A phosphonic acid appended naphthalene diimide motif for self-assembly into tunable nanostructures through molecular recognition with arginine in water

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1. Methods, Materials and Instrumentation:

All reagents were purchased either from Sigma Aldrich Chemical Co. or Merck and used as such without any further purification. All the solvents were received from commercial sources and purified by standard methods. For all aqueous spectroscopic studies, water of spectroscopy or HPLC grade was used. Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on ADVANCE 300 MHz and INNOVA 500 MHz NMR

spectrometer and all the spectra were calibrated against TMS. Mass spectrometric data were obtained by electron spray ionization (ESI-MS) technique on an Agilent Technologies 1100 Series (Agilent Chemistation Software) mass spectrometer. High-resolution mass spectra (HRMS) were obtained by using ESI-QTOF mass spectrometry. FTIR spectra were obtained on a Perkin Elmer FT-IR 400 spectrometer.

2. Sample preparation

Titration experiment of Phos-NDI with D/L-arginine: Aqueous Phos-NDI **1** solutions ($0.5x10^{-5}$ M) were prepared by dilution from 10^{-2} M solution and basify to pH 9 by aqueous NaOH (0.1 M). Followed by the mixed aggregate system, which prepared by titrating Phos-NDI ($0.5x10^{-5}$ M, pH 9) with aqueous solution of D/L-arginine (1×10^{-2} M) and pH of the solution was adjusted by addition of NaOH (0.1 M) and final pH of the solution was 9. The sample solutions were allowed to equilibrate at room temp for 2h before spectroscopic measurements.

UV-Vis Spectroscopic Spectroscopy: UV-Vis spectra were recorded in a Shimadzu 2450 UV-visible spectrometer in 1cm path length cuvette.

Fluorescence Spectroscopy: Fluorescence emission spectra were recorded in a Horiba Jobin Yvon FluoroMax®-4 –Spectrofluorometer. All experiments were performed in a quartz cell with a 1 cm path length with 384 nm excitation wavelength.

Scanning Electron Microscopy (SEM): SEM measurements were performed on an electron microscopy HITACHI S-4800, operating at an accelerating voltage of 15 kV. 10 μL of freshly prepared sample solution was dropped onto a carbon tape and the solvent was allowed to evaporate before investigation by SEM and images were collected.

Transmission electron microscopy (TEM): ***Method 1:*** TEM measurements were carried out on an Igor 1200EX, operating at an accelerating voltage of 80 kV. Freshly prepared sample (10 μL) of premixed Phos-NDI with L- or D-arginine was dropped onto a TEM grid (400-mesh

copper grid coated with carbon) sample were stained with uranyl acetate and the solvent was allowed to evaporate before introduction into the vacuum system and images were collected. ***Method 2:*** TEM samples were prepared by dropping 10 μL aliquots of Phos-NDI 1:D-or L-arg (1:2 ratio in H2O at pH 9) onto a carbon-coated grid followed by sample were stained with uranyl acetate. After about 1 min, the remaining solution was blotted off with a filter paper. TEM measurements were carried out on an Igor 1200EX, operating at an accelerating voltage of 100 kV was used to obtain the images of **Fig.'s S9 and S10**. Interestingly obtained results are similar to air dry imaging (Fig. **3c** and **4a** show in main manuscript**)**.

Circular Dichroism (CD): CD spectra were recorded on an AVIV 202 CD spectrometer under a nitrogen atmosphere. Experiments were performed in a quartz cell with a 1 mm path length over the range of 300-450 nm at 25 ^{o}C.

Dynamic Light Scattering (DLS): DLS experiments were carried out on a PD Expert instrument. A solution of (2 mL, 1 mm) the sample was prepared and equilibrated at RT for 2 h before the DLS analysis.

X-ray diffraction (XRD): XRD data were recorded on a Seifert XRD3000P diffractometer and a voltage and a current of 40 kV and 30 mA, respectively. In a typical XRD experiment, a solution of Pho-NDI **1** in water (1 mm) was repeatedly drop-cast onto a glass slide to make a thick film. The film was then dried under a high vacuum and the data were recorded from 1–308 with a sampling interval of 0.028/step.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra were collected on a Perkin Elmer FT-IR 400 at ambient temperature. The instrument was continuously purged with CO_2-free dry air. Spectra were recorded between 450 and 4000 cm^{-1}. A solution of the aggregate sample in water (50 μL, 2 mM) was isolated and the spectra of the solids were recorded.

Differential scanning calorimetry (DSC): DSC was carried out by using a DSC Q 100. 20 mg of NDI bola was placed into a large volume capsule (LVC) that was then sealed. The sample

LVC pan was placed into the DSC apparatus together with an empty LVC pan as reference. The pans were cooled to 0 °C, and aged for 30 min at this temperature. Heating scan was then recorded from 0–300 °C at a scan rate of 1 °C min^{-1}.

Thermal Gravimetric Analysis (TGA): TGA was carried out by using a TGA Q500 thermal analyzer. Measurements were carried out in platinum pans under nitrogen atmosphere (flow rate of 50 mL/ min) with a heating rate of 10 °C/min over a temperature range of 0–700 °C. The temperature calibration of the TGA equipment was carried out by use of the Curie-point calibration technique (Alumel, Ni, Perkalloy, Fe).

Atomic Force microscope (AFM): The samples were characterised using an Atomic Force Microscope (AFM) from Agilent Technologies (5500 AFM). Freshly prepared sample (10 µL) of premixed Phos-NDI with L- or D-arginine was dropped onto freshly cleaved mica and put these samples into vacuum for 2 h to remove the solvent before analysis.

4. Microscopy analysis

4.1 UV-Vis absorption spectroscopy

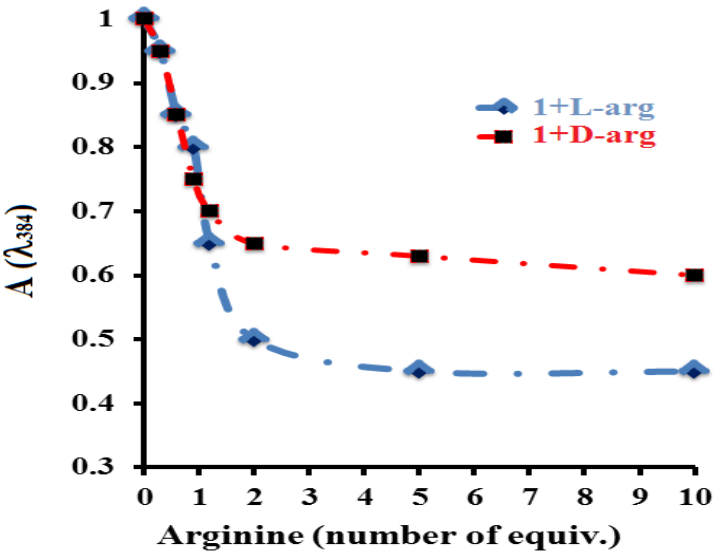


Fig. S1 Plot of number of equivalents of arginine *vs* absorption at 384 nm.

4.2. Fluorescence Spectroscopy

At pH 9 the phosphonic acid exists in it is fully deprotonated species and is able to interact with cationic guanidinium group of arginine *via* electrostatic interaction as the primary force for the association. As the addition of L- or D-arginine solution was continuously increasing, the fluorescence intensity started enhancing. The characteristic change in fluorescence intensity is attributed to the electrostatic interaction between guanidinium and phosphate groups as a result of the formation of aggregate i.e. nanobelt and nanoparticles. The change in fluorescence intensity thus consistent with the SEM images suggesting such changes originates from the formation of aggregates. Thus the concentration dependence of emission intensity is closely associated with the size of the aggregates.[S1]

Furthermore, it can be clearly seen that **1** (10^{-5} M, λ_{ext} = 384 nm), the fluorescence emission spectra show a well-resolved vibronic structure between 380-430 nm, which is characteristic of the monomer fluorescence of Phos-NDI. Upon addition of L-arginine (0-2 equiv.), a broad structureless emission along with new fluorescence emission band at 445 nm, which corresponds to the excimer fluorescence of Phos-NDS, appers in the spectra. However, such effect (excimer formation) were not observed upon addition of D-arginine at same concentration (Fig. S2b). Therefore, we believe that excimer is formed more effectively in Phos-NDI in the presence of L-arginine. However after 2 equivalent additions of L- or D-arginine over Phos-NDI 1, there were no further changes in absorption spectrum thus indicates the saturation point (Fig. S3).

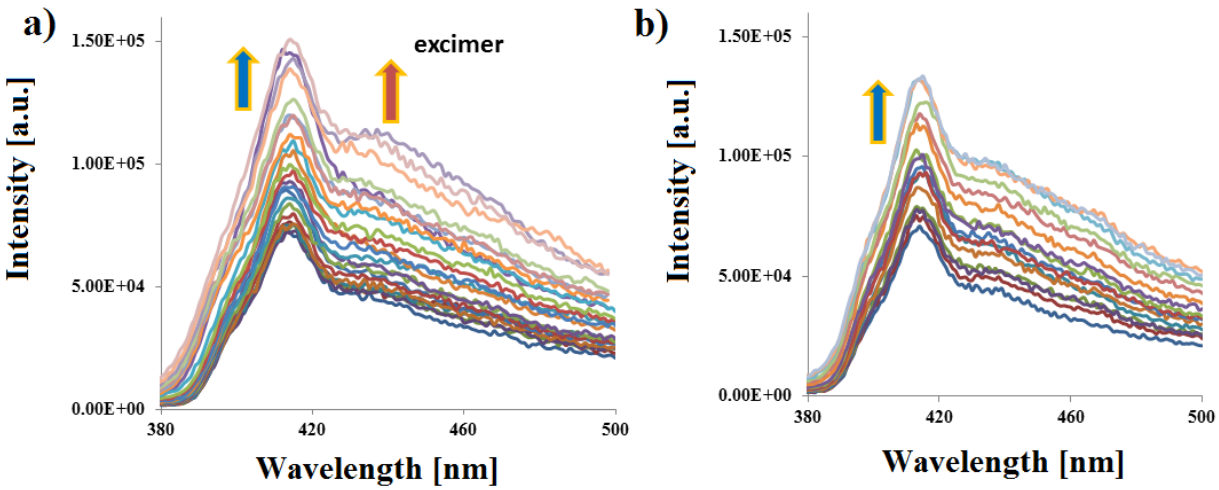


Fig. S2 Fluorescence spectra of **1** (10^{-5} M; λ_{ex} = 384 nm) upon addition of 0-10 equivalent of (a) L-Arg (10^{-2} M) and (b) D-Arg (10^{-2} M) respectively.

