J Incl Phenom Macrocycl Chem (2007) 59:143–154
DOI 10.1007/s10847-007-9307-2

ORIGINAL ARTICLE

Stable complexes of tertiary ammonia derivative of phenothiazine with tertramethylsulfonated resorcin[4]arenes obtained under substoichiometric conditions

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Received: 22 September 2006 / Accepted: 6 March 2007 / Published online: 2 June 2007

Abstract Eight water insoluble complexes of tetramethylsulfonated calix[4]resorcinarenes **1** and **2** (–CH_3 and –C_5H_{11}) with phenothiazine derivative, **3**, were obtained under substoichiometric conditions by mixing aqueous solutions of the initial reagents. It was found that complexation of cationic **3** by macrocycles was provided by both Coulomb interaction with the negative sulfonato-groups on the upper rim and by cation-π interactions with the aromatic cavity. The complexes precipitated and, therefore, were studied in organic solvents—DMSO, CD_3OD, and $CDCl_3$ using IR-, UV-, and NMR- spectroscopy. Formation of the complexes accompanied by gradual dehydratation of the host—estimated quantity of water in the complexes decreased with increase of the initial concentration of **3**. Driving forces of precipitation and complexation, the role of water coordinated by the hosts, and distribution of phenothiazine derivative between two kinds of binding sites are discussed.

Keywords Host-guest systems · Association · Multiple interactions · Self-diffusion · Phenothiazine · Resorcinarene

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Introduction

The cavities of many macromolecular compounds were and are extensively studied as prototypes imitating the hydrophobic pockets of protein molecules formed by the residues of amino acids [1]. To improve the compatibility of the macrocycles with the experimental conditions close to those in natural systems they are often functionalized with groups improving their solubility in water [2].

In case of calixresorcinarenes, we performed modification of the upper rim with four methylsulfonato-groups [3]. Obtained water soluble sodium salt of tetramethylsulfonato-calix[4]resorcinarene **1** exists in solution as a tetra-anion and forms inclusion complexes with amines (binding constant log β: log β 2.43–5.47), *N*-methylaminopyridin (log β 2.69–5.03), and amino acids (log β 1.44–2.17) [4]. Recently **1** and **2** were used for modification of peroxidase biosensor—it was utilized for selective pre-concentration of the analytes—competitive peroxidase inhibitors, promazine [5], **3**, and chloropromazine containing terminal amino-groups providing their binding with **1** [6] (Fig. 1).

In aqueous media, macrocyclic compounds with hydrophobic interior typically form inclusion host-guest complexes mimicking substrate recognition in the hydrophobic protein pockets. In case of resorcinarenes, suitable guests include aromatic and ammonium compounds that can be both protonated amines and quaternary ammonium salts. Depending on the structure and lipophilicity of the bound substrate, the complexes remain in solution or precipitate [7]. The latter takes place when hydrophilic groups providing water-solubility are taking part in competitive binding of the substrate and are no longer available for interactions with the solvent molecules. Apparently, in the isolated complexes one can expect to find both water and excess of the substrate molecules precipitated with the obtained complex.

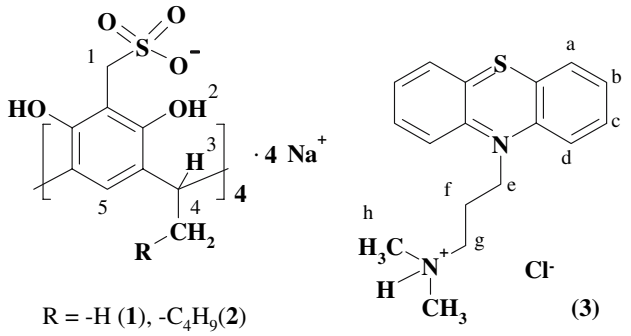

Fig. 1 Structures of the hosts **1** and **2** and promazine **3**·**HCl**

Water molecules are included in crystal structure of complexes. The resorcinarenes are known to form capsules and hexameric hydrogen-bonded spherical assemblies with participation of water molecules in wet apolar solvent [8, 9].

Here we present an extensive investigation of a variety of the complexes of **1** and **2** with promazine **3** forming precipitates in aqueous solutions.

Experimental

Materials, instruments, and method

5,11,17,23-Tetrakissulfonatomethylen-2,8,14,20-tetramethyl-calix[4]arene tetrasodium salt **1** and 5,11,17,23-tetrakissulfonatomethylen-2,8,14,20-tetra-penthylcalix[4]arene tetrasodium salt **2** were prepared according to procedures described in [3]. 10-[3-(Dimethylamino)propyl]phenothiazine **3** was used as hydrochloride salts purchased from Sigma (Germany).

Melting points of the complexes **4**–**11** were determined with heating block ''BOËTIUS'' (Dresden, Germany).

Stoichiometry determination relied on combination of ^{1}H NMR-spectroscopy and elemental analysis data.

IR-spectra on KBr tablets were recorded with Fourier IR-spectrometer ''Vector 22'' (Bruker, Germany) with the resolution of 1 cm^{-1}, number of accumulated scans of 64, and acquisition time of 16 s. UV-spectra were recorded with UV–Vis-spectrophotometer ''Specord UV–VIS'' (Carl Zeiss, Jena, Germany) for 10^{-4} M solutions in freshly distilled methanol for the light pass of 1.5 mm.

^{1}H and ^{13}C NMR experiments were performed in 10 mM solutions on a Bruker AVANCE-600 spectrometer with pulsed gradient unit producing magnetic field pulse gradients of 50 G cm^{-1} in z-direction. All experiments were carried out with 5 mm diameter broadband inverse probe head at 30 °C unless stated otherwise. Chemical shifts are reported relative to TMS internal standard with the errors less than ±0.01 ppm in ^{1}H and ±0.05 ppm in ^{13}C spectra. The ratio of integral intensities in ^{1}H NMR spectra complexes of **1&3** and **2&3** measured in DMSO at 303 K were used for determination of the stoichiometry of the complexes. In case of overlapping peaks deconvolution procedure was applied for higher accuracy of integral intensity analysis [10]. 2D NOESY experiments were performed with the mixing times of 50–600 ms with pulsed filtered gradient techniques. The pulse programs for all NMR experiments were taken from the Bruker software library.

Fourier-transform pulsed field-gradient (FT-PFG) NMR experiments were reproduced at least three times. All reported data have correlation coefficients of $\ln(I/I_0)$ vs. $\gamma^{(}\delta^{(}g^2(\Delta-\delta/3)$ higher than 0.999. The pulse gradients increments were of 0–32 G cm^{-1} and were applied in 32 steps with pulse duration of 1.2–4 ms depending on viscosity of the solvent and molecular weight of the examined samples. The pulse gradient separation was 50 ms. The error of the self-diffusion coefficients determination did not exceed 5%.

