Synthesis and anion binding properties of novel 3,12- and 3,7-bis(4′-nitrophenyl)-azo-calix[4]pyrrole receptors†

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Two novel 3,12- and 3,7-bis(4′-nitrophenyl)-azo-5,5,10,10,15,15,20,20-octamethyl calix[4]pyrroles were prepared and studied as potential anion binders for AcO$^-$ and $H_2PO_4^-$; the isomeric pair not only allowed for the colorimetric detection but also helped to discriminate the geometrically different anions.

The development of anion sensors and receptors is an important area of supramolecular chemistry due to their importance in a wide range of chemical and biological processes.[1] Colorimetric recognition has gained popularity in recent years because a shift of an absorption band is often intrinsically ratiometric, avoiding the necessity for an internal reference as well as offering the possibility of "naked eye detection".[2] Although, a few colorimetric anion sensors are able to discriminate selectively between anionic substrates of similar basicity and surface charge density, simple and reliable sensors with a high substrate affinity and selectivity for anions are desirable.

The rich and unique anion complexation behavior of 5,5,10,10,15,15,20,20-octamethylcalix[4]pyrrole (OMCP; **1**) has prompted the synthesis of a range of calixpyrrole (CP) derivatives with inherent affinities and selectivities toward anions of different sizes, shapes and basicities.[3]

Electrophilic substitution (ES) is an important method to prepare chromogenic CPs[4a–d] and their N-confused derivatives.[4e–g] However, much less attention has been paid to the synthesis of disubstituted products by ES,[5] presumably due to tedious chromatographic separations. Recently, we reported that chromogenic calix[4]pyrroles based on an azo dye skeleton are excellent sensors for fluoride.[6] In this Letter, we describe the synthesis, characterization and anion complexation behavior of two novel bisazo-calix[4]pyrroles,[7] namely, 3,12-bis(4′-nitrophenyl)-azo-5,5,10,10,15,15,20,20-octamethyl calix[4]pyrrole (**5**) and 3,7-bis(4′-nitrophenyl)-azo-5,5,10,10,15,15,20,20-octamethyl calix[4]pyrrole (**6**), using different spectroscopic tools (Fig. 1).

The cyclocondensation of acetone and pyrrole catalyzed by Amberlyst-15 in DCM afforded OMCP **1** and NC-OMCP **2**, as reported previously by us.[8] The reaction of **1** and **2** with an equimolar quantity of 4-nitrophenyldiazonium chloride afforded **3** and **4** in 15 and 21% yields, respectively. Furthermore, the reaction of **1** with two equiv. of 4-nitrophenyldiazonium chloride led to a rapid reaction, but scrambling was observed. This scrambling was supposed to be due to the formation of a variety of substitution products on the eight equally reactive pyrrole β-positions. In addition, the quantity of acid used under experimental conditions may also engender some decomposition products. Nevertheless, an analysis of the crude mixture by TLC (SiO_2, 82 : 18 hexanes/ethyl acetate) showed two components in significant concentrations that were later characterized as the bisazo-CP **5** (R_f 0.84) and bisazo-CP **6** (R_f 0.46) with the lowest contamination of **3** (R_f 0.92).

Column chromatography and subsequent preparative thin layer chromatography (PTLC) afforded **5** and **6** as red solids in 6 and 3% yields, respectively. The ESI-HRMS analysis of **5** and **6** revealed signals of m/z = 726.3388 and 726.3395, respectively, corresponding to the molecular ion $M^{\bullet+}$ as the major peak in both cases (calc. mass $M^{\bullet+}$ = 726.3391).

The ^{1}H NMR spectrum of **5** (Fig. S11, ESI†) recorded in DMSO-d_6–0.5% water at 298 K showed three singlets at δ 11.7 (2H, broad), 10.3 (1H) and 10.1 (1H), which can be assigned to the three chemically-non-equivalent NH protons. The three singlets at δ 6.93, 6.04 and 5.72, integrated as two protons each, can be assigned to the β-pyrrolic protons. In addition, the two singlets of equal intensity at δ 1.68 and 1.22 (each for 12H) are assigned to the two chemically-non-equivalent methyl protons. It is worth mentioning here that both set of resonances in **5** (–NH and β-pyrrolic) experienced significant

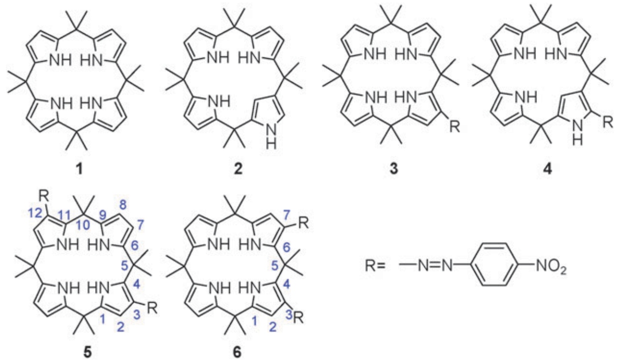

Fig. 1 The structures of OMCP (**1**), NC-OMCP (**2**), 2-arylazo-OMCP (**3**), 2-arylazo-NC-OMCP (**4**), and 3,12- (**5**) and 3,7- (**6**) disubstituted azo-OMCPs.

