

Supporting Information

First Demonstration of the Two-Step FRET in a Synthetic Supramolecular Assembly

Moorthy Suresh, Amal Kumar Mandal, E. Suresh and Amitava Das*

*CSIR-Central Salt and Marine Chemicals Research Institute (CSIR), Bhavnagar,
Gujarat, India. 364 002*

Contents

1. General experimental section	S1
2. Determination of binding constant	S2
3. Mass Spectrum for 3NPC	S3
4. ¹ H NMR spectra for (1:1) mixture of NCC₂₄O₈ and T	S4
5. ¹ H NMR spectra for (1:1) mixture of D and T	S5
6. ¹ H NMR spectra for (1:1) mixture of A and T	S6
7. Mass spectrum for (1:1) mixture of D and T	S7
8. Mass spectrum for (1:1) mixture of A and T	S8
9. Methods for determination of energy levels	S9
10. Emission spectra of D , T and (1:1) mixture of D.T	S10
11. Emission spectra of NCC₂₄O₈ , T and (1:1) mixture of NCC₂₄O₈.T on excitation at 310 nm	S11
12. UV-Vis spectra of D and A , NCC₂₄O₈ and T	S12
13. Fluorescence decay profiles	S14
14. Summary of time resolved life time details	S17
15. Details of X-ray crystallography Data	S18
16. ¹ H NMR spectrum of T	S20
17. ESI-Mass spectrum of T	S21

General Experimental Section:

1A. Chemicals.

Pyrene carbaldehyde and benzyl amine were purchased from Sigma-Aldrich (USA). Dichloromethane, Ethanol (AR Grade) and HPLC water were obtained from S.D. Fine Company (India).

Synthesis of T: 500 mg (2.2 mmol) of pyrene carbaldehyde was dissolved in 100 ml of ethanol. 230 mg (2.1 mmol) of benzyl amine was allowed to stir at room temperature overnight. After completion of reaction, a yellow precipitate was the obtained. This was isolated and collected through filtration. This was redissolved in methanol and was further rereduced with excess of NaBH₄ to achieve the corresponding amine derivative. This amine derivative was treated with concentrated HCl to isolate the chloride salt, which was isolated and redissolved in acetone for treatment with NH₄PF₆ for counter anion exchange. The hexafluorophosphate salt was separated as precipitate and collected by filtration to yield **T**. ¹H-NMR (500 MHz, CDCl₃, ppm): 8.32-8.31 (2H, m), 8.29-8.27 (2H, m), 8.22 (2H, d, *J* = 8), 8.16-8.11 (3H, m), 7.51-7.48 (5H, m), 4.94 (2H, s), 4.38 (2H, s). ESI–Ms: Calcd. For C₂₄H₂₀F₆NP: 467.12, found: 322.16 [M – PF₆]⁺.

1B. Spectroscopic Techniques.

Absorption Spectra were recorded with Varian Cary 500 Scan UV-VIS-NIR Spectrophotometer. While room temperature steady-state fluorescence and time resolved fluorescence decays were recorded with HORIBA JOBIN YVON spectrophotometer. Time resolved fluorescence measurements were done using a picosecond time resolved single photo counting technique. The Rhodamine 6G dye laser pumped by CW passively mode-locked frequency-doubled Nd:YAG was used as excitation source, which generates 1 ps pulses width. The excitation of pyrene unit in the pseudorotaxane was done at 310 nm, which is the second harmonic output of an angle-tuned KDP crystal. The half-width of the instruments response function was 40 ps. Fluorescence decays were also recorded by using an IBH time-correlated single-photon counting technique. The sample was excited using a nano LED source (pulse width < 1 ns). The fluorescence emission at magic angle (54.7 °) dispersed in a monochromator (*f* = 3 aperture) and counted by a MCP PMT (Hamamatsu R 3809). The photon signal from the MCP-PMT was fed into the CFD and

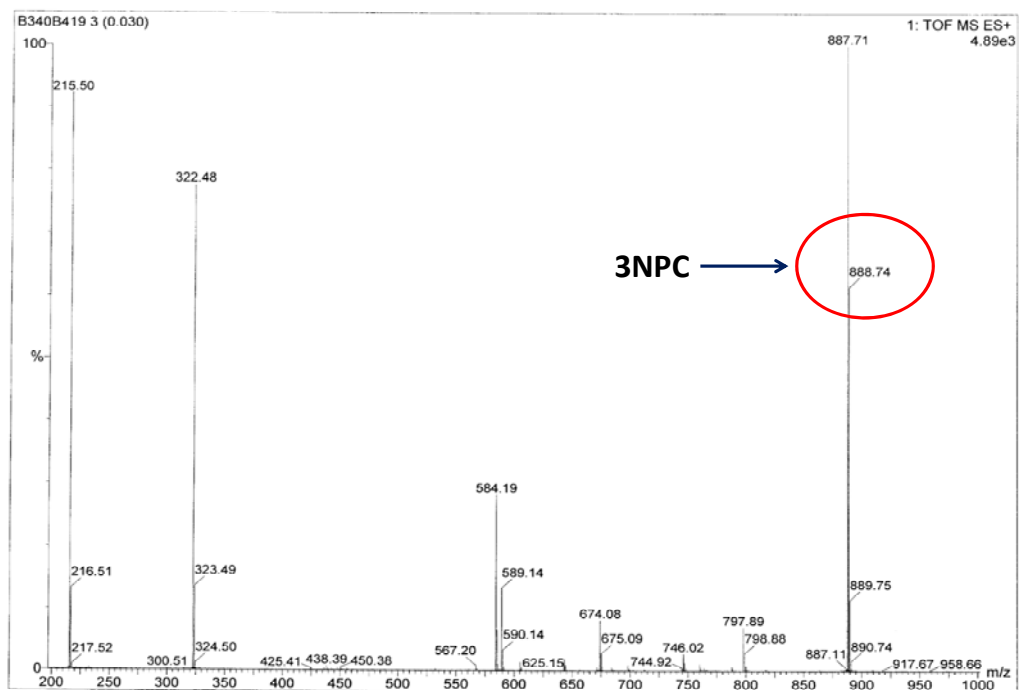
the NIM out from the CFD serves as a stop signal in the TAC. The MCP output was directly read on a rate meter. The TAC output is fed in the MCA Card (Oxford Corporation U.K) and data collection was carried out by the software (Data Station 2000) provided by IBH. For recording the lamp profile, a scatterer was placed instead of the sample and the same procedure was repeated. The response time of the instrument was around 52 ps. Association constant and thermodynamic parameters were evaluated using an isothermal calorimeter.

^1H NMR spectra were recorded on a 500 MHz FT NMR spectrometer at room temperature (RT, 25°C). The chemical shift (δ) data and coupling constant (J) values are given in parts per million (ppm) and Hertz, respectively. ESI-MS measurements were carried out on a QToF-Micro instrument. The redox values were measured by cyclic voltammetry (CV) using a bipotentiostat. A conventional three electrode cell assembly was used with a platinum disk electrode as working electrode, a platinum wire as a counter electrode and an Ag/AgCl electrode as a reference electrode. All measurements were carried out in dichloromethane/acetonitrile containing 0.1M tetraethyl ammonium tetrafluoroborate $[(\text{C}_2\text{H}_5)_4\text{NBF}_4]$ as a supporting electrolyte. The scan rate was 0.5 Vs^{-1} .

