The use of a lyotropic liquid-crystalline medium and residual dipolar coupling constants for determination of the spatial structure of thiacalix[4]arenes in solutions*

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The possibility of using an approach for the elucidation of the spatial structure of functionalized thiacalix[4]arenes based on the determination the residual dipolar coupling constants between the ^{1}H and ^{13}C nuclei separated by one chemical bond ($^1D_{CH}$) in lyotropic liquid-crystalline media (poly-γ-benzyl-L-glutamate and $CDCl_3$) is demonstrated for the first time. This approach was used to distinguish between the *cone* and *1,3-alternate* conformations of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(2-oxopropyloxy)-2,8,14,20-tetrathiacalix[4]arene. The results were confirmed by the data from 2D NMR (^{1}H—^{1}H) NOESY experiments for these compounds in an isotropic solvent ($CDCl_3$).

Key words: lyotropic liquid-crystalline media, residual dipolar coupling constants, thiacalix[4]arene, conformations, NMR.

Structural studies of relatively small organic molecules in solutions are based traditionally on both the data from conventional one-dimensional NMR spectroscopy and the use of advanced NMR techniques such as dynamic NMR [1,2] and 2D NMR.[3,4] The 2D NMR NOESY technique is suitable for determination of the Overhauser effect of magnetic nuclei separated by distances up to 5 Å and is used to elucidate the spatial structures of organic compounds in solutions. The increase in the number of atoms in molecules may preclude the use of NMR data for an adequate description of their conformations.

In this communication, we demonstrate for the first time the possibility of determination the spatial structures of thiacalix[4]arene derivatives based on the analysis of the dipolar coupling constants between the magnetic nuclei separated by one chemical bond (^1D) in anisotropic media. Since recently, lyotropic liquid-crystalline media have been actively used in NMR studies of biochemical subjects that comply with the slow motion condition ($\omega_0 \cdot \tau_c \gg 1$, τ_c is the correlation time, ω_0 is the angular velocity of the precession of magnetic nuclei).[5,6] A few examples of using this approach for determination of the conformations of organic molecules ($\omega_0 \cdot \tau_c \ll 1$) have been reported.[7,8] This approach appears more significant in view of the fact that the application of 2D NMR NOESY spectroscopy to relatively small molecules is not always efficient.[3,4] This is due to short correlation times τ_c of these molecules in solution and to fulfillment of the condition of the limiting case of fast motion, resulting in low-intensity cross-peaks in the NMR NOESY spectra.

Experimental

The ^{1}H (300 MHz) and ^{13}C (75.43 MHz) spectra of calixarene stereoisomers **1a** and **1b** were recorded in an isotropic solvent and a lyotropic liquid-crystalline medium using a Varian Unity-300 NMR spectrometer operating in the internal stabilization mode at the ^{2}H resonance line. The ^{1}H NMR spectra were measured using 10—15° pulses and delays between the pulses DL = 1—2 s; the spectrum width was SW = 15 ppm; and the accumulation number NT was 10 to 100. The ^{13}C NMR spectra were recorded using 20—30° pulses, broadband proton decoupling, and digital exponential filtration with LB = 2—4 Hz (delays between the pulses DL = 1—2 s; SW = 200 ppm; NT, from 400 to 1000). In performing 2D NMR experiments (NOESY modification), the delay between pulse sequences was three times as long as the average longitudinal relaxation time T_1

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for the protons of stereoisomers **1a** and **1b**. The spectra were recorded using a phase-sensitive procedure for 1024 points of the F2-coordinate and 256 points of the F1-coordinate; exponential filtration along both coordinates was used. The mixing time parameter τ_m was chosen to be 0.2, 0.4, 0.6, and 0.8 s. The ^{1}H NMR and ^{13}C NMR spectra were recorded for 5—10% (w/w) solutions of compounds in the corresponding solvents using Me_4Si as the internal standard. The accuracy of determination of chemical shifts was 0.01 ppm, while that for spin-spin coupling constants was 0.5 Hz.

