ANION RECOGNITION BY NEUTRAL RECEPTORS

Dmitry M. Rudkevich, Jurgen Scheerder, and David N. Reinhoudt*

Laboratory of Organic Chemistry
University of Twente
P.O. box 217
7500 AE Enschede
The Netherlands

Abstract. In this review the design of neutral receptors for anions is described. In these receptors, selective anion recognition takes place either exclusively via hydrogen bonding or by combination of a Lewis acidic UO_2-center and hydrogen bonds. An approach to neutral bifunctional receptors containing both anion and cation complexing sites is described. The results on selective $H_2PO_4^-$ anion transport and simultaneous transport of hydrophilic cations and anions through a supported liquid membrane are presented.

1. Introduction.

The recognition of cations and neutral molecules has had our attention since many years. A remaining challenge is the selective recognition and binding of anions by neutral molecules. In nature, phosphate and sulfate binding proteins are very important for the active transport systems in the cell and organelles [ref 1]. A very high selectivity in binding has been observed in prokariotic, periplasmic phosphate and sulfate binding proteins, which demonstrate $>10^5$ selectivity for complexation phosphate over sulfate and sulfate over phosphate, respectively. In those proteins the specific binding *exclusively* takes place through hydrogen bonding. Native zinc enzymes such as carboxypeptidase A (CPA) bind small inorganic anions like hydrogen phosphate via *combination* of metal-anion

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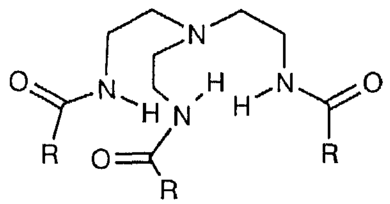
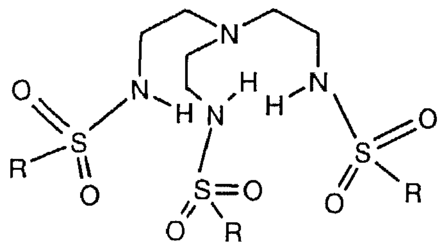
interactions and hydrogen bonding [ref 1].
The established way to recognize anions is the use of positively charged receptors. The disadvantage of these receptors is the limited selectivity due to the dominant electrostatic interactions that govern the anion recognition [ref 2]. This disadvantage can be overcome by the use of neutral anion receptors. Despite several neutral receptors incorporating Lewis acidic centers have been developed, they lack the possibility of structural variation in order to control the selectivity of the anion binding. To increase the selectivity, we have investigated neutral receptors that have, in addition to a Lewis acidic center, also directional modes of interaction via hydrogen bonds. The proper orientation of hydrogen bond donating and accepting groups enables a more selective anion recognition.

2. Anion recognition exclusively through hydrogen bonding.

In our first attempt, we have developed the tris(aminoethyl)amine based receptors which orient the hydrogen bond donating groups in a tetrahedral fashion (**1** and **2**) [ref 3]. These neutral receptors showed selective recognition of $H_2PO_4^-$ anions over Cl^- and HSO_4^- in MeCN (Table 1).



1a: R = CH_2Cl
1b: R = $(CH_2)_4CH_3$
1c: R = C_6H_5
1d: R = 4-$MeOC_6H_4$



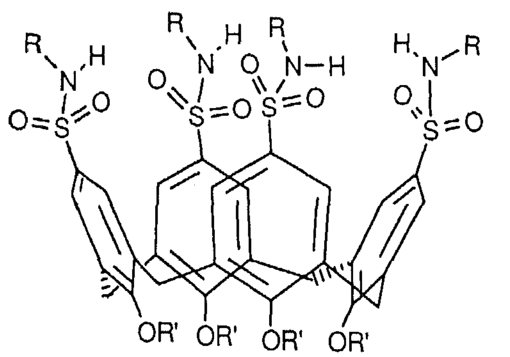
2a: R = 4-$MeOC_6H_4$
2b: R = 2-naphthyl

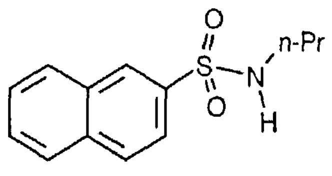
We envisaged that the positioning of hydrogen bond donating groups on a molecular platform would enable further benefit from the directional character of hydrogen bonds and consequently further control the anion complexation. As a molecular platform we have used calix[4]arenes. This versatile building block can be rigidified, and both the upper rim and the lower rim can be used to position hydrogen bond donating groups.

Table 1. *Association constants (K_{ass}, M^{-1}) of receptors 1 and 2 determined by conductometry*

	$H_2PO_4^-$	HSO_4^-	Cl^-
1a	6.1 x 10^3	1.7 x 10^2	1.7 x 10^3
1b	2.8 x 10^2	3.1 x 10^1	2.9 x 10^2
1c	8.7 x 10^2	5.6 x 10^1	1.0 x 10^2
1d	5.1 x 10^2	7.3 x 10^1	1.9 x 10^2
2a	3.5 x 10^3	7.9 x 10^1	5.4 x 10^2
2b	1.4 x 10^4	3.8 x 10^1	1.6 x 10^3

Chlorosulfonylation of calix[4]arenes at the upper rim followed by the addition of a variety of amines gave a class of sulfonamide calix[4]arenes (**3**) [ref 4]. These receptors show a remarkable preference for the complexation of HSO_4^- over $H_2PO_4^-$ and Cl^- in $CHCl_3$ (> 10^2, Table 2). Comparison of the association constants with non-cyclic reference compounds (**4** and **5**, Table 2) shows that the preorganization of the binding sites on calix[4]arene substantially increases the association constants for the anion complexation. Also the lower rim of calix[4]arenes can be used to position binding sites for anions. Functionalization of the phenolic positions with two or four (thio)urea moieties gave a class of





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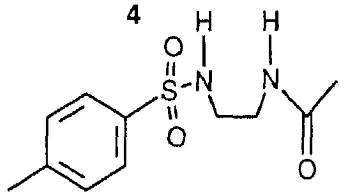

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R' = $CH_2CH_2OCH_3$
3a: R = H
3b: R = *n*-Pr
3c: R = *tert*-Bu
3d: R = $CH_2CH_2NHC(O)CH_3$

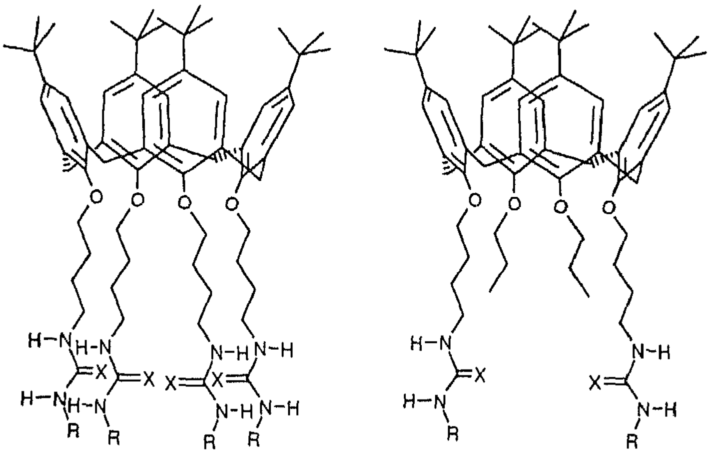
neutral calix[4]arene (thio)urea derivatives (**6-9**) [ref 5]. Despite the flexible butyl spacers, the binding sites are preorganized due to hydrogen bonding between the complementary (thio)urea moieties. The complexation behaviour was studied by ^1H NMR titration experiments and FAB mass

