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Electronic Supplementary Information

Morphology engineering: Dramatic roles of serine and threonine in supramolecular assembly

M.B. Bijesh, Rituraj Mishra, Narayanan D. Kurur and V. Haridas*

Department of Chemistry, Indian Institute of Technology Delhi, New Delh-110016, India

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Experimental section

(a)Synthesis and characterization

All the organic solvents used in the reactions were distilled or dried prior to use. Amino acid L-Serine was purchased from SRL India. Progress of reactions was monitored by thin layer chromatography (TLC) and the purification of compounds was done by silica gel column chromatography. Melting points were recorded on a Fisher-Scientific melting point apparatus. Rudolph Research Analytical Autopol® V Polarimeter was used for measuring optical rotation; where concentrations are given in gram/100 mL. 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. ^{1}H NMR data are reported as s (singlet), d (doublet), br (broad), t (triplet) and m (multiplet), dd (double doublet). High Resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF-QII model using ESI technique. Circular Dichroism (CD) spectra were recorded on AVIV Model 410 spectropolarimeter equipped with a temperature controller. CD spectra were recorded using 1mm length cell.

Microscopic studies

(b) Scanning Electron Microscopy (SEM)

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

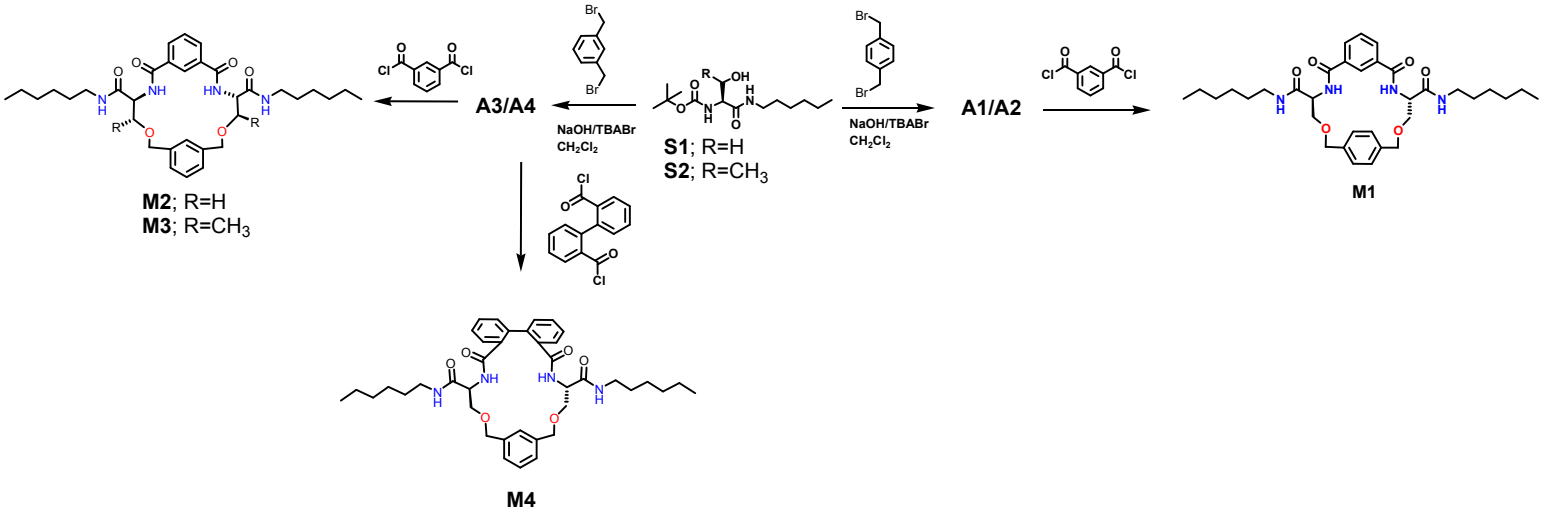
(c) Atomic Force Microscopy (AFM)

Bruker Dimension Icon atomic force microscope was used for imaging. Tapping mode is used for the analysis. About 10µl aliquot of the sample solution was transferred onto freshly cleaved mica and allowed to dry and imaged using AFM.

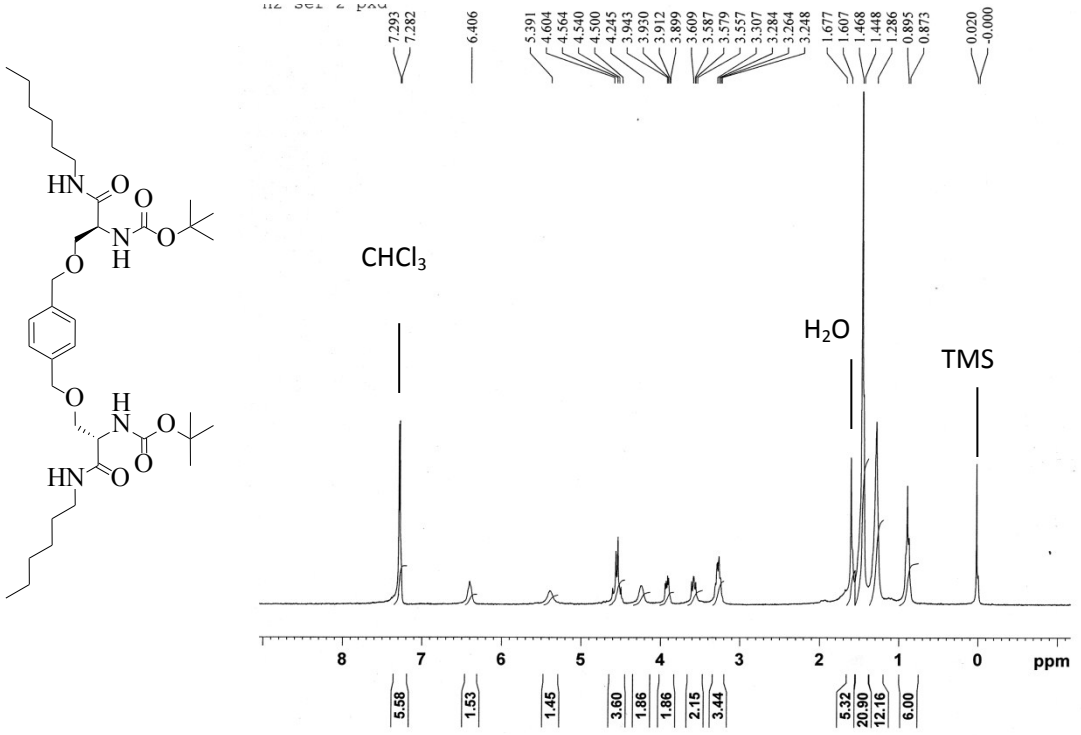
(d) High Resolution-Transmission Electron Microscopy (HR-TEM)

Samples for HR-TEM were prepared by dissolving the compound in methanol. A 2µl aliquot of the sample solution was placed on a 200 mesh copper grid and the excess fluid was removed using a filter paper and samples were viewed using a TECHNAI G2 (20S-TWIN) electron microscope.

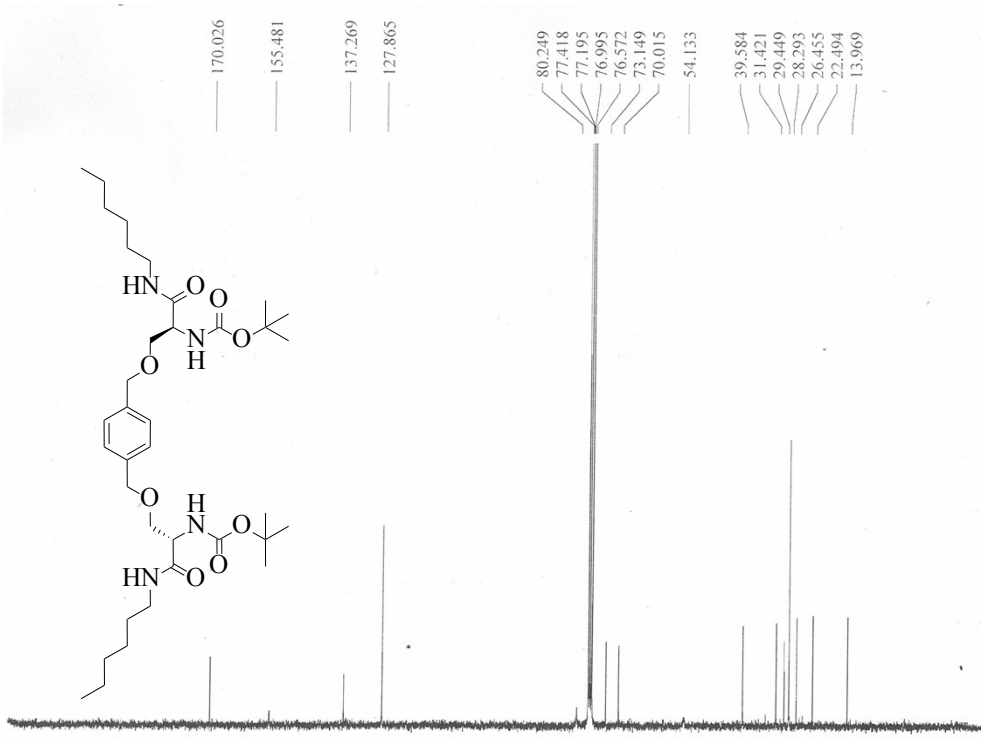
Scheme 1: Synthesis of macrocycles **M1-M4**



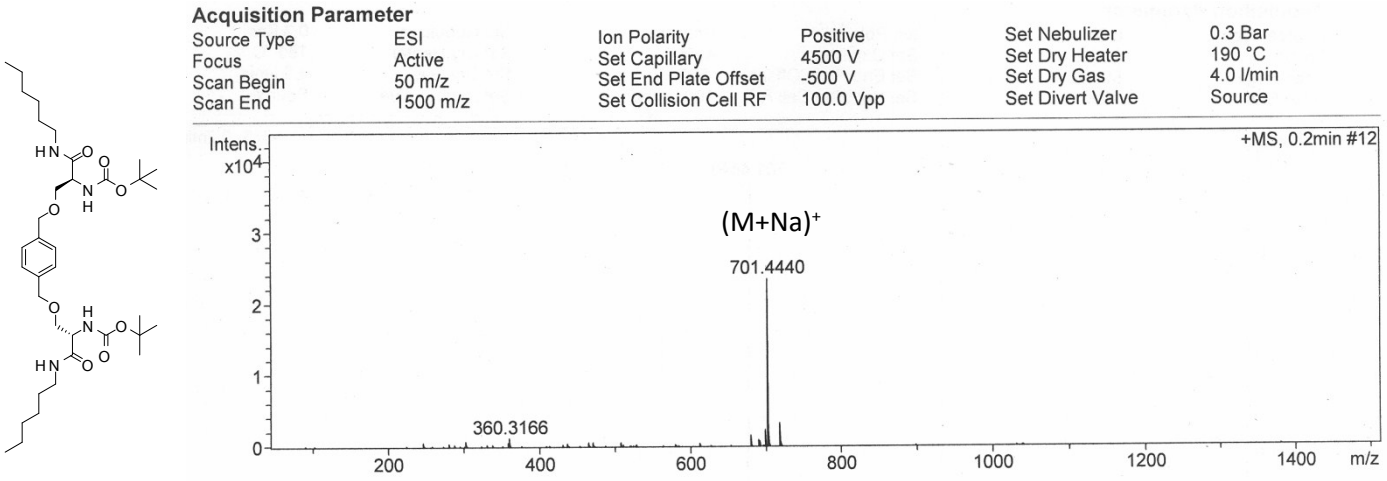
^{1}H NMR (300MHz, $CDCl_3$) spectrum of **A1**



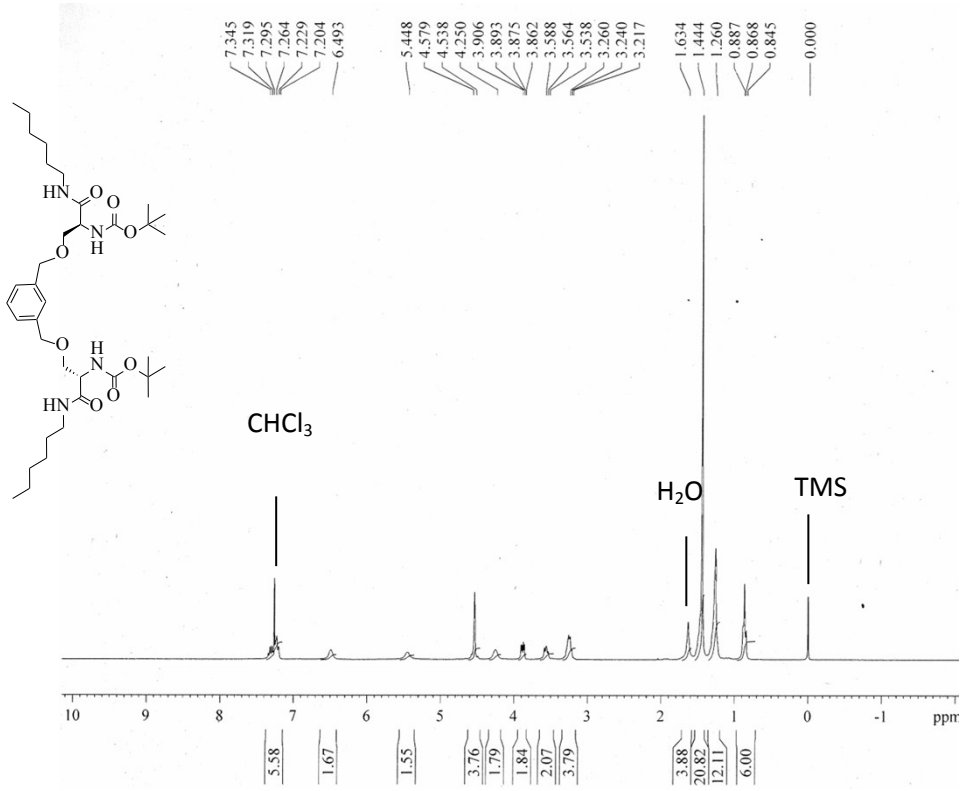
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **A1**



