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Complexation of upper rim phosphorylated calix[4]arenes with uracil derivatives in water-containing solution

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ABSTRACT: The host–guest complexation of the upper rim diisopropoxyphosphoryl derivatives of dipropoxy- or tetrapropoxycalix[4]arenes and the upper rim unsubstituted parent calixarenes with uracil and 5-amino-, 5-chloro-, 5-nitro-, 6-methyl and 6-amino-1,3-dimethyluracil in methanol–acetonitrile–tetrahydrofuran–water (15:10:5:70, v/v) solution was investigated by reversed-phase high-performance liquid chromatography. The association constants of the 1:1 host–guest complexes of the uracils with the calixarenes within the range 1200–54 300 M^{-1} were calculated from the relationship between the capacity factor of the uracil solutes and concentration of the calixarenes in the mobile phase. The association constants were dependent on the nature of uracil guests, the manner of the lower rim substitution of the calixarene skeleton and the number of the phosphoryl groups at the upper rim. Molecular dynamics (MD) simulations of host–guest interactions were performed. Based on the MD trajectories, the atomic partition to the net molecular solvent-exposed surface was analysed for the separate host and guest molecules and for the complexes.

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KEYWORDS: calixarenes; uracils; organophosphorus compounds; host–guest complexes; high-performance liquid chromatography; molecular modelling

INTRODUCTION

The vase-shaped calix[4]arenes,[1] composed of four phenolic units connected via methylene links, have been investigated as a platform for the design of artificial receptors[2,3] with capabilities similar to those of natural enzymes for recognizing a range of bioactive guest molecules such as amino acids,[3,4] dipeptides,[5] proteins,[6] choline or acetylcholine,[7] carbohydrates,[8] vitamins B (riboflavin) and B_{12} (cyanocobalamin),[9] nucleotides (cytidine, uridine, thymidine),[10] nucleotides and even DNA.[11] The receptor–substrate (host–guest) interaction of the calixarenes with biorelevant molecules is the basis of their biomedical properties.[2d,12]

In a previous study,[13] the host–guest interaction of tetrapropoxycalix[4]arene **1** (Scheme 1) with a series of bioactive uracil and adenine derivatives in a water-containing medium was investigated by reversed-phase high-performance liquid chromatography (RP-HPLC) and molecular dynamics (MD) methods. These heterocycles, being structural fragments of nucleotides, RNA and DNA, can serve as binding sites in processes of its recognition by calixarenes.

In this work, we utilized both RP-HPLC and MD methods for the analysis of the host–guest complexation of a series of uracils with dipropoxy- or tetrapropoxycalix[4]arenes (**1**–**6**) (Scheme 1) functionalized at the upper rim with hydrophilic diisopropoxyphosphoryl groups. These groups extend the host's cavity and increase the solubility of the calixarene molecules in aqueous solution.

RESULTS AND DISCUSSION

HPLC analysis of the host–guest complexation

To investigate the role of the phosphoryl groups in the host–guest interaction, stability constants of the complexes of calixarenes **2**–**6** with the uracil derivatives were determined by HPLC using methanol–acetonitrile–

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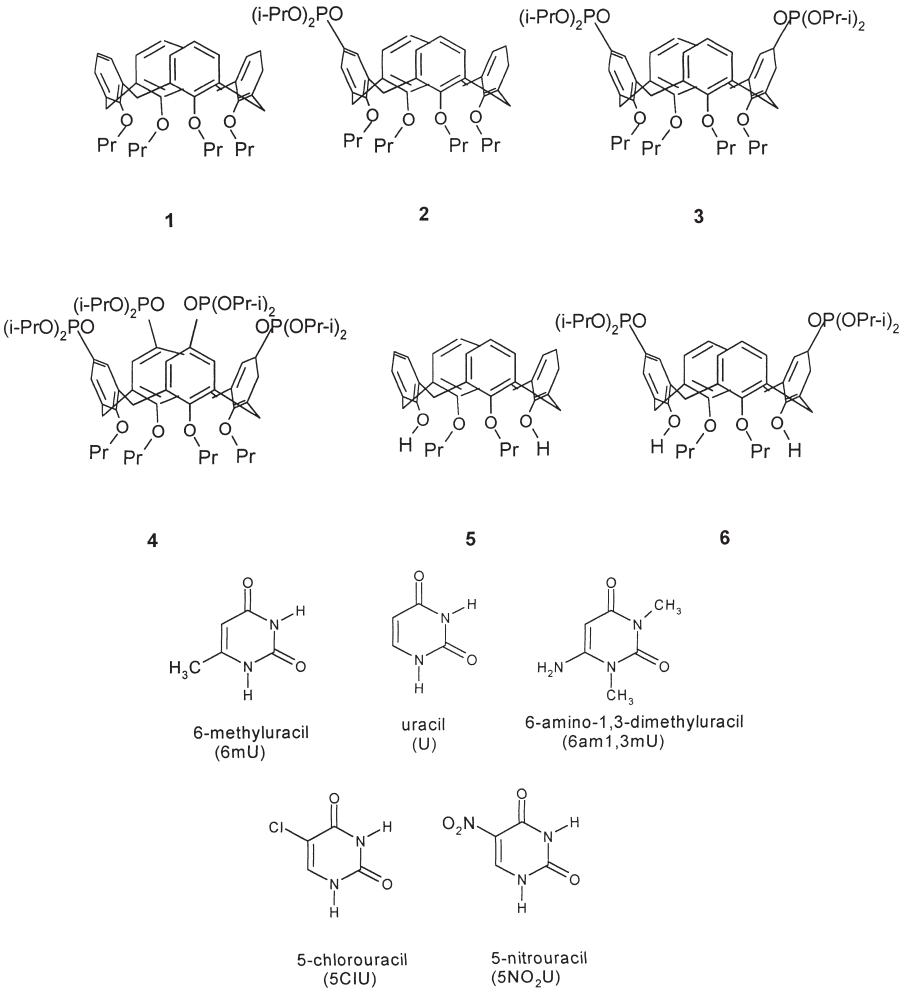

