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Catalytic Specificity Exhibited by *p*-Sulfonatocalix[*n*]arenes in the Methanolysis of *N*-Acetyl-L-amino Acids

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Specific acid catalysis of *p*-sulfonatocalix[*n*]arenes ($n = 4$, Calix-S4; $n = 6$, Calix-S6; $n = 8$, Calix-S8) was observed in the alcoholysis of *N*-acetyl-L-amino acids in methanol. The methanolysis rates of basic amino acid substrates (His, Lys, and Arg) were markedly enhanced in the presence of Calix-S*n*, as compared with rates observed with *p*-hydroxybenzenesulfonic acid (pHBS), which is a noncyclic analogue of Calix-S*n*. This catalytic effect of Calix-S*n* was not observed for the methanolysis of Phe, Tyr, and Trp substrates. On the other hand, ^{1}H NMR experiments following the effect of Calix-S*n* on *N*-acetyl-L-amino acid substrates in CD_3OD showed that the spectrum of a mixture of the His substrate with Calix-S*n* was significantly different from the combined spectra of the respective compounds. These changes in spectra support the formation of an inclusion complex of Calix-S*n* with basic amino acids. Furthermore, it was obvious that methanolysis of the His substrate catalyzed by Calix-S4 and Calix-S6 obeyed Michaelis−Menten kinetics. These results indicate that the catalytic activity of Calix-S*n* originates from its forming a complex with specific substrates (basic amino acids), similar to enzymatic reactions.

Introduction

One of the principal subjects in biomimetic chemistry has been the creation of artificial enzymes having high catalytic activity and specificity comparable to those of native enzymes. In particular, stereochemical control has been recognized as a very important subject. It has attracted considerable attention in connection with understanding the origins of catalytic specificity in proteolytic enzymes and in creating corresponding artificial enzymes. In the course of our study of esterase models, remarkably stereospecific catalysis was observed in the hydrolysis of amino acid and/or dipeptide esters carried out by functional molecular assemblies composed of surfactants and catalytic species. We emphasized that stereochemical control could be established by changing amino acid residues that were covalently introduced into substrates and catalysts (reactants),[1−9] by changing the composition of coaggregates (reaction fields),[1,2,9−15] and by regulating the temperature,[1,2,10,11,16−20] pH,[2,5] and ionic strength[11,17,21−24] of the reaction media. In particular, the authors attained almost complete L-enantioselective catalysis.[11,17,21−23] This can be attributed to optimization in the enzyme model conformation in the coaggregate systems by controlling the reaction micro-

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environment.[1,5,11,25] Furthermore, since cyclodextrins have been utilized as useful enzyme mimics,[26−32] we employed cyclodextrins as host molecules in the enantioselective[33] and diastereoselective[34] hydrolysis of amino acid and dipeptide esters. Significant stereoselective catalysis was obtained in the hydrolysis of specific substrates mediated by γ-cyclodextrins.

Calixarenes[35−38] are macrocyclic oligomers of *para*-substituted phenolic residues bridged by methylene groups. They are also useful host molecules for efficient enzyme models. Considerable effort has been devoted over the past decade to developing methods to synthesize functionalized derivatives, and to describing the conformational characteristics and inclusion properties of calixarenes as host molecules toward various guest molecules in solution.[39] Nevertheless, little has been discovered regarding the enzyme-like catalytic system of calixarenes.[40−42] To extend our study of enzyme models, we have examined the catalytic effects of *p*-sulfonatocalix[*n*]arenes (Calix-S*n*), which were originally synthesized as "water-soluble" calixarenes by Shinkai et al.,[40,42−45] for the hydrolysis of (*Z*)-amino acid esters in buffered solution. However, catalytic activity of Calix-S*n* was not observed, and the hydrolysis rates of the amino acid esters decreased in the presence of Calix-S*n* (see the Supporting Information). On the other hand, the enzyme mimetic catalysis by Calix-S*n* in the alcoholysis of amino acids in methanol was characterized.[46] Recently, it became obvious that the Calix-S*n* catalysis could be analyzed using Michaelis−Menten kinetics.

