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# DAB-Am-4 based colorimetric receptors for fluoride and pyrophosphate anions

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ABSTRACT

Flexible four armed polypropylenimine tetraamine dendrimer (DAB-Am-4) core linked through urea moieties to a chromophore (aza group) gave rise to the colorimetric sensor **1** for fluoride ions, and a fluorophore (naphthyl group) attached to a similar assembly resulted in **2** which showed an excellent association constant with pyrophosphate anions.



## 1. Introduction

Anion recognition and sensing has received considerable attention in recent years [1]. Anionic guests like pyrophosphate, phosphate, and fluoride are of particular interest as they possess important biological and medicinal properties and there have been a dearth of reports regarding their detection using colorimetric techniques [2].

The limited reports on colorimetric anion sensing and selective affinity describe the complexity of design and synthesis involved in the efforts to increase the receptor's intrinsic association as well as its specificity for the ion [3]. There are reports on urea linked aza-naphthalene derivatives as chemosensors which show visible color change for specific anions and most of such sensors are based on rigid framework of pyrene, anthracene, and naphthyl derivatives [4]. In contrast to such rigid systems, flexible dendritic exosystems have been studied as potent molecular sensors and their role as macromolecular hosts has been well documented in host–guest chemistry [5]. The peripheral groups determine many of physical properties of such exosystems, and cautious modifications in these end-groups result in interesting dendritic architectures with excellent sensing strength [6]. As they offer suitable binding sites for the guests and stabilize the complexes by non-covalent interactions like hydrogen and ionic bonding, urea based anion sensors are of utmost importance [7]. Owing to the strong and selective binding, these groups should be pre-organized to complement the target anion and minimize intramolecular interactions.

The non-covalent interactions like hydrogen bonding increases with the increase in the number of peripheral groups, thus four peripheral groups were ideally placed on four amino-terminal based DAB-Am-4 moiety which offers suitable interface for the anionic binding and makes a suitable core for the chemosensors. This flexible four armed skeleton offers appropriate distance between the urea functionalities for host–guest interaction and thus help achieve higher binding values [8]. The presence of electron withdrawing groups like nitro groups in the conjugated system enhances the charge transfer and hence induces the color change phenomenon [4a].

Keeping these features into consideration, we synthesized and now report that two highly flexible DAB-Am-4 based chemosensors equipped with a chromophore (aza) resulted in the colorimetric sensor **1** for fluoride ions, and a fluorophore (naphthyl) attached to a similar assembly resulted in **2** (Fig. 1) which showed high binding affinity with pyrophosphate anions in organic solvent.

## 2. Results and discussion

Reactions of 1:4 molar ratios of DAB-Am-4 (N,N,N′,N′-tetrakis(3-aminopropyl)-1,4-butanediamine) and 4-isocyanato-4′-nitroazobenzene or naphthyl isocyanate in acetonitrile followed

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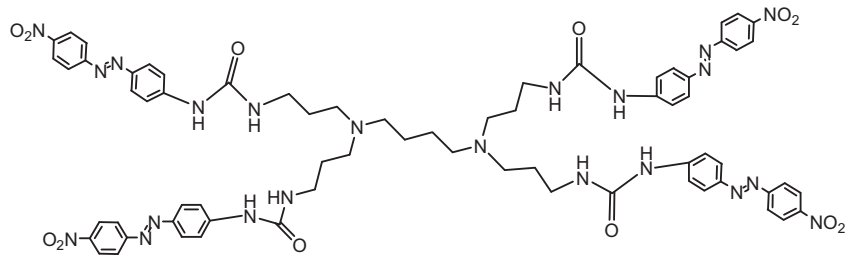
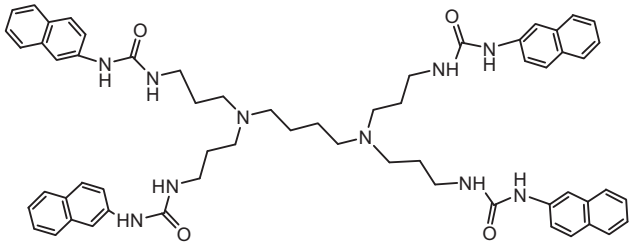
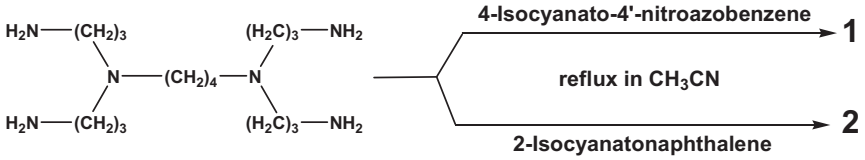


