Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **30:** 69–77, 1998.

Chiral Discrimination of Amino Acids by an Optically Active Crown Ether Studied by HPLC, Extraction and a Liquid Membrane Transport Experiments

MAŁGORZATA KOŻBIAŁ, MAREK PIETRASZKIEWICZ* and
OKSANA PIETRASZKIEWICZ
Institute of Physical Chemistry, Polish Academy of Sciences, 01224 Warsaw, Kasprzaka 44/52, Poland.

(Received: 3 December 1996; in final form: 27 March 1997)

Abstract. A chiral crown ether incorporating a methyl α-D-mannopyranoside unit displayed pronounced enantioselection of amino acids in partition liquid chromatography experiments involving solvent systems of limited miscibility: water–ethanol–2,2,4-trimethylpentane. The same system has been used for amino acid transport across a liquid membrane containing the crown ether, and in liquid–liquid extraction experiments. Remarkable enantioselection has been noted for amino acids in all the processes studied.

Key words: Crown ether, amino acids, enantioselection, membrane transport, chiral receptors, HPLC.

1. Introduction

The enantiomeric purity of optically active natural and synthetic products has been the focus of intense research, since the potential use of such products in medicine, the food industry and agriculture has been subject to increasingly rigorous standards of enantiomeric purity. There is a dual problem: on the one hand, there is a need to prepare enantiomerically pure compounds; on the other, enantiomeric purity has to be monitored by analytical methods. Supramolecular chemistry may make an outstanding contribution to the analytical problems by means of chiral recognition of optically active compounds by chiral macrocyclic molecular receptors. This branch of supramolecular chemistry has been a 'hot' topic over the past two decades, during which a great diversity of host receptors have been designed and synthesized, incorporating amino acids [1], binaphthyls [2], sugars [3], steroids [4], tartaric acid [5], etc.

Our part in this research has involved an interest in the synthesis and discriminating properties of macrocyclic crown ethers [6], diaza crown ethers [7],

* Author for correspondence.

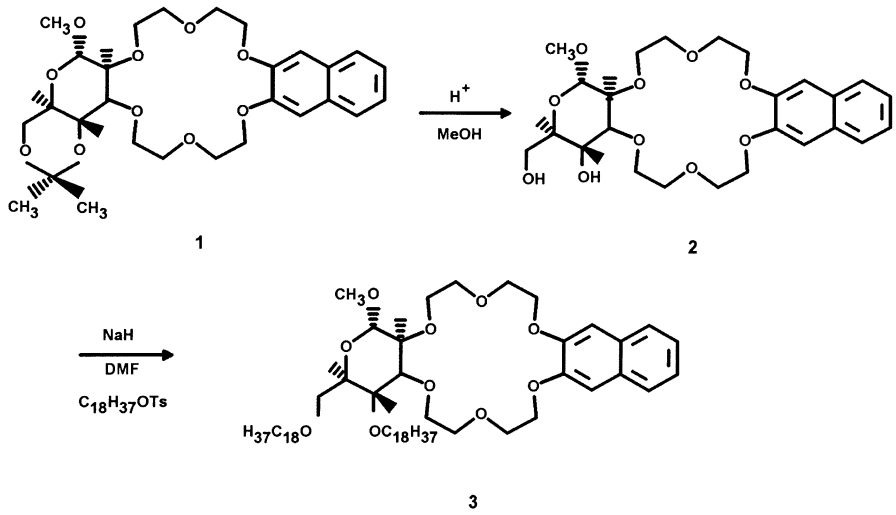

Figure 1. Reaction route leading to the lipophilic crown ether.

and cryptands [8] incorporating the methyl α-D-mannopyranoside unit as chiral auxiliary.

We now report on the enantioselection of amino acids by a chiral lipophilic crown ether in a ternary solvent system of a limited mutual miscibility: water–ethanol–2,2,4-trimethylpentane (2,2,4-TMP). Figure 1 shows the reaction route leading to the title compound. The lipophilic function was introduced for a practical reason: to be able to retain the crown ether in a nonpolar organic phase.

2. Experimental

2.1. General

All chemicals were purchased from Aldrich and used without purification, except for DMF which was distilled over CaH_2. Elemental analysis was performed at the Institute of Organic Chemistry, Polish Academy of Sciences, and the ^{1}H-NMR spectra were run on a Gemini 200 instrument.