1. Determination of binding constant:

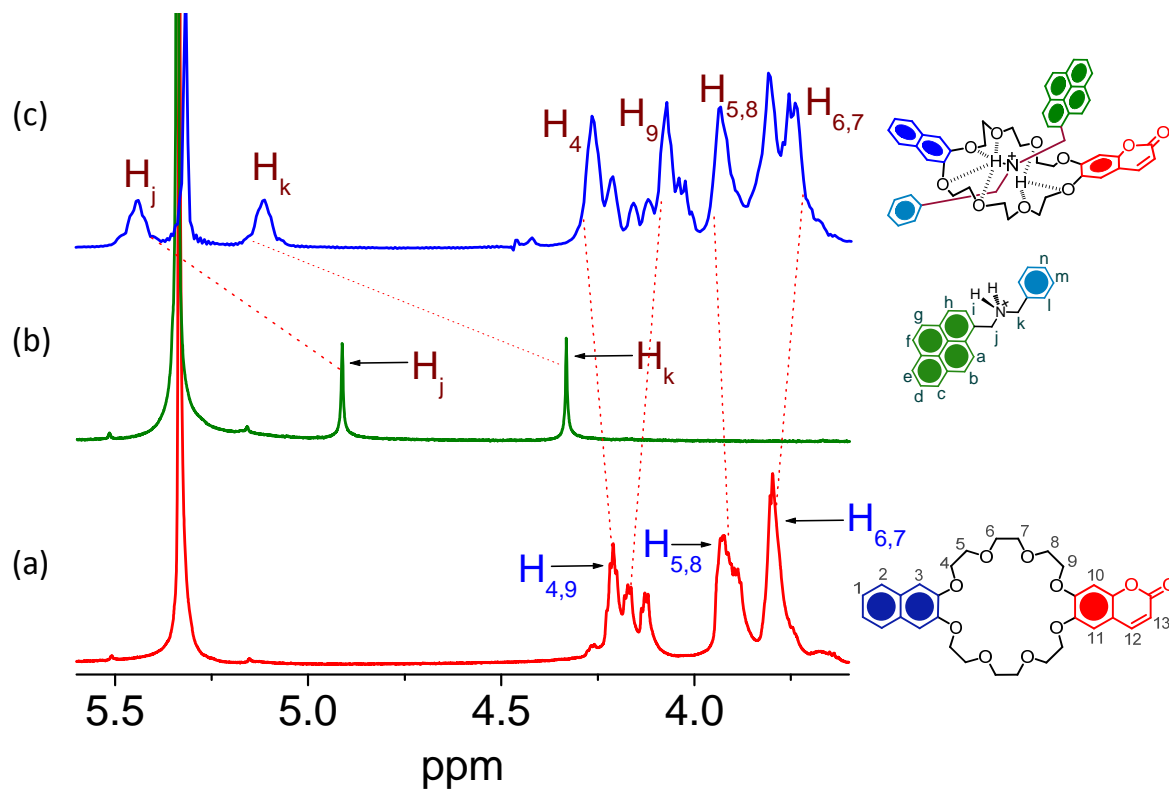
Isothermal titration calorimetry (ITC) was used to quantify the complexations of the host ($\text{NCC}_{24}\text{O}_8$) and guest (T) compounds in solution. The titration was carried out in $\text{CHCl}_3:\text{CH}_3\text{CN}$ (3:2 v/v) medium at 25 °C using an isothermal titration calorimeter (Microcal ^iTC -200) with stirring at 1000 rpm. About 200 μl of host ($\text{NCC}_{24}\text{O}_8$) solution was titrated with the guest (T) solution. A typical titration experiment consisted of 19 consecutive injections of 2 μl volume and 20 s duration each, with a 180 s interval between injections. Heat of dilution of the guest (T) were determined by injecting the guest solution into the solvent alone and the total observed heats of binding were corrected for the heat of dilution. A single set binding model fitted the binding isotherm, from where binding constant (K), binding stoichiometry (N), change of enthalpy (ΔH) and the change of entropy for the binding (ΔS) were obtained.

2. Mass Spectrum for 3NPC:



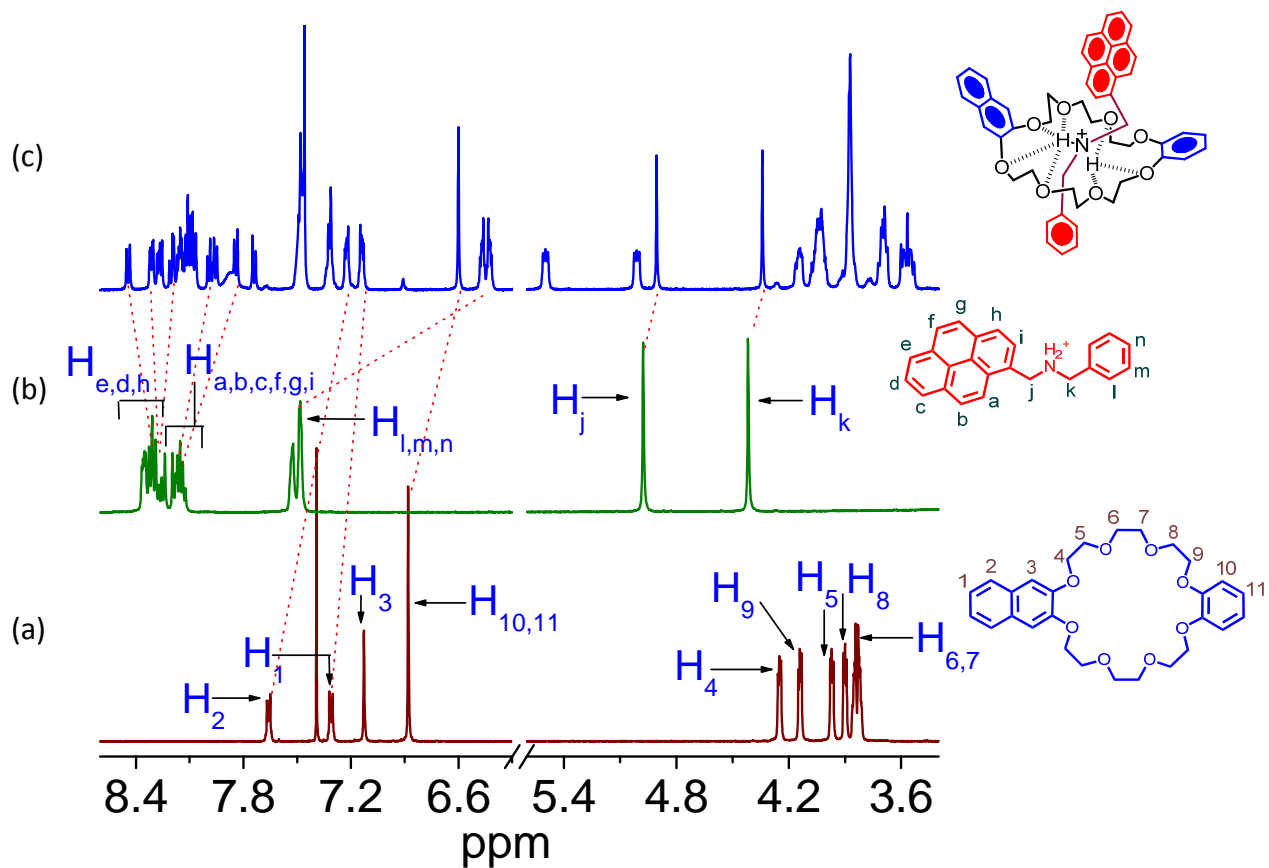
SI Figure 1: ESI-Mass spectrum for **3NPC** recorded in $\text{CHCl}_3:\text{CH}_3\text{CN}$ (4:1, v/v) in positive ion mode at room temperature.

