Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Delivery of cell membrane impermeable peptides into live cells by using head-to-tail cyclized mitochondria-penetrating peptides

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1. General Information

Materials

Ethyl(hydroxyimino)cyanoacetate (Oxyma), *N*, *N'*-Diisopropyl-carbodiimide(DIC), N, N-Dimethylformamide(DMF), Acetic anhydride, 2,6-Lutidine, Piperidine, trifluoroacetic acid (TFA), Triisopropylsilane (TIPS), m-Cresol, dichloromethane (DCM), Diethyl ether anhydrous, Acetonitrile (HPLC grade), Guanidine Hydrochloride(Gn·HCl), Sodium phosphate dibasic (Na_2HPO_4), sodium hydroxide(NaOH), hydrochloric acid (HCl), sodium($NaNO_2$), sodium 2 - Mercaptoethanesulfonate(MesNa), N, N-Diisopropylethylamine (DIPEA), Hydrazine hydrate, methanol, Tetrahydrofuran(THF), Tetrakis(triphenylphosphine)palladium, N-Methylaniline(MA), acrylic acid(AA), Sodium diethyldithiocarbamate trihydrate,Benzotrizole-1-yl-oxytripyrrolidinophosphonhexa-fluorophosphate(PyBOP), N-methylmorpholine (NMM) were purchased from Energy Chemical (Shanghai, China). All resins were purchased from Hecheng Technology (Tianjing, China).

HPLC, MALDI-TOF/MS

Reversed phase HPLC was performed on Shimadzu Prominence HPLC systems. For peptide analysis, Vydac C18 (4.6 × 250 mm) column was used, with a flow rate of 1.0 mL/min. For peptide purification, Vydac C18 (10 × 250 mm) column was used, with a flow rate of 3.0 mL/min. Solvent A: water (with 1% acetonitrile and 0.1% TFA); Solvent B: acetonitrile (with 1% water and 0.1% TFA). MALDI-TOF/MS was performed on Bruker Autoflex III MALDI-TOF mass spectrometer, α-cyano-4-hydroxycinnamic acid.

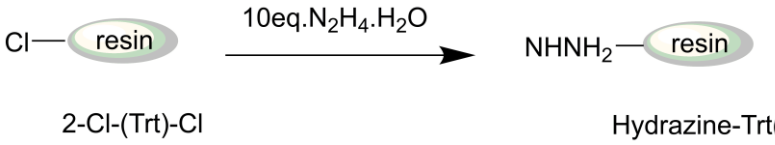
General procedure for Fmoc-SPPS

All peptides were manually synthesized by Fmoc-SPPS. The resin was swollen in DMF for 15 min. Fmoc group was removed by 20% piperidine/DMF solution (7 min plus 8 min). Then, the resin was washed with DMF (5 times). For amino acid coupling, a solution of the Fmoc-amino acid (4.5 equiv.), Oxyma (4.5 equiv.) and DIC (4.5 equiv.) in DMF was added to the resin for 40 min (55℃). Then, the resin was washed with DMF (5 times),capped with (acetic anhydride/2,6-Lutidine/DMF, 5:6:89) for 3 min,

washed with DMF (5 times), and treated with 20% piperidine/DMF. The step was repeated until the desired peptide was assembled on the resin. For Alloc deprotection, a mixture of $Pd(PPh_3)_4$ (1 equiv.) and N-Methylaniline (28 equiv.) in THF was added to the resin, avoiding of light. After 2 hours, the resin was washed with DMF (5 times), 5% sodium diethyldithiocarbamate trihydrate/DMF, DCM (5 times), and DMF (5 times), respectively.For 5(6)-Carboxyfluorescein coupling, a solution of Oxyma (4.5 equiv.), DIC (4.5 equiv.) in DMF was added to the resin. After 16 hours, the resin was washed with DMF (5 times), treated with 20% piperidine/DMF,and washed with DMF (5 times) and DCM(5times). For peptide cleavage, the resin was treated with TFA cocktail (TFA/phenol/water/Triisopropylsilane, 88:5:5:5:2) for 2 hours.The collected TFA solution was precipitated with cold Et_2O. The crude peptide was obtained by centrifugation. After purification by HPLC, the peptide was confirmed by MALDI-TOF/MS. After lyophilization, the desired peptide was obtained as a yellow powder.

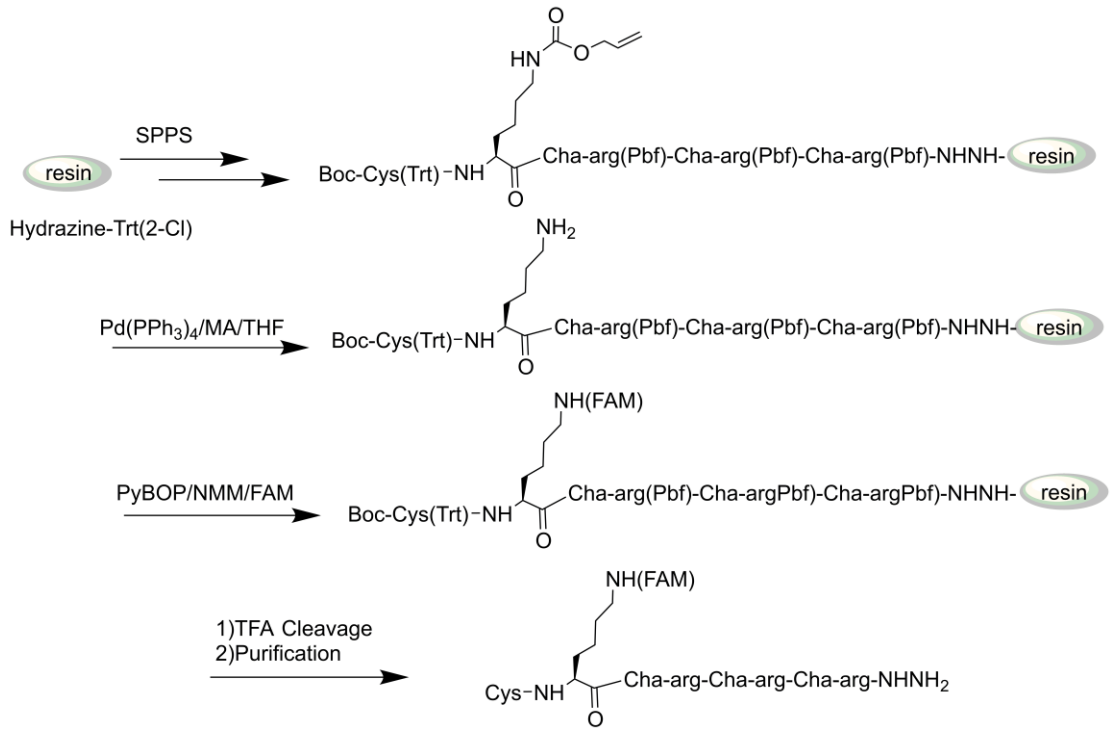
2. Synthesis of head-to-tail cyclized mitochondria-penetrating peptides

2.1 Preparation of hydrazine-Trt(2-Cl) resin

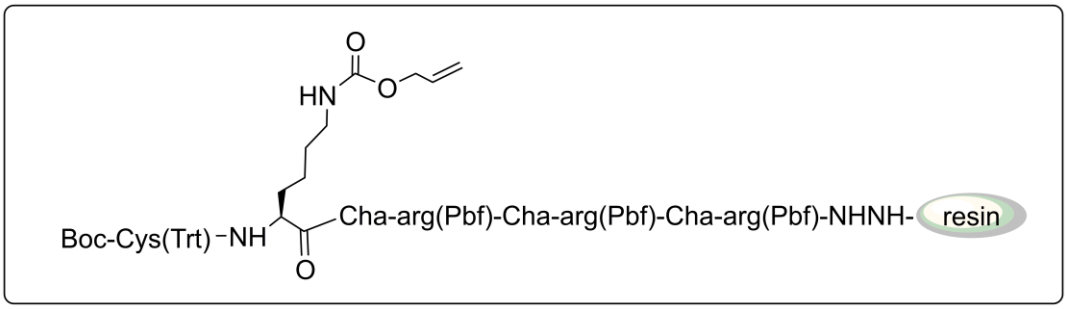


Commercial 2-Cl-(Trt)-Cl resin (200 mg, 80 μmol) was swollen for 15 min in DMF (2 mL). Under 0 °C, a mixture of N, N-Diisopropylethylamine (DIPEA) (265 μl, 1.6 mmol) and hydrazine hydrate (80 μl, 1.6 mmol) in DMF (800 μl) was added dropwise. The suspension was stirred for 90 min at rt. Then methanol (40 μl) was added to quench the reaction for 20 min. Then the resin was filtered, washed with DMF, water, DMF, methanol and ether, respectively. After that, the resin was dried for 1h at rt in vacuo.

2.2 Synthesis of FAM-modified peptide hydrazides

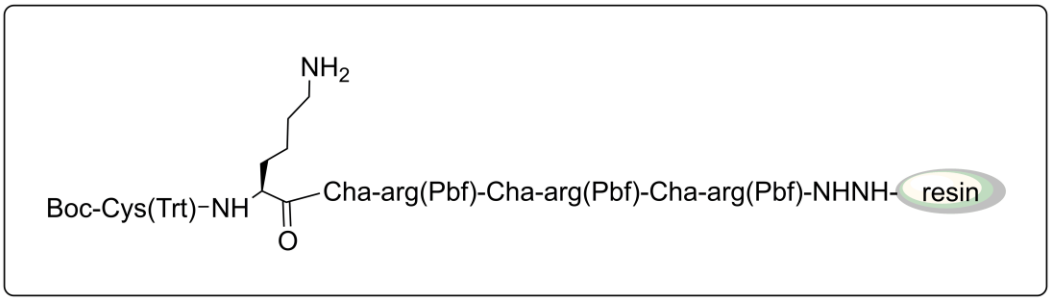


Scheme S1. The synthetic route for chemical synthesis of FAM-modified peptide hydrazide



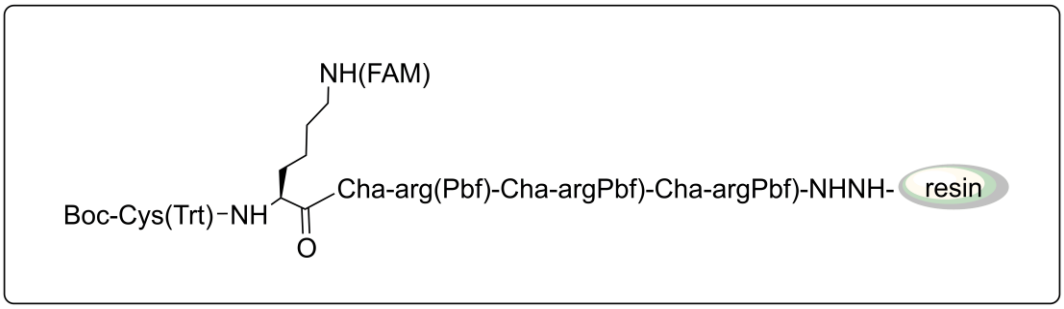
Intermediate 1

Hydrazine-Trt(2-Cl) resin (50 mg, 0.40 mmol/g) was used. The resin was swollen in DMF for 15 min.Then,the first amino acid was anchored to the hydrazine resin by using a mixture solution of Fmoc-D-Arg(Pbf)-OH (58.4 mg, 4.5 equiv), Oxyma (12.8 mg, 4.5 equiv) and DIC(13.9 μl,4.5 equiv) in 400 μl DMF (55°C). After 40 min, the resin was washed with DMF (5 times), capped with a mixture of acetic anhydride/2,6-Lutidine/DMF (5:6:89) for 3 min, and washed with DMF (5 times). After treating with 20% piperidine/DMF (7 min plus 8 min), the resin was washed again with DMF (5 times). This step was repeated until the completion of the coupling of all amino acids.



