Russian Journal of General Chemistry, Vol. 75, No. 2, 2005, pp. 278–284. Translated from Zhurnal Obshchei Khimii, Vol. 75, No. 2, 2005, pp. 305–312.

Choline Esterase Inhibitors and Synthetic Oxalic Acid Receptors Based on Calix[4]arene Derivatives

I. I. Stoikov, A. A. Khrustalev, D. Sh. Ibragimova, E. E. Stoikova, G. A. Evtyugin, I. S. Antipin, and A. I. Konovalov

Kazan State University, Kazan, Tatarstan, Russia

Received June 5, 2003

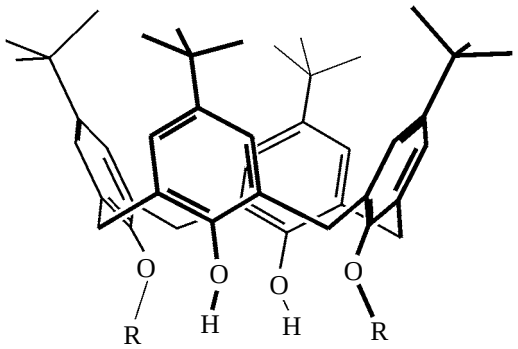
Abstract — New reversible butyrylcholine esterase inhibitors based on calix[4]arene derivatives were suggested. A series of new distally disubstituted calix[4]arenes were prepared in 60–80% yields. Some of these compounds showed properties of reversible choline esterase effectors, activating it at low concentrations and inhibiting at high concentrations. The macrocycles prepared were tested in extraction of *d*,*l*-tartaric, glycolic, *d*,*l*-mandelic, *d*,*l*-glutamic, malonic, oxalic, and succinic acids and of sodium acetate. Oxalic acid is efficiently transferred through a liquid impregnated membrane under the action of calix[4]arenes with nitrogen-containing substituents.

Various biochemical processes such as recognition, reaction, active and selective transport, and reception [1] are based on intermolecular interactions. Transformation of the genetic information into such cell functions as growth activation and regulation of enzymes [1] is based on recognition and selective binding of the protein surface with biopolymers. Active studies made in the past decade are aimed at development of synthetic small molecules whose target is protein–protein interaction [2, 3].

Relatively simple and synthetically accessible molecules capable to reversibly "switch" various functions of proteins are interesting as potential components of drugs enhancing the specificity of the active component and its selective transport in patient's body [4]. However, the high degree of solvation and large area of the protein surface complicate the designing of molecules capable to reversibly alter the protein function. Calixarenes, being low-toxic and containing several functionalization centers, show promise for development of new highly effective drugs.

We have shown recently that some distally substituted calix[4]arenes inhibit the hydrolysis of indophenyl acetate with butyrylcholine esterase, which is due to formation of host–guest complexes of calixarene with indophenyl acetate and localization of the resulting supramolecule near the active center of the enzyme [3].

To examine the influence of structural factors on the interaction of calix[4]arenes with a model protein, butyrylcholine esterase, we prepared a series of new 1,3-disubstituted (at the lower rim) *p*-*tert*-butylcalix[4]arenes containing proton-donor (**I**), proton-acceptor (**II**), and electron-acceptor (**III, IV**) groups. Dicarboxylic acid **I** was prepared by hydrolysis of the corresponding ester. Compounds **II**–**IV** were prepared by selective alkylation of *p*-*tert*-butylcalix[4]arene with the corresponding halo derivatives in acetonitrile in the presence of potassium carbonate.

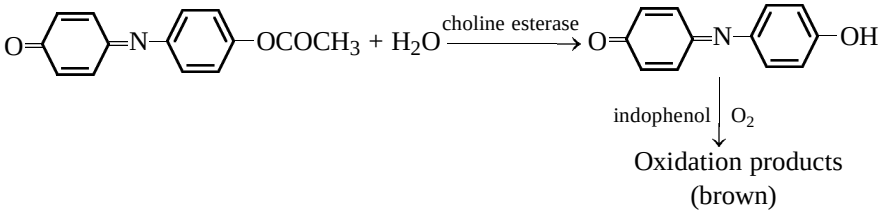


R = 4-CH_2–C_6H_4–COOH (**I**), 4-CH_2–C_5H_4N (**II**), 4-CH_2–C_6F_5 (**III**), 4-CH_2–C_6H_4O–(4-$C_6H_4NO_2$) (**IV**).

Calixarenes **I**–**IV** showed pronounced biological activity. In particular, they alter the activity of one of the key enzymes of higher animals, choline esterase, reversibly enhancing or suppressing its activity toward a specific substrate, indophenyl acetate.

Calixarenes exert an activating effect when in low concentrations and an inhibiting effect when in high concentrations (Table 1). The reversibility of the effect of calixarenes is confirmed by the independence

1070-3632/05/7502-0278



of their effect from the order of adding the reactants and by the constancy of the reaction rate change at varied time of contact of the enzyme and calixarene. The inversion of the biological effect was observed at a calixarene concentration of 1.9×10^{-5} M for **II** and **IV** at pH 7.6, 2.3×10^{-5} M for **IV** at pH 4, and 4.9×10^{-5} M for **II** at pH 4 (Fig. 1). The activating and inhibiting effects of the calixarenes are described by a common calibrating dependence in the coordinates enzymatic reaction–rate effector concentration. The only exception is calixarene **III**, which exerts no appreciable activating or inhibiting effect at pH 7.6 but behaves as an inhibitor in an acidic medium, in which $D_0/D_I = (1.005 \pm 0.020) + (0.018 \pm 0.004) \times 10^{-5}C$. The regular trends revealed allow the effect exerted by calixarenes on choline esterase to be controlled by varying the substituents and pH of the medium. This may be useful in development of cholinergic drugs.