Table 2. *Association constants (K_{ass}, M^{-1}) of receptors **3,4** and **5** determined by ^1H NMR in $CDCl_3$*

	$H_2PO_4^-$	HSO_4^-	Cl^-	NO_3^-
3b	3.5×10^2	9.7×10^2	3.6×10^2	2.4×10^2
3c	< 10	1.3×10^2	7.2×10^1	4.3×10^1
3d	-	1.0×10^5	1.3×10^3	5.1×10^2
4	1.4×10^1	1.0×10^1	1.5×10^1	< 10
5	2.6×10^2	3.5×10^2	3.3×10^2	9.9×10^1

spectrometry. The receptors showed 1:1 complexation of Cl^-, Br^-, I^-, N_3^-, and SCN^- with a selectivity for Cl^- (Table 3). A preference for hard anions (Cl^-, Br^-, or CN^-) over soft anions (I^-, or SCN^-) is found and spherical anions are bound more strong than linear anions. Despite the fact that the diurea derivative **8** has only two binding sites, the anion complexation is stronger, probably due to less extensive hydrogen bonding in the free ligands. Upon anion complexation these hydrogen bonds must be broken which is more demanding for the (tetrakis)-urea derivatives **6** and **7**. Surprisingly, the thiourea derivatives **7** and **9** show weaker anion complexation than the corresponding urea derivatives (**6e** and **8**, respectively), despite the higher acidity of the thiourea hydrogens.

The same strategy was applied using 1,3,5-trimethoxy-2,4,6-trihydroxycalix[6]arene as a building block [ref 6]. Functionalization of this derivative gave a class of anion receptors (**10** and **11**) which have a C_3 symmetry axis. A receptor with three binding sites arranged in a C_3 symmetry would be able to bind tricarboxylate anions. The anion complexation was studied by ^1H NMR titration experiments, FAB mass spectrometry, and FTIR spectroscopy. The association constants are summarized in Table 4. In all cases a 1:1 stoichiometry was found.

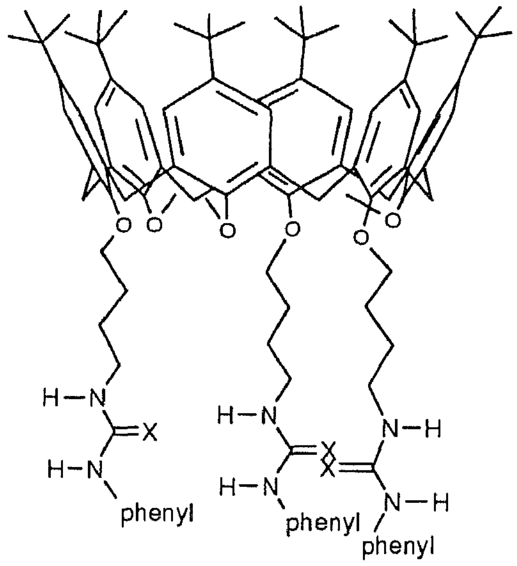


6a: X = O, R = phenyl
6b: X = O, R = *n*-propyl
6c: X = O, R = *n*-octyl
6d: X = O, R = *tert*-butyl
7: X = S, R = phenyl

8: X = O, R = phenyl
9: X = S, R = phenyl

Table 3. *Association constants (K_{ass}, M^{-1}) of receptors **6-9** determined by ^1H NMR in $CDCl_3$*

	Cl^-	Br^-	I^-	CN^-	SCN^-
6a	2.7×10^3	1.7×10^3	< 25	8.6×10^2	< 25
6b	< 25	< 25	< 25	< 25	< 25
6c	2.9×10^2	4.5×10^2	-	5.5×10^2	-
6d	2.1×10^3	1.3×10^3	-	8.0×10^1	-
7	3.4×10^2	5.8×10^2	< 25	8.6×10^2	< 25
8	7.1×10^3	2.6×10^3	6.1×10^2	1.1×10^3	< 25
9	1.3×10^3	4.9×10^2	-	6.7×10^2	370



10 X = O **11** X = S

Comparison of the association constants for the complexation of benzoate, isophthalate, and 1,3,5-benzenetricarboxylate revealed cooperativity. The values for the association constants for 1,3,5-benzenetricarboxylate, 1,2,4-benzenetricarboxylate, and 1,2,3-benzenetricarboxylate show a strong preference for the binding of the anion with the same C_3 symmetry as the host. Also, the planar 1,3,5-benzenetricarboxylate is complexed in preference over the non-planar *cis*-1,3,5-cyclohexanetricarboxylate. This is due to the fact that the carboxylate groups of *cis*-1,3,5-benzenetricarboxylate can rotate freely and complexation of this anion will be accompanied with a larger unfavourable entropy term. As for the calix[4]arene (thio)urea derivatives, the thiourea derivatives are less effective to complex anions than the corresponding urea derivatives. To investigate this effect we studied the self-association behaviour of N-(n-butyl)-N'-phenyl-thiourea and N-(n-butyl)-N'-phenyl-urea. ^{1}H NMR dilution experiments showed that the thiourea derivative is more strongly associated than the urea derivative. Upon complexation the self-association must be broken which is more demanding for the thiourea derivative (**11**) than for the urea derivative (**10**) and this will result in a preferred binding of anions by the urea receptor.

Table 4. *Association constants (K_{ass}, M^{-1}) for receptors **10** and **11** determined by ^1H NMR in $CDCl_3$*

	10	**11**
Benzoate	1.6×10^3	1.4×10^3
Isophthalate	6.9×10^4	6.4×10^3
1,3,5-$(COO^-)_3$-benzene	8.7×10^4	2.9×10^5
1,2,4-$(COO^-)_3$-benzene	2.3×10^4	2.5×10^3
1,2,3-$(COO^-)_3$-benzene	4.7×10^4	1.8×10^4
1,3,5-$(COO^-)_3$-cyclohexane	1.0×10^5	2.9×10^4
Cl^-	4.8×10^2	< 25
Br^-	1.5×10^3	3.5×10^2

However, in case of the 1,3,5-benzenetricarboxylate breaking of the hydrogen bonds is more than compensated by the cooperative binding of three carboxylate groups by the three thiourea groups, and this results in a preferred binding of this anion by the thiourea host **11**. The complexation of Cl^- and Br^- was also investigated. In contrast to the corresponding calix[4]arene (thio)urea derivatives, the Br^- is complexed more strongly than Cl^-. The better complementarity of the Br^- with the cavity formed by the three (thio)urea moieties dominates the larger affinity of the binding site for the harder Cl^-.

3. UO_2-Sal(oph)ens as Anion Receptors

Previously we have reported that metallomacrocycles and clefts containing an immobilized Lewis acidic UO_2-cation, are excellent receptors for the complexation of neutral molecules as the result of coordination of a nucleophilic group (C=O, S=O, =N-) to the uranyl center in addition to H-bond formation and π-π stacking. The uranyl cation complexed in a salophen unit prefers a pentagonal bipyramidal coordination, with the two oxygens at the apical positions and with both the four-coordinating sites of the salophene moiety and a neutral molecule in the equatorial positions. In the same way the presence of both a uranyl Lewis acidic center and additional hydrogen-bonding sites like C(O)-NH fragments in a preorganized receptor molecule should increase the selectivity and efficiency of anion complexation [ref 7].

