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Dedicated to Prof. Paweł Kafarski on the occasion of his 63rd birthday

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Abstract
A series of dipropoxy-, tripropoxy- and tetrapropoxycalix[4]arenes bearing one or two fragments of α-hydroxymethylphosphonic acid at the upper rim of the macrocycle was prepared by the reaction of the corresponding mono- and di-formylcalixarenes with sodium salts of dialkyl phosphites or with trialkyl (tristripropylsilyl)phosphites followed by dealkylation (desilylation) of the ester derivatives. The conformations of the macrocyclic skeleton and the stereoisomeric forms of the compounds obtained were investigated by 1H NMR. The resulting α-hydroxymethylphosphonic acids were found to be able to inhibit the activity of glutathione S-transferase in vitro.

Keywords: Calix[4]arenes, hydroxyphosphonic acids, synthesis, stereochemistry, glutathione S-transferase, inhibition

Introduction

Calixarenes – cyclic oligomers obtained by the condensation of para-substituted phenols with formaldehyde1 – were proposed as a useful macrocyclic platform for the design of artificial receptors of molecules and ions.2-5 Calix[4]arenes containing bio-inspiring groups at the upper or at the lower rim of the macrocycle can recognize a wide range of biologically active molecules such as amino acids, dipeptides, proteins, choline and acetylcholine, carbohydrates, riboflavin, vitamin B12, nucleotides, nucleosides and short DNA fragments.6-10 Also, the calix[4]arene
derivatives were fixed on proteins.\textsuperscript{11,12} Calixarenes are considered as promising objects of biochemical research due to their ability to simulate the substrate-receptor interaction with biomolecules.\textsuperscript{13,14}

Previously we have shown that calix[4]arenes functionalized with phosphonic, aminophosphonic and methylenebisphosphonic acid residues are effective inhibitors of alkaline phosphatases\textsuperscript{15-17} and some of protein tyrosine phosphatases.\textsuperscript{18,19} Such compounds are more active than model acyclic inhibitors due to effects of the calixarene platform. Efficiency of the inhibition significantly depends on the number of the phosphonic groups, their stereochemical configuration and size or conformation of the macrocyclic platform.\textsuperscript{15-18} The aim of this work was to design calixarene based $\alpha$-hydroxymethylphosphonic acids and characterize their properties as inhibitors of glutathione $S$-transferase (GST). This enzyme is involved in a variety of biological processes including detoxication of xenobiotics in cells\textsuperscript{20-22} and thereby can decrease effects of drugs causing multidrug resistance.\textsuperscript{22,23} Several heterocyclic compounds,\textsuperscript{24,25} etacrinic acid derivatives\textsuperscript{26} and some structural analogues of glutathione\textsuperscript{27,28} have been described as reversible or irreversible inhibitors of GST. Some endogenous compounds such as bilirubin and bile salts are able to bind to GST and inhibit their activity.\textsuperscript{23,29} As we have already noted,\textsuperscript{30} glutathione $S$-transferase can be a potential target for calixarene based phosphonic acids \textit{in vitro}. In this paper we describe the synthesis, stereochemistry and inhibition ability of a series of lower-rim substituted calix[4]arenes bearing one or two $\alpha$-hydroxymethylphosphonic acid fragments at the upper rim of the macrocycle.

\section*{Results and Discussion}

Calixarene-mono-$\alpha$-hydroxymethylphosphonates 4-6 were obtained as the precursors of the desired acids by the reaction of dipropoxy-, tripropoxy- and tetrapropoxyformylcalixarenes 1-3 with triisopropyl phosphate in the presence of anhydrous hydrogen chloride (Method A) or by the Pudovik addition of sodium salts of diisopropyl phosphate to the CHO group of 1-3 (Method B) (Scheme 1).\textsuperscript{31-33}

\begin{center}
\includegraphics[width=\textwidth]{Scheme_1.png}
\end{center}

\textbf{Scheme 1.} Synthesis of calixarene-mono-$\alpha$-hydroxymethylphosphonates 4-6.
Compounds 4-6 are colorless crystalline substances. Doublets of PCH proton (δ 4.5-4.7 ppm, \( J_{HP} \) 13 Hz) and two doublets (or two multiplets) of AB spin system of axial and equatorial methylene protons ArCH\(_2\)Ar groups are the most characteristic signals in the \(^1\)H NMR spectra of the phosphonates 4-6. The difference in chemical shifts between the axial and equatorial protons in the dipropoxycalixarene 4 (\( \Delta\delta \) 0.88 ppm) confirms the flattened cone conformation of the macrocyclic skeleton. In this conformation (symmetry \( C_{2v} \)) two opposite phenol rings possess almost coplanar orientation and two O-propylated phenol rings possess almost perpendicular orientation to the main plane of the macrocycle formed by the methylene links. Intramolecular hydrogen bonds OH…OPr at the lower rim of the macrocycle stabilize this conformation.\(^{34}\) The value \( \Delta\delta \) 1.22 ppm shows the regular cone conformation (symmetry \( C_{2v} \)) of tetrapropoxycalixarene 6.\(^{35}\) Tripropoxycalixarene 5 with \( \Delta\delta \) 1.05 ppm occupies an intermediate position between these two conformations.

Two chiral carbon atoms in calixarene-bis-\( \alpha \)-hydroxymethylphosphonates determine the formation of racemic form (RR+SS), meso form (RS) or their mixtures in the reaction products. Earlier calixarene-bis-\( \alpha \)-hydroxymethylphosphonates were synthesized as the mixtures of diastereoisomeric forms (RR+SS+RS) by the reaction of diformyldipropoxycalixarene 7 with trialkyl phosphites in the presence of anhydrous HCl.\(^{31-33}\) For synthesis of an individual diastereoisomers we introduced diformylcalixarene 7 in the Pudovik reaction. The interaction of 7 with an excess of sodium salt of dialkyl phosphites in dioxane solution at 10-15 °C for 8 h proceeds diastereoselectively and leads to the meso form of calixarene-di-\( \alpha \)-hydroxymethylphosphonates 8-10 with d.e. almost 100% (Scheme 2).

![Scheme 2. Synthesis of dipropoxycalixarene-bis-\( \alpha \)-hydroxymethylphosphonates 8-13.](image)

The reaction of diformylcalixarene 7 with trialkyl phosphites in the presence of anhydrous HCl is less diastereoselective (d.e. 4-86%) than the Pudovik reaction. The ratio of racemic and meso forms of calixarene-bis-\( \alpha \)-hydroxymethylphosphonates 11-13 is depended on the trialkyl phosphite alkyl length as well as the temperature of the reaction as shown in Table 1. The highest
diastereomeric excess (86%) was obtained by the reaction of calixarene 7 with tributyl phosphite at 10 ºC. Decreasing the temperature significantly increases the reaction time but does not improve the diastereoselectivity. Pure racemic forms of calixarene-bis-α-hydroxymethyl-phosphonates 11-13 were obtained by crystallization.

The configuration of the stereogenic carbon atoms of CH(OH)P fragments in the racemic 11-13 and meso 8-10 forms of the calixarene-bis-α-hydroxymethylphosphonates were determined from signal patterns of aromatic protons in para-unsubstituted benzene rings (see36 for a comparison). In the racemic form 11 possessing C2 symmetry axis the protons appear as three doublets of doublets. In the meso form 8 possessing C3 symmetry plane two doublets and two triplets of the protons are observed in the 1H NMR spectra (Figure 1).

