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Supporting Information

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Supplementary Information

Ditryptophan conjugation triggers morphing of biotin fibers to soft spherical structurtes

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MATERIAL AND METHODS

General- Dichloromethane, N, N-dimethylformamide, methanol, triethylamine and 1, 2-dimethoxy ethane were distilled following standard procedures prior to use. Trifluoroacetic acid, Hydrochloric acid, N-hydroxybenzotriazole, t-butyloxycarbonyl carbonate, sodium hydroxide, diethyl ether, L-amino acids, D-Biotin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N,N*-dimethyl-4-aminopyridine (DMAP) were purchased from Spectrochem, Mumbai, India, and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on JEOL-JNM LAMBDA 400 model operating at 400 and 100 MHz, respectively. HRMS mas spectra were recorded at IIT Kanpur, India, on Waters, Q-Tof Premier micromass HAB 213 mass

spectrometer using capillary voltage 2.6-3.2 kV. Elemental analyses (C, H, N) were performed on Perkin-Elmer 240-C automatic elemental analyzer.

Atomic Force Microscopy (AFM) – Fresh and aged peptide, biotin, biotin methyl ester and N-biotinyted peptide samples were imaged with an atomic force microscope (Molecular Imaging, USA) operating under the Acoustic AC mode (AAC), with the aid of a cantilever (NSC 12(c) from MikroMasch). The force constant was 0.6 N/m, while the resonant frequency was 150 kHz. The images were taken in air at room temperature, with the scan speed of 1.5-2.2 lines/sec. The data acquisition was done using PicoScan 5® software, while the data analysis was done using of visual SPM. Fresh solutions of biotin and biotin conjugates (1 mM) in 50% methanol / water were prepared and incubated at 37 °C for 0-12 days in methanol/water, prior to microscopic investigation. 10 µL aliquot of sample was transferred onto freshly cleaved mica surface and uniformly spread using a spin-coater operating at 200-500 rpm (PRS-4000). The sample-coated mica was dried for 30 minutes at room temperature, followed by AFM imaging.

Scanning Electron Microscopy (SEM) - A 20 µL aliquot of the fresh and aged sample solution (1 mM) was dried at room temperature on gold-coated copper stubs. Scanning electron microscopy images acquired on FEI QUANTA 200 microscope, equipped with a tungsten filament gun, operating at WD 10.6 mm and 20 kV.

Transmission Electron Microscopy (TEM) – A 10 µL aliquot of the peptide, biotin and biotin conjugate solution after fresh and 12 days incubation was placed on a 400 mesh copper grid. After 1 minute, excess fluid was removed and the grid was negatively stained with 2% uranyl acetate solution. Excess stain was removed from the grid and the samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV.

Fluorescence microscopy – Dye stained structures were examined under a fluorescent microscope (Leica DM2500M), provisioned with a fluorescence illuminator and a fluorescein filter (494/521 nm). This filter optimized visualization of fluorescein -treated (positive resolution) compared with untreated (negative resolution) spherical structures. 10 µM fluorescein dye solution was added directly to biotinylated conjugate solution (1 mM) or co-

incubated from day 1-12 in 50% methanol/water. 20 µL of this solution was spread on a glass slide, dried at room temperature, and imaged under a fluorescence microscope. In another experiment, pH of the 12 days co-aged solution was adjusted 4.05, 2.0 or 1.0, with 1 mM HCl. After 24 hours, 20 µL of the incubated solution was spread on a glass slide, dried at room temperature, and imaged under microscope.

Focused Ion Beam- Scanning Electron Microscopy (FIB-SEM)

Technical terms

The milling process of peptide based soft material was depends upon: beam current, accelerating voltage and mill depth to minimize surface artifacts.

Accelerating Voltage: The interaction volume of gallium (Ga^+) ions is smaller at lower voltage which may cause less damaging to the soft structure compare to higher voltage. Therefore the FIB system which is usually operates at 30 kV operates at an accelerating voltage of 20 kV in the initial milling stage. We have also used a lower accelerating voltage upto 15 kV for ion beam and electron beam.

Beam Currents: Usually the high beam current for peptide based soft structure were not applicable because, the fragile specimens may not be stable under high beam current. Due to this reason we have used a set of current in the rage of 1pA- 2.1nA and find out the lowest possible beam current which is less damaging in nature. Initially 2.1 nA current for 1 minute used for the irradiation and effect of this irradiation causing more damage to the soft specimen (see supporting information), another beam current of 81pA is also causing damage to the soft specimen under 1-2 minutes time duration. Therefore a current of 37 pA for 1 minute apply to mill the soft-specimen this gives a fine cutting and less damage to the specimen. Since the milling times were inversely proportional to beam current strength therefore we did not use this beam current for longer time which may cause sputtering and melting of the soft-specimen (melting due to the heat generated by ion irradiation). The optimal beam current of 10 pA for milling the specimen typically for 5 minutes was used for present sample. An extremely low beam current of 1 pA is also suitable for milling propose for long time duration upto 1-2h (lowest beam currents required impractically long exposure).

Mill Depth: The fragile specimen under given beam currents, accelerating voltage and longer milling periods may also show to damage. Therefore it is important to define the optimal mill depth of fragile specimen for the required time period. The optimal mill depth from 100-500 nm which gives a clear view of interior of the biotinylated microshpere.

Peptide synthesis - All *N*-biotinyted peptide were synthesized by simple solution phase fragment condensation methodologies using *t*-Boc chemistry and in the presence of HOBt. Purity of final products was checked by analytical FPLC (Amersham Pharmacia FPLC system (Akta Basic), using a µRPC C2/C18 ST 4.6/100 column (Pharmacia Biotech) 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 (Supplementary Figure 1 and 2).

Peptide Synthesis:

Synthesis of (*N-tert*-butyloxycarbonyl-L-Tryptophan)- L-Tryptophan (1.0 g, 4.89 mmol, 1equiv) was suspended in 17 mL of methanol and triethylamine (1.63 mL 1.2 equiv) as its 10 % solution in methanol was added and stirred. To this, di-tert- butyl pyrocarbonate (1.42 mL, 5.87 mmol, 1.2 equiv) was added drop-wise with constant stirring. Reaction was refluxed at 65 $^{\circ}$C for 2- 3h, followed by 30 minutes stirring at room temperature. The clear solution so obtained was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate, acidified to pH 2. The solution was saturated with sodium chloride followed by extraction with ethyl acetate (2 × 40mL). The organic layer was dried over anhydrous sodium sulphate and evaporated *in* vacuo to yield pure product (1.35 g, yield 90 %) ^{1}H NMR: (400 MHz $CDCl_3$, 25°C, TMS) δ(ppm) 1.40 (s, $^{t-}$Boc 9H); 3.04 (m, 2H, Trp βH); 4.01(m, 1H, Trp αH); 5.70 (br s, 1H, $^{t-}$Boc –NH); 6.5 (s, 1H, Trp ArH); 6.92 (m, 1H, Trp ArH); 7.01 (m, 1H, Trp ArH) 7.29 (m, 2H, Trp ArH); 8.35 (s, 1H, indole –NH). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS) δ (ppm) = 28.7, 39.2, 62.2, 79.5, 108.55, 111.30, 118.40, 118.58, 120.90, 124.40, 127.37, 136.10, 157.5, 175.10 Anal. Calcd. for $C_{16}H_{20}N_2O_4$;

Elemental Analysis: C, 63.14; H, 6.62; N, 9.20; found C, 63.20; H, 6.60; N, 9.15%.

Synthesis of (*N-tert*-butyloxycarbonyl-di-L-Trytophan methyl ester)- A solution of compound *N*-tert-butyloxycarbonyl L-Trytophan (1.0 g, 3.3 mmol, 1 equiv) in dry DCM (20 mL) was cooled to 0 ^{o}C and N-hydroxybenzotriazole (0.48 g, 3.6 mmol, 1.1 eq) was added, followed by dicyclohexylcarbodi-imide (0.81 g, 3.94 mmol, 1.2 equiv). After 1 h the reaction mixture was allowed to warm to room temperature then compound L-Trptophan methyl ester hydrochloride salt (0.91 g, 3.6 mmol, 1.1 equiv) dissolved in dimethyl formamide (10 mL) was added, followed by triethyl amine (0.54 mL, 3.94 mmol 1.2 equiv). The reaction mixture was stirred for 12 h. The precipitated dicyclohexylurea was filtered off and washed with little DCM. The filtrate was evaporated under high *vacuo*, the crude compound was acidified under ice cold condition with 1N HCl to pH 2-3 and extracted with (3 × 15 mL) dichloromethane. The organic layer was washed with 10% $NaHCO_3$ solution (3 × 10mL), and finally with saturated brine solution (2 × 10 ml), followed by drying of the organic layer over anhydrous sodium sulphate. Dichloromethane was evaporated to get the crude compound (1.8 g), which was further purified with a silica

gel column (0.5-5 % CH_3OH gradient in CH_2Cl_2) to give protected compound *N*-tert-butyloxycarbonyl di-L-Tryptophan methyl ester as a off white solid, R_f value is 0.70 (10% methanol in dichloromethane, 1.4 g, 85% yield) obtained. M.P: 165-168 °C; ^1H NMR: (400 MHz $CDCl_3$, 25°C, TMS) δ(ppm) 1.36 (s, ^{t}-Boc 9H); 3.05-3.28 (merged signals 4H, Trp β,β'); 3.57 (s, 3H, $-OCH_3$); 4.42 (br s, 1H, Trp αH); 4.78 (m, 1H, Trp α'H); 6.20 (br, s, 1H, ^{t}-Boc –NH); 6.51 (br, s, 1H, –NH); 6.88 (m, 2H, Ar-Trp-H); 7.09-7.32 (m, 8H, Ar-Trp H); 7.62 (m, 1H, indole -NH); 7.96 (m, 1H, indole -NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS) δ (ppm) = 28.56, 34.44, 34.47, 52.80, 54.70, 55.08, 80.06, 107.77, 110.36, 112.38, 112.58, 119.03, 119.88, 120.26, 120.30, 122.51, 122.57, 124.49, 125.76, 128.26, 128.53, 138.03, 138.28, 157.50, 170.05, 173.29 Anal. Calcd. for $C_{28}H_{32}N_4O_5$; Elemental Analysis: C, 66.65; H, 6.39; N, 11.10; found C, 66.58; H, 6.25; N, 11.05%.

