Synthetic receptors based on calix[4]arene functionalized at the lower rim in molecular recognition of dicarboxylic, α-hydroxycarboxylic, and α-amino acids ✾

I. I. Stoikov, L. I. Gafiullina, D. Sh. Ibragimova, I. S. Antipin, and A. I. Konovalov*

Kazan State University,
18 ul. Kremlevskaya, 420008 Kazan, Russian Federation.
Fax: +7 (843 2) 31 5416. E-mail: Ivan.Stoikov@ksu.ru

New calix[4]arenes, di- and tetrasubstituted at the lower rim, with different functional groups were synthesized. They were studied as carriers of a series of dicarboxylic and α-hydroxycarboxylic acids through a liquid impregnated membrane. The calix[4]arenes under study are capable of molecular recognition of oxalic acid in the series of structurally similar dicarboxylic and α-hydroxycarboxylic acids. The regularities found make it possible to change purposefully the receptor ability of 1,3-disubstituted calix[4]arenes by variation of the nature of substituents.

Key words: calix[4]arene, thiacalix[4]arene, membrane transport, dicarboxylic acids, α-hydroxycarboxylic acids, carboxylate anions, synthetic receptors, molecular recognition.

Processes of specific recognition, transformations, transport, and regulation in biological systems are based on intermolecular interactions.[1] Principles of molecular recognition have intensely been used recently for the creation of different artificial systems. One of the vigorously developing directions is the synthesis of relatively small receptor molecules capable of highly selective binding with substrates of a certain type.[2,3]

It should specially be mentioned that molecular recognition of carboxylic acids is a complex problem, which is more difficult than recognition of cations. At least three-point binding of a "guest" molecule by a receptor seems to be principal for recognition of these substrates.[4] Carrier molecules for biologically significant polyfunctional substrates, such as peptides, nucleosides, and dicarboxylic, α-hydroxy, and α-amino acids, should fulfill several requirements.[5] First, receptor molecules should contain several coordination sites complementary to a substrate molecule. Second, they should be rather lipophilic. Finally, a compromise between the stability of a complex formed in the membrane phase and the high ion-exchange rate at the interface is necessary.

Macrocyclic compounds, such as cyclodextrins and calixarenes, are used as a platform for the creation of receptors of dicarboxylic, α-hydroxycarboxylic, and α-amino acids. Functionalization of phenol groups, aromatic rings, and bridging fragments in the starting calix[4]arenes, *i.e.*, [1_4]-metacyclophanes, by the corresponding organic and organoelement substituents determines a high receptor ability of "host" molecules. The calixarene fragment has a sufficient conformational rigidity and can provide a required orientation of binding sites in the space. Non-toxicity of calixarenes in combination with the complexing ability provides their promising use in pharmacology to stabilize and prolong the effect of medicines and decrease their side effect and to create new convenient medicinal forms.[6]

We have previously shown[7] that the calix[4]arenes distally disubstituted at the lower rim can bind carboxylic acids and the nature of substituents has a noticeable effect on complexation.

The use of the functionalized calix[4]arenes as molecular carriers through the lipophilic membrane made it possible to study the influence of the number and nature of substituents at the lower rim, heteroatoms included in the lateral substituents or directly in the macrocycle, on the ability to molecular recognition of dicarboxylic, α-hydroxy, and α-amino acids.

Experimental

^{1}H NMR spectra were recorded on a Varian XL-300 instrument (300 MHz) using $CDCl_3$ as a solvent. Mass spectra were obtained on an MX-1310 mass spectrometer. Electroconductivity of solutions was measured with a WTW inoLab Cond Level 1 conductometric detector.

D,L-Mandelic, glycolic, D,L-tartaric, D,L-glutamic, oxalic, malonic, and succinic acids (all reagent grade) and sodium acetate (reagent grade) were used. Quantum-mechanical calculations of the complexes were carried out by the MM+ molecular

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mechanics and semiempirical PM3 methods included in the MOPAC 7.00 program package.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(*N*-benzoylaminoethoxy)calix[4]arene (1). A mixture of 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(aminoethoxy)calix[4]arene[8] (4.00 g, 5.78 mmol), benzoic anhydride (2.61 g, 11.56 mmol), and benzene (30 mL) was refluxed for 2 h. Then the solvent was removed *in vacuo*, and the dry residue was dispersed in a saturated aqueous solution of $NaHCO_3$ (150 mL) for 30 min, filtered off, and washed with water (3×50 mL). The residue dried above P_2O_5 was dissolved in a minimum amount of CH_2Cl_2 and precipitated with hexane. The white precipitate was dried *in vacuo* (0.01 Torr) at 100 °C for 12 h. The yield was 2.70 g (59%), m.p. 135—136 °C. Found (%): C, 78.91; H, 7.98; N, 2.95. $C_{62}H_{74}N_2O_6$. Calculated (%): C, 78.95; H, 7.91; N, 2.97. ^{1}H NMR ($CDCl_3$), δ: 1.12, 1.23 (both s, 18 H each, CMe_3); 3.39 (d, 4 H, $ArCH_{2(eq)}Ar$, $^2J_{H,H}$ = 14.1 Hz); 3.62 (dt, 4 H, CH_2—N, $^3J_{H,H}$ = 4.5 Hz, $^3J_{H,H}$ = 4.9 Hz); 4.03 (t, 4 H, O—CH_2, $^3J_{H,H}$ = 4.5 Hz); 4.18 (d, 4 H, $ArCH_{2(ax)}Ar$, $^2J_{H,H}$ = 14.1 Hz); 7.02, 7.07 (both s, 4 H each, ArH); 7.31 (dd, 4 H, ArH, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.1 Hz); 7.43 (tt, 2 H, ArH, $^3J_{H,H}$ = 7.4 Hz, $^4J_{H,H}$ = 1.3 Hz); 7.95 (dd, 4 H, ArH, $^3J_{H,H}$ = 7.1 Hz, $^4J_{H,H}$ = 1.3 Hz); 8.21 (t, 2 H, N—H, $^3J_{H,H}$ = 4.9 Hz); 8.39 (s, 2 H, OH). Found: *m/z* 942.5551 $[M]^+$. $C_{62}H_{74}N_2O_6$. Calculated: M = 942.5547.

