Calix[4]arene-α-hydroxyphosphonic acids. Synthesis, stereochemistry, and inhibition of glutathione *S*-transferase

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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 (tristrimethylsilyl)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 ^{1}H NMR. The resulting α-hydroxymethylphosphonic acids were found to be able to inhibit the activity of glutathione *S*-transferase *in vitro*.

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

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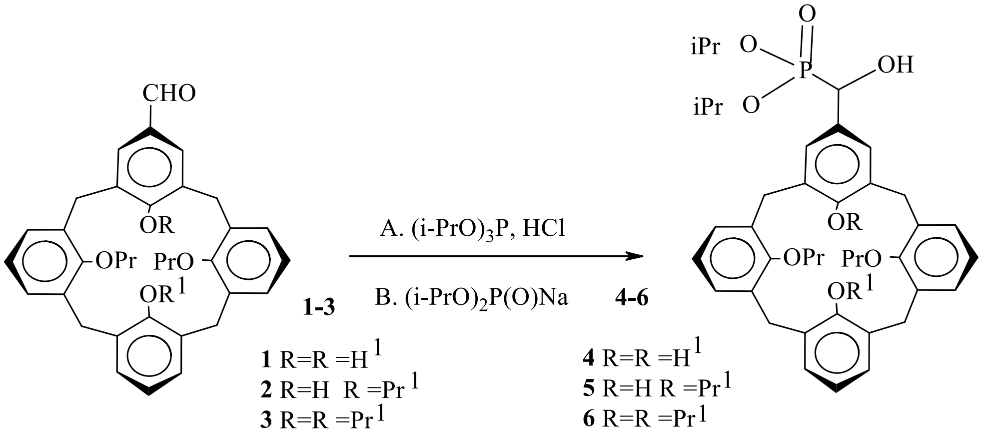
Introduction

Calixarenes – cyclic oligomers obtained by the condensation of para-substituted phenols with formaldehyde[1] – 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 B_{12}, nucleotides, nucleosides and short DNA fragments.[6-10] Also, the calix[4]arene derivatives were fixed on proteins.[11,12] Calixarenes are considered as promising objects of biochemical research due to their ability to simulate the substrate-receptor interaction with biomolecules.[13,14]

Previously we have shown that calix[4]arenes functionalized with phosphonic, aminophosphonic and methylenebisphosphonic acid residues are effective inhibitors of alkaline phosphatases[15-17] and some of protein tyrosine phosphatases.[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.[15-18] The aim of this work was to design calixarene based α-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[20-22] and thereby can decrease effects of drugs causing multidrug resistance.[22,23] Several heterocyclic compounds,[24,25] etacrinic acid derivatives[26] and some structural analogues of glutathione[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.[23,29] As we have already noted,[30] glutathione *S*-transferase can be a potential target for calixarene based phosphonic acids *in vitro*. It this paper we describe the synthesis, stereochemistry and inhibition ability of a series of lower-rim substituted calix[4]arenes bearing one or two α-hydroxymethylphosphonic acid fragments at the upper rim of the macrocycle.

Results and Discussion

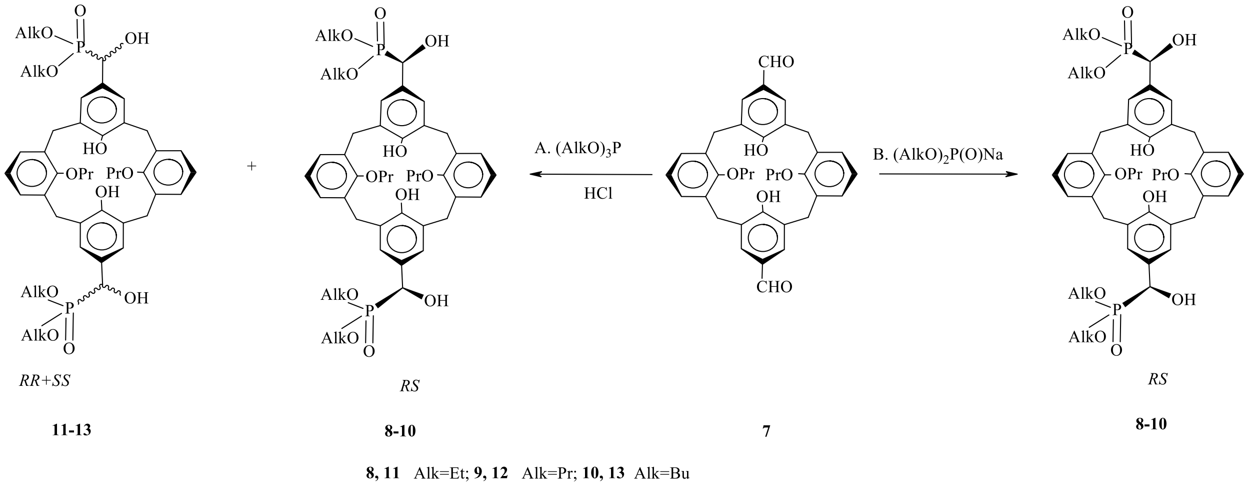
Calixarene-mono-α-hydroxymethylphosphonates **4-6** were obtained as the precursors of the desired acids by the reaction of dipropoxy-, tripropoxy- and tetrapropoxyformylcalixarenes **1-3** with triisopropyl phosphite in the presence of anhydrous hydrogen chloride (Method A) or by the Pudovik addition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



Scheme 1. Synthesis of calixarene-mono-α-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 $ArCH_2Ar$ 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** (Δδ 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 Δδ 1.22 ppm shows the *regular cone* conformation (symmetry C_{2v}) of tetrapropoxycalixarene **6**.[35] Tripropoxycalixarene **5** with Δδ 1.05 ppm occupies an intermediate position between these two conformations.

Two chiral carbon atoms in calixarene-bis-α-hydroxymethylphosphonates determine the formation of *racemic* form (*RR+SS*), *meso* form (*RS*) or their mixtures in the reaction products. Earlier calixarene-bis-α-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 ºC for 8 h proceeds diastereoselectively and leads to the *meso* form of calixarene-di-α-hydroxymethylphosphonates **8-10** with d.e. almost 100% (Scheme 2).



Scheme 2. Synthesis of dipropoxycalixarene-bis-α-hydroxymethylphosphonates **8-13**.

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-α-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 °C. Decreasing the temperature significantly increases the reaction time but does not improve the diastereoselectivity. Pure *racemic* forms of calixarene-bis-α-hydroxymethylphosphonates **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 (see[36] for a comparison). In the *racemic* form **11** possessing C_2 symmetry axis the protons appear as three doublets of doublets. In the *meso* form **8** possessing C_s symmetry plane two doublets and two triplets of the protons are observed in the ^{1}H NMR spectra (Figure 1).

