Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

Self-assembled Vesicles of Urea-Tethered Foldamer as a Hydrophobic Drug Carrier

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Materials and methods

Unless otherwise stated, all the chemicals and reagents were obtained commercially. Commercial solvents were distilled and dried by the standard protocol, prior to use. Isopropylamine, trichloroacetic acid, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC.HCl), 4-(dimethylamino) pyridine (DMAP), propargylamine, 1,8-diazabicycloundec-7-ene (DBU), $PdCl_2(PPh_3)$, cuprous iodide (CuI) and curcumin were purchased from Sigma Aldrich. Spectra/Pro Dialysis membrane (MW cutoff: 1000) was purchased from Spectrum Laboratories.

Measurements

NMR spectra were recorded in $CDCl_3$, CD_3OD and CD_3OH on AV 400 MHz and AV 500 MHz Bruker NMR spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities as singlet (s), doublet (d), quartet (q), broad singlet (bs), multiplet (m), etc. IR spectra were recorded using Bruker Alpha FTIR spectrophotometer. Melting point analysis was performed using Buchi melting point instrument B-540. HRMS data were recorded on a Thermo Scientific Q-Exactive, Accela 1250 pump.

Scanning electron microscopy

SEM measurements were performed on a FEI, QUANTA 200 3D scanning electron microscope with tungsten filament as electron source. For SEM analysis, solution of octapeptide **2** at a concentration of 1.25 mM (1 mg/0.5 mL) was prepared in organic solvent (or binary solvent mixture). 5 μL of freshly prepared each of transparent solutions were drop-casted on silicon surface and allowed to dry overnight at room temperature. Before recording of morphology, a gold film was applied by sputtering method.

Transmission electron microscopy

TEM measurements were performed on a JEOL-JEM-3010 instrument at 80 kV. For TEM analysis, 5 μL of freshly prepared 1:9 methanol-toluene solution of octapeptide **2** (0.62 mM) was drop-casted directly on a carbon coated copper grid and allowed to dry overnight at room temperature.

Atomic force microscopy

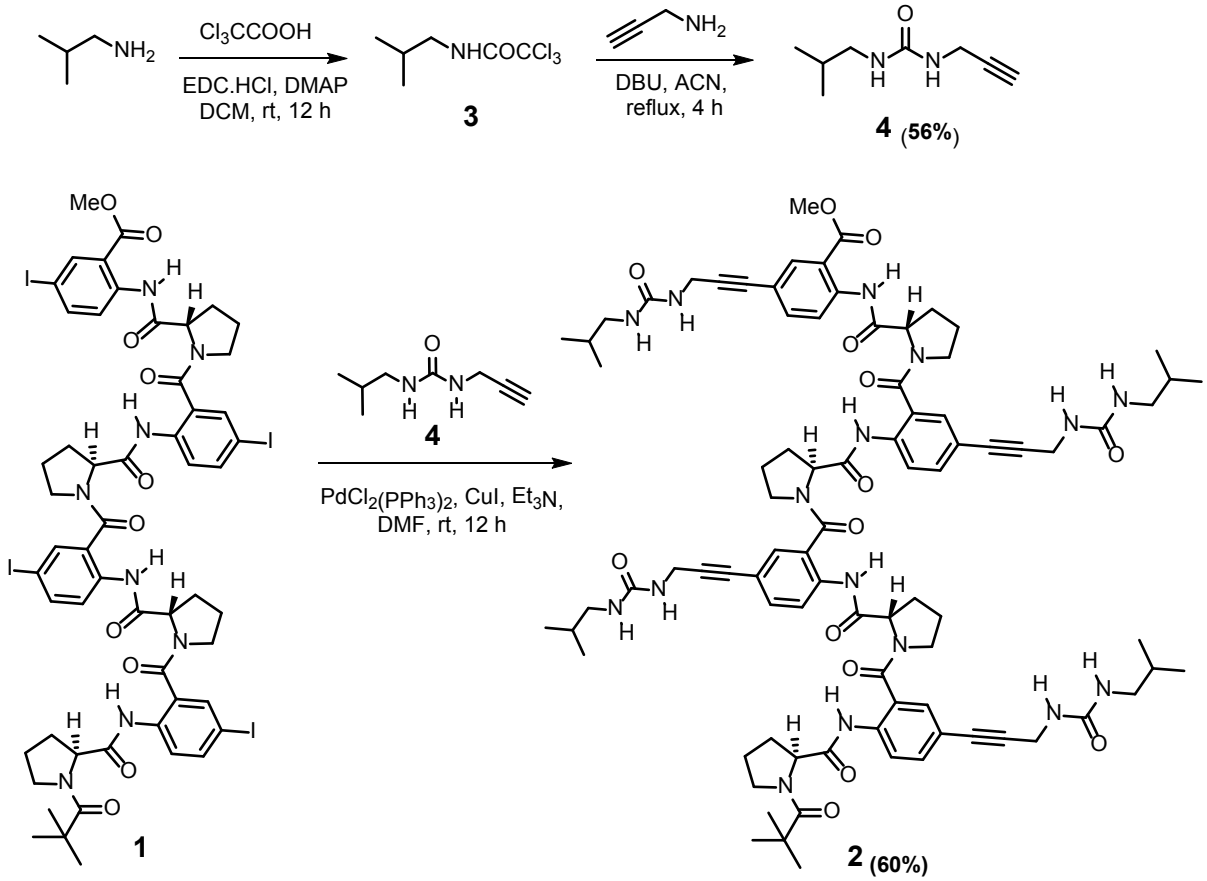
The AFM measurements were performed on a multimode scanning probe microscope equipped with a Nanoscope IV controller from Veeco Instrument Inc., Santa Barbara, CA. The imaging was done under ambient conditions in tapping-mode, using the Tap190Al probe purchased from Budget Sensors®. The 10 μm x 10 μm areas were scanned at resolution of 512 X 512 pixels. For AFM analysis, 5 μL of freshly prepared transparent 1:9 methanol-

toluene solution of octapeptide **2** (0.62 mM) was drop-casted on silicon surface and allowed to dry overnight at room temperature.

Curcumin encapsulation and release studies

The octapeptide **2** (1 mg) and curcumin (0.6 mg) were dissolved in 1 mL of methanol and kept stirring for 24 h to ensure encapsulation. The solution of octapeptide and curcumin was then taken in a dialysis membrane with a molecular weight cutoff of 1000, sealed off at the ends, and dialyzed against methanol for 1 day. Methanol was removed several times until it became colourless to ensure complete removal of unencapsulated curcumin. 10 μL of resultant solution was drop-casted on glass slide and analyzed under EVOS fluorescence microscope equipped with 100X oil immersion objective and Sony ICX285AQ color CCD camera, 1.4 megapixel (PEQLAB Ltd, UK) using green filter. For TBAF-mediated release of curcumin, 1 equivalent of TBAF was added to the methanolic solution of curcumin encapsulated octapeptide vesicles and incubated overnight followed by imaging under fluorescence microscope. The fluorescence spectral measurements were performed on a VARIAN CARY Eclipse fluorescence spectrophotometer using a slit width of 10 nm in a cuvette of path length 1 cm. The fluorescence emission spectra were recorded from 430 to 800 nm, exciting at 420 nm. The fluorescence spectra of both before and after addition of TBAF to the curcumin encapsulated methanolic solution at a concentration of 1 mg/mL were recorded.

