Supramolecular Sensing of 2,4,6-Trinitrophenol by a Tetrapyrenyl Conjugate of Calix[4]arene: Applicability in Solution, in Solid State, and on the Strips of Cellulose and Silica Gel and the Image Processing by a Cellular Phone

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Supporting Information

ABSTRACT: A calix[4]arene conjugate possessing a tetrapyrenyl moiety at its upper rim (R) is designed as a receptor for sensing trinitrophenol (TNP). To understand the role of the calix[4]arene platform and that of pyrenyl moieties in R, two other control molecules were synthesized. These are as follows: the one possessing a tetraphenyl moiety in place of tetrapyrenyl (R₁) and the other one is a p-pyrenyl-hydroxy benzene (R₂) that is devoid of the calix[4]arene platform. The R shows high sensitivity toward TNP in tetrahydrofuran (THF) over eleven other nitroaromatic compounds (NACs) studied by exhibiting large fluorescence enhancement and hence is selective to TNP over the other NACs studied. However, the control molecules R₁ and R₂ showed only marginal fluorescence enhancement, supporting the need of a calixarene platform and the presence of a tetrapyrenyl moiety in the receptor system for the selective sensing of TNP.

Further, R₁ and R₂ are not suitable for sensing, since these exhibit similar fluorescence response over several NACs studied. The binding of TNP by R has been addressed by fluorescence titration and isothermal titration calorimetry. The nature of the complexation of TNP by R has been revealed by the computational calculations, wherein the data showed the entrapment of TNP by two adjacent pyrene moieties via π–π stacking interactions. Such host–guest complexation is expected to restrict the mobility of the pyrene moieties present in R. The reduction of the flexibility of the pyrenyl moieties of R upon TNP binding is evidenced by the 1H NMR spectral study, wherein this acts as an additional evidence for the complexation. In the present study, the sensing of TNP by R has been shown in THF solution, on the surface of silica gel and the cellulose paper to result in lowest detection limits (LODs) of 1.5, 3.5, and 6.5 μM, respectively. Even the solid mixture of R and TNP showed LOD of 2.1 μmol.

Since R is expected to show supramolecular aggregation that is dependent on the guest species, the corresponding details were probed by microscopy techniques, using scanning electron microscopy, atomic force microscopy, and transmission electron microscopy methods, and significant changes in the aggregation of R upon interaction with TNP were found. Such aggregation is responsible for the observed fluorescence enhancement. Thus, the tetrapyrenyl calix[4]arene conjugate (R) acts as a sensitive and robust platform for selective detection of TNP from a mixture of nitroaromatic compounds (NACs) wherein the fluorescence intensities can be imaged and managed by a cellular phone.

INTRODUCTION

In the present social scenario, the safety and security have become major issues due to the unlawful usage of explosive substances. Among these, the nitroaromatic compounds (NACs), such as trinitrotoluene (TNT) and trinitrophenol (TNP) or the commonly known picric acid (PA), are of great relevance. TNP is commonly used in the industries based on pharmaceuticals and dyes, in addition to its use in the manufacture of rocket fuel. Thus, the TNP is not just an explosive precursor, but an environmentally hazardous compound that leads to the health problems, such as the respiratory damage and skin irritation. Thus, sensitive and selective detection of TNP is a contemporary topic for scientists to focus on. Many fluorophores reported in the literature for the detection of TNP are based on fluorescence turn-off. Such sensors are associated with disadvantages since the fluorescence quenching is challenged by the fluctuations of background fluorescence, flexibility in the excited state of the species, and strong interaction between the excited state and the lattice. Hence, the sensors that function on turn-on fluorescence are of greater utility, while these are scarce in the literature. Although there are reports for the selective recognition of TNP, supramolecular systems such as calixarene-based ones are limited and all of these receptors detect TNP by fluorescence quenching. Calixarenes can be easily functionalized on its upper or lower
rim to meet the requisite needed in providing complementary interacting sites with the guest molecules.

Design and Strategy. Therefore, for the design of such a receptor molecule, the following aspects have been used as inputs. Since the TNP has an aromatic moiety, it would be amenable to complex through π···π interactions. Hence, the pyrenyl derivatization is preferred to provide a fluorescence signal and also to form a cup-shaped structure on the upper rim of the calix[4]arene platform. The presence of the phenolic-OH groups at the lower rim would ensure the cone conformation through circular hydrogen bonding. All of these were taken into consideration in building the receptor molecule R (Figure 1), and the synthesized R was well characterized. The ability of R for sensitive recognition of TNP has been addressed by carrying out extensive spectroscopy and microscopy studies, and its selectivity was further addressed by comparing the data obtained for two other related control molecular systems (R₁: tetraphenyl calix[4]arene; R₂: p-pyrenyl hydroxy benzene) and also by comparing the data related to eleven different NAC guest molecular species. In addition, the sensing of TNP by R was demonstrated in solution, in solid mixture, and on the surface of silica gel and cellulose paper.

