NMR COMPLEXATION STUDY OF SEVERAL OXYETHYLATED CALIX[4]ARENES WITH LITHIUM, SODIUM AND POTASSIUM IONS

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The influence of calix[4]arene upper rim substitution on the complexation with Li^+, Na^+, K^+ was studied by ^1H NMR spectroscopy. Calix[4]arenes **1–4** namely 25,26,27,28-tetrakis(3-oxapentyloxy)calix[4]arene (**1**), its 5,17-diamino (**2**) and 5,17-dinitro derivative (**3**) as well as 25,26,27,28-tetrakis(3,6,9-trioxadecyloxy)calix[4]arene (**4**) having four monoalkyloligoethylene glycol chains on the lower rim have been studied. No complexation has been observed for Li^+. Two electron-donating NH_2 groups on the calixarene upper rim (compound **2**) improve the complexation ability for Na^+ and K^+ compared with parent calixarene **1**. The electron-withdrawing nitro groups in **3** have the opposite influence. It seems that the complexation of alkali metal ions studied is not significantly influenced by the increasing number of donor atoms (from eight in **1** to sixteen in **4**) available for complexation on the lower rim. The position of sodium cation in **1** . Na^+ and **4** . Na^+ is supposed to be in close proximity of phenolic oxygens based on ^1H and ^{13}C NMR data. The error analysis is given for the stability constant determination from NMR data.

Key words: Calix[4]arenes; Complexation; Alkali metals.

Calixarenes are a family of macrocycles prepared by the condensation of *para* substituted phenols and formaldehyde[1–3]. Preparative procedures have been developed for efficient synthesis of these macrocycles with four[4], six[5] or eight[6] phenolic residues in the ring in high yields. The alkyl (usually *tert*-butyl) group at the *para* position is relatively easy to remove by aluminium chloride catalyzed reaction with toluene and phenol, leaving both rims (upper-hydrogens and lower-phenolic hydroxyls) ready for further synthetic elaboration. Calixarenes bearing oligooxyethene chains have been studied as early as 1955 in a study directed to the synthesis of antituberculous surfactants[7,8] using the treatment of calixarene with ethylene oxide in different ratios under

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basic conditions[9,10]. Synthesis and structural characterization of calixarenes with 2-hydroxyethoxy lower rim functionalization (among others) have also been studied recently[11].

We are interested in a structure–complexation ability relationship study utilizing the fact that calixarenes are able to form complexes with cations, anions and neutral molecules[1–3]. In this work NMR spectroscopy was used to investigate the complexation of alkali metal cations with calix[4]arenes **1–4** (Fig. 1).

Compounds **1–3** bear four 3-oxapentyloxy groups on lower rim, compound **4** bears four 3,6,9-trioxadecyloxy groups forming extended array of oxygens – cation binding sites. It enables to study the influence of the lower rim chain length on alkali metal cation complex formation. Calixarenes **1–3**, on the other hand, differ by substitution on the upper rim of calix[4]arene skeleton; **1** is unsubstituted, while calixarenes **2** and **3** bear two electron-donating and two electron-withdrawing substituents, respectively, in positions 5 and 17 of calix[4]arene skeleton. Consequently, the second aim of this study is to investigate influence of electronic factors on complexation ability. The third aim is to determine the complexation site for cation in the complex of this type in solution.

The bottom-line of this study was our interest in mimetics of ion channels. The studied compounds are expected to orient themselves on the two immiscible liquids interface in organized way and to form something like perforated phase boundary with the ability to conduct charged species. These future applications dictated in some extent our choise of experimental conditions (solvents, anions).

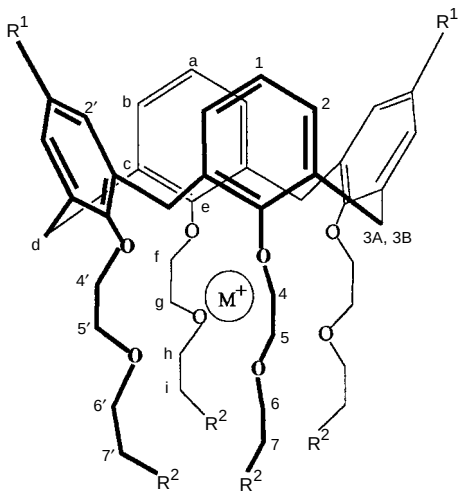

FIG. 1
Structures of systems studied including proton numbering and carbon lettering; **1** R^1,R^2 = H, **2** R^1 = NH_2, R^2 = H, **3** R^1 = NO_2, R^2 = H, **4** R^1 = H, R^2 = $O–CH_2–CH_2–O–CH_3$; M^+ = Li^+, Na^+, K^+

EXPERIMENTAL

Materials

Calixarenes **1** (ref.[12]), **2** (ref.[13]), **3** (ref.[14]) and **4** (ref.[12]) were synthesized according to the published procedures. Deuterated solvents used were $CDCl_3$ (99.8%) and CD_3OD (99.5%) from Merck. $LiNO_3$, NaSCN and KSCN, all of p.a. purity grade, were from Lachema Brno.