The dipolar coupling constants between the magnetic nuclei in stereoisomers **1a** and **1b** were determined using a solution of poly-γ-benzyl-L-glutamate (with an average number of monomeric fragments *n* of 562, Sigma) in $CDCl_3$ (13.2% w/w). The liquid-crystalline medium (poly-γ-benzyl-L-glutamate/$CDCl_3$) was prepared as described previously.[7] The existence of an ordered lamellar liquid-crystalline L_α-phase was confirmed by observing the quadrupole ^{2}H NMR splitting of a signal for chloroform $CDCl_3$ present as a part of the liquid-crystalline system.[6]

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrakis(2-oxopropyloxy)-2,8,14,20-tetrathiacalix[4]arene (1a) (*cone*). Bromoacetone (0.7 mL, 8.33 mmol) was added with vigorous stirring to a mixture of *p-tert*-butylthiacalix[4]arene[9] (1 g, 1.38 mmol) and anhydrous Na_2CO_3 (0.88 g, 8.33 mmol) in 50 mL of MeCN. The reaction mixture was refluxed with stirring for 12 h under argon. The solvent was removed under reduced pressure. 1 *M* HCl (20 mL) and $CHCl_3$ (30 mL) were added to the residue and the product was extracted for 30 min. The organic phase was separated and dried with $MgSO_4$ and the solvent was evaporated. The residue was recrystallized from 95% EtOH to give a colorless solid in a yield of 0.28 g (20%), m.p. 235—236 °C. ^{1}H NMR ($CDCl_3$), δ: 1.09 (s, 36 H, $C(CH_3)_3$); 2.36 (s, 12 H, CH_3); 5.10 (s, 8 H, OCH_2); 7.29 (s, 8 H, ArH). Found (%): C, 66.41; H, 6.98; S, 13.56. $C_{52}H_{64}O_8S_4$. Calculated (%): C, 66.07; H, 6.82; S, 13.57. MS (MALDI-TOF): 945.2 $[MH]^+$.

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrakis(2-oxopropyloxy-2,8,14,20-tetrathiacalix[4]arene (1b) (*1,3-alternate*). Bromoacetone (0.7 mL, 8.33 mmol) was added with vigorous stirring to a mixture of *p-tert*-butylthiacalix[4]arene[9] (1 g, 1.38 mmol) and anhydrous K_2CO_3 (1.15 g, 8.33 mmol) in 50 mL of MeCN. The reaction mixture was refluxed with stirring for 14 h under argon. Then the solvent was removed under reduced pressure. 1 *M* HCl (20 mL) and $CHCl_3$ (30 mL) were added to the residue and the product was extracted for 30 min. The organic phase was separated and dried with $MgSO_4$, and the solvent was evaporated. The dry residue was recrystallized from a CH_2Cl_2—95% EtOH mixture to give a colorless crystalline solidin a yield of 0.81 g (68%), m.p. 314—315 °C (*cf.* Ref. 10: m.p. 314—315 °C). ^{1}H NMR ($CDCl_3$), δ: 1.22 (s, 36 H, $C(CH_3)_3$); 1.50 (s, 12 H, CH_3); 4.47 (s, 8 H, OCH_2); 7.34 (s, 8 H, ArH). ^{1}H—^{1}H NOESY (cross-peaks): 4b/8>8/3, 4b/3, 4b/6, 8/6, 6/3. Found (%): C, 66.31; H, 6.78; S, 13.46. $C_{52}H_{64}O_8S_4$. Calculated (%): C, 66.07; H, 6.82; S, 13.57. MS (MALDI-TOF): 945.7 $[MH]^+$.

Results and Discussion

Dipolar coupling between magnetic nuclei within the molecule in solutions is known to be fully averaged due to the chaotic motion of molecules. If the molecular system occurs in a lyotropic liquid-crystalline medium, the translational and rotational motions of molecules are no longer isotropic due to collisions with magnetically oriented molecular structures.[5] This anisotropy in the molecular motion gives rise to weak dipolar couplings between the magnetic nuclei, which is manifested in the NMR spectra as the residual dipolar coupling constants. No broadening of the NMR signals is observed.[5,6]

In this case, the measured dipolar coupling constant D_{IJ} (θ,φ) between two magnetic nuclei I and J can be represented as follows:[6]

$$D_{IJ}(\theta,\varphi) = D_a^{IJ}\{(3\cos^2\theta - 1) + 3/2\, R\sin^2\theta\cos 2\varphi\}, \quad (1)$$

where

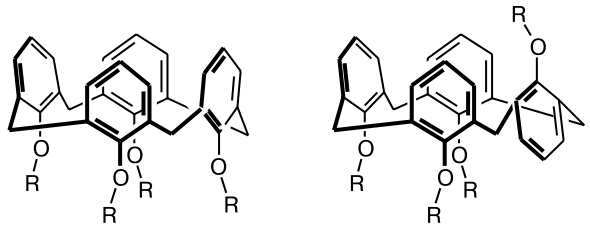
$$D_a^{IJ} = -(\mu_0 h/16\pi^3)\, S\, \gamma_I\gamma_J \{r_{IJ}^{-3}\}\, A_a. \quad (2)$$

