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Color response of tri-armed azo host colorimetric sensors and test kit for fluoride

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ABSTRACT

Five new chromogenic tripodal receptors (**2a**–**e**) containing electron withdrawing and donating groups appended to the azophenol moiety were synthesized, characterized, and their chromogenic behaviors toward various anions were investigated. These tripodal receptors showed a distinct color change only when treated with fluoride ions in CH_3CN solution. Yet, other anions such as Cl^-, Br^-, I^-, $NO_3{}^-$, $ClO_4{}^-$, AcO^-, $HSO_4{}^-$, and $H_2PO_4{}^-$ could not cause any color change. Thus, the receptors **2a**–**e** can be used as a colorimetric chemosensor for the determination of fluoride ion. In addition, ^{1}H NMR experiments were carried out to explore the nature of interaction between tripodal receptors and fluoride. Finally, analytical application and the use of test strip of the receptor **2b** to detect fluoride was also reported.

1. Introduction

Anions are ubiquitous. They have major roles in the wide range of chemical, biological and environmental processes. The rational design and synthesis of efficient sensors to selectively recognize anion analytes is an emerging field of supramolecular chemistry [1–3]. In particular, the sensing of fluoride ion has attracted growing attention [4], because of its great potential for biological and industrial applications [5], and fluoride's unique properties compared to its congeners as a result of relative size and electronegativity. The recognition and detection of the fluoride ion are of growing interest because it is associated with nerve gases, the analysis of drinking water [6], and the refinement of uranium used in nuclear weapons manufacture. Excess fluoride, however, can cause dental or skeletal fluorosis [7], and it is associated with kidney failure [8] and nephrolithiasis [9]. Further, fluoride is released upon hydrolysis of UF_6 [10] or of fluorinated organophosphorus compounds [11], which makes it an interesting tracer for these strictly supervised substances.

In view of the pharmacological and environmental importance of fluoride, there is a need to develop selective and sensitive methods for fluoride detection in environments that are not easily served by conventional ion selective electrodes. However, the number of fluorescence sensors or chemodosimeters [12–14] for fluoride anions is quite limited, in spite of its importance in dental care and the treatment of osteoporosis [15].

During the last two decades, the design of synthetic hosts for anion binding has become an important area of research in chemistry [16–24]. Because of the synthetic and structural simplicity, tripodal hosts consisting of three arms received a significant interest in binding a variety of inorganic anions [25–28]. The topology of podand-type receptors necessitates that some form of "core" is present to which the functionalized arms are attached. The tripodal synthetic receptors constitute a special class of acyclic ionophores, which consist of multi-armed ligands that can coordinate with the target anion. The tripodal molecular platform provides three arms to which ligating groups are tailored or attached. As a recognition motif, tripodal-based receptors have been reported to be used successfully as an optical sensors [29–31]. For example, simple tripodal amide based ligands reported by Reinhoudt and co-workers, were shown to bind anions with *C3* symmetry [32]. Recently, Ghosh and co-workers reported that pentafluorophenyl-substituted tripodal urea effectively binds phosphate anion through hydrogen bonding interactions from urea units [33,34]. The affinity for fluoride is boosted by the presence of a Al(III)- or Sc(III) metalloporphyrins moiety [35,36] up to two orders of magnitude with respect to the uncoordinated receptor. While a number of compounds that are able to bind fluoride ions with high affinity and selectivity have been reported [37,38], the challenges of detecting and amplifying the fluoride ion binding event to produce a measurable output still remain. Our continuing interests [39–41] in this area are colorimetric anion sensors, species that would allow the naked-eye detection of fluoride without resorting to any spectroscopic instrumentation.

Here we report the synthesis, characterization, and chromogenic studies on a tripodal ditopic Schiff base hosts (**2a**–**e**) with

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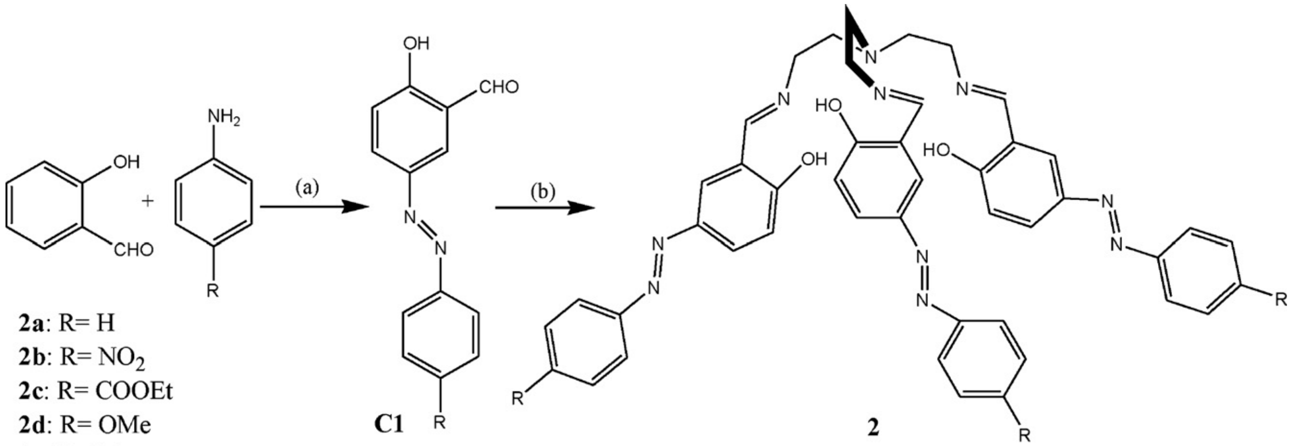

