Amplified Fluorescence Emission of Bolaamphiphilic Perylene-Azacrown Ether Derivatives Directed towards Molecular Recognition Events

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Contents:

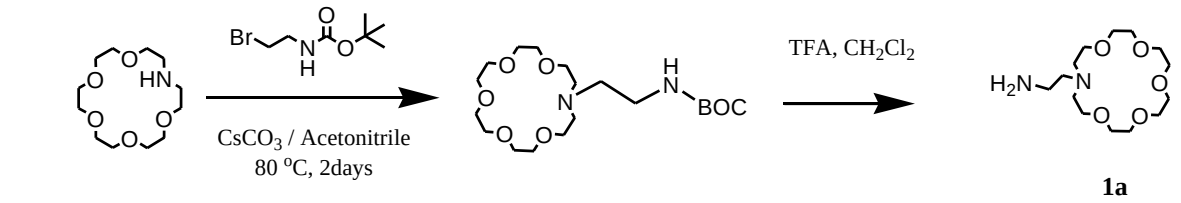
1. Experimental Section

Materials: All commercially available chemicals were of reagent grade and used as received. Tetrahydrofuran (THF) was purchased from Wako Pure Chemical, Ltd, and deoxigenated by argon gas. Metal perchlorate and hexafluoride salts, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were obtained from Sigma-Aldrich Chem. Co.. $CDCl_3$ and DMSO-d^6 containing 0.03 (*v/v*)% TMS for NMR were purchased from ACROS ORGANICS. Water was purified with a Direct-Q system (Millipore, Co.). The buffer solution of pH 7·4 (25 ^oC) was prepared by following the standard procedure. Heparin (sodium salt, 10^6 U) was purchased from Wako Pure Chemicals, protamine chloride (Salman, grade V, Histone free) was purchased from Sigma-Aldrich, and Fetal Bovine Serum (FBS) was purchased from Gibco by Life Technologies (South America). The nucleotides [adenosine mono/di/tri-phosphate (AMP, ADP, ATP), citosine triphosphate (CTP), guanosine triphosphate (GMP), and uracil triphosphate (UTP)] were purchased from Wako Pure Chemical. The synthesis of the compounds, PyC2DMA[S1] and 1,6,7,12-tetrachloro-Perylene-3,4,9,10- tetra carboxyldiimide[S2], are mentioned elsewhere.

Physical Measurements: (i) ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were recorded on a Bruker Avance 400 spectrometer. Chemical shifts were reported in ppm with the signals of TMS as an internal standard for ^{1}H-NMR and residual solvent for ^{13}C-NMR measurements; (ii) MALDI-TOF-MS measurement was carried out in Bruker autoflex; (iii) UV-vis spectra were recorded on a JASCO V-670 quipped with a peltier-type thermostatic cell holder using a quartz cell with 1 cm path length; (iv) FL spectra were recorded by Perkin-Elmer LS55 luminescence spectrophotometer at room temperature (25 °C) using quartz cell with 1 mm path length; (v) Transient PL decay profiles of the solution species at ambient temperature were measured with a Hamamatsu compact fluorescence lifetime spectrometer C11367 (Quantaurus-Tau); (v) DLS and zeta-potential measurements were conducted on the Malvern Zeta sizer Nano-ZS; (vi) Atomic force microscopy (AFM) was experimented using a JEOL JEM-2010 (acceleration voltage 120 kV) and Veeco Nanoscope IIIa (Tapping mode) (vii) Transmission electron microscopy (TEM); (viii) XRD analysis was conducted on a RIGAKU smart-lab with a copper K-alpha source, and the samples were prepared by freeze-drying method.

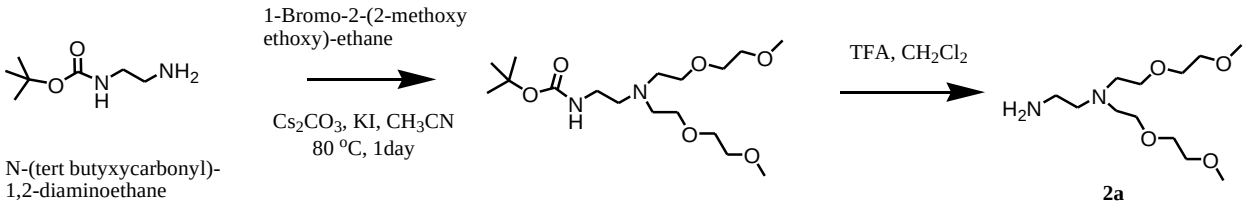
2. Multistep Synthesis and Characterization.

2-(1-Aza-18-crown-6) ethylamine, **1a**



1a

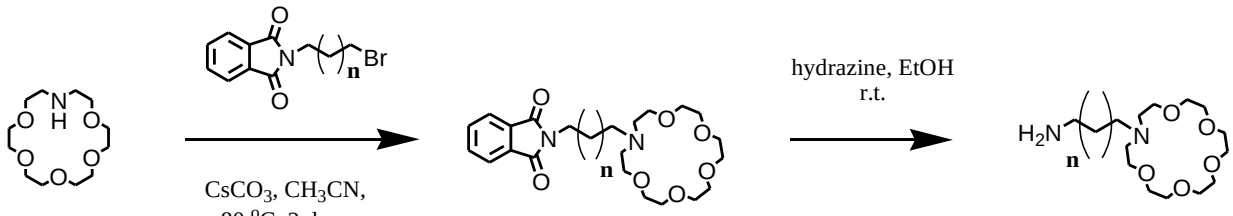
2-(*N*,*N*-bis(2-(2-methoxyethoxy)ethane) ethylamine, **2a**



N-(tert butyxycarbonyl)-1,2-diaminoethane

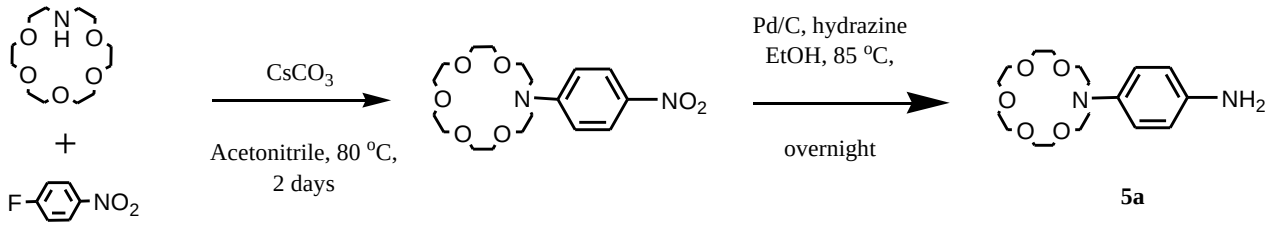
2a

n-(1-Aza-18-crown-6) alkylamine (n =3,4), **3a, 4a**



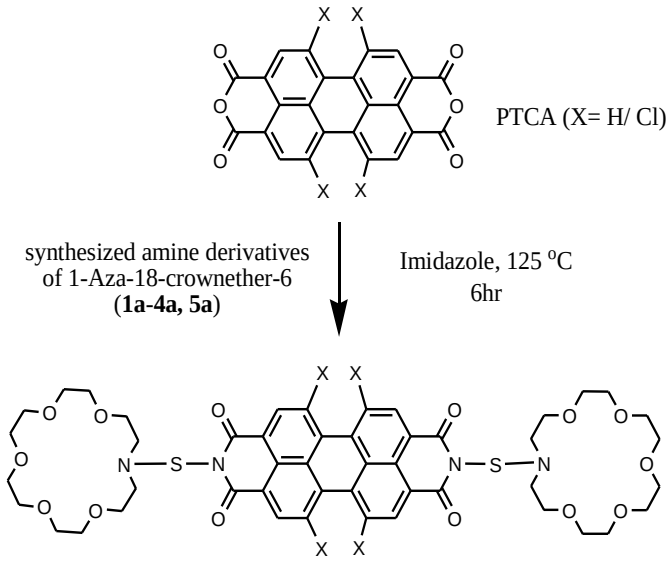
3a, 4a

1-(1-Aza-18-crown-6)-4-aminobenzence, **5b**



5a

Synthesis of Symmetrical *N*,*N'*-bis(spcacer, **S**)-perylene-3,4,9,10-tetracarboxyldiimide [X = H, Cl; S = spacer types, **1a**-**5a**]:



Synthetic Method and Characterizations

The synthesis of the compounds was performed accordingly as shown in the Scheme S1:

Synthesis of 1a: 1-Aza-18-crown-6 (2 gm, 7.6 mmole), 2-(Boc-amino)-ethylbromide (4 gm, 17.8 mmole), and Cs_2CO_3 (5 gm, 15.3 mmole) were added in 20 ml acetonitrile. The whole reaction mixture was allowed to heat at 80 °C for 2 days under argon (Ar) atmosphere. After completing, the reaction mixture was cooled to room temperature, and filtered by washing with 100 ml acetonitrile. The filtrate was evaporated under vacuum, and extracted three times with $CHCl_3$ and water. The organic layer was collected and evaporated under reduced pressure to get yellow thick viscous material. The product was purified on a silica-gel column using $CHCl_3$:MeOH (9:1) as eluent. The yellow viscous product was collected by solvent evaporation.

The crude product was dissolved in 25 ml DCM, and treated with trifluoroeacetic acid (1 ml). The solution was stirred for 3 hr at room temperature, and the mixture was extracted three times with DCM and alkaline water (pH ~11-12 using NaOH, 1N). The organic layer was dried over magnesium sulfate, and the yellow viscous product was collected after evaporating the solvent under vacuum. The produced (912 mg, 3.0 mmol) was used in the next synthetic step without further purification.

Yield: 39%

^{1}H NMR (400 MHz, d6-DMSO, ppm): δ 4.29 (t, NH_2), 3.91 (t, 6 Hz, 4H), 3.53-3.39 (m, 54H), 2.70 (t, 6 Hz, 4H). Ms (MALDI-TOF) m/z(+): Calculated; 306.22, Found; 307.14 [M-H$^+$].

Synthesis of 2a: *N*-(tert-butyxycarbonyl)-1,2-diaminoethane (1 gm, 6.2 mmole), 1-Bromo-2-(2-ethoxyethoxy)-ethane (3.4 gm, 18.7 mmole), Potassium iodide (200 mg) and Cs_2CO_3 (5gm, 15.3 mmole)

were added in 10 ml acetonitrile. The whole reaction mixture was allowed to heat at 80 ^{o}C for 1 day under the Ar-atmosphere. The next steps were followed according to previously mentioned procedure in synthesis of 1a. The final amine product was as yellow viscous oil (982 mg, 3.8 mmole).
Yield: 60%
^{1}H NMR (400 MHz, d6-DMSO, ppm): δ 6.50 (t, 5.6 Hz, NH_2), 3.49-3.47 (m, 4H), 3.43-3.40 (m, 8H), 3.24 (s, 7H), 2.95 (q, 6.4 Hz, 2H), 2.62 (t, 6.2 Hz, 4H), 2.50 (t, 6.6 Hz, 2H). Ms (MALDI-TOF) m/z (+): Calculated; 264.22, Found; 287.277 [M+Na^+].

Synthesis of 3a, 4a: 1-Aza-18-crown-6 (2 gm, 7.6 mmole), N-(n-bromoalkyl) phthalimide (5 equiv.), and Cs_2CO_3 (5 gm, 15.3 mmole) were added in 20 ml acetonitrile. The whole reaction mixture was allowed to heat at 80 ^{o}C for 3 days under Ar atmosphere. After completing, the reaction mixture was cooled to room temperature, and filtered by washing with 100 ml acetonitrile. The filtrate was evaporated under vacuum, and extracted three times with $CHCl_3$ and water. The organic layer was collected and evaporated under reduced pressure to get yellow thick viscous material. The product was purified on a silica-gel column using 4% MeOH-DCM as eluent. The yellow viscous product was collected by solvent evaporation.

The crude product was dissolved in 50 ml ethanol, and treated with hydrazine (1 ml). The solution was refluxed overnight and allowed the formation of phthalidrazide as a heavy white precipitate. The reaction mixture was then cooled to room temperature and filtered; the precipitate was carefully washed with cold EtOH. Evaporation of the filtrate under reduced pressure afforded 3a (4a) as light yellow viscous oil. Finally; washed with 1N NaOH (pH ~11-12) solution and extracted with CH_2Cl_2. The product was collected after evaporate to dryness.
Yields: 52% for **3a**, and 66 % for **4a**
^{1}H NMR (400 MHz, ppm): (**3a,** d6-DMSO) δ 4.03 (t, 6.4 Hz, 4H), 3.53-3.39 (m, 53 H), 2.58 (t, 7 Hz, 4H), 1.61 (q, 6.5 Hz, 4H); (**4a,** $CDCl_3$) δ 4.08 (t, 6.6 Hz, 4H), 3.68-3.54 (m, 51H), 2.72 (t, 6.8 Hz, 4H), 1.67 (q, 7.3 Hz, 4H), 1.50 (q, 7.4 Hz, 4H). Ms (MALDI-TOF) m/z(+): (**3a**) Calculated; 320.23, Found; 321.21 (M+H^+) /(**4a**) Calculated; 334.25, Found; 357.37 [M+Na^+].

