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ORIGINAL ARTICLE

# Complexation of calix[4]arenehydroxymethylphosphonic acids with amino acids. Binding constants determination of the complexes by HPLC method

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**Abstract** Host–Guest complexation process of calixarenehydroxymethylphosphonic acids with 10 amino acids in solution $H_2O$/MeCN (99:1) had been studied. Binding constants of the inclusion complexes from the dependence between capacity factors of the Guest and the calixarene-Host concentration in the mobile phase had been calculated. It was shown the binding constants depend on the nature of the amino acid residue, conformation of the calixarene skeleton, quantity of phosphoryl groups at the upper rim. In accordance with molecular calculation the complexation is determined by the electrostatic interactions between the positively charged nitrogen atom of amino acid and the negatively charged oxygen atom of phosphonic group of calixarene molecule, hydrogen bonds, $\pi$–$\pi$, CH–$\pi$ and solvatophobic, interactions.

**Keywords** Calixarenehydroxymethylphosphonic acids · Reversible-phase high performance liquid chromatography (RP HPLC) · Complexation · Binding constants · Amino acids · Adsorption isotherms

## Introduction

Calixarenes [1] contained preorganized bio-affine groups are able to recognize different biologically active molecules such as amino acids, dipeptides, proteins, choline and acetylcholine, carbohydrates, riboflavin, vitamin $B_{12}$, nucleotides, nucleosides and short DNA fragments [2–14]. Calix[4]arene derivatives can also be fastened on the surface of proteins [14, 15]. Due to the ability to simulate the substrate-receptor interactions with biomolecules, calixarenes are objects of biomedical research [16, 17].

Formerly it was shown that calix[4]arenes functionalized by residues of phosphonic, aminophosphonic or methylenebisphosphonic acids are effective inhibitors of alkaline phosphatases [18–20], calcium channel modulators [21, 22], antithrombotics [23]. They inhibit the ability of *P*-glycoprotein to remove from the cells anticancer drug Doxorubicin [24]. The base of these biochemical effects are supramolecular (substrate-receptor) interactions of calixarenes with amino acid fragments in the active sites of the respective protein structures. As a result of the macrocyclic effect of calixarene platform the biological activity such phosphonic acids is much higher comparatively with model acyclic compounds [18–21]. The biological activity is essentially depended from the number of phosphonic groups, their stereochemical configuration and geometrical parameters of the macrocyclic platform as well.

The aim of this work is to study the complexation of dipropoxy- and tripropoxycalix[4]arenes **1–3** modified by the fragments of hydroxymethylphosphonic acid at the upper rim of the macrocycle (Scheme 1) with a series of L-amino acids (Scheme 2) in water containing solution. Reversible-phase high-performance liquid chromatography method (RP HPLC) was used for determination of the binding constants of supramolecular complexes of the calixarenes with amino acids.

## Experimental

Calixarene-mono-α-hydroxymethyl phosphonic acids **1, 2** were obtained at two steps from dipropoxy- or tripropoxyformylcalixarenes **4, 5** respectively (Scheme 3). At the first step calixarene-mono-α-hydroxymethylphosphonates **6, 7**