2a: R= H
2b: R= NO_2
2c: R= COOEt
2d: R= OMe
2e: R= Me

Scheme 1. Synthesis of tripodal receptors (**2a–e**): (a) Na_2CO_3, $NaNO_2$/HCl, H_2O, 5 °C; (b) tris(2-aminoethyl)amine, dry methanol, 5 h, room temperature.

azophenol moiety as the end groups so as to dispose three –OH groups capable of forming H-bonding or undergoing deprotonation in the presence of anions. There are some reports [42–44] for the synthesis of phenolic-OH bearing tripodal ligands but they were not used as anion sensors. Only compound like Alizarin, which is reported [45] to sense various anions in dichloromethane, to the best of our knowledge this is the first example of a synthetic receptor, bearing hydrogen bonding moiety phenolic OH and chrmogenic moiety azo groups, i.e., azophenol being used as an anion sensor. These receptors acts as a highly selective, visually detectable optical sensor for F^- ions in CH_3CN working via internal charge transfer.

We prepared a set of five tripodal hosts **2a–e** based on a azophenol–imine platform, hosts which have an identical binding topology but differ in the level of electron influencing effect, cf. Scheme 1.

2. Experimental

2.1. Apparatus and reagents

Melting points were determined using a Thomas–Hoover capillary melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer using Me_4Si as the internal standard. The ^1H NMR chemical shift values are expressed in ppm (δ) relative to $CHCl_3$ ($\delta = 7.26$ ppm). UV–vis and fluorescence spectra measurements were performed on a JASCO V530 and a PerkinElmer LS-55 spectrofluorimeter respectively. All of the measurements were operated at 298 K. CH_3CN and DMSO of analytical grade were purchased from Merck. Deionized water (double distilled) was used throughout the experiment as an aqueous solution. All other materials for synthesis were purchased from Aldrich Chemical Co. and used without further purification. The solutions of anions were prepared from their terabutylammonium salts of analytical grade, and then subsequently diluted to prepare working solutions.

2.2. Calculation of binding constant

2.2.1. UV–vis titration

An aliquot (2 mL) of a freshly prepared solution of tripodal hosts **2a–e** in acetonitrile (1.0×10^{-5} M) was transferred into a quartz cell (1 cm width). Small portions of a stock solution of the anion was added to the solution (TBA salt in acetonitrile, 0.5 M) in an incremental fashion. The corresponding UV–vis spectra were recorded at 298 K. On the basis of 1:1 stoichiometry of the anion complexes, the binding constants were determined by the nonlinear least-squares curve-fitting method according to Eq. (1) [63–64].

$$A = A_0 + \left[\frac{(A_{\text{lim}} - A_0)}{2c_0}\right]\left\{c_0 + c_M + \frac{1}{K_s} - \left[\left(c_0 + c_M + \frac{1}{K_s}\right) - 4c_0c_M\right]^{0.5}\right\} \quad (1)$$

where c_0 is the concentration of tripodal hosts, c_M is the concentration of the anion, A_{lim} is the maximum of UV–vis absorbance at saturation, A_0 is the absorbance of free tripodal host, A is the absorbance of tripodal host with the anion, and $K_{\text{ass.}}$ is the association constant (see Supplementary data, Page S22).

2.3. Synthesis

The tripodal azophenol-imine receptors **2a–e** were prepared by a Schiff base condensation reaction between tris(2-aminoethyl)amine (1 mmol) and azo-coupling product C1 (3 mmol) with 82% yield. The azo derivatives (C1) were prepared by applying the di-azocoupling reaction of salicylaldehyde with *para*-substituted aniline at low temperature. These compounds were isolated as solids and were stable both in solid state and in solution. All of these compounds were characterized by ^1H NMR, IR, and ESI-MS. The CHN data are also in accordance with the molecular formula.

2.4. General procedure for the preparation of compounds *2a–e*

2.4.1. Preparation of 5-(4-substituted phenylazo)salicylaldehyde (*C1*)

To a solution of 4-substituted aniline derivative (5 ml; 0.05 mol) in a small quantity of water was slowly added 6 ml of 37% aq HCl solution at 0–5 °C with stirring. 20 ml of 20% aq $NaNO_2$ solution was added to the mixture and the resulting solution was stirred for 1 h, affording a bright yellow solution. Salicylaldehyde (5 ml; 0.05 ml) was dissolved in a solution comprising 18 g Na_2CO_3 and 150 ml H_2O and the resulting solution of salicylaldehyde was added dropwise to the bright yellow colored solution over 1 h. After stirring for 4 h, the reaction mixture was neutralized with HCl, the brown crude solid was filtered and recrystallized from ethanol to afford a pure yellow product.

2.4.2. Preparation of tripodal hosts (*2a–e*)

To a solution of 5-(4-substituted phenylazo)salicylaldehyde (**C1**) (0.226 g, 1 mmol) and a catalytic amount of *p*-toluenesulphonic acid in dry ethanol was added dropwise a solution of tris(2-aminoethyl)amine (0.167 g,1 mmol) in ethanol. Then the mixture was heated to boil under magnetic stirring for 2 h. During the reaction a yellow precipitate appeared which was collected by filtration, washed with ethanol and dried in vacuo. Desired solid (0.300 g) was obtained.

2.4.3. tris{2-[(2-Hydroxy-5-phenylazo-benzylidene)-amino]ethyl}amine *2a*

Orange solid. Yield: 89%; Mp = 171–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.96 (6H, t, imine–CH_2CH_2–, J = 6.2 Hz), 3.68 (6H, s, imine–CH_2–), 6.99 (3H, d, Ar, J = 9.2 Hz), 7.29 (3H, d, Ar, J = 5.1 Hz), 7.38–7.36 (9H, m, Ar), 7.64 (6H, d, J = 5.5 Hz), 7.65 (3H, s), 7.79 (3H, dd, J = 2.4 Hz), 8.21 (3H, s, –OH, –CH=N); IR: ν_{max} (KBr) cm^{-1} 3040.0, 2947.3, 2812.6, 1631.6, 1144.4; Anal. Calcd for $C_{45}H_{42}N_{10}O_3$(770.89): C, 70.11; H, 5.49; N, 18.17; Found: C, 70.31; H, 5.37; N, 17.98%.

