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Originally published:

March 2018

Spectrochimica Acta Part A 199(2018), 50-56

DOI: https://doi.org/10.1016/j.saa.2018.03.029

Chelation of heavy group 2 (radio)metals by *p-tert*-butylcalix[4]arene-1,3-crown-6 and logK determination via NMR

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A crown-bridged calix[4]arene scaffold was investigated as lead compound for the ligation of heavy alkaline earth metals such as strontium, barium, and radium, which appear to be useful for radiopharmaceutical applications in diagnosis as well as in radiotherapy. The ability of *p-tert-butylcalix[4]arene-1,3-crown-6* (**1**) in particular to chelate cations, such as group 1 and 2 metal ions or ammonium ions is well known. Also, the manifold possibilities of structural modification on the upper- and lower-rim as well as on the crown itself produce properties that may lead to a highly selective and effective chelating agent. In this work, titration experiments of the perchlorate salts of Ba^{2+}, Sr^{2+} and Pb^{2+} with ligand **1** were performed to determine their stability constants (logK = 4.7, 4.3, and 3.3, respectively) by ^{1}H NMR measurements in acetonitrile-d_3.

Introduction:

Group 2 metals and their use in radiopharmacy. Radium is the heaviest known member of the alkaline earth metals and all 33 of its isotopes are radioactive [1]. Four of these isotopes are available from the decay of primordial nuclides [2]. Two of these, radium-223 and radium-224, have suitable half-lives (^{223}Ra: 11.4 d, ^{224}Ra: 3.6 d) and nuclear decay properties that make them useful tools for alpha particle therapy [3]. Alpha particles are highly energetic (5-9 MeV) and create frequent ionization events (approx. 80 keV/µm) across a short path length (40-100 µm), which leads to a high effectiveness for tumor cell killing [4]. Both ^{223}Ra and ^{224}Ra rapidly decay *via* series of six daughter nuclides (four alpha and two beta particles) to stable ^{207}Pb and ^{208}Pb, respectively, which results in

massive energetic emissions of 28 and 27 MeV, respectively [1]. In 2013, [^{223}Ra]$RaCl_2$ (Xofigo®) became the first and, until now, the only alpha-emitting radiopharmaceutical to receive FDA and EMEA approval for clinical use.

The homologous elements barium and strontium exist as stable nuclides, but offer radioisotopes for radiopharmaceutical applications [5]. ^{131}Ba (E_γ = 496 keV, 48%) and ^{135m}Ba (E_γ = 268 keV, 16%) are both possible γ-emitters for diagnostic use and were broadly discussed as bone-scanning agents in scintigraphy [6-8]. ^{90}Sr ($E_{\beta,max}$ = 546 keV, 100%) also finds extensive application as a strong beta-emitter for superficial brachytherapy of some cancers [7,9,10]. No literature was found regarding the application of the light alkaline earth metals beryllium, magnesium, and calcium for therapy or diagnosis.

Calix[4]arenes and calix[4]crowns. Searching for suitable ligands, a group of molecular baskets termed calix[4]arenes was found to be a promising start. Calix[4]arenes are metacyclophanes having a hydrophobic cavity between the lower and upper rim, formed by four *p-tert*-butylphenol units bridged with methylene links [11]. This class of macromolecules is known for a wide range of applications [12,13], most likely due to their numerous possibilities for functionalization [14]. Calix[4]arenes act as biologically active compounds and are used as antibacterial and even anti-malarial agents or in cancer chemotherapy [15-18]. Additionally, they can be promising enzyme inhibitors or interact with amino acids [19,20]. Their ability to form inclusion compounds with neutral molecules or ions makes them useful as sensors, catalysts, ligands or, when bound to a resin, as effective separation-agents for ions [21-26].

The calix[4]arene framework can also be seen as a platform for building an optimized chelator. On the lower rim, there are four hydroxyl groups. Two can be functionalized as proton-ionizable groups to form a neutral complex with divalent cations; the remaining two can be bridged by a crown ether. With this concept, both the advantages of the electrostatic, macrocyclic, and cryptate effect are combined. Another benefit of the calix[4]crown-6 scaffold is the easy access [27,28]. To provide the ideal cavity for heavy group 2 metals, it is essential to choose a suitable crown size. The group around Bartsch focused on extraction of alkaline earth metal cations using calixcrowns [29-31]. As a result of their studies, calix[4]arene-1,3-crown-6 derivatives have been found very effective for Ba^{2+}. Barium, strontium and radium possess analogous chemical properties and their radii are of a similar range [32], therefore, these studies were considered to be a useful starting point. Additionally, these compounds showed a high selectivity for barium over the lighter alkaline earth metals or alkali metals, however, Ra^{2+} was not investigated. Van Leeuwen *et al.* [33-35] compared the efficiency of various ligands including calixarenes as ionophores for Ra^{2+} extraction in the case of nuclear waste management. They also investigated derivatives of *p-tert*-butylcalix[4]arene-1,3-crown-6 (**1**) and

showed that they have high extraction rates and suitable selectivity in contrast to simple (aza-)crown ethers. In radiopharmacy, a high stability constant of the complex is important so that a radium release and accumulation in bone tissue is minimized.

