Electronic Supplementary Information

Cyanide Modulated Fluorescent Supramolecular Assembly of Hexaphenylbenzene Derivative for Detection of Trinitrotoluene at Attogram Level

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General Experimental Procedure:

All reagents were purchased from Aldrich and were used without further purification. THF was dried over sodium and benzophenone as a indicator and kept over molecular sieves overnight before use. UV-vis spectra were recorded on a SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette (path length, 1 cm). The cell holder was thermostatted at 25°C. The fluorescence spectra were recorded with a SHIMADZU 5301 PC spectrofluorimeter. The TEM mages was recorded from Transmission Electron Microscope (TEM) - JEOL 2100F. Scanning electron microscope (SEM) images were obtained with a field-emission scanning electron microscope (SEM JEOL JSM-6610LV). ¹H was recorded on a JOEL-FT NMR-AL 300 MHz spectrophotometer using CDCl₃, DMSO-d₆ as solvent and tetramethylsilane SiMe₄ as internal standards. UV-vis studies were performed in THF and HEPES buffer (pH=6.99). Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, br = broad, singlet, d = doublet, t = triplet, m = multiplet), coupling constants J (Hz), integration, and interpretation. Silica gel 60 (60-120 mesh) was used for column chromatography. Fluorescence quantum yield was determined by using optically matching solution of diphenyl anthracene ($\Phi_{fr} = 0.90$ in cyclohexane) as standard at an excitation wavelength of 373 nm and quantum yield is calculated using the equation:

$$\Phi_{fs} = \Phi_{fr} \quad \times \quad \frac{1 - 10^{-ArLr}}{1 - 10^{-AsLs}} \quad \times \quad \frac{N_s^2}{N_r^2} \quad \times \quad \frac{D_s}{D_r}$$

 Φ_{fs} and Φ_{fr} are the radiative quantum yields of sample and the reference respectively, A_s and A_r are the absorbance of the sample and the reference respectively, D_s and D_r the respective areas of emission for sample and reference. L_s and L_r are the lengths of the absorption cells of sample and reference respectively. N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed respectively).

Procedure for analytes sensing:

UV-vis and fluorescence titrations were performed on 5.0 μ M solutions of ligand (15 μ l of THF are used to dissolve) in H₂O/EtOH (6:4, v/v). *Tert-butyl ammonium anions* (^tBu₄N⁺X⁻) where X= CN⁻, F⁻, Cl⁻, Br⁻, Γ, NO₃⁻, CH₃COO⁻, ClO₄⁻ and H₂PO₄⁻ are used. Also various nitroderivatives are examined such as picric acid (PA); 2,4,6-trinitrololuene (TNT); 2,4-dinitrotoluene (DNT); 1,4-dinitrobenzene (DNB); 1,4-dinitrobenzoic acid (DNBA); 1,4-benzoquinone (BQ); nitromethane (NM); 2,3-dimethyl-2,3-dinitrobutane (DMDNB). In titration experiments, each time a 3 ml solution of **3** was filled in a quartz cuvette (path length, 1 cm) and spectra were recorded.

Preparation of Filter Paper Test Strips:

Filter paper (5cm \times 2 cm) test strips were prepared by coating the **3-CN**⁻ ensemble (5 \times 10⁻⁶ M) followed by removal of solvent under vacuum at room temperature. The ensemble coated filter papers were then cut into 8 pieces (1 cm \times 1 cm) to get the test strips and used for the detection of explosives.

Contact Mode Visual Detection of TNT:

Aqueous samples were prepared by dissolving TNT in water/THF (9:1) mixture. The explosive solutions were spotted onto the test strips at the desired concentration level using a micropipette. A solvent blank was spotted near to the spot of each explosive. In order to ensure consistent analysis, all depositions were prepared from a 10 μ L volume, thereby producing a spot of ~0.5 cm in diameter. After solvent evaporation, the filter paper was illuminated with 365 nm UV light. The dark spots (under UV light) and red colored spots (naked eye) were identified by an independent observer, and each set of experiments was repeated three times for consistency. The detection limits were calculated from the lowest concentration of the explosive that enabled an independent observer to detect the quenching visually.