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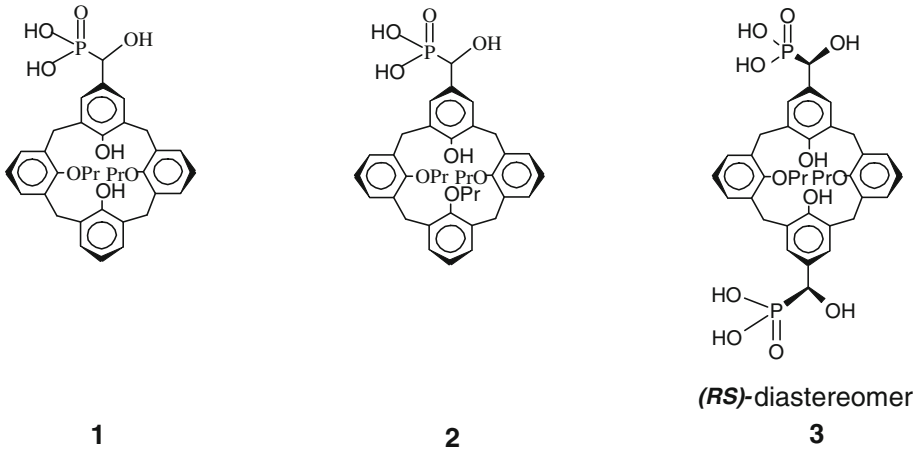
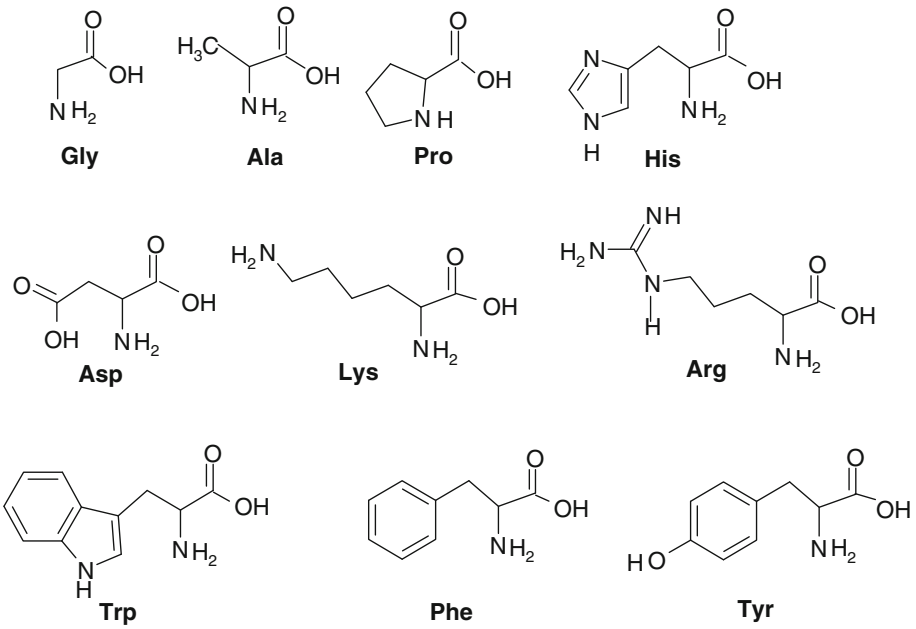