Investigations of enantiomeric discrimination involving the title ligand have been performed by transport experiments across a liquid membrane, liquid–liquid extraction and partitioning liquid chromatography. All processes were conducted at 25 °C in the ternary system water–ethanol–2,2,4-trimethylpentane, which has limited mutual miscibility. This system is represented in the form of a Gibbs' triangle in Figure 2. The compositions of the mixtures in the miscibility domain are determined by the equilibrium strings. The methodology of phase selection is represented by points A, B, and C in Figure 2. The initial phase with the composition determined by the point B (water 40%, ethanol 20%, 2,2,4-trimethylpentane 40%, w/w) separated into two phases with the compositions determined by the points

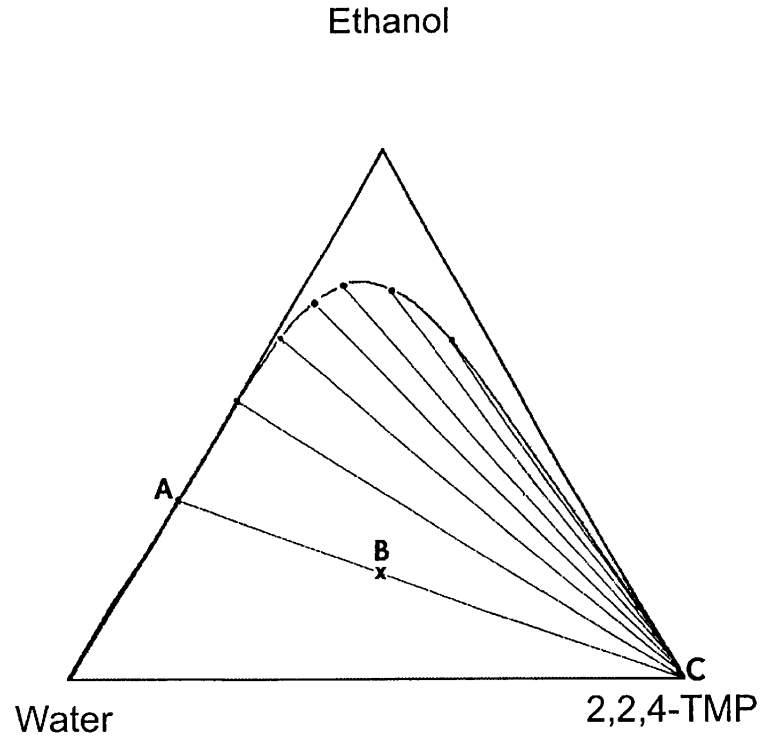

Figure 2. Phase diagram of the ternary system water–ethanol–2,2,4-trimethylpentane at 25 °C. Composition of (w/w): (A) water 67.17%, ethanol 32.77%, 2,2,4-trimethylpentane 0.06%; (B) water 40%, ethanol 20%, 2,2,4-trimethylpentane 40%; (C) water 0.08%, ethanol 1.25%, 2,2,4-trimethylpentane 98.67%.

A (water 67.17%, ethanol 32.77%, and 2,2,4-TMP 0.06%) and B (water 0.08%, ethanol 1.25%, and 2,2,4-TMP 98.67%. w/w). The compositions of the phases have been assigned according to the literature available and verified by GLC. Solvent systems of limited miscibility have been already used by Huber and coworkers [9]. A similar system has been used by Pawłowska [10] in partitioning chromatography and liquid–liquid extraction for the resolution of chiral species, using cyclodextrin derivatives as chiral auxiliaries.

2.2. EXTRACTION

The extraction experiments were conducted at 25 °C for 3 h. The polar phase (2 mL) containing an amino acid at a concentration of 10^{-3} M/L was stirred at 200 rpm with the nonpolar phase (8 mL) containing the crown ether at a concentration of 10^{-3} M/L. The concentration of the amino acids in the polar phase after extraction was determined by HPLC.