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† Electronic supplementary information (ESI) available: Experimental details, product characterization of **1–6**, copies of ^{1}H and ^{13}C NMR of **1–6**, job plots, UV-vis and ^{1}H NMR titration spectra. See DOI: 10.1039/c0nj00315h

downfield shifts (δ 2 for NH and δ 0.2–0.8 for β-pyrrolic) relative to **3**. This is presumably as a result of disubstitution. Furthermore, if the two azo units in **5** had been located opposite one another, there would have been a high degree of symmetry in the molecule and consequently only two NH resonances would have been observed. Thus, the appearance of three NH resonances in **5** leads to the conclusion that disubstitution occurred at positions 3 and 12 rather than at positions 3 and 13.

The ^{1}H NMR spectral characteristics of **6** were found to differ significantly from those of **5**. In particular, for **6** at 298 K, the ^{1}H NMR spectrum revealed only two NH resonances at δ 11.7 (broad) and 9.99, integrating as two protons each. The rest of the signals (β-pyrrolic) were nearly the same relative to **5** (Fig. S12, ESI†). Unexpectedly, only two peaks (δ 2.30, 6H and 1.49–1.52, 18H) for the methyl protons were recognized in DMSO-d_6. However, the ^{1}H NMR spectrum of **6** in CD_2Cl_2 showed three clear resonances, integrating to 6H, 12H and 6H, respectively, for the methyl protons. This assignment is in agreement with that made in the case of the bisTTF-calix[4]pyrrole (*i.e.*, two chemically-non-equivalent NH protons).[9] Further support for the proposed structure of **6**, namely one where the two azo dye units are located next to each other, came from an analysis of the ^{13}C NMR spectrum. Specifically, unlike **5**, two additional signals for the methyl and *meso*-C were observed in the ^{13}C NMR spectrum of **6**, precluding disubstitution at positions 3 and 7.

Dramatic changes in color were induced upon addition of anions as their TBA salts to solutions of **3–6** (50 μM in DMSO). Specifically, the initial orange solution of **5** turned blue, greenish-black and deep red upon the addition of 10 equiv. of F^-, $H_2PO_4^-$ and AcO^-, respectively. Compound **6** showed a similar response, manifested in a color change from yellow to blue, green and red in the presence of F^-, $H_2PO_4^-$ and AcO^-, respectively (Fig. 2). Such large color transitions are also reflected in the pronounced bathochromic displacement of the absorption bands in **5** and **6** with a 10-fold excess of anions. Interestingly, the observed color modulations of **5** and **6** are comparable with **4** for F^- and AcO^-. The addition of Cl^-, Br^-, I^- and HSO_4^- to solutions of **5** and **6** resulted in no changes of color.

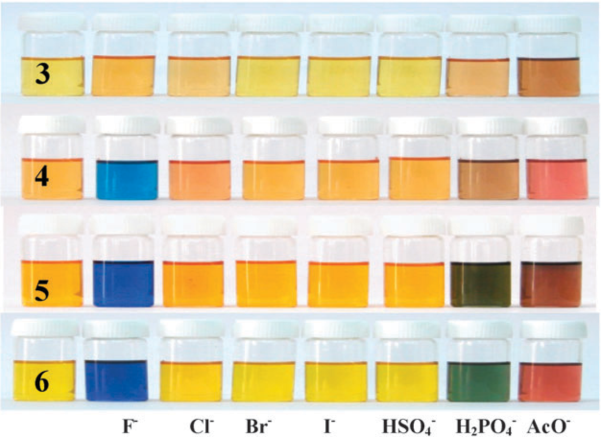

Fig. 2 Solutions of sensors **3–6** (50 μM in DMSO) in the presence of an excess of various anions (10 equiv.).