In this study, we report successful specific acid catalysis by Calix-S*n* ($n = 4$, Calix-S4; $n = 6$, Calix-S6; $n = 8$, Calix-S8) in the methanolysis of various *N*-acetyl-L-amino acids (Phe, Tyr, Trp, His, Lys, and Arg). The reactions were monitored by kinetic and ^{1}H NMR measurements. The calixarenes and amino acid substrates employed in this study are listed in the following section.

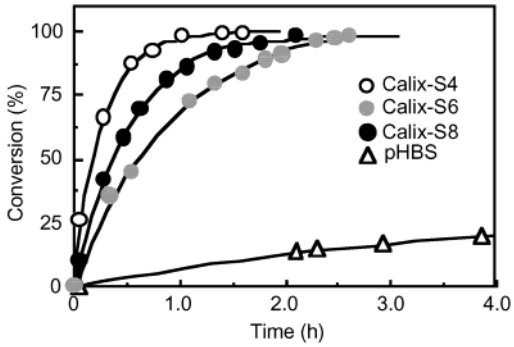
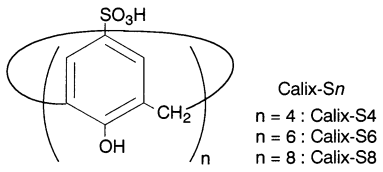
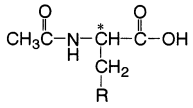
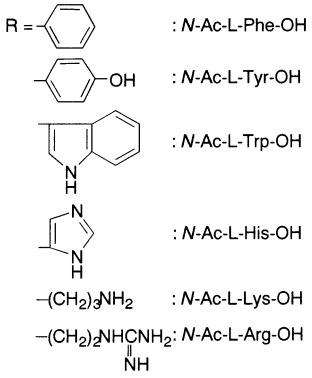


FIGURE 1. Time courses of the methanolysis of *N*-Ac-L-His-OH in the presence of pHBS or Calix-S*n* at 25 °C. [*N*-Ac-L-His-OH] = 1.0×10^{-4} M, [pHBS] = 3.0×10^{-2} M, [Calix-S4] = 7.5×10^{-3} M, [Calix-S6] = 5.0×10^{-3} M, and [Calix-S8] = 3.75×10^{-3} M.





N-Acetyl-L-amino Acid



Results and Discussion

Methanolysis of *N*-Acetyl-L-amino Acids in the Presence of Calix-S*n*. To investigate the catalytic effects of Calix-S*n* on the alcoholysis of amino acids, Calix-S*n* or pHBS, which is a noncyclic analogue of Calix-S*n*, was added to methanol solutions of various *N*-acetyl-L-amino acids (Phe, Tyr, Trp, His, Lys, and Arg). Examples of the time courses for methanolysis of *N*-Ac-L-His-OH in the presence of Calix-S*n* or pHBS are shown in Figure 1. All of the reactions obeyed pseudo-first-order kinetics. However, the methanolysis rates of *N*-acetyl-L-amino acids were extremely slow in the absence of Calix-S*n* or pHBS, so the apparent second-order rate constant (k_2), in the concentration unit of the sulfonate group of

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TABLE 1. Apparent Second-Order Rate Constants (k_2) for the Methanolysis of *N*-Acetyl-L-amino Acids in the Presence of pHBS and Calix-S*n*[a]

catalyst	substrate	k_2 (M^{-1} h^{-1})	$k_2^{Calix-Sn}/k_2^{pHBS}$
pHBS	*N*-Ac-L-Phe-OH	15	
	N-Ac-L-Tyr-OH	17	
	N-Ac-L-Trp-OH	20	
	N-Ac-L-His-OH	1.4	
	N-Ac-L-Lys-OH	1.7	
	N-Ac-L-Arg-OH	1.2	
Calix-S6	*N*-Ac-L-Phe-OH	14	0.93
	N-Ac-L-Tyr-OH	17	1.0
	N-Ac-L-Trp-OH	17	0.85
	N-Ac-L-His-OH	33	24
	N-Ac-L-Lys-OH	20	12
	N-Ac-L-Arg-OH	25	16
Calix-S4	*N*-Ac-L-His-OH	120	86
	N-Ac-L-Lys-OH	20	12
	N-Ac-L-Arg-OH	19	16
Calix-S8	*N*-Ac-L-His-OH	57	41
	N-Ac-L-Lys-OH	95	56
	N-Ac-L-Arg-OH	20	17

