Supporting Information

Stilbazolium dye based chromogenic and Red-fluorescent probe for Highly selective detection of 2,4,6-trinitrophenol in water

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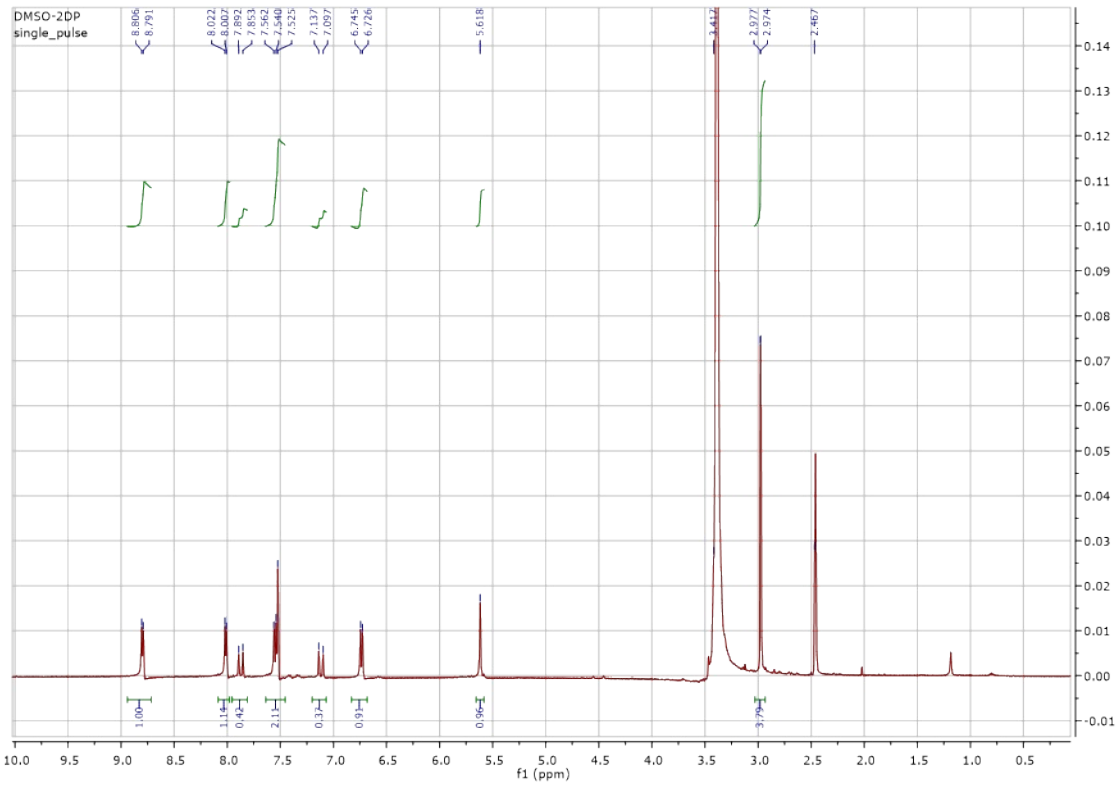

Figure: S1 ^{1}H NMR spectrum of **DMAS-DP**.

