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The effect of the solvent in the binding of anions and ion-pairs with a neutral [2]rotaxane

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In this work we report the binding properties of rotaxane **1** towards a series of tetraalkylammonium salts of Cl^-, OCN^- and NO_3^- anions in acetone and a $CHCl_3$/MeOH solvent mixture. We use ^1H NMR titrations and Isothermal Titration Calorimetry (ITC) experiments to monitor and analyze the binding processes. We compare the obtained results with those previously described by us in chloroform solution. In acetone solution, the determined binding constants for the 1:1 complexes resulted to be 1 to 3 orders of magnitude larger than those measured in chloroform, a less competitive solvent for hydrogen-bonding. The thermodynamic signatures of the binding processes in acetone, determined by ITC experiments, revealed favorable enthalpic and entropic contributions having similar magnitudes. These results suggested that solvation/desolvation processes in acetone play a significant role in the binding processes. Conversely, the addition of just 5% of methanol to chloroform solutions of **1** significantly reduces the magnitude of the binding constants of all studied ion-pairs. In this solvent mixture, the entropy term is also favorable but it does not compensate the experienced loss of binding enthalpy. Moreover, in acetone solution, the addition of the Cl^- and OCN^- tetraalkylammonium salts in excess (more than 1 equiv.) produced the immediate appearance of 2:1 complexes. Related high-stoichiometry complexes are not observed in the solvent mixture ($CHCl_3$/MeOH 95/5). In chloroform, a large excess of the salt (> 6 equiv.) is required for its formation. From the analysis of the obtained binding data we infer that, in acetone, the formed complexes are mainly anionic. However, in the $CHCl_3$/MeOH solvent mixture they are predominantly ion-paired.

Introduction

The combination of empirical and computational approaches provided important advances to our understanding of the effect of solvent in the binding strength of the complexes formed by neutral hosts and guest species.[1,2] In contrast and despite the known influence of the solvent's nature in ion pairing,[3,4] detailed studies of the effect played by the solvent in the binding affinity of charged species (e.g. anions) to neutral receptors are limited. Moreover, the obtained results are not always easy to explain or understand.[5,6,7,8,9] Most likely, the use of simple theoretical binding models in the analysis of titration data involving multiple equilibria, i.e. ion-pair dissociation, anion-binding and ion-pairing of the formed anionic complex, must take some of the blame. Typically, binding studies involving neutral molecular receptors operating through electrostatic interactions, such as hydrogen bonds, with polar neutral or charged guests are performed in non-polar organic solvents. Polar, protic or non-protic, solvents are avoided under these circumstances owing to their strong competition for hydrogen-bonding. This competition produces a negative impact on binding affinity. Polar protic solvents are used in binding studies between non-polar compounds or residues. Their interaction is mainly driven by solvophobic effects, which are maximized in water solution (hydrophobic effect). The development of selective synthetic receptors featuring high binding affinity for charged substrates, i.e. anions, in polar/protic solvents is a challenging endeavor.[10,11,12] Nevertheless, this effort has an acceptable trade-off for gaining additional knowledge and a better understanding of the binding processes occurring in biological and environmental contexts.[13]

Not surprisingly, several studies describing the binding of synthetic hosts with anions/ion-pairs showed the existence of linear free-energy relationships with different parameters associated to solvent polarity.[14,15,16,17,18,19] However, more relevant to this work are the results described by Sessler, Schmidtchen, Gale and coworkers in 2006. The authors reported the association constant values of the 1:1 complexes formed by a series of tetraalkylammonium chloride salts with *meso*-octamethyl calix[4]pyrrole in polar and non-polar organic solvents.[20] They did not find any apparent correlation between the determined binding constant values and the permittivity, dielectric constant, refractive index, or donor/acceptor strength parameters of the solvent. Instead, they detected a significant effect of the nature of the counter-cation on binding affinity. This effect was more evident in non-

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polar organic solvents such as dichloromethane. The obtained results suggested that the binding process in solution was more complex than the simple formation of a 1:1 anion:receptor complex. A few years later, the same authors described that in dichloromethane solution the *meso*-octamethyl calix[4]pyrrole acted as a heteroditopic receptor featuring cooperative binding.[21] The resulting termolecular ion-paired complex (1:1:1 anion:receptor:cation) displayed receptor-separated binding mode.[22] A likely explanation of the observed cation effect involved a stepwise binding mechanism. That is, the initial binding of the chloride (anion) induced the calix[4]pyrrole unit to adopt the cone conformation yielding a 1:1 anionic complex. Subsequently, the cation was bound in the electron-rich aromatic cavity defined by the pyrrole rings in cone conformation opposite to the bound chloride.

Polar non-protic solvents can solvate cationic species better than the non-polar counterparts. This phenomenon favors ion-pair dissociation. As a consequence, the cation effect observed in the binding of ion pairs to calix[4]pyrrole receptors is reduced in polar media. For this reason, *meso*-octamethyl calix[4]pyrrole mainly acted as a homotopic anion receptor in polar non-protic solvents.

In 2011, Flood and co-workers considered ion-pairing equilibria in the analysis of titration data derived from the binding of ion-pairs with a neutral macrocyclic triazolophane receptor (**Figure 1**).[23] In doing so, they were able to thermodynamically dissect the cation effect in the binding of ion-pairs. More recently and using the same theoretical binding model, Flood's group evaluated the effect of the solvent on the chloride binding step.[10] The obtained results evidenced the existence of an inverse relationship between the solvent dielectric constant (ε_r) and the association constant values of the 1:1 and 2:1 anionic complexes formed between chloride and the triazolophane receptor (i.e. $K\alpha 1/\varepsilon_r$). The free binding energies (ΔG) of chloride were less negative in acetone (ε_r = 20.5) or DMSO (ε_r = 46.8) solutions than in chloroform (ε_r = 4.7) or dichloromethane (ε_r = 8.9) solutions. In addition, they claimed that the binding of chloride in non-polar solvents (e.g. chloroform) was mainly driven by electrostatic contributions. In contrast, induction and dispersion forces (solvophobic effect) were the most relevant in solvents with higher dielectric constant (e.g. acetone).

Many of the studies addressing the effect of the solvent in the binding of ion-pairs with synthetic receptors focus on the quantification of binding constants (K) and the corresponding free energies of binding ($\Delta G = -RT\ \ln K$).[9,10,24] We consider that the dissection of the free energy of binding in its entropic and enthalpic terms might be useful for a better understanding of the driving forces involved in the binding event/s.[7,25,26] Furthermore, the complete thermodynamic characterization of the binding process is effortlessly obtained from ITC experiments.

