Supporting Information

Stapling of leu-enkephalin analog with bifunctional reagent for prolonged analgesic activity

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Table content

1. General information

Purification of modified analog of Leu-enkephalin and stapled analogs of Leu-enkephalin (HPLC analysis): The products were purified by a preparative reversed-phase HPLC on a Vydac C18 column (22 mm × 250 mm), using solvent systems: S1: 0.1% aqueous TFA, S2: 40% acetonitrile + 0.1% TFA, linear gradient was individually set for each compound, flow rate 7.0 mL/min, UV detection at 210 nm. The fractions were collected and lyophilized. Their identities were confirmed by LC-UV and LC-MS.

LC-MS/MS analysis: *The LC-MS analysis was performed on Shimadzu LC IT-TOF. Separation was carried out on an RP-Zorbax (50×2.1 mm, 3.5 µm) column with a gradient elution of 0-50% B in A for compound Leu-Enk and 0-80% B in A for modified analog 2 (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at room temperature over a period of 15 min (flow rate: 0.1 mL/min).* The collision energy was selected from the range 25-35 eV to obtain the best sequence coverage. Analysis for 3a and 3b were performed on column ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm) using gradient elution of 1-70% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.2 ml/min); ESI-MS/MS analysis – positive ion mode).

HPLC-DAD analysis: The HPLC-DAD analysis was performed on UHPLC Nexera equipped with PDA detector. Separation was carried out on an RP-Zorbax (50×2.1 mm, 3.5 µm) column with a gradient elution of 0-50% B in A for compound Leu-Enk. and 0-80% B in A for modified analog 2 (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at room temperature over a period of 15 min (flow rate: 0.1 mL/min). Analysis for 3a and 3b were performed on column ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm) using gradient elution of 1-70% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.2 ml/min).

NMR spectroscopy: NMR spectra (^{1}H, ^{13}C, 2D) for non-modified and stapled analogs were recorded on a high-field spectrometer Bruker 500 MHz AVANAC III NMR (^{1}H 500 MHz, ^{13}C 125.75 MHz), equipped with a broadband inverse gradient probehead. Spectra were referenced to the residual solvent signal (DMSO-d6 2.50 ppm). Standard pulse programs from the Bruker library were used for homo- and heteronuclear 2D experiments. ^{19}F NMR spectra were recorded on spektrometr Jeol JNM-ECZ500R 500 MHz (liquid probe HFX, 5 mm, 1H/19F/2H/31P-109Ag, Z-grad, ATM) using gradients sequence through a standard program from the Bruker pulse sequence library.

Circular Dichroism (CD) Spectroscopy: The measurements were carried out using J-600 Circular Dichroism Spectrophotometer equipped with a temperature control accessory of cell holder under a constant nitrogen flow. The peptides secondary structure was measured with far-UV (190-260 nm) and 1 mm of cuvette path length. Peptides concentration were 50 µM. For each CD spectrum an average of 20 scans of the same sample was collected at 25°C with a step resolution of 0.2 nm, a scan speed of 50 nm per minute and a bandwidth of 1 nm. The data were processed by Spectra Manager Analysis software provided from JASCO as follows: the spectrum of each sample was corrected to baseline, smoothed with Savitsky-Golay filter and converted to molar ellipticity.

Theoretical calculation:

DFT calculations were performed with Gaussian 16 C.01[1] suite of programs using the ωB97X-D[2] long-range corrected hybrid density functional with damped atom-atom dispersion corrections was used with a triple-ζ 6-311G(2d,2p) basis set containing polarization functions. Molecular orbital studies on

3, 3a, 3b and Leu-Enk. compounds have been done on the DFT level of theory with IEFPCM (integral equation formalism for polarizable continuum model)[3] solvent (water) approach. The IEFPCM approximation describes a solvent as a homogeneous dielectric medium with electrical permeability (ε) equal to that of a pure solvent, and the cavity size is modelled for a solvent immersed molecule. The starting structure of the peptides for DFT calculations was generated on the basis of the amino acid sequence after 45 ps simulation at 300 K, without cutoffs using BIO+ implementation of CHARMM force field.

2. Experimental section

The derivatives of amino acids for peptide synthesis, Wang-Leu-Fmoc Resin (0.70 mmol/g), and the coupling reagent (TBTU - O-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate, PyBop - (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) were purchased from NovaBiochem. The solvents for peptide synthesis (analytical grade) were obtained from Riedel de Haen (DMF) and J. T. Baker (methanol, acetonitrile). LC-MS solvents (water, acetonitrile, methanol) were purchased from ChemSolve and J.T. Baker. Other reagents used in this work were obtained from Aldrich: triisopropylsilane (TIS), 1-Hydroxybenzotriazole (HOBt), N,N-diisopropylethylamine (DIEA), hexafluorobenzene, trithiocyanuric acid (TCA), triethylamine (TEA), benzyl bromide, 1 M TEAB buffer (1M triethylammonium biscarbonate in water), and IrisBiotech: trifluoroacetic acid (TFA).

2.1 Experimental procedure

2.1.1 Synthesis of H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-enkephalin)

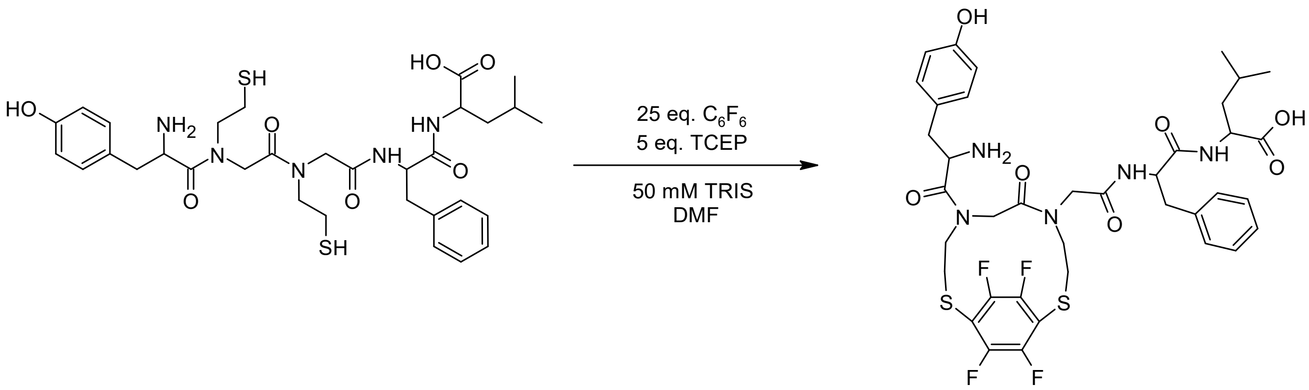
The peptide was synthesized manually on Wang-Leu-Fmoc Resin (loaded 0.7 mmol/g) according to Fmoc protocol ultrasonic agitation developed by Wołczański et al.[4] using DIEA (N,N diisopropylethylamine) (6 equiv.) and TBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate) (3 equiv.) as a coupling reagent. The progress of the coupling reaction was monitored by Kaiser test after the each step of the synthesis. After the synthesis of the whole peptide sequence the Fmoc protecting group was removed in the 25% of piperidine in DMF for 3 min in ultrasonic bath, than the peptidylresin was washed with DMF (7 x 1 min), DCM (3 x 1 min) and MeOH (3 x 1 min) and dried. Peptide was cleaved from resin with TFA : H_2O : TIS (triisopropylsilane) (95:2.5:2.5) mixture. Obtained product was purified and analyzed by LC-MS/MS.

2.1.2 Synthesis of H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1)

The peptide was synthesized manually on Wang-Leu-Fmoc Resin (loaded 0.7 mmol/g) according to Fmoc protocol ultrasonic agitation developed by Wołczański et al.[4] using DIEA (N,N diisopropylethylamine) (6 equiv.) and TBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate) (3 equiv.) as a coupling reagent. The building block N-[2-thioethyl]Gly was attached was attached in two steps based on the protocol published by Wierzbicka et al.[5] Firstly bromoacetic acid was added (5 equiv.) with 5 equiv. of DIC (diisopropylcarbodiimide) in DMF three times, each time adding a fresh portion of the reagent. The mixture was stirred for 30 min on a rotary mixer, followed by filtrations and washing with DMF (7 x 1 min). Then, 6 equiv. of the protected cysteamine (Trt-cysteamine) was added in DMF, and the mixture was stirred overnight at room temperature on a rotary mixer. Next, the peptidylresin was filtered and washed with DMF (5 x 1 min). Coupling the last amino acid residue (Tyr) to the secondary nitrogen atom required repeating the coupling reaction using PyBop (3 equiv.) as the coupling reagent. The reaction steps were monitored with both Kaiser and chloranil tests. After synthesis, the peptidylresin was washed with DMF/DCM, DCM, MeOH and dried in a desiccator. Peptide was cleaved from resin with TFA : H_2O : TIS (triisopropylsilane) (95:2.5:2.5) mixture. After evaporating trifluoracetic acid the product was lyophilized and analyzed by analytical methods: ESI-MS, ESI-MS/MS, LC-MS.

2.1.3 Procedure of stapling using hexafluorobenzene

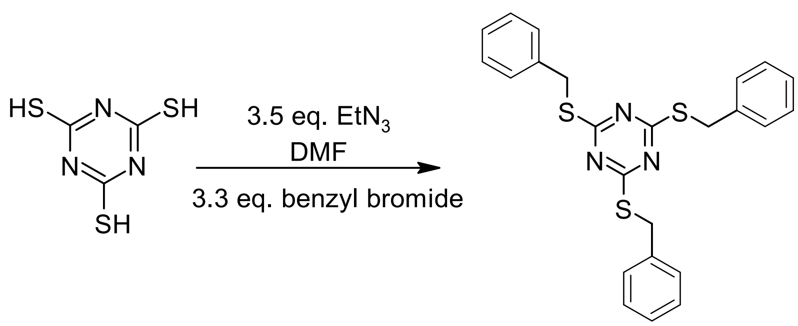
The stapling procedure was based on the modified procedure published by Spokoyny et al.[6] To a solid sample of H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1) (7.5 µmoles) in a plastic Eppendorf tube was added 1.9 mL of 100 µM solution (~ 25 equiv.) of hexafluorobenzene in DMF containing 5 equiv. of TCEP (tris(2-carboxyethyl)phosphine hydrochloride) and 1.5 mL of 50 mM solution of TRIS base (tris(hydroxymethyl)aminomethane) in DMF. The tube was vigorously mixed on a shaker for 30 seconds and left at room temperature for 4.5 hours. Then the solvents were evaporated and the sample was dissolved in water and desalted by SPE, lyophilized. The sample was subjected to purification on HPLC. Fractions containing stapled peptide product (analyzed by LC-MS/MS) were combined and lyophilized. The purity was confirmed by LC-UV. The reaction yield was 65%.



Scheme S1. The scheme of the synthesis of stapled analog **2**

2.1.4 Synthesis of S,*S'*,*S''*-tris(benzyl) trithiocyanurates – TMT(Bzl)$_3$

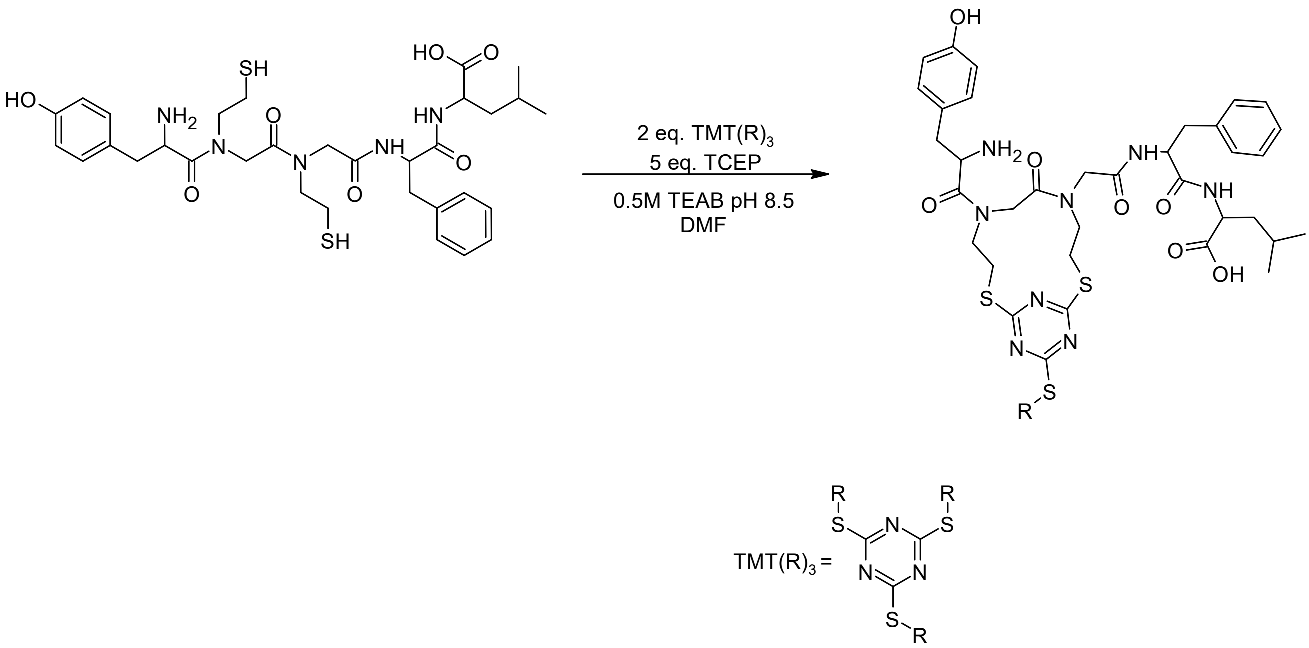
1 mL of triethylamine (about 7 mmol) was added to 352 mg of trithiocynuric acid (2 mmol) suspended in 10 mL of DMF. The resulted clear yellow solution was filtered through a syringe filter with 0.2 µm PES membrane. Then 780 µL of benzyl bromide (6.6 mmol) was added to the filtrate. The reaction mixture was stirred on a rotary shaker overnight and diluted with water to 50 mL of the volume. The precipitated product was centrifuged in a falcon tube, washed 3 times with water and lyophilized. Yield 0.86 g (96%).



Scheme S2. The scheme of the synthesis of TMT(Bzl)$_3$

2.1.5 Procedure of stapling using trithiocyjanuric acid derivative

The original stapling procedure was based on the recently published procedure for tris(alkyl) thiocyanurates transthioesterification.[7] The reaction buffer was prepared freshly before the use by dissolution of 20 mg TCEP x HCl in 1 mL of water, followed by neutralization with 1M NaOH solution added dropwise, and mixing with 1 mL of 1M TEAB buffer at pH 8.5. Simultaneously, about 25 µmoles of $TMT(R)_3$ (8.7 mg of $TMT(AcNH_2)_3$ or $TMT(Bzl)_3$) was dissolved in 2 mL of DMF. The buffer was added to 10 mg of TFA x of H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1) (12.7 µmoles) weighed in a 15 mL falcon tube, followed by addition of the solution of a thiocyanurate reagent. The resulted suspension was incubated on a rotary shaker at 40°C for an appropriate time (3 hours for $TMT(AcNH_2)_3$ or 24 hours for $TMT(Bzl)_3$). The final mixture was diluted to 10 mL by 40% ACN in water. The unreacted thiocyanurate was centrifuged at 5000 rpm at 5°C. The precipitate was washed additionally by 5 mL of 40% ACN in water. Combined supernatants were carefully acidified with TFA, evaporated under nitrogen and lyophilized. The sample was subjected to purification on HPLC. Fractions containing stapled peptide product (analyzed by LC-MS/MS) were combined and lyophilized. The purity was confirmed by LC-UV. The reaction yield was 85%.



Scheme S3. The scheme of the synthesis of stapled analogs 3a and 3b

2.2. Biological activity assay

Samples analyzed for analgesic activity were lyophilized from acetic acid three times.

2.2.1. Animals

In the present study, we used adult male Wistar rats (250-270 g) obtained from the Animal House Mossakowski Medical Research Institute, Polish Academy of Sciences. Rats were housed in cages lined with sawdust with elements of environmental enrichment in a temperature-controlled room (approximately 22°C), in a 12 : 12 h light–dark cycle. Food and water were provided *ad libitum.* All experiments were performed according to the recommendations of IASP, the NIH Guide for Care and Use of Laboratory Animals, and were approved by the Local Bioethics Committee (Warsaw, Poland).

2.2.2 Intrathecal Catheterization

Analgesic activity of compounds was measured in the tail flick test after intrathecal administration to rats, according to a protocol of Yaksh and Rudy[8] with modifications[9]. Catheters were made of silastic tubing (ID = 0.30 mm; OD = 0.64 mm) with a dead volume of 10 µl. Catheters measured a total of 11.5 cm with 7.5 cm inserted into the intrathecal space to the level of T13-L1. Rats were anesthetized with 2,5% isoflurane. The catheter was inserted through the alanto-occipital membrane and into the intrathecal space using a guide wire. Sutures were used to secure the placement of the catheter. The rats were allowed to recover from the surgery for 2-3 days. Rats exhibiting any sign of neurological or motor impairment, as evidenced by paralysis, abnormal gait, weight loss, or negligent grooming, were excluded from the study. Rats were housed separately to ensure catheter patency.

2.2.3 Tail-Flick Assay

Each experimental group consisted of 7-8 rats. Spinally mediated analgesia was assessed in the tail flick test utilizing the Tail Flick Analgesia Meter apparatus (IITC Life Science Inc., Los Angeles, CA, USA). Withdrawal latency was measured in triplicate. The cut-off latencies were set at 7 s to avoid burns. The measurements were performed before the administration of the tested compound (time 0) and 5, 15, 30, 60, and 120 min. after administration. The control group received saline (0.9% NaCl). The responses were expressed as a percentage of a maximum possible effect (%MPE), calculated as $((T1 - T0)/(T2 - T0)) \times 100$, where T0 and T1 are latencies before and after drug injection, respectively, and T2 is the cut-off time. Data from *in vivo* studies are presented as means ± SEM and were analyzed using two-way repeated measures of ANOVA followed by Tukey's multiple comparison test, using GraphPad Prism[10] (3). Significance was defined as * $p < 0.03$, ** $p < 0.001$.

2.3 Analytical data

Table S1 The list of obtained peptides

Sequence (name)	Abbreviation
H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (*N^2,N^3*-bis(2-mercaptoethyl)[Leu^5]enkephalin)	**1**
H-Tyr-*cyclo*-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (*cyclo*-[S^2,S^3-(2,3,5,6-tetrafluorophenylenyl]-*N^2*,*N^3*-bis(2-mercaptoethyl)[Leu^5]enkephalin)	**2**
H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (cyclo-[S^2,S^3-(2-carbamidomethylsulfanyl-1,3,5-triazin-4,6-diyl)]-*N^2*,*N^3*-bis(2-mercaptoethyl)[Leu^5]enkephalin)	**3a**
H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (cyclo-[S^2,S^3-(2-benzylsulfanyl-1,3,5-triazin-4,6-diyl)]-*N^2*,*N^3*-bis(2-mercaptoethyl)[Leu^5]enkephalin)	**3b**
H-Tyr-Gly-Gly-Phe-Leu-OH name: [Leu^5]enkephalin	**Leu-Enk.**

2.3.1 H-Tyr-Gly-Gly-Phe-Leu-OH ([Leu^5]enkephalin; Leu-Enk.)

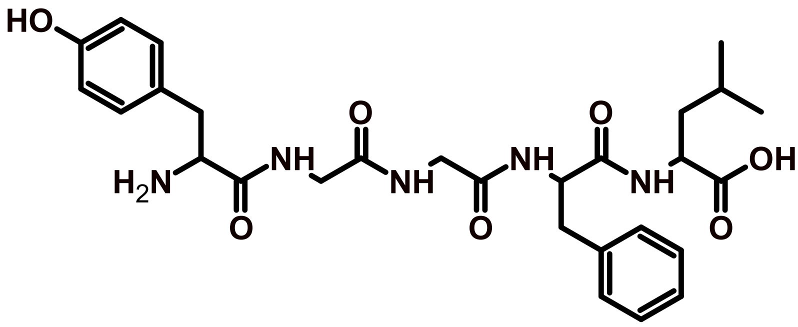


Table S2 Analytical data for H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-Enk.)

LC-IT-TOF-MS	t_R: 9.0 min
MS	*m/z*: **556.2578** (calc. for $C_{28}H_{37}N_5O_7$ $[M+H]^+$: 556.2766)
MS/MS	precursor ion at *m/z*: **556.2578** ($[M+H]^+$) daughter ions *m/z*: 538.2703 (calc. for $[M\text{-}H_2O\text{+}H]^+$: 538.2660); 425.1851 (calc. for b_4: 425.1825); 397.1907 (calc. for a_4: 397.1876); 278.1155 (calc. for b_3: 278.1141); 262.1188 (calc. for y_4b_4: 262.1180); 221.0937 (calc. for b_2: 221.0926)

2.3.2 H-Tyr-[N-(2-SEt)]Gly-[N-(2-SEt)]Gly-Phe-Leu-OH
(*N*^{2},*N*^{3}-bis(2-mercaptoethyl)[Leu^5]enkephalin; 1)

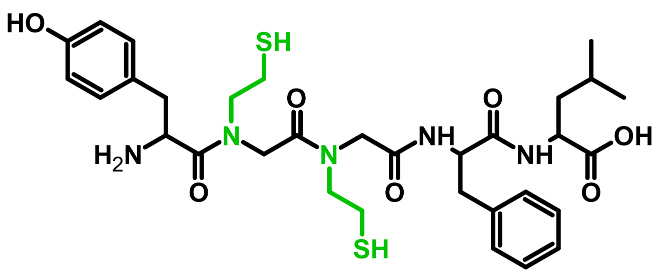


Table S3 Analytical data of H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1)

LC-IT-TOF-MS	t_R: 8.3 min
MS	*m/z*: **676.2865** (calc. for $C_{32}H_{45}N_5O_7S_2$ $[M+H]^+$: 676.2833)
MS/MS	precursor ion at *m/z*: **676.2865** ($[M+H]^+$) daughter ions *m/z*: 658.2846 (calc. for $[M\text{-}H_2O\text{+}H]^+$: 658.2727); 513.2208 (calc. for y_4: 513.2200); 398.1298 (calc. for b_3: 398.1208); 396.1956 (calc. for y_3: 396.1951); 281.0966 (calc. for b_2: 281.0960)

2.3.3 H-Tyr-*cyclo*-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (cyclo-[S^2,S^3-(2,3,5,6-tetrafluorophenylenyl]-N^2,N^3-bis(2-mercaptoethyl)[Leu^5]enkephalin; 2)

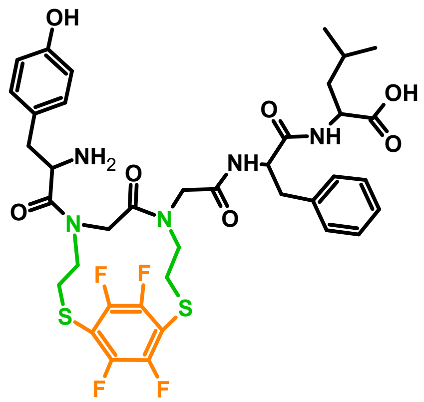


Table S4 Analytical data of H-Tyr-*cyclo*-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)

LC-IT-TOF-MS	t_R: 10.0 min
MS	*m/z*: **822.2685** (calc. for $C_{38}H_{43}F_4N_5O_7S_2$ $[M+H]^+$: 822.2613)
MS/MS	precursor ion at *m/z*: **822.2685** ($[M+H]^+$) daughter ions *m/z*: 691.1739 (calc. for b_4: 691.1672); 659.1991 (calc. for y_4: 659.1979); 544.0878 (calc. for b_3: 544.0988); 381.0386 (calc. for b_3y_4: 381.0343); 353.0451 (calc. for a_3y_4: 353.0394)

2.3.4 S, S',S''-Tris(benzyl) trithiocyanurate: TMT(Bzl)$_3$

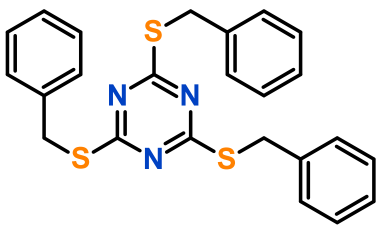


Table S5 Analytical data of TMT(Bzl)$_3$

LC-IT-TOF-MS	t_R: 18.0 min
MS	*m/z*: **448.1037** (calc. for $C_{24}H_{22}F_4N_3S_3$ $[M+H]^+$: 448.0970)
1H NMR	**(DMSO-d6, 500 MHz, 25°C) δ (ppm) =** 4.40 (s, 6H, benzyl), 7.20-7.40 (m, 15H, phenyl)
13C NRM	**^{13}C NMR (DMSO-d6, 125 MHz, 25°C) δ (ppm) =** 178.7 (triazine), 136.6 (1-phenyl), 128.9 (2-phenyl), 128.5 (3-phenyl), 127.3 (4-phenyl), 33.6 (benzyl)

