Pergamon

Tetrahedron Vol. 51, No. 1, pp. 217-230, 1995
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4020/95 $9.50+0.00

0040-4020(94)00935-X

Chiral Lipophilic Ligands. 2. Cu(II)-Mediated Transport of α-Amino Acids Across a Bulk Chloroform Membrane†

Paolo Scrimin*, Paolo Tecilla, and Umberto Tonellato*

University of Padova, Department of Organic Chemistry and Centro CNR Meccanismi Reazioni Organiche, via Marzolo 1, 35131 Padova, Italy

Abstract: A number of achiral (**1** - **4**) and chiral (**5** - **10**) 1,2-diamino ethane derivatives bearing on one or both nitrogen atoms long paraffinic chains have been synthesized and successfully tested as carriers for the transport of Cu(II) and α-amino acids across a bulk chloroform membrane from a buffered (pH=5.5) source phase to a receiving solution of EDTA. This, being a highly hydrophilic ligand with an affinity for Cu(II) several order of magnitude larger than that of the carrier, allows an effective "up-hill" co-transport of ions and amino acids. The results indicate that the transport occurs *via* the formation of a ternary complex comprising Cu(II), the lipophilic diamine, and the amino acid. The lipophilicity of the amino acid is the main factor affecting the rate of transport using the same carrier; on the other hand, for the same amino acid the best carrier is that having affinity constant for Cu(II) close to that of the amino acid transported. The rate of transport is apparently governed by the rate of release of the metal ion and the amino acid from the source phase to the organic phase. The enantioselectivities observed in the co-transport of natural amino acids employing chiral ligands are generally modest; in the best cases, the enantioselectivity ratios (the initial rates of transport of the faster enantiomer relative to the slower) are slightly higher than 2. The enantioselectivity appears to depend more on thermodynamic rather than kinetic factors.

INTRODUCTION

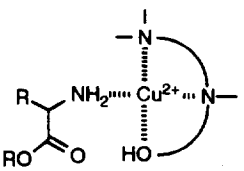
The transport of α-amino acids across membranes is a quite intriguing process which has attracted the attention of several research groups[1,2] for the obvious relevance of these substrates. Because of their bifunctional character, amino acids may be recognized by a suitable carrier through the interaction with the carboxylic (or carboxylate) group, the amine (or ammonium) or both of them. Indeed, successful transport of α-amino acids as anionic[1a], cationic[1b,c] or zwitterionic[1e] species, has been achieved. The translocation of zwitterionic species appears as the most challenging approach because of the need of a ditopic carrier. In biological systems the transport of amino acids is a complex process usually associated with the transport of cations[3]. Cations mediated transport of amino acids across membranes, with artificial carriers, has been reported using group I cations[1f,g].

In previous papers[4] we have addressed the enantioselective cleavage of α-amino acid *p*-nitrophenyl esters by chiral metallomicelles made up of chiral lipophilic ligands and divalent transition ions. Since a critical feature of the proposed mechanism was the formation of a ternary complex (**I**) comprising ligand, transition metal ion and substrate, we speculated that the formation of such a ternary complex could be exploited to translocate

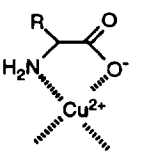
† For part 1 see ref. 4b

relatively hydrophilic species, like α-amino acids, across a bulk liquid membrane. The formation of ternary complexes in the case of Cu(II) ions is a well known process[5] and the use of lipophilic ligands as carriers of transition metal ions (Cu(II) in particular) has been described[6,7]. The transport of anions and N-protected α-amino acids as well by Cu(II) complexes of polymeric ligands has been reported[2]. The transport of the non-protected derivative proved more challenging and, in fact, these authors were less successful in the transport of these compounds. In a preliminary communication[8] we reported however, that native, non-protected α-amino acids can be successfully transported through a bulk membrane using a lipophilic Cu(II) complex as the carrier. The use of other transition metal ions (Ni(II), Zn(II)) led to a much less efficient transport[8]. The basic point behind a successful transport experiment is the formation of a lipophilic Cu(II) complex in which the first co-ordination sphere of the transition metal ion is not saturated by strong donors so that there is still room for the co-ordination of the extra ligand whose transport is pursued.

In this paper we report our results on the translocation of α-amino acids and Cu(II) ions across a bulk chloroform membrane by lipophilic ligands. The use of chiral ligands for enantioselective transport was also investigated and the results discussed.



I



II

RESULTS AND DISCUSSION

The ligands

On the basis of the arguments outlined above we designed our lipophilic ligands keeping in mind two points: i) Cu(II), in its most usual co-ordination geometry[9], has four strong co-ordination positions in the plane. Two apical positions may also be available: because of the Jahn-Teller effect[10] they are of different strength and usually weaker than those in the plane, so that they can be overlooked in a first approximation; ii) α-amino acids bind to Cu(II) ions to form chelates[11] (see **II**). Accordingly, a proper ligand must have no more than two good donor atoms[12] in order to avoid the displacement of the amino acid from the strong co-ordination positions of Cu(II). For these reasons we have chosen, synthesized and investigated 1,2-diamino ethane derivatives bearing at least one long hydrocarbon chain. Their structure is reported in Chart I.

Transport experiments with achiral carriers

The transport experiments were performed using a bulk chloroform membrane and a U-shaped transport cell (see Experimental Section). As recently pointed out by Menger and Lee[7] a liquid membrane is not a membrane at all, this latter being a well organized system; however liquid (or bulk) membranes are quite useful for the study of the partitioning of hydrophilic species between aqueous and organic phases so, their use constitutes a widespread, albeit rough, approach for carrying out transport experiments.