The self-diffusion coefficient (D_s) depends on the size of the molecule:

$$D_S = \frac{k_B T}{6\pi\eta R_h} \tag{1}$$

where k_B is Boltzmann constant, T-temperature (K), η (Pa s)—dynamic viscosity of the solvent and R_h hydrodynamic radius of the molecule.

In case of fast exchange between complexed and free forms of **3** in NMR time scale it was possible to calculate the fraction of promazine bound to **1** or **2**, p_b [9, 11, 12]:

$$p_b = \frac{D_{\text{obs}} - D_{\text{free}}}{D_{\text{complex}} - D_{\text{free}}} \tag{2}$$

where D_{obs} is the apparent (weighted average) self-diffusion coefficient of the ligand (promazine) in the complex, D_{complex} is the self-diffusion coefficient of the complex and D_{free} is the self-diffusion coefficient of free promazine in same solvent. D_{complex} is an approximation, since self-diffusion coefficient of the complex cannot be determined. However, it can be assumed equal to D_{host} (self-diffusion coefficient of the macrocycle), which will lead to a slight overestimation of the determined value of bonded ligand (p_b) [11, 12].

To correct the solvent effect imposed on self-diffusion coefficients by aggregation of **1** and **2** in solutions, hydrodynamic radii, R_h, were calculated by Einstein–Stokes equation (1).

Fraction of the molecules included into the cavity, p_q,, was estimated [13]:

$$p_q = \frac{\delta_{\text{obs}} - \delta_{\text{free}}}{\delta_{\text{guest}} - \delta_{\text{free}}} \tag{3}$$

where δ_{obs} is the chemical shift of promazine in complexes registered in different solvents, δ_{free} is the chemical shift of free promazine in same solvents (See group *h* in Table 1A in Supporting information), and δ_{guest} is the chemical shift of the guest molecules bound by the macrocycle cavity (the ''end point of titration'').

Synthesis of the complexes

Complex 4 (1&3): 10-(3-dimethylaminopropyl)phenothiazine hydrochloride **3** (0.0642 g, 0.2 mmol) dissolved in 2 ml of water were added under stirring at room temperature to tetramethylsulfonated resorcinarene **1** (0.2016 g, 0.2 mmol) dissolved in 5 ml of water. Precipitation started immediately. The reaction mixture was left for a weak at room temperature. The sediment was filtered off and dried under vacuum for 72 h at 70–72 °C. Cream-colored crystalline product **4** (0.09 g) was obtained. M.p. 188–189 °C; elemental analysis calcd (%) for $C_{36}H_{36}O_{20}S_4$•4-$C_{17}H_{21}N_2S$•$4H_2O$ (2130.68): C 58.62, H 6.05, N 5.26, S 12.03; found: C 58.97, H 5.93, Cl 0.90, N 5.66, S 12.12.

Complex 5 (1&3) was obtained as described for the complex **4** with the following amounts of the reagents: **1** 0.2016 g, 0.2 mmol; **3** 0.1284 g, 0.4 mmol. Cream-colored crystalline product **5** (0.136 g) was isolated. M.p. 166–168 °C; elemental analysis calcd (%) for $C_{36}H_{36}O_{20}S_4$•4-$C_{17}H_{21}N_2S$•$C_{17}H_{21}ClN_2S$•$4H_2O$ (2451.57): C 59.28, H 6.13, Cl 1.44, N 5.71, S 11.77; found, %: C 59.51, H 5.89, Cl 1.20, N 5.62, S 12.08.

Complex 6 (1&3) was obtained as described for the complex **4** with the following amounts of the reagents: **1** 0.2016 g, 0.2 mmol; **3** 0.2568 g, 0.8 mmol. Cream-colored crystalline product **6** (0.359 g,) was isolated. M.p. 157–158 °C; elemental analysis calcd (%) for $C_{36}H_{36}O_{20}S_4$•4-$C_{17}H_{21}N_2S$•$C_{17}H_{21}ClN_2S$•$3H_2O$ (2433.57): C 59.72, H 6.09, Cl 1.45, N 5.75, S 11.85; found: C 60.03, H 5.96, Cl 1.24, N 5.97, S 12.02.

Complex 7 (1&3) was obtained as described for the complex **4** with the following amounts of the reagents: **1** 0.2016 g, 0.2 mmol; **3** 0.321 g, 1.0 mmol. Crystalline product **7** (0.473 g) was obtained. M.p. 143–144 °C; elemental analysis calcd (%) for $C_{36}H_{36}O_{20}S4$•4-$C_{17}H_{21}N_2S$•$C_{17}H_{21}ClN_2S$•$2H_2O$ (2351.35): C 60.16, H 6.04, Cl 1.47, N 5.79, S 11.94; found: C 60.33, H 5.96, Cl 1.23, N 5.64, S 11.90.

Complex 8 (2&3): 10-(3-dimethylaminopropyl)phenothiazine hydrochloride **3** (0.1604 g, 0.5 mmol) dissolved in 2 ml of water were added under stirring to solution of tetramethylsulfonated resorcinarene **2** (0.616 g, 0.5 mmol) in 10 ml of water. Precipitation started while mixing. The precipitate was filtered off after 72 h and dried under vacuum for 30 h and thus product **8** (0.516 g) was obtained. The product forms light-brown film that did not melt below 360°C. Elemental analysis calcd (%) for $C_{52}H_{68}Na_3O_{20}S_4$•$C_{17}H_{21}N_2S$•$2H_2O$ (1531.77): C 54.10, H 6.11, N 1.82, S 10.46; found: C 54.04, H 6.10, N 2.02, S 10.70.

Complex 9 (2&3) was obtained as described for the complex **8** with the following amounts of the reagents: **2** 0.2 g, 0.16 mmol; **3** 0.104 g, 0.32 mmol. Pink crystalline product **9** (0.196 g,) was isolated. M.p. 148–151 °C; elemental analysis calcd (%) for $C_{52}H_{68}O_{20}S_4$-Na•$3C_{17}H_{21}N_2S$ (2020.61): C 61.23, H 6.53, N 4.16, S 11.10, Na 1.13, found: C 61.41, H 6.82, N 4.40, S 11.07.

Complex 10 (2&3) was obtained as described for the complex **8** with the following amounts of the reagents: **2** 0.2 g (0.16 mmol); **3** 0.208 g (0.64 mmol). Light-brown crystalline product **10** (0.353 g) was isolated. M.p. 124–126 °C; elemental analysis calcd (%) for $C_{52}H_{68}O_{20}S_4$•$4C_{17}H_{21}N_2S$ (2283.05): C 63.13, H 6.71, N 4.91, S 10.24; found: C 62.79, H 6.92, N 4.95, S 10.93.

