Supporting Information

A novel synthetic method for backbone-cyclized polypeptide POL7080 with the help of hydrophobic-support materials

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Contents

1 General remarks

^{1}H-NMR spectra were recorded on a Bruker Avance DRX600 (600MHz) spectrometer. Chemical shifts are given in parts per million (δ/ppm), downfield from tetramethylsilane (TMS) and are referenced to chloroform (7.26 ppm) as an internal standard. All coupling constants are absolute values and *J* values are expressed in Hertz (Hz). The spectra were analyzed according to first order. ^{13}C NMR spectra were recorded on a Bruker Avance DRX600 (100 MHz) spectrometer.

MS (ESI) (electrospray ionization mass spectrometry): API 4000 instrument (Applied Biosystems, Connecticut, USA). The molecular fragments are quoted as the relation between mass and charge (m/z), with the intensities being a percentage value relative to the intensity of the base signal (100%).

Routine monitoring of reactions was performed using 0.25-mm pre-coated silica GF254 plates, which were analyzed under UV light at 254 nm and fumigated by iodine vapor.

Solvent mixtures are understood as v/v. Solvents, reagents and chemicals were purchased from Sinopharm, Bidepharm, TCI and Tianjin Fuyu and were used without further purification unless stated otherwise.

2 General procedures

2.1 General Method for Diatomite-Mixing Washing

After the coupling or deprotection reaction was completed, the reaction mixture was concentrated under reduced pressure at room temperature. Then the concentrate was diluted with 20% H_2O in MeCN (400 mL) to give a precipitate. Next diatomite (8 g) was added to the reaction mixture and fully mixed. After filtered and washed with MeCN, the precipitate with diatomite was slurry-washed with THF or DCM (200 mL). After filtration, THF or DCM (20 mL) was added to the filtrate and the resultant solution could be used for the next step without further treatment.

2.2 General Method for Coupling

Amino-acid-Tag or peptide-Tag (1 equiv) was dissolved in a mixture of THF (54 mL) and DMF (9 mL). Fmoc-AA-OH (1.20 equiv), O-(1*H*-benzotriazol-1-yl)-*N, N, N′, N′*-tetramethyluronium hexafluorophosphate (HBTU, 1.20 equiv), 1-hydroxy-1*H*-benzotriazole (HOBt, 1.20 equiv), and *N, N*-diisopropylethylamine (DIPEA, 5.00 equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed. Then the reaction mixture was treated according to Method **2.1** to give a solution of the coupling product.

2.3 General Method for N-Fmoc Group Deprotection

Amino-acid-Tag or peptide-Tag (1 equiv) was dissolved in 1% (v/v) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of piperidine (1.50 equiv) in THF or DCM (60 mL). The reaction mixture was stirred at room temperature until the reaction was completed. After completion, 6 M HCl was added to the solution to adjust the pH value to 7.0. Then the reaction mixture was treated according to Method **2.1** to give a solution of the deprotected product.

3 Synthesis

3.1 Synthesis of NH_2-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe (Fragment 1)

3.1.1 Synthesis of Dipeptide fragment: Fmoc-Dab(Boc)-Dab(Boc)-OH

Tag-OH **2** (2.27 g, 3.00 mmol) was dissolved in CH_2Cl_2 (60 mL), and Fmoc-Dab(Trp)-OH (1.98g，4.5 mmol), DIC (567 mg, 4.50 mmol), and DMAP (73.2 mg, 0.600 mmol) were then added. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). The reaction mixture was treated according to Method **2.1** to give a solution to the product. The product solution was then subjected to consecutive coupling and deprotecting reactions according to Method **2.2** and **2.3** to give dipeptide fragment: Fmoc-Dab(Boc)-Dab(Boc)-O-Tag in 83.2% yield. This dipeptide fragment (3.44 g, 2.5 mmol) was then dissolved in a mixture of CH_2Cl_2, TFE, and TFA (90 mL, 100:10:1 (v/v)). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, the reaction solution was filtered and then purified by silica gel column chromatography.

ESI-MS (m/z): Calcd for $C_{33}H_{44}N_4O_9[M+H]^+$ 640.31，found 639.0

^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): δ 12.68 (s, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.74 (dt, *J* = 8.6, 4.1 Hz, 2H), 7.60–7.53 (m, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 2H), 6.76 (d, *J* = 5.2 Hz, 2H), 4.25 (dq, *J* = 21.5, 7.1 Hz, 4H), 4.08 (q, *J* = 7.7 Hz, 1H), 2.99 (dq, *J* = 55.7, 12.8, 9.7 Hz, 4H), 1.91–1.67 (m, 4H), 1.38 (d, *J* = 16.7 Hz, 18H).

^{13}C NMR (151 MHz, DMSO-d_6): δ 173.62, 172.18, 156.33, 155.99, 144.35, 141.16, 128.07, 127.51, 125.80, 120.52, 78.07 (d, *J* = 5.1 Hz), 66.24, 55.32, 52.81, 50.36, 47.15, 37.35 (d, *J* = 25.0 Hz), 32.83, 31.74, 28.71 (d, *J* = 8.5 Hz).

3.1.2 Synthesis of Alanine-Tag

Tag=O **1** (2.27 g, 3.00 mmol) was dissolved in toluene (30 mL) and H-Ala-OMe（1.25g，9 mmol), DMF (30 mL), glacial acetic acid (540 mg, 9.0 mmol), and $NaBH(OAc)_3$ (3.81 g, 18.0 mmol) were then added. The resulting reaction mixture was stirred at 60 °C until the completion of the reaction (determined by TLC). The resulting reaction mixture was filtrated and the filtrate was diluted with 20% H_2O in MeCN to give a precipitate. The precipitate was dissolved in CH_2Cl_2 and washed with sodium bicarbonate solution and brine. The organic phase was concentrated under reduced pressure, and the residue was finally purified by silica gel column chromatography.

ESI-MS (m/z): Calcd for $C_{55}H_{103}NO_4[M+H]^+$ 842.43，found 842.48

^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): δ 6.41 (d, *J* = 2.3 Hz, 1H), 6.38 (dd, *J* = 8.2, 2.3 Hz, 1H), 3.92 (dt, *J* = 12.8, 6.5 Hz, 4H), 3.74–3.63 (m, 5H), 1.83–1.72 (m, 4H), 1.44 (dq, *J* = 15.5, 7.8 Hz, 4H), 1.38–1.12 (m, 77H), 0.88 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (151 MHz, DMSO-d_6): δ 175.91, 159.66, 130.31, 120.30, 104.39, 99.76, 68.01 (d, *J* = 24.3 Hz), 55.85, 51.58, 47.17, 31.93, 29.75–29.58 (m), 29.45–29.25 (m), 26.14 (d, *J* = 19.8 Hz), 22.68, 18.96, 14.08.

3.1.3 Synthesis of Tripeptide-Tag Compound: NH_2-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe

Dipeptide fragment: Fmoc-Dab(Boc)-Dab(Boc)-OH (640 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 mL), and alanine-Tag (7.59 g, 9 mmol), HATU (1.14g，3mmol), HOAt (408 mg，3mmol) and DIPEA (645mg，5mmol) were then added. The resulting reaction mixture was stirred at 40 °C until 108 h. After being filtered, the filtrate was diluted with 20% H_2O in MeCN (200 mL) to give a precipitate. The precipitate was finally purified by silica gel column chromatography and then was treated according to Method **2.3** to give the Tripeptide-Tag: NH_2-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe (574 mg, 0.46 mmol) in 92.4% yield.