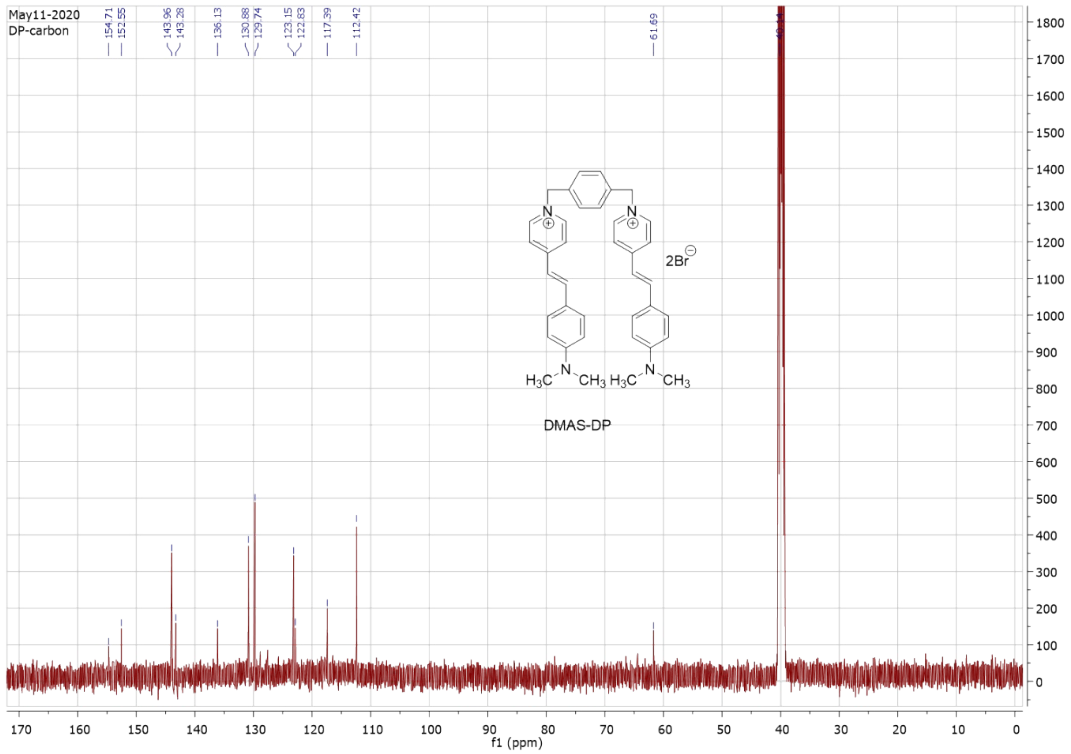

Figure: S2 ^{13}C NMR Spectrum of **DMAS-DP**.

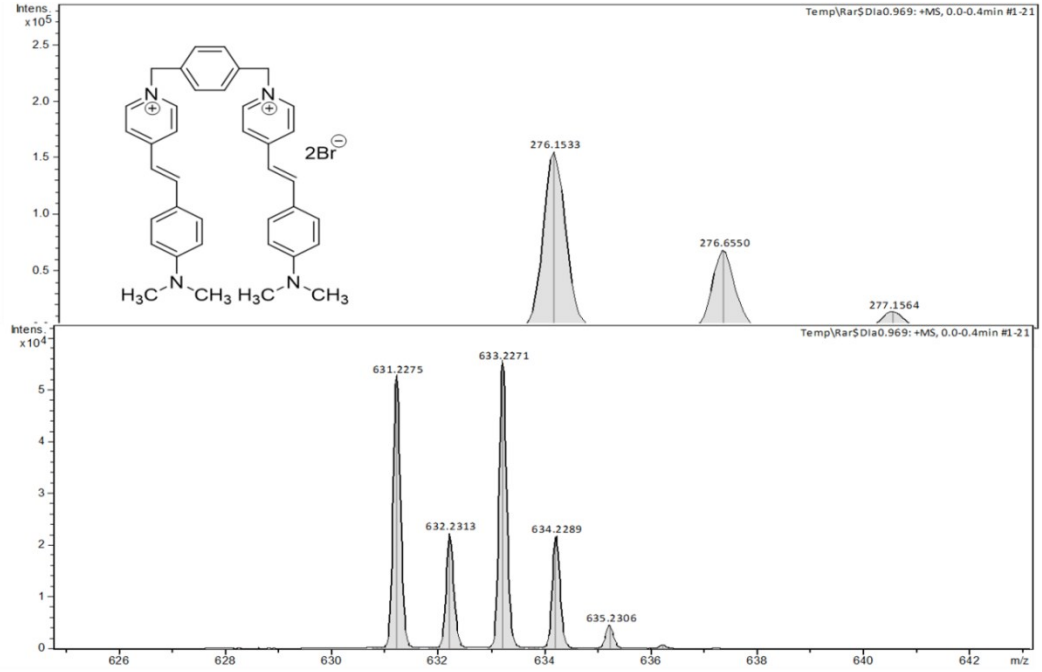

Figure: S3 HRMS spectrum of **DMAS-DP**.

2. **Experimental details**

2.1 **Materials and characterization**

Chemicals and solvents were purchased from Aldrich, spectrochem, and used without any purification. The organic solvents including DMSO, DMF, CH3CN, and THF were of HPLC grade. Compound DMAS (1) was prepared by reaction between 4-methyl pyridine and 4-(dimethylamino)-benzaldehyde as earlier reported in literature [S1]. The progress of reactions was monitored by TLC analysis, performed on glass coated silica gel GF254. 1H and 13C NMR spectra were recorded on JEOL 400 MHz FT NMR machine using DMSO-d6 as solvent and tetramethylsilane (TMS) as internal standard. The peak in Chemical shift in ppm relative to TMS as internal standard and coupling constant J in Hz, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplets). High resolution mass spectra were recorded on Bruker Micro Toff/ QII (Germany). Dynamics Light scattering (DLS) measurements were performed at 25.0 ± 0.1^{o}C by using light scattering apparatus (zeta sizer Nano ZS Malvern instrument LTD, UK). The deionized water was obtained from the ultra UV/UF Rions lab water system ultra 370 series which were used for preparing all solutions. UV-visible studies were performed on Shimadzu UV-2450 instrument machine by using slit width of 1.0 nm and matched quartz cell with 1cm path length, thermo stated at 25.0 ± 0.1^{o}C for all measurements. The fluorescence spectra were recorded by Horiba Fluorolog-3 and Perkin Elmer instrument spectrophotometer with quartz cuvette of 1cm path length. All fluorescence and absorption data scan were saved as ASCII files and further draw graph with help Microsoft Excel.

