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Chiral separation of amino acids by chiral octamide derivatives of calixarenes derived from resorcinol by impregnation on a polymeric support

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Abstract—Chiral amide derivatives of octaester calixresorcarene were synthesized and employed as chiral stationary phases for chiral discrimination of amino acid derivatives. Enantiomers of phenylglycine and tryptophan were easily discriminated as their sodium and potassium salts. In addition, phenylalanine–trytophan, phenylglycine–trytophan mixtures were separated by column chromatography.

1. Introduction

Amino acids are the most important targets for molecular recognition by artificial host compounds. This is due to their relevance in the biological world and their rich chemistry. For this reason, chemists have been studying host–guest binding of amino acids as an instrument for manipulation of their reactivity. Therefore, the design and synthesis of different kinds of synthetic macrocycles has been one of the objectives of host–guest chemistry.

Molecular recognition is a general action in biological systems. In this process, the functional groups of receptors form supramolecules with substrates by noncovalent interaction, such as hydrogen bonding, electrostatic interaction and hydrophobic interaction.[1–5] It is therefore not surprising that artificial molecular recognition systems are attracting much attention, especially for bio-molecules such as amino acids, nucleotides, peptides and proteins.[6–10] Mendoza[11] and Schmidtchen[12] have successfully used calixarenes, which are selectively functionalized at the upper or the lower rim, as receptors in the process of molecular recognition due to their special cleft structure.[13,14] Lower rim ester derivatives of calixarene with different ring sizes can recognize Na^+ and K^+ at the air–water interface.[15] The first chiral amide derivatives of octaester calixresorcarene were introduced by Iwanek[16] using chiral amines and amino alcohols as chiral auxiliaries. We used the same method for the synthesis of chiral octaester calixresorcarene as described by Iwanek.[16]

The very poor solubility of these compounds is a disadvantage for using them as chiral catalysts and also for molecular recognition studies in the solution but is a potential advantage for preparing chiral stationary phases for chiral separation, so we focused on the preparation of chiral stationary phases for the chiral separation of amino acid derivatives. Herein, we report new chiral stationary phases and the enantioselective discrimination of amino acid derivatives.

2. Result and discussion

2.1. Synthesis of chiral octaamides

Calixresorcarenes **1a** and **1b** were prepared from resorcinol, acetaldehyde[17] and benzyaldehyde.[18] The reaction of **1** with ethyl bromoacetate (1:20) was carried out in ethyl alcohol in the presence of K_2CO_3 as a base. Octaesters **2a** and **2b** were crystallized from ethanol. Heating

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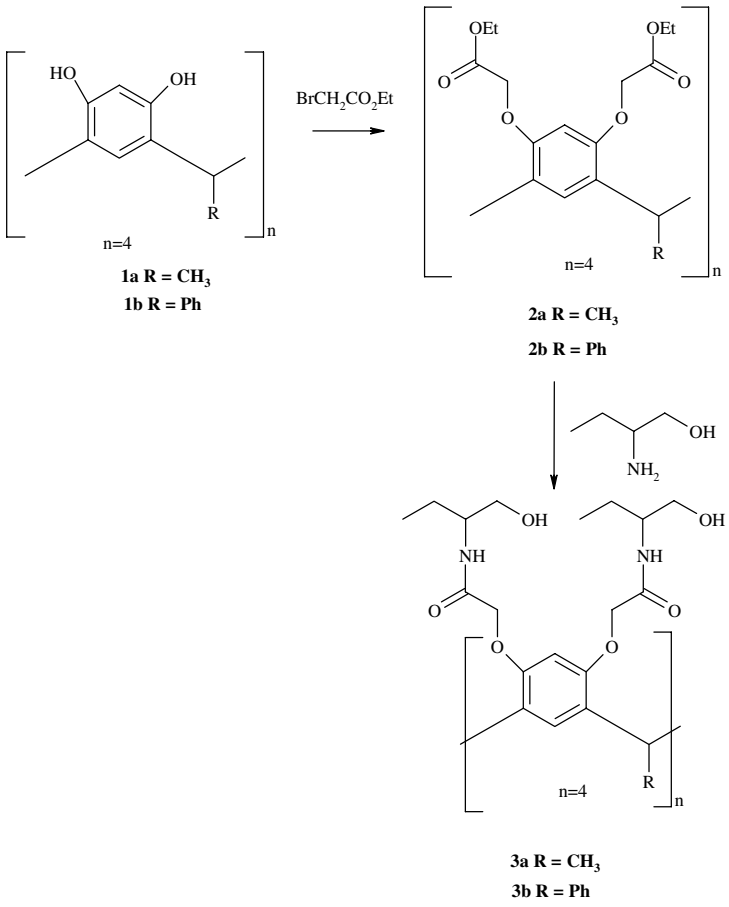

