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Multi-Functionalized Chiral Crown Ethers as Enzyme Models for the Synthesis of Peptides. Multiple Chiral Recognition in the Enzyme Model

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Abstract. A novel approach to the enzyme model for the synthesis of peptides has been established by using multi-functionalized chiral crown ethers as hosts. The new strategy consists of three key steps as follows. (1) Guest assembly: the host having one free thiol and one thioester with *N*-protected α-amino acid or peptide proceeds via rapid intra-complex thiolysis of α-amino acid ester salts to form the dithioester, and assembles two guests. (2) Amide formation: the intramolecular aminolysis occurs between the bound guests to form the amide bond. (3) Peptide chain elongation: as the thiol reactive group is regenerated, the above two reactions are repeated to elongate the peptide chain. In the present paper, we describe the multiple chiral recognition that could be achieved by the chiral crown ether in both the intra-complex thiolysis and the intramolecular aminolysis. For explanation of the chiral recognition, we propose a likely structure for the intermediate of the aminolysis.

Key words. Chiral, crown ether, enzyme model, peptide synthesis, chiral recognition, molecular recognition, thiolysis, aminolysis, thiol, thioester.

1. Introduction

One of the most important studies in the area of molecular recognition is the design and synthesis of organic molecules as models of enzymes which catalyze useful synthetic reactions. Artificial macrocycles have been expected to work as hosts to assemble guests into the host reactive sites by forming non-covalent host–guest complexes [1]. Recently, much attention has been paid to a new type of host which can assemble plural guests in a host cavity, since such a host may effect the mutual proximity between the bound guests and accelerate the synthetic reaction between them. Thus, hosts bearing multiple binding sites for plural guests have been designed [2], but few of them have been successfully applied to useful synthetic reactions.

We recently reported a novel enzyme model for the synthesis of peptides by using the multi-functionalized chiral 18-crown-6 derivatives [3]. The new hosts have achieved the assembly of plural guests by covalent bonds formed through non-covalent complexes between the host and the guest, and then enhanced the bond formation between the bound guests. This enzyme model has mimicked the general concept of enzyme catalysis, in which the reactive enzyme-substrate covalent intermediate ($E \sim\!\sim S_1$) is formed from the noncovalent complex ($E \cdot S_1$), and then reacts with the second substrate (S_2) to give the product ($S_1—S_2$) as shown in Equation (1) [4].

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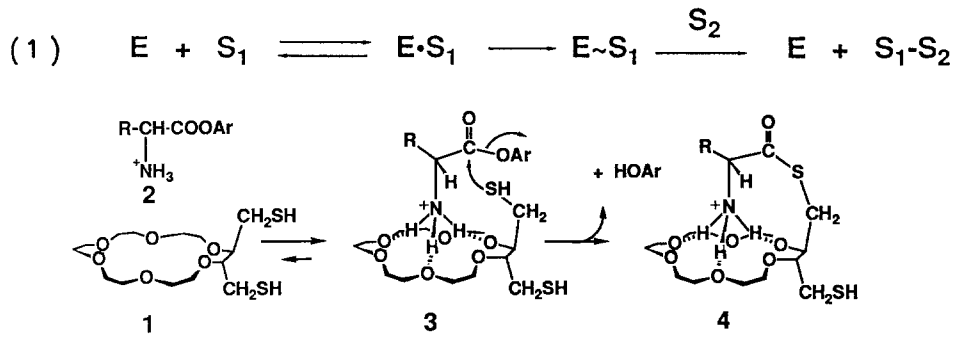
2. Design

