JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2003; **16:** 246–252
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.595

Complexation of tetrapropoxycalix[4]arene with uracil and adenine derivatives in water-containing solution

Olga Kalchenko,[1] Jarek Poznański,[2] Agnieszka Marcinowicz,[2] Sergey Cherenok,[1] Andrew Solovyov,[1] Wojciech Zielenkiewicz[2] and Vitaly Kalchenko[1]*

[1]Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanskaya Str. 5, 02094 Kyiv-94, Ukraine
[2]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, Poland

Received 16 July 2002; revised 6 November 2002; accepted 18 November 2002

ABSTRACT: The host–guest complexation of tetrapropoxycalix[4]arene with uracil, 5-amino-, 5-methyl-, 5-ethyl-, 5-chloro-, 5-nitro-, 6-methyl-, 1,3-dimethyl-, 6-amino-1-methyl- and 6-amino-1,3-dimethyluracil, adenine and 9-methyladenine 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 guests with the calixarene host within the range 3250–54300 M^{-1} were determined from the capacity factor of the guest and concentration of the calixarene host in the mobile phase. Molecular dynamic (MD) simulation of the host–guest interaction was performed. Basing on the MD trajectories atomic partition to the net molecular solvent-exposed surface was analyzed for the separated guest and host molecules and for the complex. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: uracil; adenine; host–guest complexes; reversed-phase high-performance liquid chromatography; calixarenes; molecular dynamics

INTRODUCTION

Calixarenes,[1] bowl shaped macrocyclic compounds synthesized by condensation of *para*-substituted phenols with formaldehyde, owing to their capability to recognize cations or anions or neutral organic molecules, are widely used in different branches of chemistry, physics and materials science.[2] Calixarenes demonstrate a wide spectrum of bio-activity and in the last decade have become attractive objects for bio-medical investigations (for a review of bio-medical applications of calixarenes, see Ref. 3). Supramolecular interaction of calixarenes with biorelevant molecules or ions is the basis of their bio-medical properties.

It has been reported that calix[4,6]arenes fuctionalized with sulfonyl,[4] aminophosphonyl,[5] peptido[6] and cyclopeptido[7] groups, and also calix[4]resorcinarenes bearing aminoformyl groups[8] and homooxacalix[3]arenes,[9] bind amino acids,[4,5] dipeptides,[8] proteines,[7] choline and acetylcholine ($K_A = 5 \times 10^4$–8×10^4 M^{-1}).[10] Calix[4]resorcinarene derivatives[11] and calix[4]arene boronic acids[12] bind different carbohydrates. Complexation of nucleotides and even DNA by calix[4,6,8]arenes functionalized with methylammonium groups (K_A up to 7×10^4 M^{-1}) has recently been reported.[13] It has been documented that vitamins B_2 (riboflavin) and B_{12} (cyanocobalamin)[14] and also some nucleosides such as cytidine, uridine and thymidine[15] are transported by calix[4]resorcinarenes into organic solutions. Aminocalixarenes and their metallo complexes possessing significant nucleobase specificity have been examined as bio-catalysts to create artificial enzymes.[16–18]

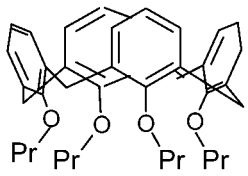
This paper presents results of the investigation of host–guest interactions of tetrapropoxycalix[4]arene (CA) with a series of uracil and adenine derivatives (Scheme 1) in a water-containing medium performed by reversed-phase high-performance liquid chromatographic (HPLC) and molecular dynamics (MD) methods.