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

Trialkyl phosphite	Temperature, °C	Ratio of the *racemic* and *meso* forms (d.e., %)
$(EtO)_3P$	10	81 : 19 (62)
	20	69 : 31 (38)
	30	52 : 48 (4)
$(PrO)_3P$	10	86 : 14 (72)
	20	79 : 21 (58)
	30	65 : 35 (30)
$(BuO)_3P$	10	93 : 7 (86)
	20	85 : 15 (70)
	30	74 : 26 (48)

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, J_{HP} 13 Hz and δ 4.9 ppm, J_{HP} 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 ^{31}P NMR spectra.

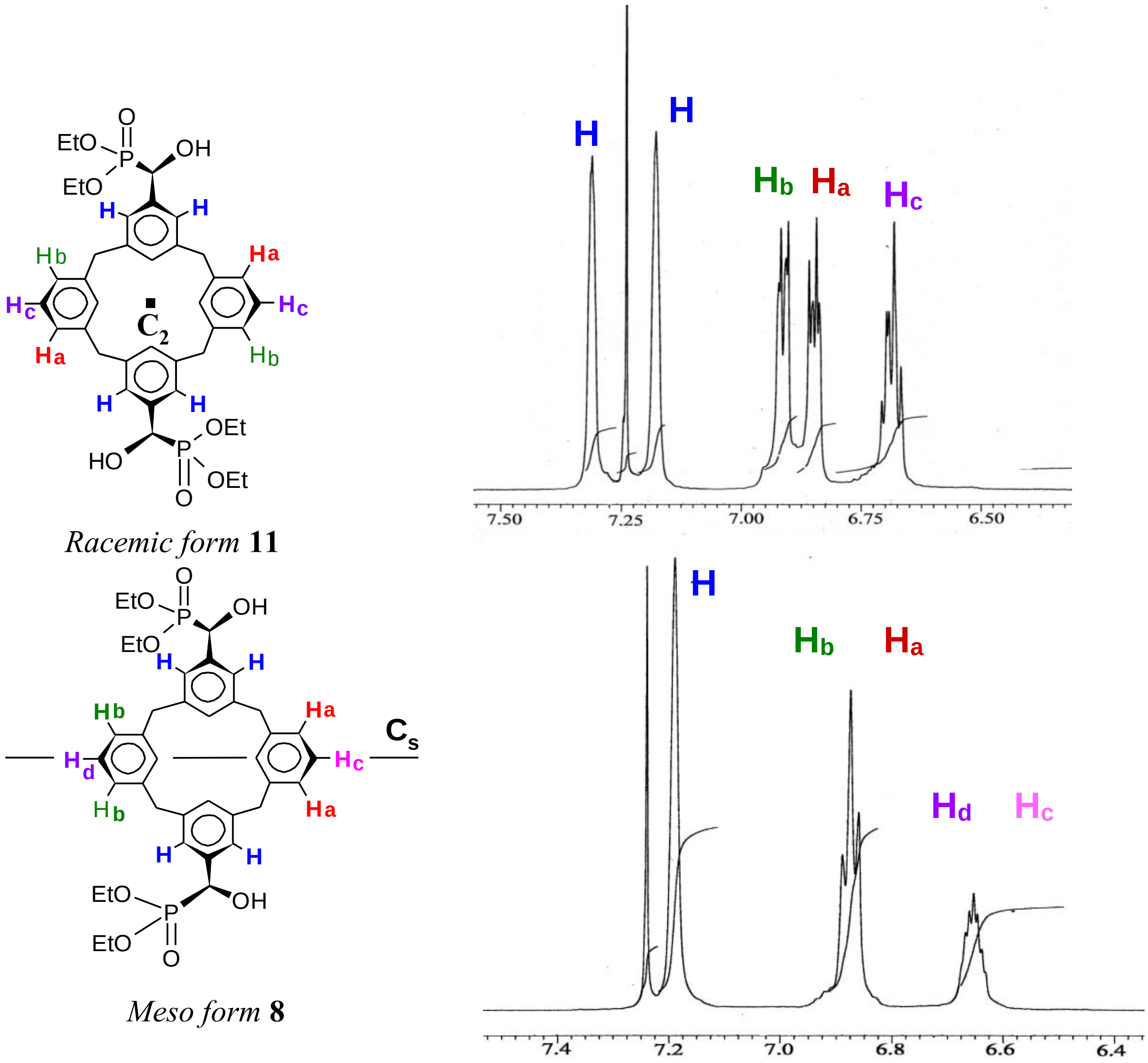
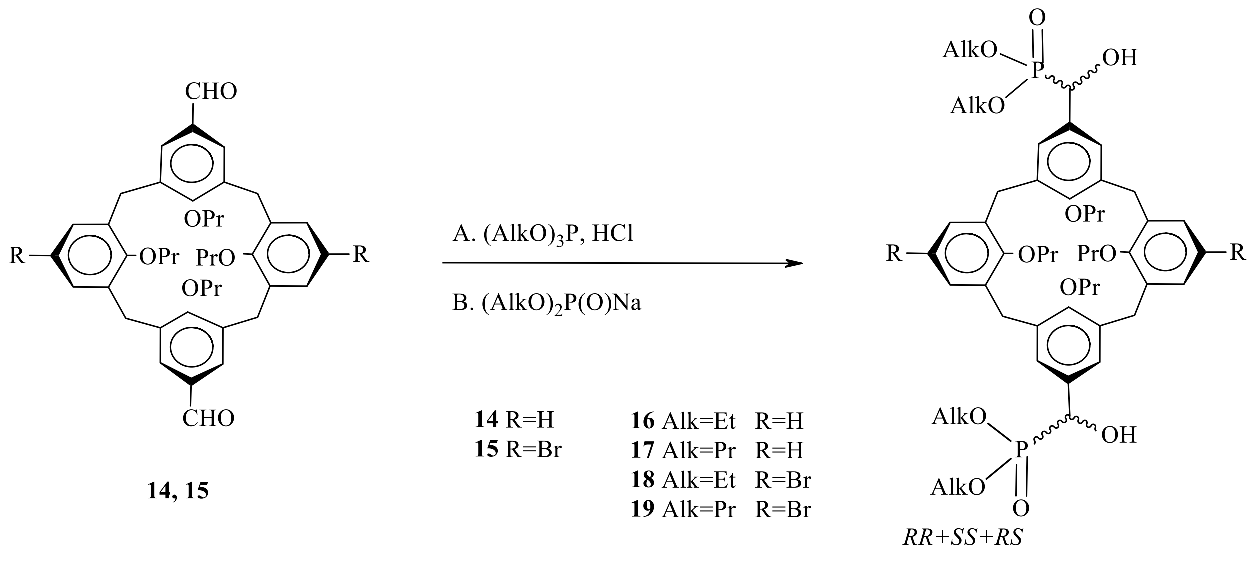


Figure 1. ^{1}H NMR spectra of aromatic protons of *racemic* **11** and *meso* **8** forms of dipropoxycalixarene-bis-α-hydroxymethylphosphonate ($CDCl_3$).