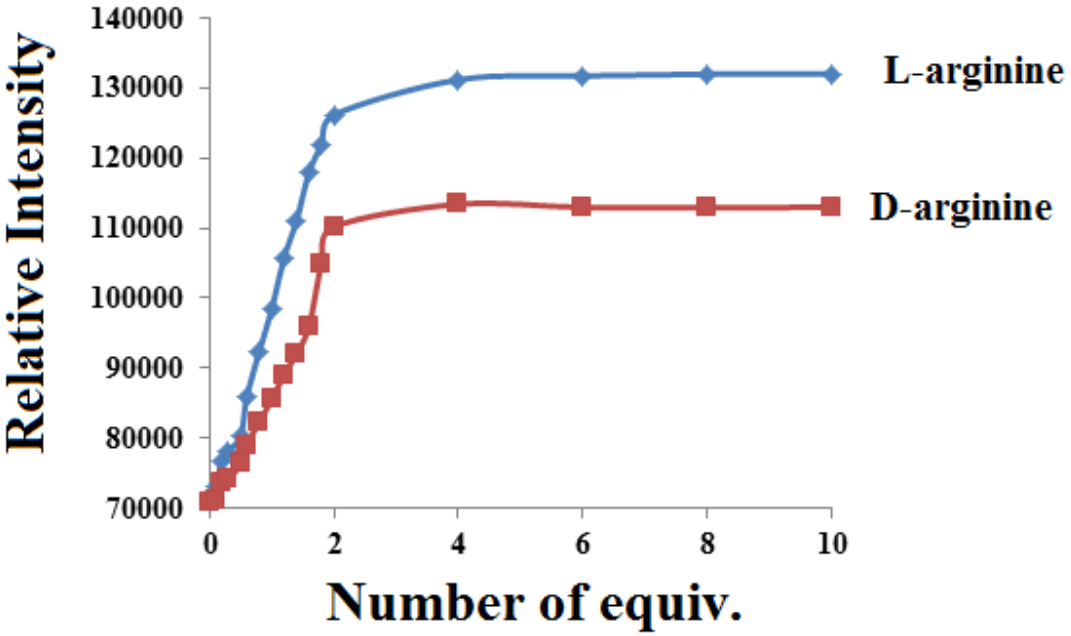
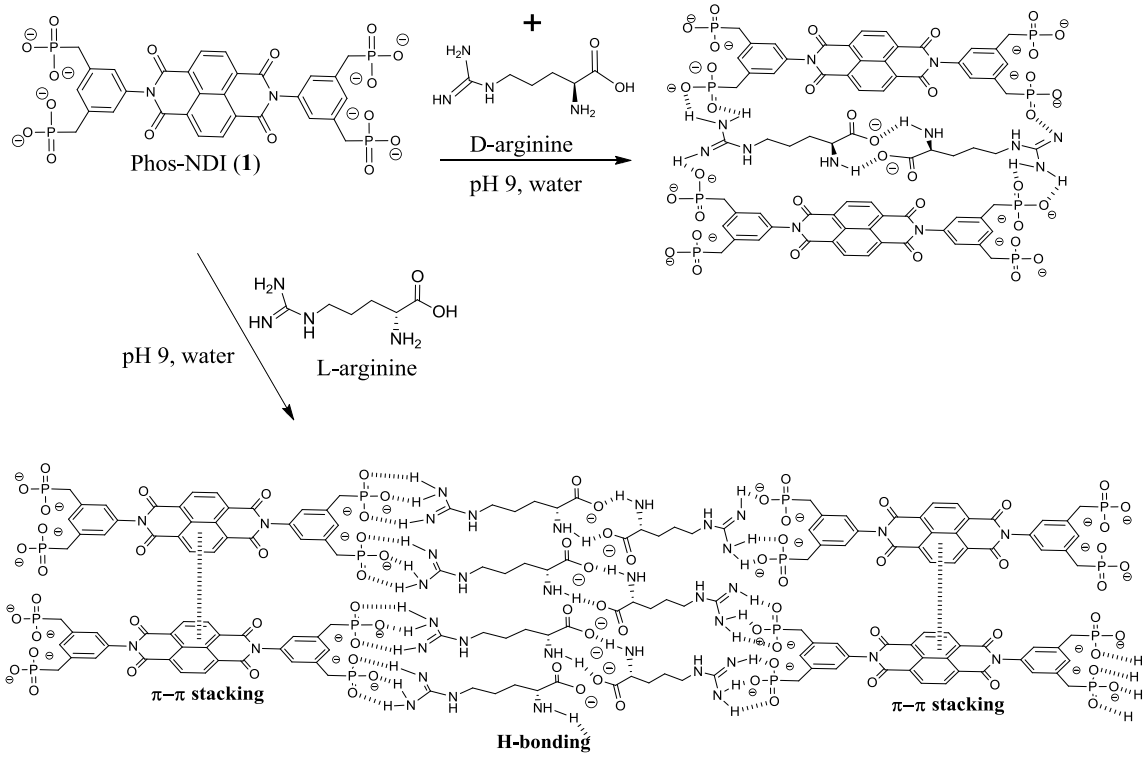


Fig. S3 Plot of number of equivalents of arginine (0-10 equivalent) *vs* relative emission intensity at 414 nm (λ_{ext} = 384 nm).



Scheme S1. Possible mode of self-assembly of Phos-NDI with D and L-arginine in water at pH 9.

4.3. SEM, TEM and AFM images

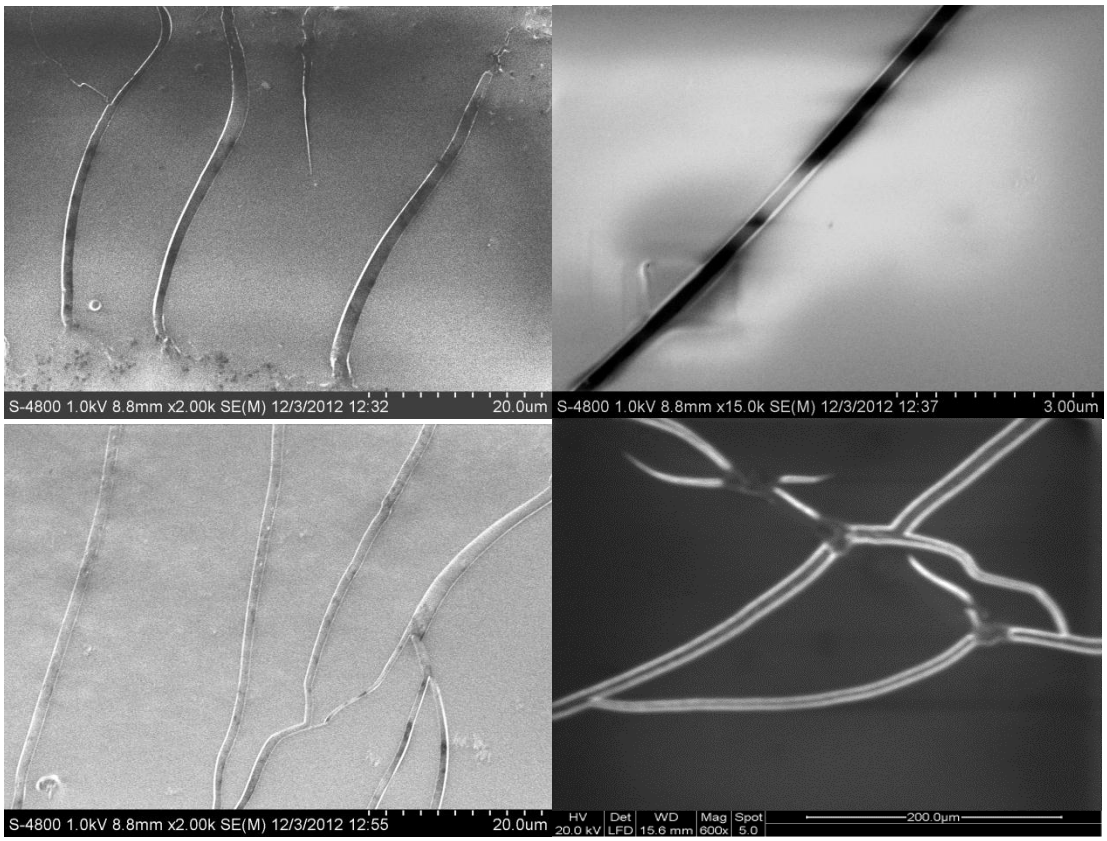


Fig. S4 SEM images of premixed solution (1:2 molar ratio) 0f **1** (1 x 10^{-4} M) with L-arginine (1.2 x 10^{-2} M) in water at pH 9.