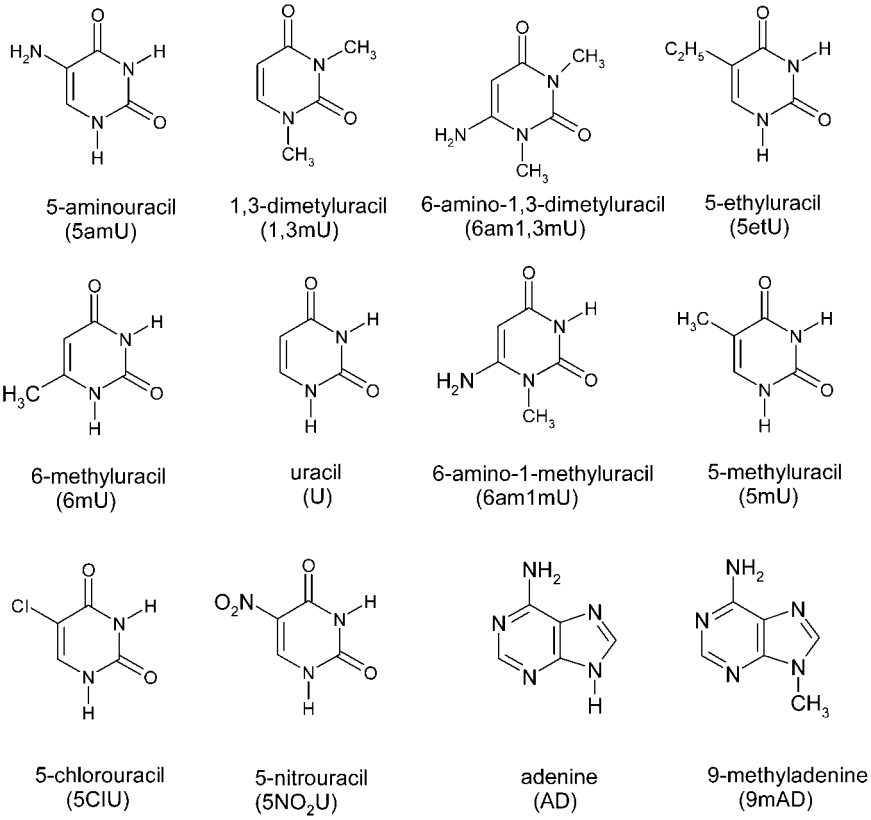
RESULTS AND DISCUSSION

Determination of stability constants

Bio-medical aspects of supramolecular host–guest interactions are usually investigated in an aqueous solution similar to those in biological processes. Unfortunately, the solubility of CA in water is too low to investigate the interaction with uracil and adenine derivatives by

**Correspondence to:* V. Kalchenko, Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanskaya Str. 5, 02094 Kyiv-94, Ukraine.
E-mail: vik@ukrpack.net
Contract/grant sponsor: Ministry of Education and Science of Ukraine; *Contract/grant number:* 03.07/00045.





Scheme 1

Table 1. 1/k' and stability constants, K_A, of the calixarene complexes with uracil and adenine derivatives

	1/k'				
	Calixarene concentration, $\times 10^{-4}$ M				
Guest	0 (control)	1.2	2.3	4.6	K_A (RSD,%)[a]
5-Aminouracil	0.691	0.968	1.212	1.686	3246 (3)
1,3-Dimethyluracil	1.248	1.935	2.697	4.673	5199 (11)
6-Amino-1,3-dimethyl-uracil	1.500	2.678	3.497	5.319	6174 (5)
5-Ethyluracil	1.337	2.715	3.869	6.410	8358 (2)
6-Methyluracil	1.390	2.817	4.311	6.329	8861 (3)
Uracil	1.670	3.502	4.968	7.813	8893 (3)
6-Amino-1-methyluracil	1.848	3.983	5.947	10.204	9698 (1)
5-Methyluracil	1.443	3.375	5.497	9.434	11804 (5)
5-Chlorouracil	1.020	2.534	3.645	6.211	11966 (5)
5-Nitrouracil	0.460	3.297	6.097	11.494	54309 (6)
Adenine	0.685	1.624	2.579	4.444	12256 (6)
9-Methyladenine	0.678	1.687	2.452	5.566	12569 (8)

[a] Relative standard deviation of the chromatographic measurements.

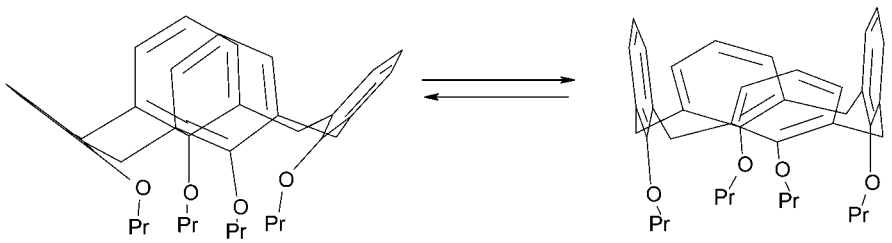


Figure 1. Flattened cone–flattened cone transformation of CA

physical methods. We have found the four-component system methanol–acetonitrile–tetrahydrofuran–water (15:10:5:70, v/v) to be the best water-containing medium for the host–guest examination.

NMR and microcalorimetry are most popular methods[19] for the determination of the stability constants of host–guest complexes. However, the solubility of CA in the above-mentioned solvent and also that of the uracil and adenine guests is still poor for the investigation of complexation by these two methods. For this reason, the much more sensitive reversed-phase HPLC method, which operates with low concentrations of the investigated compounds (10^{-4}–10^{-5} M), was used in this work.

The determination of stability constants by HPLC is based on the change in the chromatographic characteristics of the guest molecules produced, by calixarene additives (host molecules) in the mobile phase. A detailed procedure for these determination has been reported.[20]

Addition of CA to the water-containing mobile phase decreases the capacity factors, k', of uracil or adenine solutes (Table 1). The decrease confirms the formation of host–guest supramolecular complexes. The linear relationship between k' and calixarene concentration in the mobile phase (Plate 1) indicates 1:1 stoichiometry of calixarene and solute in the complexes.

In this case the stability constants of the complexes, K_A, can be calculated from the dependence of $1/k'$ values on the calixarene concentration [CA] in the mobile phase using the equation[20]

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

where k'_0 and k' are the capacity factors in the absence and presence of calixarene in the mobile phase, respectively.