Table 1. Diastereoselectivity of the reaction of diformylcalixarene 7 with trialkyl phosphites

<table>
<thead>
<tr>
<th>Trialkyl phosphite</th>
<th>Temperature, ºC</th>
<th>Ratio of the racemic and meso forms (d.e., %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(EtO)3P</td>
<td>10</td>
<td>81 : 19 (62)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>69 : 31 (38)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>52 : 48 (4)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>86 : 14 (72)</td>
</tr>
<tr>
<td>(PrO)3P</td>
<td>20</td>
<td>79 : 21 (58)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>65 : 35 (30)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>93 : 7 (86)</td>
</tr>
<tr>
<td>(BuO)3P</td>
<td>20</td>
<td>85 : 15 (70)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>74 : 26 (48)</td>
</tr>
</tbody>
</table>

The meso and racemic forms 8, 11 have also different chemical shifts of PCH protons as well as PH spin-spin coupling constants (δ 4.8 ppm, JHP 13 Hz and δ 4.9 ppm, JHP 10 Hz, respectively). Signals of phosphorus atoms of the racemic form (δ 19.0 ppm) are observed at higher field compared with those of the meso form (δ 22.1 ppm) in the 31P NMR spectra.
Figure 1. $^1$H NMR spectra of aromatic protons of racemic 11 and meso 8 forms of dipropoxycalixarene-bis-α-hydroxymethylphosphonate (CDCl$_3$).

Diastereoselectivity of the diformylcalixarene phosphorylation depends significantly on the substitution pattern of the macrocycle lower rim. In contrast to dipropoxydifomylcalixarene 7, the reactions of tetrapropoxydifomylcalixarenes 14, 15 with the sodium salts of dialkyl phosphites or with trialkyl phosphites in the presence of anhydrous hydrogen chloride lead to equivalent (1:1) mixtures of the racemic and meso forms of calixarene-bis-α-hydroxymethylphosphonates 16-19 (Scheme 3). The diastereomeric ratio is not dependent on the size of the alkyl groups of the phosphorylating agents and conditions of the reaction. The two sets of signals for both forms are observed in their $^1$H and $^{31}$P NMR spectra.

It should be noted that the reaction of tetrapropoxydifomylcalixarenes 14, 15 with an excess of the sodium salts of dialkyl phosphites does not only form calixarene-bis-α-hydroxymethylphosphonates 16-19. Phosphonate-phosphate rearrangement of one hydroxymethylphosphonate group occurs in the alkaline conditions at 20 °C for 48 h and leads to formation of phosphono-phosphates 20-22 (Scheme 4). Increasing the reaction time and temperature up to 60 °C does not cause rearrangement of the second phosphonate group and formation of the corresponding diphosphates.

Scheme 5. Synthesis of the calixarene-α-hydroxymethylphosphonic acids 26-29 by dealkylation of the corresponding esters.
The desired calixarene-α-hydroxymethylphosphonic acids 23-29 were obtained in 90-95% yields by the reaction of the corresponding esters 4-6, 8, 11, 16, 18 with trimethylbromosilane and methanol (Scheme 5).

The reaction of carbonyl compounds with trimethylsilyl phosphites is a convenient method of synthesis of α-hydroxyphosphonic acids derivatives. Calixarene-α-hydroxyphosphonic acids 25-28 were obtained in near quantitative yields by the one pot reaction of formylcalixarenes 3, 7, 14, 15 with tristrimethylsilyl phosphite (THF, room temperature, 20-30 minutes) followed by cleavage of P-O-Si bonds of the silyl esters formed with methanol (Scheme 6). One equivalent of of tristrimethylsilyl phosphite is needed for phosphorylation of tetrapropoxymonoformylcalixarene 3, four equivalents are needed for dipropoxydiformylcalixarene 7 (phenolic OH groups destroy trimethylsilyl phosphite) and two equivalents for tetrapropoxydiformylcalixarenes 14, 15.

The reaction of dipropoxydiformylcalixarene 7 with tris-trimethylsilyl phosphite is diastereoselective. The meso form of calixarene-bis-α-hydroxymethylphosphonic acids 26, identified by the \(^1\)H and \(^{31}\)P NMR spectra, was obtained exclusively after methanolysis. Similar reactions with diformyltetrapropoxycalixarenes 14, 15 led to an equivalent mixture of racemic and meso forms of bis-phosphonic acids 28, 29.

Scheme 6. One pot synthesis of calixarene-α-hydroxymethylphosphonic acids 25, 26, 28, 29.

Acids 23-29 are colorless or slightly colored solids soluble in polar solvents such as dimethylsulfoxide, alcohols or aqueous alkaline solutions. They have no clearly defined melting points, which suggests that there is formation of dimeric and polymeric associates through...
intermolecular hydrogen bonds between hydroxymethylidihydroxyphosphonic fragments. This is confirmed by peaks of molecular ions of double mass in FAB mass spectra.

**Inhibition of glutathione S-transferase**
The research *in vitro* was undertaken in order to evaluate the properties of calix[4]arene-α-hydroxymethylphosphonic acids as inhibitors of glutathione S-transferases from equine liver and human placenta. According to previous experiments, the macrocyclic α-hydroxyphosphonates showed the highest affinity for these enzymes in comparison with corresponding α-aminophosphonates or methylene bisphosphonates. The present study shows that some of calix[4]arene-α-hydroxymethylphosphonic acids have an affinity for the GST from equine liver with IC<sub>50</sub> values in the micromolar range. Among dipropoxycalix[4]arenes tested towards GST from equine liver, the calix[4]arene 23 bearing one substituent at the upper rim was more effective in comparison with bis-substituted macrocycle 26. Introduction of four propyl groups to the lower rim of calix[4]arene leads to the analogue 25 being less effective in comparison with 23. At the same time, among dipropoxycalix[4]arenes, inhibiting properties of bisphosphonate derivatives 28 and 29 are higher than of monophosphonate 25. Compound 29, which bears two bromine atoms at the upper rim, has approximately the same activity as calix[4]arene 28. As is seen from Table 2, the IC<sub>50</sub> values for tetrapropoxy substituted bisphosphorylated compound 28 are lower than these for bispropoxy substituted analogue 26.

It should be noted that impact of the calix[4]arenes on GST from equine liver is more pronounced than on placental GST. In the latter case the observed activities of bisphosphorylated compounds 26, 28 and 29 were higher than the activities of the monophosphorylated macrocycles 23 and 25 (Table 2).

**Table 2. Inhibition of glutathione S-transferases from equine liver (EL) and human placenta (HP) by calix[4]arene-α-hydroxymethylphosphonates**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(EL), μM</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(HP), μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 (R+S)</td>
<td>7.5 ± 2.3</td>
<td>*</td>
</tr>
<tr>
<td>26 (RS)</td>
<td>28 ± 5</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>25 (R+S)</td>
<td>58 ± 2</td>
<td>*</td>
</tr>
<tr>
<td>28 (RS + RR + SS)</td>
<td>6.1 ± 1.9</td>
<td>29 ± 13</td>
</tr>
<tr>
<td>29 (RS + RR + SS)</td>
<td>4.5 ± 1.1</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

*No effect at concentration of 100 μM

To elucidate the possible stereoselective effects of calix[4]arene-α-hydroxymethylphosphonates on the activity of glutathione S-transferase, the stereoisomeric forms of compounds 26 and 28 were docked computationally to the active site of GST P1-1 from human placenta. The docking calculations were performed using QXP/FLO+ program. A binding model was constructed automatically on the basis of known X-ray crystal structure of the enzyme (PDB...
The ligand was preliminary removed from the binding site of the enzyme. The phosphoryl residues of the inhibitors were in form of monoanions. Fulldock+, being the most exact in QXP/FLO+, was used as a docking method. According to the data obtained, stereoisomeric α-hydroxyphosphonates bind to the enzyme surface near to specific glutathione binding G-site. The binding free energies derived from docking (ΔE_{doc}) indicate that all stereoisomeric forms of dipropoxy[4]arene 26 and and tetrapropoxy[4]arenes 28 can exhibit almost identical affinities for the enzyme (Table 3). The possible binding modes of the inhibitors mainly include hydrophobic interactions and hydrogen bonds in only some cases. For example, calixarene-bis-α-hydroxymethylphosphonic acids 26 which was identified and used in assay as meso form occupies the active site of GST P1-1 with narrow rim of the macrocyclic skeleton being oriented toward Arg13 and (R)-α-hydroxymethylphosphonate residue being positioned near Gln51. One of the aromatic rings of calix[4]arene platform is involved in interaction with Phe8. The hydrophobic interactions were found between macrocyclic platform of this compound and residues of Tyr108, Ile 104 belonging to H-site of the enzyme (Fig. 2).

