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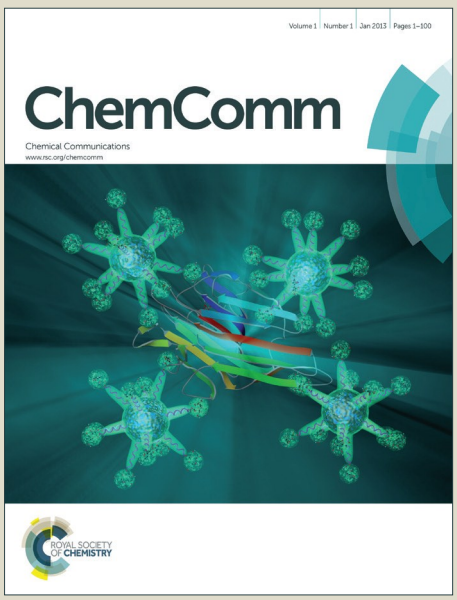

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[28]Hexaphyrin derivatives for anion recognition in organic and aqueous media

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Hexaphyrin-base anion chemosensors are reported for the first time. The meso-hexakis(ethylenediamine)-substituted [28]hexaphyrins 2 and 3 revealed strong affinity for F^-, AcO^- and $H_2PO_4^-$. Adsorption constants in aqueous media were determined on a gold piezoelectric crystal coated with 2 and 3. ^{1}H NMR titrations and molecular dynamics simulations showed the main interactions between host:guests.

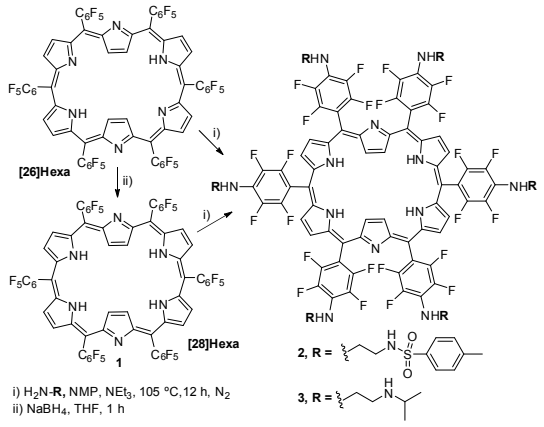
Over the last several decades, considerable efforts have been devoted to the development of efficient artificial receptors able to detect, transport or remove targeted guests.[1-7] Within this context, pyrrole-containing entities have emerged as one of the most versatile and useful anion recognition agents. They have been used in applications such as anion sensing, transport and also formation of complexes with DNA.[8] In contrast to many other entities employed for this purpose, pyrrole moieties are not particularly acidic or basic, meaning that pyrroles can be used to establish hydrogen bonds with anions under a vast variety of conditions.[9] However, rigid and planar porphyrin cavities with "hidden" NH groups lack accessibility and cannot be conveniently used to bind anions. To overcome this problem, linear oligopyrroles and porphyrinoids, including calixpyrroles, *N*-confused porphyrins and expanded porphyrins have been employed.[10-15]

Since their publication by Cavaleiro and coworkers,[16] *meso*-hexakis(pentafluorophenyl)[26]hexaphyrin (**[26]hexa**) and its congener [28]hexaphyrin **1** (Scheme 1) have been the subject of active research.[17-21] However, their binding capabilities towards anions have not yet been described. Nevertheless, it is reported the detection of silver cations by near infrared fluorescent [26]hexaphyrins and their deprotonation upon the addition of excess tetrabutylammonium fluoride.[22, 23]

As part of our studies on the synthesis of compounds with potential anion binding properties,[4, 24, 25] we report here the functionalization, structural characterization and anion binding studies of two new [28]hexaphyrins functionalized with *N*-tosylethylenediamino (**2**) and *N*-isopropylethylenediamino (**3**) groups in the *para* position of the *meso*-tetrafluorophenyl groups (Scheme 1).

The reactions between **[26]Hexa** and the two diamines were both made in NMP in the presence of triethylamine and afforded the corresponding 4-aryl substituted [28]hexaphyrins **2** and **3**. After purification by flash chromatography and crystallization from CH_2Cl_2/EtOH, the new compounds were obtained in 69% and 64% yield, respectively. Considering similar nucleophilic substitution reactions with isopropylamine,[26] the reduction of the macrocycle was anticipated. Indeed, when the reactions were performed with [28]hexaphyrin **1** and we obtained the same products.

A maximum absorption band at 619 nm, in DMF, for both compounds is consistent with the reduced form of hexaphyrins (further details are presented in the SI).