3. ^1H NMR spectra for (1:1) mixture of $\text{NCC}_{24}\text{O}_8$ and **T**:



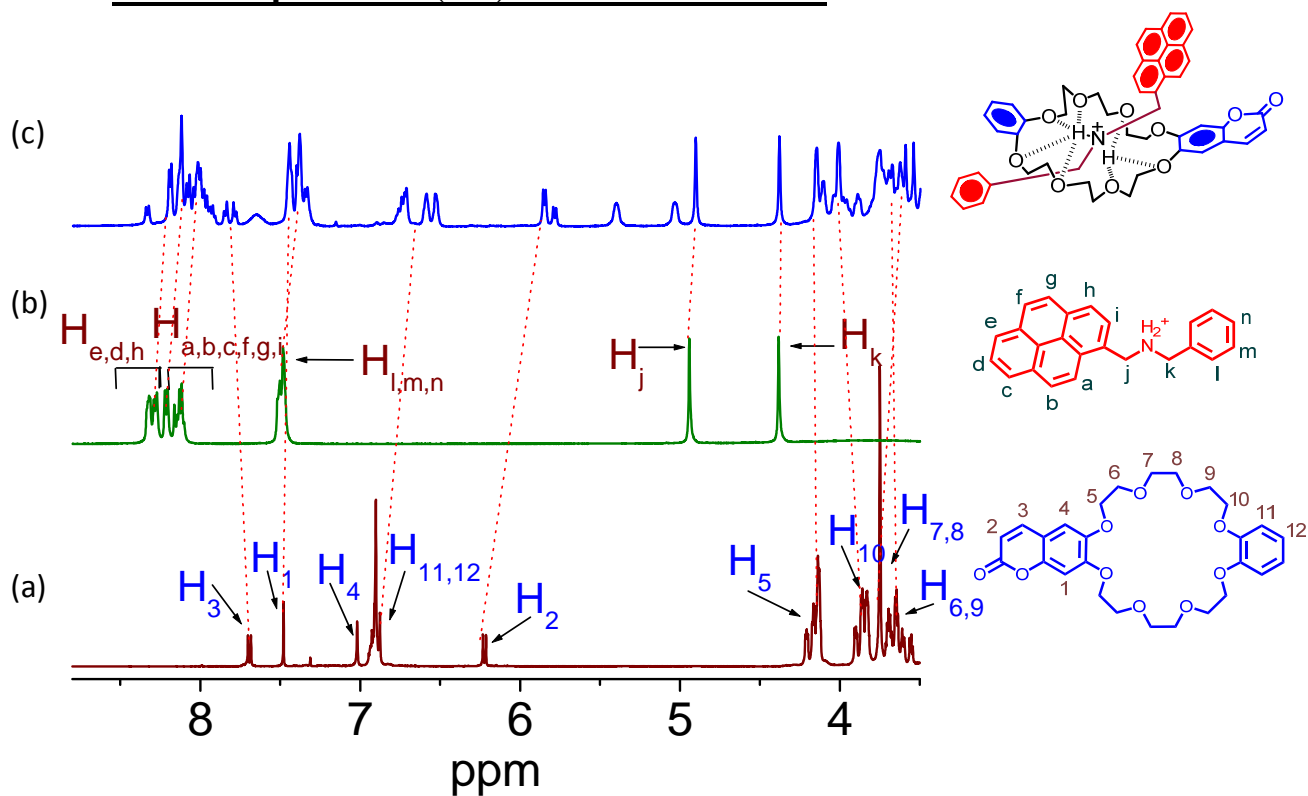
SI Figure 2: Partial ^1H -NMR spectra (500 MHz, 298 K) recorded in CD_2Cl_2 for (a) 6.38 mM $\text{NCC}_{24}\text{O}_8$, (b) 6.38 mM **T**, (c) 6.38 mM $\text{NCC}_{24}\text{O}_8$ with 6.38 mM **T**.

4. ^1H NMR spectra for (1:1) mixture of **D** and **T**:



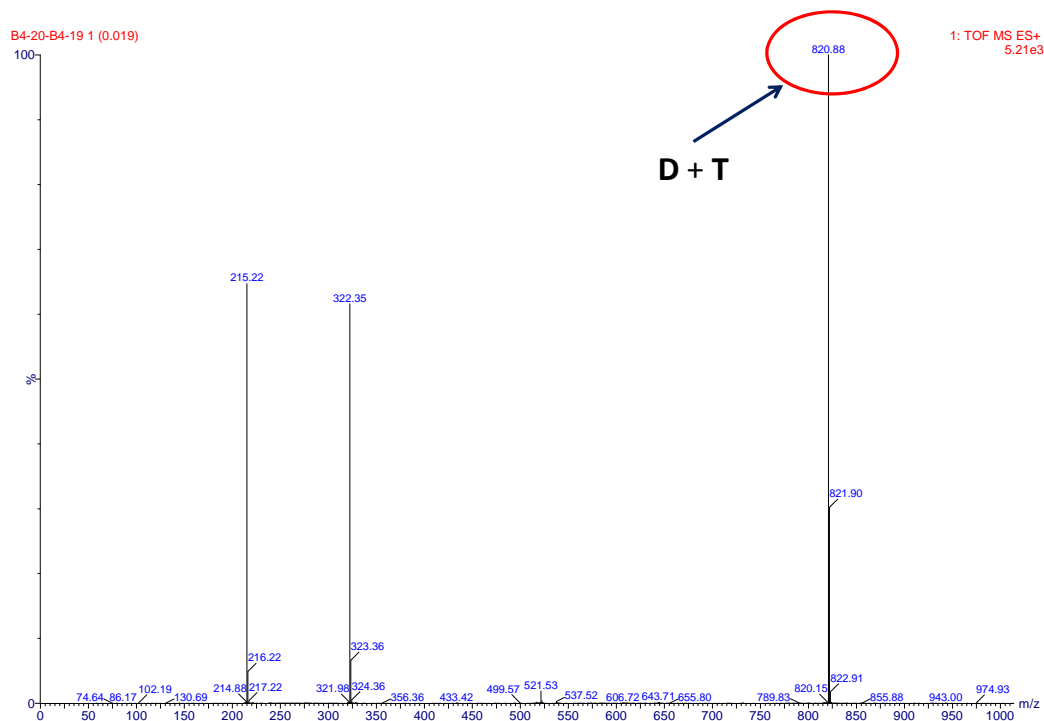
SI Figure 3: Partial ^1H -NMR spectra (500 MHz, 298 K) recorded in $\text{CDCl}_3:\text{CD}_3\text{CN}$ (4:1, v/v) for (a) 7.0 mM **D**, (b) 7.0 mM **T**, (c) 7.0 mM **D** with 7.0 mM **T**.

