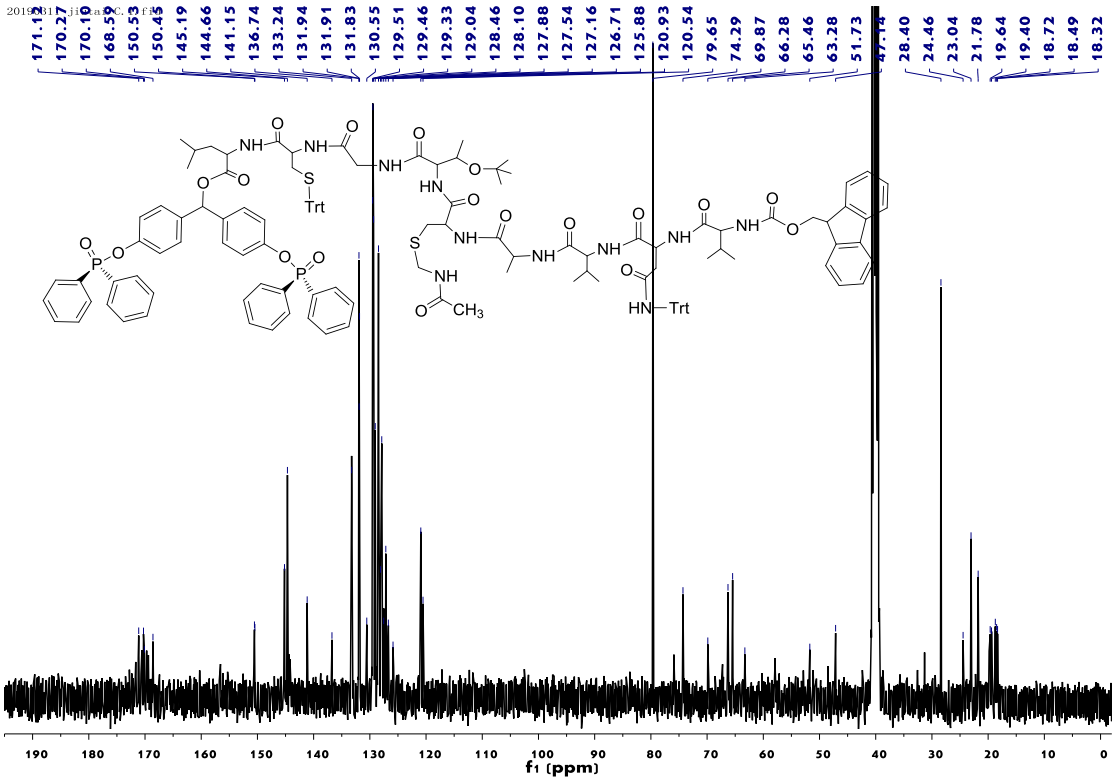
3c-10, Fmoc-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, DMSO-d_6)



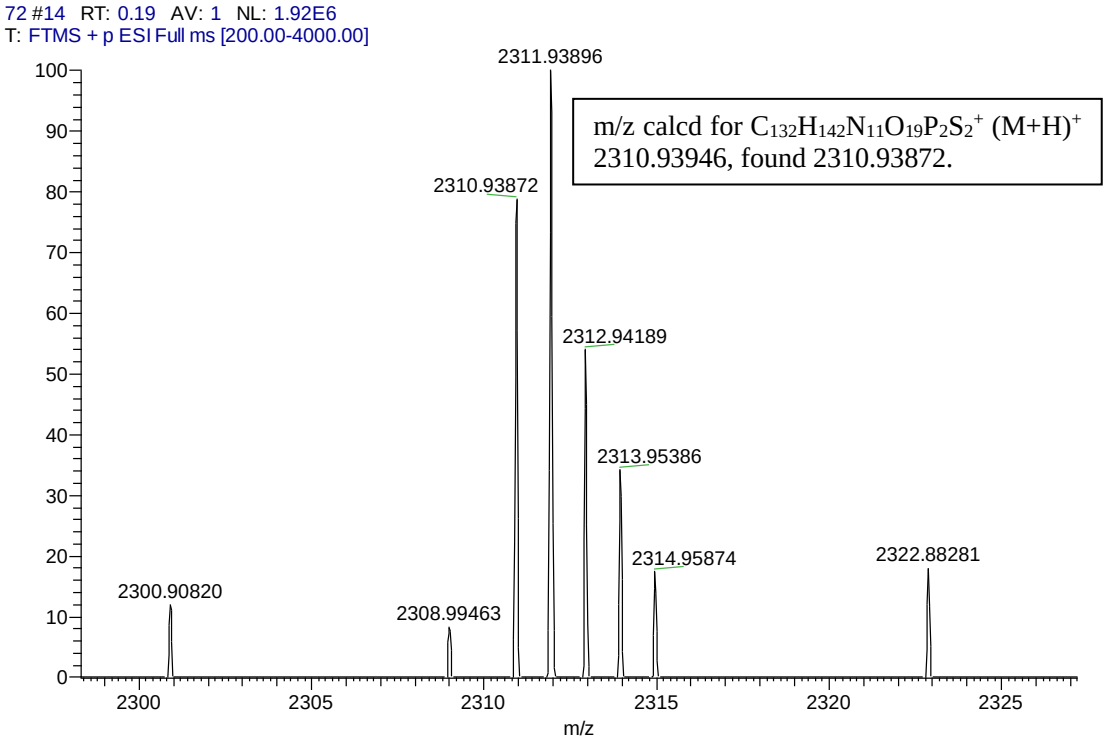
3c-10, Fmoc-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)



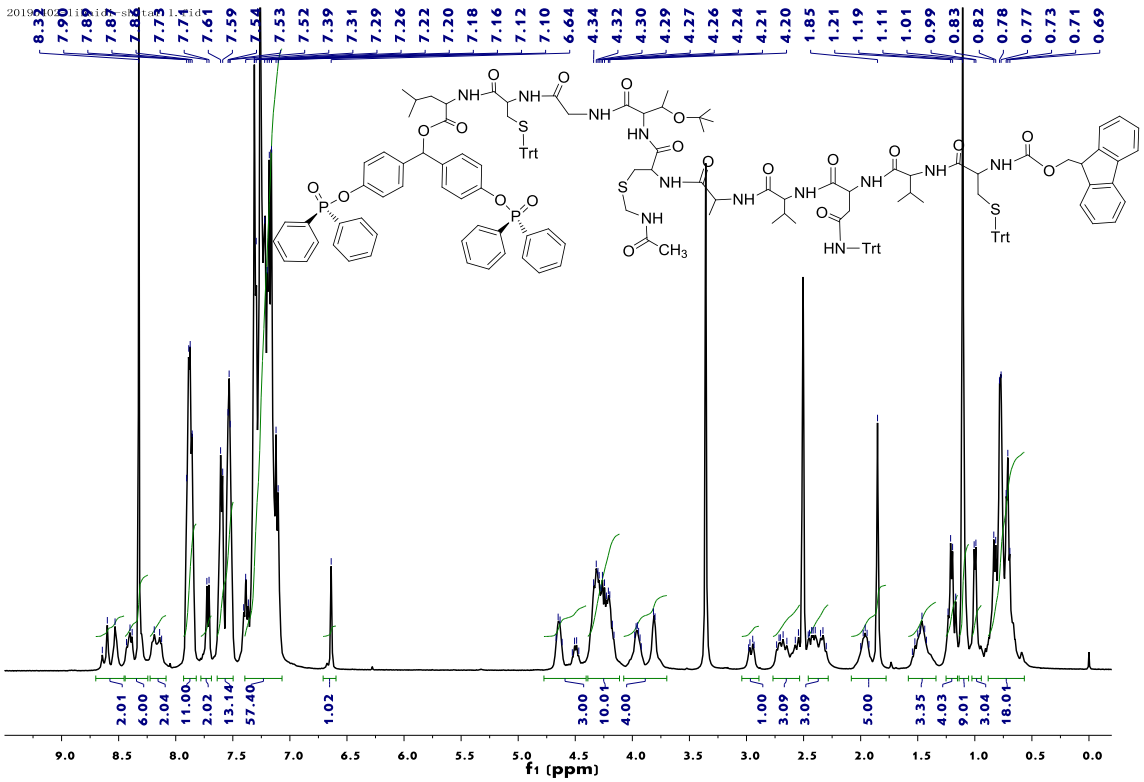
3c-11, Fmoc-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



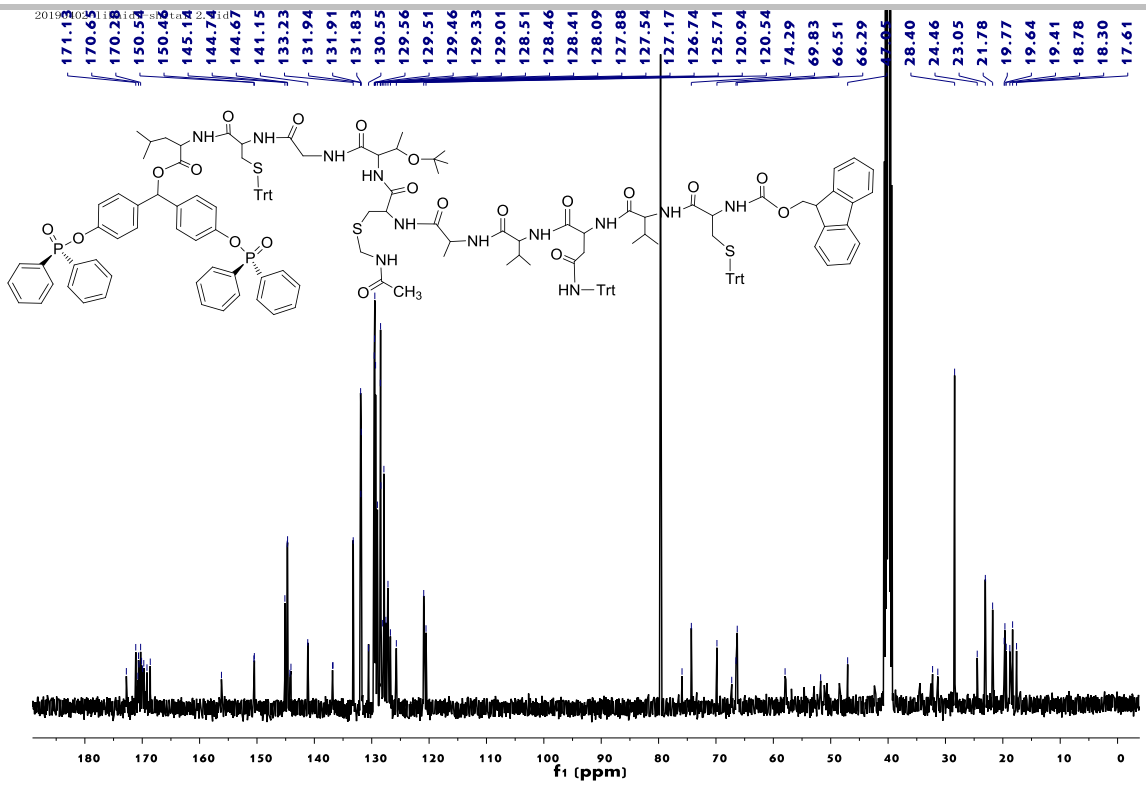
3c-11, Fmoc-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (100 MHz, DMSO-d_6)