Table 1 Ratio of the initial reagents (H:G), melting points (M.p.), yields, stoichiometry, and water quantity for the complexes of **1** and **2** with promazine **3**

Compounds	Complexes **1**&**3**				Complexes **2**&**3**			
	4	**5**	**6**	**7**	**8**[c]	**9**[c]	**10**	**11**
H:G	1:1	1:2	1:4	1:5	1:1	1:2	1:4	1:5
M.p.°C	188–189	166–168	157–158	143–144	>360	148–151	124–126	122–124
Yield %	21 (85)[a]	28 (69.4)	74 (92.3)	98 (98)	67.5 (67.5)	60 (88)	95 (95)	76.3 (88)
n[b]	4 ± 0.1	5 ± 0.1	5 ± 0.1	5 ± 0.1	1.5 ± 0.5	3 ± 0.5	4 ± 0.2	4.3 ± 0.2
N(H_2O)[d]	4	4	3	2	2	[e]	[e]	[e]

[a] Yield of the complex relative to **1** (relative to **3**)

[b] Number of promazine molecules per molecule of the macrocycle (see Experimental part)

[c] Product contains Na^+ ions

[d] The quantity of water's molecules per molecule of the macrocycle was estimated from ^1H NMR spectra in $CDCl_3$ at 253 K

[e] The quantity of water can not be estimated from ^1H NMR spectra as its peak was covered by other signals

Complex 11 (2&3) was obtained as described for the complex **8** with the following amounts of the reagents: **2** 0.2464 g (0.2 mmol); **3** 0.321 g (1 mmol). Light-brown crystalline product **11** (0.421 g) was isolated. M.p. 122–124 °C; elemental analysis calcd (%) for $C_{52}H_{68}O_{20}S_4$•$4C_{17}H_{21}N_2S$•$0.32C_{17}H_{21}ClN_2S$ (2385.72): C 63.15, H 6.70, Cl 0.48, N 5.07, S 11.18; found: C 63.46, H 7.07, Cl 0.55, N 5.11, S 11.05.

Results

Stable nearly insoluble in water complexes were obtained by mixing 1:1 aqueous solutions of **1** or **2** with **3**. The yield of the complexes calculated for 1:1 stoichiometry, however, did not exceed 21 % for macrocycle **1**. In order to increase the yield and to provide complete participation of the macrocycles in binding of promazine, an excess of **3** was taken for further preparation of the complexes. Stoichiometric ratios of **1** or **2** to **3** of 1:2, 1:4, and 1:5 were used for preparation of complexes. Unlike the initial compounds, the isolated products were insoluble in water but soluble in various organic solvents, namely, chloroform, methanol, THF, DMSO, DMF, and dioxane. The complexes show reproducible distinct melting points (Experimental part, Tables 1 and A5) decreasing with the increase of the H:G initial ratio.

Virtually, the IR-spectra of the complexes **1&3** (**6**–**8**) are represented by combination of the spectra of the individual components. The absorbance bands of SO_3^- (**1**) and $(CH_3)_2NH^+$(**3**) were found to be the most sensitive to the binding of promazine by macrocycle **1**. Upon complexation, the bands ν (SO_3^-) 1219, 1150, and 1044 cm^{-1} were shifted to 1227, 1142, and 1035 cm^{-1}, respectively. The band ν ($(CH_3)_2N$–H^+) observed in the spectra of individual **3** as a broad band well structured at high frequency region with the maximum at 2409 cm^{-1} was transformed into complex absorption contour at the area of 2730–2500 cm^{-1}. Similarly, upon binding of **3,** the shape of the band ν (OH) at 3400 cm^{-1} became a highly structured one (Fig. 2 and Table A6) [14].

The UV-spectra of the complexes **1&3** and **2&3** in methanol contain five major bands of π–π^* absorbance ascribed to initial compounds **1** and **3**. The band of the macrocycles at 287 nm remain nearly unchanged upon complexation, whereas the intensity of the bands ascribed to **3** increase and the band at 303 nm experiences bathochromic shift of 10 nm (Table A7) [15].

The ratio of integral intensities of ^{1}H NMR spectra in DMSO of the products **4** and **8** obtained with the 1:1 ratio of the initial reagents suggested a stoichiometry of the complexes of 1:4 ± 0.1 and 1:1.5 ± 0.5, revealing formation of more intricate associates than 1:1 inclusion

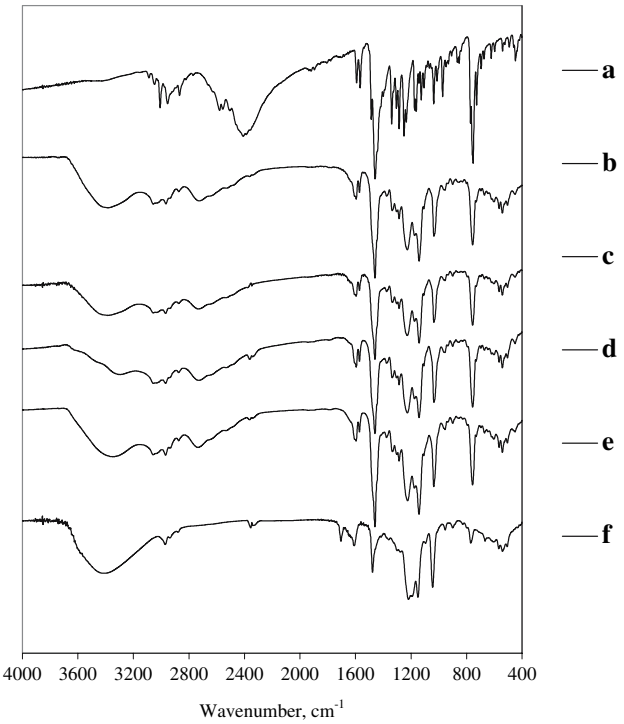

Fig. 2 IR-spectra of promazine **3** (line a), adducts **1&3** (line b-7, c-6, d-5, e-4) and calixarene **1** (f)

complexes. On the other hand, for products **5**–**7** prepared with the higher quantity of promazine, the highest achieved stoichiometry was 1:5 ± 0.1, whereas for **2**, having a tendency to aggregation in aqueous solution, the stoichiometry of isolated complexes **9**–**11** was found to be 1:3 ± 0.5 ÷ 4.3 ± 0.2.

^{1}H NMR spectra of the complexes **1&3** recorded in $CDCl_3$ consisted of significantly broadened peaks of promazine compare to those in DMSO. The degree of broadened of peaks in spectra depends on the initial ratio of component used for reception of the complexes. The smaller of broadened of peaks is observed for a complex **7**. For complex **5** signals –N(CH_3)$_2$ and $(CH_3)_2N$–CH_2–CH_2 are merged in one peak with the half-height width greater than 300 Hz, whereas the signals of $(CH_3)_2N$–CH_2 are so broadened that the peaks are not observed at all.

Cooling sample down to 253 K (temperature was limited by solubility of the complexes in $CDCl_3$) resulted in the narrowing and even splitting of the peaks. Reheating of the samples back to 303 K resulted in a drastic change of the spectra of **5** it became like to the spectra of complex **7** (Fig. 3).

Moreover, spectra of all complexes in $CDCl_3$ after cyclic temperature change 303 K → 253 K → 303 K underwent the same transformation and the respective peaks of promazine narrowed down. The subspectrum of the macrocycle **1** remains about the same in all cases.