For the complexation of anions we have first studied the simple UO_2-

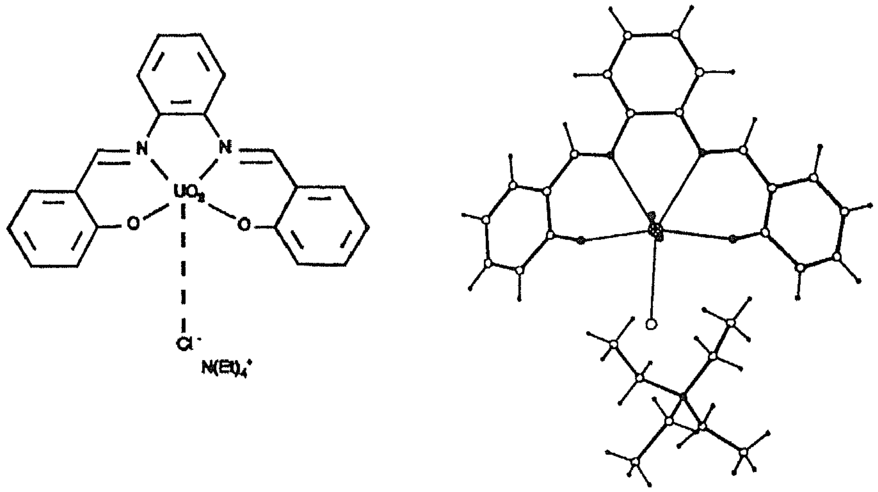


*Fig. 1. X-ray structure of the complex **12a**·Cl⁻*

containing salophen **12a**, the crystal structure of the complex **12a**· $Et_4N^+Cl^-$ is shown in Figure 1. The uranyl cation is coordinated to two oxygen atoms and two nitrogens of the salophen unit and to the *chloride anion* (U···Cl distance 2.76 Å) which clearly demonstrates the tight anion complexation. From ^1H NMR titration experiments of **12a** with $Bu_4N^+Cl^-$ in MeCN-d_3 (with 1% of DMSO-d_6) and by conductometry (see also Table 5) high K_{ass} values of 4.2 x 10^2 and 4.0 x 10^2 M^{-1}, respectively, were determined.

Synthesis of Anion Receptors and Anion Complexation in Solid State. Stirring of the uranyl containing salophen **12b** prepared from the corresponding aldehyde, 1,2-benzenediamine and $UO_2(OAc)_2·2H_2O$, with tetrabutylammonium dihydrogen phosphate $Bu_4N^+H_2PO_4^-$ in MeCN overnight, followed by evaporation of the solvent gave the corresponding anion complex as an orange powder. The negative FAB mass spectrum of the complex exhibit, in addition to a small peak of the free ligands **12b**, a very intense [**12b** + $H_2PO_4]^-$ signal, while a small [**12b** + H_2PO_4 + $Bu_4N]^-$ peak is also present. The crystal structure of the complex **12b** $Bu_4N^+H_2PO_4^-$ is shown in Figure 2. In this complex the uranium atom has the approximate pentagonal bipyramidal coordination, with the two oxygens in apical positions. In the equatorial plane besides coordination with the two nitrogens and two oxygens of the salophen moiety, the fifth coordination position is occupied by an oxygen atom of the dihydrogen

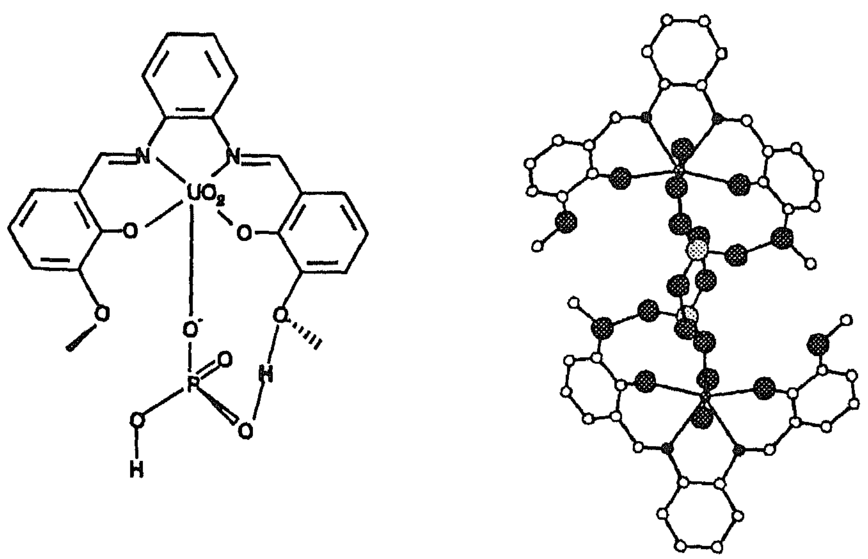
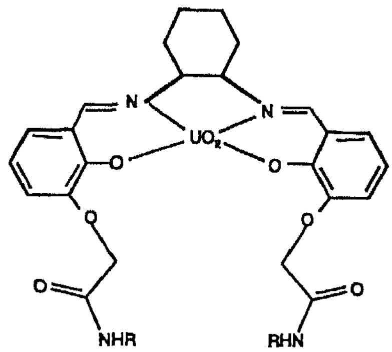
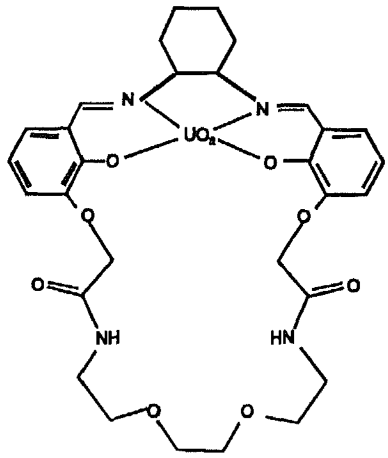


Fig. 2. X-ray structure of the complex ***12b****·$H_2PO_4^-$*

phosphate ($H_2PO_4^-$) anion (U···O-P distance 2.28(2) Å). It is evident from Figure 2 that in the solid state the complexes are arranged in centrosymmetric pairs. The "core" of the dimer consists of two $H_2PO_4^-$ anions, which are connected by two short H-bonds (O···O distance 2.52(1) Å). Another H-bond (O···O-P distance 2.68(1) Å) is formed between the $H_2PO_4^-$ anion and a methoxy oxygen of the salophen moiety. This result shows the interesting phenomenon that anion binding in this type of compounds is effected by coordination to the UO_2-cation and is augmented by hydrogen bonding between anion and ligand.