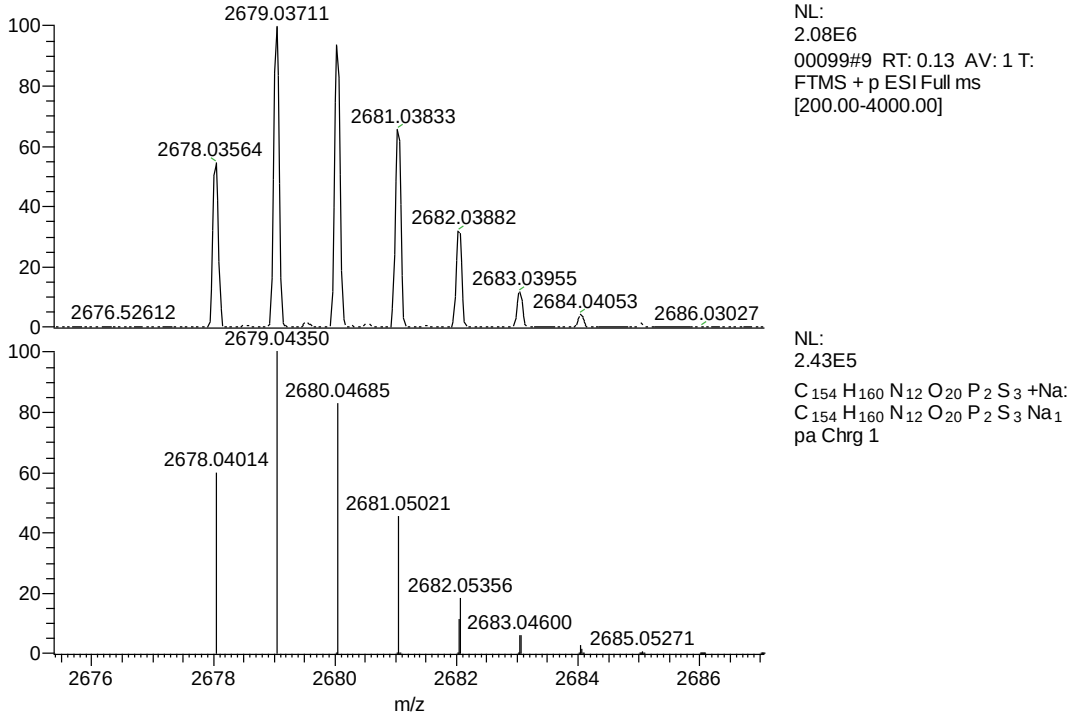
3c-11, Fmoc-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI)

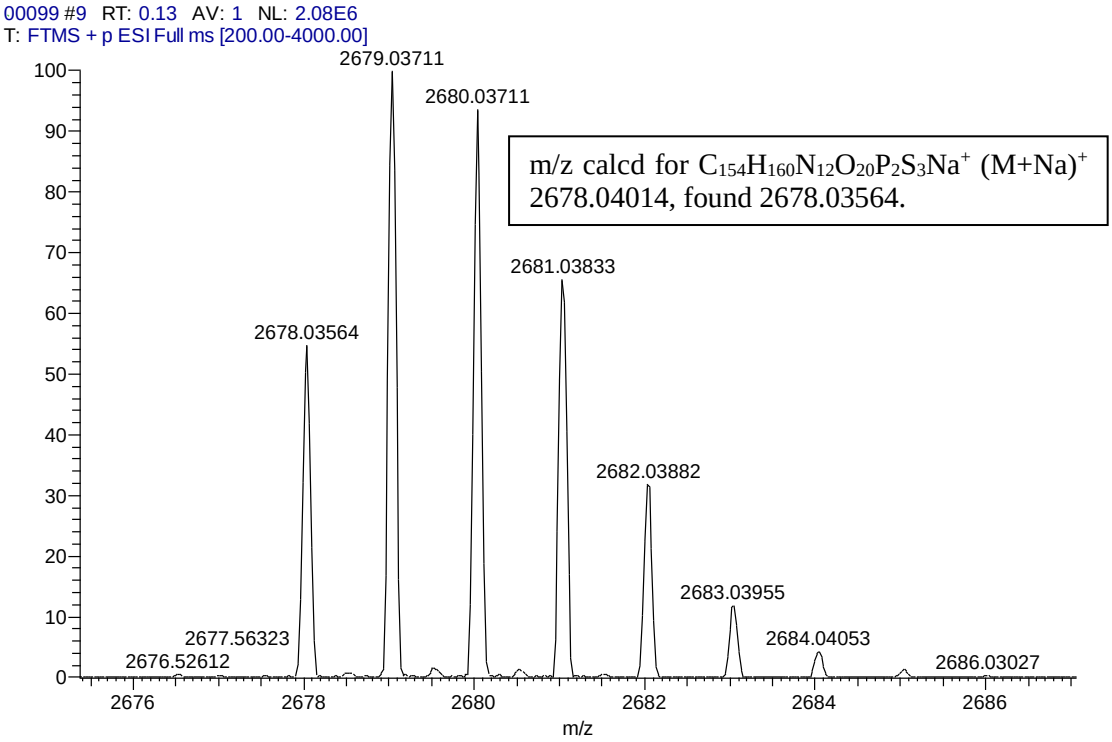


3c-12, Fmoc-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)

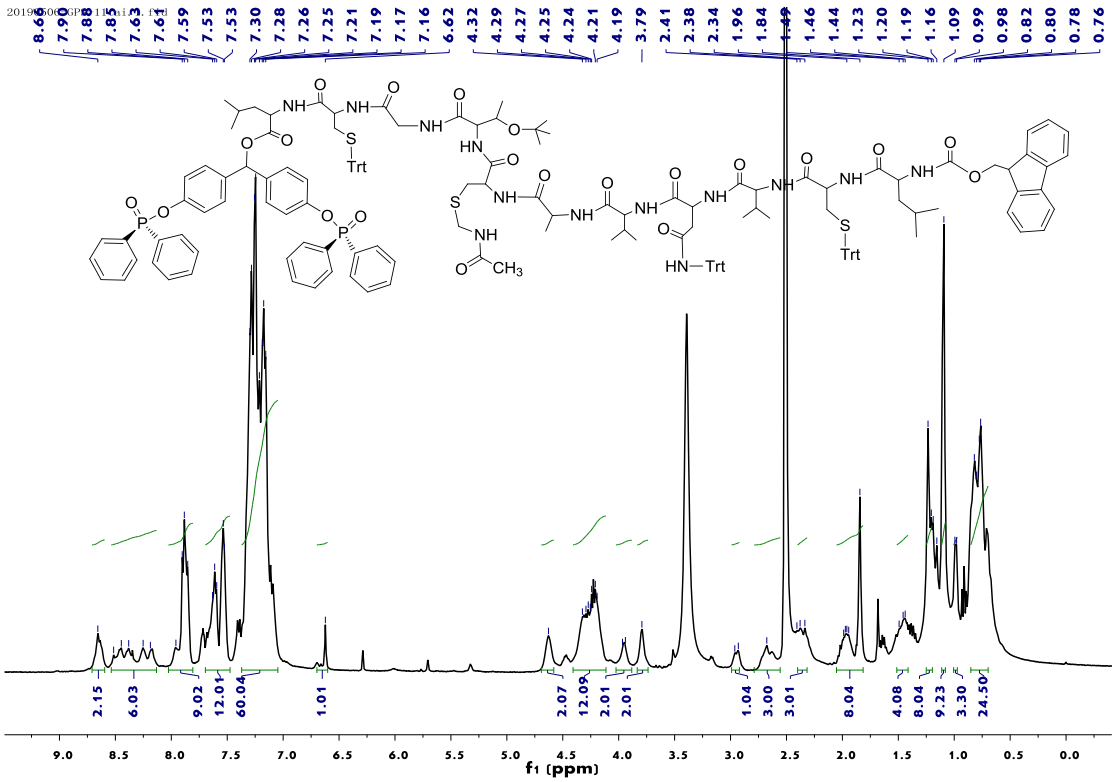


3c-12, Fmoc-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5-Fmoc ^{13}C NMR (100 MHz, DMSO-d_6)