**Scheme 1** Calix[4]arenehydroxymethylphosphonic acids



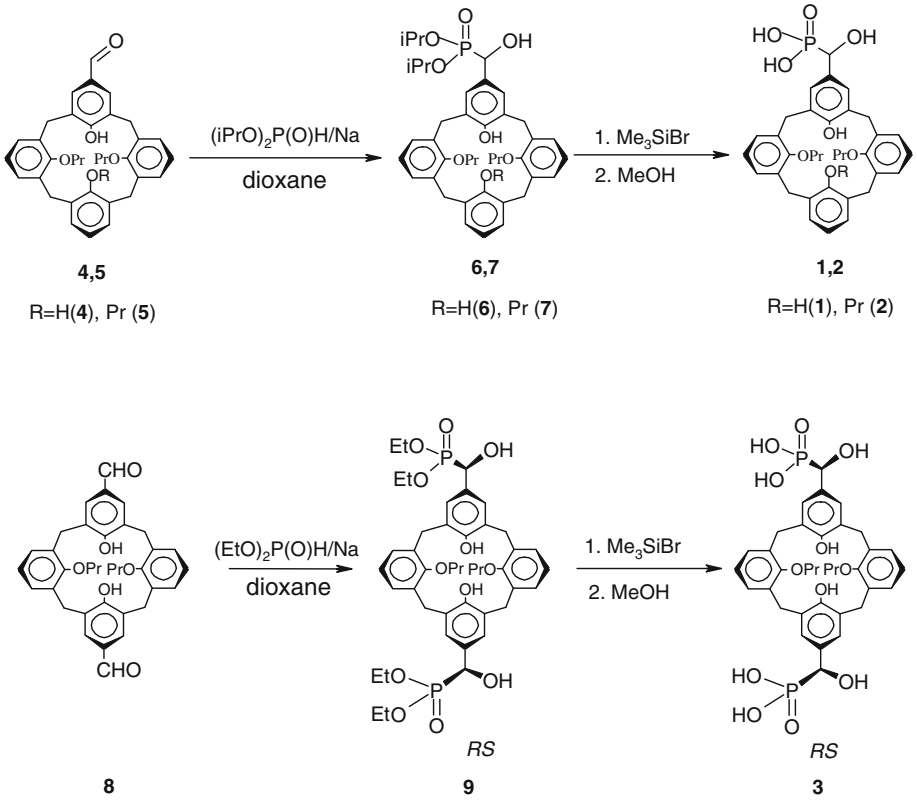

**Scheme 2** L-Amino acids

were obtained by the Pudovik addition of diisopropyl phosphite sodium salt to the CH = O group of **4, 5** in dioxane solution at 10–15 °C for 8 h**.** At the second step esters **4, 5** were transformed into acids **1, 2** by the consecutive reactions with trimethyl-bromosilane and methanol with quantitative yield [25].

Calixarene-bis-α-hydroxymethyl phosphonic acid **3** in the *RS* meso form was synthesized in the similar way by the diastereoselective Pudovik addition of diethyl phosphite sodium salt to diformylcalixarene **8** and the next dealkylation of (*RS*)-calixarene-bis-α-hydroxymethylphosphonate **9** formed by the reaction with trimethyl-bromosilane and methanol (Scheme 3).

Synthesis of calix[4]arene-α-hydroxymetylphosphonates **6, 7, 9**. General procedure

To solution of dialkylphosphite (0.3 mmol) in dioxane (5 ml) sodium metal (0.1 mmol) was cautiously added in small portions. Formylcalixarenes **4** (0.2 mmol), **5**(0.2 mmol)**, 8**(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 $Na_2SO_4$. The solvent was evaporated and the product formed was purified by the column chromatography (eluent chloroform–acetone).




**Scheme 3** Synthesis of calix[4]arenehydroxymethylphosphonic acids

Diisopropyl 26,28-dihydroxy-25,27-dipropoxycalix[4] arene-5-(α-hydroxymethylphosphonate) **6**

Colorless crystals: yield 63 %. m.p. 178–180 °C. $^1$H NMR ($CDCl_3$), $\delta$: 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, $CH_2CH_2CH_3$), 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 $CHCH_3$), 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 ($CDCl_3$), $\delta$ 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) **7**

Colorless crystals: yield 71 %. m.p. 165–167 °C. $^1$H NMR ($CDCl_3$), $\delta$: 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 $CHCH_3$), 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 ($CDCl_3$), $\delta$ 18.7. Mass spectrum (FAB) *m/z*; 746 [M + H]$^+$. Calcd for $C_{44}H_{57}O_8P$ 744.9.

Tetraethyl (*RS*) 26,28-dihydroxy-25,27-dipropoxycalix[4] arene-5,17-bis(α-hydroxymethylphosphonate) **9**

Colorless crystals: yield 65 %. m.p. 195–197 °C. $^1$H NMR ($CDCl_3$), $\delta$: 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 ($CDCl_3$), $\delta$ 22.3 Mass spectrum (FAB) *m/z*; 565[M-2HPO(OEt)$_2$ + H]$^+$, 685[M-HPO(OEt)$_2$-$H_2O$ + H]$^+$, 805[M-2$H_2O$ + H]$^+$, 823[M-$H_2O$ + H]$^+$, 841[M + H]$^+$, 863[M + Na]$^+$, 1683[2 M + H]$^+$. Calcd for $C_{44}H_{58}O_{12}P_2$ 840.8. Calcd 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.

Synthesis of calixarene-α-hydroxymethylphosphonic acids **1, 2, 3**. General procedure

To a solution of calixarene-α-hydroxymethylphosphonate **6, 7, 8** (0.1 mmol) in dry chloroform (5 ml) bromotrimethylsilane (10-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) **1**

Slightly colored crystals: yield 95 %. m.p. 270–278 °C. $^1$H NMR (DMSO-d6), $\delta$: 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), $\delta$ 16.8. Mass spectrum (FAB) *m/z*; 620$[M + H]^+$, 1238 $[2\ M + H]^+$. Calcd for $C_{35}H_{39}O_8P$ 618.65.