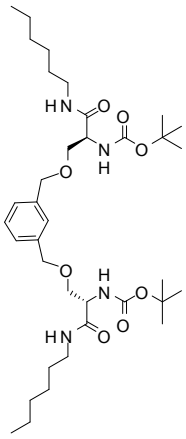
HRMS spectrum of **A1**

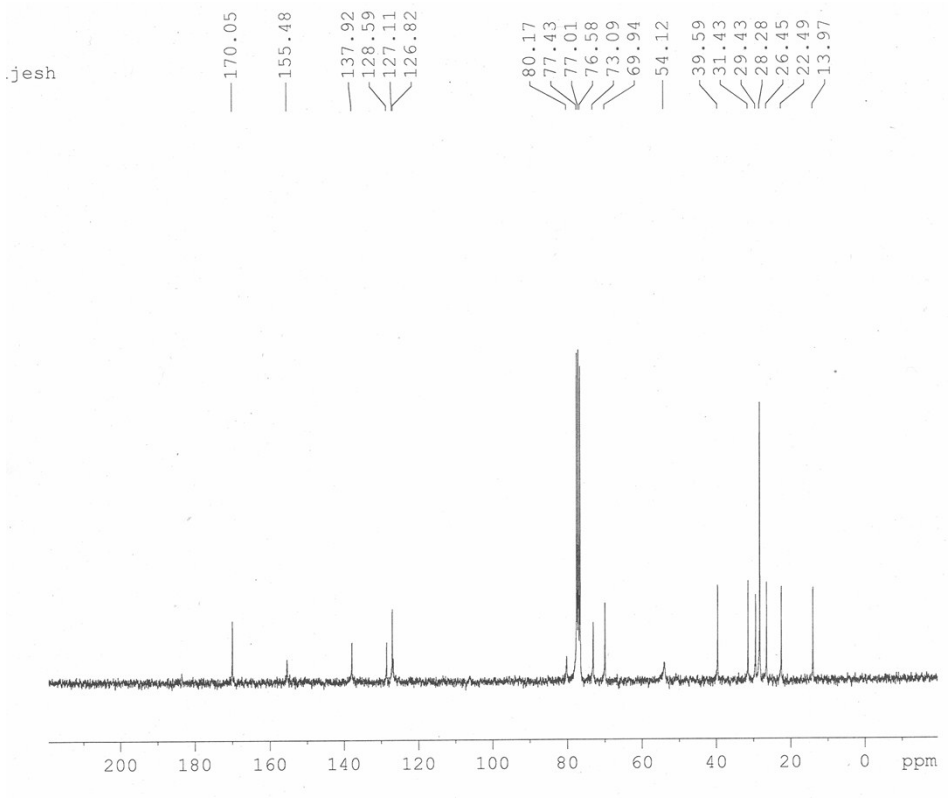


^{1}H NMR (300MHz, $CDCl_3$) spectrum of **A3**

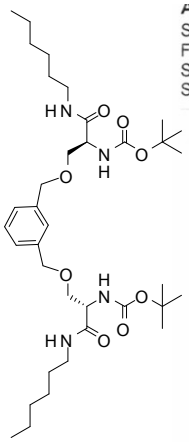


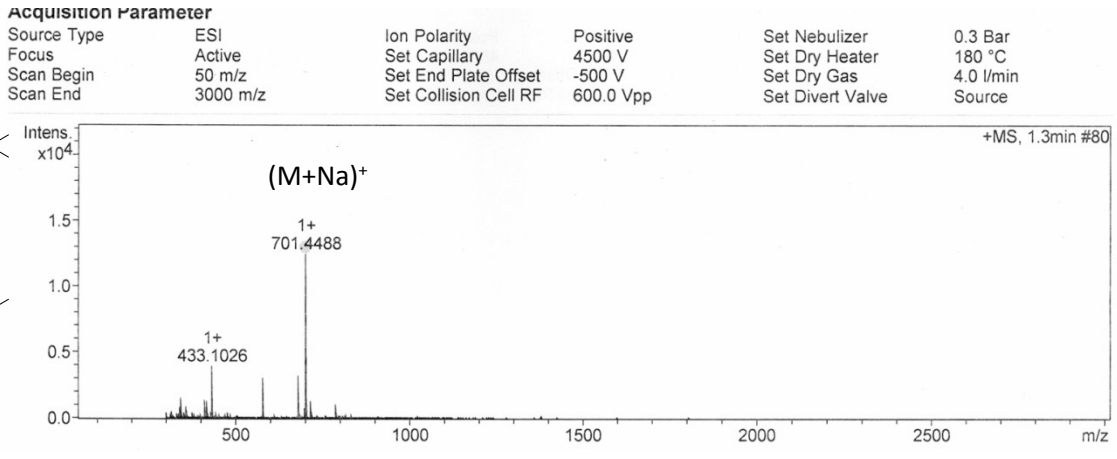
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **A3**



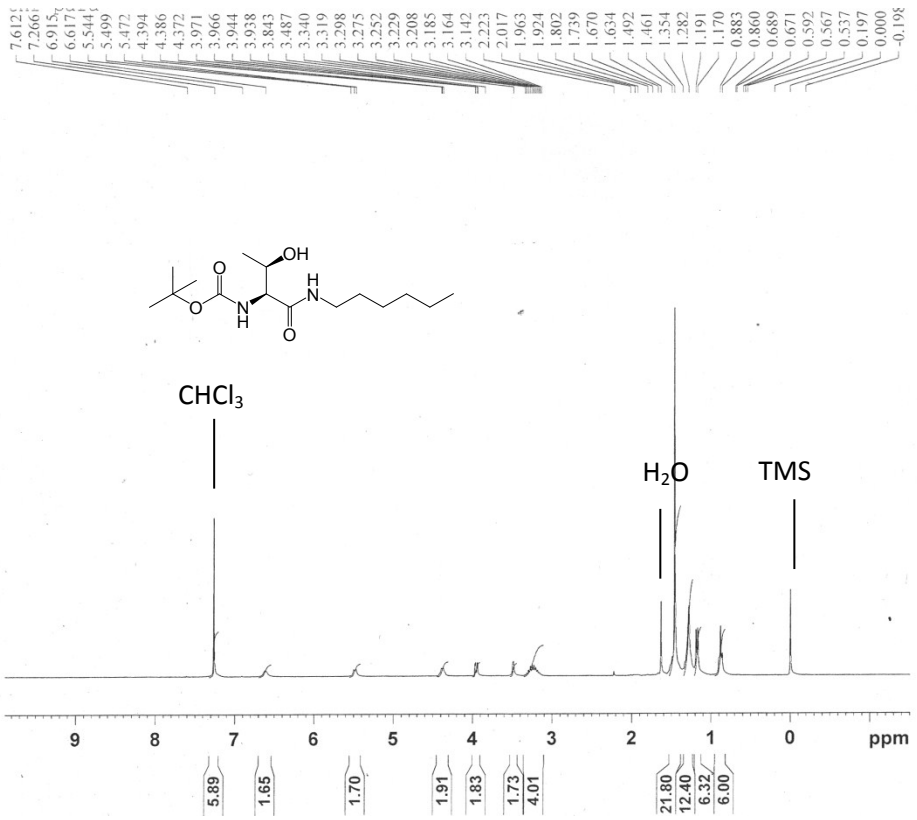


HRMS spectrum of **A3**

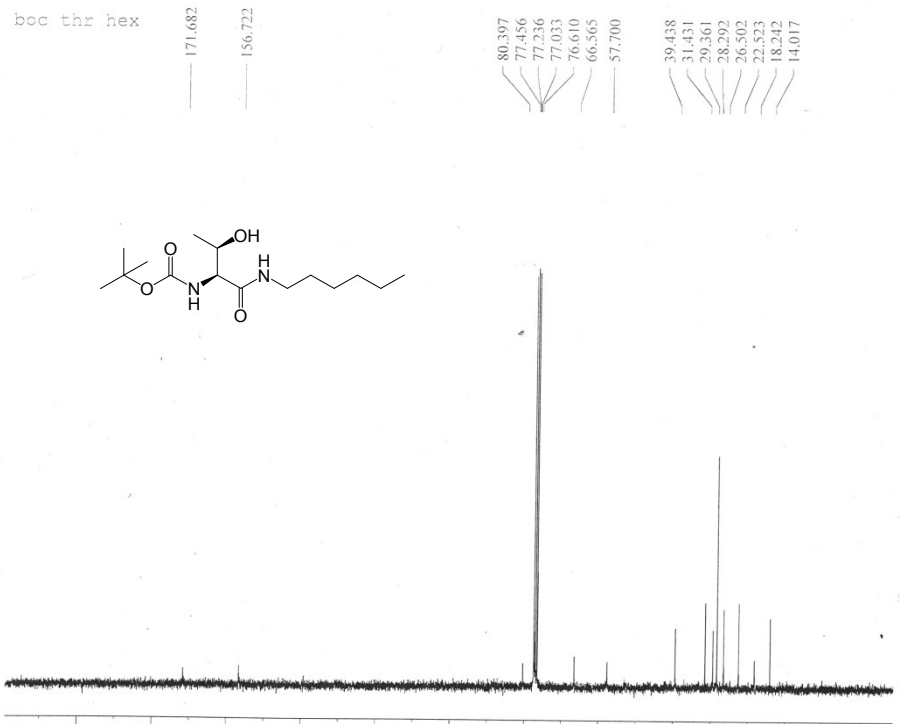




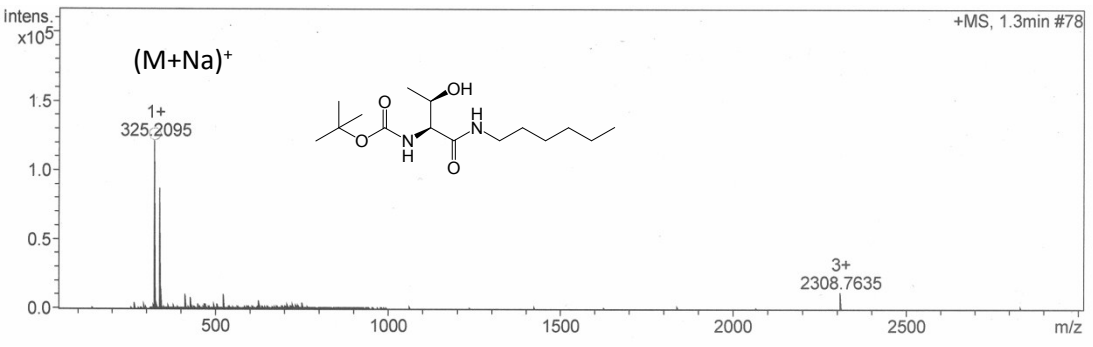
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **S2**



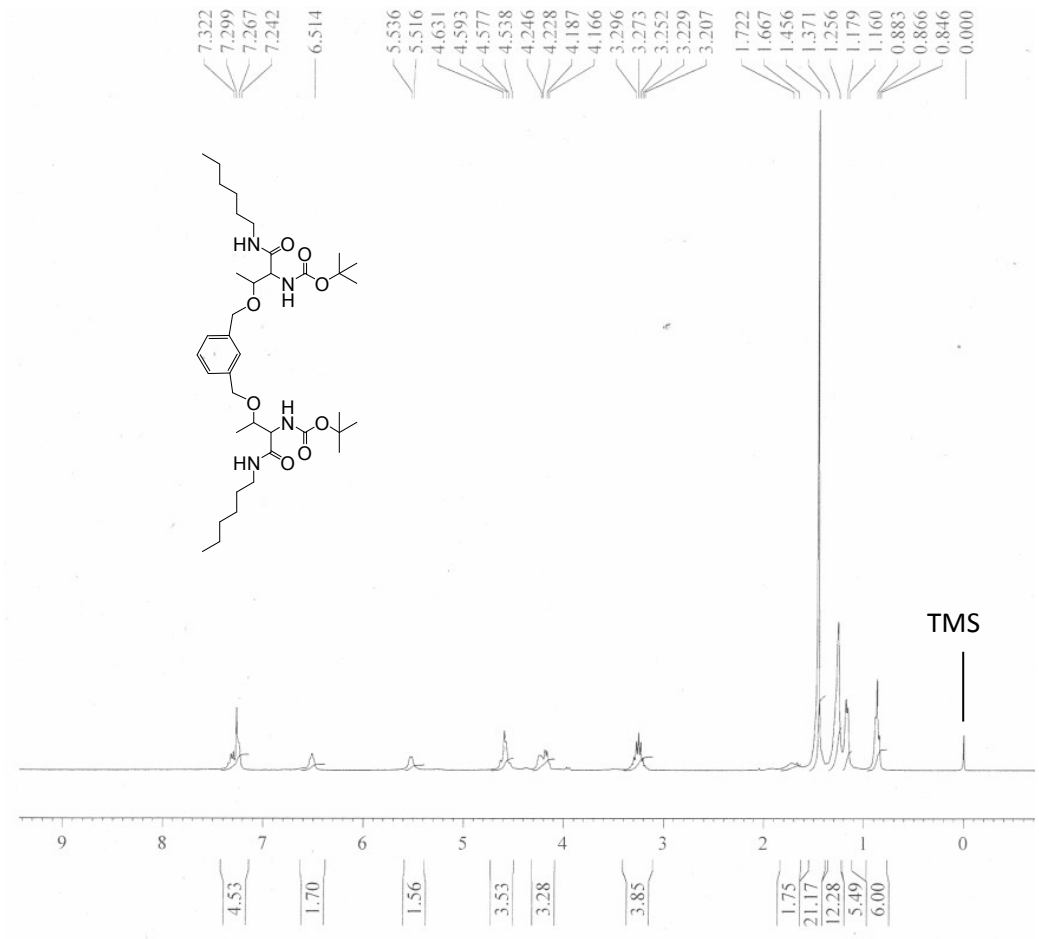
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **S2**



