Electronic Supplementary Information (ESI)

Spontaneous vesiculation: A mechanistic insight from the study of hybrid peptide molecules

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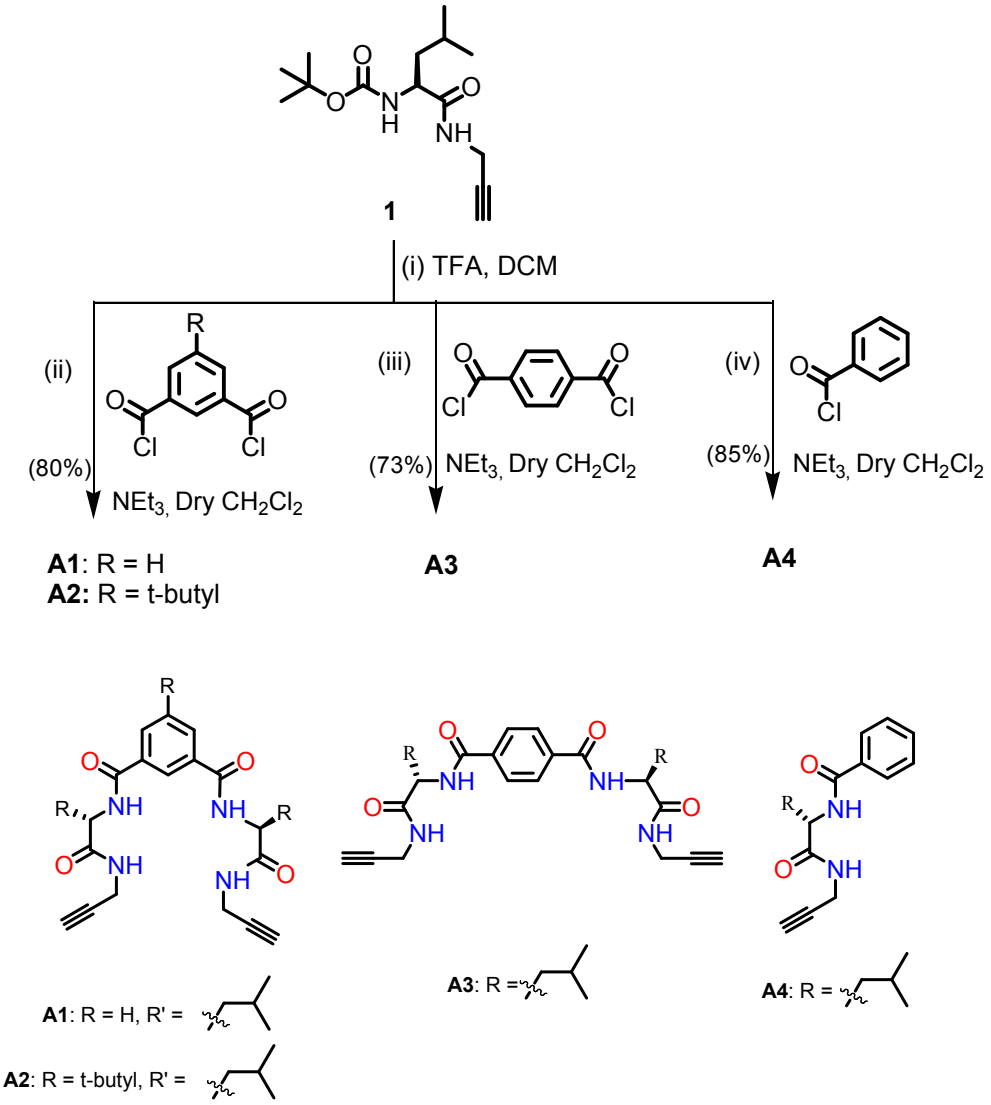
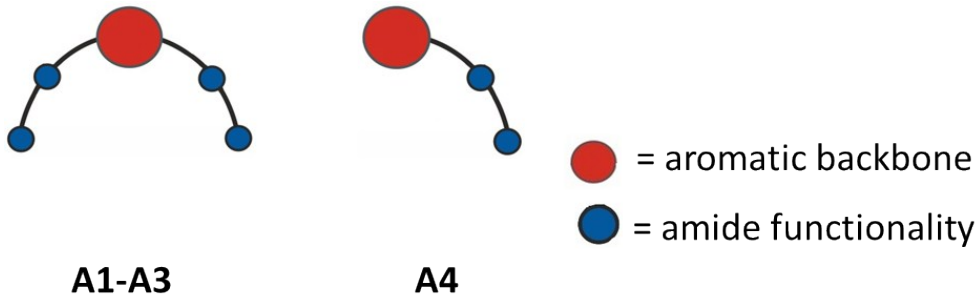
I. Synthesis and characterization:

All solvents used in the reactions were dried. Amino acid L-Leucine was purchased from SRL India. Reactions were monitored by thin layer chromatography (TLC), and purifications were done by silica gel (100-200 mesh) column chromatography. Silica gel G (Merck) was used for TLC. Melting points were recorded on a Fisher-Scientific melting point apparatus and were uncorrected. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets. ^{1}H NMR spectra were recorded on Brucker-DPX-300 spectrometer using tetramethylsilane (^{1}H) as an internal standard. High Resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF-QII model using ESI technique. Optical rotations were measured using a Rudolph Research Analytical Autopol® V Polarimeter. Specific rotation was reported with concentrations in gram/100mL.

(a)



(b)



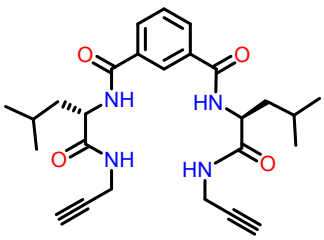
Scheme S1: (a) Synthesis of hybrid peptide molecules **A1-A4**. (b) Cartoon representation of hybrid peptide molecules **A1-A4**.

