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Late-stage Peptide and Protein Modifications Through Phospha-Michael Addition Reaction

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Experimental Procedures

General information

All reagents used in experiments were obtained from commercial companies and used without further purification.

All solvents were reagent/HPLC grade.

Anhydrous solvents were commercially available or dehydrated and distilled.

The purifications of peptides and small molecules were carried on Shimadzu LC-6AD or LC-20AD reversed-phase HPLC (RP-HPLC) (YMC C18 column, 5 μm, 20×250 mm, Japan) at a flow rate of 10 mL/min.

The purifications of proteins were carried on Shimadzu LC-20AD RP-HPLC (Proteonavi C4 column, 5 μm, 10×250 mm, Japan) at a flow rate of 4 mL/min.

The analyses of peptides and small molecules were performed on Shimadzu LC-2010A HPLC (YMC analytic C18 column, Japan, 10 μm, 4.5×150 mm) at a flow rate of 0.8 mL/min.

The analyses of proteins were performed on Shimadzu LC-2010A HPLC (Proteonavi analytic C4 column, Japan, 5 μm, 4.6×150 mm) at a flow rate of 0.8 mL/min.

Thermo Scientific UltiMate 3000 (ESI-MS) were used to identify peptides and proteins.

All the solution of HPLC was composed of solution B (80% acetonitrile/water with 0.06% trifluoroacetic acid) in solution A (water with 0.06% trifluoroacetic acid) with different ratios.

NMR spectrum were recorded on Jeol-ECA-400 spectrometer (400MHz).

All the conversion of phosphine-addition peptide and protein was estimated by UV absorption at 215 nm of the peak in RP-HPLC spectra of the reaction mixture. Conversion was defined as the percentage of phosphine-addition peptide or protein peaks versus the sum of all peptide or protein peaks.

Fluorescence of ThT was monitored by plate reader (Bioteck, Synergy 4 Plate Reader)

Protein structures: PDB: 2kkw.

Results and Discussion

^{31}P NMR analysis of compound **3b**

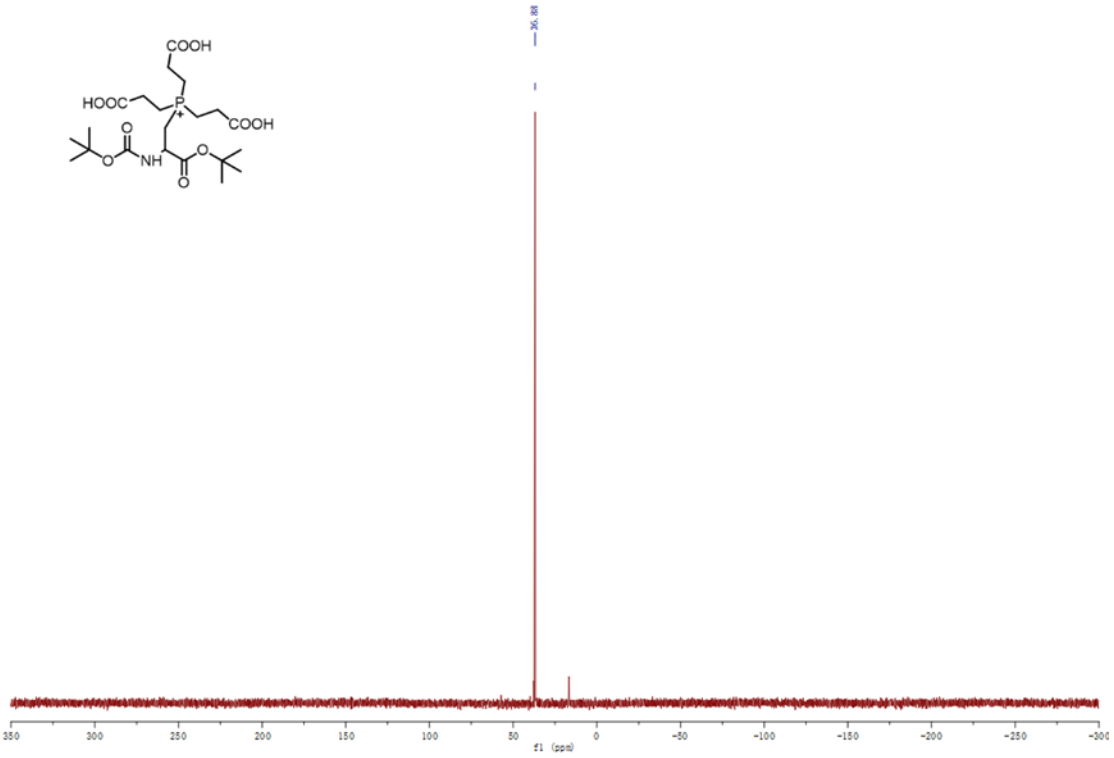


Figure S1. The chemical shift of **3b** in ^{31}P NMR (243 Hz, D_2O) analysis, which indicated that the β-phosphonium cation were formed.

Stability test

Compound **3b** (ESI-MS: m/z calculated for $C_{21}H_{37}NO_{10}P$: 494.5, $[M]^+$. Found: 494.1, $[M]^+$) was dissolved in water for 20 mM solution, then 100 μL of the solution was added in five 1.5 mL Eppendorf tubes, respectively. 100 μL of 0.1 M HCl (pH=1.0), 0.1 M NaOH (pH=13.0), 0.1 M dithiothreitol solution, 0.1 M reduced glutathione solution was added into four tubes respectively and the other one was exposure to air, incubating at 37 °C for 72 h, the solutions were analyzed by ESI-MS.

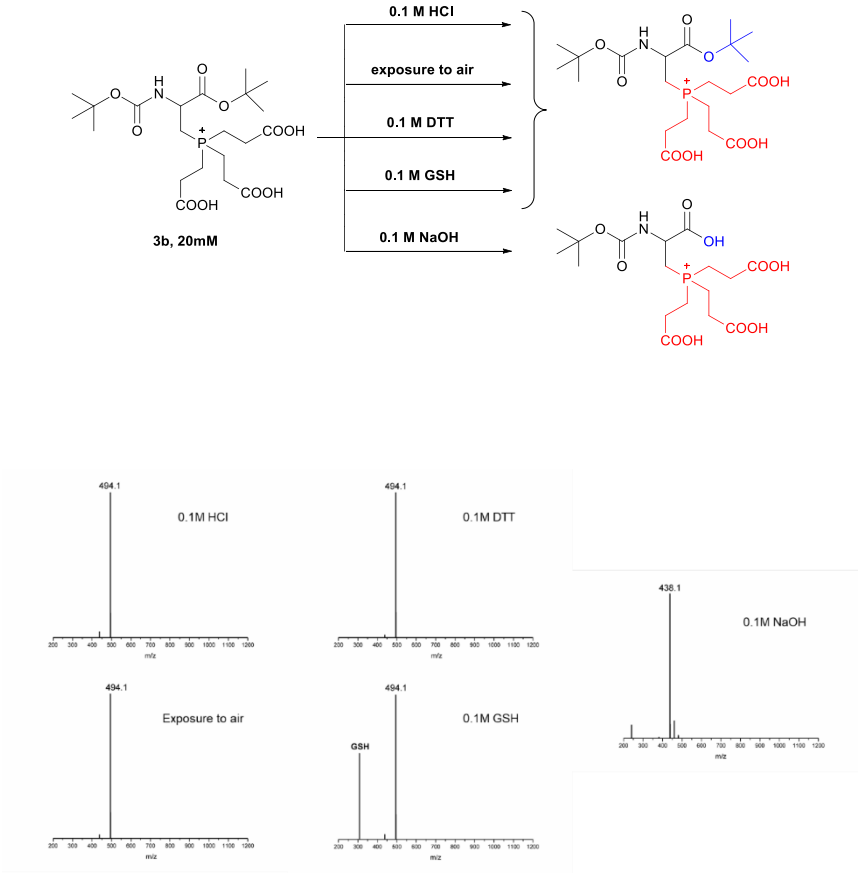


Figure S2. Stability test of compound **3b**. The solutions mass data indicated that no β-phosphonium cation degradation was happened, except in the 0.1 M NaOH condition, which indicated the tert-butyl group was hydrolyzed.

peptide **6b** (ESI-MS: m/z calculated for $C_{37}H_{54}N_8O_{17}P^+$: 913.8, $[M]^+$. Found: 913.5, $[M]^+$; 457.2, $[M+H]^{2+}$) was dissolved in water for 1 mM solution, then 100 μL of the solution was added in five 1.5 mL Eppendorf tubes, respectively. 100 μL of 0.1 M HCl (pH=1.0), 0.1 M NaOH (pH=13.0), 0.1 M dithiothreitol solution, 0.1 M reduced glutathione solution was added into four tubes respectively and the other one was exposure to air, incubating at 37 °C for 72 h, the solutions were analyzed by ESI-MS.

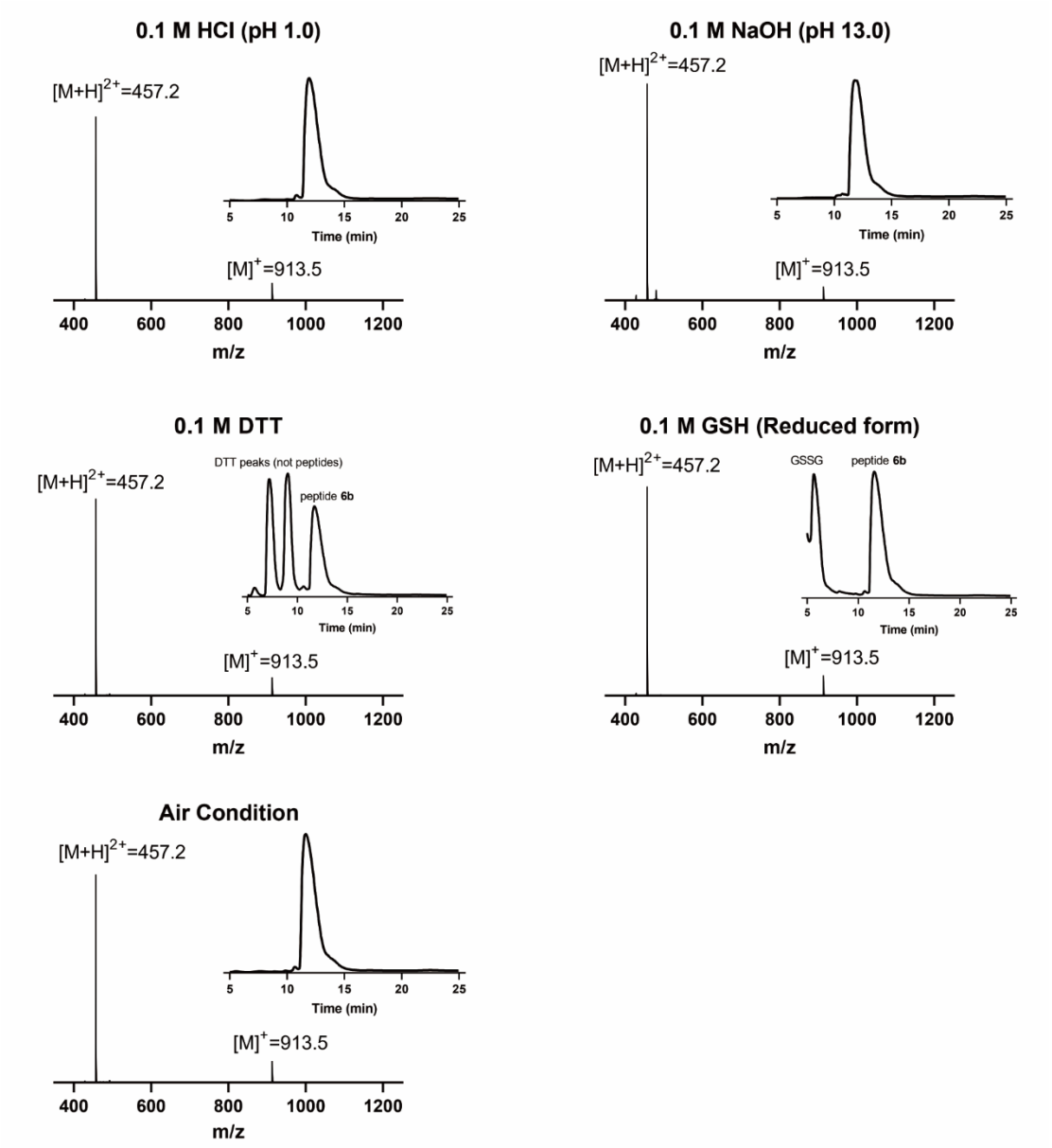


Figure S3. Stability test of compound **6b**. The solutions mass data indicated that no β-phosphonium cation degradation was happened in peptide level.

Expression and purification of recombinant proteins

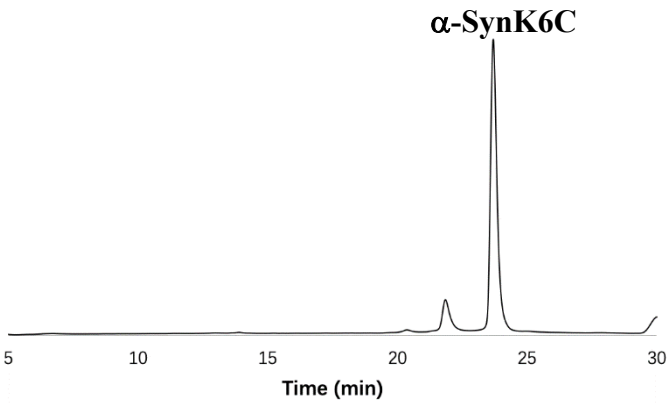
Expression and purification of recombinant WT α-Syn

WT α-syn was overexpressed and purified as previously described. [1] The E. coli BL21(DE3) was transformed with the plasmid pET22b encoding WT α-syn. The expression and extraction of recombinant WT α-syn were as follows. All the cultures were performed in LB medium with 100 μg/ml ampicillin at 37 °C. The overnight culture of transformed BL21(DE3) was induced with 100x of 100 μM IPTG. The supernatant containing WT α-syn after osmotic shock treatment was further purified with RP-HPLC with a Proteonavi column with a linear gradient of 30−70% B for 30 min at a flow rate of 4 mL/min.The purified WT α-syn was lyophilized and stored at -80 °C until use. Characterization of WT α-syn was characterized by analytical HPLC and ESI-MS.[2]

Expression and purification of recombinant α-SynK6C

The E. coli BL21(DE3) was transformed with the plasmid. The expression and extraction of recombinant α-SynK6C were the same as WT α-Syn. The supernatant from the osmotic shock treatment containing α-Syn was first treated with 1 eq TCEP·HCl to prevent disulfide bond formation, then adjusted pH=9.0 and further purified with preparative HPLC using Proteonavi column with a linear gradient of 30−70% B for 30 min at a flow rate of 4 mL/min. The purified α-SynK6C was lyophilized and stored at −80 °C until use. Characterization of α-SynK6C was characterized by analytical HPLC and ESI-MS.

a)



b)

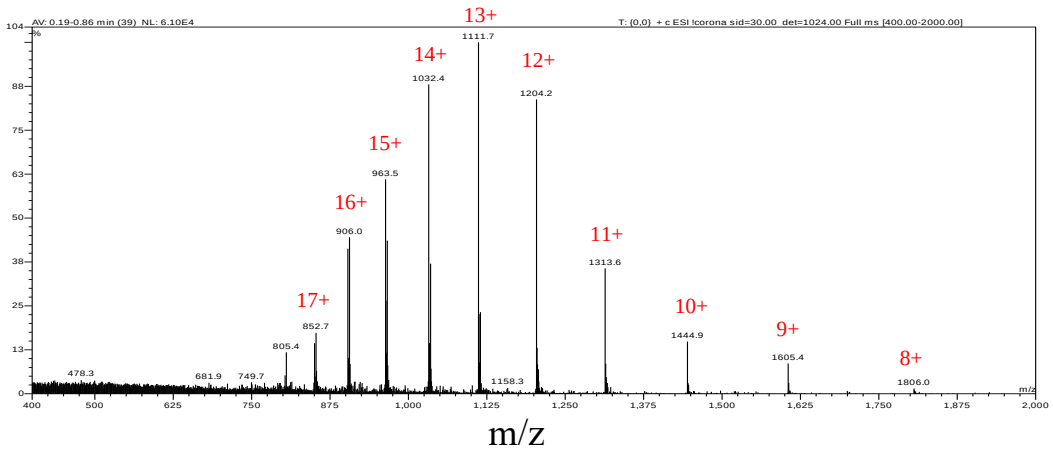
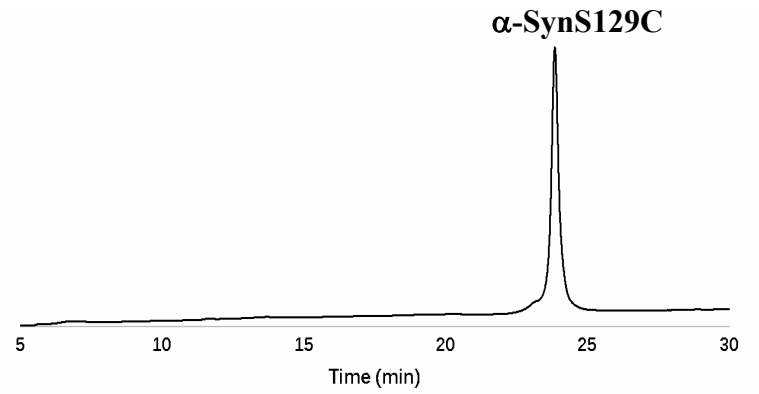


Figure S4. a) Analytic HPLC trace of α-SynK6C. (HPLC gradient is 30% to 70% of solution B in 30 min on the analytic C4 column (λ=215 nm). b) ESI-MS: Calculated: 14435, $[M+H]^+$. Found: 14439, $[M+H]^+$.

Expression and purification of recombinant α-SynS129C

The E. coli BL21(DE3) was transformed with the plasmid. The expression and extraction of recombinant α-SynS129C were the same as WT α-Syn. The supernatant from the osmotic shock treatment containing α-SynS129C was first treated with 1 eq TCEP·HCl to prevent disulfide bond formation, then adjusted pH=9.0 and further purified with preparative HPLC using Proteonavi column with a linear gradient of 30−70% B for 30 min at a flow rate of 4 mL/min. The purified α-SynS129C was lyophilized and stored at −80 °C until use. Characterization of α-SynS129C was characterized by analytical HPLC and ESI-MS.

a)



b)

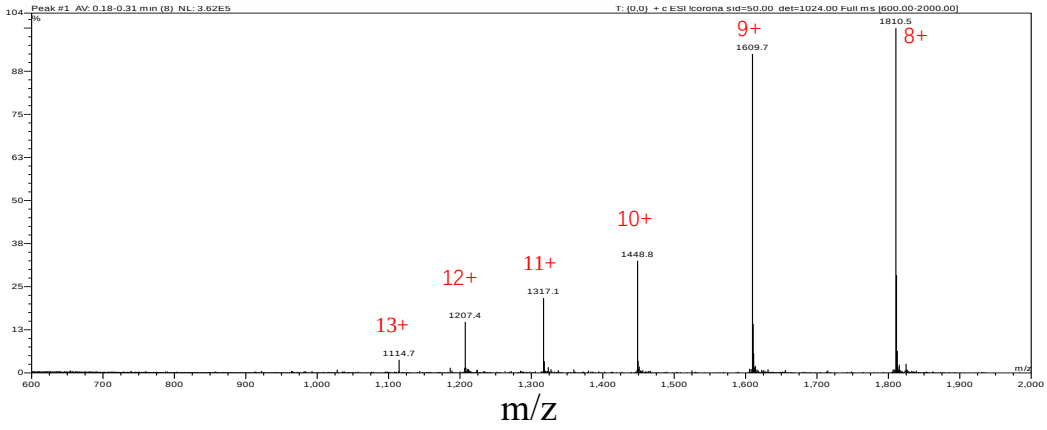


Figure S5. a) Analytic HPLC trace of α-SynS129C. (HPLC gradient is 30% to 70% of solution B in 30 min on the analytic C4 column (λ=215 nm). b) ESI-MS: Calculated: 14476, $[M+H]^+$. Found: 14478, $[M+H]^+$.

Confocal experiment of MCF-7 cells

The cell model used in confocal experiment is the human breast cancer cell MCF-7 cell line. The cell was cultured with DMEM (Dulbecco's Modified Eagle's Medium; Gibco) with 10% FBS (Fetal Bovine Serum) and 1% of penicillin-streptomycin (v/v), 37 °C and 5% CO_2.

Confocal experiment: Adding clean round coverslips to a 24-well plate, then adding 50,000 MCF-7 cells (500 μL), and placing in the cell incubator for 16 h. After removing the medium, adding 300 μL of opti-MEM medium with 5 μM peptides **5j** and **7k**, respectively. Then the medium was incubated in the cell incubator for 24 h. After incubation, the cells were incubated with 400 nM of MitoTracker Red CMXRos for 30 min, and then the cells were fixed with 4% paraformaldehyde solution for 30 min. Finally, the cells were incubated with 1 μg / mL of DAPI solution for 10 min.

ThT fluorescence assay and lag time calculation.

Protein aggregates were prepared by incubating purified proteins (65.5 μM in PBS) at 37 °C in the presence of a glass bead with constant agitation for 90 h, respectively. The final concentration of ThT was 10 μM, and WT α-Syn, protein **9a**, **9b** and **9l** were 3.5 μM, respectively. PBS solution as the control group. The ThT fluorescence (excitation, 440nm; emission, 480 nm) was measured in 96-well plates with the plate reader. The kinetics curves were fitted with sigmoidal function ($I = I_{min} + (I_{max} - I_{\min})/(1 + \exp\left[-k\left(t - t_{\frac{1}{2}}\right)\right]$) . The average lag time was resulted with $\tau_{lag} = t_{1/2} - 2/k_{max}$.

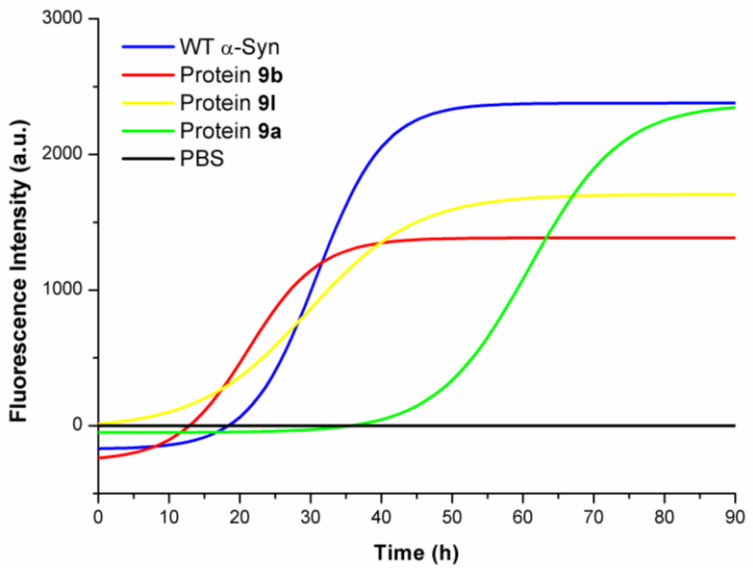


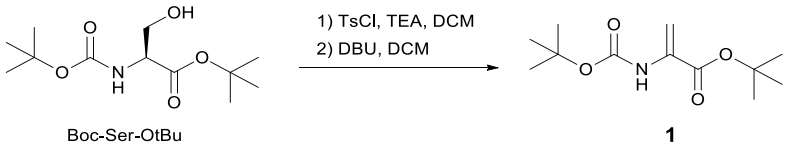
Figure S6. Fitted ThT kinetic traces of WT α-Syn, proteins **9a**, **9b** and **9l**.

Table S1. Average lag time and extra net charge of WT α-Syn, proteins **9a**, **9b** and **9l**

	WT α-Syn	protein **9a**	protein **9b**	protein **9l**
Average τlag (h)	**21.50**	**47.84**	**10.81**	**14.32**
Extra net charge(s)	**0**	**+1**	**-2**	**-1**

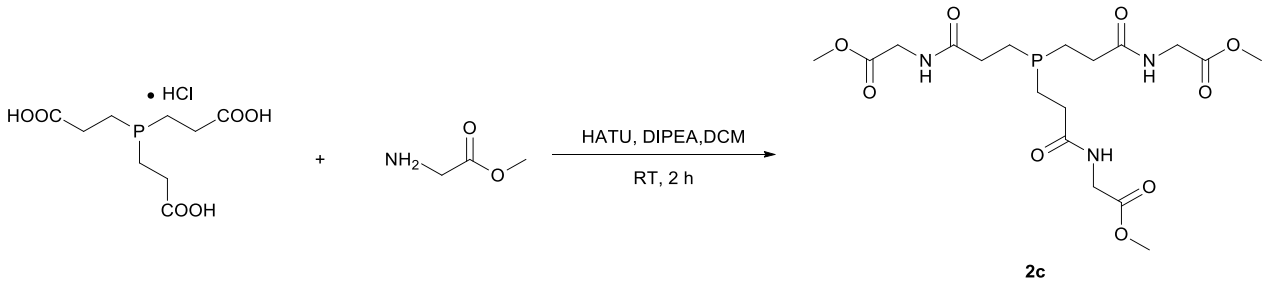
Synthesis of small moleculars

The synthesis of compound **1** was performed by the procedure previously described.[3] To a stirred solution of tert-butoxycarbonyl-L-serine tert-butyl ester (7.83 g, 30.0 mmol) in DCM (160 mL) was added trimethylamine (5.68 mL, 39.0 mmol). Methanesulfonyl chloride (3.1 mL, 39.0 mmol) was then slowly added at 0 °C, and the mixture was stirred at room temperature overnight. Another 160 mL DCM was added to the mixture and the organic phase was washed with water (150 mL×2) and saturated $NaHCO_3$ (150 mL×1), dried with $MgSO_4$ and concentrated in vacuo. The obtained residue was redissolved in DCM (100 mL), and DBU (5.5 mL, 36.0 mmol) was added at room temperature. The resultant solution was then allowed to be stirred for two more hours, and diluted with 150 mL DCM. The organic phase was washed with 1M $KHSO_4$ (150 mL×1) and brine (150 mL×1), dried over $MgSO_4$ and concentrated in vacuo. The residue was then purified by silica gel column chromatography (petrol ether/ethyl acetate = 30/1) to afford compound 1 (6.24 g, 84%) as pale yellow oil. Rf=0.64 (petrol ether/ethyl acetate = 9/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (s, 1H), 6.04 (s, 1H), 5.60 (d, 1H), 1.48 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.10, 152.69, 132.52, 104.02, 82.62, 80.46, 28.33, 27.98; ESI-MS: m/z calculated for $C_{12}H_{21}NO_4$: 244.2, $[M+H]^+$. Found: 244.2, $[M+H]^+$.



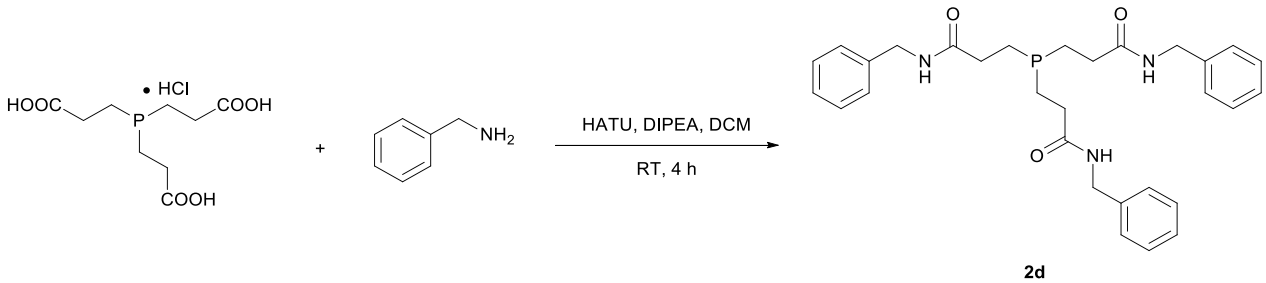
Scheme S1. Synthesis of compound **1**.

143 mg (0.5 mmol) tris(2-carboxyethyl)phosphine hydrochloride, 684.43 mg HATU (1.8 mmol, 3.6 eq) and 627 μL DIPEA (3.6 mmol, 7.2 eq) were dissolved into 6 mL DCM, then 250 mg (2.0 mmol, 4 eq) methyl 2-aminoacetate was added to the mixture. The reaction was stirred at RT for 16 h at N_2 atmosphere. The mixture then was washed by water for three times and combined the water layer. The crude product was purified with RP-HPLC and lyophilized to obtain white powder compound **2c** 110 mg with 47% yield. ^{1}H NMR (400 MHz, D_2O): δ 4.01 (s, 6H), 3.74 (s, 9H), 2.89 (m, 6H), 2.57 (m, 6H); ^{13}C NMR (100 MHz, D_2O): δ 173.47, 172.17, 54.38, 52.84, 41.27, 28.30, 17.72, 16.25, 14.99, 14.46; ESI-MS: m/z calculated for $C_{18}H_{30}N_3O_9P$: 464.4, $[M+H]^+$. Found: 464.1, $[M+H]^+$.



Scheme S2. Synthesis of compound **2c**.

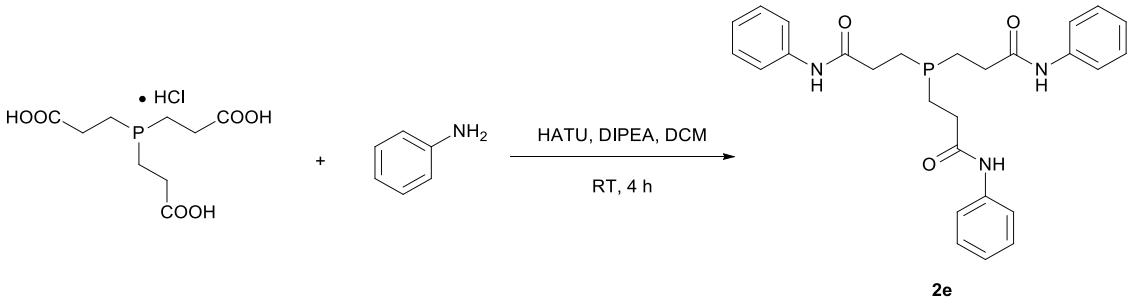
72 mg (0.25 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 342.2 mg HATU (0.9 mmol, 3.6 eq) were dissolved into 6 mL DCM, then 109 mg (4 eq, 1 mmol) benzylamine and 315 μL DIPEA (1.8 mmol, 7.2 eq) were added to the mixture. The reaction was stirred at RT for 4 h at N_2 atmosphere. The crude product was precipitated from the solution. Then the solution was concentrated in vacuo and the white precipitate was washed with acetonitrile for three times, centrifuging (4 °C, 12000 rpm, 10 min) with a high speed centrifuge to obtain white solid product **2d** 95.0 g with 73% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.31 (s, 3H), 7.17-7.29 (m, 15H), 4.21-4.23 (d, 6H), 2.18-2.24 (m, 6H), 1.60-1.64 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.48, 140.06, 128.79, 127.76, 127.24, 42.65, 32.02, 22.18; ESI-MS: m/z calculated for $C_{30}H_{36}N_3O_3P$: 518.6, $[M+H]^+$. Found: 518.1, $[M+H]^+$.



Scheme S3. Synthesis of compound **2d**.

72 mg (0.25 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 342.2 mg HATU (0.9 mmol, 3.6 eq) were dissolved into 6 mL DCM, then 93 μL (4 eq, 1 mmol) aniline and 315 μL DIPEA (1.8 mmol, 7.2 eq) were added to the mixture. The reaction was stirred at RT for 4 h at N_2 atmosphere. The crude product was precipitated from the solution. Then the solution was concentrated in vacuo and the white precipitate was washed with acetonitrile for three times, centrifuging (4 °C, 12000 rpm, 10min) with a high speed centrifuge to obtain white solid product **2e** 87.3g with 73% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.77 (s, 3H), 7.39-7.41 (m,

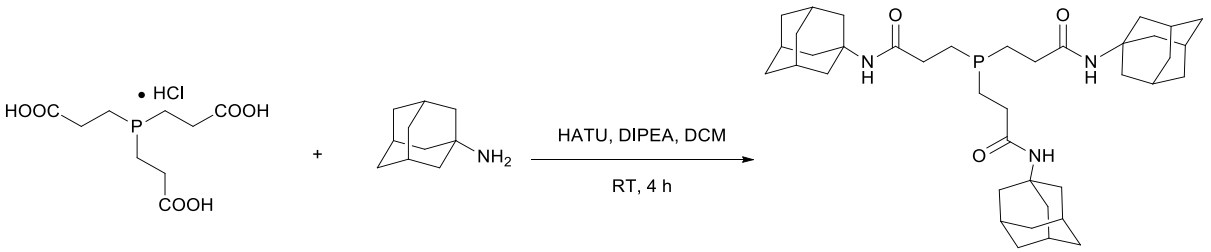
6H), 7.08-7.11 (m, 6H), 6.81-6.85 (m, 3H), 2.24-2.31 (m, 6H), 1.57-1.61 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.51, 139.81, 129.20, 123.52, 119.54, 33.17, 21.95. ESI-MS: m/z calculated for $C_{27}H_{30}N_3O_3P$: 476.5, $[M+H]^+$. Found: 476.2, $[M+H]^+$.



2e

Scheme S4. Synthesis of compound **2e**.

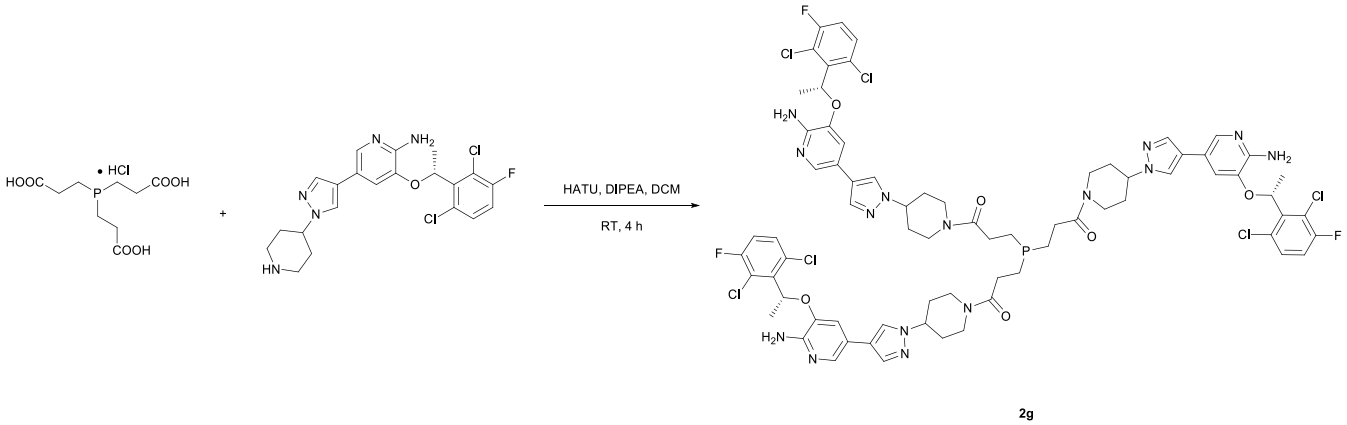
72 mg (0.25 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 342.2 mg HATU (0.9 mmol, 3.6 eq) were dissolved into 6 mL DCM, then 136 mg (3.6 eq, 0.9 mmol) amantadine and 315 μL DIPEA (1.8 mmol, 7.2 eq) were added to the mixture. The reaction was stirred at RT for 4 h at N_2 atmosphere. The solution was concentrated in vacuo and the residue was dissolved with water and DMF. The crude product was purified with RP-HPLC and lyophilized to obtain white powder compound **2f** 85.0 mg with 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.27 (s, 3H), 2.69-2.74 (m, 6H), 2.40-2.46 (m, 6H), 2.05 (s, 9H), 1.96 (s, 18H), 1.66(s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.75, 52.57, 41.34, 36.32, 30.55, 29.44, 13.89. ESI-MS: m/z calculated for $C_{39}H_{60}N_3O_3P$: 650.9, $[M+H]^+$. Found: 650.5, $[M+H]^+$.



2f

Scheme S5. Synthesis of compound **2f**.

21.5 mg (0.075 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 100 mg HATU (0.26 mmol, 3.6 eq) were dissolved into 6 mL DCM, then 105 mg (3 eq, 0.23 mmol) Crizotinib and 92 DIPEA (0.53 mmol, 7.2 eq) were added to the mixture. The reaction was stirred at RT for 4 h at N_2 atmosphere. The solution was concentrated in vacuo and the residue was dissolved with acetonitrile and DMF. The crude product was purified with RP-HPLC and lyophilized to obtain white powder compound **2g** 35.0 mg with 30% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.10 (s, 3H), 7.78 (s, 3H), 7.65 (s, 3H), 7.58 (m, 3H), 7.47 (m, 3H), 7.11 (s, 3H), 6.26 (d, 3H), 4.45 (d, 3H), 3.94 (m, 3H), 3.24 (t, 3H), 2.83 (m, 9H), 2.44- 2.38 (m, 6H), 2.20-1.56 (m, 21H). ESI-MS: m/z calculated for $C_{72}H_{75}Cl_6F_3N_{15}O_6P$: 1548.2, $[M+H]^+$. Found: 1548.0, $[M+H]^+$.

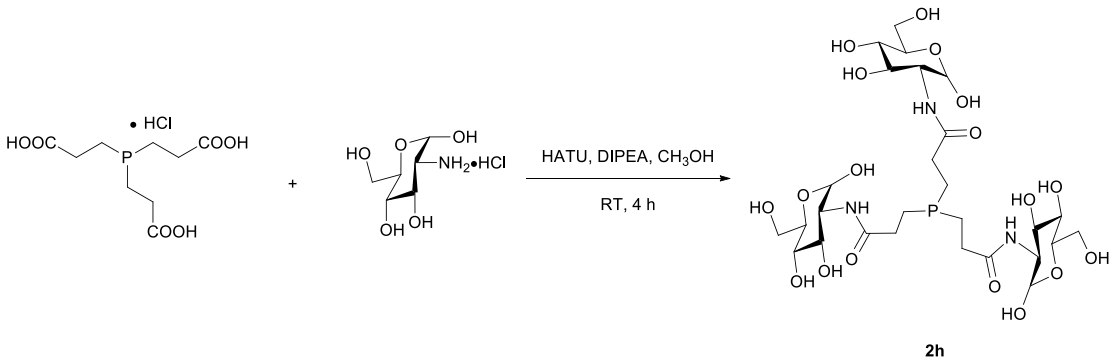


2g

Scheme S6. Synthesis of compound **2g**.

72 mg (0.25 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 342.2 mg HATU (0.9 mmol, 3.6 eq) were dissolved into 6 mL methanol, then 194 mg (3.6 eq, 0.9 mmol) D-Glucosamine hydrochloride and 315 μL DIPEA (1.8 mmol, 7.2 eq) were added to the mixture. The reaction was stirred at RT for 4 h at N_2 atmosphere. The solution was concentrated in vacuo and the residue was dissolved with 10 mL water, then washed with 6 mL DCM for three times. The water phase was washed lyophilized to obtain white solid and then purified with RP-HPLC, and lyophilized to obtain white powder compound **2h** 91.0 mg with 50% yield.

