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Enantiomeric Differentiation of Amino Acids by A Chiral Crown Ether Derived from D-Mannose Studied by the Liquid Membrane Technique

MAREK PIETRASZKIEWICZ* and MALGORZATA KOZBIAL
Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Kasprzaka 44/52, Poland

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Abstract. Chiral 18-crown-6 incorporating a D-mannopyranoside unit displays noticeable enantioselectivity in the recognition of amino acids and their sodium and potassium salts in transport experiments across a liquid membrane containing the carrier. D-phenylalanine and D-phenylglycine were transported faster than their corresponding L-enantiomers, whereas the enantioselectivity was reversed with tryptophan.

Key words. Amino acid, D-mannose, crown ether, liquid membrane.

1. Introduction

Chiral macrocyclic molecular receptors have attracted considerable interest in the past as a potential means for the resolution of enantiomeric species [1]. The interaction between a chiral host molecule and an enantiomeric guest species leads to inclusion complexes whose thermodynamic properties are different, and these differences are manifested in nonequivalent values of the stability constants for D and L forms involved in the inclusion complex. Chiral receptors may be of great importance as a practical tool for separation of enantiomeric mixtures.

Continuing our interest in this area, we have synthesized two chiral crown ethers incorporating a D-mannopyranoside unit as a chiral auxiliary, and a naphthalene moiety which might be helpful to achieve crystalline inclusion complexes with enantiomeric guest molecules for structural determination.

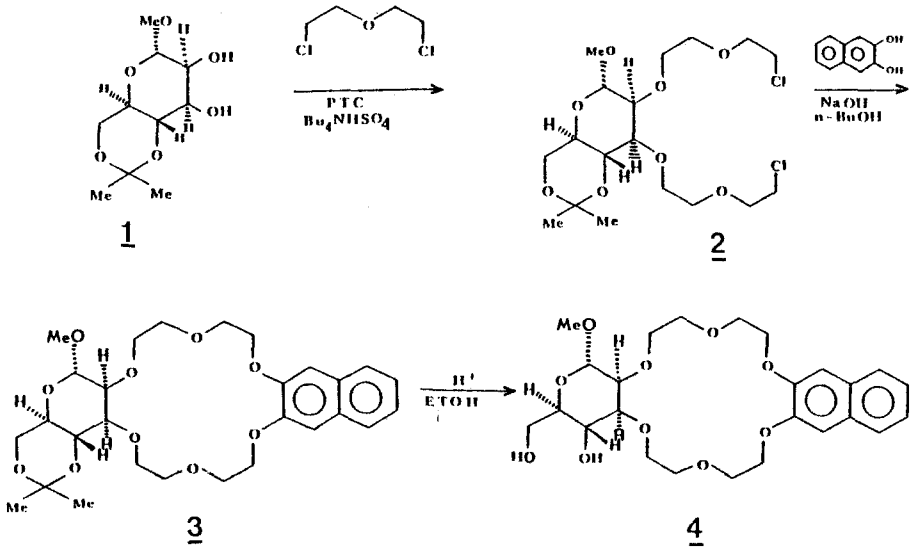
As a model binding site we selected 18-crown-6 which easily forms inclusion complexes with primary ammonium cations [2] via hydrogen bonds to three oxygen atoms of the macrocyclic ring.

2. Experimental

2.1. GENERAL

All chemicals were purchased from Fluka or Merck and used as received. NMR spectra were obtained on a Gemini Varian 200 MHz spectrometer with TMS as internal standard. The melting points were uncorrected.

* Author for correspondence.



Scheme 1

Transport experiments were run in a typical U-shaped apparatus containing a chloroform solution of the carrier as a liquid membrane. Transported species were amino acids or their sodium and potassium salts.

2.2. SYNTHESIS

The synthetic strategy consisted of a routine procedure developed by Gross and co-workers [3]. The preparation of the mannopyranoside building block **1** may be found elsewhere [4]. The synthetic pathway is outlined in Scheme 1.