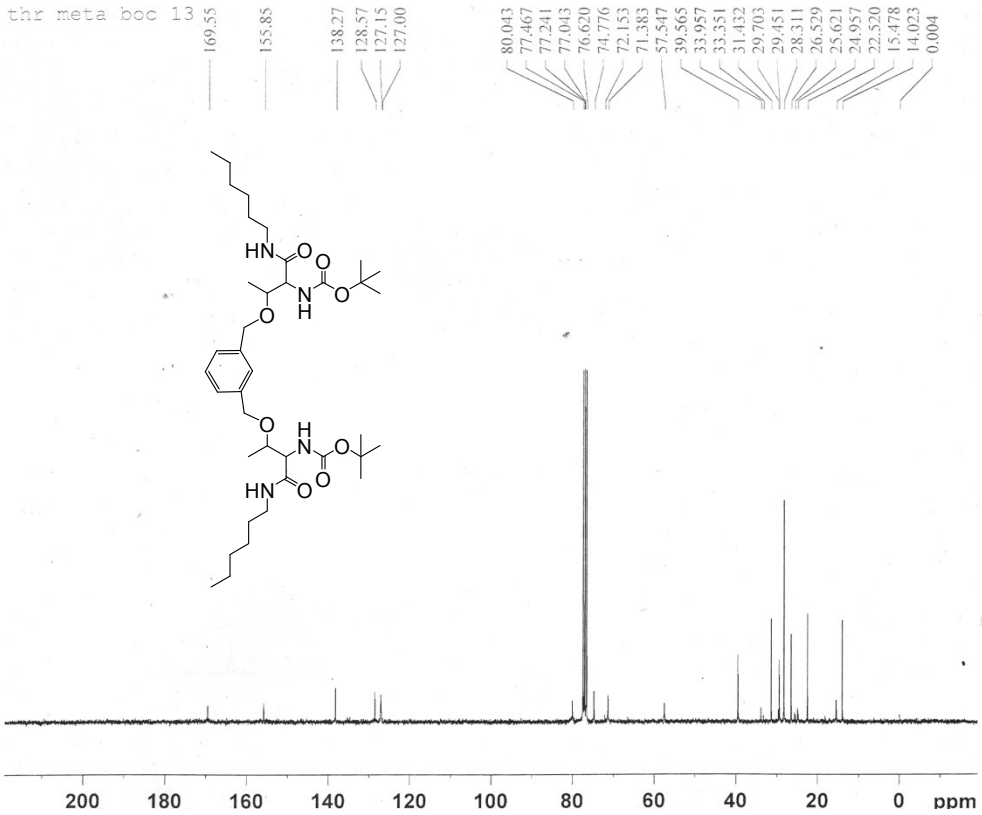
HRMS spectrum of **S2**



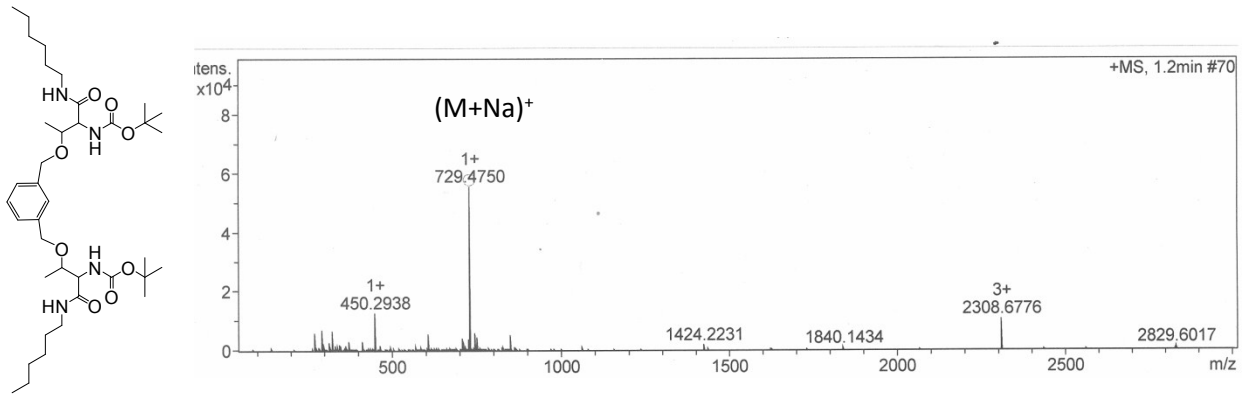
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **A4**



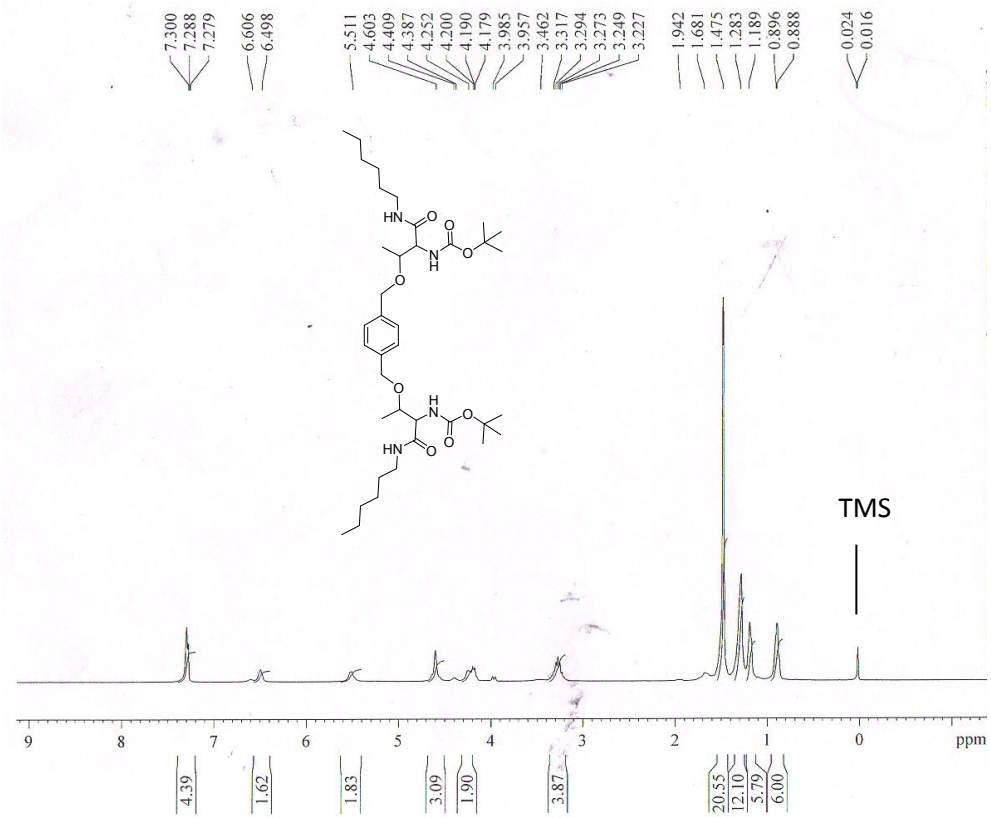
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **A4**



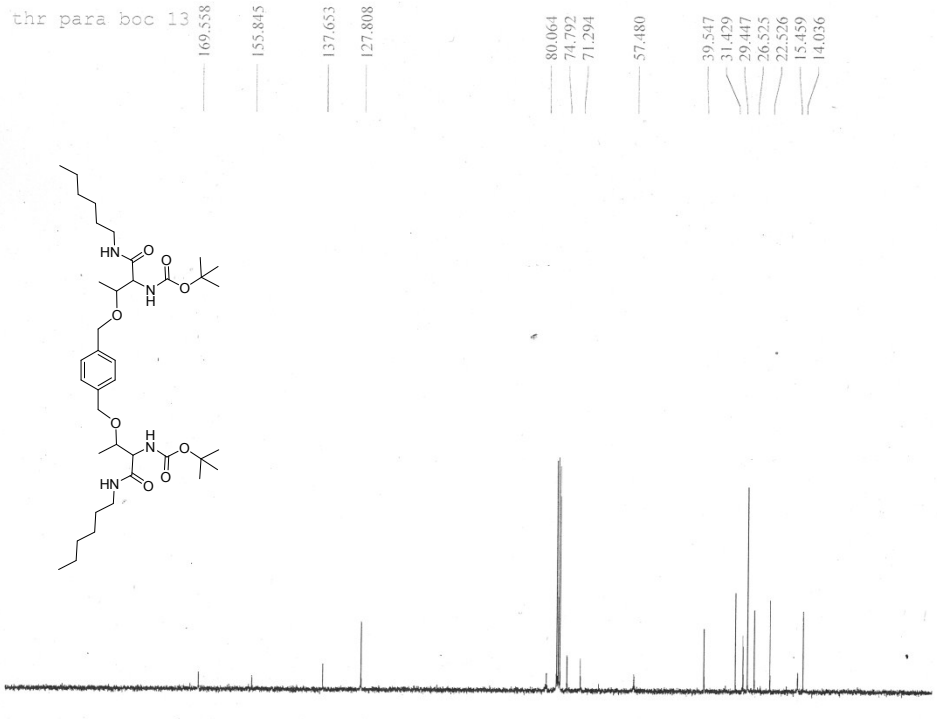
HRMS spectrum of **A4**



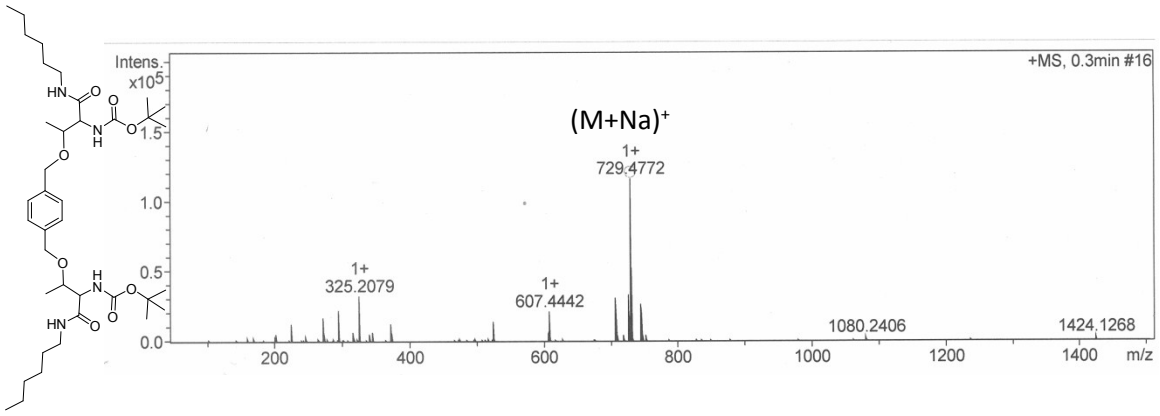
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **A2**



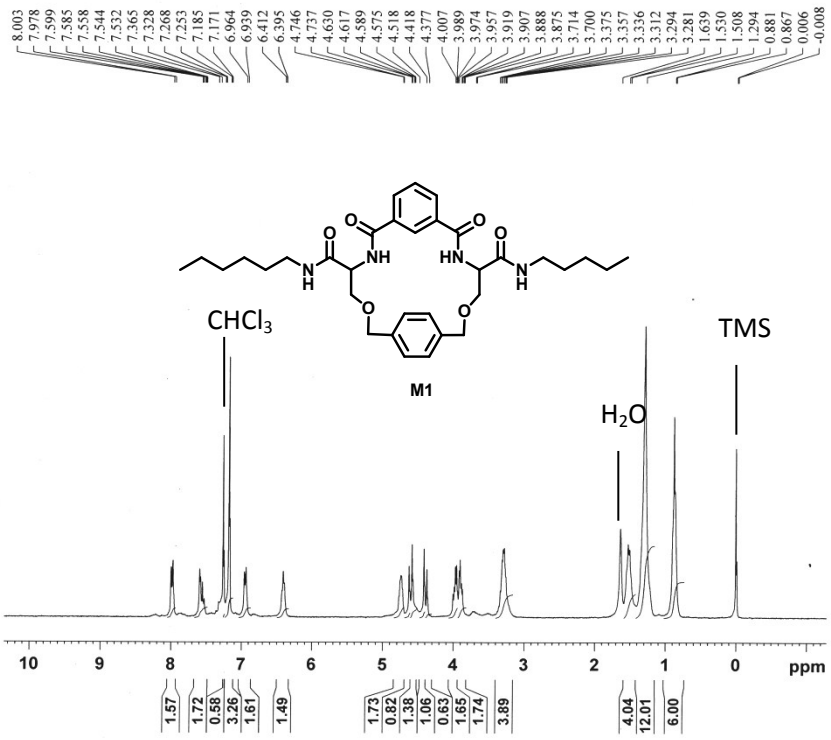
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **A2**