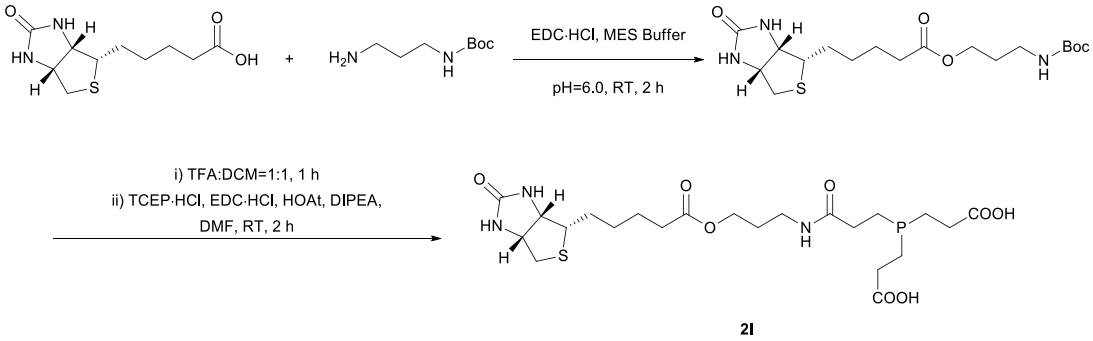
^{1}H NMR (400 MHz, D_2O): δ 5.13 (d, 2H), 4.64 (d, 1H), 3.90-3.75 (m, 6H), 3.73-3.60 (m, 6H), 3.59-3.27 (m, 6H), 2.84-2.55 (m, 6H), 2.52-2.09 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ = 173.14, 94.83, 90.82, 75.95, 73.82, 71.56, 70.72, 70.06, 69.86, 60.71, 60.54, 56.75, 54.17, 28.85, 28.56, 15.16, 14.64 ;ESI-MS: m/z calculated for $C_{27}H_{48}N_3O_{18}P$: 734.6, $[M+H]^+$. Found: 734.2, $[M+H]^+$.



2h

Scheme S7. Synthesis of compound **2h**.

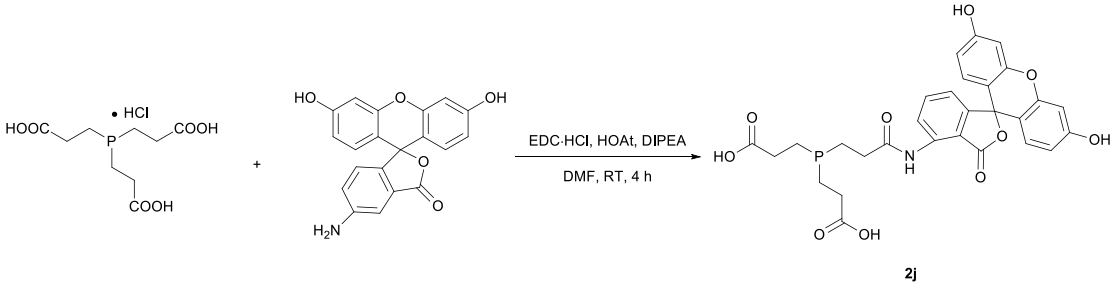
The synthesis of compound **2i** was performed similarly to the procedure previously described.[4,5] 100 mg (0.41 mmol) Biotin and 95.9mg (0.5 mmol) EDC·HCl were dissolved into 5.0 mL MES Buffer (100 mM), then 107 mg (0.61 mmol) N-Boc-1,3-diaminopropane was added to the mixture and adjust pH to 6.0. The reaction was stirred at RT for 2 h, and purified with RP-HPLC and lyophilized to obtain 40 mg white solid. The lyophilized product was dissolved in 3 mL 1:1 mixture of TFA and DCM, stirred at RT for 1 h. The solution was concentrated in vacuo to obtain the residue. 19.0 mg (0.10 mmol) EDC·HCl and 24 mg (0.18 mmol) HOAt was dissolved into 2 mL DMF and the mixture was added into the residue, stirred at RT for 2 h at N2 atmosphere. Then 86 mg (0.3 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 87.5 μL DIPEA (0.50 mmol) was dissolved into 2 mL DMF and the mixture was added into the stirred solvent and stirred at RT for another 2 h at N_2 atmosphere. Finally the solution was concentrated in vacuo to obtain the residue, dissolving with DMF and water, then the crude product was purified with RP-HPLC and lyophilized to obtain white powder compound **2i** 30.0 mg with 14% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.03 (t, 1H), 7.77 (t, 1H), 6.43 (s, 2H), 4.32-4.11 (m, 4H), 7.47 (m, 3H), 3.10-2.79 (m, 6H), 2.70-2.38 (m, 9H), 2.20-1.35 (m, 14H), 1.28 (m, 2H), 3.94 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 173.82, 172.05, 171.11, 162.78, 61.08, 59.25, 55.43, 36.59, 36.25, 35.25, 29.20, 28.62, 28.06, 26.10, 25.34, 22.97. ESI-MS: m/z calculated for $C_{22}H_{36}N_3O_8PS$: 533.6, $[M+H]^+$. Found: 533.1, $[M+H]^+$.



2l

Scheme S8. Synthesis of compound **2i**.

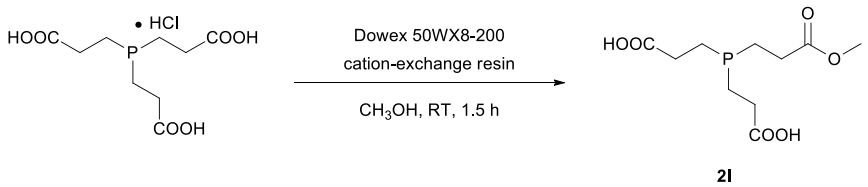
69.5 mg (0.20 mmol) Fluoresceinamine, 153.36 mg (0.80 mmol) EDC·HCl, 109 mg HOAt (0.80 mmol), 200.0 mg (0.80 mmol) tris(2-carboxyethyl)phosphine hydrochloride and 264 μL DIPEA (1.60 mmol) were dissolved into 15.0 mL DMF and the mixture was stirred at RT for 4 h at N_2 atmosphere. The solution was concentrated in vacuo and the residue was dissolved with water and DMF. The crude product was purified with RP-HPLC and lyophilized to obtain white powder compound **2j** 35.0 mg with 30% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.20 (s, 2H), 8.82 (d, 1H), 8.45 (s, 1H), 8.24 (dd,1H), 7.37 (d, 1H), 6.84 (5, 1H), 6.70 (d, 2H), 6.59-6.53 (m, 4H), 3.30 (m, 2H), 2.99 (m, 2H), 1.66–1.37 (m, 12H). ESI-MS: m/z calculated for $C_{29}H_{26}NO_{10}P$: 580.5, $[M+H]^+$. Found: 580.2, $[M+H]^+$.



2j

Scheme S9. Synthesis of compound **2j**.

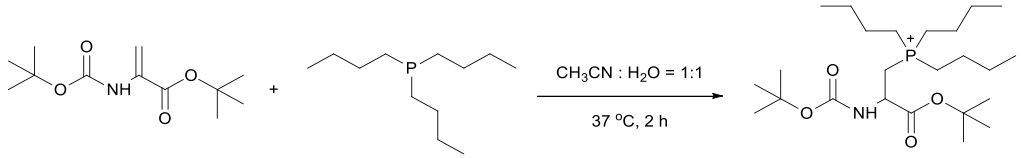
The synthesis of compound **2l** was performed by the procedure previously described.[6] Tris(2-carboxyethyl)phosphine hydrochloride (575 mg in 11 mL of methanol) was stirred at room temperature with 50 mg of treated Dowex 50WX8-200 cation-exchange resin at RT for 1.5 h. Then the resin was removed by filtration and the filtrate was concentrated to afford a pale yellow oil. The oil was dissolved in 0.6% TFA in water and purified with RP-HPLC, and lyophilized to obtain a pale oil **2l** 72mg with 12% yield. ^{1}H NMR (400 MHz, D_2O) δ = 3.67 (s, 3H), 2.81 (d, 6H), 2.52 (d, 6H); ^{13}C NMR (100 MHz, D_2O) δ = 175.87, 174.01,, 52.82, 27.77, 27.30, 14.40, 13.89. ESI-MS: m/z calculated for $C_{10}H_{17}O_6P$: 265.2, $[M+H]^+$. Found: 265.1, $[M+H]^+$.



2l

Scheme S10. Synthesis of compound **2l**.

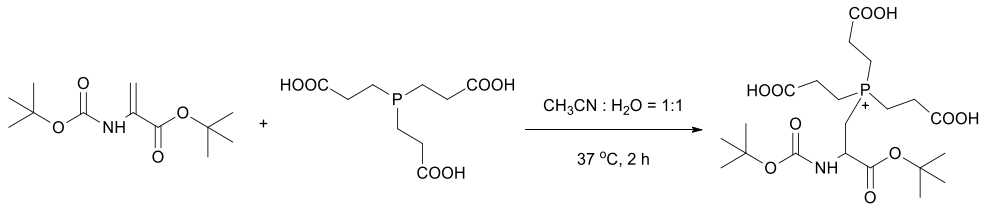
11.0 mg (0.045 mmol) compound **1** was dissolved in 400 µL 1:1 mixture of acetonitrile and sodium phosphate buffer (50 mM, pH 8.0) in a 2.0 ml Eppendorf tube, then 15 µL (1.5 eq 0.068 mmol) tributyl phosphine (**2a**) was added into the tube and vortexed at 37 °C for 2 h. The reaction was monitored with RP-HPLC and ESI-MS. ESI-MS: m/z calculated for $C_{24}H_{49}NO_4P$: 446.6, $[M+H]^+$. Found: 446.3, $[M+H]^+$.



1 **2a** **3a**

Scheme S11. Synthesis of compound **3a**.

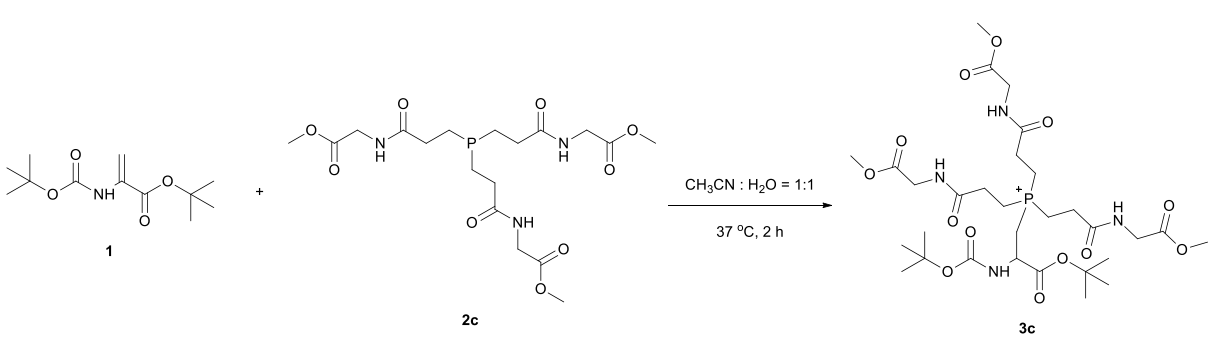
11.0 mg (0.045 mmol) compound **1** was dissolved in 400 µL 1:1 mixture of acetonitrile and sodium phosphate buffer (50 mM, pH 8.0) in a 2.0 ml Eppendorf tube, then 19mg (1.5 eq 0.068 mmol) tris(2-carboxyethyl)phosphine (**2b**) was added into the tube and vortexed at 37 °C for 2 h. The reaction was monitored with RP-HPLC and ESI-MS. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.85 (t, 1H), 3.52-3.58 (m, 6H), 3.37-3.47 (m, 6H), 2.45 (d, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.29, 174.16, 169.97, 169.82, 85.13, 82.46, 27.48, 25.77, 14.98. ESI-MS: m/z calculated for $C_{21}H_{37}NO_{10}P$: 494.5, $[M]^+$. Found: 494.1, $[M]^+$.



1 **2b** **3b**

Scheme S12. Synthesis of compound **3b**.

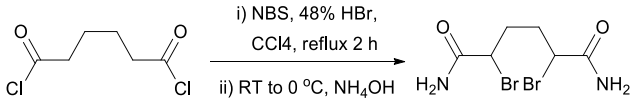
5.0 mg (0.021 mmol) compound **1** was dissolved in 500 µL 1:1 mixture of acetonitrile and sodium phosphate buffer (50 mM, pH 8.0) in a 2.0 ml Eppendorf tube, then 10 mg (1.0 eq 0.021 mmol) compound **2c** was added into the tube and vortexed at 37 °C for 2 h. The reaction was monitored with RP-HPLC and ESI-MS. ESI-MS: m/z calculated for $C_{30}H_{52}N_4O_{13}P$: 707.7, $[M]^+$. Found: 707.3, $[M]^+$.



1 **2c** **3c**

Scheme S13. Synthesis of compound **3c**.

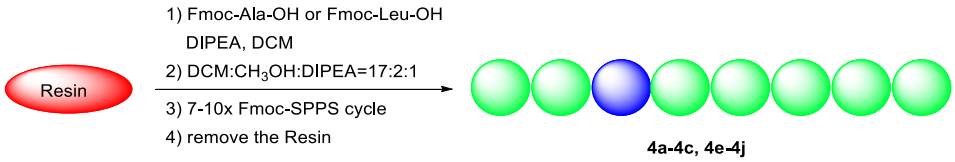
The synthesis of **DBHDA** was performed by the procedure previously described.[7] To a vigorously stirred solution of adipic dichlorid (9.20 g, 50.0 mmol) in CCl_4 (50 mL), NBS (22.30 g, 125.0 mmol) was added. 5 drops of 48% HBr aqueous solution was then slowly added into the solution, the solution was turned red and refluxed for 2 h to turn black. Then the solution was stirred at room temperature to 0 °C until all NBS was precipitated. NBS precipitation was removed by filtration and the solution was concentrated in vacuo to obtain dark red liquid, then 100 mL 25% ammonium hydroxide was cooled to 0 °C and dropped to the dark red liquid. The mixture was stirred for another 1 h and the crude product was precipitated from the solution. Filtrating the mixture to obtain black the black precipitation and was dried in drying oven at 70°C. The dried black precipitation was grinded into powder and was redissolved in 50 mL methanol and 50 mL deionized water, the solution was stirred at 60 °C for 30 minutes and cooled to 0 °C to filtrate the solution. The precipitation was washed by another 100 mL methanol and dried in drying oven to obtain 8.35 g white solid **DBHDA** with 55% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65 (s, 2H), 7.27 (s, 2H), 4.30 (m, 2H), 1.79-2.01 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.85, 169.79, 48.51, 48.23, 32.58, 32.47; ESI-MS: m/z calculated for $C_6H_{10}N_2O_2Br_2$: 302.9, $[M+H]^+$. Found: 302.9, $[M+H]^+$.



Scheme S14. Synthesis of 2,5-dibromohexanediamide (**DBHDA**).

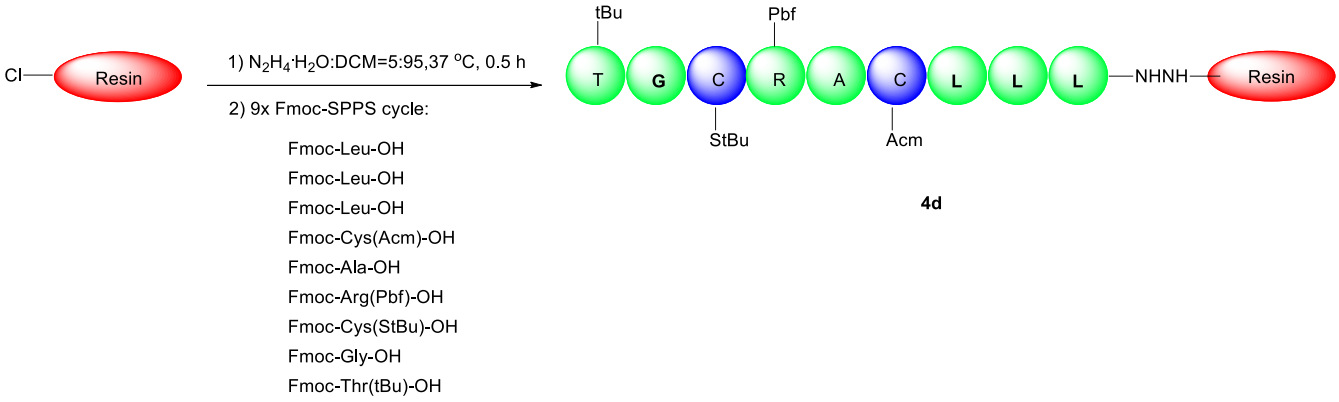
General Procedure for synthesis and purification of peptides **4a-4j**

Cysteine-containing peptides **4a-4c** and **4e-4j** were synthesized through Solid Phase Peptide Synthesis (SPPS) using Fmoc protocol. The synthesis was performed on 2-Cl Resin (loading 0.939 mmol/g) or Rink Amide MBHA Resin. The first Fmoc-amino acid (1.0 eq) was dissolved in right amount of DMF and mixed with diisopropylethylamine (DIPEA, 2.0 eq), then the solution was added into the Resin to couple the first amino acid at 37 °C for 2 h. The Resin was capped with DCM/CH_3OH/DIPEA (17/2/1, v/v/v). Fmoc group was deprotected with 20% piperidine in DMF for 15 minutes at 37 °C. Then 4.0 eq other Fmoc-amino acids were coupled with 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 4.0 eq), 1-hydroxy-7-azabenzotriazole (HOAt, 4.0 eq) and DIPEA (8.0 eq) in DMF for 1.5 h and removing Fmoc groups for 7-10 cycles. Peptide **4j** was coupled with 5-Carboxyfluorescein for another 12 h cycle. After the last Fmoc-amino acid was coupled on the resin, the Fmoc group was deprotected and the peptide was cleaved from resin with TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) or TFA/TIPS/EDT/H_2O (92.5/2.5/2.5/2.5) reagent. After precipitating with diethyl ether, the peptides were purified with RP-HPLC using C18 column and identified with ESI-MS, and then lyophilized to obtain peptide product.



Scheme S15. General Procedure for synthesis of peptides **4a-4c, 4e-4j**.

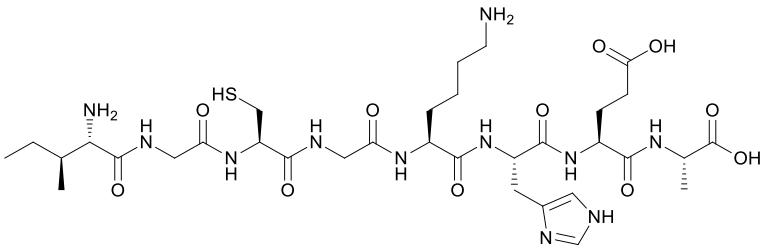
Peptide **4d** was synthesized through solid phase peptide synthesis (SPPS) using Fmoc-peptide-hydrazides protocol as previous report.[8] First the 2-Cl Resin (loading 0.939 mmol/g) was treated with $N_2H_4{\cdot}H_2O$/DCM (5/95, v/v) to afford hydrazine Resin. The synthesis steps were carried out with 9x Fmoc protocol but kept the peptide on the hydrazine Resin for the synthesis of **5d**.



Scheme S16. Synthesis of peptide **4d**.

Chemical structures and characterization of peptides **4a-4c** and **4e-4j**

a)



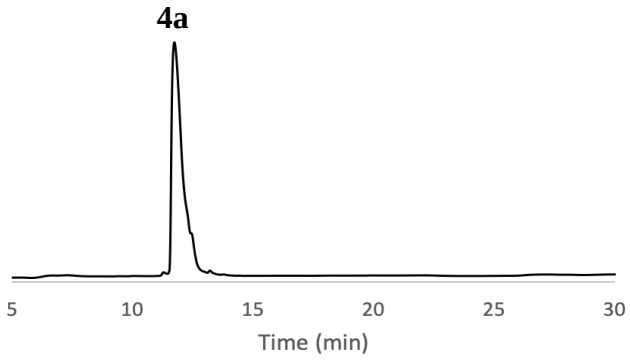
Ile-Gly-Cys-Gly-Lys-His-Glu-Ala

Chemical Formula: $C_{33}H_{55}N_{11}O_{11}S$

Exact Mass: 813.38

Molecular Weight: 813.92

b)



c)

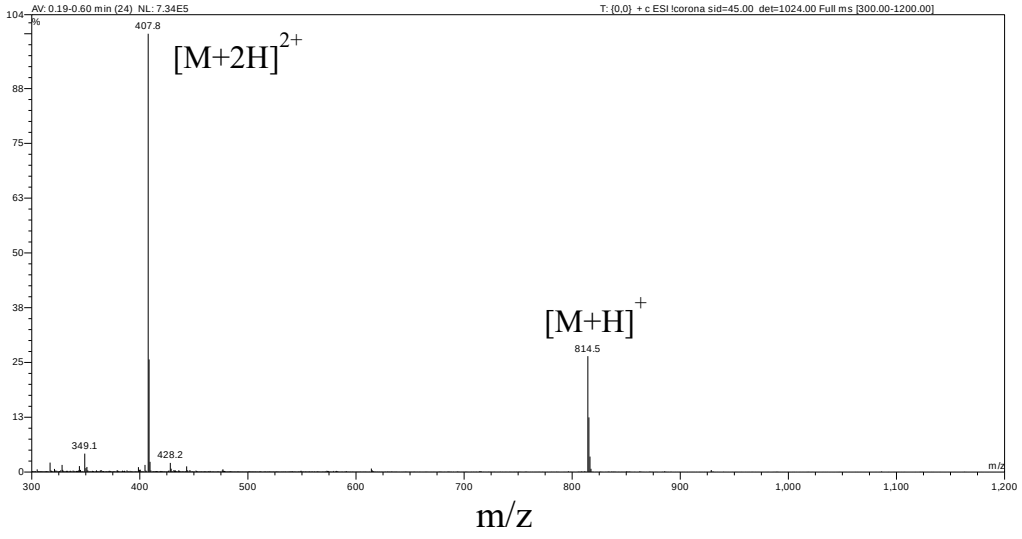
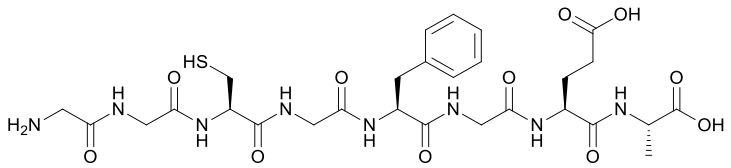


Figure S7. a) Chemical structure of peptide **4a**; b) RP-HPLC trace of peptide **4a**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{33}H_{55}N_{11}O_{11}S$: 814.9, $[M+H]^+$. Found: 814.5, $[M+H]^+$; 407.8, $[M+2H]^{2+}$.

a)



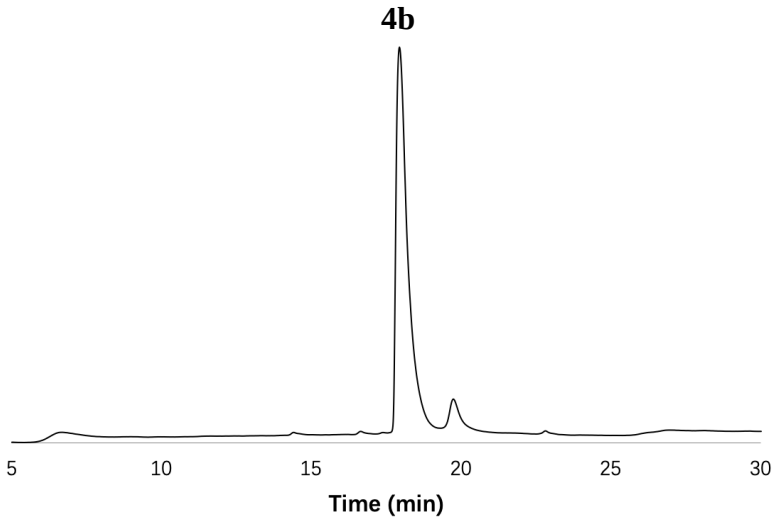
Gly-Gly-Cys-Gly-Phe-Gly-Glu-Ala

Chemical Formula: $C_{28}H_{40}N_8O_{11}S$

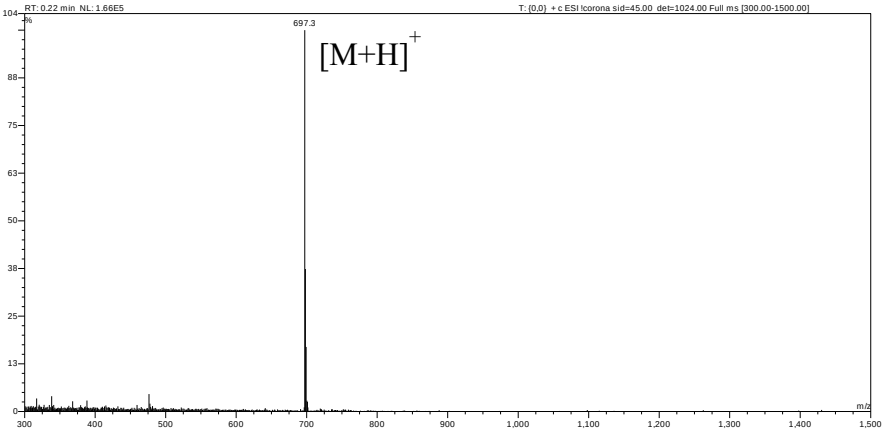
Exact Mass: 696.25

Molecular Weight: 696.73

b)



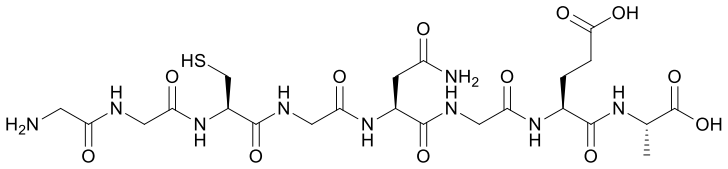
c)



m/z

Figure S8. a) Chemical structure of peptide **4b**; b) RP-HPLC trace of peptide **4b**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{28}H_{40}N_8O_{11}S$: 697.7, $[M+H]^+$. Found: 697.3, $[M+H]^+$.

a)



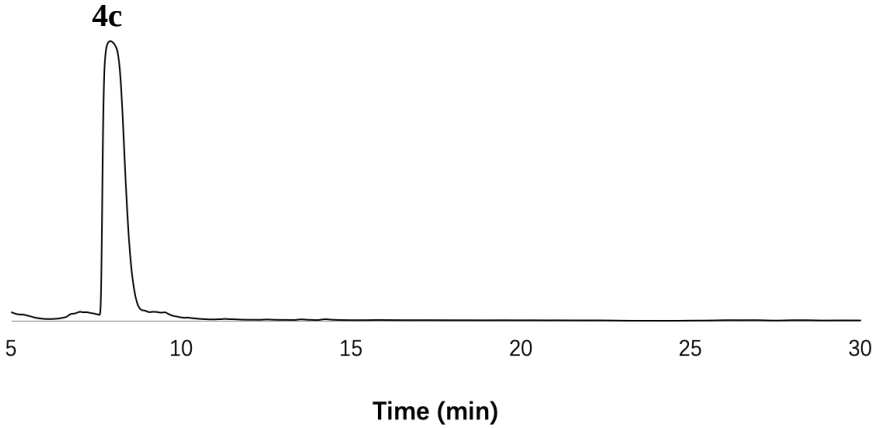
Gly-Gly-Cys-Gly-Asn-Gly-Glu-Ala

Chemical Formula: $C_{23}H_{37}N_9O_{12}S$

Exact Mass: 663.23

Molecular Weight: 663.66

b)



c)

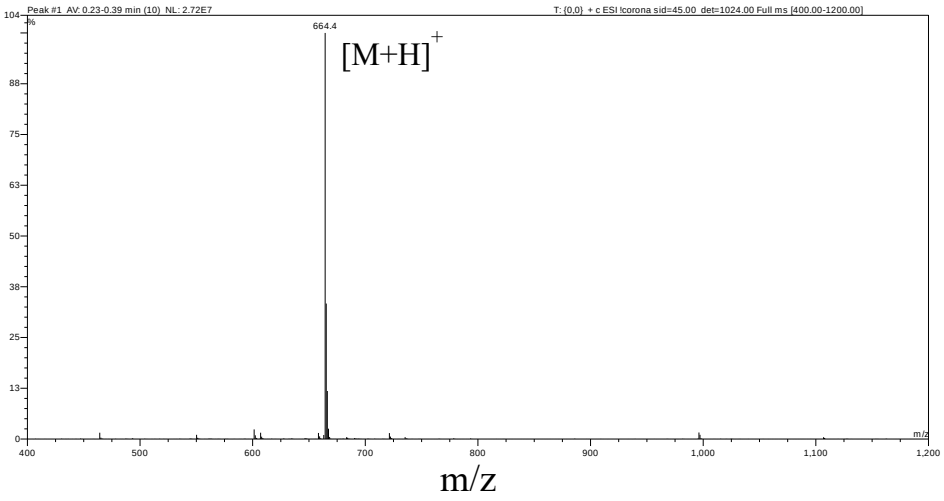
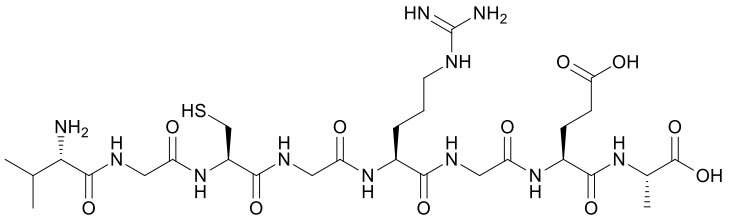


Figure S9. a) Chemical structure of peptide **4c**; b) RP-HPLC trace of peptide **4c**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{23}H_{37}N_9O_{12}S$: 664.7, $[M+H]^+$. Found: 664.4, $[M+H]^+$.

a)



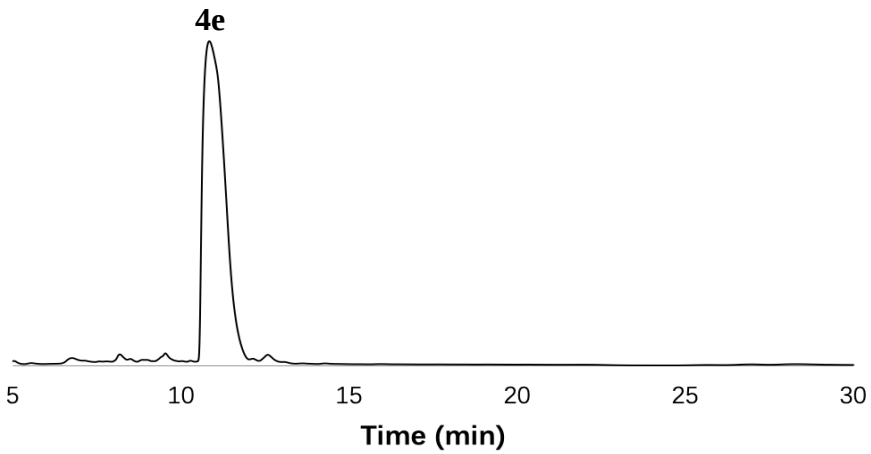
Val-Gly-Cys-Gly-Arg-Gly-Glu-Ala

Chemical Formula: $C_{28}H_{49}N_{11}O_{11}S$

Exact Mass: 747.33

Molecular Weight: 747.82

b)



c)

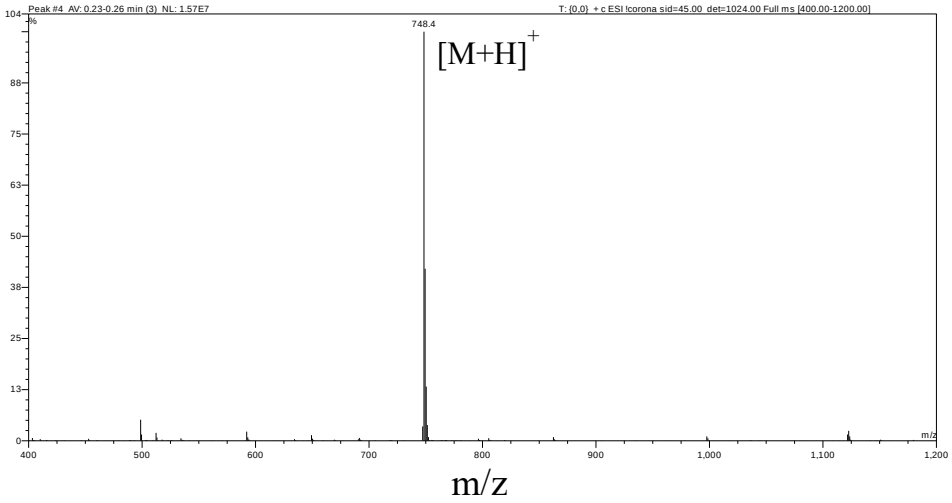
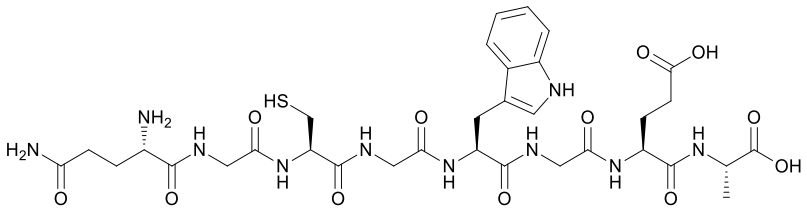


Figure S10. a) Chemical structure of peptide **4e**; b) RP-HPLC trace of peptide **4e**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{28}H_{49}N_{11}O_{11}S$: 748.8, $[M+H]^+$. Found: 748.4, $[M+H]^+$.

a)



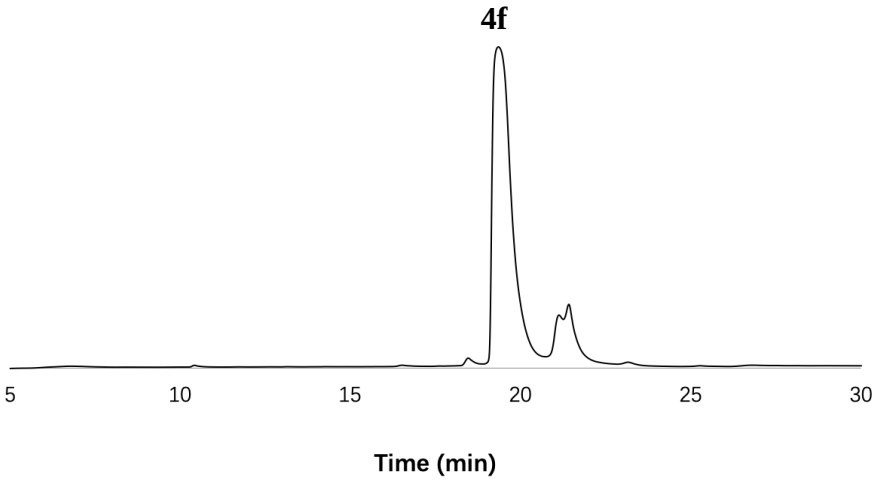
Gln-Gly-Cys-Gly-Trp-Gly-Glu-Ala

Chemical Formula: $C_{33}H_{46}N_{10}O_{12}S$

Exact Mass: 806.30

Molecular Weight: 806.84

b)



c)

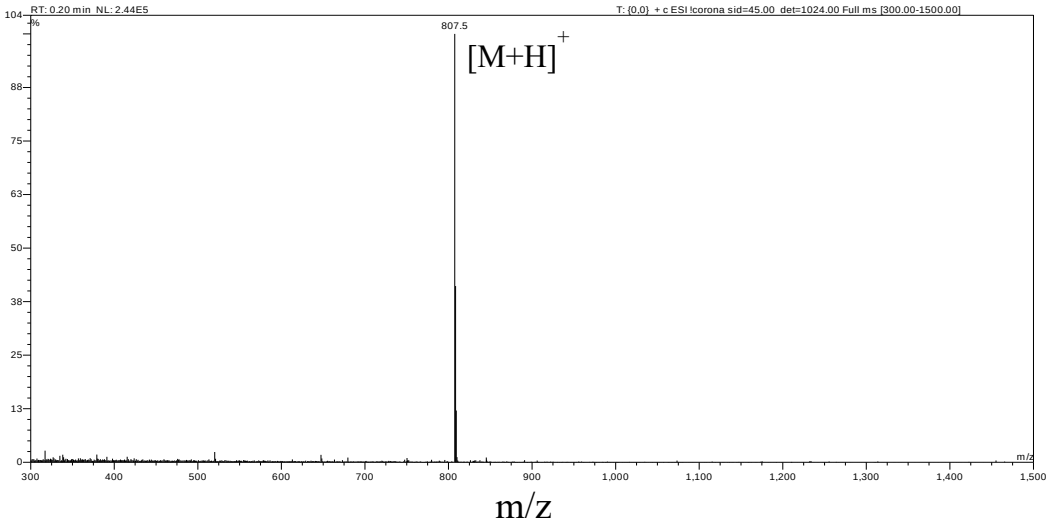
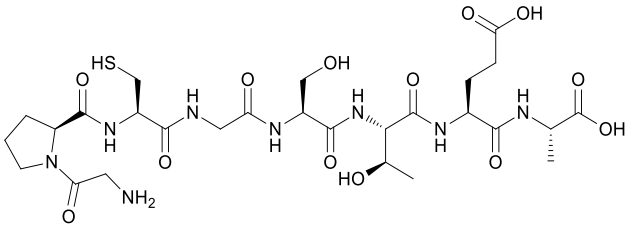


Figure S11. a) Chemical structure of peptide **4f**; b) RP-HPLC trace of peptide **4f**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{33}H_{46}N_{10}O_{12}S$: 807.8, $[M+H]^+$. Found: 807.5, $[M+H]^+$.

a)



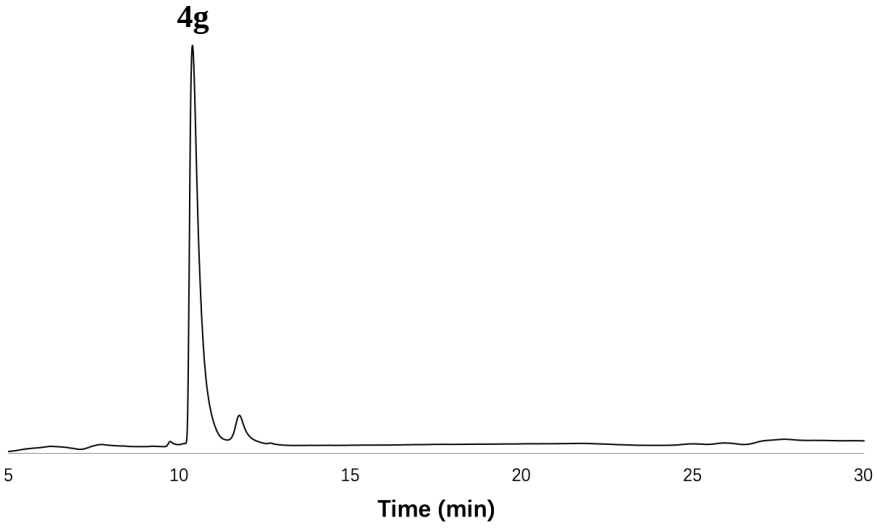
Gly-Pro-Cys-Gly-Ser-Thr-Glu-Ala

Chemical Formula: $C_{27}H_{44}N_8O_{13}S$

Exact Mass: 720.27

Molecular Weight: 720.75

b)



c)