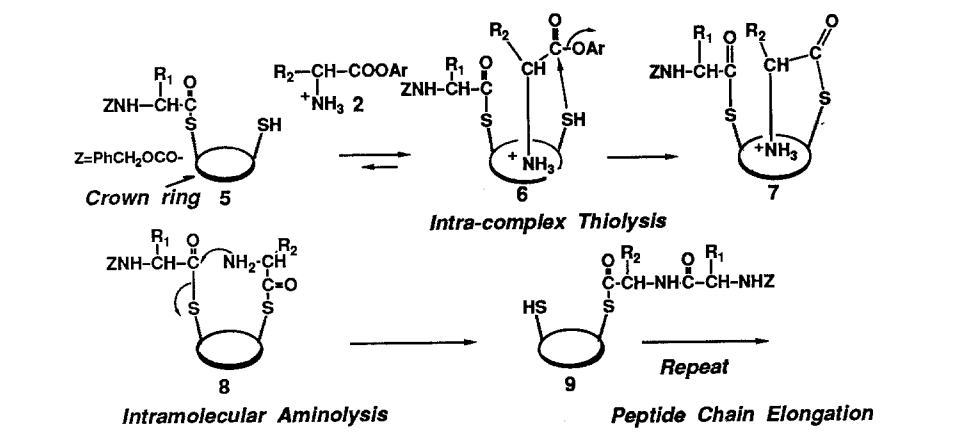
2.1. STRATEGY

It was already reported that thiol-bearing chiral crown ethers of type **1** showed rate enhancements in the thiolysis of α-amino acid *p*-nitrophenyl ester salts, due to the intra-complex nature of the reaction, forming the corresponding thioester as shown in Scheme 1 [5]. Evaluating the thioester as the reactive intermediate for nucleophile

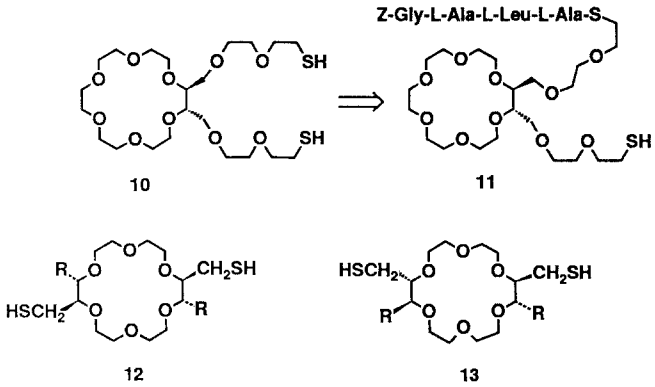


Scheme 1. Thiol protease model.

[6], a strategy for enhancement of the reaction with the second substrate was designed as shown in Scheme 2. This enzyme model contains three key steps as follows. (1) The host (**5**) having one free thiol and one thioester with *N*-protected α-amino acid or peptide can form a non-covalent complex with the guest (**2**) and undergoes intra-complex thiolysis to form another thioester (**7**). (2) After neutralization of **7**, intramolecular aminolysis occurs in the host **8** to form the amide bond (**9**). (3) As the thiol reactive group is regenerated in **9**, the above two reactions can be repeated to elongate the peptide chain. We have directed this study toward achievement of actual catlaytic activity for peptide elongation, as a final goal for the enzyme model.



Scheme 2. Enzyme model for peptide synthesis.



2.2. PREVIOUS RESULTS

In an earlier study, we pointed out some important aspects of aminolysis of thioester in our enzyme model. First, the fastest rate for aminolysis of thioester was obtained in the presence of equimolar amounts of acid and base catalysts. Second, the reaction proceeded in aprotic nonpolar solvents such as benzene, ethyl acetate, dichloromethane, and so on [7]. Thus, the peptide syntheses by the enzyme model have been performed in benzene buffered with equimolar amounts of pivalic acid and triethylamine as acid and base catalysts, respectively. Third, the superiority of intramolecular aminolysis over an intermolecular one was clearly demonstrated, despite the large membered cyclic intermediate expected for the intramolecular reaction. The host **10** could achieve the synthesis of the tetrapeptide derivative (**11**) by formal turnover of the intra-complex thiolysis and the intramolecular aminolysis, but its efficiency as an enzyme model has remained to be improved [3].

2.3. DESIGN OF THE NEW HOSTS

The *syn*-type host **12** used in the present study was designed so as to improve host efficiency, to have reactive groups on the same face (*syn*-orientation of the reactive groups), and opposite side of the crown ring. The *anti*-type host (**13**), having reactive groups on the opposite face (*anti*-orientation of the reactive groups), was used for comparison with **12**. These hosts were synthesized by an unambiguous method starting from L-tartaric acid. We expected the following two improvements for **12**. (1) In the intra-complex thiolysis step, **12** was expected to exhibit a larger rate acceleration than **10**, since the closer proximity between the ester of the guest and the thiol of the host was expected because of the short arm of the reactive group as shown in **14** vs. **15**. (2) In the intramolecular aminolysis step, **12** might afford better proximity between nucleophilic amine and electrophilic thioester, as shown in **16** vs. **17**. Substituents, R = *o*-$MeOC_6H_4OCH_2$, were introduced in order to keep the thiomethyl reactive groups perpendicular to the crown plane in the host–guest complex [5c].

