A precisely positioned chiral centre in an i, i+7 tether modulates the helicity of the backbone peptide

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1. General information

1.1 Abbreviations

Fmoc: 9-fluorenylmethyloxycarbonyl;
DIPEA: diisopropylethylamine;
MAP: 4'-Methoxyacetophenone,
FITC: Fluorescein isothiocyanate,
RT: room temperature;
SPPS: solid-phase peptide synthesis.
DMF: dimethylformamide;
DCM: dichloromethane;
TFA: trifluoroacetic acid;
TFE: 2,2,2-trifluoroethanol;
TIS: triisopropylsilane;
Et$_2$O: diethyl ether;
CD: circular dichroism;
LC-MS: liquid chromatography–mass spectrometry;
HPLC: high-performance liquid chromatography;
ESI-MS: electrospray ionization mass spectrometry;
MMP: 2-Hydroxy-4’-(2-hydroxyethoxy)-2-methylpropiophenone,
HCTU: 2-(1H-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate;

1.2 Materials

The resins and Fmoc-protected amino acid used for solid phase peptide synthesis (SPPS) and the chemical reagents were all purchased from commercial suppliers, such as GL Biochem (Shanghai) Ltd. J&K Co. Ltd., Huizhou Deep chemical technology co. LTD, Tianjin Damao Chemical Reagent Factory, Tianjin Yongda Chemical Reagent Company Limited or Shenzhen Tenglong Logistics Co. All of the reagents were used without further purification except specifically stated.

1.3. HPLC and Mass spectrometry

Peptides were purified by HPLC (SHIMAZU Prominence LC-20AT, UV detection at 220 or 254 nm) equipped with a C18 analytic column (Agilent ZORBAX SB-Aq, 4.6 × 250 mm, 5 µm, flow rate 1.0 mL/min). Filtered H$_2$O with 0.1% TFA and pure acetonitrile were used as solvents in linear gradient mixtures. Peptides were analyzed by LC-MS (SHIMAZU LC-MS 8030, ESI-MS).

1.4 Peptide Synthesis and Characterization

Peptides were synthesized on MBHA resin (loading capacity: 0.37 mmol/g) by standard Fmoc-based SPPS. At first, the resin was swelled in NMP for 30 min. Then 50% (vol/vol) morpholine in NMP was used to deprotect the Fmoc group on amine group for 30min × 2. Next the resin was washed with DCM and NMP alternatively for three time(3*1min). In coupling process, for natural amino acids, the Fmoc-protected amino acids (5.0 equiv), HCTU (4.9 equiv), DIPEA (10.0 equiv) were dissolved in NMP and mixed with resin for
2h, followed by washing with DCM and NMP for three times (3*1min). For unnatural amino acids, Fmoc-protected acids (2.5 equiv), HCTU (2.4 equiv) and DIEA (5.0 equiv) were dissolved in NMP and mixed with resin for 4h, followed by washing with DCM and NMP for several times. After we completed the synthesis of designed peptide, the intramolecular thiol-ene reaction was performed. The resins were drained and transferred to a suitable flask, mixed with 1.2eq MAP/MNP (1:1) catalyst in DMF and reacted at ultraviolet light (365nm) for 2h. Final the resins were treated with a mixture of TFA/H$_2$O/TIS (95/2.5/2.5) for 2 h and dried by blowing nitrogen. Then the peptides were precipitated with Hexane/Et$_2$O (1:1 in volume) at 4°C, isolated by centrifugation and dissolved in 40% (vol/vol) acetonitrile/ water, purified by HPLC with UV absorbance at 220 nm or 280nm and later analyzed by LC-MS.

2. Peptides preparation

2.1 Synthesis of unnatural amino acids

![Scheme 1. The chemical structure of unnatural amino acids used in this article.](image)

The synthesis process of $X_8$ was showed as an example, and $S_8$, $X_7$, $X_9$, $X_10$, $S_8$(2-Ph-S/R), $X_8$(2-Ph) were synthesized in the similar route. The procedure was referred to the technique of OKEANOS TECH CO. Ltd of China.