Table 3. Docking results (QXP|FLO+) of stereoisomers of calix[4]arene-bis-α-hydroxymethylphosphonates 26 and 28 into the active site of GST P1-1 from human placenta

<table>
<thead>
<tr>
<th>Calixarene</th>
<th>ΔE_{doc}, kJ/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 (SS)</td>
<td>-33.0</td>
</tr>
<tr>
<td>26 (RR)</td>
<td>-31.8</td>
</tr>
<tr>
<td>26 (RS)</td>
<td>-31.7</td>
</tr>
<tr>
<td>28 (SS)</td>
<td>-33.0</td>
</tr>
<tr>
<td>28 (RR)</td>
<td>-34.1</td>
</tr>
<tr>
<td>28 (RS)</td>
<td>-33.8</td>
</tr>
</tbody>
</table>

Although the glutathione S-transferases are classified into several groups, they all have highly specific glutathione binding G-site and xenobiotic binding H-site. Nonspecific H-site is capable of binding of very large substrates and shows structural plasticity among classes of these enzymes. Different affinities of calix[4]arene-α-hydroxymethylphosphonates to glutathione S-transferases from human placenta and equine liver (Table 2) may reflect some differences in the active sites of these enzymes and can be a result of more tight binding of the macrocyclic inhibitor to the enzyme from equine liver. It is known that inhibitors of glutathione S-transferases may interact with amino acid residues of both G-site and H-site. For example, kinetic studies of the enzyme from equine liver showed that S-(p-bromobenzyl)glutathione is a potent inhibitor with competitive kinetics with respect to glutathione (inhibition constant of approximately 1 μM) and mixed type of inhibition with respect to 1-chloro-2,4-dinitrobenzene (CDNB). In complex with GST P1-1, the glutathione part of the inhibitor occupies the G-site and p-bromobenzyl substituent is anchored to Phe8 and Tyr108 of the H-site. As seen in Figure 2, the residues of Phe8, Arg13 and Gln51, which are involved in glutathione binding at the G-site, contact the bulky macrocyclic inhibitor 26, and this may influence the binding of glutathione to the enzyme.
The mode of binding of macrocycle 26 may also restrict the position of small molecule of CDNB which was located at the H-site. Taking into account the results of the docking study and variability of the family of calix[n]arenes (n = 4, 5, 6, 8 and more) possessing different size and conformation of their macrocyclic skeleton one can say that functionalization of the compounds with hydroxyphosphonic acid moieties is promising way of designing new potential inhibitors of glutathione S-transferase.

**Figure 2.** The possible binding mode of (RS)-calix[4]arene-bis-α-hydroxymethylphosphonate 26 in the active site of GST P1-1.

**Conclusions**

In summary, we have synthesized a novel series of calix[4]arenes bearing one or two fragments of α-hydroxymethylphosphonic acid at the upper rim. Dipropoxy- and tetrapropoxy-derivatives of calix[4]aren α-hydroxymethylphosphonic acids were designed to explore their inhibiting effects on activity of glutathione S-transferase from equine liver and human placenta *in vitro*. Some of compounds were found to have affinity for the enzyme from equine liver in the micromolar range. The number of α-hydroxymethylphosphonic acid and propoxy groups at the calixarene platform can influence the potency of compounds towards glutathione S-transferase.

**Experimental Section**

**General.** $^1$H and $^{31}$P NMR spectra were recorded on a Varian VXR spectrometer operating at 300 MHz and 121.5 MHz respectively. The chemical shifts are reported using internal
tetramethylsilane and external 85% H₃PO₄ as references. The melting points were determined on a Boetius apparatus and are uncorrected. m-Nitrobenzyl alcohol was used as a matrix for registration of FAB-mass spectra. Analytical thin layer chromatography was carried out on Silufol plates. Column chromatography was carried out on silica gel Silufol L 40/100. Chloroform was distilled over phosphorus pentoxide. All reactions were carried out under dry argon. Formylcalix[4]arenes 1-3,7,14,15 were synthesized by the procedures described in the literature.⁴⁵

**Synthesis of calix[4]arene-α-hydroxymethylphosphonates 4-6, 11-13, 16-19 by method A. General procedure**

To a suspension of formylcalixarene 1,2,3,7,14,15 (0.1 mmol) in dioxan (5 ml) trialkyl phosphite 

(10 mmol for the each formyl group) was added. Gaseous dried hydrogen chloride was injected in the stirred reaction mixture at a temperature of 10-15 °C during 0.5 h. The solution was stirred for 1 h. The solvent was then evaporated in vacuo to form a colorless oil. The oil was washed with petroleum ether. A white solid thus obtained was dried in vacuo (0.05 mm Hg) at room temperature for 5-10 h.

**Diisopropyl 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5-(α-hydroxymethylphosphonate) (4).** Colorless crystals: yield 63%. m.p. 178-180 °C (cyclohexane). ¹H NMR (CDCl₃), δ: 0.69 (d, 3H, J 6.0 Hz, OCH₃), 1.10-1.37 (m, 9H, diastereotopic OCH₃), 1.31 (t, 6H, J 7.2 Hz, CH₂CH₃), 2.07 (m, 4H, CH₂CH₂CH₂CH₃), 3.35, 3.41 (two d, 2H+2H, J 13.4 Hz, ArCH₂eq), 3.97 (t, 4H, J 7.2 Hz, OCH₂), 4.31 (d, 4H, J 13.4 Hz, ArCH₂ax), 4.37, 4.59 (two m, 1H + 1H, diastereotopic CHCH₃), 4.78 (d, 1H, J 10 Hz, PCH), 6.65 (t, 1H, J 7.5 Hz, Ar-Hp), 6.71 (t, 2H, J 7.5 Hz, Ar-Hp), 6.90 (d, 4H, J 7.5 Hz, Ar-Hm), 7.05 (d, 2H, J 7.5 Hz, Ar-Hm), 7.19, 7.25 (two s, 2H, diastereotopic Ar-Hm), 8.24 (s, 1H, OH), 8.35 (s, 1H, OH). ³¹P NMR (CDCl₃), δ 18.5. Mass spectrum (FAB) m/z: 537[M-HPO(OPr-i)₂ + H]⁺, 685[M-H₂O + H]⁺, 704[M + H]⁺. Calcd for C₄₁H₅₁O₈P 702.8.