Molecular receptors based on mechanically interlocked topologies display superior binding properties than their non-interlocked analogues.[27,28,29] In 2017, we described the synthesis of the neutral [2]rotaxane **1** containing a bis-calix[4]pyrrole cyclic component and a 3,5-bis-amidepyridyl-*N*-oxide derivative axle. In chloroform solution, [2]rotaxane **1** was an efficient heteroditopic receptor for ion-pairs, forming kinetically and thermodynamically stable 1:1:1 ion-paired complexes (**Figure 2**).[30]

We decided to undertake this work to quantify the effect of the solvent in the binding of anions and ion-pairs with the interlocked synthetic receptor **1**. The interwoven nature of the receptor's binding site is expected to limit the access of bulk solvent molecules to its converging six hydrogen-bond donor groups. In turn, the reduction of competitive hydrogen-bonding interactions with molecules of polar solvents was expected to retain, at least in part, the high binding affinity for anions/ion-pairs displayed by **1** in chloroform solution.[31]

Herein, we report the binding properties of rotaxane **1** with a series of anions, using tetraalkylammonium salts as precursors, in acetone and $CHCl_3$/MeOH mixture solutions. We compare the obtained results with those already described in chloroform solution. We discuss the thermodynamic signatures (ΔG, ΔH and $T\Delta S$) measured for analogous binding processes occurring in different solvents: chloroform (non-polar), acetone (polar) and chloroform/methanol 95/5 mixture (polar and protic, Ɛ(chloroform/methanol 95/5) = 6.1).

Results and discussion

General considerations

The existence of ion-pairing equilibria in the binding of ion-pairs in solution causes the experimentally determined values in the form of $K_{app}(1:1)$ to be concentration dependent. For this reason and as realized by the Flood's group and others,[32,33] the accurate determination of the binding constant of the anionic complex requires the use of elaborated binding models considering all species, neutral (ion-paired) and charged, that are present in solution (**Figure 1**).

Unfortunately, the fit of titration data to elaborated binding models is not simple owing to the intrinsic difficulties of detecting species in low concentration and the lack of significant differences between spectra of anionic and ion-paired counterparts.‡ For this reason, in this manuscript, we do not consider the equilibria of the salt ion-pair dissociation and the ion-pairing yielding the neutral complexes in the quantification of the reported association constant values. The values reported here in chloroform solution correspond to experimental binding constants, $K_a(1:1)$, of the form $[H{\bullet}C^+A^-]/[H]\ [C^+A^-]$ and $K_a(2:1)$, having the form $[H{\bullet}(C^+A^-)_2]/\ [H{\bullet}C^+A^-][C^+A^-]$. This treatment implies that the ion-pair is the active component and that the complexes are fully ion-paired. Conversely, in acetone (polar solvent) and chloroform:methanol (95:5) solvent mixture, we assume that the ion-pair precursor of the anion and the resulting 1:1 and 2:1 anionic complexes are fully dissociated.ω Then, the experimentally measured binding constant are of the form $K_a(1:1)=[H{\bullet}A^-]/[H]^1$ and $K_a(2:1)=[H{\bullet}(A^-)_2]/[H{\bullet}A^-]^1$, respectively. We are aware that the latter supposition is quite improbable in low dielectric constant solvents. However, it significantly simplifies the mathematical analysis of the titration data. The existence of a good fit between experimental data and

theoretical binding model is necessary to apply the simplifications.

Elaborated binding model for 1:1:1 complex

$C^+ + A^- \rightleftharpoons C^+A^-$

$H + A^- \rightleftharpoons [H{\bullet}A^-]$ 1:1 anionic complex

$[H{\bullet}A^-] + C^+ \rightleftharpoons [H{\bullet}C^+A^-]$ 1:1:1 neutral complex

Binding models used in this work for 1:1 complexes

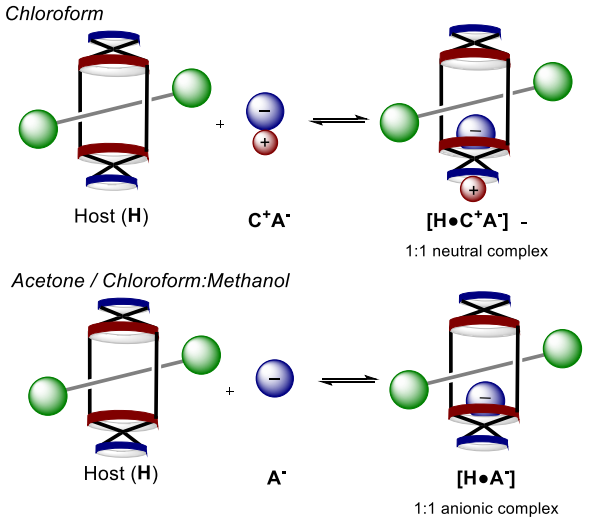

Figure 1. Top) Elaborated binding model for the 1:1:1 complexes used by Flood and co-workers in ref 10. Bottom) Binding models used for the 1:1 complexes of [2]rotaxane **1** in this work and in ref. 30.

Binding studies in acetone solution

We selected acetone as a representative example of a non-protic polar solvent. We probed the interaction of rotaxane **1** in acetone solution with a series of anions by means of ^{1}H NMR spectroscopy. We used the following tetraalkylammonium ion-pairs as anion precursors: TBA•Cl **2a** (Cl^-), TBA•OCN **2b** (OCN^-), TBA•NO_3 **2c** (NO_3^-) and MTOA•Cl **2d** (Cl^-). The effect of the counter-cation in the binding of ion-pairs in acetone solution (favoring ion-pair dissociation by preferential solvation of the cation) could be anticipated to be less important than the one we observed for **1** in chloroform solution or Schmidtchen, Gale and coworkers reported in dichloromethane for *meso*-octamethyl calix[4]pyrrole.[21]

The ^{1}H NMR spectrum of a 1 mM solution of rotaxane **1** in acetone-d_6 showed sharp and well-defined signals for most of the protons of its two molecular components: the macrocycle and the pyridyl-*N*-oxide axle (**Figure 2,** panel a). Conversely, the signal of the pyrrole NHs appeared broadened in the downfield region of the ^{1}H NMR spectrum. Most likely, this is due to the existence of a tumbling process of the pyridyl-*N*-oxide unit of the axle that alternatively hydrogen-bonds to one of the two calix[4]pyrrole identical binding sites of the macrocyclic component. This dynamic process shows intermediate dynamics for the NH signals on the ^{1}H NMR chemical shift timescale. The aromatic protons of the pyridyl-*N*-oxide moiety, $H_{1\text{-}3}$, appeared around 8.0 ppm together with the signal of the triazole proton, H_9.