28-Hydroxy-25,26,27-tripropoxycalix[4]arene-5-(α-hydroxymethylphosphonic acid) **2**

Slightly colored crystals: yield 96 %. m.p. 265–270 °C. $^1$H NMR (DMSO-d6), $\delta$: 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, 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). $^{31}$P NMR (DMSO-d6), $\delta$ 16.5. Mass spectrum (FAB) *m/z*; 662 $[M + H]^+$. Calcd for $C_{38}H_{45}O_8P$ 660.73.

(*RS*)-26,28-Dihydroxy-25,27-dipropoxycalix[4]arene-5,17-bis(α-hydroxymethylphosphonic acid) **3**

Colorless crystals: yield 95 %. m.p. 262–266 °C. $^1$H NMR ($CD_3OD$), $\delta$: 1.24 (t, 6H, J 7.5 Hz, $CH_2CH_3$), 2.04 (m, 4H, $CH_2CH_2CH_3$), 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 ($CD_3OD$), $\delta$ 16.7. Mass spectrum (FAB) *m/z*; 729 $[M + H]^+$, 752 $[M + Na]^+$, 1458 $[2\ M + H]^+$. Calcd for $C_{36}H_{42}O_{12}P_2$ 728.66.

With respect to poor solubility of calixarenehydroxymethylphosphonic acids **1–3** in the water solutions, they were analysed as monosodium (**1, 2**) or disodium (**3**) salts. Sodium salts of acids **1–3** were obtained by addition of equivalent quantity of sodium methylate to calixarene solution in methanol.

HPLC analysis

HPLC analysis (chromatograph Hitachi) was performed in isocratic conditions using the chromatographic column Zorbax CN and mobile phase $H_2O$/MeCN (99:1 v/v). UV detector was operated at 254 nm and the flow rate was 0.8 ml/min. Solutions of the amino acids with concentration $10^{-3}$–$10^{-4}$ M in $H_2O$/MeCN (99:1 v/v) mixture were used for the analysis. All chromatograms were obtained at 34 °C. The mobile phases contained calix[4]arenes **1–3** in the concentrations $0.1 \times 10^{-4}$ M–$0.34 \times 10^{-4}$ M; $0.1 \times 10^{-4}$ M–$0.5 \times 10^{-4}$ M; $0.09 \times 10^{-4}$ M–$0.34 \times 10^{-4}$ M, respectively.

Molecular modelling

The initial molecular modelling of calixarenes **1–3** and their complexes with amino acids was carried out by molecular mechanics method. After that structure obtained were optimized by semi-empirical PM3 method (software package Hyper Chem, version 8).

## Results and discussion

The binding constants of calixarene Host–Guest complexes of calixarene **3** with amino acids have been early determined by NMR and calorimetry methods in methanol solution [26]. However due to poor solubility of calixarenes **1–3** sodium salts in water and their wide signals in the NMR spectra recorded in $D_2O$ solution the both methods were ineffective for the experiments in water. So, for determination of the binding constants in the water solution we used simple and high sensitive HPLC method [27, 28]. The method includes the choice of analysis conditions for the calixarene and the Guest molecule, determination of the retention time, tr, and capacity factor, $k'$, of the Guest molecule before and after the calixarene addition to the mobile phase. The binding constant $K_A$ of the calixarene complex with the Guest molecule (the ratio 1:1) can be calculated by equation (1):

$$1/k' = 1/k'_0 + K_A \times [CA]/k'_0 \quad (1)$$

where $k'_0$ and $k'$ – are capacity factors of the Guest molecule determined in the absence and the presence of calixarene in the mobile phase.