Scheme 1

tetrahydrofuran–water (15:10:5:70, v/v) as mobile phase, as reported for tetrapropoxycalixarene (**1**).[13]

Addition of calixarenes to the mobile phase decreases the capacity factors k' of the uracil solutes (Table 1) owing to the formation of the host–guest supramolecular complexes. The linear relationship of k' versus calixarene concentration in the mobile phase (correlation coefficient 0.95–0.99) confirms the 1:1 stoichiometry of the complexes.

The stability constants K_A of the 1:1 complexes were calculated using the equation[14]

$$1/k' = 1/k_0' + K_A \times [CA]/k_0' \qquad (1)$$

where k_0' and k' are the capacity factors in the absence and presence of calixarene in the mobile phase, respectively, and [CA] is the calixarene concentration. Detailed procedures for these calculations have been reported.[14,15]

The stability constants calculated by this method are given in Table 2. In accordance with the data, the stability constant values are dependent on the structure of the guest molecule, the nature and quantity of substituents [H or P(O)(OPr-i)$_2$] at the *para*-positions of the benzene rings and the manner of the lower rim substitution (di- or tetrapropylation) that determine the conformational behaviour of the calixarene skeleton (see below). It is well documented that host–guest complexation in a water-containing medium is governed by hydrophobic effects electrostatic and π–π aromatic interactions, etc.[16]

The upper rim unsubstituted tetrapropoxycalixarene (**1**) was the best binder among tetrapropoxycalixarenes **1**–**4** ($K_A = 6200$–$54\,300\ M^{-1}$).[13] Insertion of electronegative phosphoryl groups at the upper rim (**2**–**4**) weakens the π-donor ability of the benzene rings and as a rule decreases the stability constants of the complexes compared with the unsubstituted calixarene **1** (one order of magnitude or more in the case of CA**4**·5ClU and CA**4**·5NO$_2$U complexes).

The complexing ability of the upper rim unsubstituted dipropoxycalixarene **5** ($K_A = 5640$–$41\,700\ M^{-1}$) is close to that of tetrapropoxycalixarene (**1**) (Table 2). However, in contrast to **1**, insertion of phosphoryl groups mainly increases the stability constants (twofold for **6**·6am1, 3mU and **6**·6mU complexes).

Table 1. Capacity factors of the uracil solutes (k') depending on calixarene **1**–**6** concentration ([C] $\times 10^4$ M) in the mobile phase

	1		**2**		**3**		**4**		**5**		**6**	
Guest	[C]	k'	[C]	k'	[C]	k'	[C]	k'	[C]	k'	[C]	k'
6am1,3mU	0.0	0.66	0.0	0.94	0.0	0.70	0.0	0.67	0.0	0.67	0.0	0.94
	1.2	0.37	1.8	0.36	1.2	0.50	1.2	0.41	1.2	0.36	1.5	0.36
	2.3	0.28	3.5	0.25	2.3	0.40	2.0	0.32	2.3	0.29	3.0	0.20
	4.6	0.18	7.1	0.17	5.0	0.27	4.0	0.24	4.9	0.20	4.0	0.14
6mU	0.0	0.71	0.0	0.74	0.0	0.60	0.0	0.74	0.0	0.74	0.0	0.74
	1.2	0.35	1.8	0.34	1.2	0.35	1.2	0.34	1.2	0.34	1.5	0.25
	2.3	0.23	3.5	0.26	2.3	0.27	2.0	0.26	2.3	0.26	3.0	0.14
	4.6	0.16	7.1	0.14	5.0	0.19	4.0	0.17	4.9	0.20	4.0	0.10
U	0.0	0.60	0.0	0.60	0.0	0.60	0.0	0.60	0.0	0.60	0.0	0.60
	1.2	0.29	1.8	0.35	1.2	0.35	1.2	0.30	1.2	0.35	1.5	0.24
	2.3	0.20	3.5	0.22	2.3	0.22	2.0	0.22	2.3	0.22	3.0	0.12
	4.6	0.13	7.1	0.12	5.0	0.13	4.0	0.14	4.9	0.14	4.0	0.09
5ClU	0.0	0.98	0.0	0.80	0.0	0.66	0.0	0.98	0.0	0.98	0.0	0.80
	1.2	0.40	1.8	0.38	1.2	0.46	1.2	0.84	1.2	0.43	1.5	0.33
	2.3	0.27	3.5	0.29	2.3	0.36	2.0	0.78	2.3	0.29	3.0	0.18
	4.6	0.16	7.1	0.18	5.0	0.25	4.0	0.69	4.9	0.19	4.0	0.14
$5NO_2U$	0.0	2.17	0.0	2.17	0.0	2.17	0.0	2.17	0.0	2.17	0.0	2.17
	1.2	0.30	1.8	0.38	1.2	1.33	1.2	1.49	1.2	0.38	1.5	0.34
	2.3	0.16	3.5	0.25	2.3	0.95	2.0	1.12	2.3	0.25	3.0	0.17
	4.6	0.09	7.1	0.15	5.0	0.66	4.0	0.85	4.9	0.09	4.0	0.11