2.2 **UV-Visible and Fluorescence studies**: - The stock solution of fluorescent probes **DMAS-DP** (1 mM) was prepared in DMSO and was further diluted for preparing solutions of required concentrations. For preparing 10 μM solutions of these probes, 100 μL solution of stock solution was taken in the volumetric flask and was further diluted up to 10 ml mark with HEPES buffer (0.01M, pH 7.2) or by the desired solvent. Typically, aliquots of freshly prepared standard stock solution (0.1M) of nitro aromatic compounds were prepared in DMSO and were further diluted as required.

2.3 **DLS sample preparation**: - The stock solution of **DMAS-DP** (1 mM) was prepared in DMSO and was filtered through 0.02 μM filter to remove the suspended particles. For preparing solutions for DLS experiments, 100 μl stock solution of the probe was diluted with

HEPES buffer (0.01M, pH 7.2). The solutions of fluorescent probe **DMAS-DP** (10 μM) were treated with appropriate amounts of 2,4,6-trinitrophenol (TNP) and were allowed to stand for 4 h, before determining their spectra. For DLS recording, 1 ml of each solution of these solutions was taken in glass cuvette at 25 ^{o}C and was allowed to stand for 3 min before recording.

2.4 **Detection limit [S2]:** - The detection limit for TNP and TNT was calculated on the basis of both UV-Vis and fluorescence titration studies. To determine the S/N ratio, the UV-Vis and emission spectra of **DMAS-DP** were recorded thrice to determine the standard deviation. The LOD was calculated by using equation

$LOD = 3\sigma/m$

Where σ = standard deviation of blank measurements, m is slope of straight line of plot of signal intensity versus concentration of sample.

2.5 **Quantum yield calculations [S3]**: - Fluorescence quantum yields (Φs) were calculated by using optical matched solution of rhodamine as standard and excitation wavelength at 490 nm. The quantum yield of rhodamine in ethanol under standard conditions was 0.3 and quantum yield is calculated by using this equation:-

$$\Phi_{fs} = \Phi_{fr} \times \frac{1\text{-}10^{-ArLr}}{1\text{-}10^{-AsLs}} \times \frac{N_S{}^2}{N_r{}^2} \times \frac{D_S}{D_r}$$

Φfs and Φfr are the quantum yields of sample and reference respectively, As and Ar are the absorbance of the sample and the reference respectively, Ds and Dr the respective areas of emission for sample and reference. Ls and Lr are the lengths of the absorption cells of sample and reference, respectively. Ns and Nr are the refractive indices of the sample and reference solutions.

2.6 **Binding constants and stoichiometries** [S4]: - In order to rationalize the different species being formed during titration of **DMAS-DP** with TNP or TNT, the spectral data of the titration experiments was evaluated using software SPECFIT-32. The programme performs global analysis of equilibrium and kinetic systems with singular value of decomposition and nonlinear-regression modelling by Levenberg-Marquardt method. The programme simulates the absorption or fluorescence data obtained experimentally. The

stoichiometry of the species formed, distribution of species and their association constants are determined through fit model.

2.7 **DFT calculations** [S5]: - All the calculations were performed using Gaussian 16 program package. The ground state geometry optimizations of all the compounds and their formed complexes were performed using density functional theory (DFT) with wb97xd/6-31G* level basis sets for structural optimization and then further extended to B3LYP/6-31++G** for energy calculations. The optimized structures were confirmed by absence of imaginary frequency. The UV-Vis spectra of probe **DMAS-DP** and its complex with TNP have been evaluated by TD-DFT calculations.

2.8 **Time-Resolved PL Studies**. Fluorescence lifetime studies of **DMAS-DP** (10 μM) were performed without and with TNP and TNT as analytes. The TNP and TNT concentrations of 10 -100 μM (1 to 10 equivalents) were added to the solutions of **DMAS-DP** in HEPES buffer. Pulse excitation of 444 nm and emission between 520-700 nm was measured. The time resolved photoluminescence curves were fitted with mono and biexponential species and the average lifetime was calculated.

