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New membrane carrier for glutamic acid based on *p-tert*-butylcalix[4]arene 1,3-disubstituted at the lower rim

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Calix[4]arenes 1,3-disubstituted at the lower rim were synthesized and their receptor ability toward dicarboxylic, α-hydroxy and amino acids was investigated. A new synthetic receptor for glutamic acid based on 1,3-disubstituted calix[4]arene was suggested.

Recently, attention has been focused on modeling synthetic receptors able to specifically change and control biomacromolecule functions. Simple and synthetically available molecules able to switch reversibly various functions of proteins can be requested for modeling biological processes and developing new drug delivery systems and pharmaceutical screening. The selective binding of a protein surface is mainly based on the recognition of amino and carboxylic groups of prevailing amino acid residues by means of synthetic molecular platform.[1]

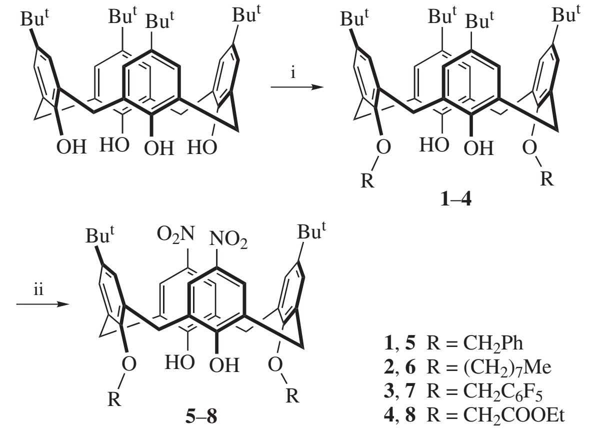
Among other synthetic receptors, calixarenes are of special interest because of their applicability to designing three-dimensional structures with various sizes of internal cavities, numbers and types of binding centers, spatial arrangements of binding groups and the ability to form asymmetric cavities and affect the balance between the hardness and flexibility of a receptor.[2,3]

Previously, it was shown[4] that di- and tetrasubstituted calix[4]arenes with various functional groups at the lower rim are able to molecular recognition of oxalic acid. The efficiency of interaction of calix[4]arene receptors with the acids depended on various factors, *i.e.* π-system area of the substituents, their structural accepting characteristics, geometrical complementarities of the binding centers, and the acid-base properties of free phenolic groups.

In this work, the molecular design of the receptor structures was performed for the recognition of biologically significant acids. For this purpose, *p-tert*-butylcalix[4]arenes **1**–**8** substituted at the lower rim were synthesized and their ability to efficient and selective transport of dicarboxylic and α-hydroxy acids through a lipophilic membrane was investigated. In addition, glutamic acid was used as a substrate to study the interaction of the compounds with amino acids. In compounds **1**–**8**, the nature of substituents at the lower rim of calixarene macrocycle and the acidity of phenolic protons were varied to affect the transport ability of the receptors toward various substrates.

Calix[4]arene derivatives **1**–**4**[5–7] disubstituted at the lower rim were synthesized with yields of 60–80% by selective alkylation[8,9] of *p-tert*-butylcalix[4]arene with appropriate alkyl halides in acetonitrile in the presence of potassium carbonate. Compounds **5**[7]–**8** were synthesized by the nitration of disubstituted calix[4]arene obtained with nitric acid in methylene chloride in the presence of acetic acid at ~20 °C.[10] The structures of compounds **6**–**8** (Scheme 1) were characterized by physical methods.[†]

The complexation ability of compounds **1**–**8** toward selected acids was investigated by membrane extraction.[‡] The experiments on the transport of dicarboxylic, α-hydroxy- and α-amino acids through lipophilic liquid membranes induced by calixarenes **1**–**8** were performed. The fluxes (j_i) through a membrane were



1–**4**

5–**8**

1, **5** R = CH_2Ph
2, **6** R = $(CH_2)_7Me$
3, **7** R = $CH_2C_6F_5$
4, **8** R = CH_2COOEt

Scheme 1 *Reagents and conditions*: i, see refs. 4–7; ii, 160 equiv. of 65% nitric acid, 100 equiv. of acetic acid, CH_2Cl_2, reflux.

[†] *General procedure of the synthesis of 5,17-di-*tert*-butyl-11,23-dinitro-25,27-disubstituted-26,28-dihydroxycalix[4]arenes* **6**–**8**. 65% Nitric acid (5.6 ml, 80 mmol) was added to 0.5 mmol of an appropriate disubstituted *p-tert*-butylcalix[4]arene dissolved in a mixture of 50 ml of CH_2Cl_2 and 2.9 ml (50 mmol) of acetic acid. The reaction mixture was stirred for 30 min at 20 °C. Then, the mixture was poured out in 50 ml of water. The organic phase was separated and dried with molecular sieves 4 Å. The solvent was evaporated to dryness, and the precipitate was recrystallized from CH_2Cl_2–ethanol.