The stability constants (3250–54300 M^{-1}) calculated by this method are given in Table 1. The stability constant values are strongly dependent on the structure of the guest molecules. Substituents at the uracil 5-position influence the K_A values as follows: amino and ethyl groups decrease but methyl, chloro and nitro groups increase the stability constants of the complexes compared with the unsubstituted uracil. The lowest stability constant is observed for 5-aminouracil and the highest for the 5-nitro derivative. 1-Methyl, 1-methyl-6-amino and 6-methyl substitutions lead to weakening of complexation, but 6-amino substitution resulted in an increase in the stability constants. To investigate the role of the substituents, molecular dynamic simulation of the host–guest complexation was performed.

Molecular modelling of the host–guest complexation

Tetrapropoxycalixarene exists in a stereochemically flexible flattened cone conformation (C_{2v} symmetry) which rapidly (on the NMR time-scale) changes the vertical and horizontal orientation of the benzene rings (Fig. 1) in solution at room temperature.[21] The free activation energy of the pseudo-rotation process determined by the variable NMR method is very low ($\Delta G‡ < 10$ kcal mol^{-1}) (1 kcal = 4.184 kJ). The regular cone

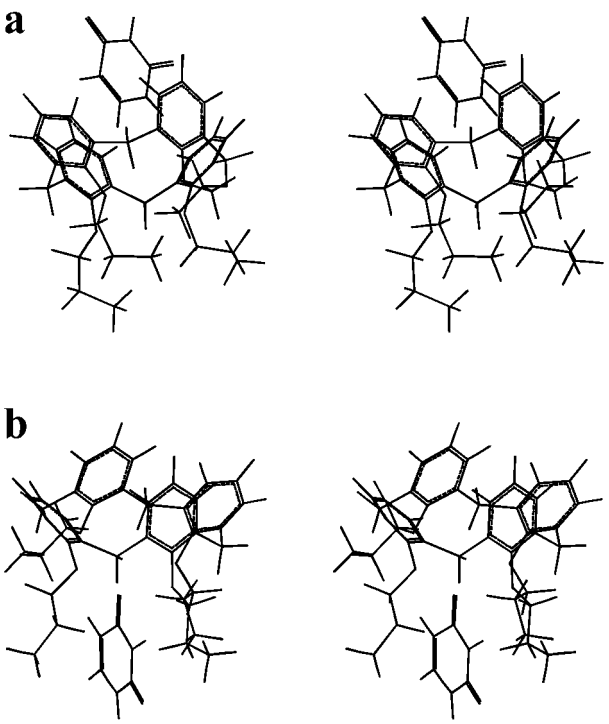


Figure 2. Stereoview of putative CA–uracil complexes. (a) Guest in the cavity formed by the benzene rings; (b) guest in the cavity formed by propoxy groups at the macrocyclic lower rim

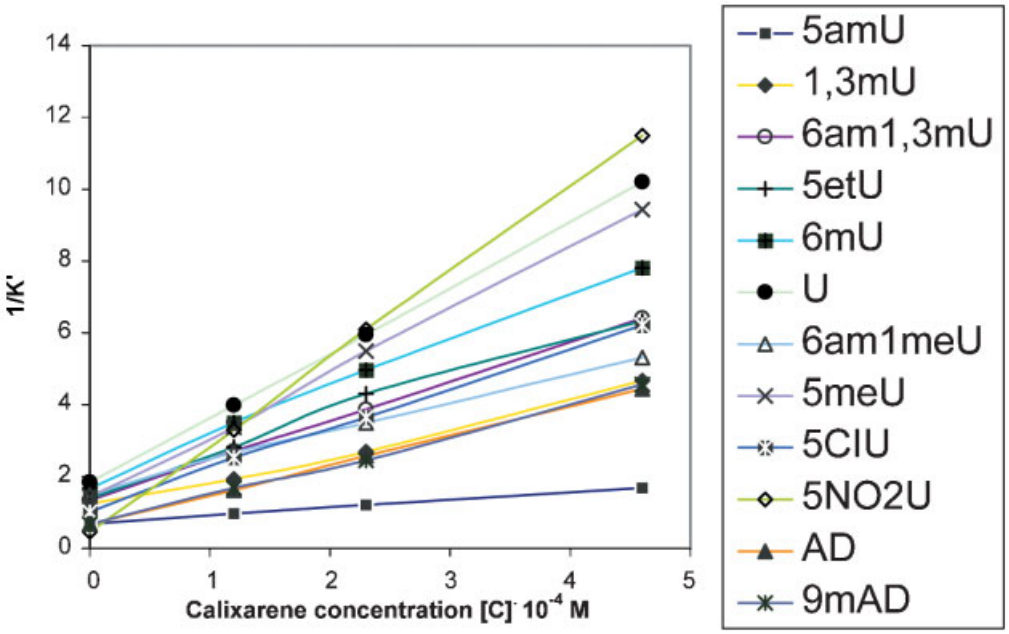


Plate 1. Plots of 1/*k*′ of guest vs CA concentration (results are summarized in Table 1)