Synthesis of (di-L-Trytophan methyl ester hydrochloride salt) - The ester ^{t}-Boc-Trp-Trp-methyl ester, (500 mg, 1.0 mmol, 1 equiv) was dissolved in 15 mL dry ethyl acetate-hydrochloric acid (1N) and set aside for 4 h under N_2 atmosphere. The precipitated product was filtered off, washed with cold distilled ethyl acetate (3 × 25ml). Off-

white solid was (456 mg, 81% yield) obtained which was dried under high vacuo. ^{1}H NMR: (400 MHz CD_3OD, 25 °C, TMS) δ(ppm) 3.29 (m, 2H, Trp ββ′H); 3.30-3.33 (m, 2H, Trp ββ′H); 3.64 (s, 3H, $-OCH_3$); 4.08 (m, 1H, Trp α, H); 4.80 (m, 1H, Trp α′ H); 6.97-7.14 (m, 4H, Ar-Trp H); 7.19 (s, 2H, Ar-Trp-H); 7.31 (d, 1H, *J* = 8.08 Hz, Ar-Trp H); 7.36 (d, 1H, *J* = 8.04 Hz, Ar-Trp H); 7.51 (d, 1H, *J* = 7.84 Hz, Ar-Trp H); 7.63 (d, 1H, *J* = 7.8 Hz, Ar-Trp H) ^{13}C NMR (100 MHz, CD_3OD, 25 °C, TMS) δ (ppm) = 31.1, 34.73, 51.9, 54.1, 55.2, 107.80, 110.41, 112.52, 112.58, 119.05, 119.91, 120.22, 120.29, 122.58, 122.87, 124.50, 125.72, 128.28, 128.52, 138.09, 138.30, 171.50, 172.31 Anal. Calcd. for $C_{23}H_{25}ClN_4O_3$; Elemental Analysis: C, 62.65; H, 5.71; N, 12.71; found C, 62.55; H, 5.62; N, 12.75%.

Synthesis of (*N*- Biotinyl-L-Tryptophan methyl ester, 1)- D-Biotin (400 mg, 1.6 mmol, 1 equiv), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC; 313 mg, 1.6 mmol, 1equiv), and *N,N*-dimethyl-4-aminopyridine (DMAP; 200 mg, 1.6 mmol, 1 equiv) were dissolved in dry DMF (25 mL) and placed in a two-neck round-bottom flask in the presence of N_2 atmosphere. L-tryptophan methyl ester hydrochloride (417 mg, 1.6 mmol, 1equiv) was added and then

the mixture was stirred for 6 h. When the reaction was complete, the solvent was evaporated in high vacuo and the residue was dissolved in CH_2Cl_2 extracted with 1N HCl (2 × 20ml). The organic layer was isolated and the product (**1**) purified by column chromatography (SiO_2; CH_2Cl_2/MeOH, 10:2). R_f value 0.6 in 10% Methanol/DCM. Yield: 568 mg (78%). M.P. 90-92 °C; $[\alpha]^{t}_{D}$ = +29.6 (c 0.25 in CH_3OH); ^{1}H NMR: (400 MHz $CDCl_3$, 25 °C, TMS) δ(ppm) 1.18-1.26 (m, 2H, CH_2); 1.49-1.59 (m, 4H, CH_2); 2.09-1.13 (t, J = 7.08 Hz, 2H, CH_2); 2.51 (d, J_{gem} = 12.96 Hz, 1H, CH_2); 2.89 (dd, J = 4.88, J_{gem} = 12.92 Hz, 1H, CH_2); 2.73-2.94 (m, 2H, Trp β, H); 3.22-3.23 (m, 1H, -CH); 3.61 (s, 3H, $-OCH_3$); 4.01-4.04 (m, 1H, -CH); 4.28 (t, J =7.56 Hz, 1H, -CH); 4.84-4.89 (m, 1H, Trp α, H); 6.29 (s, 1H, N^1H); 6.63 (d, J = 7.8 Hz, 1H, N^3H); 6.99-7.10 (m, 3H, Ar-Trp H); 7.28 (d, J = 7.84 Hz, 1H, Ar-Trp-H); 7.43 (d, J =7.8 Hz, 1H, Ar-Trp H); 7.94 (s, 1H, -NH); 8.89 (s, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS) δ(ppm) = 25.35, 27.85, 35.36, 36.58, 40.58, 52.57, 54.12, 55.53, 60.23, 62.01, 109.52, 111.57, 118.45, 119.46, 122.05, 122.89, 128.40, 138.40, 172.21, 173.24. HRMS: calculated $[M+H]^+$ 445.1910, found 445.1915.

Synthesis of (*N*- Biotinyl-L-Tryptophan, 1a) Compound **1** (265 mg, 0.6 mmol) was placed in a 25-mL round-bottom flask and

MeOH (8 mL) was added. 1N NaOH (20 mg, 1.5 equiv.) was added to the flask and the mixture was stirred at room temperature for 4 h. After the reaction was complete, the mixture was acidified using dilute sulfuric acid to pH 2–3. The hydrolyzed product that precipitated was filtered off, dried in the air, and then washed several times with acetone and CH_2Cl_2 to yield **1a** (210 mg, 82% yield). M.P. 160-163 °C; $[\alpha]^{t}_{D}$ = +24.4 (c 0.25 in CH_3OH); ^{1}H NMR: (400 MHz, DMSO-d_6, 25 °C, TMS) δ(ppm) 1.22 (m, 2H, CH_2); 1.42 (m, 2H, CH_2); 1.55 (m, 2H, CH_2); 2.06 (m, 2H, CH_2); 2.54 (d, J_{gem} = 12.20 Hz, 1H, CH_2); 2.78 (dd, J = 4.88, J_{gem} = 12.20 Hz, 1H, CH_2); 2.87-3.01 (m, 2H, Trp β, H); 3.13 (m, 1H, -CH); 4.08 (m, 1H, -CH); 4.29 (m, 1H, -CH); 4.46 (m, 1H, Trp α, H); 6.50 (s, 2H, N^1H and N^3H); 6.96-7.12 (m, 3H, Ar-Trp H); 7.30 (d, J = 7.84 Hz, 1H, Ar-Trp-H); 7.52 (d, J = 7.8 Hz, 1H, Ar-Trp H); 8.10 (d, J = 7.8 Hz, 1H, -NH); 10.87 (s, 1H, *indole* -NH); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ(ppm) = 25.12, 27.13, 27.91, 27.97, 34.81, 48.59, 52.80, 55.37, 59.24, 61.00, 109.99, 111.37, 118.17, 118.32, 120.88, 123.54, 127.20, 136.08, 162.75, 172.16, 173.61. HRMS: Calculated $[M+H]^+$ 431.1753, Found 431.1757.

Synthesis of (*N*- Biotinyl-di-L-Tryptophan methyl ester, 2)- D-Biotin (277 mg, 1.1 mmol, 1 equiv), 1-ethyl-3-(3-

dimethylaminopropyl) carbodiimidehydrochloride (EDC; 217 mg, 1.1 mmol, 1 equiv), and *N,N*-dimethyl-4-aminopyridine (DMAP; 138 mg, 1.1 mmol, 1 equiv) were dissolved in dry DMF (25 mL) and placed in a two-neck round-bottom flask in the presence of N_2 atmosphere. di-L-tryptophan methyl ester hydrochloride (500 mg, 1.1 mmol, 1 equiv) was added and then the mixture was stirred for 6 h at room temperature. When the reaction was complete, the solvent was evaporated in high vacuo and the residue was dissolved in 1N HCl (2 × 20 mL). The precipitated product was filtered off and washed several time with water and then CH_2Cl_2. The off white product (**2,** 536 mg, 75%) dried in oven at 80 °C obtained. R_f = 0.6 in 15% Methanol/DCM; M.P. 138-140 °C; $[\alpha]^{t}_{D}$ = +11.14 (c 0.35 in CH_3OH); ^{1}H NMR: (400 MHz, DMSO-d_6, 25 °C, TMS), δ(ppm) 1.18-1.29 (m, 2H, CH_2); 1.61-1.63 (m, 2H, CH_2); 1.74-1.77 (m, 2H, CH_2); 2.33 (m, 2H, CH_2); 2.49 (d, J_{gem} = 12.68 Hz, 1H, CH_2); 2.59 (dd, J = 4.88 Hz, J_{gem} = 12.68 Hz, 1H, CH_2); 2.70 (m, 1H, Trp β, H); 2.84-3.03 (m, 3H, Trp β, β'); 3.13 (m, 1H, -CH); 3.39 (s, 3H, $-OCH_3$); 3.71-3.79 (m, 1H, -CH); 4.14-4.18 (m, 1H, Trp α', H); 4.51-4.57 (m, 2H, Trp α H and -CH); 6.48 (s, 1H, N^1H); 6.52 (d, J = 7.56 Hz, 1H, N^3H); 6.71-6.89 (m, 8H, Ar-Trp H); 7.09-7.14 (dd, J_1 = 8.04 Hz, J_1 = 3.92 Hz, 2H, Ar-Trp-H); 7.38

(d, J = 7.8 Hz, 2H, -NH); 9.80 (s, 1H, indole -NH); 9.98 (s, 1H, indole –NH). ^{13}C NMR (100 MHz, DMSO-d_6,, 25 °C, TMS), δ(ppm) = 24.57, 26.42, 26.72, 30.60, 31.2, 34.44, 49.27, 51.61, 52.13, 52.60, 54.72, 59.84, 61.08, 107.88, 109.24, 110.94, 117.42, 118.10, 118.39, 120.86, 123.32, 123.46, 126.74, 126.98, 135.65, 135.90, 163.18, 171.01, 171.38, 172.59. HRMS: Calculated $[M+H]^+$ 631.2703, Found 631.2700.

Synthesis of (*N*- Biotinyl-di-L-Tryptophan, 2a)- Compound **1** (229 mg, 0.36 mmol, 1 equiv) was placed in a 25-mL round-bottom flask and MeOH (10 mL) was added.1N NaOH (21 mg, 1.5 equiv) was added to the flask and the mixture was stirred at room temperature for 4 h. After the reaction was complete, the mixture was acidified using dilute sulfuric acid to pH 2–3. The hydrolyzed product that precipitated was filtered off, dried in the air, and then washed several times with CH_2Cl_2 to yield **2a** (194 mg, 86% yield). M.P. 151-153 °C; $[\alpha]^{t}_{D}$ = +8.0 (c 0.25 in CH_3OH); ^{1}H NMR: (400 MHz, DMSO-d_6, 25 °C, TMS), δ(ppm) 1.19-1.32 (m, 2H, CH_2); 1.77-1.79 (m, 2H, CH_2); 1.86-1.90 (m, 2H, CH_2); 2.48 (m, 2H, CH_2); 2.61 (d, J_{gem} = 12.68 Hz, 1H, CH_2); 2.73 (dd, J = 5.16 Hz, J_{gem} = 12.68 Hz, 1H, CH_2); 2.88 (m, 1H, Trp β, H); 3.06-3.13 (m, 3H, Trp β, β'); 3.15 (m, 1H, -CH); 3.93-3.96 (m,

1H, -CH); 4.32-4.35 (m, 1H, Trp α', H); 4.61-4.69 (m, 2H, Trp α H and -CH); 6.61 (s, 1H, N^1H); 6.65 (d, J = 7.08 Hz, 1H, N^3H); 6.91-7.04 (m, 8H, Ar-Trp H); 7.23 (d, J = 8.08 Hz, 1H, Ar-Trp-H); 7.27 (d, J = 8.04 Hz, 1H, Ar-Trp-H); 7.52 (d, J = 7.8 Hz, 2H, -NH); 10.14 (s, 1H, indole -NH); 10.19 (s, 1H, *indole* –NH). ^{13}C NMR (100 MHz, DMSO-d_6, 25 ^{o}C, TMS), δ(ppm) = 25.10, 27.07, 30.30, 31.22, 34.91, 48.32, 52.54, 52.89, 54.82, 60.29, 61.27, 108.71, 109.54, 111.31, 118.08, 118.36, 121.03, 122.20, 123.56, 123.77, 127.33, 135.96, 136.23, 163.78, 171.26, 172.88, 173.66. HRMS: Calculated $[M+H]^+$ 617.2546, Found 617.2545.

