Efficient and Highly Selective Copper(II) Transport across a Bulk Liquid Chloroform Membrane Mediated by Lipophilic Dipeptides

Marco C. Cleij,[†,§] Paolo Scrimin,[‡] Paolo Tecilla,[†] and Umberto Tonellato*[,†]

Department of Organic Chemistry and Centro CNR Meccanismi di Reazioni Organiche, University of Padova, Via Marzolo 1, 35131 Padova, Italy, and Department of Chemical Sciences, University of Trieste, Via Giorgieri 1, 34127 Trieste, Italy

Received February 19, 1997[⊗]

Several structurally simple N-monoalkylated and -dialkylated dipeptides made of α-amino acids Gly, Phe, and Leu, **1**−**11**, were synthesized and investigated as carriers for the transport of Cu(II), Zn(II), and Ni(II) from an aqueous pH = 5.6 buffer source to a 0.1 M HCl receiving phase across a bulk chloroform membrane. The proton-driven translocation was followed during the process by analyzing the metal ion concentrations in the three phases. The transport efficiency depends on the ease of formation of a neutral complex with Cu(II) (the peptide group and carboxylic acid being deprotonated) at the source−chloroform interface and on that of its disruption by protonation at the receiving phase: the carrier's lipophilicity favors the metal ion uptake and not the release. By modulating the length of the *N*-alkyl chains and the hydrophobicity of the dipeptide moiety, a quite remarkable transport efficiency was observed for Cu(II), in most cases superior to that of the industrial extractant Kelex 100. Moreover, using L,L- and L,D-*N*-octyl-PheLeu as carriers, remarkable diastereomeric effects were observed in the rate of uptake and release of Cu(II) ion although the differences mutually compensate in the overall transport rate. Under the conditions used the carriers are much less effective in the translocation of Zn(II) and Ni(II) and their transport efficiency drops dramatically in the presence of Cu(II), the latter being favored by factors of 1.2×10^3 and $>10^4$, respectively. Such very high selectivities depend on the fact that only Cu(II) among other transition metal ions can form neutral complexes at the pH value of the source phase.

The selective transport of metal ions across a membrane is known to play an essential role in many biological processes.[1] Over the years, a large number of molecules featuring proper binding sites, particularly crown ethers, have been synthesized and demonstrated to act as selective carriers of alkali or ammonium ions across a membrane.[2] Theoretical models aimed at correlating the efficacy and selectivity of the transport with the structure of the carrier molecules have been also proposed and experimentally tested.[3] In the case of transition metal ions, although a large number of effective ligands have been characterized and utilized in selective extractions[4] from an aqueous into an organic phase, fewer studies dealing with their transport across a membrane have been reported[5−13] in spite of a growing demand for these systems in waste water treatment, medicine, and metallurgy. Investigations of the permeation of biological membranes or of closely related vesicular double layers are not easily accessible,[9] and most studies in the field have been addressed to liquid membranes, made usually of chloroform. As pointed out by Menger,[10] liquid bulk organic solvents bear very little similarity with biological membranes; however, they may be useful for evaluating the factors playing a role in the translocation from and to aqueous pools through an

*Corresponding author. Phone: +39-49-8275269. Fax: +39-49-8275239. E-mail: tonum@chor02.chor.unipd.it.
† University of Padova.
‡ University of Trieste.
§ European E.C. Research Fellow (1993−1994).
⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1997.

(1) (a) Westley, J. W.; Evans, R. H.; Williams, T.; Stempel, A. *J. Chem. Soc., Chem. Commun.* **1970**, 71. (b) Maier, C. A.; Paul, I. C. *J. Chem. Soc., Chem. Commun.* **1971**, 181. (c) Ring, K. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 345. (d) Ovchinnikov, Y. A.; Ivanov, V. T.; Shkrob, A. M. *Membrane Active Complexons*; Elsevier: Amsterdam, 1974. (e) Urry, D. W. *Top. Curr. Chem.* **1985**, *128*, 175. (f) Linder, M. C. *Biochemistry of Copper*; Plenum: New York, 1991.

(2) Selected examples: (a) Chrisstoffels, L. A. J.; Struijk, W.; de Jong, F.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1617. (b) Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425. (c) Xie, Q.; Gokel, G. W.; Hernandez, J.; Echegoyen, L. *J. Am. Chem. Soc.* **1994**, *116*, 690. (d) Pregel, M. J.; Jullien, L.; Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1637. (e) Izatt, R. M.; Gypzy, C. L.; Biernat, J. F.; Bochenska, M.; Bruening, R. L.; Bradshaw, J. S.; Christensen, J. J. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1989**, *7*, 487. (f) Masushima, K.; Kobayashi, H.; Nakatuji, Y.; Okahara, M. *Chem. Lett.* **1983**, 792. (g) Schchori, E.; Grodzinskira, J. J. *J. Appl. Polym. Sci.* **1976**, *20*, 773. (h) Choy, E. M.; Evans, D. F.; Cussler, E. L. *J. Am. Chem. Soc.* **1974**, *96*, 7085.

(3) (a) Lamb, J. D.; Izatt, R. M.; Garrick, D. G.; Bradshaw, J. S.; Christensen, J. J. *J. Membr. Sci.* **1981**, *9*, 83. (b) Izatt, R. M.; Clark, G. A.; Bradshaw, J. S.; Lamb, J. D.; Christensen, J. J. *Sep. Purif. Methods* **1986**, *15*, 21. (c) Behr, J. P.; Kirch, M.; Lehn, J. M. *J. Am. Chem. Soc.* **1985**, *107*, 241. (d) Visser, H. C.; Reinhoudt, D. N.; de Jong, F. *Chem. Soc. Rev.* **1994**, 75 and references cited therein.

(4) Reviews: (a) Freiser, H. *Anal. Sci.* **1995**, *11*, 191. (b) Freiser, H. *Chem. Rev.* **1988**, *88*, 611. (c) Lobana, T. S.; Sandhu, S. S. *Coord. Chem. Rev.* **1982**, *47*, 283. (d) Uhlig, E. *Coord. Chem. Rev.* **1982**, *43*, 299.

(5) (a) Hiratani, K.; Taguchi, K.; Ohhashi, K.; Nakayama, H. *Chem. Lett.* **1989**, 2073. (b) Hiratani, K.; Hirose, T.; Fujiwara, K.; Saito, K. *Chem. Lett.* **1990**, 1921. (c) Hiratani, K.; Taguchi, K. *Chem. Lett.* **1990**, 725. (d) Hiratani, K.; Hirose, T.; Saito, K. *J. Org. Chem.* **1992**, *57*, 7083. (e) Kasuga, K.; Hirose, T.; Takahashi, T.; Hiratani, K. *Chem. Lett.* **1993**, 2183. (f) Hiratani, K.; Sugihara, H.; Kasuga, K.; Fujiwara, K.; Hayashita, T.; Bartsch, R. A. *J. Chem. Soc., Chem. Commun.* **1994**, *319*.