References

[S1] R.C. Laird, N.P. Nguyen, S. F. Rusch, J. Baltrusaitis, L. R. MacGillivray, Non-centrosymmetric Packings Influenced by Electronic Properties of Products of Click Reactions, Cryst. Growth Des. 2014, 14, 893−896

[S2] J. Mokac, A.M. Bond, S. Mitchell, G. Scollary, A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: application to voltammetric and stripping techniques (TechnicalReport), Pure Appl. Chem. 69 (1997) 297–328.

[S3] J.N. Demas, G.A. Crosby, Measurement of photoluminescence quantum yields. Review, J. Phys. Chem. 75 (1971) 991–1024.

[S4] N. Kaur, G. Jindal, Sukhvinder, S. Kumar, Cascade recognition of Hg^{2+} and cysteine using a naphthalene based ESIPT sensor and its application in a set/reset memorized device, New J. Chem. 43 (2019) 436-443

[S5] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, Gaussian, Inc., Wallingford CT, 2016 , Gaussian 16, Revision B.01,

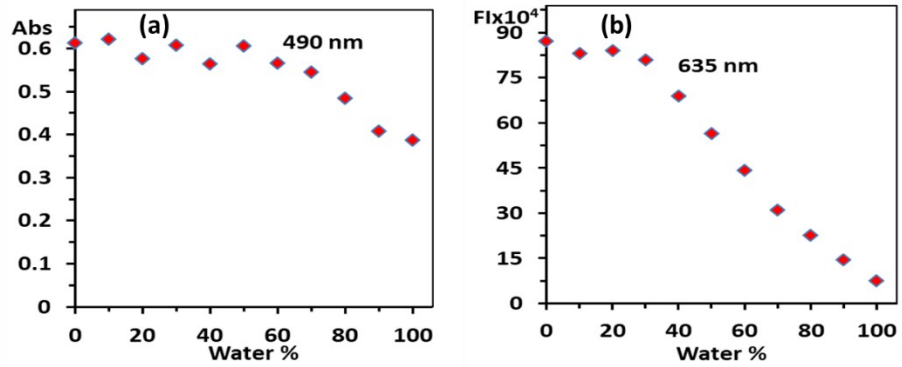

Figure S4: Effect of amount of buffer on the (a) UV-VIS and (b) fluorescence intensity of DMAS-DP (10 μM) with increasing HEPES fraction.

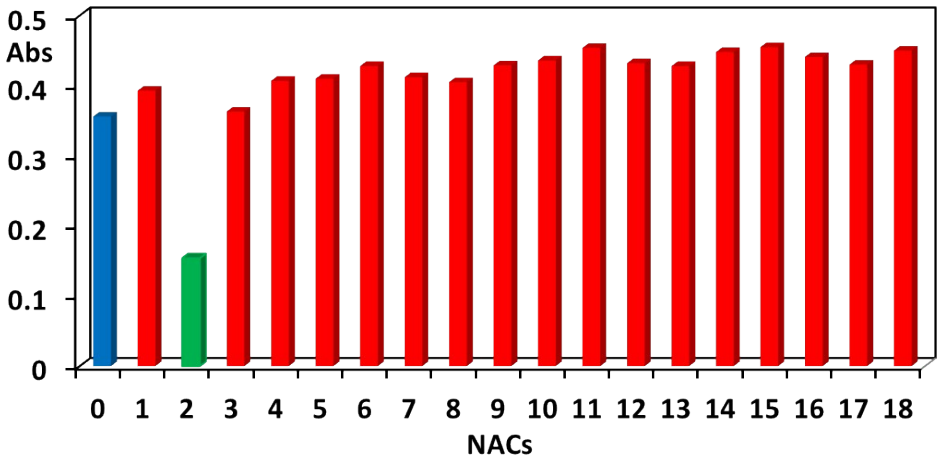

Figure S5: change in absorbance at 475 nm and spectrum of DMAS-DP on addition of different NACs. 0 = DMAS-DP; 1 = trinitrotoluene; 2 = 2,4,6-trinitrophenol; 3 = aniline; 4 = 1-chloro-3-nitroaniline; 5 = 2-nitroaniline; 6 = 2,4-dinitroaniline; 7 = 4-nitrophenol; 8 = 1-chloro-4-nitrophenol; 9

=1-chloro-2,4-dinitrobenzene; 10 = 1-chloro-2-nitrobenzene; 11 = 2-nitrophenol; 12 = 4-nitrophenol; 13 = 1-methyl-2-nitrobenzene; 14=2,4-dinitotoluene; 15=4-nitrobenzaldehyde; 16 = 2-nitrobenzaldehyde; 17 = 3,5-dintrobenzoic acid; 18 = 2,6-dinitrotoluene.