![Scheme 2. The synthesis process of unnatural amino acid $X_8$.](image)

The detailed synthesized procedures were:

- Compound 1
Potassium hydroxide (38.4 g, 0.7 mol) was dissolved in anhydrous methanol (125 ml) and heated to 60°C, then D-proline (23 g, 0.2 mol) was added into the mixture. After complete dissolution, 2-chlorobenzyl chloride (32 ml, 0.26 mol) was added dropwise. After 24 h, CH₂Cl₂ (100 ml) was added and the reaction mixture stood for 4 h. Then the mixture was filtered out and the residue was washed by CH₂Cl₂ twice. The filtrate was gathered, concentrated and crystallized in acetone to obtain compound 41 g, yield: 83%.

- **Compound 2**
  Compound 1 (24.8 g, 0.1 mol) was added into CH₂Cl₂ (200 ml) and the mixture was cooled to 0°C. Phosphorus pentachloride (30.2 g, 0.15 mol) was added dropwise and stirred for 1 h, followed by the addition of 2-aminobenzophenone (20.0 g, 0.1 mol). The reaction was stirred at r.t. for 4 h. CH₂Cl₂ was removed under vacuum and acetone was added for crystallization to obtain compound 2 (25.2 g, yield: 61%).

- **Compound 3**
  Compound 2 (25.0 g, 0.065 mol), nickel (II) nitrate hexahydrate (31.6 g, 0.11 mol) and glycine (20.5 g, 0.27 mol) were dissolved in anhydrous methanol (300 ml) and heated to 50°C. The potassium hydroxide (25.0 g, 0.47 mol) in methanol (150 ml) solution was added dropwise. After 10 h, acetic acid was added. Methanol was removed and followed by pouring the residue liquid into ice water (800 ml), and stirred at r.t. overnight to promote precipitation. The mixture was filtered out under vacuum and residue was gathered to obtain red solid compound 3 (22.0 g, yield: 75%).

- **Compound 4**
  Under N₂ atmosphere, compound 3 (20.0 g, 0.04 mol) was dissolved in DMF (200 ml), followed by the addition of powdered potassium hydroxide (21.1 g, 0.4 mol) and the reaction mixture was stirred at r.t. for 1 h. Under the condition of ice bath, 8-bromo-1-pentene (6 ml, 0.042 mol, J&K Co. Ltd) was added dropwise. Then the reaction was gradually warmed to r.t. and stirred for 4 h before the addition of 5% v/v acetic acid in water. The reaction continued to be stirred for 6 h to promote the precipitation and filtered out. The residue was gathered and washed by water for three times to obtain compound 4 (23.2 g, yield: 87%).

- **Compound 5**
  Compound 4 (23.2 g, 0.035 mol) was dissolved in methanol/CH₂Cl₂ (v/v = 50 ml/100 ml), and 3 M hydrochloric acid (100 ml) was added into the mixture. The reaction was heated to 80°C and stirred overnight until yellow/green color change was observed. Then the solvent was removed in vacuo and chloroform was used for extraction for three times to recover the ligand. The amino acid aqueous fraction was used for the next step without further purification.

- **Compound 6 (X₆)**
  Sodium bicarbonate (16.8 g, 0.2 mol) and EDTA-Na (18.6 g, 0.05 mol) were added into the aqueous fraction to remove residual nickel. After stirring for 20 minutes, sodium bicarbonate was added again to make pH value of the mixture stay at 7-8. Then the mixture was cooled to 0°C with ice bath. 9-fluorenylmethyl succinimidyl carbonate (11.7 g, 0.035 mol) was dissolved in acetonitrile (25 ml) and added dropwise into the aqueous solution. The reaction was gradually warmed to r.t. and stirred for 12 h. Acetonitrile was removed in vacuo and citric acid was added to make pH value of the mixture stay at 2-3. The reaction was extracted with ethyl acetate for three times. The organic phase was dried with anhydrous magnesium sulfate. The final
product X₈ was obtained after the purification of flash chromatography (Hexane: EA = 5:1) (5.2 g, yield: 41%)

2.2 Synthesis of the side chain of non-natural amino acids S₈(2-Ph-R) and S₈(2-Ph-S)

Scheme 3. The synthesis procedures of the unnatural amino acids S₈(2-Ph-R) and S₈(2-Ph-S).

