Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

Single-stranded DNA Detection by Solvent-Induced Assemblies of a Metallo-Peptide-Based Complex

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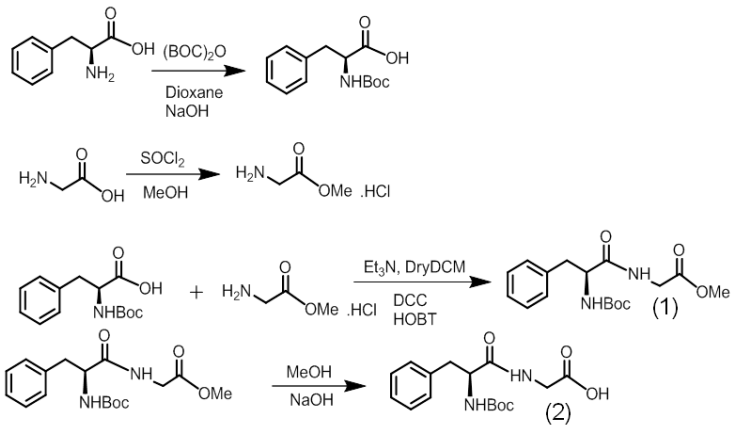
Experimental Section

Peptides synthesis: Peptides were synthesised by conventional solution-phase methods. Peptide coupling was mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The products were purified by column chromatography using silica gel (100–200 mesh) as the stationary phase and an n-hexane–ethyl acetate mixture as an eluent. The final compounds were fully characterised by Bruker 500 MHz 1H-NMR spectroscopy, and mass spectroscopy (Applied Biosystems Voyager-DE Pro MALDI-TOF and Accela Autosampler, Thermo Scientific (CCQ Fleet)).

Synthesis of BOC Phe-OH: A solution of L-phenylalanine (3.30 g, 20 mmol) in a mixture of dioxane (40 mL), water (20 mL) and 1 M NaOH (20 mL) was stirred and cooled in an ice-water bath. Di-tertbutylpyrocarbonate (4.583 g, 21 mmol) was added and stirring continued at room temperature (RT) for 6h. Then the solution was concentrated in vacuum to about 10–15 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 50 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (determined by Congo red). The aqueous phase was extracted with ethyl acetate and this was done repeatedly. The ethyl acetate extracts were pooled, washed with water, dried over anhydrous Na_2SO_4 and evaporated in a vacuum. The pure material was obtained as a waxy solid. Yield 4.45 g (16.8 mmol, 84.0%) (Scheme S1).



Scheme S1: Synthetic methodologies adopted for the synthesis of **1** and **2**.

Synthesis of NH_2-Gly-OMe Hydrochloride: 4.5g (60 mmol) of L-glycine was dissolved in 90 mL of MeOH and cooled in an ice bath. Then, 12 ml of $SOCl_2$ was added dropwise and stirred for 8h. The excess solvent was evaporated under rotary vacuum. The dried crystalline solid product obtained was L-glycine methyl ester hydrochloride. Yield 6.30 g (50.4 mmol, 85.0%) (Scheme S1).

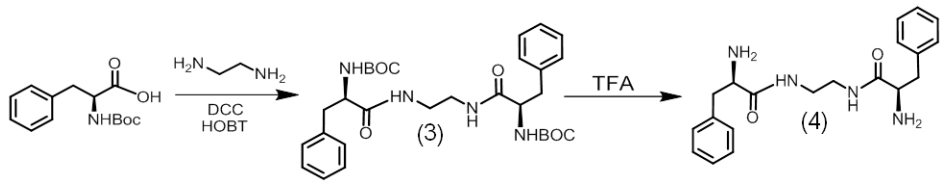
Synthesis of BOC-Phe-Gly-OMe (1): 4.0 g (15 mmol) of Boc-Phe-OH was dissolved in 40 ml dry DCM in an ice-water bath. H-gly-OMe.Hcl 2.507 g (20.0 mmol) and Et_3N 4 ml, 30 mmol) were then added to the reaction mixture, followed immediately by the addition of 3.30 g (16.0 mmol) dicyclohexylcarbodiimide (DCC) and 2.16 g (16.0 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and was stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL). The dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL) and brine (2 X 50 mL) and finally dried over anhydrous sodium sulfate. It was then evaporated under vacuum to yield Boc-Phe-Gly-OMe as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (3 : 1) as eluent. Yield: 3.65 g (10.85 mmol, 72.35%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.31-7.28 (m, 2H, ArH of Phe), 7.23-7.20 (m, 3H, ArH of Phe), 6.52 (b, 1H, NH Phe), 5.05 (b, 1H, NH Gly), 4.42-4.41 (m, 1H, C_αH, Phe) 4.07-3.91 (dd, 2H, -CH_2-Gly), 3.73 (s, 3H, OMe), 3.14-3.05 (m, 2H, C_βH, Phe) 1.39 (s, 9H, Boc). ESI-MS (m/z): [M]=336.38 (calculated); 336.59 (observed), $[M+Na+H]^+$=360.38 (calculated); 360.11 (observed); $[M+K+H]^+$ = 376.38 (calculated); 376.29 (observed), $[M+2Na]^+$=382.38 (calculated); 381.21 (observed) (Scheme S1).

Synthesis of BOC-Phe-Gly-OH (2): To 3.0 g (8.91 mmol) of Boc-Phe-Gly-OMe, 30 mL MeOH and 2M 15 mL NaOH were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After10 h, the methanol was removed under vacuum; the residue was dissolved in 50 mL of water and washed with diethyl ether (2 X 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1M HCl and extracted with ethyl acetate (3 X 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate and evaporated under vacuum to obtain the compound as a waxy solid. Yield: 2.72 g (8.46 mmol, 95%). ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 12.56 (s, 1H, COOH), 8.23 (t, 1H, NH Gly), 7.27-

7.16 (m, 5H, ArH of Phe), 6.89 (d, 1H, NH Phe), 4.23-4.17 (m, 1H, $C_{\alpha}H$, Phe) 3.86-3.72 (m, 2H, -CH_2-Gly), 3.03-2.69 (m, $C_{\beta}H$, Phe) 1.28 (s, 9H, Boc). ESI-MS (m/z): $[M+Na+H]^+$=346.35 (calculated); 346.42 (observed); $[M+K+H]^+$ = 362.35 (calculated); 362.37 (observed) (Scheme S1).