2.3.5 H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[N-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (cyclo-[S²,S³-(2-carbamidomethylsulfanyl-1,3,5-triazin-4,6-diyl)]-*N*²,*N*³-bis(2 mercaptoethyl)[Leu⁵]enkephalin; 3a)

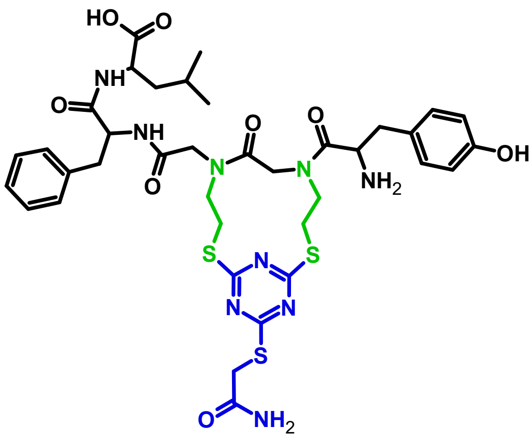


Table S6 Analytical data of H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (3a)

LC-IT-TOF-MS	t_R: 11.1 min
MS	*m/z*: **842.2767** (calc. for $C_{37}H_{48}N_9O_8S_3$ $[M+H]^+$: 842.2782)
MS/MS	precursor ion at *m/z*: **842.2767** ($[M+H]^+$) daughter ions *m/z*: 807.2425 (calc. for $b_5(z_5)$: 807.2411); 711.1870 (calc. for b_4: 711.1836); 694.1567 (calc. for $b_4(z_5)$: 694.1571); 679.2142 (calc. for y_4: 679.2149); 662.1892 (calc. for y_4-NH_3: 662.1884); 644.1782 (calc. for $b_4(y_4)$-NH_3: 644.1778); 616.1819 (calc. for $a_4(y_4)$-NH_3: 616.1829); 564.1154 (calc. for b_3: 564.1152); 536.1201 (calc. for a_3: 536.1203); 531.0931 (calc. for $b_3(y_4)$-NH_3: 531.0937); 505.0801 (calc. for $\boldsymbol{b_3(y_4)}$-$\boldsymbol{C_2H_2}$-$\boldsymbol{NH_3}$: 505.0781); 401.0490 (calc. for $b_2(y_4)$: 401.0519); 389.0717 (calc. for $\boldsymbol{b_2(y_3)}$**-HSCN-**$\boldsymbol{NH_3}$: 389.0737); 373.0547 (calc. for *a2(y4)*: 373.0570); 361.0754 (calc. for $\boldsymbol{a_2(y_3)}$**-HSCN-**$\boldsymbol{NH_3}$: 361.0787

2.3.6 H-Tyr-cyclo-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Bzl)-Phe-Leu-OH (cyclo-[S²,S³-(2-benzylsulfanyl-1,3,5-triazin-4,6-diyl)]-N²,N³-bis(2-mercaptoethyl)[Leu⁵]enkephalin; 3b)

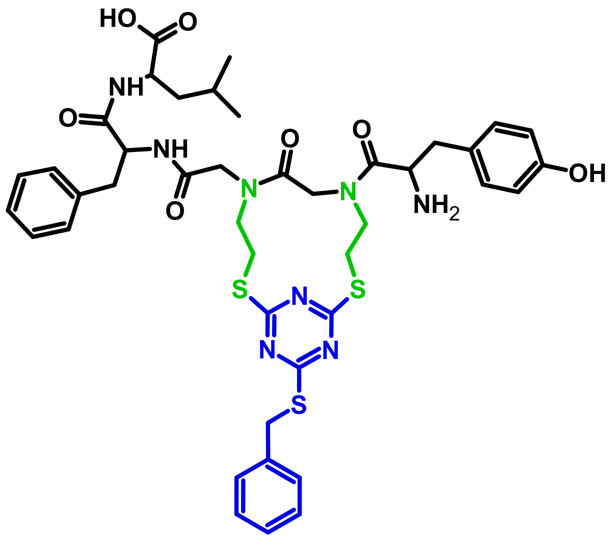


Table S7 Analytical data of H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Bzl)-Phe-Leu-OH (3b)

LC-IT-TOF-MS	t_R: 16.0 min
MS	*m/z*: **875.3023** (calc. for $C_{42}H_{51}N_8O_7S_3$ $[M+H]^+$: 875.3037)
MS/MS	precursor ion at *m/z*: **875.3023** ($[M+H]^+$) daughter ions *m/z*: 744.2070 (calc. for b_4: 744.2091); 712.2401 (calc. for y_4: 712.2404); 694.2297 (calc. for $b_4(y_4)$: 694.2298.); 666.2305 (calc. for $a_4(y_4)$: 666.2394); 597.1403 (calc. for b_3: 597.1407); 581.1403 (calc. for $b_3(y_4)$: 581.1458); 569.1503 (calc. for a_3: 569.1458); 563.2077 (calc. for **y_4-BzlSCN-HSCN**: 563.2105); 553.1507 (calc. for $a_3(y_4)$: 553.1509); 522.1612 (calc. for ***$b_3(y_4)$-C_2H_2-HSCN***: 522.1628); 434.0764 (calc. for $b_2(y_4)$: 434.0774); 406.0823 (calc. for $a_2(y_4)$: 406.0825); 375.0938 (calc. for *$b_2(y)$-HSCN*: 375.0944); 363.0398 (calc. for ***$a_2(y_4)$-C_2H_4N***: 363.0397

2.3.7 Side product - (H-Tyr-[*N*-(2-SEt])Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH)$_2$ (1a)

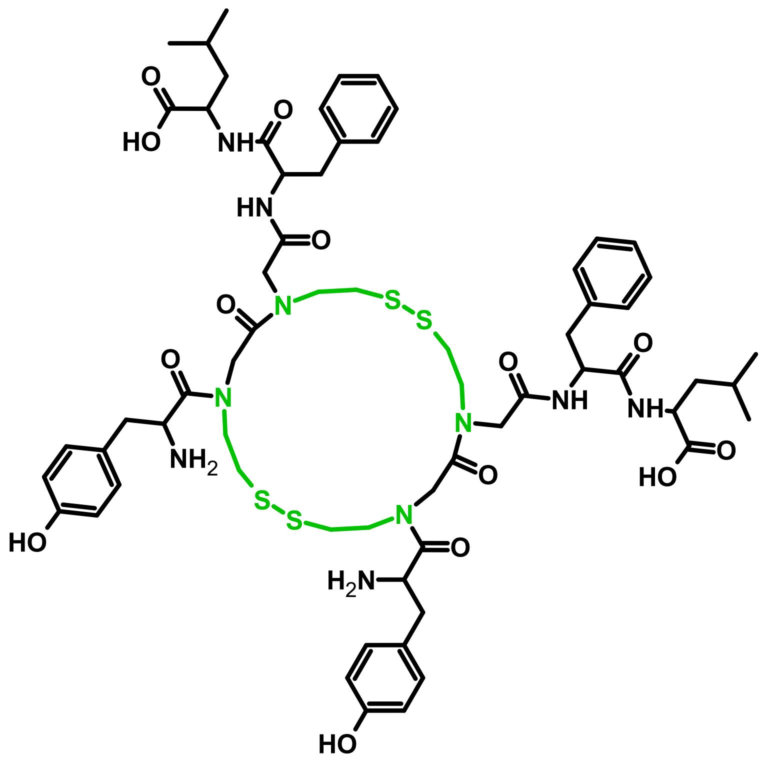


Table S8 Analytical data of (H-Tyr-[*N*-(2-SEt])Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH)$_2$ (1a)

LC-IT-TOF-MS	t_R: 8.4 min
MS	*m/z*: **674.2727** (calc. for $C_{64}H_{86}N_{10}O_{14}S_4$ $[M+2H]^{2+}$: 674.2677.)
MS/MS	precursor ion at *m/z*: **674.2727** ($[M+2H]^{2+}$) daughter ions *m/z*: 1184.4626 (calc. for y_4: 1184.4647); 906.3070 (calc. for b_3y_4: 906.3015); 888.3106 (calc. for b_3y_4 $-H_2O$: 888.2906); 279.1830 (calc. for y_2: 279.1703)

3. LC-MS, LC-MS/MS, ESI-MS analysis

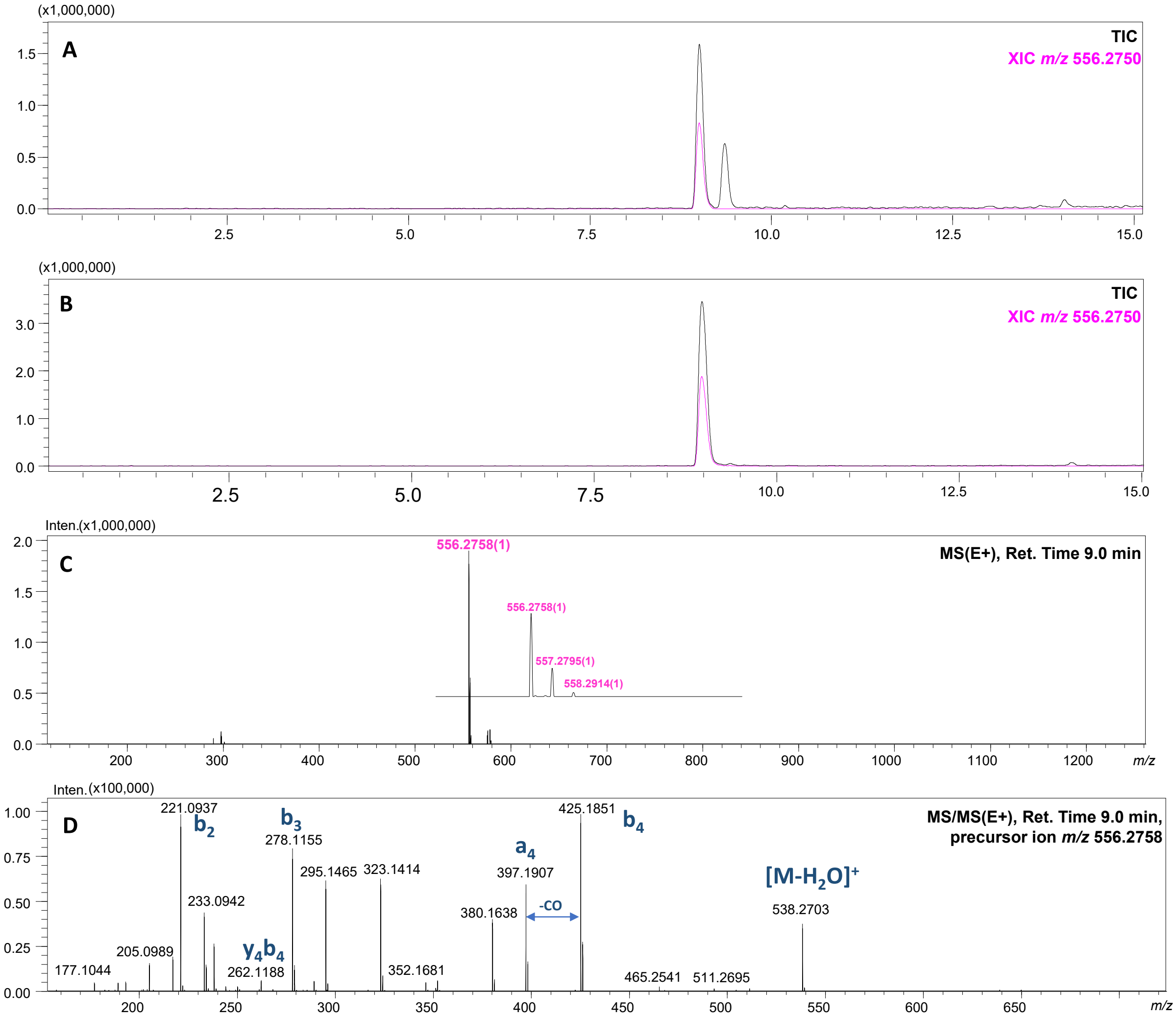


Fig. S1 LC-MS chromatogram of **H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-Enk.)** and XIC *m/z* for 556.2750 for crude product (A) and after purification (B); ESI-MS spectrum of signal with retention time 9.0 min (B); ESI-MS/MS spectrum of at *m/z* 556.2750 (C) *(Conditions for LC-MS analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-50% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min); ESI-MS/MS analysis – positive ion mode).*

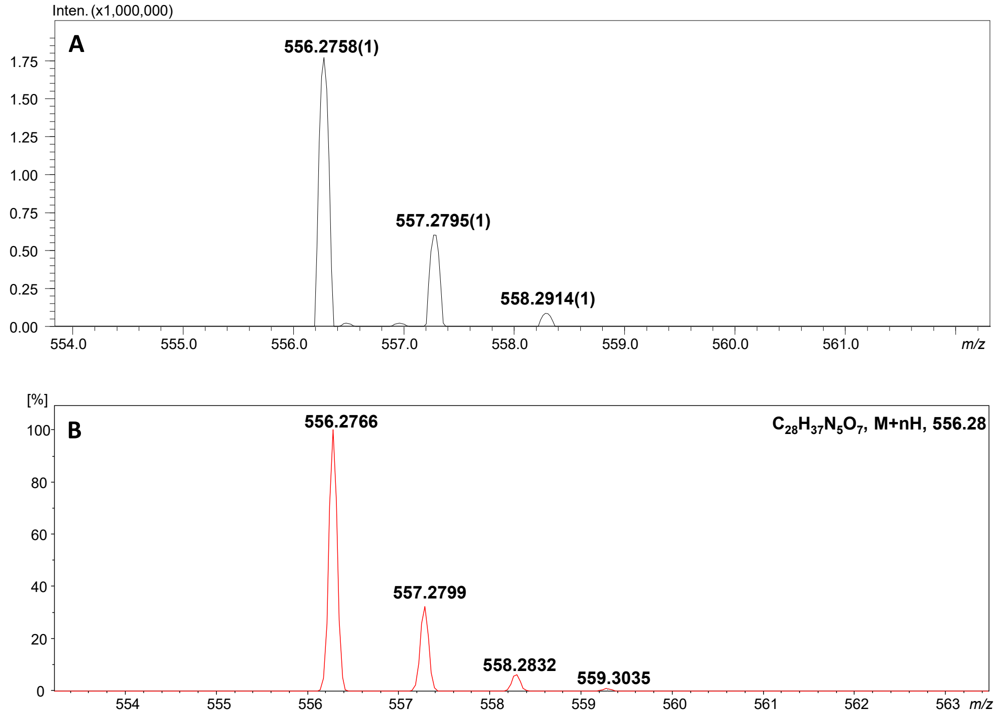


Fig. S2 ESI-MS of **H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-Enk.)** in zoom range at *m/z* 554-563 (A) and simulated for pseudomolecular ion $[M+H]^+$ where M = $C_{28}H_{37}N_5O_7$(B).

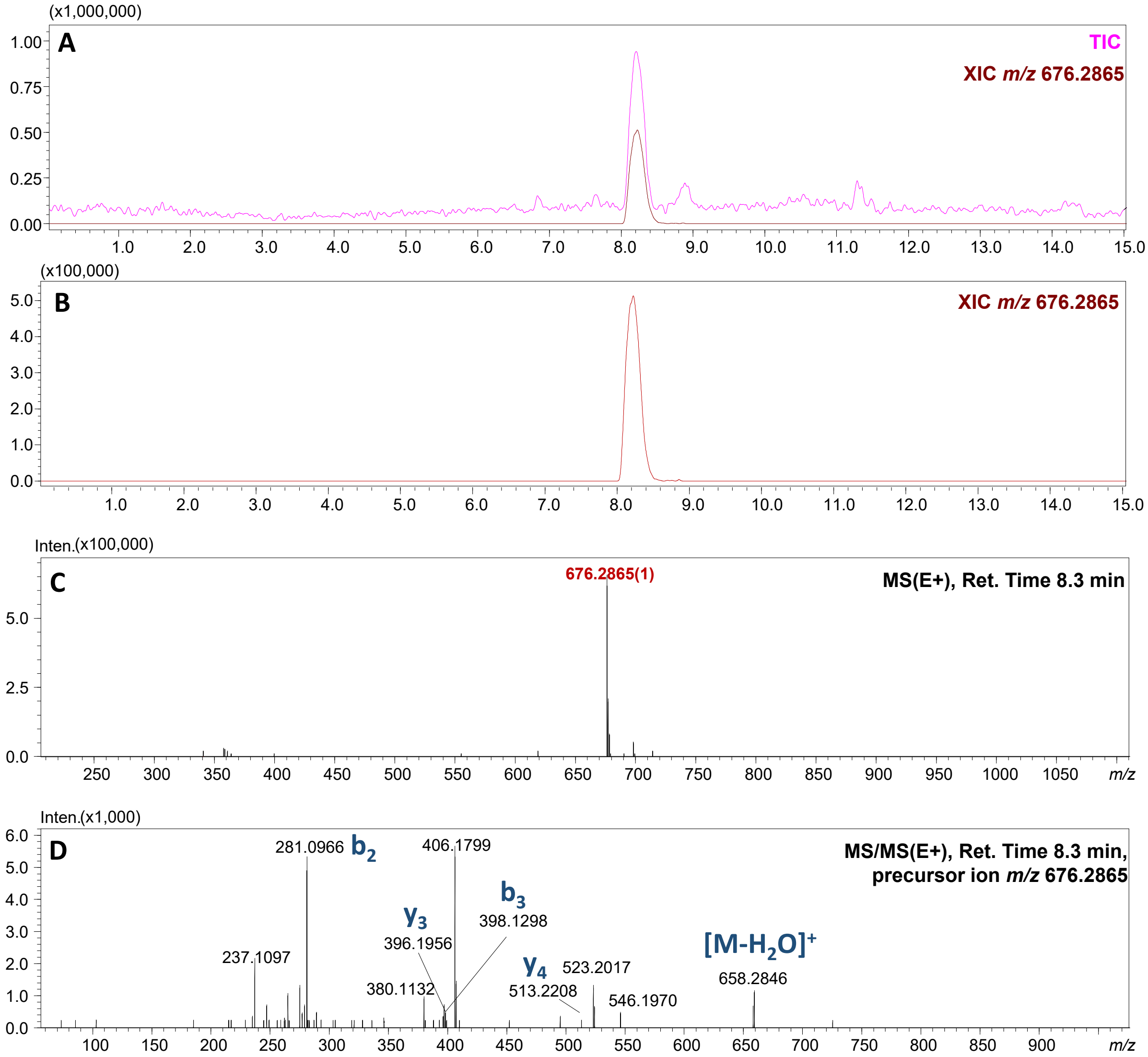


Fig. S3 LC-MS chromatogram of **H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1)** and XIC m/z for 676.2865 for crude product (A) and after purification (B); ESI-MS spectrum of signal with retention time 8.3 min (B); ESI-MS/MS spectrum of signal at *m/z* 676.2865 (C) *(Conditions for LC-MS analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-80% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min); ESI-MS/MS analysis – positive ion mode).*

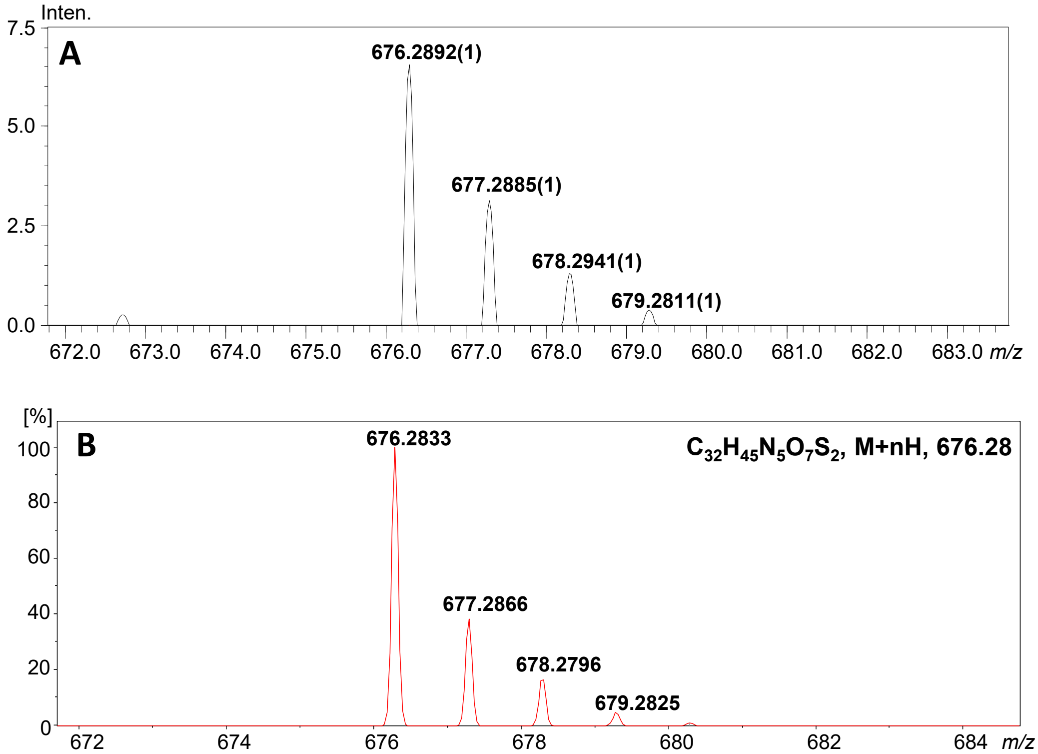


Fig. S4 ESI-MS of **H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1)** in zoom range at *m/z* 672-684 (A) and simulated for pseudomolecular ion $[M+H]^+$ where M = $C_{32}H_{45}N_5O_7S_2$(B).

