

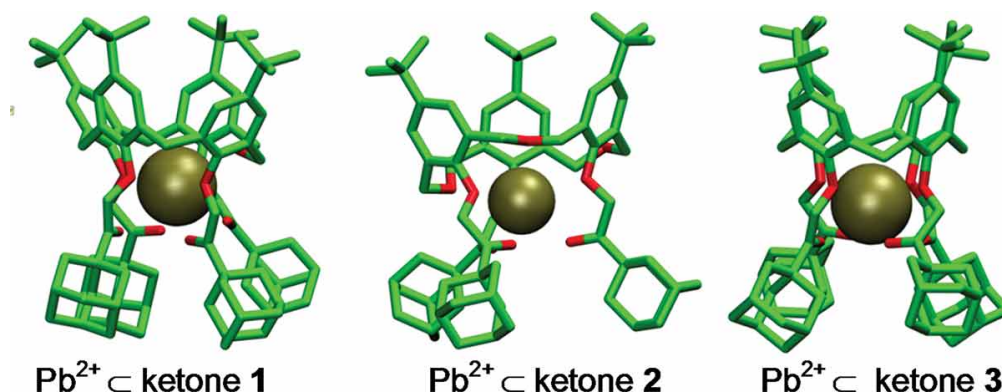
Calixarene-Based lead receptors: an NMR, DFT and X-Ray synergetic approach

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ABSTRACT

The conformational changes of the homooxa adamantyl ketones **1** and **2**, as well as of the calix[4]arene analogue derivative **3**, all in the cone conformation, upon Pb^{2+} complexation were investigated by ^1H , ^{13}C , ^{207}Pb NMR and proton spin-lattice relaxation times (T_1) experiments. The X-ray crystal structures of ketones **1** and **3** were determined. The inclusion of an acetonitrile molecule in the hydrophobic cavity of the ligands was observed in the solid state. In solution, inclusion was observed only in the case of Pb^{2+} \subset ketone **3** complex. DFT calculations were also performed to complement the NMR conformational analysis and to bring further insights to the cation complexation. The data confirmed the formation of 1:1 complexes between Pb^{2+} and the ligands, and that the cation is located inside the cavity defined by the phenoxy and carbonyl oxygen atoms. In general, the ligand conformations became closer to a regular cone upon complexation, with the binding models found for the three ketones through the NMR studies corroborated by the DFT calculations.



ARTICLE HISTORY

Received 19 May 2021

Accepted 13 July 2021

KEYWORDS

Calixarene adamantyl ketones, Pb^{2+} binding, NMR studies, DFT calculations, X-ray diffraction

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Introduction

Lately, increasing attention has been paid to the coordination chemistry of heavy metals, such as arsenic, cadmium, mercury and lead. These metals, resulting from industrial processes, are very toxic and difficult to remove, thus provoking a harmful impact on environmental quality and consequently on human health [1,2]. In fact, from soil and water contamination, these heavy metals enter into the food chain and can induce serious problems in human health. Pb(II) is one of the heavy metal ions broadly present in the environment. Even at very low concentrations, lead can cause severe damages in the human brain and nervous system, increasing the risk of neurodegenerative diseases, as well as kidney, bone and cardiovascular pathologies [3].

Calixarenes are a versatile family of macrocyclic compounds widely investigated in host-guest and supramolecular chemistry [4,5]. Their availability and relatively easy functionalization at the upper and lower rims afford a large variety of derivatives, with multiple possibilities of applications in different areas, such as extraction, sensing and recognition of relevant ions and neutral molecules [6–8]. In particular, lower rim functionalised calixarenes with carbonyl groups have shown a strong ability towards different metal cations [9,10]. Among them, certain calixarene derivatives have been recognised as useful ionophores for Pb²⁺ ion. The carbonyl and the phenoxy oxygen atoms play an important role in Pb²⁺ binding. Very recent articles report studies with calix[4]arene tetraacetic acid [11] and amide derivatives [12–14] in solvent extraction systems and electrochemical sensors for Pb²⁺ determination.

In the course of our systematic research on the binding properties of homooxacalixarenes [15] bearing ketonic carbonyl groups at the lower rim [16–21], we were interested in determine how the cation complexation would influence the calixarene conformation. Thus, the conformational analysis of the homooxa adamantyl ketones **1** and **2**, and their conformational changes upon Pb²⁺ complexation, were assessed by ¹H, ¹³C, ²⁰⁷Pb, 2D NMR experiments and proton spin-lattice relaxation times (T₁). The X-ray crystal structures of ketones **1** and **3** were determined, and DFT computational studies were performed to add further insights to the binding process. Tetraadamantyl ketone derivative [3] of the p-tert-butylcalix[4]arene (Figure 1) was also studied in this work, and the results of the three ligands were compared and discussed in order to analyse the effects of the additional oxygen bridges on the macrocycle conformational flexibility.

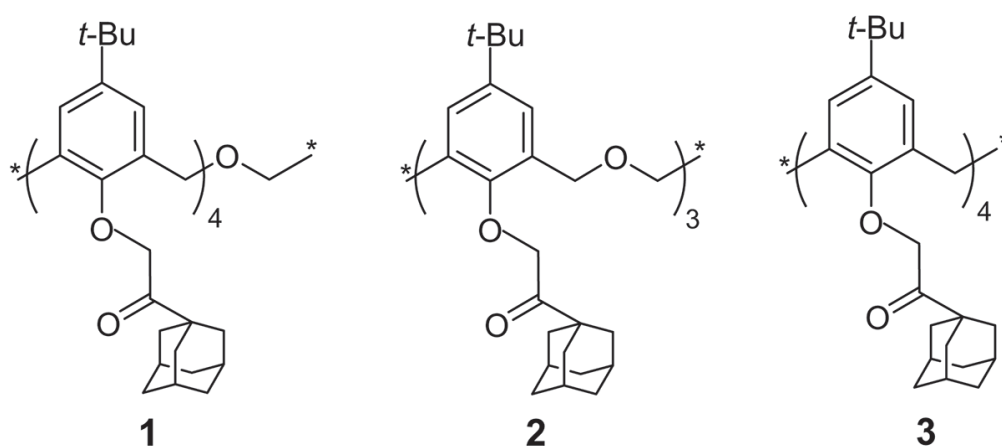


Figure 1. Chemical structures of adamantyl ketones **1**, **2** and **3**

Materials and methods

Materials

Adamantyl ketones **1**, **2** and **3** were synthesised according to the procedures reported in the literature (20, 18 and 21, respectively). Lead (II) perchlorate hydrate 98% was purchased from Sigma-Aldrich and it was dried over P₄O₁₀ under vacuum for several days before use.