Synthesis of compounds

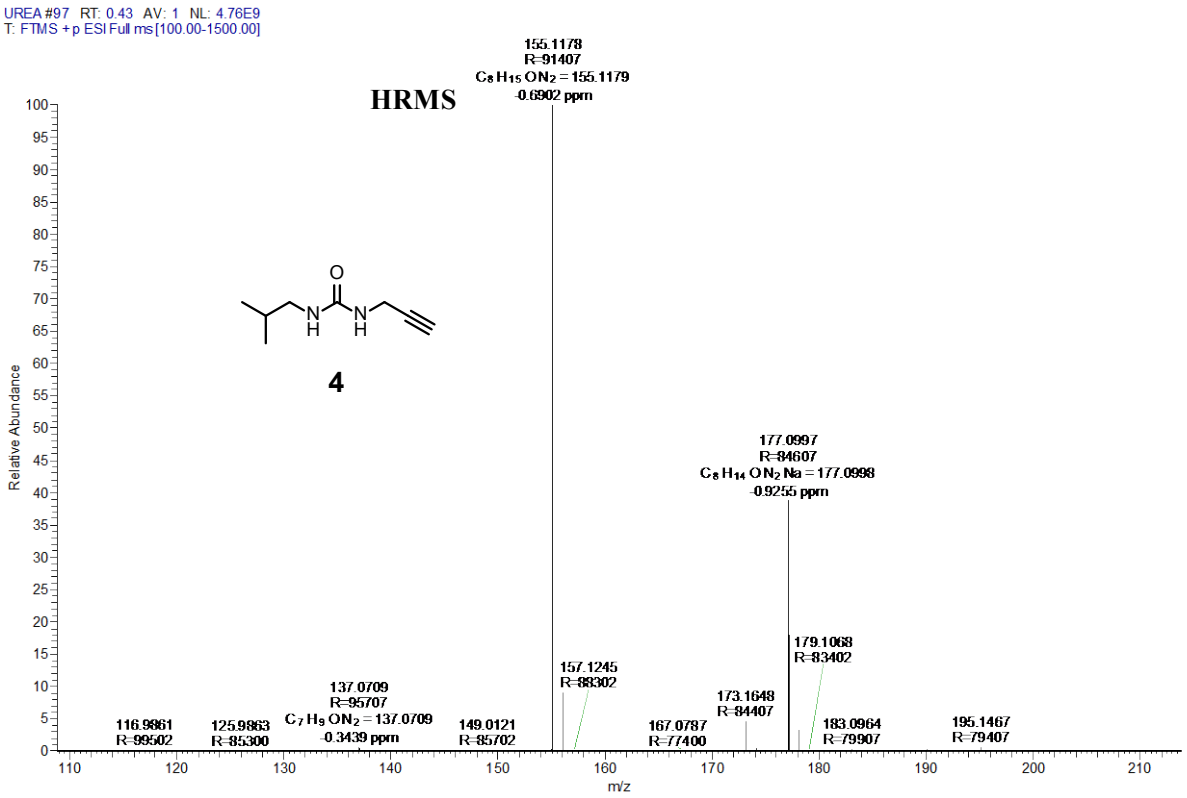


Scheme 1. Synthesis of urea-tethered Ant-Pro hybrid octapeptide **2**.

1-isobutyl-3-(prop-2-yn-1-yl) urea 4. To a solution of trichloroacetic acid (1.58 g, 9.14 mmol) and isobutyl amine (0.736 g, 10 mmol) in dry DCM (50 mL), EDC.HCl (2.63 g, 13.7 mmol) and DMAP (0.57 g, 4.57 mmol) were added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with DCM (50 mL). The organic layer was washed with water, saturated solution of $KHSO_4$ and then with brine. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product **3** was used for next step without purification. To a solution of **3** (1.58 g, 9.14 mmol) and propargyl amine (0.736 g, 10 mmol) in dry acetonitrile (50 mL), DBU (2.63 g, 13.7 mmol) was added. The reaction mixture was refluxed for 4 h. Then the reaction mixture was cooled and diluted with ethyl acetate (50 mL). The organic layer was washed with water, saturated solution of $KHSO_4$ and then with brine. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography (40:60 ethyl acetate/petroleum ether, R_f =0.5) to afford **4** as a white solid (0.86 g, 56%), m.p. 48-50 °C. IR ($CHCl_3$): ν = 3343, 3310, 3143, 3040, 1960, 2877, 1631, 1586, 1463, 1430,

1354, 1271, 1052, 917, 654. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.93 (bs, 1 H), 5.73 (bs, 1 H), 3.95 (dd, *J* = 2.4, 5.4 Hz, 2 H), 3.01-2.92 (m, 2 H), 2.19-2.13 (m, 1 H), 1.77-1.65 (m, 1 H), 0.90 (d, J = 6.8 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.74, 81.12, 70.60, 47.79, 29.986, 28.94, 20.07 ppm. HRMS: Calcd. $C_8H_{15}ON_2$, 154.1106; found 154.1108.

