# Biomimetic Studies Using Artificial Systems. IV.[1] Biomimetic Peptide Synthesis by Using Multi-Functionalized Crown Ethers as a Novel Enzyme Model. A New Concept in Mimicking of Enzyme-Catalyzed Bond-Forming Reactions[2]

Shigeki SASAKI and Kenji KOGA*

*Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.* *Received October 7, 1988*

**A novel approach to the mimicking of enzyme-catalyzed bond-forming reactions has been examined using multi-functionalized chiral crown ethers. In addition to the 18-crown-6 moiety as a binding site, the hosts have one thiol and one thio ester with an *N*-protected α-amino acid or a peptide, and have successfully achieved peptide synthesis in an enzyme-mimetic reaction mode. This new method involves the following three key reactions. (1) Intra-complex thiolysis: the host carries out the rapid intra-complex thiolysis of α-amino acid ester salts to form the dithioester, corresponding to the assembly of two guests by the host. (2) Amide formation: 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. Formal turnover of the enzyme model has been demonstrated by the synthesis of a tetrapeptide derivative by the repetition of the above processes.**

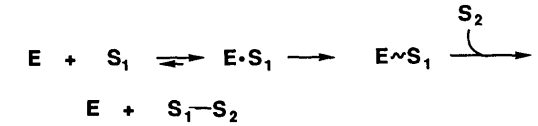
**Keywords** biomimetic synthesis; multifunctional crown ether; intra-complex thiolysis; peptide synthesis; enzyme model; intramolecular aminolysis; thio ester

During the last two decades, a variety of macrocycles have been synthesized, and their complex forming abilities have been widely investigated.[3] One of the major applications of such artificial macrocycles includes the design and synthesis of molecules which can accelerate reactions in an enzyme-mimetic reaction mode. Since the fascinating properties of enzymatic reactions, such as high reaction rate, high selectivity, and efficient catalysis with turnover, *etc.*, are facilitated by the formation of non-covalent Michaelis–Menten type complexes, an enzyme-mimetic approach has been expected to provide new strategies for performing efficient artificial reactions.

So far, such biomimetic studies have centered on the mimicking of transformation reactions such as hydrolysis,[4] acyl transfer[5,6] amino transfer,[7] hydride transfer,[8] and so on. Recently, enzyme analogues that can accelerate bond-forming reaction have become of great interest. One important approach to this problem has been to use hosts with multiple binding sites, expecting that such hosts will bring bound guests into close proximity and enhance the reaction between them. Thus, crown ethers,[9] cyclodextrins,[10] and cyclophanes[11] have been used as binding sites to construct such hosts, but few of them have been successfully applied to enzyme models for bond-forming reactions.

On the other hand, enzymes commonly employ a different strategy in that the enzyme binds one of the substrates with a covalent bond and transforms it to its activated form, then utilizes it for the subsequent bond-forming reaction with another substrate.[12] We have recently succeeded in mimicking this strategy of enzyme catalysis, performing biomimetic peptide synthesis by the use of multi-functionalized chiral crown ethers.[2] This enzyme-mimetic strategy has become recognized as a promising one to construct enzyme analogues for bond-forming reactions, as also seen in the successful results obtained by other groups.[13,14] In this paper, we report some of the details of our study on the enzyme model for the synthesis of peptides.

**Strategy** In the general concept of enzyme catalysis, the reactive covalent intermediate ($E\text{-}S_1$) is formed from the non-covalent complex ($E\cdot S_1$) and then reacts with the second substrate ($S_2$) to give the product ($S_1\text{-}S_2$) as shown in Eq. 1.[12] Characteristic of this mechanism is that the assembly of the plural guests is achieved at the enzyme active site in multiple steps. That is, both the formation of the covalent intermediate and its reaction with the second substrate are facilitated by the formation of the Michaelis–Menten complex. We tried to mimic this concept to design an enzyme analogue for peptide bond formation.




(1)

It has already been reported that thiol-bearing crown ethers of type **1** show rate enhancements in the thiolysis of α-amino acid *p*-nitrophenyl ester salts (**2**) to give the corresponding thioesters (**4**) due to the intra-complex nature of the reaction of **3**, as shown in Chart 1.[5] Based on the use of a thioester as the reactive intermediate for the amine nucleophile to form a peptide bond, a strategy to effect the assembly of the second guest was designed, employing intra-complex thiolysis, as shown in Chart 2. Thus, a novel crown ether of type **5** having one free thiol and one thioester with an *N*-protected α-amino acid or peptide was expected to work as an enzyme model for peptide synthesis.

We expected the following three steps to be involved. (i) Intra-complex thiolysis: crown ether **5** catches an α-amino acid *p*-nitrophenyl ester salt (**2**) by forming a noncovalent complex **6**. Subsequently, thiolysis occurs at an enhanced rate to give the corresponding dithio ester (**7**). Now, two substrates are forced into close proximity. (ii) Amide formation: intramolecular aminolysis ($S\rightarrow N$ acyl transfer) from **7** to **9** *via* **8** occurs at an enhanced rate, due to the intramolecular nature of the reaction, to form a peptide bond. (iii) Peptide chain elongation: as the thiol reactive group is regenerated in every cycle and the aminolysis product **9** is functionally equivalent to **5**, elongation of the peptide chain can be expected by repeating the above processes. This sequence of reactions resembles the biosynthesis of the natural cyclic peptide gramicidin S,[15] and was

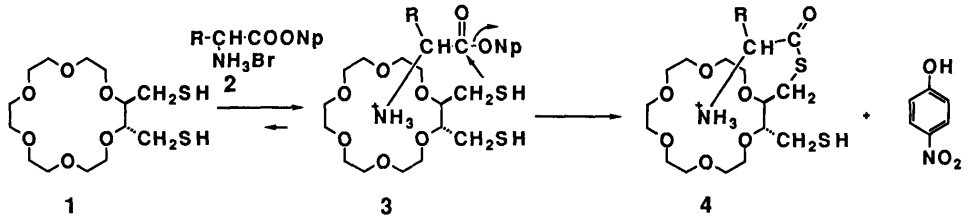


Chart 1

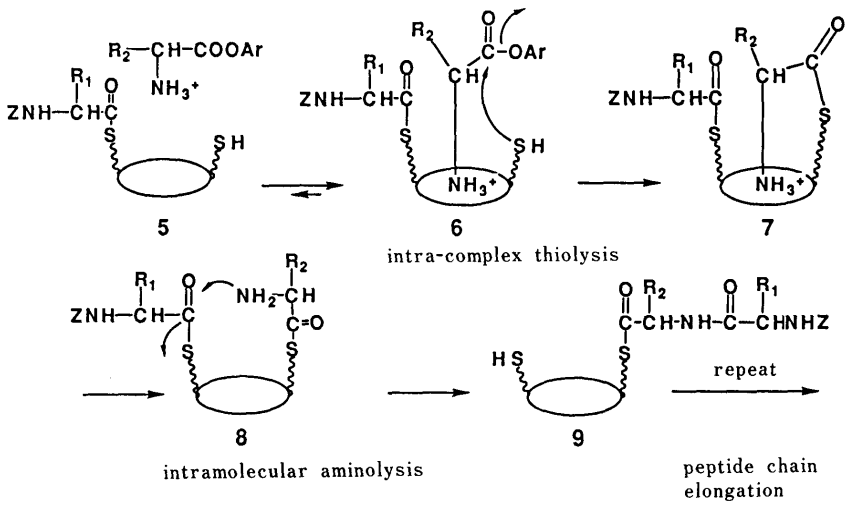


Chart 2. Enzyme Model for Peptide Synthesis

expected to perform formal turnover of the catalytic group.

It was anticipated that the intramolecular aminolysis would proceed through the large-membered cyclic intermediate because of the structural demands of the host with its 18-crown-6 unit, and that its efficiency would be the key feature of this biomimetic peptide synthesis.

## Results and Discussion

**Synthesis** The crown ethers (**1**, **10**, **11**) were prepared from the diol (**14**). Compounds **12** and **13** were obtained from the ditosylate (**16**). The acyclic reference compound **20** was synthesized from the dithiol (**19**), starting from the diol (**17**), as shown in Chart 3.