Analytical Data:

Compound **3**: Elemental anal., *calc.* for $C_{28}H_{38}O_{10}$: C 62.9 H 7.16 *found*: C 62.84 H 7.14.

^{1}H NMR ($CDCl_3$, TMS) δ : 1.38 and 1.49 [2*s*, 6H, $(CH_3)_2C$], 2.84 (5.3H, OMe), 3.42–4.39 (*m*, 22H, sugar protons, CH_2—O macrocycle) 4.57 (*d*, 1H, H–1, J_{12} = 1.5 Hz), 7.10 (*d*, 2H, aromatic).

Compound **4**: Elemental anal., *calc.* for $C_{25}H_{34}O_{10}$: C 60.71 H 6.93 *found*: C 60.68 H 6.89.

^{1}H NMR ($CDCl_3$, TMS) δ : 2.50 (*b*, 2H, OH), 3.04 (*s*, 3H, OCH_3), 3.53–4.24 (*m* 22H, sugar protons, CH_2O macrocycle), 4.63 (*d*, 1H, H–1, J_{12} = 1.6 Hz), 7.10 (*s*, 2H, aromatic), 7.28–7.36 (*m*, 2H, aromatic), 7.62–7.69 (*m*, 2H, aromatic).

The final crown ether **3** was a nicely crystallizing material and its X-ray molecular structure has been published[5].

2.3. TRANSPORT EXPERIMENTS

Transport experiments were run at room temperature (20–23°C). The chloroform bulk membrane was a carrier solution (0.01 M) and the receiving phase was pure

water. The chloroform solution was stirred magnetically at 200 rpm. Samples of the receiving phase were periodically taken for concentration measurement of the amino acids by means of HPLC.

Chromatographic experiments were carried out with a Type 302 HPLC apparatus (Institute of Physical Chemistry PAN, Warsaw, Poland) equipped with a 25 μL injector and a diode array UV detector (Sonopan Poland, 10 μL flow cell). The analytical column, 250 mm × 4 mm, was prepacked with Nucleosil 10 μm (Marchery Nagel). Eluent: 5% or 10% methanol in water. The pH was adjusted with H_3PO_4 (0.016 M) within the range 2.2–2.3.

It turned out that the presence of a protecting group, necessary at the beginning of the synthetic work, appeared to be undesirable in transport experiments. In fact, the crown ether **3** did not display any enantioselectivity in transport experiments, probably due to weak binding. On the other hand, removal of the isopropylidene group at the 4, 6-*O*-position of the sugar unit resulted in pronounced enantioselectivity. This observation may be explained by an involvement of the free hydroxyl groups as auxiliary binding groups via hydrogen bonding to amino acids, helping in this manner to orient the guest species spatially within the close proximity of the macrocyclic ring.

The results of transport experiments are collected in Table I and Figures 1–9.

3. Results and Discussion

Three amino acids were investigated as enantiomerically pure D- and L-forms, together with their sodium and potassium salts: D- and L-phenylglycine, D- and L-phenylalanine and D- and L-tryptophan. No racemates were used in transport experiments.

There are two different modes for complexation of the free amino acid and its alkali salt. In the first instance the ammonium group is assumed to form tripod-like hydrogen bonds to the three oxygen atoms of the macrocyclic ring. The sodium salt is believed to form a so-called 'cascade' complex where the cation is placed in the cavity and is ion-paired with the carboxylic group. In this second case, the efficiency of enantioselection depends strongly on the binding strength of the alkali metal cation by the crown ether binding site.

Table I shows that phenylglycine and phenylalanine are transported faster as the free amino acids than their corresponding sodium salts. D-Sodium glycinate is transported faster than L-sodium glycinate, but D-sodium phenylalaninate and the D-tryptophan sodium salt are transported slower compared to their L-enantiomers.