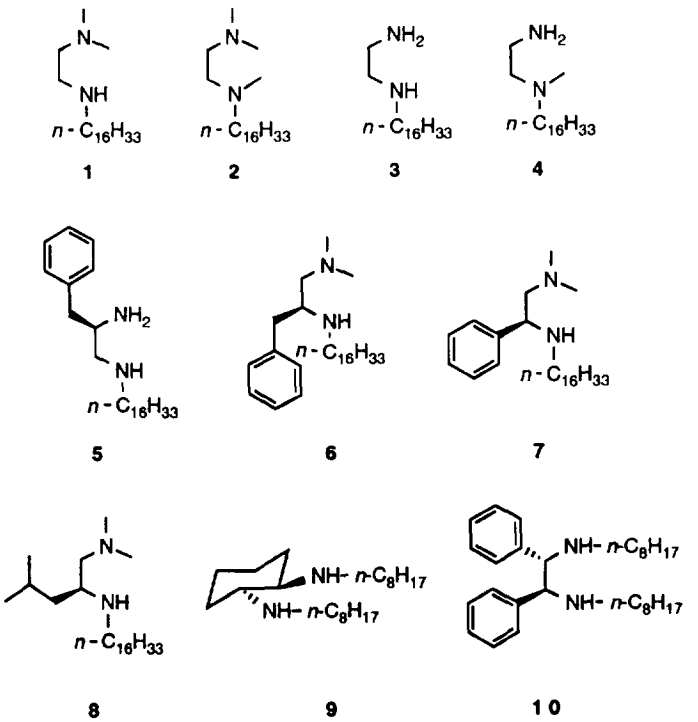


Chart I

In our case the source phase was a pH=5.5 water solution (6 mL) buffered with 0.25 M 2-morpholinoethane sulphonic acid (MES) containing the amino acid (5.0×10^{-3} M) and $Cu(NO_3)_2$ (2.0×10^{-2} M). In our early experiments[8] the receiving phase was a solution (6 mL) of 0.1 N $HClO_4$. The systematic investigation here described was however carried out using the same volume of a solution of EDTA (0.05 M, pH=6.5) for the reasons discussed below. Using $HClO_4$ in the receiving phase, the release of Cu(II) and amino acid is achieved by protonation of the carrier while, using EDTA, a much stronger ligand[13] than our lipophilic diamines, the metal ion is quite effectively stripped from the carrier and, as a consequence, the amino acid is released into the receiving phase. The chloroform solution (25 mL) contained the proper ligand at a selected concentration. A typical EDTA-driven transport experiment is illustrated in Figure 1 for ligand **1** and the amino acid phenylalanine (Phe). This Figure reports the μmoles of Phe present at different time intervals in the source and receiving phases as determined analytically (see Experimental Section); those present in the organic phase were evaluated by difference. The amount of amino acid in the receiving phase, after an induction time of *ca.* 1.5 h increases linearly while the amount in the organic phase reaches a steady concentration. Under such experimental conditions, allowing enough time, 100% transport of the amino acid can be achieved so that an "uphill" transport is possible. In the absence of Cu(II) in the source phase, no transport of the amino acid is observed. The inset of Figure 1 illustrates the outcome of an analogous experiment using $HClO_4$ in the receiving phase. When comparing Figure 1 and its inset, it appears that in the case of the proton-driven process: a) the transport rate is slower; b) the amount of amino acid in the organic phase continues to increase and never

reaches a steady concentration. Clearly EDTA is more efficient than H^+ in transferring Cu(II) (and, consequently, the amino acid) into the receiving phase and this efficiency appears related to a faster transfer of ions from chloroform to the receiving phase. Kinetic control in the rate of transfer of Cu(II) ions from the chloroform phase to an acidic receiving phase was also observed by Menger[7]. The steady amino acid concentration reached in the organic phase in the EDTA-driven experiment clearly indicates that the rate determining step is the transfer of ions at the interface between source phase and chloroform (as the stirring rate of the chloroform solution was high enough so that the rate of transport was not affected). In order to evaluate the efficiency of the different carriers and α-amino acids, initial rates were determined from the slopes of the linear portion of the plot μmoles *vs* time in the receiving phase. Table I reports the initial rates for the achiral carriers **1-4** and different amino acids.

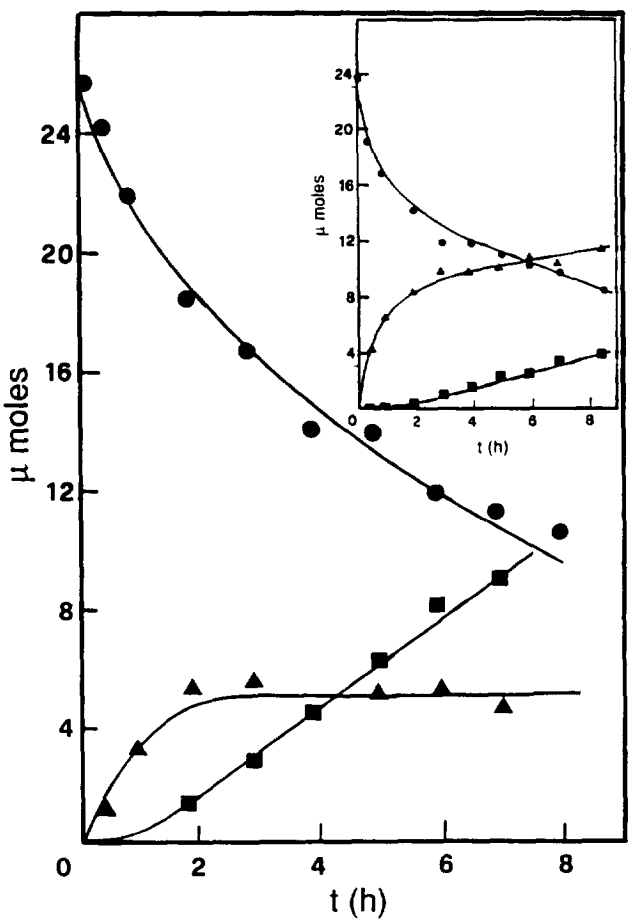


Figure 1. Amount of amino acid phenylalanine (μmoles) present in the three different phases during the transport experiment with ligand **1** using a receiving phase made up of a pH=6.5, 5 x 10^{-2} M EDTA solution. ●, Source phase; ■, receiving phase; ▲, chloroform phase. Inset: same experiment but using as a receiving phase a pH=1 $HClO_4$ solution.

Table I. Initial Transport Rates[a] of α-Amino Acid and Cu(II) Determined for the Achiral Ligands **1** - **4**.

Entry	Ligand	α-Amino Acid	Transport Rates (μmoles/h)	
			Amino Acid	Cu(II)
1	**1**	Phe	1.60	1.60
2	**1**	Trp	3.04	n.d.[b]
3	**1**	Leu	0.85	n.d.[b]
4	**1**	Ala	0.07	n.d.[b]
5	**2**	Phe	0.17	0.21
6	**3**	Phe	0.06	0.45
7	**4**	Phe	0.33	0.74

[a]Conditions: 25°C, [ligand]=$1x10^{-3}$ M; for the source and receiving phases see standard conditions in the Experimental Section; [b]n.d.: not determined.