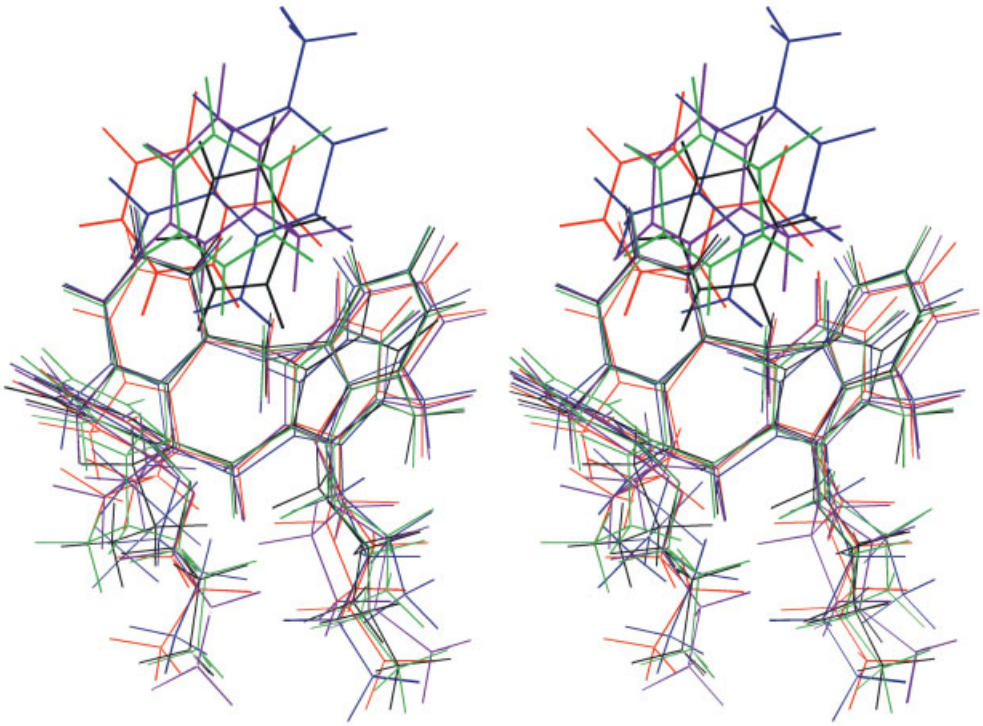


Plate 2. Stereoview of CA structures complexed with uracil (black), 5-nitrouracil (red), 5-chlorouracil (green), 6-methyluracil (purple) and 1,3-dimethyl-6-aminouracil (blue)

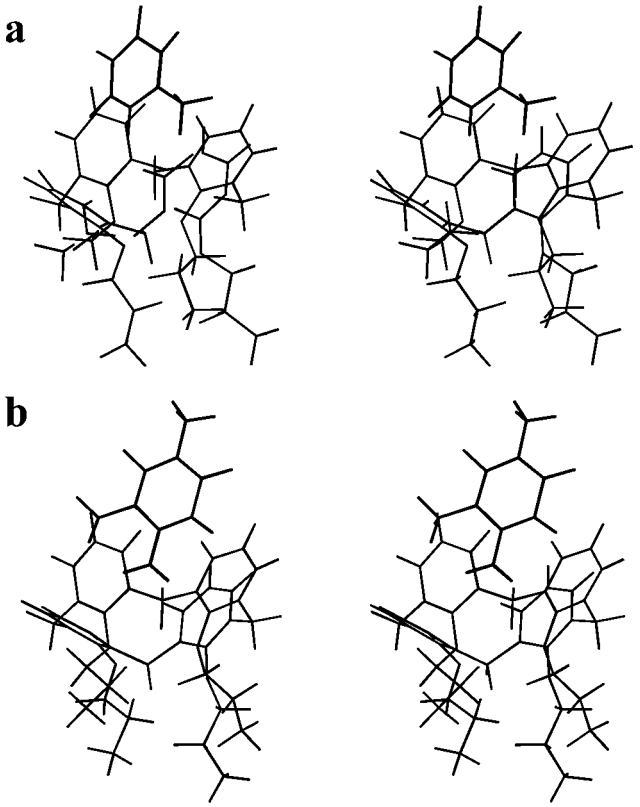


Figure 3. Stereoview of (a) CA–6-methyluracil and (b) CA–6-amino-1,3-dimethyluracil complexes

conformer (C_{4v} symmetry) is an intermediate in the process.

Preliminary analysis performed with the help of a docking module proved that the flattened cone conformation of the CA molecule shows a relatively high uracil binding ability owing to the macrocyclic cavity formed with the benzene rim [Fig. 2(a)] whereas the uracil guest included in the cavity formed by four propoxy groups at the macrocyclic lower rim induced strong deformation of the methylene link geometry in the macrocyclic skeleton [Fig. 2(b)] and in consequence upon simple minimization the inclusion complex is converted into a surface to surface complex. The MD analysis confirmed that only in the flattened cone conformation do effective host–guest interactions take place.