Here $A_a = A_{zz} - (A_{xx} + A_{yy})/2$ is the axial component of the molecule tensor **A**, which describes the predominant spatial arrangement of the molecule relative to the external magnetic field; $R = (A_{xx} - A_{yy})/A_{zz}$ is the rhombic component; A_{xx}, A_{yy}, and A_{zz} are the projections of the molecular tensor **A** on the *x*, *y*, and *z* axes of the orthogonal coordinate frame related to the molecule; θ and φ are the polar coordinates of the internuclear vector (for the nuclei I and J) connecting the principal axes of the molecular tensor and the direction of the external magnetic field; S is the order parameter reflecting the internal dynamic mobility of the internuclear vector; γ_I and γ_J are the gyromagnetic ratios for the nuclei I and J; and r_{IJ} is the distance between the magnetic nuclei. It follows from relations (1) and (2) that the observed dipolar coupling constants depend on both the properties of the medium (the components of the lyotropic system and their concentrations) and the relative positions of the magnetic nuclei in question in the magnetic field.

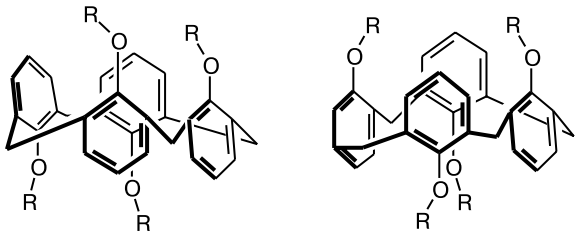
p-tert-Butylthiacalix[4]arene, first prepared by a one-step synthesis in the late 1990s, has become a new subject in the chemistry of macrocyclic compounds.[9] The replacement of the methylene bridges by sulfur atoms gives rise to new features in the chemical behavior and complexing ability of calix[4]arenes. However, this markedly complicates the problem of determination of the spatial structure of thiacalix[4]arene, due to the absence of bridging methylene groups, which allow reliable determination of the conformations of classical calixarenes by 1D NMR spectroscopy. Therefore, the solution of this problem often requires resorting to 2D NMR spectroscopy.[11]

It is known[12—14] that the conformational behavior of calix[4]arenes is discussed in terms of four idealized conformations: *cone*, *partial cone*, *1,3-alternate*, and *1,2-alternate*.

On the basis of ^{1}H and ^{13}C NMR spectroscopy of calix[4]arenes, the *partial cone* and *1,2-alternate* conformations are identified most readily. In both cases, due to



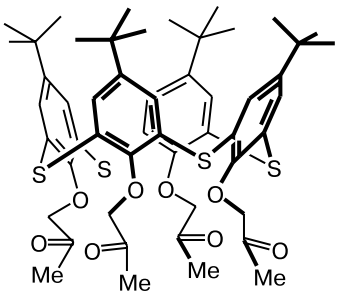
Cone *Partial cone*



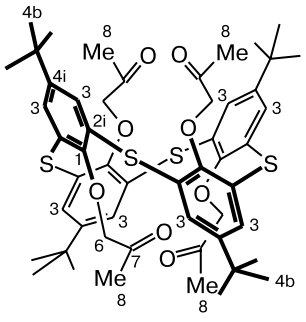
1,2-Alternate *1,3-Alternate*

the different symmetry of the conformations, the spectra exhibit characteristic signal multiplicities and integral intensities for both protons and carbon atoms of the thiacalix[4]arene core[9]. Identification of the *cone* and *1,3-alternate* conformations is the most difficult, because their NMR spectra are very simple and identical as regards the type of signals due to the symmetrical structures of these molecules.

For testing the approach for determination of the conformations of thiacalix[4]arenes based on analysis of dipolar coupling constants, we chose two stereoisomers, namely, **1a** (*cone*) and **1b** (*1,3-alternate*) as the subjects.



1a



1b

In order to prepare stereoisomers **1a** and **1b** in a pure state, we took advantage of the template effect of the K^+ and Na^+ cations in the alkylation of *p-tert*-butylthiacalix[4]arene with bromoacetone.[15] This gave the *cone* (**1a**) and *1,3-alternate* (**1b**) conformers in 20% and 68% yields, respectively. The ^1H and ^{13}C NMR chemical shifts of the stereoisomers are given in Tables 1 and 2. As noted above, the data from the ^1H and ^{13}C NMR spectra do not allow one to choose a particular conformation, either *cone* or *1,3-alternate*, for stereoisomers (**1a**) and (**1b**).