Determination of the crystallographic structures of **1** and **3**

Single crystals suitable for X-ray diffraction structure analysis were obtained by slow evaporation of solutions of the adamantyl ketones **1** or **3** in various mixtures of organic solvents. The crystallisation trials in the presence of lead acetate did not yield any structure with encapsulated Pb²⁺. Two pseudo-polymorphic forms for both adamantyl ketones **1** and **3** were obtained in the presence or absence of the co-solvent acetonitrile, which exhibits guest properties towards the calixarene cavity. Data collection was carried out at the Macromolecular crystallography XRD1 beamline of the Elettra synchrotron (Trieste, Italy), employing the rotating-crystal method with a Dectris Pilatus 2 M area detector. Single crystals were dipped in paratone cryoprotectant, mounted on a nylon loop and flash-frozen under a nitrogen stream at a 100 K. Diffraction data were indexed and integrated using the XDS package [22], while scaling was carried out with XSCALE [23]. Structures were solved using the SHELXT program [24] and structure refinement was performed with SHELXL-18/3 [25], operating through the WinGX GUI [26], by full-matrix least-squares (FMLS) methods on F². Non-hydrogen atoms were refined anisotropically, with the exception of some disordered groups. Hydrogen atoms were added at the calculated positions and refined using the riding model. Crystallographic data are reported in Table S1. Full detailed refinement procedures are reported in the Supporting Information (Figure S1).

NMR studies

The NMR experiments were performed in CDCl₃ at 25°C using a Bruker Avance III 500 spectrometer. TMS was used as internal reference for ¹H and ¹³C NMR measurements, while for ²⁰⁷Pb chemical shifts a 1.0 M solution of Pb(NO₃)₂ in D₂O was used as external calibration ($\delta = -2961.2$ ppm relative to Pb(CH₃)₄). The ²⁰⁷Pb spectra were acquired using a 90° pulse of 10.2 μ s and a relaxation delay time of 0.6 s. The NOESY and HMBC 2D NMR spectra were acquired using the Bruker standard pulse sequences. The NOESY experiments were collected as a 256x2K complex points and a mixing time of 0.6 s and the HMBC as 512x1K data points and a relaxation delay time of 1.5 s. Spin-lattice relaxation times (T₁) were determined by the inversion recovery method [27], in which 16 spectra of 32 K data points were collected, with 16 inversion recovery delay times (τ) ranging from 50 ms to 2 s. A quick T₁ estimation was performed for all samples to set the appropriate relaxation delay between 2 and 5 s. The T₁ values were obtained by fitting the data to the equation:

$$I = I_{\infty} \left(1 - 2e^{-\frac{\tau}{T_1}} \right)$$

where *I* is the magnetisation, *I*_∞ is the magnetisation at thermal equilibrium and τ is the inversion time.

The complexation studies were carried out by adding 1 equiv of the salt Pb(ClO₄)₂ (0.25 M) in CD₃OD to 0.5 mL of CDCl₃ solutions (5×10^{-3} M) of the ligands directly in the

NMR tube, the ^1H and ^{13}C spectra being recorded after the addition. For the titrations with MeCN, several aliquots (up to 10 equiv) of MeCN solution in CDCl_3 (1.25×10^{-1} M) were added to the free ligands and to the Pb^{2+} \subset ketone complexes. In the case of the ^{207}Pb spectra, the experiments were performed by adding 1 equiv of the ligands to a $\text{CDCl}_3/\text{CD}_3\text{OD}$ (10:1) solution of the salt (5×10^{-2} M) directly in the NMR tube.

DFT calculations

Stationary points were optimised with the Gaussian 09 program [28] with different functionals for comparison: B3LYP [29], BP86 [30], M06-2X [31] and wB97XD [32] functions with the 6–31 G(d,p) basis for C, N, O, H atoms and SDD for lead. A D3-Grimme correction [33] was also used associated to B3LYP and BP86 functionals. Experimental X-ray diffraction structure determination results were employed as the starting structures for geometry optimisation. All reported structures were confirmed as energy minima, with no negative eigenvalue in the Hessian matrix. The ^{13}C NMR calculations employed the Gauge-Independent Atomic Orbital (GIAO) method [34]. An average of values of equivalent atoms was assumed.

Results and discussion

X-Ray diffraction studies

The X-ray structures of two pseudo-polymorphic forms (α and β) for adamantyl ketones **1** and **3** were determined using synchrotron radiation with cryogenic techniques.

The dihomooxacalix[4]arene macrocycle **1** shows the expected cone conformation in both crystal forms (Figure 2a,b). The planes of the phenyl rings A and B (connected to the dihomooxa bridge) of **1 α** make dihedral angles of 120.6° and 62.6° with respect to the mean plane of the methylene bridging groups (Table 1). Angles greater than 90° indicate that the tert-butyl groups on the upper rims lean outwards from the centre of the cone (Figure 2a) and vice versa. Therefore, the A and B are oriented outwards and inwards, respectively. The planes of the phenyl ring C makes a dihedral angle of 148.1° with the mean plane of the methylene bridging groups, with the upper rim largely inclined outwards, while the phenyl ring D is tilted slightly outwards, with a dihedral angle of 104.3° . This distorted cone conformation, with one inward oriented phenyl ring involved in the dihomooxa bridge, appears to be a characteristic feature of dihomooxacalix[4]arenes functionalised with tert-butyl groups on the upper rim and four substituents on the lower rim, in absence of a guest [35–37]. In the presence of a guest molecule, the macrocycle necessarily opens its cup to host the molecule, thereby altering its conformation, as observed in the **1 β** form which hosts an acetonitrile molecule in the cup. In this case, the dihedral angles of phenyl rings are all oriented outwards (Table 1 and Figure 2b). The orientation of the phenyl groups is reflected in the position of the adamantyl substituents on the lower rim. In the α form, the adamantyl group of the most outwardly oriented C ring is more internal and aligned with the central axis of the molecule, while the adamantyl group of the inwardly oriented B ring protrudes more than the others (Figure 2a). Consistently, in the β form with all the phenyl rings outwardly oriented, the adamantyl groups are more closely packed (Figure 2b). The orientation of the adamantyl and carbonyl groups are mainly dictated by the torsion angle around the O-CH₂ bond (Table 1). In both crystal forms two carbonyl groups are internally oriented but on different rings (B, D for **1 α** and A, C for **1 β** , see Table 1 torsion angles close to 180°). The layer organisation of the

crystal packing of **1 α** (Figure 3a) produces two types of solvent channels along the crystallographic a axis, which contain the disordered co-crystallised solvent molecules (Figure 4a). On the contrary, the more closed packed β form shows interrupted channels along the crystallographic b axis, therefore producing enclosed cavities occupied by the solvent molecules (Figure 4b).