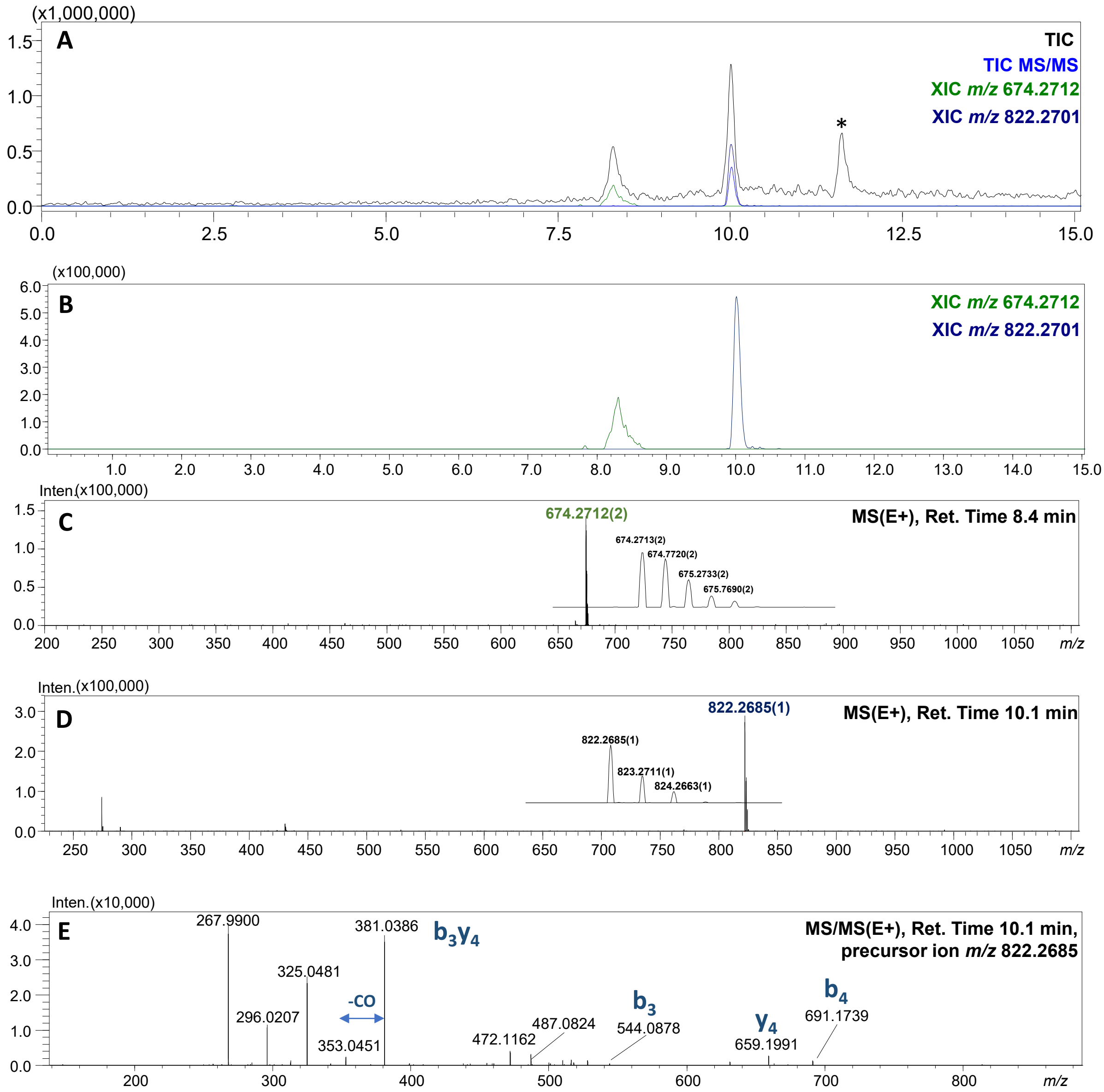


Fig. S5 LC-MS chromatogram of **H-Tyr-[*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly-Phe-Leu-OH (1)** after cyclization reaction with hexafluorobenzene and XIC *m/z* for 674.2712 for **intermolecular dimer (1a)** of compound 1 and 822.2701 for **compound 2** (A,B); ESI-MS spectrum of signal with retention time 8.3 min (C); ESI-MS spectrum of signal with retention time 10.0 min (D); ESI-MS/MS spectrum of signal at *m/z* 822.2685 (E) *(Conditions for LC-MS analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-80% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min); ESI-MS/MS analysis – positive ion mode; * - impurities in mobile phase).*

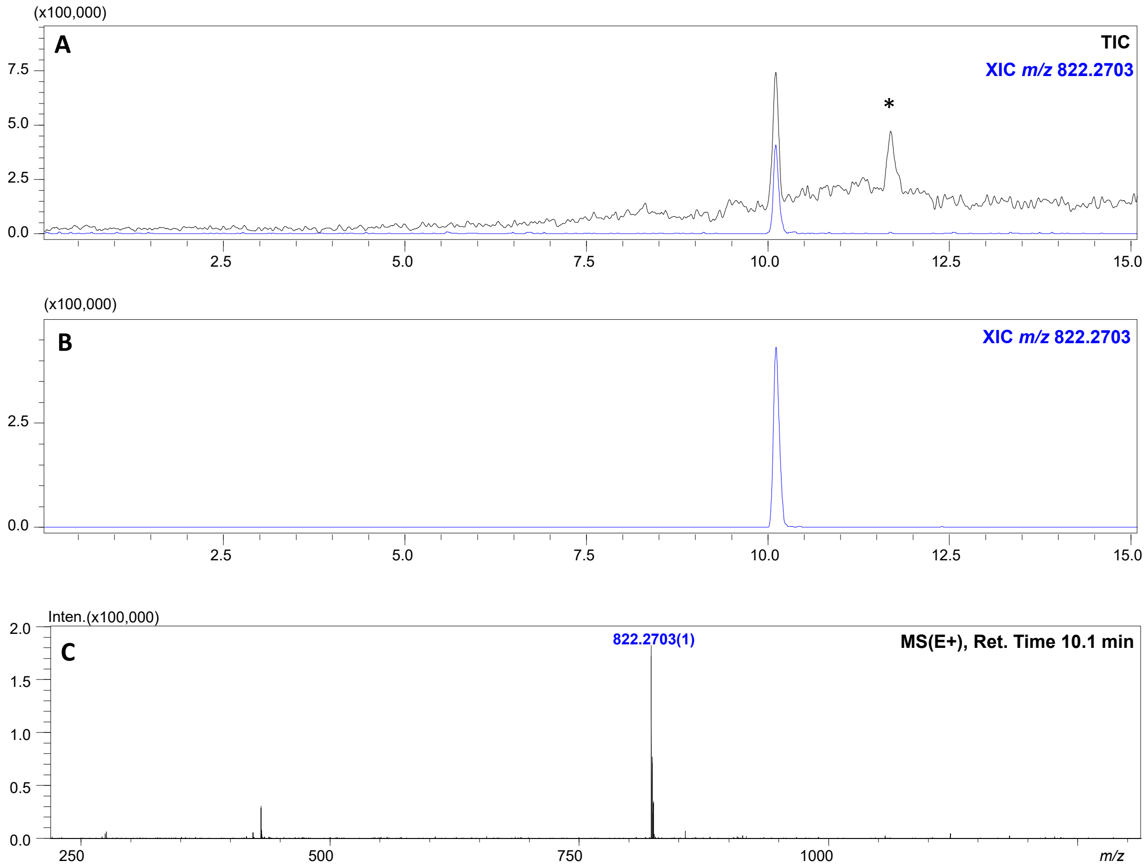


Fig. S6 LC-MS chromatogram of **H-Tyr-*cyclo*-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)** after purification and XIC *m/z* for 822.2703 (A, B); ESI-MS spectrum of signal with retention time 10.3 (C); *(Conditions for LC-MS analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-80% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min); ESI-MS analysis – positive ion mode; * - impurities in mobile phase).*

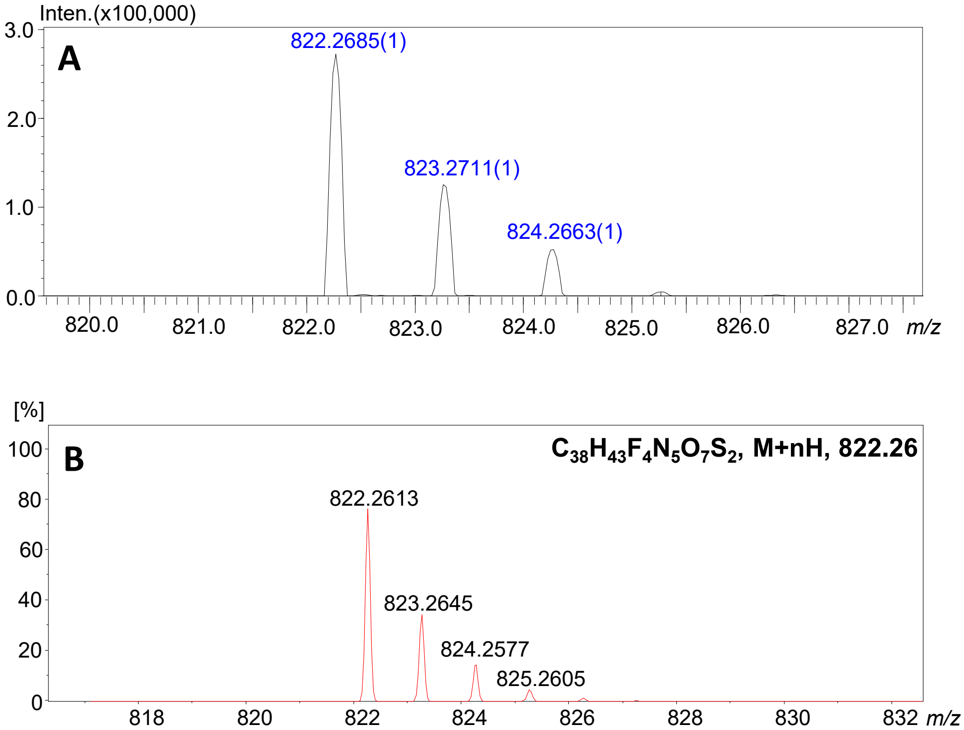


Fig. S7 ESI-MS of **H-Tyr-*cyclo*-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)** in zoom range at *m/z* 819-832 (A) and simulated spectrum for pseudomolecular ion $[M+H]^+$ where M = $C_{38}H_{43}N_5O_7S_2F_4$(B).

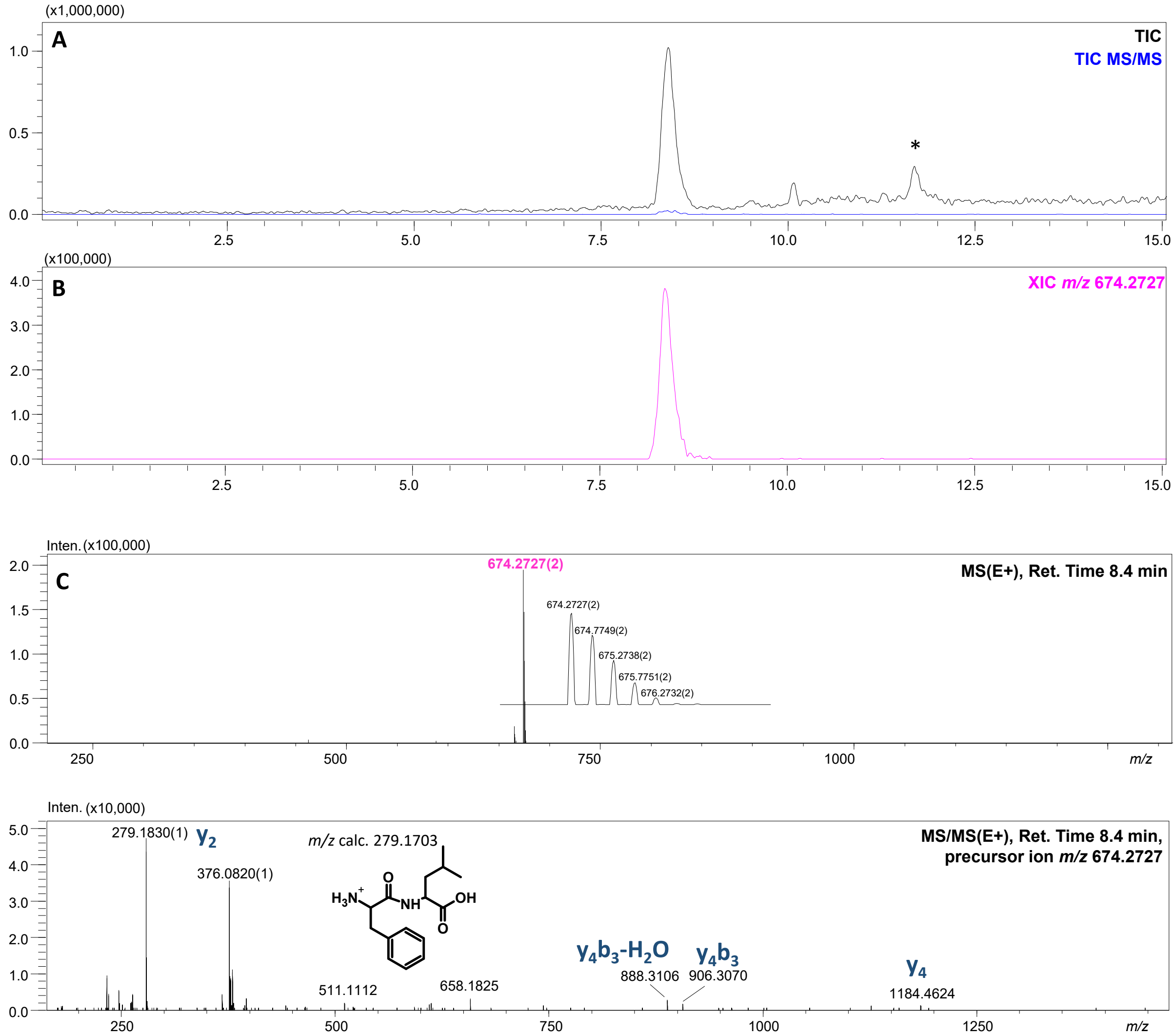


Fig. S8 LC-MS chromatogram of **intermolecular dimer of product 1 (1a)** after purification and XIC *m/z* for 674.2727 (2+) (A, B); ESI-MS spectrum of signal with retention time 10.3 (C); *(Conditions for LC-MS analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-80% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min); ESI-MS/MS analysis – positive ion mode; * - impurities in mobile phase).*

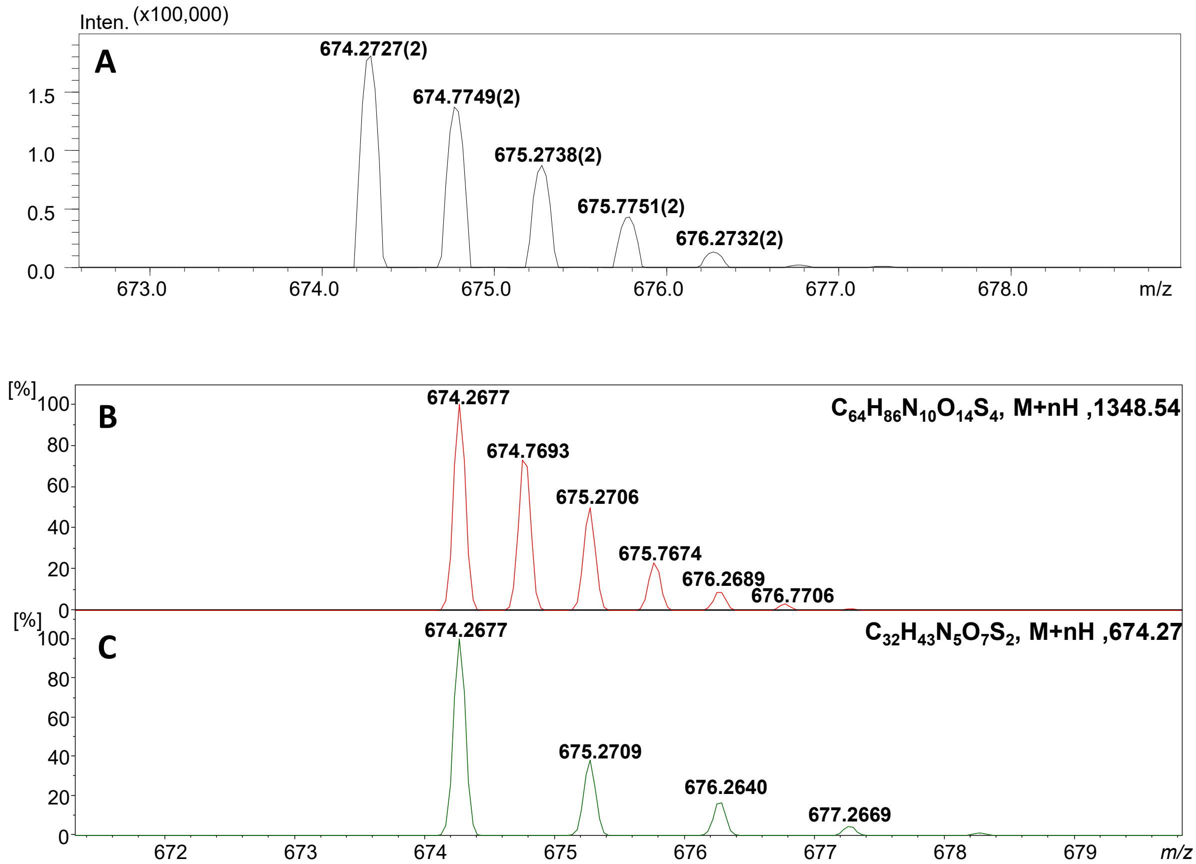


Fig. S9 ESI-MS of **intermolecular dimer of product 1 (1a)** in zoom range at *m/z* 670-680 (A) and simulated for pseudomolecular ions $[M+H]^+$ where M = $C_{64}H_{86}N_{10}O_{14}S_4$ (B) and $C_{32}H_{43}N_5O_7S_2$ (C).

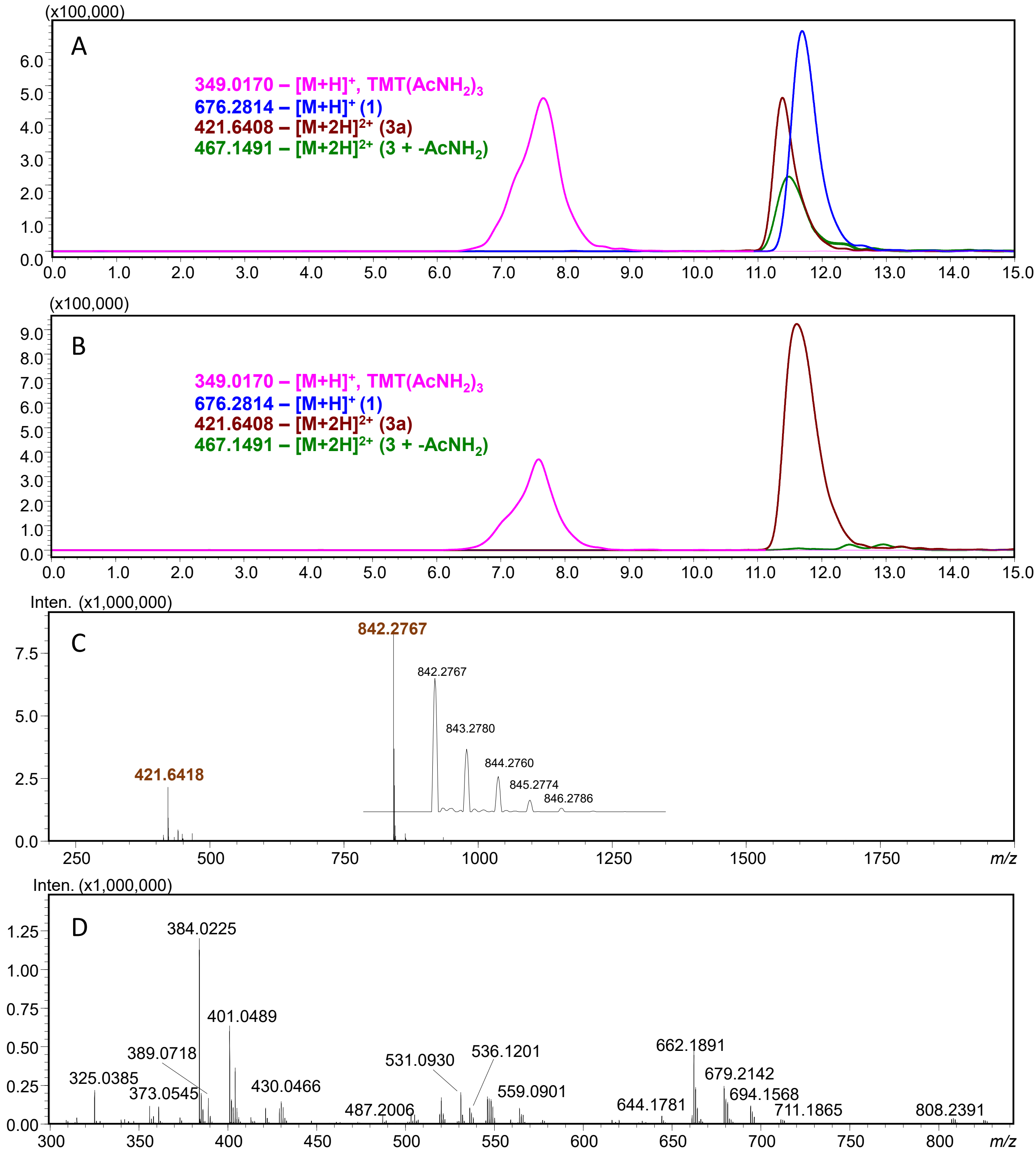


Fig.S10 Extracted LC-MS chromatograms of the main product **-** H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH **(3a)** (brown), the side product TMT(Acm)$_2$(1) (green), and substrates – 1 (blue), TMT(Acm)$_3$ (pink) after 5 minutes (A) or 3 hours (B) of the cyclization reaction; ESI-MS spectrum of signal with retention time 11.5 min (C); MS^2 spectrum of the parent ion with m/z = 842.3 (D) *(Conditions for LC-MS analysis: ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm); gradient elution of 1-70% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.2 ml/min); ESI-MS/MS analysis – positive ion mode)*

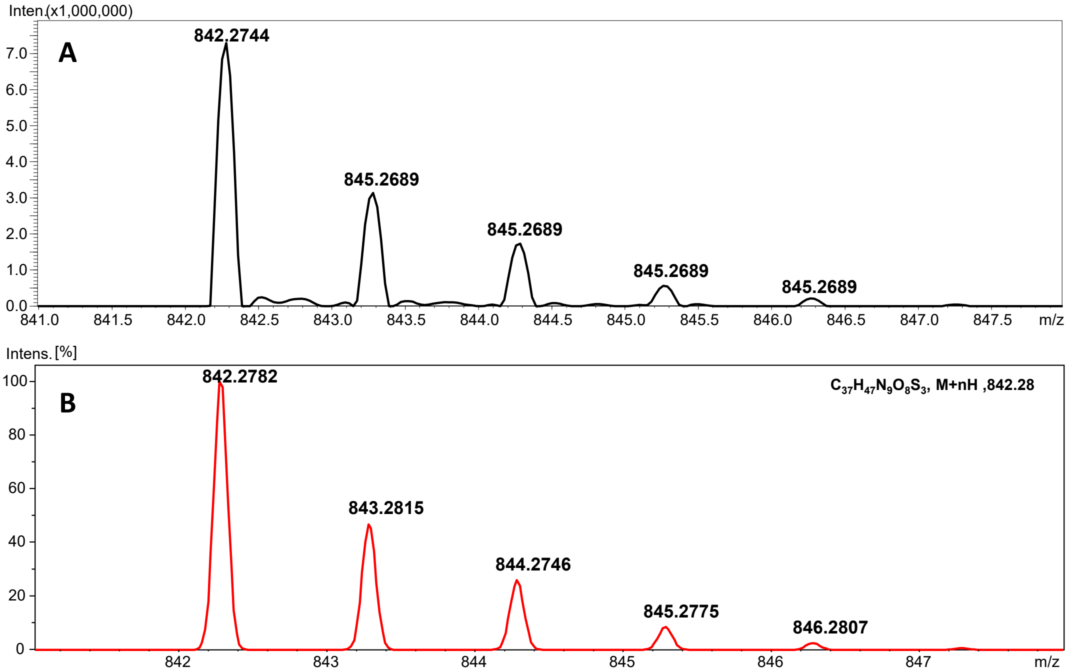


Fig. S11 ESI-MS of **H-Tyr-cyclo-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (3a)** in zoom range at *m/z* 841 – 848 (A) and simulated spectrum for pseudomolecular ion $[M+H]^+$, where M = $C_{37}H_{47}N_9O_8S_3$ (B).