Scheme 1

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Furthermore, the ^{1}H, ^{13}C and ^{19}F NMR spectra are consistent with the proposed structures (see SI). For example, the ^{19}F NMR spectra of compounds **2** and **3** doesn't show resonances between δ -174.5 and -176.0 ppm, typically appointed to the fluorine atoms at the *para*-pentafluorophenyl positions, which confirms that all *p*-F atoms of the starting hexaphyrin **1** were substituted. Also, the high-resolution mass spectra show a peak at *m/z* = 2627.5335 $(M+H)^+$ for **2** and a peak at *m/z* = 1955.7584 $(M+H)^+$ for **3**, confirming the expected structures.

The interaction of compounds **1**-**3** with anions was evaluated by UV-Vis titration with addition of aliquots of DMSO stock solutions of each anion prepared from their tetrabutylammonium salts. The perturbation in the UV-Vis spectra caused by the addition of fluoride, acetate and dihydrogen phosphate anions provided the association constants from standard non-linear curves of the changes observed in the spectra of receptors **1**-**3** in $CHCl_3$ and DMSO (table 1 and Fig. SI 1-12).

Analysis of the binding properties revealed a 1:1 host-guest complex in solutions of **1**, while compounds **2** and **3** formed a 1:2 host-guest complexes with F^-, AcO^- and $H_2PO_4^-$, indicating that the outer periphery of macrocycles **2** and **3** is playing an active role in the association with the anions (Fig. SI 13). The association constants for **1**, in DMSO, were only possible to be calculated for F^- and the resulting value was Ka = $2.19x10^3$ M^{-1}. Addition of AcO^- and $H_2PO_4^-$ do not induce significant perturbations in the UV-Vis spectrum of compound **1** (Fig. SI 3-6).

Receptors **2** and **3** show higher association constants in DMSO than in $CHCl_3$, and, in both cases, in the order $F^- > AcO^- > H_2PO_4^-$. Notably, the selectivity for AcO^- compared with $H_2PO_4^-$ drastically increases when DMSO is used as solvent, being the best association constants ratio $AcO^-/H_2PO_4^-$ = 459.92 for receptor **3**. Nitrite and nitrate (as their tetrabutylammonium salts) did not triggered any perturbation in the UV-Vis spectra of receptors **1**-**3** and for this reason no results are shown for these anions.

The obtained data reveal that solvent polarity has a significant influence on the affinity of the receptors. This suggests that the conformation of the molecule is influenced by the polarity of the solvent employed and that in more polar solvents the higher acidity of the NH protons outweighs the conformational preferences of these groups.

Table 1 - Affinity constants at 22 °C for compounds **1**-**3**.

	1[a]*	**2**[b]		**3**[b]	
	$CHCl_3$	$CHCl_3$	DMSO	$CHCl_3$	DMSO
F^-	$2.32x10^4$	$1.06x10^7$	$3.94x10^{10}$	$1.32x10^7$	$4.94x10^9$
AcO^-	$1.27x10^4$	$5.31x10^5$	$4.00x10^9$	$1.63x10^5$	$2.41x10^8$
$H_2PO_4^-$	$3.16x10^3$	$2.48x10^5$	$6.51x10^7$	$3.24x10^4$	$5.24x10^5$
$AcO^-/H_2PO_4^-$	5.44	2.14	61.44	5.03	459.92

[a] These complexes are 1:1 (hexaphyrin: anion), therefore K_a units are M^{-1}; [b] These complexes are 1:2 (hexaphyrin: anion), therefore K_a units are M^{-2}, *Ka for **1**, in DMSO, was only possible to be calculated for F^- (2.19×10^3 M^{-1}).

The ^{1}H NMR spectra of **1** in $CDCl_3$ and DMSO-d_6 clearly show the conformational differences in both media (Fig. SI 14). In fact, [28]hexaphyrins are well known for their variable structure upon different conditions, such as temperature and solvents. This has been highlighted by Osuka and co-workers in a study comprehending low temperature NMR studies and crystallization of hexaphyrins (including **1**) in different solvents.[27] Their results indicate that [28]hexaphyrins in solution exist as an equilibrium between twisted Möbius conformations and a planar rectangular conformation.

It may be anticipated that these conformation variations might influence the binding capabilities of these compounds since a more favourable conformational structure can increase their binding abilities. To better understand this behaviour, ^{1}H NMR titrations were performed with **2**, both in $CDCl_3$ and DMSO-d_6. As can be observed in figure 1, the addition of F^- to compound **2**, depending on the solvent used, leads to different changes in the ^{1}H NMR signals.