The objective of this research was to evaluate *p-tert*-butylcalix[4]arene-1,3-crown-6 (**1**) as a possible leading compound that could, upon further modification, yield a viable chelator for heavy group 2 metals in radiopharmaceutical application. Existing literature about group 2 metal ligands specifically with radium are focused mainly on extraction studies, while useful, this does not provide information about comparable stability constants [30,33,36]. Therefore, a reliable and constant method for the calculation of stability constants with barium and strontium *via* NMR spectroscopy was developed to determine the efficiency of ligand **1**. Since there is no stable radium isotope, barium is additionally used as non-radioactive surrogate, due to its related chemical behavior and size. To further guarantee the size selectivity of the basic structure, sodium and tetrabutylammonium cation were additionally investigated.

Furthermore, the interaction of ligand **1** and Pb^{2+} was studied by using ^{1}H and ^{207}Pb NMR spectroscopy. For our research, lead is also a metal of interest. On the one hand, it is the stable end product of both radium decay chains. On the other hand, ^{212}Pb is a promising β^--emitter (570 keV max β^-, 12%), and a feasible candidate for radiopharmaceutical applications, since it can also be used as an *in vivo* generator for ^{212}Bi, which is a strong alpha emitter [37,38].

Experimental Section:

General. ^{1}H NMR spectra were recorded on an Agilent DD2-400 MHz NMR spectrometer with ProbeOne at 298 K. Chemical shifts of the spectra were reported in parts per million (ppm) using TMS as internal standard. All ^{207}Pb spectra were recorded at a frequency of 125.1 MHz on an Agilent DD2-600 MHz NMR spectrometer with ProbeOne at 298 K using a 90° pulse width of 6.0 µs, a 0.157 acquisition time, and a 0.6 s delay time. A 1.0 M $Pb(NO_3)_2$ solution (natural) was used as an external standard (δ = −2965 ppm, D_2O, 25°C; relative to $PbMe_4$). For the synthesis of ligand **1,** 4-*tert*-butylcalix[4]arene (abcr, 99%), pentaethylene glycol di(*p*-toluenesulfonate) (Alfa Aesar, 95%), potassium carbonate anhydrous (Acros, 99+%), acetonitrile (Fisher Scientific, HPLC-grade), dichloromethane (Fisher Scientific, HPLC-grade), hydrochloric acid (Merck, 37%), and sodium sulfate anhydrous (Alfa Aesar, 99%) were used as obtained. Preparative column chromatography was carried out with silica gel 60 (Merck, particle size 0.040-0.063 mm), petroleum ether (Fisher Scientific, bp 40-60 °C, analytical reagent grade), and ethyl acetate (Fisher Scientific, HPLC-grade). For the preparation of the complexes, barium perchlorate (Alfa Aesar, 99%), sodium perchlorate (Alfa Aesar, 98%),

strontium perchlorate (abcr, 99.9%), and tetrabutylammonium perchlorate (Acros, 98%) were dried at room temperature under vacuum and used without further purification. Lead (II) perchlorate trihydrate (abcr, 97%) was used as obtained. The solvents used for NMR measurements were purchased from Deutero GmbH.

Synthesis of 1. *p-tert*-Butylcalix[4]arene-1,3-crown-6 (**1**) was synthesized according to the literature [29]. Briefly, a suspension of *p-tert*-butylcalix[4]arene (**2**, 1.00 g, 1.54 mmol), pentaethylene glycol di(*p*-toluenesulfonate) (924 mg, 1.69 mmol) and K_2CO_3 (256 mg, 1.85 mmol) in acetonitrile (100 mL) was refluxed under argon for 7 d. After cooling to rt, the solvent was removed and CH_2Cl_2 (50 mL) was added. The suspension was washed with 10% HCl (2 x 50 mL) and water (1 x 50 mL). The organic layer was dried over Na_2SO_4. After evaporation of CH_2Cl_2, the crude mixture was purified by column chromatography (SiO_2, petroleum ether/ ethyl acetate, gradient: 0% → 60%). The product was obtained as a colorless solid (580 mg, 50%). Analyses were in accordance with the previously published literature [29].