One of the main approaches to binding of protein surfaces with synthetic receptors is based on recognition of carboxy groups on the protein surface [5]. Designing of molecules recognizing carboxylic acids is difficult, because selective recognition should involve at least three-point binding. In this connection, it seems promising to use calix[4]arenes. The calixarene core is sufficiently rigid and allows, by choosing appropriate functional groups, construction of molecules with the required surface topology, capable of highly selective recognition of definite substrates.

Table 1. Analytical characteristics of the determination of calixarenes **I**–**IV** by the biochemical effect (0.002 M TRIS buffer solution, pH 7.6)[a]

Calix-[4]-arene	*a*	*b*	*r*	$C \times 10^5$, M[b]
I	0.998 ± 0.030	–0.014 ± 0.001	–0.9845	0.22–54.60
II	0.909 ± 0.014	0.046 ± 0.003	0.9860	0.24–10.91
IV	0.790 ± 0.011	0.084 ± 0.003	0.9980	1.19–8.24

[a] (a, b) Coefficients of the equation $D_0/D_I = a + bC \times 10^5$, where D_0 and D_I are changes in the optical density in a control run and in the presence of calixarene; *C*, calixarene concentration; and *r*, correlation coefficient. [b] Range of measurable concentrations.

We have shown previously that calix[4]arenes containing two distally arranged aromatic substituents at the lower rim are capable of molecular recognition of

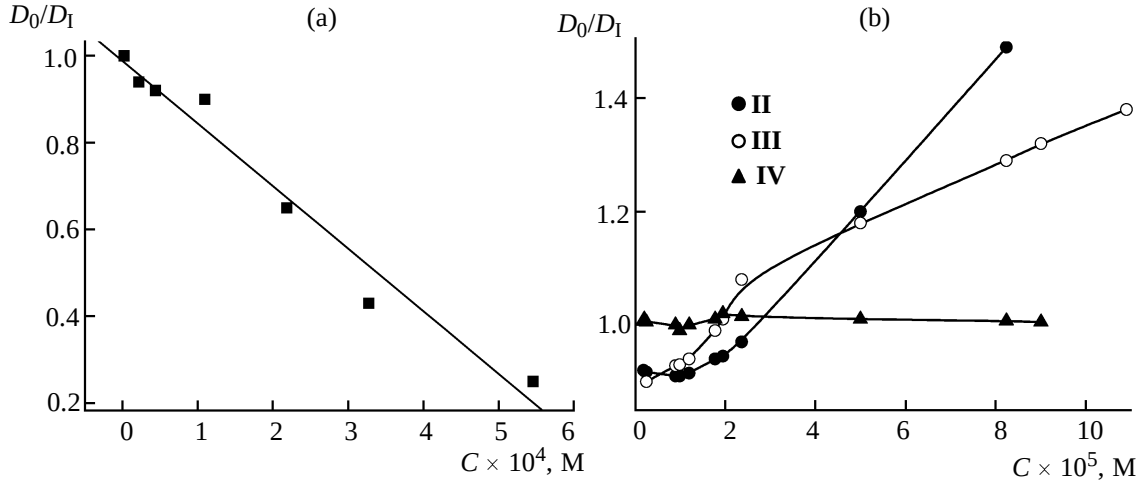
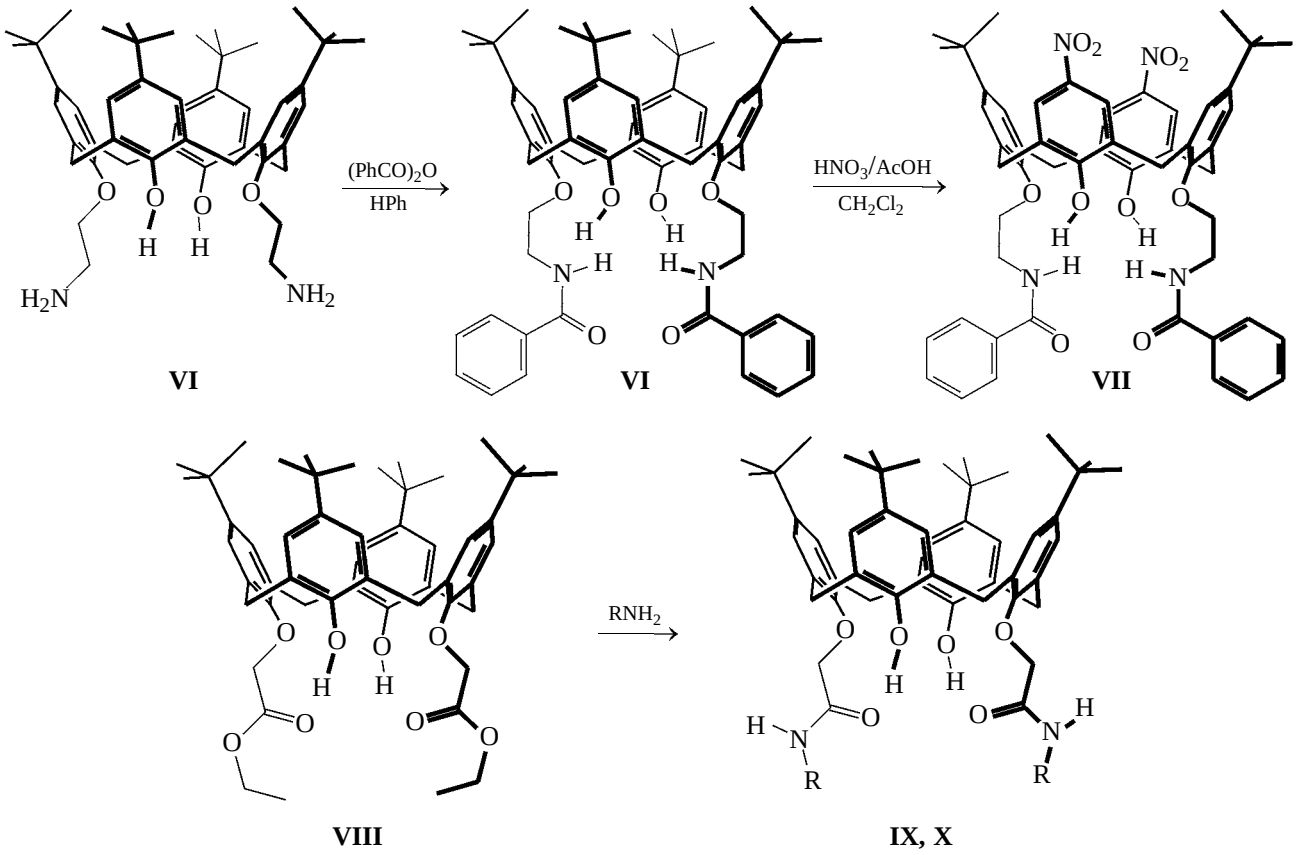


