Supporting Information

Transition metal ions mediated morphological transformation of pyridine based peptide nanostructures

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Material and methods

1.0 General: Acetonitrile (CH_3CN), dichloromethane (DCM), methanol (MeOH) and triethylamine (TEA) were distilled following standard procedures prior to use. L-Phenylalanine, Tyrosine, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl), 1-hydroxybenzotriazole and trifluoroacetic acid was purchased from Avra synthesis Pvt. Ltd. (Hyderabad, India). 2,6-Pyridinedicarboxylic acid was purchased from SRL private limited (India). Sodium bicarbonate was obtained from Merck. Sodium carbonate was purchased from Rankem; hydrochloric acid was purchased from Thermo Fisher Scientific; tri-ethyl amine was procured from S-D Fine Chem. Limited (Mumbai) and was used without further purification. $Fe(NO_3)_3.9H_2O$, $Co(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Cu(NO_3)_2.3H_2O$ and $Zn(NO_3)_2.6H_2O$ metal salts purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India

2.0 NMR Spectroscopy and NMR titration experiments: Samples were prepared by dissolving compound in deuterated solvents (DMSO-d_6 and CD_3OD. ^1H and ^{13}C-NMR spectra were recorded at 25 °C on JEOL-JNM ECS 400 and 500 MHz JEOL ECX spectrometer. 1D spectrum was recorded at a peptide concentration of 10 mg/400μL in DMSO-d_6 and CD_3OD at 298 K.

3.0 Fluorescence spectroscopy: Luminescence Cary eclipsed with 10 mm quartz cell at 25±0.1°C. All samples were excited at λ_{ex} = 270 nm wavelength. The emission spectra were recorded from 280 nm to 500 nm range. A 15 mM stock solution of each metal ion salt was prepared in water. Ethanolic solution of each peptide (20 μM) was titrated with each metal ion up to 80 μM.

4.0 FT-IR Study- FT-IR spectra with transmittance mode were recorded for peptide 1 and 2 respectively to characterize the presence of functional groups with or without metal ions. FT-IR measurements were carried out on a Bruker Alfa II ATR. Spectra were recorded in the spectral range of 4000–500 cm^{-1} at 4 cm^{-1} spectral resolution, 2 sample gain, and 32 sample/background scans using OPUS 7.0 computer software and removed unwanted noise. A 200 μM solution of peptide(s) and peptide with metal ions was deposited over the ZnSe plate and dried. The Amide I band from 1700 to 1600 cm^{-1} was deconvolated using origin software. In the fitting, the numbers of initial peak values were taken from the second derivative spectrum. The contribution of each component was calculated by measuring area of the curve of each component.[1-5]

5.0 Atomic Force Microscopy (AFM): All samples of Peptide **1** and **2** prepared at neat and cleaned dust free place over freshly cleaved muscovite mica surfaces and dried under 60W bulb for ½ an hour followed by vacuum dry. A freshly prepared solution of peptide was filled in the liquid cell for obtaining solution phase AFM images.[6-7] The samples were imaged with an atomic force microscope (INNOVA, ICON analytical equipment, Bruker, Sophisticated Instrument Centre (SIC) Dr. Harisingh Gour Central University, Sagar-M.P. AFM was operate under contact and tapping mode, with the aid of a cantilever (NSC 12(c) from MikroMasch, Silicon Nitride Tip by NanoDrive™ version 8 software. The resonant frequency of the used cantilever was ~260 kHz. The images were taken in air at 25±0.1 °C with the scan speed of 2-1.5 lines/sec. The data analysis was done using of nanoscope analysis software.

6.0 Scanning Electron Microscopy (SEM): Field emission scanning electron microscopy (FE-SEM) images were acquired on FEI QUANTA 200 microscope, equipped with a tungsten filament gun, operating at working distance 4.0 mm and 7.99 kV. A 10 μL aliquot of freshly prepared solution (in ethanol and aqueous ethanol) was placed on appropriate surface(s) and allowed to dry at room temperature for overnight followed by drying under high vacuum for another 30 minutes. The sample was gold coated for 1 min and then imaged with FE-SEM.

7.0 Transmission Electron Microscopy (TEM): A 10 μL aliquot of freshly prepared solution was placed on a 400 mesh carbon coated copper grid and excess sample was removed from the grid and the sample was dried at room temperature for 6 hours. The

samples were viewed using a FEI Titan G2 60-300 Transmission Electron Microscope (HR-TEM).

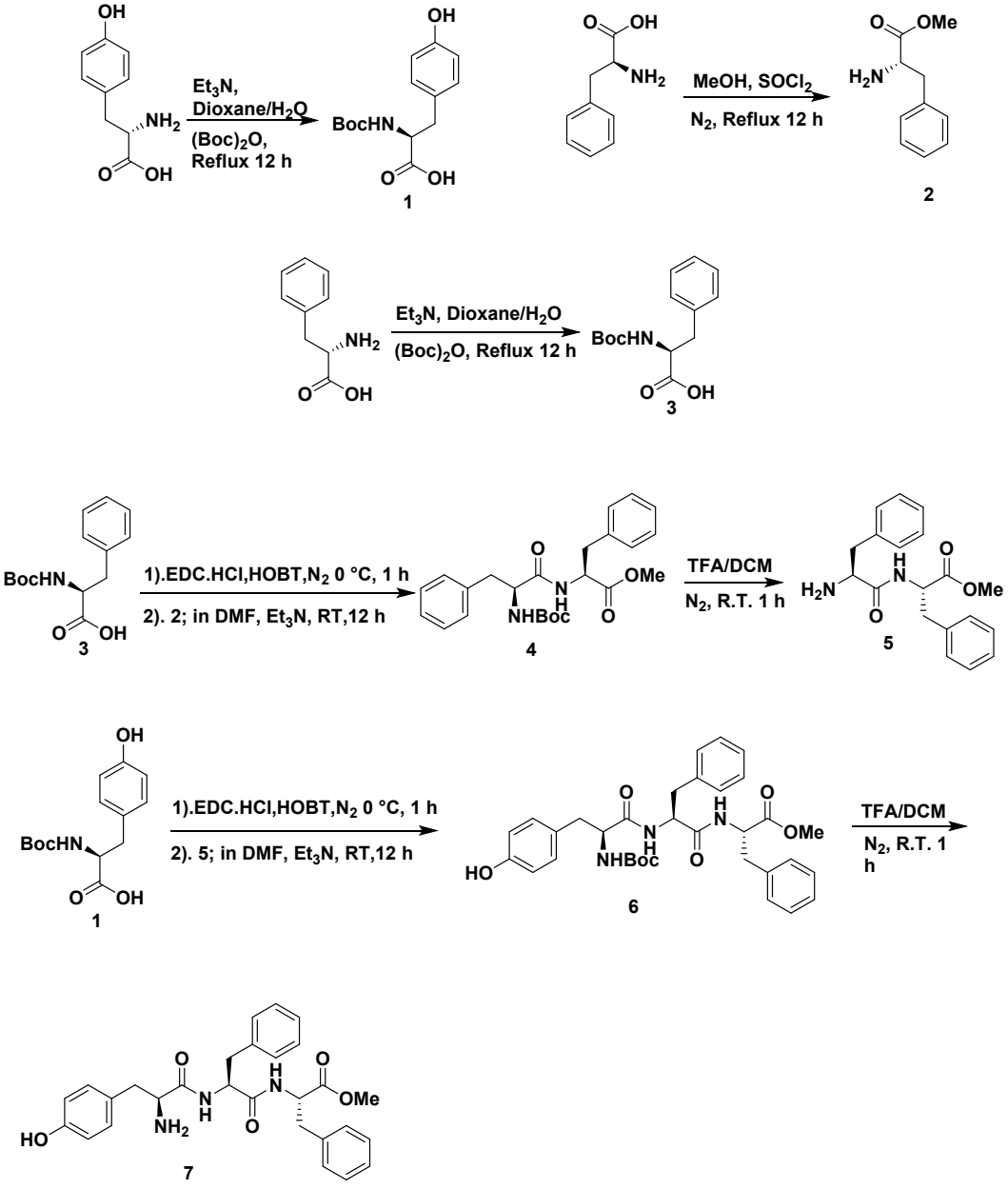
8.0 UV Studies: UV-Vis absorption spectra were recorded on Lab India UV-VIS Spectrophotometer 3000+ with 10 mm quartz cell at 25±0.1 °C. A stock solution of 20 mM of each metal ion was prepared in water. A 40 μM solution of both the peptide **1** and **2** in ethanol were titrated with each type of metal ions till 200 μM concentration by the above stock solution. To perform the titration in acidic medium the peptide solution with same concentration was acidified to pH 3 using hydrochloric acid and then titrated with metal ions.[8] After addition of metal ions solution the pH of the solution was further adjusted to pH 3 using concentrated HCl.[8]

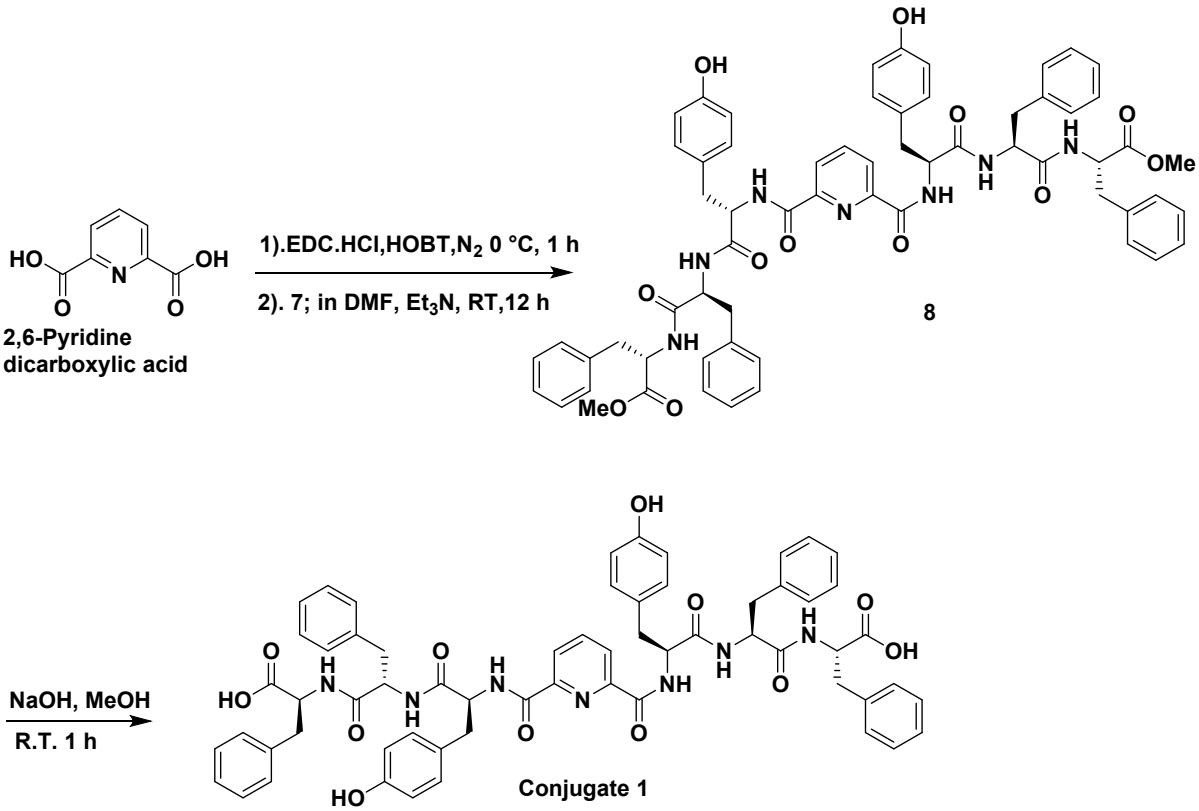
9.0 Mass spectrometry: HRMS mass spectra were recorded on Waters, Q-Tof Premier Micromass HAB 213 mass spectrometer using capillary voltage 2.6-3.2 kV.