Guest-dependent structure of the complex. MD trajectory analysis for the flattened cone CA complexes demonstrated two main types of guest binding topologies, depending on the nature of the guest. All N1-unsubstituted uracil derivatives, despite C-5 and/or C-6 substitution, exhibit a common pattern of host–guest interactions [Fig. 3(a)]. The pyrimidine ring is partially placed between two distal benzene rings of CA, enabling stacking interactions to occur. The hydrophilic part of the molecule [C2(O)N3C4(O) fragment] is solvent exposed to be hydrogen bonded with water molecules. The N1–H fragment is placed close to the center of the third benzene ring (H···Ar distance 2.65 Å in the CA–U complex) exhibiting H-bonding to the π-electron orbital as discussed previously.[22] In contrast, N1-methylation disables H-bonding and also N1–CH_3 steric repulsions decrease the ring interaction upon complexation of 1,3-dimethyl-6-aminouracil [Fig. 3(b)]. The 5- and 6-amino derivatives exhibit an additional tentative H–π bond formed by the amino group hydrogen. Detailed analysis demonstrates that the organization of the CA macrocyclic skeleton remains almost unchanged in all complexes, whereas the position of the bound guest is dependent both on the position of substitution and on the nature of the substituent (Plate 2).

Structure-related binding constant analysis. In order to reduce the force-field dependence of the performed analysis, all the energetic terms derived directly from MD simulations were neglected. Structure-related binding analysis was based on the concept of atomic solvation parameters (ASP) assuming that the solute–solvent interaction free energy ΔG is parameterized by a weighted solvent-accessible surface area.[23] In the presented analysis the ASP parameterization was reduced to two atomic types: apolar and polar (oxygen, nitrogen, exchangeable hydrogen). An additional term describing the free energy change of the H-bond donor transfer from solvent to CA rim was introduced (see Ref. 24 for a review). Thus, in the first order of approximation, the binding constant K_A was assumed to be a structure-derived function of the form

$$\begin{aligned}\ln K_A &= -\Delta\Delta G/RT \\ &= a \times \Delta S_{pol} + b \times \Delta S_{apol} + c - d \times \Delta n_{HD}\end{aligned}$$

where $\Delta\Delta G$ is the change in ASP-derived ΔG upon complexation, ΔS_{pol} and ΔS_{apol} are the net changes of polar and apolar molecular surface upon complexation (including both host and guest), Δn_{HD} is the number of guest H-bond donors transferred inside the CA cavity upon complexation, a and b are optimized coefficients scaling the free energy partition of solvent interaction with polar and apolar atoms respectively, c is a scaling factor depending on the nature of the host molecule, the value of which is common for the whole series of guests, and d is the free energy partition of the transfer single H-bond donor from the solvent to CA cavity. The results obtained demonstrate that the proposed simplified model of host–guest interaction properly describes experimentally measured binding constants (Fig. 4). For all complexes a significant reduction (~10%) of the solvent exposed surface of the apolar atoms is observed (Table 2).

In conclusion, readily available tetrapropoxycalix[4]-arene existing in the stereochemically flexible flattened cone conformation is an effective binder for bio-relevant uracil and adenine derivatives in a water-containing

Table 2. Structural data obtained from molecular dynamics analysis of CA (host) complexed with uracil derivative (guest)[a]

State	Guest	SH_{exc}	SN	SO	S_{tot}	S_{pol}	S_{apol}
Host	U	0.0	0.0	0.0	534.1	0.0	534.1
Guest		12.1	9.3	25.4	106.3	46.8	59.6
Complex		5.6	3.5	23.0	554.6	32.1	522.5
Change		6.5	5.8	2.4	85.8	14.6	71.2
Host	13mU	0.0	0.0	0.1	532.0	0.1	531.8
Guest		0.0	4.5	21.0	144.5	25.6	119.0
Complex		0.0	3.4	19.5	581.1	22.9	558.2
Change		0.0	1.2	1.6	95.4	2.8	92.7
Host	5C1U	0.0	0.0	0.0	538.0	0.0	538.0
Guest		13.2	8.7	12.5	110.9	34.4	76.6
Complex		6.6	3.8	6.3	558.3	16.6	541.7
Change		6.6	5.0	6.2	90.7	17.8	72.9
Host	5C1U	0.0	0.0	0.0	531.1	0.0	531.1
Guest		12.4	8.8	24.7	127.0	46.0	81.0
Complex		5.9	3.6	20.1	554.9	29.6	525.3
Change		6.5	5.2	4.6	103.1	16.4	86.8
Host	5etU	0.0	0.0	0.4	524.6	0.4	524.3
Guest		12.1	8.8	24.6	145.2	45.4	99.8
Complex		5.6	3.9	21.4	559.9	30.9	529.0
Change		6.5	5.0	3.5	110.0	14.9	95.1
Host	5amU	0.0	0.0	0.4	527.8	0.4	527.4
Guest		24.5	17.1	24.7	119.6	66.2	53.4
Complex		11.7	9.9	19.9	548.5	41.5	507.0
Change		12.8	7.2	5.2	98.9	25.2	73.7
Host	$5NO_2U$	0.0	0.0	0.1	541.1	0.1	541.0
Guest		12.2	12.1	49.7	131.3	74.0	57.2
Complex		5.8	6.1	35.7	572.9	47.6	525.2
Change		6.4	6.0	14.0	99.5	26.5	73.0
Host	6mU	0.0	0.0	0.1	534.7	0.1	534.6
Guest		11.2	8.6	25.7	127.7	45.4	82.3
Complex		6.0	4.2	19.3	569.3	29.4	539.9
Change		5.2	4.4	6.6	93.1	16.1	77.0
Host	6amU	0.0	0.0	0.3	534.5	0.3	534.2
Guest		24.0	16.8	26.6	121.5	67.4	54.1
Complex		5.9	4.1	25.0	558.3	35.0	523.3
Change		18.1	12.7	1.9	97.6	32.7	65.0
Host	6am1mU	0.0	0.0	0.0	539.9	0.0	539.9
Guest		16.9	14.1	24.6	138.6	55.5	83.1
Complex		5.1	5.6	24.4	581.3	35.1	546.2
Change		11.8	8.5	0.2	97.3	20.5	76.8
Host	6am13mU	0.0	0.0	0.1	530.3	0.1	530.2
Guest		11.1	12.5	20.6	155.5	44.2	111.4
Complex		0.0	3.5	19.6	591.7	23.0	568.6
Change		11.1	9.0	1.1	94.2	21.2	73.0

