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ORIGINAL ARTICLE

Complexation of amino acids derivatives in water by calix[4]arene phosphonic acids

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Abstract Series of the calix[4]arene phosphonic acids with various substituents at the lower rim was synthesized. Complexing properties of these receptors towards methyl esters of six amino acids strongly depended on the calix[4]arene conformation flexibility. The complex formation processes were monitored using ^{1}H NMR spectroscopy (deuterated phosphate buffer at pD 7.3, 22 °C) and association constant values were evaluated. Inherently mobile calix[4]arene molecule **3** occurred in *cone* conformation in aqueous solution turned out to be more effective in complexation of the basic amino acids methyl esters compared to the rigid **2** and flexible **4**. Mixed 1:2 and 2:1 (host–guest) complexes were observed for compound **1** with all amino acids methyl esters.

Keywords Amino acids · Association constants · Calix[4]arenes · Molecular recognition in water · Phosphonic acids · Supramolecular chemistry · Complex formation

Introduction

Calix[4]arenes [1] are easy available through the base-induced condensation of *p*-alkylphenols with formaldehyde. Moreover, because of their susceptibility to the chemical modifications and ipso facto access to the unique architecture of four conformers (*cone*, *partial cone*, *1,2-alternate*, *1,3-alternate*) they are often used as scaffolds for a creation of the new, sophisticated molecules with designed solubility and binding properties [2]. The supramolecular complex forming ability of the calix[4]arenes with charged or neutral molecules through the weak noncovalent interactions (e.g. hydrogen bonds, electrostatic interactions, van der Waals forces, π–π, and π–cation interactions) depends on the nature and number of the interacting groups as well as their mutual structural fitting [3]. Especially in polar solvents where solvophobic effect plays a crucial role, the aromatic cavity present in the calixarene molecule with the accompaniment of other functional groups may become very useful. Since molecular recognition of amino acids in natural environment involves multiple non-covalent interactions, the multivalent macrocyclic receptors created to this aim have to be equipped with the array of the properly arranged substituents in order to ensure effective interactions with the multifunctional guest molecules. Such selective artificial receptors offer better insight into mechanisms of interactions between biomolecules [4] and can be used for analytical purposes as well [5].

Since the non-covalent phosphate–ammonium interactions play a key role in living systems for many critical molecular recognition processes, we were interested in the complexation properties of the calix[4]arene-phosphonic acids towards the ammonium type compounds. Previously, we reported the molecular recognition of the aromatic and aliphatic amines and 1,2-amino alcohols by a macrocyclic host **1** in phosphate buffered D_2O at pD 7.3 [6]. The results have shown dependence of the binding strength on a guest geometry, the protonated form of the amino group has also appeared important. This prompted us to investigate the inclusion properties of the host **1** towards amino acids derivatives. We were extremely interested in a subject of

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how changes of a calixarene conformation flexibility thereby its hydrophobic cavity shape would influence on amino acids complexation. We therefore extended our studies to a series of calix[4]arene-phosphonic acids **1–5**, shown on the Fig. 1.

A considerable number of synthetic receptors based on a calixarene framework for amino acids derivatives has been designed and studied in organic media but only a few examples have been reported in aqueous solution. The inclusion properties of the *cone* peptidocalix[4]arenes with different conformation flexibility towards aliphatic and aromatic amino acids and their methyl esters were investigated in D_2O (pD 7.3, phosphate buffer 0.067 M) [7]. *Rigid* receptor with two di(ethylene glycol) units introduced in proximal (1,2;3,4) positions at the lower rim of the calix[4]arene skeleton was much more efficient than the *flexible* analog in all complexation processes. The aromatic molecules were better bound than the aliphatic ones with the highest association constants values $K = 110$ and 620 M^{-1} for L-Trp and L-Trp-OMe, respectively. The affinity of the *p*-sulphonatocalix[*n*]arenes ($n = 4$, 6, and 8) towards amino acids was extensively tested by means of ^{1}H NMR [8, 9], microcalorimetry [10, 11], RP-HPLC [12]. According to data reported by Douteau-Guevel et al. [8b] and Arena et al. [9] *p*-sulphonatocalix[4]arenes formed 1:1 complexes with basic amino acids more strongly (K_{ass} values for Arg and Lys complexes were 1,520 and 740 M^{-1}, respectively, phosphate buffer, pH 8) than with aliphatic or aromatic amino acids: Val, Leu, Phe, His, Trp, for which K_{ass} values were between 16 and 63 (phosphate buffer, pD 7.3). Da Silva and Coleman [13] have studied complexing properties of *p*-sulphonatocalix[*n*]arenes mono-functionalized at a phenolic oxygen towards 11 amino acids by means of ^{1}H NMR spectroscopy in unbuffered aqueous solution (pH 8.0, NaOH). Tetrasulphonatomethylcalix[4]resorcinarene [14] formed complexes with amino acids in D_2O (pD 7.2, phosphate buffer), the K_{ass} values for these complexes estimated from ^{1}H NMR experiments decreased in the order Lys > Arg > Pro > Trp > Phe and no interactions with Asp, Asn, Thr, Leu, Met were observed. The formation of complexes between derivatized cyclotetrachromotropylene host and Ala, Asp, and Lys in aqueous solution at pD 1.0 was also investigated [15]. Very recently, calix[4]arenes having α-aminophosphonic acid fragments at the upper or lower rim were described and their remarkable selectivity as carriers for zwitterionic aromatic amino acids in membrane transport were reported [16]. Quite recently, Zielenkiewicz et al. [17] have extensively studied complexing properties of the calix[4]arenes having two α-hydroxyphosphonic acid moieties distally substituted at the upper rim towards amino acids and dipeptides in methanol by means of calorimetry, NMR and UV–VIS spectroscopies. They have found that neutral aliphatic and aromatic amino acids are better bound than basic one with the 1:1 stoichiometry of formed complexes, the one exception was complex with the Ile existing in the form 1:1 and 2:1 (calixarene-amino acid). We have to also mention some related work of other groups regarding calix[4]arenes functionalized with phosphonic groups placed directly at the upper rim. Zadmard et al. [18] have presented novel type of capsules formed in polar solvents by two *cone* calix[*n*]arenes ($n = 4, 6$); the first substituted at the upper rim by phosphonic ethyl ester lithium salt groups,