10.0 High-performance liquid chromatography (HPLC): HPLC analysis was performed with Agilent technologies 1260 infinity, HPLC system equipped with a quaternary pump (G1311B), auto liquid sampler (G1329B), Diode array detector (G1315D) and analytical scale fraction collector (G1364C). Instrumental control, data acquisition, and processing were performed using ChemStation software (Agilent Technologies, Wokingham, UK). A ZORBAX Eclipse plus C18 (250 x 4.6 mm) column with 5 μm particle size at room temperature was used. Acetonitrile and water were used as mobile phase and the flow rate was 1 ml/min. Injection volume was 20 μL and effluent was measured at 220 nm and 254 nm.

11.0 Peptide synthesis and characterization: All peptide conjugates, N-Boc-protected and de-protected peptide conjugates were synthesized by established lab protocol.[9-10] The synthesis was done by using simple solution phase fragment condensation methodologies using t-Boc chemistry and in the presence of HOBt. Purity of final products was checked by analytical HPLC (mentioned above) with an applied gradient of 0.1% trifluoroacetic acid in water (eluent A) to 0.1% trifluoroacetic acid in acetonitrile (eluent B) (20-80% in 30 min). Concentration of a peptide for a typical analytical run was (1 mg/ml) and satisfactory analytical spectroscopic results were also obtained for all the samples.

12.1 Synthesis of N-tert-butyloxycarbonyl-L-Phenylalanine, L-Phenylalanine methyl ester hydrochloride and N-tert-butyloxycarbonyl-L-Tyrosine: These peptide conjugates were synthesized using standard lab protocols and similar to previous reported work followed by simple purification and characterization and used them for next synthesis steps wherever needed (scheme S1).[11-14]





Scheme S1: Synthesis scheme of conjugate 1.

12.2 Synthesis of (N-tert-butyloxycarbonyl-di-L-Phenylalanine methyl ester): N-(Boc)-L-phenylalanine (5 g, 18.8 mmol), and HOBt (2.55 g, 21.3 mmol) were dissolved in dry DMF (50 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 °C in an ice bath. A solution of EDC.HCl in DMF (4.05 g, 21.3 mmol) was then added drop wise to the reaction mixture. The reaction mixture was stirred at 0 °C for 1 h, after which, L-phenylalanine methyl ester hydrochloride (4.04 g, 18.8 mmol) was added to it followed by N-methyl morpholine (4.85 mL, 44.4 mmol). The reaction mixture was monitored and stirred for 12 h at room temperature under nitrogen atmosphere. Reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1 N HCl (3 x 30 mL), 10% $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude compound was purified through silica gel column chromatography by using hexane and ethyl acetate (80:20) solvent system to isolate pure N-tert-butyloxycarbonyl-di-L-Phenylalanine methyl ester (6.17 g, 76.76% yield). ^{1}H NMR: (400 MHz, DMSO-d_6, 25 °C), δ(ppm) = δ 8.30 (d, 1H), 7.31-7.10 (m, 10H), 6.82 (d, 1H), 4.40-4.30 (m, 1H), 4.20-4.10 (m, 1H), 3.54 (s, 3H), 3.0-2.6 (m, 4H), 1.24 (s,

9H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 ˚C) δ 172.40, 172.35 ,155.62, 138.57, 137.53, 129.66, 128.79, 128.50, 127.11, 126.68, 78.52, 56.03, 54.05, 52.38, 37.92, 37.21, 28.64.

12.3 Synthesis of Phe-Phe methyl ester TFA salt: N-tert-butyloxycarbonyl-di-L-Phenylalanine methyl ester (5 g, 9.5 mmol) was dissolved in 75% TFA-DCM and stirred for 1 h under nitrogen atmosphere. After completion of the reaction, the solvent was evaporated in vacuo and was subsequently washed with diethyl ether resulting in a white solid. The white solid then dissolved in methanol and passed through activated anion exchange resin and evaporated under reduced pressure to obtain pure (3.27 g, 85.4% yield). ^{1}H NMR (400 DMSO-d_6, 25 ˚C) δ(ppm) = 8.96 (d, 1H), 8.07 (bs, 2H), 7.30-7.18 (m, 10H), 4.55-4.48 (m, 1H), 4.15-3.94 (m, 1H), 3.57 (s, 3H), 3.08-2.85 (m, 4H); ^{13}C NMR (100 DMSO-d_6, 25 ˚C) δ(ppm) = 171.69, 168.79, 137.17, 135.19, 130.04, 129.60, 129.09, 128.94, 127.74, 127.31, 54.33, 53.64, 52.62, 37.39, 37.17.

12.4 Synthesis of Boc-Tyr-Phe-Phe methyl ester: N-(Boc)-L-Tyrosine (5 g, 17.7 mmol), and HOBt (2.9 g, 21.3 mmol) were dissolved in dry DCM (50 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 °C in an ice bath. A solution of EDC.HCl in DCM (4.05 g, 21.3 mmol) was then added drop wise to the reaction mixture. The reaction mixture was stirred at 0 °C for 1 h, after which, a solution of L-Phe-L-Phe methyl ester hydrochloride (6.4 g, 17.7 mmol) in DMF was added to it followed by N-methyl morpholine (4.85 mL, 44.4 mmol). The reaction mixture was monitored and stirred for 12 h at room temperature under nitrogen atmosphere. Reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1N HCl (3 x 30 mL), 10% $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo. The crude compound was purified through silica gel column chromatography to isolate pure Boc-Tyr-Phe-Phe methyl ester (7.5 g, 71.9% yield). ^{1}H-NMR (400 MHz, DMSO-d_6, 25 ˚C) δ(ppm) = 9.10 (s, 1H), 8.51 (d, J = 7.5 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.30-7.08 (m, 10H), 6.95-6.82 (m, 2H), 6.75 (d, J = 8.8 Hz, 1H), 6.57 (d, J = 8.4 Hz, 2H), 4.63-4.50 (m, 1H), 4.49-4.36 (m, 1H), 4.03-3.83 (m, 1H), 3.53 (s, 3H), 3.05-2.85 (m, 4H), 2.80-2.55 (m, 2H), 1.25 (s, 9H). ^{13}C-NMR (100 MHz, DMSO-d_6, 25 ˚C) δ(ppm) = 172.17, 171.95, 171.57, 156.18, 155.55, 137.92,

137.44, 130.52, 129.85, 129.53, 128.82, 128.51, 126.79, 115.28, 78.55, 56.66, 54.11, 53.72, 52.37, 38.35, 37.30, 37.15, 28.64. HRMS $[M+H]^+$ for $C_{33}H_{40}N_3O_7$: 590.2861 (calc.), 590.2861 (anal).

12.5 Synthesis of Tyr-Phe-Phe methyl ester: N-tert-butyloxycarbonyl-Tyr-Phe-Phe methyl ester (5 g, 8.47 mmol) was dissolved in 75% TFA-DCM and stirred for 1 h under nitrogen atmosphere. After completion of the reaction, the solvent was evaporated in vacuo and was subsequently washed with diethyl ether resulting in a white solid. The white solid then dissolved in methanol and passed through activated anion exchange resin and evaporated under reduced pressure to obtain pure Tyr-Phe-Phe methyl ester (3.5 g, 84.3% yield). ^{1}H NMR (400 MHz, CD_3OD, 25 ˚C) δ(ppm) = 7.31-7.11 (m, 10H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.72 (d, *J* = 8.4 Hz, 2H), 4.70-4.56 (m, 2H), 3.93 (m, 2H), 3.61 (s, 3H), 3.16-2.72 (m, 6H). ^{13}C-NMR (100 MHz, CD_3OD, 25 °C) δ(ppm) = 171.63, 171.46, 168.21, 156.93, 136.68, 136.62, 130.29, 128.99, 128.96, 128.21, 128.17, 126.59, 126.52, 124.48, 115.53, 54.66, 54.24, 54.00, 51.37, 37.71, 37.12, 36.52.

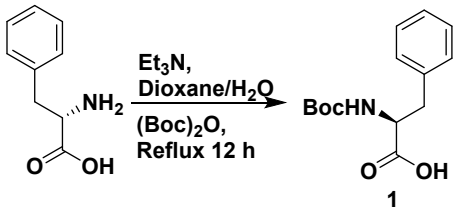
12.6 Synthesis of Pyridyl-bis-Tyr-Phe-Phe methyl ester: 2,6-Pyridinedicarboxylic acid (1.5 g, 8.98 mmol), and HOBt (2.92 g, 21.55 mmol) were dissolved in dry DMF (50 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 ˚C in an ice bath. A solution of EDC.HCl in DMF (4.12 g, 21.55 mmol) was then added drop wise to the reaction mixture. The reaction mixture was stirred at 0 ˚C for 1 h, after which, Tyr-Phe-Phe methyl ester hydrochloride (10.84 g, 17.96 mmol) was added to it followed by N-methyl morpholine (4.93 mL, 44.91 mmol). The reaction mixture was monitored and stirred for 12 h at room temperature under nitrogen atmosphere. Reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1 N HCl (3 x 30 mL), 10% $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude compound was purified through silica gel column chromatography to isolate pure Pyridyl-bis-Tyr-Phe-Phe methyl ester (6.50g, 65.26% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 25 ˚C) δ(ppm) = 9.04 (bs, 2H), 8.84 (d, *J* = 8.3 Hz, 2H), 8.45 (d, *J* = 7.4 Hz, 2H), 8.14 (d, *J* = 8.1 Hz, 2H), 8.10-8.0 (m, 3H), 7.32-6.93 (m, 24H), 6.55 (d, *J* = 8.1 Hz, 4H), 4.69-4.35 (m, 6H), 3.52 (s, 6H), 3.05-2.72 (m, 12H). ^{13}C-NMR (100 MHz, DMSO-d_6, 25 ˚C) δ(ppm) = 172.15, 171.43, 171.12, 163.49, 156.21, 149.06, 137.91, 137.48, 130.66, 129.72,

129.56, 128.80, 128.46, 128.39, 127.08, 126.69, 125.28, 115.54, 55.24, 54.19, 52.34, 38.01, 37.22, 37.03.

12.7 Synthesis of Pyridyl-bis-Tyr-Phe-Phe-OH: Pyridyl-bis-Tyr-Phe-Phe methyl ester (5 g, 4.50 mmol) was dissolved in 10 mL Methanol. A 1 M solution of Sodium hydroxide (0.72 gm, 18.02 mmol) in water was then added to the reaction mixture and stirred for 1 h. After completion of the reaction, the reaction mixture was passed through an activated ion exchange resin (pH 6) and then was dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain pure Pyridyl-bis-Tyr-Phe-Phe-OH (4.09 g, 83% yield).

^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ(ppm) = 8.14 (d, J = 7.6 Hz, 2H, C_3 & C_5-H of Pyridine), 8.05-8.01 (m, 1H, C_4-H of Pyridine), 7.26-7.07 (m, 14H, Aromatic protons of Phenylalanine and Tyrosine), 7.05-6.85 (m, 10H, Aromatic protons of Phenylalanine and Tyrosine), 6.61 (d, J = 8.4 Hz, 4H, Ortho protons of Tyrosine), 4.76 (dd, J = 8.9, 5.6 Hz, 2H, Chiral protons of outer Phenylalanine), 4.71-4.59 (m, 4H, Chiral protons of Tyrosine and inner Phenylalanine), 3.21-2.77 (m, 12H, -CH_2- Protons of all amino acids).