Synthesis of 5b: 1-Aza-18-crown-6 (1.5 gm, 5.7 mmole), 1-fluoro-4-nitrobenzene (4.2 gm, 30 mmole), and Cs_2CO_3 (5 gm, 15.3 mmole) were added in 20 ml acetonitrile and heated for 3 days at 80 ^{o}C. The reaction mixture was cooled to room temperature, and solvent was evaporated under vacuum. The reaction mixture was washed with water and extracted with $CHCl_3$. The organic layer was collected and evaporated under vacuum. Further the product was purified on a silica-gel column using 1-5% MeOH-DCM as eluent. The bright yellow colored solution was collected and dried under vacuum to get orange viscous liquid Nitro-derivative (1.8 gm).
Yield of **5a**: 89 %
^{1}H NMR (400 MHz, d6-DMSO, ppm): δ 8.09 (d, 10.8 Hz, 2H), 6.67 (d, 9.6 Hz, 2H), 3.47-3.65 (m, 48H). Ms (MALDI-TOF) m/z(+): Calculated; 384.19, Found; 407.29 [M+Na^+].

The reduction of the Nitro-derivative was carried out by following previous report [Ref. S2]. The product was dissolved in 100 ml ethanol, and PD/C 10% (200 mg, cat) was added to the solution. Hydrazine monohydrate (5 ml) was added drop-wise to the reaction mixture. After refluxing overnight, the solution was filtered through celite to remove solid materials. The filtrate was collected and dried under vacuum to get deep green colored final product, 5a. The product was carried for the next step without further purification.
Yield of **5b**: >80%
Ms (MALDI-TOF) m/z(+): Calculated; 354.22, Found; 354.28 [M].

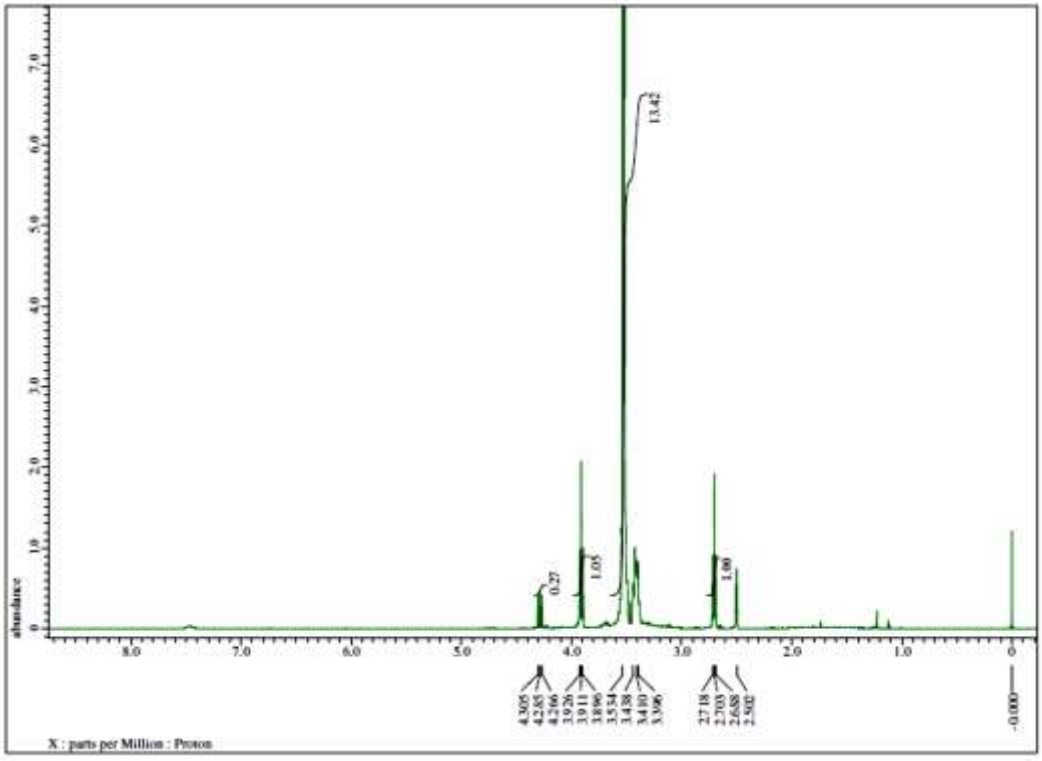
General Synthesis Procedure of Perylene-Azacrown Ether Derivatives:

Perylene-3,4,9,10- tetracarboxyldiimide (PTCA) or 1,6,7,12-tetrachloro-Perylene-3,4,9,10- tetra carboxyldiimide (1 equiv), amine derivatives (5 equiv) and imidazole (10·0 gm) were heated at 125 ^{o}C

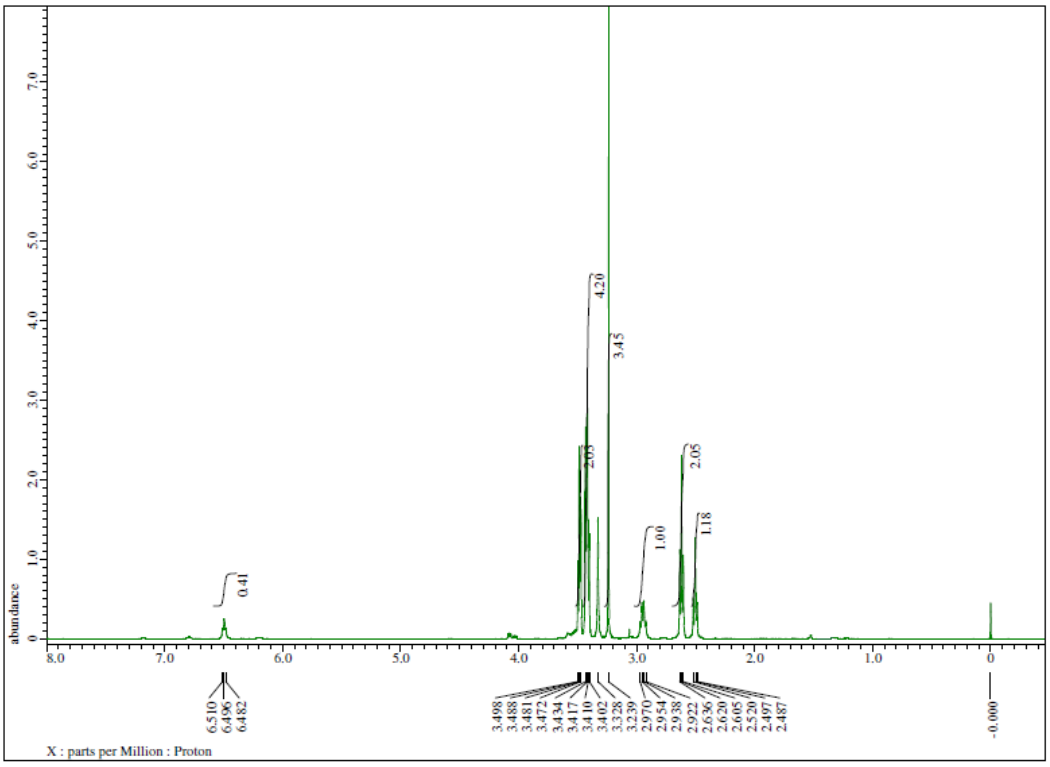
for 6 hr under Ar atmosphere. The reaction mixture was poured into 200 ml water, and extracted with $CHCl_3$ (3 × 50 ml). The organic phase was collected and evaporated under vacuum to get the crude material. The product was passed through a silica gel column by using 3-5% MeOH/ $CHCl_3$ solvent mixture as eluent. The desired product was collected and further purified by reprecipitation in $CHCl_3$/ n-hexane mixture solvent. The final products were collected solid.
Yields: for PyC2ACE (55%), PyC2EG (58%), PyC3ACE (63%), PyC4ACE (70%), Cl.PyC3ACE (55%), and PyBzACE (64%)

PyC2ACE, **1:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.66 (d, 8 Hz, 4H), 8.59 (d, 8.4 Hz, 4H), 4.56-3.47 (m, 52 H), 2.99 (b, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.3, 154.8, 134.5, 131.3, 129.3, 126.3, 123.3, 123, 70.9, 70.8, 70.5, 70.3, 54.4, 52.7, 38.4; Ms (MALDI-TOF) m/z(+): Calculated; 968.44, Found; 967.52 [M^+]; Elemental Analysis: ($C_{52}N_{64}N_4O_{14}$) Calculated; C, 64.45; H, 6.66; N, 5.78%; Found; C, 63.95; H, 7.05; N, 5.28%.
PyC2EG, **2:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.70 (d, 7.6 Hz, 4H), 8.65 (d, 8.0 Hz, 4H), 4.32 (t, 7.0 Hz, 4H), 3.58 (m, 16H) 3.51 (m, 8H), 3.35 (m, 13H), 2.90 (m, 12H, -OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.2, 156.2, 134.5, 131.3, 129.2, 126. 3, 123 (d), 71.9, 70.4, 69.9, 59.1, 54.1, 28.5; Ms (MALDI-TOF) m/z(-): Calculated; 884.42, Found; 884.75 [M]; Elemental Analysis: ($C_{48}H_{60}N_4O_{12}$) Calculated; C, 65.04; H, 6.83; N, 6.33%; Found; C, 65.82; H, 6.91; N, 6.15%.
PyC3ACE, **3:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.63 (d, 8 Hz, 4H), 8.54 (d, 7.6 Hz, 4H), 4.33 (t, 7.4 Hz, 4H), 4.23 (t, 6.4 Hz, 3H), 3.65 (m, 49H), 2.14 (q, 6.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.2, 156.2, 134.5, 131.3, 129.2, 126. 3, 123 (d), 70.9, 70.6, 70.5, 70.0, 69.8, 63.1, 48.3 (d), 37.8, 27.9; Elemental Analysis: ($C_{54}H_{68}N_4O_{14}$) Calculated; C, 65.04; H, 6.87; N, 5.62%; Found; C, 64.12; H, 6.95; N, 5.31%.
PyC4ACE, **4:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.67 (d, 8 Hz, 4H), 8.61 (d, 8 Hz, 4H), 4.26 (t, 7.2 Hz, 4H), 4.15 (t, 6.2 Hz, 4H), 3.67-3.53 (m, 48 H), 1.83 (b, 8H);); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.2, 156.3, 134.4, 129.2, 126.2, 123.1 (d), 70.8, 70.6 (d), 70.4 (d), 69.8 (d), 64.9, 48.4, 47.9, 40.2, 26.8, 24.8; Elemental Analysis: ($C_{56}H_{72}N_4O_{14}$) Calculated; C, 65.61; H, 7.08; N, 5.47%; Found; C, 66.39, H, 7.56; N, 5.29%.
ClPyC3ACE: Ms (MALDI-TOF) m/z(+): Calculated; 1146.33, Found; 1148.843 (M+2).
PyBzACE, **5:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.75 (d, 8H, 4H), 8.66 (d, 8 Hz, 4H), 7.16 (d, 9.2 Hz, 4H), 6.83 (d, 9.2 Hz, 4H), 3.78-3.69 (m, 50H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 163.9, 148.0, 134.6, 131.5, 129.5, 129.1, 126.4, 123.6, 123.1, 122.8, 111.9, 70.9 (t), 68.6, 51.5; Ms (MALDI-TOF) m/z(-): Calculated; 1064.44, Found; 1064.91 [M]; Elemental Analysis: ($C_{60}H_{64}N_4O_{14}$) Calculated; C, 67.66; H, 6.06; N, 5.26%; Found; C, 65.98; H, 5.89; N, 5.51%.