5,17-Di-*tert*-butyl-11,23-dinitro-25,27-dihydroxy-26,28-bis(*N*-benzoylaminoethoxy)calix[4]arene (2). A mixture of 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(*N*-benzoylaminoethoxy)calix[4]arene (**1**) (0.47 g, 0.5 mmol), CH_2Cl_2 (50 mL), glacial AcOH (2.9 mL, 50 mmol), and 65% HNO_3 (5 mL, 8.0 mmol) was stirred for 30 min at ~20 °C and then poured into water (50 mL). The organic layer was twice washed with water and dried with $MgSO_4$. The solvent was removed *in vacuo*, and the residue was recrystallized from a CH_2Cl_2—hexane mixture. The yield was 0.11 g (28%), m.p. 135 °C. Found (%): C, 70.39; H, 6.15; N, 6.12. $C_{54}H_{56}N_4O_{10}$. Calculated (%): C, 70.42; H, 6.13; N, 6.08. ^{1}H NMR ($CDCl_3$), δ: 1.01 (s, 18 H, CMe_3); 3.49 (d, 4 H, $ArCH_{2(eq)}Ar$, $^2J_{H,H}$ = 13.4 Hz); 3.82 (dt, 4 H, CH_2—N, $^3J_{H,H}$ = 4.8 Hz, $^3J_{H,H}$ = 5.5 Hz); 4.12 (t, 4 H, O—CH_2, $^3J_{H,H}$ = 4.8 Hz); 4.18 (d, 4 H, $ArCH_{2(ax)}Ar$, $^2J_{H,H}$ = 13.4 Hz); 6.90, 7.30 (both m, 4 H each, ArH); 7.44 (m, 2 H, ArH); 7.69 (t, 2 H, N—H, $^3J_{H,H}$ = 5.5 Hz); 7.86 (d, 4 H, ArH, $^3J_{H,H}$ = 7.5 Hz); 8.04 (s, 4 H, ArH); 8.94 (s, 2 H, OH). Found: *m/z* 920.4001 $[M]^+$. $C_{54}H_{56}N_4O_{10}$. Calculated: M = 920.3996.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(*N*-octylaminocarbonylmethoxy)calix[4]arene (3). A mixture of 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene[7] (**6**) (1.58 g, 2.16 mmol), *n*-octylamine (9.5 mL, 57.5 mmol), and NH_4Cl (0.16 g, 2.96 mmol) was stirred with refluxing for 2.5 h. Then volatiles were distilled off *in vacuo*, and the residue was washed with water (2×10 mL) and 1 *M* HCl (3×10 mL). The reaction mixture was separated by flash chromatography on silica gel L 5—40 μm (30 g) using successive elution with 10-mL portions of $CHCl_3$—Pr^iOH (2 : 1, 1 : 1, 1 : 2) mixtures. The solvent was removed *in vacuo*. The residue as a colorless glassy mixture was dried *in vacuo* (0.001 Torr) at 100 °C for 12 h. The yield was 1.10 g (60%). Found (%): C, 77.37; H, 9.85; N, 2.51. $C_{64}H_{94}N_2O_6$. Calculated (%): C, 77.80; H, 9.59; N, 2.84. ^{1}H NMR ($CDCl_3$), δ: 0.84 (t, 6 H, $\underline{Me}(CH_2)_7$, $^3J_{H,H}$ = 6.9 Hz); 1.05, 1.27 (both s, 18 H each, CMe_3); 1.36—1.10 (m, 24 H, $CH_2(C\underline{H}_2)_6Me$); 3.38 (dt, 4 H, $NHC\underline{H}_2(CH_2)_6Me$, $^3J_{H,H}$ = 5.2 Hz, $^3J_{H,H}$ = 6.7 Hz); 3.43 (d, 4 H, $ArCH_{2(eq)}Ar$, $^2J_{H,H}$ = 13.2 Hz); 4.12 (d, 4 H, $ArCH_{2(ax)}Ar$, $^2J_{H,H}$ = 13.2 Hz); 4.56 (s, 4 H, O—$C\underline{H}_2C(O)NH$); 6.94, 7.08 (both s, 4 H each, ArH); 7.86 (s, 2 H, OH); 8.84 (t, 2 H, N—H, $^3J_{H,H}$ = 5.2 Hz). Found: *m/z* 986.7121 $[M]^+$. $C_{64}H_{94}N_2O_6$. Calculated: M = 986.7112.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(pyridin-4-ylmethoxy)calix[4]arene (4). A mixture of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene (1.00 g, 1.54 mmol), anhydrous K_2CO_3 (3.14 g, 22.72 mmol), and anhydrous NaI (1.15 g, 7.67 mmol) in MeCN (150 mL) was refluxed with stirring under argon for 30 min. Then a solution of 4-chloromethylpyridine hydrochloride (0.54 g, 3.24 mol) in anhydrous MeOH (30 mL) was added to the reaction mixture for 2 h, after which the mixture was refluxed with stirring for 20 h. Then the reaction mixture cooled to 50 °C was filtered, the residue was washed with MeCN (2×30 mL), and the solvent was removed from the combined extracts. The dry residue was dispersed in water (150 mL) for 30 min, washed with water until the filtrate stopped coloring, and recrystallized from a mixture of Et_2O (50 mL) and MeCN (15 mL). The white needle-like precipitate was dried for 12 h *in vacuo* (0.01 Torr) at 100 °C. The yield was 0.79 g (62%), m.p. 108—109 °C. Found (%): C, 79.37; H, 8.15; N, 3.41. $C_{56}H_{66}N_2O_4$. Calculated (%): C, 80.93; H, 8.00; N, 3.37. ^{1}H NMR ($CDCl_3$), δ: 0.93, 1.30 (both s, 18 H each, CMe_3); 3.33 (d, 4 H, $ArCH_{2(eq)}Ar$, $^2J_{H,H}$ = 13.4 Hz); 4.25 (d, 4 H, $ArCH_{2(ax)}Ar$, $^2J_{H,H}$ = 13.4 Hz); 5.07 (s, 4 H, O—$C\underline{H}_2$Pyr); 6.79 (s, 4 H, ArH); 6.97 (s, 2 H, OH); 7.07 (s, 4 H, ArH); 7.64, 8.63 (both d, 4 H each, C_5H_4N, $^3J_{H,H}$ = 6.1 Hz). Found: *m/z* 830.5028 $[M]^+$. $C_{56}H_{66}N_2O_4$. Calculated: M = 830.5023.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(pentafluorophenylmethoxy)calix[4]arene (5). A mixture of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene (1.0 g, 1.54 mmol), the appropriate alkyl bromide (3.24 mmol), and anhydrous K_2CO_3 (1.7 g, 13.9 mmol) in MeCN (30 mL) was refluxed with stirring for 12 h. Then the solvent was removed, and the dry residue was treated with a 5 *M* solution of HCl (10 mL) and $CHCl_3$ (50 mL). The organic phase was separated, triply washed with water (30 mL), and dried above molecular sieves 4 Å. After the solvent was removed, the residue was recrystallized from a $CHCl_3$—MeOH mixture. The yield was 1.33 g (85.5%), m.p. 222 °C. Found (%): C, 68.73; H, 5.85. $C_{58}H_{58}F_{10}O_4$. Calculated (%): C, 69.04; H, 5.79. ^{1}H NMR ($CDCl_3$), δ: 0.94, 1.30 (both s, 18 H each, CMe_3); 3.28 (d, 4 H, $ArCH_{2(eq)}Ar$, $^2J_{H,H}$ = 13.09 Hz); 4.22 (d, 4 H, $ArCH_{2(ax)}Ar$, $^2J_{H,H}$ = 13.09 Hz); 5.09 (s, 4 H, O—CH_2); 6.39 (s, 2 H, OH); 6.75, 7.06 (both s, 4 H each, ArH). Found: *m/z* 1008.4179 $[M]^+$. $C_{58}H_{58}F_{10}O_4$. Calculated: M = 1008.4175.

