1. **Synthesis of calix-and binaphthocrown ethers and applications thereof**

1.1. Cyclization of p-tert-butylcalix[6]arene with diols. Conformation and complexation studies [1]

Recently, we have disclosed an unexpectedly selective diametrical alkylation and ring closure of thiacalix[4]arene (TCA) and calix[4]arene (CA) with alcohols and oligoethylene glycols under the Mitsunobu conditions. With the aid of this simple and mild method 1,3-calix[4]crown-4, 5 and -6 derivatives were accessible which otherwise were difficult to obtain. This simple method was tried to extend over calix[6]arenes but only 1,6-hexane-and 1,8-octanediols gave A,D-ring cyclized products both in cone conformation. As calix[6]crowns were not accessible in this route, further functions were tried to introduce to obtain complexing ligands. The base-promoted peralkylation of the four OH groups with allyl bromide and ethyl bromoacetate resulted in cone, partial cone and 1,2,3-alternate conformers, respectively, depending on the substituents and base used (Fig. 1). Interesting conformational interconversion of the 1,2,3-alternate tetraallyl derivative to cone was observed upon complexation with Ag^+, whereas the cone tetraester responded to Na^+ without conformational change. Unfortunately, none of the ligands exhibited complexation with amino acid derivatives or other biomolecules.

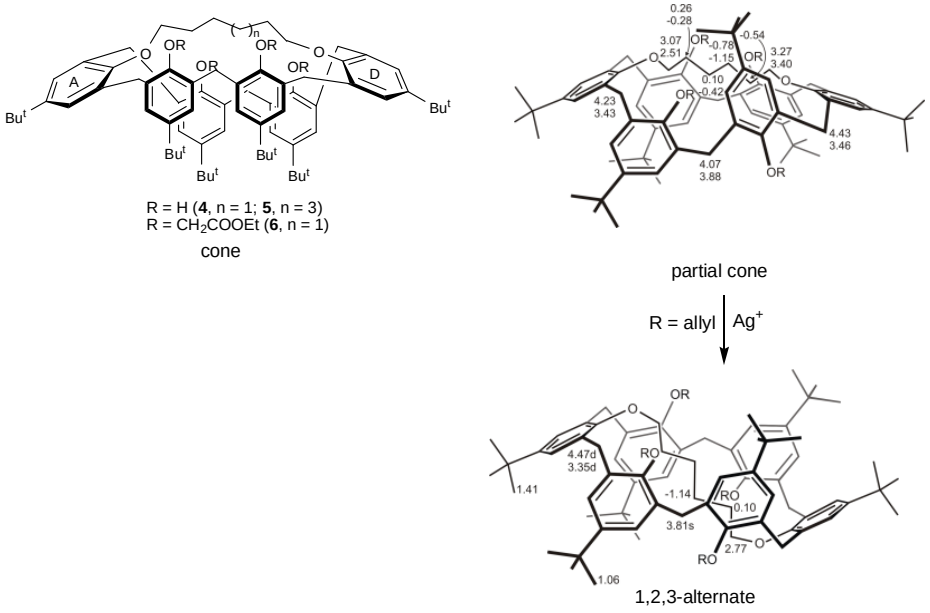
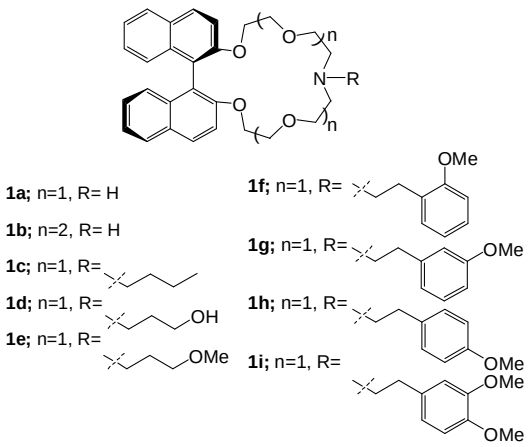
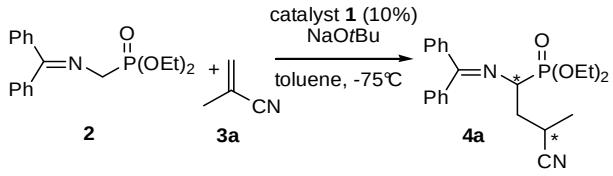