^{1}H NMR titration measurements. A solution of **1** was prepared in the appropriate deuterated solvent ($2.0 \cdot 10^{-3}$ M) and 1.0 mL was pipetted in a NMR tube. The sample was referenced to the residual solvent. Then, the complexation of cations with **1** was studied. A 0.1 M solution of the metal perchlorate was prepared in the same solvent. Next, stepwise portions (2 µL) of the respective perchlorate solution were added into the NMR tube containing the ligand, and after extensive mixing the complexation-induced shifts were recorded. At a ligand:metal ratio of 2:3, 30 µL portions of a 1.0 M perchlorate salt solution were used, and stepwise additions were continued until ligand:metal ratio of 1:6 was reached to exclude the formation of a complex with another stoichiometry. The displacements of selected ^{1}H NMR signals of ligand **1** upon addition of the perchlorate salt were used to calculate the complex stability constants. The calculations were performed using the WinEQNMR2 software.[39] The advised range for the data input covers the addition of metal to ligand from 0.1 to 0.9 equivalents. This instruction was followed and 9 points in this range were measured (steps of 0.1 equiv.) and used for the calculation. The formation of a 1:1 complex was proven by plotting the changes of selected signals against the cation to chelate ratio, observing the change of the slope at a ligand:metal ratio of 1:1.

^{207}Pb NMR titration measurements. A solution of lead (II) perchlorate trihydrate in acetonitrile-d_3 ($5.0 \cdot 10^{-2}$ M) and a solution of ligand **1** in acetonitrile-d_3 ($1.0 \cdot 10^{-1}$ M) were prepared. A 1.0 mL aliquot of the lead solution was pipetted in a NMR tube. The sample was measured and the ^{207}Pb signal determined. Next, stepwise portions of ligand **1** (40 µL, 0.08 equiv.) were added into the NMR tube containing the lead solution, and the spectra were recorded after extensive mixing.

Results and Discussion:

Initial ^{1}H NMR studies. To determine the stability constant for the complexation of Ba^{2+}, Sr^{2+}, and Pb^{2+}, a reliable method was developed using ^{1}H NMR spectroscopy. To study the influence of the solvent on the complexation and to optimize shift characterization, NMR measurements involving ligand **1** were carried out in various solvents: $CDCl_3$, DMSO-d_6, acetone-d_6, acetonitrile-d_3, and methanol-d_4. Since ligand **1** is not soluble in aqueous solutions, D_2O was not used. Afterward, stability constant measurements were performed using Ba^{2+}, Sr^{2+}, Pb^{2+}, Na^+, and Bu_4N^+ as their perchlorate salts in acetonitrile-d_3.

First, the protons of ligand **1** were assigned to their signals. The ^{1}H NMR spectra of **1** in different solvents are shown in Figure 1 and the assignment with the chemical shifts is listed in Table 1. A C_2-symmetry is found for ligand **1** which results in the number of 12 ^{1}H signals.