- **Compound 7**
  To a solution of (S)-4-benzyloxazolidin-2-one (4.80 g, 27.1 mmol, 1.00 eq) in THF (100 mL) at −78 °C was added 1.6 M n-butyllithium in hexane (18.6 mL, 30.0 mmol, 1.10 eq) dropwise over 20 minutes. The temperature of the solution was maintained at −78°C prior to and during the addition of base. Phenylacetyl chloride (4.61 g, 30.0 mmol, 1.10 eq) was added over 10 minutes and the solution was stirred at −78 °C for 2.5 hrs. The reaction was warmed to room temperature and quenched with saturated ammonium chloride (10 mL). The THF was removed under vacuum and the residual oil was taken up into dichloromethane (100 mL), washed with water (1 × 50 mL), washed with 10% sodium hydroxide (1 × 50 mL), washed with water (2 × 50 mL), washed with brine (1 × 50 mL), dried over MgSO₄, and concentrated to a white powder (7.80 g, yield: 97.5%).

- **Compound 8**
  To a solution of 0.94 M lithium diisopropyl amide (20 mL, 18.9 mmol, 1.10 eq) in THF/hexanes at −78 °C was added a solution of 7 (5.04 g, 17.1 mmol, 1.00 eq) in THF (25 mL) over 30 minutes. After allowing the solution to stir for 15 minutes at −78 °C, 6-Bromo-1-hexene (8.38 g, 51.4 mmol, 3.00 eq) was added dropwise over 30 minutes. Stirring was continued for 30 minutes at −78 °C followed by an additional stirring for 2.5 hrs at 0 °C in an ice bath. Upon the disappearance of starting material, the reaction mixture was warmed to room temperature and quenched with saturated ammonium chloride (20 mL). Volatile reaction components were removed under vacuum. The residue was dissolved in DCM (100 mL), washed with water and brine.
The organic phase was dried with anhydrous magnesium sulfate to obtain compound 8 (3.50 g, yield: 60.1%).

**Compound 9**

Compound 8 (1.06 g, 3.0 mmol, 1.0 eq) was dissolved in diethylether (25 mL) and cooled to 0°C in an ice/salt bath. To this solution was added ethanol (0.21 g, 4.5 mmol, 1.5 eq) followed by LiBH₄ (4 × 25 mg portions, 4.6 mmol, 1.5 eq). The solution was stirred for 1 hr at 0 °C and then stirred for an additional 3 hrs at room temperature. The reaction was quenched with 1 M NaOH (25 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 × 25 mL). The combined organic layers were washed with ammonium chloride (1 x50 mL), washed with water and brine. The organic phase was dried with anhydrous magnesium sulfate to obtain compound 9 (0.48 g, yield: 89.5%).

**Compound 10**

PPh₃ (1.44 g, 5.5 mmol, 1.1 eq) and imidazole (0.51 g, 7.5 mmol, 1.5 eq) was dissolved in anhydrous DCM (50 mL) and cooled to 0°C in an ice/salt bath. To this solution was added I₂ dropwise (1.4 g, 5.5 mmol, 1.1 eq). After allowing the solution to stir for 20 minutes at 0 °C, Compound 9 (0.86 g, 5.0 mmol, 1.0 eq) was added dropwise. The solution was stirred for overnight in dark. Upon the disappearance of starting material, the reaction mixture was warmed to room temperature and quenched with saturated ammonium chloride (20 mL). Volatile reaction components were removed under vacuum. The residue was dissolved in DCM (100 mL), washed with water and brine. The organic phase was dried with anhydrous magnesium sulfate to obtain compound 10 (1.23 g, yield: 88.5%).

Reference:


**2.3 Preparation of cyclic I, i+7 thiol-ether peptide**

The synthesis of cyclic thioether peptide Ac-(cyclo-1,8)-[X₈AAAAAC]-NH₂ was showed as an example below, and thioether peptides 1, 3-8 were synthesized in a similar way. The peptide synthesis was applied the Fmoc-based solid phase peptide synthesis (SPPS). The intramolecular thiol-ene reaction was used to close the side ring. Briefly, the linear peptide on resin was mixed with MAP/MMP (1:1) catalysis (1.2eq) in DMF solvent and reacted at ultraviolet light (365nm) for 2h. Then the N-terminus was capped with acetyl group. Finally, the raw peptide was cleaved from the resin by a mixture of TFA/H₂O/TIS (95/2.5/2.5), dried by
blowing nitrogen and purified by HPLC. In general, the S/R isomers in each peptide were separable with retention time difference. The purified peptides were characterized by LC-MS with ESI positive ion mode. The LC-MS figures were attached at the end of supporting information.