Synthesis of (*N-tert*-butyloxycarbonyl-L-Phenylalanine)- L-Phenylalanine (1.5 g, 9.08 mmol, 1eq.) was suspended in 30 mL of NaOH (0.39 g, 9.98 mmol, 1.1 eq.) solution and the reaction mixture was diluted with *tert*- butanol. To this, di-tert- butyl pyrocarbonate (2.63 mL, 10.89 mmol, 1.2 eq) was added drop-wise with constant stirring. Reaction mixture was stirred at room temperature for overnight. Next day the reaction mixture was extracted with pentane and the organic phase is extracted with saturated $NaHCO_3$ solution (3 x 15 mL). The combined aqueous layer was acidified to pH 2-

3 with 1N HCl solution. The acidified layer was extracted with ethyl acetate (3 x 25 mL) and washed with brine solution (2 x 15 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated to give the product (2.1 g, 87.5 % yield) ^{1}H NMR: (400 MHz $CDCl_3$, 25°C, TMS) δ(ppm) 1.34 (s, $^{t-}$Boc 9H); 2.99 (m 1H, Phe βH); 3.13 (m, 1H, Phe βH); 4.93 (m, 1H, Phe αH); 6.44 (d, J= 6.84, 1H, $^{t-}$Boc –NH); 7.10- 7.22 (m, 5H, aromatic H); 10.88 (br, s, 1H, -COOH). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS) δ (ppm)= 28.7; 39.6; 61.1; 79.5; 125.7; 127.9; 128.4; 135.2; 157.5; 177.0. Anal. Calcd. for; $C_{14}H_{19}NO_4$ Elemental Analysis: C, 63.38; H, 7.22; N, 5.28; found C, 63.22; H, 7.30; N, 5.32%.

Synthesis of (*N-tert*-butyloxycarbonyl-di-L-Phenylalanine methyl ester)- A solution of compound *N*-tert-butyloxycarbonyl L-Phenylalanine (1.5 g, 5.6 mmol, 1eq.) in dry DCM (20 mL) was cooled to 0 °C and 1-hydroxybenzotriazole (0.83 g, 6.2 mmol, 1.1 eq) was added, followed by dicyclohexylcarbodi-imide (1.39 g, 6.78 mmol, 1.2 eq). After 1 h the mixture was allowed to warm to room temperature then compound L-Phenyalanine methyl ester hydrochloride salt (1.1 g, 6.21 mmol, 1.1 eq) dissolved in dimethyl formamide (10 mL) was added, followed by

triethylamine (0.94 mL, 6.78 mmol 1.2 eq.) The reaction mixture was stirred for 12 h. The precipitated dicyclohexylurea was filtered off and washed with little DCM. The filtrate was evaporated under high vacuuo, the crude compound was acidified under ice cold condition with 1N HCL to pH 2-3 and extracted with (3 x 15 mL) dichloromethane. The organic layer was washed with 10% $NaHCO_3$ solution (3 × 10 mL), and finally with saturated brine solution (2× 10 mL), followed by drying of the organic layer over anhydrous sodium sulphate. Dichloromethane was evaporated to get the crude compound (2.2 g), which was further purified with a silica gel column (0-4 % CH_3OH gradient in CH_2Cl_2) to give protected compound *N*-tert-butyloxycarbonyl di-L-Phenylalanine methyl ester, R_f value is 0.65 (10% methanol in dichloromethane, 1.7 g, 70% yield). M.p. 114-116 °C; $[\alpha]_D^t$ = - 13.9 ° (*c* 1 in CH_3OH). ^1H NMR: (400 MHz $CDCl_3$, 25°C, TMS) δ(ppm) 1.32 (s, ^{t}-Boc 9H); 2.97 (m 4H, Phe $\beta\beta'$H); 3.6 (s, 3H, $-OCH_3$); 4.70 (m, 1H, Phe α'H); 4.85 (m, 1H, Phe αH) 6.19 (br, s, 1H, ^{t}-Boc –NH); 6.91 (br, s, 1H, –NH) 7.11- 7.23 (m, 10H, aromatic H). ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS) δ (ppm) = 28.80, 37.84, 38.07, 51.0, 55.40, 55.83, 80.06, 126.78, 126.94, 128.27, 128.37, 129.20, 129.36, 136.60, 136.75, 155.20, 169.98, 172.64

Anal. Calcd. for $C_{24}H_{30}N_2O_5$; Elemental Analysis: C, 67.59; H, 7.09; N, 6.57; found C, 67.44; H, 7.19; N, 6.61%.

Synthesis of (di-L-Phenylalnine methyl ester TFA salt) - The ester $^{t-}$Boc-Phe-Phe-methyl ester, (1.5 g, 3.5 mmol, 1 eq) was dissolved in 75% Trifluoroacetic acid-Dichloromethane (20mL) and set aside for 1 h under N_2 atmosphere. Solvent was evaporated in high vacuuo. Anhydrous ether (50 mL) was added, stirrer it for 30 min. the white solid was filtered off and washed with boiling ether (50 mL.) then cold ether. The white solid (1.29g, 83.7%) was obtained. mp: >220°C (decomposed), ^{1}H NMR: (400 Hz CD_3OD, 25°C, TMS) δ (ppm) 2.95-3.04 (m, 2H, Phe β, β' H); 3.15-3.29 (m, 2H, Phe β, β' H); 3.67 (s, 3H, $-OCH_3$); 4.05 (dd, J = 5.36, 1H, Phe α H); 4.71 (dd, J = 5.84, 1H, Phe α' H); 7.20-7.37 (m, 10H aromatic H); ^{13}C NMR (100 MHz, CD_3OD, 25°C, TMS) δ (ppm)= 38.28, 38.56, 52.34, 52.83, 55.44, 111.40, 128.06, 128.90, 129.63, 130.52, 170.02, 171.12, 176.01, Anal. Calcd. for $C_{21}H_{22}F_3N_2O_5$; C, 57.40; H, 5.05; N, 6.38 %, found C, 57.28; H, 5.12; N, 6.22%.

Synthesis of (*N*- Biotinyl-L-Phenylalanine methyl ester, 3a)-D-Biotin (250 mg, 1.0 mmol, 1 eq), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDC; 215 mg,

1.12 mmol, 1.1eq), and *N,N*-dimethyl-4-aminopyridine (DMAP; 137 mg, 1.12 mmol, 1.1 eq) were dissolved in dry DMF (25 mL) and placed in a two-neck round-bottom flask in the presence of N_2 atmosphere. L-phenylalanine methyl ester hydrochloride (242 mg, 1.12 mmol, 1.1eq) was added and then the mixture was stirred for 6 h. When the reaction was complete, the solvent was evaporated in high vacuo and the residue was dissolved in CH_2Cl_2 and extracted with 1N HCL (2 x 20ml). The organic layer was isolated and the product (**3a,** *N*-biotinyl-L-phenylalanine methyl ester) purified by column chromatography (SiO_2; CH_2Cl_2/MeOH, 10:2).Yield: (345 mg, 76%); R_f value is 0.55 in 10% Methanol/DCM; M.p. 70-80 $^{\circ}C$; $[\alpha]^{t}_{D}$ = +41.6 (c 0.25 in CH_3OH); ^{1}H NMR: (400 MHz $CDCl_3$, 25 $^{\circ}C$, TMS) δ(ppm) 1.35 (m, 2H, CH_2); 1.58-1.69 (m, 4H, CH_2); 2.15-2.19 (t, J = 6.6 Hz, 2H, CH_2); 2.69 (d, J_{gem} = 12.68 Hz, 1H, CH_2); 2.85 (m, 1H, CH_2); 2.93-3.12 (m, 2H, Phe β, H); 3.15 (m, 1H, -CH); 3.68 (s, 3H, $-OCH_3$); 4.30 (m, 1H, -CH); 4.51 (m, 1H, -CH); 4.81-4.83 (m, 1H, Phe α, H); 6.72 (br, s, 2H, N^1H and N^3H); 7.10-7.27 (m, 5H, Phe-ArH); 7.01 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 $^{\circ}C$, TMS) δ (ppm)= 25.20, 26.71, 36.52, 37.10, 41.32, 51.90, 53.14, 55.50, 60.40, 61.70, 126.11, 127.71, 128.73, 137.40, 162.90, 171.51, 172.62. HRMS: calculated $[M+H]^+$ 405.1722, found 405.1801.

Synthesis of (*N*- Biotinyl-L-Phenylalanine, 4a)-Compound **3a** (200 mg, 0.5 mmol) was placed in a 25-mL round-bottom flask and MeOH (10 mL) was added.1N NaOH (29 mg, 1.5 eq.) was added to the flask and the mixture was stirred at room temperature for ~5 h. After the reaction was complete, the mixture was acidified using 1N HCl to pH 2–3. The hydrolyzed product that precipitated was filtered off and then washed several times with water and CH_2Cl_2, dried in the air, to yield **4a** (165 mg, 85% yield). M.p. 202-204 °C; $[\alpha]^{t}_{D}$ = +18.4 (c 0.25 in CH_3OH); ^{1}H NMR: (400 MHz $CDCl_3$, 25 °C, TMS) δ(ppm) 1.35 (m, 2H, CH_2); 1.48-1.50 (m, 4H, CH_2); 2.00 (m, 2H, CH_2); 2.52 (d, J_{gem} = 12.44 Hz, 1H, CH_2); 2.77 (m, 1H, CH_2); 2.80-98 (m, 2H, Phe β, H); 3.12 (m, 1H, -CH); 4.03 (m, 1H, -CH); 4.25 (m, 1H, -CH); 4.36-4.41 (m, 1H, Phe α, H); 6.60 (br, s, 2H, N^1H and N^3H); 7.15-7.21 (m, 5H, Phe-ArH); 8.12 (d, J = 8.32 Hz, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS) δ (ppm)= 25.24, 28.01, 34.86, 36.78, 38.67, 41.32, 53.14, 55.49, 59.90, 61.87, 126.41 128.19, 129.11, 137.82, 162.79, 172.19, 173.39. HRMS: Calculated $[M+H]^+$ 392.1644, Found 392.1641.