A scrutiny of Table I reveals that the rate of transport is strongly dependent on the lipophilicity of the amino acid[14]. Using carrier **1** the initial rates of transport are in the order Trp>Phe>Leu>Ala. The fact that the rate of transport of Trp is higher than that of Phe in spite of the claimed[14a] greater hydrophobicity of the latter when partitioning between water and chloroform, may suggest some secondary interaction with the metal complex present in the case of Trp. This point was not further investigated.

In the transport process, Cu(II) is co-transported with the amino acid and the amount of ion transported was also measured: as shown in Fig. 2, in the case of carrier **1** and Phe, the rate of transport of the amino acid is, within the limit of the experimental error, the same as that of Cu(II), thus indicating a 1:1 amino acid/Cu(II) stoichiometry for the lipophilic complex. On the other hand the rates of transport of Cu(II) for the other carriers **2** - **4**, when the amino acid is Phe, are larger than those of the amino acid, as reported in Table I.

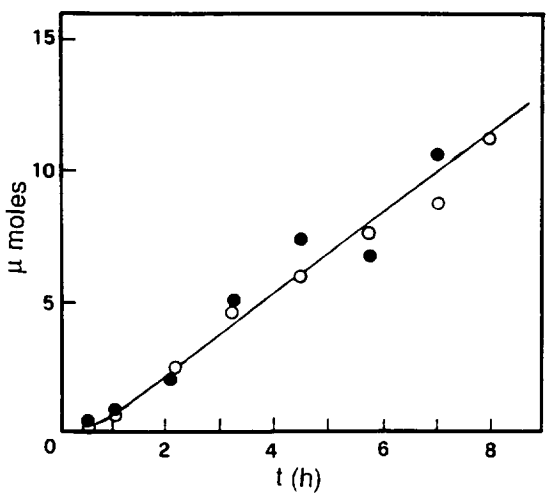
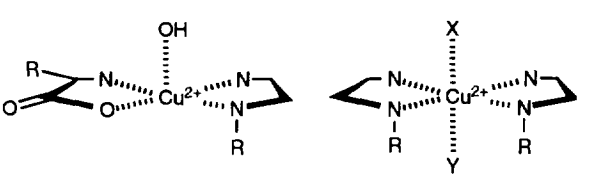


Figure 2. μMoles of amino acid, ○, and Cu(II), ●, present in the receiving phase during the transport experiment of phenylalanine with ligand **1**.

On analyzing these data it is apparent that the most efficient carrier both for the amino acid and the Cu(II) ion is ligand **1**. From the 1:1 ratio Cu(II)/amino acid for the best system and considering the geometry of complexation of Cu(II) (see above) it is conceivable to suggest that the active species in the transport process is complex **III**, comprising the lipophilic ligand, the amino acid and, for neutrality, an hydroxide ion. This latter appears a better choice than the buffer or NO_3^- (present in the source phase as counter-ion of Cu(II)) because of its higher affinity constant for the metal ion[15]. Furthermore, during the transport experiment, if the source phase is not buffered, its pH decreases slightly, in accord with the removal of hydroxide ions from it.

However, only in the case of ligand **1** the amount of Cu(II) transported is equal to that of the amino acids. With the remaining ligands, particularly **3** and **4**, the transport of the transition metal ion is more efficient than that of the amino acid. This fact may be explained considering that, beside the active complex **III**, the following competing complexes may be formed depending on the relative affinities of the ligand and amino acid for Cu(II): a) *the affinity constant of the ligand for Cu(II) is larger than that of the amino acid*; in this case the ligand may compete with the amino acid for the co-ordination to the metal ion so that a complex made of two carrier molecules and not involving the amino acid (**IV**) is formed and acts as a carrier for Cu(II) only; b) *the affinity constant of the ligand for Cu(II) is lower than that of the amino acid*; in this case the amino acid retains the metal ion in the source phase. Under both conditions the transport rate of the amino acid should be depressed and the best carrier should have an affinity constant for the metal ion very close to that of the amino acid transported. Regrettably, because of their insolubility in water, the carriers are not amenable to an easy determination of the binding constants; however, since binding constants for water soluble diamines with the same substitution pattern are available, a rough comparison can be made. Figure 3 reports a plot of the log of the transport rates for the four carriers as a function of the log of the affinity constants for Cu(II) ($\log K_{Cu}$) of the water soluble ligands taken from the literature[16]. The maximum of this curve is associated with ligand **1**, the one with an affinity constant for Cu(II) very close to that of the amino acid transported (Phe), in agreement with the hypothesis discussed above. Within the approximations of the correlation of Figure 3, these results further support the mode of transport proposed, involving complex **III** as the active species. If the rates of transport were controlled by the carrier availability at the interface between the source and organic phases, as pointed out by Menger[7], one would expect a rate of transport following the relative hydrophilicity of the head groups of the ligands (i.e. **3 > 4 > 1 > 2**), because this would result in a more amphiphilic ligand. On the contrary the actual order of the transport rates is **1 > 4 > 2 > 3**.



III IV

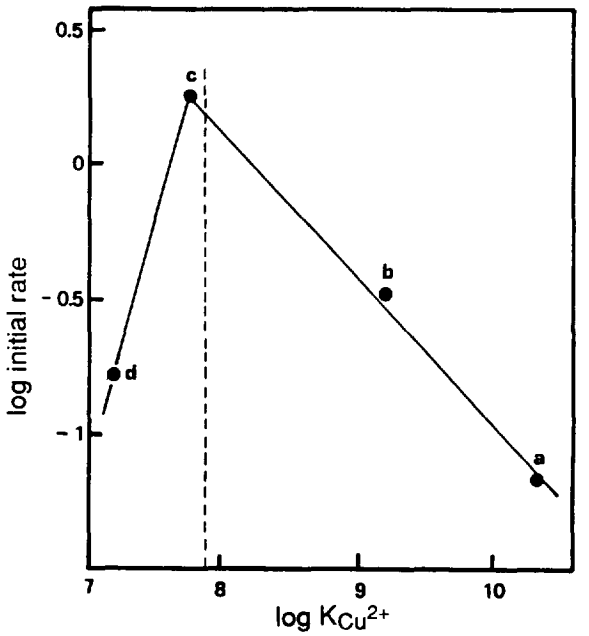
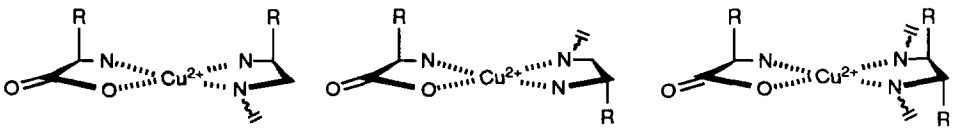