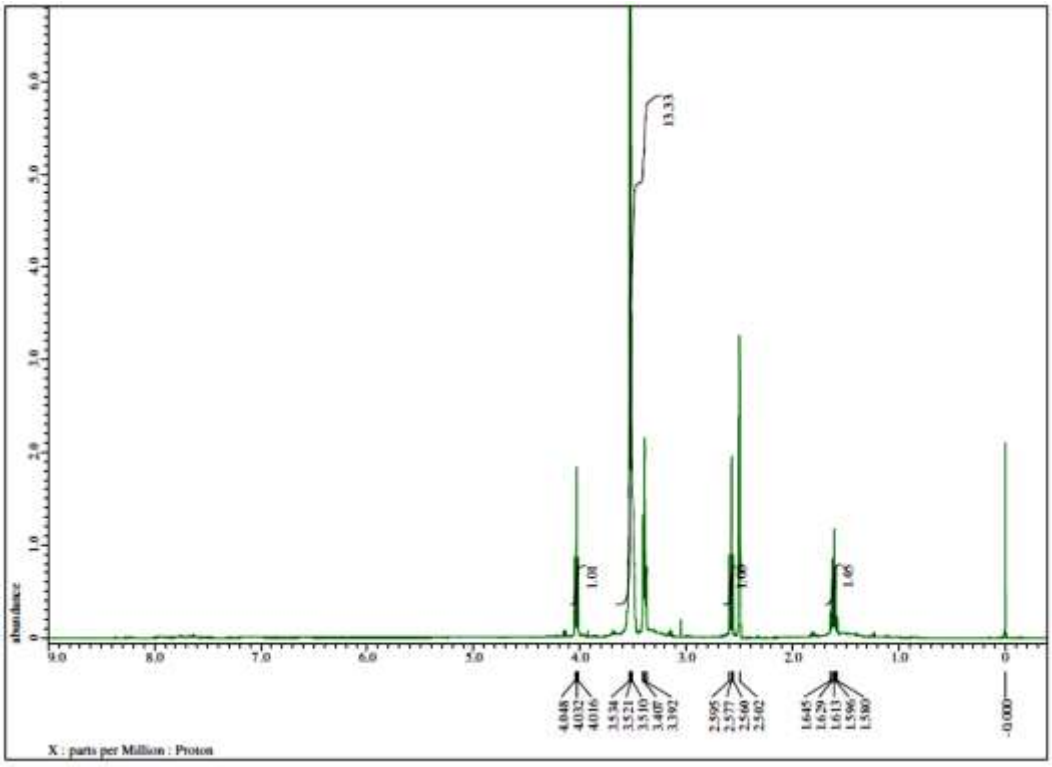
^{1}H NMR Spectra of 2-(1-Aza-18-crown-6) ethylamine, **1a.**



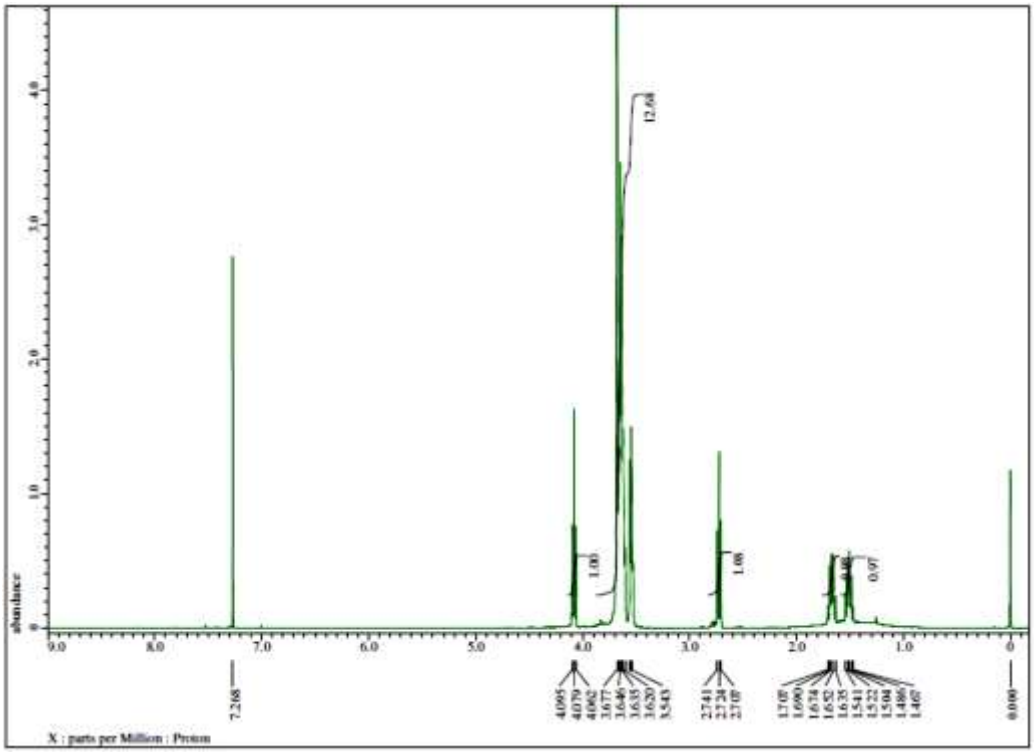
^{1}H NMR Spectra of 2-(*N*,*N*-bis(2-(2-methoxyethoxy)ethane) ethylamine**, 2a.**



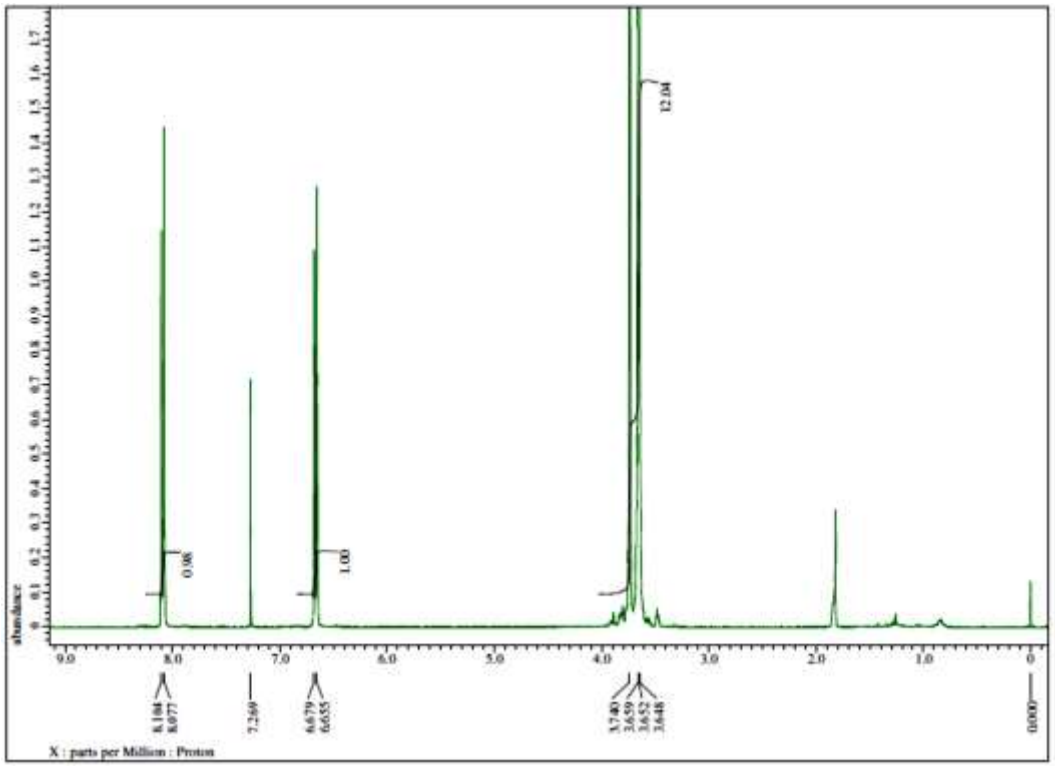
^{1}H NMR Spectra of 3-(1-Aza-18-crown-6) propylamine, **3a.**



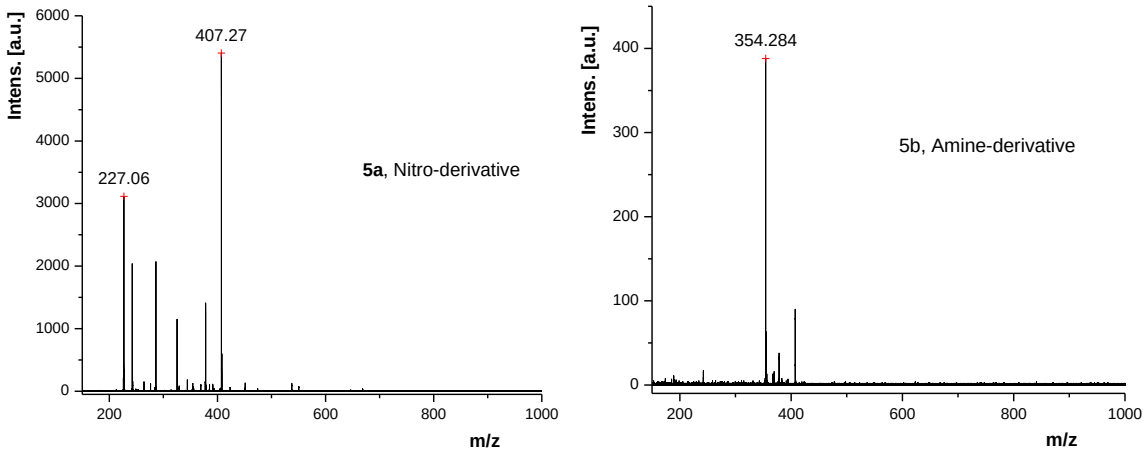
^{1}H NMR Spectra of 4-(1-Aza-18-crown-6) butylamine, **4a.**



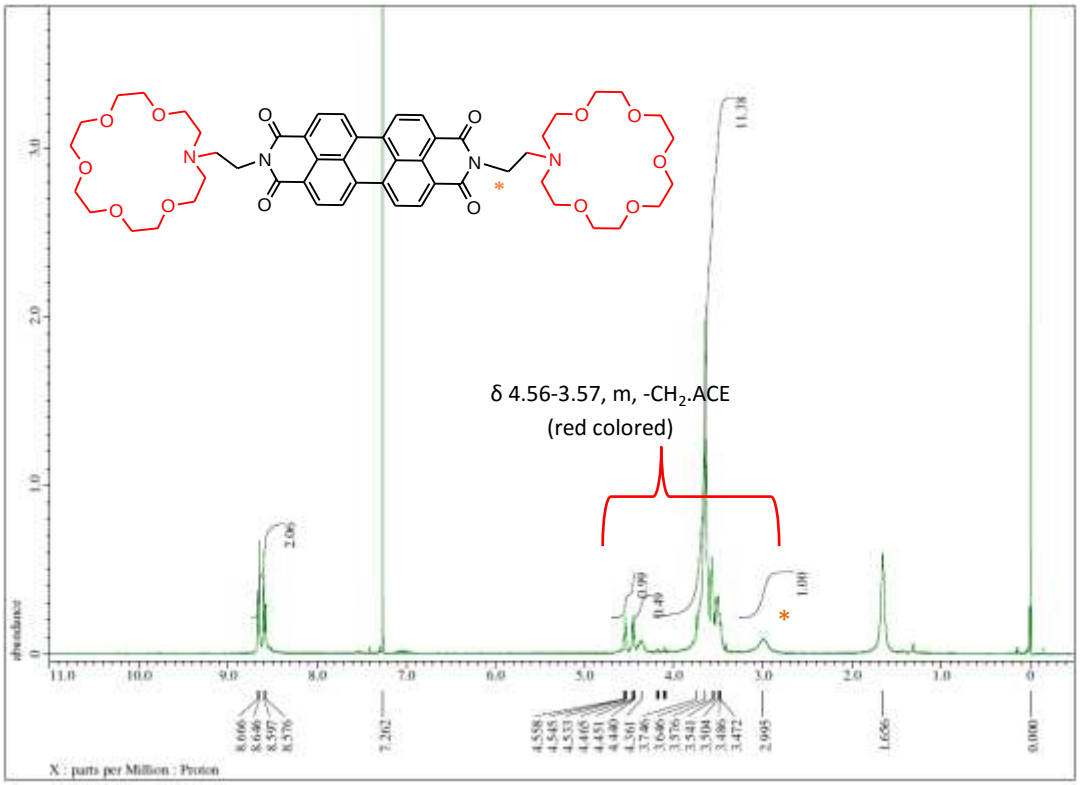
^{1}H NMR Spectra of **5a,** 1-(1-Aza-18-crown-6)-4-nitrobenzence.