The addition of 1 equiv. of TBA•Cl, **2a**, to the above solution produced the appearance of a new set of sharp signals that

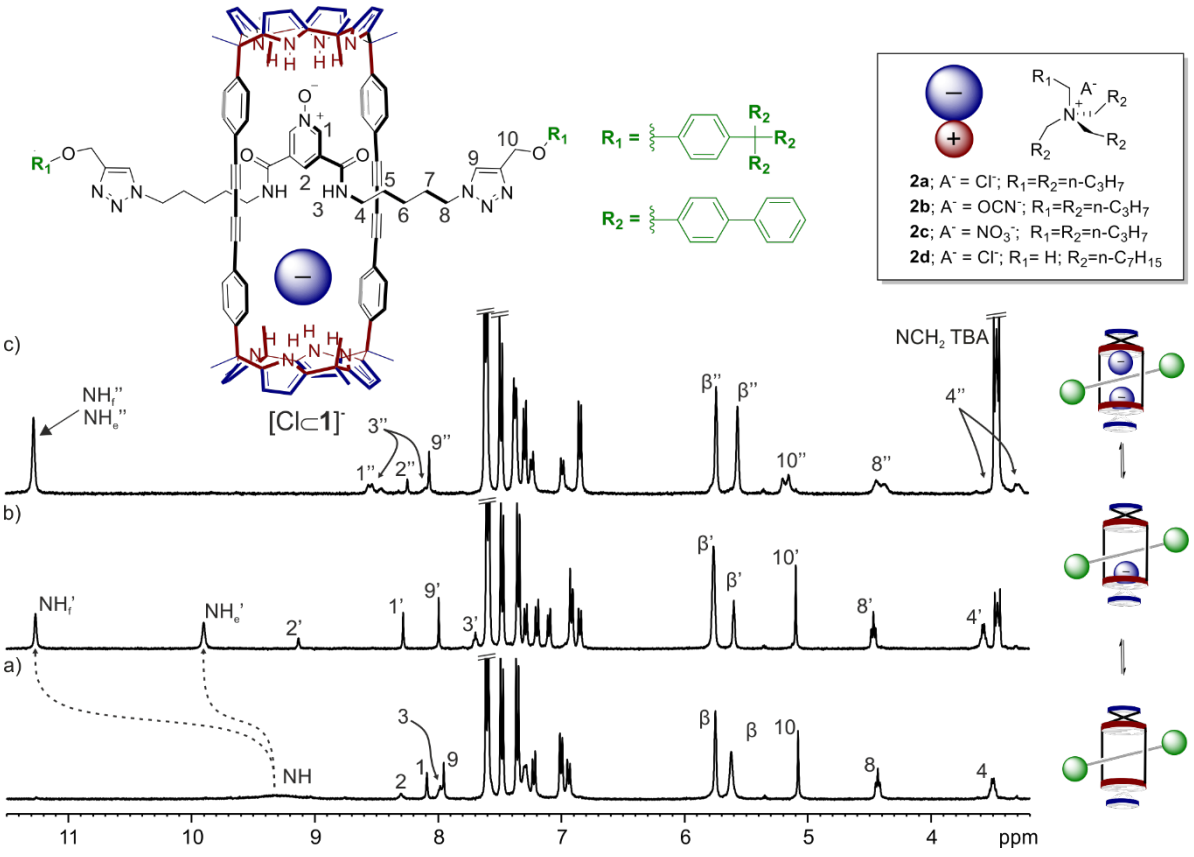

Figure 2. Top - Molecular structure of rotaxane **1** (left) and the tetraalkylammonium salt (right) used in the titration experiments. Bottom – Selected region of the ^{1}H NMR spectra (400 MHz, 298 K, Acetone-d_6) of the titration experiment of a 2 mM solution of rotaxane **1** (a) with 1 equiv. (b) and 3 equiv. (c) of **2a**. Primed letters and numbers correspond to the 1:1 complex **2a⊂1**, and doubled primed letters and numbers correspond to the 2:1 complex **2a$_2$⊂1.**

were attributed to the hydrogen atoms of bound receptor **1** (**Figure 2,** panel b). We observed two separate, downfield shifted singlets resonating at δ = 11.4 and 9.9 ppm, respectively. We assigned these signals to the pyrrole NHs of bound **1**. This splitting indicated that the two calix[4]pyrrole binding sites of the macrocyclic component in bound **1** are chemically non-equivalent. Each calix[4]pyrrole binding site participates in different hydrogen bonding interactions. The ditopic bis-amide-pyridyl-*N*-oxide unit of the lineal component hydrogen bonds its *N*-oxide group to one calix[4]pyrrole cap of the macrocycle. In turn, the chloride anion hydrogen bonds simultaneously to the two amide NHs of the axle and the four opposing NHs of the other calix[4][pyrrole cap. In the formed complex, the chemical exchange (tumbling) of the linear axle and the chloride between the two calix[4]pyrrole caps of the bound cyclic component becomes slow on the ^{1}H NMR chemical shift timescale (see **Figure 2**, for the structure and cartoon of the 1:1 complex). The aromatic protons of the pyridyl-*N*-oxide unit (H_1 and H_2) also experienced upfield shifts. The change was most noticeable for H_2, being consistent with its involvement in a hydrogen bonding interaction with the chloride. The observation of a single set of signals for the protons of receptor **1** assigned a 1:1 stoichiometry to the complex and allowed us to estimate its association constant value as larger than 10^4 M^{-1}. A ROESY experiment revealed the existence of cross-peaks, due to close spatial proximity, exclusively between the aromatic hydrogen atom *ortho* to the *N*-oxide and the calix[4]pyrrole NHs resonating at δ = 9.9 ppm. This finding allowed the unequivocal assignment of the two NH signals to the two chemically non-equivalent calix[4]pyrrole caps (Figure S4).

The addition of more than 1 equiv. of TBA•Cl, **2a**, provoked the emergence of a new set of proton signals (**Figure 2**, panel **c**). A new singlet resonating at δ = 11.4 ppm appeared and grew in intensity at the expenses of the NH signals of the calix[4]pyrrole caps of the initially formed 1:1 complex. Other proton signals of bound receptor **1** broaden or split. In the presence of 3 equiv. of **2a**, we did not detect the proton signals assigned to the initially formed 1:1 complex (**Figure 2**, panel **c**). Taken together, these observations indicated that the 1:1 complex is in equilibrium with another complex of larger stoichiometry. The two complexes are involved in a slow chemical exchange on the ^{1}H NMR chemical shift time scale. We propose that the chloride anions that are in excess not only exchange with the bound chloride in the 1:1 complex but also compete with the pyridyl-*N*-oxide group of the axle in the binding of the calix[4]pyrrole cap. The binding of the second chloride by the 1:1 complex, displaces the pyridyl-*N*-oxide unit of the axle from the macrocyclic component aromatic cavity leading to the formation of the 2:1 counterpart. An energy minimized structure for the putative 2:1 complex is shown in **Figure 3**. The bis-amide-pyridyl-*N*-oxide unit of the axle component interact simultaneously with the two bound

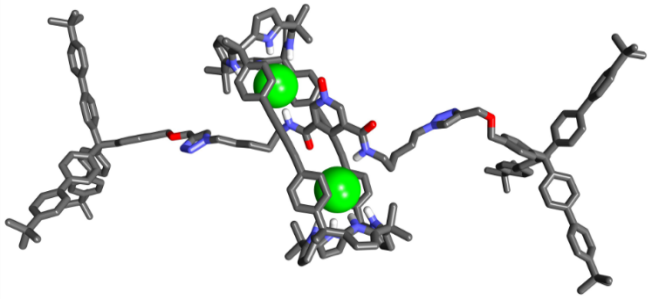

Figure 3 Energy minimized structure (MM3) of the $(Cl^-)_2$⊂**1** complex. Chloride anions are shown as CPK model while rotaxane **1** is shown with stick representation. Non-polar hydrogens are omitted for clarity.

chloride anions by forming two diverging hydrogen bonds with the amide NH protons.