Figure 3. Initial rate of transport (μmoles/h) of the amino acid phenylalanine by ligands **1** - **4** plotted against the affinity constants taken from the literature[16] for model compounds; a: ligand **3**, $\log K_{Cu}$ = 10.4 for $CH_3NH(CH_2)_2NH_2$; b: ligand **4**, $\log K_{Cu}$ = 9.2 for $(CH_3)_2N(CH_2)_2NH_2$; c: ligand **1**, log K_{Cu} = 7.7 for $(CH_3CH_2)_2N(CH_2)_2NHCH_3$; d: ligand **2**, $\log K_{Cu}$ = 7.2 for $(CH_3)_2N(CH_2)_2N(CH_3)_2$. The vertical line indicates the $\log K_{Cu}$ for the transported amino acid, phenylalanine ($\log K_{Cu}$ = 7.9).

Transport experiments with the chiral carriers

The transport experiments with the chiral carriers **5** - **10** were performed under the same conditions used for the achiral ligands. Ligands **5** - **8** have only one chiral carbon while ligands **9** and **10** have two, adjacent, chiral centers and C_2 symmetry. Table II reports the initial rate of transport for the different systems studied using a 1 x 10^{-3} M carrier concentration. The transport experiments with the two enantiomers were performed using two matching cells and usually repeated inverting the two cells: the results were reproducible within 10%. Table II indicates the enantioselectivities observed in terms of ER, *i.e.* the relative rates of the two enantiomers. The data reveal that the enantioselectivity is generally poor and the ER values do not define any clear trend. For each enantiomer at least two geometries are possible (**V**, syn; **V**, anti) having the substituents at the chiral carbons either at the same or opposite side of the co-ordination plane of Cu(II). As a consequence, any difference due to steric effects (albeit modest) may vanish out. In order to overcome such an ambiguity we synthesized and investigated also the carriers **9** and **10** with C_2 symmetry. In such cases the number of useful geometries of the ternary complex are reduced to only one (**VI**). Contrary to our expectations, very little (if any) improvement of the ER was observed. Early studies[18] on ternary complexes of α-amino acids are in agreement with a very little thermodynamic stereoselectivity unless specific interactions between the substituents in the side chains are present. In our case these interactions are limited to steric (repulsive) or π-π (attractive) between the

aromatic rings: both appear of little relevance. This could be related to the distance of the substituents in the complex, its flexibility and, as for the π-π interactions, to the low polarity of chloroform.



V, syn **V, anti** **VI**

Table II. Initial Transport Rates[a] (μmoles/h) and Enantioselectivity Ratios[b] (E R) Determined for the Chiral Ligands (**5** - **10**) and Different α-Amino Acids.

Entry	Ligand	Amino Acid	Transport Rate, (μmoles/h)		E R
			(S)	(R)	
1	**5**-(S)	Phe	0.074	0.038	1.90
2	**5**-(S)	Phg	0.011	0.020	1.80
3	**5**-(S)	Leu	0.009	0.021	2.30
4	**5**-(S)	Trp	0.029	0.049	1.70
5	**6**-(R)	Phe	0.87	0.90	1.03
6	**7**-(S)	Phe	1.13	1.30	1.15
7	**7**-(S)	Leu	0.80	0.91	1.14
8	**8**-(S)	Phe	0.077	0.073	1.04
9	**9**-(R,R)	Phe	0.33	0.38	1.15
10	**9**-(R,R)	Phg	0.061	0.120	1.97
11	**9**-(R,R)	Leu	0.076	0.112	1.47
12	**10**-(R.R)	Phe	0.198	0.233	1.18
13	**10**-(R,R)	Phg	0.04	0.063	1.60
14	**10**-(R,R)	Leu	0.011	0.016	1.45

[a]Conditions: 25°C; [ligand]=$1x10^{-3}$ M, for the source and receiving phase see the standard conditions reported in the Experimental Section. [b]ER: Relative initial rate of transport of the two enantiomers.

Table II reveals also that (with the exception of ligand **8**), comparing homogeneous groups of ligands, the enantioselectivity is somehow related to the rate of transport with the faster carriers being less selective than the slower ones. Furthermore, a plot of the ER values for ligand **7** *vs* its concentration (Figure 4) shows a remarkable increase of the enantioselectivity as the concentration of the carrier (and, hence, the rate of transport) decreases. The source of the enantioselectivity could be either kinetic (the two diastereomeric complexes formed at the interface between the source and chloroform phases move at a different rate across the interface) or thermodynamic (the two diastereomeric complexes have different formation constants). Under complete kinetic control one would expect no dependence of the ER from the carrier concentration. Under thermodynamic control

an increase of concentration of carrier should be associated with a decrease of the ER and the enantioselectivity should eventually vanish out when the carrier concentration reaches the point at which the two amino acid enantiomers are totally bound. Of course, both thermodynamic and kinetic control may be at play and, depending on the conditions, control the process; however the results, illustrated by the plot of Figure 4, clearly suggest that the major source of enantioselectivity is thermodynamic.

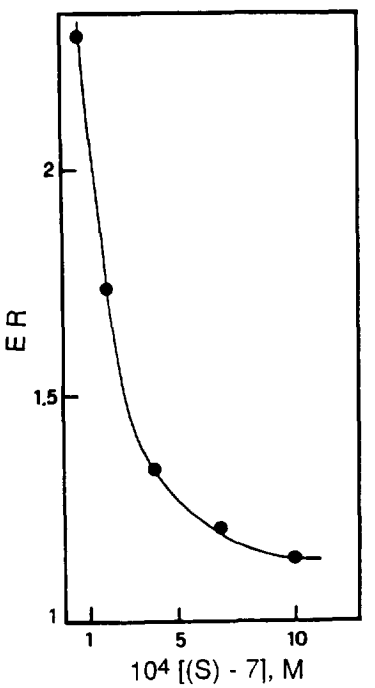


Figure 4. Change of the ER (see text) as a function of the ligand concentration in the transport experiments of the enantiomers of phenylalanine with carrier **7**.