1



2

**Fig. 1.** Flexible DAB-Am-4 based chemosensors with aza (**1**) and naphthyl (**2**) moieties as sensing units.




**Scheme 1.** General synthetic scheme of receptors **1** and **2**.

by filtration afforded **1** as red color and **2** as colorless microcrystals, respectively (Scheme 1). These two new dendrimeric receptors were characterized by NMR ($^1$H and $^{13}$C), MS (FAB) and elemental analysis.

The colorimetric anion sensing capacity of **1** was studied by a visual inspection of the color changes (Fig. 2) induced for $CH_3CN$:DMSO (9:1) solutions of receptor **1** in the presence of various anionic guests (1:1000 equiv. ratio) in the form of their tetrabutylammonium salts (Fig. 3). It was observed that the host solution undergoes significant color changes from yellow ($\lambda_{max}$ = 401 nm) to blue (636 nm) upon the addition of fluoride anion (Table 1).

This shifting is ascribed to the presence of an extended π-conjugation network involving the 4-isocyanato-4′-nitroazobenzene moiety and the urea linker which are mounted on the dendrimer. Charge transfer occurred between the electron-rich donor units and the electron-deficient acceptor units which changed the color of the host solution.

**Table 1**
Absorption shifts of 0.1875 mM of **1** upon the addition of tetrabutylammonium salts of anions (18.75 mM) as 1:1000 equivalent ratio in $CH_3CN$:DMSO (9:1, v/v) mixture.

| Anion | Absorption shift (nm) **1** (401 nm) | Δλ (nm) |
|---|---|---|
| $Cl^-$ | 410 | 9 |
| $Br^-$ | 401 | 0 |
| $I^-$ | 401 | 0 |
| $F^-$ | 433 (636)* | 32 |
| $HSO_4^-$ | 413 | 12 |
| $CH_3COO^-$ | 425 | 24 |
| $H_2PO_4^-$ | 427 | 26 |
| $HP_2O_7^{3-}$ | 446 (641)* | 45 |

* New peak.

The stable complexes are formed by virtue of hydrogen bonding as the receptor binds with the incoming anion. This causes an increase in overall electron density in the extended conjugated supramolecular system, which promotes the charge transfer between electron-rich and electron-deficient moieties. This charge transfer between the electron rich donor units (anion bound urea groups) and the electron deficient p-nitrophenyl-phenyl-diazene moiety caused the color change in the host solution [4a]. On the other hand, exposure of receptor **1** to chloride, bromide, iodide, acetate, sulfate and phosphate anionic species, did not lead to any conspicuous change in color (Fig. 2), and no significant change in absorption spectra was observed, suggesting very weak binding for these anions to the receptor. This dramatic combination of anion-specific response/non-response makes this system an effective naked-eye-detectable anion sensor under solution-phase conditions. Appearance of another peak at 636 nm in the case of fluoride anion signifies a strong affinity and selectivity of the sensor **1** towards the fluoride anion (Fig. 2). Interestingly, addition of pyrophosphate resulted in a similar but weak peak at 641 nm with intensified yellow color (For interpretation of references to color in this sentence the reader is referred to the web version of this article.).