[a] All surface values are in $Å^2$. SH_{exc} is the solvent-exposed surface of the exchangeable hydrogens; $S_{pol} = SH_{exc} + SN + SO$, $S_{apol} = S_{tot} - S_{pol}$.

Table 3. Analysis of the guest binding affinities in the terms of structural properties of the complexes

Guest	Δn_{HB}	K_{exp}	RSD (%)	K_{calc}	$\mathrm{Ln}K_{exp}$	$\mathrm{Ln}K_{calc}$	χ^2
U	1	8893	3	8157	9.09	9.01	1.0
13mU	0	5199	11	5237	8.56	8.56	3.0
5C1U	1	11966	5	13215	9.39	9.49	0.5
5mU	1	11804	5	11585	9.38	9.36	0.1
5etU	1	8358	2	9785	9.03	9.19	2.7
5amU	3	3246	3	3485	8.08	8.16	0.9
5no2U	1	54309	6	49236	10.90	10.80	3.7
6mU	1	8861	3	10534	9.09	9.26	9.3
6am1mU	2	9698	1	5964	9.18	8.69	31.5
6am13mU	2	6174	5	6532	8.73	8.78	0.5

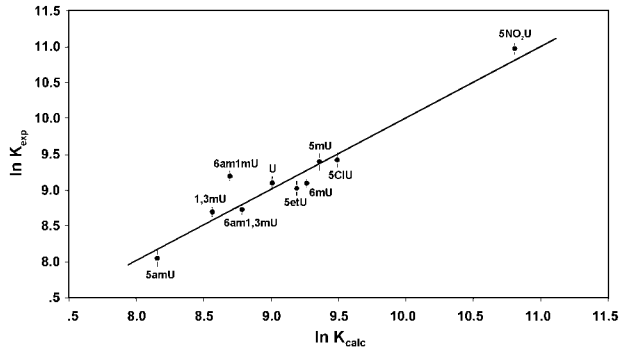


Figure 4. Structure-derived prediction of binding uracil derivatives by calixarene (results are summarized in Table 3)

medium. The inclusion of the guest in the calixarene cavity stabilizes the macrocyclic skeleton in the flattened cone conformation with C_{2v} symmetry. Hydrophobic effects and N–H–π interactions play an important role in the complexation process. Reversed-phase HPLC is a useful tool for the investigation of binding constants of poorly soluble host and guest molecules in a water-containing medium.

EXPERIMENTAL

Uracil, 5-ethyluracil, 5-chlorouracil, 6-methyluracil, 6-amino-1,3-dimethyluracil and adenine were purchased from Sigma and 6-amino-1-methyluracil from Fluka. The remaining guest molecules were kindly supplied by Professor Dr M. Draminski of the Institute of Basic Sciences, Military School of Medicine, Łódź, Poland. The compounds were throughly purified by repeated crystallization and the repeated vacuum sublimation and then carefully dried for several days before use. Tetrapropoxycalix[4]arene was synthesized by the method described previously.[25]

RP-HPLC analysis

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

The methanol–acetonitrile–tetrahydrofuran–water (15:10:5:70, v/v) mobile phase containing CA additives at concentrations of 4×10^{-4}–5×10^{-4} M was used. 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 analyzed three times. All chromatograms were obtained at 31° C. The flow-rate was 0.6 ml min^{-1}. The UV–visible detector was operated at 254 nm.

Molecular modelling

All structural calculations were carried with Builder, Biopolymer, Discover 3, DMol, Docking and Analysis modules of the InsightII (MSI) package using the cvff force-field.[26] The initial conformation of the CA molecule was built *de novo* in a cone-like conformation. Coordinates of uracil were taken from uridine. Uracil derivatives were constructed by substituent addition to the uracil skeleton. The atomic partial charges were adapted from the ESP charge distribution calculated on the basis of density functional theory[27] using DMol version 960 with the DNP basis set and BLYP functional.[28] In the case of CA, the charge distribution was adapted from data calculated for the smaller model 2,6-dimethylpropoxybenzene compound.