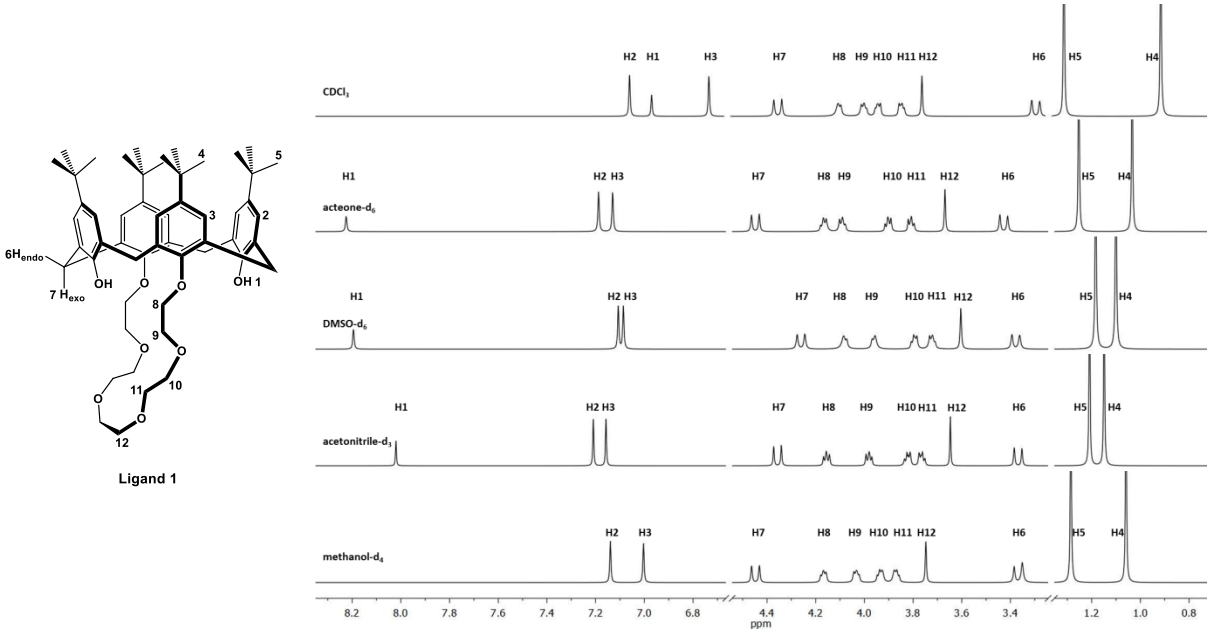

Figure 1. ^{1}H NMR spectra of ligand **1** in different solvents (solvent peaks have been removed for clarity).

Table 1. ^{1}H NMR (400 MHz,) shifts of compound **1**.

^{1}H signal**	Multiplicity [Hz]	Integral	Shift [ppm]					assignment
			$CDCl_3$	acetone-d_6	DMSO-d_6	acetonitrile-d_3	methanol-d_4	
H1	s	2	6.96	8.22	8.19	8.02	-	OH
H2	s	4	7.07	7.19	7.11	7.21	7.14	Ar-H
H3	s	4	6.74	7.13j	7.09	7.16	7.00	Ar-H
H4	s	18	0.91	1.03	1.10	1.15	1.06	^{t}Bu
H5	s	18	1.31	1.25	1.18	1.21	1.29	^{t}Bu
H6	d*	4	3.30	3.43	3.38	3.37	3.37	H_{endo}
H7	d*	4	4.36	4.45	4.26	4.36	4.45	H_{exo}
H8	m	4	4.12-4.08	4.19-4.15	4.10-4.06	4.17-4.14	4.19-4.15	CH_2O
H9	m	4	4.02-3.98	4.11-4.07	3.98-3.94	4.00-3.96	4.05-4.01	CH_2O
H10	m	4	3.95-3.92	3.92-3.88	3.81-3.77	3.84-3.81	3.96-3.91	CH_2O
H11	m	4	3.86-3.82	3.82-3.79	3.74-3.70	3.78-3.75	3.89-3.84	CH_2O
H12	s	4	3.77	3.67	3.60	3.65	3.75	CH_2O
	* ^{2}J [Hz] =		13.1	12.7	12.6	12.6	12.8	

**Is not representing the IUPAC assignment.

Next, the titration method had to be developed, which was representatively executed with Ba^{2+} as guest ion. For the calculation of stability constants, the chemical shifts had to be exactly determined [40]. To ensure this, the changes of the chemical shifts in the ^{1}H signals between ligand **1** and the Ba^{2+} complex must be sufficiently pronounced. For this reason, it had to be ascertained which solvent is most advantageous [41]. Since the solubility of the perchlorate salts is very little in $CDCl_3$ and the solubility of the formed complexes is also insufficient, this solvent could not be used. Remarkably, upon addition of barium perchlorate to a solution of **1** in DMSO-d_6 there was no change visual in the ^{1}H spectra. This can be attributed to the formation of Ba-DMSO-complexes which are described in the literature [42]. Once formed, these complexes are not interacting with the calix[4]crown anymore. The DMSO-interaction is discussed in more detail later. The use of acetone-d_6 and methanol-d_4 showed clear changes in the spectra comparing the ligand and the 1:1 complex. However, the strongest difference was obtained in acetonitrile-d_3 which makes it the favorable solvent for the titration experiment (see SI for spectra).

Consequently, all NMR titration experiments were performed in acetonitrile-d_3. Therefore, ^{1}H NMR spectra of a $2.0 \cdot 10^{-3}$ M solution (1 mL) of **1** were measured upon stepwise addition (2 µL) of a 0.1 M solution of the perchlorate (prepared in acetonitrile-d_3). Each step represents the addition of 0.1 equiv. of the metal perchlorate to ligand **1**. In Figure 2, the titration experiment with Ba^{2+} is shown. Shifts for all proton signals are observed.