**Amide Bond Formation by Inter- and Intramolecular Aminolysis of Thioester** Since rate enhancement in intra-complex thiolysis has already been shown,[5] we started this project by establishing the advantage of the intramolecular aminolysis *via* a large membered cyclic intermediate over the intermolecular one. First of all, the effects of acid and base catalysts on the intermolecular aminolysis were investigated in the reaction between *N*-carbobenzyloxyglycine thioethyl ester (Z–Gly–SEt, **22**) and L-alanine ethyl ester (H–L-Ala–OEt, **23**) forming the corresponding dipeptide (Z–Gly–L-Ala–OEt, **24**). The aminolysis was performed by using pivalic acid and triethylamine as acid and base catalysts, respectively, in benzene at 20 °C.[16] The formation of the dipeptide **24** was followed by high performance liquid chromatography (HPLC), and the second-order rate constants were obtained at various acid and base concentrations, as recorded in Table I. The significant effects of acid and base catalysts on the aminolysis of the thioester may be summarized as follows. i) The reaction was enhanced in the presence of the acid catalyst (run 2 *vs.* 1). ii) An excess of acid decreased its catalytic

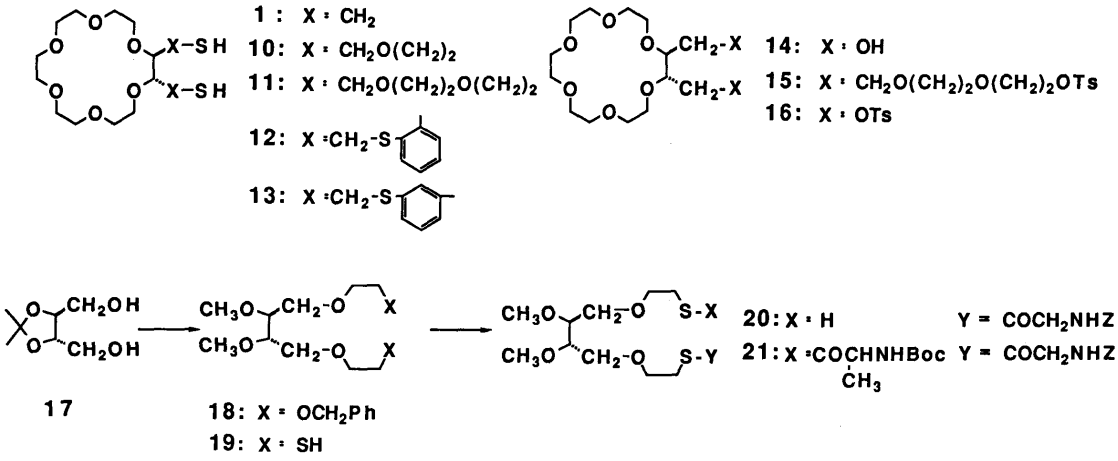
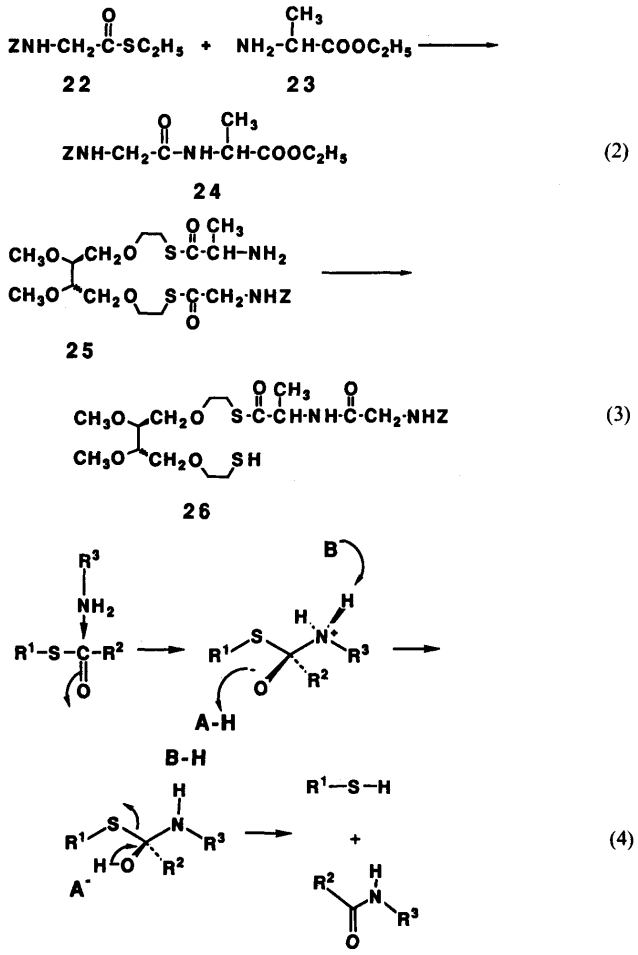


Chart 3

effect (run 4 *vs.* 3). iii) The reaction was further accelerated in the presence of base catalyst equimolar to the acid catalyst (run 6 *vs.* 3). iv) The rate constant was dependent on the concentration both of the acid and the base (run 9 *vs.* 10). These facts suggested that the aminolysis of the thioester includes a rate-determining process of protonation of and deprotonation from the polar tetrahedral intermediate as shown in Eq. 4.




Thus, inter- and intramolecular aminolyses to form the peptide bonds (**24** and **26**) were performed using 5 mM each of **22**, **23**, and **25** in benzene buffered with 5 mM each of pivalic acid and triethylamine at 20 °C. It was shown that the intramolecular aminolysis to form **26** was completed within 20 h, while about 10% of **24** was formed under the same conditions. The first-order ($k_1$) and the second-order ($k_2$) rate constants were obtained to be $k_1 = 3.36 \times 10^{-3}$ min$^{-1}$ and $k_2 = 0.0252$ M$^{-1}$ min$^{-1}$, respectively. The ratio of $k_1$ and $k_2$, which is regarded as an effective concentration of one functional group relative to the other in the intramolecular reaction,[17] was calculated to be 0.14 M, meaning that about thirty times greater effective concentration was obtained by this intramolecular aminolysis. Although the efficiency is not comparable to the intramolecular reaction *via* a 5- or 6-membered cyclic intermediate, this result has clearly demonstrated the advantage of the intramolecular aminolysis *via* the large-membered cyclic intermediate over the corresponding intermolecular reaction.

Table I. Effects of Acid and Base Catalysts on the Aminolysis of the Thioester[a]

| Run | *tert*-BuCOOH (M) | TEA (M) | $k_2 \times 10^3$ (M$^{-1}$ min$^{-1}$)[b] | Relative rate |
|---|---|---|---|---|
| 1 | 0 | 0 | 0.9 | 1 |
| 2 | 0.05 | 0 | 19 | 21 |
| 3 | 0.1 | 0 | 21 | 23 |
| 4 | 0.2 | 0 | 14 | 15 |
| 5 | 0.4 | 0 | 7.8 | 9 |
| 6 | 0.1 | 0.1 | 84 | 93 |
| 7 | 0.1 | 0.4 | 40 | 44 |
| 8 | 0.1 | 0.9 | 17 | 19 |
| 9[c] | 0.005 | 0.005 | 24 | 27 |
| 10[c] | 0.1 | 0.1 | 84 | 93 |

*a*) The aminolyses were performed by using 0.1 M each of **22** and **23** in benzene at 20 °C, and the formation of **24** was followed by HPLC by using acetanilide as an internal standard. *b*) Experimental errors were estimated to be less than 15% of recorded values. *c*) Concentrations of 5 mM each of **22** and **23** were used.

**Peptide Synthesis by Using Crown Ethers** Peptide formation *via* intramolecular aminolysis of the crown compounds was investigated by using the host of type **7** (Chart 2) with various structures of the side arm. The hosts having a Z-glycyl residue on the thiol and an L-alanyl residue on the other were synthesized as shown in Chart 4. The acylations of dithiols (**1**, **10**—**13**) were performed by using Z–Gly–OH and diethyl phosphorylcyanide (DEPC)[18] in the cases of **1**, **10**, and **11**, or by using Z–GLY–ON*p* in the cases of **12** and **13** to give mixtures of mono- (**27**—**31**) and bis(Z-glycyl)crown ethers (**32**—**36**). These mixtures were subject to intra-complex thiolysis with L-Ala–ON*p*·HBr in pyridine or acetone–pyridine to form mixtures of the unchanged hosts (**32**—**36**) and the desired dithio esters

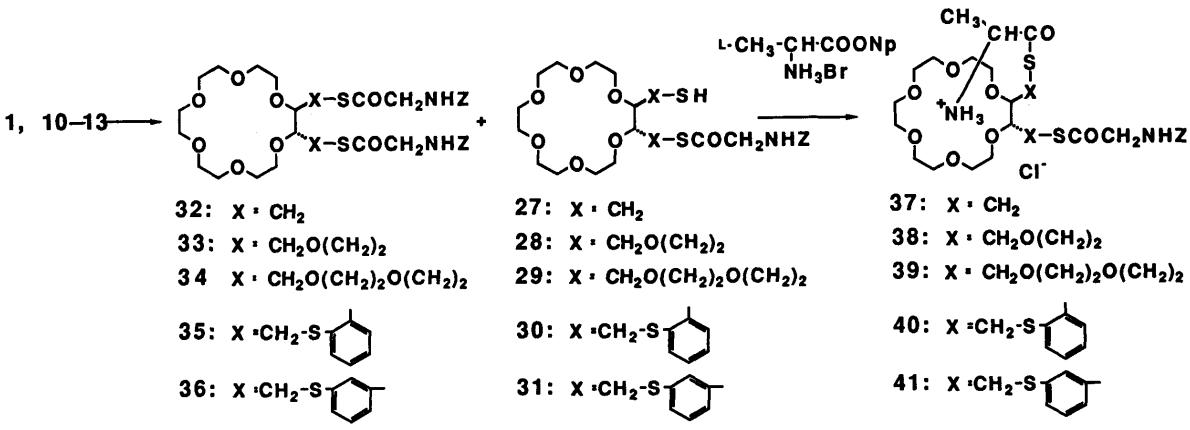
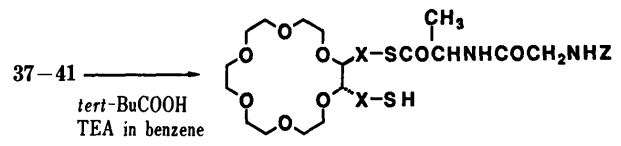



Chart 4




42: X = $CH_2$
43: X = $CH_2OCH_2CH_2$
44: X = $CH_2OCH_2CH_2OCH_2CH_2$
45: X = $CH_2$-S-
46: X = $CH_2$S-

Chart 5

TABLE II. Intramolecular Aminolysis Forming Dipeptide Derivatives

| Run | Host | Time (h) | Product | Yield (%) |
|---|---|---|---|---|
| 1 | **37** | 12 | — | 0[a] |
| 2 | **38** | 20 | **43** | 68 |
| 3 | **39** | 10 | **44** | 81 |
| 4 | **40** | — | — | 0[b] |
| 5 | **41** | — | — | 0[b] |

The reactions were performed in benzene at room temperature, using 5 mM host and 0.15 M each of triethylamine and pivalic acid. *a*) Compound **37** was recovered. *b*) Complete decomposition was observed.