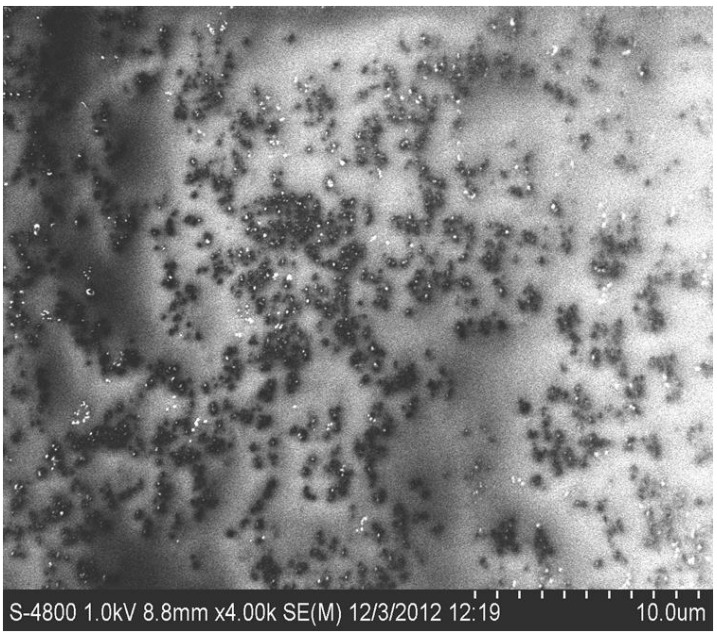


Fig. S5 SEM images of premixed solution (1:2 molar ratio) 0f **1** (1 x 10^{-4} M) with D-arginine (1.2 x 10^{-2} M) in water at pH 9.

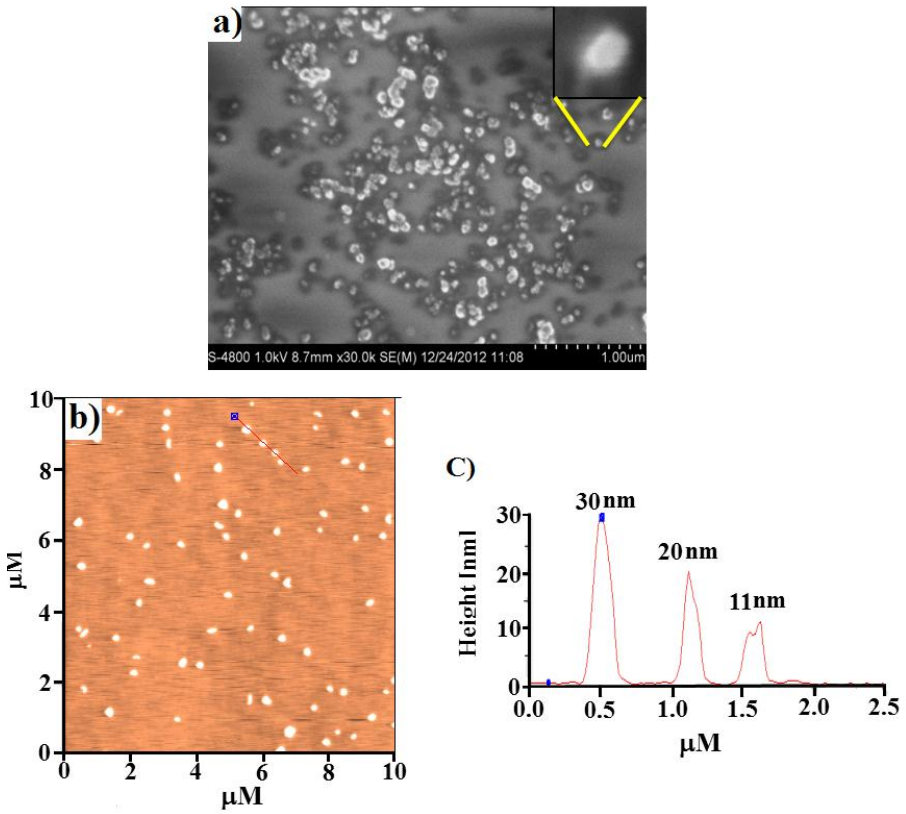


Fig. S6 (a) SEM, (b) AFM images of spherical aggregates formed from Phos-NDI **1** with D-arginine in water at pH 9. (c) Cross-section analysis of three spherical aggregates indicated in (b).

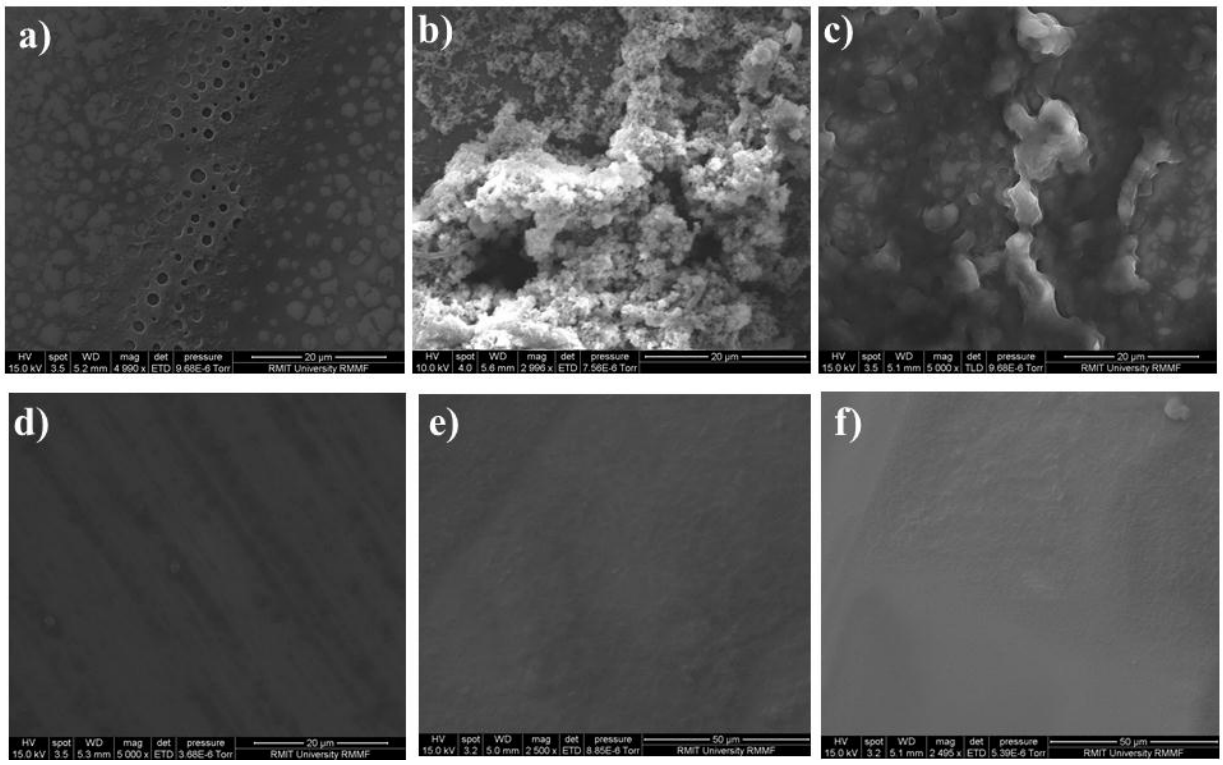


Fig. S7 SEM images of (a) only Phos-NDI **1** ($c = 10^{-4}$ M), (b) Phos-NDI **1** in the presence of L-arginine (1:1 molar ratios), (c) Phos-NDI **1** in the presence of D-arginine (1:1 molar ratios), (d) Phos-NDI **1** in the presence of L-lysine (1:2 molar ratios), (e) only L-arginine ($c = 10^{-4}$ M) and (f) D-arginine ($c = 10^{-4}$ M) in water at pH 9.

4.4. Requirement of pH 9 for the self-assembly

The phosphonic acid remains in fully protonated form with lowering the pH 3-5 whereas at pH 6.5 remains partially protonated.[S1] In these two forms, Phos-NDI is unable to interact with cationic guanidinium group *via* electrostatic interactions, thus corresponds to no particular morphological structure. Whereas above pH 9 complete deprotonation of the phosphonic acid resulted electrostatic interactions with guanidinium group. Furthermore, increase in pH doesn't change deprotonated state of phosphonic acid resulted same electrostatic interaction induces chirality *via* molecular recognition as well as nanostructure morphology.

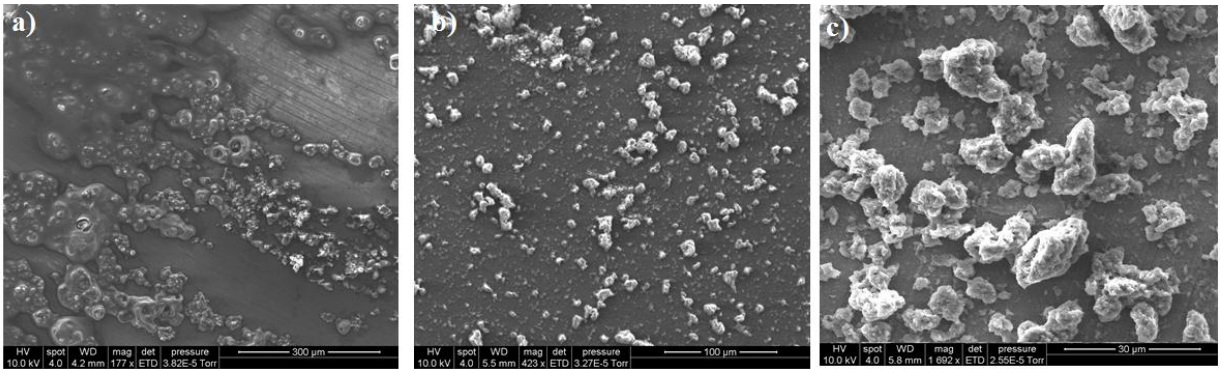


Fig. S8 SEM images of Phos-NDI **1** (10^{-4} M) with L-arginine (1:2 molar ratios) at (a) pH 4.5, (b) pH 7, and (c) pH 8 in water, respectively.