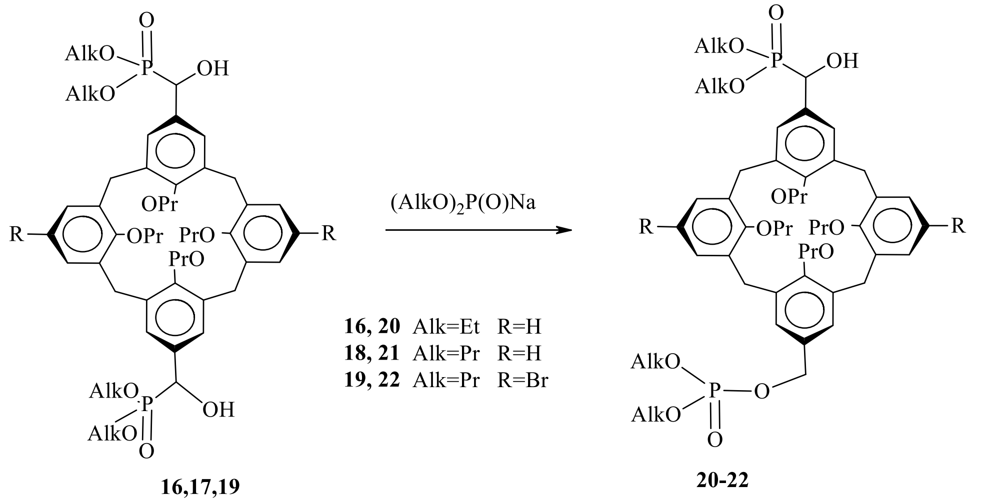
14, 15

16-19

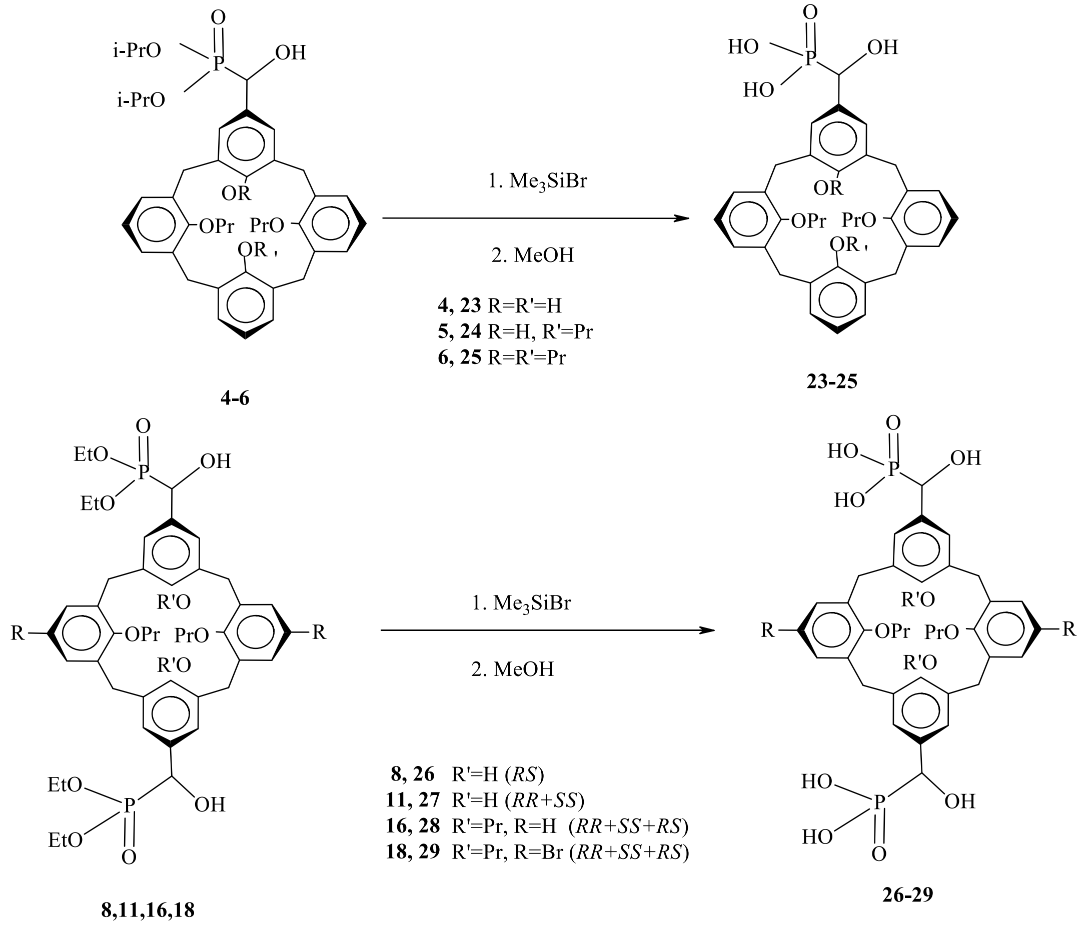
Scheme 3. Synthesis of tetrapropoxycalixarene-bis-α-hydroxymethylphosphonates **16-19.**

Diastereoselectivity of the diformylcalixarene phosphorylation depends significantly on the substitution pattern of the macrocycle lower rim. In contrast to dipropoxydiformylcalixarene **7**, the reactions of tetrapropoxydiformylcalixarenes **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 tetrapropoxydiformylcalixarenes **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 ^{o}C for 48 h and leads to formation of phosphono-phosphates **20-22** (Scheme 4). Increasing the reaction time and temperature up to 60 ^{o}C does not cause rearrangement of the second phosphonate group and formation of the corresponding diphosphates.



Scheme 4. Synthesis of calixarene-phosphonatophosphates **20-22**.

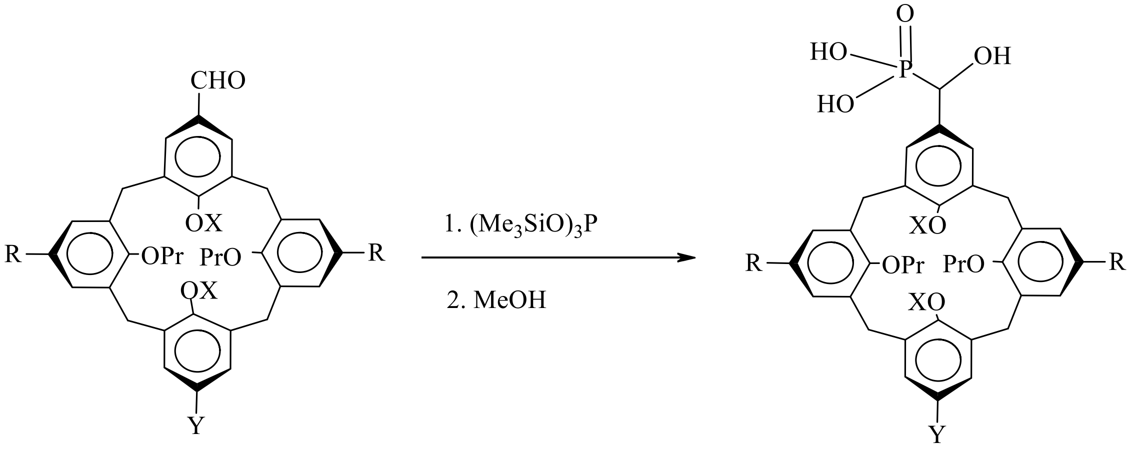


Scheme 5. Synthesis of the calixarene-α-hydroxymethylphosphonic acids **26-29** by dealkylation of the corresponding esters.