■ RESULTS AND DISCUSSION

Development of the Receptor (R) and the Control Molecules (R₁ and R₂). The synthesis of the designed receptor molecule (R) having upper-rim functionalization on calix[4]arene has been given in Scheme 1. R has been synthesized via three steps starting from p-tert-butylcalix[4]-arene (P₁) and its dealkylated form (P₂) followed by the upper-rim tetra-formyl functionalization (P₃) and its reaction with 1-amino pyrene. All of these molecules were characterized by spectroscopy techniques, such as ¹H and ¹³C NMR and electrospray ionization mass spectrometry (ESI-MS). The receptor R can be specifically and selectively used as a chemosensor for the detection of TNP among the various NACs studied. The results obtained based on R were compared with the corresponding control molecules, viz., R₁, wherein a phenyl moiety is present in place of each of the pyrene unit in R, and R₂ in which no supramolecular calix platform is present but a single unit of pyrene. The circular H-bonding present at the lower rim is expected to maintain the cone conformation. In the ¹H NMR spectrum, two doublets for the rim CH₂ protons, one at 4.52 ppm and the other at 3.85 ppm, characteristic for the cone conformation, were observed.

Interaction and Binding of R with TNP by Absorption and Fluorescence Titrations. The absorption spectrum of R exhibit bands at 235, 284, and 385 nm. Upon incremental addition of TNP to R, the absorbance at 235 and 385 nm increases by ∼12 and ∼18%, respectively, while no change is observed in the absorption band at 284 nm (Figure 2a). The plot of absorbance vs mole ratio at 385 nm (inset in Figure 2a) shows sigmoidal behavior, indicating the complex formation between R and TNP. The significant change observed in the

Scheme 1. Synthesis of the Receptor (R) and Control (R₁, R₂) Molecules

(a) Anhydrous AlCl₃, phenol, toluene, stirring at room temperature (RT) for 24 h; (b) hexamethylenetetramine, trifluoroacetic acid, reflux 24 h and then dil. HCl, stirring at RT for 6 h; (c) 1-amino pyrene, benzoic acid (catalytic amount), toluene, heated at 110 °C for 5 days; (d) aniline, benzoic acid (catalytic amount), toluene, heated at 110 °C for 3 days; (e) 1-amino pyrene, benzoic acid (catalytic amount), toluene, heated at 110 °C for 24 h.
in ESI-MS, the complex formation is also supported by ESI-MS spectra. The formation of 1:1 complex between corresponding to \([M + H]^+\) (Figure S12c), and when TNP is being present at 450 and 485 nm, which are characteristic of the pyrenyl moieties (Figure 3a). Upon incremental addition of TNP to \(R_1\), the emission intensity gradually increases up to >7-fold and the plot of relative emission intensity against the mole ratio shows sigmoidal behavior, supporting the complex formation between \(R\) and TNP (inset of Figure 3a). However, the addition of other eleven NACs to \(R\) did not show appreciable changes as can be observed from the histogram given in Figure 3d and Supporting Information Section S3. The data thus suggests that \(R\) can selectively sense TNP among all of the twelve NACs studied. This can also be visualized from the photograph of the vials containing \(\{R + NAC\}\) in which fluorescence enhancement is observed only in the case of TNP under UV light (Figure S4). The control molecule \(R_2\) shows an emission band at 335 nm. With the addition of the increasing concentration of TNP (0−6 equiv), the emission intensity gradually increases only up to ∼2.5-fold (Figure 3b) and the plot of relative emission intensity vs mole ratio shows a sigmoidal curve (inset of Figure 3b). However, the addition of eleven other NACs showed only marginal enhancement (Figure 3e) and therefore \(R_2\), though forms a complex with TNP, is unable to differentiate TNP among other NACs and hence is not an ideal sensor for TNP. The calix[4]arene platform is essential for selective sensing of TNP. Also, the calix[4]arene platform is important for the selective sensing of TNP, which can be clearly observed by comparing the emission spectra of \(R\) and \(R_2\). Hence, the presence of the four pyrenyl moieties and a calix[4]arene platform is essential for selective sensing of TNP.
Indeed, the R has all of these qualities over the control molecules, viz., R1 and R2; hence, the R is selective to TNP. The association constant (K') and limit of detection (LOD) obtained from fluorescence spectral titration data of R with TNP are (3.66 ± 0.07) × 10^4 M^-1 and (1.54 ± 0.05) μM or (0.35 ± 0.01) ppm, respectively (Figures S5 and S6). The quantum yields of R and [R + TNP] are 0.004 and 0.027, respectively, with respect to the quinine sulfate used as standard (Figure S7). The competitive fluorescence titration studies show that the presence of other eleven NACs does not interfere with the selectivity of R to sense TNP and that the fluorescence enhancement is ~7–9-fold (Figure 4a) when TNP is added to R. The complexation of TNP to R has also been proven based on the isothermal titration calorimetry (ITC) data. The ITC data shows the best fit for one-site binding (Figure 4b). The overall heat of the reaction was exothermic, and TNP binds with an association constant (K') of (1.16 ± 0.02) × 10^3, suggesting a strong binding of TNP by R. Since the ΔS value is small and negative and the ΔH is large, the ΔG for complexation becomes negative, showing its feasibility.

