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Extraction Behavior of Amino Acids by Calix[6]arene Carboxylic Acid Derivatives

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Abstract

A series of calixarene carboxylic acid derivatives were synthesized for the extraction of amino acids. A calix[6]arene carboxylic acid derivative showed the highest extractability to the target tryptophan ester. The main driving force for the complexation was the interaction between the ammonium cation of the amino acid and the oxygen atoms of the host molecule. Stripping of amino acids was also accomplished by contacting the organic solution with a fresh acidic solution. Based on slope and Job method analyses, it was confirmed that the calix[6]arene forms a 1 : 1 complex with the amino acid ester. The structure of the complex between the calix[6]arene and the amino acid was investigated by ^{1}H-NMR and CD spectra. The calix[6]arene includes a guest molecule in the cavity, and the inclusion induces the asymmetrization of the host molecule. This host compound functions as a novel recognition tool for amino acids.

Introduction

Calixarene [1] is a cyclic oligomer that is formed by plural phenolic units linked by methylene bridges. The cavity size of calixarenes is readily controlled by changing the number of the phenol units; hence the framework has attracted much attention for creating specific affinity to a target guest molecule. These features were initially utilized in designing an ionic receptor [2]. To date, various calixarenes that possess ketone, ester, amide, carboxylic acid or other functional groups have been synthesized for selectively extracting target metal ions. There are many reports related to metal ion recognition [3], but relatively little attention has been paid to the molecular recognition of organic compounds by calixarenes.

A series of calixarenes should make very suitable platforms for creating interactions to target specific organic compounds [4]. In the present study we focused on the amino acid inclusion 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.* first reported the transport of amino acids with a calixarene derivative as a mobile carrier in a bulk liquid membrane system [5]. They used calix[6]arene ethyl ester for the transport of *N*-benzoyl amino acid. The transport rate depended on the hydrophobicity of guest anions and the size of alkaline metal cations, which coexisted in the source phase. Thus the amino acids appeared to have been transported as a counteranion of the alkaline metal cations. They also utilized the carrier to transport amino acid esters directly [6]. However, the exact structure of the complex between the carrier and amino acid esters is not yet clarified.

Later, Zolotov *et al.* developed *p* − 1-adamantylcalix[8]arene ethyl ester as an extractant for various amines and amino acid esters [7]. Recently, Antipin *et al.* synthesized calix[4]arene based-aminophosphonates, at either the lower or upper rims, and utilized them as a mobile carrier for the membrane transport of zwitterionic amino acids [8]. The transport efficiency depended on where the aminophosphonate groups were introduced, and the order of the transport rate did not reflect the hydrophobicity of amino acids.

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 transport experiment [9]. However, systematically studies utilizing calixarenes for the recognition of amino acids have not yet been conducted.

In the present work, we found that calix[6]arene introducing carboxylic acids, ^{t}Oct[6]CH_2COOH, became a powerful host molecule for the quantitative extraction of amino acids in a liquid–liquid extraction system. This calixarene has six ionizable carboxylic acid groups to ensure electrostatic interaction, and a large cavity enabling the inclusion of relatively large organic compounds. The extraction be-

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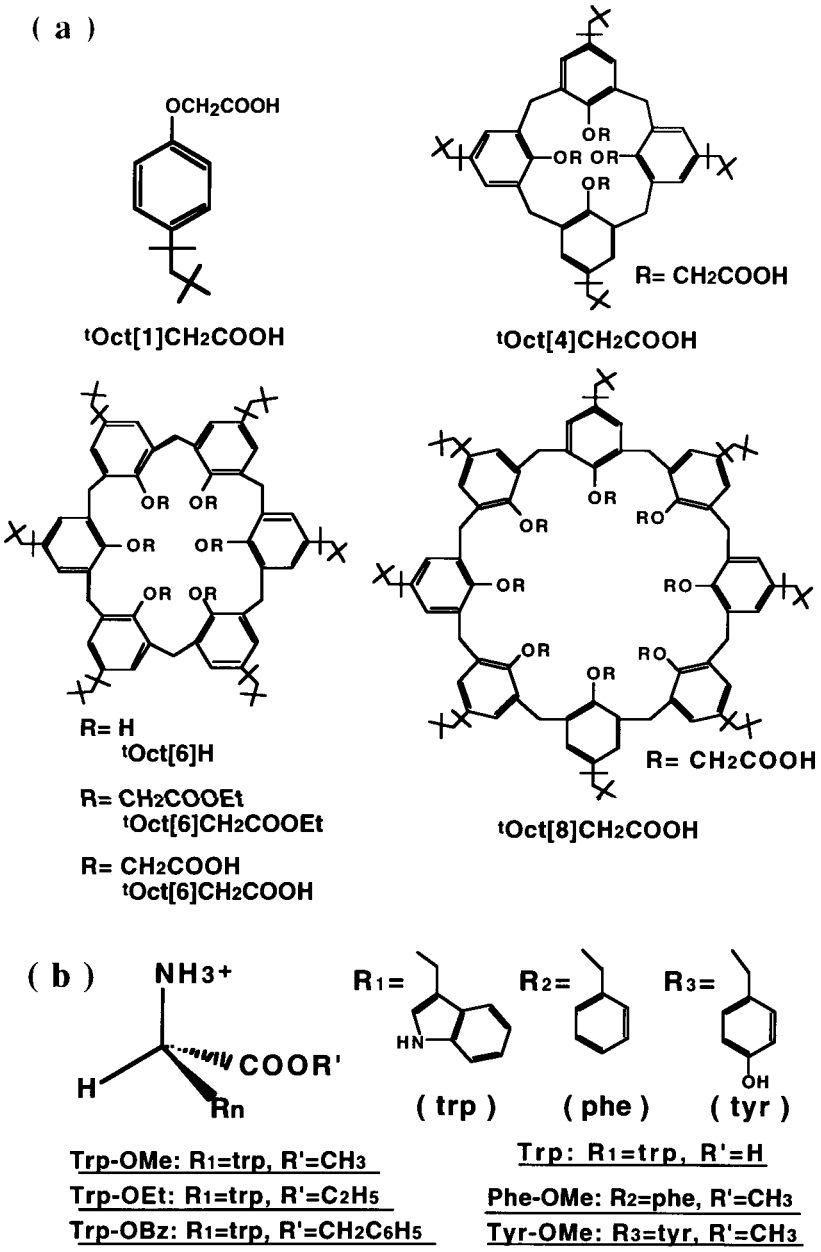

Figure 1. Molecular structures and abbreviations of (a) host molecules and (b) guest amino acid derivatives.

havior was investigated in detail by comparing it to that of several analogs to elucidate the structural effect on its recognition ability towards amino acids. The extraction mechanism was confirmed by slope analysis and the Job method. Spectroscopic analysis for the extracted complex was also performed. A few researchers have reported a complexation model of amino acids by *p*-sulfonatocalix[n]arene in an aqueous solution [10], however, there have been no attempts to observe the complex in organic media. Such information would contribute to the understanding of the inclusion phenomena of amino acids by macrocyclic compounds.