^{13}C-NMR (100 MHz, CD_3OD, 25 °C) δ(ppm) = 172.86, 171.90, 171.72, 164.10, 155.85, 148.45, 138.98, 136.82, 136.71, 130.06, 129.03, 128.95, 128.14, 127.96, 127.75, 126.49, 126.21, 125.03, 115.03, 55.01, 54.40, 53.76, 37.52, 37.06, 36.38. HRMS $[M+Na]^+$ for $C_{61}H_{59}N_7O_{12}$: 1104.4114 (calc.), 1104.4110 (anal).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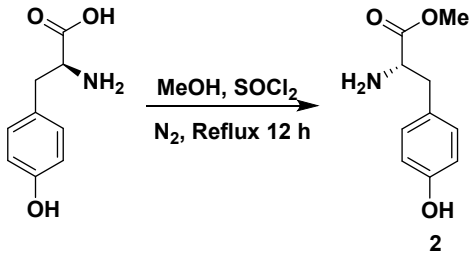
OH
BocHN

1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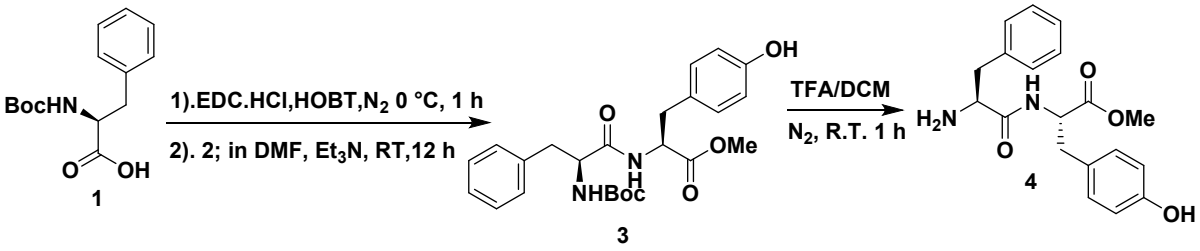
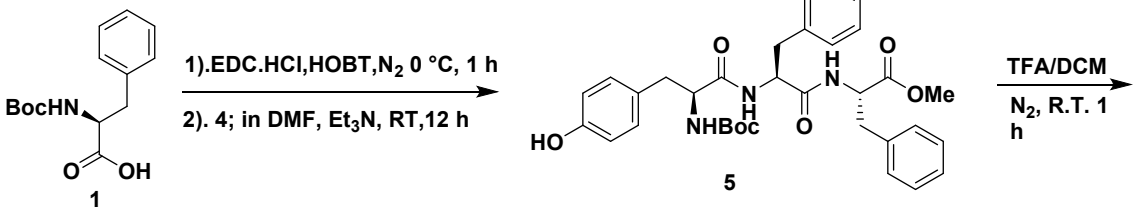
H2N
OMe
OH

2


1).EDC.HCl,HOBT,N2 0 °C, 1 h
2). 2; in DMF, Et3N, RT,12 h
TFA/DCM
N2, R.T. 1 h
BocHN
NHBoc
OMe
H2N

1 3 4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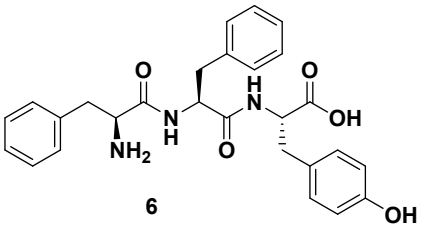
NHBoc
OMe
HO

1 5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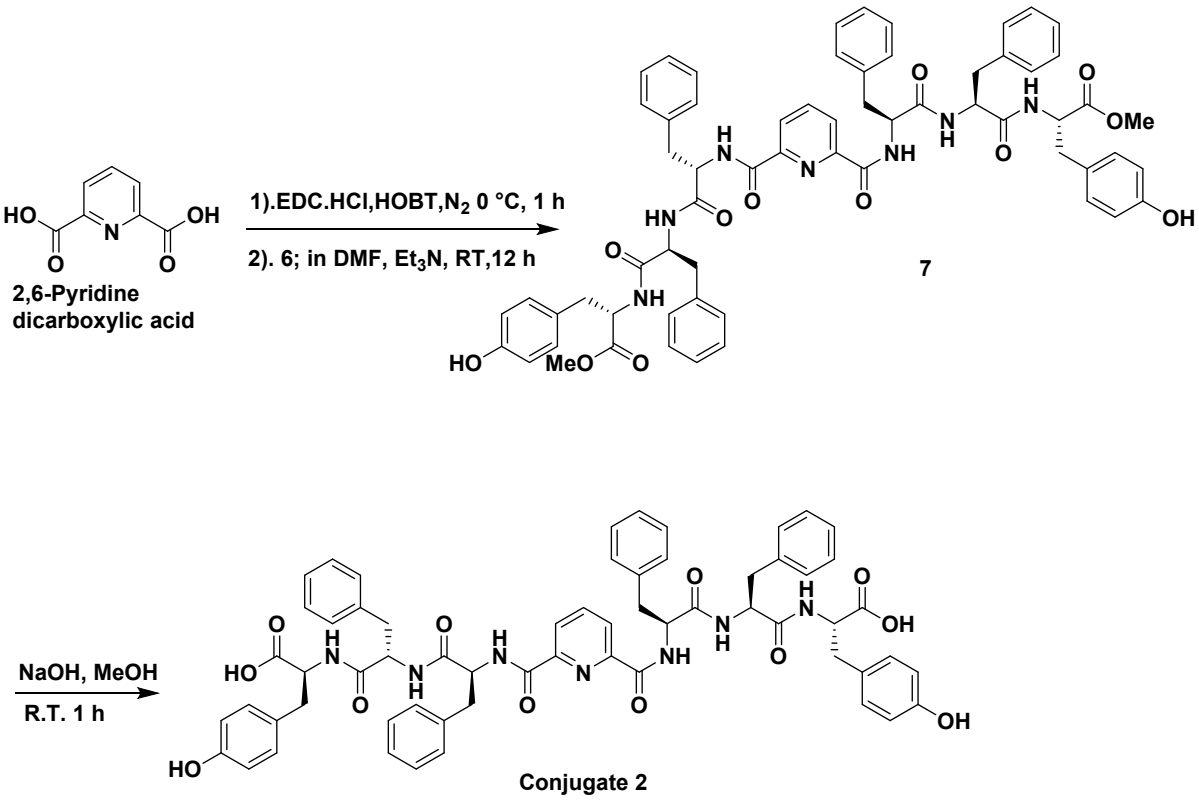
OH

6



Scheme S2: Synthesis scheme of conjugate 2.

13.1 Synthesis of (N-tert-butyloxycarbonyl-L-Phenylalanine-L-Tyrosine methyl ester): N-(Boc)-L-phenylalanine (5 g, 18.8 mmol), and HOBt (2.55 g, 21.3 mmol) were dissolved in dry DMF (50 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 °C in an ice bath. A solution of EDC.HCl in DMF (4.05 g, 21.3 mmol) was then added drop wise to the reaction mixture. The reaction mixture was stirred at 0 °C for 1 h, after which, L-Tyrosine methyl ester hydrochloride (4.35 g, 18.8 mmol) was added to it followed by N-methyl morpholine (4.85 mL, 44.4 mmol). The reaction mixture was monitored and stirred for 12 h at room temperature under nitrogen atmosphere. Reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1 N HCl (3 x 30 mL), 10% $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude compound was purified through silica gel column chromatograph to isolate pure N-tert-butyloxycarbonyl-L-Phenylalanine-L-Tyrosine methyl ester (6.3 g, 75.81% yield). ^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ 7.28-7.10 (m, 5H), 6.96 (d, J = 7.5 Hz, 2H), 6.65 (d, J = 7.5 Hz, 2H), 4.65-4.49 (m, 1H), 4.33-4.14 (m, 1H), 3.62 (s, 3H), 3.08-2.79 (m, 4H), 1.29 (s, 9H). ^{13}C NMR (125 MHz, CD_3OD, 25 °C)

δ = 172.77, 171.87, 156.14, 137.23, 129.99, 129.01, 128.03, 127.09, 126.31, 114.97, 79.33, 55.89, 54.00, 51.32, 37.84, 36.39, 27.32.

13.2 Synthesis of Phe-Tyr methyl ester TFA salt: N-tert-butyloxycarbonyl-L-Phenylalanine-L-Tyrosine methyl ester (5 g, 11.29 mmol) was dissolved in 75% TFA-DCM and stirred for 1 h under nitrogen atmosphere. After completion of the reaction, the solvent was evaporated in vacuo and was subsequently washed with diethyl ether resulting in a white solid. The white solid then dissolved in methanol and passed through activated anion exchange resin and evaporated under reduced pressure to obtain pure Phe-Tyr methyl ester (4.21 g, 84.2% yield). ^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ 7.37-7.21 (m, 5H), 7.07-6.93 (m, 2H), 6.74-6.64 (m, 2H), 4.67-4.56 (m, 1H), 4.17-3.99 (m, 1H), 3.65 (s, 3H), 3.30-2.82 (m, 4H). ^{13}C NMR (125 MHz, CD_3OD, 25 °C) δ = 171.60, 168.32, 156.19, 134.09, 129.85, 129.22, 128.74, 127.50, 127.04, 115.02, 54.48, 54.07, 51.43, 37.17, 36.25.

13.3 Synthesis of Boc-Phe-Phe-Tyr methyl ester: N-(Boc)-L-Phenylalanine (5 g, 18.8 mmol), and HOBt (3.06 g, 22.56 mmol) were dissolved in dry DCM (50 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 °C in an ice bath. A solution of EDC.HCl in DCM (4.31 g, 22.56 mmol) was then added drop wise to the reaction mixture. The reaction mixture was stirred at 0 °C for 1 h, after which, a solution of L-Phe-L-Tyr methyl ester TFA salt (8.26 g, 18.8 mmol) in DMF was added to it followed by N-methyl morpholine (5.16 mL, 47 mmol). The reaction mixture was monitored and stirred for 12 h at room temperature under nitrogen atmosphere. Reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1N HCl (3 x 30 mL), 10% $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated in vacuo. The crude compound was purified through silica gel column chromatography to isolate pure Boc-Phe-Phe-Tyr methyl ester (7.98 g, 72.54% yield). ^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ 7.43-7.05 (m, 10H), 7.05-6.85 (m, 2H), 6.73-6.57 (m, 2H), 4.70-4.47 (m, 2H), 4.28-4.11 (m, 1H), 3.65 (s, 3H), 3.10-2.73 (m, 6H), 1.25 (s, 9H). ^{13}C NMR (125 MHz, CD_3OD, 25 °C) δ = 172.60, 171.81, 171.54, 156.21, 156.11, 137.28, 136.72, 129.95, 129.16, 128.99, 128.08, 128.04, 127.15, 126.41, 126.29, 114.98, 79.35, 55.99, 54.28, 54.14, 51.30, 37.80, 37.76, 36.39, 27.32.

13.4 Synthesis of Phe-Phe-Tyr methyl ester: N-tert-butyloxycarbonyl-Phe-Phe-Tyr methyl ester (5 g, 8.47 mmol) was dissolved in 75% TFA-DCM and stirred for 1 h under nitrogen atmosphere. After completion of the reaction, the solvent was evaporated in vacuo and was subsequently washed with diethyl ether resulting in a white solid. The white solid then dissolved in methanol and passed through activated anion exchange resin and evaporated under reduced pressure to obtain pure Phe-Phe-Tyr methyl ester (3.60 g, 86.95% yield). ^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ 7.45-7.12 (m, 10H), 7.06-6.92 (m, 2H), 6.79-6.53 (m, 2H), 4.75-4.63 (m, 1H), 4.61-4.49 (m, 1H), 4.11-3.89 (m, 1H), 3.64 (s, 3H), 3.17-2.83 (m, 6H). NMR (125 MHz, CD_3OD, 25 °C) δ = 171.82, 171.45, 168.05, 156.12, 136.69, 134.11, 130.02, 129.21, 128.99, 128.79, 128.17, 127.51, 127.25, 126.53, 114.98, 54.66, 54.35, 54.07, 51.35, 37.75, 37.24, 36.42.

13.5 Synthesis of Pyridyl-bis-Phe-Phe-Tyr methyl ester: 2,6-Pyridinedicarboxylic acid (1.5 g, 8.98 mmol), and HOBt (2.92 g, 21.55 mmol) were dissolved in dry DMF (50 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 ˚C in an ice bath. A solution of EDC.HCl in DMF (4.12 g, 21.55 mmol) was then added drop wise to the reaction mixture. The reaction mixture was stirred at 0 ˚C for 1 h, after which, Phe-Phe-Tyr methyl ester hydrochloride (10.84 g, 17.96 mmol) was added to it followed by N-methyl morpholine (4.93 mL, 44.91 mmol). The reaction mixture was monitored and stirred for 12 h at room temperature under nitrogen atmosphere. Reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1 N HCl (3 x 30 mL), 10% $NaHCO_3$ (3 x 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude compound was purified through silica gel column chromatography to isolate pure Pyridyl-bis-Phe-Phe-Tyr methyl ester (6.7 g, 67.26% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 25 ˚C) δ 9.21 (s, 2H), 8.13-8.00 (m, 3H), 7.30-6.85 (m, 24H), 6.61 (d, J = 8.4 Hz, 4H), 4.69 (dd, J = 9.7, 4.7 Hz, 2H), 4.58 (dd, J = 8.9, 4.9 Hz, 2H), 4.42-4.32 (m, 2H), 3.52 (s, 6H), 3.16-2.73 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6, 25 ˚C) δ = 172.31, 171.36, 170.90, 163.47, 156.56, 148.94, 138.29, 137.92, 130.52, 129.76, 129.72, 128.67, 128.45, 127.42, 126.73, 115.60, 55.43, 54.52, 54.06, 52.31, 38.01, 37.57, 36.50.