2.4.4. tris(2-{[(2-Hydroxy-5-(4-nitrophenylazo)benzylidene]-amino}ethyl)amine *2b*

Redish brown solid. Yield: 72%; Mp = 246–250 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.99 (6H, t, imine–CH_2CH_2–, J = 6.6 Hz), 3.72 (6H, s, imine–CH_2–), 6.97 (3H, d, Ar, J = 7.2 Hz), 7.28 (3H, s, –CH=N), 7.67 (6H, d, Ar, J = 7.1 Hz), 7.78 (6H, d, Ar, J = 5.3 Hz), 8.23 (6H, d, Ar, J = 5.8 Hz), 8.49 (3H, s, –OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 57.07, 57.08, 118.88, 118.93, 122.39, 127.59, 129.37, 130.09, 150.84, 165.49, 165.52; IR: ν_{max} (KBr) cm^{-1} 3415.1, 2975.7, 1632.3, 1512.9, 1339.6; [MS-ESI]: 905.4 (M^+); Anal. Calcd for $C_{45}H_{39}N_{13}O_9$(905.89): C, 59.66; H, 4.34; N, 15.89; Found: C, 59.49; H, 4.28; N, 15.84%.

2.4.5. tris(2-{[(2-Hydroxy-5-(4-carboethoxyphenylazo)-benzylidene]amino}ethyl)amine *2c*

Orange solid. Yield: 83%; Mp = 197–200 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (9H, t, –CH_3, J = 9.5 Hz), 2.96 (6H, t, imine–CH_2CH_2–, J = 6.5 Hz), 3.67 (6H, s, imine–CH_2–), 4.43 (6H, q, J = 9.5 Hz), 6.99 (3H, d, Ar, J = 12 Hz), 7.17 (3H, s, –CH=N), 7.59 (6H, d, Ar, J = 11.2 Hz), 7.79 (6H, d, Ar, J = 11.9 Hz), 8.02 (6H, d, Ar, J = 11.2 Hz), 8.16 (3H, s, –OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.61, 55.63, 56.52, 61.36, 117.54, 119.14, 122.46, 126.74, 129.86, 130.53, 131.47, 144.76, 155.02, 160.30, 166.33, 167.26; IR: ν_{max} (KBr) cm^{-1} 3390.5, 2979.5, 2937.0, 1715.0, 1632.2; [MS-ESI]: 987.1(M^+) Anal. Calcd for $C_{54}H_{54}N_{10}O_9$ (987.08): C, 65.71; H, 5.51; N, 14.19. Found: C, 65.99; H, 5.26; N, 14.03.

2.4.6. tris(2-{[(2-Hydroxy-5-(4-methoxyphenylazo)-benzylidene]amino}ethyl)amine *2d*

Yellow solid. Yield: 82%; Mp = 193–196 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (6H, t, imine–CH_2CH_2–, J = 6.6 Hz), 5.18 (6H, s, imine–CH_2–), 3.87 (9H, s, –OCH_3), 6.89 (6H, d, Ar, J = 11.8 Hz), 7.00 (3H, d, J = 11.8 Hz), 7.24 (3H, s, –CH=N), 7.68 (6H, d, J = 11.7 Hz), 7.78 (6H, dd, J = 3 Hz), 8.20 (3H, s, –OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.84, 57.17, 86.55, 113.43, 114.88, 117.89, 118.86, 126.35, 128.32, 145.17, 147.08, 161.56, 165.15, 166.40; IR: ν_{max} (KBr) cm^{-1} 3445.7, 1634.6, 1253.5; [TOP-MS-ES$^+$]: 882.2 (M+Na)$^+$; Anal. Calcd for $C_{48}H_{48}N_{10}O_6$ (860.97): C, 66.96; H, 5.62; N, 16.27. Found: C, 66.68; H, 5.38; N, 16.02%.

2.4.7. tris(2-{[(2-Hydroxy-5-(4-methylphenylazo)-benzylidene]amino}ethyl)amine *2e*

Yellow solid. Yield: 85%; Mp = 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (9H, s, –CH_3), 2.97 (6H, t, imine–CH_2CH_2–, J = 6.5 Hz), 3.68 (6H, s, imine–CH_2–), 6.99 (3H, d, Ar, J = 11.9 Hz), 7.19 (6H, d, J = 10.8 Hz), 7.33 (3H, s, –CH=N), 7.59 (6H, d, J = 10.9 Hz), 7.79 (6H, dd, J = 3 Hz), 8.24 (3H, s, –OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.65, 55.77, 57.11, 118.46, 118.47, 118.51, 122.82, 122.84, 122.85, 126.89, 127.98, 129.68, 129.71, 166.29; IR: ν_{max} (KBr) cm^{-1} 3443.9, 2853.5, 1634.0; [MS-ESI]: 812.8 (M)$^+$; Anal. Calcd for $C_{48}H_{48}N_{10}O_3$(812.97): C, 70.92; H, 5.95; N, 17.23. Found: C, 70.65; H, 5.86; N, 17.45%.

