Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supporting Information

Self - Assembly of Isomannide-based Monoesters of C_{18}-Fatty Acids and their Cellular Uptake Studies

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ESI -1. Synthesis of isomannide-based monoesters of C_{18}-fatty acid lipids (OAIML, EAIML and SAIML)

To a mixture of fatty acid (1 mmol) and D-mannitol (1.5 mmol) was added carbon acid catalyst (20 wt% of mannitol) at room temperature. The total reaction mixture was taken in ACE glass pressure tube and heated to 180 °C by immersing in an oil bath with electrical heating under magnetic stirring for 12 h. The progress of the reaction was monitored by TLC and Gas Chromatography using HP-1 capillary column. The catalyst was separated by filtration and washed with chloroform (10 mL). The solvent was dried over anhydrous sodium sulphate and removed under reduced pressure to obtain the crude residue. The resulted crude product was subjected to silica gel column chromatography using hexane/ethyl acetate (80:20 *v/v*) as eluent to obtain pure isomannide lipids (IMls) (yield 81-85%).

ESI -2. NMR and HR-Mass spectra of the three IMLs (Spectral data)

2.1. Oleic acid isomannide lipids (OAIMLs)

Yield: 81.3%.

$[\alpha]_D^{24}$: +65.6(*c* 1.0 in $CHCl_3$).

ν_{max}/cm^{-1}: 3446 (OH), 2926 and 2856 (CH), 1737 (C=O), 1650 (C=C), 1175 (C-O).

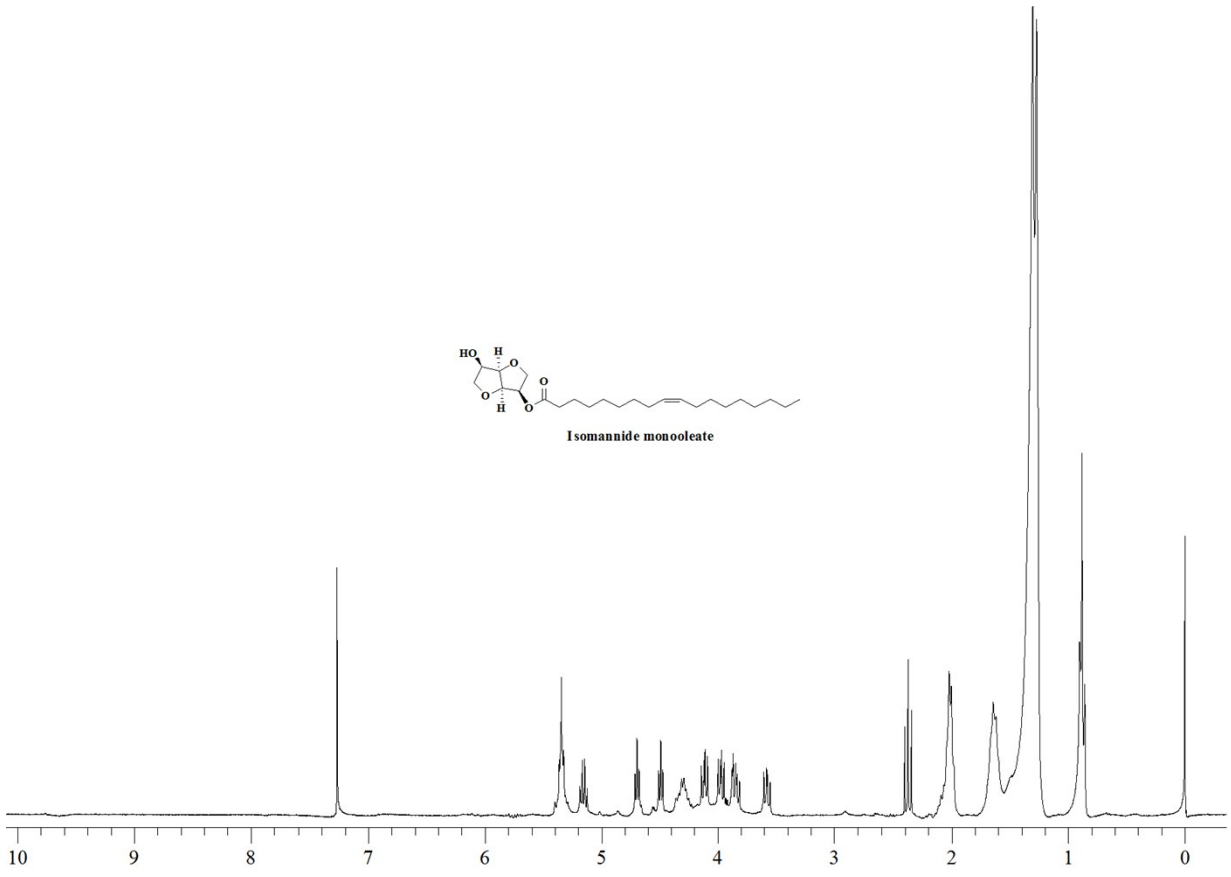


Figure S1: (a1) ^{1}H NMR Spectra of OAIML

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 6.7 Hz, 18-H), 1.23-1.40 (m, 20H, 4-H, 5-H, 6-H, 7-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H), 1.57-1.71 (2H, m, 3-H), 1.96-2.07 (4H, m, 8-H, 11-H), 2.37 (2H, td, J^1= 1.1 Hz, J^2= 7.7 Hz, 2-H), 3.57 (1H, dd, J^1= 6.9 Hz, J^2 = 9.0 Hz, OCH_2CHOH, H_b), 3.84 (1H, dd, J^1= *6.7 Hz,* J^2= 9.6 Hz, OCH_2CHOCO, H_b), 3.97 (1H, dd,J^1= 6.2 Hz, J^2 = 9.0 Hz, OCH_2CHOH, H_a), 4.11 (1H, dd, J^1= 6.4 Hz, J^2 = 9.4 Hz, OCH_2CHOCO, H_a), 4.22-4.38 (1H, m, CH_2CHOH), 4.48 (1H, t, J = 5.2 Hz, CHCHOH), 4.69 (1H, t, J = 5.0 Hz, CHCHOH), 5.12-5.18 (1H, q, CHOCO), 5.30-5.41 (2H, m, 9-H, 10-H).

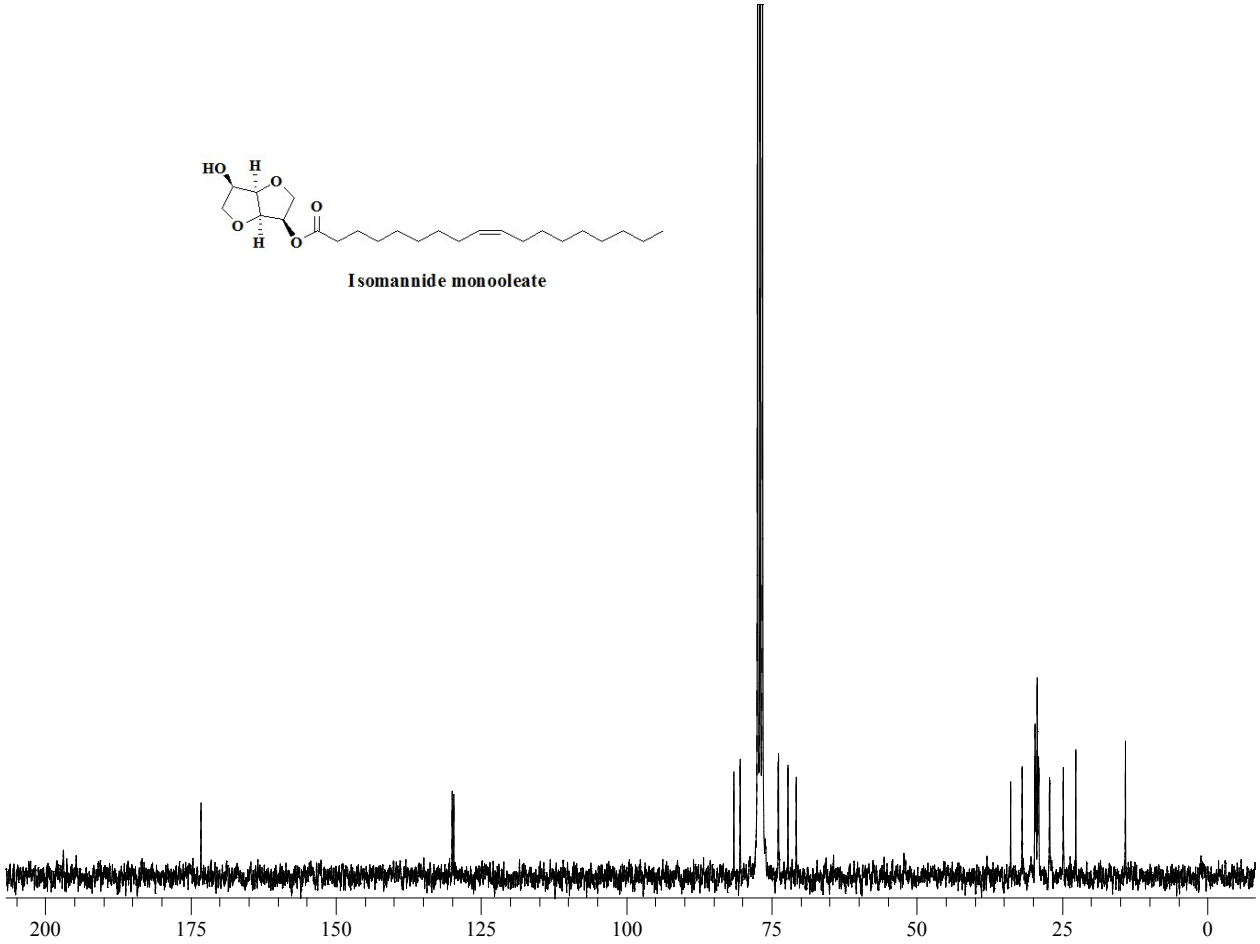
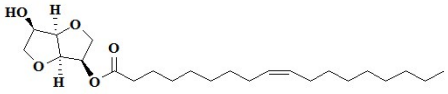


Figure S1: (a2) ^{13}C NMR Spectra of OAIML

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.11, 22.66, 24.80, 27.13, 27.18, 29.01, 29.07, 29.12, 29.29 (2 × C), 29.50, 29.65, 29.73, 31.88, 33.87, 70.76, 72.18, 73.81, 73.87, 80.41, 81.49, 129.69, 129.97, 173.19.