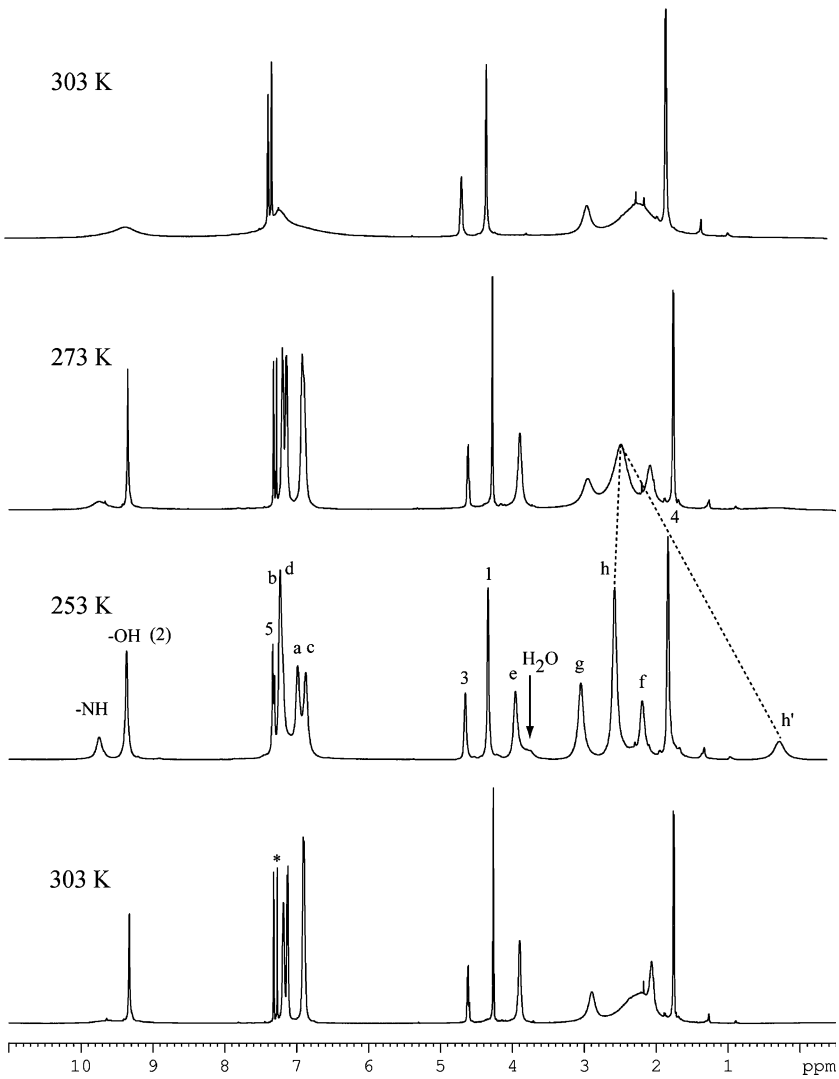

Fig. 3 Influence of cyclic temperature change 303 K → 253 K → 303 K on ^{1}H NMR spectra of complex **5** in the $CDCl_3$. The signal *h* at 253 K was split into *h* and *h′*. Signal of $CHCl_3$ is marked with*

It was reasonable to suggest, that presence of water can be responsible for the steady difference in the physical–chemical characteristics of the complexes of **1** obtained with various ratios of initial components. The water molecules are remained in the multiple ionic assembly in apolar $CDCl_3$. Unfortunately, precise determination of the amounts of water included in the complex is complicated. At 303 K water is involved in the fast exchange with the NH and OH groups and the signals are broadened. Nevertheless, the amount of water participating in the complexation can be estimated from the spectra recorded in deutero-chloroform at low temperature assuming as negligible the amount of the water in the solvent used for solution preparation because the solvent was the same (Table 1). It was reasonable to suggest that the narrowing of the signal of NMR spectra at cyclic temperature changes testifies to the changes in structure of the multiple ionic assembly of the complexes, and mainly of the complex **5**. Immediately after their dissolution in chloroform the complexes include promazine together with water molecules, which had been incorporated during preparation of complexes (Fig. 4).

On cooling, part of water is excluded from the coordination of the multiple ionic assembly. The excessive water is pushed out and left in the bulk solution after the following temperature increase. This suggestion also is supported by narrowing of the hydroxyl proton signals at cyclic temperature changes.

The high-field shift of promazine protons observed in ^{1}H NMR spectra of complexes (Table 2) in organic solvents supports the suggestion of inclusion complex formation. Splitting of the signal of $N(CH_3)_2$-group for **6** into two peaks at 2.489 and 0.175 ppm (intensities ratio ~4:1) at low temperatures in chloroform points on that peak at 0.175 ppm corresponds to **3** included into the cavity of macrocycle. The signals of other groups of aliphatic chains of phenothiazine fragment are splitted to a less extent. Changes in chemical shifts of **3** induced by aromatic

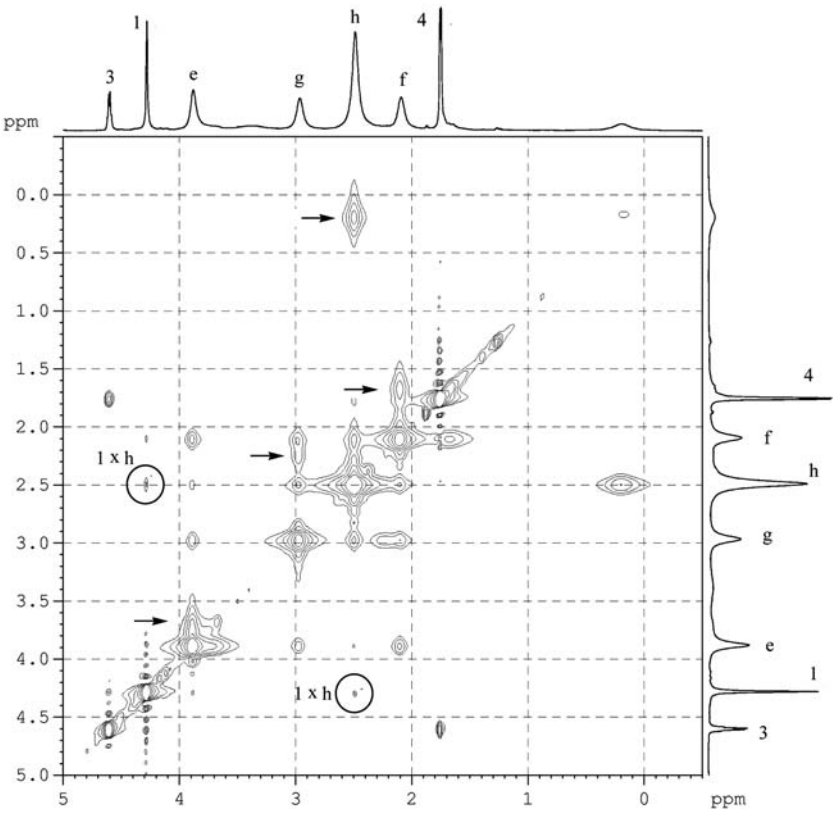

Fig. 4 The part of NOESY spectrum of **6** in $CDCl_3$ at 253 K, mixing time 125 ms. Arrows indicate the exchange cross-peaks between signals of the cavity- and upper-rim bound promazines; The $l \times h$ cross-peaks represent the NOE's between $-CH_2SO_3^-$ groups of macrocycle and $-N(CH_3)_2$ groups of promazine