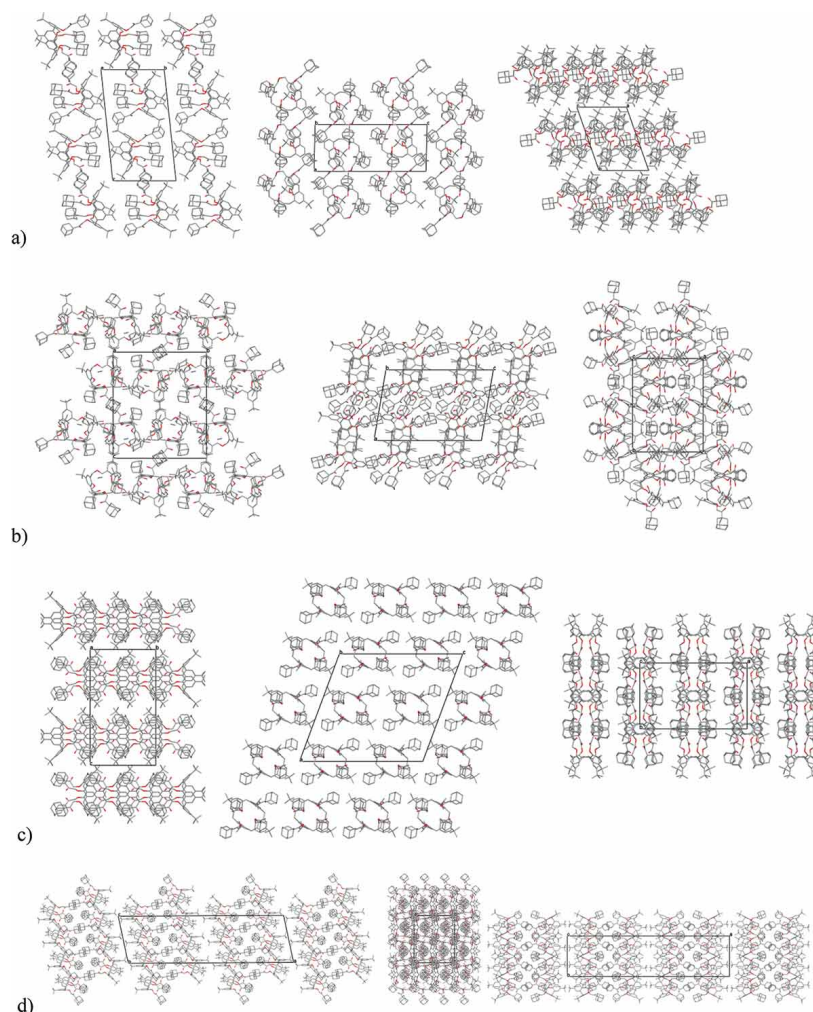


Figure 3. Crystal packing views along the a, b and c unit cell axes of the X-ray structures of a) **1 α** , b) **1 β** , c) **3 α** and d) **3 β** .

Table 1. Comparison of cone conformations: dihedral angles between corresponding aryl planes of the calixarene cones (A, B, C and D) and the mean planes of the bridging methylene carbon atoms and torsion angles (between parentheses) around the O-CH₂ bond determining the orientation of the carbonyl and adamantyl groups. See Figure 2a for the labelling scheme of the arene moieties (Table view)

	A	B	C	D
1α	120.6 (−81.5)	62.6 (−160.9)	148.1 (−58.3)	104.3 (167.7)
1β	102.0 (180.0)	141.9 (−56.6)	96.5 (168.4)	132.9 (−57.2)
3α	135.3 (50.5)	94.6 (−167.9)	135.3 (50.5)	94.6 (−167.9)
3β	112.1 (124.8)	117.9 (120.6)	111.5 (129.1)	114.6 (121.8)

The calix[4]arene macrocycle **3** shows the expected cone conformation in both pseudo-polymorphic forms (Figure 2c,d). In the α form the molecule shows a C₂ symmetry with two opposite phenyl rings more outwardly inclined (135.3°) with the other two nearly orthogonal (94.6°) with respect to the mean plane of the methylene bridging groups. In the β form, in which the molecule hosts an acetonitrile guest molecule, a pseudo C₄

symmetry is observed with the planes of the phenyl rings making similar dihedral angles, ranging from 111.5° to 117.9° (Table 1). This acetonitrile guest forms the typical host-guest CH- π interactions. The **3 β** structure shows a water molecule entrapped on the lower rim by H-bond with the phenoxy oxygen atoms (Figure 2d). This assignment is supported by the high resolution electron density maps (Figure S1f). A water molecule entrapped between the oxygen on the lower rim has been previously reported for analogous calix[4]arene [38]. Similarly to both **1** structures, in **3 α** two opposite carbonyl groups are oriented inwards (Table 1). On the contrary, in **3 β** the carbonyl groups are oriented orthogonally with respect to the symmetry axis of the molecule in a way to maintain the pseudo C_4 symmetry. This produces a dissymmetric conformation of the calix[4]arene, with the orientation of the carbonyl and adamantyl groups dictated by the torsion angle around the O-CH₂ bond ranging from 120.6° to 129.1° (Table 1). The position of the entrapped water molecule is reminiscent of the coordination of Pb²⁺ ions found in analogues calix[4]arene functionalised on the lower rim with four ketone groups, four ester groups [39] or four amide groups [40]. In these structures the lead shows eightfold oxygen coordination. In the case of the ketone and ester groups, which are more closely related to **3**, the oxygen atoms are arranged as the corners of a tetragonally compressed cube [39], while they are arranged in an intermediate shape between a cube and a square antiprism for the amide derivative [40]. Comparison of the intramolecular assembly of the adamantyl groups shows that the calix[4]arene complexed by the guest molecules is more compact than the free calix[4]arene (Figure 2c,d). In the α structure the C₂ symmetric pinched cone conformation forces two opposite adamantyl groups outwards from the symmetry axis (Figure 2c). The crystal packing of **3 α** is characterised by a 1D stacks of calix[4]arene molecules (Figure 3c). This results in a very open structure with an interconnected network of solvent accessible channels (Figure 4c). In the case of **3 β** , the head-to-head/tail-to-tail organisation of the crystal packing (Figure 3d) produces cavities, which contain the disordered co-crystallised solvent molecules (Figure 4d).