Experimental

Reagents

Figure 1 shows the structures and abbreviations of extractants and guest amino acid derivatives used in the present study. The extractants *p*-tert-octylphenoxyacetic acid [*p*-(1,1,3,3,-tetramethylbutyl) phenoxyacetic acid] (abbreviated as ^{t}Oct[1]CH_2COOH, as the monomer analog), *p*-tert-octylcalix[4]arene tetracarboxylic acid derivative [25,26,27,28-tetrakis(carboxymethoxy)-5,11,17,23-tetrakis(1,1,3,3,- tetramethylbutyl) calix[4]arene] (abbreviated as ^{t}Oct[4]CH_2COOH), and *p*-tert-octylcalix[6]arene hexacarboxylic acid derivative [37,38,39,40,41,42-hexakis(carboxymethoxy)-5,11,17,23,29,35-hexakis (1,1,3,3,-tetramethylbutyl)calix[6]arene] (abbreviated as ^{t}Oct[6]CH_2COOH) were synthesized according to

the procedures described in a previous paper [11]. The two analogues *p*-tert-octylcalix[6]arene (^{t}Oct[6]H) and *p*-tert-octylcalix[6]arene hexaethyl ester derivative (^{t}Oct[6]CH_2COOEt) were obtained as precursors of ^{t}Oct[6]CH_2COOH. Here, ^{t}Oct[4]CH_2COOH has a cone conformation. These final products were purified by recrystallization and then identified by means of FT-IR, ^{1}H NMR, and elemental analysis.

Analytical grade amino acids and amino acid ester hydrochlorides were purchased and employed without further purification as guest molecules for the solvent extraction experiments: i.e, *L*-tryptophan methyl ester hydrochloride (Trp-OMe), *L*-tryptophan ethyl ester hydrochloride (Trp-OEt), *L*-tryptophan benzyl ester hydrochloride (Trp-OBz), *L*-phenylalanine methyl ester hydrochloride (Phe-OMe), *L*-tyrosine methyl ester hydrochloride (Tyr-OMe) from Aldrich Chemical Co. (Milwaukee, WI), *L*- or *D*-leucine tert-butyl ester hydrochloride (Leu-OtBu) from Nova Biochem Co. (Darmstadt, Germany), and *L*-tryptophan (Trp) from Wako Pure Chemical Industries (Osaka, Japan).

Synthesis of p-tert-octylcalix[8]arene octacarboxylic acid derivative [43, 44, 45, 46, 47, 48, 49, 50-octakis(carboxymethoxy)-5, 11, 17, 23, 29, 35, 41, 47-octakis(1, 1, 3, 3,-tetramethylbutyl)calix[8]arene] (abbreviated as ^{t}Oct[8]CH_2COOH)

The precursors, *p*-tert-octylcalix[8]arene (^{t}Oct[8]H) and *p*-tert-octylcalix[8]arene octaethylester derivative (^{t}Oct[8]CH_2COOEt) were synthesized in a similar manner as described previously [12]. To 250 mL of tetrahydrofuran were added ^{t}Oct[8]CH_2COOEt (4.0 g, 1.6 mmol) and tetramethylammonium hydroxide aqueous solution (15%, 150.0 g, 247 mmol). The solution was refluxed for 20 hours. After cooling, 10 M hydrochloric acid (27 mL, 270 mmol) was added to the solution and refluxed for 4 more hours. After the organic layer was separated, the aqueous layer was washed with chloroform. The organic solvent was removed *in vacuo*, and the residue extracted in chloroform. The solution was washed with distilled water three times. After drying with anhydrous magnesium sulfate, the solution was dried *in vacuo*, and recrystallized with cooled methanol. The following properties were observed; white powder, m.p. 245–246 °C, 38%, TLC (SiO_2, chloroform : ethanol = 1 : 5 v/v, R_f = 0.16–0.42, tailing); IR (KBr) O—H 3410 cm^{-1}, C=O 1750 cm^{-1}; ^{1}H NMR (250 MHz, $(CDCl_2)_2$, TMS, 90 °C) 0.60–0.84 (72 H, m, $C(CH_3)_3$), 0.99–1.38 (48 H, m, $C(CH_3)_2$), 1.52–1.73 (16 H, m, C—CH_2—C), 3.60–4.50 (32 H, m, Ar—CH_2—Ar, OCH_2COO), 6.76–7.18 (16 H, m, ArH). The elemental analysis was as follows. Found: C, 73.33; H, 8.79%. Calcd for $C_{36}H_{192}O_{24}$: C, 73.88; H, 8.75%.

Extraction equilibrium of amino acids in liquid–liquid extraction

An aqueous solution was prepared by dissolving 1.0 mM amino acid or amino acid ester hydrochloride. The pH in the aqueous solution was adjusted by the concentration of hydrochloric acid. An organic solution was prepared by dissolving an extractant into chloroform. Equal volumes (5 mL) of the aqueous and organic solutions were mixed in a screw-cap polypropylene tube and gently shaken in a thermostatted water bath at 303 K. After 20 hours the extraction achieved the equilibrium state and each phase was separated [13]. The concentration of amino acids in the aqueous solution was determined by a UV-VIS spectrometer (JASCO U-best 570) to determine the degree of extraction (E [-], E = 1-[amino acid]$_{aq,eq}$/[amino acid]$_{aq,ini}$) and the distribution ratio (D [-]). The detailed experimental conditions are listed in Table 1.

