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Electronic Supporting Information (ESI†)

A Cystine Containing Cationic Lipopeptide Based Injectable Hydrogel with Antimicrobial Activities against Multi-Drug Resistant Strains and Anti-biofilm Efficacy against Methicillin-resistant *Staphylococcus aureus*

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Instrumentation

Mass and NMR Experiments:

All NMR studies (^{1}H and ^{13}C) were carried out on Bruker DPX 400 MHz or 500 MHz spectrometer at 300 K with usual concentration range between 5−10 mM in $CDCl_3$ or DMSO-D_6. Mass spectra were recorded on either with Q-Tofmicro (Waters Corporation) mass spectrometer (HRMS; positive mode electro-spray ionization) or with MALDI-TOF.

Rheological Experiments:

Rheological experiments (Frequency Sweep, Amplitude Sweep, Step-strain) were performed using Anton Paar Modular Compact Rheometer MCR 102. Hydrogel in tris-HCl buffer (0.5 % W/V) was taken at 25 °C and measurements were analyzed using parallel plate geometry (25mm diameter, 1 mm gap).

FEG-TEM Experiment:

For FEG-TEM experiment, peptide hydrogel was made at above minimum gelation concentration (5 mM). Then 10 μL of gel was taken in a screw cap vial and diluted with 1 mL of Milli-Q water to get gelator solution of 50 μM. Finally, 10 μL was drop-casted on carbon coated copper grids (300 mesh) and dried overnight by slow evaporation. Images were recorded on a UHR FEG-TEM (JEM-2100F) at 200 kV and analyzed by Image-J software.

FTIR Spectroscopy:

Fourier transformed Infrared spectroscopy study of the freeze-dried, powdered xerogel was performed by forming solid state KBr pellet using a cell with CaF_2 windows of a Nicolet 380 FT-IR spectrophotometer (Thermo-Scientific).

Vibrational Raman Spectroscopy:

For vibrational Raman spectroscopy, finely divided xerogel was used to analyze via Laser Raman Spectroscope

Powder XRD Experiment:

For wide-angle powder X-ray diffraction study (scan range: $2\theta = 10°-60°$), freeze-dried xerogel powder was taken to detect using a Bruker D8 Advance X-ray diffractometer (9 kW) having parallel beam optics attachment and operated at 35 kV voltage-30 mA current with Ni-filtered CuKα radiation and a scintillation detector with scan speed of 2 s and step size of 0.03°.

UV-Vis Spectroscopy:

Peptide gelator solutions in different concentrations were made and UV/Visible absorption spectra were recorded on Hewlett-Packard (HP model 8453) UV/Visible spectrophotometer (Varian carry 50 bio).

CD Spectroscopy:

CD spectra were taken using in a J-815 spectropolarimeter (Jasco, Japan). CD measurements with various concentrations of peptide gelators in tris-HCl buffer (pH 7.4) were carried out in the far UV region with path length of 1.0 mm, scan speed of 50 nm/min, and response time of 2.0 s. To minimize noise, each spectrum was averaged over five scans.

Materials and Methods:

FE-SEM of Bacterial Cell Morphology:

Overnight freshly cultured bacterial suspensions were incubated with peptide aggregated gelator solutions diluted to 50 μM at 37 ℃ for 16 h in LB medium. The treated and untreated bacteria suspensions were centrifuged at 6000 rpm for 5 min followed by washing the pellets twice with 0.87% NaCl solution and fixed with 2.5% glutaraldehyde. Next the samples were gradually dehydrated with ethanol solutions of different grades (30%, 50%, 70%, 90%, 100%). The specimens were then air-dried, drop-casted on sterile silicon wafers and microscopic images were taken using FE-SEM at 5 kV (Jeol Scanning Microscope JSM-6700F).

Outer Membrane Permeability Assay:

Overnight cultured bacterial cells were diluted in LB media grown at 37 ℃ for 5 hours after which suspensions were then centrifuged and washed thrice with Tris-HCl buffer (pH = 7.4) and then resuspended in tris-HCl buffer containing ANS (10 μM) with an OD at 0.05 at 600nm. The test samples were then incubated for 30min in the dark. Peptide gelator solutions were serially diluted in 96-well plates with tris HCl buffer. The incubated test bacterial samples with ANS were added equivalently with a negative control having only Bacterial cells with Tris Buffer and ANS. Triton X-100 treated bacteria solutions with ANS were tested as positive control. After incubation at 37℃ for 1 hour in dark and the fluorescence intensity values were examined using BioTek Synergy Neo microplate reader with excitation/emission wavelengths of 380/450-600nm. Each measurement was triplicated and the final results were taken in average.

Inner Membrane Permeability Assay:

Sampling procedure was same as outer membrane permeability assay using ANS as stated above. The incubated bacterial samples with PI were added equivalently after compound addition in similar serially diluted manner with a control having only Bacterial cells, Tris Buffer, and PI and similar positive control with Triton x-100 were used. The 96-well plates were incubated at 37 ℃ for 20 mins in dark and the fluorescence emission was examined using BioTek Synergy Neo microplate reader with excitation/emission wavelengths of 535/615nm. Each measurement was triplicated after which the final results were taken in average.

Cytoplasmic Membrane Potential Assay:

Overnight cultured bacteria solutions were diluted and incubated for 5 h at 37°C, followed by washing twice with HEPES buffer. Then the bacteria were re-suspended in HEPES buffer with an OD at 0.05 (at 600 nm), treated with $DiSC_3$ (5) (1.0 μM), and incubated in 96-well plates in the dark. After 18 min, the test samples were added (40 μM, 80 μM, 100 μM of treatments) and the fluorescence emission at 670 nm was measured by using the Synergy Neo microplate reader (Excitation= 622 nm / Emission= 670nm). Each measurement was triplicated, and the final results were the average of three runs.

Reactive Oxygen Species (ROS) Generation Studies:

Samples were prepared by centrifugation (at 6000 rpm for 5 min) of treated (after 6h of incubation at 37°C) and untreated (control) bacteria suspensions each of 1 mL volume in micro-centrifuge tubes and then washing and re-suspending the pellets in Hank's balanced salt solution (HBSS). 10 μL of DCFDA from stock solution (1mg/mL) was added to each micro-centrifuge tube 10 min before taking readings using a Fluoromax-3 instrument (Horiba Jobin Yvon; emission at 535 nm after excitation at 485 nm).

Live/Dead Assay:

The bacterial flocs treated by peptides (100 μM) were washed twice and re-suspended with saline. Then live/dead staining solution (SYTO 9: propidium iodide = 1:1) was loaded into the bacterial flocs and cultured for 20 min in the dark. 10 μL of the stained bacteria were placed on a glass slide, followed by covering with a coverslip and imaged using a Zeiss NLO510 laser scanning confocal microscope.

Procedure for Biocompatibility Studies:

MTT Assay:

HEK-293, HaCaT and NKE cells were seeded in 96-well plate at density of 5X103 cells/well and were allowed to attach for 24 h following which the media was replaced by compound containing media at different doses. After 24 h of incubation, Thiazolyl Blue Tetrazolium Bromide (MTT reagent) was added to the cells after dissolving in media (0.5mg/ml) and the cells were incubated for 4h. After 4h, the media was removed and the formazan crystals were dissolved in DMSO. The absorbance at 570 nm was read using a plate reader (BioTek) and the data was plotted using the Graph Pad Prism 6 software. IC_{50} values were obtained from the software statistically via student's *t*-tests.

Hemolytic Activity Assay:

To perform haemolytic activity assay, fresh human blood samples were collected from normal candidate with content following sterile medical procedure in thin tubes containing heparin as anticoagulant. 1mL of each blood sample were centrifuged at 1500 x g for 5 min and the resulting plasma fraction was removed from the samples. The pellets were washed with an equal volume (1 mL) of PBS mixing by inversion. The red blood cells (RBCs) were washed with PBS until the supernatant was clear (no red colour). After that, a volume of PBS was added, and the resulting samples were diluted 1:10 with PBS to yield an RBC

concentration of ~ 3 × 10^6 RBC/mL. The assay was performed in round bottom 96-well plate. After addition of the compounds the plate was sealed, shaken on a plate shaker for 20s, incubated at 37°C. Finally, it was centrifuged at 1500 x g for 5 min at room temperature. 60µL of supernatants were removed from all plate well and transferred to a fresh 96 well plate. This plate was then centrifuged at 1000 x g for 1 min to remove air bubble and optical density was measured at 405 nm using a plate reader. Percentage of haemolysis was calculated from absorbance (A) values with the following equation:

$$\textbf{\% haemolysis} = (A_{\text{sample}} - A_{\text{negative ctrl}}) / (A_{\text{positive ctrl}} - A_{\text{negative ctrl}}) \times 100$$

Table S1: Comparison of the behavior of the lipopeptide in different buffer systems

Buffer Type	pH Range	Observation
Acetate buffer	3.6-5.1	Transparent, clear solution obtained.
Phosphate buffer	5.9-8.0	Opaque solution with coagulated clumps appeared after a heat-cool procedure (heating up to 80-90 °C and then cooled back to room temperature).
Tris-HCl buffer	7.2-8.0	Semi-transparent solution which spontaneously formed thixotropic hydrogel followed by a heat-cool procedure.

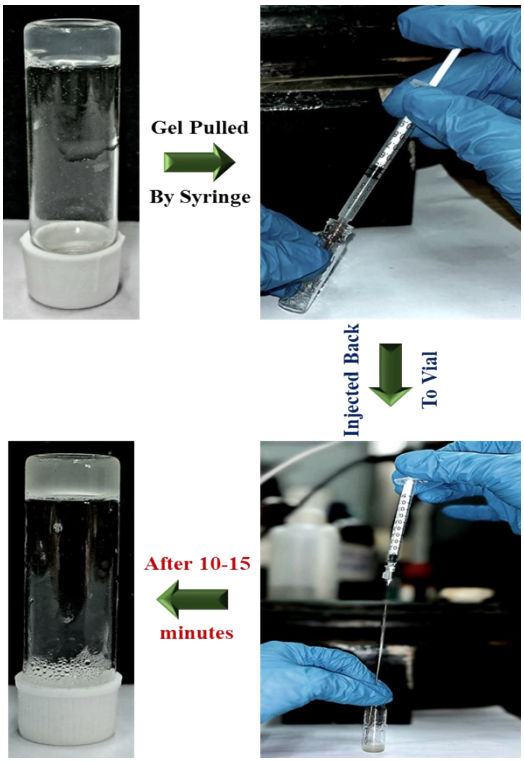


Figure S1: Injectability of the peptide hydrogel in tris-HCl buffer (pH 7.4).

