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Unique binding behaviour of water-soluble polycationic oxacalix[4]arene tweezers towards the paraquat dication†‡

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The first water-soluble polycationic oxacalix[4]arene molecular tweezers able to recognize –under pH control– the paraquat dication as a result of a delicate balance between electrostatic repulsion, Coulombic shielding and attractive π -stacking interactions are reported.

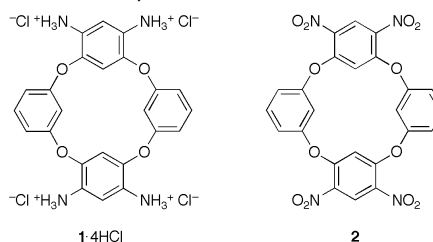
In 1966, Sommer and Staab¹ reported the synthesis of the first oxa[1₄]metacyclophane, oxacalix[4]arene (**2**),² during the course of their studies on conjugation in aromatic macrocycles. Owing to its poor solubility and limited yield, in the following years this intriguing compound was all but ignored by the growing community of supramolecular chemists, until in 2005 the group of Katz³ brought about an efficient synthetic procedure that took advantage of S_NAr reactions between aromatic diols –the so-called 'nucleophilic component'– and dihalodinitrobenzene –the 'electrophilic component'– leading, in a single step and under mild conditions, to a range of substituted oxacalix[4]arenes in high yield.⁴ In the same period, Wang and co-workers⁵ reported on the synthesis of oxacalix[2]arene[2]triazines, based on a two-step procedure reliant on the preparation of 1,3-bis(dichloro-*s*-triazinyloxy)benzene synthetic intermediates that were subsequently cyclized with aromatic diols.

However, even though oxacalix[2]arene[2]triazines have been shown to bind halide ions with remarkable efficiency by means of anion- π interactions with the heteroaromatic electron-deficient rings,⁶ scant data has been published so far on the supramolecular chemistry of the parent oxacalix[4]arenes. Shimizu and co-workers⁷ showed that a phloroglucinol-derived oxacalixarene was able to bind the ferrocenium cation in CH₂Cl₂, whereas Barbour and co-workers⁸ found that oxacalix[2]arene[2]naphthalene acted, in the solid state, as molecular tweezers for a range of crystallization

solvent molecules. In both cases, complexation was seen to take place in the π -rich cleft generated by the two aromatic moieties of the 'nucleophilic component'.

Given their proclivity for π -stacking, it was envisaged that, if oxacalix[4]arenes could be rendered water-soluble, they could somehow act as molecular tweezers toward suitable aromatic guest molecules. In this paper, we report on the first water-soluble oxacalix[4]arene,⁹ the tetraammonium derivative **1**·4HCl, capable – despite its polycationic nature– of recognizing and binding the paraquat dication under acidic conditions.

4,6,16,18-Tetraamino-2,8,14,20-tetraoxacalix[4]arene **1** was prepared by Raney-Ni-catalysed reduction of the tetranitro precursor **2**, according to a procedure recently reported by us.¹⁰ Treatment of a THF solution of **1** with aqueous 0.1 M HCl led to the formation of the tetra-hydrochloride salt **1**·4HCl.



Oxacalixarene **1**·4HCl shows a modest solubility in plain water that significantly increases upon lowering the pH. Its ¹H NMR spectrum, recorded at pH = 2.0 (10⁻² M DCl in D₂O)§ suggests that **1**·4HCl adopts a saddle-like conformation typical of oxacalix[4]arenes,¹¹ as indicated by the high field resonance of the intra-annular aromatic hydrogen atoms of the electrophilic component (δ = 6.55 ppm, see H₂ in Fig. S5, ESI†), owing to the diamagnetic shielding provided by the flanking aromatic rings.¹²

Solubility in acidic media, along with a strong dependence of the ¹H NMR spectrum on pH (Fig. S6, ESI†), confirmed that prior to any investigation on the binding potential of **1**·4HCl, it was necessary to gain a clear picture of the distribution of the different protonated species (i.e., **1**·*n*H⁺ with *n* = 0–4) over a wide range of pH (1.0 < pH < 10.0).¹³ Given the limited solubility of **1**·4HCl at pH > 2.5, UV-Vis spectroscopy was selected for the determination of the four protonation constants of tetraamine **1**. To this end, solutions of the tetraammonium tetrachloride salt in 0.1 M HCl (**1**·4HCl) in the

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† Dedicated to Professor Silvio Sammartano (Dipartimento di Scienze Chimiche, Università di Messina, Italy) on the occasion of his 70th birthday.