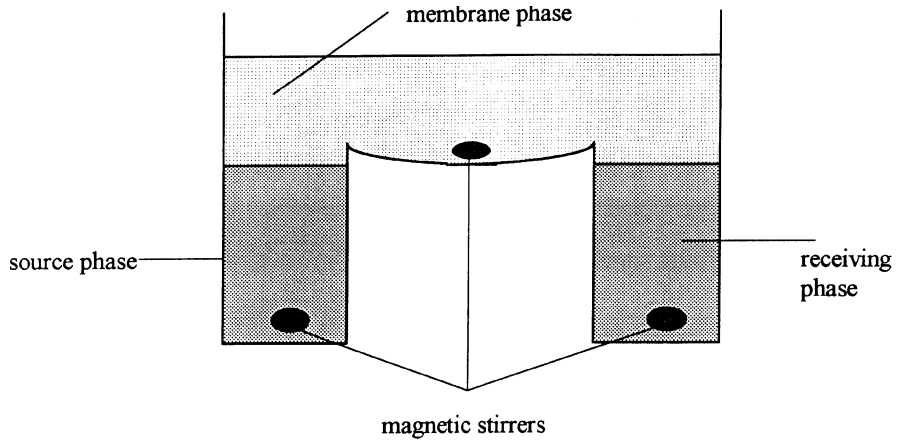

Figure 3. Custom-built set-up for transport experiments.

2.3. TRANSPORT

The transport experiments were conducted for 6 h at 25 °C in the apparatus shown in cross-section in Figure 3. The source phase (polar, 3 mL) contained the amino acid at a concentration of 10^{-2} M/L. The receiving phase was the polar phase (3 mL). The membrane phase was a nonpolar phase (8 mL) containing the chiral crown ether at a concentration of 10^{-3} M/L. The phases were stirred magnetically at 200 rpm. After 6 h the samples were analysed by means of UV spectroscopy using the polar phase as reference.

2.4. CHROMATOGRAPHY

The chromatographic experiments were conducted at 25 °C (thermostated columns). The column with dimensions 250 × 1 mm was filled with Lichrosorb RP-18, 5 μm (Merck, Darmstadt). Column preparation: 20 mL of the nonpolar phase containing the crown ether (10^{-2} M/L) was passed through the bed. The polar phase was washed out using the nonpolar phase (64 mL) saturated with the chiral crown ether (10^{-5} M/L). Flow rate: 20 μL/min. During the column preparation an evolution of pressure was observed. All investigations were performed on the microcolumn chromatograph (microbore), custom-made IChF PAN type 310 equipped with an injection valve (0.5 μL) and UV-254 detector with cell volume of 1 μL.

Phenylglycine, phenylalanine and tryptophan were purchased from Fluka and used as received. Water was doubly distilled and deionized.

2.5. SYNTHESIS OF TITLE COMPOUND **3**

The synthesis of the 4,6-*O*-isopropylidene-α-D-mannopyranoside naphthocrown **1** has been published [6]. This compound served as starting material to prepare

a lipophilic crown bearing two hydrocarbon chains in positions 4 and 6 of the mannopyranoside: **1** (3 g) was dissolved in methanol (50 mL) and one drop of trifluoroacetic acid was added to effect hydrolysis of the isopropylidene protecting group in positions 4 and 6. The progress of the hydrolysis was monitored by TLC (Merck 254F) in a hexane–acetone system (3 : 2). When the hydrolysis was complete, the acid was quenched by addition of powdered, anhydrous potassium carbonate with stirring for 2 h. Solid was removed by filtration, and the solution was evaporated to dryness. The crude product was used directly for further reaction. It was dissolved in dry DMF (40 mL), two equivalents of sodium hydride were added, and after 2 h at 40 °C solid octadecyl *p*-toluenesulfonate was added with stirring. Stirring at 60 °C was continued for 24 h, and monitored on TLC with the same conditions as above. Once the reaction was complete, the reaction mixture was treated with water (150 mL). The product was filtered off, washed with water and dried in the open air. For further purification the product was crystallized from ethanol. Overall yield 67%.

^{1}H-NMR ($CDCl_3$, TMS): δ = 0.879 (t, 6H, 2 × CH_3, $\underline{CH_3}$—$(CH_2)_{17}$); 1.256 (m, 64H, 2 × [$(CH_2)_{16}$]); 3.105 (s, 3H, OCH_3); 3.35–4.30 (m, 26H, OCH_2CH_2O, sugar protons, $O\underline{CH_2}(CH_2)_{16}$—); 4.665 (s, 1H, anomeric proton); 7.305 (q, 3H, aromatic); 7.645 (q, 3H, aromatic) ppm. $C_{61}H_{106}O_{10}$ (998, 78) *Calcd*. C 73.29% H 10.70%. *Found:*. C 73.05% H 10.93%.

3. Results and Discussion

We have used lipophilic, chiral crown ether **3** incorporating the D-mannopyranoside unit as chiral selector, and two octadecyl groups attached to oxygen atoms at positions 4 and 6 to make this compound highly lipophilic.

