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Functionalized Derivatives of Benzocrown-Ethers, V
Multiple Molecular Recognition of Zwitterionic Phenylalanine

Mihai D. Barboiu*[a,b,c], Nadine D. Hovnanian[a], Constantin Luca[b], Louis Cot[a]

[a]*Laboratoire des Matériaux et Procédés Membranaires, Ecole Nationale Supérieure de Chimie Montpellier 8, rue de l'Ecole Normale, F-34296 Montpellier, Cedex 5, France.*

[b]*University "Politehnica" Bucharest, Dept. of Analytical Chemistry, 1, Polizu, R-78126, Bucharest, Romania*

[c]*Research Centre of Macromolecular Materials and Membranes, 206, Spl. Independentei, P.O. Box 15-143, R-79611, Bucharest, Romania*

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Abstract: The synthesis of the polytopic receptor 4-(4'-benzo-18-crown-6-sulfonamido)benzo-15-crown-5 is reported. This polytopic receptor provides multiple molecular recognition of zwitterionic phenylalanine through different non-covalent bonds. Active membrane transport mechanisms by bulk liquid membranes of the phenylalanine are presented as a function of the co-transported alkali cation.

Multiple molecular recognition controls or initial specific biophysical functions are the essence of biological phenomena. An elucidation of the rules and restrictions which govern these intermolecular interactions is important for understanding and manipulation of these processes. Therefore the design and synthesis of new synthetic receptors has become an important field of supramolecular chemistry.[1-5] Molecular recognition of neutral or charged organic molecules has only recently received attention in sharp contrast to the far more advanced development of the corresponding coordination chemistry of cations.[6-8] Organic substrates are more bulkier thus, are more polarizable and more strongly solvated than cations. For these reasons the artificial receptors should have a specific geometry with an organized disposition of binding sites, to allow a strong entrapment of organic guest. Much interest in the field of molecular recognition of organic compounds has focused on the amino acids. The selective complexation via three hydrogen bonds of protonated ammonium moieties **1, 2** [9-12] or the charge interactions of carboxylate form **3** [13] can allow an enantiomeric molecular recognition of the amino acid (see Figure 1).[11-13]

The simultaneous recognition of both charged moieties of the amino acids is more difficult to realise, due to the hydrophilic nature of the zwitterionic form, which preferentially dissolves in water. This form may be complexed by combining the non-covalent interaction forces between the amino acid moieties and favourably disposed complexation sites of the receptor.[1, 14-19] Functionalized receptor **4** showed a higher selectivity for ω-amino acids as the chain length decreased.[17] Polytopic macrocyclic receptor **5**, reported by us, were able to complex the zwitterionic form of the amino acids by double non-covalent interactions[15, 16, 20] (Fig. 2).

Tritopic receptor **6** provided enantioselective molecular recognition for aromatic α-amino acids under neutral conditions.[1,14,21] Molecular mechanics calculations reveal that, in the case of supramolecular complex **7** in absence of solvent, almost half of the stabilisation energy comes from the electrostatic interaction and hydrogen bonding of the bicyclic guanidinium form. Additionally, one-third of the stabilization energy is provided by the ammonium-crown interaction and about one-sixth arises from π-π interactions (Fig. 3).

Corresponding author: e-mail: barboiu@cit.enscm.fr; Fax: +33 4 67 14 43 47

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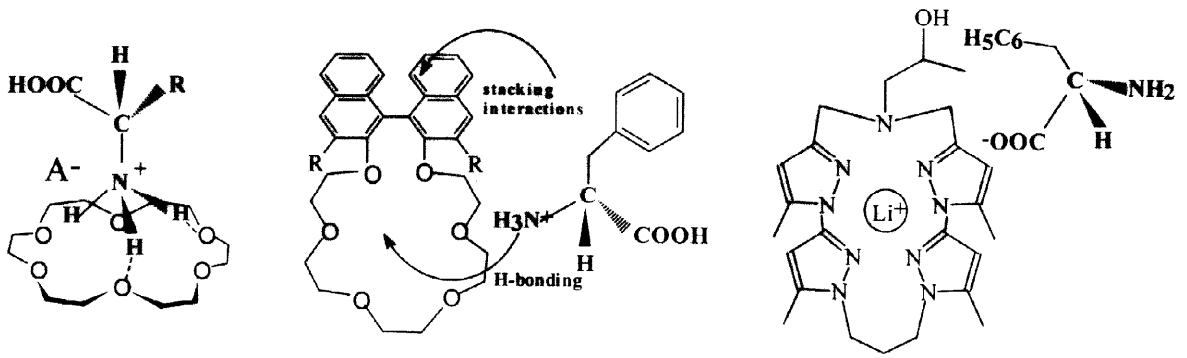


1 (S)- 2 (R=-C_6H_5) L-Phe 3

Fig. 1: Molecular recognition of ammonium (H_3N^+-R-COOH) **1**, **2** and the carboxylate (H_2N-R-COO^-) **3** amino forms of amino acids by monotopic macrocyclic receptors.

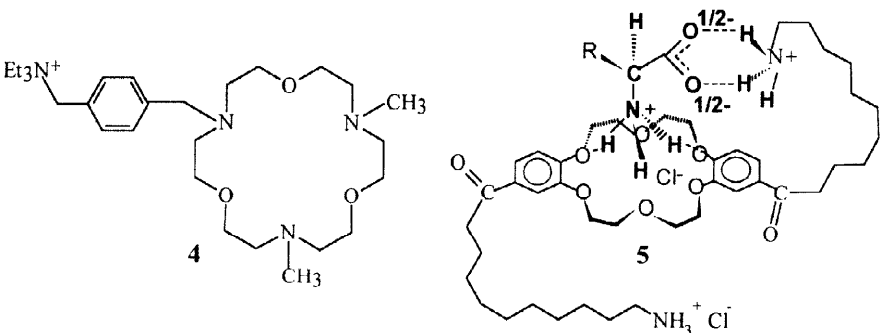


Fig. 2: Molecular recognition of zwitterionic amino acids by ditopic macrocyclic receptors **4** and **5**.

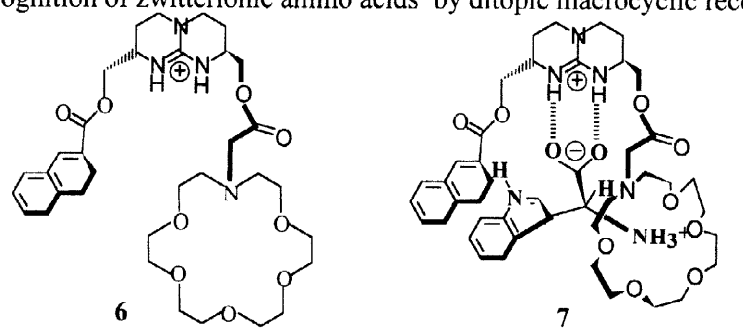
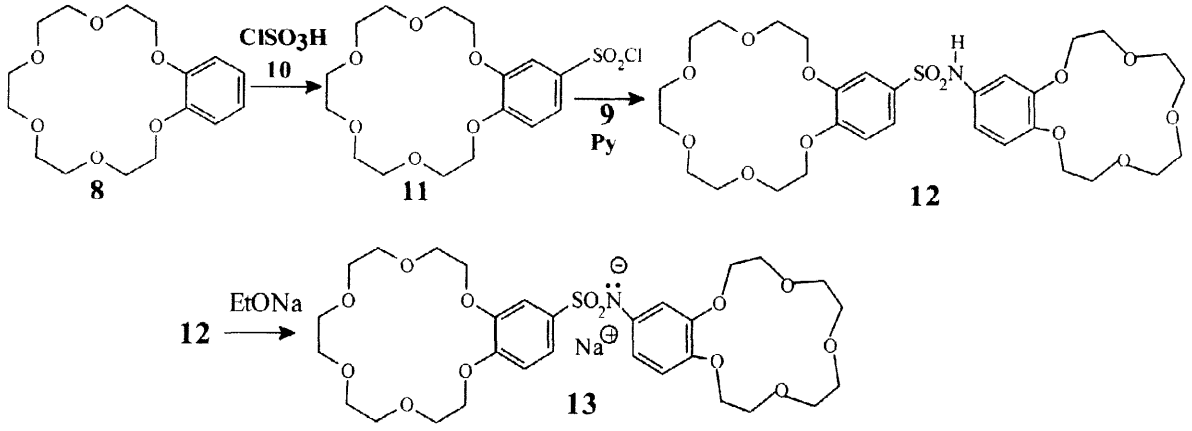