Scheme 1. Preparation of chiral octamides.

Table 1. Some fundamental frequencies (in cm^{-1}) of Amberlite XAD-16 and calixresorcarene impregnated resin

Amberlite XAD-16	Chiral octamide	Assignments
—	3406	OH, NH stretching
3106, 3022	3028, 3015	Aromatic C–H stretching
2928, 2852	2962	Aliphatic C–H stretching
—	1677	C=O stretching
1510, 1488	1510, 1486	C=C ring stretching
—	1407	OH bending
—	1103, 1062	C–O stretching

2a and **2b** with (*R*)-(−)-2-amino-1-butanol gave the chiral octamides (Scheme 1). The IR absorption frequencies of XAD-16 and the chiral octamide impregnated resin are given in Table 1. The IR spectrum of the loaded Amberlite XAD-16 was compared with that of free Amberlite XAD-16. Sharp carbonyl stretching vibration at 1677 cm^{-1} and the OH and NH stretching vibration at 3406 cm^{-1} were observed for the chiral octamide loaded resin. Additional bands observed were at 1407, 1103 and 1062 cm^{-1}, which belong to OH bending and C–O stretching groups, respectively.

2.2. Impregnation process

Portions of 0.1 g portion of dry Amberlite XAD-16 were added to 10.0 ml of chiral octamide solution and stirred for 24 h. The impregnated resin was separated by filtration through a sintered glass funnel and washed with water to remove the solvent. The resin was used as an air-dried product. The chiral octamide content in the impregnated solution was determined by spectrophotometric measurement at 282 nm and also gravimetrically, by weighing the dry ligand residue after removing the resin and evaporation of the solution. The amount of impregnated resin was calculated from the material balance.

In this study, the chiral octamides containing methyl or phenyl groups were used. These chiral molecules were impregnated with Amberlite XAD-16 or Amberlite XAD-7 or silica gel. However, the chiral octamide containing methyl group, **3a**, did not impregnate the resins and the chiral octamide containing a phenyl group was impregnated with Amberlite XAD-16 only seen. So, Amberlite XAD-16 impregnated with the chiral octamide containing a phenyl group was used as support material.

2.3. Molecular recognition

The experimental results show that the L-enantiomers of PhGlyNa and TrpNa were easily discriminated from the D-enantiomers. The retention time of the L-enantiomers of PhGlyNa and TrpNa were shorter than that for the D-enantiomers. This indicated that the D-enantiomers of PhGlyNa and TrpNa were interacting more tightly

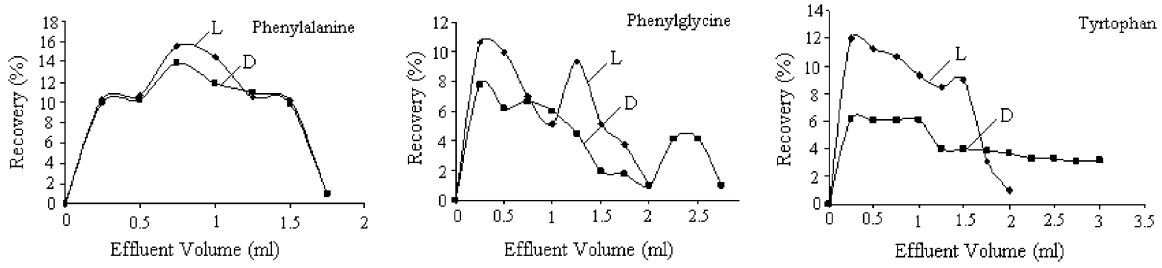

Figure 1. Separation of D- and L-forms of amino acids sodium salts on resin.