All processes were run in similar ternary solvent systems. However, the enantioselectivity in various experiments (extraction, transport, chromatography) is different (Tables I and II). Extraction and transport experiments involved single enantiomers, whereas chromatographic separations were run for enantiomeric pairs of amino acids.

The highest enantioselection for all amino acids was observed in extraction experiments, for which higher partition coefficients were observed for the L enantiomers, with the following order of enantioselection: PhAla > Trp > PhGly. Transport experiments indicated that the better extracted L forms of PhGly and Trp are significantly more slowly transported. The L-PhAla is modestly faster in transport over its D counterpart. These relationships indicate that the rate of enantioselective transport depends on the release step of a given enantiomer from the membrane to the receiving phase. Taking into account our earlier studies we can conclude that the use of a ternary solvent system gave better efficiencies in extraction and transport, when compared with a pure immiscible solvent. Figures 4–6 display enantiomeric differentiation of amino acids during extraction and transport experiments.

Table I. Liquid–liquid extraction and transport data through a liquid membrane.

Amino acid	k_a	$\times 10^{-2}$	α_E	J (mol m^{-2} s^{-1}) $\times 10^{-6}$	α_T
L-PhGly	13.90		2.78[a]	4.32	1.36[c]
D-PhGly	5.00			5.88	
L-PhAla	46.20		4.13[a]	7.84	1.25[b]
D-PhAla	11.20			6.27	
L-Trp	18.80		11.60[a]	9.04	2.08[c]
D-Trp	1.62			18.82	

[a] $\alpha_E = k_a \quad (L)/k_a \quad (D)$.
[b] $\alpha_T = J(L)/J(D)$.
[c] $\alpha_T = J(D)/J(L)$.

Table II. Capacity and enantioseparation factors for amino acids investigated in a liquid–liquid ternary system (HPLC).

Amino acid	k' without chiral agent	k' with chiral agent	α
L-PhGly	0.56	6.44	
D-PhGly	0.56	6.78	1.05
L-PhAla	0.78	3.62	
D-PhAla	0.78	3.84	1.06
L-Trp	0.78	49.6	
D-Trp	0.78	Not eluted	–

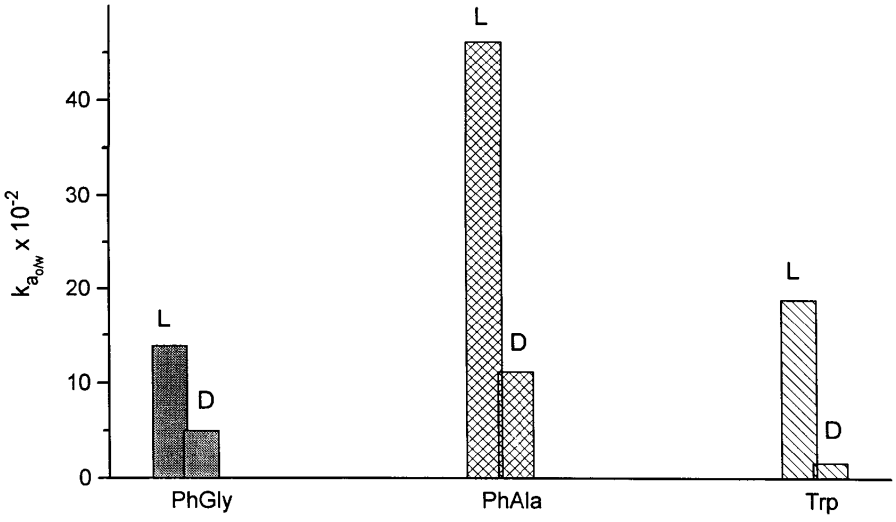

Figure 4. Bar plots of partition coefficients for amino acids.