13.6 Synthesis of Pyridyl-bis-Phe-Phe-Tyr-OH: Pyridyl-bis-Phe-Phe-Tyr methyl ester (2 g, 1.80 mmol) was dissolved in 5 mL Tetrahydrofuron/Water (3:1). Aqueous solution of Lithium hydroxide (0.46 gm, 10.80 mmol) was then added to the reaction mixture

and stirred for 3h. After completion of the reaction, reaction mixture was concentrated in vacuo, redissolved in ethyl acetate. The organic layer was then washed with 1 N HCl (3 x 30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude compound was purified through silica gel column chromatography to isolate pure Pyridyl-bis-Phe-Phe-Tyr-OH (1.70 g, 87.62% yield).

^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ 8.13 (d, J = 7.6 Hz,, 2H, C_3&C_5-H of Pyridine), 8.05 - 8.01 (m, 1H, C_4-H of Pyridine), 7.29 - 6.80 (m, 24H, Aromatic protons of Phenylalanine and Tyrosine rings), 6.67 (d, J = 8.4 Hz, 4H, Ortho protons of Tyrosine rings), 4.84 - 4.80 (m, 2H, Chiral protons of Tyrosine), 4.68 (dd, J = 9.0, 5.2 Hz, 2H, Chiral protons of Phenylalanine), 4.57 (dd, J = 7.8, 5.5 Hz, 2H, Chiral protons of Phenylalanine), 3.24 - 2.96 (m, 8H, -CH_2- Protons of Phenylalanine), 2.95 - 2.77 (m, 4H, -CH_2- Protons of Tyrosine).

^{13}C NMR (100 MHz, CD_3OD, 25 ˚C) δ = 173.07, 171.75, 171.71, 164.11, 156.01, 148.38, 138.99, 137.09, 136.74, 130.08, 129.03, 128.97, 128.18, 127.95, 127.50, 126.39, 126.21, 125.04, 114.94, 54.73, 54.39, 54.10, 37.57, 37.01, 36.30. HRMS $[M+Na]^+$ for $C_{61}H_{59}N_7O_{12}$: 1104.4114 (calc.), 1104.4057 (anal).

Figures

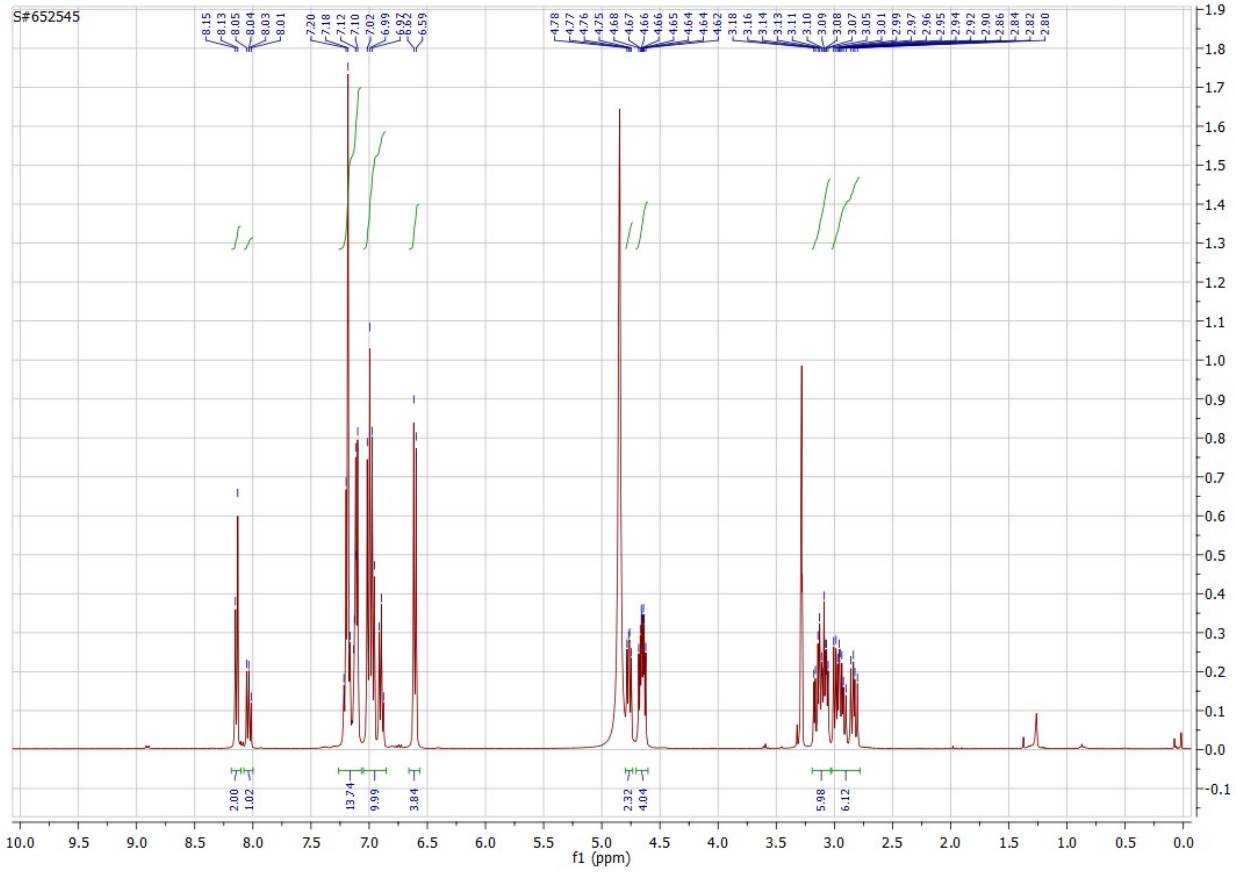


Figure S1. ^{1}H-NMR spectrum of Conjugate 1, Pyridyl-bis-Tyr- Phe-Phe-OH; Py(YFF)$_2$.

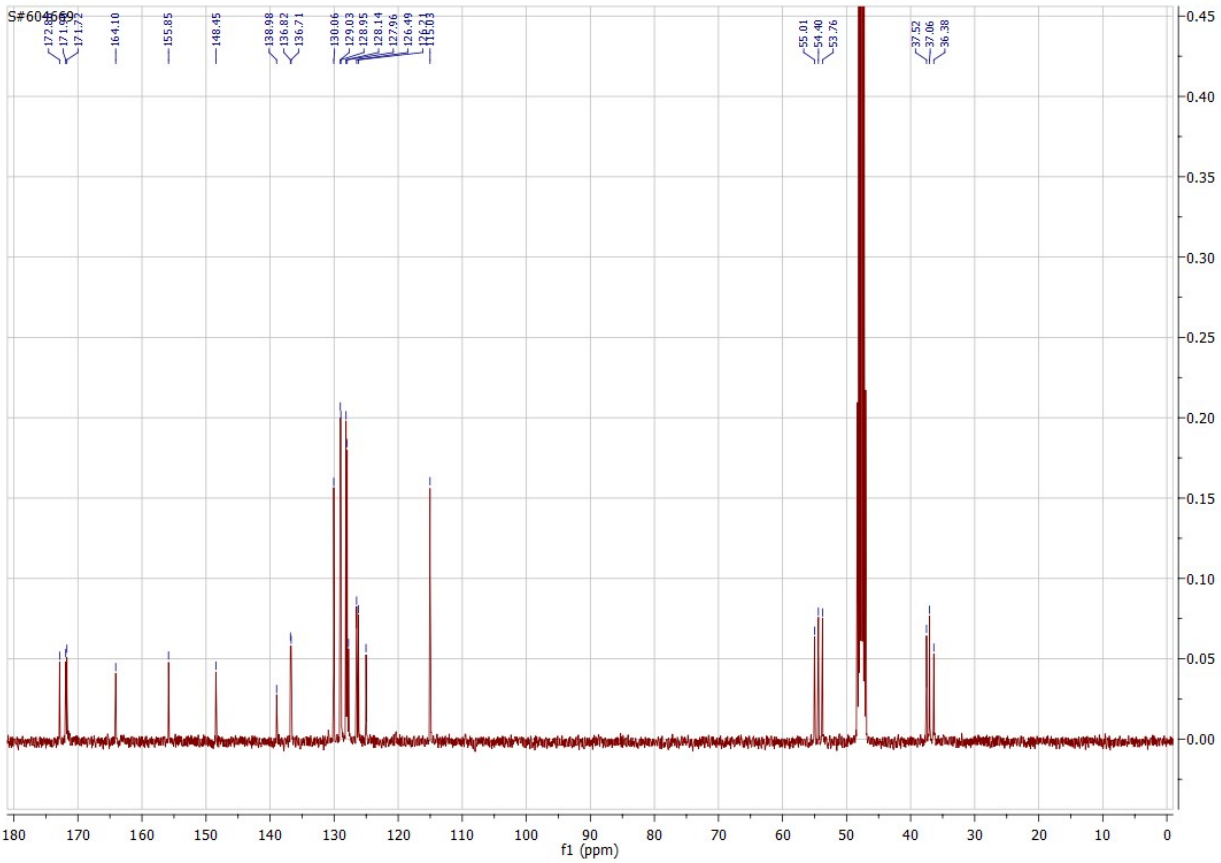


Figure S2. ^{13}C-NMR spectrum of Conjugate 1.

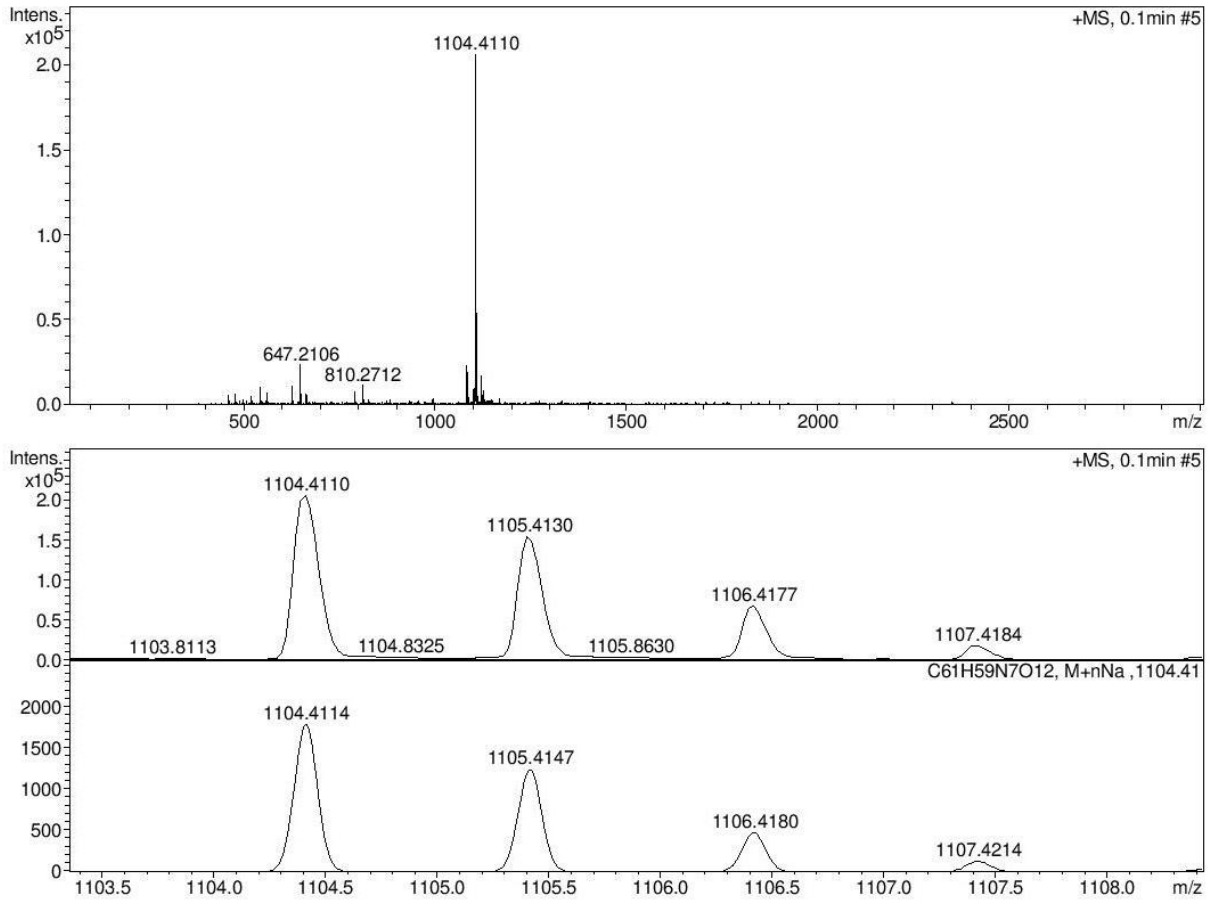


Figure S3. ESI-HRMS spectrum of conjugate 1.