Table 2. Stability constants K_A of calixarene **1**–**6** complexes with uracil derivatives

	K_A (RSD, %)					
Uracil guest	**1**[a]	**2**	**3**	**4**	**5**	**6**
6am1,3mU	6200 (5)	7750 (15)	3400 (9)	4700 (14)	5640 (13)	12300 (14)
6mU	8860 (3)	5950 (9)	5100 (13)	7750 (5)	6250 (17)	14450 (13)
U	8900 (3)	4900 (19)	6800 (8)	6900 (19)	6500 (3)	12300 (16)
5ClU	12000 (5)	5400 (14)	3500 (6)	1200 (14)	9200 (13)	11100 (13)
$5NO_2U$	54300 (6)	22200 (15)	5150 (9)	4100 (11)	41700 (9)	41400 (11)

[a] Data from Ref. 13.

The relationship between the K_A of the complexes and structure of the calixarene host and uracil guest molecules is complicated. In order to understand the nature of the complexation, MD analysis of the interaction was performed.

Molecular modelling of the host–guest complexation

The calixarenes investigated can be separated into two families, **1**–**4** and **5** and **6**, depending on the conformational behaviour of their macrocyclic skeletons. Tetrapropoxycalixarenes **1**–**4** exist in a stereochemically flexible flattened cone conformation, which rapidly changes the quasi-vertical and quasi-planar orientation of the benzene rings in solution at room temperature.[17] The cone conformation is an intermediate in the exchange.

Tetrapropoxycalixarenes **1**–**4** exist in a stereochemicaly flexible flattened cone conformation. A degenerate process flattened cone–flattened cone transformation is realized for the upper rim unsubstituted or tetraphosphorylated compounds **1** and **4**. For monophosphorylcalixarene **2** (C_s symmetry) or diphosphorylcalixarene **3** (C_{2v} symmetry), sterically less restricted conformations with quasi-planar phosphorylated benzene rings are preferable according to the MD analysis. In contrast to stereochemically flexible tetrapropoxycalixarenes **1**–**4**, the flattened cone conformation of dipropoxycalixarenes **5** and **6** is rigidified by bifurcated intramolecular hydrogen bonds of OH groups with proximal oxygen atoms at the lower rim.[18] The stereochemical rigidity of calixarenes **5** and **6** in the flattened cone conformation is confirmed by the decrease in the $\Delta\delta$ parameter of the axial and equatorial protons of the methylene links to 0.78–0.82 ppm compared with 1.22–1.32 ppm in flexible tetrapropoxycalixarenes **1**–**4**.[14]

MD trajectory analysis of the complexation demonstrates the flattened cone conformation for all calixarenes and two types of guest binding topologies, depending on the nature of the guest (Plate 1). All N1-unsubstituted