(**37**—**41**). The mixtures were dissolved in aqueous hydrochloric acid, and the neutral bis(Z-glycyl)crown ethers (**32**—**36**) were extracted with a less polar organic solvent such as diethyl ether, then the hydrochlorides (**37**—**41**) were extracted with a polar organic solvent such as $CHCl_3$.[19]

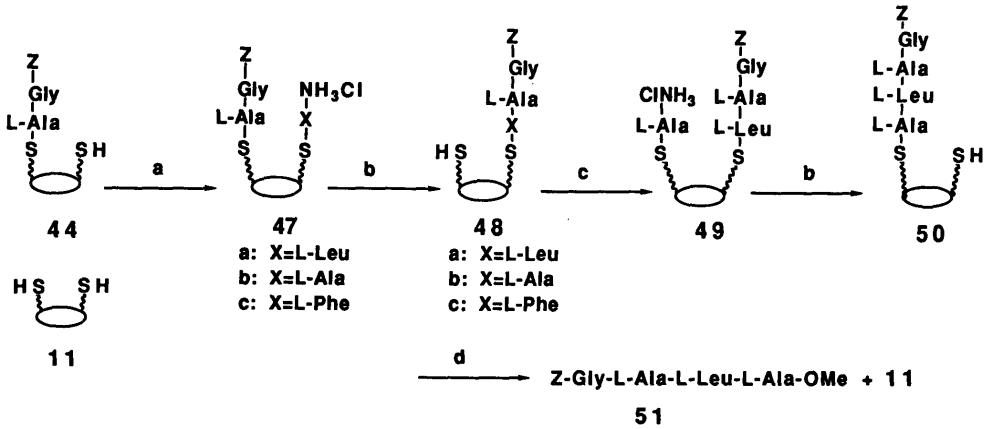
The intramolecular aminolysis of the hosts having various side arm structures to give the *S*-(Z-glycyl-alanyl)-crown ethers was examined as shown in Chart 5. The results are summarized in Table II. Only the hosts having long side arms (**38**, **39**) gave the desired aminolysis products **43** and **44** in 68% and 81% yields, respectively. The reactions of the hosts **42**, **45**, **46** did not take place, although **45** and **46** were expected to have more reactive arylthio esters rather than alkylthio esters. Probably, their cyclic intermediates were highly strained. The fact that the aminolysis reaction was much affected by the strain of the expected cyclic intermediate, coupled with the comparison between the inter- and intramolecular reaction, has shown that the amide bond formation by the host proceeded in the intramolecular reaction mode. At this stage of the study, the host **11** was shown to have the most suitable structure to check the formal turnover to achieve peptide chain elongation.

**Synthesis of Tri- and Tetrapeptide Derivatives by the Host** As a demonstration of formal turnover of our enzyme model for the synthesis of a peptide, the elongation of the peptide chain was achieved by repeating the intra-complex thiolysis and the intramolecular aminolysis as shown in Chart 6. *S*-(Z–Glycyl-L-alanyl)crown ether **44** obtained above underwent rapid intra-complex thiolysis with L-leucine *p*-nitrophenyl ester hydrobromide (L-Leu–ONp·HBr) in pyridine at room temperature for 30 min to give **47a** as the hydrochloride in 87% yield after extraction with $CHCl_3$ from the 1 N aqueous HCl solution. Then the intramolecular aminolysis was carried out in benzene at room temperature for 2 d to give *S*-(Z-glycyl-L-alanyl-L-leucyl)crown ether (**48a**) in 58% yield. Similarly, *S*-(Z-glycyl-L-alanyl-L-alanyl)crown ether (**48b**) and *S*-(Z-glycyl-L-alanyl-L-phenylalanyl)crown ether (**48c**) were obtained in 45% and 55% yields based on **44**, respectively. The corresponding tripeptide derivatives, Z–Gly–L-Ala–L-Leu–OMe, Z–Gly–L-Ala–L-Ala–OMe and Z–Gly–L-Ala–L-Phe–OMe, were isolated in 86%, 78%, and 49% yields based on **48a**, **b**, **c**, respectively, after methanolysis using $K_2CO_3$ as a base.

The thiolysis between **48a** and L-Ala–ONp·HBr was again performed in pyridine at room temperature for 30 min to give **49** in 82% yield as the hydrochloride. The subsequent intramolecular aminolysis of **49** in benzene–ethyl acetate at room temperature for 3 d gave *S*-(Z-glycyl-L-alanyl-L-leucyl-L-alanyl)crown ether (**50**) in 60% yield. The tetrapeptide derivative **51** was isolated along with the parent host **11** from **50** after methanolysis by using $K_2CO_3$ as a base in 47% and 60% yields, respectively.[20]

## Conclusion

We have developed a novel approach to mimicking of enzyme-catalyzed bond-forming reactions, performing the biomimetic synthesis of peptides by using multi-functionalized crown ethers. These crown ethers have the following characteristics as an enzyme model: they have the




(a) L-Leu–ONp·HBr, pyridine (b) *tert*-BuCOOH, TEA, benzene (c) L-Ala–ONp·HBr, pyridine (d) $K_2CO_3$, MeOH

Chart 6

18-crown-6 moiety as a single binding site for the primary ammonium cation, and dithiol provides two reactive sites for two substrates. Amino acid derivatives as substrates are assembled in the hosts by the formation of thioesters *via* prior intra-complex thiolysis. Peptide bond formation, as well as peptide chain elongation by repeating the intra-complex thiolysis followed by the intramolecular aminolysis, has been realized. This study has also established a new strategy for mimicking enzymatic covalent catalysis by showing that thiol reactive groups can be used repeatedly for elongation of the peptide chain. The effect of the structure of the reactive groups on the efficiency of the intramolecular aminolysis raises the possibility of designing more efficient hosts.[21]

## Experimental

Melting points were measured on a Büchi 510 melting point apparatus, and are uncorrected. Optical rotations were recorded on a JASCO DIP-181 polarimeter. Infrared (IR) spectra were recorded on a JASCO IRA-1 or a JASCO DS-402G infrared spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured on a JEOL JNM-OS 100 high resolution NMR spectrometer ($^1$H, 100 MHz), or a JEOL FX100 Fourier-transform NMR spectrometer ($^1$H 100 MHz), or a Hitachi R-24 high resolution NMR spectrometer ($^1$H, 60 MHz). Chemical shifts are reported in $\delta$ values in ppm with tetramethylsilane (TMS) as an internal standard in $CDCl_3$. Coupling constants ($J$) are reported in hertz (Hz). Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOL JMS-01 SG-2 and JEOL JMS-DX300 mass spectrometers, respectively.

**(+)-(2*S*,3*S*)-2,3-Bis(8-tosyloxy-3,6-dioxaoctyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (15)** A solution of **14**[5a,b,d] (5.0 g, 15.4 mmol) in dimethylformamide (DMF) (10 ml) was added to a suspension of NaH (50% in oil, 1.7 g, 35.4 mmol) in DMF (20 ml) during 1 h under argon at room temperature, and the mixture was stirred at 40 °C for 1 h. This solution was added to a solution of diethylene glycol ditosylate (21 g, 50 mmol) in DMF (20 ml) at room temperature, and the mixture was stirred at the same temperature for 17 h. The mixture was poured into ice, and extracted with AcOEt (1 l × 2). The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated to give a crude oil (7.9 g), which was chromatographed (silica gel, benzene : acetone = 5 : 1) to afford a colorless oil (2.23 g, 18%). $[\alpha]_D^{20}$ +1.03 ($c$ = 2.1, $CHCl_3$). IR $\nu_{max}^{film}$ cm$^{-1}$: 1600, 1100. NMR $\delta$: 2.44 (6H, s, ArC$\underline{H}_3$), 3.2—4.0 (38H, m, -C$\underline{H}_2$O-, -OC$\underline{H}$-), 4.13 (4H, t, $J$ = 5 Hz, -C$\underline{H}_2$OTs), 7.30 and 7.75 (8H, ABq, $J$ = 8 Hz, Ar$\underline{H}$). *Anal.* Calcd for $C_{36}H_{56}O_{16}S_2$: C, 53.45; H, 6.98. Found: C, 53.16; H, 6.93.

**(+)-(2*S*,3*S*)-2,3-Bis(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (11)** A solution of **15** (2.0 g, 2.47 mmol) in EtOH (10 ml) was added to a suspension of KSCOPh (1.0 g, 5.67 mmol) in EtOH (5 ml) under argon at room temperature, and the mixture was stirred under reflux for 1 h, and then cooled in ice bath. After the precipitates were filtered off, a solution of 5 N NaOH (1.5 ml) was added to the filtrate under argon at room temperature, and the mixture was stirred at 40 °C for 1 h. The reaction mixture was acidified with 6 N HCl (1.5 ml) at 0 °C, and the solvent was evaporated off to give a crude oil, which was chromatographed (alumina, benzene : AcOEt = 2 : 1) to afford a pale yellow oil **11** (1.21 g, 85%). $[\alpha]_D^{20}$ +0.5° ($c$ = 1.98, $CHCl_3$). IR $\nu_{max}^{film}$ cm$^{-1}$: 2560, 1110. NMR $\delta$: 1.6 (2H, t, $J$ = 8.2 Hz, -S$\underline{H}$), 2.67 (4H, dt, $J$ = 8.2, 6.1 Hz, -C$\underline{H}_2$SH), 3.5—4.0 (38H, m, -C$\underline{H}_2$-, -C$\underline{H}$O-). HRMS Calcd for $C_{22}H_{44}O_{10}S_2$: 532.2374. Found: 532.2374.