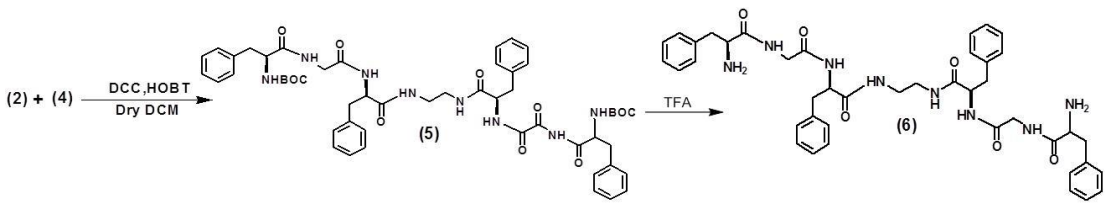
Synthesis of BOC-Phe-NH-CH_2-CH_2-NH-Phe-Boc (3) and NH_2-Phe-NH-CH_2-CH_2-NH-Phe-NH_2 (4): 3.0 g (11.27 mmol) of Boc-Phe-OH were dissolved in 40 ml dry DCM in an ice-water bath. Ethylenediamine (340 mg) (5.63 mmol) was then added to the reaction mixture, followed immediately by the addition of 2.79 g (13.5 mmol) dicyclohexylcarbodiimide (DCC) and 1.82 g (13.5 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and was stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL) and the dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL) brine (2 X 50 mL) and dried over anhydrous sodium sulfate; finally it was evaporated under vacuum to yield **BOC-Phe-NH-CH_2-CH_2-NH-Phe-Boc (3)** as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (4: 1) as eluent. Yield: 2.04 g (3.61 mmol, 64.18%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.34-7.30 (m, 3H, ArH of Phe), 7.19-7.17 (m, 2H, ArH of Phe), 5.77 (b, 1H, NH Phe), 5.10 (b, 1H, NH Ethelynediamine), 4.77-4.11 (m, 1H, $C_{\alpha}H$, Phe) 3.18-3.16 (m, 2H, -CH_2-Ethylenediamine), 3.02-2.93 (m, 2H, $C_{\beta}H$ Phe) 1.41 (s, 9H, Boc). ESI-MS (m/z): $[M+Na+2H]^+$=579.67 (calculated); 579.74 (observed); $[M+K+H]^+$ = 595.67 (calculated); 596.10 (observed).

Next, 2 g (3.60 mmol) of compound **3** were dissolved in 25 mL of DCM in an ice bath. Then, 6 mL of TFA were added and stirred for 2h. The progress of the reaction was monitored by TLC. After the reaction was completed, all solvents were evaporated in a rotary evaporator. The product was then dissolved in water, neutralized with NaHCO3 solution, extracted with ethyl acetate, dried over anhydrous sodium sulphate and evaporated by rotary evaporator to obtain an oily product **4**, which was immediately used for the next reaction. Yield: 1.194 g (3.36 mmol, 93.6%). ESI-MS (m/z): $[M+Na+2H]^+$=379.44 (calculated); 379.93 (observed); $[M+2Na]^+$ = 400.44 (calculated); 400.25 (observed) (Scheme S2).



Scheme S2: Synthetic methodologies adopted for the synthesis of **3** and **4**.

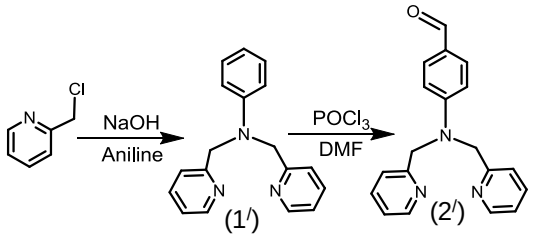
Synthesis of BOC-Phe-Gly-Phe-NH-CH_2-CH_2-NH-Phe-Gly-Phe-Boc (5) and NH2-Phe-Gly-Phe-NH-CH_2-CH_2-NH-Phe-Gly-Phe-NH_2 (6): 2.554 g (7.90 mmol) of Boc-Phe-Gly-OH were dissolved in 30 ml dry DCM in an ice-water bath. Compound **4** (1.274 g 3.6 mmol) was then added to the reaction mixture, followed immediately by the addition of 1.96 g (9.48 mmol) dicyclohexylcarbodiimide (DCC) and 1.28 g (9.48 mmol) of HOBt. The reaction mixture was allowed to warm-up to RT and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (50 mL) and the dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 X 50 mL), brine (2 X 50 mL), 1 M sodium carbonate (3 X 50 mL), brine (2 X 50 mL), dried over anhydrous sodium sulfate and evaporated in a vacuum to yield **BOC-Phe-Gly-Phe-NH-CH_2-CH_2-NH-Phe-Gly-Phe-Boc (5)** as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (4: 1) as eluent. Yield: 2.09 g (2.20 mmol, 62.21%). ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.11-8.05 (m, 2H, NH Phe and Gly), 7.28-7.23 (m, 8H, ArH Phe), 7.22-7.18 (m, 4H, ArH Phe), 6.97 (d, *J*=8.3Hz, 1H, NH Phe), 4.46-4.42 (m, 1H, C_αH, Phe) 4.22-4.17 (m, 1H, C_αH Phe), 3.83-3.78 (dd, 1H, CH_2 Gly), 3.69-3.63 (dd, 1H, CH_2 Gly) 3.09-2.96 (m, 4H, C_βH Phe), 2.87-2.36 (m, 2H, CH_2 EDA), 1.30 (s, 9H, Boc). ESI-MS (m/z): $[M+2H]^+$=965.13 (calculated); 965.61 (observed); $[M+H_3O]^+$=982.13 (calculated); 982.60 (observed); $[M+Na+H]^+$ = 987.14 (calculated); 987.44 (observed) (Scheme S3).



Scheme S3: Synthetic methodologies adopted for the synthesis of **5** and **6**.

Next, 1.5 g (1.58 mmol) of compound **5** was dissolved in 20 mL of DCM in an ice bath. Then, 4 mL of TFA were added and stirred for 2h. The progress of the reaction was monitored by TLC. After the reaction was completed, all solvents were evaporated in a rotary evaporator. The product was then dissolved in water, neutralised with $NaHCO_3$ solution, extracted with ethyl acetate, dried over anhydrous sodium sulphate, and evaporated by a rotary evaporator to obtain an oily product **6**, which was immediately used for the next reaction. Yield: 1.103 g (1.47 mmol, 93.3%). ESI-MS (m/z): [M]=762.89 (calculated); 762.75 (observed); $[M+Na]^+$=785.89 (calculated); 785.80 (observed); $[M+K]^+$ = 801.89 (calculated); 801.62 (observed) (Scheme S3).

Synthesis of Phenyl-bis-pyridin-2ylmethyl-amine (1′): To a solution of 2-chloromethylprydine hydrochloride (2 g, 12 mmol) in H_2O (0.5 ml), aniline (0.558 g, 6 mmol), 5 N NaOH (6 ml) and hexadecytrimethylammonium chloride (20 mg) were added under N_2 protection. The mixture was stirred vigorously for 24h at RT. It was then extracted with CH_2Cl_2, and the extract was washed with H_2O and dried with MgSO4. After the solvent was evaporated, the desired product was obtained as a beige solid via column chromatography (silica, CH_2Cl_2/AcOEt, 4/1, v/v). Yield: 850.6 g (3.08mmol, 51.2%). ^1H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 8.60 (d, *J*= 6.8 Hz, 2H ArH), 7.63 (t, *J*= 7.6 Hz, 2H ArH), 7.28 (d, *J*= 8 Hz, 2H ArH), 7.19-7.15 (m, 4H ArH), 6.74-6.70 (m, 3H ArH), 4.84 (s, 4H -CH2-). ESI-MS (m/z): $[M+ H]^+$=276.14 (calculated); 276.17 (observed) (Scheme S4).