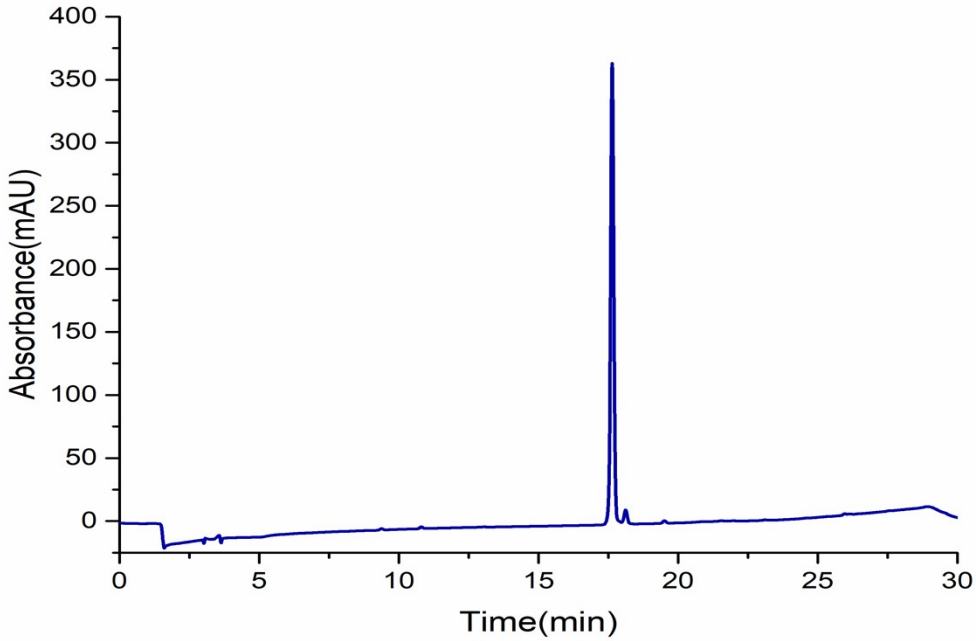


Figure S4. Analytical HPLC spectrum of Conjugate 1.

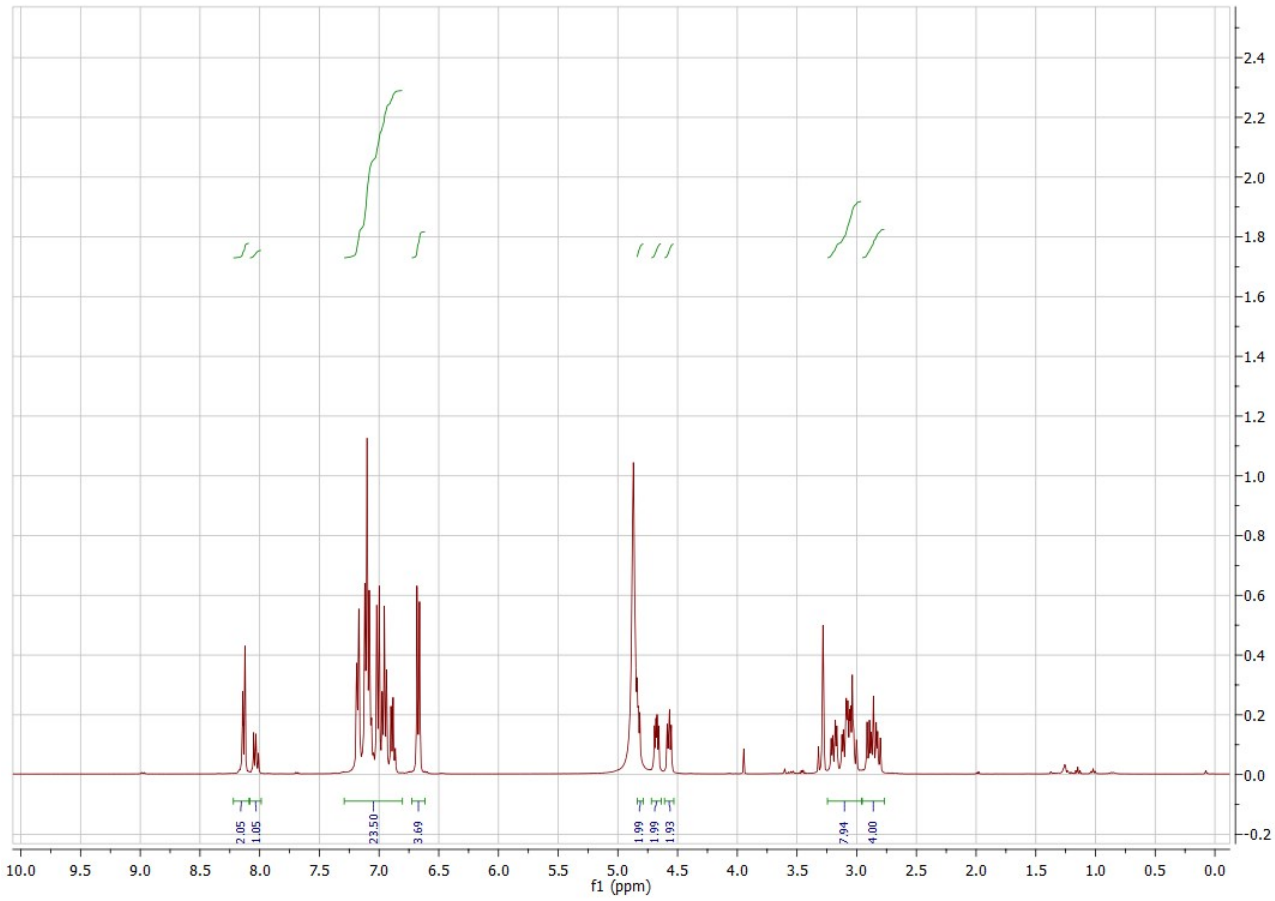


Figure S5. ^{1}H-NMR spectrum of Conjugate 2 Pyridyl-bis- Phe-Phe-Tyr-OH, Py(FFY)$_2$.

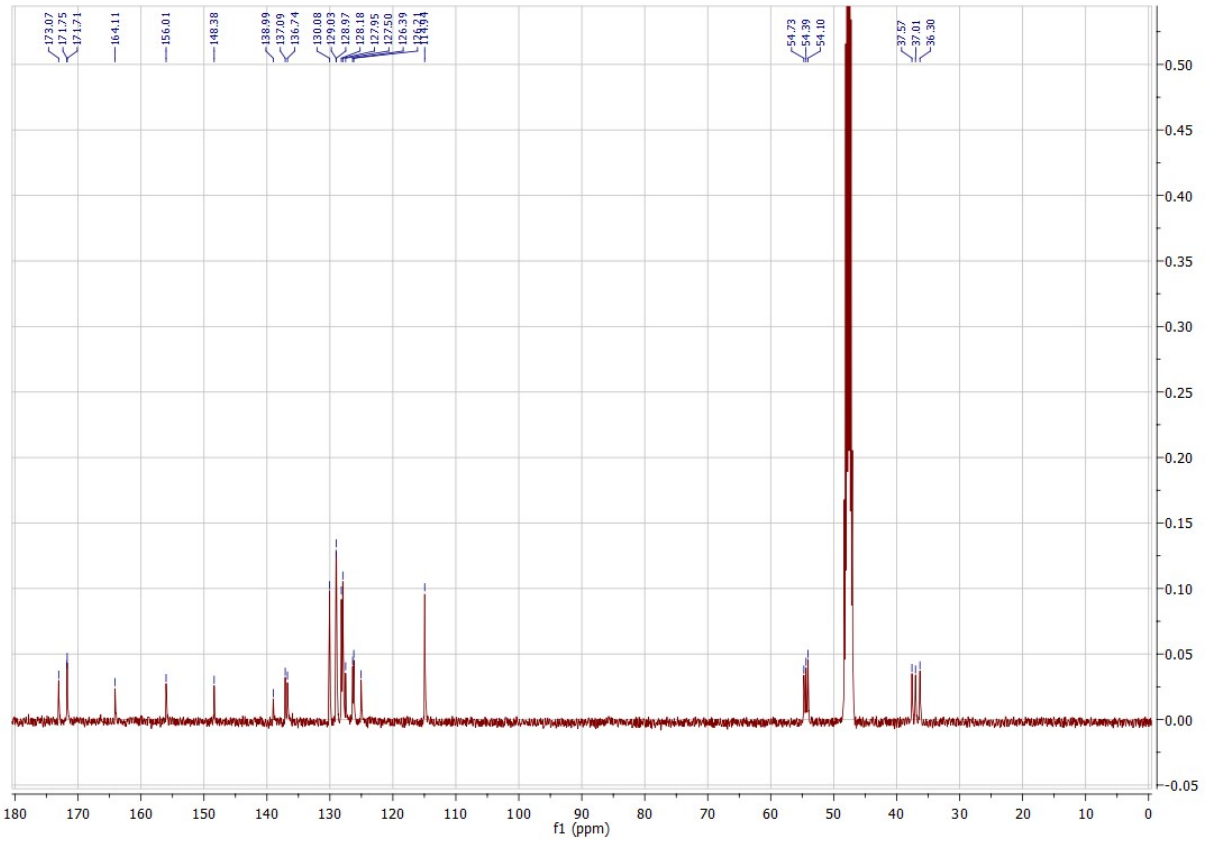


Figure S6. ^{13}C-NMR spectrum of Conjugate 2, Py(FFY)$_2$.

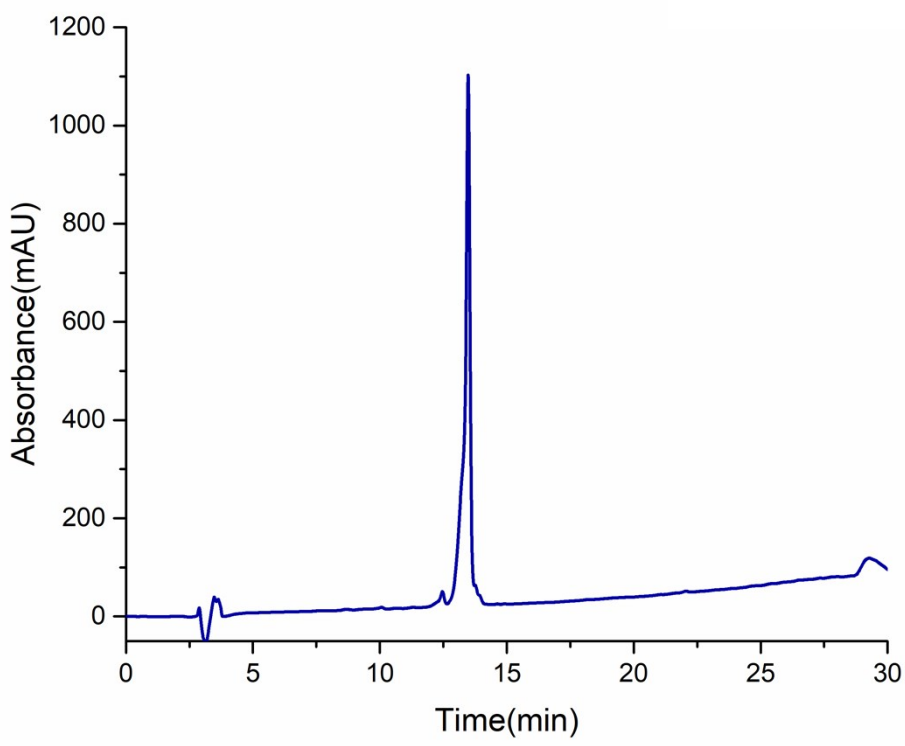


Figure S7. Analytical HPLC spectrum of Conjugate 2.