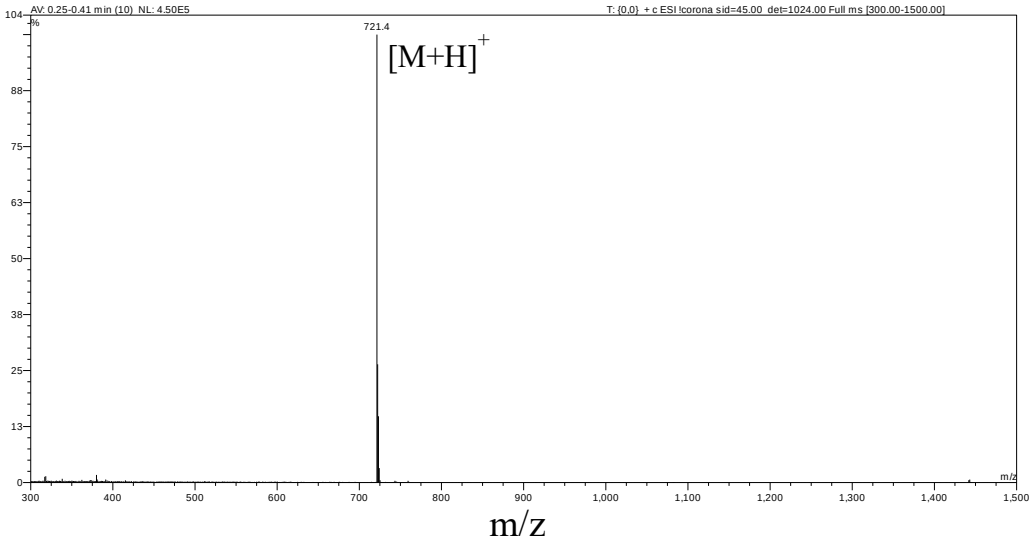
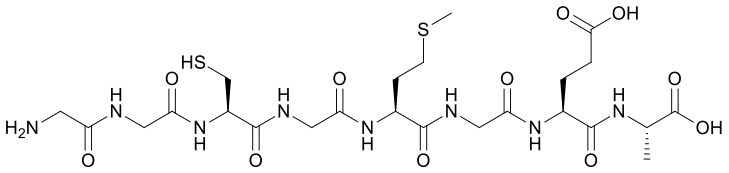


Figure S12. a) Chemical structure of peptide **4g**; b) RP-HPLC trace of peptide **4g**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{27}H_{44}N_8O_{13}S$: 721.8, $[M+H]^+$. Found: 721.4, $[M+H]^+$.

a)



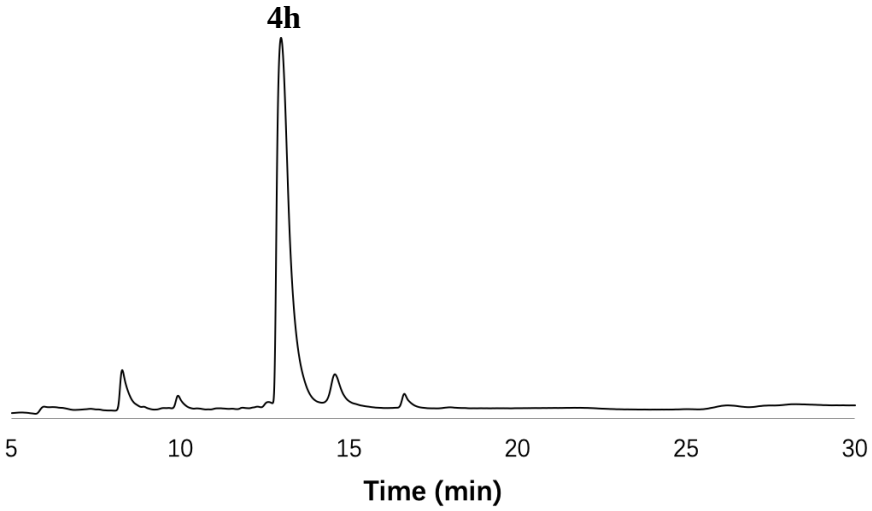
Gly-Gly-Cys-Gly-Met-Gly-Glu-Ala

Chemical Formula: $C_{24}H_{40}N_8O_{11}S_2$

Exact Mass: 680.23

Molecular Weight: 680.75

b)



c)

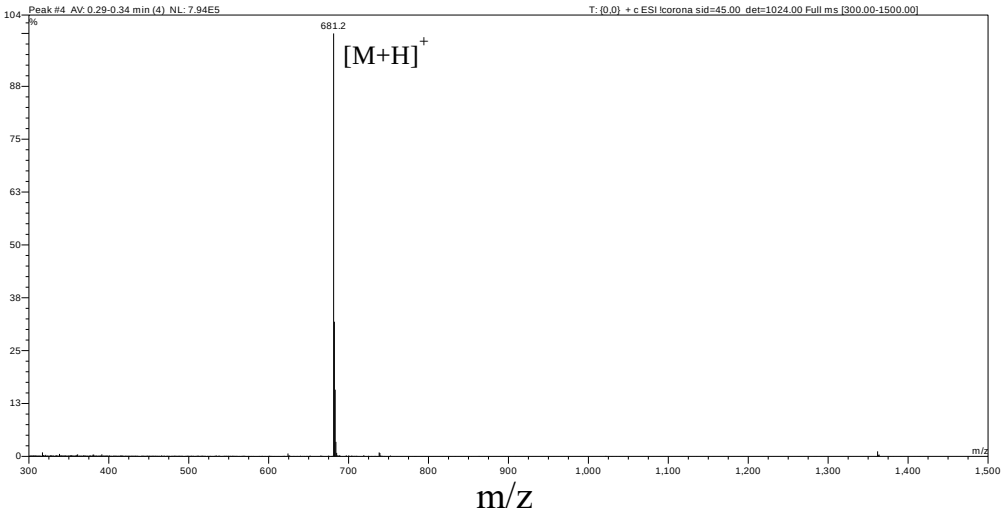
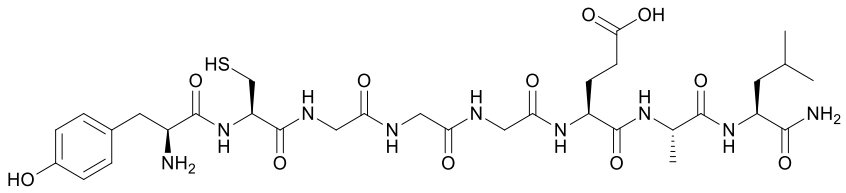


Figure S13. a) Chemical structure of peptide **4h**; b) RP-HPLC trace of peptide **4h**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{24}H_{40}N_8O_{11}S_2$: 681.8, $[M+H]^+$. Found: 681.2, $[M+H]^+$.

a)



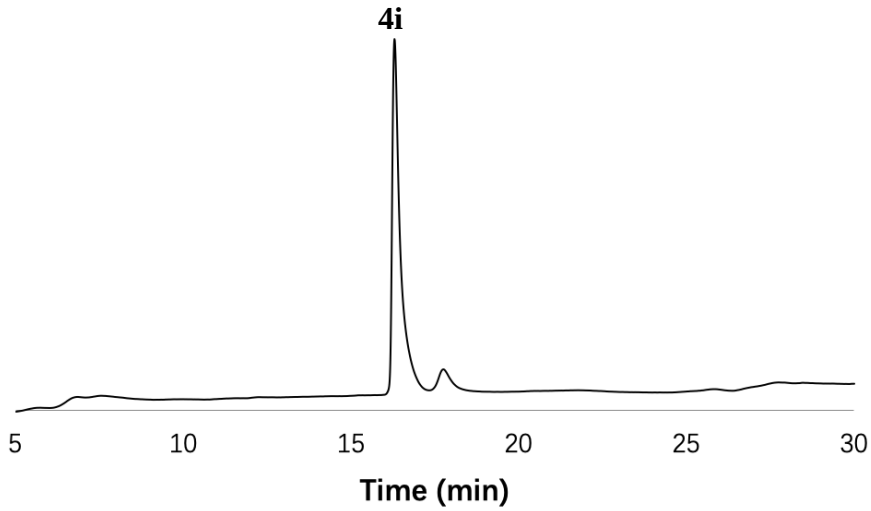
Tyr-Cys-Gly-Gly-Gly-Glu-Ala-Leu-$CONH_2$

Chemical Formula: $C_{32}H_{49}N_9O_{11}S$

Exact Mass: 767.33

Molecular Weight: 767.85

b)



c)

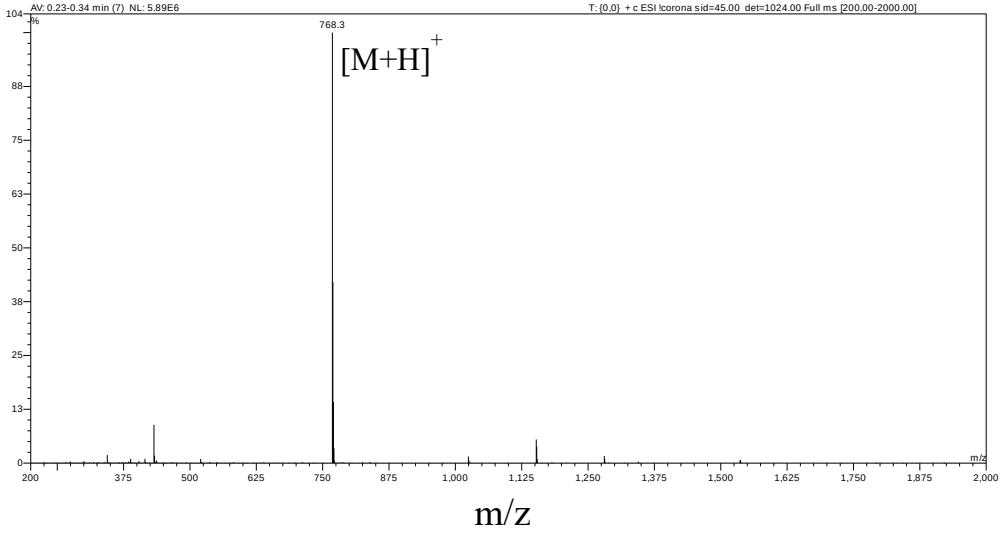
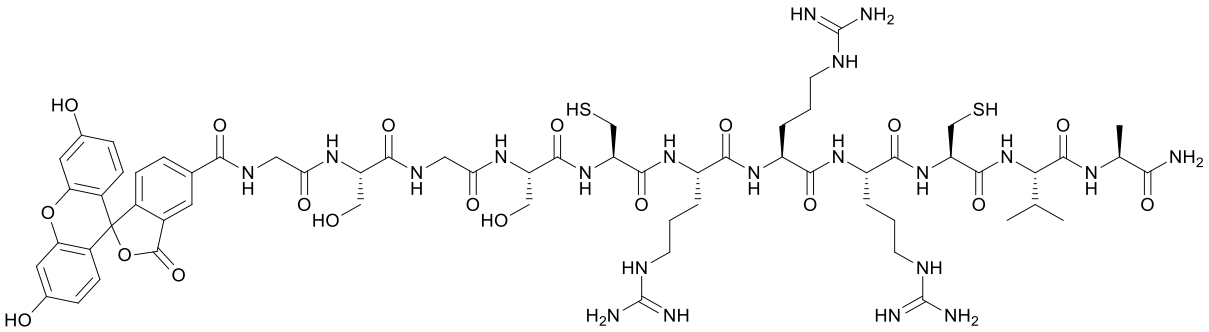


Figure S14. a) Chemical structure of peptide **4i**; b) RP-HPLC trace of peptide **4i**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{32}H_{49}N_9O_{11}S$: 768.9, $[M+H]^+$. Found: 768.3, $[M+H]^+$.

a)



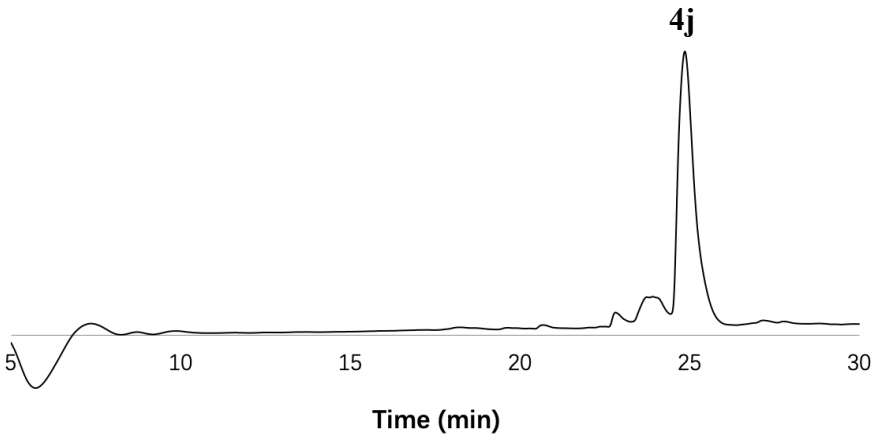
FAM-Gly-Ser-Gly-Ser-Cys-Arg-Arg-Arg-Cys-Val-Ala-$CONH_2$

Chemical Formula: $C_{63}H_{89}N_{21}O_{19}S_2$

Exact Mass: 1507.61

Molecular Weight: 1508.64

b)



c)

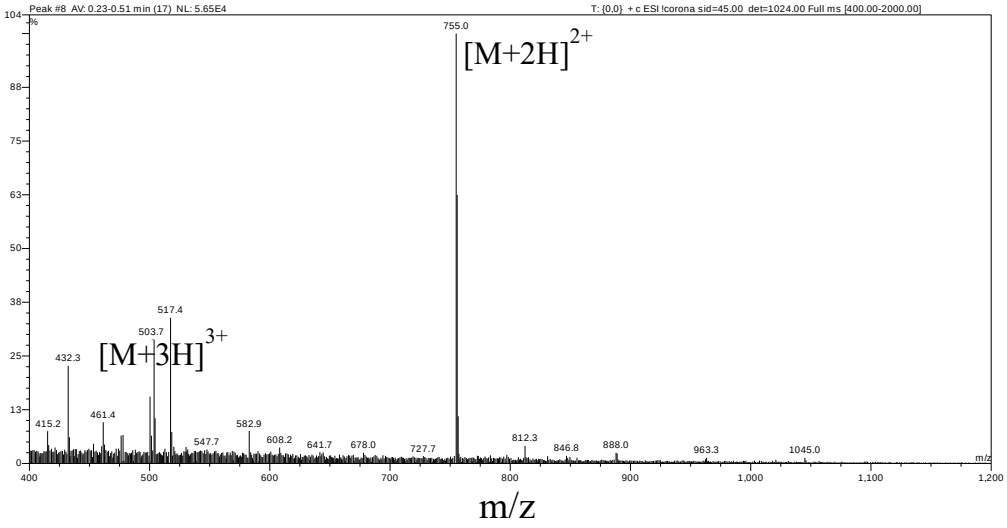


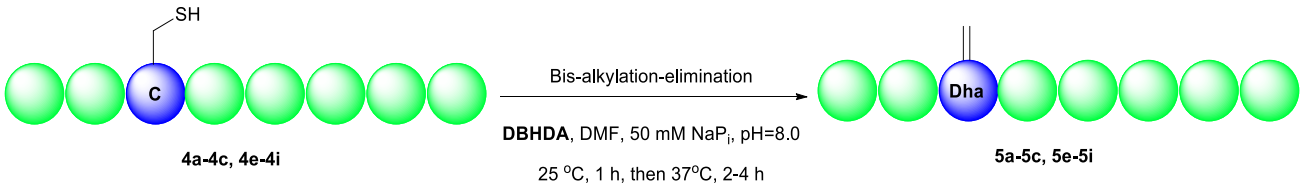
Figure S15. a) Chemical structure of peptide **4j**; b) RP-HPLC trace of peptide **4j**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{63}H_{89}N_{21}O_{19}S_2$: 755.3, $[M+2H]^{2+}$. Found: 755.0, $[M+2H]^{2+}$; 503.7, $[M+3H]^{3+}$.

General Procedure for synthesis and purification of Dha peptides **5a-5j**

All the Dha residues in the peptide sequences were synthesized by Bis-alkylation-elimination from cysteine, as previous works reported. [7,9]

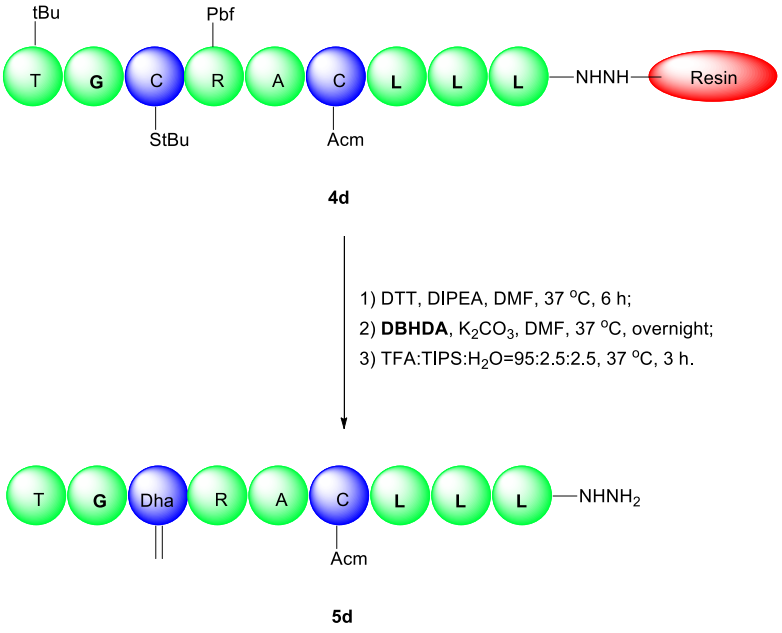
General Procedure for synthesis and purification of single Dha peptides **5a-5c** and **5e-5i**

1.0 mg peptide with 0.2 eq tris(2-carboxyethyl)phosphine hydrochloride was dissolved into 1 mL NaP_i buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 30-50 eq 2,5-dibromohexanediamide (DBHDA) was dissolved into moderate amount DMF to make it completely dissolved. The DMF solution was added into the NaP_i buffer solution and the mixture was vortexed about 30 s by Vortex to mix well. The tube was vortexed at 25 °C for 1 h, then 37 °C for 2-4 h. Finally taking supernatant after centrifuging the solution to purify the crude peptide with RP-HPLC and ESI-MS, and then lyophilized to obtain peptide product.



Scheme S17. General Procedure for synthesis of single Dha peptides **5a-5c, 5e-5j**.

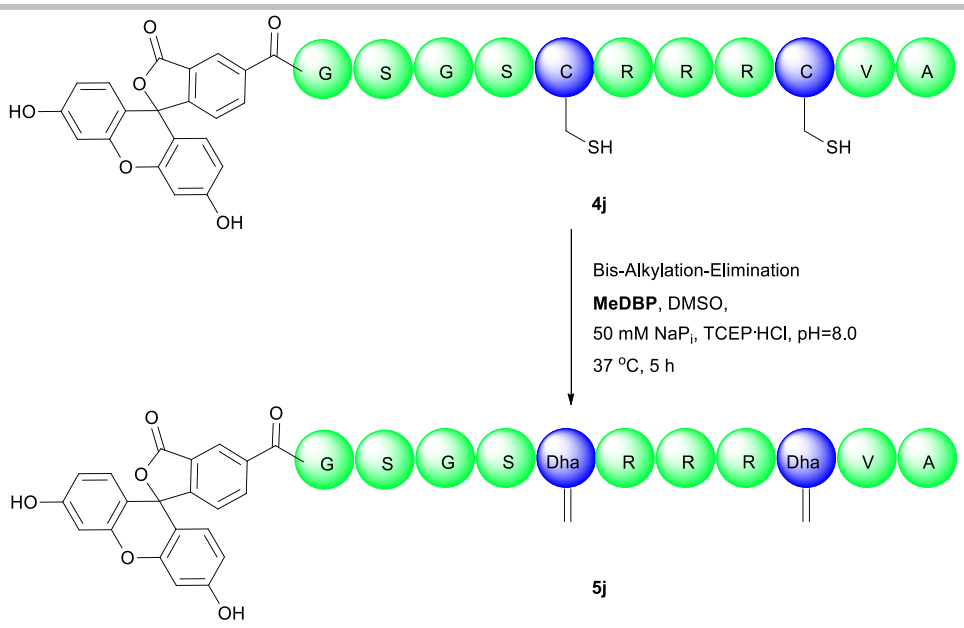
Peptide **5d** was synthesized with Resin **4d** with Bis-alkylation-elimination and Fmoc-peptide-hydrazides protocol as previous report.[8] After the peptide was cleaved from resin and precipitated with diethyl ether, purifing the crude peptide with RP-HPLC and ESI-MS, and then lyophilized to obtain peptide product.



Scheme S18. Synthesis of peptide **5d**.

Synthesis and purification of multiple Dhas peptide **5j**

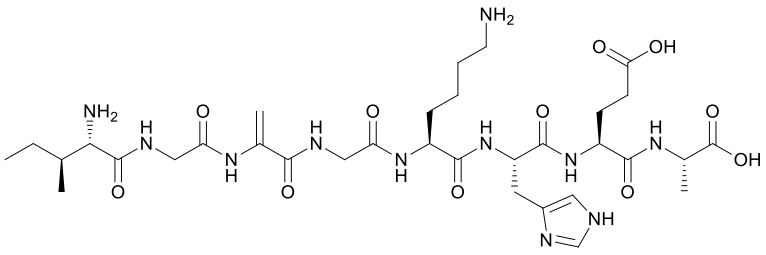
1.0 mg peptide with 0.2 eq tris(2-carboxyethyl)phosphine hydrochloride was dissolved into 1 mL NaP_i buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 50 eq Methyl 2,5-dibromopentanoate (**MeDBP**) was dissolved into 350 μL DMSO. The DMSO solution was added into the NaP_i buffer solution and the mixture was vortexed about 30 s by Vortex to mix well. The tube was vortexed at 37 °C for 4 h. Finally taking supernatant after centrifuging the solution to purify the crude peptide with RP-HPLC and ESI-MS, and then lyophilized to obtain peptide product.



Scheme S19. Synthesis of peptide **5j**.

Chemical structures and characterization of peptides **5a-5j**

a)



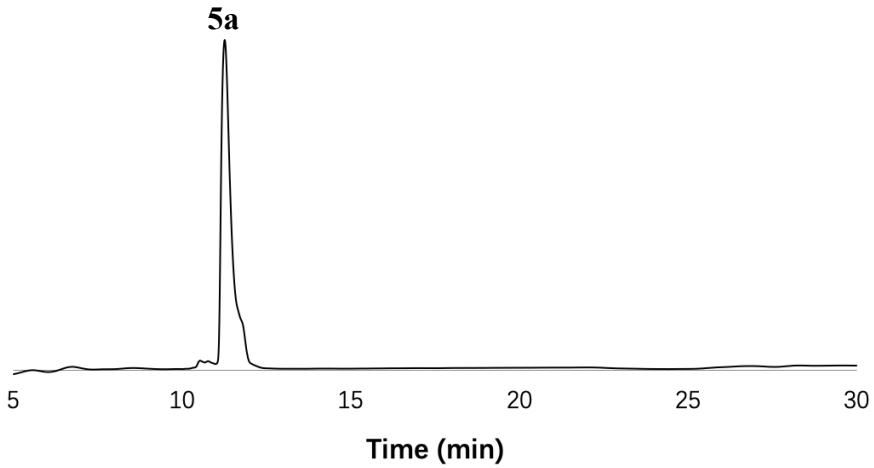
Ile-Gly-Dha-Gly-Lys-His-Glu-Ala

Chemical Formula: $C_{33}H_{53}N_{11}O_{11}$

Exact Mass: 779.39

Molecular Weight: 779.84

b)



c)

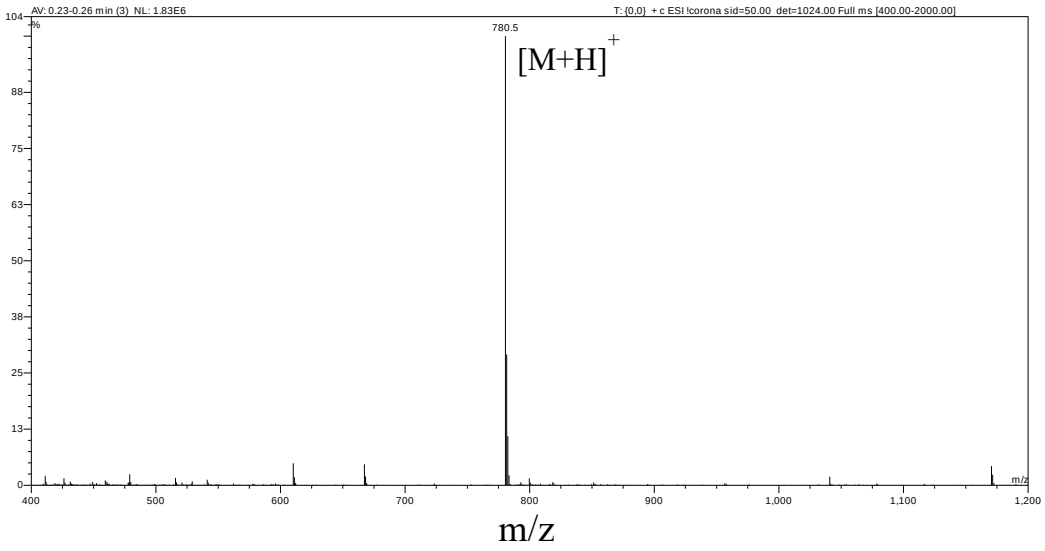
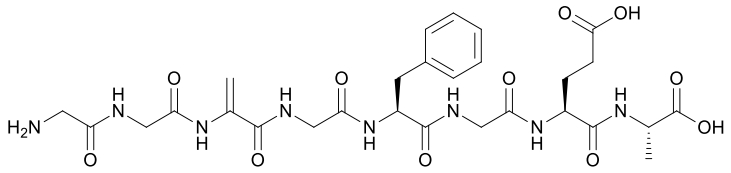


Figure S16. a) Chemical structure of peptide **5a**; b) RP-HPLC trace of peptide **5a**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{33}H_{53}N_{11}O_{11}$: 780.8, $[M+H]^+$. Found: 780.5, $[M+H]^+$.

a)



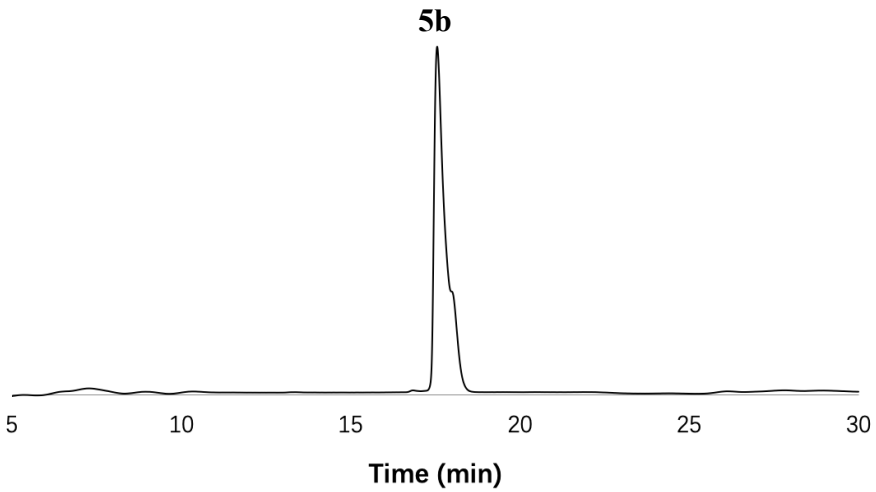
Gly-Gly-Dha-Gly-Phe-Gly-Glu-Ala

Chemical Formula: $C_{28}H_{38}N_8O_{11}$

Exact Mass: 662.27

Molecular Weight: 662.65

b)



c)

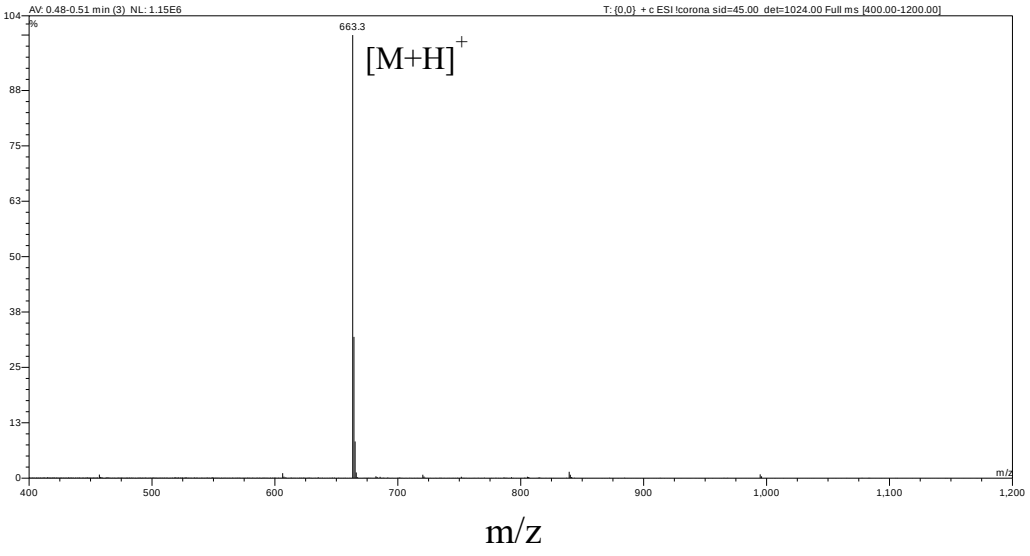
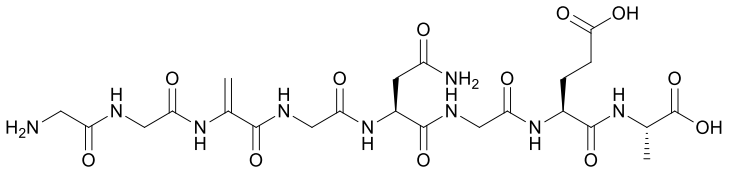


Figure S17. a) Chemical structure of peptide **5b**; b) RP-HPLC trace of peptide **5b**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{28}H_{38}N_8O_{11}$: 663.7, $[M+H]^+$. Found: 663.3, $[M+H]^+$.

a)



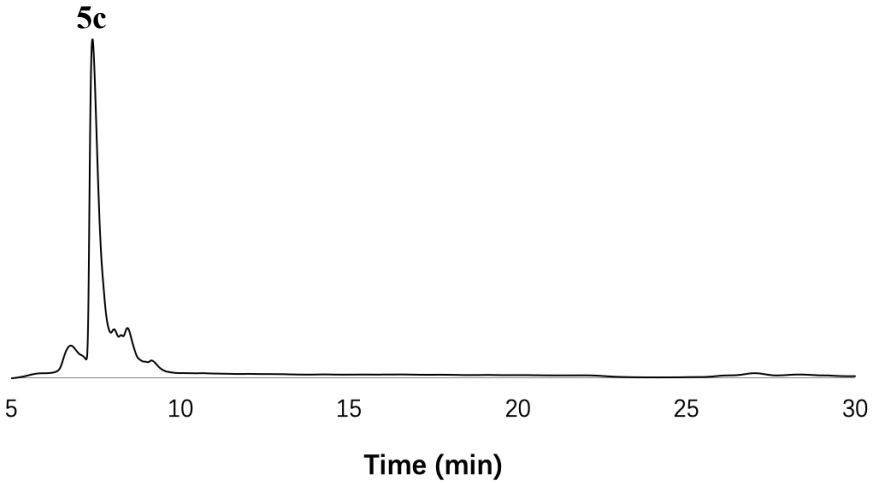
Gly-Gly-Dha-Gly-Asn-Gly-Glu-Ala

Chemical Formula: $C_{23}H_{35}N_9O_{12}$

Exact Mass: 629.24

Molecular Weight: 629.58

b)



c)

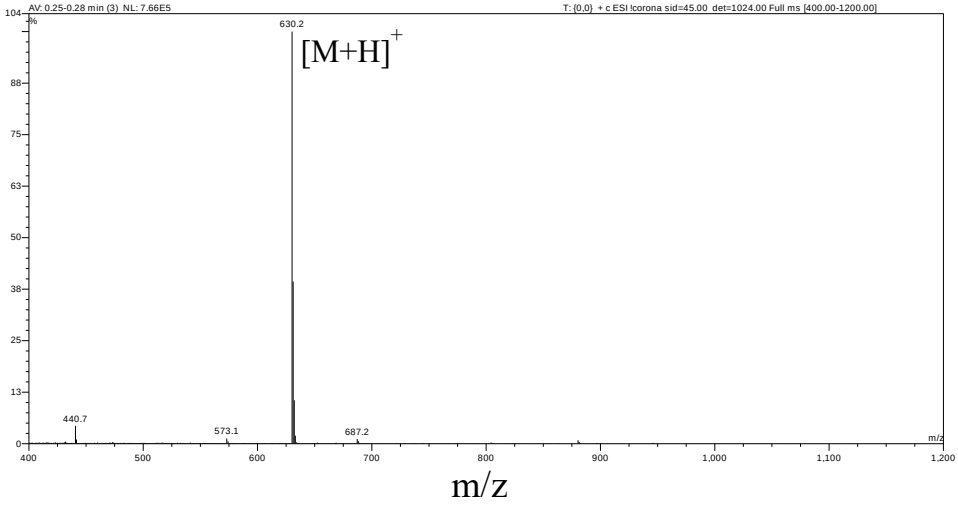
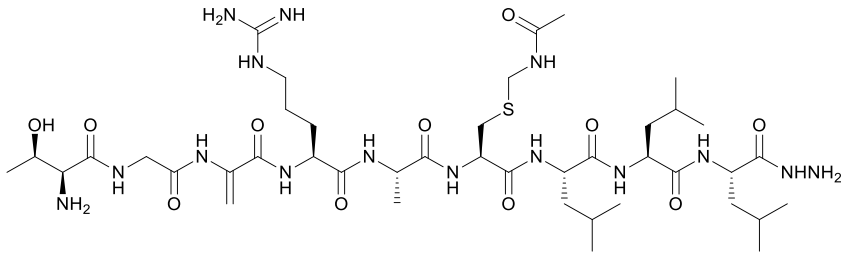


Figure S18. a) Chemical structure of peptide **5c**; b) RP-HPLC trace of peptide **5c**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{23}H_{35}N_9O_{12}$: 630.6, $[M+H]^+$. Found: 630.2, $[M+H]^+$.

a)



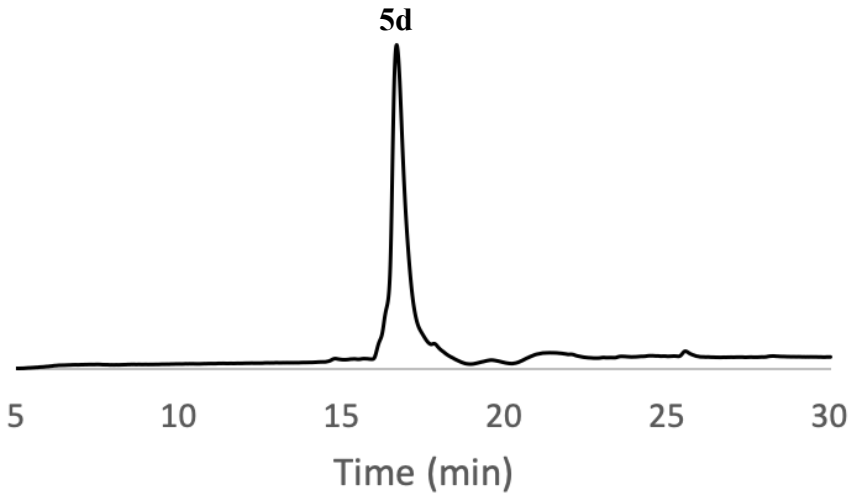
Thr-Gly-Dha-Arg-Ala-Cys(Acm)-Leu-Leu-Leu-CONHNH$_2$

Chemical Formula: $C_{42}H_{77}N_{15}O_{11}S$

Exact Mass: 999.56

Molecular Weight: 1000.22

b)



c)

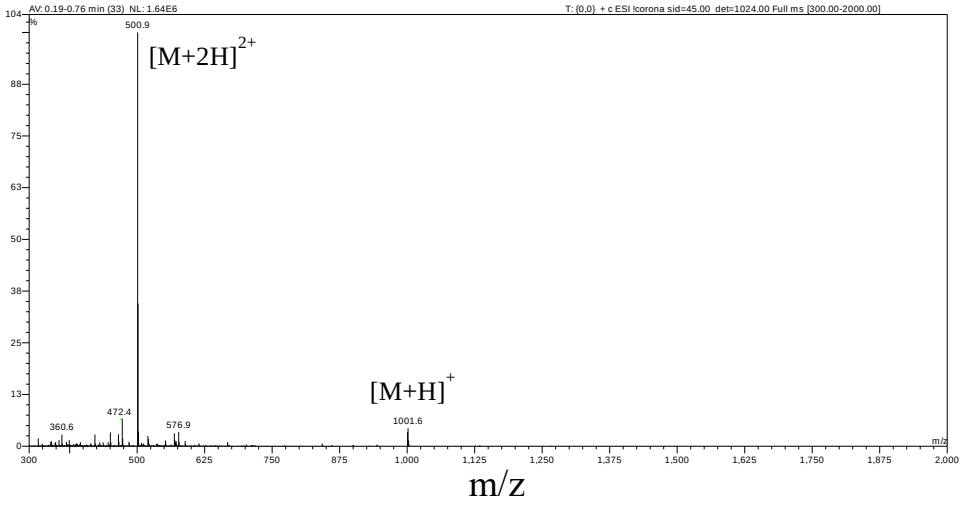
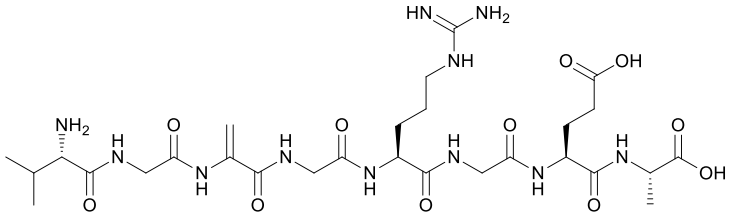


Figure S19. a) Chemical structure of peptide **5d**; b) RP-HPLC trace of peptide **5d**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{42}H_{77}N_{15}O_{11}S$: 1001.2, $[M+H]^+$. Found: 1001.6, $[M+H]^+$; 500.9, $[M+2H]^{2+}$.

a)



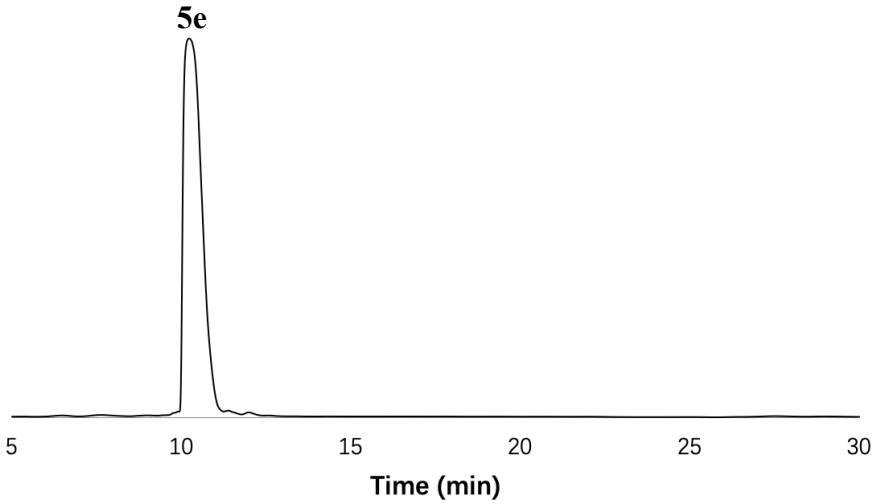
Val-Gly-Dha-Gly-Arg-Gly-Glu-Ala

Chemical Formula: $C_{28}H_{47}N_{11}O_{11}$

Exact Mass: 713.35

Molecular Weight: 713.74

b)



c)