The non-symmetric time average binding geometry proposed for the 2:1 complex would serve to explain the downfield shift experienced by the calix[4]pyrrole NHs, as well as the broadening and splitting of some of the proton signals of the axle component.

The chemical shift changes experienced by the protons of receptor **1** when titrated with **2a** in acetone-d_6 solution were almost analogous to those observed previously in chloroform-*d*.[30] It is worth noting than in chloroform-*d* solution the diagnostic proton signals of the formation of the 2:1 complex were present in low intensity even in the presence of a large excess of **2a**. Another significant difference of the titrations performed in the two solvents, was the lack of chemical shift changes for the methylene protons *alpha* to the nitrogen atom of the tetrabutylammonium cation (N-CH_2, TBA^+) in acetone-d_6 solution (**Figure 2**). We interpreted this observation as a result of the reduced involvement of the counter-cation in the formation of the anionic complexes in acetone-d_6. The TBA^+ cation is better solvated in acetone-d_6 (stronger ion-dipole interactions) than in chloroform-*d* solution. This fact provokes a better dissociation of the ion-pairs in acetone-d_6 supporting the formation of the 1:1 and 2:1 complexes of chloride with receptor **1** mainly as anionic species.

Not surprisingly, we also detected reduced complexation-induced shifts for the methylene protons alpha to the nitrogen of the methyl-trioctylammonium cation when MTOA•Cl, **2d**, was used as chloride precursor for the titrations of receptor **1** in acetone-d_6 solution compared to chloroform-*d* solution (Figure S1).

It is well established that in non-polar solvents anionic calix[4]pyrrole complexes bind the $MTOA^+$ cation in the shallow and electron rich cavity opposite to the bound anion more strongly than the TBA^+ counterpart. The binding affinity difference due to a better fit of the methyl group of the $MTOA^+$ cation almost disappears in acetone-d_6 solution. These results support the formation of weakly ion-paired complexes in acetone-d_6 solution.

Table 1. Values for the association constants, free energies of complexation, and for the enthalpy and entropy components for the interactions between rotaxane **1** and the different ammonium salts **2a, 2b** and **2d** in chloroform, acetone and 95/5 mixture of chloroform/methanol determined by ITC experiments at288 K. Association constant values are reported in M^{-1}; free energies, enthalpy and entropic contributions are expressed in kcal mol^{-1}. Errors are reported as standard deviations of two independent experiments.

Solvent	entry	Ion Pair	$K_{a(1:1)} \times 10^{-5}$	$K_{a(2:1)} \times 10^{-5}$	$\Delta G_{(1:1)}$	$\Delta G_{(2:1)}$	$\Delta H_{(1:1)}$	$\Delta H_{(2:1)}$	$-T\Delta S_{(1:1)}$	$-T\Delta S_{(2:1)}$
Chloroform	1	2a	0.5±0.2	n.d.	-6.4±0.2	n.d.	-6.3±0.4	n.d.	-0.1±0.4	n.d.
	2	2b	7.9±0.2	-	-8.0±0.02	-	-11.7±1.7	-	3.7±1.7	-
	3	2c	0.4±0.1	-	-6.3±0.1	-	-10.7±2.1	-	4.4±2.1	-
	4	2d	158±16	n.d.	-9.8±0.06	n.d.	-9.6±0.3	n.d.	-0.2±0.3	n.d.
Acetone	5	2a	480[a]	0.064±0.01	-10.1	-5.0±0.1	-5.8	-6.1±0.2	-4.3	1.1±0.2
	6	2b	30	n.d.	-8.8	n.d.	-5.9±0.6	n.d.	-2.9±0.1	n.d.
	7	2c	0.76±0.4	-	-6.6±0.6	-	-2.9±0.3	-	-3.7±0.7	-
	8	2d	700[a]	0.35±0.07	-10.3	-6.0±0.1	-5.2	-5.4±0.1	-5.1	-0.6±0.1
$CHCl_3$/ MeOH	9	2a	0.14±0.06	-	-5.4±0.2	-	-2.6±0.2	-	-2.8±0.3	-
	10	2b	4.1±0.8	-	-7.4±0.5	-	-5.8±0.01	-	-1.5±0.4	-
	11	2d	1.08±0.09	-	-6.6±0.05	-	-6.0±0.5	-	-0.6±0.5	-

[a] Estimated and fixed value during the manual fit to determine $K_{a(2:1)}$.

We also investigated the binding properties of rotaxane **1** in acetone-d_6 solution using polyatomic anions of different size and shape, cyanate (cylindrical) and nitrate (trigonal), by means of ^{1}H NMR spectroscopy (Figure S2 and S3). The salt precursors of the two anions contained the TBA^+ counter-cation. The incremental addition of TBA•OCN, **2b**, to a millimolar solution of **1** in acetone-d_6 produced similar results to the ones described above for TBA•Cl, **2a**. The anionic $[OCN\subset\mathbf{1}]^-$ complex was quantitively formed in the presence of 1 equiv. of the ion-pair. The addition of increasing amounts of the salt also induced the emergence of the proton signals diagnostic of the $[(OCN)_2\subset\mathbf{1}]^{2-}$complex. Notably, while the ^{1}H NMR titration of **1** with TBA•NO_3, **2c** in acetone-d_6 evidenced the quantitative formation of the $[NO_3\subset\mathbf{1}]^-$ 1:1 complex (Figure S3), the addition of increasing amounts of **2c** (up to 6 equiv.) did not produce relevant changes to the proton signals of bound **1**. This result indicated that the formation of the $[(NO_3)_2\subset\mathbf{1}]^{2-}$ did not take place to a significant extent in the range of concentrations used of the **2c** salt. The nitrate anion is less competitive for the binding with the second calix[4]pyrrole site of the macrocycle component than cyanate or chloride. Shape, size or the reducing hydrogen-bonding capabilities of the nitrate can be invoked to explain the result.

We performed Isothermal Titration Calorimetry (ITC) experiments in acetone solution in order to fully characterize thermodynamically the investigated binding complexes. The ITC data derived from the titration of rotaxane **1** (5.0×10^{-4} M) with **2a**, TBA•Cl, (17×10^{-3} M) in acetone solution showed two sigmoidal curves with inflection points centered approximately at **2a/1** molar ratio of 1 and 2 (**Figure 4,** top panel). A similar behavior was obtained for the titration of **1** with **2d**, MTOA•Cl. We manually fit the thermograms to a theoretical binding model that considers the formation of a 1:1 and 2:1 complex (two sets of sites, Microcal Software). The thermodynamic constants fixed and returned from the fits are summarized in Table 1 (entries 5 and 8).