When $CDCl_3$ is used as solvent, the signals corresponding to the NH protons of the tosylamide groups disappear, even when fluoride is added in less than 1 equiv. However, when the same titration is done in DMSO-d_6, moderate negative Δδ values (0.8-1.1 ppm) are observed for the external NH protons. Examples of receptors with enhanced binding effects over polar solvents can be found in literature.[28] Furthermore, these results also demonstrate that anion complexation with hydrogen-bonding receptors in a competitive solvent is enhanced by solvent molecules which can be incorporated into the binding motif.[29-31] In this way, competitive solvent adds to the overall complexation energy and thereby strengthens binding rather than weakens it, as it is commonly believed.

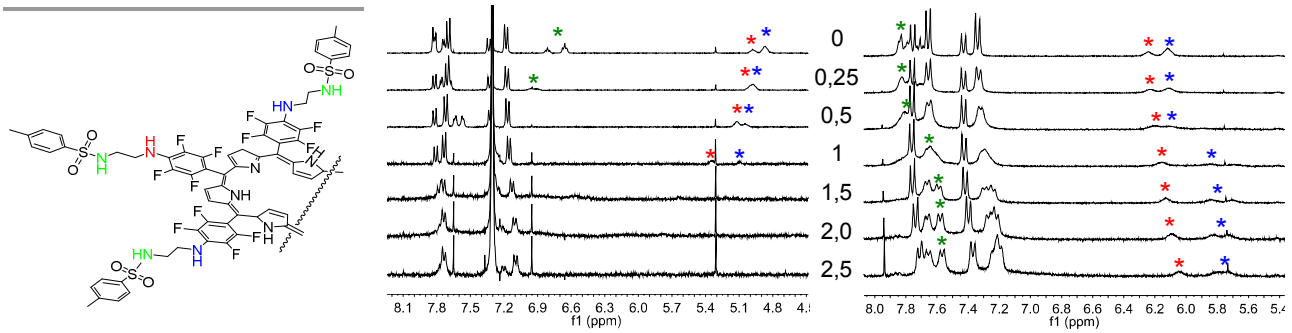

Figure 1 - ^{1}H NMR spectra of compound **2** in $CDCl_3$ (left) and DMSO (right) upon addition of F^-. NH signals: *NHTs, *2 NHC_6F_4 and *4 NHC_6F_4.

Addition of F^- to a $CDCl_3$ solution of **1** results in large differences in the ^1H NMR signals of **1**. For instance, the internal NH protons and the 8 external β-pyrrolic protons have a positive shift, suggesting a variation in the conformation of **1** while this compound interacts with anions by hydrogen bonding (Fig. SI 15).

Various attempts were made to obtain diffraction grade single crystals of the above anion complexes, but without success. However, in order to model the interactions between the hexaphyrin and the anions, molecular dynamics simulations were carried out using GROMACS 5.0.4[32] and GROMOS 54A7[33] force field. For that, new parameters were generated for hexaphyrins **2** and **3** in order to accurately describe their dynamical behaviour in $CHCl_3$ and DMSO. For such, pyrrole rings, fluorinated rings, R-groups, tetrabutylammonium and acetate topologies were validated using liquid phase properties. Here, the same protocol as described in Caleman *et. al.*[34] was applied and all topologies were only accepted as useful when density and enthalpy of vaporization values were, at least, 90% of experimental values. When possible, parameters already implemented in molecules described in GROMOS54A7 were also used. With each group properly validated, torsional profiles were generated for each new bond to better describe hexaphyrin's conformation using the same method as Pol-Fachim *et. al.*[35] For more details see the theoretical section in the SI.

In order to characterize the possible anion binding modes, radial distribution functions (RDF) were calculated between hexaphyrins' nitrogen atoms and the center of mass of acetate's oxygen atoms. Accordingly, the presence of acetate at r = 0.3 nm in the first solvation shell around nitrogen atoms from inner pyrrole rings and outer R-groups clearly indicates a stable hydrogen bond during simulation time (Fig. 2) for both hexaphyrins **2** and **3**. Regarding to solvent effects, acetate anions were able to reach its binding modes in $CHCl_3$ within few nanoseconds. However, it was not possible to observe similar interactions in DMSO within the same time scale. Thus, in order to test the consistence of our model, different binding modes observed in $CHCl_3$ were selected, had the solvent removed and re-solvated with DMSO.

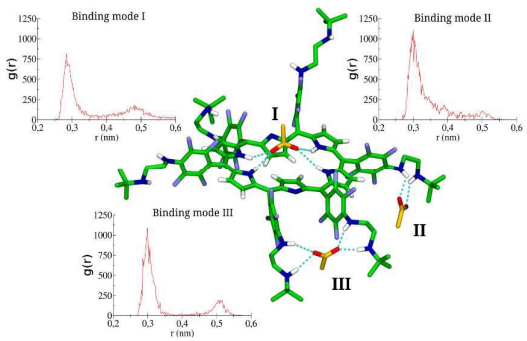

Figure 2 – Representative binding modes of **3** and AcO^- obtained in $CHCl_3$ and DMSO. Each binding mode was obtained from independent simulations and combined for a single illustration. Radial distribution function was calculated between hexaphyrin N atoms and acetate oxygen atoms, indicating stable hydrogen bonds throughout the simulation time.