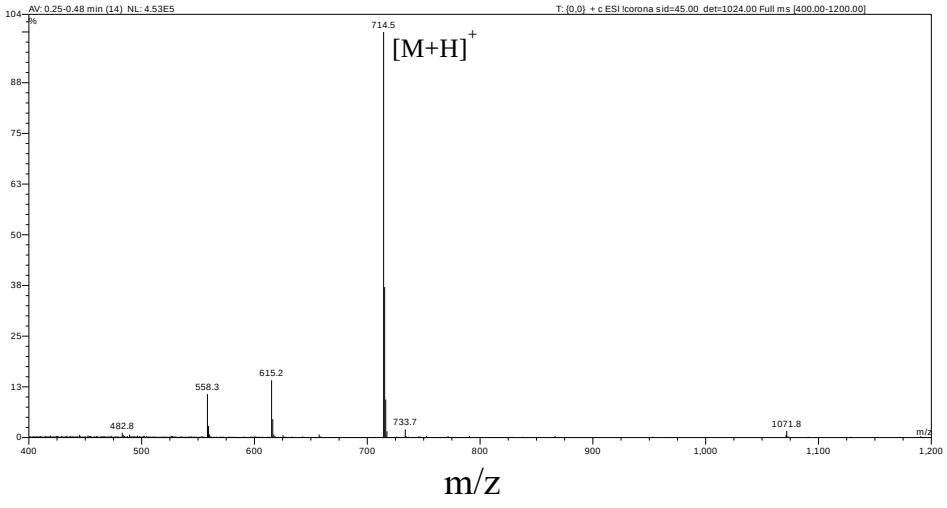
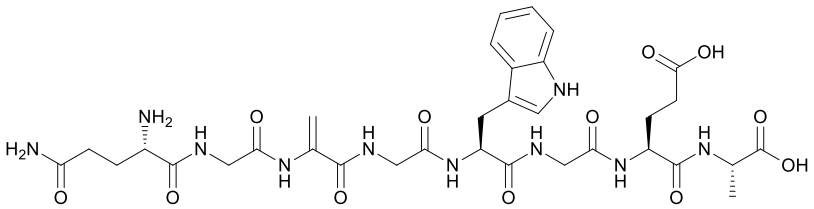


Figure S20. a) Chemical structure of peptide **5e**; b) RP-HPLC trace of peptide **5e**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{28}H_{47}N_{11}O_{11}$: 714.7, $[M+H]^+$. Found: 714.5, $[M+H]^+$.

a)



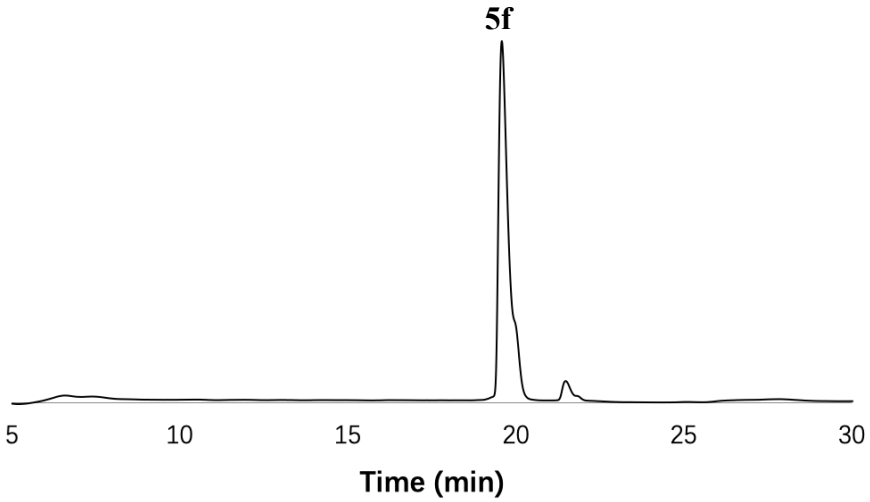
Gln-Gly-Dha-Gly-Trp-Gly-Glu-Ala

Chemical Formula: $C_{33}H_{44}N_{10}O_{12}$

Exact Mass: 772.31

Molecular Weight: 772.76

b)



c)

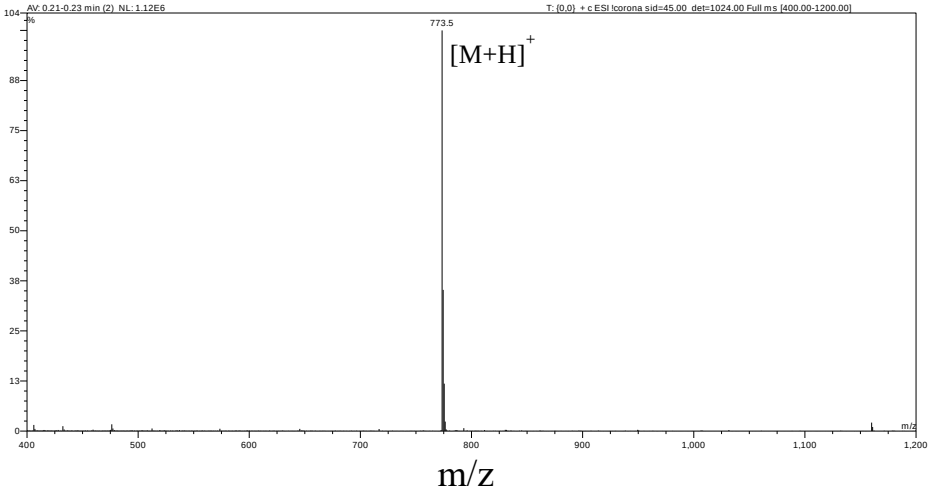
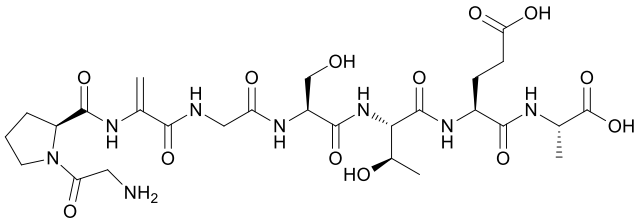


Figure S21. a) Chemical structure of peptide **5f**; b) RP-HPLC trace of peptide **5f**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{33}H_{44}N_{10}O_{12}$: 773.8, $[M+H]^+$. Found: 773.5, $[M+H]^+$.

a)



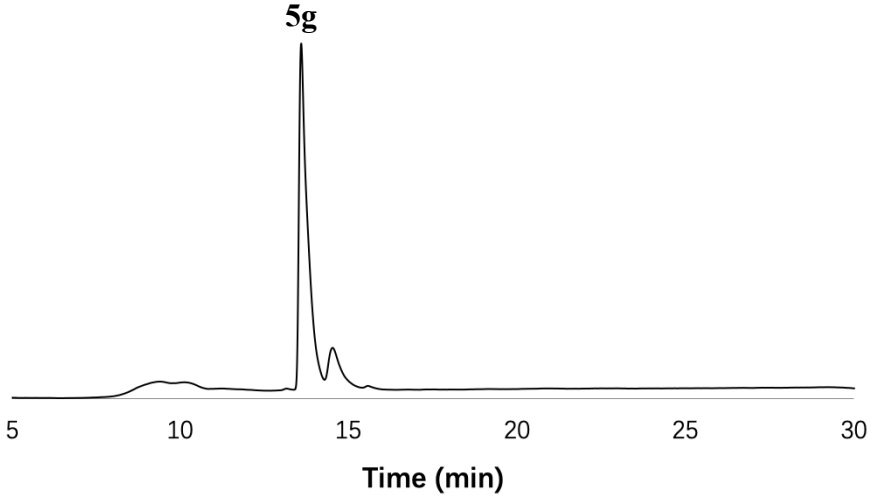
Gly-Pro-Dha-Gly-Ser-Thr-Glu-Ala

Chemical Formula: $C_{27}H_{42}N_8O_{13}$

Exact Mass: 686.29

Molecular Weight: 686.67

b)



c)

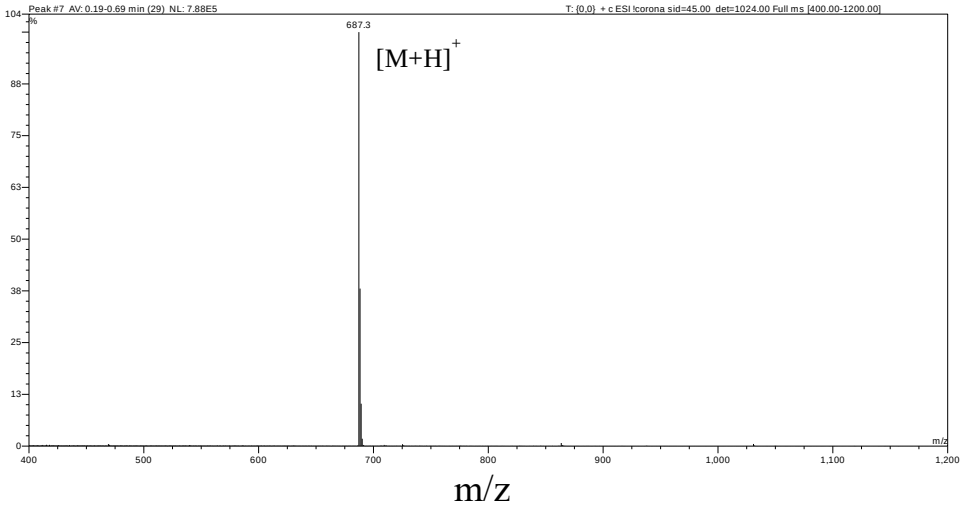
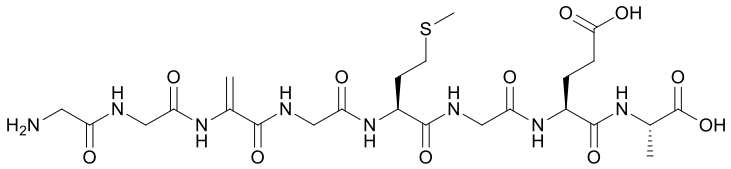


Figure S22. a) Chemical structure of peptide **5g**; b) RP-HPLC trace of peptide **5g**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{27}H_{42}N_8O_{13}$: 687.7, $[M+H]^+$. Found: 687.3, $[M+H]^+$.

a)



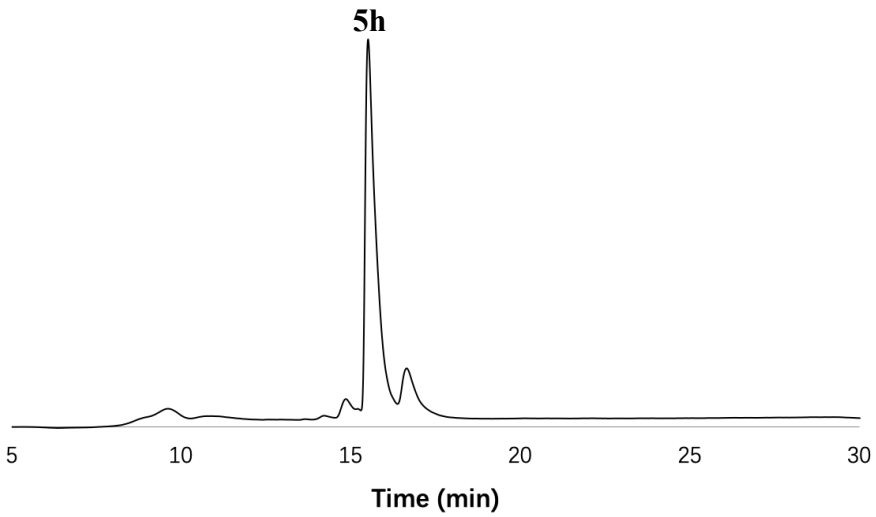
Gly-Gly-Dha-Gly-Met-Gly-Glu-Ala

Chemical Formula: $C_{24}H_{38}N_8O_{11}S$

Exact Mass: 646.24

Molecular Weight: 646.67

b)



c)

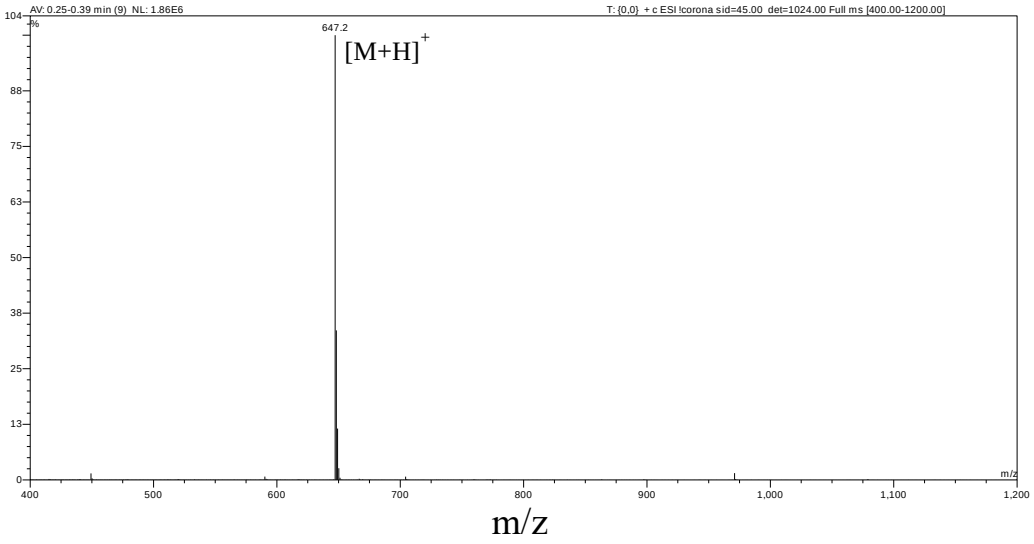
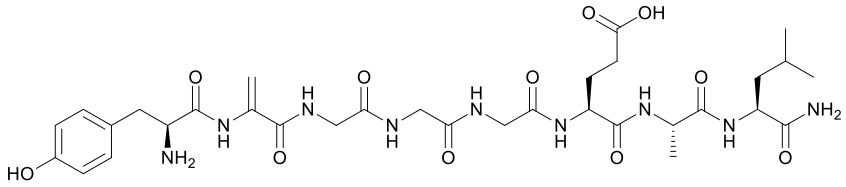


Figure S23. a) Chemical structure of peptide **5h**; b) RP-HPLC trace of peptide **5h**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{24}H_{38}N_8O_{11}S$: 647.7, $[M+H]^+$. Found: 647.2, $[M+H]^+$.

a)



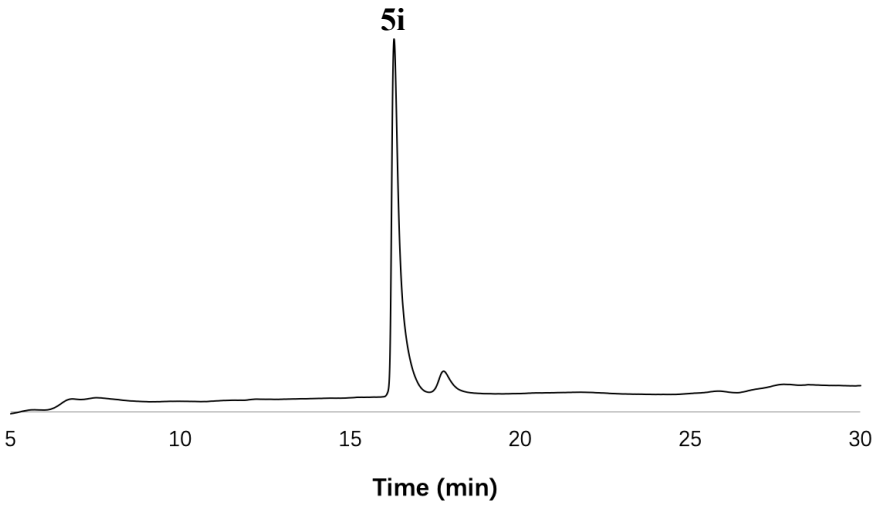
Tyr-Dha-Gly-Gly-Gly-Glu-Ala-Leu-$CONH_2$

Chemical Formula: $C_{32}H_{47}N_9O_{11}$

Exact Mass: 733.34

Molecular Weight: 733.77

b)



c)

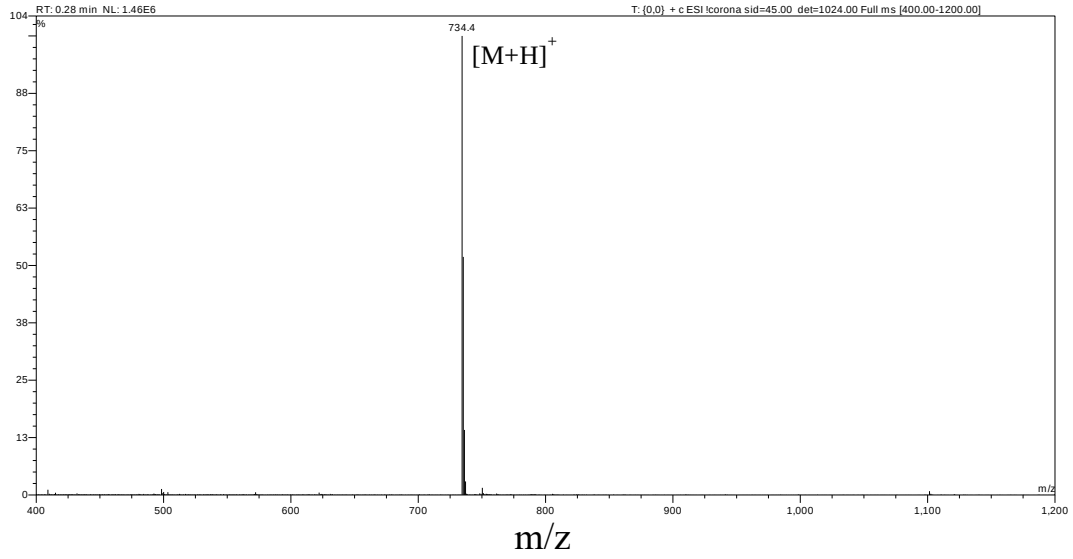
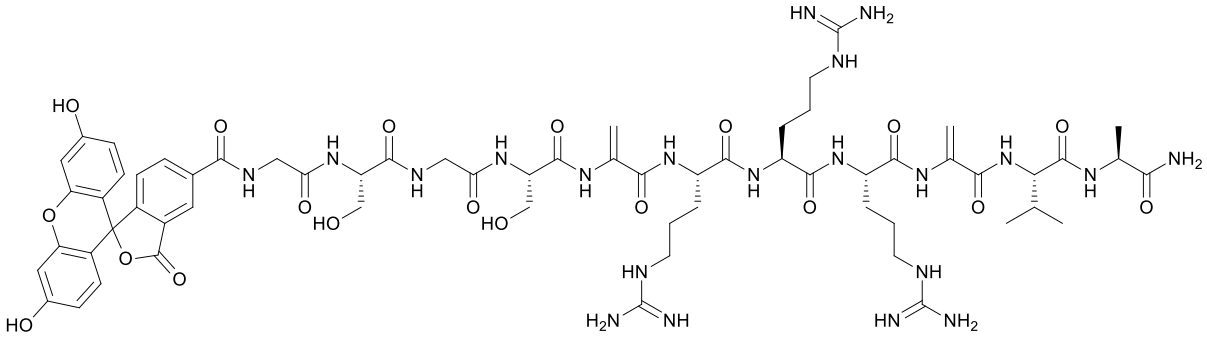


Figure S24. a) Chemical structure of peptide **5i**; b) RP-HPLC trace of peptide **5i**. (HPLC gradient is 5% to 40% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{32}H_{47}N_9O_{11}$: 734.8, $[M+H]^+$. Found: 734.4, $[M+H]^+$.

a)



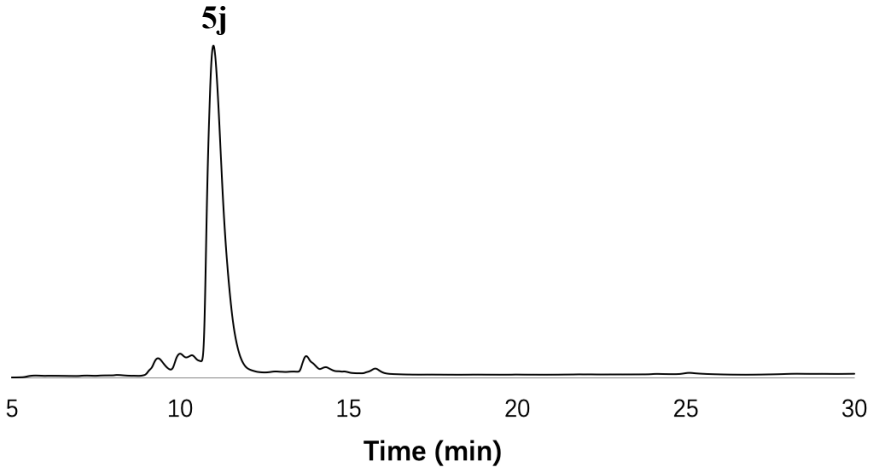
FAM-Gly-Ser-Gly-Ser-Dha-Arg-Arg-Arg-Dha-Val-Ala-$CONH_2$

Chemical Formula: $C_{63}H_{85}N_{21}O_{19}$

Exact Mass: 1439.63

Molecular Weight: 1440.48

b)



c)

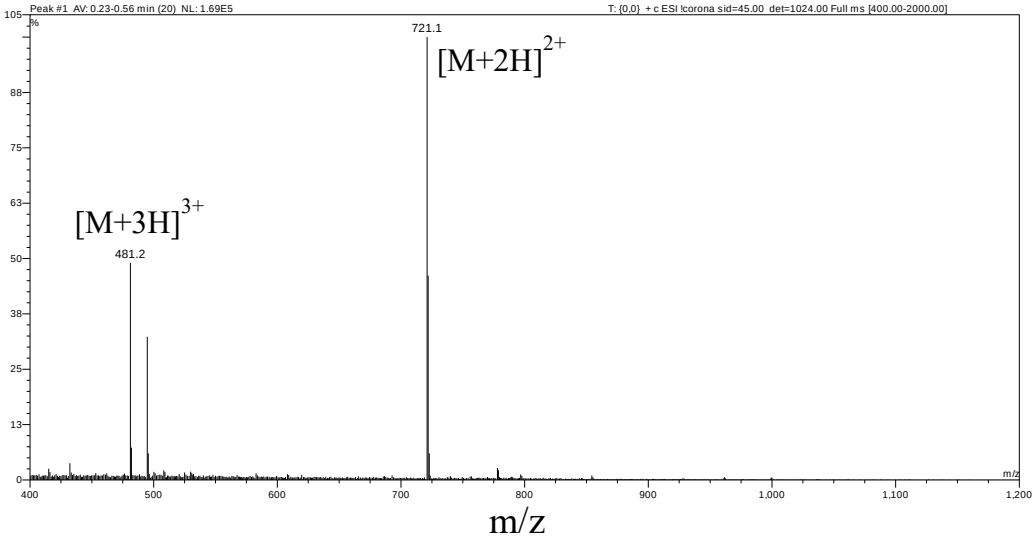
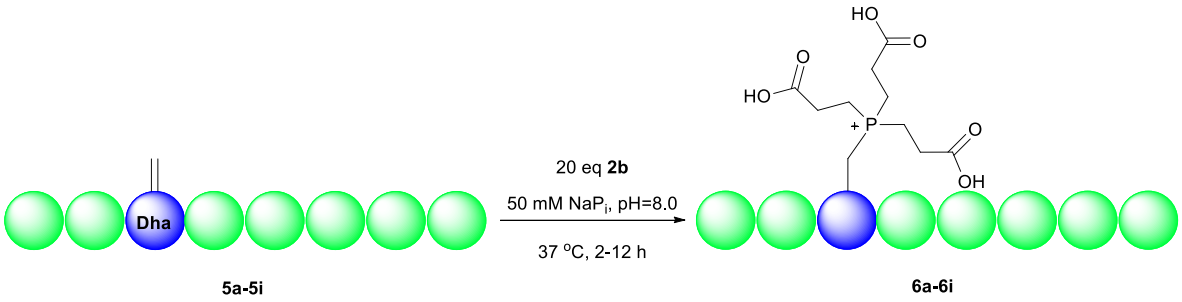


Figure S25. a) Chemical structure of peptide **5j**; b) RP-HPLC trace of peptide **5j**. (HPLC gradient is 20% to 60% of solution B in 30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{63}H_{85}N_{21}O_{19}$: 721.3, $[M+2H]^{2+}$. Found: 721.1, $[M+2H]^{2+}$; 481.2, $[M+3H]^{3+}$.

General Procedure for synthesis of TECP-addition peptides **6a-6i**.

1.0 mg peptide **5a-5i** was dissolved into 1.0 mL NaP_i buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 20 eq TECP (**2b**) was dissolved into the peptide solution, the pH of the solution was adjusted by 2M NaOH solution to 8.0. The tube was vortexed at 37 ℃ for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

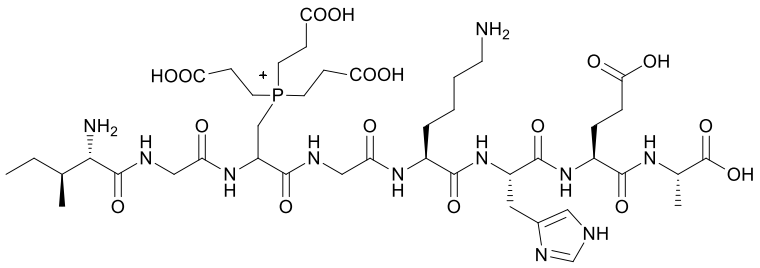


5a-5i **6a-6i**

Scheme S20. General Procedure for synthesis of peptides **6a-6i**.

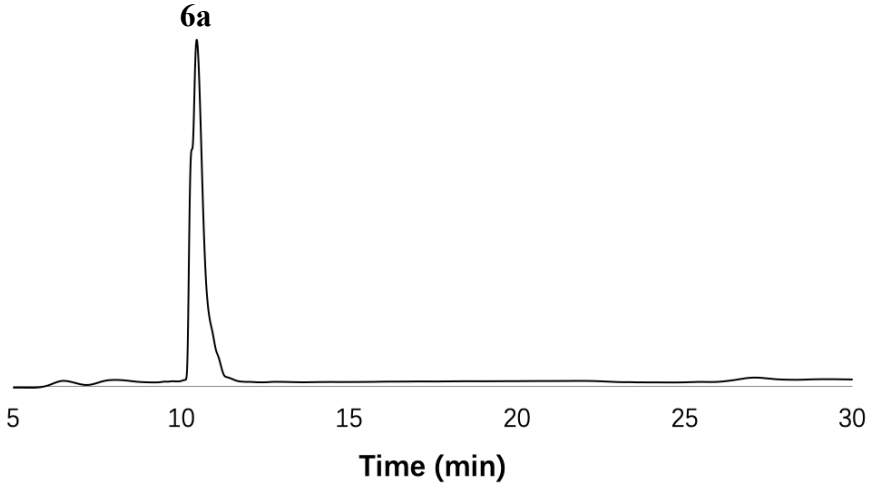
Chemical structures and characterization of peptides **6a-6i**

a)



Chemical Formula: $C_{42}H_{69}N_{11}O_{17}P^+$
Exact Mass: 1030.46
Molecular Weight: 1031.03

b)



c)

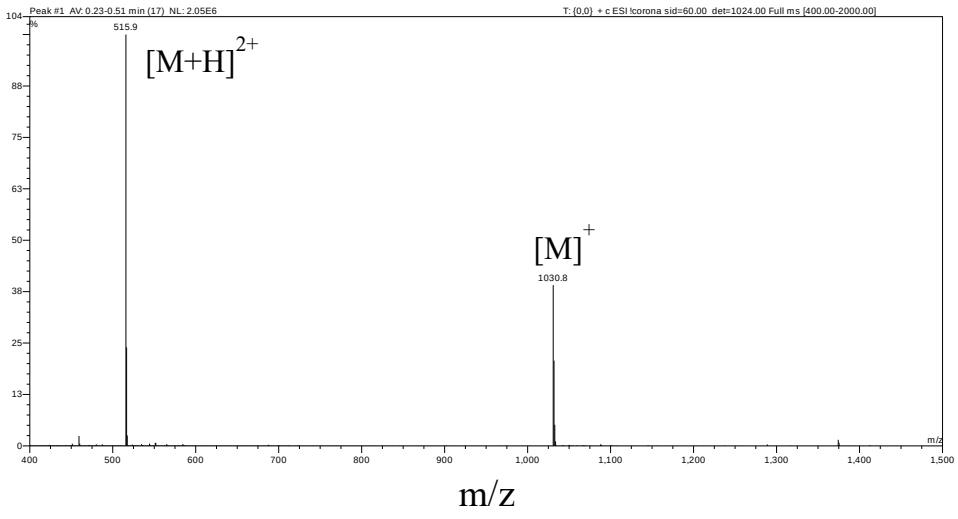
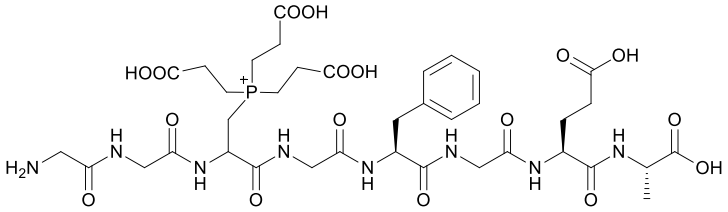


Figure S26. a) Chemical structure of peptide **6a**; b) Analytic HPLC trace of peptide **6a**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{42}H_{69}N_{11}O_{17}P^+$: 1031.0, $[M]^+$. Found: 1030.8, $[M]^+$; 515.9, $[M+H]^{2+}$.

a)

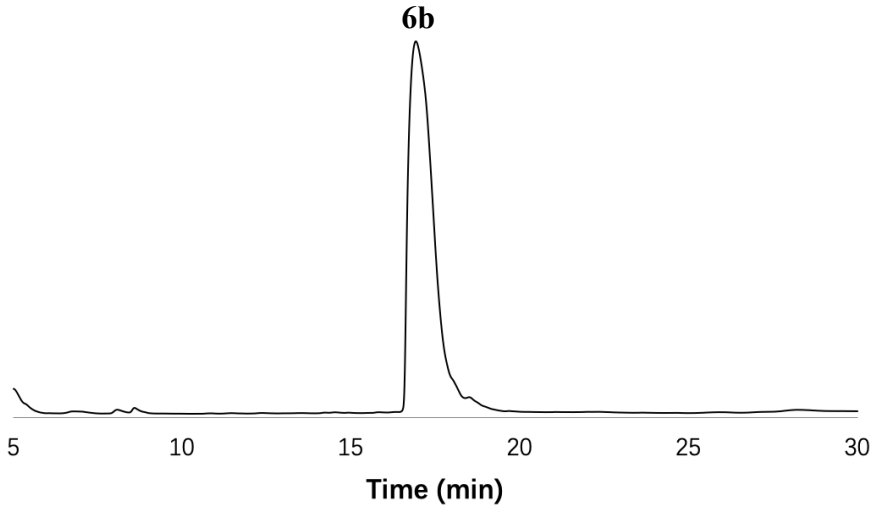


Chemical Formula: $C_{37}H_{54}N_8O_{17}P^+$

Exact Mass: 913.33

Molecular Weight: 913.84

b)



c)

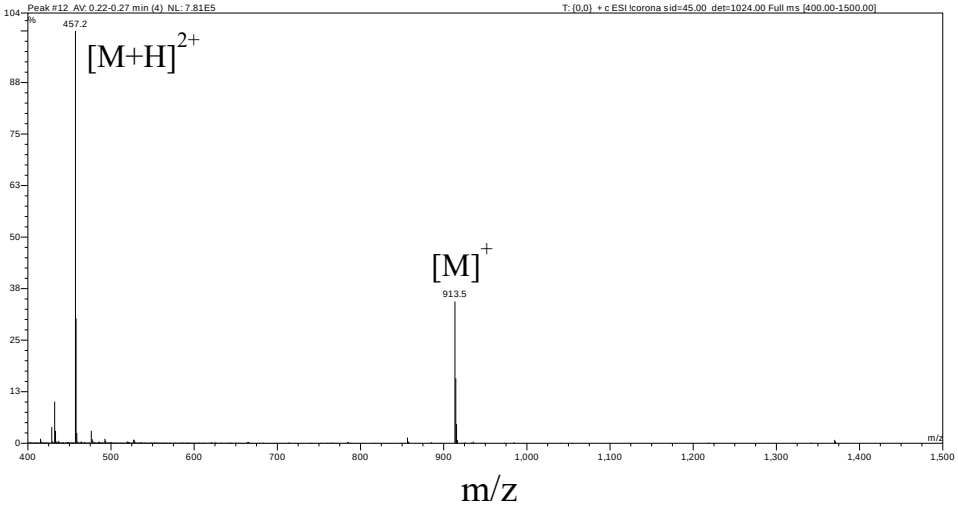
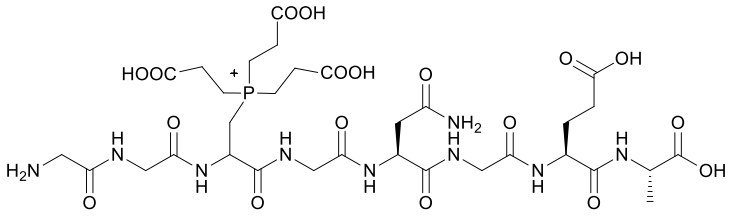


Figure S27. a) Chemical structure of peptide **6b**; b) Analytic HPLC trace of peptide **6b**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{37}H_{54}N_8O_{17}P^+$: 913.8, $[M]^+$. Found: 913.5, $[M]^+$; 457.2, $[M+H]^{2+}$.

a)

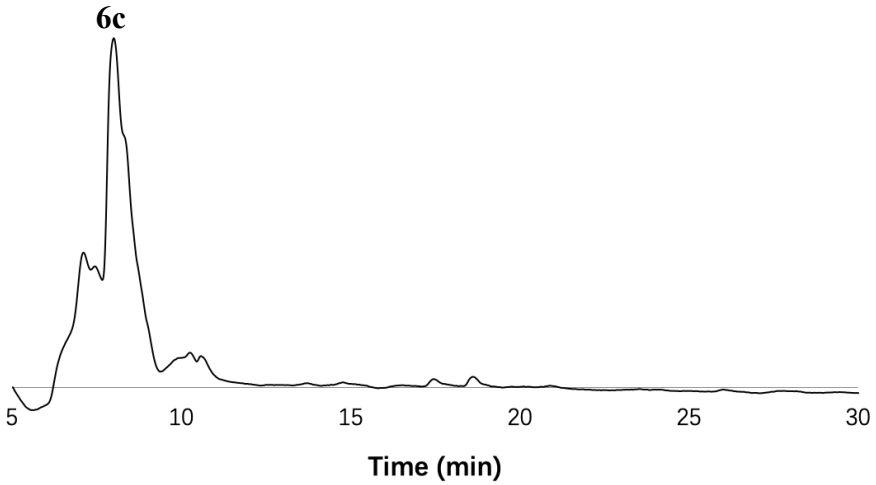


Chemical Formula: $C_{32}H_{51}N_9O_{18}P^+$

Exact Mass: 880.31

Molecular Weight: 880.77

b)



c)

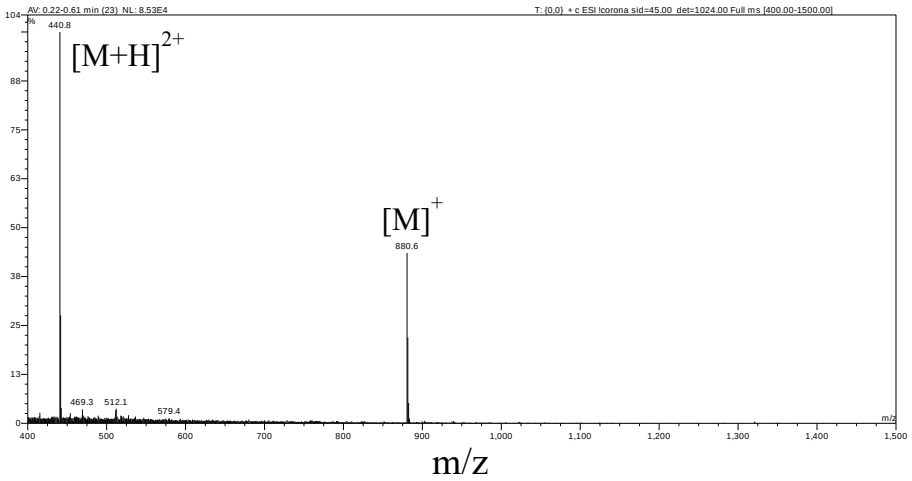
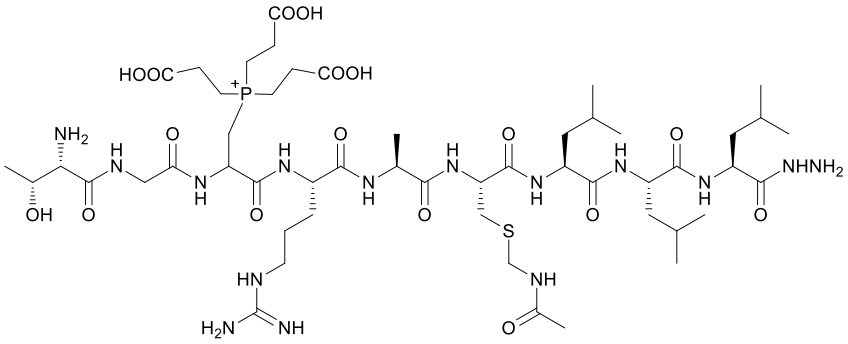


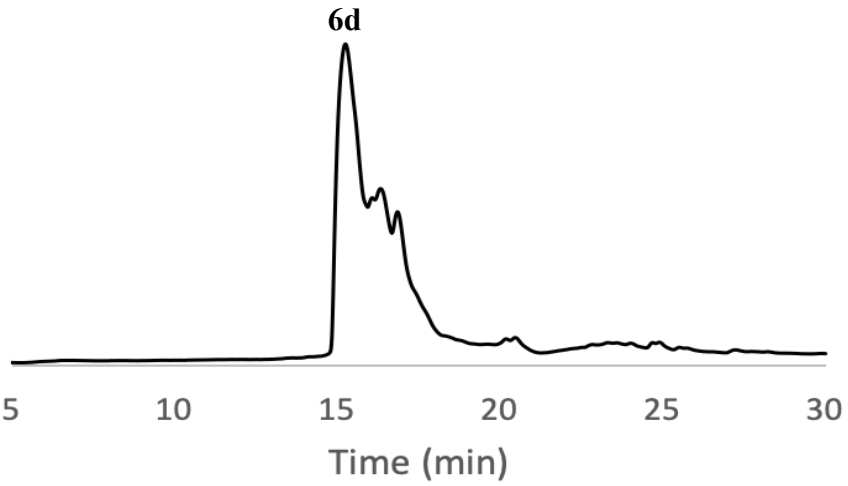
Figure S28. a) Chemical structure of peptide **6c**; b) Analytic HPLC trace of peptide **6c**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{32}H_{51}N_9O_{18}P^+$: 880.8, $[M]^+$. Found: 880.6, $[M]^+$; 440.8, $[M+H]^{2+}$.