Isomannide monooleate

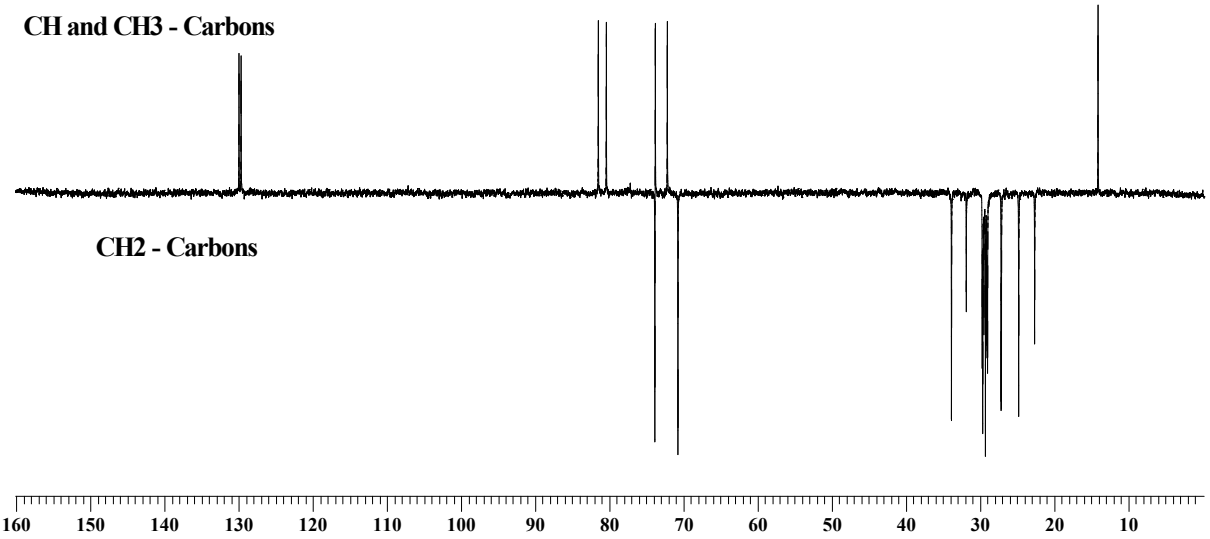


Figure S1: (a3) DEPT -135 NMR Spectra of OAIML

DEPT-135 NMR (125 MHz, $CDCl_3$):δ 14.09 (CH_3), 22.65 (CH_2), 24.80 (CH_2), 27.13 (CH_2), 27.17 (CH_2), 29.00 (CH_2), 29.06 (CH_2), 29.12 (CH_2), 29.28 (2 × CH_2), 29.48 (CH_2), 29.65 (CH_2), 29.72 (CH_2), 31.87 (CH_2), 33.86 (CH_2), 70.76 (CH_2), 72.19 (CH), 73.80 (CH), 73.87 (CH_2), 80.41 (CH), 81.49 (CH), 129.68 (CH), 129.96 (CH).

2.1. HR - Mass Spectra of OAIML

HR - MS [ESI] $^{+}$**:** calcd for $C_{24}H_{43}O_5$ $[M+H]^+$, 411.3105; found, 411.3110.

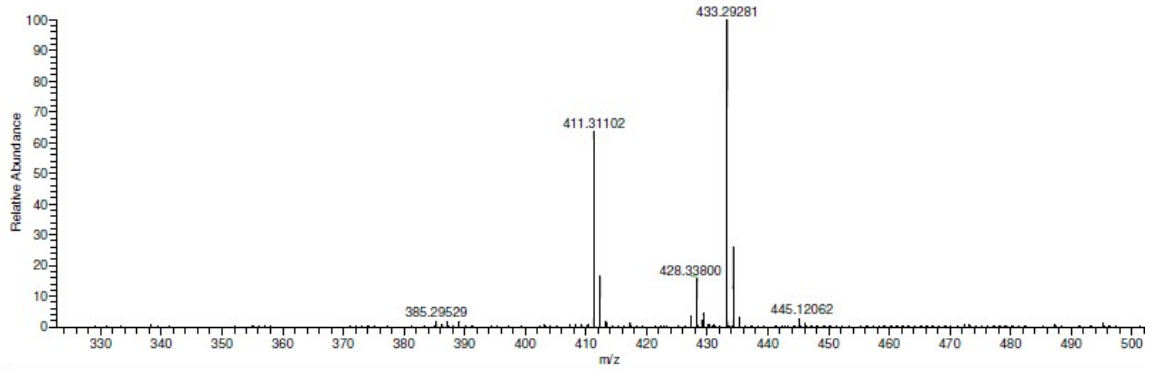


Figure S1: (a4) HR - Mass Spectra of OAIML.

2.2. Elaidic acid isomannide lipids (EAIMLs):

Yield:82.7%.

$[\alpha]_D^{24}$:+73.8(*c* 0.55 in $CHCl_3$).

ν_{max}/cm^{-1}:3460 (OH), 2925, 2854 (CH), 1742 (C=O), 1629 (C=C), 1171 (C-O), 967 (*trans*-C-H out-of-plane bend).

Isomannide monoeladiate

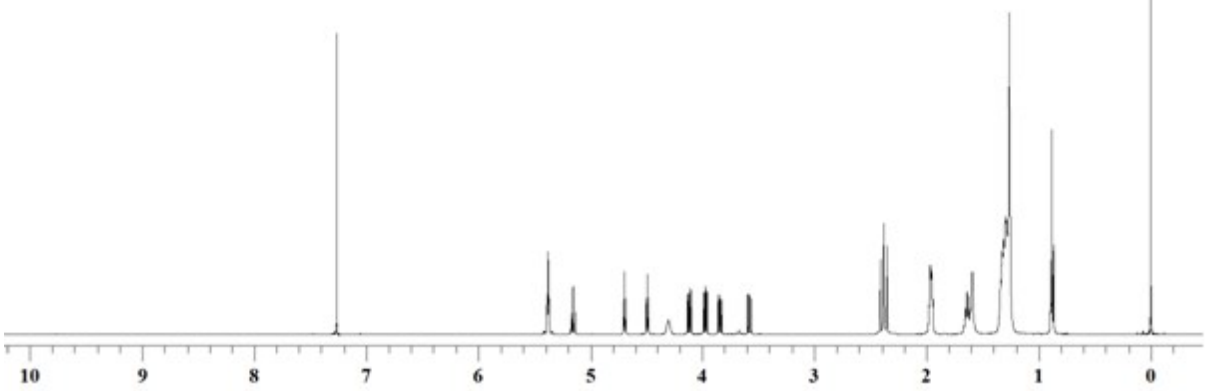
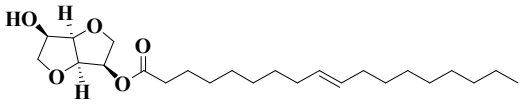


Figure S1: (b1) ^{1}H NMR Spectra of EAIML

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 7.0 Hz, 18-H), 1.22-1.36 (m, 20H, 4-H, 5-H, 6-H, 7-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H), 1.56-1.67 (2H, m, 3-H), 1.93-1.99 (4H, m, 8-H, 11-H), 2.37 (2H, td, J^1 = 2.4 Hz, J^2 = 7.4 Hz, 2-H), 3.57 (1H, dd, J^1= 7.0 Hz, J^2 = 9.1 Hz, OCH_2CHOH, H_b), 3.84 (1H, dd, J^1=6.5Hz, J^2= 9.4 Hz, OCH_2CHOCO, H_b), 3.97 (1H, dd,J^1= 6.1 Hz, J^2 = 9.1 Hz, OCH_2CHOH, H_a), 4.11 (1H, dd, J^1= 6.2 Hz, J^2 = 9.3 Hz, OCH_2CHOCO, H_a), 4.27-4.33 (1H, m, CH_2CHOH), 4.49 (1H, t, J = 5.3 Hz, *CH*CHOH), 4.70 (1H, t, J = 5.1 Hz, *CH*CHOH), 5.13-5.18 (1H, q, *CH*OCO), 5.36-5.39 (2H, m, 9-H, 10-H).



