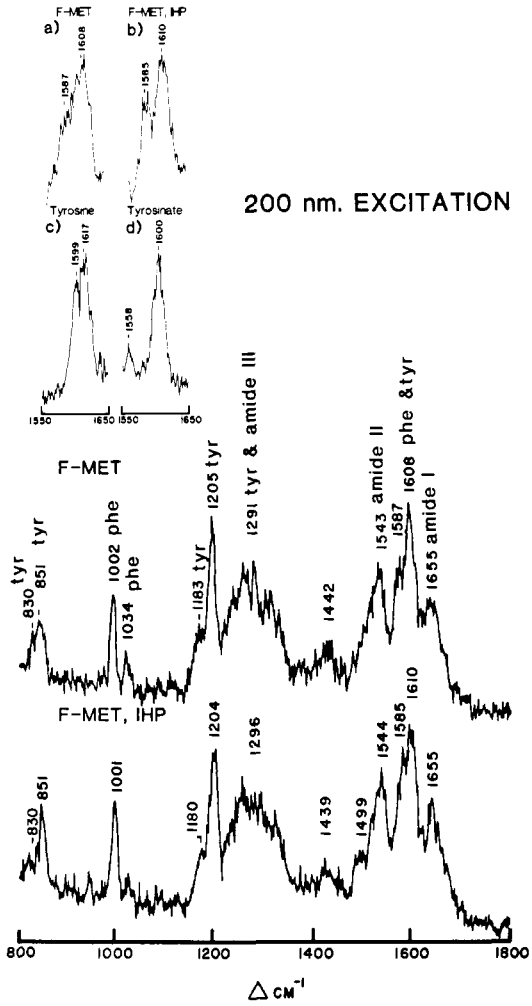


Figure 2. As for Figure 1, but with 200-nm excitation. Each spectrum is the sum of 20 scans collected with 0.05 Å/s accumulation time. Inset: comparison of the high-frequency region of the 200-nm excited RR spectra of 200 μM metHb(F^-) without (a) and with (b) IHP, 600 μM tyrosine, pH 7.0 (c), and 600 μM tyrosinate, pH 12.0 (d). The protein spectra are each the sum of 30 scans, while the amino acid spectra are each the sum of 20 scans.

to the 270-nm region, analogous to the shift for nucleotide bases stacked in nucleic acid duplexes from the 260 nm to longer wavelength transitions.[14]

The 200-nm RR spectra (Figure 2) show strong enhancement of Tyr modes but also contributions from peptide vibrations (amide I, II, and III) as well as from Phe.[9] Hb A contains six pairs of symmetry-related tyrosines. One of these, α42, is H bonded to a negatively charged carboxylate group (Asp β99) in the T state, but an uncharged backbone carbonyl in the R state; the other tyrosines do not change their H-bonding significantly.[13,15] Pronounced alterations in the metHb(F^-) UV absorption at 279 and 287 nm upon IHP addition have been associated with tyrosine H-bond effects.[5] They can likewise account for the change in the 1600-cm^{-1} region of the 200-nm RR spectrum, which is highlighted in the inset of Figure 2, where it is compared with the 200-nm RR spectra of tyrosine and tyrosinate.[10] Ionization of tyrosine shifts the 1617- and 1599-cm^{-1} bands (ν_{8a} and ν_{8b}) to 1600 and 1558 cm^{-1}. Addition of IHP to metHb(F^-) produces a change in the 1608-cm^{-1} band shape and an apparent augmentation in the 1587-cm^{-1} shoulder. Both of these bands contain Phe as well as Tyr contributions,[9,10] but computer subtraction of the two spectra indicates a shift in the Tyr mode from 1617 and 1599 cm^{-1} to 1605 and 1582 cm^{-1}. While better data will be needed to confirm these values, they are suggestive of strong H bonding of Tyr protons, producing shifts in the direction of those seen upon deprotonation, as expected for a strong H bond to a carboxylate group. It may also be significant that the intensity ratio of the 830-851-cm^{-1} tyr doublet changes appreciably upon IHP addition (Figure 2). This doublet has been shown[15] to be sensitive to H bonding, but the relationships established for visible excitation are inapplicable to UV-RR spectra because of specific intensity changes at resonance;[10] the UV systematics have yet to be worked out.

