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Design and Synthesis of New Chiral Calix[4]arenes as Liquid Phase Extraction Agents for α-Amino Acid Methylesters and Chiral α-Amines

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Abstract

The article describes the synthesis and extraction properties of new (S)-(-)-1-phenylethylamine substituted *p-tert*-butylcalix[4]arene/calix[4]arene. These compounds have been synthesized *via* nucleophilic substitution reactions involving 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetra(2-hydroxyethoxy)calix[4]arene (**4a**), or 5,11,17,23-tetra-*H*-25,26,27,28-tetra(2-hydroxyethoxy)calix[4]arene (**4b**) with (S)-(-)-1-phenylethylamine in dry THF. The extraction properties of ligands **5a** and **5b** towards the some selected α-amino acid methylesters and chiral α-amines are also reported. It has been observed that receptor **5a** was an excellent ionophore for α-amino acid methylesters/α-amines and good extractant than **5b**. However, both of the ligands did not display any selectivity towards the configurations of this species.

Introduction

Calixarene derivatives as important receptors of supramolecular hosts have the ability to form complexes with several charged or uncharged guest species. The calixarenes formed by condensation of phenols with formaldehydes have properties, which make them interesting receptors especially in enzyme mimics, catalysis, separation of organic compounds, field effect transistors, liquid membranes, chemical sensors and binding biological substrates [1–20]. Much work has been dedicated to the synthesis of calixarenes modified on the upper [1, 2] and lower rims [21]. These derivatives present the ability to form complexes with different compounds. There are studies dedicated to the molecular inclusion of biological substrates, like biogenic amines, amino acid methylesters, and peptides by these receptors [22–26]. Comparing with cyclodextrins, the calixarene molecules exhibit large sterical flexibility. Chiral recognition of amino acids by calixarenes has been investigated [27, 28]. The interesting studies have been realized by using calixarene compounds for separation of positional isomers in capillary electrophoresis [29].

A series of calixarenes should make very suitable platforms for creating interactions to target specific organic compounds [30–33]. In the present work we focused on the amino acid extraction ability of a cyclic ligand. Amino acids are well known to play many important roles in biochemistry, and now the separation of amino acids is a key technology in the downstream processing in the bioindustrial complex. In 1990, Chang *et al*. [34] first reported the transport of amino acids with a calixarene derivative as a mobile carrier in a bulk liquid membrane system. They used calix[6]arene ethyl ester for the transport of *N*-benzoyl amino acid. Later, Zolotov *et al*. [35] developed *p*-1-adamantyl-calix[8]arene ethyl ester as an extractant for various amines and amino acid esters. The complex of calix[4]arene with benzylammonium cation has been studied by gas phase ion molecule reactions [36, 37]. A series of carboxylic acid derivatives were used as host molecules for the quantitative extraction of amino acid methylesters in a liquid-liquid extraction system [38]. Also *p-tert*-butylcalix[n]arenes ($n = 6$ and 8) were investigated as carriers in liquid membrane transport experiments [39]. Chiral separation of amino acids was also attempted with calixarenes. Okada *et al*. designed a calix[4]arene analog having chiral pendant groups and performed chiral recognition of L-amino acid derivatives in a liquid membrane experiment [40].

In the present work, we reported that *p-tert*-butylcalix[4]arene and *p-H*-calix[4]arene introducing (S)-(-)-1-phenylethylamine, became a useful host molecule for the quantitative extraction of α-amino acid methylesters and α-phenylethylamines in a liquid–liquid extraction system. The extraction behavior of ligands was investigated by comparing to elucidate the structural effect on its recognition ability towards amino acid methylesters.

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Experimental

Materials and general methods

Melting points were determined on a Gallenkamp apparatus in a sealed capillary and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 250 MHz spectrometer in $CDCl_3$ with TMS as internal standard. IR spectra were recorded on a Perkin Elmer 1605 FTIR spectrometer as KBr pellets. UV–Vis. spectra were obtained on a Shimadzu 160A UV–Visible recording spectrophotometer. Elemental analyses were performed on a Leco CHNS-932 analyzer.

Analytical TLC was performed on precoated silica gel plates (SiO_2, Merck PF_{254}), while silica gel 60 (Merck, particle size 0.040–0.063 mm, 230–240 mesh) was used for preparative column chromatography. Generally, solvents were dried by storing them over molecular sieves (Aldrich; 4 Å, 8–12 mesh). Acetone and CH_2Cl_2 was distilled from $CaSO_4$ and $CaCl_2$, respectively. Dry THF was distilled from the ketyl prepared from sodium and benzophenone. All aqueous solutions were prepared with deionized water that had been passed through a Millipore Milli-Q Plus water purification system.