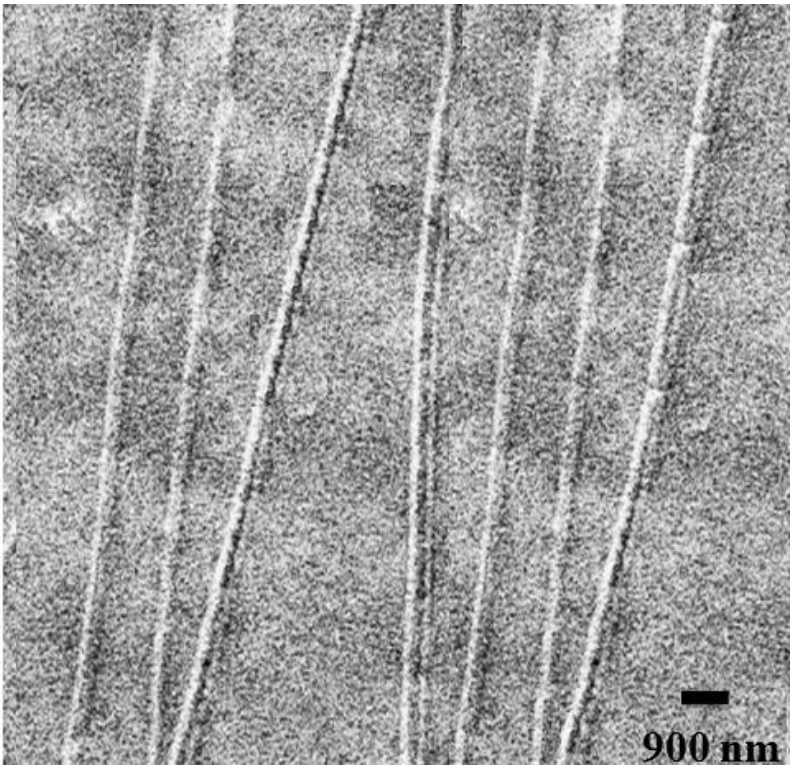


Fig. S9 (a) TEM micrograph of the mixture of **1** with L-arginine (1:2 ratio, $0.5x10^{-5}$ M), in water at pH 9, deposited onto the carbon-coated copper grid and blotted with filter paper.

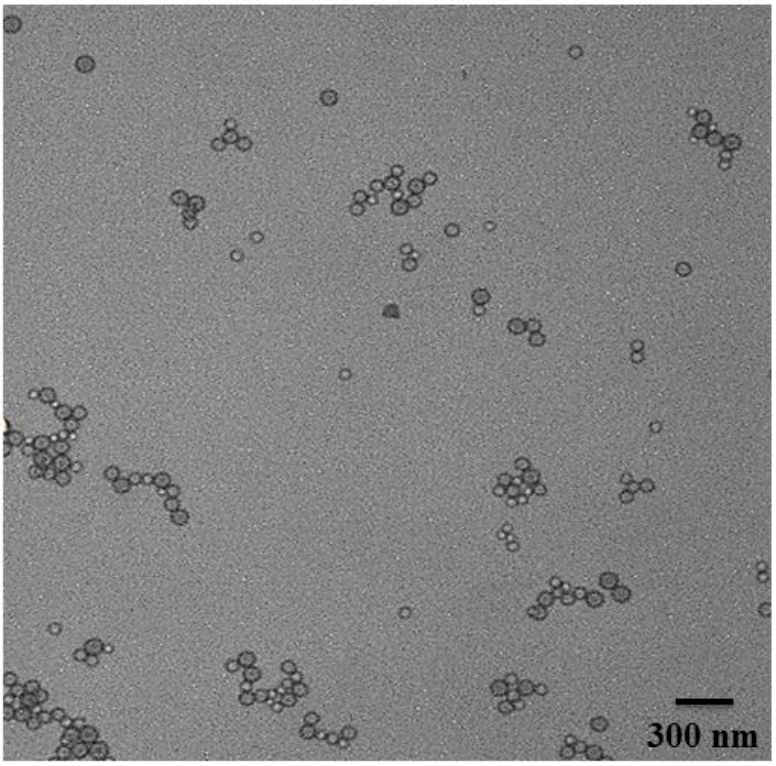


Fig. S10 (a) TEM micrograph of the mixture of **1** with D-arginine (1:2 molar ratio, $0.5x10^{-5}$ M), in water at pH 9, deposited onto the carbon-coated copper grid and blotted with filter paper.

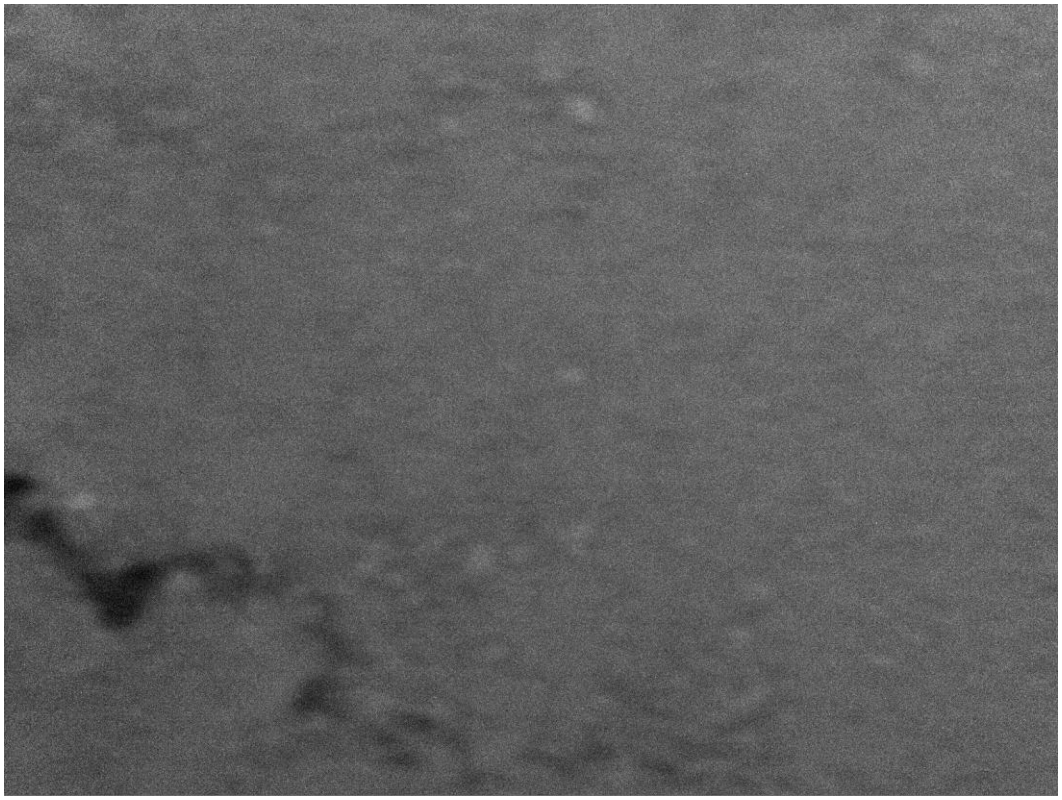


Fig. S11 TEM micrograph of the **1** ($0.5x10^{-5}$ M), in water at pH 9, deposited onto the carbon-coated copper grid and blotted with filter paper.

X-ray diffraction (XRD) of 1 with D-arginine:

X-ray diffraction (XRD) patterns of Phos-NDI-D-arginine nanoparticles shown in Figure S11. Low angle XRD pattern of Phos-NDI **1** with D-arginine (1:2 molar ratios) displayed amorphous aggregates.