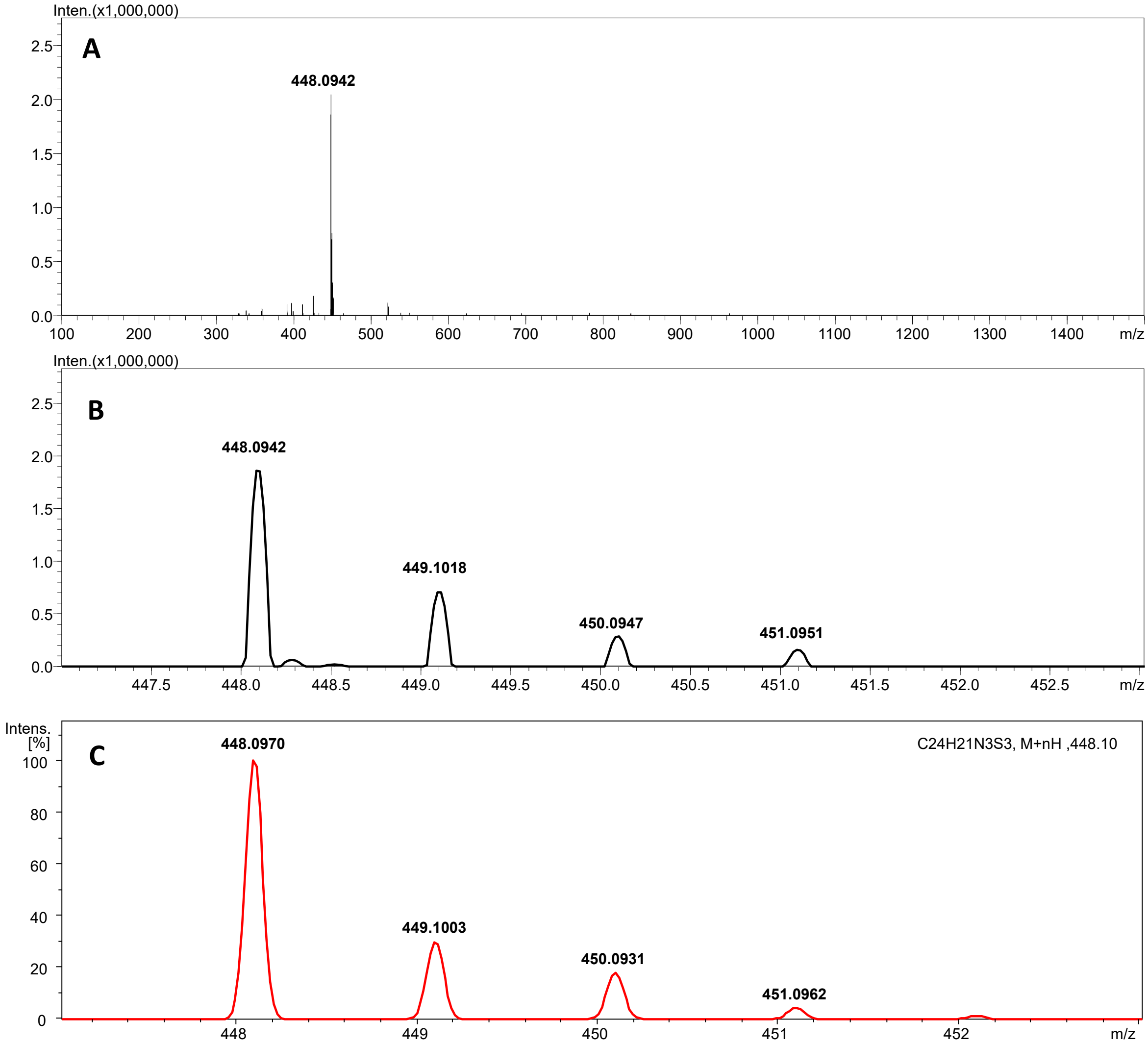


Fig. S12 ESI-MS spectrum of $TMT(Bzl)_3$ in a wide range (A), in a zoom range at m/z 447 – 453 (B) and a simulated spectrum for pseudomolecular ion $[M+H]^+$, where M = $C_{24}H_{21}N_3S_3$ (C)

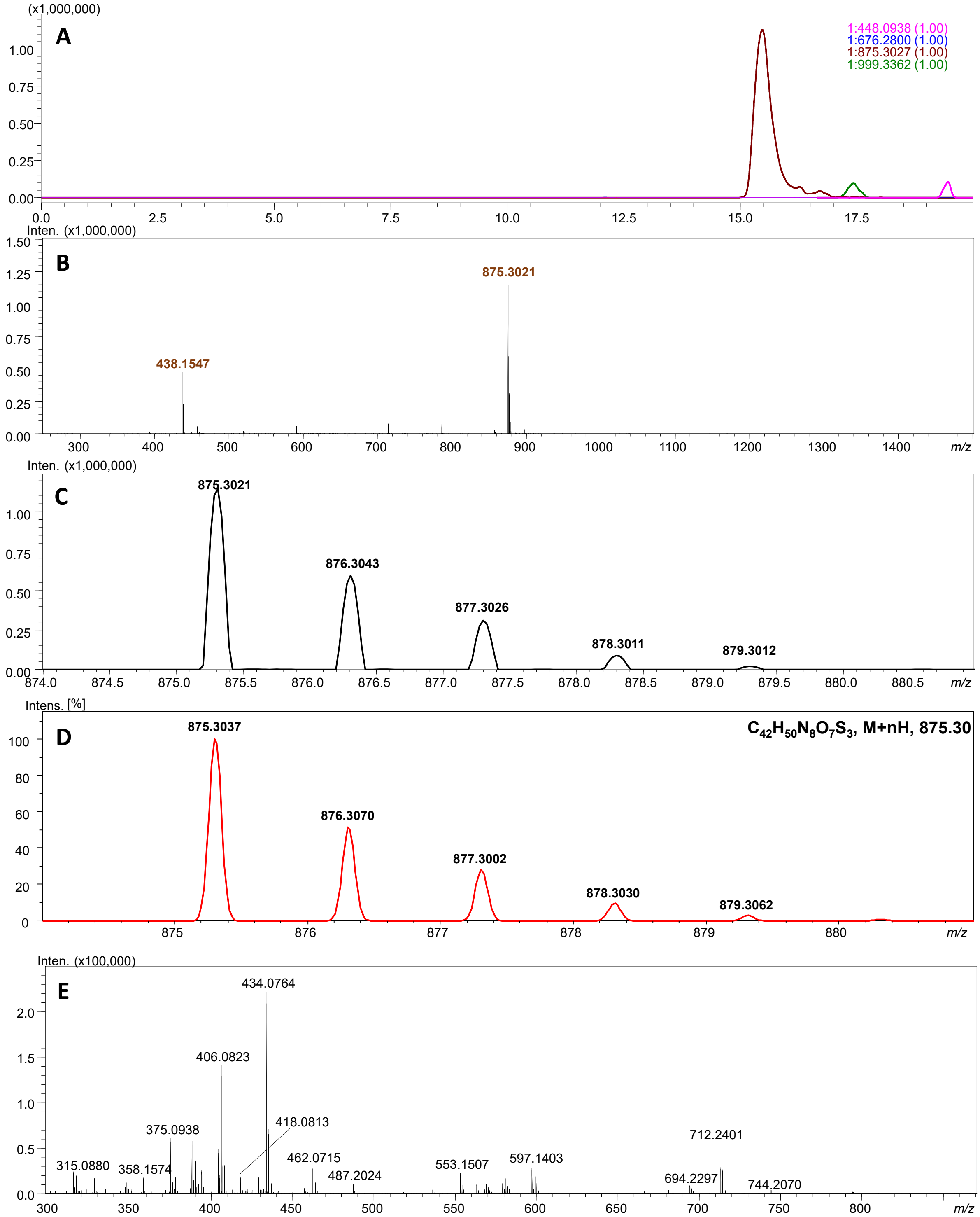


Fig. S13 Extracted LC-MS chromatograms of the main product - **H-Tyr-cyclo-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Bzl)-Phe-Leu-OH (3b) (brown),** the side product TMT(Bzl)$_2$(1) (green), and substrates – 1 (blue), TMT(Acm)$_3$ (pink) after 24 hours of the cyclization reaction (B); ESI-MS spectrum of signal with retention time 16.0 min (C); ESI-MS spectrum in zoom range at *m/z* 874 – 881 (C); simulated spectrum of pseudomolecular ion $[M+H]^+$, where M = $C_{42}H_{50}N_8O_7S_3$ (D); MS^2 spectrum of the parent ion with m/z = 875.3 (E) *(Conditions for LC-MS analysis: ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm); gradient elution of 1-70% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.2 ml/min); ESI-MS/MS analysis – positive ion mode)*

4. LC-UV analysis

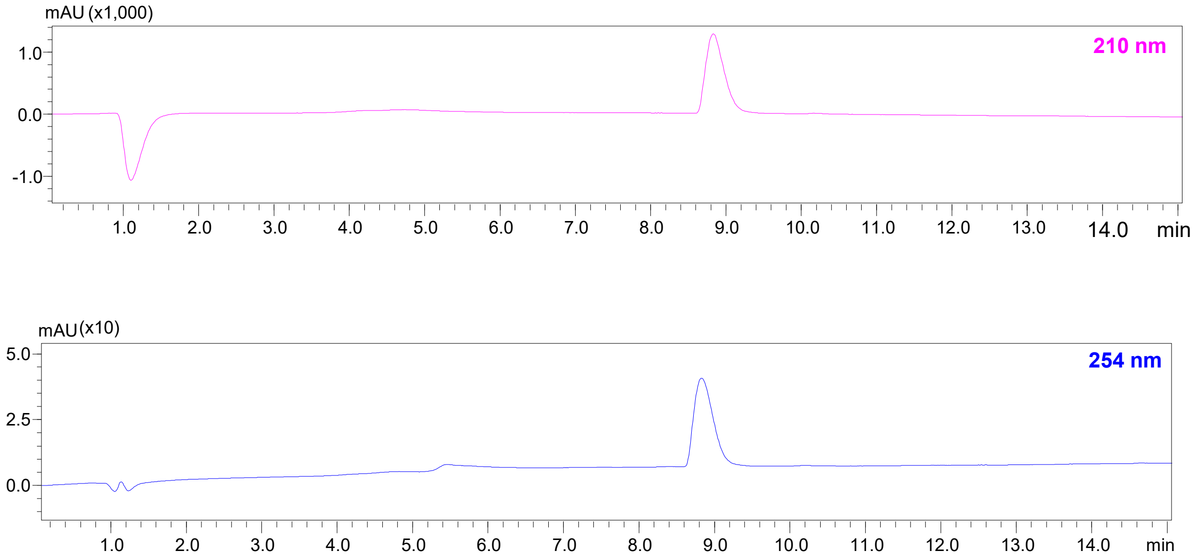


Fig. S14 LC-UV chromatogram of **H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-Enk.)** *(Conditions for LC-UV analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-50% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min).*

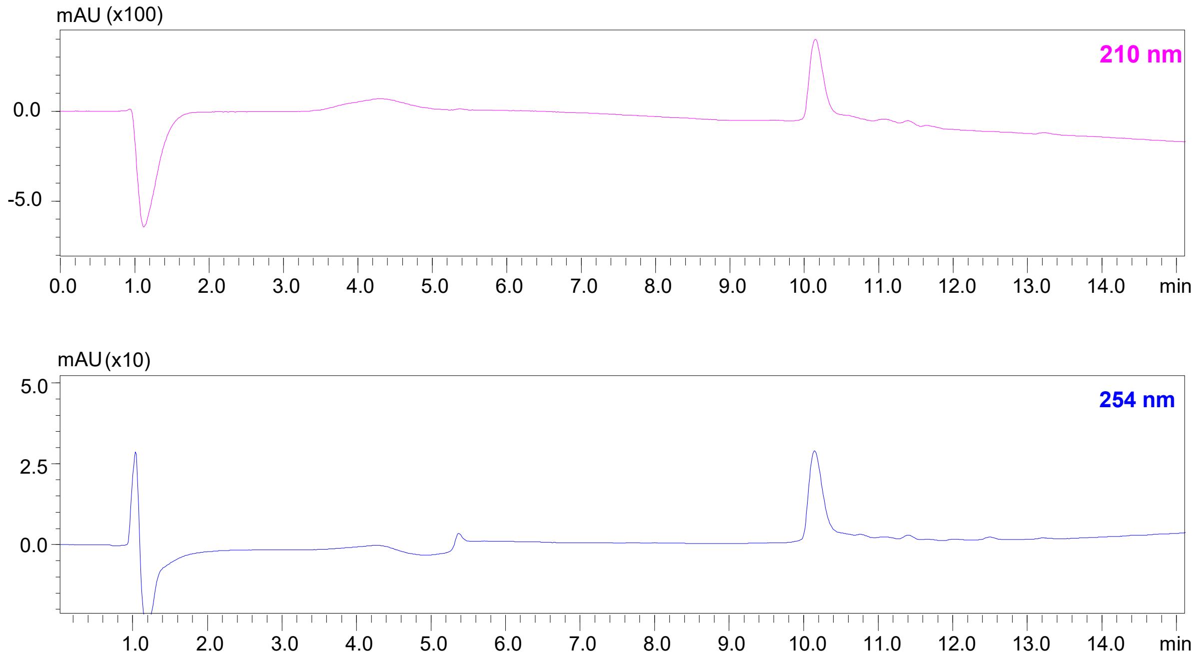


Fig. S15 LC-UV chromatogram of **H-Tyr-cyclo-4FB([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)** *(Conditions for LC-UV analysis: RP-Zorbax column (50 × 2.1 mm, 3.5 µm); gradient elution of 0-80% B in A (A = 0.1% HCOOH in water; B = 0.1% HCOOH in MeCN) at RT in 15 min, 15-20 min washing and stabilizing the column (flow rate: 0.1 ml/min).*

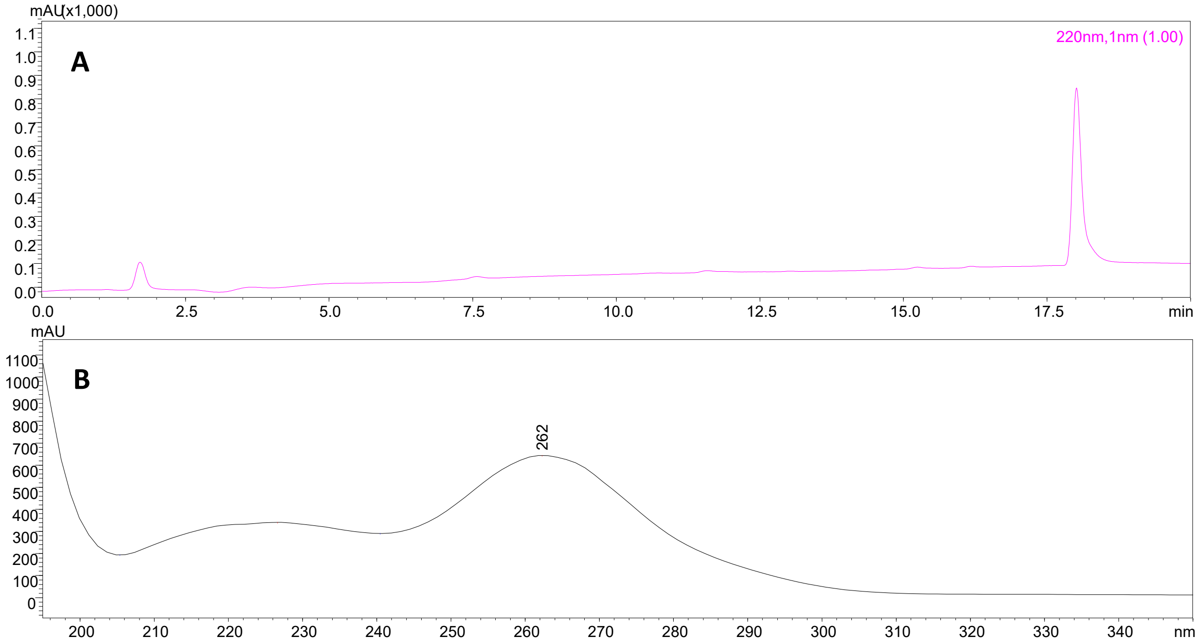


Fig. S16 LC-UV chromatogram (A) and extracted UV absorption spectrum (B) of **TMT(Bzl)$_3$** *(Conditions for LC-UV analysis: column -ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm); detection - DAD, 220 nm; gradient elution – 20-100% B in A in 15 min, 100% B for 5 min; eluent A – 0.1% formic acid in the water, eluent B – 0.1% formic acid in acetonitrile; flowrate 0.2 mL/min).*

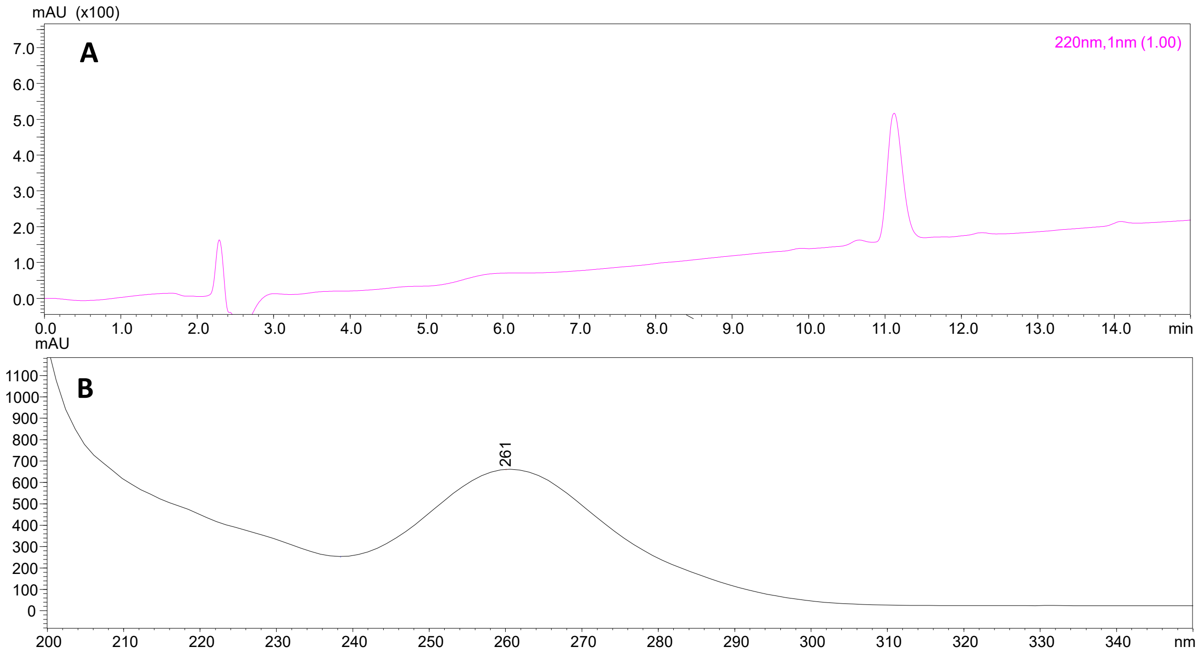


Fig. S17 LC-UV chromatogram (A) and extracted UV absorption spectrum (B) of **H-Tyr-cyclo-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (3a)** *(Conditions for LC-UV analysis: ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm); gradient elution of 1-70% B in A at RT in 15 min, 15-20 min washing and stabilizing the column; eluent A – 0.1% formic acid in the water, eluent B – 0.1% formic acid in acetonitrile; flow rate: 0.2 ml/min).*

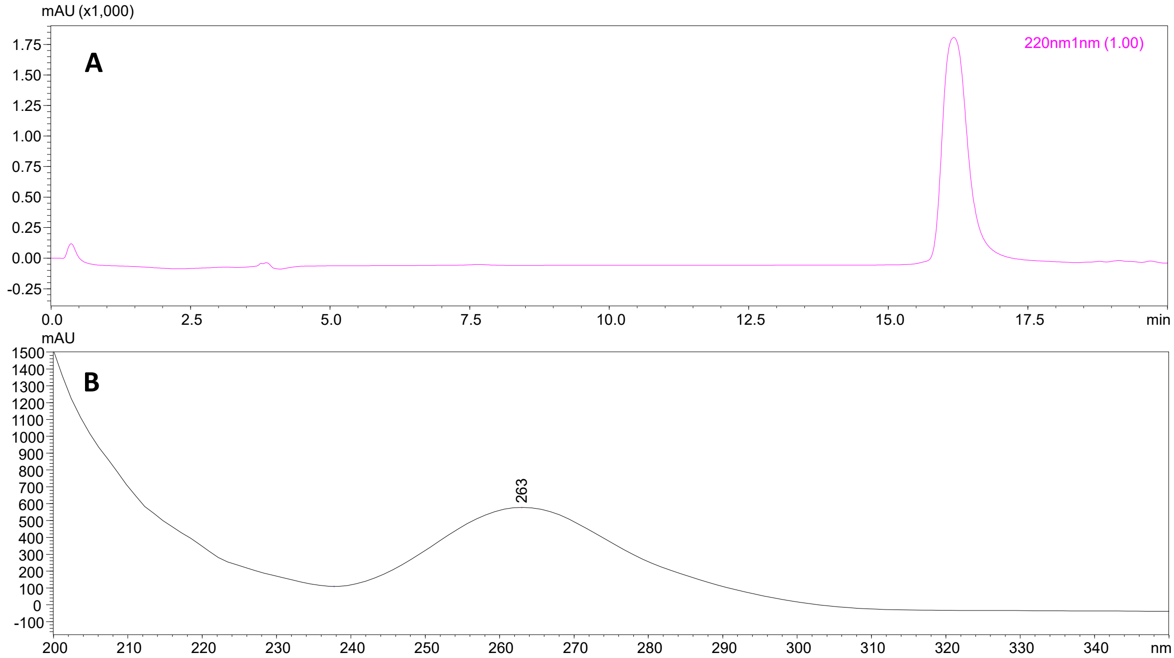


Fig. S18 LC-UV chromatogram (A) and extracted UV absorption spectrum (B) of **H-Tyr-cyclo-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Bzl)-Phe-Leu-OH (3b)** *(Conditions for LC-UV analysis: ReproSil-XR 120 C18-MS (3 µm, 100 x 2 mm); gradient elution of 1-70% B in A at RT in 15 min, 15-20 min washing and stabilizing the column; eluent A – 0.1% formic acid in the water, eluent B – 0.1% formic acid in acetonitrile; flow rate: 0.2 ml/min).*

5. CD analysis

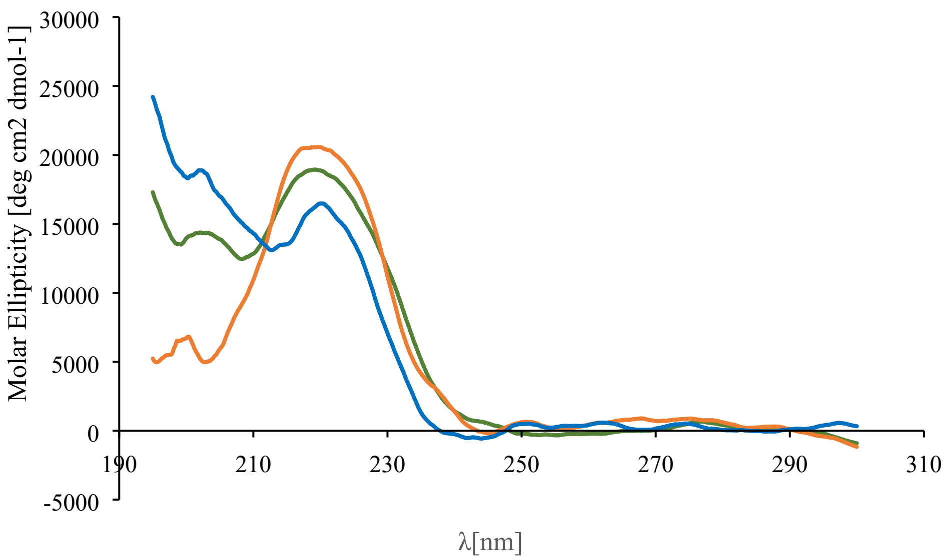


Fig. S19 CD spectra for Leu-Enkephalin (solvents: H_2O (blue); TFE (orange), MeCN (green))

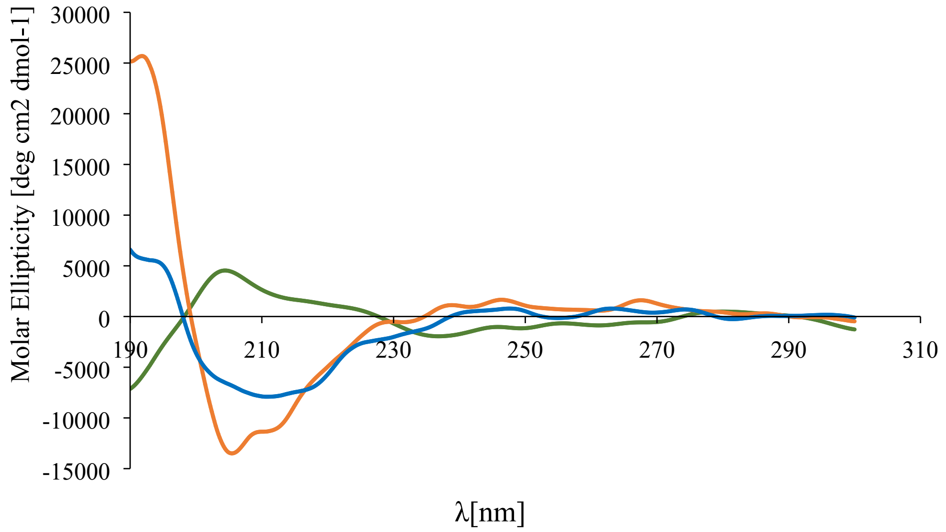


Fig. S20 CD spectra for 1a (solvents: H_2O (blue); TFE (orange), MeCN (green))