These results demonstrate the feasibility of establishing conformational markers in the UV Raman spectra of hemoglobin, associated with the environment of tryptophan and tyrosine residues. These should be useful in studies of the structural dynamics associated with hemoglobin cooperativity.

Acknowledgment. We thank Dr. R. P. Rava for initiating this project and for useful discussions. Prof. M. F. Perutz kindly read the manuscript and made helpful comments. This work was supported by NSF Grant CHE-8106084 and NIH Grant GM33576.

(14) (a) Saracchi, M.-T.; Guschlbauer, W. *Eur. J. Biochem.* **1973**, *34*, 232-240. (b) Rimai, L.; Maher, V. Am.; Gill, D.; Salmeen, I.; McCormick, J. J. *Biochim. Biophys. Acta* **1974**, *361*, 155-165.

(15) Shaanan, B. *J. Mol. Biol.* **1983**, *171*, 31.

(16) Siamiwiza, M. N.; Lord, R. C.; Chen, M. C.; Takamatsu, T.; Harada, I.; Matsuura, H.; Shimanouchi, T. *Biochemistry*, **1975**, *14*, 4870.

(17) Asher, S. A.; Vickey, L. E.; Schuster, T. M.; Sauer, K. *Biochemistry* **1977**, *16*, 5849-5856.

Functionalized Crown Ethers as an Approach to the Enzyme Model for the Synthesis of Peptides†,1

Shigeki Sasaki, Mitsuhiko Shionoya, and Kenji Koga*

Faculty of Pharmaceutical Sciences
University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113, Japan
Received December 3, 1984

Formation of molecular complexes between organic hosts and guests has been achieved in several artificial systems[2] and successfully applied to enzyme models in the sense that host-guest complexes are formed prior to the reactions. However, only a limited number of such enzyme models have been designed for bimolecular synthetic reactions.[3] Here we wish to report a novel type of host as an approach to the enzyme model for the synthesis of peptides.

We tried to design novel hosts based on the general concept of enzyme catalysis,[4] in which the reactive covalent intermediate ($E-S_1$) is formed from the noncovalent complex ($E\cdots S_1$) and then reacts with the second substrate (S_2) to give the product (S_1-S_2) as shown in eq 1. Previous papers have reported that thio-bearing

$$E + S_1 \rightleftharpoons E\cdots S_1 \rightarrow E-S_1 \xrightarrow{S_2} E + S_1-S_2 \qquad (1)$$

† This paper is dedicated to Professor Shun-ichi Yamada on the occasion of his 70th birthday.

(1) A part of this work was presented at the Second International Kyoto Conference on New Aspects of Organic Chemistry, Kyoto, Japan, Aug 1982.