Fig. 1. Effect of calixarenes (a) **I** and (b) **II**–**IV** on the rate of product accumulation in hydrolysis of indophenyl acetate effected with choline esterase. Photometric detection; (D_0, D_I) optical densities of the solution in the absence and in the presence of calixarene, respectively. Measurement time 5 min; (*C*) calixarene concentration.

carboxylic acids [7]. To recognize substrates containing two carboxy groups, we introduced into the lower rim of calix[4]arene additional binding centers, amide groups, capable to interact with both proton-donor and proton-acceptor fragments of the compounds.

To vary such factors as the distance between the amide and hydroxy groups of calix[4]arene, sequence of atom binding in amide groups, lipophilicity, and acid–base properties of phenolic groups, we prepared a series of new calix[4]arene derivatives: **VI**, **VII**, **IX**, and **X**. By the reaction of diamine **V** [8] with benzoic anhydride in benzene, we prepared diamide **VI**; its subsequent selective *ipso* nitration with a mixture of nitric and acetic acids yielded dinitro derivative **VII**. Diamides **IX** and **X** were prepared by the reaction of **VIII** with excess amine at 110°C. In the ^{1}H NMR spectra of **I**–**X**, the bridging methylene protons of the macrocycles give two doublets, suggesting the *cone* conformation of the calixarenes [7].



VI **VI** **VII**

VIII **IX, X**

R = $(CH_2)_7CH_3$ (**IX**), CH_2Ph (**X**).

The complexing properties of the compounds prepared were evaluated by the membrane transport of substrates containing a carboxy group in the acidic [dicarboxylic acids (tartaric, oxalic, malonic, succinic), α-hydroxy acids (glycolic, mandelic)] or ionized (sodium acetate, glutamic acid) form. The lipophilic membrane was a solution of calixarene **I**–**IV**, **VI**, **VII**, or **IX** in *o*-nitrophenyl octyl ether **XI**, impregnated into pores of a Teflon matrix [7]. Compound **X** is poorly soluble, and, therefore, it was not used as a membrane-transfer agent.

The initial flows (j) and enhancement factors of the transfer ($\varepsilon = j/j_0$) of the substrates through a liquid impregnated membrane are given in Table 2. In the head row of the table, we also give the pK_a values [9] and the log octanol–water partition coefficients (log$P$) [10] characterizing the hydrophilicity of the compounds.