5. ^1H NMR spectra for (1:1) mixture of A and T:



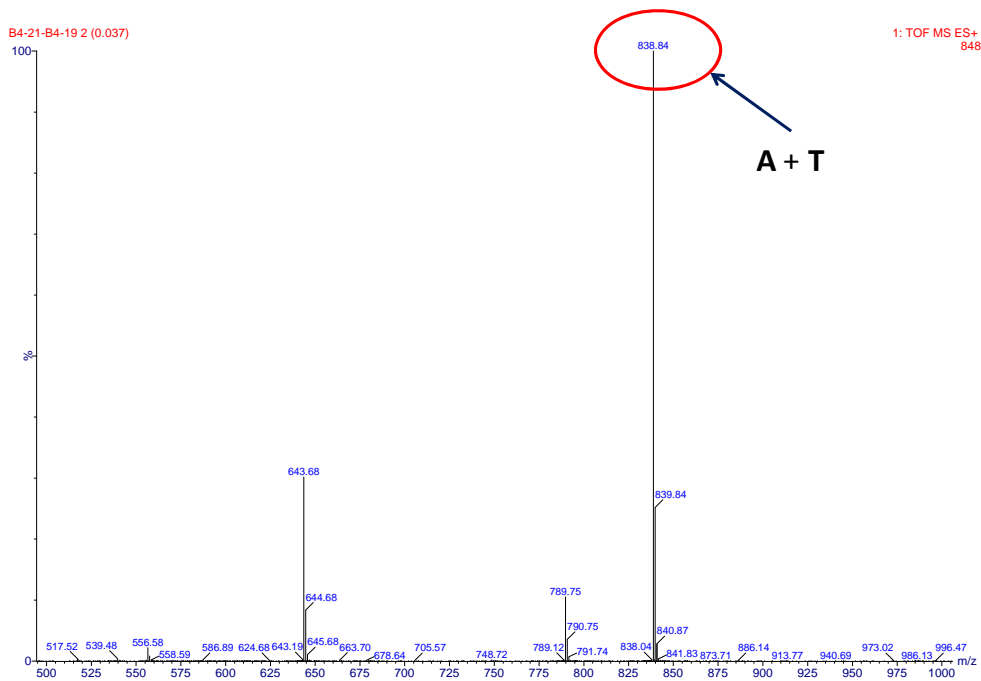
SI Figure 4: Partial ^1H -NMR spectra (500 MHz, 298 K) recorded in $\text{CDCl}_3:\text{CD}_3\text{CN}$ (4:1, v/v) for (a) 7.0 mM A, (b) 7.0 mM T, (c) 7.0 mM A with 7.0 mM T.

6. Mass spectra for (1:1) mixture of D and T:



SI Figure 5: ESI-Mass spectrum for (1:1) mixture of **D** and **T** recorded in $\text{CHCl}_3:\text{CH}_3\text{CN}$ (4:1, v/v) in positive ion mode at room temperature.

7. Mass spectra for (1:1) mixture of A and T:



SI Figure 6: ESI-Mass spectrum for (1:1) mixture of **A** and **T** recorded in $\text{CHCl}_3:\text{CH}_3\text{CN}$ (4:1, v/v) in positive ion mode at room temperature.

8. Methods for determination of energy levels:

The most accurate methods to characterize organic materials for energy band are cyclic voltammetry (CV) and differential pulsed voltammetry (DPV) technique.^[1] The oxidation potentials can be measured by cyclic voltammetry and then the HOMO and LUMO values are calculated. A conventional three electrode cell assembly was used with a platinum disk electrode as working electrode, a platinum wire as a counter electrode and an Ag/AgCl electrode as a reference electrode. All measurements were carried out in dichloromethane/acetonitrile medium containing 0.1M tetraethyl ammonium tetrafluoroborate $[(C_2H_5)_4NBF_4]$ as a supporting electrolyte. The scan rate was 0.5 Vs^{-1} . The corresponding HOMO and LUMO levels were calculated using E_{ox} and the estimations was done with the empirical relation $E_{HOMO} = [(E_{ox} - E_{1/2(\text{ferrocene})}) + 4.8] \text{ eV}$.^[2] Ferrocene (Fc) was used as external standard. It shows two peaks at 0.37 and 0.44 V and hence the $E_{1/2(\text{ferrocene})}$ for Fc/Fc^+ couple is equal to 0.41 V which can be used in equation to calculate the E_{HOMO} .

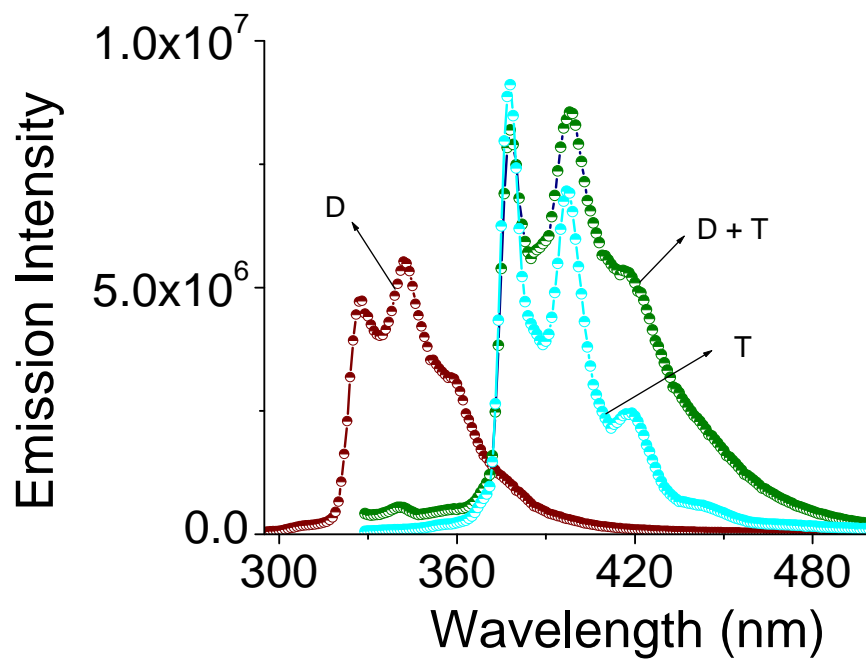
The band gap energy (E_{0-0}) values were obtained from the intersection of the normalised absorption and emission spectra by using the following equation.

$$E_{0-0} = \{1240/(\lambda_{\text{intersecting}})\} \text{ eV}.$$

Ref. 1: (a) Ibrahim, A.; Konkin, A.; Roth, H. K.; Egbe, D. A. M.; Klemm, E.; Zhokhavets, U.; Gobsch, G.; Sensfuss, S. *Thin Solid Film* **2005**, *474*, 201-210. (b) Hwang, S.W.; Chen, Y. *Macromolecules* **2002**, *35*, 5438-5443. (c) Tian, H.; Yang, X.; Pan, J.; Chen, R.; Liu, M.; Zhang, Q.; Hagfeldt, A.; Sun, L. *Adv. Funct. Mater.* **2008**, *18*, 3461–3468.