The binding studies of receptor **1** were carried out using UV-titration with the $HP_2O_7^{3-}$ and $F^-$ in $CH_3CN$:DMSO (9:1). The base peak ($\lambda_{max}$ = 401 nm) of receptor **1** was slightly red-shifted upon complexation with $HP_2O_7^{3-}$ and new absorption maxima developed at 635 nm (Fig. 5). Significant bathochromic shift for absorption maxima in the visible region upon the anion

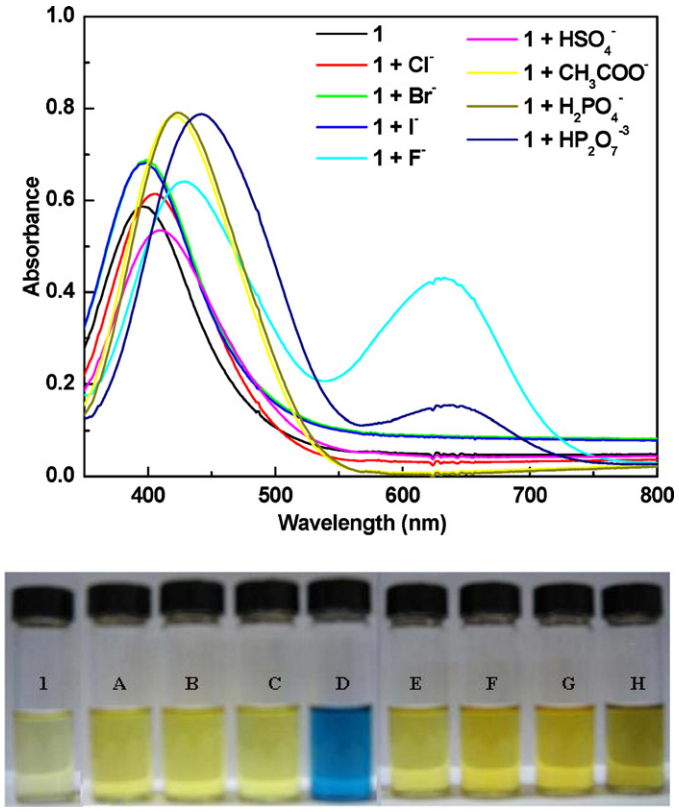



**Fig. 2.** (a) Absorption spectra of 0.01875 mM of **1** upon the addition of tetrabutylammonium salts of anions (18.75 mM) as 1:1000 equivalent ratio in $CH_3CN$:DMSO (9:1, v/v) mixture. Visual color changes for only **1** (free host), **1** + $F^-$ and **1** + $HP_2O_7^{3-}$ are shown. (b) Visual features of **1** upon the addition of tetrabutylammonium salts of anions (**1** = free receptor, A = **1** + $Cl^-$; B = **1** + $Br^-$; C = **1** + $I^-$; D = **1** + $F^-$; E = **1** + $HSO_4^-$; F = **1** + $CH_3COO^-$; G = **1** + $H_2PO_4^-$; H = **1** + $HP_2O_7^{3-}$).

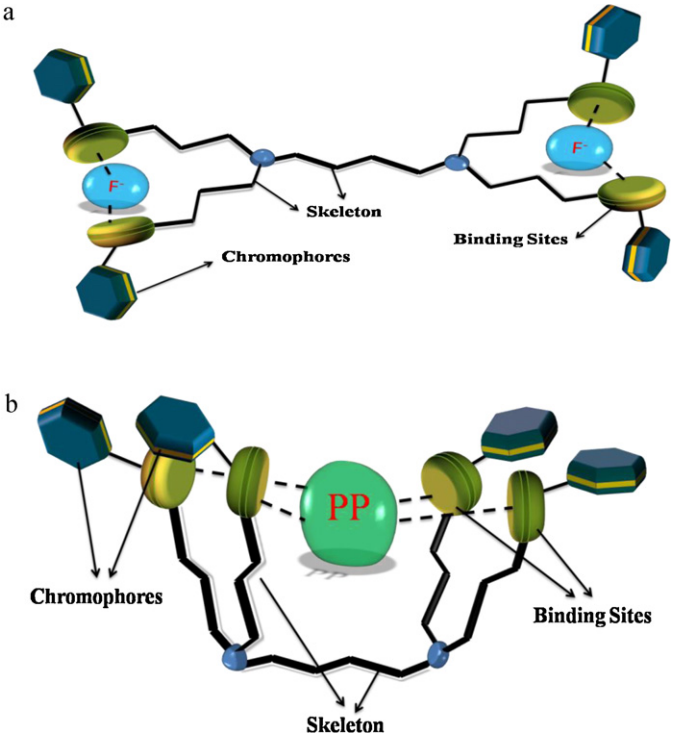



**Fig. 3.** (a) Proposed 1:2 binding of fluoride anions to the receptors. (b) Proposed 1:1 binding of pyrophosphate anion to the receptors, causing major conformational changes to the sensor.