We determined the spatial structures of stereoisomers **1a** and **1b** using an approach based on comparison of the dipolar coupling constants between the ^{13}C and ^1H nuclei separated by one chemical bond in an isotropic solvent ($CDCl_3$) and in a lyotropic medium (poly-γ-benzyl-L-glutamate in $CDCl_3$). The carbon chemical shifts (δ_C) and direct spin-spin coupling constants ($^1J_{CH}$) for both stereoisomers obtained by ^{13}C NMR spectroscopy without radiofrequency proton decoupling are listed in Table 2. The invariability of carbon chemical shifts (δ_C) for each stereoisomer on passing from the isotropic solvent to the lyotropic liquid-crystalline medium (LLCM) suggests that the spatial structures of these compounds are identical in these two media. The dipolar coupling constants ($^1D_{CH}$)

Table 1. ^1H NMR chemical shifts (δ_H, relative to Me_4Si) of thiacalix[4]arene stereoisomers **1a** and **1b** in $CDCl_3$

Stereoisomer	CH	CH_2	CH_3	CMe_3
1a	7.29	5.10	1.55	1.08
1b	7.34	4.47	1.55	1.22

Table 2. Data on the ^{13}C NMR spectra relative to Me_4Si and spin-spin coupling constants for thiacalix[4]arene stereoisomers **1a** and **1b** in the isotropic solvent and in the lyotropic liquid-crystalline medium

Stereo-isomer	Solvent	δ (^1J/Hz)			
		CH	CH_2	CH_3	CMe_3
1a	$CDCl_3$	135.2 (159.5)	79.5 (145.0)	26.6 (127.9)	31.8 (125.7)
	LLCM	135.2 (170.0)	79.6 (165.0; 168.0)	26.8 (96.0)	32.0 (117.5)
1b	$CDCl_3$	130.0 (160.0)	74.4 (146.2)	27.4 (128.5)	31.7 (125.6)
	LLCM	130.5 (217.4)	74.4 (257.0; 256.0)	28.0 (90.6)	32.1 (112.7)

determined from the difference between the observed spin-spin coupling constants ($^1J_{CH} + {}^1D_{CH}$) for the magnetic nuclei of the molecules dissolved in the LLCM and in the isotropic solvent ($^1J_{CH}$)[6] were as follows:

Assign-ment	$^1D_{CH}$	$^1D_{CH_2(\alpha)}$	$^1D_{CH_2(\beta)}$	$^1D_{Me}$	$^1D_{CMe_3}$
			Hz		
1a	+10.5	+20.0	+23.0	−31.9	−8.2
1b	+57.4	+110.8	+109.8	−38.0	−12.9

The resulting dipolar coupling constants ($^1D_{CH}$) were analyzed using the PALES software.[16] Using expression (1), this program relates the values of the observed constants to the arrangement of the internuclear vector (the C—H chemical bond) with respect to the magnetic field within the framework of the known conformation of the molecule under study. A linear correlation between the observed and calculated (given the specified spatial structure) dipolar coupling constants serves as the criterion for the correspondence of the calculated structure to the real one.

As the input data for the PALES program, we used the atomic coordinates of the conformers **1a** and **1b** calculated by the MOPAC 93 program (PM3 method). Analysis of the experimental dipolar coupling constants showed the total lack of correlation between the observed and calculated constants in the case where conformation *1,3-alternate* was ascribed to stereoisomer **1a** and the *cone* conformation was attributed to stereoisomer **1b** (the root-mean-square deviation from the linearity was $2.7 \cdot 10^2$ or higher). The change in the input data attained by changing the starting conformations gave the only possible match: the *cone* conformation for stereoisomer **1a** and the *1,3-alternate* conformation for stereoisomer **1b**. Only in this case, was the full compliance between the observed and calculated dipolar coupling constants observed (Fig. 1 and 2).