Fluxional Behavior of R by 1H NMR Spectroscopy. The broad features observed in the room temperature (RT) 1H NMR spectrum suggest fluxional behavior for the receptor molecule R, which has been ascertained by measuring 1H NMR spectra from RT down to −40 °C in CDCl3. 1H NMR spectral traces obtained in this temperature range are overlaid in Figure 5 for comparison. At room temperature, the R shows broad peaks corresponding to the pyrene protons as well as the phenyl ring protons of the calix core. Upon lowering the temperature of the sample, all of these broad peaks were sharpened and split, suggesting the presence of more than one conformation. This can be easily understood from the spectra obtained in the temperature range of −20 to −40 °C. The presence of more than one conformer is also understood from the bridged –CH2 proton pattern observed by lowering the temperature.

TNP Binding by R Using 1H NMR Titration. 1H NMR titrations were performed to support the binding of TNP to R. During the titration, the concentration of R was kept constant and the [TNP]/[R] mole ratio was increased. Upon gradual addition of TNP to R, the broad peaks observed for the pyrene protons in the range 7.6–8.8 ppm starts sharpening and splitting, thereby suggesting that TNP interacts with the pyrene moieties, and as a result, the flexibility of the arms at the upper rim is hindered (Figure 6). Even the peaks corresponding to the phenyl moiety (~7.4 ppm) of the calix platform are sharpened, supporting the fact that the flexibility of the calix rim is dampened. The spectral sharpening observed for {R + TNP} is reminiscent of the low temperature spectrum obtained for R. The sharpening of the peaks upon addition of TNP further supports that the flexibility of the pyrene arms is reduced upon addition of TNP due to the binding of TNP through π-π interactions, and the presence of such interactions was delineated by the density functional theory (DFT) computational studies as given in this paper. When a similar titration was carried out between R1 and TNP (Figure 7), the 1H NMR spectra showed no significant sharpening even at one equivalent addition of TNP, supporting the fact that there is no considerable interaction present between the phenyl moieties in R1 and TNP, unlike that observed between R and TNP.

Sensing of TNP by R in the Solid State. The sensing of TNP by R in the solid state was studied by fluorescence microscopy. Based on the solution studies, R was shown to selectively sense TNP among other NACs. To understand the
The components together without using any solvent. R alone shows feeble blue fluorescence emission. Upon addition of increasing equivalents of TNP, the intensity of blue fluorescence gradually increases, as can be seen from Figure 8a–f, and at the highest equivalents, it shows ∼6-fold enhancement (Figure 8k). The limit of detection of TNP in the solid state by R is 2.1 micromole (Figure S8). Similar experiments carried out with the control molecules, viz., R2 and R3, showed no significant change in the fluorescence intensity even after mixing 5 equiv of TNP (Figure 8g–j). Thus, TNP can be selectively sensed by R even in the solid state just by grinding both the components together without using any solvent.

Sensing of TNP by R on Silica Gel and on Celulose Paper Strips. The fluorescence intensity of the receptor (R) increases in the presence of TNP as shown in solution and in the solid powder. To demonstrate its sensitivity on a silica gel strip, the strips were drop-casted with R followed by adding the THF solution of TNP (0–6 equiv) and the fluorescence spectra were measured. With increasing concentration of TNP, the intensity of the emission band at 425 nm gradually increases, as can be seen from Figure 9a. The plot of I/I0 versus mole ratio shows a maximum 8-fold fluorescence enhancement (Figure 9c), which is marginally greater than that observed even in the solution phase owing to the orientation of R on the silica surface as shown in Figure 9e. Similar experiments were carried out using cellulose paper strips. In the case of the cellulose paper, the emission maximum of R is red-shifted by 10 nm as compared to the same on the silica gel strip owing to the variation in the nature of the surface interactions present in these two cases; however, the enhancement in the fluorescence intensity is the same (Figure 9b,d). The lowest detection limits of TNP are 3.5 and 6.5 μM, respectively, in the case of silica gel and cellulose paper (Figure S8).