Isomannide monoeladiate

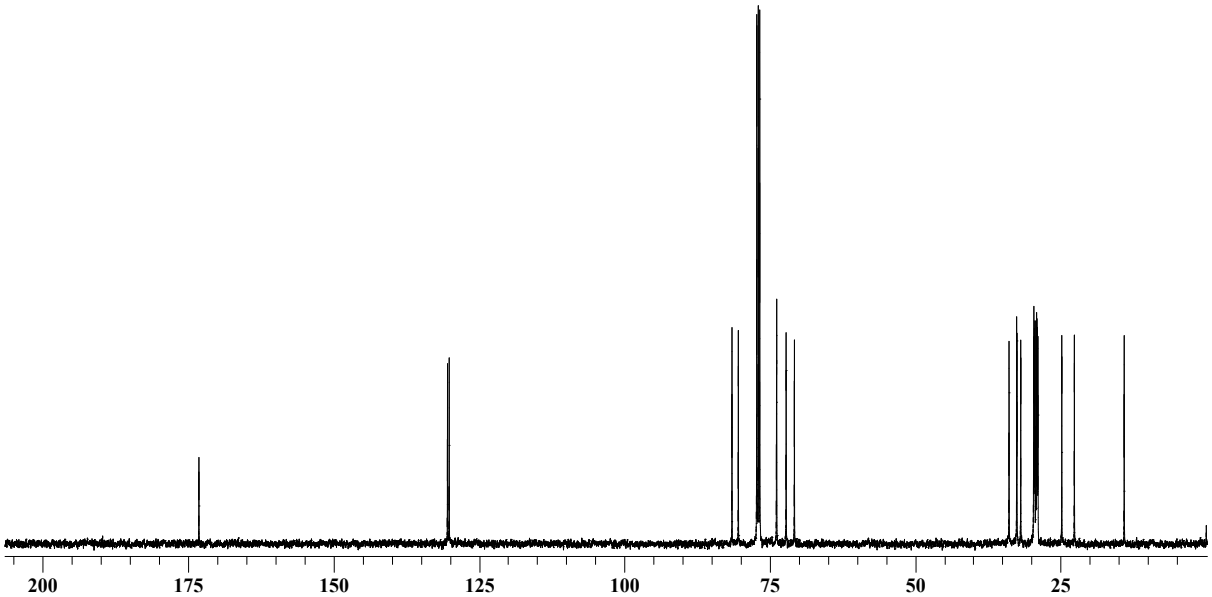


Figure S1: (b2) ^{13}C NMR Spectra of EAIML

^{13}C NMR (125 MHz, $CDCl_3$):δ 14.08, 22.64, 24.81, 28.90, 28.98, 29.06, 29.14, 29.27, 29.44, 29.51, 29.61, 31.86, 32.50, 32.56, 33.87, 70.78, 72.20, 73.82, 73.86, 80.42, 81.52, 130.14, 130.43, 173.17.

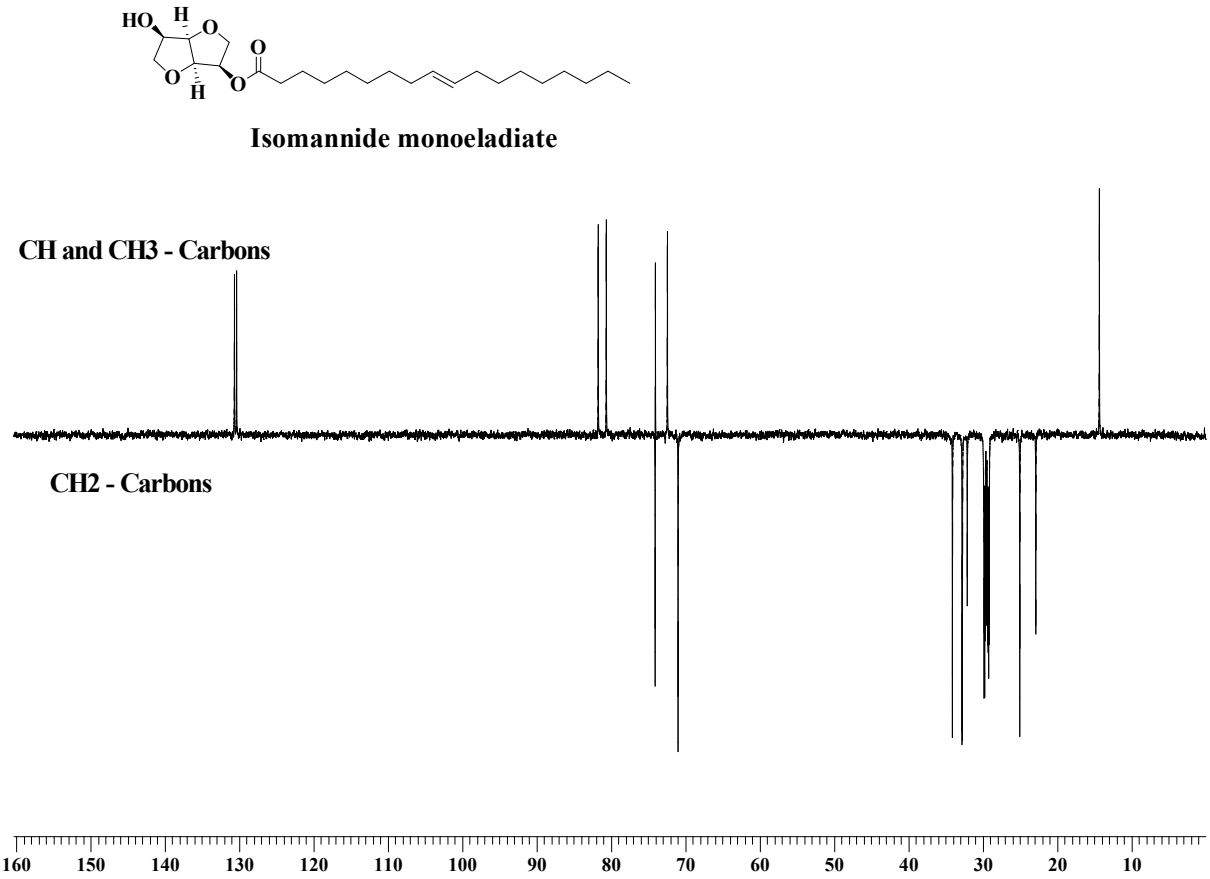


Figure S1: (b3) DEPT -135 NMR Spectra of EAIML

DEPT-135 NMR (125 MHz, $CDCl_3$):δ 14.08 (CH_3), 22.63 (CH_2), 24.79 (CH_2), 28.89 (CH_2), 28.97 (CH_2), 29.06 (CH_2), 29.14 (CH_2), 29.27 (CH_2), 29.44 (CH_2), 29.51 (CH_2), 29.60 (CH_2), 31.85 (CH_2), 32.51 (CH_2), 32.56 (CH_2), 33.86 (CH_2), 70.75 (CH_2), 72.18 (CH), 73.80 (CH), 73.83 (CH_2), 80.40 (CH), 81.49 (CH), 130.13 (CH), 130.42 (CH).

2.2. HR - Mass Spectra of EAIML

HR - MS $[ESI]^+$: calcd for $C_{24}H_{43}O_5$ $[M+H]^+$, 411.3105; found, 411.3098.

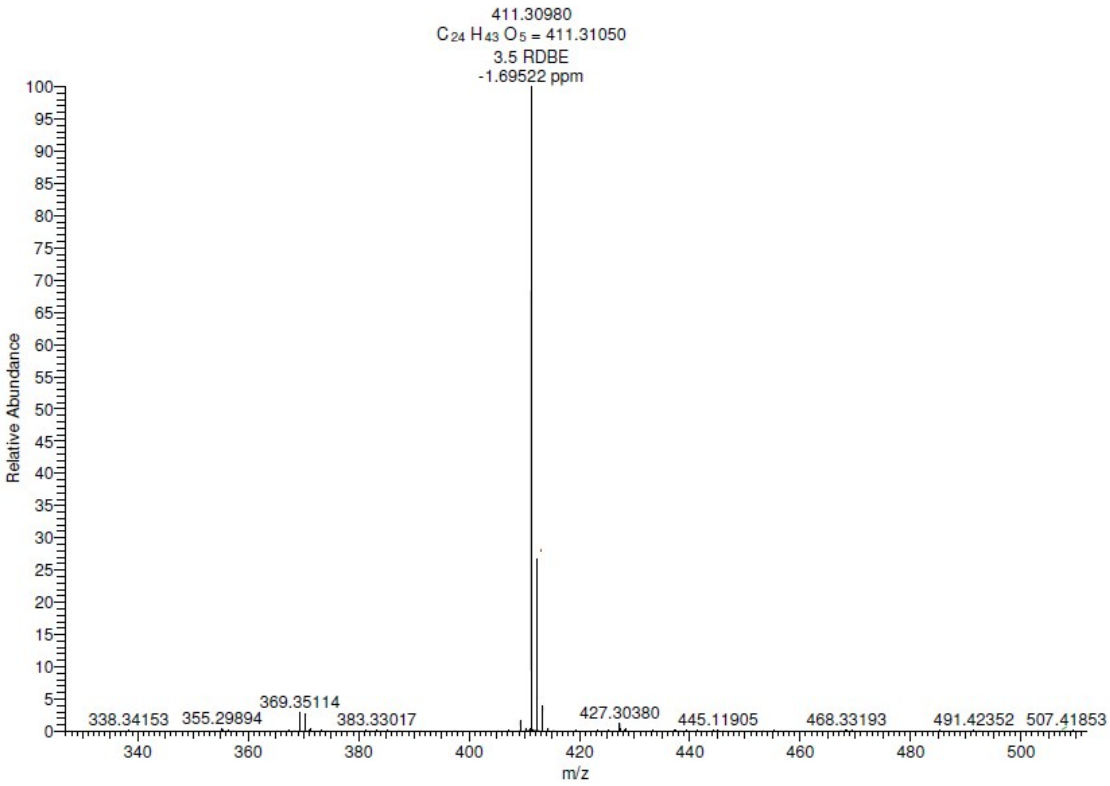


Figure S1: (b4) HR - Mass Spectra of EAIML**.**

2.3. Stearic acid isomannide lipids (SAIMLs):

Yield: 85.1%.