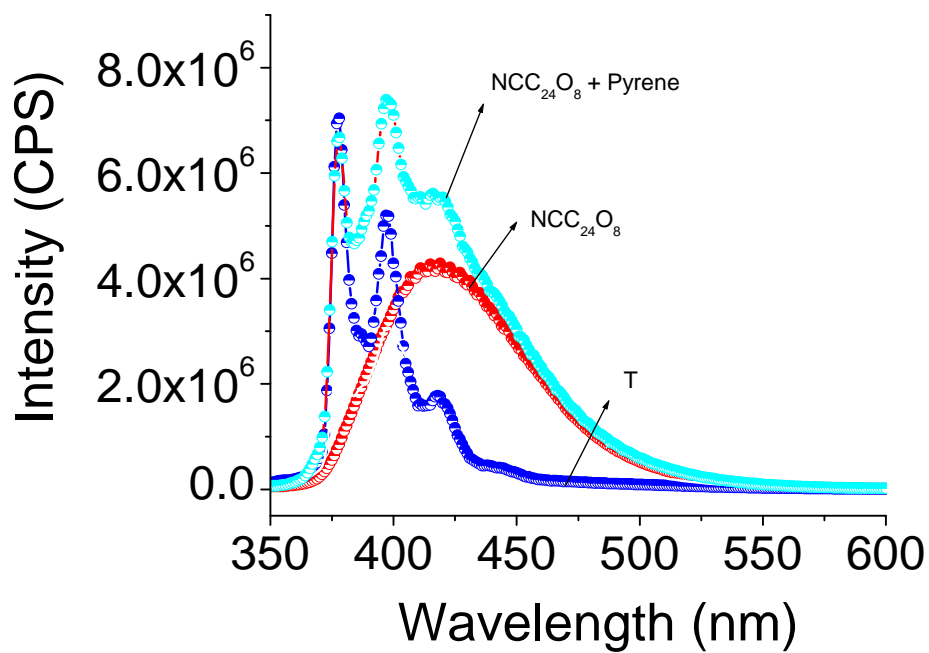
Ref. 2: (a) Pommerehne, J.; Vestweber, H.; Guss, W.; Mahrt, R. F.; Bassler, H.; Prorsch, M.; Daub, J. *Advanced Materials* **1995**, *7*, 551-554. (b) Shafiee, A.; Salleh, M. M.; Yahaya, M. *Sains Malaysiana* **2011**, *40*, 173–176.

9. Emission spectra of D, T and (1:1) mixture of D.T:



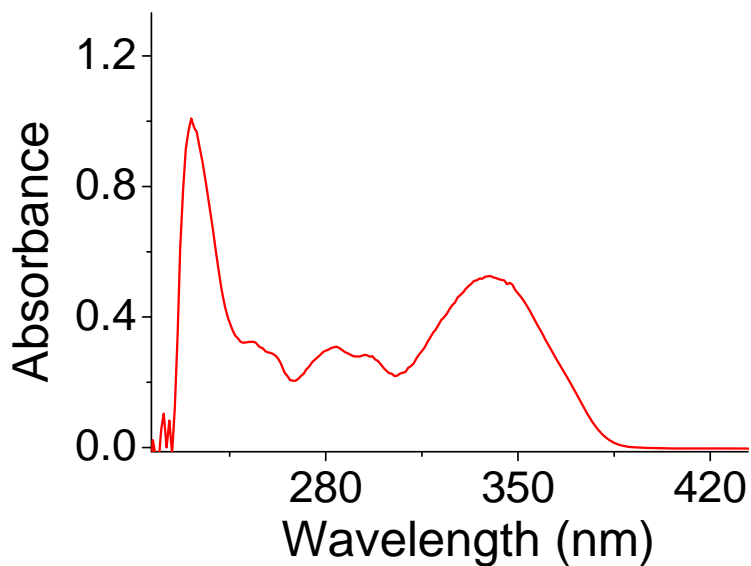
SI Figure 7: Steady state fluorescence spectrum of **D** (λ_{Exc} 280 nm), **T** (λ_{Exc} 310 nm) and 1:1 mixture of **D** and **T** (λ_{Exc} 310 nm) in dichloromethane solution.

10. Emission spectra of $\text{NCC}_{24}\text{O}_8$, **T and (1:1) mixture of $\text{NCC}_{24}\text{O}_8$.**T** on excitation at 310 nm:**

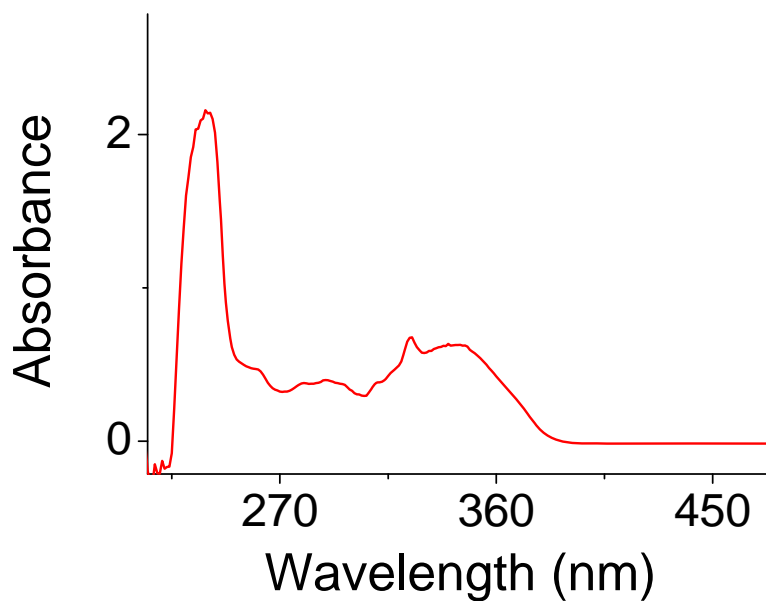


SI Figure 8: Steady state fluorescence spectrum of $\text{NCC}_{24}\text{O}_8$ (λ_{Exc} 340 nm), and **T** (λ_{Exc} 310 nm) and 1:1 mixture of $\text{NCC}_{24}\text{O}_8$ and **T** (λ_{Exc} 310 nm) in dichloromethane solution.