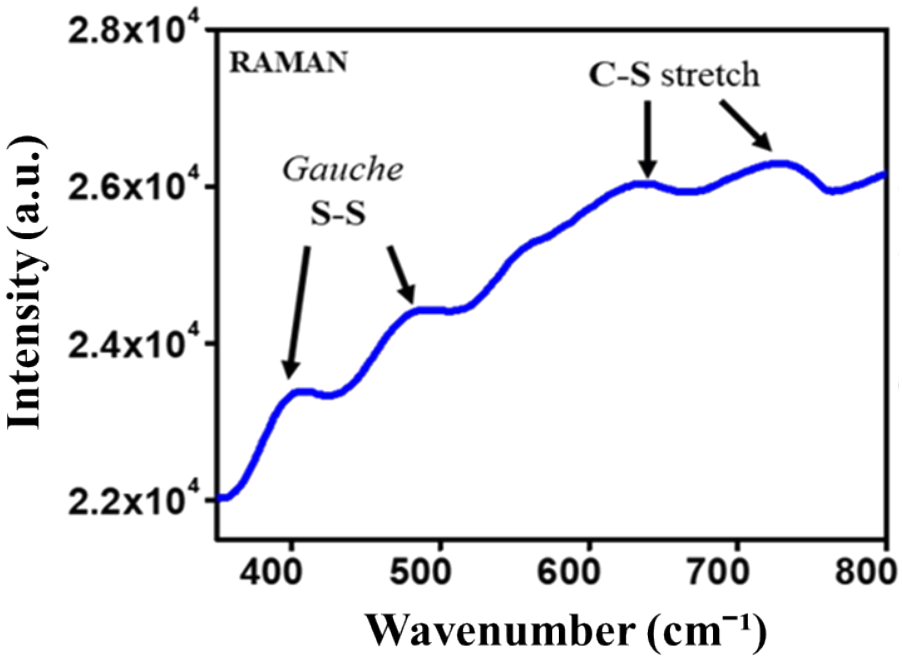


Figure S2: Vibrational Raman spectra of the peptide xerogel where characteristic peaks are marked with arrows.

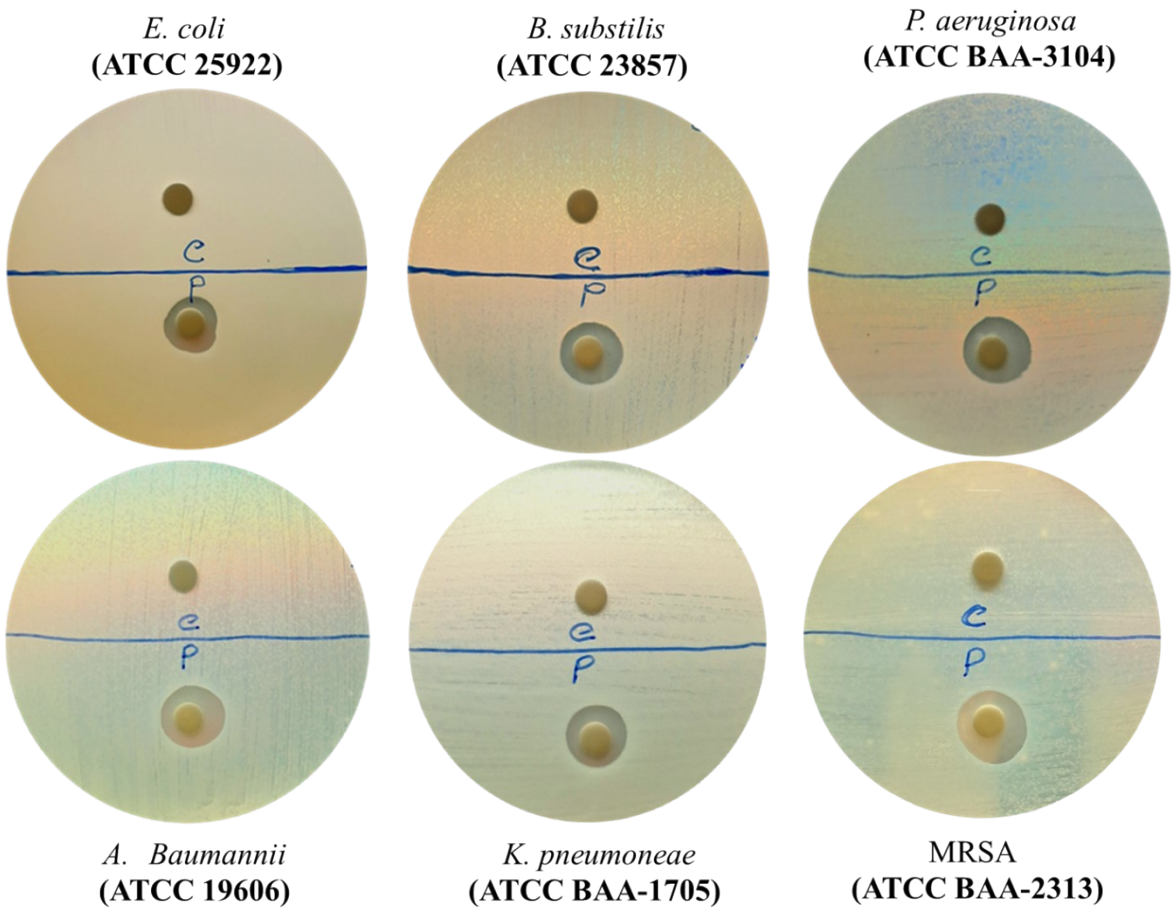


Figure S3: Optical Images of disc diffusion assay performed on Muller-Hinton Agar (MHA) plates inoculated separately with different ATCC bacteria strains. Here, C stands for control (only tris-HCl buffer solution of pH 7.4) and P refers to peptide self-assembled solution in the same buffer (100 μM).

Table S2: Zone of inhibition and minimum inhibitory concentration (MIC) values of the peptide gelator against several Gram-positive and Gram-negative bacteria strains.

	Bacteria Strains	**Zone of Inhibition diameter (In mm)**	**MIC values (In µM)**
Gram-positive	Methicillin resistant *S. aureus* **(ATCC BAA-2313)**	18	22.5
	B. substilis **(ATCC 23857)**	16	26.8
Gram-negative	*K. pneumoneae* **(ATCC BAA-1705)**	16	34.5
	E. coli **(ATCC 25922)**	15	46.8
	P. aeruginosa **(ATCC BAA-3104)**	17	32.4
	A. baumannii **(ATCC 19606)**	16	40.2

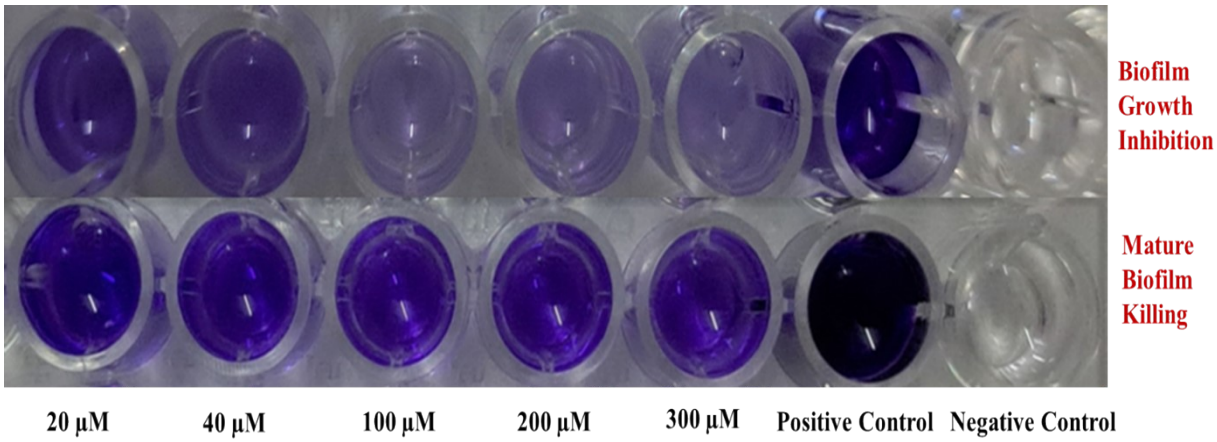


Figure S4: Optical images of Crystal Violet (CV) assay to ascertain MRSA biofilm inhibition and mature MRSA biofilm reduction ability of the peptide gelators (AP1). Here, Positive Control- untreated biofilm suspension stained with CV; Negative Control- peptide gelator solutions only.

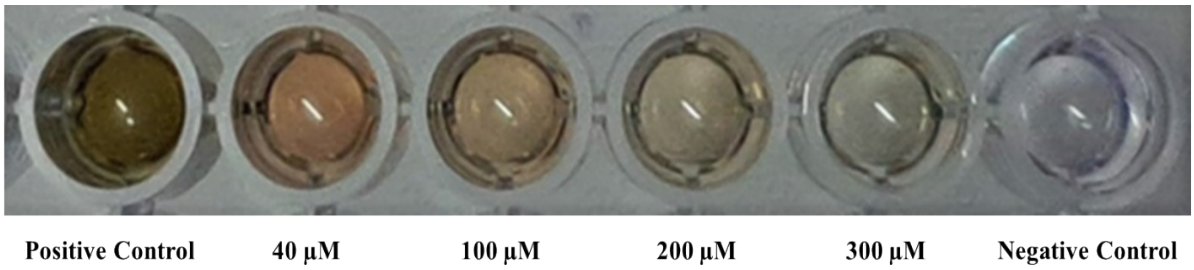


Figure S5: Optical image of phenol-sulphuric acid assay to evaluate EPS reduction ability of the peptide gelators (AP1). Here also, Positive Control- untreated EPS; Negative Control- peptide gelator solutions only.

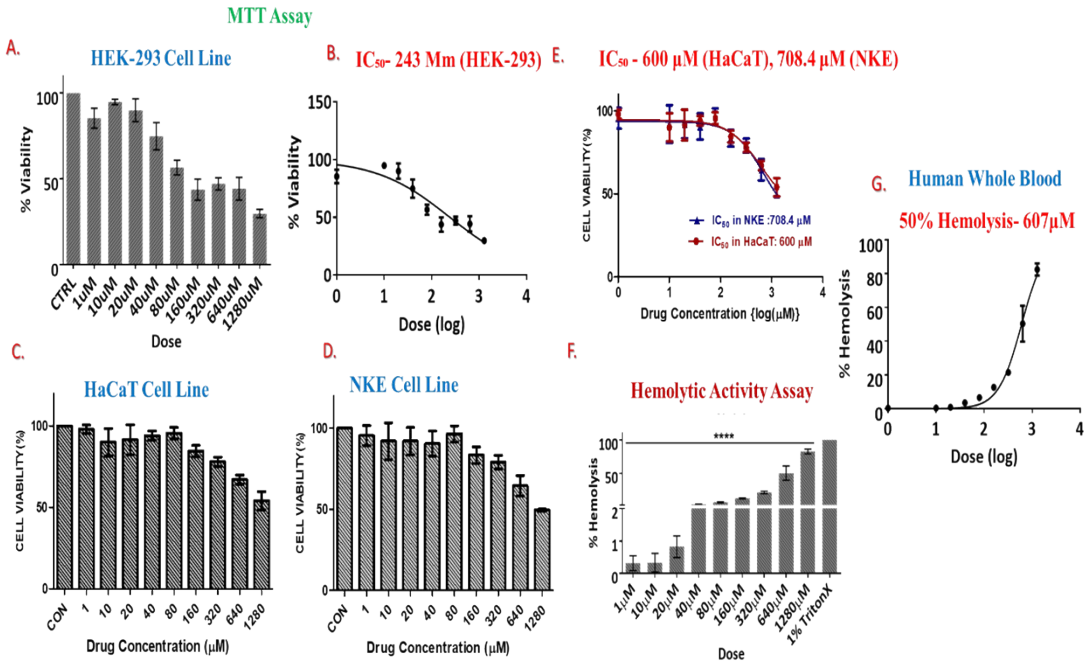


Figure S6: (A), (B) represents MTT assay data to test for biocompatibility of the peptide AP1 using HEK-293 cell line in terms of plots of % cell viability against dosage concentration from which 50% cell viability was quantified as IC_{50} value of 243 µM; (C)-(E) represents MTT assay data to test for biocompatibility of the peptide AP1 against HaCaT and NKE cell lines in terms of plots of % cell viability against dosage concentrations from which 50% cell viability was quantified as IC_{50} value- 600 µM, 708.4 µM respectively; (G),(F) represents data for haemolytic activity of the peptide gelator solution with fresh human blood where % haemolysis was plotted against concentration from which 50% haemolysis was calculated as 607 µM.