The magnitude of the binding constant for the first binding event $K_{a(1:1)}$, formation of the $[Cl\subset\mathbf{1}]^-$ anionic complex, in the titrations of **1** with the two salts, **2a** and **2d**, could only be estimated as larger than 10^7 M^{-1}. It was not possible to determine more accurate values because the very dilute concentrations ([**1**] = 1.0×10^{-6}-1.0×10^{-7} M) that are required produced a release of heat that was below the detection limit of our instrument (0.25 µcal, Microcal VP-ITC). Notably, the binding constant values of the binding events of chloride with receptor **1** are strongly correlated. Thus, the accurate determination of the second binding constant, $K_{a(2:1)}$, demanded fixing the values of $K_{a(1:1)}$ and $\Delta H_{(1:1)}$. We took in consideration the reduced effect played by the cation in acetone solution and assigned a two-fold difference to the estimated values of $K_{a(1:1)}$ (1:1 complex) in the titrations of **1** with **2a** and **2d**. The estimated $K_{a(1:1)}$ values are indicated in Table 1. In contrast, the $\Delta H_{(1:1)}$ values were determined by visual analysis of the thermograms. The first binding of chloride to **1** was highly enthalpically driven and for a $K_{a(1:1)}$ larger than 10^7 M^{-1} it must also be highly favored entropically. Next, we calculated the stepwise binding constants for the formation of the 2:1 complex, $[(Cl)_2\subset\mathbf{1}]^{2-}$, in the titrations of **1** with **2a** and **2d** by manually fitting the binding isotherm while fixing the values of $K_{a(1:1)}$ and $\Delta H_{(1:1)}$. The obtained fits were good and returned constant values, $K_{a(2:1)}$, that were approximately three orders of magnitude smaller (~10^4 M^{-1}) than the estimates of $K_{a(1:1)}$. The decrease in affinity for the binding of the second chloride is associated with a noticeable reduction of the entropic term. In contrast, the enthalpy term showed very similar values to those of the binding of the first chloride (**Table 1**).

Most likely, the large difference observed in the entropic values of the two binding events relates to the dissimilar solvation/desolvation processes. The binding of the first anion requires the desolvation of the host's cavity with the concomitant release of solvent molecules to the bulk solution. In contrast, in the second binding event the incoming chloride displaces the *N*-oxide bound to the tetra-pyrrole core requiring additional solvation. We expected differences in the enthalpy values of the two binding events. However, in polar

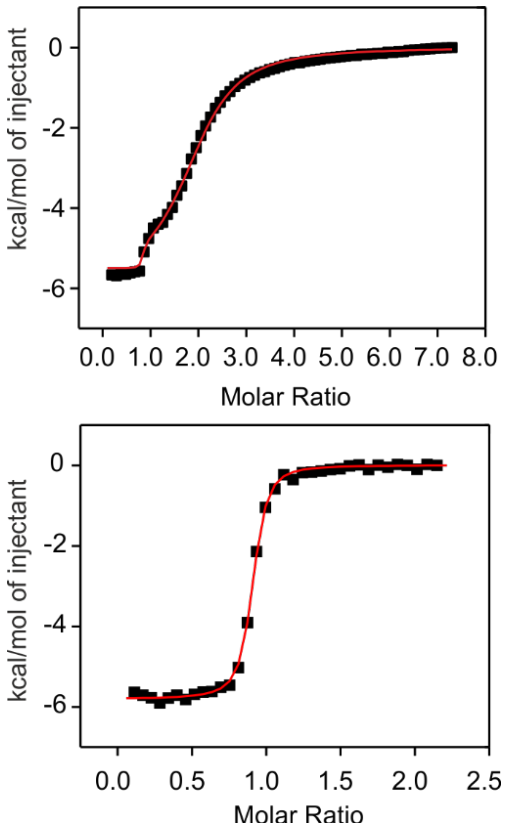

Figure 4 Binding isotherm or thermograms (integrated and normalized heat data vs molar ratio) of the calorimetric titration of receptor **1** with **2a** (top) and **2b** (bottom) in acetone. The data was fit to a theoretical binding model considering a 2:1 and 1:1 complexes and a 1:1 complex, respectively.

solvents solvation/desolvation effects also impact the enthalpy terms in ways that are difficult to predict or explain.

Owing to the reduced cation effect in acetone solution, the thermodynamic constants calculated for the formation of the $[Cl\subset\mathbf{1}]^-$ and $[(Cl)_2\subset\mathbf{1}]^{2-}$ complexes are similar independently of the salt used as source of chloride: TBA•Cl, **2a**, or MTOA•Cl, **2d** (Figure S11). This result gives an indication of the quality and fit of the data analysis. As discussed in the introduction, the observed small differences are the result of the use of a simplified theoretical model (not considering the dissociation of the ion-pairs: salt and complexes) in the mathematical analyses of the titration data.

In addition, the obtained results are fully consistent with the hypothesis that, in acetone solution, the salt and the 1:1 and 2:1 complexes are dissociated. That is, in acetone solution anionic complexes are mainly formed. The TBA^+ and $MTOA^+$ counter-cations are not significantly involved in ion-pairing processes.

The results described above are significantly different from the ones previously reported by us for the analogous titrations of **1** performed in chloroform solution. In chloroform, and in the range of concentrations used for the ITC experiments, we observed the exclusive formation of the 1:1 complex. Furthermore, the determined association constant values of the chloride complex were highly dependent on the salt precursor used as chloride source. The titration with MTOA•Cl assigned a stability constant to the 1:1 complex that was three orders of magnitude larger than for TBA•Cl (**Table 1** entries 1 and 4). From this finding we draw the following conclusions: 1) in a non-polar solvent i.e. chloroform, rotaxane **1** acts as a heteroditopic receptor; 2) the methyl group of $MTOA^+$ cation is a better fit than the butyl substituents of the TBA^+ cation for the electron-rich shallow aromatic cavity of calix[4]pyrrole chloride complexes; and 3) ion-paired complexes are predominantly formed in chloroform solution.[21,34]

Going back to acetone solution, the ITC experiments of rotaxane **1** (1.2 - 5.3×10^{-4} M) with **2b** (OCN^-) and **2c** (NO_3^-)(1.2 – 7.3×10^{-3} M) produced single sigmoidal binding isotherms with inflection points centered at molar ratio of **1**/**2b** and **1**/**2c** = 1 (**Figure 4,** bottom panel and S10).