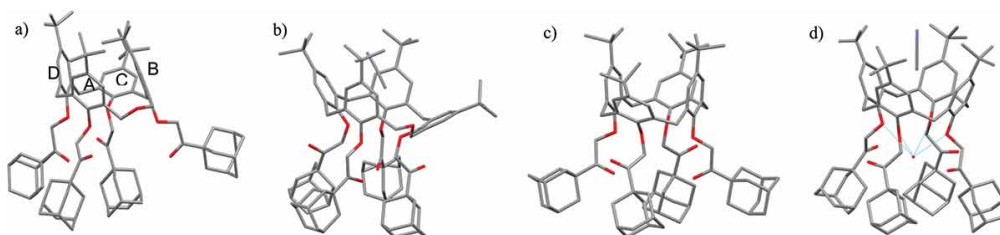


Figure 2. Stick representation of X-ray structures of a) **1 α** , b) **1 β** , c) **3 α** and d) **3 β** . The atomic species are represented in CPK colours. Hydrogen atoms are omitted for clarity

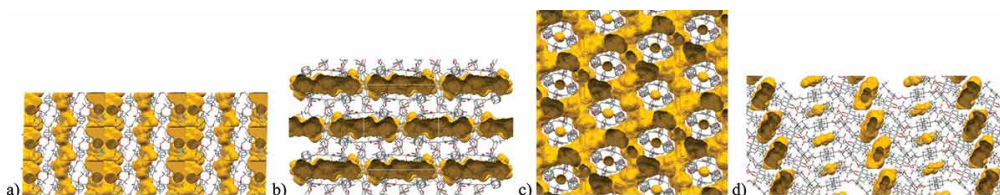


Figure 4. Accessible void volume of X-ray structures of a) **1 α** , b) **1 β** , c) **3 α** and d) **3 β** .

NMR and DFT conformational analysis

Proton and carbon-13 studies

The ionophoric properties of homooxacalixarene ketone derivatives were previously reported [17,18], showing that these ligands form 1:1 complexes with heavy metal cations, such as Pb^{2+} . Limitations in the solubility of adamantyl ketone derivatives prevented however the determination of their stability constants. The ^1H NMR data indicated that the cations were encapsulated inside the cavity formed by the phenoxy and carbonyl oxygen atoms. Following this line of research and to gain further information on the cation binding behaviour of the ligands, namely concerning the binding sites, homooxa ketones **1** and **2**, as well as calix[4]arene ketone **3** were studied through ^1H , ^{13}C , ^{207}Pb and T_1 relaxation NMR experiments.

Proton NMR data of the free and complexed ligands **1**, **2** and **3** were obtained and are in agreement with the results previously reported [17,18,41]. Similarly, ^{13}C NMR data were obtained. All the assignments were confirmed by DEPT, and by NOESY and HMBC 2D NMR experiments.

Concerning dihomooxa ketone **1**, the conformational analysis in CDCl_3 indicated a molecule with a symmetry plane, with the following characteristic ^1H and ^{13}C signals of a cone conformation: two singlets for the tert-butyl protons, three AB quartets (in a 2:2:1 ratio) for the CH_2 bridge protons and two pairs of doublets for the aromatic protons of the calixarene framework (Figure 5a) [42], as well as two ArCH_2Ar resonances around 32 ppm [43]. Protons corresponding to rings A and B (Figure 5c) were identified by the NOESY experiments (Figure S2). Upon Pb^{2+} complexation, all the proton (Figure 5b) and carbon-13 chemical shifts (Table S2) in ligand **1** are affected. The highest upfield shift variations were observed for the bridging axial methylene protons (ArCH_2Ar) and the oxygen bridge equatorial and axial methylene protons (CH_2OCH_2), while the largest downfield changes were observed for the aromatic protons, the methylene protons of the OCH_2CO groups and for the equatorial methylene protons of the ArCH_2Ar bridges.

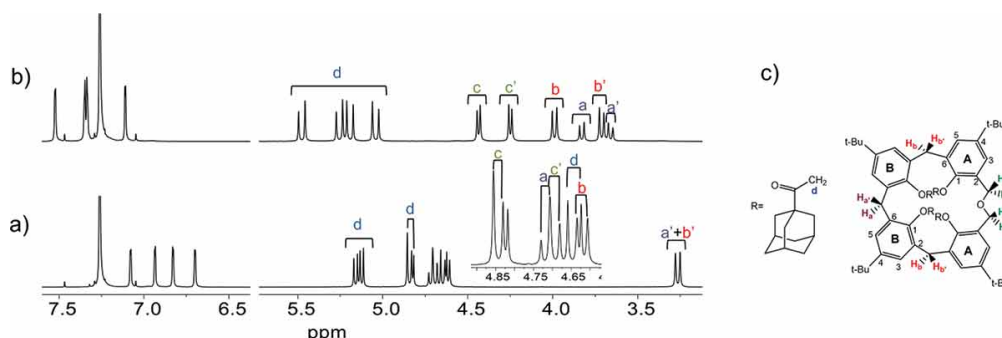


Figure 5. Partial ^1H NMR spectra (500 MHz, CDCl_3 , 25°C) of: a) Ad ketone **1**, insert: 4.5–4.9 ppm region expansion; b) **1** + 1 eq of $\text{Pb}(\text{ClO}_4)_2$; c) Rings and atoms identification in **1**.

Gutsche ([4a]) established that the difference in the chemical shifts between the axial and the equatorial protons of the ArCH_2Ar bridges indicates the degree of flattening of the cone conformation. A value around 0.9 ppm means a regular cone and zero a regular 1,3-alternate conformation. Upon Pb^{2+} complexation, the $\Delta\delta_{\text{Hax-Heq}}$ decreases from 1.39 ppm to 0.22 ppm (in average), indicating that the pendant arms of ketone **1** move closer together, resulting in a more flattened cone conformation. In addition, both axial and equatorial methylene protons of CH_2OCH_2 bridge move upfield and with the same value, suggesting significant conformational changes on the oxygen bridge upon complexation.

	C	CH ₃	CH	CH ₂	C	CH ₂ OCH ₂	ketone 2 ArOCH ₂		Ar1	Ar2	Ar3	Ar4
	t-Bu			Ad				CO	Aromatic ring			
	C	CH ₃	CH	CH ₂	C	CH ₂ OCH ₂	ArOCH ₂		Ar1	Ar2	Ar3	Ar4
NMR	0.3	-0.4	-0.4	-0.6 0.1	1.2	1.4	4.3	7.9	-3.6	-0.3	4.0	4.5
DFT	0.7	-1.2	-1.7	-1.0	3.4	1.2	-2.9	16.5	-13.2	-3.7	4.9	11.3
						ketone 3						
NMR	-0.2	-1.0	-1.2	-1.3 1.0	0.4	-3.8	6.8	7.0	-6.0	0.1	0.7	4.1
DFT	0.1	-1.3	-1.4	-0.1	2.2	-4.9	3.2	13.2	-11.8	-1.6	2.8	11.3

$$*\Delta\delta \text{ (ppm)} = \delta (\text{Pb}^{2+} \text{ c ligand}) - \delta (\text{ligand})$$