3.1.4 Synthesis of NH_2-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe (Fragment 1)

Tripeptide-Tag (574 mg, 0.46 mmol) was dissolved in CH_2Cl_2 (10 mL). Fmoc-Trp(Boc)-OH (316mg, 0.6 mmol),

HBTU (228mg, 0.6 mmol), HOBt (81mg, 0.6 mmol) and DIPEA (323g, 2.5mmol) were then added. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After being filtered, the filtrate was diluted with 20% H_2O in MeCN (200 mL) to give a precipitate. Then the precipitate was treated according to Method **2.3** to give Fragment 1 (597 mg, 0.4 mmol) an 89.8% yield.

ESI-MS (m/z): Calcd for $C_{89}H_{153}N_7O_{13}[M+H]^+$ 1528.15， found 1528.73

^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): δ 8.13 (s, 1H), 8.03 (d, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.48 (s, 1H), 7.33 (dt, *J* = 15.5, 7.3 Hz, 2H), 7.25 (d, *J* = 12.2 Hz, 3H), 7.09 (d, *J* = 8.3 Hz, 1H), 6.52–6.40 (m, 2H), 5.37–5.29 (m, 1H), 5.22 (s, 1H), 4.95 (t, *J* = 9.1 Hz, 1H), 4.46 (d, *J* = 9.6 Hz, 3H), 4.30 (dd, *J* = 13.9, 7.3 Hz, 1H), 3.93 (q, *J* = 6.5, 6.1 Hz, 4H), 3.79 (dd, *J* = 9.7, 3.9 Hz, 1H), 3.63 (d, *J* = 17.4 Hz, 3H), 3.41–3.28 (m, 3H), 2.91 (td, *J* = 14.3, 13.5, 8.2 Hz, 3H), 2.16–1.88 (m, 3H), 1.76 (h, *J* = 6.2, 5.3 Hz, 5H), 1.67 (s, 10H), 1.45 (s, 10H), 1.42 (s, 2H), 1.34 (s, 7H), 1.25 (s, 76H), 0.88 (t, *J* = 6.9 Hz, 8H).

^{13}C NMR (151 MHz, DMSO-d_6): δ 171.81, 129.87 (d, *J* = 117.3 Hz), 124.67, 124.23, 122.64, 119.24, 116.38, 115.33, 104.74, 99.67, 83.71, 79.38, 68.12, 54.78, 54.46, 52.12, 50.73, 45.42, 36.65 (d, *J* = 34.0 Hz), 33.19, 31.91, 30.44, 29.74–29.62 (m), 29.59, 29.45, 29.38, 29.33 (d, *J* = 2.3 Hz), 29.03, 28.47, 28.34, 28.22, 26.09, 26.02, 22.67, 14.12 (d, *J* = 11.7 Hz).

3.2 Synthesis of Fmoc-Ile-Dab(Boc)-Orn(Boc)-d-Dab(Boc)-Dab(Boc)-OH (Fragment 2)

Tag-OH **2** (2.27 g, 3.00 mmol) was dissolved in CH_2Cl_2 (60 mL), and then Fmoc-Dab(Trp)-OH (1.98 g, 4.5mmol), DIC (567 mg, 4.50 mmol), and DMAP (73.2 mg, 0.600 mmol) were added in turn. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). Then the reaction mixture was treated according to Method **2.1** to give a solution of the target product. The product solution was then subjected to the consecutive coupling and deprotecting reactions according to Method **2.2** and **2.3** to give Fragment 2-Tag (4.0 g, 2.1 mmol) in 70.3% yield in over thirteen steps. Fragment 2-Tag (5.05 g, 2.1 mmol) was then dissolved in a mixture of CH_2Cl_2, TFE, and TFA (60 mL, 100:10:0.8 (v/v)). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, the solution was filtered, and then DIPE was added to give Fragment 2 (2.34 g, 2.0 mmol) in 81.0% yield as a precipitate.

ESI-MS (m/z): Calcd for $C_{58}H_{89}N_9O_{16}[M-H]^-$ 1166.64, found 1166.45

^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): δ 8.16 (t, *J* = 9.1 Hz, 2H), 8.08–7.96 (m, 2H), 7.89 (s, 1H), 7.88 (s, 1H), 7.73 (dd, *J* = 12.8, 7.6 Hz, 2H), 7.41 (q, *J* = 5.8, 3.8 Hz, 3H), 7.32 (td, *J* = 7.5, 2.7 Hz, 2H), 6.70 (dt, *J* = 18.7, 5.8 Hz, 4H), 4.39–4.19 (m, 7H), 3.92 (t, *J* = 8.3 Hz, 1H), 3.02–2.87 (m, 8H), 2.51 (p, *J* = 1.8 Hz, 2H), 1.85 (dd, *J* = 35.4, 7.5 Hz, 1H), 1.72 (qd, *J* = 14.8, 14.4, 7.6 Hz, 2H), 1.67–1.55 (m, 2H), 1.37 (t, *J* = 6.1 Hz, 36H), 1.27 (dd, *J* = 9.7, 6.6 Hz, 10H), 1.12 (tq, *J* = 14.3, 7.7, 7.0 Hz, 1H), 0.82 (d, *J* = 7.2 Hz, 6H).

^{13}C NMR (151 MHz, DMSO-d_6): δ 173.52, 171.79 (d, *J* = 21.8 Hz), 171.52, 158.74 (d, *J* = 36.1 Hz), 156.46, 155.99 (d, *J* =10.3 Hz), 144.30 (d, *J* = 30.1 Hz), 141.17, 128.05, 127.48, 125.77, 120.49 (d, *J* = 3.4 Hz), 78.08, 77.85, 66.11, 59.47, 54.07, 52.91, 50.60, 50.25, 47.20, 42.29, 37.19, 36.95, 29.92, 28.70 (d, *J* = 4.8 Hz), 26.23, 24.91, 18.52, 17.19, 15.78, 12.80, 11.33.

3.3 Synthesis of Fmoc-Ser(tBu)-d-Pro-Pro-Thr(tBu)-Trp(Boc)-OH (Fragment 3)

Tag-OH **2** (2.27 g, 3.00 mmol) was dissolved in CH_2Cl_2 (60 mL), and then Fmoc-Trp(Boc)-OH (2.37 g, 4.50 mmol), DIC (567 mg, 4.50 mmol), and DMAP (73.2 mg, 0.600 mmol) were added in turn. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). Then the reaction mixture was treated according to Method **2.1** to give a solution of the target product. The product solution was then subjected to the consecutive coupling and deprotecting reactions according to Method **2.2** and

2.3 to give Fragment 3-Tag in 66.7% yield (3.56 g, 2.01 mmol). The Fragment 3-Tag was dissolved in a mixture of CH_2Cl_2, TFE, and TFA (60 mL, 100:10:1 (v/v)), and was stirred at room temperature until the reaction was completed (30 min). After the reaction suspension was filtered, and then DIPE was added to give Fragment 3 (1.60 g, 1.57 mmol) in 78.5% yield as a precipitate.

ESI-MS (m/z): Calcd for $C_{56}H_{72}N_6O_{12}[M-H]^-$ 1019.52，found 1019.20.

^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): δ 8.12 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.84 (d, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.46 (s, 1H), 7.42 (td, *J* = 7.5, 1.1 Hz, 2H), 7.35 (td, *J* = 7.5, 1.1 Hz, 2H), 7.28 (s, 1H), 7.19 (d, *J* = 7.4 Hz, 1H), 6.28 (s, 2H), 5.02–4.90 (m, 1H), 4.60–4.32 (m, 3H), 4.32–4.07 (m, 3H), 3.97–3.64 (m, 1H), 3.60–3.50 (m, 1H), 3.49–3.35 (m, 4H), 3.19 (s, 2H), 2.92–2.73 (m, 1H), 2.52 (p, *J* = 1.8 Hz, 1H), 2.05 (qd, *J* = 11.7, 10.9, 5.2 Hz, 2H), 1.85–1.72 (m, 2H), 1.61 (s, 9H), 1.25–1.19 (m, 2H), 1.16–0.94 (m, 23H).

