Supporting Information

Proline-Derived Transannular *N*-Cap for Nucleation of Short α-Helical Peptides

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General Information

1. Abbreviations

Fmoc, 9-fluorenylmethyloxycarbonyl; HCTU, 2-(1H-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate; HATU, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate ; DIPEA, diisopropylethylamine; DMF, dimethylformamide; DCM, dichloromethane; THF, tetrahydrofuran; DEAD, diethyl azodicarboxylate; DPPA, diphenylphosphoryl azide; HOBt, 1-Hydroxybenzotriazole; DIC, N,N'-Diisopropylcarbodiimide; NMDA, 1,3-Dimethylbarbituric acid; PyBOP, benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; NMM, 4-Methylmorpholine; tBu, tert-butyl; Boc, di-tert-butyl dicarbonate ester; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; TIS, triisopropylsilane; Et_2O, diethyl ether; LC-MS, liquid chromatography–mass spectrometry; NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; CD, circular dichroism; ESI-MS, electrospray ionization mass spectrometry; RP-HPLC, reserved-phase high performance liquid chromatography; RT, room temperature; SPPS, solid-phase peptide synthesis.

2. Materials

The materials used for solid phase peptide synthesis were purchased from commercial suppliers including Energy Chemical Co., 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 reagents were used without further purification unless otherwise stated.

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_2O (containing 0.1% TFA) and pure acetonitrile (containing 0.1% TFA) were used as solvents in linear gradient mixtures. Peptides were analyzed by LC-MS (SHIMAZU LC-MS 8030, ESI-MS).

4. Peptide Synthesis and Characterization

Peptides were synthesized on Rink Amide MBHA resin (loading capacity: 0.54 mmol/g) (GL Biochem Ltd.) *via* standard Fmoc-based solid-phase peptide synthesis. Firstly, the resin was pre-swelled with NMP for 30 min. The Fmoc group was removed by 50% (vol/vol) morpholine in NMP for 30min × 2. Then the resin was washed sequentially with DCM and NMP (5x). For natural amino acids, Fmoc-protected amino acids (5.0 equiv according to initial loading of the resin) and HCTU (4.9 equiv) were dissolved in DMF, followed by DIPEA (10.0 equiv). The mixture was pre-activated for 1 min and added to the resin for 2 h, followed by washing with DCM and NMP (5 times). For unnatural amino acids, Fmoc-protected acids (3.0 equiv) and HCTU (2.9 equiv) were dissolved in DMF, followed by DIPEA(6.0 equiv). Peptides were N-terminally acetylated with a solution of acetic anhydride and DIPEA in NMP (1:4: 20 in volume) for 30min × 2. Final resins were treated with a mixture of TFA/H_2O /TIS (95/2.5/2.5) for 2 h and concentrated under a stream of nitrogen. The crude peptides were then precipitated with Hexane/Et_2O (1:1 in volume) at 4°C, isolated by centrifugation then dissolved in 25% (vol/vol) water/acetonitrile, purified by analytic reversed phase HPLC and analyzed by LC-MS. Acetylated peptides were quantified by their UV absorbance at 280 nm.

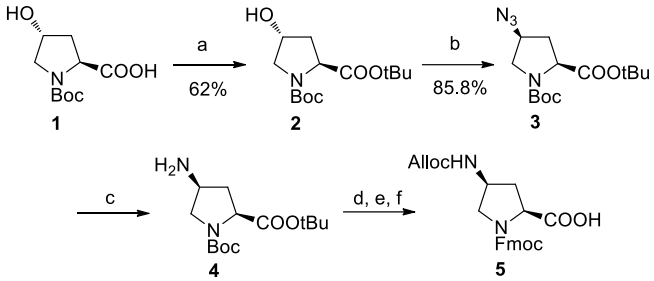
5. NMR Spectroscopy

1D NMR spectra were recorded on Bruker Avance-III 400MHz spectrometer in $CDCl_3$ or DMSO-*d6*. Chemical

shifts (δ) are reported in parts per million (ppm) with TMS ($CDCl_3$). 2D NMR spectra were recorded on Bruker Avance-III 600 MHz spectrometer with a TXI probe in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K. Data are reported in the following order: chemical shifts are given (δ); multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and app (apparent).

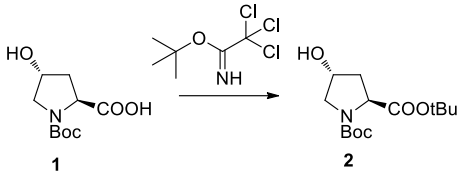
Experimental section

1. Synthesis of proline derivatives



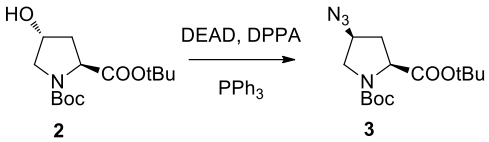
Scheme S1 Synthetic route of proline derivatives a) tert-Butyl 2,2,2-trichloroacetimidate，24 h; b) DEAD, DPPA,THF, 24 h; c) 10% Pd/C, H_2, MeOH, 4 h; d) Alloc-Cl, 25% K_2CO_3; e) TFA/DCM, 30min; f) Fmoc-OSu, $NaHCO_3$, overnight.

1.1 Synthesis of ***tert-Butyl N^{α}-Boc-4-trans-hydroxy-L-prolinate***



Compound **2** was synthesized according to the previous method reported by Lei Liu et al [1].Compound **1** (1.0 g, 4.3 mmol) was dissolved in dry CH_2Cl_2 (26 mL) and tetrahydrofuran (6.5 mL). Tert-butyl 2,2,2-trichloroacetimidate (1.8 mL, 9.89 mmol) was slowly added. Then the mixture was stirred overnight under Nitrogen protection. The reaction was concentrated in vacuo, then taken up in EtOAc, washed with saturated sodium bicarbonate and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography (SiO2, 2:1 Petroleum ether/EtOAc) to yield **2** (0.8g, yield 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.44 (s, 1 H), 4.34-4.18 (m, 1 H), 3.69-3.33 (m, 2 H), 2.35-2.13 (m, 1 H), 2.08-1.93 (m, 1 H), 1.45 (3s, 18 H).

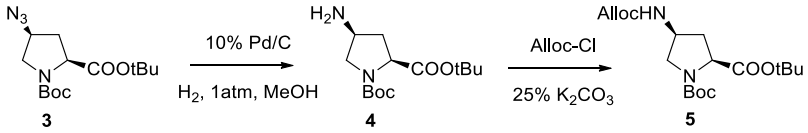
1.2 Synthesis of ***N-Boc-cis-3-azido-L-proline tert-butyl ester***



Compound 3 was synthesized according to previous method reported by Franca Zanardi *et. al.* [2]. A solution of DEAD (1.3 ml, 8.4 mmol) and DPPA (1.8 mL, 8.4 mmol) in dry THF (16 mL) was added dropwise over 30 min to a solution of proline **2** (0.8 g, 2.8mmol) and PPh_3 (2.2 g, 8.4mmol) in dry THF (24 mL) at 0 °C under argon. The mixture was stirred for 24 h at room temperature. After addition of EtOH (10 mL), the solvent was concentrated to dryness in vacuo. The residue was purified by silica gel flash chromatography (hexanes/EtOAc 20:1 to 10:1) to afford azidoproline **3** (0.75 g, 85.8%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) major rotamer: δ = 4.15-4.09 (m, 2 H), 3.42 (m, 1 H), 3.39 (m, 1 H), 2.35-2.43 (m, 1 H), 2.10 (m, 1 H), 1.41-1.37 (m, 18 H).

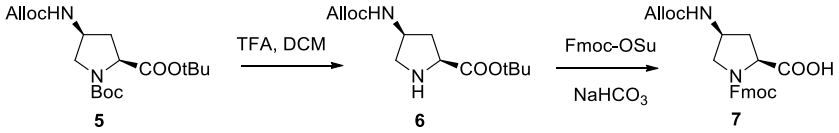
1.3 Synthesis of ***(2S,4S)-di-tert-butyl 4-((((allyloxy)carbonyl)amino)pyrrolidine-1,2-dicarboxylate***



To a solution of **1** (1 g, 3.8 mmol) in MeOH was added 10% Pd/C (90mg) and the suspension was stirred under balloon pressure of H_2 for 5 h. TLC indicated complete consumption of starting material and the reaction mixture was filtered through Celite. The filtrate was concentrated and crude product **4** was used for the next step without further purification.

To a solution of **4** in 1,4-dioxane was added 25% K_2CO_3(1.5 ml) and the suspension was stirred under nitrogen at pH 8. Then Alloc-Cl (372 µl, 3.5 mmol) was added dropwise into the suspension at 0 °C under nitrogen. The mixture was stirred for 24 h at room temperature. The reaction was concentrated in vacuo, then taken up in EtOAc, washed with brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was used without further purification.

1.3 Synthesis of ***(2S, 4S)-Alloc-4-amino-1-Fmoc-pyrrolidine-2-carboxylic acid***

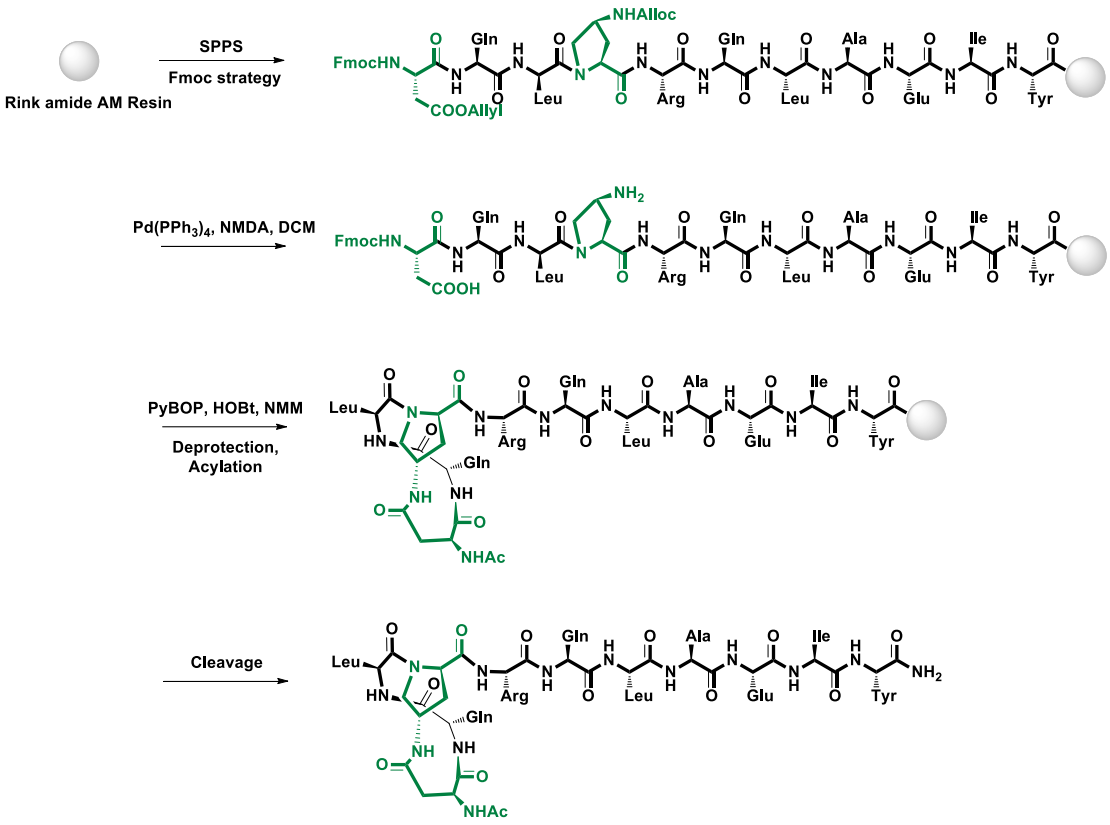


Compound **5** (1.2 g, 3.2 mmol) was dissolved in CH_2Cl_2 (30 mL) and TFA (30 ml) was added dropwise at 0 °C. The mixture was stirred for 3 h. After consumption of starting material, the mixture was concentrated in vacuo and washed with diethyl ether 3 times to afford compound **6** without further purification.

Compound **6** was dissolved in water (20 ml), and adjusted the pH of the mixture to pH 8 with $NaHCO_3$.The CH_3CN solution of Fmoc-OSu (1.6 g, 4.7 mmol) was added dropwise to the mixture at 0 °C. The reaction was stirred for 24 h at room temperature. CH_3CN was removed under reduced pressure and aqueous solution was acidified with citric acid solid. The product was extracted with EtOAc and the combined EtOAc layer was dried over anhydrous MgSO4. The product was purified by flash column chromatography (SiO2, Petroleum ether/EtOAc 2:1) to product **7**. ^1H NMR (DMSO-d6, 400MHz): δ = 7.90(m, 2 H), 7.65 (m, 3 H), 7.38 (m, 2 H), 7.29 (m, 2 H), 5.86 (m, 1 H), 5.21 (d, 1 H), 5.15 (d, 1 H), 4.41(m, 2 H), 4.22-4.08 (m, 5 H), 3.71 (m, 1 H), 3.55 (m, 1 H), 2.51 (m,1 H), 2.08 (m, 1 H).