**Diisopropyl 28-hydroxy-25,26,27-tripropoxycalix[4]arene-5-(α-hydroxymethylphosphonate) (5).** Colorless crystals: yield 71%. m.p. 165-167 °C (cyclohexane). ¹H NMR (CDCl₃), δ: 0.71 (d 3H, J 6.0 Hz, OCH₃), 1.12-1.39 (m, 9H, diastereotopic OCH₃), 1.29 (m, 9H, CH₂CH₃), 2.07 (m, 6H, CH₂CH₂CH₂CH₃), 3.29, 3.36 (two d, 2H+2H, J 13.4 Hz, ArCH₂eq), 3.97 (m, 6H, J 7.2 Hz, OCH₂), 4.35 (d, 4H, J 13.4 Hz, ArCH₂ax), 4.35, 4.61 (two m, 1H + 1H, diastereotopic CHCH₃), 4.75 (d, 1H, J 10 Hz, PCH), 6.68 (t, 1H, J 7.5 Hz, Ar-Hp), 6.75 (t, 2H, J 7.5 Hz, Ar-Hp), 6.87 (d, 4H, J 7.5 Hz, Ar-Hm), 7.08 (d, 2H, J 7.5 Hz, Ar-Hm), 7.20, 7.24 (two s, 2H, diastereotopic Ar-Hm), 8.35 (s, 1H, OH). ³¹P NMR (CDCl₃), δ 18.7. Mass spectrum (FAB) m/z: 746 [M + H]⁺. Calcd for C₄₄H₇₁O₈P 744.9.

**Diisopropyl 25,26,27,28-tetrapropoxycalix[4]arene-5-(α-hydroxymethylphosphonate) (6).** Colorless crystals: yield 68%. m.p. 134-136 °C. ¹H NMR (CDCl₃), δ: 0.68 (d 3H, J 6.0 Hz, OCH₃), 1.14-1.37 (m, 9H, diastereotopic OCH₃), 1.04 (m, 12H, CH₂CH₃), 2.02 (m, 8H, CH₂CH₂CH₃), 3.19 (d, 4H, J 12.9 Hz, ArCH₂eq), 3.90 (m, 8H, OCH₂), 4.41 (d, 4H, J 12.9 Hz, ArCH₂ax), 4.31, 4.57 (two m, 1H + 1H, diastereotopic CHCH₃), 4.75 (d, 1H, J 10 Hz, PCH),
Tetraethyl (RR+SS) 26, 28-dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (11). Colorless crystals: yield 45%. m.p. 192-194 °C (cyclohexane). $^1$H NMR (CDCl$_3$), δ: 0.84 (m, 6H, CH$_3$), 1.29 (t, 12H, J 7.5 Hz, CH$_2$CH$_3$), 2.04 (m, 4H, CH$_2$CH$_2$CH$_3$), 3.38, 3.40 (two d, 2H + 2H, J 14.0 Hz, ArCH$_2$eq), 3.54, 3.82 (two m, 2H + 2H, diastereotopic POCH$_2$), 3.97 (t 4H, J 6.2 Hz, OCH$_2$), 4.16 (m, 4H, POCH$_2$), 4.26, 4.28 (two d, 2H + 2H, J 14.0 Hz, ArCH$_2$ax), 4.75 (t, 2H, J 13.0 Hz, PCH), 6.69, 6.71 (two dd, 1H + 1H, J 8.0 Hz, J 2.0 Hz, p-ArH), 6.89, 6.93 (two dd, 2H + 2H, J 8.0 Hz, J 2.0 Hz, m-ArH), 7.21 (d, 4H, J 2.0 Hz, m-ArH), 7.33 (d, 4H, J 2.0 Hz, m-ArH), 8.49 (s, 2H, OH). $^{31}$P NMR (CDCl$_3$), δ 79.4. Mass spectrum (FAB) m/z: 843.8[2M + H]$^+$, 863[M + Na]$^+$, 1683[2M + H]$^+$. Calcd for C$_{47}$H$_{63}$O$_8$P 840.8. Calculated for C$_{44}$H$_{58}$O$_{12}$P$_2$: %: C 62.83, H 6.96, P 7.37. Found, %: C 63.05, H 6.81, P 7.19.

Tetrapropyl (RR+SS) 26, 28–dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (12). Colorless crystals: yield 58%. m.p. 179-180 °C (cyclohexane). $^1$H NMR (CDCl$_3$), δ: 1.03, 1.16 (two t, 6H+6H, J 6.0 Hz, diastereotopic OCH$_2$CH$_2$CH$_3$), 1.31 (t, 6H, J 7.5 Hz, CH$_2$CH$_3$), 1.94 (m, 8H, OCH$_2$CH$_2$CH$_3$), 2.05 (m, 4H, CH$_2$CH$_2$CH$_3$), 3.35, 3.39 (two d, 2H + 2P, J 14.0 Hz, ArCH$_2$eq), 3.55, 3.82 (two m, 2H + 2H, diastereotopic POCH$_2$), 3.94 (t 4H, J 6.2 Hz, OCH$_2$), 4.16 (m, 4H, POCH$_2$), 4.27, 4.29 (two d, 2H + 2H, J 14.0 Hz, ArCH$_2$ax), 4.75 (d, 2H, J 13.0 Hz, PCH), 6.70, 6.77 (two dd, 1H + 1H, J 8.0 Hz, J 2.0 Hz, p-ArH), 6.89, 6.94 (two dd, 2H + 2H, J 8.0 Hz, J 2.0 Hz, m-ArH), 7.21 (d, 4H, J 2.0 Hz, m-ArH), 7.33 (d, 4H, J 2.0 Hz, m-ArH), 8.49 (s, 2H, OH). $^{31}$P NMR (CDCl$_3$), δ 19.4. Mass spectrum (FAB) m/z: 898[M + H]$^+$, 863[M + Na]$^+$, 1795[2M + H]$^+$. Calcd for C$_{48}$H$_{66}$O$_{12}$P$_2$ 896.98. Calculated for C$_{48}$H$_{66}$O$_{12}$P$_2$: %: C 64.27, H 6.96, P 6.91. Found, %: C 64.27, H 6.96, P 7.83.

Tetrabutyl (RR+SS) 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (13). Colorless crystals: yield 65%. m.p. 171-172 °C (cyclohexane). $^1$H NMR (CDCl$_3$), δ: 1.02, 1.17 (two t, 6H+6H, J 6.0 Hz, diastereotopic OCH$_2$CH$_2$CH$_3$), 1.28 (t, 6H, J 7.5 Hz, CH$_2$CH$_3$), 1.95 (m, 8H, OCH$_2$CH$_2$CH$_3$), 2.51 (m, 4H, CH$_2$CH$_2$CH$_3$), 3.38, 3.40 (two d, 2H + 2P, J 14.0 Hz, ArCH$_2$eq), 3.54, 3.81 (two m, 2H + 2H, diastereotopic POCH$_2$), 3.97 (t 4H, J 6.2 Hz, OCH$_2$), 4.16 (m, 4H, POCH$_2$), 4.26, 4.28 (two d, 2H + 2H, J 14.0 Hz, ArCH$_2$ax), 4.75 (d, 2H, J 13.0 Hz, PCH), 6.70, 6.72 (two dd, 1H + 1H, J 8.0 Hz, J 2.0 Hz, p-ArH), 6.88, 6.92 (two dd, 2H + 2H, J 8.0 Hz, J 2.0 Hz, m-ArH), 7.20 (d, 4H, J 2.0 Hz, m-ArH), 7.34 (d, 4H, J 2.0 Hz, m-ArH), 8.51 (s, 2H, OH). $^{31}$P NMR (CDCl$_3$), δ 19.1. Mass spectrum (FAB) m/z: 934[M - H$_2$O + H]$^+$, 954[M + H]$^+$, 976[M + Na]$^+$, 1683[2M + H]$^+$. Calcd for C$_{52}$H$_{74}$O$_{12}$P$_2$ 953.08. Calculated for C$_{52}$H$_{74}$O$_{12}$P$_2$: %: C 65.53, H 7.83, P 6.50. Found, %: C 65.56, H 7.85, P 6.43.