Table 1. Experimental conditions for amino acid extraction

Aqueous phase	Volume:	5 mL
	Amino acid:	Trp, Trp-Ome, Trp-Oet, Trp-Obz, Phe-Ome, or Tyr-Ome (1.0 mol/m^3)
	pH: 1.0–5.5:	(hydrochloric acid media)
Organic phase	Volume:	5 mL
	Solvent:	Chloroform
	Extractant:	^{t}Oct[4]CH_2COOH (5.0 mol/m^3)
		^{t}Oct[6]CH_2COOH (5.0 mol/m^3)
		^{t}Oct[8]CH_2COOH (5.0 mol/m^3)
		^{t}Oct[1]CH_2COOH (30.0 mol/m^3)
Temperature	303 K	
Contact time	20 hours	

Stripping test

Trp-OMe was selected for the stripping test. After forward extraction was performed from the aqueous to the organic phase (50 mL/50 mL), 0.85 mM of Trp-OMe was transferred to the organic phase at an equilibrium pH of 3.1. The organic phase was divided into 5 mL, and each solution was contacted with a 5 mL fresh aqueous solution containing each mineral acid. Both phases were mixed and gently shaken at 303K for 20 hours. The stripping solution was separated from the organic phase and the Trp-OMe concentration was quantified to calculate the stripping ratio (= 100 × [amino acid]$_{aq,eq}$/[amino acid]$_{org,ini}$).

^{1}H-NMR and ICD spectral measurement for the host-guest complex

Procedure for ^{1}H NMR study

Trp-OMe at desired concentration (0, 0.4, 1, or 10 equivalent to the extractant) as the guest cation was dissolved into 8 mL D_2O, and 10.0 mM ^{t}Oct[6]CH_2COOH was dissolved into 4 mL $CDCl_3$ to which tetramethylsilane was added as an internal standard. Both phases were mixed and vigorously shaken by a mechanical vibrator at 298 K for 1 hour. After each solution was separated, ^{1}H NMR measurement of the organic phase was performed at 273 and 303 K by a JEOL GSX 400 spectrometer.

Procedure for ICD study

Aqueous solutions were prepared by dissolving 5.0 mM L- or D-Leu-OtBu (10 equivalent to the extractant) as the guest cation and organic solutions were prepared by dissolving 0.5 mM ^{t}Oct[6]CH_2COOH into chloroform. Equal volumes (5 lM) of the aqueous and the organic solutions were contacted and vigorously shaken by a mechanical vibrator at 298 K for 1 hour. After each phase was separated, CD measurement for the organic solution was conducted at 298 K with a JASCO J-820 spectropolarimeter.

Results and discussion

Extraction equilibrium of amino acid esters

The extraction experiments were carried out with an acidic solution. An amino group is protonated and exists as a cationic species under the present pH conditions; therefore calixarene carboxylic acid should interact with the cationic species of amino acid esters.

The extraction abilities of various extractants (^{t}Oct[n]CH_2COOH (n = 1, 4, 6, 8)) for Trp-OMe were compared under the same operational conditions (Figure 2). Here, the initial concentrations of ^{t}Oct[4]CH_2COOH, ^{t}Oct[6]CH_2COOH, and ^{t}Oct[8]CH_2COOH were 5 mM, while the concentration of ^{t}Oct[1]CH_2COOH was 30 mM to adjust the number of functional carboxyl groups to that of ^{t}Oct[6]CH_2COOH. Calix[6]arene carboxylic acid derivative ^{t}Oct[6]CH_2COOH provided the highest affinity toward Trp-OMe in the entire acidic region among the host molecules tested in this study. Calix[8]arene ^{t}Oct[8]CH_2COOH was also able to extract the guest cation, though its performance was second to ^{t}Oct[6]CH_2COOH, while the monomer analog ^{t}Oct[1]CH_2COOH did not show any extraction ability compared to that of ^{t}Oct[6]CH_2COOH. The results indicate that the macrocyclic structure of calixarenes is essential for the recognition of an amino acid ester. However, in the case of a cyclic tetramer ^{t}Oct[4]CH_2COOH, Trp-OMe was rarely extracted. These results suggest that the cavity size of calixarene is one of the most important factors for successfully entrapping the amino acid ester.

The effect of functional groups introduced at the lower rim of the calix[6]arene platform on the extraction of Trp-OMe was also examined. Unsubstituted calix[6]arene ^{t}Oct[6]H, and the ethyl ester ^{t}Oct[6]CH_2COOEt did not afford any extraction ability for amino acid esters under the same experimental conditions. This result shows that introducing carboxylic acid to the calixarene platform is a key factor creating an affinity to amino acid esters. The carboxylic acid derivative ^{t}Oct[6]CH_2COOH is an acidic extractant, therefore the electrostatic interaction enables the transfer of an amino acid ester to the organic phase without any promoters. In a previous report, calix[6]arene ester facilitated the transport of amino acid esters in a bulk liquid membrane system, the carrier realizing the transport of amino acid esters by accompanying with the counter perchlorate anion [6]. Based on the above results, we concluded that ^{t}Oct[6]CH_2COOH, which did not require any promoters for the extraction of amino acids, and is thus one of the best extractants presently available.

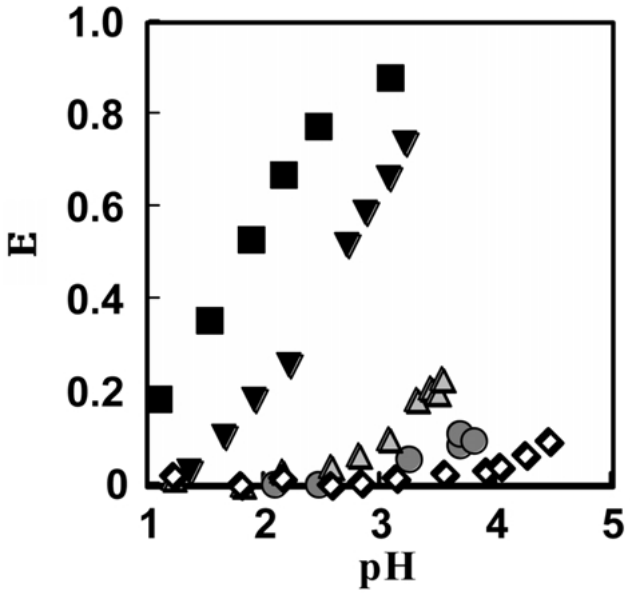

Figure 2. Extracton profile of TrP-OMe with various ^{t}Oct[n]CH_2COOH (n = 1, 4, 6, 8): extractant free (◇), ^{t}Oct[1]CH_2COOH (△), ^{t}Oct[4]CH_2COOH (○), ^{t}Oct[6]CH_2COOH (■), ^{t}Oct[8]CH_2COOH (▲).