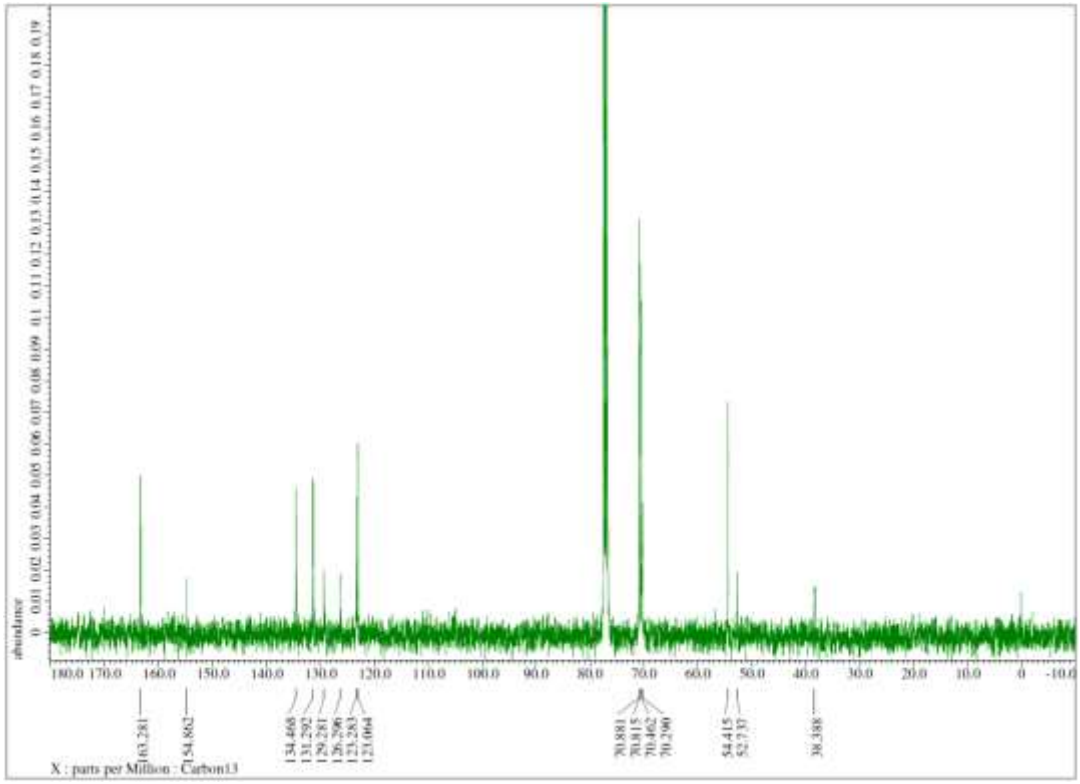


Ms (MALDI-TOF) of **5a** and **5b.**

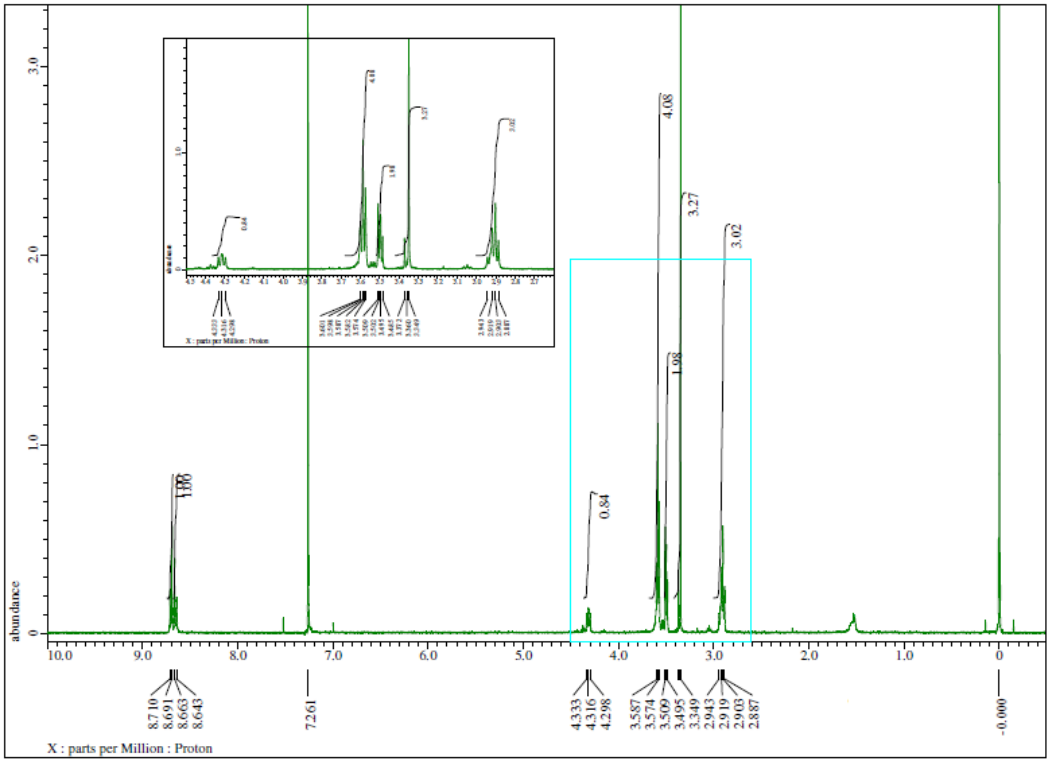


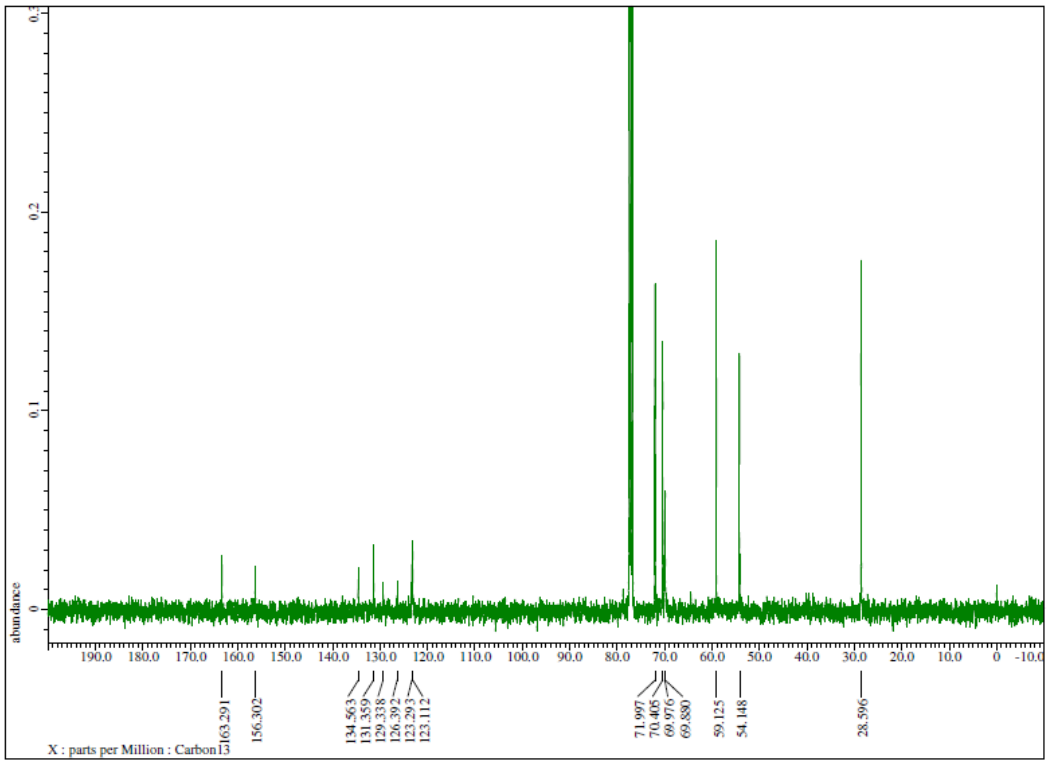
^{1}H, ^{13}C NMR Spectra of **1**:



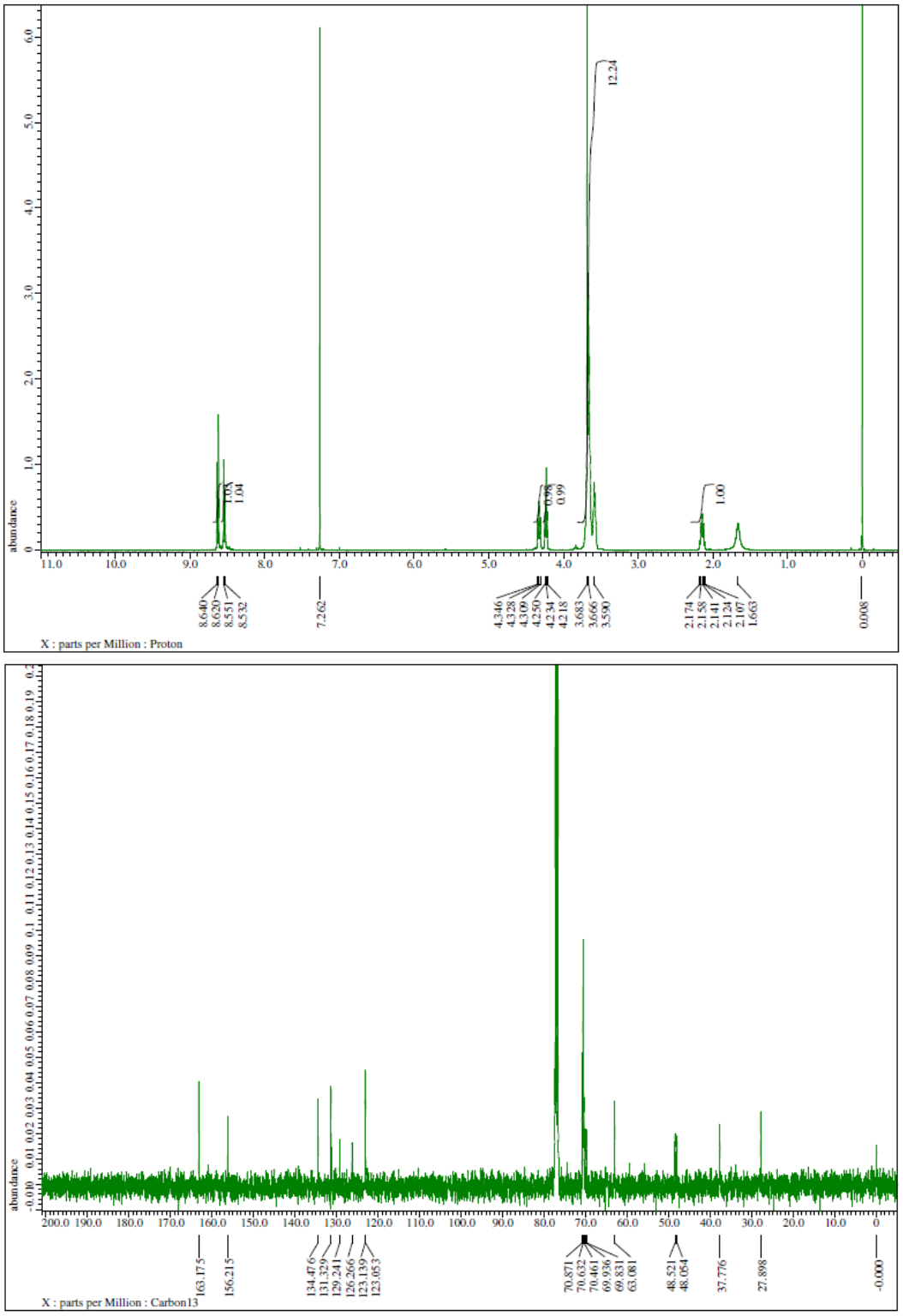


^{1}H, ^{13}C NMR Spectra of PyC2EG, **2.**

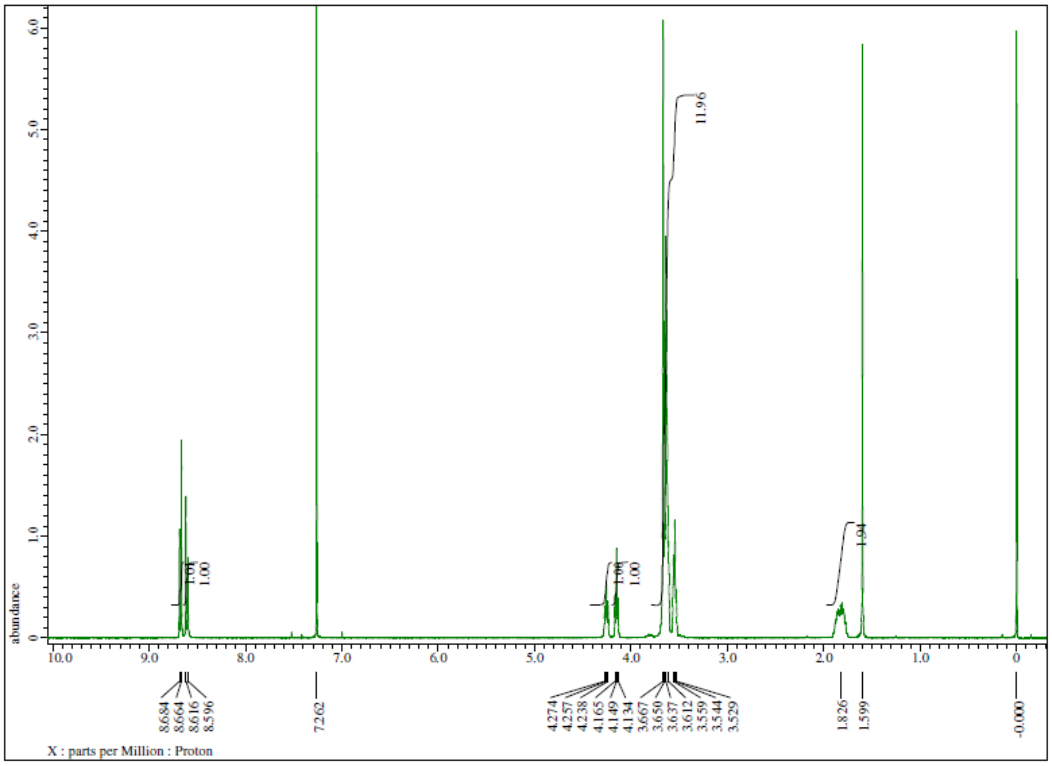


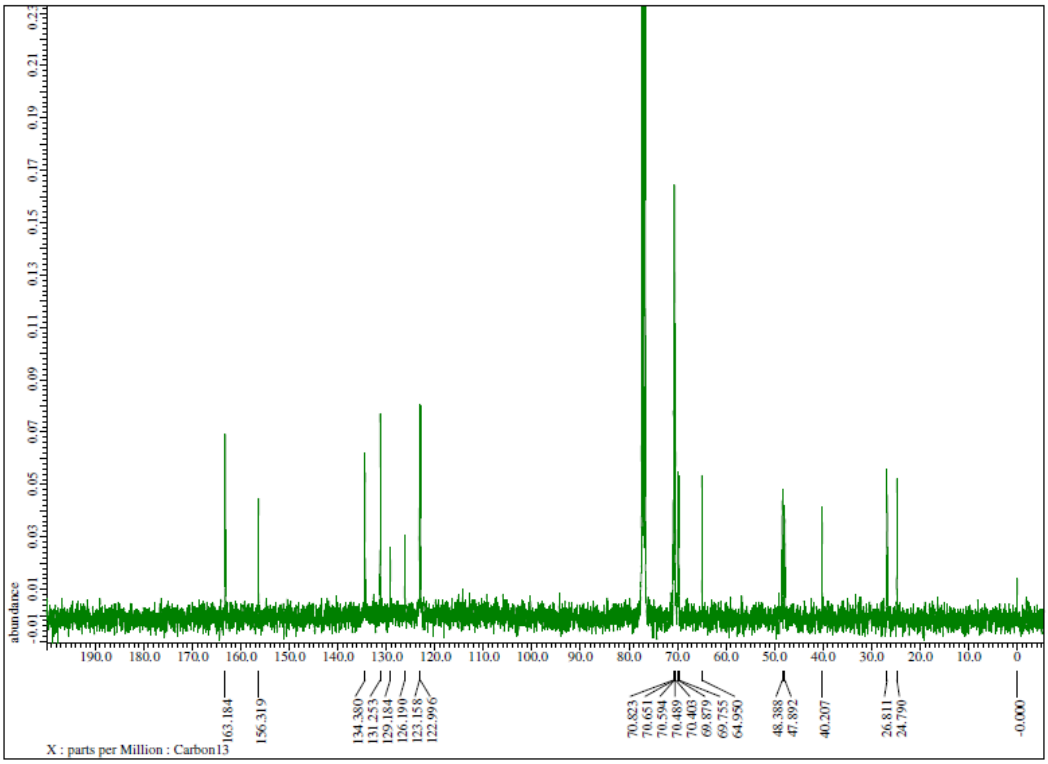


^{1}H, ^{13}C NMR Spectra of PyC3ACE, **3**.