**(+)-(2*S*,3*S*)-2,3-Bis(2-mercaptophenylthiomethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (12)** *o*-Dithiophenol (2.2 g, 15.5 mmol) was added to a degassed solution of **16**[5a,b,d] (4.2 g, 6.7 mmol) in 1 N aqueous KOH–EtOH–tetrahydrofuran (THF) (13 ml–30 ml–10 ml), and the mixture was stirred at 50 °C for 2 h, then cooled to room temperature. The mixture was acidified with 1 N aqueous HCl, then extracted with AcOEt (300 ml × 3). The combined extracts were washed with brine and dried over $Na_2SO_4$, then evaporated to give a crude oil, which was chromatographed (silica gel, AcOEt : hexane = 1 : 4—3 : 2) to give a colorless solid with mp 106—107 °C (1.3 g, 43%). IR $\nu_{max}^{film}$ cm$^{-1}$: 2530, 1450, 1470, 1110. NMR $\delta$: 3.09 (2H, dd, $J$ = 23, 6.5 Hz, -C$\underline{H}_2$SH), 3.32 (2H, dd, $J$ = 23, 4.5 Hz, -C$\underline{H}_2$SH), 3.4—4.9 (22H, m, -C$\underline{H}_2$-, -C$\underline{H}$O-), 4.22 (2H, s, S$\underline{H}$), 7.0—7.50 (8H, m, Ar$\underline{H}$). HRMS Calcd for $C_{26}H_{36}O_6S_4$: 572.1395. Found: 572.1342.

**(+)-(2*S*,3*S*)-2,3-Bis(3-mercaptophenylthiomethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (13)** The same procedure as described for the synthesis of **12** using *m*-dithiophenol and **16** gave a colorless caramel in 27% yield. IR $\nu_{max}^{film}$ cm$^{-1}$: 2600, 1450, 1470, 1110. NMR $\delta$: 3.09 (2H, dd, $J$ = 23, 6.5 Hz, -C$\underline{H}_2$SH), 3.32 (2H, dd, $J$ = 23, 4.5 Hz, -C$\underline{H}_2$SH), 3.4—4.9 (22H, m, -C$\underline{H}_2$-, -C$\underline{H}$O-), 4.22 (2H, s, S$\underline{H}$), 7.0—7.50 (8H, m, Ar$\underline{H}$). HRMS Calcd for $C_{26}H_{36}O_6S_4$: 572.1395. Found: 572.1377.

**(5*S*,6*S*)-1,10-Dibenzyloxy-5,6-dimethoxy-3,8-dioxadecane (18)** A solution of (+)-(2*S*,3*S*)-2,3-*O*-isopropylidenethreitol (**17**)[22] (1.62 g, 10 mmol) in DMF (10 ml) was added to a suspension of NaH (60% in oil, 0.96 g, 24 mmol) in DMF (10 ml) under argon during 30 min at room temperature. A solution of 1-benzyloxy-2-tosyloxyethane (6.65 g, 22 mmol) in DMF (10 ml) was added to the reaction mixture, and the whole was stirred at room temperature for 2 d. The reaction mixture was poured into water (50 ml), and extracted with AcOEt (200 ml). The AcOEt layer was separated and washed successively with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, and then dried over $MgSO_4$. The AcOEt was evaporated to give a crude oil. A solution of this oil in EtOH–10% aqueous HCl (40 ml–10 ml) was stirred at room temperature for 2 h, then the solvent was evaporated off. The residue was dissolved in $CHCl_3$ (100 ml), and dried over $MgSO_4$. The $CHCl_3$ was evaporated off to give a crude oil (3.5 g), which was purified by column chromatography (silica gel, hexane : AcOEt = 1 : 1—2 : 1) to afford a colorless oil (3.07 g, 79%). IR $\nu_{max}^{film}$ cm$^{-1}$: 3440, 1100. NMR $\delta$: 3.0 (2H, br, -O$\underline{H}$), 3.3—4.0 (4H, -C$\underline{H}_2$O-, -C$\underline{H}$O-), 4.52 (4H, s, -C$\underline{H}_2$Ar), 7.29 (10H, s, -Ar$\underline{H}$). MS $m/z$: 390 ($M^+$), 299 ($M^+ - C_7H_7$), 207 ($M^+ - C_{14}H_{14}$), 148 ($C_6H_{12}O_5^+$), 108 ($C_4H_8O^+$).

**(5*S*,6*S*)-5,6-Dimethoxy-1,10-dimercapto-3,8-dioxadecane (19)** A solution of **18** (2.77 g, 7.09 mmol) was added to a suspension of NaH (60% in oil, 0.74 g, 18.5 mmol) in DMF (15 ml) under argon at room temperature, and the mixture was stirred at 50 °C for 1 h. Methyl iodide (1.1 ml, 17.7 mmol) was added at room temperature, and the mixture was stirred at the same temperature for 2 d, then poured into water, and extracted with AcOEt. The extract was washed successively with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$, then evaporated to give a crude oil (3.0 g, quantitative), which was used for the next reaction without purification. A mixture of the crude oil and 10% Pd–C (300 mg) in EtOH (50 ml) was stirred under $H_2$ at room temperature for 2 h. The catalyst was filtered off and the filtrate was evaporated to dryness to afford a crude oil, which was dissolved in pyridine (15 ml) and treated with tosyl chloride (3.26 g, 17.1 mmol) at 0 °C overnight. The mixture was poured into 10% aqueous HCl, and extracted with AcOEt. The extract was washed successively with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$, then evaporated to give a crude oil (3.56 g, 91%). This tosylate was converted to **19** by the same procedure as described for the preparation of **11** to afford a colorless oil (0.91 g, 48% overall yield). $bp_{5\ mmHg}$ 175 °C. IR $\nu_{max}^{film}$ cm$^{-1}$: 2560, 1100. NMR $\delta$: 1.56 (2H, t, $J$ = 8 Hz, -S$\underline{H}$), 2.70 (4H, dt, $J$ = 8, 6 Hz, -C$\underline{H}_2$SH), 3.48 (6H, s, -OC$\underline{H}_3$), 3.3—3.8 (10H, m, -C$\underline{H}_2$-, -C$\underline{H}$O-). MS $m/z$: 271 ($M^+ + 1$), 270 ($M^+$). *Anal.* Calcd for $C_{24}H_{30}O_6S_2$: C, 44.42; H, 8.20. Found: C, 44.37; H, 8.18.

**(+)-(5*S*,6*S*)-5,6-Dimethoxy-*S*-(*N*-carbobenzyloxyglycyl)-1,10-dimercapto-3,8-dioxadecane (20)** Triethylamine (0.19 g, 1.85 mmol) was added to a solution of **13** (0.5 g, 1.85 mmol), Z–Gly–OH (0.38 g, 1.85 mmol), and DEPC (0.30 g, 1.85 mmol) in DMF (10 ml) at −20 °C, and the whole was stored at −20 °C overnight. The mixture was diluted with AcOEt–benzene (1 : 1), and washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$, then evaporated to give a crude oil, which was chromatographed (silica gel, hexane : AcOEt = 5 : 1—1 : 1) to afford a colorless oil (0.34 g, 40%). $[\alpha]_D^{20}$ +10.2° ($c$ = 0.6, $CHCl_3$). IR $\nu_{max}^{film}$ cm$^{-1}$: 3360, 1730, 1690. NMR $\delta$: 1.56 (1H, t, $J$ = 8 Hz, -S$\underline{H}$), 2.69 (2H, dt, $J$ = 8, 6 Hz, -C$\underline{H}_2$SH), 3.12 (2H, t, $J$ = 6 Hz, -CH$_2$SCO-), 3.46 (6H, s, -OC$\underline{H}_3$), 3.3—3.8 (10H, m, -OC$\underline{H}_2$-, -C$\underline{H}$O-), 4.12 (2H, d, $J$ = 5.4 Hz, -C$\underline{H}_2$NH-), 5.12 (2H, s, -C$\underline{H}_2$Ar), 5.56 (1H, br, -N$\underline{H}$), 7.30 (5H, s, -Ar$\underline{H}$). *Anal.* Calcd for $C_{20}H_{31}N_1O_7S_2$: C, 52.04; H, 6.77; N, 3.06. Found: C, 51.99; H, 6.81; N, 2.83.

The bis(Z-glycyl) derivative of **19** was also separated (0.14 g, 12%). IR $\nu_{max}^{film}$ cm$^{-1}$: 3360, 1730, 1690. NMR $\delta$: 3.08 (4H, t, $J$ = 6 Hz, -CH$_2$SCO-), 3.42 (6H, s, -OC$\underline{H}_3$), 3.3—3.8 (10H, m, -OC$\underline{H}_2$-, -C$\underline{H}$O-), 4.04 (4H, d, $J$ = 5.4 Hz, -C$\underline{H}_2$NH-), 5.09 (4H, s, -C$\underline{H}_2$Ar), 5.88 (2H, br, -N$\underline{H}$), 7.30 (10H, s, -Ar$\underline{H}$). *Anal.* Calcd for $C_{30}H_{40}N_2O_{10}S_2$: C, 55.20; H, 6.18; N, 4.29. Found: C, 54.91; H, 6.09; N, 3.99.