[a] Conditions: 25 °C, [*N*-Acetyl-L-amino Acid] = 1.0×10^{-4} M, [pHBS] = 3.0×10^{-2} M, [Calix-S6] = 5.0×10^{-3} M, [Calix-S4] = 7.5×10^{-3} M, [Calix-S8] = 3.75×10^{-3} M.

Calix-S*n* or pHBS, was evaluated according to eq 1. The

$$k_2 = k_1/[\text{sulfonate unit}] \quad (1)$$

rate constants in the presence of pHBS or Calix-S*n* are summarized in Table 1.

Each rate constant for the methanolysis of *N*-Ac-L-Phe-OH, *N*-Ac-L-Tyr-OH, and *N*-Ac-L-Trp-OH in the presence of Calix-S6 was almost identical with that in the presence of pHBS ($k_2^{Calix-S6}/k_2^{pHBS}$ values are nearly 1). Therefore, there was no significant difference between the Calix-S6 and pHBS catalysts in the methanolysis of these amino acids. In contrast, the methanolysis rates of *N*-Ac-L-His-OH, *N*-Ac-L-Lys-OH, and *N*-Ac-L-Arg-OH were enhanced in the presence of Calix-S6 relative to rates measured in the presence of pHBS ($k_2^{Calix-S6}/k_2^{pHBS} = 12-24$). Interestingly, catalytic rates for the basic *N*-acetyl-L-amino acids in the presence of pHBS were significantly smaller than those seen for pHBS plus the other three substrates. The rate constants for methanolysis of the basic amino acids His, Lys, and Arg in the presence of Calix-S4 and Calix-S8 are shown in Table 1. All of these rate constants were also greater than those measured in the presence of pHBS. In particular, the methanolysis of *N*-Ac-L-His-OH was most dramatically accelerated by Calix-S4 ($k_2^{Calix-S4} = 120$ M^{-1} h^{-1}, $k_2^{Calix-S4}/k_2^{pHBS} = 86$). These results suggest that the catalytic activity of Calix-S*n* depends on the structure of the substrates and that Calix-S*n* acts as an efficient catalyst in the methanolysis of basic amino acid substrates.

^{1}H NMR Spectroscopic Studies. ^{1}H NMR measurement is one of the most useful means for studying molecular interactions in solution. Thus, ^{1}H NMR measurements were undertaken to explore the catalytic properties of Calix-S*n* in the methanolysis of basic amino acids. Figures 2 and 3 show the ^{1}H NMR spectra of *N*-Ac-L-His-OH in the presence of pHBS and Calix-S4, respectively, in CD_3CD at room temperature. With respect to the spectra of *N*-Ac-L-His-OH, the chemical shifts of the C2 and C4 protons of the imidazole (Im) ring were

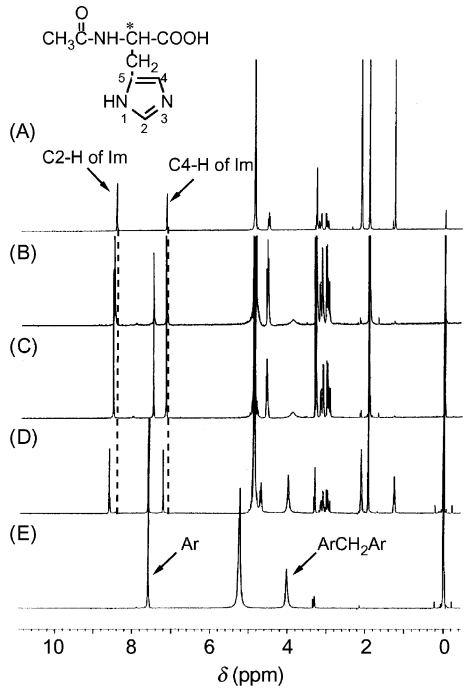


FIGURE 2. ^{1}H NMR spectra of *N*-Ac-L-His-OH alone (A) and in a mixture with Calix-S4 (B−D) and of Calix-S4 alone (E) in CD_3OD at room temperature. [*N*-Ac-L-His-OH]:[Calix-S4] = 1:0.025 (B), 1:0.05 (C), and 1:0.25 (D).