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetrakis(2-oxoprop-1-yloxy)calix[4]arene (8). A mixture of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene (1 g, 1.54 mmol) and K_2CO_3 (1.26 g, 9.25 mmol) in MeCN (70 mL) was refluxed with stirring for 1 h. Then bromoacetone (0.8 mL, 9.25 mmol) was added with vigorous stirring. The reaction mixture was stirred for 9.5 h at the boiling temperature of the solvent and filtered through the Celite layer 1.5 cm thick. The precipitate was washed with $CHCl_3$ (3×20 mL), and the solvent was removed under a reduced pressure. 1 *M* HCl (20 mL) and $CHCl_3$ (30 mL) were added to the residue, and the mixture was ex-

tracted for 30 min. The organic layer was separated and dried with $MgSO_4$, and the solvent was removed. The dry residue was recrystallized from a $CHCl_3$—EtOH mixture, and a white crystalline substance was obtained. The yield was 1.21 g (90%), m.p. 216—217 °C (Ref. 9: m.p. 204—207 °C). Found (%): C, 77.30; H, 8.18. $C_{56}H_{72}O_8$. Calculated (%): C, 77.03; H, 8.31. ^{1}H NMR ($CDCl_3$), δ: 1.07 (s, 36 H, CMe_3); 2.21 (s, 12 H, Me); 3.18 (d, 4 H, $ArCH_{2(eq)}Ar$, $^2J_{H,H}$ = 13 Hz); 4.81 (d, 4 H, $ArCH_{2(ax)}Ar$, $^2J_{H,H}$ = 13 Hz); 4.88 (s, 8 H, O—CH_2); 6.80 (s, 8 H, ArH). Found: *m/z* 872.5223 $[M]^+$. $C_{56}H_{72}O_8$. Calculated: M = 872.5227.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene[7] (6), 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(ethoxycarbonylmethoxy)calix[4]arene[10] (7), and 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(ethoxycarbonylmethoxy)-2,8,14,20-tetrathiacalix[4]arene[11] (9) in the cone conformation were synthesized according to previously described procedures.[7,10,11]

Experiments on membrane transfer. Transfer rates of substrates through liquid impregnated membranes were measured in a glass vertical diffuse cell, whose temperature was maintained constant, with a movable cylinder.[12] The Millipore Type FA porous Teflon filters (thickness 1 μm, pore size 100 nm, porosity 85%) served as hydrophobic matrices. They were reinforced with a capron mesh and impregnated with the liquid matrix. The ratio of volumes of the feeding and receiving phases was 5 : 1, which provided the equal levels of solutions to eliminate osmotic acid transfer. Experiments on mass transfer were carried out under normal conditions (25 °C). A liquid membrane was a 0.05 *M* solution of carriers **1—9** in an organic solvent or a pure solvent (*o*-nitrophenyl octyl ether).

Procedure of experiments on membrane transfer. The starting solution of a substrate was placed in the external vessel with a constant temperature, and the internal vessel was filled with bidistilled water. Source and receiving solutions were magnetically stirred. The concentrations of substances were determined from the conductance of the solutions. The calibrating plots were reproduced three times. Experiments on membrane transfer were triply reproduced under the same conditions. The error of determination of the mass transfer flux j_i was 10%.

UV experiments on complexation. Electronic absorption spectra were recorded on a Perkin—Elmer Lambda-35 spectrometer (l = 1 cm). The molar absorption coefficient of calix[4]arene **4** (ε = 5526.3 mol^{-1} L cm^{-1} in CH_2Cl_2) was measured at the wavelength λ_{max} = 259.03 nm. Solutions of carboxylic acids, which were used as substrates, in CH_2Cl_2 do not absorb in this spectral region.[13]

Procedure of experiments on measuring the stability constant.[14] A solution of receptor **4** in CH_2Cl_2 with a concentration of $1 \cdot 10^{-4}$ mol L^{-1} was added to 1000-fold excess of carboxylic acid, and the solution was magnetically stirred for 4 h. Then the solution was filtered to remove uncomplexed acid. Electronic absorption spectra of the starting solution of macrocycle **4** and the complexes were recorded, as well as solutions of the complexes diluted by 1.25, 1.43, 1.67, 2.00, 2.50, 3.33, 5.00, and 10.00 times. The results were processed as described previously.[15]

Results and Discussion

Based on analysis of approaches to design of synthetic receptors of carboxylic acids using *m*-cyclophane platforms,[2—7] we proposed two models (**A** and **B**, Fig. 1) for molecular recognition of dicarboxylic, α-hydroxy, and α-amino acids by 1,3-disubstituted calix[4]arenes. The basic distinction of these schemes is the different orientation of a substrate with respect to the "host" molecule, namely, interaction of the "docking" (**A**) or "tweezers" (**B**) type. In the first model (see Fig. 1, **A**), an acid is recognized simultaneously by the phenylic hydroxyls of the macrocycle and functional groups of the lateral substituents (R). In another scheme (see Fig. 1, **B**), the R substituents at the lower rim of calix[4]arene operate as a "tweezers" or a "two-handed" podand, performing the ditopic interaction of binding sites of the "host" with both functions of the "guest." We chose *m*-cyclophanes **1—9** to accomplish both types of interaction ("docking" and "tweezers").