Instrumentation

A Varian NMR spectrometer, model Gemini 300 HC, with working frequency of 300.075 MHz, deuterium lock, temperature of 298 K and 5 mm NMR tubes were used for all measurements. The one-dimensional ^1H NMR (δ in ppm; J in Hz) spectra were acquired using a 45 degree pulse (10 μs), a spectral width of 4 500 Hz, an acquisition time 1.778 s, a repetition delay of 2 s and typically 16 accumulations. The one-dimensional ^{13}C NMR spectra (δ, ppm) were measured with working frequency 75.462 MHz using standard APT technique (relaxation delay 2 s).

Preparation of Samples

The corresponding amount of calixarene was weighted accurately and dissolved in 0.6 ml of deuterated solvent to obtain the calixarene concentration around 0.05 mol/l. A mixture of $CDCl_3$ and CD_3OD was used because of insolubility of inorganic salts in $CDCl_3$. The ratio of both components was 2 : 1 ($CDCl_3$: CD_3OD, v/v) for measurements of systems containing NaSCN, NaBr, $LiNO_3$ and 1 : 1 (v/v) for those containing KSCN. Typically ^1H NMR spectrum of pure ligand was measured first, then weighted amounts of corresponding salt were added gradually to the same sample tube and the spectrum was taken after each addition. The accuracy of the differential weighing on the analytical balance was better than 0.1 mg. Usually ten additions of salt were used corresponding to about 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 molar equivalents of alkali metal as compared to calixarene. Higher additions of inorganic salts were limited by solubility of inorganic salts used. For systems containing calixarene **2** the experiments had to be carried out using concentration of calixarene ten times lower (c = 0.005 mol/l). In this case the needed additions of solid salts would be very small and therefore the measurement was performed using the addition of salt stock solutions. Stock solutions of salts with concentration 0.5 ml/l were prepared in the same mixtures of solvents as used for calixarene itself and defined volumes of this stock solution were added. Appropriate corrections were made for sample dilution. For higher additions (10–50) equivalents of a given salt weighing of solid salts was used again. Tetramethylsilane (TMS) was added to the solution of calixarene to test if the chemical shifts of deuterated solvents are constant.

THEORETICAL

All experiments were performed at 298 K. At this temperature fast exchange between two sites corresponding to free and complexed forms of calixarene occurs, i.e. in the spectra of partially complexed calixarene only one averaged signal for each individual proton is observed. Then the difference δ_{dif} of the chemical shift of a given signal corresponding to the partially complexed calixarene δ_{obs} and the chemical shift of this signal for the free, uncomplexed form of calixarene δ_{free} is given in Eq. (*1*)

$$\delta_{dif} = \delta_{obs} - \delta_{free} = \frac{c_{LM}}{c_1}\,\delta_{cis} \quad , \qquad (1)$$

where δ_{cis} is the complexation induced shift (CIS), i.e. the difference of chemical shift of an individual proton of calixarene in fully complexed state and its free form, c_1 is the initial concentration of ligand and c_{LM} is the actual concentration of the complex in equilibrium. Only the 1 : 1 complex stoichiometry (calixarene–cation, i.e. ligand–metal) was taken into account.

There is no definitive proof, that compounds **1–4** can form only 1 : 1 complexes with alkali metal cations. Our assumption of exclusive 1 : 1 complex stoichiometry is based on following facts. First, all calixarenes **1–4** are fixed in cone conformation. As calix[4]arenes provide "soft" π-donor cavity composed of benzene ring as well as "hard" oxygen cavities constructed on lower rim[1,2,15], it is well known that only the softest cesium cation can be complexed by upper rim of reinforced cone of calix[4]arene skeleton[16]. Moreover, it was proved that very similar compounds (namely 25-bromoalkoxy-5,11,17,23-tetra-*tert*-butyl-26,27,28-tripropoxycalix[4]arenes) which are alco stable in cone conformation are able to form only 1 : 1 complexes with lithium and sodium cations in $CDCl_3$–CD_3CN 4 : 1 solution[17]. As all our compounds possess very flexible cavity we believe that they are able to form only 1 : 1 complexes with sodium and potassium cations using an array of hard oxygen donors at lower rim of calixarene unit in cone conformation.