The extraction behavior of various amino acid esters with ^{t}Oct[6]CH_2COOH is shown in Figure 3. It should be noted that amino acid esters themselves distribute to the organic phase, to some extent, without a host molecule (empty plots). Various tryptophan esters were quantitatively extracted to the organic phase with ^{t}Oct[6]CH_2COOH (Figure 3a). The order of the extraction depended on the hydrophobicity of the ester groups; Trp-OBz > Trp-OEt > Trp-OMe ≫ Trp. The zwitterion of Trp was about 20% extracted at optimal conditions. At the higher pH, the carboxylic acid group in Trp is negatively charged due to the dissociation of carboxylic acid, therefore, the extraction of Trp was depressed. The extraction behavior of some other amino acid methyl esters was also investigated (Figure 3b). The order was Trp-OMe > Phe-OMe > Tyr-OMe. The tendency was consistent with the order of the values of log *P* (listed in parentheses), which is a quantitative indicator for the hydrophilic/lipophilic balance of organic compounds [14]. Thus, the extraction efficiency was also related to the hydrophobicity of the guest amino acid esters.

To confirm the amino acid stripping method is also important for its separation or the concentration. The stripping of tryptophan ester from the organic phase into a fresh aqueous phase was examined and the results are summarized in Table 2. Tryptophan methyl ester was well transferred in low pH conditions, and the stripping efficiency was dependent on the concentration of mineral acids.

Determination of complex species

The complexation mechanism between Trp-OMe and ^{t}Oct[6]CH_2COOH was investigated by slope analysis and the Job Ostomissiensky method [15].

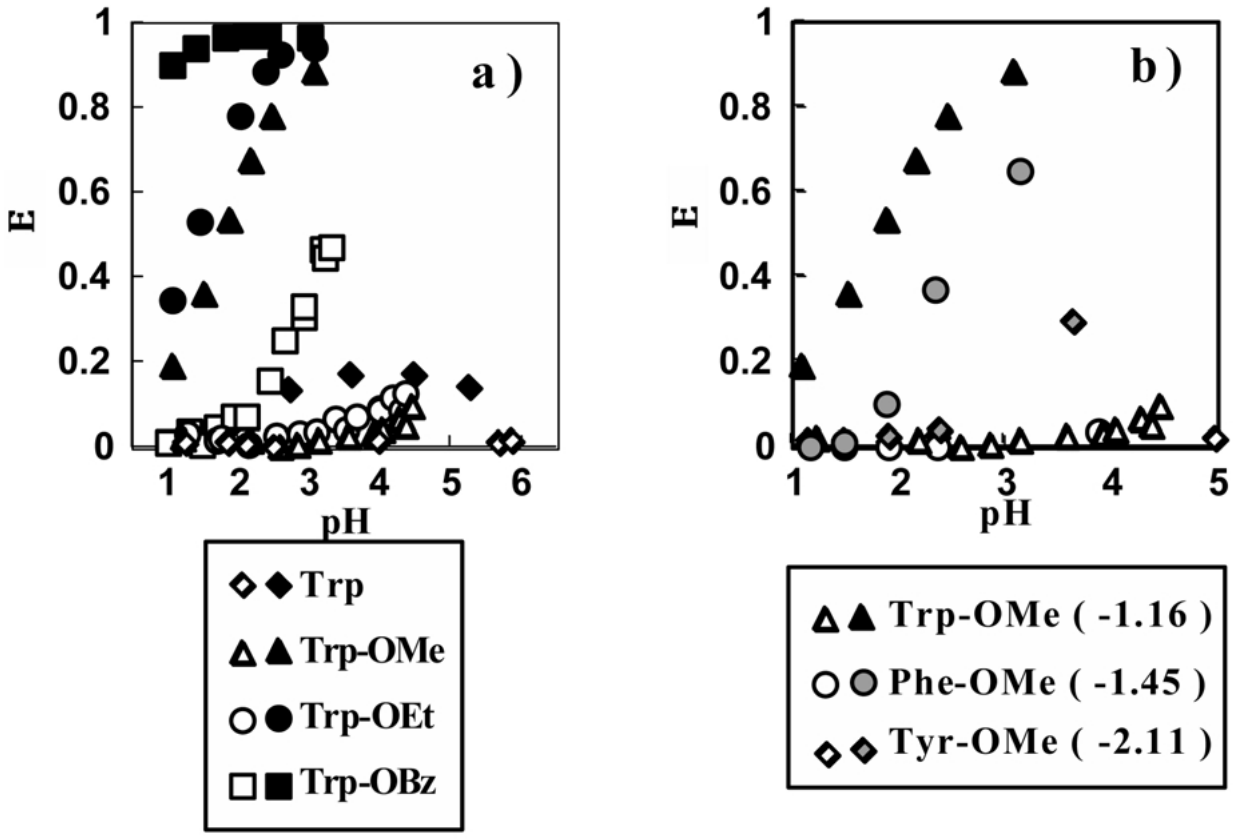

Figure 3. Extraction profile of amino acid and amino acid esters with ^{t}Oct[6]CH_2COOH: open symbols; extractant free, filled symbols; ^{t}Oct[6]CH_2COOH 5 mM. (a) Tryptophan and tryptophan esters; (b) amino acid methyl esters. Parenthesis show log *P* value for each amino acid.

Table 2. Stripping of Trp-Ome from the organic phase

Acid	Acid concentration (mol/m^3)	Stripping ratio (%)
HCl	100	87
HNO_3	100	79
$HClO_4$	100	87
H_2SO_4	50	70
H_2SO_4	100	79
H_2SO_4	1000	95

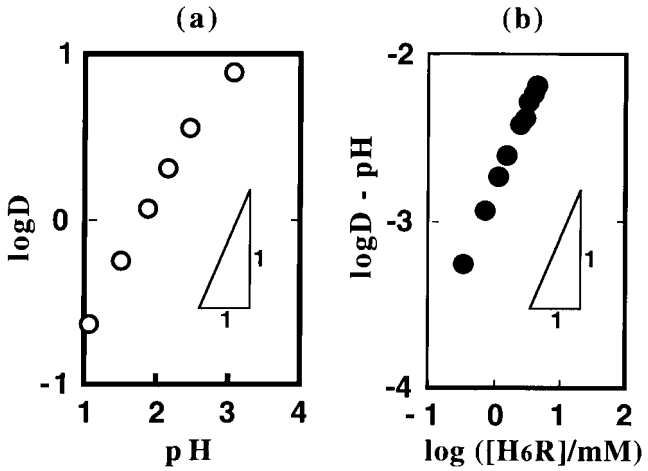

Figure 4. Slope analysis of Trp-OMe extraction with ^{t}Oct[6]CH_2COOH (H_6R). (a) Relationship between the logarithmic distribution ratio of Trp-Ome and the pH. (b) Relationship between log *D*-pH and log[H_6R] for Trp-OMe extraction.