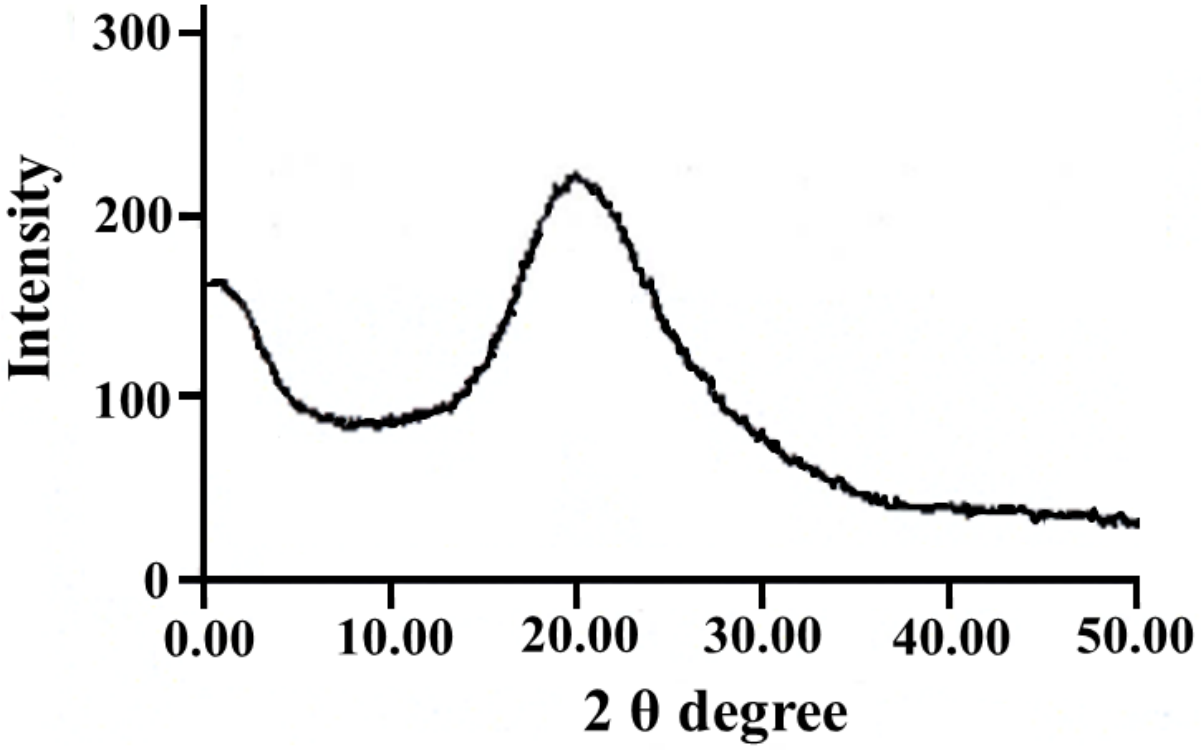


Fig. S12 XRD pattern of particles (film prepared by evaporating premixed solution of **1** with D-arginine (1:2 molar ratio) from water on glass slide for 2 h, and plotted against the angle 2θ at 298K.

4.5. TGA/DTG and DSC analysis

The thermal behaviour of Phos-NDI **1** was examined using TGA/DTG (Fig. S11). TGA analysis of **1** indicated that below 270 ^{o}C, ~10 % **wt.** loss was observed in the nitrogen atmosphere. This mass loss is due to the desorption of adsorbed water and occluded solvent.

Phos NDI 1 is thermally stable up to a temperature of 270^{o}C in nitrogen. The mass loss above 270 ^{o}C observed in DTG thermogram attributed to the oxidation of organic groups. The **Phos NDI 1** showed only the melting transition in their DSC thermogram (Fig. S12). In DSC curve the oxidative decomposition was observed above 270 ^{o}C.

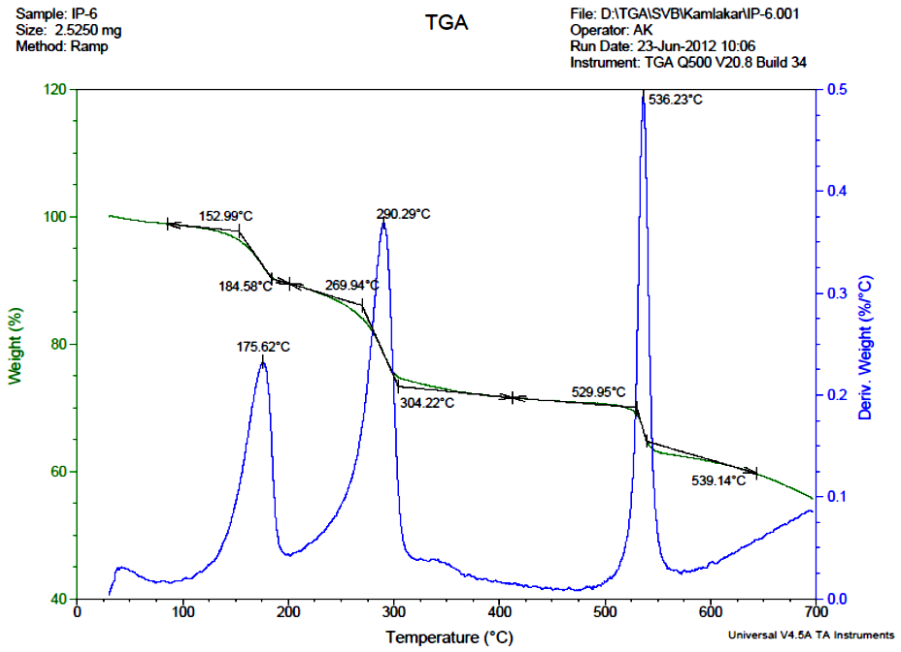


Fig. S13 TGA/DTG thermogram of **1.**

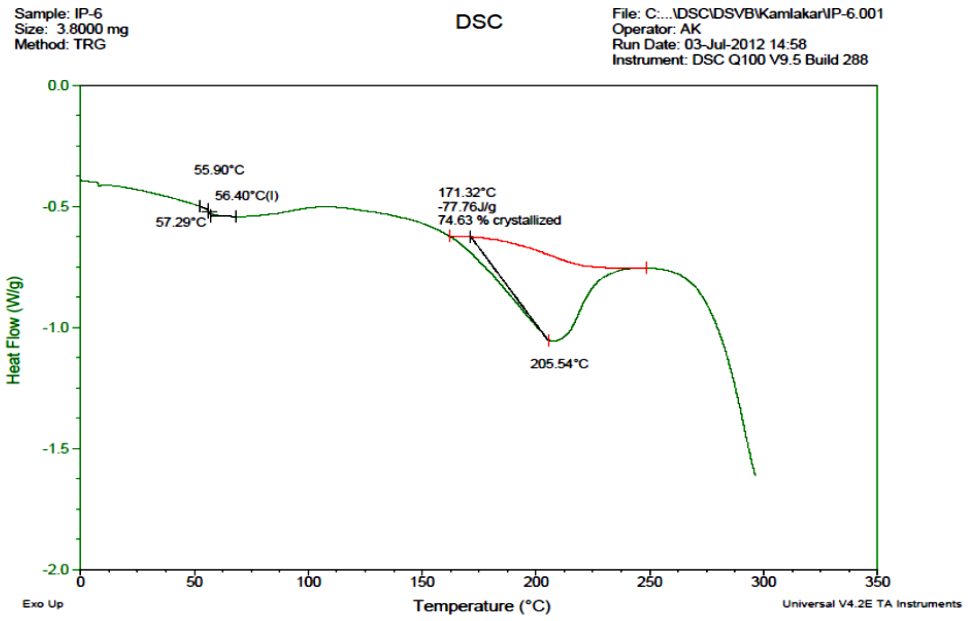


Fig. S14 DSC of **1**.

4.6. FTIR spectroscopy

In particular, FTIR peak at 3468 cm^{-1} belongs to O-H stretching of phosphoric acid groups of **1**, which was shifted to 3365 cm^{-1} when **1** was assembled with arginine. It was further noted that C=O stretching vibrational band at 1630 cm^{-1} was broadened in the presence of arginine. The decrease of wavenumber and broadening of peaks confirms the formation of strong hydrogen bonding.

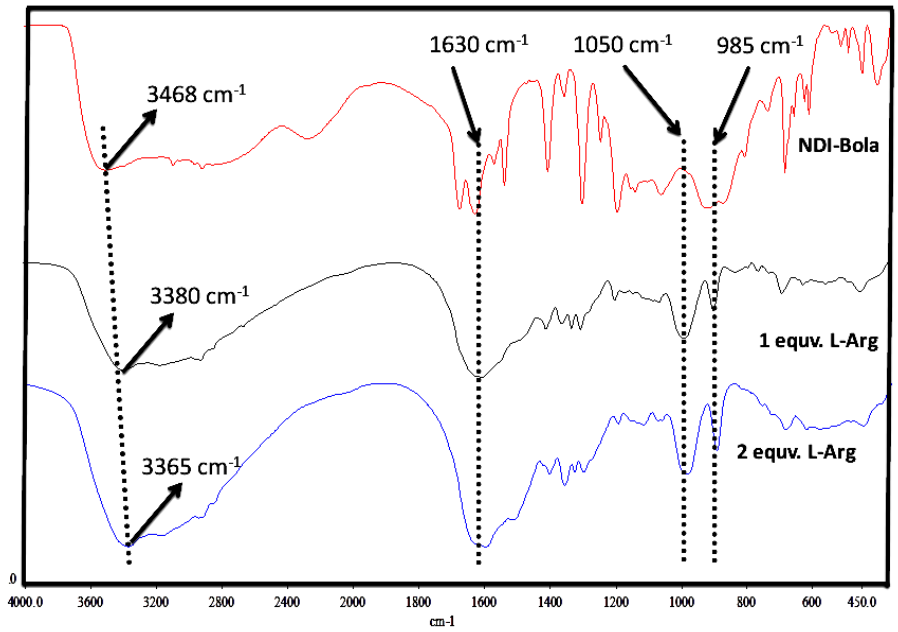


Fig. S15 FTIR spectra of powder and assemblies on two equivalents addition of L-arginine to **1**.