^{13}C NMR (151 MHz, DMSO-d_6): δ 173.05 (d, *J* = 188.3 Hz), 149.55, 143.07, 139.90, 137.91, 135.05, 129.35, 127.71, 124.43, 123.97, 122.70, 121.80, 120.44, 119.90, 114.92, 110.04, 83.70, 74.20 (d, *J* = 3.0 Hz), 73.15 (d, *J* = 22.6 Hz), 67.33, 63.33, 60.12, 57.92 (d, *J* = 17.0 Hz), 53.90, 47.19 (d, *J* = 20.8 Hz), 45.50, 32.61, 29.86–28.95 (m), 28.66–28.08 (m), 27.92–27.34 (m), 25.14–24.57 (m), 22.64 (d, *J* = 31.6 Hz), 19.00.

3.4 Synthesis of POL7080

3.4.1 Synthesis of the linear Fmoc-protected-POL7080-Tag

Fragment 1 (0.5 mmol, 1 equiv) was dissolved in a mixture of THF (9 mL) and DMF (1 mL), and Fragment 2 (1.5 equiv), DMT-MM (1.5 equiv), and DIPEA (5.00 equiv) were then added. The reaction mixture was stirred at 40 °C until the reaction was completed (2 h). After completion, the reaction solution was diluted with 20% H_2O in MeCN to give a precipitate. Then the precipitate was dissolved in THF (10 mL), and piperidine (12.0 equiv) and DBU (3.00 equiv) were added. The reaction mixture was stirred at 40 °C until the reaction was completed (15 min). 6 M HCl was added to the reaction mixture to adjust the pH value to 7.0, and diluted with 20% H_2O in MeCN to give a precipitate. After subsequent couplings of Fragment 3 according to the method mentioned above, the linear Fmoc-protected-POL7080-Tag was given in 79.7% yield as a light-brown solid.

3.4.2 Synthesis of the linear protected peptide-tag precursor: NH_2-Ser(tBu)-d-Pro-Pro-Thr(tBu)-Trp(Boc)-Ile-Dab(Boc)-Orn(Boc)-d-Dab(Boc)-Dab(Boc)-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)-OH

The linear Fmoc-protected-POL7080-Tag (689 mg, 0.25 mmol) was then dissolved in THF (11.4 mL) and 1 M LiOH (8.6 mL). The reaction mixture was stirred at room temperature until the reaction was completed (10 h). 6 M HCl was added to the reaction mixture to adjust the pH value to 7.0, and was filtrated. The filtrate was diluted with 20% H_2O in MeCN to give a precipitate in 84% yield.

3.4.3 Synthesis of the cyclized-protected-POL7080-Tag

Cyclo-[Ser(tBu)-d-Pro-Pro-Thr(tBu)-Trp(Boc)-Ile-Dab(Boc)-Orn(Boc)-d-Dab(Boc)-Dab(Boc)-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)]

To a solution of the linear protected peptide-tag precursor (677 mg, 0.21 mmol) in 10% DMF in THF (350 mL), DMT-MM (277 mg, 1 mmol), and DIPEA (1.55 g, 4 mmol) were added. The resulting reaction mixture was stirred at 40 °C until the reaction was completed (30 min). After being filtered, the filtrate was diluted with 20% H_2O in MeCN to give a precipitate of 71.4% yield.

3.4.4 Synthesis of POL7080

The cyclized-protected-POL7080-Tag (480 mg, 0.15 mmol) was dissolved in 2.5% TIS and 2.5% H_2O in TFA (10 mL). The reaction mixture was stirred at room temperature until the reaction was completed (2 h). After completion, the reaction suspension was filtered, and then DIPE at -20 ^oC was added to the filtrate to give POL7080 quantitatively (218 mg, 0.140 mmol) in 93.6% yield, which was further purified by RP-HPLC using H_2O-MeCN.

ESI-MS (m/z): Calcd for $C_{77}H_{112}N_{22}O_{16}[M+2H]^{2+}$ 777.92, found 777.62.

4 In vitro antibacterial assay

The MIC values of the test compounds were determined in a 96-well microplate using the standard broth microdilution procedures recommended by CLSI. Bacterial strains were incubated on Mueller Hinton Agar (MHA) medium at 37 °C for 20 h and then log-phase bacteria were suspended in 20 mL sterile water to make a 0.5 McFarland standard inoculum. The standard inoculum was diluted 10 times for further use. The experimental bacteria strains included *B. subtilis* ATCC9372, *S. aureus* ATCC25923, *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *P. aeruginosa* PAO1. To be specific, the test compounds and control drugs were prepared in sterile Mueller Hinton (MH) broth or LB medium by using a 2-fold dilution method with concentrations ranging from 128 to 0.25 μg/mL. Then 10 μL of diluted standard inoculum (1.5×10^7 CFU/mL) was added to the above solutions and 96-well plates were incubated aerobically for 20 h at 37 °C. After 20 h, turbidity was observed and the last well with no growth of bacteria was recorded to represent the MIC value of the test compound, expressed in μg/mL.

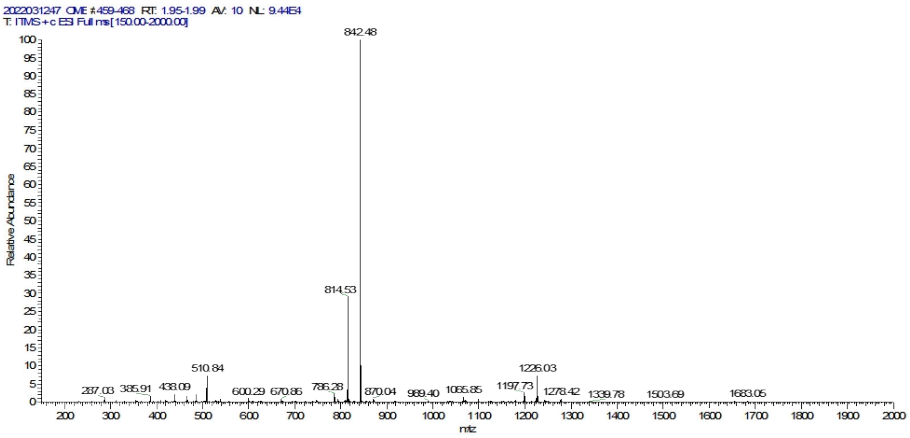
Table S1. POL7080 with its minimum inhibitory concentration against Gram-positive and negative strains.