General procedure for the synthesis of A1-A4:

Compound 1 and A2 were synthesized based on the reported procedure.[1]

Compound **1** was dissolved in 50% TFA in dry CH_2Cl_2 (~ 4mL), and stirred for 4h at 0 °C. It was subjected to vacuum to obtain the amine. Amine was then dissolved in dry CH_2Cl_2 (~50 mL), added triethylamine (2 equivalents) stirred for 5 minutes, and the corresponding acid chloride in dry CH_2Cl_2 (~50 mL) was added dropwise over 20 minutes. The reaction mixture was stirred for 12h at 0 °C, diluted with CH_2Cl_2 (50 mL), washed sequentially with 2N H_2SO_4, $NaHCO_3$ and water. The organic layer was collected, dried over anhyd. Na_2SO_4 and evaporated to the yield desired product.

Data of **A1**:



Yield: 80 %.

Appearance: White solid

Mp: 276-278 ˚C.

$[\alpha]_D^{34}$: -3 (c = 0.1, CH_3OH)

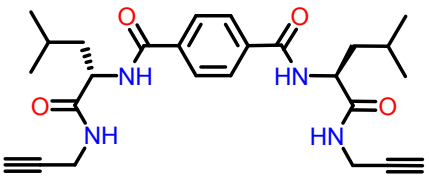
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (br d, 12H), 1.70-1.98 (br m, 2H+4H), 2.22 (s, 2H), 4.05 (q, 4H), 4.72 (m, 1H), 7.10-7.40 (br m, 2H+1H), 7.66 (br s, 2H), 7.81 (d, J = 7.5 Hz, 2H), 8.06 (s, 1H).

^{13}C NMR (75 MHz, DMSO-d_6): δ 21.9, 23.5, 24.9, 28.5, 52.2, 73.3, 81.6, 127.4, 128.5, 130.7, 134.7, 166.5, 172.4.

IR (KBr): 3298, 3072, 2957, 2350, 1647, 1522, 1248, 1170, 1034 cm^{-1}.

HRMS: Calcd for $C_{26}H_{34}N_4O_4Na$ m/z =489.2472, found m/z = 489.2472.

Data of **A3**



Yield: 85 %.

Appearance: White solid

Mp: 217-219 ˚C.

$[\alpha]_D^{34}$: +1 (c = 0.1, CH_3OH)

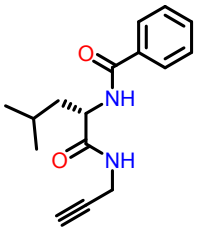
^{1}H NMR (300 MHz, DMSO-d_6): δ 0.73-.98 (br m, 12H), 1.49-1.78 (br m, 2H+4H), 3.10 (s, 2H), 3.87 (d, J = 3 Hz, 4H), 4.522 (br m, 2H), 7.98 (s, 4H), 8.47 (m, 2H), 8.59 (d, J = 8.1 Hz, 2H).

^{13}C NMR (75 MHz, DMSO-d_6): δ 21.4, 23.1, 24.4, 28.0, 51.7, 72.9, 81.2, 127.4, 136.3, 165.7, 172.0.

IR (KBr): 3281, 3069, 2952, 2369, 1631, 1544, 1336, 1288, 1117 cm^{-1}.

HRMS: Calcd for $C_{26}H_{34}N_4O_4Na$ m/z =489.2472, found m/z = 489.2474.

Data of **A4**



Yield: 85 %.

Appearance: White solid

Mp: 147-149 ˚C.

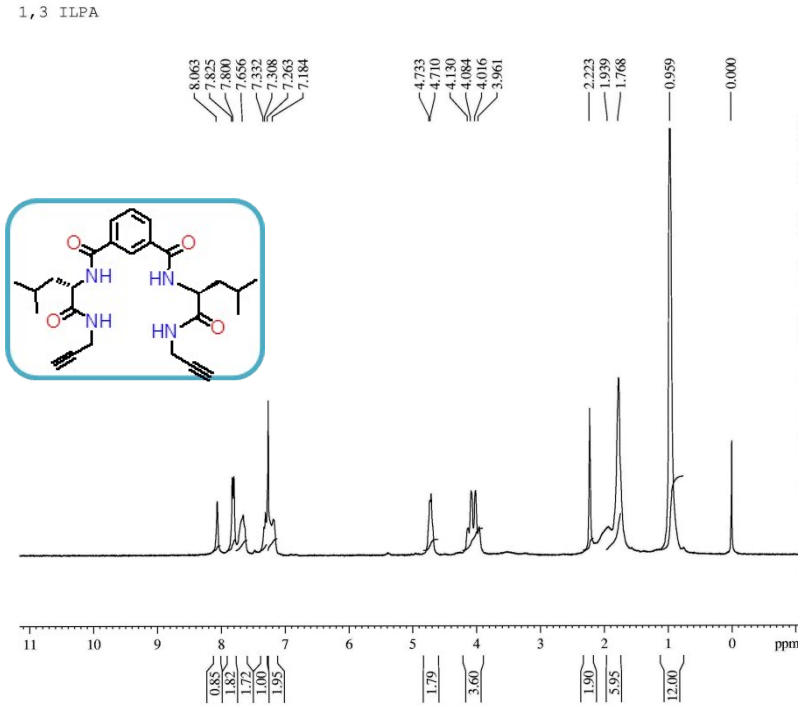
$[\alpha]_D^{34}$: +9 (c = 0.1, CH_3OH)

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (br d, 6H), 1.60-1.90 (br m, 1H+2H), 2.20 (s, 1H), 4.03 (q, 4H), 4.73 (m, 1H), 6.70 (m, 4H), 7.43 (t, 2H), 7.50 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 7.2 Hz, 2H).