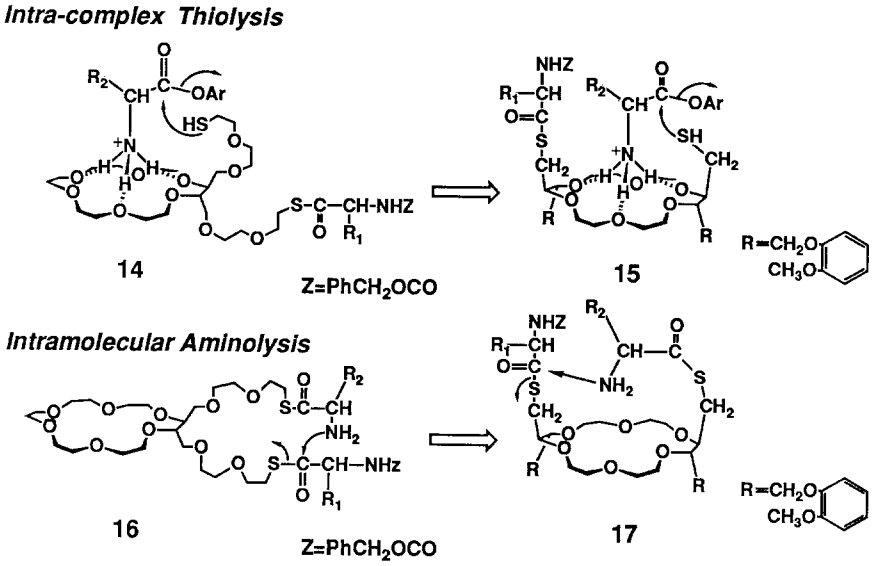


Fig. 1. Design of new host.

3. Intra-complex Thiolysis

3.1. RATE ENHANCEMENT OF THIOLYSIS BY CROWN HOSTS

Rate enhancements of thiolysis by thiol-bearing crown ethers were compared by kinetic experiments, using **18** [5a] as the reference host with long side arms, and the results are summarized in Table I. As shown in the column of enhancement ratio, large rate enhancements by the crown ethers have been clearly demonstrated in comparison with the rate constants by the acyclic dithiol (**19**), (entry 2~4 vs. 1). Particular advantages of the mercaptomethyl reactive group of **12** and **13** over the reactive group with the long side arm of **18** were shown by the fact that an enhancement ratio of only 5 was obtained by **18** but rates of more than 150 were obtained by the hosts **12** and **13** in the thiolysis of D-phenylalanine guest (entry 6~12).

3.2. CHIRAL RECOGNITION BY CROWN HOSTS

As shown in the column of D/L ratio in Table I, **12** and **13** showed chiral recognition by the factors of $k_D/k_L = 4.5$, 4.0, and 5.2 for phenylalanine guest by **12**, **13**, and **20**, respectively. The reaction by **20**, which has a free thiol and a thioester with *Z*-glycine, exhibited high chiral recognition with the ratio of 10 for valine guest. In contrast, **18** showed no chiral recognition. Here, superiority of the structure of the thiomethyl reactive group of **12** over that of **18** has been clearly demonstrated in the intra-complex thiolysis. It seems reasonable that the *syn*-type **12** and the *anti*-type **13** showed the same preference for D-guests, because both hosts have the same chirality of the thiomethyl reactive groups.

The previous study showed that chiral recognition by the chiral crown ethers occurs not in the complex formation but in the intra-complex thiolysis, and that

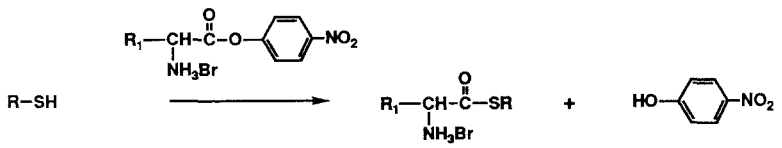
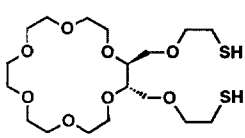


Table I. Chiral recognition in the intra-complex thiolysis[a]