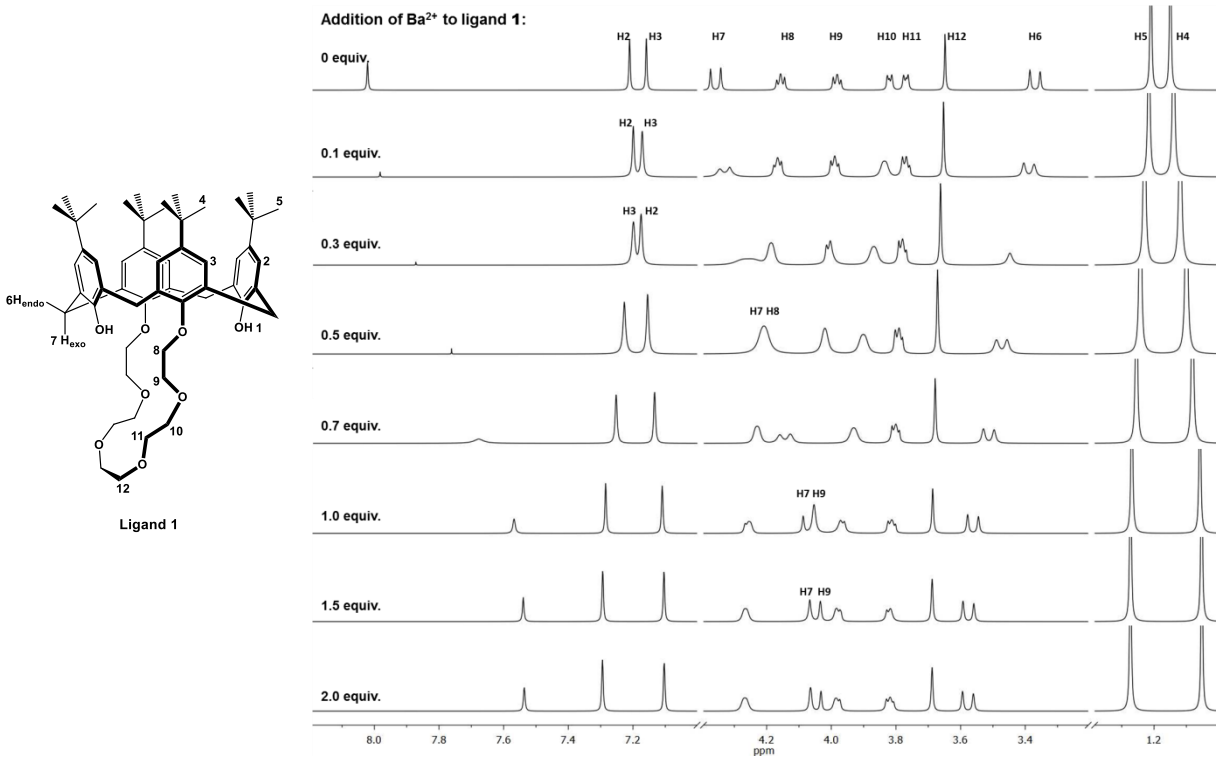

Figure 2. ^{1}H NMR spectra of **1** at different $Ba(ClO_4)_2$ concentrations measured in acetonitrile-d_3.

The differences of the chemical shifts $\Delta\delta_{H2\text{-}H3}$, $\Delta\delta_{H7\text{-}H6}$ and $\Delta\delta_{H5\text{-}H4}$ upon Ba^{2+} addition indicate a significant alteration in the orientation of the aromatic rings. $\Delta\delta_{H7\text{-}H6}$ decreased from 0.99 ppm (ligand) to 0.47 ppm (1:1 complex). The shifting of these doublets is related to a twisting of the aromatic rings [43]. The general presence of the doublets proves the fixed cone conformation, since a free rotation of the aryl rings would cause only a singlet [44]. The distance between the doublets H6 and H7 results from the orientation of the protons into or out of the aromatic ring current and gives information about the cone flattening [45]. The difference in the chemical shift between the axial (H7) and equatorial (H6) protons ($\Delta\delta_{H7\text{-}H6}$) gets smaller when the aromatic rings rotate (Figure 3). It can be deduced from Figure 2 that the two non-bridged aromatic rings turn, so that the hydroxyl groups point towards the Ba^{2+} ion which is located on the lower rim. This is explained by Figure 4. When ring B rotates at a certain angle, the *meta*-aryl proton will face ring A frontally. The proton will perceive a lower magnetic field and this will be noticed as a high field shift in NMR measurements. If the proton moves to the outside of ring A, it will perceive a higher magnetic field and this will be noticed as a down field shift. The *tert*-butyl group behaves contrary. When the *meta*-aryl proton of ring B faces ring A, the *tert*-butyl group directs to the outside of ring A and will perceive an increase of the magnetic field strength this results in a displacement of the ^{1}H signal to down field. For this reason, H2 and H4 are down field but H3 and H5 high field shifted.