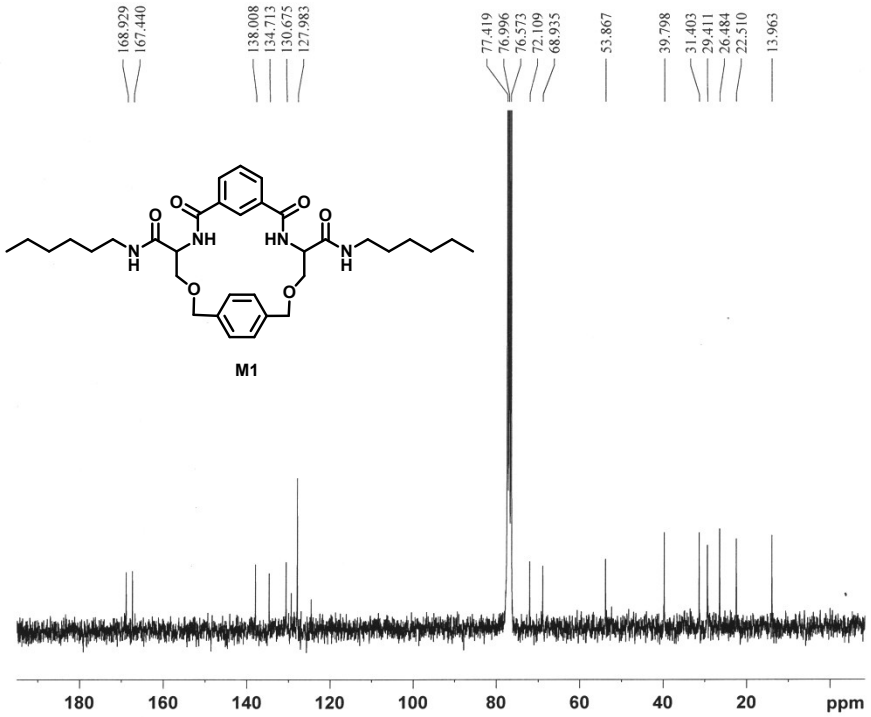
HRMS spectrum of **A2**



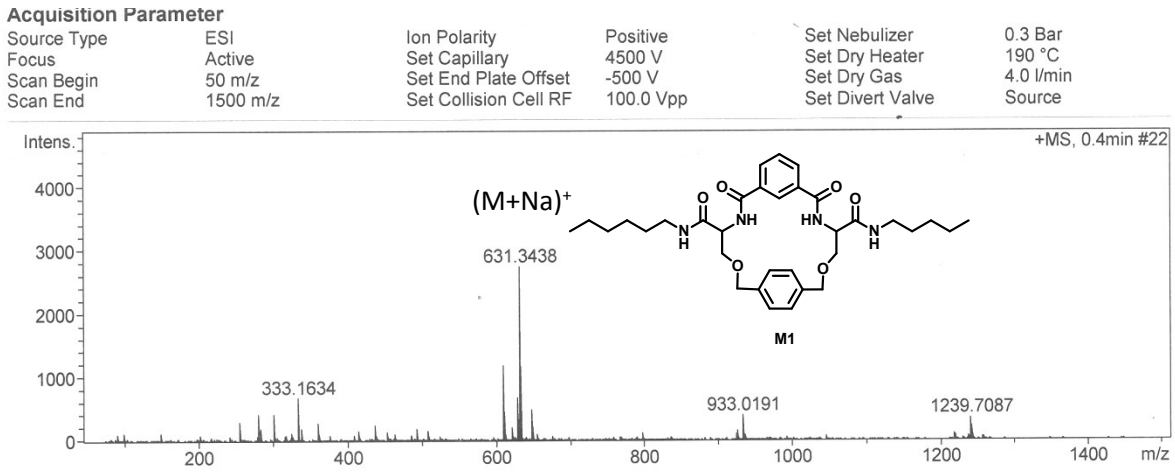
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **M1**



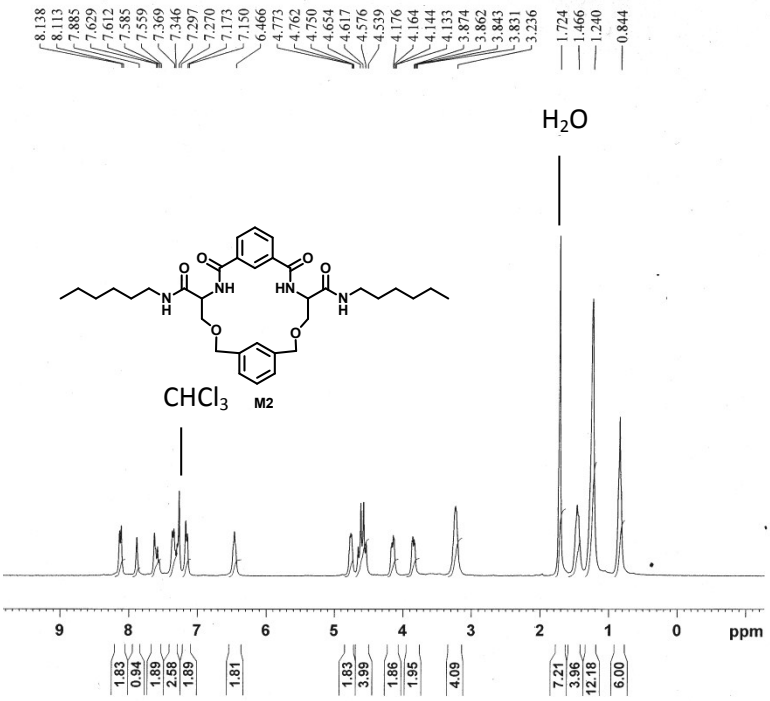
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **M1**



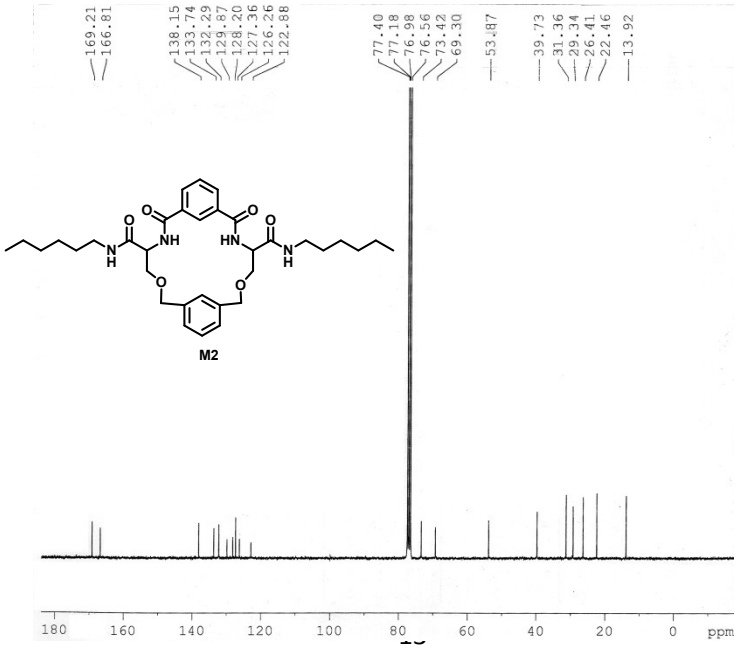
HRMS spectrum of **M1**



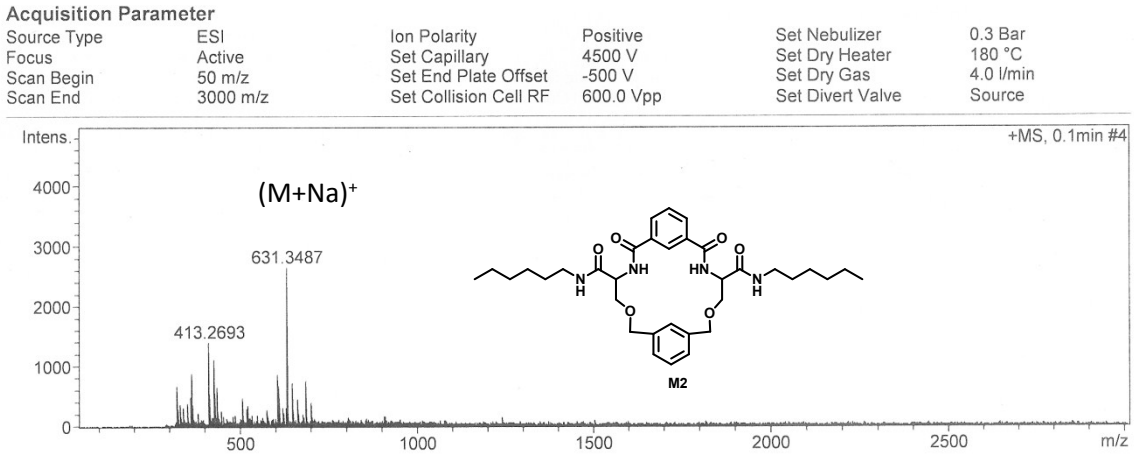
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **M2**



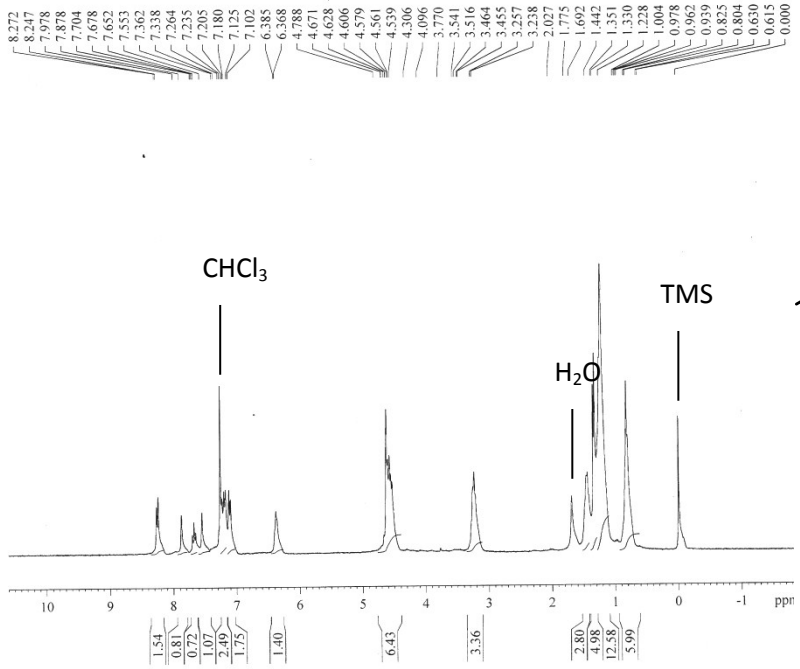
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **M2**

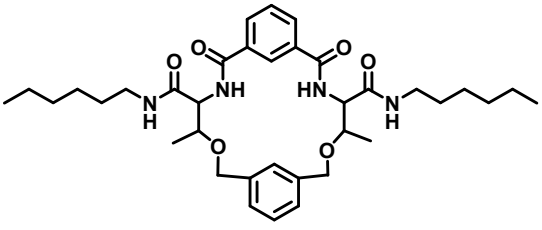


HRMS spectrum of **M2**

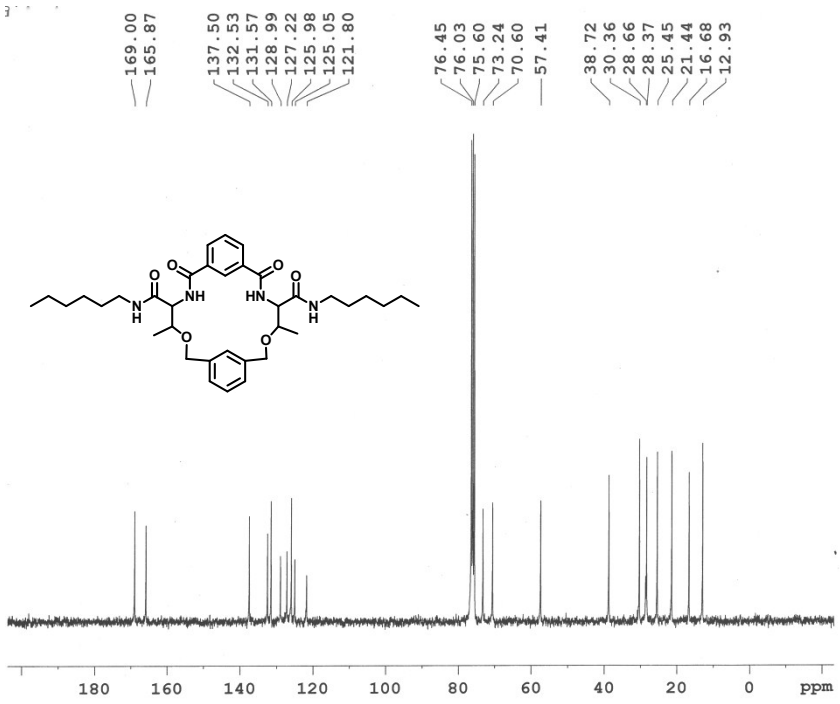


^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **M3**

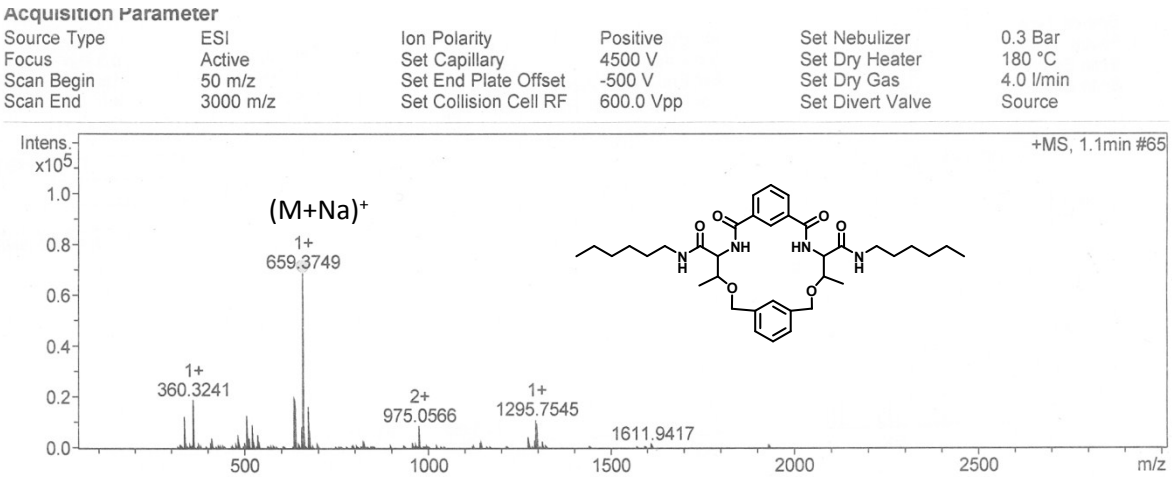




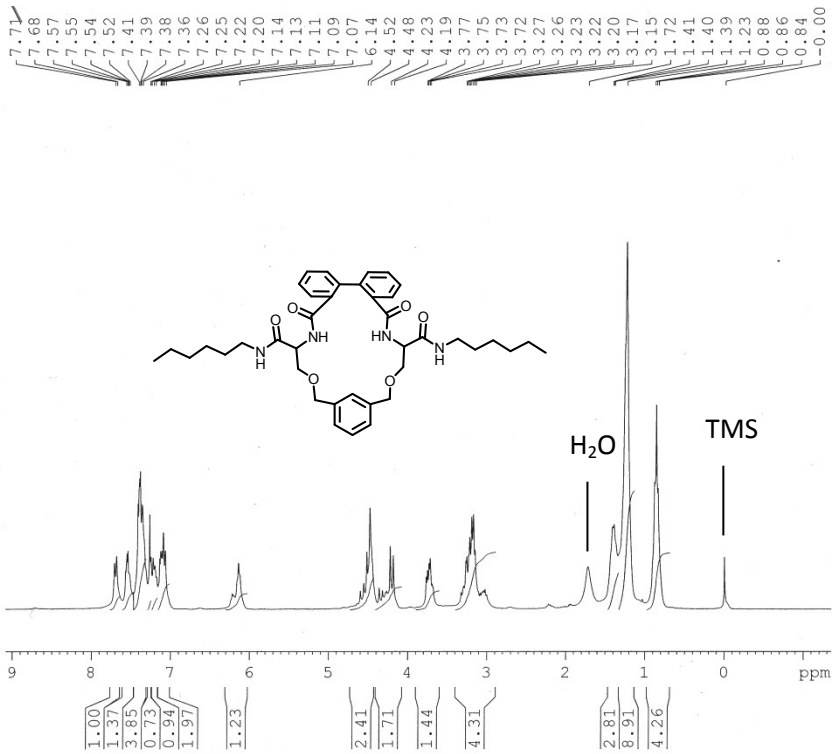
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **M3**



