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Highly selective naked-eye anion sensors based on thioureido or amido calix[4]arenes

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Abstract: Calix[4]arene-thiourea and -tetraamide naked-eye receptors do not show any tendency to self-aggregation and are highly sensitive towards small monoanions; association constants in DMSO for halogenides (chloride to iodide) and HSO_4^- are <200 M^{-1}. Basic anions deprotonate both receptors leading to a high and selective optical readout. Binding constants for carboxylates, fluoride, and dihydrogen phosphate are three orders of magnitude higher (~10^5 M^{-1}) in case of the tetrathiourea receptor.

Keywords: amide; anion receptor; calix[4]arene; naked-eye sensor; thiourea.

1 Introduction

Recognition of anions for industrial, biological, or environmental purposes is still a challenge in today's supramolecular chemistry and has attracted considerable attention [1–10]. For the detection of small anions, many recognition moieties have been exploited and incorporated into a plethora of molecular scaffolds over the last decade. Most common receptors contain amide [11–13], urea [14], thiourea [15–18], imidazolium [19, 20], triazole [21, 22], or pyrrole units [23–26] and, therefore, rely on hydrogen bond interactions of the Y–H···X^- type. Especially, ureas or thioureas show very good performance as recognition units for anions, such as carboxylates and phosphates. Thioureas are more acidic compared to ureas and therefore broadly used as organo-catalysts. In this application, polar groups in the substrate are activated by hydrogen bonding towards the (thio)urea groups [27, 28]. Similarly, thioureas recognize anions utilizing two main binding motifs [29–31]: (a) binding of weakly basic anions by bifurcated simultaneous hydrogen bonding between both NH and the anion, (b) two-step binding of basic anions. The weakly acidic thiourea is first deprotonated and then hydrogen bonds are formed between the deprotonated receptor and the protonated anion. This binding geometry is usually observed for basic anions such as fluoride or acetate (cf. Fig. 3).

A colorimetric sensor based on two *p*-nitrophenyl substituted thiourea units on a cyclohexane scaffold has been proposed [32], and this simple receptor exhibits a high selectivity towards cyanide over other monoanions such as halogenides, carboxylates, or dihydrogen phosphate. Here, we report on the synthesis and supramolecular characterization of calixarene **1** decorated with four *p*-nitrophenyl thiourea recognition units and calixarene **2** bearing four amide binding sites (Scheme 1). In this way, multiple recognition sites are prearranged to fit an appealing geometric array for possibly cooperative binding of more than one thiourea to spherical, planar, or tetrahedral anions [33].

2 Results and discussion

The syntheses of receptors **1** and **2** start with the easily accessible 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene [34, 35]. Pure receptor **1** was obtained by reacting 4-nitrophenylisothiocyanate with the tetraaminocalix[4]arene scaffold in chloroform in high yield after simple precipitation and filtration. Reaction of 4-nitrobenzoyl chloride and the tetraamine under similar, non-optimized conditions yielded tetraamide **2.**

Before we could investigate possible anion binding of receptor **1** or **2**, it was necessary to inspect the self-aggregation behavior of these structures. Structurally similar urea-calixarene derivatives are well known for their capsule-like dimerization [36]. Therefore, we performed dilution experiments. In the concentration range, which could be followed by ^{1}H NMR spectroscopy (0.25–2.5 mM), no concentration-dependent changes of

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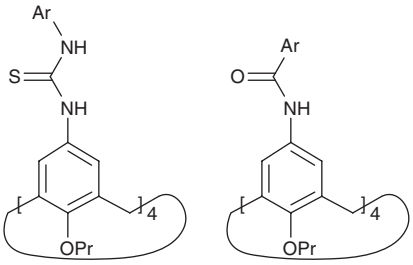


Scheme 1: Calix[4]arene-based receptors **1** and **2**.

chemical shifts could be observed. Similarly, at lower concentration ranges (0.75–10 μM), the UV/Vis spectra clearly obeyed the Lambert–Beer law. This rules out any dimerization/aggregation processes under conditions similar to the situations used later for the anion binding.