Entry	Host	Guest (R_1=)	k_ψ, 10^{-4} s^{-1}	Enhancement ratio[b]	D/L Ratio
1	19	H	5.2	1	
2	18	H	340	65	
3	12	H	244	47	
4	13	H	198	38	
5	19	L-CH_2Ph	0.65		
6	19	D-CH_2Ph	0.73	1	1.2
7	18	L-CH_2Ph	3.9		
8	18	D-CH_2Ph	3.4	5.2	1.2
9	12	D-CH_2Ph	25		
10	12	D-CH_2Ph	114	156	4.5
11	13	L-CH_2Ph	29		
12	13	D-CH_2Ph	115	158	4.0
13	20	H	170		
14	20	L-CH_3	260		
15	20	D-CH_3	780		3.0
16	20	L-CH_2Ph	30		
17	20	D-CH_2Ph	160		5.2
19	20	L-$CH_2(CH_3)_2$	2.4		
20	20	D-$CH_2(CH_3)_2$	24		10

[a] Intra-complex thiolysis was carried out in CH_2Cl_2-EtOH (95:5) buffered with 0.02 M pyridine and 0.01 M AcOH (pH = 5.4 in H_2O) at 25°C, using 5 mM of host (10 mM of 20, entry 13–20) and 0.1 mM of guest, and released *p*-nitrophenol was followed photometrically at 320 nm.
[b] Enhancement ratios were calculated using the rate constants by 19 for glycine guest (entry 2–4) and phenylalanine guest (entry 7–12) as standard values.



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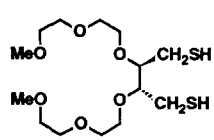

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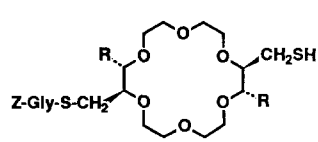
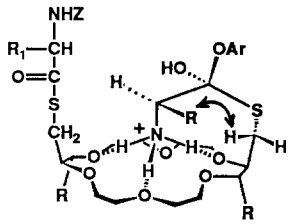


20

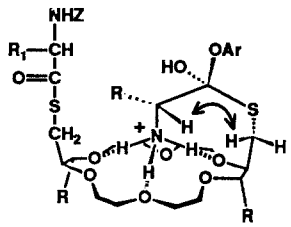
conformational restriction of the thiomethyl reactive group in the tetrahedral intermediates seems to play an important role in the chiral recognition [5c]. Such plausible tetrahedral intermediates of the intra-complex thiolysis are depicted in Figure 2, where the conformation of the thiomethyl was assumed to be *anti* to the adjacent C—C bond of the crown ring [7]. In the intermediate with the L-guest, there seems to be large steric repulsion between the hydrogen of the thiomethyl group and the α-substituent of the guest as depicted by the arrow in **21**. By contrast, in the intermediate with the D-guest, such steric repulsion between two hydrogens seems to be much smaller as depicted in **22**. Thus, the faster thiolysis of D-guests may be explained by the existence of a more stable tetrahedral intermediate with D-guests.



21

With L-guest

(Less stable)



22

With D-guest

(More stable)

Fig. 2. Tetrahedral intermediates in thiolysis.

3.3. SELECTIVE MONOACYLATION OF THE HOSTS THROUGH THE INTRA-COMPLEX THIOLYSIS

The enhanced thiolysis has been applied to the selective monoacylation of the hosts. The thiolyses of *p*-nitrophenyl ester salts of α-amino acids were performed in slightly acidic solution (CH_2Cl_2 buffered with pyridine–acetic acid, pH = 5.0) to form the monoacylated host selectively. Protection of the amine could be achieved cleanly and rapidly by using carbobenzyloxychloride in the presence of pyridine. The results are summarized in Table II. The intra-complex thiolysis could be also applied to the formation of dithioester under the similar thiolysis condition, to give the desired dithioesters rapidly as shown in Table III. It should be noted that **20** exhibited the largest reaction rate for D-alanine guest and almost the same rates for glycine and D-phenylalanine guests, in both kinetic and preparative experiments.