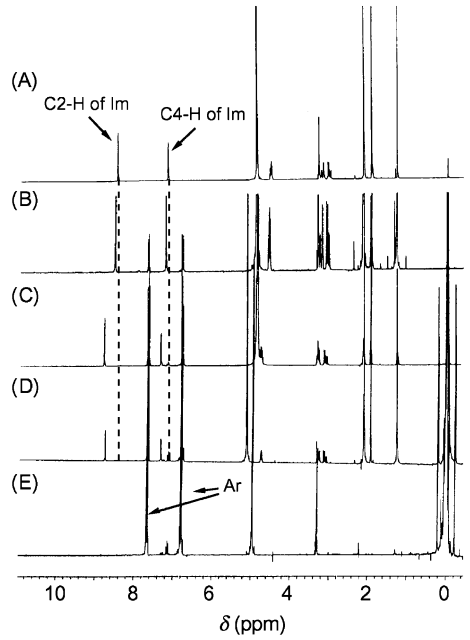


FIGURE 3. ^{1}H NMR spectra of *N*-Ac-L-His-OH alone (A) and in a mixture with pHBS (B−D) and of pHBS alone (E) in CD_3OD at room temperature. [*N*-Ac-L-His-OH]:[pHBS] = 1:0.1 (B), 1:1 (C), and 1:4 (D).

observed to shift to lower magnetic fields in the presence of pHBS or Calix-S4. These types of spectral changes were not observed for the mixtures of *N*-Ac-L-Phe-OH, *N*-Ac-L-Tyr-OH, or *N*-Ac-L-Trp-OH with Calix-S*n*. The shifts to lower magnetic fields were apparently caused by protonation of the imidazole nitrogen with sulfonate protons of pHBS or Calix-S*n*. Figure 4 shows the chemical shifts for the C2 and C4 protons as a function of the

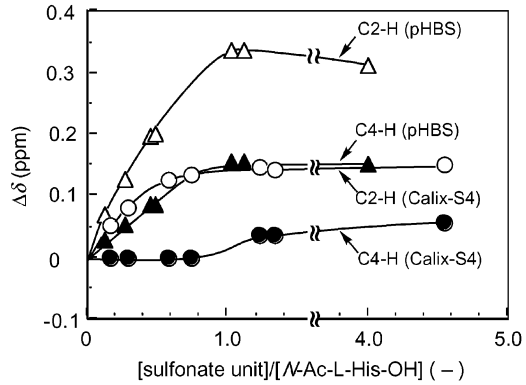
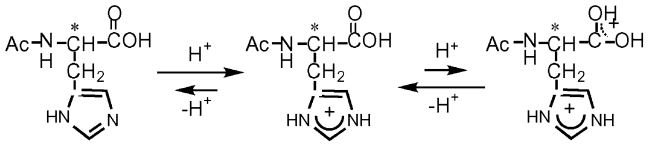


FIGURE 4. Chemical shifts of C2−H and C4−H of the imidazole ring of *N*-Ac-L-His-OH as a function of the [sulfonate unit]/[*N*-Ac-L-His-OH] ratio in CD_3OD at room temperature. $\Delta\delta = \delta$(*N*-Ac-L-His-OH + Calix-S4 (or pHBS)) − δ(*N*-Ac-L-His-OH).