Scheme 4. The synthesis process of cyclic peptides 1-8. The cyclization efficiency of all peptides is more than 90%.

2.4 Preparation of cyclic i, i+7 sulfoxide peptide

Cyclic octapeptide was projected to 5% H\textsubscript{2}O\textsubscript{2}, then stirred at r.t. for 2hrs. The mixture was used for HPLC purification without further treatment, the S/R isomers were separated as their retention time is different.

Scheme 5. The oxidation of thiol-ether peptide to sulfoxide peptide were completed with incubation in 5% H\textsubscript{2}O\textsubscript{2}.

2.5 Preparation of i, i+7 tethering CIH peptides 11-S/R

Scheme 6. The synthesis process of cyclic peptides 11-S/R. The cyclization efficiency of all peptides is more than 80%. Peptide 12-S/R was synthesized in a similar route.
Reference:


3. Chemical structures, chemical formula and calculated molecular weight of peptides

**Sulfoxide chiral center peptides**

**7+2**

Chemical Formula: $C_{32}H_{55}N_9O_{10}S$
Molecular Weight: 757.91

**8+2**

Chemical Formula: $C_{33}H_{57}N_9O_{10}S$
Molecular Weight: 771.93

**9+2**

Chemical Formula: $C_{34}H_{59}N_9O_{10}S$
Molecular Weight: 785.96

**7+3**

Chemical Formula: $C_{35}H_{61}N_9O_{10}S$
Molecular Weight: 799.99

**8+3**

Chemical Formula: $C_{36}H_{63}N_9O_{10}S$
Molecular Weight: 814.01

**9+3**

Chemical Formula: $C_{32}H_{55}N_9O_{10}S$
Molecular Weight: 757.91

**10+2**

Chemical Formula: $C_{33}H_{57}N_9O_{10}S$
Molecular Weight: 771.93

**10+3**

Chemical Formula: $C_{34}H_{59}N_9O_{10}S$
Molecular Weight: 785.96

**2+7**

Chemical Formula: $C_{35}H_{61}N_9O_{10}S$
Molecular Weight: 799.99

**2+8**

Chemical Formula: $C_{35}H_{61}N_9O_{10}S$
Molecular Weight: 799.99
4. Experimental section

CD analysis

All peptides were dissolved in deionized H₂O for CD measurements. The spectra were obtained on an applied photophysics chirascan Circular Dichroism Spectrometer at 20°C using the following standard measurement parameters: wavelength, 185-260 nm; step resolution, 0.5 nm; speed, 20 nm/sec; accumulations, 10; response, 1 sec; bandwidth, 1 nm; path length, 0.1 cm. Every sample was scanned twice and the final CD spectrum was averaged and smoothed. Variable temperature CD scans for peptide 9-R were collected from 5°C to 65°C at 5°C intervals.
NMR measurement

NMR data were recorded on a Bruker AVANCE III 400 (or 500) MHz spectrometer. DMSO-d$_6$ was used for 1H NMR to characterize the peptides. NMR data were processed using Topspin 3.0. Temperature coefficients were used as tools to characterize the propensity for exchangeable protons to form intramolecular hydrogen bonds (IMHBs). For this experiment, the peptides were dissolved in 9:1 H$_2$O: D$_2$O. The protection of the IMHBs decreases the temperature dependence of the chemical shift of the exchangeable protons. This ultimately results in a smaller value of $\Delta\delta/T$ compared to non-IMHB donors. Generally, the cutoff value of $\Delta\delta/T$ for IMHBs is solvent dependent. In aqueous solution, values of $\Delta\delta/T$ less than 4.5 ppb/K usually indicate hydrogen bonding.

2D NMR data were collected on a Bruker Avance III 500MHz spectrometer with a TXI probe. Watergate pulse sequence with gradients were used for water suppression in 1D and 2D 1H spectrum. 2D 1H-1H TOCSY and NOESY spectra were acquired with mixing time of 100ms and 300ms, respectively. The TOCSY and NOESY spectra were acquired with a width of 10ppm and 13C spectra width of 100ppm, and size of 1024×400 complex points. All the 2D NMR spectra were processed by Topspin® to final 2048×1024 complex points, and analyzed by CCPNMFR software. $^3$J(NH-Hα) couplings were measured from 1D-1H spectrum. Temperature dependence for amide NH chemical shifts were measured from 2D TOCSY spectra recorded at temperature ranges from 5°C to 65°C with 5°C interval. At each temperature, the sample were allowed to equilibrate for 15min, and the chemical shifts were calibrated with standard 4,4-dimethyl-4-silapantane-1-sulfonic acid(DSS).