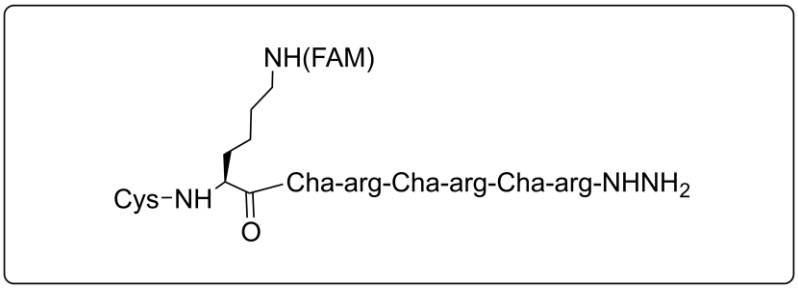
Intermediate 2

Deprotection of Alloc protecting group was performed by using a solution of Pd $(PPh_3)_4$ (24 mg, 1.0 equiv) and N-Methylaniline (60 μL, 28 equiv) in DMF (1.2 mL, rt, 2 hours). Then, the resin was washed with DMF (5 times), 5% sodium diethyldithiocarbamate trihydrate/DMF, DCM (5 times), and DMF (5 times), affording resin-bound intermediate 2.



Intermediate 3

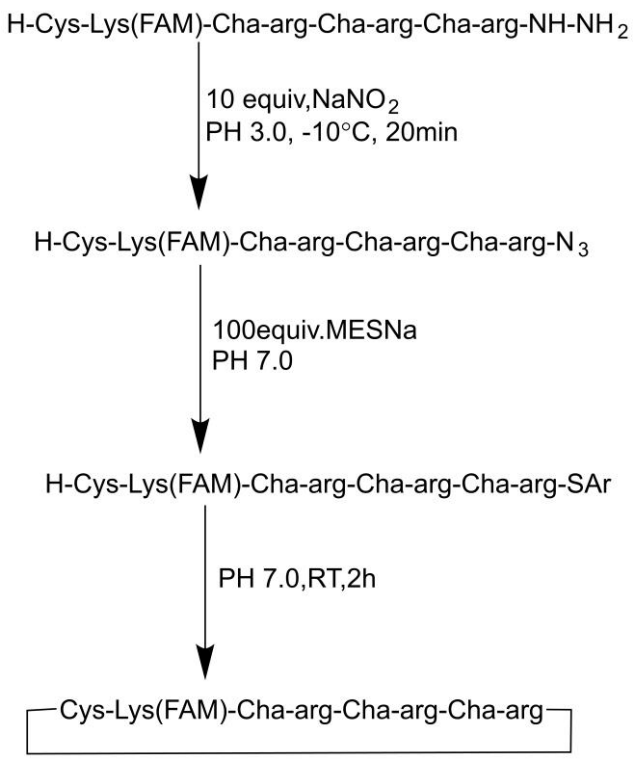
For 5(6)-Carboxyfluorescein coupling, a solution of the 5(6)-carboxyfluorescein (37.5 mg, 5.0 equiv), PyBOP (52 mg, 5.0 equiv), NMM (44 μl, 10 equiv) in DMF was added to the resin for 16 hours. Then, the resin was washed with DMF (5 times), treated with 20% piperidine/DMF (10 min plus 10 min) to remove resin-bound FAM. Finally,the resin was washed with DMF (5 times), DCM (5times), affording resin-bound intermediate 3.



Intermediate 4

(FAM-modified MPP-1 peptide hydrazide) The peptide was cleaved from resin by using cocktail reagent (TFA/phenol/water/Triisopropylsilane, 88:5:5:5:2) for 2 hours. The collected TFA solutions were precipitated with cold Et_2O. The crude peptide was obtained by centrifugation. Analysis and purification of the crude peptide was carried out by HPLC (a linear gradient from 5 % to 90 % Solvent B in 42 min, C18). After lyophilization, 2.8 mg of FAM-modified MPP-1 peptide hydrazide was obtained as a yellow powder in an isolated yield of 16.0 %.

2.3 cyclization of peptide hydrazide



FAM-modified MPP-1 peptide hydrazide (1.3 mg, 0.8 μmol) was dissolved in 1 mL cyclization buffer[a] containing 0.2 mL DMF. Under -10 °C, 80 μL of the oxidative solution[b] (100 mM) was added dropwise. The reaction mixture was kept at -10°C and stirred for 20 min. After adding 400 μL of MESNa solution[c] (200 mM), the reaction mixture was slowly adjusted to pH 7.0 with NaOH (2.0 M). The reaction was stirred for 4 h at room temperature,and monitored by HPLC. The formed **cyclic MPP-1** was purified by using HPLC. After lyophilization, the desired peptide was obtained as a yellow powder.

The cyclization buffer[a]: 6.0 M Gn·HCl, 0.2 M Na_2HPO_4, pH 3.0.

The oxidative solution[b]: 7 mg $NaNO_2$ (100 mM) in neat water.

MESNa solution[c]: 16.4 mg MESNa was dissolved in 0.5 mL cyclization buffer (pH adjusted to 7.0).

cyclic MPP-1 :cyclo [C-K(FAM)-*Cha*-r-*Cha*-r-*Cha*-r]

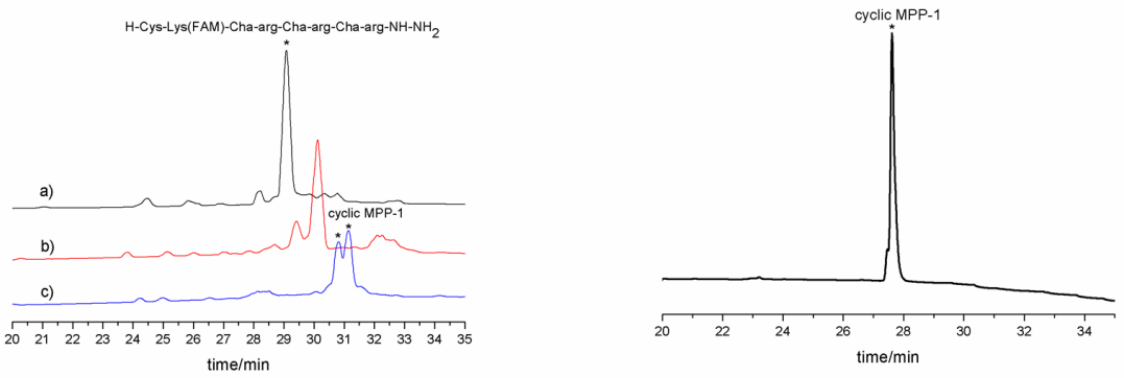


Figure S1. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-1**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) at 30 min (after addition of MESNa), (gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min), d) purified **cyclic MPP-1**(gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min), FAM=5(6)-carboxyfluorescein.

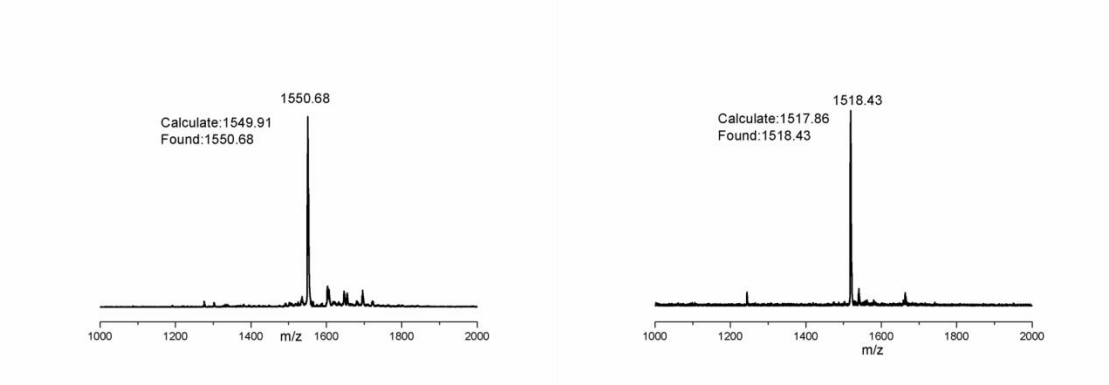


Figure S2. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-1**(left) and the desired **cyclic MPP-1** (right).

2.4 Synthesis of cyclic MPP-1', cyclic MPP-2, cyclic MPP-3, cyclic MPP-4, cyclic MPP-5, cyclic MPP-6, cyclic MPP-7, cyclic MPP-8, cyclic MPP-9, and cyclic MPP-10

Of note: **cyclic MPP-1(R), cyclic MPP-2, cyclic MPP-3, cyclic MPP-4, cyclic MPP-5, cyclic MPP-6, cyclic MPP-7, cyclic MPP-8, cyclic MPP-9, and cyclic MPP-10** were prepared according to the same protocol as **cyclic MPP-1** using 50 mg hydrazine-Trt(2-Cl) resin. Analysis and purification were carried out by HPLC (a linear gradient from 2% to 90% Solvent B in 42 min, C18).

(1) cyclic MPP-1':cyclo [C-K(FAM)-*Cha*-R-*Cha*-R-*Cha*-R]

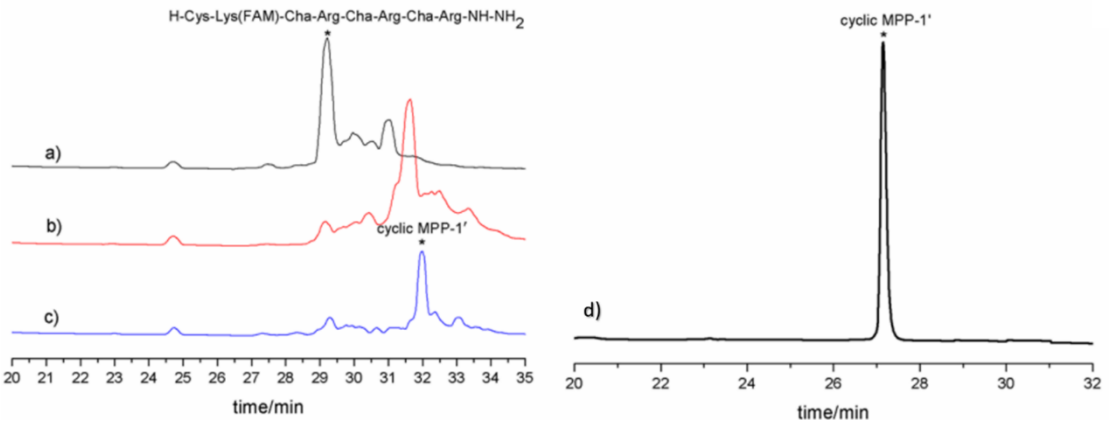


Figure S3. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-1'**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa, gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min), d) purified **cyclic MPP-1'** (gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min), FAM=5(6)-carboxyfluorescein.