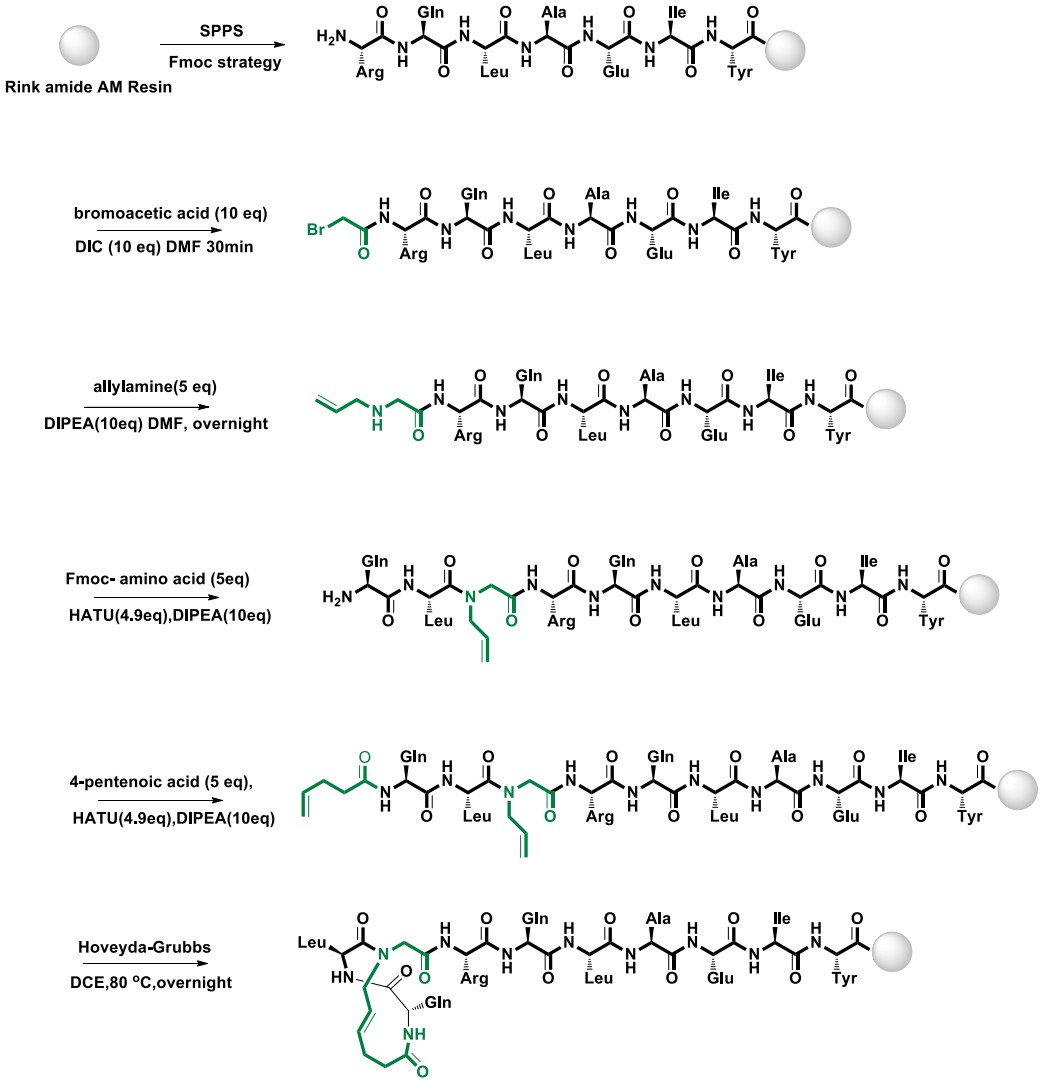
2. Represent example for synthesis of proline-capped peptides

2.1 Synthesis of Bak^{pro}



SPPS was performed by standard protocol mentioned in general information. Upon completion of peptide assembly, Allyl and Alloc protecting groups were deprotected by $Pd(PPh_3)_4$ (0.05 eq) and NMDA (4.0 eq) dissolved in DCM . Then peptide cyclization was performed by PyBOP (2.4 eq), HOBt (2.4 eq) and NMM (4.0 eq) in DMF. Peptides were cleaved by TFA/TIS/H_2O(95:2.5:2.5) and precipitated in diethyl ether. After that, the resulting residue was then dried in vacuo and dissolved in 25% (vol/vol) CH_3CN/H_2O. Crude peptides were purified on reverse phase-high performance liquid chromatography (RP-HPLC) confirmed by LC-MS (SHIMAZU LC-MS 8030, ESI-MS).

2.2 Synthesis of Bak^{HBS}



HBS-derived peptides were synthesized according to the previous method reported by the Arora *et. al.*[4]. Fmoc amino acids (and 4-petenoic acid) (5 equiv) were activated by HCTU(4.9 equiv), DIPEA(10 equiv) in DMF. Fmoc were deprotected by treatment with 50% (vol/vol) morpholine in NMP for 30min × 2. The bis-olefin peptide containing resin was thoroughly washed with DMF and DCM respectively, and dried under vacuum overnight. Then the resin was placed in a round flask with 20 mol% of Hoveyda Grubbs II catalyst. To this flask was added 2 ml of freshly distilled dichloroethane, then purged with nitrogen and the resin allowed to swell for 10 min. All reactions were carried out at 80°C overnight. Then the resin was washed with DMF (5x) and DCM (5x), dried under vacuum. Peptides were cleaved with TFA/TIS/H_2O(95:2.5:2.5) and precipitated in diethyl ether. The resulting residue was then dried in vacuo and dissolved in 25% (vol/vol) CH_3CN/H_2O. Crude peptides were purified on reverse phase-high performance liquid chromatography (RP-HPLC) confirmed by LC-MS (SHIMAZU LC-MS 8030, ESI-MS).

3. Circular Dichroism Spectroscopy (CD)

CD spectra were detected by Chirascan Plus Circular Dichroism Spectrometer (Applied photophysics) at 25 ºC . Peptides were dissolved in 300 µl ddH_2O at a final concentration of 0.14 mM. Parameters used in the experiment are as followed: wavelengths from 250 to 190 nm were measured with resolution of 0.5 nm at a scan speed of 20 nm/sec. Each sample was scanned twice and the averaged spectrum was smoothed using Pro-Data Viewer by Applied Photophysics with smooth window of 10. CD data are presented as mean residual elipticity [θ] in deg•cm^2 •$dmol^{-1}$. Concentrations were determined by their UV absorbance of Tyrosine at 280 nm reported by Gregory L Verdine [3]. Concentration of each sample: absorption (OD) at 280 nm × dilution factor ÷1 (cm) ÷ 1490 ($1cm^{-1}M^{-1}$) ×1000 (mMM^{-1})= concentration (mM). Percent helicity was calculated based on the equation described by Arora[5]: Helicity% = $[\theta]_{222}/[\theta]_{max}\times100$, where $[\theta]_{max} = (-44\,000 + 250T)(1 - k/n)$ for k = 4.0 and n = number of amino acid residues in the peptide (including linker), T = 20 ˚C.

3. 2-D NMR Experiments

Sample was prepared by dissolving dried peptide in phosphate buffer (pH 5.0) containing 20% TFE-d3 at 298K. All NMR data were recorded on a Bruker Avance III 600 MHz spectrometer equiped with a TXI probe. Water suppression was achieved using watergate pulse sequence with gradients in 1D and 2D 1H spectrum. The mixing time was 80 ms in 2D 1H-1H TOCSY and 350 ms in 2D 1H-1H ROESY. All the 2D 1H-1H spectra were acquired with a 10 ppm spectra width and 2048×256 complex points. 2D 1H-13C HSQC and HMBC spectra were acquired with 1024×400 complex points, and the spectra width of 10 ppm for 1H and 100 ppm for 13C. All the 2D NMR spectra were processed to final 2048×1024 complex points by TopSpin® , and analyzed by CCPNMR software. 3J(NH-Hα) couplings were extracted from 1D 1H spectrum. 2D TOCSY spectra were used to measured temperature dependence for amide NH chemical shifts.The TOCSY spectra were recorded at temperature ranges from 288K to 313K with a 5K interva and a equilibration time of 15 min. The final amide NH chemical shifts were calibrated with standard 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS).

5. Stability Assays

1). Guanidine·HCl denaturation: Dried peptides were dissolved in ddH_2O. Then the peptide stocks were equally divided and lyophilized. Each sample was added to 0.5 M, 1 M, 2 M, 3 M guanidine·HCl to a final peptide concentration of 145 µM. The samples were then incubated overnight at room temperature before CD spectra were carried out as described above.

2). Thermo denaturation: Temperature experiments were carried out by Chirascan Plus Circular Dichroism Spectrometer equipped with a temperature controller. The temperature varied from 25˚C to 85˚C in 5˚C increments with a rate of 1 ˚C /min, thus desired temperatures were 25 ˚C, 35 ˚C, 45 ˚C, 55 ˚C, 65 ˚C, 75 ˚C. CD spectra were automatically recorded at every desired temperature. The data were processed as general method mentioned above.

6. TFE Titration Experiment

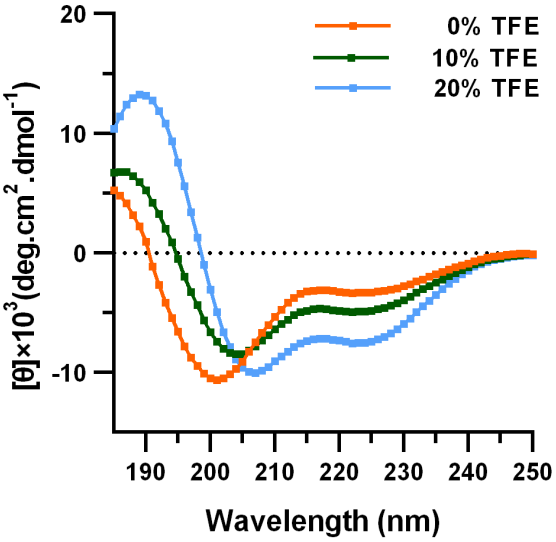
The dried peptides were dissolved in ddH_2O. The peptide stocks were equally divided to 50 µl per tube. Each sample was added to 0%, 10%, 20% aqueous TFE (a helix inducing solvent) at 25 ˚C.

Tables and Figures

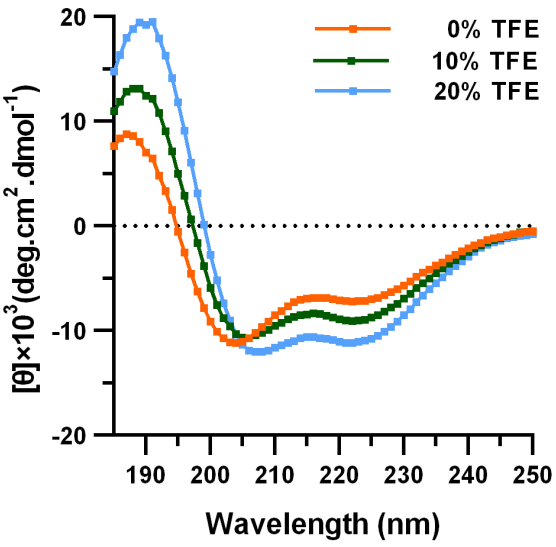
Supporting Table S1: Peptide sequences and MS data. Calculated and Found m/z are presented as $[M+1H]^{1+}$ / $[M/2+H]^{+}$ / $[M/3+H]^{+}$

Peptide	Sequence	Chemical Formula	Calculated m/z	Found m/z
Bak^{linear}	Ac-DQLPRQLAEIY-NH_2	$C_{62}H_{99}N_{17}O_{19}$	1385.73	1387.9
Bak^{pro}	D*QL*P**RQLAEIY-NH_2	$C_{62}H_{98}N_{18}O_{18}$	1382.73	1384.8
Bak^{HBS}	Z*QLG*RQLAEIY-NH_2	$C_{60}H_{98}N_{16}O_{15}$	1282.74	1284.0
EK^{linear}	Ac-DEKPYEKEEKEKKRKE-NH_2	$C_{92}H_{152}N_{26}O_{32}$	2134.11/1067.56/712.04/534.28	1067.8/712.4/534.6
EK^{pro}	D*EK*P**YEKEEKEKKRKE-NH_2	$C_{92}H_{151}N_{27}O_{31}$	2131.11/1066.06/711.04/533.53	1066.70/711.4/555.8
EK^{HBS}	Z*EKG*YEKEEKEKKRKE-NH_2	$C_{89}H_{147}N_{25}O_{29}$	2031.08/1016.04/677.69/508.52	1016.5/678/508.7
ER^{linear}	Ac-DILPRLLQY-NH_2	$C_{55}H_{90}N_{14}O_{14}$	1171.68/586.34	1171.8/586.7
ER^{pro}	D*IL*P**RLLQY-NH_2	$C_{55}H_{89}N_{15}O_{13}$	1168.68/584.84	1168.8/585.1
ER^{HBS}	Z*ILG*RLLQY-NH_2	$C_{52}H_{85}N_{13}O_{11}$	1068.65/534.83	1068.8/535.1
HIF^{linear}	Ac-DELPRALDQY-NH_2	$C_{55}H_{85}N_{15}O_{19}$	1260.61/630.81	1260.7/631.1
HIF^{pro}	D*EL*P**RALDQY-NH_2	$C_{55}H_{84}N_{16}O_{18}$	1257.61/629.31	1257.7/629.6
HIF^{HBS}	Z*ELG*RALDQY-NH_2	$C_{52}H_{80}N_{14}O_{16}$	1157.59/579.30	1157.7/579.5
ER$^{pro\text{-}2}$	Ac-D*RK(FAM)*P**ILRRLLQGW-NH_2	$C_{98}H_{139}N_{27}O_{22}$	1024.03/683.01/512.51	1024.5/683.5/513
ER$^{HBS\text{-}2}$	Z*RK(FAM)G*ILRRLLQGW-NH_2	$C_{95}H_{135}N_{25}O_{20}$	974.03/649.69/487.51	974.5/650.1/487.9