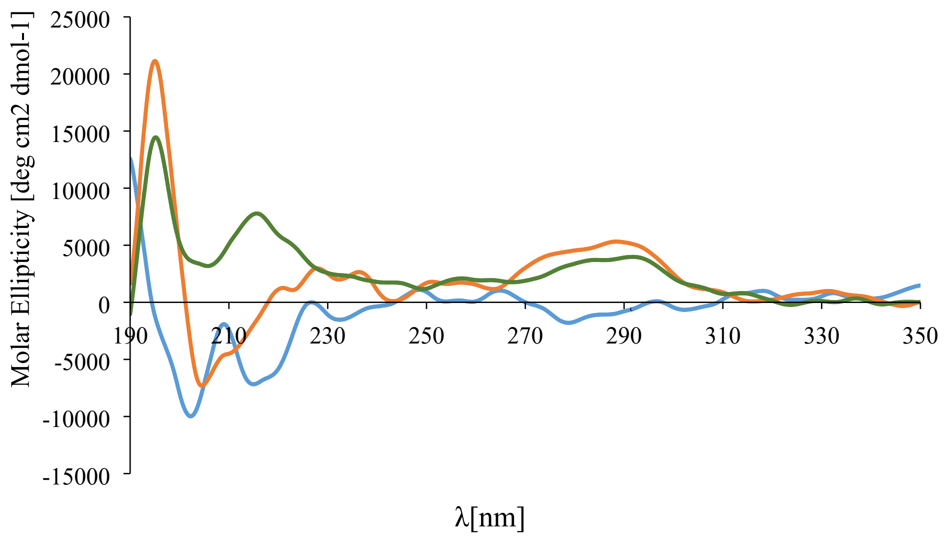


Fig. S21 CD spectra for 2 (solvents: H_2O (blue); TFE (orange), MeCN (green))

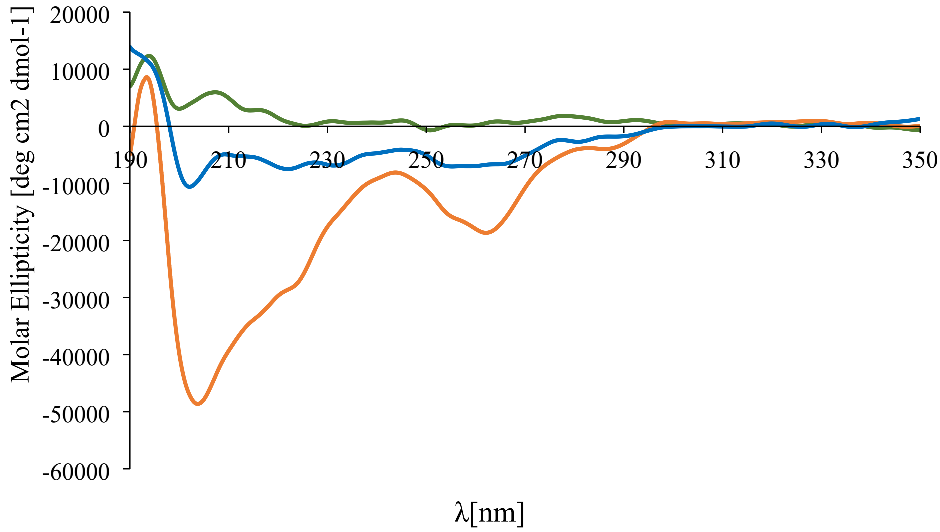


Fig. S22 CD spectra for 3a (solvents: H_2O (blue); TFE (orange), MeCN (green))

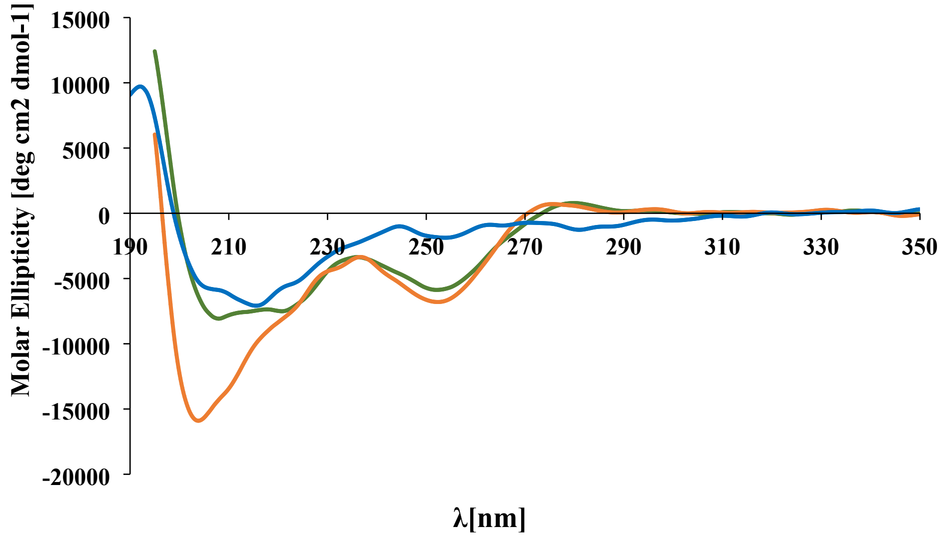


Fig. S23 CD spectra for 3b (solvents: H_2O (blue); TFE (orange), MeCN (green))

6. NMR spectra

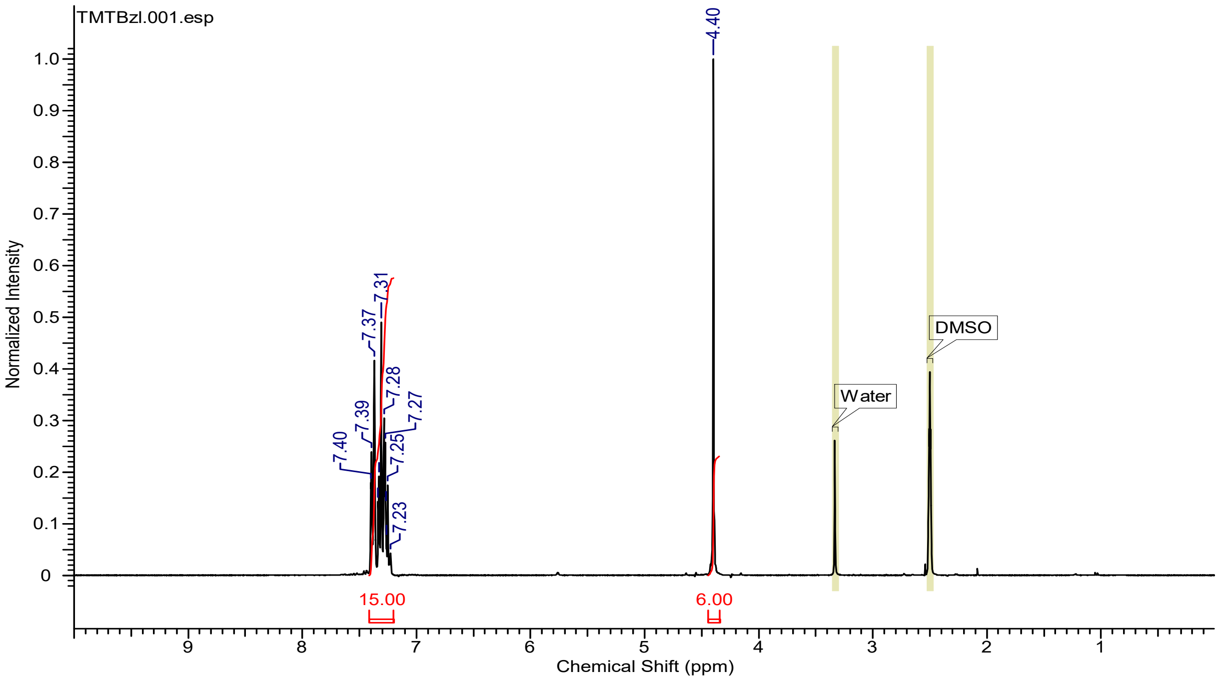


Fig. S24 [1]H NMR spectrum of TMT(Bzl)$_3$

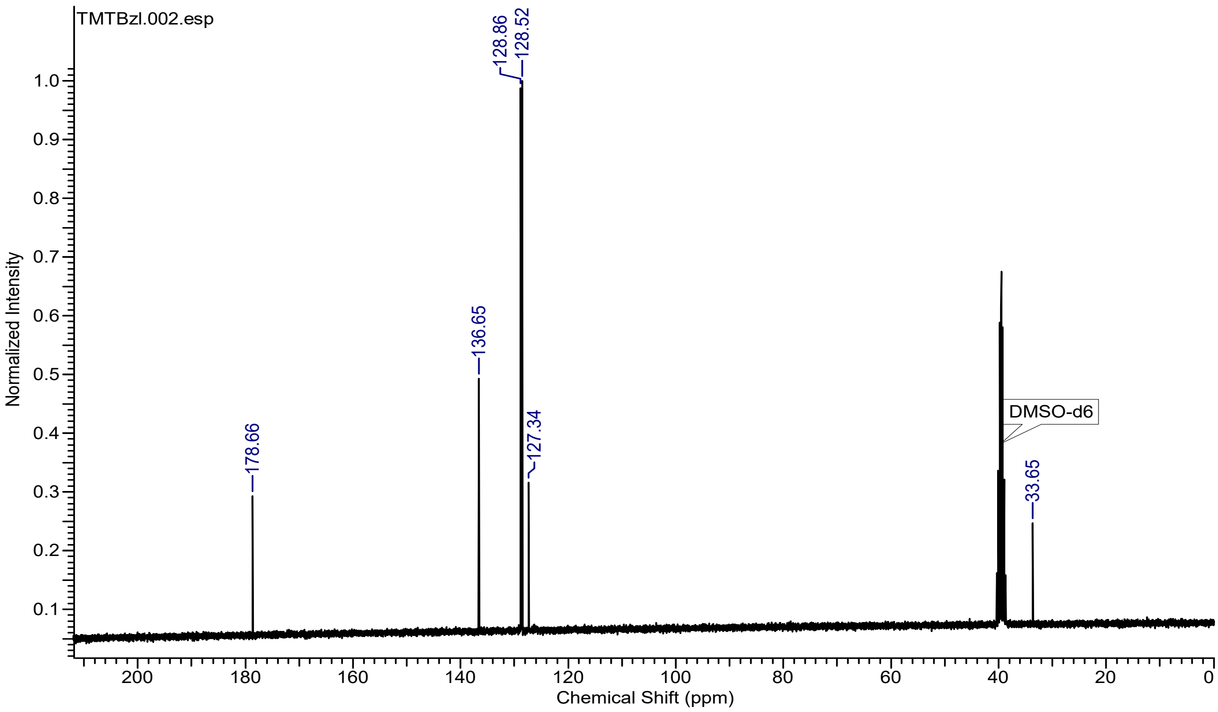


Fig. S25 [13]C NMR spectrum of TMT(Bzl)$_3$

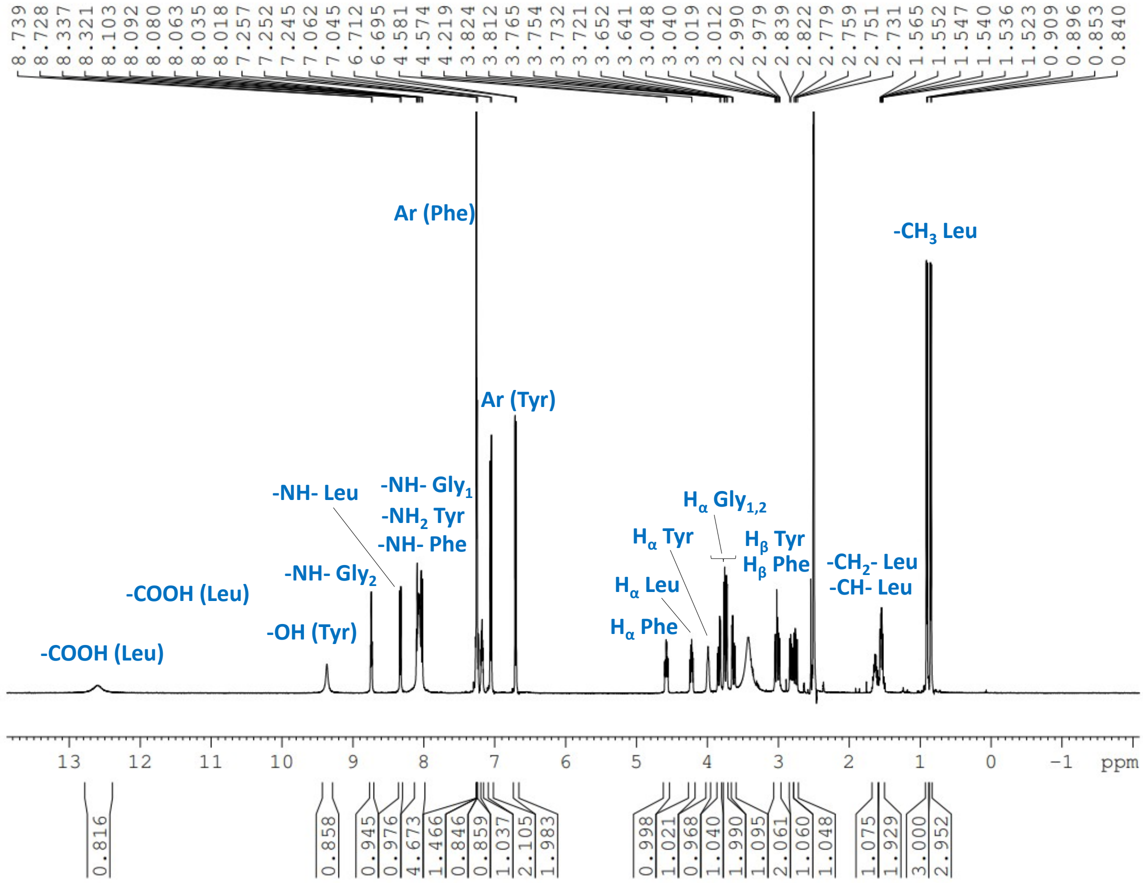


Fig. S26 ^{1}H NMR spectrum of Leu-enkephalin (Leu-Enk.)

Table S9 ^{1}H NMR (500 MHz, 300K, DMSO) analysis of Leu-enkephalin (Leu-Enk.)

	Shifts (ppm)				Coupling constants (*J* in Hz)				
	H_α	H_β	NH	Other	$^{2}J_{\alpha1\alpha1}$	$^{2}J_{\alpha\beta}$	$^{2}J_{\beta\beta}$	$^{3}J_{NH\text{-}H\alpha}$	Other
Tyr1	4.0	3.02 2,78	8.07	9.36 Ar: 6.7; 7.05			14.4		$J_{o.m}$ = 8.5
Gly2	3.75; 3.85	-	8.09					5.5	
Gly3	3.75; 3.63	-	8.74					6.0	
Phe4	4.58	3.02 2.78	8.02	Ar: 7.25		3.75 9.20		8.5	
Leu5	4.22	1.55	8.32	$-C^{\delta}H_3$ 0.87 $-C^{\gamma}H$ 1.62		11.8 1.1		7.9	$J_{CH\text{-}CH_3}$ = 6.5

^{13}C NMR (assignment based on HSQC and ^{13}C) (150 MHz, 300K, DMSO) δ = 21.9, 23.5, 24.8, 36.8, 38.3, 40.4, 42.1, 42.2, 50.8, 54.1, 54.21, 115.9, 125.3, 126.8, 128.5, 129.7, 131, 168.7, 169.1, 171.7, 174.4.

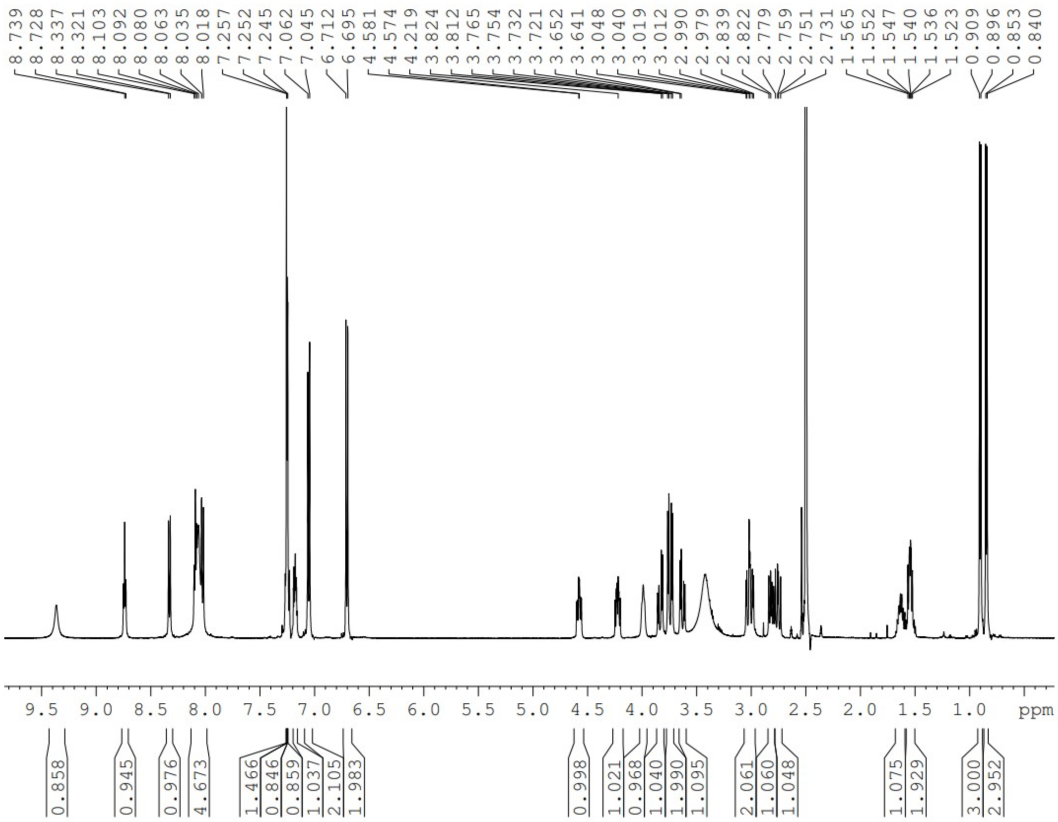


Fig. S27 [1]H NMR spectrum of Leu-enkephalin (Leu-Enk.) (zoom)

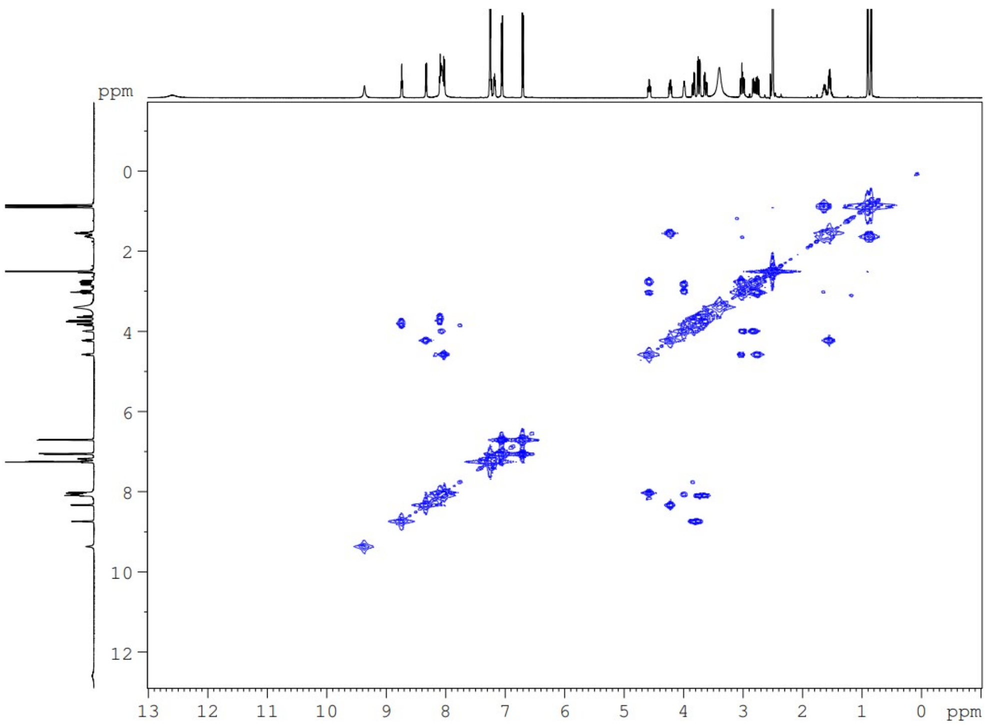


Fig. S28 COSY spectrum of Leu-enkephalin (Leu-Enk.)

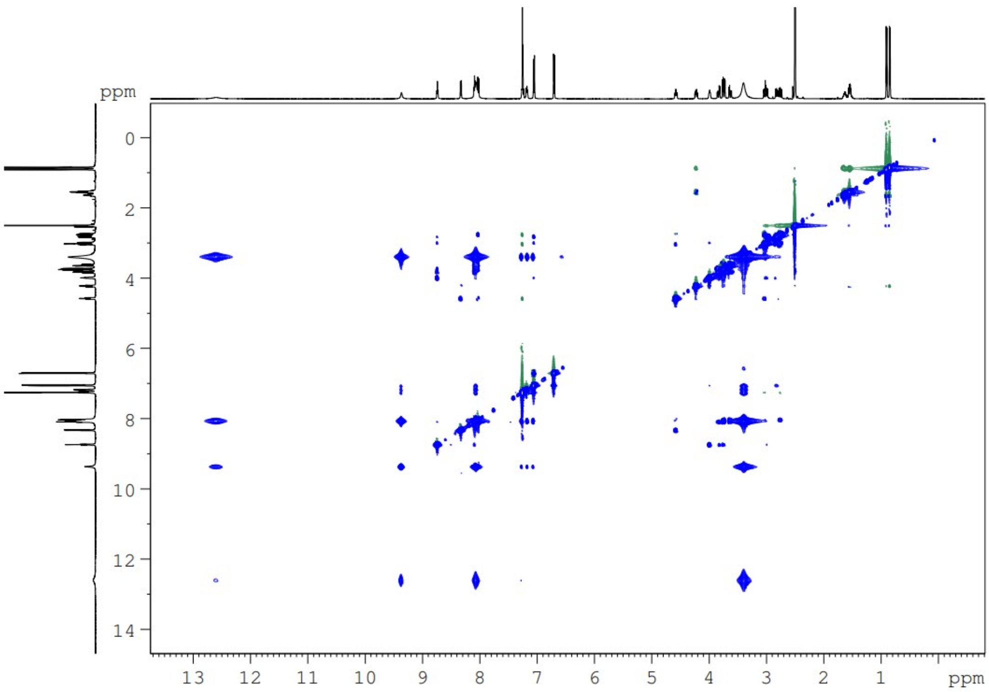


Fig. S29 2D ^{1}H-^{1}H-NOESY NMR spectrum of Leu-enkephalin (Leu-Enk.)