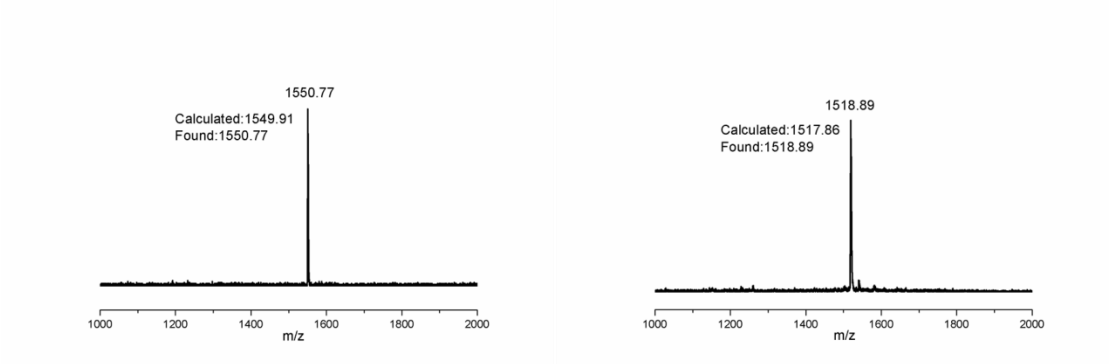


Figure S4.MALDI-TOF/MS spectrum ofthe linear peptide hydrazide of **cyclic MPP-1'**(left) and the desired **cyclic MPP-1'** (right).

(2)cyclic MPP-2:cyclo [C-K(FAM)-r-*Cha*-r-*Cha*-r]

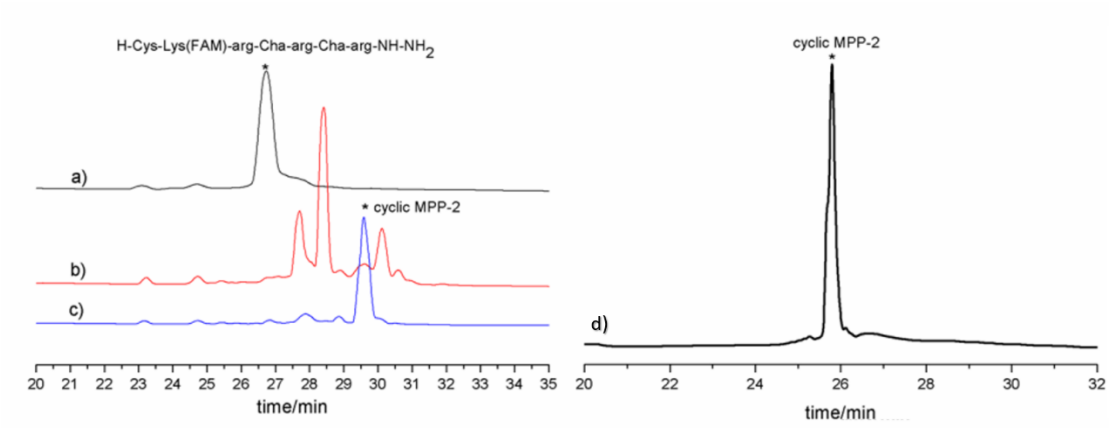


Figure S5. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-2**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa, gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min), d) purified **cyclic MPP-2**(gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min). FAM=5(6)-carboxyfluorescein.

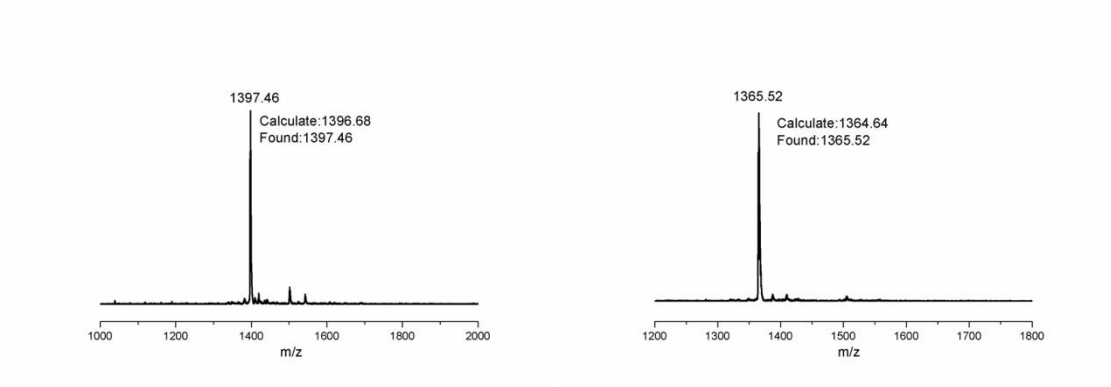


Figure S6.MALDI-TOF/MS spectrum ofthe linear peptide hydrazide of **cyclic MPP-2** (left) and **cyclic MPP-2**(right).

(3) cyclic MPP-3:cyclo [C-K(FAM)-r-*Cha*-r-*Cha*-r-*Cha*-r]

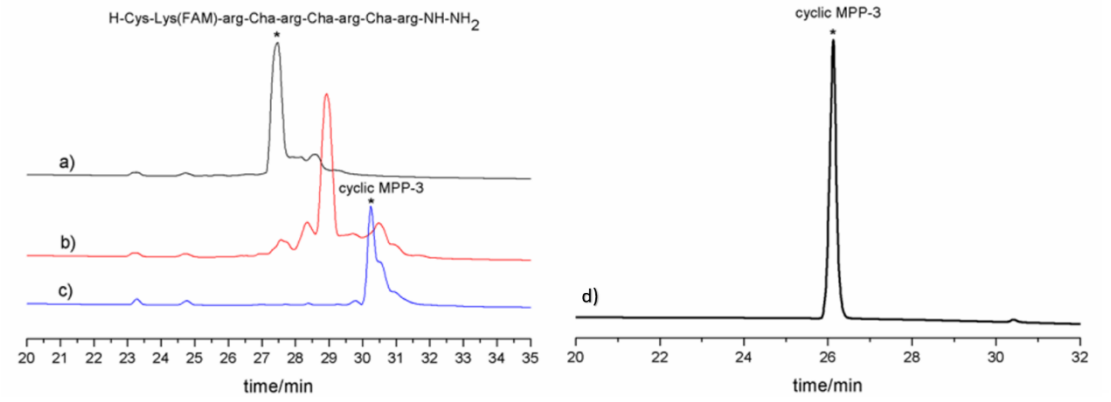


Figure S7. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-3**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa, gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min), d) purified **cyclic MPP-3**(gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min).FAM=5(6)-carboxyfluorescein.

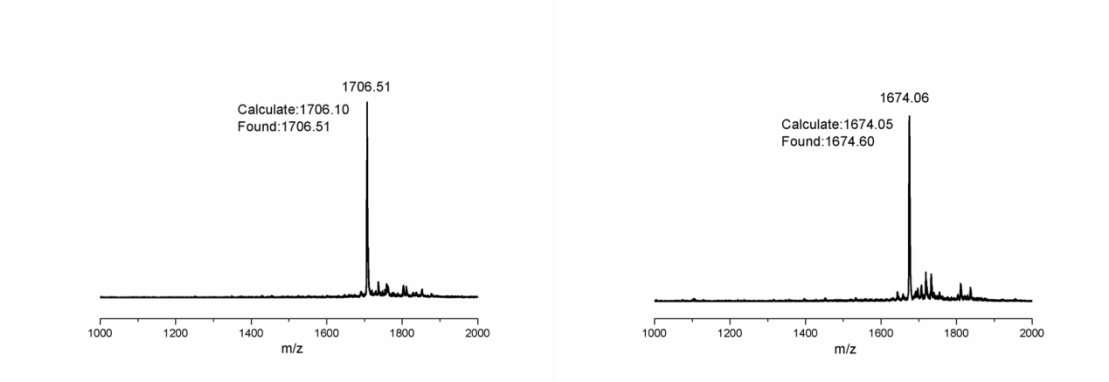


Figure S8.MALDI-TOF/MS spectrum ofthe linear peptide hydrazide of **cyclic MPP-3** (left) and **cyclic MPP-3**(right).

(4) cyclic MPP-4:cyclo [C-K(FAM)-R-*Cha*-r-*Cha*-r]

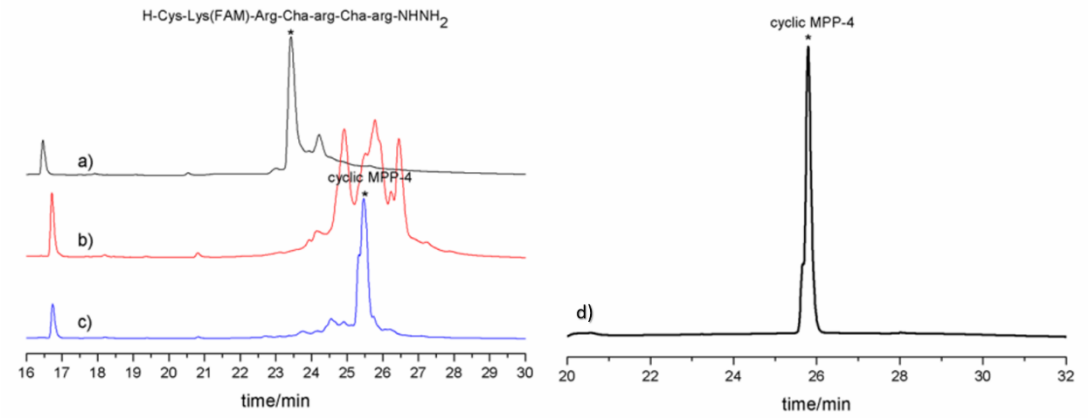


Figure S9. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-4**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa), d) purified **cyclic MPP-4**. Gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min.FAM=5(6)-carboxyfluorescein.

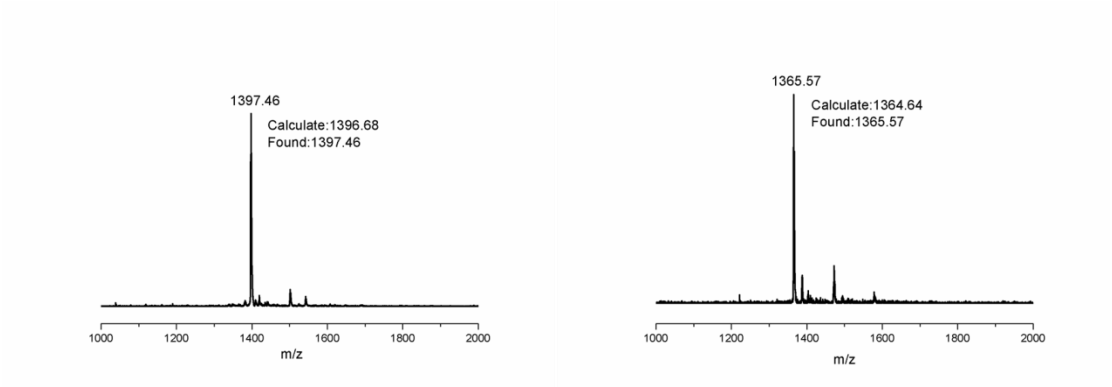


Figure S10.MALDI-TOF/MS spectrum of the linearpeptide hydrazide of **cyclic MPP-4** (left) and the desired **cyclic MPP-4**(right).