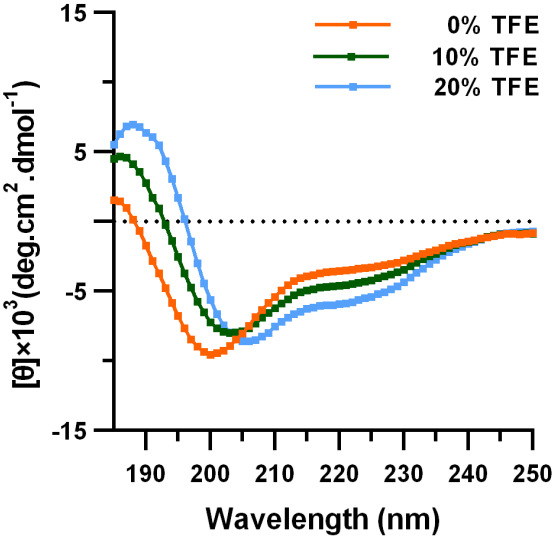
where Z*, G*, D*, *P** denote cyclizing amino acids



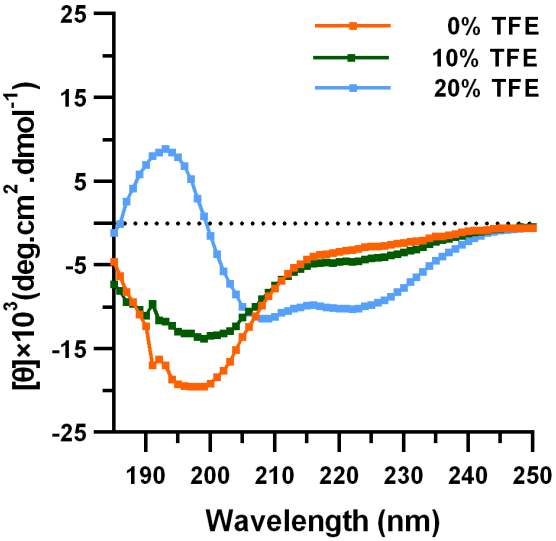
Supporting Figure S1: Circular Dichroism Spectra (CD) for **linear** EK peptide (Ac-DEKPYEKEEKEKKRKE-NH_2) in ddH_2O with increasing percentage of TFE at 25 °C



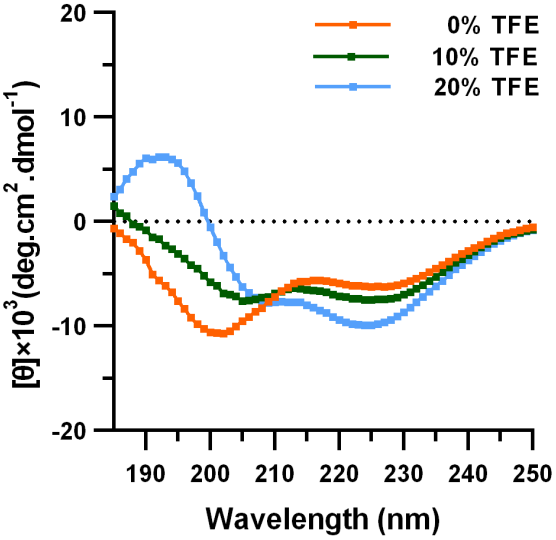
Supporting Figure S2: Circular Dichroism Spectra (CD) for **Proline-capped** EK (D*EK*P**YEKEEKEKKRKE-NH_2) peptide in ddH_2O with increasing percentage of TFE at 25 °C. (D* and *P** denote the cyclized amino acids.)



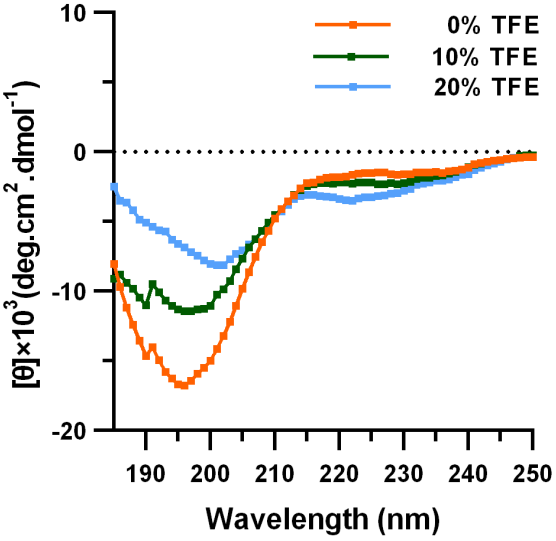
Supporting Figure S3: Circular Dichroism Spectra (CD) for **HBS-derived** EK peptide (Z*EKG*YEKEEKEKKRKE-NH_2) in ddH_2O with increasing percentage of TFE at 25 °C. (Z* and G* denote the cyclized amino acids.)



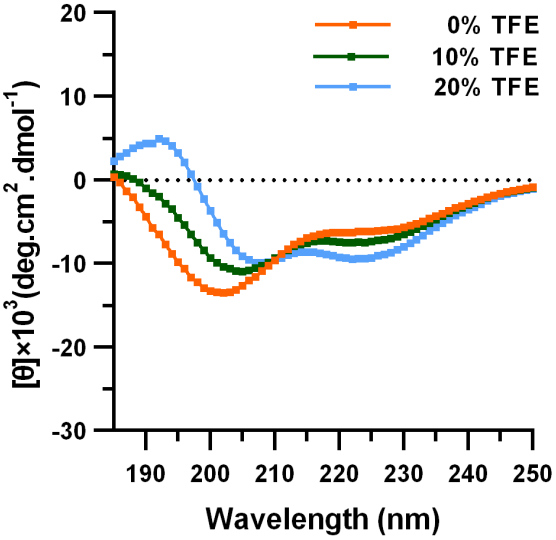
Supporting Figure S4: Circular Dichroism Spectra (CD) for **linear** ER peptide (Ac-DILPRLLQY-NH_2) in ddH_2O with increasing percentage of TFE at 25 °C



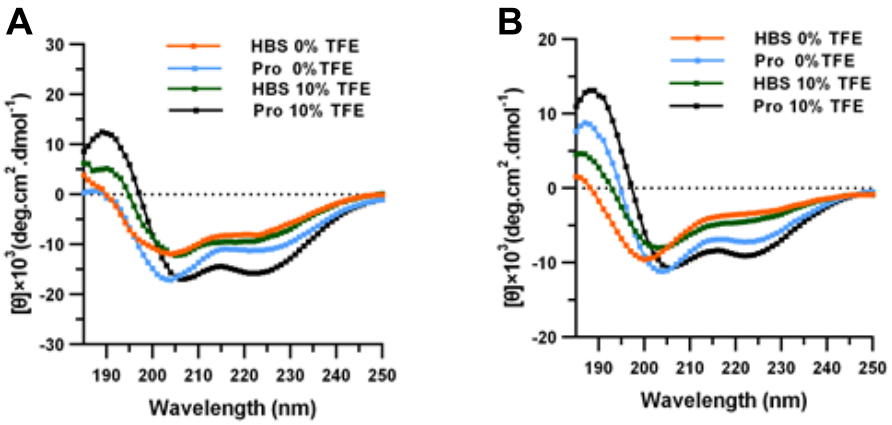
Supporting Figure S5: Circular Dichroism Spectra (CD) for **Proline-capped** ER peptide (D*IL*P**RLLQY -NH_2) in ddH_2O with increasing percentage of TFE at 25 °C. (D* and *P** denote the cyclized amino acids.)



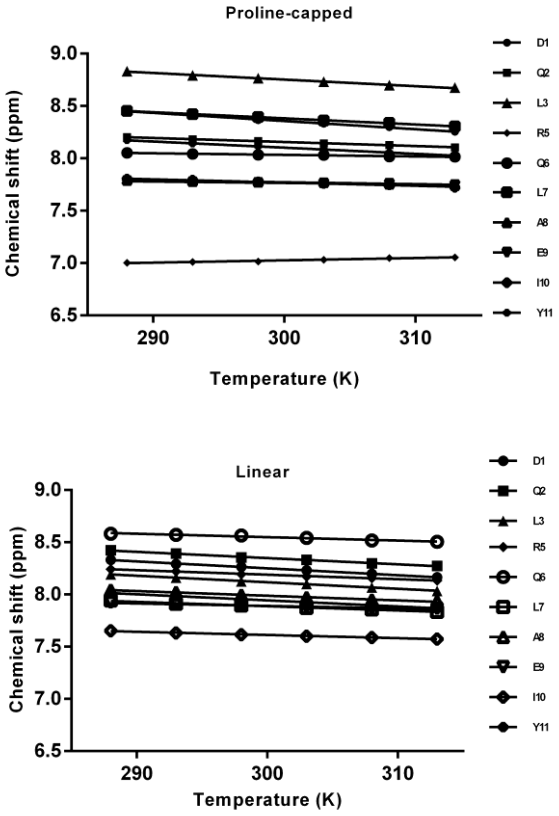
Supporting Figure S6: Circular Dichroism Spectra (CD) for **linear** HIF peptide (Ac-DELPRALDQY-NH_2) in ddH_2O with increasing percentage of TFE at 25 °C



Supporting Figure S7: Circular Dichroism Spectra (CD) for **Proline-capped** HIF peptide (D*EL*P**RALDQY-NH_2) in ddH_2O with increasing percentage of TFE at 25 °C. (D* and *P** denote the cyclized amino acids.)



Supporting Figure S8: Circular Dichroism Spectra (CD) for Proline-capped and HBS-derived Bak peptide (A) and EK peptide (B) in ddH_2O and 10% TFE at 25 °C. (Sequence: Bak (*X*QL*X*RQLAEIY) EK (*X*EK*X*YEKEEKEKKRKE-NH_2), where *X* denote the cyclized amino acids in different method.)

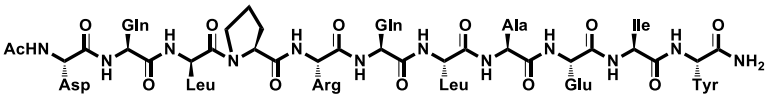


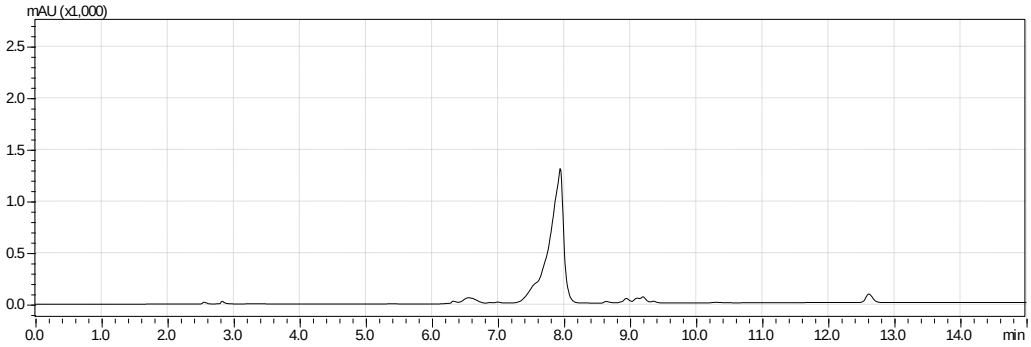
Supporting Figure S9: Temperature dependence for amide NH chemical shifts