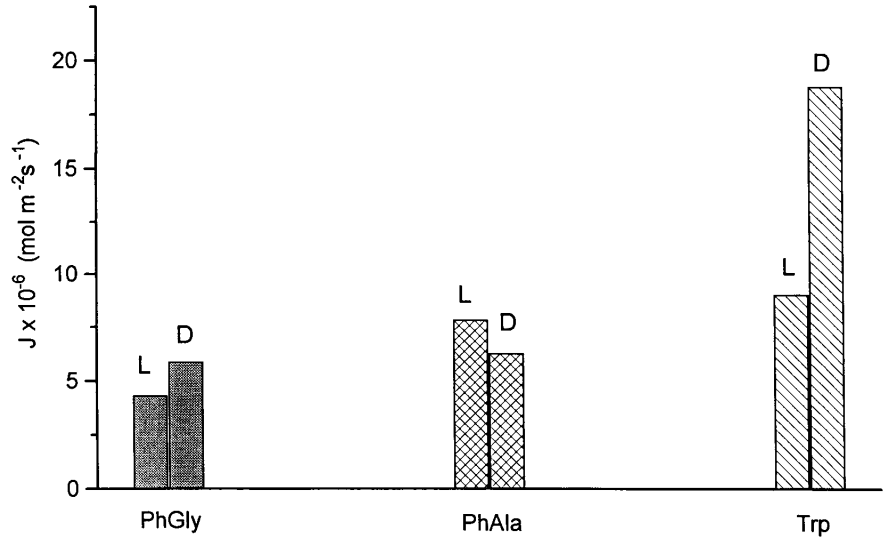

Figure 5. Bar plots of fluxes of amino acids.

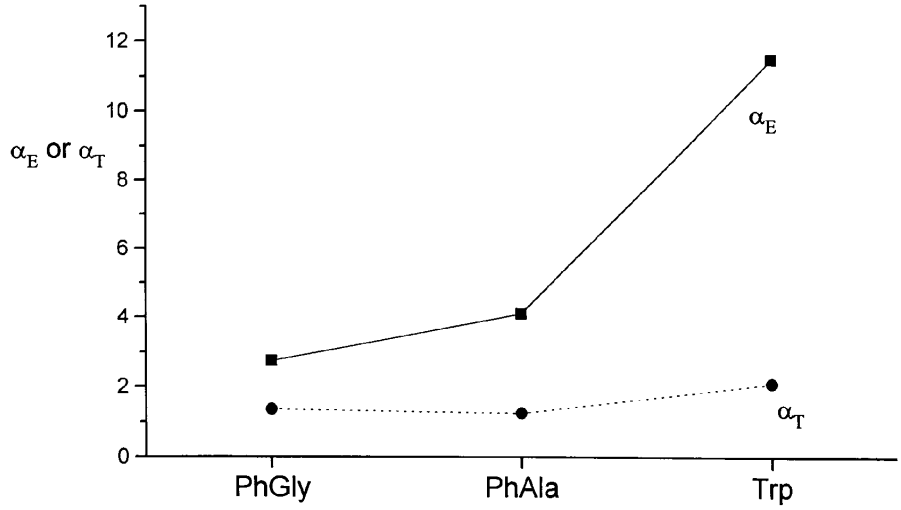

Figure 6. Enantiomeric differentiation for liquid–liquid extraction of amino acids and during transport.

In the chromatographic experiments the L enantiomers possessed a shorter retention time, which suggested weaker complexation in this case.

It seems that the comparison of transport and chromatographic processes might be speculative, since it is hard to predict the orientation of the lipophilic crown on the surface of the RP-18 bed coated with a nonpolar phase. Nevertheless, it is clear that both processes may be complementary tools for further chiral discrimination

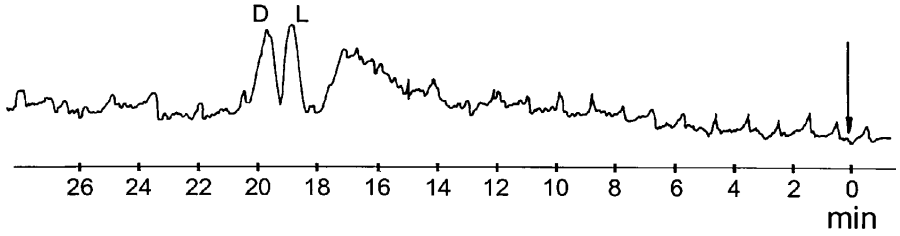

Figure 7. HPLC chromatogram of enantiomer separation of DL-phenylglycine. The concentration of the crown ether in a stationary phase: 10^{-2} M/L. Flow rate: 20 μL/min at 25 °C.

Figure 8. HPLC chromatogram of enantiomer separation of DL-phenylalanine. Experimental conditions as in the Figure 7.

studies. Figures 7 and 8 display the separation of enantiomeric pairs of phenylglycine and phenylalanine using liquid–liquid chromatography with a chiral crown ether.