In a first screening experiment, various monoanions were added to separate DMSO solutions of the yellow receptor molecules **1** and **2** (Fig. 1). Basic anions (fluoride, acetate, benzoate, dihydrogen phosphate) exhibited a distinct color change from yellow to red in case of the tetrathiourea **1**. The addition of fluoride resulted in a deep red, nearly black solution. In contrast, the addition of

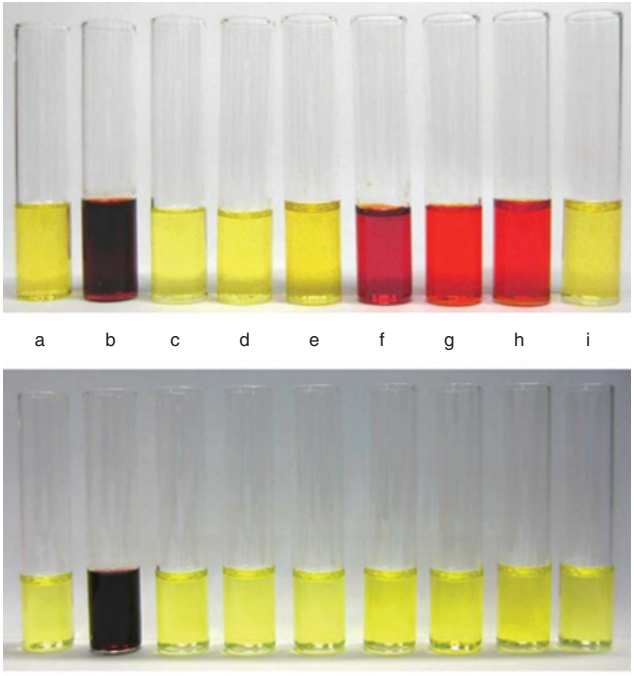


Fig. 1: Color change by the addition of various anions to receptors **1** (top) and **2** (bottom). a–f = no guest, F^-, Cl^-, Br^-, I^-, AcO^-, BzO^-, $H_2PO_4^-$, HSO_4^-.

the other halide ions or hydrogen sulfate gave no optical changes. The optical response of amide **2** was more selective. Here, only fluoride gave a deep-red color.

After this qualitative test for anion binding, anion recognition was quantified by standard ^1H NMR and UV/Vis spectroscopy on DMSO solutions at room temperature. All anions were added in the form of their tetrabutylammonium (TBA) salts.

Job plot analysis (Fig. 2, top) clearly supported a 1:1 stoichiometry for binding of chloride, bromide, iodide (data not shown), and hydrogen sulfate with thiourea **1** despite the available four binding sites in this receptor. This was further supported by the fact that the binding isotherms of these four anions could be fitted to a 1:1 binding model. Although more than one thiourea molecule and multiple H···X^- interactions are involved [11], the binding of the higher halogens is weak, as expected, and follows the usual trend (F^- >) Cl^- > Br^- > I^- [37].

Because of the distinct optical readout of receptor **1** with carboxylates, dihydrogen phosphate, and fluoride, the binding could be monitored at low concentrations using UV/Vis spectroscopy (Fig. 3). The addition of up to 10 equivalents of more basic anions (fluoride, acetate, benzoate, or dihydrogen phosphate) to a solution of receptor **1** gave rise to a new absorption band at 460–480 nm reflecting binding of the anion to one thiourea moiety. In case of benzoate or dihydrogen phosphate, fitting the spectral changes using a 1:1 binding model gave very good fits; the association constants K_{ass} are in the usual range for thiourea-based receptors (~10^5 M^{-1}).

Subtle changes in the basicity of the anion shifted the binding mode from a direct binding of the anions toward a two-step mechanism. Job plot analysis of the basic anions fluoride and acetate performed at the relatively high concentrations necessary for NMR studies indicates a totally different, erratic binding (Fig. 2, middle) compared to all anions discussed so far. Here it became obvious that the total amount of host and guest is important for the observed behavior. At the high concentrations used for the NMR measurement, no clear 1:1 motif is operational. (Multiple) deprotonation of the host by the basic anions now plays a dominant role and interferes with the pure 1:1 host–guest interaction. However, at the lower concentrations used for UV/Vis measurements (Fig. 2, bottom), a clean 1:1 stoichiometry is apparent by the Job plot analysis. Here, the total concentrations of anions are low and side reactions are suppressed.