CONCLUSION

We have shown that lipophilic ligands may act as carriers of Cu(II) ions and α-amino acids across a bulk chloroform membrane. Conditions for improving the efficiency of the release of the ions into the receiving phase have been found and some of the parameters influencing the rate of transport have been identified. From the point of view of the amino acid the main factor appears to be its lipophilicity, *i.e.* its availability at the interface between the source and organic phases. From the point of view of the carrier the main factor appears to be its affinity constant for the metal ion. This should be very close to that of the amino acid transported.

Enantioselectivities are modest though among the highest reported so far if one excludes the very peculiar case found by Cram[1b,c] using a crown ether-based system, which, to date, is still the best reported (ER ≈ 10). Steric factors appear to be of little relevance perhaps due to the flexibility of the complexes. The enantioselectivity effects observed in the cleavage of chiral amino acid esters by metallomicelles made of similar lipophilic ligands were much higher[4]; however in that case the geometrical and solvation requirements appeared to be more stringent than in the transport systems described here. The present investigation apparently indicate that, in the absence of further interactions[19] (hydrogen bonding or electrostatic, for instance) between carrier and amino acid the chances to improve the ER, up to a point of possible applications, are very tenuous.

We think that the present study brings new knowledge and helps understanding the transport phenomena. The translocation across natural or artificial membrane is a very complex process and the principles governing it are far from having been satisfactorily evaluated or even fully identified. Other parameters influencing the rate of transport, including an improved design of the transport apparatus and the use of supported membranes[20], have not been examined in the present study and, consequently, there is room for further investigation aimed to realize a more effective system.

EXPERIMENTAL

General. $Cu(NO_3)_2$ was analytical grade material. Ethylendiaminotetracetate, sodium salt (EDTA), 2-morpholinoetanesulphonic acid (MES), N,N-dimethyl-1,2-diaminoethane, (L)-phenylalanilol, (L)-phenylglycinol, (L)-leucinol were commercial materials used as received. *n*-Hexadecylaldehyde (m.p. 34-34 °C) was obtained by reduction of methyl-hexadecylcarboxylate ($LiAlH_4$) and oxidation of the resulting alcohol with pyridinium chlorochromate. Solvents used throughout have been purified according to standard procedures[21]. Compound **2** has been recently described[22].

All NMR spectra were recorded on a Bruker instrument operating at 200 MHz; chemical shifts, (δ, ppm) are refferred to internal $(CH_3)_4Si$. GC-MS spectra were recorded on a Hewlett-Packard 5970 instrument using a 15 m AllTech capillary column of polymethylsiloxane. Internal diameter of the column was 0.25 mm and the flux of the carrier gas (He) was 1mL/min. Elemental analyses were performed by the "Laboratorio di Microanalisi" of our Department. The enantiomeric purity of all chiral compounds was checked by ^{1}H-NMR using as shift reagent (S)-(+)-2,2,2-trifluoro-1-(9-antranyl)-ethanol: it was, in all cases, over 95 %.

N,N-Dimethyl-N'-n-hexadecyl-1,2-diaminoethane (**1**). n-Hexadecylaldehyde (1 g, 4.16 mmol) was dissolved in a dry THF solution containing N,N-dimethyl-1,2-diaminoethane (370 mg, 4.16 mmol) and freshly activated 4 Å molecular sieves. The solution was stirred overnight at room temperature under a nitrogen atmosphere. The resulting imine was added, after evaporation of the THF, to a methanol solution of $NaBH_4$ (315 mg, 8.3 mmol) and allowed to react 4 h at room temp. Usual work-up and purification on a SiO_2 column ($CHCl_3/CH_3OH$, 4:1) gave 406 mg of pure **1** as an oil. ^{1}H-NMR ($CDCl_3$): 0.88 (t, 3 H, $(CH_2)_nCH_3$); 1.27 (m, 26 H, $(CH_2)_{13}$); 1.48 (m, 2 H, $NCH_2CH_2(CH_2)_n$); 1.98 (br s, 1H, NH); 2.22 (s, 6 H, $N(CH_3)_2$); 2.41 (t, 2 H, $CH_2N(CH_3)_2$); 2.61 (t, 2 H, $NCH_2(CH_2)_n$); 2.69 (t, 2 H, $NHCH_2$). GC-MS: T_i=80°C; T_{max}=270°C; grad. 15°C/min; solvent $CHCl_3$. Retention time: 12.42 min.; m/z (%): 58 (100), M+ < 2%. Anal. Calcd. for $C_{20}H_{44}N_2$: C, 76.01; H, 15.03; N, 8.96%. Found: C, 75.91; H, 15.18; N, 8.85%.

N-n-Hexadecyl-1,2-diaminoethane, dihydrochloride (**3**·2HCl). This compound was prepared following the procedure described for the N-octyl analogue[17]. Accordingly, 1-bromohexadecane (1 g, 3.3 mmol) was dissolved in ethanol (50 mL) containing 1,2-diaminoethane (1 mL, 15 mmol) and the solution kept in a sealed pressure vial at 75°C overnight. Work up of the reaction mixture afforded, after column chromatography (SiO_2, $CHCl_3/CH_3OH$, 8:2), 426 mg (45% yield) of **3** as an oil. The compound was converted in the dihydrochloride by dissolving it in dioxane, adding a few drops of concentrated HCl solution and filtering off the solid obtained. ^{1}H-NMR (CD_3OD): 0.85 (t, 3 H, CH_3); 1.27 (m, 26 H, $(CH_2)_{13}$); 1.65 (m, 2 H, $NCH_2CH_2(CH_2)$n); 3.0 (t,

2 H, $NCH_2(CH_2)n$); 3.24 (s, 4 H, NCH_2CH_2N, overlapping with CD_2H signal of the solvent). Anal. Calcd. for $C_{18}H_{40}N_2 \cdot 2HCl$: C, 60.48; H, 11.84; N, 7.84%. Found: C, 60.25; H, 11.91; N, 7.72%.