‡ Electronic Supplementary Information (ESI) available: Full experimental details, extended discussion on the determination of protonation and association constants, additional figures for both NMR experiments and DFT calculations. See DOI: 10.1039/x0xx00000x

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$3 \times 10^{-5} - 5 \times 10^{-6}$ M range) were titrated with a 0.1 M NaOH solution (ESI†). The analysis of the absorbance data, recorded at different pH values in the $\lambda = 250-330$ nm range, made the determination of four independent protonation constants possible (Table 1, ESI†) according to eqs (1) and (2) and led, in turn, to the distribution diagram depicted in Fig. 1 (see Fig. S3 for the full $1.0 < \text{pH} < 10.0$ interval).

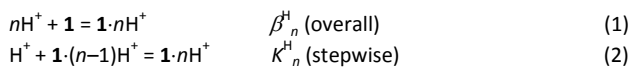


Table 1 Protonation constants ($T = 298.15$ K, $I = 0.1$ M) of oxcalix[4]arene **1**.

	$n = 1$	$n = 2$	$n = 3$	$n = 4$
$\log \beta_n^{\text{H}}$	4.39 ± 0.04	8.19 ± 0.04	10.74 ± 0.06	12.58 ± 0.06
$\log K_n^{\text{H}}$	4.39 ± 0.04	3.80 ± 0.04	2.55 ± 0.06	1.84 ± 0.06

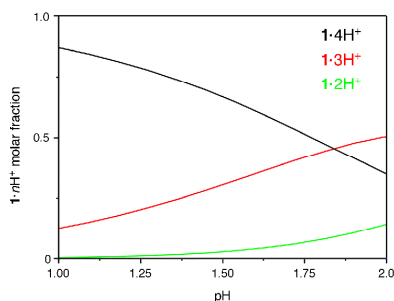


Fig. 1 Distribution diagram of the $\mathbf{1}\cdot n\text{H}^+$ protonated species at $[\mathbf{1}\cdot n\text{HCl}] = 1 \times 10^{-3}$ M, $I = 0.1$ M and $T = 298.15$ K, in the $1.0 < \text{pH} < 2.0$ interval.

A ^1H NMR ($[\mathbf{1}\cdot n\text{HCl}] = 10^{-3}$ M in $\text{D}_2\text{O}/\text{DCI}$, $\text{pH} = 1.6$) preliminary screening was carried out to gain information on the affinity of the oxcalixarene for a range of different neutral and charged guests. Rather surprisingly, upon addition of the dicationic methyl viologen (paraquat, PQT^{2+}) dichloride,¹⁴ the resonances belonging to the positively charged macrocycle (H_5 and H_1 , in particular) underwent small but significant, guest concentration-dependent shifts. A 2D ROESY spectrum carried out on a 1:4 host-to-guest solution (Fig. 2) shed light on the topological features of this complexation event, showing NOE cross-peaks between the H_α and H_β of the paraquat unit and the H_1 , H_4 and H_5 hydrogen atoms of the receptor, indicating that the guest molecule nestles within the cleft generated by the two π -rich aromatic units of $\mathbf{1}\cdot n\text{H}^+$.

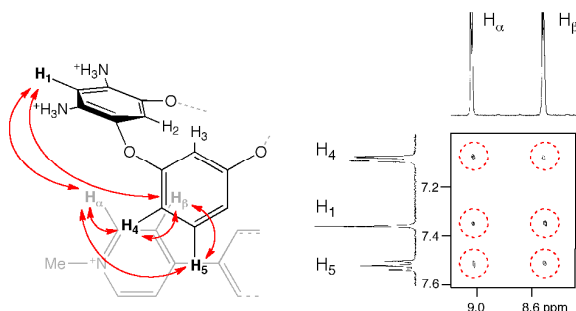


Fig. 2 Section of the 2D ROESY spectrum (500 MHz, 298 K, D_2O , $\text{pH} = 1.6$) of the $\text{PQT}^{2+} \subset \mathbf{1}\cdot n\text{H}^+$ complex: $[\mathbf{1}\cdot n\text{HCl}] = 10^{-3}$ M; $[\text{PQT}\cdot 2\text{Cl}] = 4 \times 10^{-3}$ M.

The host-guest system was found to be in fast exchange regime on the NMR timescale. Being aware that at $\text{pH} = 1.6$ there are no

less than three protonated forms of **1** (see Fig. 1), and that as a result the observed resonances are the weighted averages of up to six species (the 'free' receptors $\mathbf{1}\cdot 4\text{H}^+$, $\mathbf{1}\cdot 3\text{H}^+$ and $\mathbf{1}\cdot 2\text{H}^+$, along with $\text{PQT}^{2+} \subset \mathbf{1}\cdot 4\text{H}^+$, $\text{PQT}^{2+} \subset \mathbf{1}\cdot 3\text{H}^+$ and $\text{PQT}^{2+} \subset \mathbf{1}\cdot 2\text{H}^+$), five ^1H NMR titrations at different fixed pH values (in the $1.0 < \text{pH} < 2.0$ range) were carried out (Figs. S7–S11), with the aim of gathering sufficient data to unravel the multiple equilibrium and quantify all the microconstants relating to the individual host-guest events. At each pH a different K_{app} was determined (Table 2, see the ESI† for full details) according to eq. (3):