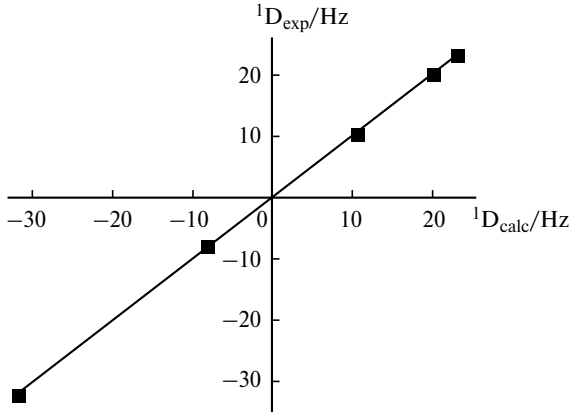


Fig. 1. Relationship between the observed dipolar coupling constants ($^1D_{CH}$) for stereoisomer **1a** dissolved in the lyotropic liquid-crystalline medium and the calculated values of these constants for stereoisomer **1a** in the *cone* conformation.

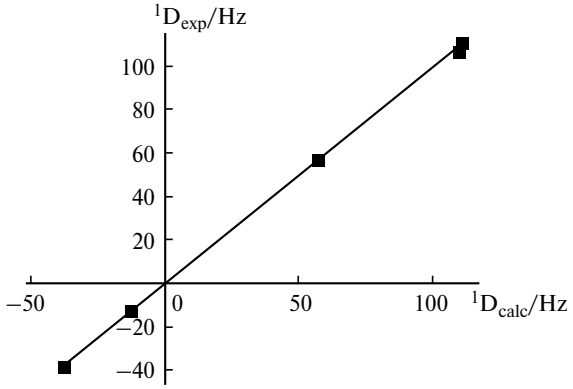


Fig. 2. Relationship between the observed dipolar coupling constants ($^1D_{CH}$) for stereoisomer **1b** dissolved in the lyotropic liquid-crystalline medium and the calculated values of these constants for stereoisomer **1b** in the 1,3-*alternate* conformation.

The validity of the approach to determine the thiacalix[4]arene conformations based on the analysis of the residual dipolar coupling constants is confirmed by 2D NMR ($^1H—^1H$) NOESY experiments carried out for stereoisomers **1a** and **1b**. For the compounds in question dissolved in an isotropic solvent ($CDCl_3$), cross-peaks were detected in 2D NMR spectra (with varied mixing time τ_m); these results could also be interpreted only with the assumption that stereoisomer **1a** occurs in the *cone* conformation, while stereoisomer **1b**, in the *1,3-alternate* conformation. The 2D NMR NOESY spectra of stereoisomer **1a** exhibited cross-peaks between the proton signals of the $—CMe_3$ group and the CH group of the benzene ring, and also cross-peaks between the signals for the protons of the methylene and methyl groups of the $CH_2C(O)Me$ fragment located on the lower rim of the

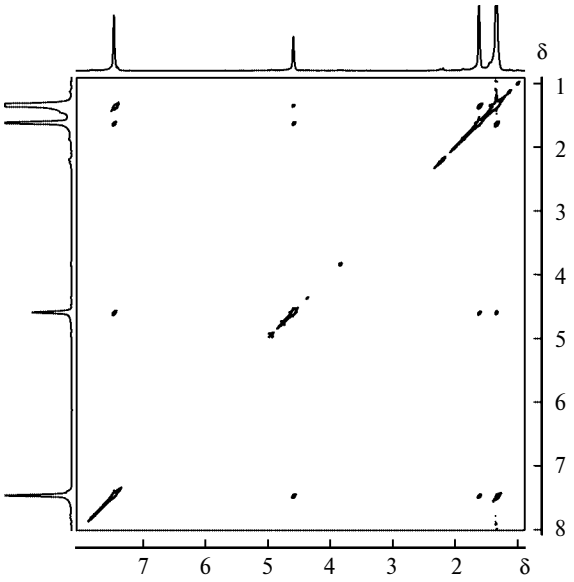


Fig. 3. 2D NMR NOESY spectrum of stereoisomer (**1b**) of thiacalix[4]arene in a $CDCl_3$ solution (τ_m = 0.8 s).

macrocycle. In addition to the above cross-peaks, the 2D NMR NOESY spectrum of stereoisomer **1b** (Fig. 3) contains cross-peaks between the signals for the protons of the benzene ring and the signals for the protons of the methylene and methyl groups of the $CH_2C(O)Me$ fragment and the cross-peaks between the signals for the protons of the CMe_3 group and the methyl group of the $C(O)CH_3$ fragment. This spectral pattern can be interpreted by assuming that these groups of protons are arranged in space in such a way that the distance between them is shorter than 5 Å, which is possible only in the *1,3-alternate* conformation.

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