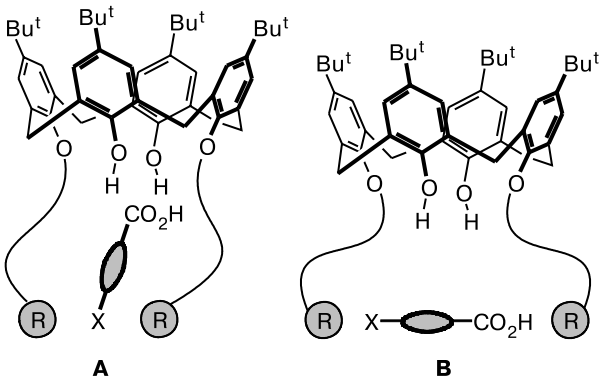
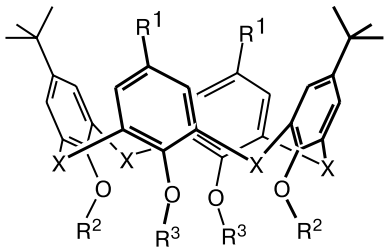


Fig. 1. Models assumed for binding of organic acids by calix[4]arenes 1,3-disubstituted at the lower rim: complexes **A** and **B**.

Compounds **1—9**, which are calix[4]arene and thiacalix[4]arene derivatives, were synthesized to vary such characteristics as the lipophilicity, acid-base properties of phenol groups, number of fragments at the lower rim (di- and tetrasubstituted), nature of functional groups of substituents, macrocycle size (on going from calixarene to its thia analog), sequence of atoms in the amide groups, and distance between the amide and hydroxy groups of the macrocycle.

The reaction of benzoic anhydride with 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(aminoethoxy)calix[4]arene[8] in benzene gives compound **1**. Product **2** was synthesized by the nitration of macrocycle **1** with nitric acid at room temperature.[16] The selective alkylation[17] of *p*-*tert*-butylcalix[4]arene and *p*-*tert*-butylthiacalix[4]arene with the corresponding haloderivatives resulted in a series of compounds **4—9** in the cone conformation[18] in 60—90% yields. Diamide **3** was obtained by heating of **6** with excess amine. The compositions and the structures of the synthesized compounds were confirmed by the data of ^{1}H NMR spectroscopy, mass spectrometry, and elemental analysis. The cone con-



1—9

Compound	X	R^1	R^2	R^3
1	CH_2	Bu^t	CH_2CH_2NH-C(O)-Ph	H
2	CH_2	NO_2	$CH_2CH_2NHC(O)Ph$	H
3	CH_2	Bu^t	$CH_2C(O)NH(CH_2)_7Me$	H
4	CH_2	Bu^t	4-CH_2Pyr	H
5	CH_2	Bu^t	$CH_2C_6F_5$	H
6	CH_2	Bu^t	$CH_2C(O)OEt$	H
7	CH_2	Bu^t	$CH_2C(O)OEt$	$CH_2C(O)OEt$
8	CH_2	Bu^t	$CH_2C(O)Me$	$CH_2C(O)Me$
9	S	Bu^t	$CH_2C(O)OEt$	$CH_2C(O)OEt$

formation[18] for compounds **1—8** was established from signals of the bridging methylene protons of the macrocycles appeared as a spin AB system in the ^{1}H NMR spectra.

To estimate a possibility of formation of different types of complexes, we calculated the models proposed for binding of organic acids by 1,3-disubstituted calix[4]arenes by the molecular mechanics (MM+) and semiempirical PM3 methods (see Fig. 1). Simulation of the complexes of *m*-cyclophanes **1—9** with the substrates showed that both the first (**A**) and second types of binding (see Fig. 1, **B**) are possible for interaction with different substrates.

The complexing ability of synthesized *m*-cyclophanes **1—9** with respect to some carboxylic acids was studied by the membrane extraction method. We carried out experiments on induced (by calix[4]arenes **1—9**) transport of dicarboxylic, α-hydroxy, and α-amino acids through lipophilic liquid membranes. The flux values measured for substrates **10—17** (glutamic acid (**10**), sodium acetate (**11**), and tartaric (**12**), oxalic (**13**), glycolic (**14**), malonic (**15**), succinic (**16**), and mandelic (**17**) acids) are presented in Table 1.

Liquid membranes were 0.05 *M* solutions of compounds **1—9** in *o*-nitrophenyl octyl ether impregnated in pores of Teflon filters.[12] The membrane extractions of glutamic acid **10** and sodium acetate **11** were studied to estimate the influence of the carboxylate functions on the mass transfer of carboxylic acids by the carriers studied. The initial concentration of substrates **10—17** in the source phase was 0.1 mol L^{-1}.

The fluxes (j_i) through the membrane were calculated from the initial linear regions of the time plot of the concentration of the transferred substance in the receiving phase. Table 1 also contains the fluxes of the "blank" experiment (j_0) in which the membrane was the pure solvent, *o*-nitrophenyl octyl ether. The incorporation of carriers **1—9** into the membrane phase gives different enhancement factors for transfer ($\varepsilon = j_i/j_0$) of substrates **10—17** through the liquid impregnated membranes (Fig. 2). It should be noted that there was no dependence between log*P* of the "guest" (*P* is the partition constant of acids in the two-phase octanol—water system, which characterizes lipophilicity) and the enhancement factors for the studied acids (Table 2). Insignificant enhancement factors are observed in the case of the most lipophilic substance among the substrates studied (mandelic acid).