**(+)-(5*S*,6*S*)-5,6-Dimethoxy-*S*-(*N*-carbobenzyloxyglycyl)-*S'*-(*N*-carbo-*tert*-butoxy-L-alanyl)-1,10-dimercapto-3,8-dioxadecane (21)** Triethylamine (0.073 ml, 0.48 mmol) was added to a solution of **20** (200 mg,

0.43 mmol), DEPC (85 mg, 0.48 mmol), and *N*-carbo-*tert*-butoxy-L-alanine (97 mg, 0.48 mmol) in DMF (2 ml) at −20 °C, and the mixture was stored at −20 °C overnight. The mixture was poured into water, and extracted with AcOEt. The extract was washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$, then evaporated to give a crude oil, which was chromatographed (silica gel, hexane : AcOEt = 1 : 1) to afford a colorless oil (190 mg, 69%). $[\alpha]_D^{20}$ +1.04° ($c$ = 1.57, $CHCl_3$). IR $\nu_{max}^{film}$ $cm^{-1}$: 3360, 1730, 1690. NMR $\delta$: 1.35 (3H, d, $J$ = 7 Hz, $-CHC\underline{H}_3$), 1.45 (9H, s, $CC\underline{H}_3$), 3.06 (2H, t, $J$ = 6 Hz, $-C\underline{H}_2S-$), 3.15 (2H, t, $J$ = 6 Hz, $-C\underline{H}_2S-$), 3.45 (6H, s, $-OC\underline{H}_3$), 3.3—3.8 (10H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.05 (2H, d, $J$ = 6 Hz, $-C\underline{H}_2NH-$), 4.0—4.5 (1H, m, $-C\underline{H}NH-$), 5.13 (1H, br, $-NH-$), 5.85 (1H, br, $-NH-$), 7.36 (5H, s, $Ar\underline{H}$). *Anal.* Calcd for $C_{28}H_{44}N_2O_{10}S_2$: C, 53.14; H, 7.01; N, 4.43. Found: C, 53.44; H, 7.11; N, 4.36.

**(+)-(5*S*,6*S*)-5,6-Dimethoxy-*S*-(*N*-carbobenzyloxyglyxyl-L-alanyl)-1,10-dimercapto-3,8-dioxadecane (26)** A solution of **25** (130 mg, 0.2 mmol) in AcOEt (2 ml) containing 2.4 N HCl was stirred at room temperature for 30 min, then the solvent was removed *in vacuo*. The residue was dissolved in benzene containing triethylamine (TEA) (0.057 ml, 0.41 mmol) and AcOH (0.024 ml, 0.41 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was evaporated off to give a crude oil, which was chromatographed (silica gel, benzene : AcOEt = 3 : 2) to afford **26** (77 mg, 71%) as a colorless oil. $[\alpha]_D^{20}$ +1.6 ($c$ = 1.3, $CHCl_3$). IR $\nu_{max}^{film}$ $cm^{-1}$: 3350, 1730, 1700. NMR $\delta$: 1.35 (3H, d, $J$ = 8 Hz, $-CHC\underline{H}_3$), 1.55 (1H, t, $J$ = 8 Hz, $-S\underline{H}$), 2.66 (2H, dt, $J$ = 8, 6 Hz, $-C\underline{H}_2SH$), 3.03 (2H, t, $J$ = 6 Hz, $-C\underline{H}_2SCO-$), 3.25—3.8 (10H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 3.45 (6H, s, $-OC\underline{H}_3$), 3.92 (2H, d, $J$ = 6 Hz, $-C\underline{H}_2NH-$), 4.72 (1H, dq, $J$ = 8, 8 Hz, $-C\underline{H}NH-$), 5.17 (2H, s, $-C\underline{H}_2Ar$), 6.55 (1H, d, $J$ = 8 Hz, $-N\underline{H}-$), 7.36 (5H, s, $-Ar\underline{H}$). *Anal.* Calcd for $C_{23}H_{36}N_2O_8S_2$: C, 51.86; H, 6.81; N, 5.26. Found: C, 51.54; H, 6.86; N, 4.97.

**Kinetic Measurement of Inter- and Intramolecular Aminolysis** Intermolecular Aminolysis: The intermolecular aminolyses were carried out by using 0.1 M or 5 mM each of Z-Gly-SEt (**22**) and L-Ala-OEt (**23**) in benzene containing various concentrations of pivalic acid and TEA at 20 °C. The aminolysis product (Z-Gly-L-Ala-OEt, **24**) was quantitatively analyzed by HPLC (Waters, Radial Pak B, 60% AcOEt in hexane, monitored at 254 nm) by using acetanilide as an internal standard: **24** and acetanilide gave the retention times of 6.9 and 4.1 min, respectively, at the flow rate of 2 ml/min. Experimental errors were estimated to be less than 15% of the recorded rate constants.

Intramolecular Aminolysis: **21** (77.2 mg, 0.122 mmol) was treated with 2.4 N HCl in AcOEt (3 ml, 7.2 mmol) at room temperature for 40 min, then the solvent was evaporated off. The resulting **25**·HCl was dried *in vacuo*, and neutralized with TEA (0.017 ml, 0.122 mmol) in benzene (10.0 ml), then the precipitates were filtered off to afford a clear benzene solution containing 12.2 mM **25**. This solution was used for the kinetic experiment without further purification. The intramolecular aminolysis was performed at 20 °C in benzene containing 5 mM each of **25**, pivalic acid, and TEA. The product **26** was quantitatively analyzed by HPLC (Waters, Radial Pak, B, 67% AcOEt in hexane, monitored at 254 nm) by using acetanilide as an internal standard: **26** and acetanilide gave retention times of 4.7 and 2.0 min, respectively at the flow rate of 3.5 ml/min.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl)-*S*′-L-alanyl-2,3-bis(mercaptomethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (37)** TEA (0.023 ml, 0.17 mmol) was added to a solution of **1** (40 mg, 0.11 mmol), Z-Gly-OH (33 mg, 0.16 mmol), and DEPC (0.025 ml, 0.17 mmol) in DMF (2 ml) at 0 °C, and the mixture was stirred at the same temperature for 1 h, then at room temperature for 1 h. The mixture was poured into water, and extracted with AcOEt. The extract was washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$, then evaporated to give a crude oil (105 mg). L-Ala-ON*p*·HBr (39 mg, 0.13 mmol) was added to a solution of the above oil in acetone–pyridine (1 ml–0.09 ml), and the mixture was stirred at room temperature for 20 min. The reaction mixture was shaken with 1 N HCl–ether (10 ml–20 ml), and the organic layer was separated, then extracted with 1 N HCl (100 ml × 2). The combined 1 N HCl layers were extracted with $CHCl_3$ (30 ml × 3), and the combined $CHCl_3$ extracts were filtered through a filter paper, and then evaporated off to give crude **37** (37.5 mg, 52%) as a colorless oil. The *N*-benzoyl derivative was prepared in 80% yield by the reaction using **37** (20 mg), benzoyl chloride (0.01 ml), and TEA (0.01 ml) in dry $CHCl_3$ (2 ml) at room temperature for 30 min. IR $\nu_{max}^{film}$ $cm^{-1}$: 3400, 1720, 1690, 1670. NMR $\delta$: 1.53 (3H, d, $J$ = 7 Hz, $-CHC\underline{H}_3$), 3.10 (4H, t, $J$ = 6 Hz, $-C\underline{H}_2-$), 3.2—4.0 (22H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 3.92 (2H, d, $J$ = 4 Hz, $-C\underline{H}_2NH-$), 4.90 (1H, dt, $J$ = 6, 7 Hz, $-NH\underline{H}-$), 5.12 (2H, s, $-C\underline{H}_2Ar$), 5.8 (1H, br, $-N\underline{H}-$), 6.82 (1H, br, $-N\underline{H}-$), 7.32 (5H, s, $-Ar\underline{H}$), 7.2—7.9 (5H, m, $-COAr\underline{H}$).

The first ether layer was washed successively with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$, then the solvent was evaporated off to give a crude oil, which was chromatographed (silica gel, ether) to afford pure bis(Z-glycyl)crown ether **32** (20.7 mg, 25%) as a colorless caramel. IR $\nu_{max}^{film}$ $cm^{-1}$: 3400, 1700. NMR $\delta$: 3.1 (4H, t, $J$ = 6 Hz, $-C\underline{H}_2-$), 3.2—4.0 (22H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 3.92 (4H, d, $J$ = 4 Hz, $-C\underline{H}_2NH-$), 5.12 (4H, s, $-C\underline{H}_2Ar$), 5.4 (2H, br, $-N\underline{H}-$), 7.3 (10H, $-Ar\underline{H}$). *Anal.* Calcd for $C_{34}H_{46}N_2O_{12}S_2 \cdot H_2O$: C, 53.95; H, 6.39; N, 3.70. Found: C, 53.90; H, 6.44; N, 3.33.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl)-*S*′-L-alanyl-2,3-bis(4-mercapto-2-oxabutyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (38)** **10**[5a,b] (0.3 g, 0.68 mmol) was converted to **38** (160 mg, 32%) by the same procedure as described for the synthesis of **37** except that pyridine (14 ml) was used instead of acetone–pyridine, and that the pyridine was evaporated off before extraction. IR $\nu_{max}^{film}$ $cm^{-1}$: 3000, 1720, 1690, 1100. NMR $\delta$: 1.56 (3H, d, $J$ = 6.8 Hz, $-CHC\underline{H}_3$), 3.11 (4H, t, $J$ = 6 Hz, $-C\underline{H}_2-$), 3.2—4.0 (30H, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 3.9—4.4 (3H, m, $-C\underline{H}_2NH-$, $-C\underline{H}NH-$), 5.15 (2H, s, $-C\underline{H}_2Ar$), 6.3 (1H, t, $J$ = 6 Hz, $-CH_2N\underline{H}$), 7.35 (10H, s, $-Ar\underline{H}$), 7.2—7.8 (3H, br, $N\underline{H}_3$).