SCHEME 1



[sulfonate unit]/[*N*-Ac-L-His-OH] ratios. The $\Delta\delta$ values increased along with the increasing ratios in both systems, but the values in the presence of Calix-S4 were smaller than in the presence of pHBS. On the other hand, for the spectra of Calix-S4, the $ArCH_2Ar$ methylene proton signal of Calix-S4 was broadened in the presence of an excess amount of *N*-Ac-L-His-OH (Figure 2B,C). These observations may be attributable to the inclusion of *N*-Ac-L-His-OH in the Calix-S4 cavity; that is, the fixed conformation led to broadening of the methylene peak.[38,40,44] It is plausible that the imidazole moiety of *N*-Ac-L-His-OH not only was protonated but also experienced a ring current effect due to its proximity to Calix-S4, so that the chemical shifts to lower magnetic fields were less pronounced relative to those for pHBS.

It is well-known that the acid-catalyzed methanolysis of carboxylic acids proceeds via formation of several cationic intermediates. In the methanolysis of *N*-Ac-L-Phe-OH, *N*-Ac-L-Tyr-OH, and *N*-Ac-L-Trp-OH with Calix-S*n* and pHBS as catalysts, these sulfonic acids would act as "simple" acid catalysts. On the other hand, in the methanolysis of *N*-Ac-L-His-OH and other basic amino acids, the imidazole or amino groups in the side chains seem to be protonated, and the resulting positive charge may prevent the formation of cationic intermediates, as shown in Scheme 1, so that the rate enhancement is even smaller than that seen for other substrates in the presence of pHBS. In contrast, it is noteworthy that the methanolysis rate of basic amino acids was extremely enhanced in the presence of Calix-S*n*. Calix-S*n* seems to promote complex formation with basic amino acids and to thereby induce stabilization of cationic intermediates with the anionic sulfonate groups.

Kinetic Analysis Based on Michaelis−Menten Principles. The ^{1}H NMR study suggested that specific catalysis by Calix-S*n* proceeds via complex formation with basic amino acid substrates. Therefore, we attempted to analyze the catalysis of Calix-S*n* on the basis of Michaelis−Menten kinetics. The effect of Calix-S4, Calix-S6, and pHBS concentrations on the pseudo-first-order rate constant k_t for the methanolysis of *N*-Ac-L-His-OH was examined, and the results are shown in Figure 5. With respect to pHBS, the k_t value increased linearly along with the concentration of pHBS. The apparent catalytic constant k_{H^+} for the methanolysis by pHBS, obtained from the slope, was 1.8 M^{-1} h^{-1}. On the other hand, k_t values for Calix-S4 and Calix-S6 increased rapidly, almost reaching saturation above [sulfonate unit] = 0.03 M (the corresponding concentration of Calix-S*n*, [Calix-S4] = 7.5×10^{-3} M, [Calix-S6] = 5.0×10^{-3} M). These kinetic behaviors are typical for enzymatic catalysis, so the data were treated by kinetic analysis on the basis of Michaelis−Menten principles[32] (Scheme 2). Under the experimental conditions of [catalyst] ≫ [substrate], the observed first-order rate constant $k_{t,Calix-Sn}$ for the methanolysis in the presence of Calix-S*n* is given by

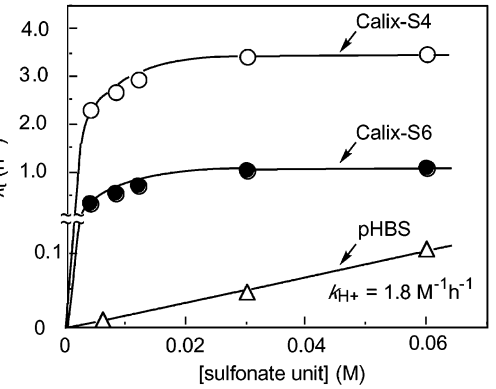
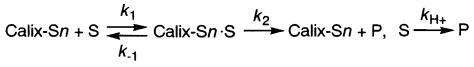


FIGURE 5. Concentration dependence of Calix-S*n* and pHBS in the unit of the sulfonate group on the pseudo-first-order rate constants (k_t) for the methanolysis of *N*-Ac-L-His-OH.