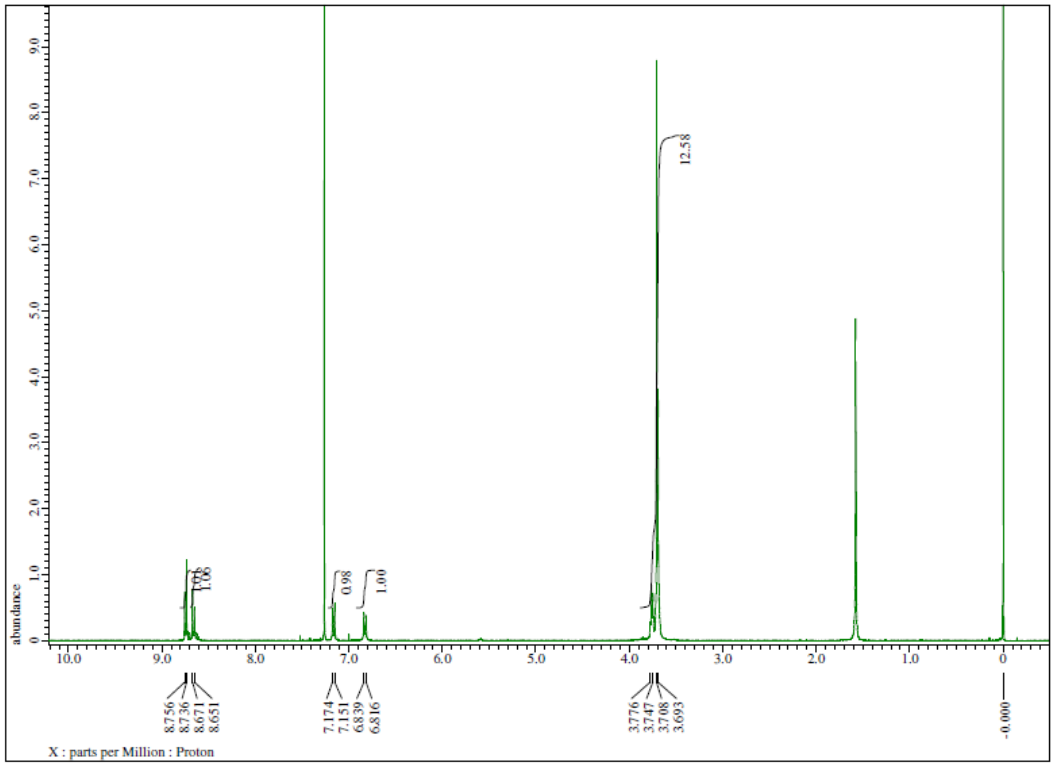


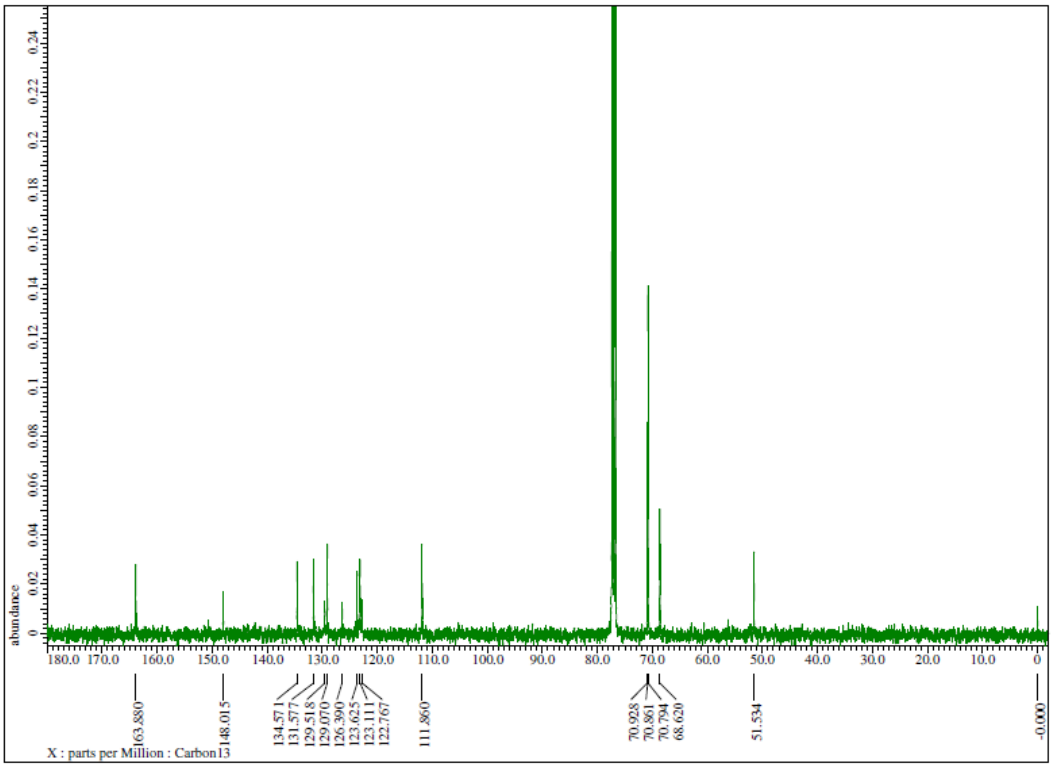
^{1}H, ^{13}C NMR Spectra of PyC4ACE, **4.**



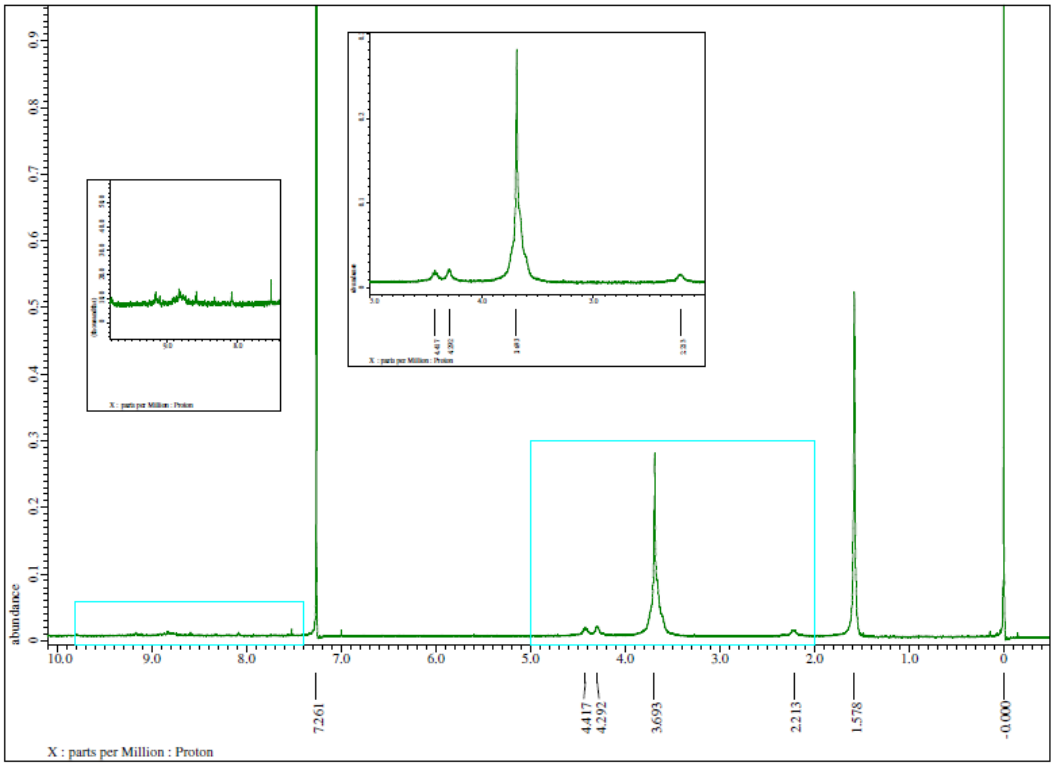


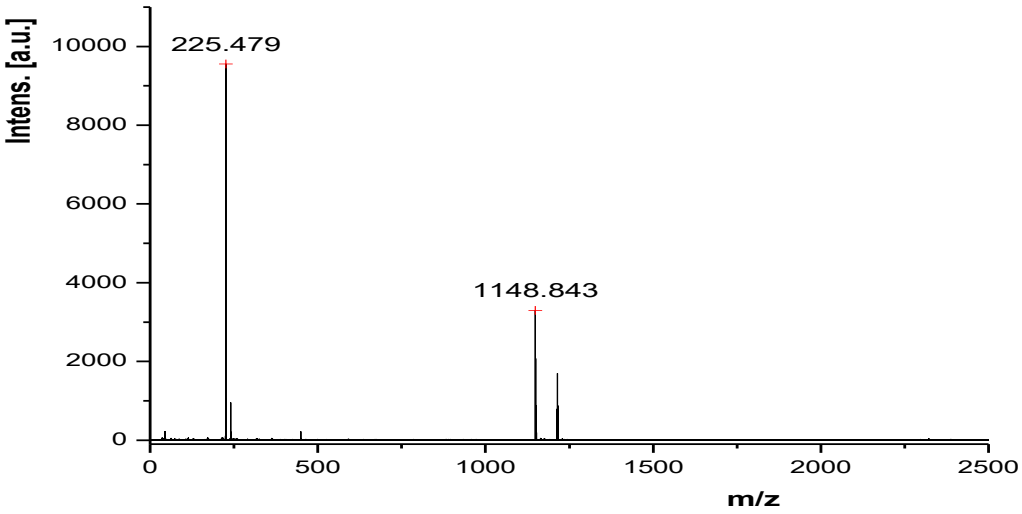
^{1}H, ^{13}C NMR Spectra of PyBzACE, **5.**



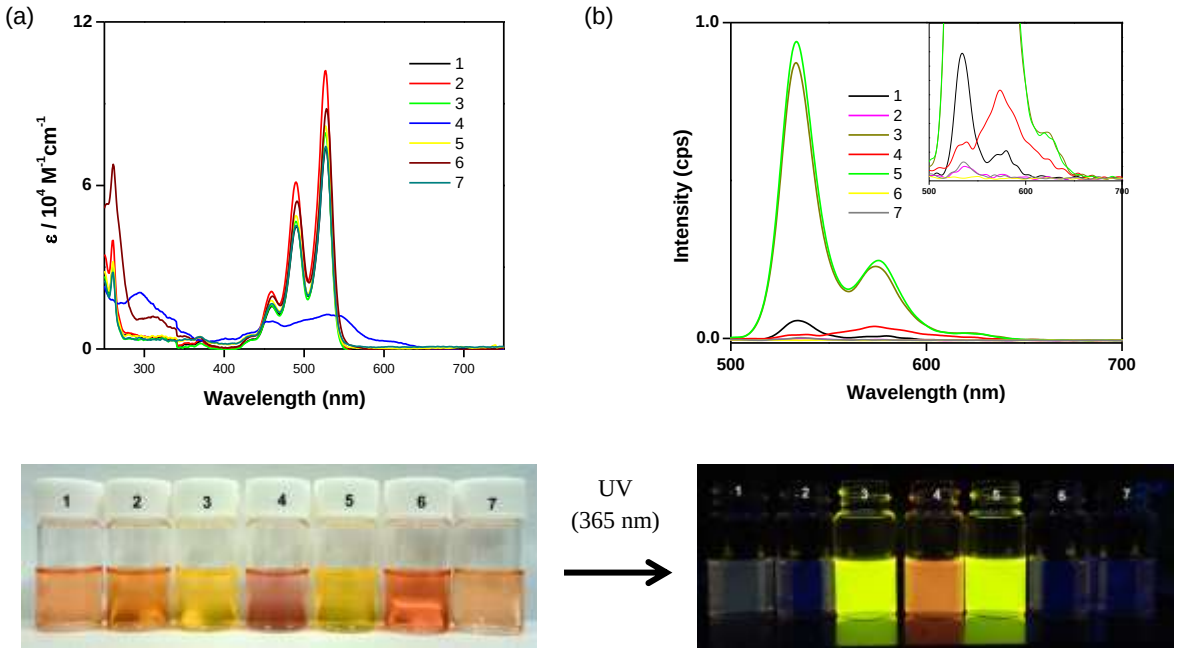


1H NMR and Ms (MALDI-TOF) m/z(+) Spectra of ClPyC3ACE**.**





3. Comparative spectroscopic studies of all the synthesized perylene-derivatives.



SI Figure 1. (a) Absorbance, and (b) fluorescence spectra of the synthesized ACE-derivatives in chloroform at 10·0 μM concentration, and 20 ^{o}C temperature [inset (a): Zoomed FL graph].
(below): The visual images of the solutions in $CHCl_3$ under normal light and under UV light at 365 nm. [Compounds: (1) PyC2ACE, (2) PyC2EG, (3) PyC3ACE, (4) ClPyC3ACE, (5) PyC4ACE, (6) PyBzACE, and (7) PyC2DMA]