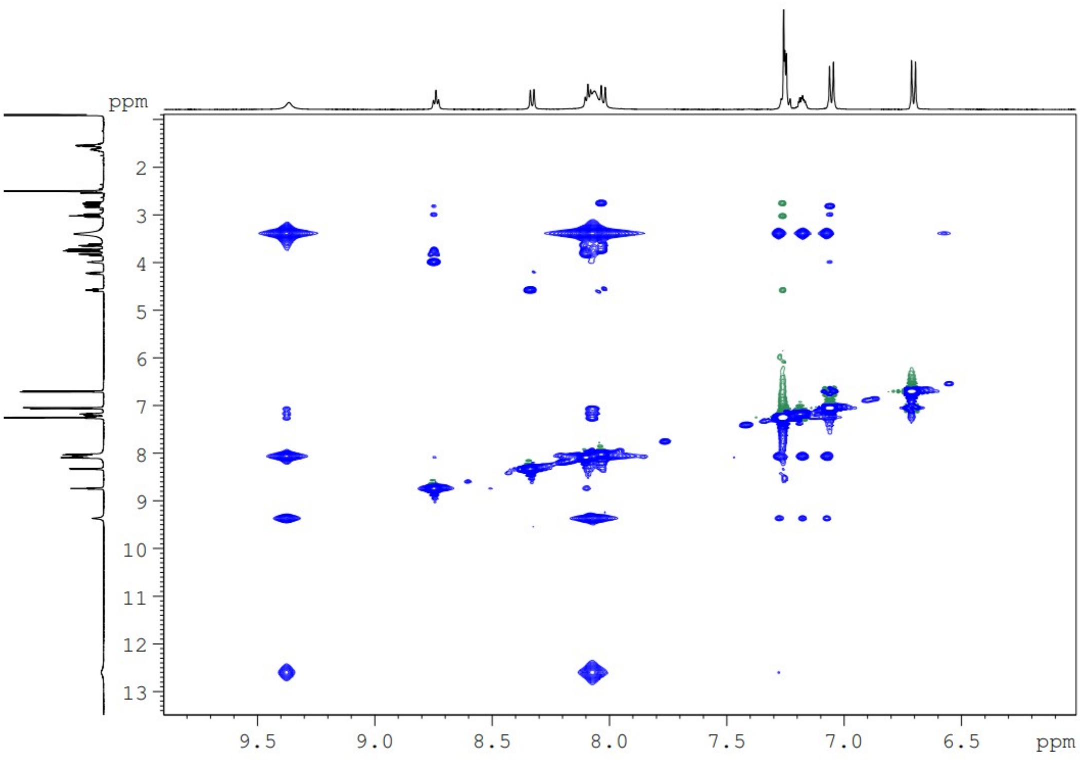


Fig. S30 2D ^{1}H-^{1}H-NOESY NMR spectrum of Leu-enkephalin (Leu-Enk.) (zoom)

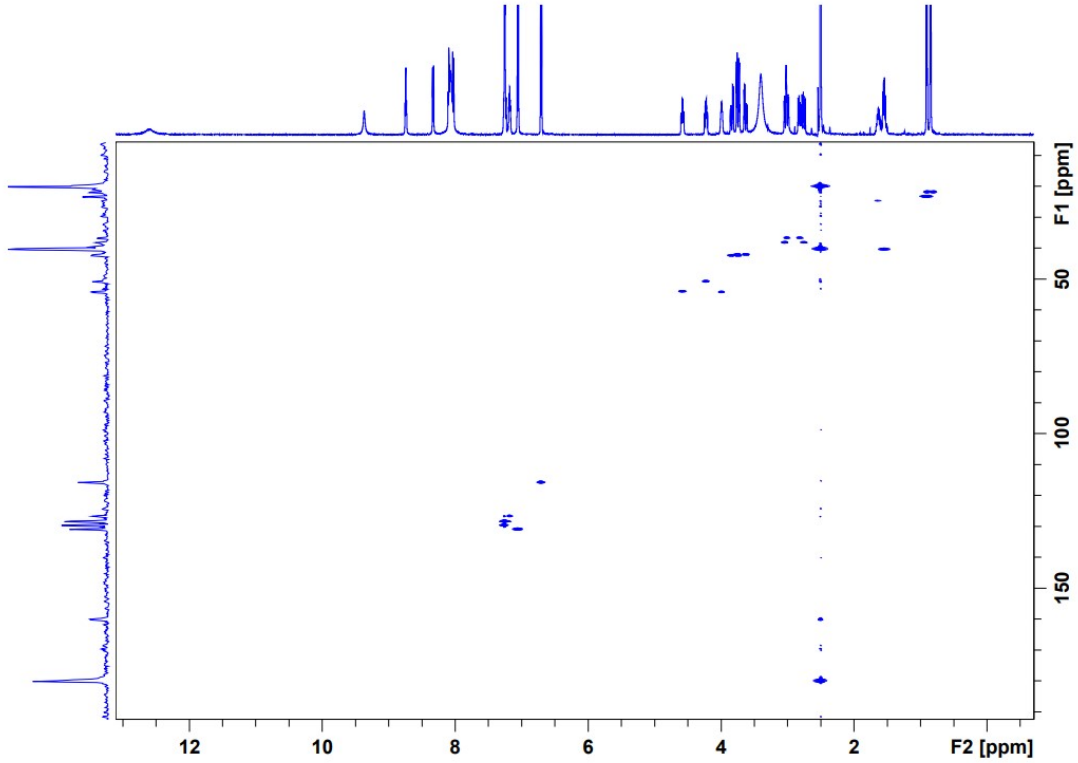


Fig. S31 HSQC spectrum of Leu-enkephalin (Leu-Enk.)

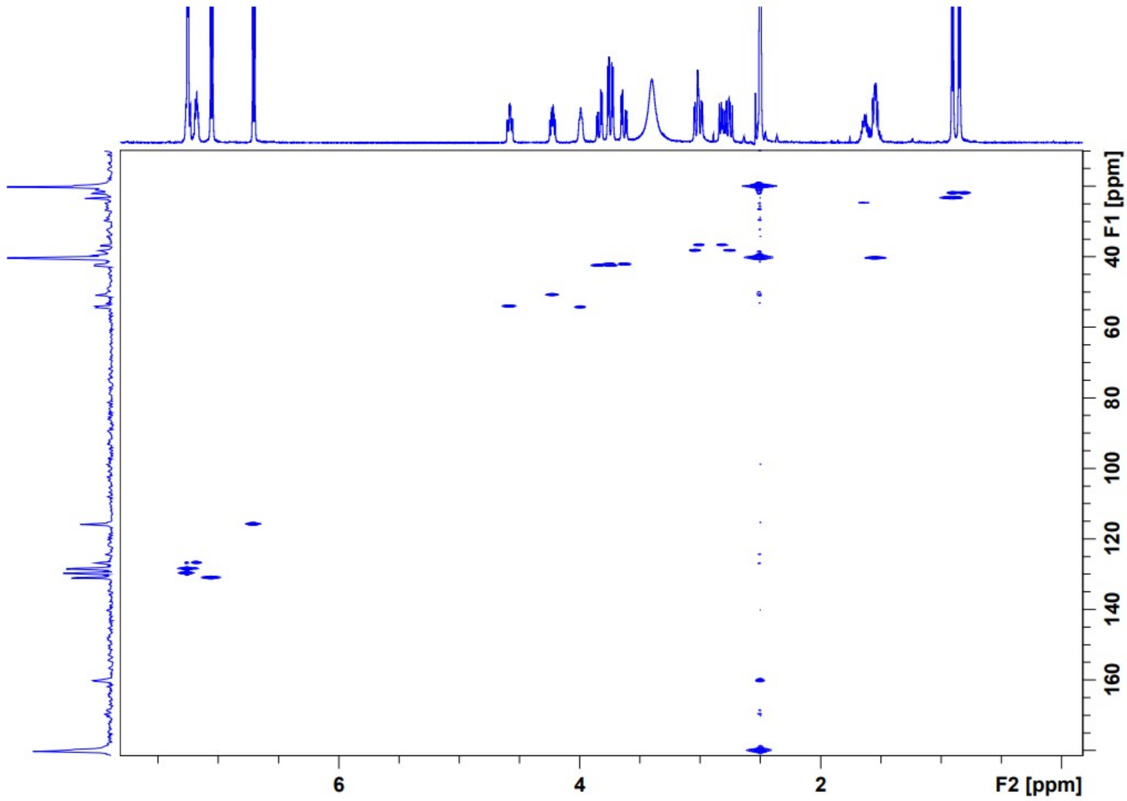


Fig. S32 HSQC spectrum of Leu-enkephalin (Leu-Enk.) (zoom)

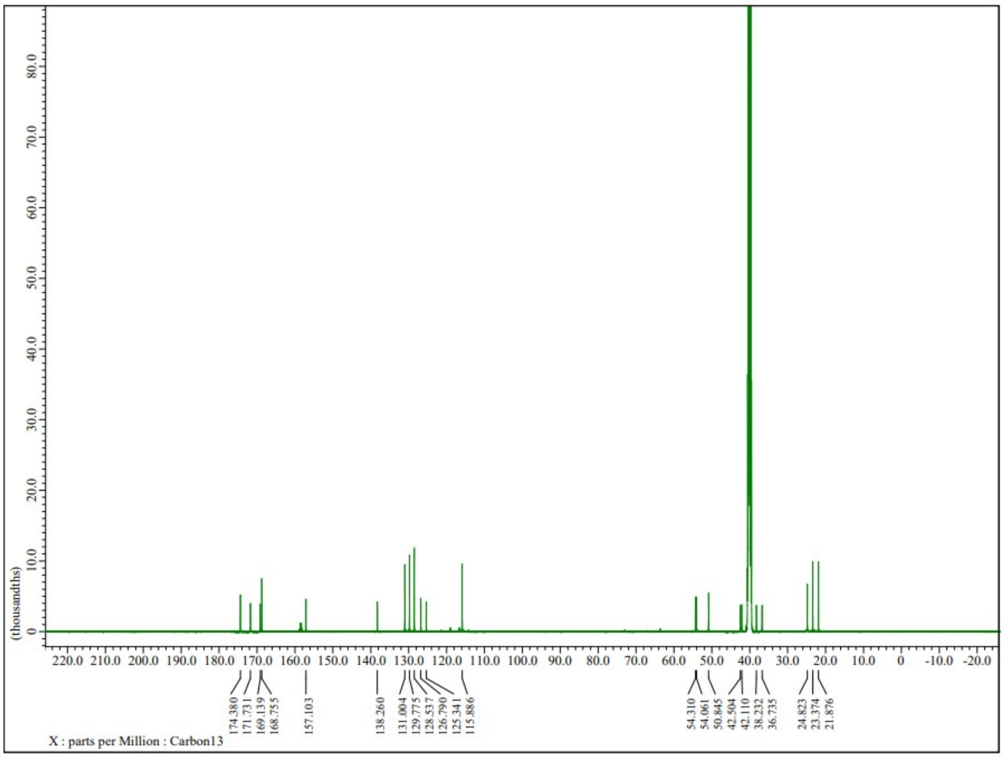


Fig. S33 ^{13}C NMR spectrum of Leu-enkephalin (Leu-Enk.)

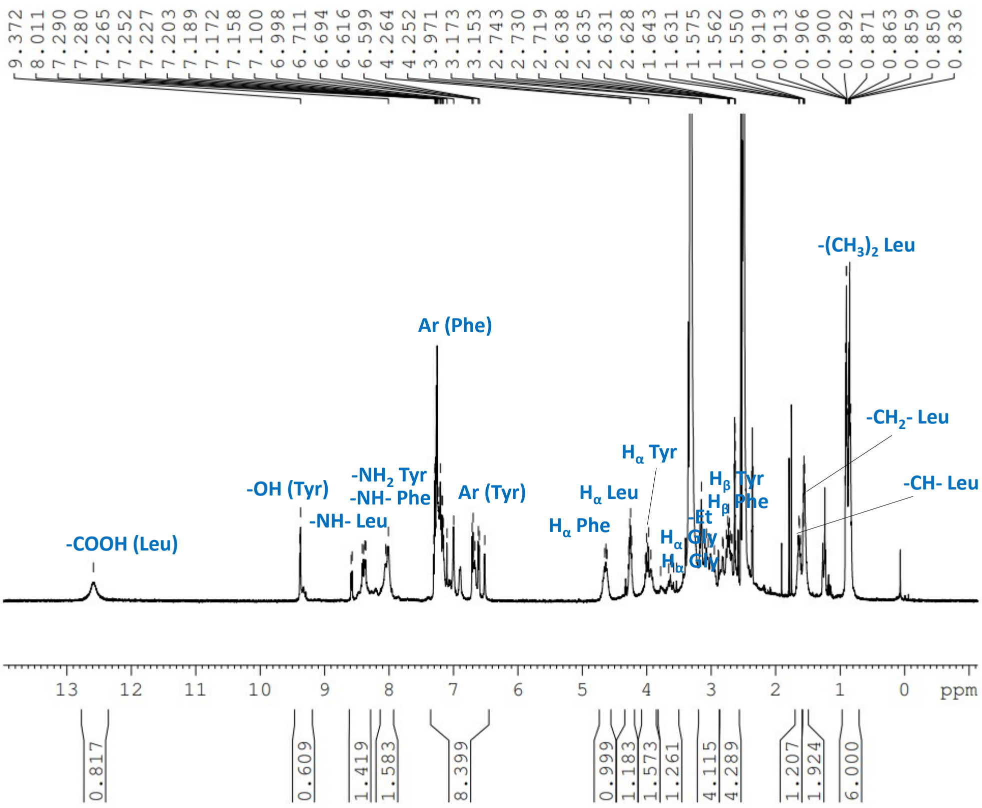


Fig. S34 ^{1}H NMR spectrum of H-Tyr-cyclo-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)

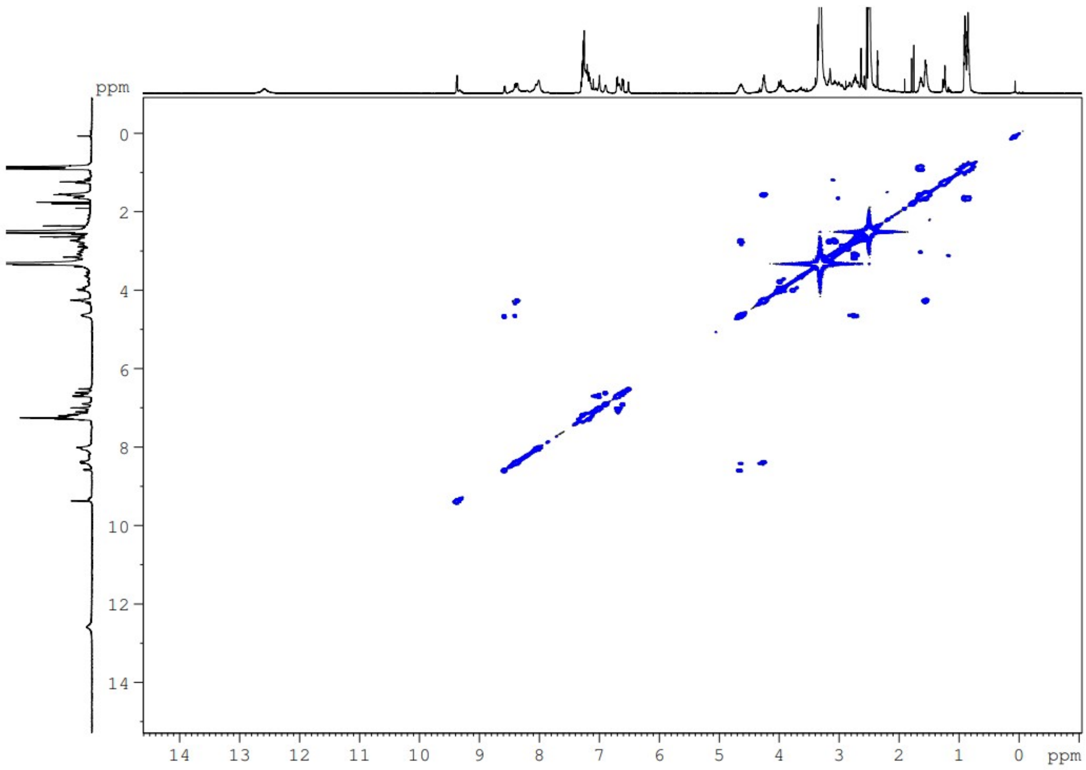


Fig. S35 COSY spectrum of H-Tyr-cyclo-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)

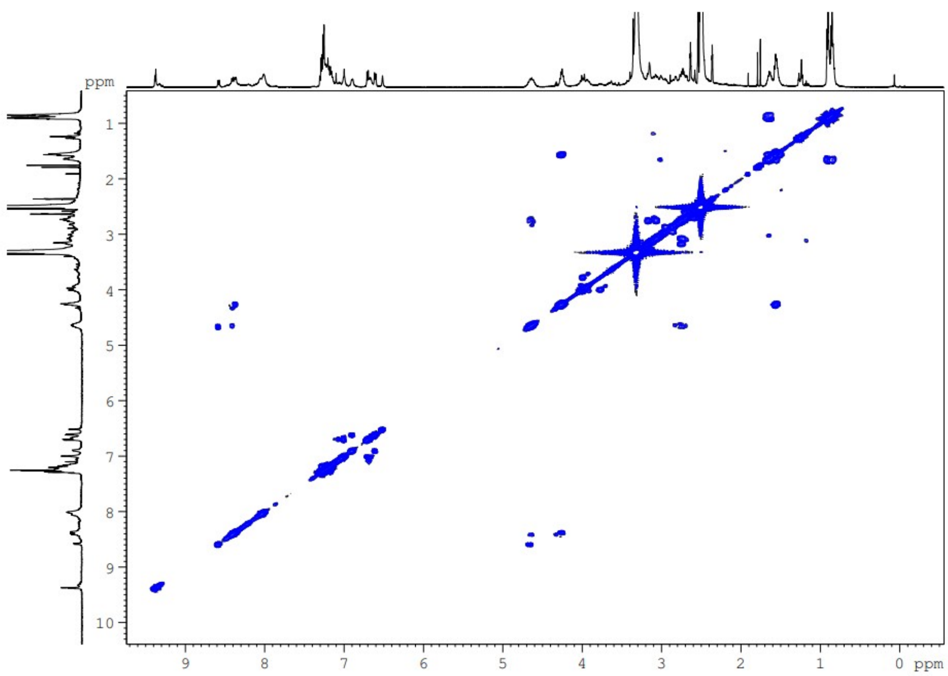


Fig. S36 COSY spectrum of H-Tyr-cyclo-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2) (zoom)

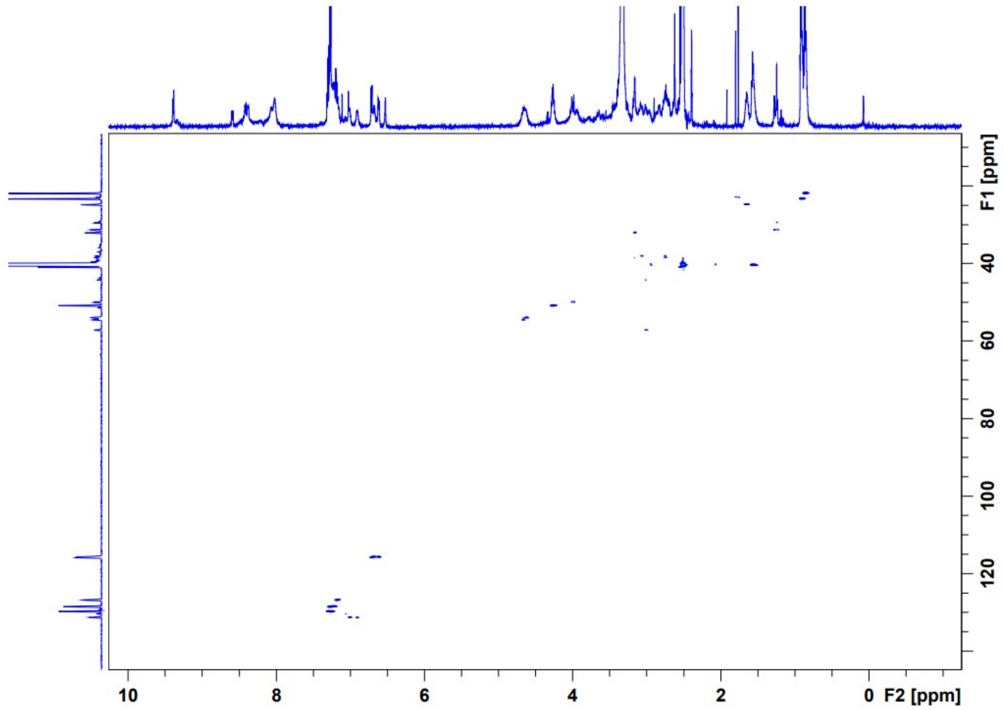


Fig. S37 HSQC spectrum of H-Tyr-cyclo-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)

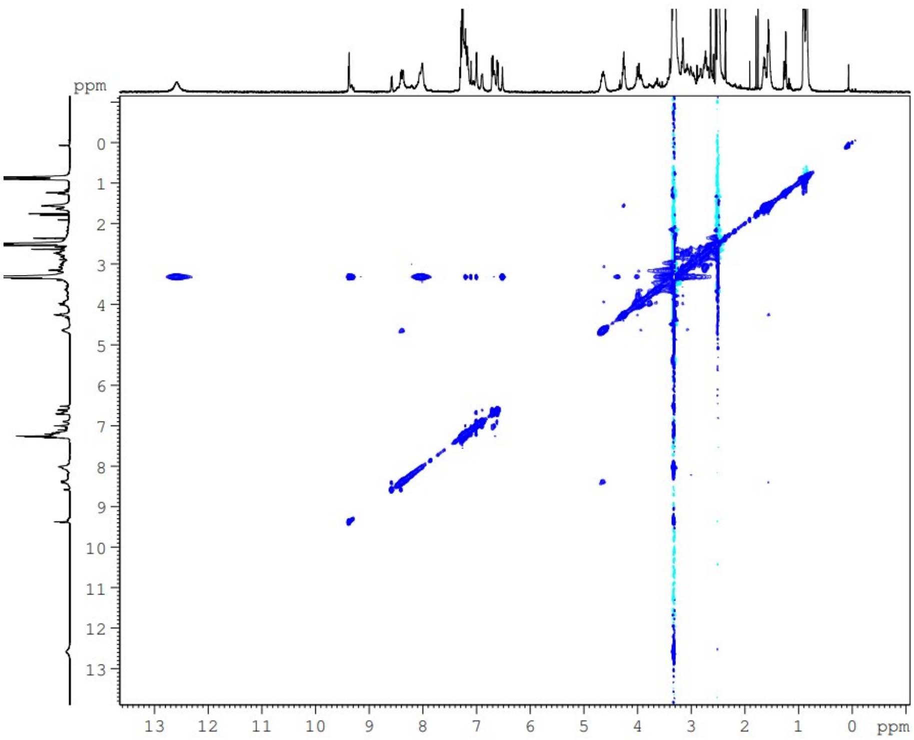


Fig. S38 NOESY spectrum of H-Tyr-cyclo-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2)

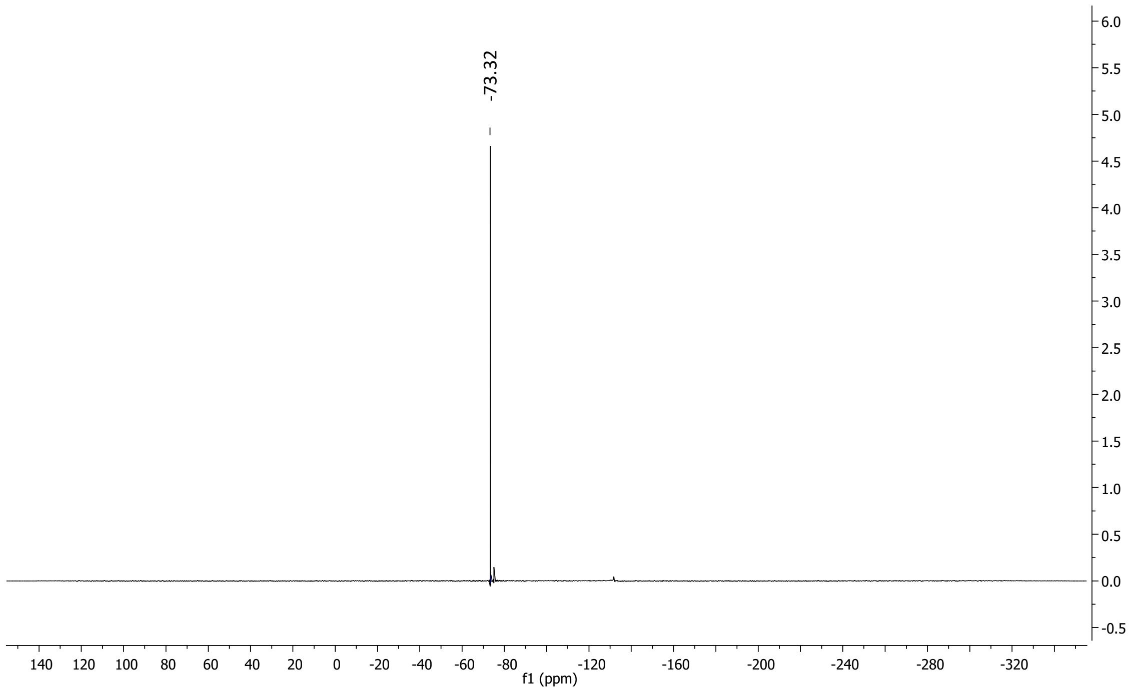


Fig. S39 [19]F NMR spectrum of H-Tyr-cyclo-4FB([*N*-(2-SEt)]Gly-[*N*-(2-SEt)]Gly)-Phe-Leu-OH (2) (zoom)