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.[37,38] 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 dipropoxydiformyl-calixarene **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**.



3, 7, 14, 15

3: R=Y=H,X=Pr
7: R=X=H, Y=CHO
14: R=H, Y=CHO, X=Pr
15: R=Br, Y=CHO, X=Pr

25, 26, 28, 29

25: R=Y=H,X=Pr
26: R=X=H, Y=CH(OH)P(O)(OH)$_2$ (*RS)*
28: R=H, X=Pr, Y=CH(OH)P(O)(OH)$_2$ *(RS+RR+SS)*
29: R=Br, X=Pr, Y=CH(OH)P(O)(OH)$_2$ (*RS*+*RR*+*SS*)

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 hydroxymethyldihydroxyphosphonic 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,[30] 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_{50} 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_{50} 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

Inhibitor	IC_{50}(EL), μM	IC_{50}(HP), μM
23 (*R*+*S*)	7.5 ± 2.3	*
26 (*RS*)	28 ± 5	77 ± 10
25 (*R*+*S*)	58 ± 2	*
28 (*RS* + *RR* + *SS*)	6.1 ± 1.9	29 ± 13
29 (*RS* + *RR* + *SS*)	4.5 ± 1.1	34 ± 6

*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.[39] A binding model was constructed automatically on the basis of known X-ray crystal structure of the enzyme[40] (PDB code: 20GS.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 dipropoxycalix[4]arene **26** and and tetrapropoxycalix[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-hydroxy-methylphosphonates **26** and **28** into the active site of GST P1-1 from human placenta

Calixarene	ΔE_{doc}, kJ/mol
26 (*SS*)	-33.0
26 (*RR*)	-31.8
26 (*RS*)	-31.7
28 (*SS*)	-33.0
28 (*RR*)	-34.1
28 (*RS*)	-33.8

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.[41] 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).[42] 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.[43] As seen in Figure 2, the residues of Phe8, Arg13 and Gln51, which are involved in glutathione binding at the G-site,[44] 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.

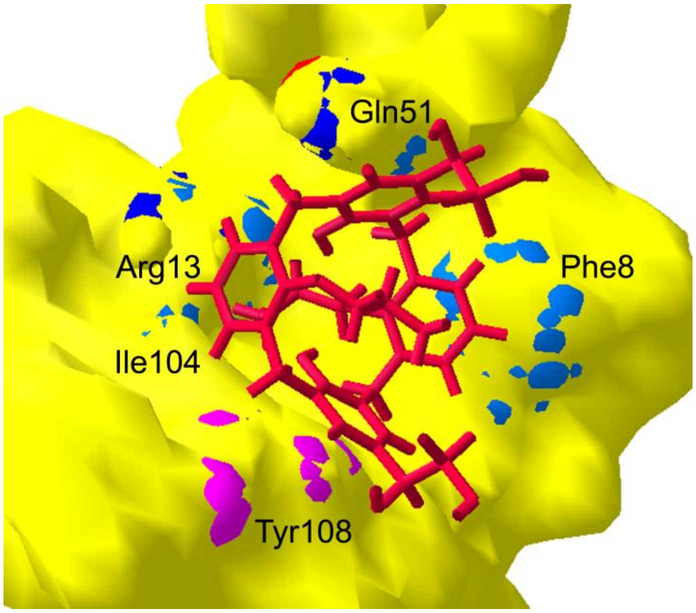


Figure 2. The possible binding mode of (*RS*)-calix[4]arene-bis-α-hydroxymethylphosphonate **26** in the active site of GST P1-1.

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-hydroxymetylphosphonic 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% H_3PO_4 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 ºC (cyclohexane). ^{1}H NMR ($CDCl_3$), δ: 0.69 (d, 3H, J 6.0 Hz, OCHCH_3), 1.10-1.37 (m, 9H, diastereotopic OCHCH_3), 1.31 (t, 6H, J 7.2 Hz, CH_2CH_3), 2.07 (m, 4H, CH_2C$H_2$$CH_3$), 3.35, 3.41 (two d, 2H+2H, J 13.4 Hz, ArCH_{2eq}), 3.97 (t, 4H, J 7.2 Hz, OCH_2), 4.31 (d, 4H, J 13.4 Hz, ArCH_{2ax}), 4.37, 4.59 (two m, 1H + 1H, diastereotopic C*H*CH_3), 4.78 (d, 1H, J 10 Hz, PCH), 6.65 (t, 1H, J 7.5 Hz, ArH-p), 6.71 (t, 2H, J 7.5 Hz, ArH-p), 6.90 (d, 4H, J 7.5 Hz, ArH-m), 7.05 (d, 2H, J 7.5 Hz, ArH-m), 7.19, 7.25 (two s, 2H, diastereotopic ArH-m), 8.24 (s, 1H, OH), 8.35 (s, 1H, OH). ^{31}P NMR ($CDCl_3$), δ 18.5. Mass spectrum (FAB) *m/z;* 537[M-HPO(OPr-i)$_2$ + H]$^+$, 685[M-H_2O + H]$^+$, 704[M + H]$^+$. Calcd for $C_{41}H_{51}O_8P$ 702.8.

Diisopropyl 28-hydroxy-25,26,27-tripropoxycalix[4]arene-5-(α-hydroxymethylphosphonate) (5). Colorless crystals: yield 71%. m.p. 165-167 ºC (cyclohexane). ^{1}H NMR ($CDCl_3$), δ: 0.71 (d 3H, J 6.0 Hz, OCHCH_3), 1.12-1.39 (m, 9H, diastereotopic OCHCH_3), 1.29 (m, 9H, CH_2CH_3), 2.07 (m, 6H, CH_2CH_2CH_3), 3.29, 3.36 (two d, 2H+2H, J 13.4 Hz, ArCH_{2eq}), 3.97 (m, 6H, J 7.2 Hz, OCH_2), 4.35 (d, 4H, J 13.4 Hz, ArCH_{2ax}), 4.35, 4.61 (two m, 1H + 1H, diastereotopic C*H*CH_3), 4.75 (d, 1H, J 10 Hz, PCH), 6.68 (t, 1H, J 7.5 Hz, ArH-p), 6.75 (t, 2H, J 7.5 Hz, ArH-p), 6.87 (d, 4H, J 7.5 Hz, ArH-m), 7.08 (d, 2H, J 7.5 Hz, ArH-m), 7.20, 7.24 (two s, 2H, diastereotopic ArH-m), 8.35 (s, 1H, OH. ^{31}P NMR ($CDCl_3$), δ 18.7. Mass spectrum (FAB) *m/z;* 746 [M + H]$^+$. Calcd for $C_{44}H_{57}O_8P$ 744.9.