a)



Chemical Formula: $C_{51}H_{93}N_{15}O_{17}PS^+$
Exact Mass: 1250.63
Molecular Weight: 1251.41

b)



c)

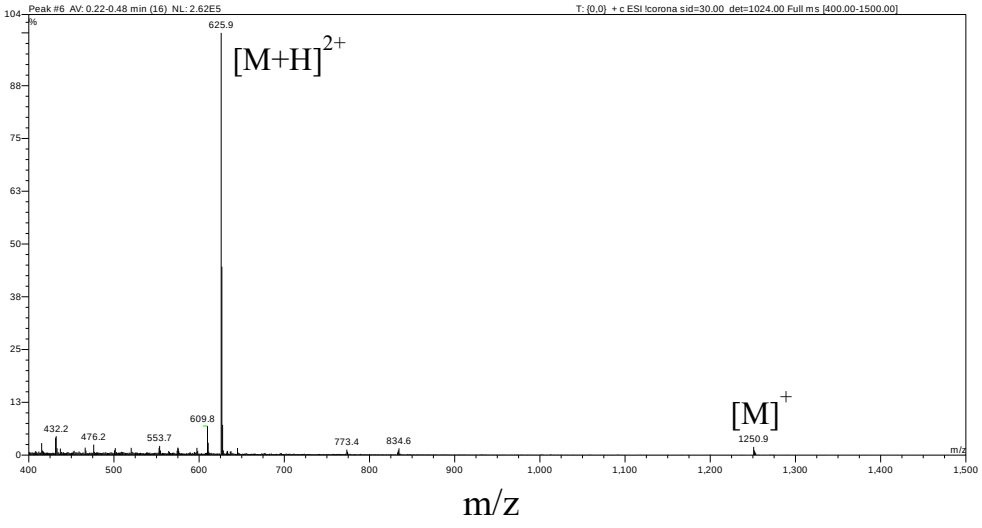
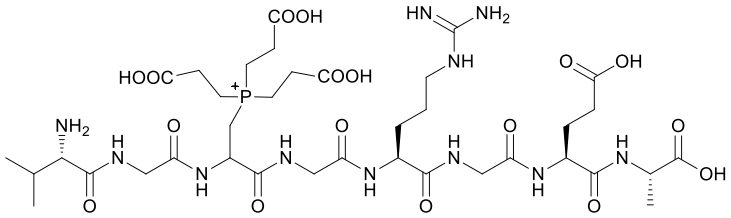


Figure S29. a) Chemical structure of peptide **6d**; b) Analytic HPLC trace of peptide **6d**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{51}H_{93}N_{15}O_{17}PS^+$: 1251.4, $[M]^+$. Found: 1250.9, $[M]^+$; 625.9, $[M+H]^{2+}$.

a)

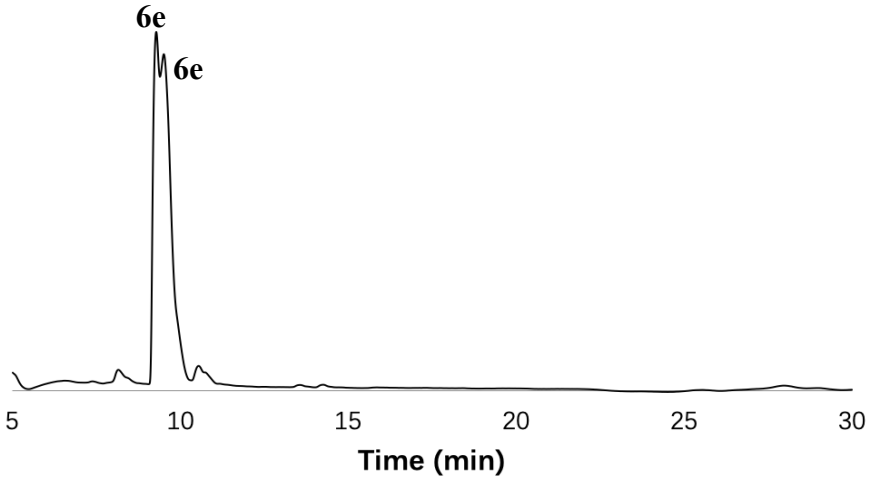


Chemical Formula: $C_{37}H_{63}N_{11}O_{17}P^+$

Exact Mass: 964.41

Molecular Weight: 964.93

b)



c)

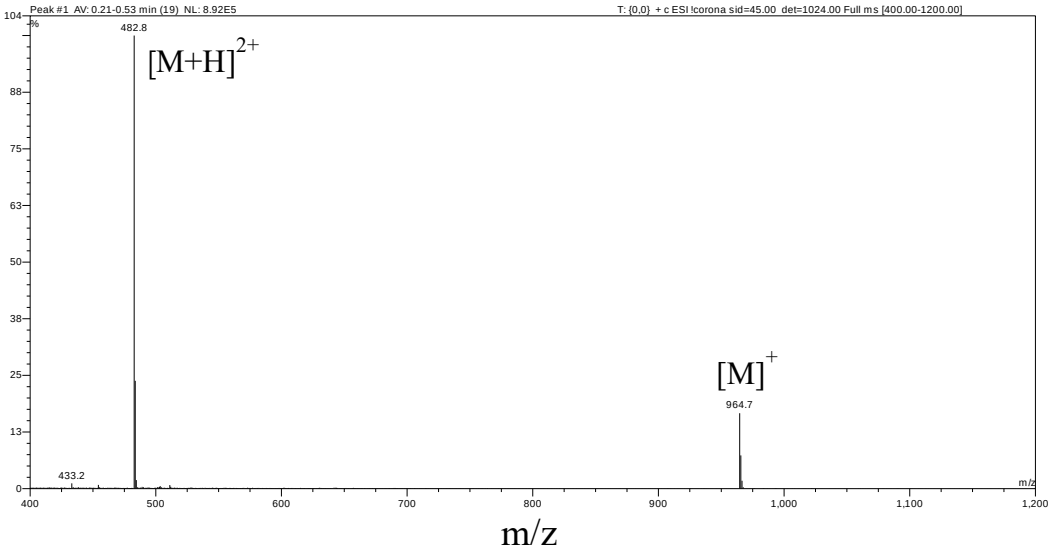
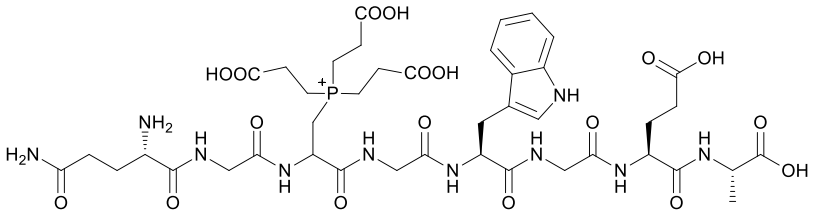


Figure S30. a) Chemical structure of peptide **6e**; b) Analytic HPLC trace of peptide **6e**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{37}H_{63}N_{11}O_{17}P^+$: 964.9, $[M]^+$. Found: 964.7, $[M]^+$; 482.8, $[M+H]^{2+}$.

a)

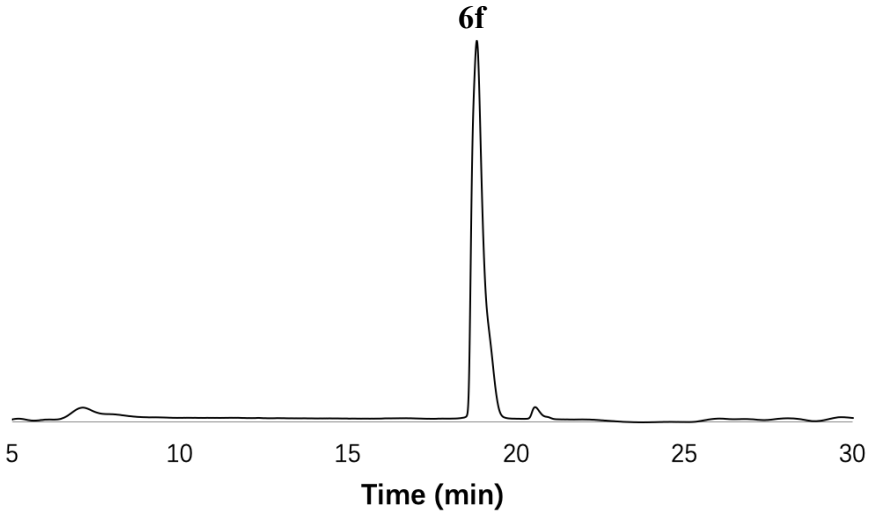


Chemical Formula: $C_{42}H_{60}N_{10}O_{18}P^+$

Exact Mass: 1023.38

Molecular Weight: 1023.96

b)



c)

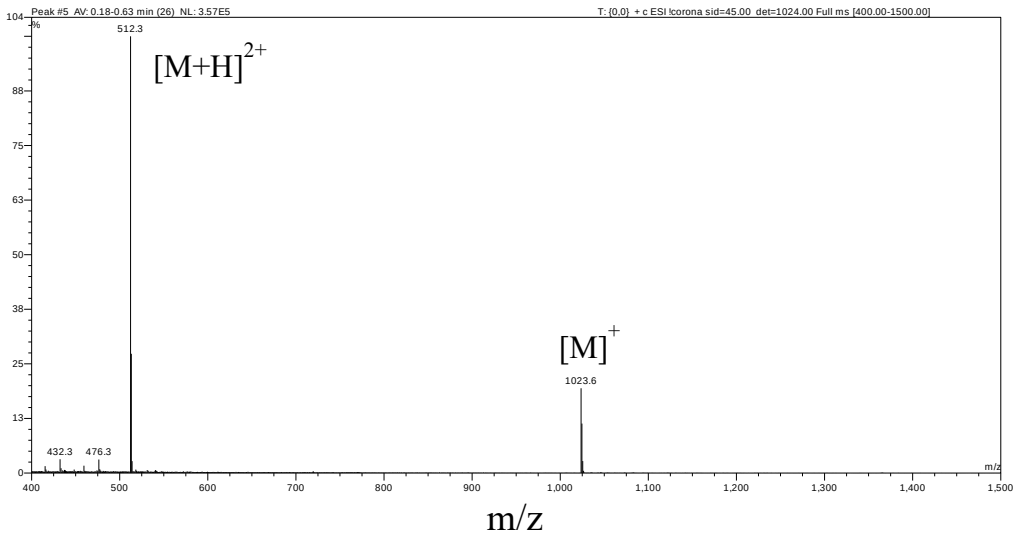
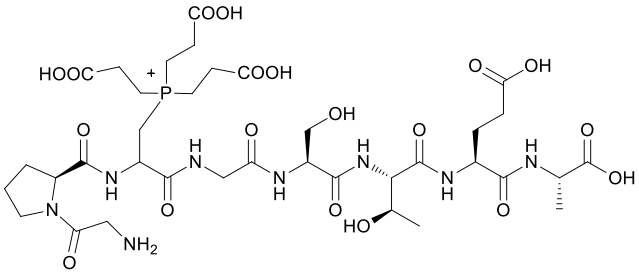


Figure S31. a) Chemical structure of peptide **6f**; b) Analytic HPLC trace of peptide **6f**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{42}H_{60}N_{10}O_{18}P^+$: 1024.0, $[M]^+$. Found: 1023.6, $[M]^+$; 512.3, $[M+H]^{2+}$.

a)

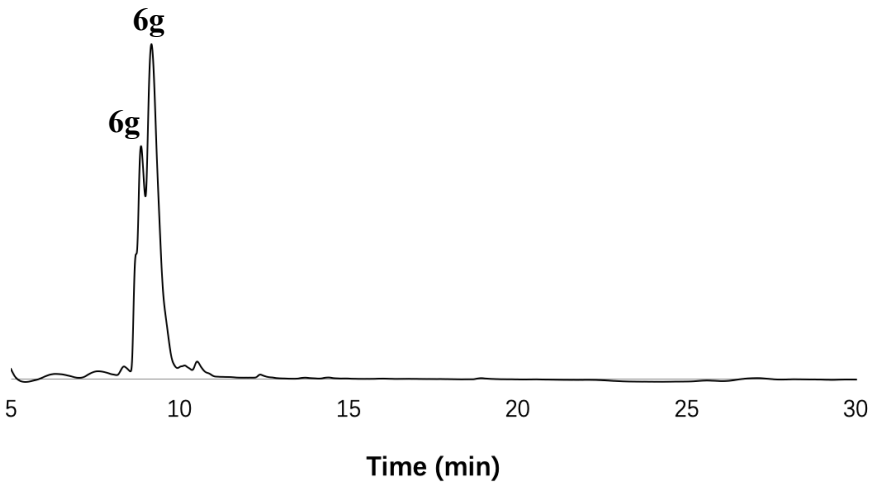


Chemical Formula: $C_{36}H_{58}N_8O_{19}P^+$

Exact Mass: 937.36

Molecular Weight: 937.86

b)



c)

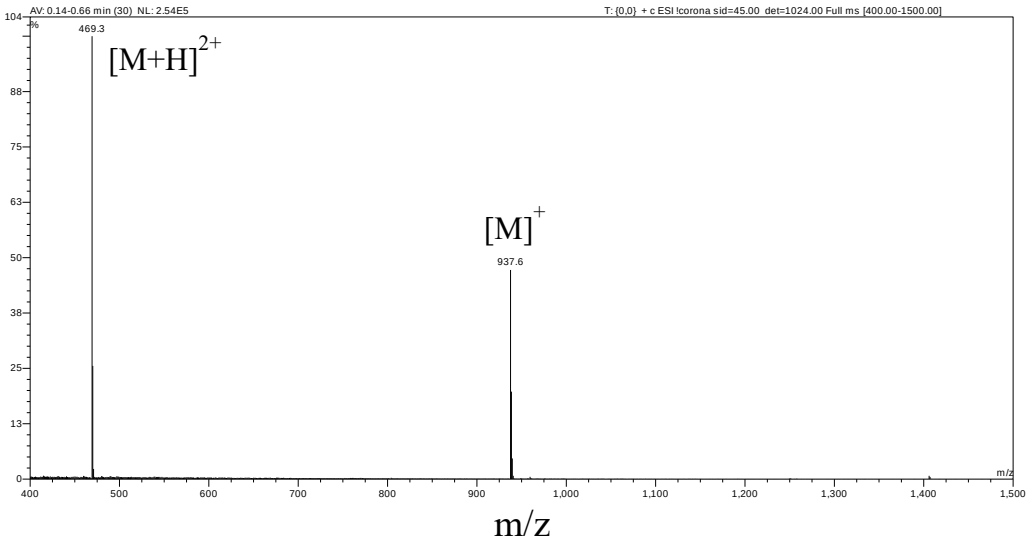
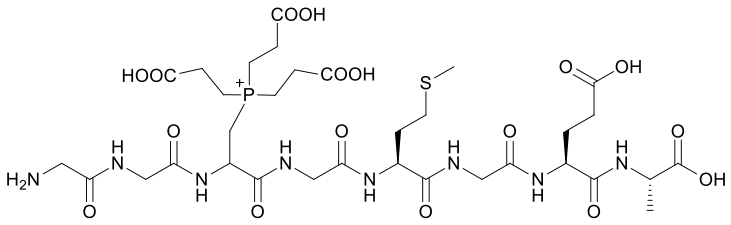


Figure S32. a) Chemical structure of peptide **6g**; b) Analytic HPLC trace of peptide **6g**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{36}H_{58}N_8O_{19}P^+$: 937.9, $[M]^+$. Found: 937.6, $[M]^+$; 469.3, $[M+H]^{2+}$.

a)

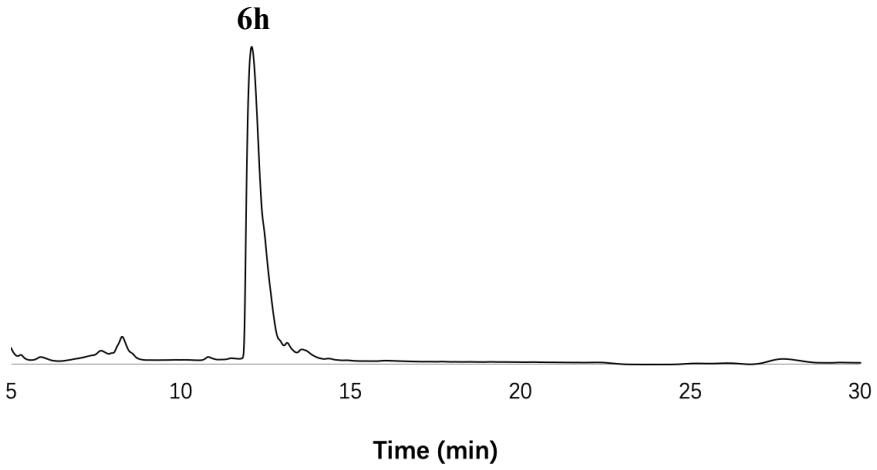


Chemical Formula: $C_{33}H_{54}N_8O_{17}PS^+$

Exact Mass: 897.31

Molecular Weight: 897.86

b)



c)

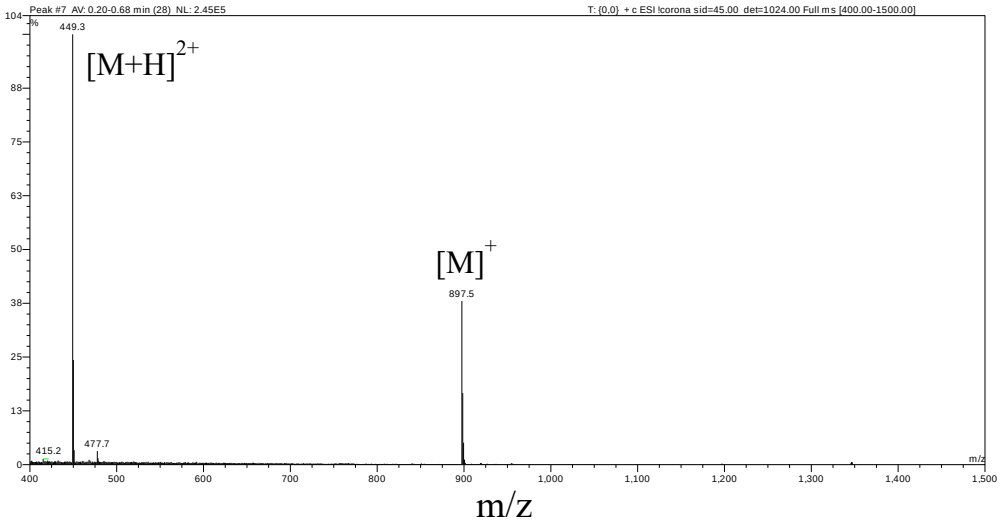
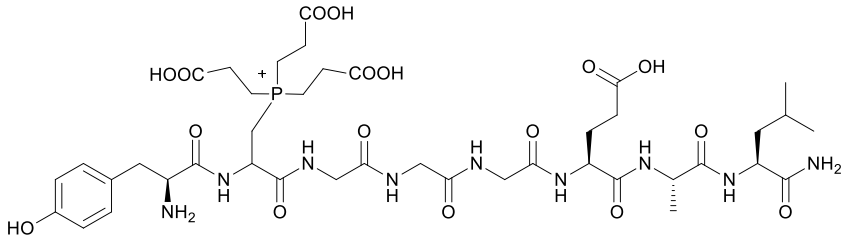


Figure S33. a) Chemical structure of peptide **6h**; b) Analytic HPLC trace of peptide **6h**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{33}H_{54}N_8O_{17}PS^+$: 897.9, $[M]^+$. Found: 897.5, $[M]^+$; 449.3, $[M+H]^{2+}$.

a)

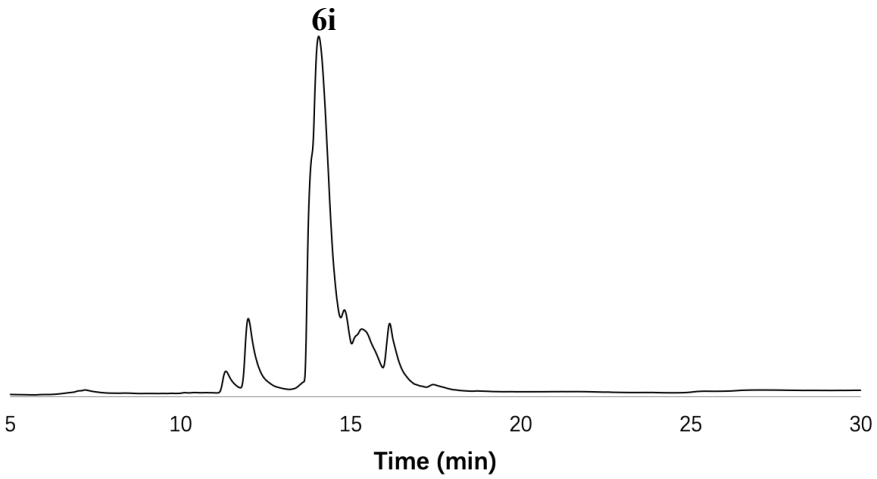


Chemical Formula: $C_{41}H_{63}N_9O_{17}P^+$

Exact Mass: 984.41

Molecular Weight: 984.96

b)



c)

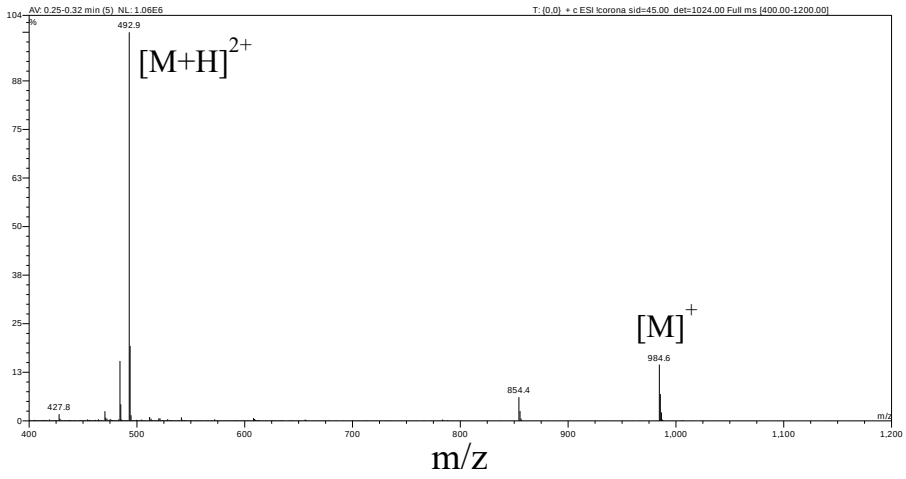
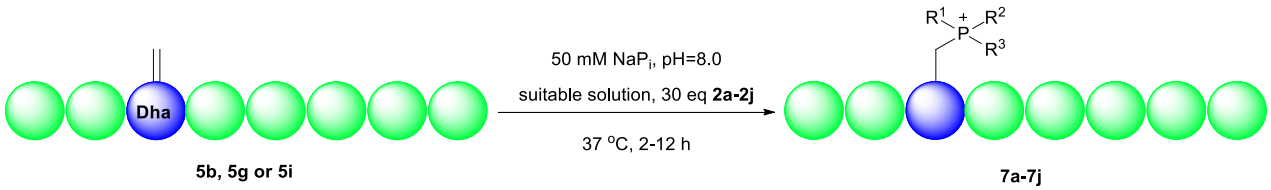


Figure S34. a) Chemical structure of peptide **6i**; b) Analytic HPLC trace of peptide **6i**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{41}H_{63}N_9O_{17}P^+$: 985.0, $[M]^+$. Found: 984.6, $[M]^+$; 492.9, $[M+H]^{2+}$.

General Procedure for Synthesis of phosphine-addition peptides **7a-7j**.

1.0 eq of Dha peptide **5b**, **5g** or **5i** was dissolved into 150-200 μL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq phosphine **2a-2j** was dissolved into 50-100 μL suitable solutions. The phosphine solution was added into the peptide solution and the tube was vortexed at 37 °C for 2-12 h, then monitoring the reaction with analytic HPLC and ESI-MS.



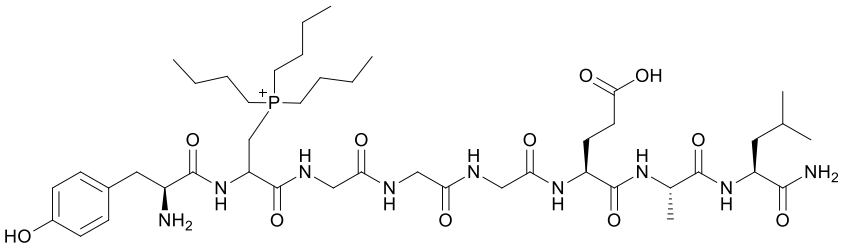
Scheme S21. General Procedure for synthesis of peptides **7a-7j. (7b** = **6i)**

Synthesis and characterization of peptides **7a-7k**

Synthesis and characterization of peptide **7a**

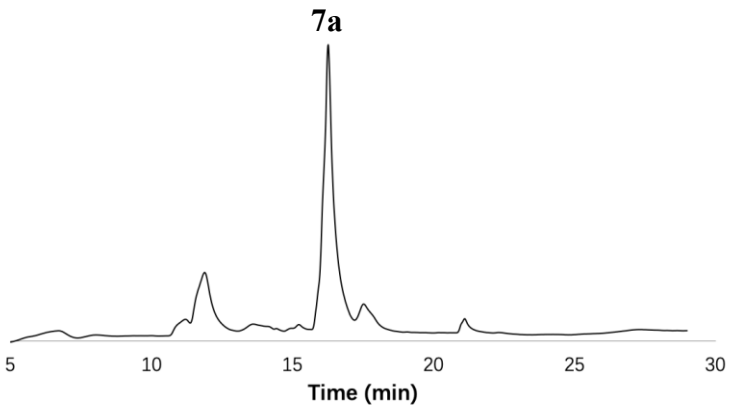
0.2 mg peptide **5i** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq tributyl phosphine **2a** was dissolved into 100 µL acetonitrile. The acetonitrile solution was added into the peptide solution and the tube was vortexed at 37 °C for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{44}H_{75}N_9O_{11}P^+$
Exact Mass: 936.53
Molecular Weight: 937.09

b)



c)

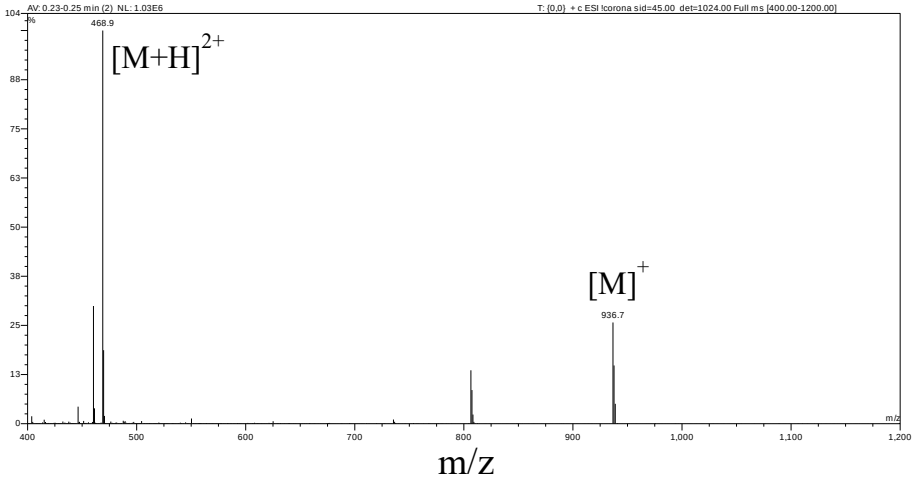
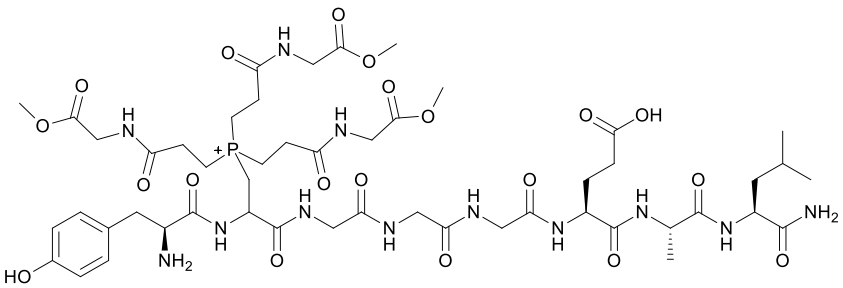


Figure S35. a) Chemical structure of peptide **7a**; b) Analytic HPLC trace of peptide **7a**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{44}H_{75}N_9O_{11}P^+$: 937.1, $[M]^+$. Found: 936,7, $[M]^+$; 468.9, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7c**

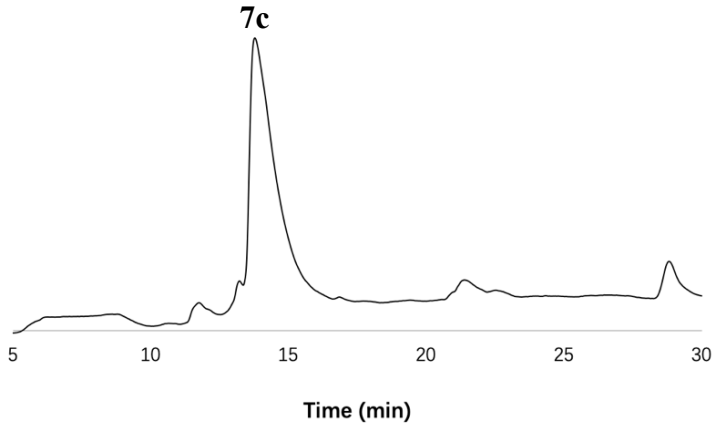
0.2 mg peptide **5i** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2c** was dissolved into the tube. The tube was vortexed at 37 °C for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{50}H_{78}N_{12}O_{20}P^+$
Exact Mass: 1197.52
Molecular Weight: 1198.20

b)



c)

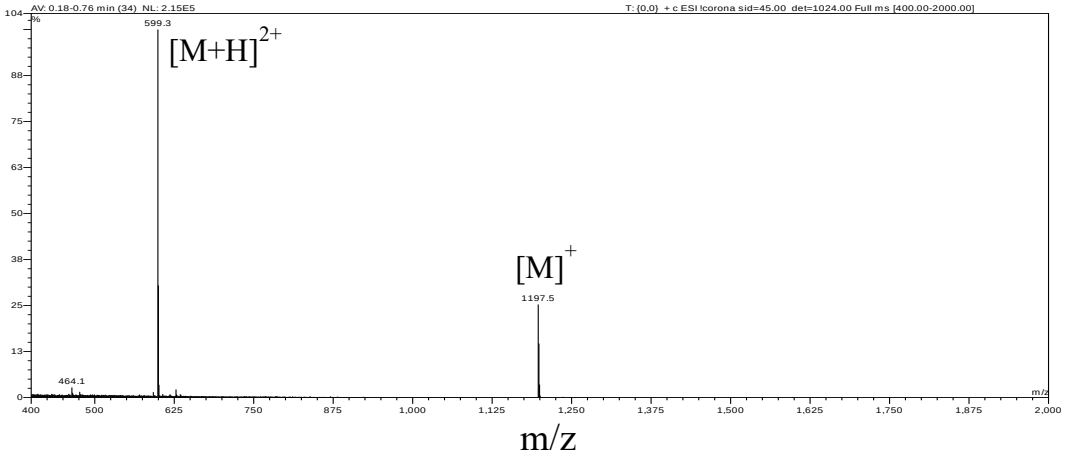
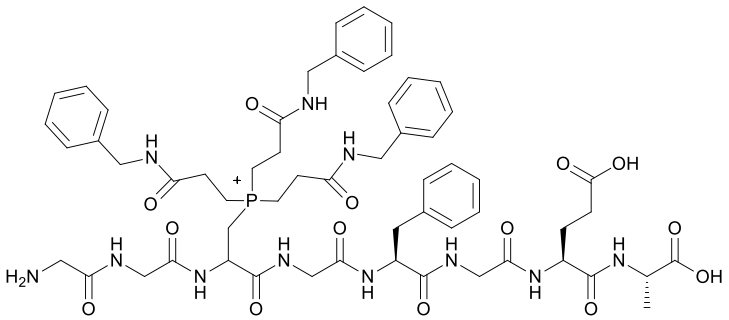


Figure S36. a) Chemical structure of peptide **7c**; b) Analytic HPLC trace of peptide **7c**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{50}H_{78}N_{12}O_{20}P^+$: 1198.2, $[M]^+$. Found: 1197.5, $[M]^+$; 599.3, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7d**

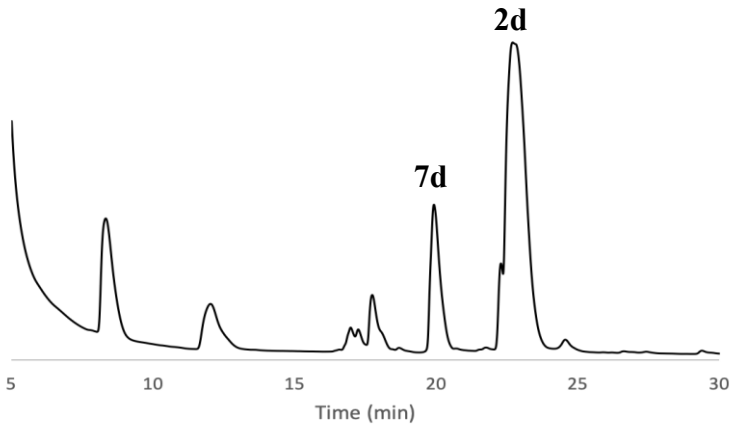
0.2 mg peptide **5b** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2d** was dissolved into 200 µL DMF. The DMF solution was added into the peptide solution and the tube was vortexed at 37 °C for 12 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{58}H_{75}N_{11}O_{14}P^+$
Exact Mass: 1180.52
Molecular Weight: 1181.25

b)



c)

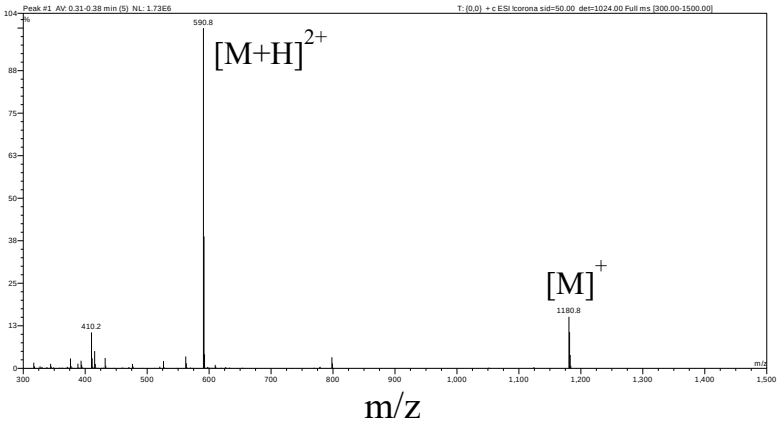
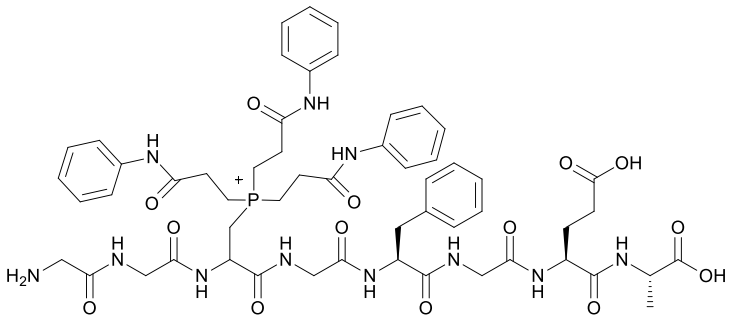


Figure S37. a) Chemical structure of peptide **7d**; b) Analytic HPLC trace of peptide **7d**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{58}H_{75}N_{11}O_{14}P^+$: 1181.3, $[M]^+$. Found: 1180.8, $[M]^+$; 590.8, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7e**

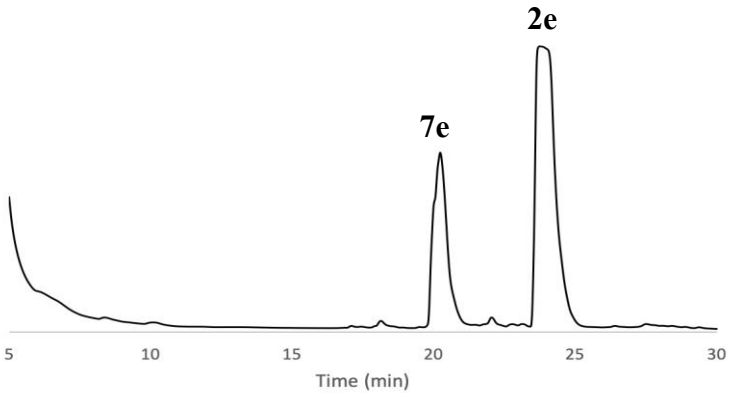
0.2 mg peptide **5b** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2e** was dissolved into 200 µL DMF. The DMF solution was added into the peptide solution and the tube was vortexed at 37 °C for 12 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{55}H_{69}N_{11}O_{14}P^+$
Exact Mass: 1138.48
Molecular Weight: 1139.17

b)



c)

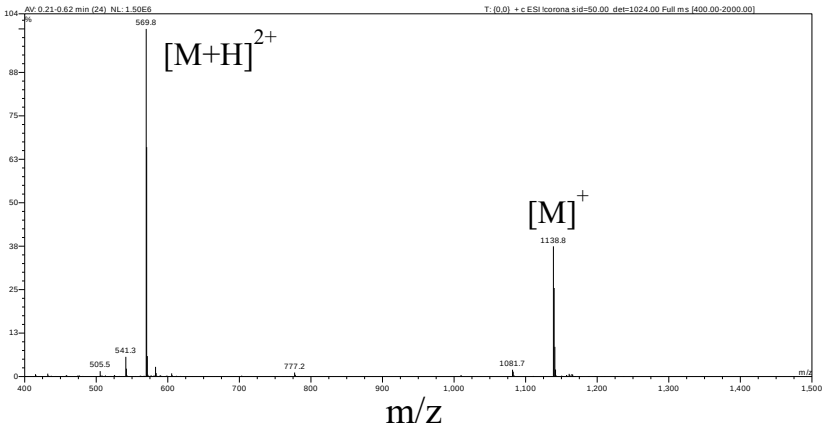
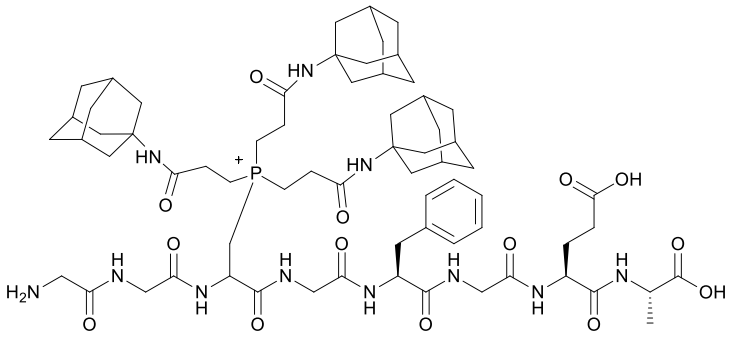