SCHEME 2



$$k_{t,Calix-Sn} = (k_{t,RSO_3H} + K_b k_2[\text{Calix-S}n])/(1 + [\text{Calix-S}n]) \quad (2)$$

$$k_{t,RSO_3H} = k_{H^+}[RSO_3H] \quad (3)$$

$$K_b = k_1/k_{-1} = [\text{Calix-S}n\cdot\text{S}]/([\text{Calix-S}n][\text{S}]) \quad (4)$$

Here, k_{t,RSO_3H} is the pseudo-first-order rate constant for acid-catalyzed methanolysis measured in the presence of pHBS. The binding constant K_b of the Calix-S*n*·substrate complex and the rate constant k_2 for the complexed substrate were determined by the least-squares method from a modified Lineweaver−Burk plot between $1/(k_{t,Calix-Sn} - k_{t,RSO_3H})$ and 1/[Calix-S*n*], as shown in eq 5. The plots

$$1/(k_{t,Calix-Sn} - k_{t,RSO_3H}) = 1/k_2 + 1/(K_b k_2[\text{Calix-S}n]) \quad (5)$$

of $1/(k_{t,Calix-Sn} - k_{t,RSO_3H})$ against 1/[Calix-S*n*] show straight lines (Figure 6), indicating that the catalysis of Calix-S*n*

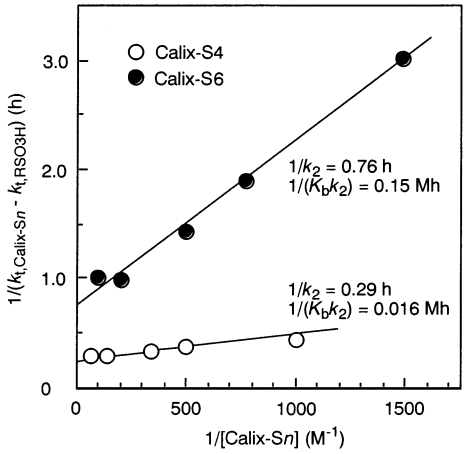


FIGURE 6. Lineweaver−Burk plots for the methanolysis of *N*-Ac-L-His-OH catalyzed by Calix-S4 and Calix-S6.

TABLE 2. Kinetic Parameters for the Methanolysis of *N*-Ac-L-His-OH Catalyzed by Calix-S*n*[a]

catalyst	K_b (M^{-1})	k_2 (h^{-1})	k_2K_b (M^{-1} h^{-1})
Calix-S4	1.8×10^3	3.5	6.3×10^3
Calix-S6	5.1×10^2	1.3	6.6×10^2

[a] Conditions: 25 °C, [*N*-Ac-L-His-OH] = 1.0×10^{-4} M, [Calix-S4] = $1.0 \times 10^{-3} \sim 1.5 \times 10^{-2}$ M, [Calix-S6] = $6.7 \times 10^{-4} \sim 1.0 \times 10^{-2}$ M.

proceeds via the formation of a 1:1 complex, and the binding constant and rate constant obtained from the linear relationship are presented in Table 2. The binding constant and rate constant for Calix-S4 were 1.8×10^3 M^{-1} and 3.5 h^{-1}, while the values for Calix-S6 were 5.1×10^2 M^{-1} and 1.3 h^{-1}, respectively. The binding affinity (reflected by K_b) and reaction activity (reflected by k_2) for the Calix-S4 are 3.5-fold and 2.7-fold larger than those for Calix-S6, respectively. The differences in catalytic activity between Calix-S4 and Calix-S6 in the methanolysis of *N*-Ac-L-His-OH depend on both binding and reaction processes, and the analysis clarified that Calix-S4 forms a more stable and reactive complex with *N*-Ac-L-His-OH as compared with Calix-S6. The ring size of the Calix-S*n* species plays an important role in their catalytic activity. Calixarenes adopt some preferred conformaitons depending on their ring size; for example, rigid calix[4]arenes (cyclic tetramers) adopt a "cone" conformation, and the more flexible calix[6]arenes (cyclic hexamers) adopt "winged" or "hinged" conformations.[35,36,40] It is likely that the conformation and cavity size of Calix-S4 are more amenable to complex formation with *N*-Ac-L-His-OH, and as shown schematically in Figure 7, such a stereochemical arrangement may induce stabilization of the cationic intermediates with the anionic sulfonate groups.