Synthetic scheme of compound 3:



Synthesis of HPB based derivative **3**, (i) K_2CO_3 (aq.), $PdCl_2(PPh_3)_2$, THF, 80 °C; (ii) Dry THF : Methanol (4:6), 80 °C.

Synthesis of compound 1b:

To a solution mixture of di(4-bromophenyl)benzene **1a** (0.4 g, 0.578 mmol) and **2a** (0.33 g, 2.60 mmol) in THF (15 ml) were added K₂CO₃ (2.073 g, 15 mmol), distilled H₂O (2.1 mL), and [Pd(PPh₃)₄] (0.243 g, 0.346 mmol) under N₂ atmosphere. The reaction mixture was degassed with nitrogen for three times and was refluxed at 110 °C under the inert atmosphere for 24 hrs. The toluene was then removed under vacuum, and the residue so obtained was treated with water, extracted with chloroform, and dried over anhydrous Na₂SO₄. The organic layer was evaporated, and compound was purified by column chromatography using (85:15) (Hexane/Ethylacetate) as an eluent to give compound **1b**, recrystallized from methanol in 55% yield as brown solid; mp: 215 °C; ¹H NMR (300 MHz, CDCl₃ ppm). δ = 3.64 (br, S, 4 H, NH), 6.63 (d, J = 8.4 Hz, 4H), 6.71 (d, J = 8.7 Hz, 4H), 6.84-6.89 (m, 16H, Ar-H), 7.05 (d, J = 8.4 Hz, 4H, Ar-H), 7.14-7.24 (m, 4H, Ar-H), 7.39-7.54 (m, 4H, Ar-H);). ¹³C NMR (CDCl₃, 75.45 MHz, ppm) 115.21, 124.29, 125.09, 126.51, 126.59, 127.49, 131.42, 137.21, 138.61, 140.12, 140.27, 140.40, 140.68, 145.36; ESI/MS, *m/z* = 739.3042 [M+Na]⁺. Elemental analysis: Calculated for C₅₄H₄₀N₂: C 90.47; H 5.62; N 3.91; Found: C 90.47 %; H 5.62 %; N 3.91%.

Synthesis of compound 3:

A clear solution of compound **1b** (0.05 g, 0.07 mmol) and **2b** (0.03 g, 0.17 mmol) in dry THF: MeOH (4:6) was stirred at 70 °C. After 24hrs, the reaction mixture turned turbid. The reaction mixture was concentrated under the reduced pressure and dry methanol was poured into it, solid appears. The solid was filtered and recrystallized from methanol to afford the yellow coloured compound **3** (0.065 g, 85%);; mp: >280 °C; ¹H NMR (300 MHz, DMSO-d₆) $\delta = 15.82$ (d, J = 4 Hz, 2H, -O-H), 9.63 (d, J = 4 Hz, 2H, C=NH), 8.46 (d, J = 6.5 Hz, 2H), 7.90 (d, J = 3.5 Hz, 2H), 7.76 (d, J = 6.0 Hz, 2H), 7.61 (s, 4H), 7.51 (t, J = 5.5 Hz, 6H), 7.32 (d, J = 6.0 Hz, 4H), 7.06 (d, J = 6.0 Hz, 4H), 6.85-6.90 (m, 24H). ¹³C NMR (CDCl₃, 300 MHz) $\delta = 118.85$, 120.35, 122.40, 123.52, 125.02, 125.26, 126.62, 126.73, 127.87, 128.09, 128.13, 131.44, 132.04, 133.25, 136.8, 140.53, 147.75, 153.81, 170.83, MALDI TOF MS, $m/z = 1025.4335 [M+H]^+$. Elemental analysis: Calculated for C₇₆H₅₂N₂O₂: C 89.03; H 5.11; N 2.73; Found: C 89.03 %; H 5.11 %, N 2.73 %.



Fig. S1: Absorbance spectra of compound 3 (5 μ M) showing the variation of absorbance intensity in Water/Ethanol mixture with different water fraction; Inset showing the absorbance intensity of 3 in ethanol.