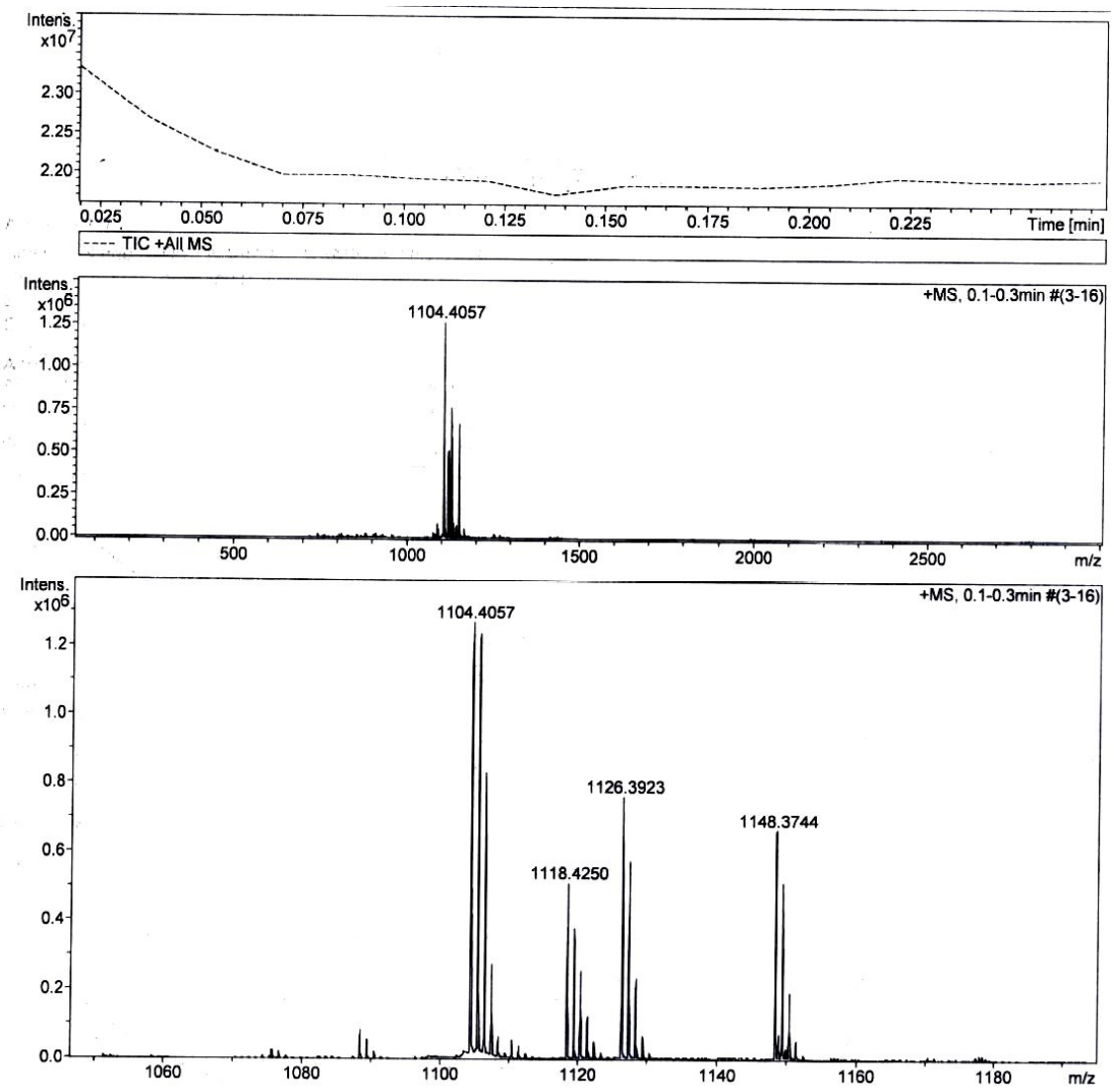


Figure S8. ESI-HRMS spectrum of conjugate 2. HRMS $[M+Na]^+$ for $C_{61}H_{59}N_7O_{12}Na$: 1104.4114 (calc.), 1104.4057 (anal). And the additional peaks are expected for; 1118.4250 for $[C_{61}H_{60}N_7O_{12}.HCl]^+$, 1126.3923 for $[C_{61}H_{58}N_7O_{12}.2Na]^+$, and 1148.3744 for $[C_{61}H_{57}N_7O_{12}.3Na]^+$.

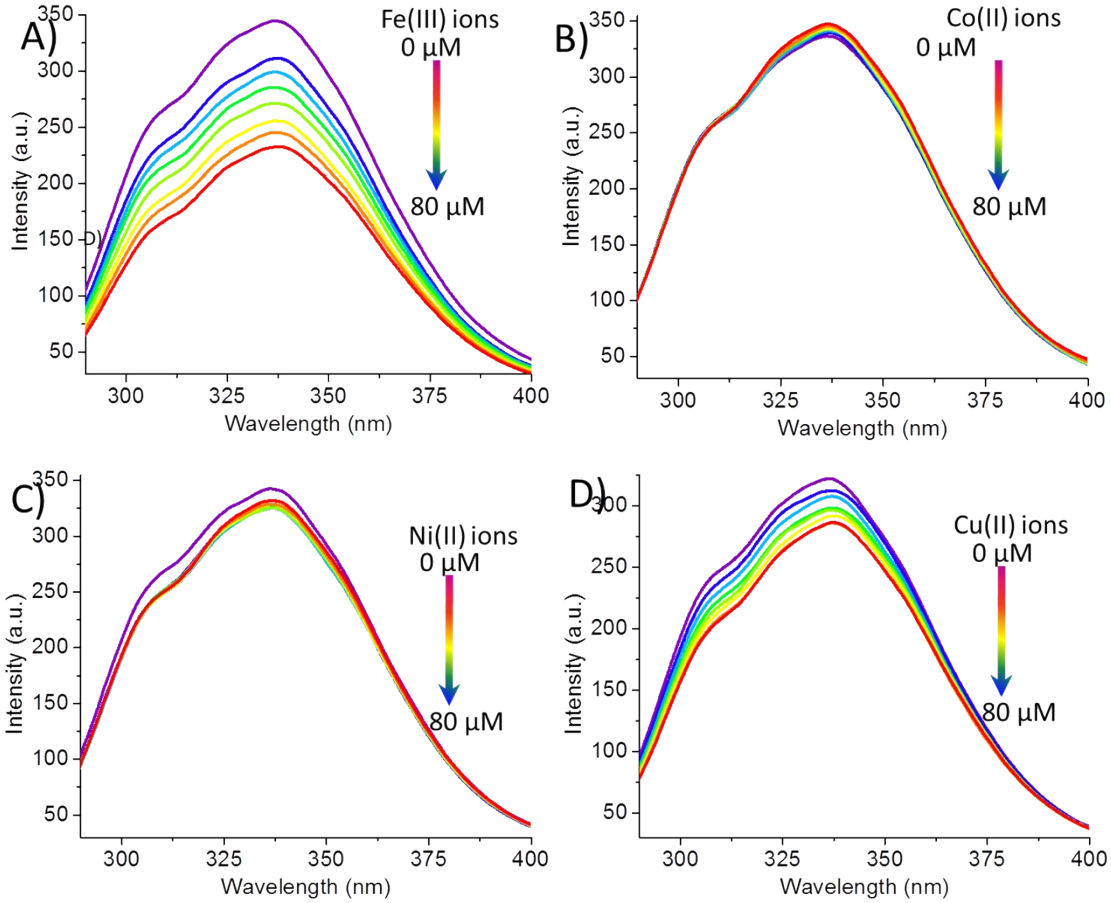


Figure S9. Emissions spectra of peptide conjugate **1** with gradual addition of metal ions, A) Fe(III), B) Co(II), C) Ni(II) and D) Cu(II) ion. The concentration of peptide was 20 µM.

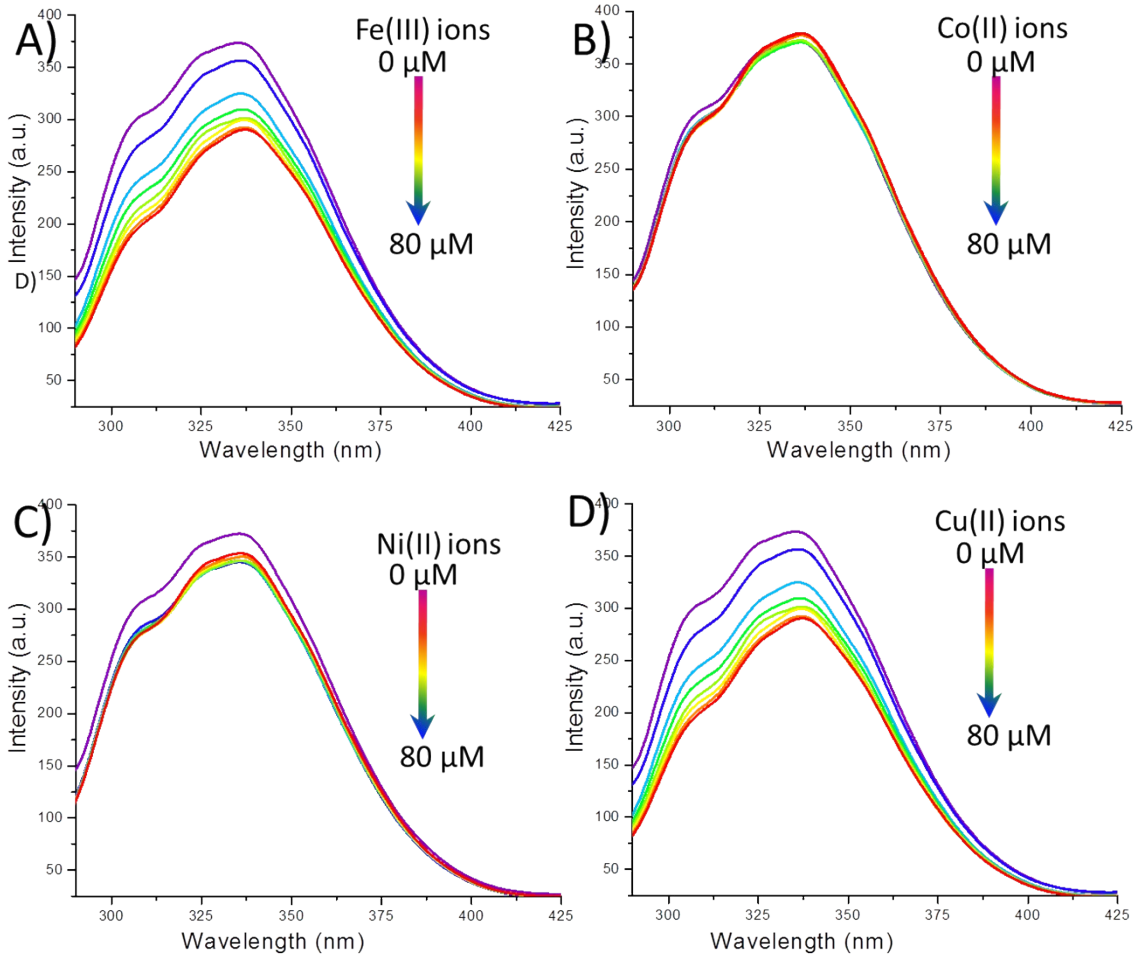


Figure S10. Emissions spectra of peptide conjugate **2** with gradual addition of metal ions, A) Fe(III), B) Co(II), C) Ni(II) and D) Cu(II) ion. The concentration of peptide was 20 µM.