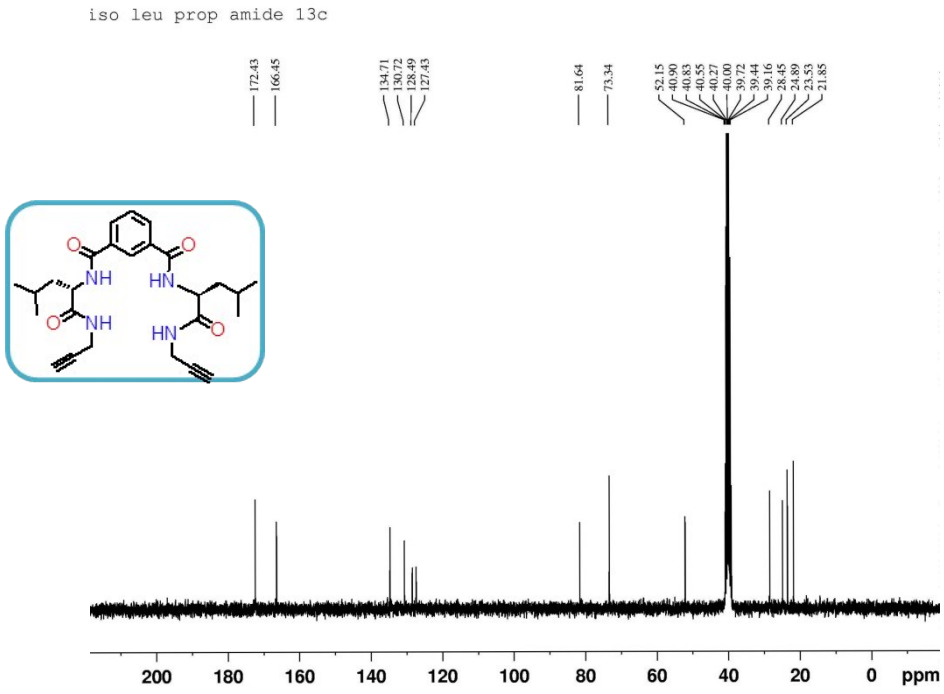
^{13}C NMR (75 MHz, $CDCl_3$): δ 22.3,22.9, 24.9, 29.1, 52.1, 71.5, 79.3, 127.2, 128.5, 131.8, 133.7, 167.6, 172.2.

IR (KBr): 3285, 3071, 2957, 2350, 1653, 1557, 1247, 1176 cm^{-1}.

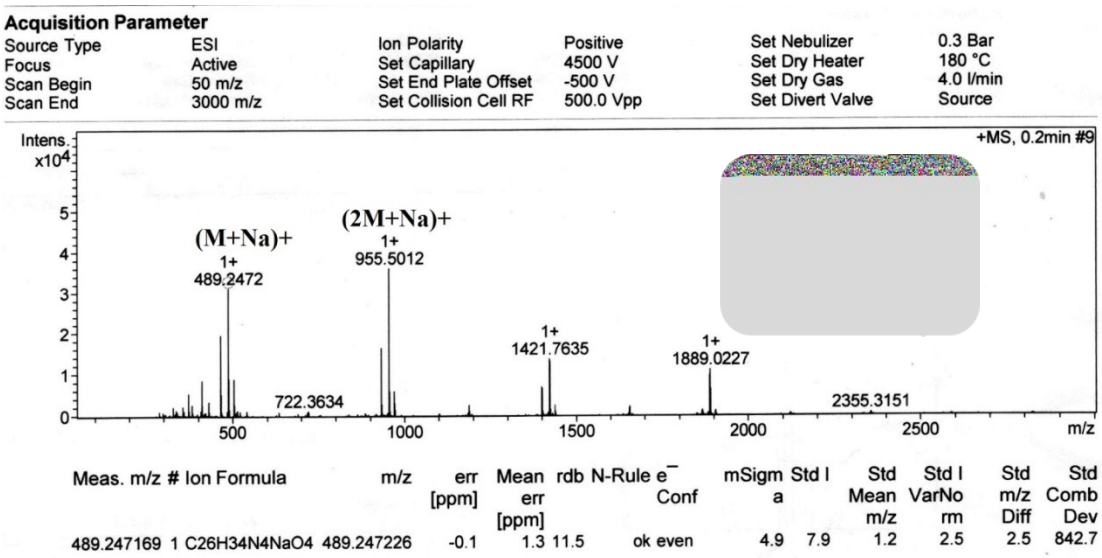
HRMS: Calcd for $C_{30}H_{42}N_4O_4Na$ m/z = 295.1417 found m/z = 295.1420



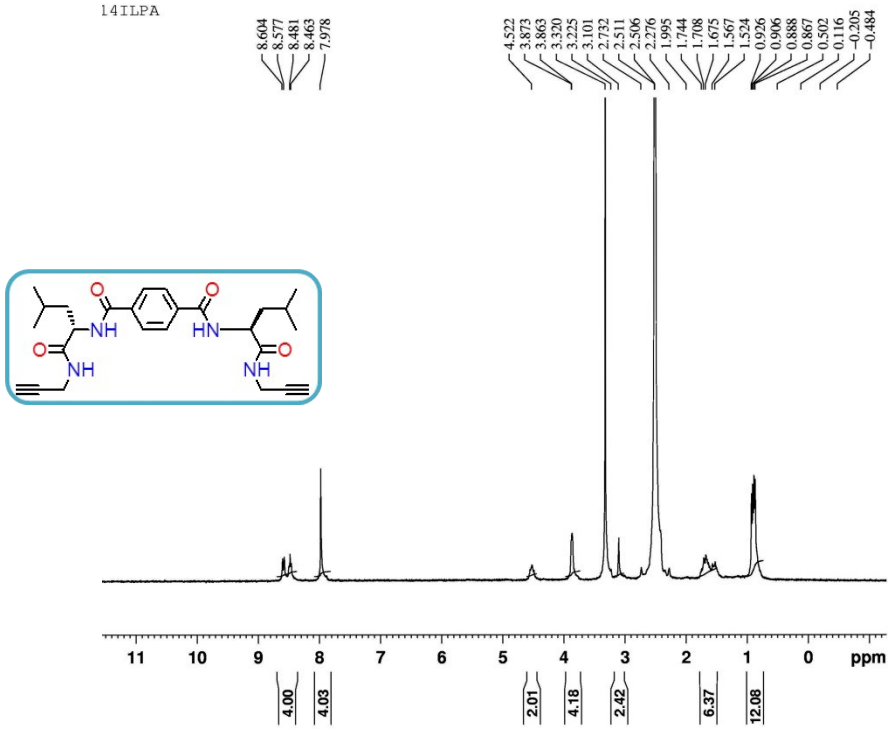
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of A1