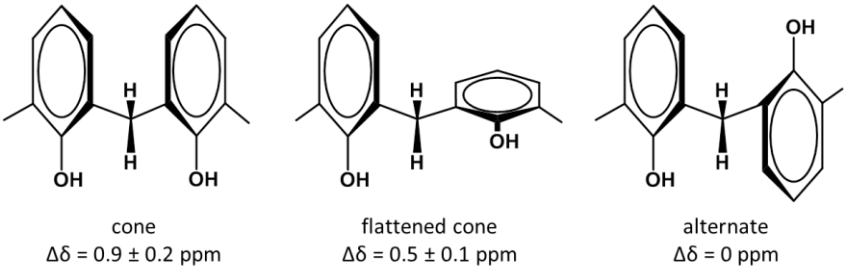

Figure 3. Conformational dependence of $\Delta\delta_{H6\text{-}H7}$ [11]**.**

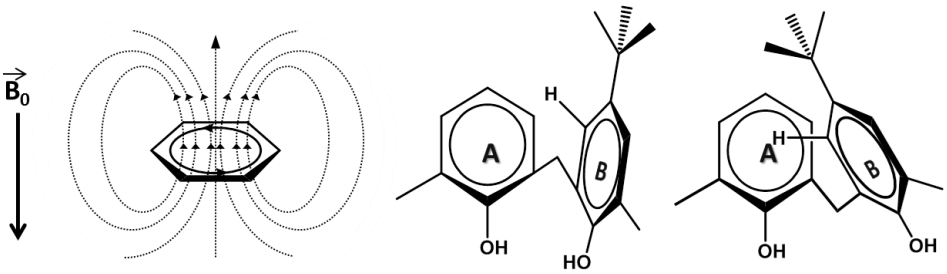

Figure 4. Conformational dependence of $\Delta\delta_{H2\text{-}H3}$ and $\Delta\delta_{H4\text{-}H5}$.

Not only an interaction with the calix cone can be determined but also clear changes in chemical shifts of the crown signals (H8-H12) are observed due to the coordination of Ba^{2+} to the ether oxygen atoms. The strongest movements occurred for the chemical shifts of the crown protons H8, H9 and H10, whereas only a slight movement was found for the signals H11 and H12. This indicates that the Ba^{2+} is bound more closely to lower calix rim, strongly interacting with the aromatic ether oxygens but hardly with the upper ether oxygens of the crown.

For evaluation of the data, the four ^{1}H signals of H2, H3, H4 and H5 were used since these singlets were easy to follow upon Ba^{2+} addition. When the ^{1}H shifts are plotted against the equivalents of Ba^{2+}, Figure 5 is obtained. All curves show a change of the slope at the ratio of 1:1, indicating the formation of a 1:1 complex.

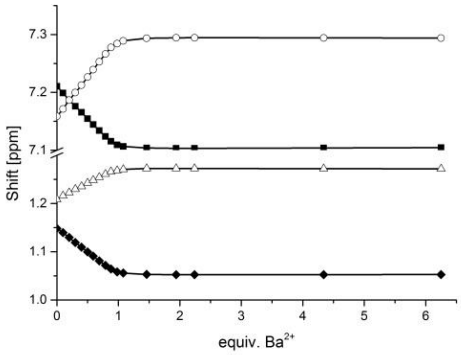

Figure 5. Shifts of four selected ^{1}H NMR signals of **1** at different $Ba(ClO_4)_2$ concentrations measured in acetonitrile-d_3. ○: shift of H2, ■: shift of H3, △: shift of H5, ◆: shift of H4.

The same titration method was applied to the perchlorate salts of Sr^{2+}, Pb^{2+}, Na^+ and Bu_4N^+. For the small Na^+ and the bulky tetrabutylammonium ion, no changes in the spectra were observed, indicating that **1** shows size selectivity for the cations of interest. Furthermore, these two experiments prove that there is no interaction between the perchlorate and ligand **1**. For the divalent metals Sr^{2+} and Pb^{2+} incorporation into the calix[4]arene-crown cavity was observed as well, and the formation of a 1:1 complexes was confirmed (see SI).

After the ^{1}H NMR shifts upon Ba^{2+}, Sr^{2+} and Pb^{2+} addition were measured, the stability constants were calculated [39]. The logK values for the 1:1 complex of ligand **1** with Ba^{2+}, $Sr^{2+}$and Pb^{2+} calculated for different ^{1}H signals are listed in Table 2.

Table 2. Stability constants for ligand **1** determined by ^{1}H NMR titration experiment in acetonitrile-d_3.

cation	stability constants, determined by selected ^{1}H signals			
	H2	H3	H4	H5
Ba^{2+}	4.6 ± 0.1	4.9 ± 0.2	4.6 ± 0.1	4.8 ± 0.2
Sr^{2+}	-	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.2
Pb^{2+}	-	-	3.3 ± 1	3.2 ± 1

For the calculation of the stability constant of the Pb^{2+}-**1** complex, the signal H2 did not move significantly enough and signal H3 was too broad to be used. The calculation was performed with the chemical shifts of signals H4 and H5 only, but due to the shape of these signals with high inaccuracy.

