Electronic Supporting Information

Cationic and amphiphilic Peptide-Based Hydrogels with Dual Activities as Anticancer and Antibacterial Agents

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Instrumentations

NMR experiments

All NMR spectra were collected by using Bruker DPX500 MHz spectrometer and Bruker Ultrasheild Plus- 400 MHz spectrometer at 300 K. Compounds were taken for this experiment in the range of 1–10 mM in $CDCl_3$ and DMSO-d_6.

MASS spectrometry

Mass spectra were recorded in a Quadruple-TOF (Q-TOF) micro-^{TM} mass spectrometer system (Waters Corporation) by positive mode electron spray ionisation method.

FT-IR spectroscopy

FT-IR spectroscopic analyses were carried out by using Nicolate 380 FT-IR spectrophotometer (Thermo Scientific). FTIR spectra were recorded using a cell with CaF_2 windows.

Rheology study

The rheological experiments were performed by using an Anton Paar Modular Compact Rheometer MCR 102. Different gels of 0.5 wt % were taken individually and analysed at 25°C using a parallel plate geometry (25mm diameter, 1 mm gap).

Fluorescence Spectroscopy

The photoluminescence (PL) spectra of hydrogel samples for ROS generation studies were collected from a Fluoromax-3 instrument (HorivaJovin Yvon). The quartz cell of 1 cm path length was used for this experiment.

Powder X-ray Diffraction

X-ray diffraction performances of xerogel were carried out by placing the sample on a glass plate. Experiments were carried out using an X-ray diffractometer (Bruker D8 Advance) with

a parallel beam optics attachment. The instrument was operated at 35 kV voltages and 30 mA current using Ni-filtered CuKα radiation and the instrument was calibrated with a standard silicon sample before use. Samples were scanned from 2º to 50º (2θ) in the step scan mode (step size 0.03°, present time 2s) and diffraction patterns were recorded using a scintillation scan detector.

Field Emission Scanning Electron Microscopic Study (FE-SEM)

FE-SEM experiments were performed by placing a small portion of gel sample on a microscope cover glass. Then, these samples were dried first in air and then in vacuum and coated with platinum for 90 sec at 10 kV voltages and 10 μA current. The average thickness of the coating layer of platinum was 3 to 4 nm. After that micrographs were taken by using a Jeol Scanning Microscope JSM-6700F.

Field Emission Gun Transmission Electron Microscope (FEG-TEM)

FEG-TEM images were recorded on a UHR-FEG-TEM JEM-2100F at 200 kV. During FEG-TEM experiment. Hydrogels were made at minimum gelation concentration (MGC) and then 10 μL of gel (concentration of gelators are 33.8 μM, 50.0 μM, 69.4 μM) was taken in a screw cap vial and diluted with 1 mL Milli-Q water. Then, a drop of dilute solution was placed on a carbon coated copper grid (300 mesh) and dried by slow evaporation. The grid was then allowed to dry in a vacuum for two days and then images were taken.

Dynamic Light-Scattering (DLS) and Zeta Potential Study

Zen 3690 Zetasizer (Malvern Instrument Ltd.) was used for measurement of mean hydrodynamic diameter and zeta potential. The scattering intensity was at 173° angle and Malvern Zetasizer software was used for data analysing to determine the Zeta potential.

Table S1. Measurement of thermal stability of freshly prepared hydrogel in ultrapure water and gel forming kinetics.

Gelators	MGC(W%/V)	Gel forming time after cooling (min)	T_{gel}(°C)
CP1	2	15	66
CP2	3	25	64
CP3	4	35	60

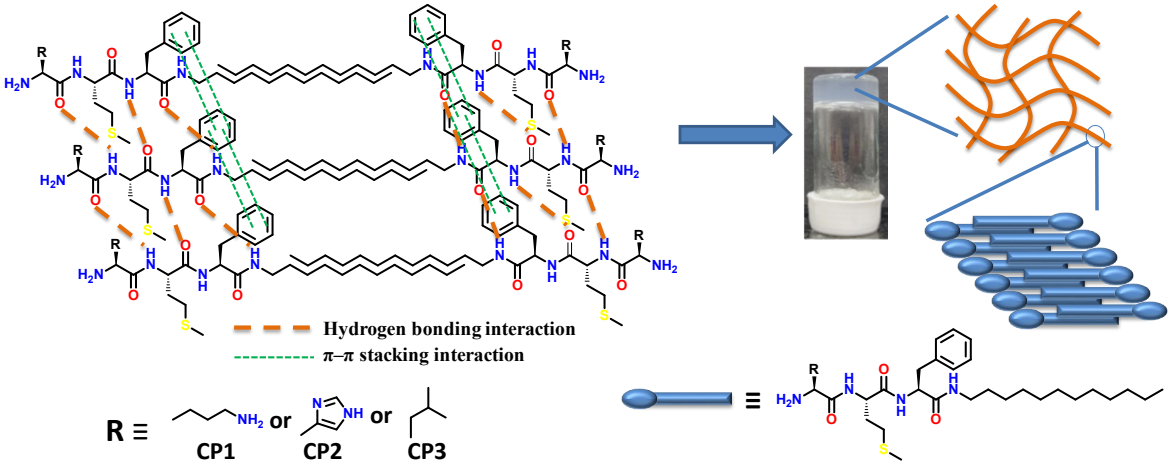


Fig. S1 Tentative model of the gelators **CP1**, **CP2** and **CP3** packing arrangement during self-assembly.

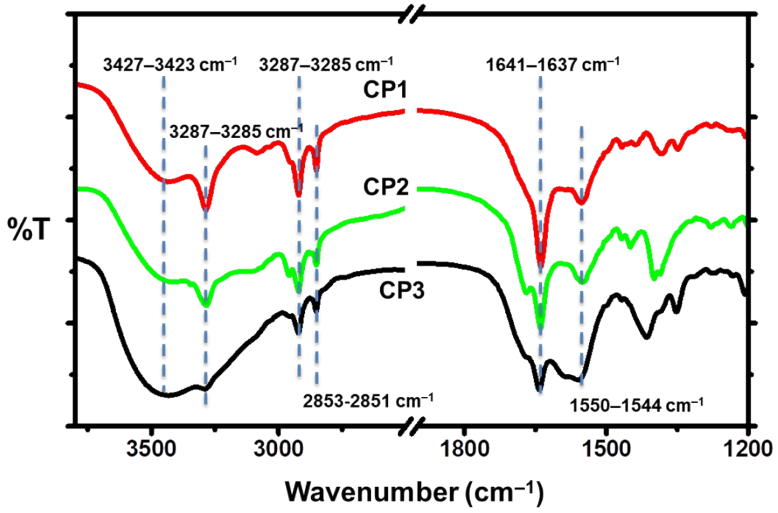


Fig. S2 FT-IR pattern of the xerogels obtained from the hydrogels formed by the peptide gelators **CP1** (red), **CP2** (green), and **CP3** (black).

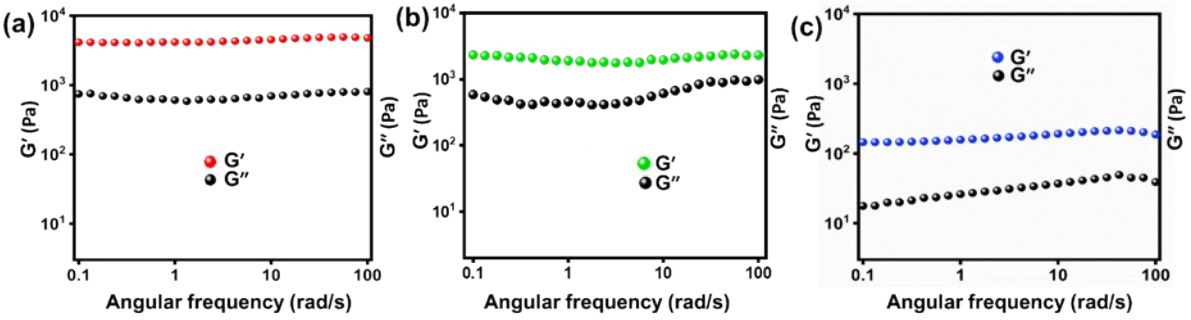


Fig. S3 Frequency-sweep experiments of hydrogels formed by the peptide gelators (a) **CP1**, (b) **CP2** and (c) **CP3** at constant 0.5 wt %.

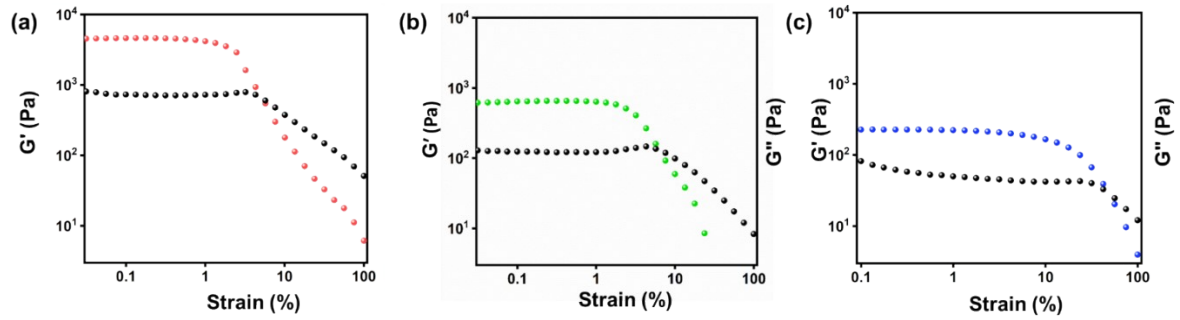


Fig. S4 Amplitude-sweep experiments of hydrogels formed by the peptide gelators: (a) **CP1** hydrogel, (b) **CP2** hydrogel, (c) **CP3**, at constant 0.5 wt %.