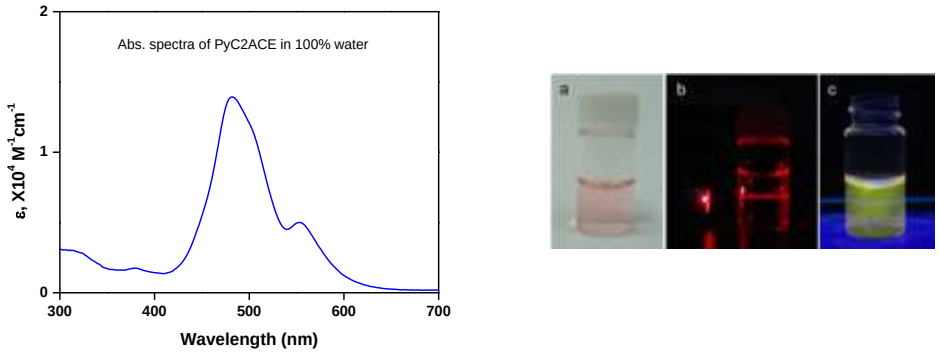
4. Investigation of water solubility.

Hydrophilic-lyophilic balance (HLB): Griffin's method[S4] for non-ionic surfactants as described in the 1954 work as follows:

$$HLB = 20 * M_h/M$$

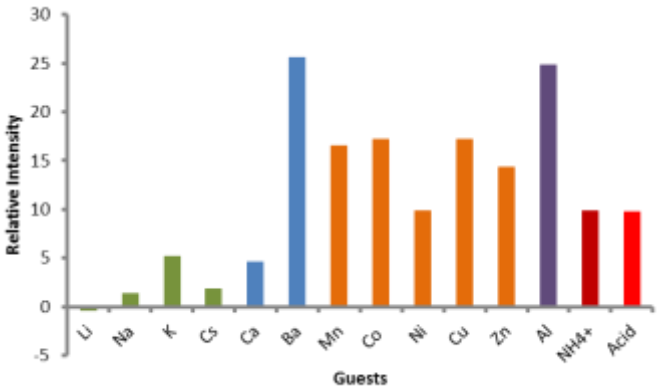
Where M_h is the molecular mass of the hydrophilic portion of the molecule, and M is the molecular mass of the whole molecule, giving a result on a scale of 0 to 20.
An HLB value of 0 corresponds to a completely lipophilic/hydrophobic molecule, and a value of 20 corresponds to a completely hydrophilic / lyophobic molecule

Solubility of PyC2ACE in water: PyC2ACE was taken in a glass vial and calculated amount of water was added to prepare 1·0 mM stock solution. The mixture was then sonicated for 5 minutes in order to make it dispersed, and the final solution was appeared as stable, limpid, and bright red colored. The absorbance spectrum was measured at 10·0 μM after by dilution with water.



SI Figure 2. (left) Absorbance spectra of PyC2ACE in 100% aqueous solution at 10·0 μM concentration and 25 ^{o}C; (right) Images of the physical state of the solution in different conditions, (a) under normal light, (b) Rayleigh's scattering measured by using red-laser (635 nm), and (c) fluorescence image under UV-light (at 365 nm).

5. Binding Study of PyC2ACE with cationic guests.

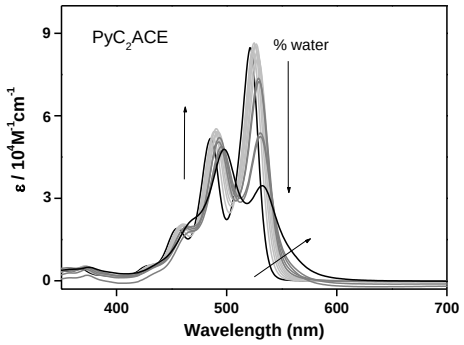


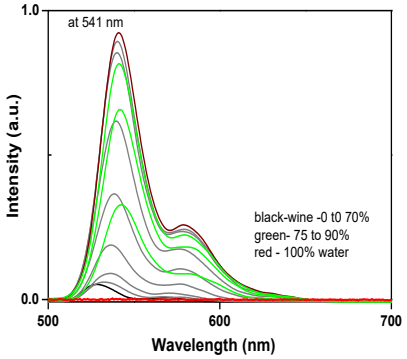
SI Figure 3. Relative fluorescence enhancement suppressing the PeT effect of PyC2ACE fluorophore (10 μM, THF) in presence excess amount (10 times) of different cationic guests (100 μM).

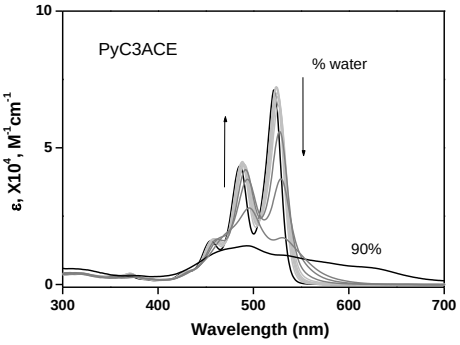
6. One-pot synthesis and characterization of the induced aggregates.

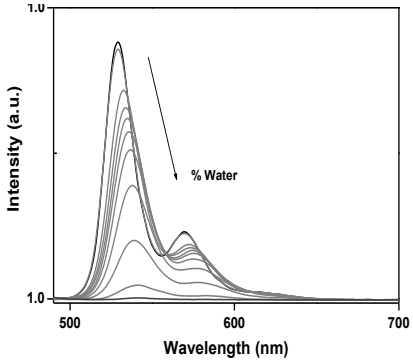
ONPs synthesis: 120·0 μM of a 0·25 mM stock solution in THF of a perylene-derivative was taken in a glass vial and requisite amount of THF was added for dilution. Further the calculated amount of deionized water was syringed into the THF solution to make final volume of 3 ml and followed by vigorous mixing in a vortex. The solutions were aged for 1 hr. before any further study.

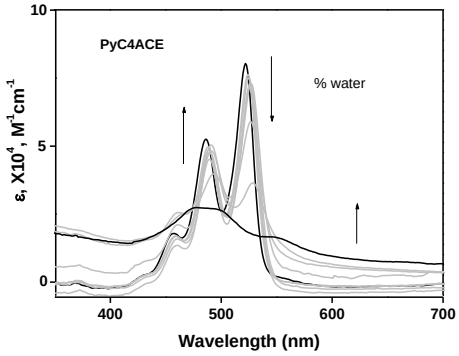
Spectral data were collected at different $f_{w,\%}$. The aggregates formed at different $f_{w,\%}$ were considered for spectroscopic measurements.

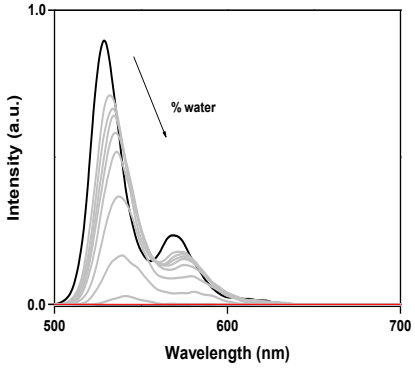


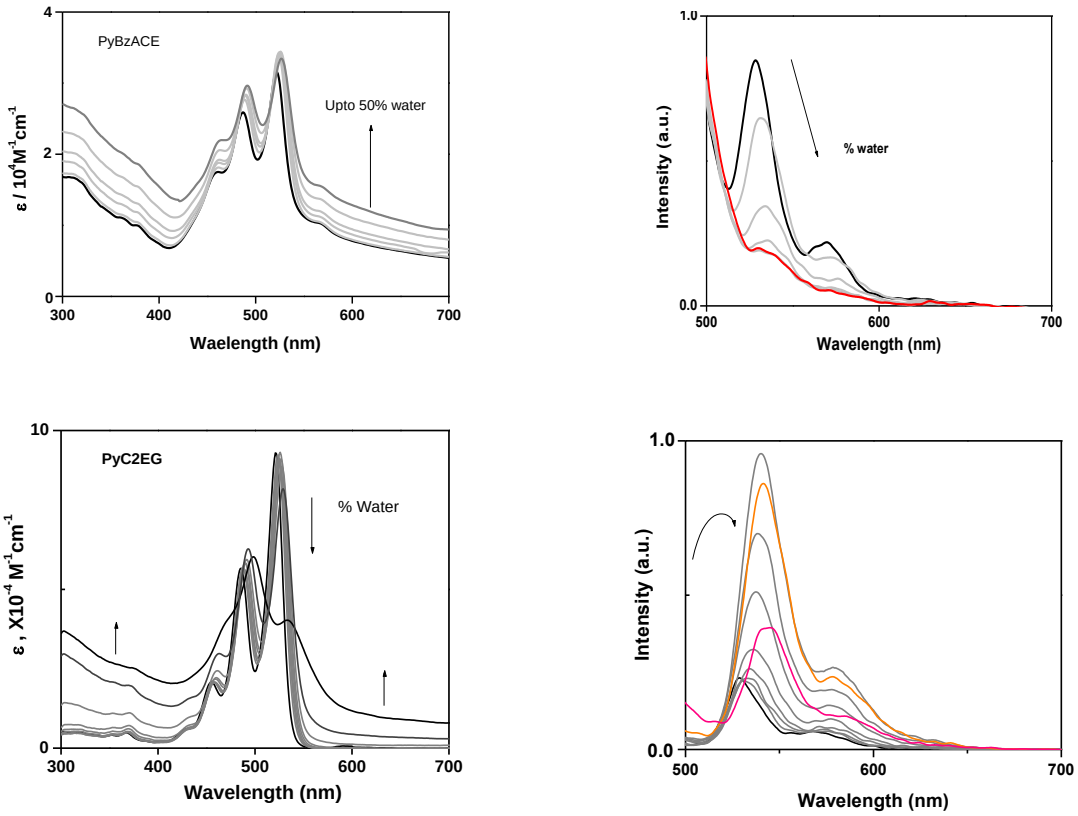








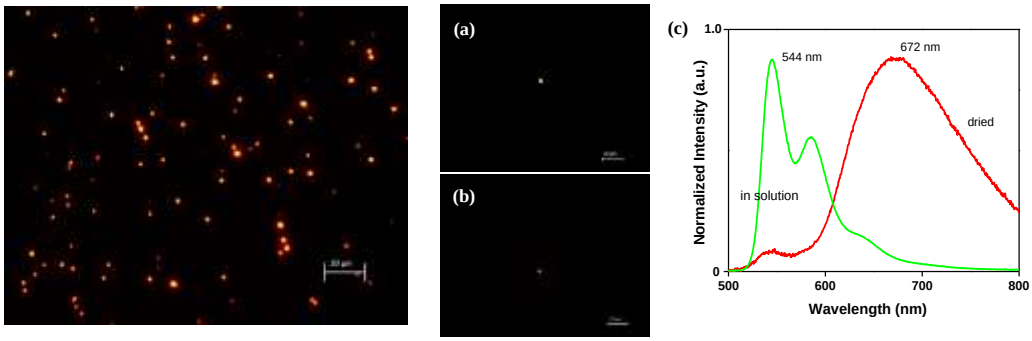




SI Figure 4. Absorbance (left) and fluorescence (right) spectral change was monitored against the solvent ratio change (water-THF) in a homogeneous solution of perylene derivatives at a constant concentration of 10·0 μM and at 20 ^{o}C.

7. Fluorescence image and single particle characterization.

The sample solution was placed on a glass slide covered with a thin square glass at a 0·05 mm gap, followed by solvent evaporation under vacuum.



SI Figure 5. (left) The collective image of dried PyC2ACE OPNs in $f_{w,70\%}$ at 10·0 μM and 20 ^{o}C. Single particle characterization by excitation of 450-490 nm: (a) solution state image; (b) dried state image, and (c) their corresponding FL spectra.