Table 2 Complexation induced shifts in ^1H NMR spectra (303 K) protons $N(CH_3)_2$ group (*h*) of promazine in complexes **6** and **10** and the fraction of promazine bonded in the cavity (p_q)

	6 (**1**&**3**)			**10** (**2**&**3**)	
Solvent	DMSO	CD_3OD	$CDCl_3$	DMSO	$CDCl_3$
$\Delta\delta = \delta_{obs} - \delta_{free}$	–0.118	–0.416	–0.295, –2.47[a]	–0.234	–0.243, –2.34[a]
p_q	0.05	0.17	0.20	0.10	0.20

[a] The value of induced shift for up-field signal at 253 K

See also group h in Table 1A in Supporting information

currents of the macrocycle strongly depend on remoteness from $-N(CH_3)_2$ group: $(CH_3)_2N-CH_2-$ (0.75 ppm), $(CH_3)_2N-CH_2-CH_2-$ (0.40 ppm), $(CH_3)_2N-CH_2-CH_2-CH_2-$ (0.21 ppm). The exchanging sets these groups are identified by appearance of appropriate cross-peaks in NOESY spectra (Fig. 3). Since experiments were carried in different solvents and at different temperatures at the same complexes, it allowed unambiguously attribution of considered cross-peaks in NOESY spectra to exchanging cross-peaks between different binding sites of promazine. Strong dependence of the CISs of methylene protons of promazine aliphatic chain on their distance from $N(CH_3)_2$-group indicate deep immersion of the ammonia head into macrocycle cavity. Since CIS($\delta_{guest}-\delta_{free}$ = –2.47 ppm) of **3** included into the cavity are induced exclusively by aromatic currents of the macrocycles, which in turn are defined by its geometry and the nature of the aromatic substituents in the complexes, one can assume an independence of the latter on the solvent that causes CIS of **3** to be independent on the solvent as well. Fraction of the molecules included into the cavity, p_q, in this case can be estimate by Eq. (3) (Table 2).

It is worth noting that the NMR titration shifts are sensitive mainly to the inclusion-type complex formation.

However, it should be noted that in the examined complexes the molecules can be bound in a different manner - not forming an inclusion complex but, for example, via Coulomb interactions between negatively charged sulfonato-groups of the host and $HN^+(CH_3)_2$-group of promazine. Such interaction would not significantly affect chemical shifts of either compound in NMR spectra. Therefore, an additional NMR in impulse gradient magnet field was carried out in order to determine and compare self-diffusion coefficients of free and complexed hosts and **3** [9, 11, 12].

Assuming fast exchange on the NMR time scale, the observed (measured) diffusion coefficient (D_{obs}) is a weighted average of the free and bound diffusion coefficients (D_{free} and D_{compl}, respectively) and can, therefore, be used to calculate the bound fraction p_b, as shown in equation (2), in the same way that chemical shifts are used. The most important difference between the two methods is that in many cases a complete titration to find D_{compl} for the guest is not a necessity with the former method. This is true in cases where there is a large difference between the molecular weight of the host and the guest (usually the guest has a significantly lower molecular weight) and, hence, one can predict, a priori, that the D_{compl} value of the complex will be very similar to the diffusion coefficient value of the much larger host. The following distinction corresponds to the difference of D_{obs} in the complex where macrocycle-promazine association is realized as both, electrostatic, cation-π interactions, and hydrogen bonding [9, 11, 12].

Self-diffusion coefficients of the complexed (D_{compl}) and free **1**, **2**, and **3** (D_{free}) in the different solvents were obtained by FT-PFG NMR (Table 4). Since macrocycles incline to self-association and the viscosities of the used solvents are significantly different this inevitably affects the self-diffusion coefficients. For this reason, we additionally calculated the hydrodynamic radii by Einstein–Stokes equation (1). Obtained self-diffusion coefficients, the hydrodynamic radii of free and associated macrocycles (R_h^{free} and R_h^{compl}) as well as the fractions of bound **3** p_b (See Experimental part) see in Table 3.

Resorcinarene **1** forms dimer in aqueous solutions at concentrations higher than 2×10^{-1} M [16]. Preparation of the complexes **1&3** and the following study were carried out at 0.1÷10 mM solutions, where self-diffusion coefficient of **1** remains unchanged ($D_s = 3.5 \times 10^{-10}$ m^2/s) within the experimental error. And more, the hydrodynamic radius R_h (7.91 Å), calculated from self-diffusion coefficients at those concentrations agrees with theoretically hydrodynamic radius of monomers (R_h^{theor} = 7.44 Å) calculated on the bead model approximation [17]. Therefore calixarene **1** is non-aggregated in the used water conditions.The hydrodynamic radius of resorcinarene **1** in complexes **1&3** was not very different from that of free macrocycle indicating that in the examined solution the complexes are not aggregated.

Resorcinarene **2** is indeed aggregated in its aqueous solutions in the concentration range used for preparation of the complexes **2&3**. However, in the course of the study of complex **10** in organic solvents, namely DMSO and $CDCl_3$, it was found that the hydrodynamic radius of **2** is close to that of its monomer 8.5 Å (R_h^{theor} is 8.39Å) obtained in 10^{-4} M solutions, where **2** was found to exist in a monomeric form. Therefore, it was concluded that **2&3** complexes, unlike initial **2**, in examined solutions exist in monomeric form.

The increase of the calculated hydrodynamic radius of the macrocycle in the chloroform solution of the complex **6** (10.00 Å) in comparison with individual value of **1** in water (7.91 Å) results from rather low dissociation of the complexes in this solvent (Table 1).

Resorcinarene **2** is indeed aggregated in its aqueous solutions in the concentration range used for preparation of the complexes **2&3**. However, in the course of the study of complex **10** in organic solvents, namely DMSO and $CDCl_3$, it was found that the hydrodynamic radius of **2** is close to that of its monomer 8.5 Å (R_h^{theor} is 8.39Å) obtained in 10^{-4} M solutions, where **2** was found to exist in a monomeric form. Therefore, it was concluded that **2&3** complexes, unlike initial **2**, in examined solutions exist in monomeric form.