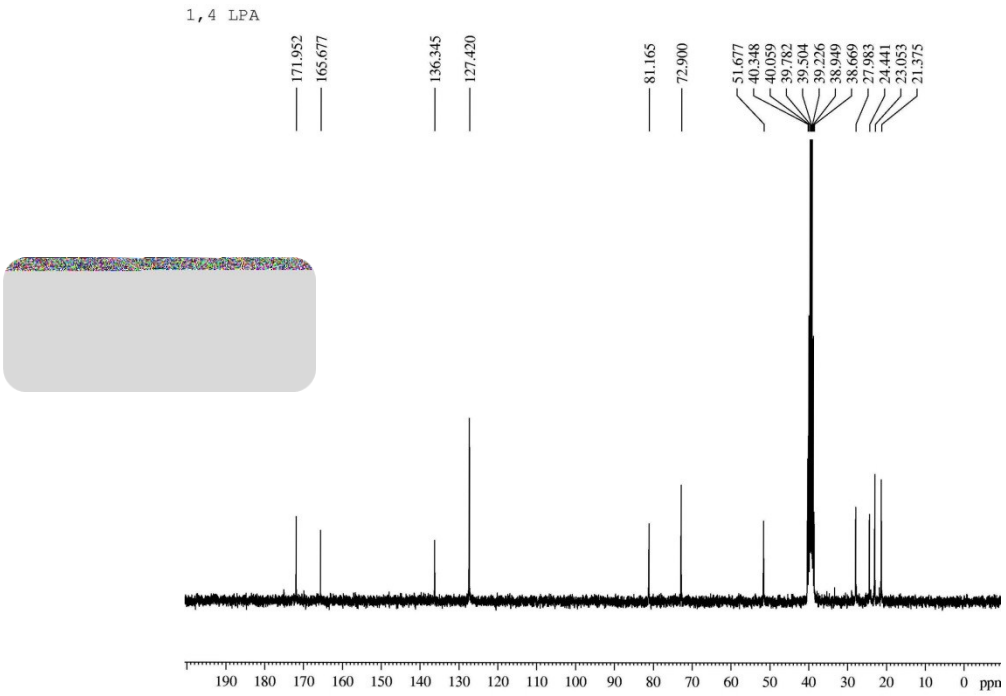
^{13}C NMR (75 MHz, DMSO-d_6) spectrum of A1



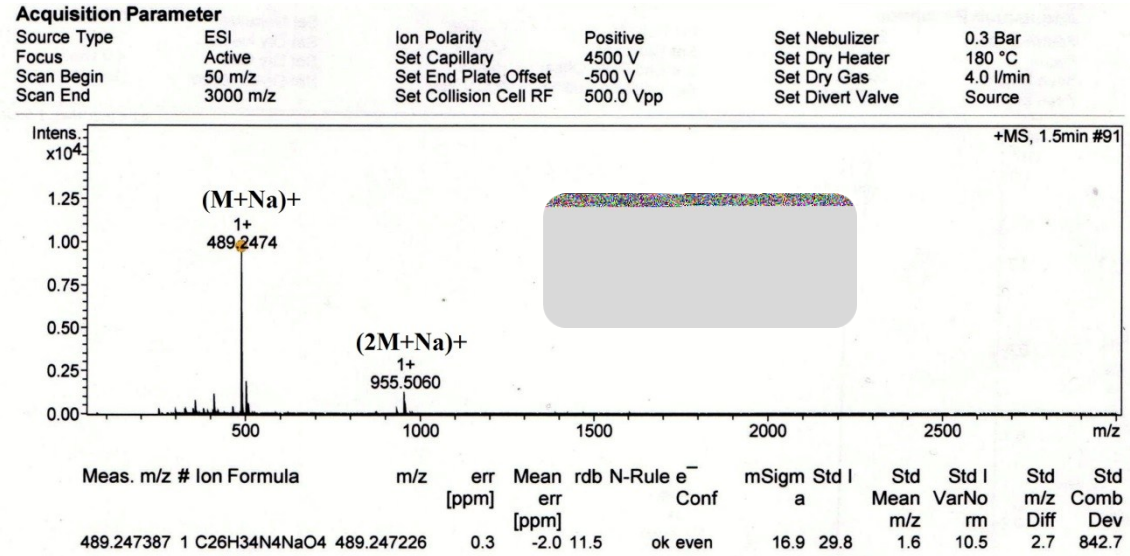
Mass spectrum of A1



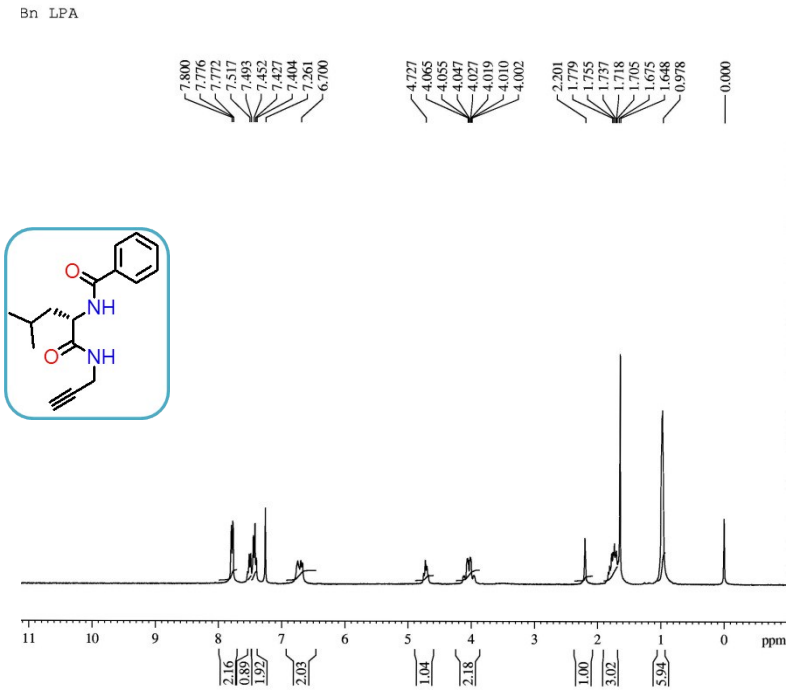
^{1}H NMR (300 MHz, DMSO-d_6) spectrum of A3



