Supporting Information

Chemoselective Nitrile Oxide-Alkyne 1,3-Dipolar Cycloaddition Reactions from Nitroalkane Tethered Peptides

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1. Materials and Methods:

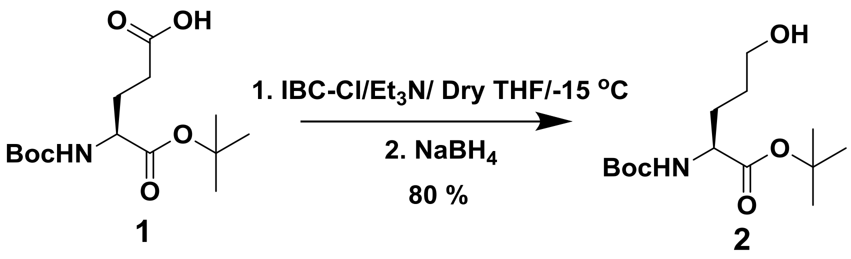
All the amino acids, phenylacetylene, activated Pd/C, triphenylphosphine, imidazole, solid iodine, pyridine, isovaleric acid, Rink amide resin, HBTU, HOBt, EDC.HCl, TFA, DIPEA, IBC-Cl, Cbz-Cl, triethylamine, sodium nitrite, $CuSO_4.5H_2O$, sodium ascorbate and solvents were obtained from the commercial sources. For all nitrile oxide-alkyne cycloaddition reaction dry THF (dried over sodium) was used. MeOH was distilled before use. Ethylacetate/hexane or MeOH/DCM solvent systems were used to run TLC. Column chromatography was performed on silica gel (120-200 mesh). Final peptides were purified by reverse phase HPLC (C_{18} column, MeOH/H_2O 70:30-95:5 as a gradient with flow rate 2.0 mL/min). ^1H NMR and ^{13}C NMR spectra were recorded on 400 MHz and 100 MHz respectively using the residual solvent signal as internal standards ($CDCl_3$). Chemical shifts (δ) reported in parts per million (ppm) and coupling constants (J) reported in Hz. Mass of pure peptides was confirmed by MALDI/TOF.

2. Synthesis of Nitro Amino Acid 4:

Synthesis of the compound 2 (*tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-5-hydroxypentanoate:

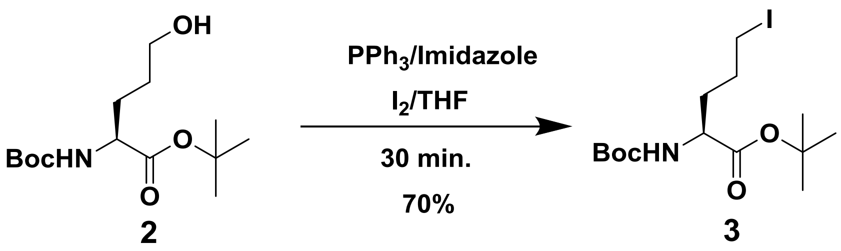
Commercially available BocNH-Glu (COOH)-O^{t}Bu (4.54 g, 15 mmol) was dissolved in 30 mL dry THF. Then the reaction mixture was cooled to -15 $^\circ$C by salt ice combination and triethylamine (2.06 mL, 15 mmol) was added to this. After 5 minutes, isobutyl chloroformate (3 mL, 22.5 mmol) was added drop wise under nitrogen atmosphere and the reaction mixture was stirred for about 30 minutes. Then, $NaBH_4$ (2.85 g, 75 mmol) was added to the reaction mixture and stirred for another 30 minutes. The progress of the reaction was monitored by TLC. After the completion of the reaction (~30 minutes), THF solvent was evaporated under reduced pressure and the excess $NaBH_4$ was quenched using 10% HCl solution. After that, the reaction mixture was extracted with ethyl acetate (3 × 50 mL). Then the organic layer was washed with 10% HCl solution (3 × 50 mL), 10% Na_2CO_3 (3 × 50 mL), brine solution (3 × 50 mL) and dried over anhydrous Na_2SO_4. The crude compound was purified through column chromatography in EA/hexane solvent system to get pure compound **2**. Yield: 3.4 g (80%).

$[\alpha]_D^{25}$ ($CHCl_3$, c 1.0): +9.6; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 5.20 (d, *J* = 8 Hz, 1H), 4.20-4.12 (m, 1H), 3.63 (t, *J* = 8Hz, 2H), 2.49 (bs, 1H), 1.87 – 1.80 (m, 1H), 1.72-1.54 (m, 3H), 1.43 (s, 9H), 1.41 (s, 9H). **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 172.07, 155.67, 82.03, 79.84, 62.12, 53.72, 29.71, 28.41, 28.32, 28.07. **HRMS** m/z calculated value for $C_{14}H_{27}NO_5$ is [M+Na$^+$] 312.1786 and observed 312.1793.



Synthesis of compound 3 (*tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-5-iodopentanoate):

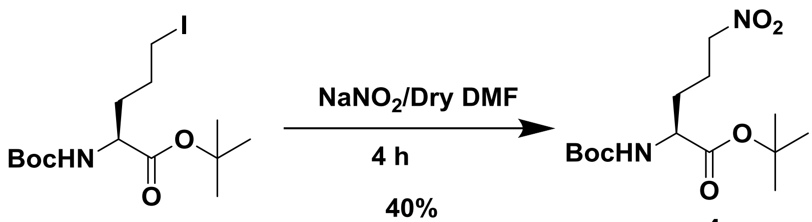
Compound **2** (2.9 g, 10 mmol) was dissolved in 30 mL dry THF under nitrogen atmosphere. Then triphenyl phosphine (3.93 g, 15 mmol), imidazole (1.00 g, 15 mmol) and solid iodine (3.70 g, 15 mmol) were added respectively. The progress of the reaction was monitored by TLC. After completion of the reaction, THF solvent was evaporated under reduced pressure and the crude product was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with 10% $Na_2S_2O_3$ solution (3 × 50 mL) and brine solution (3 × 50 mL). Then the organic layer was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified through silica gel column chromatography using EA/hexane solvent system to get pure compound **3**. Yield: 2.8 g (70%).



Synthesis of the compound 4 (*tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-5-nitropentanoate):

Compound **3** (4.00 g, 10 mmol) was dissolved in 5 mL dry DMF. To the solution, $NaNO_2$ (1.7 g, 25 mmol) was added and the reaction mixture was stirred for about 4 h. The completion of the reaction was monitored by TLC. Then DMF was evaporated, and the compound was dissolved in ethyl acetate. The organic layer was washed with 10% $Na_2S_2O_3$ solution (3 × 50 mL), brine solution (3 × 50 mL) and dried over anhydrous Na_2SO_4. Then the combined organic layer was evaporated under reduced pressure. The crude compound was purified through silica gel column chromatography using EA/hexane solvent system to get pure compound **4**. Yield: 1.27 g (40%).

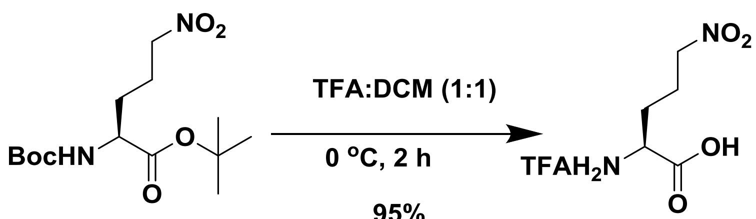
mp (^{0}C): 53-55; $[\alpha]_D^{25}$ ($CHCl_3$, c 1.0): +11.6; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 5.13 (d, *J* = 8 Hz, 1H), 4.42 (t, *J* = 8Hz, 2H), 4.24 – 4.19 (m, 1H), 2.11 – 2.02 (m, 2H), 1.95 – 1.86 (m, 1H), 1.76 – 1.66 (m, 1H), 1.47 (s, 9H), 1.44 (s, 9H). **^{13}C** NMR (100 MHz, Chloroform-*d*) δ 171.18, 155.52, 82.76, 80.17, 75.05, 53.17, 29.97, 28.44, 28.10, 23.29. **HRMS** m/z calculated value for $C_{14}H_{26}N_2O_6$ is [M+Na$^+$] 341.1688 and observed 341.1690.



3 4

Synthesis of the compound 5 ((*S*)-2-amino-5-nitropentanoic acid):

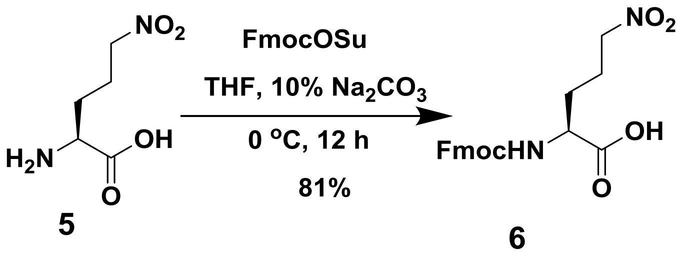
Compound 4 (1.00 g, 3.14 mmol) was dissolved in 5 mL DCM. To this solution 5 mL trifluoroacetic acid was added at 0 °C. The completion of the reaction was monitored by TLC. After completion of the reaction (~2 h), the trifluoroacetic acid was coevaporated 5 times with DCM under reduced pressure. Then the compound 5 was precipitated by adding cold diethyl ether. Yield: 486 mg (95%).



4 5

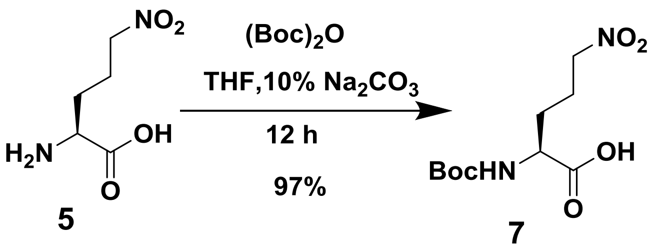
Synthesis of the compound 6 ((*S*)-2-((((9H-fluoren-9-yl)methoxy) carbonyl)amino)-5-nitropentanoic acid):

Compound **5** (1.00 g, 6.17 mmol) was dissolved in 30 mL THF and the solution was cooled to 0 °C. To this solution 30 mL of 10% Na_2CO_3 was added followed by FmocOSu (2.00 g, 6 mmol). Then the reaction mixture was stirred for about 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, THF solvent was evaporated under reduced pressure and the reaction mixture was acidified with 10% HCl solution. Then the reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with brine solution (3 × 50 mL). Then the organic layer was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to get compound **6**. Yield: 1.92 g (81%).



Synthesis of the compound 7 ((*S*)-2-((*tert*-butoxycarbonyl)amino)-5-nitropentanoic acid):

Compound **5** (1.00 g, 6.17 mmol) was dissolved in 30 mL THF. To this solution, 30 mL of 10% Na_2CO_3 was added followed by Boc anhydride (1.45 mL, 6.17 mmol). Then the reaction mixture was stirred for about 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, THF solvent was evaporated under reduced pressure and the reaction mixture was acidified with 10% HCl solution. Then the reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with brine solution (3 × 50 mL). Then the organic layer was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to get compound **7**. Yield: 1.70 g (97%).



3. Crystal structure analysis of Nitro Amino Acid (4):

Crystals of Nitro Amino Acid **4** were grown by slow evaporation from a solution of aqueous methanol. A single crystal (0.1 × 0.05 × 0.03 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100K temperature on a Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å), ω-scans (2θ = 56.80), for a total of 84907 independent reflections. Space group P 21, a = 9.289(4), b = 20.701(8), c = 10.602(6), β = 115.928(10), V = 1833.5(15) $Å^3$, monoclinic, Z = 4 for chemical formula C_{14} H_{26} N_2 O_6, with two molecules in asymmetric unit; ρcalcd = 1.153 gcm^{-3} , μ = 0.090 mm^{-1} , F

(000) = 688. The structure was obtained by direct methods using SHELXS-97. The final R value was 0.0713 (wR2 = 0.1463) 9147 observed reflections (F0 ≥ 4σ (|F0|)) and 409 variables, S = 0.910. The largest difference peak and hole were 0.276 and -0.310eÅ^3, respectively.

4. ORTEP Diagram of Nitro Amino Acid (4):

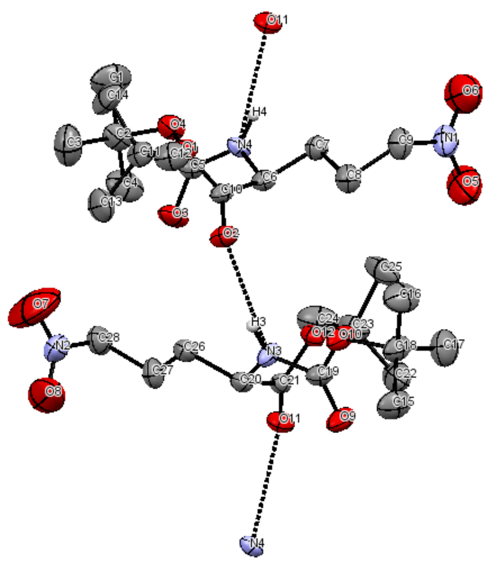


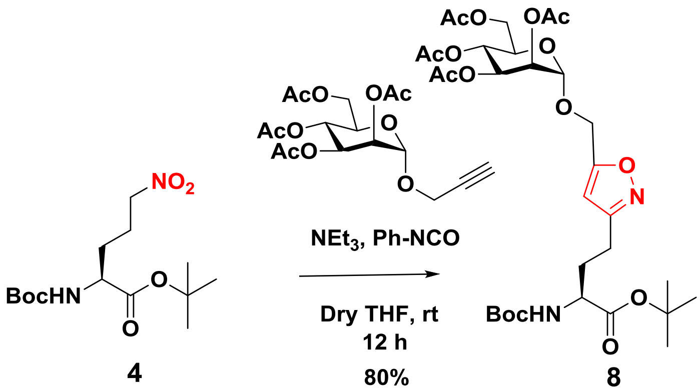
Figure S1: ORTEP diagram of compound **4**. H-bonds are shown in dotted lines. H-atoms are omitted for clarity. Ellipsoids are drawn at 50% probability. (CCDC No 1548954)

5. 1,3-Dipolar Cycloaddition Reaction with *in situ* generated nitrile oxide form Nitro Amino Acid 4:

Synthesis of compound **8** ((*2R,3R,4S,5S,6R*)-2-(acetoxymethyl)-6-((3-((*S*)-4-(*tert*-butoxy)-3-((*tert*-butoxycarbonyl)amino)-4-oxobutyl)isoxazol-5-yl)methoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate):

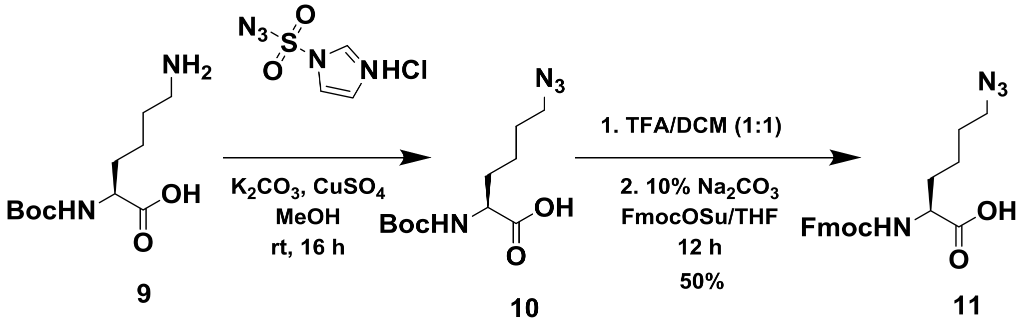
To the solution of compound **4** (0.47 g, 1.5 mmol) in dry THF (2 mL), propargylated mannose (2.80 g, 7.5 mmol) and phenyl isocyanate (0.81 mL, 7.5 mmol) were added at 0 $^\circ$C. Triethylamine (1.04 mL, 7.5 mmol) was added to the above solution drop wise at room temperature and the reaction mixture was stirred for about 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, urea (by-product) was filtered through celite using EtOAc (50 mL). The filtrate was washed with 5% HCl (3 × 20 mL), 10% Na_2CO_3 (3 × 20 mL), brine solution (3 × 20 mL) and dried over anhydrous Na_2SO_4. The crude product was purified through silica gel chromatography using EA/hexane solvent system to get pure compound **8**. Yield: 0.82 g (80%).

$[\alpha]_D^{25}$ ($CHCl_3$, c 1.0): +37.8; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 6.16 (s, 1H), 5.28 – 5.23 (m, 4H), 5.15 (d, *J* = 8 Hz, 1H), 4.90 (s, 1H), 4.70 (d, *J* = 12 Hz, 1H), 4.61 (d, *J* = 12 Hz, 1H), 4.25 (dd, *J* = 8Hz, 4Hz, 2H), 4.09 – 3.99 (m, 3H), 2.78 – 2.64 (m, 2H), 2.12 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.44 (s, 9H), 1.41 (s, 9H). **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 171.33, 170.65, 169.99, 169.88, 169.74, 167.23, 163.10, 155.48, 103.73, 97.33, 82.37, 79.89, 69.28, 69.08, 68.89, 65.94, 62.31, 60.11, 53.66, 31.46, 28.37, 28.05, 22.38, 20.88, 20.78, 20.73, 20.69. **HRMS** m/z calculated value for $C_{31}H_{46}N_2O_{15}$ is $[M+H^+]$ 687.2976 and observed 687.2991



6. Synthesis of Fmoc-Lys(N_3)-OH (N2-(((9*H*-fluoren-9-yl) methoxy) carbonyl)-N6-diazo-L-lysine, Compound 11):

Fmoc-Lys (N_3)-OH was synthesised using reported protocol.[1] Briefly, the Boc-protected L-lysine (2.50 g, 10.15 mmol) was dissolved in MeOH (50 mL). To this solution K_2CO_3 (2.23 g, 18.09 mmol), copper(II) sulfate pentahydrate (253 mg, 1.01 mmol) and imidazole-1-sulfonyl azide HCl salt (3.18 g, 15.17 mmol) were added. After stirring for about 16 h at room temperature, half of the solvent was evaporated under *vacuum* before the solution was acidified with 2M HCl. The reaction mixture was extracted with DCM (3×100 mL), and the organic layer was dried over anhydrous $MgSO_4$ and concentrated under *vacuum*. The crude product was then dissolved in DCM (100 mL) and extracted with 5% aq. $NaHCO_3$ (3×100 mL). The combined aqueous layers were washed twice with DCM (100 mL), acidified with 1M HCl and extracted with DCM (3×100 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *under* reduced pressure to afford the product as a colour less oil. The Boc protected L-Lys (N_3)-OH was used for the solution phase peptide synthesis. For the solid phase peptide synthesis, the Boc group was deprotected using 1:1 TFA/DCM, and it was further protected with Fmoc group and purified through silica gel column chromatography to get the final Fmoc protected L-Lys(N_3)-OH. Yield: 2.00 g (50%). **HRMS** m/z calculated value for $C_{21}H_{22}N_4O_4$ is [M+Na$^+$] 417.1538 and observed 417.1534.