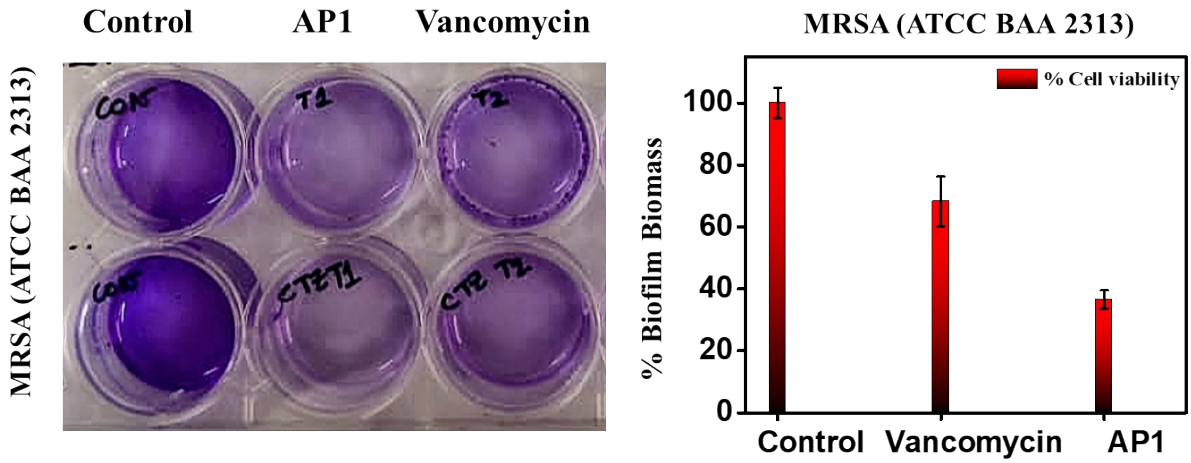
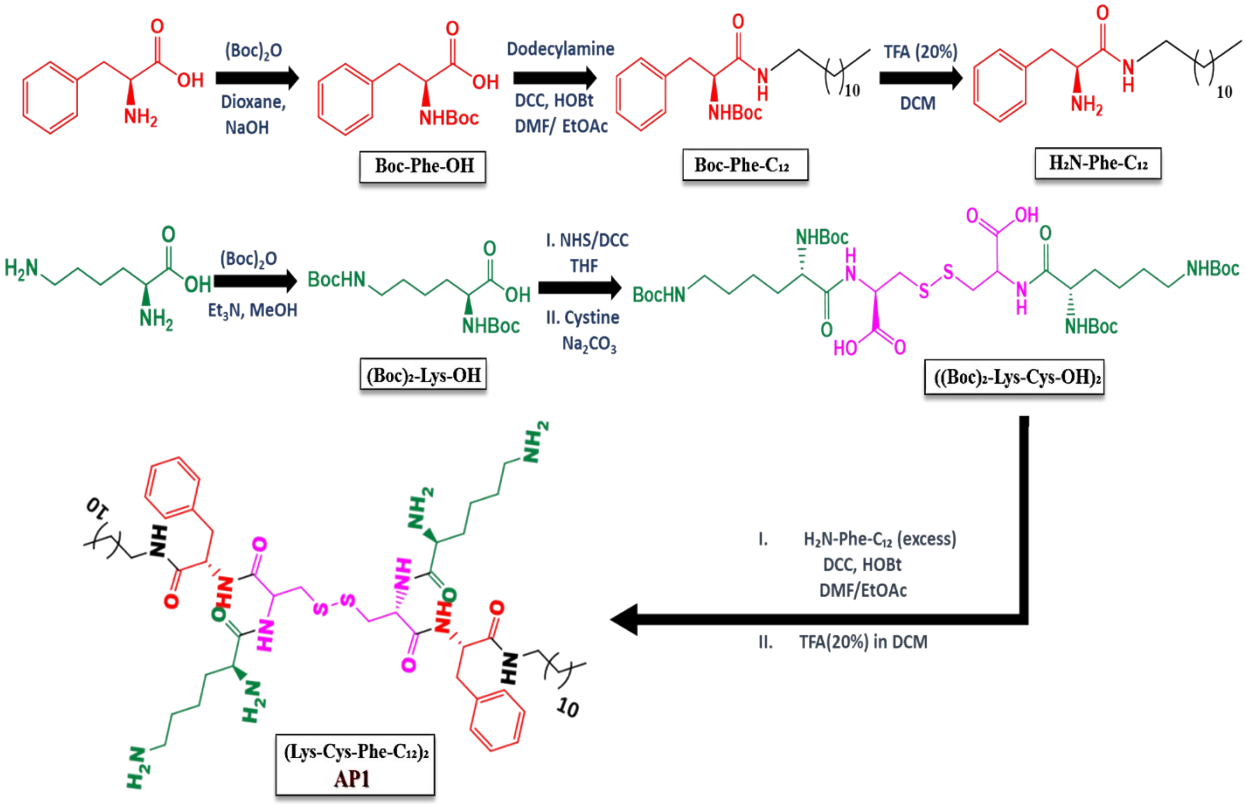


Figure S7: Optical images of Crystal Violet (CV) assay along with % biofilm biomass reduction to ascertain comparison between MRSA biofilm inhibition with lipopeptide gelator AP1 (50 µM) and Vancomycin (1 µg/mL). Each experiment was performed twice.

Schematic Representation of Lipopeptide (AP1) Synthesis



Detailed Synthetic Procedures and Characterisations

Synthesis of $((Boc)_2Lys\text{-}CyS\text{-}OH)_2$:

In a 250 mL r.b. flask, 3.04 g (8.8 mmol) of $(Boc)_2$-Lys-OH was taken in tetrahydrofuran (THF) and 1.2 g (10 mmol) of N-hydroxysuccinimide was added which was stirred for 2 h in which DCC was added (2.1 g; 10 mmol) followed by stirring for 16 h. Then, after filtering the N, N-dicyclohexyl urea (DCU) by product, cystine (0.9 g; 4 mmol) was added after being dissolved in 1(N) Na_2CO_3 solution and the whole reaction mixture was stirred for 24 h. Evaporating the THF in vacuum, the aqueous mixture was extracted in ethyl acetate after neutralizing with 1(N) HCl, dried over anhydrous Na_2SO_4 and evaporated in vacuum to obtained colourless sticky crude which was purified by silica gel column chromatography (chloroform: methanol = 7:3 as eluent). The white solid product was then thoroughly characterized by NMR and high-resolution mass spectrometry (HRMS).

Yield: 2.9 g (3.2 mmol, 80%)

HRMS (m/z): Calculated for $C_{38}H_{68}N_6O_{14}S_2$ (M): 896.42; Found: 897.49 $[M + H]^+$

^{1}H NMR (500 MHz, DMSO-d_6) δ (in ppm) 12.94 (s, 2H), 8.14 (d, J = 181.2 Hz, 2H), 6.95 (d, J = 51.4 Hz, 2H), 6.79 – 6.63 (m, 2H), 4.32 (d, J = 103.8 Hz, 2H), 3.82 (d, J = 47.5 Hz, 2H), 2.90 – 2.73 (m, 8H), 1.65 – 1.48 (m, 3H), 1.47 – 1.39 (m, 4H), 1.38 (d, J = 5.4 Hz, 36H), 1.24 (t, J = 11.2 Hz, 4H).

^{13}C NMR (101 MHz, DMSO) δ (in ppm) 172.08, 162.29, 155.53, 155.25, 79.16, 78.06, 77.30, 54.35, 40.19, 40.13, 39.98, 39.93, 39.77, 39.72, 39.51, 39.30, 39.09, 38.88, 35.77, 31.60, 29.17, 28.26, 28.18, 22.75.

Synthesis of Boc-Phe-C_{12}:

In a 250 mL round bottom flask, Boc-Phe-OH (2.3g, 9 mmol) was dissolved in 20 mL DMF and 40 mL of EtOAc. Then, 9 mmol (1.7 g) of dodecylamine was added followed by addition of 1.8 g (13.5 mmol) HOBt and then DCC (2.8 g, 13.5 mmol) and the whole reaction mixture

was stirred for 24 h. After which it was filtered to separate N, N dicyclohexyl urea (DCU) followed by extraction into ethyl acetate by working up with 1(N) HCl (2 × 30 mL), 1 (N) Na_2CO_3 (2 × 30 mL) and brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuum. A yellowish white material was obtained as an impure material, which was subsequently purified using silica gel column in pet ether and ethyl acetate (85:15) as eluent. After purification white colour powder was obtained which was characterized by NMR and HRMS.

Yield: 3.3 g (7.7 mmol; 85%)

HRMS (m/z): Calculated for $C_{26}H_{44}N_2O_3$ (M): 432.34; Found: 433.31 $[M + H]^+$

^{1}H NMR (500 MHz, $CDCl_3$) δ (in ppm) 7.34 – 7.21 (m, 5H), 4.27 (t, J = 7.6 Hz, 1H), 3.16 (t, J = 6.7 Hz, 2H), 3.13 – 2.99 (m, 2H), 1.44 (s, 9H), 1.37 – 1.13 (m, 20H), 0.90 (t, J = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ (in ppm) 170.89, 155.37, 136.89, 129.33, 128.67, 126.93, 80.14, 77.34, 77.03, 76.71, 56.15, 39.48, 38.82, 31.92, 29.66, 29.63, 29.59, 29.50, 29.35, 29.34, 29.25, 28.29, 26.77, 22.69, 14.13.

Synthesis of H_2N-Phe-C_{12}:

3.3g (7.7 mmol) of Boc-Phe-C_{12}, 5 mL of 20% TFA in dichloromethane was added and the removal of the Boc group was monitored by TLC. After 2 h, TFA was removed under vacuum and the residue was taken extracted in ethyl acetate (2 × 30 mL) after neutralizing by saturated Na_2CO_3 solution. The ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated in vacuum to obtain the white colourless sticky product which was purified by column chromatography using basic alumina with chloroform and methanol (9:1) as eluent. NMR and HRMS spectra of the purified compound were recorded for Characterization.

Yield: 2.3g (7 mmol, 91%)

HRMS (m/z): Calculated for $C_{21}H_{36}N_2O$ (M): 332.28; Found: 333.28 $[M + H]^+$

^{1}H NMR (500 MHz, DMSO-d_6) δ (in ppm) 7.76 (t, J = 5.7 Hz, 1H), 7.31 – 7.14 (m, 5H), 3.01 (dp, J = 22.7, 6.4 Hz, 2H), 2.88 (dd, J = 13.3, 5.4 Hz, 1H), 2.61 (dd, J = 13.3, 7.9 Hz, 1H), 1.24 (d, J = 8.4 Hz, 20H), 0.86 (t, J = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ (in ppm) 174.02, 138.01, 129.32, 128.69, 126.79, 77.36, 77.04, 76.72, 56.49, 41.10, 39.13, 31.93, 29.66, 29.64, 29.61, 29.58, 29.55, 29.36, 29.32, 26.95, 22.70, 14.13, 1.03.