It seems that the free amino acids form stronger complexes with the host molecule than their corresponding sodium salts. This fact may be attributed to relatively weak binding of the sodium cation, so the overall stability of the cascade complex is not particularly high. A distinct enantioselection of the D-enantiomer over the L-enantiomer may be interpreted on the assumption that complex formation occurs on the same side of the macrocyclic ring as the sugar unit, ensuring effective non-bonded interactions responsible for chiral recognition.

The tryptophan case is different, L-Tryptophan is transported faster than its D-form, and the sodium salts of D- and L-tryptophan are transported faster than the free tryptophan, so this behaviour is opposite to that displayed by D- and L-phenylglycine and phenylalanine.

Table I. Enantiomeric resolution of amino acids and their sodium salts transported across a liquid membrane (after 75 h)

Amino acid	$C_{75} \times 10^{-6}$ (mole/L) concentration of enantiomer in receiving phase after 75 h	$\alpha_{75} = \frac{C_{D75}}{C_{L75}}$
D-phenylglycine	910	3.29
L-phenylglycine	277	
D-phenylglycine Na	367	1.70
L-phenylglycine Na	216	
D-phenylalanine	301	1.25
L-phenylalanine	241	
D-phenylalanine Na	127	0.45
L-phenylalanine Na	280*	
D-tryptophan	359	0.27
L-tryptophan	1340	
D-tryptophan Na	642	0.41
L-tryptophan Na	1550	

* Extrapolated value.

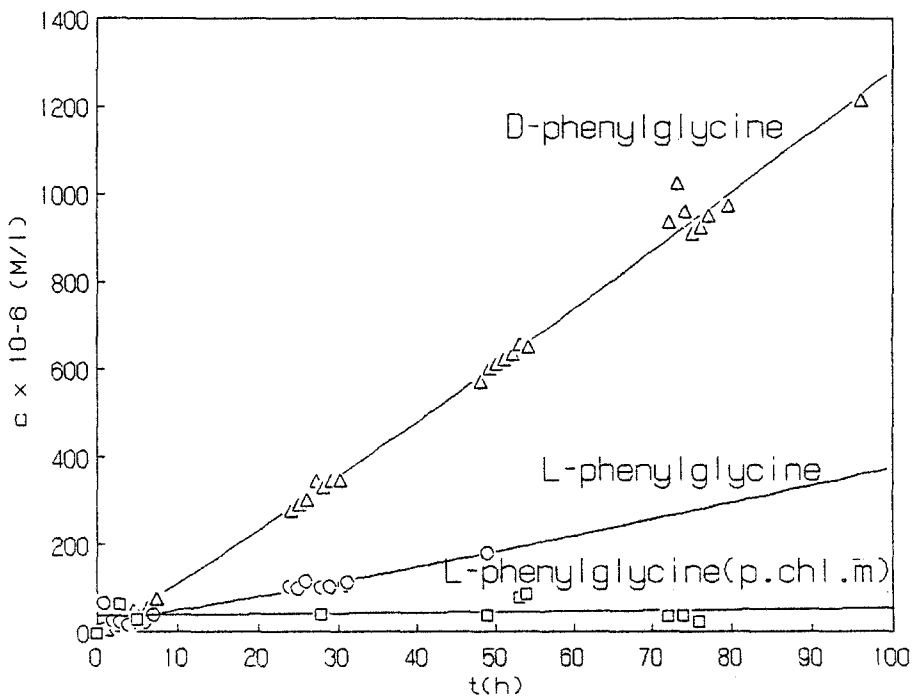


Fig. 1.