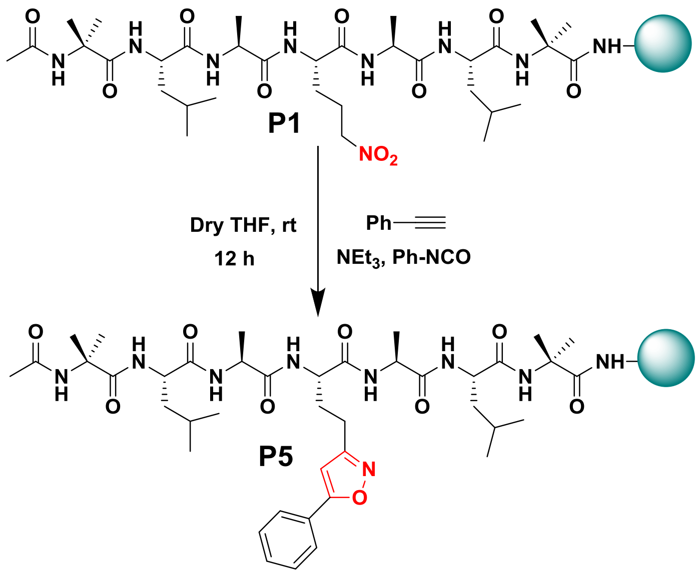
7. 1, 3-Dipolar Cycloaddition Reaction with Peptide P1 on Resin:

Solid phase synthesis of the heptapeptide (P1):

The heptapeptide was synthesized in 0.2 mmole scale on Rink amide resin by standard solid phase synthesis protocol. The coupling reactions were performed by HOBt/HBTU and DIEA combination in NMP solvent. The Fmoc group deprotection was carried out using 20% piperidine in DMF. After synthesis of the peptide, the *N*-terminus amine was protected using acetic anhydride in pyridine. Then the resin was used for further reactions without purification. **MALDI TOF/TOF-** *m/z* calculated for crude compound $C_{33}H_{59}N_9O_{10}$ [M+Na$^+$] 764.42 and observed 764.08.

Transformation of P1 to P5 through 1, 3-dipolar cycloaddition reaction on resin:

Resin (25 mg, 0.015 mmol) was taken in a 10 mL RB flask. To this 2 mL of dry THF was added under N_2 atmosphere. Then it was cooled to 0 $^{\circ}$C. After that phenylacetylene (110 µL, 1 mmol) was added to the reaction, followed by triethylamine (139 µL, 1 mmol), and phenyl isocyanate (112 µL, 1 mmol). The reaction mixture was stirred for about 12 h. After completion of the reaction (monitored by MALDI-TOF mass), the resin was filtered through sintered funnel. Then the peptide was cleaved from the resin using cocktail mixture of TFA/TIPS/Phenol (90:5:5). After completion of the reaction (~2h), the resin was filtered through sintered funnel and the filtrate was evaporated under reduced pressure and precipitated by adding cold diethyl ether. The precipitate was dissolved in MeOH and purified by reverse phase HPLC on a C_{18} column using MeOH/H_2O gradient system starting from 70% MeOH and 30% H_2O and reached 95% MeOH in 35 min. **MALDI TOF/TOF-** *m/z* calculated for $C_{41}H_{63}N_9O_9$ [M+Na$^+$] 848.46 and observed 848.45.



8. Orthogonal 1,3-Dipolar Cycloaddition Reaction of Peptide P2 on Resin:

Solid phase synthesis of the heptapeptide (P2):

The heptapeptide was synthesized in 0.2 mmol scale on Rink Amide Resin by standard solid phase synthesis protocol. The coupling reactions were performed using HOBt/HBTU and DIEA combination in NMP solvent. The Fmoc group was deprotected by 20% piperidine in DMF. After the synthesis, the free amine of the peptide was protected by isovaleric acid. Then the resin was used for further reactions without purification. **MALDI TOF/TOF-** *m/z* calculated for crude compound $C_{44}H_{80}N_{12}O_{10}$ [M+Na$^+$] 959.60 and observed 959.81.

Transformation of P2 to P6 through 1, 3-dipolar cycloaddition reaction on resin:

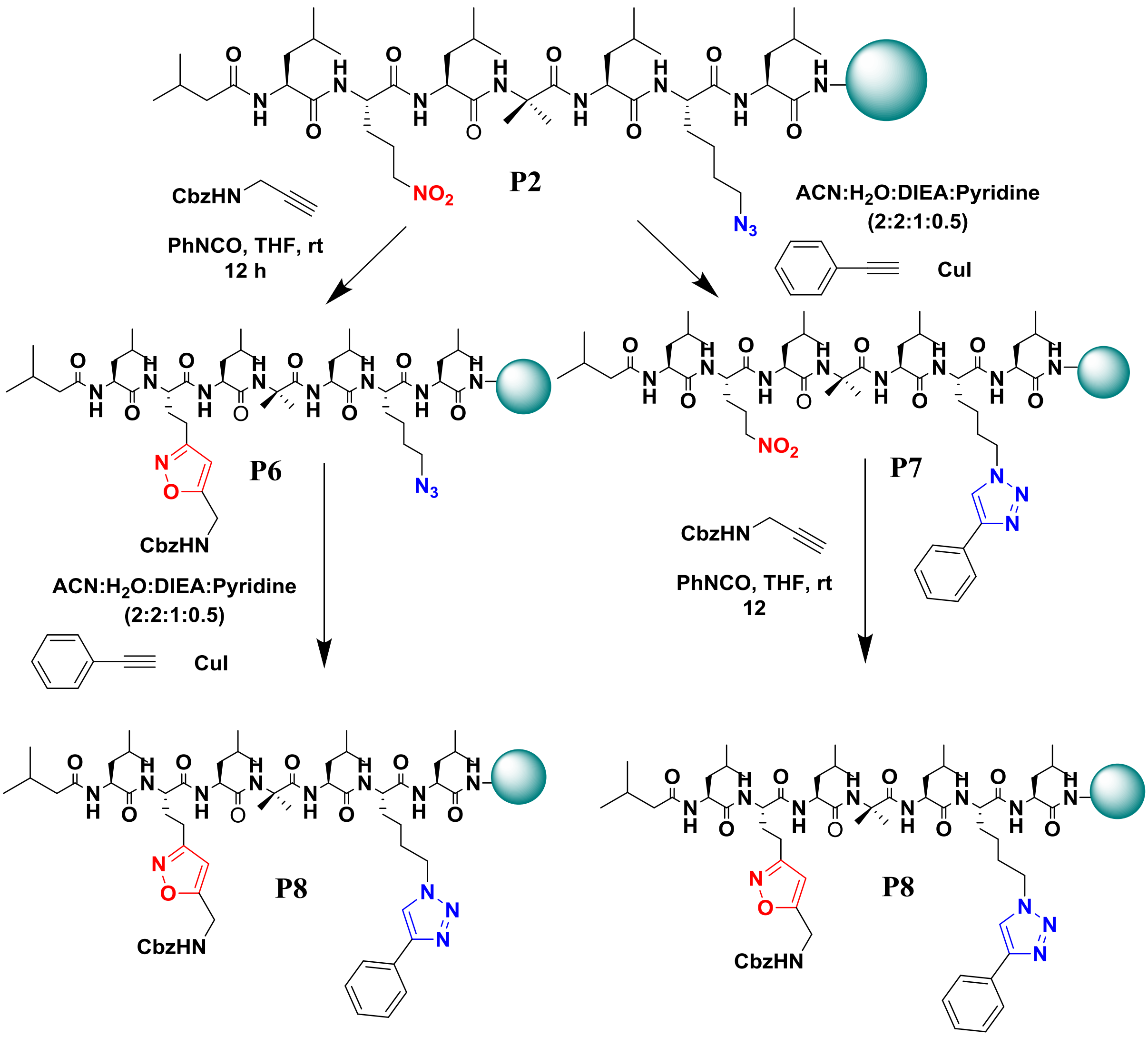
Resin (25 mg, 0.015 mmol) was taken in a 10 mL RB flask. To this 3 mL dry THF was added under N_2 atmosphere. Then reaction mixture was cooled to 0 $^{\circ}$C. After that Cbz protected propargylamine (189 mg, 1mmol) was added, followed by triethylamine (139 µL, 1 mmol), and phenyl isocyanate (112 µL, 1 mmol). Then the reaction mixture was stirred for about 12 h. After completion of the reaction (monitored by MALDI-TOF mass), the resin was filtered through sintered funnel. Then the peptide was cleaved from the resin by a cocktail mixture of TFA/TIPS/Phenol (90:5:5). After completion of the reaction (~2 h), the resin was filtered through sintered funnel and the filtrate was evaporated under reduced pressure and precipitated by adding cold diethyl ether. The precipitate was dissolved in MeOH and purified by reverse phase HPLC on a C_{18} column using MeOH/H_2O gradient system starting from 70% MeOH and 30% H_2O and reached 95% MeOH in 50 min.**MALDI TOF/TOF-** *m/z* calculated for $C_{55}H_{89}N_{13}O_{11}$ [M+K$^+$] 1146.64 and observed 1144.53.

Transformation of P2 to P7 through CuAAC on resin:

The resin of protected heptapeptide **P2** (25 mg, 0.015 mmol) was suspended in 2 mL of acetonitrile/ water/ DIEA/ pyridine (4:4:1:0.5) mixture. To this solution, phenylacetylene (109.8 μL, 1 mmol) was added, followed by a catalytic amount of CuI. The reaction mixture was stirred overnight at room temperature; the solution was filtered and washed with 5% HCl, an excess of DMF and dichloromethane. The peptide was cleaved from the resin using a cocktail mixture of TFA/TIPS/Phenol (90:5:5) and purified by reverse HPLC using a C_{18} column using MeOH/H_2O gradient system starting from 70% MeOH and 30% H_2O and reached 95% MeOH in 35 min. **MALDI TOF/TOF-** *m/z* calculated for $C_{52}H_{86}N_{12}O_{10}$ $[M+Na^+]$ 1061.65 and observed 1061.40.

Cycloaddition reaction between nitrile oxide –alkyne in the presence of triazole (P8):

Resin of peptide **P7** (25 mg, 0.015 mmol) was taken in a 10 mL RB flask. To this 2 mL dry THF was added under N_2 atmosphere. Then it was cooled to 0 $^{\circ}$C. After that Cbz protected propargylamine (189 mg, 1mmol) was added, followed by triethylamine (139 µL, 1 mmol), and phenyl isocyanate (112 µL, 1 mmol). Then the reaction mixture was stirred for about 12 h. After completion of the reaction (monitored by MALDI-TOF mass), the resin was filtered through sintered funnel. Then the peptide was cleaved from the resin by a cocktail mixture of TFA/TIPS/Phenol (90:5:5). After completion of the reaction (~2 h), the resin was filtered through sintered funnel and the filtrate was evaporated under reduced pressure and precipitated by adding cold diethyl ether. The precipitate was dissolved in MeOH and purified by reverse phase HPLC on a C_{18} column using MeOH/H_2O gradient system starting from 70% MeOH and 30% H_2O and reached 95% MeOH in 35 min. **MALDI TOF/TOF-** *m/z* calculated for $C_{52}H_{83}F_5N_8O_{11}$ $[M+Na^+]$ 1232.71 and observed 1237.35.



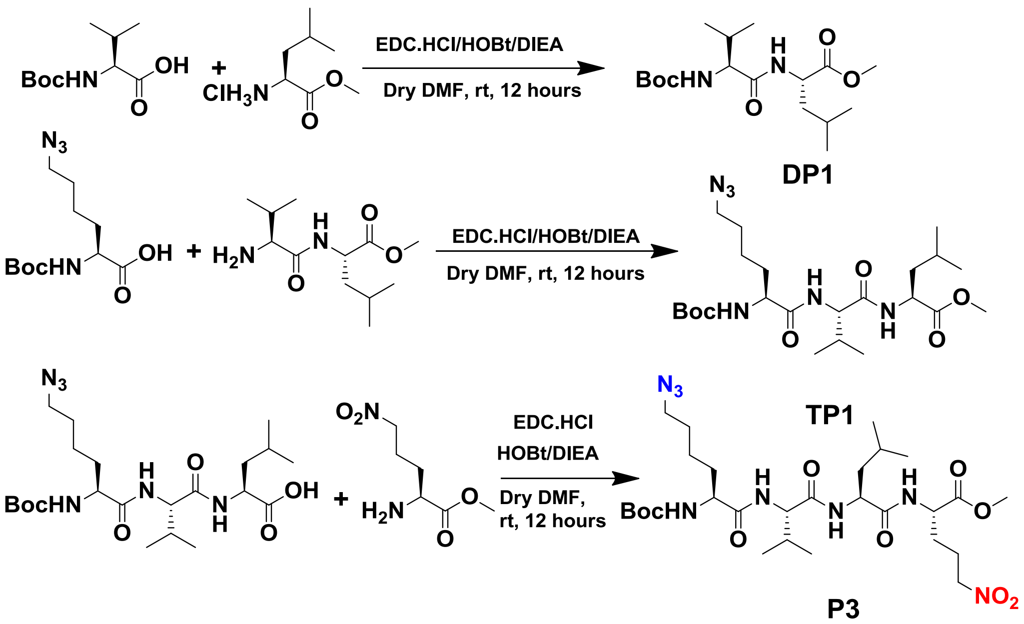
9. Orthogonal 1,3-dipolar cycloaddition reactions on Peptide P3 in Solution:

Synthesis of the tetrapeptide (P3):

The tetrapeptide **P3** was synthesized by the 1+2+1 strategy. First, the Boc-protected valine was coupled with the methyl ester of leucine using EDC.HCl/HOBt as a coupling reagent and DIEA as a base. Then, the Boc group of dipeptide BocNH-Val-Leu-OMe (**DP1)** was deprotected by TFA/DCM at 0 °C, and coupled with BocNH-Lys(N_3)-OH. After that, the tripeptide BocNH-Lys (N_3)-Val-Leu-OMe (**TP1**) was hydrolysed with 1*N* NaOH in MeOH and coupled with methyl ester of compound **4** by EDC.HCl/HOBt as a coupling reagent and

DIEA as a base in dry DMF to get the **P3**. The crude tetrapeptide **P3** was purified through silica gel column chromatography using EA/hexane solvent system.

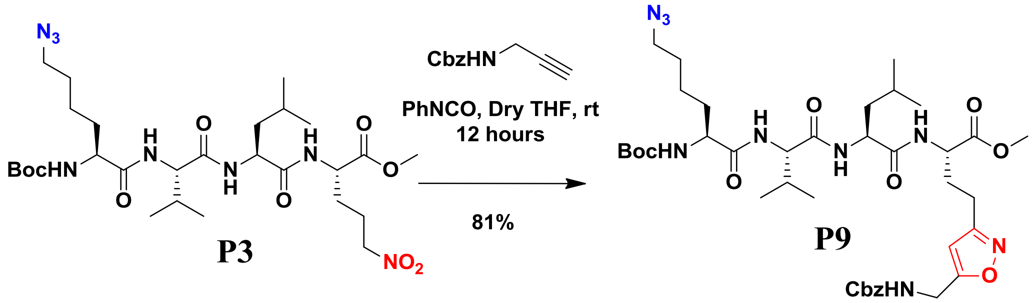
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (d, *J* = 8 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 6.59 (d, *J* = 8 Hz, 1H), 4.98 (d, *J* = 8 Hz, 1H), 4.60-4.53 (m, 2H), 4.41 (t, *J* = 6.9 Hz, 2H), 4.19 (dd, *J* = 6.8, 4.5 Hz, 1H), 3.94-3.90 (m, 1H), 3.74 (s, 3H), 3.31 (t, *J* = 6.6 Hz, 2H), 2.40-2.33(m, 1H), 2.07 – 1.94 (m, 2H), 1.88 – 1.79 (m, 2H), 1.66-1.60 (m, 9H), 1.45 (s, 9H), 0.99 (d, *J* = 8 Hz, 2H), 0.93-0.89 (m, 10H). **MALDI TOF/TOF**- *m/z* calculated for $C_{28}H_{50}N_8O_9$ [M+K$^+$] 681.33 and observed 681.10.



Cycloaddition reaction with *in situ* generated nitrile oxide in solution: Synthesis of P9 from P3

The tetrapeptide **P3 (**160 mg, 0.25 mmol) was dissolved in dry THF under N_2 atmosphere and cooled to 0 °C. To this solution, Cbz protected propargylamine (236 mg, 1.25 mmol) was added, followed by phenyl isocyanate (135 µL, 1.25 mmol) and triethylamine (173 µL, 1.25 mmol). The reaction mixture was stirred for about 12 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the urea was filtered through filter paper, and the solvent THF was evaporated under reduced pressure. The crude product was dissolved in MeOH, and the compound was purified by reverse phase HPLC on a C_{18} column using MeOH/H_2O gradient system starting from 70% MeOH and 30% water and reached 95% MeOH in 35 min.   Yield: 164 mg (81%).