Octapeptide 2. To a solution of **1**[1] (0.150 g, 0.1 mmol) and **4** (0.09 g, 0.6 mmol) in DMF (2 mL), $PdCl_2$ $(PPh_3)_2$ (0.007 g, 0.01 mmol), CuI (0.002 g, 0.01 mmol) and Et_3N (4 mL) were added under an inert atmosphere. The reaction mixture was cooled using liquid N_2 and argon was purged. Then the reaction mixture was warmed to room temperature and again purged with argon. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (20 mL), and washed with water, saturated solution of $KHSO_4$ and then with brine. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography (0.8:92 methanol/ethyl acetate, R_f 0.4) to afford **2** as a yellow solid (0.096 g, 60%), m.p. 182-185 °C. $[\alpha]^{26}_D$ = –35.18° (*c* = 0.1, MeOH). IR (nujol): ν = 3390, 2840, 1704, 1641, 1624, 1577, 1462, 1374, 1160, 680. ^{1}H NMR (400 MHz, CD_3OH): δ = 11.99 (s, 1 H), 10.55 (s, 1 H), 10.46 (s, 1 H), 10.14 (s, 1 H), 8.89-8.86 (d, *J* = 8.5 Hz, 1 H), 8.40 (s, 1 H), 8.32-8.29 (m, 1 H), 8.22-8.20 (d, *J* = 7.9 Hz, 1 H), 7.97-7.89 (m, 4 H), 7.83-7.77 (m, 4 H), 6.65 (bs, 4 H), 6.50 (bs, 4 H), 4.34 (bs, 4 H), 4.10-3.87 (m, 8 H), 3.89 (s, 3 H), 3.79-3.77 (m, 1 H), 3.71-3.70 (m, 4 H), 3.66 (bs, 3 H), 2.81-2.77 (m, 1 H), 2.67-2.58 (m, 3 H), 2.47-2.45 (m, 3 H), 2.37-2.30 (m, 7 H), 2.12-2.00 (m, 10 H), 1.57 (s, 9 H), 1.48-1.46 (m, 4 H), 1.25-1.21 (m, 24 H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 179.33, 173.7, 173.09, 173.03, 172.90, 170.93, 170.14, 169.76, 169.51, 160.94, 160.88, 160.76, 141.28, 138.31, 136.37, 136.20, 135.30, 134.96, 134.84, 132.27, 131.85, 131.80, 130.07, 129.36, 124.54, 124.32, 124.04, 121.85, 121.02, 120.87, 120.64, 119.76, 117.92, 88.81, 88.56, 82.05, 82.00, 81.91, 81.67, 71.48, 64.59, 64.10, 63.04, 62.76, 53.84, 51.73, 51.52, 51.33, 50.16, 40.06, 31.44, 31.28, 31.20, 31.13, 31.03, 30.62, 30.35, 30.29, 27.92, 27.14, 26.35, 26.26, 20.56 ppm; HRMS: Calcd. $C_{86}H_{109}O_{14}N_{16}Na$ 1612.8201; found 1612.8145.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4
177.0997
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179.1068
R=83402
157.1245
R=88302
137.0709
R=95707
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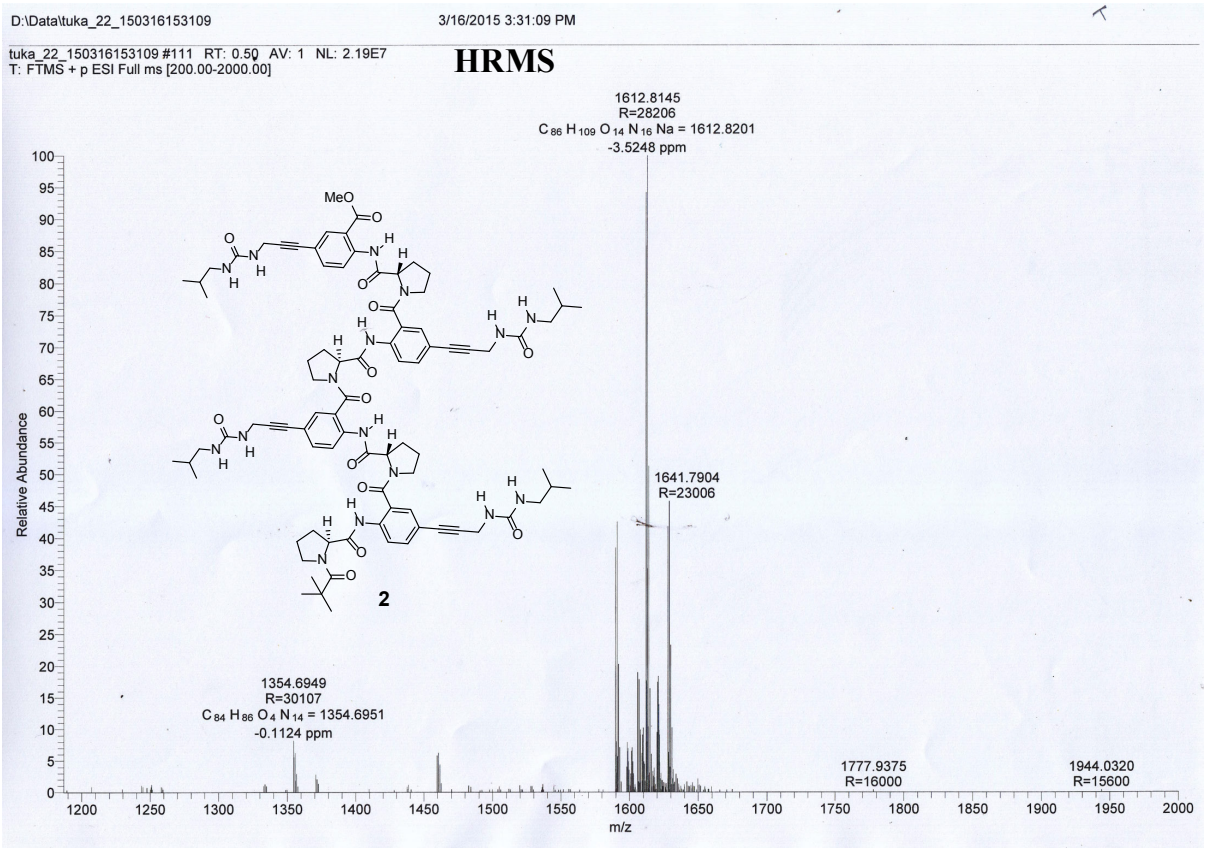
Relative Abundance
m/z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2
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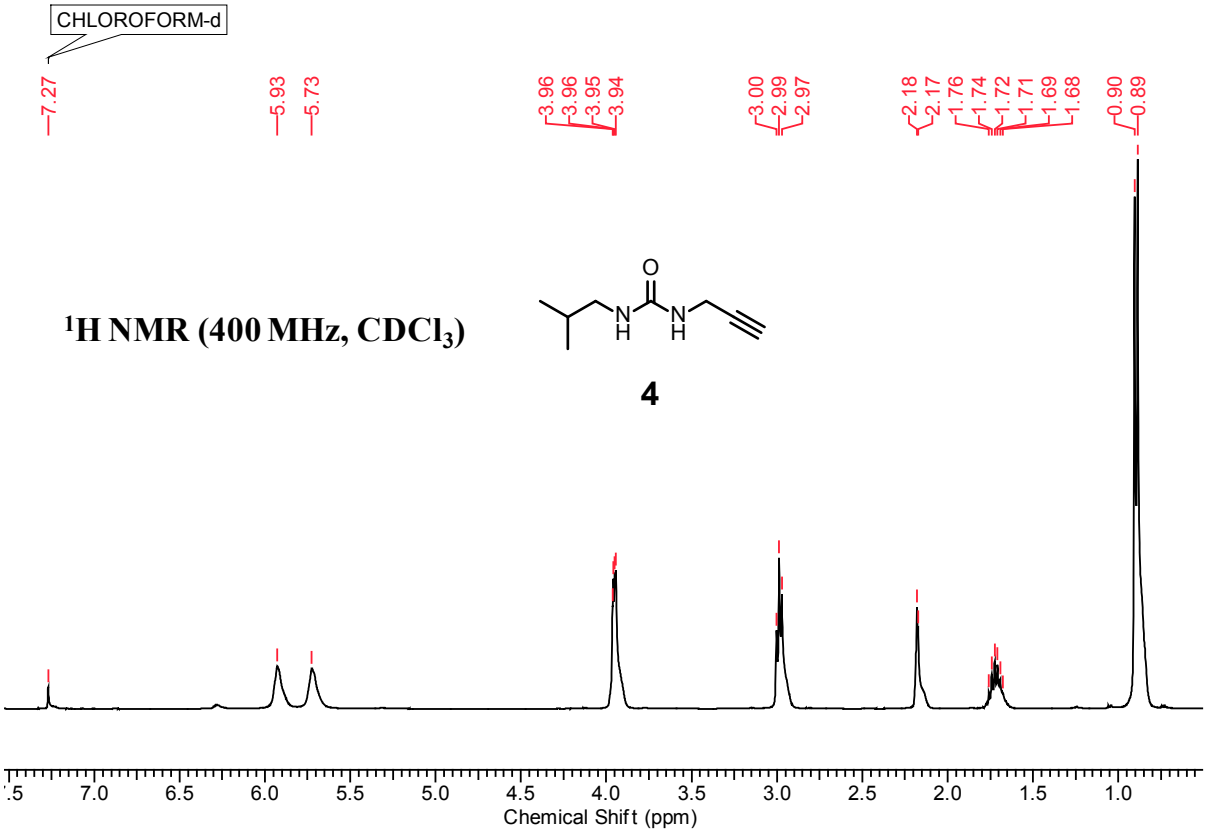
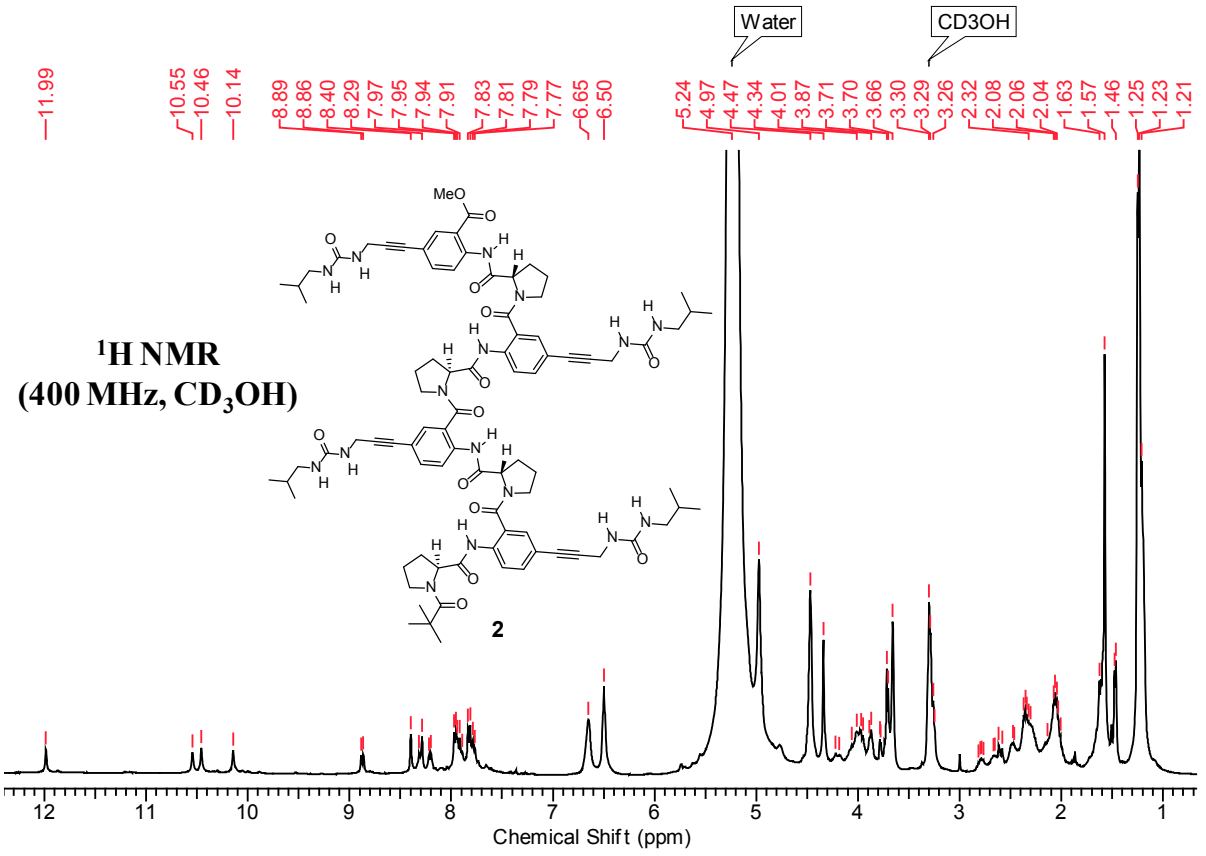
m/z