Addition of calix[4]arenes **I**–**IV**, **VI**, **VII**, and **IX** to the membrane phase affects the rate of the substrate transfer differently. For the majority of the macro-

Table 2. Initial flows [$j^a \pm \delta$,[b] mol h^{-1} m^{-2}] and enhancement factors of the transfer ($\varepsilon = j/j_0$, in parentheses) of a series of organic substrates through a liquid impregnated membrane (25°C)

Comp. no	*d,l*-Tartaric acid: log*P* –1.96, pK_a 2.89	Glycolic acid: log*P* –1.02, pK_a 3.83	*d,l*-Mandelic acid: log*P* 0.64, pK_a 3.37	*d,l*-Glutamic acid: log*P* –4.6, pK_a 4.33	Oxalic acid: log*P* –1.88, pK_a 1.25	Malonic acid: log*P* –0.49, pK_a 1.38	Amber acid: log*P* –0.47, pK_a 4.21	Succinic acetate: log*P* –4.24
I	$(1.6 \pm 0.1) \times 10^{-9}$ (1.0)	$(3.4 \pm 0.3) \times 10^{-9}$ (1.0)	$(5.4 \pm 0.5) \times 10^{-9}$ (1.0)	$(1.0 \pm 0.1) \times 10^{-9}$ (1.0)	$(1.8 \pm 0.1) \times 10^{-9}$ (1.0)	$(1.0 \pm 0.1) \times 10^{-9}$ (1.0)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)
II	$(1.7 \pm 0.1) \times 10^{-9}$ (1.0)	$(18.1 \pm 1.5) \times 10^{-9}$ (3.5)	$(2.01 \pm 0.1) \times 10^{-6}$ (3.7)	$(1.0 \pm 0.1) \times 10^{-9}$ (1.0)	$(5.4 \pm 0.3) \times 10^{-7}$ (299.0)	$(3.55 \pm 0.3) \times 10^{-8}$ (3.6)	$(52.3 \pm 3.5) \times 10^{-9}$ (11.4)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)
III	$(1.6 \pm 0.1) \times 10^{-9}$ (1.0)	$(3.4 \pm 0.3) \times 10^{-9}$ (1.0)	$(5.4 \pm 0.5) \times 10^{-7}$ (1.0)	$(1.0 \pm 0.1) \times 10^{-9}$ (1.0)	$(1.8 \pm 0.1) \times 10^{-7}$ (1.0)	$(1.0 \pm 0.1) \times 10^{-8}$ (1.0)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)
IV	$(1.6 \pm 0.1) \times 10^{-9}$ (1.0)	$(3.4 \pm 0.3) \times 10^{-9}$ (1.0)	$(5.4 \pm 0.5) \times 10^{-7}$ (1.0)	$(1.0 \pm 0.1) \times 10^{-9}$ (1.0)	$(6.8 \pm 0.5) \times 10^{-7}$ (3.8)	$(1.0 \pm 0.1) \times 10^{-8}$ (1.0)	$(5.9 \pm 0.5) \times 10^{-9}$ (1.3)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)
VI	$(1.1 \pm 0.09) \times 10^{-8}$ (6.9)	$(9.2 \pm 0.8) \times 10^{-9}$ (2.7)	$(12.3 \pm 1.2) \times 10^{-7}$ (2.3)	$(1.0 \pm 0.1) \times 10^{-9}$ (1.0)	$(8.8 \pm 0.5) \times 10^{-8}$ (48.9)	$(8.4 \pm 0.5) \times 10^{-8}$ (8.4)	$(15.6 \pm 1.2) \times 10^{-9}$ (3.4)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)
VII	$(2.2 \pm 0.2) \times 10^{-9}$ (1.4)	$(7.5 \pm 0.6) \times 10^{-9}$ (2.2)	$(9.2 \pm 0.9) \times 10^{-7}$ (1.7)	$(25.4 \pm 1.8) \times 10^{-9}$ (25.0)	$(2.7 \pm 0.2) \times 10^{-8}$ (15.0)	$(4.1 \pm 0.4) \times 10^{-8}$ (4.0)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)	$(4.6 \pm 0.4) \times 10^{-9}$ (1.0)
IX	$(1.9 \pm 0.2) \times 10^{-9}$ (1.2)	$(14.1 \pm 1.1) \times 10^{-9}$ (4.1)	$(19.3 \pm 1.5) \times 10^{-7}$ (3.6)	$(5.8 \pm 0.4) \times 10^{-9}$ (4.5)	$(1.8 \pm 0.1) \times 10^{-9}$ (1.0)	$(1.0 \pm 0.1) \times 10^{-8}$ (1.0)	$(5.0 \pm 0.4) \times 10^{-9}$ (1.1)	$(48.3 \pm 3.3) \times 10^{-9}$ (10.5)
XI[c]	$(1.6 \pm 0.1) \times 10^{-9}$	$(3.4 \pm 0.3) \times 10^{-9}$	$(5.4 \pm 0.3) \times 10^{-7}$	$(1.0 \pm 0.1) \times 10^{-9}$	$(1.8 \pm 0.1) \times 10^{-9}$	$(1.0 \pm 0.1) \times 10^{-8}$	$(4.6 \pm 0.4) \times 10^{-9}$	$(4.6 \pm 0.3) \times 10^{-9}$

[a] Membrane area *S* 9.616 cm^2. [b] *p* 95%. [c] Without transfer agent (j_0).

cycles, *j* and ε increase with an increase in pK_a (except data for oxalic acid). This trend is apparently associated with the acid–base interaction of the acid with the receptor. It should be noted that there is no correlation between the acid lipophilicity (log*P*) and enhancement factor of substrate transfer. With the most lipophilic substrate, mandelic acid, the enhancement factors are insignificant.

