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Chiral Macrocycles, Part II: Transport of Amino Acid Li^+ Salts

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Abstract. Complexation of amino acids in both their zwitterionic and Li^+ salt forms by macrocycles, and carrier-mediated transport of the Li^+ salts through a CH_2Cl_2 membrane have been investigated: the transport study of four amino acids by a new series of tetrapyrazolic macrocycles with functionalized sidearms shows wide variations of the transport rates depending on both the macrocyclic sidearm and the amino acid structure.

Key words: Macrocycles, amino acids, transport, complexation, artificial liquid membrane.

Introduction

The transport of amino acids through lipophilic membranes plays a primordial role in biochemical processes [1]. The design of synthetic carriers capable of extracting and transporting these species has become an important research field in supramolecular chemistry [2–14]. In order to realize the necessary molecular recognition, several receptors have been prepared [15–20] but not yet tried in transport experiments. Some of these structures have been designed to realize chiral recognition [3, 12, 16–20].

Receptor and amino acid may interact in four ways to form stable complexes and in fact these four possibilities have been investigated: in neutral solution the amino acid may be complexed in its neutral form (coordination of the amino group and hydrogen bonding of the acid part) [15] or more generally in its zwitterionic form [7, 9, 10, 16, 18, 20] for which thermodynamic studies have been undertaken [21], complexation being weak; in acidic medium the amino acid is bound through the ammonium ion [3]. In basic medium it is complexed through the carboxylate function [4–6, 11, 12, 17, 19]. Complexation and transport of the different forms have been investigated as a function of pH [2, 8, 13, 14]. From all these data, we may conclude that for such bifunctional substrates, a better recognition is expected when two or three point interactions are present; most of the best structures correspond to a convergent arrangement of the binding sites belonging to the same molecule as is the case for macrocycles bearing functionalized sidearms [12, 15, 18]. This is why

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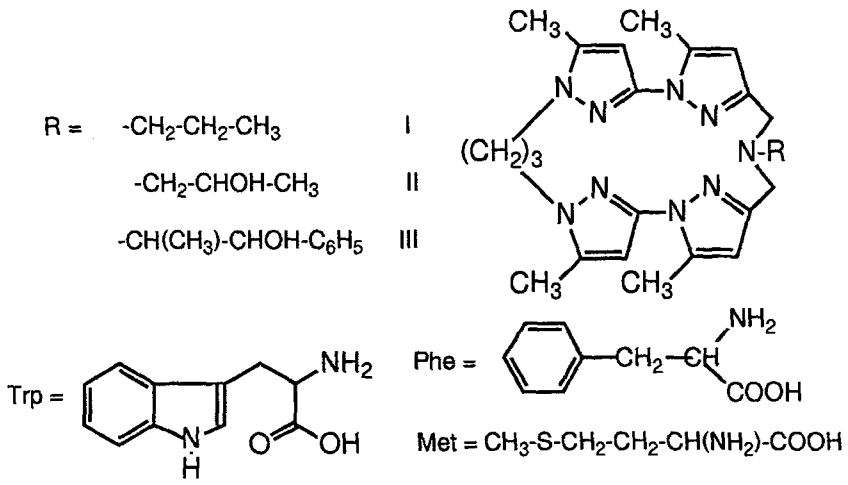

Fig. 1. Macrocycles **I–III**.

we have been interested in designing structures capable of achieving molecular recognition involving a lateral discrimination and transport of amino acids as carboxylate salts; until now some results on the efficient transport of these anionic species have been given [4, 6] but none concerning their molecular or chiral recognition.

As receptors we have chosen macrocycles containing two bipyrazolic subunits and a functionalized sidearm for which we have previously reported [22] a remarkable efficiency and selectivity for the extraction and transport of alkali cations through an artificial liquid membrane. In Part I of this paper [25], we have investigated the effect of the sidearm function on the transport rates of a series of lithium phenylacetates including mandelate; we have shown that small variations of the substrate structure could have a strong effect on the transport rates.

The aim of the present work is to show that macrocyclic structures such as **I**, **II** and **III** (Figure 1) may be efficient in selectively complexing and transporting amino acids.

Experimental

The syntheses of the macrocycles **I–III** have been described in Part I of this work. NMR spectra have been obtained with a 250 AC Bruker spectrometer; chemical shifts are given in ppm from TMS.

The complexation behaviour of the amino acids in their zwitterionic form has been studied by NMR spectroscopy on solutions of the mixture amino acid/macrocycle **II** (4×10^{-2} M/2.10^{-2} M in $CDCl_3$, CD_3OD and a 45/55 mixture of them).