The simplest model of complex-formation reaction between ligand L and metal cation M (L + M → LM) assumes that the mixture of true species in equilibrium (L, M, LM) forms an ideal solution. The standard equilibrium constant K in terms of relative concentrations (reference state is unit concentration c_0 = 1 mol/l) can be defined as given in Eq. (*2*).

$$K = \frac{c_{LM}/c_0}{(c_1/c_0 - c_{LM}/c_0)(c_2/c_0 - c_{LM}/c_0)} = \frac{c_{LM}}{(c_1 - c_{LM})(c_2 - c_{LM})} \quad , \qquad (2)$$

where c_2 is the initial concentration of metal. For the constant ligand concentration c_1 the expression for K can be given in the form of Eq. (*3*).

$$Kc_1 = \frac{\alpha}{(1-\alpha)(n-\alpha)} \quad , \qquad (3)$$

where $\alpha = c_{LM}/c_1$ is the degree of complexation and $n = c_2/c_1$ is the number of molar equivalents of metal ion.

To evaluate K it is necessary to measure the dependence $\alpha(n)$ in a wide range of α. Generally, the range 0.2–0.8 is recommended for all methods of stability constant

determination as relative error of K is proportional to the term $[\alpha(1 - \alpha)]^{-1}$ (ref.[18]). For the limits $\alpha \to 0$ and $\alpha \to 1$ the relative error of K tends to infinity. These two requirements are not sufficient. The relative error of K can be expressed (supposing constant c_1 and negligible error in n) as given in Eq. (*4*).

$$\mathrm{d} \log K = \left(\frac{1}{\alpha} + \frac{1}{1-\alpha} + \frac{1}{n-\alpha} \right) \mathrm{d}\alpha = E \, \mathrm{d}\alpha \qquad (4)$$

From this equation it is clear that not only for $\alpha \to 0$ and $\alpha \to 1$ but also for $\alpha \to n$ the relative error of stability constant tends to infinity. Besides two recommended conditions $\alpha > 0.2$ and $(1 - \alpha) > 0.2$ (i.e. $\alpha < 0.8$) also the condition $(n - \alpha) > 0.2$ should be included. In the NMR experiment the value of α is not determined directly, in fact the dependence $\delta_{dif}(n)$ is measured and $\alpha = \delta_{dif}/\delta_{cis}$. For the precise determination of δ_{cis} the last part of dependence $\delta_{dif}(n)$ (for high enough values of n) is very important, as for $\alpha \to 1$ the measured δ_{dif} is approaching the value of δ_{cis}. Therefore the dependence $\alpha(n)$ is measured also for $\alpha > 0.8$ although it means high relative error of K.

In Fig. 2 the contour lines are given for factor $E = 4.5$, 6 and 10. The minimal value of $E = 4$ is reached for n tending to infinity and $\alpha = 0.5$. It means that the relative error of K is at least four times higher than the absolute error of α, i.e. the uncertainty of ± 0.01 in the determination of complexation degree causes at least 4% error in K. In Fig. 2 also the dependences $\alpha(n)$ are given for different values of Kc_1. Supposing constant calixarene concentration ($c_1 = 0.05$ mol/l) the curves represent different values of K : K = 1 (*a*), K = 10 (*b*), K = 100 (*c*), and $K \to \infty$. For each value of K also curves with stability constants by 10% higher and 10% lower are given. The accuracy about ±10% is mostly considered as acceptable for stability constant determination. It is apparent

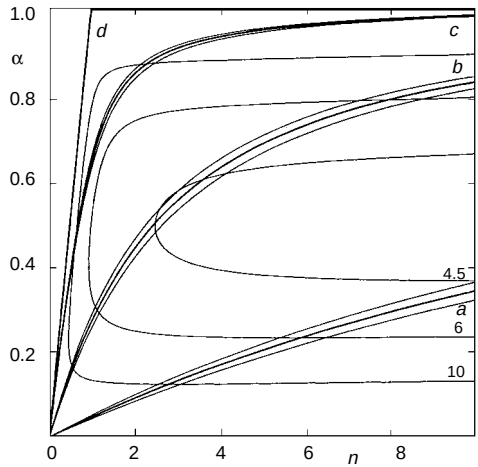

FIG. 2
The model dependences of complexation degree (α) on the number of equivalents of added metal cation (n) for the stoichiometry 1 : 1 and ligand concentration 0.05 mol/l. Curves correspond to: $K = 1 \pm 0.1$ (*a*), $K = 10 \pm 1$ (*b*), $K = 100 \pm 10$ (*c*), and $K \to \infty$ (*d*). The contour lines (4.5, 6, 10) are given for the constant value of E (d ln $K = E$ dα)

from the Fig. 2 that high values of factor E mean insensitivity of measured dependences $\alpha(n)$ to K. Relatively large changes of K result in small changes of $\alpha(n)$. Therefore relatively small experimental errors in measurement of $\alpha(n)$ cause high error of the calculated K. So the whole curve *c* is placed in the region with high values of E and it can be seen that $\alpha(n)$ is very insensitive to K compared with curves *a* and *b*. The curve *d* corresponds to the full complexation ($\alpha = n$ for $n < 1$ and $\alpha = 1$ for $n = 1$ and $n > 1$). This curve represents a limiting case when no information about the value of stability constant can be obtained. On the other hand this curve contains information about stoichiometry of the complex which is used in the method of molar ratio[18] – the measurement is performed with high enough concentration of c_1 to approach the limiting curve *d*.