In order to obtain anion receptors which contain a combination of both UO_2-cation and C(O)-NH amido functionalities as additional binding sites, cleft-type molecules **13a-j** and metallomacrocycle **14** containing a polyether bridge were designed [ref 7]. The crystal structure of the complex of ligand **13e** with $Bu_4N^+H_2PO_4^-$ is shown in **Figure** 3. As in the case of the complex of **12b** with $H_2PO_4^-$ (Figure 2), the $H_2PO_4^-$ anion is tightly coordinated to the UO_2-center (U···O-P distance 2.28(2) Å) in addition to a H-bond formation with the acetoxy oxygen of the salen moiety (O···O-P distance 2.84(2) Å). However, in this case two additional H-bonds between the amido groups of the ligand and the complexed anion are present (N···O-P distance 2.79(2) Å) which clearly shows the participation of C(O)-NH fragments in anion complexation. As in Figure 2 the $H_2PO_4^-$ complexed to the UO_2-cation forms a H-bonded associate with the second $H_2PO_4^-$ anion (O···O distance 2.48(2) Å) which in this case is not complexed itself by ligand **13e**.





13a: R = H
13b: R = $4\text{-}MeC_6H_4$
13c: R = $n\text{-}C_{18}H_{37}$
13d: R = $4\text{-}(n\text{-}C_8H_{17}O)\text{-}C_6H_4$
13f: R = $C(O)NH\text{-}n\text{-}(CH_2)_8O\text{-}C_6H_4\text{-}2\text{-}NO_2$
13j: R = $(CH_2)_2NHSO_2\text{-}4\text{-}MeC_6H_4$

14

The covalent combination of a UO_2-center and C(O)NH functionalities was also immobilized at the upper rim of a rigid and lipophilic calix[4]arene platform, and consequently calixarene based UO_2-containing anion receptor **15** was prepared [ref 8].
Stirring of a mixture of the ligands **12b**, **13a,b** and **e**, **14**, or **15** and $Bu_4N^+H_2PO_4^-$ in MeCN overnight followed by evaporation of solvent gave the corresponding anion complexes as orange powders. In all cases the negative FAB mass spectra of the solid complexes exhibit, in addition to small peaks of the free ligands, very intense [Ligand + Anion]⁻ signals, while small [Ligand + Salt]⁻ peaks are also present. In the ^1H NMR spectra of all complexes significant changes of the host were found for the NH amido, the HC=N (except ligand **15**), and the $CH_2C(O)$ signals which clearly indicate the presence of a guest anion in the cavity.
In the ^{31}P NMR spectra of $H_2PO_4^-$ complexes with **12b**, **13a**, **13b**, and **14**

signals of $H_2PO_4^-$ are shifted downfield (1.9-2.3 ppm) in comparison with free $H_2PO_4^-$.

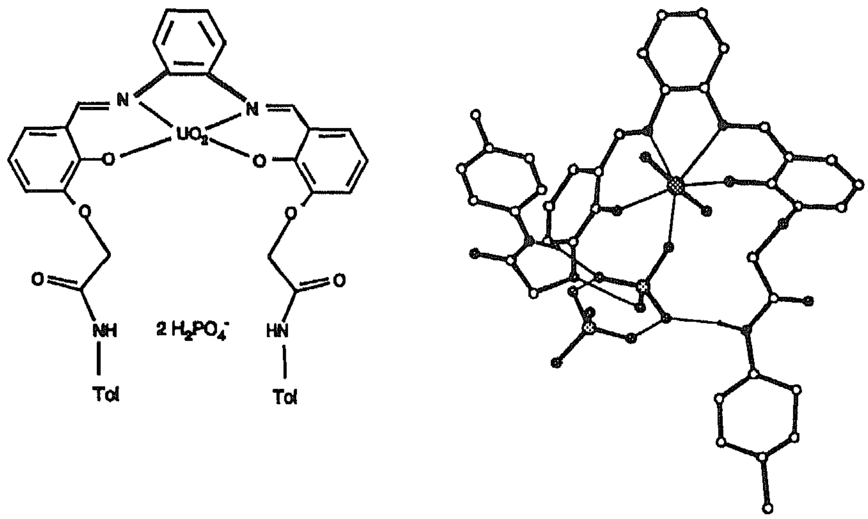
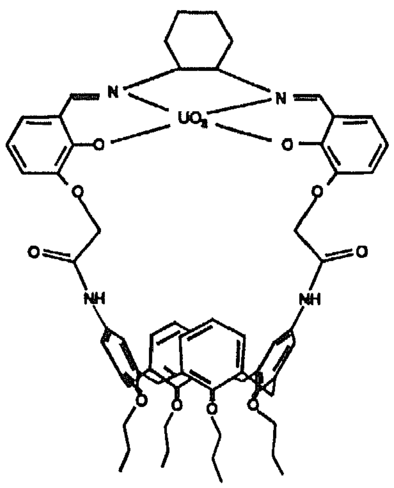


*Fig. 3. X-ray structure of the complex **13e**·2$H_2PO_4^-$*



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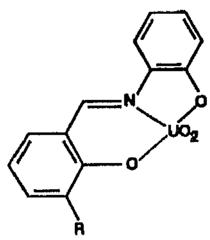


16a: R=H
16b: R=$OCH_2C(O)NH$-C_6H_4Me-4
16c: R=$O(CH_2)_2O$-C_6H_4-$C(O)NH_2$-2

In receptors **13a-e**, **14**, and **15** the uranyl center contains only one vacant position. The so-called "naked salophens" **16a-c** which have two vacant positions for complexation with guests were also synthesized.

The crystal structure of the complex **16a** · $2Bu_4N^+H_2PO_4^-$ is presented in Figure 4 and clearly shows that binding of two $H_2PO_4^-$ anions takes place (U···O-P distance 2.33 Å).

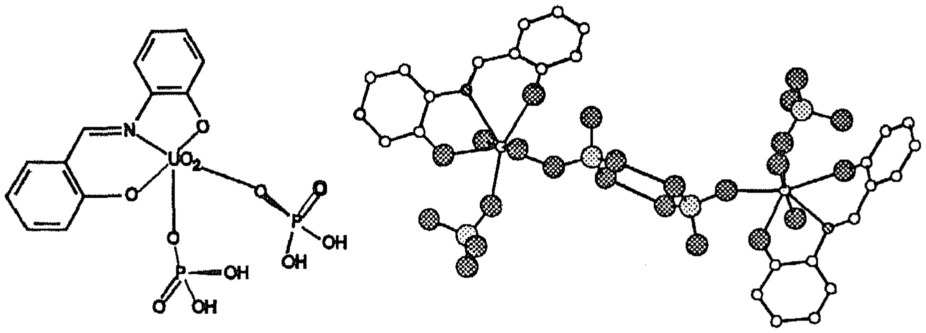


*Fig. 4. X-ray structure of the complex **16a**·$2H_2PO_4^-$*

As in previous cases phosphate anions form H-bonded dimers with phosphates complexed by another molecule of **16a** (O···O distance 2.53 Å). At the same time due to the unique fact that two phosphates are complexed by the UO_2-cation, molecules of the complex are organized as a H-bonded ribbon [ref 7].

The first neutral ditopic receptor **17** for adenosine monophosphate (AMP^{2-}) employing the combination of a Lewis acid-anion interaction and complementary base-pairing, has been prepared [ref 9].

In receptor **17** both a UO_2-center and a thymine fragment participate in the nucleotide anion complexation. Solid complex of **17** with the 3'-AMP^{2-} anion exhibits in the negative FAB mass spectrum an intense (ca 55%) signal of [**17** + 3'-AMP + $Bu_4N]^-$ (Figure 5).