Fig. 3: Molecular recognition of L-Tryptophan by the tritopic receptor **6**.

In this paper, we proposed a new type of multiple molecular recognition of zwitterionic phenylalanine. We report the preparation of unsymmetric bis-macrocyclic sulfonamide: 4-(4'-benzo-18-crown-6-sulfonamido)benzo-15-crown-5, **12**. Our interest is to use this molecule as a polytopic receptor for multiple molecular recognition of zwitterionic phenylalanine by combining different non-covalent interaction types. Synthesis of different solid supramolecular complexes of **12** with the phenylalanine and their characterization by IR, UV-Visible, ^{1}H-NMR and ^{13}C-NMR spectroscopy as well as U-tube membrane transport experiments will be presented.

The macrocylic receptors used in the syntheses were the well-known, commercially derivatives: benzo-18-crown-6 (**8**) and 4-aminobenzo-15-crown-5 (**9**). Treatment of **8** with chlorosulfonic acid (**10**) led to the 4-(4 chlorosulfonyl)benzo-18-crown-6 (**11**) which upon reaction with (**9**) gave the unsymmetric bis-macrocyclic derivative: 4-(4'-benzo-18-crown-6-sulfonamido)benzo-15-crown-5 (**12**). Sodium salt **13** was synthesised by

treating bis-macrocyclic compound **12** with EtONa in EtOH (Scheme 1). Reaction of **12** with phenylalanine (*Phe*) or with phenylalanine in the presence of sodium picrate or sodium tetraphenylborate afforded supramolecular complexes **14**, **15** and **16**, respectively.



Scheme 1

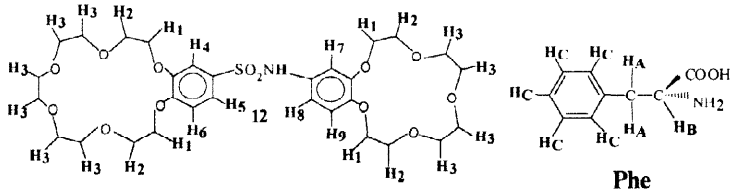
Compounds **11-13**, as well as supramolecular complexes **14-16** reported herein, were characterized by elemental analyses, IR and ^{1}H or ^{13}C NMR spectroscopy. Elemental analysis data for compounds **11-16** were within ±0.4% of theoretical values calculated for the proposed formulae, thus confirming the 1:1 stoichiometry of the bis-macrocyclic compound **12** and phenylalanine or metal ion in complexes **14-16**.

In the IR spectrum of **11**, the ν_{SO2Cl} bands appeared at ν= 1150 and 1373 cm^{-1}. For macrocyclic bis-benzenesulfonamide **12** characteristic sulfonamide IR vibrations appeared at: $\nu_{SO2\ sym}$= 1135 cm^{-1}, $\nu_{SO2\ as}$= 1347 and ν_{NH}= 3235 cm^{-1}. In the IR spectra of **13** and supramolecular complexes **14-16**, these sulfonamide bands were shifted to the lower frequencies: 1110-1125 cm^{-1} and 1321-1336 cm^{-1}, respectively. A change in the ether vibrations are also observed on going from the uncomplexed bis-benzenesulfonamide to the supramolecular complexes in symmetrical ($\nu_{C\text{-}O\text{-}CAr\ sym.}$= 1266 cm^{-1} to 1271 cm^{-1}) and in asymmetrical ($\nu_{CH2asym.}$= 2939 cm^{-1} to 2923 cm^{-1}) stretchings. These observations possibly are attributed to the complexation of ammonium (18-crown-6) or sodium (15-crown-5) moieties within the macrocyclic cavities. In the IR spectra of the supramolecular complexes, the characteristic bands of the amino acid could be detected: a broad absorption band was observed in the range 2400-3600 cm^{-1} (arising from asymmetrical and symmetrical combination stretchings of the NH_3^+, OH and CH groups) and a strong carboxylate (COO^-) anion vibration band at ν= 1618-1641 cm^{-1}. The characteristic bands of picrate anion (ν_{NO2sym}= 1336 cm^{-1} and ν_{NO2as}=1517 cm^{-1}) and strong tetraphenylborate bands ($\nu_{B\text{-}C6H5}$= 710-737 cm^{-1}) were also observed in the IR spectra of supramolecular complexes **15** and **16**.

The interactions between macrocyclic bis-benzenesulfonamide **12**, the amino acid and associated counter-ions are also supported by the ^{1}H NMR spectra of new supramolecular complexes **14-16**. (Table 1) In the proton NMR spectrum of **12**, the signals for the crown-ether protons were reduced to three types: the H_1 and H_2 protons α or β to the aromatic rings and the H_3 protons from the non-vicinal bridges of crown moieties (see Table 1). The aromatic proton signals were at δ=6.55-7.36. The sulfonamide proton resonate as a singlet at δ=

9.1. Upon complexation with the phenylalanine the ethylenic protons signals of the ligand undergo chemical shift changes of varying magnitudes (Table 1). Broadened, merged and split signals of the macroring protons are the result of anisotropic effects and the reduction of the conformational flexibility by complexation of the bis-macrocyclic by the cation.

Table 1. Proton NMR signals of **12** and the chemical shift changes complexes **14-16** in CD_3OD at 25 °C.



Phe

Species	**12**	**14**; **12**+Phe [a]	**15**; **12**+Phe+PiNa [a]	**16**; **12**+Phe+$NaBPh_4$ [a]
Proton	**δ, ppm**	**Δδ, ppm**	**Δδ, ppm**	**Δδ, ppm**
H_1	3.97-4.11	0	-0.061	+0.1115
H_2	3.78	-0.0205[e]	-0.0205	+0.1115
H_3	3.54-3.65	-0.1125	-0.0715	0
H_4	6.91-6.95	-0.051	-0.0825	-[c]
H_5	7.14	-[b]	-[b]	-[c]
H_6	7.25-7.30	-0.0625	-0.0825	-[c]
H_7	6.54-6.59	0	-0.04	-0.051
H_8+H_9	6.69-6.73	-0.0205	-0.051	0
	Phe			
H_A	3.16-3.30	+0.232	+0.264	+0.243
H_B	3.99-4.03	+0.713	+0.8125	+0.7125
Hc	7.34-7.47	+0.264	+0.184	-[d]

[a] The symbols represent Phe-Phenylalanine; PiNa-Sodium Picrate, $NaBPh_4$- Sodium tetraphenylborate. [b] overlapping with the aromatic protons of the Phe. [c] overlapping with the aromatic protons of Phe and BPh_4^-. [d] overlapping with the aromatic protons of the BPh_4^-. [e] the "-"refers to downfield and the positive values to upfield shifts.