Synthesis of $((Boc)_2$Lys-Cys-Phe-$C_{12})_2$:

2.9 g (3.2 mmol) $((Boc)_2$Lys-Cys-$OH)_2$ was taken in a 250 mL round bottom flask and dissolved in 10 mL DMF and 20 mL of EtOAc after which 2.3g (7 mmol) of H_2N-Phe-C_{12} was added into it and stirred to dissolve. Then, 5 mmol (0.7 g) of HOBt was added followed by addition of 5 mmol of DCC (1.1 g) and the whole reaction mixture was kept under stirring for 48 h. After filtering out N, N dicyclohexyl urea (DCU), the reaction mixture was extracted in ethyl acetate followed by working up with 1(N) HCl (2 × 30 mL), 1(N) Na_2CO_3 (2 × 30 mL) and brine (2 × 30 mL). Then, after drying over anhydrous Na_2SO_4 and evaporated in vacuum, the crude product was obtained. It was subsequently purified by silica gel column chromatography using chloroform and methanol (7:3) as eluent. The pure product was characterized by ^{1}H, ^{13}C NMR spectroscopy and MALDI-TOF mass spectrometry.

Yield: 3.8 g (2.5 mmol, 78%)

HRMS (m/z): Calculated for $C_{80}H_{136}N_{10}O_{14}S_2$ (M): 1524.97; Found: 1548.83 $[M + H + Na]^{2+}$

^{1}H NMR (500 MHz, DMSO-d_6) δ (in ppm) 8.08 (dd, J = 32.3, 7.9 Hz, 4H), 7.89 (s, 2H), 7.26 – 7.14 (m, 10H), 6.94 (d, J = 7.8 Hz, 2H), 6.73 (d, J = 8.0 Hz, 2H), 4.54 (s, 2H), 4.50 – 4.44 (m, 2H), 3.94 (s, 2H), 3.05 (t, J = 6.7 Hz, 2H), 2.95 (dq, J = 12.9, 7.3, 6.3 Hz, 5H), 2.85 (dt, J = 21.4, 5.3 Hz, 5H), 1.60 – 1.38 (m, 8H), 1.38 – 1.30 (m, 35H), 1.23 (d, J = 10.4 Hz, 43H), 0.85 (t, J = 6.8 Hz, 6H).

^{13}C NMR (126 MHz, DMSO) δ (in ppm) 172.91, 170.61, 169.72, 156.01, 137.84, 129.57, 128.47, 126.72, 78.69, 77.75, 54.87, 54.52, 52.38, 40.58, 40.49, 40.42, 40.33, 40.25, 40.16, 40.08, 39.99, 39.91, 39.83, 39.66, 39.49, 39.01, 38.39, 31.98, 31.77, 29.66, 29.54, 29.49, 29.46, 29.33, 29.23, 29.19, 28.73, 28.66, 26.76, 23.27, 22.56, 14.41.

Synthesis of (Lys-Cys-Phe-C_{12})$_2$ (Abbreviated as AP1):

In a 100 mL round bottom flask, 2 g (1.3 mmol) of ((Boc)$_2$Lys-Cys-Phe-C_{12})$_2$ was taken and 20% TFA in dichloromethane was added followed by stirring for 8 h. Then, TFA was removed under vacuum and the residue was then taken in water (10 mL) and neutralized by using ammonium hydroxide solution. Then the aqueous mixture was lyophilized using freeze dryer and yellowish powder of crude product was obtained which was then subsequently purified via high performance liquid chromatography (HPLC). HPLC was performed using a Shimadzu SPD-20A system C_{18} Waters SPHERISORB (4.6x250mm) analytical column, employing gradient systems 10-90 % ,0.1%TFA-MeCN/ 0.1%TFA-water for 25 min and then 90-10% ,0.1%TFA-MeCN/ 0.1%TFA-water for 10min. Finally, the pure peptide was characterized by ^{1}H, ^{13}C NMR and MALDI-TOF mass spectrometric analysis.

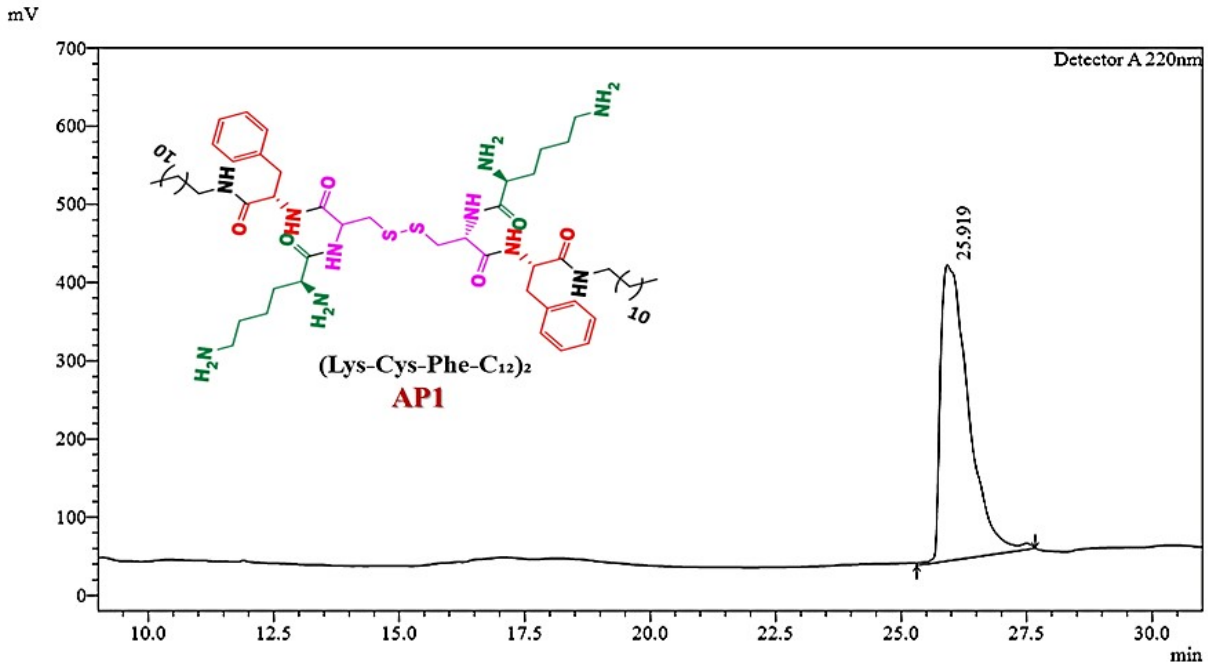
Yield: 1.3 g (1.2 mmol, 90%)

HRMS (m/z): Calculated for $C_{60}H_{104}N_{10}O_4S_2$ (M): 1124.76; Found: 1126.62 $[M + 2H]^{2+}$, 1148.62 $[M + H + Na]^{2+}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (in ppm) 8.28 (s, 4H), 8.09 (t, J = 5.7 Hz, 4H), 7.89 (s, 2H), 7.23 – 7.19 (m, 10H), 4.62 (td, J = 8.4, 4.9 Hz, 2H), 4.52 (q, J = 8.0 Hz, 2H), 3.09 (dt, J = 13.0, 5.8 Hz, 4H), 3.00 – 2.77 (m, 8H), 2.74 (d, J = 8.1 Hz, 4H), 1.69 (dt, J = 11.4, 6.4 Hz, 4H), 1.52 (q, J = 7.5 Hz, 4H), 1.23 (d, J = 8.5 Hz, 44H), 0.85 (t, J = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, DMSO-d_6) δ (in ppm)170.79, 169.19, 158.77, 137.80, 129.64, 128.47, 126.77, 118.89, 116.50, 54.55, 52.69, 52.38, 40.54, 40.45, 40.37, 40.28, 40.20, 40.11, 39.94,

39.78, 39.61, 39.44, 38.92, 38.36, 31.76, 31.00, 29.53, 29.48, 29.31, 29.22, 29.17, 26.95, 26.78, 22.55, 21.52, 14.40.



Peak Table

Detector A 220nm

Peak#	Ret. Time	Area	Height	Area%
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Total		14815199	378427	100.000

Figure S7: HPLC chromatogram of the synthesized peptide AP1.