complexation is presumably due to the charge-transfer phenomenon [9]. This also suggests that the excited state would be more stabilized by the anion binding [1b,7a]. The association constant for $HP_2O_7^{3-}$ by receptor **1** in DMSO is calculated to be 1180 $M^{-1}$ from the UV-titration. However, this association constant would be related to the deprotonation of the urea groups rather than the binding constant which can be studied from the NMR titration. The job plot analysis for receptor **1** with the $HP_2O_7^{3-}$ anion shows a maximum at a mole fraction of 0.5. This signifies that the host binds the guest in 1:1 ratio (Fig. 3a). On the other hand, the UV-titration data of receptor **1** with the $F^-$ anion show 1:2. This indicates that two urea groups act as cooperative binding sites (Fig. 3b). This can be explained as the DAB core being flexible enough to orient itself to tightly enclose the large pyrophosphate anion, while small anions ($F^-$, $Cl^-$, $Br^-$, and $I^-$) were not bound in this fashion (Fig. 4). The binding study of receptor **2** was carried out using $^1H$ NMR titration with various anions ($F^-$, $CH_3COO^-$, $HSO_4^-$, $H_2PO_4^-$ and $HP_2O_7^{3-}$) in DMSO-$d_6$ by monitoring the changes in chemical shift of the urea –NH proton. Addition of one equimolar amount of anion $HP_2O_7^{3-}$ as tetrabutylammonium salt to receptor

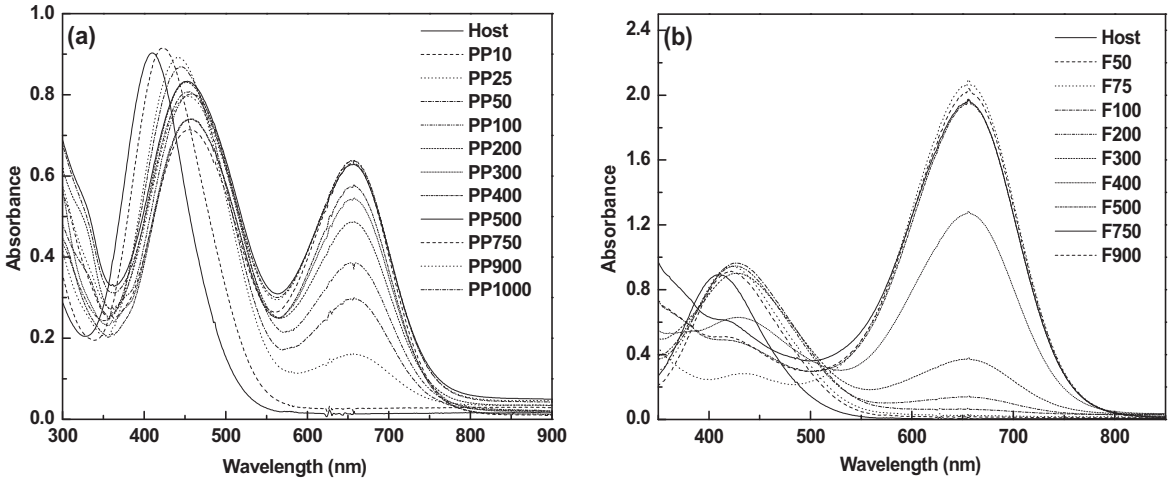



**Fig. 4.** UV–vis titration spectra for receptor **1** at room temperature (10 μM) with the increasing concentration of (a) $HP_2O_7^{3-}$ and (b) $F^-$ in $CH_3CN$:DMSO (9:1).

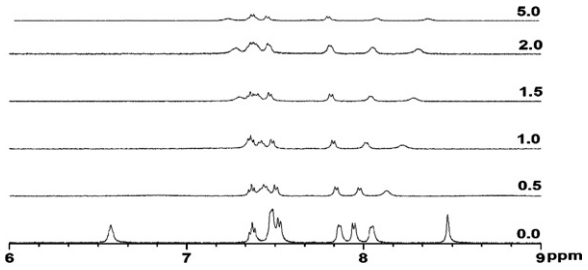



**Fig. 5.** The partial $^1$H NMR spectra of compound **2** in the presence of tetrabutylammonium fluoride in DMSO-$d_6$.