It is necessary to use suitable concentration of calixarene and suitable additions of alkali metal in order to cover the range of α from about 0.2 to $\alpha \to 1$. The curve *b* is suitable for evaluation of K, i.e. the concentration of calixarene of 0.05 mol/l is suitable for the determination of K value around 10. The curve *a* has narrow range of measured α, therefore it would be necessary to continue with additions up to 100 equivalents of salt for reaching $\alpha = 0.8$. Another possibility is to increase the concentration of ligand, e.g. for 10 times higher concentration, i.e. $c_1 = 0.5$ mol/l and $K = 1$ it corresponds to the shape of curve *b*. As mentioned above, the determination of K is very inaccurate for the curve *c*. Supposing K value around 100 the concentration of calixarene should be decreased by one order of magnitude to $c_1 = 0.005$ mol/l to obtain again the shape of curve *b*. Such an experiment only (curve *c*) can be used to prove the stoichiometry (1 : 1 in this case).

Another fact which contributes to the accuracy of stability constant determination is the error of determination of δ_{dif} which was estimated to be 0.5 Hz. As mentioned above, the relative error of K is at least four times higher then the absolute error of α. If δ_{dif} becomes smaller the error of its determination becomes higher and consequently the error of stability constant K is also higher tending to infinity when the values δ_{dif} are negligible. For small δ_{dif} (in the order of 1 Hz) the error of its determination is comparable with the value measured. That is why usually only those protons exhibiting the highest value of CIS are taken into account for calculation of the stability constant. It is, however, more correct to consider as much protons as possible. In this work we have used the second approach. For each measurement (with known ligand concentration c_1) the dependences $\delta_{dif}(n)$ were obtained for all protons except those with overlapping signals and those with very small values of δ_{dif}. From these input data the stability constants K and δ_{cis} values were calculated. The objective function given as the sum of squares of differences between experimental and calculated values of δ_{dif} was minimized to find the best values of parameters K and δ_{cis} within their 95% confidence intervals and the standard deviation of fit[19]. Averaged value of stability constant was then determined as the weighted arithmetic mean (weights w were the the reciprocal

values of square roots of standard deviations of each particular stability constant) from the values of stability constants obtained for individual protons (Eq. (*5*)).

$$K_{\mathrm{av}} = \frac{\sum w_i K_i}{\sum w_i} \qquad (5)$$

The 95% confidence interval for these averaged stability constants was also calculated and is given by Eq. (*6*),

$$\sigma(K_{\mathrm{av}}) = F \left(\frac{\sum w_i (K_i - K_{\mathrm{av}})^2}{(m-1) \sum w_i} \right)^{1/2} , \qquad (6)$$

where F is the Student coefficient and m is the number of particular K values. These averaged stability constants were used to compare the complexation abilities of the investigated calix(4)arenes **1–4**.

The calculated values of stability constants have in general only limited physical meaning. The assumption of ideal behaviour of the system calixarene–cation in $CDCl_3$–CD_3OD solvent represents an inevitable simplification because the activity coefficients of all species are not at disposal. Therefore only values of K calculated for similar systems can be compared. In this case the K values obtained for systems containing similar calixarenes, identical cation and identical solvent were compared.

RESULTS

The ^1H NMR spectra of calixarenes **1–4** in $CDCl_3$–CD_3OD (2 : 1, v/v) mixture are given in Table I. Differences between chemical shifts of free calixarene obtained from measurement in two solvent mixtures namely $CDCl_3$–CD_3OD 2 : 1 and 1 : 1 were negligible. The chemical shift of proton signal of chloroform depends on the composition of the $CDCl_3$–CD_3OD mixture, however. The complexation experiments with changing sample volume (it increases from 0.6 ml to about 0.7 ml during the additions of anion stock solutions) were performed with addition of small amount of TMS and it was found that the position of the chloroform signal is stable. It was thus used for referring of the spectra in the complexation experiments with constant volume.