HRMS, MALDI-TOF, ^{1}H NMR, ^{13}C NMR data

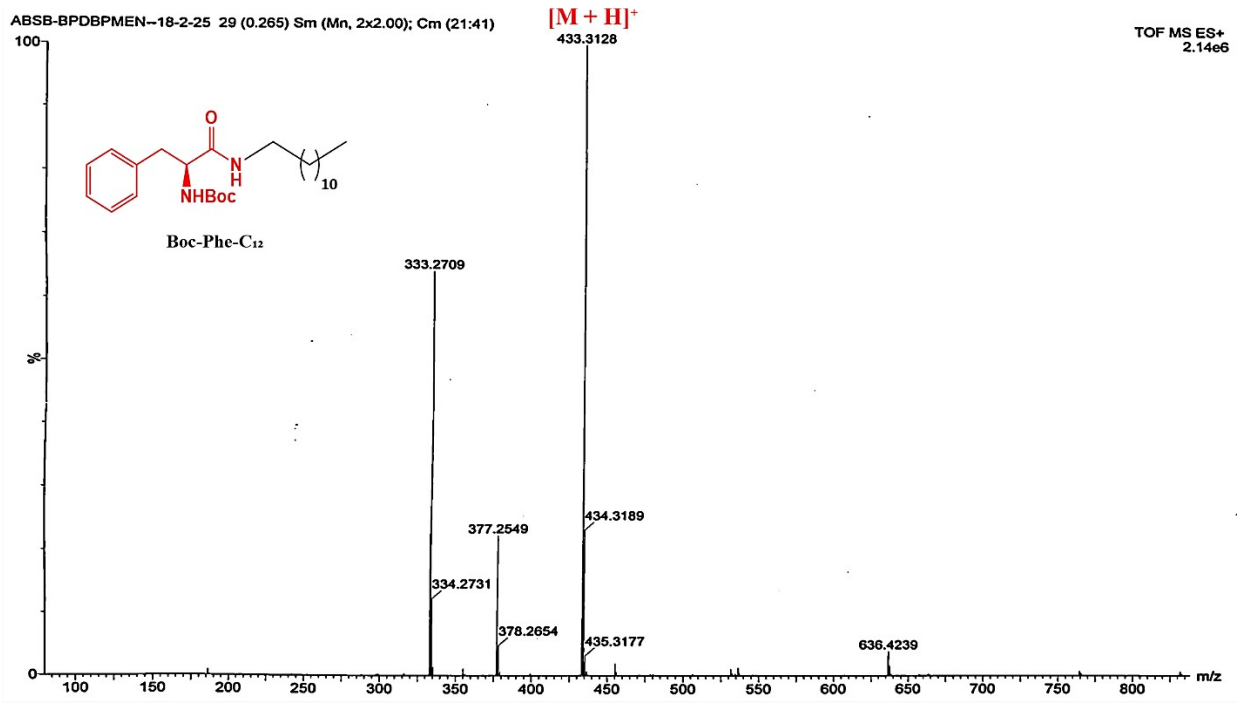


Figure S8: HRMS Spectra of Boc-Phe-C_{12}

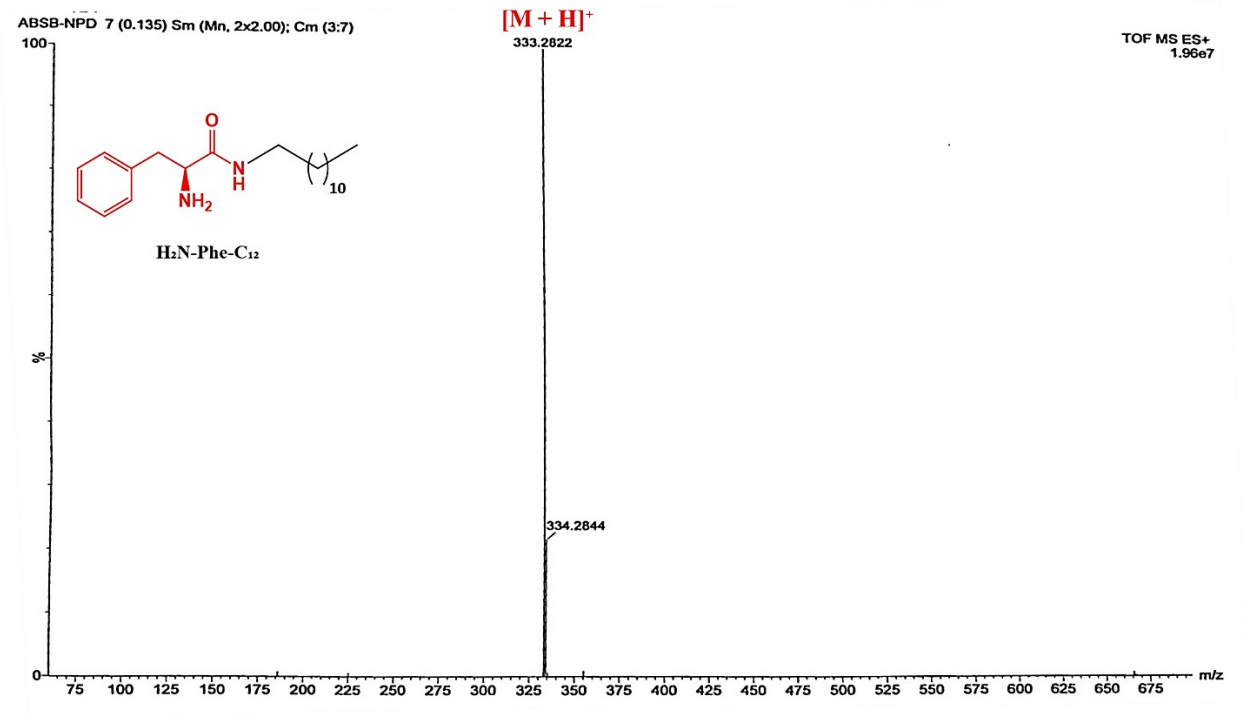


Figure S9: HRMS Spectra of NH_2-Phe-C_{12}

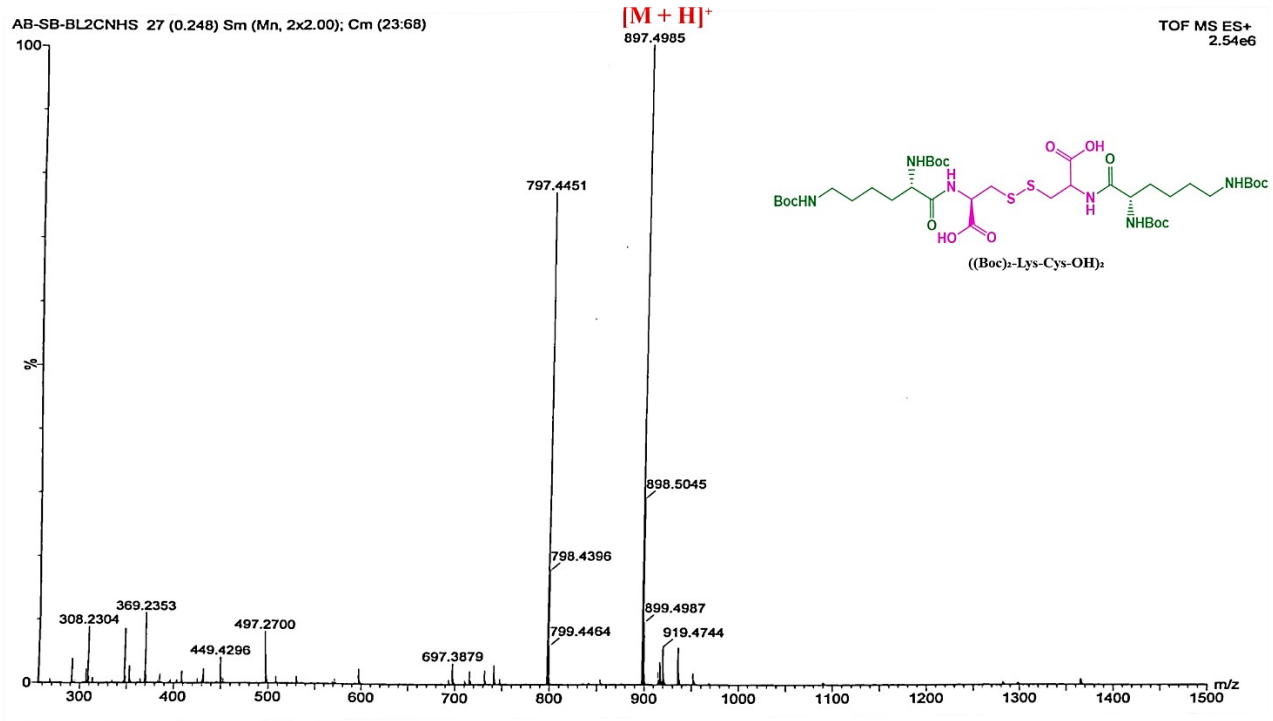


Figure S10: HRMS Spectra of $((Boc)_2Lys\text{-}CyS\text{-}OH)_2$

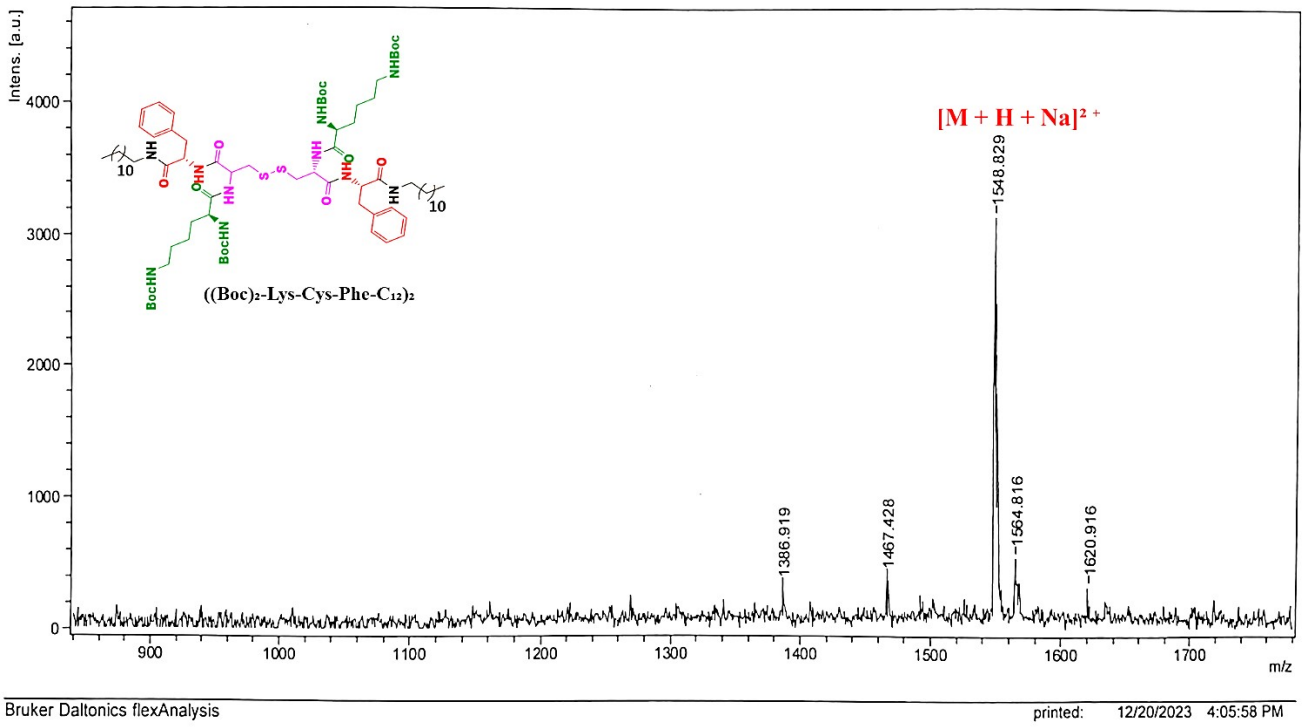


Figure S11: HRMS Spectra of $((Boc)_2Lys\text{-}Cys\text{-}Phe\text{-}C_{12})_2$

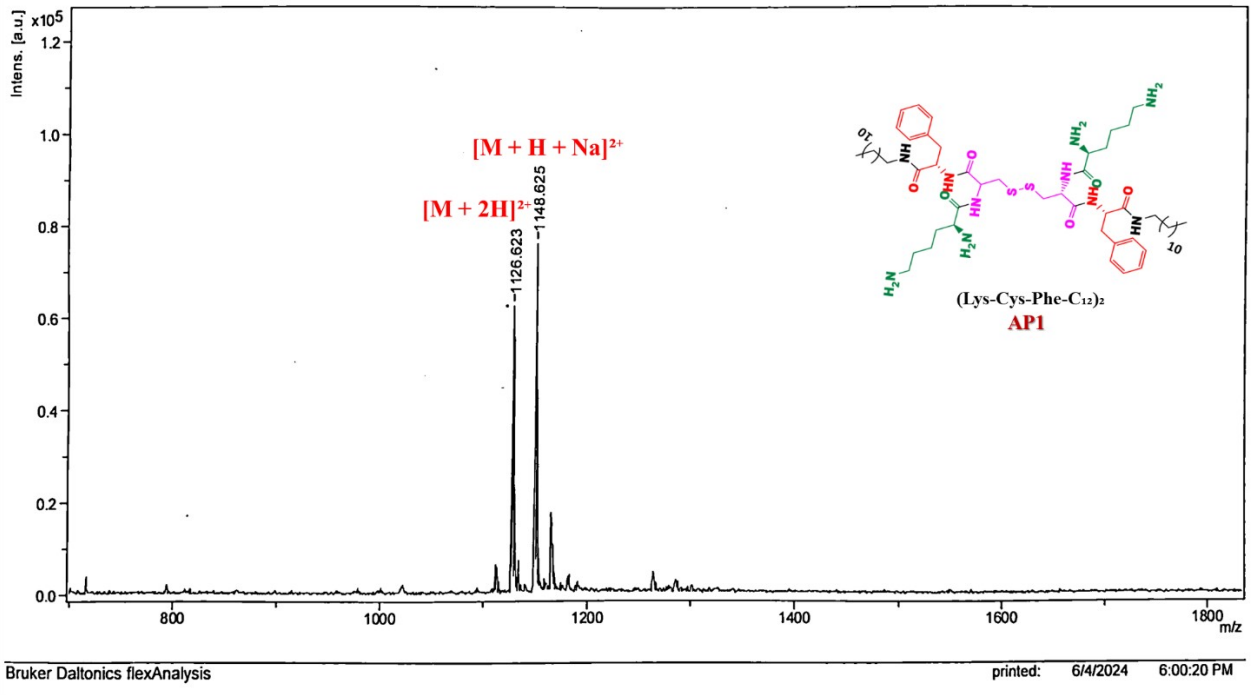
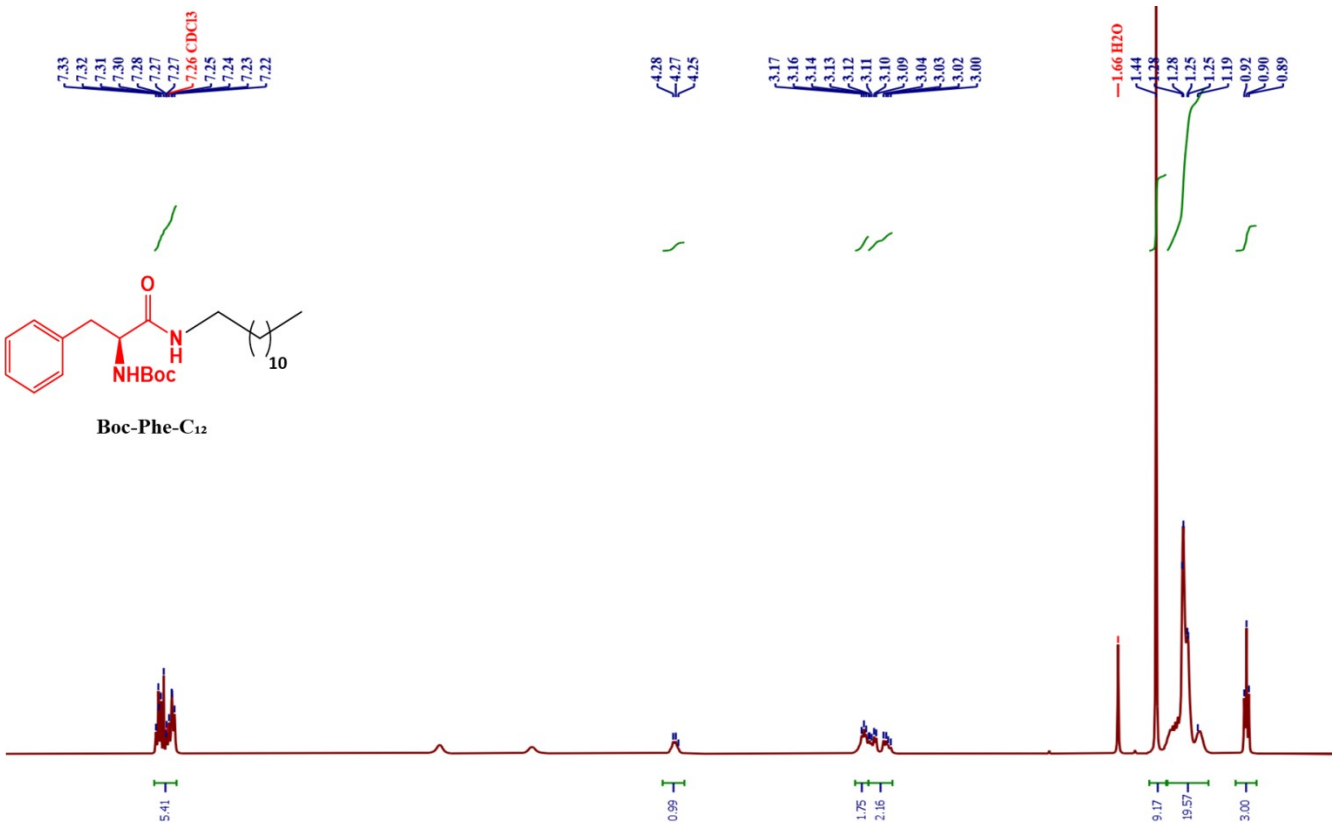