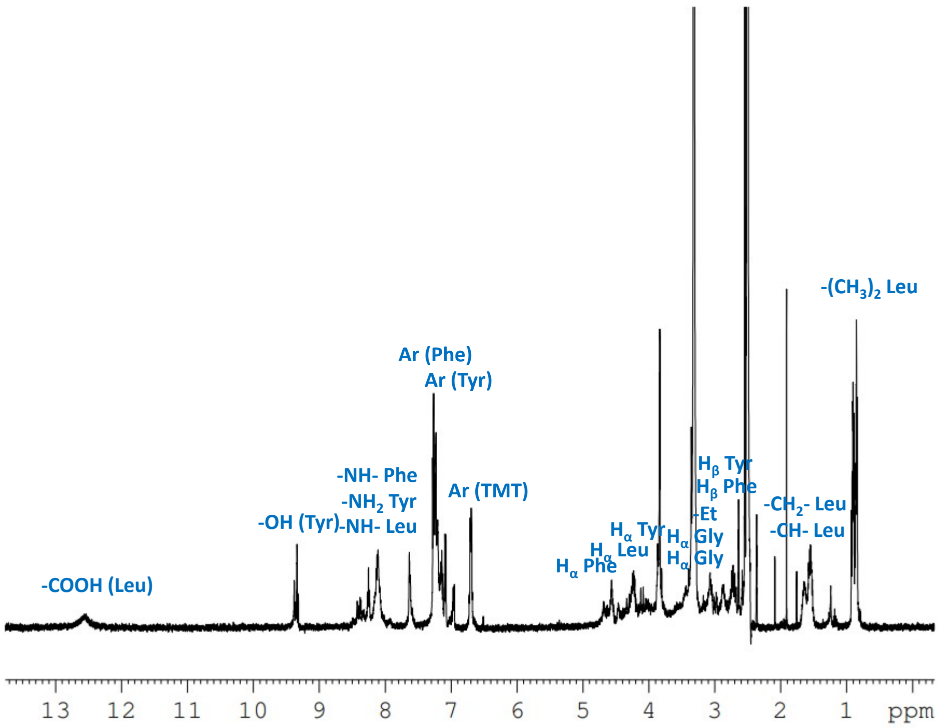


Fig. S40 [1]H NMR spectrum of H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (3a)

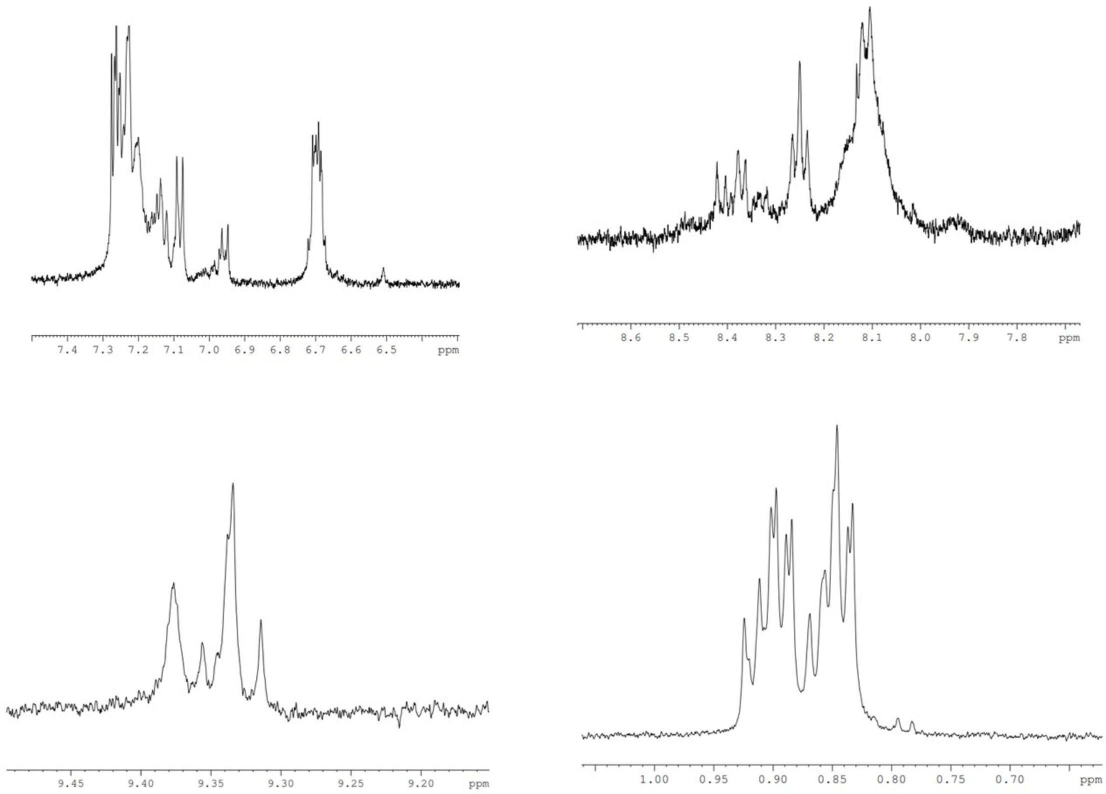


Fig. S41 [1]H NMR spectra of H-Tyr-*cyclo*-TMT([*N*-(2-SEt])]Gly-[*N*-(2-SEt)]Gly)(Acm)-Phe-Leu-OH (3a) (zoom regions)

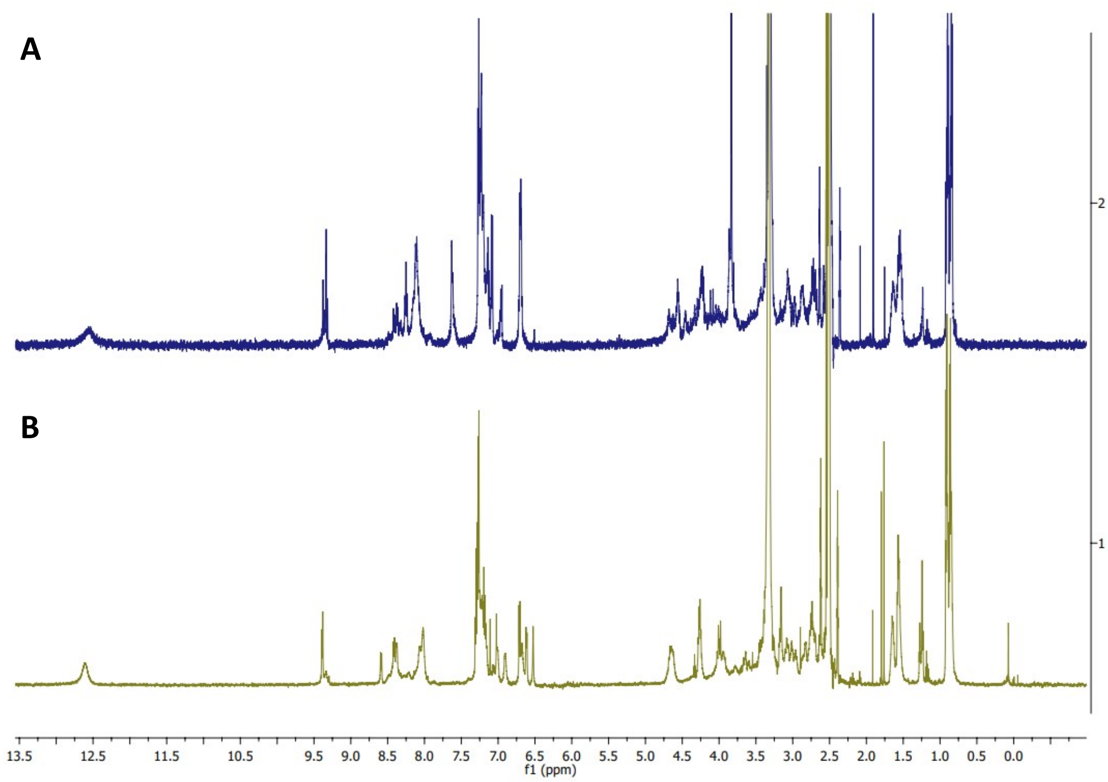


Fig. S42 Comparison of [1]H NMR spectra for stapled analogs 3a (A - navy) and 2 (B - green)

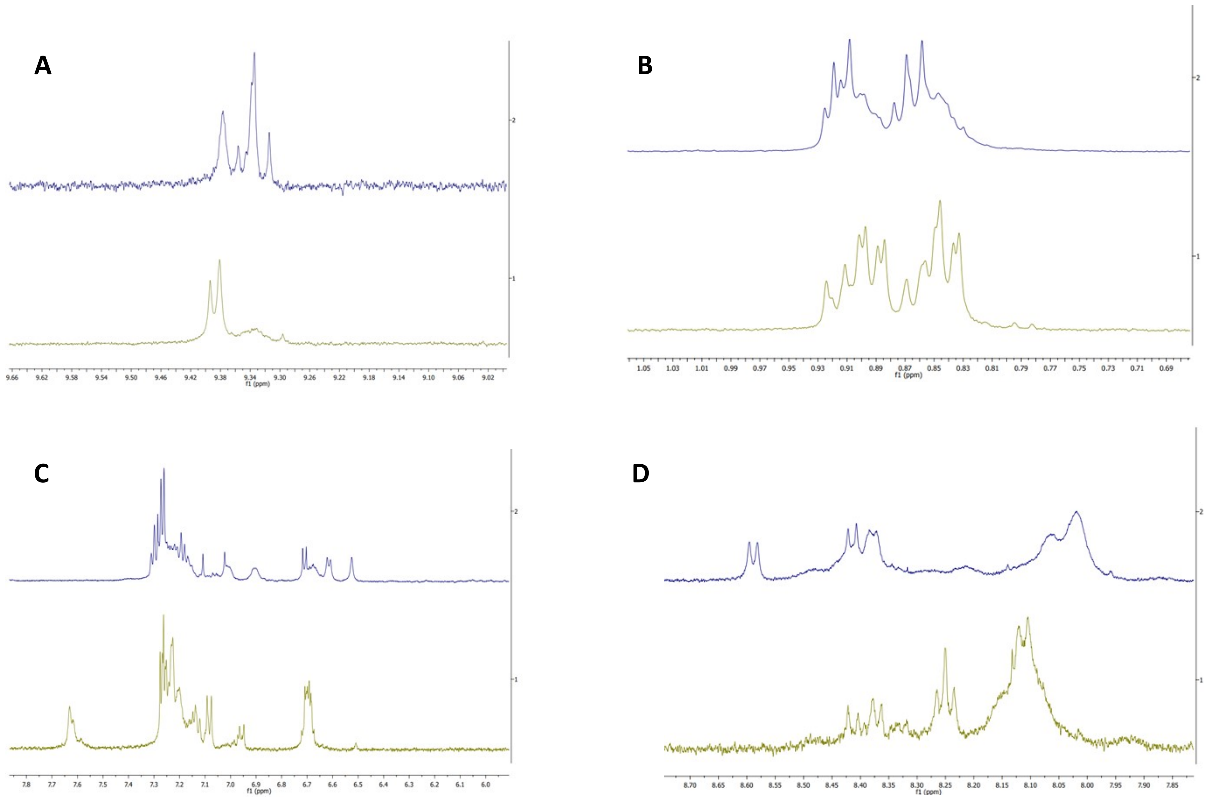


Fig. S43 Comparison of ^{1}H NMR spectra for stapled analogs 3a (navy) and 2 (green) (zoom regions: A - -OH (Tyr); B – $(\text{-CH}_3)_2$ (Leu); C – Aromatic Phe and Tyr; D – amide region)

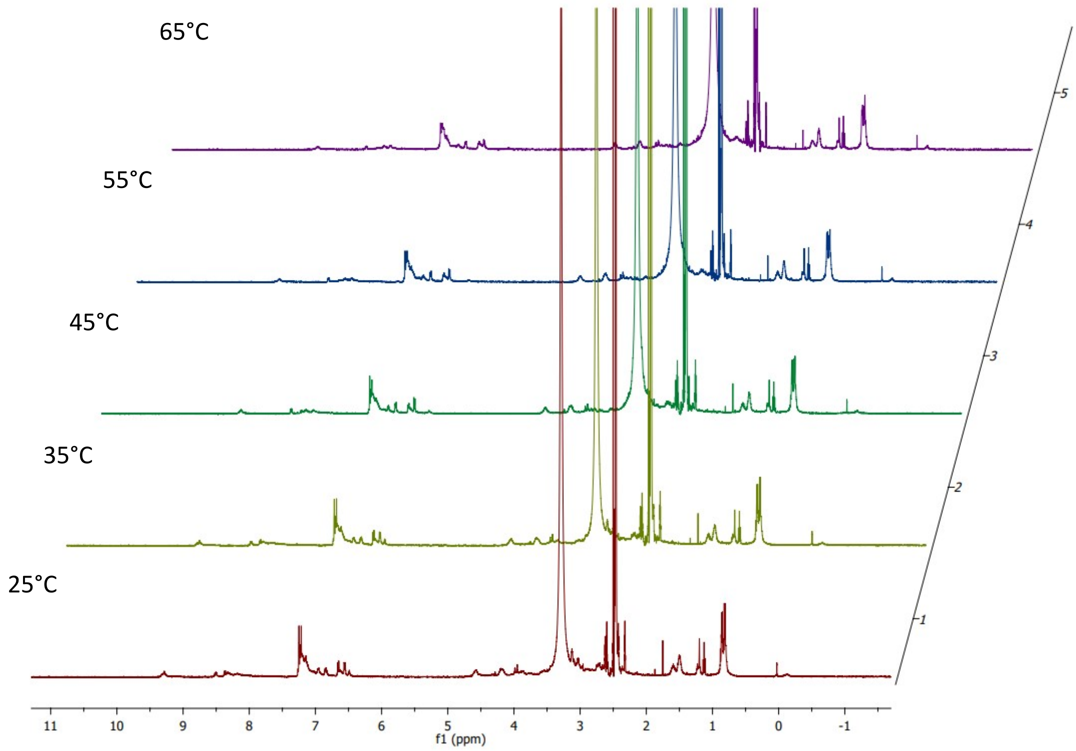


Fig. S44 VT ^{1}H NMR spectra for stapled analog 2

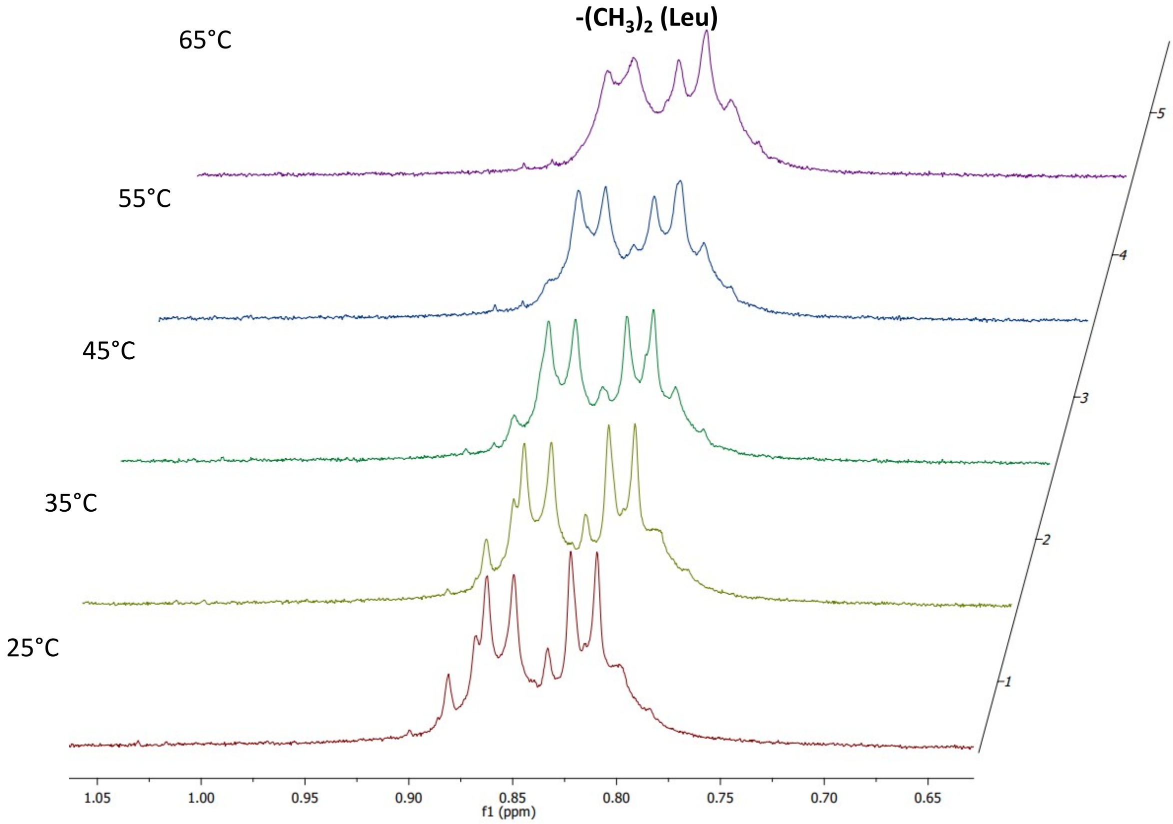


Fig. S45 VT [1]H NMR spectra for stapled analog 2 (zoom)

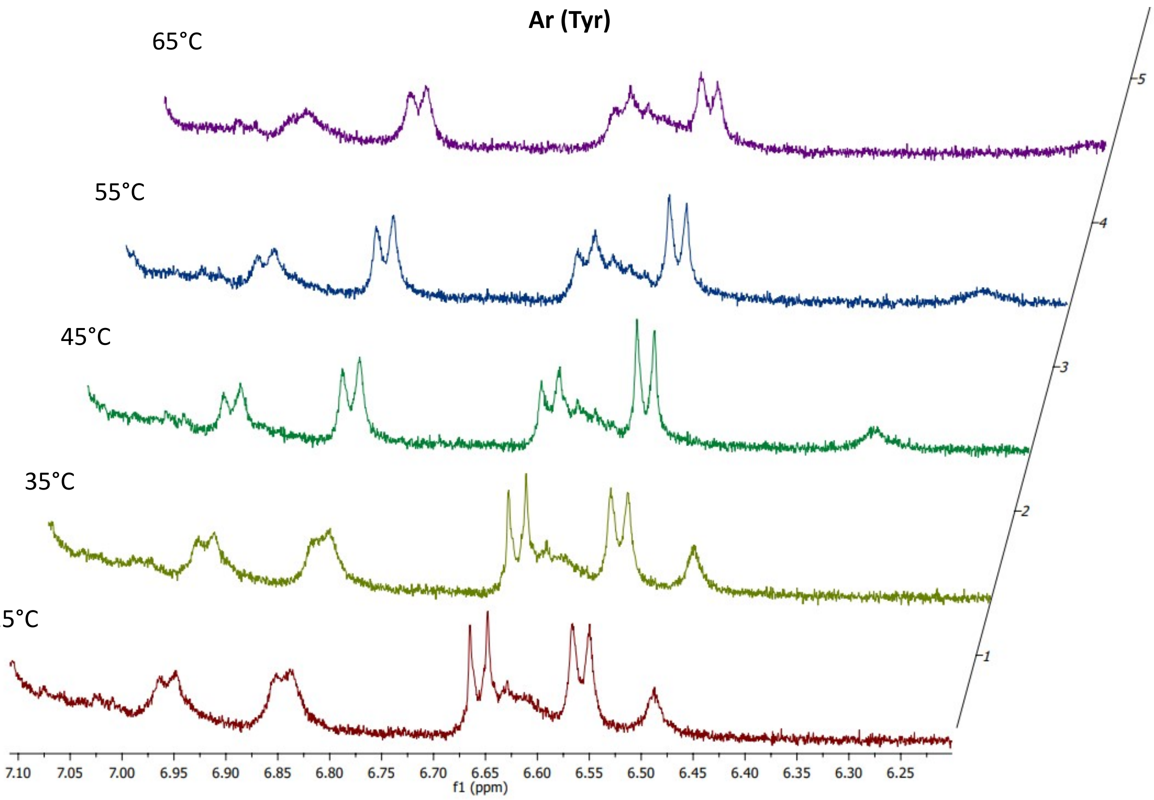


Fig. S46 VT [1]H NMR spectra for stapled analog 2 (zoom)