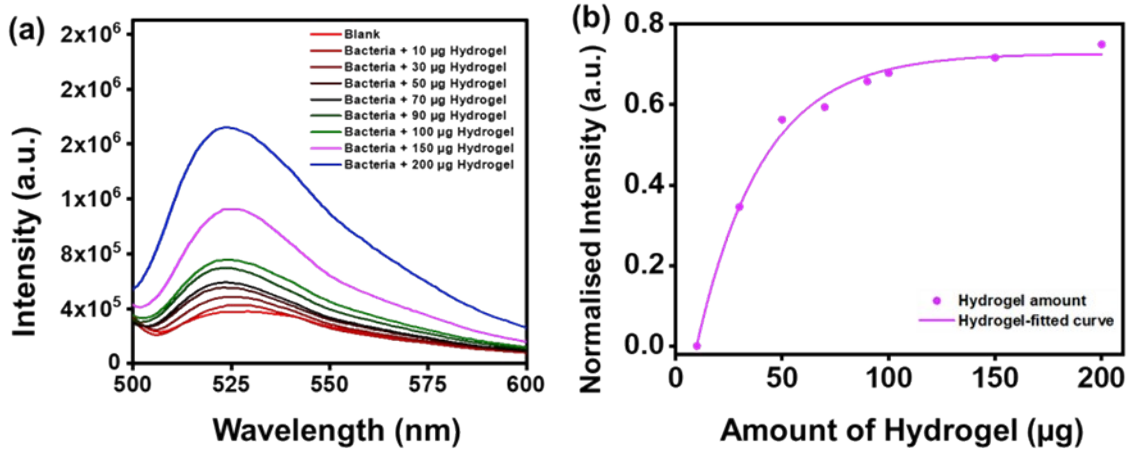


Fig. S5 (a) Fluorescence spectra of 2′,7′-dichlorofluorescein (DCF) probing the intra cellular ROS generation in *E.coli* when treated to different concentrations of **CP1** hydrogel in HBSS. (b) Comparative analysis of intra-cellular ROS generated in *E.coli* in the presence of **CP1** hydrogels. The intensity is normalized with respect to the highest intensity point of the exp eriment.

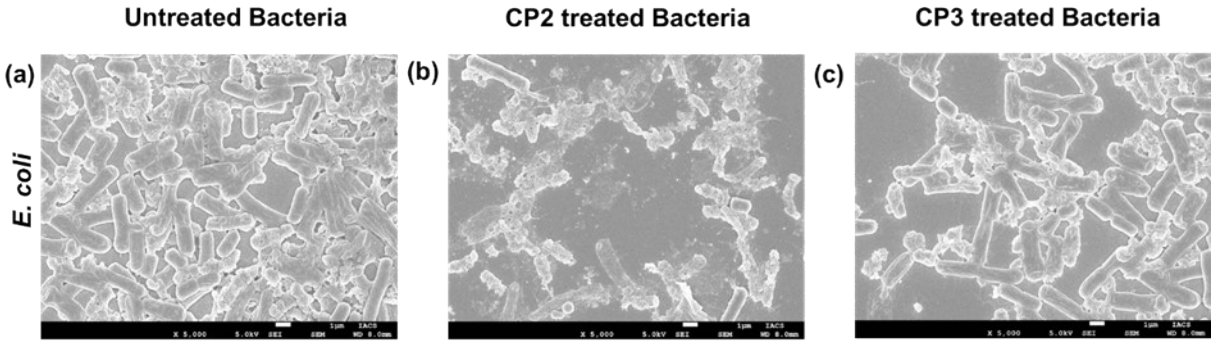


Fig. S6 SEM images of live bacteria: (a) *E. coli* (untreated bacteria) (b) CP2 treated bacteria (c) CP3 treated bacteria.

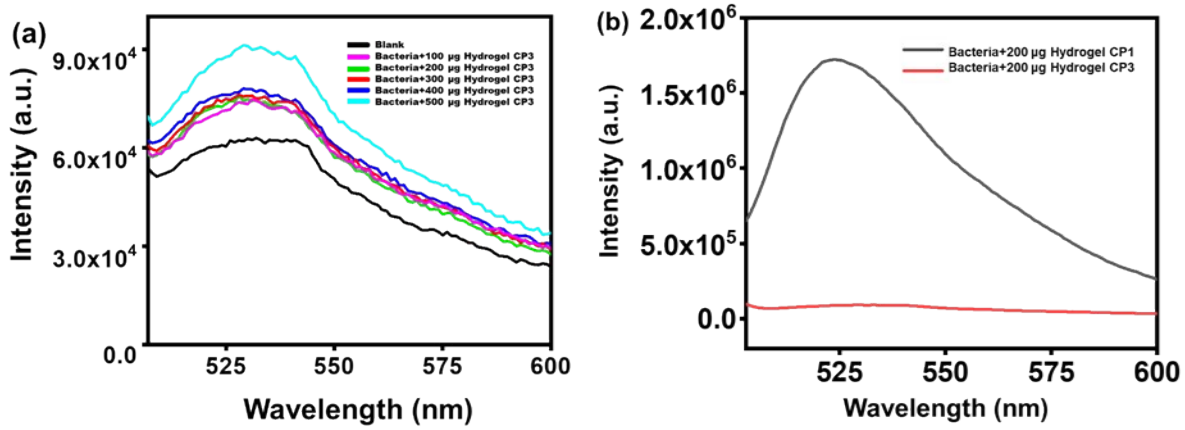


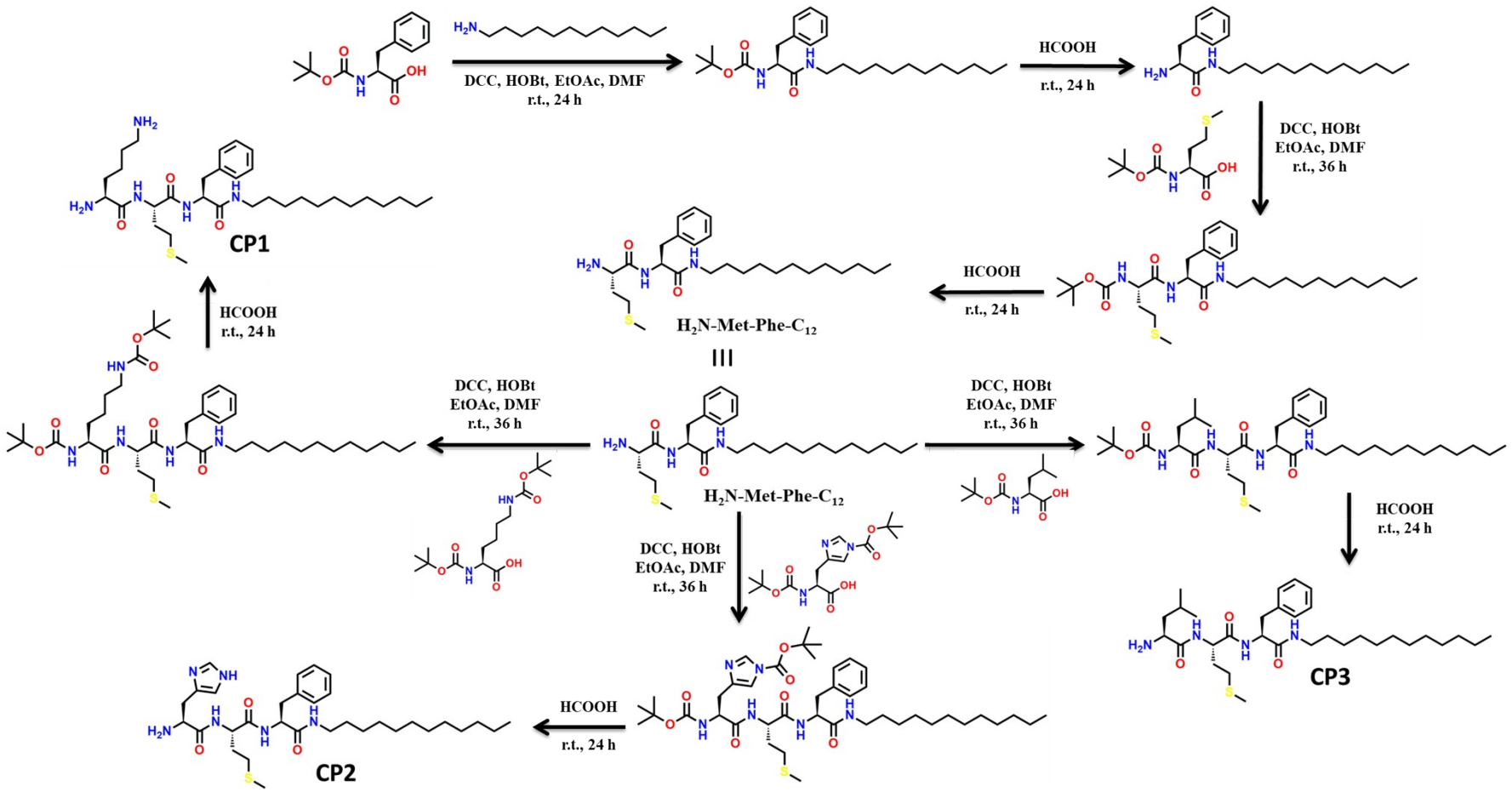
Fig. S7 (a) Fluorescence spectra of 2′,7′-dichlorofluorescein (DCF) probing the intra cellular ROS generation in *E.coli* when treated to different concentrations of CP3 hydrogel in HBSS.