Sensing of TNP in Different Sources of Water. To demonstrate a real sample application of the probe molecules R to sense TNP, water samples were collected from different sources, such as ground, rain, sea, Milli-Q, and distilled water, and were spiked with TNP (50 μM). The fluorescence spectra were recorded for all of these water samples spiked with TNP. R shows enhancement in fluorescence by ∼3.5–4-fold in all of the cases (Figures 10a and S9), showing that it is possible to extend the application potential of R to real water samples.

Influence of pH on TNP Sensing. The influence of pH on sensing TNP by R has been demonstrated in PBS buffer at pH values ranging from 3 to 12. Across this pH range, the fluorescence enhancement is almost comparable and lies in the range of ∼3–4.5-fold (Figure 10b), supporting the fact that the R can be used in a wide range of conditions for sensing TNP.

TNP-induced Changes in the Supramolecular Features by Microscopy. To study the changes induced in the microscopy features of R upon interaction with TNP, scanning electron microscopy (SEM) study was performed. R exhibits

Figure 7. 1H NMR spectra obtained during the titration of R1 with 0–1 equiv (i–vi) of TNP in DMSO-d6. TNP shows only one peak at 8.32 ppm.

Figure 8. Fluorescence microscopy images are shown for the “bright field”, under the ‘blue filter’, and overlap of these two as “merged”. The images for R are given under (a). Those for (R + xTNP) were given under (b) x = 0.5, (c) x = 1, (d) x = 3, (e) x = 4, and (f) x = 5. The images for R2 and {R2 + 5TNP} are given under (g) and (h), respectively. Similarly, the images for R3 and {R3 + 5TNP} are given under (i) and (j), respectively. (k) Histogram of relative fluorescence intensity (I/I0) vs mole ratio [TNP]/[R]. (l) Red bars in the histogram correspond to the relative fluorescence intensity (I/I0) for (i) R1, (ii) R2, and (iii) R3 upon grinding with 5 equiv of TNP. The black bars correspond to the controls measured without TNP.
Herein, microscopy data reveal that the supramolecular features of elongated fiberlike features, which form bundles upon addition of TNP (Figure 11b,e). All of these 17 structures showed CEs in the range −40 to −46 kcal/mol, where the highest complexation energy was observed with the structure obtained from the frame of 3984. The final structure obtained from this frame is shown in Figure 12 and was further analyzed for the interactions present between R and TNP. Figure 12 clearly supports that TNP is sandwiched between two consecutive pyrene moieties labeled as “B” and “C”. The corresponding centroid-to-centroid distances between TNP and the pyrene moieties (B and C) are 3.300 and 3.317 Å, respectively, supporting the presence of strong π···π interactions between the pyrenes and the TNP. In addition, “O” of one of the ortho-nitro groups interacts with the π face of one of the other two pyrene rings with a O···π distance of 3.029 Å. Even after the complexation of R by TNP, all of the four lower-rim phenolic-OH···O hydrogen bonds (Table 1) were retained, supporting the cone conformation for the calix[4]arene platform.

**CONCLUSIONS AND COMPARISONS**

Sensing of TNP by R in Solution, in the Solid State, and on the Surface of Silica Gel and Cellulose Paper. An upper-rim-derivatized calix[4] arene with a tetrapyrene moiety (R) has been synthesized in three steps and demonstrated for its selective detection of trinitrophenol (TNP). The control molecules R₁ and R₂ have also been synthesized to prove that the design of the receptor molecule R possessing tetrapyrenyl groups at the upper rim and the calix[4]arene platform in the cone conformation is essential for sensing TNP. Upon interaction with TNP, the fluorescence emission is enhanced by >7-fold in the case of R while it is much lower in the case of R₁ and R₂ supporting the fact that the presence of the calix[4]arene platform and the pyrenyl moieties at the upper rim is essential for sensitive detection. The TNP can be distinguished from other NACs only in the case of R and not in the case of R₁ and R₂ by fluorescence emission study. This provides clear-cut differentiation between the role of the receptor molecule versus that of the control ones. The binding of TNP is proven by ITC and the rigidity of R upon TNP binding by 1H NMR. The details of the complexation between spherical particle-like morphology of diameter (272 ± 42) nm (Figure 11a). The addition of TNP leads to the aggregation of these particles to give long chainlike aggregates as evident from the SEM micrographs shown in Figure 11d. The size of the particles present in these chains is reduced by one-third to give (98 ± 22) nm. The control molecule R₁ shows twisted elongated fiberlike features, which form bundles upon addition of TNP (Figure 11b,e). R₂ also showed fibril-like features, which were further bundled to give twisted ropelike structures upon interaction with TNP (Figure 11c,f).

The results observed in AFM and TEM agree well with those observed in SEM. Thus, the spherical particles of R aggregate in the presence of TNP as observed in both AFM and TEM. Even in the case of R₁ and R₂, the density of the fibrils increases considerably upon interaction with TNP as observed from AFM and TEM (Figure 11g–r). All of the microscopy data reveal that the supramolecular features of R, R₁, and R₂ exhibit recognizable changes in their morphology upon interaction with TNP as proven based on SEM, AFM, and TEM.