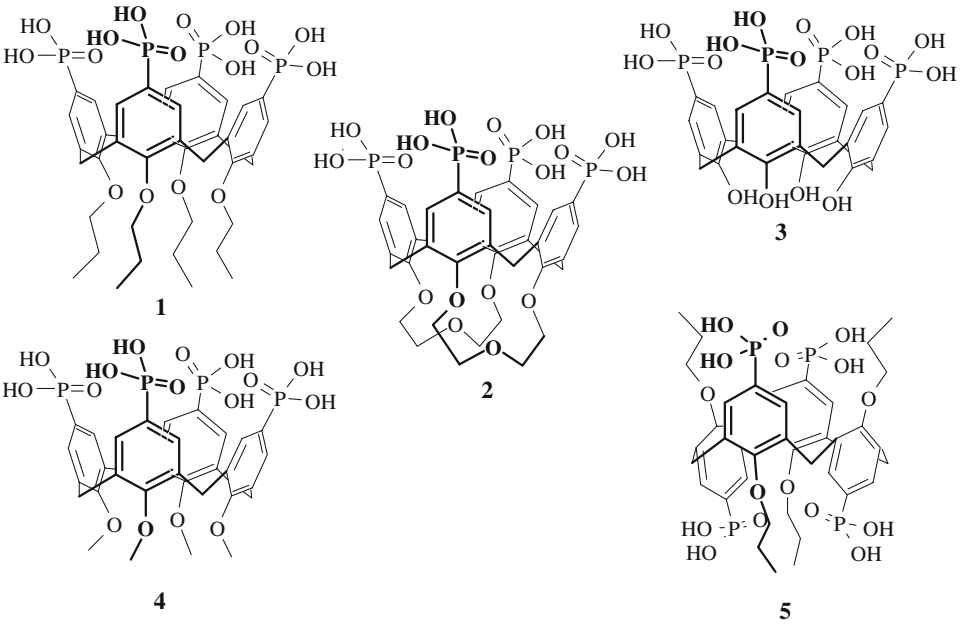


Fig. 1 The calix[4]arene phosphonic acids **1–5** used for complexation of amino acids derivatives in water

while the second consisted of ammonium cations. Inclusion of the Phe and other organic molecules into the capsule cavity in methanol was investigated [19]. Since the capsules were far more stable than the complex with the guest molecule, $\sim 10^5$ vs. $\sim 10^3$ M^{-1} in methanol-d_4, authors claimed that guest molecule was included inside the anionic half-sphere after opening the capsule. However, recently, calixarene tetraphosphonate was classified as specific receptor for basic amino acids, with preference for arginine, binding constants in methanol ranged from 7×10^2 for Ac-Lys-OMe to 1×10^4 for Ts-Arg-OMe. Consequently, that host molecule was used in lipid monolayers for recognition of basic protein surfaces [20]. Some other macrocyclic receptors for amino acids studied in aqueous solution were reported [21]. Ternary *exo* complexes formation between 25,27-diphosphoryloxy-calix[4]arene, amino acids, and metal salts was observed by means of ES/MS and ^{1}H NMR techniques by Perret et al. [22]. We now present synthesis and binding properties of the calix[4]arene phosphonic acids with different calix[4]arene skeleton flexibility as well as conformations.

Experimental

General

All moisture-sensitive reactions were performed under an argon atmosphere. Melting points were uncorrected. The ^{1}H, ^{13}C, ^{31}P NMR spectra were recorded on a Varian Gemini 500 spectrometer operating at 499.8, 125.7, and 202.3 MHz, respectively, for ^{1}H, ^{13}C, and ^{31}P. IR spectra were measured on a Perkin-Elmer 580. Mass spectra were recorded on a Bruker BIFLEX 3 Mass Spectrometer (MALDI-TOF technique), where 2,5-dihydroxybenzoic acid (DHB), 4-hydroxy-α-cyanocinnamic acid (4HCCA), 6-aza-2-thiothymine (ATT) were used as matrix. Reactions were monitored by TLC on precoated silica gel plates (SiO_2, E. Merck, $60F_{254}$). Flash column chromatography were performed on silica gel 60 (SiO_2, E. Merck, particle size 0.040–0.064 mm, 230–400 mesh).

Synthesis

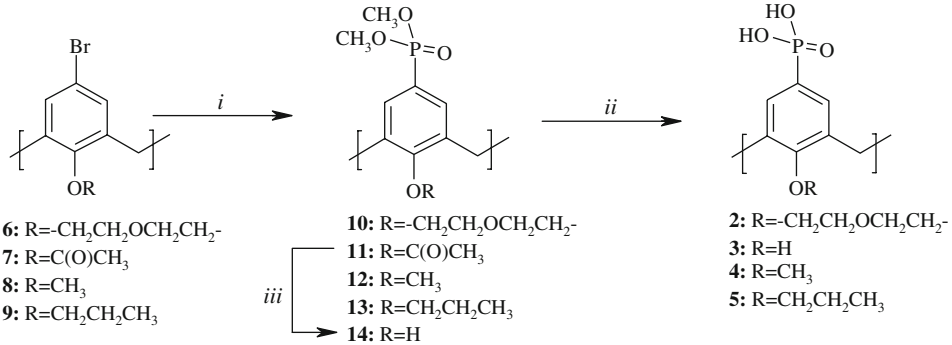
Compounds **10**–**14** and **2**–**5** were obtained as shown on the Scheme 1. Compound **1** was obtained as reported previously [6], compounds **6**, **8** and **9** were obtained according to the literature [23].

5,11,17,23-Tetrabromo-25,26,27, 28-tetraacetoxycalix[4]arene—1,3-alternate (**7**)

To the clear solution of the tetrabromocalix[4]arene [24] (1 g, 1.35 mmol) and DMAP (6.6 mg, 0.054 mmol) dissolved in dry pyridine (100 mL) acetic anhydride (1.52 mL, 16 mmol) was added at once. The reaction mixture was stirred at ambient temperature for 3 days, then the solvent was evaporated in vacuo to dryness and the residue was purified by flash chromatography ($CHCl_3$) affording 0.75 g (yield 61%) of **7** as a white solid; m.p. >330 °C; R_f ($CHCl_3$) 0.75; IR (KBr) ν [cm^{-1}] = 1755 (C=O); MS (MALDI-TOF, CCA matrix) m/z = 904.6 [4, $(M + H)^+$], 906.6 [15, $(M + 2 + H)^+$], 908.6 [19, $(M + 4 + H)^+$], 928.3 [70, $(M + 2 + Na)^+$], 930.3 [100, $(M + 4 + Na)^+$], 932.3 [64, $(M + 6 + Na)^+$]; ^{1}H NMR ($CDCl_3$) δ = 1.75 (s, 12 H, C(O)CH_3), 3.66 (s, 8 H, ArCH_2Ar), 7.23 (s, 8 H, Ar); ^{13}C NMR ($CDCl_3$) δ = 20.0 (*C*H_3), 36.8 (Ar*C*H_2Ar), 118.5, 132.0, 134.6, 147.0, 167.3 (*C*=O); Anal. Calcd for $C_{36}H_{28}O_8Br_4$ (M_r = 908.22): C, 47.61; H, 3.11. Found: C, 47.63; H, 3.44.

General procedure for synthesis of dimethyl calix[4]arene phosphonates (**10**–**13**)

The suspension of bromocalix[4]arene derivative (5 mmol) and anhydrous $NiCl_2$ (0.26 g, 2 mmol, for **10**, **12** and **13**) or (1.62 g, 12.5 mmol, for **11**) in diphenyl ether (100 mL) placed in a 250 mL roundbottom flask, equipped with magnetic stirrer, condenser, thermometer and septum, was stirred and heated to 210–220 °C. At this temperature trimethyl phosphite (7.1 mL, 60.2 mmol) was added, dropwise, over a period of 15 min. The reaction mixture was maintained at 210–220 °C for an additional 2 h. After



Scheme 1 Synthesis of compounds **2**–**5**; *i*: $P(OCH_3)_3$, $NiCl_2$, diphenyl ether, 210 °C; *ii*: 1. $BrSi(CH_3)_3$, CH_2Cl_2; 2. CH_3OH; *iii*: CH_3ONa, CH_3OH

evaporation of the solvent the residue was purified by column chromatography using ($CHCl_3$/CH_3OH) as eluent to give pure product as white solid.