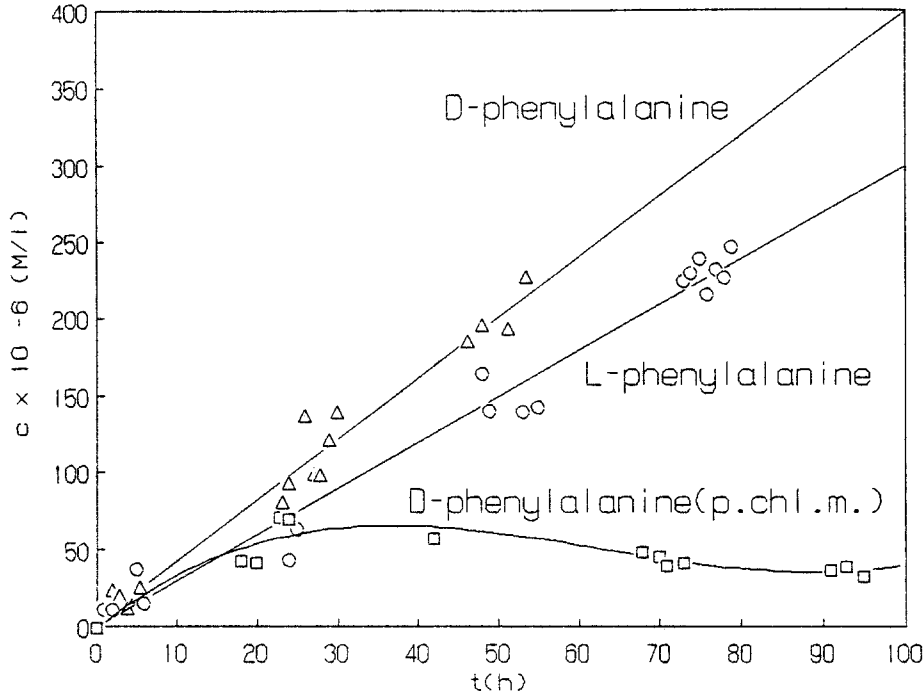
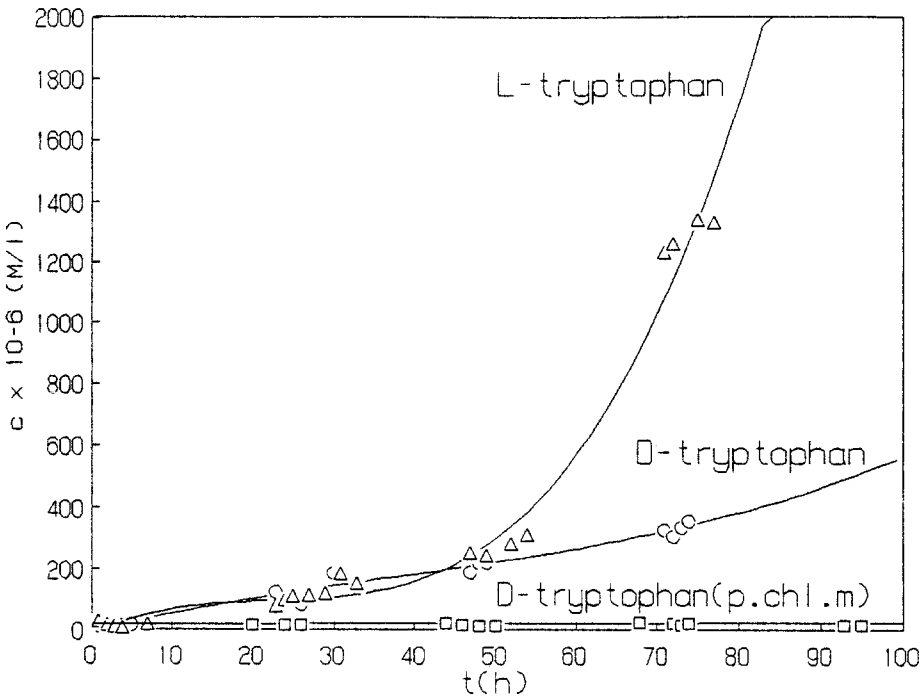


Fig. 2.