Figure 4 shows the effects of pH (open symbols) and the extractant concentration (filled symbols) on the extraction of Trp-OMe. Extraction experiments were performed in the low pH conditions (less than pH 3.5) in which the amino acid ester is not self-distributed. A slope of unity was obtained for the relationship between the distribution of tryptophan ester and pH (Figure 4a), indicating that one hydrogen ion was released from the extractant by complexing with the tryptophan ester. Further, the slope of the distribution on the extractant concentration (Figure 4b) indicated that one extractant molecule participates in the complex formation.

Moreover, the stoichiometry for the extraction complex was confirmed by the Job method. As shown in Figure 5, when the ratio of ^{t}Oct[6]CH_2COOH/Trp-OMe was unity, the concentration of the tryptophan ester in the organic phase ($[Trp\text{-}OMe]_{org.eq.}$) reached a maximum value. Based on the result, it was elucidated that a 1 : 1 complex was formed by the extraction.

In the results, the proton-exchange reaction of an amino acid ester with ^{t}Oct[6]CH_2COOH is expressed by the following equation:

$$HA^{+} + H_6R = (HA)H_5R + H^{+}$$

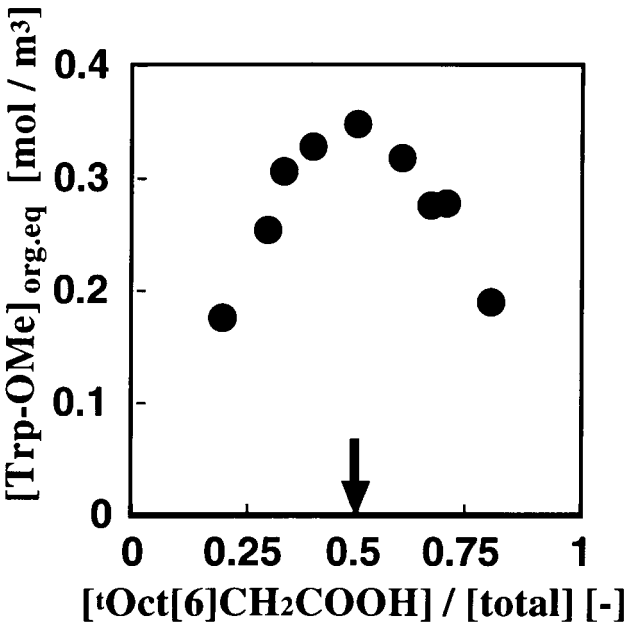

Figure 5. Job's plot of complexation between ^{t}Oct[6]CH_2COOH and Trp-Ome.

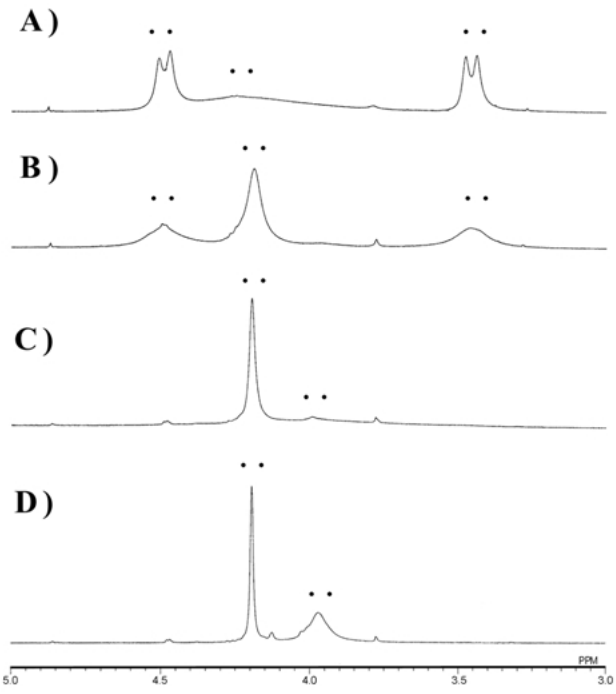

Figure 6. Partial ^{1}H NMR spectra of ^{t}Oct[6]CH_2COOH; 400 MHz, $(CDCl_2)_2$: (A) 30 °C, (b) 60 °C, (c) 90 °C, (d) 120 °C. ● and ○ denote the signals for $ArCH_2Ar$ and OCH_2CO, respectively.

where H_6R denotes ^{t}Oct[6]CH_2COOH, and HA^+ denotes a protonated amino acid ester.

The protonated amino group in the amino acid ester is well-fitted to the pseudocavity of ^{t}Oct[6]CH_2COOH [6], which is formulated by carbonyl groups. Furthermore, the C6 symmetry formed in ^{t}Oct[6]CH_2COOH is stereochemically favorable for interaction with the amino group of amino acids [16]. Therefore, ^{t}Oct[6]CH_2COOH accomplished a high recognition to Trp-OMe.

In contrast, the monomer analog ^{t}Oct[1]CH_2COOH cannot include an amino acid. The tetramer ^{t}Oct[4]CH_2COOH has a smaller cavity than that of ^{t}Oct[6]CH_2COOH and the structure is not of C_3 symmetry. This means that it is difficult to include an amino acid in the cavity. These inconveniences cause a lower extractability for ^{t}Oct[1]CH_2COOH and ^{t}Oct[4]CH_2COOH. Furthermore, calix[8]arene ^{t}Oct[8]CH_2COOH, which has a little larger cavity to include the amino acid, is inferior to calix[6]arene in terms of the strict recognition of the amino acid, and its (C_8) symmetry is also unfavorable for interacting with the protonated amino group. Therefore, the extractability of ^{t}Oct[8]CH_2COOH was shown to be next to ^{t}Oct[6]CH_2COOH. It was confirmed that the specific interaction between ^{t}Oct[6]CH_2COOH and the NH_3^+ group was essential for the strict recognition of amino acid esters.

Conformational properties of calix[6]arene hexacarboxylic acid and the geometry of the complex from ^{1}H NMR study

Recently, some researchers have reported the recognition of organic amines using calix[6]arene esters as receptors [4], and discussed how the guest cation is recognized. The recognition of primary amines by calix[6]arenes is often related to the ammonium cation – crown ether complex system [6]. These host compounds have an ideal structure and symmetry to form tripodal hydrogen bonding with the protonated ammonium group. In addition, two complexation geometries have been reported in previous papers: *endo*-calix complex, which is formed by inclusion of a guest cation into the cavity of calixarene, and *exo*-calix complex, which is formed without inclusion of a guest molecule into the cavity (see Ref. 4a). Here, the complex structure was directly observed by ^{1}H NMR to get information about the complexation mechanism.