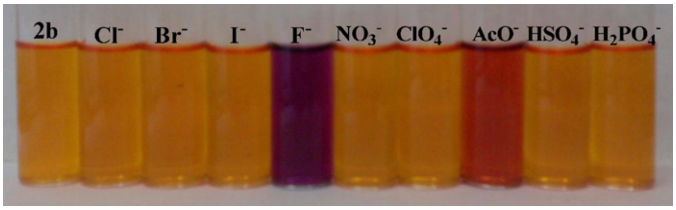

Fig. 1. The visible color changes observed for **2b** in MeCN solution (1.0×10^{-5} M) upon addition of 5 equivalent of anions as TBA salts: from left to right: free **2b**, Cl^-, Br^-, I^-, F^-, NO_3^-, ClO_4^-, AcO^-, HSO_4^-, and $H_2PO_4^-$.

3. Results and discussion

3.1. Anion sensing

The recognition properties of the tripodal hosts **2a**–**e** toward different anions were studied by the several methods such as the naked-eye experiment, the UV–vis titration, ^{1}H NMR titration and test kit. The response of the naked-eye experiment of tripodal receptors **2a**–**e** toward a set of anions (F^-, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, HSO_4^-, and $H_2PO_4^-$) was studied in CH_3CN. In this experiments, all the tripodal hosts **2a**–**e** (1.0×10^{-5} M in CH_3CN) gave birth to dramatic color changes from light pale yellow to orange (**2a** and **2c**–**e**) and tripodal host **2b** displays noticeable deep color change from yellow to deep purple in the presence of 5 equiv. of F^- (Fig. 1).

However, addition of a large excess of Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, HSO_4^-, and $H_2PO_4^-$ could not result in any color changes of the receptors **2a**–**e** at all. Furthermore, the color changes of the tripodal hosts **2a**–**e** would disappear upon addition of excess water. It has been very clear that the protic solvent [46] such as H_2O would compete with the anionic guests for the bonding sites of hosts and therefore, could disturb hydrogen bonding interactions between hosts and guests. So the results observed indicated that visible color changes were most probably due to the deprotonation of phenolic OH followed by hydrogen bonding to the addition of fluoride ions. The receptor bound anions, hydrogen bonds were constructed to form stable complexes, and the electron density in the supramolecular system was much increased to enhance the charge-transfer interactions between the electron rich and electron-deficient moieties, which resulted in a visible color change [47].

In this context, the chromogenic diazo unit is introduced at the 5-position of salicylaldehye to achieve direct resonance between them [48–54]. As shown in Fig. 2, the electron-withdrawing nitro or ester group at the end increases not only the degree of π-conjugation but also the hydrogen bond donor ability of the phenolic OH. The strong hydrogen bonding to, or deprotonation/protonation of the phenolic moiety might modulate the electronic properties of the chromophore [55] and give rise to large color changes.

The binding ability of the tripodal receptors **2a**–**e** with a series of anions such as F^-, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, HSO_4^-, and $H_2PO_4^-$ in CH_3CN was monitored by UV–vis absorption spectroscopy at room temperature. The anions were added as tetrabutylammonium salts to the CH_3CN solutions of the receptors **2a**–**e** ($\sim 1.0 \times 10^{-5}$ M). The UV–vis absorption spectra of tripodal receptors **2a**–**e** in CH_3CN are dominated by one strong absorption bands at 356, 410, 366, 360 and 352 nm respectively. The UV–vis titrations were carried out in CH_3CN at a concentration level of 1.0×10^{-5} M

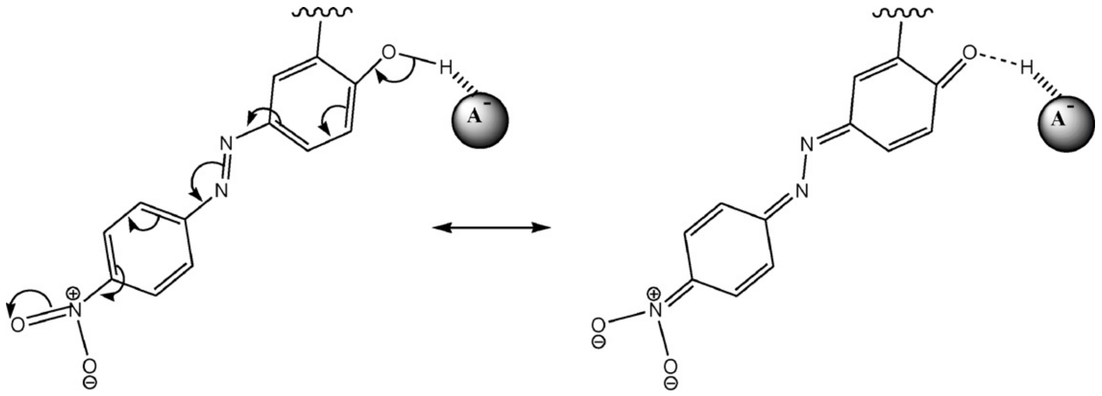

Fig. 2. Resonance structures of phenolic OH and chromogenic dizo scaffold.