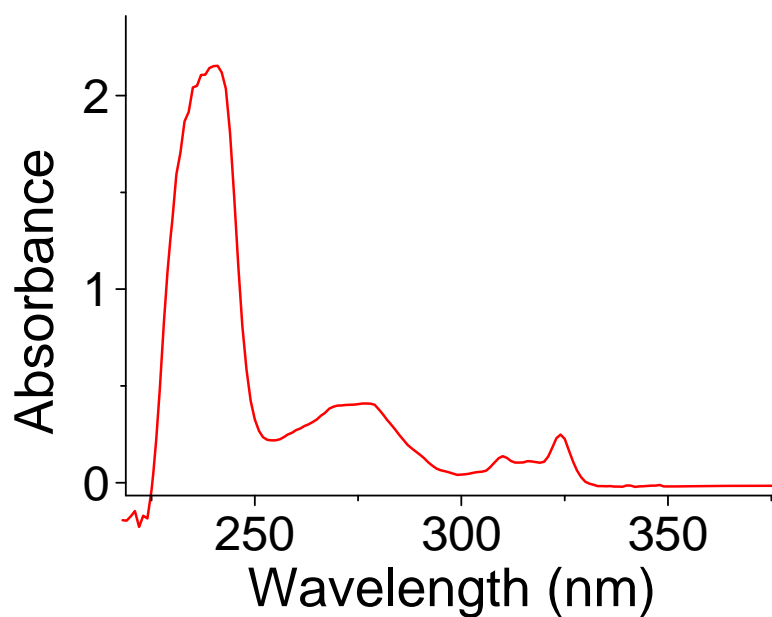
11. UV-Vis spectra of D and A, $\text{NCC}_{24}\text{O}_8$ and T:



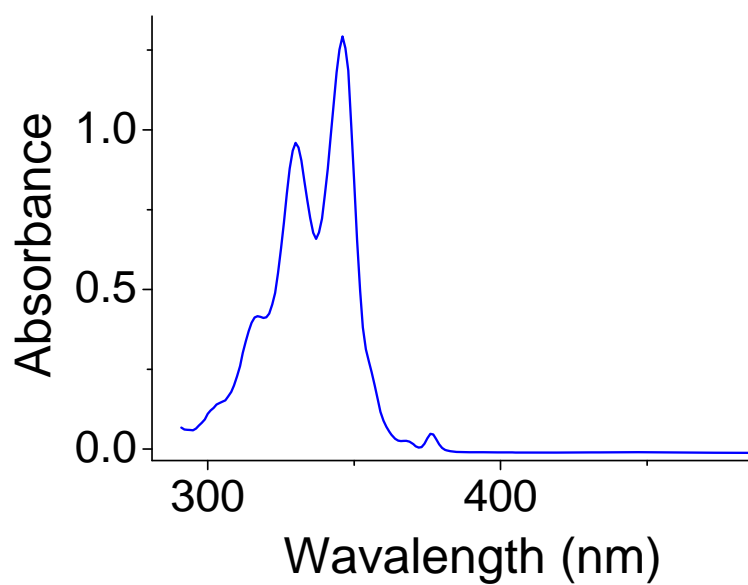
SI Figure 9: UV-Vis spectrum of coumarin crowns (A) (5.8×10^{-5} M) in dichloromethane at room temperature.



SI Figure 10: UV-Vis spectrum of $\text{NCC}_{24}\text{O}_8$ (6.7×10^{-5} M) in dichloromethane at room temperature.

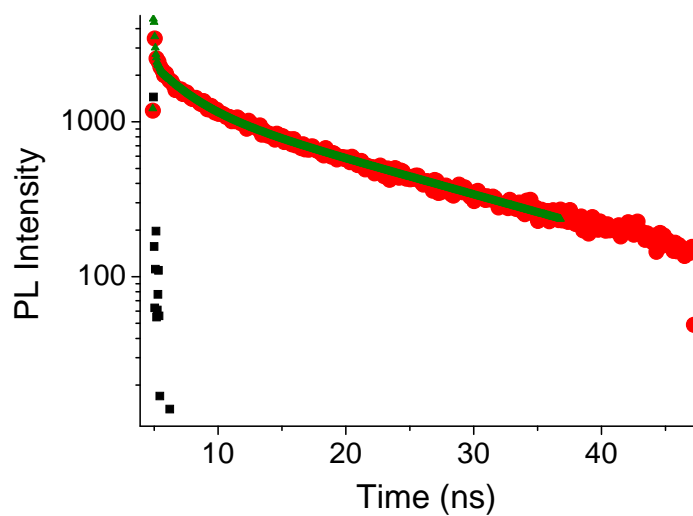


SI Figure 11: UV-Vis spectrum of **D** (6.0×10^{-5} M) in dichloromethane at room temperature.

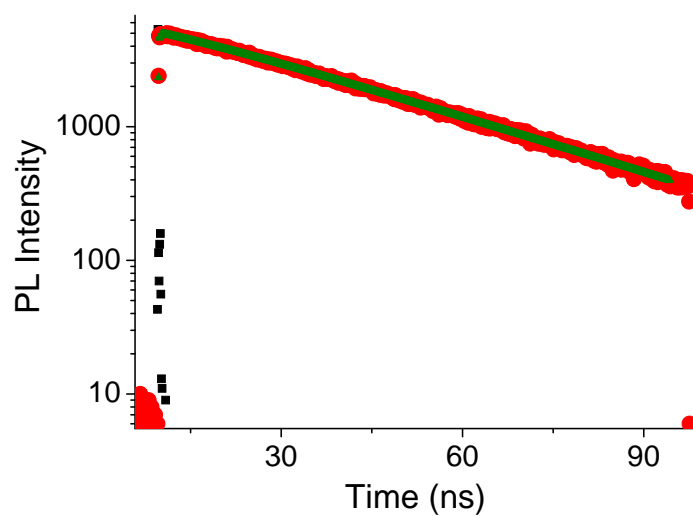


SI Figure 12: UV spectrum of **T** (3.0×10^{-5} M) in dichloromethane at room temperature.

