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Nitroxide Radicals as Probes for Exploring the Binding Properties of the Cucurbit[7]uril Host

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Abstract: EPR spectroscopy was used for the first time to explore the binding properties of cucurbit[7]uril (CB7), a representative member of the cucurbituril family. Evidence for the formation of a complex between nitroxide radicals and the host system in an aqueous solution was provided by large changes in the nitrogen hyperfine splitting, attributed to the different polar environments experienced by the included radical. In the presence of alkali cations, the EPR spectra of benzyl *tert*-butyl nitroxide were characterised by new signals attributed to the radical hosted in the CB7 cavity in which one metal cation is in close contact with the nitroxidic oxygen. The formation of the coordination complex results in a substantial increase in the electron spin density on the nitrogen in inverse order with respect to the size of the cation owing to increased localisation of negative charge on the oxygen atom from bonding to the alkali cation. The EPR spectra showed selective line-broadening effects as a result of metal exchange between bulk water and the coordination complex. Analysis of the EPR linewidth variations allowed us to measure the corresponding kinetic rate constants for the first time. NMR spectroscopy showed that this behaviour is not peculiar to nitroxides but is also exhibited by the related carbonyl compounds. These data allowed us to quantify the template effect and to reach the conclusion that, in the presence of a guest having a coordinating lone pair, the formation of ternary metal-guest-CB complexes must be taken into account when discussing the complexation behaviour of cucurbituril derivatives in the presence of salts.

Keywords: cucurbiturils · EPR spectroscopy · host–guest chemistry · molecular dynamics · nitroxide

Introduction

Cucurbit[*n*]urils (CB*n*, *n* = 5–8, 10) are a new family of cyclic pumpkin-shaped molecules consisting of *n* glycoluril units and characterised by the presence of a hydrophobic cavity that is accessible through two identical carbonyl-fringed portals.[1] The isolation and characterisation of the first member of this family, cucurbit[6]uril, was reported in 1981 by Mock.[2] In the meantime, the groups of Kim,[3a] Day[3b] and Isaacs[3c] have expanded the family of cucurbituril hosts by reporting the preparation and purification of the CB6 homologues CB5, CB7, CB8 and CB10. Very recently, the isolation and characterisation of inverted CB6 and CB7, containing a single glycoluril unit directed into the CB cavity, has also been reported.[4]

The host–guest chemistry of CB*n* has been studied extensively.[5–7] Similar to cyclodextrins (CDs), the hydrophobic interior of CB*n* provides a potential site for the inclusion of various small molecules, including gas encapsulation,[8] dyes[9] and pharmaceuticals.[6f] Unlike CD, the polar carbonyl groups at the portals also allow CB*n* to bind ions through charge–dipole interactions.[10] Buschmann and co-workers have published a number of papers on the inclusion complexes of CB5 and its decamethylated derivative (as well as CB6) and have reported association constants for the inclusion of alkali, alkaline earth and transition metal ions.[11]

The rigid structure and the capability of forming complexes with molecules and ions make CB attractive not only as an encapsulating agent, but also as a building block for supramolecular assemblies. Actually, the recognition properties of the CB*n* family are potentially useful in developing pseudorotaxanes,[12] molecular machines,[13] self-sorting systems,[14] self-assembling vesicles[15] and dendrimers.[16]

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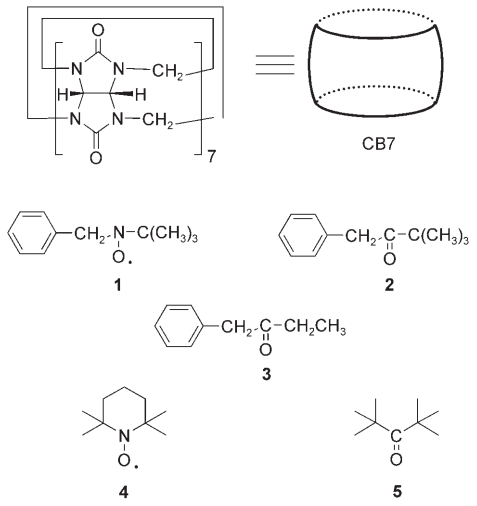
One severe drawback of CBs as a synthetic receptors is their poor solubility in both aqueous and organic solutions. Two approaches can be envisaged to overcome this problem. The first involves the preparation of CB*n* derivatives with enhanced solubility in water and organic solvents. This problem is addressed by the synthesis of new derivatives with improved solubility properties.[17] A simpler approach consists of adding alkali cation salts to an aqueous solution of CBs. In this way, alkali cations being complexed by both ureido rims provide a significant increase of CB solubility in aqueous solutions.[10] Under this condition, however, the amount of host having at least one portal of CB uncomplexed, which is essential for guest complexation, decreases resulting in a reduction of the value of the apparent equilibrium constant for the complexation of charged organic guests.[5l, 18]

In the last few years, we have shown that the formation of a host–guest complex can be studied very conveniently with EPR spectroscopy by the use of an appropriate radical probe.[19] Benzyl *tert*-butyl nitroxide and related dialkyl nitroxides were found to be very suitable probes to investigate host–guest interactions in cyclodextrins,[20] calixarenes,[21] micelles[22] and protected nanoparticles.[23] Evidence for the formation of paramagnetic complexes between these radicals and the host systems was provided by large spectral changes caused by the different environments experienced by the radical guest, and to conformational changes occurring upon complexation. In most cases, the EPR spectra also showed a strong linewidth dependence on temperature, indicating that the lifetime of nitroxides in the associated and free form is comparable to the EPR timescale; this enabled us to measure the rate constants for the association and dissociation processes.

On this basis, we decided to employ EPR spectroscopy to explore the binding properties of the cucurbit[7]uril host. In particular, we set out to characterise the picture describing the interplay between the association of metal ions and the inclusion of an organic guest taking CB7 as the model host. The selection of CB7 as the host is dictated by two relevant factors: firstly, the employed nitroxide fits the CB7 cavity tightly; secondly, the moderate solubility of CB7 in water (30 mM)[1c] is sufficient to allow us to compare the complexation behaviour of CB7 both in the presence and in the absence of salts. It will be shown that the reported nitroxides are particularly suitable probes for studying the complexation behaviour of CB7 by EPR spectroscopy because the formation of complexes with a different geometry and/or stoichiometry give rise to additional EPR lines easily distinguishable from those of the free radical. In particular, we were able for the first time, to detect in aqueous solution the formation of a coordination complex between alkali cations and a nitroxide–cucurbit[7]uril inclusion complex, thus demonstrating a template effect for metal cations. We used NMR spectroscopy to show that this template effect is not peculiar to nitroxides but is also exhibited by other molecules having an oxygen lone pair (Scheme 1).