(6) Di Casa, M.; Fabbrizzi, L.; Perotti, A.; Poggi, A.; Riscassi, R. *Inorg. Chem.* **1986**, *25*, 3984.

(7) (a) Tsukube, H.; Adachi, H.; Morosawa, S. *J. Chem. Soc., Perkin Trans. I* **1989**, 89. (b) Maruyama, K.; Tsukube, H. *J. Am. Chem. Soc.* **1980**, *102*, 3246.

(8) (a) Kishii, N.; Araki, K.; Shiraishi, S. *J. Chem. Soc. Dalton Trans.* **1985**, 373. (b) Kishii, N.; Araki, K.; Shiraishi, S. *J. Chem. Soc., Chem. Commun.* **1984**, 103.

(9) (a) Moss, R. A.; Byeong, D. P.; Scrimin, P.; Ghirlanda, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1627. (b) Ghirlanda, G.; Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Org. Chem.* **1993**, *58*, 3025. (c) Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Am. Chem. Soc.* **1992**, *114*, 5086. (d) Kragten, U. F.; Roks, M. F. M.; Nolte, R. J. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1275.

(10) Menger, F. M.; Lee, J. J. *J. Org. Chem.* **1993**, *58*, 1909.

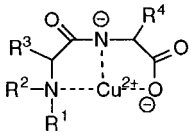
(11) Ameerunisha, S.; Zacharias, P. S. *Polyhedron* **1994**, *13*, 2327.

(12) (a) Scrimin, P.; Tonellato, U.; Zanta, N. *Tetrahedron Lett.* **1988**, *29*, 4967. (b) Scrimin, P.; Tecilla, P.; Tonellato, U. *Tetrahedron* **1995**, *51*, 217.

(13) Kimura, E.; Dalimunte, C. A.; Yamashita, A.; Machida, R. *J. Chem. Soc., Chem. Commun.* **1985**, 1041.

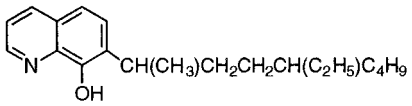
S0022-3263(97)00325-3 CCC: $14.00

Chart 1



Carrier*	R^1	R^2	R^3	R^4
1	C_2H_5	C_2H_5	H	H
2	C_4H_9	C_4H_9	H	H
3	C_6H_{13}	C_6H_{13}	H	H
4	C_8H_{17}	C_8H_{17}	H	H
5	$C_{12}H_{25}$	$C_{12}H_{25}$	H	H
6	C_8H_{17}	C_8H_{17}	$CH_2C_6H_5$	H
7	C_8H_{17}	C_8H_{17}	H	$CH_2CH(CH_3)_2$
8	C_8H_{17}	C_8H_{17}	H	$CH_2C_6H_5$
9	C_8H_{17}	C_8H_{17}	$CH_2C_6H_5$	$CH_2C_6H_5$
10	C_8H_{17}	H	$CH_2C_6H_5$	$CH_2CH(CH_3)_2$
11	C_8H_{17}	H	$CH_2C_6H_5$	$CH_2CH(CH_3)_2$

*All chiral amino acid residues have *L*- configuration with the exception of the Leu residue in **11** which has *D*- configuration.



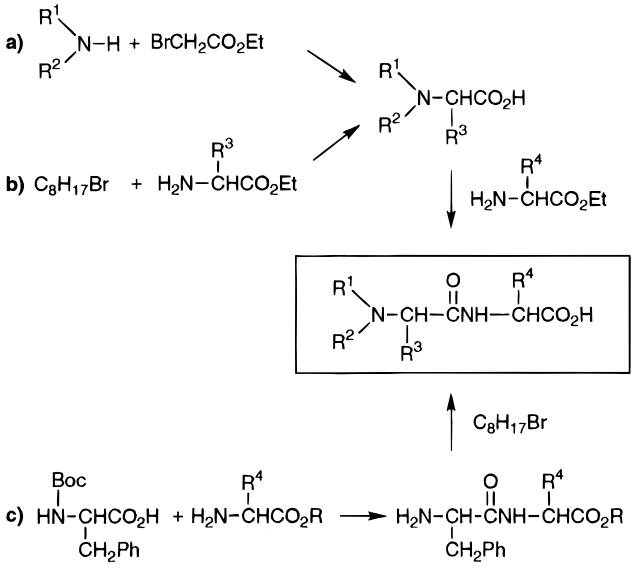
Kelex 100

organic phase. From the published studies, also from this laboratory,[12] the transport through liquid membranes is a more complicated process than it may appear, involving a subtle interplay of many factors that are not easy to estimate: thus it has been shown that a ligand that is very efficient and selective in extracting transition metal ions from an aqueous phase into an organic phase may be extremely poor in its transport ability[5a,b] and vice versa.[5b,c]

In this paper, we report our efforts to produce simple ligands that are highly effective and selective carriers in the transport of Cu(II) across a bulk chloroform membrane. We focused our attention on simple dipeptides, like glycylglycine, which are known to strongly bind Cu(II) in aqueous solution to give a neutral complex in which the amido group is deprotonated[14−16] also under slightly acidic conditions. The dipeptide coordinates with its amino and amide nitrogens and with a carboxylate oxygen to three of the four square-planar coordination sites of the Cu(II) ion as schematically represented in Chart 1. Deprotonation of the amide group under slightly acidic or neutral pH only occurs upon coordination with Cu(II) and not with Ni(II), Zn(II), or Co(II).[16] Therefore, dipeptide derivatives are potential candidates for the selective transport of Cu(II) across a membrane, and their selectivity may be modulated on changing the pH of the solution.

On these premises, we synthesized and investigated the dipeptides listed in Chart 1. Compounds **1**−**5** are GlyGly derivatives with the amino group alkylated with a pair of paraffinic chains of increasing (from ethyl to *n*-dodecyl) length and, hence, lipophilicity. The structures of compounds **6**−**9** are just variations of that of **4** aimed at increasing its lipophilicity: in **6** the N-terminal amino acid is Phe, in **7** and **8** the C-terminal amino acids are Leu and Phe, respectively, and in **9** both amino acids of the dipeptide are Phe. A special case is that of **10** and **11**: they are the L,L and L,D diastereomers of the *N*-octyl-PheLeu and were conceived to verify the possible effect of diastereoselectivity on the Cu(II) transport. Moreover, to compare the effectiveness of these carriers to that of extractants used in industry, we included in our study the active component of the industrial extractant Kelex 100,[17] *i.e*, the 7-(4-ethyl-1-methyloctyl)-8-hydroxyquinoline.

Scheme 1



Results and Discussion

Synthesis of the Carriers. Scheme 1 shows the synthetic routes followed to obtain the ligands. Route a, involving as a first step the alkylation of the proper symmetrically substituted amine with the ethyl ester of bromoacetic acid, was convenient for the synthesis of **1**−**5**, and **7**, and **8**. Compound **6** was obtained following route b through the dialkylation of the ethyl ester of *L*-phenylalanine with octyl bromide. Compounds **9**−**11** were prepared via route c, by reacting the Boc-protected phenylalanine with the phenylalanine or leucine ethyl ester and, after deprotection, by alkylating the amino group of the dipeptide with octyl bromide. The yields of the latter step were very low (<25%) due to the competitive formation of diketopiperazines; however, since the optical purity of the diastereomeric ligand was relevant, we preferred this route because of the absence of any significant racemization (as ensured by the ^{1}H-NMR analysis of the products).