^{1}H NMR (400 MHz, $CDCl_3$)

4



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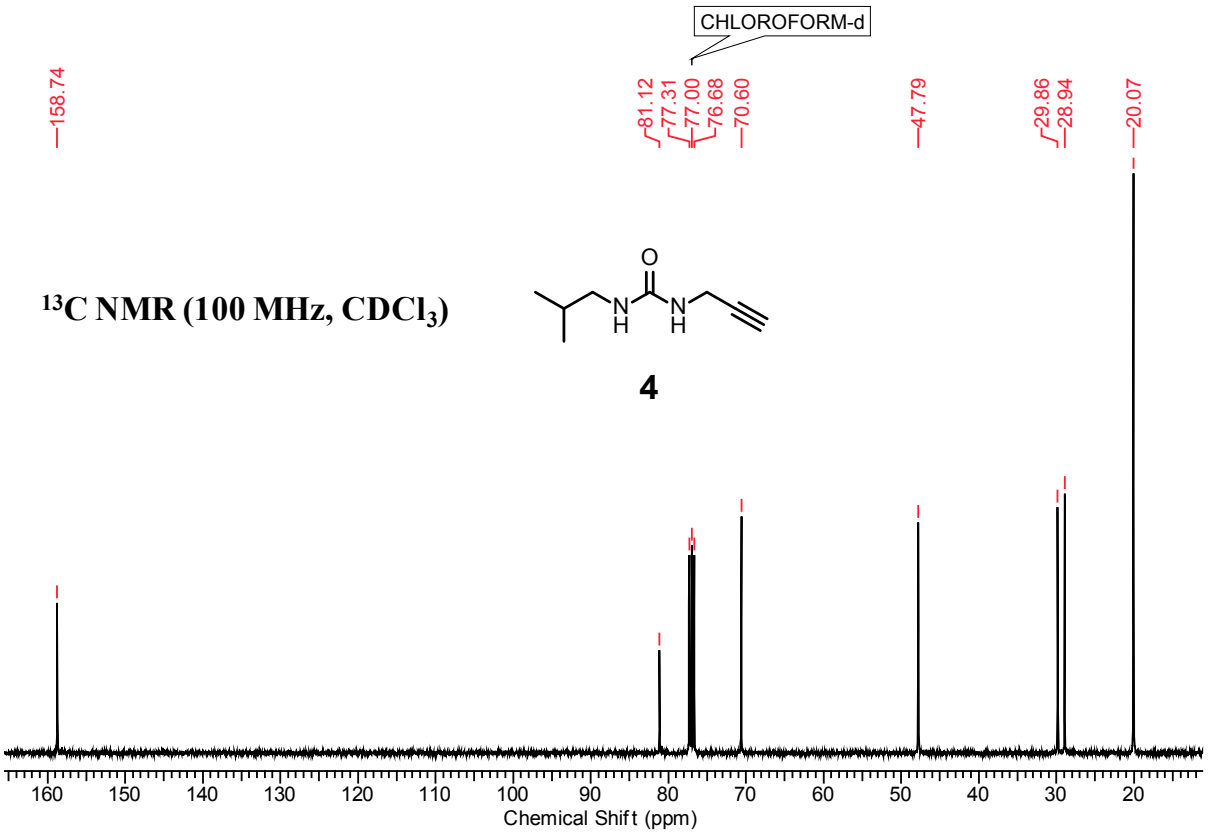
2



^{13}C NMR (100 MHz, $CDCl_3$)