With regard to the hexahomotrioxa ketone **2**, its cone conformation with C_{3v} -symmetry is reflected by its ^1H and ^{13}C NMR spectra. The former spectrum shows one AB quartet for the CH_2 bridge protons, and one singlet for the tert-butyl, aromatic and methylene protons of the OCH_2CO groups. In addition, a series of multiplets for the adamantyl groups is also present. The latter spectrum exhibits a pattern containing five downfield resonances arising from the carbonyl and aromatic carbon atoms, two midfield resonances arising from the methylene carbon atoms of the OCH_2CO and CH_2OCH_2 groups, and six upfield resonances arising from the adamantyl and tert-butyl groups. As observed before for ketone **1**, the addition of Pb^{2+} cation affects all the proton and carbon-13 chemical shifts in ligand **2** (Table S3). The largest variations are recorded for the aromatic protons and the methylene protons of the OCH_2CO groups, which move downfield, and by the oxygen bridge equatorial methylene protons, which move upfield. The $\Delta\delta_{\text{Hax-Heq}}$ increases from 0.26 ppm to 0.52 ppm upon Pb^{2+} complexation. If Gutsche criterion is also applicable to the CH_2OCH_2 bridges, it indicates that the phenyl groups in ketone **2** are more flattened than those in ketone **1**, and stand up when the cation enters into the binding cavity, resulting in a more regular cone conformation. Concerning ^{13}C chemical shifts, the largest downfield differences are observed for the carbonyl and OCH_2CO carbon atoms, while an upfield shift is observed for the aromatic carbon atom 1 (Table 3, Figure 7). The spectroscopic data indicate a similar binding mode to the one observed before for ketone **1**, with Pb^{2+} bound through metal-oxygen interactions inside the ionophoric cavity.

Finally, calix[4]arene ketone **3** also presents symmetric proton and carbon-13 NMR spectra, compatible with a cone conformation. Upon Pb^{2+} complexation, the largest downfield variations are recorded for the aromatic protons and the bridging equatorial methylene protons, while the highest upfield change is observed for the bridging axial methylene protons (Table S4). In this case, the $\Delta\delta_{\text{Hax-Heq}}$ decreases from 1.72 ppm to 0.57 ppm being, among the three ligands, the closest value to 0.9 ppm, indicating that ketone **3** adopts a more symmetric cone conformation upon complexation. In the case of the ^{13}C chemical shift variations upon complexation (Table S4), the highest values are recorded for both carbonyl and methylene carbon atoms of the OCH_2CO groups, which move downfield, and for the aromatic carbon atom Ar1, which moves upfield, being all similar in absolute value (Table 3, Figure 7). This suggests that Pb^{2+} cation should be centred inside the ionophoric cavity, interacting equally with the eight oxygen atoms.

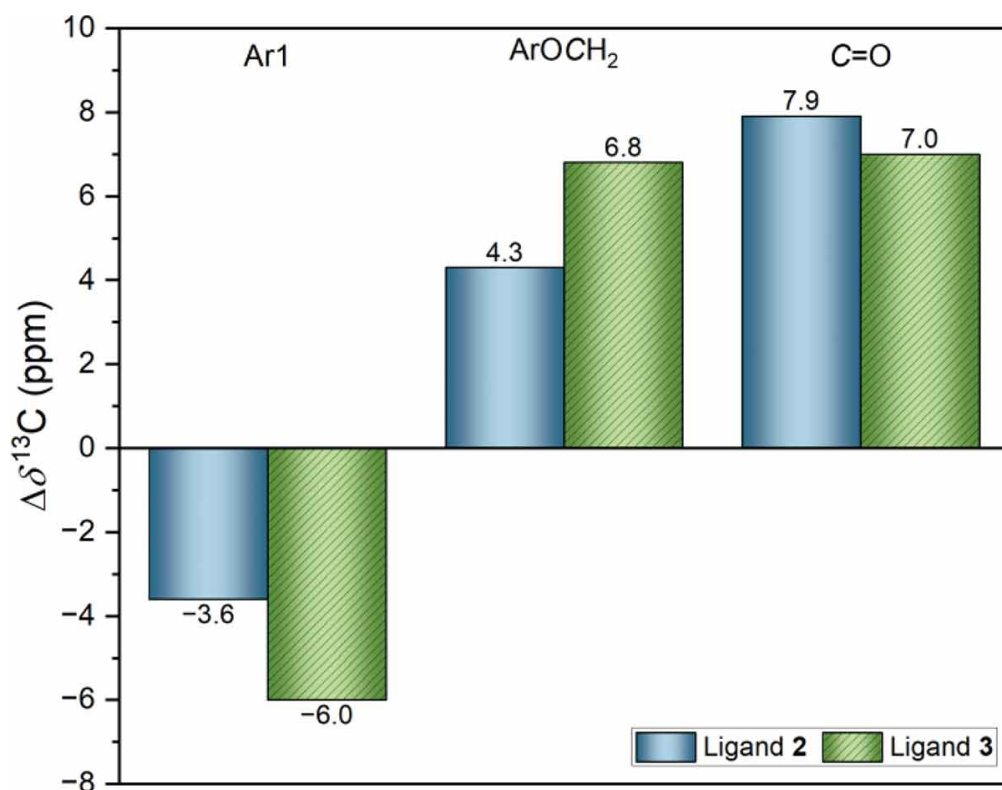


Figure 7. Largest ^{13}C chemical shift differences for ligands **2** and **3** upon Pb^{2+} complexation [$\delta(\text{Pb}^{2+} \leftarrow \text{ligand}) - \delta(\text{ligand})$]

The X-ray results have shown the inclusion of an acetonitrile molecule in the hydrophobic cavity of ligands **1** and **3**, and the impact on their conformations. Thus, to see if the MeCN had also a role in the complexation process in solution, as described for calix[4]arene analogues [39,44], additional ^1H NMR experiments were carried out. Free and complexed ligands **1** and **3** were titrated with MeCN in CDCl_3 . No effects were observed for ligand **1** (free or complexed) and for free ligand **3** (Figure S3a). The chemical shift of the MeCN protons remained unchanged at 2.0 ppm during the titration (up to 7 equiv). In the case of $\text{Pb}^{2+} \leftarrow \text{ketone } \mathbf{3}$ complex (1:1), a small effect could be detected: the MeCN resonance appeared at 1.85 ppm in the beginning of the titration and slightly shifted to 1.91 ppm after the addition of 10 equiv of MeCN (Figure S3b). This corresponds to a small upfield shift ($\Delta\delta = -0.25$ ppm) relative to its normal position in CDCl_3 ($\delta = 2.10$ ppm), and indicates some inclusion of this molecule in the hydrophobic cavity of ligand **3**. However, the proton signals of **3** remained almost unchanged throughout the titration. This result may indicate that, in this case, the affinity of the complex for MeCN is slightly higher than that of the free ligand, due to a better preorganisation of the hydrophobic cavity of the former.