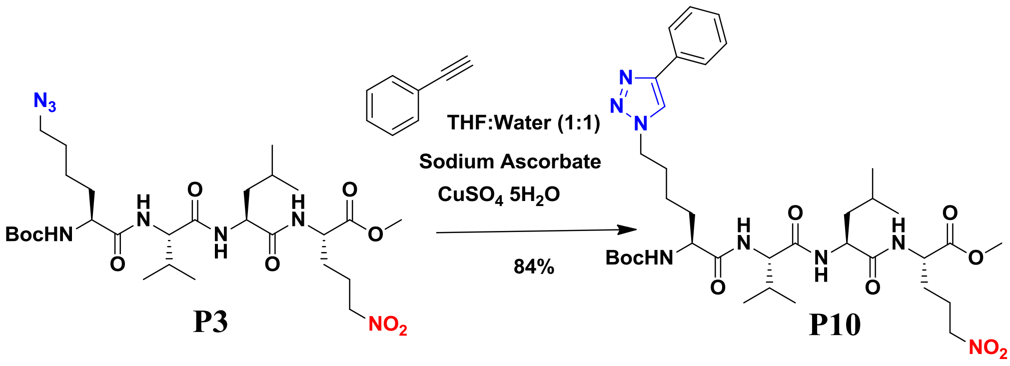
¹H NMR (400 MHz, Chloroform-*d*) δ 7.34 (s, 4H), 7.02 (d, *J* = 8 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 6.8 Hz, 1H), 6.06 (s, 1H), 5.53 (s, 1H), 5.12 (s, 2H), 4.98 (d, *J* = 8 Hz, 1H), 4.52 (dd, *J* = 5.4, 5.4 Hz, 1H), 4.22 – 4.06 (m, 1H), 3.96-3.92 (m, 1H), 3.72 (s, 1H), 3.25 (t, *J* = 6.6 Hz, 1H), 2.70 (dt, *J* = 15.1, 8.3 Hz, 1H), 1.84 – 1.70 (m, 1H), 1.59 (s, 15H), 1.43 (s, 3H), 0.96 (d, *J* = 6.9 Hz, 1H), 0.92 – 0.84 (m, 4H). **MALDI TOF/TOF**- *m/z* calculated for $C_{39}H_{59}N_9O_{10}$ [M+Na$^+$] 836.42 and observed 836.24.



Copper (I) catalyzed azide-alkyne cycloaddition reaction on P3: Transformation of P3 into P10

The tetrapeptide **P3** (160 mg, 0.25 mmol) was dissolved in 2 mL of THF/H_2O (1:1) mixture. To this solution phenyl acetylene (53 µL, 0.50 mmol) was added followed by $CuSO_4$, $5H_2O$ (125 mg, 0.5 mmol) and sodium ascorbate (50 mg, 0.25 mmol). Then the reaction mixture was stirred for overnight at room temperature. After completion of the reaction (confirmed by TLC), solvent THF was evaporated and the compound was extracted with ethyl acetate (3 × 25 mL).Then the organic layer was washed with brine solution (3 × 25 mL) and dried over anhydrous Na_2SO_4. After that, the combined organic layer was evaporated under reduced pressure and dissolved in MeOH. Then the crude compound was purified by reverse phase HPLC on a C_{18} column MeOH/H2O gradient system starting from 70% MeOH and 30% water and reached 95% MeOH in 35 min. Yield: 156 mg (84%).

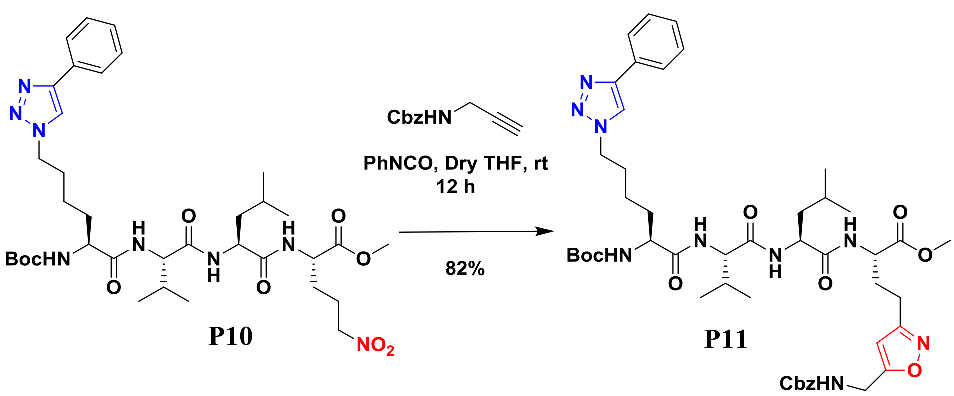
¹H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.34 (m, 5H), 7.01 (d, *J* = 8 Hz, 2H), 6.85 (d, *J* = 8 Hz, 2H), 6.62 (d, *J* = 8 Hz, 2H), 6.09 (s, 1H), 5.52 (bs, 1H), 5.15 (s, 2H), 4.99 (bs, 1H), 4.65-4.59 (m, 1H), 4.48 (d, *J* = 8 Hz, 2H), 4.20 (dd, *J* = 8, 4Hz, 1H), 4.15-4.11 (m, 2H), 3.75 (s, 3H), 3.28 (t, *J* = 4Hz, 2H), 2.38-2.16 (m, 4H), 1.85-1.76 (m, 2H), 1.59 (bs, 4H), 1.45 (s, 9H), 1.27 (bs, 4H), 0.98 (d, *J* = 8 Hz, 1H), 0.94 – 0.88 (m, 10H). **MALDI TOF/TOF**- *m/z* calculated for $C_{36}H_{56}N_8O_9$ [M+Na$^+$] 767.40 and observed 767.51.



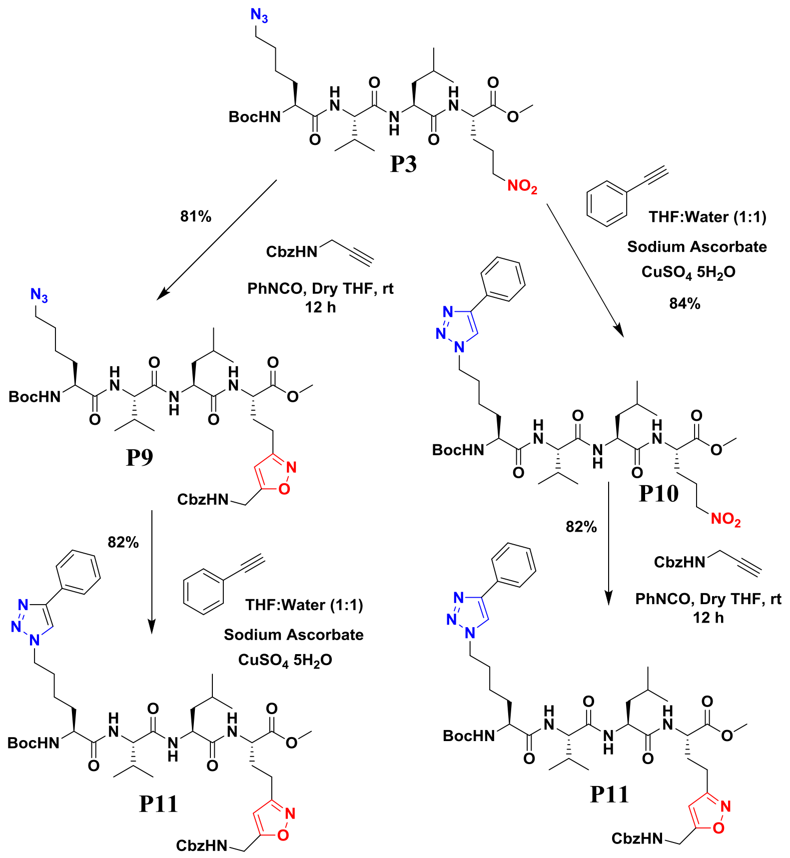
Cycloaddition reaction between nitrile oxide-alkyne in presence of triazole (P11):

Click conjugated tetrapeptide **P10** (111 mg, 0.15 mmol) was dissolved in dry THF under N_2 atmosphere and cooled to 0 $^{\circ}$C. To this solution Cbz protected propargylamine (141 mg, 0.75 mmol) was added followed by phenyl isocyanate (81 µL, 0.75 mmol) and triethylamine (104 µL, 0.75 mmol). The reaction mixture was stirred for about 12 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the urea was filtered through filter paper and solvent THF was evaporated under reduced pressure. The crude product was dissolved in MeOH and purified by reverse phase HPLC on a C_{18} column using MeOH/ H_2O gradient system starting from 70% MeOH and 30% water and reached 95% MeOH in 35 min. Yield: 112 mg (82%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 – 7.83 (m, 2H), 7.81 (s, 1H), 7.45 – 7.33 (m, 8H), 7.11 (d, *J* = 8 Hz, 1H), 6.98 (d, *J* = 8 Hz, 1H), 6.67 (d, *J* = 8Hz, 1H), 6.03 (s, 1H), 5.90 (t, *J* = 4Hz, *J* = 8Hz 1H), 5.15 (s, 2H), 5.11 (bs, 1H), 4.64 - 4.59 (m, 2H), 4.46 (d, *J* = 8 Hz, 2H), 4.42-4.37 (m, 2H), 4.20-4.17 (m, 1H), 3.98 (bs, 1H), 3.74 (s, 3H), 1.95 – 1.84 (m, 4H), 1.66 (b, 10H), 1.44 (s, 9H), 0.98 – 0.96 (m, 2H), 0.94 - 0.90 (m, 10H). **MALDI TOF/TOF-** m/z calculated for $C_{47}H_{65}N_9O_{10}$ [M+Na$^+$] 938.47 and observed 938.26.



Overall Scheme:



10. Selective copper (I) catalyzed cycloaddition reaction between alkyne and azide in the presence of nitroalkane functionality in P4:

Synthesis of the Peptide P4:

The heptapeptide was synthesized using solution phase peptide synthesis through 2+3+2 strategy. All coupling reactions were performed using EDC.HCl/HOBt coupling reagents in the presence of base DIEA. The crude peptide was purified by reverse phase HPLC on C_{18} column using MeOH/H_2O gradient system. **MALDI TOF/TOF**-*m/z* calculated for $C_{47}H_{82}N_8O_{13}$ [M+Na$^+$] 989.58 and observed 989.67.

11. Crystal structure analysis of peptide P4:

Crystals of Peptide **P4** were grown by slow evaporation from a solution of aqueous methanol. A single crystal (0.1 × 0.06 × 0.02 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100K temperature on a Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ = 0.71073 Ǻ), ω-scans (2θ = 57.77), for a total of 102587 independent reflections. Space group P 21, a = 14.885(6), b = 20.472(7), c =

18.004(7), β = 91.102(13), V = 5485(4) $Å^3$, monoclinic, Z = 4 for chemical formula C_{47} H_{82} N_8 O_{13}, with two molecules in asymmetric unit; ρcalcd = 1.171 gcm^{-3} , μ = 0.085 mm^{-1} , F (000) = 2096. The structure was obtained by direct methods using SHELXS-97. The final R value was 0.1140 (wR2 = 0.2096) 27312 observed reflections (F0 ≥ 4σ (|F0|)) and 1253 variables, S = 0.919. The largest difference peak and hole were 0.291 and -0.286e$Å^3$, respectively.

12. ORTEP diagram of peptide P4:

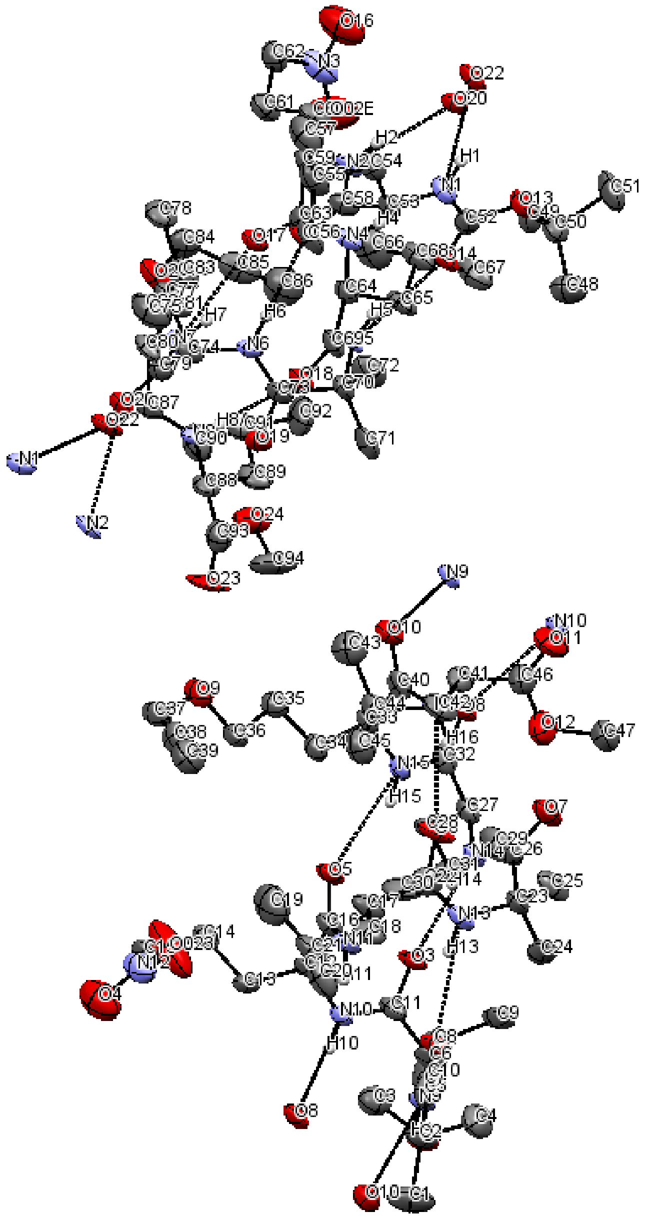


Figure S2: ORTEP diagram of peptide **P4**. H-bonds are shown in dotted lines. H-atoms are omitted for clarity. Two molecules are found in the asymmetric unit. Ellipsoids are drawn at 50% probability. (CCDC No 1548955).

13. Torsion angles and H-bond parameters of peptide P4:

Table S1: Torsional angle parameters of **P4** (molecule A in the asymmetric unit)

Peptide P4	**ϕ (deg)**	**Ψ (deg)**
Leu 1	-59.0	-35.6
Nitro Amino Acid 2	-60.8	-47.2
Leu 3	-69.5	-39.4
Aib 4	-60.2	-43.3
Leu 5	-70.7	-47.7
Alkyne Amino Acid 6	-64.6	-37.0
Leu 7	-126.3	-39.2

Torsional angle parameters of **P4** (molecule B in the asymmetric unit)

Peptide P4	**ϕ (deg)**	**Ψ (deg)**
Leu 1	-62.1	-32.0
Nitro Amino Acid 2	-61.2	-48.6
Leu 3	-71.8	-33.3
Aib 4	-59.8	-46.8
Leu 5	-68.7	-48.8
Alkyne Amino Acid 6	-62.9	-37.4
Leu 7	-125.3	-40.3

Table S2: Hydrogen bond parameters of **P4**

Intramolecular H-bonds **(**molecule A in the asymmetric unit)

Donor (D)	**Acceptor (A)**	**D….A (Å)**	**DH….A (Å)**	**NH….O (deg)**
N5	O14	3.06	2.23	162.4
N6	O15	2.95	2.10	168.2
N7	O17	3.02	2.22	155.8
N8	O18	2.98	2.229	146.0

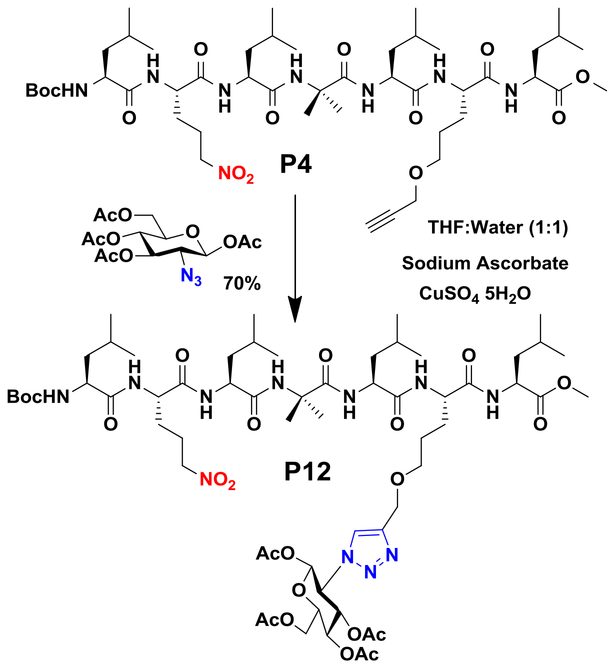
Intramolecular H-bonds (molecule B in the asymmetric unit)

Donor (D)	**Acceptor (A)**	**D….A (Å)**	**DH….A (Å)**	**NH….O (deg)**
N13	O2	3.04	2.20	164.8
N14	O3	2.93	2.10	164.1
N15	O5	3.03	2.23	154.4
N16	O6	2.95	2.20	145.2

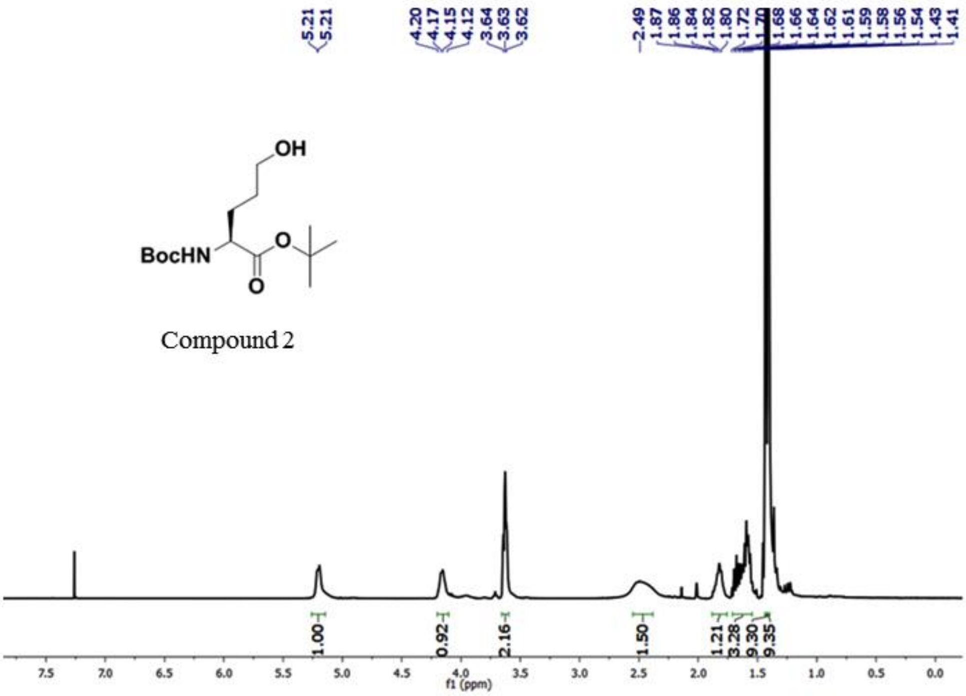
Synthesis of P12 from P4: Selective cycloaddition reaction between alkyne with 2-deoxy-azido-1,3,4,6-tetraacetyl glucopyranoside