(5) cyclic MPP-5:cyclo [C-K(FAM)-r-*Cha*-R-*Cha*-r]

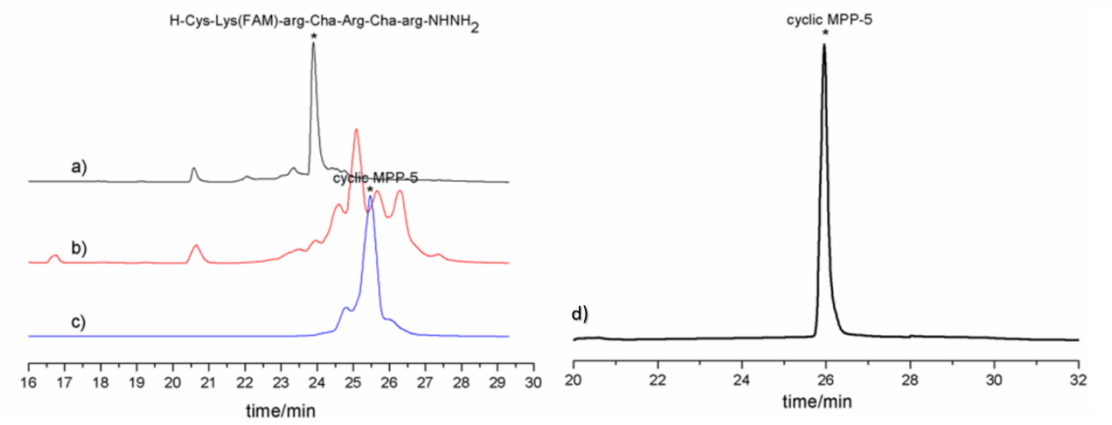


Figure S11. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-5**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa), d) purified **cyclic MPP-5.** Gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min.FAM=5(6)-carboxyfluorescein.

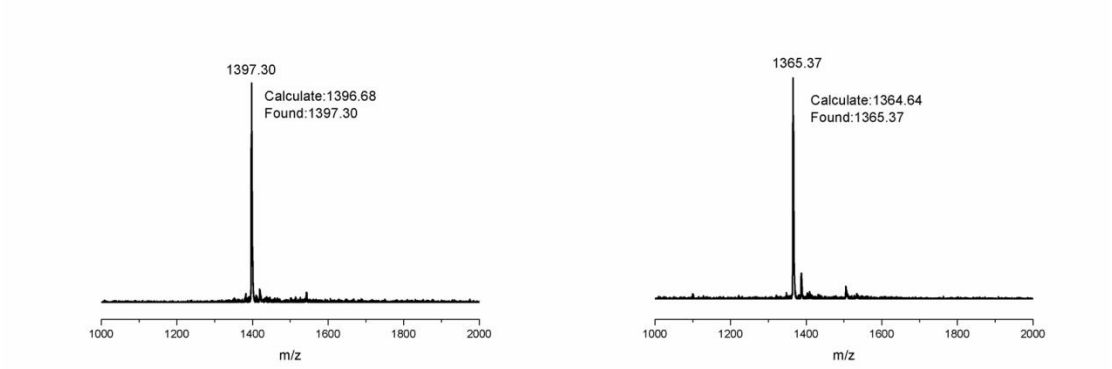


Figure S12. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-5**(left) and the desired**cyclic MPP-5**(right).

(6) cyclic MPP-6:cyclo [C-K(FAM)-r-*Cha*-r-*Cha*-R]

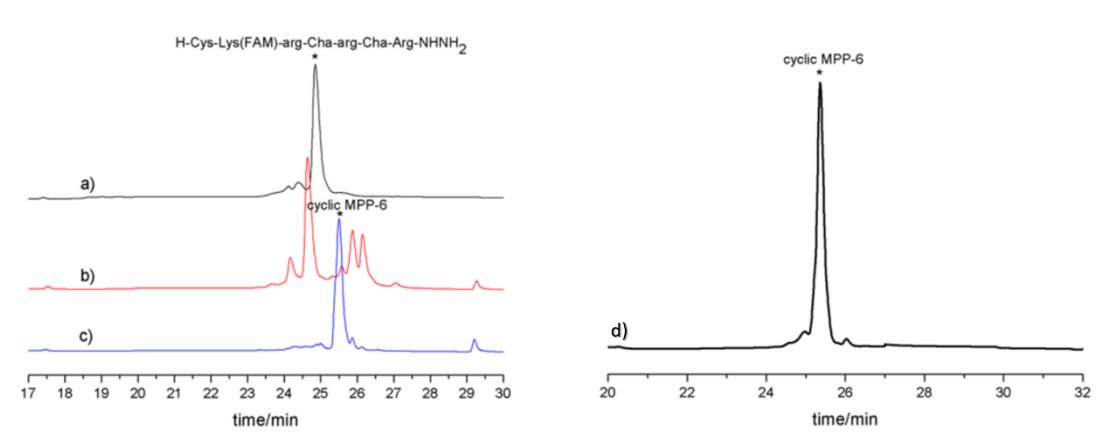


Figure S13. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-6**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 120 min (after addition of MESNa), d) purified **cyclic MPP-6.**Gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min.FAM=5(6)-carboxyfluorescein.

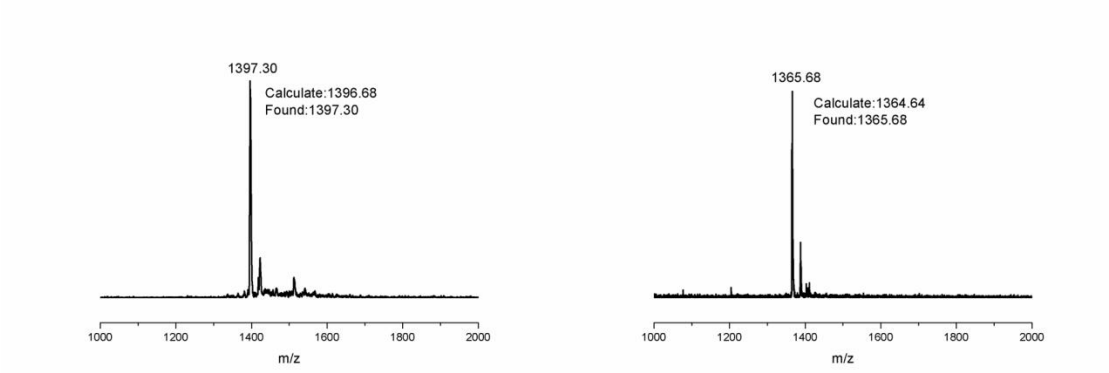


Figure S14. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-6** (left) and the desired **cyclic MPP-6** (right).

(7) cyclic MPP-7:cyclo [C-K(FAM)-R-*Cha*-R-*Cha*-r]

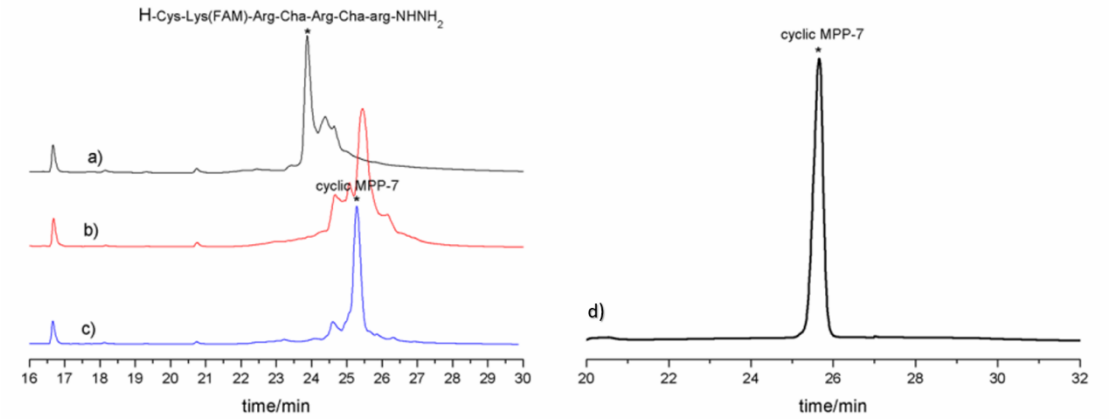


Figure S15. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-7**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) at 30 min (after addition of MESNa), d) purified

cyclic MPP-7.Gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min. FAM=5(6)-carboxyfluorescein.

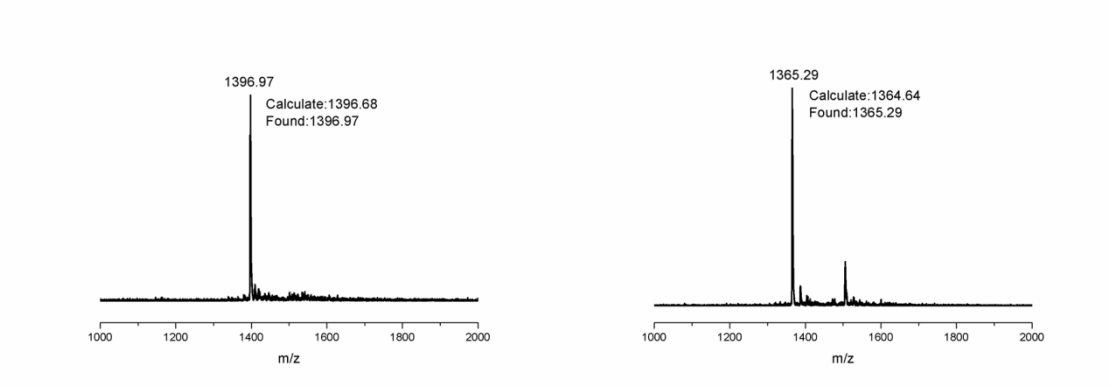


Figure S16. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-7** (left) and the desired **cyclic MPP-7**(right).

(8) cyclic MPP-8:cyclo [C-K(FAM)-R-*Cha*-r-*Cha*-R]

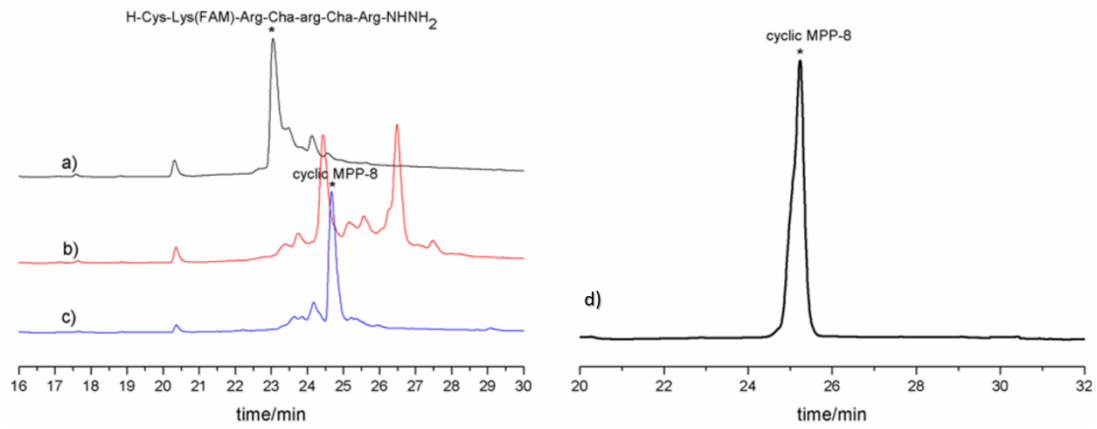


Figure S17. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-8**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa), d) purified **cyclic MPP-8**.Gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min. FAM=5(6)-carboxyfluorescein.

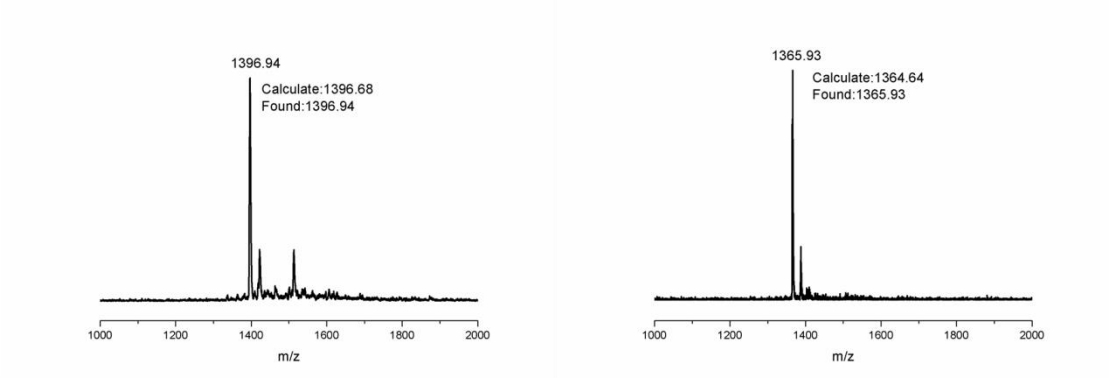


Figure S18. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-8**(left) and the desired **cyclic MPP-8** (right).