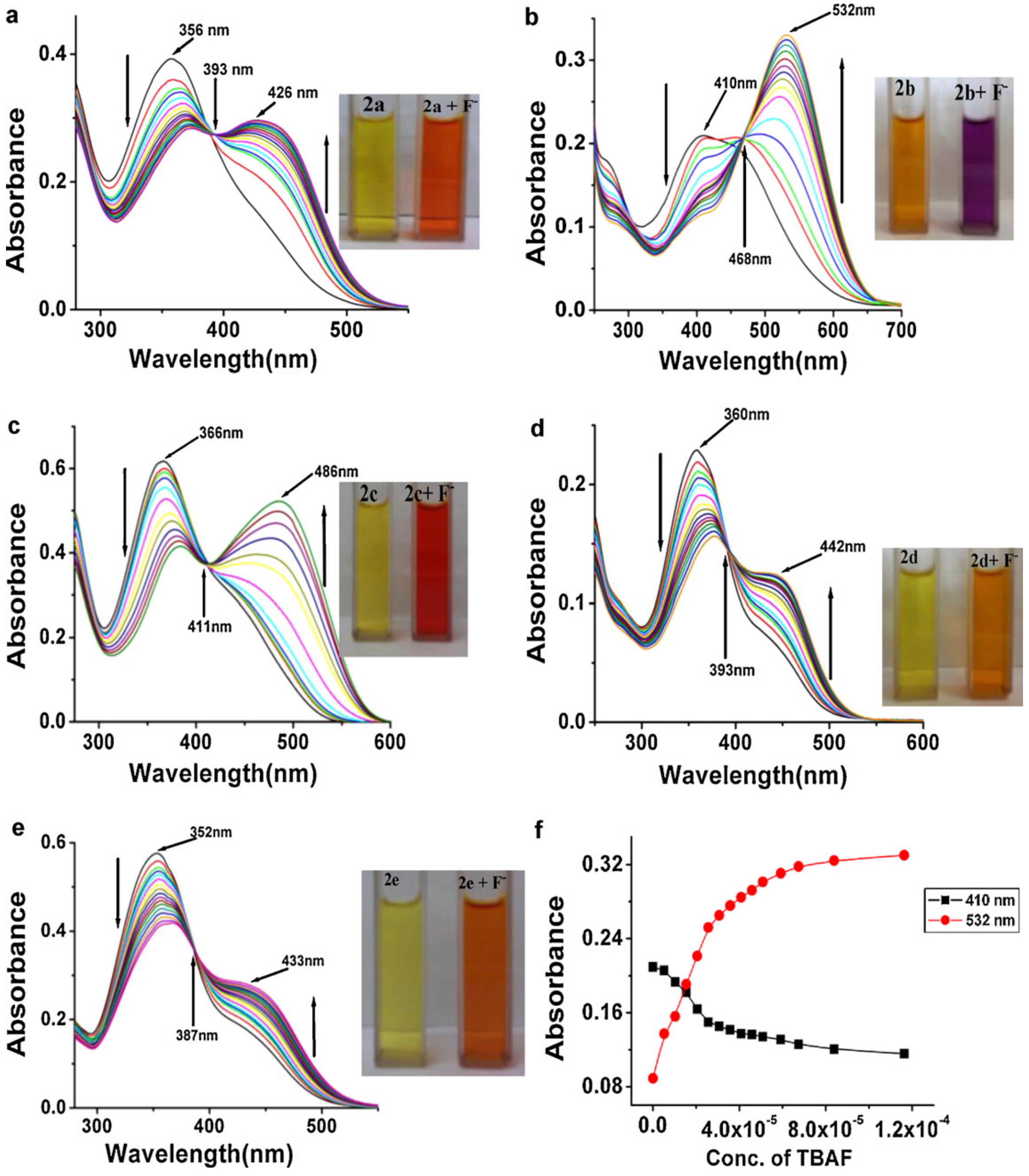

Fig. 3. (a–e) A family of UV–vis spectra of tripodal hosts **2a–e** were taken in the course of the titration of CH_3CN solution of **2a–e** ($c \approx 1.0 \times 10^{-5}$ M) with a standard solution of n-Bu_4NF ($c = 3.44 \times 10^{-4}$ M) at 25 °C, inset shows the visual color change on addition of F^- ion. (f) Showing plot of tripodal host **2b** of absorption versus concentration of F^- showing decrease and increase in absorption of the 410 nm and 532 nm bands, respectively.

Table 1
Data obtained from the UV–vis spectra upon titration of tripodal receptors (**2a**–**e**) with n-$Bu_4N^+F^-$ in CH_3CN.[a]

	Tripodal receptor, λ_{max} (nm)	Complex, λ_{max} (nm)	Bathochromic shift, $\Delta\lambda$ (nm)	Isosbestic point (nm)	K_{ass} (M^{-1})	Detection limit (M)
2a	356	426	70	393	1.913×10^3	1.02×10^{-5}
2b	410	532	122	468	3.3311×10^4	1.00×10^{-5}
2c	366	486	120	411	1.477×10^3	1.01×10^{-5}
2d	360	442	82	393	1.6×10^3	1.03×10^{-5}
2e	352	433	81	387	9.243×10^3	1.05×10^{-5}

[a] $c = 3.44 \times 10^{-4}$.

upon addition of F^- as tetrabutylammonium ($c = 3.44 \times 10^{-4}$ M) and the spectra were shown in Fig. 3a–e. When increasing the concentration of F^-, all these tripodal receptors **2a**–**e**, a new red shifted absorption band at 426, 532, 486, 442 and 433 nm respectively were gradually enhanced, while the intensity of absorption at 356, 410, 366, 360 and 352 nm respectively were decreased correspondingly. A clear isosbestic points were observed at 393, 468, 411, 393 and 387 nm for **2a**–**e** respectively.

The appearance of a single isosbestic point indicates the presence of only two species, neutral tripodal host and its anion in the solution, Fig. 3f shows the gradual decrease and increase in the concentration of these two species with increase in equivalents of TBAF, respectively. The negative charge brought about by the anion induced deprotonation increases the dipole moment and stabilizes the excited state causing a red shift of **2a**–**e** tripodal hosts $\Delta\lambda = 70, 122, 120, 82$ and 81 nm respectively. These results (Table 1) demonstrate that a complex formation of **2a**–**e** with F^- anion is taking place via hydrogen bonding electrostatic interactions. Addition of F^- ion brings significant changes in the spectra, on the other hand, no change was observed with Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, HSO_4^-, and $H_2PO_4^-$ ions in CH_3CN (see Supplementary data, Page S16).

