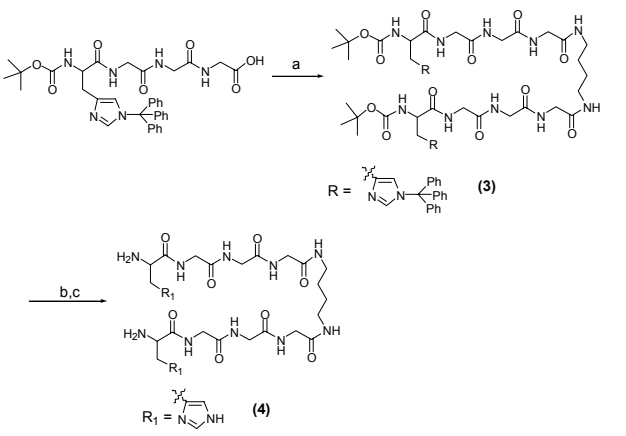
††**Supporting information**



Reagents and reaction conditions: a) HOBt, DCC, 1,4-diamino butane, 20 h,DMF-DCM, yield 53%; b) 95% TFA in DCM, 3 h, RT, yield 70%, c) strong anion exchange resin

Scheme 1. Synthesis of $(HGGG)_2$ DAB

N,N'-bis-(t-butoxycoarbonyl-L-prolyl-N^{im}(trityl)-L-histidyl-glycyl-glycyl-glycine)-1,4-diaminobutane (1): HOBt (0.648 g, 4.8 m moles, 1 eq) was added to a pre-cooled solution of *t*-butoxycarbonyl-L-prolyl-N^{im}(tirtyl)-L-histidyl-glycyl-glycyl-glycine (3.66 g, 4.8 mmol, 1 eq) in anhydrous dichloromethane (55 mL). DCC (1.09 g, 5.28 mmol, 1.1 eq) in dry DCM (1 mL) was added drop-wise to the peptide solution with constant stirring. Stirring was continued for 10 min at 0˚C and then for 50 min at room temperature. After this time, 1,4-diaminobutane (0.24 mL, 2.35 mmol, 0.5 eq) in dry DCM (0.2 mL) was drop-wise added at room temperature with constant stirring and it was continued for 20 h. The precipitated dicyclohexylurea was filtered off after this time and washed with dichloromethane (4 x 5 mL). Combined organic layers were washed with 2N HCl (3 x 40 mL), 10% $NaHCO_3$ (3 x 40 mL) and finally with saturated brine solution and dried over anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure and the crude product was purified by silica gel column using dichloromethane/methanol (93:7) to afford pure. [R_f 0.6 (6% methanol in DCM), Yield 61% (2.3 g), m.p.=141-143˚ C, $[\alpha]_D^{25}$ = -28º (c=1 in methanol, 25º C), FAB MS (M+3)=1585]. ^{1}H NMR: (400 MHz, [D]$CHCl_3$, 25˚C, TMS) δ = 1.16 (s, 18 H, ^{t}Boc); 1.44 (br s, 4H, Linker's –CH_2CH_2); 1.93 (m, 4 H, Pro γ H); 2.24 (appeared as br s, 4 H, Pro β H); 2.84-2.886 (dd, *J(H,H)*= 4.8 Hz and 10.2 Hz, 2 H, His β H); 3.06-3.13 (br dd, 6 H, overlapping signals for His β H and Linkers –CH_2N); 3.41-3.45 (m, 4 H, overlapping signal for Pro δ H and Gly –CH_2); 3.53-3.58 (dd, *J(H,H)* = 6.4 Hz and 11.6 Hz, 2 H, Pro δ H); 3.69-3.84 (m, 6 H, overlapping signals of Gly CH_2); 3.90-3.96 (dd, *J(H,H)* = 5.2 Hz and 17.2 Hz, 2 H, Gly –CH_2); 4.05-4.08 (dd, *J(H,H)* = 4.8 Hz and 8.0 Hz, 2 H, Pro α H); 4.13-4.19 (dd, *J(H,H)* = 7.2 Hz and 17.2 Hz, 2 H, Gly –CH_2); 4.46-4.50 (q, *J(H,H)* = 5.2 Hz, 2 H, His α H); 6.56 (s, 2 H, His ring's H); 6.97 (br d, 12 H, Ar C-H); 7.24-7.29 (m, 24 H, overlapping signals for Ar C-H, His ring's C-H, and –NH); 7.39 (br s, 2 H, -NH); 7.70 (br s, 4 H, -NH); 9.04 (d, *J(H,H)* = 6.6 Hz, 2 H, -NH). The ^{13}C (100 MHz, [D] $CHCl_3$, 25˚C, TMS) δ = 24.72, 26.31, 27.73, 28.36, 30.2, 38.92, 43.14, 43.19, 43.32, 47.24, 54.37, 61.88, 75.59, 81.22, 120.53, 128.16, 128.37, 129.66, 136.15, 138.24, 141.90, 156.36, 169.29, 169.95, 170.37, 172.53, 174.79.