No changes in chemical shifts were observed for additions of Li^+. On the other hand, Na^+ and K^+ additions to the calix[4]arenes **1–4** caused significant changes in chemical shifts, except the system **3** + KSCN where the changes were subtle. The system **1** + NaSCN was measured twice with freshly prepared samples. The dependences of the observed changes of chemical shifts for all groups of equivalent protons for the **1** + NaSCN system are given in Fig. 3. The calculated values of stability constants and CIS with their 95% confidence intervals and the standard deviation of fit are given for repeated

TABLE I
^{1}H NMR spectra of calixarenes **1–4**

Proton	**1**			**2**			**3**			**4**		
	m	δ	*J*	m	δ	*J*	m	δ	*J*	m	δ	*J*
1	t	6.585	7.5	t	6.679	7.5	t	6.782	7.1	t	6.584	7.0
2	d	6.648	7.5	d	6.810	7.5	d	6.817	7.1	d	6.628	7.0
2′				s	5.936		s	7.449				
3A	d	4.516	13.5	d	4.444	13.4	d	4.625	13.7	d	4.490	13.5
3B	d	3.177	13.5	d	3.069	13.2	d	3.299	13.7	d	3.148	13.5
4	t	4.124	5.7	t	4.160	5.9	t	4.185	5.3	t	4.132	5.4
4′				t	3.996	5.5	t	4.254	4.8			
5	t	3.895	5.7	t	3.882	5.8	t	3.856	5.0	t	3.896	5.4
5′				t	3.836	5.8	t	3.873	5.0			
6	q	3.597	7.0	q	3.591	7.0	q	3.578	7.0	(t)	[a]	
6′				q	3.559	7.0	q	3.572	7.0			
7	t	1.239	7.0	t	1.215	7.0	t	1.223	7.0	(t)	[a]	
7′				t	1.202	7.0	t	1.231	7.0			
8										(t)	[a]	
9										(t)	[a]	
10										s	3.370	

[a] Not resolved signals in the region 3.5–3.7 ppm.

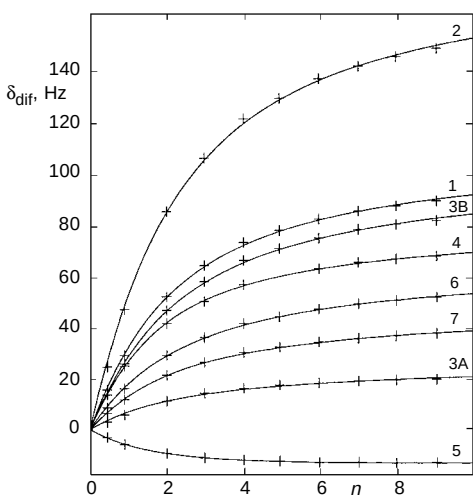

FIG. 3
System **1** + NaSCN, protons 1–7: measured (points) and fitted (curves) dependences of changes of chemical shift (δ_{dif}) on the number of equivalents of added cation (*n*), temperature 298.15 K, ligand concentration 0.05035 mol/l

measurements (shown as **1** + NaSCN, set 1, and **1** + NaSCN, set 2) in Table II. The CIS values obtained in both measurements are in good agreement. The averaged values of *K* manifest an acceptable reproducibility.

The influence of the anion of the salt used for additions of Na^+ was examined using sodium bromide. The averaged value of *K* and also the CIS values are different than those obtained for **1** + NaSCN. The results obtained for **1** + NaBr and **1** + KSCN system are given in Table II. For the latter system the changes in chemical shift for proton 3A of calixarene were very small – in order of 1 Hz. In such a case the optimization procedure collapses because the response variable is a random number. This proton had to be excluded.

As has been already mentioned above, the mixture $CDCl_3$–CD_3OD (1 : 1) was used for experiments with KSCN. Therefore the results obtained for complexations with NaSCN are not fully comparable with those obtained with KSCN.

The system **2** + NaSCN was also measured for the concentration of calixarene about 0.05 mol/l. In Fig. 4 the dependence $\delta_{dif}(n)$ measured for proton in position 1 is shown. It was found that the stability constant is high (the order of 100). This combination of *K* and calixarene concentration c_1 represents the case (as discussed in Theoretical) when the product Kc_1 is too high and the determination of *K* is very inaccurate. The standard deviations of *K* were comparable with the calculated values of *K*. On the other hand, from this experiment the 1 : 1 stoichiometry can be clearly proved while the 1 : 2 (ligand : metal ion) stoichiometry (dotted line in Fig. 4) can be excluded.

To determine the *K* for system **2** + NaSCN value the experiment was carried out with the initial calixarene concentration about 0.00578 mol/l which was changed by consequent additions of NaSCN solution to 0.00437 mol/l while for the last 2 additions (up to 30 equivalents) solid NaSCN was used.