Figure S12: HRMS Spectra of $(Lys\text{-}Cys\text{-}Phe\text{-}C_{12})_2$



Boc-Phe-C_{12}

Figure S13: ^{1}H NMR Spectra of Boc-Phe-C_{12}

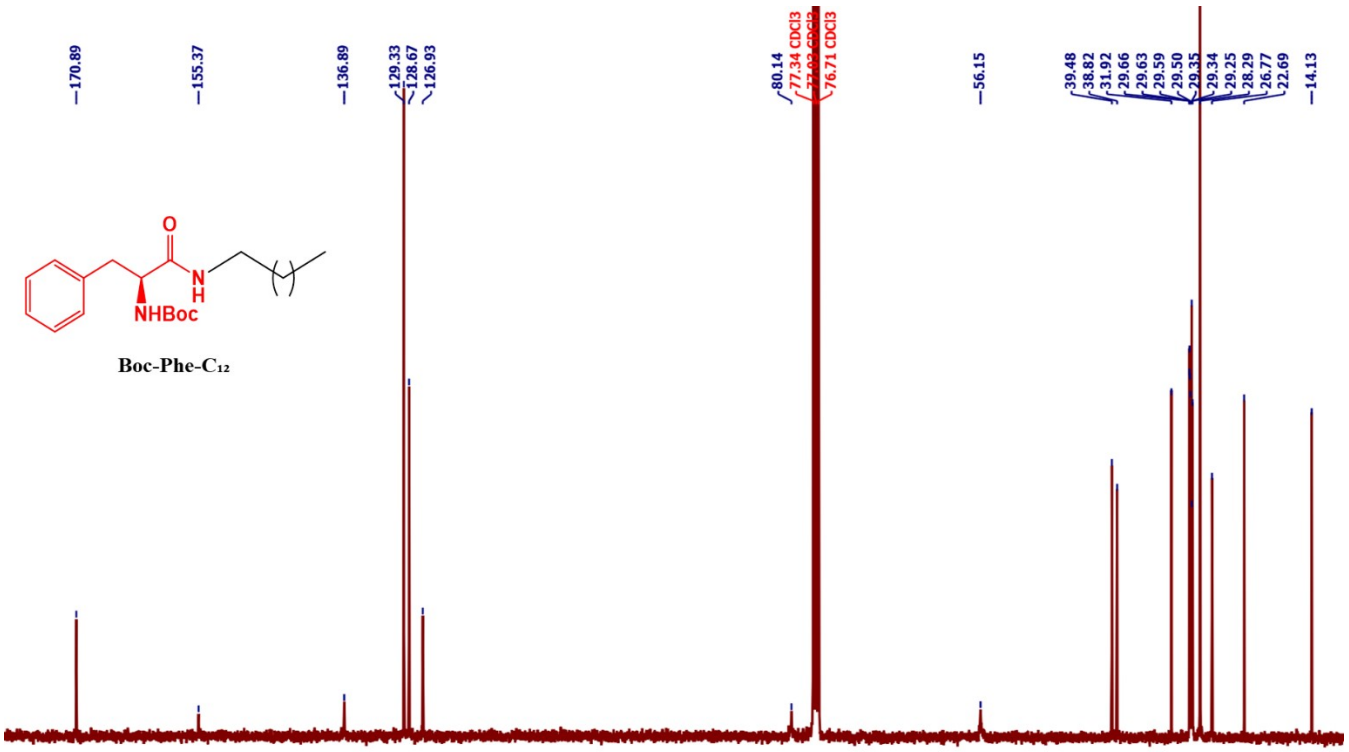


Figure S14: ^{13}C NMR Spectra of Boc-Phe-C_{12}

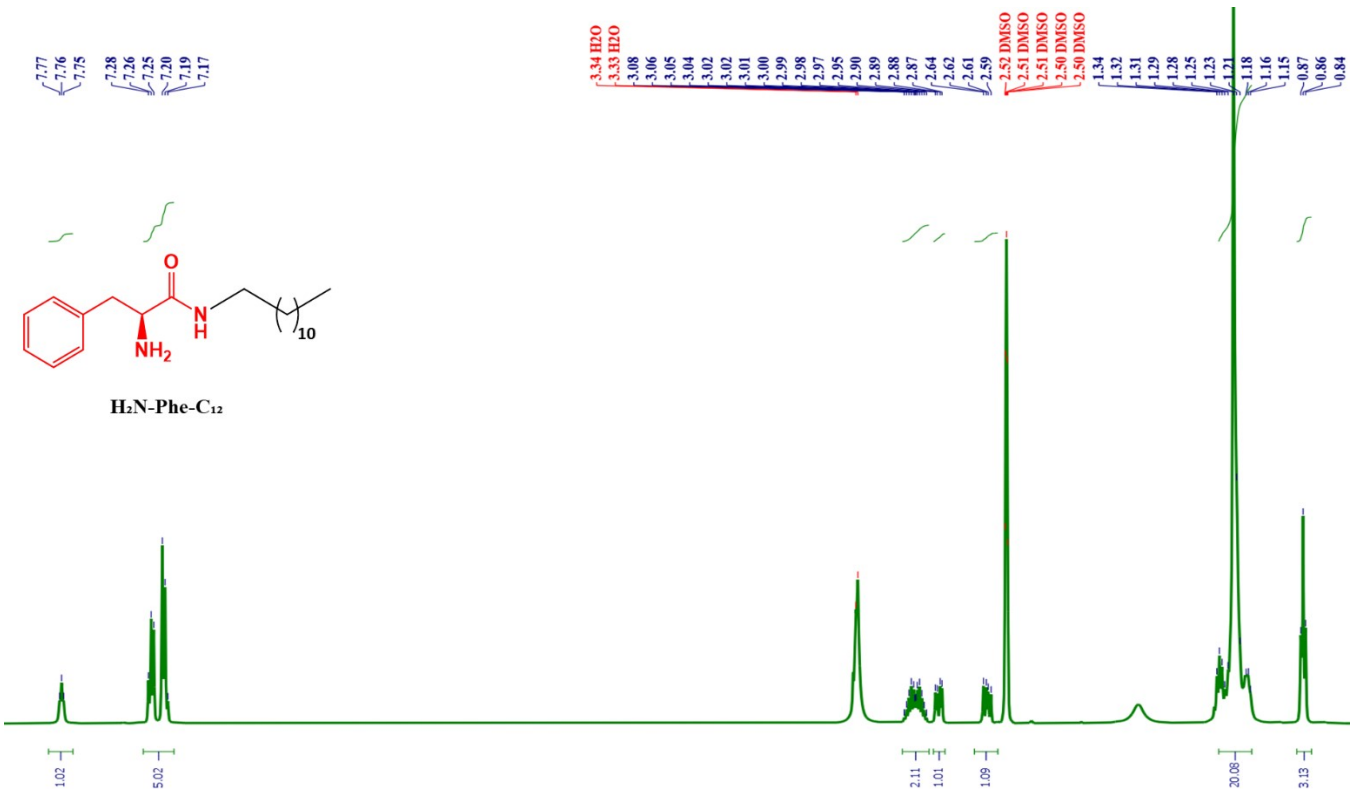


Figure S15: ^{1}H NMR Spectra of NH_2-Phe-C_{12}

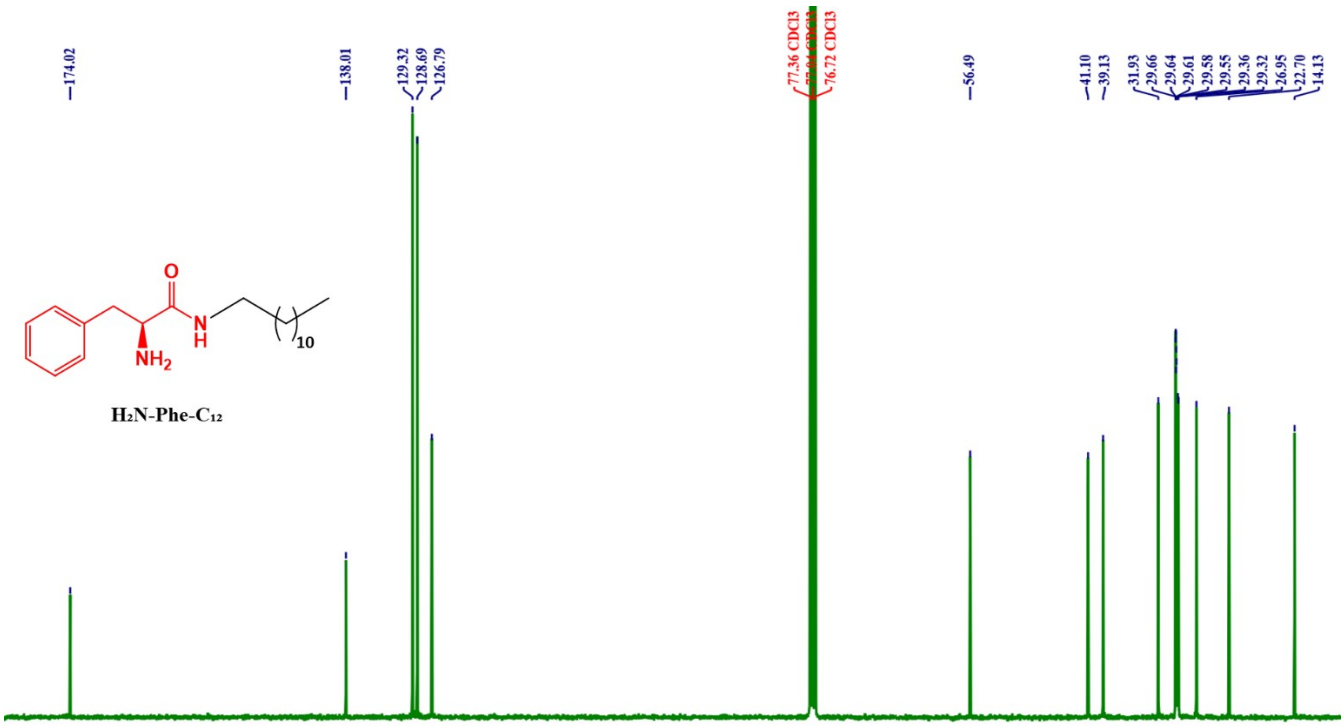