5,11,17,23-Tetrakis(dimethoxyphosphoryl)-25, 26–27,28-bis-crown-3-calix[4]arene—cone (**10**)

Yield 42%; m.p. 236–238 °C; R_f ($CHCl_3$/CH_3OH, 15:1) 0.10; IR (KBr) ν [cm^{-1}] = 772, 826, 1026 (P–O–C), 1127, 1236/1267 (P=O), 1458 (P-Ph); MS (MALDI-TOF, DHB matrix) m/z = 997.2 [100, $(M + H)^+$], 1019.2 [83, $(M + Na)^+$], 1035.2 [40, $(M + K)^+$]; ^{1}H NMR ($CDCl_3$) δ [ppm] = 3.32 (d, J_{HH} = 12.21 Hz, ArCH_2Ar, 2H), 3.38 (d, J_{HH} = 12.70 Hz, ArCH_2Ar, 2H), 3.56 (d, $^3J_{PH}$ = 10.74 Hz, POCH_3, 12H), 3.57 (d, $^3J_{PH}$ = 11.23 Hz, POCH_3, 12H), 3.93–4.39 (m, OCH_2CH_2, 16H), 4.54 (d, J_{HH} = 12.70 Hz, ArCH_2Ar, 2H), 5.22 (d, J_{HH} = 12.21 Hz, ArCH_2Ar, 2H), 7.39 (d, $^3J_{PH}$ = 10.74 Hz, Ar, 4H), 7.41 (d, $^3J_{PH}$ = 11.23 Hz, Ar, 4H); ^{13}C NMR ($CDCl_3$) δ [ppm] = 29.4, 30.4 (ArCH$_2$Ar), 52.57 (d, $^2J_{CP}$ = 5.9 Hz, POCH$_3$), 52.61 (d, $^2J_{CP}$ = 5.5 Hz, POCH$_3$), 73.6, 76.1 (OCH$_2$$CH_2$), 121.4 (d, $^1J_{CP}$ = 190.6 Hz, Ar), 132.2 (d, $^2J_{CP}$ = 10.9 Hz, Ar), 133.0 (d, $^2J_{CP}$ = 10.5 Hz, Ar), 135.4 (d, $^3J_{CP}$ = 21.0 Hz, Ar), 135.6 (d, $^3J_{CP}$ = 16.0 Hz, Ar), 158.9 (d, $^4J_{CP}$ = 3.4 Hz, Ar); ^{31}P NMR ($CDCl_3$) δ [ppm] = 22.37; Anal. Calcd for $C_{44}H_{56}O_{18}P_4 \times 2H_2O$ (M_r = 1032.83): C, 51.17; H, 5.86. Found: C, 51.01; H, 5.77.

5,11,17,23-Tetrakis(dimethoxyphosphoryl)-25,26,27, 28-tetraacethoxycalix[4]arene—1,3-alternate (**11**)

Yield 55%; m.p. 339 °C dec. (in sealed tube); R_f ($CHCl_3$/CH_3OH, 20:1) 0.18; IR (KBr) ν [cm^{-1}] = 762, 828, 1028 (P–O–C), 1118, 1180 (C(O)–O), 1252 (P=O), 1460 (P-Ph), 1755 (C=O); MS (MALDI-TOF, ATT matrix) m/z = 1025.4 [100, $(M + H)^+$], 989.3 [48, $(M–CH_3C(O)–CH_3 + Na)^+$], 1047.4 [16, $(M + Na)^+$]; ^{1}H NMR ($CDCl_3$) δ [ppm] = 1.71 (s, C(O)CH_3, 12H), 3.79 (s, ArCH_2Ar, 8H), 3.79 (d, $^3J_{PH}$ = 11.23 Hz, POCH_3, 24H), 7.53 (d, $^3J_{PH}$ = 13.18 Hz, Ar, 8H); ^{13}C NMR ($CDCl_3$) δ [ppm] = 19.9 (CH_3), 37.2 (ArCH$_2$Ar), 52.8 (d, $^2J_{CP}$ = 5.9 Hz, POCH$_3$), 125.0 (d, $^1J_{CP}$ = 193.1 Hz, Ar), 133.0 (d, $^2J_{CP}$ = 10.5 Hz, Ar), 133.3 (d, $^3J_{CP}$ = 16.4 Hz, Ar), 151.4, 167.3 (C=O); ^{31}P NMR ($CDCl_3$) δ [ppm] = 20.74; Anal. Calcd for $C_{44}H_{52}O_{20}P_4 \times H_2O$ (M_r = 1042.78): C, 50.68; H, 5.22. Found: C, 50.60; H, 4.96.

5,11,17,23-Tetrakis(dimethoxyphosphoryl)-25, 26,27,28-tetramethoxycalix[4]arene (**12**)

Yield 64%; m.p. 114–116 °C (from acetone–hexane); R_f ($CHCl_3$/CH_3OH, 15:1) 0.17; IR (KBr) ν [cm^{-1}] = 772, 827, 1024 (P–O–C), 1120, 1238/1260 (P=O), 1463 (P-Ph); MS (MALDI-TOF) m/z = 913.1 [100, $(M + H)^+$]; ^{1}H NMR (DMSO, 403 K) δ [ppm] = 3.57 (bs, OCH_3, POCH_3, 36H), 3.81 (bs, ArCH_2Ar, 8H), 7.38 (bs, Ar, 8H); ^{13}C NMR (DMSO, 403 K) δ [ppm] = ~34 (bs, ArCH$_2$Ar), 52.8 (d, $^2J_{CP}$ = 6.1 Hz, POCH$_3$), 60.5 (OCH$_3$), 121.5 (d, $^1J_{CP}$ = 183.9 Hz, Ar), 133.5, 134.8, 161.6; ^{31}P NMR (DMSO, 403 K) δ [ppm] = 22.24; Anal. Calcd for $C_{40}H_{52}O_{16}P_4 \times 2H_2O$ (M_r = 948.76): C, 50.64; H, 5.95. Found: C, 50.82; H, 5.90.