Fig. S2: Fluorescence spectra of compound 3 (5 μ M) showing the variation of fluorescence intensity in Water/Ethanol mixture (0 to 60% volume fraction of water in Ethanol); λ_{ex} = 322 nm.



Fig. S3: (A) Fluorescence spectra of compound **3** showing the variation of fluorescence intensity with different concentration of **3** in ethanol; λ_{ex} = 322nm; (B) Variation in quantum yield value with the variation of water fractions (0 to 99% volume fraction of water in Ethanol); λ_{ex} = 322nm.



Fig. S4: Change in absorption spectra of compound **3** (5 μ M) with the addition of CN⁻ ions (200 equivalent) in H₂O/EtOH (6:4) mixture. Inset showing enlarge UV spectra of compound **3** (5 μ M) with the addition of CN⁻ ions in H₂O/EtOH (6:4) mixture in the range of 500-900 nm.



Fig. S5: Fluorescence spectra showing reversibility of CN⁻ ion coordination to receptor **3** by Cu²⁺ ions in H₂O/EtOH (6:4, v/v), λ_{ex} = 322 nm; blue line, free **3** (5 µM); yellow line, **3** + 200 equiv. CN⁻; red line, **3** + 200 equiv. CN⁻ + 300 equiv. Cu²⁺; green line, **3** + 200 equiv. CN⁻ + 300 equiv. Cu²⁺ + 250 equiv. CN⁻. This can prove that weak hydrogen bonding occurs between hydroxyl group of compound **3** and CN⁻ ions.

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10.0 9.5

9.0



Fig. 6a: Overlay ¹H NMR spectra of compound **3** and titration with CN^{-} and TNT respectively in dmsod₆.

8.5 8.0 7.5 7.0 6.5 6.0

 1 1

ppm

Compound 3	Compound 3+CN ⁻	$\Delta \delta_1 = \delta_3 - \delta_C$	Compound 3+CN ⁻	$\Delta \delta_1 = \delta_T - \delta_C$
(δ ₃)	$(\delta_{\rm C})$		+TNT (δ_{T})	
15.82(-OH) 🔺	Signal disappear		Signal disappear	
9.36(N=CH) +	9.02	0.34	9.27	0.25
8.46(d) ★	8.13(Broad)	0.33	8.35(broad)	0.22
7.90(d) (aromatic)	7.46(broad)	0.44	7.70(d, but broad)	0.26
7.76(d) (aromatic)	7.21(broad)	0.55	7.45(d, but broad)	0.24

Fig. S6b: Change in chemical shift (δ) value of compound **3** and after addition of CN⁻ and TNT respectively in dmso-d₆.



Fig. S7: (A) Compiled fluorescence spectra of compound **3** (5 μ M) with different anions in H₂O/EtOH (6:4, v/v); λ_{ex} = 322 nm; (B) Showing (a) Selectivity of **3** towards CN⁻ ions upon addition of different anions and (b) Competitive selectivity of **3** towards CN⁻ in the presence of different anions in H₂O/EtOH (6:4, v/v), λ_{ex} = 322 nm.



Fig. S8: (A) Histograms showing the fluorescence response of **3** (5 μ M) with CN⁻ ion (200 equiv.) in the presence of blood serum in H₂O/EtOH (6:4, v/v) at pH = 7.0, λ_{ex} = 322nm. The **3-CN**⁻ complex was titrated with varying amounts (μ L) of serum. (a)10 μ L; (b) 20 μ L; (c) 30 μ L; (d) 40 μ L; (e) 50 μ L. (B) Fluorescence spectra of compound **3** in response to NaCN in tap water, λ_{ex} = 322 nm.

Detection Limit of CN⁻ ions:



Fig. S9: Showing the fluorescence intensity of compound **3** at 450 nm as a function of CN⁻ ions concentration.

To determine the detection limit, fluorescence titration of compound **3** with cyanide ions was carried out by adding aliquots of cyanide solution (in equiv.) and the fluorescence intensity as a function of CN^{-} ions added was then plotted. From this graph the concentration at which there was a sharp change in the fluorescence intensity multiplied with the concentration of receptor **3** gave the detection limit.