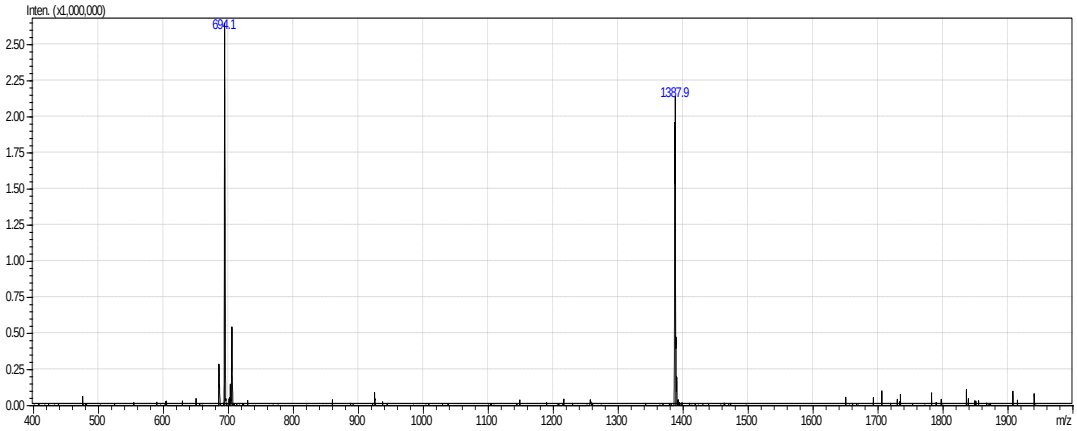
Appendix

HPLC Traces and MS Spectra

Bak^{linear} **Ac-DQLPRQLAEIY-NH$_2$**

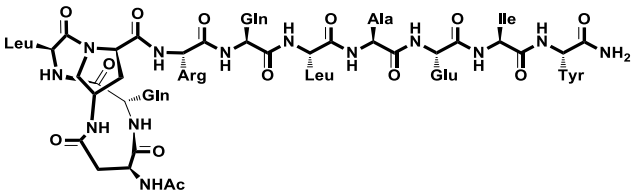


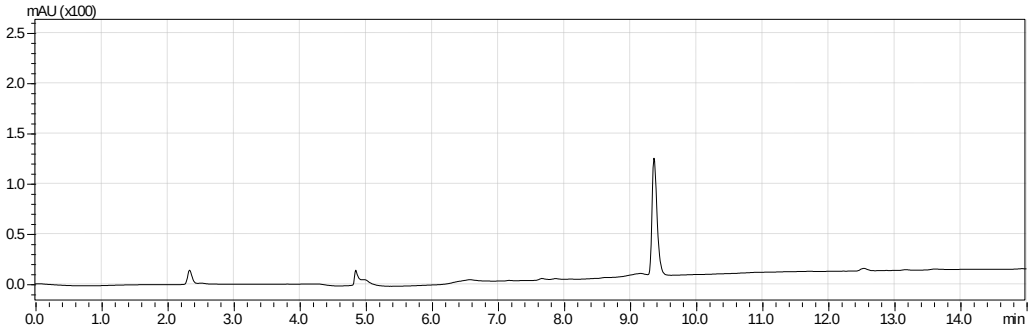


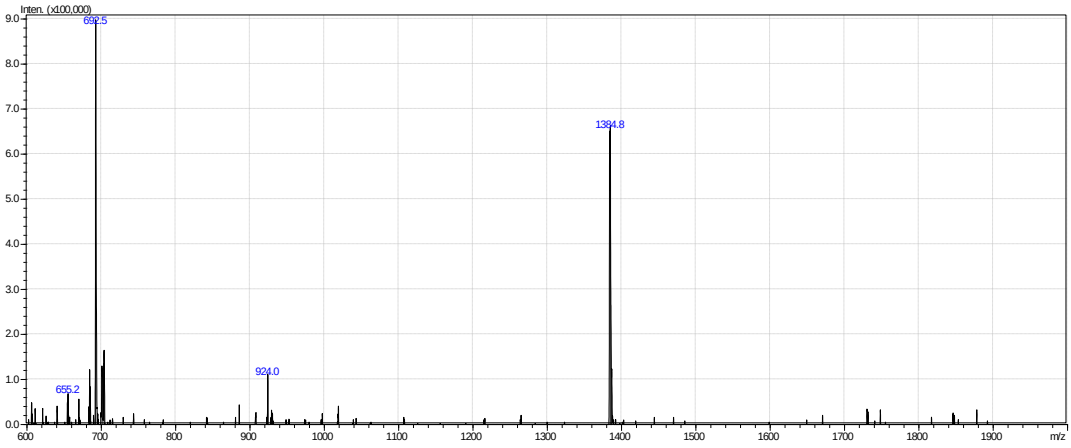


Bak^{pro}

D*QL*P*RQLAEIY-NH$_2$

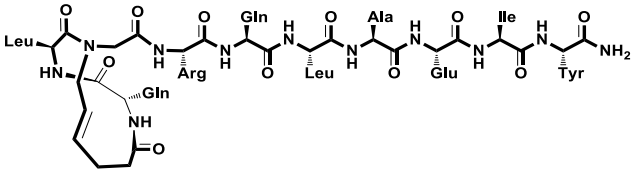


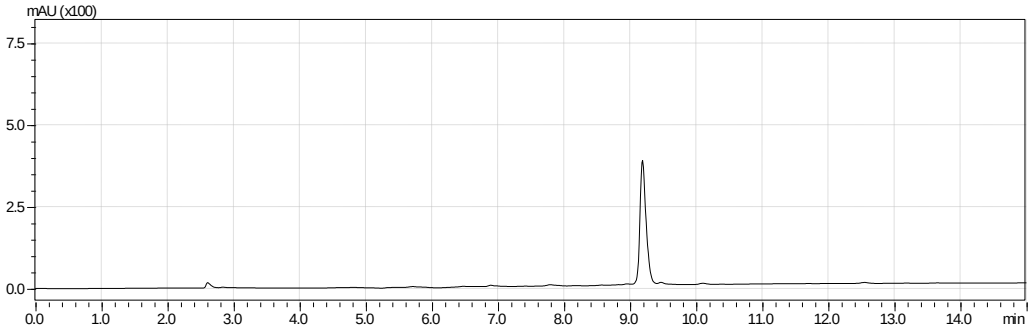


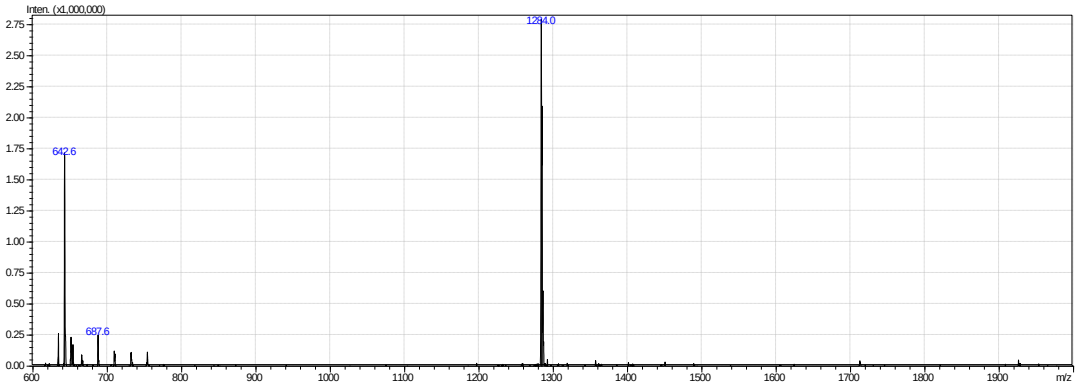


Bak^{HBS}

Z*QLG*RQLAEIY-NH$_2$

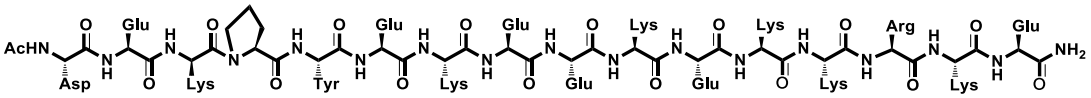


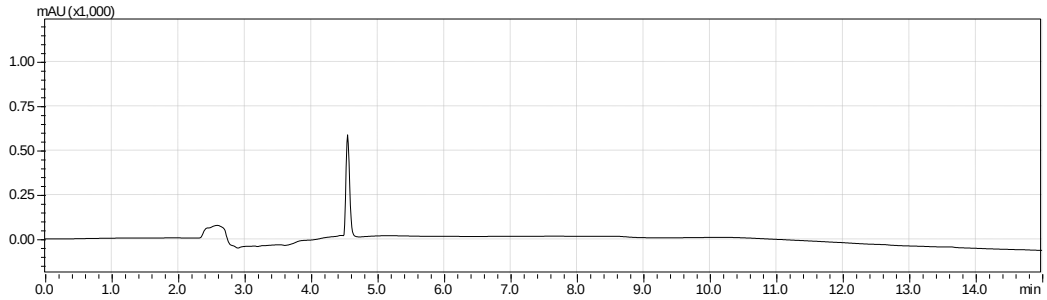


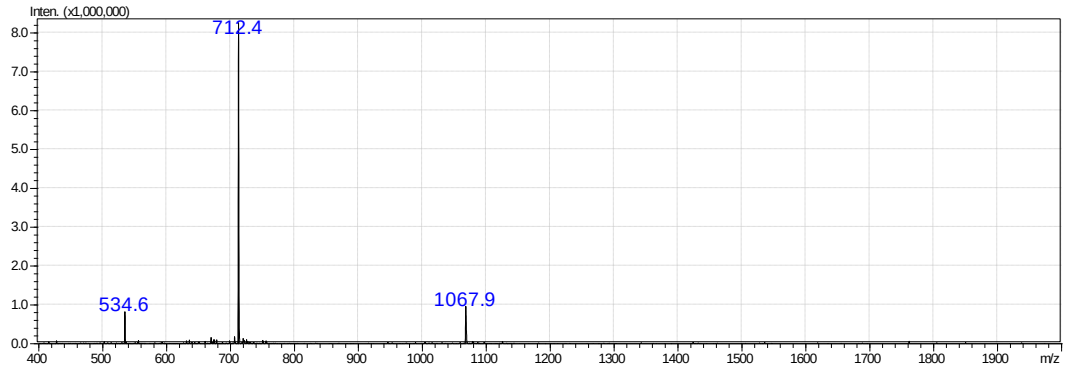


EK$^{\textbf{linear}}$

Ac-DEKPYEKEEKEKKRKE-NH$_2$

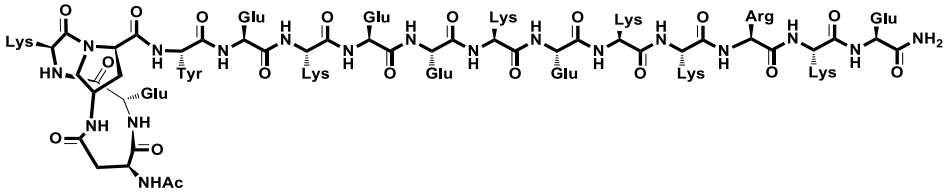


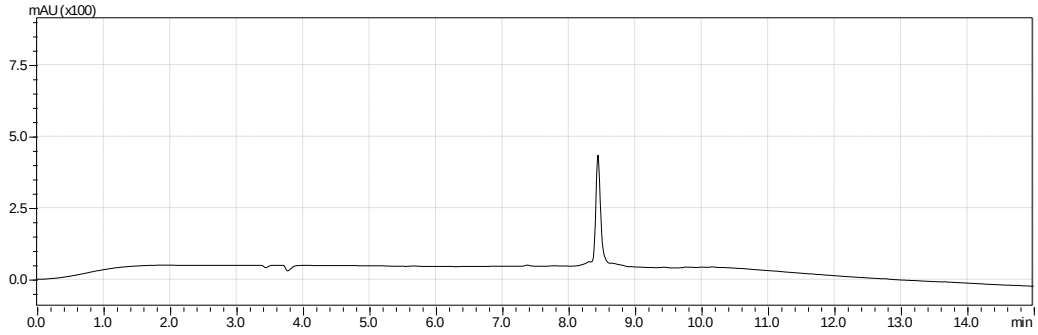


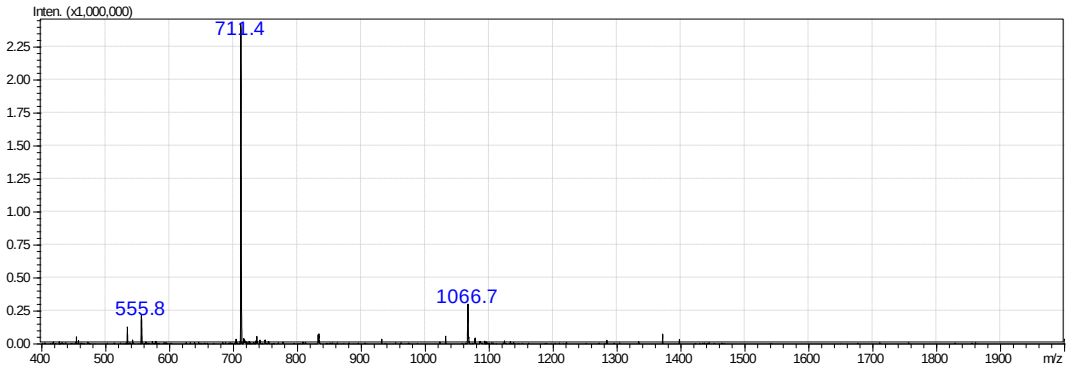


EK^{pro}

D*EK*P*YEKEEKEKKRKE-NH$_2$