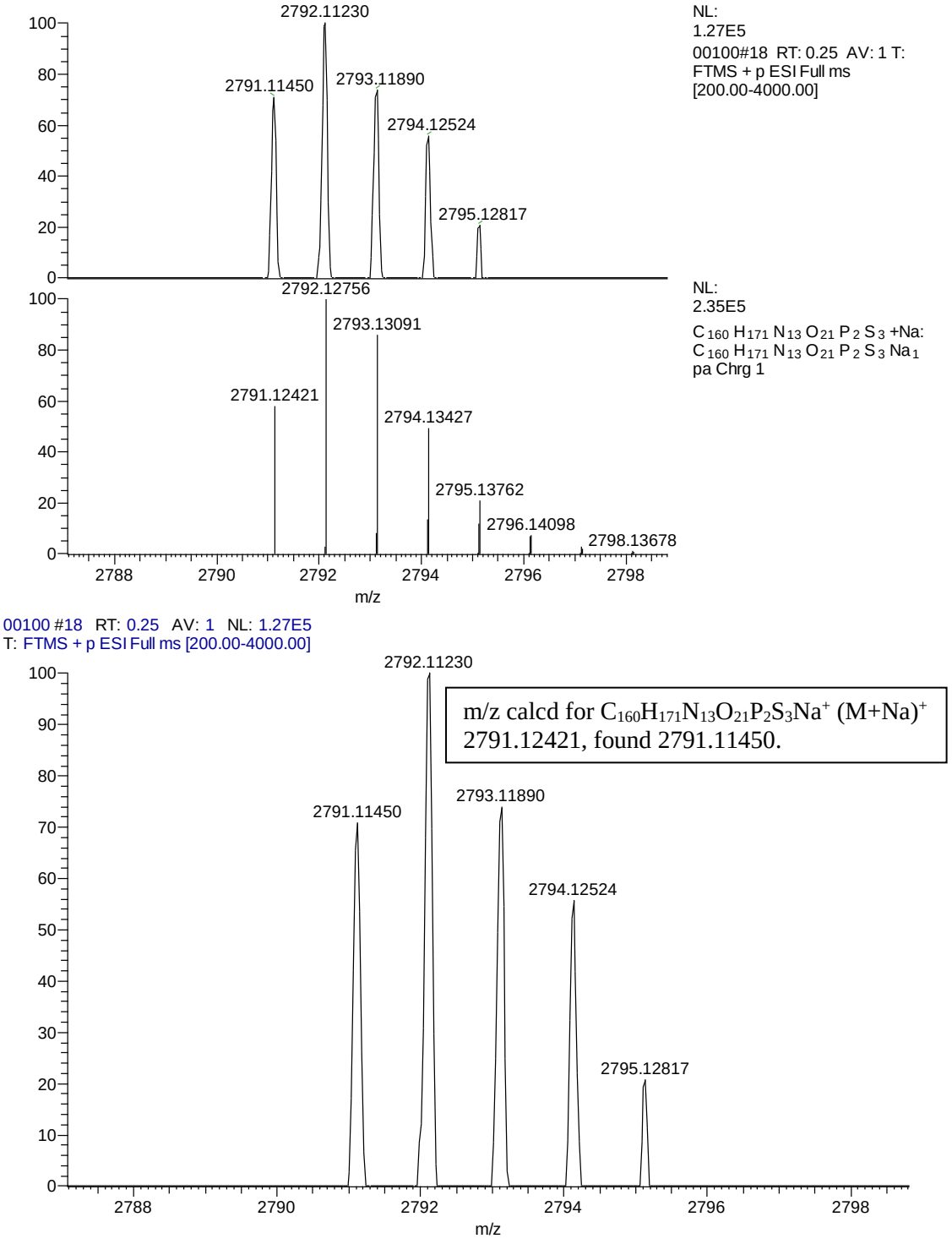




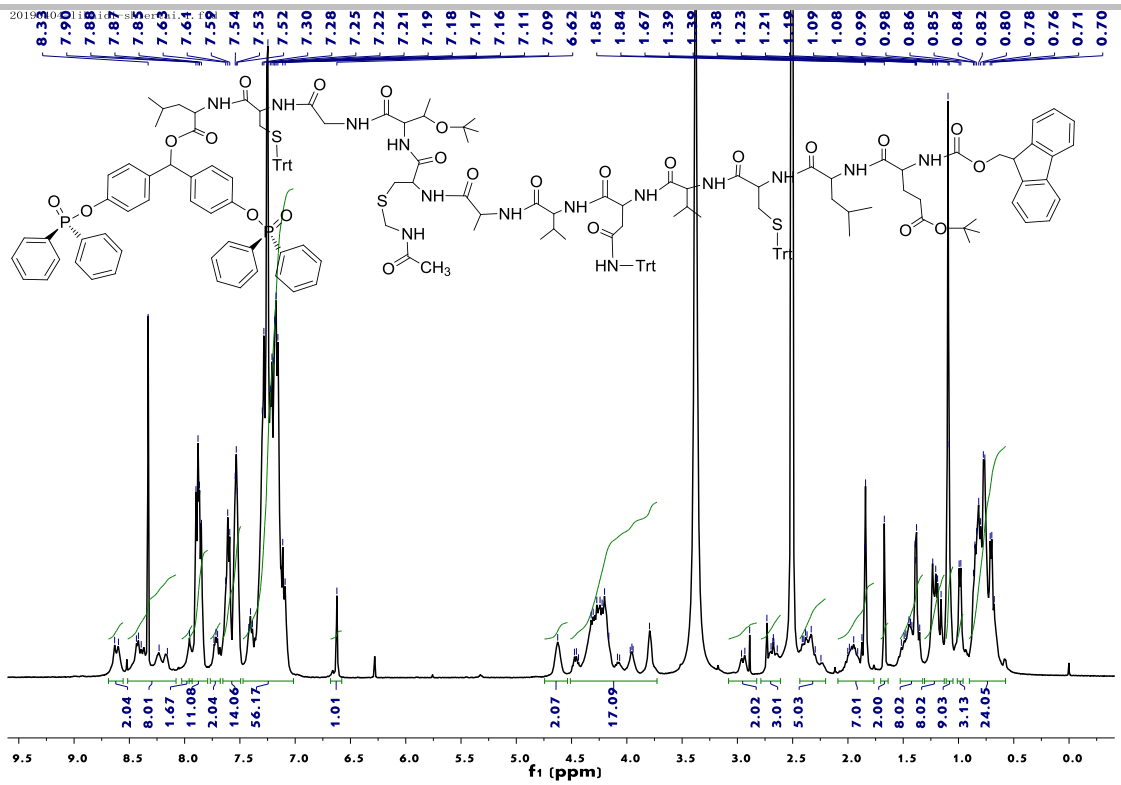
3c-12, Fmoc-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5HRMS (ESI) m/z calcd for $C_{154}H_{160}N_{12}O_{20}P_2S_3Na^+$ $(M+Na)^+$ 2678.04014, found 2678.03564.



3c-13, Fmoc-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)

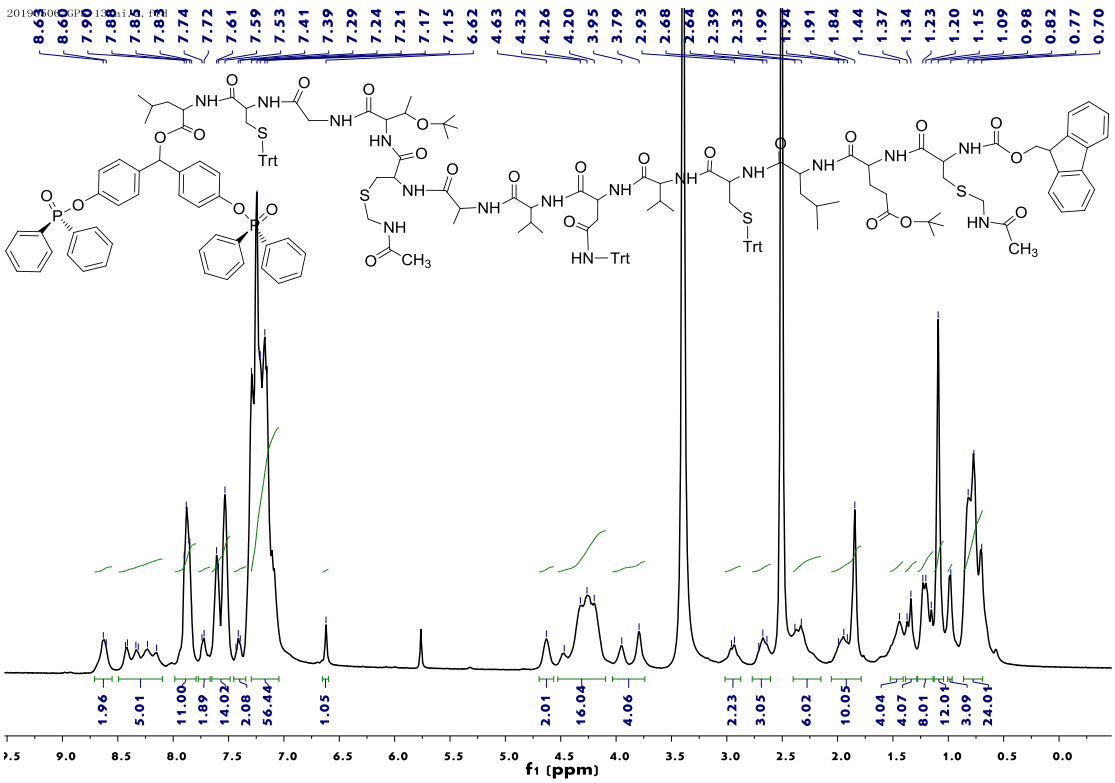


3c-13, Fmoc-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 HRMS (ESI) m/z calcd for $C_{160}H_{171}N_{13}O_{21}P_2S_3Na^+$ $(M+Na)^+$ 2791.12421, found 2791.11450.

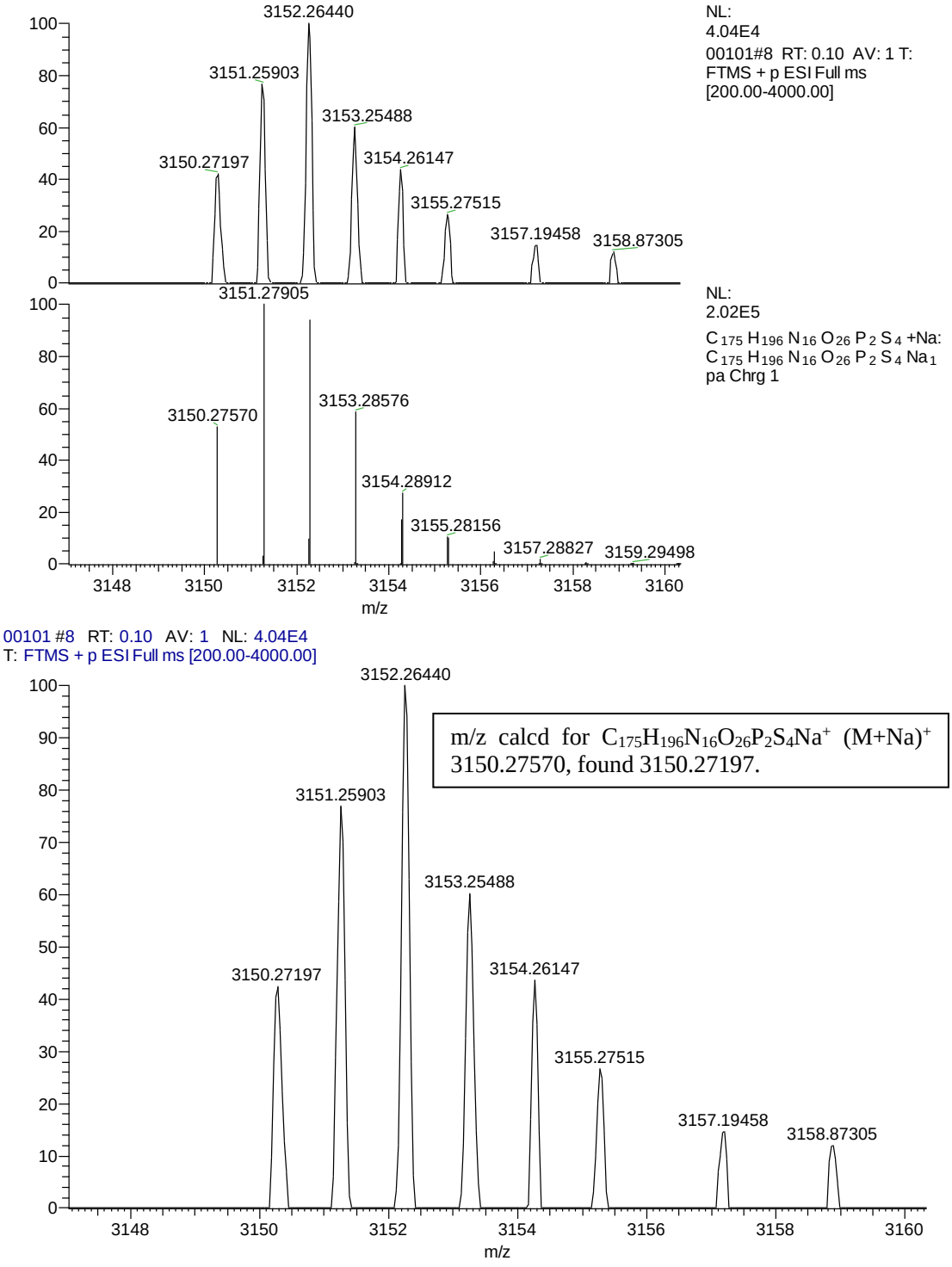


3c-14, Fmoc-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5

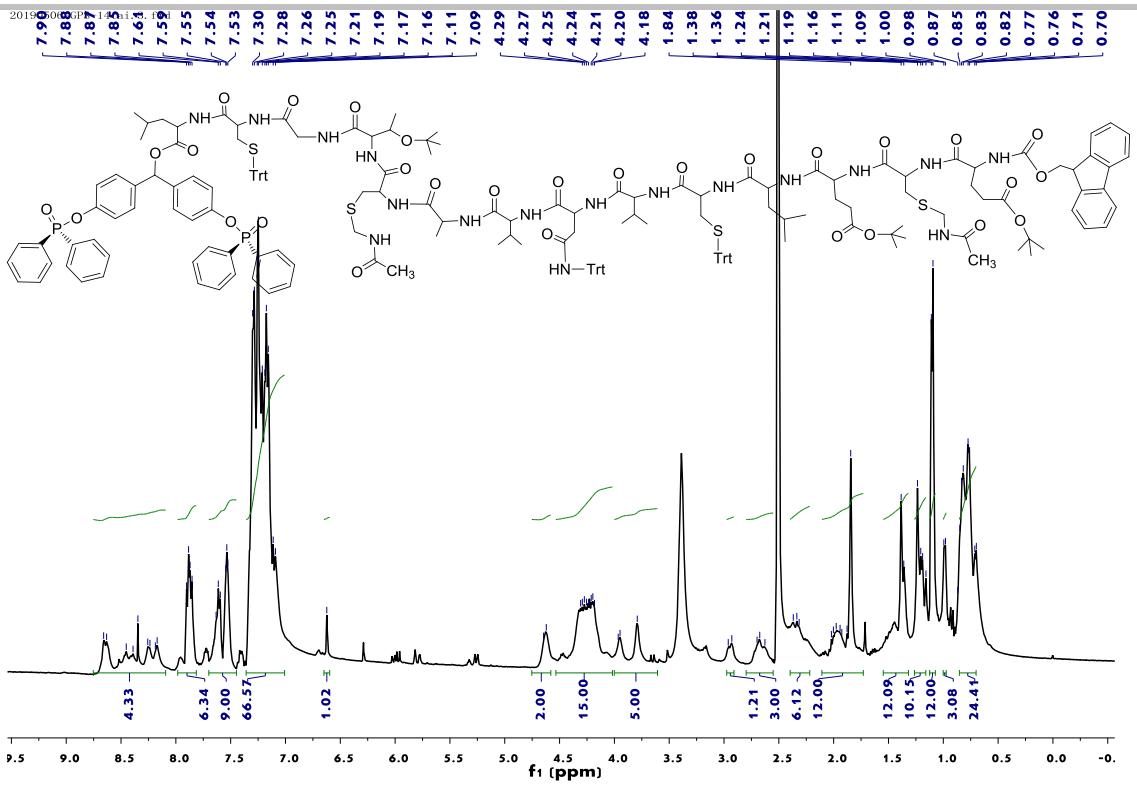
^{1}H NMR (400 MHz, DMSO-d_6)