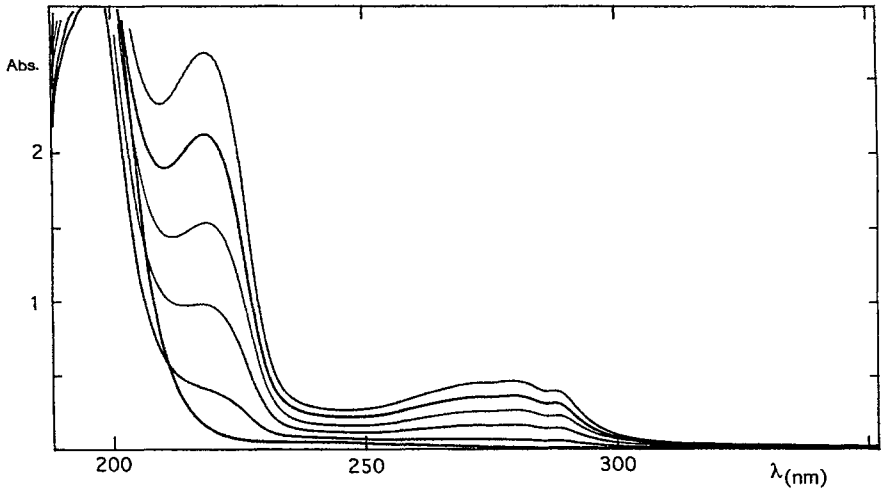

Fig. 2. Variation of the absorbance of the receiving phase versus time for the transport of Li^+ tryptophane by macrocycle **II**.

Transport experiments have been performed as described in Part I [25], using the same experimental setup except for the concentration of the two phases which have been modified:

1. Source phase: aqueous solution of amino acid lithium salt 0.5M
2. Membrane phase: macrocycle 10^{-3} M in CH_2Cl_2 (50 mL)

Transport has been followed by UV spectrometry as described in Part I [25]; transport rates have been extracted from the linear part of the O.D. vs time curve using Equation 1; UV spectra and comparison of the transport rates at different wavelengths show only the presence of the expected amino acid in the receiving phase. Correlation coefficients of the linear regression are greater than 0.99; examples of spectra with the resulting regressions are given in Figures 2 and 3.

$$v = \mathrm{d(O.D.)}/\mathrm{d}t \times \mathrm{Vol}/\varepsilon_\lambda \tag{1}$$

where Vol = volume of the receiving phase = 5 mL; ε_λ = molar extinction coefficient at a chosen wavelength λ.

Each experiment has been repeated at least three times: results were reproducible within 3–5%.

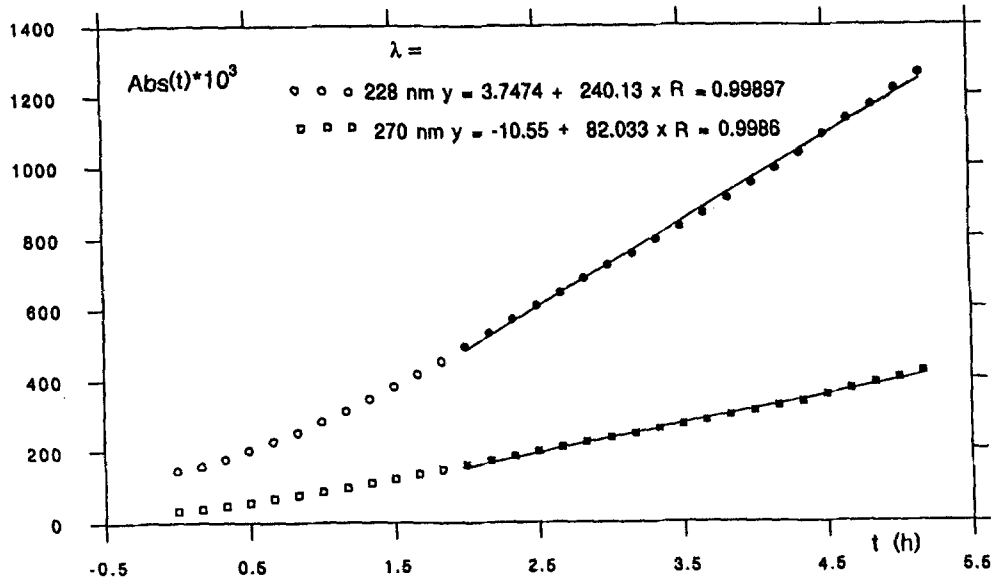

Fig. 3. Absorbance vs. time plot at two different wavelengths extracted from Figure 2.