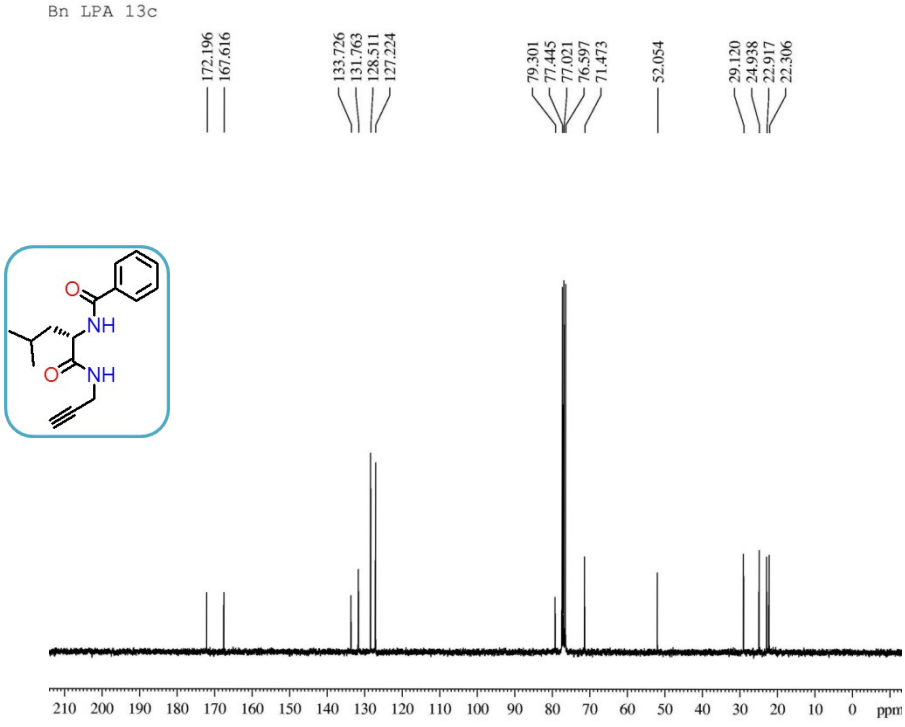
^{13}C NMR (75 MHz, DMSO-d_6) spectrum of A3



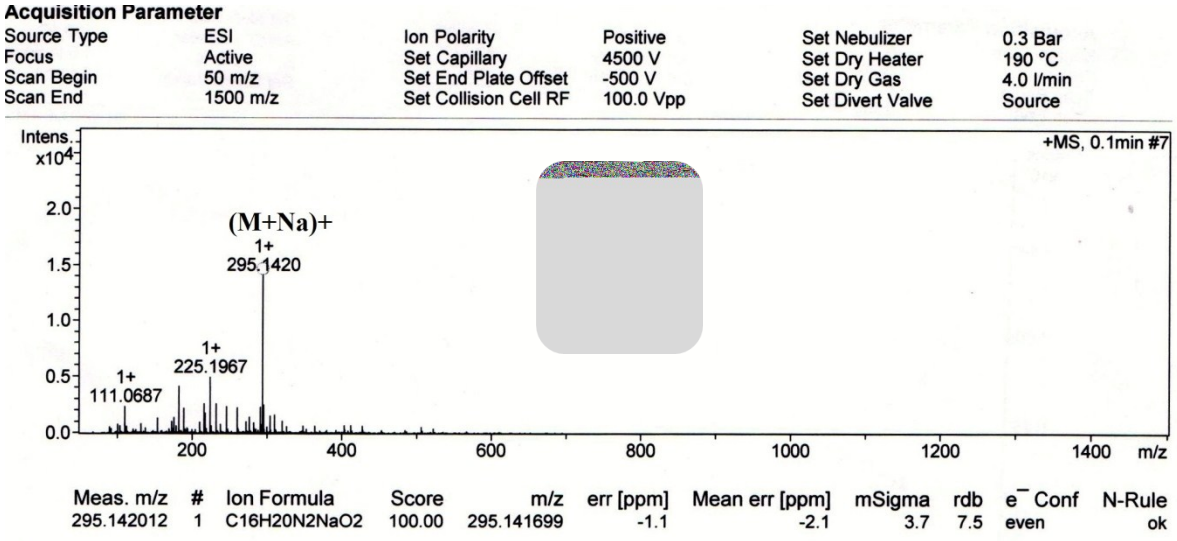
Mass spectrum of A3



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of A4



^{13}C NMR (75 MHz, $CDCl_3$) spectrum of A4



Mass spectrum of A4

III. Microscopic studies:

(a) Scanning Electron Microscopy (SEM)

A 10 µl solution of the compound was put on a fresh piece of glass, which is attached to a stub via carbon tape. The sample was allowed to evaporate at room temperature and coated with ~10nm of gold. All the samples were analyzed using ZEISS EVO 50 SEM.

(b) Atomic Force Microscopy (AFM)

Bruker Dimension Icon atomic force microscope was used for imaging the samples. About 10 µl aliquot of the sample solution was placed on the freshly cleaved mica and allowed to dry. It was then imaged using tapping mode AFM.

(c) High Resolution-Transmission Electron Microscopy (HR-TEM)

Samples for HR-TEM were prepared by dissolving the compound in 1:1methanol and chloroform mixture. A 2 µl aliquot of the sample solution was placed on a 200 mesh copper grid allowed to dry at room temperature and samples were viewed using a TECHNAI G2 (20S-TWIN) electron microscope.