Table 2 shows that calix[4]arene **II** with pyridine fragments is the most effective and selective transfer agent. It transfers oxalic acid (the strongest acid in the examined series) with an enhancement factor of 299.0, whereas with succinic acid the enhancement factor is only 11.4. The results of the membrane extraction reveal a significant influence of not only acid–base interaction (oxalic acid, being the strongest, is transferred better), but also geometric matching of the transfer agent and substrate molecules.

Macrocycle **VI** appeared to be the next after **II** in the efficiency and selectivity; it enhances the oxalic acid flow by a factor of 48.9. The enhancement factors of the transfer ε of its nearest homologs, malonic and succinic acids, are as low as 8.4 and 3.4, respectively. The selectivity of **VI** toward oxalic acid is apparently due to formation of a complex in which both carboxy groups of oxalic acid interact with two amide fragments of the transfer agent. With an increase in the acidity of phenolic hydroxyls (compound **VII**), the transport selectivity changes: Oxalic acid is transferred with ε 15, and glutamic acid, with ε 25. Diamide **IX** with the shortest spacer and different orientation of the amide fragments selectively transfers sodium acetate through a lipophilic membrane (ε 10).

Macrocycles **I** and **III** containing acceptor substituents at the lower rim of the calixarene form another group of transfer agents. Addition of these calixarenes to the membrane phase does not affect the rate of transfer of any of the substrates studied. Apparently, interaction of substrates with only the hydroxy groups at the lower rim of the calixarene is insufficient for

binding of carboxylic acids and their extraction into the membrane phase [7]. In the case of calix[4]arenes disubstituted at the lower rim and containing aromatic groups in lateral fragments, the OH–π hydrogen bonding contributes to the acid–transfer agent complexation [7]. Apparently, the strength of such a bond will decrease on introducing electron-withdrawing groups into aromatic substituents of calix[4]arene. We found that addition of 4-(4'-nitrophenyloxy)benzyl derivative **IV** into the membrane phase slightly (by a factor of 1.3–3.3) enhanced the transport of the examined substrates, compared to the blank experiment. Such a behavior suggests formation of a transfer agent–substrate complex whose energy is insufficient to compensate the energy consumption for the desolvation of the strongly hydrophilic substrates in water and their transfer to the lipophilic phase of the membrane.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer (300 MHz). The chemical shifts were determined relative to the residual proton signals of the solvent ($CDCl_3$). The electron impact mass spectra with determination of the exact masses of molecular and fragment ions were taken on an MKh-1310 mass spectrometer (60 eV, 200–450°C).

The inhibiting effect of calixarenes on native choline esterase was studied photometrically with an AKI-Ts-01 colorimetric analyzer equipped with a 530-nm color filter [3]. Butyrylcholine esterase from horse blood serum (EC 3.1.1.8, class 4, specific activity 16 units mg^{-1}) was purchased from the Biomed Research and Production Association (Perm, Russia); indophenyl acetate, from Sigma (concentration in the measurement cell 0.03472 mg ml^{-1}); and TRIS buffer solution (analytically pure grade, 0.002 M), from Reakhim (Russia). Calixarene solutions were prepared by dissolving a weighed portion of the substance in acetone to obtain a 1 mg ml^{-1} solution and diluting with the buffer solution to the required concentration. The rate of the choline esterase reaction was determined from the amount of the product, indophenol, formed by enzymatic hydrolysis of indophenyl acetate. The reaction was performed in a cell of a standard kit for immunochemical studies.

The reaction rate was determined from the slope of the initial linear portion of the kinetic curve in the solution optical density–time coordinates. The activating and inhibiting effects were estimated from the relative change in the reaction rate in the presence of calixarenes, which were added directly to the measurement cell containing the enzyme solution before or simultaneously with adding the substrate.

The membrane extraction of substrates with calixarenes was studied following the procedure from [7]. The electrical conductivity of solutions was measured with a WTW inoLab-Cond Level-1 conductometer. The following chemicals (all chemically pure grade) were used: *d,l*-mandelic, glycolic, *d,l*-tartaric, *d,l*-glutamic, oxalic, malonic, and succinic acids and sodium acetate. The rate of substrate transport across liquid membranes was measured in a temperature-controlled vertical glass diffusion cell with a mobile cylinder. A hydrophobic matrix (Millipole Type-FA porous Teflon filters, thickness 1 μm, pore size 100 nm, 85% porosity, reinforced with polycaprolactam gauze) was impregnated with a liquid membrane. The substance concentrations were determined from the electrical conductivities of the solutions. The volume ratio of the feeding and receiving phases was 5 : 1, which provided the same level of solutions to eliminate osmotic transfer. The mass transfer experiments were performed under standard conditions (25°C). The initial substrate solutions were prepared by dissolving an accurately weighed substrate portion in double-distilled water just before the measurements; the solutions were used within a day. The liquid membrane was a solution of calix[4]arene **VI**–**X** in an organic diluent or a straight diluent (*o*-nitrophenyl octyl ether **XI**).