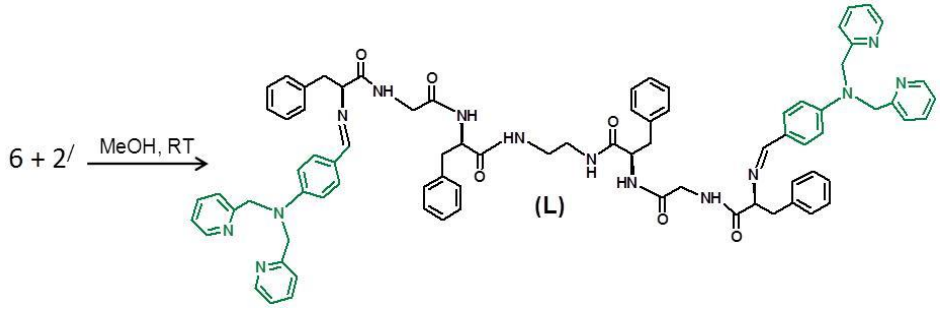


Scheme S4: Synthetic methodologies adopted for the synthesis of **1′**and **2′**.

Synthesis of 4-(Bis-Pyridin-2-ylmeythyl-amino-benzaldehyde (2′): $POCl_3$ (1 ml, 17 mmol) was added to the solution of DMF (2 ml, 26 mmol) in 2 portions within 30

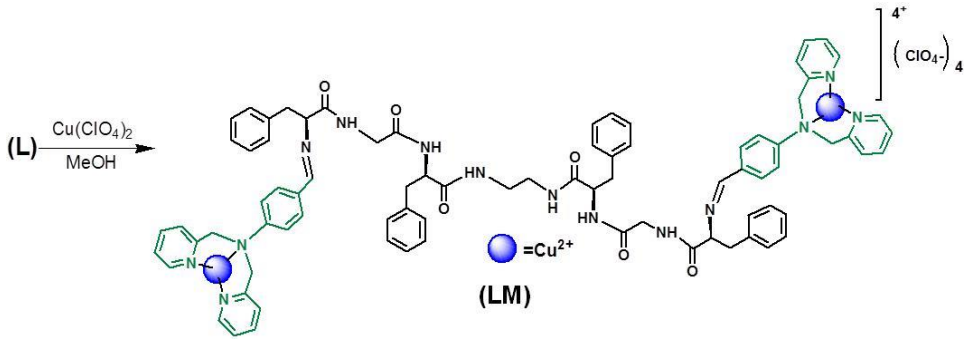
min, and cooled in an ice bath. Then, the solution was stirred for 30 min. Compound **1**/ (0.800 g, 2.89 mmol) in DMF (1.25 ml) was added in portions within 20 min. The mixture was heated for 3 h at 90 ºC, poured into H_2O (5ml), and then neutralized to pH 6-8 with K_2CO_3 along with stirring. The mixture was extracted with CH_2Cl_2, and dried with Na_2SO_4. Via column chromatography (silica, petroleum:acetone, 5:3, v/v), the desired product was obtained as a yellow sticky oil. Yield: 294.3 mg (0.97 mmol, 33.5%). ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 9.75 (s, 1H, -CHO), 8.60 (d, *J*= 5.8 Hz, 2H ArH), 7.68-7.61 (m, 4H ArH), 7.20 (d, *J*= 7.8 Hz, 4H ArH), 6.78 (d, *J*= 8.4 Hz, 2H ArH), 4.89 (s, 4H -CH2-). ESI-MS (m/z): [M]=303.13 (calculated); 303.33 (observed) (Scheme S4).

Synthesis of L: 2/ (200 mg, 0.65 mmol) was added to a solution of **6** (490.2 mg, 0.655 mmol) dissolved in 20 ml of methanol. The resulting mixture was stirred for 8 h. After the reaction was completed (confirmed by TLC), the reaction mixture was filtered off and a light brown oily residue was obtained upon removal of the solvent from the filtrate under vacuum. This oily residue upon treatment with n-hexane yielded a light brownish solid precipitate, which was collected by decantation as well as proper washing and drying to afford **L** as a pure product. Yield: 518.6 mg, 60.5%. 9.04 (d, J= 6.0Hz, 2H ArH of DPA based receptor), 8.59 (s, 1H N=CH), 8.13-8.11(m, 2H, NH Phe and Gly), 7.71-7.66 (m, 14H ArH), 7.22 (d, *J*= 7.8 Hz, 4H ArH), 6.73 (d, *J*= 8.2 Hz, 2H ArH), 5.32 (s, 4H, -CH2- of DPA), 4.12-4.07 (m, 1H, C_αH Phe), 3.89-3.84 (m, 1H, C_αH Phe), 3.54-3.16 (m, 2H, CH_2 Gly), 2.38-2.35 (m, 4H, C_βH Phe), 2.12-2.09 ((m, 2H, CH_2 EDA), ESI-MS (m/z): [M/2]=666.32 (calculated); 666.37 (observed); $[M+2H]^+$=1335.58 (calculated); 1335.64 (observed) (Scheme S5).



Scheme S5: Synthetic methodologies adopted for the synthesis of **L**.

Synthesis of LM: L (200 mg, 0.15 mmol) was dissolved in 20 mL of methanol and then a solution of $Cu(ClO_4)_2.6H_2O$ (138 mg, 0.372 mmol) in 5 mL HPLC water was added in a dropwise manner into it. The resulting solution was stirred for 10h at RT. The desired compound was then precipitated in a pure form by slow evaporation of the solvent at RT. The precipitate was filtered, washed with cold water and dried. Yield: 160 mg, 57.5%. ESI-MS (m/z): $[M+2H]^+$=1854.86 (calculated); 1855.31 (observed) (Scheme S6).



Scheme S6: Synthetic methodologies adopted for the synthesis of **LM**.

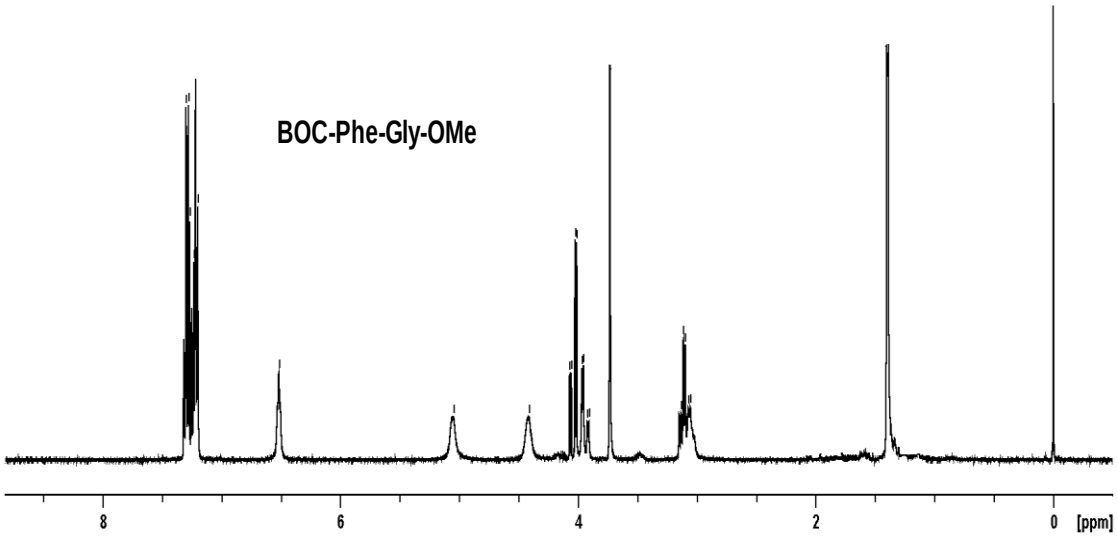


Figure S1. ^1H NMR ($CDCl_3$, 400 MHz, δppm) of Boc-Phe-Gly-OMe (1).