The following α-amino acid methylester hydrochlorides obtained from Aldrich and chiral amines obtained from Merck at the highest purity commercially available were used in this study: L-phenylalanine methylester hydrochloride (L-PheOMe), D-phenylalanine methylester hydrochloride (D-PheOMe), L-alanine methylester hydrochloride (L-AlaOMe), D-alanine methylester hydrochloride (D-AlaOMe), (R)-(+)-1-phenylethylamine (R-(+)-1-PEA) and (S)-(-)-1-phenylethylamine (S-(-)-1-PEA) (Scheme 1).

Scheme 2 illustrates the successive synthetic steps of the extractants (**1a–5a** and **1b–5b**) used. Compounds **1a–4a** and **1b–4b** were prepared according to literature methods [41, 42].

Synthesis

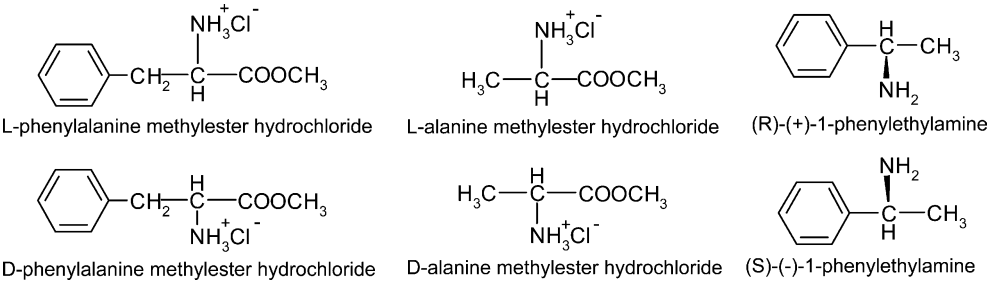
The preparation of compounds **5a** and **5b** is carried out as following the general procedure: (S)-(-)-1-phenylethylamine (4.10 mmol) was added to a solution of **4a/4b** (1.00 mmol) dissolved in dry THF (20 mL). After the mixture was stirred at room temperature for 5 h, distilled water (10 mL) was added to the solution. The mixture was extracted with dichloromethane, washed with distilled water (2 × 50 mL), brine (2 × 50 mL), and then dried over magnesium sulfate. The solvent was evaporated under vacuo, and recrystallized with dichloromethane to give **5a/5b** as white crystals.

5,11,17,23-tetra-tert-*butyl-25,26,27,28-tetra(S-(-)-(1)-phenylethylaminoethoxy)-calix[4]arene (5a)*
Yield 85%; mp: 178 °C; IR (KBr): 3302 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.99 (s, 36 H, *tert*-butyl), 2.35 (s, 16H, CH, CH_3), 2.92 (d, 4H, 12.8 Hz, $ArCH_2Ar$), 4.02 (t, 8H, CH_2N), 4.15 (d, 4H, 12.8 Hz, $ArCH_2Ar$), 4.32 (t, 8H, OCH_2), 7.03 (m, 4H, phenylethylamine-H_{para}), 7.19 (s, 8H, ArH), 7.26 (m, 8H, ArH, phenylethylamine-H_{ortho}), 7.68 (m, 12H, NH and phenylethylamine-H_{meta}). Anal. Calcd for $C_{84}H_{108}N_4O_4$: C, 81.51; H, 8.79; N, 4.53. Found: C, 81.24; H, 8.98; N, 4.24.