Scheme 1.

Results and Discussion

EPR studies on benzyl *tert*-butyl nitroxide (1): The EPR spectrum at 298 K of benzyl *tert*-butyl nitroxide (**1**), produced by the reaction of the magnesium salt of monoperoxyphthalic acid (0.8 mM) with benzyl *tert*-butyl amine (0.8 mM) in water, is shown in Figure 1a. The spectrum is

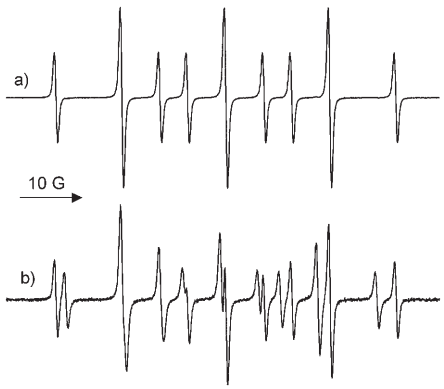


Figure 1. EPR spectra of **1** at 298 K recorded in water a) in the absence and b) in the presence of CB7 (7.0 mM).

straightforwardly interpreted on the basis of the coupling of the unpaired electron with the nitrogen nucleus and with the two equivalent benzylic protons. The spectroscopic parameters are reported in Table 1. In the presence of CB7 (7 mM), the observed EPR spectrum shows additional signals (Figure 1b) that were assigned to the same radical included in the host cavity (CB7@**1**). Experimental evidence that these new signals are attributable to the radical hosted in the cucurbituril cavity in equilibrium with the free nitroxide is obtained by changing the amount of CB7 present in solution. In particular: i) the ratio between the signals of the complexed and free radical increases with increasing concentration of the dissolved CB7 in solution; ii) strong dilu-

Table 1. EPR parameters of nitroxides at 298 K in water (1 G = 0.1 mT).

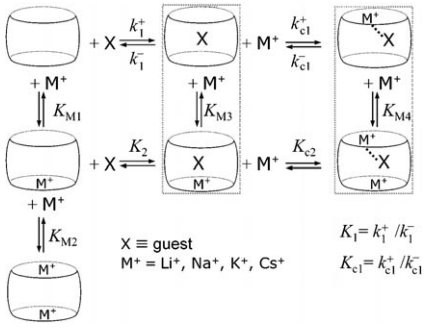
Nitroxide	a(N) [G]	$a(2H_\beta)$ [G]	g factor
1	16.80	10.70	2.0056
CB7@**1**	15.60	9.57	2.0061
CB7@**1**(Li^+)	17.12	14.82	2.0058
CB7@**1**(Na^+)	17.08	15.28	2.0059
CB7@**1**(K^+)	16.64	14.65	2.0060
CB7@**1**(Cs^+)	16.52	13.90	2.0061
4	17.30	–	2.0056
CB7@**4**	16.20	–	2.0064

tion of the most concentrated solution in CB7 (13 mM) leads only to the signals produced by the unbound species.

The spectra of radical **1** recorded in the presence of CB7 do not show any evidence of selective line-broadening up to 50 °C, this being an indication that the exchange between the nitroxide included in CB7 and the corresponding unbound species is slow on the EPR time-scale (residence time of **1** in the CB cavity $>10\ \mu s$). We have previously reported that, in the presence of β-cyclodextrin (β-CD), the EPR spectra of benzyl *tert*-butyl nitroxide are characterised by selective line-broadening as a result of exchange between the nitroxide included in β-CD and the corresponding unbound species, occurring with rates comparable to the EPR time-scale.[20b] Although the equatorial width of CB7 is larger than that of β-CD (the mean diameters of the internal cavity are 7.3 and 6.5 Å, respectively[1c,24]), the portals guarding the entry to CB are much narrower than the cavity itself. This results in constrictive binding that produces significant steric barriers to guest association and dissociation.[5l]

Table 1 reports the EPR spectroscopic parameters of the nitroxide radicals **1** and **4**. The values of the nitrogen splitting, a(N), and of β-proton splitting, $a(2H_\beta)$, decrease significantly upon inclusion into the less polar environment of the CB7 host cavity, giving rise to the remarkable differences in the resonance frequencies for the $M_I(2H_\beta) = \pm 1$ lines of the included and free species. Nitroxide **1** can be included in the CB7 cavity in two different ways, either from the *tert*-butyl side or from the phenyl side. Nevertheless, only one species,[25] identified as the 1:1 inclusion complex, is detected. Information on the preferred geometry of the complex is obtained from the values of the spectroscopic parameters. It is well established that the nitrogen hyperfine splitting constant, a(N), of nitroxides is sensitive to the polarity of the environment where they are dissolved.[26] The significant reduction in a(N), with respect to the values in water, that accompanies complexation of **1** by CB7 indicates that the NO group is quite deeply included in the internal apolar environment of the host cavity. This complex geometry is expected to be found if inclusion takes place from the *tert*-butyl side, while inclusion from the phenyl side is expected to leave the NO group exposed to bulk water because of the larger distance between the radical centre and the aromatic ring. We could, therefore, hypothesize that in the paramagnetic complex nitroxide **1** is located with the *N*-*tert*-butyl group well inside the CB7 cavity.

The EPR results demonstrate the formation of a stable inclusion complex between nitroxide **1** and CB7. However, attempts to obtain the true equilibrium constant (K_1, Scheme 2) were unsuccessful. Actually, the ratio between free and included radical strongly depends on the initial concentrations of the amine and the Mg salt of the peracid employed to generate the nitroxide radical, indicating that the radical precursors strongly compete with the radical for the internal cavity of CB7.[27]



Scheme 2.