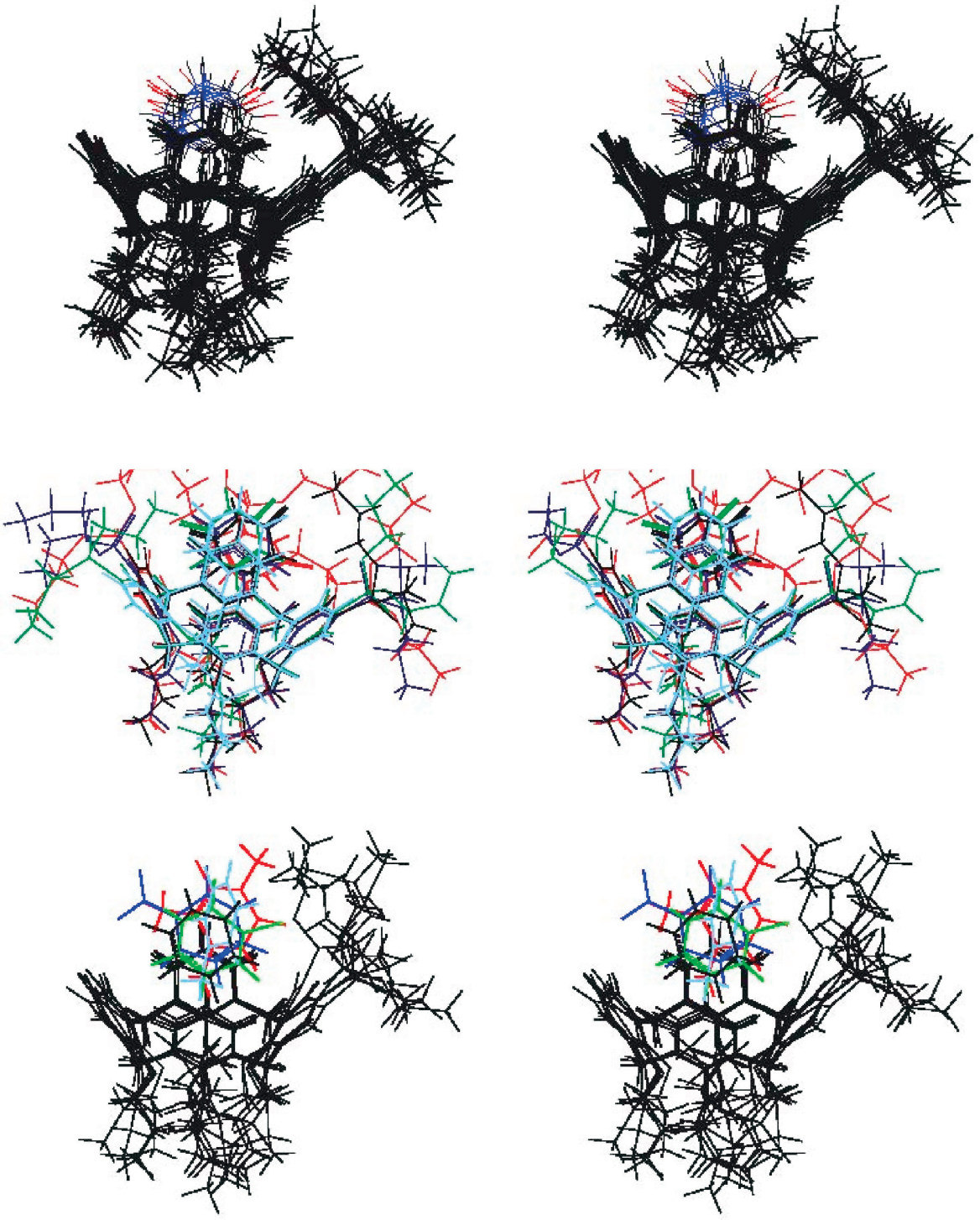

Plate 1. Stereoview of the host–guest complexes. Top: evolution of the complexes of calixarene **3** with uracil. Uracil oxygen atoms are marked red, and nitrogen blue, respectively. Middle: representation of complexes of uracil with calixarenes **2**–**6**: **2** (green), **3** (black), **4** (red), **5** (light blue) and **6** (blue). Bottom: representation of complexes of calixarene **3** with uracils U (black), $5NO_2U$ (blue), 5ClU (green), 6mU (light blue), 6am1,3mU (red) superimposed using the calixarene CH_2 bridging group

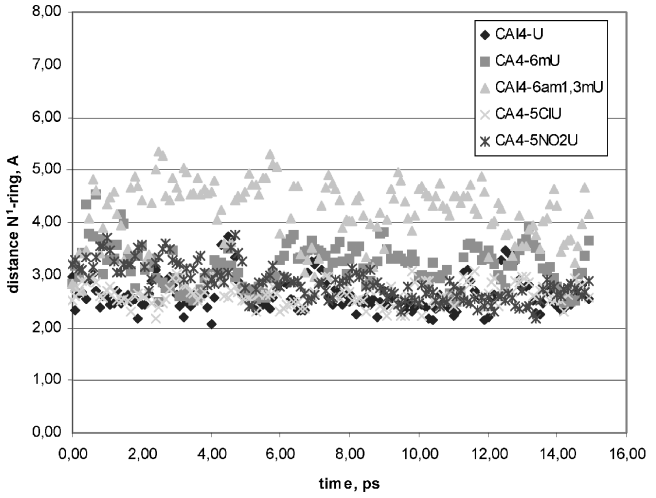

Figure 1. Distances between uracil N1 atom and calixarene **4** benzene ring

uracil derivatives (6mU, 5ClU, $5NO_2U$) exhibit a common pattern of host–guest interaction (Fig. 1, bottom). The heterocycle ring is partially placed between two nearly parallel benzene rings of the calixarene skeleton, permitting a number of van der Waals contacts and stacking interactions. The hydrophilic part of the guest molecule [C2(O)N3C4(O) fragment] is oriented outside the rim, allowing hydrogen bonding with the solvent molecules. The N1-H nitrogen, placed close to the centre of the third ('horizontal') benzene ring, exhibits H-bonding to the π-electron orbital where the distance between the nitrogen and the centre of the benzene ring is in the range 2.5–3.5 Å.[19]

Figure 1 shows how the distance between the uracil nitrogen atom and the centre of the benzene ring of the calixarene changes on complexation. In the complexes, the C5–C6 side of the uracil molecule is protected from the solvent by interaction with three other rings. Hence both stacking and hydrophobic interactions stabilize the complex, whereas the N1-H group is involved in additional H-bonding to the benzene π-electron orbital. Taking into account the N–H$\cdots\pi$ interactions, it is clear that the strong decreasing K_A for the complexes of tetraphosphorylcalixarene **4** with 5-chloro- or 5-nitrouracil molecules possessing highly acidic NH protons is caused by the electron-accepting phosphoryl groups lowering the π-donor ability of the calixarene benzene rings.

N1-methylation (6am1,3mU) disabled H-bonding as well as N1–CH_3 steric repulsions decrease the rings' interaction upon complexation. The putative NH–π interaction of 6-amino group is characteristic for all 6am1,3mU complexes. Perhaps in the case of calixarene **2** the interactions stabilize the complex ($K_A = 7750\ M^{-1}$), causing binding constants that are relatively high in comparison to the uracil complexes ($K_A = 4900\ M^{-1}$).