Fig. 1 Conformation of A,D-alkylene bridged calix[6]arenes and peralkylated derivatives

1.2. 1,1'-Binaphtho(monoazacrown) ether organocatalysts for asymmetric Michael additions [2]

The enantioselective synthesis of α-aminophosphonates of biological relevance (peptide mimics, antibacterials, antihypertensive and anti-HIV agents, etc.) was elaborated with $NaOBu^t$ base-promoted Michael addition of a *N*-protected α-aminophosphonate onto acrylic acid derivatives in the presence of novel (*R*)-BINOL-appended azacrown lariate ether catalysts (Scheme 1). High diastereo-and enantioselectivities were achieved with methacrylonitrile when catalyst **1i** (anti/syn = 99.3/0.7%, ee. 96/46%) was used. In this way a series of α-aminophosphonic acids have become available without optical resolution of the racemic compounds.





Entry	Catalyst	Conversion (%)[b]	Yield (%)[c] (*anti/syn*)[d]	*ee* (%)[d,e]
1	**1a**	89	78 (74/26)	44/22
2	**1b**	87	75 (58/42)	8/7
3	**1c**	81	72 (55/45)	32/19
4	**1d**	30	23 (58/42)	87/17
5	**1e**	94	85 (88/12)	84/77
6	**1f**	96	87 (83/17)	86/67
7	**1g**	93	81 (92/8**)**	94/35
8	**1h**	92	81 (93/7)	94/51
9	**1i**	94	86 (99.3/0.7)	96/46

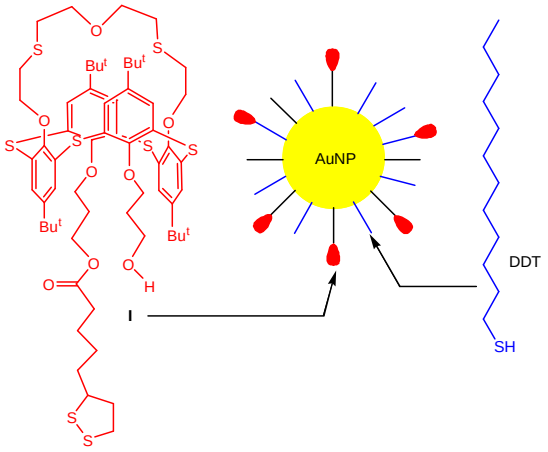
Scheme 1. (*R*)-BINOL-based chiral catalysts and the enantioselective addition of N-protected aminophosphonate **2** on methacrylonitrile **3a**

1.3. Ionophore-gold nanoparticle conjugates and solid-state ion-channels for potentiometric sensing [3,4]

Ionophores incorprated in hydrophobic membrane matrices (often plasticized PVC) has led to ion-selective electodes (ISEs) having worldwide applications in clinical analysis most frequently for blood electrolyte measurements. The general disadvantages of traditional ISEs of this kind (loss of membrane components to the blood, extraction of sample matrix components to the membrane, etc.) can partly be overcome by covalent immobilization of the ionophore to the membrane phase affording ion-selective membrane (ISM). A new concept of the non-covalent immobilization of ionophores onto the surface of inert gold nanoparticle (AuNP, d = 5.5 nm) carrier was worked out by the Gyurcsányi's group in our University. To prove the feasibility and utility of this idea in the potentiometric determination of cations, an ionophore capable of binding to AuNP by non-covalent interacion was required. We synthesized a 1,3-alt thiacalix[4]dithiacrown-5 ligands **I** comprising a sufficiently long chain with dithiolane endgroup necessary for immobilization via Au-S bond. Though this molecules exhibited Ag^+ selectivity in PVC ISE (this cation is not found in blood), it proved to be suitable model to verify the new concept of immobilization. ISM made of IP-AuNP conjugate mixed with PVC membrane coctail containing ionohore **I** and DDT (Scheme 1) exhibited excellent Ag^+ selectivity over a series of cations (Table 1). Moreover, the diffusion coefficient of IP-AuNP lowered by four orders of magnitude that means the loss of ionophore (leakage) was significantly restricted as compared to conventional PVC membranes.