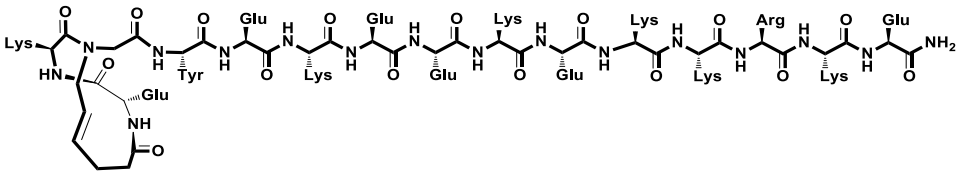


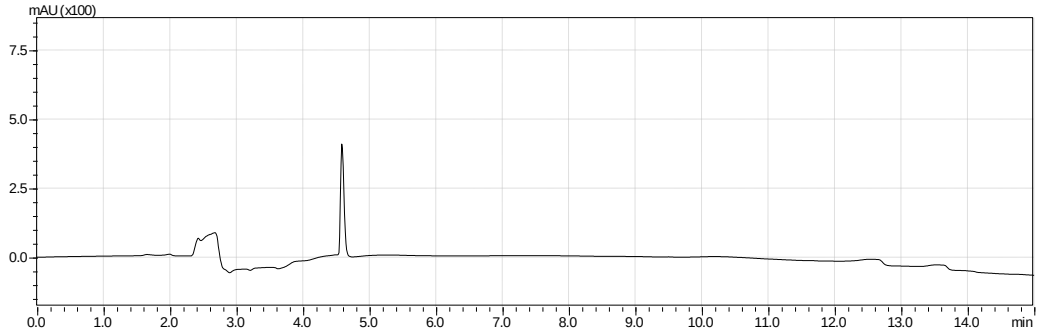


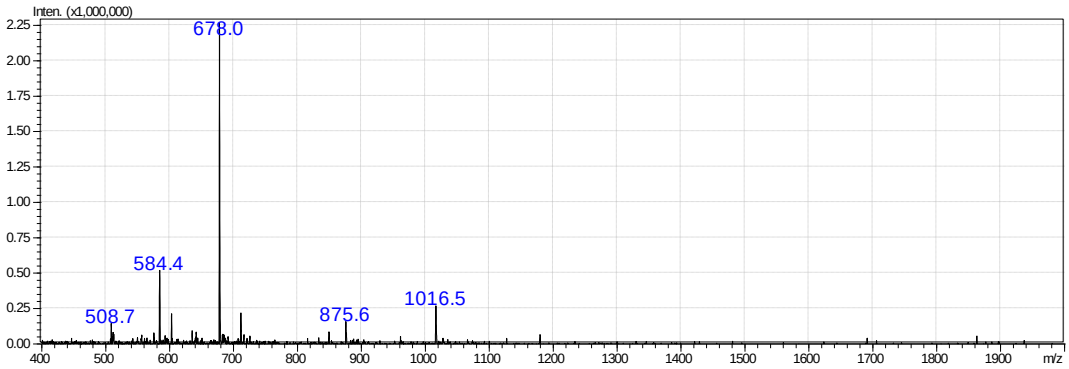


EK^{HBS}

Z*EKG*YEKEEKEKKRKE-NH_2

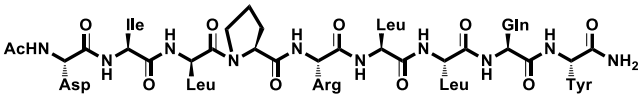


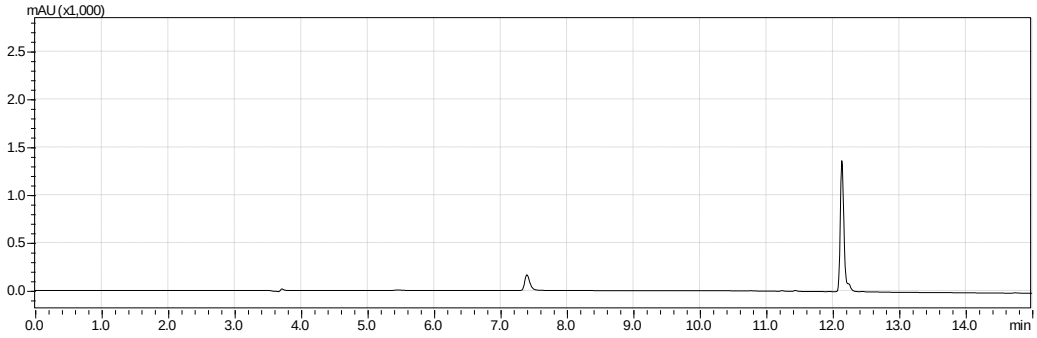


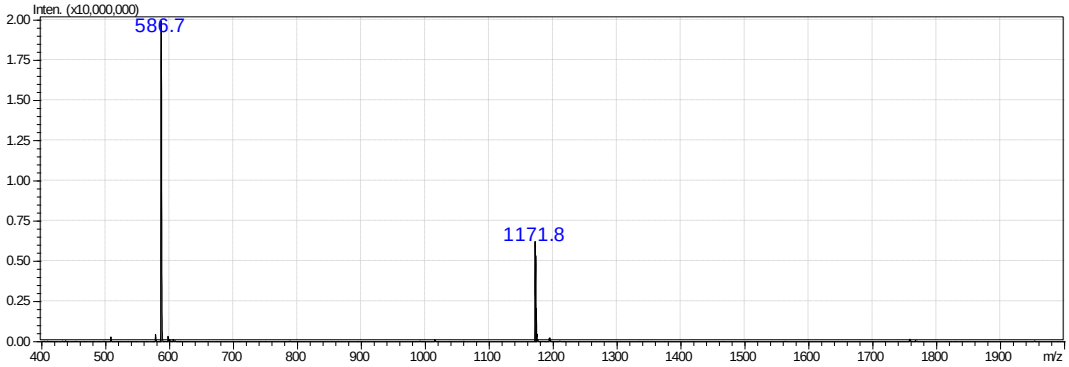


ER^{linear}

Ac-DILPRLLQY-NH$_2$

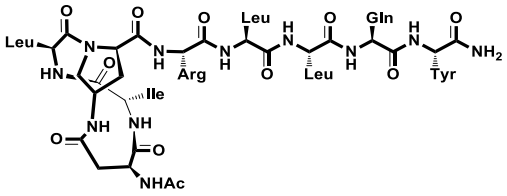


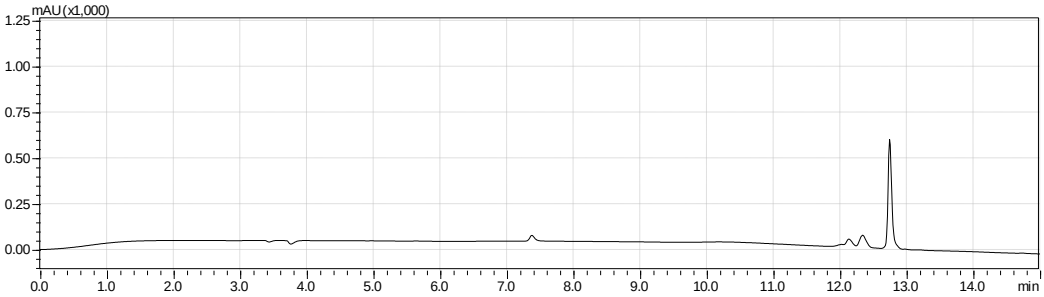


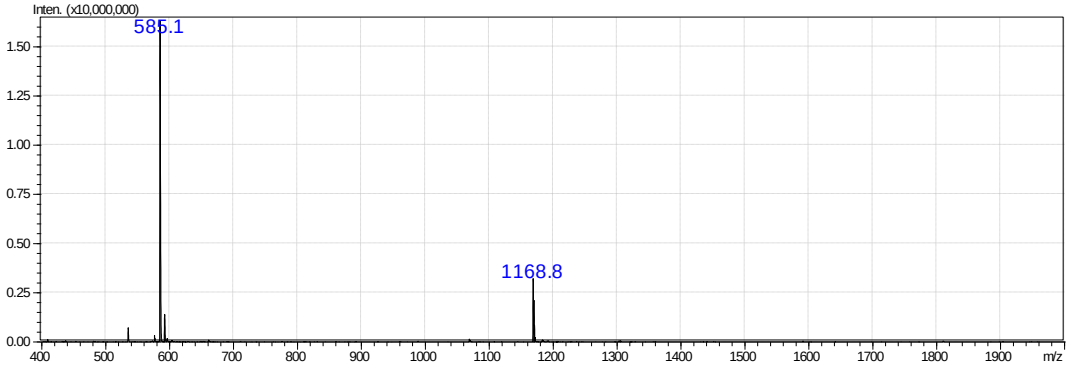


ER^{pro}

D*IL*PRLLQY-NH$_2$**

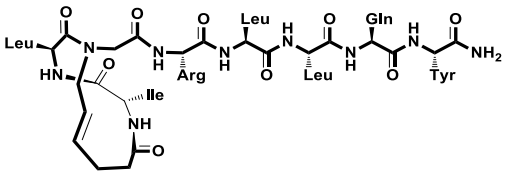


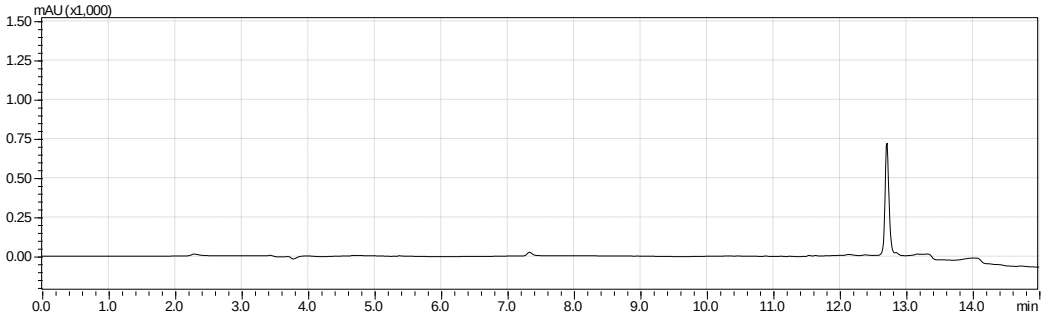


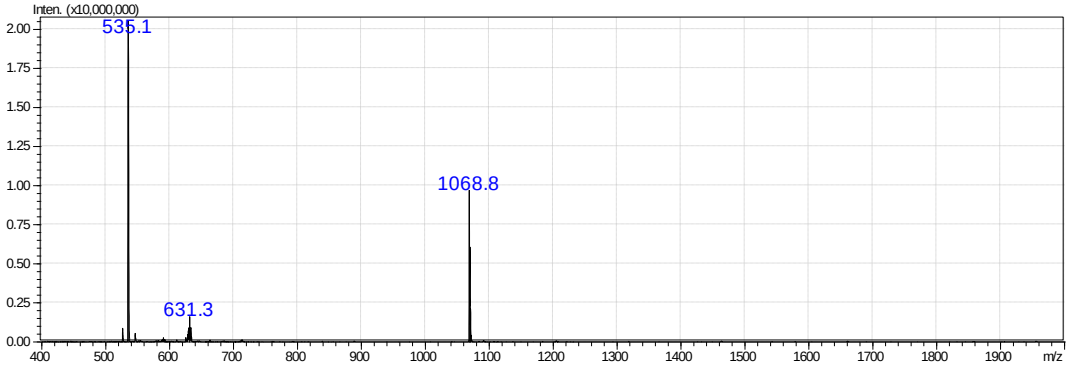


ER^{HBS}

Z*ILG*RLLQY-NH$_2$

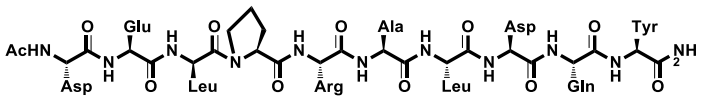


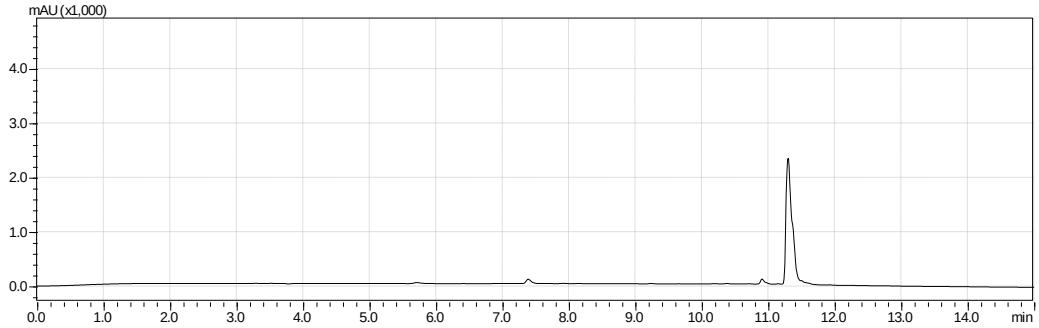


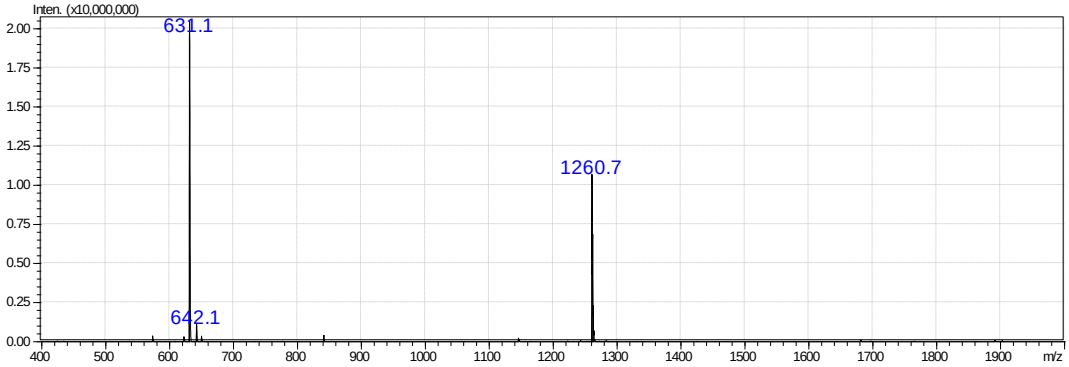