Figs. 1–3. Plots of the D and L amino acid concentration in the receiving phase as a function of time (p.chl.m. = pure chloroform membrane without crown ether).

Tryptophan thus behaves differently from the other amino acids. Therefore, complexation of the sodium salt of D- and L-tryptophan may be a more complicated phenomenon, involving not only cascade binding but also hydrogen bonding between the amino group and the free hydroxyl groups of the ligand as well as a π-stacking interaction between the indole nucleus and the naphthalene unit of the chiral crown ether.

The potassium salts of the amino acids were transported across the liquid membrane in the following manner: D-tryptophan was transported faster than its L-form, L-phenylalanine faster than D-phenylalanine, and D-phenylglycine faster than L-phenylglycine. Although these results are difficult to interpret in a rational way, we can say that the size of the cation involved plays a crucial role in enantioselection of enantiomeric carboxylates. It seems therefore that the stereochemistry of the inclusion complex involving an ion pair is strongly dependent on the cation size.

Comparison of the transport rates of the amino acid sodium and potassium salts is as follows:

	Na salts	K salts
tryptophan	D > L	L > D
phenylglycine	D > L	D > L
phenylalanine	L > D	L > D

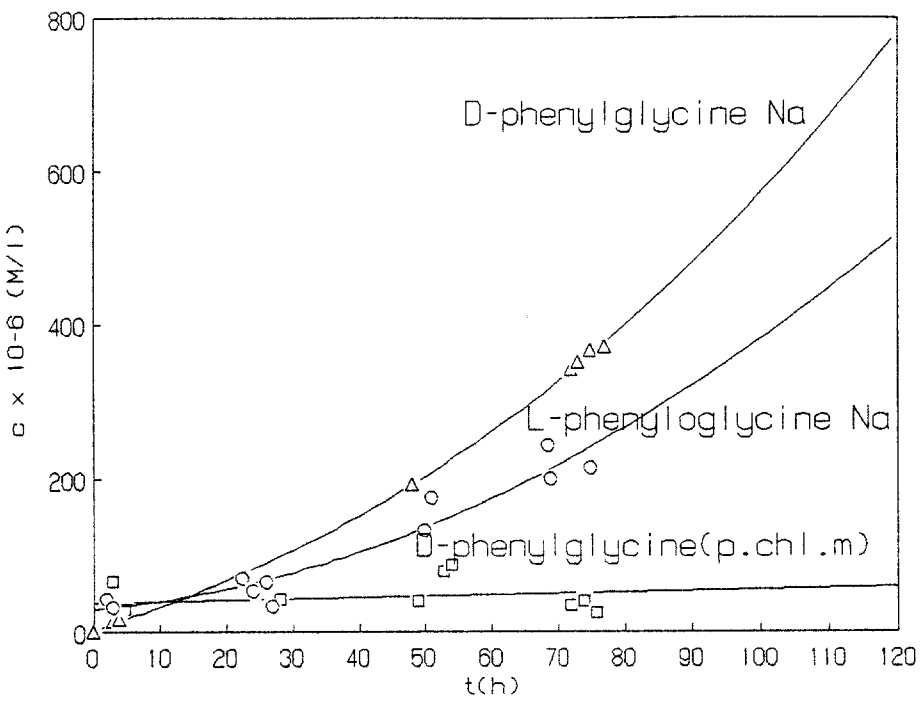


Fig. 4.