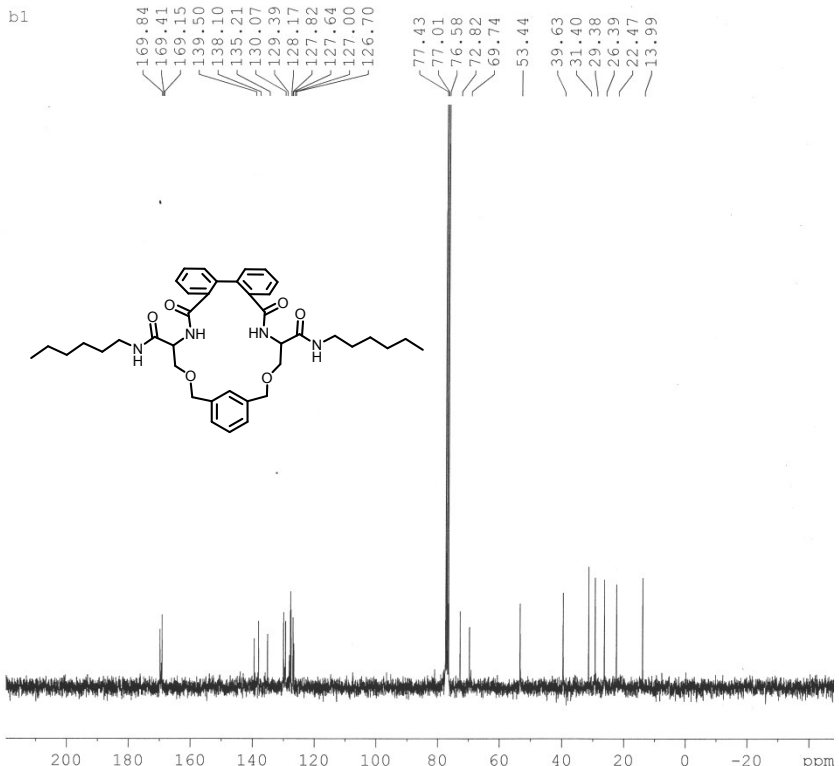
HRMS spectrum of **M3**



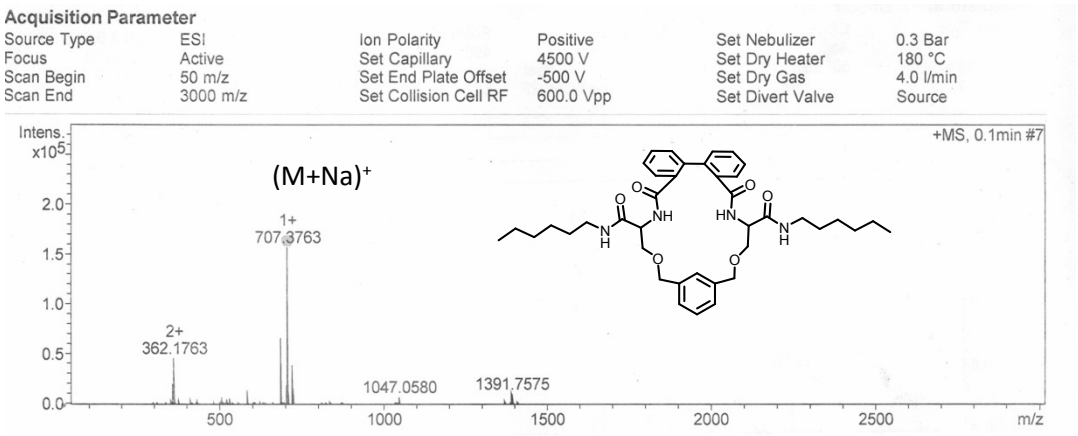
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **M4**



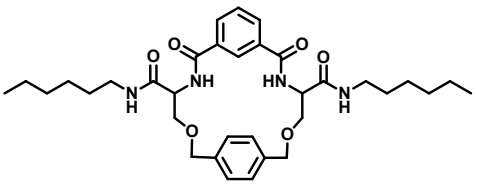
^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **M4**



HRMS spectrum of **M4**



Compound **M1**



M1

To an ice-cooled solution of **A1** (0.42 g, 0.62 mmol) in dry CH_2Cl_2 (0.7 mL) was added trifluoroacetic acid (0.7 mL, 9.13 mmol) and the reaction mixture was left stirred for 4 h, it was subjected to high vacuum to remove the solvent and the resulting amine was dissolved in dry CH_2Cl_2 (50 mL), added NEt_3 (0.43 mL, 3.1 mmol) followed by drop-wise addition of benzene 1, 3 dicarbonyldichloride (0.126 g, 0.62 mmol) over a period of 2 h. The reaction mixture was left stirred for 24 h and washed sequentially with 2 N H_2SO_4, $NaHCO_3$ and water. The organic layer was collected, dried over anhyd. Na_2SO_4 and evaporated to yield 0.412 g of the crude material which was chromatographed over silicagel (100-200 mesh) and eluted with $CHCl_3$:CH_3OH (9:1) to yield 0.15 g of the pure product.

%Yield: 40

Appearance: White crystalline solid

Melting point: 210 ^oC

$[\alpha]_D$: 28^o (c = 0.05g/ 100 mL CH_3OH)

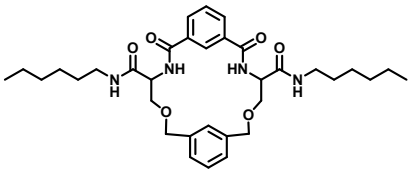
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (br t, 6H), 1.29 (br s, 12H), 1.52 (m, 4H), 3.33 (m, 4H), 3.91 (m, 4H), 4.30-4.69 (m, 4H), 4.74 (br m, 2H), 6.40 (br m, 2H), 6.95 (d, 2H, *J* = 7.5 Hz), 7.18 (s, 4H), 7.56 (m, 2H), 7.99 (d, 2H, *J* = 7.5 Hz)

^{13}CNMR (75MHz, $CDCl_3$): δ 13.96, 22.51, 26.48, 29.41, 31.40, 39.80, 53.87, 68.94, 72.11, 125.0, 127.98, 130.0, 130.68, 134.71, 138.01, 167.44, 168.93

IR (KBr): 3294, 3247, 2928, 2859, 1655, 1635, 1547, 1464, 1360, 1299, 1236, 1115, 1023 cm^{-1}

HRMS calcd for $C_{34}H_{48}N_4O_6Na$, m/z = 631.3472, obtained m/z = 631.3438.

Compound **M2**



To an ice-cooled solution of **A3** (0.325 g, 0.479 mmol) in dry CH_2Cl_2 (0.55 mL) added trifluoroacetic acid (0.55 mL, 7.19 mmol) and the reaction mixture was left stirred for 4 h and the solvent was removed under high vacuum to obtain the amine. It was then dissolved in dry CH_2Cl_2 (50 mL), added NEt_3 (0.33 mL, 2.37 mmol) and benzene 1, 3 dicarbonyldichloride was added drop wise over a period of 2 h. The reaction mixture was left stirred for 24 h and washed sequentially with 2 N H_2SO_4, $NaHCO_3$ and water. The organic layer was collected, dried over anhyd. Na_2SO_4 and evaporated to yield 0.34 g of the crude material which was chromatographed over silicagel (100-200 mesh) and eluted with EtOAc:Hexane (4:1) to yield 0.14 g of the pure product.

%Yield: 48

Appearance: White solid

Melting point: 219-220 ^oC

$[\alpha]_D$: 76^o (c = 0.075g/ 100 mL CH_3OH)

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (br t, 6H), 1.24 (br m, 12H), 1.47 (br m, 4H), 3.24 (br m, 4H), 3.85 (m, 2H), 4.15 (m, 2H), 4.59 (q, 4H, J = 12.3 Hz), 4.76 (br t, 2H), 6.47 (br s,

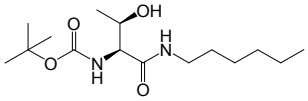
2H), 7.16 (d, 2H, *J* = 6.9 Hz), 7.3 (s, 1H), 7.36 (d, 2H, *J* = 6.0 Hz), 7.60 (m, 2H), 7.89 (s, 1H), 8.13 (d, 2H, *J* = 7.5 Hz)

^{13}CNMR (75 MHz, $CDCl_3$): δ 13.92, 22.46, 26.41, 29.34, 31.36, 39.73, 53.87, 69.30, 73.42, 122.88, 126.26, 127.36, 128.20, 129.87, 132.29, 133.74, 138.15, 166.81, 169.21

IR (KBr): 3309, 3056, 2926, 2858, 1657, 1535, 1469, 1365, 1298, 1249, 1156, 1108, 1014 cm^{-1}

HRMS calcd for $C_{34}H_{48}N_4O_6Na$, m/z = 631.3472, obtained m/z = 631.3487.

Compound **S2**



To a solution of Boc L-Threonine (2.83 g, 12.9 mmol) in CH_2Cl_2 (100 mL) added N-hydroxysuccinimide (2.29 g, 19.4 mmol), DCC (4.0 g, 19.4 mmol), hexylamine (2.7 mL, 19.4 mmol) NEt_3 (2.7 mL. 19.4 mmol) and was left stirred for 24h. The reaction mixture was filtered, and washed sequentially with 0.2 N H_2SO_4, $NaHCO_3$ and water. The organic part was collected, dried over anhyd.Na_2SO_4 and evaporated to yield the crude product. Crude product was then chromatographed over silicagel (60-120 mesh) with EtOAc: Hexane (6:4) to yield 2.76 g of the pure product.

%Yield: 71

Appearance: White semi solid

$[\alpha]_D$: -13.2^o (c = 0.083g/ 100 mL CH_3OH)

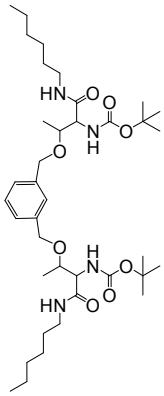
^{1}HNMR (300MHz, $CDCl_3$): δ 0.87 (br t, 3H), 0.18 (m, 3H), 1.28 (br s, 6H), 1.46 (s, 11 H), 3.24 (m, 2H), 3.49 (s, 1H), 3.96 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz), 4.38 (m, 1H), 5.49 (m, 1H), 6.62 (br s, 1H)

^{13}CNMR (75 MHz, $CDCl_3$): δ 14.02, 18.24, 22.53, 26.50, 28.29, 29.36, 31.43, 39.44, 57.70, 66.57, 80.40, 156.72, 171.68

IR (KBr): 3359, 2928, 2861, 1692, 1646, 1555, 1503, 1371, 1337, 1296, 1289, 1169, 1072 cm^{-1}

HRMS calcd for $C_{15}H_{30}N_2O_4Na$, m/z = 325.2103, calcd m/z = 325.2095

Compound **A4**



To an ice cooled and well stirred solution of **S2** (1.5 g, 4.96 mmol) in CH_2Cl_2 (100 mL), added 10 mL NaOH (2g/5 mL), TBABr (0.3 g, 0.93 mmol) and left stirred for 20 minutes. Afterwards, *m*-xylylene dibromide (0.655 g, 2.48 mmol) was added and the reaction mixture was left stirred at room temperature for 18 h. The reaction mixture was washed several times with water and the organic layer was dried over anhyd.Na_2SO_4 and evaporated to yield the crude product. The crude mixture was chromatographed over a short column of silicagel (100-200 mesh) and eluted with EtOAc: Hexane (2:8) to yield 0.241 g of **A4**.

%Yield: 14

Appearance: pale yellow semisolid

$[\alpha]_D$: 1.11^o (c = 0.09g/ 100 mL CH_3OH)

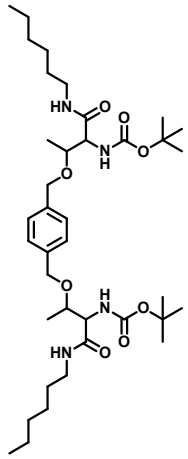
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (br t, 6H), 1.17 (br d, 6H), 1.26 (br m, 12H), 1.46 (s+ m, 22 H), 3.25 (m, 4H), 4.1-4.3 (m, 4H), 4.59 (m, 4H), 5.53 (br s, 2H), 6.51 (br s, 2H), 7.2-7.4 (m, 4H)

^{13}CNMR (75 MHz, $CDCl_3$): δ 14.02, 15.48, 22.52, 26.53, 28.31, 29.70, 31.43, 39.57, 57.55, 71.38, 74.78, 80.04, 127.01, 127.15, 128.57, 138.27, 155.85, 169.55

IR (KBr): 3327, 3209, 3120, 2929, 2862, 1688, 1650, 1532, 1374, 1305, 1248, 1170, 1108, 1057 cm^{-1}

HRMS calcd for $C_{38}H_{66}N_4O_8Na$ m/z = 729.4778, obtained m/z = 729.4750

Compound **A2**



To an ice cooled and well stirred solution of **S2** (1.11 g, 3.67 mmol) in CH_2Cl_2 (100 mL), added 10 mL NaOH (2g/5 mL), TBABr (0.2 g, 0.62 mmol) and stirred for 20 minutes. Afterwards, *p*-xylylene dibromide (0.483 g, 1.83 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was washed several times with water and the organic layer was dried over anhyd.Na_2SO_4 and evaporated to yield the crude product. The crude mixture was chromatographed over a short column of silicagel (100-200 mesh) and eluted with EtOAc: Hexane (1:4) to yield 0.33 g of **A2**.