Calixarenes **1–3** (sodium salts) and amino acids in the analysis conditions choosen were registered on the

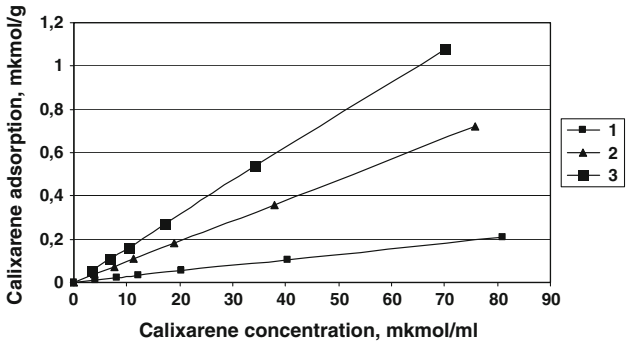



**Fig. 1** Adsorption isotherms of sodium salts of calixarenephosphonic acids **1–3** ($R^2 = 0.99$)

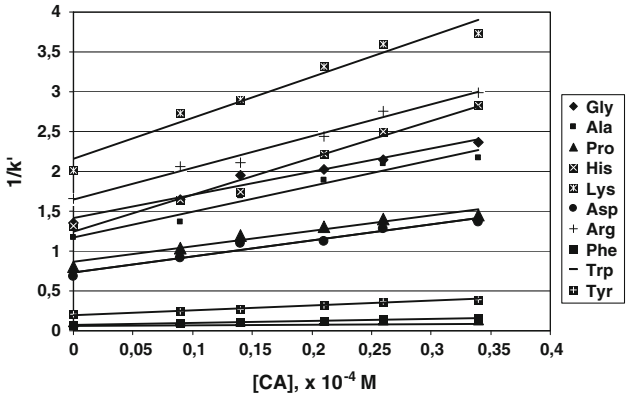



**Fig. 2** Dependence of $1/k'$ versus calixarene **3** concentration in the mobile phase for Gly, Ala, Pro, His, Lys, Asp, Arg, Phe, Trp, Tyr ($R^2 = 0.96–0.99$)

chromatograms by sharp peaks. Linear adsorption isotherms of the calixarenes (Fig. 1) indicate on their reversible sorption on the Zorbax CN surface.

An addition of the calixarene to the mobile phase decreases the capacity factor values of amino acids. The linear character plots of $1/k'$ versus the calixarene concentration (Fig. 2) ($R^2 = 0.93–0.99$) testifies formation of the Host–Guest supramolecular complexes with 1:1 stoichiometry and allows correct calculation $K_A$ values by equation (1).

The binding constants $K_A$ and free Gibbs energy $\Delta G$ ($\Delta G = -RT \ln K_A$) for calixarene **1–3** complexes with the amino acids are presented in Table 1.

The binding constants are vary from 15,873 $M^{-1}$ ($\Delta G = -24.52$ kJ/mol) to 48,189 $M^{-1}$ ($\Delta G = -27.33$ kJ/mol) and increase in the order **2** < **1** < **3** (Table 1). The constants are greater than the binding constants of the aminoacids complexes with calixarene **3** obtained by calorimetry method in methanol solution [26] and the binding constants of the amino acids complexes with *p*-sulphonatocalix[4]arene obtained by HPLC method in $H_2O$/MeOH/$CF_3COOH$ (97/3/0.5 v/v) solution [29]. It should be noted no differences (within the experimental errors) was observed in binding constants for complexes of calixarene **3** (*RS*-diastereomer) with D-amino acids.

It's evident, that efficiency of the complexation of calixarenephosphonic acids **1–3** depends on the nature of amino acids, the number of phosphonic groups at the upper rim, as well as propyl groups at the lower rim which determines conformation of the macrocycle.

Macrocyclic skeleton conformation of calixarenes **1–3** was determined by $^1H$ NMR method and precised by molecular modelling. In the NMR spectra signals of methylene groups $ArCH_2Ar$ are observed as doublets of axial and equatorial protons of AB spin system ($^2J_{HH} = 13$ Hz) with the difference between their chemical shifts $\Delta\delta$ 0.85–1.1 ppm. That indicates calixarenes **1–3** are in the *cone*-conformation stabilized by intramolecular hydrogen bonds OH···OPr at the