Synthesis of (*N*- Biotinyl-di-L-Phenylalanine methyl ester, 3)- D-Biotin (250 mg, 1.0 mmol, 1 eq), 1-(3-

dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDC; 215 mg, 1.12 mmol, 1.1eq), and *N*,*N*-dimethyl-4-aminopyridine (DMAP; 138 mg, 1.12 mmol, 1.1 eq) were dissolved in dry DMF (25 mL) and placed in a two-neck round-bottom flask in the presence of N_2 atmosphere. Di-L-phenylalanine methyl ester hydrochloride (495 mg, 1.12 mmol, 1.1eq) was added and then the mixture was stirred for 6 h at room temperature. When the reaction was complete, the solvent was evaporated in high vacuo and the residue was dissolved in 1N HCl (2x 30ml). The precipitated product was filtered off and washed several time with water and then CH_2Cl_2. The off white product (**3,** 400 mg, 70%) dried in oven at 80 °C obtained. R_f = 0.56 in 10% Methanol/DCM; M.p. 140-142 °C; $[\alpha]^{t}_{D}$ = +23.6 (c 0.25 in CH_3OH); ^{1}H NMR: (400 MHz DMSO-d_6, 25 °C, TMS) δ(ppm) 1.11-1.15 (m, 2H, CH_2); 1.33-1.39 (m, 2H, CH_2); 1.49-1.54 (m, 2H, CH_2); 1.99 (t, J = 7.36 Hz, 2H,CH_2); 2.57 (d, J_{gem} = 12.44 Hz, 1H, CH_2); 2.66 (m, 1H, Phe β H); 2.81 (dd, J = 5.12 Hz, J_{gem} = 12.44 Hz, 1H, CH_2); 2.91-3.05 (m, 3H, Phe β, β'); 3.15 (m, 1H, -CH); 3.58 (s, 3H, $-OCH_3$); 4.05-4.08 (m, 1H, -CH); 4.28 (t, J = 7.32 Hz, 1H, Phe α', H); 4.44-4.56 (m, 2H, Phe α H and -CH); 6.37 (bs, 2H, N^1H and N^3H); 7.15-7.28 (m, 8H, Ar-Phe H); 7.93 (d, J = 8.52 Hz, 1H, -NH); 8.43 (d, J = 7.56 Hz, 1H, -NH). ^{13}C NMR (100 MHz, DMSO-

d_6, 25 °C, TMS) δ (ppm)= 25.17, 27.94, 34.91, 36.58, 37.53, 42.3, 51.86, 53.31, 53.61, 55.40, 59.18, 60.98, 126.18, 126.60, 127.96, 128.28, 129.05, 129.15, 137.02, 137.89, 162.71, 171.64, 171.76, 171.88. HRMS: Calculated $[M+H]^+$ 553.2485, Found 553.2482.

Synthesis of (*N*- Biotinyl-di-L-Phenylalanine, 4)- Compound **3** (165 mg, 0.3 mmol, 1 eq) was placed in a 25-mL round-bottom flask and MeOH (10 mL) was added.1N NaOH (17 mg, 1.5 eq.) was added to the flask and the mixture was stirred at room temperature for 6 h. After the reaction was complete, the mixture was acidified using 1N HCl to pH 2–3. The hydrolyzed product that precipitated was filtered off, dried in the air, and then washed several times with water and then CH_2Cl_2 to yield **4** (123 mg, 76% yield). M.p. 160-163 °C; $[\alpha]^{t}_{D}$ = +20.33 (c 0.3 in CH_3OH); ^{1}H NMR: (400 MHz DMSO-d_6, 25 °C, TMS) δ(ppm) 1.11-1.12 (m, 2H, CH_2); 1.34 (m, 2H, CH_2); 1.51-1.53 (m, 2H, CH_2); 1.99 (t, J =6.84 Hz, 2H,CH_2); 2.56 (d, J_{gem} = 12.44 Hz, 1H, CH_2); 2.70 (m, 1H, Phe β H); 2.83 (dd, J = 4.16 Hz, J_{gem}= 12.44 Hz, 1H, CH_2); 2.90-3.09 (m, 3H, Phe β, β'); 3.15 (m, 1H, -CH); 3.95-4.08 (m, 1H, -CH); 4.3 (m, 1H, Phe α', H); 4.40-4.51 (m, 2H, Phe α H and -CH); 6.49 (bs, 2H, N^1H and N^3H); 7.15-7.25 (m, 8H, Ar-Phe H); 8.06 (d, J = 8.28 Hz, 1H, -NH); 8.43 (d, J= 7.84 Hz,

1H, -NH). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm)= 25.12, 27.90, 34.93, 36.57, 37.43, 42.3, 53.46, 53.55, 55.32, 59.22, 60.98, 126.09, 126.41, 127.89, 128.13, 129.13, 129.15, 137.41, 137.97, 162.73, 171.48, 171.85, 172.64. HRMS: Calculated $[M+H]^+$ 539.2328, Found 539.2329.

Synthesis of (D-Biotin methyl ester) - D-Biotin (250 mg, 1.0 mmol, 1 eq), was dissolved in dry Methanol (10mL) and placed in a two-neck round-bottom flask in the presence of N_2 atmosphere. Concentrated H_2SO_4 (150 µL) was added and the reaction mixture was refluxed for 12 h. The solvent was evaporated in high vacuo and the residue was washed with anhydrous ether, an off-white solid material was isolated. Yield (220 mg, 83%). M.p. 148-150 °C; $[\alpha]^t_D$ = +73.2 (c 0.25 in CH_3OH). ^1H NMR: (400 MHz $CDCl_3$, 25 °C, TMS) δ(ppm) 1.39-1.44 (m, 2H, CH_2); 1.60-1.71 (m, 4H, CH_2); 2.30 (t, J=7.56, 2H,CH_2); 2.71 (d, J_{gem} = 12.62, 1H, CH_2); 2.89 (dd, J= 4.88, J_{gem}= 12.68, 1H, CH_2); 3.10-3.15 (m, 1H, -CH); 3.63 (s, 3H, -OCH_3); 4.27-4.30 (m, 1H, -CH); 4.48-4.51 (m, 1H, -CH); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS) δ (ppm)= 24.78, 25.7, 28.38, 33.70, 40.58, 51.58, 55.50, 60.32, 62.11, 164.26, 174.38.

Synthesis of (*N-tert*-butyloxycarbonyl-Tyrosine) - Tyrosine (2.71 g, 15 mmol, 1eq.) was suspended in a mixture of 20 mL of dioxane and 1N NaOH (20 mL) and stirred. To this, di-tert- butyl pyrocarbonate (3.9 g, 17 mmol, 1.2 eq) was added drop-wise with constant stirring for 14 h at room temperature. Next the solvent was evaporated in vacuo at 45 $^{\circ}$C and the residue was dissolved in dichloromethane, acidified with 1N HCl, to pH 2 and extracted. The water layer was saturated with sodium chloride followed by extraction with dichloromethane (2 × 50 mL). Both the organic layers was combined and dried over anhydrous sodium sulphate and evaporated in vacuo to yield pure solid product (3.9 g, yield 92 %), which was directly used for further reaction. ^{1}H NMR (400 MHz, $CDCL_3$, TMS, 25°C): δ(ppm) 1.40 (s, $^{t-}$Boc 9H); 2.87-3.01 (m, 2H, -CH_2); 4.50 (m, 1H, -CH); 5.04 (d, *J* = 7.8 Hz, $^{t-}$Boc -NH); 6.69 (d, *J* = 7.56 Hz, 2H, aromatic H); 6.97 (d, *J* = 7.56 Hz, aromatic H). ^{13}C NMR (100 MHz, $CDCL_3$, TMS, 25°C) δ (ppm) = 28.30, 37.09, 54.49, 80.48, 115.65, 127.59, 130.55, 155.00, 175.22.

Synthesis of (*N-tert*-butyloxycarbonyl L-Tyrosine-Tyrosine methyl ester) - A solution of compound N-tert-butyloxycarbonyl Tyrosine (2.87 g, 10 mmol, 1 eq.) in dry DCM (30 mL) was cooled to 0 $^{\circ}$C and 1-hydroxybenzotriazole

(1.38 g, 10 mmol, 1 eq) was added, followed by dicyclohexylcarbodiimide (2.32 g, 11 mmol, 1.1 eq). After 1 h the mixture was allowed to warm to room temperature then compound Tyrosine methyl ester hydrochloride salt (2.37 g, 10 mmol, 1 eq) dissolved in dimethyl formamide (10 mL) was added, followed by triethyl amine (1.56 mL, 11 mmol 1.1 eq.) The reaction mixture was stirred for 24 h. The precipitated dicyclohexylurea was filtered off and washed with little DCM. The filtrate was evaporated under high vacuo, the crude compound was acidified under ice cold condition with 1N HCl to pH 2-3 and extracted with (3 × 20 mL) dichloromethane. The organic layer was washed with 10% $NaHCO_3$ solution (3 × 20 mL), and finally with saturated brine solution (2 × 15 mL), followed by drying of the organic layer over anhydrous sodium sulphate. Dichloromethane was evaporated to get the crude compound (4.5 g), which was further purified with a silica gel column (1-4 % CH_3OH gradient in CH_2Cl_2) to give protected compound N-tert-butyloxycarbonyl L-tyrosyl-tyrosine methyl ester as a yellow solid, M.P. 52-55 °C R_f = 0.70 (10% methanol in dichloromethane, 3.95 g, 84 % yield). ^1H NMR: (400 MHz $CDCl_3$, 25 °C, TMS) δ (ppm) 1.38 (s, ^{t}-Boc 9H); 2.83-2.94 (m, 4H, $-CH_2$, Tyr β, β′ H); 3.63 (s, 3H, $-OCH_3$); 4.26

(m, 1H, Tyr α H); 4.70 (m, 1H, Tyr α′ H); 5.24 (bs, 1H, ^{t}-Boc -NH); 6.62-6.65 (t, *J* = 8.28 Hz, 4H, aromatic H); 6.75-6.77 (d, *J* = 8.32, 4H, aromatic H); 6.89 (bs, 1H, -NH); 7.99 (s, 2H, Tyr –OH). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS) δ (ppm) = 28.28, 3704, 37.63, 52.35, 53.46, 56.06, 80.41, 155.71, 127.14, 127.95, 130.31, 130.46, 155.24, 155.59, 171.22, 171.59. Anal. Calcd. for $C_{24}H_{30}N_2O_7$; Elemental Analysis: calculated C, 62.87; H, 6.59; N, 6.11, found C, 62. 72; 6.55; N, 6.02.

Synthesis of (H_2N-Tyr-Tyr-OCH_3.TFA) salt - Boc-Try-Tyr-OMe, (1.0 g, 2.1 mmol, 1 eq) was dissolved in 75% Trifluoroacetic acid - Dichloromethane (15 mL) and set aside for 1 h under N_2 atmosphere. Solvent was evaporated in high vacuum, washed with diethyl ether (3 × 50 mL). Brownish viscous product was obtained which was washed with boiling ether (50 mL) then cold ether. Yield 0.95 g, which was directly used for next reaction.