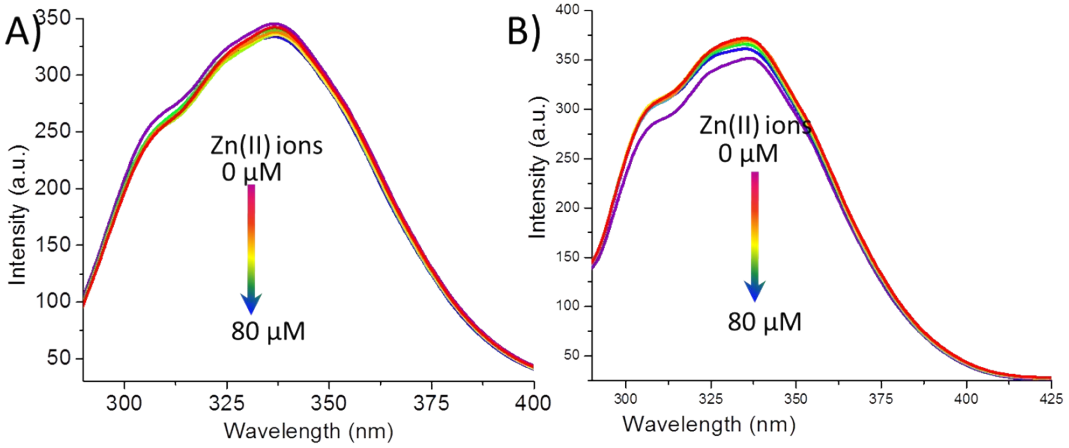


Figure S11. Emissions spectra of A) peptide conjugate **1** with gradual addition of Zn(II)metal ions, B) peptide conjugate **2** with gradual addition of Zn(II)metal. The concentration of peptides was 20 µM.

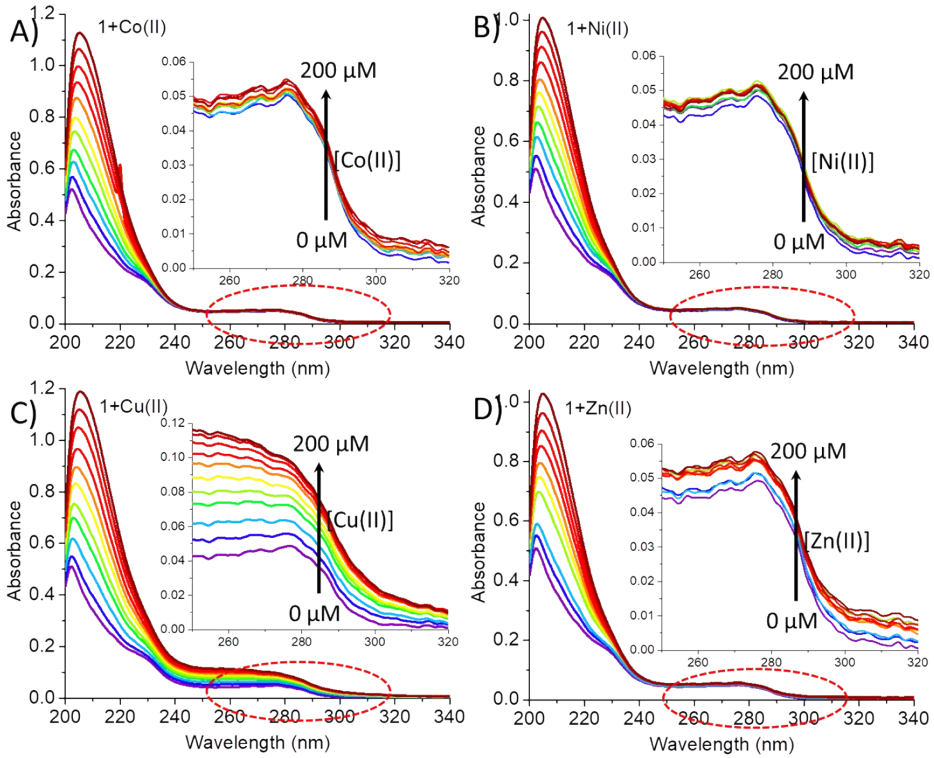


Figure S12. UV/Vis titration spectra of **1** (40 μM) with gradual addition of different metal ions: A) Co(II), B) Ni(II), C) Cu(II), and D) Zn(II).

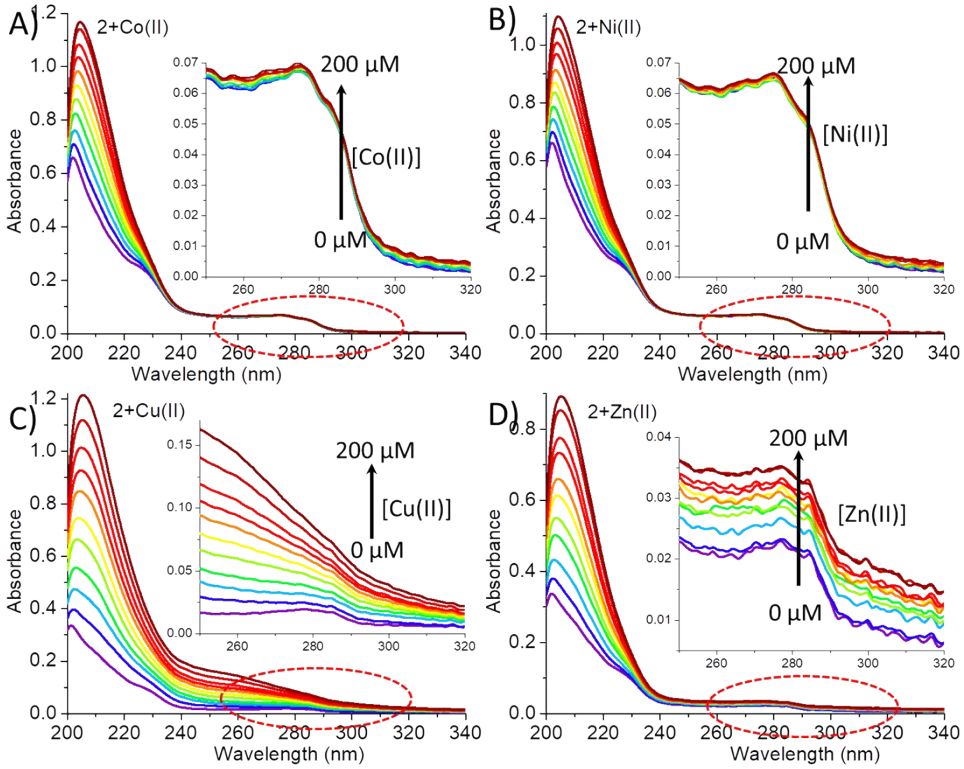


Figure S13. UV/Vis titration spectra of **2** (40 μM) with gradual addition of different metal ions: A) Co(II), B) Ni(II), C) Cu(II), and D) Zn(II).

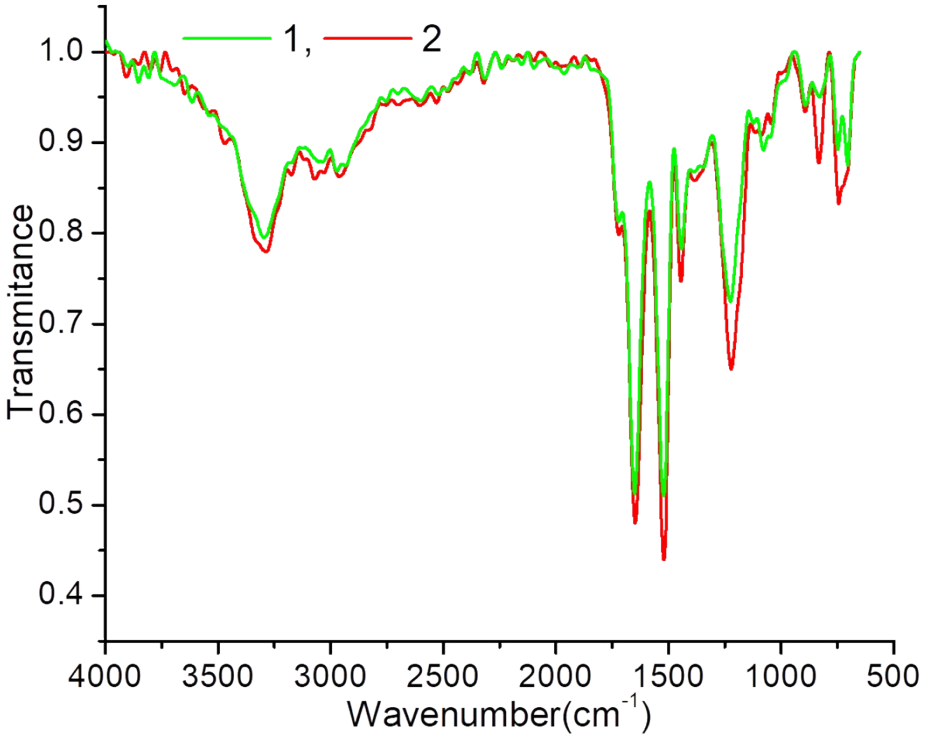


Figure S14. FT-IR spectra of compound **1** and **2**.

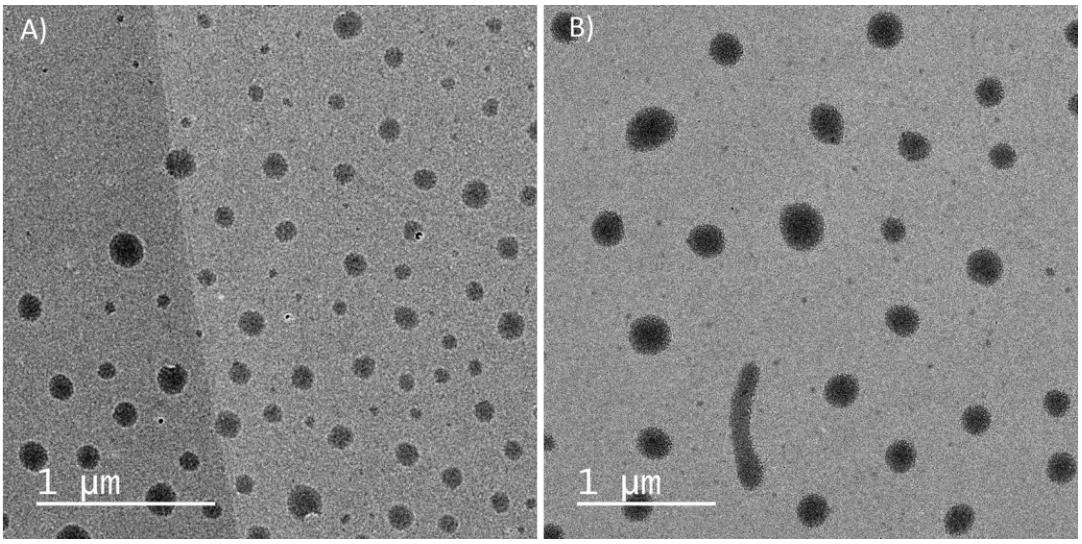


Figure S15. TEM images; A) Peptide **1** and b) Peptide **2**.