EPR studies of 2,2,6,6-tetramethyl piperidine-*N*-oxyl (TEMPO, 4): When CB7 is added to a solution of TEMPO in water, the high-field line in the EPR spectrum splits into two well-separated components that we assign to the free and complexed radical exchanging slowly on the EPR time-scale (Figure 2). The complexed guest showed distinctly

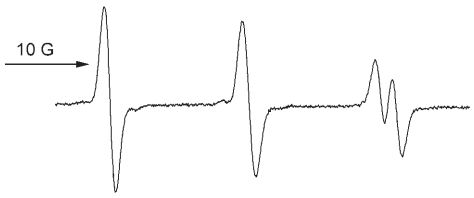


Figure 2. EPR spectrum of **4** (0.1 mM) recorded in the presence of CB7 (0.20 mM) at 298 K.

smaller nitrogen hyperfine splitting and larger g-factor values than the corresponding free species (Table 1). Although this effect is expected because of the less polar environment experienced by the NO group within the CB7 cavity,[26] the spectral resolution of the signals due to the free and included radical is much higher than that generally observed in water with other macrocyclic host, such as cyclodextrins or calixarenes.[19]

This observation can be justified by assuming that the NO group of the nitroxide is deeply immersed in the CB7 cavity, thus experiencing the hydrophobic environment of the inner part of the cavity. In fact, calculated molecular models for the CB7@**4** complex (AMBER Force Field as provided in the Macromodel 7.0 package) show that the guest is inserted in a symmetrical fashion with the NO group lying on the

plane passing through the equatorial carbon–carbon bonds of the host and with the geminal methyl groups pointing toward the carbonyl portals (Figure 3).

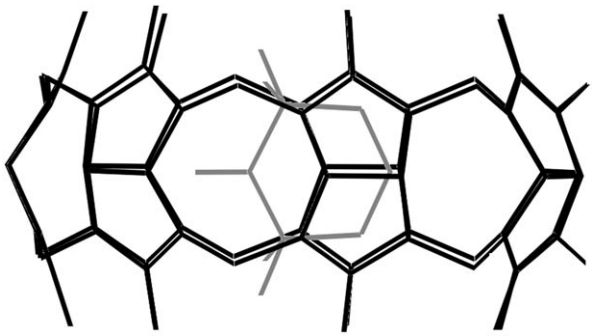


Figure 3. Molecular mechanics-minimised structure of the CB7@**4** complex. Hydrogen atoms have been omitted for clarity.

Further evidence for the correct assignment of the geometry of the CB7@**4** complex is obtained by measuring NOE interactions in the complex formed by CB7 and di-*tert*-butyl ketone **5**, an alicyclic sterically hindered diamagnetic analogue of TEMPO. ROESY spectra of aqueous solutions of **5** containing a large excess of CB7 show significant NOE interactions (Supporting Information) of the methyl protons of the *tert*-butyl groups of the guest molecule with the methylene hydrogen of the macrocyclic host pointing toward the carbonyl portals and resonating at $\delta = 5.77$ ppm. This demonstrates that the guest is lodged in the host cavity with the methyl groups very close to the carbonyl rims.

Simulation of the EPR spectra recorded at different concentrations of the macrocyclic host provides a value of $25000 \pm 2000\ \text{M}^{-1}$ for the binding constant K_1 for TEMPO complexation (Table 2). This is one order of magnitude larger than that measured for the same guest with β-cyclodextrin ($2950\ \text{M}^{-1}$).[28] The huge enhancement of the affinity constant should be attributed to the larger equatorial width of CB7 that allows total inclusion of the paramagnetic guest inside the internal cavity as previously discussed. On the other hand, the narrower internal cavity of β-CD cannot easily accommodate the radical guest with the N–O bond perpendicular to the long axis of the macrocylic ring, thus leaving the N-O group in the complexed radical exposed to bulk water.

Table 2. Stability and kinetic parameters for guest complexation by CB7 at 298 K determined by EPR and NMR.

Guest (**X**)	k^+_1 [$\text{M}^{-1}\text{s}^{-1}$]	k^-_1 [$\text{M}^{-1}\text{s}^{-1}$]	K_1 [M^{-1}]	K_2 [M^{-1}]	K_{M1} [M^{-1}]	K_{M2} [M^{-1}]
2	slow[a]	slow[a]	27340			
3	4.6×10^6	1000	4600	470[b]		
4			25000	6150[c]		
K^+					600[c]	53[c]

[a] Relative to the NMR time-scale. [b] From numerical fitting of the experimental dependence of the CIS on the K^+ concentration. [c] From the decrease in the [CB7@**4**]/[**4**] ratio observed by EPR in the presence of K^+ cations.

NMR studies on 2 and 3: On account of the similar geometries adopted around the N–O and C=O bonds, we also decided to investigate the behaviour of *tert*-butyl benzyl ketone (**2**), the diamagnetic analogue of **1**, upon complexation with CB7 by recording the corresponding ^{1}H NMR spectra. The addition of small portions of CB7 (0–1.0 mM) to a solution containing **2** (0.8 mM) led to progressive replacement of all guest resonances (Figure 4) by new signals attributed to the ketone included in the CB cavity.

As proof for the formation of an inclusion complex, the doublets corresponding to the host's methylene protons of the portals in the ^{1}H NMR spectrum of CB7@**2** split into two sets centred at $\delta = 5.78$, 5.77, 4.16 and 4.18 ppm, reflecting the differences between the two portals created by the interaction of the unsymmetrical guest with the inner cavity of the host.[29] The formation of an inclusion complex is also suggested by the enhanced solubility of benzyl *tert*-butyl ketone in aqueous media containing CB7.

The magnitude of the chemical-induced shifts (CIS) of the guest proton strongly depends on the nature of the group they belong to. While complexation results in a considerable upfield shift of the aliphatic protons of **2** (≈ -0.8 ppm), the aromatic protons do not significantly change their resonance frequency apart from a $+0.30$ CIS experienced by H_{ortho}. Because it has been generally observed that inclusion inside the cucurbituril internal cavity leads to upfield shifts of the included guest protons,[30] the experimental CIS strongly suggest that the complex CB7@**2** is adopting a geometry in which the *tert*-butyl residue is deeply immersed in the host cavity while the guest's phenyl ring is further away and interacts with the carbonyl oxygens on the host portal. This conclusion is in agreement with the EPR results reported for the corresponding nitroxide **1**.