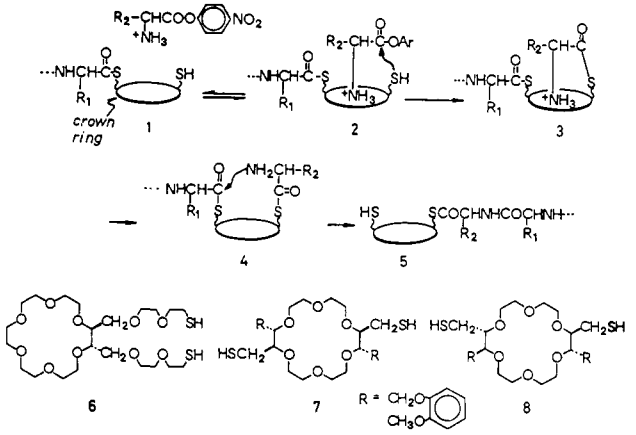
(2) (a) Cyclodextrins: Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer-Verlag: New York, 1977. Breslow, R. *Science (Washington, D.C.)* **1982**, *218*, 532. Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66. Komiyama, M.; Bender, M. L. In "The Chemistry of Enzyme Action"; Page, M. I., Ed.; Elsvier: Amsterdam, 1984; Chapter 14. (b) Crown ethers: de Jong, F.; Reinhoudt, D. N. "Stability and Reactivity of Crown Ether Complexes"; Academic Press: London, 1981. Stoddart, J. F. In "The Chemistry of Enzyme Action"; Page, M. I., Ed.; Elsevier: Amsterdam, 1984; Chapter 15. (c) Cyclophanes: Odashima, K.; Koga, K. In "Cyclophanes"; Keehn, P. M., Rosenfeld, S. M., Eds.; Academic Press: New York, 1983; Vol. 2, Chapter 11. Sutherland, I. *Ibid.* Vol. 2, Chapter 12. Tabushi, I.; Yamamura, K. *Top. Curr. Chem.* **1983**, *113*, 145. Murakami, Y. *Ibid.* **1983**, *115*, 107.

(3) (a) Murakami, Y.; Aoyama, K.; Dobashi, K.; Kida, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3633. (b) Tabushi, I.; Kuroda, Y.; Shimokawa, K. *J. Am. Chem. Soc.* **1979**, *101*, 1614. (c) Kotzyba-Hibert, F.; Lehn, J. M.; Viering, P. *Tetrahedron Lett.* **1980**, *21*, 741. (d) Rideout, D. C.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *102*, 7816. (e) Tabushi, I.; Yamamura, K.; Fujita, K.; Kawakubo, H. *J. Am. Chem. Soc.* **1979**, *101*, 1019. (f) Breslow, R.; Campbell, P. *J. Am. Chem. Soc.* **1969**, *91*, 3085.

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

0002-7863/85/1507-3371$01.50/0

Scheme I



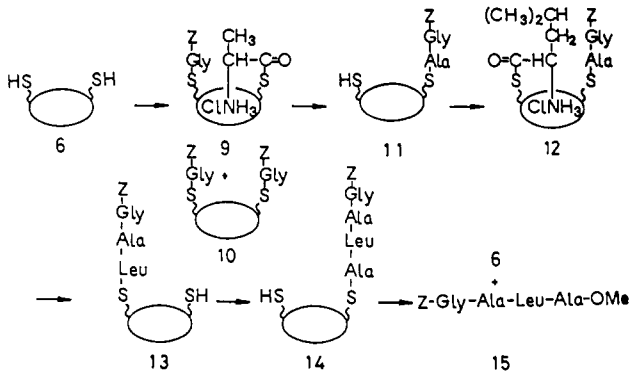
crown ethers showed rate enhancement in the thiolysis of α-amino ester salts to give the corresponding thio esters.[5] Evaluating such a thio ester as a reactive covalent intermediate, a novel type of crown ether (**1**), having one thiol and one thio ester with an N-protected α-amino acid or peptide, was designed as an enzyme model for the synthesis of peptides as shown in Scheme I.

We expected the following: (1) Crown ether **1** catches an α-amino acid *p*-nitrophenyl ester salt by forming a noncovalent complex **2**. Subsequently, thiolysis occurs at an enhanced rate[5] to give the corresponding bis(thio ester) **3**. Now, two substrates are forced to be assembled in proximity. (2) In **3**, aminolysis (S–N acyl transfer) occurs at an enhanced rate, due to the intramolecular nature of the reaction, to form a peptide bond.[6,7] The aminolysis product **5** has one regenerated thiol and one thio ester with an N-protected peptide and therefore is functionally equivalent to **1**. (3) Further elongation of peptide chain can be achieved by repeating the above processes, which formally means turnover.