Anion Recognition in Solution. The complexation of anions in solution was studied first by conductometry. Already the simple UO_2-salophens **12a,b** show strong binding of different anions (e.g Cl^-, $H_2PO_4^-$, NO_2^-) in MeCN-DMSO, 99:1 solutions (Table 5). In all cases a preference for $H_2PO_4^-$ binding was observed. The binding of anions by metalloclefts **13a,b** and **d** and metallomacrocycle **14**, which contain amido C(O)-NH functionalities, is remarkably strong. The influence of C(O)-NH moieties that are able to form H-bonds with anions complexed is demonstrated (exept $H_2PO_4^-$) by comparing the K_{ass} values of compounds **12b** and **13a,b** and **d** (Table 5).

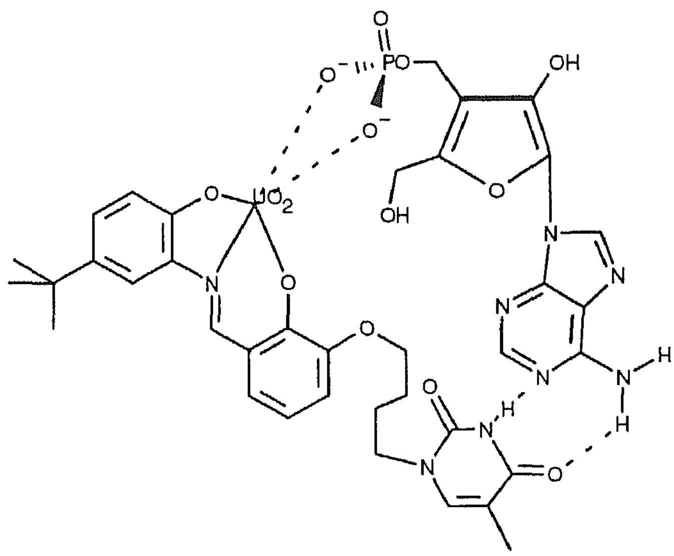


*Fig. 5. Proposed structure of the complex **17**·AMP^{2-}*

More preorganized ligands (**13b**, **13d** and **14**) exhibit a very strong (K_{ass} > 10^5 M^{-1}) and selective complexation of $H_2PO_4^-$. Compound **13b** shows for $H_2PO_4^-$ selectivities of > 10^2 over Cl^- and > 10^3 over NO_2^-. For HSO_4^- and SCN^- K_{ass} values of < 3 x 10^2 M^{-1} were obtained for all ligands.

The complexation of anions in solution was also studied by NMR spectroscopy. The addition of $Bu_4N^+H_2PO_4^-$ to solutions of **12b**, **13a,b,d,e**, and **14** in DMSO-d_6 gave two sets of HC=N, $CH_2C(O)$, and NH signals, both for the free and complexed ligand, indicating that the formation of kinetically stable (on the NMR time scale) complexes with $H_2PO_4^-$ takes place even in pure DMSO-d_6. In case of **15** only the shift of C(O)NH-protons was observed. The contribution of the C(O)NH···$H_2PO_4^-$ hydrogen bond interaction to the anion complexation is clearly reflected in the markedly downfield shifts Δδ 0.4-0.9 ppm of the NH signals for **13a,b,d,e**, **14**, and **15**. From dilution experiments the K_{ass} values of $H_2PO_4^-$ binding were calculated (Table 6). The influence of C(O)NH-moieties that are able to form H-bonds is demonstrated by comparing the K_{ass} values of compounds **12b**, in which binding mainly takes place via electrostatic interaction, and functionalized salens **13a,b,d** and **e** (Table 6).

More preorganized ligands **13b**, **13d**, **13e**, and **14** exhibit a very strong

(K_{ass} > 10^3 M^{-1}) complexation of $H_2PO_4^-$. Under the same conditions, complexation with Cl^-, HSO_4^-, SCN^-, and ClO_4^- was not observed. No changes in the ^1H NMR spectra of ligands **13a-e**, **14**, and **15** were found after addition of tetraalkylammonium salts of these anions which indicates that very *selective* $H_2PO_4^-$ recognition takes place.

Table 5. *Association constants (K_{ass}, M^{-1}) of functionalized sal(oph)ens determined by conductometry in MeCN-DMSO (99:1)*[a]

Anion	$H_2PO_4^-$	Cl^-	NO_2^-
12a	1.5×10^4	4.0×10^2	3.1×10^2
12b	2.0×10^4	< 300	< 300
13a	1.9×10^4	4.0×10^3	8.9×10^2
13b	> 10^5	1.7×10^3	4.5×10^2
13c[b]	8.0×10^3	< 5.0	< 5.0
13d	> 10^5	2.9×10^3	4.7×10^3
14	> 10^5	1.2×10^4	1.5×10^3

[a]Tetrabutylammonium salts were used.
[b]Determined by ^1H NMR in $CDCl_3$-DMSO-d_6 (9:1) due to low solubility in MeCN and DMSO.

Table 6. *Association constants (K_{ass}, M^{-1}) for $H_2PO_4^-$ anion complexation determined with ^1H NMR in DMSO-d_6*[a]

Ligand	K_{ass}, M^{-1}
12b	5.1×10^2
13a	8.4×10^2
13b	8.0×10^3
13c	(8.0×10^3)[b]
13d	2.0×10^3
13e	1.5×10^3
14	1.8×10^3
15	3.5×10^2

[a]Tetrabutylammonium salt was used.
[b]Determined by ^1H NMR in $CDCl_3$-DMSO-d_6 (9:1) due to low solubility in DMSO.

Analogously in the ^{31}P NMR spectra the addition of $Bu_4N^+H_2PO_4^-$ to solutions of **12b, 13a,b,d,e,** and **14** in DMSO-d_6 gave two signals ($\Delta\delta$ 1.9-2.3 ppm) of $H_2PO_4^-$ both for the free and complexed anion; the phosphate peak of the complex shifting downfield.

Since the "naked" salophens **16a-c** contain two free positions for coordination with guests, the complexation of the dianions of malonate and succinate was investigated. The K_{ass} values for **16a-c** and disodium salts of malonate and succinate dianions were determined by ^1H NMR titration experiments in DMSO-d_6 (Table 7). The HC=N signals of both the free and complexed ligand could be observed separately at 9.50-9.55 ppm and 9.40-9.47 ppm, respectively. Under the same conditions complexation with sodium acetate was not observed. From Table 7 it is clear that in the cases of **16b** and **16c** the contribution of the C(O)NH···dianion hydrogen bond interaction increases the strength of binding significantly. In the ^1H NMR spectra, NH signals of both free ligands **16b** and **16c** and their complexes can be separately observed at 10.50 ppm and 11.34 ppm for **16b**, respectively, and at 7.97, 7.45 ppm and 8.05, 7.85 ppm for **16c**, respectively. The previously described UO_2-salens like **13a,b,** and **14** do not or otherwise very weakly bind dianions as was concluded from the fact that no shifts in the ^1H NMR spectra have been observed after addition of malonate or succinate salts.

Table 7. *Association constants (K_{ass}, M^{-1}) of "naked" receptors determined with ^1H NMR in DMSO*[a]

Dianion	**16a**	**16b**	**16c**
Malonate	8.0×10^1	2.2×10^2	*b*
Succinate	1.7×10^2	4.6×10^2	1.5×10^2

[a]Disodium salts were used.
[b]No visible changes observed in ^1H NMR spectra.