Table 3 Self-diffusion coefficients D ($\times 10^{-10}$ m^2 s^{-1}), hydrodynamic radii R_h (Å), and the fraction of bound promazine (p_{bD}) from diffusion data for complexes **6** and **10**

	Solvent	**6** (**1**&**3**)				**10** (**2**&**3**)		
		H_2O	DMSO	CD_3OD	$CDCl_3$	H_2O	DMSO	$CDCl_3$
Macrocycles	$D_{1,2}^{free}$ [a]	3.50	1.40	[b]	[b]	1.30	1.37	[b]
	$D_{1,2}^{compl}$		1.40	5.70	4.40		1.40	3.40
	R_h^{free} [c]	7.91	9.25			21.0	9.54	
	R_h^{compl} [c]		9.53	7.85	10.00		9.50	13.00
Promazine	D_3^{free} [d]	5.80	2.90	11.20	12.70	5.68	2.94	12.92
	D_3^{compl}		2.30	7.7	4.60		2.33	4.26
	p_b^e		0.39	0.63	0.98		0.38	0.91

[a] Data obtained for Na^+ salts of **1** and **2**

[b] **1** and **2** are insoluble in these solvents

[c] Hydrodynamic radius of the macrocycles (Å) calculated according to Eq. 1

[d] Data obtained with promazine hydrochloride **3**

[e] Fraction of bound promazine calculated according to Eq. 3

Discussion

In order to obtain a complete image from the results of extensive studies of the complexes **1&3** and **2&3**, the stoichiometry, driving forces of precipitation, and driving forces of the complexation have to be considered along with the role of water molecules included into the complexes and distribution of promazine between two kinds of the binding sites of **1** and **2**.

Complexes **4** and **8** were prepared with the idea of 1:1 inclusion complexes in mind. According to ^{1}H NMR data, they, however, turned out to be 1:4 ± 0.1 (**4**), and 1:1.5 ± 0.5 (**8**) (Table 1).

The raise of promazine concentration vs. macrocycle increased the yield of the complexes and resulted in the complexes of reproducible stoichiometry 1:5 ± 0.1 and 1:4.3 ± 0.2. That in turn corresponds to successful competition of **3** with water molecules for binding sites of the macrocycles (see below).

Apparently, *the driving force of the complexes precipitation* is participation of SO_3^- groups of the macrocycles in binding with $NH(CH_3)_2^+$ groups of promazine suppressing partly their solvation by water and self-assembling of macrocycle **2**.

$((CH_3)_2N–H^+)$ band. Inclusion of promazine into aromatic cavity, resulting in up-field CIS of $–CH_2–CH_2–CH_2–N(CH_3)_2$ proton signals, also increased hydrophobic surface of the complexes leading to their instant precipitation (Fig. 5)

Mixing of substoicheometric ratios of **1** or **2** and **3** resulted in formation of crystallohydrates that were transformed into ionic complexes when H:G ratio approached stoichiometric one. For **9**,**10** (**2&3)** complexes, where host is known to form aggregates in aqueous solutions, formation of ionic complexes disturbs their self-aggregation. Mixing of substoichiometric 1:1 ratios of initial reagents **2** and **3** yields complexes containing Na^+ (complex **8, 9**).

The *driving forces of promazine coordination* rely on Coulomb interactions with sulfonato-groups on the upper rim of the macrocycle and cation-π interactions with the aromatic cavities of **1** and **2**. Existence of cation-π interactions (inclusion in cavity) were proved by CIS of the signals of promazine hydrogen atoms of $–CH_2–CH_2–CH_2–N(CH_3)_2$ group in both DMSO and $CDCl_3$ (Table 2). Existence of two binding modes of **3** by **1** and **2**, namely by cavity and by its upper rim (outer-sphere coordination), was proved by means Fourier-transform

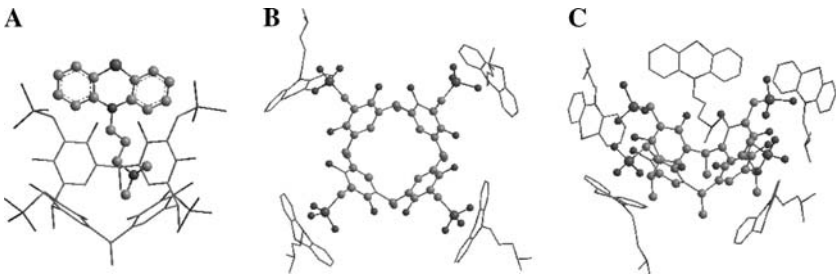

Fig. 5 Models of associates: A-inclusion into the cavity, B- and C-binding by the upper rim

Corresponding interactions appear on the one hand as shifts of SO_3^- bands in IR-spectra of the complexes and on the other hand as a complication of the contour of the *v* pulsed field-gradients (FT-PGSE) NMR and respective calculations (Tables 3 and 4). At room temperature outer-sphere coordinated molecules of promazine underwent

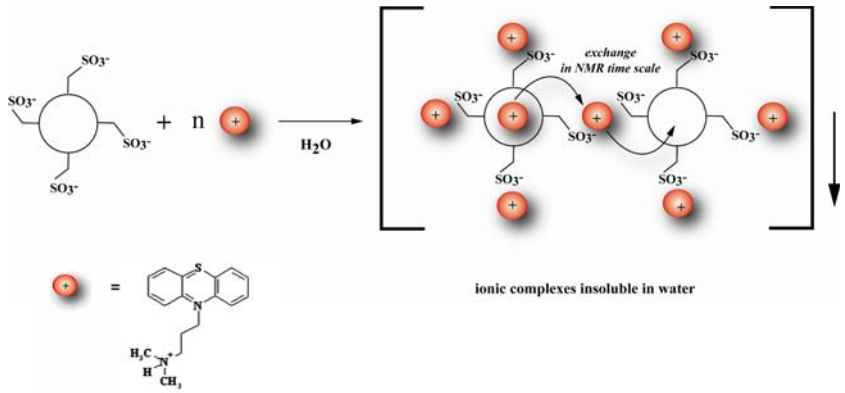

Table 4 Distribution of the bound promazine among the binding sites of **1** and **2** in different organic solvents

Solvent	**6** (**1&3**)			**10** (**2&3**)	
	DMSO	CD_3OD	$CDCl_3$	DMSO	$CDCl_3$
Total binding fraction, p_b	0.39	0.63	0.98	0.38	0.91
In the cavity, p_q	0.05	0.17	0.20	0.10	0.20
On the rim, $(p_b - p_q)$	0.34	0.46	0.78	0.22	0.71

an exchange with those coordinated by cavity in NMR time scale.

The role of water molecules isolated with the products is represented by two major functions—stabilization of the macrocycle conformation (hydrogen bond seam [18]) and participation in exchange interactions providing coordination of **3** by resorcinarenes. Water content found in the isolated complexes and respective melting points were reproducible through repetitive preparation of the complexes **1&3** and **2&3** indicating its involvement in coordination of **3** (Table 1). Narrowing of ^{1}H NMR peaks NH- and OH-groups of **1&3** in $CDCl_3$ after exposure to low temperature suggests partial release of coordinated water.