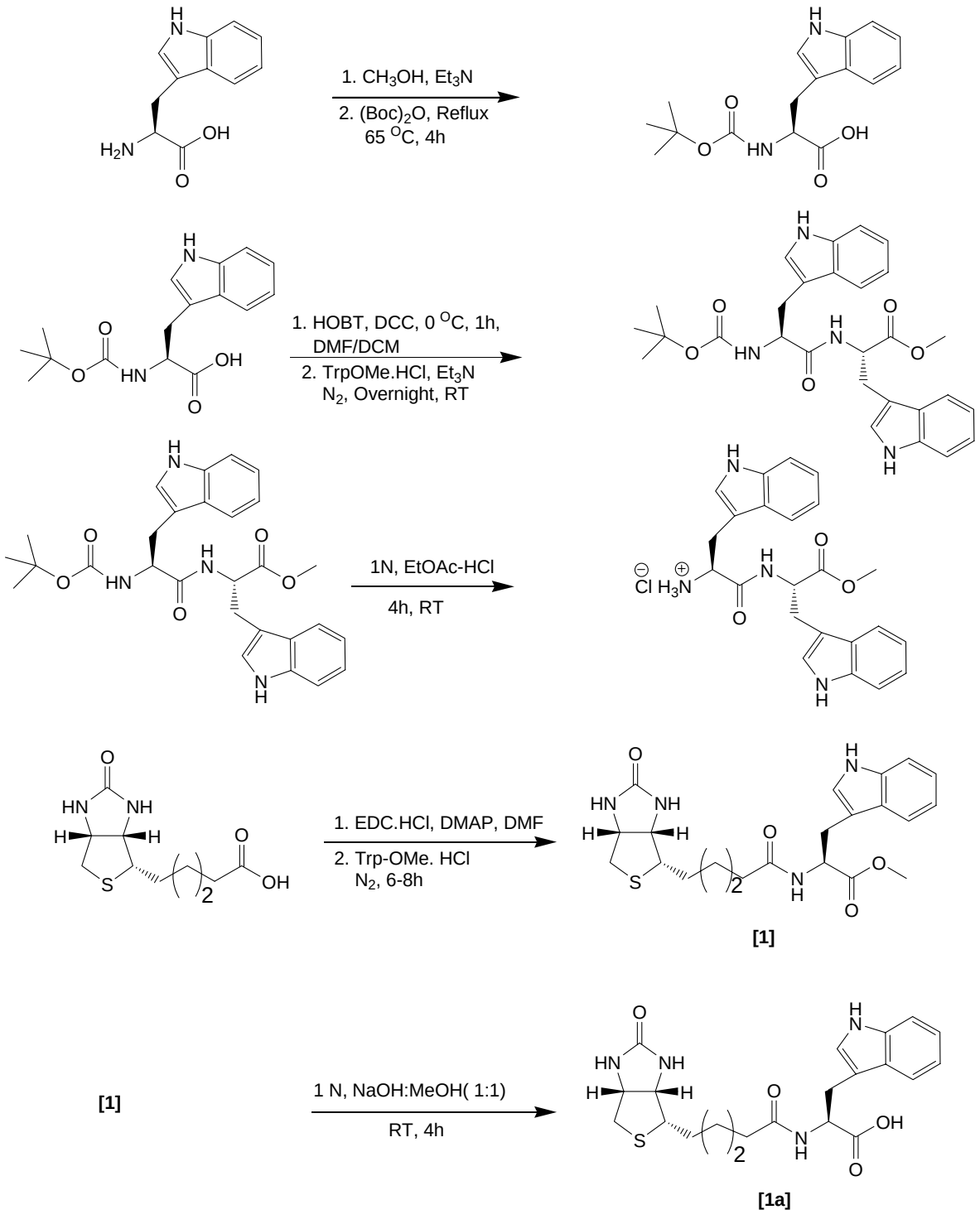
Synthesis of (Biotinylated-Tyr-Tyr-methyl ester, 5)- D-Biotin (157 mg, 0.64 mmol, 1 eq.), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC; 147 mg, 0.77 mmol, 1.2 eq.), and N,N-dimethyl-4-aminopyridine (DMAP; 94 mg, 0.77 mmol, 3 eq.) were dissolved in dry DMF

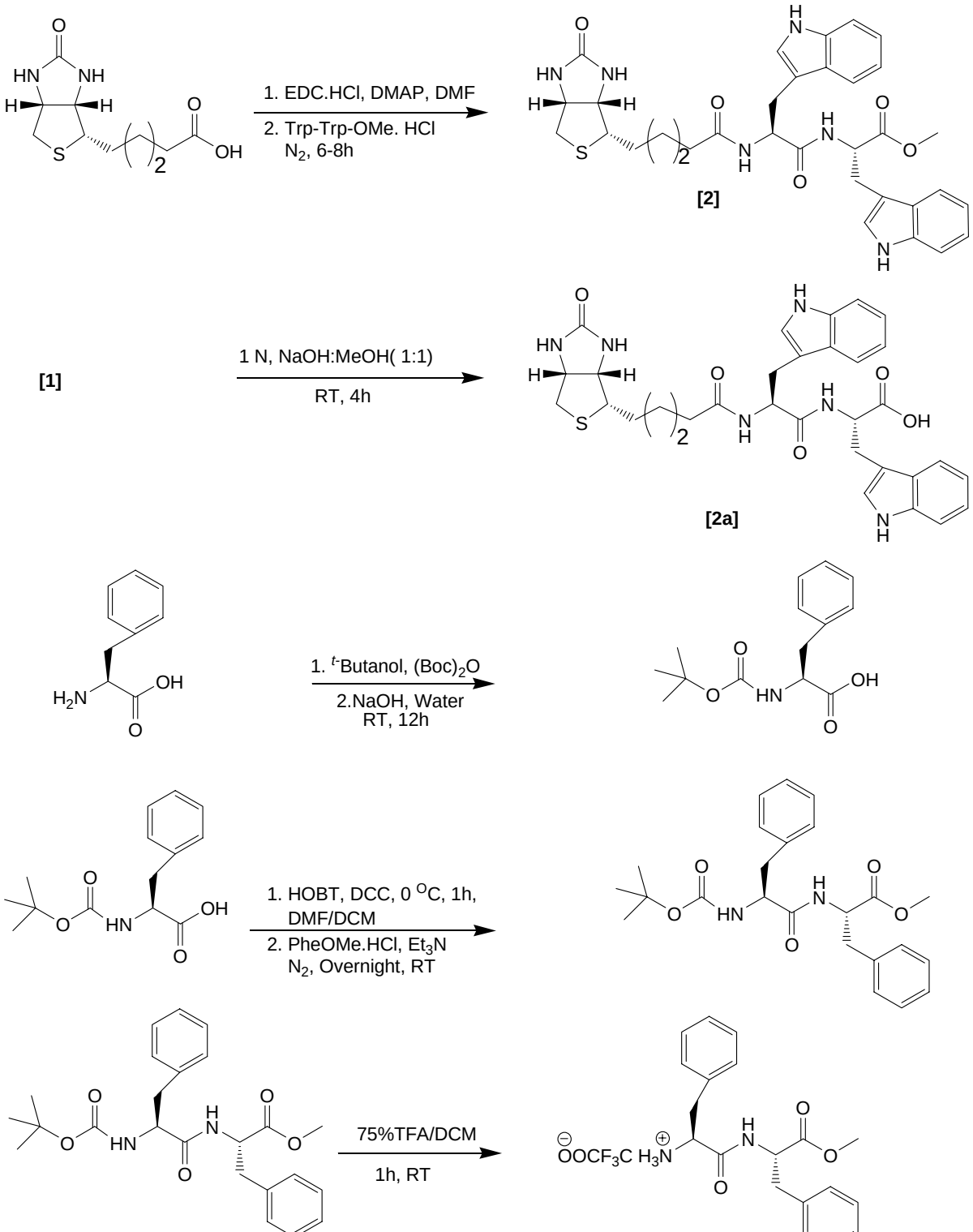
(25 mL) and placed in a two-neck round-bottom flask in the presence of N2 atmosphere. H_2N-Tyr-Tyr-methyl ester. TFA salt (295 mg, 0.64 mmol, 1eq.) was added and then the mixture was stirred for 6 h, when the reaction was complete, the solvent was evaporated in high vacuum and the residue was triturated with water (2 × 20 mL), hot water (2 × 20 mL) and 1N HCl (2 × 20 mL) respectively. The white yellow solid product was isolated. R_f value 0.45 in 10 % methanol/dichloromethane. Yield: 300mg (79%). M.P. 165 -167 °C; ^{1}H NMR: (400 MHz DMSO-d_6, 25 °C, TMS) δ(ppm) 1.15-1.22 (m, 2H, -CH_2); 1.36-1.38 (m, 2H, -CH_2); 1.52 (m, 2H, -CH_2); 1.97-1.99 (m, 2H, -CH_2); 2.54-2.57 (d, J_{gem} = 12.20 Hz, 1H, -CH_2); 2.79 (dd, J = 4.12, J_{gem} = 11.58 Hz, 2H, -CH_2); 3.00 (m, 2H, -CH_2); 3.55 (m, 2H, -CH, and -CH_2); 3.57 (s, 3H, -OCH_3); 4.06 - 4.07 (m, 2H, -CH); 4.27- 4.28 (m, 1H, -CH); 4.35 (m, 1H, -CH); 6.40 (bs, 2H, -N^{1}H and -N^{3}H); 6.60-6.64 (t, J = 8.56 Hz, 4H aromatic H); 6.95-6.99 (t, J = 7.08 Hz, 4H aromatic H): 7.90 (bs, 2H, –NH); 8.45 (bs, 2H, Tyr -OH); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ(ppm) = 25.02, 27.83, 34.89, 35.89, 36.64, 53.55, 55.20, 59.19, 60.95, 114.70, 114.99, 126.83, 127.80, 129.86, 155.62, 155.95, 162.61, 171.47, 171.79. HRMS: calculated $[M+H]^+$ = 585.2383, found 585.2394 (33% intensity) and $[M+Na]^+$ = calculated 607.2202, found 607.2200(100% intensity).

Synthesis of (*N*- Biotinyl-di-L-Tryosine, 6)- Compound **5** (200 mg, 0.34 mmol, 1 equiv) was placed in a 25-mL round-bottom flask and MeOH (10 mL) was added. 1N NaOH (20 mg, 1.5 equiv) was added to the flask and the mixture was stirred at room temperature for 4 h. After the reaction was complete, the mixture was acidified using dilute sulfuric acid to pH 2–3. The hydrolyzed product that precipitated was filtered off, dried in the air, and then washed several times with CH_2Cl_2 to yield **6** (175 mg, 89% yield). ^{1}H NMR: (400 MHz DMSO-d_6, 25 °C, TMS) δ(ppm) 1.15-1.21 (m, 2H, -CH_2); 1.34-1.37 (m, 2H, -CH_2); 1.52 (m, 2H, -CH_2); 1.97-1.99 (m, 2H, -CH_2); 2.52-2.57 (d, J_{gem} = 12.34 Hz, 1H, -CH_2); 2.79-2.83 (dd, J = 4.25, J_{gem} = 12.31 Hz, 2H, -CH_2); 3.15 (m, 2H, -CH_2); 3.38-3.55(m, 2H, -CH and -CH_2); 4.07 - 4.12 (m, 2H, -CH); 4.27- 4.30 (m, 1H, -CH); 4.35-4.37 (m, 1H, -CH); 6.44 (bs, 2H, -N^{1}H and -N^{3}H); 6.59-6.64 (t, J = 8.52 Hz, 4H aromatic H); 6.95-6.97 (t, J = 8.08 Hz, 4H aromatic H): 8.01-8.03 (d, J = 8.08, 1H, amide-NH,); 8.14-8.8.16 (d, J = 8.00 Hz 1H, amide-NH), 9.15 (bs, 2H, Tyr –OH). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C, TMS) δ(ppm) = 28.28, 29.34, 29.68, 37.07, 37.63, 40.19, 52.35, 53.46, 60.40, 60.98, 115.71, 127.14, 127.95, 130.31, 130.46, 155.24, 155.59, 161.50,

171.22, 171.59. HRMS: calculated $[M+Na]^+$ = 593.2046, found 593.2044.