As it can be seen in the Table 1, in the case of supramolecular complex **14**, significant downfield shifts occur for the aromatic protons of the benzo-18-crown-6 moiety. This fact can be understood in terms of electrodonating interactions of bis-macrocyclic receptor with the amino acid, which results in a magnetic deshielding effect.[22] All these downfield effects are caused by hydrogen bondings of the ammonium moiety of the amino acid by the oxygen atoms of the macroring. In the ^{1}H-NMR spectrum of bis-macrocyclic sulfonamide sodium salt **13**, the downfield shifts of aromatic protons of the 15-crown-5 moiety were explained by a specific complexation of the Na^+ ion in the 15-crown-5 cavity (see experimental). On the other hand, in the ^{1}H-NMR spectra of ternary supramolecular complexes **15** and **16** the downfield effects of the aromatic protons of the 18-crown-6 moiety (due to the complexation of ammonium moiety) were accompanied by supplementary downfield shifts of aromatic protons of the15-crown-5 moiety (due to the complexation of the Na^+ cation). Similar downfield shifts were observed when functionalized azacrown-ethers formed complexes with Na^+ and K^+.[22] The upfield effect observed for the H_1 and H_2 protons of the ethylenic bridges could be caused by the association of the supramolecular cationic aggregate with the tetraphenylborate counter-ion, which increases the electron density of these moieties. This can be understood from the electron-donating point of view. An opposite downfield effect of the H_1-H_3 protons was evidenced in the case of electron-accepting picrate anion (Table 1). Large upfield chemical shift changes in the phenylalanine ^{1}H NMR spectra can be seen upon

complexation with bis-macrocyclic receptor (Table 1). The complexation of phenylalanine into a macrobicyclic architecture increases its electron density. The upfield shifts of aromatic protons of phenylalanine can be attributed to π-π-stacking interactions with macrocyclic aromatic rings.[18] In the ^{1}H NMR spectra of ternary supramolecular complexes **15** and **16** the characteristic signals of the picrate (δ= 8.67) and of the tetraphenylborate (δ=6.68-6.86 and δ=6.90-6.94) anions were also observed.

Multiple molecular recognition of zwitterionic amino acid by the polytopic macrocyclic receptor was also supported by the ^{13}C NMR spectra. (Fig. 4)

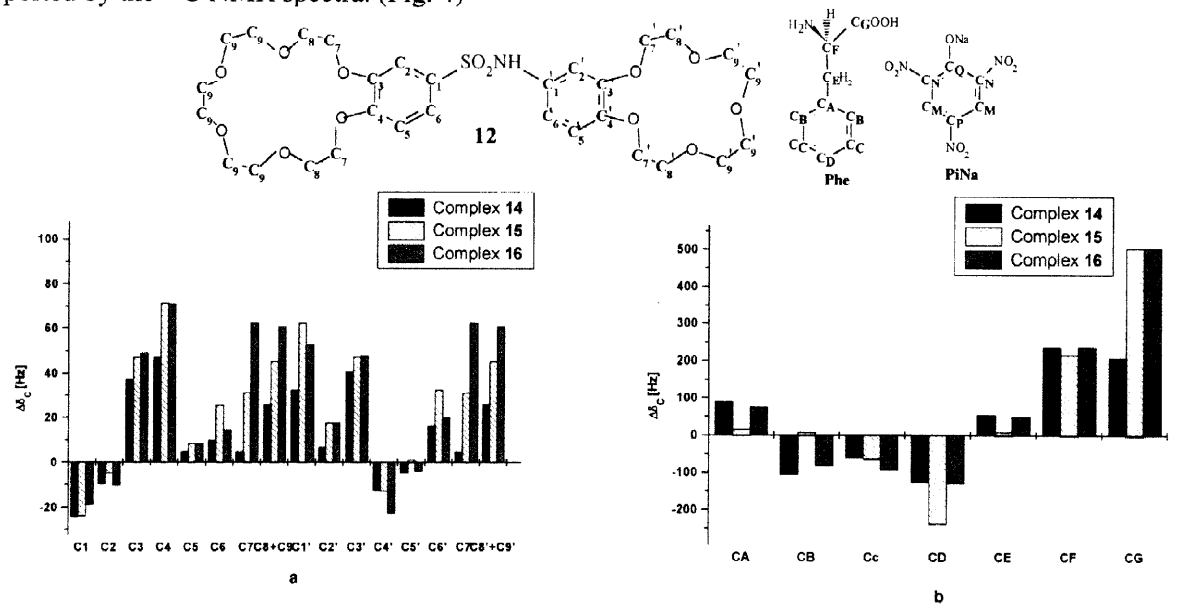


Fig. 4: Chemical induced shifts Δδ, Hz of (a) macrocyclic and (b) phenylalanine carbon atoms upon complexation.

Upon complexation with phenylalanine, upfield shifts occur for the ethylenic carbons of bis-macrocyclic receptor in an opposite way with the downfield effect observed in the ^{1}H NMR spectra of the supramolecular complexes. Complexation of cationic species ($-NH_3^+$ and Na^+) in macrocyclic cavities increase the electron density at the carbon atom probably due to a cooperative effect: electron accepting properties of complexed oxygen atom and electron donating or hyperconjugative properties of geminal hydrogen atoms. Significant upfield shifts for the signals of the ethylenic carbons of the 15-crown-5 macroring (Fig.4a) and for the carboxylate carbon (C_G) (Fig. 4b) were observed in the ^{13}C NMR spectra on going from simple supramolecular complex **14** to the ternary supramolecular complexes **15** and **16**. Significant upfield shifts for the carboxylate carbon (C_G) due to charge interactions and opposite downfield shifts for phenylalanine aromatic carbons (C_C,C_B,C_D), attributed to π-π stacking interactions with the aromatic moieties of macrocyclic receptor were evidenced. The other carbons (C_A,C_E,C_F,C_G) presented upfield shifts, explained by the electron donating properties of the complexed amino acid (Fig.4b).