Analysis of the MD trajectories demonstrates relatively small changes of the complex organization during the simulation (Plate 1, top). The complexes organization of C5-substituted uracil derivatives (5ClU, $5NO_2U$) is similar, with only small differences arising from steric interactions of the substituents with the calixarene benzene rings. The C6 substitution (6am1,3mU or 6mU) causes relatively larger deviations of the complex structure, because of the C6 substituents localized deeper inside the pocket (Plate 1, middle).

Detailed analysis demonstrates that the organization of calixarene macrocyclic skeleton remains unchanged in all complexes, whereas the orientation of the guest bound is dependent on both the position of substitution and the nature of the substituent (Plate 1, bottom). The smallest changes are observed for uracil and its 5-substituted derivatives.

Binding constant analysis

Semiquantitative structure-related binding analysis is based on the concept of atomic solvatation parameterization (ASP), which assumes that the solute–solvent interactions are proportional to the weight by atom type solvent-exposed solute surfaces. Thus, in the first order of approximation, the binding constant K_A is expected to be a function in the form

$$\ln K_A = -\Delta\Delta G/RT = a \times \Delta S_{pol} + b \times \Delta S_{npol} + c - d \times \Delta n_{HD}$$

where $\Delta\Delta G$ is the change in ASP-derived ΔG upon complexation, ΔS_{pol} and ΔS_{npol} are net changes of polar and apolar molecular surface upon complexation (including both host and guest), Δn_{HD} is the number of the guest H-bond donors placed inside the calixarene cavity upon complexation, a and b are coefficients scaling the free energy partition of water interaction with polar and apolar atoms, respectively, c describes the effect of other interactions common for the whole series of guests and d is the penalty for the transfer of the single H-bond donor from the solvent to calixarene cavity (Tables 3–5).

Table 3. Structural data obtained from MD analysis of uracil derivatives[a]

Uracil	SH_{exc}	SN	SO	S_{tot}	S_{pol}	S_{apol}
U	11.98	9.01	25.90	84.94	46.89	38.05
6mU	11.35	8.50	25.94	97.13	45.79	51.34
6am1,3mU	11.22	12.41	21.87	118.28	45.51	72.78
5ClU	12.33	8.96	25.07	92.15	42.37	49.78
$5NO_2U$	12.19	12.37	49.91	100.10	74.47	25.63

[a] SH_{exc}, solvent-exposed surface of the exchangeable hydrogens; SN, SO, molecular surface of polar (hydrogen, nitrogen and oxygen) atoms of the molecule skeleton (Å^2); S_{tot}, total molecular surface of the molecule (Å^2); S_{pol}, S_{apol}, total polar and apolar surface, respectively (Å^2); $S_{pol} = SH_{exc} + SN + SO$; $S_{apol} = S_{tot} - S_{pol}$.

Table 4. Structural data obtained from molecular dynamics analysis of calixarenes **1**–**6**[a]

Calixarene	SH_{exc}	SN	SO	S_{tot}	S_{pol}	S_{apol}
1	0.00	0.00	0.06	346.25	0.06	346.19
2	0.00	0.00	38.59	541.90	38.59	503.31
3	0.00	0.00	18.55	441.40	18.55	422.85
4	0.00	0.00	66.50	592.63	66.50	526.13
5	0.31	0.00	1.75	322.00	2.05	319.94
6	0.48	0.00	40.85	503.47	41.33	462.14

[a] Symbols as in Table 3.

We achieved a good correlation between the experimental data obtained by HPLC analysis and calculated data using molecular modeling for complexes of particular calixarenes and derivatives of uracil (Fig. 2). Unfortunately, no common model for all calixarene series was obtained. In fact, there is no correlation of binding constants for uracil complexed with different calixarene molecules. The latter simply indicates the importance of the electrostatic partition to the binding propensity of the calixarene molecules. Quantum mechanical calculations proved that the electron density distribution is significantly modified by upper rim substituents.

In order to overcome the electrostatic problem, we decided to analyse the substituent effect on binding propensity. Thus, for each calixarene the ASP-derived free energy partition was related to the level obtained for the uracil complex. The final relation was postulated in the form

$$\ln K_A/\ln K_{uracil} = a \times \Delta\Delta S_{pol} + b \times \Delta\Delta S_{npol} - d \times \Delta\Delta n_{HD}$$

where $\Delta\Delta$ means the difference between the appropriate parameter change upon complexation obtained for uracil and a given uracil derivative. The results obtained are equally correct for all calixarenes and uracil derivatives excluding nitro derivatives, for which strong electrostatic interactions between the NO_2 group and the calixarene skeleton exists. Moreover, for all calixerenes complexed with 6am1, 3mU, we did not obtain the stable complex in the postulated form in which strong electrostatic interactions involving 2O were found. Taking into account these deviations, we divided three series: the complex with the nitro derivatives of uracil, the 6-amino-1,3-dimethyl derivatives of uracil, and other derivatives of uracil. For this proposed series, the correlation coefficients are fairly good. Since the nitro and amino groups are rich in free electrons, they could involve other specific interactions with calixarene rings. This is the interpretation and explanation of the other behaviours of these two series of guest compounds compared with the derivatives of uracil with a methyl group (Fig. 3).