$$K_{\text{app}} = \frac{[\text{PQT}^{2+} \subset \mathbf{1}\cdot n\text{H}^+]}{[\text{PQT}^{2+}][\mathbf{1}\cdot n\text{H}^+]} \quad (3)$$

where $[\mathbf{1}\cdot n\text{H}^+]$ and $[\text{PQT}^{2+} \subset \mathbf{1}\cdot n\text{H}^+]$ refer to the total concentration of all the differently protonated species of the host present at the equilibrium in the free and complexed form, respectively.

Data in Table 2 show that $\log K_{\text{app}}$ peaks at $\text{pH} = 1.48$ (Fig. S4, ESI†). This evidence, along with the marked $\log K_{\text{app}}$ decrease observed on lowering the pH, indicates that in the $0.97-1.48$ range –where the only two oxcalixarene species present are the tetra- and triprotonated forms (Fig. 1)– only the tricationic macrocycle $\mathbf{1}\cdot 3\text{H}^+$ is able to significantly interact with the methyl viologen dication. In addition, the similar $\log K_{\text{app}}$ drop seen on going from 1.48 to 1.99 –the interval in which the formation of $\mathbf{1}\cdot 2\text{H}^+$ begins– suggests that the dicationic form does not contribute to guest complexation either: on increasing the pH to 2, precipitation of the receptor is observed. The combination of the K_{app} values (as a function of pH) with the protonation constants of the receptor within a 1:1 binding model, yields the association constant of the $\text{PQT}^{2+} \subset \mathbf{1}\cdot 3\text{H}^+$ complex ($K_3 = 253 \pm 50 \text{ M}^{-1}$).

Table 2 Dependence on pH of the $\log K_{\text{app}}$ of the interaction between $\mathbf{1}\cdot n\text{HCl}$ and $\text{PQT}\cdot 2\text{Cl}$ at $T = 298.15$ K.^a

[DCI]	pH	$\log K_{\text{app}}^a$	% of formation				
			$\mathbf{1}\cdot 4\text{H}^+$	$\mathbf{1}\cdot 3\text{H}^+$	$\mathbf{1}\cdot 2\text{H}^+$	$\mathbf{1}\cdot \text{H}^+$	1
0.100	0.97	n.d. ^b	87.06	12.58	0.35	–	–
0.075	1.35	1.44 ± 0.03^c	74.40	24.08	1.41	0.01	–
0.050	1.48	2.29 ± 0.02	67.85	29.62	2.52	0.01	–
0.025	1.59	1.70 ± 0.04	61.56	34.62	3.80	0.02	–
0.010	1.99	n.d. ^{b,d}	34.97	50.55	14.25	0.23	–

^a Average of two measurements. ^b n.d.: not detected (see the ESI† for full details). ^c 95% Confidence interval (C.I.). ^d Precipitation of the oxcalixarene prevented measurement of the $\log K_{\text{app}}$.

A deeper insight into the structural features of $\mathbf{1}\cdot n\text{H}^+$ –and its proclivity to act as molecular tweezers towards paraquat– was gathered by means of a geometry optimization study carried out at the density functional level of theory (B3LYP functional, 6-31G(d) basis set)†. All the possible protonation states were examined (Fig. 3), including the two constitutional isomers of the $\mathbf{1}\cdot 2\text{H}^+$ species (i.e., a C_2 and a C_s symmetric one, depending on the protonation pattern on the opposite amino-bearing rings)‡.

All the protonated species investigated possess the typical oxcalix[4]arene saddle-shaped conformation. Progressive protonation, however, has a dramatic effect on the interplanar angles between the pairs of aromatic rings facing each other. The free base **1** is seen with almost coplanar amino-bearing rings (30.8°) and wide-open dioxaphenylene rings (76.0°). The first protonation

step has almost no effect on the overall conformation of $1\cdot\text{H}^+$, but once the second proton comes into play ($1\cdot 2\text{H}^+$), electrostatic repulsion drives the two ammonium-bearing rings away from each other, forcing a marked narrowing of the interplanar angle between the electron-rich rings. Repulsion increases with additional protonation, resulting in a further narrowing of the interplanar angle (38.7° and 34.7° for $1\cdot 3\text{H}^+$ and $1\cdot 4\text{H}^+$, respectively) that effectively preorganises the molecular tweezers for paraquat complexation (in the case of $1\cdot 3\text{H}^+$ a cleft with an edge-to-edge distance of 6.6 \AA allows for a close-to-ideal match with the *ca.* 3.4 \AA thick paraquat guest). However, even though $1\cdot 2\text{H}^+$, $1\cdot 3\text{H}^+$ and $1\cdot 4\text{H}^+$, all appear to be suitable receptors for paraquat, only $1\cdot 3\text{H}^+$ is able to efficiently recognize the dicationic guest, thus finding the best trade-off between host-guest positive charge repulsion and π - π attraction: $1\cdot 2\text{H}^+$ is not soluble enough, whereas in the case of $1\cdot 4\text{H}^+$ –even in the presence of the highest chloride concentration– repulsion clearly overcomes attractive interactions.