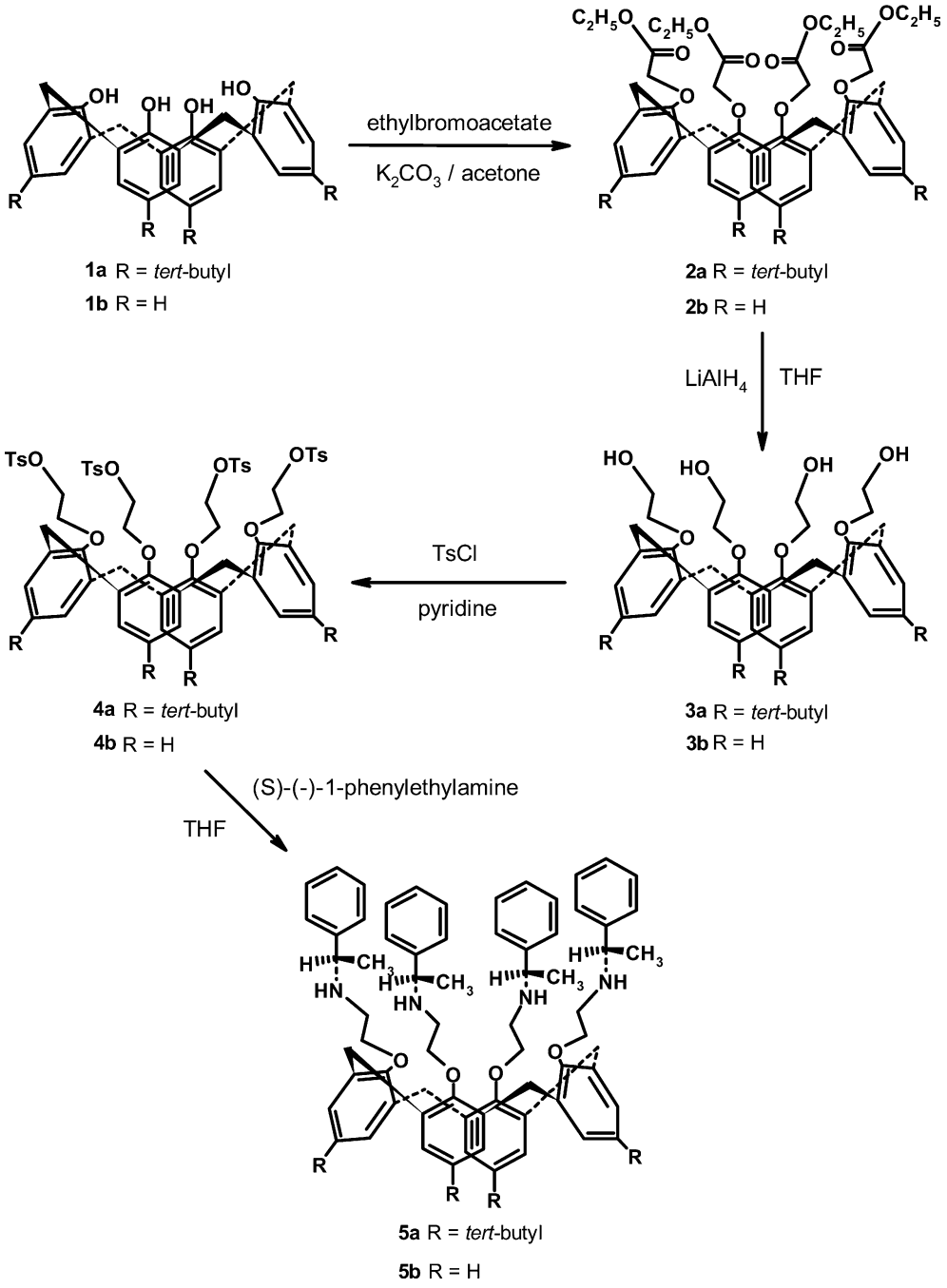
5,11,17,23-tetra-H-25,26,27,28-tetra(S-(-)-(1)-phenylethylaminoethoxy)calix[4]-arene (5b)
Yield 77%; mp: 125 °C; IR (KBr): 3304 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.35 (s, 12H, CH_3), 2.37 (s, 4H, CH), 2.98 (d, 4H, $J = 13.6$ Hz, $ArCH_2Ar$), 4.05 (t, 8H, CH_2N), 4.22 (d, 4H, $J = 13.6$ Hz, $ArCH_2Ar$), 4.31 (t, 8H, OCH_2), 6.48 (s, 12H, ArH and phenylethylamine-H_{para}), 7.24 (d, 8H, $J = 8.1$ Hz, ArH, phenylethylamine-H_{ortho}), 7.68 (d, 12H, $J = 8.2$ Hz, NH and phenylethylamine-H_{meta}). Anal. Calcd for $C_{68}H_{72}N_4O_4$: C, 80.92; H, 7.19; N, 5.55. Found: C, 80.57; H, 7.38; N, 5.23.

Analytical procedure

Picrate extraction experiments were performed following Pedersen's procedure [43]. A 10 mL of a 2.0×10^{-5} M aqueous picrate and 10 mL of 1.0×10^{-3} M solution of calixarene **(5a/5b)** in CH_2Cl_2 were vigorously agitated in a stoppered glass tube with a mechanical shaker for 2 min, then magnetically stirred in a thermostated water-bath at 25 °C for 1 h, and finally left standing for an additional 30 min. The concentration of picrate ion remaining in the aqueous phase was then determined spectrophotometrically at 357 nm. Blank experiments showed that no picrate extraction occurred in the absence of calixarene. The percent extraction (E%) has been calculated as:



Scheme 1. The chemical structures of some selected α-amino acid methylesters and chiral α-amines used in experiments.