5,11,17,23-Tetrakis(dimethoxyphosphoryl)-25,26,27, 28-tetrapropoxycalix[4]arene—1,3-alternate (**13**)

Yield 54%; m.p. 295–302 °C; R_f ($CHCl_3$/CH_3OH, 15:1) 0.23; IR (KBr) ν [cm^{-1}] = 780, 825, 1026 (P–O–C), 1124, 1236/1256 (P=O), 1458 (P-Ph); MS (MALDI-TOF, DHB matrix) m/z = 1024.7 [100, $(M + H)^+$], 1046.7 [31, $(M + Na)^+$], 1062.6 [8, $(M + K)^+$]; ^{1}H NMR ($CDCl_3$) δ [ppm] = 0.93 (t, J_{HH} = 7.33 Hz, OCH$_2$CH$_2$CH_3, 12H), 1.72 (sq, J_{HH} = 7.33 Hz, J_{HH} = 8.30 Hz, OCH$_2$CH_2CH$_3$, 8H), 3.61 (t, J_{HH} = 8.30 Hz, OCH_2CH$_2$CH$_3$, 8H), 3.69 (d, $^3J_{PH}$ = 11.23 Hz, POCH_3, 24H), 3.72 (s, ArCH_2Ar, 8H), 7.47 (d, $^3J_{PH}$ = 13.18 Hz, Ar, 8H); ^{13}C NMR ($CDCl_3$) δ [ppm] = 10.1 (OCH$_2$CH$_2$$CH_3$), 22.9 (OCH$_2$$CH_2CH_3$), 37.0 (Ar$CH_2$Ar), 52.5 (d, $^2J_{CP}$ = 5.5 Hz, POCH$_3$), 73.7 (OCH$_2$$CH_2CH_3$), 119.4 (d, $^1J_{CP}$ = 191.8 Hz, Ar), 133.8 (d, $^3J_{CP}$ = 16.8 Hz, Ar), 134.5 (d, $^2J_{CP}$ = 10.9 Hz, Ar), 160.5 (d, $^4J_{CP}$ = 3.8 Hz, Ar); ^{31}P NMR ($CDCl_3$) δ [ppm] = 23.64; Anal. Calcd for $C_{48}H_{68}O_{16}P_4 \times 0.5H_2O$ (M_r = 1033.35): C, 55.78; H, 6.74. Found: C, 55.60; H, 6.73.

Synthesis of 5,11,17,23-Tetrakis(dimethoxyphosphoryl)-25,26,27,28-tetrahydroxycalix[4]arene (**14**)

A calix[4]arene **11** (2.0 g, 1.95 mmol) was added to a solution of sodium (0.54 g, 23.4 mmol) in dry methanol (150 mL) and the resulting suspension was stirred for 7 h at ambient temperature, after 1.5 h reaction mixture became a clear solution. After evaporation of the solvent in vacuo, the residue was subsequently acidified with 2% HCl (50 mL) until white solid precipitated. The suspension was four times extracted of $CHCl_3$ (4 × 100 mL). The organic layer was successively washed with water (50 mL), brine (50 mL), and dried over $MgSO_4$. The residue after evaporation of the solvent was purified by flash column chromatography to give pure compound **14** as white solid.

Yield 83%; m.p. >300 °C; R_f ($CHCl_3$/CH_3OH, 5:1) 0.20; IR (KBr) ν [cm^{-1}] = 787, 826, 1029 (P–O–C), 1208 (P=O), 1447 (P-Ph), 3200–3600 (O–H); MS (MALDI-TOF, 4HCCA matrix, positive) m/z = 857.0 [100, $(M + H)^+$], 879.0 [8, $(M + Na)^+$], 1084.0 [31, $(M + 4HCCA + K)^+$], (negative) m/z = 855.3 [100, $(M–H)^-$]; ^{1}H NMR (D_2O) δ

[ppm] = 3.06 (d, $^3J_{PH}$ = 10.74 Hz, POCH_3, 24H), 3.51 (d, J_{HH} = 8.80 Hz, ArCH_2Ar, 4H), 4.24 (d, J_{HH} = 8.80 Hz, ArCH_2Ar, 4H), 7.32 (d, $^3J_{PH}$ = 13.67 Hz, Ar, 8H); ^{13}C NMR (D_2O) δ [ppm] = 31.6 (ArCH$_2$Ar), 52.6 (d, $^2J_{CP}$ = 5.3 Hz, POCH$_3$), 114.1 (d, $^1J_{CP}$ = 196.1 Hz, Ar), 131.2 (d, $^2J_{CP}$ = 16.8 Hz, Ar), 132.5 (d, $^3J_{CP}$ = 12.2 Hz, Ar), 158.5; ^{31}P NMR (D_2O) δ [ppm] = 26.26; Anal. Calcd for $C_{36}H_{44}O_{16}P_4 \times 2H_2O$ (M_r = 892.18): C, 48.44; H, 5.42. Found: C, 48.18; H, 5.04.

General procedure for synthesis of calix[4]arene phosphonate acids (**2**–**5**)

A subsequent ester **10**, **12**–**14** (1 mmol) was dissolved in dry methylene chloride (4 mL) and cooled to 5 °C (in the case of **14** a suspension was resulted, which disappeared after the addition of BrTMS), then BrTMS (4 mL) was added. Reaction mixture was stirred overnight at ambient temperature under argon. The solvent and excess of the BrTMS were evaporated in vacuo and residue was taken up with CH_3OH (2 mL) and stirred overnight at ambient temperature. The solvent was removed to leave **2**–**5** as white powders.

5,11,17,23-Tetrakis(dihydroxyphosphoryl)-25, 26-27,28-bis-crown-3-calix[4]arene—cone (**2**)

Yield 90%; m.p. 240 °C dec.; IR (KBr) ν [cm^{-1}] = 971 (P–O), 1126, 1271 (P=O), 1459 (P-Ph); MS (MALDI-TOF, ATT matrix, positive) m/z = 885.4 [100, (M + H)$^+$], 870.0 [57, (M–O + H)$^+$], 852.1 [10, (M–2O + H)$^+$], 1769.2 [28, (2M + H)$^+$], 1753.6 [9, (2M–O + H)$^+$], 1738.1 [6, (2M–2O + H)$^+$]; ^{1}H NMR (D_2O, NaOD) δ [ppm] = 3.12 (d, J_{HH} = 12.21 Hz, ArCH_2Ar, 2H), 3.17 (d, J_{HH} = 12.21 Hz, ArCH_2Ar, 2H), 3.58–4.07 (m, OCH_2CH_2, 16H), 4.22 (d, J_{HH} = 12.21 Hz, ArCH_2Ar, 2H), 4.58 (d, J_{HH} = 12.21 Hz, ArCH_2Ar, 2H), 7.24 (d, $^3J_{PH}$ = 9.76 Hz, Ar, 4H), 7.26 (d, $^3J_{PH}$ = 9.76 Hz, Ar, 4H); ^{13}C NMR (D_2O, NaOD) δ [ppm] = 29.2, 30.0 (ArCH$_2$Ar), 74.6, 75.8 (OCH$_2$$CH_2$), 130.6 (d, $^3J_{CP}$ = 9.9 Hz, Ar), 131.4 (d, $^3J_{CP}$ = 9.2 Hz, Ar), 134.9 (d, $^2J_{CP}$ = 13.7 Hz, Ar), 135.0 (d, $^2J_{CP}$ = 13.0 Hz, Ar), 136.8 (d, $^1J_{CP}$ = 168.6 Hz, Ar), 155.0; ^{31}P NMR ($CDCl_3$) δ [ppm] = 12.37; Anal. Calcd for $C_{36}H_{40}O_{18}P_4 \times 5H_2O$ (M_r = 974.17): C, 44.36; H, 5.17. Found: C, 44.59; H, 5.02.