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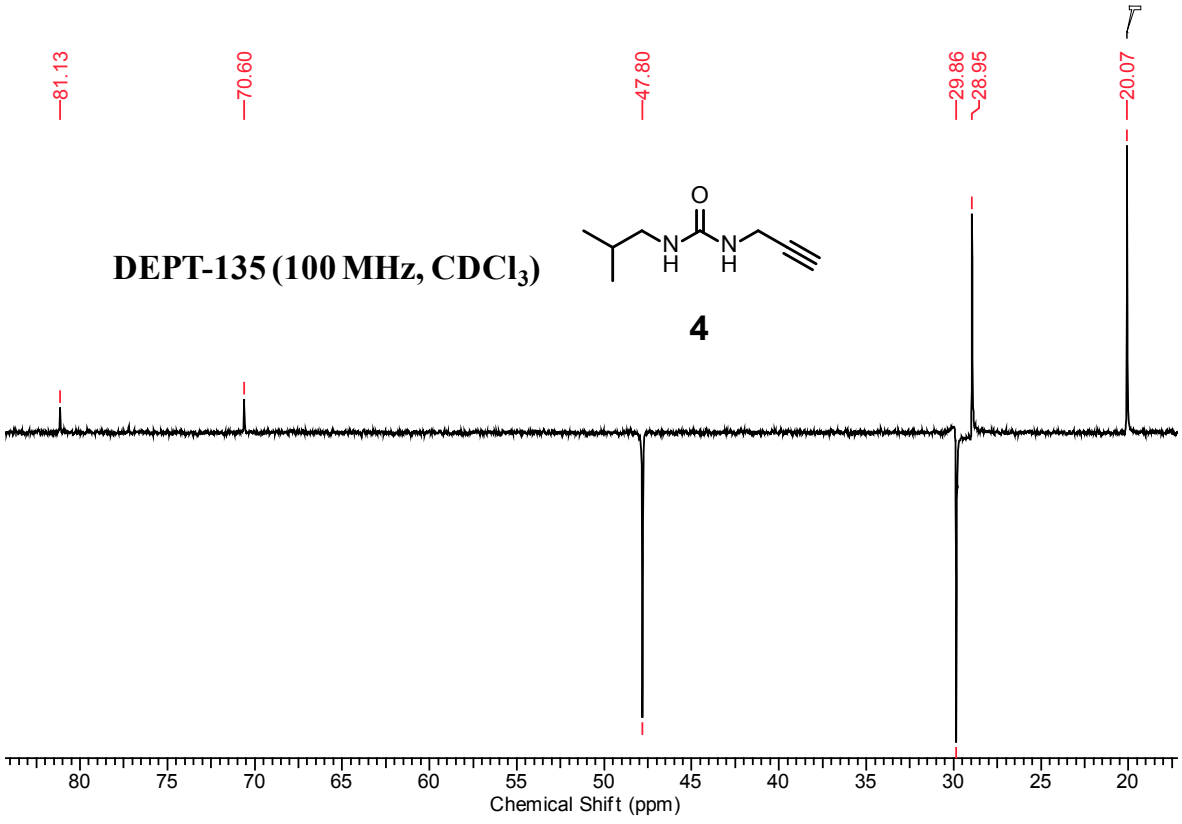
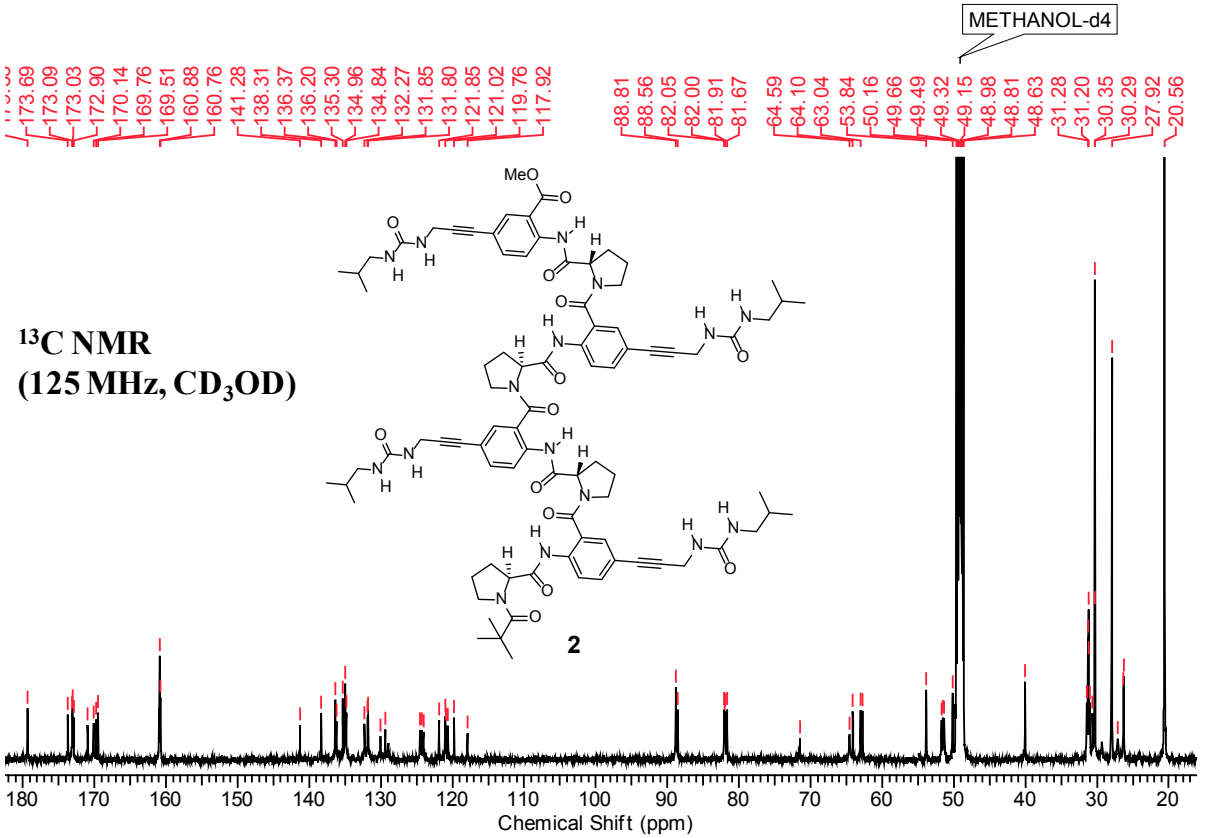
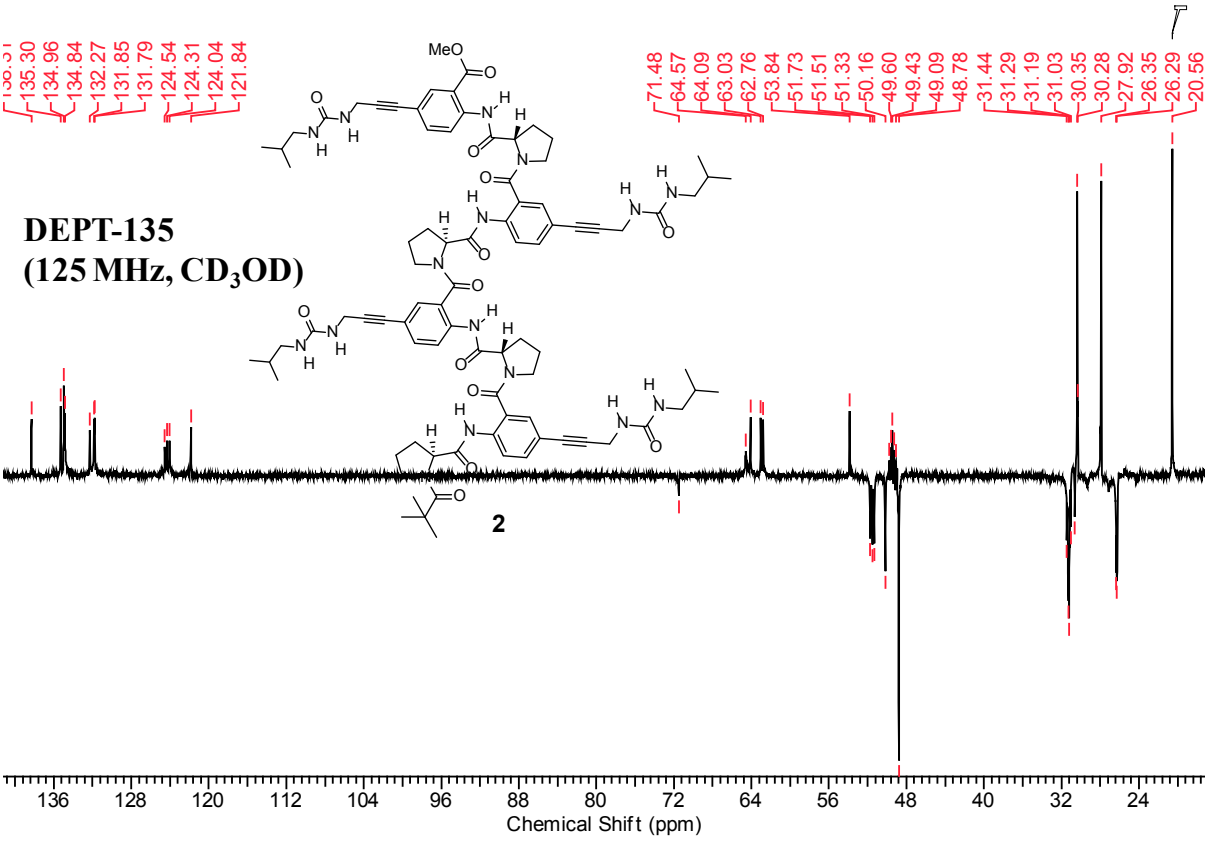


DEPT-135 (100 MHz, $CDCl_3$)

4



^{13}C NMR
(125 MHz, CD_3OD)



DEPT-135
(125 MHz, CD_3OD)

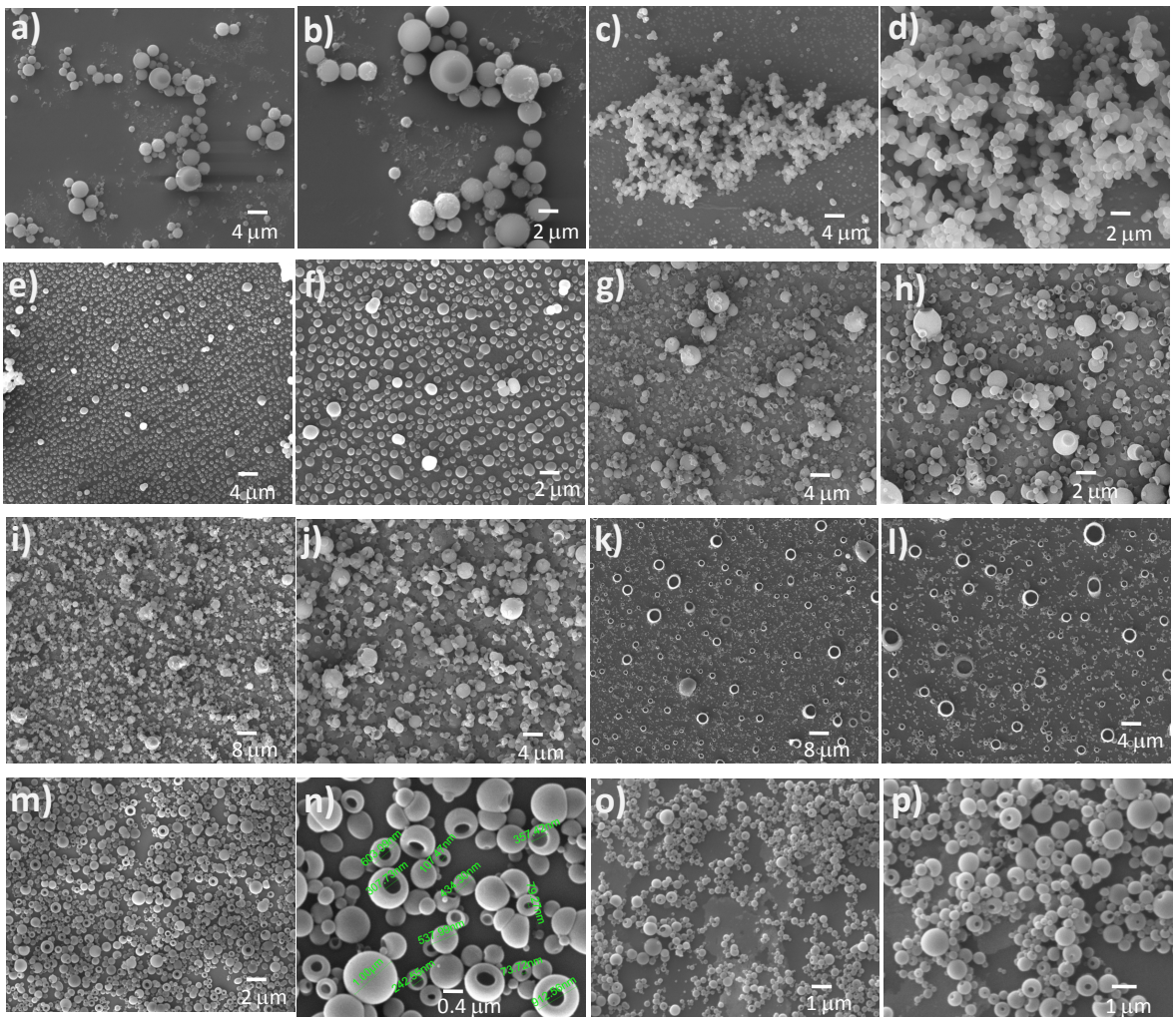


Fig. S1 Self-assembly of octapeptide **2**. Large area SEM images of octapeptide **2** at a concentration of 1 mg/0.5 mL: (a, b) methanol, showing the association of particles to generate twins, triplets, or even multiplets; (c, d) 9:1 methanol-toluene; (e, f) 8:2 methanol-toluene; (g, h) 6:4 methanol-toluene; (i, j) 5:5 methanol-toluene; (k, l) 2:8 methanol-toluene; (m, n) 1:9 methanol-toluene and (o, p) 0.8:9.2 methanol-toluene.