N,N'-bis-(L-prolyl-L-histidyl-glycyl-glycyl-glycine)-1, 4-diaminobutane **(2):** A mixture of trifluoroacetic acid (95%) in dichloromethane (12 mL) was added to conjugate 1 (2.02 g, 1.3 mmol), at room temperature with constant stirring under calcium filled guard-tube protection. Stirring was continued for 3 h at room temperature. After this time, the solvent was evaporated under reduced pressure and resulting residue was triturated with diethyl ether. The white solid so formed was washed with dichloromethane to yield the peptide (1.54 g) as its trifluoroacetate salt. This was passed through strong anion exchange resin (Dowex 1-X8, J. T. Baker, USA) and eluted with 50% aqueous methanol, followed by recrystallization from cold isopropyl alcohol and diethyl ether to give pure 1. [R_f (methanol:acetic acid:water= 1:0.25:0.42) = 0.35, Yield = 80%, m.p.=193-195˚C, $[\alpha]_D^{25}$ = -18º (c = 1 in methanol, 25ºC), FAB MS (M+2)= 900]. ^{1}H NMR (400 MHz, [D]H_2O, 25˚C, TMS) δ = 1.35 (br s, 4H, Linker's –CH_2CH_2); 1.47-1.63 (m, 6H, Pro γ, β H); 1.95 (m, 2H, Pro β H); 2.72-2.84 (m, 4H, His β H); 2.90-3.06 (m, 6H, overlapping signals for linker's –CH_2NH and Pro δ H); 3.23 (appeared as br t, 2H, Pro δ H); 3.63-3.67 (dd, J(H, H) = 5.2 Hz and 8.4 Hz, 2H, His α H); 3.76 (br s, 4H, Gly –CH_2); 3.84 (d, *J*(H, H) = 5.2 Hz, 4H, Gly –CH_2); 3.869 (br s, 4H, Gly –CH_2); 4.47-4.51 (dd, J(H, H) = 6.4 Hz and 8 Hz, 2H, Pro α H); 6.87 (s, 2H, His ring's H); 7.56 (s, 2H, His ring's H). The ^{13}C (100 MHz, [D]H_2O, 25˚C) δ = 25.68, 26.43, 30.17, 38.20, 41.98, 42.19, 42.59, 46.48, 51.85, 60.08, 127.52, 127.25, 135.25, 168.46, 169.81, 171.49, 174.18.