NMR (CDCl3), δ: 0.84, 1.02 (two m, 6H+6H, OCH2CH3), 1.09, 1.19 (two m, 6H+6H, CH2CH2CH2CH3), 1.93 (m, 8H, CH2CH2CH3), 3.10, 3.15 (two d, 2H+2H, J 13.2 Hz, ArCH2eq), 3.71-4.15 (m, 16H, OCH2), 4.42, 4.45 (two d, 2H+2H, J 13.2 Hz, ArCH2ax), 4.55, 4.63 (two d, 1H+1H, J 10.9 Hz and J 11.3 Hz, PCH), 6.43 (s, 1H, ArH), 6.49 (t, 1H, J 7.6 Hz, ArH), 6.20 (m, 6H, ArH), 6.68 (m, 5H, ArH), 6.81 (m, 3H, ArH). 31P NMR (CDCl3), δ 20.8. Mass spectrum (FAB) m/z: 769[M-H2O-HPO(O2C)3]+, 907[M -H2O +H]⁺, 926[M +H]⁺. Calcd for C50H70O12P2 925.03. Calculated for C50H70O12P2, %: C 64.92, H 7.63, P 6.70. Found, %: C 64.96, H 7.66, P 6.61.

Tetrapropyl (RR+SS+RS) 25,26,27,28-tetrapropoxy calix[4]arene-5,17-bis(α-hydroxymethyl-phosphonate) (17). Colorless crystals: yield 82 %. m.p. 102-107 °C. 1H NMR (CDCl3), δ: 0.87, 0.98 (two m, 6H+6H, OCH2CH2CH3), 1.12, 1.1.23 (two m, 6H+6H, CH2CH2CH2CH3), 1.85-2.05 (m, 16H, CH2CH2CH3), 3.12, 3.14 (two d, 2H+2H, J 13.2 Hz, ArCH2eq), 3.69-4.18 (m, 16H, OCH2), 4.43, 4.44 (two d, 2H+2H, J 13.2 Hz, ArCH2ax), 4.51, 4.68 (two d, 1H+1H, J 10.9 Hz and J 11.3 Hz, PCH), 6.42 (s, 1H, ArH), 6.41 (t, 1H, J 7.6 Hz, ArH), 6.22 (m, 6H, ArH), 6.65 (m, 5H, ArH), 6.79 (m, 3H, ArH). 31P NMR (CDCl3), δ 20.5. Mass spectrum (FAB) m/z: 965[M -H2O +H]⁺, 982[M +H]⁺, 1003[M + Na]⁺. Calcd for C54H78O12P2 981.14. Calculated for C54H78O12P2, %: C 66.10, H 8.01, P 6.31. Found, %: C 66.21, H 7.95, P 6.21.

Tetraethyl (RR+SS+RS) 25,26,27,28-tetrapropoxy calix[4]arene-11,12-dibromo-5,17-bis(α-hydroxymethylphosphonate) (18). Colorless crystals: yield 75 %. m.p. 144-146 °C. 1H NMR (CDCl3), δ: 0.85, 0.95 (two t, 6H + 6H, J 6.0 Hz, OCH2CH3), 1.23 (m, 12H, CH2CH3), 1.96 (m, 8H, CH2CH2CH3), 3.14, 3.16 ( two d, 4H, J 13.0 Hz, ArCH2eq), 3.51-4.2 (m, 16H, POCH2+OCH2), 4.39, 4.41 (two d, 4H, J 13.0 Hz, ArCH2ax), 4.79, 4.85 (two d, 1H + 1H, J 10.7 Hz and J 11.3 Hz PCH), 6.45,6.49 (two s, 1H+1H, ArH), 6.69, 6.70 ( two s, 1H + 1H, ArH), 6.93 (s, 2H, ArH), 7.01, 7.15 (two s, 1H+1H, ArH). 31P NMR (CDCl3), δ 19.6. Mass spectrum (FAB) m/z: 1084 [M +H]⁺, 1106[M + Na]⁺, 2166[2M +H]⁺. Calcd for C50H68Br2O12P2 1082.84. Calculated for C50H68Br2O12P2, %: C 55.46, H 6.33, P 5.72. Found, %: C 55.57, H 6.21, P 5.77.

Tetrapropyl (RR+SS+RS) 25,26,27,28-tetrapropoxy calix[4]arene-11,12-dibromo-5,17-bis(α-hydroxymethylphosphonate) (19). Colorless crystals: yield 75 %. m.p. 135-138 °C. 1H NMR (CDCl3), δ: 0.86, 0.92 (two t, 6H + 6H, J 6.0 Hz, OCH2CH2CH3), 1.22 (m, 12H, CH2CH3), 1.95-2.02 (m, 16H, CH2CH2CH3), 3.12, 3.17 (two d, 4H, J 13.0 Hz, ArCH2eq), 3.55-4.2 (m, 16H, POCH2+OCH2), 4.37, 4.44 (two d, 4H, J 13.0 Hz, ArCH2ax), 4.81, 4.82 (two d, 1H + 1H, J 10.7 Hz and J 11.3 Hz, PCH), 6.46,6.48 (two s, 1H + 1H, ArH), 6.67, 6.68 (two s, 1H + 1H, ArH), 6.94 (s, 2H, ArH), 7.02, 7.12 (two s, 1H + 1H, ArH). 31P NMR (CDCl3), δ 19.2. Mass spectrum (FAB) m/z: 1140[M +H]⁺, 1162[M + Na]⁺, 2279[2M +H]⁺. Calcd for C54H76Br2O12P2 1139. Calculated for C54H76Br2O12P2, %: C 56.95, H 6.73, P 5.44. Found, %: C 56.07, H 6.61, P 5.53.
Synthesis of calix[4]arene-α-hydroxymethylphosphonates 4, 5, 8-10, 16,18,19 by method B. General procedure

To solution of dialkyl phosphite (0.3 mmol) in dioxane (5 ml) sodium metal (0.1 mmol) was cautiously added in small portions. Formylcalixarene 1(0.2 mmol), 2(0.2 mmol), 7(0.1 mmol), 14,15 (0.1 mmol) was added to the resulting solution. The reaction mixture was stirred at 10-15 °C for 8 h and was quenched with water (100 ml). The product was extracted with chloroform. Organic phase was dried over Na2SO4. The solvent was evaporated and the product formed was purified by the column chromatography (eluent chloroform – acetone, 10:1).


Tetraethyl (RS) 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (8). Colorless crystals: yield 65 %. m.p. 195-197 °C. 1H NMR (CDCl₃): δ(ppm) 1.01, 0.99, 1.17 (two t, 6H + 6H, J 6.0 Hz, diastereotopic OCH₂CH₂), 1.30 (t, 6H, J 7.5 Hz, CH₂CH₃), 2.07 (m, 4H, CH₂CH₂CH₂CH₃), 3.39 (d, 4H, J 13.0 Hz, ArCH₂eq), 3.74, 3.93 (two m, 4H, 4H + 4H, diastereotopic POCH₂), 3.97 (t, 4H, J 6.2 Hz, OCH₂). 4.29 (d, 4H, J 13.0 Hz, ArCH₂ax), 4.86 (d, 2H, J 10.0 Hz, PCH), 6.67,6.68 (two t, 2H, J 8.0 Hz, p-ArH), 6.89, 6.91 (two d, 4H, J 8.0 Hz, m-ArH), 7.21 (s, 4H, m-ArH), 8.33 (s, 2H, OH). 31P NMR (CDCl₃): δ 22.3 Mass spectrum (FAB) m/z: 565[M -2HPO(OEt)₂ + H]⁺, 685[M -HPO(OEt)₂ -H₂O +H]⁺, 805[M -2H₂O +H]⁺, 823[M -H₂O +H]⁺, 841[M +H]⁺, 863[M + Na]⁺, 1683[2M +H]⁺. Caled for C₄₄H₅₈O₁₂P₂: 840.8. Calculated for C₄₄H₅₈O₁₂P₂, %: C 62.83, H 6.96, P 7.37. Found, %: C 63.09, H 6.82, P 7.21.