The addition of fluoride or acetate to receptor **1** did not only increase the absorption at 460–480 nm but also changed the habitus of these spectra. Two isosbestic points (around 400 and 310 nm) are obvious and are an indication

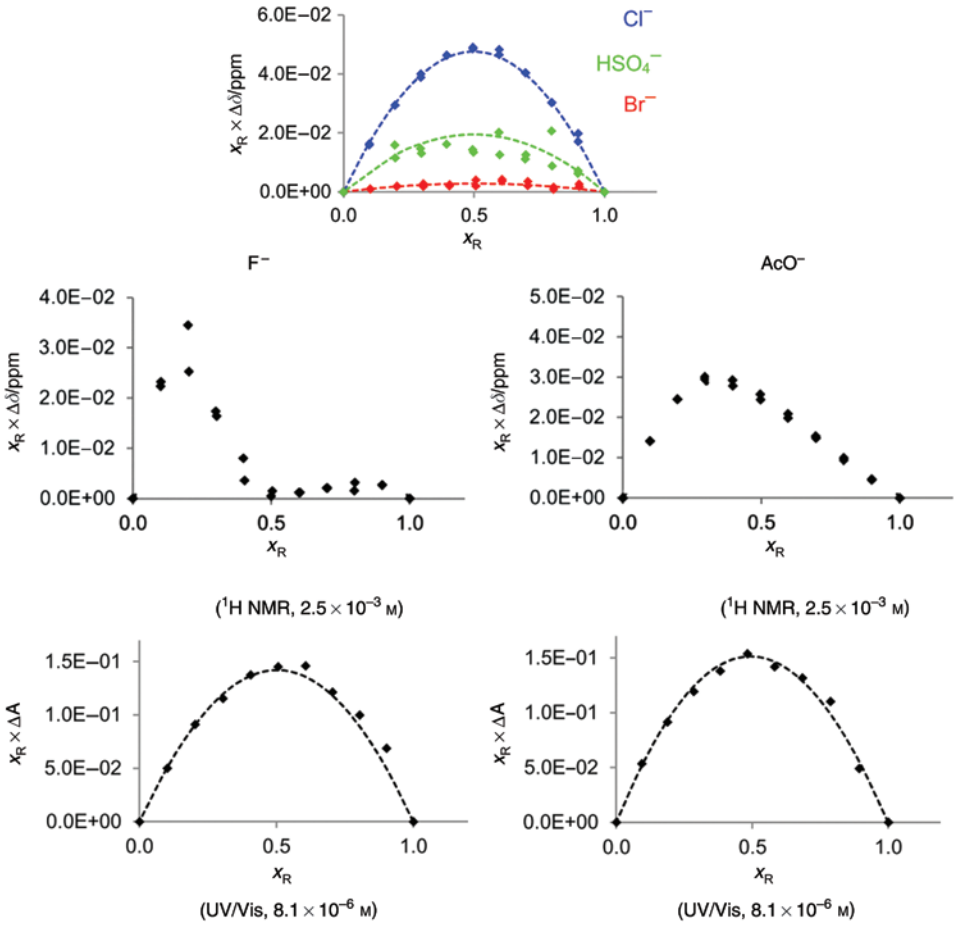


Fig. 2: Job plot analyses of the binding stoichiometry of host **1** with various anions in DMSO; spectroscopic methods and total concentrations of host and guest are given in the plots. (Dashed lines are added to guide the eye and indicate a 1:1 complex; x_R = mole fraction of the receptor. For NMR measurements $\Delta\delta$ (NH), for UV/Vis spectra the spectral changes at the individual maxima in the rage 460–480 nm are analyzed.)

for the aforementioned two-step deprotonation-binding process [30]. Similar optical changes could be observed when host **1** was deliberately deprotonated using tetrabutylammonium hydroxide. As depicted in Scheme 2, basic anions first deprotonate one of the four thiourea units and the resulting N^-/NH binding motif recognizes the carboxylic acid or HF [29–31]. However, it was not possible to evaluate which of the two possible NH protons is abstracted.

Quantitative evaluation of the binding of F^- and acetate was performed assuming a 1:1 binding isotherm. This estimation yielded association constants similar to dihydrogen phosphate.

When the UV/Vis spectra for titrations of receptor **1** with fluoride or acetate are compared with the data for titrations with dihydrogen phosphate, clear differences become apparent (Fig. 3). For dihydrogen phosphate, the proton transfer – usually indicated by the existence of isosbestic points – from the receptor to the guest (Scheme 2 and Fig. 3) seems to be negligible. Additionally, no protonated guest molecules could be detected by ^{1}H NMR spectroscopy. This indicates that dihydrogen phosphate is bound to receptor **1** via hydrogen bonds to the thiourea NH functions. For fluoride and acetate, the only slightly higher basicity leads to clear deprotonation of host **1** [38]. In case of benzoate as guest, the changes during the addition of the anion are not as distinct as with fluoride or acetate; the absorption band at 475 nm develops only slowly. This can be explained by the basicity of the anions (pK_a in DMSO [39, 40]: HF: 15, HOAc: 12.3, $PhCO_2H$: 11.1, H_3PO_4: 10.1, pK_a for **1** should be ~12 [30]), which indicates that benzoate does not fully deprotonate receptor **1.** Anion basicity clearly correlates with the response in UV/Vis titrations with weakly basic ($H_2PO_4^-$), intermediately basic (benzoate), and sufficiently basic (F^-) anions.