**Complexation of R and TNP by Computational Studies.** All of the DFT, molecular dynamics (MD), and ONIOM computational calculations were performed as per the details given in the Experimental Section, and the sequence of these operations can be noted from Scheme 2.

The optimized structure for R and TNP were used to build a model structure for its 1:1 complex, which was subjected to 10 ns MD simulations from which 22 trajectories were manually selected by keeping the experimental outcome in mind and their complexation energies (CEs) were varied from −35 to −51 kcal/mol as obtained based on single-point calculations. These 22 structures were subjected to ONIOM calculations, which resulted in the convergence in the case of 17 structures that are similar in nature. All of these 17 structures showed CEs in the range −40 to −46 kcal/mol, where the highest complexation energy was observed with the structure obtained from the frame of 3984. The final structure obtained from this frame is shown in Figure 12 and was further analyzed for the interactions present between R and TNP. Figure 12 clearly supports that TNP is sandwiched between two consecutive pyrene moieties labeled as “B” and “C”. The corresponding centroid-to-centroid distances between TNP and the pyrene moieties (B and C) are 3.300 and 3.317 Å, respectively, supporting the presence of strong π···π interactions between the pyrenes and the TNP. In addition, “O” of one of the ortho-nitro groups interacts with the π face of one of the other two pyrene rings with a O···π distance of 3.029 Å. Even after the complexation of R by TNP, all of the four lower-rim phenolic-OH···O hydrogen bonds (Table 1) were retained, supporting the cone conformation for the calix[4]arene platform.
R and TNP were computed by the computational calculations, and it was observed that this results in a host–guest complexation via trapping the TNP between the two consecutive pyrenyl moieties by π···π interactions, which is
expected to restrict the flexibility of the pyrenyl arms. The interaction of \( R \) is also studied in the solid powder mixture by fluorescence microscopy and on the surface of silica gel and cellulose paper by fluorescence spectroscopy. The complexation of TNP by \( R \) shows fluorescence enhancement irrespective of whether the interaction is in solution, powder mixture, or on the surface of silica gel or on cellulose. However, the limit of detection in each of these cases differs and is 1.5, 3.5, and 6.5 \( \mu \text{M} \), respectively, in solution, silica gel strips, and cellulose paper, and is 2.1 \( \mu \text{mole} \) in the solid powder mixture (Figure 13c). Even the naked eye detection of TNP on the \( R \)-coated silica gel strips gives a detection limit of 21.5 \( \mu \text{M} \) (Figure 13a,b) as can be noticed from the picture taken under UV light using a cellular phone.

**Comparison between \( R \) and the Literature Reports on Calixarenes as Sensors for TNP.** Calixarene-based chemosensors reported in the literature for sensing TNP are given in Table 2. This table clearly reveals that all of these sensors function through fluorescence quenching and the studies are mostly in the organic solvents. Only the calixarene-based conjugate reported in this paper shows fluorescence enhancement upon addition of TNP, while the binding strength and the detection limits are comparable with those reported in the literature. To our knowledge, ours is the first example where the sensing of TNP is shown under four different conditions, viz., solution, powder mixture, silica gel surface, and cellulose surface.

**Table 1. Table Providing the Metric Data for the H-bonds Exhibited at the Lower rim of \( R \)**

| H-bonds | D--H (\( \text{Å} \)) | A--H (\( \text{Å} \)) | D···A (\( \text{Å} \)) |
|---------|-----------------|-----------------|-----------------|
| O1--H···O2 | 1.025 | 1.790 | 2.757 |
| O2--H···O3 | 1.021 | 1.912 | 2.832 |
| O3--H···O4 | 1.024 | 1.817 | 2.775 |
| O4--H···O1 | 1.021 | 1.900 | 2.802 |

*Here, “D” stands for the donor and “A” stands for the acceptor of hydrogen.*

**Figure 13.** (a) Photograph taken by a cellular phone under the UV light of \((R+xTNP)\) spotted on a silica gel sheet, where \( x = 0, 0.5, 1, 1.5, 2, 3, 4, \) and 5 (from left to right) equivalents. (b) Histogram of relative fluorescence intensity \((I/I_0)\) vs mole ratio of \([\text{TNP}]/[R]\) obtained from the photograph given under (a), where the intensities were obtained by ImageJ software. \( I/I_0 \) values were shown upon subtraction using appropriate controls. (c) Bar diagram of the limit of detection of TNP by \( R \) under different conditions as labeled in the figure.