Diisopropyl 25,26,27,28-tetrapropoxycalix[4]arene-5-(α-hydroxymethylphosphonate) (6). Colorless crystals: yield 68%. m.p. 134-136 ºC. ^{1}H NMR ($CDCl_3$), δ: 0.68 (d 3H, J 6.0 Hz, OCHCH_3), 1.14-1.37 (m, 9H, diastereotopic OCHCH_3), 1.04 (m, 12H, CH_2CH_3), 2.02 (m, 8H, CH_2CH_2CH_3), 3.19 (d, 4H, J 12.9 Hz, ArCH_{2eq}), 3.90 (m, 8H, OCH_2), 4.41 (d, 4H, J 12.9 Hz, ArCH_{2ax}), 4.31, 4.57 (two m, 1H + 1H, diastereotopic C*H*CH_3), 4.75 (d, 1H, J 10 Hz, PCH), 6.44-7.04 (m, 11H, ArH. ^{31}P NMR ($CDCl_3$), δ 21.4. Mass spectrum (FAB) *m/z;* 788 $[M + H]^+$. Calcd for $C_{47}H_{63}O_8P$ 786.97. Calculated for $C_{47}H_{63}O_8P$: C 71.73, H 8.07, P 3.94. Found, C 71.82, H 8.16, P 3.83%.

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 ºC (cyclohexane). ^{1}H NMR ($CDCl_3$), δ: 0.84 (m, 6H, CH_3), 1.29 (t, 12H, J 7.5 Hz, $CH_2C\mathit{H}_3$), 2.04 (m, 4H, $CH_2CH_2C\mathit{H}_3$), 3.38, 3.40 (two d, 2H + 2H, J 14.0 Hz, $ArCH_{2eq}$), 3.54, 3.82 (two m, 2H + 2H, diastereotopic $POC\mathit{H}_2$), 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, $ArCH_{2ax}$), 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, *м*-ArH), 7.21 (d, 4H, J 2.0 Hz, *м*-ArH), 7.33 (d, 4H, J 2.0 Hz, *м*-ArH), 8.49 (s, 2H, OH). ^{31}P NMR ($CDCl_3$), δ 19.4. Mass spectrum (FAB) *m/z;* 841.8$[M +H]^+$, 863$[M + Na]^+$, 1683$[2M +H]^+$. Calcd for $C_{44}H_{58}O_{12}P_2$ 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 ºC (cyclohexane). ^{1}H NMR ($CDCl_3$), δ: 1.03, 1.16 (two t, 6H+6H, J 6.0 Hz, diastereotopic $OCH_2CH_2C\mathit{H}_3$), 1.31 (t, 6H, J 7.5 Hz, $CH_2C\mathit{H}_3$), 1.94 (m, 8H, $OCH_2CH_2C\mathit{H}_3$), 2.05 (m, 4H, $CH_2CH_2C\mathit{H}_3$), 3.35, 3.39 (two d, 2H + 2P, J 14.0 Hz, $ArCH_{2eq}$), 3.55, 3.82 (two m, 2H + 2H, diastereotopic $POC\mathit{H}_2$), 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, $ArCH_{2ax}$), 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, *м*-ArH), 7.21 (d, 4H, J 2.0 Hz, *м*-ArH), 7.33 (d, 4H, J 2.0 Hz, *м*-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 ºC (cyclohexane). ^{1}H NMR ($CDCl_3$), δ: 1.02, 1.17 (two t, 6H+6H, J 6.0 Hz, diastereotopic $OCH_2CH_2C\mathit{H}_3$), 1.28 (t, 6H, J 7.5 Hz, $CH_2C\mathit{H}_3$), 1.95 (m, 8H, $OCH_2CH_2C\mathit{H}_3$), 2.51 (m, 4H, $CH_2CH_2C\mathit{H}_3$), 3.38, 3.40 (two d, 2H + 2P, J 14.0 Hz, $ArCH_{2eq}$), 3.54, 3.81 (two m, 2H + 2H, diastereotopic $POC\mathit{H}_2$), 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, $ArCH_{2ax}$), 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, *м*-ArH), 7.20 (d, 4H, J 2.0 Hz, *м*-ArH), 7.34 (d, 4H, J 2.0 Hz, *м*-ArH), 8.51 (s, 2H, OH). ^{31}P NMR ($CDCl_3$), δ 19.1. Mass spectrum (FAB) *m/z;* 934$[M –H_2O+H]^+$, 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.

Tetraethyl (*RR*+*SS*+*RS*) 25,26,27,28-tetrapropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (16). Colorless crystals: yield 85 %. m.p. 110-116 ºC. ^{1}H

NMR ($CDCl_3$), δ: 0.84, 1.02 (two m, 6H+6H, OCH_2CH_3), 1.09, 1.19 (two m, 6H+6H, $CH_2CH_2CH_3$), 1.93 (m, 8H, $CH_2CH_2CH_3$), 3.10, 3.15 (two d, 2H+2H, J 13.2 Hz, $ArCH_{2eq}$), 3.71-4.15 (m, 16H, OCH_2), 4.42, 4.45 (two d, 2H+2H, J 13.2 Hz, $ArCH_{2ax}$), 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). ^{31}P NMR ($CDCl_3$), δ 20.8. Mass spectrum (FAB) m/z; 769[M-H_2O–$HPO(OC_2H_5)_2$+H]$^+$, 907[M -H_2O +H]$^+$, 926[M +H]$^+$. 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 64.96, H 7.66, P 6.61.