Heptapeptide **P4** (95 mg, 0.1 mmol) was dissolved in 2 mL of THF/H_2O (1:1) mixture. To this solution, 2-deoxy-azido-1,3,4,6-tetraacetyl glucopyranoside (112 mg, 0.3 mmol) was added followed by $CuSO_4$, $5H_2O$ (75 mg, 0.3 mmol) and sodium ascorbate (50 mg, 0.25 mmol). Then the reaction mixture was stirred for about 12 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, solvent THF was evaporated and the compound was extracted with ethyl acetate (3 × 25 mL). Then the organic layer was washed with brine solution (3 × 25 mL) and dried over anhydrous Na_2SO_4. The combined organic layer was evaporated under reduced pressure and crude was dissolved in MeOH. The crude compound was purified by reverse phase HPLC on a C_{18}

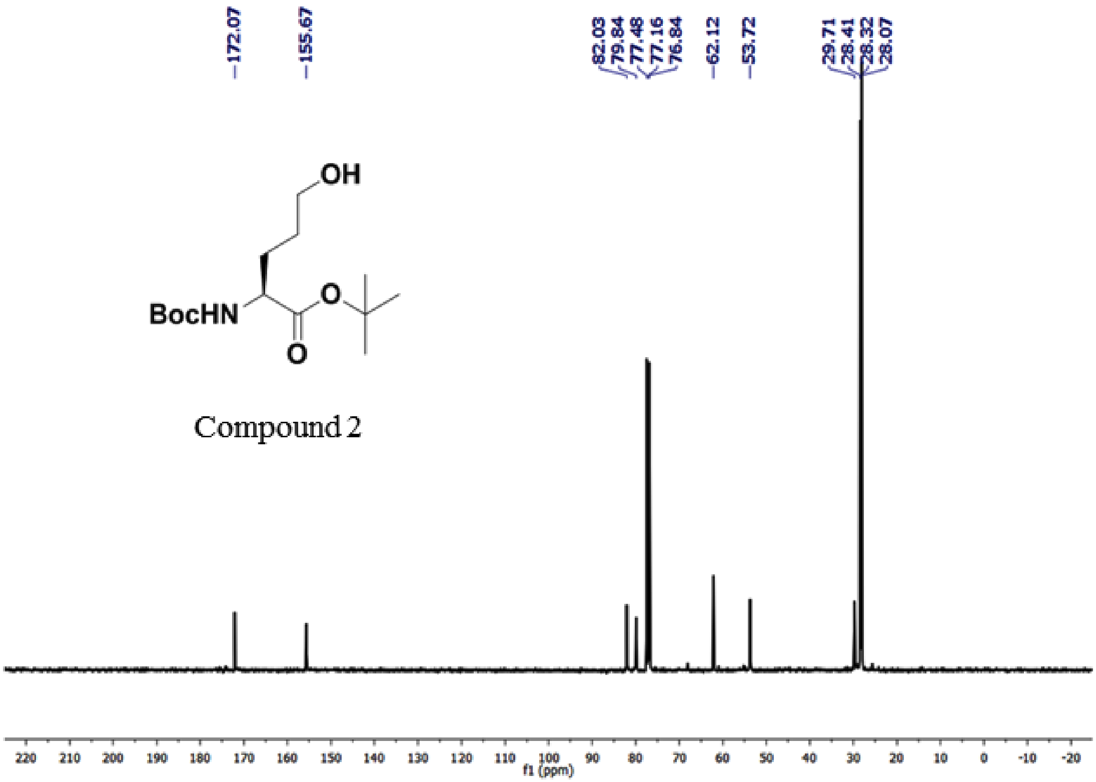
column using MeOH/H_2O gradient system staring from 70% MeOH and 30% water and reached 95% MeOH in 20 min. Yield: 94 mg (70%). **MALDI TOF/TOF**- *m/z* calculated for $C_{60}H_{99}N_{11}O_{22}$ [M+Na^+] 1362.70 and observed 1363.00.



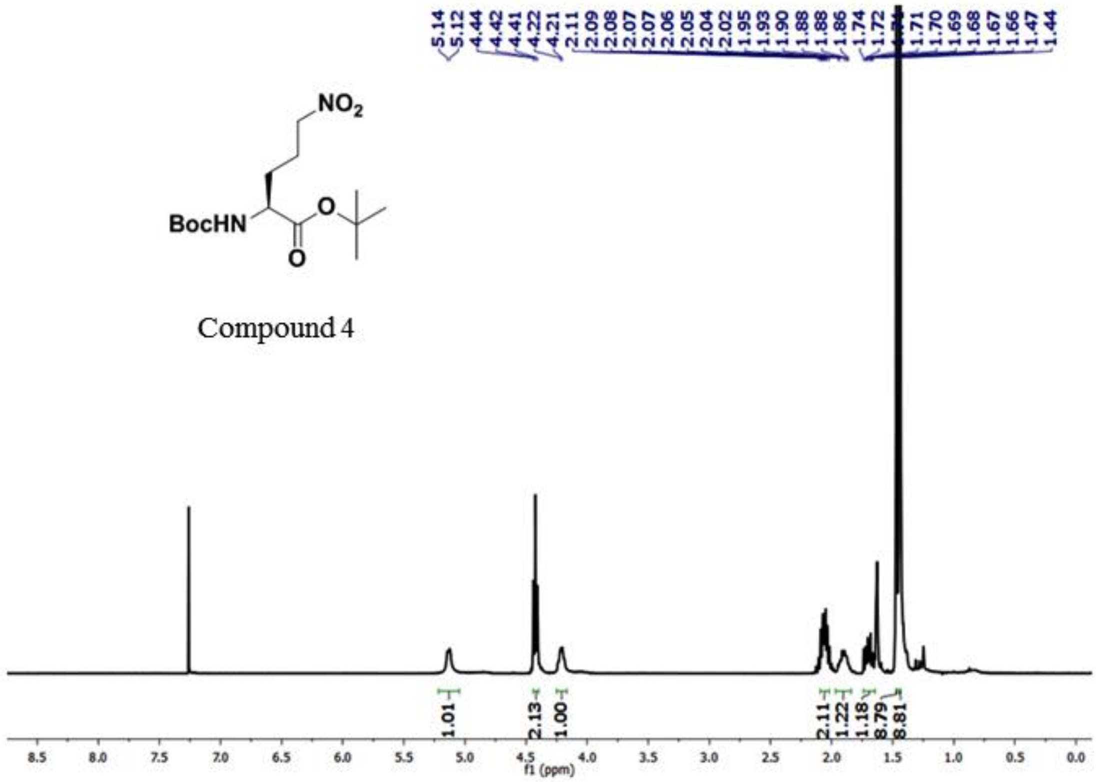
14. ^{1}H and ^{13}C NMR Spectra:



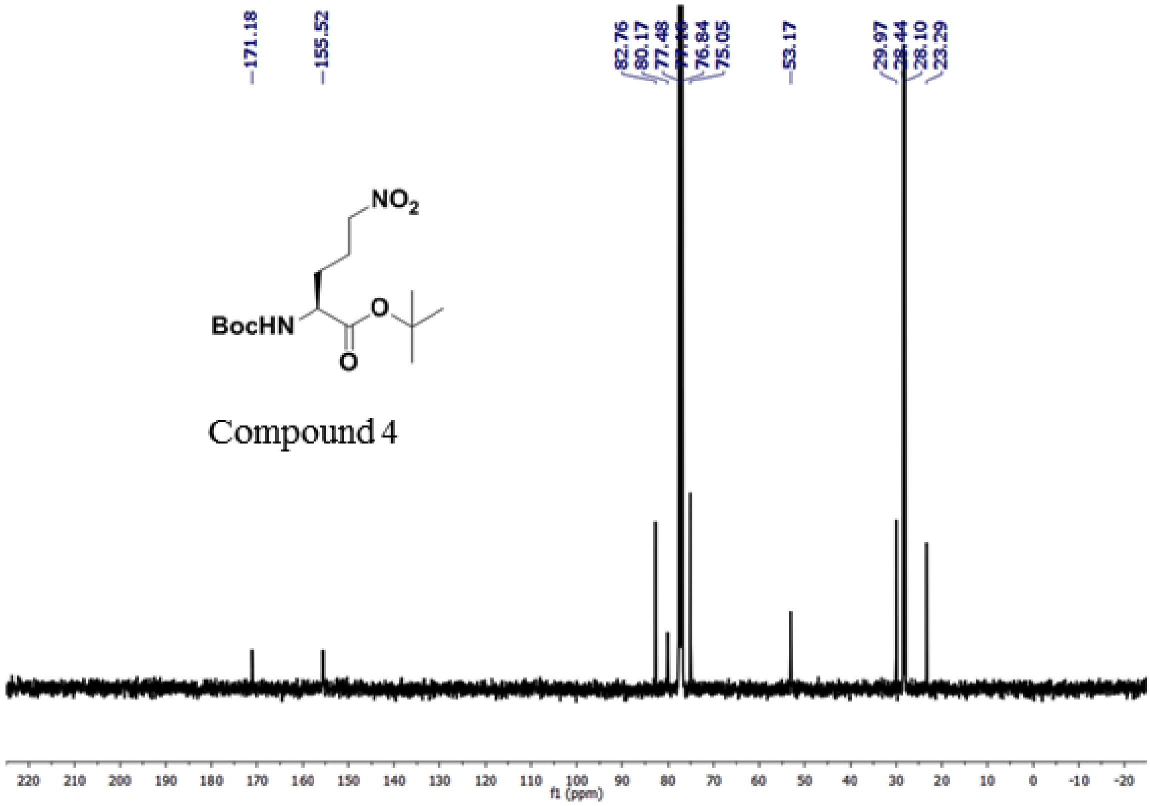
Compound 2



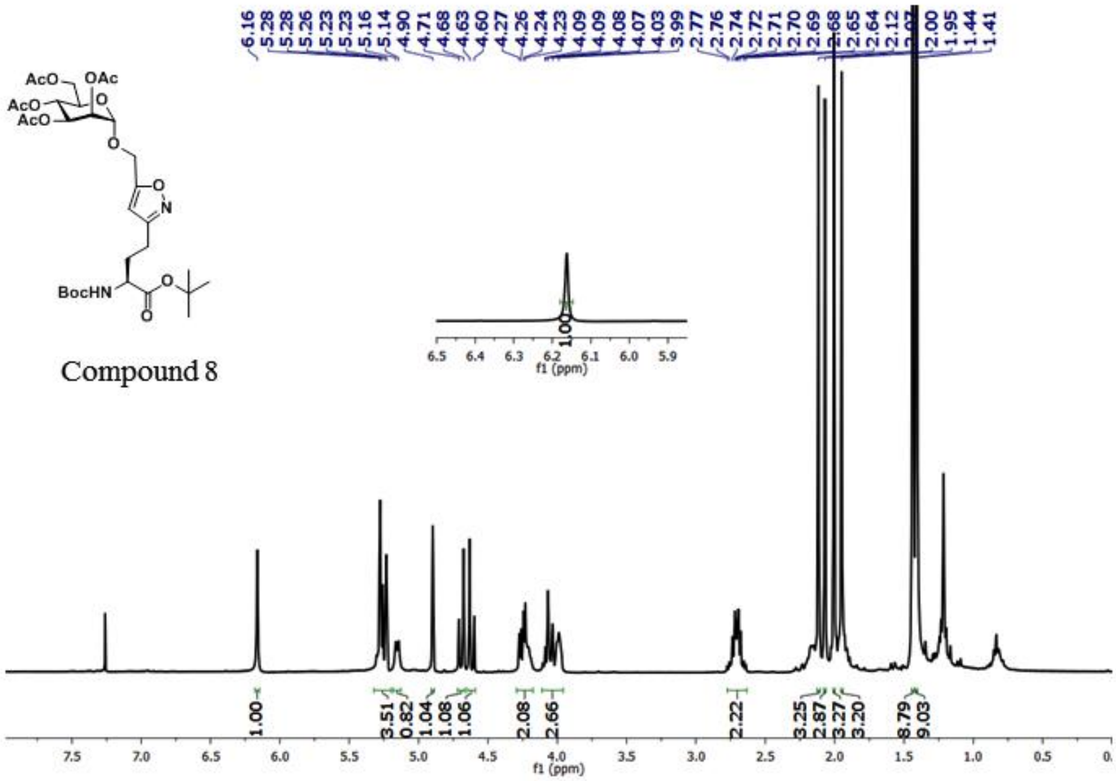
Compound 2



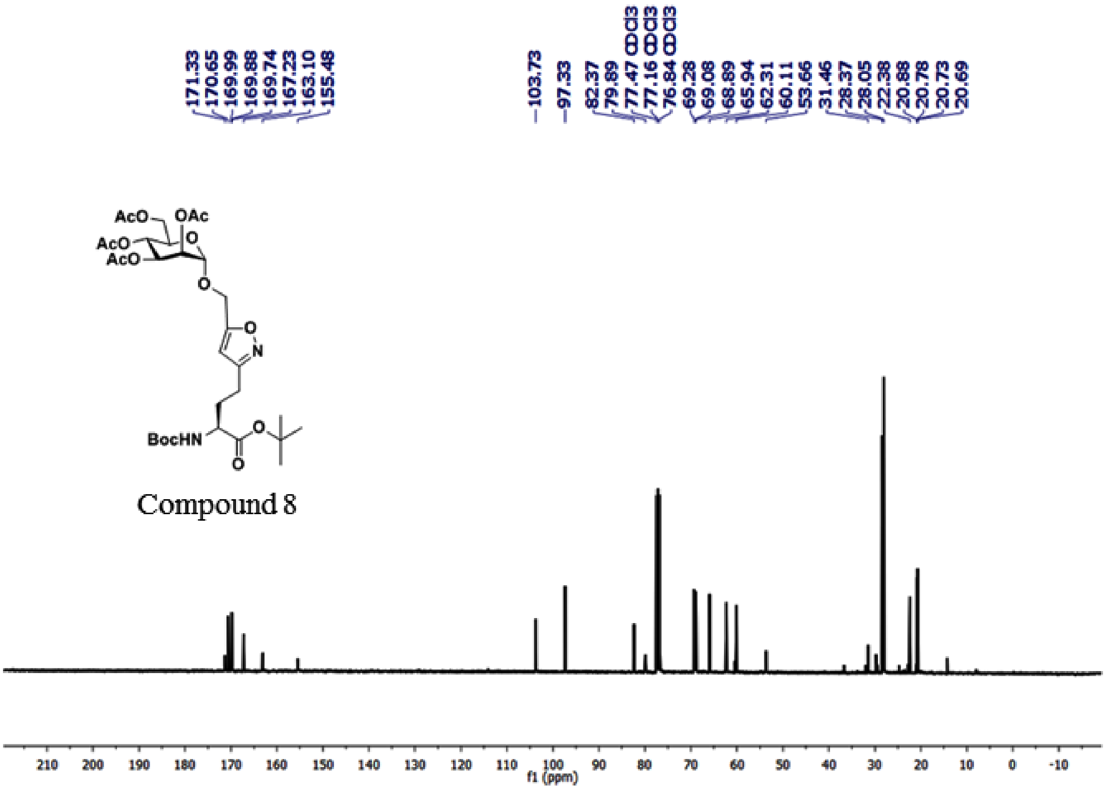
Compound 4



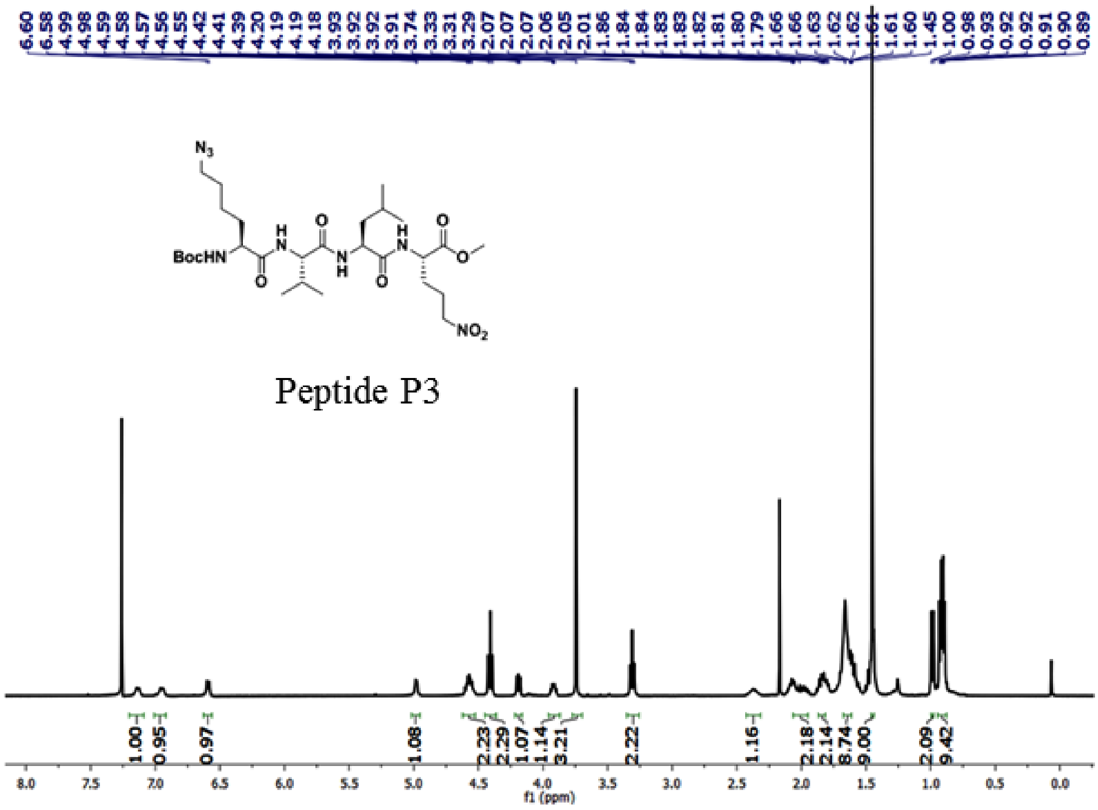
Compound 4



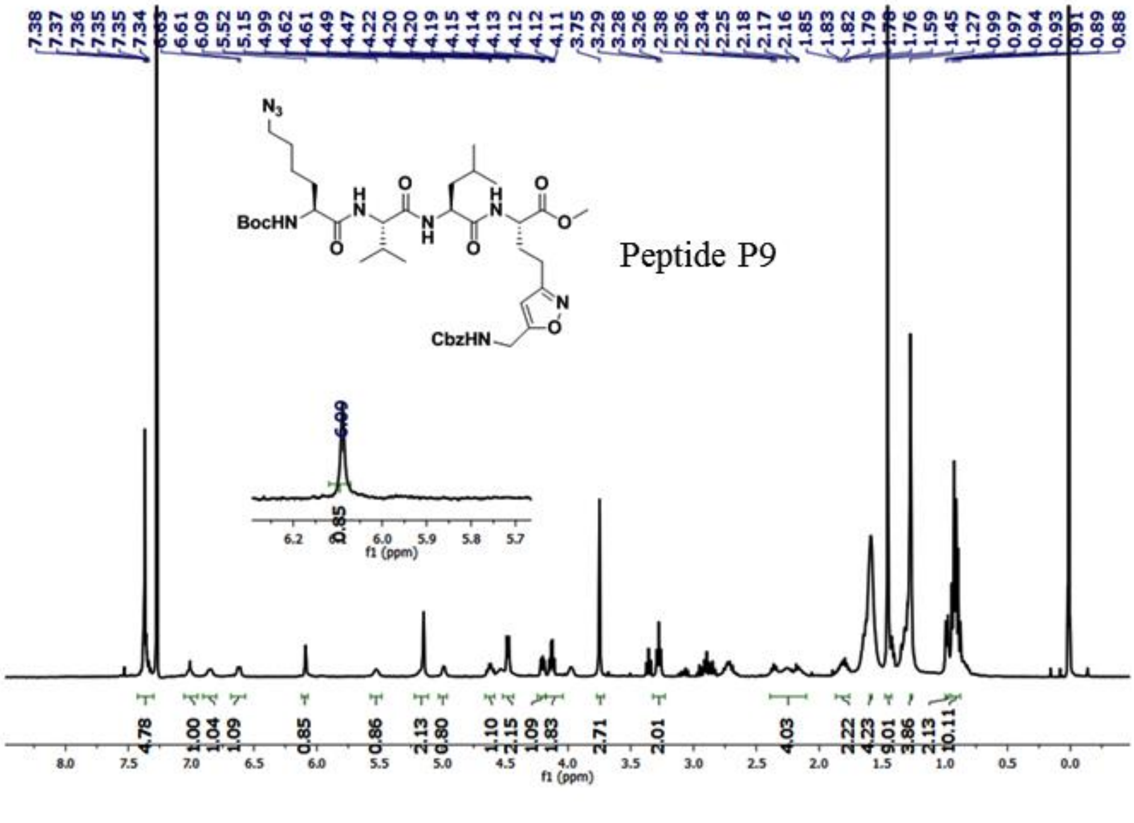
Compound 8



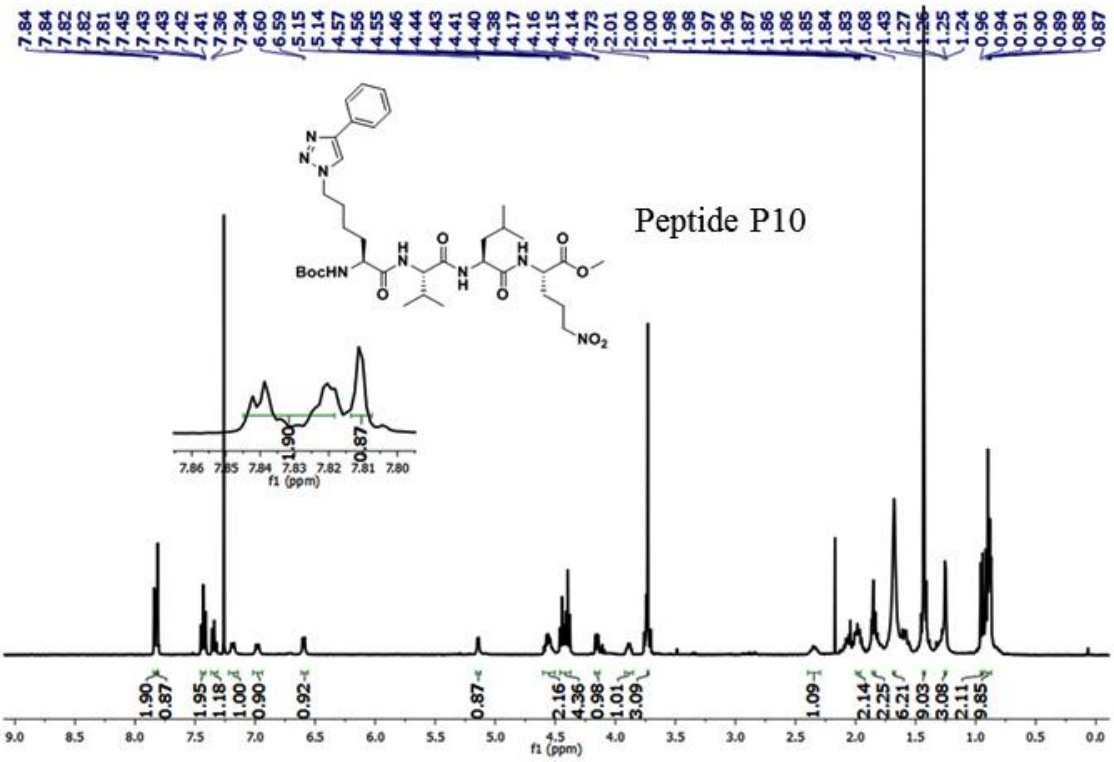
Compound 8



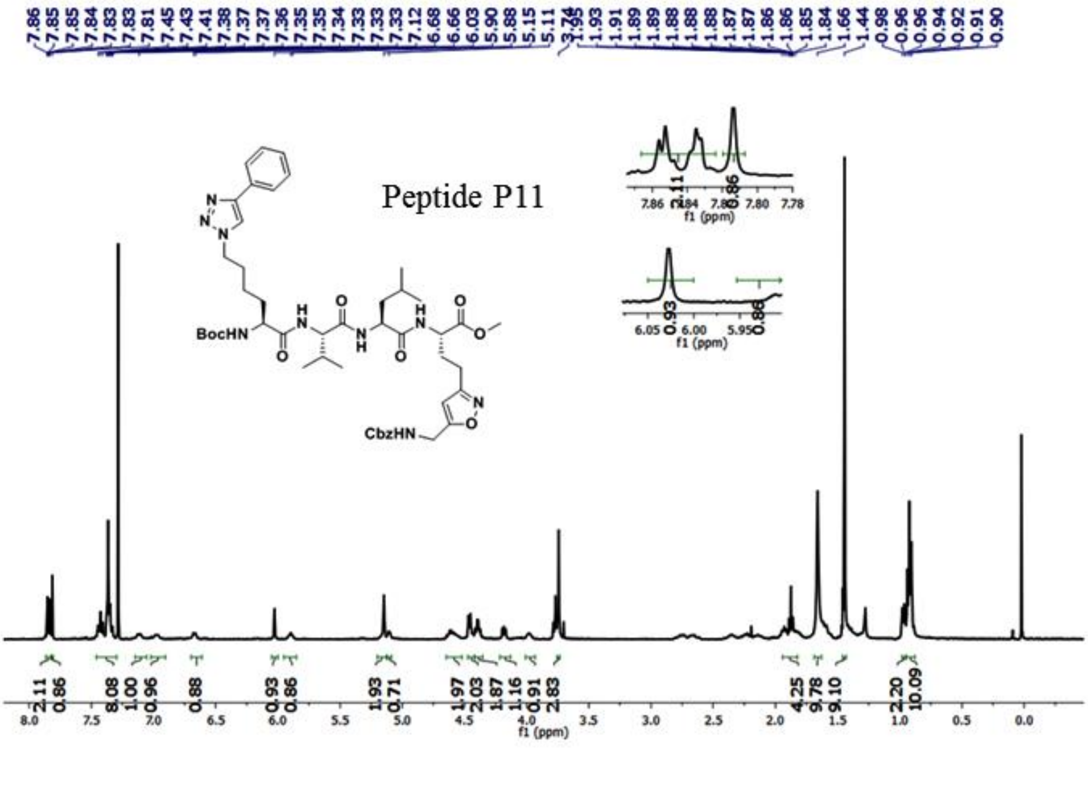
Peptide P3



Peptide P9

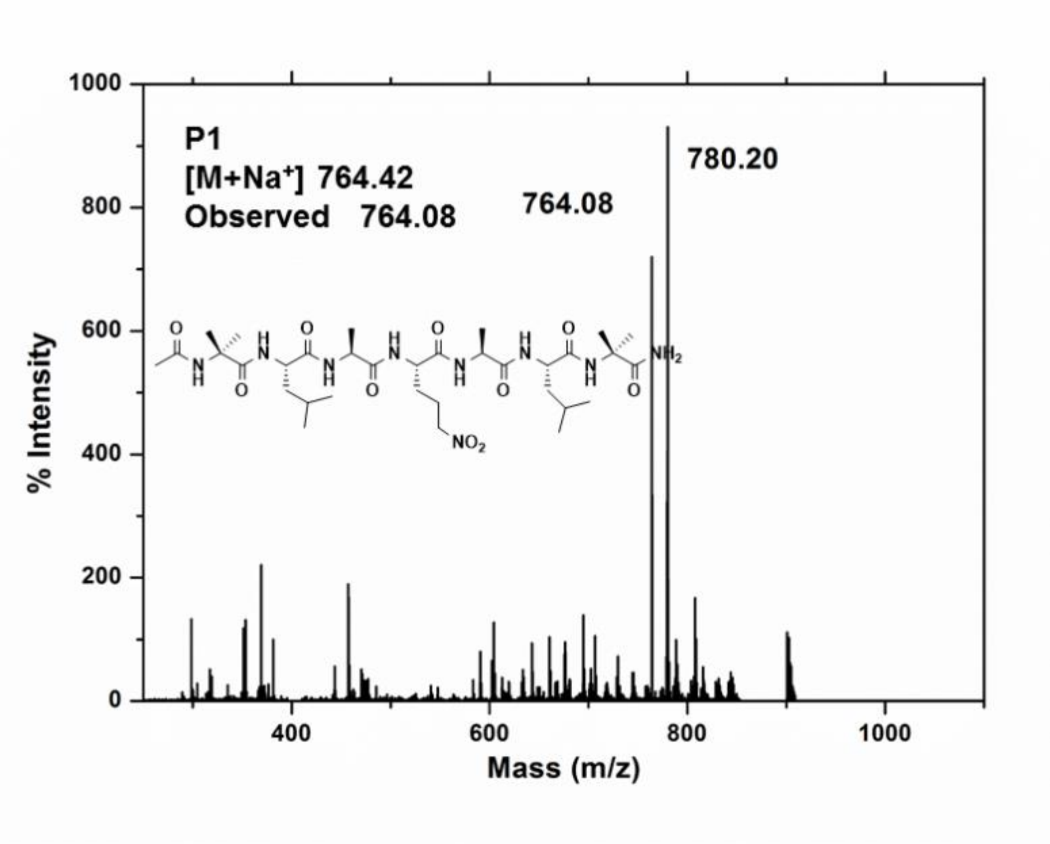


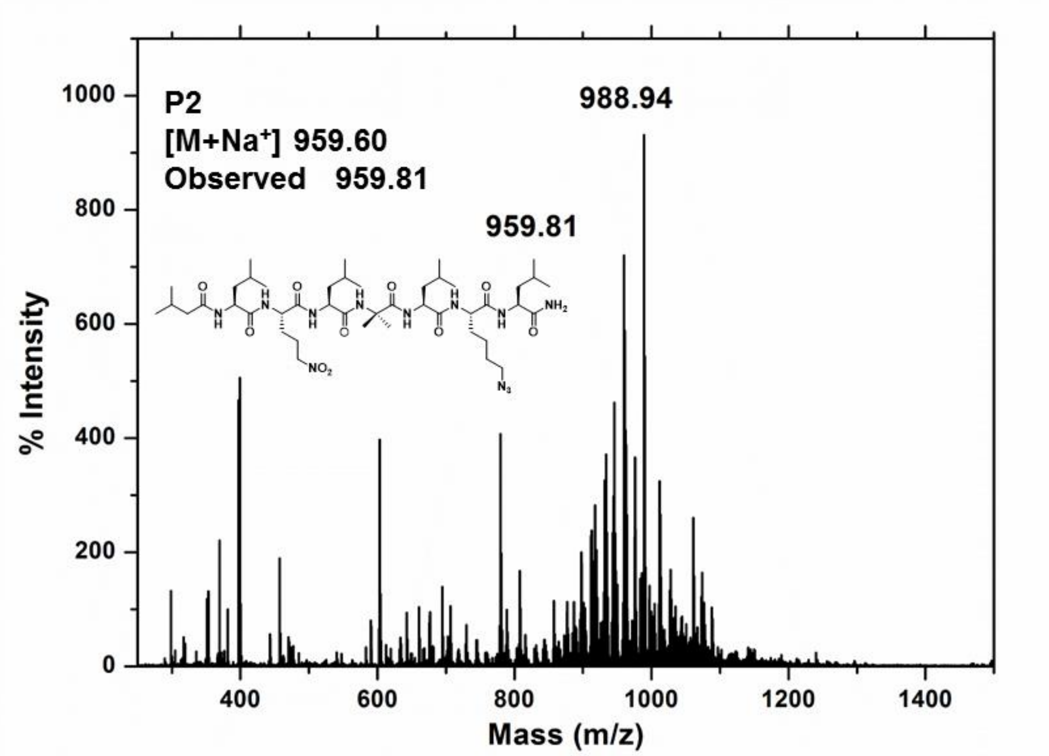
Peptide P10
BocHN
NO2
f1 (ppm)

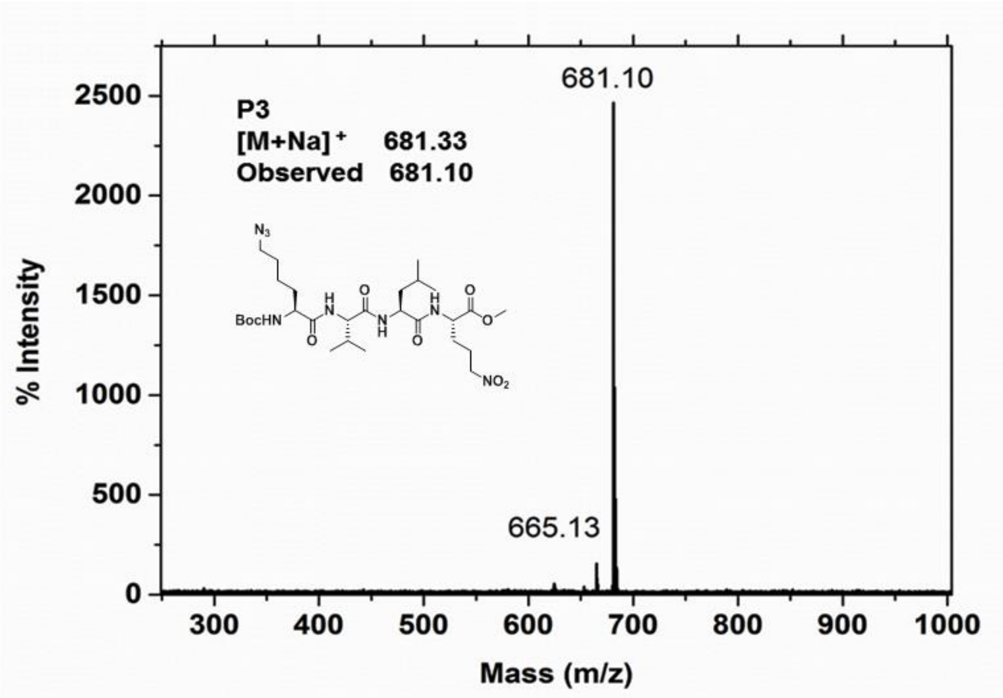


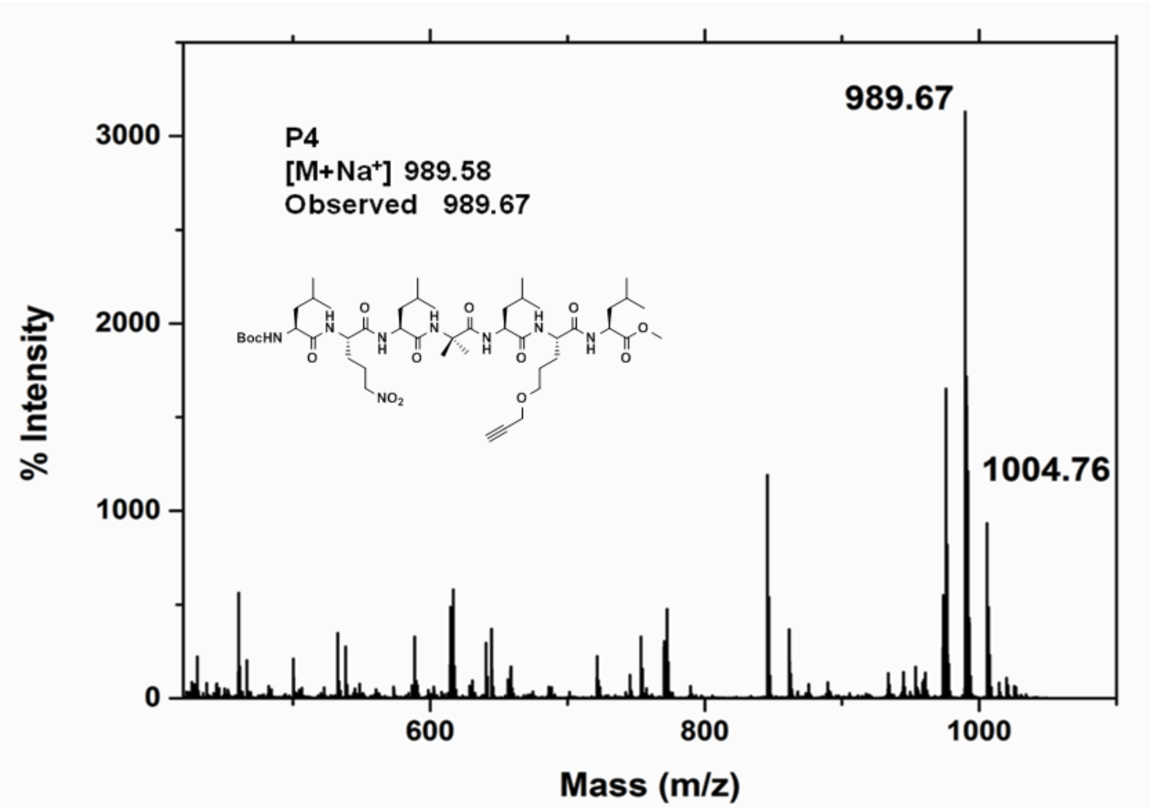
Peptide P11
BocHN
CbzHN
f1 (ppm)