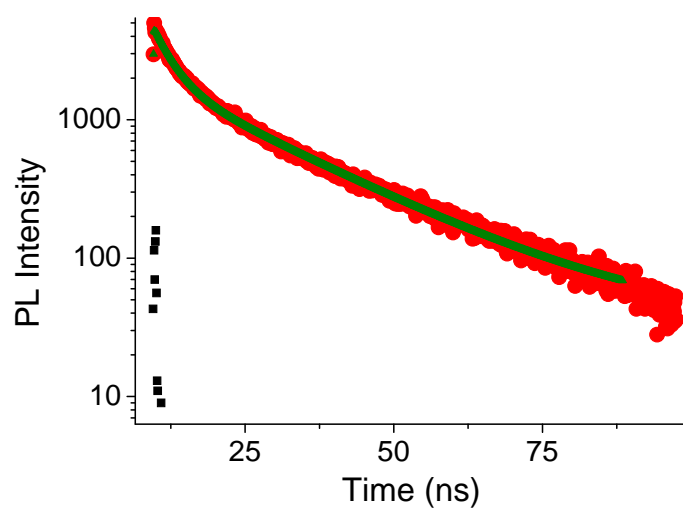
12. Fluorescence decay profile:



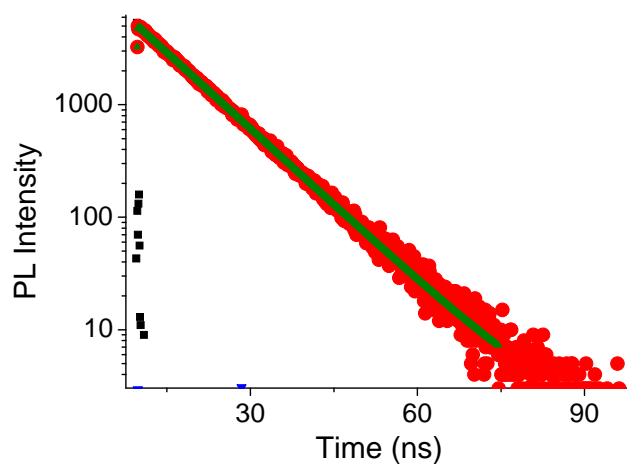
SI Figure 13: Time-resolved fluorescence decay profile of $\text{NCC}_{24}\text{O}_8$ monitored at 320 nm in dichloromethane using the 260 nm excitation.



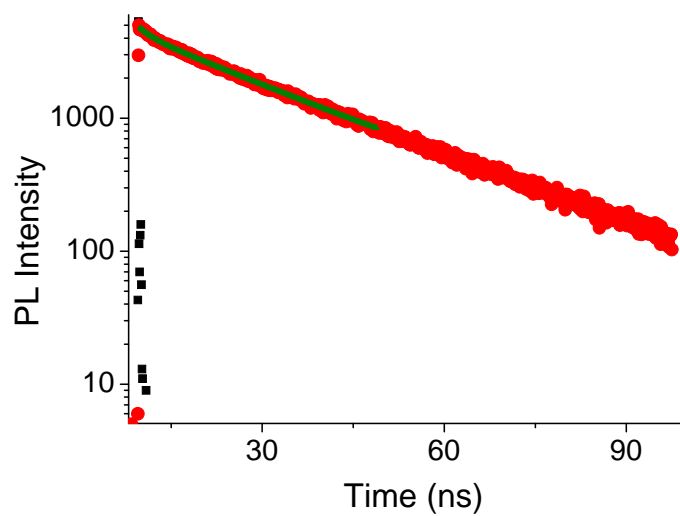
SI Figure 14: Time-resolved fluorescence decay profile of 1:1 mixture of $\text{NCC}_{24}\text{O}_8$ and T monitored at 378 nm in dichloromethane using the 260 nm excitation.



SI Figure 15: Time-resolved fluorescence decay profile of $\text{NCC}_{24}\text{O}_8$ and **T** monitored at 470 nm in dichloromethane using the 260 nm excitation.



SI Figure 16: Time-resolved fluorescence decay profile of **T** monitored at 378 nm in dichloromethane using the 310 nm excitation.



SI Figure 17: Time-resolved fluorescence decay profile of 1:1 mixture of **D** and **T** monitored at 378 nm in dichloromethane using the 260 nm excitation.

13. Table S1. Summary of time resolved life time details:

Excitation	280 nm			310 nm		340 nm
Emission	340/320 nm	378 nm	420 nm	378 nm	420/470 nm	470 nm
D	9.61 ± 0.017 (100%) at 340 nm					
T				23.4 ± 0.2 (100%)		
A						0.9 ns
NCC₂₄O₈	$\tau_1 = 0.32 \pm 0.002$ at 320 nm (95.65%) $\tau_2 = 8.68 \pm 0.02$ at 320 nm (4.35%)		$\tau_1 = 0.32 \pm 0.005$ at 470 nm (-60.82%) $\tau_2 = 0.85 \pm 0.01$ at 470 nm (160.82%)			
D+T	8.0 ± 0.26 at 340 nm (100%)		$\tau_1 = 11.0$ ns at 420 nm (-5%) $\tau_2 = 31.8$ ns at 420 nm (105%)			
T+A				0.952 ns (average 3 life time components)	0.95 ns at 470 nm and 0.94 at 420 nm (average two decay constants)	
3NPC	$\tau_1 = 3.3 \pm 0.1$ (0.7%) at 320 nm $\tau_2 = 0.31$ ns (99.33) Laster excitation: 260 nm	$\tau_1 = 9.81$ ns (-3.67%) $\tau_2 = 21.24$ ns $\tau_3 = 2.4$ ns	$\tau_1 = 0.97$ ns (93.8%) $\tau_2 = 6.06$ ns (6.2%)	1.2 ns (average 3 life time components)	1.1 ns at 470 nm (average of three decay constants)	

14. Details of X-ray crystallography Data:

Summary of crystallographic data and details of hydrogen bonding interactions for compound **3NPC** is given in Table S1 and S2 respectively. Single crystals of the compound with suitable dimensions were chosen under an optical microscope and immersed in paratone oil before being mounted on a glass fiber for data collection. Intensity data for all the three crystals were collected using MoK α ($\lambda=0.71073$ Å) radiation on a Bruker SMART APEX diffractometer equipped with CCD area detector at 110K. The data integration and reduction were processed with SAINT³ software. An empirical absorption correction was applied to the collected reflections with SADABS⁴. The structures were solved by direct methods using SHELXTL⁵ and were refined on F² by the full-matrix least-squares technique using the program SHELXL-97⁶. The solvent molecule present in the lattice along with the compound was highly disordered and their contribution to the diffraction data has been removed using the SQUEEZE subroutine as implemented in PLATON.⁷ A potential solvent accessible volume of 943.8Å⁵ with 304 electron counts per unit cell worth of scattering were located in the void. Refinement of **1** using the modified reflection data set apparently improved the R-factor. All non-hydrogen atoms were refined anisotropically till convergence is reached. Hydrogen atoms attached to the organic moieties either located from the difference Fourier map or stereochemically fixed in all the three complexes.

Ref 3: G.M. Sheldrick, SAINT 5.1 ed., Siemens Industrial Automation Inc., Madison, WI, 1995.

Ref 4: SADABS, Empirical Absorption Correction Program, University of Göttingen, Göttingen, Germany, 1997.

Ref 5: G. M. Sheldrick, SHELXTL Reference Manual: Version 5.1, Bruker AXS, Madison, WI, 1997.

Ref 6: G. M. Sheldrick, SHELXL -97: Program for Crystal Structure Refinement, University of Göttingen, Göttingen, Germany, 1997.

Ref 7: A. L. Spek, J. Appl. Crystallogr., 2003, 36, 7.