Tetrapropyl (*RR*+*SS*+*RS*) 25,26,27,28-tetrapropoxycalix[4]arene-5,17-bis(α-hydroxymethyl-phosphonate) (17). Colorless crystals: yield 82 %. m.p. 102-107 °C. ^1H NMR ($CDCl_3$), δ: 0.87, 0.98 (two m, 6H+6H, $OCH_2CH_2CH_3$), 1.12, 1.1.23 (two m, 6H+6H, $CH_2CH_2CH_3$), 1.85-2.05 (m, 16H, $CH_2CH_2CH_3$), 3.12, 3.14 (two d, 2H+2H, J 13.2 Hz, $ArCH_{2eq}$), 3.69-4.18 (m, 16H, OCH_2), 4.43, 4.44 (two d, 2H+2H, J 13.2 Hz, $ArCH_{2ax}$), 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). ^{31}P NMR ($CDCl_3$), δ 20.5. Mass spectrum (FAB) m/z; 965[M -H_2O +H]$^+$, 982[M +H]$^+$, 1003[M + Na]$^+$. Calcd for $C_{54}H_{78}O_{12}P_2$ 981.14. Calculated for $C_{54}H_{78}O_{12}P_2$, %: 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-tetrapropoxycalix[4]arene-11,23-dibromo-5,17-bis(α-hydroxymethylphosphonate) (18). Colorless crystals: yield 75 %. m.p. 144-146 °C. ^1H NMR ($CDCl_3$), δ: 0.85, 0.95 (two t, 6H + 6H, J 6.0 Hz, OCH_2CH_3), 1.23 (m, 12H, CH_2CH_3), 1.96 (m, 8H, $CH_2CH_2CH_3$), 3.14, 3.16 (two d, 4H, J 13.0 Hz, $ArCH_{2eq}$), 3.51-4.2 (m, 16H, $POCH_2$+OCH_2), 4.39, 4.41 (two d, 4H, J 13.0 Hz, $ArCH_{2ax}$), 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), ^{31}P NMR ($CDCl_3$), δ 19.6. Mass spectrum (FAB) *m/z;* 1084 [M +H]$^+$, 1106[M + Na]$^+$, 2166[2M +H]$^+$. Calcd for $C_{50}H_{68}Br_2O_{12}P_2$ 1082.84. Calculated for $C_{50}H_{68}Br_2O_{12}P_2$, %: 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-tetrapropoxycalix[4]arene-11,23-dibromo-5,17-bis(α-hydroxymethylphosphonate) (19). Colorless crystals: yield 75 %. m.p. 135-138 °C. ^1H NMR ($CDCl_3$), δ: 0.86, 0.92 (two t, 6H + 6H, J 6.0 Hz, $OCH_2CH_2CH_3$), 1.22 (m, 12H, CH_2CH_3), 1.95-2.02 (m, 16H, $CH_2CH_2CH_3$), 3.12, 3.17 (two d, 4H, J 13.0 Hz, $ArCH_{2eq}$), 3.55-4.2 (m, 16H, $POCH_2$+OCH_2), 4.37, 4.44 (two d, 4H, J 13.0 Hz, $ArCH_{2ax}$), 4.81, 4.82 (two d, 1H + 1H, J 10.7 Hz and J 11.3 Гц, 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), ^{31}P NMR ($CDCl_3$), δ 19.2. Mass spectrum (FAB) *m/z;* 1140[M +H]$^+$, 1162[M + Na]$^+$, 2279[2M +H]$^+$. Calcd for $C_{54}H_{76}Br_2O_{12}P_2$ 1139. Calculated for $C_{54}H_{76}Br_2O_{12}P_2$, %: 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-hydroxymetylphosphonates 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**(0.2mmol)**, 7**(0.1 mmol)**, 14,15** (0.1mmol) was added to the resulting solution. The reaction mixture was stirred at 10-15 ^{o}C for 8 h and was quenched with water (100 ml). The product was extracted with chloroform. Organic phase was dried over Na_2SO_4. The solvent was evaporated and the product formed was purified by the column chromatography (eluent chloroform – acetone, 10:1).

Diisopropyl 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5-(α-hydroxymethyl-phosphonate) (**4**)**.** Colorless crystals: yield 63%. m.p. 178-180 ^{o}C. Physico-chemical and spectral parameters are identical to the substance obtained by method A.

Diisopropyl 28-hydroxy-25,26,27-tripropoxycalix[4]arene-5-(α-hydroxymethyl-phosphonate) (5). Colorless crystals: yield 71%. m.p. 165-167 ^{o}C. Physico-chemical and spectral parameters are identical to the substance obtained by method A.

Tetraethyl (*RS*) 26,28-dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (8). Colorless crystals: yield 65 %. m.p. 195-197 ^{o}C. ^{1}H NMR ($CDCl_3$), δ:□0.99, 1.17 (two t, 6H + 6H, J 6.0 Hz, diastereotopic OCH_2CH_3), 1.30 (t, 6H, J 7.5 Hz, CH_2CH_3), 2.07 (m, 4H, $CH_2CH_2CH_3$), 3.39 (d, 4H, J 13.0 Hz, $ArCH_{2eq}$), 3.74, 3.93 (two m, 4H + 4H, diastereotopic $POCH_2$), 3.97 (t, 4H, J 6.2 Hz, OCH_2), 4.29 (d, 4H, J 13.0 Hz, $ArCH_{2ax}$), 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, *м*-ArH), 7.21 (s, 4H, *м*-ArH), 8.33 (s, 2H, OH). ^{31}P NMR ($CDCl_3$), δ 22.3 Mass spectrum (FAB) *m/z;* 565$[M-2HPO(OEt)_2 + H]^+$, 685$[M -HPO(OEt)_2 -H_2O +H]^+$, 805$[M -2H_2O +H]^+$, 823$[M -H_2O +H]^+$, 841$[M +H]^+$, 863$[M + Na]^+$, 1683$[2M +H]^+$. Calcd for $C_{44}H_{58}O_{12}P_2$ 840.8. Calculated for $C_{44}H_{58}O_{12}P_2$, %: 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 ^{o}C. ^{1}H NMR ($CDCl_3$), δ: 1.01, 1.17 (two t, 6H + 6H, J 6.0 Hz, diastereotopic $OCH_2CH_2CH_3$), 1.30 (t, 6H, J 7.5 Hz, CH_2CH_3), 1.95 (m, 8H, $OCH_2CH_2CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_3$), 3.40 (d, 4H, J 14.0 Гц, $ArCH_{2eq}$), 3.73, 3.94 (two m, 4H + 4H, diastereotopic $POCH_2$), 3.98 (t, 4H, J 6.2 Hz, OCH_2), 4.29 (d, 4H, J 14.0 Hz, $ArCH_{2ax}$), 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, *м*-ArH), 7.22 (s, 4H, *м*-ArH), 8.32 (s, 2H, OH). ^{31}P NMR ($CDCI_3$), δ 22.1 Mass spectrum (FAB) *m/z*; 898$[M+H]^+$, 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 64.21, 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 ^{o}C. ^{1}H NMR ($CDCl_3$), δ: 1.02, 1.16 (two t, 6H + 6H, J 6.0 Hz, diastereotopic $OCH_2CH_2CH_2CH_3$), 1.30 (t, 6H, J 7.5 Hz, CH_2CH_3), 1.95 (m, 8H, $CH_2CH_2CH_2CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_3$), 2.53 (m, 8H, $CH_2CH_2CH_2CH_3$), 3.41 (d, 4H, J 14.0 Hz, $ArCH_{2eq}$), 3.74, 3.93 (two m, 4H + 4H, diastereotopic $POCH_2$), 3.97 (t, 4H, J 6.2 Hz, OCH_2), 4.29 (d, 4H, J 14.0 Гц, $ArCH_{2ax}$), 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, *м*-ArH), 7.23 (c, 4H, *м*-ArH), 8.33 (c, 2H, OH). ^{31}P NMR ($CDCl_3$), δ 21.9. Mass spectrum (FAB) *m/z;* 934[M -H_2O +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.

Tetraethyl (*RR*+*SS*+*RS*) 25,26,27,28-tetrapropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonate) (16). Colorless crystals: yield 75 %. m.p. 112-116 ºC. Physicochemical and spectral parameters are identical to the substance obtained by method A.

Tetraethyl (*RR*+*SS*+*RS*) 25,26,27,28-tetrapropoxycalix[4]arene-11,23-dibromo-5,17-bis(α-hydroxymethylphosphonate) (18). Colorless crystals: yield 72 %. m.p. 143-146 ºC. Physicochemical and spectral parameters are identical to the substance obtained by method A.