**Table 1** Values of $K_A$* and free Gibbs energy ($\Delta G$) of calixarenes **1–3** complexes with the amino acids

| Guest | Calixarene | | | | | |
|---|---|---|---|---|---|---|
| | **1** | | **2** | | **3** | |
| | $K_A$, $M^{-1}$ | $\Delta G$, kJ/mol | $K_A$, $M^{-1}$ | $\Delta G$, kJ/mol | $K_A$, $M^{-1}$ | $\Delta G$, kJ/mol |
| Gly | 27,727 | −25.30 | 20,888 | −24.60 | 31,234 | −25.60 |
| Ala | 21,203 | −24.64 | 21,299 | −24.65 | 47,299 | −26.62 |
| Pro | 38,243 | −26.10 | 15,873 | −23.92 | 40,082 | −26.21 |
| His | 31,199 | −25.60 | 28,174 | −25.34 | 48,189 | −26.67 |
| Lys | 32,485 | −25.69 | 29,295 | −25.44 | 42,572 | −26.36 |
| Asp | 28,807 | −25.40 | 21,954 | −24.73 | 29,947 | −25.49 |
| Arg | 27,419 | −25.28 | 23,567 | −24.90 | 35,992 | −25.95 |
| Phe | 26,581 | −25.20 | 26,927 | −25.23 | 30,185 | −25.51 |
| Trp | 23,414 | −24.89 | 20,795 | −24.59 | 27,490 | −25.28 |
| Tyr | 26,885 | −25.23 | 17,546 | −24.17 | 20,983 | −24.61 |

* Relative standard deviation RSD is 7.7–27.49 %

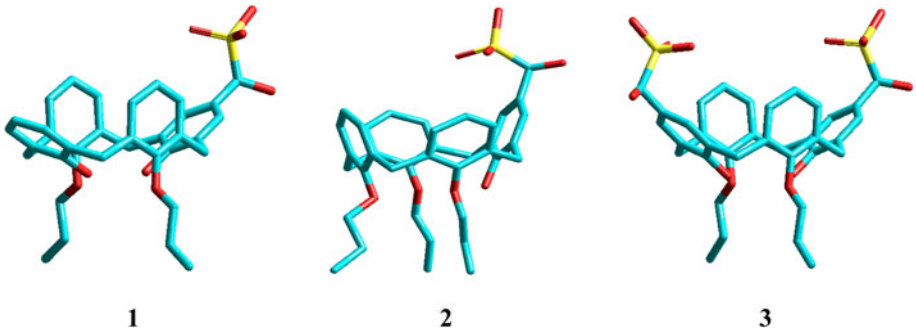



**Fig. 3** Energy minimized structures of calixarenes **1–3**

lower rim of the macrocycle [30]. Structures of calixarenes **1–3** optimized by molecular modelling are presented in Fig. 3.

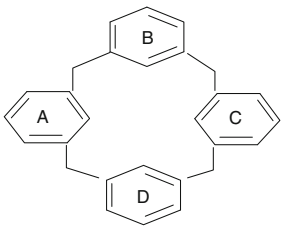
The inclination (dihedral angles) of benzene rings A, B, C, D relatively to the main calixarenes **1, 2, 3** macrocycle plane formed by $CH_2$ links are presented in Table 2.

Macrocyclic skeleton of dipropoxycalixarenemonophosphonic acid **1** and diphosphonic acid **3** has symmetry $C_{2v}$ (*flattened-cone* conformation). Aromatic rings with phenolic OH groups are oriented "coplanar" but the propylated rings are oriented "perpendicular" to the main plane of the macrocycle. Dihedral angles between "coplanar" rings A, C are 109.986° in calixarene **1** and 96.432° in calixarene **3**. Dihedral angles between "perpendicular" rings B, D are respectively 3.04° and 16.044°.

In tripropoxycalixarene acid **2** the propylated rings B, D have "coplanar" orientation. Two another A, C rings have "perpendicular" orientation. For that *cone* of the macrocyclic skeleton is more regular compared to calixarenes **1, 3**. Dihedral angles between "coplanar" rings B, D and "perpendicular" rings A, C are 64.851° and 34.633° relatively.