(b) Comparative analysis of intra-cellular ROS generation in *E.coli* in the presence of CP1 and CP3 hydrogels at a fixed concentration 200 µg/m.

Table S2. Reason for buffer selection and clarification for choosing concentration.

Gelators	MGC (W%/V)	Buffer	Reason for buffer selection	Clarification on the chosen concentration
CP1	2 mg	Tris buffer pH=7.46	Lysine is basic amino acid, at this pH both $-NH_2$ groups are protonated and –COOH group is in deprotonated form, this zwitterionic structure helps to soluble in this buffer.	It is the minimum amount of gelator, which is needed for gelation. Below this concentration gel is not formed and above this concentration the gelator precipitate out in the solution.
CP2	3 mg	Acetate buffer pH=4.6	Presence of imidazole moiety in the side chain of histidine, it is basic in nature. So acidic buffer is needed to make it soluble.	It is the minimum amount of gelator, which is needed for gelation. Below this concentration gel is not formed and above this concentration the gelator precipitate out in the solution.
CP3	4 mg	2% DMSO in Acetate buffer pH=4.6	Leucine has a hydrophobic group in the side chain. It is nonpolar in nature. Due to this, 2% DMSO is used in acidic medium.	It is the Minimum gelation concentration (MGC), below this concentration there is no gel formation by this gelator.

Schematic Representation of Synthesis of Amphiphilic Peptide Gelators CP1, CP2 and CP3



Synthetic Procedure of Amphiphilic Gelators CP1, CP2 and CP3

Synthesis of Boc-Phe-OH: 3.3g (20 mM) phenyl alanine was taken in a 250 mL round bottom flask. 20 mL 1 (N) NaOH and 25 mL dioxane was added to it and cooled to 0°C. 4.8 g (22 mM) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 12 h at room temperature. Then dioxane was removed by reduced pressure. The resulting mixture was acidified with 1 (N) HCl solution and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The ethyl acetate (EtOAc) extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the white coloured solid product.

Yield: 4.8g (18 mM, 90%)

Synthesis of Boc-Phe-C_{12}: Boc-Phe-OH (4.8g, 18 mM) was dissolved in 10 mL DMF and 10 mL of EtOAc in a 250 mL round bottom flask. The mixture was cooled to 0°C in an ice water bath. 2.7g (19.8 mM) HOBt was added to it. Then H_2N-C_{12} (3.3g, 18mM dissolved in 15 mL ethyl acetate) followed by DCC (4g, 19.8mM) was added to the reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for 24 h. The reaction mixture was filtered to separate N, N-dicyclohexyl urea (DCU). The organic layer was washed with 1 (N) HCl (3 × 30 mL), brine (1 × 30 mL) and brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A yellowish white material was obtained, purified using silica gel in pet ether and ethyl acetate (85:15) as eluent. After purification white colour powder was obtained. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 6.5g (15 mM, 83%)

HRMS (m/z): Calculated for $C_{26}H_{44}N_2O_3$ (M): 432.3352, 433.3430 $(M+H)^+$, 455.3250 $(M+Na)^+$, Obtained mass: 433.0827 $(M+H)^+$

^{1}H NMR (400MHz, $CDCl_3$, TMS, 25°C): δ7.32–7.28 (m, 2H, aromatic), 7.24–7.21 (m, 3H, aromatic), 5.79 (br, 1H, NH), 5.16 (br, 1H, NH), 4.32–4.26 (m, 1H, α-CH of Phe), 3.19–3.14 (m, β-CH_2 of Phe), 3.12–3.03 (m, β–CH_2 of Phe C=O), 1.43 (s, 9H, 3-CH_3 of boc), 1.27 (br, 20H, 10-CH_2 of dodecyl amine), 0.92–0.88 (m, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (101 MHz, $CDCl_3$, TMS, 25 °C): δ 171.0, 155.5, 137.0, 129.3, 128.7, 127.0, 80.2, 56.2, 39.2 (d, *J*= 63.5 Hz), 31.9, 29.8 – 27.5 (m), 28.4, 26.9, 22.8, 14.2.

Synthesis of H_2N-Phe-C_{12}: 4.3g (10 mM) of Boc-Phe-C_{12}, 5 mL of formic acid was added and the removal of the Boc group was monitored by TLC. After 24 h, formic acid was removed under vacuum. The residue was taken in water (10 mL) and pH was maintained by using saturated sodium carbonate solution. The resulting aqueous layer was extracted with ethyl acetate (3 × 40 mL).The ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the white colourless sticky product. A white material was obtained after purification in basic alumina in chloroform and methanol (9:1) as eluent. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 3g (9 mM, 90%)

HRMS (m/z): Calculated for $C_{21}H_{36}N_2O$ (M): 333.2906 $(M+H)^+$, Obtained mass: 333.1806 $(M+H)^+$.

^{1}H NMR (400MHz, DMSO-d_6, TMS, 25°C): δ 8.37 (s, 1H, NH of amide bond), 7.28–7.24 (m, 2H, aromatic), 7.20–7.16 (m, 3H, aromatic), 5.50 (br, 2H, NH_2), 3.43–3.40 (m, 1H, α-CH of Phe), 3.05–2.99 (m, 2H, β-CH_2 of dodecyl amine), 2.92–2.87 (m, 1H, β-CH of Phe), 2.70–

2.64 (m, 1H, β-CH of Phe), 1.32–1.14 (m, 19H, 10-CH_2 of dodecyl amine), 0.87–0.83 (t, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (101 MHz, DMSO-d_6, TMS, 25°C): δ 173.0, 165.0, 138.2, 129.3, 128.0, 126.1, 55.8, 40.6, 38.3, 31.3, 29.0, 28.9, 28.7, 26.3, 22.1, 13.9.

Synthesis of Boc-Met-OH: 1.5g (10 mM) methionine was taken in a 250 mL round bottom flask. 10 mL 1 (N) NaOH and 15 mL dioxane was added to it and cooled to 0°C. 2.6 g (12 mM) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 12 h at room temperature. Then dioxane was removed by reduced pressure. The resulting mixture was acidified with 1 (N) HCl solution and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain sticky product.

Yield: 2.2g (9 mM, 90%)

Synthesis of Boc-Met-Phe-C_{12}: Boc-Met-OH (2.2g, 9mM) was dissolved in 10 mL DMF and 10 mL of EtOAc in a 250 mL round bottom flask. The mixture was cooled to 0°C in an ice water bath. 1.35g (10mM) HOBt was added to it. Then H_2N-Phe-C_{12} (3g, 9mM dissolved in 15 mL ethyl acetate) followed by DCC (2g, 10 mM) was added to the reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for 36 h. The reaction mixture was filtered to separate N, N-dicyclohexyl urea (DCU). The organic layer was washed with 1 (N) HCl (3 × 30 mL), brine (1 × 30 mL) and brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A yellowish white material was obtained, purified using silica gel in chloroform and methanol (97:3) as eluent. After purification white colour powder was obtained. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 4g (7 mM, 77.8%)

HRMS (m/z): Calculated for $C_{31}H_{53}N_3O_4S$ (M): 564.3835 $(M+H)^+$, 586.3654 $(M+Na)^+$, 602.3394 $(M+K)^+$, Obtained mass: 564.3925 $(M+H)^+$, 586.3733 $(M+Na)^+$, 602.3517 $(M+K)^+$.

^{1}H NMR (400MHz, $CDCl_3$, TMS, 25°C): δ 7.33 (q, *J* = 8.4 Hz, 3H, aromatic), 7.27 (d, *J* = 8.3 Hz, 2H, aromatic), 6.83 (s, 1H, NH), 6.08 (s, 1H, NH), 5.31 (br, 1H, NH), 4.64 (d, *J* = 7.3 Hz, 1H, α-CH of Phe), 4.22 (d, *J* = 6.6 Hz, 1H, α-CH of Met), 3.21–3.17 (m, 2H, β-CH_2 of Phe), 3.08 (d, *J* = 7.5 Hz, 2H, β-CH_2 of dodecyl amine C=O), 2.52 (t,*J* = 7.2 Hz, 2H, β-CH_2 of Met), 2.10 (s, 3H, S-$CH_3$of Met), 1.88 (s, 2H, γ-CH_2 of Met), 1.43 (s, 9H, 3-CH_3 of boc), 1.28 (s, 20H, 10-CH_2 of dodecyl amine), 0.92–0.89 (t,*J* = 6.9 Hz, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (101 MHz, $CDCl_3$, TMS, 25°C): δ 171.3, 170.2, 136.7, 129.4, 128.8, 127.1, 80.6, 54.5, 39.8, 38.3, 32.0, 31.3, 31.0, 30.3, 29.7, 29.6, 29.4, 29.3, 28.3, 26.9, 22.8, 15.4, 14.2.