4. Conclusions

The application of the ternary system of water–ethanol–2,2,4-trimethylpentane for the studies of enantioselectivity of the chiral crown ether as chiral discriminator for amino acids, gave better separation possibilities than those with binary systems. During the extraction of amino acids from water to 2,2,4-trimethylpentane containing a lipophilic crown ether, the crown, or the amino acid, precipitated at the interface of water and the organic solvent. The application of a ternary solvent system eliminated precipitation at the interface, and allowed for the estimation of the partition coefficient of the amino acids investigated between the polar and nonpolar phases. We have not detected the partitioning of the crown ether from the nonpolar phase to the polar phase. A particularly important advantage of the ternary systems is the possibility of modelling extraction selectivity, transport across a liquid membrane, and chromatographic processes by adjustment of the compositions of the particular phases, giving changes in polarity, solubilization abilities for the species involved, etc., in order to attain the highest selectivity of a given process. The literature data indicated that in partitioning chromatography with the applica-

tion of ternary systems of limited miscibility the results obtained are more reliable, because the selectivity of this process depends very little on the method of column preparation. Additionally, the amount of the crown ether is remarkably small. Since the enantiomeric purity is the focus of many commercial applications, the development of appropriate tools is essential. The ternary system presented in this work has remarkable possibilities in this respect. Work is in progress in this direction.

5. List of Symbols

J flux of transported amino acids after 6 h (mol m^{-2} s^{-1})
k_a partition coefficient of amino acids extracted from the polar phase to the nonpolar phase $k_a = c_a / c_a$
c_a concentration of amino acids in the non-polar phase (M)
c_a concentration of amino acids in the polar phase (M)
α_E ratio of partition coefficients: higher/lower coefficient
α_T ratio of fluxes: higher/lower flux
k' capacity factor (HPLC)
α enantioseparation factor (HPLC)

Acknowledgement

This work was supported by the Institute of Physical Chemistry, Polish Academy of Sciences.

References

1. M. Žinić and V. Škarić: *J. Org. Chem.* **53**, 2582 (1988); D.J. Chadwick, J.A. Clife, and I.O. Sutherland: *J. Chem. Soc., Chem. Commun.* 992 (1981).
2. D.J. Cram, R.C. Helgeson, S.C. Peacock, J.L. Kaplan, L.A. Domier, D. Moreau, K. Koga, J.M. Mayer, Y. Chao, M.G. Siegel, D.H. Hoffman, and G.D.Y. Sogah: *J. Org. Chem.* **43**, 1930 (1978); E.P. Kyba, G.W. Gokel, F. de Jong, K. Koga, L.R. Sousa, M.G. Siegel, L. Kaplan, G.D.Y. Sogah, and D.J. Cram: *J. Org. Chem.* **42**, 4173 (1977).
3. D. Curtis, D.A. Laidler, J.F. Stoddart, and J.F. Jones: *J. Chem. Soc., Perkin Trans. I* 1756 (1976); D.A. Laidler and J.F. Stoddart: *J. Chem. Soc., Chem. Commun.* 979 (1976).
4. G.W. Gokel: *Crown Ethers and Cryptands* (Monographs in Supramolecular Chemistry, Ed. J.F. Stoddart), The Royal Society of Chemistry, Cambridge, England (1991).
5. J.-P. Behr, J.M. Girodeau, R.C. Hayward, J.-M. Lehn, and J.-P. Sauvage: *Angew. Chem.* **87**, 813 (1975).
6. J. Żukowski, M. Pawłowska, and M. Pietraszkiewicz: *Chromatographia* **32**, 83 (1991); M. Pietraszkiewicz and M. Koźbiał: *J. Incl. Phenom.* **14**, 339 (1992).
7. M. Pietraszkiewicz: *J. Incl. Phenom.* **5**, 177 (1987); M. Pietraszkiewicz and J.F. Stoddart: *J. Chem. Soc., Perkin Trans. 2*, 1559, (1985).
8. M. Pietraszkiewicz and N. Spencer: *J. Coord. Chem A.* **27**, 115 (1992).
9. J.F.K. Huber, M. Pawłowska, and P. Markl: *Chromatographia* **19**, 19 (1984); J.F.K. Huber, M. Pawłowska, and P. Markl: *J. Chromatogr.* **500**, 257 (1990).
10. M. Pawłowska and J. Lipkowski: *J. Chromatogr.* **547**, 59 (1991).