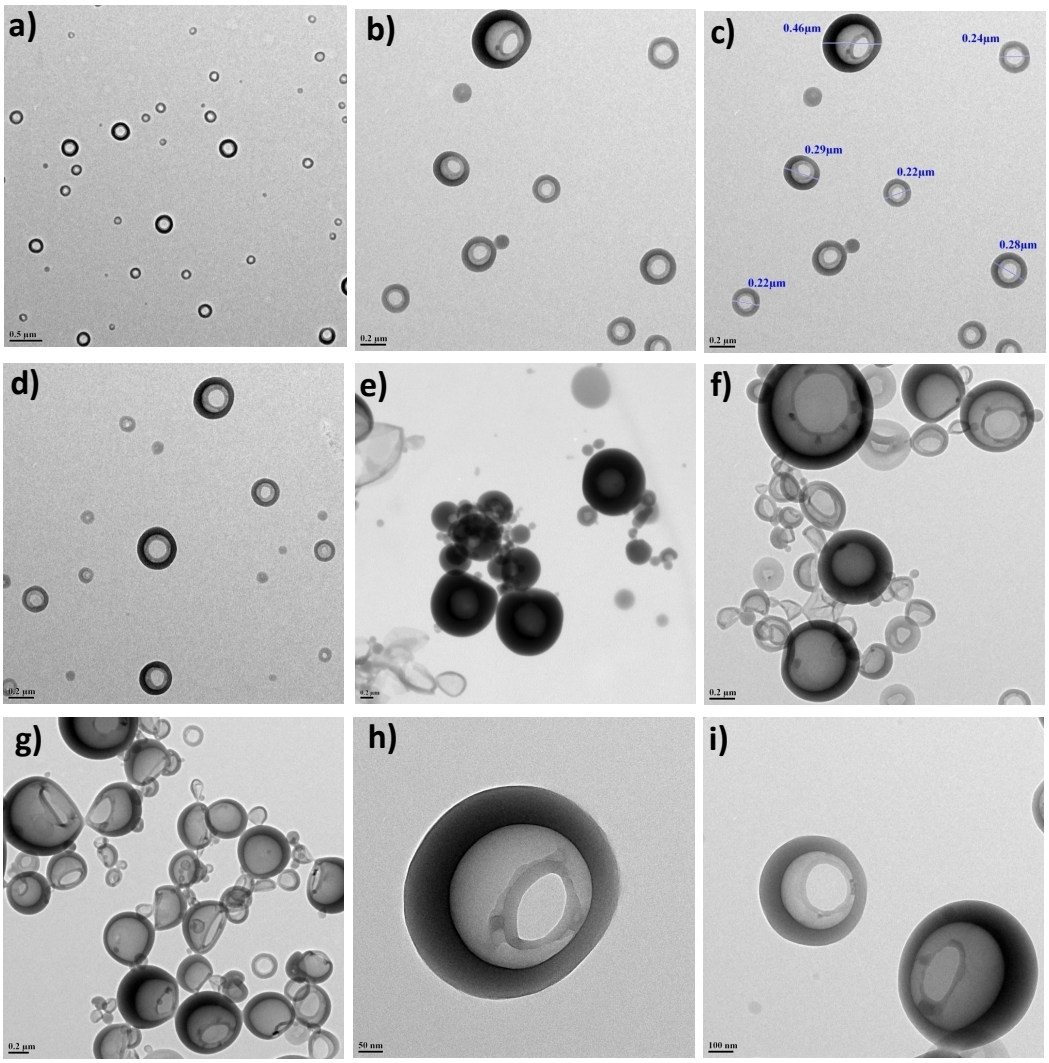


Fig. S2 Self-assembly of octapeptide **2** at a concentration of 1 mg/mL from 1:9 methanol-toluene solvent mixture. TEM images and scale bars are: (a) 0.5 µm; (b-g) 0.2 µm; (h) 50 nm and (i) 100 nm.

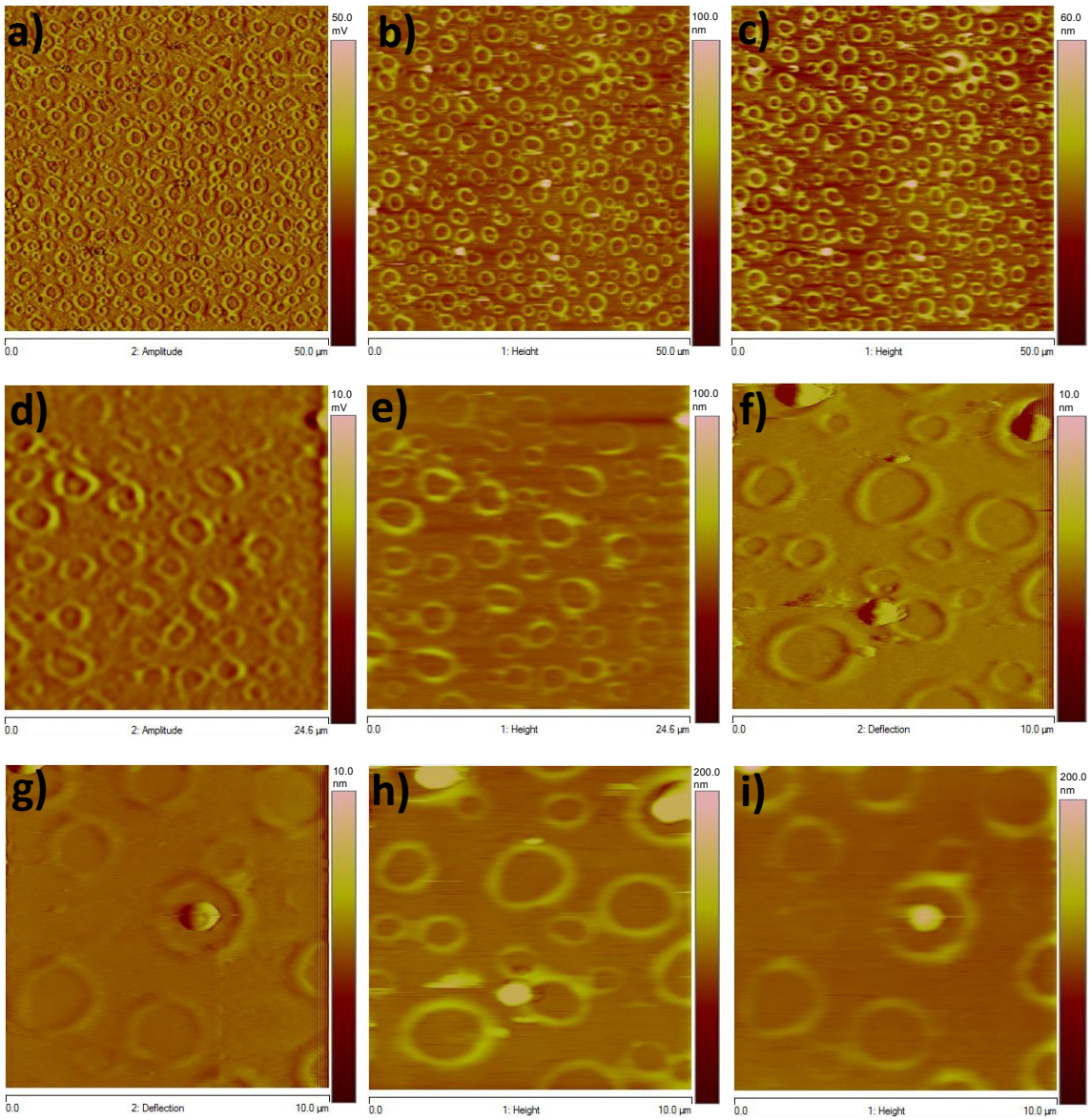


Fig. S3 AFM images of octapeptide **2** at a concentration of 1 mg/mL in 1:9 methanol-toluene solvent mixture.