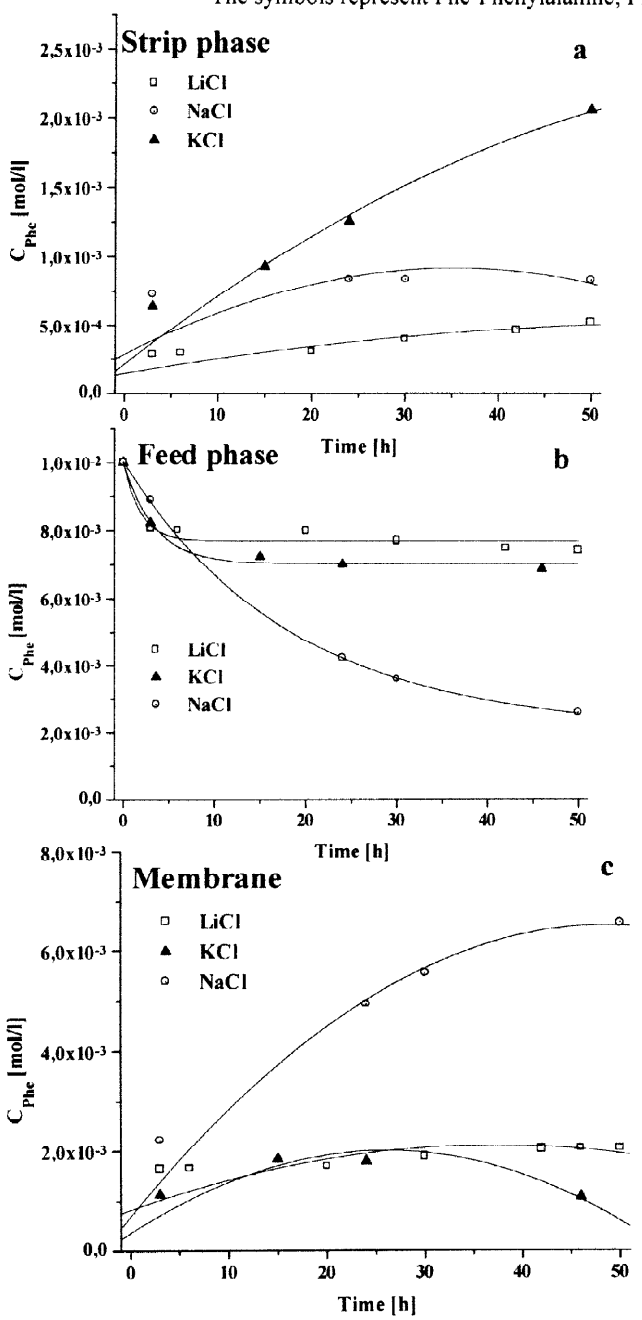
U-tube membrane transport experiments of phenylalanine ($CHCl_3$) (Table 2) have also confirmed multiple molecular recognition of zwitterionic amino acids by polytopic macrocyclic receptor **12**. (Fig.5)

By varying the co-transported alkali cation added into the feed phase (Li^+, Na^+ or K^+), we observed different transport time-dependent profiles of phenylalanine concentration in the strip, (Fig.5a) the feed (Fig.5b) and the membrane phase (Fig.5c).

Table 2. Amino acid transport rates (K_t) by liquid membranes using **12** (10^{-3} M, $CHCl_3$) as carrier,as a function of co-transported alkali cation (Li^+, Na^+ or K^+) .

	Feed Phase (aq.)	Strip phase (aq.)	k_t (10^{-3} mol cm^{-2} h^{-1})
1.	10^{-2} M Phe [a] +10^{-2} M LiCl[b]	10^{-4} M LiOH[c]	10.1
2.	10^{-2} M Phe +10^{-2} M NaCl[b]	10^{-4} M LiOH[c]	4.4
3.	10^{-2} M Phe +10^{-2} M KCl[b]	10^{-4} M LiOH[c]	66.8
4.	10^{-2} M Phe +10^{-2} M PiNa [a] [b]	10^{-4} M LiOH[c]	66.1
5.	10^{-2} M Phe+10^{-4} M KOH[c]	10^{-4} M LiOH[c]	139

[a] The symbols represent Phe-Phenylalanine; PiNa-Sodium Picrate; [b] pH=pI= 5.91; [c] pH=10



Firstly, at feed/membrane interface a proton exchange reaction between the zwitterionic amino acid and macrocyclic bis-benzenesulfonamide **12** is not possible due to the low difference between the pK_a values of these species ($pK_{a,\ Phe}$=9.24 mol/l, $pK_{a,\ 12}$ = 8.29 mol/l- see experimental). Thus, at this interface the amino acid is transferred in zwitterionic form.

Lithium cation is not complexed by the macrocyclic cavities of **12** due to the large size difference of the ionic diameter (d_{Li}^+= 1.20 Å) and the diameters of the cavity of the cyclic polyethers ($d_{benzo\text{-}15\text{-}crown\text{-}5}$ = 1.72-1.84 Å and $d_{benzo\text{-}18\text{-}crown\text{-}6}$= 2.67-2.86 Å)[23]. By using Li^+ as competitive transport partner (Table 2, entry 1), a slow transport of the amino acid by the membrane was observed. The amino acid is transported by complexation of the ammonium moiety by the 18-crown-6 cavity, whereas the carboxylate moiety is compensated by the Li^+ cation.

Fig. 5: L-Phenylalanine concentration profiles in the strip a), the feed b) and the membrane phase c) in U-. tube membrane transport experiments as a function of the nature of co-transported alkali cation.

Sodium cation (d_{Na^+}= 1.90 Å) is strongly complexed by the 15-crown-5 cavity, but is too small to completely fill the 18-crown-6 cavity.[23] Transport concentration profiles versus time of the amino acid in the presence of Na^+ proved a slow transport rate (Table 2, entry 2) and a strongly accumulation of the amino acid in the membrane phase (Fig. 5c) probably due to the *high stability* of **[12-Phe-Na$^+$]Cl$^-$** supramolecular complex. A *synergetic multiple supramolecular effect* is evidenced here by selective competitive complexation of Na^+ in the 15-crown-5 macroring which induces a preferential conformation of a new three points interaction model of the amino acid with polytopic receptor: a) $-NH_3^+{}_{Phe}$-18-crown-6; b) $COO^-{}_{Phe}$-[15-crown-5 : Na^+] and c) π-π stacking interactions of phenyls moieties (Fig. 6).

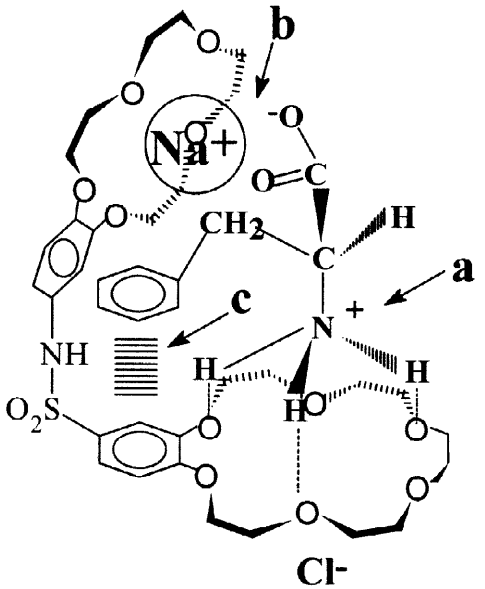


Fig. 6: Multiple molecular recognition of zwitterionic phenylalanine by combining different non-covalent interactions: a) hydrogen bonding, b) charge interactions and c) π-π stacking interactions.

Potassium cation (d_{K^+}= 2.66 Å) is one most strongly complexed alkali metal ions[23] by the 18-crown-6 or the 15-crown-5 (a sandwich type conformation) derivatives. In our transport experiments a high transport rate of the phenylalanine was observed by using this ion as co-transported cation (Table 2, entry 3). Probably, the amino acid is transported as the counter-ion of a supramolecular sandwich complex as a result of complexation of the potassium cation by **12**. This fact is confirmed by an increasing transport rate of the anionic form of phenylalanine (H_2N-R-COO^- K^+) at high pH (Table 2, entry 5) (Fig. 7).