Synthesis of H_2N-Met-Phe-C_{12}: 4g (7 mM) of Boc-Met-Phe-C_{12}, 4 mL of formic acid was added and the removal of the Boc group was monitored by TLC. After 24 h, formic acid was removed under vacuum. The residue was taken in water (10 mL) and pH was maintained by using saturated sodium carbonate solution. The resulting aqueous layer was extracted with ethyl acetate (3 × 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the white colourless sticky product. A white material was obtained after purification in basic alumina in chloroform and methanol (9:1) as eluent. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 2.8g (6 mM, 86%)

HRMS (m/z): Calculated for $C_{26}H_{45}N_3O_2S$ (M): 486.3130 $(M+Na)^+$, Obtained mass: 486.2356 $(M+Na)^+$.

^{1}H NMR (400MHz, DMSO-d_6, TMS, 25°C): δ 8.24 (s, 1H, NH of amide bond), 7.98 (s, 1H, NH of amide bond), 7.27–7.24 (m, 2H, aromatic), 7.21–7.18 (m, 3H, aromatic), 4.50 (d, *J* = 6.4 Hz,1H, α-CH of Phe), 4.25 (s, 2H, NH_2 group), 3.34 (dd, *J* = 7.7, 4.9 Hz, 1H, α-CH of Met), 3.04–2.93 (m, 2H, β-CH_2 of Phe), 2.84 (dd, *J* = 13.6, 8.7 Hz, 1H, α-CH of Met), 2.41–2.37 (m, γ-CH_2 of Met), 2.00 (s, 3H, S-CH_3 of Met), 1.80–1.71 (m, 1H, β-CH of Met), 1.64-1.57 (m, 1H, β-CH of Met), 1.25 (br, 20H, 10-CH_2 of dodecyl amine), 0.88–0.84 (t, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (100 MHz, DMSO-d_6, TMS, 25°C): δ 173.2, 170.9, 138.0, 129.6, 128.4, 126.7, 54.0, 53.8, 38.9, 38.6, 34.1, 31.7, 29.7, 29.5, 29.4, 29.2, 29.1, 26.7, 22.5, 15.0, 14.4.

Synthesis of $(Boc)_2$-Lys-OH: 1.46g (10 mM) of lysine was taken in a 250 mL round bottom flask. 70 mL methanol was added in the round bottom flask and suspension was formed. 3mL triethyl amine was added in the suspension after 10 min. 6.5g (30 mM) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 12 h at room temperature. When the reaction was completed, the reaction mixture turned into a clear solution. Then methanol and triethyl amine were completely evaporated and a white coloured solid was obtained. Water was added and pH was maintained by 10% citric acid. The aqueous layer was extracted with chloroform (3 × 40 mL). The chloroform extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the white coloured sticky product.

Yield: 2.2g (9 mM, 90%)

Synthesis of $(Boc)_2$-Lys-Met-Phe-C_{12}: ($(Boc)_2$-Lys-OH (2.2g, 9mM) was dissolved in 10 mL DMF and 20 mL of EtOAc in a 250 mL round bottom flask. The mixture was cooled to 0–4 °C in an ice water bath. 1.35g (10 mM) HOBt was added to it. Then H_2N-Met-Phe-C_{12} (4.2g, 9 mM dissolved in 15 mL ethyl acetate) followed by DCC (2g, 10 mM) was added to the reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for 48 h. The reaction mixture was filtered to separate N, N-dicyclohexyl urea (DCU). The organic layer was washed with 1 (N) HCl (3 × 30 mL), brine (1 × 30 mL) and brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A yellowish sticky material was obtained, purified using silica gel in chloroform and methanol (95:5) as eluent. After purification white colour sticky was obtained. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 4.7g (6 mM, 66.7%)

HRMS (m/z): Calculated for $C_{42}H_{73}N_5O_7S$ (M): 792.5309 $(M+H)^+$, 814.5128 $(M+Na)^+$, Obtained mass: 792.2891 $(M+H)^+$, 814.2665 $(M+Na)^+$.

^{1}H NMR (400MHz, $CDCl_3$ at 25°C): δ 7.54 (s, 1H, NH of amide bond), 7.36–7.33 (m, 3H, aromatic), 7.31–7.28 (m, 2H, aromatic), 6.61 (s, 1H, NH of amide bond), 5.72 (s, 1H, NH of amide bond), 4.86 (br, 2H, NH of amide bond), 4.51 (br, 1H, α-CH), 4.04 (br, 1H, α-CH), 3.53 (br, 1H, α-CH), 3.17–3.09 (m, 4H, β-CH), 2.98 (d, 2H, β-CH), 2.45 (br, 2H, β-CH), 2.07 (s, 3H, S-CH_3 of Met), 2.13 (m, 2H, γ-CH), 1.54 (d, J = 6.4 Hz, 20H, 6-CH_3 of boc and δ-CH), 1.35 (s, 23H, 10-CH_2 of dodecyl amine), 0.98 (t, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (101 MHz, $CDCl_3$, TMS, 25°C): δ 171.3, 170.2, 155.8, 136.7, 129.4, 128.8, 127.1, 80.6, 54.5, 39.8, 38.3, 36.5, 34.0, 32.0, 31.3, 31.0, 30.3, 29.7, 28.7, 29.6, 29.4, 29.3, 28.3, 26.9, 25.8, 25.0, 22.8, 15.4, 14.2.

Synthesis of H_2N-Lys-Met-Phe-C_{12} (CP1): 4.7g (6mM) of $(Boc)_2$-Lys-Met-Phe-C_{12}, 5mL of formic acid was added and the removal of the Boc group was monitored by TLC. After 24 h, formic acid was removed under vacuum. The residue was taken in water (10 mL) and pH was maintained by using ammonium hydroxide solution. Then the aqueous mixture was lyophilized and white coloured powder was obtained. A white powder was purified in basic alumina in chloroform and methanol (9:1) as eluent. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 3g (5 mM, 83%)

HRMS (m/z): Calculated for $C_{32}H_{58}N_5O_3S$ (M): 592.4260 $(M+H)^+$, 614.4080 $(M+Na)^+$, Obtained mass: 592.6530 $(M+H)^+$, 614.6428 $(M+Na)^+$.

^{1}H NMR (500MHz, DMSO-d_6 at 25°C): δ 8.37 (s, 1H, NH of amide bond), 8.20 (d, *J* = 8.3 Hz, 1H, NH of amide bond), 7.94 (t, *J* = 5.6 Hz, 1H, NH of amide bond), 7.22-7.15 (ddt, *J* = 20.5, 14.4, 7.5 Hz, 5H, aromatic), 4.44 (br , 4H, free NH_2), 4.30 (d, *J*= 6.6 Hz, 2H, α CH of Phe and Met), 3.22 (dq, *J* = 13.0, 6.5 Hz, 1H, α CH of Lys), 3.06 – 3.00 (m, 2H,β-CH), 2.95-2.91 (m, 2H, β-CH), 2.74-2.70 (m, 4H, β and γ CH), 2.34 – 2.31 (m, 2H, δ CH), 2.00 (s, 3H, S CH_3), 1.82 (q, *J* = 7.1 Hz, 1H), 1.75 (q, *J* = 8.6 Hz, 1H), 1.60 – 1.55 (m, 3H), 1.54-1.48 (m, 2H, γ CH of Met), 1.23 (s, 21H, 11-CH_2 of dodecyl amine), 1.20 (d, *J* = 17.1 Hz, 4H), 0.85 (t, *J* = 6.7 Hz, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (101 MHz, DMSO-d_6 at 25°C): δ 171.1, 170.9, 138.1, 129.6, 128.4, 126.7, 54.5, 54.4, 52.2, 39.0, 38.9, 38.2, 34.1, 32.7, 31.7, 29.8, 29.5, 29.4, 29.4, 29.3, 29.21, 29.1, 27.5, 26.7, 22.5, 22.5, 15.0, 14.4.

Synthesis of $(Boc)_2$-His-OH: 1.55g (10 mM) of Histidine was taken in a 250 mL round bottom flask. 70 mL methanol was added in the round bottom flask and suspension was formed. 3mL triethyl amine was added in the suspension after 10 min. 6.5g (30 mM) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 12 h at room temperature. When the reaction was completed, the reaction mixture turned into a clear solution. Then methanol and triethyl amine were completely evaporated and a white coloured solid was obtained. Water was added and pH was maintained by 10% citric acid. The aqueous layer was extracted with chloroform (3 × 40 mL). The chloroform extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the white coloured sticky product.

Yield: 2.3g (9 mM, 90%)

Synthesis of $(Boc)_2$-His-Met-Phe-C_{12}: $(Boc)_2$-His-OH (2.3g, 9mM) was dissolved in 10 mL DMF and 20 mL of EtOAc in a 250 mL round bottom flask. The mixture was cooled to 0°C in an ice water bath. 1.35g (10 mM) HOBt was added to it. Then H_2N-Met-Phe-C_{12} (4.2g, 9 mM dissolved in 15 mL ethyl acetate) followed by DCC (2g, 10 mM) was added to the reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for 48 h. The reaction mixture was filtered to separate N, N-dicyclohexyl urea (DCU). The organic layer was washed with 1(N) HCl (3 × 30 mL), brine (1 × 30 mL) and brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A yellowish sticky material was obtained, purified using silica gel in chloroform and methanol (95:5) as eluent. After purification white colour sticky was obtained. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 4.8g (6 mM, 66.7%)

HRMS (m/z): Calculated for $C_{42}H_{68}N_6O_7S$ (M): 801.4948 $(M+H)^+$, 823.4768 $(M+Na)^+$, Obtained mass: 801.4733 $(M+H)^+$, 823.4454 $(M+Na)^+$.