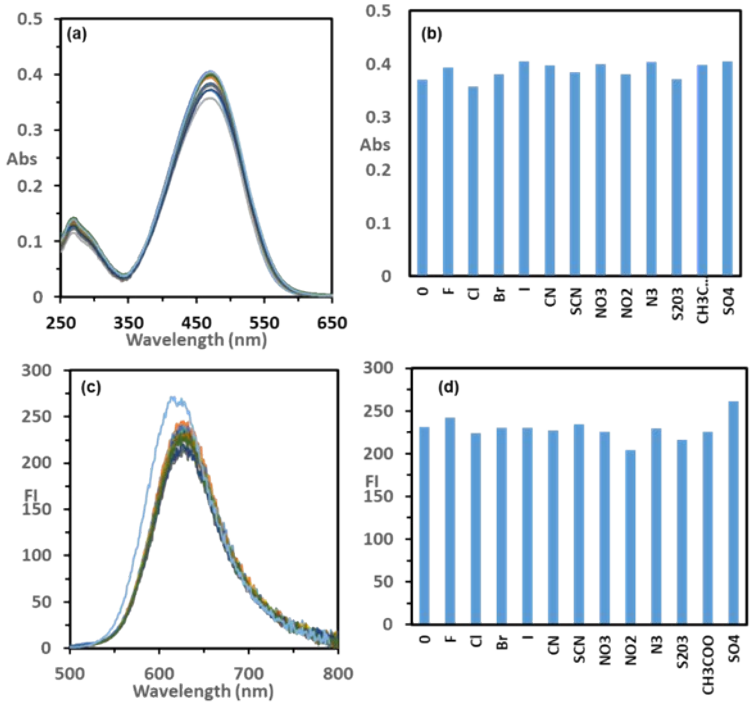

Figure S6: (a) The change in absorption spectrum and (b) absorption intensity of DMAS- DP on addition of various anions; (c) the change in fluorescence spectrum and (d) fluorescence intensity of DMAS-DP in presence of various anions in HEPES buffer

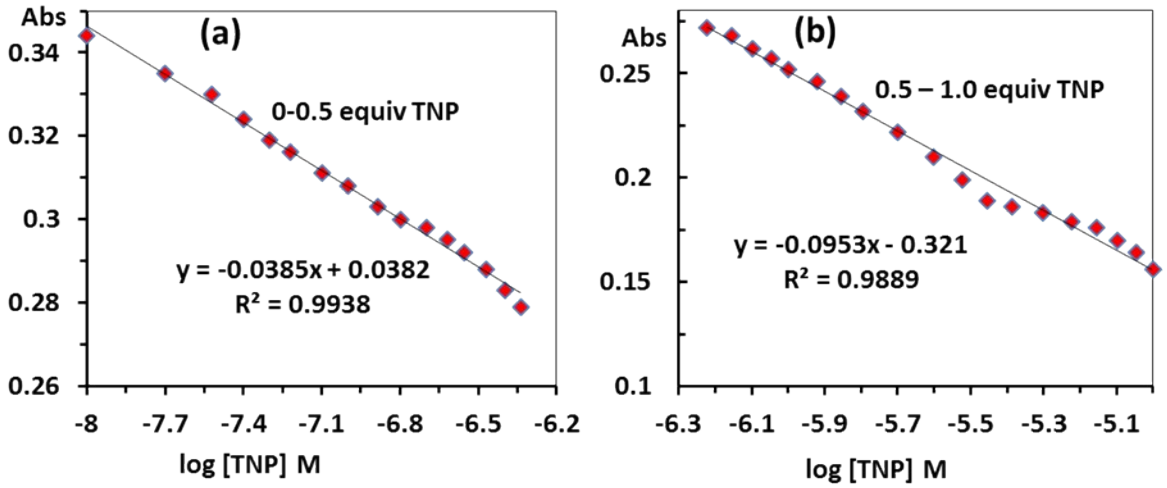

Figure S7: Plots of absorbance of DMAS-DP against log [TNP] in HEPES buffer (0.1 % DMSO) showing linear change in absorbance between (a) 0 to 0.5 equivalents of TNP; (b) 0.5 to 2 equivalents of TNP