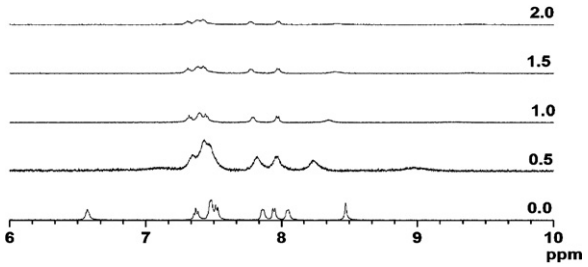



**Fig. 6.** The partial $^1$H NMR spectra of compound **2** in the presence of tetrabutylammonium $H_2PO_4^-$ in DMSO-$d_6$.

**Table 2**
Binding constants ($K_a$) and experimental Gibbs free energies ($\Delta G_{expt}$) for the interaction of **2** with anions.[a]

| Anion | $K_a$ ($M^{-1}$) | | $\Delta G_{expt}$ (kcal/mol) |
|---|---|---|---|
| | $K_1$ | $K_2$ | |
| $F^-$ | $>10^5$ | 12,000 | >7/5.56 |
| $CH_3COO^-$ | 19,400 | 4220 | 5.85/4.95 |
| $HSO_4^-$ | 165 | 19,000 | 3.02/5.84 |
| $H_2PO_4^-$ | $>10^5$ | 24,100 | >7/5.97 |
| $HP_2O_7^{3-}$ | 71,800 | – | 6.62 |

[a] $K_a$ and $\Delta G_{expt}$ were measured using $^1$H NMR titration in DMSO-$d_6$ (with ± 10% errors). The values of $K_1$ for $F^-$ and $H_2PO_4^-$ would not be so reliable because they could involve more complex binding patterns than the 1:2 binding which we assumed in the calculations.

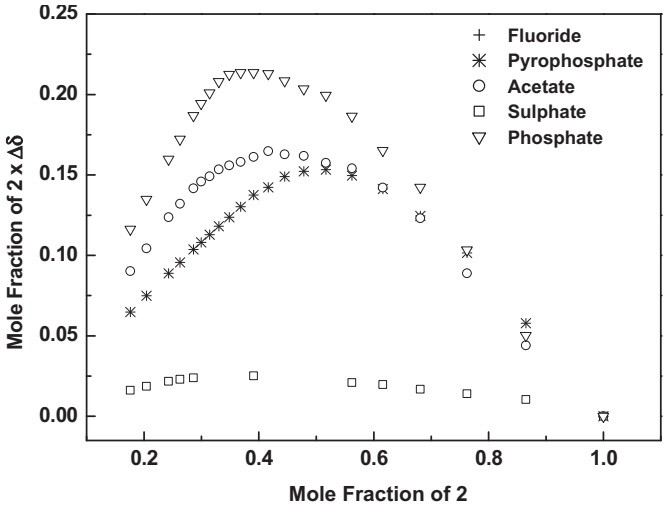



**Fig. 7.** $^1$H NMR titration job plot of **2** (1.0 mM) with various anions (4.0 mM) in DMSO-$d_6$.

**2** in DMSO-$d_6$ induced a downfield chemical shift upto 0.319 ppm. Further addition caused no significant shift in the spectrum as a whole (Fig. 6).