To complement the previous studies, DFT optimisations of the $\text{Pb}^{2+} \leftarrow \text{ketone}$ complexes in the presence of an acetonitrile molecule in the hydrophobic cavity were performed. Overall, there are very little structural differences between the structures with and without acetonitrile (Figure S4). The RMSD fluctuations, for each ligand, between mono and di-complexed structures are 0.114. Lead coordination sphere remains unchanged despite the presence of a solvent molecule sitting in the ligand hydrophobic cavity.

Quantum Mechanical calculations

Geometry optimisations were performed on free and complexed ligands with four different DFT functionals (B3LYP, BP86, M06-2X and wB97XD). The M06-2X functional was selected as in general it showed the best performance for the calculation of the ^{13}C NMR

chemical shifts. Similar results with this functional and calix[4]arene derivatives have been reported [45]. A comparison between experimental and theoretical chemical shifts provides practical information on the chemical structure and conformation of the macrocycles and their complexes. The ^{13}C chemical shifts obtained with all functionals used are given in Tables S5-S7, and they are only slightly dependent on the functional, being in agreement with the experimental values (see Tables 2 and 3). In a few cases, variations larger than 2 ppm were observed.

Independently from the chosen functional, the ΔE complexation energies [$\Delta E = E(\text{complex}) - E(\text{free ligand}) - E(\text{Pb}^{2+})$] follow the same order for the three ligands: ketone **1** > ketone **3** > ketone **2**, thus being ketone **1** the best ligand for Pb^{2+} cation (Table S8). The ΔE values obtained with M06-2X functional are -254.2, -237.4 and -225.6 kcal mol $^{-1}$ for ketones **1**, **3** and **2**, respectively, showing quite large differences in the complexation energies. Functionals without dispersion correction present little differentiation among the ligand energies, while in the case of functionals wB97XD and M06-2X, including already dispersion corrections to some degree, and for the B3LYP and BP86 functionals with the Grimme D3 correction, the differences are higher (Table S8). It should be noted that dispersion correction is important to obtain accurate NMR chemical shifts, especially for systems presenting weak intra- or intermolecular contacts [46].

A structural analysis of the different complexes shows significant differences depending on the ligand. Snapshots of the optimised Pb^{2+} complexes are given Figure 8 and Figures S5 to S7, and the most relevant distances, especially $\text{Pb}^{2+} \cdots \text{O}$ ones, are given in Figure 9 and Table 4 and S9.

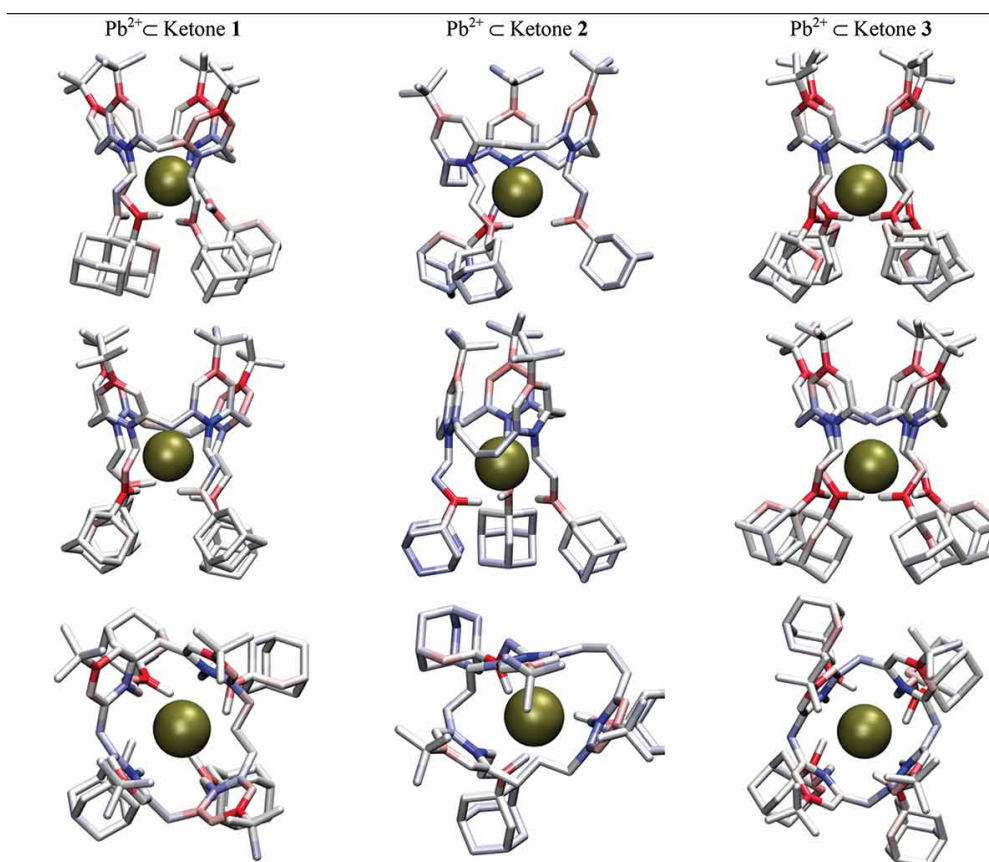


Figure 8. Structures of the Pb^{2+} c ketone complexes calculated at the M06-2X DFT level. ^{13}C NMR chemical shift variations [$\delta(\text{Pb}^{2+} \text{ c ketone}) - \delta(\text{ketone})$], negative in blue and positive in red (front, orthogonal and top views)

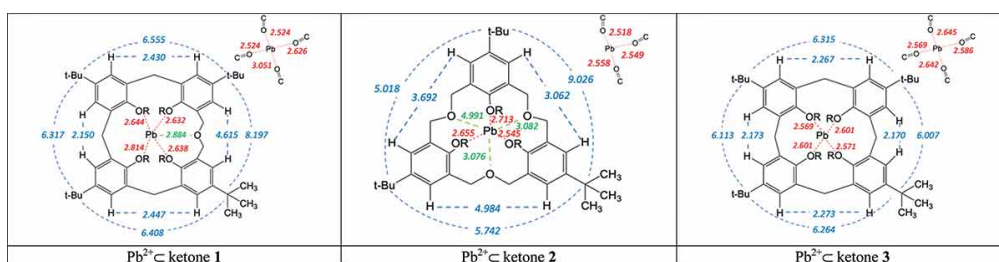


Figure 9. Relevant distances (in Å) in structures of the Pb^{2+} ketone complexes calculated at the M06-2X DFT level; R = CH_2COAd