Tetrapropyl (RS) 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (9). Colorless crystals: yield 71 %. m.p. 182-184 °C. 1H NMR (CDCl₃): δ: 1.01, 1.17 (two t, 6H + 6H, J 6.0 Hz, diastereotopic OCH₂CH₂CH₃), 1.30 (t, 6H, J 7.5 Hz, CH₂CH₃), 1.95 (m, 8H, OCH₂CH₂CH₂CH₃), 2.07 (m, 4H, CH₂CH₂CH₂CH₃), 3.40 (d, 4H, J 14.0 Гц, ArCH₂eq), 3.73, 3.94 (two m, 4H + 4H, diastereotopic POCH₂), 3.98 (t, 4H, J 6.2 Hz, OCH₂), 4.29 (d, 4H, J 14.0 Hz, ArCH₂ax), 4.83 (d, 2H, J 10.0 Hz, PCH), 6.66 (t, 2H, J 8.0 Гц, p-ArH), 6.91 (d, 4H, J 8.0 Hz, m-ArH), 7.22 (s, 4H, m-ArH), 8.32 (s, 2H, OH). 31P NMR (CDCl₃), δ 22.1 Mass spectrum (FAB) m/z: 898[M+H]⁺, 1795[2M +H]⁺. Caled for C₄₈H₆₆O₁₂P₂: 896.98 Calculated for C₄₈H₆₆O₁₂P₂, %: C 64.27, H 6.96, P 6.91. Found, %: C 64.21, H 6.99, P 7.85.

Tetrabutyl (RS) 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (10). Colorless crystals: yield 76 %. m.p. 170-172 °C. 1H NMR (CDCl₃), δ: 1.02, 1.16 (two t, 6H + 6H, J 6.0 Hz, diastereotopic OCH₂CH₂CH₂CH₃), 1.30 (t, 6H, J 7.5 Hz, CH₂CH₃), 1.95 (m, 8H, CH₂CH₂CH₂CH₃), 2.07 (m, 4H, CH₂CH₂CH₂CH₃), 2.53 (m, 8H, CH₂CH₂CH₂CH₃), 2.41 (d, 4H, J 14.0 Hz, ArCH₂eq), 3.74, 3.93 (two m, 4H + 4H, diastereotopic POCH₂), 3.97 (t, 4H, J 6.2 Hz, OCH₂), 4.29 (d, 4H, J 14.0 Гц, ArCH₂ax), 4.83 (d,
2H, J 10.0 Hz, PCH), 6.64 (t, 2H, J 8.0 Гц, p-ArH), 6.90 (d, 4H, J 8.0 Hz, m-ArH), 7.23 (c, 4H, m-ArH), 8.33 (c, 2H, OH). $^{31}$P NMR (CDCl$_3$), $\delta$ 21.9. Mass spectrum (FAB) m/z: 934[M - H$_2$O + H]$^+$, 954 [M + H]$^+$, 976 [M + Na]$^+$. Calcd for C$_{52}$H$_{74}$O$_{12}$P$_2$ 953.08 Calculated for C$_{52}$H$_{74}$O$_{12}$P$_2$, %: C 65.53, H 7.83, P 6.50. Found, %: C 65.56, H 7.85, P 6.43.


**Synthesis of phosphate phosphate substituted calixarenes 20-22. General procedure**

To solution of diethyl phosphite (4 mmol) in dioxane (5 ml) sodium metal (0.4 mmol) was cautiously added in small portions. Formylcalixarene 14, 15 (0.2 mmol) was added to the resulting solution. The reaction mixture was stirred at 20 °C for 48 h and was quenched with water (100 ml). The product was extracted with chloroform. Organic phase was dried over Na$_2$SO$_4$. The solvent was evaporated and the product formed was purified by the column chromatography (elucent chloroform – acetone, 10:1).

**Diethyl 25,26,27,28-tetrapropoxyxicalx[4]arene-5-(diethoxyphosphoryloxymethyl)-17-(α-hydroxymethylphosphonate) (20).** Colorless crystals: yield 45 %. m.p. 61-63 °C. $^1$H NMR (CDCl$_3$), $\delta$: 0.87, 1.07 (two t, 6H + 6H, J 7.5 Hz, OCH$_2$CH$_3$), 1.12, 1.15, 1.26, 1.36 (four t, 3H+3H+3H+3H, J 7.2 Hz CH$_2$CH$_3$), 1.86, 1.96 (m, 4H+4H, CH$_2$CH$_2$CH$_3$), 3.10, 3.12, 3.16 (three d, 1H+1H+2H, J 13.0 Hz, ArCH$_2$eq), 3.58, 3.68 (two t, 2H + 2H, J 7.2 Hz, OCH$_2$), 3.75-4.05 (m, 14H, OCH$_2$), 4.25, 4.27 (two d, 0.5H+0.5H, J$_{HH}$ 10.3 Гц, diastereotopic ArCH$_2$OP), 4.38, 4.40 (two d, 0.5H+0.5H, J$_{HH}$ 10.3 Hz, diastereotopic ArCH$_2$OP), 4.42, 4.44 (two d, 2H+2H, J 13.0 Hz, ArCH$_2$ax), 5.00, 5.04 (two d, 0.5H+0.5H, J 11.2 Hz, PCH), 5.92, 6.04 (two d, 1H+1H, J 2 Hz, m-ArH), 6.14, 6.51 (two dd, 1H+1H, J 1.6 Hz, J 2 Hz, m-ArH), 6.85, 6.97 (two t, 1H+1H, J 7.2 Hz, p-ArH), 7.15 (m, 4H, m-ArH). $^{31}$P NMR (CDCl$_3$), $\delta$ -1.39, $\delta$ 17.5. Mass spectrum (FAB) m/z: 926[M + H]$^+$, 948[M + Na]$^+$. Calcd for C$_{50}$H$_{70}$O$_{12}$P$_2$ 925.03. Calculated for C$_{50}$H$_{70}$O$_{12}$P$_2$, %: C 64.92, H 7.63, P 6.70. Found, %: C 64.85, H 7.68, P 6.59.

**Dipropyl 25,26,27,28-tetrapropoxyxicalx[4]arene-5-(dipropoxyphosphoryloxymethyl)-17-(α-hydroxymethylphosphonate) (21).** Colorless crystals: yield 52 %. m.p. 55-57 °C. $^1$H NMR (CDCl$_3$), $\delta$: 0.85, 1.09 (two t, 6H + 6H, J 7.5 Hz, OCH$_2$CH$_2$CH$_3$), 1.13, 1.15, 1.25, 1.34 (four t, 3H+3H+3H+3H, J 7.2 Hz CH$_2$CH$_3$), 1.81-2.05 (m, 16H, CH$_2$CH$_2$CH$_3$), 3.11, 3.13, 3.15 (three d, 1H+1H+2H, J 13.0 Hz, ArCH$_2$eq), 3.57, 3.65 (two t, 2H + 2H, J 7.2 Hz, OCH$_2$), 3.76-4.06 (m, 14H, OCH$_2$), 4.26, 4.28 (two d, 0.5H+0.5H, J$_{HH}$ 10.3 Hz, diastereotopic ArCH$_2$OP), 4.39, 4.41 (two d, 0.5H+0.5H, J$_{HH}$ 10.3 Hz, diastereotopic ArCH$_2$OP), 4.43, 4.42 (two d, 2H+2H, J 13.0 Hz,
ArCH₂ₐ), 5.01, 5.05 (two d, 0.5H+0.5H, J 11.2 Hz, PCH), 5.93, 6.04 (two d, 1H+1H, J 2 Hz, m-ArH), 6.15, 6.52 (two dd, 1H+1H, J 1.6 Hz, J 2 Hz, m-ArH), 6.84, 6.98 (two t, 1H+1H, J 7.2 Hz, p-ArH), 7.16 (m, 4H, m-ArH). 3¹P NMR (CDCl₃), δ -1.73, δ 17.1. Mass spectrum (FAB) m/z; 982[M +H]⁺, 1004[M + Na]⁺. Calcd for C₅₃H₇₈O₁₂P₂ 981.13. Calculated for C₅₄H₇₈O₁₂P₂, %: C 66.10, H 8.01, P 6.31. Found, %: C 66.19, H 7.92, P 6.39.