Serum stability

Standard solutions of peptides (1 mg/mL) were prepared in PBS. Stability in human serum was carried out by incubation of peptides at a final concentration of 100 µM (25% serum) at 37 °C. Aliquots (10 µL) were taken periodically at 0, 30, 60min, 2h, 4h, 8h, 24h, and then 100 µl 12% trichloroacetic acid in H$_2$O/CH$_3$CN (1:3) was added and cooled to 4 °C for 30 min to precipitate serum proteins. The decanted supernatant was analyzed by LC-MS with a 4.6 × 250 mm2 Zorbax SB C18 5 µm column, using a 3% per minute linear gradient from 10%- 90% acetonitrile over 27 min. The amount of starting material left in each sample was quantified by determination of total ion counts for the molecular ion.

Flow cytometry analysis

Hela cells were grown in DMEM medium with 10% FBS (v/v) in imaging dishes (50000 cells/well) in 37°C, 5% CO$_2$ incubator for two days(50,000 cells per well). Cells were treated with fluoresceinated peptides (5 µM) for up to 2 hours at 37°C. After washing with media, the cells were exposed to trypsin (0.25%; Gibco) digestion (5-10 min, 37°C), washed with PBS, and resuspended in PBS. Cellular fluorescence was analyzed using a BD FACS Calibur flow cytometer (Becton Dickinson).

Fluorescence polarization

FITC (fluorescein isothiocyanate) labelled peptides (10nM) were incubated with MDM2 17-125 in binding assay buffer (140 mM NaCl, 50 mM, Tris pH 8.0) at room temperature for 1 hours. Fluorescence polarization experiments were performed in 96-well plates (Perkin Elmer Optiplate-96F) on plate reader (Perkin Elmer, Envision, 2104 multilabel reader). Concentrations of the peptides were determined by 494 nm absorption of FITC. $K_d$ values were determined by nonlinear regression analysis of dose response curves using Origin pro 9.0.

Cell Imaging

Hela cells were cultured with DMEM with 10% FBS (v/v) in imaging dishes (50000 cells/well) in 37°C, 5% CO$_2$ incubator for one day until they were about 80% adherent. Peptide were first dissolved in DMSO to make a 1 mM stock solution and then added to cells to a final concentration of 5 µM. The cells were incubated with peptides for 1 hour at 37°C. After incubation, cells were washed 3 times with PBS and then stained with
1µg/ml Hoechst 33258 (Invitrogen, CA) in PBS for 5 minutes. Then washed 3 times with PBS and images of peptide localization in cells were taken on PerkinElmer confocal microscopy. Image processing was done using Volocity software package (Zeiss Imaging).

5. Supplementary Table

Table 1. Mass data for peptides 1-14

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<th>Entry</th>
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<td>S/R 832.03</td>
<td>832.50</td>
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12-PDI linear Ac-LTFQ'EYWAQLTSA-NH₂ | 1668.81 | 834.40 |
13-PDI+4  Ac-LTF-cyclo[CYWS₆(Ph-R)]QLTSAA-NH₂ | 1774.06 | 887.00 |
14-PDI+7  Ac-LTF-cyclo[PEWAQLS₆(Ph-R)]SAA-NH₂ | 1786.11 | 893.85, 1786.95 |
13-PDI+4-FITC FITC-[A-LTF-cyclo[CYWS₆(Ph-R)]QLTSA-NH₂ | 2193.48 | 1097.50 |
14-PDI+7-FITC FITC-[A-LTF-cyclo[PEWAQLS₆(Ph-R)]SAA-NH₂ | 2212.53 | 1106.26 |
6. Supplementary Figures

Figure S1. CD spectra of thioether peptides 1-8 (≈100 μM) shown as a random coil/ non-helical structure in water at 20°C.