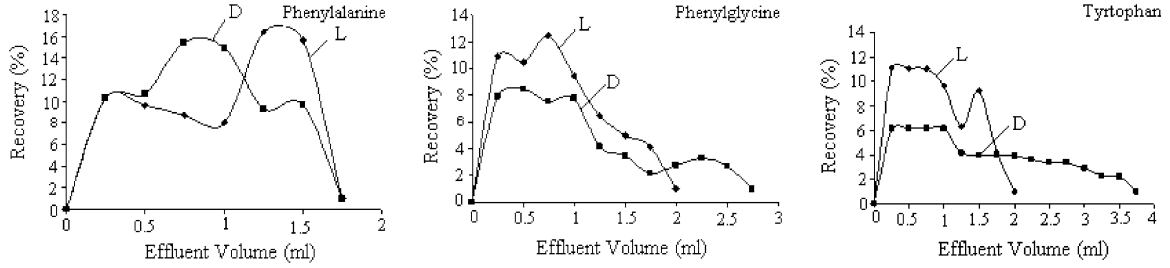

Figure 2. Separation of D- and L-forms of amino acids potassium salts on resin.

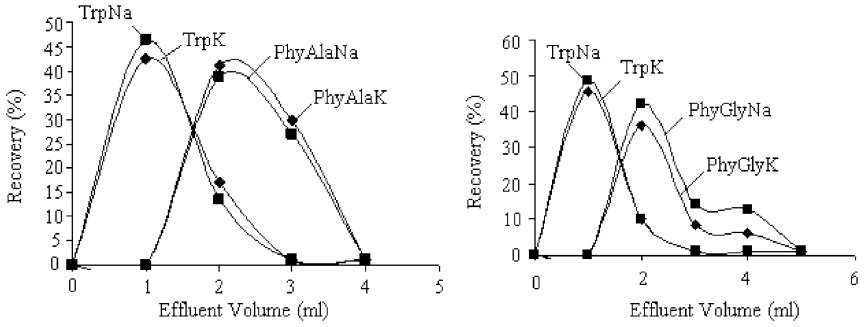

Figure 3. Separation of D-forms of amino acids sodium and potassium salts on resin.

with the stationary phase than the L-enantiomers. In the case of PhAlaNa, there is no sign for discrimination between the L-enantiomer and the D-enantiomer. This indicated that the L- and D-enantiomers of PhAlaNa interact with the stationary phase to the same degree (Fig. 1). The same results were observed in the case of PhGlyK and TrpK. The retention time of the L-enantiomers of PhGlyK and TrpK were shorter than the D-enantiomers. Although the observed discrimination was not great as for PhGlyK and TrpK, the D-enantiomer of PhAlaK was discriminated from L-enantiomer. The retention time of the D-enantiomer of PhAlaK was shorter than that for the L-enantiomer. This result was not observed in the case of PhAlaNa, indicating that the importance of the cation involved in molecular recognition (Fig. 2). After getting these results, we decided to prepare mixtures of different amino acids (L-PhGlyNa-L-TrpNa, L-PhGlyK-L-TrpK, D-PhGlyNa-D-TrpNa, D-PhGlyK-D-TrpK). The mixture of phenylalanine and phenylglycine was not employed because they have same λ_{max}. The experimental result shows that L-PhAlaNa was easily separated from L-TrpNa. The same result was observed for the D-enantiomers. When the cation was changed to potassium, there was no change, again L- and D-enantiomers of PhAla were easily discriminated from Trp. This situation was the same for the mixture of PhGly and Trp. This result indicated that there was no effect of the cation involved in the recognition of different amino acid enantiomers but the structure of the amino acid has a profound effect on recognition. In all cases, the retention time of tryptophan was shorter than that for phenylalanine and phenylglycine. This is mostly due to bulkiness of trytophan compared with phenylalanine and phenylglycine (Figs. 3 and 4).