Table 1. Potentiometric selectivity coefficients of ISMs based on the free and AuNP-conjugated ligand **I** determined by separate solution method at 1 mM level

Ions (J)	log $K_{Ag/J}$ (unbiased)		log $K_{Ag/J}$ (conventional)	
	Ligand **I**	IP-AuNP	Ligand **I**	IP-AuNP
Mg^{2+}	-11.6	-7.4	-6.4	-5.8
Pb^{2+}	-10.8	-6.6	-5.1	-4.8
K^+	-8.4	-4.9	-5.2	-4.9
Na^+	-10.1	-5.7	-5.3	-5.1



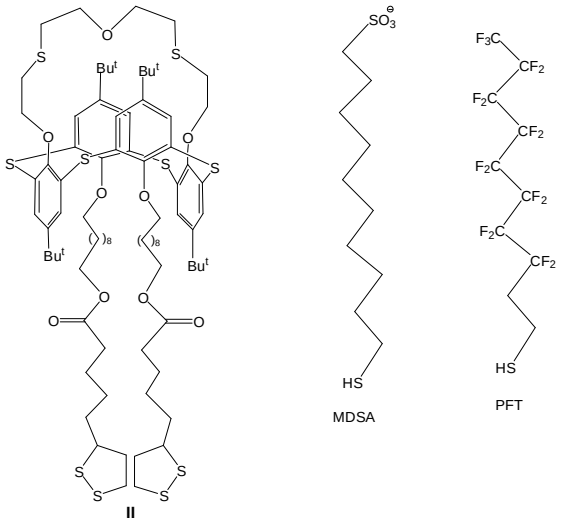
Scheme 1 Schematic representation of the ionophore-Au nanoparticle conjugates and chemical structure of the surface modifying compounds (ligand **I** and 1-dodecanethiol, DDT).

Using the selectivity filter of biological ion-channels (ICs) as inspiration, solid state ICs based on ionophore modified nanopore arrays were introduced for the first time to use for potentiometric sensing of small inorganic

cations. Gold nanopores formed by electroless deposition of gold onto the surface of polycarbonate track-etch membranes were used with randomly distributed straight cylindrical pores ($6x10^8$ pores/cm^2 with nominal d = 15-80 nm). For proof of principle, thiacalixarene derivative **II** immobilized on the wall of nanopores was used to induce Ag^+ selectivity. In addition, the sensor fabrication required negative sites generated with mercaptodecanesulfonate (MDSA) to induce a proper potentiometric response, and a perfluorinated thiol (PFT) to confer hydrofobicity to the Au nanopores. Both additives were immobilized via Au-S bond on the surface of nanopores. With this new Ag^+ sensing device excellent selevtivities exceeding six orders of magnitude were determined for a range of interfering cations (Scheme 2).

Table 2. Potentiometric selectivity coefficients log $K_{Ag/J}$ of nanopore-based ISEs with and without ionophore modification

Ions (J)	Ligand **II**/MDSA/PFT (11:10:1)	MDSA/PFT (10:1)
K^+	-6.1	-1.0
Cs^+	-6.5	-0.9
H^+	-6.0	-0.2
Et_4N^+	-6.1	-1.2

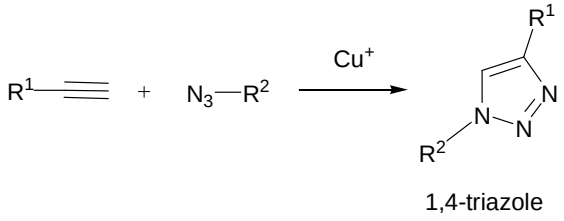


Scheme 2. Components of nanopore-based ISMs: ligand **II**, 1-mercaptodecane-10-sulfonate (MDSA), 1*H*,1*H*, 2*H*, 2*H*-perfluoro-1-decanethiol (PFT)

The new concept of inducing ion selectivity seems to be extendable to biologically relevant analytes, thereby it might have a great impact on the development of new sensing devices.

2. Azide-alkyne click reaction (CuAAC) in the syntheses of sensing systems

Over the past decade the Cu(I)-catalyzed dipolar azide-alkyne cycloaddition (CuAAC) leading to 1,2,3-triazoles (Scheme 1) has attracted enormous interest as one of the most powerful and valuable examples of „click chemistry. Due to its simplicity, selectivity, high yields, etc. almost unlimited applications have been reported mostly on linking optical markers to peptides, proteins, DNS and various derivatizations of macromolecules. E.g. in calixarene chemistry this method represents an easy access to sophisticated large molecules, such as hibrid calix[4]arenes with carbohydrate and amino acid moieties, glycoclusters, water soluble derivatives, calixarene-based cavitands and nanotubes, multicalixarenes etc.