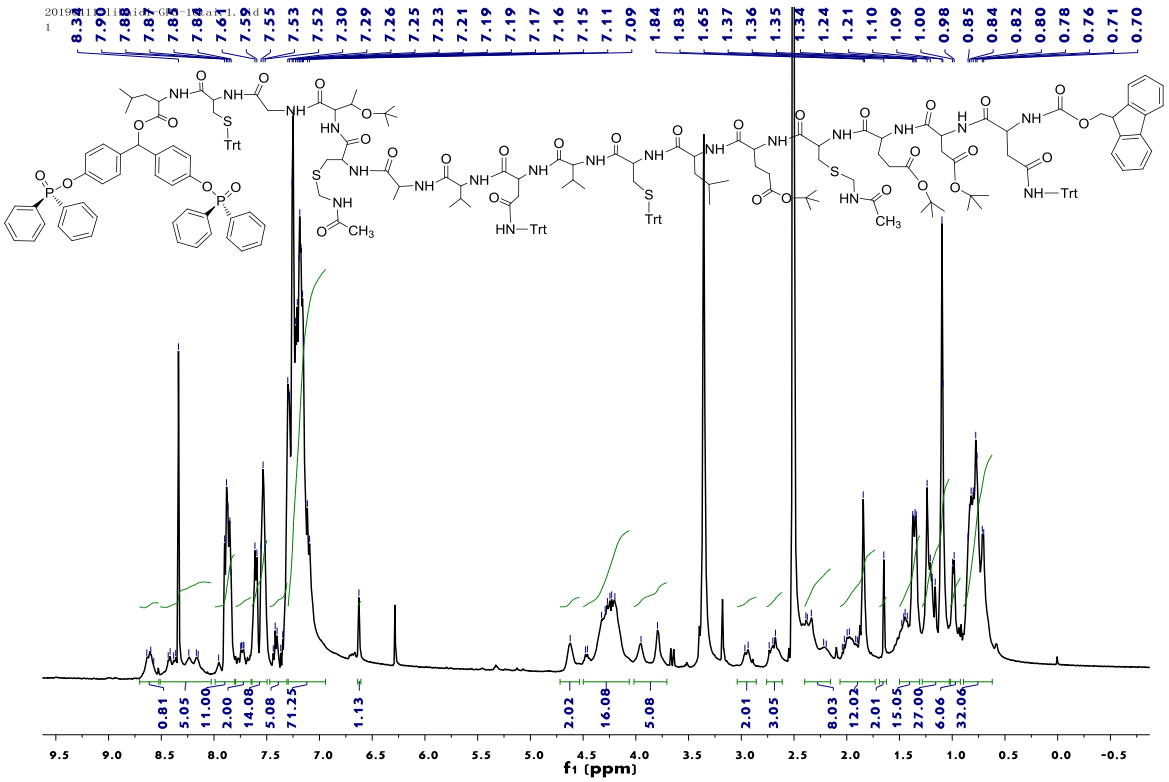
3c-15, Fmoc-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



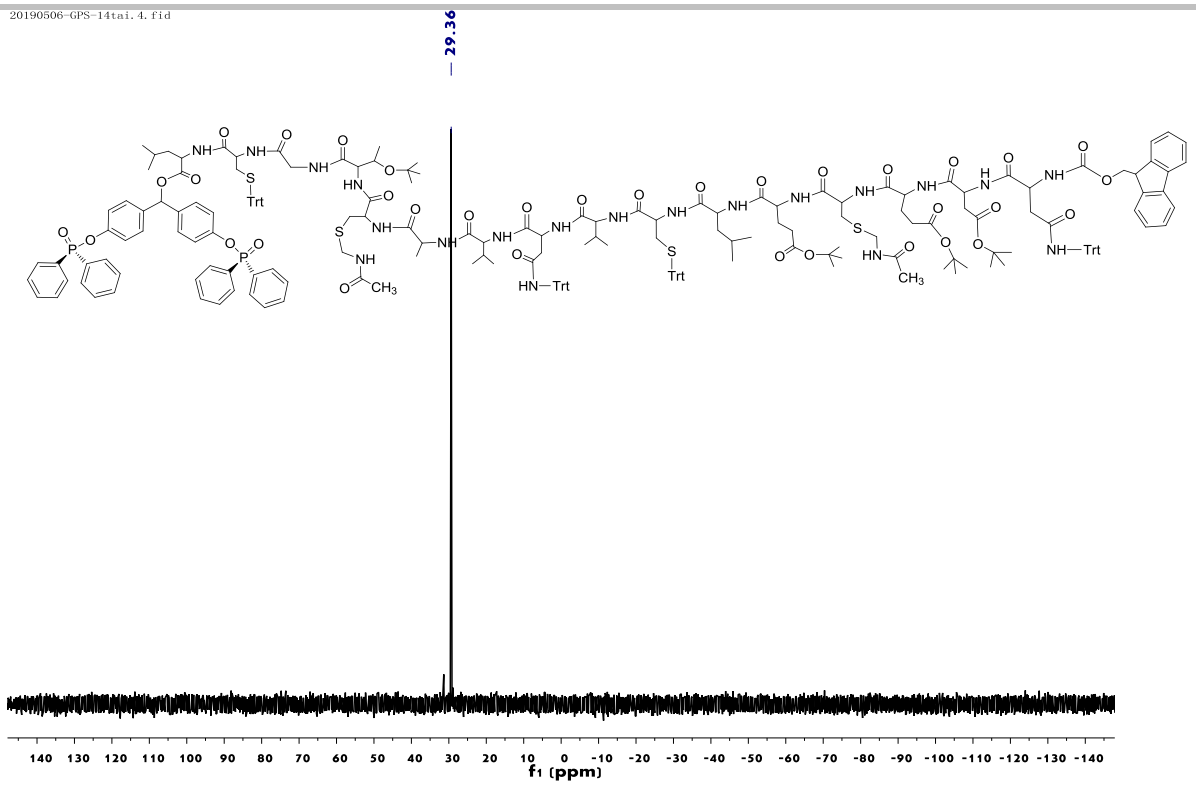
3c-15, Fmoc-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys (Trt)-Leu-DDK-5 HRMS (ESI) m/z calcd for $C_{175}H_{196}N_{16}O_{26}P_2S_4Na^+$ $(M+Na)^+$ 3150.27570, found 3150.27197.



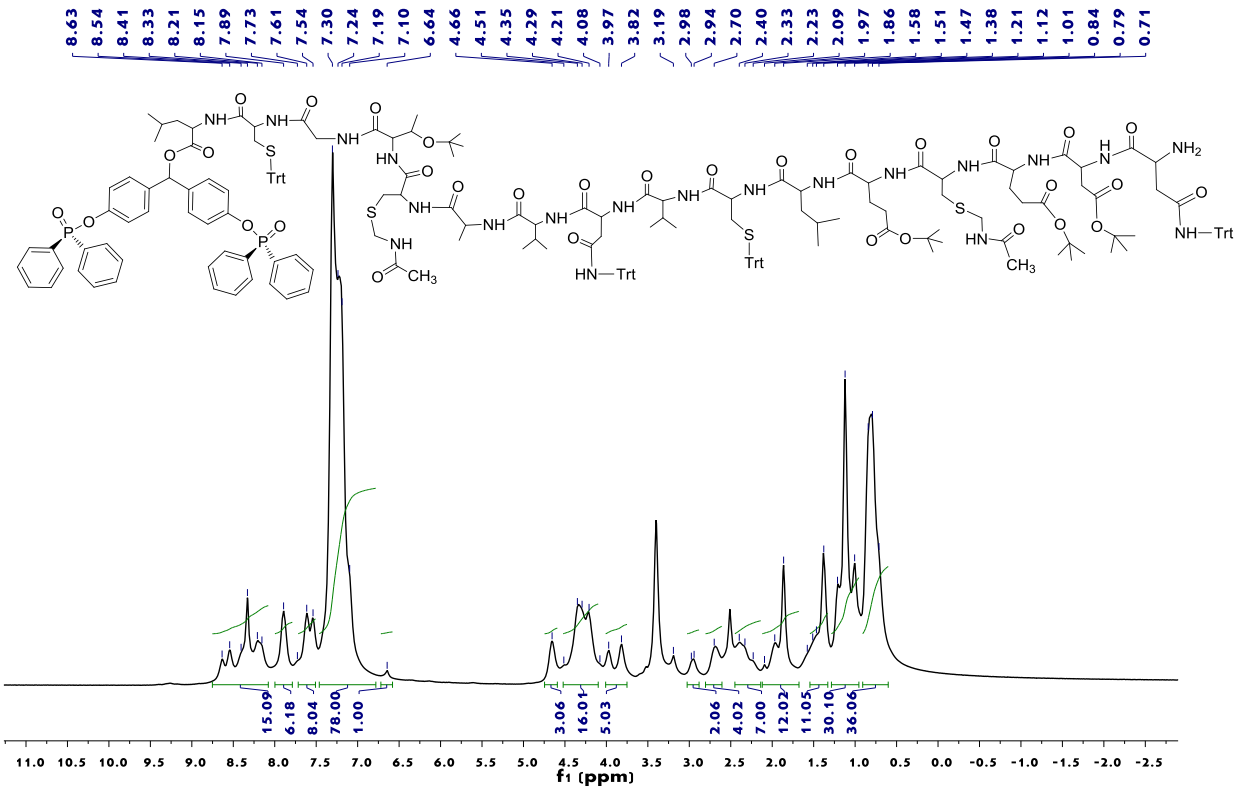
3c-16, Fmoc-Glu(tBu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



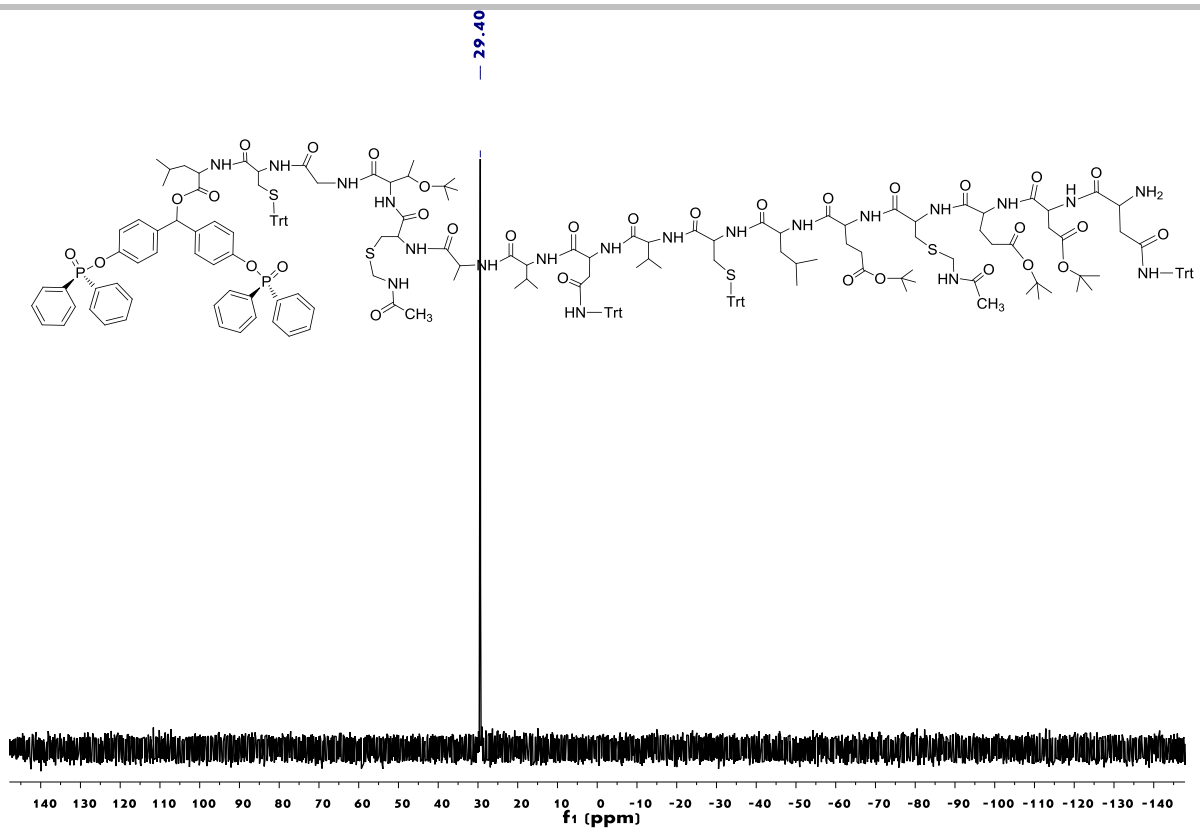
3c-17, Fmoc-Asn(Trt)-Asp(tBu)-Glu(tBu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{1}H NMR (400 MHz, DMSO-d_6)