Conclusions

(a) With respect to the methanolysis of *N*-acetyl-L-amino acids catalyzed by Calix-S*n* in methanol, the methanolysis rate of basic amino acids (His, Lys, and Arg) was enhanced in the presence of Calix-S*n* as compared with that in the presence of its noncyclic analogue pHBS. It was particularly notable that a marked rate enhancement for the methanolysis of His substrates was obtained in the presence of Calix-S4. (b) ^{1}H NMR results following the reaction of *N*-acetyl-L-amino acids and Calix-S*n* in CD_3OD revealed that the spectrum of the mixture of the His substrate plus Calix-S*n* was significantly different from the combined spectra of the respective compounds. These changes in the spectra suggest the formation of an inclusion complex of Calix-S*n* with basic amino acids. (c) Methanolysis of the His substrate catalyzed by Calix-S4 and Calix-S6 obeyed Michaelis−Menten kinetics. The results show that the high catalytic activity of Calix-S4 in the methanolysis of His substrates derives from both binding and reaction processes.

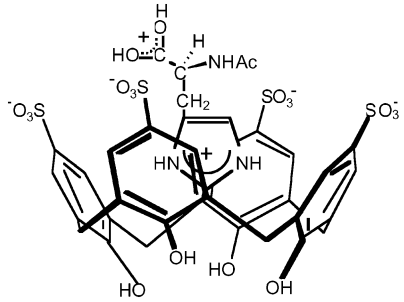


FIGURE 7. A plausible mechanism for the complexation of Calix-S4 with *N*-Ac-L-His-OH.

This is the first example of a marked substrate-specific catalysis by calixarenes in the alcoholysis of amino acids. It is significant that the catalysis of calixarenes proceeds via complex formation with specific substrates, and that the accompanying stereochemical rearrangements may induce stabilization of the reaction intermediates as in catalysis by native enzymes.

Experimental Section

Materials. Calix-S*n*[40,42−45] was obtained commercially and used after purification by cation-exchange column chromatography and drying over P_2O_5 in a vacuum desiccator for 24 h. The water content of Calix-S*n* was determined by thermogravimetry/differential thermal analysis (TG/DTA) measurements. pHBS and all of *N*-acetyl-L-amino acids were obtained commercially and used without further purification.

Kinetic Measurements. Methanolysis rates for *N*-acetyl-L-amino acids were monitored by measuring the yield of ester products on an HPLC column at room temperature. Typical conditions were as follows: gel-packed column (4 mm $\phi \times$ 250 mm); eluent, CH_3CN/H_2O (2:8 (v/v)); flow rate, 0.50 mL/min; detector, UV (212 nm). Under the conditions of [Calix-S*n* (or pHBS)] $\gg$ [*N*-acetyl-L-amino acid], the reaction followed a pseudo-first-order rate law with the methanolysis rate constant (k_1) calculated by eq 6, where S_t and S_∞ denote peak areas of ester formation at time t and at infinite time, respectively.

$$\log(S_\infty - S_t) = -k_1 t/2.303 + \log S_\infty \qquad (6)$$

^{1}H NMR Measurements. ^{1}H NMR spectra in CD_3OD were measured at room temperature (using the internal standard TMS) with a 270 MHz NMR apparatus. The concentration of *N*-Ac-L-His-OH was maintained constant (5.0×10^{-2} M) while that of Calix-S4 or pHBS was varied.

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Supporting Information Available: Kinetic results of the hydrolysis of (*Z*)-amino acid (Ala, Leu, Phe, Trp, and Lys) esters in buffered solutions, ^{1}H NMR spectra of the Lys ester and pHBS or Calix-S8 in 15% (v/v) CD_3CN-D_2O, and ^{1}H NMR spectra of *N*-acetyl-L-amino acids (Phe, Tyr, Trp, and His) and Calix-S6 in CD_3OD. This material is available free of charge via the Internet at http://pubs.acs.org.

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