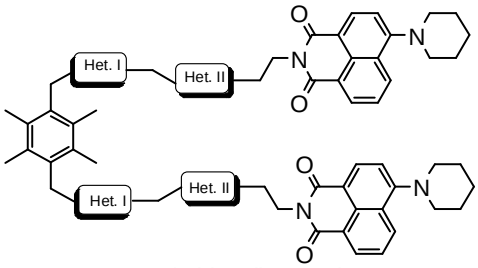


Scheme 1 General representation of CuAAC click reaction

2.1.Fluorescent receptors for sensing of nucleoside polyphosphates [5,6,8]

Development of fluorescent sensing systems capable of detection of nucleoside polyphosphates under physiological conditions still has been an important topic of biosensor research. Aminonaphthalimide-based two-

armed fluorescent imidazolium/triazole receptors (**1**, **2**) were synthesized to investigate how the sensing of nucleoside polyphosphates is influenced by the number and order of heterocyclic constituents of the receptor arms. Our measurements revealed that at least one imidazolium and one triazole unit by arms is required for the efficient binding with the triphosphate moiety of GTP and ATP by ionic and H-bonding interactions. Another important finding was to establish the position of the cationic imidazolium subunit (**2** vs **1**) to achieve high sensitivity of sensing without affecting the GTP and ATP selectivity (Fig. 1). These observations can be utilized in designing new naphthalimide-based chemosensors comprising similar binding sites.



Het.1 = 1,3-imidazolium and
Het.2 = 1,4-(1,2,3-triazole) (**1**)
Het.1 = 1,4-(1,2,3-triazole) and
Het.2 = 1,3-imidazolium (**2**)

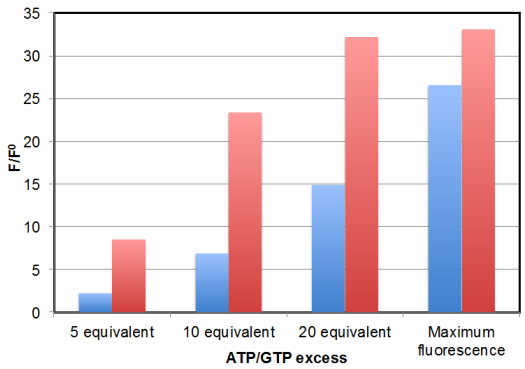


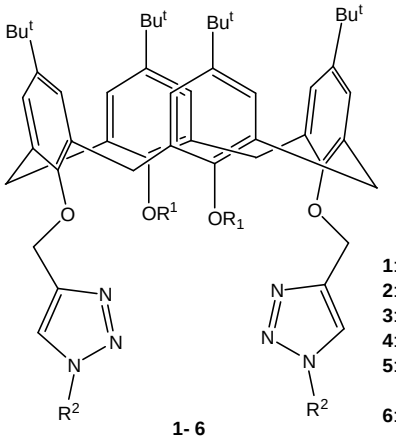
Fig. 1 Structure of receptors **1** and **2**, and fluorescent responses of **2** to ATP (blue) and GTP (red)

2.2. Triazole-linked calix[4]arene-based potentiometric ion selective electrodes [7,9,12]

A series of 1,2,3-triazole-linked calix[4]arene ionophores comprised of different *O*-donor groups (OH, COOEt, $CONEt_2$) attached either to the lower rim of calix or to the triazole moieties were synthesized to explore their ion-selectivity by competitive ESI-MS measurements and for the first time, in potentiometric transduction (Fig. 1). PVC membrane electrodes (ISEs) were fabricated and masured their potentiometric selectivities toward a series of mono-and divalent metal ions (Table 1). Structure-ionselectivity relationship and the structural requirements of the coordination sphere for selective binding were established. ISEs made of calix[4]arene-bis-triazoles were found generally to exhibit distinct Ag^+ selectivity in the order **3**≈**4**>**2**>**1** indicating the beneficial effect of the carboxamide or ester groups in the complexing site.