After a careful equilibration protocol and a subsequent 10 ns simulation, we were able to observe the maintenance of the same binding mode observed in simulations in $CHCl_3$, so as the same RDF profile (Fig. SI 17). The difficulty of this event might be due to the fact that molecular dynamics are based on classical mechanics and, therefore, do not count with polarization effects that could enhance polar interactions, as described in literature, lowering the energetic barrier to the interaction.[31]

In addition, DMSO solutions of hexaphyrins **2** and **3** combined with anions (F^-, AcO^- and $H_2PO_4^-$) show naked-eye colour changes (Fig. SI 18). No colour changes are observed after the addition of bromide, nitrate, and nitrite anions. The colour changes observed are thus directly related with the values of the affinity constants.[4, 36]

In order to test the ability of receptors **1**-**3** to interact with anions in aqueous solutions, piezoelectric quartz crystal gold electrodes coated with each hexaphyrin were prepared (see details in SI).[4]

By adding different amounts of anion, while keeping the temperature at 22 ºC, an isotherm of the responses of the sensor (frequency decrease) vs. the anion concentration was obtained (Figs. SI 21-23). The experiments with the different anions were made at the pH at which 99.9% of the selected anion was in the desired form (F^-, AcO^- and HPO_4^{2-}). The assumption of a Langmuir model endorsed the estimation of the adsorption constants for several anions, which are displayed on table 2. From that it is possible to conclude that both *N*-tosylethylenediamino and *N*-isopropylethylenediamino groups increased the adsorption equilibrium constants of [28]hexaphyrin with the studied anions (F^-, AcO^- and $H_2PO_4^-$). Besides the introduction of tosyl groups the adsorption constants for fluoride experienced a much larger increase than for the other ions.

The interaction of hexaphyrin compounds with all anions was fast and reversible. Figure SI 20 shows the interaction, which can be followed by the frequency shift of the quartz crystal, when a 0.5 mL of a 5.0×10^{-5} mol L^{-1} solution of fluoride anion was injected in a constant flow of water that was passing over the coated quartz crystal face (see Fig. SI 19).[37] As can be seen, the frequency was decreasing while fluoride was attaching to the hexaphyrin, and increasing while it was washed away by the water flow. The complete process including the adsorption and complete desorption lasted 80 s (Fig. SI 20).

Table 2 Adsorption constants at 22 °C of the piezoelectric sensors made with hexaphyrins **1**-**3** for the anion in aqueous solutions, prepared from the corresponding sodium salts.

	1	**2**	**3**
F^-	9.0×10^6	7.3×10^7	2.2×10^7
AcO^-	1.4×10^7	5.1×10^7	4.2×10^7
HPO_4^{2-}	1.1×10^7	5.3×10^7	4.4×10^7

In order to study the influence of pH on the equilibrium, experiments with fluoride, which have been made at pH 6.7 were repeated at pH 9.7 (the pH used in the HPO_4^{2-}

experiments) and no significant differences in the results (α = 0.05) were found (see Fig. SI 24). No sensor is specific towards one of the ions, although their selectivity regarding the studied anions did change with the chemical groups present in the hexaphyrin receptor. However all sensors showed a remarkable stability along time, to which contributes the insolubility of [28]hexaphyrins **1**-**3** in water.

In conclusion, we present for the first time the use of *meso*-aryl [28]hexaphyrins as anion receptors. The new compounds are able to interact with anions in chloroform and DMSO. Conversely, the expected, results demonstrate that anion complexation with hydrogen-bonding receptors in a competitive solvent is enhanced. These compounds, specially compounds **2** and **3** in DMSO, exhibit a pronounced constant ratio Ka(AcO^-)/Ka($H_2PO_4^-$) of 61.44 and 459.92, respectively.

Sensors based on piezoelectric crystals coated with compounds **1**-**3** were successfully implemented and showed a remarkable stability, reversibility and sensitivity to several anions in aqueous solutions. Adsorption constants based on Langmuir model were higher for sensors coated with **2** and **3**, than for sensors coated with **1**. Furthermore the increase affinity/adsorption constants is dependent of the peripheral groups attached to the macrocycle, which shows that hexaphyrin **1** can serve as a convenient precursor for the construction of efficient and selective anion receptors that work in both organic and aqueous environments.

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