Figure S38. a) Chemical structure of peptide **7e**; b) Analytic HPLC trace of peptide **7e**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{55}H_{69}N_{11}O_{14}P^+$: 1139.2, $[M]^+$. Found: 1138.8, $[M]^+$; 569.8, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7f**

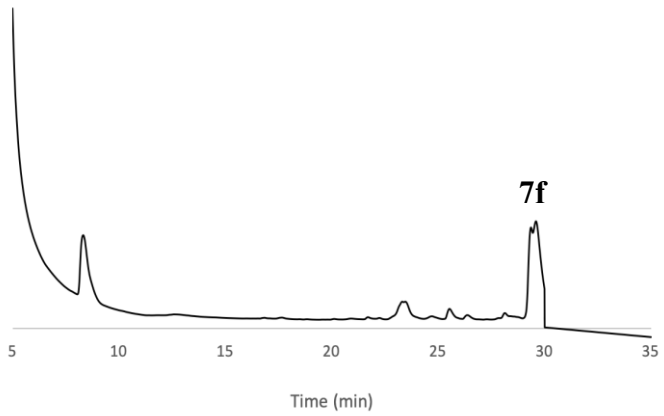
0.2 mg peptide **5b** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2f** was dissolved into 200 µL DMF. The DMF solution was added into the peptide solution and the tube was vortexed at 37 °C for 12 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{67}H_{99}N_{11}O_{14}P^+$
Exact Mass: 1312.71
Molecular Weight: 1313.54

b)



c)

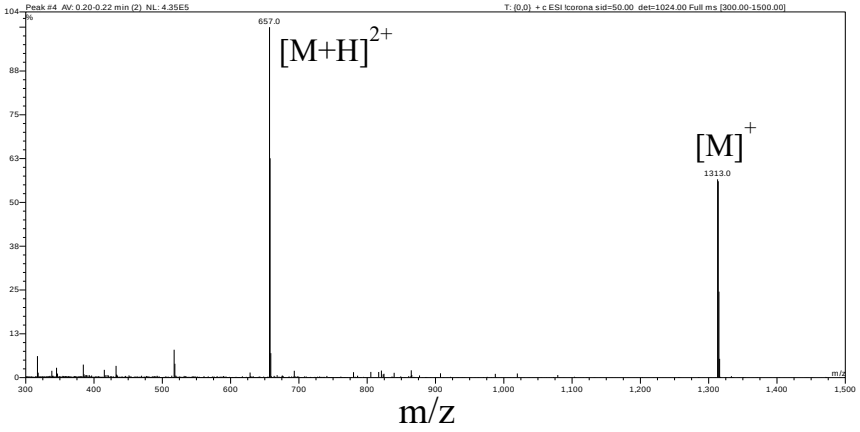
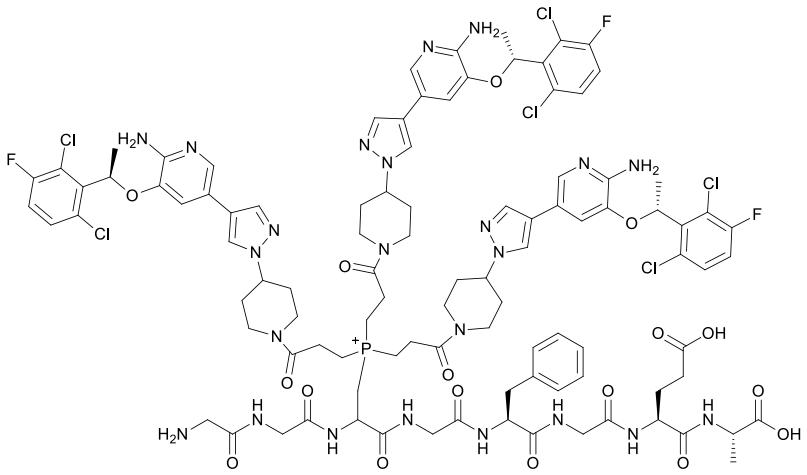


Figure S39. a) Chemical structure of peptide **7f**; b) Analytic HPLC trace of peptide **7f**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{67}H_{99}N_{11}O_{14}P^+$: 1313.5, $[M]^+$. Found: 1313.0, $[M]^+$; 657.0, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7g**

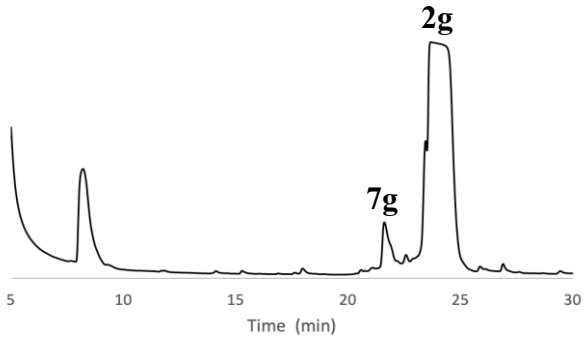
0.15 mg peptide **5b** was dissolved into 150 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2g** was dissolved into 300 µL DMF. The DMF solution was added into the peptide solution and the tube was vortexed at 37 °C for 12 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{100}H_{114}Cl_6F_3N_{23}O_{17}P^+$
Exact Mass: 2206.66
Molecular Weight: 2210.81

b)



c)

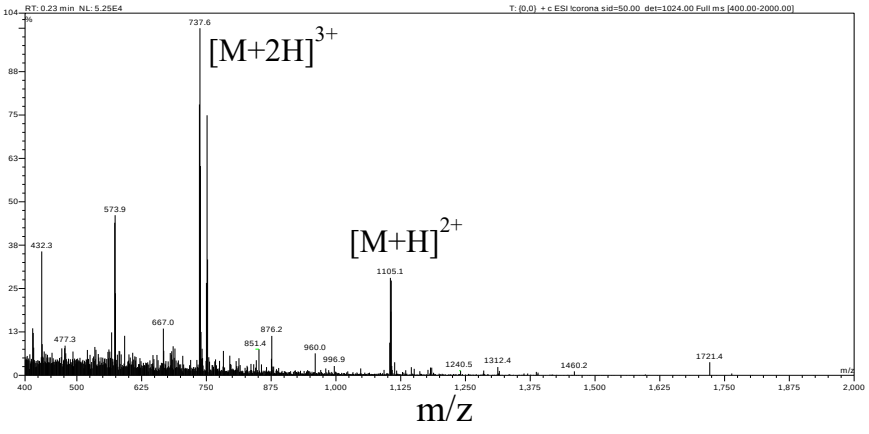
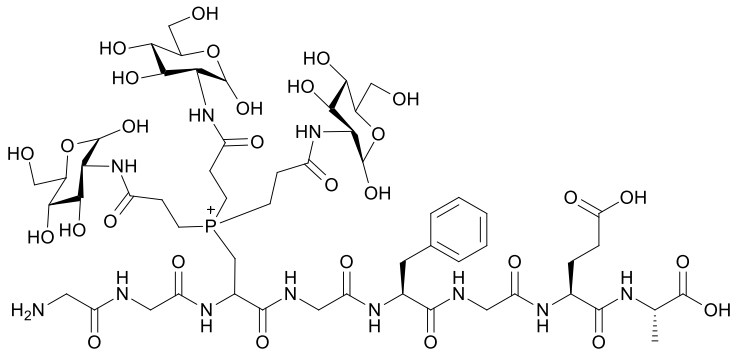


Figure S40. a) Chemical structure of peptide **7g**; b) Analytic HPLC trace of peptide **7g**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{100}H_{114}Cl_6F_3N_{23}O_{17}P^+$: 2210.8, $[M]^+$. Found: 1105.1, $[M+H]^{2+}$; 737.6, $[M+2H]^{3+}$.

Synthesis and characterization of peptide **7h**

0.2 mg peptide **5b** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2h** was dissolved into the tube. The tube was vortexed at 37 °C for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)

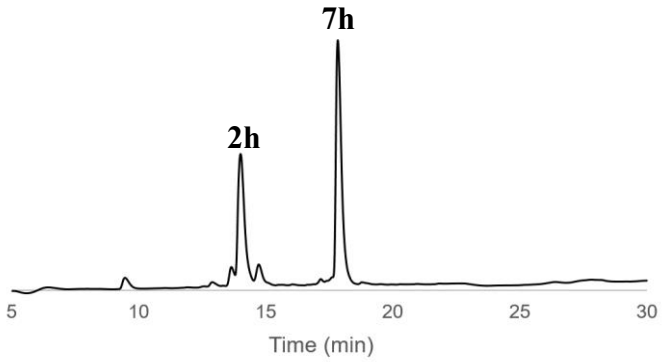


Chemical Formula: $C_{55}H_{87}N_{11}O_{29}P^+$

Exact Mass: 1396.54

Molecular Weight: 1397.31

b)



c)

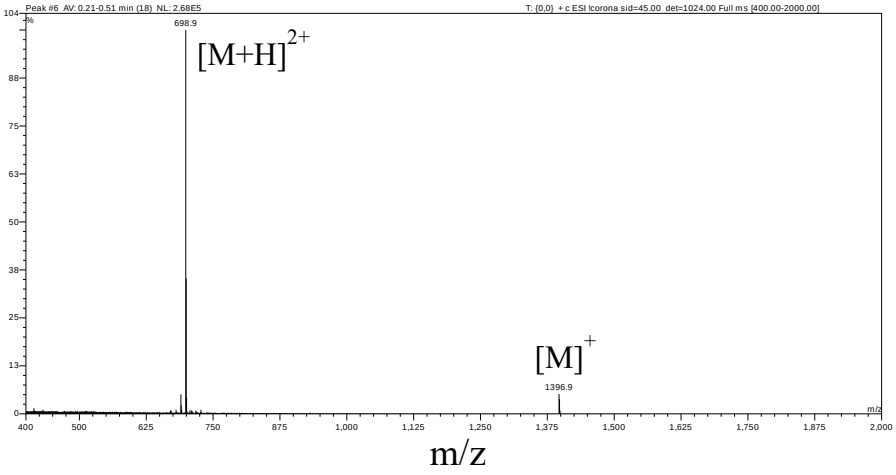
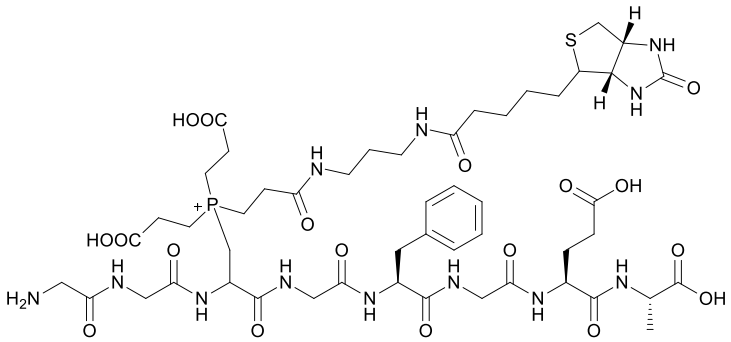


Figure S41. a) Chemical structure of peptide **7h**; b) Analytic HPLC trace of peptide **7h**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{55}H_{87}N_{11}O_{29}P^+$: 1397.3, $[M]^+$. Found: 1396.9, $[M]^+$; 698.9, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7i**

0.2 mg peptide **5b** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound 2i was dissolved into the tube. The tube was vortexed at 37 °C for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)

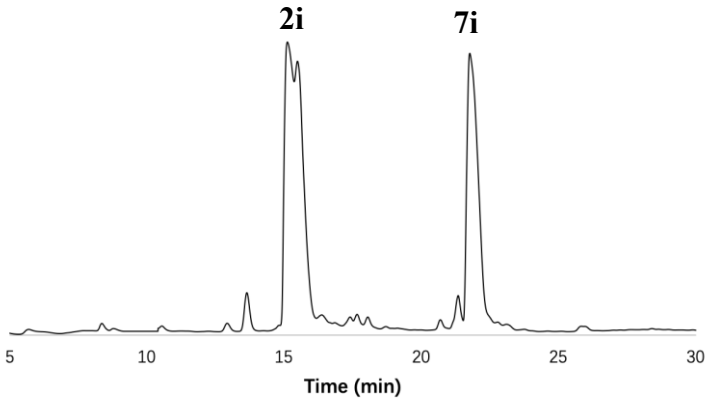


Chemical Formula: $C_{50}H_{76}N_{12}O_{18}PS^+$

Exact Mass: 1195.49

Molecular Weight: 1196.25

b)



c)

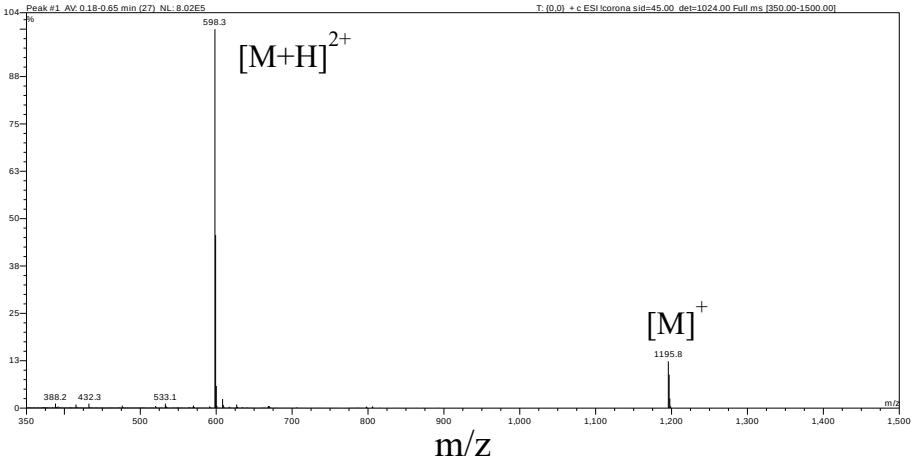
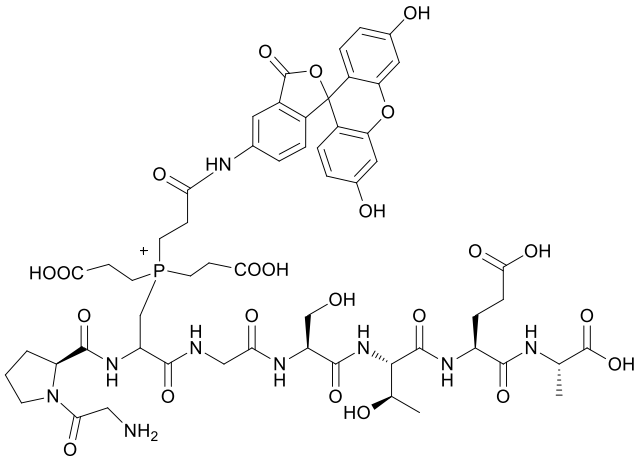


Figure S42. a) Chemical structure of peptide **7i**; b) Analytic HPLC trace of peptide **7i**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{50}H_{76}N_{12}O_{18}PS^+$: 1196.3, $[M]^+$. Found: 1195.8, $[M]^+$; 598.3, $[M+H]^{2+}$.

Synthesis and characterization of peptide **7j**

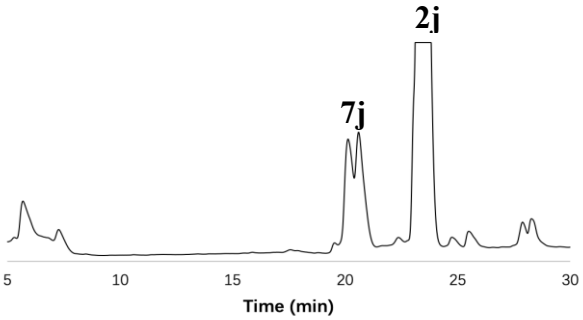
0.2 mg peptide **5g** was dissolved into 200 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 30 eq compound **2j** was dissolved into 50 µL DMF. The DMF solution was added into the peptide solution and the tube was vortexed at 37 °C for 6 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



Chemical Formula: $C_{56}H_{69}N_9O_{23}P$
Exact Mass: 1266.42
Molecular Weight: 1267.17

b)



c)

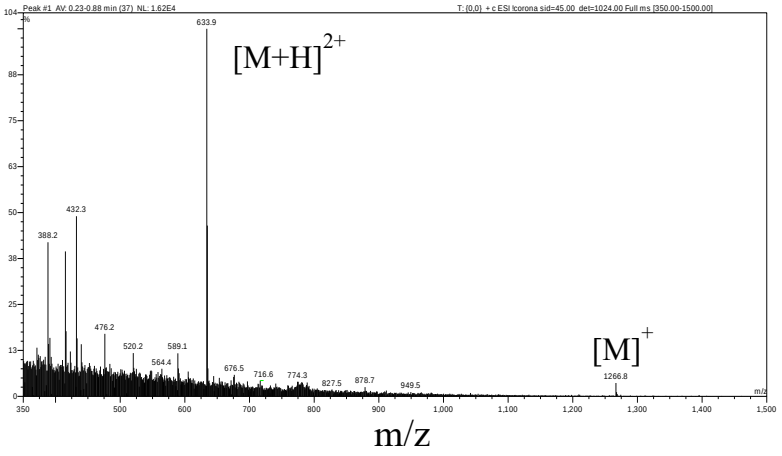
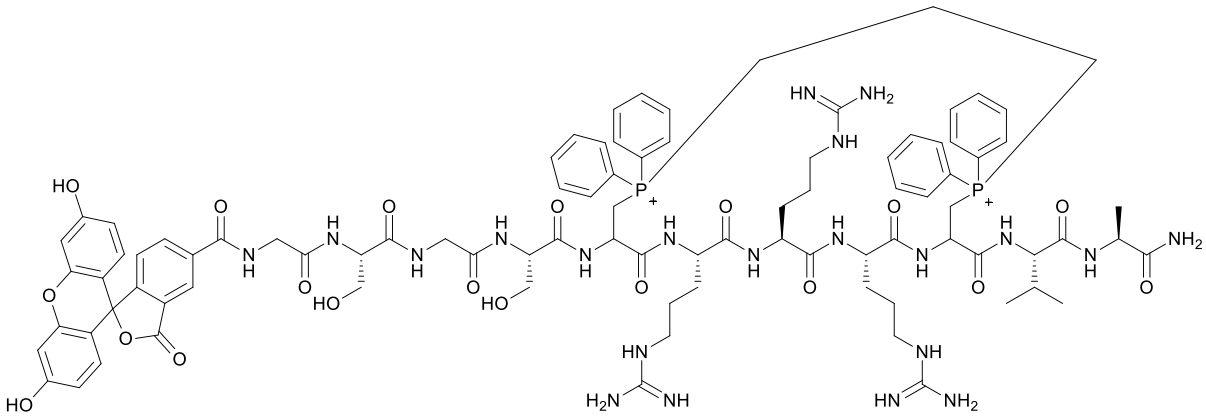


Figure S43. a) Chemical structure of peptide **7j**; b) Analytic HPLC trace of peptide **7j**. (HPLC gradient is 5% to 40% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{56}H_{69}N_9O_{23}P$+: 1267.2, $[M]^+$. Found: 1266.8, $[M]^+$; 633.9, $[M+H]^{2+}$.

Synthesis and characterization of staple peptide **7k**

1.0 mg peptide **5j** was dissolved into 100 μL deionized water in a 1.5 mL Eppendorf tube, then 10.0 mg (35 eq) 1,3-Bis(diphenylphosphino)propane was dissolved into 500 μL DMF. The DMF solution was added into the peptide solution and the tube was vortexed at 37 °C overnight, then purifying the crude peptide with analytic HPLC and ESI-MS, and then lyophilized to obtain peptide product.

a)

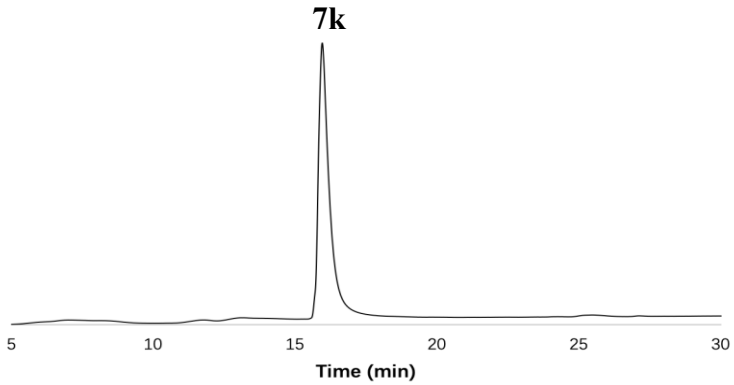


Chemical Formula: $C_{90}H_{113}N_{21}O_{19}P_2^{2+}$

Exact Mass: 1853.80

Molecular Weight: 1854.94

b)



c)

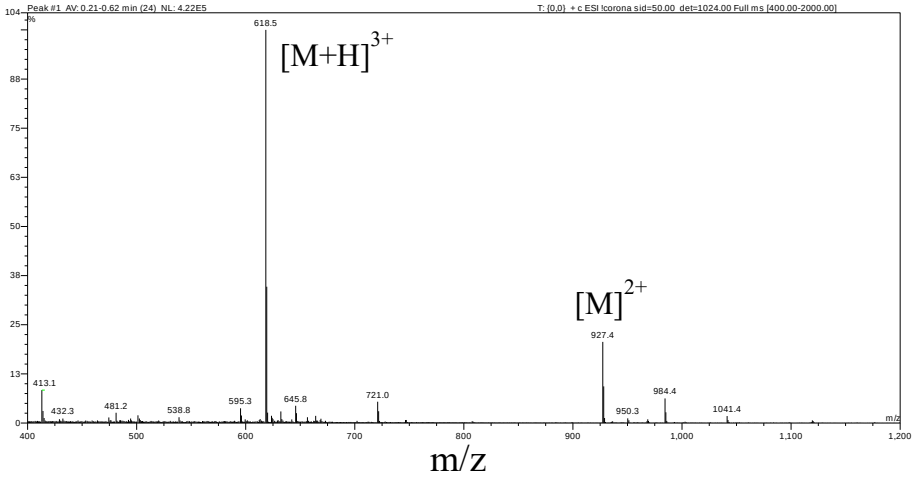


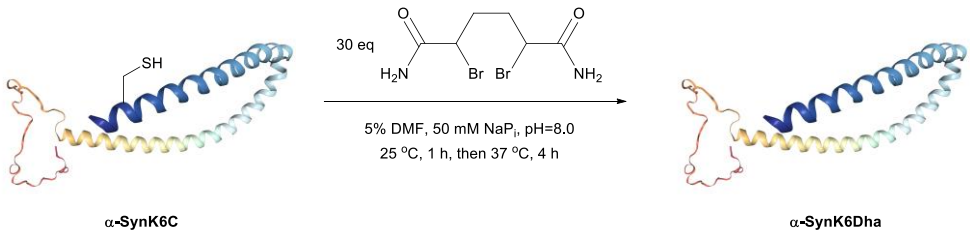
Figure S44. a) Chemical Structure of staple peptide **7k**; b) Analytic HPLC trace of peptide **7k**. (HPLC gradient is 20% to 60% of solution B in 30 min on the Analytic C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{90}H_{113}N_{21}O_{19}P_2^{2+}$: 927.5, $[M]^{2+}$. Found: 927.4, $[M]^{2+}$; 618.5, $[M+H]^{3+}$.

Synthesis and characterization of proteins

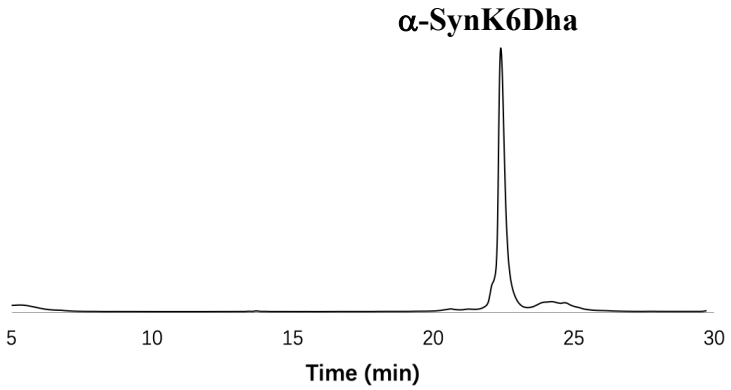
Synthesis and characterization of α-SynK6Dha

α-SynK6Dha was synthesized via Bis-alkylation-elimination from α-SynK6C. 1.0 mg α-SynK6C with 0.2 eq tris(2-carboxyethyl)phosphine hydrochloride was dissolved into 1.0 mL NaP_i buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 10.4 mg (500 eq) 2,5-dibromohexanediamide (**DBHDA**) was dissolved into 100 μL DMF to make it completely dissolved. The DMF solution was added into the NaP_i buffer solution and the mixture was vortexed about 30 s by Vortex to mix well. The tube was vortexed at 25 °C for 1 h, then 37 °C for 4 h. Finally taking supernatant after centrifuging the solution to purify the crude protein with RP-HPLC, and then lyophilized to obtain protein product. Characterization was assessed with Analytic HPLC and ESI-MS.

a)



b)



c)

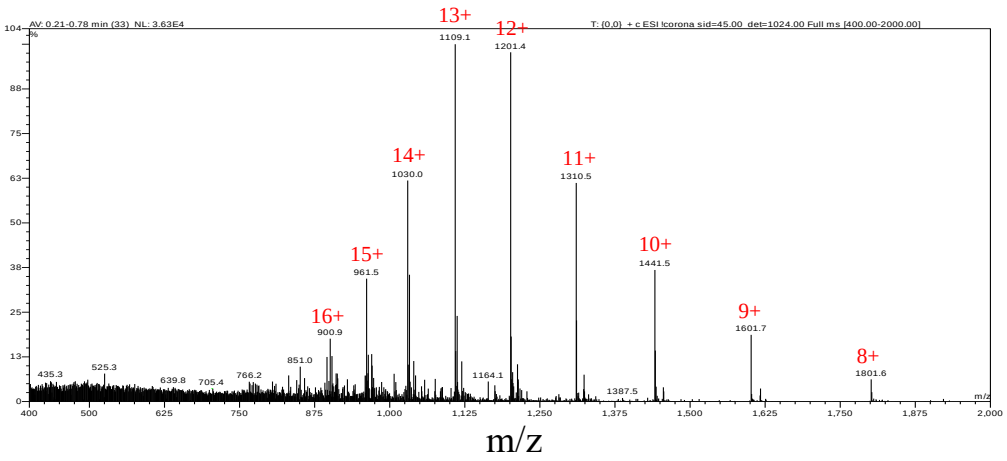
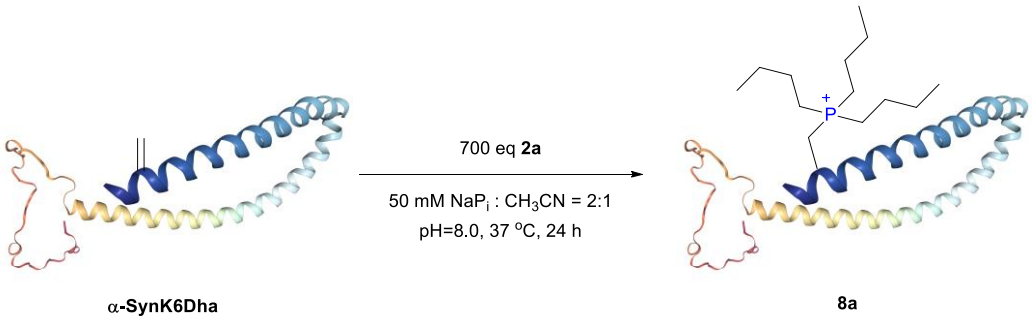


Figure S45. a) Synthesis of α-SynK6Dha; b) Analytic HPLC trace of α-SynK6Dha. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for α-SynK6Dha: 14401, $[M+H]^+$. Found: 14404, $[M+H]^+$.

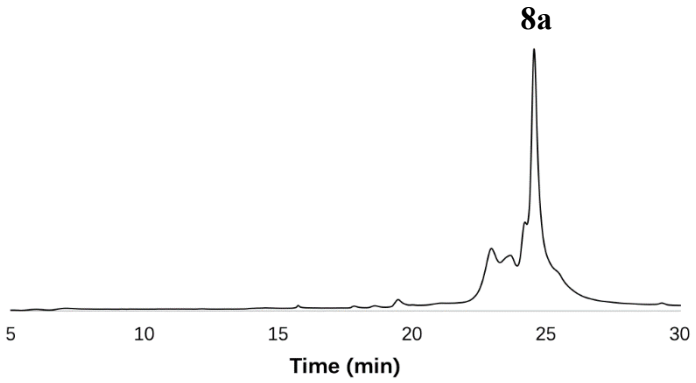
Synthesis and characterization of protein **8a**

0.1 mg α-SynK6Dha was dissolved into 100 µL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 1.2 µL (700 eq) TBUP (**2a**) was dissolved into 50 µL acetonitrile. The acetonitrile solution was added into the peptide solution and the tube was vortexed at 37 °C for 24 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



b)



c)

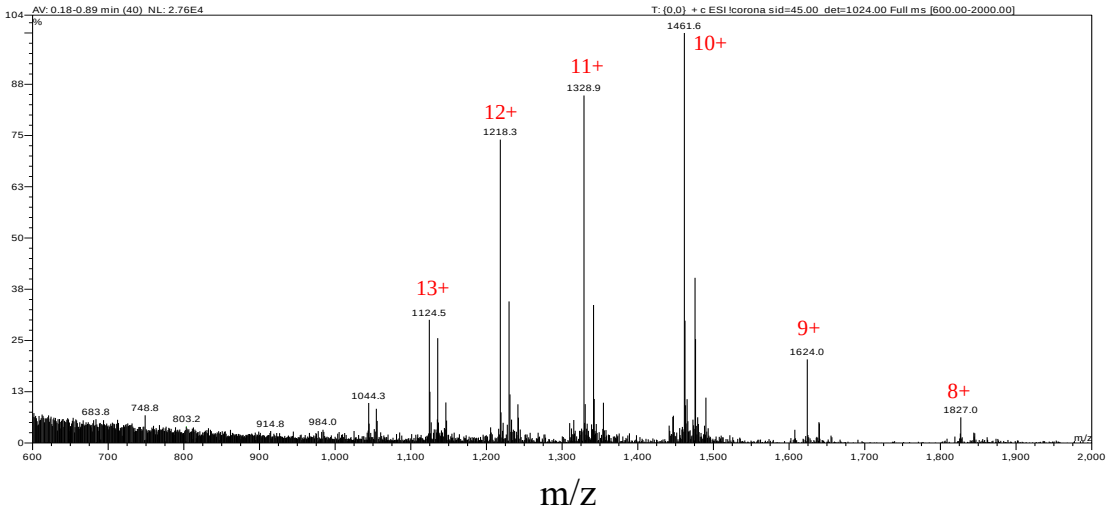
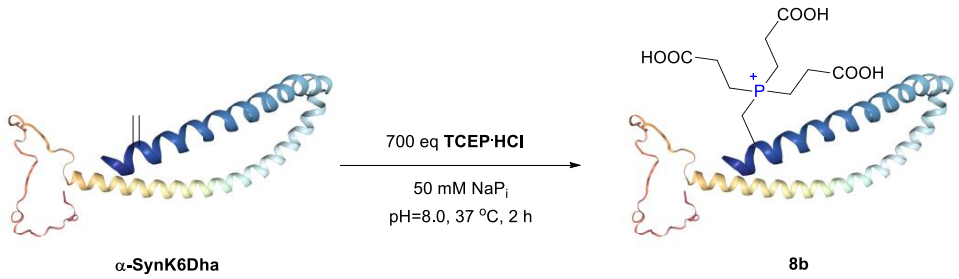


Figure S46. a) Synthesis of protein **8a**; b) Analytic HPLC trace of protein **8a**. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for protein **8a**: 14603, $[M]^+$. Found: 14606, $[M]^+$.

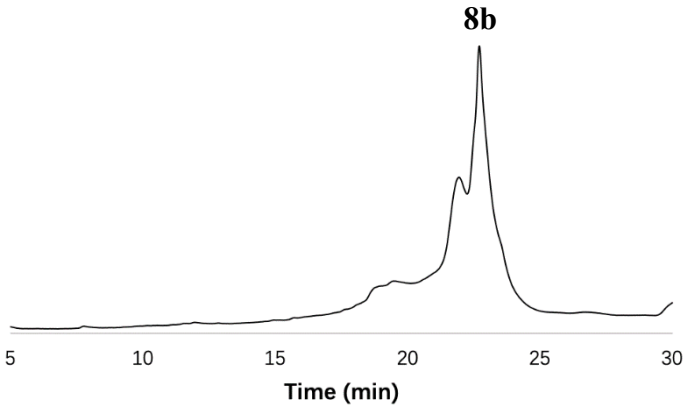
Synthesis and characterization of protein **8b**

0.1 mg α-SynK6Dha was dissolved into 100 μL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 1.0 mg (700 eq) TECP·HCl was dissolved into the tube, the pH of the solution was adjusted by dilute 2M NaOH solution to 8.0. The tube was vortexed at 37 °C for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



b)



c)

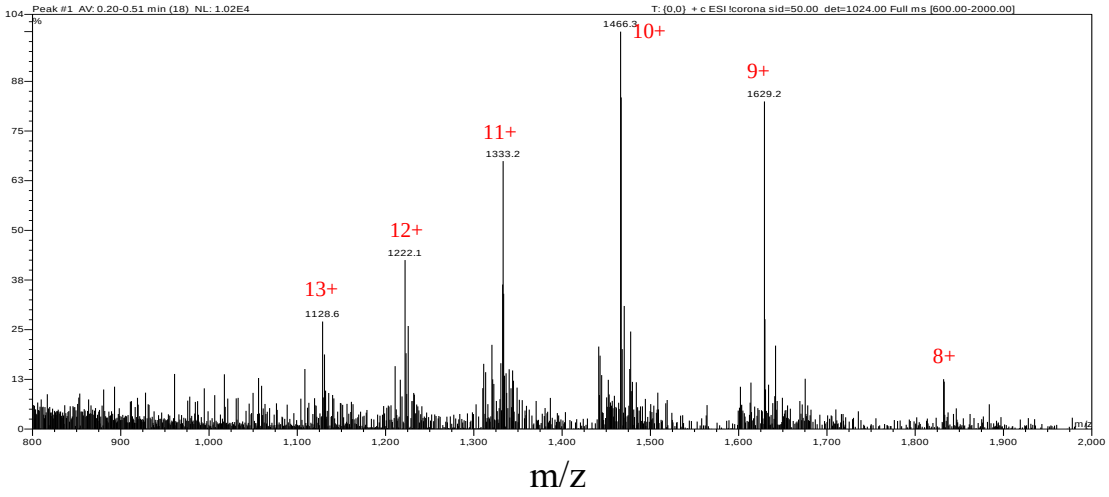
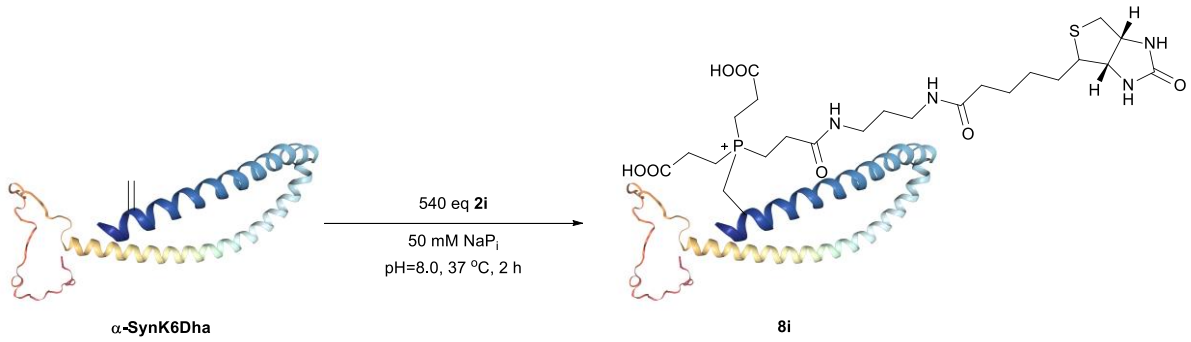


Figure S47. a) Synthesis of protein **8b**; b) Analytic HPLC trace of protein **8b**. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for protein **8b**: 14651, $[M]^+$. Found: 14653, $[M]^+$.

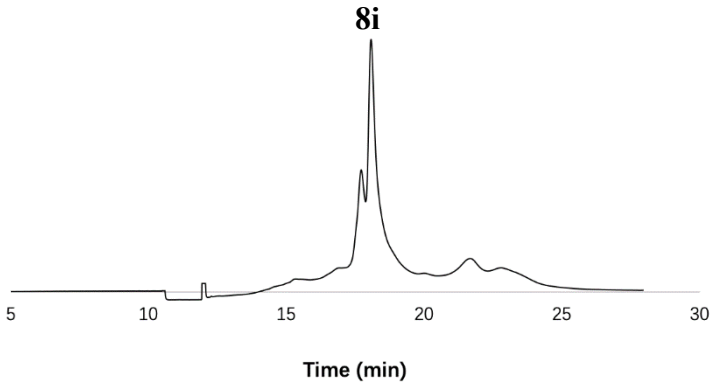
Synthesis and characterization of protein **8i**

0.1 mg α-SynK6Dha was dissolved into 100 μL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 2.0 mg (540 eq) compound **2i** was dissolved into the tube. The tube was vortexed at 37 °C for 2 h, then monitoring the reaction with analytic HPLC and ESI-MS.

a)



b)



c)

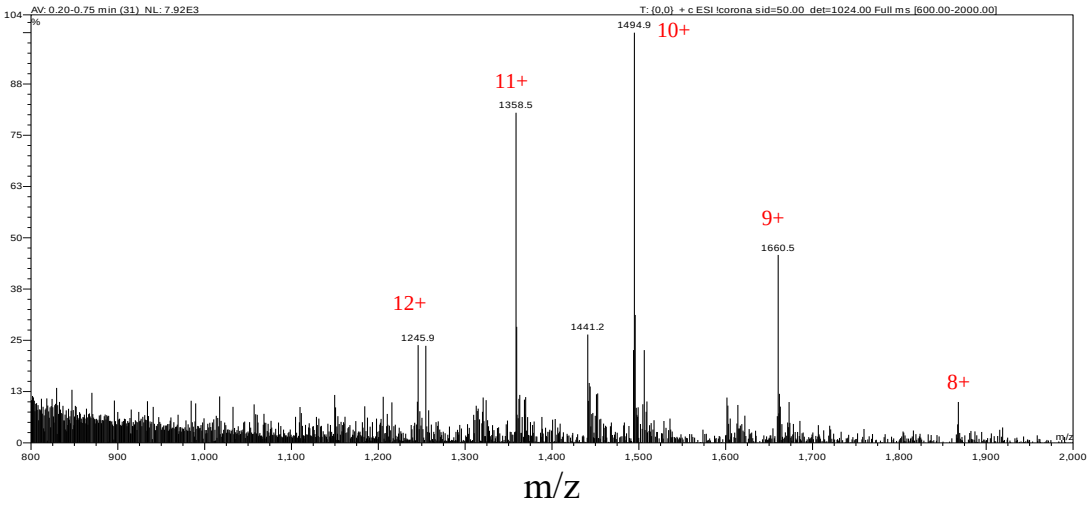
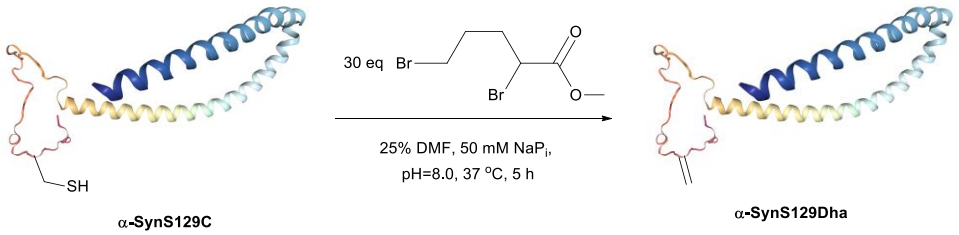


Figure S48. a) Synthesis of protein **8i**; b) Analytic HPLC trace of protein **8i**. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for protein **8i**: 14936, $[M]^+$. Found: 14939, $[M]^+$.