Equation used for calculating detection limit (DL):

 $DL = CL \times CT$ CL = Conc. of Ligand; CT = Conc. of Titrant at which change observed.

Thus; DL = $0.011 \times 5 \times 10^{-6} = 55 \times 10^{-9} = 55$ nM = 1.43 ppb.



Fig. S10: Photographs of (A) SEM and (B) TEM images of compound **3** in $H_2O/EtOH$ (6:4, v/v) showing the irregular shaped nanoaggregates.



Fig. 11: Fluorescence spectra shows (A) CN⁻ ions in absence of **3** did not exhibit any response towards TNT TNT (10 equiv.); (B) The aggregates of **3** in absence of cyanide ions did not show any respond to TNT (10 equiv.).



Fig. S12: (A) Showing (a) Selectivity of **3-CN**⁻ ensemble towards TNT upon addition of different nitroderivatives and (b) Competitive selectivity of **3-CN**⁻ ensemble towards TNT (2 equiv.) in the presence of other nitroderivatives (10 equiv.) in H₂O/EtOH (6:4, v/v), λ_{ex} = 322 nm. (B) Shows the Stern-Volmer plot between quenching of fluorescence of **3-CN**⁻ with the concentration of TNT, inset showing the stern-Volmer plot at lower concentarion (1.2 equiv.).



Fig. S13a: (A) The cyclic voltammogram of **3** in DMSO (10^{-3} M) using ferrocene as internal standard shows the change in potential values with the addition of CN⁻ ions; (B) The change in potential values of **3** with the addition of CN⁻ ions and then TNT. $E_{HOMO} = [-4.8 \text{ eV} - (E)_{0x}(V)] \text{ eV}$, $E_{LUMO} = [-4.8 \text{ eV} - (E)_{red}(V)] \text{ eV}$].

Compound	E _{Red} (v)	LUMO(eV)	HOMO(eV)
Compound 3	-1.20	-3.60	-3.75
Compound 3 + CN ⁻	-1.1059	-3.6941	-3.90
Comp. 3 + CN ⁻ + TNT	-1.15	-3.65	-3.9077

Fig. S13b: HOMO and LUMO values of compound 3 in presence of CN⁻ and TNT.



Fig. S14: (A) The change in fluorescence of **3** (5 μ M) in H₂O/EtOH (6:4, v/v) on exposing to the vapors of solid TNT after 0 min, 1 min, 2 min, 3 min, 4 min, 5 min at λ_{ex} = 322 nm; Inset showing 27% fluorescence quenching within 5 minutes; (B) the fluorescence intensity of compound **3** at 450 nm as a function of CN⁻ ions concentration. DL = 9 × 10⁻⁹ × 5 × 10⁻⁶ = 45 × 10⁻¹⁵ M = 10.21 ppq; (1 × 10⁻⁹ equiv. = 1.5 μ I of 10⁻¹¹(M) solution of TNT).



Fig. S15: Spectral overlap of absorption spectrum of TNT and fluorescence spectrum of nanoaggregates of compound **3-CN**⁻ formed in H₂O/EtOH (6:4, v/v) mixture.



Fig. S16: Photographs (with naked eye) of compound **3-CN**⁻on test strips (A) before and (B) after dipping into aqueous solution of TNT (10^{-3} M); photographs (under 365 nm UV light) of **3-CN**⁻ on test strips (C) before and (D) non-fluorescence after dipping into aqueous solution of TNT (10^{-3} M).



Fig. S17: Paper strips of **3-CN**⁻ ensemble (A) Naked eye detection of TNT in aqueous medium in different concentrations of TNT (i) 10^{-3} M, (ii) 10^{-5} M, (iii) 10^{-7} M, (iv) 10^{-9} M, (v) 10^{-11} M, (vi) 10^{-13} M, (vii) 10^{-15} M; (B) The change in the fluorescence of **3-CN**⁻ ensemble in presence of TNT (under 365 nm UV light); The change in color (C) and fluorescence (D) of **3-CN**⁻ after contact with solid TNT; Thumb impression on **3-CN**⁻ paper strip (E) before and (F) after rubbing with TNT.