N-n-Hexadecyl-N-methyl-1,2-diaminoethane (**4**). N-*n*-Hexadecyl-N-methyl-2-aminoethanol (2.6 g, 8.7 mmol) was dissolved in $SOCl_2$ (50 mL) and the solution stirred overnight at room temperature. After evaporation of the $SOCl_2$ (Caution: reacts exothermically with water!) the crude chloride derivative was dissolved in a 30% ethanolic solution of NH_3 (80 mL), introduced in a pressure vial and kept overnight at 70° C. Evaporation of the solvent and treatment with a Na_2CO_3 solution gave the crude free base which was purified by column chromatography on basic alumina ($CHCl_3/CH_3OH$, 1:1). The resulting pure **4** was isolated as an oil (1.04 g). ^{1}H-NMR (CD_3OD): 0.94 (t, 3 H, $(CH_2)_nCH_3$); 1.35 (m, 26 H, $(CH_2)_{13}$); 1.62 (m, 2 H, $NCH_2CH_2(CH_2)_n$); 2.50 (s, 3 H, NCH_3); 2.67 (br t, 2 H, $NCH_2(CH_2)_n$); 2.89 (t, 2 H, NCH_2); 3.16 (t, 2 H, NCH_2). Anal. Calcd. for $C_{19}H_{42}N_2$: C, 76.40; H, 14.18; N, 9.42%. Found: C, 76.31; H, 14.31; N, 9.35%.

(S)-1-Benzyl-1-amino-2-n-hexadecylaminoethane (**5**). The *n*-hexadecylamide of (S)-phenylalanine (860mg, 2.2 mmol), prepared following standard procedures for peptide synthesis, was reduced with $LiAlH_4$ (1g, 26.4 mmol) in dry THF at reflux for 16 h. Work-up of the reaction mixture gave the crude diaminoderivative. Purification by column chromatography over SiO_2 ($CHCl_3/CH_3OH$, 1:1) yielded 540 mg of pure **5** having m.p. = 44-45 °C and $[\alpha]_D$ = + 7.1 (c = 1, ethanol). ^{1}H-NMR ($CDCl_3$): 0.88 (br t, 3 H, CH_3); 1.27 (m, 26 H, $(CH_2)_{13}$); 1.17 (m, 2 H, $NCH_2CH_2(CH_2)_n$); 1.73 (br s, 2 H, NH_2); 2.42-2.86 (m, 6 H, 2 NCH_2 and CH_2Ph); 3.14 (m, 1 H, CH); 7.14-7.37 (m, 5 H, Ph). GC-MS: T_i=80°C; T_{max}=270°C; grad. 15°C/min; solvent: $CHCl_3$. Retention time: 17.50 min.; m/z (%): 375 (M^+, 2); 44 (100). Anal. Calcd. for $C_{25}H_{46}N_2$: C, 80.15; H, 12.40; N, 7.50%. Found: C, 81.40; H, 12.57; N, 7.31%.

General procedure for the synthesis of compounds **6 - 8**. Hexadecylaldehyde (640 mg, 2.66 mmol) and the proper substituted alaninol (2.64 mmol) were dissolved in dry THF containing freshly activated 4 Å molecular sieves and stirred overnight at room temperature under a nitrogen atmosphere. Filtration through a celite pad and evaporation of the solvent gave a diastereomeric mixture of 1,3 oxazolidine as ascertained by ^{1}H-NMR analysis (C_2H δ_{CDCl3} = 4.47, t). This crude material was reduced with $NaBH_4$ (3 mmol) in methanol (100 mL) over a period of 15 h at room temperature. Standard work-up of the reaction mixture gave the crude chiral 2-amino ethanol. This was purified by column chromatography ($CHCl_3/CH_3OH$; 10:1) to give the pure material characterized by ^{1}H-NMR and GC-MS. The 2-aminoethanol derivative (1.3 mmol) was then dissolved in freshly distilled $SOCl_2$ and stirred at room temperature for 36 h. The crude material obtained after evaporation of the $SOCl_2$ (Caution: $SOCl_2$ reacts vigorously with water!) was dissolved in a 5.6 M ethanolic solution of dimethylamine, sealed in a pressure vial and kept at 65°C for 15 h. Evaporation of the solvent and extraction with diluted NaOH gave, after anhydrification and evaporation of the solvent, the crude 1,2-diamino derivative. This was then purified by column chromatography over silica ($CHCl_3/CH_3OH$; 10:1).
The following compounds were obtained according to the above procedure:

(R)-1,2-diamino-(N,N-dimethyl-N'-n-hexadecyl)-2-benzylethane (**6**), oil (solid below 5°C), $[\alpha]_D$ = +3.53 (c = 1.3, ethanol). ^{1}H-NMR ($CDCl_3$): 0.87 (br t, 3 H, $(CH_2)_nCH_3$); 1.22 (m, 26 H, $(CH_2)_{13}$); 1.5 (br t, 2 H, $NCH_2(CH_2)_n$); 2.17 (s, 6 H, $N(CH_3)_2$); 2.27-3.12 (m, 7 H, $2NCH_2$, CH_2Ph and CH); 7.15-7.35 (m, 5 H, Ph). GC-MS: T_i=150°C; T_{max}=270°; grad. 20°C/min. Retention time: 10.52 min; m/z (%): 344 (100); M+ < 2%. Anal. Calcd for $C_{27}H_{50}N_2$: C, 82.69; H, 11.11; N, 6.20%. Found: C, 82.41; H, 11.28; N, 6.09%.

(S)-1,2-diamino-(N,N-dimethyl-N'-n-hexadecyl)-2-phenylethane (**7**), m. p. 36-37°C, $[\alpha]_D$ = -3.58 (c = 1, $CHCl_3$), 11.93 (c = 0.2, ethanol). ^{1}H-NMR ($CDCl_3$): 0.87 (br t, 3 H, $(CH_2)_nCH_3$); 1.27 (m, 26 H, $(CH_2)_{13}$); 2.17 (s, 6 H, $N(CH_3)_2$); 2.60 (m, 2 H, $NCH_2CH_2(CH_2)_n$); 2.80 (m, 2 H, $NCH_2(CH_2)_n$); 2.95-3.12 (m, 2 H, NCH_2CHN); 3.75 (m, 1 H, CH); 7.18-7.40 (m, 5 H, Ph). GC-MS: T_i=150°C; T_{max}=270°C; grad. 20°C/min. Retention time: 10.94 min.; m/z(%): 134(100), M+ <2%. Anal. Calcd. for $C_{26}H_{48}N_2$: C, 80.34; H, 12.45; N, 7.21%. Found: C, 79.37; H, 12.68; N, 6.52%.