15. MALDI TOF/TOF Mass Spectra:

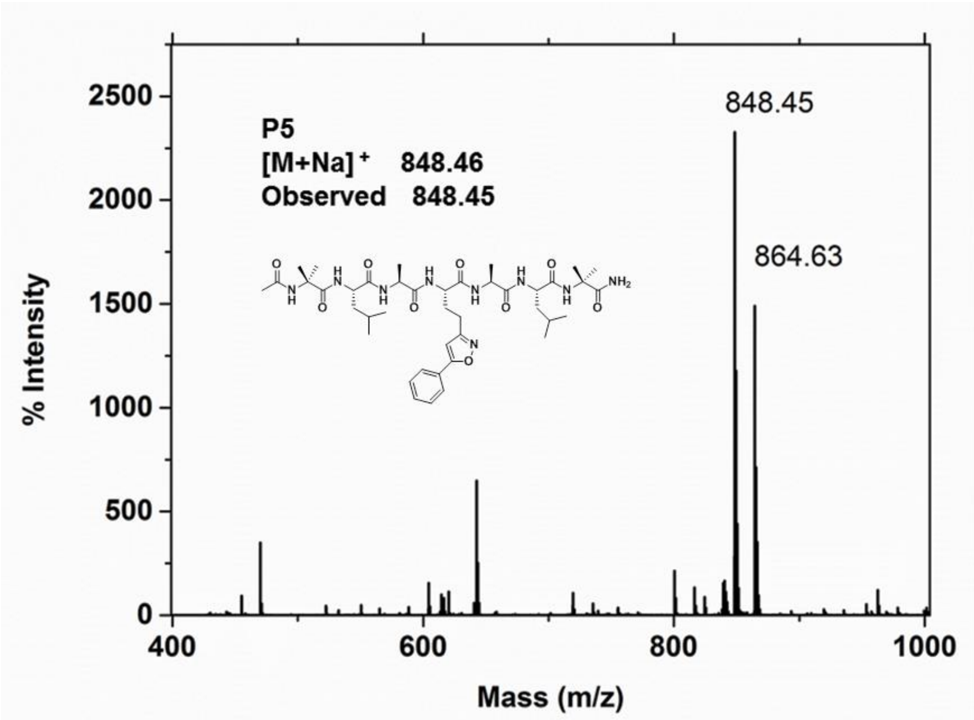



P2
[M+Na⁺] 959.60
Observed 959.81
988.94
959.81
% Intensity
Mass (m/z)

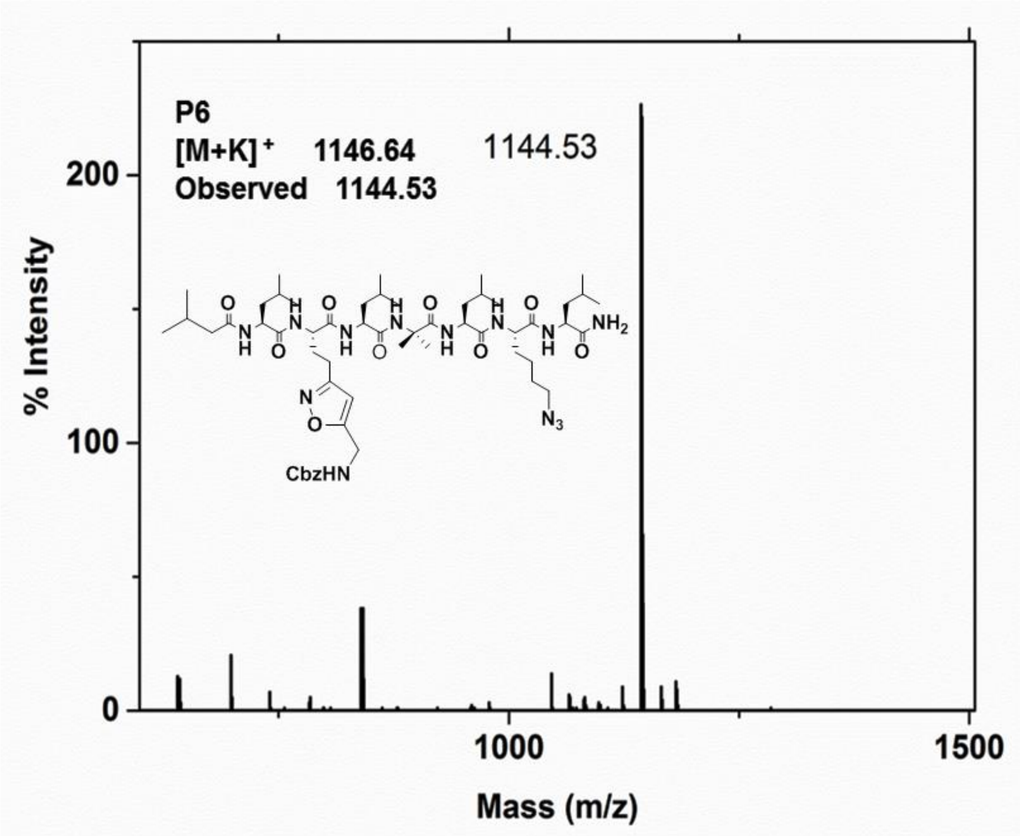

P3
[M+Na]⁺ 681.33
Observed 681.10
681.10
665.13
BocHN
% Intensity
Mass (m/z)

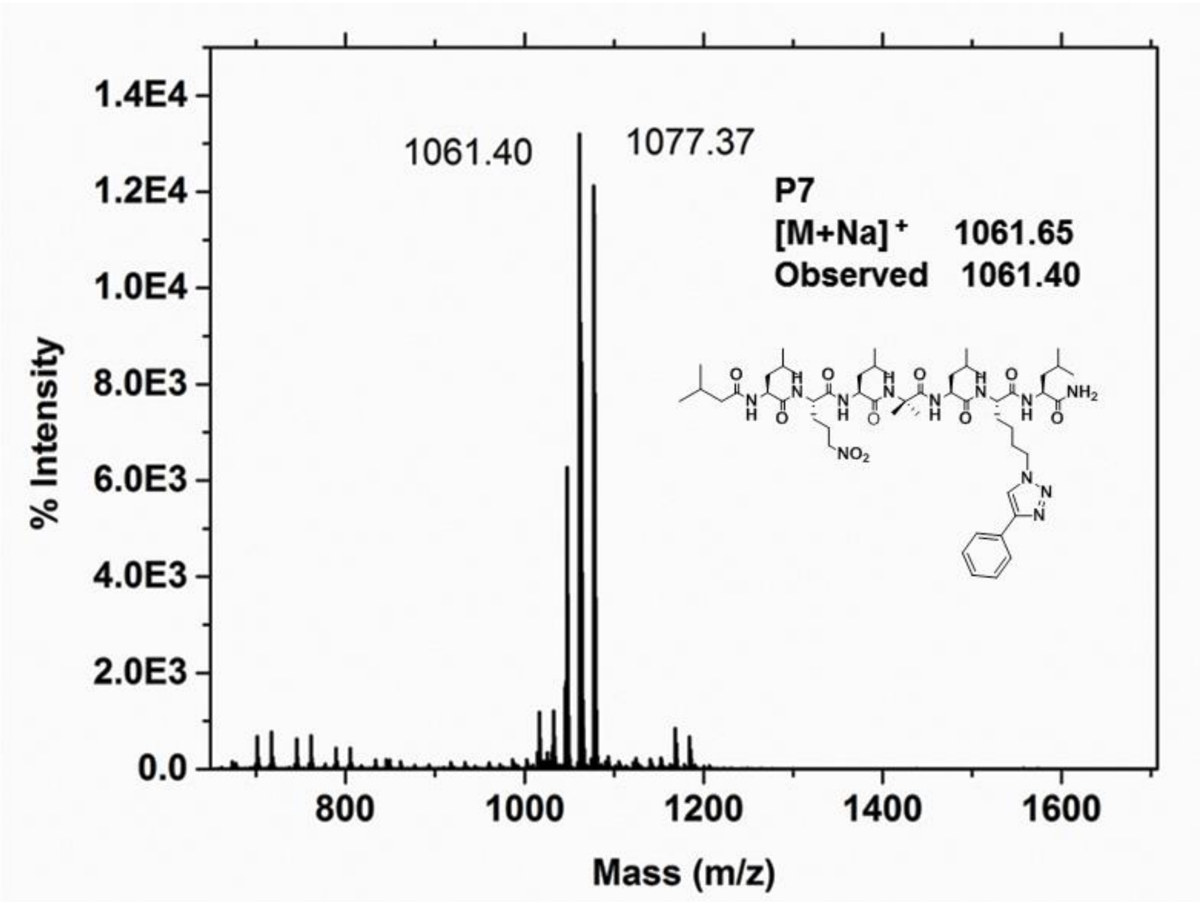


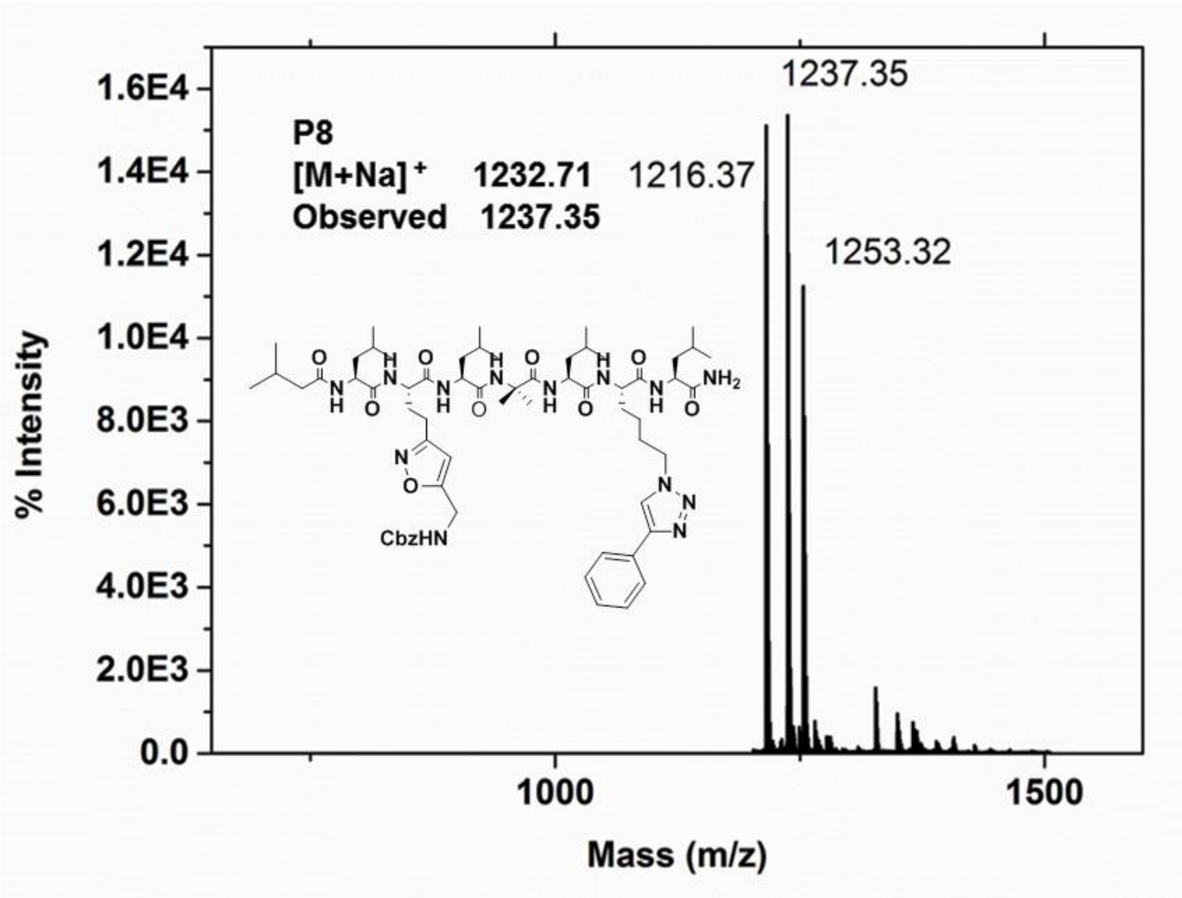
989.67
1004.76
P4
[M+Na+] 989.58
Observed 989.67
BocHN
NO_2
% Intensity
Mass (m/z)
0
1000
2000
3000
600
800
1000