Compounds	MIC: μg/mL				
	[a]*P. aeruginosa* POA1	[b]*E. coli* ATCC25922	[c]*P. aeruginosa* ATCC27853	[d]*S. aureus* ATCC25923	[e]*B. subtilis* ATCC9372
POL7080$_{sample}$	0.25	≥128	0.25	128	64
CIP[f]	≤0.25	≤0.25	1	≤0.25	0.5
VAN[g]	>128	128	>128	0.5	1

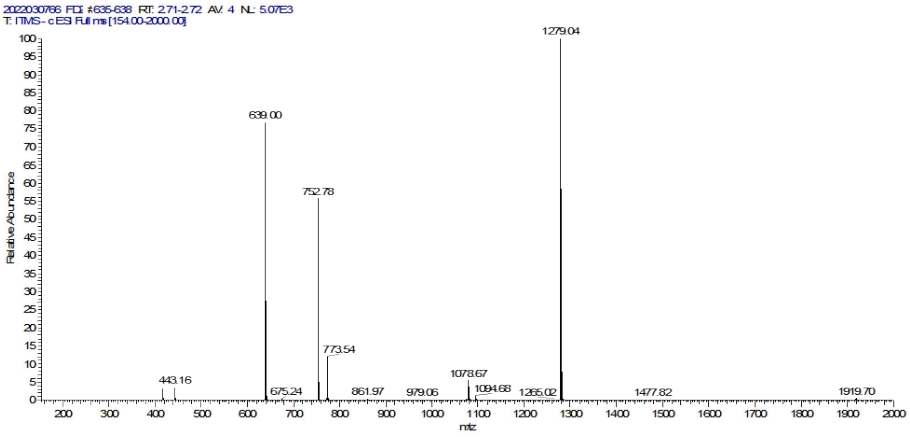
[a]*P. aeruginosa PAO1*: *Pseudomonas aeruginosa* CGMCC 1.12483, wild type strain;
[b]*E. coli* ATCC25922: *Escherichia coli* ATCC2592, penicillin-susceptible strain;
[c]*P. aeruginosa* ATCC27853: *Pseudomonas aeruginosa* ATCC27853, penicillin-susceptible strain, not characterized.
[d]*S. aureus* ATCC25923: *Staphylococcus aureus* ATCC25923, erythromycin-susceptible strain;
[e]*B. subtilis* ATCC9372: *Bacillus subtilis* ATCC9372, penicillin-susceptible strain;
[f]CIP: ciprofloxacin (Adamas life, China); [g]VAN: vancomycin(Meryer, China).

5 Mass Spectra

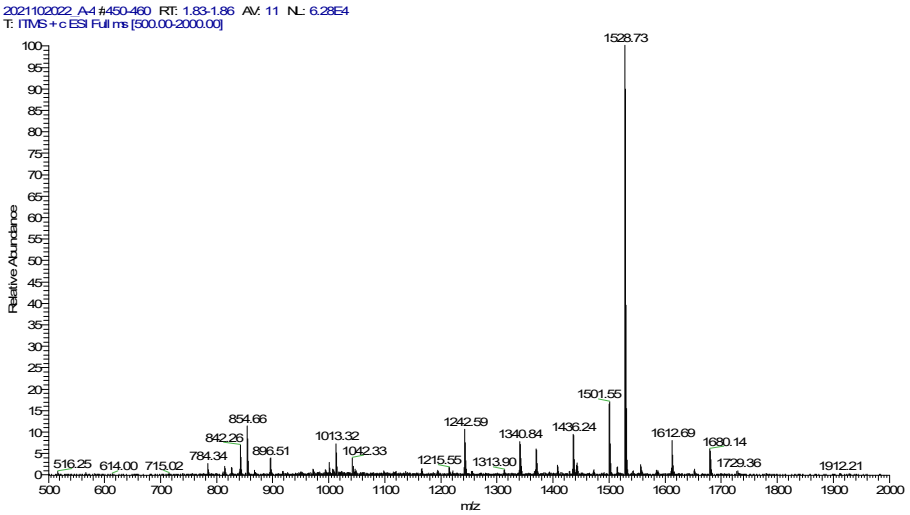
5.1 Alanine-Tag



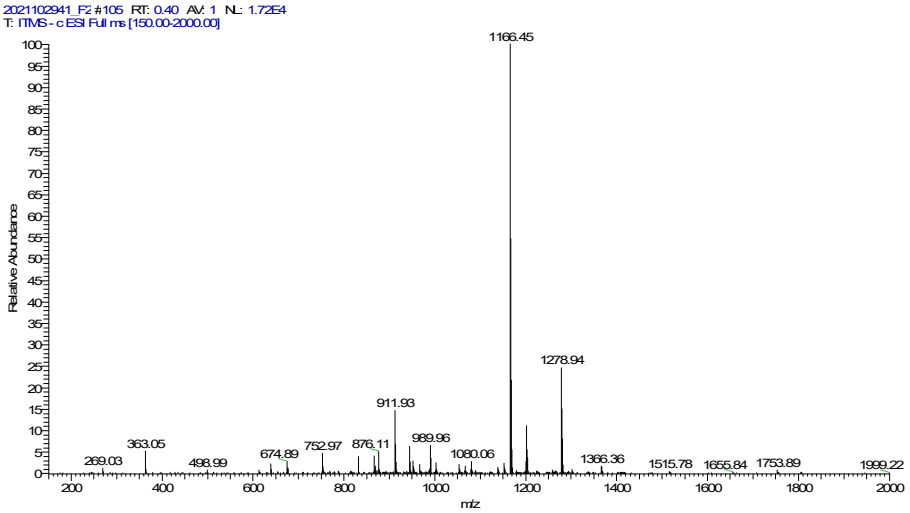
5.2 Dipeptide fragment: Fmoc-Dab(Boc)-Dab(Boc)-OH



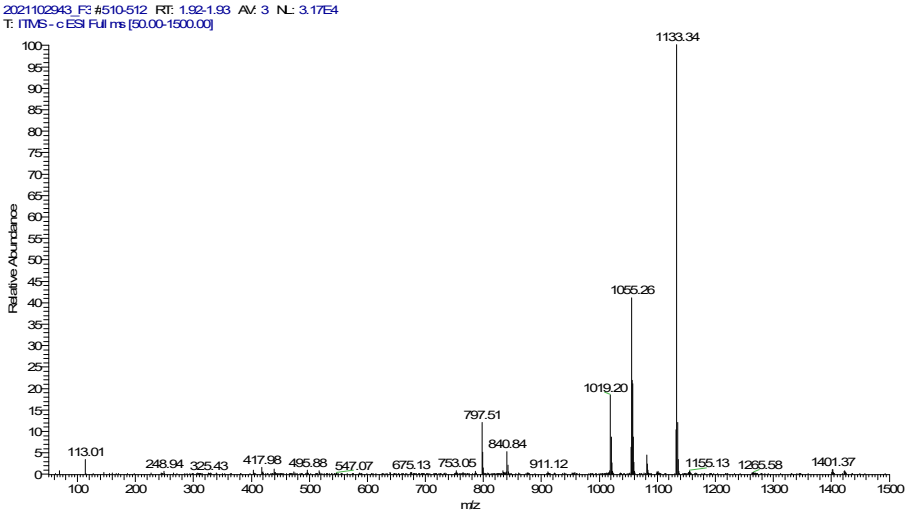
5.3 NH_2-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe (Fragment 1)



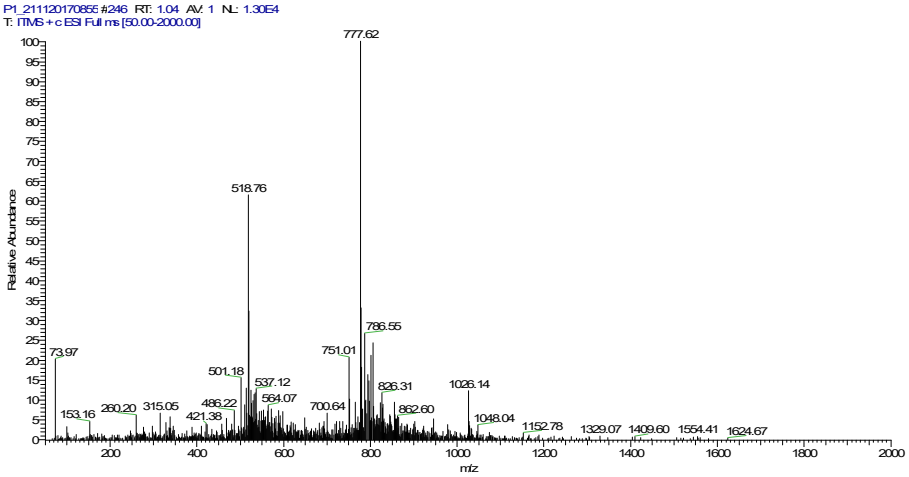
5.4 Fmoc-Ile-Dab(Boc)-Orn(Boc)-d-Dab(Boc)-Dab(Boc)-OH (Fragment 2)