Supplementary Scheme 1

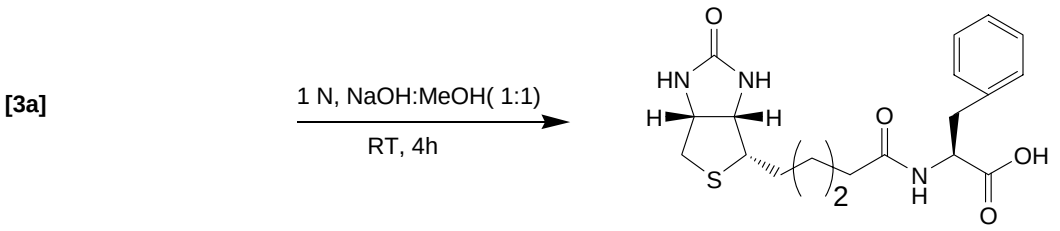



1. EDC.HCl, DMAP, DMF
2. Trp-Trp-OMe. HCl
N2, 6-8h
[2]
[1]
1 N, NaOH:MeOH(1:1)
RT, 4h
[2a]
1. t-Butanol, (Boc)2O
2.NaOH, Water
RT, 12h
1. HOBT, DCC, 0 OC, 1h,
DMF/DCM
2. PheOMe.HCl, Et3N
N2, Overnight, RT
75%TFA/DCM
1h, RT

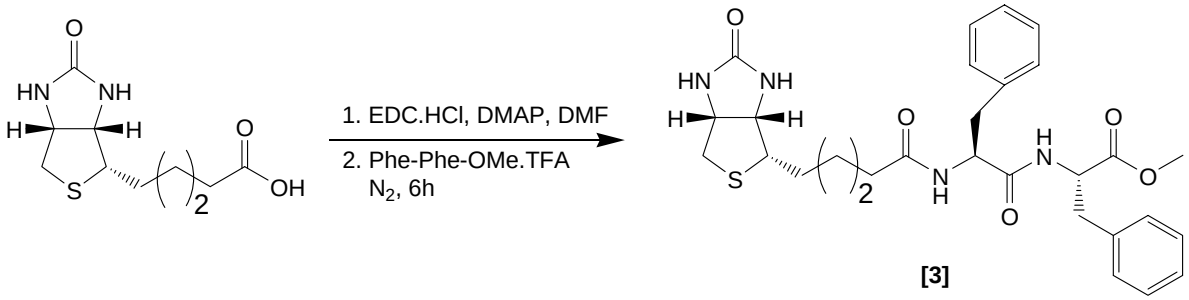

1. EDC.HCl, DMAP, DMF
2. Phe-OMe. HCl
N2, 6h

[3a]

[3a]

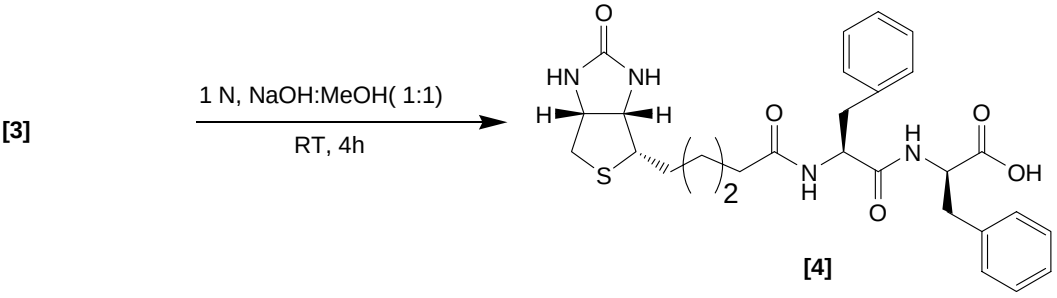

1 N, NaOH:MeOH(1:1)
RT, 4h

[4a]

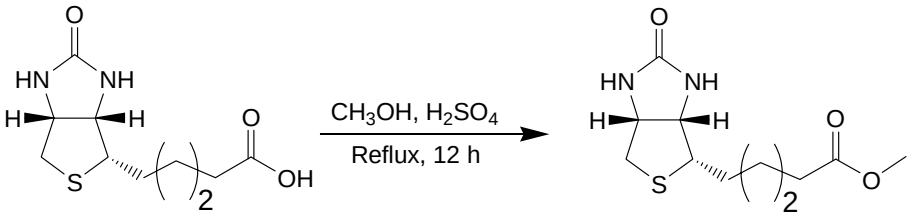

1. EDC.HCl, DMAP, DMF
2. Phe-Phe-OMe.TFA
N2, 6h

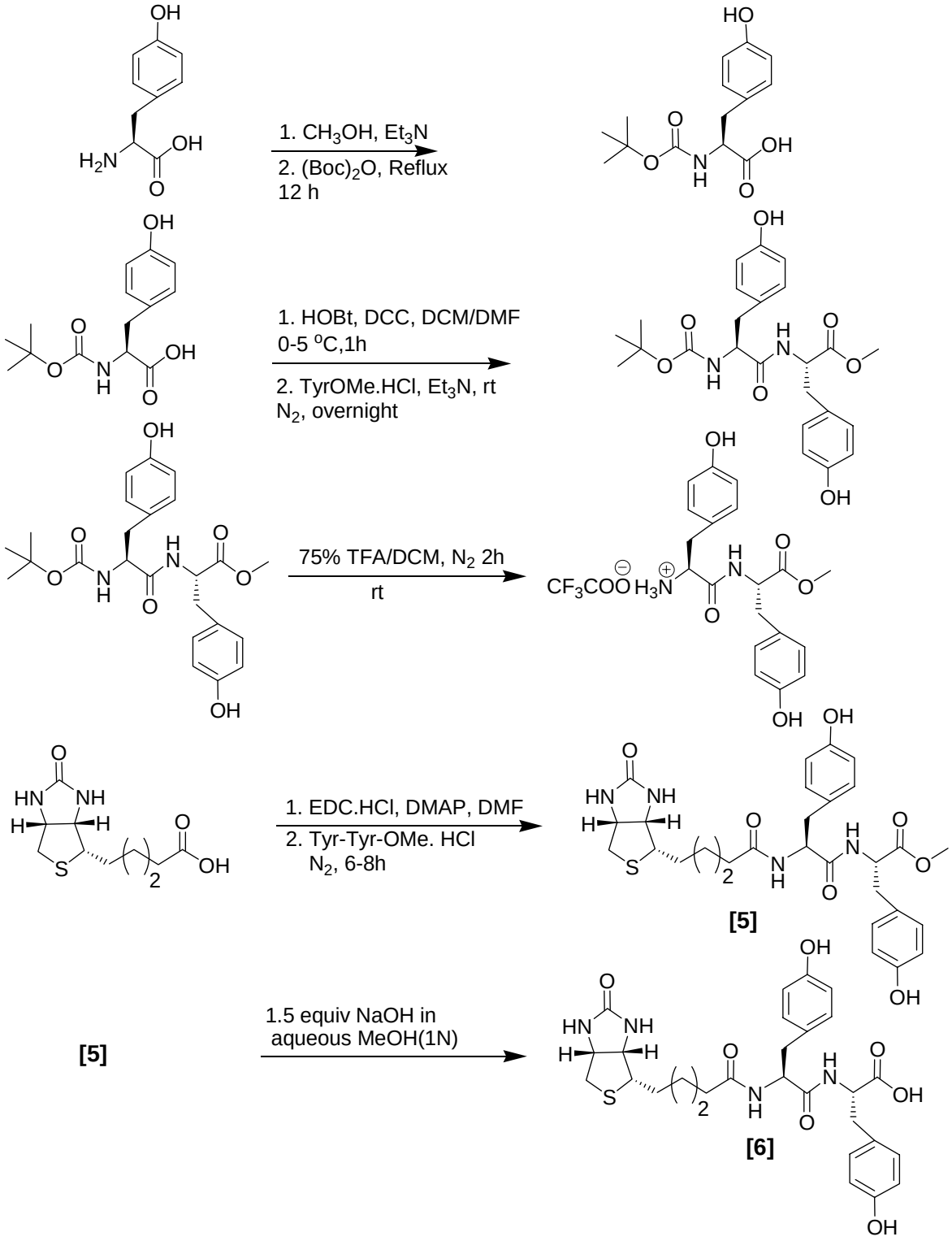
[3]

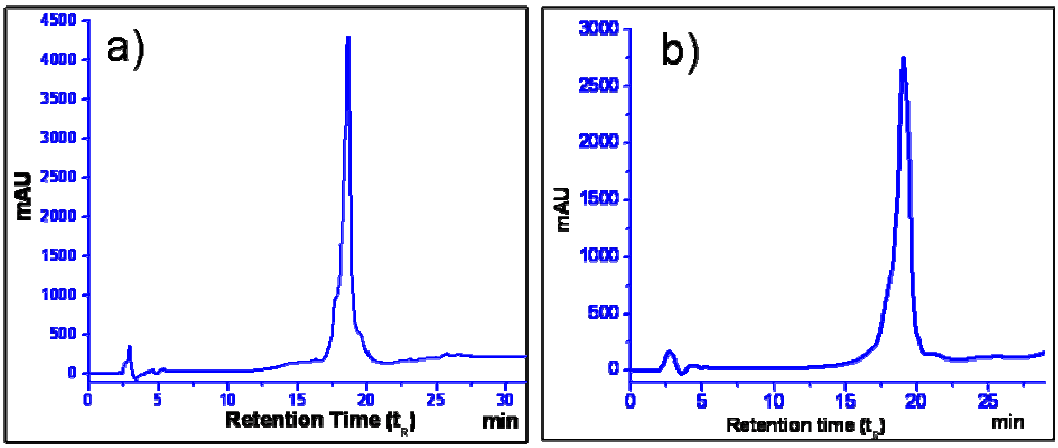
[3]


1 N, NaOH:MeOH(1:1)
RT, 4h

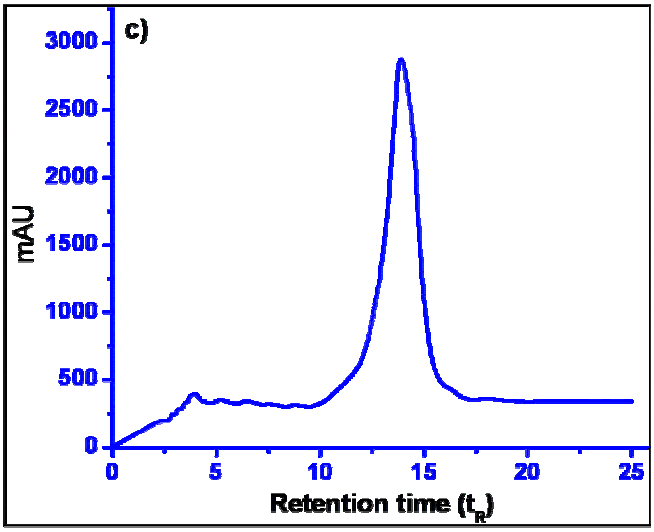
[4]