The fit of the titration data to a simple 1:1 theoretical binding model was good (one set of sites, Microcal Software, Figures S10-S11). The thermodynamic constants returned from the fits are summarized in **Table 1** (entries 6 and 7). The fact that for these polyatomic anions 2:1 complexes were not formed to a significant extent under the range of concentrations used in the ITC experiments of **1** is in agreement with the observations made in the titrations monitored by ^1H NMR spectroscopy.

Remarkably, all the association constant values determined in acetone for the 1:1 anionic complexes of the investigated anions (Cl^-, OCN^- and NO_3^-) were larger than those of the 1:1:1 ion-paired counterparts formed in chloroform.[p] We propose that in acetone, the superior dissociation of the salt together with the reduced solvation energy of the anion may explain the observed result.

In the specific case of OCN^- and NO_3^- we also detected significant differences in the enthalpic and entropic binding components in the two solvents (Table 1, entries 2, 3, 6 and 7). In chloroform, the binding is mainly driven by enthalpy. In acetone, the entropic component is larger and favors binding. Solvation/desolvation processes are more relevant in the binding process in acetone solution.

We use TBA^+ salts as anion sources for the study of the binding selectivity in the 1:1 complexes of receptor **1**. Interestingly, receptor **1** shows an inversion of selectivity in the two solvents. In acetone, the trend of binding affinities is $Cl^- > {}^-OCN > NO_3^-$ (**Table 1**, entries 5, 6 and 7). Conversely, in chloroform, the OCN^- salt forms a more stable complex than the Cl^- counterpart (**Table 1**, entries 1 and 2). The latter complex being almost isoenergetic with that of NO_3^- (**Table 1**, entries 2 and 3).

The anion selectivity expressed by receptor **1** in acetone solution agrees with the strength of the electrostatic interactions stabilizing the complex. Chloride having the larger charge density (smaller size for the same charge) produced stronger hydrogen-bonding (ion-dipole) interactions. In contrast, in chloroform, 1:1:1 neutral ion-paired complexes are formed through a stepwise binding process. We hypothesized that the superior fit of the OCN^- anion in the cylindrical polar cavity of **1** becomes more relevant in the series of neutral complexes.

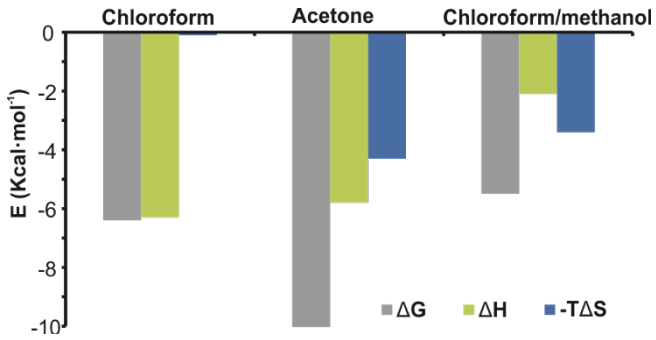

Figure 5 Thermodynamic parameters (free energy, and enthalpy and entropy contribution) for the formation of 1:1 complexes in the titrations of **1** with **2a**, TBA•Cl. The calculated values were derived from the ITC experiments performed in chloroform and acetone solution, as well as in a chloroform/MeOH (95/5) solvent mixture. See text for details on the nature of the formed 1:1 complexes.

The effect played by the solvent in the binding of ion-pairs by receptor **1** is analogous to that of the simple *meso*-octamethyl calix[4]pyrrole. In dichloromethane, Sessler and coworkers reported that the association constant for the complex of the *meso*-octamethyl calix[4]pyrrole with TBACl using a 1:1 theoretical binding model was $K_a = 3.5 \times 10^2$ M^{-1}.[35] A few years later, Namor et al., using an analogous theoretical binding model, determined that the binding constant of the anionic chloride complex was two orders of magnitude larger in acetonitrile $K_a = 5.0 \times 10^4$ M^{-1}.[36] Results in different acetonitrile mixtures and DMSO also revealed larger binding constants for *meso*-octamethyl calix[4]pyrrole compared to dichloromethane.[37,38,39] Sindelar and co-workers reported a similar trend in binding affinities of chloride with neutral bambusuril (H) hosts. In chloroform, the binding constant of the 1:1 [H•Cl^-]●TBA complex was determined to be $K_a = 2.5 \times 10^4$ M^{-1} and one order of magnitude larger in acetonitrile solution, $K_a = 5.6 \times 10^5$ M^{-1}, corresponding, most likely, to the 1:1 anionic analogue [H•Cl^-].[14]

In striking contrast with the results above, Flood and coworkers, using a more elaborated binding model that considers the ion-pairing equilibria, reported a decrease in binding affinity for the binding of chloride to shape-persistent macrocyclic triazolophane receptors when changing from non-polar to polar, non-protic solvents. The complexation of chloride with the triazolophane in chloroform (1:1 anionic complex) was energetically more favored than in polar solvents such as acetone or acetonitrile.[10] The authors attributed this difference to the electrostatics screening exerted by the polar solvents.

Taken together, these results suggest that the effect of the solvent in the binding affinity of neutral hosts with anions, when moving from non-polar to non-protic polar solvents, cannot be easily generalized or predicted and may strongly depend on the host's structure.

It is worthy to note that the binding constant values referred above, which were determined in different solvents, were derived using identical theoretical binding models. Nevertheless, the comparison of the anion binding constant values determined for a receptor in a polar solvent with those of the ion-pair in a non-polar counterpart is not fair. The polarity of the solvent dictates the formation of the complex mainly as anionic or ion-paired species. The cation should have no effect on the direct binding of the anion with the receptor. However, it may modulate the binding affinity of the ion-paired complex, especially in the case of heteroditopic receptors like the calix[4]pyrroles. In addition, the introduction of the interfering ion-pairing equilibria in the analysis of titration data for ion-pairs binding ~~in non-polar solvents~~ results in association constant values that are different to those determined using a simple 1:1 model.

Binding studies in chloroform/methanol solvent mixture.

Next, we set out to explore the binding properties of rotaxane **1** in a protic polar solvent. Protic polar solvents, like methanol, are strong competitors for host-guest hydrogen bonding interactions and efficiently solvate anionic and cationic charged species. These solvent properties often translate in a diminution of the free energy of binding of the complexes, involving neutral hosts and polar/charged species, that are formed in polar protic solvents in comparison to polar non-protic and non-polar counterparts.

Rotaxane **1** is not soluble in MeOH but it dissolves in mixtures of chloroform containing reduced amounts of MeOH. The addition of 5% (v/v) of methanol-d_3 to a mM chloroform-*d* solution of rotaxane **1** did not provoke significant changes in its ^{1}H NMR spectrum. The addition of incremental amounts of tetraalkylammonium salts **2a**, **2b** and **2d** to the above solution produced the appearance of a new set of proton signals for the receptor (Figures S7-S9). This new set of signals was diagnostic of the formation of 1:1 complexes. Specifically, the pyrrole NHs of **1** involved in hydrogen bonding interactions with the anion and the *N*-oxide group of the axle resonated at δ = 10.7 and 9.5 ppm, respectively. (Figure S7-S9). Noticeably, we did not observe any evidence for the formation of the 2:1 complex in the range of concentrations used for the ^{1}H NMR titrations.