*5,17-Di-*tert*-butyl-11,23-dinitro-25,27-dioctyloxy-26,28-dihydroxycalix[4]arene* **6**: yield 0.19 g (44%), yellow powder, mp 156–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 [t, 6H, $OCH_2(CH_2)_6Me$, $^3J_{HH}$ 6.5 Hz], 1.11 (s, 18H, CMe_3), 1.25–1.50 [m, 16H, $O(CH_2)_2CH_2(CH_2)_4Me$], 1.67 [tt, 4H, $O(CH_2)_2CH_2(CH_2)_4Me$, $^3J_{HH}$ 7.4 Hz, $^3J_{HH}$ 7.6 Hz], 2.08 [tt, 4H, $OCH_2CH_2(CH_2)_5Me$, $^3J_{HH}$ 6.8 Hz, $^3J_{HH}$ 7.6 Hz], 3.48 (d, 4H, $ArCH_{2ax}Ar$, $^2J_{HH}$ 13.2 Hz), 4.02 [t, 4H, $OCH_2(CH_2)_6Me$, $^3J_{HH}$ 6.8 Hz], 4.28 (d, 4H, $ArCH_{2eq}Ar$, $^2J_{HH}$ 13.2 Hz), 6.97 (s, 4H, H_{Ar}), 8.04 (s, 4H, H_{Ar}), 9.44 (s, 2H, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 22.6, 25.9, 29.3, 29.4, 29.9, 31.1, 31.5, 31.9, 34.2, 124.3, 126.2, 128.7, 131.3, 139.8, 148.5, 149.7, 159.6. IR (vaseline oil, ν/cm^{-1}): 3238 (OH), 1208 (COC). Found (%): C, 74.55; H, 8.56; N, 3.36. Calc. for $C_{52}H_{70}N_2O_8$ (%): C, 73.38; H, 8.29; N, 3.29.

*5,17-Di-*tert*-butyl-11,23-dinitro-25,27-bis(pentafluorophenyl)-26,28-dihydroxycalix[4]arene* **7**: yield 0.23 g (46%), yellow powder, mp 238–239 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (s, 18H, CMe_3), 3.26 (d, 4H, $ArCH_{2ax}Ar$, $^2J_{HH}$ 13.2 Hz), 4.21 (d, 4H, $ArCH_{2eq}Ar$, $^2J_{HH}$ 13.2 Hz), 5.08 (s, 4H, OCH_2), 6.35 (s, 2H, OH), 6.73 (s, 4H, H_{Ar}), 7.05 (s, 4H, H_{Ar}). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.0, 31.1, 34.2, 64.4, 124.4, 126.5, 128.4, 130.8, 139.9, 149.1, 149.4, 159.0. IR (vaseline oil, ν/cm^{-1}): 3389 (OH), 2964 (CH), 1261 (COC).

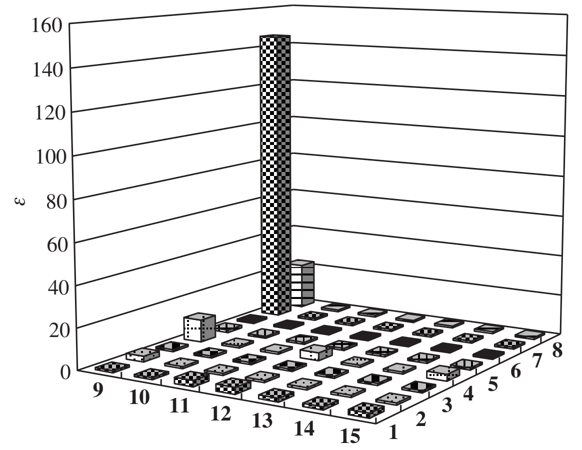


Figure 1 Enhancement factor for the transport of organic substrates **9**–**15** through the liquid impregnated membrane containing carriers **1**–**8**.

calculated from initial linear pieces of the time dependence of the concentration of the transferred substance to accepting phase. The fluxes (j_0) with the membrane impregnated by a pure solvent (*o*-nitrophenyl octyl ether) were used in a blank experiment.

The measurements of the flux enhancement factor ($\varepsilon = j_i/j_0$) of glutamic **9**, tartaric **10**, oxalic **11**, glycolic **12**, malonic **13**, succinic **14** and mandelic **15** acids are illustrated in Figure 1. The inclusion of compounds **1**–**8** in the membrane phase results in different enhancement factors for the transport ($\varepsilon = j_i/j_0$) of substrates **9**–**15** through the liquid impregnated membranes.