Decrease of the fraction of bound promazine, p_b, in the CD_3OD and DMSO reflected competition between the organic solvents and **3** for the binding sites of **1** and **2**. This is in a good agreement with the increase of polarity of the solvents in a row $CDCl_3$–CD_3OD–DMSO. More detailed consideration of this competition is given in Table 4 illustrating distribution of the bound promazine between the binding sites of the macrocycles. Decrease of the fraction of the inclusion binding mode correlates with the ability of the solvent to participate in CH-π -interactions with the aromatic cavity.

Conclusions

Since the conditions of the complex preparation were substoichiometric, nearly instant precipitation of the complexes points out on domination of the driving forces of precipitation, namely, development of the hydrophobic surface of the complexes that preferably leads to formation of the precipitate of complexes network, where neighbor resorcinarenes can connect via promazines coordinated by their upper rims. Existence of two binding modes of **3** by **1** and **2**, namely by cavity and by its upper rim (outer-sphere coordination). Interaction of **2** is accompanied by disturbing of macrocycles self-aggregation. Decrease of the melting points of the obtained complexes **1&3** and **2&3** are accompanied by increase of the amount of promazine used for preparation of the ionic complexes and water content for **1&3** (ionic complexes are melting at lower temperatures than corresponding crystallohydrates).

Acknowledgments We thank Collaboration linkage grant of NATO (PCT.CLG.979178) and Russian Foundation of Basic Research for financial support of this work (RFBR 06-03-32189a and 05-03-32558-a)). We are also grateful to Prof. Wolf D. Habicher and Mrs. Margaret Grüner from TU Dresden for active support of the experimental part of the present research.

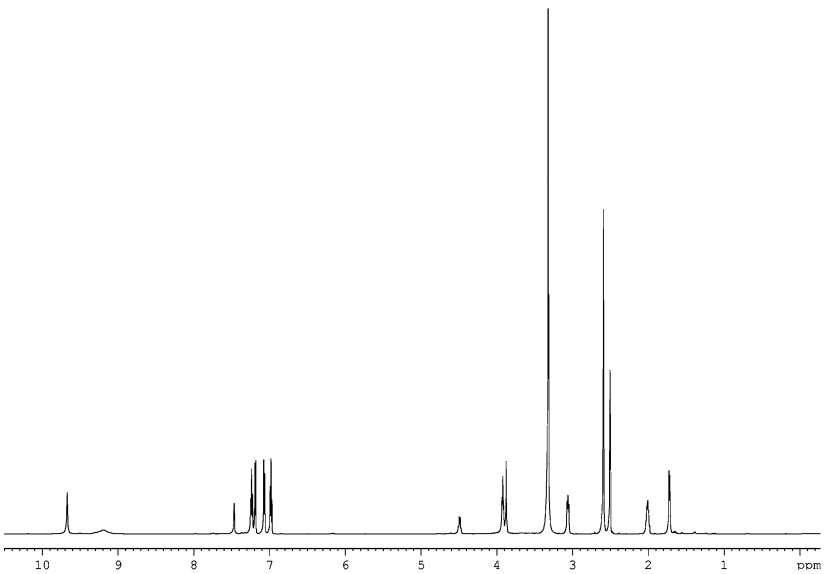

Fig. A1 ^{1}H NMR spectrum (600 MHz, 30 °C) of **6,** 10 mM in DMSO-d6

Appendix

Table A1 Chemical shifts δ(ppm) of protons of free and complexed **3**

Solvent	Groups							
	a	*b*	*c*	*D*	*e*	*f*	*g*	*h*
				3				
H_2O	7.03	7.27	7.03	7.23	4.02	2.13	3.16	2.69
DMSO	7.08	7.29	6.98	7.19	3.96	2.06	3.14	2.71
CD_3OH	7.07	7.21	6.99	7.19	4.11	2.21	3.12	2.79
$CDCl_3$	7.00	7.24	6.92	7.20	4.10	2.41	3.12	2.64
				6 **(*1*&*3* (*1:4*))**				
DMSO	7.07	7.24	6.97	7.18	3.92	2.02	3.06	2.59
CD_3OH	7.02	7.23	6.95	7.15	3.98	2.06	2.99	2.38
$CDCl_3$	6.94	7.19	6.91	7.16	3.89	2.09	2.96	2.34
				10 **(*2*&*3* (*1:4*))**				
DMSO	7.06	7.16	6.96	7.22	3.89	1.98	2.98	2.48
$CDCl_3$	9.96	7.19	6.89	7.13	4.27	2.09	2.93	2.4

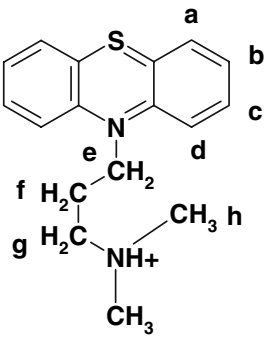

Table A2 Chemical shifts δ(ppm) of protons of macrocycles **1** and **2** and their complexes

Solvent	Ph	CH	CH_2–SO_3	CH_3
		6 (**1**&**3** (1:4))		
H_2O[a]	6.82	4.50	4.16	1.46
DMSO	7.47	4.50	3.88	1.72
CD_3OD	7.44	4.65	4.19	1.35
$CDCl_3$	7.33	4.63	4.27	1.77
		10 (**2**&**3**(1:4))		
H_2O[a]	6.87	4.31	4.26	0.70
DMSO	7.37	4.24	3.89	0.87
$CDCl_3$	7.32	4.35	4.32	0.96

[a] Sodium salts of macrocycles of **1** and **2**

Table A3 ^{13}C NMR-spectra of **1** and **3** and their complexes **4–7** in DMSO-d_6, δ, ppm

N	1	3	4	5	6	7
$C^{1,5}$	125.9		127.7	127.8	127.8	127.8
$C^{2,4}$	149.7		149.7	149.8	149.8	149.8
C^{3}	109.2		109.1	109.2	109.2	109.2
C^{6}	123.0		125.8	125.9	125.9	125.9
C^{7}	29.6 d		28.5	28.6	28.6	28.6
C^{8}	20.3		20.2	20.3	20.3	20.3
C^{9}	48.1		48.0	48.1	48.2	48.1
$C^{10,21}$		144.2	144.4	144.5	144.5	144.5
$C^{11,20}$		115.8	115.9	116.0	116.0	116.0
$C^{12,19}$		127.5	127.7	127.8	127.8	127.8
$C^{13,18}$		122.5	122.8	122.9	122.9	122.9
$C^{14,17}$		127.0	127.2	127.3	127.4	127.4
$C^{15,16}$		123.6	123.9	124.0	124.2	124.2
C^{22}		43.7	43.6	43.6	43.6	43.6
C^{23}		21.2	21.6	21.7	21.7	21.7
C^{24}		53.9	54.5	54.6	54.6	54.6
C^{25}		41.7	42.2	42.4	42.4	42.4