CH3OH, H2SO4
Reflux, 12 h

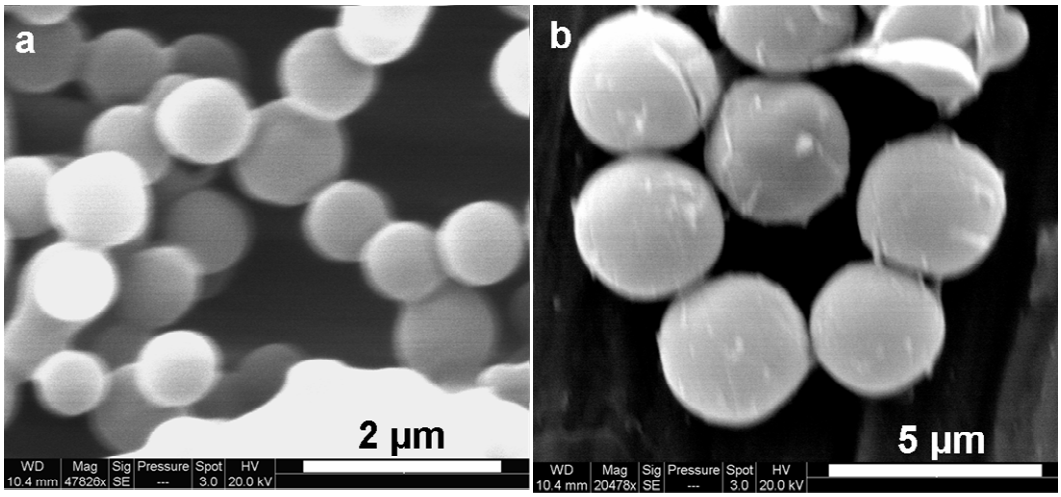

1. CH3OH, Et3N
2. (Boc)2O, Reflux
12 h
1. HOBt, DCC, DCM/DMF
0-5 °C,1h
2. TyrOMe.HCl, Et3N, rt
N2, overnight
75% TFA/DCM, N2 2h
rt
1. EDC.HCl, DMAP, DMF
2. Tyr-Tyr-OMe. HCl
N2, 6-8h
[5]
1.5 equiv NaOH in
aqueous MeOH(1N)
[5]
[6]



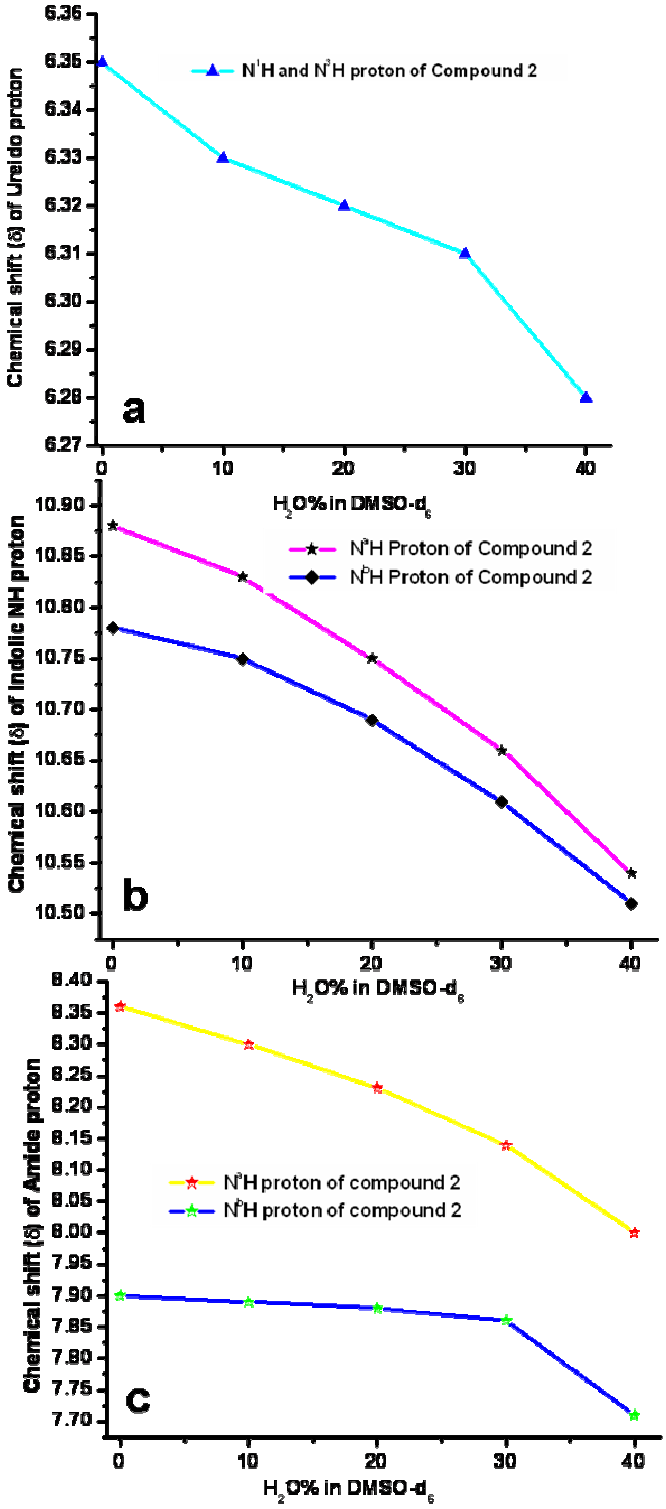
Supplementary figure 1 FPLC Chromatogram. **a**, compound **1. b**, compound **3.** eluent A: 0.1% TFA / H_2O, eluent B: 0.1% TFA / acetonitrile; Flow rate: 0.5 ml /min. linear gradient was 20-100% B and sample concentration was 1mg/ml.



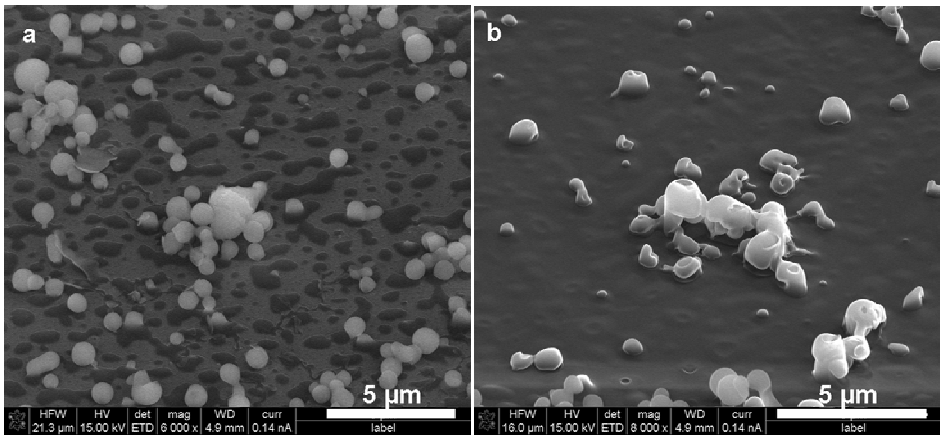
Supplementary figure 2 FPLC Chromatogram. **c**, compound **5.** eluent A: 0.1% TFA / H_2O, eluent B: 0.1% TFA / acetonitrile; Flow rate: 0.5 ml /min. linear gradient was 10-100% B and sample concentration was 1mg/ml.



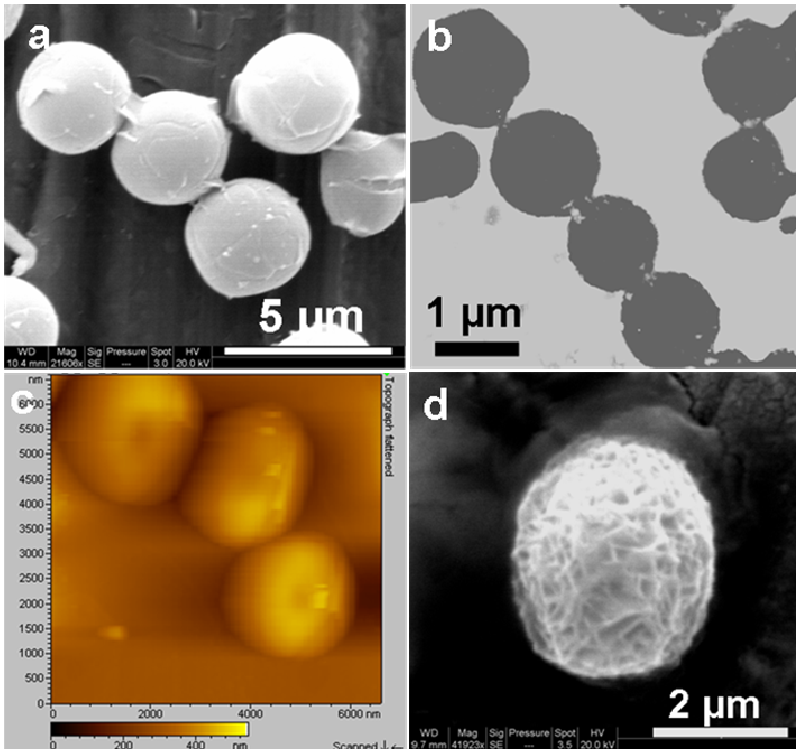
Supplementary figure 3 SEM micrograph of 12 days aged 1 mM solution of **a**, compound **1** and **b**, compound **2a**



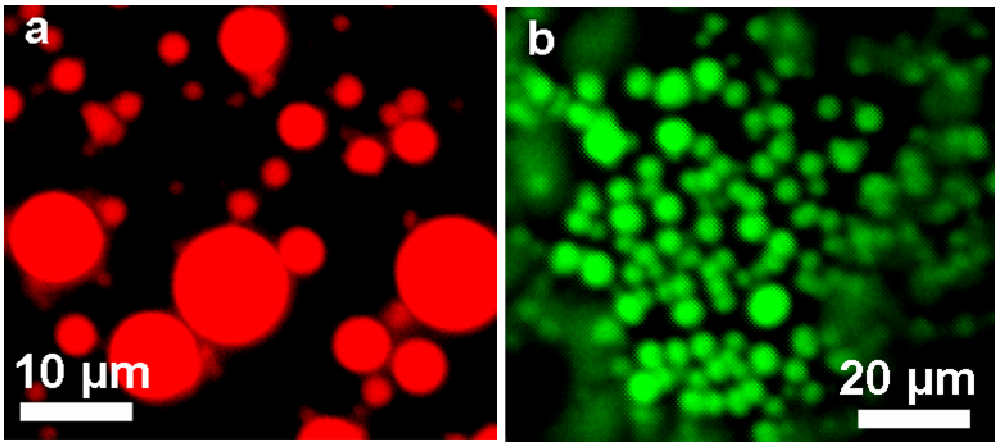
Supplementary figure 4 The chemical shifts changes in the protons of compound **2**. **a**, ureido. **b**, indolic and **c**, amido moieties in solution containing various ratios (v/v) of DMSO-d_6 and H_2O, concentration was 7.5 mg/mL.