Table 4. Relevant distances (in Å) for free ketones and their Pb^{2+} complexes calculated at the M06-2X DFT level (Table view)

	ketone 1	ketone 2	ketone 3	Pb^{2+} c 1	Pb^{2+} c 2	Pb^{2+} c 3
$\text{C}_{\text{t-Bu}} \cdots \text{C}_{\text{t-Bu}}$ in adjacent rings	6.470	5.321	6.489	6.317	5.018	6.007
	6.600	5.363	6.807	6.408	5.742	6.113
	6.652	9.295	6.851	6.555	9.026	6.264
	9.094		7.521	8.197		6.315
$\text{H}_{\text{arom}} \cdots \text{H}_{\text{arom}}$ in adjacent rings	2.573	3.196	2.664	2.150	3.062	2.170
	2.682	3.517	2.735	2.430	3.692	2.172
	2.854	4.878	3.013	2.447	4.984	2.267
	5.073		3.034	4.615		2.273

Free ketone **1** has an asymmetrical conformation due to the presence of the CH_2OCH_2 bridge, showing three shorter (~ 6.57 Å) and one longer (~ 9.09 Å) $\text{t-Bu} \cdots \text{t-Bu}$ distances. This leads to a flattened cone conformation, with one short (5.337 Å) and one long (12.722 Å) $\text{t-Bu} \cdots \text{t-Bu}$ distances between two opposite aryl groups. The two closest aryl groups have an almost parallel orientation. Concerning free ketone **2**, it exhibits also an asymmetrical conformation, with two shorter (~ 5.34 Å) and one longer (9.30 Å) $\text{t-Bu} \cdots \text{t-Bu}$ distances, leading to a distorted cone conformation. Finally, ketone **3**, with a symmetrical structure, presents also a flattened cone conformation, with a short (5.324 Å) and a long (12.230 Å) $\text{t-Bu} \cdots \text{t-Bu}$ distances between two opposite aryl groups (see Figures S5 to S7).

The Pb^{2+} complexation tightens the ligand upper rim, as shown by the shorter $\text{t-Bu} \cdots \text{t-Bu}$ and $\text{H}_{\text{arom}} \cdots \text{H}_{\text{arom}}$ distances in the complexes compared to those of the corresponding free ligands (Table 4). The complexed ligand **1** leads to an asymmetrical structure, with a flattened cone conformation. Pb^{2+} cation is surrounded by nine oxygen atoms, at a distance less than 3.05 Å: three of the four carbonyl oxygen atoms are the closest, at less than 2.6 Å, and the phenoxy oxygen atoms sit at about 2.6–2.9 Å (included the CH_2OCH_2 one): This may explain the highest complexation energy obtained with ketone **1**. Ketone **3** shows a symmetrical structure, being the cation positioned in the centre of the cavity created by the four + four oxygen atoms of the pendant arms. All the $\text{Pb}^{2+} \cdots \text{oxygen}$ distances are lower than 2.65 Å, which may also explain the higher interaction energy compared to ketone **2**, with only three pendant arms. The influence of the complexation on the ligand conformation is quite large in this case, changing ketone **3** from a flattened cone conformation to a square cone conformation upon complexation, as illustrated in Figure 8 and S7. Both $\text{t-Bu} \cdots \text{t-Bu}$ and $\text{H}_{\text{arom}} \cdots \text{H}_{\text{arom}}$ distances decrease, with variations up to 1.2 Å. The cone conformation of the complexed ligand **2** remains distorted and asymmetrical, although in the sphere of coordination around lead, composed by six

oxygen atoms from the three pendant arms, the $\text{Pb}^{2+} \cdots \text{O}$ distances are very similar and the shortest among the three studied complexes: around 2.5 Å for $\text{Pb} \cdots \text{O}_{\text{C=O}}$ and from 2.5 to 2.7 Å for $\text{Pb}^{2+} \cdots \text{O}_{\text{phenoxy}}$. The sphere of coordination is completed by two CH_2OCH_2 bridge oxygens sitting at ~3.1 Å (Figure 9).

^{207}Pb NMR studies

The ^{207}Pb NMR experiments were performed to confirm the coordination environment of Pb^{2+} cation in both ketones **1** and **3**, since the ^{207}Pb nucleus has a large chemical shift range (~17,000 ppm), being very sensitive to changes in its coordination sphere [47]. ^{207}Pb NMR studies with calix[4]arene-crowns-6 in acetonitrile have been reported [48]. Thus, ^{207}Pb spectra were obtained after the addition of the ligands to a $\text{CDCl}_3/\text{CD}_3\text{OD}$ (10:1) solution of $\text{Pb}(\text{ClO}_4)_2$.

The ^{207}Pb NMR resonances of the complexed ketones **1** and **3** display sharp peaks at -3148.8 and -2738.8 ppm, respectively (Figure 10). This result shows that oxygen donor atoms produced large shielding effects in ^{207}Pb resonances and that the coordination number of Pb^{2+} ion should be the same, in agreement with DFT results for both complexes. However, the presence of the CH_2OCH_2 oxygen bridge in ketone **1**, an additional although weaker coordination site, may account for the further shielding effect observed in this case.

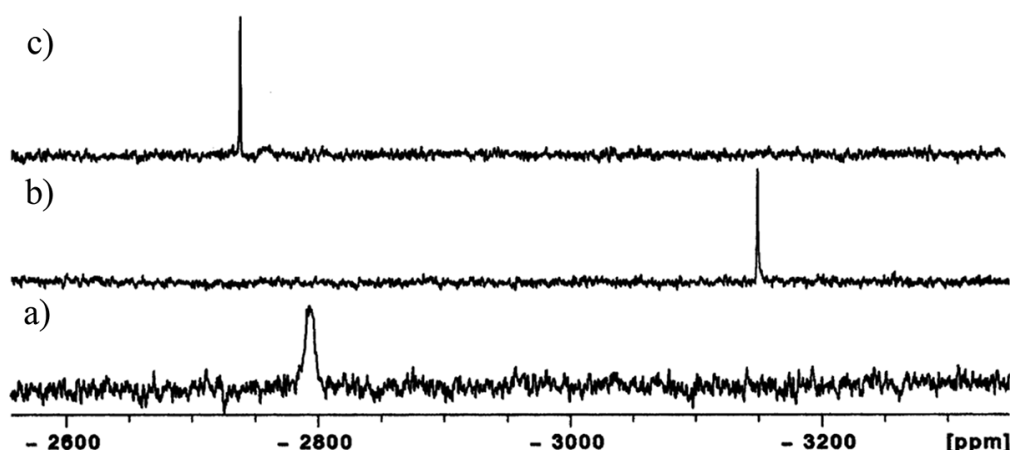


Figure 10. ^{207}Pb NMR spectra in $\text{CDCl}_3/\text{CD}_3\text{OD}$ (10:1) of: a) $\text{Pb}(\text{ClO}_4)_2$, b) 1:1 $\text{Pb}^{2+} \subset \mathbf{1}$ complex and c) 1:1 $\text{Pb}^{2+} \subset \mathbf{3}$ complex

Proton relaxation studies

The proton spin-lattice relaxation times (T_1) of the free and complexed calixarenes were also determined to access the conformational changes of the ligands **1**, **2** and **3** upon Pb^{2+} ion complexation. The T_1 relaxation is mainly due to intramolecular dipolar interactions between the protons of the receptor in the free and bound species.