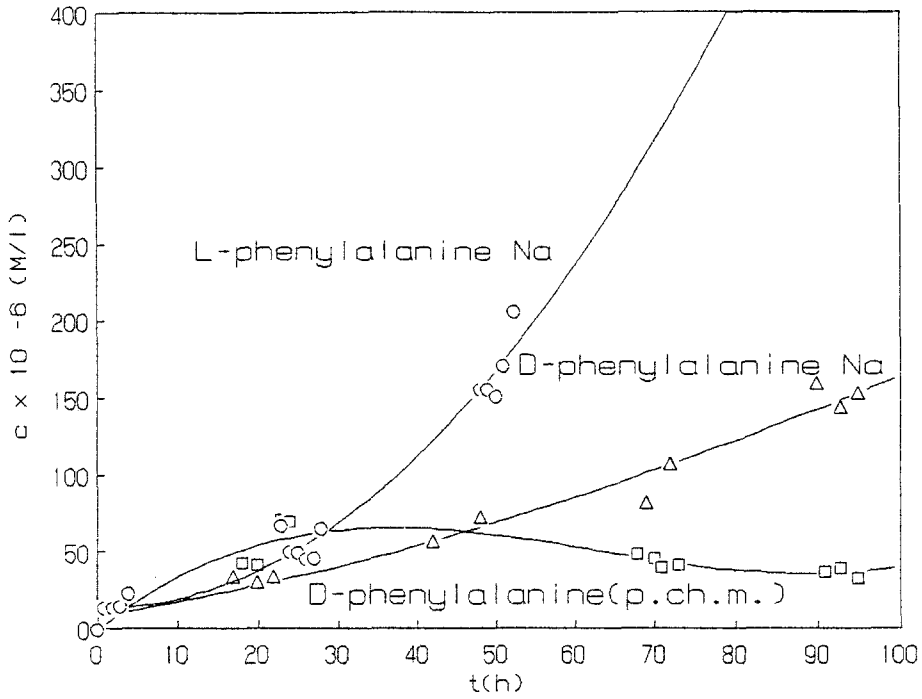
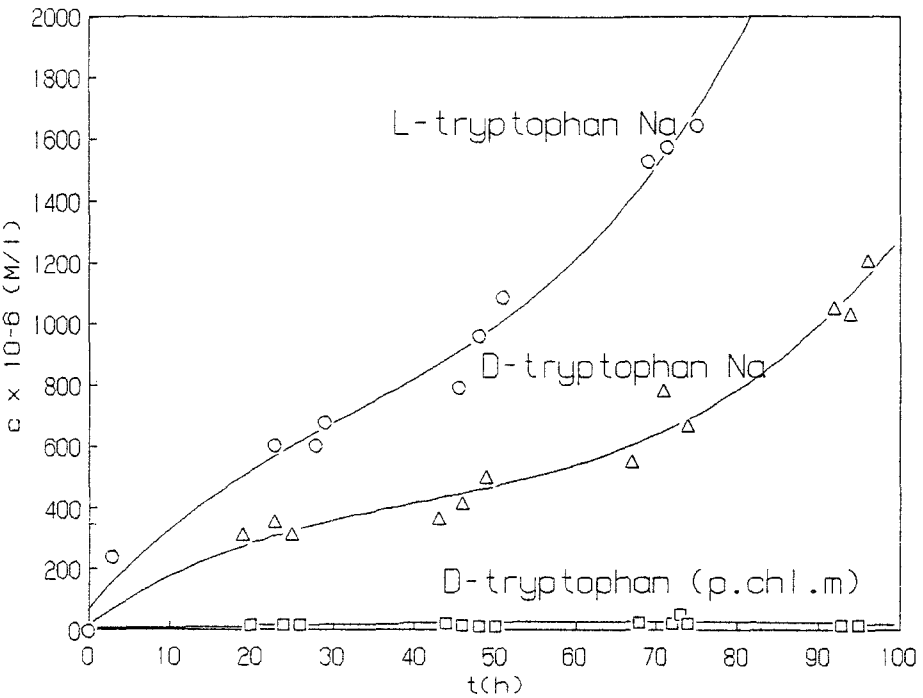


Fig. 5.



Figs. 4–6. Plots of the D and L amino acid sodium salt concentration in the receiving phase as a function of time (p.chl.m. see Figs. 1–3).