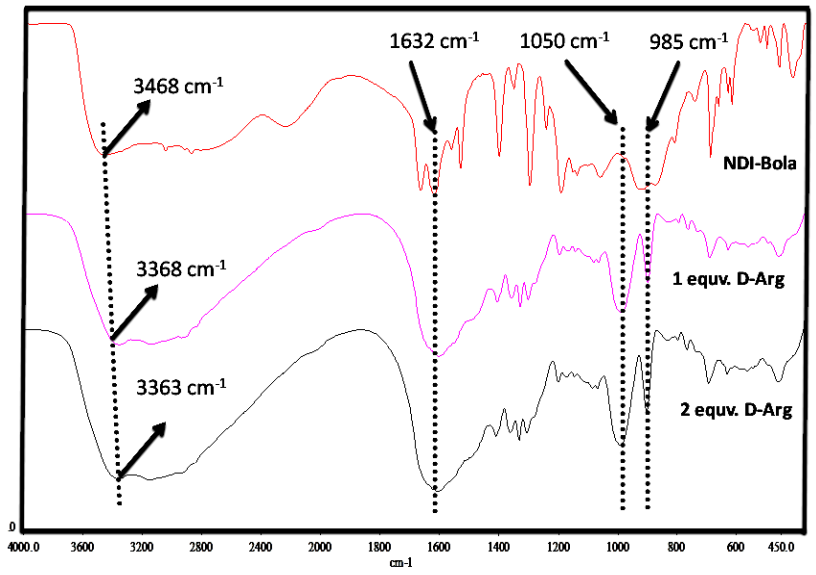
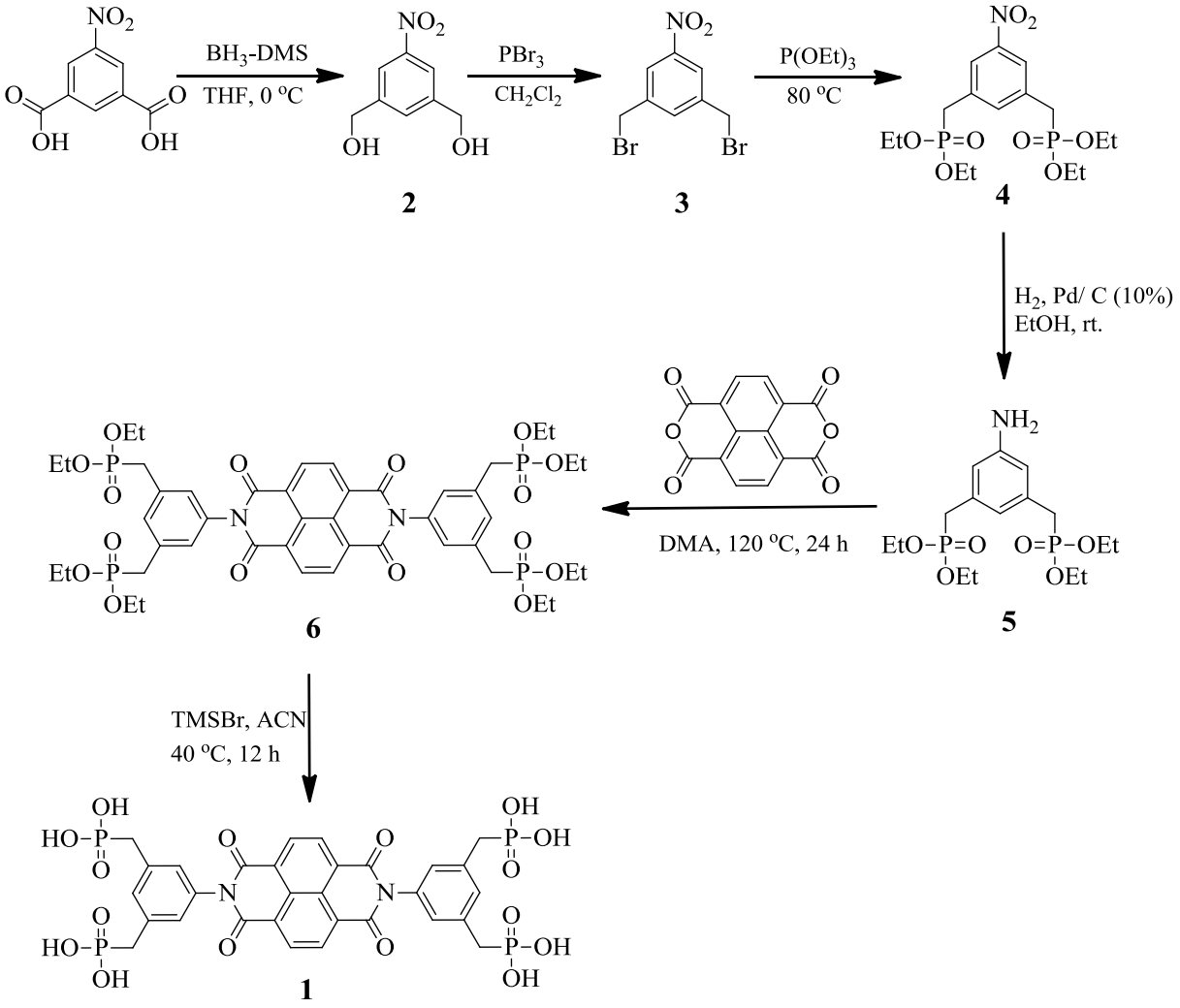


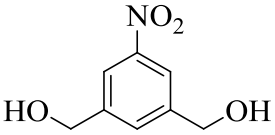
Fig. S16 FTIR spectra of powder and assemblies on two equivalents addition of D-arginine to **1.**

5. Synthesis of Phos-NDI target molecule



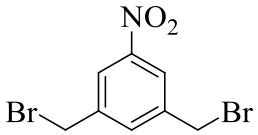
Scheme S1: Synthetic route for the preparation of Phos-NDI **1**

Synthesis of 3, 5-bis-hydroxymethylnitrobenzene:[S2]



A solution of 5-nitroisophthalic acid (10.0 gm, 47.36 mmol) in 100 mL anhydrous THF was cooled to 0 ^{o}C and 2.0 M BH_3-THF (4 equiv.) was added drop wise over the period of 1h. The reaction mixture was allowed to warm slowly to room temperature and stirred for another 48 h. Methanol (100 mL) was added drop wise into the reaction mixture and the reaction mixture was filtered. The filtrate evaporated with a rotary evaporator and the residue was re-dissolved in ethyl acetate (500 mL), washed with saturated $NaHCO_3$, water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and evaporated resulting yellow solid, which was further purified by flash chromatography on silica (elution with 1:1—1:5 Hexane/Ethyl acetate) to afford pure 3,5-bis-hydroxymethylnitrobenzene **2** as a white solid (7.68 g, 88.6 % yield). mp 90–92 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.83 (s, 4H), 7.72 (s, 1H), 8.16 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 61.6, 118.3, 129.2, 143.2, 147.0. IR (KBr, ν cm^{-1}): 1342, 1457, 1536, 2918, 3069, 3203 and 3292. ESI-MS (*m/z %*): 206 (22) [M + H], 159 (100) $[M + Na - NO_2]^{+}$.

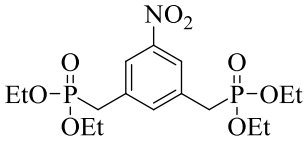
Synthesis of 3, 5-bis-bromomethylnitrobenzene:[S2]



A solution of PBr_3 (6.42 mL, 68.28 mmol) in 10 mL anhydrous CH_2Cl_2 was added slowly to a stirred solution of compound **2** (5.0 g, 27.31 mmol) in 30 mL anhydrous CH_2Cl_2 at 0 ^{o}C. The reaction mixture was further stirred for 1h at 0 ^{o}C followed by room temperature overnight. Then it was poured onto crushed ice and extracted with diethyl ether (300 mL). The organic phase was washed with saturated $NaHCO_3$ solution, brine, and dried over Na_2SO_4. Removal of the solvent and recrystallization with EtOAc-hexane gave **3** as analytical pure faint yellow solid (8.02 g, 95.7 % yield). mp 102–104 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.52 (s, 2H), 7.75 (s, 1H), 8.19 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 30.5, 123.6, 135.2, 140.3,

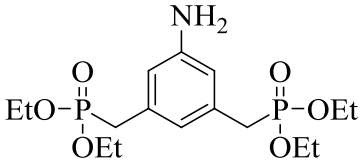
148.4. IR (KBr, ν cm^{-1}): 687, 1214, 1360, 1530, 2975, 3037, and 3068. ESI-MS (*m/z %*): 283 (100$[M + Na - NO_2]^+$.

Synthesis of Tetraethyl 5-nitrobenzene-1,3-bis(methylphosphonate):[S3]



A mixture of compound **3** (5.0 g, 16.29 mmol) and triethyl phosphite (15 mL) was stirred at 80 ^{0}C for 5h. The reaction mixture was cooled and the excess triethyl phosphite was evaporated *in vacuo*. The residue was stirred in light petroleum ether (b.p. 40-50 ^{0}C) for 1h. The precipitates were filtered, washed with hot hexane and recrystallized with EtOAc/hexane to afford **4** as a pure white solid (6.33 g, 91.8 % yield). mp 74–76 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.26 (t, J = 6.9 Hz, 12H), 3.18 (d, J_{HP} = 21.9 Hz, 4H) 4.02- 4.12 (m, 8H), 7.58 (s, 1H), 8.06 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.2, 32.3, 34.2, 62.3, 123.0, 134.2, 137.1, 148.1. IR (KBr, ν cm^{-1}): 1026, 1219, 1352, 1534, 1622, and 2985. ESI-MS (*m/z %*): 424 (100) $[M + H]^+$. HRMS: calculated for $C_{16}H_{27}O_8NNaP_2$ = 446.1104 Found (ESI^+) [MH+ Na] = 446.1112.