Job plot analysis showed a maximum at a mole fraction of 0.5, inferring the 1:1 binding stoichiometry. The association constant ($K_a$) of 71,800 $M^{-1}$ (Table 2) was calculated with the HOSTEST [10] program with a 1:1 binding profile (Fig. 7). However, the calculated association constant of $HP_2O_7^{3-}$ with **1** (1180 $M^{-1}$) using the UV absorption spectra is comparatively much smaller than that calculated from $^1$H NMR titration. Even though there are generally certain variations in the association constants depending on the method and concentration that we used for the titration, such a large variation is not anticipated. Therefore, we certainly believe that the change in the absorption spectra of **1** with the increasing concentration of $HP_2O_7^{3-}$ reflects the deprotonation of one or two urea groups. Therefore, this calculated value, more appropriately, seems to correspond to the inverse of deprotonation constant of **1** in the presence of $HP_2O_7^{3-}$. On the other hand, the $^1$H NMR titration curves were fit to 1:2 receptor-anion stoichiometry with all the other anions ($F^-$, $CH_3COO^-$, $HSO_4^-$ and $H_2PO_4^-$) and the binding constants were found to be $K_1 \gg K_2$ (except for the $HSO_4^-$) (Table 2). Another interesting point noticed was the upfield shift of the $-CH_2$ protons of the core with the increase in the anion concentration. The shift of these protons shows the conformational change during the reorientation of the flexible DAB structure and the peripheral groups. From the binding study experiments, we conclude that both receptors **1** and **2** are selective sensors for the $HP_2O_7^{3-}$.

## 3. Conclusion

In conclusion, we have synthesized a new class of easy-to-prepare four armed dendrimer based neutral receptors (**1** and **2**) which could effectively recognize biologically important anions such as $F^-$ and $HP_2O_7^{3-}$. We have also developed an interesting methodology that allows a simple colorimetric detection of anions employing these receptors. To the best of our knowledge, the receptor **1** is the first naked-eye-detectable chemosensor containing four-armed dendritic core that permits discrimination between different anionic substrates as a result of disparate change in color. The NMR calculations are in good agreement with chemical shift changes. Depending upon the size of the anion, the conformational changes and the reorientation of the flexible DAB structure has been observed. Molecular sensors of this kind are likely to be particular benefit in field tests and other situations, where sophisticated instrumentation might be lacking. Receptors **1** and **2** also prove to be very important contribution to the fast growing field of supramolecular anion recognition and sensing as selective sensors for the $HP_2O_7^{3-}$ anion along with receptor **1** being the selective colorimetric sensor for $F^-$.

## 4. Experimental

All new compounds were fully characterized with standard spectroscopic techniques. Microanalyses were performed on a Carlo 1102 elemental analysis instrument. Absorption spectra were recorded using a Shanghai 756 MC UV–vis spectrometer. $^1$H and $^{13}$C NMR spectra were performed on a Bruker Avance DPX500 (500 MHz) spectrometer at 298 K. High resolution mass spectra were obtained on a Micromass Platform II mass spectrometer. All reagents and solvents were purchased from Aldrich and used as such.

### *4.1. General procedure for the synthesis of receptor* 1 *and* 2

The DAB-Am-4 (N,N,N′,N′-tetrakis(3-aminopropyl)-1,4-butanediamine) was refluxed with corresponding isocyanates in dry acetonitrile at 90 °C for 8 h under inert atmosphere. The titled

compounds were isolated, purified and characterized by NMR ($^1$H and $^{13}$C), MS (FAB) and elemental analysis.

### 4.2. N1,N1,N4,N4-tetrakis(3-(N′-(1-(4-nitrophenyl)-2-phenyldiazenyl)-ureido)propyl)butane-1,4-diamine (1)

$^1$H NMR (500 MHz, $(CD_3)_2SO$, 25 °C) $\delta$ 9.11 (s, 4H, C–NH–CO–), 8.36 (t, 8H, ArH, $J$ = 10.78, 9.16 Hz), 7.95 (dd, 4H, CH=CH–, $J$ = 7.97, 9.64, 9.54 Hz), 7.86 (t, 4H, Ar–CH=CH–, $J$ = 9.48, 8.68 Hz), 7.51 (m, 16H, ArH), 6.45 (d, 4H, Ar–CH=CH–, $J$ = 14.34 Hz), 3.16 (d, 8H, –C–CH–N, $J$ = 23.84 Hz), 2.49 (t, 8H, –CH–N–CH–, $J$ = 17.67 Hz), 2.36 (s, 4H, –CH–N–CH–), 1.59 (d, 8H, –C–CH–C–, $J$ = 30.5 Hz), 1.39 (s, 4H, –C–CH–CH–C–); $^{13}$C NMR (125 MHz, $(CD_3)_2SO$) $\delta$ 26.4, 27.2, 40.2, 52.2, 54.8, 122.1, 123.8, 125.6, 125.8 139.2, 148.6, 151.3, 154.7, 156.3 FAB MS ($m/e$): 1389.6 (100.0%), 1390.4 (68.6%), 1392.18 (31.4%), elemental analysis for ($C_{68}H_{72}N_{22}O_{12}$) calcd: C, 58.78; H, 5.22; N, 22.18; O, 13.82 Found: C, 58.85; H, 5.62; N, 21.01; O, 13.12.