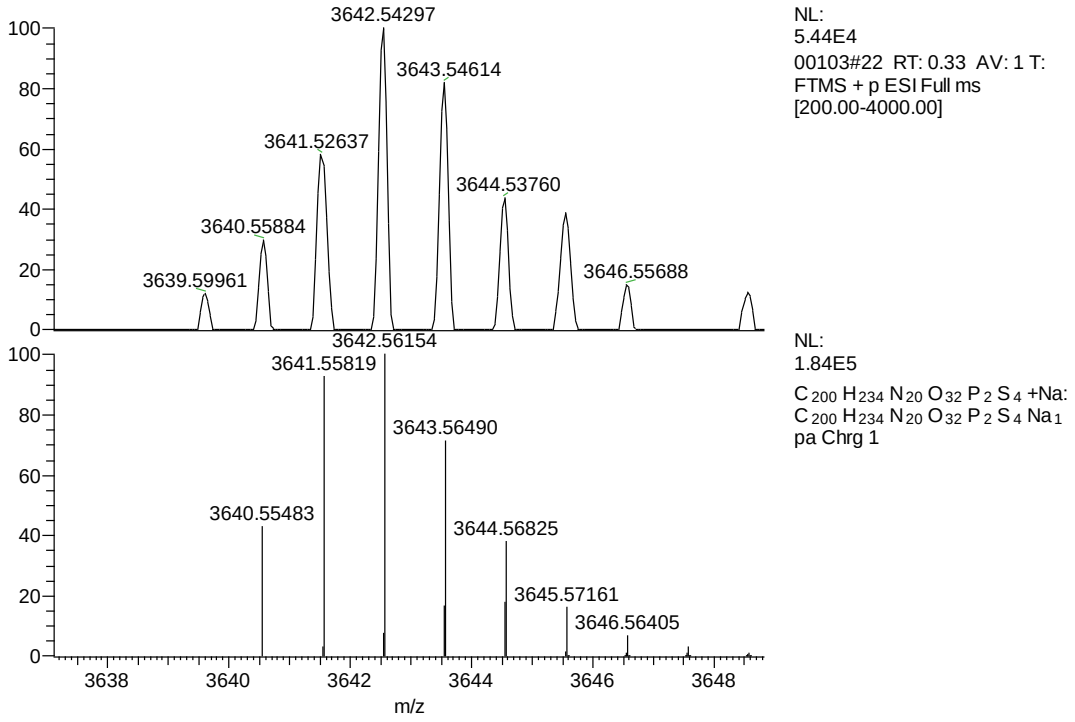
3c-17, Fmoc-Asn(Trt)-Asp(tBu)-Glu(tBu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{31}P NMR (162 MHz, DMSO-d_6)



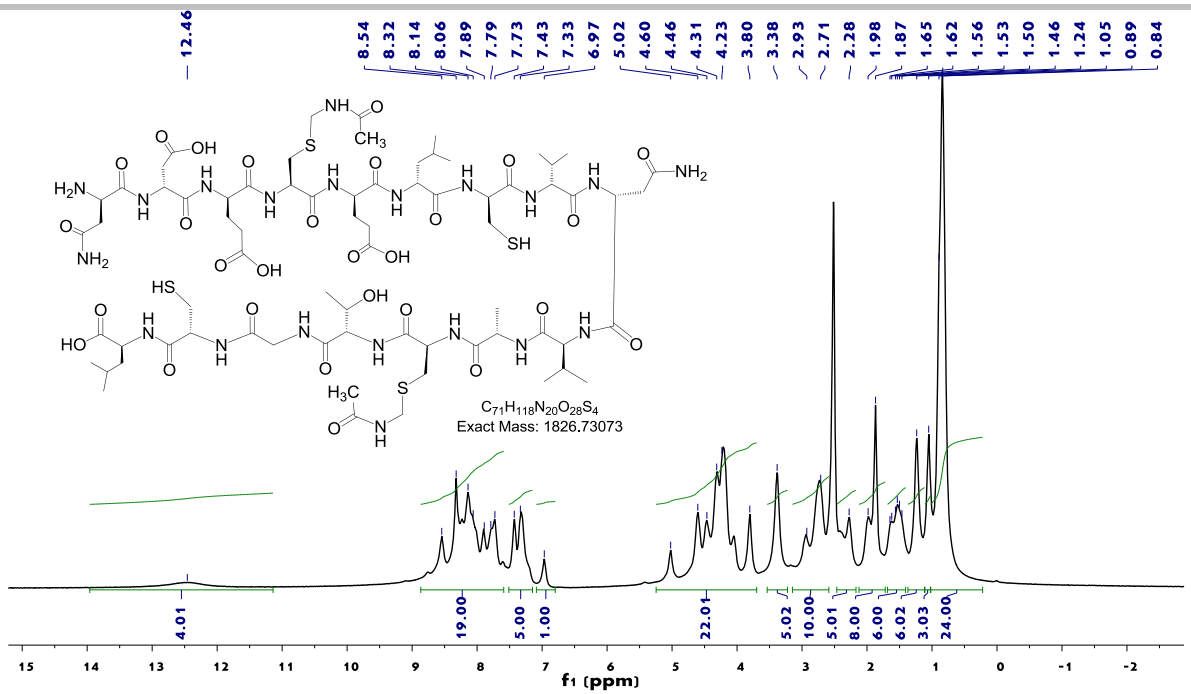
3c-18, (*de*-Fmoc-16) H-Asn(Trt)-Asp(tBu)-Glu(tBu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{13}C NMR (400 MHz, DMSO-d_6)



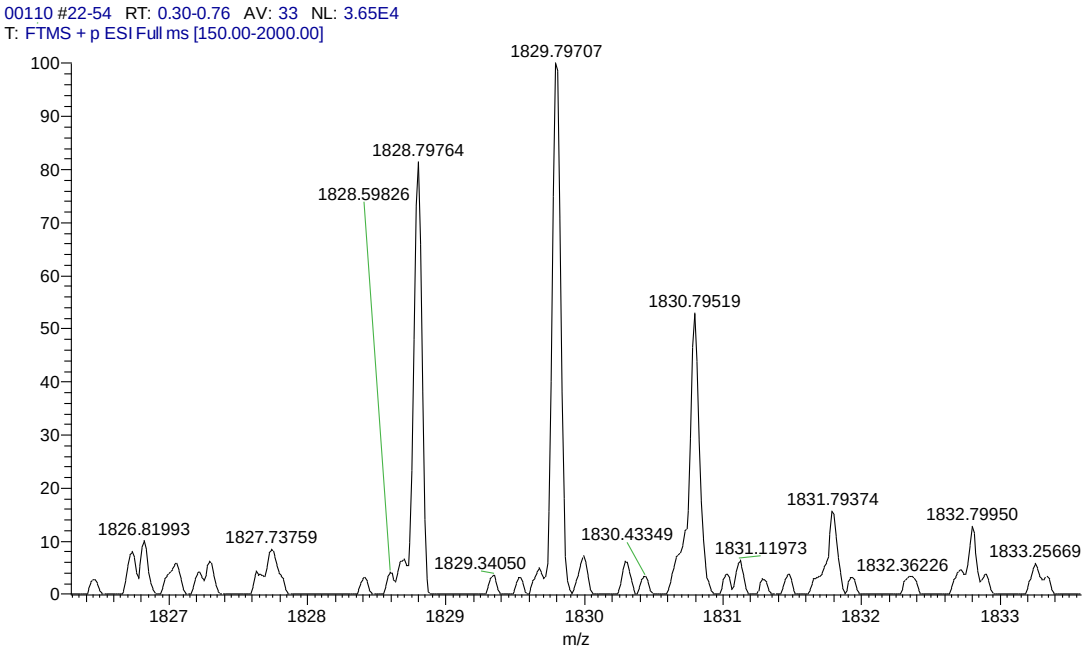
3c-18, (*de*-Fmoc-16) H-Asn(Trt)-Asp(tBu)-Glu(tBu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5 ^{31}P NMR (162 MHz, DMSO-d_6)



3c-18, (*de*-Fmoc-16) H-Asn(Trt)-Asp(tBu)-Glu(tBu)-Cys(Acm)-Glu(tBu)-Leu-Cys(Trt)-Val-Asn(Trt)-Val-Ala-Cys(Acm)-Thr(tBu)-Gly-Cys(Trt)-Leu-DDK-5: HRMS (ESI) m/z calcd for $C_{200}H_{234}N_{20}O_{32}P_2S_4Na^+$ $(M+Na)^+$ 3640.55483, found 3640.55884.