5,11,17,23-Tetrakis(dihydroxyphosphoryl)-25, 26,27,28-tetrahydroxycalix[4]arene (**3**)

Yield 75%; m.p. 267 °C dec; MS (MALDI-TOF, DHB matrix, positive) m/z = 744.8 [100, (M + H)$^+$], 766.8 [16, (M + Na)$^+$], 1488.4 [8, (2M + H)$^+$], (negative) m/z = 743.0 [100, (M–H)$^-$], 1487 [13, (2M–H)$^-$]; ^{1}H NMR (D_2O, phosphate buffer, pD 7.3, 275 K) δ [ppm] = 3.57 (bs, ArCH_2Ar, 4H), 4.36 (bs, ArCH_2Ar, 4H), 7.48 (d, $^3J_{PH}$ = 12.70 Hz, Ar, 8H); ^{13}C NMR (D_2O, phosphate buffer, pD 7.3, 275 K) δ [ppm] = 31.9 (ArCH$_2$Ar), 126.3 (d, $^1J_{CP}$ = 180.5 Hz, Ar), 130.7 (d, $^2J_{CP}$ = 15.1 Hz, Ar), 131.0 (d, $^3J_{CP}$ = 10.9 Hz, Ar), 154.9; ^{31}P NMR (D_2O, phosphate buffer, pD 7.3, 275 K) δ [ppm] = 13.83; Anal. Calcd for $C_{28}H_{28}O_{16}P_4 \times 3H_2O \times$ HBr (M_r = 879.4): C, 38.24; H, 4.01. Found: C, 38.20; H, 3.96.

5,11,17,23-Tetrakis(dihydroxyphosphoryl)-25, 26,27,28-tetramethoxycalix[4]arene (**4**)

Yield 91%; m.p. 282 °C dec.; IR (KBr) ν [cm^{-1}] = 1004 (P–O), 1121 (P=O), 1465 (P-Ph); MS (MALDI-TOF, DHB matrix, positive) m/z = 801.1 [100, (M + H)$^+$], 823.1 [16, (M + Na)$^+$], 1601.4 [3, (2 M + H)$^+$]; ^{1}H NMR (D_2O, NaOD)—for main *paco* conformer δ [ppm] = 2.92 (s, OCH_3), 3.15–3.40 (bs, OCH_3),3.53 (d, J_{HH} = 14.16 Hz, ArCH_2Ar), 3.84 (s, ArCH_2Ar), 4.08 (d, J_{HH} = 14.16 Hz, ArCH_2Ar), 7.34 (d, J_{PH} = 11.72 Hz, Ar), 7.47 (d, J_{PH} = 11.72 Hz, Ar); ^{13}C NMR (D_2O, NaOD) δ [ppm] = 29.9 (ArCH$_2$Ar), 35.0 (ArCH$_2$Ar), 61.4 (OCH$_3$), 131.8, 133.4 (d, $^2J_{CP}$ = 13.7 Hz, Ar), 134.5 (d, $^2J_{CP}$ = 13.7 Hz, Ar), 136.9 (d, $^1J_{CP}$ = 167.1 Hz, Ar), 157.1; ^{31}P NMR (D_2O, NaOD) δ [ppm] = 11.78; Anal. Calcd for $C_{32}H_{36}O_{16}P_4 \times 3H_2O$ (M_r = 854.56): C, 44.98; H, 4.95. Found: C, 44.73; H, 4.72.

5,11,17,23-Tetrakis(dihydroxyphosphoryl)-25,26,27, 28-tetrapropoxycalix[4]arene—1,3-alternate (**5**)

Yield 74%; m.p. 271 °C dec.; IR (KBr) ν [cm^{-1}] = 997 (P–O), 1124, 1262 (P=O), 1458 (P-Ph); MS (MALDI-TOF, ATT matrix, positive) m/z = 913.3 [100, (M + H)$^+$], 935.4 [24, (M + Na)$^+$], 897.8 [22, (M–O + H)$^+$]; ^{1}H NMR (D_2O, NaOD) δ [ppm] = 0.54 (t, J_{HH} = 7.33 Hz, OCH$_2$CH$_2$CH_3, 12H), 1.34 (sq, J_{HH} = 7.33 Hz, J_{HH} = 8.06 Hz, OCH$_2$CH_2 CH$_3$, 8H), 3.33 (t, J_{HH} = 8.06 Hz, OCH_2CH$_2$CH$_3$, 8H), 3.72 (s, ArCH_2Ar, 8H), 7.32 (d, $^3J_{PH}$ = 11.23 Hz, Ar, 8H); ^{13}C NMR (D_2O, NaOD) δ [ppm] = 7.8 (OCH$_2$CH$_2$$CH_3$), 20.8 (OCH$_2$$CH_2CH_3$), 35.9 (Ar$CH_2$Ar), 73.3 (O$CH_2CH_2CH_3$), 131.2 (d, $^3J_{CP}$ = 9.2 Hz, Ar), 133.1 (d, $^2J_{CP}$ = 13.7 Hz, Ar), 135.3 (d, $^1J_{CP}$ = 167.1 Hz, Ar), 153.6; ^{31}P NMR (D_2O, NaOD) δ [ppm] = 11.47; Anal. Calcd for $C_{40}H_{52}O_{16}P_4 \times 2.5H_2O$ (M_r = 957.73): C, 50.11; H, 5.95. Found: C, 50.14; H, 5.82.

Complexation experiments

All complexation processes were performed in deuterated phosphate buffer (0.2 M at pD 7.3). The guest concentration was kept constant (1×10^{-3} M) while the host concentration was varied from 0.5×10^{-3} to 15×10^{-3} M for **3** and

from 2×10^{-3} to 20×10^{-3} M for the others. Chemical shifts (δ, ppm) were internally referenced to TSP-d_4 [2,2,3,3-d_4-3-(trimethylsilyl)propionic acid sodium salt].

Data analysis

Data obtained from the ^{1}H NMR titration experiments for each investigated complex were analyzed depending on the stoichiometry of formed complex (Jobs plot) by fitting them to the corresponding equations presented below [25] and association constant values were estimated:

1:1 binding model

$$\Delta\delta_{obs} = \frac{\Delta\delta_{nas}}{2[G]_t} \times \left\{ \frac{1}{K_1} + [H]_t + [G]_t - \sqrt{\left(\frac{1}{K_1} + [H]_t + [G]_t\right)^2 - 4[H]_t[G]_t} \right\}$$

1:2 binding model

$$\Delta\delta_{obs} = \frac{\Delta\delta_{nas1}K_1[H] + \Delta\delta_{nas2}K_1K_2[H]^2}{1 + K_1[H] + K_1K_2[H]^2}$$

2:1 binding model

$$\Delta\delta_{obs} = \frac{\Delta\delta_{nas1}K_1[H] + 2\Delta\delta_{nas2}K_1K_2[G][H]}{1 + K_1[H] + 2K_1K_2[G][H]}$$