Synthesis and characterization of α-SynS129Dha

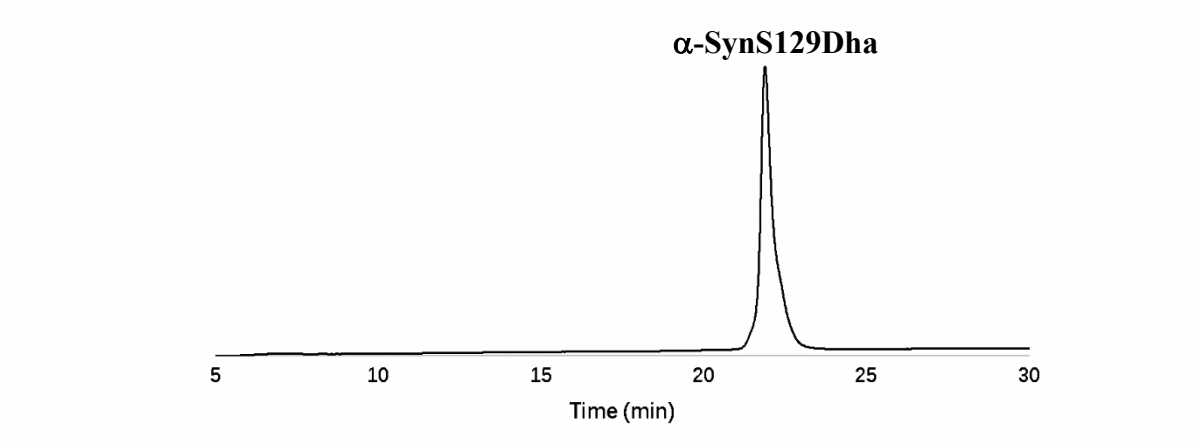
α-SynS129Dha was synthesized via Bis-alkylation-elimination from α-SynS129C. 1.0 mg α-SynS129C with 0.2 eq tris(2-carboxyethyl)phosphine hydrochloride was dissolved into 1.0 mL NaP_i buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 10.0 μL (500 eq) Methyl 2,5-dibromopentanoate (**MeDBP**) was dissolved into 350 μL DMSO. The DMSO solution was added into the NaP_i buffer solution and the mixture was vortexed about 30 s by Vortex to mix well. The tube was vortexed at 37 °C for 5 h. Finally taking supernatant after centrifuging the solution to purify the crude protein with RP-HPLC, and then lyophilized to obtain protein product. Characterization was assessed with Analytic HPLC and ESI-MS.

a)



α-SynS129C α-SynS129Dha

b)



c)

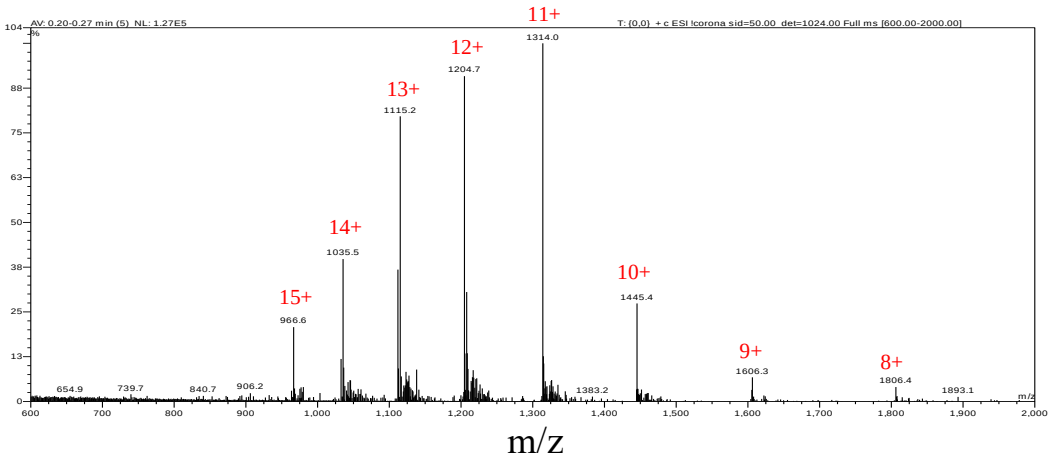
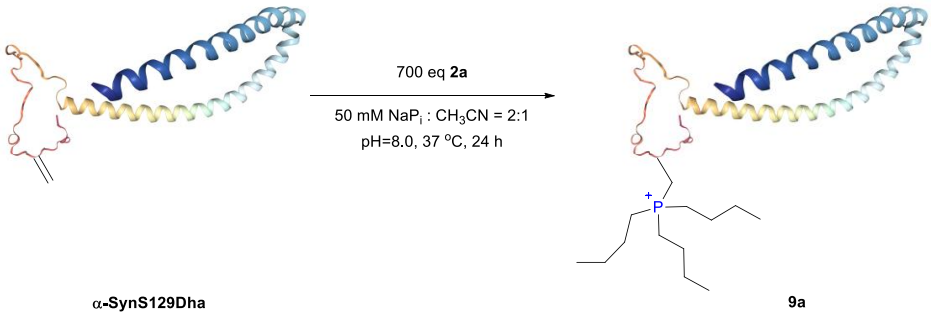


Figure S49. a) Synthesis of α-SynS129Dha; b) Analytic HPLC trace of α-SynS129Dha. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for α-SynS129Dha: 14442, $[M+H]^+$. Found: 14444, $[M+H]^+$.

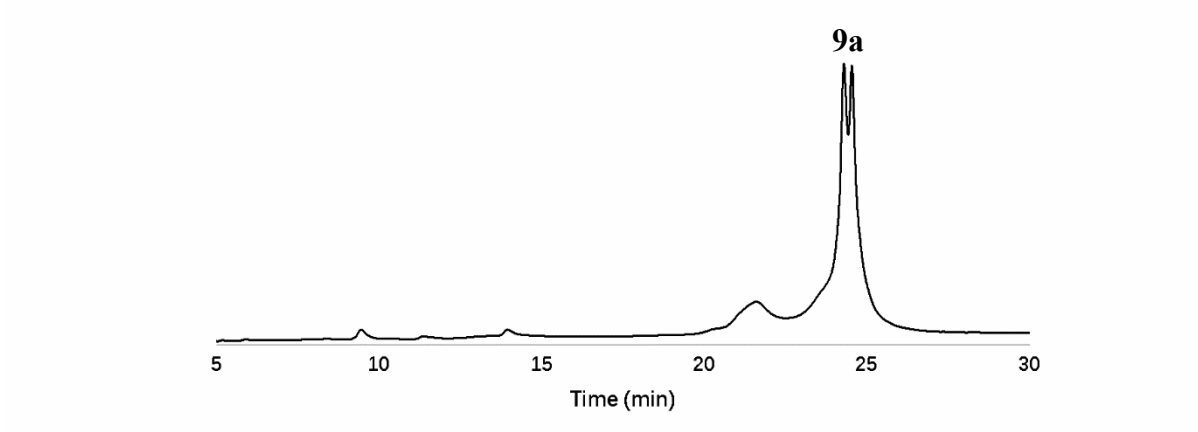
Synthesis and characterization of protein **9a**

0.1 mg α-SynS129Dha was dissolved into 100 μL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 1.2 μL (700 eq) tributyl phosphine (**2a**) was dissolved into 50 μL acetonitrile. The acetonitrile solution was added into the peptide solution and the tube was vortexed at 37 ℃ for 24 h, then purifying the crude protein with RP-HPLC, and then lyophilized to obtain protein product. Characterization was assessed with Analytic HPLC and ESI-MS.

a)



b)



c)

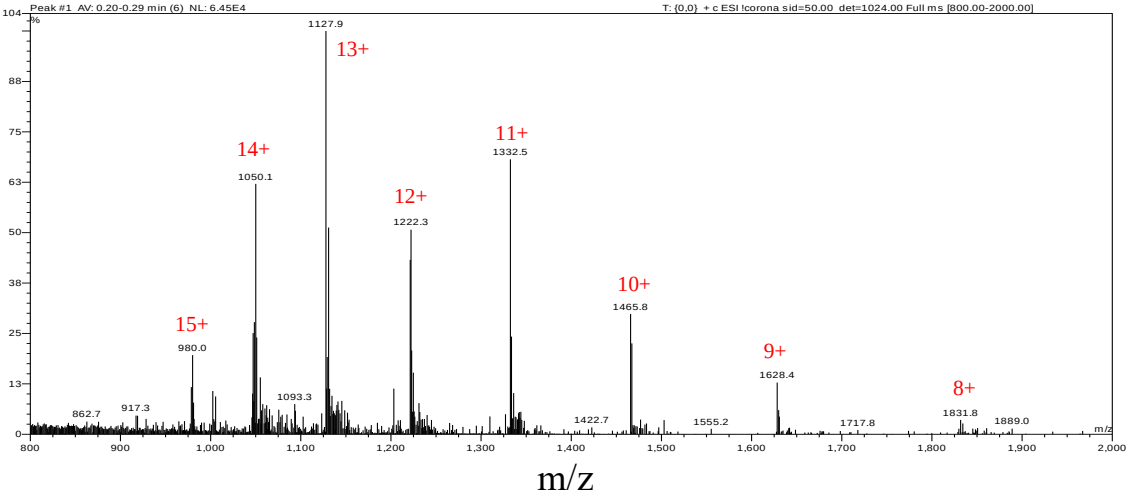
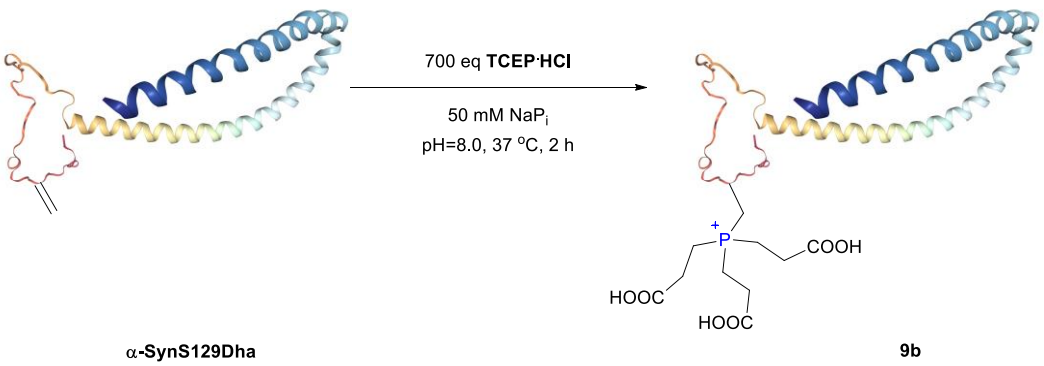


Figure S50. a) Synthesis of protein **9a**; b) Analytic HPLC trace of protein **9a**. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for protein **9a**: 14644, $[M]^+$. Found: 14646, $[M]^+$.

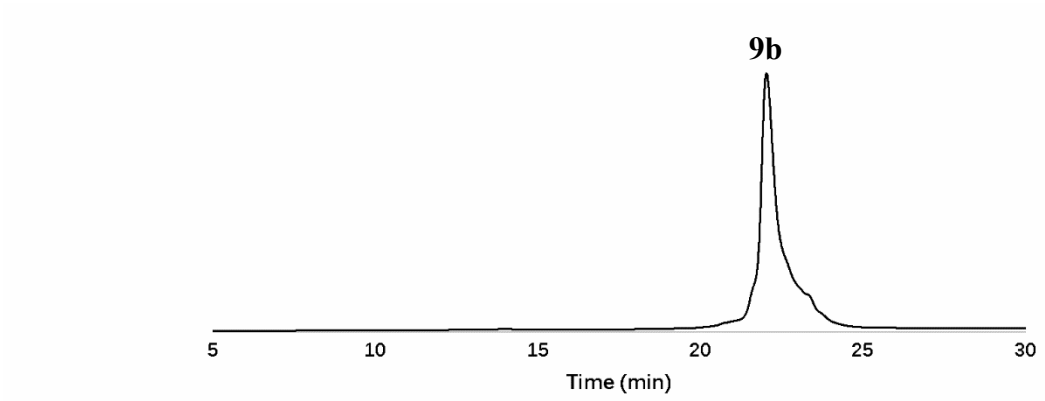
Synthesis and characterization of protein **9b**

0.1 mg α-SynS129Dha was dissolved into 100 μL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 1.0 mg (700 eq) tris(2-carboxyethyl)phosphine hydrochloride was dissolved into the tube, the pH of the solution was adjusted by dilute 2M NaOH solution to 8.0. The tube was vortexed at 37 ℃ for 2 h, then purifying the crude protein with RP-HPLC, and then lyophilized to obtain protein product. Characterization was assessed with Analytic HPLC and ESI-MS.

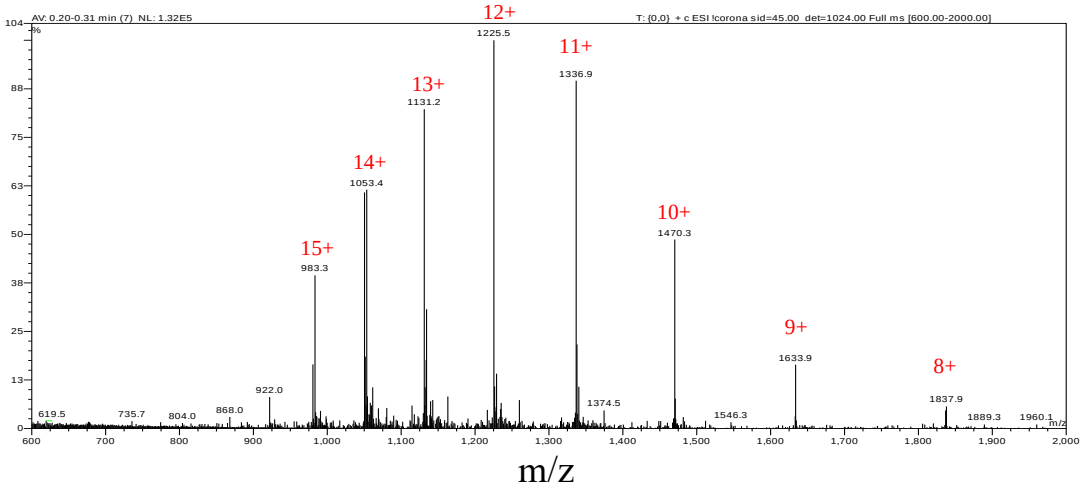
a)



b)



c)



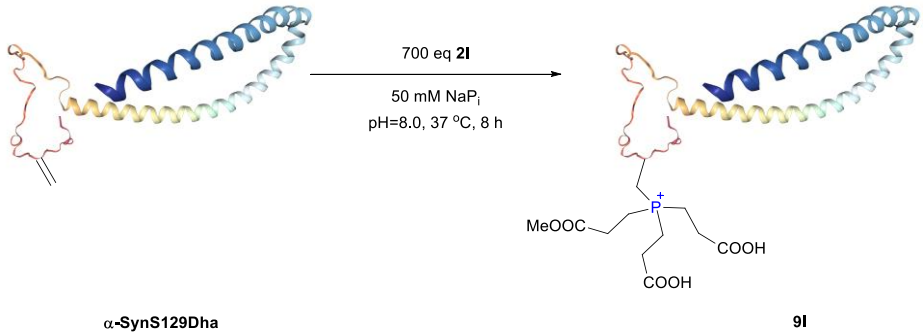
m/z

Figure S51. a) Synthesis of protein **9b**; b) Analytic HPLC trace of protein **9b**. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for protein **9b**: 14693, $[M]^+$. Found: 14693, $[M]^+$.

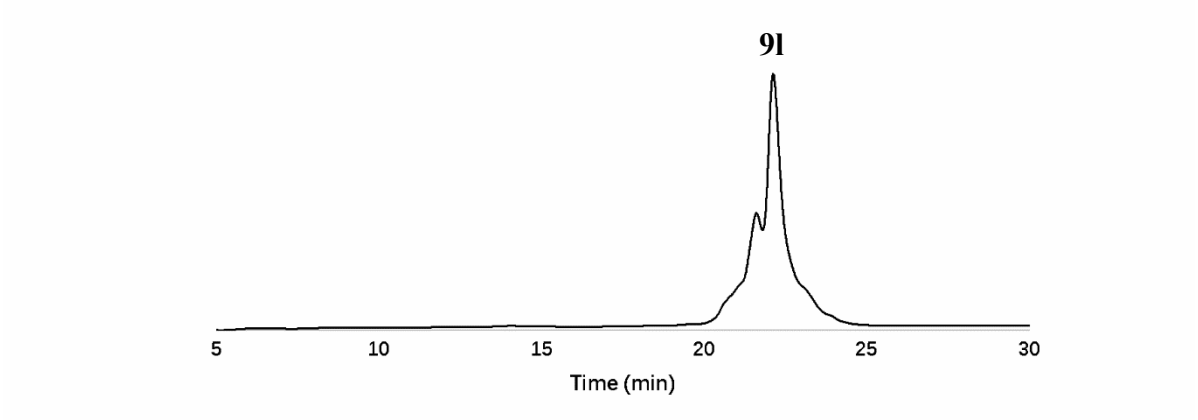
Synthesis and characterization of protein **9l**

0.1 mg α-SynS129Dha was dissolved into 100 μL NaP_i buffer (50 mM, pH 8.0) in a 1.5 mL Eppendorf tube, then 1.0 mg (700 eq) compound **2l** was dissolved into water in another 1.5 mL Eppendorf tube, the pH of the compound **2l** solution was adjusted by dilute 2M NaOH solution to 8.0, then the two tubes of solution were mixed together. The tube was vortexed at 37 ℃ for 2 h, then purifying the crude protein with RP-HPLC, and then lyophilized to obtain protein product. Characterization was assessed with Analytic HPLC and ESI-MS.

a)



b)



c)

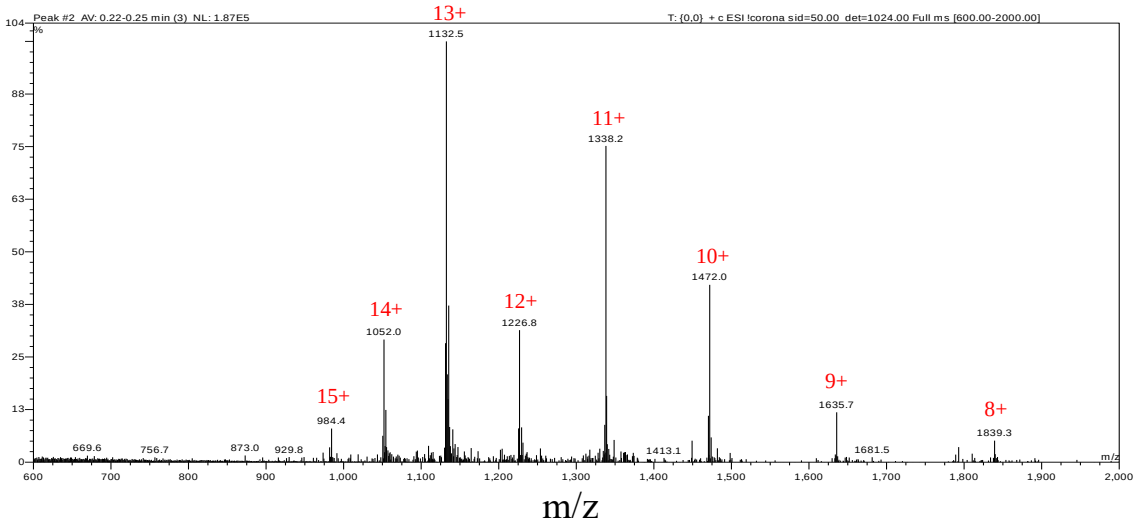
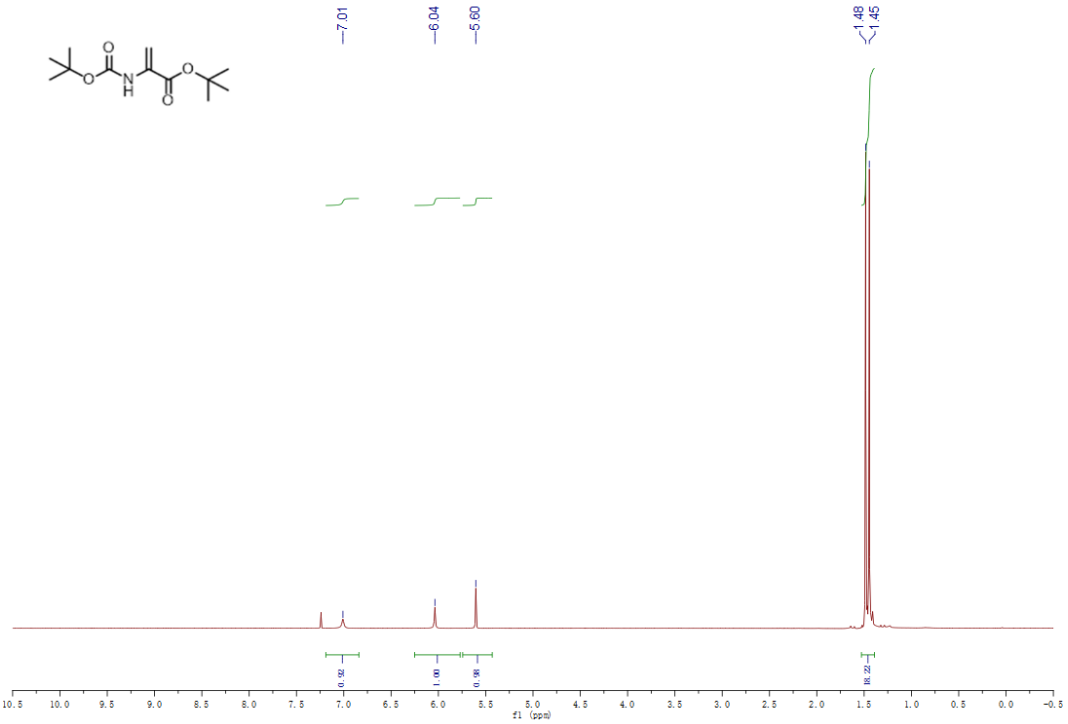


Figure S52. a) Synthesis of protein **9l**; b) Analytic HPLC trace of protein **9l**. (HPLC gradient is 30% to 70% of solution B in 30 min on the Analytic C4 column (λ=215 nm). c) ESI-MS: m/z calculated for protein **9l**: 14707, $[M]^+$. Found: 14710, $[M]^+$.

NMR data

Compound **1**

a)



b)

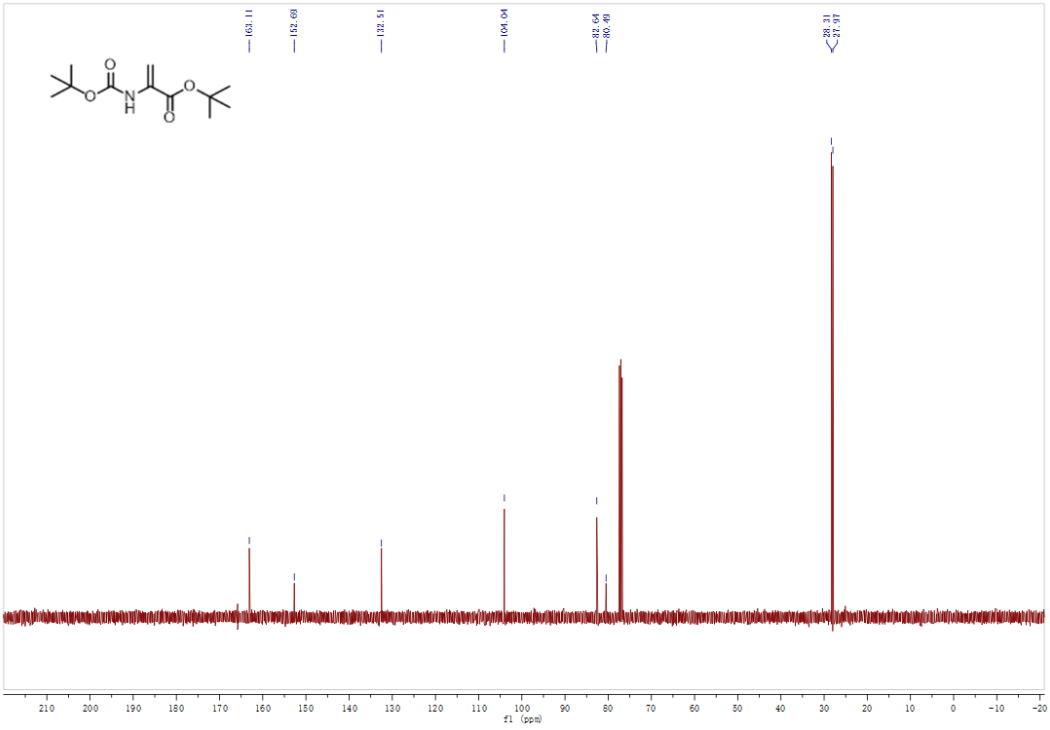
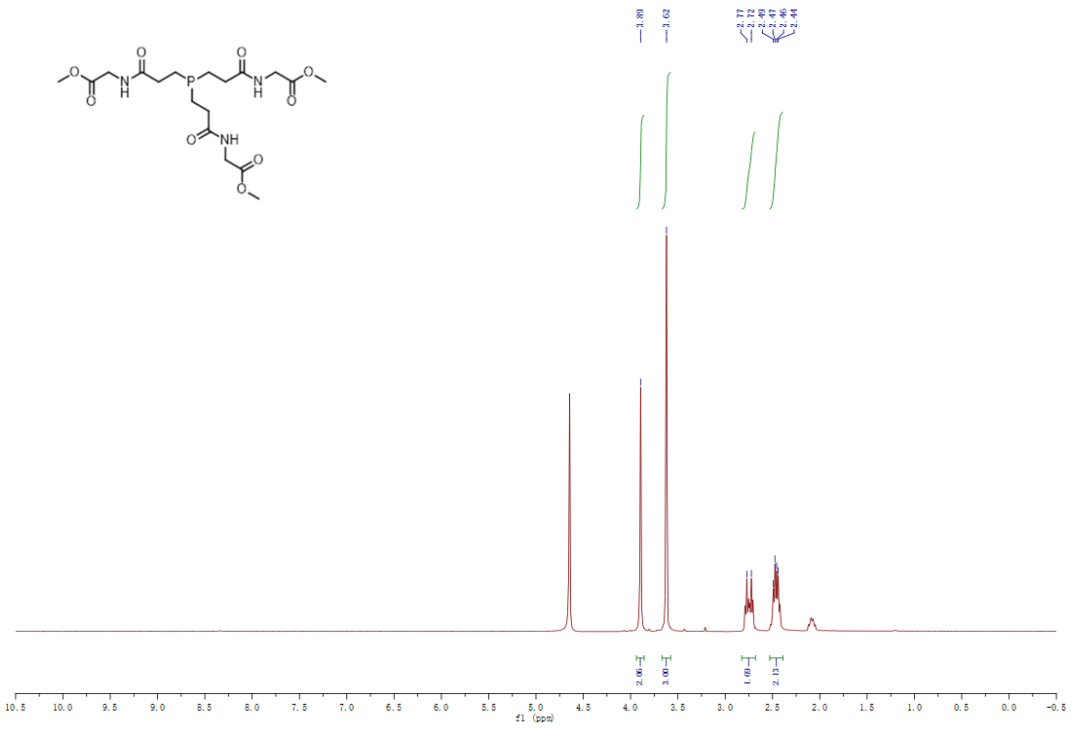


Figure S53. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **1**; b) ^{13}C NMR (100 MHz, $CDCl_3$) of compound **1**.

Compound **2c**

a)



b)

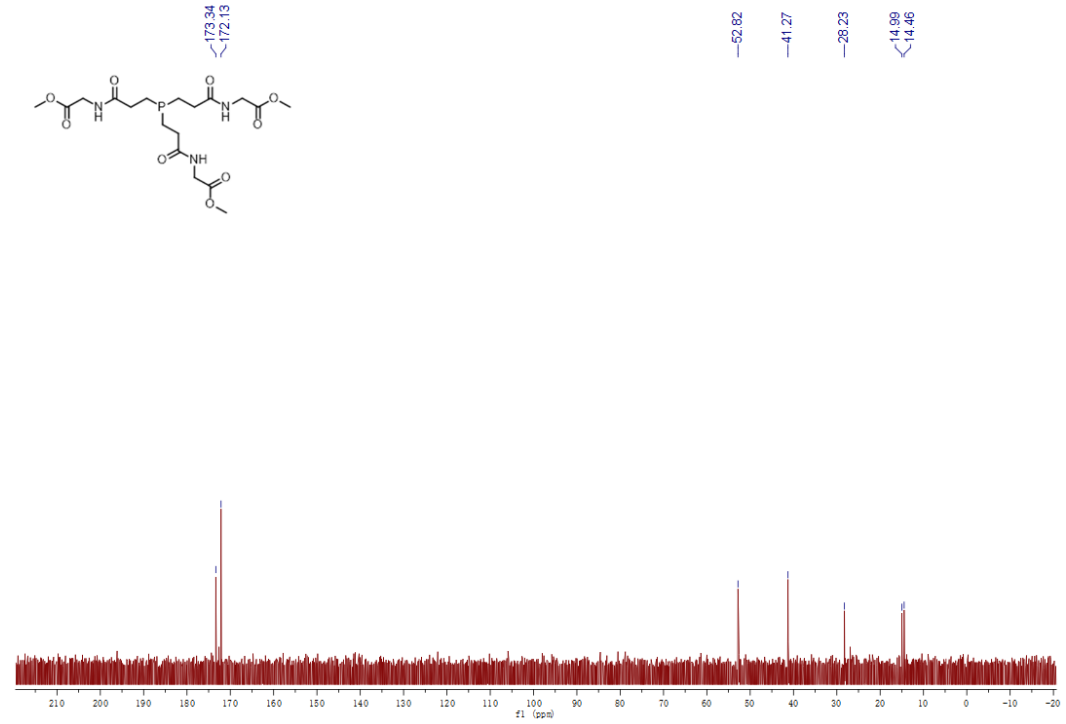
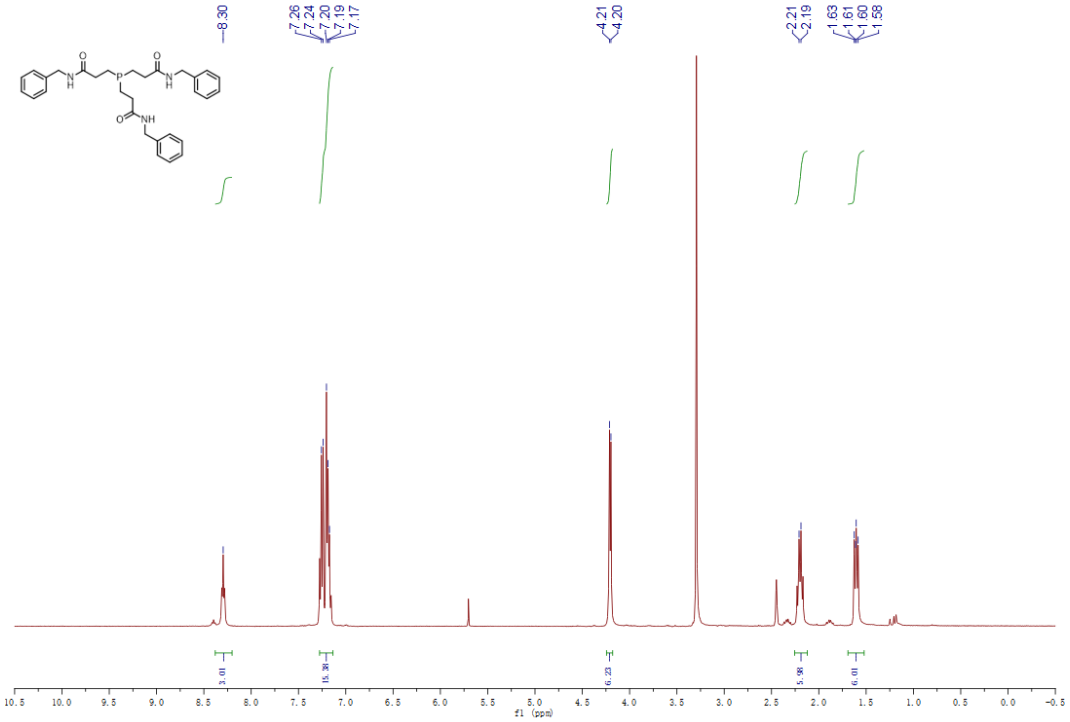


Figure S54. a) ^{1}H NMR (400 MHz, D_2O) of compound **2c**; b) ^{13}C NMR (100 MHz, D_2O) of compound **2c**.

Compound **2d**

a)



b)

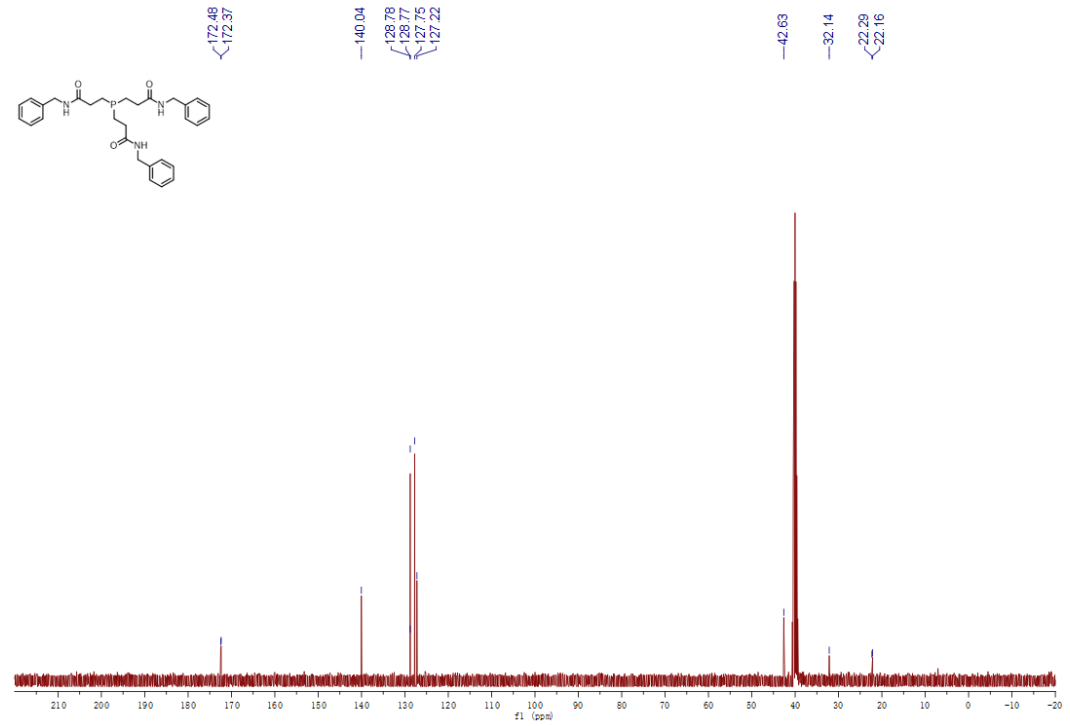
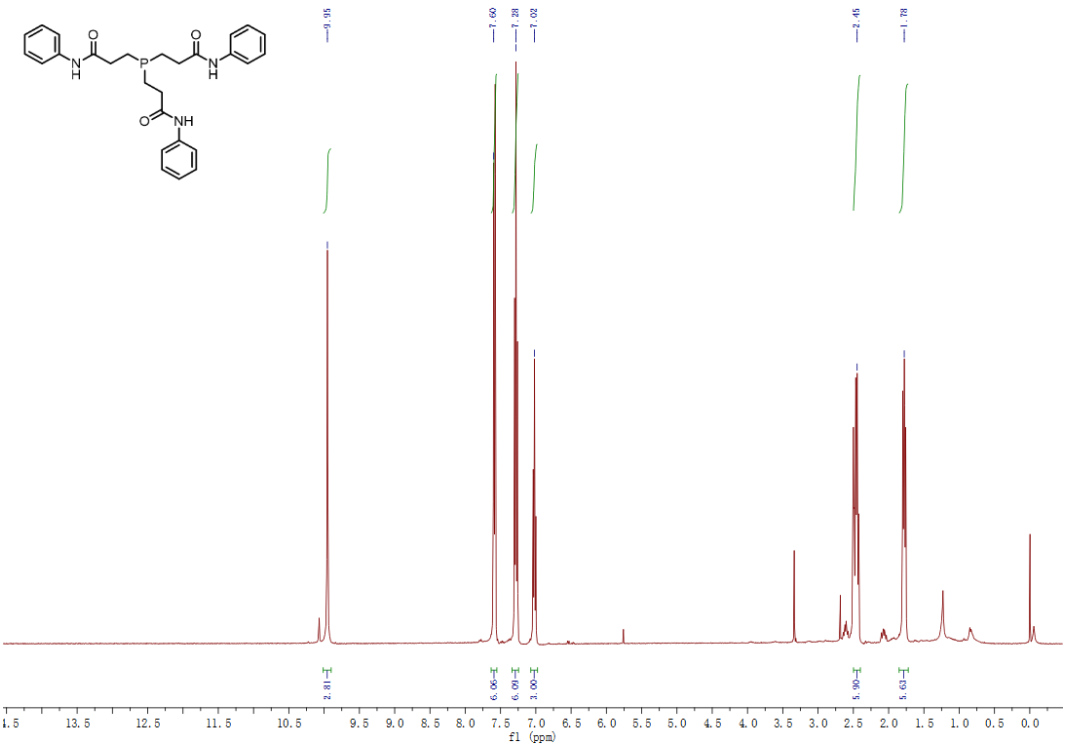


Figure S55. a) ^{1}H NMR (400 MHz, DMSO-d$_6$) of compound **2d**; b) ^{13}C NMR (100 MHz, DMSO-d$_6$) of compound **2d.**

Compound **2e**

a)



b)