Bis(Z-glycyl)crown ether (**33**) was isolated as a colorless oil (265 mg, 48%). IR $\nu_{max}^{film}$ $cm^{-1}$: 3400, 1720, 1100. NMR $\delta$: 3.06 (4H, t, $J$ = 6 Hz, $-C\underline{H}_2S-$), 3.35—4.0 (30H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.06 (4H, d, $J$ = 7 Hz, $-C\underline{H}_2NH-$), 5.10 (4H, s, $-C\underline{H}_2Ar$), 5.7 (2H, br, $-N\underline{H}$), 7.27 (10H, s, $-Ar\underline{H}$).

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl)-*S*′-L-alanyl-2,3-bis(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (39)** **11** (0.95 g, 18 mmol) was converted to **39** (437 mg, 33%) by the same procedure as described for the synthesis of **37** except that pyridine (35 ml) was used instead of acetone–pyridine, and that the pyridine was evaporated off before extraction. IR $\nu_{max}^{film}$ $cm^{-1}$: 3400, 3000, 1720, 1690, 1100. NMR $\delta$: 1.53 (3H, d, $J$ = 7.1 Hz, $-CHC\underline{H}_3$), 3.07 (4H, t, $J$ = 5 Hz, $-C\underline{H}_2-$), 3.2—4.0 (38H, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.12 (2H, d, $J$ = 6 Hz, $-C\underline{H}_2NH-$), 4.0—4.4 (1H, m, $-C\underline{H}NH-$), 5.13 (2H, s, $-C\underline{H}_2Ar$), 6.64 (1H, t, $J$ = 6 Hz, $-CH_2N\underline{H}$), 7.33 (10H, s, $-Ar\underline{H}$), 7.2—7.7 (3H, br, $N\underline{H}_3$).

Bis(Z-glycyl)crown ether (**34**) was isolated as a colorless oil (711 mg, 44%). IR $\nu_{max}^{film}$ $cm^{-1}$: 3450, 1720, 1690, 1100. NMR $\delta$: 3.09 (4H, t, $J$ = 6 Hz, $-C\underline{H}_2S-$), 3.2—4.0 (38H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.08 (4H, d, $J$ = 7 Hz, $-C\underline{H}_2NH-$), 5.12 (4H, s, $-C\underline{H}_2Ar$), 5.8 (2H, br, $-N\underline{H}$), 7.33 (10H, s, $-Ar\underline{H}$). *Anal.* Calcd for $C_{42}H_{62}N_2O_{16}S_2$: C, 55.12; H, 6.83; N, 3.06. Found: C, 54.83; H, 6.84; N, 2.91.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl)-*S*′-L-alanyl-2,3-bis(2-mercaptophenylthiomethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (40)** Z-Gly-ON*p* (21 mg, 0.064 mmol) was added to a solution of **12** (42 mg, 0.073 mmol) and TEA (0.01 ml, 0.072 mmol) in ethanol (10 ml), and the mixture was stirred at room temperature for 30 min, then the solvent was evaporated off. The residue was dissolved in acetone (10 ml), and pyridine (0.018 ml, 0.22 mmol) and L-Ala-ON*p*·HBr (21 mg, 0.072 mmol) were added to this solution, and the solution was stirred at room temperature for 15 min. The mixture was acidified with ice-cold 6 N aqueous HCl (50 ml), and extracted with benzene (50 ml). The organic phase was extracted with 6 N aqueous HCl (10 ml). The combined aqueous layers were extracted with AcOEt (50 ml × 2), and the combined AcOEt extracts were evaporated to give a crude oil (19.2 mg, 30%), which was benzoylated in 75% yield with benzoyl chloride–TEA in $CHCl_3$. IR $\nu_{max}^{film}$ $cm^{-1}$: 3440, 1720, 1708, 1660, 1100. NMR $\delta$: 1.59 (3H, d, $J$ = 7.0 Hz, $-CHC\underline{H}_3$), 2.92—3.40 (4H, m, $-C\underline{H}_2-$), 3.4—3.9 (22H, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.20 (2H, d, $J$ = 6 Hz, $-C\underline{H}_2NH-$), 4.90—5.28 (1H, m, $-C\underline{H}NH-$), 5.12 (2H, s, $-C\underline{H}_2Ar$), 5.90 (1H, t, $J$ = 6 Hz, $N\underline{H}$), 6.94 (1H, d, $J$ = 8 Hz, $-CHN\underline{H}$), 7.32 (5H, s, $-Ar\underline{H}$), 7.0—7.6 (11H, m, $Ar\underline{H}$), 7.68—7.92 (2H, m, $Ar\underline{H}$).

Bis(Z-glycyl)crown ether (**35**) was isolated as a colorless oil (17.2 mg, 21%). IR $\nu_{max}^{film}$ $cm^{-1}$: 3350, 1720, 1710, 1100. NMR $\delta$: 2.93—3.33 (4H, m, $-C\underline{H}_2S-$), 3.3—3.92 (22H, m, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.12 (4H, d, $J$ = 8 Hz, $-C\underline{H}_2NH-$), 5.08 (4H, s, $-C\underline{H}_2Ar$), 5.82 (2H, br, $-N\underline{H}$), 7.27 (10H, s, $-Ar\underline{H}$), 6.99—7.52 (8H, m, $Ar\underline{H}$).

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl)-*S*′-L-alanyl-2,3-bis(3-mercaptophenylthiomethyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (41)** **13** gave crude **41** in 32% yield by the same procedure as described for the synthesis of **40**. *N*-Benzoyl derivative of **41**: IR $\nu_{max}^{film}$ $cm^{-1}$: 3440, 1720, 1708, 1100. NMR $\delta$: 1.59 (3H, d, $J$ = 7.0 Hz, $-CHC\underline{H}_3$), 2.82—3.40 (4H, m, $-C\underline{H}_2-$), 3.3—4.0 (22H, $-C\underline{H}_2O-$, $-C\underline{H}O-$), 4.20 (2H, d, $J$ = 6 Hz, $-C\underline{H}_2NH-$), 4.90—5.28 (1H, m, $-C\underline{H}NH-$), 5.12 (2H, s, $-C\underline{H}_2Ar$), 5.90 (1H, t, $J$ = 6 Hz, $N\underline{H}$), 6.94 (1H, d, $J$ = 8 Hz, $-CHN\underline{H}$), 7.32 (5H, s, $-Ar\underline{H}$), 7.0—7.6 (11H, m, $Ar\underline{H}$), 7.68—7.92 (2H, m, $Ar\underline{H}$).

Bis(Z-glycyl)crown ether (**36**) was isolated as a colorless oil in 21% yield.

IR $\nu_{max}^{film}$ cm$^{-1}$: 3350, 1720, 1710, 1100. NMR $\delta$: 2.82—3.30 (4H, m, $-CH_2S-$), 3.30—4.0 (22H, m, $-CH_2O-$, $-CHO-$), 4.12 (4H, d, $J$=8 Hz, $-CH_2NH-$), 5.15 (4H, s, $-CH_2Ar$), 5.75 (2H, br, $-NH$), 7.34 (10H, s, $-ArH$), 5.15 (4H, s, $-CH_2Ar$), 5.75 (2H, br, $-NH$), 7.34 (10H, s, $-ArH$), 7.01—7.50 (8H, m, $ArH$).

**Peptide Synthesis by Sing Crown Ethers. General Procedure** A solution of crown ether hydrochloride (1 mmol), pivalic acid (30 mmol), and TEA (31 mmol) in benzene (200 ml) was stirred at room temperature. After the reaction was completed, the reaction mixture was diluted with AcOEt (200 ml), and washed successively with 1 N HCl (70 ml), saturated $NaHCO_3$ (70 ml) and brine (70 ml), then dried over $MgSO_4$. The solvent was evaporated off, and the residue was purified by column chromatography (silica gel deactivated with $H_2O$, ether–$H_2O$) to give a product.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl-L-alanyl)-2,3-bis(4-mercapto-2-oxabutyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (43)** The reaction of **37** was carried out at room temperature for 20 h to give **42** in 68% yield after purification. IR $\nu_{max}^{film}$ cm$^{-1}$: 3320, 1720, 1690. NMR $\delta$: 1.38 (3H, d, $J$=7 Hz, $-CHCH_3$), 1.58 (1H, t, $J$=8 Hz, $-SH$), 2.68 (2H, dt, $J$=8, 7 Hz, $CH_2SH$), 3.08 (2H, t, $J$=6 Hz, $-CH_2SCO-$), 3.2—4.0 (30H, m, $-CH_2O-$, $-CHO-$), 3.92 (2H, d, $J$=6 Hz, $-CH_2NH-$), 4.71 (1H, dq, $J$=8, 7 Hz, $-CHNH-$), 5.14 (2H, s, $-CH_2Ar$), 5.78 (1H, br, $-NH-$), 6.88 (1H, d, $J$=8 Hz, $-NH-$), 7.35 (5H, s, $-ArH$).