N,N'-bis-[N^{im}(trityl),N$^{\alpha}$(tert-butoxycarbonyl)-L-histidyl-glycyl-glycylglycine]1,4-diaminobutane **(3)**: *N^{im}(trityl),N$^{\alpha}$(tert-butoxycarbonyl)-L-histidyl-glycyl-glycyl-glycine* (2.77 g, 4.1 mmol) was dissolved in dry DCM and DMF mixture (45 mL DCM, 17 mL DMF) and cooled to 0ºC. To this HOBt (0.56 g, 4.1 mmol), DCC (0.85 g, 4.1 mmol) in DCM (1 mL) were added dropwise, with vigorous stirring. Stirring was continued for 50 min at 0º C. After this time, 1,4-diaminobutane (0.20 mL, 2 mmol) in DCM (0.5 mL) was added dropwise. Then the reaction was stirred for 20 h at room temperature. The reaction mixture was filtered and the solvent was evaporated under pressure. The residue was redissolved in DCM and washed with 10% sodium bicarbonate, with 2N HCl, and finally with brine solution. The DCM layer was dried over anhydrous Na_2SO_4 and the organic layer was evaporated. The crude compound was purified over silica gel column chromatography by using methanol and DCM system (14:86) to give 1.5 g of pure compound [Yield 52%, R_f (8% methanol in DCM) = 0.5, m.p. 186-188˚C]. ^{1}H NMR (400 MHz, [D] $CHCl_3$, 25˚C, TMS) δ (ppm) = 1.38 (s, 18 H, t-Boc H); 1.51 (br s, 4 H, linker's –CH_2CH_2); 3.03-3.19 (m, 8 H, overlapping signals for linker's CH_2NH- and His □ H); 3.80-4.01 (m, 12 H, overlapping signals for Gly –CH_2); 4.37 (br s, 2 H, His □ H); 5.73 (br s, 2 H, t-Boc-NH); 6.65 (s, 2 H, His ring's H); 7.082-7.10 (m, 12 H, 12 H, trityl H); 7.33-7.35 (m, 22 H, overlapping signals for trityl H, His ring's H and –NH); 7.59 (br s, 2 H, -NH); 7.79 (br s, 2 H, -NH). ^{13}C NMR (100 MHz, [D] $CHCl_3$, 25˚C, TMS) δ (ppm) = 26.05, 28.30, 30.34, 39.02, 42.90, 43.42, 43.78, 54.34, 75.53, 80.15, 120.70, 128.17, 128.24, 129.61, 135.52, 138.61, 141.96, 155.66, 169.52, 170.02, 171.43, 173.04. FAB MS (M+2) = 1390.

N,N'-bis-(L-histidyl-glycyl-glycyl-glycine)1,4-diaminobutane **(4)**: Compound **3** (0.745 g, 0.54 mmol), was treated with 95% TFA in DCM (3 mL) at room temperature for 2h. Solvent was evaporated and the residue was triturated with diethyl ether (10 mL). The resulting white solid was dissolved in minimum amount of methanol and reprecipitated with dropwise addition of diethyl ether. This compound was redissolved in 50% aq. methanol and passed through strong anion exchange resin. The column was washed with 50% aq. methanol and the solvent was evaporated. The compound was washed with methanol and dried in vacuum to give 0.242 g of pure compound. [Yield = 64%, R_f (methanol:acetic acid:water; 1:0.25:0.42) = 0.25; m.p. 195-197˚C (Dec)]. ^{1}H NMR (400 MHz, [D] H_2O, 25˚C, TMS) δ (ppm) = 1.33 (br s, 4 H, linker's –CH_2CH_2); 2.79-2.82 (appeared as two br d, 4 H, His □ H); 3.58 (t, J (H,H)= 6.4 Hz, 4 H, His □ H); 3.75 (s, 4H, Gly –CH_2); 3.813 (s, 4H, Gly –CH_2); 3.85 (s, 4 H, Gly –CH_2); 6.83 (s, 2 H, His ring's H); 7.54 (s, 2H, His ring's H). ^{13}C NMR (100 MHz, [D] H_2O, 25˚C, TMS) δ (ppm) = 26.38, 32.61, 39.66, 43.25, 43.26, 43.29, 55.34, 118.03, 128.98, 136.83, 171.802, 172.78, 173.14, 178.4. FAB MS (M+2) = 706.