Initially, the conformational property of ^{t}Oct[6]CH_2COOH was investigated by ^{1}H NMR measurement. Calix[6]arene is known to possess eight possible conformational isomers [1c, 17], but the conformations are not so rigid due to the rotation of the phenyl units; except for several calix[6]arene derivatives, which were immobilized by the multi-point bridging at the lower rim [18]. Furthermore, the most stable conformation of calix[6]arenes differs from the functional groups introduced at the lower rim, and a guest complexation sometimes causes a conformational change. For instance, a *p*-tert-butylcalix[6]arene hexaester, which has a 1,2,3-alternate conformation in the absence of a metal cation, changes to a cone conformation by complexation with a K^+ ion [19]. Thus, attention needs to be paid to the original conformation of the extractant, which might change by the entrapment of an amino acid. Figure 6 shows the result of ^{1}H-NMR measurement for the $ArCH_2Ar$ methylene proton of ^{t}Oct[6]CH_2COOH (3-5 ppm) in the absence of the guest cation from 0 °C to 120 °C (the spectra were observed in $(CDCl_2)_2$). For ^{t}Oct[6]CH_2COOH, the ^{1}H NMR spectrum at 30 °C was just consistent with that of the regular cone conformation (a pair of doublets for the ArCH2Ar proton). These results were different from that of the calix[6]arene hexaester derivative

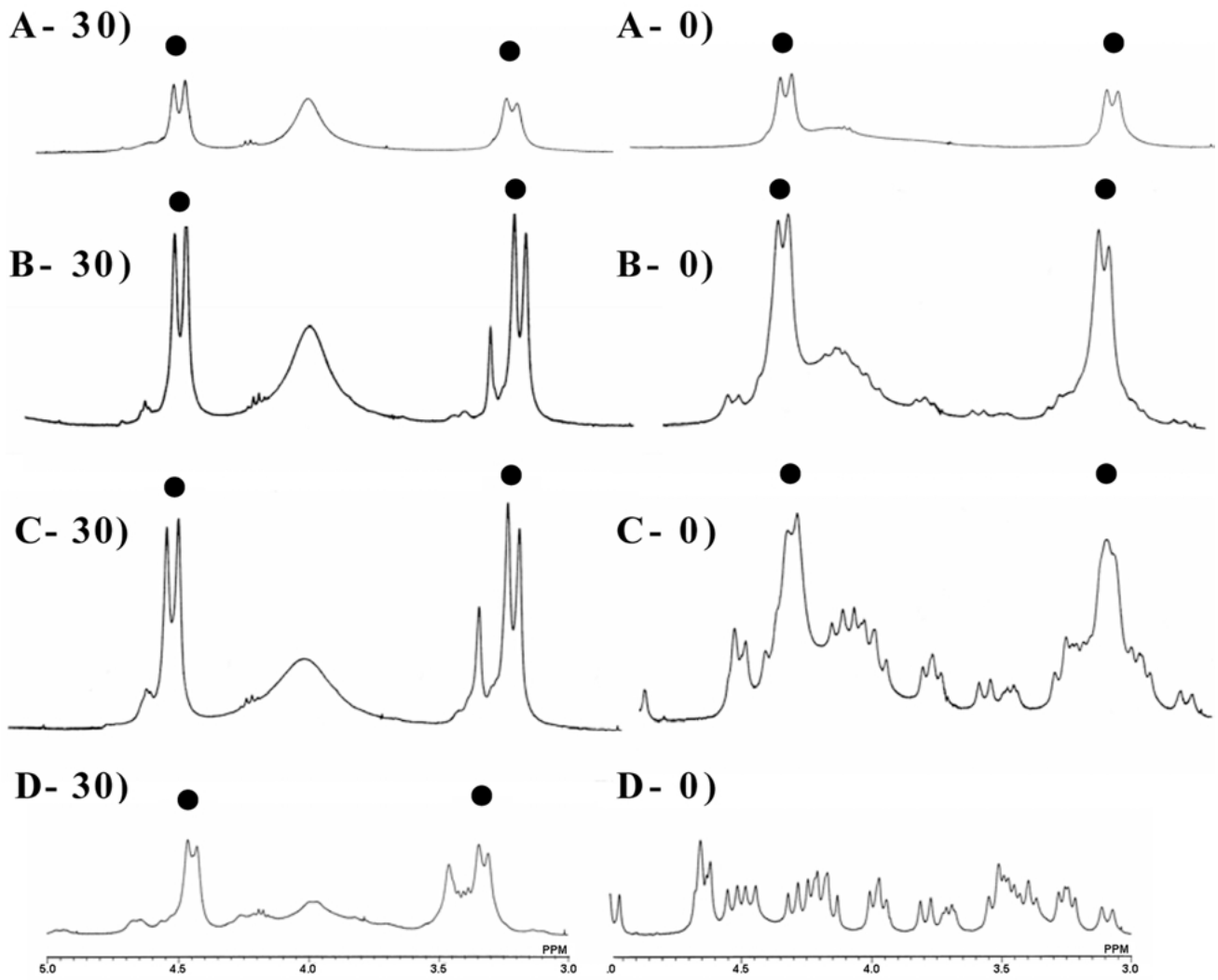

Figure 7. ^{1}H NMR spectra of the complex between ^{t}Oct[6]CH$_2$COOH and Trp-OMe: 3 ~5 ppm, 400 MHz, $CDCl_3$: X-30; 30 °C, X-0; 0 °C [^{t}Oct[6]CH$_2$COOH] : [Trp-OMe] = 1 : 0 (A-30, A-0), 1 : 0.4 (B-30, B-0) 1 : 0 (C-30, C-0), 1 : 10 (D-30, D-0). ●, ArC$\underline{H}_2$Ar.

that takes a 1,2,3-alternate conformation. It was deduced that six carboxyl groups in ^{t}Oct[6]CH$_2$COOH provide an intermolecular hydrogen bonding network to make the cone structure the most stable conformation, while the ArCH$_2$Ar methylene proton was coalesced at 90 °C, suggesting that the conformation of ^{t}Oct[6]CH$_2$COOH is not immobilized.