¹³C NMR (CDCl₃, 300 MHz, ppm) of compound 1b:



Mass spectrum of compound 1b:



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¹H NMR (DMSO-D₆, 300 MHz, ppm) of compound 3:



Expanded ¹H NMR Spectra of compound 3:



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¹³C NMR (CDCl₃, 300 MHz, ppm) of compound 3:



Mass Spectrum of compound 3:



Mass Spectrum of 3-CN⁻ complex:



Table 1: Comparison of **3** with previous reports of CN⁻ ions detector:

S.No.	Publication	Detection limit	Anion induced self- assembly	Practical applications of ensemble	Sensing ability of ensemble in blood serum	Sensing ability of ensemble in tap water, ground water	Interference with other anions
1	Progont	55 mM	Vac	Vag	Vac	Vec	No
1	Manuscript	55 IIVI	Tes	Tes	Tes	Tes	INU
2	ACS Appl. Mater. Interfaces., 2012, 4 , 4387–4392	800 nM	No	No	No	No	No
3	<i>Org. Lett.</i> , 2011, 13 , 14, 3730–3733	328 nm	No	No	No	No	No
4	Inorg. Chem., 2012, 51 , 7174–7184	5 μΜ	No	No	No	No	No
5	J. Mater. Chem., 2012, 22 , 5581	3.0 µM	No	No	No	Yes	No
6	Analyst, DOI:10.1039/C2A N35886G	2.3 µM	No	No	Yes	No	No
7	<i>Tetrahedron.</i> , 2011 ,67 , 5678- 5685	0.276 μM	No	No	No	No	No
8	<i>J. Mater. Chem.</i> , 2012, 22 , 1747-1750	0.14 µM	No	No	No	No	No
9	<i>Org. Lett.</i> , 2011, 13 , 5056–5059	2.0 µM	No	No	No	No	No
10	<i>Org. Biomol.</i> <i>Chem.</i> , 2012, 10 , 555-560	37.50 nM	No	No	No	No	Yes

Table 2: Comparison of nanoaggregates of 3-CN	r with previous reports of TNT detector:
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S.No.	Publication	Detection limit	Naked eye	$K_{SV}(M^{-1})$	Phase
			detection		
			(colorimetric		
			Change)		
1	Present Manuscript	10.21 ppq (23 ag/cm ²⁾	Yes	2.27×10^{5}	Solution and Vapor
2	<i>Chem. Commun.</i> , 2012, 48 , 9903–9905	33 ppt	Yes	-	Solution
3	<i>J. Mater. Chem.</i> , 2012, 22 , 4720–4727	2.84 ppb	Yes	1.52×10^4	Solution
4	<i>Chem. Commun.,</i> 2012, 48 , 4633–4635	136 ppb	No	1×10^5	Solution
5	<i>J. Mater. Chem.</i> , 2012, 22 , 2129–2132	-	No	-	Vapor, Solid
6	Analyst, 2012, 137 , 1771	0.227 ppb	Yes	-	Solution
7	<i>J. Mater. Chem.</i> , 2011, 21 , 9130–9138	1 ppb	Yes	1.97×10^4	Vapor, Solid and solution
8	J. Am. Chem. Soc., 2011, 133 , 8424–8427	5 ng/mm ²	Yes	-	Solution
9	Anal. Chem., 2011, 83 , 1401–1407	22.7 ppt	No	-	Solid and solution
10	Anal. Chem., 2011, 83 , 30–37	181.6 ppt	Yes	1× 10 ⁶	Solid and solution
11	Adv. Mater., 2010, 22 , 1900–1904	60 ppb	No	-	Vapor and solution
12	<i>J. Am. Chem. Soc.</i> 2009, 131 , 1390–1391	1 pg/mm ²	Yes	-	Vapor and solution
13	Angew. Chem. Int. Ed., 2008, 47 , 8601 – 8604	0.1135 ppt	Yes	-	solution
14	Biosensors & Bioelectronics, 2012, 3 , 1, 1000115	0.1 ppb	Yes	-	Vapor and solution