(S)-1,2-diamino-(N,N-dimethyl-N'-n-hexadecyl)-2-(2-methylpropyl)ethane (**8**), oil, $[\alpha]_D$ = + 37 (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.89 (overlapping t and d, 9 H, $CH_3(CH_2)_n$ and $C(CH_3)_2$); 1.25 (m, 26 H, $(CH_2)_{13}$); 1.48 (m, 2H, $NCH_2CH_2(CH_2)_n$); 1.62 (m, 1 H, CH *i*Pr); 2.05-2.32 (m, 2 H, CH_2 *i*Pr); 2.20 (s, 2 H, $N(CH_3)_2$); 2.40-2.80 (m, 5 H, 2 NCH_2 and NCH). GC-MS: T_i=150°C; T_{max}=270°C; grad. 20°C/min. Retention time: 7.63 min.; m/z(%): 310(100); 368 (M+, 2). Anal. Calcd. for $C_{24}H_{52}N_2$: C, 78.16; H, 14.21; N, 7.63%. Found: C, 77.97; H, 14.32; N, 7.51%.

General procedure for the synthesis of ligands **9** and **10**. The 1,2 diamine (500 mg) and two equivalents of triethylamine were dissolved in 50 mL of THF (occasional heating favours dissolution). To this solution, one equivalent of octanoylchloride was added in *ca* 30 min. The solution was let to stir (protected from moisture, $CaCl_2$) overnight at room temperature. After evaporation of the solvent the solid material was taken up with CH_2Cl_2 (100 mL), extracted with a 10% solution of $NaHCO_3$, and the organic solution was evaporated after drying with Na_2SO_4. The yield of crude diamide was usually >90% (^{1}H-NMR). This diamide was dissolved in dry THF to which $LiAlH_4$ (1 M soln. in THF, a fourfold stoichiometric excess) was added. The reaction mixture was refluxed overnight under dry N_2 and worked-up as usual[23] to yield the crude diamine. Final purification by column chromatography (SiO_2, AcOEt/EtOH, 3:2 for **9** and $CH_2Cl_2 \rightarrow CH_2Cl_2/CH_3OH$ 20:1 for **10**) afforded the analytically pure diamine:

(R,R)-1,2-dioctylaminocyclohexane (**9**), oil, $[\alpha]_D$ = -63.5 (c = 4.8, $CHCl_3$). ^{1}H-NMR, ($CDCl_3$): 0.85 (m, 6 H, 2 $(CH_2)_nCH_3$); 1.25 (m, 24 H, 2 $(CH_2)_6$); 0.85-1.68 (m, 8 H, 4 cyclohexane CH_2); 2.05 (m, 4 H, 2 $NCH_2(CH_2)$n); 2.38 2.69 (m, 2 H, cyclohexane H_1 and H_2). Anal. Calcd. for $C_{22}H_{46}N_2$: C, 78.01; H, 13.69; N, 8.30%. Found: C, 77.61; H, 13.76; N, 8.19%.

(R,R)-1,2-diphenyl-1,2-dioctylaminoethane (**10**), oil, $[\alpha]_D$ = -4.0 (c = 1, $CHCl_3$). ^{1}H-NMR, ($CDCl_3$): 0.87 (m, 6 H, 2 $(CH_2)_nCH_3$); 1.22 (m, 16 H, 2 $(CH_2)4$); 1.42 (m, 4 H, 2 NCH_2CH_2); 2.06 (br s, 2 H, 2 NH); 2.38 (m, 4 H, 2 $NCH_2(CH_2)_n$); 3.61 (m, 2 H, 2 CHPh); 7.08 (m, 10 H, 2 Ph). Anal. Calcd. for $C_{30}H_{48}N_2$: C, 82.48; H, 11.08; N, 6.44%. Found: C, 82.11; H, 11.19; N, 6.28%.

Transport experiments. For these experiments two "U"-shaped matching cells have been used. The internal diameter of the glass tube was 1.6 cm. This tube was immersed in a thermostatted water solution and kept at 25.0 ± 0.5 °C. The water phases were mechanically stirred with two rotating teflon bars while the chloroform solution was magnetically stirred at 400 rpm. Composition of the solutions of the three phases was: source phase, 6 mL of H_2O, pH = 5.5, 0.25 M MES buffer, [amino acid] = $5x10^{-3}$ M, $[Cu(NO_3)_2]$ = $2x10^{-2}$ M; liquid membrane, 20 mL of $CHCl_3$ containing the ligand; receiving phase, 6 mL of H_2O contaning [EDTA] = $5x10^{-2}$ M, pH = 6.5. At time intervals 100 µL (2x100 µL in the case of the contemporaneous analysis of the amino acid and Cu(II)) of source and receiving phase were withdrawn and subject to the analysis of the amino acid or Cu(II).

Amino acids analysis. 100 µL of the solution to be analyzed were added to 0.9 mL of a $2.5x10^{-2}$ M EDTA solution and 1 mL of ninhydrine reagent[24] (see below for its preparation). The solution was warmed to 70°C for 20 min and its absorbance determined at 570 nm. The µmoles of amino acid, $(n_i)_{AA}$, were determined using the following equation:

$$(n_i)_{AA} = \frac{(A_i \times V_i + \sum_n A_n \times \Delta V) \times 20}{\varepsilon} \times 10^6$$

where ε is the molar extinction coefficient of the amino acid-ninhydrine derivative at 570 nm, A_i the absorbance at time i, V_i is the volume of the analyzed phase at time i, ΔV is the volume withdrawn (usually $1x10^{-4}$ L).
The ninhydrin reagent was prepared immediately before its use by dissolving 1 g of ninhydrine and 150 mg of hydridantine in 37.5 mL of 2-methoxyethanol. To this solution, 12.5 mL of acetate buffer (I=0.1), pH=5.36, was subsequently added.

The μmoles of Cu(II) were determined with a Perkin Elmer 360 Atomic Absorbtion instrument using a multielement lamp. Each sample (100 μL) was diluted with 0.9 mL of water and subsequently subject to the analysis. The μmoles of Cu(II) transported, $(n_i)_{Cu}$ have been calculated using the following equation:

$$(n_i)_{Cu} = \frac{(ppm_i \times V_i + \sum_n \Delta V \times ppm_n)}{MW_{Cu}} \times 10^4$$

where ppm_i is the amount (mg/L) of Cu(II) in the i sample; V_i is the volume (liters) of the phase analyzed at time i; ΔV is volume withdrawn ($1x10^{-4}$ or $2x10^{-4}$ L).