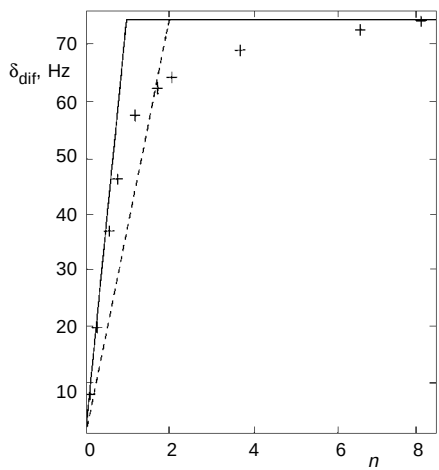

FIG. 4
System **2** + NaSCN. Proof of 1 : 1 stoichiometry, proton 1; ligand concentration 0.05 mol/l. Dotted line corresponds to 1 : 2 (ligand : metal cation) stoichiometry

TABLE II

Calculated stability constants *K*, CIS values (Hz) and standard deviations of fit σ_{fit} (Hz) for the systems **1** + NaSCN, set 1, and **1** + NaSCN, set 2 (both systems measured in $CDCl_3$–CD_3OD 2 : 1), **1** + NaBr and **1** + KSCN (both systems measured in $CDCl_3$–CD_3OD 1 : 1)

Proton	**1** + NaSCN, set 1			**1** + NaSCN, set 2			**1** + NaBr			**1** + KSCN		
	K	CIS	σ_{fit}	*K*	CIS	σ_{fit}	*K*	CIS	σ_{fit}	*K*	CIS	σ_{fit}
1	12.6 ± 0.9	105.4 ± 2.0	0.7	13.5 ± 1.2	109.0 ± 2.7	1.1	23.9 ± 5.1	54.3 ± 3.2	1.1	15.6 ± 1.3	76.1 ± 2.4	1.0
2	12.0 ± 1.0	177.6 ± 4.1	1.4	12.8 ± 1.0	181.4 ± 4.8	1.6	22.1 ± 4.9	91.7 ± 5.9	1.8	13.8 ± 0.9	152.4 ± 4.3	2.5
3A	12.6 ± 3.2	21.7 ± 1.5	0.5	11.7 ± 1.6	24.4 ± 1.0	0.4	29.7 ± 19.2	6.5 ± 1.1	0.4		[a]	
3B	11.7 ± 1.0	97.8 ± 2.2	0.7	12.4 ± 1.1	100.6 ± 2.6	1.0	22.7 ± 5.2	48.4 ± 3.2	1.0	13.7 ± 1.4	83.3 ± 3.5	1.7
4	16.5 ± 3.1	76.3 ± 1.6	0.7	12.7 ± 1.4	76.6 ± 2.4	1.0	20.1 ± 4.6	34.8 ± 2.1	0.7	11.8 ± 1.8	29.1 ± 1.9	0.4
5	19.0 ± 1.5	–17.2 ± 0.3	0.1	31.2 ± 8.9	–15.0 ± 0.9	0.5	20.9 ± 2.8	–16.6 ± 0.6	0.2	26.6 ± 6.5	–17.4 ± 1.3	0.6
6	11.4 ± 0.8	61.0 ± 1.3	0.4	11.4 ± 1.0	64.6 ± 1.7	0.6	24.5 ± 6.3	28.4 ± 1.6	0.7	13.1 ± 0.8	60.1 ± 1.6	0.3
7	11.9 ± 1.0	43.4 ± 1.0	0.3	11.6 ± 1.1	46.6 ± 1.3	0.5	25.9 ± 6.9	20.0 ± 1.1	0.5	13.6 ± 0.9	47.5 ± 1.3	0.2

[a] Negligible changes of chemical shifts.

TABLE IV

Calculated stability constants *K*, CIS values (Hz) and standard deviations of fit δ_{fit} (Hz) for the systems **3** + NaSCN, **4** + NaSCN and **4** + KSCN measured in $CDCl_3$–CD_3OD 2 : 1

Proton	**3** + NaSCN			**4** + NaSCN			**4** + KSCN		
	K	CIS	σ_{fit}	*K*	CIS	σ_{fit}	*K*	CIS	σ_{fit}
1	1.36 ± 0.56	55.5 ± 14.2	1.0	11.8 ± 2.4	76.3 ± 4.4	1.6	10.4 ± 2.3	27.5 ± 1.8	0.6
2	1.22 ± 0.51	81.7 ± 21.7	1.4	10.6 ± 2.8	128.4 ± 6.9	2.5	14.0 ± 3.6	33.3 ± 2.1	0.8
2′	3.50 ± 0.44	–47.8 ± 2.6	0.6						
3B				10.8 ± 4.7[a]	69.1 ± 13.5[a]	1.4	6.6 ± 1.0	37.0 ± 2.0	0.5

[a] Only 7 points (not resolved signals in the spectra of later additions).