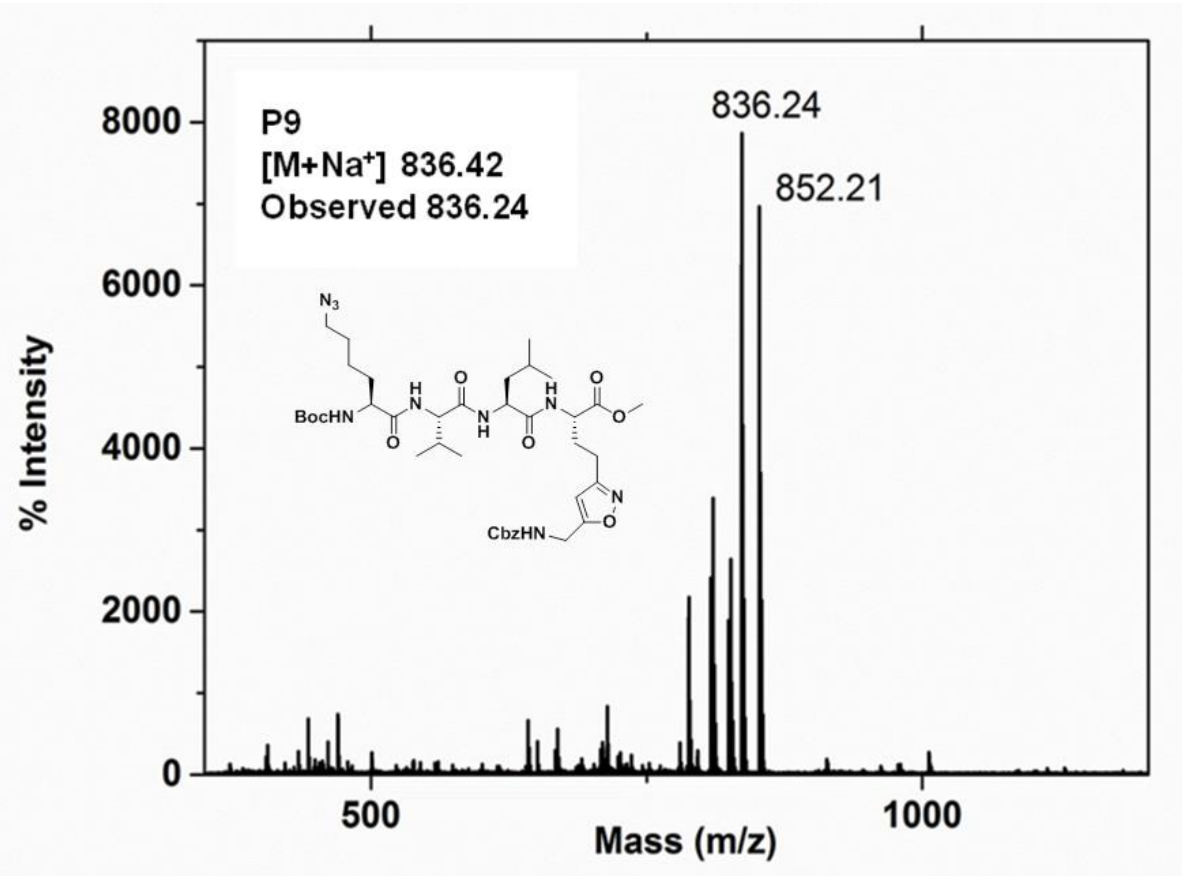


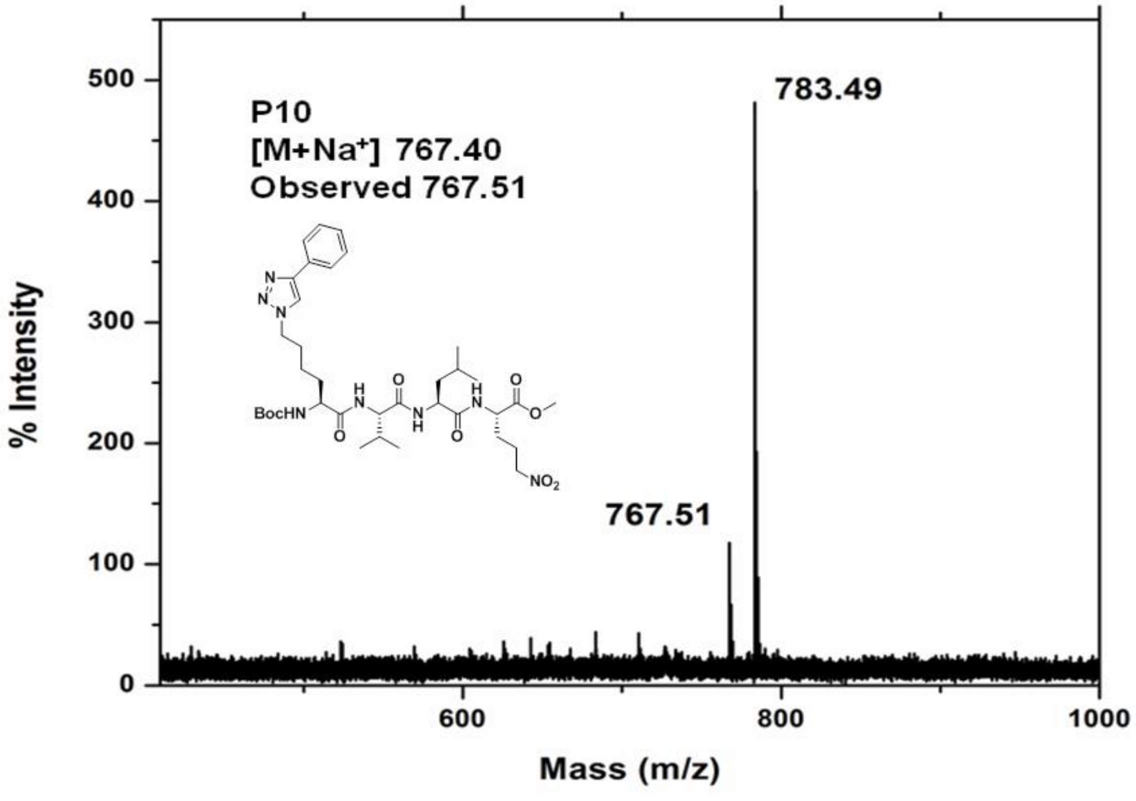
848.45
864.63
P5
[M+Na]+ 848.46
Observed 848.45
NH_2
% Intensity
Mass (m/z)
0
500
1000
1500
2000
2500
400
600
800
1000

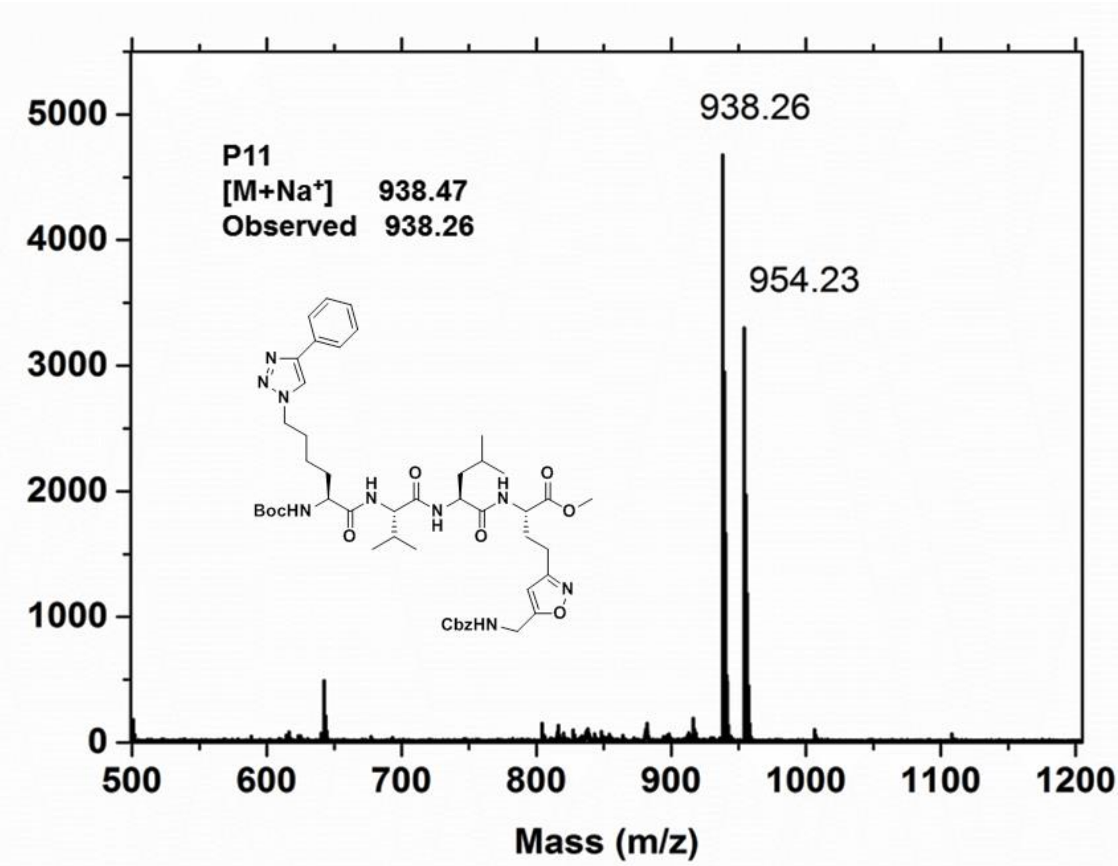

P6
[M+K]+ 1146.64
Observed 1144.53
1144.53
% Intensity
Mass (m/z)
CbzHN

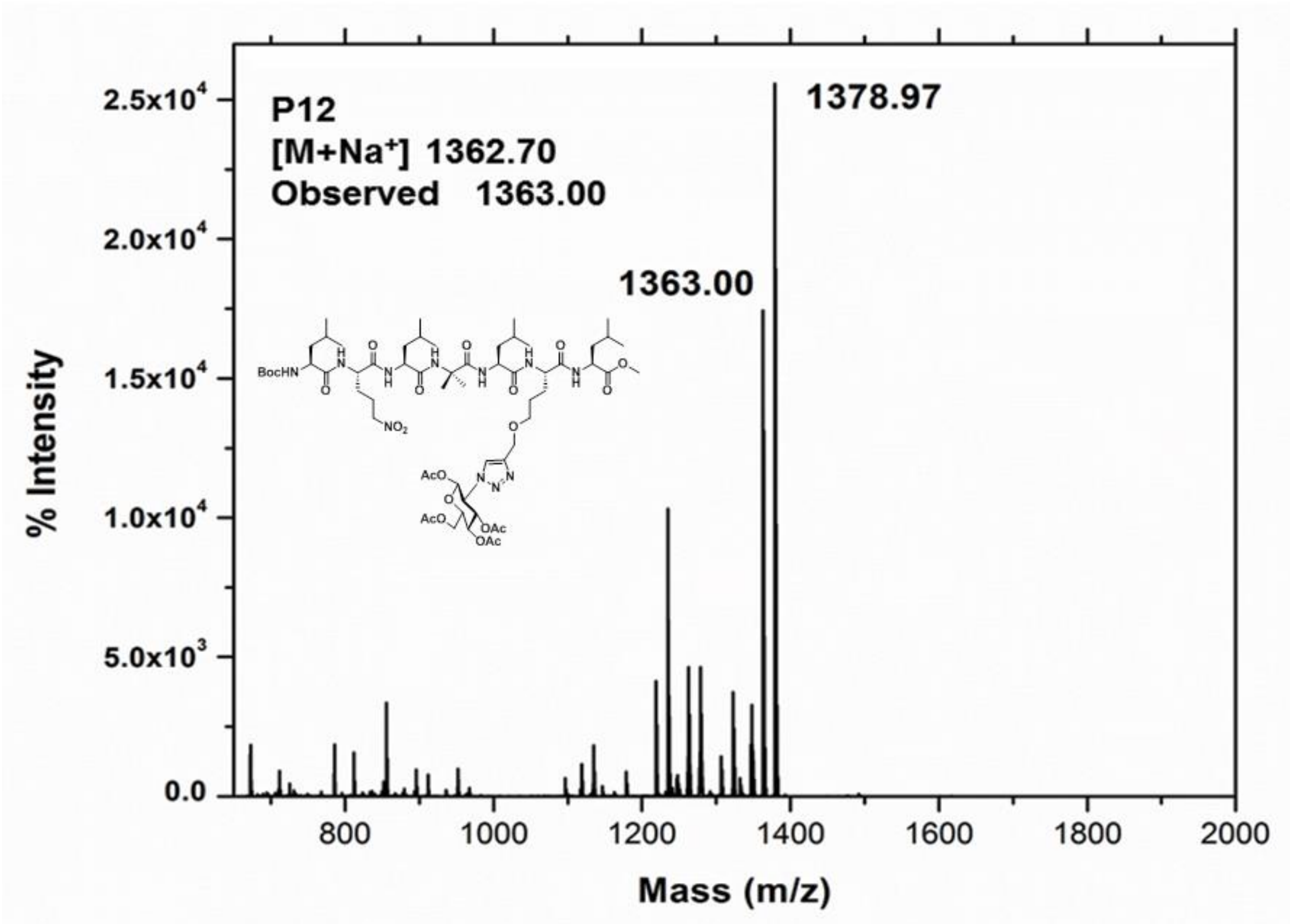

1061.40
1077.37
P7
[M+Na]+ 1061.65
Observed 1061.40
% Intensity
Mass (m/z)


P8
[M+Na]+ 1232.71
Observed 1237.35
1237.35
1216.37
1253.32
CbzHN
NH2
% Intensity
Mass (m/z)
1.6E4
1.4E4
1.2E4
1.0E4
8.0E3
6.0E3
4.0E3
2.0E3
0.0
1000
1500


P9
[M+Na+] 836.42
Observed 836.24
836.24
852.21
N3
BocHN
CbzHN
% Intensity
Mass (m/z)
8000
6000
4000
2000
0
500
1000

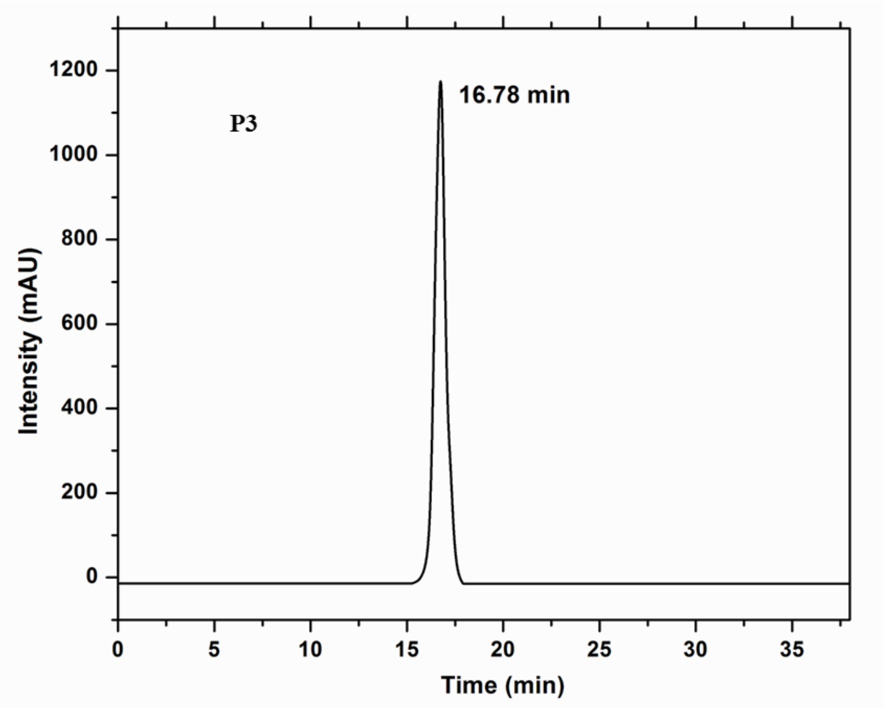

P10
[M+Na+] 767.40
Observed 767.51
783.49
767.51
BocHN
NO_2
% Intensity
Mass (m/z)
0
100
200
300
400
500
600
800
1000

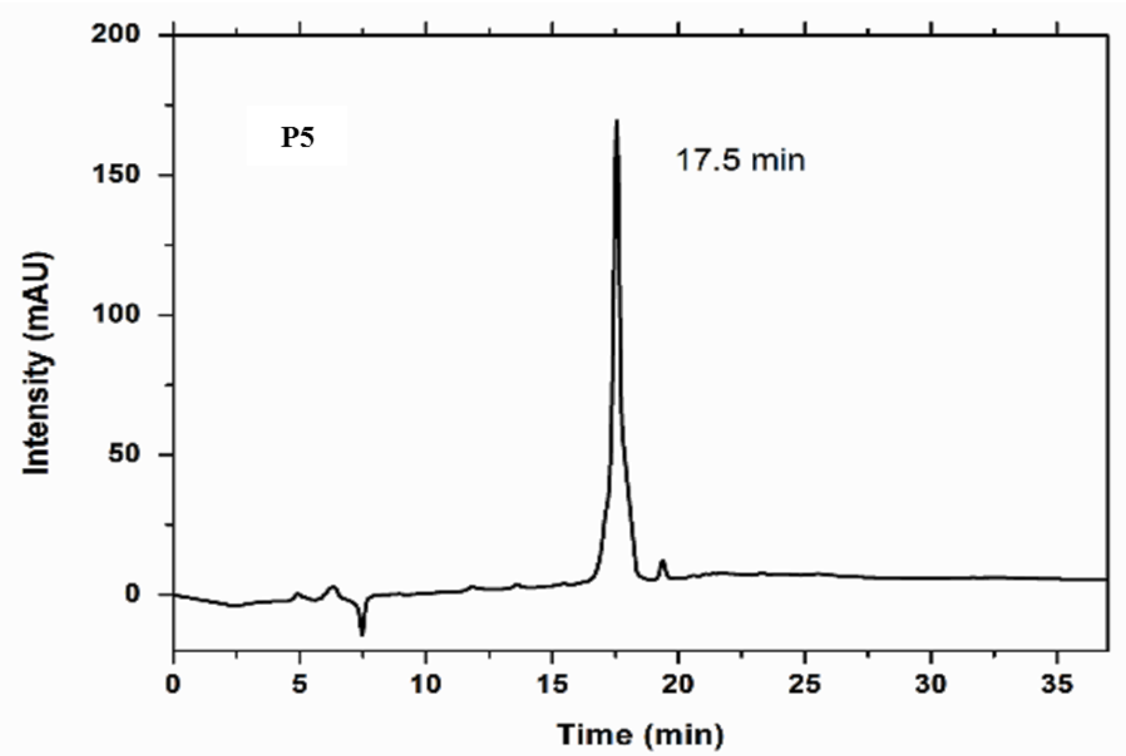

P11
[M+Na+] 938.47
Observed 938.26
938.26
954.23
BocHN
CbzHN
Mass (m/z)
0
1000
2000
3000
4000
5000
500
600
700
800
900
1000
1100
1200

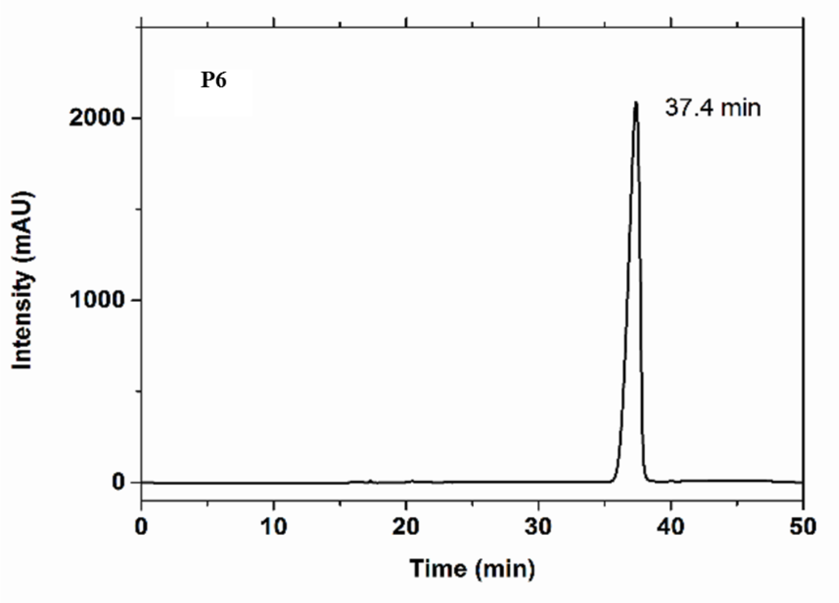


P12
[M+Na+] 1362.70
Observed 1363.00
1378.97
1363.00
BocHN
NO_2
AcO
OAc
% Intensity
Mass (m/z)

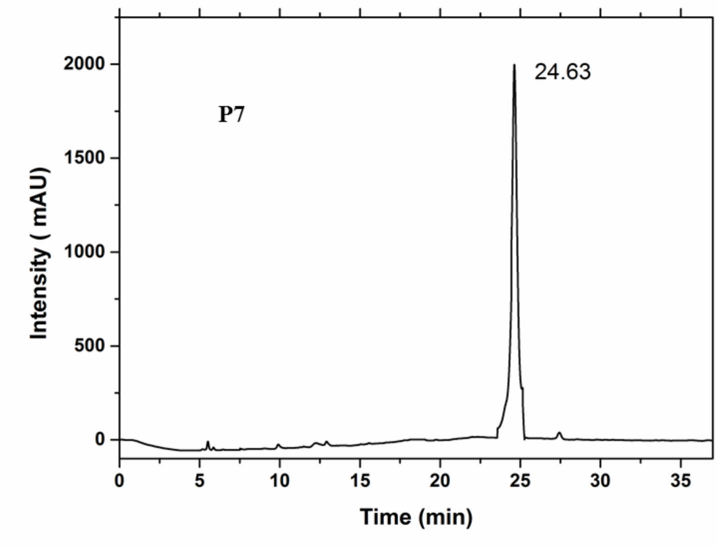
16. HPLC Traces:



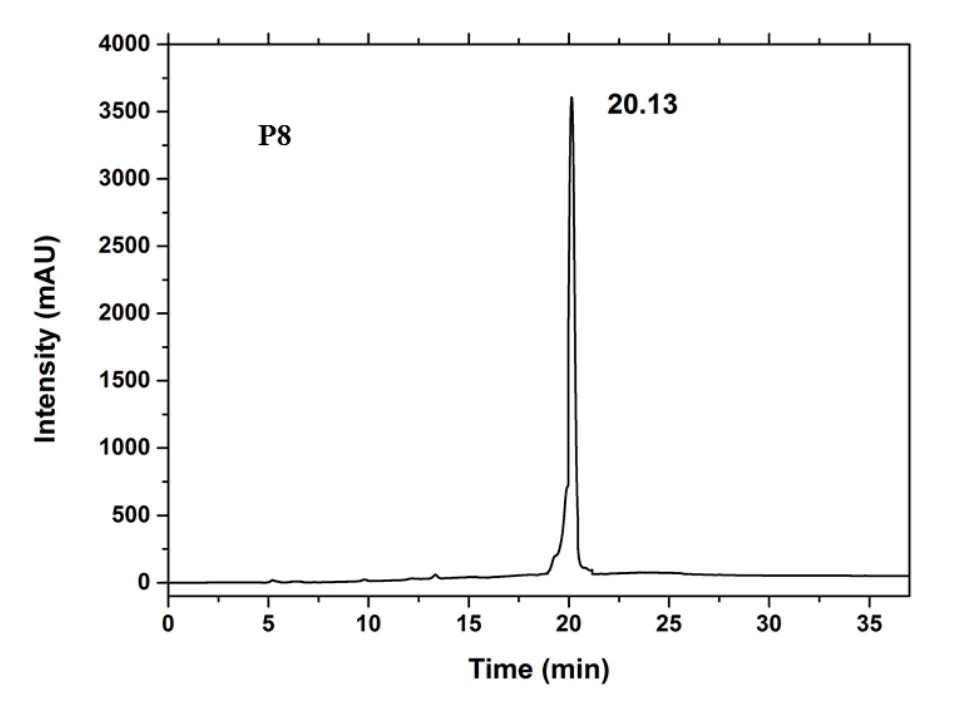


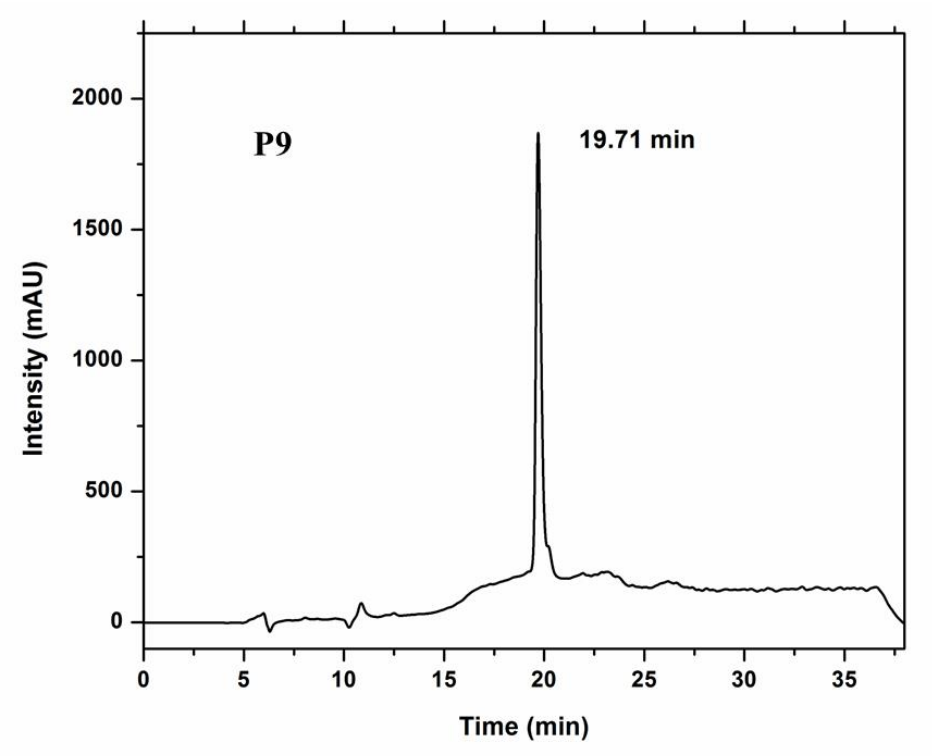


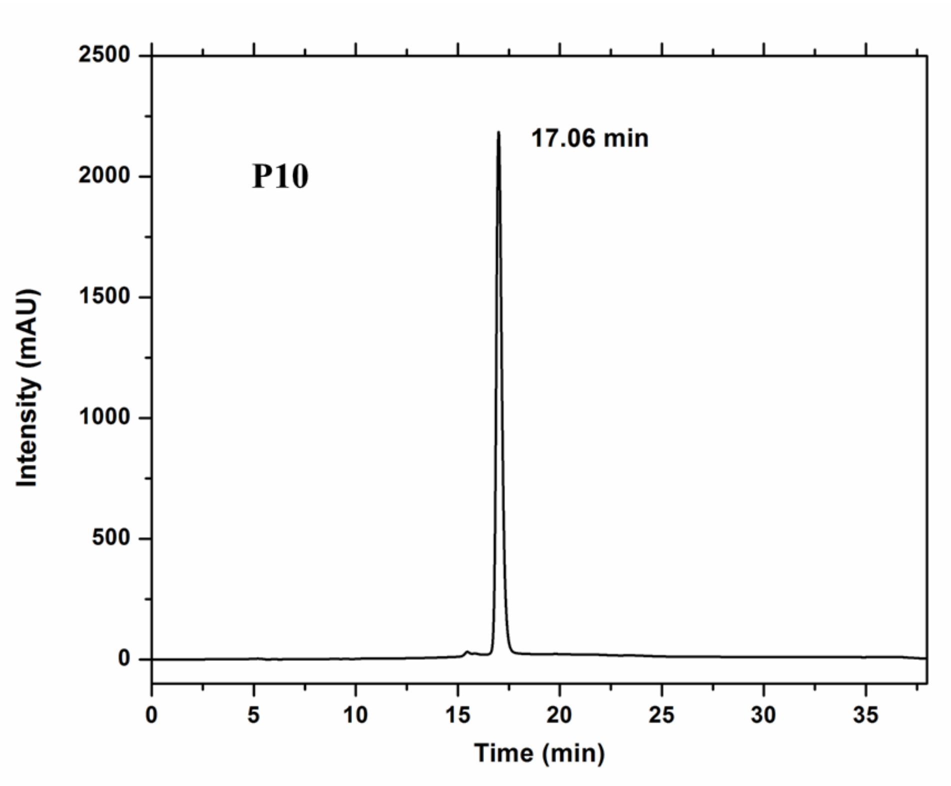
P6
37.4 min
Intensity (mAU)
2000
1000
0
0
10
20
30
40
50
Time (min)

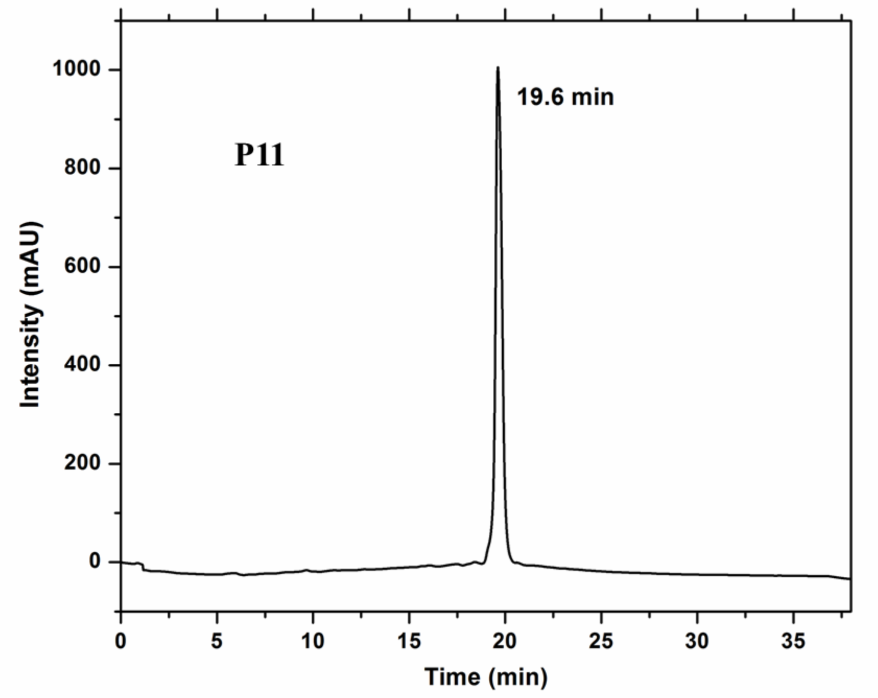


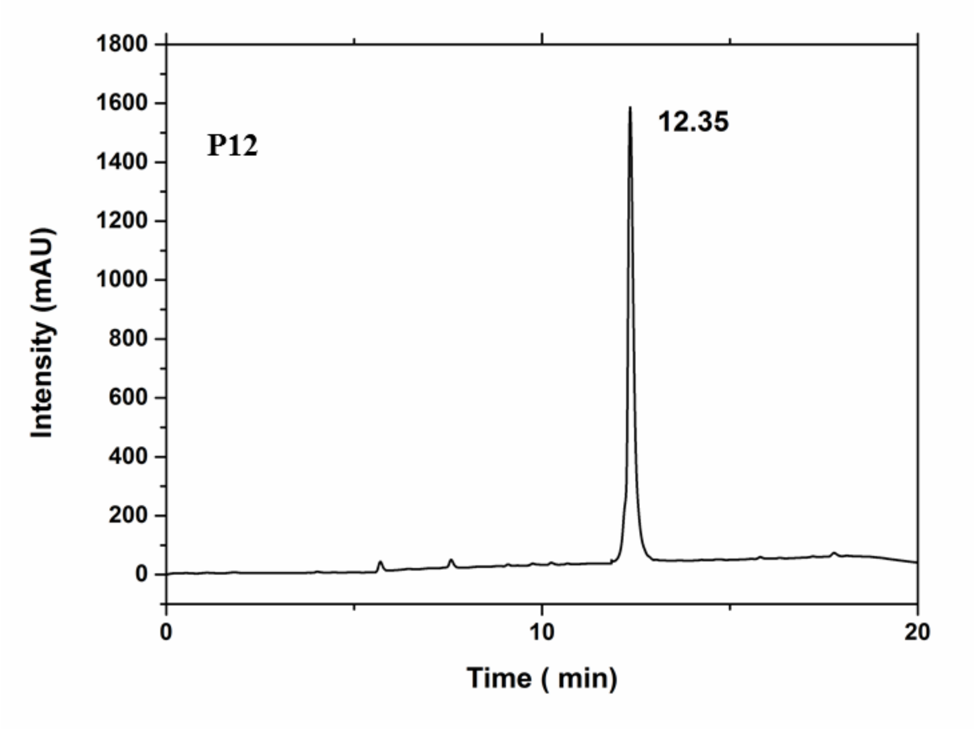
P7
24.63
Intensity (mAU)
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
Time (min)


P8
20.13
Intensity (mAU)
Time (min)
0
500
1000
1500
2000
2500
3000
3500
4000
5
10
15
20
25
30
35

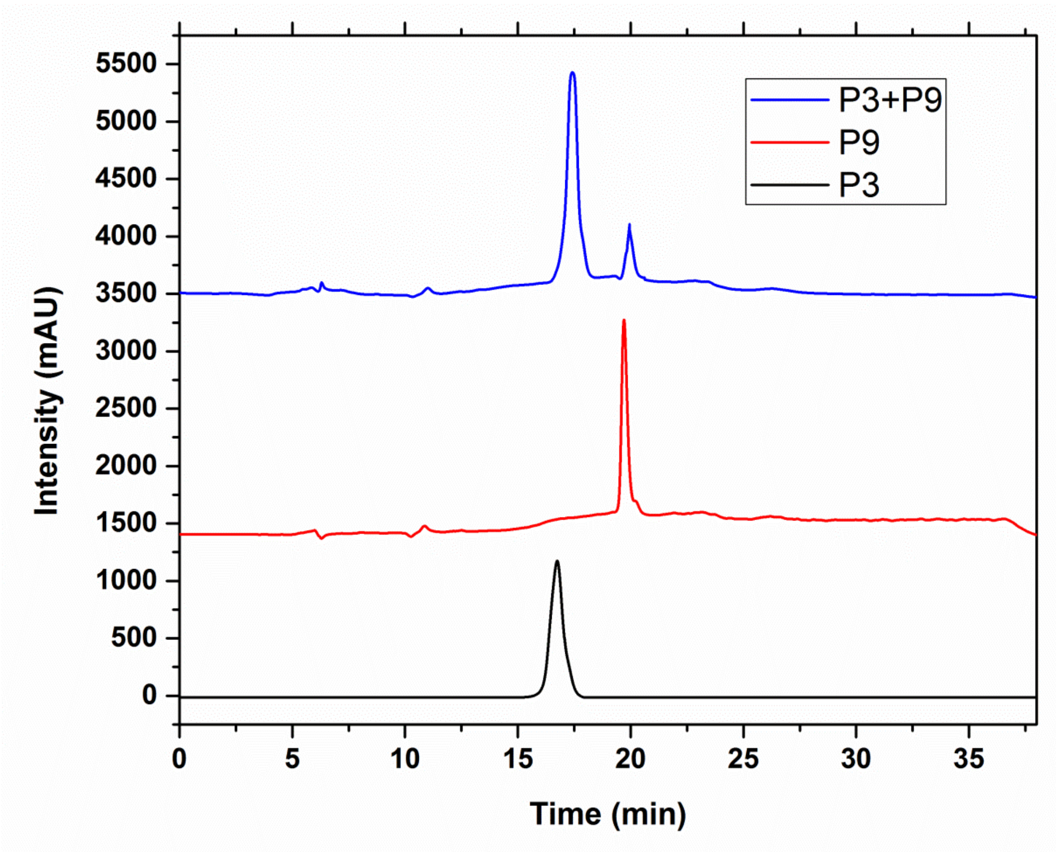

P9
19.71 min
Intensity (mAU)
Time (min)
0
500
1000
1500
2000
5
10
15
20
25
30
35


P10
17.06 min
Intensity (mAU)
Time (min)
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35

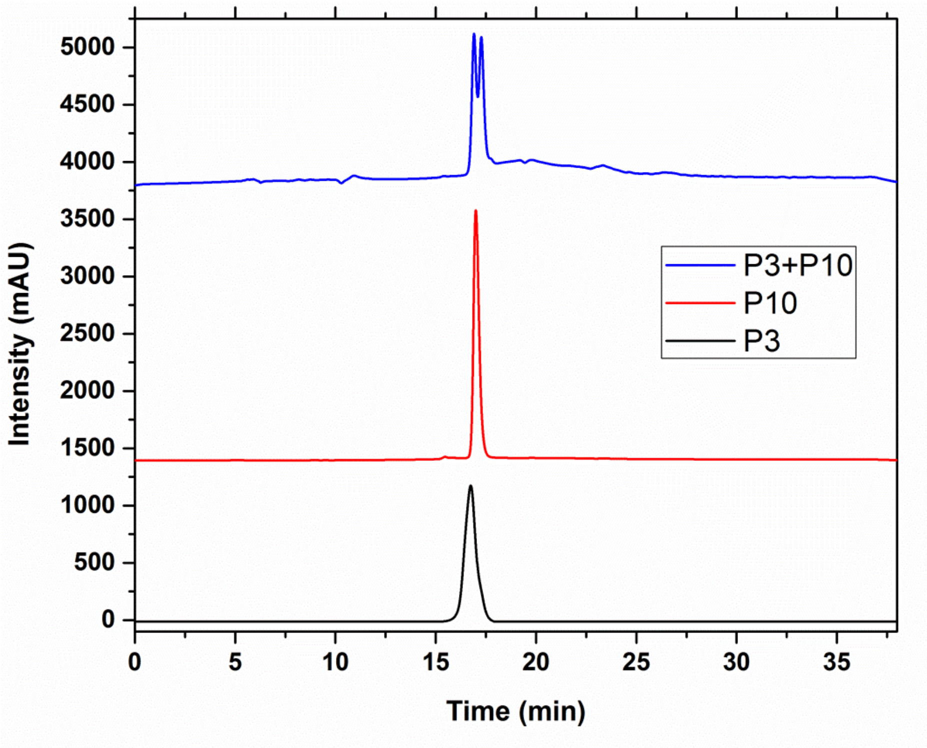

P11
19.6 min
Intensity (mAU)
Time (min)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35



17. Co-injected HPLC chromatograms of P3 and P9



18. Co-injected HPLC chromatograms of P3 and P10



19. SI References:

1. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797.
2. Gopi, H. N.; Tirupula, K. C.; Baxter, S.; Ajith, S.; Chaiken, I. M. *ChemMedChem* **2006**, *1*, 54.
3. Mukaiyama, T.; Hoshino, T. *J. Am. Chem. Soc.* **1960**, *82*, 5339.
4. SHELXS-97: Sheldrick, G. M.; *Acta Crystallogr. Sect A.* **1990**, *46*, 467. b) Sheldrick, G. M. ; SHELXL-97, Universität Göttingen (Germany) **1997**.
5. Spek, A. L.; *Acta Crystallogr.* **1990**, *A46*, C34.