The constructed CA structure, relaxed upon 1 ns *in vacuo* molecular dynamics with distance-dependent permeability ε set to $4.5r$, were used as the targets for docking of the uracil molecule. According to known hydrophobic/hydrophilic properties of the uracil skeleton,[29] the complex was built in a form protecting the C5–C6 uracil side from the solvent accessibility. Two structural types of complex (Fig. 2) were built and tuned by 1 ns *in vacuo* MD followed by 10 ps MD with explicit water molecules (periodic boundary conditions, 25 Å cubic box) in the NPT ensemble ($T = 300$ K, $p = 0.1$ GPa).

Two mentioned CA–uracil complexes were additionally analyzed using the SYBYL 6.7.1 package (Tripos, St. Louis, MO, USA) with either TRIPOS[30] or Amber 4.0[31] force-field by 15 ns MD in a 25 Å cubic water box. For all force-fields used in calculations the general structure of the complex remained unchanged.

The initial conformations of the substituted uracil complexes were obtained from the water-solvated flattened cone CA–uracil complex using the perturbation procedure.[32] Finally, 15 s MD simulations were performed for uracil and its nine derivative complexes. The last 10 ps of each trajectory were analyzed in 1 ps frames.

Solvent-exposed surfaces were calculated using GEPOL 12.1 software.[33] Based on the MD trajectories, atomic partition to the net molecular solvent-exposed surface was analyzed for the separated guest and host molecules and also for the complex.

Acknowledgements

This work was performed in accordance with the scientific collaboration between the Institute of Organic

Chemistry, National Academy of Sciences of Ukraine and the Institute Physical Chemistry Polish Academy of Sciences. The authors of the Kiev team thank the Ministry of Education and Science of Ukraine for support of this work through grant 03.07/00045. All molecular simulations were carried on using computer resources of the Interdisciplinary Center for Mathematical and Computational Modelling, Warsaw University, Poland (grant G23-5).