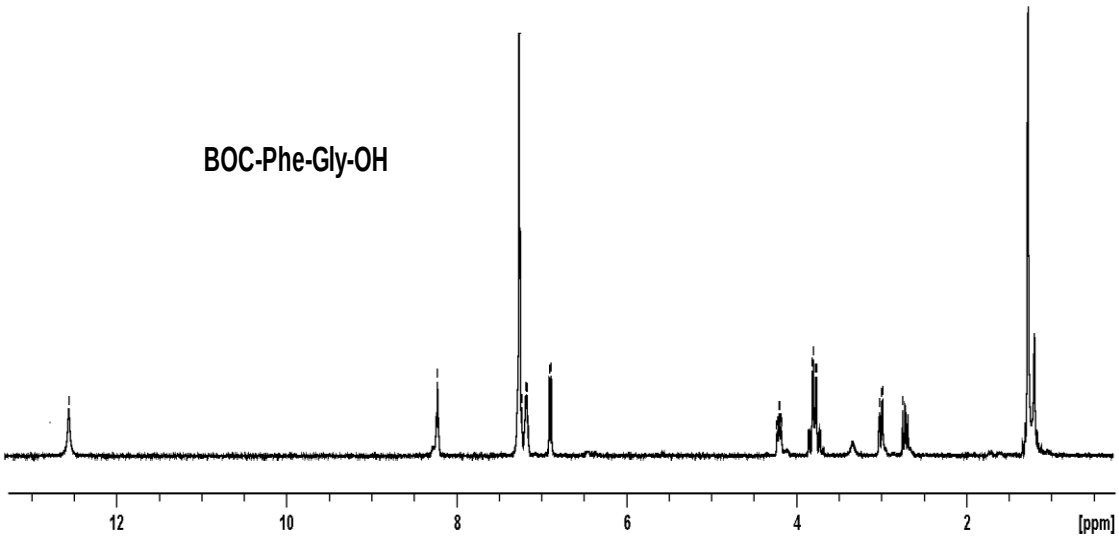


Figure S2. ^{1}H NMR (DMSO-d_6, 400 MHz, δppm) of Boc-Phe-Gly-OH (2).

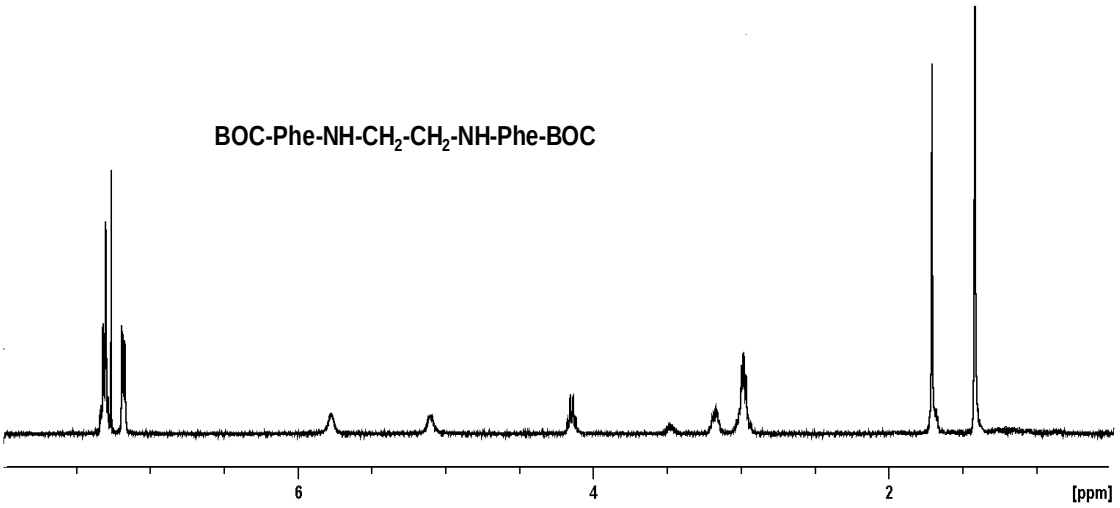


Figure S3. ^{1}H NMR ($CDCl_3$, 400 MHz, δppm) of Boc-Phe-NH2-CH2-CH2-NH2-Phe-Boc (3).

Phenyl-bis-pyridin-2ylmethyl-amine

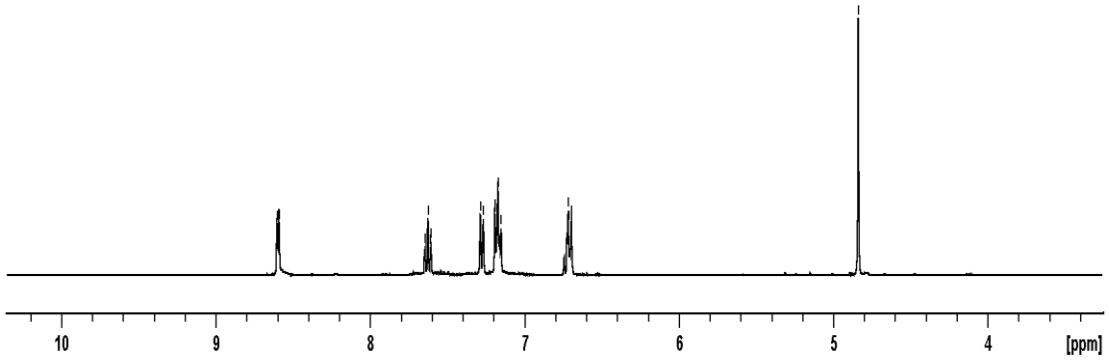


Figure S4. ^{1}H NMR ($CDCl_3$, 400 MHz, δppm) of Phenyl-bis-pyridin-2ylmethyl-amine (**1**/).