that is selective to TNT over TNP and also several other NACs.\(^{30}\) The similarity between \( L \) and the present receptor \( R \) is the imine moiety that is being fixed in a five-membered ring in \( L \) (resulting in an imidazole moiety), while it is free in \( R \), but both have aromatic moieties that differ in their size. These differences resulted in a cup-shaped structure in the case of \( L \), and it expands in the presence of three TNT guest species where each TNT interacts with one benzimidazole moiety through weak \( \pi \cdots \pi \) interactions in addition to \( \text{H-bonding} \) between the O of the nitro group and the \( \text{−NH} \) of the imidazole moiety. On the other hand, in the case of \( R \), the pyrene moieties enjoy free rotation to adjust their conformation and lead to a rigid structure upon binding by TNP. The rigidity arises from the binding of TNP since both the adjacent pyrene moieties exhibit stronger \( \pi \cdots \pi \) interactions. As the present receptor \( R \) lacks an imidazole \( \text{−NH} \), the \( R \) is deprived of extending H-bonding interactions with the guest.
NAC. All of these chemical followed by structural differences present between L and R lead to the diversity in the selective sensing of NACs (Figure 14); while it is TNT in the case of L, it is TNP in the case of R, wherein both these cases are guided by the specific interactions.

**Table 2. Comparison between Parameters of R and the Literature Reports on Calixarenes as Sensors for TNP**

| probe                     | interaction                | solvent   | fluorescence Response | LOD (μM) | binding constant (μM) | ref |
|---------------------------|----------------------------|-----------|-----------------------|----------|-----------------------|-----|
| aminophthalimide-appended calix[4] arene | H-bonding, dipole–π, and π···π | THF       | quenching             | 0.3      | 4.51 × 10^3           | 26  |
| anthryl calix[4]arene     | π···π                       | CH₂CN     | quenching             | nil      | 3.75 × 10^3           | 27  |
| hexahomotrioxa calix[3] arene | charge transfer            | CH₂CN     | quenching             | 0.3      | 2.23 × 10^3           | 28  |
| tetraphenyl-ethylenic-calixarene | charge transfer       | H₂O/THF   | quenching             | 0.1      | 1.7 × 10^3            | 29  |

In this paper, we have shown that the TNP sensing can be performed in solution, the solid state, or on oxophilic surfaces, such as silica gel or cellulose paper, and all of the events can be performed in solution, the solid state, or on oxophilic surfaces, such as silica gel or cellulose paper, and all of the events can be monitored by a cellular phone for taking the images and by software ImageJ for processing the data. Therefore, there exists a wide scope for the utility of R in the sensitive and selective detection of TNP present in the samples collected from different sources where the image handling and the data management are trivial.

**EXPERIMENTAL SECTION**

**Instrumentation.** ^1^H and ^13^C NMR spectra were recorded on a 400 MHz NMR spectrometer. The ESI-MS spectra were recorded on a Bruker maXis Impact instrument, using electrospray ionization (ESI) in a positive ion mode method. The steady-state fluorescence spectra were measured on a Horiba Scientific Instrument/Fluoromax-4 instrument. The absorption spectra were measured on Shimadzu UV-NIR-3600. Elemental analyses were carried out on a Thermo Finnigan FLASH EA 1112 series CHNS instrument. The SEM, AFM, and TEM images of the samples were measured on JSM-7600F working at 5 kV, Nano Surf Flex AFM, and JEOL TEM 2100F instruments, respectively. All of the solvents used were of HPLC grade and were dried by following standard procedures immediately before use.

**Synthesis and Characterization of the Receptor Molecule (R).** The precursors P₁ and P₂ were synthesized according to the procedure reported in the literature, and the characterization data has been given in the Supporting Information (Figures S10 and S11). A mixture of P₁ (0.2 g, 0.373 mmol) and 1-aminopyrene (0.648 g, 2.982 mmol) in 25 ml of toluene in the presence of catalytic amount of benzoic acid (10 mg) was heated at 110 °C in a Dean Stark apparatus for 5 days. The reddish-yellow product was precipitated out, and this was filtered under hot conditions, washed with diethyl ether, and dried under vacuum. The product (R) was collected. Yield 54% (0.268 g); ^1^H NMR (400 MHz; CDCl₃): δ (ppm) 8.53 (s, 4H, imine-H), 8.51 (s, 4H, pyrene Ar-H), 8.04 (dd, J₁ = 7.2 Hz, J₂ = 6.0 Hz, 8H, pyrene Ar-H), 7.98 (s, 8H, pyrene Ar-H), 7.93 (s, 8H, Calix-Ar-H), 7.82 (t, J = 7.6 Hz, 4H, pyrene Ar-H), 7.69 (d, J = 9.2 Hz, 8H, pyrene Ar-H), 7.57 (d, J = 8.0 Hz, 4H, pyrene Ar-H), 4.52 (br s, 4H, bridge −CH₂−), 3.85 (d, J = 10 Hz, 4H, bridge −CH₂−), ^13^C NMR (125 MHz; DMSO-d₆): δ (ppm) 161.1, 159.1, 146.2, 131.0, 130.8, 130.8, 129.6, 128.2, 127.3, 127.2, 126.5, 126.4, 126.1, 125.9, 124.6, 124.4, 124.3, 121.3, 115.9, 32.1; ESI-MS (HRMS) chemical Formula: C₉₆H₆₀N₄O₄ [M + H]^+ calculated m/z at 1334.47, observed m/z at 1334.47; Elemental analysis for C₉₆H₆₀N₄O₄ (observed/calculated) C = 60.42/60.36, H = 4.22/4.16, N = 4.78/4.64.