The formation of these hydrogen bonds affects the electronic properties of the chromophore, resulting in a color change with a subsequent new intraligand or internal charge transfer (ICT) band involving between the F^--bound hydroxyl group and electron deficient azo moiety.

Judging from the titrations, the strong binding of fluoride allowed the Job's plot method [56] to be used in the determination of the binding stoichiometry, which was found to be a 1:1 host-to-anion complexation (Fig. 4a). On the basis of 1:1 stoichiometry, the corresponding binding constants (K_{ass}) were calculated in CH_3CN, which was shown in Table 1, based on the UV–vis titration data. The data showed that all tripodal receptors **2a**–**e** has higher selectivity for fluoride than other anions.

Parallel investigations were carried out with a series of other anions (Cl^-, Br^-, I^-, $NO_3{}^-$, $ClO_4{}^-$, AcO^-, $HSO_4{}^-$, and $H_2PO_4^-$). The addition of other anions did not result in significant absorbance spectral changes during UV–vis titration and no noticeable changes in color was observed in the cases of Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, AcO^-, HSO_4^-, and $H_2PO_4^-$ even at high concentration up to 100 equiv each (see Supplementary data). Next, we evaluated the UV–vis behavior of **2b** and **2c** toward counter cations such as K^+, Na^+, and $NH_4{}^+$ fluoride under the same experimental conditions but no spectral change was observed (see Supplementary data, Page S32). It has been noted that only the deprotonation of tripodal receptor **2b** took place by basic anion AcO^-, and development of the light orange color (Fig. 1) of receptor **2b** was observed after large excess addition and due to presence of more electron withdrawing nitro group which enhance acidity of azophenol moiety. No deprotonation took place in the presence of less basic anion such as, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, HSO_4^- and $H_2PO_4^-$. However, a full understanding of the principles that govern anion recognition has not been achieved. It became clear early on that multiple hydrogen-bonding interactions are necessary in high-affinity anion binding sites. Charge and shape complementarity [57–58] between the host and the anionic guests are also extremely important. Here tripodal receptors **2a**–**e** was able to bind fluoride ion more strongly than other anions as phenolic OH moiety being particularly appropriate for effective binding of the smaller anion (Fig. 4b and c). The deprotonation occurred at higher concentration of acetate than fluoride, this is due to the higher electronegativity, smaller size and higher basicity of the F^- ions that makes them to bind strongly with the tripodal receptors (**1a**–**e**) [59]. That was why F^- could be recognized selectively from the other anions.

3.2. Sensing mechanism

^1H NMR spectroscopy is of immense value in the understanding of receptor–substrate interaction and is capable of providing a revealing picture of the details of the interaction between tripodal receptors and anions. Fig. 5a and b displayed a representative spectrum of tripodal receptors **2b** and **2c** recorded when NMR titrations were carried out in DMSO-d_6, with increasing amounts of tetrabutylammonium fluoride.

Before the addition of F^-, the ^1H NMR chemical shift of the OH proton of receptors **2b** and **2c** were δ 8.25 and 8.21 ppm, respectively. Upon addition of 1.0 equiv $[Bu_4N]^+F^-$, the peak which were assigned to the O–H protons, shifted downfield and finally disappear, indicating the formation of a host–guest hydrogen-bonding complex [60–62] between tripodal host and fluoride ions. At the same time, deprotonation of the receptor can possibly occur, and indeed, we observed such deprotonation and the formation of HF_2^- in the ^1H NMR spectra of **2b** and **2c** as a new signal at δ 13.54 ppm. Consequently, we propose that the F^- recognition occurs by the initial hydrogen bonding of the anion to the receptor, followed by deprotonation which brings electron density onto the π-conjugated framework through bond propagation thus causing a shielding effect and inducing small upfield shift of aromatic protons.

The fluoride and acetate induced deprotonation process is fully reversible as evidenced from the addition of CH_3OH and H_2O. The addition of polar protic solvent methanol results gradual decrease of absorbance in UV–vis studies and reverse color change from orange to yellow (see Supplementary data, Page S15). So the receptor, unfortunately cannot be used in fully aqueous medium. This is presumably because protic solvent competes for F^- or AcO^- with the OH moiety, moreover, the presence of a relatively high amount of protic solvent disfavors the formation of the deprotonated tripodal receptors **2a**–**e**. However, in water-containing medium, no color changes were observed upon addition of organic bases such as triethylamine and the excess addition of $[Bu_4N]^+OH^-$ can deprotonate tripodal receptors **2a**–**e** and induce a color change. The deprotonation of the tripodal receptors were confirmed by Bronsted acid–base reaction between **2c** and [n-Bu_4]NOH (see Supplementary data). A stepwise increase in the concentration of the TBAOH produces results analogous to those found in the case of F^- ion and other ions are less basic than OH^- ($pK_a = 30$ in CH_3CN).