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis[4'-(carboxy)benzyloxy]calix[4]arene I. A mixture of 1.00 g of 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis[4'-(carboxyethyl)benzyloxy]calix[4]arene [7], 10 ml of 10% aqueous NaOH, and 10 ml of dimethyl sulfoxide in 100 ml of ethanol was refluxed for 36 h until the starting compound fully dissolved. Then the solvent was removed in a vacuum, 30 ml of water was added to the residue, concentrated HCl was added to pH 1, and the mixture was stirred for 30 min. The precipitate was filtered off and washed on a glass frit with water to neutral reaction of the filtrate. The colorless crystalline precipitate was recrystallized from ethanol. Yield 0.90 g (71%), mp 168–169°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.03 s [18H, $C(CH_3)_3$], 1.31 s [18H, $C(CH_3)_3$], 3.42 d (4H, $ArCH_{2e}Ar$, $^2J_{HH}$ 12.6), 4.39 d (4H, $ArCH_{2a}Ar$, $^2J_{HH}$ 12.6), 5.16 s (4H, OCH_2Ar), 6.93 s (4H, ArH), 7.10 s (4H, ArH), 7.79 s (2H, OH), 7.91 d (4H, $CH_2C_6H_4CO_2H$, $^3J_{HH}$ 8.4), 7.97 d (4H, $CH_2 \cdot C_6H_4CO_2H$, $^3J_{HH}$ 8.4). Found, %: C 78.19; H 7.93. $C_{60}H_{68}O_8$. Calculated, %: C 78.57; H 7.47. Found *m/z* 916.4919 $[M]^+$. Calculated *M* 916.4914.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(4'-pyridylmethoxy)calix[4]arene II. A mixture of 1.00 g of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene, 3.14 g of anhydrous potassium carbonate, and 1.15 g of anhydrous sodium

iodide in 150 ml of acetonitrile was refluxed with stirring under argon for 30 min. Then a solution of 0.54 g of 4-chloromethylpyridine hydrochloride in 30 ml of anhydrous methanol was added over a period of 2 h, after which the mixture was refluxed with stirring for an additional 20 h. Then the mixture was cooled to 50°C and filtered on a glass frit; the precipitate was washed with acetonitrile (2 × 30 ml), and the solvent from the combined filtrates was removed. The dry residue was dispersed in 150 ml of water for 30 min, filtered off on a glass frit, and washed with water until the filtrate became colorless, after which it was recrystallized from a mixture of 50 ml of diethyl ether and 15 ml of acetonitrile. The colorless needle-like precipitate was dried in a vacuum (0.01 mm Hg) at 100°C for 12 h. Yield 0.79 g (62%), mp 108–109°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.93 s [18H, $C(CH_3)_3$], 1.30 s [18H, $C(CH_3)_3$], 3.33 d (4H, ArCH_{2e}Ar, $^2J_{HH}$ 13.4), 4.25 d (4H, ArCH_{2a}Ar, $^2J_{HH}$ 13.4), 5.07 s (4H, OCH_2Pyr), 6.79 s (4H, ArH), 6.97 s (2H, OH), 7.07 s (4H, ArH), 7.64 d (4H, $CH_2C_5H_4N$, $^3J_{HH}$ 6.1), 8.63 d (4H, $CH_2C_5H_4N$, $^3J_{HH}$ 6.1). 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. Found *m/z* 830.5028 $[M]^+$. Calculated *M* 830.5023.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-disubstituted calix[4]arenes III and IV *(general procedure)*. A mixture of 1.54 mmol of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene, 3.24 mmol of appropriate alkyl bromide, and 13.90 mmol of anhydrous potassium carbonate in 30 ml of acetonitrile was refluxed with stirring for 12 h. Then the solvent was removed in a vacuum, and the dry residue was treated with 10 ml of 5 M HCl and 50 ml of $CHCl_3$. The organic phase was separated, washed with distilled water (3 × 30 ml), and dried over 4 Å molecular sieves. The solvent was removed on a rotary evaporator, and the residue was recrystallized from chloroform–methanol.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(pentafluorophenylmethoxy)calix[4]arene III. Yield 1.33 g (86%), mp 222°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.94 s [18H, $C(CH_3)_3$], 1.30 s [18H, $C(CH_3)_3$], 3.28 d (4H, ArCH_{2e}Ar, $^2J_{HH}$ 13.1), 4.22 d (4H, ArCH_{2a}Ar, $^2J_{HH}$ 13.1), 5.09 s (4H, OCH_2), 6.39 s (2H, OH), 6.75 s (4H, ArH), 7.06 s (4H, ArH). Found, %: C 68.73; H 5.85. $C_{58}H_{58}F_{10}O_4$. Calculated, %: C 69.04; H 5.79. Found *m/z* 1008.4179 $[M]^+$. Calculated *M* 1008.4175.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis[4'-(4''-nitrophenyloxy)benzyloxy]calix[4]-arene IV. Yield 1.60 g (94%), mp 128°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.95 s [18H, $C(CH_3)_3$], 1.30 s [18H, $C(CH_3)_3$], 3.31 d (4H, ArCH_{2e}Ar, $^2J_{HH}$ 13.3), 4.28 d (4H, ArCH_{2a}Ar, $^2J_{HH}$ 13.3), 5.06 s (4H, OCH_2Ar), 6.80 s (4H, ArH), 7.08 s (4H, ArH), 7.15 s (2H, OH), 6.97 d (4H, $CH_2C_6H_4O$, $^3J_{HH}$ 8.9), 7.03 d (4H, $CH_2C_6H_4O$, $^3J_{HH}$ 8.9), 7.71 d (4H, $OC_6H_4NO_2$, $^3J_{HH}$ 8.8), 8.16 d (4H, $OC_6H_4NO_2$, $^3J_{HH}$ 8.8). Found, %: C 75.98; H 6.89; N 2.61. $C_{70}H_{74}N_2O_{10}$. Calculated, %: C 76.20; H 6.76; N 2.54. Found *m/z* 1102.5340 $[M]^+$. Calculated *M* 1102.5344.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis[2'-(benzoylamino)ethoxy]-26,28-dihydroxycalix[4]arene VI. A mixture of 4.00 g of 5,11,17,23-tetra-*tert*-butyl-25,27-bis(2'-aminoethoxy)-26,28-dihydroxycalix[4]arene **V** [8], 2.61 g of benzoic anhydride, and 30 ml of benzene was refluxed for 2 h. Then the solvent was removed in a vacuum, the dry residue was dispersed for 30 min in 150 ml of a saturated solution of sodium carbonate, the suspension was filtered on a glass frit, and the precipitate was washed with water (3 × 50 ml) and dried over P_2O_5. The resulting product was dissolved in a minimal amount of dichloromethane and precipitated with hexane. The colorless precipitate was dried in a vacuum (0.01 mm Hg) at 100°C for 12 h. Yield 2.70 g (59%), mp 135–136°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (J, Hz): 1.12 s [18H, $C(CH_3)_3$], 1.23 s [18H, $C(CH_3)_3$], 3.39 d (4H, ArCH_{2e}Ar, $^2J_{HH}$ 14.1), 3.62 d.t (4H, CH_2N, $^3J_{HH}$ 4.5, $^3J_{HH}$ 4.9), 4.03 t (4H, OCH_2, $^3J_{HH}$ 4.5), 4.18 d (4H, ArCH_{2a}Ar, $^2J_{HH}$ 14.1), 7.02 s (4H, ArH), 7.07 s (4H, ArH), 7.31 d.d (4H, ArH, $^3J_{HH}$ 7.4, $^3J_{HH}$ 7.1), 7.43 t.t (2H, ArH, $^3J_{HH}$ 7.4, $^4J_{HH}$ 1.3), 7.95 d.d (4H, ArH, $^3J_{HH}$ 7.1, $^4J_{HH}$ 1.3), 8.21 t (2H, NH, $^3J_{HH}$ 4.9), 8.39 s (2H, OH). 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. Found *m/z* 942.5551 $[M]^+$. Calculated *M* 942.5547.