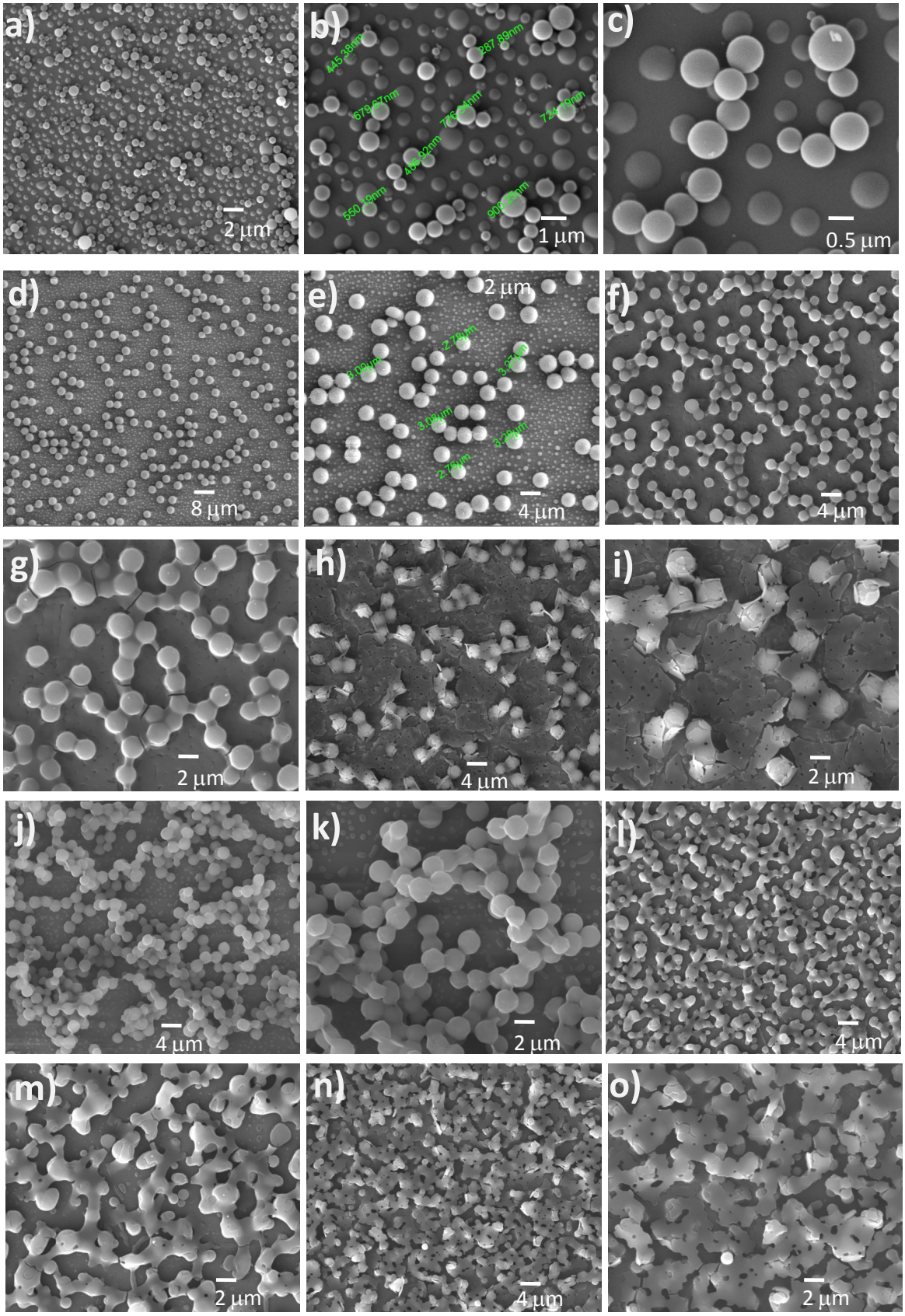


Fig. S4 Self-assembly of octapeptide **2** at a concentration of 1 mg/0.5 mL. SEM images from different solvent ratios: (a-c) TFE; (d,e) 9:1 TFE-DMSO; (f,g) 8:2 TFE-DMSO; (h,i) 5:5 TFE-DMSO; (j,k) 2:8 TFE-DMSO; (l,m) 1:9 TFE-DMSO and (n,o) DMSO.

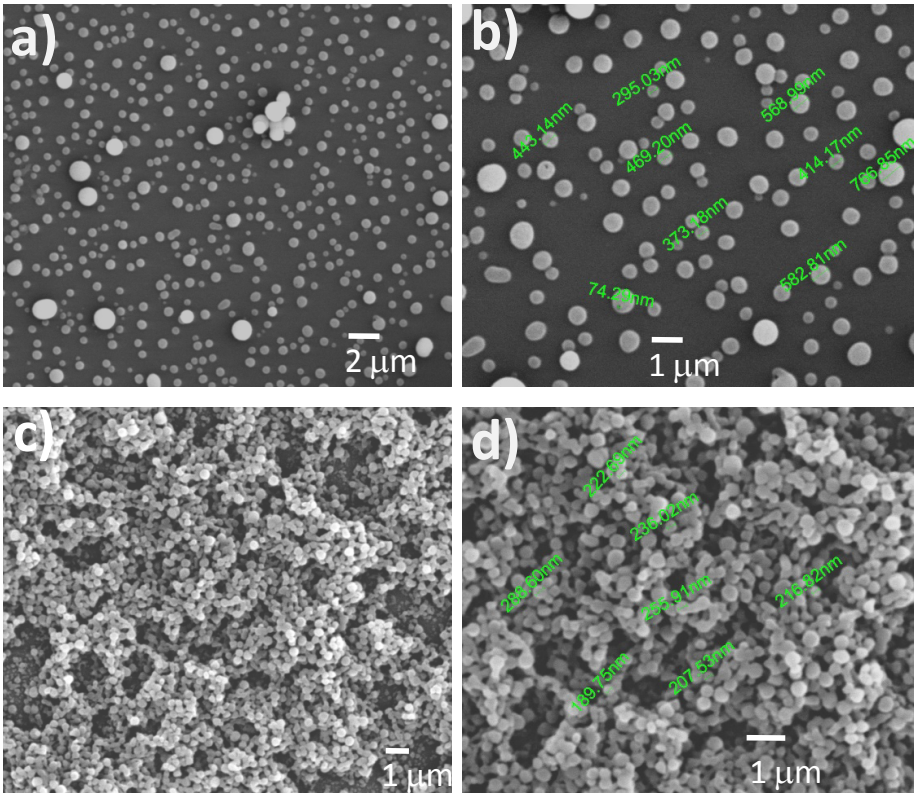


Fig. S5 Self-assembly of octapeptide **2** at a concentration of 1 mg/0.5 mL. SEM images from different solvent mixtures: (a,b) 5:5 methanol-chloroform and (c,d) 5:5 methanol-water.