Table 1 Potentiometric ionselectivities of ISEs fabricated from ligands **3-5** for further evaluation

Ions, *J*	log $K_{Ag/J}$ **3**	log $K_{Na/J}$ **4**	log $K_{Cu/J}$ **5**
Cs^+	-8,06	-3,59	-
K^+	-7,89	-3,36	-8,14
Na^+	-4,5	0	-3,77
Li^+	-7,49	-3,08	-
Ca^{2+}	-8,26	-3,78	-8,74
Mg^{2+}	-8,27	-3,78	-
Pb^{2+}	-6,35	-2,15	-4,12
Cu^{2+}	-6,65	-2,55	0
Zn^{2+}	-8,17	-3,69	-
H^+	-5,49	-3,05	-6,54
Ag^+	0	3,8	-4,42



1- 6

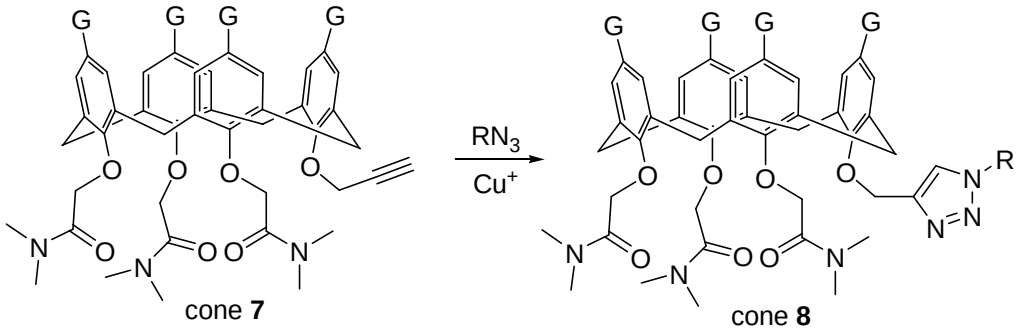
1: R^1 = H, R^2 = $(CH_2)_6OH$
2: R^1 = H, R^2 = CH_2COOEt
3: R^1 = CH_2CONEt_2, R^2 = $(CH_2)_6OH$
4: R^1 = CH_2CONEt_2, R_2 = CH_2COOEt
5: R^1 = 1-R^2-4-methylene-1,2,3-triazole, R^2 = $(CH_2)_6OH$
6: R^1 = 1-R^2-4-methylene-1,2,3-triazole, R^2 = CH_2COOEt

Fig.1 Structure of triazole-linked calix[4]arenes and selected potentiometric ionselectivities

In contrast, calix[4]arene-tetratriazole **5** comprised only *sp*^{2} N-donor atoms displayed excellent Cu^{2+} selectivity over a series of alkali-, alkaline earth- and transition metal ions. A unique feature of the outstanding Ag^+ selective electrodes made of **3** and **4** was recognized by suggesting their potential application as Na^+ ISEs in systems not

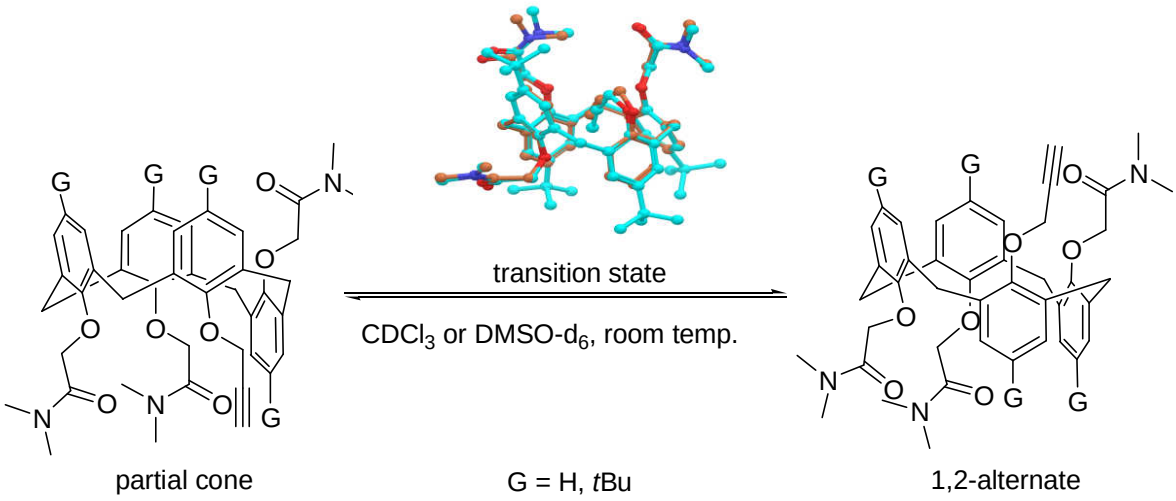
containing silver ions (Table 1). Synthetic efforts to prepare thiocarboxamide analogues probably capable of recognizing environmentally important ions (Pb^{2+} and Cd^{2+}) are under progress.