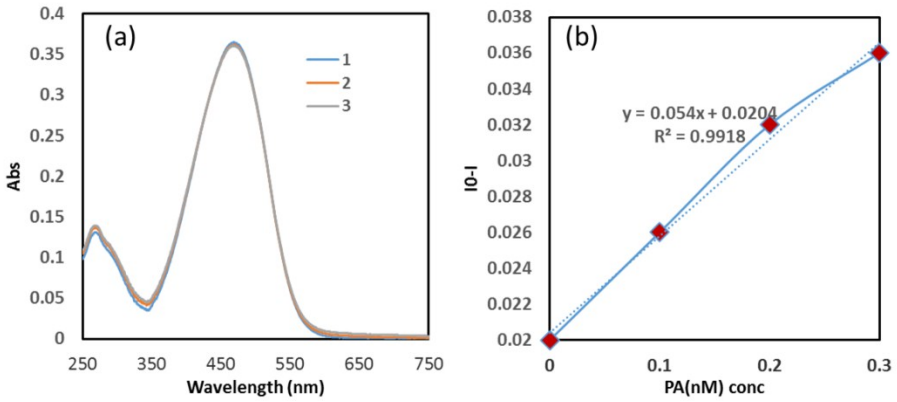

Figure S8: (a) Uv-Vis spectrum of DMAS-DP determined thrice. (b) Titration of DMAS-DP with TNP

Calculation of Standard Deviation and detection limit

Detection limit (DL) – 3σ/m

σ = standard deviation, m = slope

σ - 0.0017, slope - 0.054

DL - 3* 0.0017/0.054

= 0.1 nM

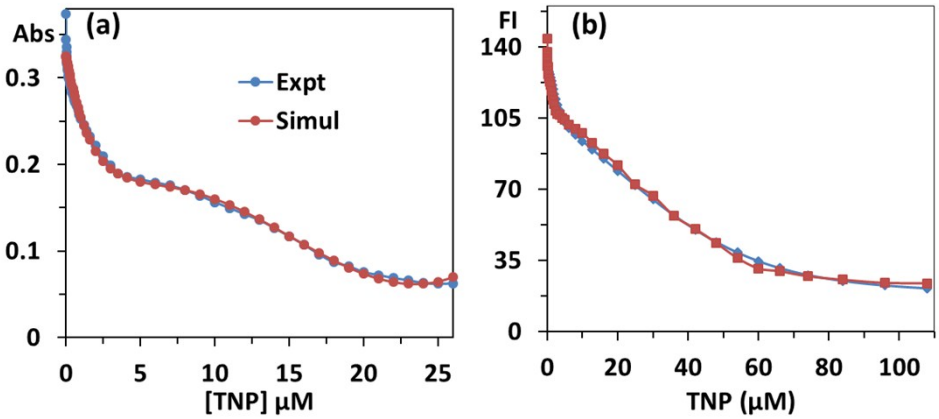

Figure S9: (a) Plot of absorbance and fluorescence (b) of DMAS-DP against [TNP] and its fit model

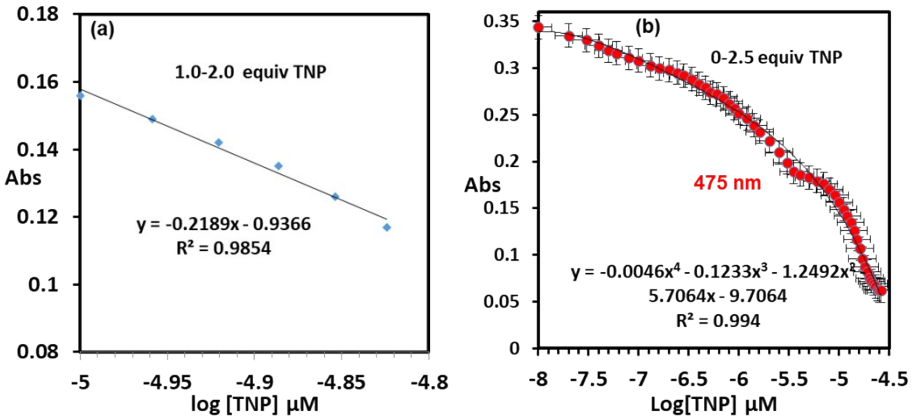

Figure S10: The plots absorbance of **DMAS-DP** against log [TNP] in HEPES buffer (0.1 % DMSO) in absorbance of (a) linear change with 1.0 to 2.0 equivalents of TNP; (b) non-linear change in absorbance with 0 to 2.5 equivalents of TNP