**Dipropyl 25,26,27,28-tetrapropoxyalix[4]arene-11,23-dibromo-5-(dipropoxyphosphoryloxyethyl)-17-(α-hydroxymethylphosphonate) (22).** Colorless crystals: yield 57 %. m.p. 75-77 °C. ¹H NMR (CDCl₃), δ: 0.88, 1.08 (two t, 6H + 6H, J 7.5 Hz, OCH₂CH₃), 1.13, 1.15, 1.27, 1.35 (four t, 3H+3H+3H+3H, J 7.2 Hz CH₂CH₃), 1.88, 1.95 (m, 4H+4H, CH₂CH₂CH₃), 3.11, 3.13, 3.17 (three d, 1H+1H+2H, J 13.0 Hz, ArCH₂eq), 3.57, 3.69 (two t, 2H + 2H, J 7.2 Hz, OCH₂), 3.74-4.04 (m, 14H, OCH₂), 4.26, 4.28 (two d, 0.5H+0.5H, JHH 10.3 Гц, diastereotopic ArCH₂OP), 4.35, 4.35 (two d, 0.5H+0.5H, JHH 10.3 Hz, diastereotopic ArCH₂OP), 4.41, 4.45 (two d, 2H+2H, J 13.0 Hz, ArCH₂ax), 5.01, 5.06 (two d, 0.5H+0.5H, J 11.2 Hz, PCH), 5.91, 6.05 (two d, 1H+1H, J 2 Hz, m-ArH), 6.16, 6.53 (two dd, 1H+1H, J 1.6 Hz, J 2 Hz, m-ArH), 7.15 (m, 4H, m-ArH). ³¹P NMR (CDCl₃), δ -0.95, δ 18.7. Mass spectrum (FAB) m/z; 1084[M +H]⁺. Calcd for C₅₀H₆₈Br₂O₁₂P₂ 1082.82. Calculated for C₅₀H₆₈Br₂O₁₂P₂, %: C 55.46, H 6.33, P 5.72. Found, %: C 55.60, H 6.38, P 5.59.

**Synthesis of calixaren-α-hydroxymethylphosphonic acids 23-29 by dealkylation of corresponding esters. General procedure**

To a solution of calixaren-α-hydroxymethylphosphonate 4-6, 8, 11, 16, 18 (0.1 mmol) in dry chloroform (5 ml) bromotrimethylsilane (ten-fold molar excess per each dialkoxyphosphonate group) was added. The reaction mixture was stirred at room temperature for 48 h and then was concentrated under reduced pressure. The residue was dissolved in absolute methanol (15 ml), the resulting mixture was stirred at 50 °C for 2 h, and then concentrated and dried in vacuo (0.05 mm Hg) at room temperature for 10 h.

**26,28-Dihydroxy-25,27-dipropoxyalix[4]arene-5-(α-hydroxymethylphosphonic acid) (23).** Slightly colored crystals: yield 95 %. m.p. 270-278 °C. ¹H NMR (DMSO-d₆), δ: 1.18 (t, 6H, J 7.2 Hz, CH₂CH₃), 2.15 (m, 4H, CH₂CH₂CH₂CH₃), 3.26, 3.35 (two d, 2H+2H, J 13.4 Гц, ArCH₂eq), 4.05 (t, 4H, J 7.2 Hz, OCH₂), 4.36 (d, 4H, J 13.4 Hz, ArCH₂ax), 4.54 (d, 1H, J 10 Hz, PCH), 6.61 (t, 1H, J 7.5 Hz, ArH-p), 6.61 (t, 2H, J 7.5 Hz, ArH-p), 6.92 (d, 4H, J 7.5 Hz, ArH-m), 6.98 (d, 2H, J 7.5 Hz, ArH-m), 7.19, 7.25 (two s, 2H, diastereotopic ArH-m), 3¹P NMR (DMSO-d₆), δ 16.8. Mass spectrum (FAB) m/z; 620[M +H]⁺, 1238 [2M + H]⁺. Calcd for C₅₃H₇₉O₈P 618.65.

**28-Hydroxy-25,26,27-tripropoxyalix[4]arene-5-(α-hydroxymethylphosphonic acid) (24).** Slightly colored crystals: yield 96 %. m.p. 265-270 °C. ¹H NMR (DMSO-d₆), δ: 1.31 (m, 9H, CH₂CH₃), 2.03 (m, 6H, CH₂CH₂CH₃), 3.29-3.35 (m, 4H, ArCH₂eq), 4.06 (m, 6H, OCH₂), 4.28 (d, 4H, J 13.4 Hz, ArCH₂ax), 4.54 (d, 1H, J 10 Hz, PCH), 6.71 (t, 1H, J 7.5 Hz, ArH-p), 6.72 (t, 2H, J 7.5 Hz, ArH-p), 6.72 (d, 4H, J 7.5 Hz, ArH-m), 7.14 (d, 2H, J 7.5 Hz, ArH-m), 7.16, 7.20 (two s, 2H, diastereotopic ArH-m). ³¹P NMR (DMSO-d₆), δ 16.5. Mass spectrum (FAB) m/z; 662 [M + H]⁺. Calcd for C₃₈H₄₅O₈P 660.73.
25,26,27,28-Tetrapropoxy calix[4] arene-5-(α-hydroxymethylphosphonic acid) (25). Colorless crystals: yield 94 %. m.p. 247-251 ºC. 1H NMR (DMSO-d6): δ: 0.98 (m, 12H, CH₂CH₂CH₃), 1.96 (m, 8H, CH₂CH₂CH₃), 3.21 (d, 4H, J 12.9 Hz, ArCH₂eq), 3.96 (m, 8H, OCH₂), 4.45 (d, 4H, J 12.9 Hz, ArCH₂ax), 4.63 (d, 1H, J 10 Hz, PCH), 6.35-6.92 (m, 11H, ArH). 31P NMR δ (DMSO-d6) 17.2. Mass spectrum (FAB) m/z: 704 [M+H]+. Calcd for C₄₁H₅₁OsP 702.81.

(RS)-26,28-Dihydroxy-25,27-dipropoxy calix[4] arene-5,17-bis(α-hydroxymethylphosphonic acid) (26). Colorless crystals: yield 95 %. m.p. 262-266 ºC. 1H NMR (CD3OD), δ: 1.24 (t, 6H, J 7.5 Hz, CH₂CH₃), 2.04 (m, 4H, CH₂CH₂CH₃), 3.42 (d, 4H, J 13.0 Hz, ArCH₂eq), 3.98 (t, 4H, J 6.2 Hz, OCH₂), 4.24 (d, 4H, J 13.0 Hz, ArCH₂ax), 4.81 (d, 2H, J 10.0 Hz, PCH), 6.65, 6.67 (two t, 2H, J 8.0 Hz, p-ArH), 6.85, 6.87 (two d, 4H, J 8.0 Hz, m-ArH), 7.21 (s, 4H, m-ArH). 31P NMR (CD3OD), δ 16.7. Mass spectrum (FAB) m/z: 729 [M +H]+, 752 [M + Na]+, 1458 [2M +H]+. Calcd for C₃₆H₄₂O₁₂P₂ 728.66.