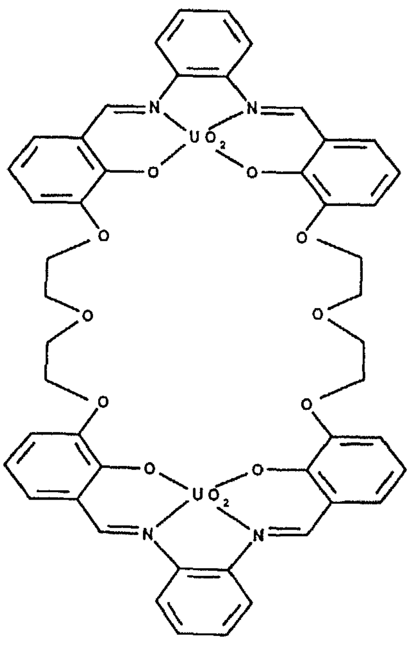
Titration of 3'-AMP^{2-} (as tetrabutylammonium salt) with **17** in DMSO-d_6 exhibited the appearance of an upfield HC=N singlet at 9.34 ppm at the expense of the free host singlet at 9.50 giving association constant of K_{ass} 1.2×10^3 M^{-1} [ref 9].

Dinuclear UO_2-sal(oph)en metallomacrocycles **18** and **19**, containing flexible and rigid cavities, respectively, form strong solution complexes with dicarboxylate anions such as terephthalate, succinate and fumarate in DMSO-d_6; weaker interactions between these hosts and the shorter malonate and monoanionic benzoate were observed (Table 8) [ref 10].

Table 8. *Association constants (K_{ass}, M^{-1}) of dinuclear receptors **18** and **19** determined with ^1H NMR in DMSO[a]*

Anion	**18**	**19**
Succinate[a]	5.0×10^3	2.7×10^3
Malonate[a]	4.5×10^2	1.7×10^2
Fumarate[a]	$> 10^5$	1.8×10^4
Terephthalate[b]	1.4×10^4	2.0×10^4
Benzoate[b]	2.0×10^2	*c*

[a]Tetrabutylammonium salts were used.
[b]Tetraethylammonium salts were used.
[c]No visible changes observed in the ^1H NMR spectra.



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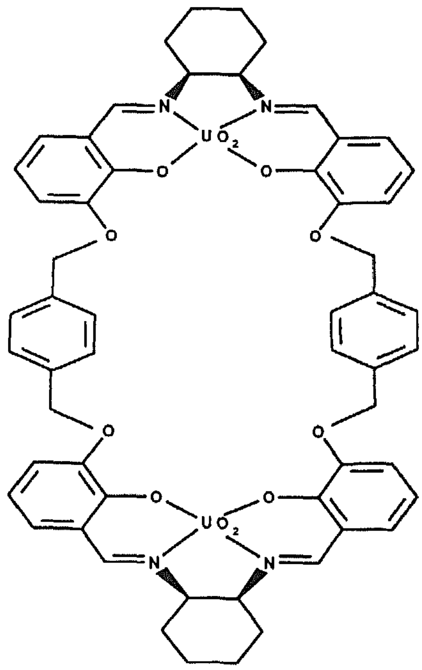


19

spectrum even in DMSO-d_6 as a solvent, showing the N-H signals of the free and complexed **20** at 10.47 and 11.21 ppm, respectively. Complexation of potassium cation K^+ by receptor **20** was studied by picrate extraction experiments to give in $CHCl_3$ a value of log K_{ex} = 5.3 for the 1:1 **20** · K^+Pic^- complex. The complexation of K^+ and $H_2PO_4^-$ ions by **20** has also been investigated by cyclic voltammetry in DMSO with 2% of H_2O using tetrabutylammonium tetraphenylborate as a supporting electrolyte. Addition of $Bu_4N^+H_2PO_4^-$ to a solution of **20** causes a clear shift of both cathodic and anodic peaks toward more negative potentials. This effect is accompanied by a systematic increase of the peak separation, from 85 to 100 mV, and by a decrease of the normalized peak heights. The results suggest that a labile, electroinactive complex with $H_2PO_4^-$ is formed. Assuming 1:1 stoichiometry, a K_{ass} value of 1.3 x 10^3 M^{-1} for $H_2PO_4^-$ was obtained which is in a good agreement with the ^1H NMR measurements. Addition of potassium tetraphenylborate to the DMSO solution of receptor **20** gave no changes in the electrochemical behavior, probably due to the fact that the crown ether moieties in **20** are situated rather far from the electroactive UO_2-center. However, an indirect procedure for the determination of complexation constants, based on competition between Tl^+ and K^+ cations gave a K_{ass} value of 1.0 x 10^2 M^{-1} for K^+. Finally, FAB-MS was used which is an established technique for investigation of non-covalent binding of cationic and anionic complexes. In the positive FAB-MS spectrum (*m*-nitrobenzyl alcohol as a matrix) of the 1:1 complex of receptor **20** and KH_2PO_4, prepared by mixing of the host and guest in MeCN with 10% of water followed by evaporation of solvents, an intense peak corresponding to [**20** + K]$^+$ was observed. The corresponding negative FAB-MS spectrum of the same sample showed an intense peak of [**20** + H_2PO_4]$^-$, while a signal of the [**20** + H_2PO_4 + K]$^-$ was also present, and this clearly proves the complexation of the salt.

Calix[4]arene Based Bifunctional Receptor for NaH_2PO_4 [ref 8]. In this paragraph the first calix[4]arene based bifunctional receptor **21** is described. This compound contains a combination of a UO_2-Lewis acidic center and C(O)NH groups which is known to act as anionic binding site for $H_2PO_4^-$. Besides calixarene **21** contains also four preorganized ester fragments which are known to complex alkali metal cations with a high selectivity for Na^+.

A study of the binding ability of receptor **21** has been carried out using the general strategy we described for the simple bifunctional molecule **20**. In this way it was found that receptor **21** selectively binds $H_2PO_4^-$ anions. From ^1H NMR dilution experiments with $Bu_4N^+H_2PO_4^-$ in

DMSO-d_6 an association constant K_{ass} of 3.9 x 10^2 M^{-1} was calculated. The contribution of the C(O)NH···$H_2PO_4^-$ hydrogen bond interaction to the anion complexation can be seen from a significant downfield shift of the C(O)NH protons of ca 0.4 ppm upon complexation. Only minor shifts were observed upon dilution experiments with tetrabutylammonium salts of Cl^-, HSO_4^- and ClO_4^- anions which indicates their weak binding (K_{ass} < 10 M^{-1}). In the negative FAB mass spectrum of the 1:1 complex of **21** with $Bu_4N^+H_2PO_4^-$, prepared by mixing of host and guest in MeCN, an intense peak corresponding to [**21** + $H_2PO_4]^-$ was observed. Moreover, in the positive FAB mass spectrum of the 1:1 complex of **21** and $Na^+H_2PO_4^-$, prepared by mixing of host and guest in MeCN-H_2O, 10:1, an intense peak corresponding to [**21** + Na$]^+$ was observed, while the corresponding negative FAB mass spectrum of the same sample yielded an intense peak for [**21** + $H_2PO_4]^-$, which proves the complexation of both cation and anion in one bifunctional receptor molecule.