Figure S16: ^{13}C NMR Spectra of NH_2-Phe-C_1

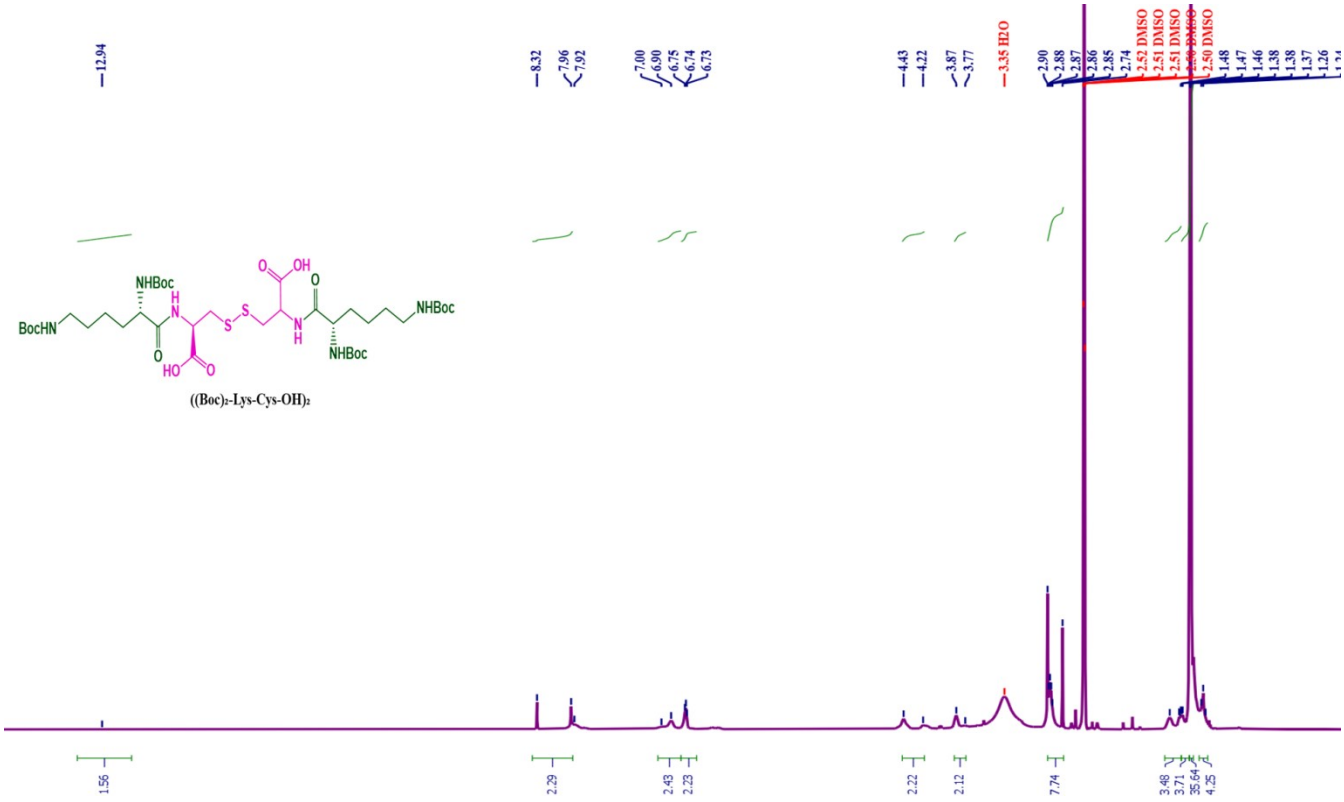


Figure S17: ^{1}H NMR Spectra of $((Boc)_2Lys\text{-}CyS\text{-}OH)_2$

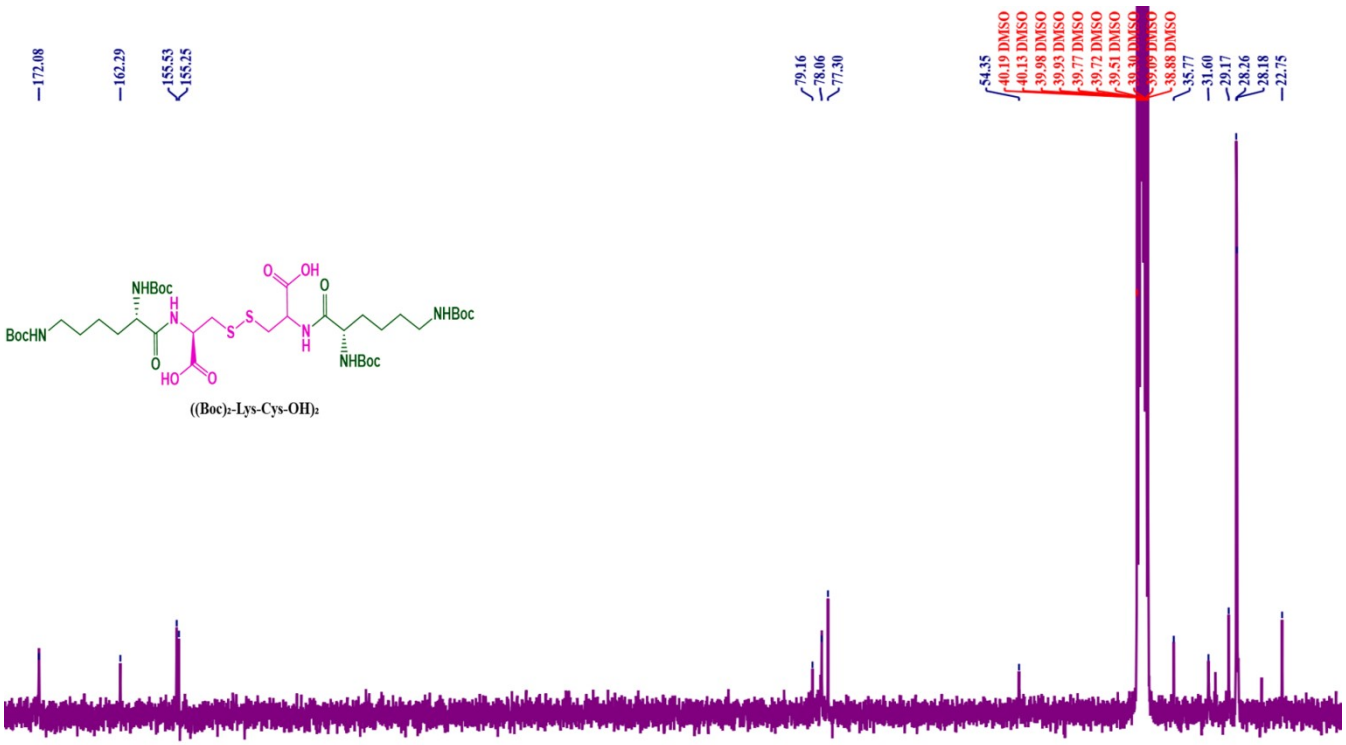


Figure S18: ^{13}C NMR Spectra of $((Boc)_2Lys\text{-}CyS\text{-}OH)_2$

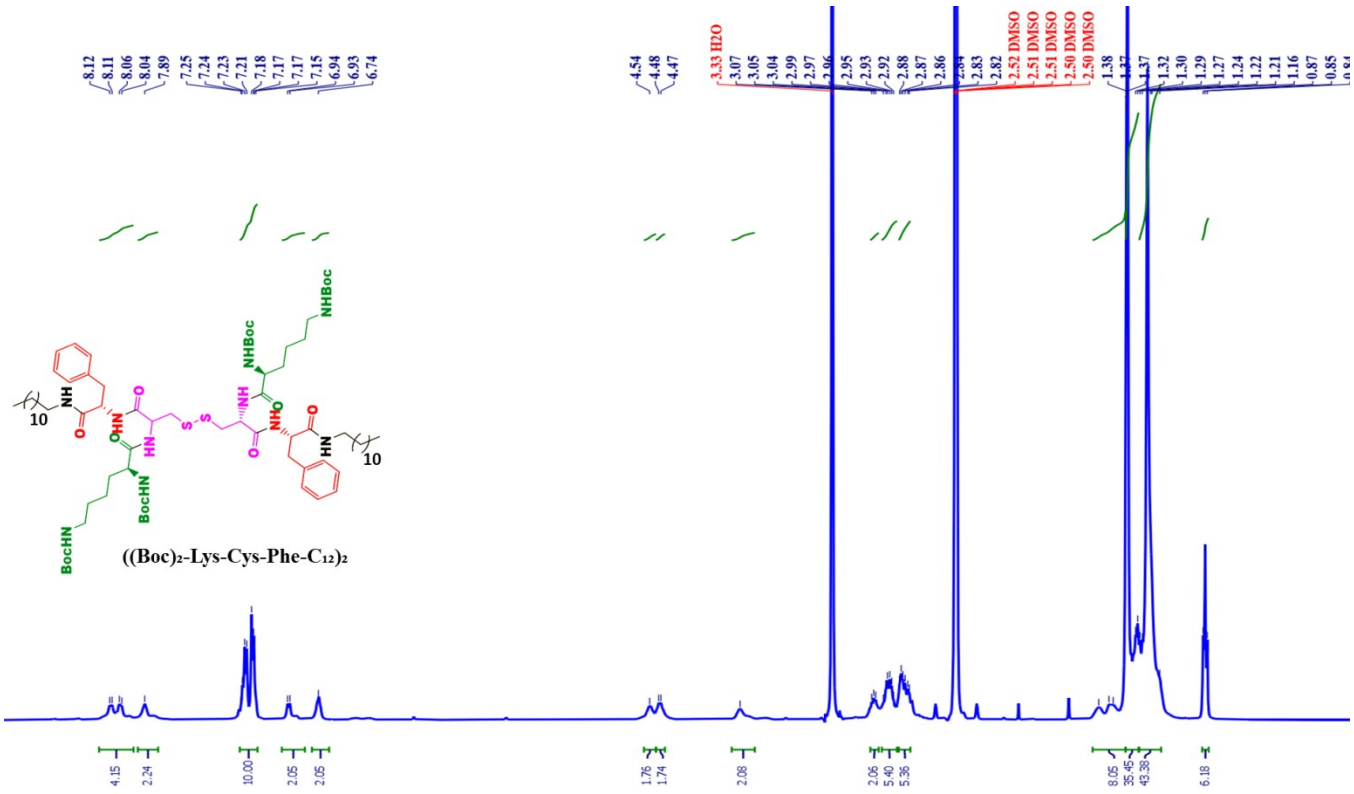


Figure S19: ^{1}H NMR Spectra of $((Boc)_2Lys\text{-}Cys\text{-}Phe\text{-}C_{12})_2$