**Synthesis and Characterization of the Control Molecule (R₁).** The precursor molecule P₃ (0.25 g, 0.466 mmol) and aniline (3.4 ml, 37.2 mmol) were mixed along with catalytic amount of benzoic acid (10 mg), and the reaction was allowed to proceed as per details given in the case of R. The progress of the reaction was monitored through checking the TLC. The reaction yielded a yellow product (R₁). Yield 72% (0.28 g); ^1^H NMR (500 MHz; DMSO-d₆): δ (ppm) 8.44 (s, 4H, imine-H), 7.78 (s, 8H, Calix-Ar-H), 7.37 (t, J = 6.5 Hz, 8H, Ph Ar-H), 7.25-7.19 (m, 12H, Ph Ar-H), 4.39 (br s, 4H, bridge −CH₂−), 3.54 (br s, 4H, bridge −CH₂−), ^13^C NMR (125 MHz; DMSO-d₆): δ (ppm) 159.9, 130.7, 130.5, 129.3, 129.0, 125.8, 120.5, 117.9, 115.5, 31.61; ESI-MS (HRMS) chemical Formula: C₉₆H₆₀N₄O₄ [M + H]^+ calculated m/z at 837.3437, observed m/z at 837.3435; Elemental analysis for C₉₆H₆₀N₄O₄ (observed/calculated) C = 80.14/80.36, H = 5.35/5.30, N = 6.86/6.69. The corresponding data is given in Figure S12.

Figure 14. Schematic structures for (a) L (ref 30) and (b) R. Computational structures for the complexes, (c) (L + 3TNT) and (d) (R + TNP).
(500 MHz; DMSO-d$_6$): $\delta$ (ppm) 10.24 (s, 1H, phenolic-OH), 8.76 (s, 1H, imine-H), 8.64 (d, $J =$ 9.0 Hz, 1H, pyrene Ar-H), 8.29 (d, $J =$ 8.0 Hz, 1H, pyrene Ar-H), 8.25 (d, $J =$ 7.5 Hz, 2H, Ph Ar-H), 8.18-8.03 (m, 4H, pyrene Ar-H), 8.01 (d, $J =$ 8.5 Hz, 2H, Ph Ar-H), 7.89 (d, $J =$ 8.0 Hz, 1H, pyrene Ar-H), 6.99 (d, $J =$ 8.5 Hz, 2H, pyrene Ar-H); $^{13}$C NMR (125 MHz; DMSO-d$_6$): $\delta$ (ppm) 160.9, 160.8, 145.5, 131.1, 131.0, 130.9, 128.6, 127.9, 127.3, 126.8, 126.4, 125.9, 124.9, 124.8, 124.6, 124.5, 124.1, 115.8, 115.8; ESI-MS (HRMS) chemical formula C$_{23}$H$_{15}$NO (observed/calculated) C = 86.19/85.96, H = 4.79/4.70, N = 4.61/4.36. The corresponding data is given in Figure S14.

Absorption and Fluorescence Studies of R with NACs. Absorption and fluorescence titrations were carried out using tetrahydrofuran (THF). All of the titrations were carried out in a 1 cm quartz cell, and the concentration of R, R$_1$, or R$_2$ was kept constant at 4 $\mu$M. The concentrations of NACs were varied from 0 to 24 $\mu$M. The twelve different NACs used in the present study are nitrobenzene, 1,3-dinitrobenzene, 3-nitrotoluene, 4-nitrotoluene, 1,3-dinitrotoluene, 2,4,6-trinitrotoluene (TNT), 3-nitropheno1, 2,4-dinitrophenol, 2,4,6-trinitrophenol (TNP), o-nitro chlorobenzene, p-nitro benzoic acid, and 3-nitrophenol, 2,4-dinitrobenzene, 3-nitrotoluene, 4-nitrotoluene, 1,3-dinitrotoluene.