In other words, deprotonation of the receptor by the anion leads to strong optical response. Therefore, the acidity of the host should determine the selectivity of hosts for naked-eye sensing of the most basic anion (F^-). Therefore,

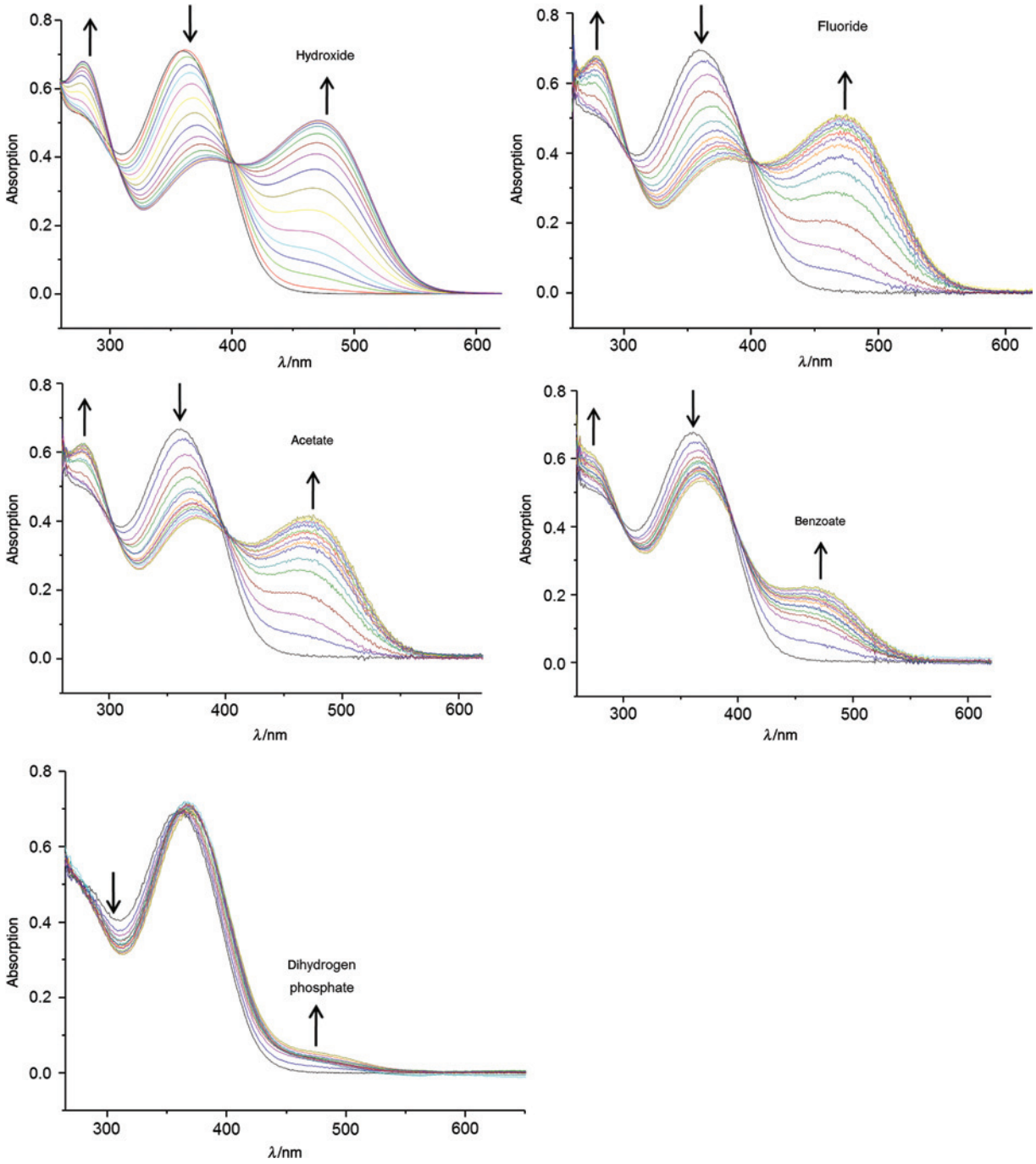
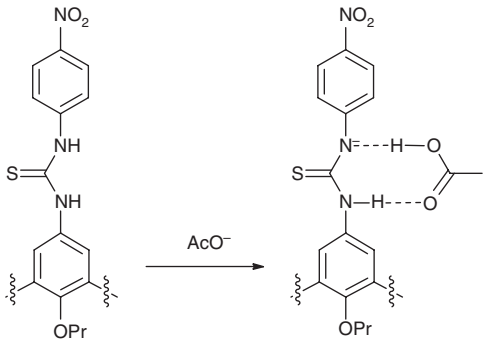