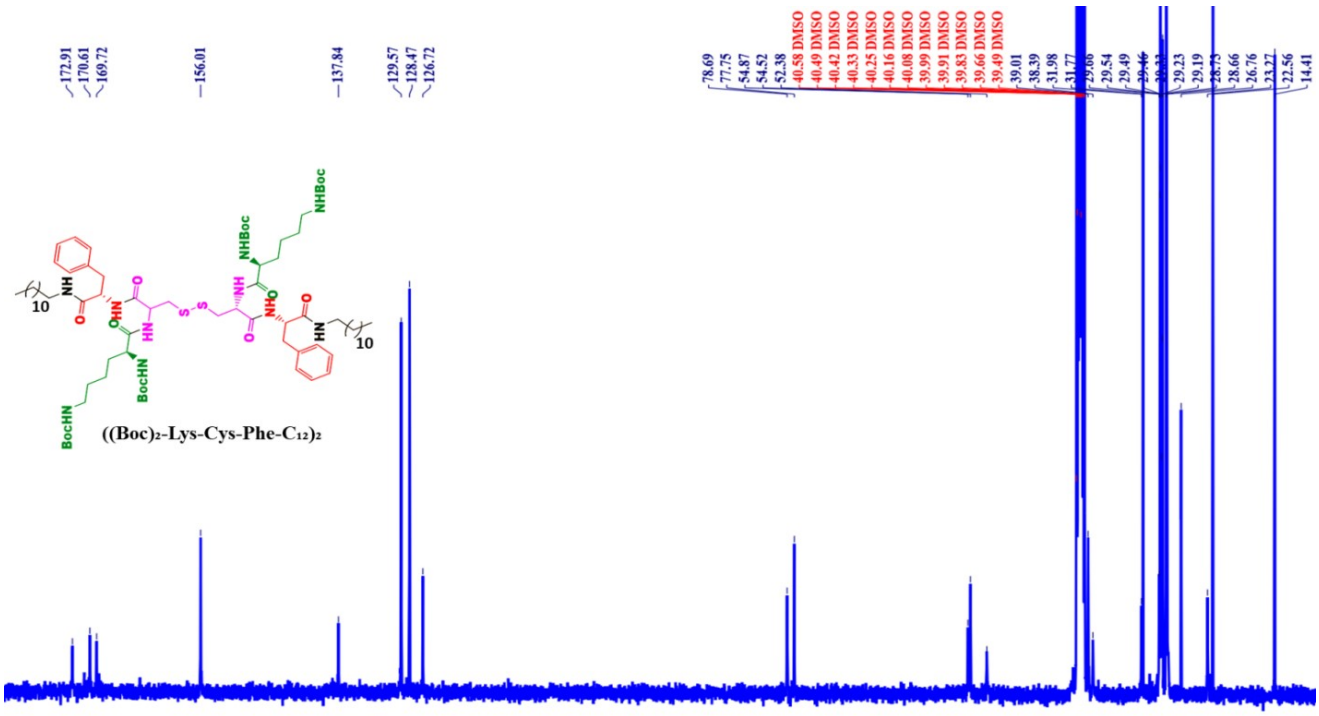


Figure S20: ^{13}C NMR Spectra of ((Boc)$_2$Lys-Cys-Phe-C$_{12}$)$_2$

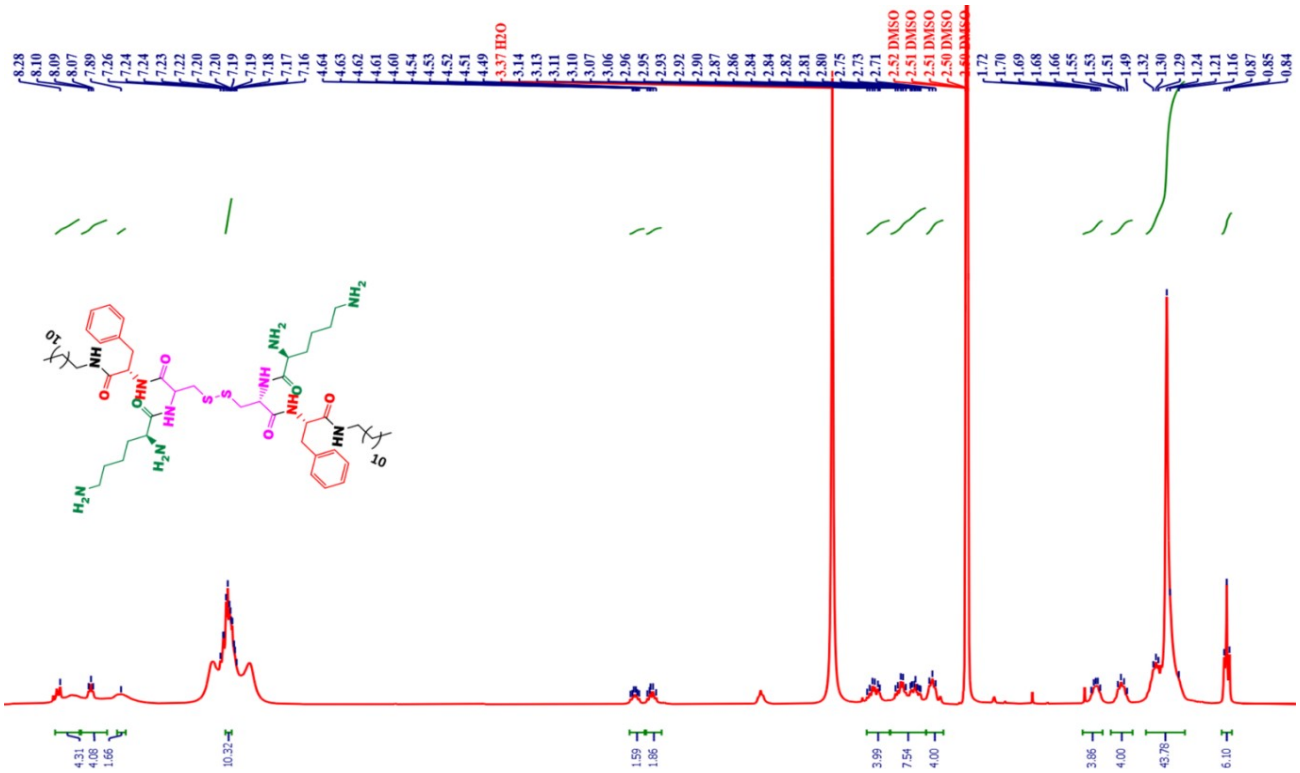


Figure S21: ^{1}H NMR Spectra of (Lys-Cys-Phe-C$_{12}$)$_2$

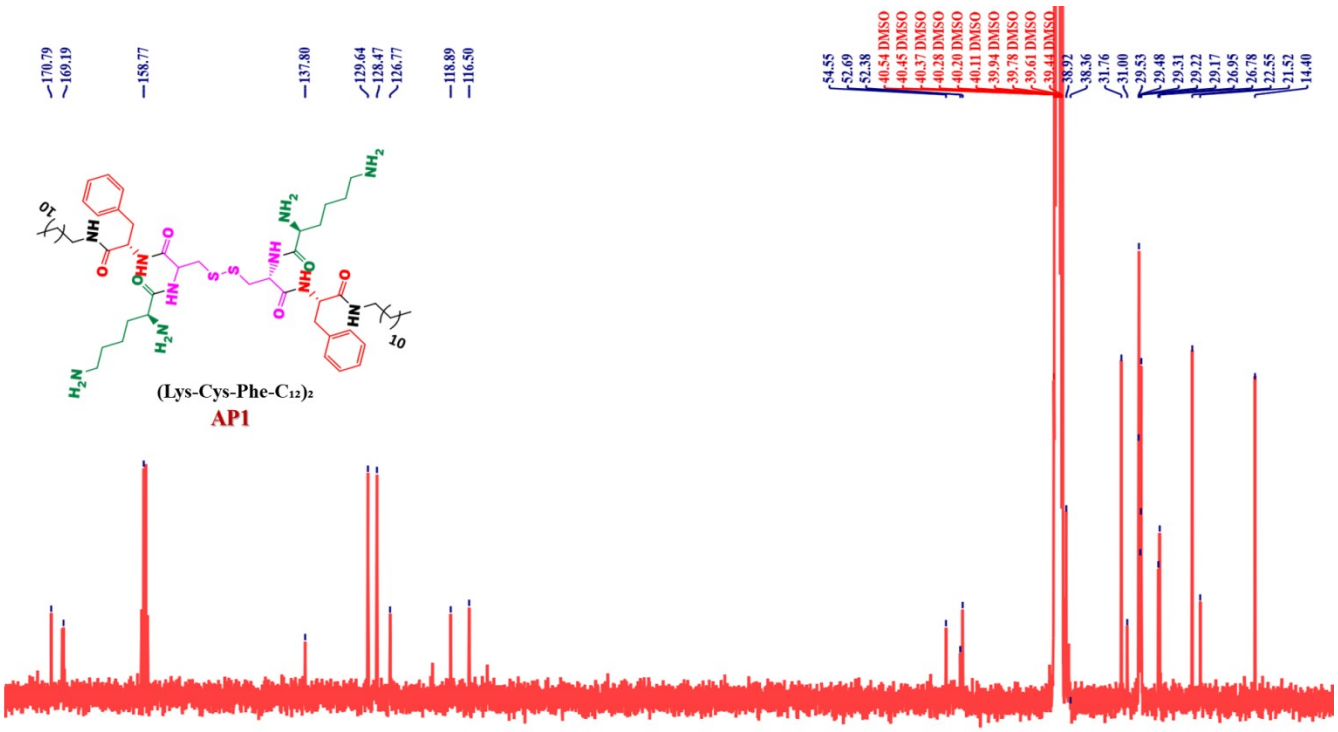


Figure S22: ^{13}C NMR Spectra of $(Lys\text{-}Cys\text{-}Phe\text{-}C_{12})_2$