When an excess amount of the guest is present, the signals of the free and bound guests are simultaneously observed, revealing that the rate of exchange between these species is slow on the NMR time-scale. Integration of the separate signals for the free and complexed guest protons as a function of the CB7 concentration provides the corresponding associ-

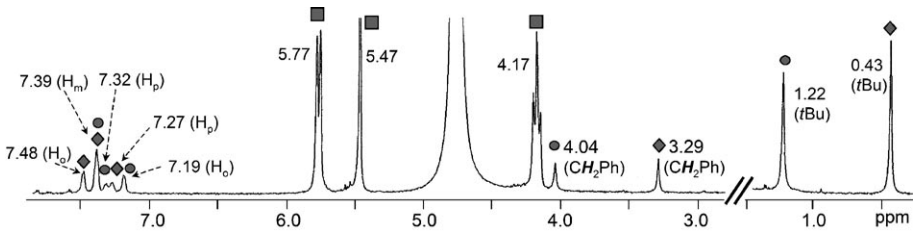


Figure 4. 600 MHz ^{1}H NMR spectrum of benzyl *tert*-butyl ketone (**2**, 0.8 mM) in the presence of CB7 (0.48 mM). Assignment: ● free guest, ◆ complexed guest, ■ complexed host. All spectra were recorded at 298 K in D_2O solutions with residual HOD as an internal standard (4.76 ppm).

ation equilibrium constant (K_1) as $27340 \pm 1490\,\text{M}^{-1}$. This value supports the conclusion that benzyl *tert*-butyl ketone forms a stable inclusion complex with CB7.

The behaviour of ethyl benzyl ketone (**3**) in the presence of CB7 was also investigated by ^{1}H NMR. In this case, in contrast to what was previously observed with **2**, all the guest signals shift toward a lower frequency upon complexation by CB7. However, the most pronounced upfield CIS (see the Supporting Information) are exhibited by the aromatic and methylene protons of the guest, suggesting that ketone **3** is included inside the inner cavity of the host with the aromatic ring.

As a remarkable manifestation of this difference, complex CB7@**3** is characterised by a typical dynamic ^{1}H NMR spectrum, indicating that the kinetics of exchange between the free and complexed guest is comparable on the NMR timescale (see the Supporting Information). A complete NMR line-shape analysis[31] of the CB7-dependent coalescence of the resonance of free and complexed **3** as the guest exchange between these two environments yield the rate constants for the complexation process in water at 298 K as $4.6 \times 10^6\,\text{M}^{-1}\text{s}^{-1}$ for the ingression rate (k_1^+) and $1000\,\text{s}^{-1}$ for the egression rate (k_1^-) ($K_1 = 4600\,\text{M}^{-1}$). The faster rate of exchange of **3** with respect to **2** can be rationalised in terms of a weaker steric hindrance of the less bulky phenyl group with the portals during exchange.

Molecular dynamic simulations: In order to obtain a more detailed picture of the geometry of CB7@**1** and the corresponding diamagnetic CB7@**2** complex,[32] stochastic dynamics (SD) simulations were performed with the AMBER* force field of the Macromodel 7.0 program. The computational approach taken in this study is to dock the guest molecule inside the host cavity, minimise the energy of the complex and then carry out standard equilibrations and production runs to derive averaged energies and distances.[33]

Molecular dynamics (Table 3) results indicate that complexation from the phenyl and *tert*-butyl side are equally energetically favourable processes. In both cases, the main contribution to complexation arise from van der Waals attractions that are only partially compensated by the nonbonded repulsion in the stretching and bending energy values. Nevertheless, only the complex in which the *tert*-butyl group is inserted inside the cavity (Figure 5) shows a calculated distance between the carbonyl carbon of the guest and the plane passing through the equatorial hydrogens of CB7, compatible with the experimental value of a(N) found by EPR analysis of CB7@**1**.

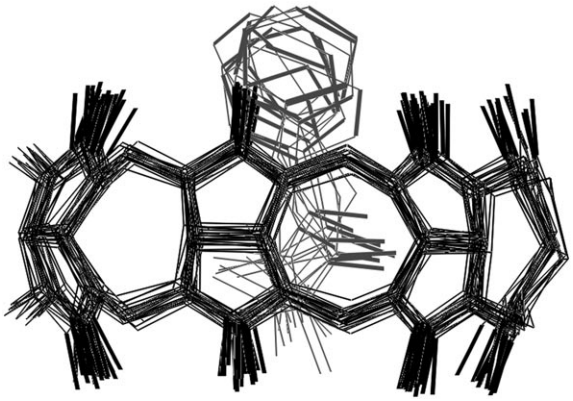


Figure 5. Clustered molecular display. Dynamics of the inclusion complex CB7@**2**. Drawings include 20 structures that refer to the 2000 ps simulation. Hydrogen atoms have been omitted for clarity.

Table 3. Calculated averaged components of energies [kJ mol^{-1}] and distances [Å] at 298 K for the inclusion complexes CB7@**2**.

	Total E	E_{stretch}	E_{bend}	E_{tor}	E_{VDW}	E_{electro}	E_{solv}	$\langle d \rangle$[b]
2	110	18	47	29	22	5	−11	–
CB7	543	133	684	187	−100	79	−440	–
CB7 + **2**	653	151	732	216	−78	84	−451	–
CB7@$\mathbf{2}_{t\text{But}}$[a]	599	151	742	216	−170	72	−412	1.396
CB7@$\mathbf{2}_{\text{Ph}}$[a]	597	151	741	215	−170	74	−414	3.755

[a] Labels refer to the two different orientations of the complex (see text). [b] Distances between the carbonyl carbon of the guest and the plane passing through the equatorial hydrogens of CB7.

Effect of alkali cations on the complexation of TEMPO (4): It is well known that alkali metal cations bind to the carbonyl portals of the CB*n* family. As such, one might expect that the presence of alkali cations would simply reduce the concentration of free CB7 by the formation of CB7@M^+ giving rise to a reduction of the apparent value of K_1 for the host–guest complex.[51,18] This is indeed what we observed with TEMPO. Figure 6 reports the plots of the apparent binding constant K_{EPR} for the CB7@**4** complex as a function of K^+ ion concentration.