HIF^{linear}

Ac-DELPRALDQY-NH$_2$

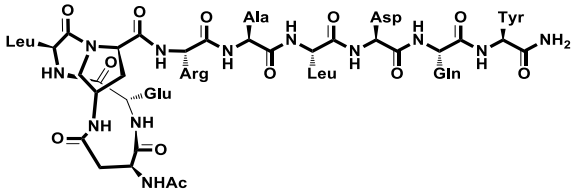


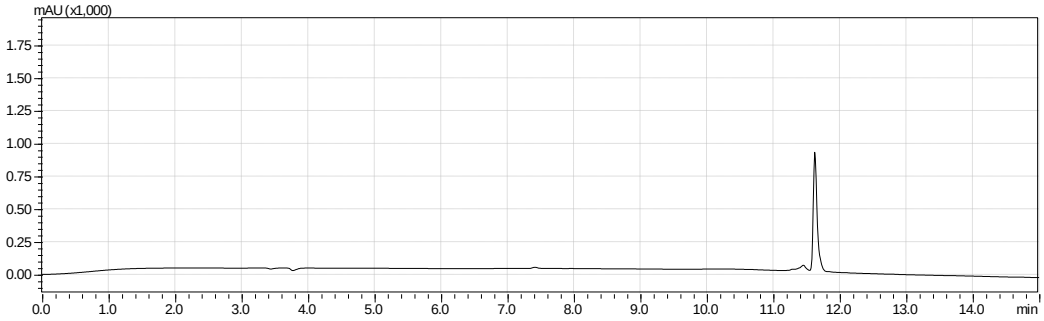


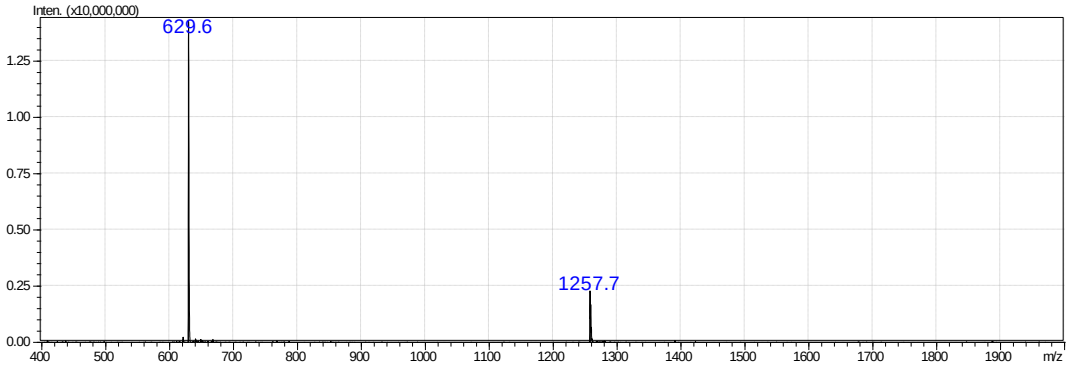


HIF^{pro}

D*EL*P*RALDQY-NH$_2$

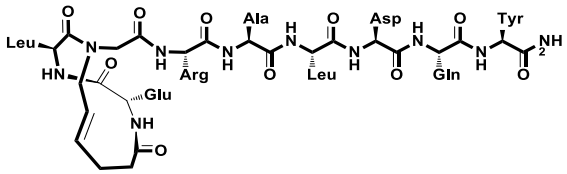


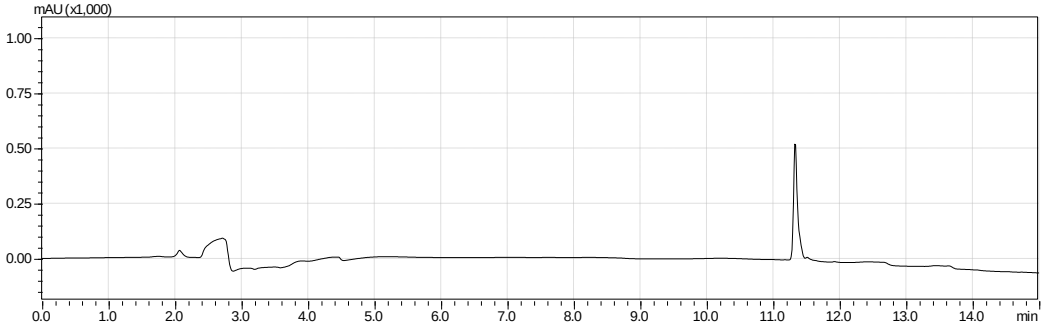


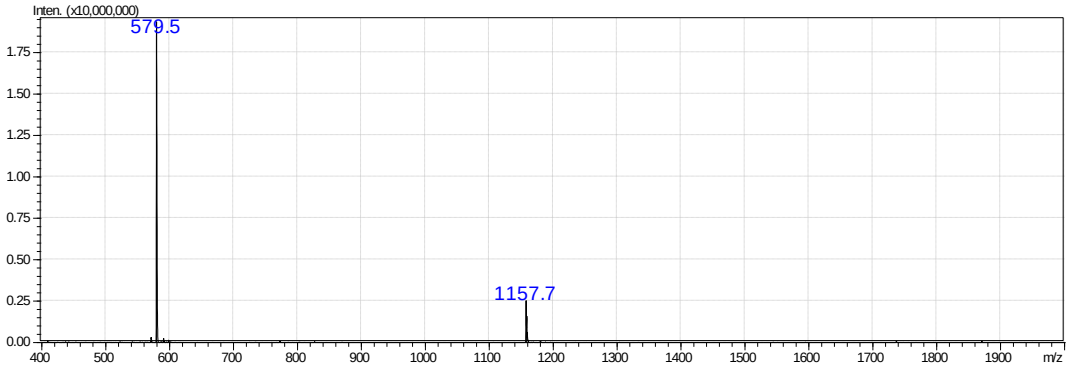


HIF^{HBS}

Z*ELG*RALDQY-NH_2

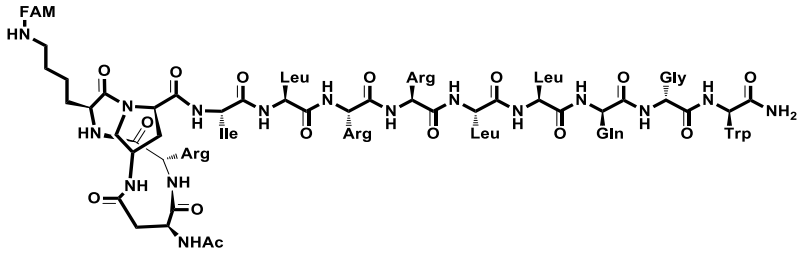


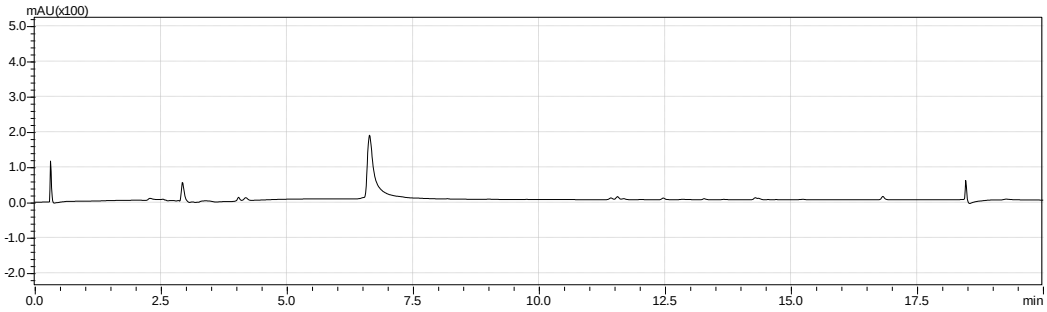


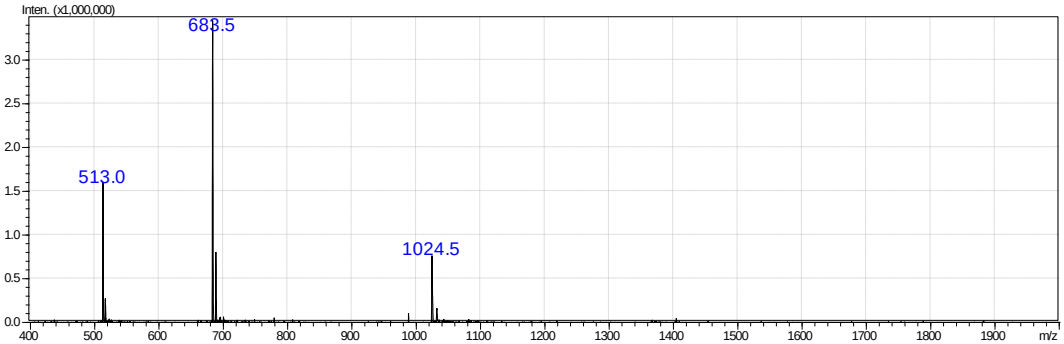


ER^{pro-2}

D*RK*PILRRLLQGW-NH$_2$**

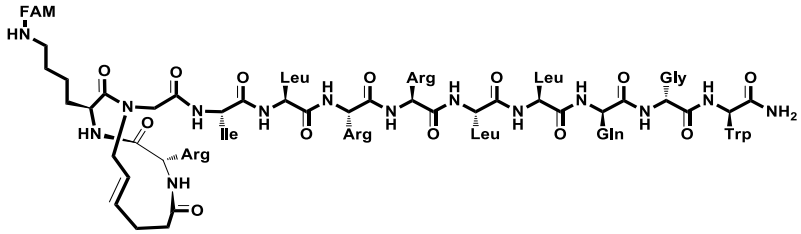


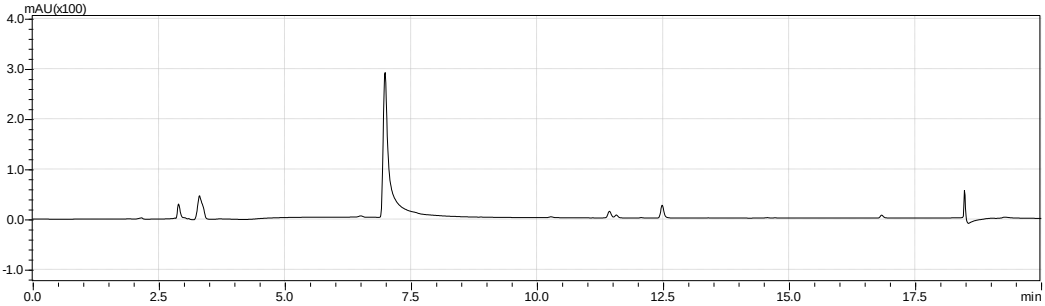


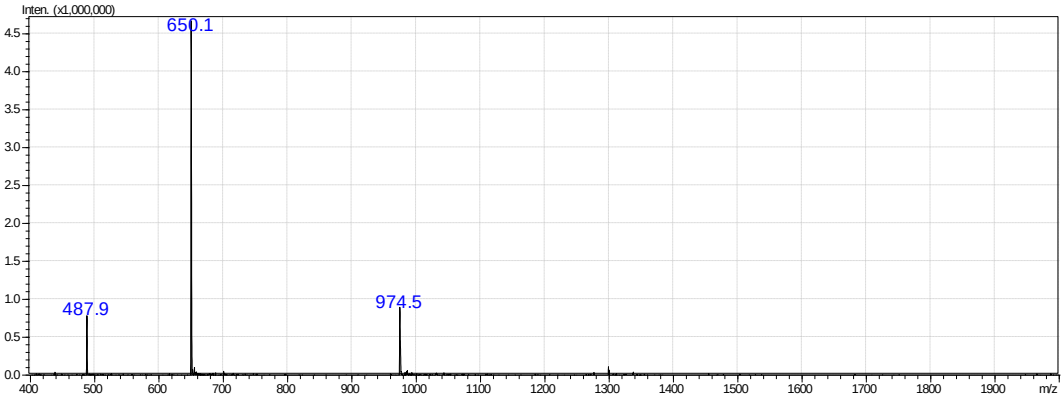


ER^{HBS-2}

Z*RKG*ILRRLLQGW-NH_2



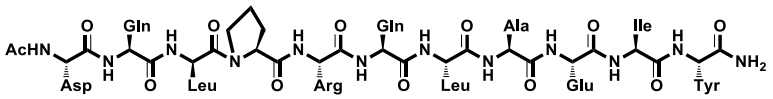


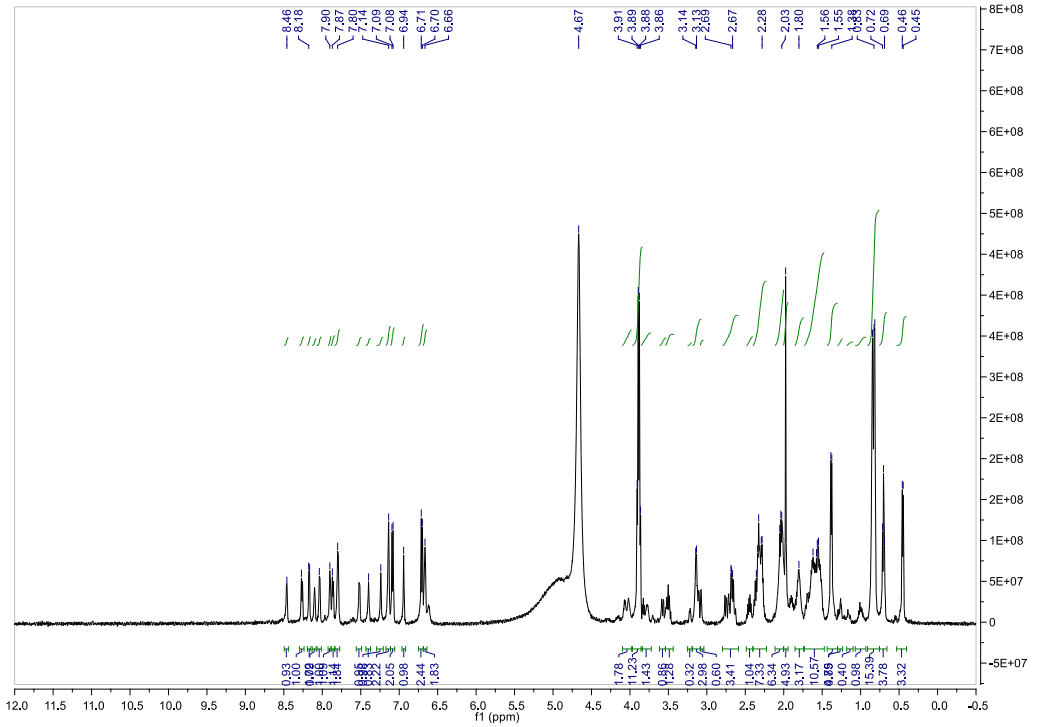