5,17-Di-*tert*-butyl-11,23-dinitro-25,27-bis-[2'-(benzoylamino)ethoxy]-26,28-dihydroxycalix[4]-arene VII. A mixture of 0.47 g of 5,11,17,23-tetra-*tert*-butyl-25,27-bis[2'-(benzoylamino)ethoxy]-26,28-dihydroxycalix[4]arene **VI**, 50 ml of dichloromethane, 2.9 ml of glacial acetic acid, and 5.6 ml of 65% nitric acid was stirred for 30 min at room temperature, after which it was poured into 50 ml of water. The organic layer was washed with two portions of water and dried over magnesium sulfate. The solvent was removed in a vacuum. The residue was recrystallized from a dichloromethane–hexane mixture. Yield 0.11 g (28%), mp 135°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.01 s [18H, $C(CH_3)_3$], 3.49 d (4H, ArCH_{2e}Ar, $^2J_{HH}$ 13.4), 3.82 d.t (4H, CH_2N, $^3J_{HH}$ 4.8, $^3J_{HH}$ 5.5), 4.12 t (4H, OCH_2, $^3J_{HH}$ 4.8), 4.18 d (4H, ArCH_{2a}Ar, $^2J_{HH}$ 13.4), 6.90 s (4H, ArH), 7.30 m (4H, ArH), 7.44 m (2H, ArH), 7.69 t (2H, NH, $^3J_{HH}$ 5.5), 7.86 d (4H, ArH, $^3J_{HH}$ 7.5), 8.04 s (4H, ArH), 8.94 s