To study the ability of calix[4]arene to bind carboxylic acids only due to the interaction with two hydroxy groups at the lower rim, we synthesized *m*-cyclophane **5** with two pentafluorophenyl groups, which cannot bind substrates **10—17** because of the electron-deficient character of the substituents and their inability to manifest either proton-donor or proton-acceptor properties. The incorporation of **5** into the membrane phase did not change the transfer rates of all substrates under investigation. Therefore, interactions of the substrates only with the hydroxy groups

Table 1. Mass transfer fluxes (j_i/kmol m^{-2} s^{-1}) of substrates **10—17** through the liquid impregnated membrane (25 °C)[a]

Substrate	j_i									
	j_0[b]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
10	$2.8\cdot10^{-12}$	$2.8\cdot10^{-12}$	$3.5\cdot10^{-11}$	$2.8\cdot10^{-12}$	$2.8\cdot10^{-12}$	$2.8\cdot10^{-12}$	$3.2\cdot10^{-11}$	$2.8\cdot10^{-12}$	$2.8\cdot10^{-12}$	$2.8\cdot10^{-12}$
11	$1.3\cdot10^{-11}$	$1.3\cdot10^{-11}$	$1.3\cdot10^{-11}$	$1.8\cdot10^{-10}$	$2.8\cdot10^{-12}$	$1.3\cdot10^{-11}$	$3.0\cdot10^{-10}$	$1.3\cdot10^{-11}$	$1.3\cdot10^{-11}$	$1.3\cdot10^{-11}$
12	$4.4\cdot10^{-12}$	$2.2\cdot10^{-10}$	$1.7\cdot10^{-11}$	$4.4\cdot10^{-12}$	$4.6\cdot10^{-12}$	$4.4\cdot10^{-12}$	$4.4\cdot10^{-12}$	$4.4\cdot10^{-12}$	$4.9\cdot10^{-12}$	$2.3\cdot10^{-11}$
13	$5.0\cdot10^{-12}$	$1.7\cdot10^{-11}$	$5.0\cdot10^{-12}$	$1.2\cdot10^{-11}$	$1.4\cdot10^{-9}$	$5.0\cdot10^{-12}$	$5.0\cdot10^{-12}$	$5.0\cdot10^{-12}$	$3.0\cdot10^{-10}$	$7.7\cdot10^{-10}$
14	$9.4\cdot10^{-12}$	$5.3\cdot10^{-11}$	$9.4\cdot10^{-12}$	$4.2\cdot10^{-11}$	$5.0\cdot10^{-11}$	$9.4\cdot10^{-12}$	$3.6\cdot10^{-11}$	$9.4\cdot10^{-12}$	$2.0\cdot10^{-11}$	$4.3\cdot10^{-11}$
15	$2.9\cdot10^{-11}$	$1.9\cdot10^{-10}$	$6.7\cdot10^{-11}$	$2.9\cdot10^{-11}$	$9.9\cdot10^{-11}$	$2.9\cdot10^{-11}$	$2.8\cdot10^{-11}$	$2.9\cdot10^{-11}$	$6.3\cdot10^{-11}$	$1.5\cdot10^{-10}$
16	$1.3\cdot10^{-11}$	$4.4\cdot10^{-11}$	$1.3\cdot10^{-11}$	$3.0\cdot10^{-11}$	$1.4\cdot10^{-11}$	$1.3\cdot10^{-11}$	$1.4\cdot10^{-11}$	$1.3\cdot10^{-11}$	$1.3\cdot10^{-11}$	$5.5\cdot10^{-11}$
17	$1.5\cdot10^{-9}$	$3.5\cdot10^{-9}$	$1.5\cdot10^{-9}$	$4.1\cdot10^{-9}$	$2.8\cdot10^{-12}$	$1.5\cdot10^{-9}$	$4.8\cdot10^{-9}$	$1.5\cdot10^{-9}$	$1.5\cdot10^{-9}$	$3.3\cdot10^{-10}$

[a] The error in determination of the mass transfer fluxes is ±10%. Surface area of the membrane $S = 9.616$ cm^2.
[b] The flux of acid through the membrane containing no carrier (kmol m^{-2} s^{-1}).

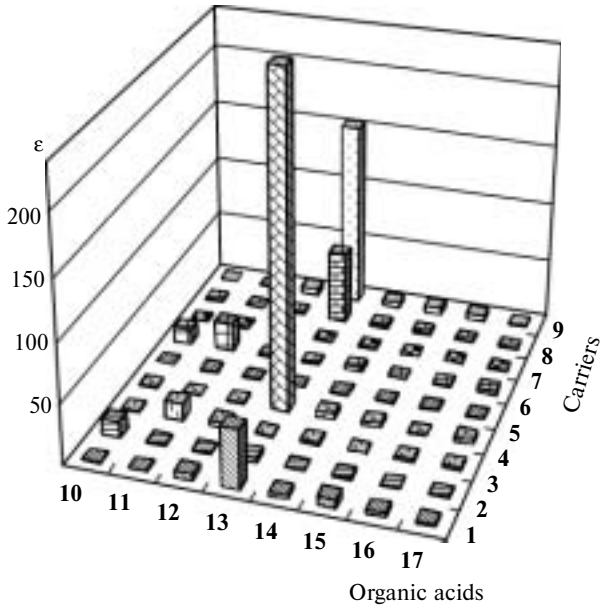


Fig. 2. Enhancement factors of flux for several organic substrates **10—17** through the liquid impregnated membrane containing carriers **1—9**.

Table 2. Acid equilibrium constants and lipophilicity of substrates **10—17**

Substrate	pK_a	log$P$
Glutamic acid (**10**)	4.33	−4.6
Sodium acetate (**11**)	—	−4.24
Tartaric acid (**12**)	3.03	−1.96
Oxalic acid (**13**)	1.25	−1.88
Glycolic acid (**14**)	3.83	−1.02
Malonic acid (**15**)	2.85	−0.49
Succinic acid (**16**)	4.21	−0.47
Mandelic acid (**17**)	3.37	0.64

at the lower rim of 1,3-disubstituted calix[4]arene are insufficient for binding and extraction of hydrophilic carboxylic acids into the lipophilic membrane phase.