4-(Bis-Pyridin-2-ylmeythyl)-amino-benzaldehyde

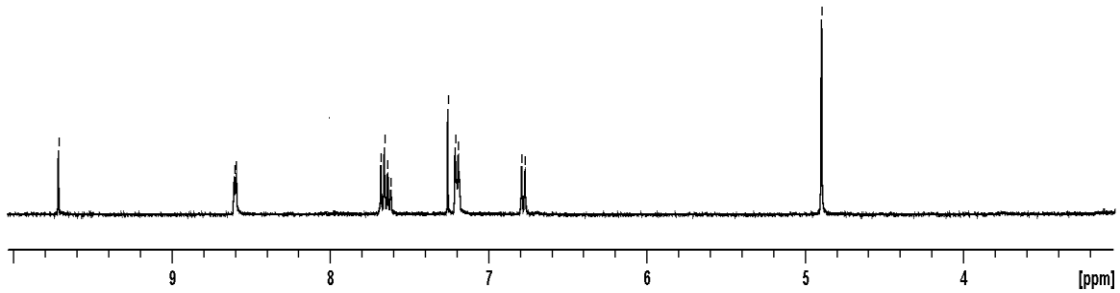


Figure S5. ^{1}H NMR ($CDCl_3$, 400 MHz, δppm) of 4-(Bis-Pyridin-2-ylmeythyl-amino-benzaldehyde **(2/)**.

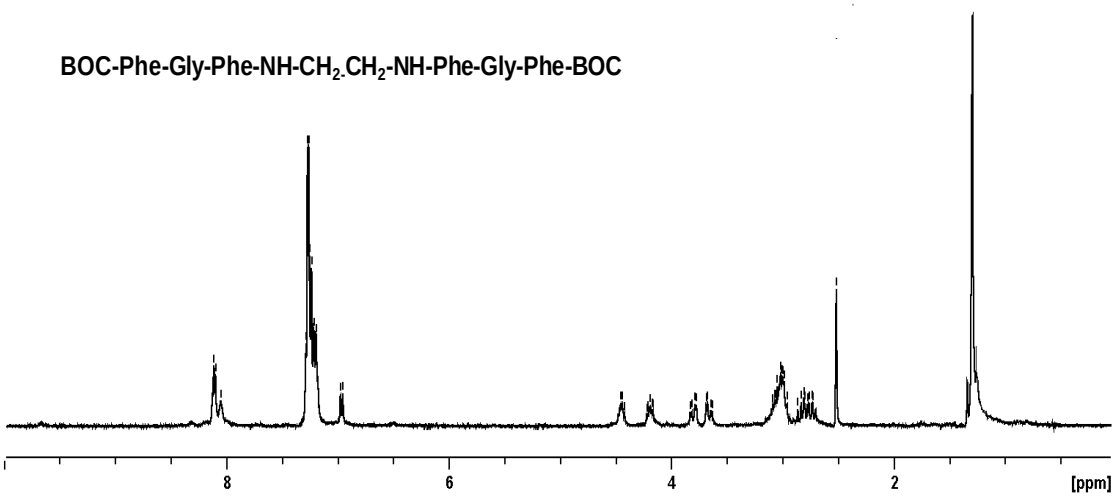


Figure S6. ^{1}H NMR (DMSO d_6, 400 MHz, δppm) of BOC-Phe-Gly-Phe-NH-CH_2-CH_2-NH-Phe-Gly-Phe-Boc **(5)**.

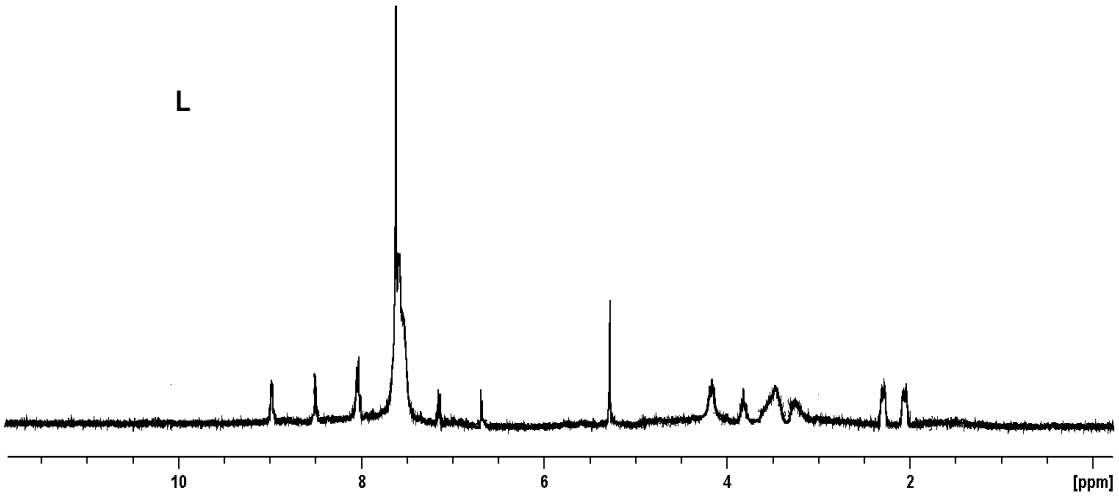


Figure S7. ^{1}H NMR ($CDCl_3$, 400 MHz, δppm) of **(L)**.