^{1}H NMR (400MHz, $CDCl_3$ at 25°C): δ 7.95–7.89 (m, 2H, aromatic), 7.44 (br, 1H, NH of amide bond), 7.30–7.26 (m, 2H, aromatic), 7.24–7.20 (m, 3H, aromatic), 6.56 (s, 1H, NH of amide bond), 6.00 (s, 1H, NH of amide bond), 4.85 (s, 1H, NH of amide bond), 4.41 (dq, *J* = 51.7, 5.7 Hz, 1H, α-CH), 4.28–4.23 (m, 1H, α-CH), 3.53–3.49 (m, 1H, α-CH), 3.25–3.18 (m, 2H, β-CH), 3.02–2.91 (m, 4H, β-CH), 2.31–2.08 (m, 4H, β-CH_2 of dodecyl amine C=O), 2.02 (s, 3H, S-CH_3 of Met), 1.96–1.92 (m, 2H, γ-CH_2 of Met), 1.64 (s, 9H, 3-CH_3 of boc), 1.47 (s, 9H, 3-CH_3 of boc), 1.26 (s, 20H, 10-CH_2 of dodecyl amine), 0.92-0.88 (t, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (100 MHz, $CDCl_3$, TMS, 25°C): δ170.9, 170.8, 146.7, 138.3, 137.0, 137.2, 129.0, 126.7, 115.4, 86.2, 80.6, 55.3, 54.7, 54.1 39.9, 37.1, 32.0, 29.9, 29.8, 29.7, 29.5, 29.4, 28.5, 28.0, 27.0, 22.8, 15.2, 14.2.

Synthesis of H_2N-His-Met-Phe-C_{12} (CP2): 4.7g (6mM) of $(Boc)_2$-His-Met-Phe-C_{12}, 5mL of formic acid was added and the removal of the Boc group was monitored by TLC. After 24 h, formic acid was removed under vacuum. The residue was taken in water (10 mL) and pH was maintained by using ammonium hydroxide solution. Then the aqueous mixture was lyophilized and white coloured powder was obtained. A white powder was purified in basic alumina in chloroform and methanol (9:1) as eluent. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 3g (5 mM, 83%)

HRMS (m/z): Calculated for $C_{32}H_{52}N_6O_3S$ (M): 601.3900 $(M+H)^+$, 623.3719 $(M+Na)^+$, Obtained mass: 601.3876 $(M+H)^+$, 623.3651 $(M+Na)^+$.

^{1}H NMR (400MHz, DMSO-d_6 at 25°C): δ 8.45 (d, *J* = 8.4 Hz, 1H, NH of aromatic histidine), 8.23 (s, 2H, aromatic histidine), 7.78 (s, 1H, NH of amide bond), 7.52 (s, 1H, NH of amide bond), 7.23-7.16 (ddt, *J* = 19.6, 14.1, 7.3 Hz, 5H, aromatic), 6.85 (s, 1H, NH of amide bond), 4.47 (td, *J* = 8.8, 5.3 Hz, 1H, α CH), 4.26 (t, *J* = 6.5 Hz, 1H, α CH), 3.51 (dd, *J* = 7.4, 4.9 Hz, 2H, NH_2), 3.07 – 2.96 (m, 4H, β CH_2), 2.89 – 2.77 (m, 2H, β CH_2), 2.68 (dd, *J* = 14.5, 7.4 Hz, 1H), 2.27 (t, *J* = 7.9 Hz, 2H, γ CH_2), 1.98 (s, 3H, S-CH_3), 1.81 – 1.80 (m, 2H), 1.70 1.67 (dq, *J* = 15.1, 7.8 Hz, 1H), 1.23 (s, 20H), 0.86 (t, *J* = 6.8 Hz, 3H, -CH_3 of dodecyl amine).

^{13}C NMR (101 MHz, DMSO-d_6 at 25 °C): δ 173.5, 170.6, 170.3, 137.8, 134.8, 128.9, 127.9, 126.16, 54.4, 53.9, 52.0, 38.4, 37.3, 31.9, 31.8, 31.2, 29.1, 28.9, 28.9, 28.8, 28.8, 28.6, 26.1, 22.0, 14.5, 13.8.

Synthesis of Boc-Leu-OH: 1.3g (10 mM) leucine was taken in a 250 mL round bottom flask. 10 mL 1 (N) NaOH and 15 mL dioxane was added to it and cooled to 0°C. 2.6 g (12 mM) di-tert-butyl dicarbonate (Boc anhydride) was added to the reaction mixture and stirred for 12 h at room temperature. Then dioxane was removed by reduced pressure. The resulting mixture was acidified with 1 (N) HCl solution and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtained sticky product.

Yield: 2g (9 mM, 90%)

Synthesis of Boc-Leu-Met-Phe-C_{12}: (Boc)-Leu-OH (2g, 9mM) was dissolved in 10 mL DMF and 20 mL of EtOAc in a 250 mL round bottom flask. The mixture was cooled to 0–4 °C in an ice water bath. 1.35g (10 mM) HOBt was added to it. Then H_2N-Met-Phe-C_{12} (4.2g, 9 mM dissolved in 15 mL ethyl acetate) followed by DCC (2g, 10 mM) was added to the reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for 48 h. The reaction mixture was filtered to separate N, N-dicyclohexyl urea (DCU). The

organic layer was washed with 1 (N) HCl (3 × 30 mL), brine (1 × 30 mL) and brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A yellowish sticky material was obtained, purified using silica gel in chloroform and methanol (96:4) as eluent. After purification, we obtained white colour sticky compound as our desired product. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 4g (6 mM, 66.7%)

HRMS (m/z): Calculated for $C_{37}H_{64}N_4O_5S$ (M): 677.4678 $(M+H)^+$, Obtained mass: 677.3328 $(M+H)^+$.

^{1}H NMR (400MHz, $CDCl_3$ at 25°C): δ 7.47–7.41 (m, 1H, NH of amide bond), 7.33–7.25 (m, 3H, aromatic), 7.22–7.20 (m, 2H, aromatic), 7.13–7.10 (m, 1H, NH of amide bond), 6.44 (br, 1H, NH of amide bond), 4.93 (br, 1H, NH of amide bond), 4.78 (br, 1H, α-CH), 4.45–4.43 (m, 1H, α-CH), 4.04–3.99 (m,1H, α-CH), 3.21–3.14 (m, 2H, β-CH_2), 2.99–2.94 (m, 2H, β-CH_2), 2.43–2.38 (m, 2H, β-CH_2), 2.11–2.10 (m, 2H, γ-CH_2 of Met), 2.06 (s, 3H, S-CH_3 of Met), 2.01–1.97 (m, 2H, β-CH_2 of Met), 1.70–1.60 (m, 3H, β-CH_2 and γ-CH of Leu), 1.46 (s, 9H, 3-CH_3 of boc), 1.28 (s, 20H, 10-CH_2 of dodecyl amine), 0.99–0.95 (m, 6H, 2-CH_3 of Leu), 0.92–0.89 (t, 3H, -CH_3 of dodecyl amine).

13C NMR (100 MHz, $CDCl_3$, TMS, 25°C): δ 170.6, 156.3, 136.6, 129.2, 128.9, 128.7, 128.5, 127.0, 80.9, 55.0, 54.0, 39.8, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 28.4, 28.3, 26.8, 26.7, 24.8, 24.7, 22.9, 22.7, 21.7, 15.1, 14.1.

Synthesis of H_2N-Leu-Met-Phe-C_{12} (CP3): 4g (6 mM) of $(Boc)_2$-Leu-Met-Phe-C_{12}, 5 mL of formic acid was added and the removal of the Boc group was monitored by TLC. After 24 h, formic acid was removed under vacuum. The residue was taken in water (10 mL) and pH was maintained by using ammonium hydroxide solution. Then the aqueous mixture was lyophilized and white coloured powder was obtained. A white powder was purified in basic

alumina in chloroform and methanol (9:1) as eluent. NMR and HRMS spectra of the purified compound were recorded using nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry to characterize the specific compound.

Yield: 2.8g (5 mM, 83%)

HRMS (m/z): Calculated for $C_{32}H_{56}N_4O_3S$ (M): 577.4151 $(M+H)^+$, Obtained mass: 577.5940 $(M+H)^+$.

^{1}H NMR(400MHz, DMSO-d_6 at 25°C): δ 8.06 (dd, *J* = 13.2, 8.2 Hz, 1H, NH of amide bond), 7.89 (dt, *J* = 20.1, 5.6 Hz, 1H, NH of amide bond), 7.21 (dp, *J* = 15.4, 7.4 Hz, 5H, aromatic), 4.45 (td, *J* = 8.6, 5.5 Hz, 1H, α-CH), 4.28 (d, *J* = 6.3 Hz, 1H, α-CH), 3.19 (q, *J* = 5.0 Hz, 1H, α-CH), 2.97 (dtd, *J* = 29.7, 14.3, 5.8 Hz, 3H), 2.86 – 2.71 (m, 1H), 2.35 (t, *J* = 7.9 Hz, 1H), 2.01 (s, 3H, S CH_3), 1.83 (dq, *J* = 14.7, 7.6 Hz, 1H), 1.74 (d, *J* = 18.9 Hz, 2H), 1.24 (s, 22H), 0.86 (ddt, *J* = 9.9, 6.6, 4.0 Hz, 9H, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6 at 25°C): δ 171.3, 171.1, 170.8, 138.4, 138.1, 129.5 128.4, 126.7, 126.6, 54.5, 54.4, 53.5, 53.4, 53.0, 52.0, 44.3, 38.9, 31.7, 29.54, 29.5, 29.4, 29.2, 28.5, 26.7, 24.4, 23.7, 23.4, 22.5, 22.0, 15.0, 14.8, 14.4.