REFERENCES

1. (a) Böhmer V. *Angew. Chem., Int. Ed. Engl.* 1995; **34**: 713–745; (b) Gutsche CD. *Calixarenes Revisited*. Royal Society of Chemistry: Cambridge, 1998.
2. (a) Diamond D, McKervey MA. *Chem. Soc. Rev.* 1996; 15–24; (b) Lumetta GJ, Rogers RD, Gopalan AS (eds). *Calixarenes for Separations*. American Chemical Society: Washington, DC, 2000; (c) Mandolini L, Ungaro R (eds). *Calixarenes in Action*. Imperial College Press: Singapore, 2000; (d) Asfari Z, Boehmer V, Harowfield J, Vicens J (eds). *Calixarenes 2001*. Kluwer: Dordrecht, 2001.
3. Sansone F, Segura M, Ungaro R. In *Calixarenes 2001*, Asfari Z, Boehmer V, Harowfield J, Vicens J (eds). Kluwer: Dordrecht, 2001; 496–512.
4. Selkti M, Coleman AW, Nicolis I, Douteau-Guevel N, Villian F, Tomas A, De Rango C. *Chem. Commun.* 2000; 161–162.
5. Antipin IS, Stoikov II, Pinkhassik EM, Fitseva NA, Stibor I, Konovalov AI. *Tetrahedron Lett.* 1997; **38**: 5865–5868.
6. Sansone F, Barboso S, Casnati A, Sciotto D, Ungaro R. *Tetrahedron Lett.* 1999; **40**: 4741–4744.
7. (a) Hamuro Y, Calama MC, Park HS, Hamilton AD. *Angew. Chem., Int. Ed. Engl.* 1997; **36**: 2680–2683; (b) Park HS, Lin Q, Hamilton AD. *J. Am. Chem. Soc.* 1999; **121**: 8–13.
8. Zielenkiewicz W, Pietraszkiewicz O, Wszeliaka-Rylik M, Pietraszkiewicz M, Roux-Desgrandes G, Roux AH, Groiler J-PE. *J. Solution Chem.* 1998; **27**: 121–134.
9. (a) Okada Y, Kasai Y, Nishimura J. *Tetrahedron Lett.* 1995; **36**: 555–558; (b) Araki K, Inada K, Shinkai S. *Angew. Chem., Int. Ed. Engl.* 1996; **35**: 72–74.
10. Lehn J-M, Meric R, Vigneron J-P, Cesario M, Guilhem J, Pascard C, Asfari Z, Vicens J. *Supramol. Chem.* 1995; **5**: 97–103.
11. Fujimoto K, Miyata T, Aoyama Y. *J. Am. Chem. Soc.* 2000; **122**: 3558–3559.
12. Ohseto F, Yamamoto H, Yatsumoto H, Shinkai S, *Tetrahedron Lett.* 1995; **36**: 6911–6912.
13. Shi Y, Schneider H-J. *J. Chem. Soc., Perkin Trans. 2* 1999; 1797–1803.
14. (a) Aoyama Y, Tanaka T, Toi H, Ogoshi H. *J. Am. Chem. Soc.* 1988; **110**: 634–635; (b) Kurihara K, Ohto K, Tanaka T, Aoyama Y, Kunitake T. *J. Am. Chem. Soc.* 1991; **113**: 444–450.
15. Kobayashi K, Asakawa Y, Kato Y, Aoyama Y. *J. Am. Chem. Soc.* 1992; **114**: 10307–10313.
16. (a) Wilcox CS, Hamilton AD (eds). *Molecular Design and Bioorganic Catalysis*. NATO ASI Series. Kluwer: Dordrecht, 1996; 478; (b) Schneider U, Schneider H-J. *Chem. Ber.* 1994; **127**: 2455–2469.
17. (a) Wilcox CS. *Chem. Rev.* 1996; **96**: 2435–2458; (b) Molenveld P, Kapsabelis S, Engbersen JFJ, Reinhoudt DN. *J. Am. Chem. Soc.* 1997; **119**: 2948–2949; (c) Cuevas F, Di Stefano S, Magrans JO, Prados P, Mandolini L, De Mendoza J. *Chem. Eur. J.* 2000; **6**: 2338–3234.
18. Molenveld P, Engbersen JFJ, Reinhoudt DN. *Angew. Chem., Int. Ed. Engl.* 1999; **38**: 3189–3191.
19. Wang T, Bradshaw JS, Izatt RM. *J. Heterocycl. Chem.* 1994; **31**: 1097–1114.
20. (a) Kalchenko OI, Lipkowski J, Kalchenko VI, Vysotsky MA, Markovsky LN. *J. Chromatogr. Sci.* 1998; **36**: 269–273; (b) Lipkowski J, Kalchenko OI, Slowikowska J, Kalchenko VI, Lukin OV, Markovsky LN, Nowakowski R. *J. Phys. Org. Chem.* 1998; **11**: 426–435; (c) Kalchenko OI, Solovyov AV, Lipkowski J, Kalchenko VI. *J. Inclusion Phenom. Macrocycl. Chem.* 1999; **34**: 259–266; (d) Kalchenko OI, Solovyov AV, Lipkowski J, Kalchenko VI. *J. Chem. Res. (S)*. 1999; 60–62; (e) Kalchenko OI, Perret F, Morel Desroisiers N, Coleman AW. *J. Chem. Soc., Perkin Trans. 2* 2001; 258–263.
21. (a) Ikeda A, Tsuzuki H, Shinkai S. *J. Chem. Soc., Perkin Trans. 2* 1994; 2073–2080; (b) Arduini A, Fabbi M, Mantovani M, Mirone L, Pochini A, Secci A, Ungaro R. *J. Org. Chem.* 1995; **60**: 1454–1457; (c) Arduini A, McGregor W, Paganuzzi D, Pochini A, Secci A, Ugozzoli F, Ungaro R. *J. Chem. Soc., Perkin Trans. 2* 1996; 839–846.
22. Cheney J, Cheney BV, Richards G. *Biochim. Biophys. Acta.* 1987; **954**: 137–139.
23. (a) Juffer AH, Eisenhaber F, Hubbard SJ, Walther D, Argos P. *Protein Sci.* 1995; **4**: 2499–2509; (b) Wang JM, Wang W, Huo SH, Lee M, Kollman, PA. *J. Phys. Chem. B* 2001; **105**: 5055–5067.
24. Vajda S, Sippl M, Novotny J. *Curr. Opin. Struct. Biol.* 1997; **7**: 222–228.
25. Arduini A, Casnati A. In *Macrocycle Synthesis. A Practical Approach*, Parker D (ed.) Oxford University Press: Oxford, 1996; 159.
26. Maple JR, Dinur V, Hagler A. *Proc. Natl. Acad. Sci. USA* 1988; **85**: 5350–5354.
27. (a) Hohenberg P, Kohn W. *Phys. Rev. B* 1964; **136**: 864–871; (b) Kohn W. *Phys. Rev. A* 1965; **140**: 1133–1138.
28. Becke AD. *J. Chem. Phys.* 1993; **98**: 5648–5652.
29. (a) Zielenkiewicz W, Poznański J. *J. Solution Chem.* 1997; **27**: 543–549; (b) Zielenkiewicz W. *Pure Appl. Chem.* 1999; **71**: 1285–1290.
30. Clark M, Cramer RD III, Van Opdenbosch N. *J. Comput. Chem.* 1989; **10**: 982–1012.
31. Weiner SJ, Kollman PA, Nguyen DT, Case DA. *J. Comput. Chem.*, 1986; **7**: 230–252.
32. Singh UC, Brown FK, Bash PA, Kollman PA. *J. Am. Chem. Soc.* 1987; **109**: 1607–1614.
33. Silla E, Villar F, Nilsson O, Pascual-Ahuir JL, Tapia O. *J. Mol. Graphics* 1990; **8**: 168–172.