**Z–Gly–L-Ala–OEt** A mixture of **43** (23 mg, $3.2\times10^{-5}$ mol) and $K_2CO_3$ (1 mg, $7.2\times10^{-6}$ mol) in EtOH (3 ml) was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt (100 ml), and washed successively with 10% citric acid, $NaHCO_3$, and brine, then dried over $MgSO_4$. The solvent was evaporated off to give a crude oil, which was chromatographed (silica gel, ether, then AcOEt) to afford Z–Gly–L-Ala–OEt (6.9 mg, 70%) and the disulfide of **10** (6.0 mg, 26%). The dipeptide was purified again by HPLC (Waters, Radial Pak B, 67% AcOEt in hexane) to afford a pure sample (4.1 mg) as a colorless oil. $[\alpha]_D^{20}$ +6.8 ($c$=0.4, $CHCl_3$). Auth. ($[\alpha]_D^{20}$ +7.04 ($c$=1.05, $CHCl_3$)). Its NMR ($CDCl_3$) spectrum was identical with that of an authentic sample.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl-L-alanyl)-2,3-bis(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (44)** The reaction of **38** was carried out at room temperature for 10 h to give **44** in 81% yield after purification. IR $\nu_{max}^{film}$ cm$^{-1}$: 3320, 1720, 1680. NMR $\delta$: 1.38 (3H, d, $J$=7 Hz, $-CHCH_3$), 1.60 (1H, t, $J$=8 Hz, $-SH$), 2.67 (2H, dt, $J$=8, 7 Hz, $-CH_2SH$), 3.07 (2H, t, $J$=6 Hz, $-CH_2SCO-$), 3.4—4.0 (38H, m, $-CH_2O-$, $-CHO-$), 3.92 (2H, d, $J$=6 Hz, $-CH_2NH-$), 4.68 (1H, dq, $J$=8, 7 Hz, $-CHNH-$), 5.13 (2H, s, $-CH_2Ar$), 5.86 (1H, br, $-NH-$), 6.98 (1H, d, $J$=8 Hz, $-NH-$), 7.34 (5H, s, $-ArH$).

**Z–Gly–L-Ala–OMe** A mixture of **44** (41.5 mg, $5.22\times10^{-5}$) and $K_2CO_3$ (3 mg, $2.2\times10^{-5}$ mol) in MeOH (1 ml) was stirred at room temperature for 1.5 h. The reaction mixture was worked up by the same procedure as described for the preparation of Z–Gly–L-Ala–OEt to give a crude oil (17 mg), which was chromatographed (silica gel, AcOEt : hexane=2:3) to afford Z–Gly–L-Ala–OMe (9.7 mg, 63%) as a colorless oil. $[\alpha]_D^{20}$ −8.3° ($c$=1, AcOEt). Lit. ($[\alpha]_D^{20}$ −8.1 ($c$=1, AcOEt)). Auth. ($[\alpha]_D^{20}$ −8.8 ($c$=, AcOEt)). Its NMR and HRMS spectra were identical with those of the authentic sample.

As the synthesis of crown ethers (**47a, b, c** and **48a, b, c**) and the isolation of tripeptide derivatives were performed under almost the same conditions, only the synthesis of **48a** is described.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl-L-alanyl)-*S'*-L-leucyl-2,3-bis(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (47a)** L-Leu–ON*p*·HBr (35 mg, 1.14 mmol) was added to a solution of **44** (83 mg, $1.0\times10^{-4}$ mol) in pyridine (2.1 ml), and the whole was stirred at room temperature for 15 min. The pyridine was removed *in vacuo*, and the residue was shaken with 1 N HCl–AcOEt (20 ml–20 ml). The AcOEt layer was separated, and extracted with 1 N HCl (20 ml × 3). The combined 1 N HCl layers were extracted with $CHCl_3$ (20 ml × 2). The $CHCl_3$ extract was filtered through a filter paper, then evaporated to give crude **47a** (85.7 mg, 87%) as a yellow caramel. IR $\nu_{max}^{film}$ cm$^{-1}$: 3000. NMR $\delta$: 0.95 (6H, d, $J$=4 Hz, $-CH(CH_3)_2$), 1.42 (3H, d, $J$=7 Hz, $-CHCH_3$), 1.2—2.0 (3H, m, $-CH_2CH-$), 2.9 (4H, t, $J$=6 Hz, $-CH_2SCO-$), 3.1—4.1 (38H, m, $-CH_2O-$, $-CHO-$), 3.9 (2H, d, $J$=6 Hz, $-CH_2NH-$), 4.50 (1H, dq, $J$=6.6, 7 Hz, $-CHNH-$), 5.06 (2H, s, $-CH_2Ar$), 6.5—7.4 (3H, br, $-NH_3-$), 7.2 (5H, s, $-ArH$), 8.6 (2H, br, $-NH-$).

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl-L-alanyl-L-leucyl)-2,3-bis(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (48a)** Intramolecular aminolysis of **47a** (85.7 mg, $8.7\times10^{-5}$ mol) was carried out at room temperature for 2 d to give crude **48a** (47.7 mg, 58%). IR $\nu_{max}^{film}$ cm$^{-1}$: 3320, 1720, 1680, 1100. NMR $\delta$: 0.84 (6H, br, $-CH(CH_3)_2$), 1.2—1.8 (7H, m, $-CHCH_3$, $-CH_2CH-$, $-SH$), 2.60 (2H, dt, $J$=7, 6 Hz, $-CH_2SH$), 2.99 (2H, t, $J$=6 Hz, $-CH_2SCO-$), 3.2—4.0 (38H, m, $-CH_2O-$, $-CHO-$), 3.82 (2H, d, $J$=6 Hz, $-CH_2NH-$), 4.3—4.7 (2H, m, $-CHNH-$), 5.04 (2H, s, $-CH_2Ar$), 5.83 (1H, br, $-NH-$), 7.1 (2H, br, $-NH-$), 7.24 (5H, s, $-ArH$).

**Z–Gly–L-Ala–L-Leu–OMe** A mixture of **48a** (9.5 mg, $1.05\times10^{-5}$ mol) and $K_2CO_3$ (3 mg, $2.2\times10^{-5}$ mol) in MeOH (0.7 ml) was stirred at room temperature for 1 h. The reaction mixture was worked up by the same procedure as used for the preparation of Z–Gly–L-Ala–OEt, and the crude product was purified by HPLC (Waters, Radial Pak B, 80% AcOEt in hexane) to afford Z–Gly–L-Ala–L-Leu–OMe (3.7 mg, 86%) as a colorless oil. HRMS and NMR spectra were identical with those of an authentic sample.

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl-L-alanyl-L-leucyl)-*S'*-L-alanyl-2,3-bis(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane Hydrochloride (49)** **48a** (32 mg, $3.52\times10^{-5}$ mol) gave crude **49** (29.3 mg, 82%) by the same procedure as described for the preparation of **39**. NMR $\delta$: 0.9 (6H, d, $J$=4 Hz, $-CH(CH_3)_2$), 1.58 (3H, d, $J$=7 Hz, $-CHCH_3$), 1.62 (3H, d, $J$=7 Hz, $-CHCH_3$), 1.6—1.96 (3H, m, $-CH_2CH-$), 2.7—3.1 (4H, t, $J$=6 Hz, $-CH_2SCO-$), 3.1—4.1 (38H, m, $-CH_2O-$, $-CHO-$), 3.82 (2H, d, $J$=6 Hz, $-CH_2NH-$), 4.0 (1H, br, $-CHNH-$), 4.4—4.7 (2H, m, $-CHNH-$), 5.11 (2H, s, $-CH_2Ar$), 7.33 (5H, s, $-ArH$), 7.5 (3H, br, $-NH_3$), 8.4 (3H, br, $-NH-$).

**(2*S*,3*S*)-*S*-(*N*-Carbobenzyloxyglycyl-L-alanyl-L-leucyl-L-alanyl)-2,3-bis-(7-mercapto-2,5-dioxaheptyl)-1,4,7,10,13,16-hexaoxacyclooctadecane (50)** The intramolecular aminolysis of **49** (29.3 mg, mol) was carried out in benzene–AcOEt (3:1) at room temperature for 3 d to give crude **50** (17.1 mg, 60%). IR $\nu_{max}^{film}$ cm$^{-1}$: 3320, 1720, 1685, 1675, 1100. NMR $\delta$: 0.84 (6H, br, $-CH(CH_3)_2$), 1.34 (6H, d, $J$=6 Hz, 2 × $-CHCH_3$), 1.4—1.8 (3H, m, $-CH_2CH-$), 2.5—2.7 (2H, m, $-CH_2SH$), 2.8—3.1 (2H, m, $-CH_2SCO-$), 3.2—4.0 (38H, m, $-CH_2O-$, $-CHO-$), 3.82 (2H, d, $J$=6 Hz, $-CH_2NH-$), 4.2—4.7 (3H, m, $-CHNH-$), 5.04 (2H, s, $-CH_2Ar$), 5.7 (1H, br, $-NH-$), 6.9 (3H, br, $-NH-$), 7.24 (5H, s, $-ArH$).