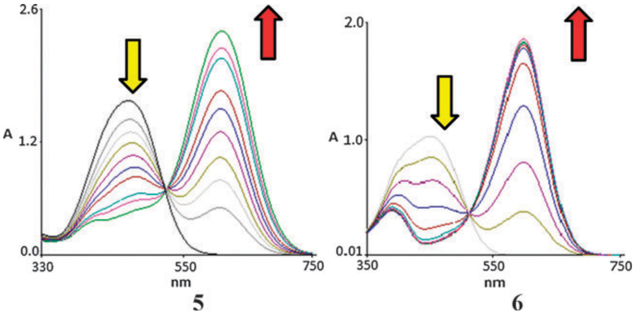

Fig. 3 Absorption spectroscopic titrations of sensors **5** and **6** (50 μM in DMSO) with F^-.

UV-vis titrations were performed to determine the binding constants (Table 1) for complex formation between the anions and the investigated azo sensors. Upon addition of F^- into a DMSO solution of **5**, the characteristic absorption peak at 463 nm decreased gradually and a new maxima at 608 nm appeared. Likewise, the addition of F^- to **6** shifted the absorption peak from 450 to 597 nm ($\Delta\lambda_{max}$ = ~145–140 nm, Fig. 3). On the contrary to what observed with F^-, the degree of red shift, as well as absorption intensity, determined for **5** and **6** with $H_2PO_4^-$ and AcO^- were significantly low. The addition of Cl^-, Br^-, I^- and HSO_4^- did not cause any noticeable spectral change, even in the presence of a high excess of anions.

A 1 : 1 stoichiometry (Fig. S15, ESI†) was determined in all cases by the continuous variation method. The data presented in Table 1 are in good agreement with those reported previously,[4b] and reveals that isomeric pair **5** and **6** display an augmented affinity and selectivity toward AcO^- and $H_2PO_4^-$ relative to their counterparts **3** and **4**, indicative of the positive effect of disubstitution on anion binding.

In general, the results reflect the expectation that the attachment of nitrophenyl azo substituents at two β-positions of OMCP should enhance the acidity of the pyrrole NH, which in turn increases the availability of the NHs for hydrogen bonding and the affinity of the sensor toward anions. Potentially, the increased pyrrole NH acidity may result in pyrrole deprotonation, specifically with highly basic F^-[10a–d] as well as with less basic AcO^-, BzO^- and $H_2PO_4^-$, as demonstrated in several ureas and thioureas having nitro substituents.[10e–h]

To obtain more clarity of the F^- response, the UV-vis spectra of **5** and **6** were recorded in the presence of Bu_4NOH. As expected, the spectra of **5** and **6** in the presence of F^- were almost identical with those of the free ligands in the presence of hydroxide, suggesting deprotonation. Furthermore,

Table 1 Affinity constants (K_a)[a] for **3**, **4**, **5** and **6** (M^{-1}), and anionic substrates in DMSO (50 μM, 0.5% water at 22 °C)

Anion[b]	Sensor **3**	Sensor **4**	Sensor **5**	Sensor **6**
Cl^-	750 (735)[c]	< 50 (43)[c]	78	85
HSO_4^-	89	< 50	191	151
$H_2PO_4^-$	3100	295	9560	8497
AcO^-	8470	14230	19700	19234

[a] Determined from absorption spectroscopic titrations; see ref. 11. [b] F^- was excluded due to its special behaviour, as described in the text. [c] Determined by NMR titration; errors are < ±15%.

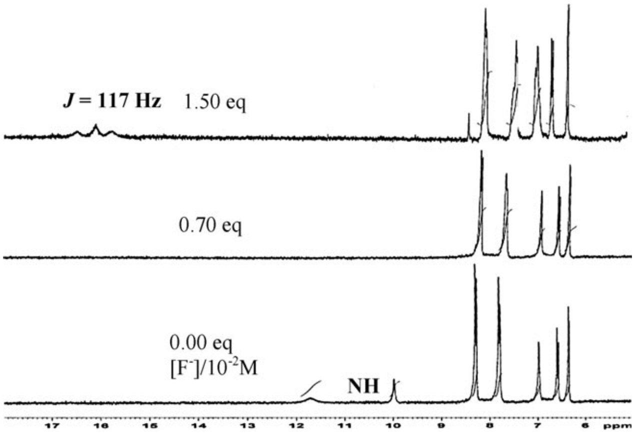

Fig. 4 ^{1}H NMR titration of sensor **6** (10 mM) in DMSO-d_6 with F^-.