### 4.3. N1,N1,N4,N4-tetrakis(3-(N′-naphthyl-ureido)propyl)butane-1,4-diamine (2)

$^1$H NMR (500 MHz, $(CD_3)_2SO$, 25 °C) $\delta$ 8.47 (s, 4H, C–NH–CO–), 8.05 (d, 4H, ArH, $J$ = 8.33 Hz), 7.95 (d, 4H, Ar–CH=CH–, $J_{ortho}$ = 7.25 Hz), 7.86 (d, 4H, Ar–CH=CH–, $J$ = 4.45 Hz), 7.51 (d, 12H, Ar–CH–CH–, $J$ = 7.96 Hz), 7.38 (t, 4H, Ar–CH=CH–, $J$ = 7.79, 7.66 Hz), 6.57 (s, 4H, Ar–CH=CH–), 3.16 (d, 8H, C–CH–N, $J$ = 5.72 Hz), 2.42 (t, 8H, –CH–N–CH–, $J$ = 16.01 Hz), 2.36 (s, 4H, –CH–N–CH–), 1.59 (s, 8H, –C–CH–C–), 1.39 (s, 4H, –C–CH–CH–C–); $^{13}$C NMR (125 MHz, $(CD_3)_2SO$) $\delta$ 25.3, 28.2, 40.2, 51.9, 54.3, 117.5, 120.6, 122.2, 126.2, 126.5, 126.7, 129.2, 134.5, 136.0, 156.5. FAB MS ($m/e$): 993.7 (100.0%), 994.55 (75.2%), 995.55 (27.7%); elemental analysis for ($C_{60}H_{68}N_{10}O_4$) calcd: C, 72.55; H, 6.90; N, 14.10; O, 6.44; Found: C, 71.44; H, 6.98; N, 13.70; O, 6.34.

### 4.4. 4-Isocyanato-4′-nitroazobenzene

To a 50 mL three-neck round-bottom flask quipped with a reflux condenser fitted with a calcium chloride drying tube, a thermometer, and a magnetic stirring bar was added 4-amino-4′-nitroazobenzene (Disperse Orange 3, 2.06 mmol, 500 mg) in 25 mL freshly distilled THF and trichloromethyl chloroformate (4.14 mmol, 0.5 mL). The mixture was stirred and heated at 55–60 °C for 10 h. The reaction was monitored by TLC and the heating discontinued after 10 h. The resulting precipitate was filtered off and the product was obtained as red crystals in 80% yield; mp 109–110 °C; IR (KBr) 2255, 1736, 1595, 1522, 1341 cm$^{-1}$; Anal. Calcd for ($C_{13}H_8N_4O_3$): C, 58.19; H, 3.01; N, 20.89. Found: C, 58.20; H, 3.02; N, 20.90; MS ($m/z$) 268.08; $^1$H NMR (500 MHz, $(CD_3)_2SO$, 25 °C) $\delta$ 6.71 (d, 2H, –N–CH=CH–, $J_{ortho}$ = 8.36 Hz), 7.75 (d, 2H, –CH=CH–N–, $J_{ortho}$ = 8.46 Hz), 7.92 (d, 2H, –N–CH=CH–, $J_{ortho}$ = 8.60 Hz), 8.36 (d, 2H, –CH=CH–N–, $J_{ortho}$ = 8.38 Hz); $^{13}$C NMR (125 MHz, $(CD_3)_2SO$) $\delta$ 121.41, 123.93, 124.11, 125.36, 125.72, 135.82, 149.86, 150.62, 158.81.

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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.jfluchem.2011.12.016.

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