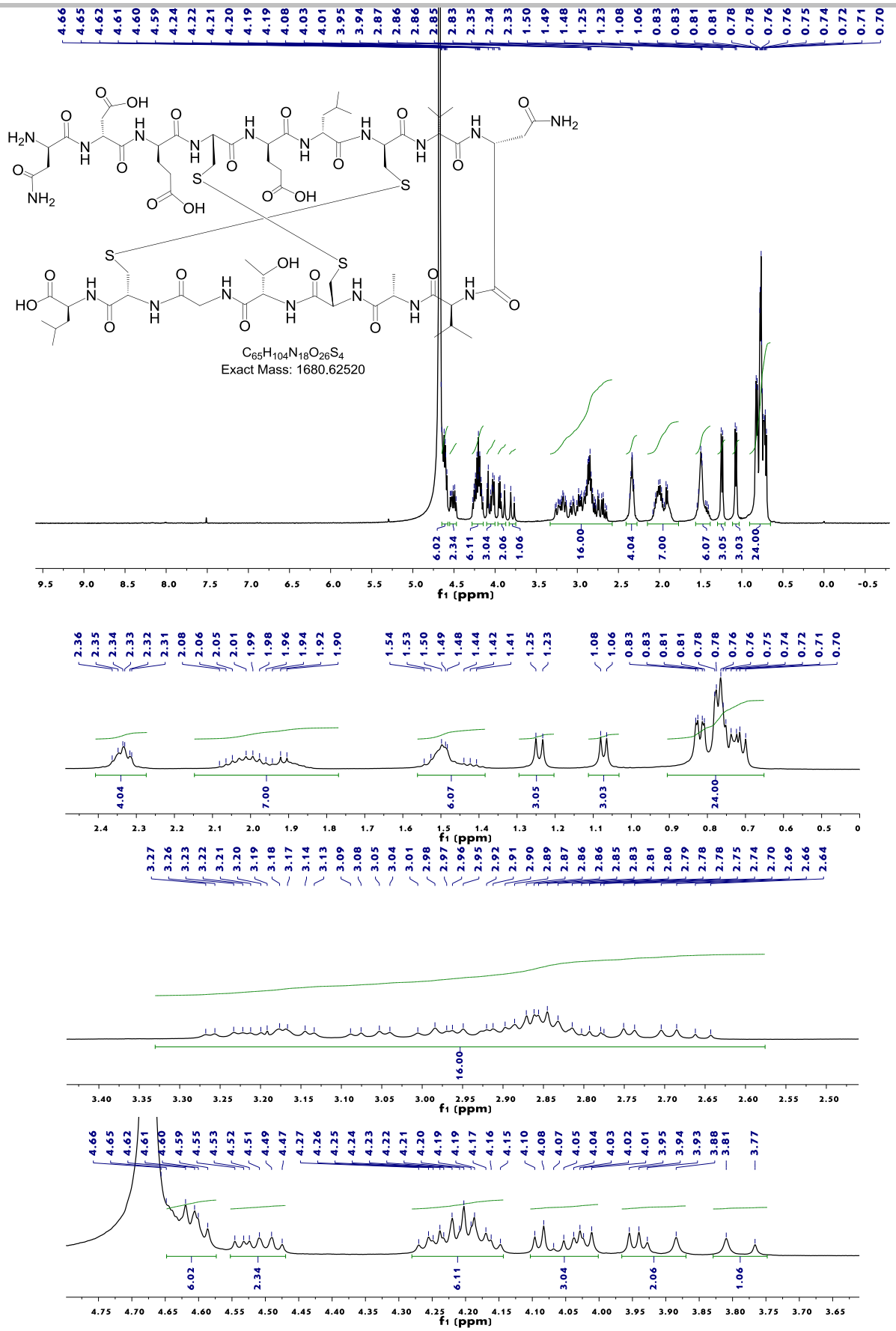


H-Asn-Asp-Glu-Cys(Acm)-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys(Acm)-Thr-Gly-Cys-Leu-OH ^{1}H NMR (400 MHz, DMSO-d_6)

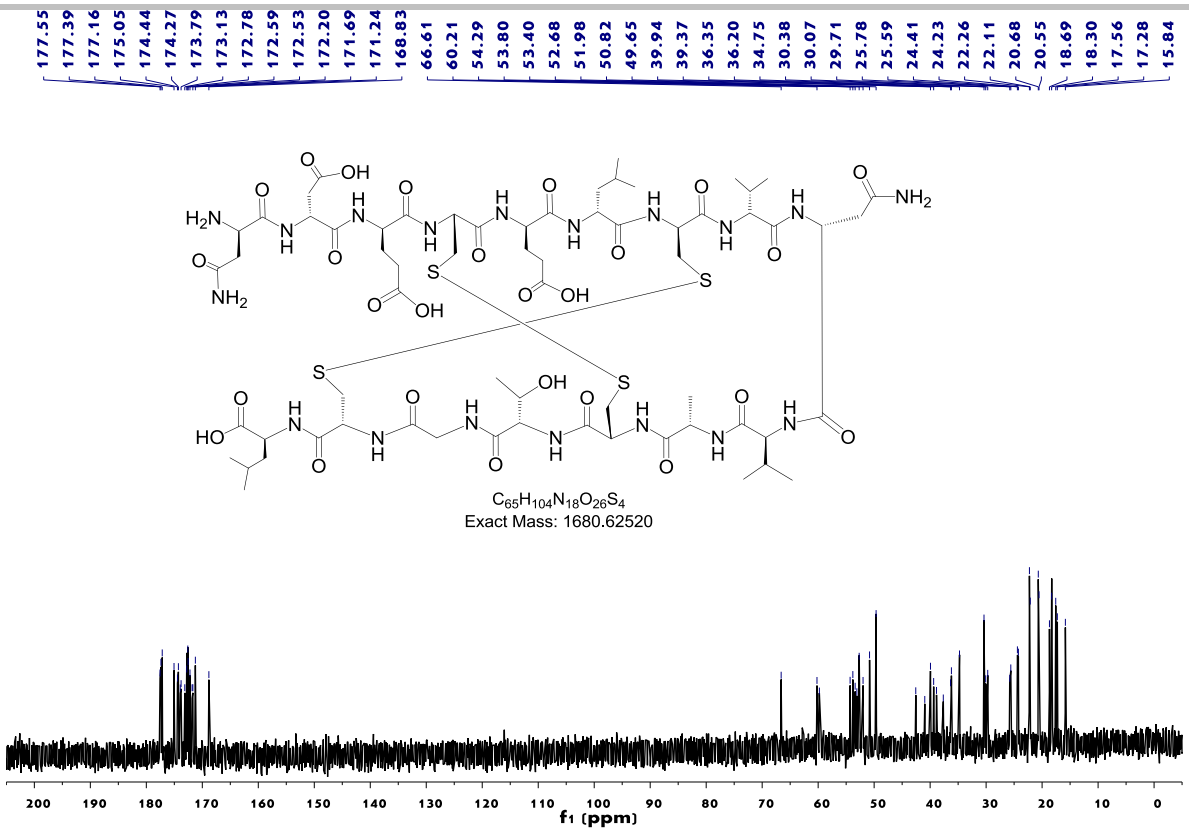


H-Asn-Asp-Glu-Cys(Acm)-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys(Acm)-Thr-Gly-Cys-Leu-OH

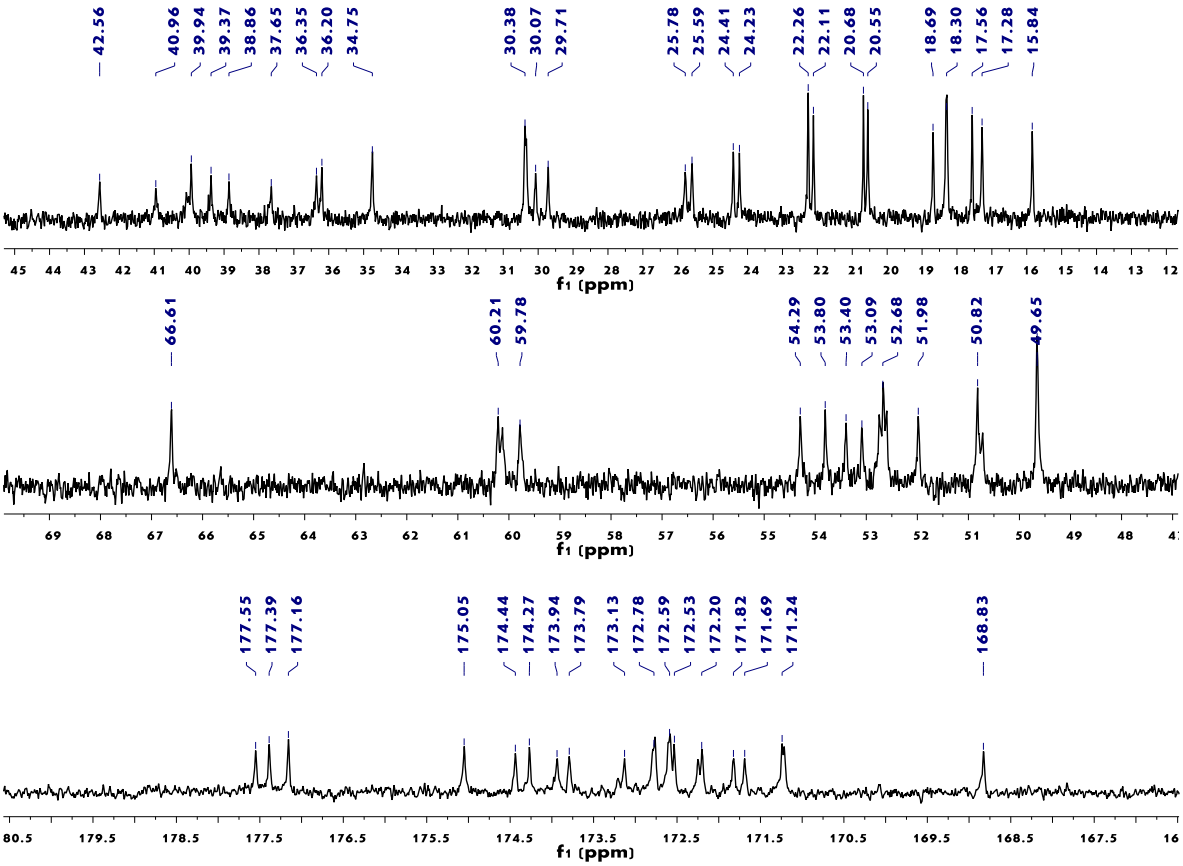
HRMS (ESI) m/z calcd for $C_{71}H_{119}N_{20}O_{28}S_4^+$ $(M+H)^+$ 1827.73800, found 1827.73759.



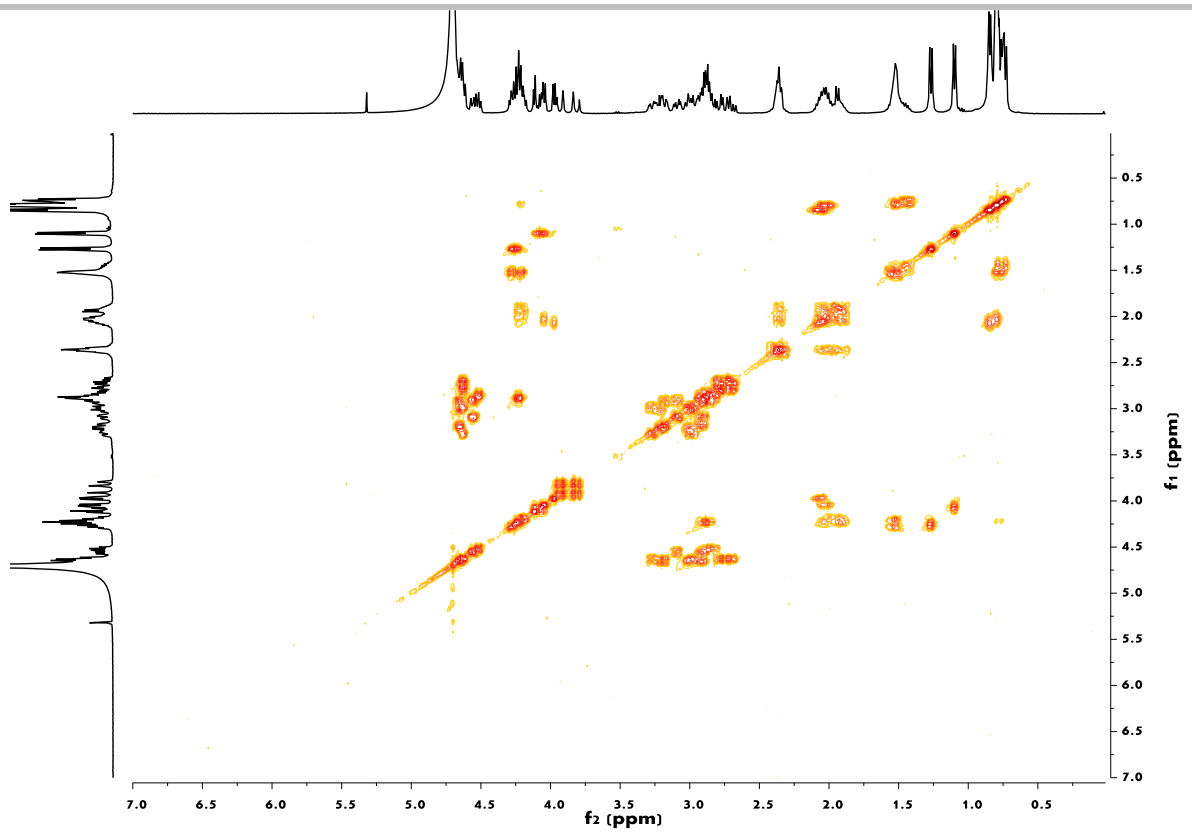
Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{1}H NMR (400 MHz, D_2O)