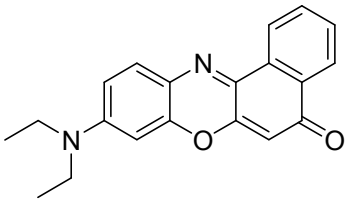
(d) Confocal Microscopy

Individual cover slips were mounted on the stage of an inverted microscope (Olympus), equipped with an water immersion objective (N.A.=1.2, 60x), both of these being components of our custom-built confocal microscope (NANONICS, Israel). The tracer dye, fluorescein, was excited with the 488 nm line of a CW argon-ion laser (Modu-Laser, Model: Stellar Pro. Select) while NR and R6G were excited by the 514 nm laser line. The laser was guided onto the dichroic using a single mode fiber. The fluorescence from the

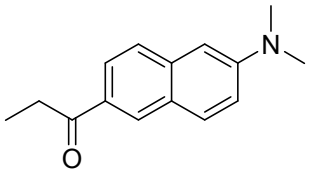
samples was collected with the same objective and focused onto an avalanche photo diode (Model: SPCM-AQRH-14, CANADA), fitted with a 50 μm confocal pinhole, using a non-polarizing beamsplitter. The samples were scanned using a separate *x-y* closed loop piezo scanner (Nanonics Imaging). For all experiments, piezo scanning and data acquisition were controlled with a HV Piezo Driver (Nanonics Imaging) and software (NWS11). For image acquisition the size of the filed of view, resolution points and time exposure per points were 80 x 80 μM, 300 and 3 ms/point respectively.

Materials

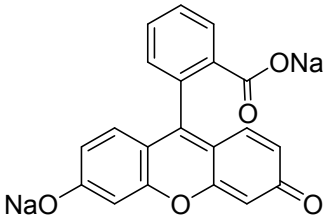
Nile red (9-Diethylamino-benzo[α]phenoxazin-5-one), PRODAN (1-(6-Dimethylamino-naphthalen-2-yl)-propan-1-one) and fluorescein sodium salt **(**2-(3-Oxo-6-oxydo-3H-xanthén-9-yl)benzoate di sodium**)** and rhodamine 6G were purchased from Sigma-Aldrich Chemical Co. (USA) and were used as received.



(A) Nile red (NR)



(B) PRODAN



(C) Fluorescein sodium salt

Preparation of solutions for confocal imaging

For confocal imaging experiments different concentrations (0.25 mM, 0.5 mM and 1 mM) of **A2** were dissolved in chloroform-ethanol (1:1) mixture after weighing out the appropriate amounts using a Precisa XB 120A (Sweden) analytical balance to get the desired concentration. Fluorescein dye (100 μM) was mixed with each of the solutions and incubated for sufficient time for proper equilibration. A few microliters of these solutions of **A2** were placed on washed cover slips and

allowed to dry. Subsequently, the excess dye was washed away with sufficient amount of water and then the cover slip was dried again prior to imaging. A similar methodology was also followed for the NR and R6G imaging studies.

Dye encapsulation studies

Stock solutions of nile red (NR) and PRODAN were prepared in methanol. The concentrations of NR and PRODAN were measured with a UV-Vis spectrophotometer (Model UV-2600, Shimadzu) in the range of 300-750 nm (for NR) and 200 - 500 nm (for PRODAN). The molar extinction coefficients of NR[2] and PRODAN[3] used were as follows: 45,000 M^{-1} cm^{-1} at 552 nm and 18,400 M^{-1} cm^{-1} at 360 nm in methanol respectively. The solutions of **A2** with various concentrations (0.25 mM, 0.5 mM and 1mM) were prepared by following the abovementioned procedure. Appropriate amounts of the stock solution of the dyes were added to 1 ml of **A2** containing solutions to get the desired concentrations. Absorption spectra of NR and PRODAN in presence of varying concentrations of **A2** were acquired by using 1 cm quartz cuvettes, with temperature being maintained at 25 ºC. The concentrations of NR and PRODAN used were 5 μM and 7 μM respectively.

(e) **Fluorescence Measurements**

All the steady-state fluorescence experiments were carried out on the FLS900 spectrofluorometer (Edinburgh, UK). Emission spectra of nile red containing solutions of **A2** were obtained upon excitation at 500 nm and spectra were collected from 520 to 800 nm. The PRODAN containing solutions of **A2** were excited at 340 nm and the emission spectra were recorded from 360 to 650 nm. The concentrations of NR and PRODAN were kept at 5 μM and 7 μM respectively. The

fluorescence measurements were carried out by using 1 cm quartz cuvettes and the temperature was maintained at 25 ºC using a Peltier based cooler (Quantum Northwest).