In the initial phase of the titration, the methylene protons *alpha* to the nitrogen atom of the TBA^+ counteraction, for salts **2a** and **2b**, as well as the methyl group of the $MTOA^+$ analogue for **2d**, moved slightly upfield in comparison to the free salts' chemical shifts in the same solvent mixture (Figures S7 and S9). This result supports the involvement of the cations in ion-paired complexes formed in solution. However, the addition of incremental amounts of the salts produced a quick reversion of the chemical shifts towards values that are almost coincident with those of the salt free in solution.[λ] This behavior demonstrates that the chemical exchange between the free and the bound counter-cation is fast on the ^{1}H NMR chemical shift timescale and that the counter-cations are involved to a reduced extent in ion-paired complexes. Notably, in chloroform solution downfield shifts of the counter-cations proton signals required the addition of more than 1 equiv. of salt. In short, in chloroform-*d* solution containing 5% of methanol-d_3, receptor **1** produced mainly non-ion-paired 1:1 complexes.

We used ITC experiments for the accurate assessment of the association constant values of the mainly 1:1 anionic

complexes (Figures S12 and S13). The determined thermodynamic variables for the three studied salts, **2a**, **2b** and **2d**, are summarized in **Table 1** (entries 9, 10 and 11). All titrations (rotaxane **1** = [1-5 × 10^{-4} M] and **2** = [1 × 10^{-2}-5 × 10^{-3} M]) were exothermic and produced a single sigmoidal thermogram. We obtained good fits of the titration data to a simple 1:1 binding model. In agreement with the ^{1}H NMR titration experiments, the formation of 2:1 complexes was not detected in the range of concentrations used for the ITC experiments (Figures S12-S13). Most likely, the amount of MeOH present in the solution mixture strongly solvates the anions. The anion must be desolvated before engaging in hydrogen-bonding interactions with receptor **1**. The high energetic cost required for anion desolvation impacts negatively in its binding properties (*vide infra*).

From the analysis of the determined thermodynamic constant (**Table 1**, entries 9, 10 and 11) and its comparison with those previously determined in pure chloroform solution (**Table 1**, entries 1, 2 and 4), we deduced the following: 1) in the 95:5 $CHCl_3$/MeOH solvent mixture, the binding constant value for the 1:1 complex using, MTOA•Cl, **2d** as chloride source is almost eight-fold larger than the one calculated using, TBA•Cl, **2a**. This result indicates the existence of a reduced cation effect in the solvent mixture owing to the predominant anionic nature of the chloride complex. Please, recall that in pure chloroform ion-paired complexes are mainly formed and this provoked a difference in binding constants of three orders of magnitude when two different salts were used as chloride precursors (**Table 1**, entries 1 and 4). 2) All binding processes in the $CHCl_3$/MeOH 95/5 solution mixture are enthalpically and entropically favored exhibiting enthalpy-entropy compensation effects.[40] The large gains in entropy differ from the negative or small positive entropy values measured for the formation of the analogous ion-paired complexes in pure chloroform (**Figure 5**). 3) The magnitudes of all binding constants determined for the 1:1 complexes in the $CHCl_3$/MeOH solvent mixture are lower than in pure chloroform (**Table 1**, entries 9, 10, 11 and 1, 2, 4.). The larger difference is observed for **2d** for which the binding constant of the 1:1 complex is two orders of magnitude smaller in the $CHCl_3$/MeOH 95/5 solvent mixture than in pure chloroform. We assign this result to the superior solvation properties of MeOH for polar and charged species.

Remarkably, the trend in anion binding selectivity mirrors the one observed in pure chloroform: K_a[OCN⊂**1**]$^-$ > K_a[Cl⊂**1**]$^-$.

Conclusions

Rotaxane **1** acts as an efficient receptor of anions in acetone and chloroform/methanol solutions. We were able to thermodynamically characterize the formed complex using ITC experiments. Using ^{1}H NMR titrations experiments we determine the binding geometry of the complexes and their stoichiometry. The tetraalkylammonium salts used as source of the anions, as well as the complexes formed by the anions with receptor **1**, are significantly more dissociated in acetone and chloroform/methanol solutions than in pure chloroform.

In acetone, the magnitudes of the association constants of the complexes formed by rotaxane **1** and the studied anions are larger than those previously determined in chloroform solution. The large and favorable entropy terms calculated for the binding processes occurring in acetone solution suggested that solvation/desolvation effects play a significant role in the complex formation. Remarkably, the binding selectivity displayed by rotaxane **1** in the formation of 1:1 complexes with the anions (using TBA^+ salts as precursors) in acetone solution ($K_{Cl^-⊂1}$ > $K_{OCN^-⊂1}$ > $K_{NO_3^-⊂1}$) is inverted compared to that measured in chloroform ($K_{TBAOCN⊂1}$ > $K_{TBACl⊂1}$ ≈ $K_{TBANO_3⊂1}$). We attributed this result to the larger dissociation of the ion-pairs in acetone and the relevance of the ion-dipole interactions in the formed anionic complexes.

In acetone, the addition of more than 1 equiv. of the ion-pairs TBA•OCN, TBA•Cl and MTOA•Cl to the rotaxane **1** solution produced the formation of higher stoichiometry 2:1 complexes. The binding geometry of these complexes feature two anions coordinated to the opposed caps of bis-calix[4]pyrrole macrocycle. The axle component of **1** is displaced from the aromatic cavity of the macrocycle establishing diverging hydrogen bonds between the amides and the anions.

In chloroform/methanol 95:5 solvent mixture, the formed 1:1 complexes are mainly of anionic nature. Their binding constant values diminished in comparison to those measured in pure chloroform and acetone. Moreover, in the solvent mixture, the 1:1 complexes did not yield the high-stoichiometry 2:1 counterparts in the presence of an excess of the ion-pair.

The high thermodynamic stability determined for the 1:1 complexes of rotaxane **1** with mono- and polyatomic anions in polar solvents bodes well for the development of a water-soluble version able to bind anions also in water solution.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

‡ Our attempts on using a more elaborated binding model were unsuccessful due to the limited changes in the spectra along the titration experiments.

ω The extent of ion-pair dissociation can be experimentally determined. For example, conductivity measurements assigned a K_d = 6 ×10^{-5} M (295 K) for TBA•Cl in CH_2Cl_2 solution and K_d = 1.7 × 10^{-3} M in acetone. These values support the larger dissociation of

TBA•Cl in acetone solution at 1 mM concentration. See references 41, 42 and 43 for details.

ρ We used the same theoretical binding model to fit the data in both solvents: chloroform and acetone. However, it is worth noting here that the species distribution might be different in both solvents: highly dissociated anionic complexes in acetone and neutral ion-paired complexes in pure chloroform.