Stability constants for all three metal ions were calculated, whereupon the choice of a specific ^{1}H signal plays no significant role for the result. Overall, the highest stability constant was calculated for Ba^{2+} (approx. 4.7) followed by Sr^{2+} (approx. 4.3) and Pb^{2+} (approx. 3.3).

As mentioned before, Ba^{2+} appears to be located close to the lower rim of the calix, strongly interacting with the aromatic ether oxygens and barely with the top of the crown. Upon complexation, the ^{1}H signals for the hydroxyl protons H1 are high field shifted (Figure 2). This is not caused by the ring current effect, but by the diminishing of the hydrogen bonds between the hydroxyl groups and the aromatic ether oxygens.

Table 3. Comparison of the Δδ of ligand **1** and its 1:1 complexes (negative values represent the crossing of the two peaks).

Compound	$\Delta\delta_{H2-H3}$	$\Delta\delta_{H7-H6}$	$\Delta\delta_{H5-H4}$
Ligand 1	0.05	0.99	0.06
Ba^{2+}-1:1 Complex	-0.19	0.47	0.22
Sr^{2+}-1:1 Complex	-0.11	0.43	0.15
Pb^{2+}-1:1 Complex	-0.08	0.49	0.14

In contrast, Sr^{2+} shows a different interaction with **1**. It can be looked up in the titration experiment data (SI) that the ^{1}H signal H2, which shifts strongly for Ba^{2+} (Figure 2), only slightly shifts upon Sr^{2+} addition. Δδ's listed in Table 3 reveal that the Sr^{2+}-**1** complex is not comparably pinched to the Ba^{2+}-**1** complex. The H2 signal refers to the *meta*-protons of the non-bridged aromatic rings. This indicates that Sr^{2+} is not situated directly at the lower rim of the calix cone, and not actively influencing the orientation of the aromatic rings, like Ba^{2+} is. It is supposed to sit lower in the crown and the wrapping of the crown around Sr^{2+} leads to a pinch of exclusively the two attached aromatic rings. As a result, the *meta*-aryl protons referring to the H3 signal are facing the other two aromatic rings which is confirmed by the down field shift of signal H3. The rotation of the rings can be confirmed by the $\Delta\delta_{H7-H6}$ value. Other than barium, strontium induces significant shifts for the ^{1}H crown signals H11 and H12, proving that it is situated in the lower crown. These facts confirm a different binding mode. The reason why Sr^{2+} is not incorporated into the calix cone might be the significant smaller ionic radius. For a similar interaction as Ba^{2+}, Sr^{2+} would demand a greater pinch of the cone. Considering the necessary tension of the cone, it is comprehensible that interaction of the strontium and the aromatic ether oxygens is not advantageous. This is resulting in a stronger interaction with the ether oxygens in the middle of the crown. It is notable that only the smaller radius, considering similar chemical properties of both ions, results in a decrease of the stability constant. Since the radius of Ra^{2+} is slightly bigger than the radius of Ba^{2+} it might result in a small increase of the complex stability.

Pb^{2+} has a similar size to Sr^{2+}, but different chemical properties. Upon addition of lead perchlorate to ligand **1** the proton signals of the measured spectra appeared very broad and almost vanished (for titration experiment data see SI). Interestingly, ^{1}H signal H2 was the only one, which was barley

affected upon the titration, like it is for Sr^{2+}. As soon as reaching the 1:1 ratio, the signals are again sharply defined. This behavior indicates a fast exchange of Pb^{2+} between the free ligands and the complex during the timescale of a ^{1}H measurement. The titration also reveals a strong interaction with the crown, indicating that Pb^{2+} is situated only in the crown, and the wrapping of the crown around Pb^{2+} leads to the pinch of the cone, comparable to Sr^{2+}. Comparing the Δδ's in Table 1 the pinch is least distinct for this guest ion. Pb^{2+} shows a low stability constant in this study, the calix scaffold seems to have no significant effect on the ion.

^{207}Pb NMR studies. Since ^{207}Pb (natural abundance: 22.6%) has a medium sensitivity NMR spin-½ nucleus [46,47], ^{207}Pb NMR is a valuable and helpful tool to describe the chemical situation in the Pb-calix complex. Thus, ^{207}Pb spectra were recorded upon ligand **1** addition to a 1 M $Pb(ClO_4)_2$ solution. In contrast to the ^{1}H nucleus, the relaxation time for the ^{207}Pb nucleus is very short. During this short time range no exchange process of the lead ion between ligand **1** and the complex Pb^{2+}-**1** was observed. As a result, the received spectra (Figure 6) show two signals: a decreasing signal for the free Pb^{2+}at δ = -3380 ppm and an increasing signal for the Pb-calix-complex downfield shifted at δ = -3100 ppm. A slight shift for the free Pb^{2+} signal is noticed and is dependent on the free metal ion concentration (see SI and [48]). This data confirms the formation of a 1:1 complex, but it can not be used for the calculation of the stability constant.