5. Theoretical calculation

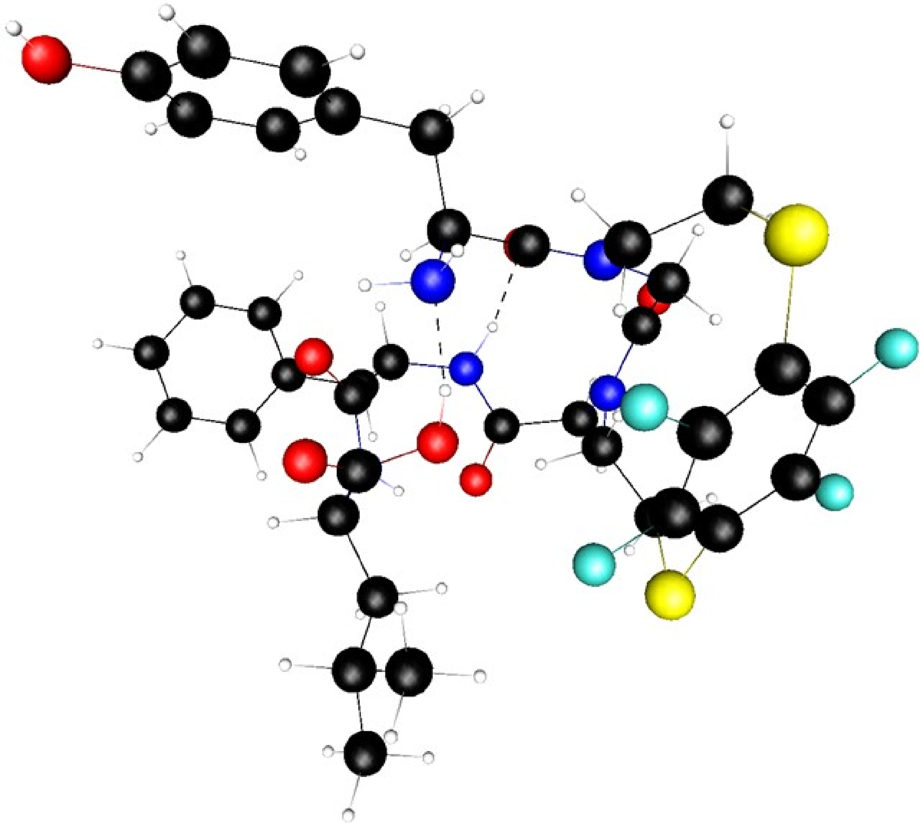


Fig. S47 The structure of **2**

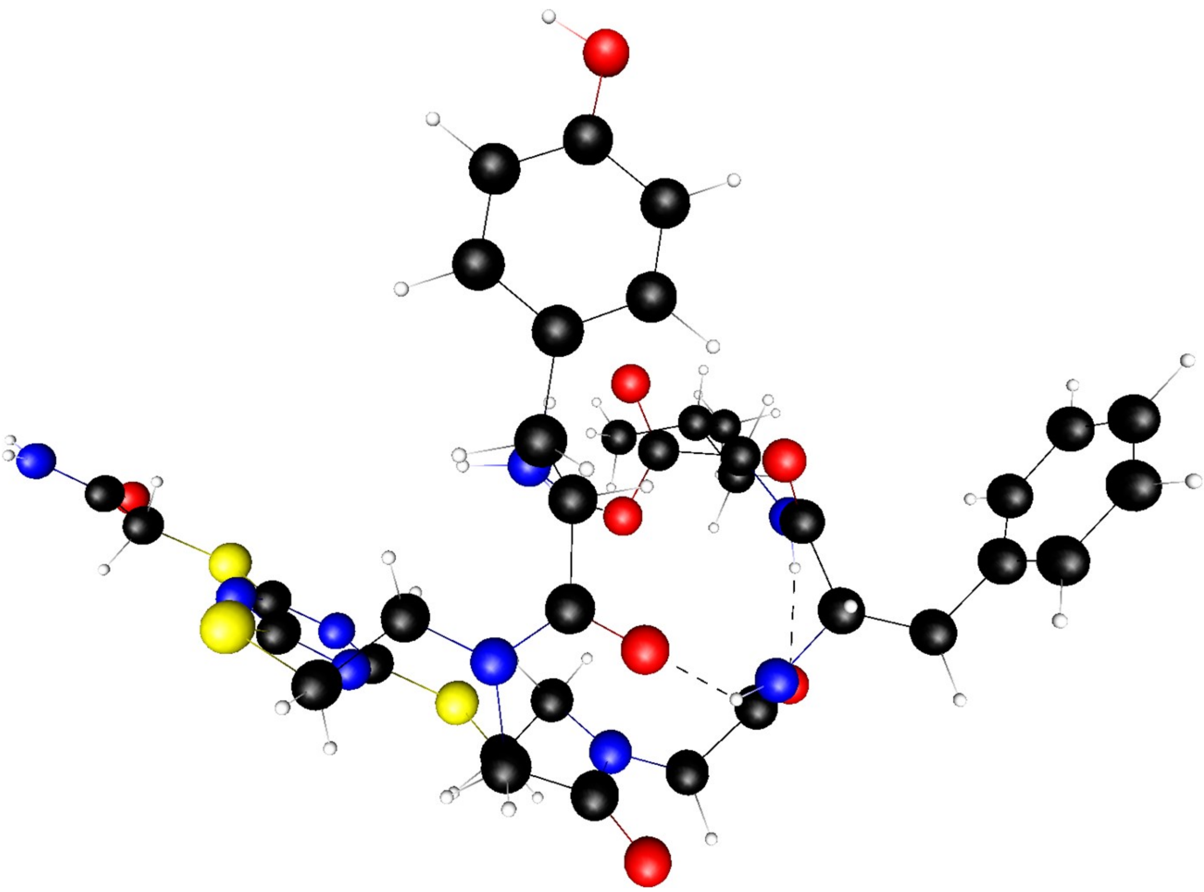


Fig. S48 The structure of **3a**

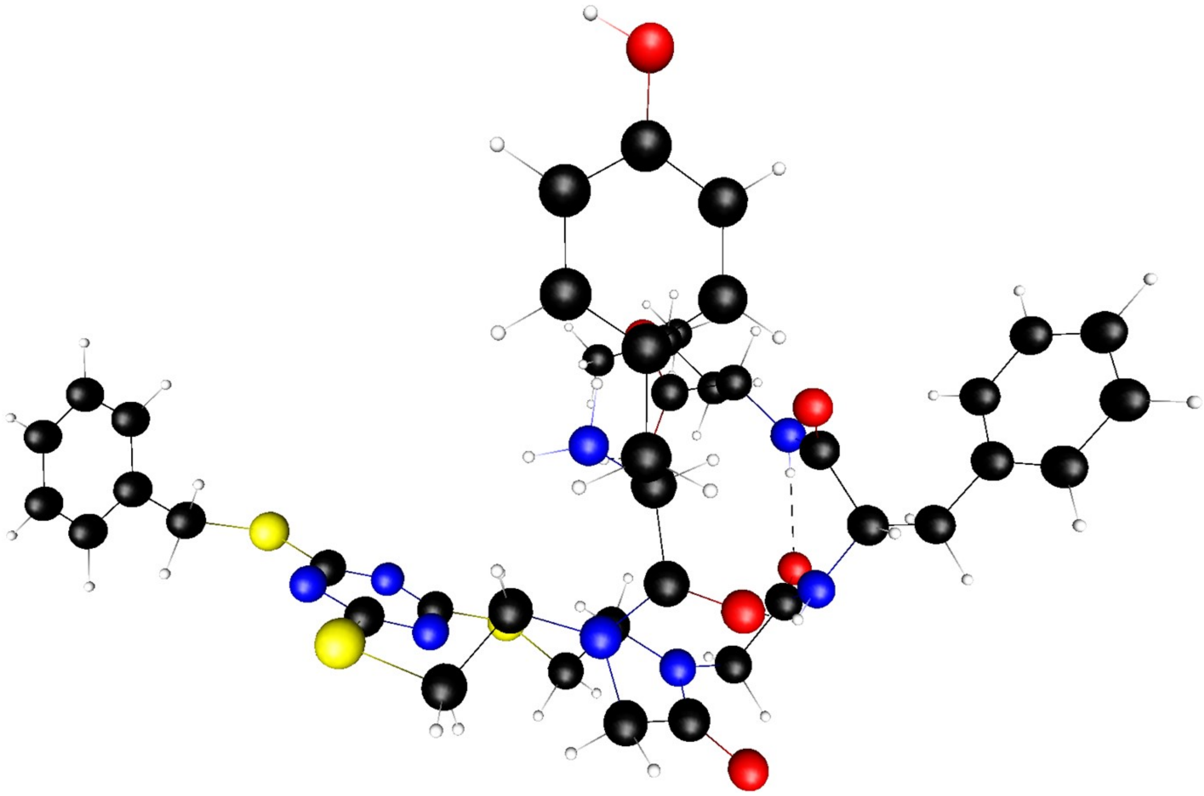


Fig. S49 The structure of **3b**

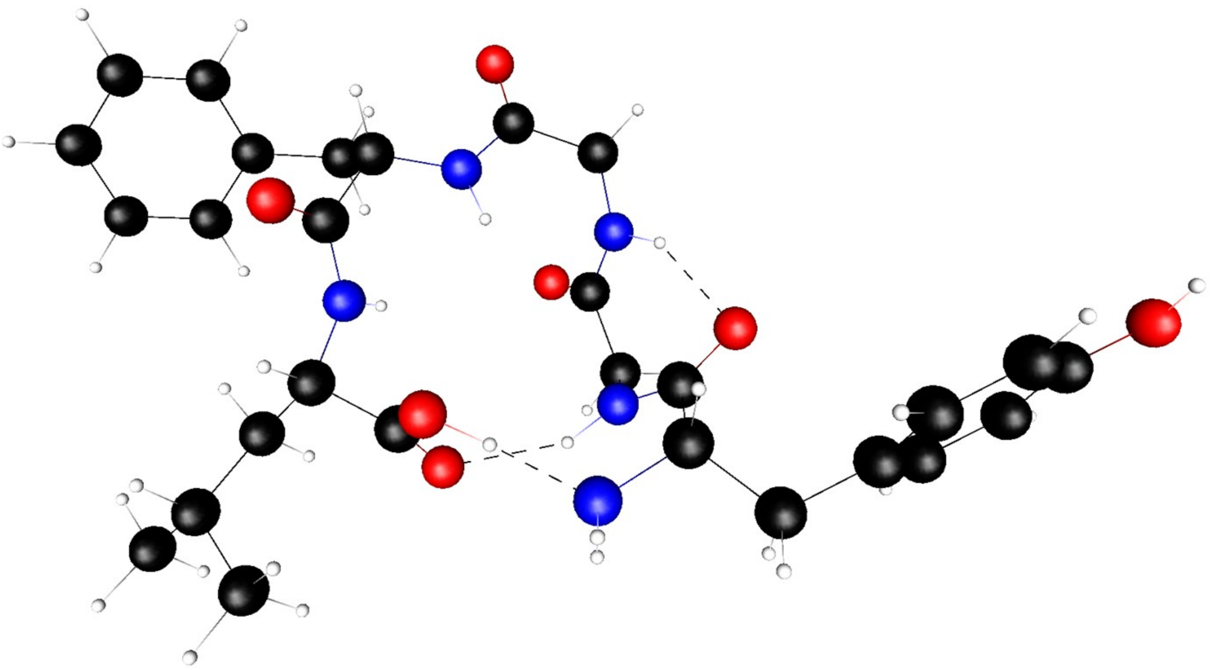


Fig. S50 The structure of **Leu-Enk.**

Table S10 The backbone dihedrals Ψ (NCCN) and Φ (CNCC) presented in deg.

No.	Amino acid residue									
	1		2		3		4		5	
	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ
2	-	-36.9	-30.8	-61.0	-115.6	55.1	74.1	-61.7	-41.8	-
3a	-	-37.9	-49.2	-49.8	-95.7	45.8	73.2	-66.7	-174.8	-
3b	-	-37.4	-50.1	-48.8	-96.3	46.5	73.4	-66.5	-51.2	-
Leu-Enk.	-	129.8	-151.8	170.8	94.2	8.9	85.2	-78.0	73.5	-

Table S11 The hydrogen bonds. Proton - proton donor (H..PA) and proton donor – proton acceptor distances in Angstroms, PD-H..PA angle in deg.

Residue	H..PA [Å]	PD..PA [Å]	PD-H..PA[deg]	Fragment
2				
Tyr..Phe	1.906	2.879	159.9	N-H..O (β-turn)
$C_{terminus}$..$N_{terminus}$	1.696	2.649	156.7	O-H..N
Gly2-Leu	1.975	2.893	149.3	N-H..O (γ-turn)
3a				
Tyr..Phe	2.086	2.974	145.6	N-H..O (β-turn)
$C_{terminus}$..$N_{terminus}$	1.485	2.532	156.5	O..H-N
Gly2-Leu	1.967	2.886	149.5	N-H..O (γ-turn)
3b				
Tyr..Phe	2.089	2.974	145.2	N-H..O (β-turn)
$C_{terminus}$..$N_{terminus}$	1.490	2.534	156.4	O..H-N
Gly2-Leu	1.971	2.888	149.3	N-H..O (γ-turn)
Leu-Enk.				
Tyr..Leu	1.973	2.904	151.8	N-H..O
$C_{terminus}$..$N_{terminus}$	1.651	2.667	176.5	O-H..N
Tyr..Gly2	1.963	2.878	148.9	N-H..O (γ-turn)

Table S12 The cartesian coordinates of 2, 3a, 3b and Leu-Enk. molecules

2			
C	-0.62514600	2.19515600	-0.65517500
H	-1.53114200	1.59331400	-0.71519300
C	0.24892600	1.54093100	-1.73387400
O	-0.33946300	1.01970800	-2.67910900
C	-0.99792800	3.63133100	-1.07331400
H	-1.32107600	3.60392200	-2.11483100
H	-0.11812900	4.27633900	-1.02565100
N	1.59887300	1.52199700	-1.66314500
C	2.35746500	0.73931800	-2.64027700
H	2.58178200	1.33846700	-3.52030000
C	1.70077600	-0.52786300	-3.20220800
O	1.54176200	-0.61365700	-4.40611100
N	1.36541500	-1.54270100	-2.35377500
C	0.57959100	-2.64605100	-2.87431600
H	0.46469100	-2.50052800	-3.94493300
C	-0.79135200	-2.71531000	-2.20423700
O	-1.16744500	-3.74380100	-1.64759400
N	-1.50562400	-1.58227600	-2.27923300
H	-1.03714600	-0.75320300	-2.62725200
C	-2.80348600	-1.33192500	-1.66712300
H	-3.12027900	-0.36126700	-2.04288100
C	-2.67952900	-1.12725300	-0.15329100
O	-2.98763100	-0.06188800	0.36474800
C	-3.87670900	-2.36439400	-2.04832300
H	-3.94367000	-2.37744400	-3.13682900
H	-3.56512200	-3.35508600	-1.72697300
N	-2.19639800	-2.16666900	0.54842500
H	-1.87518100	-2.97408500	0.02806600
C	-1.81177200	-2.03138300	1.94095200
H	-2.69939100	-1.96969600	2.57142700
C	-1.06174800	-0.71851300	2.15075400
C	-0.98965000	-3.26412900	2.33121600
H	-1.61550400	-4.12883300	2.09921300
H	-0.11179500	-3.32708600	1.68056300
O	-0.10250200	-0.53510800	1.25870400
C	2.34296600	2.35044600	-0.70671500
H	1.71003900	3.17443700	-0.40387200

H	2.54694900 1.75029500 0.17517500
C	1.78550400 -1.66240000 -0.96501600
H	2.20913900 -0.71805700 -0.64134500
H	0.91734500 -1.83151300 -0.33042500
C	2.80235000 -2.79680300 -0.79724300
H	2.31896800 -3.77033900 -0.85920200
C	3.64021500 2.97430300 -1.23928400
H	4.11956000 2.38892900 -2.01574300
H	3.46474200 3.96886100 -1.64253400
S	4.87461900 3.21566800 0.10386400
C	4.94852200 1.50702200 0.57355900
S	3.60532100 -2.85127700 0.84425600
H	3.56408600 -2.74875700 -1.57062800
C	-2.09104700 4.18592400 -0.19773900
C	-1.82013900 5.11312100 0.80056400
C	-3.40311700 3.73351600 -0.33349400
C	-2.82095000 5.58573800 1.63839100
H	-0.80964100 5.48057100 0.93028400
C	-4.41202400 4.19211300 0.49312500
H	-3.64123600 3.00520500 -1.09933600
C	-4.12207800 5.12336300 1.48678000
H	-2.58934200 6.31120800 2.40813800
H	-5.42767700 3.83797600 0.38161800
O	-5.14742800 5.54158000 2.27271700
H	-4.82223600 6.17110100 2.91898800
H	1.07138500 -3.60103500 -2.70613300
C	-5.20778200 -2.00616800 -1.43840500
C	-6.01119800 -1.02479500 -2.01415400
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C	-7.21383400 -0.65883100 -1.42820100
H	-5.69181000 -0.54532300 -2.93168600
C	-6.83758800 -2.24947200 0.32927800
H	-5.01641800 -3.37370400 0.19920300
C	-7.63001800 -1.27035700 -0.25266500
H	-7.82794800 0.10272900 -1.89012900
H	-7.15546400 -2.73230400 1.24375300
H	-8.56799000 -0.98635700 0.20511800
C	-0.55111800 -3.34436900 3.79986600
H	-1.35980800 -2.94378800 4.41857700
C	-0.33655300 -4.80653500 4.19072700
H	-0.01670800 -4.88937200 5.23012800
H	-1.25035300 -5.38949100 4.06821200

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H	0.60000000	-1.47938500	3.87304500
H	1.00852500	-2.64063400	5.12742300
H	3.30500200	0.45410300	-2.18370800
O	-1.30888500	0.05131500	3.04830600
H	-0.98649100	2.16698800	1.33136200
H	0.09766200	0.44650700	1.18040800
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N	-0.17252500	2.06074700	0.73448000
C	4.24938000	1.04162000	1.67880800
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C	4.31043600	-1.22908200	0.85913000
C	5.44785900	0.55544000	-0.30622600
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F	3.14833600	-0.65524200	2.82696700
F	6.13531900	0.93022100	-1.38111600
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F	5.59903000	-1.63373700	-1.07755400
			3a
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C	0.37825200	-1.80253400	-3.37260000
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C	-0.52443800	-2.83600500	-2.69805400
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C	-2.58513900	-3.21146500	-0.12724100
O	-2.99284900	-3.52420300	0.98805800
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H	-4.49222600	-0.84636200	-1.45604100

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C	-5.51319500	-2.14752900	-0.09570100
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C	-1.93763200	6.12096100	-0.26687700

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C	-8.72348900	0.64626200	0.53936700
H	-9.02517300	0.53010800	-1.58224600
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H	-1.86986200	1.06575800	4.89948400
C	-1.79178000	-0.59386400	6.24512900
H	-1.23546900	-0.08075100	7.03087400
H	-2.85282100	-0.54888800	6.49485300
H	-1.48915700	-1.64422700	6.25351600
C	-0.00873100	0.04792800	4.60829200
H	0.38039700	-0.97308900	4.58123700
H	0.23662300	0.52944100	3.66416300
H	0.51475700	0.58337900	5.40205100
H	1.38239100	-2.20963500	-3.40386600
S	7.20021200	-0.37061800	1.63995700
C	8.00698600	0.96181500	0.71535100
H	8.30280500	0.60345600	-0.26809900
H	7.31541800	1.79062600	0.58150500
C	9.22544400	1.40769700	1.50560900
O	-1.17973600	1.87679800	1.95182100
H	-0.35200200	2.32600900	0.04423900
H	-0.09481100	0.72485300	0.13863200
H	0.94852700	1.79562000	-0.81457700
N	-0.00461000	1.57159900	-0.55702100
O	9.53795500	0.88932000	2.56476900
H	9.64168700	2.82805800	0.07258500
N	9.91997500	2.40849100	0.94029000
H	10.73710200	2.75944800	1.40717700
3b			

C	1.44366700	1.10137700	1.91513400
H	2.37536800	1.09138000	1.35025100
C	1.41491900	-0.30977400	2.51155600
O	2.50607600	-0.82355500	2.72825200
C	1.53778000	2.18778600	2.99075700
H	2.36462100	1.91401700	3.64690800
H	0.63528800	2.20256900	3.60369200
N	0.26852400	-0.97512900	2.78532500
C	0.42658800	-2.36187300	3.21379200
H	0.88683100	-2.40202900	4.19787600
C	1.31609100	-3.24430800	2.33581500
O	2.18125600	-3.91604600	2.87340100
N	1.12896900	-3.26331300	0.98869000
C	2.07942200	-4.00783200	0.18069700
H	2.53521900	-4.78408700	0.79110800
C	3.18316600	-3.12946600	-0.40715200
O	3.50595100	-3.25340500	-1.58542000
N	3.75611900	-2.27326900	0.45233200
H	3.33879200	-2.16934700	1.36521500
C	4.77874100	-1.28194700	0.13837900
H	5.07020700	-0.85303900	1.09415500
C	4.17314400	-0.10662400	-0.63843300
O	4.09860600	1.00779300	-0.13190900
C	6.03124600	-1.87763000	-0.52230900
H	6.39445900	-2.67466500	0.12765900
H	5.76942700	-2.32855700	-1.47618700
N	3.72273200	-0.38653300	-1.87051200
H	3.68526500	-1.36593500	-2.12554800
C	2.85740300	0.53738900	-2.58214300
H	3.41026200	1.45141500	-2.79797600
C	1.66698800	0.93934500	-1.69348000
C	2.43059400	-0.12544200	-3.89253700
H	3.34921700	-0.39178700	-4.42103400
H	1.91665300	-1.06417600	-3.66168000
O	1.07486900	-0.01139600	-1.08755800
C	-1.05487500	-0.34026700	2.71335100
H	-0.96680500	0.67862800	3.08415000
H	-1.39459500	-0.31764200	1.67897800
C	-0.02640900	-2.72964100	0.27573100
H	-0.40561800	-1.84682800	0.76886600
H	0.30835400	-2.40302500	-0.70642900
C	-1.13477600	-3.77509800	0.16413400

H	-0.73576800	-4.72164900	-0.19835700
C	-2.14780800	-1.01881000	3.53100000
H	-2.24387200	-2.07632300	3.31657200
H	-2.00510000	-0.88480300	4.60052500
S	-3.75780300	-0.28636600	3.13573200
C	-4.03970300	-0.98063200	1.54493100
C	-3.46922700	-2.25261400	-0.19753500
N	-3.16767300	-1.83990200	1.03175000
N	-4.53730200	-1.88793900	-0.89435500
N	-5.15286700	-0.55140200	0.95911900
S	-2.42105400	-3.37011100	-1.04801600
H	-1.60989300	-3.95759100	1.12531400
C	-5.34949000	-1.04386800	-0.25859900
C	1.76516900	3.54773900	2.37540900
C	0.84514900	4.57420900	2.54727100
C	2.88926300	3.79407900	1.58542700
C	1.03695800	5.81915600	1.96544700
H	-0.04037400	4.40544400	3.14711100
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C	2.16454300	6.04931700	1.18720000
H	0.30960800	6.60721500	2.11401200
H	3.96253400	5.21427300	0.38311900
O	2.41295100	7.24061400	0.58797300
H	1.70869100	7.85870900	0.79258000
H	1.57023000	-4.47528800	-0.65759100
C	7.09419400	-0.82502500	-0.70864500
C	7.92769800	-0.46622700	0.34807800
C	7.23473800	-0.16210100	-1.92472800
C	8.88059900	0.52977500	0.19388400
H	7.83088200	-0.97554200	1.29948600
C	8.18614900	0.83626800	-2.08290200
H	6.58905100	-0.42853100	-2.75205200
C	9.01170100	1.18518400	-1.02345500
H	9.52297700	0.79326100	1.02361000
H	8.28323200	1.34037600	-3.03524600
H	9.75506500	1.96141800	-1.14562200
C	1.54843300	0.72489600	-4.81783100
H	1.88525400	1.76352200	-4.74692800
C	1.72546900	0.26725100	-6.26558300
H	1.10145500	0.85396500	-6.94120500
H	2.76265600	0.36509100	-6.58934800

H	1.43792600	-0.78195000	-6.37236100
C	0.06831100	0.67394600	-4.43333200
H	-0.29926100	-0.35410100	-4.48427500
H	-0.11056500	1.04924600	-3.42818900
H	-0.52525800	1.27497000	-5.12416100
H	-0.54823000	-2.83081200	3.28292100
S	-6.77020800	-0.58689200	-1.16848700
C	-7.61206800	0.55236400	-0.00995500
H	-7.81554700	0.00628200	0.90671800
H	-6.93100000	1.37008400	0.20801000
C	-8.87654900	1.03763300	-0.66242000
C	-10.07042600	0.34256600	-0.49076600
C	-8.86793300	2.18376800	-1.45258900
C	-11.23705000	0.78772600	-1.09474500
H	-10.08595600	-0.54886400	0.12382500
C	-10.03358500	2.63021700	-2.05742200
H	-7.94378900	2.73131100	-1.58964100
C	-11.22063200	1.93252300	-1.87986100
H	-12.15963400	0.24213900	-0.94979000
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H	-12.13043000	2.28152900	-2.34918400
O	1.36459800	2.13918500	-1.61709300
H	0.67774400	2.26237200	0.39596700
H	0.46616500	0.68335100	0.08091700
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			Ref.
N	2.42419300	2.49609800	-0.76054100
H	2.68202800	3.07488000	-1.55170700
C	3.39170600	1.39840200	-0.65262400
H	3.44580500	0.91224400	-1.62598600
C	2.95694400	0.31463000	0.32658800
O	3.54298800	-0.76541200	0.33603700
C	4.79547800	1.92494000	-0.27178400
H	4.98957100	2.77393200	-0.93056600
H	4.74742900	2.32200400	0.74471800
C	5.92606900	0.93701000	-0.38762800
C	6.56463400	0.43113700	0.73952400
H	6.23749700	0.74482800	1.72290900
C	6.37160800	0.51257500	-1.63530600
H	5.90006000	0.89582600	-2.53230700
C	7.61023800	-0.47116600	0.63428500

H	8.10236900	-0.85883300	1.51584800
C	7.41314200	-0.39291100	-1.76064600
H	7.74587800	-0.71002800	-2.74142400
C	8.03684400	-0.88936100	-0.62057700
O	9.06553800	-1.77629300	-0.67472500
H	9.26454000	-1.98095200	-1.59008600
N	1.96849200	0.59803400	1.18922800
C	1.65611300	-0.25798000	2.31535600
H	2.58912300	-0.61744300	2.75381000
H	1.13032300	0.33326900	3.05835800
C	0.78718500	-1.48229100	2.03235900
O	-0.14262600	-1.78002000	2.76358800
N	1.13240200	-2.21322500	0.94725600
C	0.51365100	-3.49148000	0.68964300
H	1.13055000	-4.04188000	-0.01738200
H	0.44385400	-4.07992100	1.60491300
C	-0.90208100	-3.43787400	0.12280900
O	-1.56165600	-4.45977200	0.01974000
N	-1.34096100	-2.21948200	-0.23286300
H	-0.68087800	-1.46259500	-0.17110100
C	-2.68725700	-1.94649000	-0.70388200
H	-2.89190800	-2.52663700	-1.60258100
C	-2.75210700	-0.47771800	-1.12446000
O	-3.04941500	-0.14297500	-2.25523500
C	-3.75178800	-2.27633100	0.36450000
H	-3.72729800	-3.35244100	0.52363500
H	-3.46881600	-1.80578600	1.30699100
C	-5.12629400	-1.82557700	-0.05414600
C	-5.70288600	-0.68789900	0.50310700
H	-5.17056800	-0.14596000	1.27470700
C	-5.82771200	-2.51505100	-1.04089100
H	-5.39494500	-3.40576400	-1.47996000
C	-6.94987600	-0.24453200	0.08406300
H	-7.38333000	0.64059100	0.53035300
C	-7.07325100	-2.07497200	-1.46325500
H	-7.60568300	-2.62241500	-2.22955800
C	-7.63738400	-0.93613400	-0.90290100
H	-8.60892100	-0.59274700	-1.23173200
N	-2.44822000	0.39616600	-0.13673000
H	-2.25071900	0.04744200	0.78722200
C	-2.33669800	1.83059500	-0.34476700
H	-2.59464700	2.00765300	-1.38628200

C	-0.85324100	2.12814700	-0.14396600
O	-0.16240300	2.06554400	-1.25446100
C	-3.25829200	2.61294300	0.58221400
H	-4.20585700	2.07336800	0.62296900
H	-2.83831900	2.60820100	1.59169400
C	-3.53515900	4.05237400	0.13620700
H	-4.01162800	4.00231600	-0.84806500
C	-4.51771900	4.70758100	1.10457800
H	-4.78114400	5.71282900	0.77353700
H	-5.43722800	4.12645400	1.18984500
H	-4.07684600	4.78751800	2.10126900
C	-2.26797500	4.89648200	0.00157600
H	-2.52295800	5.92514200	-0.25590100
H	-1.70871900	4.90925000	0.93919400
H	-1.60561300	4.52391700	-0.78144300
O	-0.36559900	2.31030700	0.95616500
H	0.82689400	2.19436800	-1.05319500
H	2.49062500	3.10562400	0.04898100
H	1.39183800	1.42184700	1.07413700
H	2.01833400	-1.98857900	0.51100

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