8. Morphology characterization.

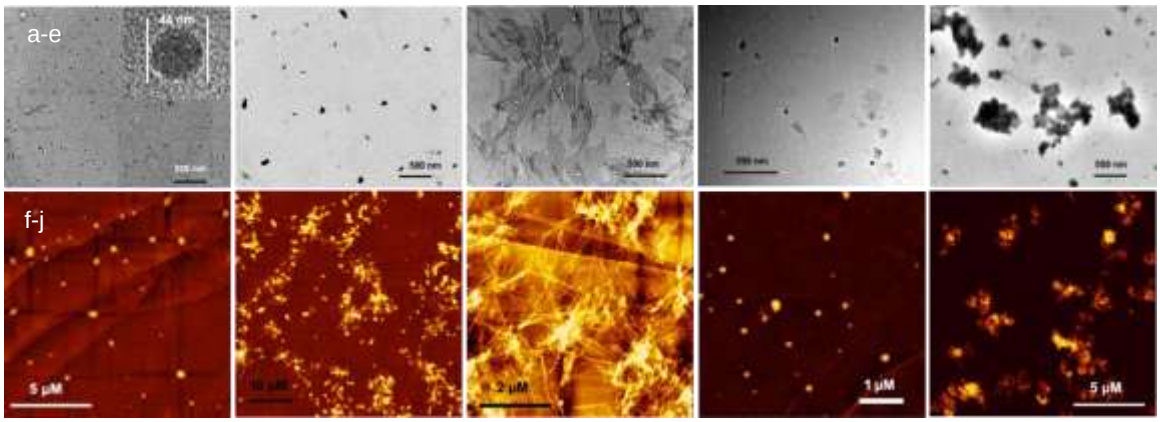
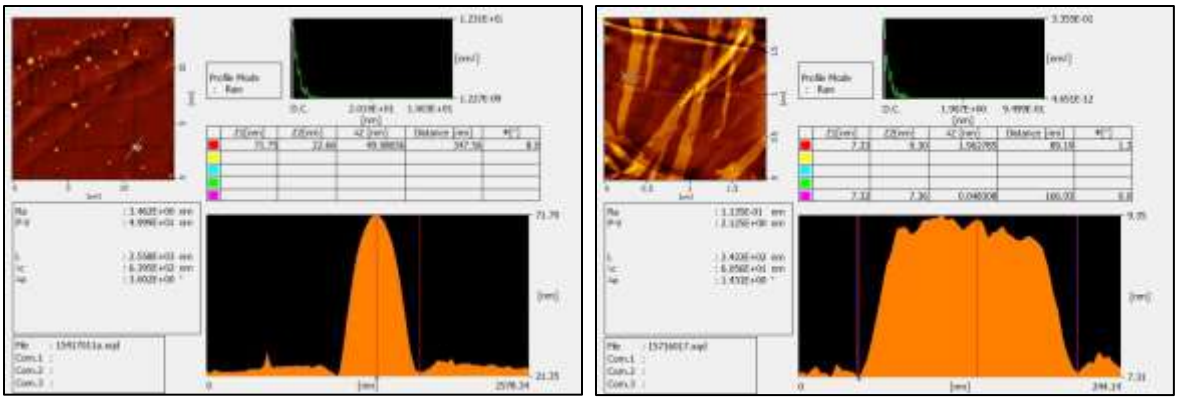


Figure 6. (a-e) TEM and (f-j) AFM images were observed for the nano-aggregates of PyC2ACE, PyC2EG, PyC3ACE, PyC4ACE, and PyBzACE derivatives prepared at $f_{w,70\%}$, 10·0 μM and 20 ^{o}C, then aging for 1 hr [inset of Figure 4a: TEM image of a single-nanoparticle having diameter of 44 nm].

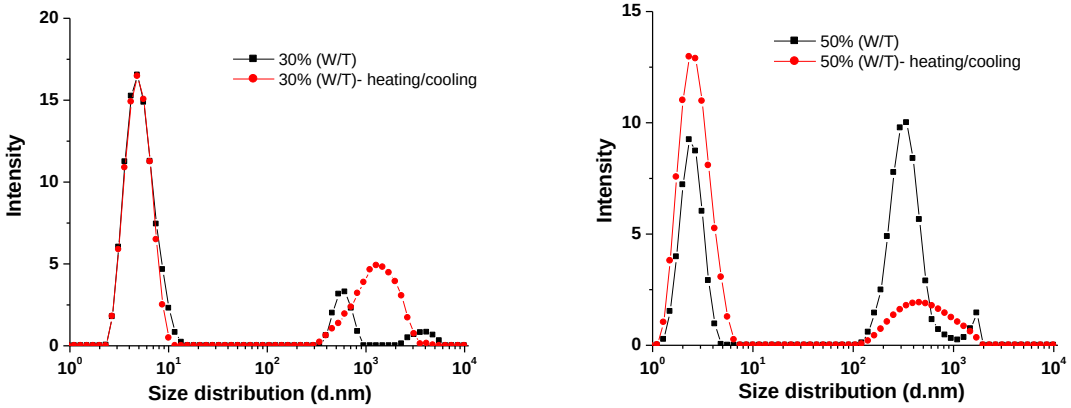
9. Size evolution of ONPs and nanosheet.

The sample solutions were placed on the HOPG surface by drop-cast method, and dried under vacuum.



SI Figure 7. Characterization of the AFM images: (left) PyC2ACE nanoparticle, and (right) PyC3ACE nanosheet.

10. Temperature dependency of bimodal distribution.

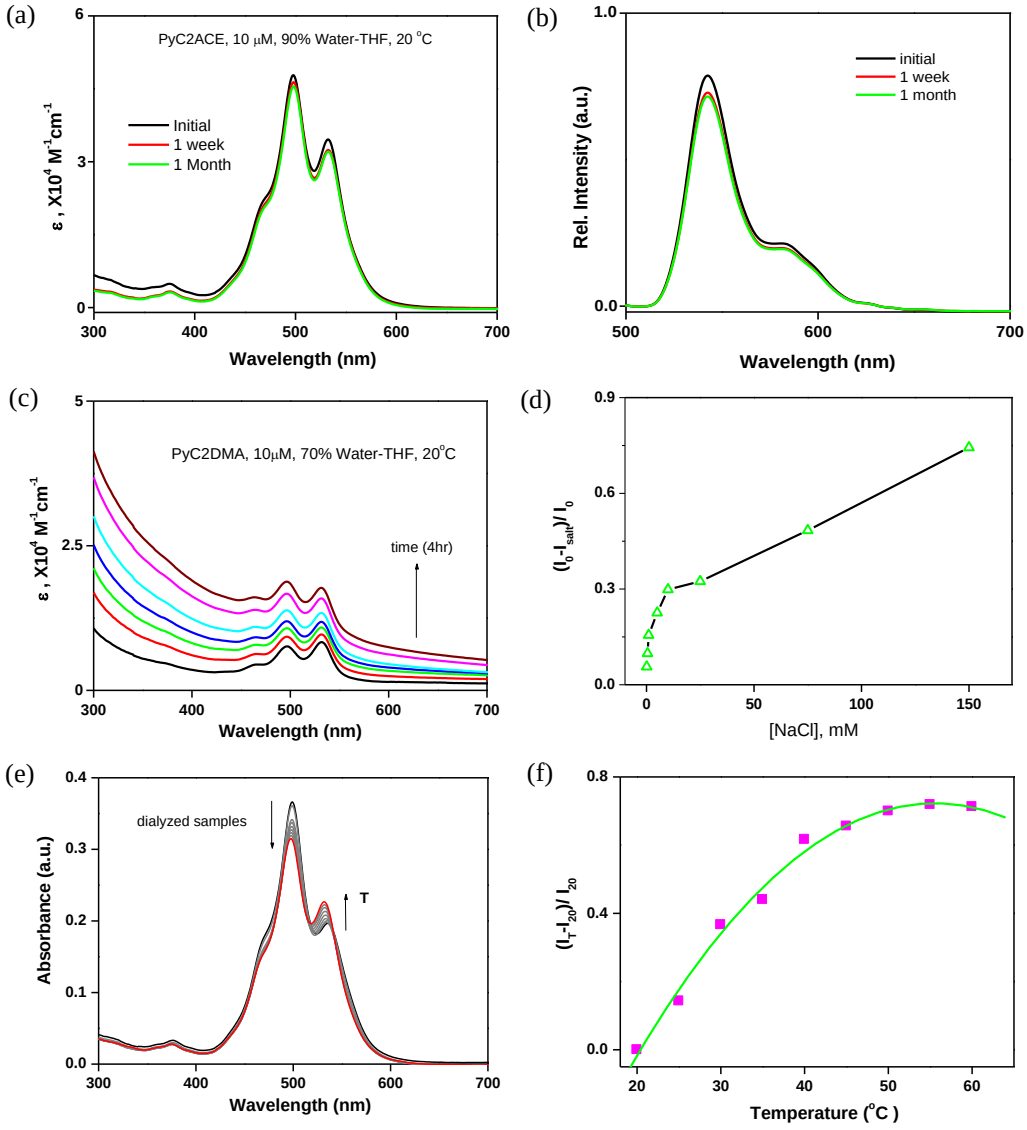


SI Figure 8. Particle size distribution is plotted against the data collected in DLS experiment for PyC2ACE in $f_{w,30\%}$ and $f_{w,50\%}$ at 10·0 μM and 20 ^{o}C, after simple solvent mixing and following heating-cooling method.

The $f_{w,30\%}$ and $f_{w,50\%}$ solutions were heated at 50 ^{o}C for 5 minutes and cooled to room temperature. The bimodal distributions at $f_{w,<50\%}$ were reconfirmed under thermodynamic stability allowing the heating-cooling method. At lower $f_{w,\%}$, the molecules seem to form an oligomer-higher aggregate equilibrium carrying FL OFF/ON balance which eventually compromised at a critical solvent mixture (i.e., $f_{w,70\%}$).

11. Colloidal stability.

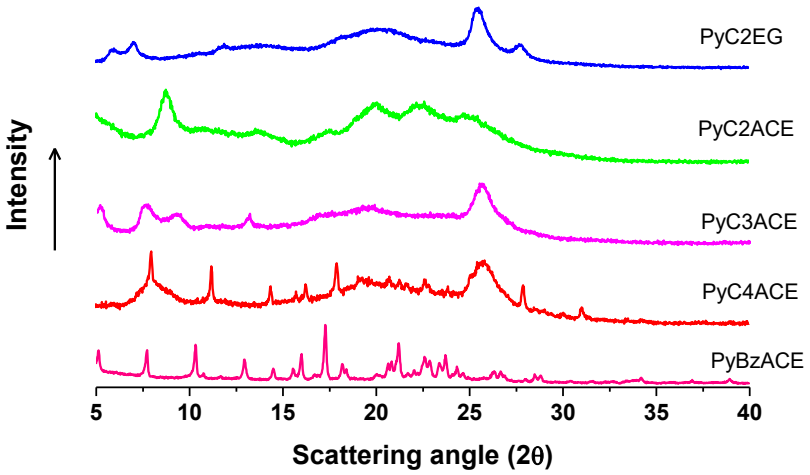
PyC2ACE shows long stability monitored over a month under similar conditions, while PyC2DMA in $f_{w,70\%}$ is very much unstable at 20 ^{o}C. The crown ether rings supposed to have a crucial role in solution stability; it forms a water dispersed surface of the aggregate particles. Change in the absorbance ratio (A_{0-0}/A_{0-1}) depicts dissociation or loosening of the aggregate constraint which is resulted out as a fluorescence enhancement.



SI Figure 9. (a-b) Absorbance and FL spectra evaluation of the PyC2ACE ONPs from initial to 1 month, (c) Absorbance spectra of PyC2DMA in $f_{w,70\%}$ over 4 hrs., and (d-f) Absorbance and relative FL spectra of PyC2ACE in $f_{w,90\%}$ in presence of salt and temperature as external stimuli.

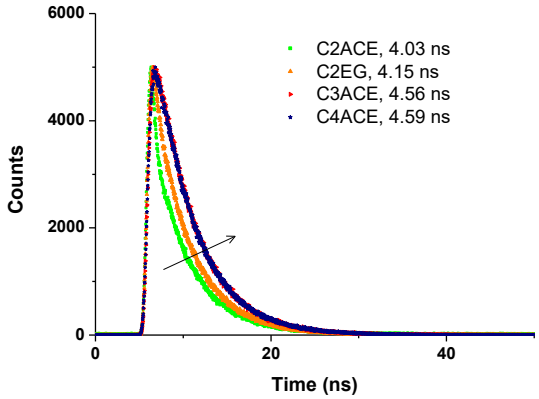
12. Wide angle X-ray scattering (WAXS).

The samples were prepared at f_w = 50% (for PyBzACE) and 70% (for PyC2EG, PyC2ACE, PyC3ACE, and PyC4ACE), and further carried out for fridge-dry to get dry powdered samples suitable for the measurement.



SI Figure 10. WAXS spectra were measured with the powdered samples of different perylene-derivatives within the range of 2θ = 5-40 at 20^{o}C.

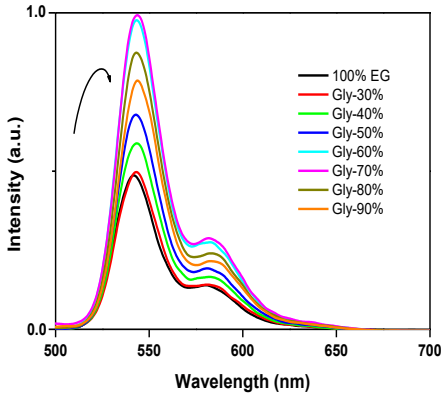
13. Excited state lifetime measurement.



SI Figure 11. Fluorescence decay profiles were measured for the samples at 10·0 μM concentration, and 20 ^{o}C.