5.5 Fmoc-Ser(tBu)-d-Pro-Pro-Thr(tBu)-Trp(Boc)-OH (Fragment 3)

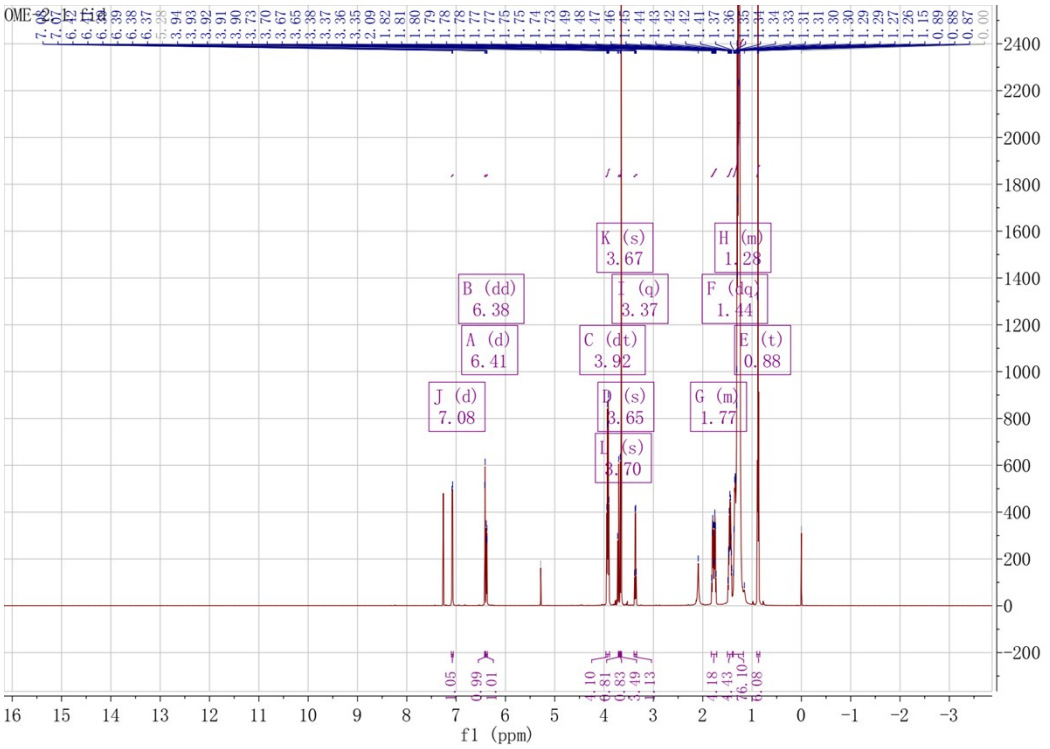


5.6 POL7080

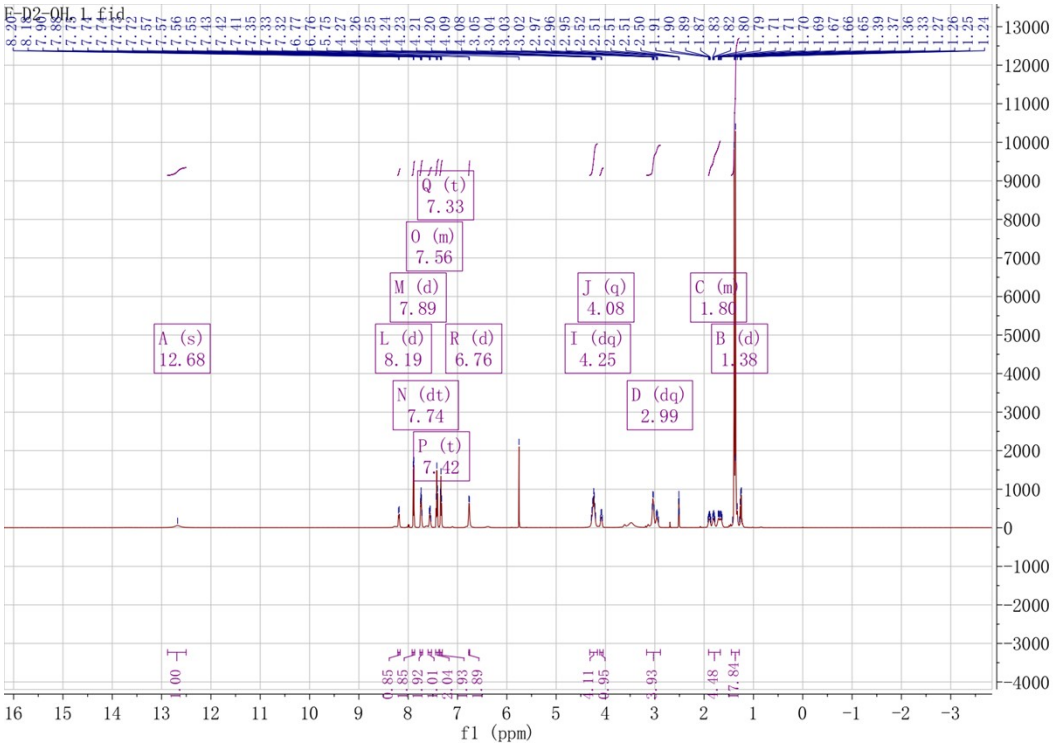


6 ^{1}H NMR Spectra

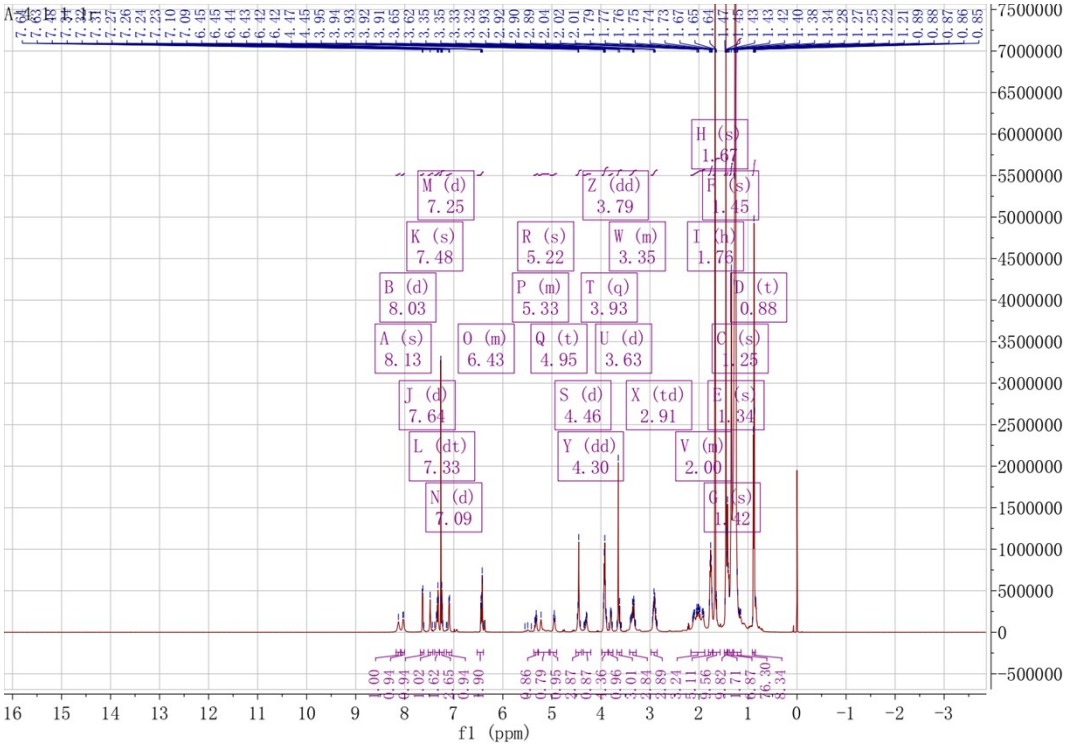
6.1 Alanine-Tag compound



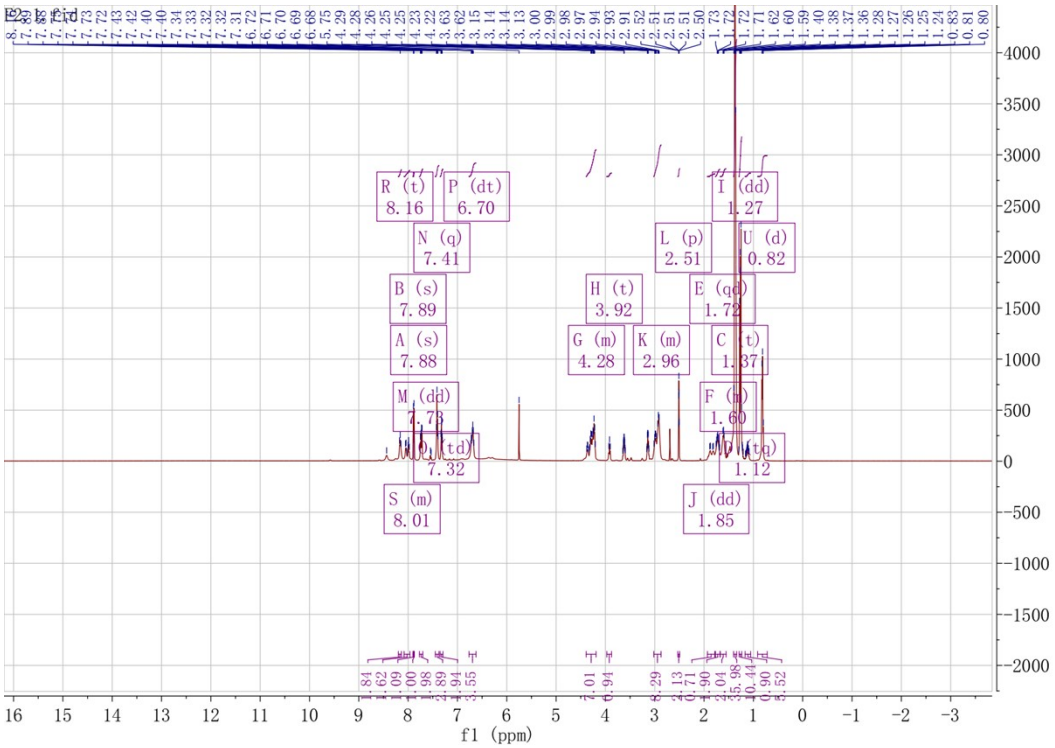
6.2 Dipeptide fragment: Fmoc-Dab(Boc)-Dab(Boc)-OH



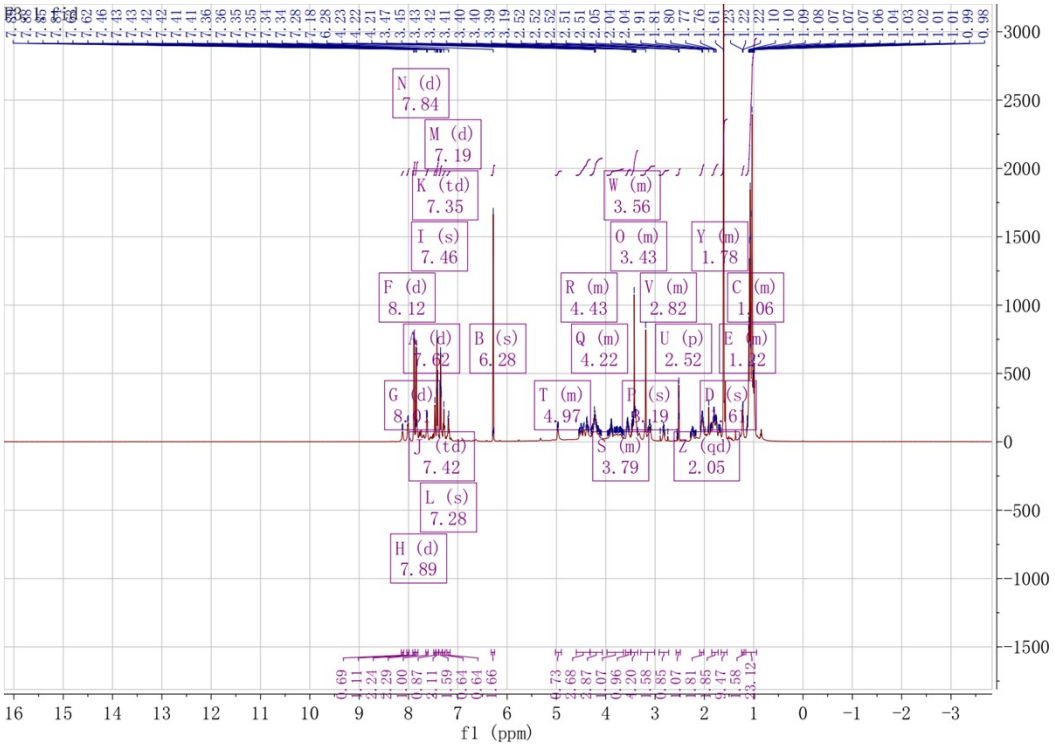
6.3 NH_2-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe (Fragment 1)