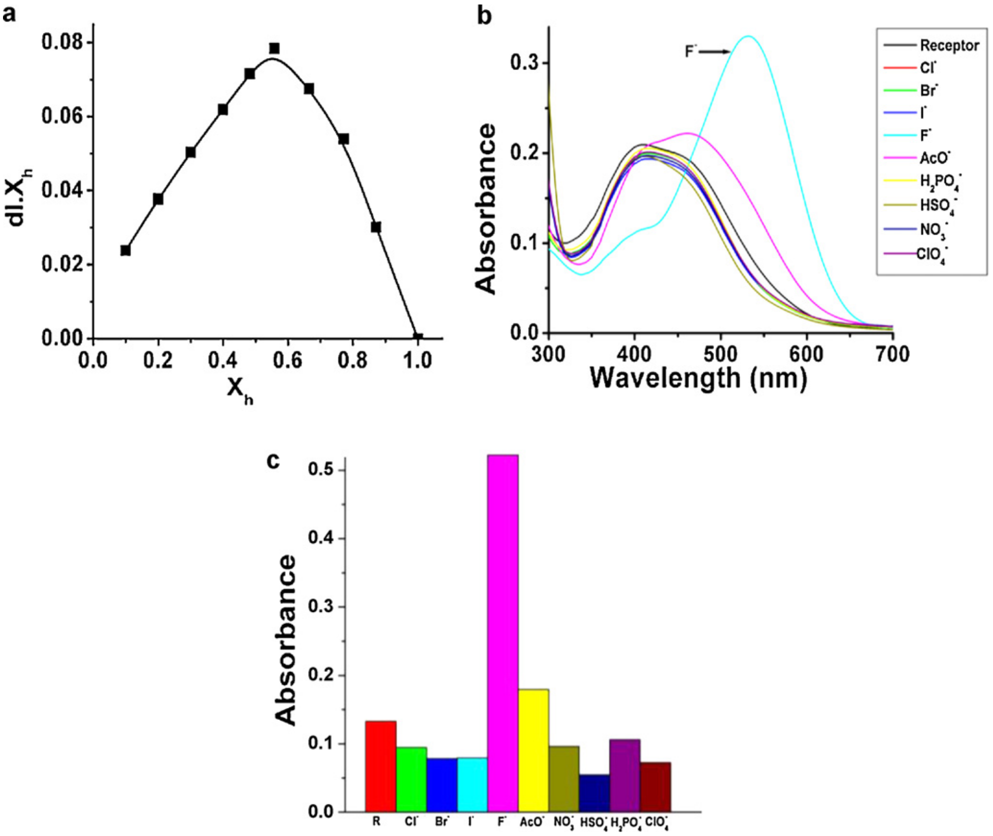

Fig. 4. (a) Job's plot of tripodal host **2b** (10 μM) with fluoride. (b) UV–vis absorption spectra of **2b** (20.0 μM) upon addition of a particular anion salt in CH_3CN. (c) UV–vis absorbance increases of tripodal host **2b**, in CH_3CN, in relation to the free host, after addition of 25 eqiv. several anions.

It shows that in more polar solvent like DMSO, the deprotonation is being favored, as it is a very good proton acceptor [38]. It corroborates the fact that the receptors provides selectivity for F^- ion which is solely based upon its deprotonation and is related to the factors (a) intrinsic acidity of the receptor (b) basicity of the anion, and (c) polarity of the solvent.

4. Sensor performance

Generally, receptors for anions based solely on hydrogen bonding interactions cannot serve as efficient sensors in aqueous media, due to the strong solvent competition. To avoid the competing solvation effect of water [16,31], we prepared a test strips of **2b** for

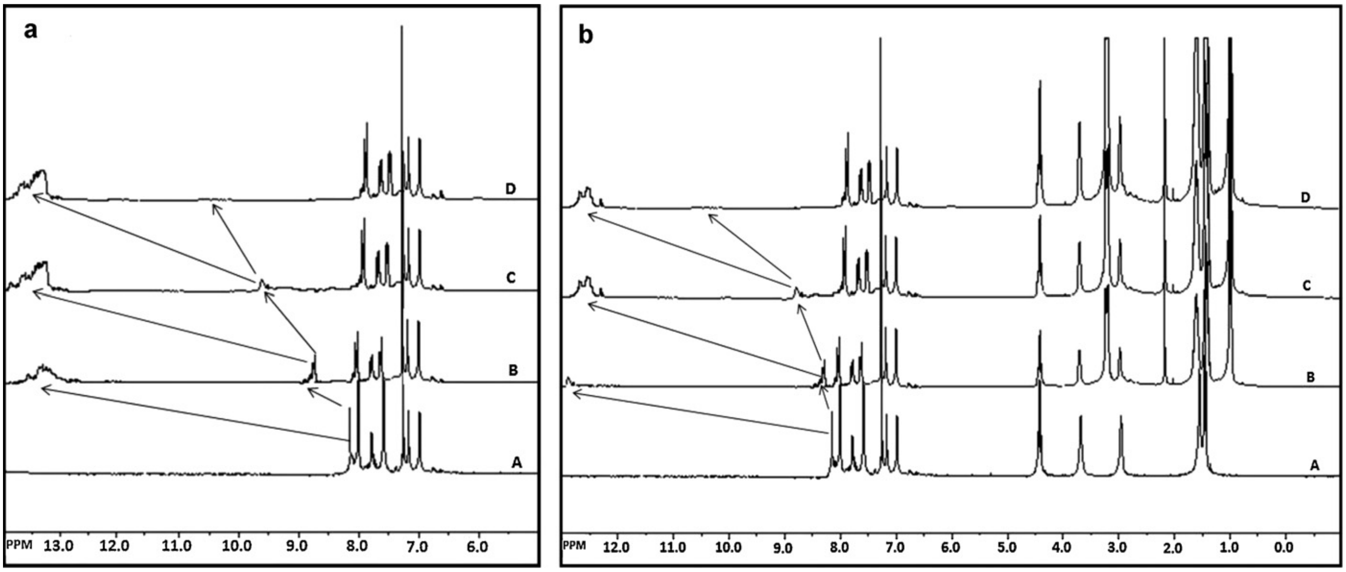

Fig. 5. (a) Partial ^1H NMR (400 MHz) spectra of tripodal host **2b** in DMSO-d_6, (A) in the absence, (B) presence of 1.0 equiv, (C) presence of 2.0 equiv and (D) 3 equiv of n-Bu_4NF. (b) ^1H NMR (400 MHz) spectra of tripodal host **2c** in DMSO-d_6, (A) in the absence, (B) presence of 1.0 equiv, (C) presence of 2.0 equiv and (D) 3 equiv of n-Bu_4NF.