∧ The maximum upfield shift of the alpha methylene proton signals of the TBA^+ cation is larger in chloroform than in chloroform/methanol mixture.

1 C. A. Hunter, *Angew. Chem., Int. Ed.*, 2004, **43**, 5310-5324.
2 J. L. Cook, C. A. Hunter, C. M. R. Low, A. Perez-Velasco and J. G. Vinter, *Angew. Chem., Int. Ed.*, 2007, **46**, 3706-3709.
3 Bjerrum, N. *K. Dan. Vidensk. Selsk*. **1926**, *7*, No. 9
4 Y. Marcus and G. Hefter, *Chem. Rev. (Washington, DC, U. S.)*, 2006, **106**, 4585-4621.
5 C. J. Pedersen, *J. Am. Chem. Soc.*, 1967, **89**, 7017-7036.
6 S. J. Pike, J. J. Hutchinson and C. A. Hunter, *J. Am. Chem. Soc.*, 2017, **139**, 6700-6706.
7 F. Sommer, Y. Marcus and S. Kubik, *ACS Omega*, 2017, **2**, 3669-3680.
8 A. Shokri and S. R. Kass, *Chem. Commun. (Cambridge, U. K.)*, 2013, **49**, 11674-11676.
9 T. J. Sherbow, H. A. Fargher, M. M. Haley, M. D. Pluth and D. W. Johnson, *J. Org. Chem.*, 2020, **85**, 12367-12373.
10 Y. Liu, A. Sengupta, K. Raghavachari and A. H. Flood, *Chem*, 2017, **3**, 411-427.
11 S. Kubik, *Chem. Soc. Rev.*, 2010, **39**, 3648-3663.
12 M. J. Langton, C. J. Serpell and P. D. Beer, *Angew. Chem., Int. Ed.*, 2016, **55**, 1974-1987.
13 Y. Inoue and M. V. Rekharsky, in *Supramolecular Chemistry: From Molecules to Nanomaterials*, eds. P. A. Gale and J. W. Steed, John Wiley & Sons, Chichester, UK, 2012, vol. 1, ch. Solvation Effects in Supramolecular Recognition, pp. 117-133.
14 T. Fiala, K. Sleziakova, K. Marsalek, K. Salvadori and V. Sindelar, *J. Org. Chem.*, 2018, **83**, 1903-1912.
15 C. G. Swain, M. S. Swain, A. L. Powell and S. Alunni, *J. Am. Chem. Soc.*, 1983, **105**, 502-513.
16 M. M. Reif and P. H. Hünenberger, *J. Phys. Chem. B*, 2016, **120**, 8485-8517.
17 C. Reichardt, *Chem. Rev. (Washington, DC, U. S.)*, 1994, **94**, 2319-2358.
18 J. P. Cerón-Carrasco, D. Jacquemin, C. Laurence, A. Planchat, C. Reichardt and K. Sraïdi, *J. Phys. Org. Chem.*, 2014, **27**, 512-518.
19 T. J. Sherbow, H. A. Fargher, M. M. Haley, M. D. Pluth and D. W. Johnson, *J. Org. Chem.*, 2020, **85**, 12367-12373.
20 J. L. Sessler, D. E. Gross, W.-S. Cho, V. M. Lynch, F. P. Schmidtchen, G. W. Bates, M. E. Light and P. A. Gale, *J. Am. Chem. Soc.*, 2006, **128**, 12281-12288.
21 D. E. Gross, F. P. Schmidtchen, W. Antonius, P. A. Gale, V. M. Lynch and J. L. Sessler, *Chem.--Eur. J.*, 2008, **14**, 7822-7827.
22 S. K. Kim and J. L. Sessler, *Chem. Soc. Rev.*, 2010, **39**, 3784-3809.
23 Y. Hua, R. O. Ramabhadran, E. O. Uduehi, J. A. Karty, K. Raghavachari and A. H. Flood, *Chem.--Eur. J.*, 2011, **17**, 312-321.
24 P. D. Beer, *Chem. Commun. (Cambridge, U. K.)*, 1997, 2377-2378.
25 A. F. Danil de Namor, M. Shehab, R. Khalife and I. Abbas, *J. Phys. Chem. B*, 2007, **111**, 12177-12184.
26 A. F. Danil de Namor and R. Khalife, *Phys. Chem. Chem. Phys.*, 2010, **12**, 753-760.
27 J. F. Stoddart, *Angew. Chem., Int. Ed.*, 2017, **56**, 11094-11125.
28 S. F. M. van Dongen, S. Cantekin, J. A. A. W. Elemans, A. E. Rowan and R. J. M. Nolte, *Chem. Soc. Rev.*, 2014, **43**, 99-122.
29 M. J. Langton and P. D. Beer, *Acc. Chem. Res.*, 2014, **47**, 1935-1949.
30 J. R. Romero, G. Aragay and P. Ballester, *Chem. Sci.*, 2017, **8**, 491-498.
31 Y. Liu, F. C. Parks, E. G. Sheetz, C.-H. Chen and A. H. Flood, *J. Am. Chem. Soc.*, 2021, **143**, 3191-3204.
32 S. Roelens, A. Vacca and C. Venturi, *Chem.--Eur. J.*, 2009, **15**, 2635-2644.
33 J. W. Jones and H. W. Gibson, *J. Am. Chem. Soc.*, 2003, **125**, 7001-7004.
34 C. J. Borman, R. Custelcean, B. P. Hay, N. L. Bill, J. L. Sessler and B. A. Moyer, *Chem. Commun. (Cambridge, U. K.)*, 2011, **47**, 7611-7613.
35 P. A. Gale, J. L. Sessler, V. Král and V. Lynch, *J. Am. Chem. Soc.*, 1996, **118**, 5140-5141.
36 A. F. Danil de Namor and M. Shehab, *J. Phys. Chem. B*, 2003, **107**, 6462-6468.
37 P. Anzenbacher, A. C. Try, H. Miyaji, K. Jursikova, V. M. Lynch, M. Marquez and J. L. Sessler, *J. Am. Chem. Soc.*, 2000, **122**, 10268-10272.
38 S. Camiolo and P. A. Gale, *Chem. Commun. (Cambridge, U. K.)*, 2000, 1129-1130.
39 B. Turner, A. Shterenberg, M. Kapon, K. Suwinska and Y. Eichen, *Chem. Commun. (Cambridge, U. K.)*, 2001, 13-14.
40 J. D. Dunitz, *Chem. Biol.*, 1995, **2**, 709-712.
41 S. Alunni, A. Pero and G. Reichenbach, *J. Chem. Soc., Perkin Trans. 2*, 1998, 1747-1750.
42 L. G. Savedoff, *J. Am. Chem. Soc.*, 1966, **88**, 664-667.
43 R. M. Fuoss and T. Shedlovsky, *J. Am. Chem. Soc.*, 1949, **71**, 1496-1498.