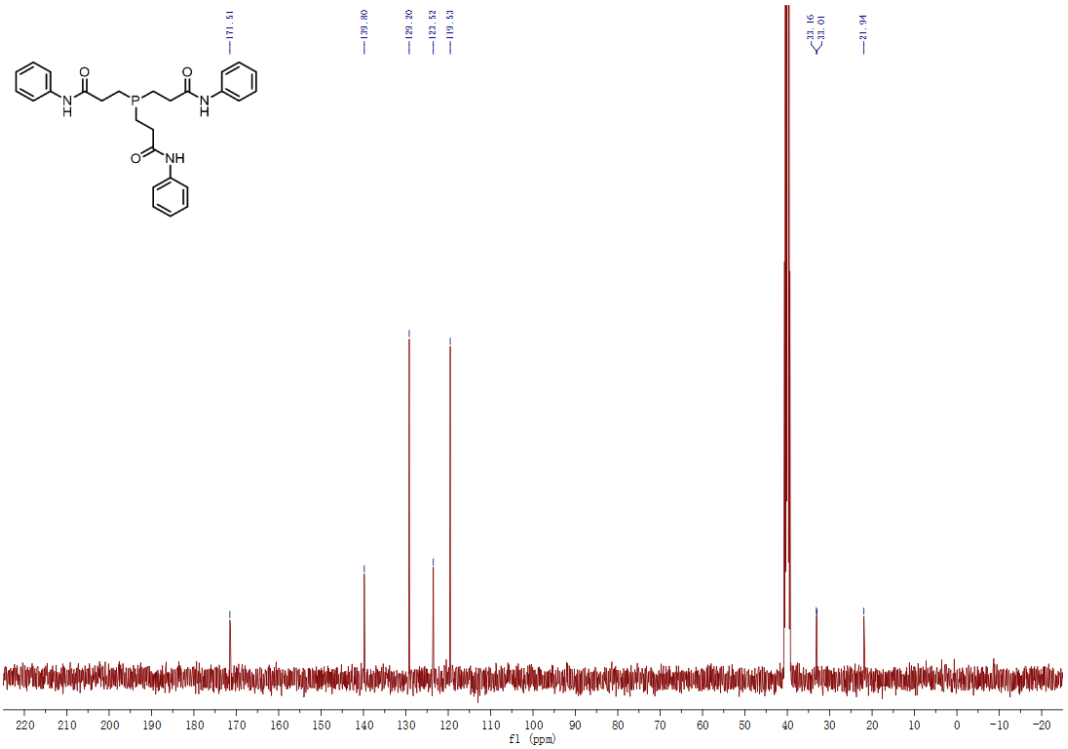
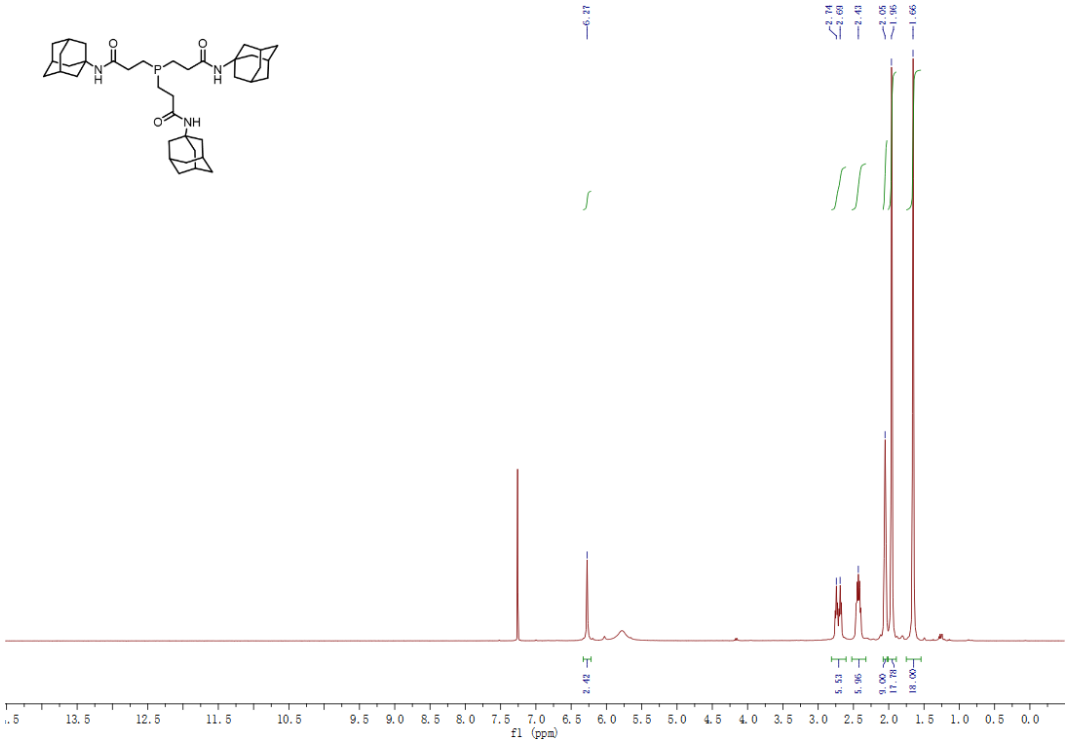


Figure S56. a) ^{1}H NMR (400 MHz, DMSO-d$_6$) of compound **2e**; b) ^{13}C NMR (100 MHz, DMSO-d$_6$) of compound **2e.**

Compound **2f**

a)



b)

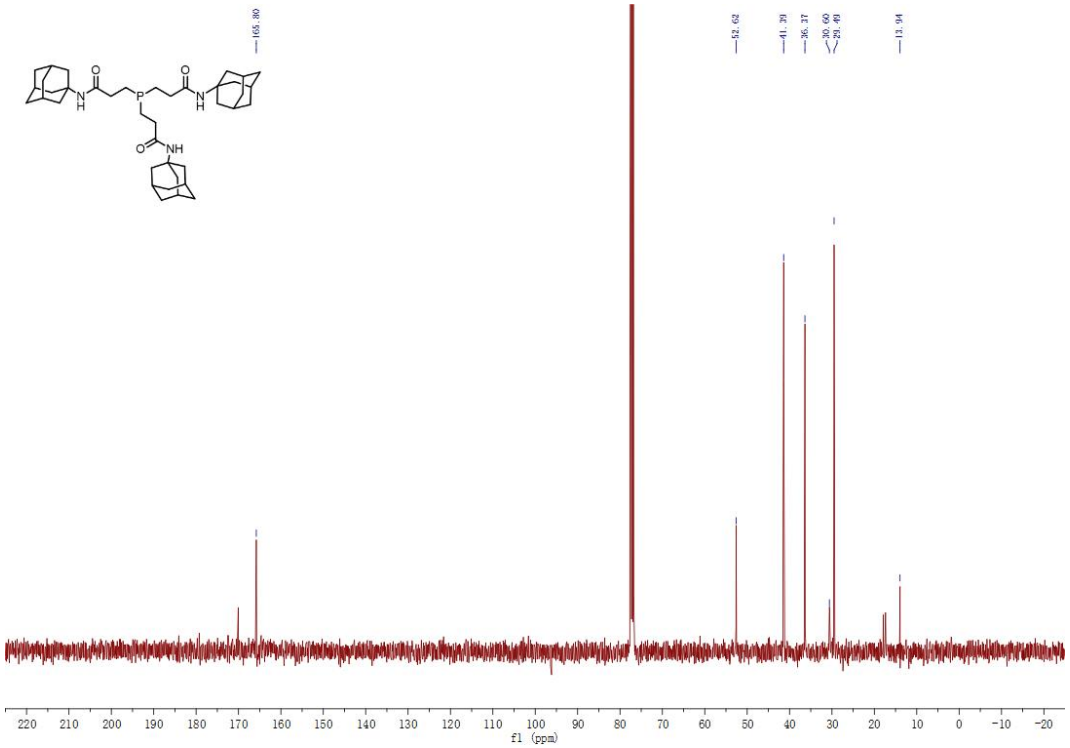


Figure S57. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **2f**; b) ^{13}C NMR (100 MHz, $CDCl_3$) of compound **2f.**

Compound **2g**

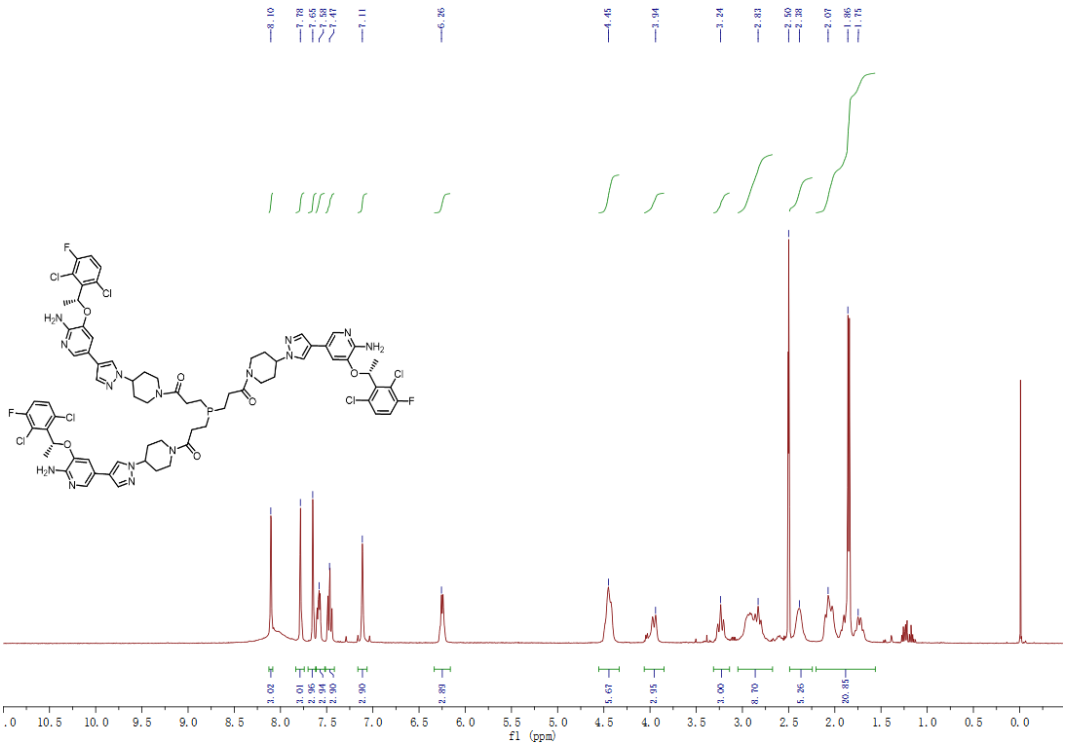
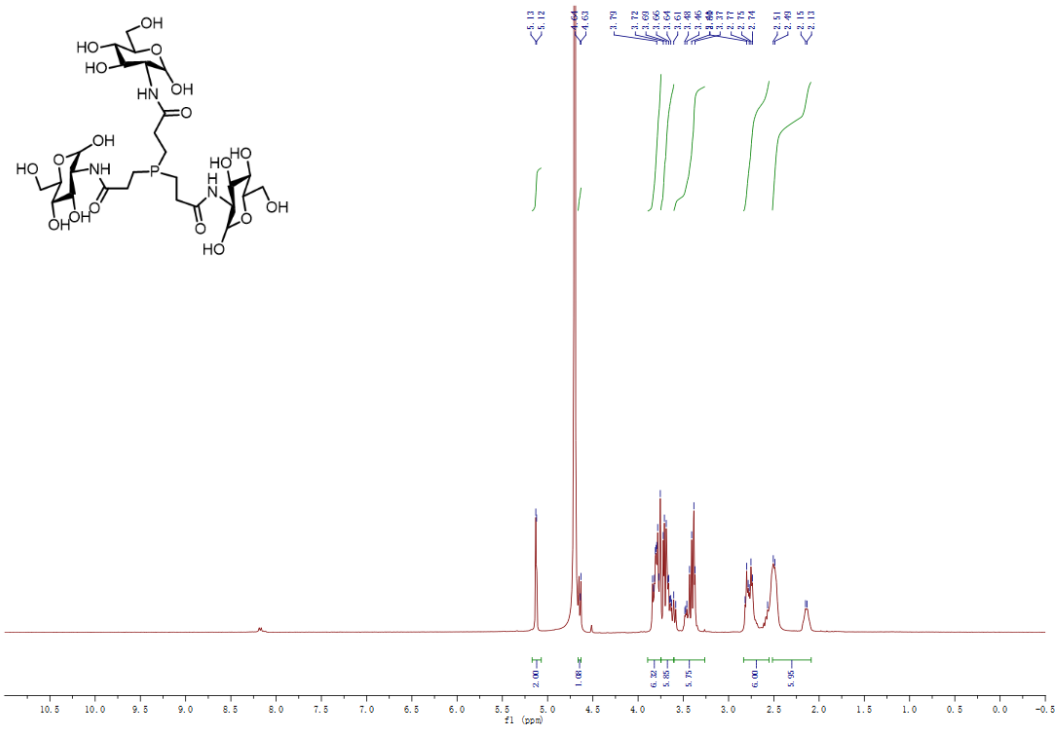


Figure S58. ^{1}H NMR (400 MHz, DMSO-d$_6$) of compound **2g.**

Compound **2h**

a)



b)

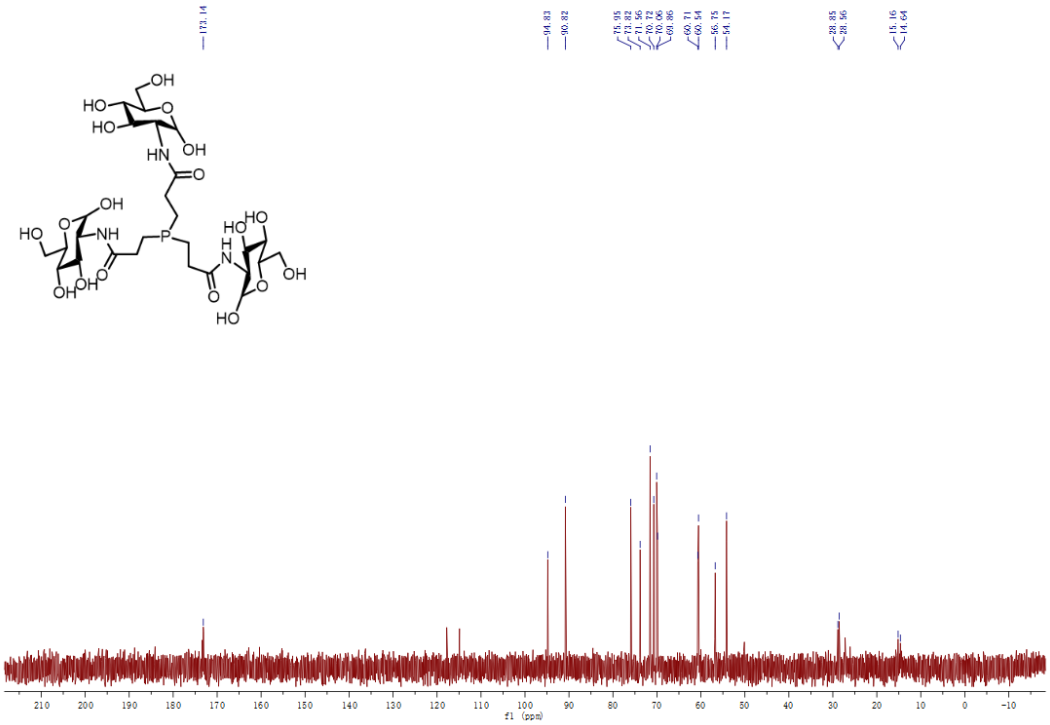
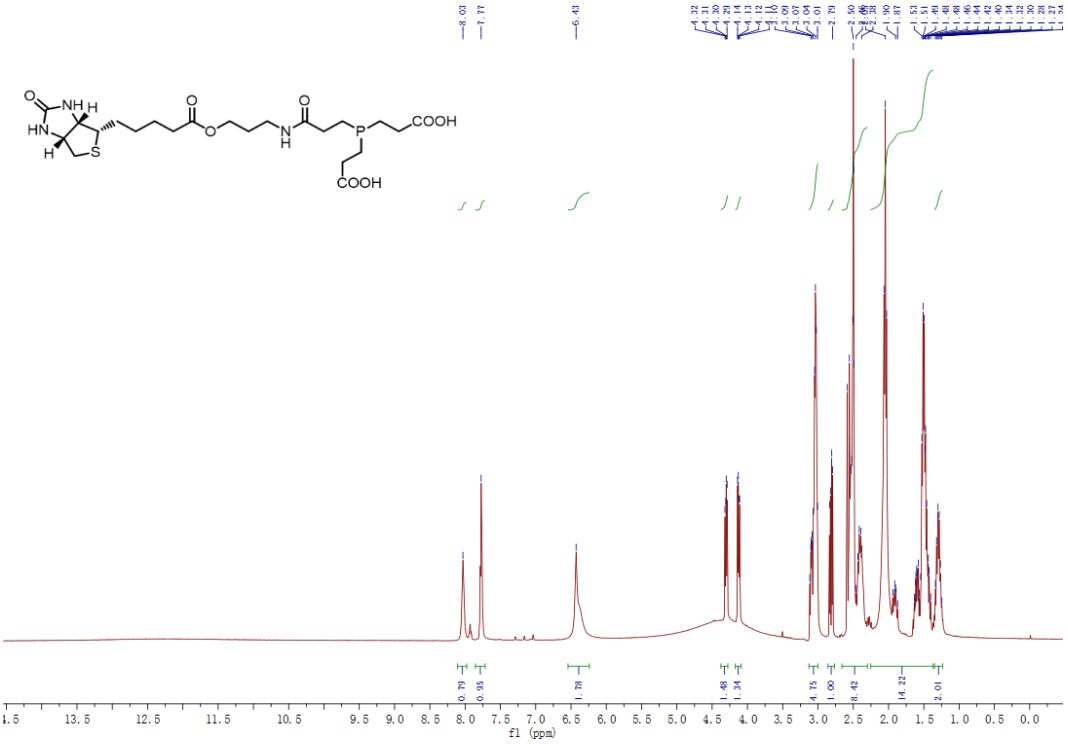


Figure S59. a) ^{1}H NMR (400 MHz, D_2O) of compound **2h**; b) ^{13}C NMR (100 MHz, D_2O) of compound **2h.**

Compound **2i**

a)



b)

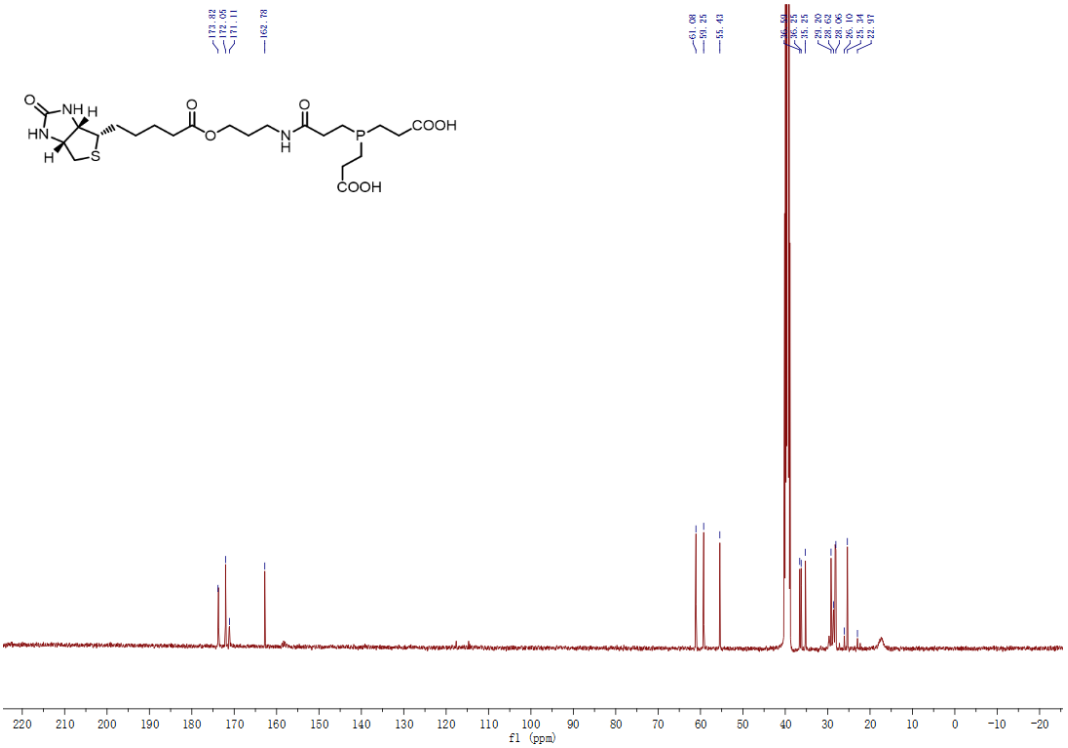


Figure S60. a) ^{1}H NMR (400 MHz, DMSO-d$_6$) of compound **2i**; b) ^{13}C NMR (100 MHz, DMSO-d$_6$) of compound **2i.**

Compound **2j**

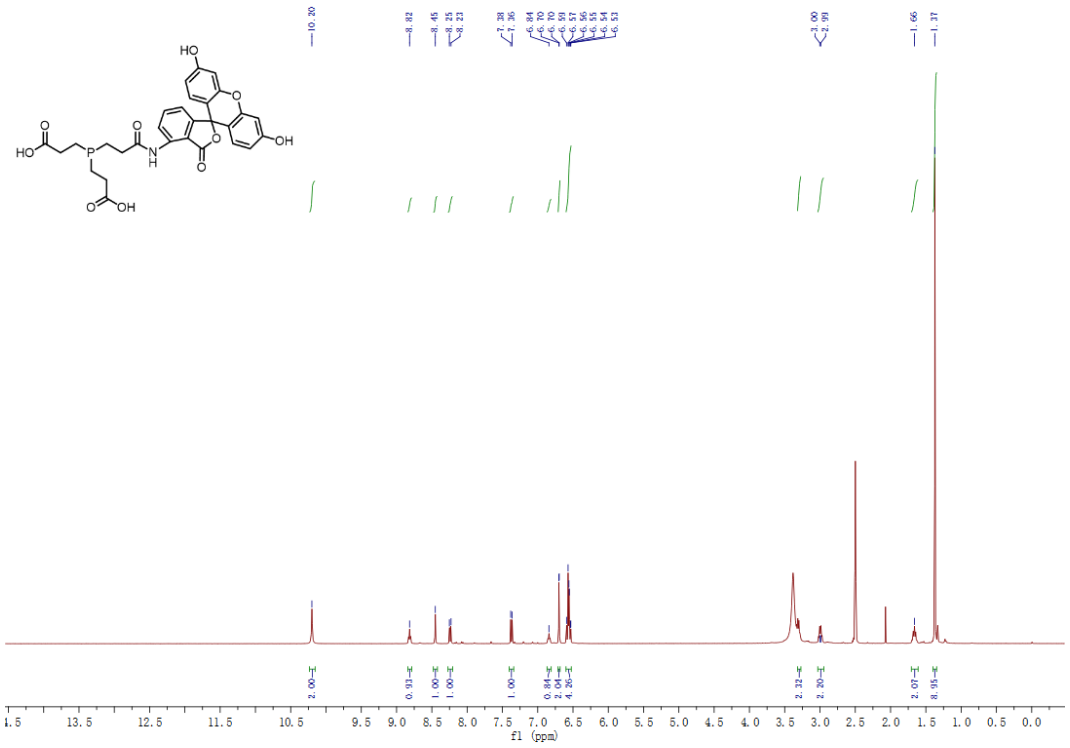
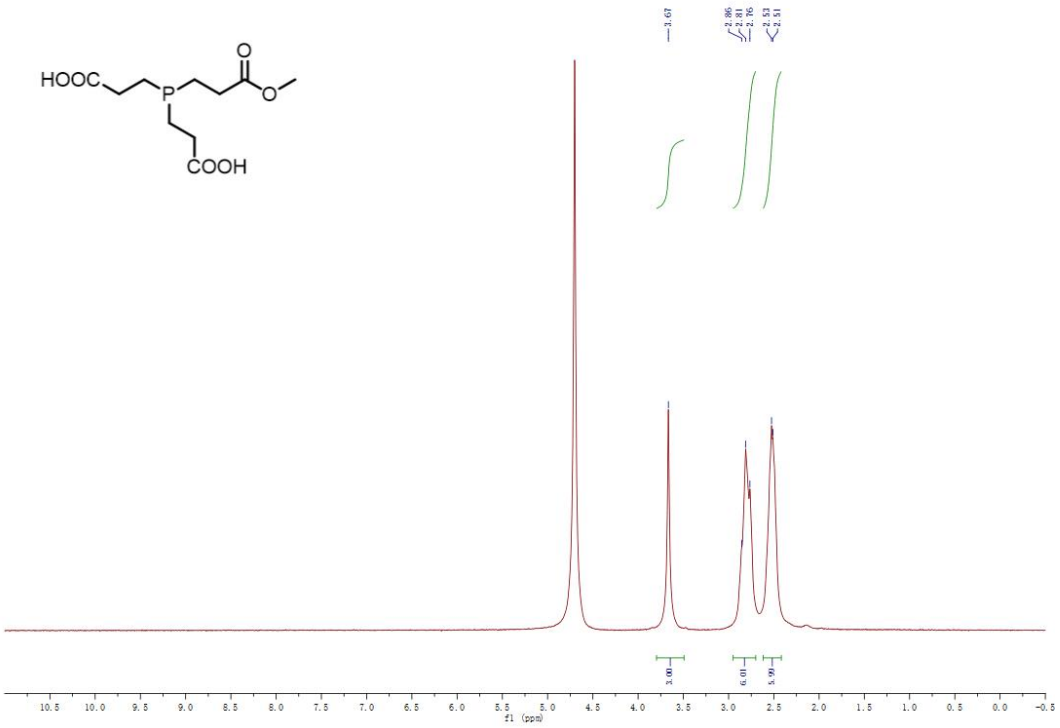


Figure S61. ^{1}H NMR (400 MHz, DMSO-d$_6$) of compound **2j.**

Compound **2l**

a)



b)

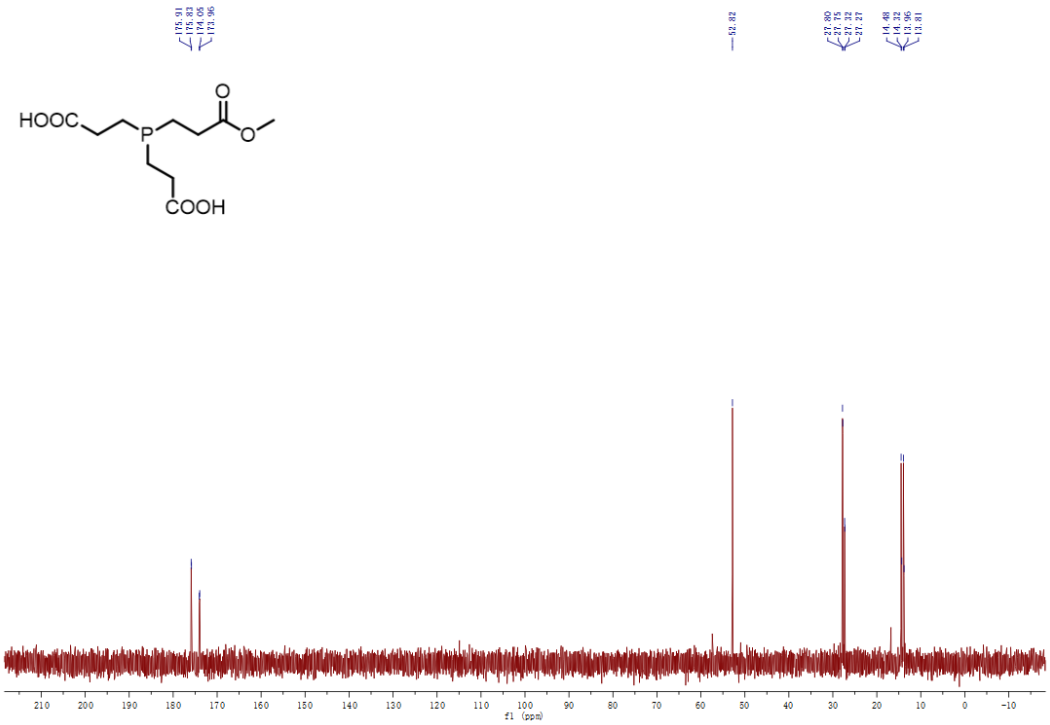
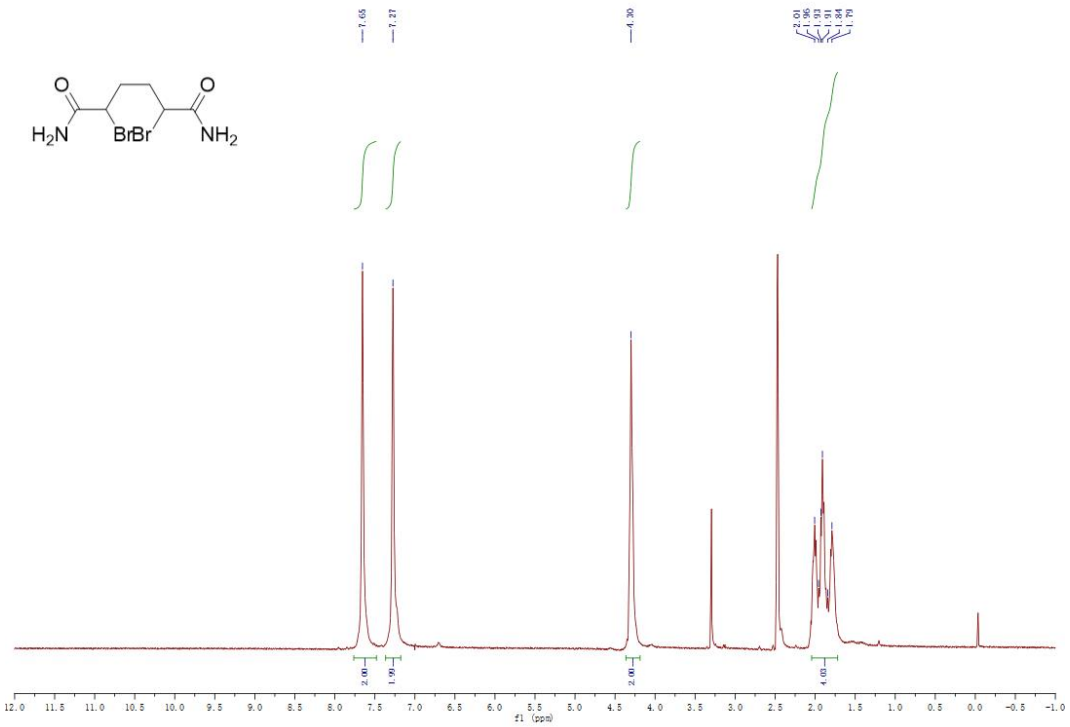


Figure S62. a) ^{1}H NMR (400 MHz, D_2O) of compound **2l**; b) ^{13}C NMR (100 MHz, D_2O) of compound **2l.**

DBHDA

a)



b)

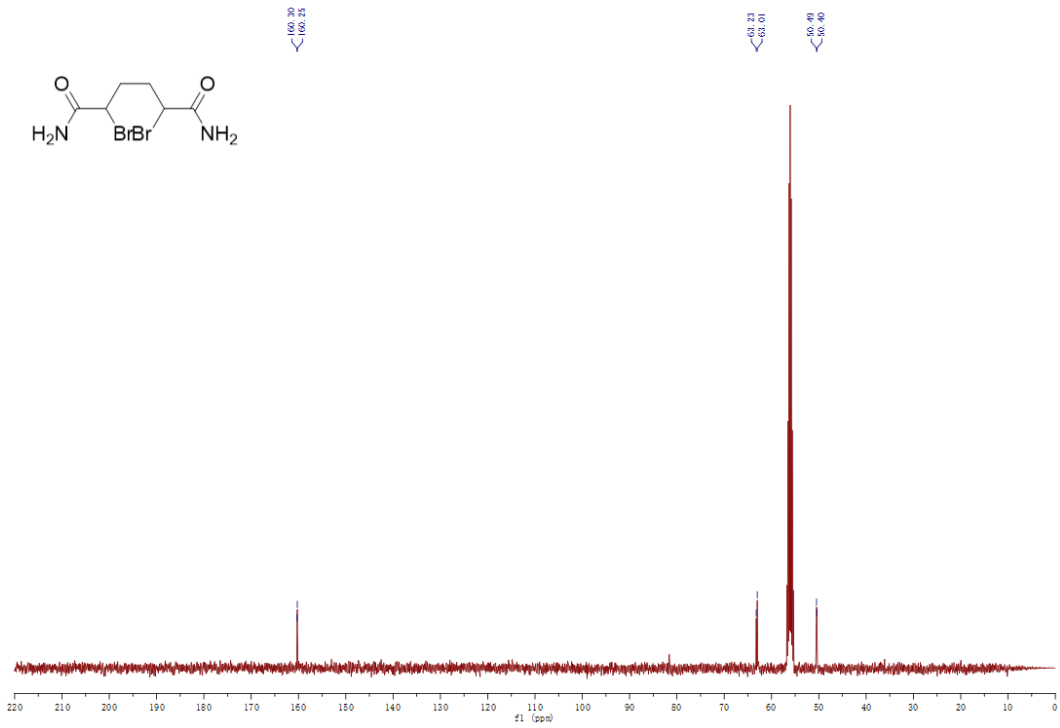
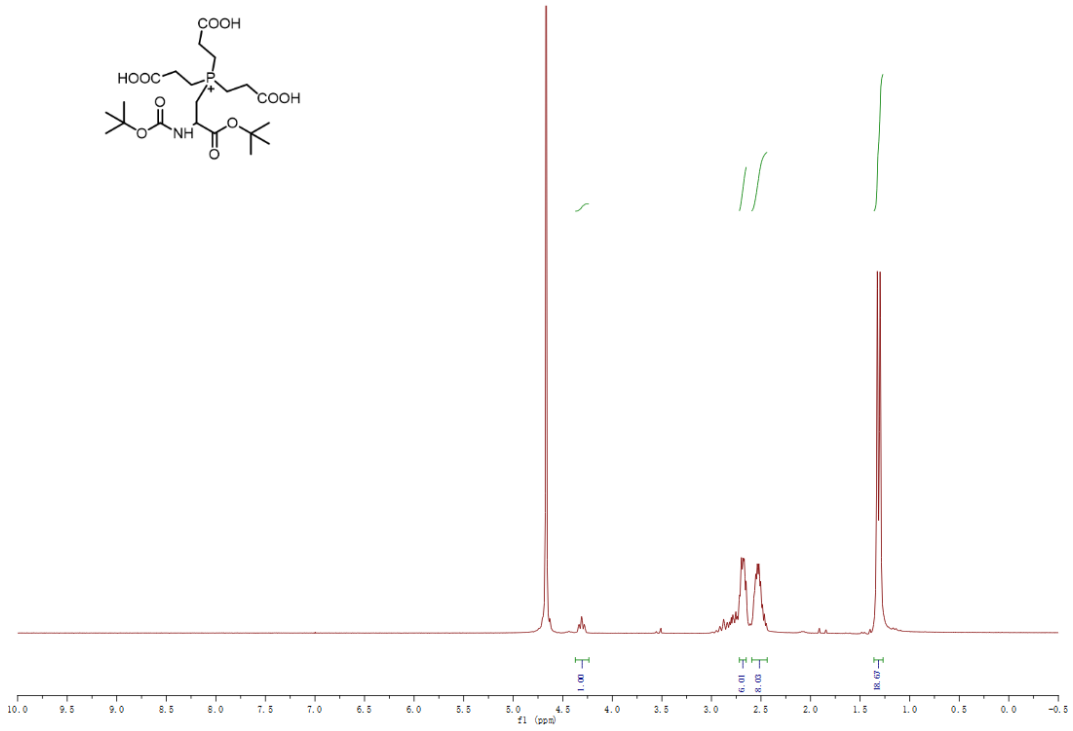


Figure S63. a) ^{1}H NMR (400 MHz, DMSO-d$_6$) of **DBHDA**; b) ^{13}C NMR (100 MHz, DMSO-d$_6$) of **DBHDA.**

Compound **3b**

a)



b)

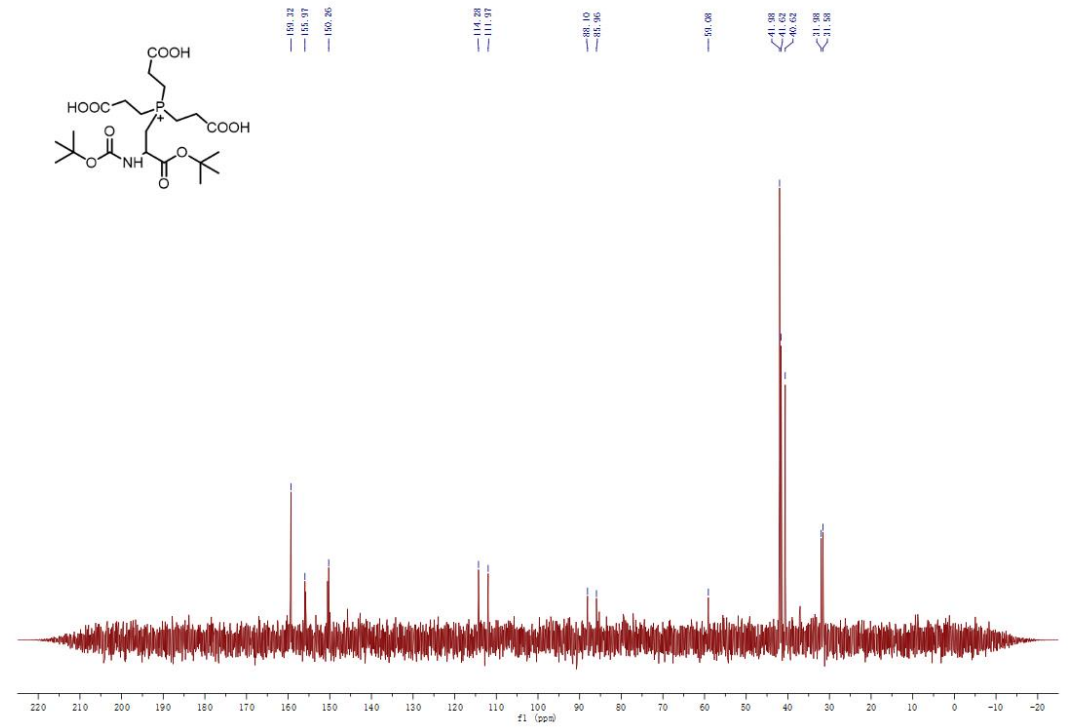


Figure S64. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **3b**; b) ^{13}C NMR (100 MHz, $CDCl_3$) of compound **3b.**

References

[1] C. Huang, G. Ren, H. Zhou and C. C. Wang, *Protein Expr. Purif.*, 2005, **42**, 173-177.

[2] M. R. Ma, Z. W. Hu, Y. F. Zhao, Y. X. Chen and Y. M. Li, *Sci. Rep.*, 2016, **6**, 37130.

[3] Y. Q. Yang, M. C. Ji, Z. Lu, M. Jiang, W. W. Huang, X. Z. He, *Synth. Commun.* 2016, **46**, 977−982.

[4] J. Bos and T. W. Muir, *J. Am. Chem. Soc.*, 2018, **140**, 4757-4760.

[5] Y. J. Lee, Y. Kurra and W. R. Liu, *ChemBiochem*, 2016, **17**, 456-461.

[6] D. J. Cline, S. E. Redding, S. G. Brohawn, J. N. Psathas, J. P. Schneider and C. Thorpe, *Biochemistry*, 2004, **43**, 15195-15203.

[7] J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernandez-Gonzalez, G. J. L. Bernardes, L. Griffin, H. Hailu, C. J. Schofield and B. G. Davis, *Chem. Sci.*, 2011, **2**, 1666-1676.

[8] H. Chen, Y. Zhang, Q. Q. Li, Y. F. Zhao, Y. X. Chen and Y. M. Li, *J. Org. Chem.*, 2018, **83**, 7528-7533.

[9] P. M. Morrison, P. J. Foley, S. L. Warriner and M. E. Webb, *Chem. Commun.*, 2015, **51**, 13470-13473.