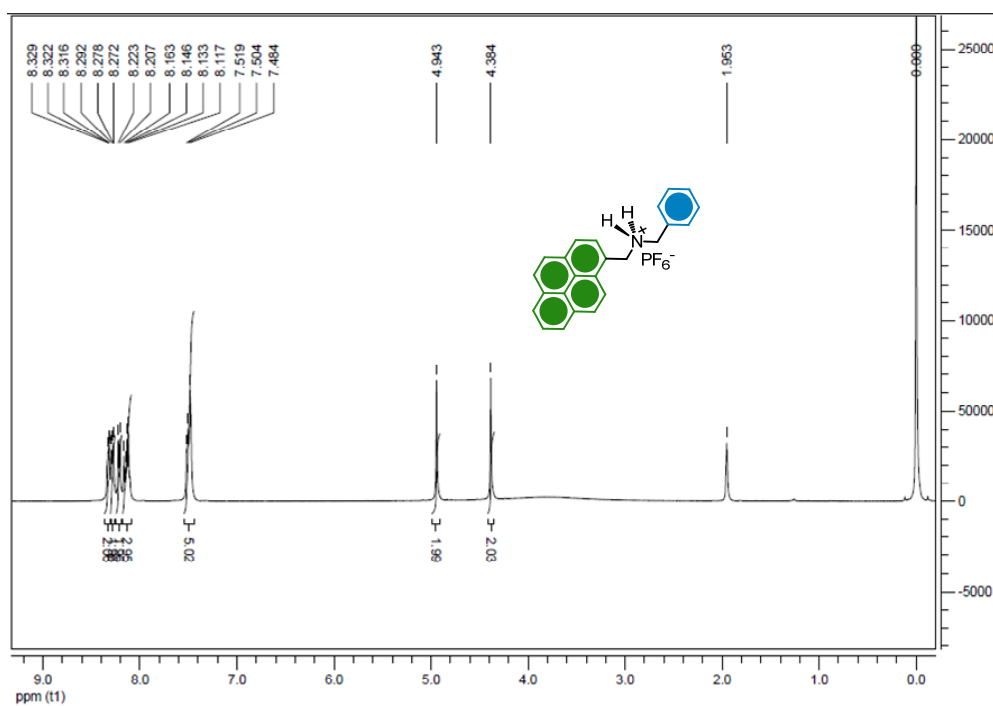
Table S2. Crystal Data and Refinement Parameters for Compound 3NPC

Identification code	Compound 3NPC
Chemical formula	C ₅₅ H ₅₄ N ₁ O ₁₀ P ₁ F ₆
Formula weight	1033.96
Crystal Colour	Colourless
Crystal Size (mm)	0.24 x 0.12 x 0.05
Temperature (K)	110(2)
Crystal System	Monoclinic
Space Group	P2 ₁ /n
a(Å)	4.0847(11)
b(Å)	15.7003(12)
c(Å)	25.3994(19)
α(°)	90.0
β(°)	91.481(2)
γ(°)	90.0
Z	4
V(Å ³)	5614.8(7)
Density (Mg/m ³)	1.223
Absorption Coefficient(mm ⁻¹)	0.123
F(000)	2160
Reflections Collected	25490
Independent Reflections	8770
R _(int)	0.0700
Number of parameters	688
S(Goodness of Fit) on F ²	1.059
Final R1, wR2 (I>2σ(I))	0.1183/0.2930
Weighted R1, wR2(all data)	0.1771/ 0.3256

Table S3. Hydrogen bonding Parameters in Compound 3NPC

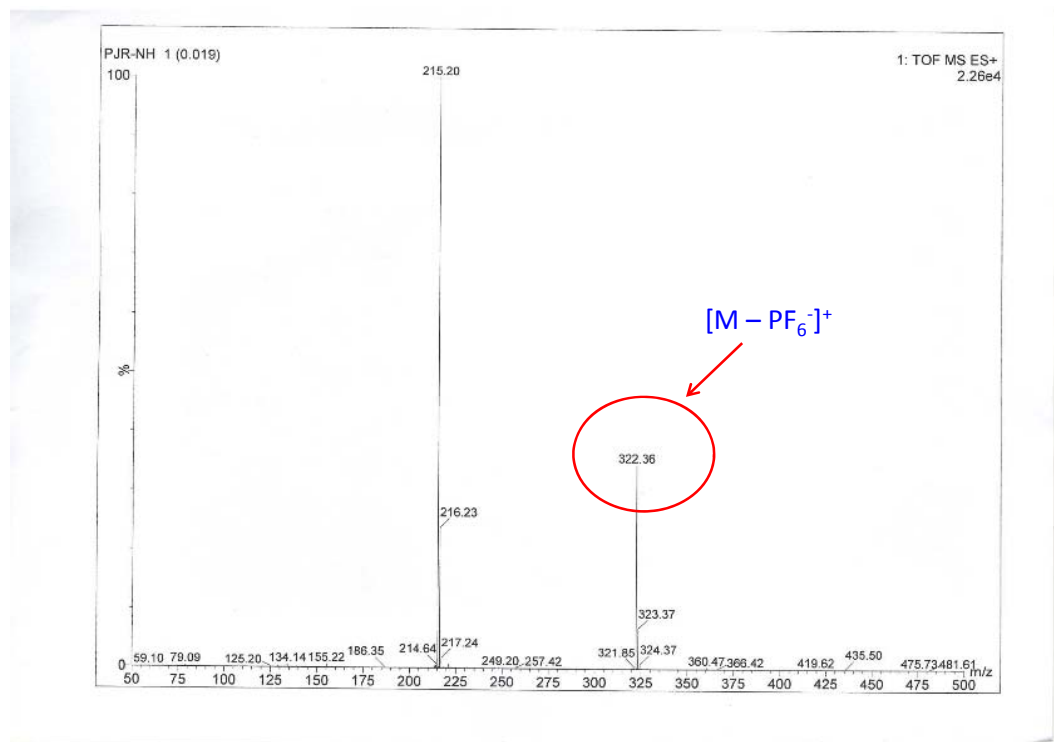
Compound 3NPC			
D-H...A	d(H...A) (Å)	d(D...A) (Å)	∠ D-H...A (°)
N(1)-H(1A)...O(4) ¹	2.26	3.160(5)	175
N(1)-H(2B)...O(8) ¹	2.11	3.010(6)	179
C(49)-H(49A)...O(3) ¹	2.32	3.089(7)	136
C(4)-H(4)...F(2) ²	2.42	3.298(14)	157
C(4)-H(4)...F(4) ²	2.49	3.263(13)	141
C(7)-H(7)...F(2) ³	2.52	3.425(13)	165
Symmetry code : 1. x,y,z; 2. -1+x,y,z; 3. 1/2-x,1/2+y,1/2-z			

15. ^1H NMR spectrum of **T**:



SI Figure 18: ^1H -NMR spectra of **T** (500 MHz, 298 K) recorded in CDCl_3 at room temperature.

17. ESI-Mass spectrum of T:



SI Figure 19: ESI-Mass spectrum for **T** recorded in acetonitrile in positive ion mode at room temperature.