Transport and Related Experiments. The experimental setup for the transport experiments is shown in Figure 1. A U-shaped glass apparatus, the inner diameter of the arms being 16.5 mm, was placed in a thermostatted bath kept at 25 °C. The transport experiments were carried out using the following procedure. The bulk liquid membrane made of a 1.0 mM solution of the ligand in 20 mL of $CHCl_3$ separated the two aqueous phases I and II. Aqueous phase I was a 5.0 mM solution

(14) Dobbie, H.; Kermack, W. O. *Biochem. J.* **1955**, *59*, 246.
(15) Datta, S. P.; Rabin, B. R. *Trans. Faraday Soc.* **1956**, *52*, 1117, 1123.
(16) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.
(17) Boumezioud, M.; Tondre, C. *Polyhedron* **1988**, *7*, 513.

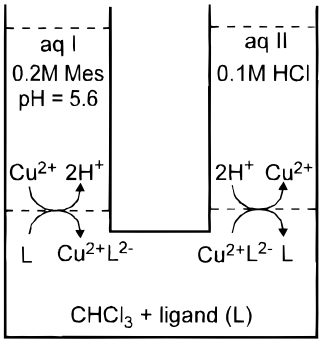


Figure 1. Experimental setup used in the transport experiments. See also the text.

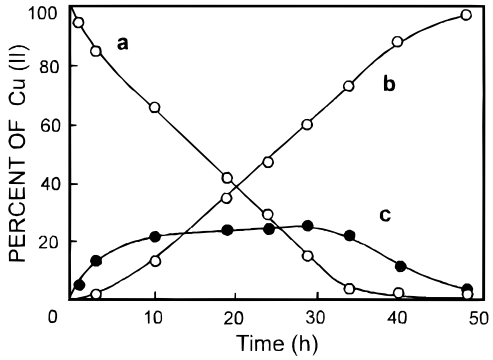


Figure 2. Percent of initial Cu(II) in aqueous phase I (curve a), aqueous phase II (curve b), and $CHCl_3$ phase (curve c) during a transport experiment with **4**. See also the text and for conditions Table 1.

(6.0 mL) of $M(NO_3)_2$ (M being Cu^{2+}, Zn^{2+}, or Ni^{2+}) in 0.2 M MES buffer at pH = 5.6,[18] and aqueous phase II was a 0.1 M HCl solution (6.0 mL). All three phases were stirred at such a rate as to ensure efficacy and smoothness at the interfaces. Under these conditions, the carrier transports the M^{2+} ions from aqueous phase I to II (where it is protonated) and protons from aqueous phase II to I. Thus, the process is driven not only by a difference in M^{2+} concentration but also by a difference in proton concentration: as a consequence, more than 50% of the M^{2+} ions can be transported and, as long as the pH difference of the aqueous phases is maintained, all the ions can be translocated (see *infra* and Figure 2).

Associated to the transport experiments we carried out simple extraction experiments and in particular two sequential runs, referred to as (i) and (ii), using simple glass test tubes of inner diameter 16.5 mm kept at 25 °C. (i) A 1 mM solution of ligand in $CHCl_3$ (20 mL) was equilibrated against aqueous donor phase I (6 mL), and the amount of Cu(II) extracted in $CHCl_3$ was determined. (ii) The thus obtained $CHCl_3$ solutions of metal complex were then exposed to the receiving acid aqueous phase II (6 mL, in the absence of phase I), both phases were stirred, and the rate (see below) of release of metal ion into the aqueous phase was measured. Since ligands **1** and **2** are too hydrophilic, the rate of release of metal ion was measured using preformed solutions of their copper complex in chloroform. In each case, the metal ion concentrations were determined by means of atomic absorption spectroscopic analyses.

(18) A reviewer suggested that the actual transported species could be $Cu(OH)^+$. However at pH = 5.6, which is the highest pH value used in the present work, copper ion is present approximately 94% as Cu^{2+} and only 6% as $Cu(OH)^+$ (see Basolo, F.; Pearson, R. G. *Mechanism of Inorganic Reaction*; J. Wiley and Sons: New York, 1967). In view of that, we omitted the presence of the latter species when drawing Figure 1. Moreover, studies with similar ligands[16] have shown that the most stable complex formed is neutral and involves Cu^{2+} and not $Cu(OH)^+$.

Table 1. Results of Cu(II) Transport, Extraction, and Decomplexation Experiments Obtained with Ligands 1−11 at 25 °C[a]

carrier	$T_{24\ (\%/24\ h)}$[b]	$k_{1\ (\%/h)}$[c]	$k_{2\ (\%/h)}$[d]	E[e]
1	0	0	38	nd[f]
2	9	1	38	nd[f]
3	24	3	23	0.67
4	47	8	18	0.69
5	49	8	17	0.70
6	53	11	22	0.67
7	54	12	17	0.69
8	23	19	7	0.73
9	9	4	7	0.47
10	67	11	28	0.91
11	66	24	19	0.94
Kelex 100	39	6	nd[f]	nd[f]

[a] For conditions, see text. [b] Percent of initial Cu(II) that is transported after 24 h. [c] Initial rate of Cu(II) extraction into the $CHCl_3$ phase expressed as the percentage of ligand that has formed a complex with Cu(II) in the $CHCl_3$ phase per hour. See also the text. [d] Initial rate of release of Cu(II) from $CHCl_3$ into phase II in the decomplexation experiments. The numbers give the percentage of the total amount of Cu(II) that is released per hour. See also the text. [e] The fraction of ligand in the $CHCl_3$ phase that has formed a complex with Cu(II) when equilibrated against phase I. See also the text. [f] Not determined.

Transport of Cu(II) Ions. Preliminary extraction tests, using the standard aqueous phase I and the chloroform solution, indicated that ligands **1** and **2** and their Cu(II) complexes partition quite favorably in water, **3** is an intermediate case (interestingly the free ligand is more water soluble than its complex which almost entirely resides in the $CHCl_3$ phase), and all the other ligands **4**−**11** and their complexes are virtually absent in the aqueous phase after equilibration.