In the case of 1:1 complexes K values were obtained using theoretical titration curve for the 1:1 binding model. In 1:2 and 2:1 models association constant values K_1, K_2, as well as $\Delta\delta_{sat1}$, $\Delta\delta_{sat2}$ were estimated according to the following algorithm: first, the equilibrium concentrations of all species participated in complexation were determined numerically on the basis given parameters of K_1, K_2, equations defining them and equations of mass balance ($[G]_t = [G] + [GH] + [GH_2]$, $[H]_t = [H] + [GH] + 2[GH_2]$ for 1:2 complexes and $[G]_t = [G] + [GH] + 2[G_2H]$, $[H]_t = [H] + [GH] + [G_2H]$ for 2:1 complexes) as well. Next, the experimental data were fitted into the appropriate functions with simultaneous adjusting $\Delta\delta_{sat1}$, $\Delta\delta_{sat2}$ parameters. The best fit of the parameters were determined by minimizing the value of $\Sigma(\Delta\delta_{obs} - \Delta\delta_{cal})^2$ which indicated the degree of agreement of the experimental with calculated values. Standard deviations of all K values obtained by fitting data to the theoretical equations were lower than 20% in each case.

Results and discussion

Synthesis

In our studies we have put emphasis on a geometrical fit possibility of the guest molecule within the flexible or rigid host interior. We have exerted calix[4]arene skeleton whose structural properties depending on the substituents present at the lower rim could be easily predicted. Therefore, we have chosen five hosts, presented in the Fig. 1; three models in *cone* conformation **1**–**3**, two of them blocked chemically and one thanks to the presence of the intramolecular hydrogen bonds. Compound **4**, with completely flexible calix[4]arene skeleton substituted by methyl groups on the phenolic oxygens, and compound **5**, blocked in *1,3-alternate* conformation by means of *n*-propyl groups, were also obtained in order to receive a comparison to the models with well preorganized aromatic cavities. Calix[4]arene **1** was reported by us previously [6]. The calix[4]arene-phosphonic acids **2**, **4**, and **5** were obtained in two steps, according to the Scheme 1. In each case reaction of known calix[4]arene bromo-derivative [23] and trimethyl phosphite was carried out in the presence of the nickel chloride (II) as catalysts in diphenyl ether at 210 °C. Yields of obtained products were in the range 42–64%. Further treatment of the phosphonic dimethyl esters **10**, **12**, **13** with trimethylsilyl bromide and following methanolysis led to suitable acids **2**, **4**, **5**, respectively.

In the case of the compound **3** reaction of *p*-bromocalix[4]arene containing unsubstituted hydroxyl phenolic groups with trimethyl phosphite in the presence of the catalyst failed, therefore the synthesis was performed using acetyl protective groups. The *p*-bromocalix[4]arene **7** was obtained from *p*-bromocalix[4]arene [24] and acetic anhydride in the presence of DMAP in anhydrous pyridine. The product **7** in *1,3-alternate* conformation was isolated in 61% yield. Attaching of the dimethylphosphonic ester groups at the upper rim of the calix[4]arene **7** was successful only when 2.5 eq. of the nickel chloride (II) was added (when less amount of $NiCl_2$ was used only starting material was recovered). In next step acetyl groups were removed (the way *iii* in the Scheme 1) and after treatment of the compound **14** with $BrSi(CH_3)_3$ and further methanolysis the calix[4]arene phosphonic acid **3** was obtained in 46% overall yield.

Study of complexing properties

The synthesized calix[4]arene-phosphonic acids were tested in aqueous solution as potential receptors towards the amino acids methyl esters. All host molecules were soluble in phosphate buffer solution at pD 7.3 up to 20 mM/dm^3. The complexation processes were clarified by monitoring the host induced chemical shifts of the guest protons using ^{1}H NMR spectroscopy technique. For each complex the titration experiment was performed in which guest concentration was kept constant ($c_G = 1$ mM), while host concentration was varied. The complexation-induced ^{1}H

NMR chemical shifts (CICS) of α-protons and also side-chain protons of studied amino-acid guests in the presence of the hosts **1–5** are presented in the bar diagram in the Fig. 2. The presence of upfield shifts for any guests studied with hosts was minus signed and was an indication of a possible host–guest interaction as well. The stoichiometries of all complexes formed by **1–5** with the guests were determined from continues variation plots (Job's plots) [25].

The estimated association constant values for all investigated complexes with the use of adequate binding model in each case are presented in the Table 1.

Compounds **1–3** are fixed in *cone* conformation, however, the shape of each cavity is various due to the different substituents present at the lower rim. Compound 1 fixed by means of four *n*-propyl groups adopts a *flattened cone* conformation (C_{2v} symmetry) because of a presence of the residual conformational mobility. The host 2 rigidified by two short di(ethylene glycol) bridges introduced in proximal (25,26-27,28) positions at the lower rim of calix[4]arene was assumed to be the best receptor for complexation processes because of rigid, open cavity with highly preorganized phosphonic moieties at the upper rim. Receptor **3**, according to the ^{1}H NMR spectra in aqueous solution, also adopts *cone* conformation, probably due to the presence very strong intramolecular hydrogen bonding between phenolic hydroxyl groups [26]. The temperature-depended ^{1}H NMR spectra in D_2O at the lowest applied temperature (2 °C) have shown two wide peaks centered at $\delta = 4.36$ and 3.57 ppm, unfortunately, no splitting into doublets was observed. The coalescence temperature (T_c) for the $ArCH_2Ar$ protons appeared at 26.0 °C (±0.5 °C) and $\Delta\nu = 196.7$ Hz, this allowed us to estimate a barrier inversion for *cone*, $\Delta G^{\ddagger} = 58.08 \pm 0.13$ kJ mol^{-1} [27]. Taking into consideration that the methylene protons are coupled, suitable coupling constant (estimated value $J = 12$ Hz) was included in calculation, notwithstanding, its contribution into the $\Delta G^{\ddagger}$ here was negligible (when J value was omitted, the $\Delta G^{\ddagger}$ value changed by −0.03 units). Host 4 with the methyl groups at the lower rim is very flexible and may adopt all possible conformations. Indeed, the ^{1}H NMR spectrum in D_2O have shown more than one conformer, nevertheless, conformer *paco* predominantly appeared. The receptor 5 obtained in *1,3-alternate* conformation possesses two separated binding places and rendered access to the hydrophobic cavity.