6.4 Fmoc-Ile-Dab(Boc)-Orn(Boc)-d-Dab(Boc)-Dab(Boc)-OH (Fragment 2)

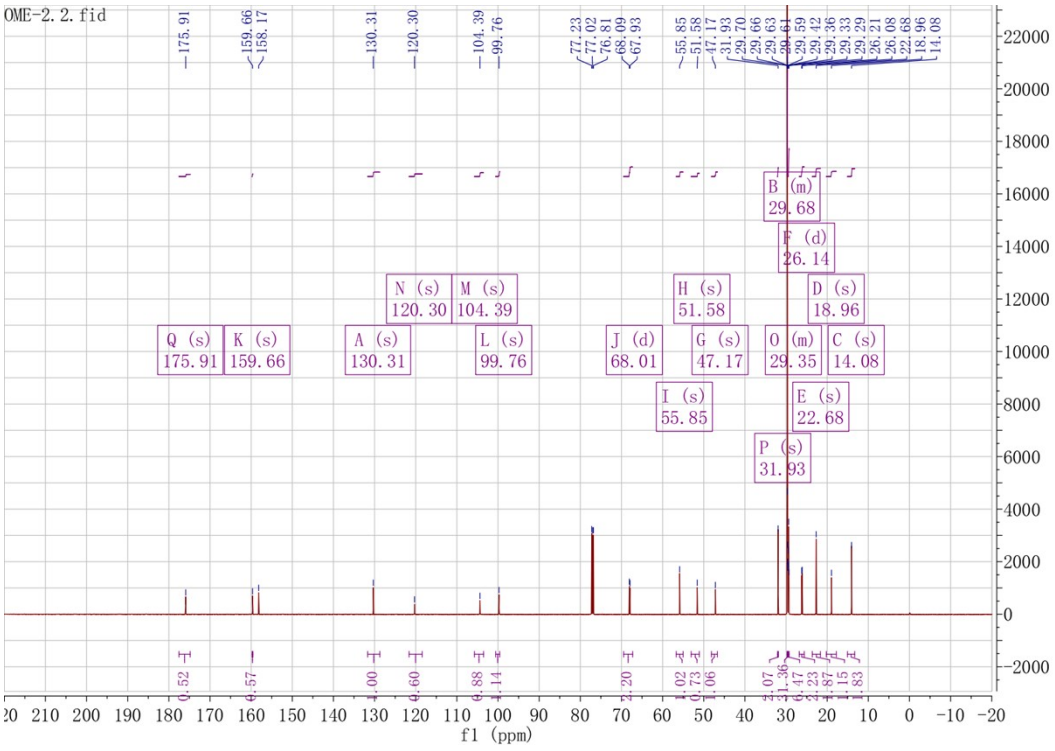


6.5Fmoc-Ser(tBu)-d-Pro-Pro-Thr(tBu)-Trp(Boc)-OH (Fragment 3)