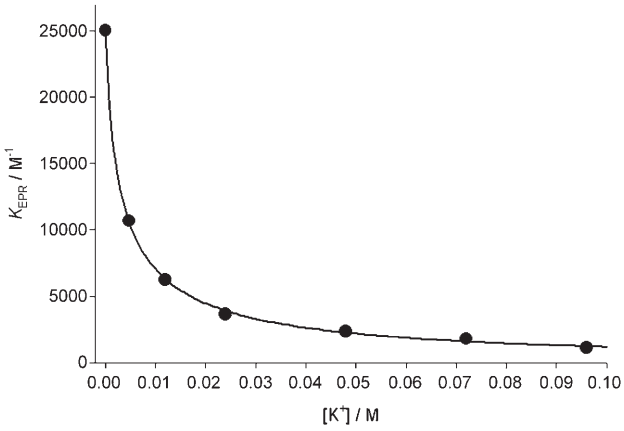


Figure 6. Dependence of the apparent binding constant K_{EPR} for the CB7@**4** complex on the potassium concentration. Numerical fitting of the experimental data was obtained with Equation (1).

On the basis of the five equilibria (K_1, K_2, K_{M1}, K_{M2}, K_{M3}) reported in Scheme 2 and following the procedure reported by Nau and co-workers for the complexation of protonated cyclohexylmethylamine by CB6,[5l] the concentration ratio between the free and complexed radical in the presence of potassium cations is given by Equation (1):

$$\frac{[CB7@X]}{[X]} = [CB7]\frac{K_1 + K_{M1}K_2[M^+]}{1 + K_{M1}[M^+] + K_{M1}K_{M2}[M^+]} \quad (1)$$

where X≡**4** and $[CB7]=[CB7]_0-[CB7@X]$. We now employ the known value for K_1 and a non-linear least-squares fitting to Equation (1) of the experimental data dependence on potassium concentration (Figure 6) to obtain K_2, K_{M1} and K_{M2} (Table 2).[34]

To the best of our knowledge, K_{M1} and K_{M2} represent the first determinations of stability constant for the complex formation between CB7 and K^+ ions. While the value of the equilibrium constant for the mono-complexation of K^+ (K_{M1}) is rather similar to that reported by Buschmann and co-workers[11] for CB6 (560 M^{-1}), that for the coordination of two potassium ions by the opposite portals of CB7 (65 $M^{-1)}$ is considerable larger respect to the upper limit of 20 M^{-1} estimated for CB6.[11] This binding enhancement can be presumably attributed to the possibility for the two cations to be located at a larger distance because of the higher dimension of CB7, thus reducing the electrostatic repulsion between the two positive charges.

The value obtained for K_2 (6150 M^{-1}) is somewhat smaller than that expected exclusively on the basis of the reduction from two to one of the binding sites available for association ($K_1/2=12\,500$ M^{-1}). The fact that the simultaneous complexation of **4** and one potassium ion by CB7 is less favoured than predictable on a purely statistical basis, indicates the presence of steric repulsion between the included radical and the potassium ion associated with the carbonyl portal.

Effect of alkali cations on complexation of nitroxide 1: Unexpected results were observed for the EPR spectroscopic study of the complexation of **1** with CB7 in the presence of alkali cations. When an alkali chloride, MCl (M = Li, Na, K or Cs) is added to a solution containing 10 mM CB7 the EPR signals of CB7@**1** complex decrease and eventually disappear, while the signals of the free species together with those of a new species become visible (Figure 7). This unpredicted species is identified as the radical hosted in the CB7 cavity in which one metal cation is in close contact with the nitroxidic oxygen, CB7@**1**(M^+), on the basis of the following experimental evidence.

1) The nitrogen hyperfine splitting, a(N), increases inversely with cation size, being 16.52 G in the presence of Cs^+ and rising monotonically to 17.12 G in the presence of Li^+ (relative to 15.60 G for the complex in the absence of an alkali-metal cation). This trend is a clear indication that the presence of the metal induces a rather remarkable gain in the odd-electron (spin) population (from ≈6% for Cs^+ to ≈10% for Li^+)[36] at the nitrogen centre. At the same time, the g factor shows an opposite trend, decreasing when passing from the binary complex CB@**1** to the ternary complex in the presence of Li^+. Since the hyperfine splittings and g factors of EPR spectra are known to be sensitive to environmental perturbations, the reported behaviour is expected for nitroxide molecules forming a coordination complex with a metal cation in close contact with the nitroxidic oxygen. The metal cation acting as a Lewis acid site caused a shift of charge towards the oxygen atom and consequently a shift of spin density towards the nitrogen atom in inverse order with cation size.[37] Similar variations of the EPR parameters have been reported for di-*tert*-butyl nitroxide included in Y zeolites after ion exchange with a series of alkali-metal ions.[38]
2) The molar ratio between the coordinated and free radical, obtained from the EPR spectra, strongly depends on the nature of the metal cation and increases on increasing the concentration of the dissolved MCl salt. At high concentrations of alkali salt, the spectrum of CB7@**1**(M^+) becomes dominant (Figure 7c and Supporting Information).
3) The spectra of **1** recorded in the presence of different alkali cations (0.2 M) and in the absence of CB7 are identical to those obtained in pure water.
4) When the EPR spectrum recorded at 298 K contains both signals of CB7@**1**(M^+) and CB7@**1** (Figure 7a), the wing lines of each β-proton triplet are significantly broader than the central lines. This is attributed to the exchange of the metal cation between the aqueous phase and CB7@**1**(M^+), which occurs with a rate comparable to the EPR time-scale. Under the above conditions, the species detected by EPR are in equilibrium as reported in Scheme 3.