(9) cyclic MPP-9:cyclo[C-K(FAM)-r-*Cha*-R-*Cha*-r]

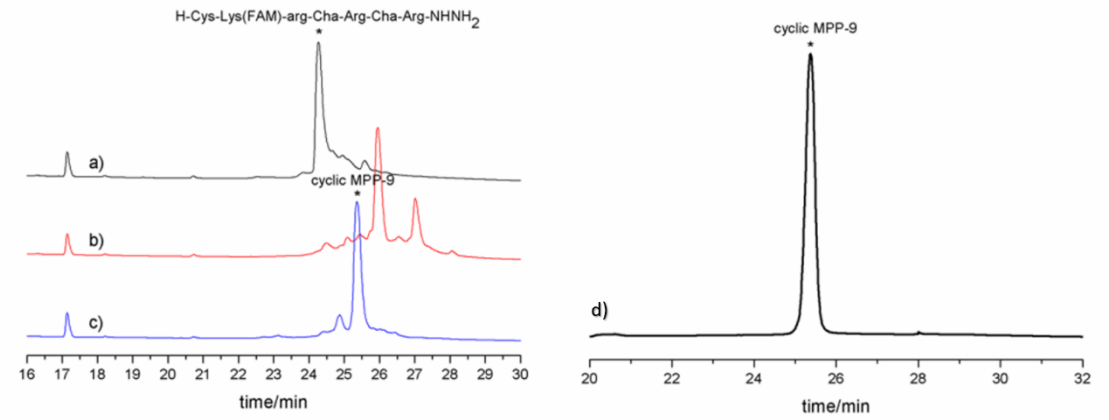


Figure S19. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-9**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa), d) purified **cyclic MPP-9**.Gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min. FAM=5(6)-carboxyfluorescein.

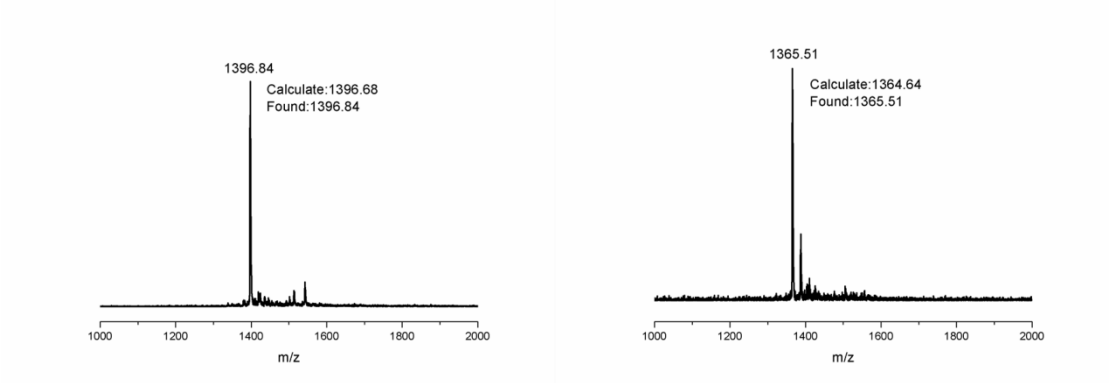


Figure S20. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-9**(left) and the desired **cyclic MPP-9**(right).

(10) cyclic MPP-10:cyclo [C-K(FAM)-R-Cha-R-Cha-R]

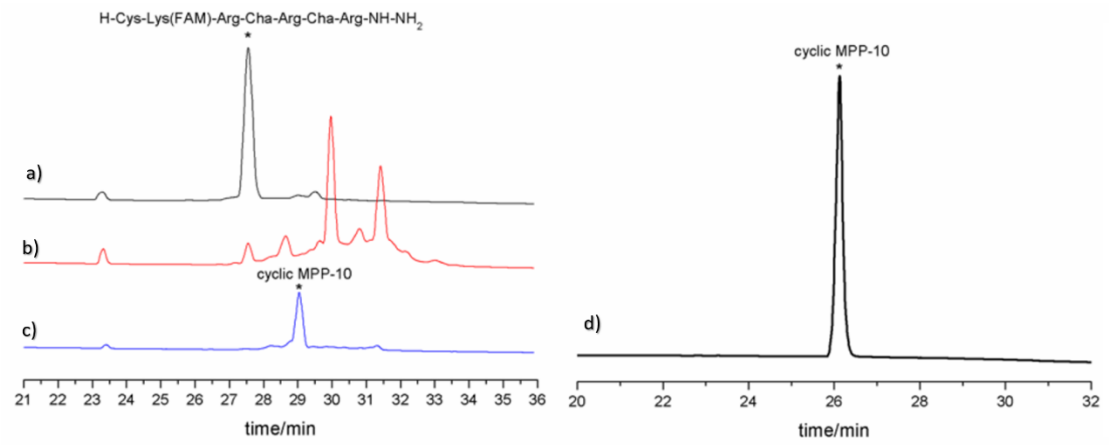


Figure S21. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-10**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa,gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min), d) purified **cyclic MPP-10** (gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min). FAM=5(6)-carboxyfluorescein.

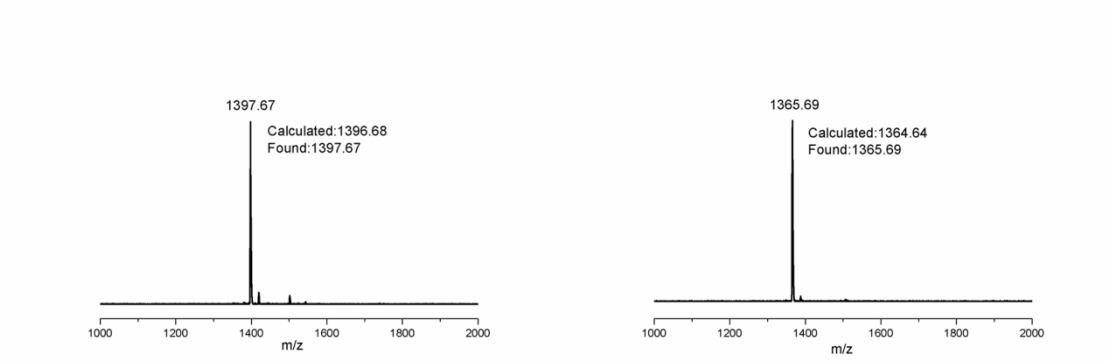


Figure S22. MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-10** (left) and the desired **cyclic MPP-10**(right).

3. Synthesis of MPP-1 and MPP-2

MPP-1 and **MPP-2** were synthesized by 15 mg(5 μmol) of rink amide resin by general Fmoc-based SPPS. The amino acids wereassembled on the rink amide resin according to the DIC/Oxyma-based method.Then, Alloc protecting group of the Lysine side chain was removed by palladium reagent. The released amino group wasconjugated to5(6)-carboxyfluorescein by DIC. Finally, the desired peptide was cleavaged from the resin by TFA solution. Analysis and purification were carried out by using HPLC and MS. After lyophilization, the desired was obtained as a yellow powder.

(1)MPP-1: Cys-Lys(FAM)-Cha-r-Cha-r-Cha-r

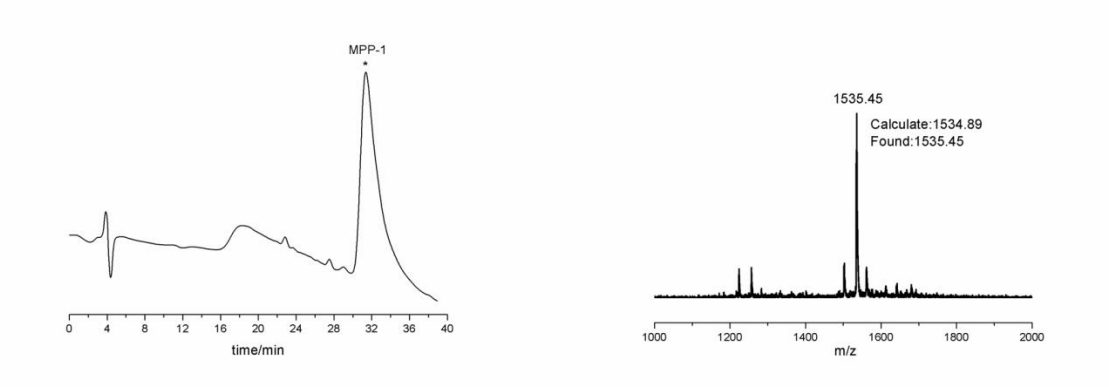


Figure S23. HPLC and MALDI-TOF/MS of **MPP-1**. Analytical RP-HPLC chromatogram (left) and MALDI-TOF/MS spectrum (right), gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min.FAM=5(6)-carboxyfluorescein.

(2) MPP-2: Cys-Lys(FAM)-r-Cha-r-Cha-r

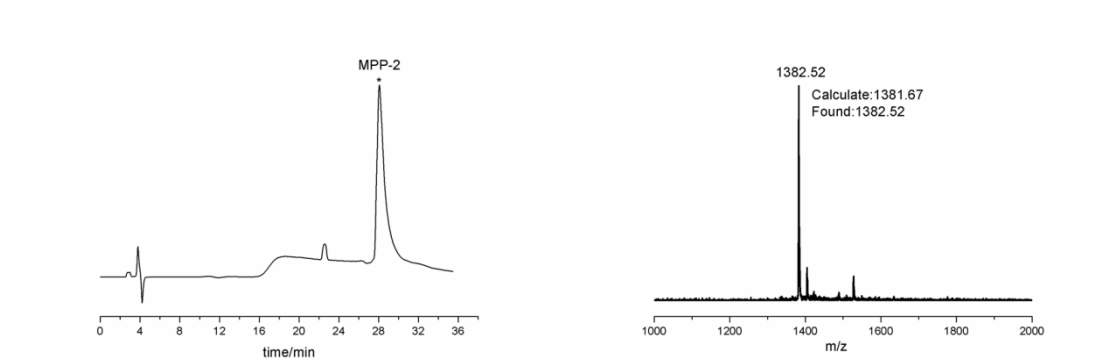


Figure S24. HPLC and MALDI-TOF/MS of the **MPP-2**. Analytical RP-HPLC chromatogram (left) and MALDI-TOF/MS spectrum (right), gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min.FAM=5(6)-carboxyfluorescein.

4.Synthesis ofFAM-$^{\beta}$AGWIYA

FAM-$^{\beta}$AGWIYA and FAM-FYFYFY were prepared according to general Fmoc-based SPPS using 15 mg(5 μmol) rink amide resin. The amino acids were assembled on the rink amide resin according to the DIC/Oxyma-based method. The 5(6)-carboxyfluorescein was coupled to the N-terminal amino group.Finally, the desired peptide were cleavaged from the resin by TFA solution. Analysis and purification were carried out using HPLC and MS. After lyophilization, the desired peptides were obtained as a yellow powder.FAM=5(6)-carboxyfluorescein.