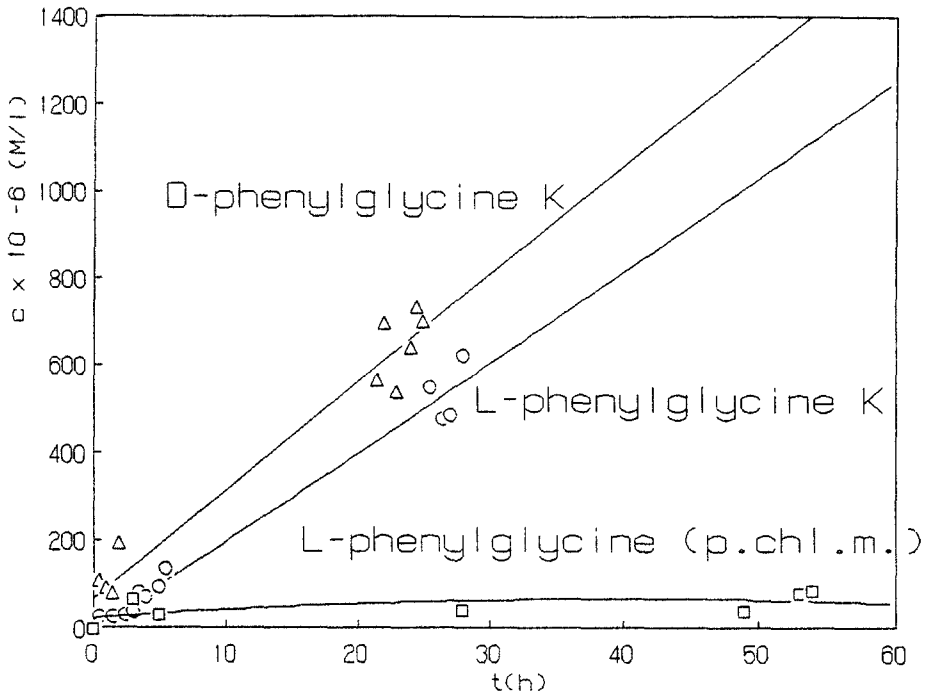

c x 10 -6 (M/l)
1400
1200
1000
800
600
400
200
0
D-phenylglycine K
L-phenylglycine K
L-phenylglycine (p.chl.m.)
0
10
20
30
40
50
60
t(h)

Fig. 7.