(2H, OH). 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. Found *m/z* 920.4001 $[M]^+$. Calculated *M* 920.3996.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(*N*-octylcarbamoylmethoxy)-26,28-dihydroxycalix[4]arene IX. A mixture of 1.58 g of 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(ethoxycarbonylmethoxy)-calix[4]arene **VIII** [11], 9.5 ml of octylamine, and 0.16 g of ammonium chloride was stirred at the boiling point of octylamine for 2.5 h. Then excess octylamine was distilled off, and the residue was washed with water (2 × 10 ml) and 1 M HCl (3 × 10 ml). The mixture was separated by flash chromatography on 30 g of silica gel L 50/40 (first eluent 10 ml of chloroform–isopropanol, 2 : 1; second, 10 ml of chloroform–isopropanol, 1 : 1; and third, 10 ml of chloroform–isopropanol, 1 : 2). The solvent was removed in a vacuum. The reaction product, colorless glassy mass, was dried in a vacuum (0.001 mm Hg) at 100°C for 12 h. Yield 1.10 g (60%). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.84 t [6H, C*H*$_3$(CH$_2$)$_7$, $^3J_{HH}$ 6.9], 1.05 s [18H, C(CH$_3$)$_3$], 1.27 s [18H, C(CH$_3$)$_3$], 1.36–1.10 m [24H, CH$_2$(C*H*$_2$)$_6$CH$_3$], 3.38 d.t [4H, NHC*H*$_2$(CH$_2$)$_6$CH$_3$, $^3J_{HH}$ 5.2, $^3J_{HH}$ 6.7], 3.43 d (4H, ArC*H*$_{2e}$Ar, $^2J_{HH}$ 13.2), 4.12 d (4H, ArC*H*$_{2a}$Ar, $^2J_{HH}$ 13.2), 4.56 s [4H, OC*H*$_2$C(O)NH], 6.94 s (4H, ArH), 7.08 s (4H, ArH), 7.86 s (2H, OH), 8.84 t (2H, NH, $^3J_{HH}$ 5.2). 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. Found *m/z* 986.7121 $[M]^+$. Calculated *M* 986.7112.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(*N*-benzylcarbamoylmethoxy)-26,28-dihydroxycalix[4]arene VI. A mixture of 0.5 g of 5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(ethoxycarbonylmethoxy)-calix[4]arene **VIII** [11], 3 ml of benzylamine, and 0.05 g of ammonium chloride was stirred at the boiling point of benzylamine for 1 h. Then the mixture was poured into 10 ml of water, and the resulting gelatinous mass was washed on a glass frit with 1 M HCl (5 × 10 ml). The white precipitate was recrystallized from dichloromethane–hexane. Yield 0.20 g (38%), mp 266°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.97 s [18H, C(CH$_3$)$_3$], 1.27 s [18H, C(CH$_3$)$_3$], 3.28 d (4H, ArC*H*$_{2e}$Ar, $^2J_{HH}$ 13.3), 3.79 d (4H, ArC*H*$_{2a}$Ar, $^2J_{HH}$ 13.3), 4.35 s [4H, OC*H*$_2$·N(O)NH], 4.52 d (4H, NHC*H*$_2$Ph, $^3J_{HH}$ 4.3), 6.80 s (4H, ArH), 6.99 s (2H, OH), 7.00 s (4H, ArH), 8.91 t (2H, NH, $^3J_{HH}$ 4.3). Found, %: C 78.37; H 8.05; N 3.01. $C_{62}H_{74}N_2O_6$. Calculated, %: C 78.95; H 7.91; N 2.97. Found *m/z* 942.5551 $[M]^+$. Calculated *M* 942.5547.

ACKNOWLEDGMENTS

The study was financially supported by the RF Ministry of Education (project no. PD 02-1.3-95), Basic Research and Higher Education (BRHE) program (grant REC-007), and Russian Foundation for Basic Research (project nos. 02-03-32888, 02-03-32934, and 03-03-96185).

REFERENCES

1. Elliott, W.H. and Elliott, D.C., *Biochemistry and Molecular Biology*, Oxford Univ. Press, 1997; 2001, 2nd ed. Translated under the title *Biokhimiya i molekulyarnaya biologiya*, Moscow: Nauka/Interperiodica, 2002, p. 444.
2. Park, H.S., Lin, Q., and Hamilton, A.D., *J. Am. Chem. Soc.*, 1999, vol. 121, no. 1, p. 8.
3. Stoikova, E.E., Evtugyn, G.A., Beljakova, S.V., Khrustalev, A.A., Stoikov, I.I., Antipin, I.S., Budnikov, H.C., and Konovalov, A.I., *J. Inclus. Phenom.*, 2001, vol. 39, no. 3/4, p. 339.
4. Peczuh, M.W., Hamilton, A.D., Sanchez-Quesada, J., Mendoza, J. de, Haack, T., and Giralt, E., *J. Am. Chem. Soc.*, 1997, vol. 119, no. 39, p. 9327.
5. Mandolini, L. and Ungaro, R., *Calixarenes in Action*, Imperial College Press, 2000, p. 271.
6. Asfani, Z., Bohmer, V., Harrowfield, J., Vicens, J., and Saadioui, M., *Calixarenes 2001*, Dordrecht: Kluwer Academic, 2001, p. 683.
7. Antipin, I.S., Stoikov, I.I., Khrustalev, A.A., and Konovalov, A.I., *Izv. Ross. Akad. Nauk, Ser. Khim.*, 2001, no. 11, p. 2038.
8. Wolf, N.J., Georgiev, E.M., Yordanov, A.T., Whittlesey, B.R., Koch, H.F., and Roundhill, D.M., *Polyhedron*, 1999, vol. 18, p. 885.
9. Dawson, R., Elliott, D., Elliott, W., and Jones, K., *Data for Biochemical Research*, Oxford: Clarendon, 1986, p. 544.
10. Stoikov, I.I., Repeikov, S.A., Antipin, I.S., and Konovalov, A.I., *Heteroatom Chem.*, 2000, no. 11, p. 518.
11. Collins, E.M., McKervey, M.A., Madigan, E., Moran, M.B., Owens, M., Ferguson, G., and Harris, S.J., *J. Chem. Soc., Perkin Trans. 1*, 1991, no. 3, p. 3137.