Scheme 2. Synthesis of compounds **1a–5a** and **1b–5b**.

$$(E\%) = A_o - A/A_o \times 100 \quad (1)$$

where A_o and A are the initial and final concentrations of the metal picrate before and after the extraction, respectively.

To prepare the ammonium picrates, an aqueous solution of α-amino acid methylester hydrochloride salt was treated with a saturated Na_2CO_3 solution and extracted three times with dichloromethane. The organic phase was dried over $MgSO_4$. The solvent was evaporated until dryness under nitrogen atmosphere to give pure α-amino acid methylester. Then the α-amino acid methylester (or chiral amine) and picric acid in the molar ratios of 1:1 were dissolved in deionized water. Thus, the stock solution was diluted to 2.0×10^{-5} M and it was used in extraction experiments.

Results and discussion

Synthesis

We have interested in the synthesis of calix[4]arene-based ionophores having chiral binding sites in order to estimate their extraction ability towards α-amino acid methylesters and α-phenylethylamines through the two

phase solvent extraction systems. The synthesis of **1a–5a** and **1b–5b** was conducted as shown in Scheme 1. Compounds **1a–4a** and **1b–4b** were synthesized according to previously literature methods [28, 29]. After preparation of **4a**, it was reacted with 4.1 equivalent of (S)-(-)-1-phenylethylamine in dry THF at room temperature for 5 h to obtain **5a** in 85% yield. Also **5b** was prepared by using same procedure in 77% yield.

The new compounds have been characterized by a combination of ^{1}H NMR, IR and elemental analysis. The IR spectra of **5a** and **5b** show an amine bands at 3302, 3304 cm^{-1}, respectively. From the ^{1}H NMR data, these compounds (**5a/5b**) were confirmed to be present in the cone conformation by detailed study of the ^{1}H NMR spectrum (doublets at δ 2.92 ppm, 4.15 ppm, $J = 12.8$ Hz and at δ 2.98 ppm, 4.22 ppm, $J = 13.6$ Hz for $ArCH_2Ar$ protons respectively).

Two-phase solvent extraction studies

In this study it was carried out two-phase solvent extraction experiments to examine extraction behavior of α-amino acid methylesters and α-phenylethylamines from the aqueous phase into the organic phase (dichloromethane) by using novel chiral calix[4]arene derivatives **5a** and **5b**. The results of the picrate extraction studies are summarized in Table 1. These data were obtained by using a dichloromethane solution of the ligands **5a** and **5b** to extract ammonium picrates from the aqueous solution. The equilibrium concentration of picrate in aqueous phase was determined spectrophotometrically. From the data given in Table 1, it was observed that all α-amino acid methylesters were highly extracted by **5a**, whereas they were extracted in less extraction ratios by **5b**. This implies that the better preorganization of fixed **5a** in the cone conformation in solution. By contrast, **5b** is significantly more flexible than **5a** and it less extraction ability toward α-amino acid methylesters as extracted species. The conformation of **5a** has significantly less flexibility because the *tert*-butyl groups lock it in the cone conformation in the liquid phase.

In this study it was used α-amino acid methylesters having different molecular size to investigate effect of size on their extraction efficiency and observed that ammonium picrate extraction ratios did not change. Besides, we thought that both **5a** and **5b** could selectively extract α-amino acid methylesters above mentioned as to their configurations. Because they have four chiral (S)-(-)-1-PEA groups that can be provide selectivity. From the extraction results it was observed that **5a** and **5b** did not display any selectivity towards α-amino acid methylesters.

Table 1. Extraction percentage of selected α-amino acid methylesters with **5a** and **5b**[a]

Ligand	L-PheOMe	D-PheOMe	L-AlaOMe	D-AlaOMe
5a	95.2	93.5	96.1	95.3
5b	27.4	28.3	26.7	25.8

[a]Aqueous phase, [ammonium picrate] = 2.0×10^{-5} M; organic phase, dichloromethane, [ligand] = 1.0×10^{-3}; at 25 °C, for 1 h.

Table 2. Extraction percentage of (R)-(+)-1-PEA and (S)-(-)-1-PEA with **5a** and **5b**[a]

Ligand	(R)-(+)-1-PEA	(S)-(-)-1-PEA
5a	95.3	96.1
5b	53.5	55.7

[a]Aqueous phase, [ammonium picrate] = 2.0×10^{-5} M; organic phase, dichloromethane, [ligand] = 1.0×10^{-3}; at 25 °C, for 1 h.