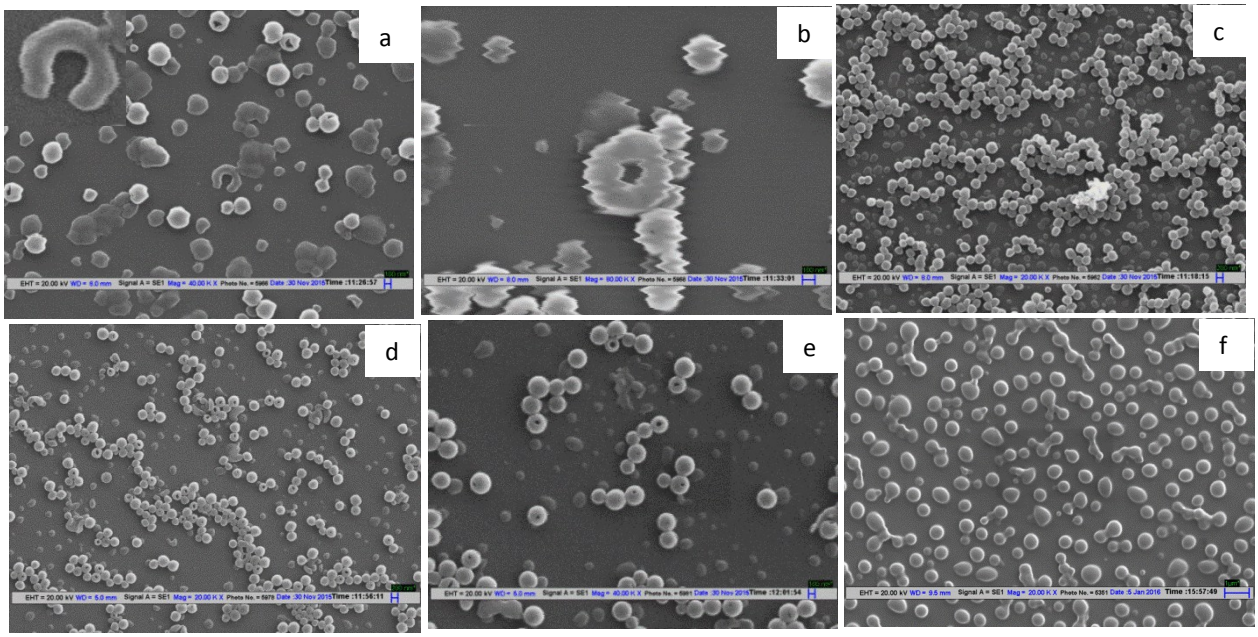


Figure S1: SEM images of (a-b) **A1** at 0.5 mM (Inset: showing single magnified hemi-toroid). (c) **A1** at 1 mM (d-e) **A2** at 0.5 mM. (f) **A2** at 1 mM.

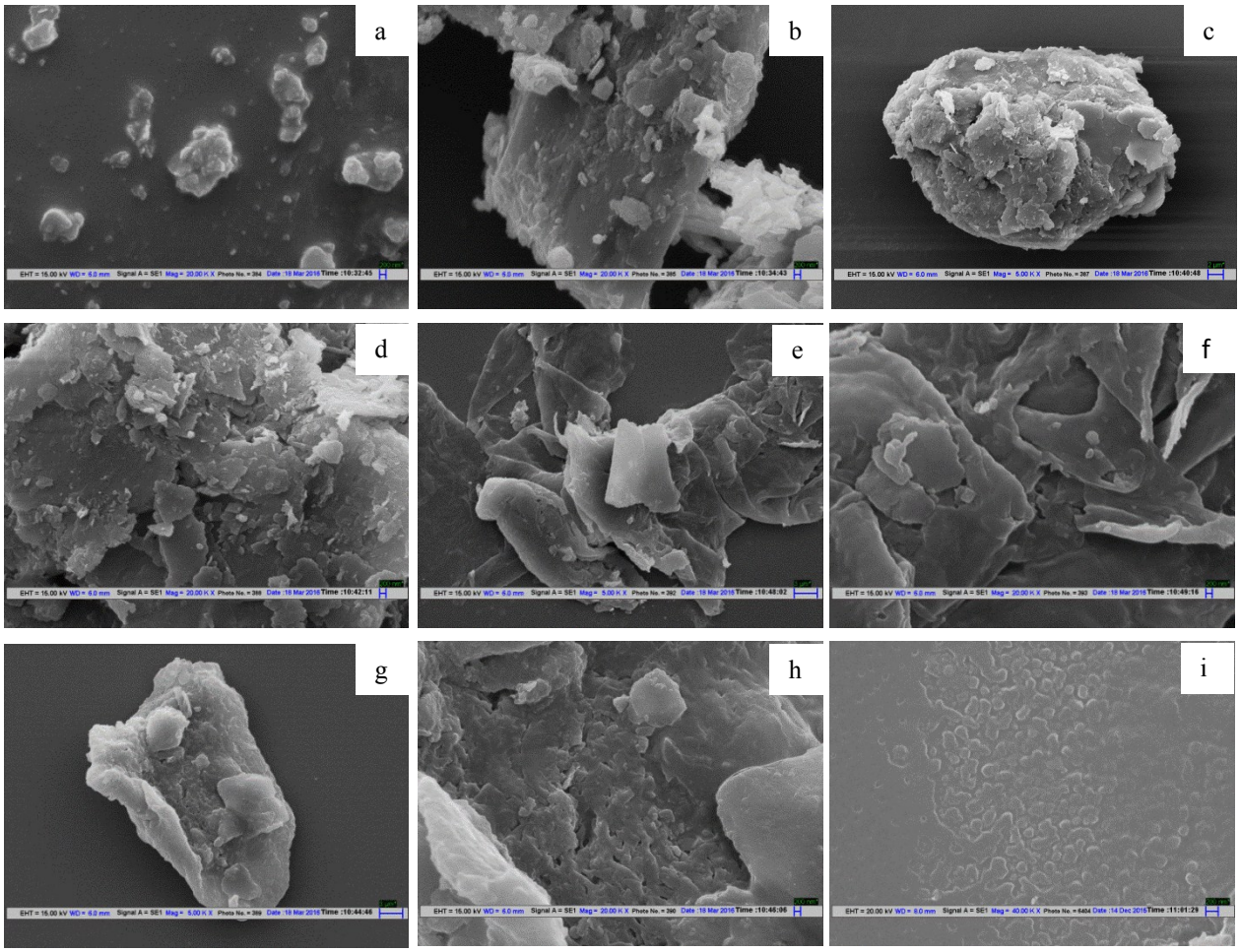


Figure S2: SEM image of **A2** in (a-b) THF: Tetra hydrofuran at 0.5 mM and 1 mM (c-d) Ethyl acetate: $CH_3COOC_2H_5$ at 0.5 mM and 1 mM (e-f) Actetoitrile: CH_3CN at 0.5 mM and 1 mM (g-h) Isopropanol: $(CH_3)_2CHOH$ at 0.5 mM and 1 mM (i-j) Chloroform: $CHCl_3$ (1 mM).