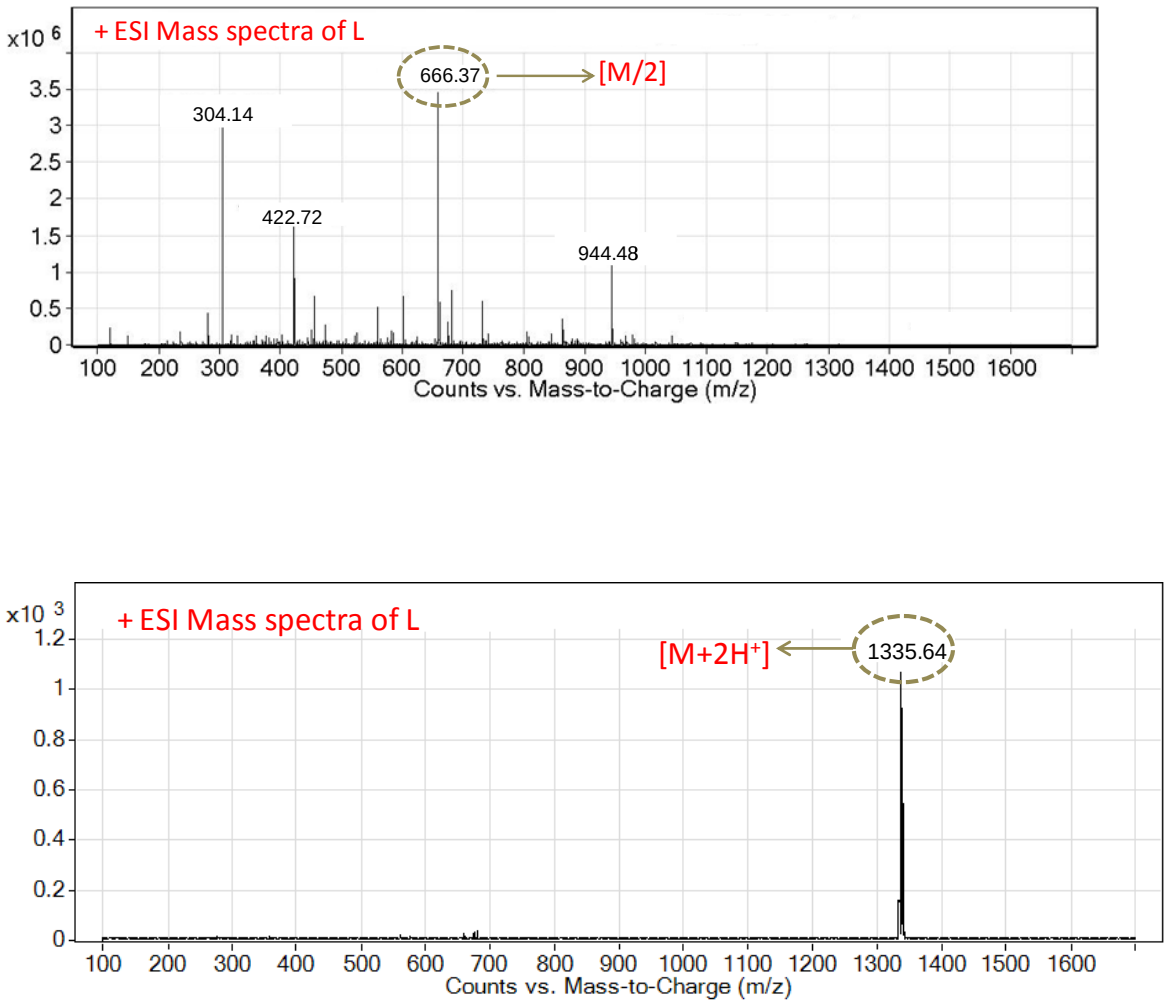


Figure S8. ESI Mass spectra of **L**.

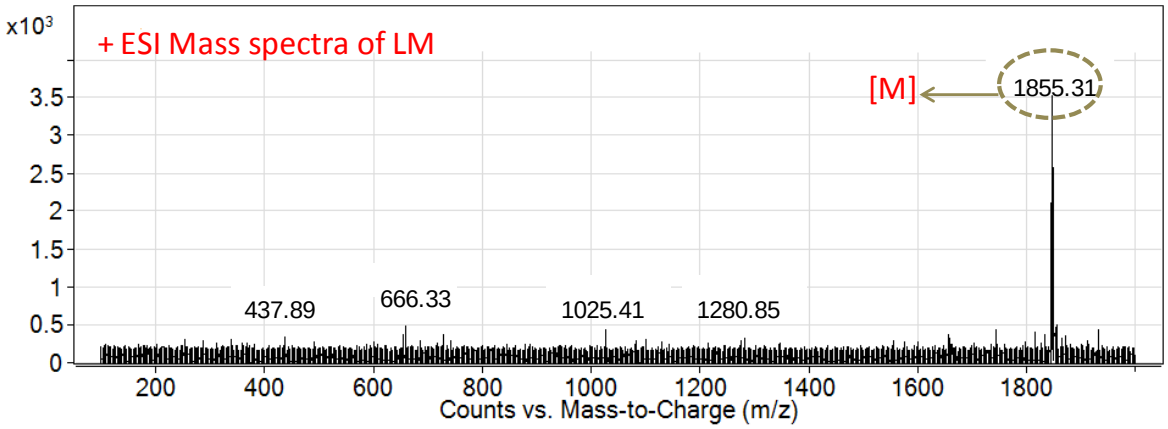


Figure S9. ESI Mass spectra of **LM**.

The UV-Vis absorption spectra of **L** displayed two absorption maxima at 260 and 340 nm. The first peak at 260 nm can be attributed to a intracomponent charge transfer (CT) transition and the second absorption maxima at 340 nm can be attributed to an intercomponent CT transition with the 2,2/-dipicolylamine (DPA)-based receptor moiety as an acceptor and the substituted moiety as the donor fragment.[1] For **LM** the absorption maximum corresponds to an intercomponent CT transition shifted towards shorter wavelengths from 340 nm to 320 nm. Coordination to Cu^{2+} was not expected to favour the intercomponent ligand-based CT transition and thus, it leads the shift of absorption maxima towards a shorter wavelength. Actually the Cu^{2+}, d^9 system promotes the MLCT from Cu^{2+} to the DPA acceptor moiety and makes the intercomponent ligand-based CT less favourable.[2]

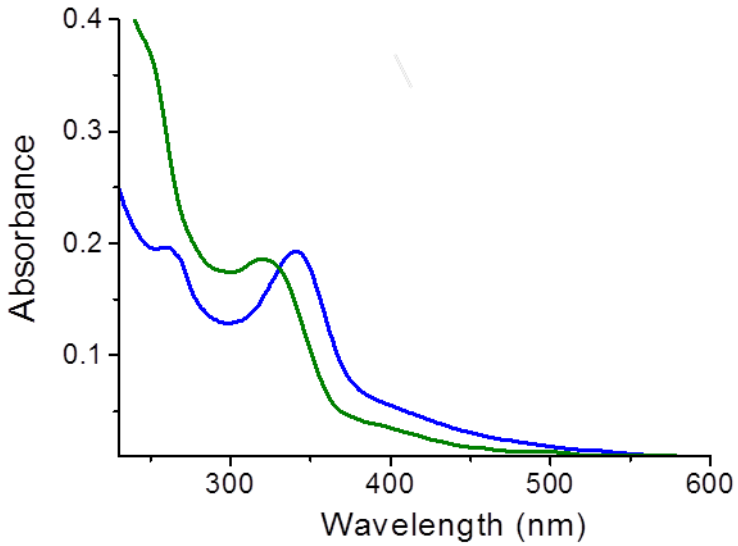


Figure S10. UV-Vis absorbance spectra of **L** (blue line**)** and **LM** (green line)**.**

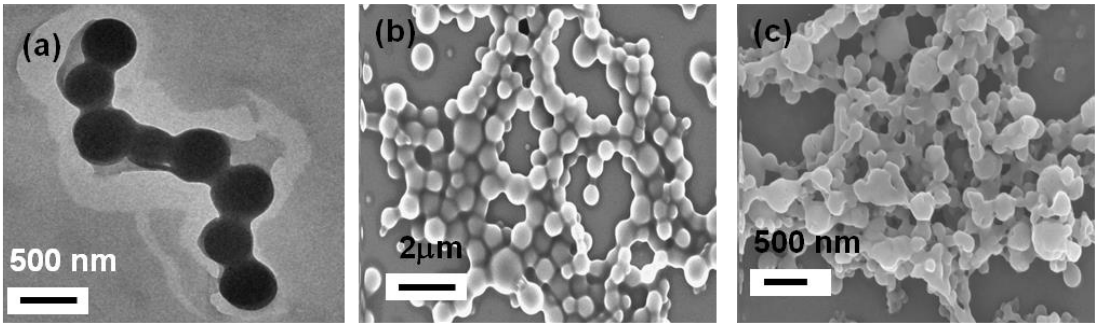


Figure S11. (a) TEM micrograph of self-assembled structures formed by **LM** in 50% ethanol. HR-SEM micrographs of (b) self-assembled structures formed by **LM** in water and (c) **L** in water.