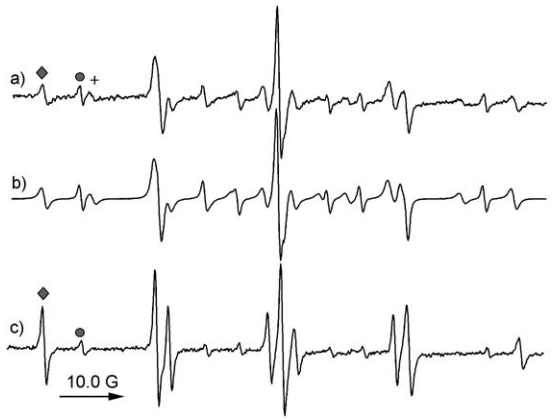
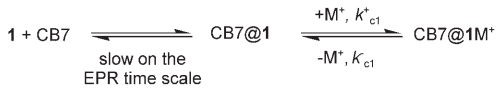


Figure 7. EPR spectra of **1** at 298 K recorded in water in the presence of CB7 (13.0 mM) and a) 1 mM NaCl and c) 400 mM NaCl. + and ◆ refer to the signals of the two complexed guests CB7@**1** and CB7@**1**(Na^+), respectively, ● free guest in water. Spectrum b represents the theoretical simulation of spectrum a obtained with the spectroscopic parameters reported in Table 1 and $k^+_{cl}=2.5\times10^{10}\,M^{-1}s^{-1}$ and $k^-_{cl}=3.0\times10^{7}\,s^{-1}$ for the metal-exchange process.



Scheme 3.

Simulation of the recorded exchange-broadened EPR spectra (Figure 7b) using well-established procedures based on the density matrix theory[39] and assuming a three-jump model as illustrated in Scheme 3, led to the determination of the rate constants k^+ and k^- for metal coordination by CB7 containing the radical probe (Table 4).[40]

Table 4. Stability and kinetic parameters for M^+ exchange at 298 K.

Guest (**X**)	M^+	$10^{-8}k^+_{c1}$ [$M^{-1}s^{-1}$]	$10^{-6}k^-_{c1}$ [s^{-1}]	K_{c1} [M^{-1}]	K_{c2} [M^{-1}]	K_{c1}/K_{M1}
1	Li^+	2.3	6.0	38		
	Na^+	250	30	833		
	K^+	130	11	1180		1.97
	Cs^+	30	45	66		
2	K^+			1300	[a]	2.17
3	K^+			85	5.2	0.14
4	K^+			≈0	≈0	0

[a] Undetermined (see text).

Analysis of the EPR spectra shows that the life-time of the ternary complex is independent of the concentration of solvated cations. Thus, the dominant exchange process occurs through a monomolecular decomplexation mechanism, as shown in Scheme 3. The variation in the selectivity patterns exhibited by CB7@**1** with respect to the different metal cations is determined by the changing balance between the solvating power of the solvent, namely, the solvation energy of M^+, and the binding energy of CB7@**1**. In the present case, the most favoured energy balance is shown by cations of intermediate size (Na^+ and K^+), which is similar to the results reported for the complexation of metal cations by free CB6.[11]

The very fast rates of exchange between CB7@**1** and CB7@**1**(M^+) rules out the possibility that the radical guest undergoes a substantial modification in the spatial position adopted inside the CB7 cavity during the coordination process and suggests that the geometry of the ternary CB7@**1**(M^+) complex should be similar to that predicted for the CB7@**1** complex in the absence of the metal cation. Accordingly, a plausible structure for CB7@**1**(M^+) is that reported in Figure 8, in which the *tert*-butyl group of the guest is included in the host molecule and the metal ion is coordinated by the nitroxidic oxygen and two neighbouring portal oxygen atoms.

The increase in the molar ratio between the coordinated and free radical, observed when the concentration of dissolved MCl is increased, lead to the conclusion that the binding of the metal ions is favoured in the presence of the encapsulated nitroxide. A measure of the effectiveness of this *templation* is given by the ratio between the affinity of the metal cation for CB7 measured in the absence (K_{M1})

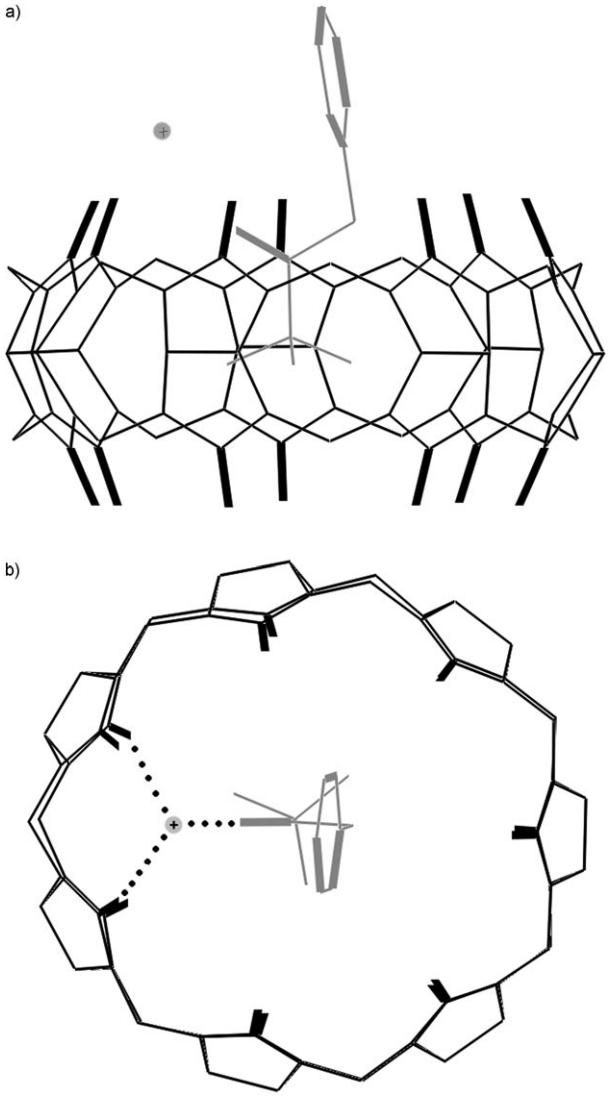


Figure 8. Plausible structure of the ternary CB7@**1**(Na^+) complex. a) side view, b) top view.

and in the presence (K_{C1}) of the radical guest. In the case of K^+, this value is equal to 1.97, confirming the existence of an additional stabilisation through the coordination of the metal cation by the nitroxidic oxygen of the encapsulated paramagnetic guest.