quantitative NMR titrations essentially confirmed this. For instance, upon the gradual addition of rigorously dried F^- to a DMSO-d_6 solution of **6**, a new 1 : 2 : 1 triplet ($\delta \sim 16$, $^1J_{HF} \approx 117$ Hz)[12a] signal originated at the expense of the pyrrolic NHs (Fig. 4) that could be attributed to the bifluoride ion (FHF^-). Indeed, in recent years, such kinds of HF_2^- recognition (deprotonation) have been exploited in a variety of fluorogenic/chromogenic anion sensors.[12b–e] On the contrary to that observed with **6**, in the ^{1}H NMR titration experiment between **5** and F^-, HF_2^- formation was not recognized; however, the aromatic protons engendered a significant upfield shift. Again, this suggests that the NH protons in **5** undergo deprotonation, as reported in the literature.[12e,13]

The ^{1}H NMR titrations of **5** and **6** with Y-shaped AcO^- and tetrahedral $H_2PO_4^-$ in DMSO-d_6 showed different patterns. It was found that only after the first addition of AcO^-, the two NHs of **5** at δ 11.7 disappeared while other two NH signals at δ 10.3 and 10.1 moved closer and underwent a continuous downfield shift (*ca.* 1.3 ppm) with increasing AcO^-, consistent with the formation of **5**–AcO^- hydrogen bonds (Fig. 5).‡

This behavior is consistent with two sequential steps, *i.e.* upon the first addition of fluoride, deprotonation occurs at the most acidic azo-substituted pyrrolic NH groups. The resulting deprotonated form may be stabilized by strong intramolecular interactions, and localization of the negative charge on the pyrrole nitrogen may lead to a greater degree of stabilization. The second addition of fluoride causes hydrogen bonding with the other NHs.

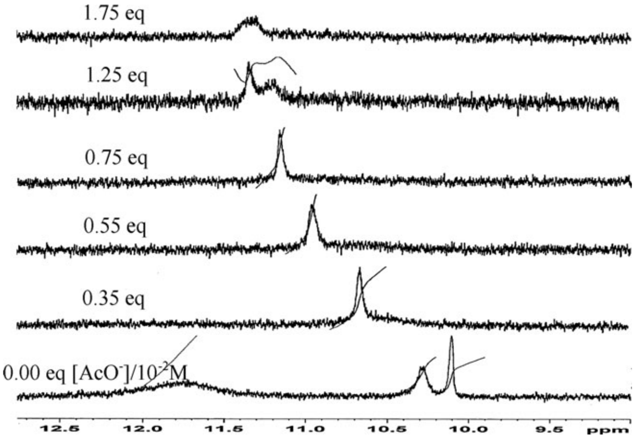

Fig. 5 The ^{1}H NMR titration profile (selected region, only NHs) of sensor **5** (10 mM) in DMSO-d_6 in the presence of AcO^-.

On careful examination of the titration spectra, it was observed that the β-pyrrolic protons of **5** and **6** became broadened and experienced a significant upfield shifting ($\Delta\delta_{\beta\text{-pyrrolic}} = 0.05$ to 0.1), since anion binding to the pyrrole NHs is expected to increase the electron density on the pyrrole ring and engender upfield shifts in the β-pyrrolic CH signals. The observed spectral shifts are consistent with the structural changes that cause the respective protons to move away from the CP plane upon anion binding. The experimental observations suggest that **5** and **6** attain a preferential cone conformation upon anion binding, which is in agreement with the various theoretical studies reported previously.[14] The exact nature of these structural changes, however, must await a more detailed analysis in the solid state.

Since the solvent effect§ on the binding of different ions to CP has been the subject of several experimental[15a–d] and theoretical studies,[15e] the ^{1}H NMR titrations of **5** and **6** were performed with AcO^- in CD_2Cl_2. The titration experiments showed the expected shifts of the protons, consistent with the significant conformational changes taking place.

In summary, the synthesis, characterization and anion complexation of bisazo-calix[4]pyrroles have been undertaken under different conditions. The isomeric pairs allow for the colorimetric detection of F^-, $H_2PO_4^-$ and AcO^- ions in DMSO, as well as helping to discriminate these geometrically different anions, which remains a challenge in supramolecular anion coordination chemistry. The selectivity trends in the binding affinities of the studied anions for sensors **5** and **6** in DMSO were determined to be: $AcO^- > H_2PO_4^- > HSO_4^- > Cl^-$. The observed binding order can be rationalized based on guest basicity. Anion binding with isomeric disubstituted calix[4]pyrroles may be extended to organic catalysis, the mimicking of enzyme reactions and the development of sensors for environmental chemistry.

Notes and references

‡ A small but definite downfield shift (*ca.* δ 0.7) of the NHs were observed upon addition of $H_2PO_4^-$.

§ Associating solvents like H_2O and MeOH show high competition between ion solvation and ion complexation.

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