Fig. 3: Changes in the UV/Vis spectra of host **1** upon the addition of anions in DMSO (addition of 5 μL aliquots of guest to 3 mL host solution). [H] = 9.9–9.6 × 10^{-6} mol L^{-1} (considering dilution), [G] = 0.0–9.6 × 10^{-5} mol L^{-1}; variation [G]:[H] = 0 → 10.

we hypothesized that the weaker acidic tetraamide **2** could be a more selective receptor for fluoride compared to **1**. Receptor **2** indeed shows selective optical response for fluoride (Fig. 1) because fluoride is the only anion tested that is able to deprotonate this host. All observed binding constants for the interaction of receptor **2** with the less basic anions (Table 1) are lower by at least one order of magnitude compared to binding with **1**.

In summary, two easily accessible chromogenic anion sensors based on the calix[4]arene scaffold are reported. Anion binding occurs via two different binding modes depending on the basicities of the host and anion. Receptor **1** is very sensitive; a fluoride concentration of 7 × 10^{-5} mol L^{-1} can be easily detected by the naked eye using a 10^{-4} mol L^{-1} solution of host **1**. Furthermore, receptor **2** is highly selective for the fluoride anion making it



Scheme 2: Probable binding mode of acetate to host **1**.

a good candidate for applications, e.g. for detection of fluoride in the environment.

3 Experimental section

3.1 Synthesis of receptor 1

A solution of 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (2.00 g, 30.6 mmol) in anhydrous chloroform (20 mL) was added to a suspension of 4-nitrophenyl isothiocyanate (2.43 g, 135 mmol) in anhydrous chloroform (30 mL). After stirring at ambient temperature for 5 min, the solution became turbid and receptor **1** precipitated in the form of a yellow solid. After stirring for 12 h, the solid was filtered off and suspended in boiling THF for 2 h. The pure receptor **1** (4.03 g, 28.5 mmol, 93 %) was obtained by filtration of the cold suspension. NMR measurements were performed with fresh solutions in [D_6]DMSO due to slow decomposition of receptor **1**. M.p. 190–191 °C. – IR (KBr disk, neat): ν = 3343 (w), 3185 (w), 2963 (w), 1596 (m), 1556 (m), 1508 (m), 1336 (s), 1263 (m), 1217 (m), 1111 (w), 1000 (w), 961 (w), 850 (w), 748 (w), 699 (w) cm^{-1}. – ^{1}H NMR (400.13 MHz, [D_6]DMSO): δ = 0.99 (t, J = 7.4 Hz, 12 H), 1.91 (sext, J = 7.5 Hz, 8 H), 3.19 (d, J = 13.1 Hz, 4 H), 3.82 (t, J = 7.3 Hz, 8 H), 4.36 (d, J = 12.8 Hz, 4 H), 6.92 (s, 8 H), 7.76 (d, J = 9.1 Hz, 8 H), 8.10 (d, J = 9.2 Hz, 8 H), 9.79 (s, 4 H), 10.01 (s, 4 H) ppm. – ^{13}C NMR (100.62 MHz, [D_6]DMSO): δ = 178.2, 153.5, 146.2, 142.1, 134.4, 132.6, 124.2, 123.4, 121.3, 76.6, 30.4, 22.8, 10.2 ppm. – Anal. for $C_{68}H_{68}N_{12}O_{12}S_4$: calcd. C 59.46, H 4.99, N 12.24; found: C 59.36, H 5.16, N 12.19 %.