Using compound **2**, the influence of alkyl substituents on the mass transfer of the investigated acids promoted by calixarenes was explored. Recently, we have shown[4] that carriers based on 1,3-disubstituted calix[4]arene with aromatic, pentafluorophenyl and ester fragments at the lower rim demonstrated low transport ability referred to blank experiment. It was found that carrier **2** was also inefficient and did not show any transport abilities toward almost all of the investigated substrates. The results confirmed the conclusion[4] that the interaction of the substrates with 1,3-disubstituted calix[4]arene, which contained only hydroxyl groups at the lower rim, were insufficient for binding and extracting hydrophilic carboxylic acids into a lipophilic membrane phase.

To estimate the influence of acidity of free hydroxyl groups on the efficiency of interaction with calix[4]arenes **9**–**15**, two *tert*-butyl substituents in the *p*-position against hydroxyl groups were replaced by electrophilic nitro groups. The replacement of two *tert*-butyl substituents by nitro groups in compounds **1**, **2** and **4** does not lead to changes in the rate of mass transfer for such acids as oxalic, tartaric, glycolic, malonic, succinic and mandelic for carriers **5**, **6** and **8**. For glutamic acid, it was observed that the acceptor groups promoted complexation and hence increased the flux rate. In case of compound **8**, the acceleration of the transport of glutamic acid through lipophilic liquid membrane by a factor of 23 was observed. Probably, this can be related to the interaction of the functional groups of the substrates both with ester and hydroxylic fragments of the receptor.

The results obtained for two structurally similar compounds **3** and **7** are of particular interest. The inclusion of carrier **3** into the membrane did not result in an increase in the flow rates of the investigated substrates.[4] However, an opposite result was observed for calix[4]arene **7**. The replacement of two *tert*-butyl substituents by electrophilic nitro groups made it possible to obtain an efficient receptor for glutamic acid. Compound **7** showed an increase in the mass transfer of glutamic acid by a factor of 146.

Thus, the kinetics of membrane transport and the fluxes of the substrates through the liquid impregnated membranes was established in this investigation. An efficient carrier for glutamic acid was found in the series of 1,3-disubstituted calix[4]arenes. The experimental relationships make it possible to shift directionally the receptor abilities of 1,3-disubstituted calix[4]arenes by varying their substituents. The introduction of nitro groups at the upper rim of calix[4]arene resulted in a novel synthetic receptor for glutamic acid.

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*5,17-Di-*tert*-butyl-11,23-dinitro-25,27-bis(ethoxycarbonylmethoxy)-26,28-dihydroxycalix[4]arene* **8**: yield 0.22 g (55%), yellow powder, mp 185–186 °C (CH_2Cl_2–EtOH) [lit.,[11] 198–200 °C ($CHCl_3$–light petroleum)]. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.11 (s, 18H, CMe_3), 1.36 (t, 6H, $CH_2\mathit{Me}$, $^3J_{HH}$ 7.1 Hz) 3.48 (d, 4H, $ArC\mathit{H}_{2ax}Ar$, $^2J_{HH}$ 13.3 Hz), 4.35 (q, 4H, $C\mathit{H}_2Me$, $^3J_{HH}$ 7.1 Hz), 4.49 (d, 4H, $ArC\mathit{H}_{2eq}Ar$, $^2J_{HH}$ 13.3 Hz), 4.79 (s, 4H, OCH_2), 7.03 (s, 4H, H_{Ar}), 7.99 (s, 4H, H_{Ar}), 8.97 (s, 2H, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 31.1, 31.7, 34.2, 61.6, 72.1, 124.4, 126.4, 128.8, 131.6, 140.0, 148.9, 150.4, 158.8, 169.2. IR (vaseline oil, ν/cm^{-1}): 3313 (OH), 1759 (CO), 1205 (COC). Found (%): C, 65.94; H, 6.21; N, 3.22. Calc. for $C_{44}H_{50}N_2O_{12}$ (%): C, 66.15; H, 6.31; N, 3.51.

[‡] *General method for membrane extraction.* The fluxes of the substrate transport through the liquid impregnated membranes were measured in a glass thermostated vertical diffusion cell with movable cylinder. Porous Teflon filters Millipore Type FA (thickness, 1 µm; pore size, 100 nm; porosity 85%; the filters were reinforced with a carbon net) were used as a hydrophobic matrix of impregnated liquid membrane. The volumes ratio between source and receiving phases was 5:1. This provided equal levels of the solutions necessary for eliminating osmotic transport of an acid. The mass transport measurements were carried out under standard conditions (25 °C). The initial substrate solutions were prepared from accurately weighed specimens in twice-distilled water, the pH of all the acid solutions was about 2. The source and receiving solutions were agitated with a magnetic stirrers. The concentrations of the compounds were determined conductometrically. A pure solvent (*o*-nitrophenyl octyl ether) or a 0.05 M solution of carrier **1**–**8** in *o*-nitrophenyl octyl ether were used as a liquid membrane. The concentration of substrates **9**–**15** in the source phase was 0.1 mol dm^{-3}. The membrane extraction experiments were performed in three parallel runs. The RSD of the mass transfer flux j_i did not exceed 10%.