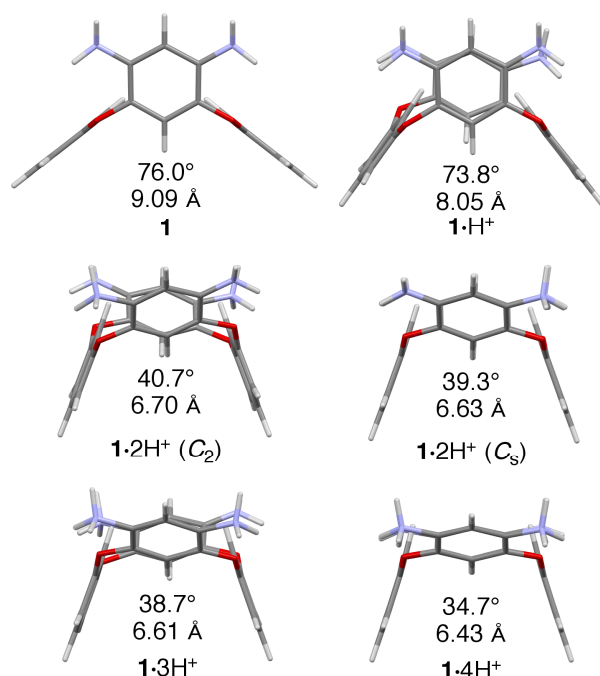


Fig. 3 Calculated gas-phase geometries (DFT, B3LYP/6-31G(d)) of oxacalixarene $1\cdot n\text{H}^+$ ($n = 0-4$). Arene-arene interplanar angles and $\text{C}_5\cdots\text{C}_5$ distances are reported for each species. See the ESI† for additional views (Fig. S14).

In conclusion, we have described the first water-soluble oxacalix[4]arene molecular tweezers and shown that, despite being positively charged, when in the right protonation state they are able to overcome unfavourable repulsive effects and recognize and bind the dicationic guest, methyl viologen. The hidden properties of this almost half a century old macrocycle have finally been unveiled. The strategy outlined in this communication may turn the plethora of nitro-substituted heteroatom-bridged calixarenes lying dormant in the literature into a full set of tuneable molecular tweezers: in spite of their venerable age, these rather neglected macrocycles may still have a prominent role to play in the ever-evolving field of host-guest chemistry.

Notes and references

§ In the presence of deuterated solvents/reagents, pH values were obtained from pD measurements by applying the appropriate correction. For easier comparison between NMR and UV-Vis data, throughout the manuscript pD values are reported as the corresponding pH values. See: R. G. Bates, *Determination of pH: theory and practice*, John Wiley & Sons, New York, 1973.

¶ Neutral aromatic hydrocarbons (e.g., naphthalene, anthracene, pyrene, etc.) as well as acidic (naproxen) and anionic (sodium tosylate) potential guests were tested.

|| The log K_{app} decrease observed on going from pH = 1.48 to 1.59 depends also on the decrease in Cl^- ion concentration. Chloride anions most probably reduce the electrostatic screening distance, allowing for attractive host-guest interactions to overcome Coulombic repulsion between cationic $1\cdot 3\text{H}^+$ and PQT^{2+} rings.¹⁵ However, given that ammonium ions are known to bind to chloride counterions even in water,^{13,16} it is reasonable to envisage that ion-paired species may contribute, to some extent, to oxacalixarene/paraquat binding, beyond mere Coulombic shielding, by means of $-\text{NH}_3^+\cdots\text{Cl}^-\cdots\text{PQT}^{2+}$ interactions. The active role played by Cl^- was confirmed by $1\cdot n\text{HCl}/\text{PQT}\cdot 2\text{Cl}$ competitive binding experiments carried out in the presence of HBr, CF_3COOH or HClO_4 in excess. In these cases, the probe resonances of the host underwent very small complexation-induced shifts, indicating a much lower degree of complex formation, if any (Fig. S13, ESI†).

¥ Protonation of both nitrogen atoms on the same ring is deemed unlikely, since protonation of the first amino group makes the second one considerably less basic than those on the opposite ring.¹³

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