Then we carried out a systematic analysis of the transport of Cu(II) using carriers **1**-**11** and Kelex 100. The main results are shown in Table 1 where the data are reported in a rather straightforward fashion in the following terms. (a) T_{24} is the percentage of the total amount of Cu ions transported in 24 h from aqueous phase I to phase II. The T_{24} values range from 0 for **1** up to 67 for **10**, and it is here emphasized that many of the new ligands are considerably more efficient in the transport of Cu(II) than Kelex 100 (T_{24} = 39). (b) k_1, here referred to as the initial rate of uptake, indicates the percentage of ligand that forms a complex and transfers the metal ion from the donor phase (I) into the $CHCl_3$ phase per hour at the beginning of the transport experiment. These rates range from 0 for **1** to 24 for **11**. (c) k_2, here referred to as the initial rate of release and measured in the associated experiments described above as (ii), is the fraction of metal ion initially contained in the $CHCl_3$ which is released per hour into the acidic aqueous phase II. The rates of release change from 7 for ligands **8** and **9** to 38 for **1** and **2**. (d) E, the extraction efficiency value, is the ratio of the ligand concentration and that of the Cu(II) that is extracted from aqueous phase I (pH = 5.6) into the $CHCl_3$ phase and measured in the associated experiment described above as (i). The E values range from 0.47 for **9** to 0.94 for **11**.

Figure 2 shows the changes of the amount of Cu(II) in the three phases in a transport experiment (note that the ion is fully translocated at the end of the experiment, after *ca.* 50 h) using **4** as a carrier, and this diagram can

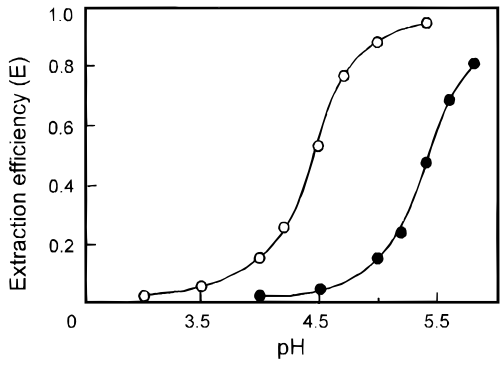


Figure 3. Extraction efficiency of Cu(II) into the $CHCl_3$ phase by **4** at different pH values in the absence (○) and presence (●) of 0.2 M MES buffer. See also the text.

be taken as typical also for carriers **5**−**11**. In the first few hours, the amount of Cu(II) ions in phase I drops rapidly and likewise increases in the $CHCl_3$ phase so that little transport occurs. Then, a steady state situation is reached in which the amount of Cu(II) in the $CHCl_3$ phase changes very little whereas the decrease in the donating phase and the increase in the receiving phase are constant and equal. Finally, when all the Cu(II) is extracted from aqueous phase I, the concentration of the metal ions in the $CHCl_3$ phase drops and the rate of release into aqueous phase II slows down. This plot shows that the rate of extraction from the donor aqueous phase I is zeroth order in Cu(II) concentration in phase I. Even at a very low Cu(II) concentration the rate of extraction remains constant. This implies that at these Cu(II) concentrations the rate of extraction of Cu(II) does not depend on the rate of complex formation at the aqueous phase I−$CHCl_3$ interface and that the amount of complex at this interface is constant. Thus by all evidence, rate determining for the process of metal ion extraction from aqueous phase I to chloroform is the transfer (or dehydration) of the hydrophilic part of the amphiphilic complex through the interface of the two phases.

Further experiments were then carried out in order to complement the results shown in Table 1 and to gain insight into the system investigated. We measured the E values in the case of **4** using buffered (MES, 0.2 M) and unbuffered donating aqueous solutions of $Cu(NO_3)_2$ (5 mM) of different pHs. The results are illustrated in Figure 3 and indicate a quite substantial effect of the buffer. Assuming that the effective chelating agent is the ligand in its deprotonated form, the curves of Figure 3 appear as titration curves, and one may estimate that in the absence of buffer the pK_a of the amido group is 4.5 and that in the presence of the buffer the apparent pK_a increases to 5.4. Whatever the reasons for such an effect, this means that in the presence of 0.2 M MES, as in the standard transport experiments (Table 1), the extraction efficiency (E = 0.69) is not as large as in the absence of buffer. We also noticed that the T_{24} and k_1 values increased when the buffer concentration was reduced to 0.1 M. However, we preferred to use the higher buffer concentration since this guarantees a stable pH and a steady efficiency of the system for at least 3 days. As a matter of fact, the pH gradient is of paramount importance: we observed that if the receiving phase was a 0.2 M aqueous solution of MES buffer of pH = 5.6 (just as the donating phase) the k_2 value for **4** drops from 18 to 0.2, indicating that at this pH value the protonation of the complex in the receiving phase is the rate-limiting step in the transport process. On the other hand, when the pH of the receiving phase was varied between 1 and 2, no relevant changes in the k_2 values were observed thus indicating that, in this pH range, the transfer of the chelating part of the complex to the receiving phase (or hydration) becomes rate determining for the release of Cu(II). On the whole, in the standard conditions, both semiprocesses of the transport, uptake of Cu(II) at the donating phase and release at the receiving phase, appear to be controlled by the transfer of the complex between the interfaces of the different phases, the uptake of the metal ion being slightly slower than its release. The finding that the transport is rate-limited by diffusion across the membrane interfaces is in agreement with what was already reported in the case of alkali[3] and also Cu(II)[10] ions.

Interestingly, in another set of experiments employing ligand **4**, the rate of extraction of Cu(II) from aqueous phase I to $CHCl_3$ was found to be first order in ligand concentration at least up to a concentration of 10 mM, 10 times larger than that used under the standard conditions (see Table 1). Also the rate of release of Cu(II) from $CHCl_3$ into aqueous phase II is first order in complex concentration in the $CHCl_3$ phase at least up to 10 mM. These observations indicate that both the free ligand and the complex are not yet saturated at the $CHCl_3$−water interfaces also with a carrier concentration at least 10 times larger than those used so that, by increasing the ligand concentrations, a very high Cu(II) flux can be achieved. The transport efficiency depends also on the nature of the bulk membrane. Using CCl_4 the T_{24} value for **4** was reduced to 23 as compared to 47 with $CHCl_3$. The presence of 10% (v/v) 1-octanol in $CHCl_3$ also resulted in a less efficient transport for **4** (T_{24} = 35).

The above results and in particular the analysis of the data of Table 1 allow the following general considerations concerning the transport of Cu(II) ions using **1**-**11**. In the case of the dialkylated carriers **1**−**7**, the rate of extraction k_1 increases as the lipophilicity increases, and vice versa, the increase of lipophilicity results in a decrease in the rate of release k_2. As a consequence of the balance of the lipophilicity/hydrophilicity effects, the most effective carriers are ligands **6** and **7** with a T_{24} value of *ca.* 54. Considering the diglycine ligands, the effect of the increase in size of the alkyl chains from two ethyl (**1**) up to two dodecyl (**5**) groups indicates that a plateau value for T_{24}, k_1 and k_2 is reached when two octyl chains are attached to the *N*-terminus as in **4** and further elongation to two dodecyl residues is not relevant. On the other hand, as pointed out above, when the chains are shorter than two octyl residues, the carrier and its complex are soluble in water so that the formation and the decomposition of the complex occur not only at the interface but also in solution thus impairing the extraction on the one hand (k_1 = 0 for **1**) and highly favoring the release on the other hand (k_2 = 38 for **1** and **2** and can be taken as the upper limit for this type of carrier). In the case of ligands **4** and **5** the processes of the extraction and release of Cu(II) can be assumed to be virtually confined to the $CHCl_3$−water interfaces, and the k_1 and k_2 values seem to stabilize around 8 and 17, respectively. The results obtained using the N-dialkylated ligands **6**−**9**, when compared to their structurally related parent compound **4**, indicate that the increase in apparent hydrophobicity brings about unexpected ef-