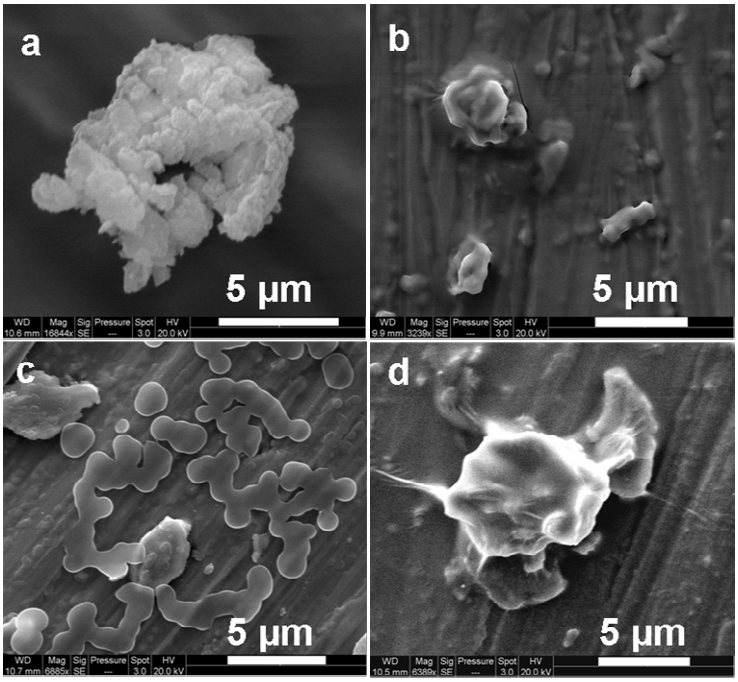
Supplementary figure 5 Focused ion beam (FIB) images of compound **1** on HOPG surface before and after irradiation at 20 kV with current 2.1 nA for 4 min.



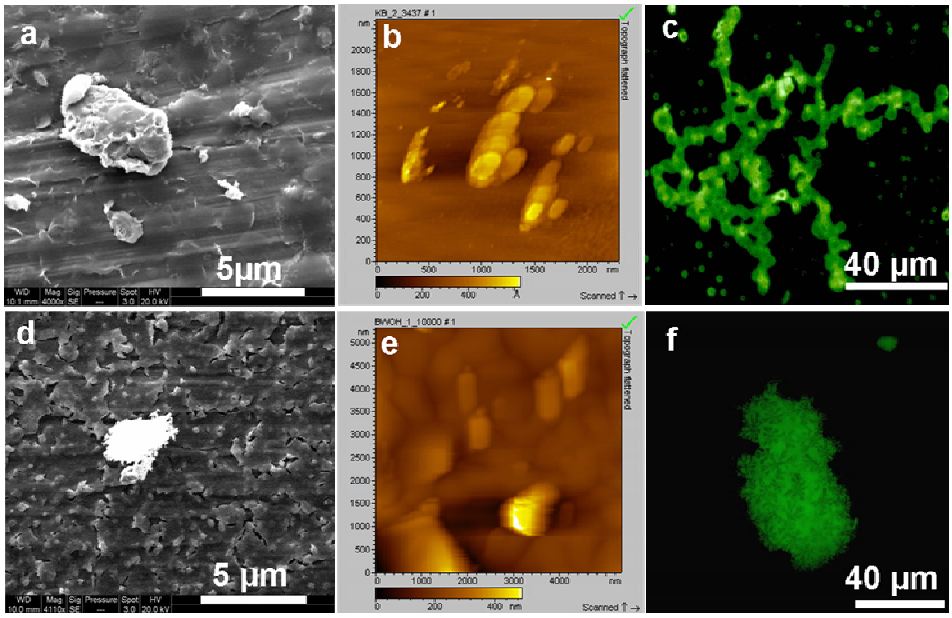
Supplementary figure 6 Fresh 1 mM solution of compound **2a**. **a**, SEM micrograph. **b**, TEM micrograph **c**, AFM micrograph and **d**, disrupted microsphere of compound **2** upon addition of sodium bicarbonate.



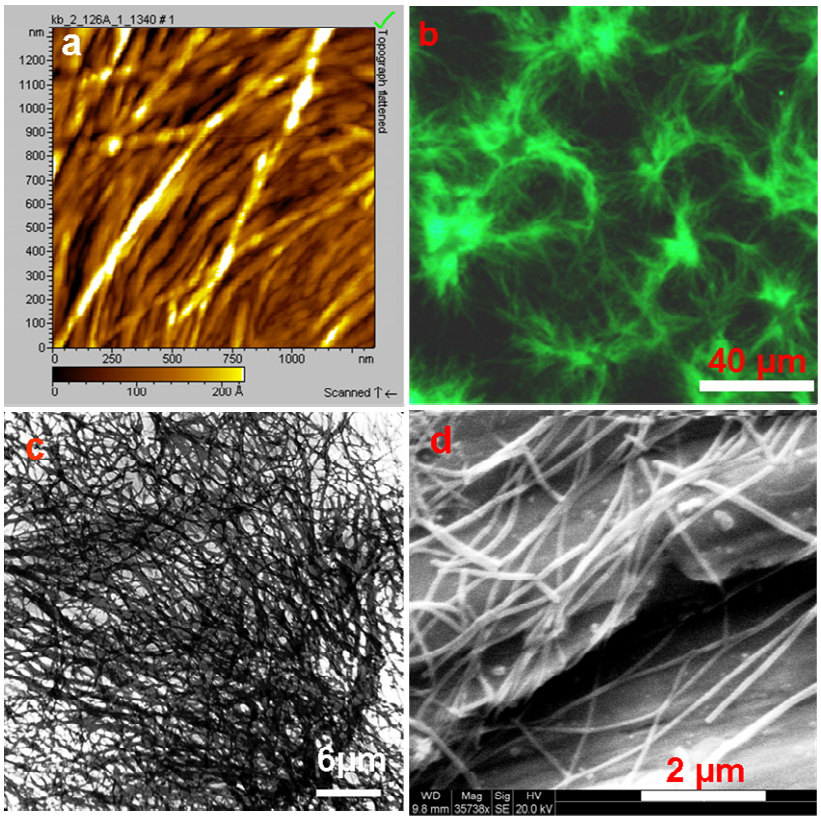
Supplementary figure 7 Fluorescent micrographs of adsorbed **a,** Rhodamine B and **b,** Fluorescein dye from microsphere of fresh sample of compound **2a.** Uniform, bright red and green circular structures indicative of adsorbed dye from compound **2a.**



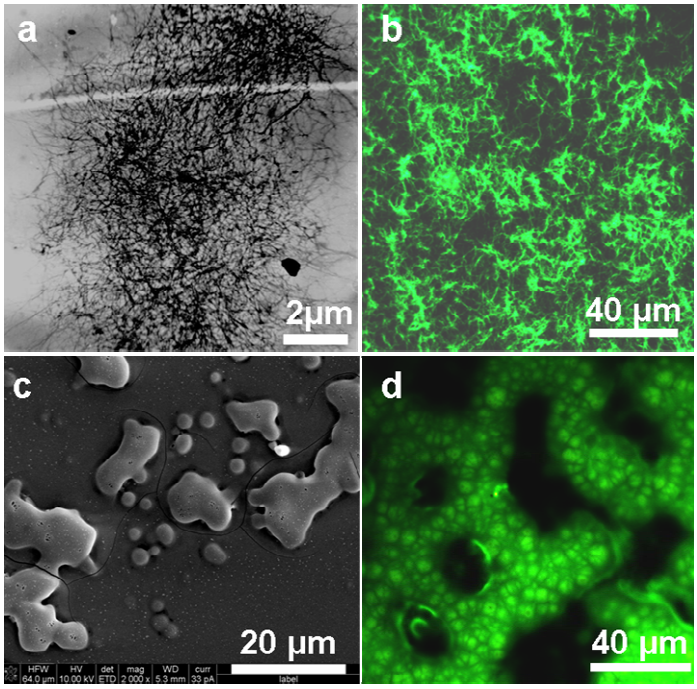
Supplementary figure 8 SEM micrograph of **a,** co-incubation of D-Biotin with NH_2-Trp-Trp-OH. **b,** co-incubation of D-Biotin with NH_2-Trp-OH. **c,** co-incubation of D-Biotin with NH_2-Trp-OCH_3 and **d,** NH_2-Trp-Trp-OH.



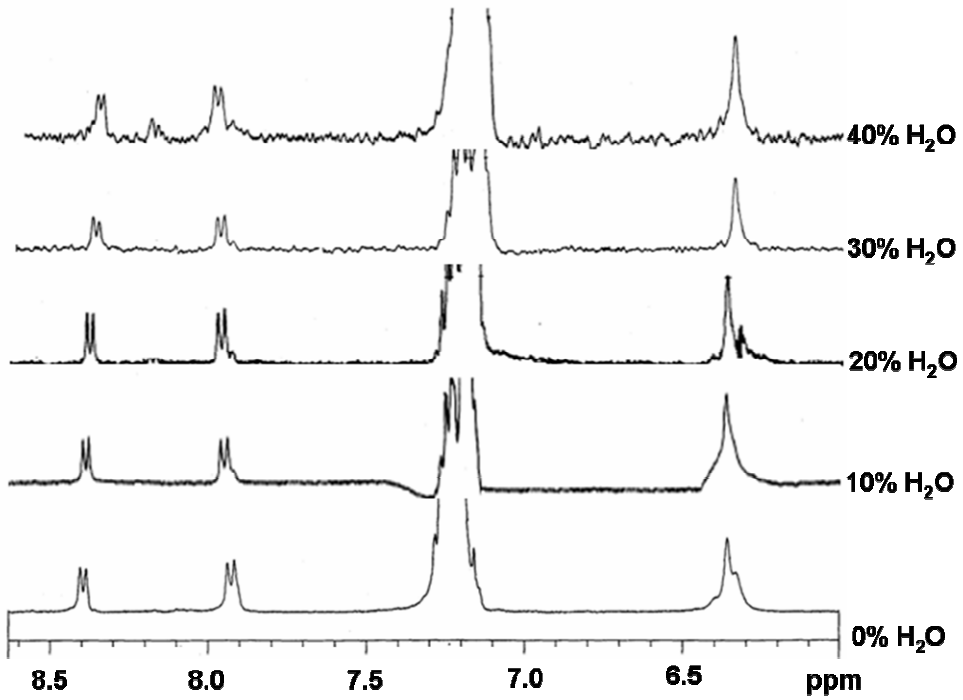
Supplementary figure 9 Microscopic images of **a**, D-Biotin-Trp-OH (SEM) **b**, AFM micrograph of D-Biotin-Trp-OH, **c**, Fluorescent micrograph of D-Biotin-Trp-OH; **d**, SEM micrograph of D-Biotin-Trp-OCH_3, **e**, AFM micrograph of D-Biotin-Trp-OCH_3 and **f**, Fluorescent micrograph of D-Biotin-Trp-OCH_3.



Supplementary figure 10 Microscopic images of Biotinylated di-phenylalnine methyl ester (**3**). **a,** AFM micrograph of 1 mM fresh sample of **3** in 50% aqueous methanol on mica surface. **b,** Optical fluorescent micrographs showing interaction of Fluorescein dye with fibers of compound **3. c,** Transmission electron micrograph of fresh sample of compound **3. d,** SEM micrograph of compound **3** on copper surface.



Supplementary figure 11 Microscopic images of Biotinylated di-phenylalanine (**4**), Biotinylated di-tyrosine methyl ester (**5**) and Biotinylated di-tyrosine (**6**). **a,** TEM micrograph of 1 mM fresh sample of **4** in 50% aqueous methanol. **b,** fluorescent micrograph of compound **4**. **c,** SEM micrograph of fresh sample of compound **5. d,** Optical fluorescent micrographs of 5 hours aged sample of compound **6.**



Supplementary figure 12 The proton NMR spectra of compound **3** in various ratios of DMSO-d_6 and H_2O (v/v), not showing any changes in chemical shift of ureido, aromatic and amido protons.