Figure 7 shows ^{1}H NMR spectra for the ArCH$_2$Ar methylene proton of the ^{t}Oct[6]CH$_2$COOH—Trp-OMe complex (3–5 ppm) at 30 °C and 0 °C, varying the Trp-OMe loading ratio. When the complexation was observed at 0 °C (A-0, B-0, C-0, D-0 in Figure 7), the new complicated signals, which were assigned to the ^{t}Oct[6]CH$_2$COOH—Trp-OMe complex, appeared to be different from the original guest-free ^{t}Oct[6]CH$_2$COOH. All the host molecules of ^{t}Oct[6]CH$_2$COOH contributed to the complex formation, when Trp-OMe was excessively present (Figure 7, D-0; Trp-OMe was 10 equiv.). On the other hand, when the sample was observed at 30 °C (Figure 7, A-30, B-30, C-30, D-30), the ArCH$_2$Ar resonance spectrum showed a broadened pattern. It is noted that the ArCH$_2$Ar proton signal (Figure 7, D-30) retains a pair of doublets after complexation. That is, ^{t}Oct[6]CH$_2$COOH maintains the cone configuration after the complexation with Trp-OMe.

The partial ^{1}H—^{1}H COSY spectrum (3–5.5 ppm) was also measured to assign in detail the complicated resonance for the complex (the sample in Figure 7, D-0). As shown in Figure 8, the complicated signals for the complex were independently coupled with each other. This result indicates that the methylene protons of ArCH$_2$Ar take a magnetically unequivalent state by including the guest amino acid ester retaining the cone conformation.

An asymmetrization model for complexation with Trp-OMe is shown in Figure 9. ^{t}Oct[6]CH$_2$COOH forms a 1 : 1 complex with Trp-OMe, which is included into the cavity. ^{t}Oct[6]CH$_2$COOH retains the cone conformation and its carboxyl groups electrostatically interact with the protonated amino group. Inclusion of Trp-OMe into the cavity would cause (i) a strain on the macrocyclic structure and (ii) an interconversion of the phenol unit ascribed to steric hindrance, as well as (iii) specific host–guest interaction (e.g., $\pi - \pi$ interaction) to the host compound. Consequently, ^{t}Oct[6]CH$_2$COOH whose original structure

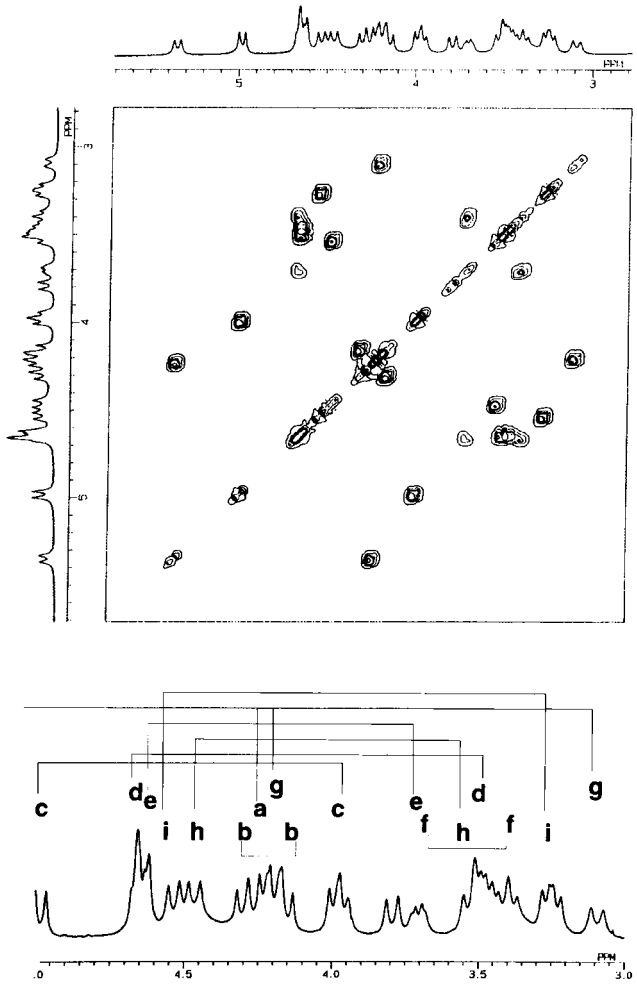

Figure 8. ^{1}H—^{1}H COSY spectrum and assignment of coupling for ^{t}Oct[6]CH_2COOH—Trp-Ome complex (3–5.5 ppm, 0 °C).

is of C_6 symmetry would change the asymmetrical structure. The structure of calix[6]arene is not rigid, therefore the structure for the complex looks symmetrical on the ^{1}H–NMR spectrum at 30 °C. At a low temperature (0 °C) where molecular dynamics are suppressed, the asymmetrization of calixarene could be observed. Based on the result, we confirmed that Trp-OMe was included deep into the calixarene cavity (*endo*-complex), because such an asymmetrization would not be observed if the complex took the *exo*-form.

Induced CD generated by the asymmetrization of host compound included amino acids

Recently, Morozumi and Shinkai have established a technique to investigate the chirality of quaternary ammonium compounds and amino acids using CD spectroscopy [10a, 20]. This is based on the phenomenon that when a chiral guest is included in the cavity of water-soluble calix[n] arenes (1n), the benzene array of 1n is deformed asymmetrically reflecting the chirality and the shape of the guest molecule. As the result, induced circular dichroism (ICD) spectra are observed.

In a former section, we interpreted that the complicated ^{1}H-NMR spectrum for the complex is observed from the asymmetrization of the calix[6]arene including the amino acid ester. If the host compound becomes asymmetric by the complexation, the induced CD spectra of calixarene should appear. With this expectation, the ^{t}Oct[6]CH_2COOH–(*L*- or *D*-)Leu-O^{t}Bu (leucine tert-butyl ester) complex was prepared in chloroform and the CD spectrum was measured. Leu-O^{t}Bu is spectroscopically silent in the measurement region, therefore if an induced CD is observed, the CD activity should have been originated from the host molecule.

The CD spectra are shown in Figure 10. Mirror spectra for ^{t}Oct[6]CH_2COOH in chloroform were observed by complexing with *L*- or *D*-Leu-O^{t}Bu. These results support the asymmetric rearrangement of ^{t}Oct[6]CH_2COOH that is generated by including a chiral amino acid into the cavity. The symmetric spectra for *L*- and *D*-guests suggest that each isomer is deformed in a reciprocal manner.

Conclusions

Inclusion phenomena of amino acid esters by a cyclic ligand calixarene were investigated in a liquid–liquid extraction system. The hexamer calix[6]arene introduced carboxylic acids were found to be one of the best host compounds currently available for the recognition of tryptophan ester. The high affinity being created by the fit between the cavity of the cyclic ligand and the guest molecule. The stoichiometry and the structure of the calix[6]arene-tryptophan ester complex was elucidated by systematical studies of the solvent extraction and by spectroscopic analysis. The host compound formed a 1 : 1 complex with a guest amino acid, and asymmetrization was induced by including a chiral guest. The induced CD spectrum of calixarene, which is generated by including a chiral guest, is interesting for the detection of the chiral structure for spectroscopically-silent guest molecules.