According to the ^{1}H NMR spectra from titration experiments almost all guests were included inside the apolar cavity of the receptor **1**. The signals of guests protons were shifted upfield due to the experienced diamagnetic shielding caused by aromatic rings of the host. Stoichiometries, determined for complexes of **1** with amino acids methyl esters indicated that multiple equilibria were involved in complexation processes and they were different for Trp-OMe and other guests. The maximum on a Job's plot for Trp-OMe—**1** complex appearing in region 0.6–0.66 value of molar ratio indicated 1:2 host–guest complex formation (Fig. 3). The CICS either aromatic or aliphatic protons were significant, $\Delta\delta = -0.323$ and −0.836 ppm, respectively, however, it is a little probability that two such big guest molecules could be included inside the calix[4]-arene cavity. However, the significant K_1 value for Trp-OMe suggests the tight inclusion complex formed through the π–π interactions between aromatic rings of the

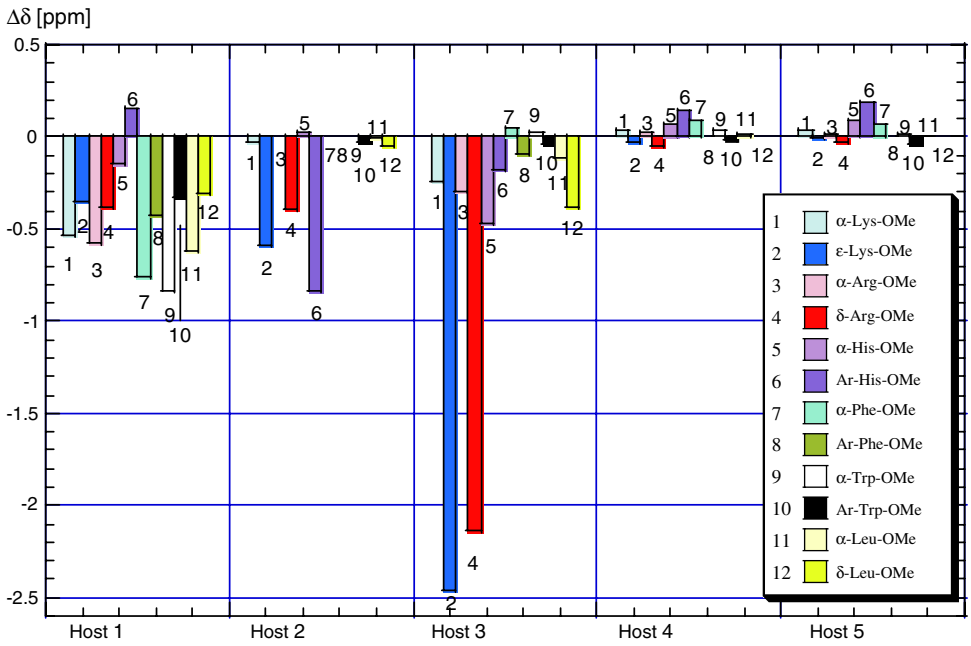


Fig. 2 CICS of the guests α- and side chain protons (ε- for Lys-OMe, δ- for Arg-OMe, Ar- for His-OMe, Phe-OMe and Trp-OMe, CH_3-aliphatic for Leu-OMe, respectively) relative to used hosts **1–5**

Table 1 Association constant values (K in M^{-1}) for the complexation of the amino acids methyl esters by the hosts **1–5** in D_2O (pD 7.3, 0.2 M phosphate buffer), at 22 °C

1*	2***	3*	4	5**
Lys-OMe*2HCl				
(1:2)	(1:1)	(1:1)	ε-H (1:1)	(1:1)
$K_1 = 420$	$K_1 = 170$	$K_1 = 600$	$K_1 = 70$	$K_1 = 110$
$K_2 = 170$				
Arg-OMe*2HCl				
(1:2)	(1:1)	(1:1)	δ-H (1:1)	(1:1)
$K_1 = 2{,}000$	$K_1 = 120$	$K_1 = 600$	$K_1 = 44$	$K_1 = 140$
$K_2 = 400$				
His-OMe*2HCl				
(1:2)	(1:1)	(1:1)	α-H (1:1)	(1:1)
$K_1 = 150$	$K_1 = 30$	$K_1 = 200$	$K_1 = 34$	$K_1 = 10$
$K_2 = 150$				
Phe-OMe*HCl				
(1:2)	(1:1)	(1:1)	α-H (2:1)	(2:1)
$K_1 = 300$	$K_1 = 150$	$K_1 = 135$**	$K_1 = 100$	$K_1 = 15$
$K_2 = 170$			$K_2 = 10$	$K_2 = 20$
Trp-OMe*HCl				
(2:1)	n.s.	(1:1)	Ar–H (1:1)	(2:1)
$K_1 = 3{,}000$		$K_1 = 152$**	$K_1 = 37$	$K_1 = 30$
$K_2 = 50$				$K_2 = 55$
Leu-OMe*HCl				
(1:2)	n.s.	n.s.	α-H (1:1)	(2:1)
$K_1 = 200$			$K_1 = 200$	$K_1 = 10$
$K_2 = 200$				$K_2 = 15$

Standard deviation of the K values was lower than 20%

n.s.—No saturation; * K_1, K_2—average values obtained for all guest protons without OCH_3; ** K_1, K_2—values obtained for guest α-proton; *** K_1—values obtained for ε-protons of lysine, δ-protons of arginine, α-protons of phenylalanine and tryptophan, aromatic protons of histidine

calix[4]arene **1** and indolyl ring of the Trp-OMe with the accompaniment of the electrostatic interactions between ammonium and phosphonic groups. Probably effective π-stacking of the tryptophan indolyl moieties and benzene rings of calix[4]arene took place.

Different situation was in the case of the remaining guest molecules which formed 2:1 (host–guest) complexes with **1**. The inclusion of the Lys-OMe, Arg-OMe, Phe-OMe and Leu-OMe molecules inside the interior of the calixarene **1** proceeded in moderate extent ($\Delta\delta < -0.9$ ppm, see Fig. 2) and was unspecific, the CICS for α-protons were in the range $-0.5 < \Delta\delta < -0.8$ ppm, additionally almost all side-chain protons of the guest molecules (the one exception is His-OMe) were included inside the hydrophobic cavity with similar CICS $-0.3 < \Delta\delta < -0.5$ ppm. According to this we cannot say about selectivity of the host **1**. Thus, in complexation processes both ammonium moiety and side-chain functional group of amino acid guest interact with two calixarene molecules. It is possible, that guest molecule is continuously transferred from one host molecule to another forming capsule-like structure for a moment in which guest has a possibility to move along the interior of the "capsule". The host **1** forms more stable complexes with Arg-OMe than with Lys-OMe or His-OMe probably due to different character of their side chain groups. The flat guanidinium group of Arg-OMe is larger and "softer" than the spherical ammonium group of Lys-OMe and less hydrophilic than the imidazolium group of His-OMe, therefore may be better fitted into the calix[4]arene cavity with the aid of interactions with anionic phosphonic groups.

The possibility of the host **3** to adopt *cone* conformation caused that all guest molecules could be placed inside the hydrophobic interior of the calixarene molecule. Nevertheless, we do not observed significant interactions with phenylalanine and tryptophan methyl esters whose aromatic protons were only slightly shifted upfield without achieving the saturation, whereas the α-protons were shifted downfield as a result of the most probable electrostatic interactions. The Leu-OMe protons were shifted higher but, unfortunately, they also did not achieved a saturation point. Compound **3** has shown better affinity towards all basic amino acids than the others, all guests protons were strongly shifted upfield. Moreover, the ammonium, guanidinium, and imidazolium groups of Lys-OMe, Arg-OMe, and His-OMe, respectively, were specifically bound inside the calix[4]arene cavity (the CICS for protons laying near those side-chain functional groups were the highest $\Delta\delta = -2.464$, -2.134, -1.009 ppm, respectively). A typical titration profile is reported in Fig. 4.