Acknowledgments.

Experimental work, at different stages of the investigation, has been performed by Drs. E. Renzi, S. Poli, and G. Cozzi: their contribution is gratefully acknowledged. The authors are indebted with Mr. E. Castiglione for technical support. Funding was provided by the C.N.R. (Rome) in the frame of the "Progetto Finalizzato Chimica Fine II" and the Ministry of the University and Scientific and Technological Research (MURST).

REFERENCES

1. a) Behr, J.-P.; Lehn, J.-M. *J. Am.Chem. Soc.* **1973**, *95*, 6108; b) Newcomb, M.; Helgeson, R. C.; Cram, D. J. *J. Am. Chem. Soc.* **1974**, *96*, 7367; c) Newcomb, M.; Toner, J. L.; Helgeson, R. C.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 4941; d) Tsukube, H. *Tetrahedron Lett.* **1981**, *22*, 3981; e) Rebek, J.; Askew, B.; Nemeth, D.; Parris, K. *J. Am. Chem. Soc.* **1987**, *109*, 2432; f) Kataoka, H.; Katagi, T. *Tetrahedron* **1987**, *43*, 4519; g) Zinic, M.; Frkanec, L.; Skaric, V.; Trafton, J.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1990**, 1726; h) Chang, S.-K.; Hwang, H.-S.; Son, H.; Youk, J.; Kang, Y. S. *J. Chem. Soc., Chem. Commun.* **1991**, 217; i) Mohler, L. K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 7037.
2. a) Maruyama, K.; Tsukube, H.; Araki, T.; *Tetrahedron Lett.* **1981**, *22*, 2001; b) Maruyama, K.; Tsukube, H.; Araki, T. *J. Am. Chem. Soc.* **1982**, *104*, 5197.
3. For a review of biochemical amino acid transport systems, see: Ring, K. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 345.
4. a) Fornasier, R.; Scrimin, P.; Tonellato, U.; Zanta, N. *J. Chem. Soc., Chem. Commun.* **1988**, 716; b) Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Org. Chem.*, **1994**, *59*, 4194.
5. Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 394.
6. a) Maruyama, K.; Tsukube, H.; Araki, T. *J. Am. Chem. Soc.* **1980**, *102*, 3246; b) Kishii, N.; Araki, K.; Shiraishi, S. *J. Chem. Soc., Chem. Commun.* **1984**, 103; c) DiCasa, M.; Fabbrizzi, L.; Perotti, A.; Poggi, A.; Tundo, P. *Inorg. Chem.* **1985**, *24*, 1610; d) Kimura, E.; Dalimunte, C. A.; Yamashita, A.; Machida, R. *J. Chem. Soc., Chem. Commun.* **1985**, 1041; e) DiCasa, M.; Fabbrizzi, L.; Perotti, A.; Poggi, A.; Riscassi, R. *Inorg. Chem.* **1986**, *25*, 3984.
7. Menger, F. M.; Lee, J-J. *J. Org. Chem.* **1993**, *58*, 1909.
8. Scrimin, P.; Tonellato, U.; Zanta, N. *Tetrahedron Lett.* **1988**, *29*, 4967.
9. Hathaway, B. J. in *Comprehensive Coordination Chemistry*; Wilkinson, G.; Gillard, R. D.; McCleverty, J. Eds, Pergamon Press: Oxford, 1987; Chapter 53, pp 596-651.

10. Ref. 9, pp 690-698.
11. Demaret, A.; Abello, L.; Fourati, M.; Lapluye, G. *J. Chem. Res.* **1978**, *9*, 354.
12. The ligand we have used in our preliminary investigation (ref. 8) has formally three donor atoms. However there are clear evidences suggesting that one of them may be easily displaced (see ref. 4b) to make room for a stronger donor.
13. The affinity costant for the 1:1 Cu(II)-EDTA complex is $10^{18.8}$ M^{-1}; see: Freiser, H.; Fernando, Q. *Ionic Equilibria in Analytical Chemistry*, Wiley: New York, 1963; p. 314.
14. a) Radzicka, A.; Wolfenden, R. *Biochemistry* **1988**, *27*, 1664; b) Adachi, M.; Harada, M.; Shioi, A.; Sato, Y. *J. Phys. Chem.* **1991**, *95*, 7925.
15. The difference in affinity constants exceeds 6 order of magnitude; see: Smith, R. M.; Martell, A. E. *Critical Stability Constants*, Plenum Press: New York, 1975, vol 4.
16. The association costants values of 1,2-diamino-N-methyl-ethane, 1,2-diamino-N,N-dimethyl-ethane, 1,2-diamino-N,N-diethyl-N'-methyl-ethane, 1,2-diamino-N,N-dimethyl-N',N'-dimethyl-ethane and phenylalanine reported in Figure 3 were taken from: Smith, R. M.; Martell, A. E., *Critical Stability Constants*, Plenum Press: New York, 1975, vol. 2; the change of the length of one hydrocarbon chain has only a minor effect on the binding constants, see reference 17.
17. Butvin, P.; Skoumal, M.; Radl, Z.; Peterka, V.; Majer, J. *Chem. Papers*, **1988**, *42*, 475.
18. Pettit, L. D.; Hefford, J. W. in *Metal Ions in Biological Systems* Sigel, H. Ed., Dekker: New York, 1979, vol. 9, Chapter 6.
19. Evidences of such interactions have been reported in polar solvents; see: a)Yamauchi, O.; Odani, A. *J. Am. Chem. Soc.* **1985**, *107*, 5938; b) Sigel, H. *Pure Appl. Chem.* **1989**, *61*, 923.
20. For a refernce on supported liquid membranes see: Stolwijk, T. B.; Sudholter, E. J. R.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1987**, *109*, 7042.
21. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd Edt, Pergamon Press: Oxford, 1980.
22. Lim, Y. Y.; Tan, E. H. L.; Gan, L. H. *J. Colloid. Interface Sci.***1993**, *157*, 442.
23. Fieser, M.; Fieser, L. F. *Reagents for Organic Synthesis*, Wiley: New York, 1967, vol. 1, pp.581-584.
24. Mc Caldin, D. J. *Chem. Rev.* **1960**, *60*, 39.

(*Received in UK* 13 *September* 1994; *revised* 20 *October* 1994; *accepted* 21 *October* 1994)