It was of interest to study macrocycles with nitrogen-containing functional fragments (amide (**1—3**) and pyridine (**4**)), because the most part of presently known models of receptors for organic acids are nitrogen-containing compounds.[2,3]

The complexing ability of carrier **4** with the pyridine fragments at the lower rim was preliminarily studied by UV spectroscopy in CH_2Cl_2 (Fig. 3). The absorption spectrum of *m*-cyclophane **4** is a superposition of the absorption spectra of the benzene rings of the macrocycle and monosubstituted pyridine. The binding of organic acids **10—17** by calix[4]arene **4** was shown to be accompanied by an increase in the intensity of the absorption bands of the receptor (n→π-transition) with maxima at 259 and 263 nm and the hypsochromic shift of the bands. The

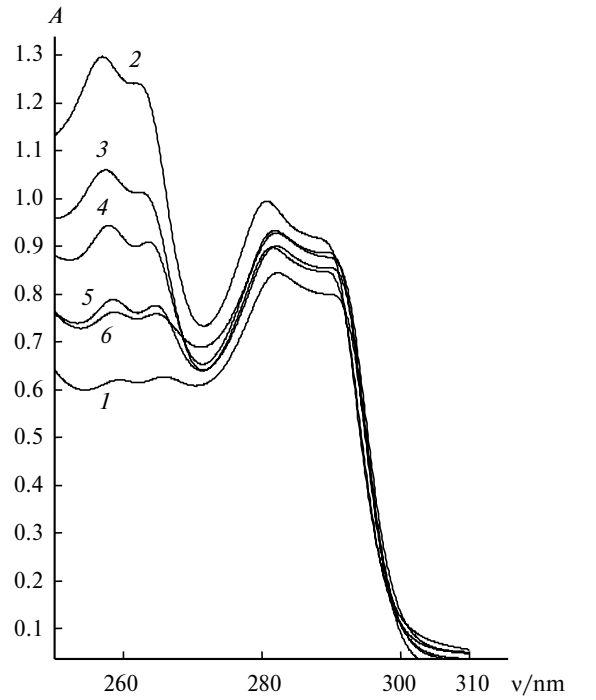


Fig. 3. UV spectra of solutions of calix[4]arene **4** (*1*) and its 1 : 1 complexes with some organic acids: oxalic (*2*), malonic (*3*), glycolic (*4*), tartaric (*5*), and succinic (*6*).

changes in the UV spectrum of calix[4]arene **4** upon interaction with several acids are caused by the involvement of a lone electron pair of the nitrogen atom (by the change in the hybridization of the sp^2-hybrid orbital)[13] in the interaction with the carboxy group of the substrate. The changes in the absorption spectra of calix[4]arene **4** in the presence of several organic acids are presented in Fig. 3. The absorption intensity decreases in the series oxalic > malonic > glycolic > tartaric, succinic acids, which is related to weakening of the interaction between the substrate and receptor. The fluxes of the acids through the membrane decrease in the same sequence, *i.e.*, the stability of the complexes and transport properties change in parallel. This dependence indicates that the extraction of acids from the aqueous phase to the lipophilic organic membrane is the limiting step of mass transfer.

The stability constants of the complexes with calix[4]arene **4** formed in CH_2Cl_2 were determined for oxalic and glycolic acids by the dilution method[14]: $(1.2\pm0.15)\cdot10^5$ and $(2.8\pm0.4)\cdot10^4$ mol L^{-1}, respectively. According to the published data,[5] the stability constant obtained for acid **13** ranges within the magnitudes characteristic of efficient carriers in the dynamic transfer process of substances though the liquid membrane. In fact, the histogram (see Fig. 2) shows that 1,3-disubstituted calix[4]arene **4** with the pyridine fragments was found to be the most efficient and selective carrier. It transfers the strongest acid (oxalic) with a flux enhancement factor

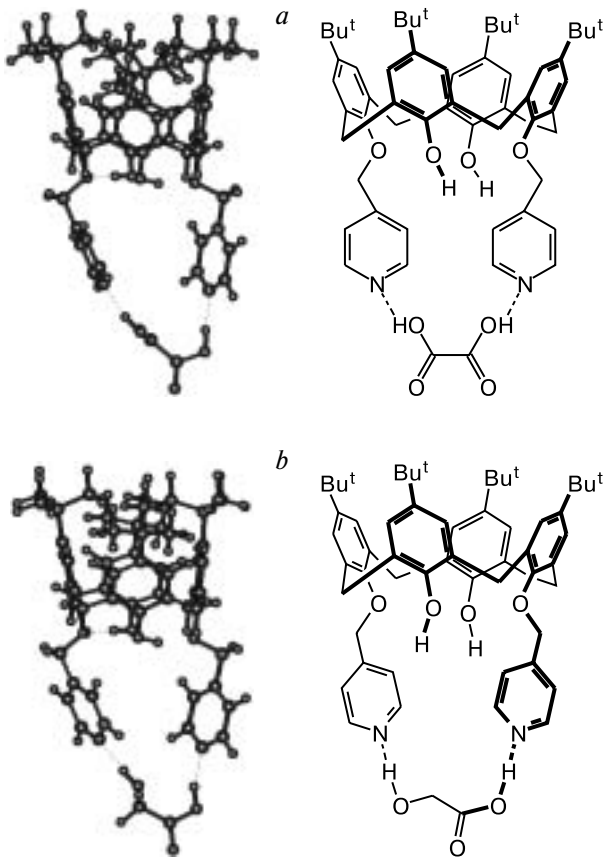


Fig. 4. Results of PM3 simulation of the 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(pyridin-4-ylmethoxy)calix[4]arene complexes with oxalic (*a*) and glycolic (*b*) acids.

of 277, while for the next substrate (glycolic acid) the flux enhancement factor is only 5.

The results of simulation of oxalic acid—calix[4]arene **4** complex are presented in Fig. 4. Both carboxyl groups of the substrate can form hydrogen bonds with the nitrogen atoms of the pyridine ring. Although this type of bonding (see Fig. 1, **B**) is also possible for malonic and succinic acids, the results of membrane extraction indicate that the geometric complementarity of the binding sites and strength of the acids are primary in the case. Oxalic acid, being the strongest, conformationally more rigid, and more preorganized, is more efficiently transferred.