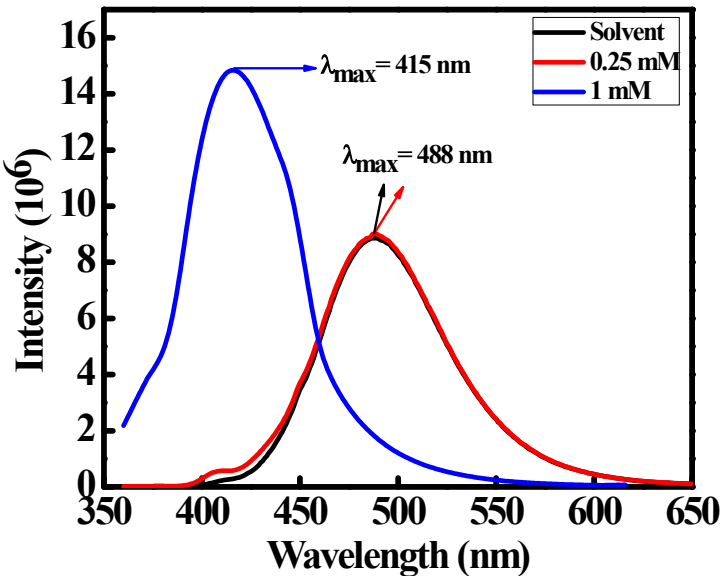


Figure S3: Emission spectra of PRODAN as a function of the concentration of **A2** as indicated in the figure legend. λ_{exc} = 340 nm.

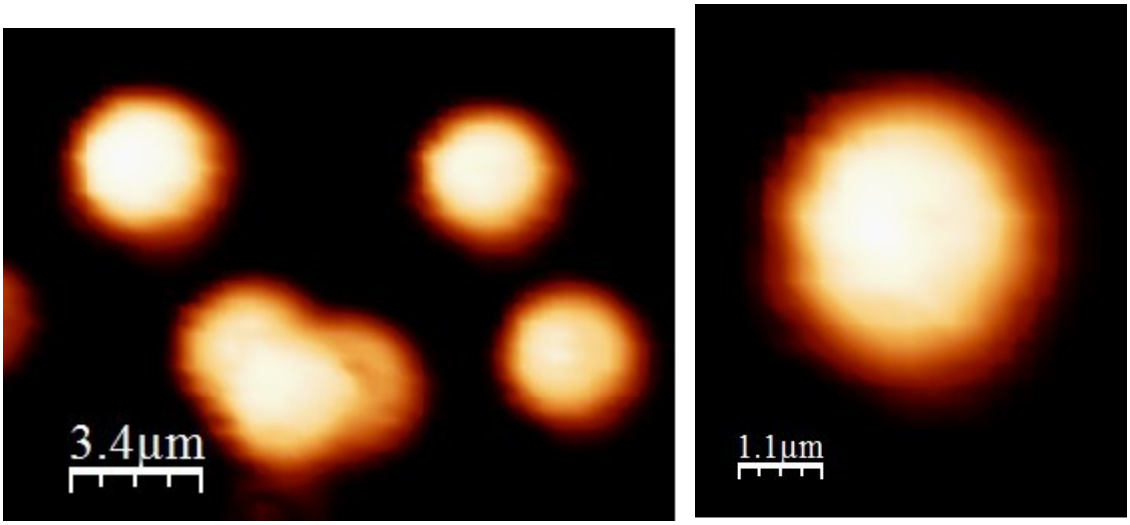


Figure S4: Confocal fluorescence images of the vesicles formed from 1 mM **A2** with the dye used here being R6G. Since R6G has no special affinity for partitioning into hydrophobic regions, thus the whole vesicle remains uniformly illuminated very similar to that observed for fluorescein

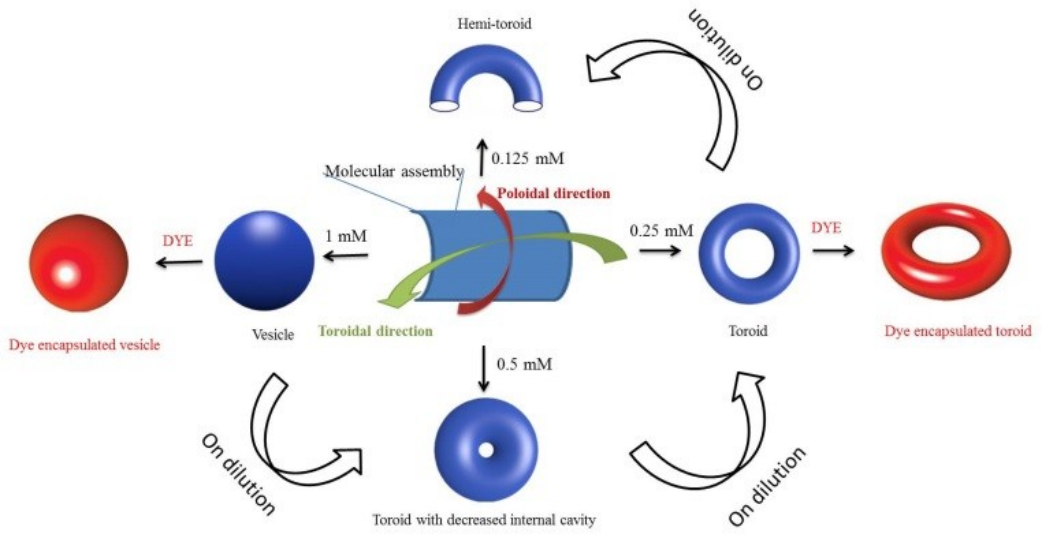


Figure S5: Schematic representation of the mechanism describing self-assembly at different concentrations and showing their encapsulation studies in red color (toroid and vesicle) and effect of dilution on self-assembly.

References:

1. V. Haridas, A. R. Sapala and J. P. Jasinski, *Chem Commun*, 2015, **51**, 6905–6908.

2. M. Adeli, H. Namazi, F. Du, S. H¨onzke, S. Hedtrich, J. Keilitza and R. Haag, *RSC Adv.*, 2015, **5**, 14958-14966.

3. G. Weber and F. J. Farris, Biochemistry, 1979, **18**, 3075-3078.