fects: in particular it appears that the presence of a phenyl group as the R^4 substituent induces a sharp decrease in k_2 and hence in T_{24}. Such an adverse effect is magnified by the presence of two phenyl groups in **9**: in this case both k_1 and k_2 are low indicating a small extraction affinity of the complex (the E value is the lowest in the series) for the $CHCl_3$ phase. We are at odds in explaining such an effect. Although it may be the result of a slightly higher pK_a of the peptide group due to substituent effects, the small E value may be related to the fact that in the complex with **9** the two very bulky phenyl groups of the dipeptide face each other, being located on the same side of the coordination plane. We argue that the interaction between the aromatic group may lead to steric repulsion in the $CHCl_3$ phase and retard the extraction into the bulk membrane. That stereochemical factors are at play in the extraction and transport of Cu(II) is indicated by the results obtained in the case of single-tailed diastereomeric ligands **10** and **11**. These appear the most effective carriers of the series in terms of the T_{24} values (66 and 67) due to the optimal balance between the rate of extraction and that of release. Their case deserves a further look.

Cu(II) Transport by Diastereomeric Carriers. The extraction efficiency (E) at pH = 5.6 of the diastereomeric carriers is remarkably larger than that of the N-dialkylated dipeptides, and this may be due to the lower pK_a of the amido group. Although the T_{24} values are identical, the k_1 and k_2 values are different: the L,D stereoisomer **11** is much faster in extracting Cu(II) from aqueous phase I than the L,L stereoisomer **10**; on the other hand, **11** is much slower in the release of Cu(II) into aqueous phase II than **10**. Thus the differences in k_1 and k_2 almost completely compensate, at least after 24 h. A picture of the transport course is shown in Figure 4a that reports for both diastereomers the amount of Cu(II) present in aqueous phases I and II as a function of time. In the case of **11**, due to the high extraction rate, the amount of metal ions in the donating phase rapidly decreases and is always lower than in the case of **10**. On the other side, in the receiving phase II, the amount of Cu(II) is almost similar for both carriers and the slope in the steady state regime is slightly greater for **11** than for **10**. The diastereoselectivity effect is clearly illustrated in Figure 4b where the amount of Cu(II) in the $CHCl_3$ phase is plotted as a function of time. Using **11**, up to 33% of the ion resides in the $CHCl_3$ phase, whereas no more than 13% is present with **10**.

The diastereomeric effect may be explained as follows. It has been reported that *in water* the Cu(II) complexes of lipophilic L,L dipeptides are more stable than the complexes of the diastereomeric L,D dipeptides.[16,19] In the first case, assuming the formation of square-planar complexes, the lipophilic side chains of the amino acid residues are located on the same side of the coordination plane and close to each other; in the second case, with the L,D diastereomer, these side chains are located on opposite sides of the coordination plane. The larger stability in water of the L,L complexes is attributed to the close proximity and hence to the positive (micellar-like) hydrophobic interaction. Thus apparently, the hydration mantle of the L,D complex is less stable, and its complex is relatively more lipophilic than the L,L complex. This may account for the higher k_1 values observed for the L,D stereoisomer **11** since the extraction of Cu(II) from phase I into the $CHCl_3$ phase is driven by lipophilicity and for the smaller k_2 rate since the release of Cu(II) into aqueous phase II is retarded by a higher lipophilicity of the complex.

(19) Nakon, R.; Angelici, R. J. *J. Am. Chem. Soc.* **1974**, *96*, 4178.

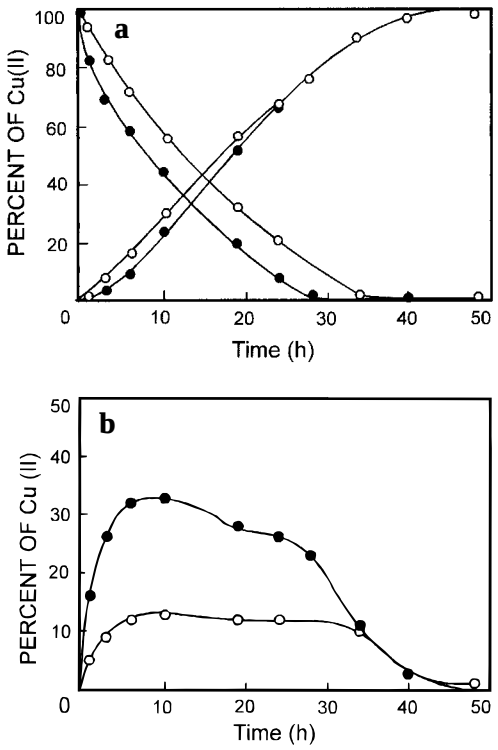


Figure 4. (a) Percent of initial Cu(II) in aqueous phases I and II during the transport experiments with carriers **10** (○) and **11** (●). (b) Percent of initial Cu(II) in the $CHCl_3$ phase (symbols as in panel a).

Selective Transport of Cu(II). We investigated the transport efficiency of Zn(II) and Ni(II) using carrier **4** in the absence and presence of Cu(II). Figure 5a shows that in the absence of Cu(II), the translocation of Zn(II) occurs at a moderate extent, the T_{24} value being 2.5 as compared with the T_{24} for Cu(II) of 47 (see Table 1), and the rate remains constant for 3 days. Thus, under these conditions the selectivity in terms of the ratio T_{24}(Cu)/T_{24}(Zn) is 19. We did not observe any accumulation of Zn(II) in the $CHCl_3$ phase which means that k_2 is much larger than k_1. Under which form Zn(II) is present in the $CHCl_3$ membrane is only a matter of speculation: perhaps, the carboxylate groups of two ligand molecules form an ion-pair with the metal ion. In the presence of Cu(II), the transport of Zn(II) is almost totally suppressed whereas the transport of Cu(II) is essentially unaffected (*cf.* Figures 5a and 3). The amount of Zn(II) that is transported after 24 h amounts to only 0.04%, and this gives a selectivity factor T_{24}(Cu)/T_{24}(Zn) = 1200. The transport of Zn(II) remains inhibited as long as even very low amounts of Cu(II) are present in the donating phase: after 40 h, when there is only 1% Cu(II) left in aqueous phase I, 0.08% Zn(II) has been transported as compared to 88% for Cu(II). Only when the donating phase is almost depleted of the latter ion does the rate of transport of Zn(II) start to increase.