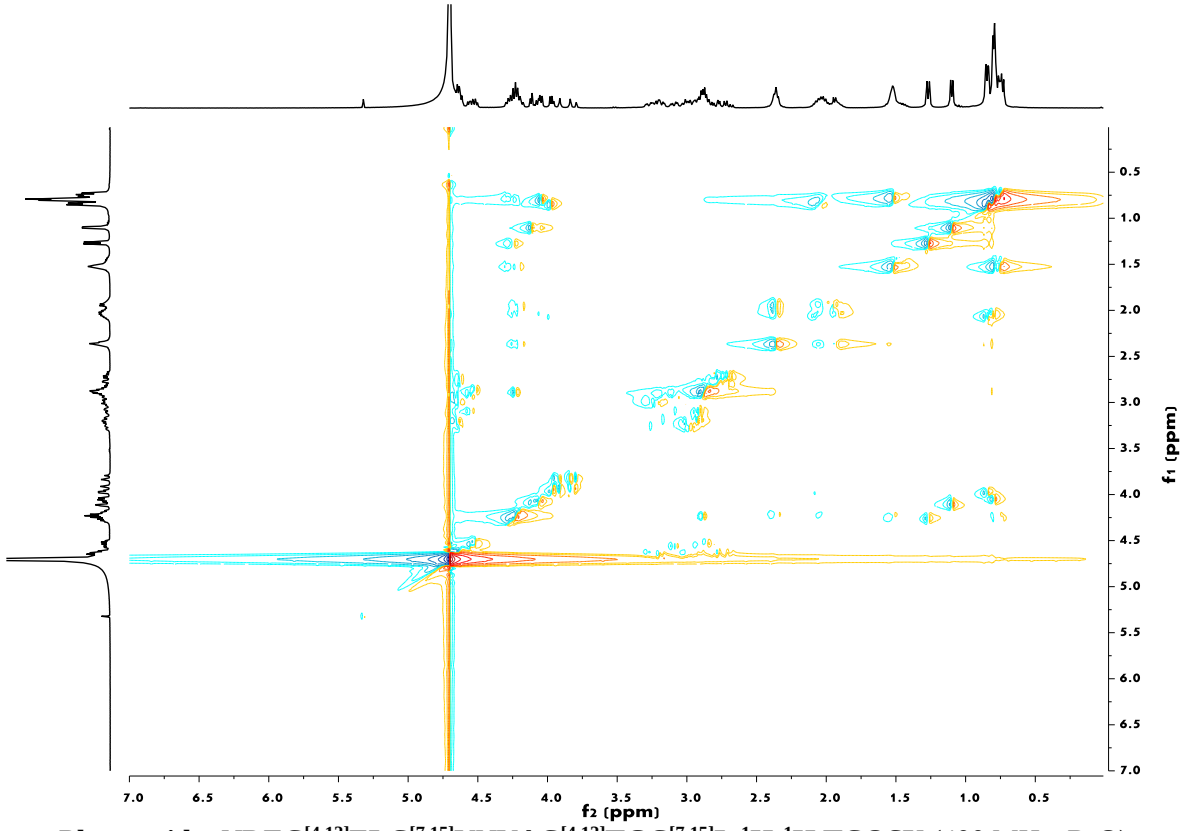
Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{13}C NMR (100 MHz, D_2O)



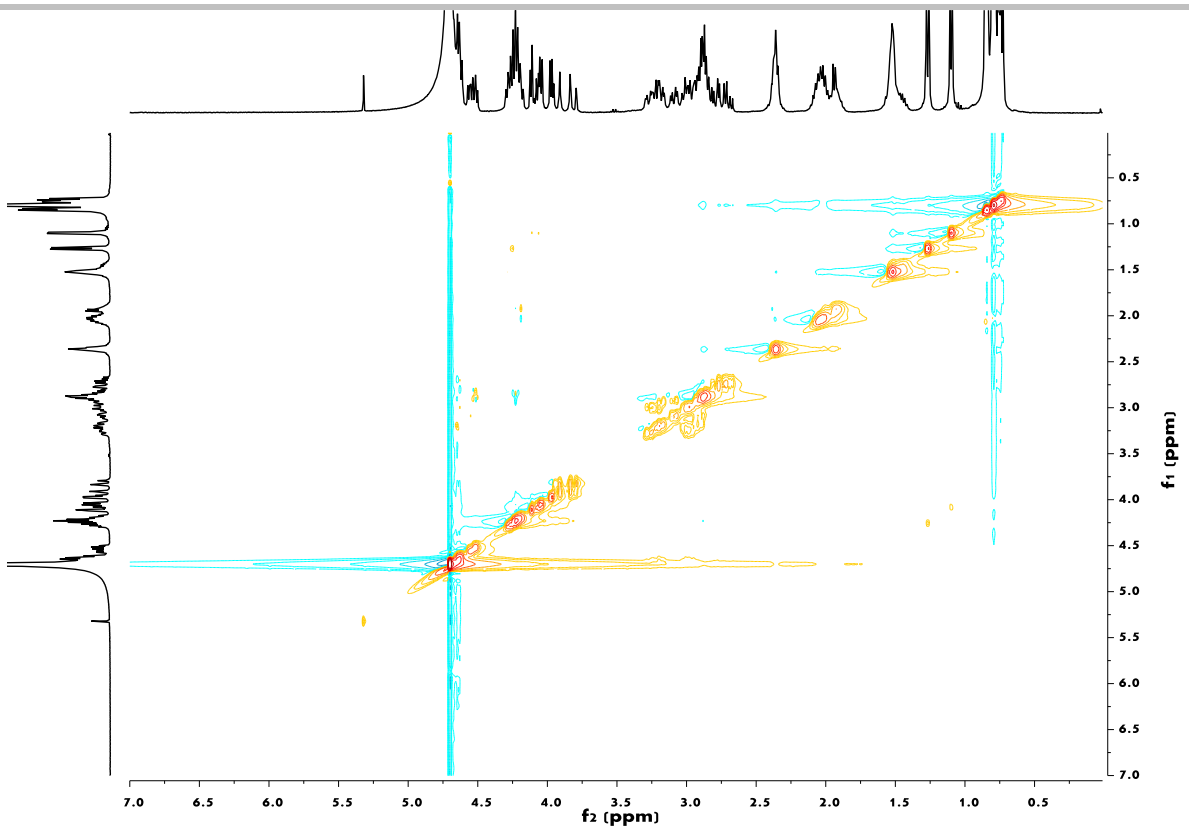
Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{13}C NMR (100 MHz, D_2O)



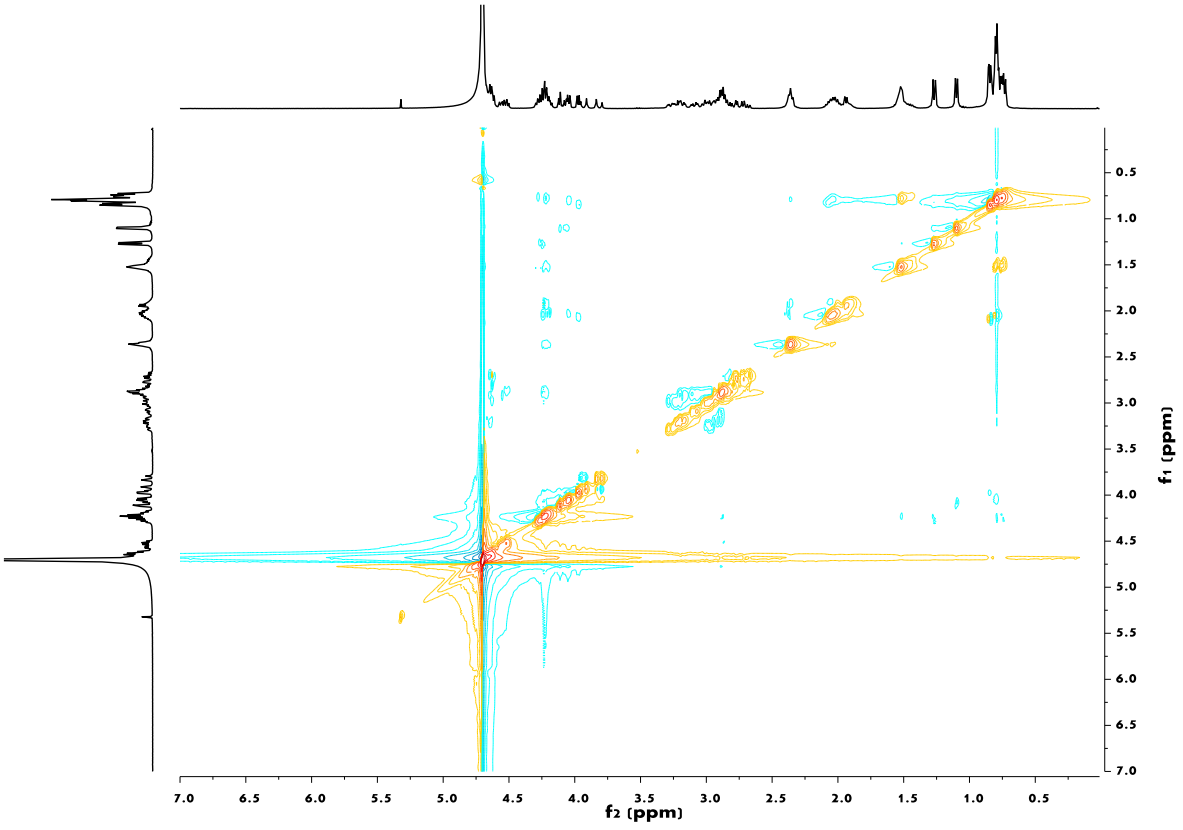
Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{1}H-^{1}H COSY (400 MHz, D_2O)



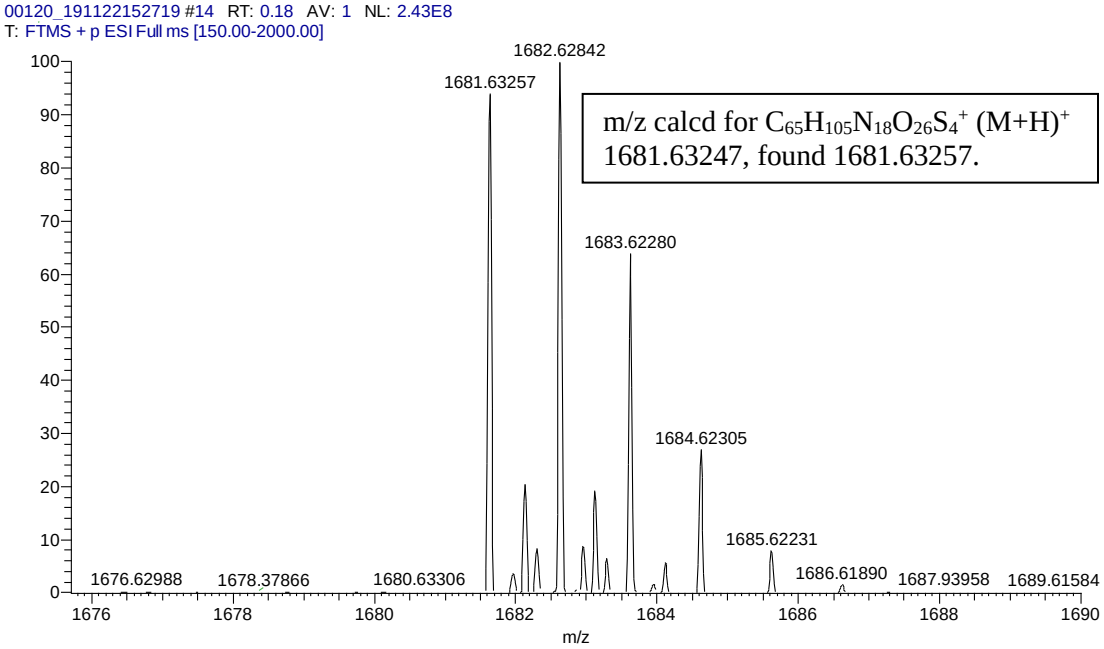
Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{1}H-^{1}H TCOSY (400 MHz, D_2O)



Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{1}H-^{1}H NOSEY (400 MHz, D_2O)

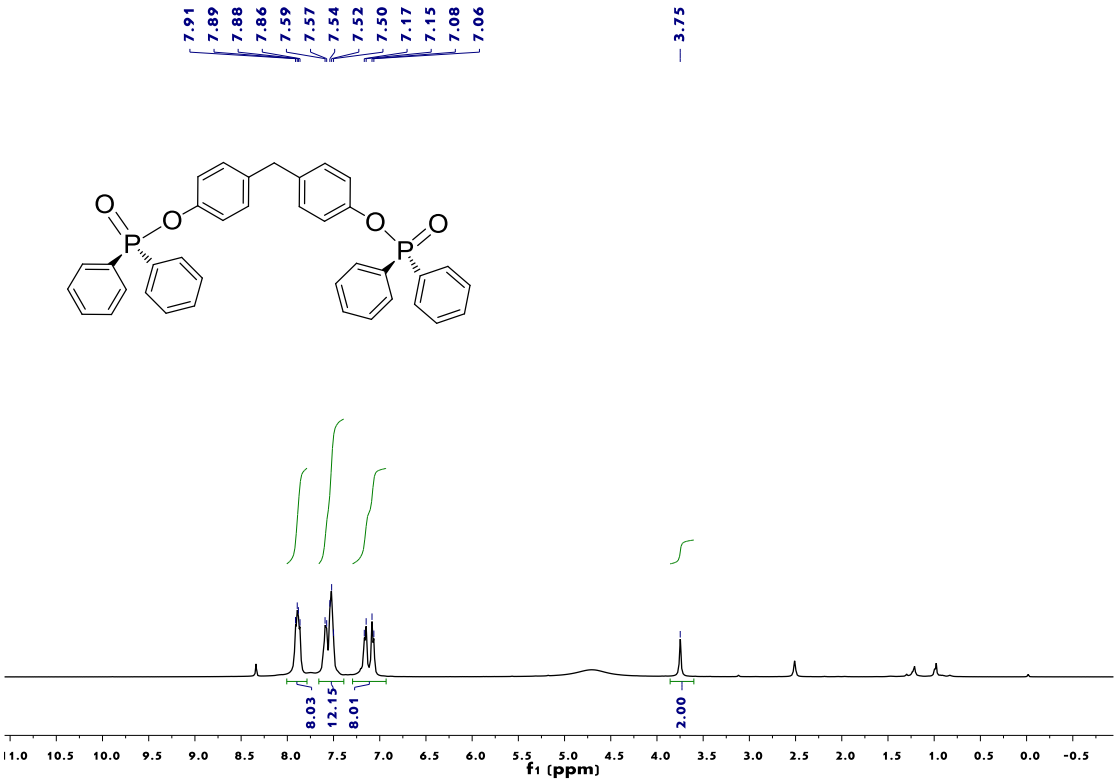


Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L ^{1}H-^{1}H ROSEY (400 MHz, D_2O)

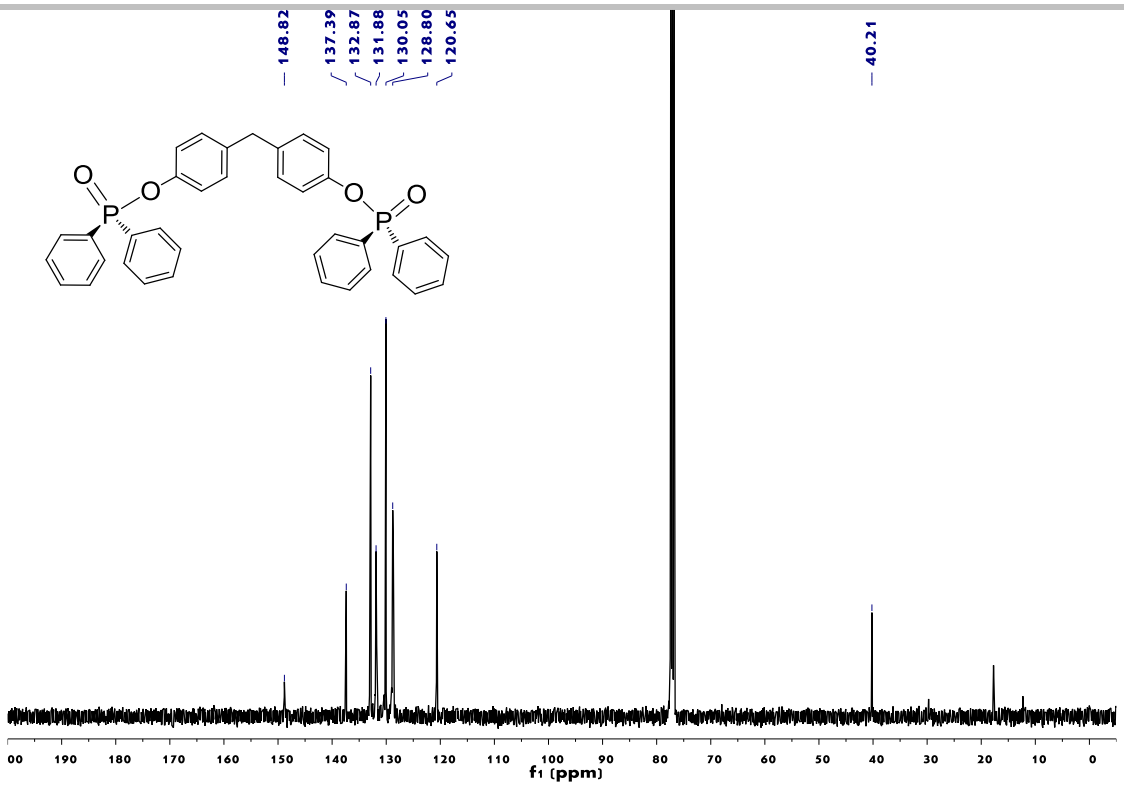


Plecanatide: NDEC[4,12]ELC[7,15]VNVAC[4,12]TGC[7,15]L

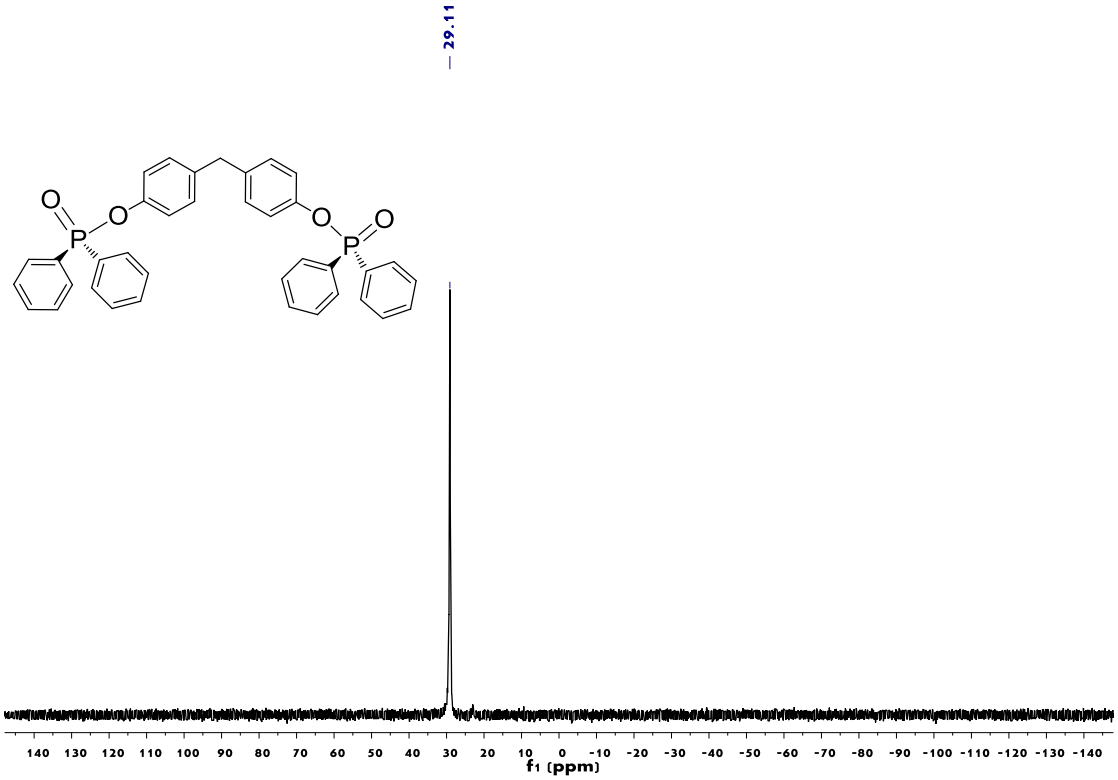
HRMS (ESI) m/z calcd for $C_{65}H_{105}N_{18}O_{26}S_4^+$ $(M+H)^+$ 1681.63247, found 1681.63257.



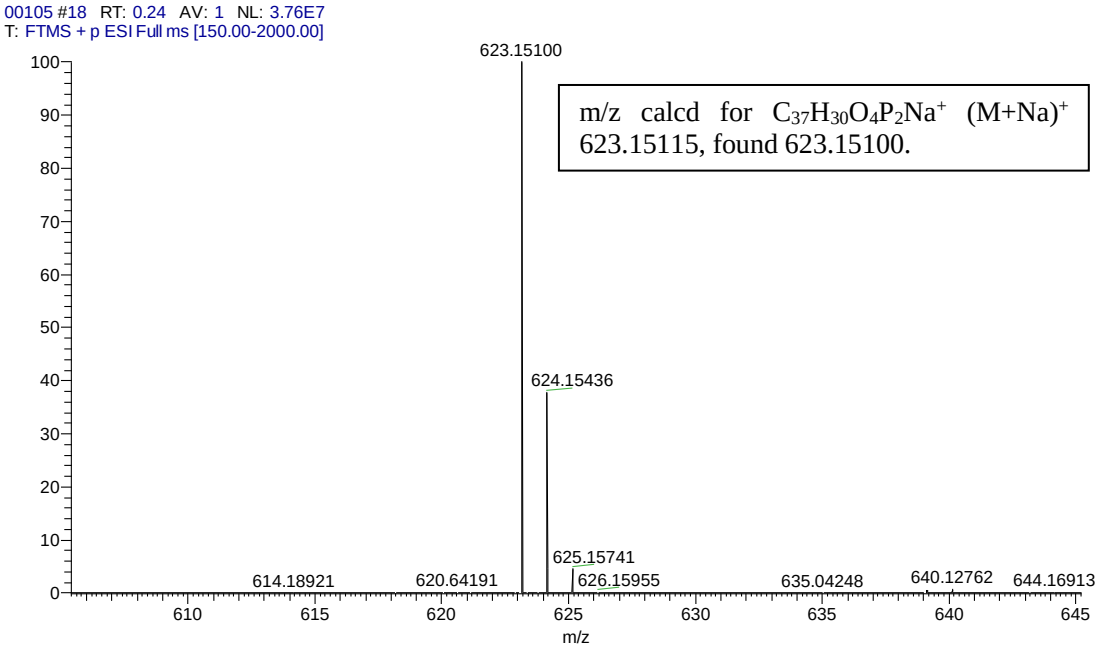
GPS5-Residue ^{1}H NMR (400 MHz, DMSO-d_6)



GPS5-Residue ^{13}C NMR (100 MHz, $CDCl_3$)



GPS5-Residue ^{31}P NMR (162 MHz, DMSO-d_6)



GPS5-Residue HRMS (ESI)