MP:42-44 °C.

$[\alpha]_D^{24}$: +45.5(*c* 0.65 in $CHCl_3$).

ν_{max}/cm^{-1}: 3434 (OH), 2919 and 2851 (CH), 1740 (C=O), 1173 (C-O).

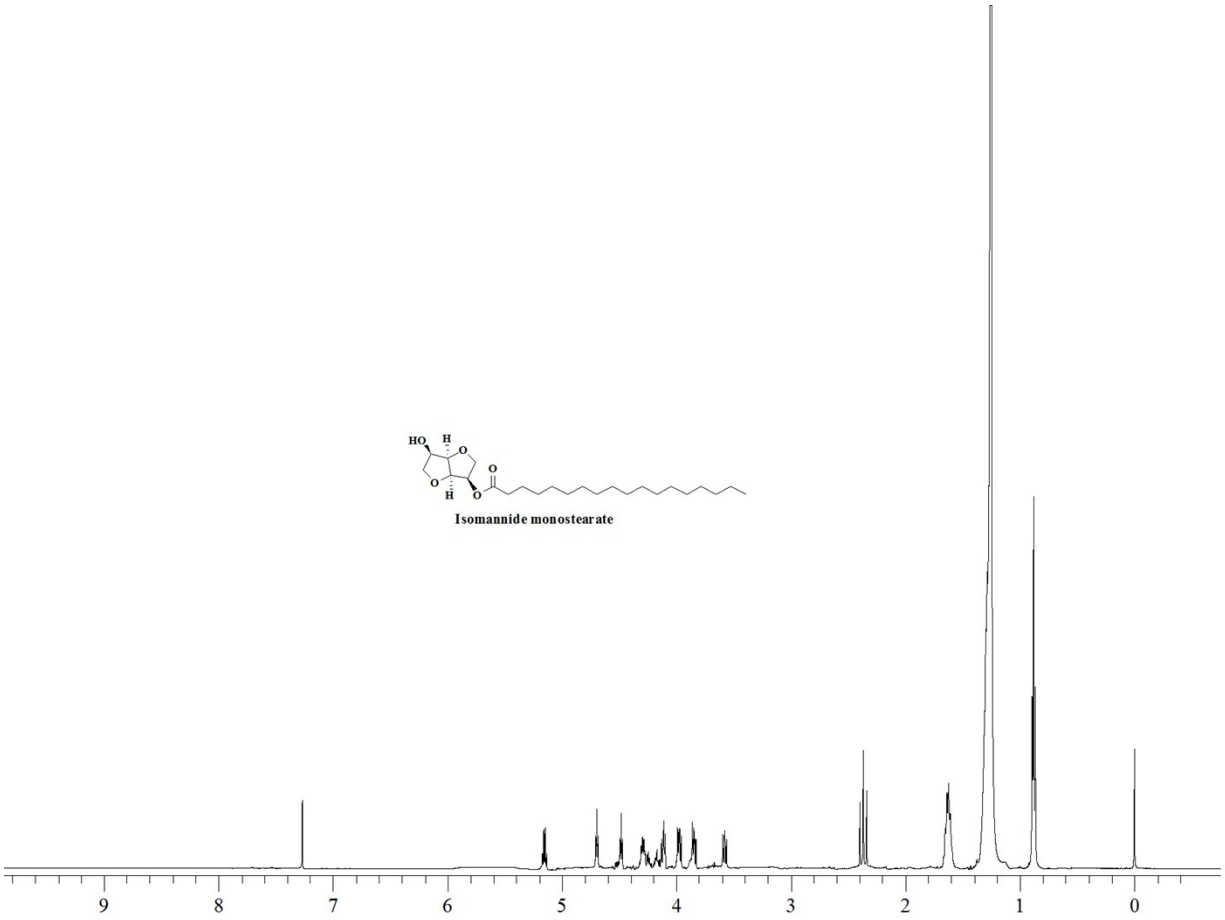


Figure S1: (c1) ^{1}H NMR Spectra of SAIML

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 7.1 Hz, 18-H), 1.21-1.35 (28H, m, 4-H, 5-H, 6-H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H), 1.58-1.67 (2H, m, 3-H), 2.37 (2H, td, J^1 = 2.4 Hz, J^2= 7.5 Hz, 2-H), 3.57 (1H, dd,J^1= 7.0 Hz, J^2 = 9.1 Hz, OCH_2CHOH, H_b), 3.84 (1H, dd,J^1=6.4 Hz, J^2= 9.3 Hz, OCH_2CHOCO, H_b), 3.97 (1H, dd,J^1= 6.2 Hz, J^2 = 9.1 Hz, OCH_2CHOH, H_a), 4.11 (1H, dd, J^1= 6.4 Hz, J^2 = 9.4 Hz, OCH_2CHOCO, H_a), 4.23-4.32 (1H, m, CH_2*CH*OH), 4.48 (1H, t, J = 5.3 Hz, *CH*CHOH), 4.69 (1H, t, J = 5.1 Hz, *CH*CHOCO), 5.13-5.18 (1H, q, *CH*OCO).

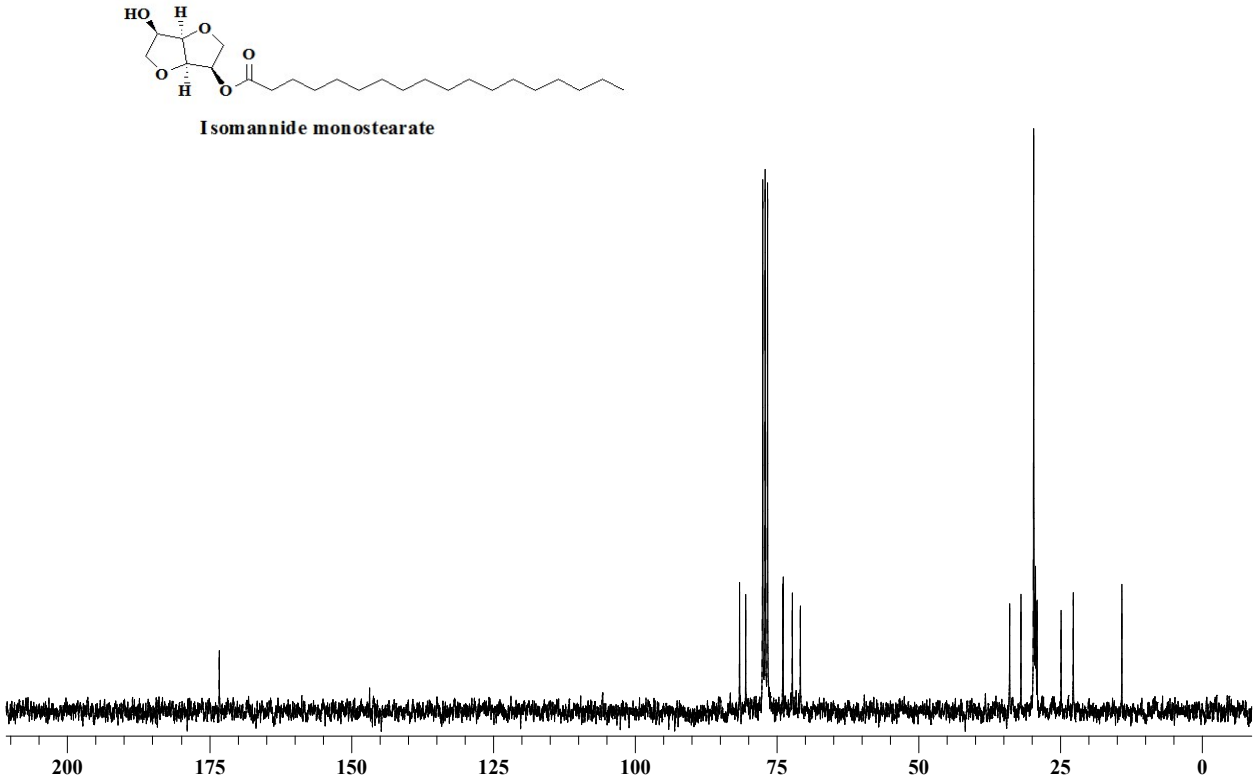


Figure S1: (c2) ^{13}C NMR Spectra of SAIML

^{13}C NMR (75 MHz, $CDCl_3$): δ 14.11, 22.67, 24.82, 29.04, 29.23, 29.34, 29.43, 29.66 (8 × C), 31.90, 33.89, 70.77, 72.20, 73.81, 73.87, 80.43, 81.50, 173.21.