2.3. New substituent size limitation of the „O-through-the-annulus" rotation among calix[4]arenes [11]
To expand the choice of triazole-linked calix[4]carboxamides ionophores type **8** were attempted to synthesize (Scheme 1). During the preparation of starting material *cone* **7** (alkylation of monopropargyl-calix[4]arenes with *N,N*-dimethylchloroacetamide promoted by Cs_2CO_3), we obtained a mixture of conformers instead, comprising partial cone **7** as the major component.



Scheme 1 Synthesis designed for the preparation of ionophore *cone* **8**

^{1}H NMR measurements revealed the partial cone-1,2-alternate interconversion of **7** (G = H, *t*Bu) at room temperature in DMSO and $CDCl_3$ as well, due to the unknown mobility of the propargyloxy group in *O*-through-the-annulus rotation (Scheme 2), which has been limited so far to substituents not bulkier then ethyl or cyanomethyl groups. The conversion rate was as follows: G = *t*Bu>> H and $CDCl_3$>>DMSO-d_6.
Conformational search was carried out to explore the conformational space of *O*-propargyl-calix[4]arene carboxamides **7** and four states with minimized *paco* and *1,2-alt* conformation were subsequently optimized using density functional theory (MPW1K (6-31g**)) calculation and two close conformational analogs of the transition state were optimized.



Scheme 2 Proposed mechanism for the *paco-1,2-alt* rotation

Calculations for the *paco-1,2-alt* rotation in detail
The cyanomethoxy group (activation energy Ea=110.5 kJ/mol in DMSO) was reported over ethyl to allow *O*-through-the annulus rotation, therefore we compared its steric requirement with that of the propargyloxy group.
Rotating moieties were modelled by 2-phenoxyacetonitrile and phenyl-propargyl-ether. Comparison of the electron densities

revealed subtle steric enlargement -presented as electron density isosurfaces- of the propargyl derivative. In Fig. 1 electron density isosurfaces (0.001 electron/bohr3, MPW1K 6-31g**) of 2-phenoxyacetonitrile (blue) and phenyl-propargyl-ether (red) are shown.

Fig. 1 Comparison of the steric requirements

Table 1 Results of thermochemical calculations (kJ/mol, relative to paco)

R	kJ/mol	TS	1,2-alt
H	ΔU	98	-16
	ΔH	97	-16
	ΔG	211	-16
	ΔG (in DMSO)	-	6
	ΔG (in $CHCl_3$)	-	-2
*t*Bu	ΔU	81	-20
	ΔH	81	-20
	ΔG	150	-18
	ΔG (in DMSO)	-	-2
	ΔG (in $CHCl_3$)	-	-9

Table 2 Gase phase energies obtained by different computational methods

Gas phase energy (kJ/mol)		MPW1K	M06-2x	B3LYP	X3LYP	LMP2
H	E_a	144	130	148	148	152
	ΔE(conf.)	-17	-10	-24	-24	-40
*t*Bu	E_a	118	104	127	128	114
	ΔE(conf.)	-20	-19	-24	-24	-39
	ΔE_a	26	26	21	20	39

- Harmonic vibration frequencies and thermochemical properties were calculated on all the six states (Table 1).

Table 3 Solvation energies (kJ/mol)

R	paco	1,2-alt	Δ
		$CHCl_3$	
H	-87	-74	14
*t*Bu	-77	-69	9
		DMSO	
H	-125	-104	21
*t*Bu	-111	-96	15

- The size of the basis does not have significant impact on the energy, therefore we selected 6-31g** .
- The six (MPW1K) geometries were further calculated by other quantum-mechanical approaches (Table 2)
- According to gas-phase calculations the 1,2-alt conformation is preferred over the paco (range from -15 to -25 kJ/mol energy difference).
- The effect of chloroform and DMSO solvents were calculated by implicit solvent model (Poisson-Boltzmann solver: Table 1 and 3) Considering solvation energies partial cone isomer is preferred in both chloroform and DMSO. The energy difference is lower in chloroform.