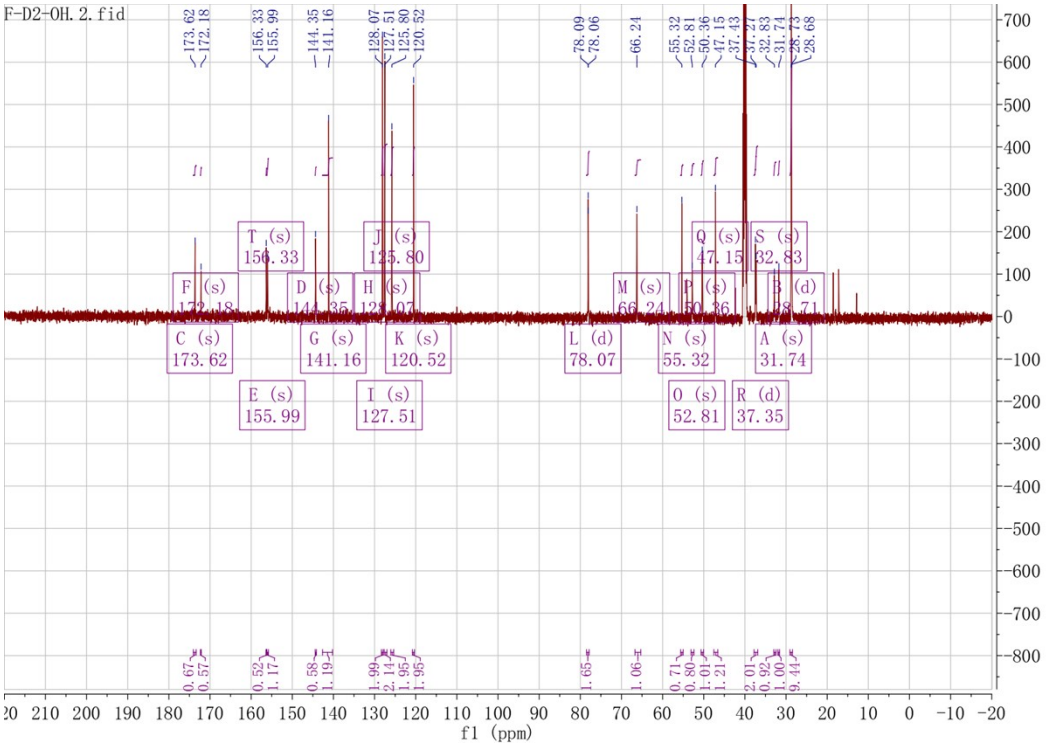


7 ^{13}C NMR Spectra

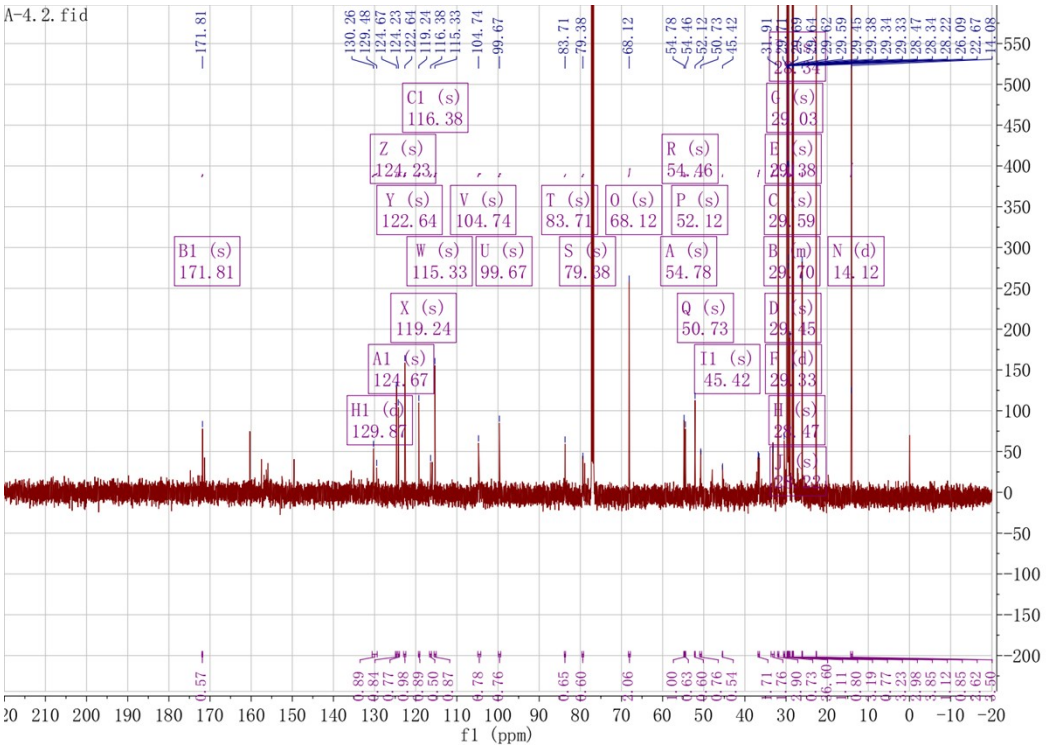
7.1 Alanine-Tag compound



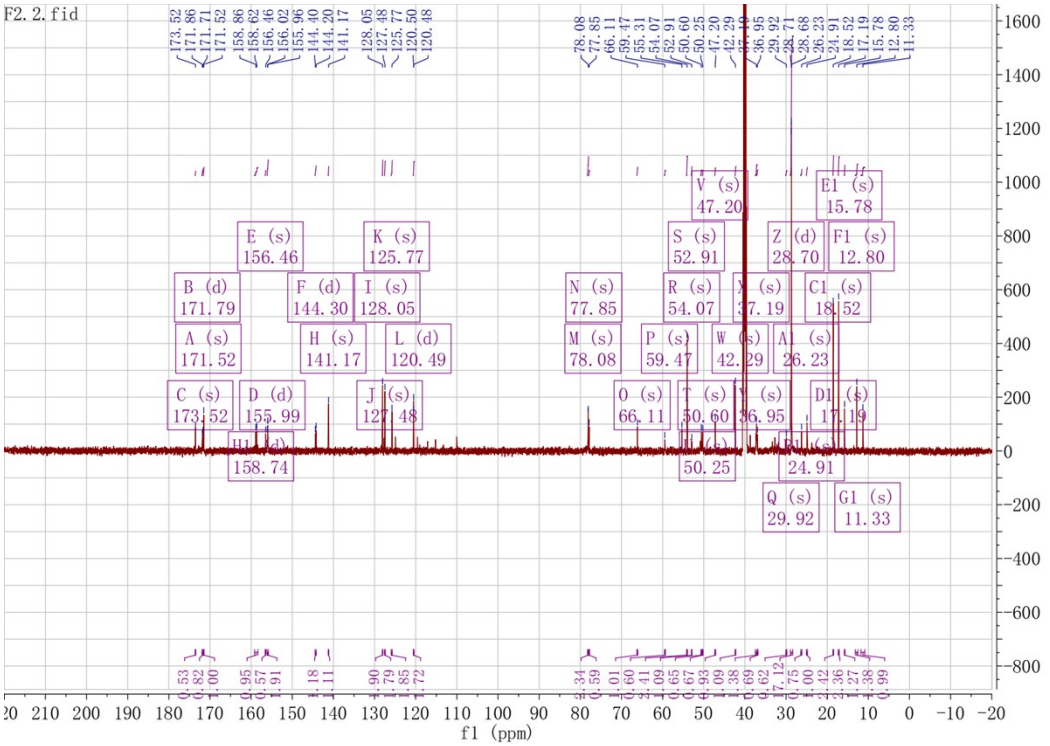
7.2 Dipeptide fragment: Fmoc-Dab(Boc)-Dab(Boc)-OH



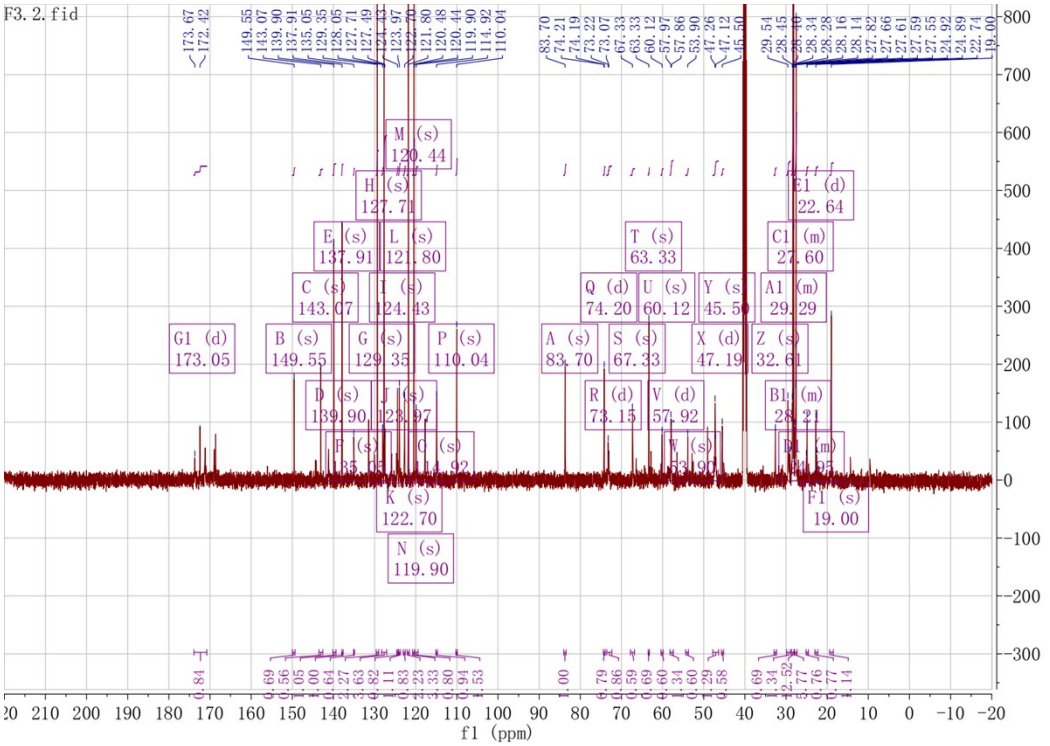
7.3 NH_2-Trp(Boc)-Dab(Boc)-Dab(Boc)-Ala(Tag)-OMe (Fragment 1)



7.4 Fmoc-Ile-Dab(Boc)-Orn(Boc)-d-Dab(Boc)-Dab(Boc)-OH (Fragment 2)

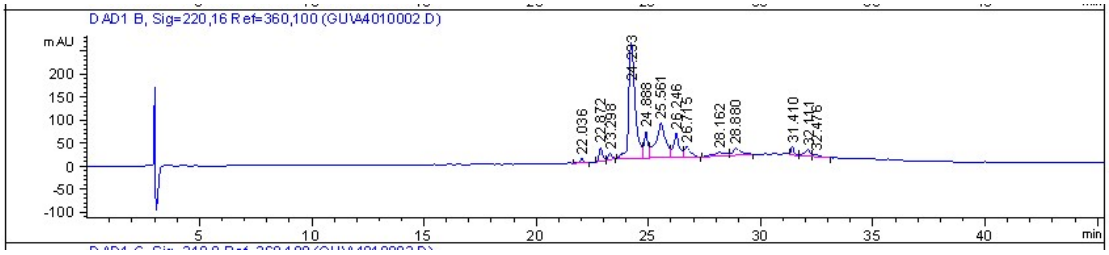


7.5 Fmoc-Ser(tBu)-d-Pro-Pro-Thr(tBu)-Trp(Boc)-OH (Fragment 3)



8 HPLC Chromatograms

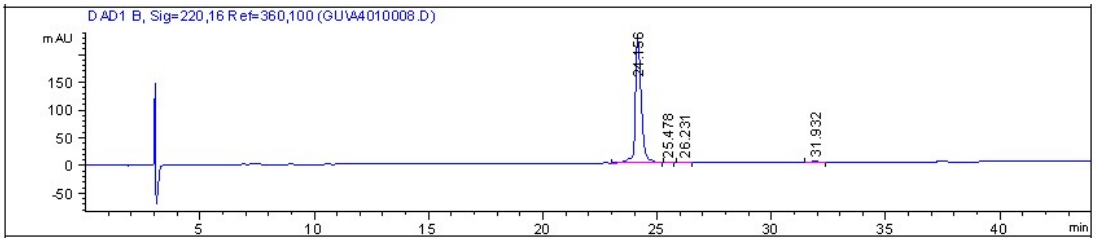
8.1 POL7080 (Crude product)



Signal 2: DAD1 B, Sig=220,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	22.036	BB	0.1626	99.87595	9.50220	1.4010
2	22.872	BV	0.1674	326.42609	29.90014	4.5790
3	23.298	VB	0.1661	153.61734	13.98832	2.1549
4	24.233	MM	0.2915	4141.89795	236.77815	58.1011
5	24.888	MM	0.1123	233.92746	34.71904	3.2815
6	25.561	MM	0.3203	1026.42749	53.40821	14.3984
7	26.246	MM	0.1793	403.40512	37.50291	5.6588
8	26.721	MM	0.2340	195.41948	13.92096	2.7413
9	28.881	MM	0.2163	100.60927	7.75088	1.4113
10	31.410	VV	0.1370	179.92319	19.25148	2.5239
11	32.111	VV	0.2493	267.24866	15.18539	3.7489
Totals :				7128.77799	471.90769	

8.2 POL7080



Signal 2: DAD1 B, Sig=220,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	24.156	BB	0.2805	4072.24878	221.48596	98.0036
2	25.478	BB	0.1963	15.11040	1.15760	0.3637
3	26.231	BB	0.2039	20.00856	1.34247	0.4815
4	31.932	BB	0.2227	47.83363	3.08865	1.1512
Totals :				4155.20137	227.07467	