14. Effects of viscosity change for PyC2ACE.

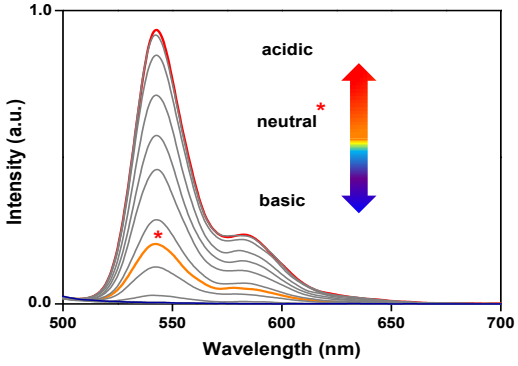
The effect of viscosity change was measured in fluorescence spectroscopy by preparing stock solution of PyC2ACE in ethylene glycol (EG, η = 1·61*10^{-2} Pa.s), and then gradual increasing the fraction of glycerol (Gly, a high viscous liquid, η = 1·41 Pa.s)



SI Figure 12. The relative FL intensity of PyC2ACE (10·0 μM) was plotted with the increasing solvent viscosity by the addition of glycerol.

15. Effects of pH change of the dialyzed sample.

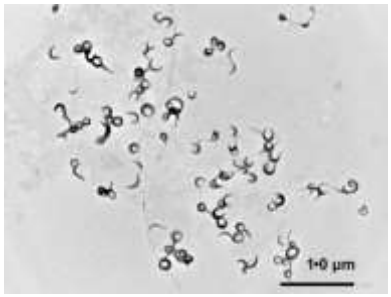
The pH of the dialyzed sample solution was found as 7·0 (measured with a pH meter, Horiba compact pH Meter B-212, against standard pH solutions). The pH dependency was measured by adding HCl or NaOH (1·0-10·0 mM) in 950 μl stock sample solution, and finally volume make-up to 1·0 ml by adding deionized water. (The data are presentation as mean values collected from the three different experimental sets.)



SI Figure 13. FL responses of the dialyzed ONPs in different pH conditions were plotted.

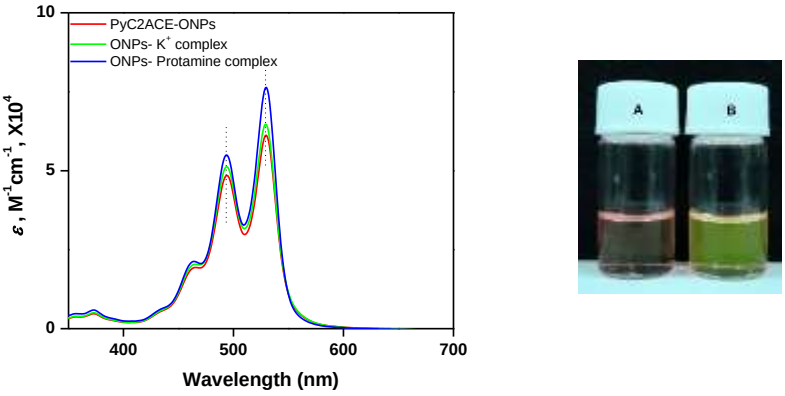
16. Morphology of ONPs / K^+-ion complex.

K^+-ions can form complex with ONPs, and as soon as K^+-ion was added to ONPs solution at $f_{w,70\%}$ the complexes start to dissociate out from the aggregated state. This phenomenon is seems to be like corrosion of ONPs by K^+-ions.



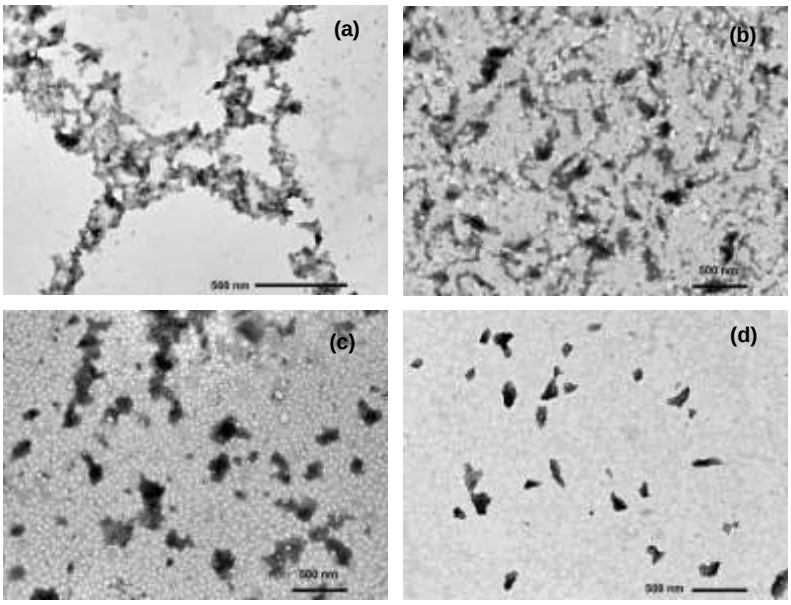
SI Figure 14. The TEM image of the ONPs was observed in the presence of K^+-ions.

16. ONPs interaction with K^+-ion or Pro in solution.



SI Figure 15. Absorbance spectral change of the PyC2ACE-ONP (6·8 µM) solution in absence and in the presence of K^+-ions or **Pro** in in $f_{w,90\%}$ and 1·0 mM HEPES buffer at 20 ^oC. Corresponding visual color change under the normal light of the hybrid solution in the absence (A) and presence (B) of **Pro**.

18. TEM images of different ONPs / polymer (molecule) hybrids.

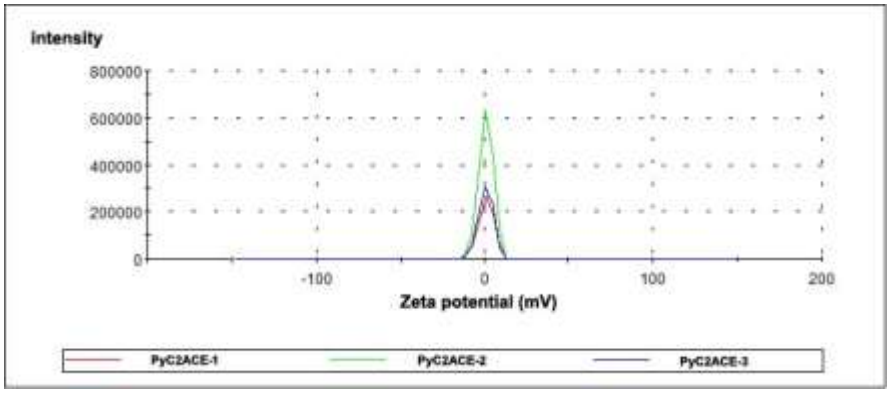


SI Figure 16. TEM images of ONPs –Polymer/ small molecule hybrid: Polymer/ small molecules are, (a) **Pro** (b) poly-lysine (c) poly-amine, and (d) arginine.

19. Zeta-potential (ζ).

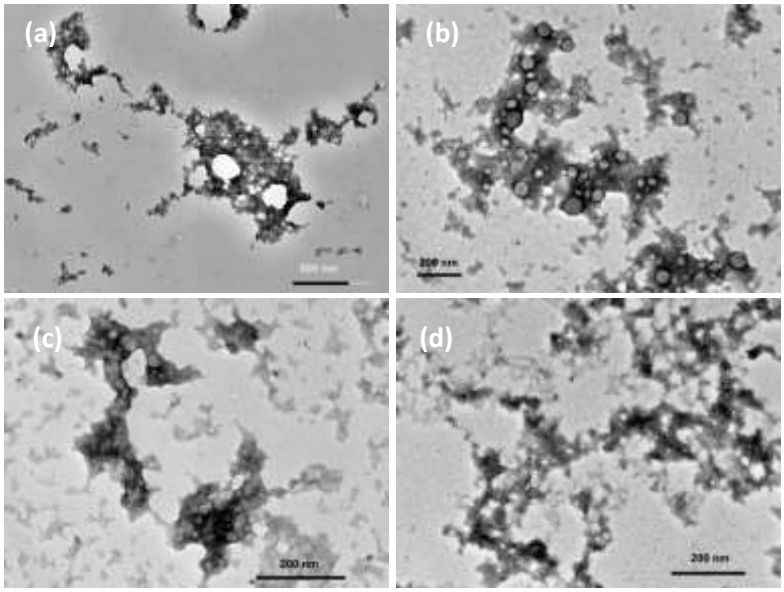
The potential value of PyC2ACE ONPs was measured for dialyzed samples and ONP-**Pro** hybrid at $f_{w,70\%}$ and 20 ^{o}C.

	Potential (mV)	Mobility(μmcm/Vs)	Conduction(mS/cm)
PyC2ACE-1	0.774	0.0549	0.0599
PyC2ACE-2	0.767	0.05443	0.0614
PyC2ACE-3	0.825	0.05854	0.0609

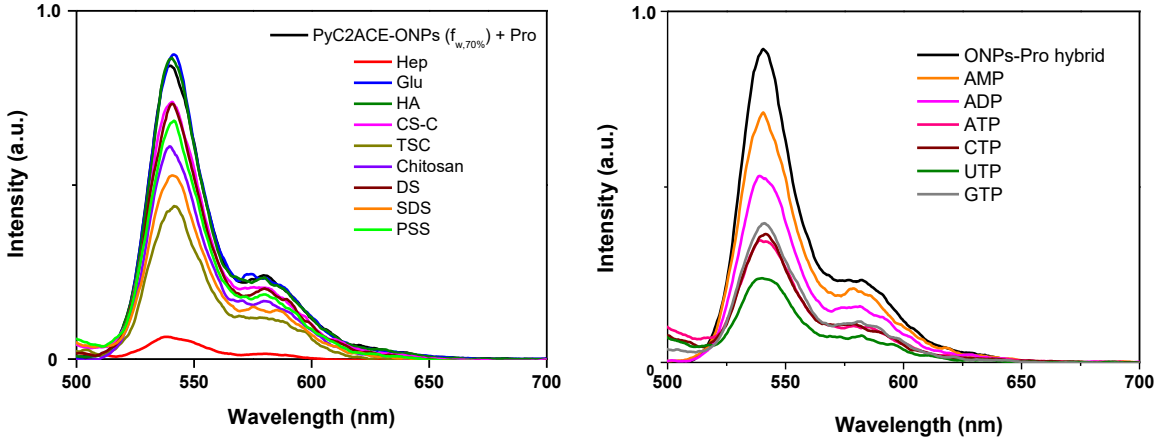


20. Application of the ONPs-Pro hybrid for recognition.

Several other cationic guests were introduced to the hybrid state with maximum FL intensity in order to check its recognition property, and the resulted solutions were carried out for checking of the morphology.



SI Figure 17. TEM images of ONPs –**Pro**-guest hybrid states: guests are (a) trisodium citrate (b) dextran (c) SDS (d) poly-styrene.



SI Figure 18. The FL intensity change of ONPs-**Pro** hybrid solution in the presence of different anionic guests (state II).

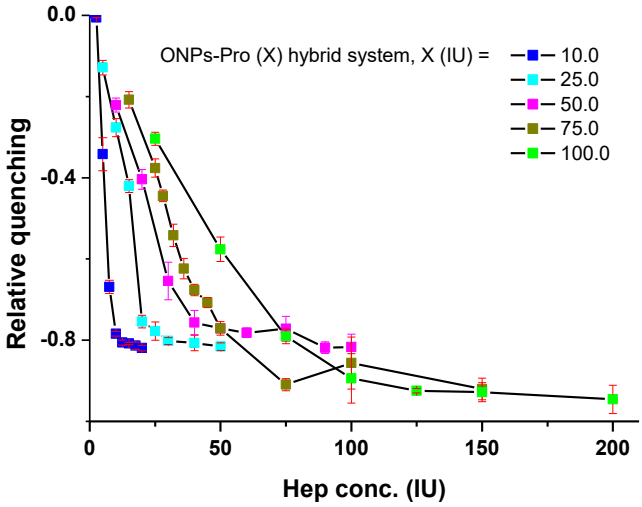


Figure 19. Equivalency check: first ONPs-**Pro** complex was prepared and then **Hep** was added to check the saturation point

20. Fluorescence quantum yield Measurement.

FL quantum yield (Φ_f) of PyC2ACE at 10·0 μM concentration in THF and in $f_{w,70\%}$ solutions were measured by using Rhodamin B (Φ_f = 0.49 in ethanol (D =1.36 at 20 ^{o}C)) as standard. Φ_f was calculated from the integrated intensities under the emission band for five different samples using the following equation:

$$\Phi_S = \Phi_S \times \frac{I_S A_R n_s^2}{I_R A_S n_R^2}$$

Where Φ_S is the FL quantum yield of the sample, Φ_R is for the standard, I_s and I_R are the integrated emission intensities of the sample and the standard, respectively, A_S and A_R are the absorbance values of the sample and the standard at excitation wavelength (530 nm), respectively, and η_S and η_R are the refractive indexes of the corresponding solutions (pure solutions were measured).

21. Photostability:

The ONPs solution ($f_{w,70\%}$, 10·0 μM) of PyC2ACE was exposed to natural light for one week, and the photostability was confirmed by the absorbance and FL spectra.

22. SI References.

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3. S. Li, M. Wei, X. Huang, X.-J. Yang, B. Wu, *Chem. Commun.* **2012**, *48*, 3097.
4. W. C. Griffin, *Journal of the Society of Cosmetic Chemists* **1954**, *5*, 249.