Tetrapropyl (*RR*+*SS*+*RS*) 25,26,27,28-tetrapropoxycalix[4]arene-11,23-dibromo-5,17-bis(α-hydroxymethylphosphonate) (19). Colorless crystals: yield 68 %. m.p. 135-138 ºC. Physicochemical and spectral parameters are identical to the substance obtained by method A.

Synthesis of phosphonate 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 ºC for 48 h and was quenched with water (100 ml). The product was extracted with chloroform. Organic phase was dried over Na_2SO_4. The solvent was evaporated and the product formed was purified by the column chromatography (eluent chloroform – acetone, 10:1).

Diethyl 25,26,27,28-tetrapropoxycalix[4]arene-5-(diethoxyphosphoryloxymethyl)-17- (α-hydroxymethylphosphonate) (20). Colorless crystals: yield 45 %. m.p. 61-63 ºC. ^{1}H NMR ($CDCl_3$), δ: 0.87, 1.07 (two t, 6H + 6H, J 7.5 Hz, OCH_2CH_3), 1.12, 1.15, 1.26, 1.36 (four t, 3H+3H+3H+3H, J 7.2 Hz CH_2CH_3), 1.86, 1,96 (m, 4H+4H, $CH_2CH_2CH_3$), 3.10, 3.12, 3.16 (three d, 1H+1H+2H, J 13.0 Hz, $ArCH_{2eq}$), 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 $ArCH_2OP$), 4.38, 4.40 (two d, 0.5H+0.5H, J_{HH} 10.3 Hz, diastereotopic $ArCH_2OP$), 4.42, 4.44 (two d, 2H+2H, J 13.0 Hz, $ArCH_{2ax}$), 5.00, 5.04 (two d, 0.5H+0.5H, J 11.2 Hz, PC*H*), 5.92, 6.04 (two d, 1H+1H, J 2 Hz, *м*-Ar*H*), 6.14, 6.51 (two dd, 1H+1H, J 1.6 Hz, J 2 Hz, *м*-Ar*H*), 6.85, 6.97 (two t, 1H+1H, J 7.2 Hz, *p*-Ar*H*), 7.15 (m, 4H, *м*-Ar*H*). ^{31}P NMR ($CDCl_3$), δ -1.39, δ 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-tetrapropoxycalix[4]arene-5-(dipropoxyphosphoryloxymethyl)-17-(α-hydroxymethylphosphonate) (21). Colorless crystals: yield 52 %. m.p. 55-57 ºC. ^{1}H NMR ($CDCl_3$), δ: 0.85, 1.09 (two t, 6H + 6H, J 7.5 Hz, $OCH_2CH_2CH_3$), 1.13, 1.15, 1.25, 1.34 (four t, 3H+3H+3H+3H, J 7.2 Hz CH_2CH_3), 1.81-2.05 (m, 16H, $CH_2CH_2CH_3$), 3.11, 3.13, 3.15 (three d, 1H+1H+2H, J 13.0 Hz, $ArCH_{2eq}$), 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 $ArCH_2OP$), 4.39, 4.41 (two d, 0.5H+0.5H, J_{HH} 10.3 Hz, diastereotopic $ArCH_2OP$), 4.43, 4.42 (two d, 2H+2H, J 13.0 Hz,

$ArCH_{2ax}$), 5.01, 5.05 (two d, 0.5H+0.5H, J 11.2 Hz, PC*H*), 5.93, 6.04 (two d, 1H+1H, J 2 Hz, *м*-Ar*H*), 6.15, 6.52 (two dd, 1H+1H, J 1.6 Hz, J 2 Hz, *м*-Ar*H*), 6.84, 6.98 (two t, 1H+1H, J 7.2 Hz, *p*-Ar*H*), 7.16 (м, 4H, *м*-Ar*H*). ^{31}P NMR ($CDCl_3$), δ -1.73, δ 17.1. Mass spectrum (FAB) *m/z;* 982[M +H]$^+$, 1004[M + Na]$^+$. Calcd for $C_{54}H_{78}O_{12}P_2$ 981.13. Calculated for $C_{54}H_{78}O_{12}P_2$, %: C 66.10, H 8.01, P 6.31. Found, %: C 66.19, H 7.92, P 6.39.

Dipropyl 25,26,27,28-tetrapropoxycalix[4]arene-11,23-dibromo-5-(dipropoxyphosphoryloxymethyl)-17-(α-hydroxymethylphosphonate) (22). Colorless crystals: yield 57 %. m.p. 75-77 ºC. ^{1}H NMR ($CDCl_3$), δ: 0.88, 1.08 (two t, 6H + 6H, J 7.5 Hz, OCH_2CH_3), 1.13, 1.15, 1.27, 1.35 (four t, 3H+3H+3H+3H, J 7.2 Hz CH_2CH_3), 1.88, 1,95 (m, 4H+4H, $CH_2CH_2CH_3$), 3.11, 3.13, 3.17 (three d, 1H+1H+2H, J 13.0 Hz, $ArCH_{2eq}$), 3.57, 3.69 (two t, 2H + 2H, J 7.2 Hz, OCH_2), 3.74-4.04 (m, 14H, OCH_2), 4.26, 4.28 (two d, 0.5H+0.5H, J_{HH} 10.3 Гц, diastereotopic $ArCH_2OP$), 4.35, 4.35 (two d, 0.5H+0.5H, J_{HH} 10.3 Hz, diastereotopic $ArCH_2OP$), 4.41, 4.45 (two d, 2H+2H, J 13.0 Hz, $ArCH_{2ax}$), 5.01, 5.06 (two d, 0.5H+0.5H, J 11.2 Hz, PC*H*), 5.91, 6.05 (two d, 1H+1H, J 2 Hz, *м*-Ar*H*), 6.16, 6.53 (two dd, 1H+1H, J 1.6 Hz, J 2 Hz, *м*-Ar*H*), 7.15 (m, 4H, *м*-Ar*H*). ^{31}P NMR ($CDCl_3$),), δ -0.95, δ 18.7. Mass spectrum (FAB) *m/z;* 1084[M +H]$^+$. Calcd for $C_{50}H_{68}Br_2O_{12}P_2$ 1082.82. Calculated for $C_{50}H_{68}Br_2O_{12}P_2$, %: C 55.46, H 6.33, P 5.72. Found, %: C 55.60, H 6.38, P 5.59.

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

To a solution of calixarene-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-dipropoxycalix[4]arene-5-(α-hydroxymethylphosphonic acid) (23). Slightly colored crystals: yield 95 %. m.p. 270-278 ºC. ^{1}H NMR (DMSO-d6), δ: 1.18 (t, 6H, J 7.2 Hz, CH_2CH_3), 2.15 (m, 4H, $CH_2CH_2CH_3$), 3.26, 3.35 (two d, 2H+2H, J 13.4 Гц, $ArCH_{2eq}$), 4.05 (t, 4H, J 7.2 Hz, OCH_2), 4.36 (d, 4H, J 13.4 Hz, $ArCH_{2ax}$), 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), ^{31}P NMR (DMSO-d6), δ 16.8. Mass spectrum (FAB) *m/z;* 620[M + H]$^+$, 1238 [2M + H]$^+$. Calcd for $C_{35}H_{39}O_8P$ 618.65.