The calix[6]arene carboxylic acid is expected to be a novel tool for recognition of various biomolecules that have an amino group. Solvent extraction of nucleosides, dopamine, and other valuable amines by calix[6]arene are now under way. Calix[n]arenes enable the solubilization of various biomolecules in an organic medium, and this property will create a new function for biomolecules.

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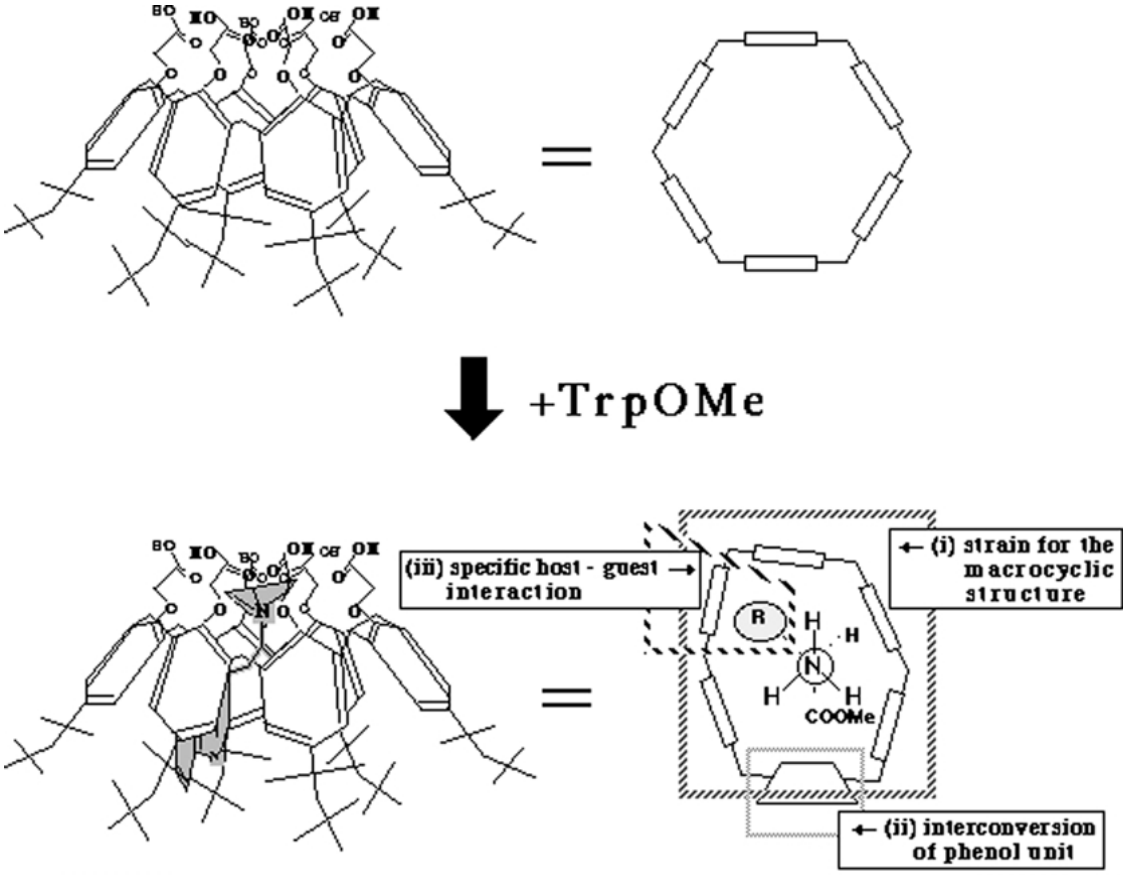

Figure 9. Asymmetrization of ^{t}Oct[6]CH_2COOH by including Trp-Ome into the cavity.

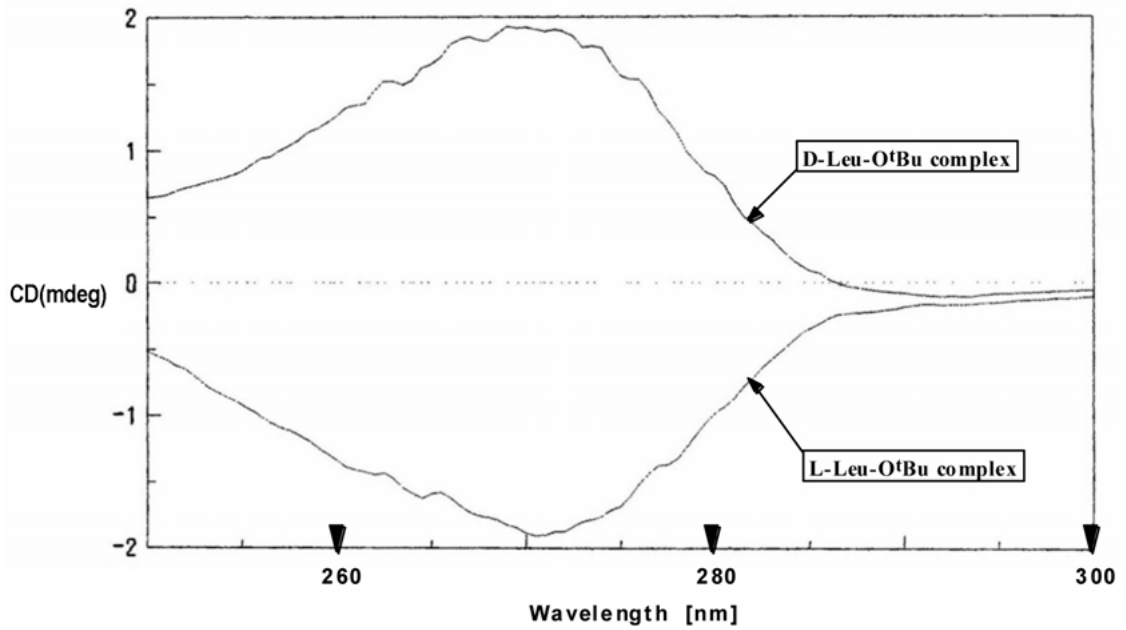

Figure 10. ICD spectra of ^{t}Oct[6]CH_2COH complexed with *L*- and *D*-Leu-O^{t}Bu at 25 °C.

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