A very similar behavior was observed for the transport of Ni(II) in the absence and presence of Cu(II) as shown in Figure 5b, but the selectivities are much higher. In the absence of Cu(II), T_{24}(Ni) amounts to 0.10% and the ratio T_{24}(Cu)/T_{24}(Ni) is 460. In the presence of Cu(II), T_{24}(Ni) is <0.01% (we could not detect any Ni(II) in the

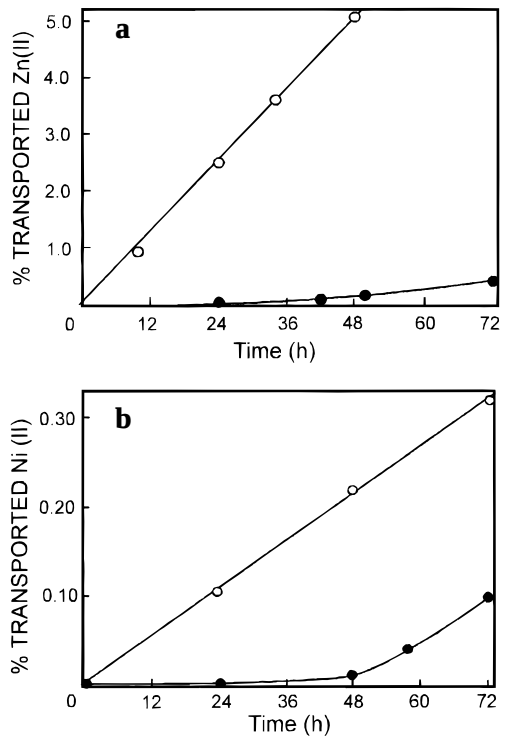


Figure 5. (a) Percent of initial Zn(II) transported by **4** in the absence (○) and presence (●) of Cu(II). (b) Percent of initial Ni(II) transported by **4** in the absence (○) and presence (●) of Cu(II). Conditions are as described for the transport experiments in Table 1; the initial concentration of $Cu(NO_3)_2$, $Zn(NO_3)_2$, and $Ni(NO_3)_2$ in phase I was 5.0×10^{-3} M.

receiving phase II) which gives a selectivity T_{24}(Cu)/T_{24}(Ni) > 5000. Only after 48 h when 97% Cu(II) was translocated could we detect the presence of 0.01% Ni(II) in phase II, and this allows to estimate a selectivity ratio T_{48}(Cu)/T_{48}(Ni) = 10^4. After 48 h, when all the Cu(II) has disappeared from aqueous phase I, the transport rate of Ni(II) increases as in the absence of the other ion.

Thus carrier **4** under the conditions used is extremely selective in the transport of Cu(II) from a mixture of other transition metal ions such as Zn(II) or Ni(II) also when it is present in relatively small amounts. Quite likely, the observed selectivity is due to a substantial change in the mode of transport: through a neutral, relatively stable complex in the case of Cu(II) and through saltlike ionic species in the case of the other transition metal ions, as long as the aqueous donating phase is a neutral or slightly acidic solution.

Conclusions

The long-chained dipeptides **4-11** are very effective and highly selective carriers of Cu(II) through a chloroform membrane. They are an interesting breed of compounds, structurally simple and relatively easy to modify. Although a quantitative analysis similar to that developed for crown ethers is not yet possible since it requires the determination of not easily accessible parameters such as the formation constants of the lipophilic carrier−metal ion complexes, the present study brought to light some interesting features which may allow to better understand their functions and the design of proper carrier molecules. The most delicate factor is the proper balance of the lipophilicity/hydrophilicity features of the carrier and its complexes: the lipophilicity favors the metal ion uptake and disfavors the release, this being kinetically rather than thermodynamically controlled. Such a point is illustrated by the limiting case of the two diastereomeric carriers **10** and **11** for which there are no arguments to indicate any difference in lipophilicity and hence in the partitioning between the aqueous and chloroform phases (as a matter of fact, the *E* values of Table 1 are the same); yet, their complexes are taken up and released at different *rates* due likely to a subtle difference in the lipophilicity of the complexes and the consequent ease of uptake in and release from the organic phase. What we learned also from this study is that the chemical changes at the interface play a fundamental role in the transport of the metal ion. In the present case, the carrier facing the donor phase with its hydrophilic portion may easily form a *neutral* lipophilic complex with Cu(II) that is transferred to the bulk chloroform and from this to the donor phase where it undergoes protonation thus releasing the metal ion and a neutral free carrier which turns over. In the case of Zn(II) or Ni(II) complexation with involvement of the amide moiety at the pH used is unlikely, and the ion can be taken out into the organic phase only as an ionic system at a rate which cannot compete with that of the Cu(II) complex. This point deserves further attention, and studies are under way in this laboratory to further investigate the selectivity also toward other metal ions and to exploit the effect of the pH of the donating phase to control the transport of different ions by modulating the acidity of the source and receiving phases.

Experimental Section

General Methods and Materials. Melting points are uncorrected. ^{1}H-NMR spectra were recorded on a 200 MHz spectrometer, and chemical shifts in ppm are reported relative to internal Me_4Si. Microanalyses were performed by the Laboratorio di Microanalisi of our department. $Cu(NO_3)_2$, $Zn(NO_3)_2$, and $Ni(NO_3)_2$ were analytical-grade products. Metal ion stocks solutions were titrated against EDTA following standard procedures.[20] The 2-morpholinoethanesulfonic acid buffer (MES) was used as supplied by Fluka. Kelex 100 was a gift of Dr. C. Tondre, Laboratoire d'Etude des Solutions Organiques et Colloidales (LESOC), UACNRS 406, Universite de Nancy I, B.P. 239, 54506 Vandoeuvre les Nancy, France. The active component 7-(4-ethyl-1-methyloctyl)-8-hydroxyquinoline was purified according to a method described in the literature.[17]

General Procedure for the Synthesis of Carriers 1−8. Route a: Scheme 1, Used for Carriers 1− 5, 7, and 8. The proper dialkylamine (16.6 mmol) (diethylamine, dibutylamine, dihexylamine, dioctylamine, or didodecylamine) and 3.5 g (21 mmol) of bromoacetic acid ethyl ester were dissolved in 10 mL of ethanol and heated for 8 h at 60 °C. The reaction mixture was then concentrated in vacuo and the residue dissolved in $CHCl_3$ and washed with a Na_2CO_3 solution. The $CHCl_3$ layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (silica gel, $CHCl_3$). The yield amounted to *ca.* 65%. The thus obtained dialkylglycine ethyl ester was hydrolyzed with NaOH in ethanol/water (5:1, v/v). Subsequently, 25 mL of water was added, and the pH of the solution was adjusted to pH = 6.0 with HCl. The solution was concentrated in vacuo, and the dry residue was extracted with ethanol/$CHCl_3$ = 1:1 (v/v). A solid material was removed by filtration, and the organic solution was concentrated in vacuo giving the different dialkylglycine in an almost quantitative yield. The products were used without further purification.

(20) Holzbecher, Z. *Handbook of Organic Reagents in Inorganic Analysis*; Wiley: Chichester, 1976.