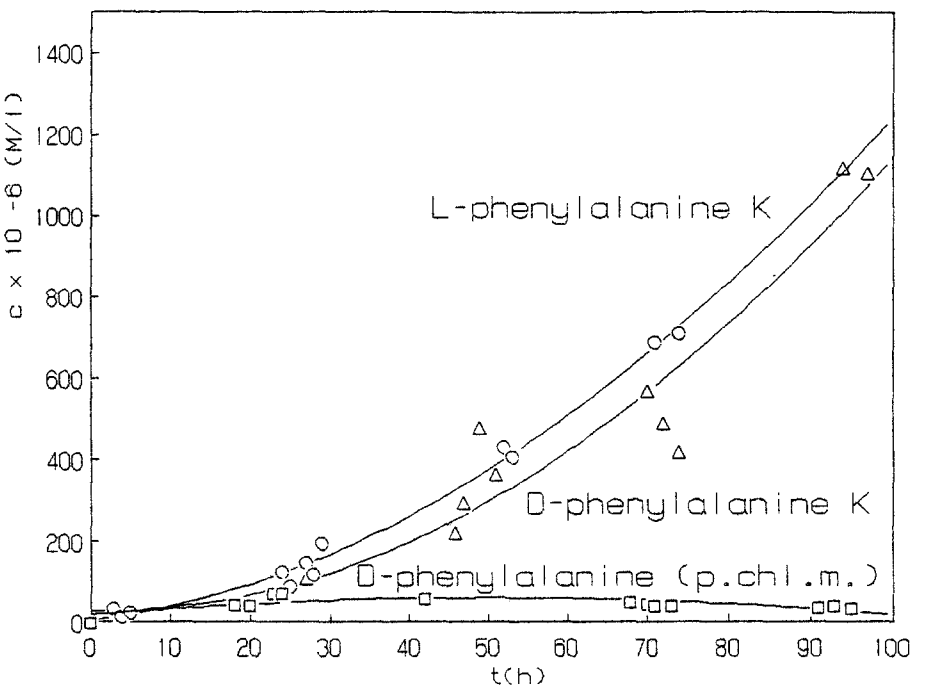
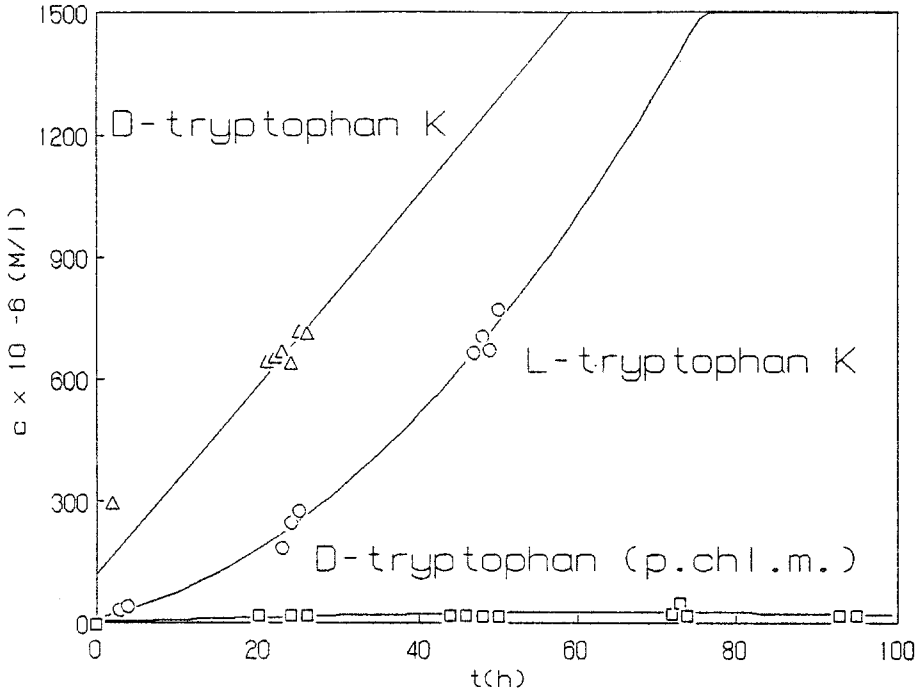

c x 10 -6 (M/l)
1400
1200
1000
800
600
400
200
0
L-phenylalanine K
D-phenylalanine K
D-phenylalanine (p.chl.m.)
0
10
20
30
40
50
60
70
80
90
100
t(h)

Fig. 8.



Figs. 7–9. Plots of the D and L amino acid potassium slats concentration in the receiving phase as a function of time (p.chl.m. see Figs. 1–3).

It seems that there is no general pattern governing transport rates as a function of the cation size. The differences may be due to differences in stereochemical features of the cascade complex involving sodium or potassium cations.

4. Conclusions

The chiral crown ether proved to be a good chiral carrier for either free amino acids and their alkali metal salts in transport experiments. It was essential to have the two hydroxyl groups at positions 4 and 6 unprotected, since they contributed distinctly to the overall binding and enantioselection. The protected crown ether did not display enantioselectivity during transport experiments.

The models of complexation for the free amino acids and their sodium salts are so far tentative and we hope to prepare the solid complexes for X-ray structural studies to get a detailed view of the stereochemistry of the inclusion complexes. Work in this direction is being pursued.

The title compound was recently successfully applied in chromatographic separations of enantiomers [6].

Acknowledgement

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