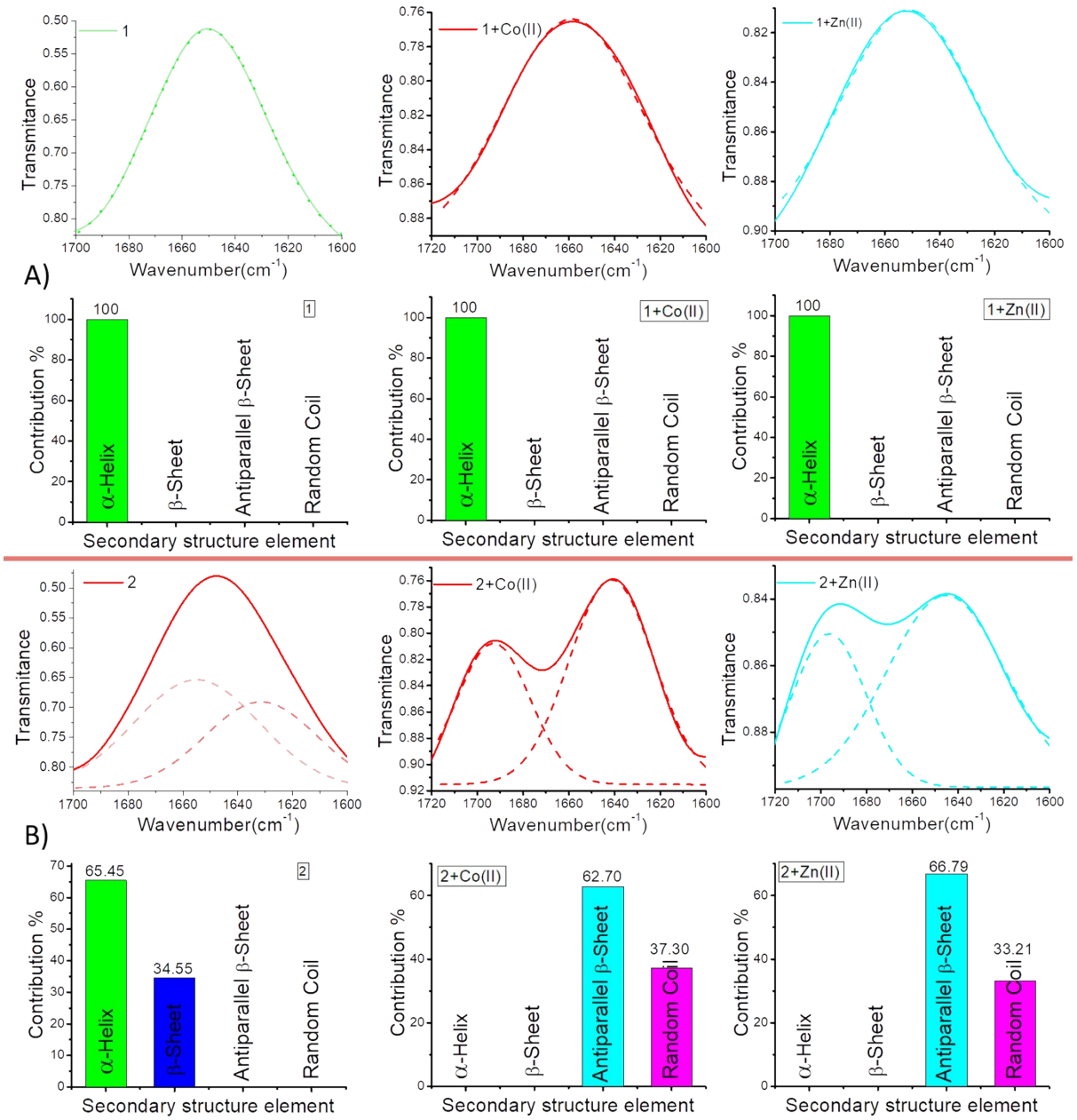


Figure S16. The FT-IR deconvolution analysis of peptide **1(A, Top panel)** and **2(B, Bottom panel)** with Co(II) and Zn(II) ions respectively in the amide I region, ranging from 1,600 to 1,700 cm^{-1}. The spectra were fitted by multiple Gaussian peaks; deconvoluted spectra indicate each type of secondary structures component such as α-helix, β-sheets, anti-parallel β-sheets and random coil.

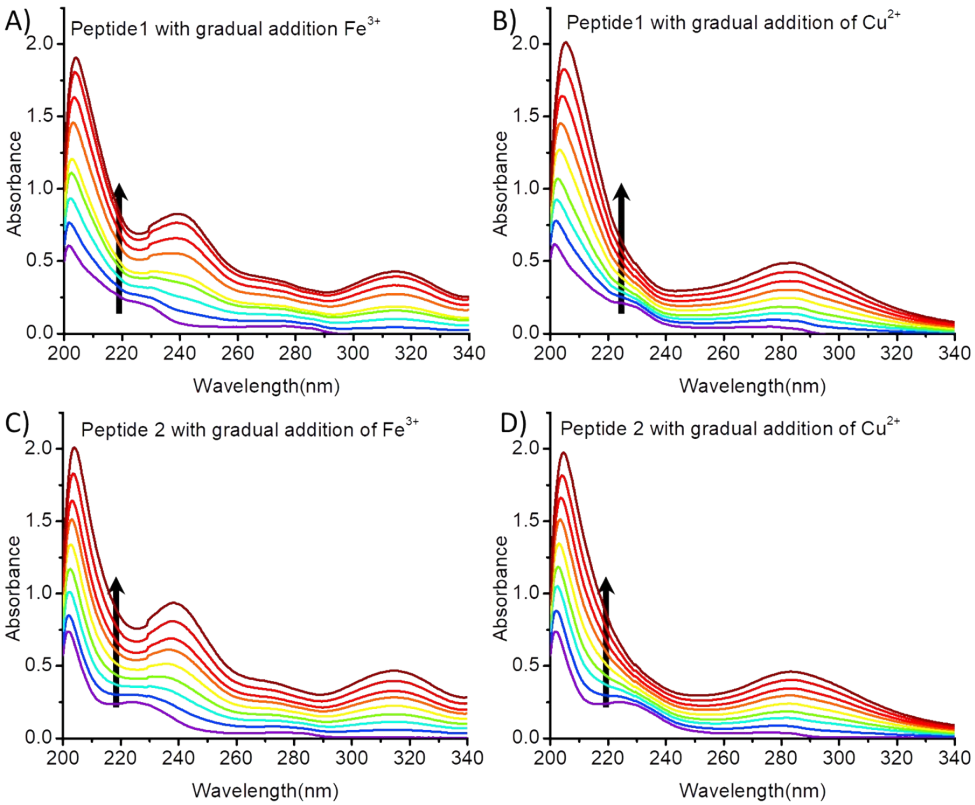


Figure S17. UV-Vis titration in acidic medium A) & B) Peptide 1 with Fe(III) & Cu(II) respectively and C) & D) Peptide 2 with Fe(III) & Cu(II) respectively.

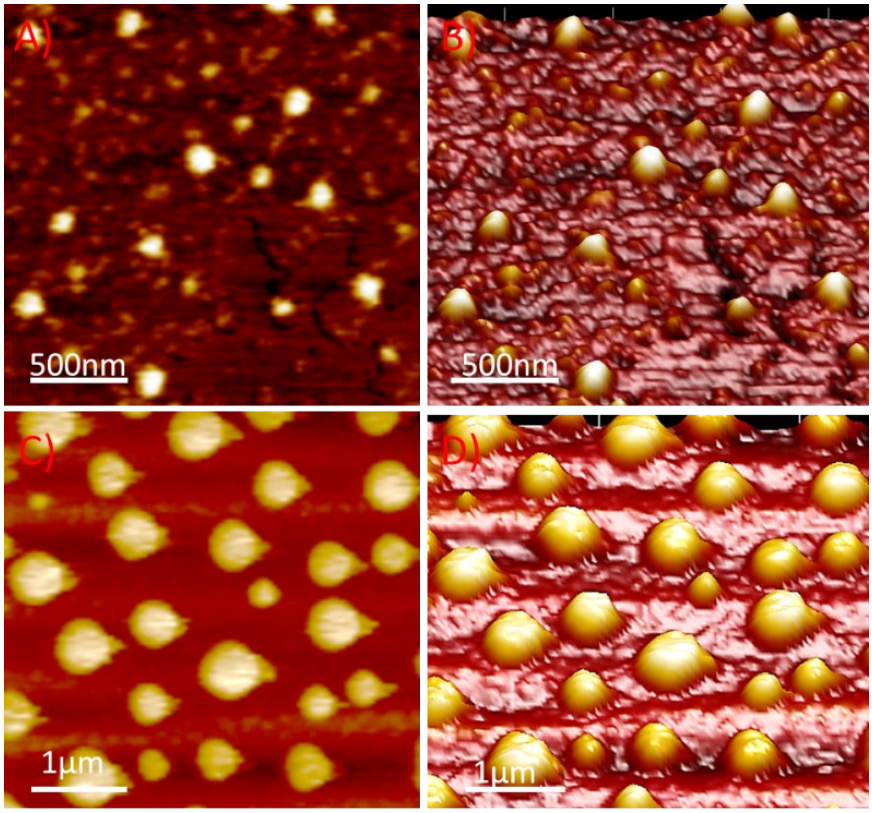


Figure S18. Liquid cell AFM images A) 2D & B) 3D image of peptide **1** and C) 2D & D) 3D image of peptide **2**.

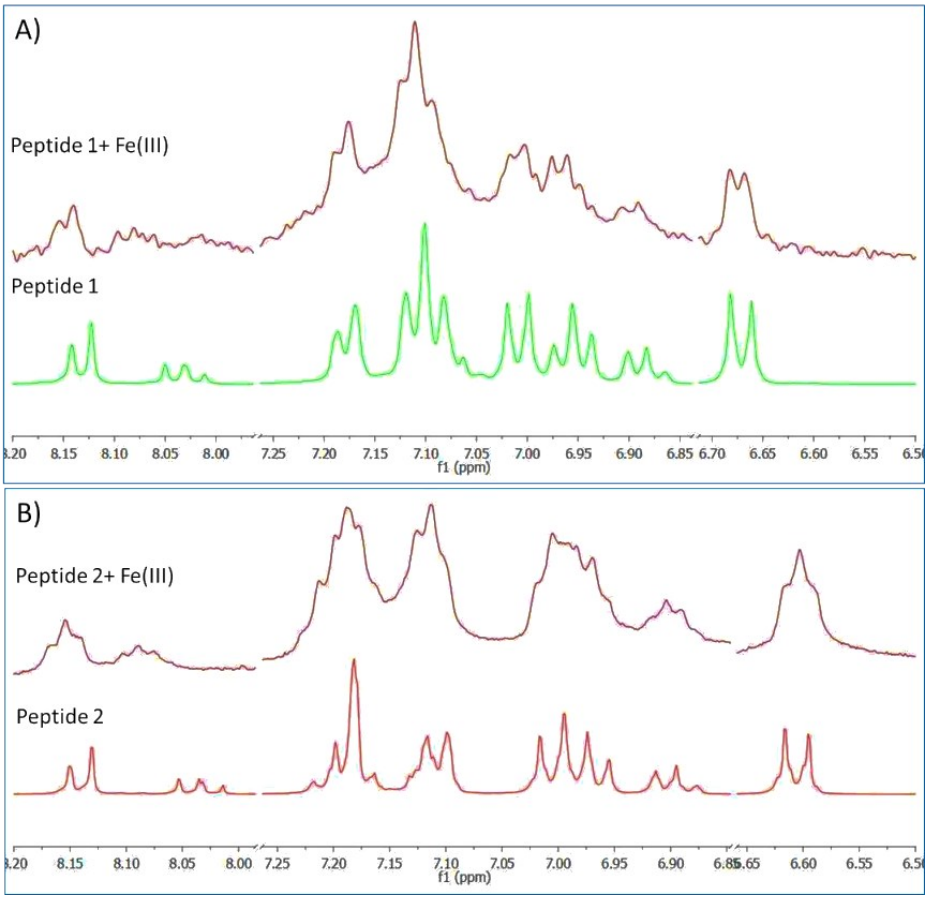


Figure S19: ^{1}H-NMR spectra of compound 1 (A) and 2 (B) in the presence of Fe(III) ions in CD_3OD

	Structure	Wavenumber	Peak	Percent
	β-Sheet	1610-1640 cm^{-1}		
$Py(YFF)_2$	Random coil	1640-1650 cm^{-1}	-	-
(1)	α-Helix	1650-1660 cm^{-1}	1650 cm^{-1}	100%
	Antiparallel β-Sheet	1660-1695 cm^{-1}	-	-

Table S1. The percentage contributions of β-sheet, α-helix, antiparallel β-sheet and random coil pattern of secondary structure of **1**.

	Structure	Wavenumber	Peak	Percent
	β-Sheet	1610-1640 cm^{-1}	1631 cm^{-1}	34.55%
$Py(FFY)_2$	Random coil	1640-1650 cm^{-1}	-	-
(2)	α-Helix	1650-1660 cm^{-1}	1654 cm^{-1}	65.45%
	Antiparallel β-Sheet	1660-1695 cm^{-1}	-	-

Table S2. The percentage contributions of β-sheet, α-helix, antiparallel β-sheet and random coil pattern of secondary structure of **2**.

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