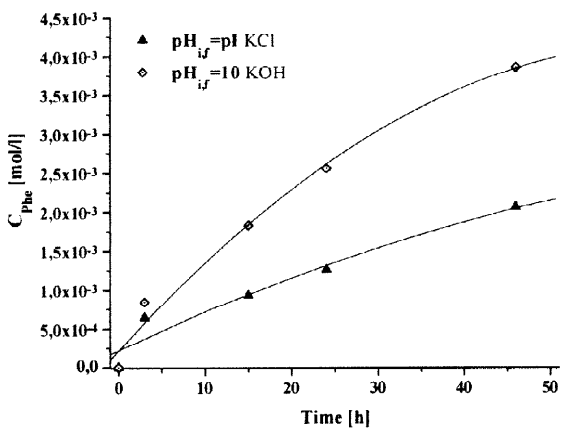


Fig. 7: L-Phenylalanine concentration profiles in the strip phase as a function of pH value of the feed phase (K^+ as co-transported cation.)

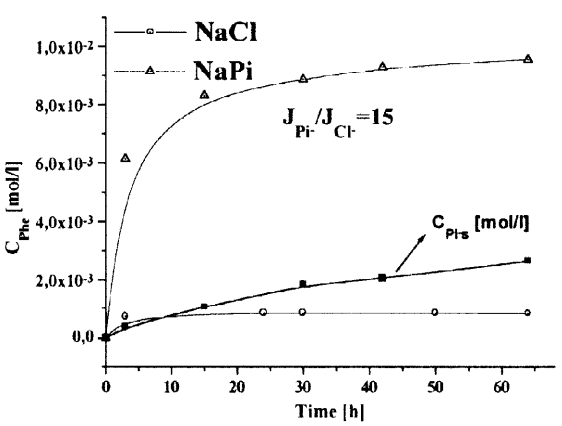


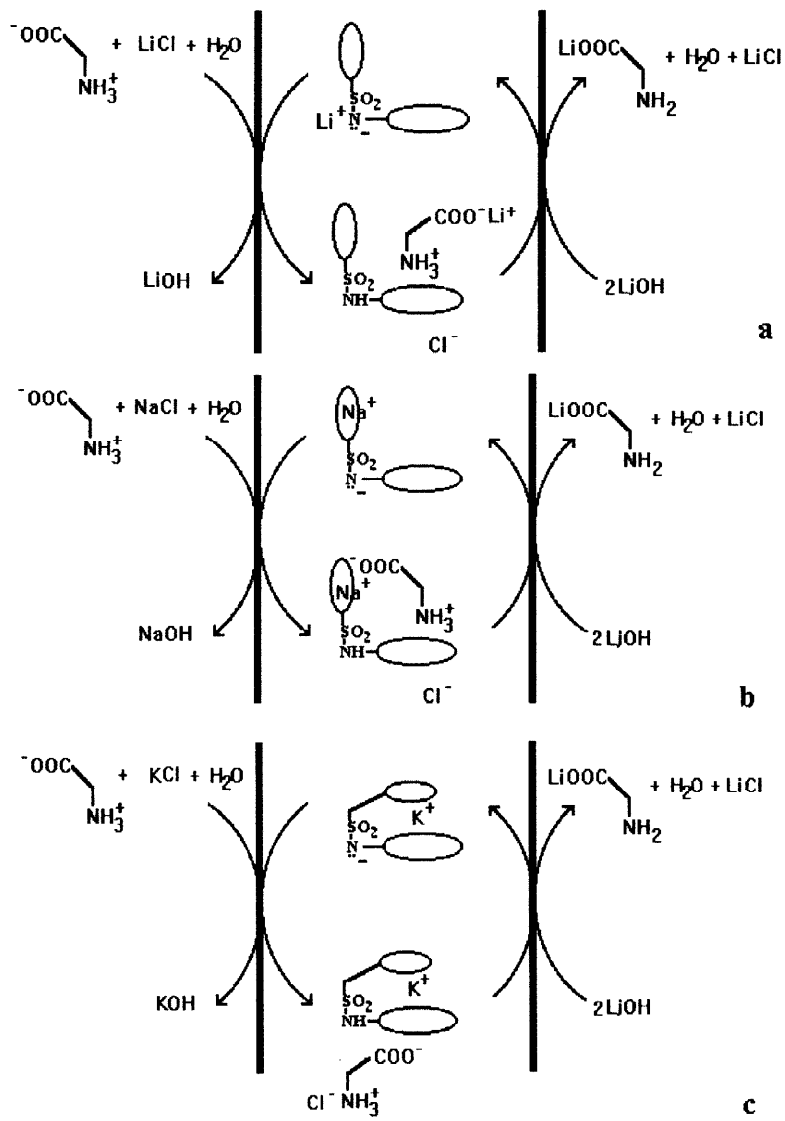
Fig. 8: Concentration profiles in the strip phase as function co-transported counter ion (Cl^- or Pi^-= Picrate) (C_{Pi^-s}= concentration of picrate anion in the strip phase).

The competitive transport rate of the phenylalanine-Na^+ by the liquid membrane increased in the presence of picrate anion in the feed phase (Fig.8). Probably, the lipophilic nature of picrate anion (co-

transported in the strip phase) increases the amount of supramolecular complex in the membrane, releasing the amino acid in the strip.

Taken together, the above results lead us to propose the transport mechanism outlined in Fig. 9. In particular, protonated bis-macrocyclic sulfonamide **12** binds the zwitterionic amino acid and alkali chloride at the feed/membrane interface forming the neutral complex mentioned above, which then diffuses through the membrane. The zwitterion and alkali chloride are released in the more basic strip phase, where the amino acid reacts with LiOH to form the amino acid lithium salt. Deprotonation of the sulfonamide moiety also facilitates also product release. Bis-macrocyclic sulfonamide salt **13** travels back to the feed phase where it is protonate to regenerate carrier **12** ready to effect zwitterionic amino acid transport again.

In accord with the proposed mechanism are: the absence of co-transported Na^+ or K^+ in the strip phase and the increase of pH in the feed phase (~0.1 pH units over 50 h) accompanied by a decrease of pH in the strip phase (~3 pH units over 50 h).



An interesting effect of the amino acid binding was observed from Job plots (dependence on supramolecular complex concentration as a function of substrate-receptor ratio) obtained by UV-Visible spectroscopic measurements following the absorbance at 282.5 nm (no absorbance for phenylalanine) (Fig. 10). As shown in Figure 10, the Job plot evidenced a 1:1 stoichiometry of **12** : phenylalanine. A second molecule of phenylalanine is associated as a counter-zwitterion, to compensate the charge of the sodium supramolecular complex **13** : phenylalanine leading to a 1:2 stoichiometry. This fact can be seen only in the UV spectrum, where phenyl-alanine has no absorbance.

Fig. 9: Proposed U-tube membrane transport mechanism using macrocyclic derivative **12** as carrier as function of the nature of co-transported alkali cation:
a) Li^+, b) Na^+, c) K^+.