Effect of potassium cations on the complexation of ketones 2 and 3: In order to check whether the template effect is peculiar to the radical probe **1** or is also present in diamagnetic molecules having an oxygen lone pair, we recorded NMR spectra of **2** and **3** in the presence of CB7 and potassium cations. Addition of KCl to solutions containing exclusively the CB7@**2** complex, that is, in the presence of a large excess of CB7 respect to the ketone, give rise to remarkable changes in the corresponding ^1H NMR spectra (Figure 9). For instance, by keeping the CB concentration constant (8.4 mM) and increasing the amount of KCl up to 0.2 M, all signals gradually underwent downfield shifts. No signals attributable

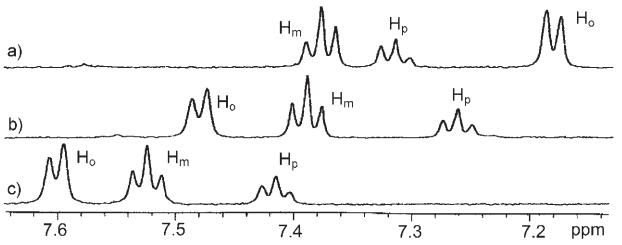


Figure 9. Phenyl region of ^{1}H NMR spectra of benzyl *tert*-butyl ketone (**2**; 0.8 mM). a) In water, b) CB7 (8.4 mM), c) CB7 (8.4 mM), KCl (0.24 M).

to the free guest species are observed under the above conditions. Similarly to the results obtained by EPR of the related radical **1**, the new signals were attributed to a new inclusion complex in which one metal cation is in close contact with the guest carbonylic oxygen thus providing an additional stabilisation through ion–dipole interactions. As expected, only the concentration-weighted averaged signals are observed for the two complexes CB7@**2** and CB7@**2**(K^+), this being an indication that the coordinated K^+ cations are exchanging too rapidly with those in solution on the NMR time-scale.

The expression for the concentration ratio of the two different complexes in the presence of a metal cation is given by Equation (2).

$$\frac{[\mathrm{CB7@X(M)}]}{[\mathrm{CB7@X}]} = [\mathrm{CB7}][\mathrm{M^+}]\frac{K_1K_{C1} + K_{M1}K_2K_{C2}[\mathrm{M^+}]}{1 + K_{M1}[\mathrm{M^+}] + K_{M1}K_{M2}[\mathrm{M^+}]} \quad (2)$$

where X≡**2** or **3**, $[\mathrm{CB7}] = [\mathrm{CB7}]_0 - [\mathrm{CB7@X}] - [\mathrm{CB7@X(M)}]$ and K_{c1}, K_{c2} (Scheme 1) represent the two equilibrium constants for the coordination of the metal ion by the carbonyl group of the guest.[34]

Non-linear least-squares fitting of the experimental dependence of the CIS on the K^+ concentration (Figure 10a) by employing the known values for K_1, K_{M1} and K_{M2} in Equation (2) is possible; however, the resulting errors in the values of K_{c1}, K_{c2} and K_2 are too large. However, variation of the product $K_{c2}K_2$ from the statistical upper limit $(K_1K_{c1})/2$ to zero, give rise to the same value for the equilibrium constant K_{c1} (1300 M^{-1}). This value, which represents a measure of the template effect, is very close to that found for radical **1**, thus confirming the similarity in the behaviour of the two probes.

A more complicated behaviour is found with ketone **3**. The initial increase in the amount of KCl up to ≈0.01–0.05 M produced a high-field shift of the signals of the aliphatic protons, while those of the aromatic hydrogens remained at the same resonating frequency. A subsequent addition of metal salt produces, instead, a down-field shift of all signals. As an example, Figure 10b shows the complexation-induced chemical shift (CIS) of the ethyl hydrogens observed when the concentration of K^+ is increased to 0.22 M ([CB7] = 12.0 mM). This finding can be only be explained by assuming the simultaneous presence of the guest in the free and in the CB7@**2** and CB7@**2**(K^+) complexed forms that

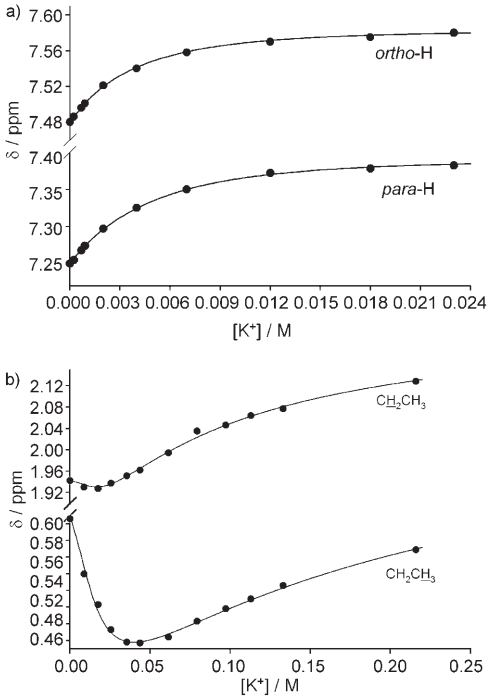


Figure 10. Plot of the complexation-induced chemical shift (CIS) of the hydrogens for a) **2** and b) **3** versus the concentration of K^+ in the presence of CB7 at concentrations of 8.4 and 12.0 mM, respectively. The lines represent the theoretical dependence of the CIS on the potassium ion concentration calculated by numerically fitting the experimental data to Equations (1) and (2) with δ(CB7@**2**)*para*-H = 7.59 ppm, δ(CB7@**2**)*ortho*-H = 7.39 ppm, δ(CB7@**3**)CH_2 = 1.58 ppm and δ(CB7@**3**)CH_3 = 0.29 ppm.

are exchanging rapidly on the NMR time-scale to produce concentration-weighted averaged signals for the three different species. Inserting the known values for K_1, K_{M1} and K_{M2}, a non-linear least-squares fitting of the experimental dependence of the CIS on the K^+ concentration by means of Equations (1) and (2) affords the values of K_{c1}, K_{c2} and K_2 (Table 2 and Table 4).