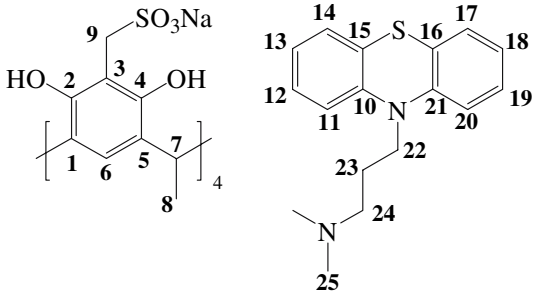

Table A4 ^{13}C NMR-spectra of **2**, **3,** and complexes **8–10** in DMSO-d_6 δ, ppm

N	2	3	8	9	10
$C^{1,5}$	127.3		124.7	123.7	124.7
$C^{2,4}$	152.1		149.9	149.9	149.9
C^{3}	110.3		108.9	108.9	108.9
C^{6}	125.1		122.9	122.7	122.9
C^{7}	36.4		34.1	34.1	34.1
C^{8}	15.4		13.9	13.9	13.9
C^{8a}	28.9		27.7	27.7	27.7
C^{8b}	31.3		31.5	31.5	31.5
C^{8c}	33.7		33.0	32.9	32.9
C^{8d}	23.9		22.1	22.1	22.1
C^{9}	49.1		48.1	48.1	47.9
$C^{10,21}$		144.3	144.3	144.3	144.3
$C^{11,20}$		115.8	115.8	115.8	115.8
$C^{12,19}$		127.5	127.6	127.6	127.6
$C^{13,18}$		122.6	122.6	122.6	122.6
$C^{14,17}$		127.0	127.1	127.1	127.1

Table A4 continued

N	2	3	8	9	10
$C^{15,16}$		123.6	123.7	123.7	123.7
C^{22}		43.7	43.5	43.5	43.5
C^{23}		21.2	21.6	21.4	21.6
C^{24}		53.9	54.4	54.3	54.4
C^{25}		41.7	42.2	42.0	42.2

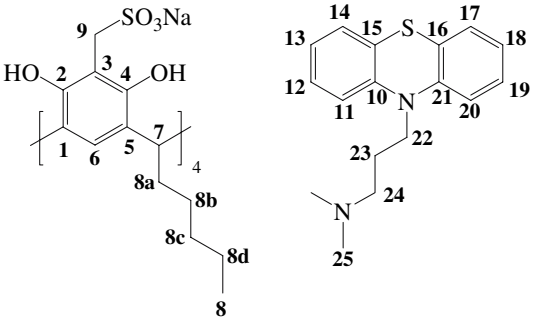

Table A5 Solubility[a] and melting point (°C) of initial compounds **1** and **3** and their complexes (**1**&**3**)

Solvents	1	3	4	5	6
Benzene	n[b]	n	n	n	n
Toluene	n	n	n	n	n
Chloroform	n	s	n	s	s
Methylene chloride	n	s	n	n	n
Acetone	n	s	n	s(t°)	s(t°)
1,4-Dioxan	n	s	s	s	s
THF	n	s	s	s	s
Methanol	n	s	s	s	s
Ethanol	n	s	n	s	s
Acetonitrile	n	s	s(t°)	s(t°)	s
DMSO	s	s	s	s	s
DMF	s	s	s	s	s
Water	s	s	n	n	n

[a] The amount of the compounds taken for solubilization corresponded to final 3% solution

[b] n-insoluble; s-soluble; s(t°)-soluble at heating

Table A6 IR-spectra of **1, 2** and **3** and their complexes **1**&**3** (**4–7**) and **2**&**3** (**8–10**) (KBr)

N	1	3	4–7	2	8–10
$\nu_{SO_3^-}$	1219	–	1227	1198	1229–1215
$\nu_{SO_3^-}$	1150	–	1142	1151	1148–1142
$\nu_{SO_3^-}$	1044	–	1035	1046	1045–1031
ν_{NH^+}	–	2409	2730-2500		2730–2400
ν_{OH}	3300	–	3450-3400	3395	3450–3400

Table A7 UV-spectra of **1, 2, 3** and their complexes **1**&**3** (**4–7**) and **2**&**3** (**8–10**) (0.1 mmol l^{-1}, MeOH) (λ, nm; log ε)

N	1&3						2&3			
	1	3	4	5	6	7	2	3	8	9	10
1	211; 4.85	206; 4.39	206; 4.76	206; 4.96	206; 5.14	207; 5.24	208; 4.70	206; 4.39	208; 5.02	209; 5.18	208; 5.37
2	238 *sh.*; 4.29	238 *sh.*; 4.23	–	–	–	–	238 *sh.*; 4.19	238 *sh.*; 4.23	–	–	–
3	–	253; 4.522	253; 4.56	253; 4.86	253; 5.02	253; 5.17[a]	–	253; 4.522	254; 4.65	254; 5.15	254; 5.34
4	287; 4.07	–	289; 3.93	288; 4.14	289; 4.28	288; 4.44[a]	287; 3.93	–	289; 4.29	290; 4.47	291; 4.61
5	297 *i.*; 3.77	–	297 *sh.*; 3.86	297 *sh.*; 4.11	296 *sh.*; 4.24	299 *sh.*; 4.40[a]	296 *i.*; 3.75	–	299 *sh.*; 4.16	298 *sh.*; 4.41	297 *sh.*; 4.59
6	–	303; 3.63	315 *sh.*; 3.65	316 *sh.*; 3.90	316 *sh.*; 4.02	315 *sh.*; 4.20[a]	–	303; 3.63	333 *sh.*; 3.63	321 *sh.*; 4.17	321 *sh.*; 4.35

sh.-shoulder; *i.*-inflexion;

[a] average value from three measurements

Table A8 Stoichiometry of ionic complexes of the macrocycle **2** (**2**&**3**) determined from ^{1}H NMR spectra in DMSO (30 °C)

Initial stoichiometry of mixing	**8** (1:1)	**9** (1:2)	**10** (1:4)	**11** (1:5)
Molecules of 3 per macrocycle	1.5 ± 0.5	3 ± 0.5	4 ± 0.2	4.3 ± 0.2

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14. Position of $\nu_{SO_3^-}$ bands changes from 1219, 1150, and 1044 cm^{-1} in spectrum of **1** to 1227, 1142, and 1035 cm^{-1} in complexes **1**&**3**. The absorbance of $\nu_{(CH_3)_2NH^+}$ observed in the spectrum of **3** as a wide band well structured in the high frequency part and the maximum at 2409 cm^{-1} is converted into the complex absorbance outline at 2730–2500 cm^{-1}. The intensity of ν_{OH} band at 3300 cm^{-1} attributed to atmospheric water absorbed by macrocycle **1** is somewhat decreased for complexes **1&3**.
15. Intensity and position of the characteristic band of resorcinarene, 287 nm, remains unaffected by complexation, while absorbance band of phenotiazine at 303 nm in complexes **1&3** and **2&3** undergoes batochromic shift of 10 nm.
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