***N,N*-Diethylglycine:** ^{1}H-NMR (CD_3OD) δ 1.25 (t, 6 H), 3.18 (q, 4 H), 3.55 (s, 2 H). This is a hygroscopic compound.

***N,N*-Dibutylglycine:** ^{1}H-NMR (CD_3OD) δ 0.85 (t, 6 H), 1.3 (m, 4 H), 1.65 (m, 4 H), 3.05 (t, 4 H), 3.55 (s, 2 H); mp = 118 °C.

***N,N*-Dihexylglycine, *N,N*-Dioctylglycine, and *N,N*-Didodecylglycine:** The ^{1}H-NMR spectra closely resemble the spectrum of *N,N*-dibutylglycine with the exception of the signal at 1.3 ppm which has an intensity of 12 H, 20 H, and 36 H, respectively. The melting points are 81, 74, and 101 °C respectively.

Route b: Scheme 1, Used for Carrier 6. To a vigorously stirred solution of 3 g of L-Phe-OEt·HCl in 50 mL of water and 50 mL of $CHCl_3$ was added a concentrated Na_2CO_3 solution. The $CHCl_3$ layer was separated, dried over Na_2SO_4, and concentrated in vacuo. To 2.0 g (10.4 mmol) of the thus obtained H-L-Phe-OEt dissolved in 10 mL of ethanol was added 6.0 g (31 mmol) of 1-bromooctane, and the reaction mixture was heated for 12 h at 75 °C. Subsequently 50 mL of $CHCl_3$ was added, and the solution was washed with a Na_2CO_3 solution. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in 10 mL of ethanol and heated for 12 h. This procedure was repeated three more times. The crude product thus obtained was purified by column chromatography (silica gel, $CHCl_3$/hexane = 1:3). The *N,N*-dioctyl-L-phenylalanine ethyl ester was hydrolyzed as described above. The final yield amounted to 38%: ^{1}H NMR (CD_3OD) δ 0.9 (t, 6 H), 1.3 (m, 20 H), 1.65 (m, 4 H), 2.95 and 3.15 (2 m, 4 H), 3.3 (AB quartet, 2 H), 4.0 (t, 1 H), 7.3 (m, 5 H); mp = 74 °C; $[\alpha]^{20}_D = +1$ ($c = 1.0$, MeOH).

To 3.0 mmol of the thus obtained dialkyl amino acids and 6.0 mmol of NEt_3 in 20 mL of $CHCl_3$ was added 1 equiv of isobutyl chloroformate at 0 °C. After stirring for 3 min, a well-stirred solution of 4.5 mmol of the proper amino acid ethyl ester hydrochloride (glycine, L-leucine, or L-phenylalanine ethyl ester hydrochloride) and 5 mmol of NEt_3 in 15 mL of $CHCl_3$ was added at 0 °C. The ice bath was removed, and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was concentrated in vacuo and the residue dissolved in 40 mL of $CHCl_3$. This solution was washed with Na_2CO_3, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, $CHCl_3$). The thus obtained *N,N*-dialkyl dipeptide ethyl esters were hydrolyzed with NaOH in 25 mL of ethanol/water = 10:1. To this solution was added 50 mL of water and the pH was adjusted to 6 with HCl. Compounds **6**−**8** precipitated from solution at pH = 6 and were collected by filtration. In the case of compounds **1**-**5** the solution was concentrated in vacuo and the residue dissolved in $CHCl_3$. The solid material was removed by filtration, and the $CHCl_3$ solution was concentrated in vacuo to yield the pure products. The overall yield amounted to 80%. Compound **1**-**5**, **7**, and **8** are very viscous oils; only **6** is a white solid.

***N,N*-Diethylglycylglycine (1):** ^{1}H-NMR (CD_3OD) δ 1.3 (t, 6 H), 3.2 (q, 4 H), 3.82 and 3.87 (2s, 4 H). Anal. Calcd for $C_8H_{16}N_2O_3$: C, 51.05; H, 8.57; N, 14.88. Found: C, 50.91; H, 8.69; N, 14.43.

***N,N*-Dibutylglycylglycine (2):** ^{1}H-NMR (CD_3OD) δ 0.9 (t, 6 H), 1.3 (m, 4 H), 1.7 (m, 4 H), 2.87 (t, 4 H), 3.6 and 3.75 (2s, 4 H). Anal. Calcd for $C_{12}H_{24}N_2O_3$: C, 58.99; H, 9.90; N, 11.47. Found: C, 58.33; H, 10.00; N, 11.21.

***N,N*-Dihexylglycylglycine (3), *N,N*-Dioctylglycylglycine (4), and *N,N*-Didodecylglycylglycine (5).** The ^{1}H-NMR spectra of these compounds closely resemble the spectrum of **4** with exception of the signal at 1.3 ppm which has an intensity of 12 H, 20 H, and 36 H, respectively. Anal. Calcd for $C_{16}H_{32}N_2O_3$: C, 63.96; H, 10.74; N, 9.32. Found: C, 64.09; H, 10.61; N, 9.39. Anal. Calcd for $C_{20}H_{40}N_2O_3$: C, 67.37; H, 11.31; N, 7.86. Found: C, 67.14; H, 11.48; N, 7.79. Anal. Calcd for $C_{28}H_{56}N_2O_3$: C, 71.74; H, 12.04; N, 5.98. Found: C, 71.34; H, 12.11; N, 5.90.

***N,N*-Dioctyl-L-phenylalanylglycine (6):** ^{1}H-NMR (CD_3OD) δ 0.9 (t, 6 H), 1.3 (m, 20 H), 1.65 (m, 4 H), 3.05 (m, 4 H), 3.15 (d , 2 H), 3.65 (AB quartet, 2 H), 3.93 (t, 1 H), 7.25 (m, 5 H), mp = 105 °C; $[\alpha]^{20}_D = +30.6$ ($c = 1.0$, MeOH). Anal. Calcd for $C_{27}H_{46}N_2O_3$: C, 72.60; H, 10.38; N, 6.27. Found: C, 72.51; H, 10.42; N, 6.22.

***N,N*-Dioctylglycyl-L-leucine (7):** ^{1}H-NMR (CD_3OD) δ 0.9 (m, 12 H), 1.3 (m, 20 H), 1.6 (m, 7 H), 2.9 (t, 4 H), 3.65 (AB quartet), 4.4 (m, 1 H); $[\alpha]^{20}_D = +6.3$ ($c = 1.0$, MeOH). Anal. Calcd for $C_{24}H_{48}N_2O_3$: C, 69.86; H, 11.72; N, 6.79. Found: C, 69.54; H, 11.81; N, 6.72.

***N,N*-Dioctylglycyl-L-phenylalanine (8):** ^{1}H-NMR (CD_3OD) δ 0.9 (t, 6 H), 1.3 (m, 20 H), 1.55 (m, 4 H), 2.85 (t, 4 H), 3.2 (m, 2 H), 3.6 (AB quartet, 2 H), 4.6 (m, 1 H), 7.25 (m, 5 H), $[\alpha]^{20}_D = +11.8$ ($c = 1.0$, MeOH). Anal. Calcd for $C_{27}H_{46}N_2O_3$: C, 72.60; H, 10.38; N, 6.27. Found: C, 72.43; H, 10.46; N, 6.19.