Figure S2. The HPLC chromatogram of peptide 2-A/B and 7-A/B. Δt indicates the retention time difference between epimers A and B.
**Figure S3.** The HPLC chromatogram of peptide **S1-A/B** (Ac-γC(O)AAAAAASγ-NH₂) and peptide **S2-A/B** (Ac-γC(O)AAAAAASγ-NH₂).

![HPLC chromatogram](image1)

**Figure S4.** The CD spectra of peptide **S1/2-A/B** mixture (~20μM) was recorded in H₂O at 20°C.

![CD spectra](image2)

**Figure S5.** NH-αCH and NH-βCH region of 2D-NOESY spectrum of **9-B** (at 500MHz in H₂O with 10% D₂O at 20°C)

![2D-NOESY spectrum](image3)
Figure S6. (A) Temperature coefficient ($\Delta\delta/T$) of peptide 9-B (Ac-X$_8$ELARALC(O)-NH$_2$). The chemical shift of amide NH in each amino acid and a linear curve was fit. The slope means the temperature coefficient. (B) The selected $^1$H NMR region of peptide 9-B in different temperatures.

Figure S7. The HPLC chromatogram of peptide 11-S, 11-R (Ac-$\varepsilon_{10}$CAAAAAA S$_8$(2-Ph)-NH$_2$).

Figure S8. The CD spectra of peptide S3-A/B (Ac-X$_8$(2-Ph)AAAAAAC-NH$_2$) mixture (~20μM) was recorded in H$_2$O at 20°C.
**Figure S9.** (A) Dependence on temperature (5-65°C) of mean residue ellipticity at 222 nm for peptide 11-R in H$_2$O. (B) Variation in molar ellipticity of 11-R (~20 μM) at 222 nm with increasing [Guanidine·HCl] at 20°C. The peptide concentration used was approximately 20 μM.

**Figure S10.** The original flow cytometry analysis data of peptides 12-14. The cells were analysed after incubation with peptides (5μM) for 2 hours at 37°C.
7. Appendix

7.1) The NMR data of peptide 9-B.

<table>
<thead>
<tr>
<th>NO.</th>
<th>Residue</th>
<th>NH</th>
<th>Ha</th>
<th>Hb</th>
<th>H(sidechain)</th>
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<tr>
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<td>3.87</td>
<td>2.07</td>
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<tr>
<td>2</td>
<td>E</td>
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<td>4.04</td>
<td>2.51</td>
<td>2.07</td>
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<tr>
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<tr>
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</table>

The H chemical shifts assignment of peptide 9-B.

NH region of NOESY spectrum of 9-B (500MHz in H₂O with 10% D₂O at 20°C)
TOCSY spectrum for peptide 9-B (500MHz in H$_2$O with 10% D$_2$O at 20°C)

7.2) The HPLC chromatogram in different time points in the in vitro serum stability assays.

For peptide 13-PDI-i/i+4

For peptide 13-PDI-i/i+7
7.3) Mass spectra for selected peptides

The MS spectra of peptide 1 (7+2) before oxidation

The MS spectra of peptide 1 (7+2) after oxidation
The MS spectra of peptide 2 (8+2) before oxidation
The MS spectra of peptide 2 (8+2) after oxidation

2-A

1- B
The MS spectra of peptide 3 (9+2) before oxidation

The MS spectra of peptide 3 (9+2) after oxidation

3-A

3-B
The MS spectra of peptide 4 (10+2) before oxidation

The MS spectra of peptide 4 (10+2) after oxidation

4-A
The MS spectra of peptide 5 (7+3) before oxidation

The MS spectra of peptide 5 (7+3) after oxidation

5-A
The MS spectra of peptide 6 (8+3) before oxidation
The MS spectra of peptide 7 (9+3) before oxidation

The MS spectra of peptide 7 (9+3) after oxidation

7-A

7-B
The MS spectra of peptide 8 (10+3) before oxidation

The MS spectra of peptide 8 (10+3) after oxidation

8-A
The MS spectra of peptide 9 before oxidation

The LC spectra and MS spectra of peptide 9 after oxidation
The MS spectra of peptide 10-S
The MS spectra of peptide 10-R

The LC spectra and MS spectra of Peptide 11-S

The LC spectra and MS spectra of Peptide 11-R
The MS spectra of peptide S1 before oxidation

The MS spectra of peptide S1 after oxidation

S1-A/B

The MS spectra of peptide S2 before oxidation
The MS spectra of peptide S2 after oxidation
S2-A/B