(RR+SS)-26,28-Dihydroxy-25,27-dipropoxy calix[4] arene-5,17-bis(α-hydroxymethylphosphonic acid) (27). Colorless crystals: yield 97 %. m.p. 254-258 ºC. 1H NMR (CD3OD), δ: 1.26 (t, 6H, J 7.5 Hz, CH₂CH₃), 2.08 (m, 4H, CH₂CH₂CH₃), 3.42, 3.44 (two d, 2H+2H, J 14.0 Hz, ArCH₂eq), 3.85 (t, 4H, J 6.2 Hz, OCH₂), 4.21, 4.21 (two d, 2H+2H, J 14.0 Hz, ArCH₂ax), 4.69 (d, 2H, J 13.0 Hz, PCH), 6.64, 6.68 (two dd, 1H+1H, J 8.0 Hz, J 2.0 Hz, p-ArH), 6.84, 6.91 (two dd, 2H+2H, J 8.0 Hz, J 2.0 Hz, m-ArH), 7.23 (d, 4H, J 2.0 Hz, m-ArH), 7.33 (d, 4H, J 2.0 Hz, m-ArH), 31P NMR (CD3OD), δ 15.2. Mass spectrum (FAB) m/z: 729 [M +H]+. Calcd for C₃₆H₄₂O₁₂P₂ 728.66.

(RR+SS+RR)-25,26,27,28-Tetrapropoxy calix[4] arene-5,17-bis(α-hydroxymethylphosphonic acid) (28). Colorless crystals: yield 95 %. m.p. 225-230 ºC. 1H NMR (DMSO-d6), δ: 1.24 (t, 8H, J 7.5 Hz, CH₂CH₃), 1.95, 2.04 (two m, 4H+4H, CH₂CH₂CH₃), 3.42 (d, 4H, J 13.0 Hz, ArCH₂eq), 3.98 (t, 8H, J 7.5 Hz, OCH₂), 4.24 (d, 4H, J 13.0 Hz, ArCH₂ax), 4.81 (d, 2H, J 10.0 Hz, PCH), 6.65, 6.67 (two t, 2H, J 8.0 Hz, p-ArH), 6.85, 6.87 (two d, 4H, J 8.0 Hz, m-ArH), 7.21 (s, 4H, m-ArH), 31P NMR (DMSO-d6), δ 16.7, 17.1. Mass spectrum (FAB) m/z: 813.3 [M +H]+, 835 [M + Na]+, 1626 [2M +H]+. Calcd for C₄₂H₅₄O₁₂P₂ 812.31.

(RR+SS+RS)-25,26,27,28-Tetrapropoxy calix[4] arene-11,23-dibromo-5,17-bis(α-hydroxymethylphosphonic acid) (29). Colorless crystals: yield 97 %. m.p. 239-244 ºC. 1H NMR (DMSO-d6), δ: 1.13, 1.26 (two t, 6H+6H, J 7.5 Hz, CH₂CH₃), 1.85, 1.98 (two m, 4H+4H, CH₂CH₂CH₃), 3.42, 3.44 (two d, 2H+2H, J 13.0 Hz, ArCH₂eq), 3.85, 4.12 (two t, 4H+4H, J 6.2 Hz, OCH₂), 4.21, 4.21 (two d, 2H+2H, J 13.0 Hz, ArCH₂ax), 4.73 (d, 2H, J 11.3 Hz, PCH), 6.25, 6.31 (two s, 2H+2H, ArH), 7.11, 7.31 (two s, 2H+2H, ArH), 31P NMR (DMSO-d6), δ 21.9, 22.3. Mass spectrum (FAB) m/z: 972 [M +H]+, 994 [M + Na]+, 1943 [2M +H]+. Calcd for C₄₂H₅₂Br₂O₁₂P₂ 970.61.

**One pot synthesis of calixarene-α-hydroxymethylphosphonic acids 25,26,28,29**
To a solution of formylcalixarene 3, 7, 14, 15 (0.1 mmol) in dry THF (5 ml) tris-trimethylsilyl phosphite (0.13 mmol for 3, 0.4 mmol for 7 and 0.25 mmol for 14, 15) was added. The reaction mixture was stirred at room temperature for 8 h and then was concentrated under reduced pressure and dried in vacuo (0.05 mm Hg) at room temperature for 8 h. The residue was
dissolved in absolute methanol (15 ml). The resulting mixture was stirred at 50 °C for 2 h, and then concentrated and dried in vacuo (0.05 mm Hg) at room temperature for 10 h.

\textbf{25,26,27,28-Tetrapropoxycalix[4]arene-5-(a-hydroxymethylphosphonic acid) (25).} Colorless crystals: yield 94\%. m.p. 247-251 °C. Physico-chemical and spectral parameters are identical to the substance obtained by dealkylation of the corresponding ester (see \textit{supra}).

\textbf{(RS)-26,28-Dihydroxy-25,27-dipropoxycalix[4]arene-5-(a-hydroxymethylphosphonic acid) (2).} Colorless crystals: yield 95\%. m.p. 262-266 °C. Physico-chemical and spectral parameters are identical to the substance obtained by dealkylation of the corresponding ester (see \textit{supra}).

\textbf{(RR+SS+RS)-25,26,27,28-Tetrapropoxycalix[4]arene-5,17-bis(a-hydroxymethylphosphonic acid) (28).} Colorless crystals: yield 95\%. m.p. 226-230 °C. Physico-chemical and spectral parameters are identical to the substance obtained by dealkylation of the corresponding ester (see \textit{supra}).

\textbf{(RR+SS+RS)-25,26,27,28-Tetrapropoxycalix[4]arene-11,23-dibromo-5,17-bis(a-hydroxymethylphosphonic acid) (29).} Colorless crystals: yield 97\%. m.p. 239-244 °C. Physico-chemical and spectral parameters are identical to the substance obtained by dealkylation of the corresponding ester (see \textit{supra}).

\textbf{Effect of inhibitors on the activity of glutathione S-transferase}

Glutathione S-transferases from equine liver and human placenta were purchased from Sigma. According to the known method, 1-chloro-2,4-dinitrobenzene and L-glutathione were used as substrates of enzyme reaction.\textsuperscript{25} The activity of the enzymes were determined spectrophotometrically at 340 nm (where the product of reaction absorbs) using a molar extinction coefficient of 9600 M\textsuperscript{-1}cm\textsuperscript{-1}. The influence of the calix[4]arene-a-hydroxymethylphosphonic acids on the rate of S-conjugate formation catalyzed by glutathione S-transferase was determined in 0.1 M Na-phosphate buffer at pH 6.5. The standard assay mixture contained 1 mM L-glutathione, 1mM CDNB and 0.1 mM EDTA and several concentrations of inhibitor. Compounds 23, 25, 26, 28 and 29 were preliminary dissolved in DMSO (the concentration of DMSO in reaction mixture was 2.5 vol.\%). The mixture with substrates and inhibitor was incubated for 5 min at 25 °C, and the reaction was initiated by adding 5 \textmu l or 10 \textmu l of the enzyme (1 mg/ml). The IC\textsubscript{50} values (inhibitor concentration that inhibits 50\% of the enzyme activity) were determined in the presence of various amounts of calix[4]arene derivatives. The results given in Table 2 are presented as mean ± S.D.

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References