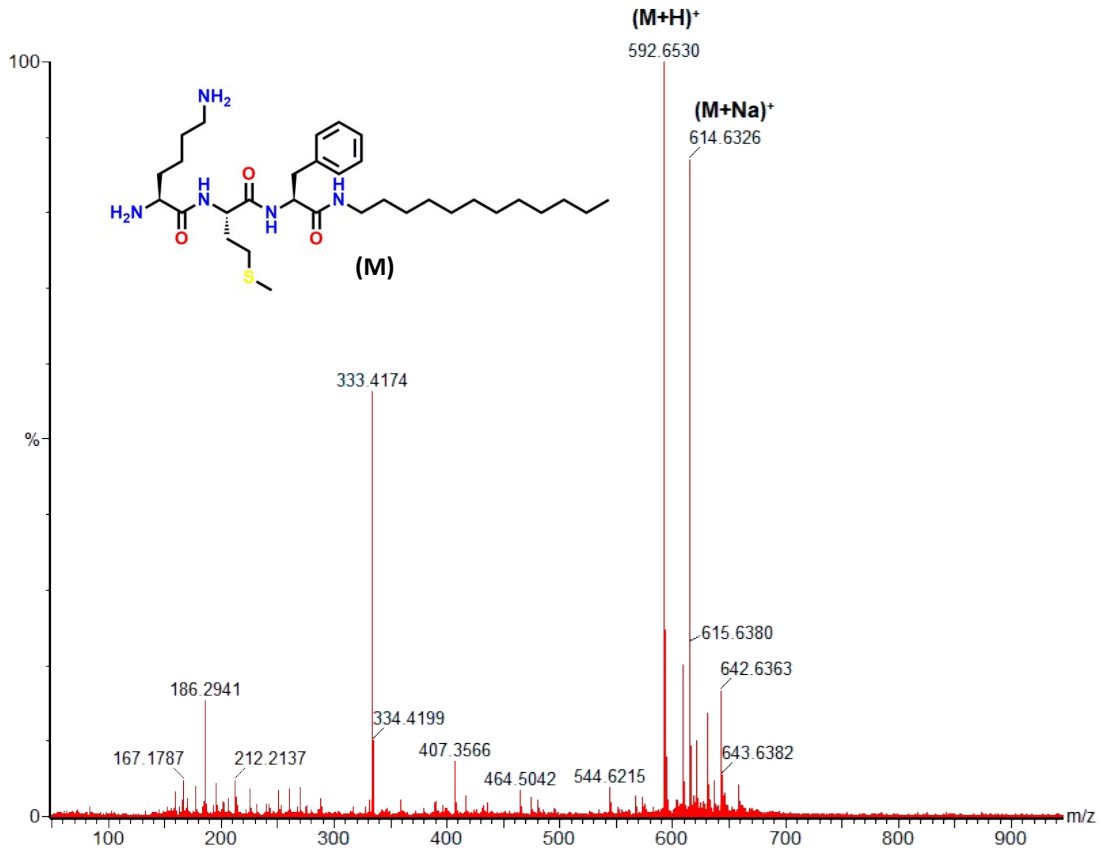


Figure S8. HR-MS spectrum of spectrum of H_2N-Lys-Met-Phe-C_{12}(**CP1**).

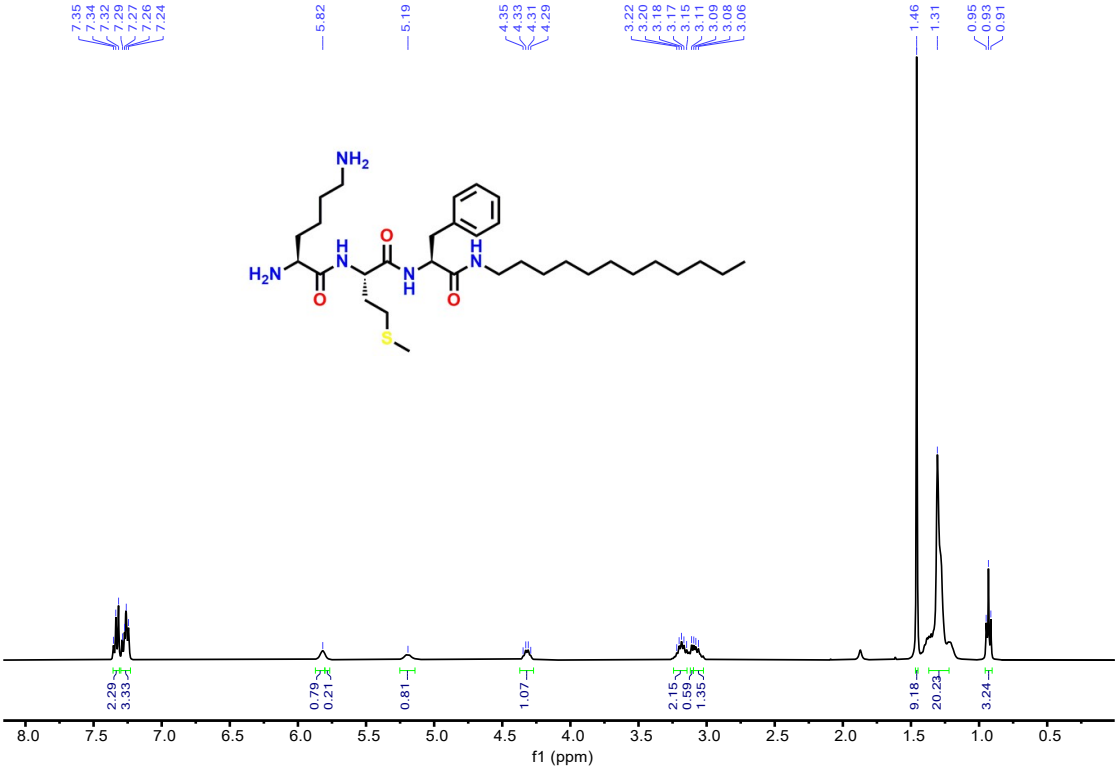


Figure S9. ^{1}H-NMR spectrum of H_2N-Lys-Met-Phe-C_{12}(**CP1**) in DMSO-d_6.

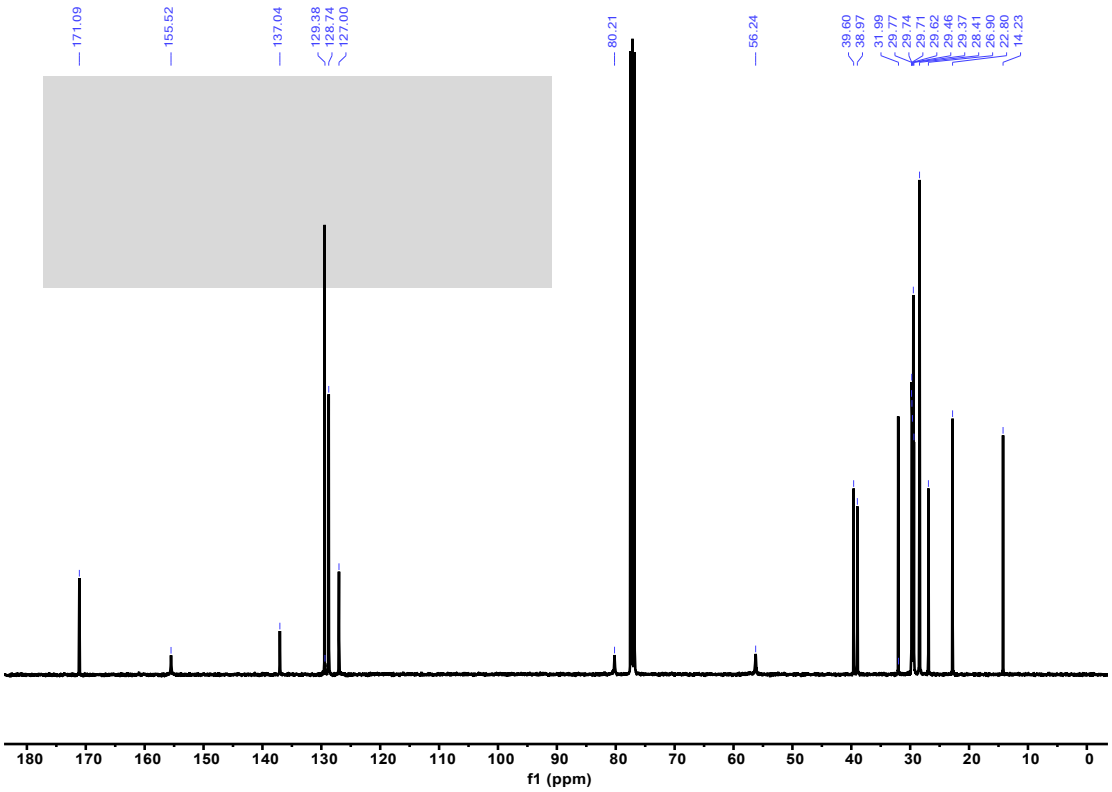


Figure S10. ^{13}C-NMR spectrum of H_2N-Lys-Met-Phe-C_{12}(**CP1**) in DMSO-d_6.