ENANTIOSELECTIVITY OF TRANSPORT

The conditions of transport are similar to those described previously. The amino acid Li^+ salt has been used as the racemic mixture, the macrocycle being in its chiral form, *R*. The kinetics have been followed as described earlier, by UV spectrometry: 1 mL of the receiving phase was removed every day and split into five 200 μL aliquots to be chromatographed on a chiral column (Crownpack – Daicel supplied by J.T. Baker, France) under the following conditions: $T = 2°C$, flow rate = 0.5 mL/min, mobile phase: perchloric acid 0.01 M [23].

Results and Discussion

COMPLEXATION OF PHENYLALANINE IN ITS ZWITTERIONIC FORM

We used ^1H NMR spectroscopy to examine the interactions of an amino acid in its usual zwitterionic form under neutral conditions. The NMR spectra of a mixture of macrocycle **II** as its *R*-enantiomer with the racemic phenylalanine have been recorded under three conditions:

- In $CDCl_3$, because of the total insolubility of the amino acid, no complexation has been observed.
- In CD_3OD (Table I), macrocycle **II** shows some affinity towards phenylalanine: first, phenylalanine is more soluble than in CD_3OD alone and its aromatic protons are slightly upfield shifted; second, some protons of the macrocycle, mainly those in the position α to the sp^3 nitrogen atom, are shifted downfield; but, as evaluated by NMR integration, the uptake of phenylalanine by the macrocycle does not exceed 10%. In order to obtain some information about the kind of complexation occuring, we studied the interaction between macrocycle **II** and phenylalanine methylester chlorhydrate (Table I); the important downfield shifts ($\sim$ +0.3 ppm) observed for the CH_2 protons α to the sp^3 nitrogen atom show that the amino acid derivative interacts with the macrocycle. This could be indicative of a *trans*-protonation on the macrocyclic sp^3 nitrogen atom, but we cannot rule out the complexation of the NH_3^+ group by the macrocyclic sp^2 nitrogen atoms as usually reported in the case of crown ethers and cryptands.
- In a $CDCl_3/CD_3OD$ mixture.

The same effects as those reported in the case of CD_3OD alone have been observed when phenylalanine is added to the solution containing the macrocycle; viz. the amount of phenylalanine incorporated in the macrocycle again does not exceed 10%.

COMPLEXATION OF THE PHENYLALANINE LI^+ SALT

Considering the disappointing results obtained in the complexation of phenylalanine itself, we have chosen to study the behaviour of its Li^+ salt because it is an easier technique with a larger field of application than e.g. the use of ester derivatives.

The ^1H NMR spectrum of a mixture of phenylalanine Li^+ salt and macrocycle **II** in $CDCl_3$ shows the presence of a signal at 7.13 ppm belonging to the aromatic protons of phenylalanine, although the phenylalanine Li^+ salt alone is not soluble enough to be detected by NMR spectroscopy; moreover we have observed broadening and shifts for all the macrocyclic protons, either downfield for the cavity protons or upfield for the sidearm ones. Such a behaviour has been observed in the case of complexation of this same kind of macrocycle (sidearm, R = Me or CH_2CH_2Py) with the salt LiBr [22]: the broadening observed corresponds to a slowing down of the Li^+ exchange; the signals observed are the average positions of protons belonging to the free and complexed macrocycles. In our case the upfield shifts observed for the sidearm protons show that the sidechain hydroxyl group is involved in the complexation possibly through a hydrogen bond with the amino group of the counter anion. The percentage of macrocycle complexing the phenylalanine salt reaches a limit of 40%, a value showing the efficiency of our system [20].

TABLE I. ^1H chemical shifts of macrocycle **II**, under different conditions.

	Hpz	CH_3pz	CH_2Nsp^2	CH_2	CH_2Nsp^3	a	b	c
				$CDCl_3$				
A	5.80	2.19	4.06	2.54 m	3.7 db (J = 13.5Hz)	2.49 b	3.95 b	1.23 d
	5.89	2.34			3.80	2.90 b		
B	5.91	2.30	4.17 b	~2.50	3.83 d	v.b.	3.90 b	1.16 b
	6.00	2.40			3.87 d			
				CD_3OD				
C	6.06 s	2.47 s	4.36 m	2.70	3.95 d	2.89 q	4.30 m	1.43
	6.14 s	2.52 s			3.99 d	3.09 q		
D	6.06 s	2.48 s	4.36 m	2.69	3.99 d	2.94	4.30 m	1.43
	6.13 s	2.51 s			4.02 d	3.12 q		
E	6.08 s	2.49 s	4.35 m	2.70	4.23 d			1.47
	6.22 s	2.51 s			4.30 d	3.20 m		

a, b, c refer to the N–C_a–C_b–C_c positions of the protons on the sidearm.
s = singlet, d = doublet, t = triplet, b = broadened, m = multiplet.
A: Macrocycle **II** alone in $CDCl_3$.
B: Macrocycle **II** + Li^+ Phe in $CDCl_3$.
C: Macrocycle **II** alone in CD_3OD.
D: Macrocycle **II** + Phe in CD_3OD.
E: Macrocycle **II** + chlorhydrate of the DL Phe methyl ester in CD_3OD.