The selectivities of macrocycles **1—3**, which bear the amide function at the lower rim, differ substantially. The incorporation of carrier **1** into the membrane increases the fluxes for all substrates studied. In the case of oxalic acid (**13**) (see Fig. 2), the transfer rate 50-fold increased, while those for hydroxycarboxylic (glycolic (**14**) and mandelic (**17**)), dicarboxylic (malonic (**15**), and succinic (**16**)) acids increased only by at most 7 times. This agrees well with the results of molecular simulation (Fig. 5), because a complex of type **B** (see Fig. 1) can be formed only in the case of oxalic acid when both carboxy groups of the acid are bound with the amide groups. The distance between the lateral binding sites of macrocycle **1** corresponds to the substrate length, and the carboxy groups of compound **13** form hydrogen bonds with the amide substituents of the receptor (see Fig. 5). When a complex of type **A** is formed (see Fig. 1), only the amide fragments of calix[4]arene and only one carboxy group of the acid can be involved in binding due to discrepancy between the interaction sites of receptor **1** and acid **13**. According to the simulation results, this structure is less favorable than the preceding one. It is most likely that the sharp increase in the oxalic acid flux (almost 50-fold) is related precisely to the formation of a complex of type **B**.

Malonic acid (**15**) ($\varepsilon = 7$) is the next substance in efficiency of transfer by *m*-cyclophane **1** through the liquid membrane. In the case of malonic acid, the calculations showed that one carboxy group of this substrate can be involved in hydrogen bonding with the hydroxy groups of calix[4]arene, while the second carboxy group can form hydrogen bonds with both amide fragments. It should be noted that the interaction of both carboxy groups of succinic acid (**16**) with "host" **1** *via* both **A** and **B** types is hindered for steric reasons ($\varepsilon = 3$). Therefore, when binding occurs *via* the "docking" type (see Fig. 1, **A**), the interaction with malonic acid is the most efficient. Thus, molecular simulation showed that the formation of a complex by substrate binding *via* the "tweezers" type (see Fig. 1, **B**) is possible only for oxalic acid, and complexes can be formed *via* the "docking" type (**A**) with an increase in the length of dicarboxylic acid.

To confirm the binding scheme **A** for carrier **1**, we synthesized calix[4]arene **2** containing nitro groups at the upper rim of the macrocycle in order to change the acid-base properties of phenol groups. The incorporation of compound **2** into the membrane phase changes the fluxes substantially compared to those for carrier **1**. For *m*-cyclophane **2**, the enhancement factors of transfer for substrates **11—17** turned out to be close to unity, *i.e.*, the carrier exerts no effect on mass transfer. This can be explained by the fact that the hydroxy functions of compound **1** are involved in substrate binding (*i.e.*, scheme **A** takes place) as proton acceptors rather than proton donors. At the same time, an increase in the acidity of the phenol groups in **2** increased the flux of glutamic acid (**10**) by 25 times, which is related to the enhancement of interaction with the carboxylate group. It is most likely that the carboxylate function binds to the acidic phenol groups as proton donors, and the ammonia and carboxy groups of the acid are bound to the amide fragments of calix[4]arene *via* scheme **A**.

The influence of the sequence of atoms in the lateral amide fragments in 1,3-disubstituted calix[4]arenes on the complementarity to substrates **10—17** was studied for macrocycle **3**. We synthesized *m*-cyclophane **3** with a different sequence of atoms in the amide groups and a