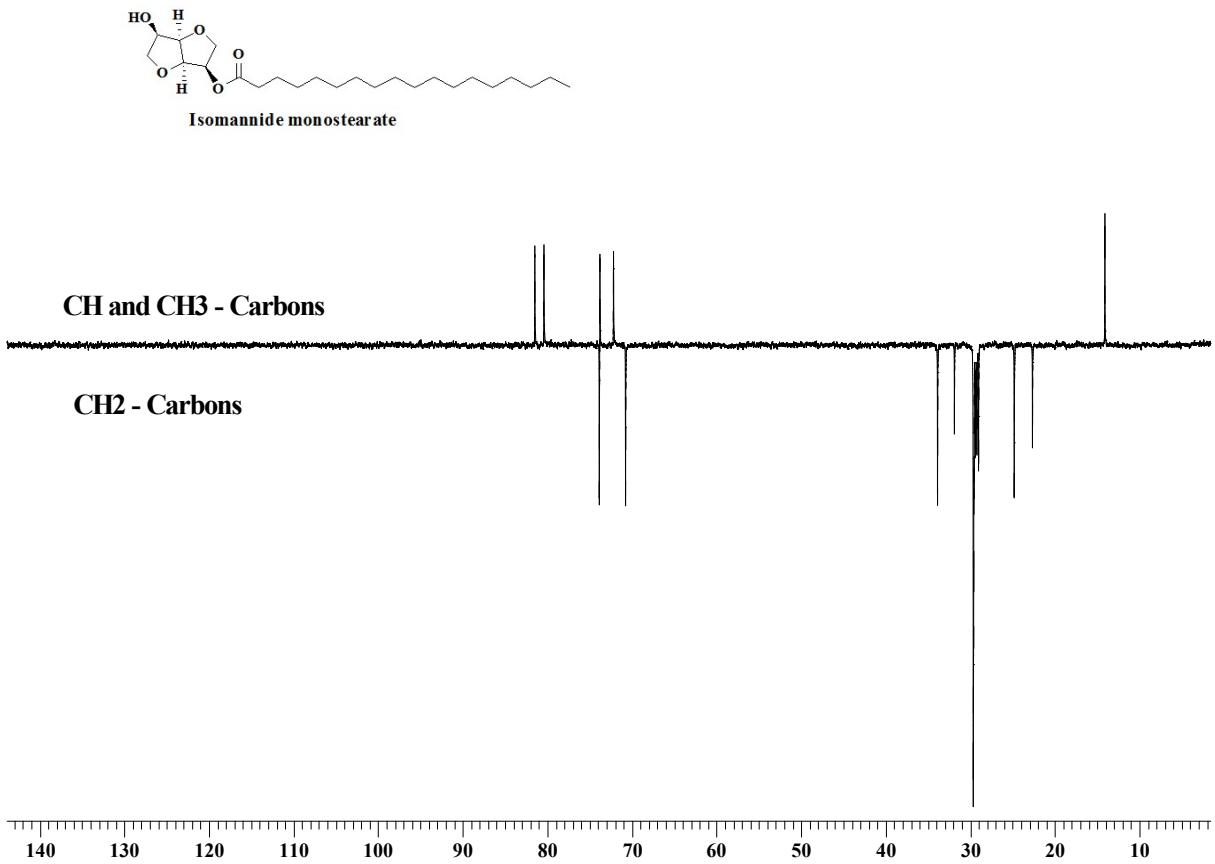


Figure S1: (c3) DEPT -135 NMR Spectra of SAIML

DEPT-135 NMR (125 MHz, $CDCl_3$): δ 14.09 (CH_3), 22.66 (CH_2), 24.81 (CH_2), 29.03 (CH_2), 29.21 (CH_2), 29.33 (CH_2), 29.42 (CH_2), 29.56 (CH_2), 29.65 (7 × CH_2), 31.89 (CH_2), 33.88 (CH_2), 70.77 (CH_2), 72.18 (CH), 73.79 (CH), 73.87 (CH_2), 80.41 (CH), 81.49 (CH).

2.3. HR - Mass Spectra of SAIML

HR - MS [ESI] $^{+}$: calcd for $C_{24}H_{44}O_5Na$ $[M+Na]^+$, 435.3081; found, 435.3083.

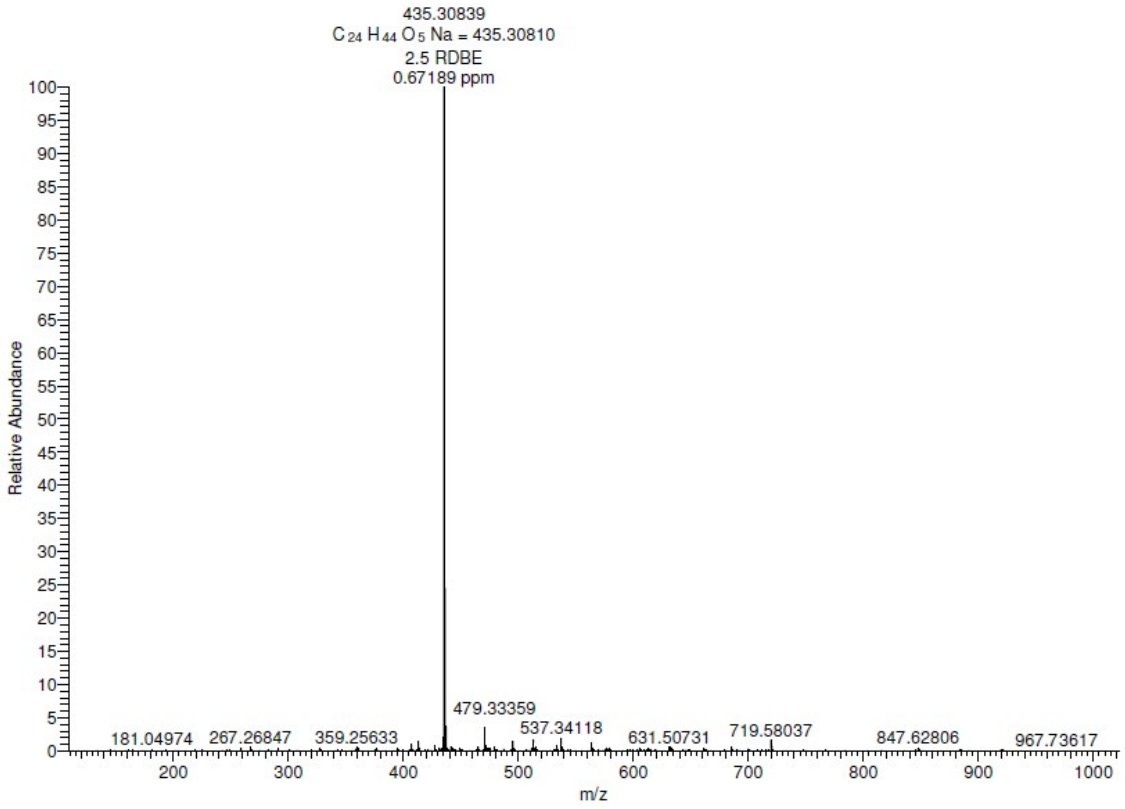


Figure S1: (c4) HR - Mass Spectra of SAIML.

ESI - 3. Preparation of self - assembled structures:

The self - assembled structures were prepared by sonicating a suitable amount of IMLs in water. In a typical experiment, 1 mg of OAIMLs was taken in a cleaned, dried glass sample vial and to that 1mL of Milli Q water was added and the solution was sonicated. The sonication was continued (~3 min) till to get the typical haziness which indicates the formation of microstructures. The same procedure is followed in the case of EAIMLs (1 mg/mL) also. Considering the more hydrophobic character of SAIMLs, the self - assembly was done by taking 0.6mg/mL and the same procedure as described above was repeated to get the supramolecular structures.

ESI - 4. Fluorescent intensity calculation:

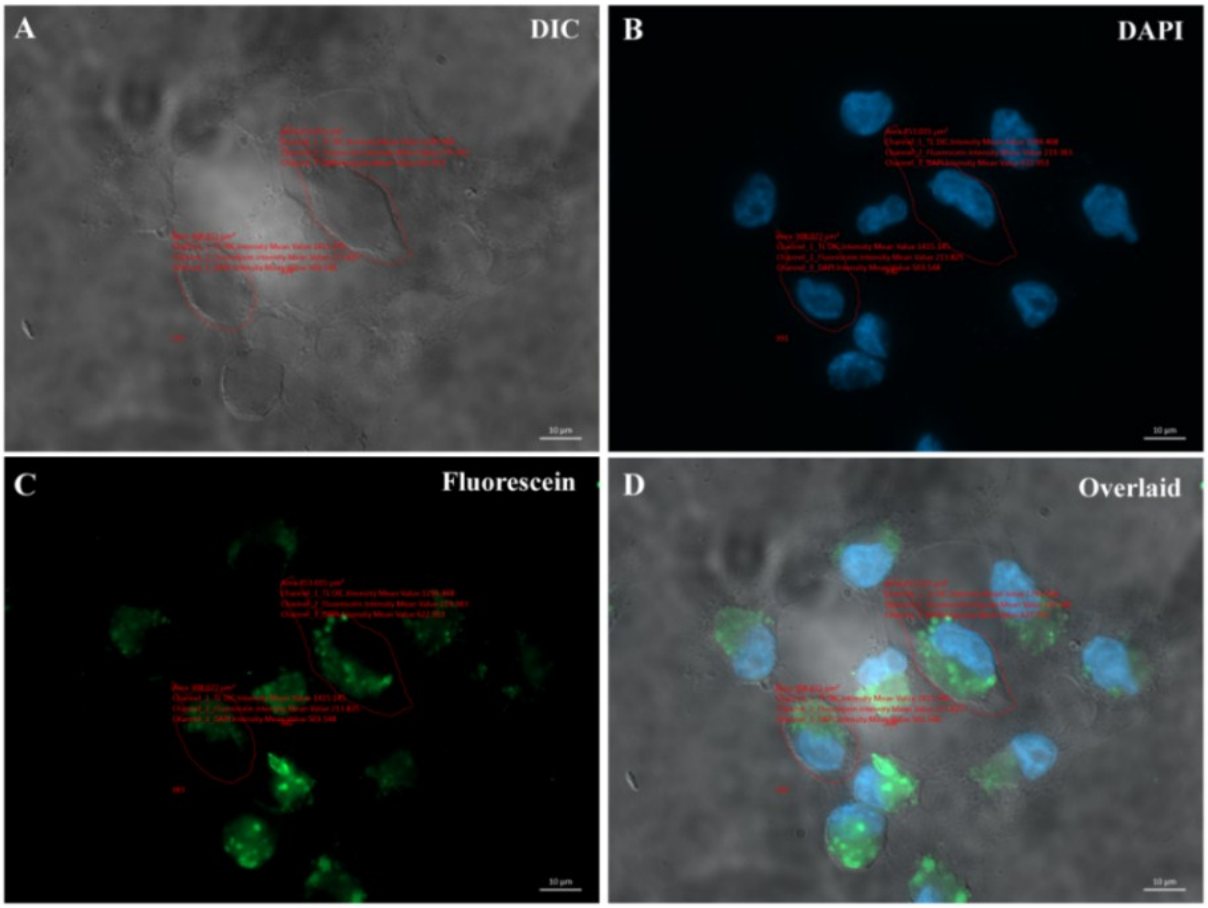


Figure S2: One of the selected images for the calculation of quantification of loaded dye. (A) DIC image, (B) cells with DAPI, (C) cells loaded with fluorescein dye and (D) is the overlaid image of the all the dyes.

ESI - 5. CMC determination of IMLs:

CMC of the self - assembled structures (for OAMLs and EAMLs) was determined using pyrene as a fluorescence probe. The experiment was started by preparing a stock solution ($5x10^{-7}$M) of pyrene. Fluorescence spectra were recorded at room temperature by using a Varian Cary Eclipse fluorescence spectrophotometer. Fluorescence measurements were taken at an excitation wavelength of 330 nm and the emission monitored from 340 to 600 nm. Excitation and emission slit widths were both maintained at 5.0 nm and spectra were collected at a scan speed of 100 nm min. A curve of intensity versus log[C] was plotted. The point where tangents curve crossed was determined as CMC. The cmc values of OAIML and EAIML are found to be 5.6 x 10^{-6} M and 3.16 x 10^{-6} M respectively.

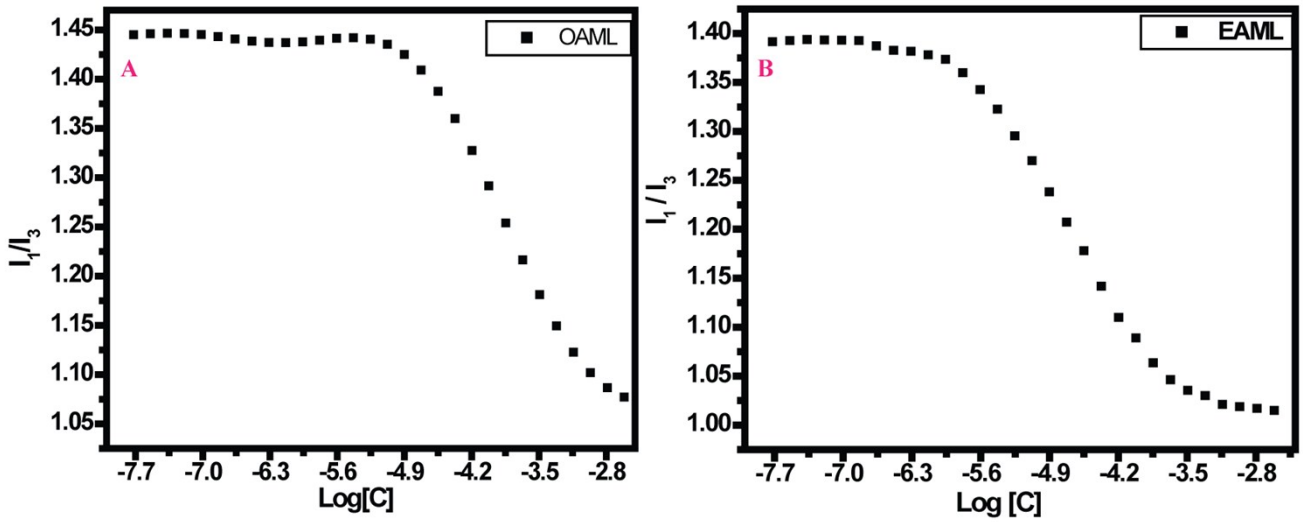


Figure S3: Plot of I_1/I_3 from pyrene versus log (conc.) for CMC determination of (A) OAIML and (B) EAIML respectively.

ESI - 6. Optical microscope images:

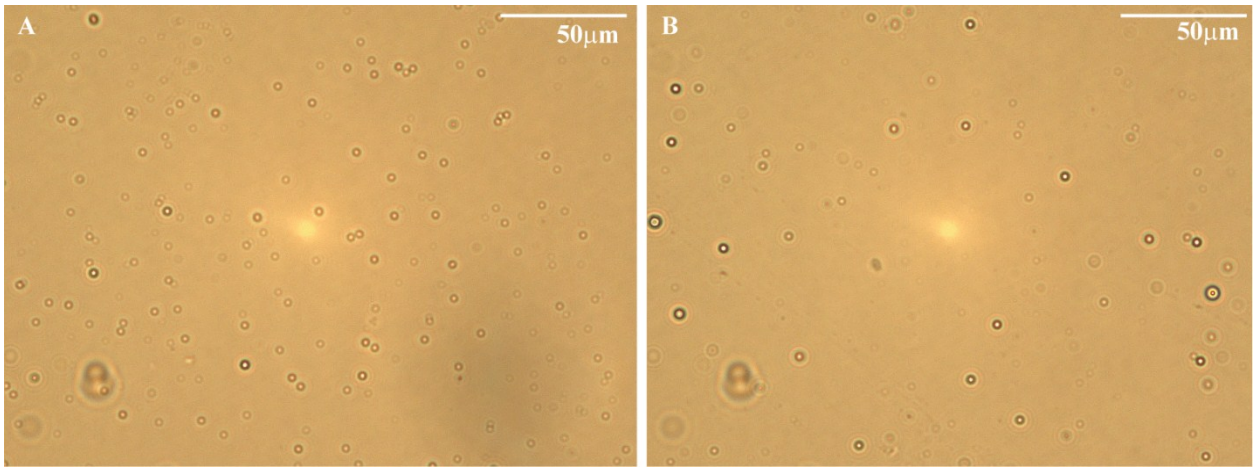


Figure S4: The optical microscope images of (A) OAIML and (B) EAIML at 500 μg/mL concentration respectively.

ESI -7. Dye encapsulation:

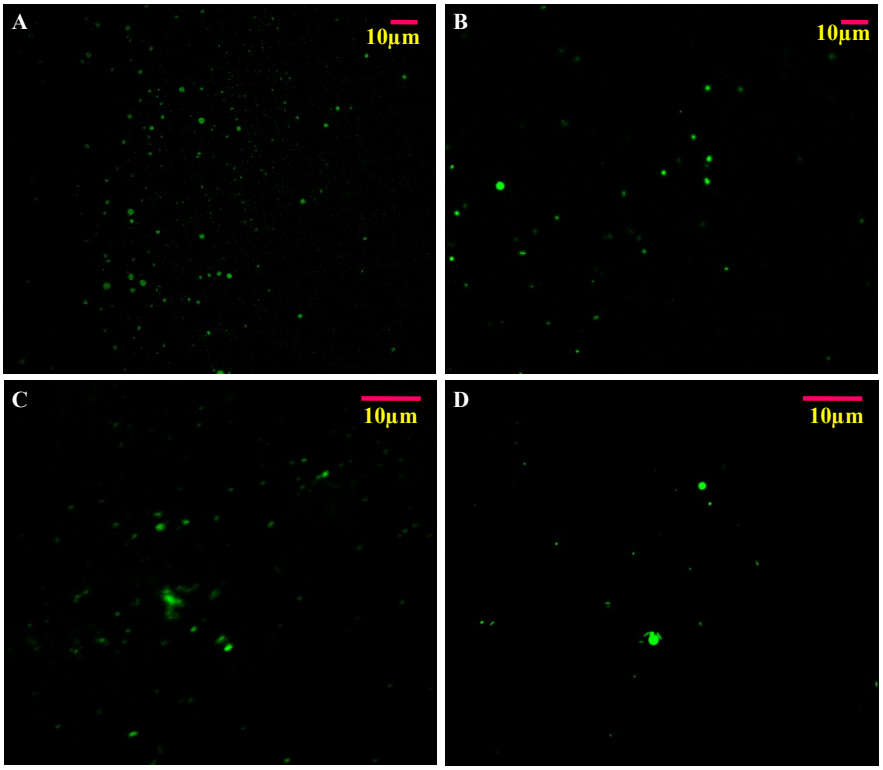


Figure S5: The confocal images of fluorescein encapsulated self - assembled structures of (A, B) at 1mg/mL and (C, D) at 500 μg/mL of OAIML and EAIML respectively.