5. Anion Carrier Mediated Membrane Transport of Phosphate; Selectivity of $H_2PO_4^-$ over Cl^-

Since last two decades numerous papers have appeared on the selective transport of salts through supported liquid membranes (SLM) mediated by neutral cation carriers. In transport assisted by neutral cation carriers (CC) such as simple crown ethers, calixarenes, or natural ionophores like valinomycin, anions affect the transport rates because of the different dehydration energies. Therefore, lipophilic anions like ClO_4^-, NO_3^-, or SCN^- are often used as the counterion. Much less lipophilic anions like Cl^- or $H_2PO_4^-$ have only been transported by charged anion carriers via an ion-exchange mechanism, and the transport selectivity follows the Hofmeister series: ClO_4^- > I^- > SCN^- > NO_3^- > Br^- > Cl^- >> CO_3^{2-}, $H_2PO_4^-$, SO_4^{2-}. Here we report the selective transport of $H_2PO_4^-$ over Cl^- through SLM, either exclusively by an anion receptor or by a combination of anion and cation receptors [ref 12]. The receptors exhibit selectivity opposite to the order of dehydration energies of the anions in the Hofmeister series.

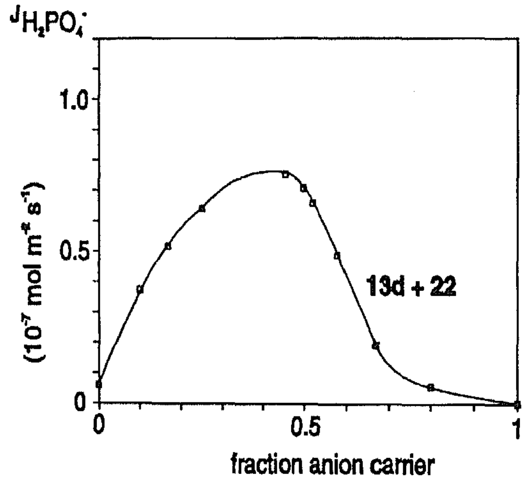
Anion receptors **13d,f,j**, described previously, were used in SLM with *o*-nitrophenyl-*n*-octyl ether (NPOE) as the membrane solvent immobilized in an Accurel/1E-PP support (Table 9). Receptors **13d,f,j** are already effective on their own as $H_2PO_4^-$ carriers in the transport of KH_2PO_4, although the fluxes are low. Surprisingly, despite strong $H_2PO_4^-$ binding detected for **13d** by ^{1}H NMR (DMSO) and conductometry (MeCN:DMSO=99:1), transport of KH_2PO_4 through NPOE could not be detected.

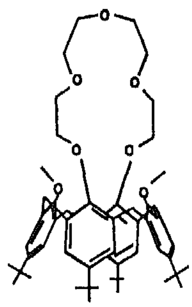
Table 9. *Effect of the combination of anion carriers and cation carrier* ***22*** *on KH_2PO_4 flux*

Carrier(s)[a]	J $(KH_2PO_4)^b$, 10^{-8} mol m^{-2} s^{-1}
13d	< 0.2
13f	3.2
13j	5.1
13d + 22	7.2
13f + 22	5.0
13j + 22	12.5

[a][Anion carrier] = [Cation carrier] = 0.02 M.
[b]$[KH_2PO_4] + [K_2HPO_4]$ = 0.2 M; pH_s = 6.8

To improve the flux a K^+ selective cation carrier, calix[4]arene crown-5 **22**, was added to the membrane solution for the simultaneous facilitation of the potassium ion transport (Table 9). The flux of KH_2PO_4 mediated by the combination of carriers **13j** and **22** is the highest in the series (12.5 x 10^{-8} mol m^{-2} s^{-1}) and higher than obtained with any of the anion carriers alone. The absolute concentrations of the 1:1 combination of carriers **13d** and **22** have also been varied. A steady increase of the flux was observed. The fact that receptor **13d** alone was not effective as a carrier for KH_2PO_4, but only in combination with **22**, prompted us to measure the KH_2PO_4 flux as a function of their concentration ratio [**13d**]/[**22**] (Figure 6).





22

Fig. 6. Transport of KH_2PO_4 mediated by the combination of carriers ***13d*** *and* ***22*** *as a function of the percentage of anion carrier. [**13d**] = [**22**] = 0.04 M; $[KH_2PO_4]_s + [K_2HPO_4]_s$ = = 0.2 M; pH_s = 6.8*

The optimum flux is reached at about equal concentrations of **13d** and **22**. Finally, we have investigated the transport selectivity for $H_2PO_4^-$ over Cl^- in competition experiments from a source phase that contained 1 x 10^{-3} M of $KH_2PO_4^-$ and 1 x 10^{-1} M of KCl by the combination of anion receptors **13d,f,j** and carrier **22** (Table 10).

Table 10. *transport selectivity for $H_2PO_4^-$ over Cl^-*

Anion carrier(s)[a]	J $(H_2PO_4^-)^b$, 10^{-8} mol m^{-2} s^{-1}	J $(Cl^-)^c$, 10^{-8} mol m^{-2} s^{-1}	S
13d	2.19	7.60	29
13f	1.81	4.75	38
13j	7.52	5.25	143

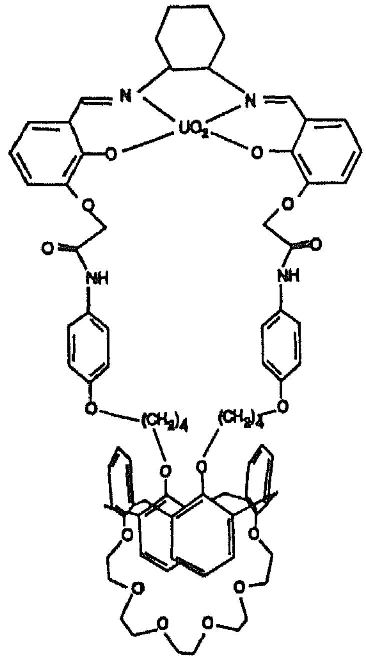
[a][Anion carrier] = [Cation carrier] = 0.02 M.
[b]$[KH_2PO_4]_s$ = 0.001 M, no K_2HPO_4 added.
[c]$[KCl]_s$ = 0.1 M.

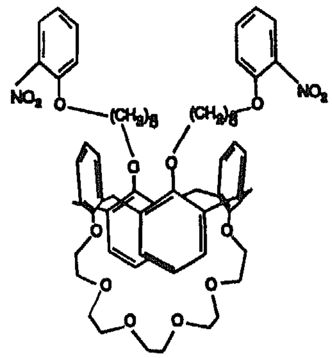
The selectivity, S, is defined as the ratio of the fluxes divided by the ratio of source phase anion concentrations. All anion carriers show transport selectivity for $H_2PO_4^-$ over Cl^-, which is opposite to the dehydration energy according to the Hofmeister series. The transport selectivity for phosphate over chloride increases as a function of the anion receptor in the order **13d** < **13f** < **13j** up to a value of about 140.

6. Simultaneous Transport of Cations and Anions through a Supported Liquid Membrane.

Previously we have described the neutral bifunctional receptors for the *simultaneous complexation* of hydrophilic anions and cations in organic media. We have also investigated the *simultaneous transport* of cations and anions through SLM assisted by a neutral bifunctional receptor [ref 13].