%Yield: 25.4

Appearance: pale yellow solid

Melting point: 115 ^oC

$[\alpha]_D$: 17.77^o (c = 0.068g/ 100 mL CH_3OH)

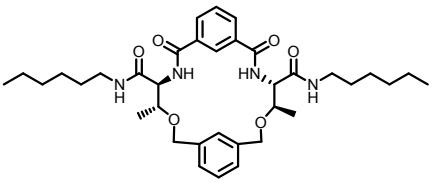
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (br t, 6H), 1.19 (br d, 6H), 1.28 (br m, 12H), 1.48 (s+m, 22H), 3.27 (m, 4H), 4.15-4.3 (m, 4H), 4.60 (m, 4H), 5.51 (br s, 2H), 6.50 (br t, 2H), 7.29 (br s, 4H).

^{13}CNMR (75 MHz, $CDCl_3$): δ 14.04, 15.46, 22.53, 26.53, 28.0, 29.45, 31.43, 39.45, 57.48, 71.29, 74.79, 80.06, 127.81, 137.65, 155.85, 169.6

IR (KBr): 3336, 3104, 2926, 2864, 1695, 1649, 1535, 1372, 1302, 1248, 1173, 1103, 1053, 1018 cm^{-1}

HRMS calcd for $C_{38}H_{66}N_4O_8Na$ m/z = 729.4778, obtained m/z = 729.4772

Compound **M3**



To an ice-cooled solution of **A4** (0.211 g, 0.298 mmol) in dry CH_2Cl_2 (0.3 mL) added trifluoroacetic acid (0.3 mL, 3.9 mmol) and the reaction mixture was left stirred for 4 h after which the volatiles were removed under high vacuum and the resulting amine was dissolved in dry CH_2Cl_2 (50 mL) added NEt_3 (0.5 mL, 3.6 mmol) and benzene 1, 3 dicarbonyldichloride (0.06 g, 0.296 mmol) was added dropwise over a period of 2 h. The reaction mixture was left stirred for 24 h and 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 yield 0.18 g of the crude material which was chromatographed over silicagel (100-200 mesh) and eluted with EtOAc: Hexane (4:1) to yield 0.018 g of the pure product.

%Yield: 9.5

Appearance: white solid

Melting point: 214 ^oC

$[\alpha]_D$: 115.3^o (c = 0.085g/ 100 mL CH_3OH)

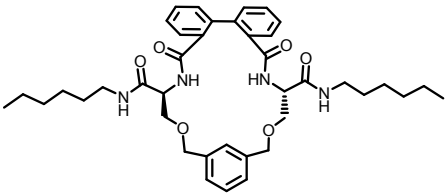
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83 (br t, 6H), 1.23 (br d, 12H), 1.34 (m, 6H), 1.44 (br m, 4H), 3.25 (br m, 4H), 4.5-4.58 (m, 8H), 6.38 (br t, 2H), 7.13 (d, 2H, *J* = 6.9 Hz), 7.29 (m, 3H), 7.57 (s, 1H), 7.68 (t, 1H, *J* = 7.8 Hz), 7.9 (s, 1H), 8.27 (d, 2H, *J* = 7.5 Hz)

^{13}CNMR (75 MHz, $CDCl_3$): δ 12.93, 16.68, 21.44, 25.45, 28.37, 30.36, 38.72, 57.41, 70.60, 73.24, 121.80, 125.05, 125.98, 127.22, 128.99, 131.57, 132.53, 137.50, 165.87, 169.00

IR (KBr): 3276, 2945, 1642, 1543, 1456, 1341, 1092 cm^{-1}

HRMS calcd for $C_{36}H_{52}N_4O_6$ Na, m/z = 659.3785, obtained m/z = 659.3749

Compound **M4**



M4

To an ice-cooled solution of **A3** (0.4 g, 0.59 mmol) in dry CH_2Cl_2 (1 mL) added trifluoroacetic acid (1 mL, 13.1 mmol) and the reaction mixture was left stirred for 4 h. Afterwards, reaction mixture was subjected to high vacuum and the resulting amine was dissolved in dry CH_2Cl_2 (50 mL), added NEt_3 (1 mL, 7.1 mmol) and freshly prepared diphenicacid dichloride (163.9 mg, 0.59 mmol) was added dropwise over a period of 12 h and was left stirred for 24 h. The reaction mixture was washed sequentially with 2 N H_2SO_4, $NaHCO_3$ and water. The organic layer was collected, dried over anhyd. Na_2SO_4 and evaporated to yield 0.56 g of the crude material which was chromatographed over silicagel (100-200 mesh) and eluted with EtOAc:Hexane (4:1) to yield 0.15 g of the pure product.

% yield: 35

Appearance: Yellow semi solid

$[\alpha]_D$: -25.2° (c = 0.083g/ 100 mL CH_3OH)

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 6H, *J* = 6.0 Hz), 1.23 (br s, 12H), 1.40 (br m, 4H), 3.19 (m, 6H), 3.73 (m, 2H), 4.2-4.7 (m, 6H), 6.14 (br m, 2H), 7-7.45 (m, 10H), 7.55 (d, 2H, *J* = 3.0 Hz), 7.69 (d, 2H, *J* = 9.0 Hz)

^{13}C NMR (75 MHz, $CDCl_3$): δ 13.99, 22.47, 26.39, 29.38, 31.40, 39.63, 53.44, 69.74, 72.82, 126.70, 127.00, 127.64, 127.82, 128.17, 129.39, 130.07, 135.21, 138.10, 139.50, 169.15, 169.84

IR (KBr): 3309, 3065, 2927, 2860, 1641, 1549, 1461, 1363, 1304, 1254, 1114 cm^{-1}

HRMS calcd for $C_{40}H_{52}N_4O_6Na$ m/z = 707.3785, obtained m/z = 707.3763

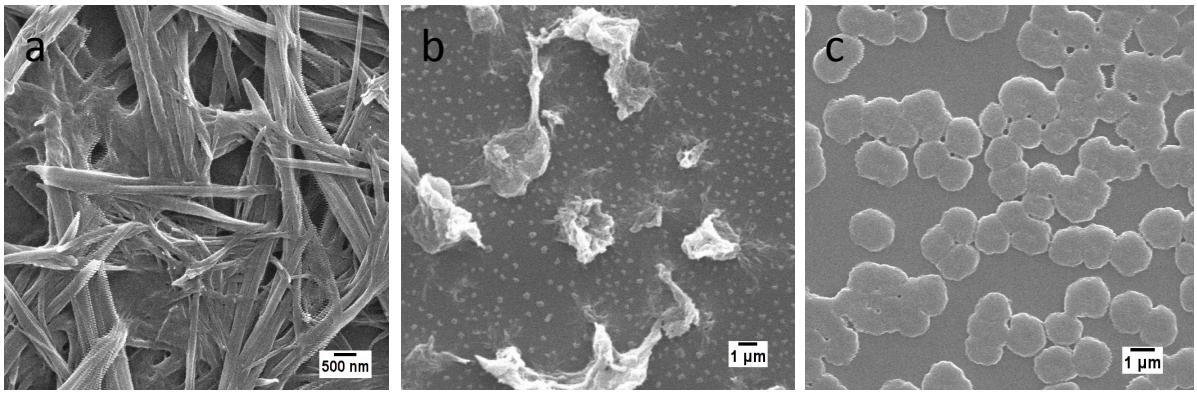


Figure S1: SEM image of 1 mM solution of **M1** in (a) Ethylacetate (b) Chloroform: Methanol (1:1) (c) isopropanol

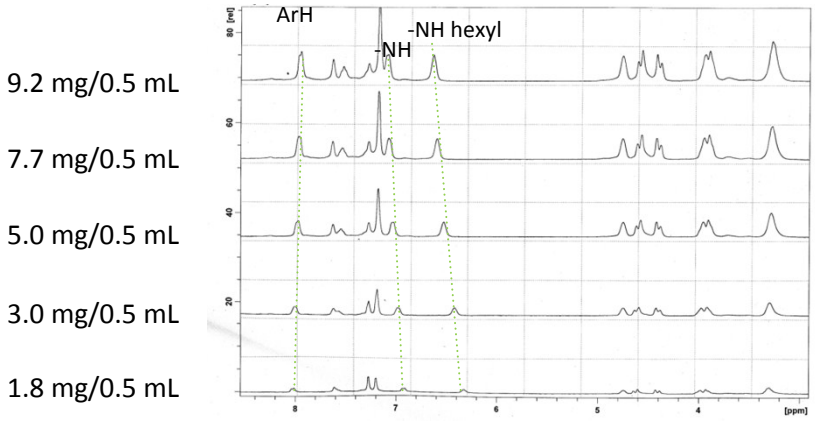


Figure S2: Concentration-dependent NMR spectra of **M1**

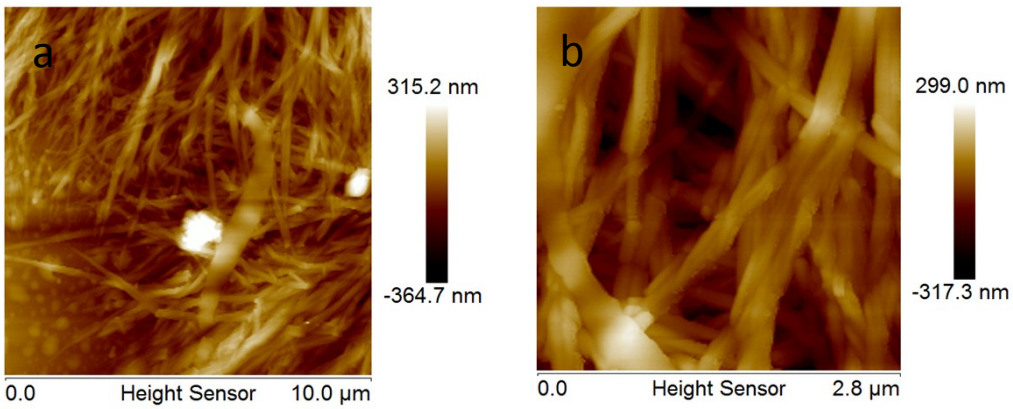


Figure S3: AFM images of **M1** at (a) low magnification (b) high magnification

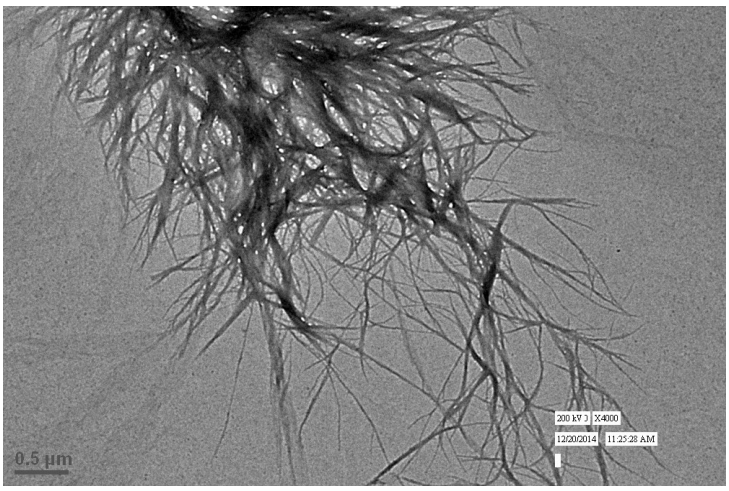


Figure S4: HR-TEM image of 1 mM solution of **M1** in methanol low magnification (Scale bar 500 nm).

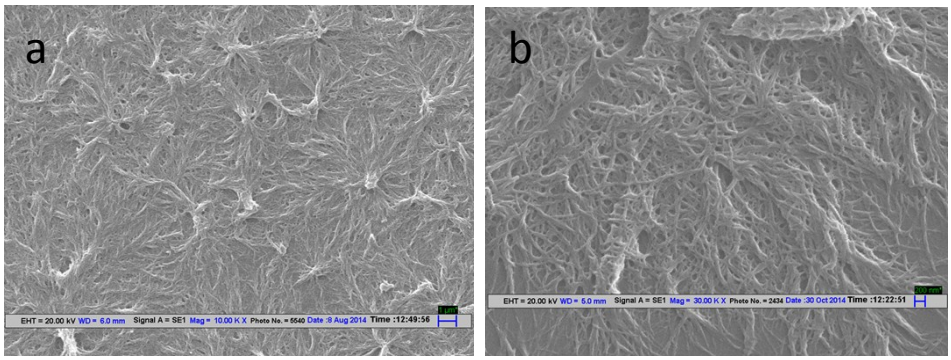


Figure S5: SEM image of (a) 0.1 mM solution of **M2** in methanol scale bar 1 µm and (b) enlarged image, scale bar 200 nm.