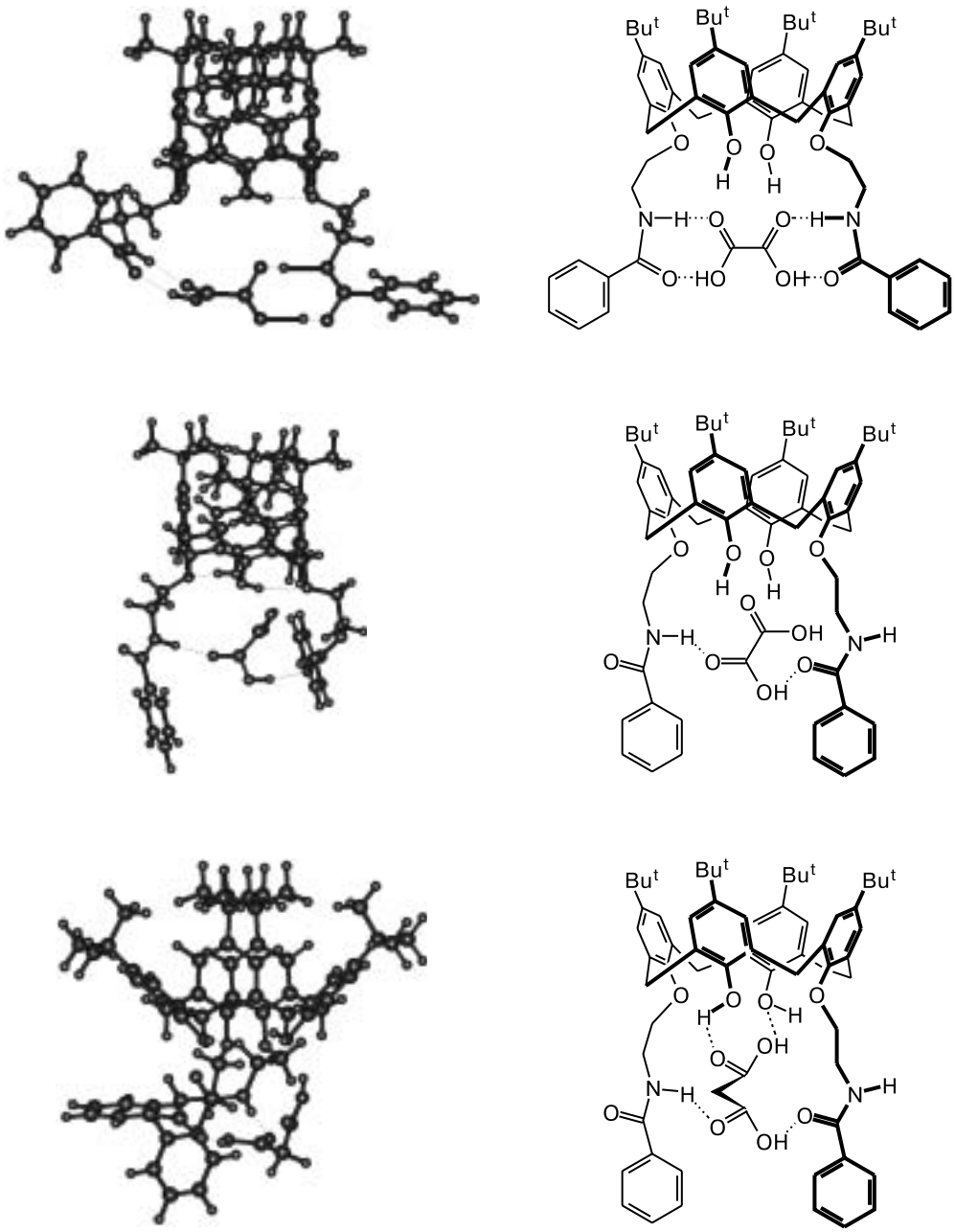


Fig. 5. Results of PM3 simulation of the 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(*N*-benzoylaminoethoxy)calix[4]arene complexes with malonic and oxalic acids.

shorter distance between the amide and hydroxy groups of the macrocycle. It turned out that diamide **3** with the shortest spacer and different orientation of the amide fragments does not accelerate the transfer of the substrates under study through the lipophilic liquid membrane, except for sodium acetate, *i.e.*, it is efficient for binding of the carboxylate function. It should be noted that we have previously shown[7] that calix[4]arene **6** containing the proton-acceptor (ester) groups at the lower rim manifested a capability of transferring substrates containing the carboxylate function, *viz.*, glutamic acid and sodium acetate. To understand the mechanism of transfer of sodium acetate, it is important to answer the question how the transfer of substrate **11** through the lipophilic membrane by synthetic receptor **6** occurs: due to the interaction with the acid anion or metal cation? The fact favoring the interaction of calix[4]arene **6** exactly with the acetate anion is the absence of its influence on the flux of sodium bromide through the membrane,[7] although the lipophilicities (log*P*) of the Br^- and $MeCO_2^-$ anions are close.

Additional experiments were carried out to study a possibility of involvement of sodium cations in the transfer of the substrates under investigation by calix[4]arenes through the lipophilic membrane. It is well known that, unlike the 1,3-disubstituted derivatives, calix[4]arenes tetrafunctionalized at the lower rims (tetraesters, tetraketones) are more efficient and selective receptors for alkali metal cations.[6,19] As compared to tetraesters, tetraketones based on calix[4]arenes are stronger extragents of Na^+.[19—21] Therefore, it was of interest to study the transfer of both sodium acetate **11** and substrates **10**, **12—17** by tetraether **7** and tetraketone **8**.

Our experiments on membrane extraction demonstrated the complete absence of any effect of calix[4]arenes **7** and **8** on the transfer of compound **11** through the lipophilic membrane. This additionally indicates that the transfer of compound **11** by macrocycles **2** and **6** is caused by binding of the carboxylate group of the substrate with the receptor. Thus, it can be concluded that 1,3-disubstituted calix[4]arenes containing the $OCH_2C(O)XAlk$ fragment (X = O or NH) at the lower rim are capable of molecular recognition of the carboxylate group.

The study of tetraether **7** and tetraketone **8** as carriers showed the fundamental difference in their transport properties in the case of transfer of carboxylic acids under study. Compound **7** turned out to be inactive as a carrier of substrates **12—17**, *i.e.*, the macrocycle does not bind these organic acids. At the same time, the incorporation of compound **8** into the liquid membrane almost 60-fold increases the oxalic acid flux, and a high selectivity is observed. The enhancement factors of flux for other substrates do not exceed 2.3. Taking into account that the energy of formation of a hydrogen bond between acetone and the hydroxy group of the ROH compound is somewhat higher than that of hydrogen bond formation between the hydroxy group of ROH and ethyl acetate ($-\Delta G_{25\,^\circ C}$ 1.8 and 1.4 kcal mol^{-1}, respectively),[21] we can assert that the high energy of hydrogen bond formation between the hydroxy groups of the substrates and the substituents of calixarene **8** makes a certain contribution to complexation. However, such an insignificant increase in the proton-acceptor ability of the carbonyl group cannot induce a sharp change in the transfer properties of tetraketone **8** compared to tetraether **7**. It can be assumed that the mutual steric repulsion of the methyl groups in compound **8** allows the proton-acceptor carbonyl groups of acid **13**, which is the smallest in size, to interact. All these factors result in the appearance of the transport capability of carrier **8**. It is most likely that an increase in the size of the pseudo-cavity, formed by four carbonyl groups on going from **7** to **8**, favors binding of oxalic acid.

Therefore, we focused our attention on the thia analog of *p-tert*-butylcalix[4]arene: the change in the bond lengths[13] from 1.54 Å (C—C) to 1.77 Å (C—S) increases the macrocycle size. To confirm the basic influence of exactly the distance between the carbonyl groups on the complementarity of calix[4]arenes, tetrasubstituted at the lower rim, to oxalic acid, we synthesized tetraester based on *p-tert*-butylcalix[4]arene (compound **9**).

The replacement of the methylene bridges in carrier **7** by sulfur atoms (carrier **9**) increases the size of the pseudocavity formed by four ester fragments. As a result, thiacalix[4]arene **9** is capable of efficient and selective transfer of oxalic acid (enhancement factor of flux is 154).

Thus, the kinetic study of mass transfer of the substrates by 1,3-disubstituted calix[4]arenes through the liquid impregnated membranes showed that the interaction of the substrates only with the hydroxy groups at the lower rim of calix[4]arene is insufficient for binding carboxylic acids. Variation of the nature of substituents in the lateral fragments of 1,3-disubstituted calix[4]arenes makes it possible to construct selective receptors for compounds containing both the carboxyl and carboxylate functions. The basic difference in transfer properties of synthetic receptors based on the calix[4]arene platform and its thia analog was shown for the calix[4]arenes tetrasubstituted at the lower rim as an example.

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