**Z–Gly–L-Ala–L-Leu–L-Ala–OMe (51)** A mixture of **50** (17 mg, $1.74\times10^{-5}$ mol) and $K_2CO_3$ (1 mg, $7.2\times10^{-6}$ mol) in MeOH (2 ml) was stirred under argon at room temperature for 1.5 h. The reaction mixture was worked up by the same procedure as described for the preparation of Z–Gly–L-Ala–OEt to give a crude oil (13 mg), which was purified by column chromatography (silica gel, AcOEt–hexane, then AcOEt) to afford Z–Gly–L-Ala–L-Leu–OMe (2.6 mg, 37%), the tetrapeptide **51** (3.9 mg, 47%), and **11** (5.7 mg, 60%). HRMS and NMR spectra of the tri- and tetrapeptide were identical with those of authentic samples.

**Authentic Samples** Authentic samples synthesized by the conventional method[23] have the following physical properties:

Z–Gly–L-Ala–OMe: mp 63—64 °C. $[\alpha]_D^{20}$ −8.8° ($c$=1.0, AcOEt) (lit.[24] mp 62—64 °C. $[\alpha]_D^{20}$ −8.1°).

Z–Gly–L-Ala–OEt: mp 65.5—66.5 °C. $[\alpha]_D^{20}$ +7.0° ($c$=1.05, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{20}N_2O_5$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.17; H, 6.61; N, 8.91.

Z–Gly–L-Ala–L-Leu–OMe: mp 106—107 °C (lit.[25] 111.5—113.5 °C). $[\alpha]_D^{20}$ −43° ($c$=0.2, MeOH). *Anal.* Calcd for $C_{20}H_{29}N_3O_6$: C, 58.95; H, 7.17; N, 10.31. Found: C, 58.68; H, 7.16; N, 10.09.

Z–Gly–L-Ala–L-Ala–OMe: mp 135—136 °C. $[\alpha]_D^{20}$ −52.9° ($c$=0.87, MeOH). *Anal.* Calcd for $C_{17}H_{24}N_3O_6$: C, 55.73; H, 6.60; N, 11.47. Found: C, 53.48; H, 6.33; N, 11.19.

Z–Gly–L-Ala–L-Phe–OMe: mp 135.5—136.5 °C. $[\alpha]_D^{20}$ −24.8° ($c$=1.0, MeOH). (lit.[26] mp 134 °C. $[\alpha]_D^{19}$ −24.8° ($c$=1.0, MeOH)).

Z–Gly–L-Ala–L-Leu–L-Ala–OMe: mp 185—187 °C. $[\alpha]_D^{20}$ −60.2° ($c$=0.88, MeOH). *Anal.* Calcd for $C_{23}H_{34}N_4O_7\cdot0.5H_2O$: C, 56.66; H, 7.24; N, 11.49. Found: C, 56.88; H, 7.15; N, 11.22.

**Acknowledgements** We are grateful to the Science and Technology Agency, Japan, for financial support.

**References and Notes**

1) Part III: K. Odashima, A. Itai, Y. Iitaka and K. Koga, *J. Org. Chem.*, **50**, 4478 (1985).
2) Preliminary communication: S. Sasaki, M. Shionoya and K. Koga, *J. Am. Chem. Soc.*, **107**, 3371 (1985).
3) General topics: *a*) F. Vögtle and E. Weber (ed.), "Host Guest Complex Chemistry I, II, and III," Springer-Verlag, Berlin, 1984; *b*) R. M. Izatt and J. J. Christensen (ed.), "Synthesis of Macrocycles," John Wiley & Sons, New York, 1987; Cyclodextrins: *c*) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer-Verlag, New York, 1977; *d*) R. Breslow, *Science*, **218**, 532 (1982); *e*) I. Tabushi, *Acc. Chem. Res.*, **15**, 66 (1982); *f*) V. T. D'Souza and M. L.

Bender, *ibid.*, **20**, 146 (1987); Crown ethers: *g*) D. J. Cram, *Angew. Chem. Int. Ed. Engl.*, **25**, 1039 (1986); *h*) J.-M. Lehn, *ibid.*, **27**, 89 (1988); *i*) J. F. Stoddard, "The Chemistry of Enzyme Action," ed. by M. I. Page, Elsevier, Amsterdam, 1984, Chapter 15; Cyclophanes: *j*) K. Odashima and K. Koga, "Cyclophanes," Vol. 2, ed. by P. M. Keehn and S. M. Rosenfeld, Academic Press, New York, 1983, Chap. 11; *k*) I. Tabushi and K. Yamamura, *Topics in Current Chemistry*, **113**, 145 (1983); *l*) Y. Murakami, *ibid.*, **115**, 107 (1983).

4) H. W. Hosseini, J.-M. Lehn, L. Maggiora, K. B. Mertes and M. P. Mertes, *J. Am. Chem. Soc.*, **107**, 537 (1987), and also see refs. 3*c*, *e*, *f*, *k*, *l*.

5) Using crown ethers: *a*) T. Matsui and K. Koga, *Tetrahedron Lett.*, **19**, 1115 (1978); *b*) *Idem*, *Chem. Pharm. Bull.*, **27**, 2295 (1979); *c*) S. Sasaki and K. Koga, *Heterocycles*, **12**, 1305 (1979); *d*) S. Sasaki, M. Kawasaki and K. Koga, *Chem. Pharm. Bull.*, **33**, 4247 (1985); *e*) Y. Chao and D. J. Cram, *J. Am. Chem. Soc.*, **98**, 1025 (1976); *f*) Y. Chao, G. R. Weisman, G. D. Sogah and D. J. Cram, *ibid.*, **101**, 4948 (1979); *g*) D. J. Cram and H. E. Katz, *ibid.*, **105**, 135 (1983); *h*) D. J. Cram, P. Y.-S. Lam and S. P. Ho, *ibid.*, **108**, 839 (1986); *i*) J. M. Lehn and C. Sirlin, *J. Chem. Soc., Chem. Commun.*, **1978**, 949.

6) Using water-soluble cyclophanes: *a*) F. Diederich, G. Schürmann and I. Chao, *J. Org. Chem.*, **53**, 2744 (1988); *b*) H.-J. Schneider and A. Junker, *Chem. Ber.*, **119**, 2815 (1986).

7) *a*) R. Breslow, A. W. Czarnik, M. Lauer, R. Leppkes, J. Winkler and S. Zimmerman, *J. Am. Chem. Soc.*, **108**, 1969 (1986); *b*) I. Tabushi, Y. Kuroda, M. Yamada and H. Higashimura, *ibid.*, **107**, 5545 (1985).

8) *a*) R. M. Kellogg, *Angew. Chem. Int. Ed. Engl.*, **23**, 4238 (1985) and references cited therein; *b*) G. R. Newkome and C. R. Martston, *J. Org. Chem.*, **50**, 4238 (1985).

9) *a*) A. D. Hamilton, J.-M. Lehn and J. L. Sessler, *J. Am. Chem. Soc.*, **108**, 5158 (1986), and references cited therein; *b*) I. O. Sutherland, *Chem. Soc. Rev.*, **15**, 63 (1986).

10) I. Tabushi, Y. Kuroda and K. Shimokawa, *J. Am. Chem. Soc.*, **101**, 1614 (1979).

11) *a*) C.-F. Rai, K. Odashima and K. Koga, *Tetrahedron Lett.*, **26**, 5179 (1985); *b*) Y. Murakami, J. Kikuchi and H. Tenma, *J. Chem. Soc., Chem. Commun.*, **1985**, 753; *c*) Y. Murakami, J. Kikuchi and T. Hirama, *Chem. Lett.*, **1987**, 161.

12) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, 1969, Chapter 2.

13) M. W. Hosseini and J.-M. Lehn, *J. Am. Chem. Soc.*, **109**, 7047 (1987).

14) H.-D. Lutter and F. Diederich, *Angew. Int. Ed. Engl.*, **25**, 1125 (1986).

15) F. Lipmann, *Acc. Chem. Res.*, **6**, 361 (1973).

16) *a*) Peptide bond formation has been reported by using thioesters of *N*-protected α-amino acids, S. Yamada, Y. Yokoyama and T. Shioiri, *Experientia*, **32**, 967 (1976); *b*) Proton transfer was discussed as a rate determining step in aminolysis of thioesters, W. P. Jencks, in ref. 9, Chapter 10.

17) W. P. Jencks, in ref. 9, Chapter 1.

18) S. Yamada, Y. Yokoyama and T. Shioiri, *J. Org. Chem.*, **39**, 3302 (1974).

19) Selective monoacylations of dithiol-bearing crown ethers have been successfully achieved *via* intra-complex thiolysis, S. Sasaki, K. Ohta and K. Koga, Proc. 12th Int. Symp. on Macrocyclic Chemistry, Hiroshima, July 1987, p. 230.

20) The racemization problem during this peptide synthesis will be discussed elsewhere.

21) *a*) K. Koga and S. Sasaki, *Pure Appl. Chem.*, **60**, 539 (1988); *b*) S. Sasaki and K. Koga, *J. Inclusion Phenomena*, in press.

22) *a*) P. J. Feit, *J. Med. Chem.*, **7**, 14 (1964); *b*) K. Mori, *Tetrahedron*, **30**, 4223 (1974).

23) S. Yamada, Y. Kasai and T. Shioiri, *Tetrahedron*, **32**, 2211, 2854 (1976).

24) J. H. Jones, B. Liberek and G. T. Young, *J. Chem. Soc., C*, **1967**, 2371.

25) W. Holley and A. D. Holley, *J. Am. Chem. Soc.*, **74**, 3069 (1952).

26) H. Breckman and M. Spirngorun, *Tetrahedron Lett.*, **1965**, 837.