3.2 Synthesis of receptor 2

Under inert gas, 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (1.40 g, 2.15 mmol, 1.0 eq.) was dissolved in dichloromethane (DCM) (4 mL) and Et_3N (1.20 mL, 870 mg, 8.60 mmol, 4.0 eq.) was added dropwise at 0 °C. After the dropwise addition of a solution of 4-nitrobenzoyl chloride (1.60 g, 8.60 mmol, 4.0 eq.) in DCM (4 mL), the reaction mixture was stirred for additional 16 h at room temperature. During that time, a yellow precipitate formed which was filtered off, washed with DCM, and dried in vacuo. The raw product was recrystallized four times from $CHCl_3$-MeOH yielding 558 mg (447 µmol, 21 %) pure product as a shiny yellow powder. M.p. 231–234 °C. – IR (KBr disk, neat): ν = 3419 (br), 3282 (w) (N–H); 3106 (w), 3098 (w), 3083 (w), 3074 (w), 3026 (m), (Ar–H); 2963 (br), 2924 (m), 2871 (br) (C–H); 1657 (m), 1652 (m) (C=O); 1599 (s) (C=C); 1519 (s), 1516 (s) (N=O); 1490 (w); 1478 (m); 1464 (s); 1451 (m); 1418 (m); 1383 (w); 1346 (s) (N=O); 1322 (w); 1302 (m); 1271 (m); 1215 (s); 1104 (m) (C–O); 1065 (m); 1033 (m); 1004 (m); 962 (w); 926 (w); 865 (m), 849 (s) (Ar–H); 777 (w); 761 (m); 713 (s); 692 (m); 653 (w); 583 (m); 534 (m); 504 (m); 453 (m) cm^{-1}. – ^{1}H NMR (300.13 MHz, [D_6]DMSO): δ = 1.00 (12 H, t, J = 7.3 Hz, CH_3), 1.89–2.01 (8 H, m, CH_2CH$_3$), 3.22 (4 H, d, J = 12.8 Hz, ArCH_2Ar), 3.86 (8 H, t, J = 7.2 Hz, OCH_2), 4.46 (4 H, d, J = 12.8 Hz, ArCH_2Ar), 7.25 (8 H, s, Ar*H*), 8.04 (8 H, d, J = 8.7 Hz, C*H*CHCNO$_2$), 8.20 (8 H, d, J = 8.7 Hz, CHC*H*CNO$_2$), 10.17 (4 H, s, N*H*) ppm. – ^{13}C NMR (100.62 MHz, [D_6]DMSO): δ = 10.20 (*C*H$_3$), 22.71 (*C*H$_2$CH$_3$), 31.00 (ArCH$_2$Ar), 76.56 (OCH$_2$), 120.71, 123.27, 129.03, 132.77, 134.26, 140.54, 148.84, 152.82 (Ar*C*), 163.03 (*C*O) ppm. – MS (MALDI-TOF, dhb): m/z = 1273 [M+Na+2H]$^+$, 1249 [M+H]$^+$

Table 1: Association constants of hosts **1** and **2** with various anions in DMSO.

Host	Guest[a]	K_{ass} (M^{-1})[b]	Method
1	Cl^-	215	^{1}H NMR
1	Br^-	25	^{1}H NMR
1	I^-	<5	^{1}H NMR
1	HSO_4^-	90	^{1}H NMR
1	$H_2PO_4^-$	7×10^4	UV/Vis
1	$PhCOO^-$	9×10^4	UV/Vis
1	H_3COO^-	7×10^4	UV/Vis
1	F^-	6×10^4	UV/Vis
2	Cl^-	6	^{1}H NMR
2	Br^-	<5	^{1}H NMR
2	I^-	<5	^{1}H NMR
2	HSO_4^-	400	^{1}H NMR
2	$PhCOO^-$	600	^{1}H NMR
2	H_3CCOO^-	100	^{1}H NMR

[a]All guest anions were added as TBA salts. Concentration range: ^{1}H NMR: c(host) = 2.5×10^{-4} mol L^{-1}, $c(X^-) = 0.0–2.5 \times 10^{-2}$ mol L^{-1}; UV/Vis: c(host) = $9.9–9.6 \times 10^{-6}$ mol L^{-1} (considering dilution), $c(X^-) = 0.0–9.6 \times 10^{-5}$ mol L^{-1}.

[b]Based on a 1:1 host/guest binding motif, errors ±20 %. ChemEqui [41] was used for quantitative evaluation.

(calcd. 1248.4 for $C_{68}H_{64}N_8O_{16}$). – Anal. for $C_{68}H_{64}N_8O_{16} \times {}^1/_2 CHCl_3$: calcd. C 62.85, H 4.97, N 8.56; found: C 63.12, H 5.24, N 8.72 %.

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