The MS spectra of peptide S3-A/B

12-PDI-linear
13-PDI-i, i+4

14-PDI-i, i+7
13-PDI-i, i+4-FITC
14-PDI-i, i+7-FITC

[Graphs and data plots]

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<td>90</td>
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</table>

[Graphs and data plots]
7.4) NMR spectra of unnatural amino acids

**$^1$H NMR of S$_8$(Ph-)$^R$**

$^1$H NMR (300 MHz, CDCl$_3$, 25°C, TMS): $\delta$ 7.77 (d, $J = 3$ Hz, 2H), 7.57 (t, $J = 3$ Hz, 2H), 7.40 (t, $J = 6$, 3 Hz, 2H), 7.32 (t, $J = 3$ Hz, 3H), 7.27 (d, $J = 6$ Hz, 2H), 7.15(d, $J = 3$ Hz, 3H), 5.73 (t, $J = 6$ Hz, 1H), 4.95 (s, 1H), 4.90 (dd, $J = 3$, 6 Hz, 2H), 4.37 (d, $J = 3$ Hz, 1H) 4.32(s, 1H), 4.20 (t, $J = 1.5$ Hz, 1H), 2.31 – 2.16 (m, 1H), 1.97 (d, $J = 3$ Hz, 3H), 1.62 (m, 2H), 1.32 (m, 5H).

**$^{13}$C NMR of S$_8$(Ph-)$^R$**
$^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ = 174.7, 156.9, 143.8, 141.4, 138.9, 128.7, 127.7, 126.9, 120.2, 114.3, 67.2, 52.9, 47.5, 42.5, 37.0, 33.5, 28.9, 27.0.

$^1$H NMR of X$_7$

$^1$H NMR (300 MHz, CDCl$_3$, 25°C, TMS): $\delta$ 7.88 (d, $J$ = 6 Hz, 2H), 7.71 (d, $J$ = 3 Hz, 2H), 7.41 (t, $J$ = 3, 6 Hz, 3H), 7.32 (t, $J$ = 3, 6 Hz, 2H), 5.77 (q, $J$ = 3 Hz, 1H), 4.98 (s, 1H), 4.94 (d, $J$ = 9 Hz, 1H) 4.26 (t, $J$ = 6, 12 Hz, 3H), 3.88 (t, $J$ = 1.5 Hz, 1H), 2.20 (m, 2H), 1.60 (m, 2H), 1.40 (q, $J$ = 1.5 Hz, 2H), 1.23 (m, 4H).

$^1$H NMR of X$_8$
$^1$H NMR (300 MHz, CDCl$_3$, 25°C, TMS): $\delta$ 7.76 (d, $J = 6$ Hz, 2H), 7.58 (d, $J = 3$ Hz, 2H), 7.39 (d, $J = 6$ Hz, 2H), 7.32 (d, $J = 6$ Hz, 2H), 7.26 (s, 1H), 5.77 (q, $J = 3$ Hz, 1H), 5.32 (d, $J = 3$ Hz, 1H), 5.11 (t, $J = 12$, 6 Hz, 2H) 4.42 (t, $J = 6$, 1.5 Hz, 2H), 4.22 (t, $J = 1.5$ Hz, 1H), 2.25-2.15 (m, 2H), 1.78 (q, $J = 1.5$ Hz, 2H), 1.43(m, 2H), 1.31 (m, 4H), 1.28 (m, 2H).

$^1$H NMR of X$_{10}$

$^1$H NMR (300 MHz, CDCl$_3$, 25°C, TMS): $\delta$ 7.77 (d, $J = 3$ Hz, 2H), 7.60 (d, $J = 6$ Hz, 2H), 7.41 (t, $J = 3$, 6 Hz, 2H), 7.32 (t, $J = 6$, 3 Hz, 2H), 7.27 (s, 1H), 5.76-5.68 (m, 1H), 5.56 (d, $J = 1.5$ Hz, 1H), 4.98 (t, $J = 15$, 6 Hz, 2H) 4.41 (m, 2H), 4.23 (t, $J = 3$ Hz, 1H), 2.3-2.23 (m, 2H), 2.18 (m, 2H), 2.06 (q, $J = 1.5$ Hz, 2H), 1.62(m, 4H), 1.28 (m, 2H), 1.24 (m, 4H)