Finally, the presented results demonstrate that the proposed simplified model of host–guest interaction

Table 5. Structural data obtained from MD analysis of complexes of calixarene hosts **2**–**6** with uracil guests[a]

Host	guest	SH_{exc}	SN	SO	S_{tot}	S_{pol}	S_{apol}	ΔS_{pol}	ΔS_{apol}
2	U	7.55	5.15	58.58	545.15	71.29	473.86	14.19	67.50
3		6.32	4.09	36.78	434.28	47.19	387.09	18.25	73.81
4		0.11	3.20	60.63	528.94	57.54	471.40	55.85	92.78
5		6.23	4.33	24.77	337.96	35.33	302.63	13.61	55.35
6		6.59	3.18	57.00	487.35	66.77	420.57	21.44	79.61
2	6mU	2.51	3.47	46.59	545.81	52.57	493.25	31.82	61.41
3		9.31	5.48	30.93	446.32	45.72	400.60	18.62	73.60
4		0.80	2.75	64.37	533.89	62.42	471.47	49.87	106.01
5		7.28	5.91	24.29	345.68	37.47	308.21	10.37	63.07
6		3.75	4.14	53.91	525.71	61.80	463.20	25.41	64.19
2	6am1,3mU	4.93	7.19	44.87	541.08	56.98	484.09	27.11	92.00
3		0.17	3.51	28.43	458.66	32.11	426.55	31.94	69.08
4		0.00	3.02	57.20	537.15	57.22	479.92	54.78	118.99
5		0.41	3.86	22.16	360.48	26.42	334.05	21.14	58.66
6		4.97	5.90	51.42	527.16	62.28	464.88	24.55	70.04
2	5CIU	5.16	3.47	51.94	544.29	60.57	483.72	20.39	69.38
3		5.75	4.38	32.01	445.89	42.14	403.75	18.77	68.88
4		1.39	2.96	58.74	533.00	57.16	475.83	51.71	100.08
5		5.77	3.67	17.05	337.65	26.49	311.16	17.93	58.57
6		6.07	3.93	53.06	508.55	63.06	445.49	20.63	66.43
2	$5NO_2U$	5.70	5.23	59.13	536.68	70.06	466.62	42.99	62.32
3		3.62	4.57	48.75	442.89	56.93	385.95	36.08	62.53
4		1.47	4.95	66.47	530.52	63.00	467.52	77.97	84.25
5		6.09	6.67	39.50	343.23	52.25	290.98	24.27	54.59
6		6.19	6.31	61.44	496.74	73.93	422.81	41.87	64.96

[a] Symbols as in Table 3. $\Delta S_{pol} = S_{pol}guest + S_{pol}host - S_{pol}complex$; $\Delta S_{apol} = S_{apol}guest + S_{apol}host - S_{apol}complex$.

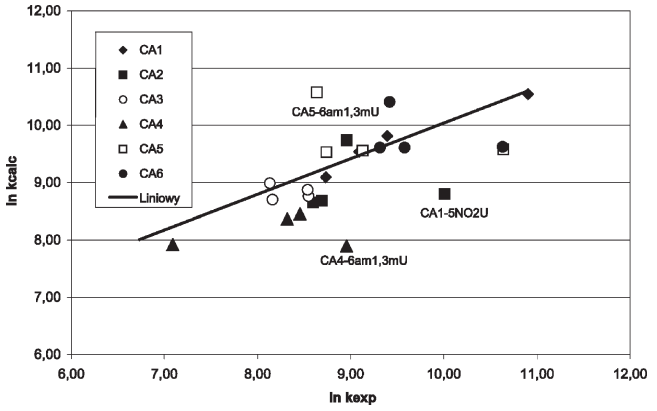

Figure 2. Correlation between calculated (calc) and experimental (exp) data for $\ln K_A$ for the calixarene–uracil complexes

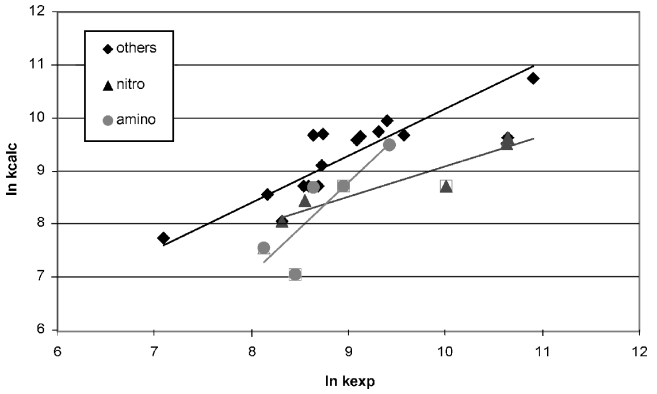

Figure 3. Correlation between calculated (calc) and experimental (exp) data for $\ln K_A$ for calixarenes **1**–**6** and uracil derivative complexes in terms of three series: nitro and amino derivatives of uracil and other uracils

satisfactorily describes experimentally measured binding constants.

CONCLUSION

Di- and tetrapropoxycalix[4]arenes functionalized at the upper rim with hydrophilic diisopropoxyphosphoryl groups are effective binders for biorelevant uracil derivatives in a water-containing medium. The phosphoryl groups decrease complexation with uracil derivatives in the case of tetrapropoxycalixarenes, but increase the complexation for dipropoxycalixarenes. Hydrophobic effects, NH–π and π–π interactions and van der Waals forces play important roles in the complexation process.

EXPERIMENTAL

Materials

Uracils were purchased from Sigma and were thoroughly purified by repeated crystallization and then carefully dried for several days before use.