These results show that inherent mobility of the host **3**, which is stabilized in *cone* conformation by means of intramolecular hydrogen bonds, is important factor for the complexation of the amino acids derivatives. That molecule forms cavity with the proper shape of a half-sphere which can preferentially hold spherical ammonium or flat guanidinium and even very hydrophilic histidinium cation. We cannot exclude that one of the hydroxyl group at the lower rim of calix[4]arene skeleton, that should be dissociated in experimental conditions, can assist binding process through the additional electrostatic interactions. Moreover, the temperature-depended ^{1}H NMR spectra of **3** performed in the presence of the 2 eq. of Lys-OMe have shown that T_c values for $ArCH_2Ar$ protons of **3** was enhanced up to 42.0 °C (±2 °C). That experiment supports the inclusion of the guest molecule inside the calix[4]arene *cone* cavity. Unfortunately, we could only estimate the T_c value because of overlapping signals of calix[4]arene methylene protons with signals of α-proton and methyl ester of Lys-OMe. On the other hand, the most rigid compound **2** formed much weaker complexes with investigated

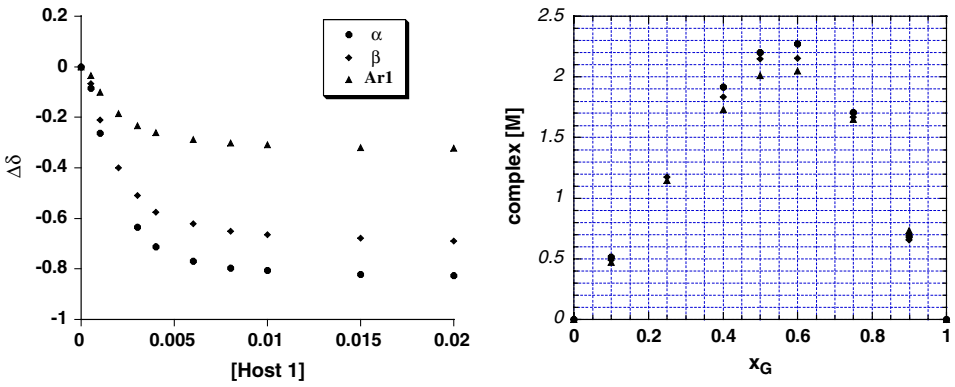


Fig. 3 Left: Complexation-induced upfield shifts of Trp-OMe protons versus concentration o the host 1; Right: Job's plot for complex **1**—Trp-OMe, complex concentration is plotted versus guest molar fraction

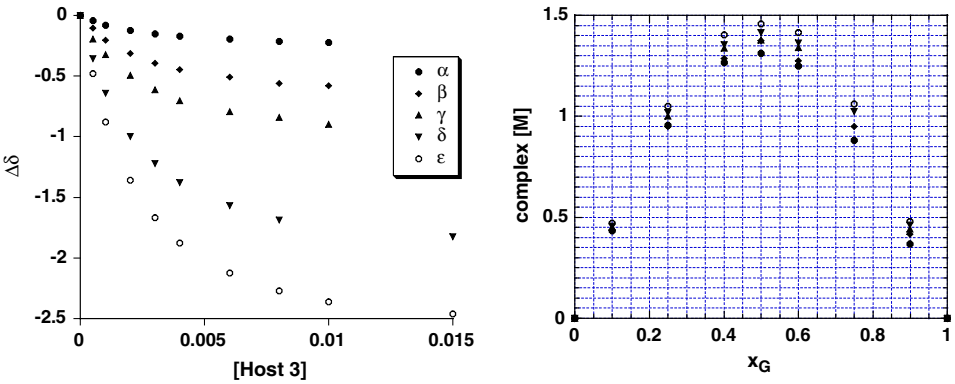


Fig. 4 Left: Complexation-induced upfield shifts of Lys-OMe protons versus concentration of the host 3; Right: Job's plot for complex 3-Lys-OMe, complex concentration is plotted versus guest molar fraction

amino acids derivatives. Similarly to the **3** the host **2** also included guest molecules disregards the apolar aromatic or aliphatic moieties in relation to charged groups appearing in Lys-OMe, Arg-OMe and His-OMe. The association constant values were relatively lower for **2** than those obtained for **3**. This was also seen on the CICS values obtained for the guest protons which experienced much weaker influence of the diamagnetic shielding by aromatic rings. In these cases the Lys-OMe, Arg-OMe and His-OMe were also specifically included inside the cavity by the side chain groups, i.e. ammonium group of the Lys-OMe, guanidinium group of the Arg-OMe and imidazolium group of the His-OMe, whereas the α-protons were the least affected (in the case of the host **2** the α-protons were not affected at all) (Fig. 2). In the case of Phe-OMe and Trp-OMe only electrostatic deshielding of their α-protons were observed.

On the other hand calixarenes **4** and **5** had relatively small influence on the CICS for all guests (ca. $\Delta\delta < 0.2$ ppm). According to the ^{1}H NMR spectra side chain protons of the amino acids methyl esters were slightly shifted upfield, while all α-protons are shifted downfield. Both hosts formed weak complexes with investigated guests. Results obtained for receptor **4** from Jobs plots were difficult to unambiguous interpretation of stoichiometry of complexes. We can only assume that guest molecules weak interact with hydrophobic part of the very flexible calixarene 4 which occurs as a mixture of various conformations in solution. Because of that the observations of these systems were complicated. However, uncertainty of the estimated association constants was so large that this is of less consequence. A problem in the determination of *K* values has been that the shift changes were small. Receptor **5** has two independent binding places able to electrostatic interactions with charged guests. However, inclusion of the guest molecule was not observed. Guest access to the narrow and long cavity of **5** is difficult because of the large functional groups with different character present at both side of calixarene molecule, apolar alkyl chains can act repulsively on coming guest molecule.

Conclusion

The most interesting properties were noticed for receptors **1**, **2**, and **3**. Since the receptor **1** did not show any remarkable selectivity towards investigated amino acids methyl esters, the latter two have shown selectivity for basic amino-acid methyl esters, i.e. Lys-OMe, Arg-OMe, and His-OMe. These dicationic molecules were hardly bound inside the calix[4]arene **3** cavity in a specific mode by inclusion functional group of the amino acid side chain. The rigidifying of the calix[4]arene *cone* structure by modification of

the lower rim of the calix[4]arene skeleton caused that complexation properties of the very rigid molecule **2** were much lower but with preservation of the selectivity and specificity in binding mode. Extremely different situation was in the case of the host **1** which cavity is not spherical in solution but may adopt flattened or pinched cone conformation in which aromatic rings of calix[4]arene skeleton are closer to maximize the π–π interactions. This caused that flat residues of amino-acids such as guanidinium of arginine and aromatic residue of tryptophan were preferentially included inside the host interior. Comparison of the complexing properties of all studied receptors suggests that the affinity of the calixarene phosphonic acids towards amino acid esters stems mostly from interaction with the cavity of the calixarene. Moreover the guests structure has a significant impact on the affinity.

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