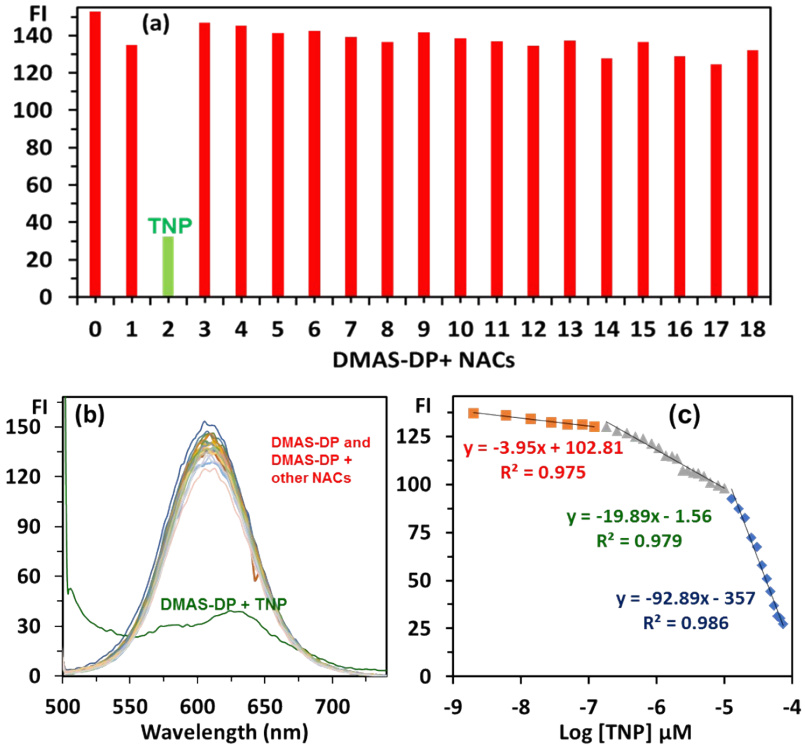

Figure S11: (a) The fluorescence intensity of **DMAS-DP** in the presence of different NACs. (b) The fluorescence spectra of **DMAS-DP** in the presence of different NACs; 0 = **DMAS-DP**; 1 = trinitrotoluene; 2 = 2,4,6-trinitrophenol; 3 = aniline; 4 = 1-chloro-3-nitroaniline; 5=2-nitroaniline; 6 = 2,4-dinitroaniline; 7 = 4-nitrophenol; 8 = 1-chloro-4-nitrophenol; 9 = 1-chloro-2,4-dinitrobenzene; 10 = 1-chloro-2-nitrobenzene; 11 = 2-nitrophenol; 12 =4-nitrophenol; 13 = 1-methyl-2-nitrobenzene; 14=2,4-dinitotoluene; 15 = 4-nitrobenzaldehyde; 16 = 2-nitrobenzaldehyde; 17 = 3,5-dintrobenzoic acid; 18 = 2,6-dinitrotoluene (c) the plot of fluorescence intensity against log[TNP]

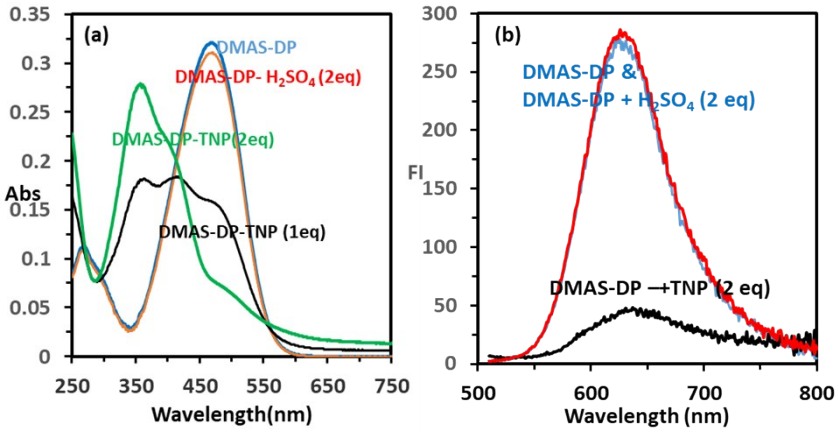

Figure S12: (a) The comparison of change in the absorption spectrum with addition of H_2SO_4 (2 equiv.), TNP (1 equiv.) and TNP (2 equiv.); (b) The comparison of change in the fluorescence spectrum of **DMAS-DP** with addition of H_2SO_4 (2 equiv.), TNP (2 equiv.).