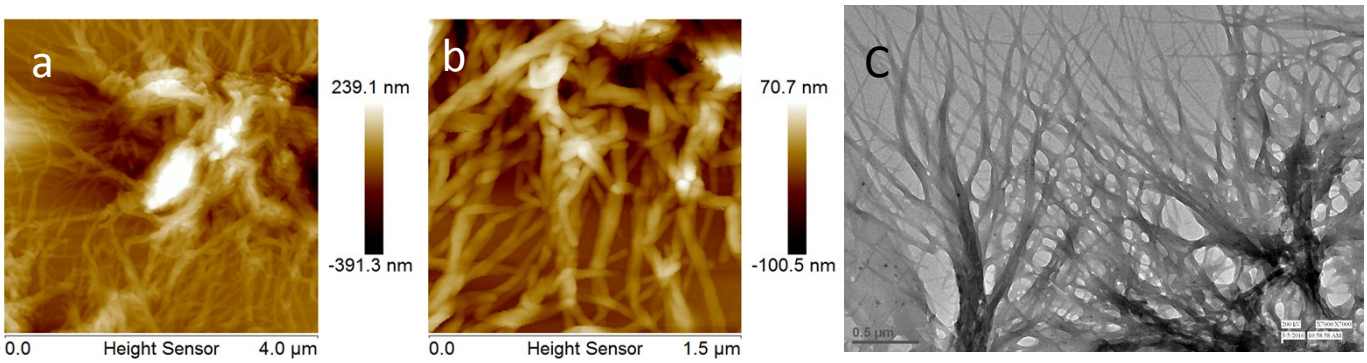


Figure S6: AFM images of 1 mM solution of **M2** in methanol (a) low magnification (b) high magnification (c) HRTEM image of **M2** (1 mM, methanol)

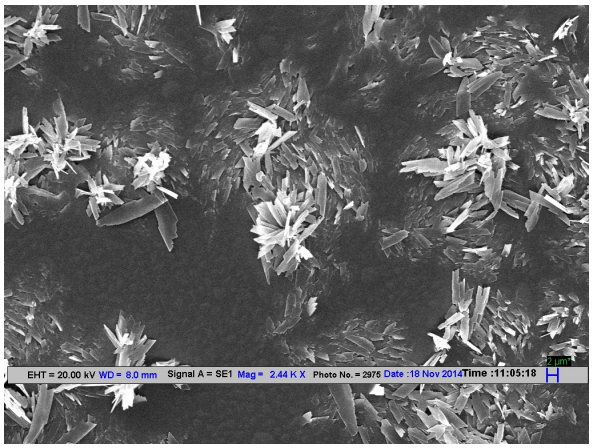


Figure S7: SEM image of **A1** (1 mM, CH_3OH)

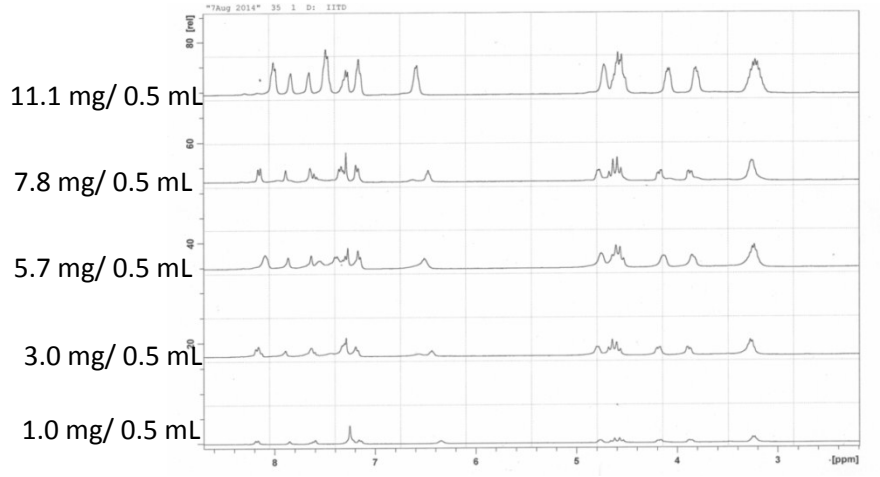


Figure S8. Concentration-dependent NMR spectra of **M2** in $CDCl_3$

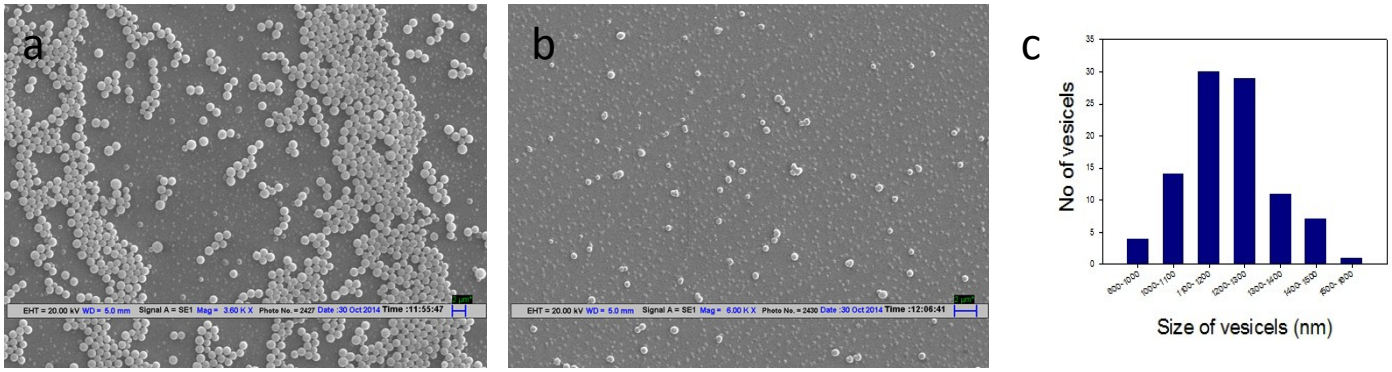


Figure S9: (a) SEM image of 1 mM solution of **M3** in CH_3OH, scale bar 2 μm (b) SEM image of 0.1 mM solution of **M3** in CH_3OH, scale bar 2 μm (c) Histogram showing the size distribution of vesicles.

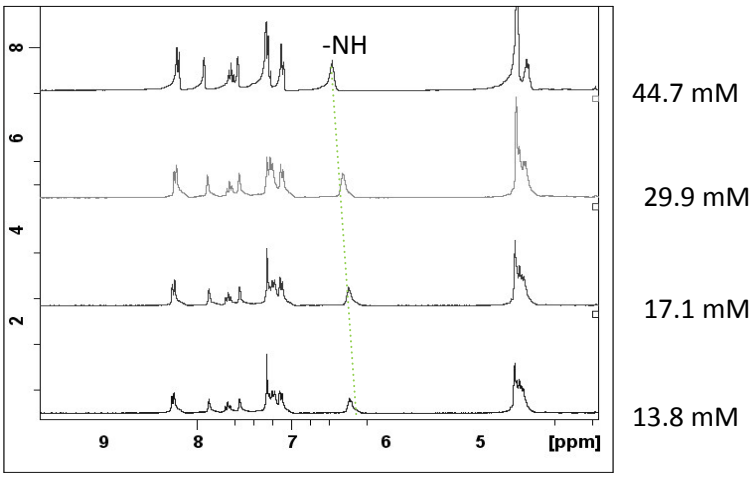


Figure S10: Concentration-dependent ^1H NMR (300 mHz) spectra of **M3** in $CDCl_3$

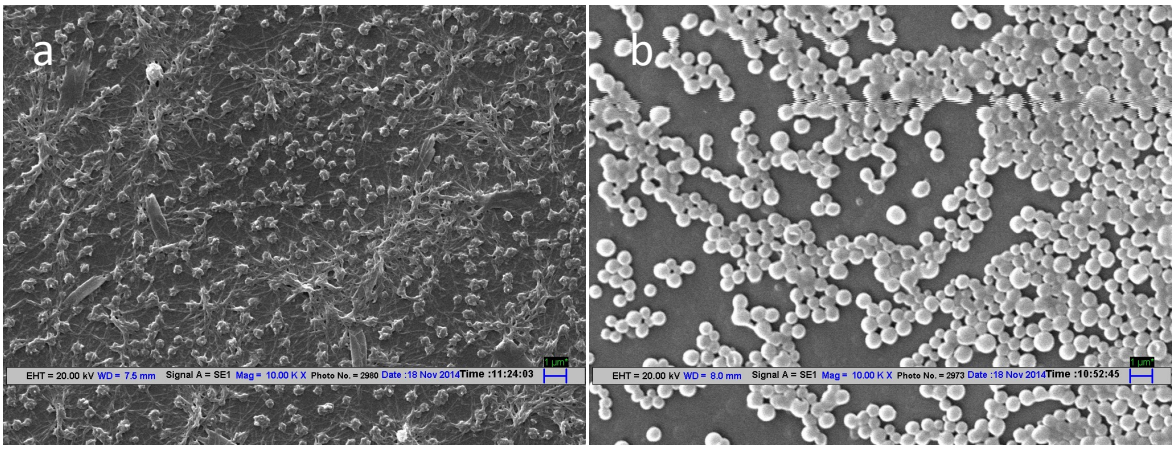


Figure 11: SEM images of 1 mM solution of (a) **A2** scale bar 1 μm (b) **A4** scale bar 1 μm. Both samples are prepared in methanol.

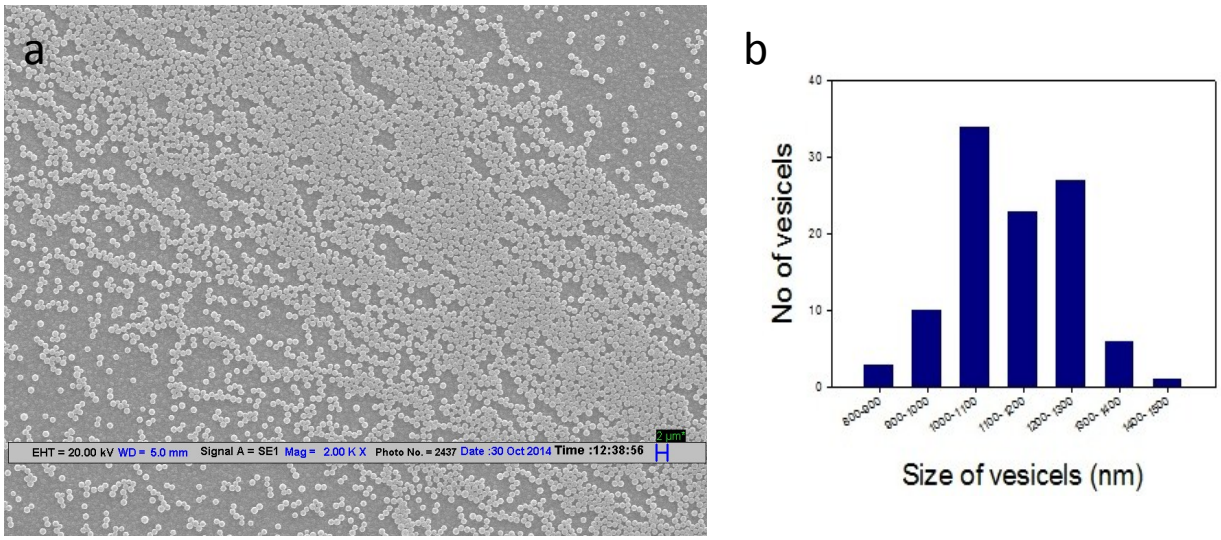


Figure S12: (a) Low magnification SEM image of **M4**, scale bar 2 µm (b) Histogram showing the size distribution of vesicles.

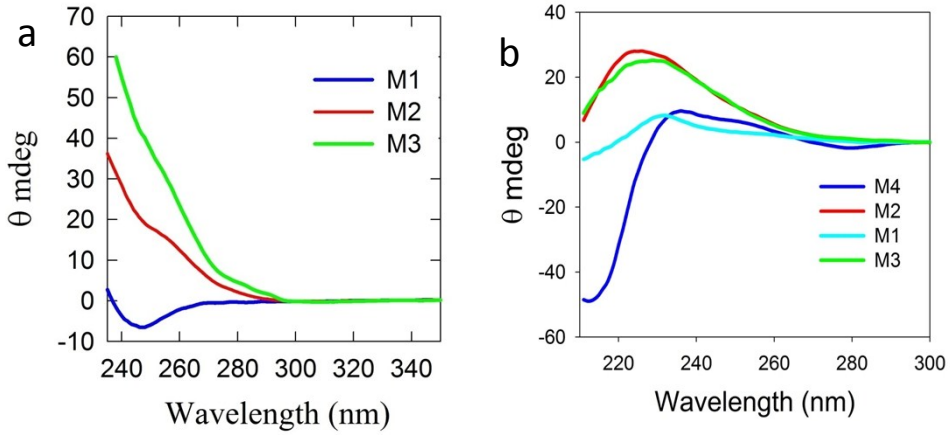


Figure S13: (a) 0.5 mM solution of **M1**-**M3** in $CHCl_3$ (b) 0.2 mM solution of **M1**-**M4** in methanol

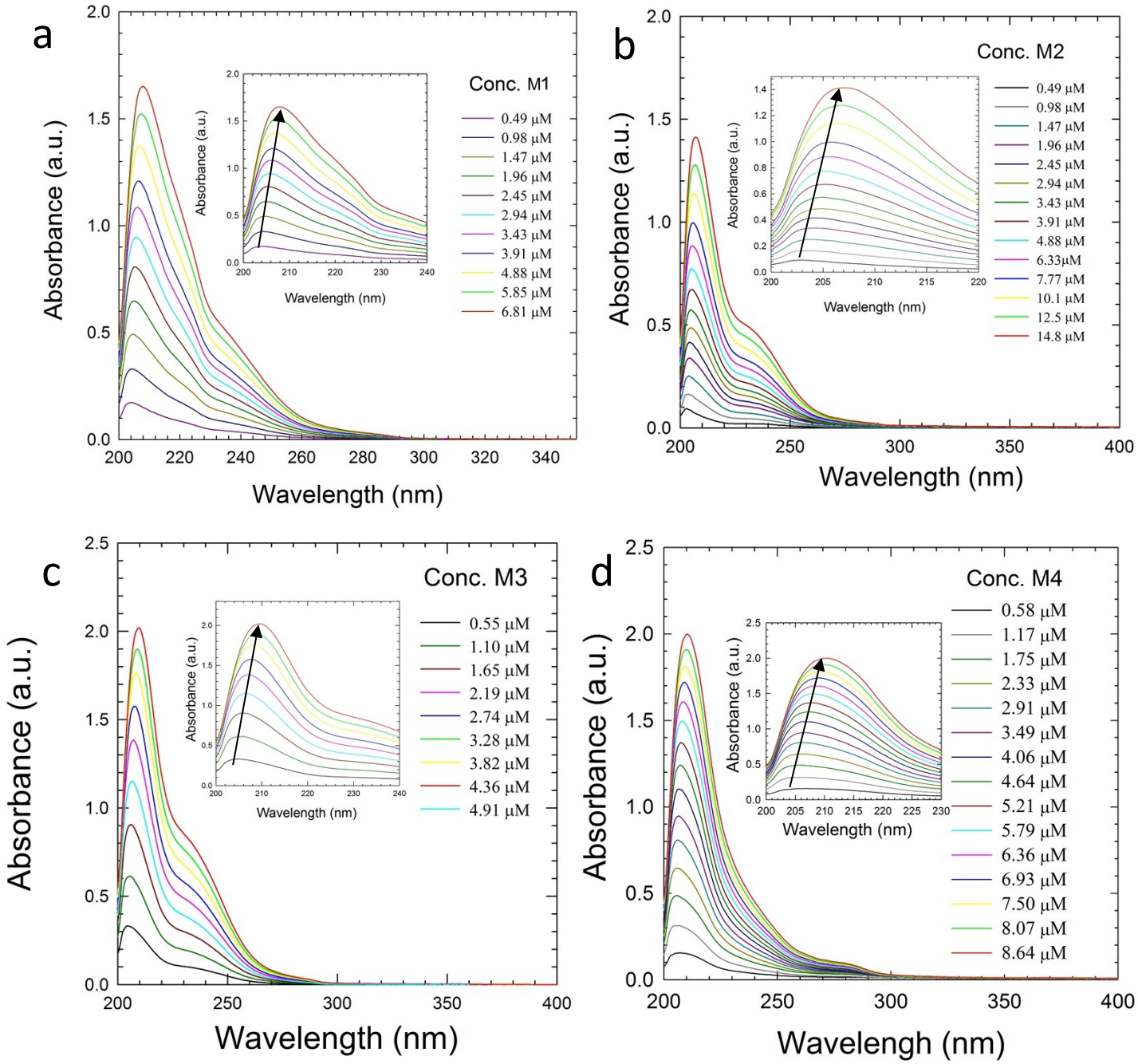


Figure S14: Concentration-dependent UV/Vis spectrum of (a) **M1** (b) **M2** (c) **M3** (d) **M4** in methanol. Inset shows the expanded spectra showing the red-shift of the absorption maximum.