Transmission electron microscopy: A solution of **2** and $(HGGG)_2$DAB (100 μL, 1 mM) in 50% aq. trifluoroethanol was aged for 7 days. This solution (100 μL) was concentrated to remove trifluoroethanol and triply distilled water (100 μL) was added to this residue, followed by sonication (TPC-25, Telsonic Technologies) for 15 seconds. 8 μL of this solution was transferred onto Formvar (Fluka, Switzerland) coated copper grids (SPI supplies, West Chester, USA, 200 mesh) and dried. These grids were negatively stained with 2% uranyl acetate, dried and subsequently examined under JEOL 2000FX-II electron microscope, at an operating voltage of 100 kv.

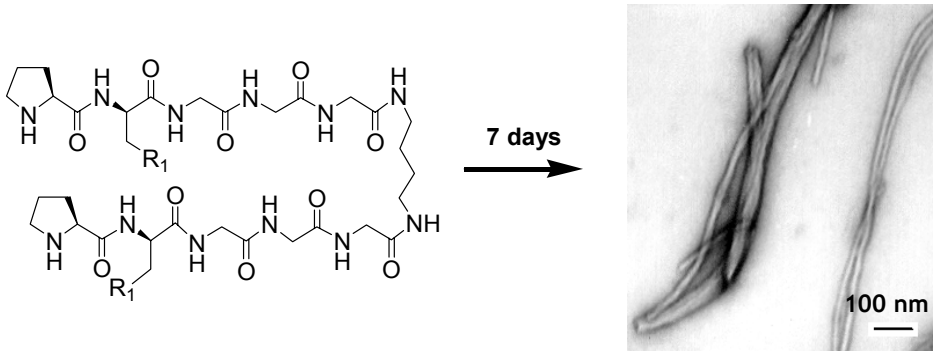
Optical microscopy: Congo red (3 μM dye in 100 mM NaCl) was added to an aged solution of **2** and the mixture was left for 6 h at room temperature. Then 50 μL of this solution was transferred on to glass slide and dried, then viewed under optical microscopy (AX10 Lab, Zeiss) with cross-polarized light (500x), which is interfaced with a PC. Images were obtained and processed by using Image-Pro Plus software.

Atomic force microscopy: The samples were imaged with an atomic force microscope (Molecular Imaging, USA), operated in Acoustic AC mode (AAC) with the aid of cantilever (NSC12(c), MikroMasch). The force constant was 0.6 N/m, while the resonant frequency used was 150 kHz. The images were taken in air at room temperature, with a scan speed of 1.5-2.2 lines/sec. The data acquisition was performed by PicoScan 5 software and the analysis was done with the aid of visual SPM. 10 μL of 7 days aged solution of **2** (1 mM) was transferred to a freshly cleaved mica piece, followed by uniform spreading of the sample with the aid of a spin-coater operating at 200-500 rpm (WS-400M, Laurell Technologies). Mica piece was dried for 30 min at room temperature, followed by AFM imaging.

TEM images of L-prolyl-L-histidyl-glycyl-glycyl-glycine methyl ester trifluoroacetate*:* 100 μL of seven days aged solution of the parent pentapeptide (1 mM) in 50% of aqueous trifluoroethanol was concentrated to remove TFE. To this, 100 μL of deionized distilled water was added and sonicated the mixture for 30 sec. Then this solution was transferred on to formvar coated copper grid, stained with 2% uranyl acetate and dried. The grid was studied under transmission electron microscope operating at 100 kv. The observed fibril morphology was different in terms of the diameter, from the aged solution of *bis*-pentapeptide **2**. The observed diameter was nearly 150 nm as shown in the figure 1. No fibrillar bundles and network were observed.

Figure 1. TEM image of 7 days aged solution of PHGGG-Ome

Self-Aggregation of Reverse Bis Peptide Conjugate Derived from the Unstructured Region of the Prion Protein



*C. Madhavaiah and Sandeep Verma**