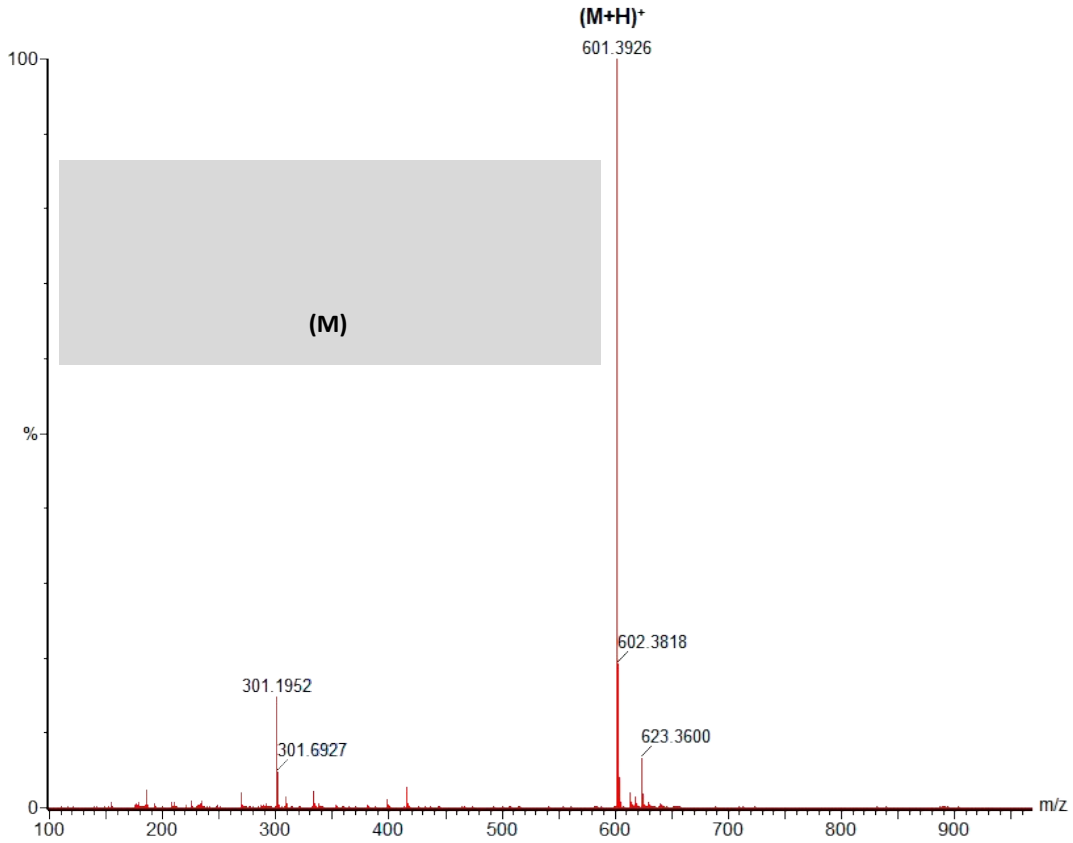


Figure S11. HR-MS spectrum of spectrum of H_2N-His-Met-Phe-C_{12}(**CP2**).

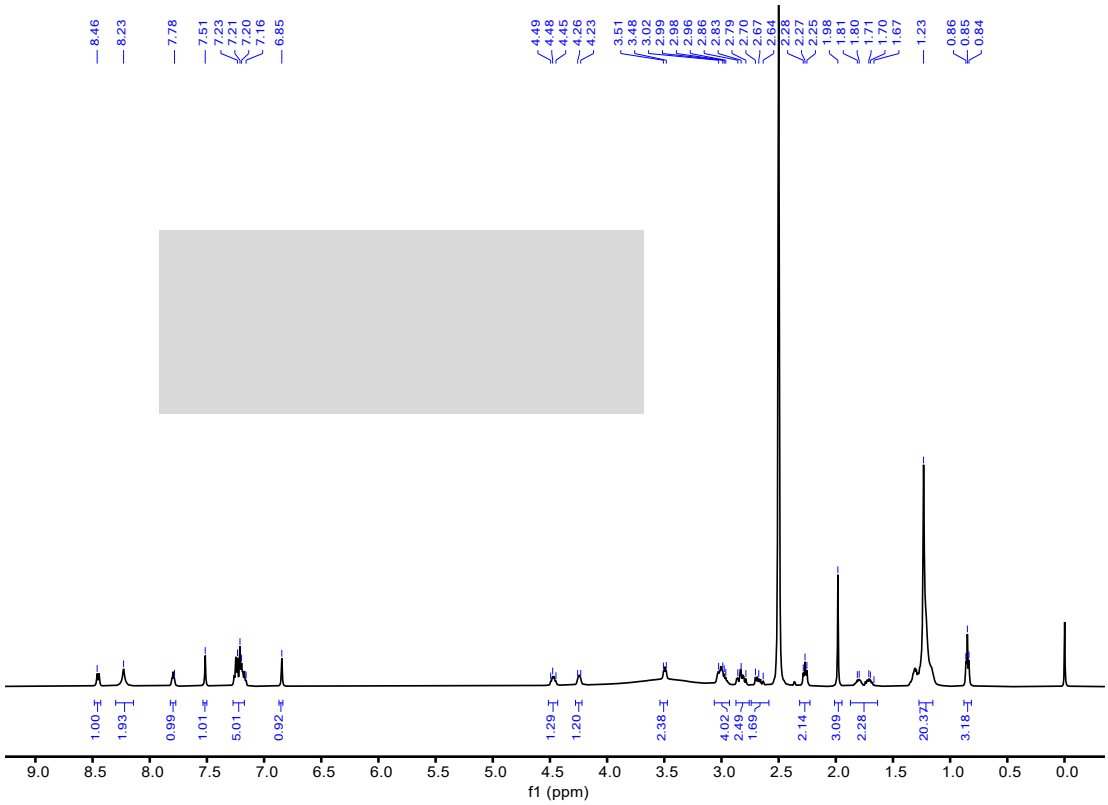


Figure S12. ^{1}H-NMR spectrum of H_2N-His-Met-Phe-C_{12}(**CP2**) in DMSO-d_6.

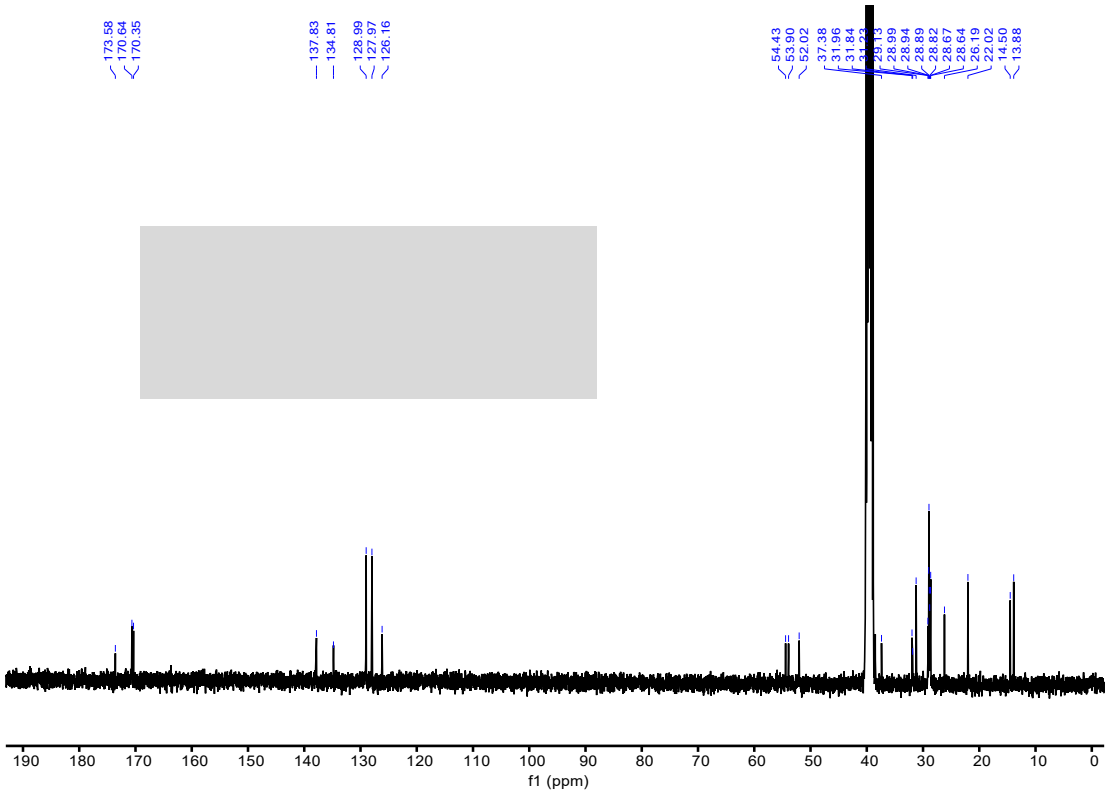


Figure S13. ^{13}C-NMR spectrum of H_2N-His-Met-Phe-C_{12}(**CP2**) in DMSO-d_6.