ESI - 8. Optical microscope images at 40 °C:

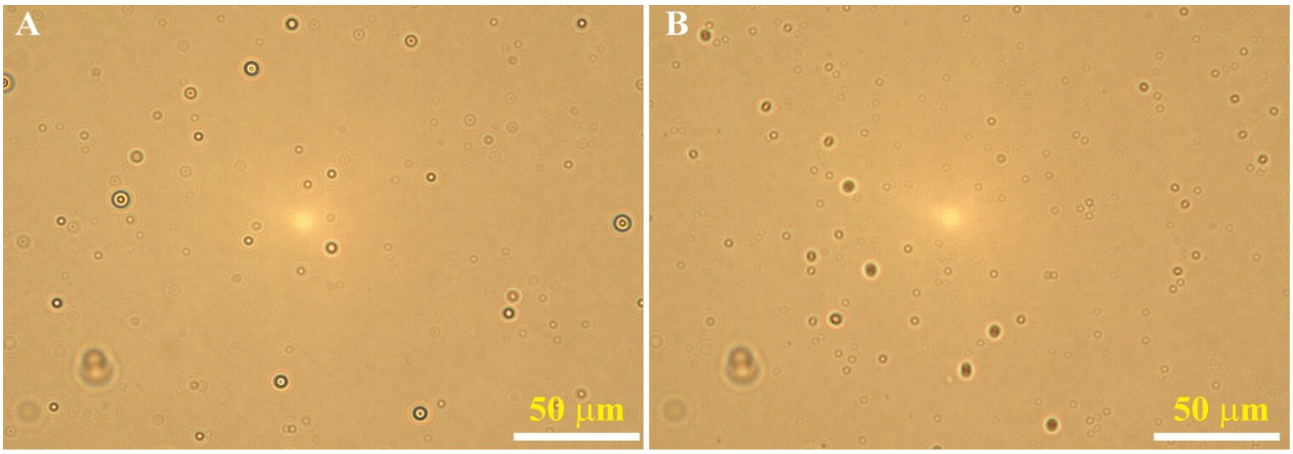


Figure S6: The optical microscope images of (A) OAIML and (B) EAIML at 40 °C (500 µg/mL concentration) respectively.

ESI - 9. UV - Vis spectra of dye encapsulated IMLs:

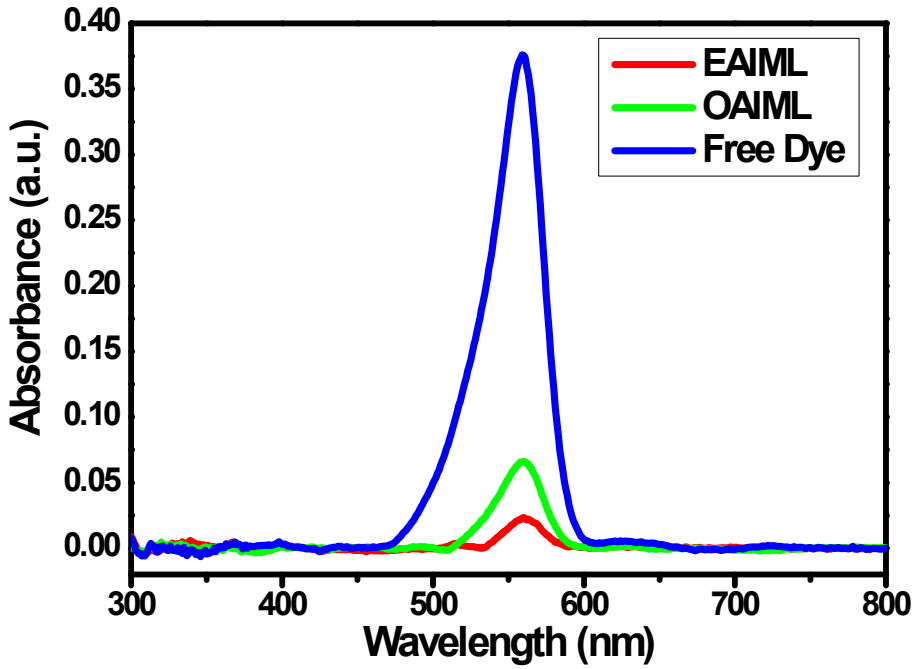


Figure S7: The UV - Vis spectra of EAIML and OAIML loaded with fluorescein dye.

ESI - 10. Fluorescence spectra of IMLs:

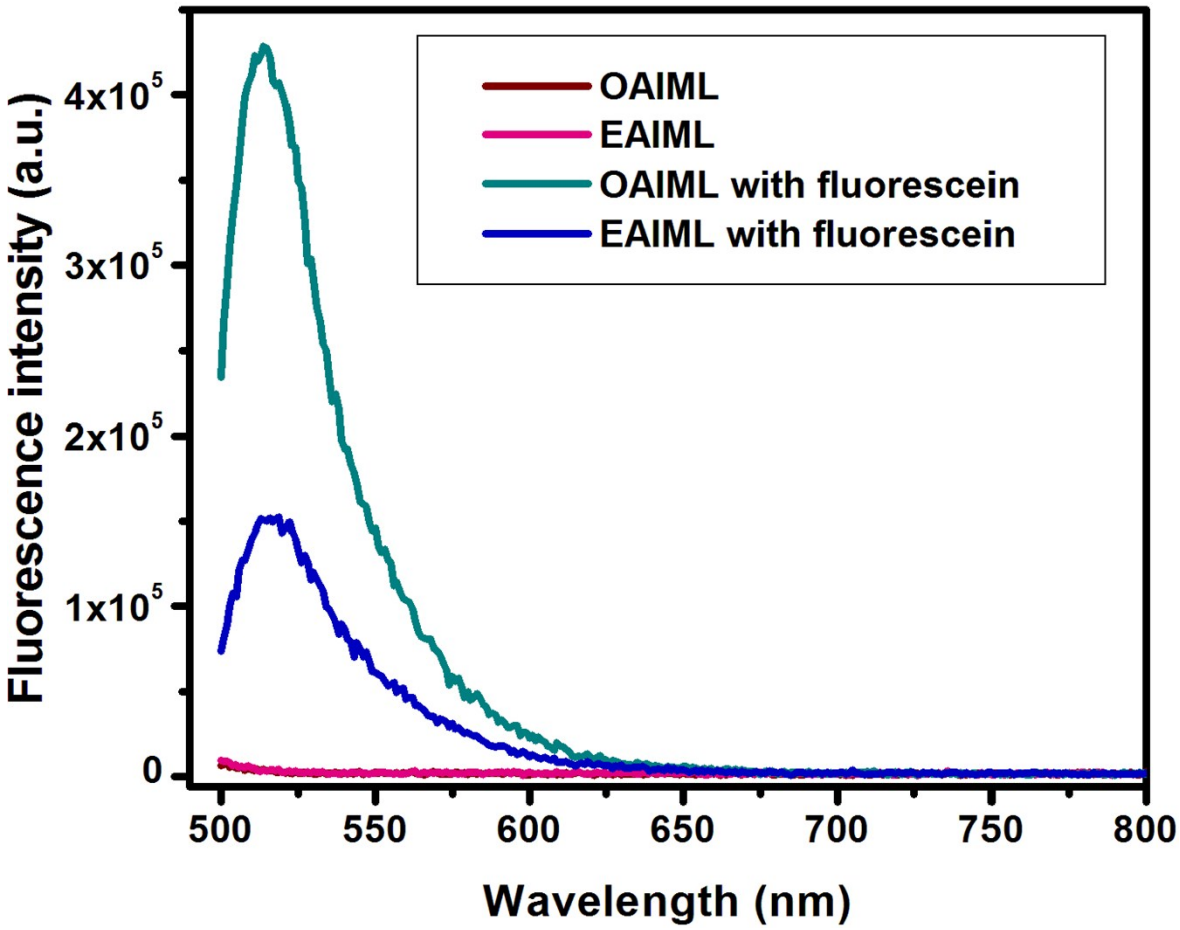


Figure S8: The fluorescence spectra of IMLs, and dye encapsulated to IMLs.
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ESI - 11. Fluorescence intensity quantification:

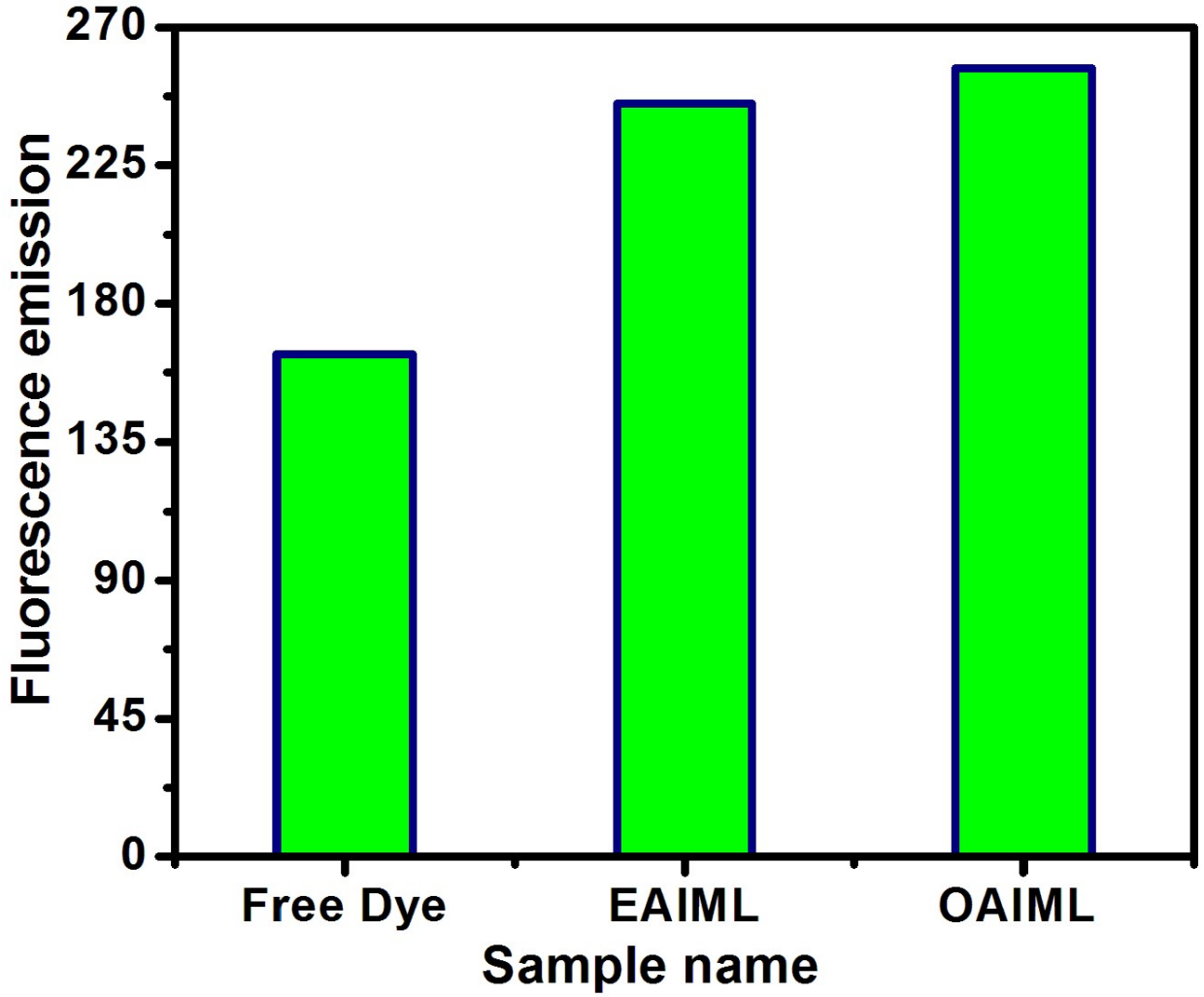


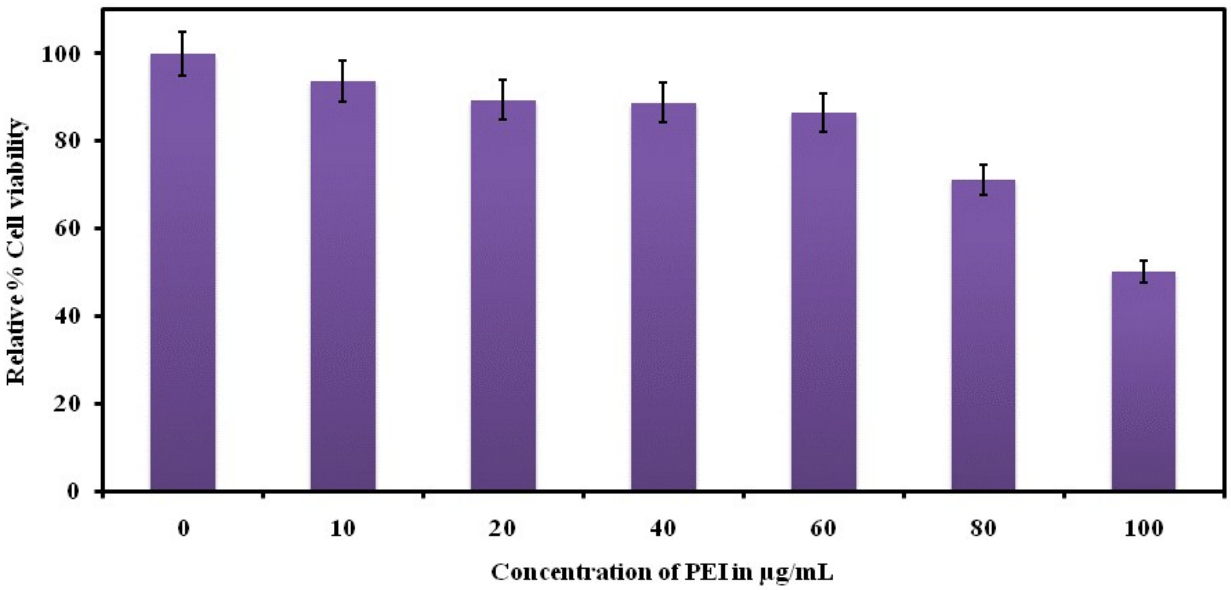
Figure S9: Average fluorescence intensity of fluorescein loaded free dye, OAIML, and EAIML in MDA-MB-231 cells where n=50, quantified by imaging software by ZEN PRO 2012 (From Carl ZEISS).

ESI - 12. Cell viability with PEI as a negative control (MTT Assay):

The cell viability assay was analyzed using colorimetric method which is based on MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). MDA-MB-231 cells were seeded in a flat bottomed 96-well plate at a density of 1 x10^4 cells/well in DMEM containing 10% FBS. The plate was incubated at 37 °C with 5% CO_2 atmosphere for 24 hours. After incubation media was replaced with PEI (1mg/mL in DMEM containing 10% FBS). The different concentration of sample (10, 20, 40, 60, 80, 100 µg/mL) were added to make final volume 100 µL/well and incubated for 40hrs at 37 °C with 5% CO_2 atmosphere. After incubation media was replaced with

filter sterilized MTT (0.45 mg/mL) prepared in DMEM containing 10% FBS and further incubated for 4hrs at 37 °C with 5% CO_2 atmosphere. After incubation the MTT reagent was replaced with100 µL/well DMSO solvent. Addition of DMSO dissolves the formazan crystals formed by reaction of sample with MTT and developed color was measured at 550 nm using a microtitre plate reader (Veroscan, Thermo Scientific). The cell viability was calculated as a percentage relative to untreated control cells.

Figure S10: The cell viability test with PEI as a negative control



ESI - 13. Cell viability Test:

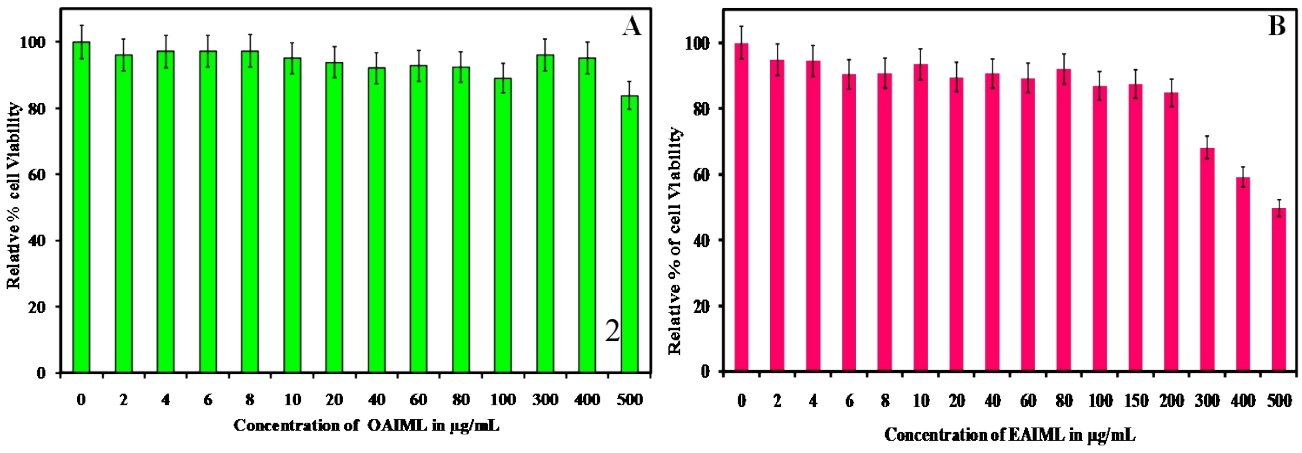


Figure S11: The plot of % of cell (MDA - MB-231) viability against different concentrations of (A) OAIML and (B) EAIML.