To clarify the nature of the Host–Guest interaction the molecular modelling of the complexes of the most effective binding agent—calixarene **3** with Phe, Tyr and Ala was carried out (Fig. 4). In all cases the electrostatic contacts of

**Table 2** The inclination (dihedral angles) of benzene rings A, B, C, D of calixarenes **1, 2, 3** and their complexes to the main macrocycle plane




| Calixarene | Dihedral angles (°) | | | |
|---|---|---|---|---|
| | A | B | C | D |
| **1** | 146.981 | 91.742 | 143.005 | 91.301 |
| **2** | 100.546 | 122.572 | 114.087 | 122.279 |
| **3** | 138.453 | 98.106 | 137.979 | 97.938 |
| **3**-Phe | 152.910 | 97.477 | 143.034 | 97.781 |
| **3**-Tyr | 148.073 | 98.736 | 140.323 | 98.264 |
| **3**-Ala | 126.797 | 111.887 | 132.891 | 111.697 |

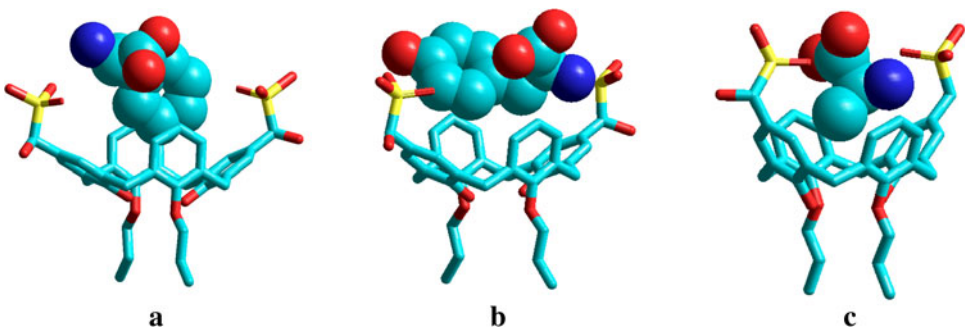



**Fig. 4** Energy minimized structures of calixarene **3** complexes with amino acids Phe (**a**), Tyr (**b**) and Ala (**c**)

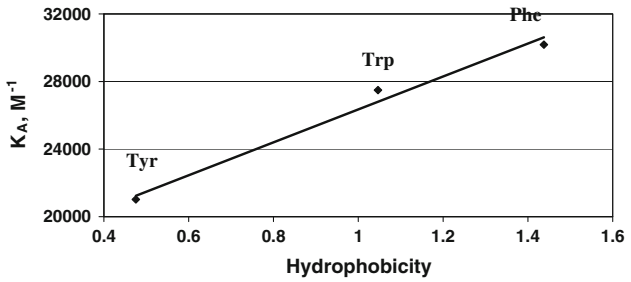



**Fig. 5** Correlation of $K_A$ calixarene **3** complexes with Tyr, Trp, Phe residue hydrophobicity ($R^2 = 0.98$)

positively charged nitrogen atom of the amino acids with a negatively charged oxygen atoms of the calixarene phosphonic group are observed (N···O distances are within 2.91–3.41 Å). The complexes are additionally stabilized by CH–$\pi$ and $\pi$–$\pi$ interactions due to including of methyl (Ala) or phenyl (Phe) group of the amino acid in the molecular cavity of calixarenes. It is interesting to note that Tyr binds with the both phosphonic groups of calixarene **3**. One group forms an electrostatic link with amino acid $NH_3^+$ group, and another one—hydrogen bond with the Ar-OH terminal group (Fig. 4b).

As seen from the benzene rings slope angles of macrocyclic skeleton in compound **3** (Table 2) the complexation of Phe and Tyr leads to flattening the calixarene *cone*, while Ala makes the *cone* more regular (Table 2).

Hydrophobic interactions between the Host and Guest molecules influences the complex stability in water solutions. This is clear to see from the dependence of calixarene **3** $K_A$ values from hydrophobicity residues of Tyr, Trp and Phe [31] (Fig. 5). The more hydrophobicity the more $K_A$ value of the calixarene complex.

## Conclusion

Calix[4]arenemethylphosphonic acids form stable supramolecular Host–Guest complexes with amino acids in water solutions. Binding constants of the complexes determined by HPLC and molecular modelling data show that the complex stability is strongly depended on the nature of the amino acid residue, calixarene skeleton conformation, and the number of phosphonic groups at the upper rim of the macrocycle. In accordance with molecular modelling data the complexation efficiency is determined by electrostatic contacts of positively charged nitrogen atom of the amino acid with the negatively charged oxygen atom of phosphonic group of calixarene, $\pi$–$\pi$, CH–$\pi$ and hydrophobic interactions.

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