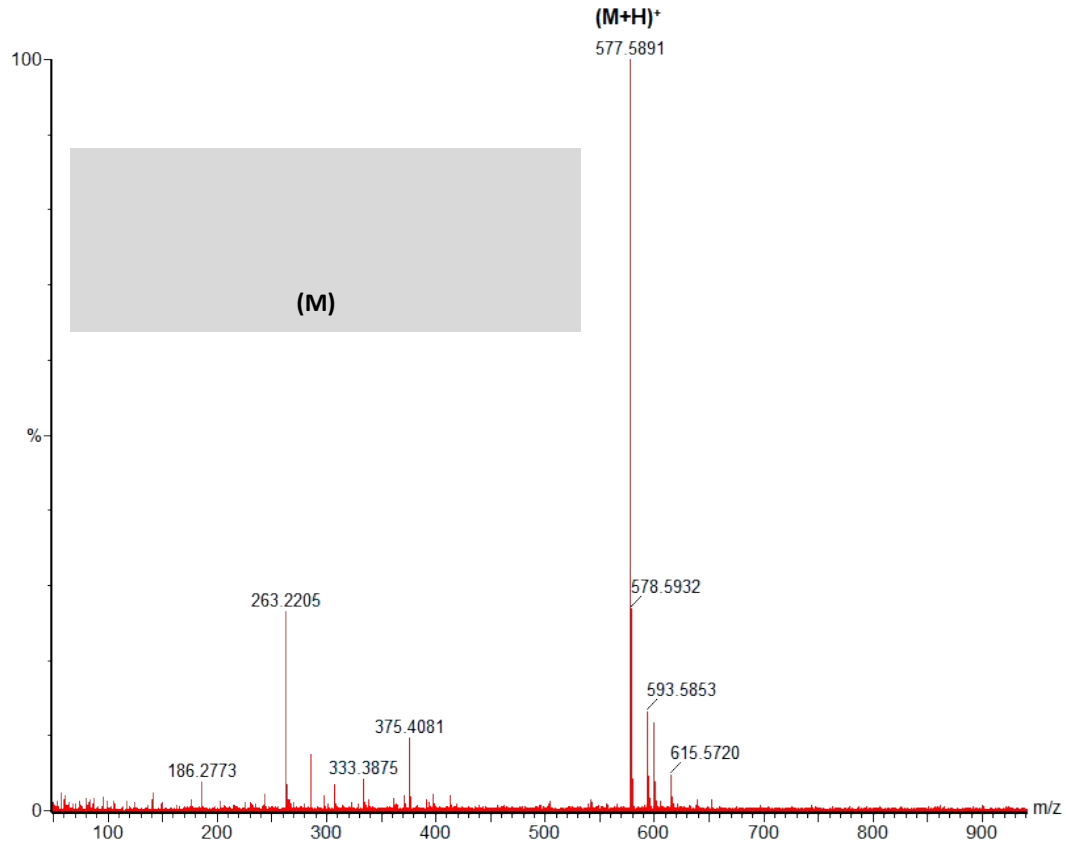


Figure S14. HR-MS spectrum of spectrum of H_2N-Leu-Met-Phe-C_{12}(**CP3**).

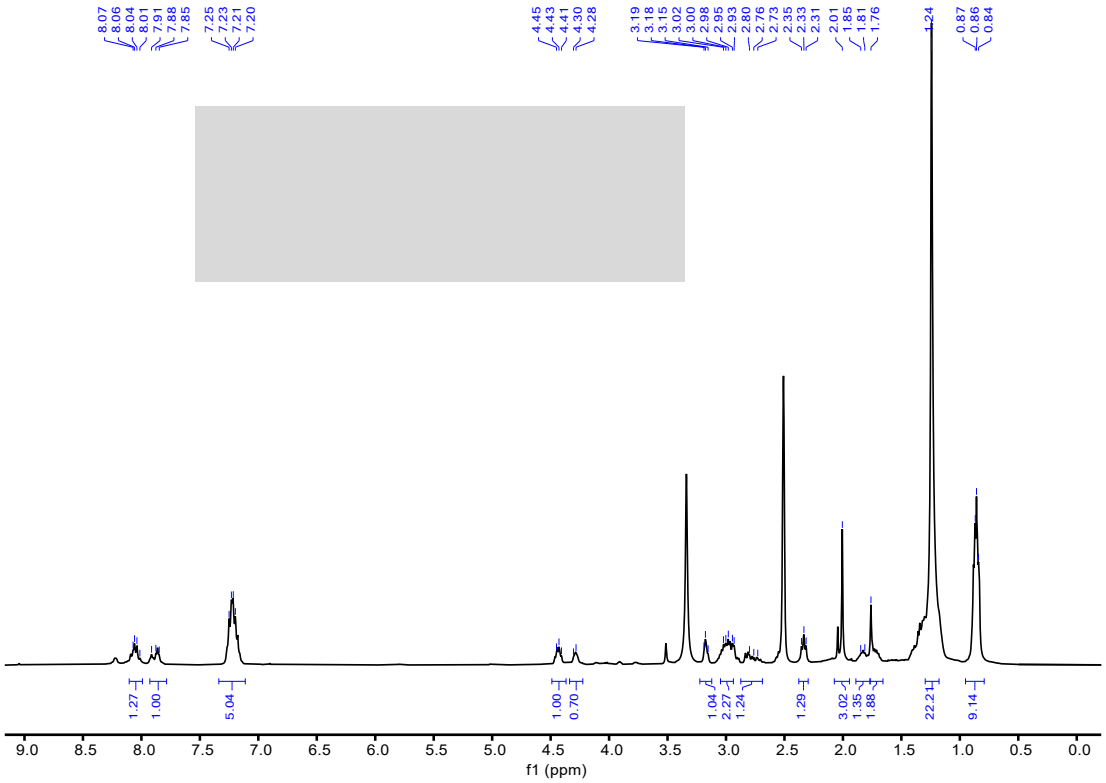


Figure S15. ^{1}H-NMR spectrum of H_2N-Leu-Met-Phe-C_{12}(**CP3**) in DMSO-d_6.

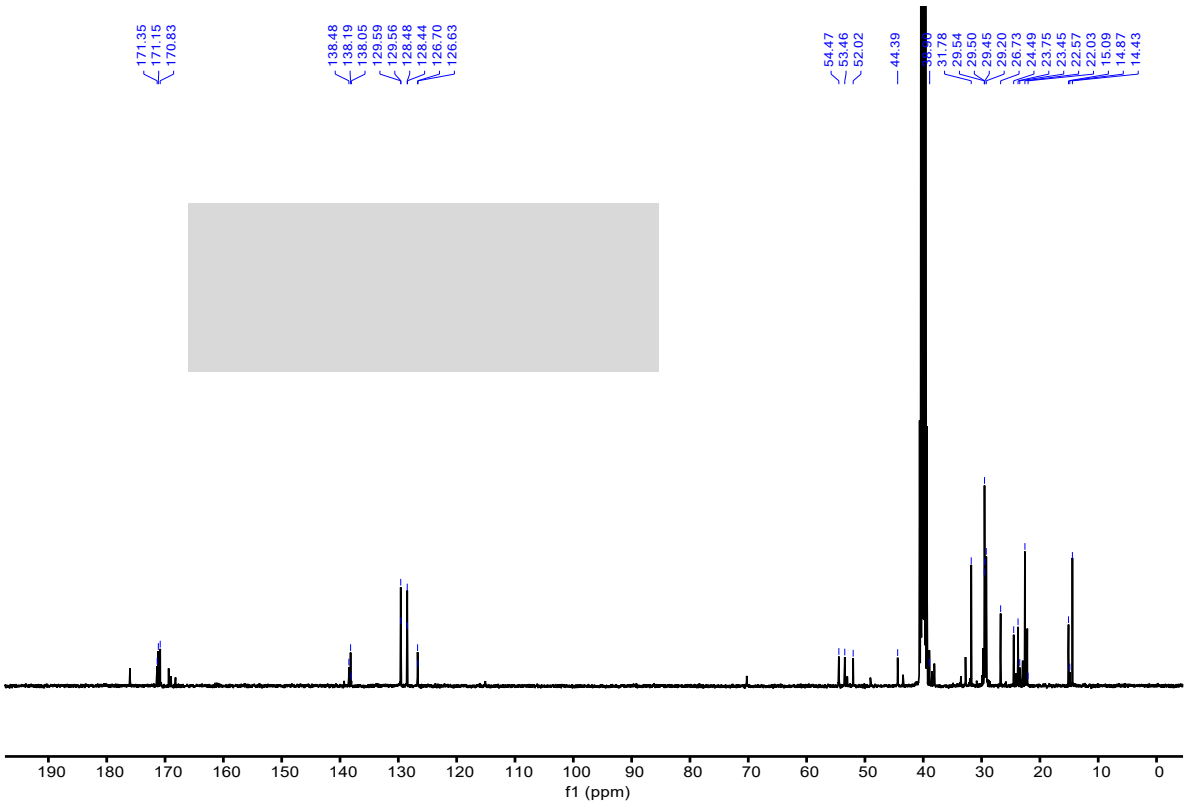


Figure S16. ^{13}C-NMR spectrum of H_2N-Leu-Met-Phe-C_{12}(**CP3**) in DMSO-d_6**.**