The phenylalanine complexed by the bis-macrocyclic ligand contributes an increasing of the molar extinction coefficient of the supramolecular complex (i.e. $\varepsilon_{257.5,12}$ = 9850 l mol^{-1} cm^{-1}, $\varepsilon_{257.5,12+Phe}$=13500 l mol^{-1} cm^{-1}, calculated as mean ± standard error, from four determinations) probably due to the above mentioned π-π stacking interactions

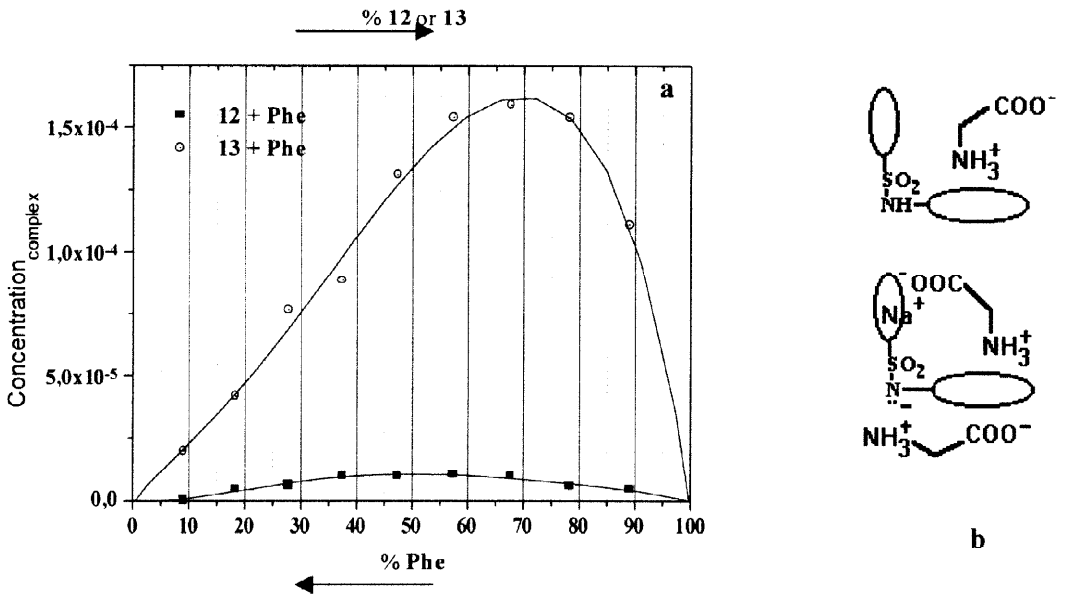


Fig. 10: Job plots a) and proposed supramolecular complexes b)

These results taken together lead us to conclude that macrocyclic derivative **12** constitutes a receptor for zwitterionic phenylalanine using multiple molecular recognition. Simultaneous complexation of the ammonium moiety of the amino acid by the benzo-18-crown-6 cavity and of the sodium ion in the benzo-15-crown-5 cavity induces a *stereogenic supramolecular effect* allowing charge interactions of the carboxylate moiety with Na^+-15-crown-5 and π-π stacking interactions between the aromatic ring of the phenylalanine and aromatic moieties of **12** as shown in Figure 6. This multiple molecular recognition model leads to a new neutral, stable lipophilic supramolecular architecture. Different complexation abilities of phenylalanine and the alkali salts by this receptor provide the basis for new U-tube active transport mechanisms as a function of the nature of the alkali cation (Li^+, Na^+ or K^+).

The approach illustrated here could provide a generalized basis for the design of other polytopic receptors capable of selectively binding and transporting other organic substrates. Work is in progress to assess selective complexation and transport through membranes of chiral amino acids.

Experimental Section

Melting points are uncorrected using a heating-plate microscope. IR spectra was done on KBr pellets on a Nicolet ZDXFTIR instrument. NMR spectra was done on a Bruker AC200 spectrometer (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) using CD_3OD as solvent and chemical shifts are reported as δ values relative to Me_4Si as

internal standard. Elemental analysis were done on a Carlo Erba CHNS Elemental Analyser, Model 1106. Benzo-18-crown-6, chlorosulphonic acid, phenyalanine, LiOH, HCl (36.5%) were purchased from Merck. 4-aminobenzo-15-crown-5, sodium tetraphenylborate, sodium chloride, sodium picrate were purchased from Fluka and were used without purification. All other reagents were analytical grade and were used as received.

Preparation of 4-(4'-benzo-18-crown-6-sulfonamido)benzo-15-crown-5, *(12)* : To a stirred and cooled (-10 °C- -5°C) solution of 2.76 g (8.84 10^{-3} mol) of **8** in 10 ml of dry $CHCl_3$, 6 ml of $ClSO_3H$ (10.5 g, 9.01 10^{-2} mol) were added dropwise and the resulting solution was stirred overnight and then refluxed for 5 h. The reaction mixture was washed with Li_2CO_3 and water, dried over Na_2SO_4 and concentrated in vacuo. The light brown oil was recrystallized from ether to give 2.25 g (5.48 10^{-3} mol, 62%) of **11** which was dissolved in 30 ml of $CHCl_3$. A solution of 1.55 g (5.48 10^{-3} mol) of **9** and 0.5 g (6.33 10^{-3} mol) of dry pyridine in $CHCl_3$ (30 ml) was added dropwise at 5 °C and the resulted mixture was stirred at room temperature for 3 h and refluxed for 5 h. The solvent was evaporated in vacuo and the crude product was recrystallized from EtOH/$CHCl_3$ to give 3 g (4.54 10^{-3} mol, 83%) of **12** as a white solid; m.p. 72-73 °C (from EtOH/$CHCl_3$); IR (KBr): ν = 601, 959, 1100, 1135, 1266, 1347, 1387, 1467, 1523, 1618, 1630, 2882, 2935, 3235, 3436, 3486, cm^{-1}; ^1H NMR (CD_3OD) : δ = 3.54-3.56 (m, 20 H, O-C*H*$_2$-C*H*$_2$-O), 3.78 (m, 8 H, -C*H*$_2$ -CH_2O), 3.97-4.11 (m, 8 H, CH_2 -C*H*$_2$O), 6.54-6.59 (m, 1 H, C*H*$_7$), 6.69-6.73 (m, 2 H, C*H*$_{8,9}$), 6.91-6.95 (m, 1 H, C*H*$_4$), 7.14 (m, 1 H, C*H*$_5$), 7.25-7.30 (m, 1 H, C*H*$_6$); ^{13}C NMR (CD_3OD, see Fig. 4): δ = 70-70.66 (m, C_8, C_9, C_8', C_9'), 71.52-71.95 (m, C_7, C_7'), 110, 85 (s, C_2'), 112.75 (s, C_6'), 113.17 (s, C_5), 115.70 (s, C_5'), 116.76 (s, C_6), 122.54 (s, C_2), 132.27 (s, C_1), 132.57 (s, C_4'), 148.29 (s, C_1'), 149.67 (s, C_3), 150.64 (s, C_3'), 153.81 (s, C_4). Anal. calcd. for $C_{30}H_{43}NO_{13}S$ (657.43): C, 54.78; H, 6.59; N, 2.13; O, 31.62; S, 4.87. Found C, 55.01; H, 6.27; N, 2.13; O, 31.96; S, 4.48.