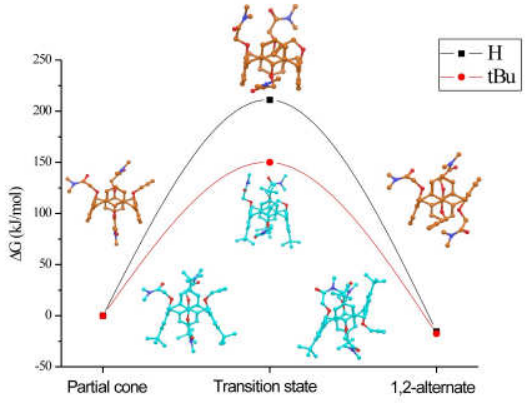


Fig. 2 The free energy profile of the isomerization in gas phase

Activation energy of R = tBu conversion is lower compared to that of R = H derivative, in good agreement with the NMR measurements.

Scientific papers

[1]. Grün, A.; Balázs, B.; Bitter, I. Cyclization of p-tert-butylcalix[6]arene with diols under the Mitsunobu protocol. A conformational study of the peralkylated derivatives. *Lett. Org. Chem.* **2009**, 311-314
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[3]. Jágerszki, Gy.; Grün, A.; Bitter, I.; Tóth, K.; Gyurcsányi, R. E. Ionophore-modified nanopores as potentiometric ion-selective membranes *Chem. Commun.* **2010**, 607-609
[4]. Jágerszki, Gy.; Takács, Á.; Bitter, I.; Gyurcsányi, R. E. Solid-State Ion Channels for Potentiometric Sensing *Angew. Chem. Int. Ed.* **2011**, *50*, 1656-1659
[5]. Bojtár M, Czirok J. B., Baranyai P., Bitter I. Synthesis and evaluation of novel fluorescent nucleoside-poliphosphate sensors *Period. Polytechn. Chem. Eng.* **55**: 69-70 (2011)
[6]. J. B. Czirok, M. Bojtár, P. Baranyai, L. Drahos, M. Kubinyi and I. Bitter, Aminonaphthalimide-based dipodal imidazolium/triazole receptors for fluorescent sensing of GTP and ATP, *Sensors and Actuators* **2012** (submitted, under revision)
[7]. J. B. Czirok, Gy. Jágerszki, K. Tóth, Á. Révész, L. Drahos and I. Bitter, Synthesis and preliminary potentiometric evaluation of triazole-linked calix[4]arene ionophores *J. Incl. Phenom. Macrocycl. Chem.* **2012** (manuscript draft before submission)

Lectures (posters)

8. J. B. Czirok, M. Bojtár, L. Drahos, P. Baranyai, M. Kubinyi, I. Bitter, Synthesis of methylene-bis(imidazolium/1,2,3-triazole) podands for fluorescent sensing of nucleoside polyphosphates, 4th European Conference on Chemistry for Life Sciences, Budapest (Magyar Kémikusok Egyesülete), 2011. p. 321 (poster)
9. J. B. Czirok, I. Juhász, A. Simon, Gy. Jágerszki, R. E. Gyurcsányi, I. Bitter, Synthesis, characterization and potentiometric evaluation of triazole-modified calix[4]arene ionophores Mátrafüred 2011: International Conference on Electrochemical Sensors 2011. p. 114 (poster)
10. Bojtár M., Czirok J. B., Baranyai P., Kubinyi M., Bitter I., Fluoreszcens nukleozid-polifoszfát-receptorok szintézise és vizsgálata XXXIV. Kémiai Előadói Napok, Szeged: Magyar Kémikusok Egyesülete, 2011. p. 81 (oral)
11. Czirok, J. B.; Tarcsay, A.; Mezei, P. D.; Simon, A.; Bitter, I., Size matters: Conformational conversion of *O*-propargyl-calix[4]arene derivatives Balticum Organicum Syntheticum, Tallin 2012. július 1-4.(poster)
12. J. B. Czirok, I. Juhász, P. D. Mezei, A. Simon, Gy. Jágerszky, R. E. Gyurcsányi, L. Drahos and I. Bitter Synthesis and potentiometric evaluation of triazole-linked calix[4]arene ionophores, 4th EuCheMS Chemistry Conference, Prága 2012. augusztus 26-30. (poster)