Similarly the system **2** + KSCN was measured: the initial calixarene concentration 0.00507 mol/l was diluted by additions of stock solution of salt to final total concentration of ligand in sample 0.00340 mol/l. Last additions (up to 50 equivalents of salt)

TABLE III
Calculated stability constants *K*, CIS values (Hz) and standard deviations of fit (Hz) for the systems **2** + NaSCN and **2** + KSCN measured in $CDCl_3$–CD_3OD 2 : 1

Proton	**2** + NaSCN			**2** + KSCN		
	K	CIS	σ_{fit}	*K*	CIS	σ_{fit}
1	199.7 ± 33.4	66.3 ± 2.2	1.3	23.4 ± 1.0	69.3 ± 1.5	0.4
2	182.4 ± 32.8	107.1 ± 4.0	2.4	20.9 ± 0.5	120.6 ± 1.5	0.4
2′	167.8 ± 36.1	170.3 ± 7.9	4.5	19.9 ± 0.6	161.2 ± 2.5	0.6
3B	182.7 ± 39.1	73.1 ± 3.2	1.9	20.4 ± 0.8	76.0 ± 1.4	0.4
4	187.3 ± 39.1	40.2 ± 1.8	1.0	23.7 ± 1.1	32.7 ± 0.8	0.2
4′	176.6 ± 28.4	73.4 ± 2.5	1.5	23.2 ± 1.0	36.4 ± 0.8	0.2
5	308.6 ± 71.0	–14.1 ± 0.5	0.4	19.5 ± 0.6	–25.3 ± 0.4	0.1
5′	218 ± 78.5	–16.8 ± 1.3	0.6	25.4 ± 2.2	–7.2 ± 0.3	0.1

TABLE V
Averaged values of stability constants for the systems measured in $CDCl_3$–CD_3OD 2 : 1 (A) and in $CDCl_3$–CD_3OD 1 : 1 (B)

A		B	
System	*K*	System	*K*
1 + NaSCN, set 1	12.5 ± 1.7	**1** + KSCN	13.7 ± 1.2
1 + NaSCN, set 2	12.3 ± 1.0	**2** + KSCN	20.9 ± 1.3
1 + NaBr	22.1 ± 1.6	**3** + KSCN	[a]
2 + NaSCN	188 ± 24	**4** + KSCN	6–17[b]
3 + NaSCN	1–4[b]		
4 + NaSCN	11.4 ± 4.7		

[a] Negligible changes of chemical shifts for all protons, probably low value of *K*. [b] Very different values of particular constants.

were added in solid form. The results for **2** + NaSCN and **2** + KSCN systems are given in Table III. The accuracy is much better in these experiments than in previous ones with calixarene concentration 0.05 mol/l. For some protons the changes of chemical shifts were very low or the signals were overlapped.

The system **3** + NaSCN represents the opposite case compared with the system **2** + NaSCN. Here the value of stability constant is low and therefore the product Kc_1 is also low. It would be necessary to continue with additions to reach higher concentration degree or to perform this experiment using higher c_1 than 0.05 mol/l. Both ways are unfortunately not possible due to the limited solubility of NaSCN in mixed solvent. The changes of chemical shifts were high enough only for protons 1, 2 and 2′. Results thus obtained are summarized in Table IV.

The results for systems **4** + NaSCN and **4** + KSCN are given in Table IV only for three protons due to signal overlapping (Table I) or due to very low changes of their chemical shifts.

The calculated values of stability constants with their 95% confidence intervals for all measured systems are summarized in Table V.

For systems **3** + NaSCN and **4** + KSCN where the values of constants calculated from shifts of individual protons differ substantially only the range of these values is given. It should be admitted here that accuracy of stability constants obtained for systems containing calixarenes **3** and **4** is very low – in fact only the order of stability constants was determined. In spite of poor accuracy it is clear that the stability constant for calixarene **3** is significantly lower compared with that for **1** while the stability constants for **4** are of the same order as those of **1**.