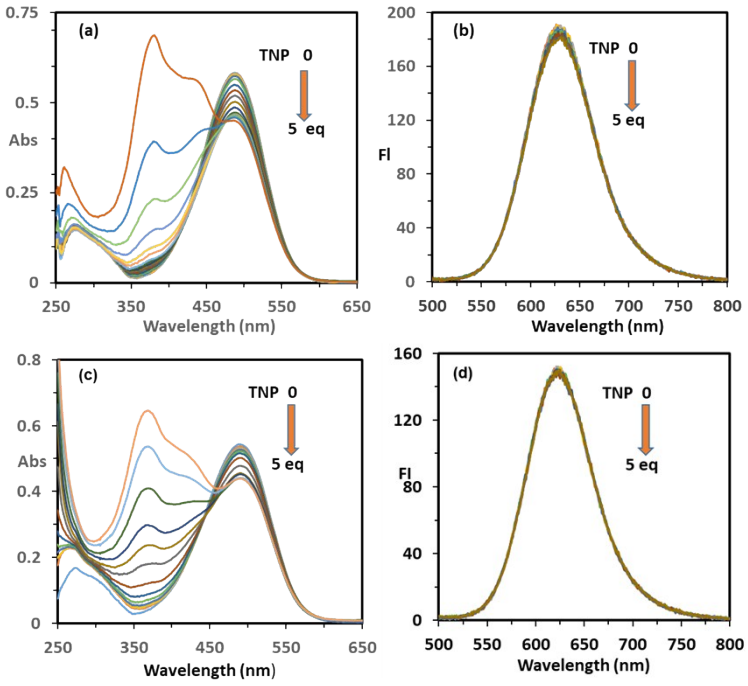

Figure S13: (a and b) The effect of TNP on the absorption and fluorescence spectra of **DMAS-DP** in DMSO; (c and d) the effect of TNP on the absorption and fluorescence spectra of **DMAS-DP** in DMSO-HEPES (1:1).

Table S1: The comparison of present work with literature reports on 2,4,6-trinitrophenol detection in terms of solvent, limit of detection using fluorescence and absorbance channels

Publication	Fluorophore	solvent	λ_{em} (nm)	LOD FL	λ_{abs} (nm)	LOD Abs
Present	Single molecule	Water	615	3 nM	490	0.1 nM
Anal. Chim. Acta. 2017, 965, 111-122	Single molecule	CH_3CN:H_2O (4:1)	410	500 nM	330	No
Anal.Chim.Acta. 2017, 976 ,74-83	Single molecule	H_2O-THF (9:1)	425	2.5 nM	335	No
ACS Appl. Mater. Interfaces 2017, 9, 13415−13421	Nanodots	ethanol	500	250 nM	375	No
Sensors and Actuators B 2017, 242, 535–544	Single molecule	MeOH-H_2O (8:2)	490	100 μM	400	No
Sensors and Actuators B 2017, 245, 665–673	Single molecule	acetonitrile	400	1 μM	345	No
ACS Omega 2018, 3, 3248−3256	Single molecule	water	460	2.6 μM	330	No
Chem. Asian J. 2017, 12, 830 – 834	Single molecule	THF/Water (80: 20)	500	----	400	No
Dyes and Pigments (2017), 143, 463-469	Single molecule	water	525	370 nM	395	No
J. Org. Chem. 2017, 82, 13359−13367	Single molecule	water	425	210 nM	295	yes
Talanta 2017 174 462–467	Single molecule	CH_3CN	420	2.42 μM	335	No
J. Mater. Chem. C, 2017, 5, 4788--4796	Single molecule	DCM	460	21.5 μM	360	No
ACS Sustainable Chem. Eng. 2019, 7, 819−830	MOF	water	437, 673	40 nM	--	No
Anal. Chem. 2019, 91, 13244−13250	Single molecule	DMSO	530	63 nM	---	No
RSC Adv., 2019, 9, 26043–26050	Single molecule	THF-water 2:8	549	1.26 μM	---	No

Table S2. The electronic excitation wavelength (nm), oscillator strengths and compositions of the low-lying singlet excited states of DP compound

S.No.	Transition	Symmetry	λ (nm)	f	Orbitals Contribution. (%)
			DMAS-DP		
1	S_0-S_1	Singlet-A	466	3.0086	H-1→L+1 (43 %), H→L (45 %)
2	S_0-S_2	Singlet-A	448	0.0121	H-1→L (45 %), H→L+1 (44 %)
			DMAS-DP(TNP)$_2$		
1	S_0-S_1	Singlet-A	387.4	0.0382	H-1→L+1 (22 %), H-1→L+2 (22 %), H→L (25 %), H→L+1 (25 %)
2	S_0-S_2	Singlet-A	387.3	0.000	H-1→L (26 %), H-1→L+1 (25 %), H→L (25%), H→L+1 (25 %)
3	S_0-S_3	Singlet-A	369.0	2.8388	H-9→L+1 (45 %), H-8→L (45 %)

Table S3: Recovery of TNP from soil samples

Sr. No	Conc actual (nM)	Absorbance with PA	Absorbance with Soil sample	Amount recovered	Recovery %
1	5	0.526	0.529	5.028	100.57
2	10	0.520	0.519	9.980	99.80
3	20	0.514	0.514	20.00	100
4	30	0.505	0.509	30.237	100.79
5	40	0.5	0.49	39.200	98
6	50	0.491	0.476	48.472	96.95