Our synthetic strategy was based on the attachment of both cation and anion binding sites to the rigid lipophilic calix[4]arene platform The covalent combination of a Lewis acidic UO_2-center and amido C(O)NH moieties provides an excellent receptor site for dihydrogen phosphate $H_2PO_4^-$ and chloride Cl^- anions, and that the calix[4]arene crown-6 (1,3-alternate) fragment is capable of thhe selective complexation of cesium ion Cs^+. Thus, bifunctional receptor **23** has been prepared and used as a carrier to investigate the transport of hydrophilic cesium chloride (CsCl) and the more lipophilic cesium nitrate ($CsNO_3$) [ΔG^0_{tr} (X^-, $H_2O \rightarrow MeCN$)





23 24

42.1 and 21.0 kJ/mol for Cl^- and NO_3^-, respectively] across a supported liquid membrane composed af a porous polymeric support (Accurel) impregnated with *o*-nitrophenyl *n*-octyl ether (NPOE). For comparison, the same expperiment was performed with the receptors **13c** and **24** that have either only anion or cation binding sites, respectively (Table 11).
The transport processes for $CsNO_3$ and CsCl are different; NO_3^- is a much more lipophilic than Cl^- and only NO_3^- can easily follow the complexed Cs^+ cation through the hydrophobic membrane, even in the absence of anion carrier. With the cation carrier **24** a high flux of $CsNO_3$ (5.5×10^{-7} mol m^{-2} s^{-1}) was observed but the anion receptor **13c**, which is not selective for NO_3^-, did not transport $CsNO_3$. The flux was very low (0.02×10^{-7} mol m^{-2} s^{-1}) and comparable with the (blank) flux obtained without carrier. It implies that, probably, in case of **24** *only* the cation binding site is responsible for the transport.

Table 11. *Salt fluxes[a-c] trough SLM measured for different carriers[d,e] in NPOE*

Carrier	$CsNO_3$ flux	CsCl flux
13c	0.02	0.07
24	5.50	0.42
23	0.89	1.20

[a]Salt concentration 0.1 M.
[b]Fluxes in 10^{-7} mol m^{-2} s^{-1} after 24 h at 298 K.
[c]Blank fluxes of the salts in NPOE, for CsCl, 0.05 x 10^{-7} mol m^{-2} s^{-1}, and for $CsNO_3$, 0.02 x 10^{-7} mol m^{-2} s^{-1}.
[d]Carrier in membrane, 0.01 M.
[e]No leakage of receptors was observed in blank experiments.

The transport of CsCl by the monofunctional carriers **13c** (anion) and **24** (cation) exhibits low flux values of 0.07 x 10^{-7} mol m^{-2} s^{-1} and 0.42 x 10^{-7} mol m^{-2} s^{-1}, respectively. Obviously, when one of the ionic species is complexed, the uncomplexed counter-ion can not sufficiently penetrate the lipophilic membrane.

However, a significant flux (1.20 x 10^{-7} mol m^{-2} s^{-1}) was observed for bifunctional carrier **23** with CsCl, which is much higher than the corresponding ones for the monofunctional carriers **13c** and **24**. At the same time, carrier **23** showed a surprisingly low flux of $CsNO_3$ (0.89 x 10^{-7} mol m^{-2} s^{-1}) when compared with the flux observed for cation receptor **24** (5.50 x 10^{-7} mol m^{-2} s^{-1}). This proves that: (i) *both* anion and cation binding sites of **23** are involved in the complexation; and (ii) the presence of only an anion or a cation binding site in the receptor molecule is *not sufficient* for effective transport of a *hydrophilic* salt such as CsCl. But more important is that this suggests a *preference* of hydrophilic CsCl over lipophilic $CsNO_3$.

These results indicate the unique feature of receptors in which both binding sites are *covalently* linked.

7. Conclusions.

Approaches to *neutral anion receptors* which are able to *selectively* complex anionic species either by hydrogen bonds or by combination of Lewis acidic UO_2 center and amido functionalities are developed. A new concept has been described of so called *bifunctional* receptors which contain both anion and cation binding sites and therefore are able to complex *simultaneously* anionic and cationic species in apolar solvents. the calix[4]arene platform (cone and 1,3-alternate conformations) and calix[6]arene platform have been successfully applied for the immobilization of (cation and) anion binding sites in the preparation of different types of anion and bifunctional receptors. The results on selective transport of $H_2PO_4^-$ anions through a supported liquid membrane assisted by anion receptors, and simultaneous transport of hydrophilic cations and anions assisted by a neutral bifunctional receptor are described. This is the first example of carrier mediated co-transport, in which the anion and cation of a hydrophilic salt are bound and transported simultaneously through a membrane.

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9. References.

[1] (a) Leucke, H.; Quiocho, F. A. *Nature* **1990**, *347*, 402-406. (b) He, J. J.; Quiocho, F. A. *Science* **1991**, *251*, 1479-1481. (c) Pflugrath, J. W.; Quiocho, F. A. *J. Mol. Biol.* **1988**, *200*, 163-180. (d) Kanyo, Z. F.; Christianson, D. W. *J. Biol. Chem.* **1991**, *266*, 4264-4268. (e) Thatcher, G. R. J.; Cameron, D. R.; Nagelkerke, R.; Schmitke, J. *J. Chem. Soc., Chem. Comun.* **1992**, 386-388. (f) Mangani, S.; Ferraroni, M.; Orioli, P. *Inorg. Chem.* **1994**, *33*, 3421-3423.

[2] For a recent review see: Dietrich, B. *Pure Appl. Chem.* **1993**, *65*, 1457-1464.

[3] Valiyaveettil, S.; Engbersen, J. F. J.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 900-901.

[4] Morzherin, Y.; Rudkevich, D. M.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1993**, *58*, 7602-7605.

[5] Scheerder, J.; Fochi, M.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem.* **1994**, *59*, 7815-7820.

[6] Scheerder, J.; Engbersen, J. F. J.; Casnati, A.; Ungaro, R.; Reinhoudt, D. N., submitted for publication.

[7] (a) Rudkevich, D. M.; Stauthamer, W. P. R. V.; Verboom, W.; Engbersen, J. F. J.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1992**, *114*, 9671-9673. (b) Rudkevich, D. M.; Verboom, W.; Brzozka, Z.; Palys, M. J.; Stauthamer, W. P. R. V.; van Hummel, G. J.; Franken, S. M.; Harkema, S.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1994**, *116*, 4341-4351.

[8] Rudkevich, D. M.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1994**, *59*, 3683-3686.

[9] Lacy, S. M.; Rudkevich, D. M.; Verboom, W.; Reinhoudt, D. N. *Tetrahedron Lett.* **1994**, *35*, 5953-5956.

[10] Lacy, S. M.; Rudkevich, D. M.; Verboom, W.; Reinhoudt, D. N. *J Chem. Soc., Perkin Trans. 2* **1995**, 135-139.

[11] Rudkevich, D. M.; Brzozka, Z.; Palys, M.; Visser, H. C.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 467-468.

[12] Visser, H. C.; Rudkevich, D. M.; Verboom, W.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1994**, *116*, 11554-11555.

[13] Rudkevich, D. M.; Mercer-Chalmers, J. D.; Verboom, W.; Ungaro, R.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6124-6125.