TABLE VI
^{13}C NMR spectrum of ligand **1** and changes of chemical shifts corresponding to 90% complexation with NaSCN

Carbon	δ	Δδ
a	122.56	+3.74
b	128.54	+1.00
c	135.33	+0.31
d	31.17	–1.02
e	156.67	–4.79
f	73.39	+2.88
g	70.11	–1.99
h	66.75	+0.45
i	15.36	–0.23

DISCUSSION

^{1}H NMR spectra of all ligand–metal systems studied contain only one signal for each individual proton. It means that all structures (different conformations of free ligand and complexes) are in dynamic equilibrium with the time-constant much lower than the NMR time-scale; this situation is usually referred as "fast exchange" (ref.[20]). The NMR signals measured represent weighted average of all forms present in equilibrium. The cone conformation of calixarene skeleton of **1–4** is stable enough thanks to four lower rim substituents[1–3]. The actual shape of the cone, however, is far from being rigid[3,12]. The oligooxyethylene chains are also flexible, as documented by the study of other podand-like structures[21]. It is reflected by ^{1}H NMR interaction constants measured for oligoxyethylene moiety. They are close to values known from completely relaxed oligoethylene glycols. The interaction constant of 5.7 Hz was measured for protons in positions 4 and 5 of free calixarene **1** (Table I). The value of interaction constant decreases gradually during the complexation with NaSCN reaching the value of 4 Hz for the last addition corresponding to the degree of complexation of about 0.82. This decreasing could correspond to the change of *anti* position of both oxygens in completely relaxed dioxyethylene unit into *gauche* orientation of these oxygens prepared for the interaction with cation. It can be concluded that in any of compounds **1–4** the lower rim substituent does not adopt preorganized conformation. It is very probably that cation in complexed state is located within pseudo-cavity formed by eight oxygen atoms (four "phenolic" and four "pure" ether) as evidenced by broadening of signals belonging to protons in positions 4,5 and 4′,5′ as well. On the other hand, the chemical shifts and shapes of signals belonging to protons 6, 7, 8, and 9 (unresolved multiplet) in ligand 4 seems to be almost unaffected by the complexation. This reflects both the conformational mobility of long oligooxyethylene chains and relatively restricted mobility of calix[4]arene cone. The pseudo-cavity is therefore preferentially formed by eight oxygen atoms close to the lower rim of calixarene.

To support this assumption further, we have compared the ^{13}C NMR spectrum of free calixarene **1** with that of system **1** + NaSCN measured for additions of 7.8 equivalents of salt (corresponding to 90% of complexed ligand). The results are shown in Table VI.

The largest change of chemical shift corresponds to the carbon in positions (e) (Fig. 1). This carbon influences strongly carbon atom (a) at *para* position and weakly carbons at *meta* and *ortho* positions (b) and (c) by facile transfer through aromatic benzene ring. As both carbons between the lower rim oxygens (f) and (g) are strongly influenced contrary to carbons (h) and (i), our assumption that pseudo-cavity formed by eight oxygens at lower rim is strongly supported. Moreover, it is in accordance with the fact that lengthening of oligoethylene chains on lower rim has almost no effect on the complexation ability toward cations studied.

The stability constant measurements is strongly dependent on the anion used (compare Table II). The reason for it is believed to be relatively high concentration of active

species in NMR samples. The association and solvation of dissolved salts in such concentration is definitely influencing the data obtained. The use of lower (milimolar) concentrations on the other hand is not possible for technical reasons (the measurement would be very time-demanding).

As far as the cation used is concerned, we have included only first three alkali metal cations as more "soft" cations (especially cesium) should tend to complex at softer upper rim, too. The insolubility of Rb^+ and Cs^+ salt in the solvent used prevent us, on the other hand, from trying preliminary experiments with these cations. The differences between the complexation with Na^+ and K^+ are not significant for calixarenes **1** and **4**. The value of the stability constant is significantly higher for the **2** + NaSCN system than for the **2** + KSCN system. It is again in line with the "hardness" of both cations. For calixarene **3** the changes in chemical shifts were very small, especially for the **3** + KSCN system.

From the comparison of all the data obtained the following conclusions can be drawn:

1. Ligands **1–4** form 1 : 1 complexes with sodium and potassium cations in solutions of intermediate polarity (chloroform–methanol mixtures).

2. Amino substitution (calixarene **2**) on the upper rim of calixarene has significant positive influence on the complexation ability compared with the unsubstituted calixarene **1**. It is likely due to the increasing electron density on "phenolic" oxygen atoms. The influence of nitro (substitution calixarene **3**) is opposite.

3. The length of the ether chains (calixarene **4**) has no significant influence on the complexation ability.

4. It is very likely that Na^+ and K^+ are complexed within pseudo-cavity formed by eight oxygen atoms. Complexation is accompanied by increasing rigidity of the group $Ar–O–CH_2–CH_2–O–$ evidenced by change in interaction constants of protons within this structure. Moreover, ^{13}C NMR data of ligand 1 and its complex with NaSCN support strongly this assumption.

The next step in the development of podand-like calix[4]arene ligands for complexation of cations in polar media will be the synthesis and complexation study of compounds with lipophilic alkyl (arylalkyl) groups in the oligoethylene chain where the formation of pseudo-cavity should be facilitated by hydrophobic forces. The role of upper rim substitution (*tert*-butyl, adamantyl) is to be included, too.

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