Fluorescence Spectral Study of TNP in Different Sources of Water. The water samples were collected from different sources, such as ground, rain, sea, Milli-Q, and distilled water and were spiked with TNP (50 $\mu$M). The fluorescence spectra were recorded for all of these water samples spiked with TNP in which R (10 $\mu$M) was added. The twelve different TNP solutions of 2 mM in distilled water and were spiked with TNP (50 $\mu$M). Then, R and TNP solutions were taken into PBS buffer at different pH values (3 to 12) to obtain their final concentrations of 10 and 50 $\mu$M, respectively. The fluorescence spectra for R and (R + TNP) were recorded in the PBS buffer at different pH values to study the fluorescence response for sensing TNP by R as a function of pH. The pH was adjusted using 5 mM phosphate buffer saline (PBS).

Computational Details. The initial model structure for the receptor R was prepared starting from a known crystal structure as per the steps given in Figure S15. The geometry optimization of this model structure for R was carried out in a cascade fashion, viz., initially with PM6 followed by the range-separated functional wB97XD in combination with the double-$\zeta$ quality basis set like 6-31G(d,p).

For the MD simulation study, an automated topology builder has been used to generate the force fields for the receptor R and TNP from the obtained equilibrium coordinates at the wB97XD/6-31G(d,p) level of geometry optimization. After obtaining the force field, the gas phase MD simulations for the 1:1 complex {R···TNP} were initiated by placing TNP in the close proximity of R in different orientations. Both these initial structures lead to similar final conformation for 10 ns simulations as can be noticed from Figure S16. Thus, for further analysis, only one complex, where TNP is kept at the center of the R, has been considered. The {R···TNP} complex thus generated was subjected to 10 ns MD simulations using 2 fs time steps as performed through the GROMACS 4.6.7 version. The resultant trajectories were recorded at a regular interval of 2 fs during the simulations. We employed the microcanonical ensemble (N.V.E.) to represent the R···TNP complex, where the $N$, $V$, and $E$ stands for the number of molecules, volume, and energy respectively. These are used to study the structural and dynamic features of the {R···TNP} complexes. In the MD simulations, the R is trapped between two consecutive pyrene rings and stays intact till the end of the simulation as can be seen from Figure S17. Among these, a total of 22 structurally distinct trajectories/conformations for the {R···TNP} complex were further subjected to the single-point energy calculation at the wB97XD/6-31G(d,p) level of theory. Figure S18 is a representation for the variation of different complexation energies (CEs).

The complexation energy (CE) varies from $-35$ to $-51$ kcal/mol among these 22 trajectories, of which the trajectory
obtained at a time step of 3984 exhibits the highest value of 
−51 kcal/mol. All of these 22 structures have similar structural 
and energetic features, where TNP is sandwiched between the 
two consecutive pyrene rings. All of these 22 structures were 
进一步 subjected to two layered ONIOM calculations,37 where 
the upper layer is treated at the wb97XD/6-31G(d,p) level and 
the lower layer is treated with the semiempirical method, PM6, using GAUSSIAN 09 software packages.38
Among the 22 structures, only 17 structures were converged, 
and these are further improved with 6-311G(d,p), a triple-ζ quality basis set, and BSSE corrections.39 The CE calculated 
from the super molecular approach is obtained from the 
wb97XD/6-31G(d,p)/ONIOM(wb97XD/6-31G-(d,p):PM6) level. At this stage, the CEs vary in the range −40 
to −46 kcal/mol. Thus, based on the MD simulations and DFT studies, the {R−TNP} complex obtained from the 3984 
frame of the MD simulations was considered and discussed in the Results and Discussion section.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02855.
Characterization data (i.e., 1H NMR, 13C NMR, and ESI-MS) of the precursors (viz., P2 and P3) and the receptors (viz., R1 and R2), absorption and fluorescence spectra of R with different NACs, Job plot of R with TNP, plots for determination of binding constant, limit of detection and quantum yields of R + TNP, preparation of R from a similar type of known crystal structure, initial and final structures of (R + TNP) in MD simulations, snapshots of structures of {R + TNP} at different time frames in MD simulations and variation of complexation energies of {R + TNP} using the wb97XD/6-31G(d,p) level of theory (PDF)

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The authors declare no competing financial interest.

ACKNOWLEDGMENTS
C.P.R. acknowledges financial support from the DST/SERB {EMR/2014/000985} for J. C. Bose National Fellowship {SB/ S2/JCB-066/2015} and IIT Bombay for Institute Chair Professorship. S.K.D. acknowledges CSIR for the award of Senior Research Fellowship {09/087/0796/2014-EMR-1}. A.U. acknowledgesUGC for the award of Senior Research Fellowship {ref No. 21/12/2014(II) EU-V; Serial No. 2121410051}. We thank Sirilata Polepalli for helping with the ITC experiment. We acknowledge the services provided by
the central facilities of IIT Bombay, viz., SEM, AFM, TEM, and CHN analyzer.

DEDICATION
We dedicate this paper to Professor C.N.R. Rao, F.R.S., on his 85th birthday.

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