28-Hydroxy-25,26,27-tripropoxycalix[4]arene-5-(α-hydroxymethylphosphonic acid) (24). Slightly colored crystals: yield 96 %. m.p. 265-270 ºC. ^{1}H NMR (DMSO-d6), δ: 1.31 (m, 9H, CH_2CH_3), 2.03 (m, 6H, $CH_2CH_2CH_3$), 3.29-3.35 (m, 4H, $ArCH_{2eq}$), 4.06 (m, 6H, OCH_2), 4.28 (d, 4H, J 13.4 Hz, $ArCH_{2ax}$), 4.54 (d, 1H, J 10 Hz, PC*H*), 6.71 (t, 1H, J 7.5 Hz, Ar*H*-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). ^{31}P NMR (DMSO-d6), δ 16.5. Mass spectrum (FAB) *m/z;* 662 [M + H]$^+$. Calcd for $C_{38}H_{45}O_8P$ 660.73.

25,26,27,28-Tetrapropoxycalix[4]arene-5-(α-hydroxymethylphosphonic acid) (25). Colorless crystals: yield 94 %. m.p. 247-251 ºC. ^{1}H NMR (DMSO-d6) δ: 0.98 (m, 12H, CH$_2$CH$_2$C*H*$_3$), 1.96 (m, 8H, CH$_2$C*H*$_2$CH$_3$), 3.21, (d, 4H, J 12.9 Hz, ArCH$_{2eq}$), 3.96 (m, 8H, OC*H*$_2$), 4.45 (d, 4H, J 12.9 Hz, ArCH$_{2ax}$), 4.63 (d, 1H, J 10 Hz, PCH), 6.35-6.92 (m, 11H, ArH). ^{31}P NMR δ (DMSO-d6) 17.2. Mass spectrum (FAB) *m/z*; 704 [M+H]$^+$. Calcd for $C_{41}H_{51}O_8P$ 702.81.

(*RS*)-26,28-Dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonic acid) (26). Colorless crystals: yield 95 %. m.p. 262-266 ºC. ^{1}H NMR (CD$_3$OD), δ: 1.24 (t, 6H, J 7.5 Hz, CH$_2$C*H*$_3$), 2.04 (m, 4H, CH$_2$C*H*$_2$CH$_3$), 3.42 (d, 4H, J 13.0 Hz, ArCH$_{2eq}$), 3.98 (t, 4H, J 6.2 Гц, OCH$_2$), 4.24 (d, 4H, J 13.0 Hz, ArCH$_{2ax}$), 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, *м*-ArH), 7.21 (s, 4H, *м*-ArH). ^{31}P NMR (CD$_3$OD), δ 16.7. Mass spectrum (FAB) *m/z*; 729 [M +H]$^+$, 752 [M + Na]$^+$, 1458 [2M +H]$^+$. Calcd for $C_{36}H_{42}O_{12}P_2$ 728.66.

(*RR*+*SS*)-26,28-Dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonic acid) (27). Colorless crystals: yield 97 %. m.p. 254-258 ºC. ^{1}H NMR (CD$_3$OD), δ: 1.26 (t, 6H, J 7.5 Hz, CH$_2$C*H*$_3$), 2.08 (m, 4H, CH$_2$C*H*$_2$CH$_3$), 3.42, 3.44 (two d, 2H+2H, J 14.0 Hz, ArC*H*$_{2eq}$), 3.85 (t, 4H, J 6.2 Гц, OC*H*$_2$), 4.21, 4.21 (two d, 2H+2H, J 14.0 Hz, ArC*H*$_{2ax}$), 4.69 (d, 2H, J 13.0 Hz, PC*H*), 6.64, 6.68 (two dd, 1H+1H, J 8.0 Hz, J 2.0 Hz, *p*-Ar*H*), 6.84, 6. 91 (two dd, 2H+2H, J 8.0 Hz, J 2.0 Hz, *м*-ArH), 7.23 (d, 4H, J 2.0 Hz, *м*-ArH), 7.33 (d, 4H, J 2.0 Hz, *м*-ArH), ^{31}P NMR (CD$_3$OD), δ 15.2. Mass spectrum (FAB) *m/z;* 729 [M +H]$^+$. Calcd for $C_{36}H_{42}O_{12}P_2$ 728.66.

(*RR*+*SS*+*RS*)-25,26,27,28-Tetrapropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonic acid) (28). Colorless crystals: yield 95 %. m.p. 225-230 ºC. ^{1}H NMR (DMSO-d6), δ: 1.24 (t, 8H, J 7.5 Hz, CH$_2$C*H*$_3$), 1.95, 2.04 (two m, 4H+4H, CH$_2$C*H*$_2$CH$_3$), 3.42 (d, 4H, J 13.0 Hz, ArCH$_{2eq}$), 3.98 (t, 8H, J 7.5 Hz, OC*H*$_2$), 4.24 (d, 4H, J 13.0 Hz, ArCH$_{2ax}$), 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, *м*-ArH), 7.21 (s, 4H, *м*-ArH). ^{31}P 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 $C_{42}H_{54}O_{12}P_2$ 812.31.

(*RR*+*SS*+*RS*)-25,26,27,28-Tetrapropoxycalix[4]arene-11,23-dibromo-5,17-bis(α-hydroxymethylphosphonic acid) (29). Colorless crystals: yield 97 %. m.p. 239-244 ºC. ^{1}H NMR (DMSO-d6), δ: 1.13, 1.26 (two t, 6H+6H, J 7.5 Hz, CH$_2$C*H*$_3$), 1.85, 1.98 (two m, 4H+4H, CH$_2$C*H*$_2$CH$_3$), 3.42, 3.44 (two d, 2H+2H, J 13.0 Hz, ArC*H*$_{2eq}$), 3.85, 4.12 (two t, 4H+4H, J 6.2 Hz, OC*H*$_2$), 4.21, 4.21 (two d, 2H+2H, J 13.0 Hz, ArC*H*$_{2ax}$), 4.73 (d, 2H, J 11.3 Hz, PC*H*), 6.25, 6.31 (two s, 2H+2H, Ar*H*), 7.11, 7. 31 (two s, 2H+2H, ArH), ^{31}P NMR (DMSO-d6), δ 21.9, 22.3. Mass spectrum (FAB) *m/z*; 972 [M +H]$^+$, 994 [M + Na]$^+$, 1943 [2M +H]$^+$. Calcd for $C_{42}H_{52}Br_2O_{12}P_2$ 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.

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

(*RS*)-26,28-Dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonic acid) (2). Colorless crystals: yield 95%. m.p. 262-266 ºC. Physico-chemical and spectral parameters are identical to the substance obtained by dealkylation of the corresponding ester (see *supra*).

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

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

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.[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 $M^{-1}cm^{-1}$. The influence of the calix[4]arene-α-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 μl or 10 μl of the enzyme (1 mg/ml). The IC_{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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