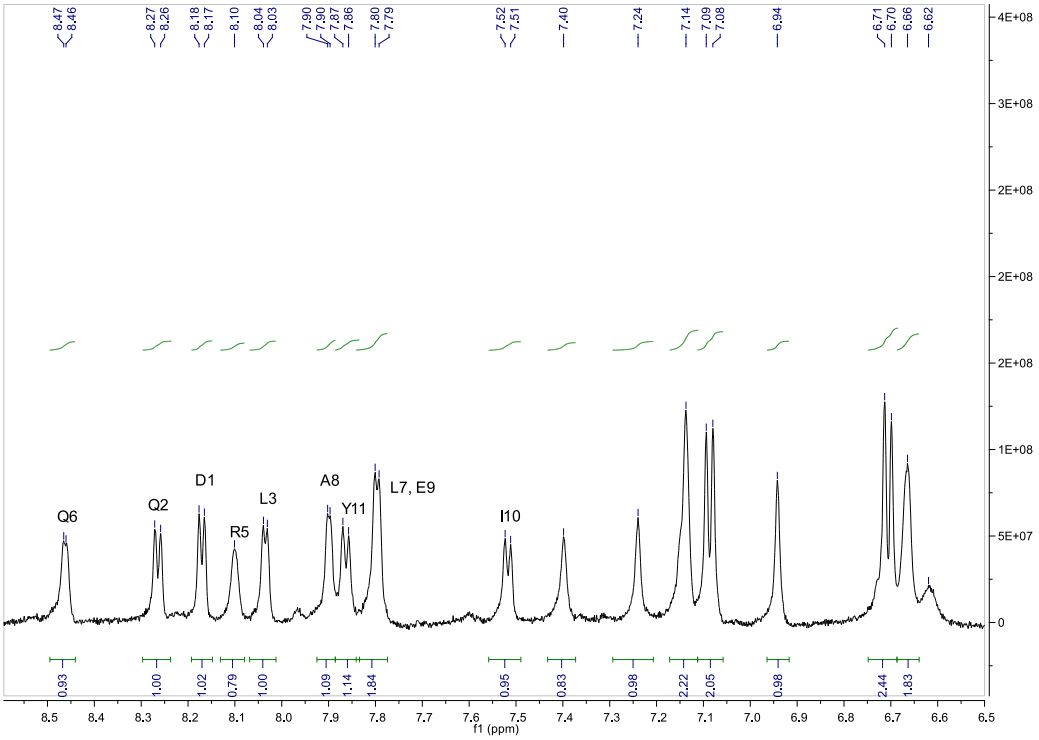
NMR Spectra

Bak^{linear}

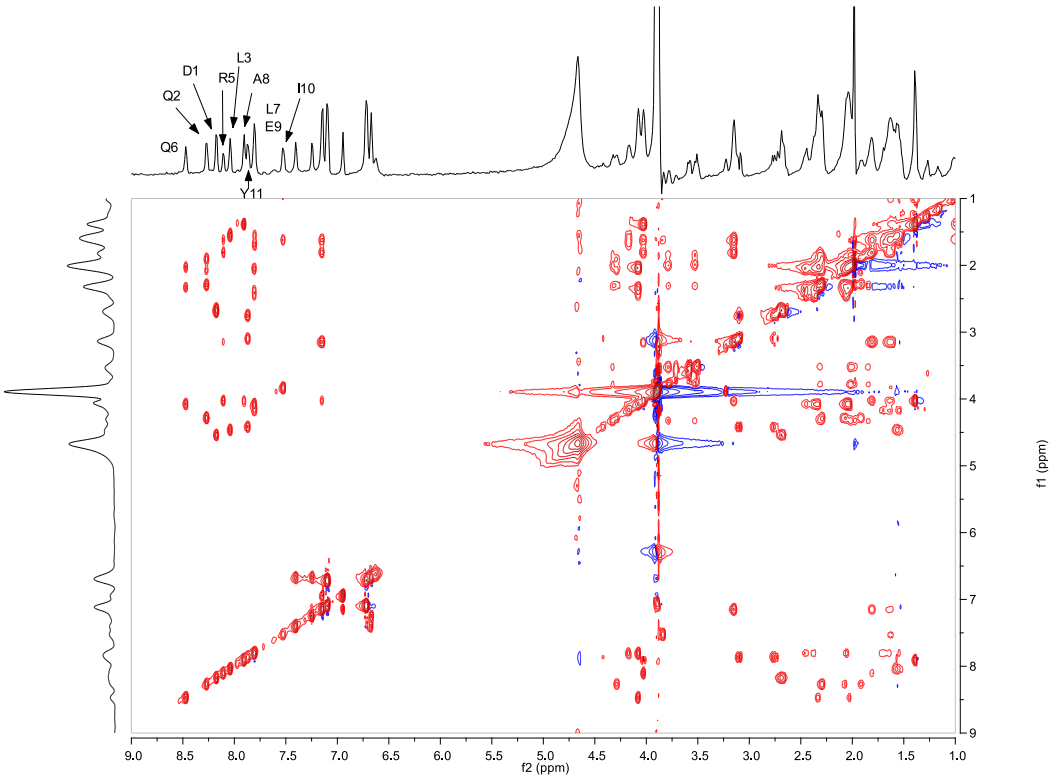
Ac-DQLPRQLAEIY-NH$_2$



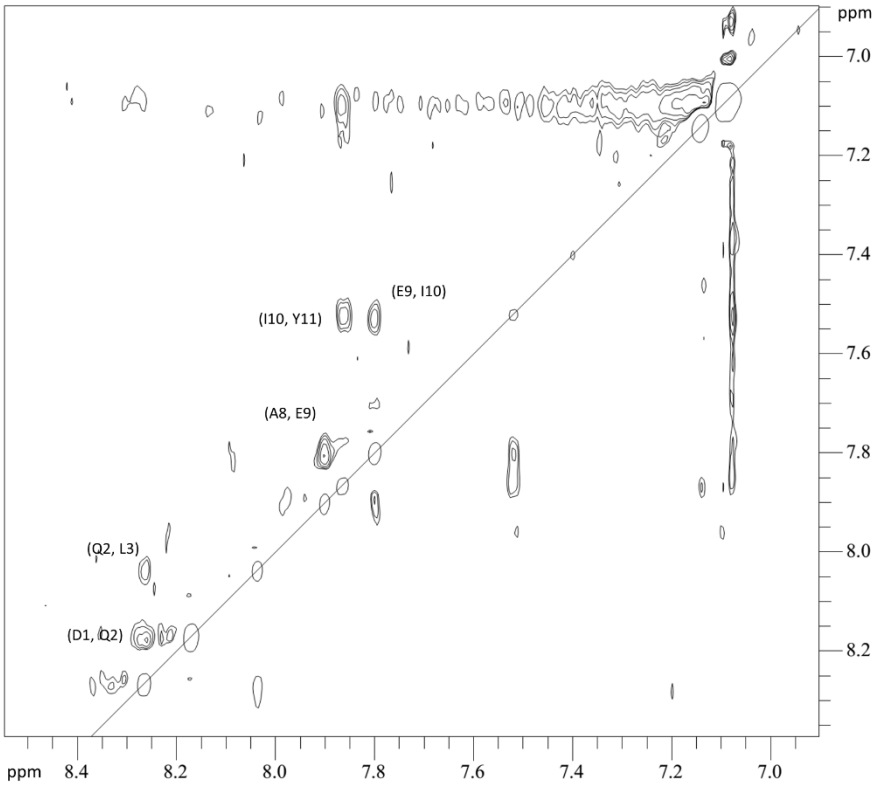




^{1}H NMR spectrum of **Bak^{linear}** (at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)

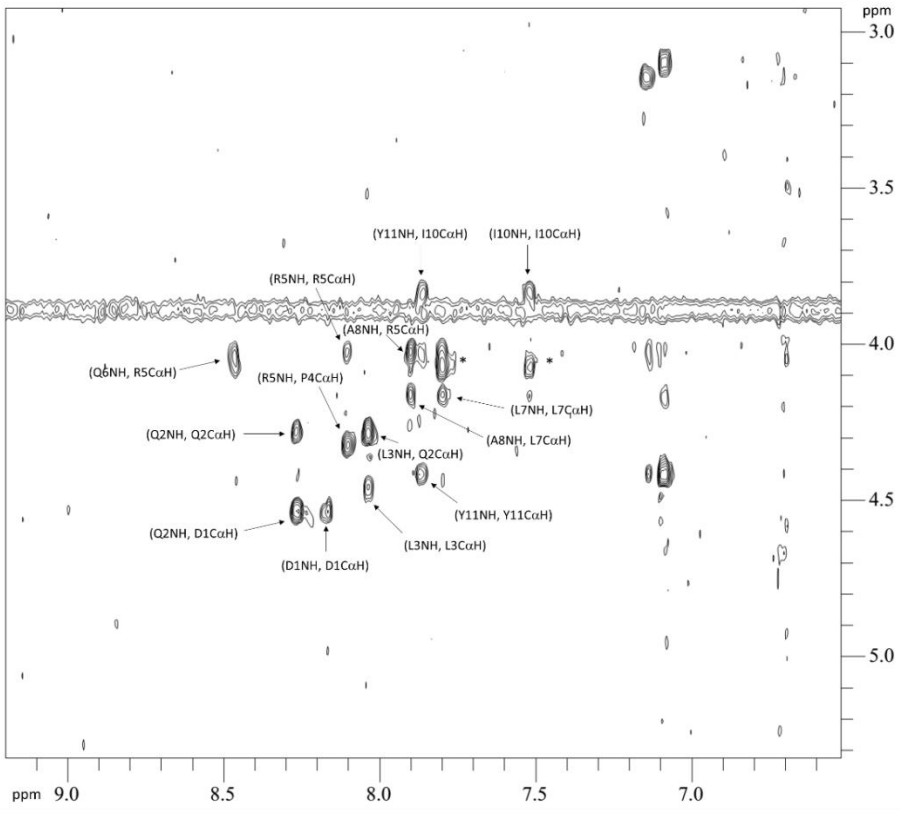


TOCSY spectrum of **Bak**$^{\textbf{linear}}$ (at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)



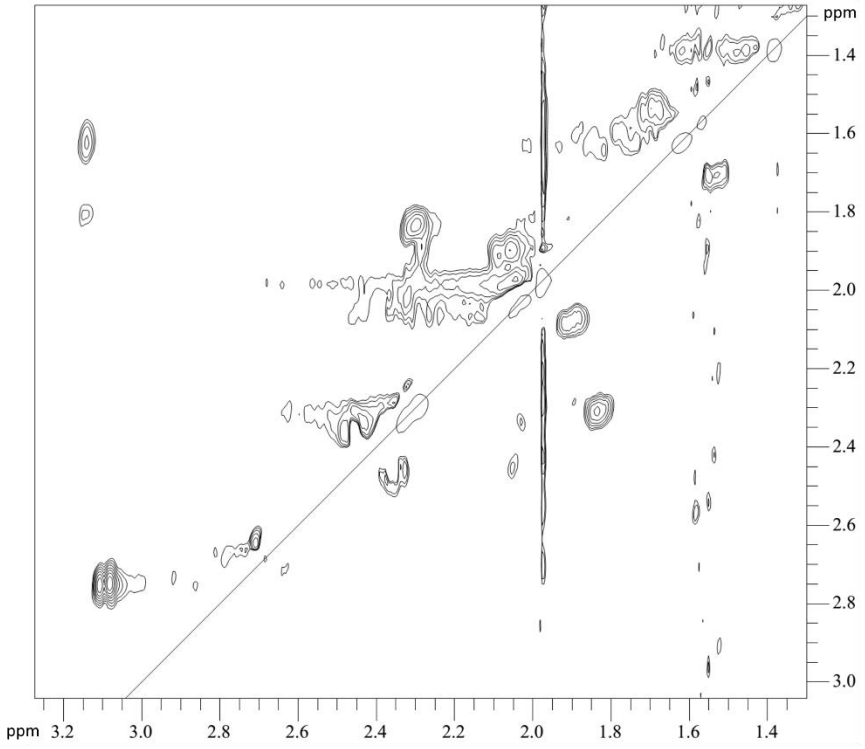
NH region of ROESY spectrum of **Bak**$^{\textbf{linear}}$

(at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)



NH-αCH region of 2D-ROESY spectrum of **Bak**$^{\textbf{linear}}$

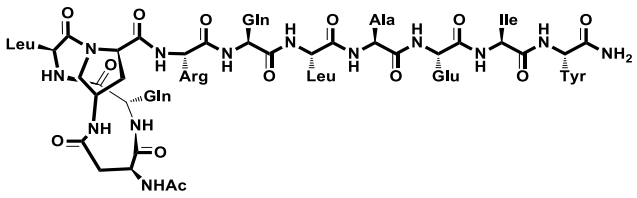
(at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K) Asterisk indicates peak overlap.