(1)FAM-$^{\beta}$AGWIYA (Note: N-terminal amino acid is beta-alanine**.)**

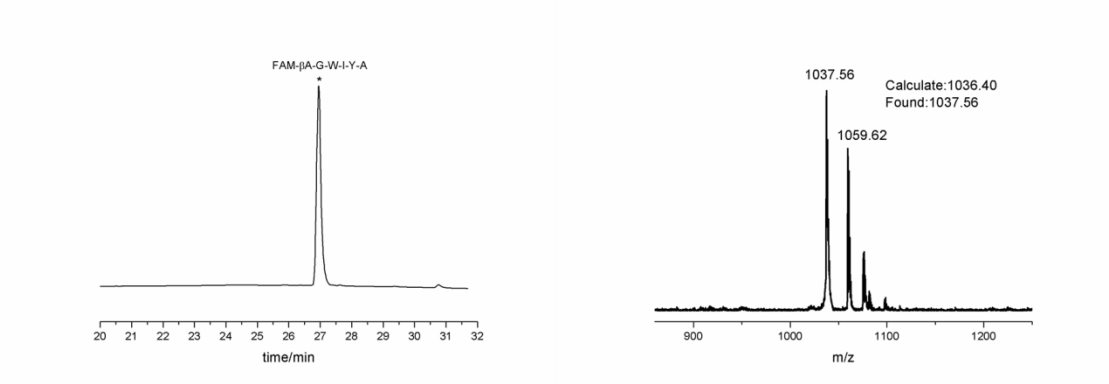


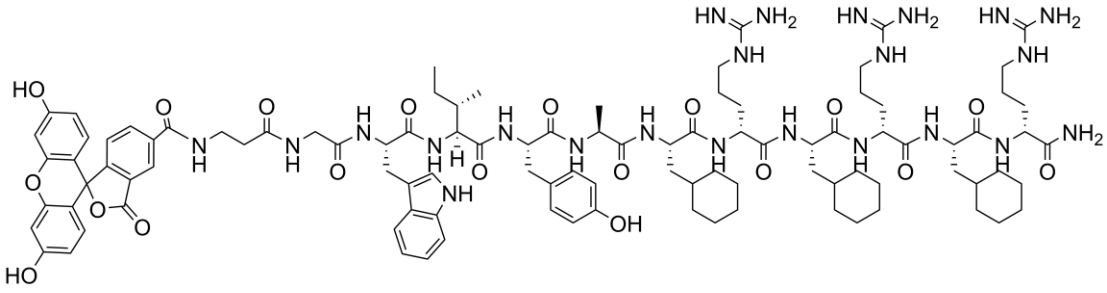
Figure S25. HPLC and MALDI-TOF/MS of the **FAM-$^{\beta}$AGWIYA**. Analytical RP-HPLC chromatogram (left) and MALDI-TOF/MS spectrum (right), gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min.

5. Synthesis of MPP-modified peptides

FAM-$^{\beta}$AGWIYA-MPP were prepared according to general procedure for Fmoc-SPPS using 15 mg(5 umol) rink amide resin.The amino acids were assembled on the rink amide resin according to the DIC/Oxyma-based method.The 5(6)-carboxyfluorescein was coupled to the N-terminal amino group. Finally, the desired peptides were cleavaged from the resin by TFA solution. Analysis and purification were carried out using HPLC and MS. After lyophilization, the desired peptides were obtained as a yellow powder. FAM=5(6)-carboxyfluorescein.

(1)FAM-$^{\beta}$AGWIYA-MPP (Note: N-terminal amino acid is beta-alanine**.)**

Chemical Structure:



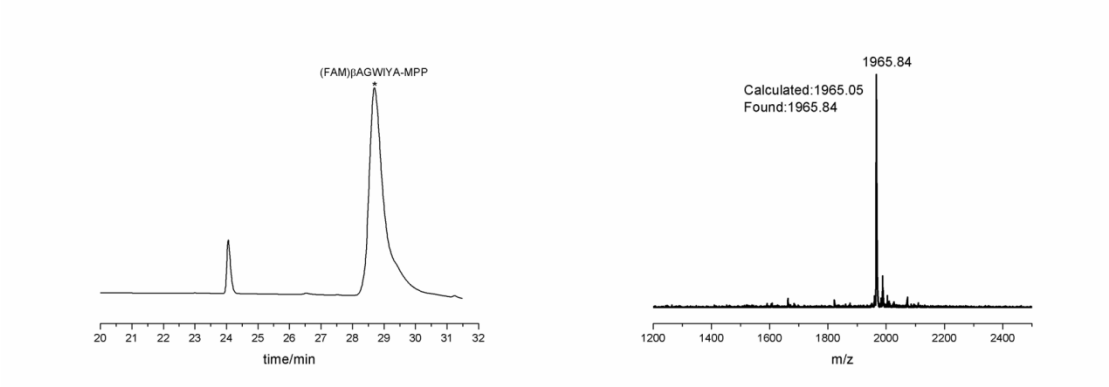


Figure S26. HPLC and MALDI-TOF/MS of the **FAM-$^{\beta}$AGWIYA-MPP**. Analytical RP-HPLC chromatogram (left) and MALDI-TOF/MS spectrum (right), gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min.

(2) CyclicMPP-11: Cyclo[Cys-Lys(FAM-$^{\beta}$AGWIYA)-r-*Cha*-r-*Cha*-r]**(**Note: N-terminal amino acid is beta-alanine**.)**

Chemical Structure:

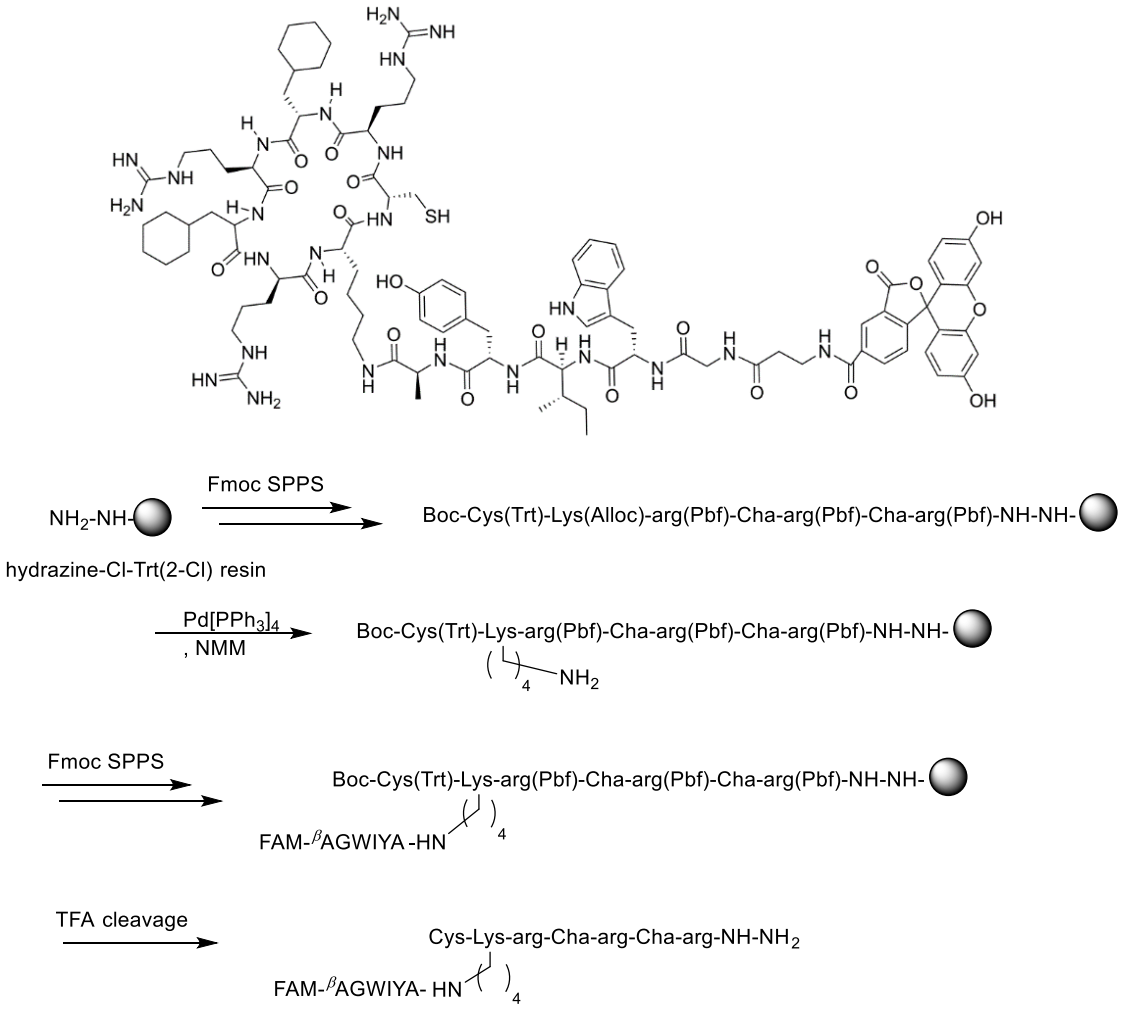


Figure S27. Chemical synthesis of the linear peptide hydrazide of **cyclic MPP-11**.FAM=5(6)-carboxyfluorescein.

The linear peptide hydrazide of**cyclic MPP-11**was synthesized by 50 mg hydrazine-Trt(2-Cl) resin (20 μmol)by general Fmoc-based SPPS, as shown in Figure S28. Analysis and purification were carried out using HPLC and MS. After lyophilization, the linear peptide hydrazide of **cyclic MPP-11** was obtained as a yellow powder. The cyclization of peptide hydrazide was performed by native chemical liagtion.

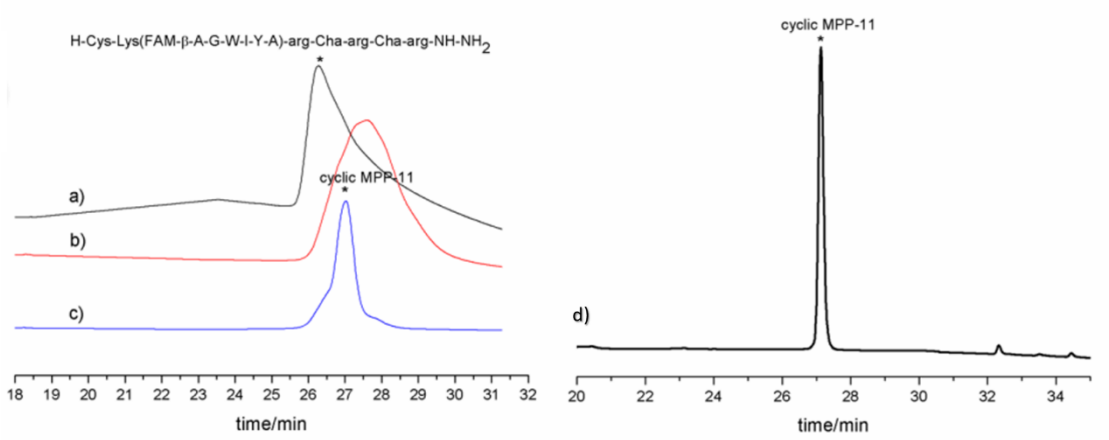


Figure S28. Time course of cyclization of the linear peptide hydrazide of **cyclic MPP-11**. (a) 0 min, (b) 20 min (after addition of $NaNO_2$), (c) 30 min (after addition of MESNa),d) purified **cyclic MPP-11.** Gradient: 5-5% Solvent B in 5 min, then 5-32% Solvent B in 10 min, then 32-80% Solvent B in 25 min, then 80-90% Solvent B in 10 min.