An analysis of the data (Tables S10-S12) shows a general decrease of T_1 upon complexation in agreement with the increase of the molecular weight of the complexes. For the three ligands, at the upper rim level, the decrease is more pronounced for the aromatic protons than for the $-\text{CH}_2-$ bridge protons, as consequence of the conformational changes that decrease the average distances between adjacent aromatic protons in the $\text{Pb}^{2+} \subset$ ketone complexes (Figure 11, S8 and S9). This effect is confirmed by the average distances taken from the DFT optimised structures (Table S9), as for example 3.30/2.91 Å and 2.86/2.22 Å for ketones **1** and **3** and their Pb^{2+} complexes, respectively. It was also observed that in case of ketone **2** the T_1 values of the aromatic protons are not so

sensitive to conformational changes upon complexation as they are in the case of ligands **1** and **3**, probably because in **2** the average distances are longer, 3.86/3.91 Å for **2** and its Pb^{2+} complex, respectively. In the macrocycle lower rim, there is also a significant decrease in the T_1 average values of the adamantyl groups of ketones **1** and **3** upon complexation that may be related with the loss of mobility of the pendant arms upon Pb^{2+} coordination by both the phenoxy and the carbonyl oxygen atoms.

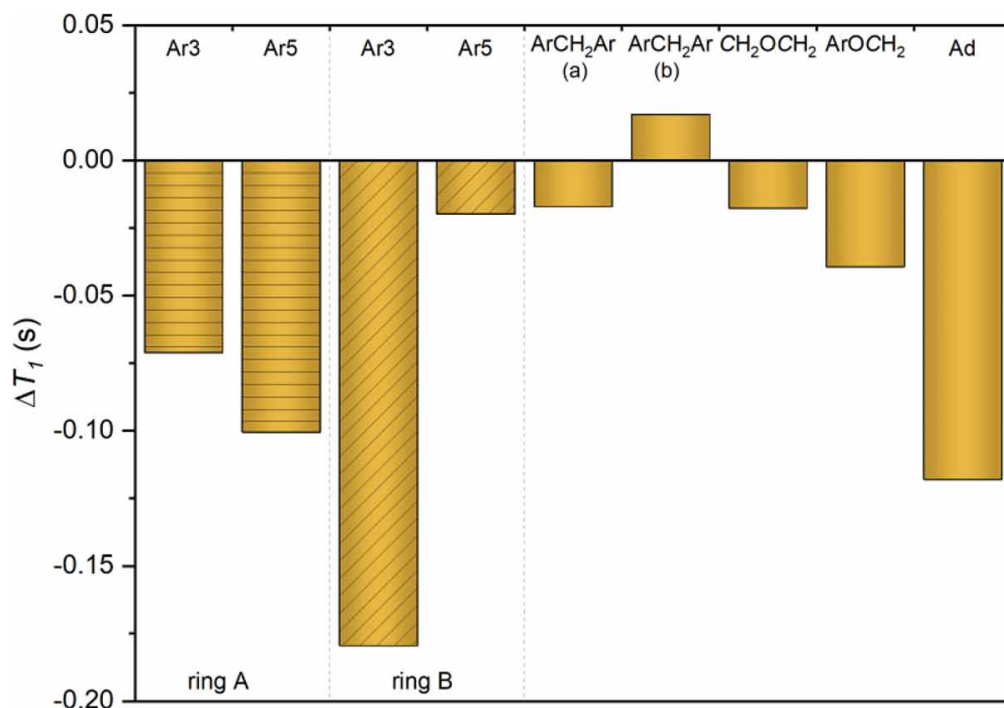


Figure 11. Relevant T_1 variation for ligand **1** upon complexation [$T_1(\text{Pb}^{2+} \subset \text{ligand } \mathbf{1}) - T_1(\text{ligand } \mathbf{1})$]

Conclusions

The conformational changes of adamantyl ketones **1**, **2** and **3** upon Pb^{2+} complexation were established by NMR measurements in chloroform. The conformations of these 1:1 complexes deduced from ^1H and ^{13}C NMR experiments showed that Pb^{2+} ion is encapsulated in the cavity composed of the phenoxy and carbonyl oxygen atoms. In the case of dihomooxa ligand **1**, the data indicate a more flattened cone conformation upon complexation, with also some interaction of the CH_2OCH_2 oxygen bridge with the cation. For the hexahomotrioxa ligand **2**, the phenyl groups seem more flattened compared to those of ligand **1**, and stand up after ion binding, resulting in a more regular cone conformation. Finally, calix[4]arene ligand **3** presents the more symmetric cone conformation upon complexation, with Pb^{2+} centred inside the ionophoric cavity and interacting equally with the eight oxygen atoms. The inclusion of a MeCN molecule in the hydrophobic cavity of the free and complexed ligands was also investigated by ^1H NMR, but only in the case of $\text{Pb}^{2+} \subset \text{ketone } \mathbf{3}$ complex such evidence was observed. These binding modes were, in general, corroborated by the DFT calculations. A good agreement was also obtained between DFT predicted and experimental ^{13}C chemical shifts. The ΔE complexation energies indicated that ketone **1** is the best Pb^{2+} ligand, apart from the functional used. The ^{207}Pb NMR experiments further support the proposed Pb^{2+} coordination mode, and a general decrease of T_1 values upon complexation is in agreement with the conformational changes observed for the three ligands.

The X-ray diffraction of the two pseudo-polymorphic crystals for both **1** and **3** derivatives has confirmed the cone conformation and revealed an interesting role of the acetonitrile guest molecule, with consequences on the conformation of the adamantyl and carbonyl pendant groups. In particular, in the dihomooxa ketone **1** the guest molecule open the cup, while in the calix[4]arene ketone **3** the pinched cone conformation becomes more symmetric.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work has received funding from Fundação para a Ciência e a Tecnologia, Project ref. UIDB/00100/2020, and infrastructure Project ref. 022161 (co-financed by FEDER through COMPETE 2020, POCI, PORL and FCT through PIDDAC).

Supplementary material

Supplemental data for this article can be accessed [here](#)

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