3. Experimental

3.1. General information

All chemicals were reagent grade unless otherwise specified. Standard amino acid solutions (10^{-3} mol l^{-1}) were prepared by dissolving the required amount in doubly deionized water. Water used throughout the work was deionized by a Millpore Milli-Q system. The Amberlite XAD-16 resin (styrene–divinylbenzene copolymer, surface area: 800 m^2 g^{-1}, pore diameter: 10 nm and bead size: 20–60 mesh) was supplied by Sigma. It was purified with 4 M HCl solution, after elimination of chlorides by washing with distilled water, with an ethanol–water (1:1) solution and finally with water again. Then, the resin

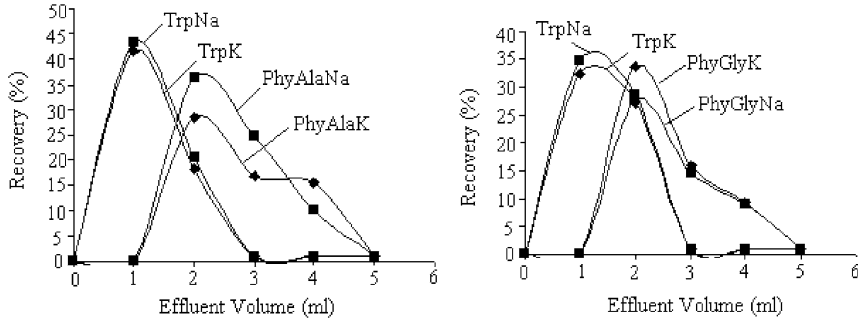

Figure 4. Separation of L-forms of amino acids sodium and potassium salts on resin.

Table 2. The adsorption amounts of amino acids on the sorbent

Aminoacid	%	μmol/g	Stripping volume (ml)
L-PhyAlaNa	70.23	35.11	1.75
D-PhyAlaNa	66.17	33.09	1.75
L-PhyAlaK	66.66	33.33	1.75
D-PhyAlaK	70.00	35.00	1.75
L-PhyGlyNa	53.70	26.85	2.00
D-PhyGlyNa	51.23	25.62	2.60
L-PhyGlyK	58.75	29.37	2.00
D-PhyGlyK	57.08	28.54	2.75
L-TrpNa	60.26	30.13	2.00
D-TrpNa	58.13	29.06	3.00
L-TrpK	61.25	30.62	2.00
D-TrpK	61.03	30.52	3.75

Experimental conditions: resin 0.1 g; volume of solution passed, 10 ml; amino acid, pH 10.5.

was dried in a vacuum oven at 60 °C and stored in a polyethylene bottle.

Perkin–Elmer Spectrum BX Fourier Transform IR spectrometer was used to record the IR spectra of KBr discs, in the range 4000–700 cm^{-1}, 30 co-added interferograms were scanned at 2 cm^{-1} resolution. UV-1601 model Shimadzu UV–vis spectrophotometer in the range of 200–400 nm with 30 mm quartz cells was used to determine amino acids. For solid phase experiments, Varian cartridges (plastic container, 1.0 cm × 10.0 cm) equipped with 20 μm polypropylene frits were used.

3.2. Synthesis

Chiral octamide derivatives were synthesized by reaction of octaester **2** with (*R*)-(−)-2-amino-1-butanol according to the literature procedure.[16]

3.3. Column experiments

0.1 g of the chiral octamide on Amberlite XAD-16 was first wetted with 5 ml methanol and stirred for 10 min, and then 5 ml of doubly deionized water was added and stirred for 10 min. Lastly, the mixture was transferred to the polyethylene column and 25 ml of methanol–water (10:90) passed through the column. A sample solution containing amino acid (phenylalanine or phenylglycine or tryptophan as their potassium or sodium salts) was taken and the pH was adjusted to ~10.5 and passed through the above column (Table 2). Then, the amino acids were stripped from the column with doubly deionized water and determined spectrophotometrically at 253 nm (for phenylalanine and phenylglycine) and 280 nm (for tryptophan). All runs were carried out at ambient temperature (23–25 °C).

Acknowledgements

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