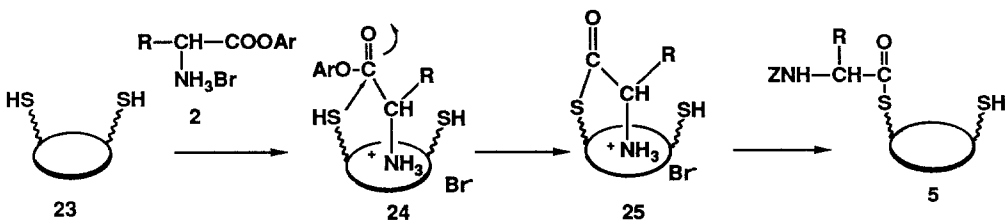


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Table II. Monoacylation of the hosts through the intra-complex thiolysis

Parent Host	Guest (R=)	Thiolysis Time (min)	Yield of 5 (%)
12	H	60	76
13	H	60	71
12	L-CH_3	<10	81
12	D-CH_3	<10	68

Thiolysis was performed in CH_2Cl_2 buffered with 10 mM each of pyridine and acetic acid at room temperature using 2 mM each of host and guest. Protection was carried out in CH_2Cl_2 in the presence of 0.03 M each of pyridine and carbobenzyloxy chrolide and 0.01 M of host at room temperature for 1 hour.

Table III. Dithioester formation by thiolysis (**5** → **6** → **7**)

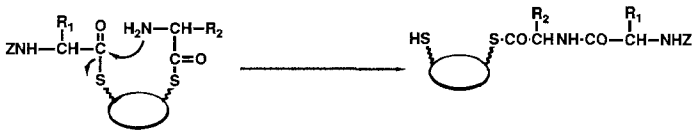
Parent Host	Guest R_1=	R_2=	Time (min)	Yield of 7 (%)
12	H	D-CH_3	10	81
12	H	L-CH_3	10	83
12	H	D-CH_2Ph	60	80
12	H	L-CH_2Ph	60	82

The thiolysis was performed in CH_2Cl buffered with 20 mM of pyridine and 10 mM of acetic acid using 2 mM each of host and guest at room temperature

4. Intramolecular Aminolysis

4.1. CHIRAL RECOGNITION IN THE INTRAMOLECULAR AMINOLYSIS

The intramolecular aminolysis was carried out in benzene buffered with pivalic acid and triethylamine, which function as general acid and base catalysts in aminolysis. The reaction rates were examined by quantitative analysis of the disappearance of the starting host (**8**), and results are compared in Table IV. The aminolysis by using **12** as a parent host, *Z*-glycine as the electrophilic guest, and D-alanine as the nucleophilic guest showed the fastest reaction rate so far obtained. The same reaction using L-alanine as the nucleophilic guest gave a much smaller reaction rate by the factor of $k_D/k_L = 4.8$. In the case of the reaction using phenylalanine as the nucleophilic guest, a chiral recognition factor of $k_D/k_L = 4.2$ was obtained. It is interesting that *syn*-type **12** showed the common preference for D-guest in both the intra-complex thiolysis and intramolecular aminolysis. Although *anti*-type **13** showed chiral selection in the thiolysis, chiral recognition was not observed in the aminolysis.



8 R_1=H **9**

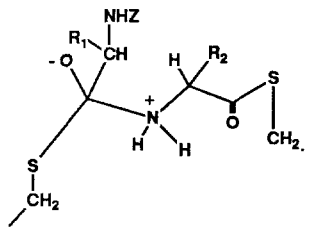
Table IV. Chiral recognition in intramolecular aminolysis

Parent Host	R_2	k, 10^{-3} min^{-1}	$t_{1/2}$ (hr)	D/L Ratio
12	D-CH_3	11.3	1.0	4.8
12	L-CH_3	2.4	4.8	
12	D-CH_2Ph	4.2	2.8	4.2
12	L-CH_2Ph	1.0	11.6	
13	D-CH_3	4.9	2.4	
13	L-CH_3	4.7	2.4	1.0

The intramolecular aminolysis was carried out in benzene buffered with 0.15 M each of triethyl amine and pivalic acid at 27°C using 1 mM of host, and the disappearance of 8 was quantitatively followed by HPTLC.

4.2. LIKELY STRUCTURE FOR THE INTERMEDIATE OF AMINOLYSIS

For consideration of chiral recognition by the *syn*-type **12**, the stability of the dipolar tetrahedral structure (**26**) was considered as the rate-determining intermediate for aminolysis of thioester [8]. The tetrahedral intermediate (**26**) formed across



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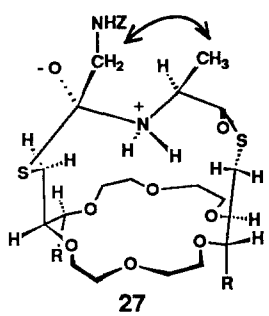
Dipolar tetrahedral intermediate of aminolysis