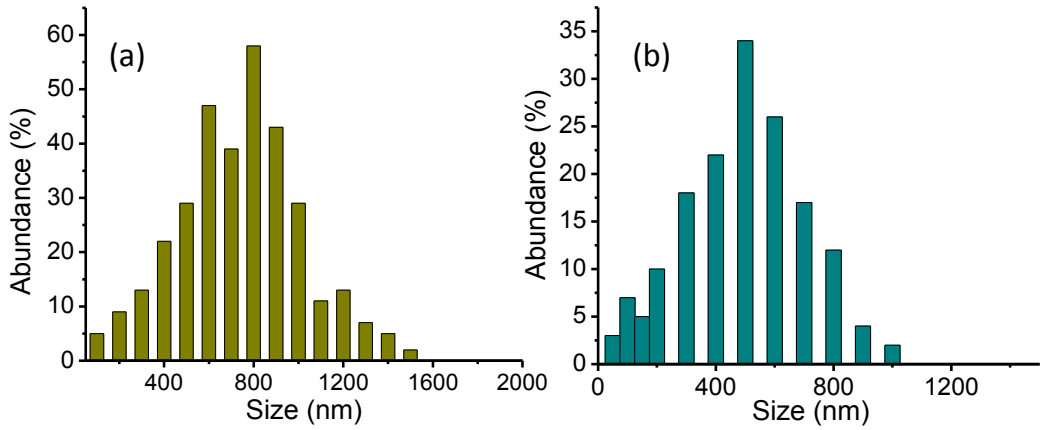


Figure S12. Size distribution obtained from the DLS measurement for the spherical particles formed by (a) **LM** in methanol and (b) **L** in 50% ethanol.

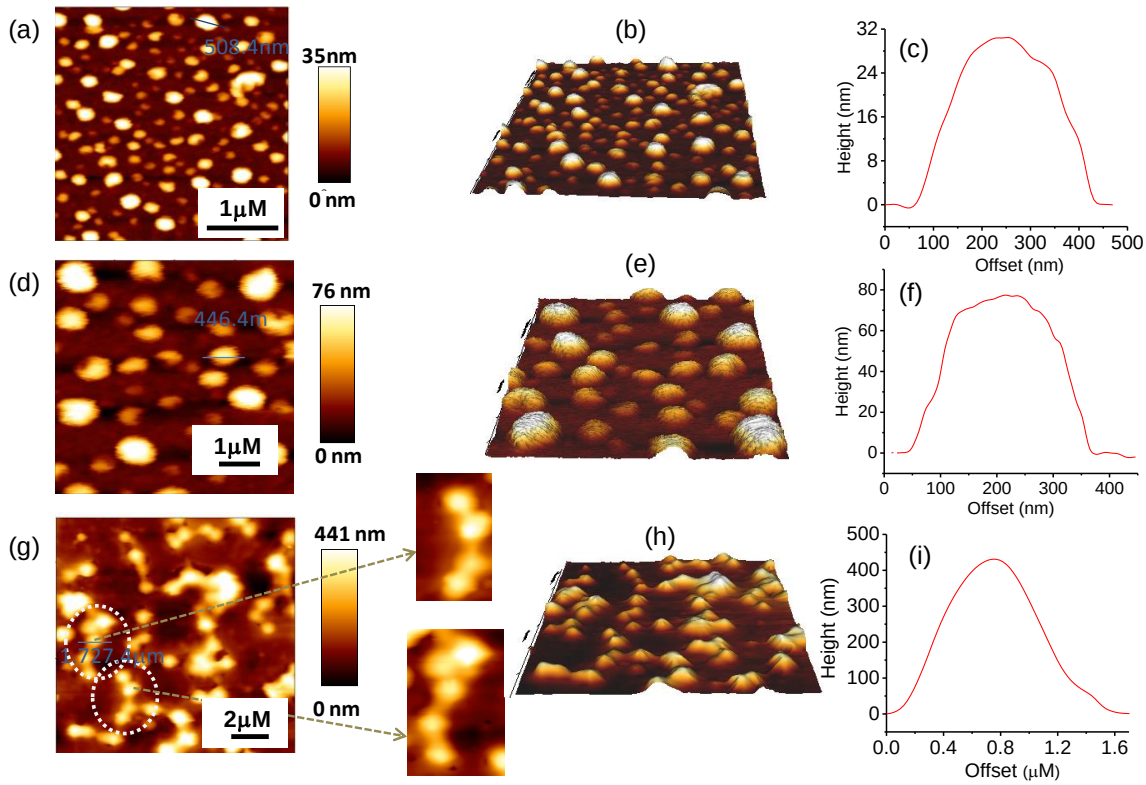


Figure S13. (a) AFM topographic analysis: Two-dimensional representation (Left panel), three-dimensional representation (Middle panel) and height analysis (Right panel) of the structures formed by **L** and **LM** under different condition of self-assembly (a, b, c) condition 2 (**L** in 50% Ethanol). (d, e, f) condition 4 (**LM** in MeOH). (g, h, i) condition 5 (**LM** in 50% Ethanol) (inset shows the connection between the nanospheres by neck formation).

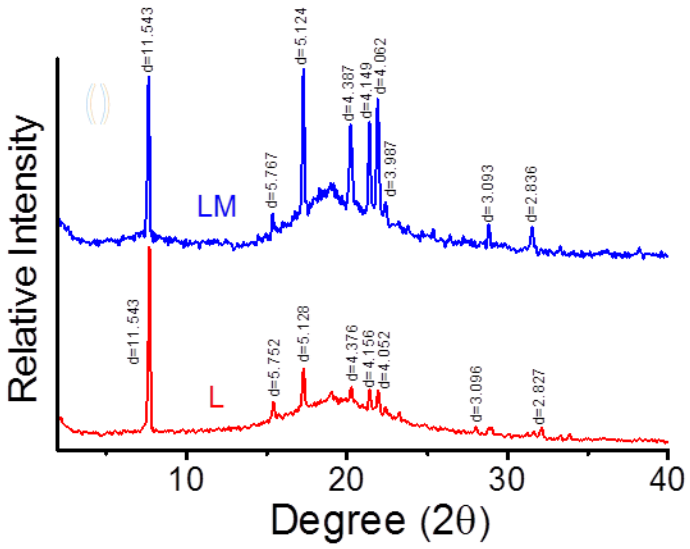


Figure S14. X-ray diffraction pattern of the self-assembled structure formed by **L** and **LM** in water.

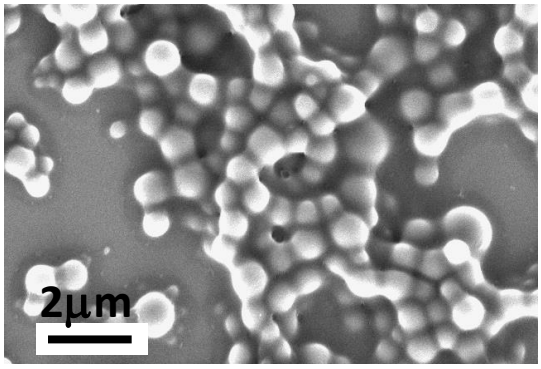


Figure S15. Representative HR-SEM micrographs of the self-assembled structures formed by **LM** in water (condition 6) after storing the solution for one month at RT and under atmospheric pressure.