Preparation of 4-(4'-benzo-18-crown-6-sulfonamido)benzo-15-crown-5, sodium salt *(13)*: To a stirred solution of 1 g (1.52· 10^{-3} mol) of **12** in 25 ml of EtOH, 0.1 g (1.52· 10^{-3} mol) of EtONa in 25 ml EtOH was added dropwise and the resulting solution was stirred 3 h and then refluxed for 2 h. The reaction mixture was concentrated in vacuo, the crude product dissolved in $CHCl_3$ (25 ml) was washed with water. The solvent was then evaporated in vacuo and the crude product was recrystallized from EtOH to give 0.92 g (1.35 10^{-3} mol, 89%) of **13** as a light brown solid; m.p. 118-120 °C (from EtOH); IR (KBr): ν = 601, 959, 1100, 1125, 1266, 1328, 1387, 1467, 1523, 1618, 1630, 2882, 2935, 3436, 3486 cm^{-1}; ^1H NMR (CD_3OD): δ = 3.61-3.69 (m, 20 H, O-C*H*$_2$-C*H*$_2$-O), 3.74 (m, 8 H, -C*H*$_2$ -CH_2O), 4.03-4.11 (m, 8 H, CH_2 -C*H*$_2$O), 6.57-6.62 (m, 1 H, C*H*$_7$), 6.78-6.82 (m, 2 H, C*H*$_{8,9}$), 6.91-6.95 (m, 1 H, C*H*$_4$), 7.14 (m, 1 H, C*H*$_5$), 7.25-7.30 (m, 1 H, C*H*$_6$); ^{13}C-NMR (CD_3OD, see Fig. 4): δ = 69.44-69.54 (m, C_8, C_9, C_8', C_9'), 71.13-71.25 (m, C_7, C_7'), 110, 50 (s, C_2'), 112.10 (s, C_6'), 113.00 (s, C_5), 115.68 (s, C_5'), 116.25 (s, C_6), 122.63 (s, C_2), 132.74 (s, C_1), 132.82 (s, C_4'), 147.10 (s, C_1'), 148.72 (s, C_3), 149.64 (s, C_3'), 152.41 (s, C_4). Anal. calcd. for $C_{30}H_{42}NO_{13}S^-Na^+$ (679.71): C, 53.01; H, 6.23; N, 2.06; O, 30.60; S, 4.72. Found C, 53.38; H, 6.32; N, 2.04; O, 31.00; S, 4.34.

General Crystallization Procedure of Supramolecular Complexes *14*, *15* and *16*: 0.1 g (1.52 10^{-4} mol) of **12** and 0.025 g (1.52 10^{-4} mol) of phenylalanine were dissolved in 25 ml of THF and refluxed for 2 h. Subsequently, the reaction mixture was stirred overnight at room temperature. For ternary supramolecular complexes **15** and **16**, 0.0346 g (1.52 10^{-4} mol) of sodium picrate and 0.052 g (1.52 10^{-4} mol) of sodium tetraphenylborate respectively, was added. The resulted mixture was diluted with a small amount of hexane. Crystal formation was slow, but a good harvest of crystals suitable for X-Ray analysis was not obtained. The yields were : 60 % (**14**), 62 % (**15**) and 69 % (**16**).

12/ L-Phenylalanine complex, 14: m.p. 187-190 °C; IR (KBr): ν = 707, 868, 959, 1110, 1125, 1271, 1336, 1387, 1412, 1457, 1512, 1588, 1608, 1633, 2880, 2923, 3040, 3075, 3439 cm^{-1}; ^1H NMR (CD_3OD): δ = 2.93-3.28 (m, 2 H, C_6H_5C*H*$_2$-, Phe), 3.28-3.31 [d, 1 H, -C*H*(NH_3^+)COO^-] 3.63-3.73 (m, 20 H, O-C*H*$_2$-C*H*$_2$-O), 3.74-3.80 (m, 8 H, -C*H*$_2$ -CH_2O), 3.93-4.11 (m, 8 H, CH_2 -C*H*$_2$O), 6.55-6.60 (m, 1 H, C*H*$_7$), 6.71-6.75 (m, 2 H, C*H*$_{8,9}$), 6.97-7.01 (m, 1 H, C*H*$_4$), 7.08-7.20 (m, 6 H, C*H*$_5$+ C*H*$_{Phe}$), 7.31-7.36 (m, 1 H, C*H*$_6$); ^{13}C NMR (CD_3OD, see Fig. 4): δ = 38.32 (s, C_E), 57.74 (s, C_F), 69.81-69.99 (m, C_8, C_9, C_8', C_9'), 71.85-71.95 (m, C_7, C_7'), 110.71 (s, C_2'), 112.42 (s, C_6'), 113.07 (s, C_5), 115.79 (s, C_5'), 116.56 (s, C_6), 122.73 (s, C_2), 128.30 (s, C_D), 130.38 (s, C_B), 129.80 (s, C_C), 132.76 (s, C_1), 132.82 (s, C_4'), 137.02 (s, C_A), 147.64 (s, C_1'), 148.92 (s,

C_3), 149.83 (s, C_3'), 152.88 (s, C_4), 172.88 (s, C_G). Anal. calcd. for $C_{30}H_{43}NO_{13}S$ + $C_9H_{11}NO_2$ (822.92): C, 56.92; H, 6.61; N, 3.40; O, 29.16; S, 3.90. Found C, 57.00; H, 6.44; N, 3.23; O, 28.95; S, 3.54.

12/ L-Phenylalanine/ sodium picrate complex, 15: m.p. 127-129 °C; IR (KBr): ν = 712, 974, 1104, 1126, 1271, 1336, 1517, 1573, 1618, 1635, 2882, 2927, 3040, 3098, 3441 cm^{-1}; ^1H NMR (CD_3OD): δ = 2.90-3.18 (m, 2 H, $C_6H_5CH_2$-, Phe), 3.18-3.20 [d, 1 H, -$CH(NH_3^+)COO^-$] 3.61-3.70 (m, 20 H, O-CH_2-CH_2-O), 3.74-3.80 (m, 8 H, -CH_2 -CH_2O), 4.03-4.18 (m, 8 H, CH_2 -CH_2O), 6.57-6.63 (m, 1 H, CH_7), 6.76-6.80 (m, 2 H, $CH_{8,9}$), 6.99-7.03 (m, 1 H, CH_4), 7.16-7.24 (m, 6 H, CH_5+ CH_{Phe}), 7.33-7.37 (m, 1 H, CH_6), 8.67 (s 2 H, CH-picrate); ^{13}C NMR (CD_3OD, see Fig. 4) : δ = 39.22 (s, C_E), 58.22 (s, C_F), 69.22-69.76 (m, C_8, C_9, C_8', C_9'), 71.00-71.26 (m, C_7, C_7'), 110.50 (s, C_2'), 112.10 (s, C_6'), 113.00 (s, C_5), 115.68 (s, C_5'), 116.25 (s, C_6), 122.63 (s, C_2), 126.7 (s, C_M, picrate), 128.14 (s, C_B), 129.82 (s, C_C), 130.47 (s, C_D), 132.74 (s, C_1), 132.82 (s, C_4'), 134.13 (s, C_n, picrate), 138.50 (s, C_A), 144.06 (s, C_P), 147.05 (s, C_1'), 148.74 (s, C_3), 149.70 (s, C_3'), 152.39 (s, C_4), 153.6 (s, C_Q, picrate), 166.98 (s, C_G). Anal. calcd. for $C_{30}H_{43}NO_{13}S$ + $C_9H_{11}NO_2$ + $C_6H_2N_3O_7^-$ Na^+ (1074.01): C, 50.32; H, 5.26; N, 6.52; O, 32.77; S, 2.98. Found C, 50.64; H, 5.22; N, 6.43; O, 32.60; S, 3.14.