DOSY EXPERIMENTS

All DOSY experiments were performed on BRUKER AVANCE-III 400 MHz instrument at 298 K. The spectra were recorded with **ledbpgp2s** pulse sequence which is composed of stimulated echo and LED (Longitudinal Eddy-current Delay) sequences with bipolar gradient pulse. Gradient strength was calibrated using 1-HDOSY pulse sequence with a diffusion delay of 60 ms. Depending upon the concentration of sample length of the diffusion gradient varied between 1ms-2ms to get a 95 % reduction in intensity. The gradient amplitude was ramped linearly in 16 steps from 2% to 95%. DOSY data were processed using the automation program **proc_dosy**. DOSY experiments were performed for **M1**-**M4** in various concentrations.

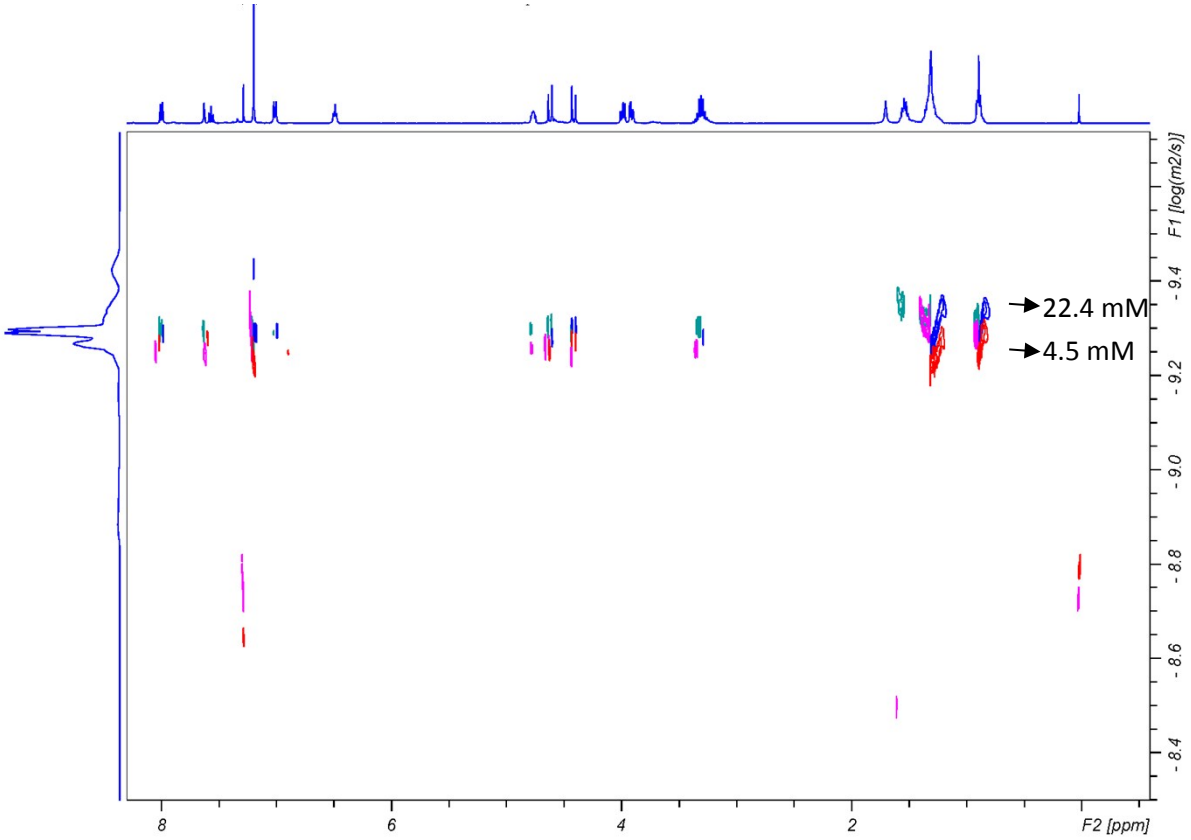
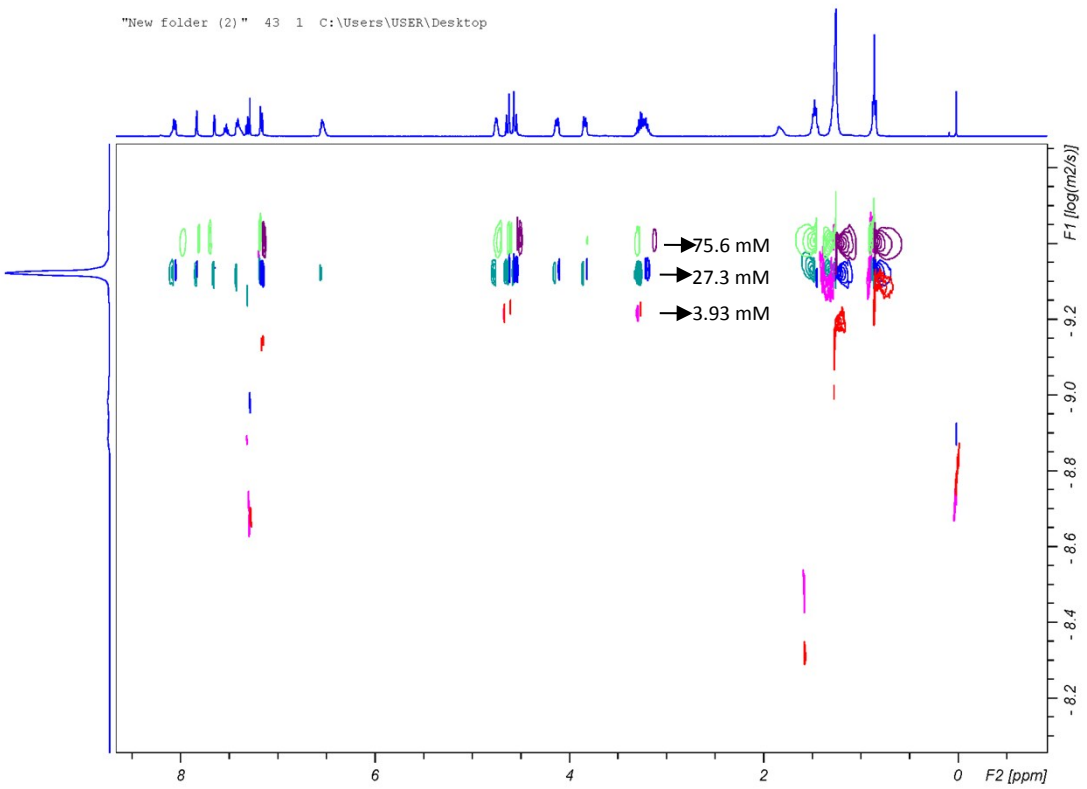


Figure S15: Concentration-dependent DOSY spectrum (400 MHz, $CDCl_3$) of **M1** (25°C)



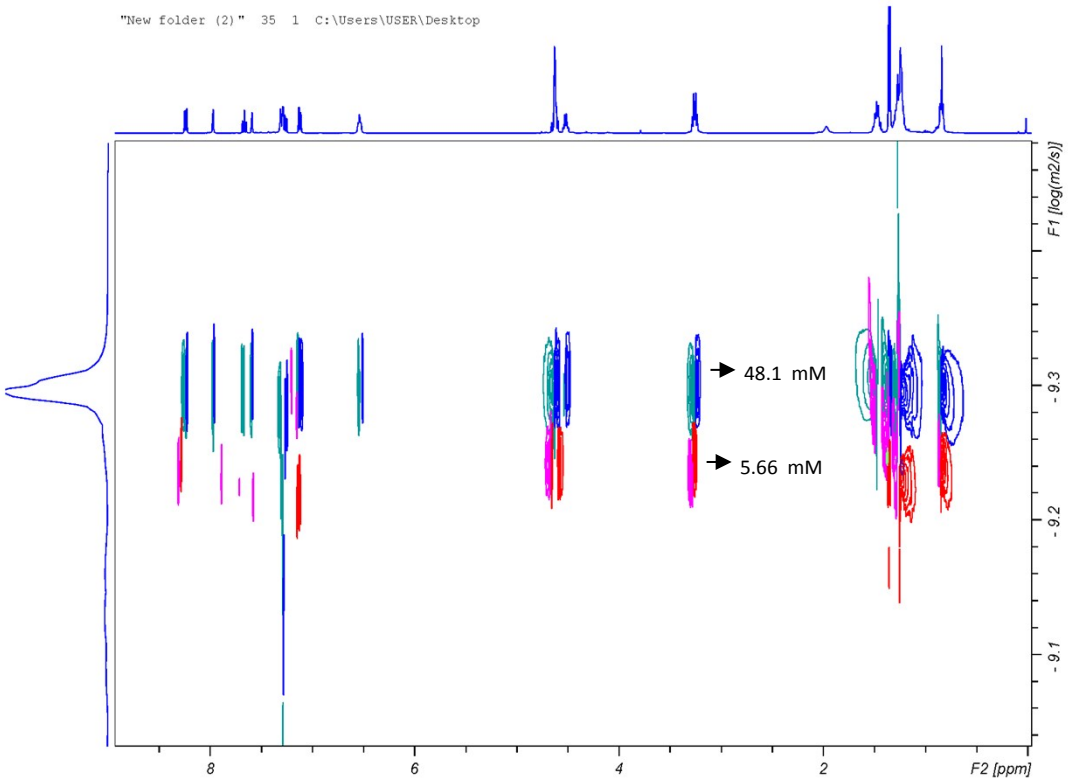


Figure S17: Concentration-dependent DOSY spectrum (400 MHz, $CDCl_3$) of **M3** (25°C)

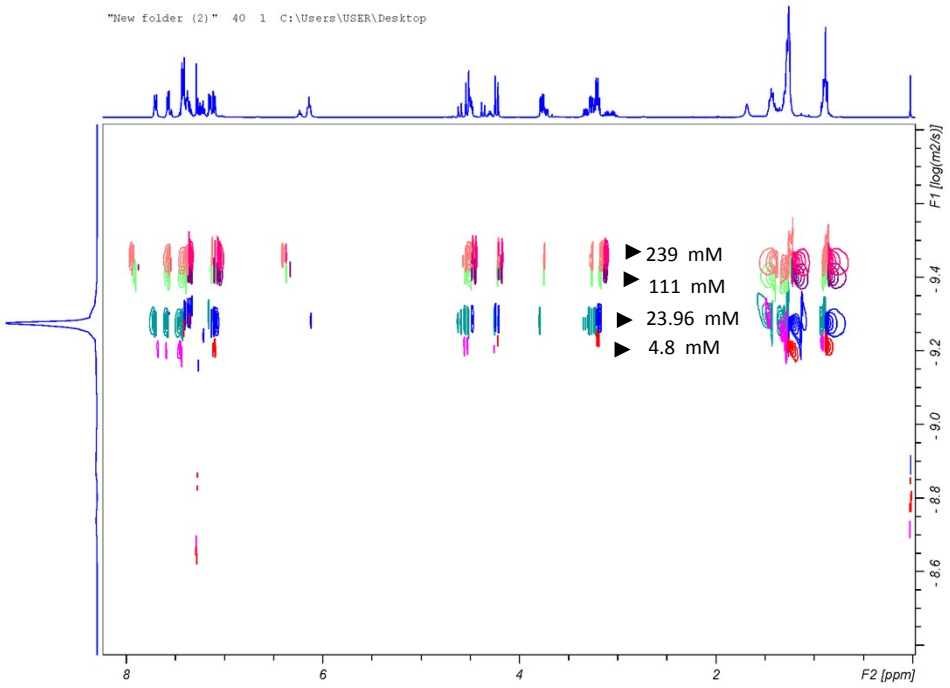


Figure S18: Concentration-dependent DOSY spectrum (400 MHz, $CDCl_3$) of **M4** (25°C)

Entry	*Compound*	*Concentration (mM)*	*D (m^2/s)*
1	**M1**	4.5	5.596×10^{-10}
2	**M1**	22.4	5.0536×10^{-10}
1	**M2**	3.94	5.9361×10^{-10}
2	**M2**	27.3	4.7479×10^{-10}
3	**M2**	75.6	3.9021×10^{-10}
1	**M3**	5.66	5.8641×10^{-10}
2	**M3**	15.7	5.7637×10^{-10}
3	**M3**	25.8	5.0176×10^{-10}
4	**M3**	48.1	4.9465×10^{-10}
1	**M4**	4.8	6.1052×10^{-10}
2	**M4**	23.96	5.2893×10^{-10}
3	**M4**	111	3.8976×10^{-10}
4	**M4**	239	3.5522×10^{-10}

Table S1: Diffusion coefficients (D) of **M1-M4** at 25°C from DOSY spectrum (400 MHz, $CDCl_3$).