The ratio between the affinity of the metal cation for CB7 in the absence (K_{M1}) and in the presence (K_{C1}) of the organic guest found by the numerical analysis, 0.14, indicates that the effectiveness of *templation* is lower with the carbonylic guest **3**, than with **2**. A possible explanation of this result is that in the pre-organised CB@**3** complex the carbonyl group of the guest is not located in the optimum position to permit the coordination of the metal cation. The consequent change in the geometry of the complex, required to ensure contact between the guest and the cation, leads to a reduction of the favourable complexation energy, which is only partially compensated by the additional ion–dipole interaction. As expected, the complexation of a second potassium ion by the CB7@**3** complex, K_{c2}, is even less favoured by about one order of magnitude because of the electrostatic repulsion between the two positive charges.

Finally, the value obtained for K_2 (470) is about five times smaller than that estimated exclusively on the basis of the

reduction of the number of binding sites available for association ($K_1/2 = 2300\,M^{-1}$), this being an indication that the potassium ion associated with the carbonyl portal makes the complexation of the guest more difficult because of partial steric repulsion.

Conclusion

The use of EPR and NMR spectroscopies together with molecular dynamics provides a detailed description of supramolecular complexes formed by CB7 in solution. In particular, we were able for the first time to detect in aqueous solution the formation of a coordination complex between alkali cations and a nitroxide–CB7 inclusion complex.[41] The characteristic time-scale of EPR spectroscopy allowed us to determine the exchange rates of the metal cation between the aqueous phase and the nitroxide–CB7 inclusion complex. We employed NMR to demonstrate that this behaviour is not peculiar to nitroxides but is also exhibited by other molecules having an oxygen lone pair.

These results lead to the conclusion that, in the presence of guests having a coordinating lone pair, the formation of ternary metal-guest–CB complexes must be taken into account when discussing the complexation behaviour of cucurbituril derivatives in the presence of salts. In particular, based on the efficacy of the template effect, we can consider three different situations:

1) $K_{C1} > K_{M1}$ (**1** and **2**): The coordination process does not require for the guest molecule a significant modification of the spatial position inside the CB7 cavity with respect to that assumed in the complex in the absence of the metal cation. The additional ion–dipole interaction leads to an increase in the affinity of the metal cation for the CB cavity. The complex ternary metal-guest–CB complex is the most stable and abundant species in solution.
2) $K_{C1} < K_{M1}$ (**3**): The coordinating group of the guest in the pre-organised CB@**3** complex is not located in the optimum position to permit coordination of the metal cation. The consequent change in the geometry of the complex, required to ensure contact between the guest and the cation, gives rise to a reduction in the favourable complexation energy that is only partially compensated by the additional ion–dipole interaction. Nevertheless, a sizeable amount of the ternary complex is present in solution.
3) $K_{C1} = 0$ (**4**): In this case, metal coordination requires a dramatic reorganization of the complex geometry with a large loss of guest–host complexation energy. This is the case for TEMPO, in which the presence of the methyl groups prevents the tilting of the radical guest inside the cavity, which is necessary to allow coordination of the alkali metal ion by the nitroxidic oxygen. Similar behaviour is also expected to be shown by organic guests that do not posses a good coordinating atom such as protonated amines,[5l] or methyl viologen.[18]

In conclusion, we have shown that EPR spectroscopy can add to the palette of analytical methods that afford useful information on cucurbituril-based supramolecular complexes.

Experimental Section

Materials: Benzyl *tert*-butyl ketone[41] and cucurbit[7]uril[3a] were prepared following literature procedures. All the other products are commercially available and were used as received.

EPR spectroscopy: EPR spectra were recorded on a Bruker ESP300 spectrometer equipped with an NMR gaussmeter for field calibration and a Hewlett Packard 5350B microwave frequency counter for the determination of the *g* factors, which were referred to that of the perylene radical cation in concentrated H_2SO_4 ($g = 2.00258$). The sample temperature was controlled with a standard variable-temperature accessory and was monitored before and after each run by means of a copper-constantan thermocouple. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. Digitised EPR spectra were transferred to a personal computer and were analysed by means of digital simulations carried out with a program developed in our laboratory and based on a Monte Carlo procedure.[20b] Nitroxide **1** is generated by mixing a solution of the corresponding amine (≈ 0.8 mM) with a solution of the magnesium salt of monoperoxyphthalic acid (Aldrich, technical grade; ≈ 0.8 mM). In order to achieve a sufficiently large steady-state radical concentration (≈ 0.05 mM), the mixed solution was heated at 40 °C for 1 min. Aliquots from a concentrated CB7 solution were added to the solution of nitroxide to yield the required concentrations. Samples were then transferred in capillary tubes (1 mm i.d.) and the EPR spectra was recorded immediately.

NMR spectroscopy: All spectra were recorded at 298 K on Varian Inova spectrometers operating at 400 and 600 MHz, respectively. NMR experiments were performed in D_2O solutions with residual HOD as an internal standard ($\delta = 4.76$ ppm). Titrations of ketones with CB7 were carried out on a Varian Inova 600 at 298 K. Equilibrium constants were calculated with an iterative procedure based on a least-squares minimisation by means of the Gauss-Newton-Marquardt algorithm.

Dynamic simulations: SD simulations were carried out with the MacroModel 7.0 program. The N–O bond was modelled by the C=O bond owing to their similar geometries and the lack of corresponding parameters in the AMBER* force field.[43] Extended non-bonded cutoff distances were set to 8 and 20 Å for the van der Waals and electrostatic interactions, respectively. All C–H and O–H bond lengths were held fixed by means of the SHAKE algorithm. Translational and rotational momentum were removed every 0.1 ps. The calculation began with a seven-fold symmetric CB7 with the guest molecule docked in its interior. The origin of a Cartesian reference frame was placed at the centre of mass of the CB7 with the *z* axis aligned with the C7 symmetry axis of CB7. The guest was translated along the *z* axis in order to have the quaternary carbon (*tert*-butyl complex) or the centre of mass of the phenyl ring located in the plane passing through the equatorial hydrogens of CB7. At the end of this procedure, minimisation of the ensemble led to the starting geometry for SD simulations. The simulations were run at 298 K with time steps of 1 fs and an equilibrium time of 500 ps before each dynamic run. The total simulation time was set to 5000 ps in order to achieve full convergence.

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[30] CIS of ≈ -1.0, -0.8 and -0.7 ppm have been found in the presence of CB6 for 1,5-diaminopentane,[1a] THF,[5d] and cyclohexylmethylammonium ion,[5l] respectively.

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