We performed further study to investigate affinity of **5a** and **5b** towards to chiral amines ((R)-(+)-1-PEA and (S)-(-)-1-PEA) and also their configurations. The results were summarized in Table 2. These values were compared with those of the chiral α-amino acid methylesters reflecting these results similarity. **5a** showed high extractability chiral amines than the compound **5b**.

The extraction data for **5b** was analyzed by a classical slope analysis method. Assuming the extraction of an ammonium cation ($R\text{-}NH_3^+$) by the receptor **5b** according to the following equilibrium:

$$[R{-}NH_3^+]_{aq} + [Pic^-]_{aq} + x[L]_{org} \rightleftharpoons [R{-}NH_3Pic(L)_x]_{org}$$

The extraction constant K_{ex} is then defined by:

$$K_{ex} = \frac{[R{-}NH_3Pic(L)_x]}{[R{-}NH_3^+][Pic^-][L]^x} \qquad (2)$$

Equation (2) can be rewritten as

$$\log D_A = \log K_{ex}Pic + x \log[L] \qquad (3)$$

where the distribution ratio D_A is defined as ratio of the concentrations of the ammonium cation ($R{-}NH_3^+$) in the two phases:

$$D_A = [R{-}NH_3Pic(L)_x]org/[R{-}NH_3^+]_{aq} \qquad (4)$$

Consequently a plot of log D_A versus log [L] leads to a straight line whose slope allows the stoichiometry of the extracted species to be determined.

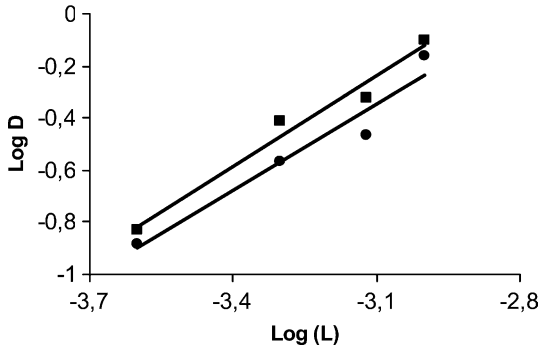


Figure 1. Log D *versus* log [L] for the extraction of D-AlaOMe (●) and (S)-(-)-1-PEA (■) by **5b** from an aqueous phase into dichloromethane phase at 25 °C.

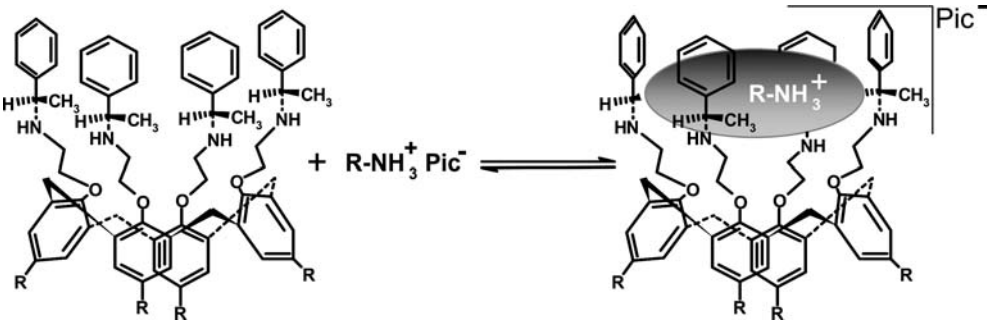


Figure 2. The proposed interactions of **5a** or **5b** moieties with an ammoinum cation belong to amino acid or amine.

Figure 1 represents the extraction into dichloromethane at different concentrations of **5b** for the ammonium ion. A linear relationship between log D_A *versus* log [L] is observed with a slope for ammonium ion by **5b** which equals 1.10 and 1.15 respectively, suggesting that **5b** forms a 1:1 complex with an ammonium cation (Figure 2). The analytical data of **5b** show that the complexation reaction takes place according to the following equilibrium:

$$(L)_{org} + (R{-}NH_3^+Pic^-)_{aq} \overset{K_{ex}}{\rightleftharpoons} (L, R{-}NH_3^+Pic^-)_{org} \quad (5)$$

By using Equation 3 for **5b**, log K_{ex} has the value 3.34 ± 0.2 and 3.07 ± 0.2, respectively.

Conclusions

In this study, synthesis and extraction ability of chiral α-phenylethylamine derivatives of *p-tert*-butylcalix[4]arene/calix[4]arene has been demonstrated. The results have showed that compound **5a** is better extractant than compound **5b** for both α-amino acid methylesters and α-amines, and there is no any selectivity in extraction phenomena by using these chiral calix[4]arene derivatives. It is important to note that fixed cone conformation plays major role the guest inclusion in the calixarene cavity.

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