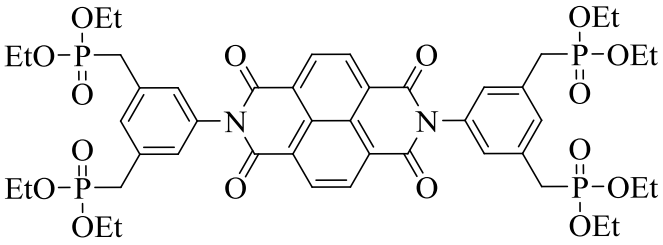
Synthesis of Tetraethyl 1-aminobenzene-3,5-bis(methylphosphonate):[S3]



A solution of compound **4** (5.0 g, 11.81 mmol) and 10 mg of 10% Pd/C in 20 mL of ethanol was stirred at 25 ^{o}C for 12h under H_2 atmosphere. The mixture was filtered through a Celite bed, washed with 30 mL ethanol and purified by flash chromatography ($CHCl_3$: MeOH = 20/1) to give **5** as a thick yellow oil (4.60 g, 99.18 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23 (t, J = 6.9 Hz, 12H), 3.00 (d, J_{HP} = 21.9 Hz, 4H) 3.59 (bs, 2H), 3.96- 4.07 (m, 8H), 6.54- 6.57 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.2, 32.4, 34.2, 62.08, 115.02, 121.2, 132.4,

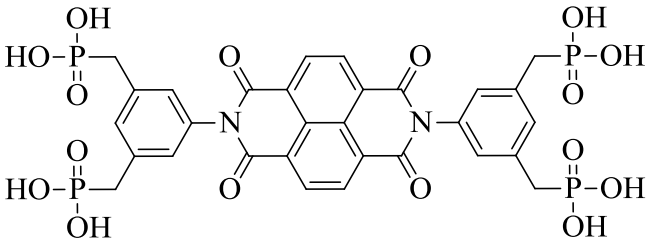
146.7. IR (KBr, ν cm^{-1}): 1053, 1275, 1555, 2913 and 3340. ESI-MS (*m/z %*): 394 (100) $[M + H]^+$, 416 (62) $[M + Na]^+$. HRMS: calculated for $C_{16}H_{29}O_6NNaP_2$ = 416.1362 Found (ESI^+) [M + Na] = 416.1372.

Synthesis of NDI phosphonate ester (6):



To a suspension of 1,4,5,8- naphthelenetetracarboxylic dianhydride (1.0 g, 3.73 mmol) in 20 mL *N, N'*-dimethyl acetamide (DMA) was added compound **5** (4.4 g, 11.19 mmol) under nitrogen atmosphere. The mixture was heated to 120 ^oC with vigorously stirring and a clear solution was obtained, which was further stirred at 120 ^oC for 8 h. The dark colored reaction mixture was cooled to room temperature and poured into pre-cooled 100 mL of 1N aqueous HCl. The resulting suspension was extracted with dichloromethane (2 × 100 mL) and the organic layer was separated. The organic layer was washed with brine solution (2 × 50 mL), dried over anhydrous $MgSO_4$ and evaporated using rotary evaporator to obtain crude material which was purified by column chromatography (silica gel (100-200 mesh size), $CHCl_3$: MeOH, 8:2), to afford **6** (2.36 g, 62.1 %) as a dark brown semisolid. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.26 (t, 24 H), 3.21- 3.26 (dd, 8H, *J* =22 Hz), 4.03- 4.09 (m, 16H), 7.24 (s, 4H), 7.41 (s, 2H), 8.8 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.2, 32.5, 34.3, 62.3, 126.8, 126.9, 128.3, 131.1, 131.8, 133.3, 134.8, 162.5. IR (KBr, ν cm^{-1}): 770, 962, 1025, 1055, 1247, 1349, 1672, 1712, 2913, 2984 and 3449. ESI-MS (*m/z %*): 1043 (100) $[M + 2H + Na]^+$. HRMS: calculated for $C_{46}H_{58}O_{16}N_2NaP_4$ = 1041.2634, Found (ESI^+) [M + Na] = 1041.2686.

Synthesis of Phos-NDI target molecule (1):



In an oven-dried round bottom flask, TMSBr (5.10 mL, 39.10 mmol) was added dropwise to a cooled solution of compound **6** (1.66 g, 1.63 mmol) in 15 mL anhydrous acetonitrile at 0 ^{o}C under nitrogen atmosphere. After 40 min stirring, the ice bath was removed and the reaction mixture was stirred at 40 ^{o}C for 18 h. The solvent was evaporated under reduced pressure and 40 mL of methanol was added to the residue and was stirred for 4 h at RT. The precipitates were filtered and washed with methanol to give titled product **1** as a white powder (0.91 g, 70 %). mp > 300 ^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6) δ : 4.05-4.04 (broad, 8H), 7.39-7.43 (m, 6H), 8.7 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ : 41.87, 43.82, 146.38, 148.15 148.36, 150.54, 151.05, 152.45, 152.59, 182.02. IR (KBr, ν cm^{-1}): 965, 984, 1040, 1252, 1454, 1581, 1674, 1714, 2276, 2914, 2987, 3165, and 3378. HRMS: calculated for $C_{30}H_{26}O_{16}N_2Na_2P_4$ = 794.0233 (M^+), Found (ESI^+) $[M+Na]^+$ = 817.4265. Elem. Anal.: for $C_{30}H_{26}N_2O_{16}P_4$: C, 45.36; H, 3.30; N, 3.53. Found: C, 45.31; H, 3.28; N, 3.52. Exact Mass:

6. Spectra of target molecules

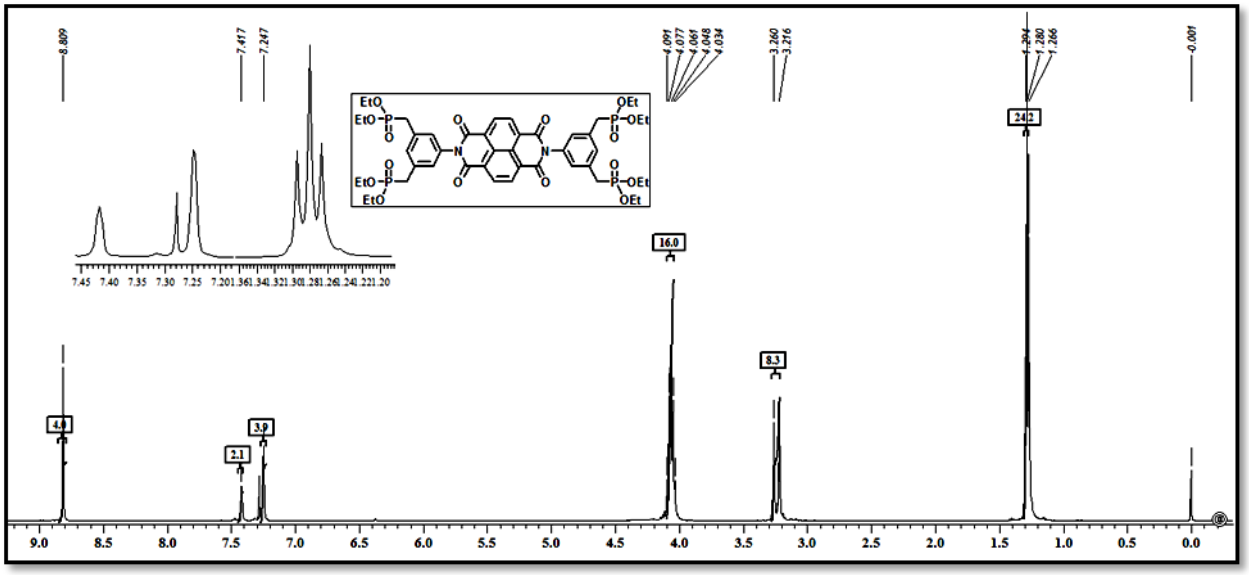


Fig. S17 ^{1}H NMR spectrum of NDI phosphonate ester **6** in $CDCl_3$.

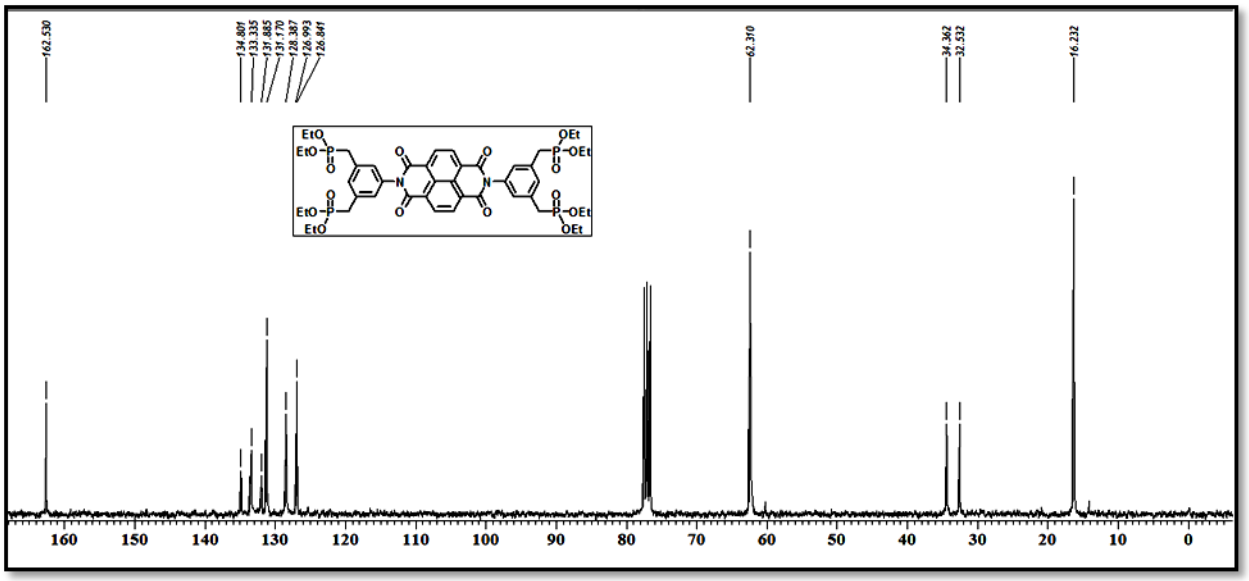


Fig. S18 ^{13}C NMR spectrum of NDI phosphonate ester **6** in $CDCl_3$.