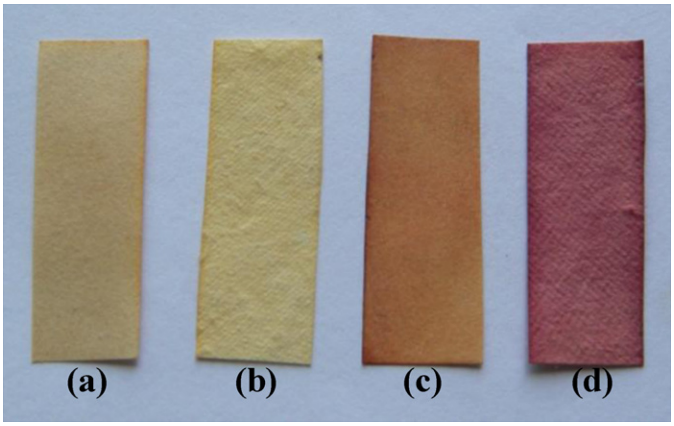

Fig. 6. Photographs of the test kits with **2b** for detecting fluoride ion in neutral aqueous solution with different F⁻concentrations. Left to right: (a) none (only **2b**), (b)10 mg/L, (c) 0.1 g/L, (d) 10 g/L.

inspecting F⁻ in aqueous environments by putting a filter paper ($2.0 \times 0.5\,cm^2$) into the acetonitrile solution of **2b** (2.0×10^{-3} M) and then drying it by two way (i) exposure to air and (ii) in vacuum (oven temperature 90–100 °C, 70 mmHg). The test strips containing **2b** were utilized to sense different anions. For detecting the fluoride anion in water, a test strips was immersed in the test aqueous fluoride containing solution (2 mM of **2b**) for several seconds and then exposed to air or in vacuum to remove water. An immediate obvious color change was observed only with fluoride solution. Fig. 6 exhibits the color changes of the test papers with different fluoride concentrations at pH ≈7.

When the fluoride ion concentration was increased, the color of the test strips changed from yellow to deep purple and it easily differentiable by naked eyes. Clearly, the test paper can detect F⁻ in aqueous solution at a low limit about 100 ppm (0.1 g/L). Other anions such as Cl⁻, Br⁻, I⁻, HSO_4^- and NO_3^- did not cause any detectable changes. In fact, sensing F⁻ in natural aqueous environments without any spectroscopic instrumentation has been very useful in preventing fluorosis caused by the fluoride toxicity [7] in undeveloped regions. However, since many other anions such as chloride were present in most water at much higher concentrations than fluoride, it was important to know that chloride (or other common anions) did not cause color changes at concentrations normally found in water. Consequently, the easy-to-prepare test kit can be utilized to roughly and quantitatively detect and estimate the concentration of fluoride ions.

In order to further develop its real-life application, we design the toothpaste experiment accordingly, we prepared the samples that contained the commercially available toothpaste 20 mg/ml, 2.0×10^{-3} M of F⁻ (as tetrabutylammonium salts) and 2×10^{-5} M of receptor **2b** in DMSO. Then, the UV–vis absorbance method for the mixture solutions were measured and compared to a toothpaste-free F− solution. It was shown (Fig. 7) that the signal from the F⁻ contaminated toothpaste solution (b) was stronger than that from the one without toothpaste (a), indicating the concentration of fluoride in solution (b) is higher than in (a). In this way receptor **2b** may be used in qualitative detecting fluoride in toothpaste. But detecting fluoride in the protic solution is called for further research.

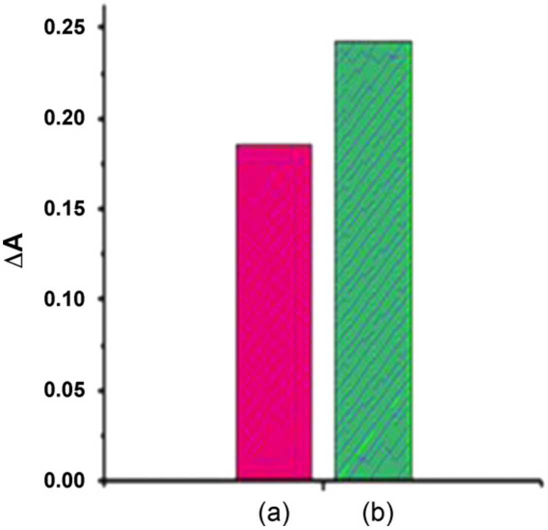

Fig. 7. The proof of concept for fluoride detection in toothpaste. (a) **2b** + TBAF; (b) **2b** + TBAF + toothpaste ($\Delta A = A_{host} + A_{guest} - A_{host\,only}$).

5. Conclusions

In conclusion, chromogenic tripodal receptors **2a**–**e** were synthesized in good yields via diazo coupling and Schiff's base condensation. Among them, the tripodal receptor **2b** has higher sensitive for the fluoride and there is a more prominent color change that can be observed by the naked-eyes. The tripodal receptor has been designed to provide anion recognition through H-bonding interactions employing phenolic–OH groups. However the results show that the deprotonation rather than the H-bonding is the key factor triggering the chromogenic effect. This deprotonation is being facilitated by the high intrinsic acidity of phenolic–OH groups, highly basic F⁻and OH⁻ions. The easy-to-prepare test kit based on the tripodal system provides a convenient and reliable detection of fluoride ions in everyday applications. Moreover, the receptor **2b** could detect the fluoride in toothpaste conveniently even at low concentration of F⁻, so it is expected to have many applications in the detection of anions in real-life.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.08.040.

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