Tetrapropoxycalix[4]arene (**1**) and dipropoxycalix[4]-arene (**5**) were synthesized by the method described previously.[18,20] The upper rim phosphorylated calix[4]-arenes **2**–**4** and **6** were synthesized by the nickel-catalysed Arbuzov reaction of the appropriate mono-, di- and tetrabromo derivatives of tetrapropoxycalix[4]arenes or dibromodipropoxycalix[4]arene with triisopropylphosphite using the previously reported procedure.[21]

General procedure for the preparation of calix[4]arenes phosphonates 2–4 and 6

A solution of mono-, di- and tetrabromotetrapropoxycalix[4]arenes (0.01 mmol) in triisopropyl phosphite (5 ml) in the presence of $NiBr_2$ (0.002 mmol for each bromine atom of calixarene) was refluxed for 1 h. The reaction mixture was evaporated under vacuum (0.05 mm, 100 °C) to give an oil. The oil was dissolved in methylene chloride and washed first with NH_4OH solution and then with water. The organic phase was dried over Na_2SO_4. The solvent was evaporated and the resulting compound was purified by crystallization (**3**, **4** and **6**) or column chromatography (**2**).

*5-Diisopropoxyphosphonyl-25,26,27,28-tetrapropoxycalix[4]arene (**2**).* Purification by column chromatography [CH_2Cl_2–acetone (5:1)], R_f 0.4. White solid: yield 60%; m.p. 127–129 °C; ^{1}H NMR ($CDCl_3$), δ 0.98, 1.05 (two t, 6H + 6H, *J* 7.5 Hz, C*H*$_3$CH$_2$CH$_2$O), 1.19, 1.36 [two d, 6H + 6H, *J* 7.5 Hz, diastereotopic (C*H*$_3$)$_2$CHO], 1.95 (m, 8H, J 7.5 Hz, CH$_3$C*H*$_2$CH$_2$O), 3.15, 3.2 (two d, 2H + 2H, *J* 13.5 Hz, ArCH$_{2eq}$), 3.80 (t, 4H, *J* 7.5 Hz, CH$_3$CH$_2$C*H*$_2$O), 3.88, 3.93 (two t, 2H + 2H, *J* 7.5 Hz, CH$_3$CH$_3$C*H*$_2$O), 4.42, 4.46 (two d, 2H + 2H, *J* 13.5 Hz, ArCH$_{2ax}$), 4.58 (m, 2H, CH$_3$C*H*), 6.44 (m, 6H, Ar*H*-*m* + Ar*H*-*p*), 6.68 (t, 1H, *J* 7.0 Hz, Ar*H*-*m*), 7.28 (d, 2H, J_{PH} 13 Hz Ar*H*-*m*); ^{31}P NMR, δ 18.6; MS (CI), *m*/*z* 757 (M^+, 100%). M calculated 756.97. Anal. Calcd for $C_{46}H_{61}O_7P$: C, 72.00; H, 8.12; P, 4.09. Found: C, 72.32; H, 8.14; P, 3.88%.

*5,17-Bis(diisopropoxyphosphonyl)-25,26,27,28-tetrapropoxycalix[4]arene (**3**).* Purification by crystallization from hexane. Yellow solid: yield 60%; m.p. 159–162 °C; ^{1}H NMR ($CDCl_3$), δ 0.90 (t, 6H, *J* 7.0 Hz, C*H*$_3$CH$_2$CH$_2$O), 1.10 (t, 6H, *J* 7.0 Hz, C*H*$_3$CH$_2$CH$_2$O), 1.37 [two d, 12H + 12H, *J* 7.0 Hz, (C*H*$_3$)$_2$ CHO diastereotopic], 1.93 (m, 8H, CH$_3$C*H*$_2$CH$_2$O), 3.22 (d, 4H, *J* 13.0 Hz, ArCH$_{2eq}$), 3.69 [m, 4H, (CH$_3$)$_2$C*H*O], 4.14 (m, 8H, CH$_3$CH$_2$C*H*$_2$O), 4.46 (d, 4H, *J* 13.0 Hz, ArCH$_{2ax}$), 6.11 (d, 4H, *J* 7.2 Hz, Ar*H*-*m*), 6.23 (t, 2H, *J* 7.2 Hz, Ar*H*-*p*), 7.56 (t, 4H, J_{PH} 13.2 Hz, Ar*H*-*m*); ^{31}P NMR ($CDCl_3$), δ 20.1; MS (CI), *m*/*z* 865 (M^+, 100%). M calculated 865.01. Anal. Calcd for $C_{48}H_{66}O_{10}P_2$: P, 7.16. Found: P, 7.08%.

*5,11,17,23-Tetrakis(diisopropoxyphosphonyl)-25,26,27,28-tetrapropoxycalix[4]arene (**4**).* Purification by crystallization from hexane. White solid: yield 70%; m.p. 200–205 °C; ^{1}H NMR ($CDCl_3$), δ 0.99 (t,12H, J 7.5 Hz, $CH_3CH_2CH_2O$), 1.08, 1.27 [two d, 12H + 12H, J 7.5 Hz, diastereotopic $(CH_3)_2CHO$], 1.97 (m, 8H, $CH_3CH_2CH_2O$), 3.31 (d, 4H, J 13.0 Hz, $ArCH_{2eq}$), 3.93 (t, 8H, J 7.5 Hz, $CH_3CH_2CH_2O$), 4.48 (d, 4H, J 13.0 Hz, $ArCH_{2ax}$), 4.55 [m, 4H, $(CH_3)_2CHO$], 7.28 (d, 8H, J_{PH} 13 Hz, ArH-*m*); ^{31}P NMR, δ 17.3; MS (CI), *m/z* 1249 (M^+, 100%). M calculated 1247.88. Anal. Calcd for $C_{64}H_{100}O_{16}P_4$: C, 61.53; H, 8.07; P, 9.92. Found: C, 61.78; H, 8.08; P, 9.75%.