12/ L-Phenylalanine/ sodium tetraphenylborate complex, 16: m.p. 126-128 °C; IR (KBr): ν = 710, 737, 974, 1099, 1126, 1268, 1336, 1462, 1517, 1573, 1613, 1635, 2882, 2922, 3040, 3063, 3421, 3481 cm^{-1}; ^1H NMR (CD_3OD): δ = 2.92-3.20 (h, 2 H, $C_6H_5CH_2$-, Phe), 3.28-3.32 [d, 1 H, -$CH(NH_3^+)COO^-$] 3.54-3.65 (m, 20 H, O-CH_2-CH_2-O), 3.65-3.69 (m, 8 H, -CH_2 -CH_2O), 3.97-4.01 (m, 8 H, CH_2 -CH_2O), 6.59-6.65 (m, 1 H, CH_7), 6.68-6.73 (m, 2 H, $CH_{8,9}$), 6.99-7.40 (m, 28 H, CH_4, CH_5, CH_{Phe}, CH_6, CH-tetraphenylborate).^{13}C NMR (CD_3OD, see Fig. 4): δ = 38.04 (s, C_E), 57.74 (s, C_F), 69.00-69.24 (m, C_8, C_9, C_8', C_9'), 70.25-70.75 (m, C_7, C_7'), 110.50 (s, C_2'), 112.35 (s, C_6'), 113.00 (s, C_5), 115.78 (s, C_5'), 116.47 (s, C_6), 122.74 (s, C_2), 126.37-126.48 (m, $C_{phenylborate}$), 128.45 (s, C_D), 129.93 (s, C_B), 130.42 (s, C_C), 132.64 (s, C_1), 133.02 (s, C_4'), 137.30 (s, C_A), 147.23 (s, C_1'), 148.69 (s, C_3), 149.87 (s, C_3'), 152.40 (s, C_4), 167.00 (s, C_G). Anal. calcd. for $C_{30}H_{43}NO_{13}S$ + $C_9H_{11}NO_2$ + $BC_{24}H_{20}^-Na^+$ (1074.01): C, 64.94; H, 6.40; N, 2.40; O, 20.60; S, 2.75. Found C, 64.58; H, 6.27; N, 2.80; O, 20.88; S, 2.63.

Determination of Protonation Constant: The protonation constant of **12** was determined by potentiometric titration in distilled H_2O at 25 °C. The titration of a 10^{-2} M solution of **13** was carried out at a constant ionic strength of 0.10 M $(CH_3)_4NCl$, using a solution of 10^{-2} HCl. The pH values (error ±0.01pH) were measured using a MeterLab™ of Radiometer pH apparatus, equipped with a pHC 3005-8 combined electrode. The protonation constant of **12** calculated as mean ± standard error from five determinations is log K_a= 8.29±0.05 mol/l.

Transports experiments: Facilitated transport experiments were performed with magnetic stirring in a conventional U-tube glass cell at room temperature. Feed phase was a 25 ml of 10^{-2} M phenylalanine (pH=pI_{Phe} = 5.91) in 10^{-2} M MeCl (Me: Li^+, Na^+or K^+) or 10^{-2} M sodium picrate (PiNa). The membrane phase consisted of 10^{-3} M of **12** in chloroform (25 ml) placed at the bottom of the tube. The strip phase consisted of 25 ml of 10^{-4} M LiOH, (pH=10). Aliquots (1 ml) of both aqueous solution were withdrawn at appropriate intervals and diluted by a factor of 5. The pH of the both sides was monitored using a MeterLab™ Radiometer pH meter equipped with a pHC 3005-8 combined electrode. The concentration of phenylalanine (λ=257.6 nm, ε=225) and sodium picrate (λ=360 nm, ε=13300) on both sides of the membrane were measured by a UV-VIS spectrophotometer (UNICAM 8600 UV/VIS). Each experiment was repeated at least twice and the results were consistent within ±10%.

REFERENCES

1. T.H. Webb, C.S. Wilcox, *Chem. Soc. Rev.* **1993**, *22*, 383-395.

2. J.M. Lehn, *Angew. Chem.* **1988**, *100*, 91-114; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89-112.

3. J.M. Lehn, *La Chimie Supramoléculaire. Concepts et Perspectives* (in French), De Boeck & Larcier, Paris, Bruxelles, **1997**, pp. 2-54.

4. X.X. Zhang, J.S. Bradshaw, R.M. Izatt, *Chem. Rev.* **1997**, *97*, 3313-3361.

5.G.W. Gokel, E. Abel, Complexation of Organic Cations in *Comprehensive Supramolecular Chemistry, Vol* 1 (Eds.: J.L.Atwood, J.E.D. Davies, D.D.MacNicol, F.Vögtle, J.M.Lehn), Pergamon, Oxford, **1996**, pp.511-534.

6. J.S. Bradshaw, R.M. Izatt, *Acc. Chem. Res.*, **1997**, *30*, 338-345.

7. J.S. Bradshaw, *J. Incl. Phenom.*, **1997**, *29*, 221-246.

8. F. Vögtle, *Supramolecular Chemistry*, John Wiley & Sons, New York, **1991**, pp. 9-116.

9. L. Mutihac, C. Luca, *Rev. Roum. Chim.*, **1991**, *36*, 85-90.

10. L. Mutihac, R. Mutihac, C. Luca, *Rev. Roum. Chim.* **1991**, *36*, 551-561.

11. M. Newcomb, J. L. Toner, R. C. Hegeson, D.J. Cram, *J. Am. Chem. Soc.* **1979**, *101*, 4971-4974.

12. M. Pietraszkiewicz, M. Kozibial, O. Pietraszkiewicz, *J. Membr. Sci.* **1998**, *138*, 109-113.

13. S. Badouche, L. Jaquet, M.A. Lobo-Recio, C. Marzin, G. Tarrago, *J. Incl. Phenom.* **1993**, *16*, 81-89.

14. A. Galàn, D. Andreu, A.M. Echavarren, P. Prados, J de Mendoza, *J. Am. Chem. Soc.* **1992**, *114*, 1511-1512.

15. M. Barboiu, C.T. Supuran, C.Luca, G. Popescu, C. Barboiu, *Liebiegs. Ann.* **1996**, 959-963.

16. M. Barboiu, C. Luca, C.T. Supuran, A. Scozzafava, F. Briganti, N. Hovnanian, L. Cot, *Liebiegs. Ann.* **1997**, 1853-1859.

17. F.P. Schmidtchen, *J. Org. Chem.* **1986**, *51*, 5161-5168.

18. J. Rebek Jr., D. Nemeth, *J. Am. Chem. Soc.*, **1985**, *107*, 6738-6739.

19. Y. Nakatsuji, M. Muraoka, M. Wada, H. Morita, A. Masuyama, T. Kida, J. Ikeda, *J. Org. Chem.*, **1997**, *62*, 6231-6235.

20. M. Barboiu, *Studies of Heteropolysiloxane Membranes Containing New Macrocyclic Polyethers. Application in Facilitated Transport of* Ag^{+}/Cu^{2+} *and Amino Acids*, Ph.D. Thesis, University of Montpellier II, Montpellier, France, **1998**.

21. C. Seel, A. Galan, J. de Mendoza, *Top. Curr. Chem.* **1995**, *175*, 101-132.

22. A.V. Bordunov, J.S. Bradshaw, X.X. Zhang, N.K. Dalley, X. Kou, R.M. Izatt, *Inorg. Chem.* **1996**, *35*, 7229-7240.

23. N.K. Dalley, in *Synthetic Multidentate Macrocyclic Compounds*, (Eds. R.M. Izatt, R.M. Christiansen), New York, Academic Press, **1978**, pp. 207-243.

24. M. Barboiu, N. Hovnanaian, C. Luca, G. Popescu, L. Cot, *Eur. J. Org. Chem.* **1998**, 1705-1708.

25. Part IV: M. Barboiu, C.T. Supuran, C. Guran, P. Diaconescu, M. Bojin, V. Iluc, L. Cot, *Metal Based Drugs*, **1999**, in press