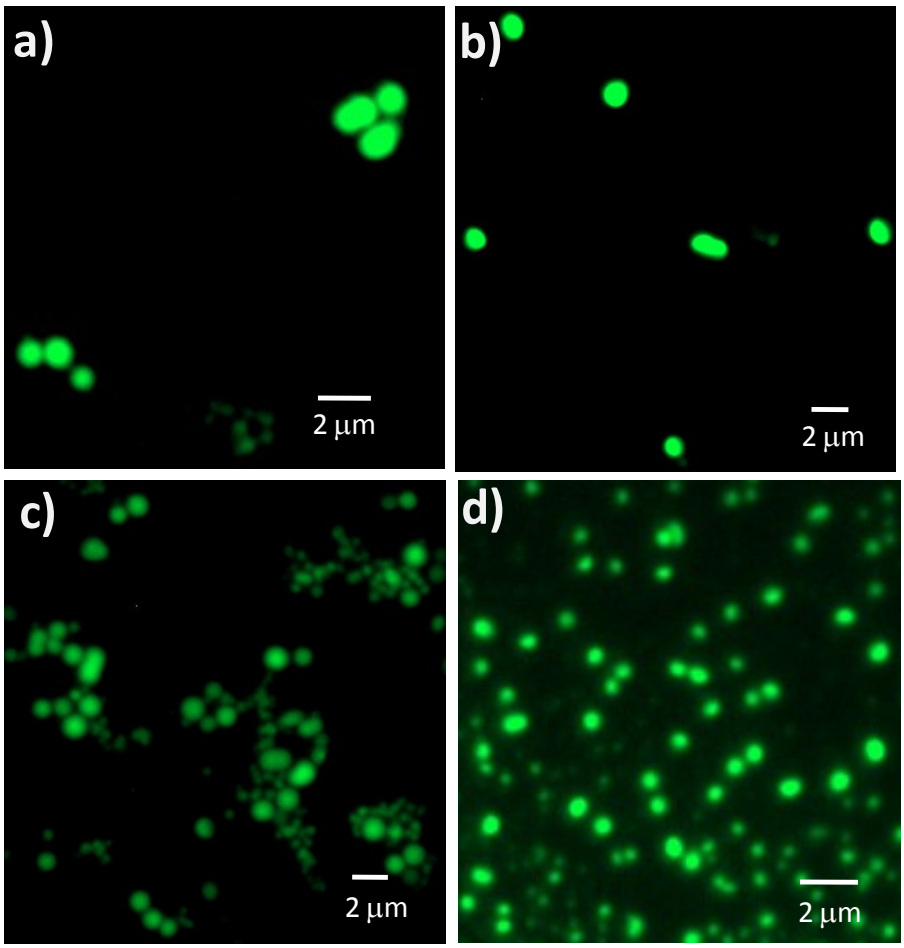


Fig. S6 Fluorescence microscopic images of curcumin-loaded vesicles showing bright green fluorescence of curcumin.

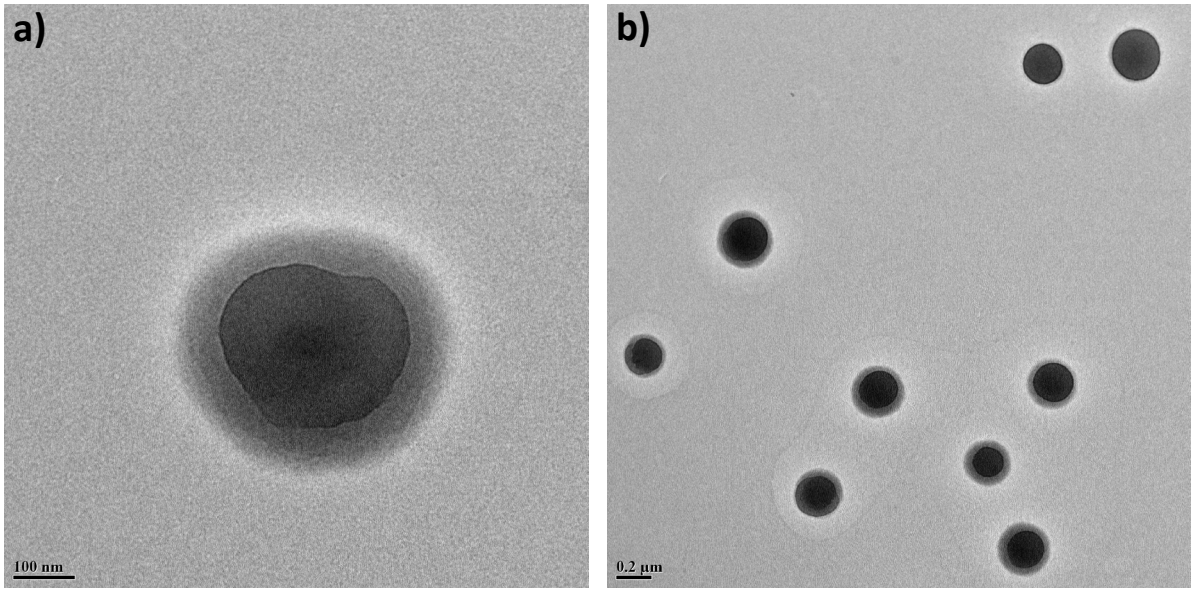


Fig. S7 TEM images of curcumin-loaded vesicles.

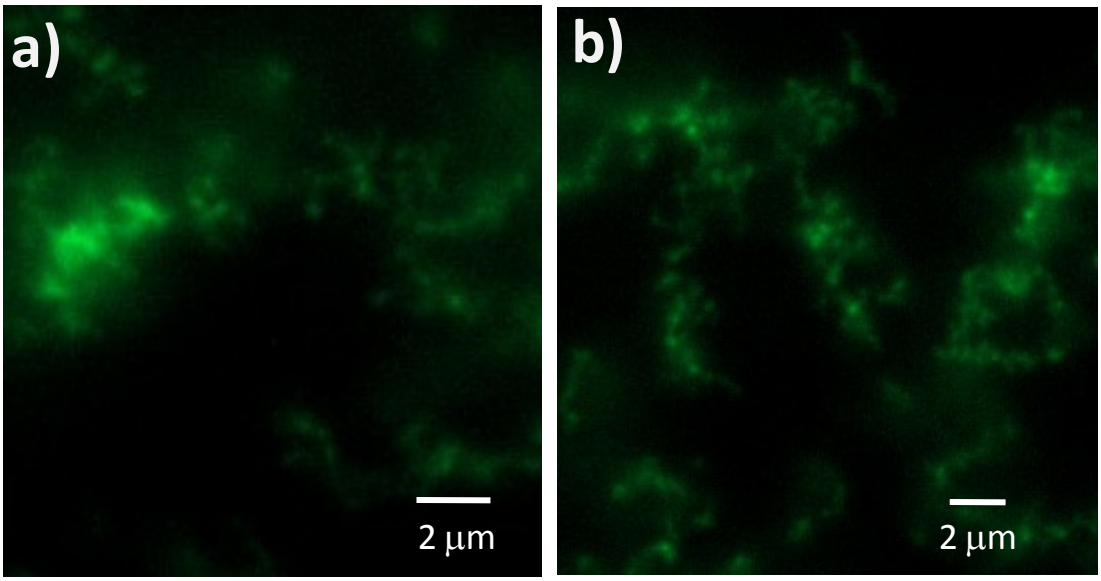


Fig. S8 Fluorescence microscopic images of ruptured vesicles after addition of TBAF.

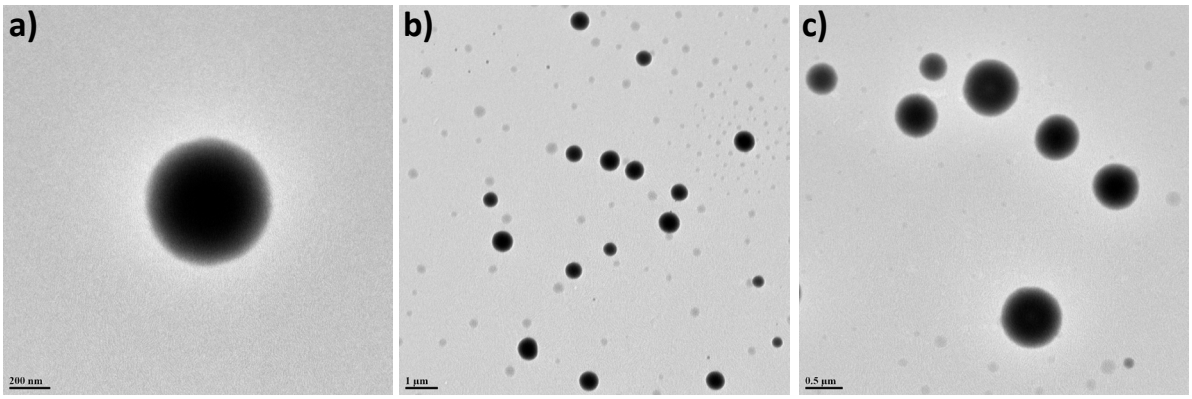


Fig. S9 TEM images of the spherical assembly formed by compound **1** in MeOH at a concentration of 1 mg/1 mL.

Reference:

1. S. S. Kale, A. S. Kotmale, A. K. Dutta, S. Pal, P. R. Rajamohanan and G. J. Sanjayan, *Org. Biomol. Chem.,* 2012, **10**, 8426.