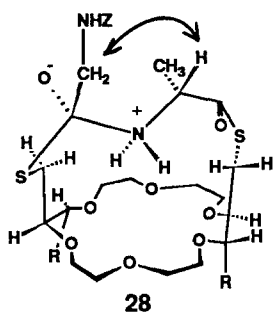
the crown ring seems to have significant conformational restriction, first by the tetrahedral nature of the ammonium cation, and second as a result of the conformational preference of thiomethyl groups, such as is discussed in the section on intra-complex thiolysis. Taking account of such conformational restriction, **27** and **28**, which have a common structure except the chirality of the nucleophilic guests, were proposed as likely structures for tetrahedral intermediates from investigation using molecular models. In these structures, all tetrahedral centers are thought to have a *gauche* relationship with the adjacent center. A structure, in which the residue of the electrophilic thioester is supposed to take a position close to the methylene of the thiomethyl group, should suffer larger steric repulsion than **27** and **28**. Using **27** and **28** as likely structures for intermediates, differences in stability between the two intermediates formed from L- and D-guests may be explained as follows. In the intermediate with L-guest (**27**), there seems to exist steric repulsion between the α-substituent of the nucleophilic guests and the methylene of the glycine unit. In contrast, such steric repulsion seems to be smaller in the intermediate with a D-guest (**28**) as depicted by the arrow.



27

The syn type host with L-guest

(Less stable)

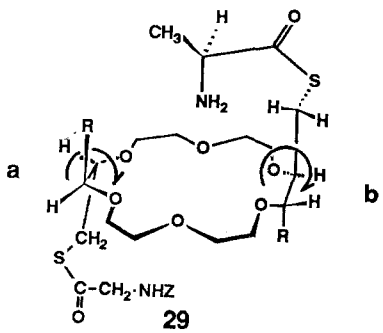


28

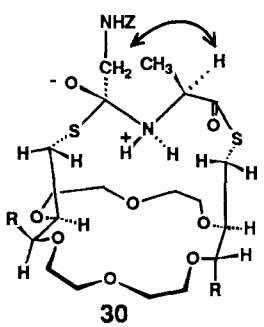
The syn type host with D-guest

(More stable)

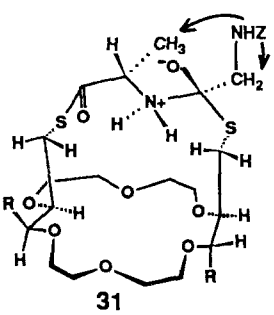
The same assumption on the conformation of the dipolar intermediates was also applied to the reaction of the *anti*-type host. Rotation around a C—C bond of the crown ring is needed so that the amine nucleophile can attack the thioester as shown in **29**. In the case of the aminolysis by L-alanine as the nucleophilic guest, rotations around the C—C bonds of the crown ring as depicted by **a** or **b** give two different intermediates such as **30** and **31**, respectively. The similar steric restriction of the tetrahedral intermediate as discussed above was applied to that of the *anti*-type host. In **31** there seems to be steric repulsion, as depicted by the arrow, whereas such repulsion seems to be much smaller in **30**. In the case of the intermediates with a D-guest, similarly, both less and the more stable structures may be formed. Thus, the *anti*-type host may form stable intermediates with both D- and L-guests, resulting in no chiral recognition.



The anti-type host with L-guest



More stable intermediate



Less stable intermediate

The fact that *syn*-**12** gave only double the reaction rate compared with *anti*-**13** showed that the conformational orientation of the thiomethyl reactive groups, that was expected to be advantageous in the intramolecular aminolysis for *syn*-type host, was not efficient enough to assemble the two reactive groups into proximity. We expect that design of a more efficient host may be facilitated from the insight into the structures for the intermediates of the aminolysis.

5. Conclusion

As an approach to the enzyme model for a synthetic reaction, we have established a new strategy by using multi-functionalized chiral crown ethers. Our enzyme model has the characteristics of a model for catalysis of a synthetic reaction in that the host has a single binding site and two reactive sites for plural guests and assembles the guests by covalent bonds formed through the intra-complex reaction. The present study has revealed that the chiral host could achieve multiple chiral recognition in both the intra-complex thiolysis and the intramolecular aminolysis, and that we could assume likely structures for the intra-complex thiolysis and intramolecular aminolysis as well. The achievement of the multiple chiral recognition has suggested the possibility of constructing a new type of enzyme model which catalyzes the synthesis of optically active peptide by using racemic guests.

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