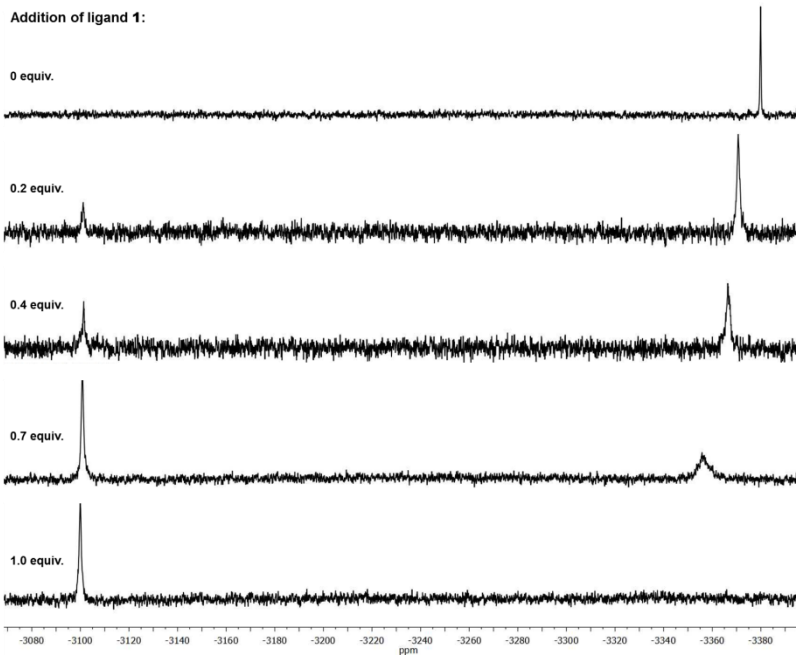

Figure 6. ^{207}Pb NMR spectra at different ligand concentrations measured in acetonitrile-d_3.

To confirm the previously mentioned complexation of Pb^{2+} with DMSO, an additional experiment was examined. The ^{207}Pb NMR titration was repeated with stepwise addition of DMSO to a 1 M $Pb(ClO_4)_2$ solution instead of ligand **1** (Figure 7, see also SI). A strong shift of the Pb^{2+} signal upon DMSO addition was observed. This proves the formation of Pb^{2+}-DMSO complexes which are also expected for barium and strontium [42]. This fact makes it impossible to perform the titration experiments in DMSO-d_6, as discussed previously.

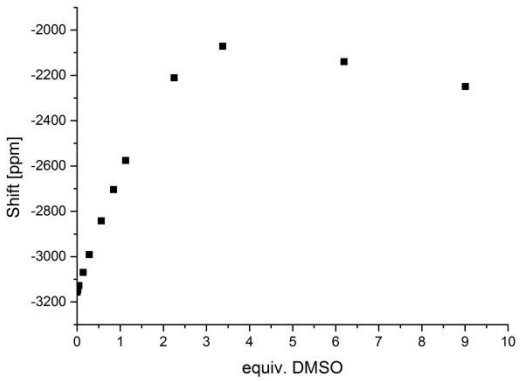

Figure 7. Shift of the ^{207}Pb NMR signal upon DMSO addition to a 1 M $Pb(ClO_4)_2$ solution in acetonitrile-d_3.

Conclusion:

Titration experiments of Ba^{2+}, Sr^{2+} and Pb^{2+} with ligand **1** were successfully performed and the stability constants were determined by NMR measurements in acetonitrile-d_3. Insights were derived from the data obtained, whereby structure-interaction-relationships of the complexes could be revealed. Compound **1** shows promising properties for Ba^{2+} which is not only a metal of radiopharmaceutical interest, but also serves as a surrogate for Ra^{2+}. Sr^{2+} does not have the same binding mode, most likely due to its smaller ionic radius. Strontium resembles the behavior of lead, and appears to interact exclusively with the ether crown moiety of **1**. Since the homologous radium has only a slightly larger radius than barium, it can be assumed that the synergetic effect of **1** is similar for both metals. Several modifications are in progress to increase the stability constants to an appropriate level for radiopharmaceutical applications. The two free hydroxyl groups offer the possibility of additional functions to increase the stability for heavy group 2 metals.

Additionally, the complexation behavior of Pb^{2+} was followed using ^{207}Pb NMR and the influence of DMSO as ligand on Pb^{2+} was investigated.

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