*5,17-Bis(diisopropoxyphosphonyl)-25,27-dipropoxycalix[4]arene (**6**).* Purification by crystallization from hexane. Yellow solid: yield 55%; m.p. 180–181 °C; ^{1}H NMR ($CDCl_3$), δ 1.23, 1.41 [two d, 6H + 6H, J 7.0 Hz, diastereotopic $(CH_3CH)_2O$], 1.37 (t, 6H, J 7.0 Hz, $CH_3CH_2CH_2O$), 2.09 (m, 4H, $CH_3CH_2CH_2O$), 3.50 (d, 4H, J 13.0 Hz, $ArCH_{2eq}$), 4.03 (t, 4H, J 7.0 Hz, $OCH_2CH_2CH_3$), 4.32 (d, 4H, J 13.0 Hz, $ArCH_{2ax}$), 4.64 [m, 4H, $(CH_3)_2CHO$], 6.83 (t, 2H, J 7.0 Hz, Ar*H-p*), 7.01 (d, 4H, J 7.0, Ar*H-m*), 7.57 (d, 4H, J_{PH} 13.2 Hz, Ar*H*), 9.00 (s, 2H, OH); ^{31}P NMR, δ 17.68; MS (CI), *m/z* 838 (M^+, 100%). M calculated 836.95. Anal. Calcd for $C_{46}H_{62}O_{10}P_2$: C, 66.01; H, 7.47; P, 7.40. Found: C, 65.86; H, 7.32; P, 7.21%.

RP-HPLC analysis

The LC system consisted of an HPP 4001 *high-pressure pump* (Laboratorni Pristroje, Prague, Czech Republic) connected to a Rheodyne (Berkeley, CA, USA) 7120 sample injector with a 0.5 µl loop and an LCD 2563 ultraviolet–visible detector (Laboratorni Pristroje) operated at 254 nm. The column (150 × 3.3 mm i.d.) was packed with Separon SGX NH_2 (5 µm) (Lachema, Brno, Czech Republic).

The mobile phase consisted of methanol–acetonitrile–tetrahydrofuran–water (15:10:5:70, v/v) containing calixarene additives at concentrations of 4×10^{-4}–5×10^{-4} M. Samples of the guest solutions for injections were prepared so as to give a concentration of 10^{-5} M using a solvent identical with the mobile phase. The amount of sample injected was 0.5 µl. Each of the samples was analysed three times. All chromatograms were obtained at 31 °C. The flow-rate was 0.6 ml min^{-1}.

Molecular modelling

All structural calculations were carried on using Builder, Biopolymer, Dmol, Discover 3, docking and analysis modules from the InsightII package (MSI/Accelerys, San Diego, CA, USA) using the cvff force-field (consistent valence force field).[22] Initial conformations of all investigated calixarene molecules were built *de novo* in the cone conformation. Uracil derivatives were constructed by substituent addition to the uracil skeleton adapted from uridine coordinates. The atomic partial charges were taken from ESP charge distribution calculated on the background of density functional theory[23] using DMol with the BLYP functional.[24] In the case of **4**, the number of atoms exceeded the DMol boundaries (>166), so the molecule was divided into two symmetrical parts and then the charges were calculated.

All calixarene molecules were relaxed upon 1 ns *in vacuo* molecular dynamics. Electrostatic repulsions between phosphoryl substituents drive the calixarene conformation to maximize the separation of the groups. Thus the molecules with at least two phosphoryl groups exhibit a conformation in which the substituted benzene rings are in the 'horizontal' positions (Fig. 1).

According to our previous investigation and the known hydrophobic/hydrophilic properties of the uracil skeleton,[25] the complexes were built in the form protecting the apolar side from solvent accessibility. The complexes of uracil guest with the calixarene host (flattened cone) were solvated in 25 Å cubic box water TIPS3 molecules. The organization of the solvating water was tuned by 1000 steps of energy minimization followed by 15 ps MD using periodic boundary conditions (PBC) in the NPT ensemble ($T = 300$ K, $p = 0.1$ GPa) with constrained calixarene and uracil molecules. The optimization protocol was repeated with the complexed molecules released. Finally, 30 ps molecular dynamics in the NPT ensemble ($T = 300$ K, $p = 0.1$ GPa) were performed. The 1 ps snapshots of the final 20 ps of MD trajectory were analysed.

The initial conformations of the substituted uracil complexes were obtained based on the final result of the MD of suitable host–guest solvated complexes using perturbation methods as presented previously.

Solvent-exposed surfaces were calculated using GEPOL 12.1 software. Based on the MD trajectories, atomic partition to the net molecular solvent-exposed surface was analysed for the separate guest and host molecules and for the complex. For each complex, 20 structures taken from MD were analysed, giving the average change of a given type of parameter and its standard deviation.

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