General Procedure for the Synthesis of Carriers 9−11. Route c: Scheme . To 1.5 g (5.7 mmol) of Boc-L-Phe-OH and 6.0 mmol of NEt_3 in 20 mL of $CHCl_3$ was added 5.7 mmol of isobutyl chloroformate at 0 °C. After stirring for 3 min, a well-stirred solution of 7.0 mmol of the proper amino acid ester hydrochloride (L-phenylalanine ethyl ester hydrochloride, L- or D-leucine methyl ester hydrochloride) and 6.0 mmol NEt_3 in 10 mL of $CHCl_3$ was added at 0 °C. The ice bath was removed, and the solution was stirred for 3 h at 25 °C. The solution was concentrated in vacuo, and the residue was dissolved in 25 mL of $CHCl_3$, washed first with a dilute HCl solution and then with a Na_2CO_3 solution, dried over Na_2SO_4, and concentrated in vacuo. The solid residue was dissolved in 10 mL of trifluoroacetic acid and stirred for 3 h. The solution was concentrated in vacuo, and to the remaining oil was added 50 mL of diethyl ether while stirring vigorously. The resulting precipitate was collected by filtration, washed with ether, and dried in vacuo. The thus obtained trifluoroacetate salts of the dipeptide esters were used without further purification. The overall yield amounted to 60%.

H-L-Phe-L-Phe-OEt·CF_3COOH: ^{1}H-NMR ($CDCl_3$) δ 1.15 (t, 3 H), 3.1 (d, 2 H), 3.4 (m, 2 H), 4.05 (q, 2 H), 4.58 (m, 1 H), 4.73 (m, 1 H), 7.25 (m, 10 H), 7.48 (d, 1 H), 8.6 (br s, 3 H).

H-L-Phe-L-Leu-OMe·CF_3COOH: ^{1}H-NMR (CD_3OD) δ 0.9 (2d, 6 H), 1.7 (m, 3 H), 3.2 (m, 2 H), 3.75 (s, 3 H), 4.15 (2d, 1 H), 4.55 (t, 1 H), 7.35 (m, 5 H).

H-L-Phe-D-Leu-OMe·CF_3COOH: ^{1}H-NMR (CD_3OD) δ 0.85 (2d, 6 H), 1.2 (m, 1 H), 1.5 (m, 2 H), 3.1 (m, 2 H), 3.72 (s, 3 H), 4.15 (t, 1 H), 4.4 (2d, 1 H), 7.35 (m, 5 H).

The thus obtained dipeptides were dissolved in 30 mL of water and 30 mL of $CHCl_3$. To this vigorously stirred mixture was added a Na_2CO_3 solution. The $CHCl_3$ layer was separated, dried over Na_2SO_4, and concentrated in vacuo; 1.0 g of the residue and 2 mL of 1-bromooctane were dissolved in 4.0 mL of ethanol and heated for 12 h at 70 °C. The reaction mixture was concentrated in vacuo; the residue was dissolved in 20 mL of $CHCl_3$, washed with Na_2CO_3, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, CH_2Cl_2). In the case of the phenylalanylphenylalanine dipeptide, the crude product was again dissolved in 4.0 mL of ethanol and heated for a further 12 h at 70 °C. This procedure was repeated three more times. The purification was performed by column chromatography (silica gel, CH_2Cl_2/petroleum ether = 1:1). The thus obtained dipeptide esters were hydrolyzed with NaOH in 20 mL of ethanol/water (9:1); 40 mL of water was added, and the pH was adjusted to 6 with a 1.0 M HCl solution. For **10** and **11** the precipitate was collected by filtration, washed with water, and dried in vacuo. In the case of **9** the resulting emulsion was extracted with 25 mL of $CHCl_3$. The $CHCl_3$ layer was separated and concentrated in vacuo leaving **9** as a viscous oil.

***N,N*-Dioctyl-L-phenylalanyl-L-phenylalanine (9):** ^{1}H-NMR (CD_3OD) δ 0.9 (t, 6 H), 1.3 (m, 24 H), 2.55 (m, 4 H), 3.0 (d, 2 H), 3.15 (m, 2 H), 3.78 (t, 1 H), 4.5 (dd, 1 H), 7.2 (m, 10 H), yield = 13%; $[\alpha]^{20}_D = +2.8$ ($c = 1.0$, MeOH). Anal. Calcd for $C_{34}H_{52}N_2O_3$: C, 76.08; H, 9.76; N, 5.22. Found: C, 75.92; H, 9.81; N, 5.15.

***N*-Octyl-L-phenylalanyl-L-leucine (10):** ^{1}H-NMR (CD_3OD) δ 0.95 (m, 9 H), 1.3 (m, 10 H), 1.65 (m, 5 H), 2.8 (m, 2 H), 3.2 (AB quartet, 2 H), 3.95 (t, 1 H), 4.38 (m, 1 H), 7.35 (m, 5 H), yield = 14%; mp = 204 °C; $[\alpha]^{20}_D = -9$ ($c = 0.3$, MeOH). Anal. Calcd for $C_{23}H_{38}N_2O_3$: C, 70.73; H, 9.81; N, 7.17. Found: C, 70.51; H, 9.88; N, 7.20.

***N*-Octyl-L-phenylalanyl-D-leucine (11):** ^{1}H-NMR (CD_3OD) δ 0.75 (2d, 6 H), 0.85 (m, 1 H), 0.95 (t, 3 H), 1.4 (m, 12 H), 1.7 (m, 2 H), 3.05 (t, 2 H), 3.15 (AB quartet, 2 H), 3.95 (dd, 1 H), 4.15 (dd, 1 H), 7.3 (m, 5 H); yield = 25%; mp = 210 °C; $[\alpha]^{20}_D$ = +79 (c = 0.4, MeOH). Anal. Calcd for $C_{23}H_{38}N_2O_3$: C, 70.73; H, 9.81; N, 7.17. Found: C, 70.61; H, 9.84; N, 7.21.

Transport Experiments. These experiments have been performed using the apparatus and solutions described in the previous section. The water phases were mechanically stirred with two rotating Teflon bars, while the chloroform solution was magnetically stirred at 400 rpm. At time intervals 100 μL portions of source and receiving phase were withdrawn, diluted with 1.9 mL of water, and subsequently subjected to the analysis. The micromoles of metal ion were determined with a Perkin Elmer 360 atomic absorption instrument using a multielement lamp.

Acknowledgment. The authors thank Mr. E. Castiglione for technical assistance. Financial support by the Ministry of the University and Scientific and Technological Research (MURST) and by the European Economic Community for Dr. M. C. Cleij's fellowship under the network Science Programme, Contract SC1*-CT92-0764, is gratefully acknowledged.

JO9703257