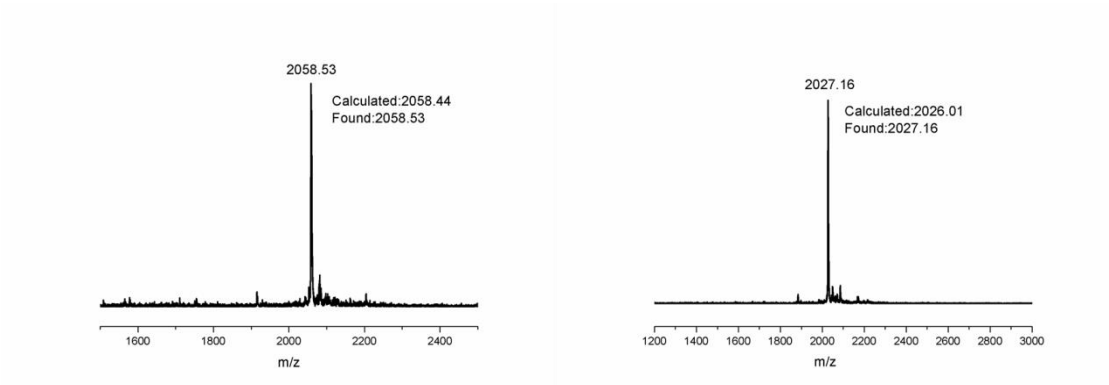
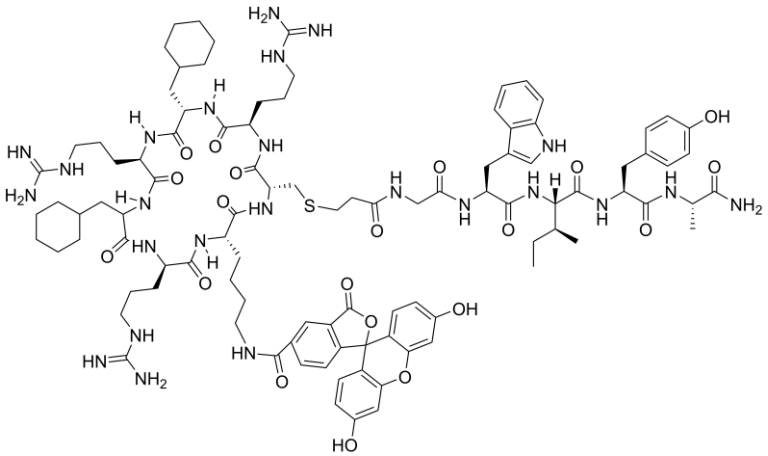


Figure S29.MALDI-TOF/MS spectrum of the linear peptide hydrazide of **cyclic MPP-11** (left) and the desired **cyclic MPP-11**(right).

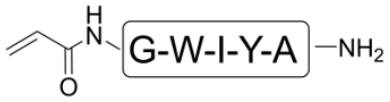
(3) Cyclic MPP-12: Cyclo [Cys(GWIYA)-Lys(FAM)-r-*Cha*-r-*Cha*-r]

Chemical Structure:



N-terminal acrylic acid-modified peptide with sequence of GWIYA was synthesized by general Fmoc-based SPPS using 60 mg (20 μmol) rink amide resin. Specifically, the amino acids were assembled on the rink amide resin according to the DIC/Oxyma-based method. Then, acrylic acid was coupled to Gly by DIC/Oxyma-based method. Finally, the desired peptide was cleavaged from the resin by TFA solution. Analysis and purification were carried out by using HPLC and MS. After lyophilization, the desired was obtained as a white powder.

Acrylic acid-modified peptide:



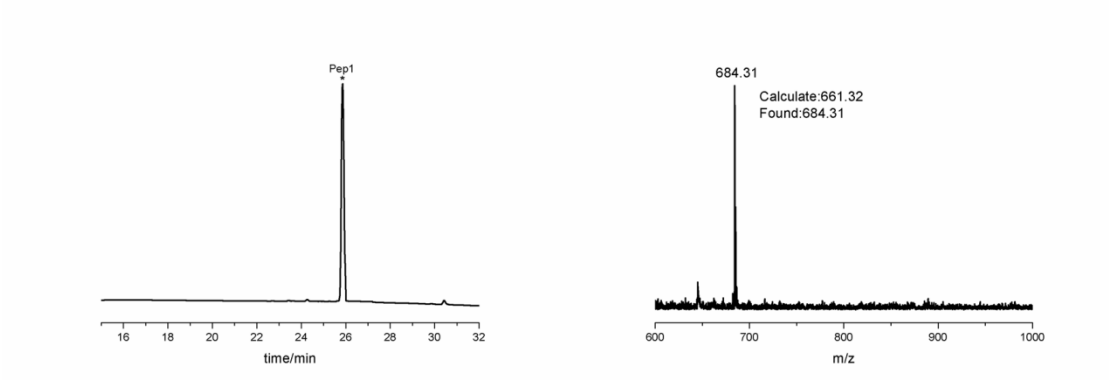
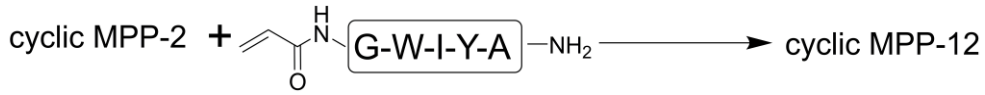


Figure S30. HPLC and MALDI-TOF/MS of the **Pep1**. Analytical RP-HPLC chromatogram (left) and MALDI-TOF/MS spectrum (right), gradient: 2-2% Solvent B in 2 min, then 2-90% Solvent B in 28 min.



Acrylic acid-modified peptide (1.7 mg) was dissolved in 1.6 ml the buffer[a], **cyclic MPP-2** (2.4 mg) was also dissolved in 1 ml the buffer[a]. Then, **cyclic MPP-2 was** added to Pep1 buffer. The reaction was stirred for 20 h at room temperature, and monitored by HPLC. The formed **cyclic MPP-12** was purified by using HPLC. After lyophilization, the desired peptide was obtained as a yellow powder.

The buffer[a]: DMF:H_2O=2:1 adjusted to pH 8.0 with NH_4CO_3 (1.0 M)

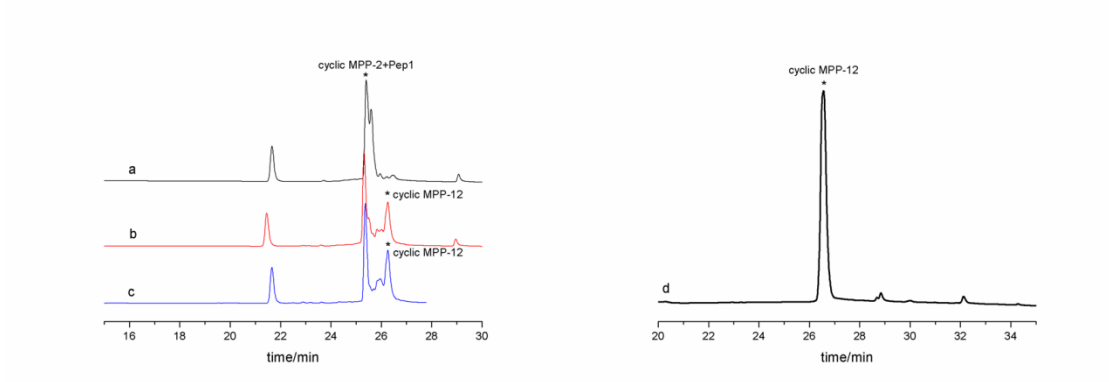


Figure S31. Time course of Click chemistry of Pep1 and **cyclic MPP-12**. (a) 0 min, (b) 20 h, (c) 40 h, (d) purified **cyclic MPP-12.**

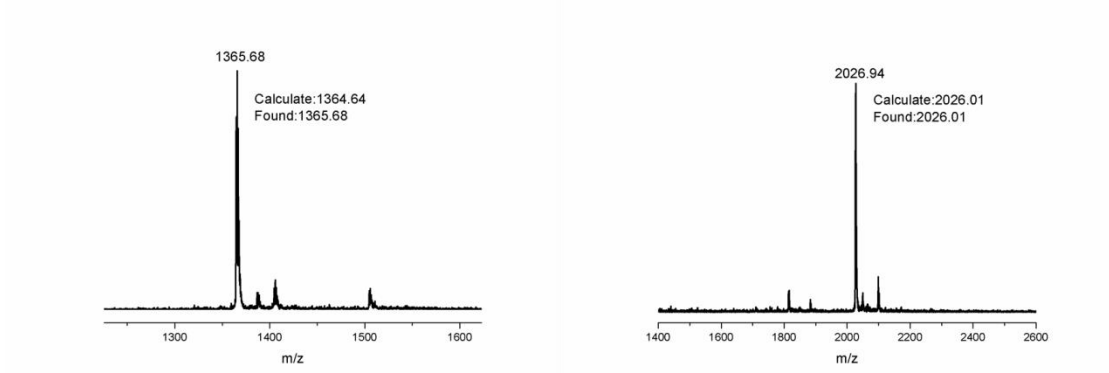


Figure S32. MALDI-TOF/MS spectrum of the desired **cyclic MPP-2** (left) and **cyclic MPP-12** (right)

6. Confocal images of HeLa cells treated with cyclic MPPs

The corresponding peptide was dissolved in DMSO to a final concentration of 1mM. HeLa cells were incubated in DMEM buffer, containing DMEM/FBS/AK (9:1:0.1). After 2-3 days, HeLa cells were transferred to 1 mLdishes forincubation of another day. 10 μL of the stored peptide solution was directly added toHeLa cells to provide a final

concentration of 10μM.(For 20 μMpeptide, 20 μL of the stored peptide solution was added to HeLa cells.)Then, HeLa cells were incubated for 1 hour in a CO_2 incubator (37 ℃, 5%CO_2). 0.5μl of commercial MitoTracker red was added to HeLa cells. After 15 minutes, HeLa cells were washed three times with PBS buffer solution. Then, 1 mL DMEM buffer was added to HeLa cells.The treated HeLa cells were imaged under confocal fluorescence microscope. Green channel (excitation wavelength of 488 nm, the emission band of 510-560 nm) for the signal of fluorescein. Red channel (excitation wavelength of 559 nm, emission wavelength of 580-620 nm) for the signal of MitoTracker Red.