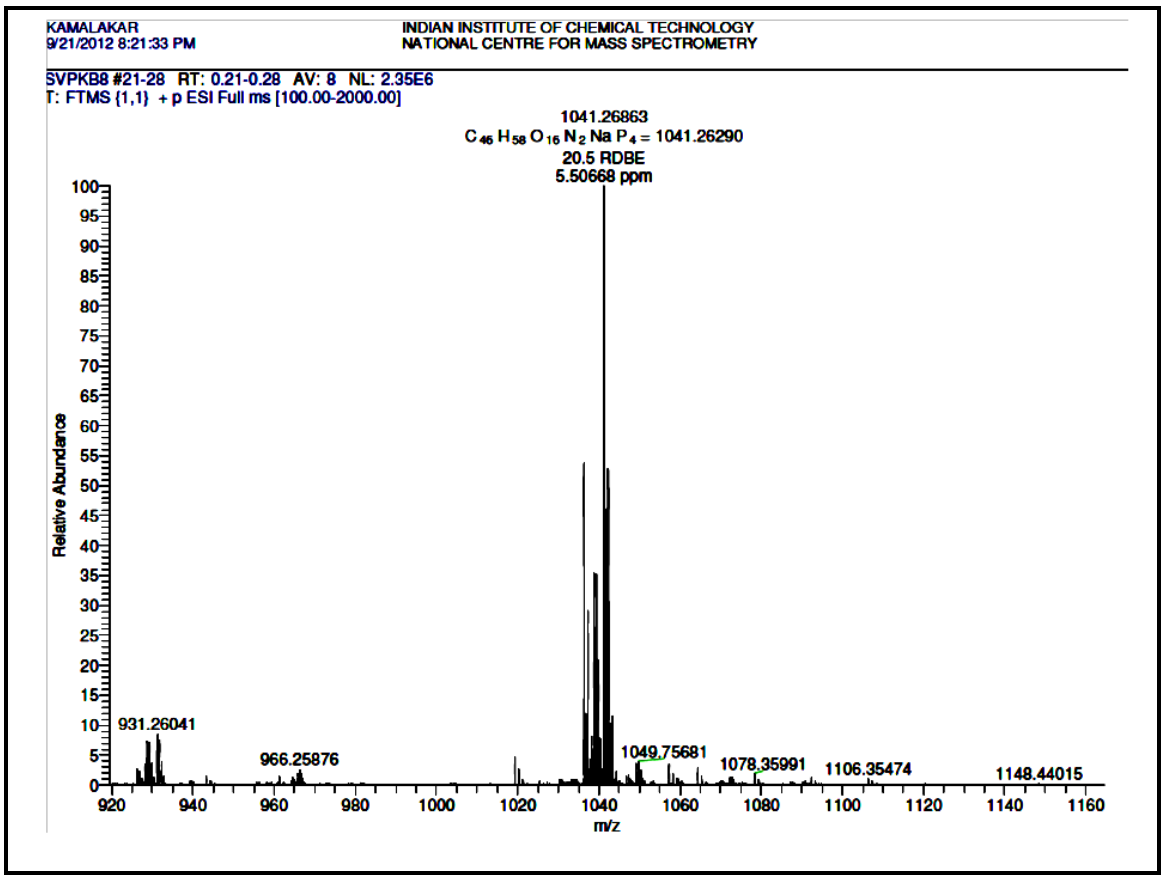


Fig. S19 HRMS spectrum of NDI phosphonate ester **6**.

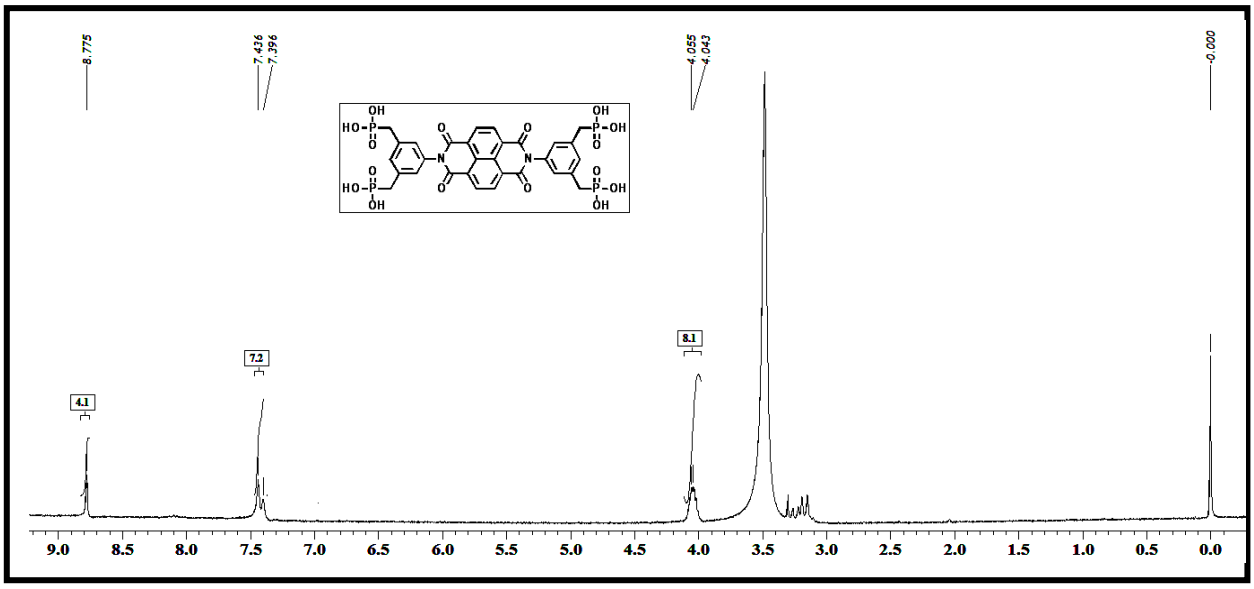


Fig. S20 ^{1}H NMR of Phos-NDI **1** in DMSO-d6.

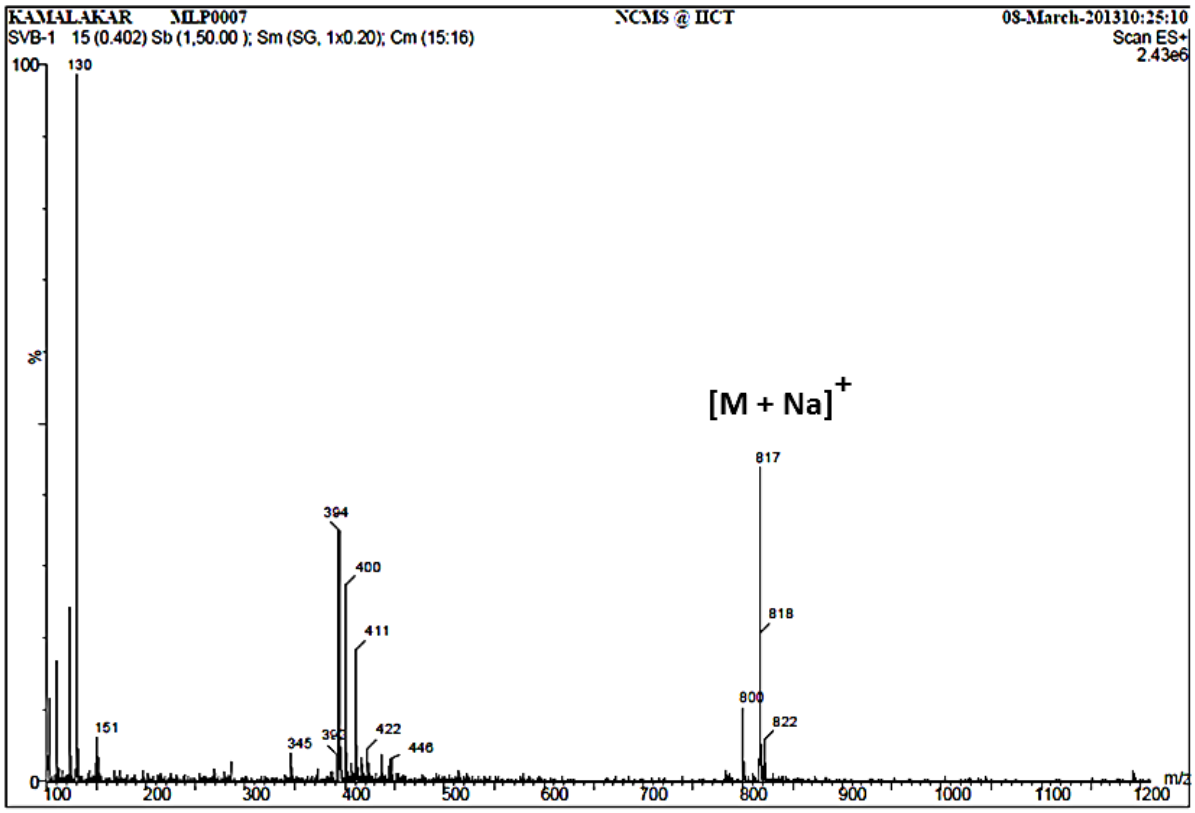


Fig. S21 HRMS spectrum of Phos-NDI **1**.

7. References

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