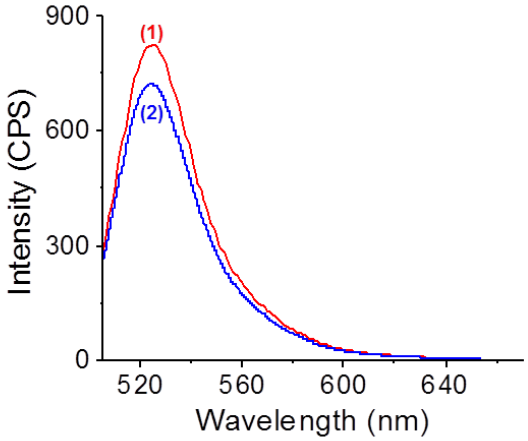


Figure S16. Fluorescence spectra of **P1** (50nM)/**T1**(400nM) conjugate (1) in the absence and (2) presence of **LM** (0.016 mM).

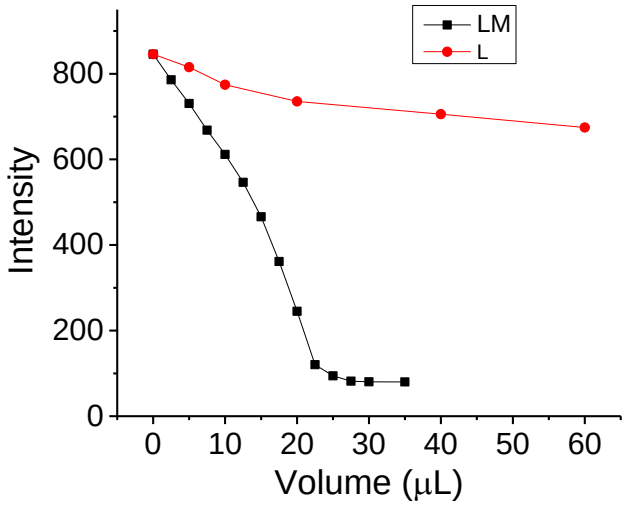


Figure S17. Effect of dosage of **L** (stock solution 0.750 mM) and **LM** (stock solution 0.659 mM) on the emission intensity of probe DNA (**P1**) (50 nmol) in Tris-HCl buffer (pH=7.4).

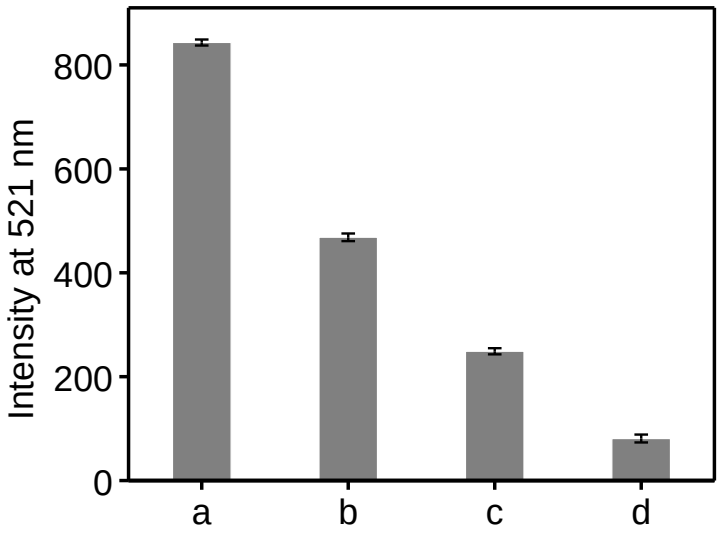


Figure S18. The emission intensity of the probe DNA (**P1**) (50 nM) at 521 nm under different conditions (λ_{Ext} = 494 nm, λ_{Mon} = 521nm): (a) **P1** Only; (b) **P1** + **LM** in methanol; (c) **P1** + **LM** in 50% ethanol; (d) **P1** + **LM** in water (incubation time =30 min).

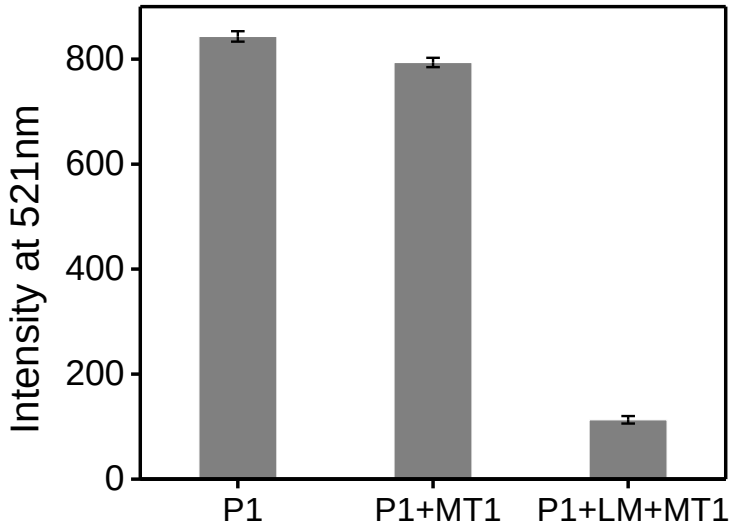


Figure S19. Emission intensity of the (a) probe DNA (**P1**) (50 nM); (b) **P1** (50 nM) + single base mismatch DNA (**MT1**, 1μM) and (c) **P1** (50 nM) +**MT1** (1μM) + **LM** (0.016 mM). (λ_{Mon} =521 nm, λ_{Ext} = 494 nm).

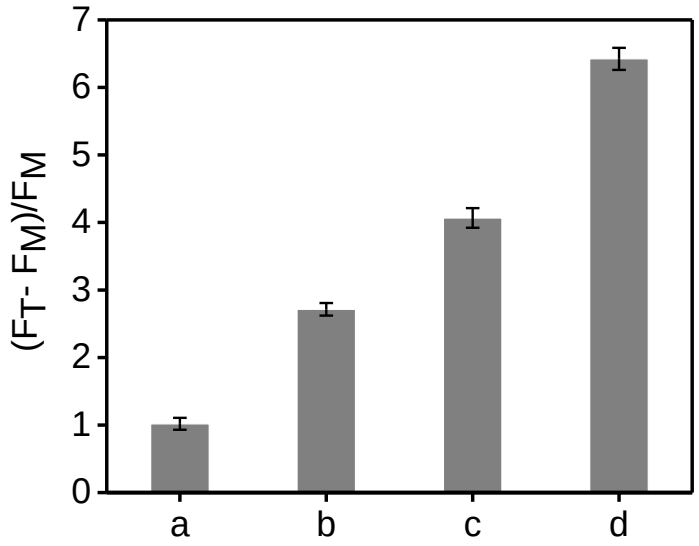


Figure S20. Changes in the emission intensity (F_T/F_M -1) of the probe DNA (**P1**) (50 nM) in the presence of **LM** (0.016 mM) and polyacrylic acid (PA; 10 μM) with or without different concentrations of **T1** (a) without **T1**; (b) with **T1** (100 nM); (c) with **T1** (200 nM); (d) with **T1** (500 nM) (λ_{Mon} =521 nm, λ_{Ext} = 494 nm).

References

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2. A. K. Mandal, M. Suresh, P. Das, M. Baidya, S. K. Ghosh, A. Das, *Org. Lett.* **2012**, *14*, 2980.