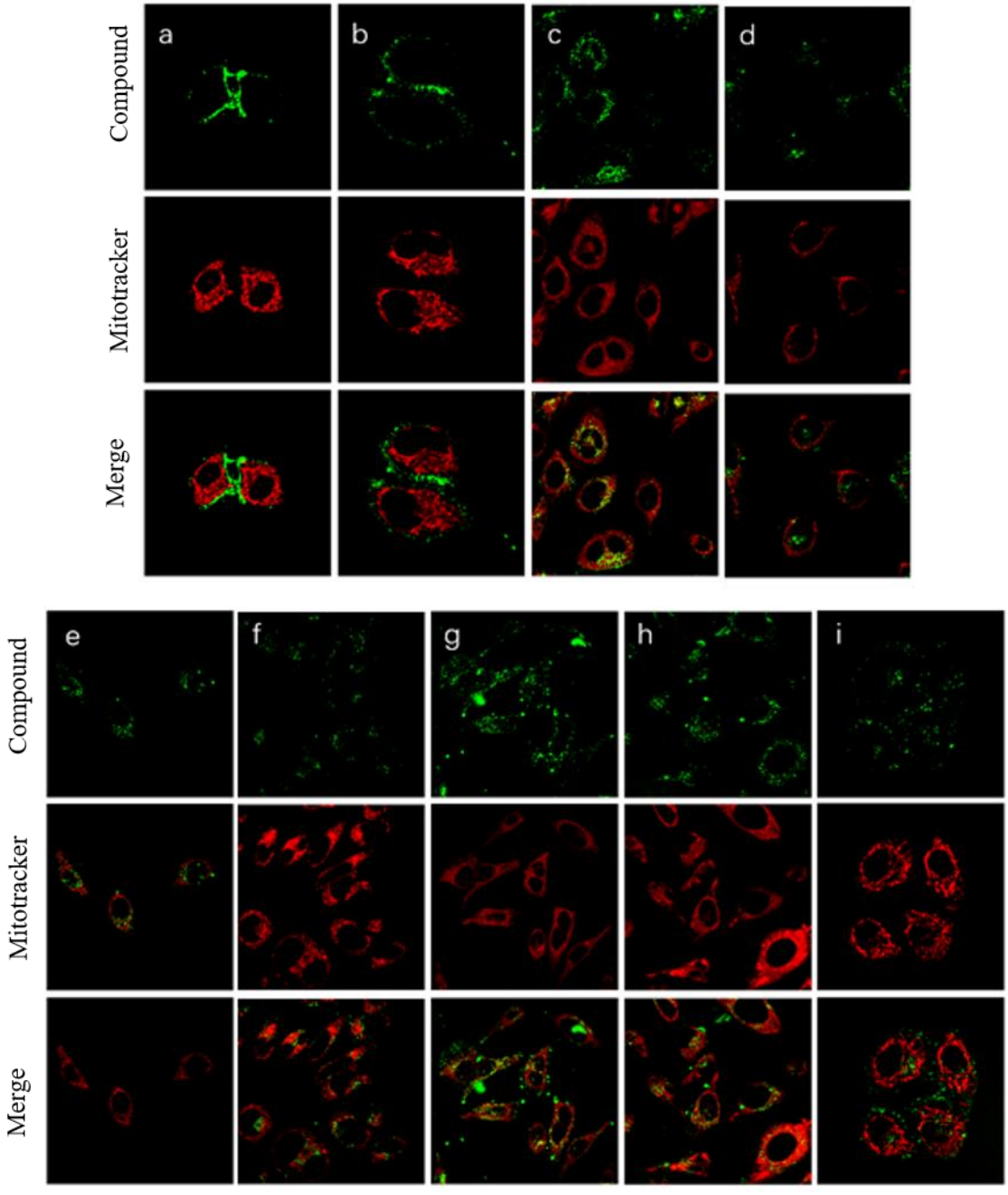


Figure S33. Live cell images of HeLa cells treated with (a) 10 μM of **MPP-1**, (b) 10 μM of **MPP-2**,(c) 10 μM of **cyclic MPP-4**, (d) 10 μM of**cyclic MPP-5**, (e) 10 μM of**cyclic MPP-6**, (f) **cyclic MPP-7**, (g) **cyclic MPP-8**, (h) **cyclic MPP-9**,(i) **cyclic MPP-10**, incubation of one hour.

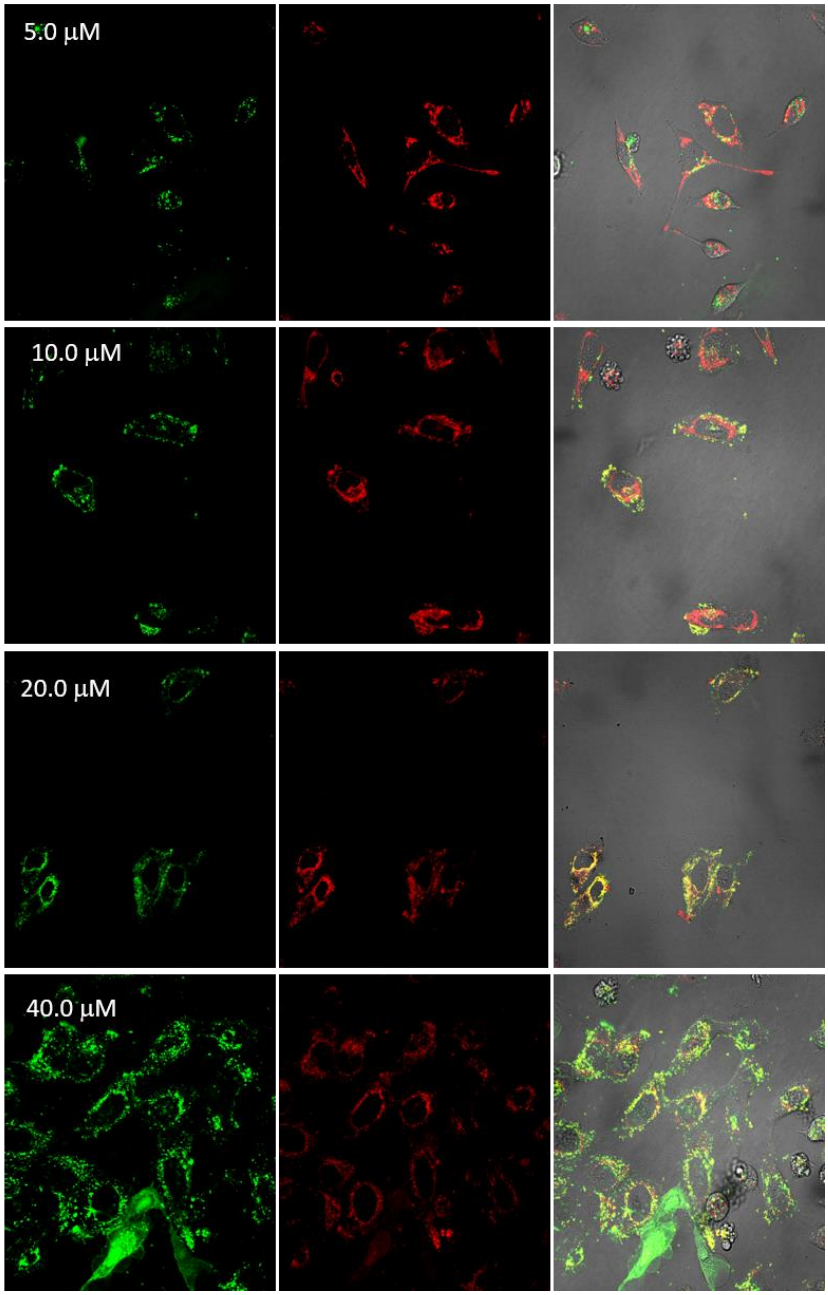


Figure S34. Live cell images of HeLa cells treated with 5 μM, 10 μM, 20 μM, and 40 μM of **cyclicMPP-2**, incubation of one hour.

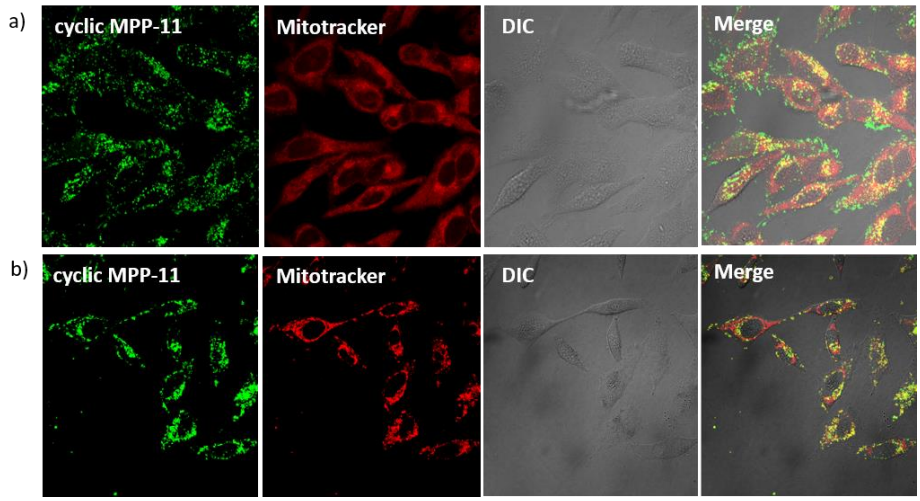


Figure S35. Live cell images of HeLa cells treated with (a) 10 μM of **cyclicMPP-11**, (b) 20 μM of **cyclicMPP-11**, incubation of three hours.

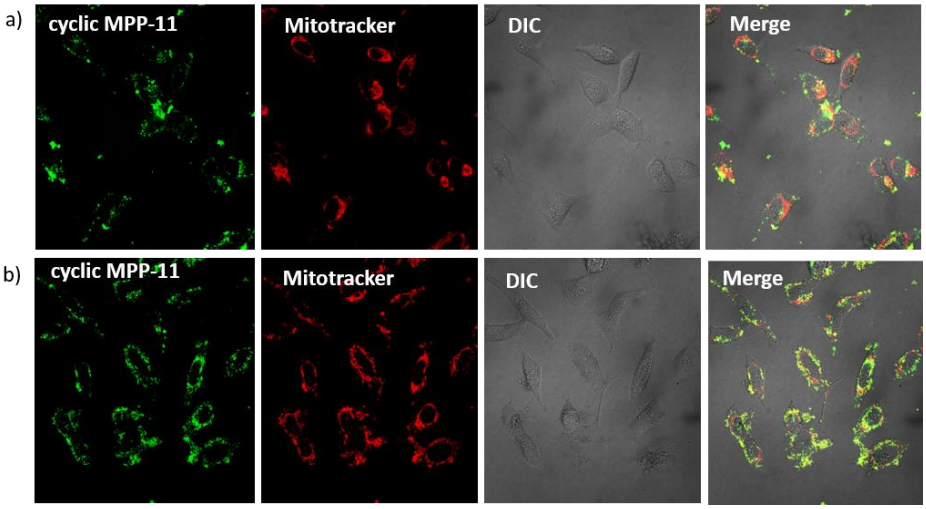


Figure S36. Live cell images of HeLa cells treated with 20 μM of **cyclicMPP-11** with incubation time of (a) one hour, (b) two hours.

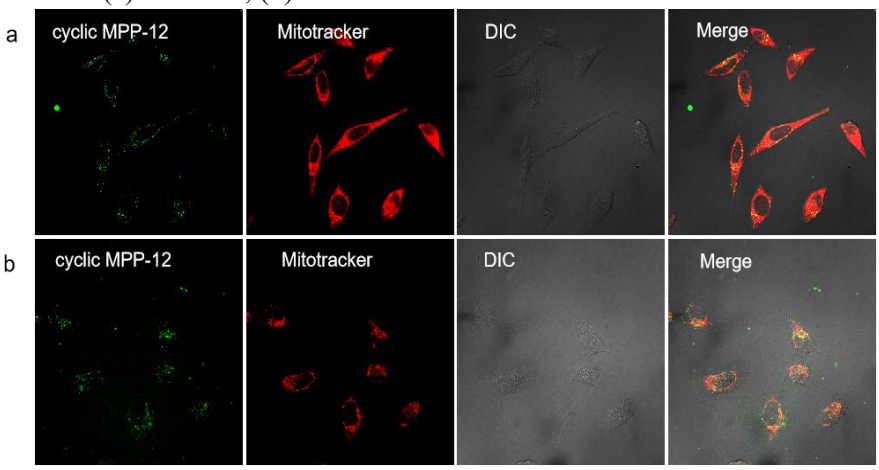


Figure S37. Live cell images of HeLa cells treated with 20 μM of **cyclicMPP-12** with incubation time of (a) one hour, (b) three hours.