KINETICS OF AMINO ACID SALT TRANSPORT

The transport was carried out through a CH_2Cl_2 membrane separating two aqueous solutions as described in Part I [25].

The transport rates, gathered in Table II, are lower than those obtained in Part I [25] with the mandelate derivative, most probably due to a poorer fit of the amino acid salt to the receptor, in terms of geometry, hindrance and stabilizing interactions; the most efficient transport was for the amino acid having the structure closest to phenylacetic acid.

Before discussing individually the behaviour of each amino acid, some general comments can be made about the transport rates gathered in Table II. We observe a transport rate enhancement for the hydroxy-bearing sidearm macrocycle, compared to the propyl one: as for the mandelate transport previously reported, the polar group may enhance the transport in several ways: by stabilizing directly the cation inside the cavity or favouring the approach of the macrocycle at the interfaces, but also

TABLE II. Transport rates in 10^{-8} mol/h, P' = Rekker's constant [24].

Macrocycle	Phenylalanine Phe	Tryptophane Trp	Methionine
I	48	12	12
II	84	17	16
III	77	19	12
P'	2.24	2.31	1.08

by creating a hydrogen bond inside the host–guest structure: a comparison of the structure of the amino acid and mandelic acid Li^+ salts shows that both the amino group and the hydroxy group are α to the carboxylate and are expected to form a hydrogen bond with the macrocyclic sidearm.

TRANSPORT OF PHENYLALANINE AND TRYPTOPHANE SALTS

As tryptophane and phenylalanine have a similar hydrophilic character as reflected in the Rekker's constant P', it is possible to discuss the difference in their transport ability in terms of structural effects. The transport rate decrease observed for the tryptophane salt by comparison with the phenylalanine one, may be explained by the steric enhancement of the indolic group relatively to the phenyl one. If we focus on the increase of transport rate observed for macrocycles II and III bearing a hydroxy sidearm, we observe that it is still present for tryptophane in spite of the steric effect, but at a smaller extent than for phenylalanine, showing that the steric factor has a more drastic influence on the transport efficiency than on its selectivity. Likewise, a slight effect on the lipophilicity of the macrocycle sidearm can still be observed, in accord with the interpretation made for phenylalanine.

TRANSPORT OF THE METHIONINE SALT

Methionine is more hydrophilic than the two other amino acids considered here, but also much less hindered, so that a compensation of those two factors explains its rate values close to those observed for tryptophane.

No significant increase of the transport rate due to the hydroxy group has been observed: the absence of an aromatic group in the amino acid structure could lead to a conformation of the supramolecular complex in which the hydroxy group effect is inhibited.

TRANSPORT OF THE HISTIDINE SALT

We have chosen this amino acid to probe the effect of its high hydrophilic character, as reflected by a P' value of -0.23, on the transport rate: no measurable transport could be detected.

TABLE III. Percentage of D enantiomer transported by the chiral macrocycles **II** and **III**.

Macrocycle	Phe (%D)	Trp (%D)
II	50.8 ± 0.1	49. ± 0.5
III	51.4 ± 1	49.7 ± 0.5

ENANTIOSELECTIVITY OF TRANSPORT

In Part I of this paper [25], we have obtained a satisfying chiral recognition of Li^+ mandelate and, as the transport rate of the amino acids has been shown to be strongly dependent on the nature of the sidearm, we could expect to observe an influence of the chirality of the sidearm on this transport rate.

We have carried out transport experiments under the same conditions as those described previously in Part I [25], using the chiral form of the macrocycle and a racemic source phase. We have chosen the two amino acids for which the transport rate is most dependent on the macrocycle sidearm structure: tryptophane and phenylalanine.

The percentages of the D enantiomers for Phe and Trp salts measured in the receiving phase are listed in Table III for the two chiral macrocycles **II** and **III**. These values show a slight enantioselectivity of transport, although the enantiomeric excess is too close to the experimental error to be really significant.

Conclusion

The amino acids to be transported have been chosen for their lipophilic character, with a range of lipophilicity varying from phenylalanine to histidine, and for their different steric bulk. The goal of the present work was not to understand in detail the multiparameter relationship between the structure of the side arm and the transport rate, which has been better investigated in Part I [25] with carboxylic acid probes, but to show that our macrocycles were able to transport amino acids. In addition, the examination of the transport rate values shows some significant variations depending on the amino acid structure, which allows one to distinguish between them.

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