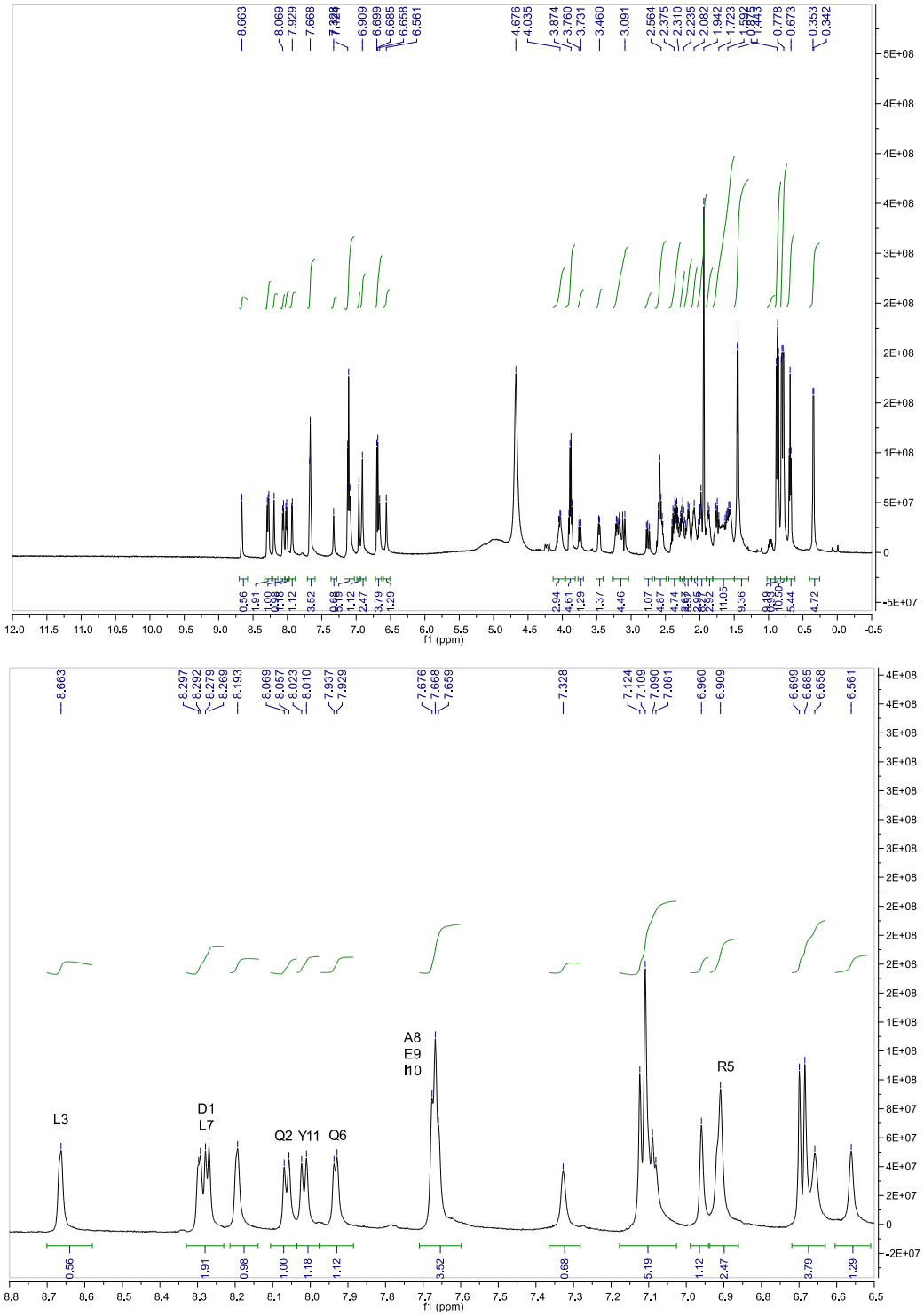


Aliphatic region of 2D-ROESY spectrum of **Bak**$^{\textbf{linear}}$

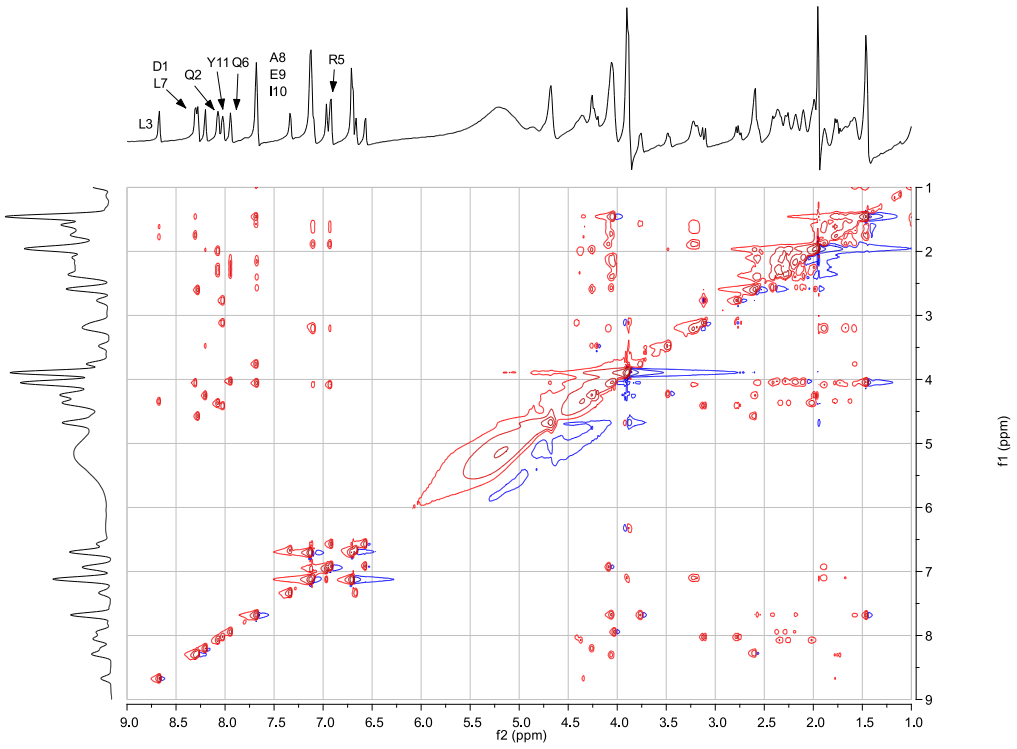
(at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)

Bak^{pro} **Ac-(cyclo-1, 3)-[DQLP*RQLAEIY]-NH$_2$**

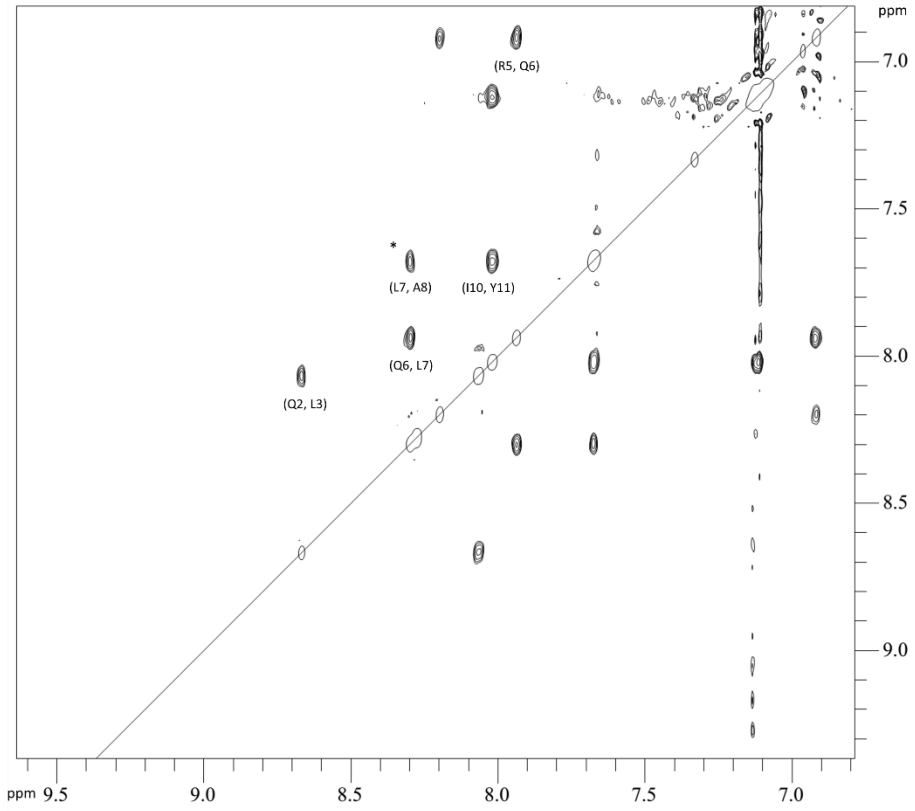




^{1}H NMR spectrum of **Bak^{pro}** (at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)

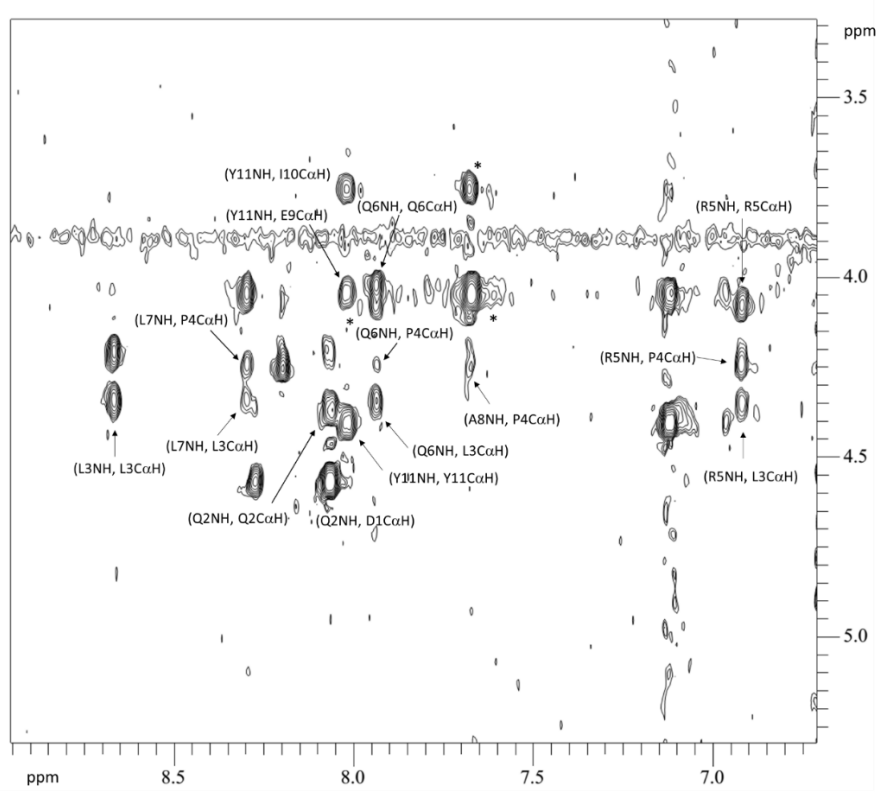


TOCSY spectrum of **Bak^{pro}** (at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)



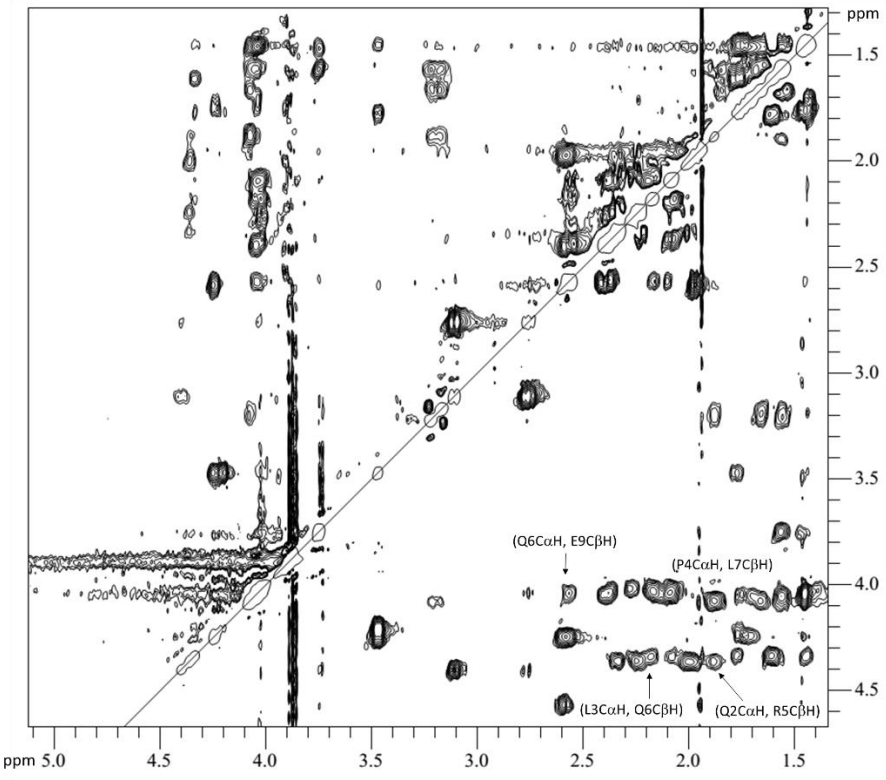
NH region of ROESY spectrum of **Bak^{pro}**

(at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)



NH-αCH region of 2D-ROESY spectrum of **Bak^{pro}**

(at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K) Asterisk indicates peak overlap.



Aliphatic region of 2D-ROESY spectrum of **Bak^{pro}**

(at 600 MHz in phosphate buffer (pH 5.0) with 20% TFE-*d3* at 298K)

Reference

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