Novel crown ethers having two thiol groups with long chains in vicinal positions (**6**),[8b,c] with short chains on the same side (**7**),[8a,c] and on the opposite side (**8**)[8a,c] were synthesized from L-(+)-tartaric acid by unequivocal methods.[9] Rate enhancement in thiolysis by these crown ethers is expected from the previous result.[5] Preliminary studies have shown that the intramolecular aminolysis via large-membered transition states (22-, 18-, and 19-membered cyclic transition states were expected for **6**, **7**, and **8**, respectively, in the step from **3** to **5** in Scheme I) occurs at enhanced rate compared with the corresponding intermolecular aminolysis.[10]

An example is shown in Scheme II. The host **6** was acylated with Z-Gly-OH (DEPC[11] and TEA in DMF, −5 °C, 1 h), and the resulting mixture of mono- and diacylated crown ethers was treated with L-Ala-ONp·HBr (room temperature, 30 min).[12] The

Scheme II



desired crown ether hydrochloride **9**[8c] was isolated in 33% overall yield from **6**.[13] Intramolecular aminolysis of **9** was carried out in benzene (room temperature, 10 h)[14] to produce *S*-(Z-glycyl-L-alanyl)crown ether **11**[8c] in 81% yield. The crown ether **11** was again treated with L-Leu-ONp·HBr (room temperature, 30 min),[12] and the crown ether hydrochloride **12**[8c] was isolated in 87% yield. The intramolecular aminolysis was again carried out in benzene (room temperature, 2 day)[14] to give *S*-(Z-glycyl-L-alanyl-L-leucyl)crown ether **13**,[8c] (58%). The same thiolysis with L-Ala-ONp·HBr (room temperature, 30 min)[12] and the intramolecular reaction (benzene–ethyl acetate, room temperature, 3 day)[14] gave the *S*-(Z-glycyl-L-alanyl-L-leucyl-L-alanyl)crown ether 14[8c] in 49% yield from **13**. The tetrapeptide Z-Gly-L-Ala-L-Leu-L-Ala-OMe (**15**)[15] was isolated in 47% yield after methanolysis (K_2CO_3 in MeOH, room temperature, 1 h) followed by purification using column chromatography (silica gel, ethyl acetate–hexane).

The host **7** and **8** have also shown the similar reactivities in both thiolysis and aminolysis. The tripeptide Z-Gly-L-Ala-L-Leu-OMe isolated by methanolysis (Na_2CO_3 in MeOH, room temperature, 2 h) from *S*-(Z-glycyl-L-alanyl-L-leucyl) derivative[8c] of **8** was found to contain ca. 2% of its diastereomer.[16] In the intramolecular aminolysis step[14] to give *S*-(Z-glycyl-L-alanyl)crown ethers, it is shown that the rate of the reaction using **8** (6.5 h, 94%) is faster than those of **6** (10 h, 81%) and **7** (8.5 h,[17] 66%). It is also recognized that the addition of NaSCN (3 equiv to crown ether) into the reaction mixture of the intramolecular aminolysis[14] has strong effects, resulting in complete inhibition of the reaction using **8**, with some accerelation of that using **7**.[18] This result shows that the complexation ability of crown ether with metal cation is useful also in the control (acceleration and inhibition) of the intramolecular aminolysis.

Thus, peptide bond synthesis by means of intracomplex thiolysis followed by intramolecular aminolysis and peptide bond elongation by repeating the above two processes have been realized by the novel crown ethers. These compounds have a single binding site (18-crown-6 moiety for primary ammonium ion or metal cation) and two reaction sites (two thiol groups for two substrates) and may be regarded as artificial enzyme mimics for the synthesis of peptides. Further studies for more efficient crown ethers are now in progress.

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

(5) (a) Chao, Y.; Cram, D. J. *J. Am. Chem. Soc.* **1976**, *98*, 1025. Chao, Y.; Weisman, G. R.; Sogah, G. D. Y.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 4948. (b) Matsui, T.; Koga, K. *Tetrahedron Lett.* **1978**, 1115. Matsui, T.; Koga, K. *Chem. Pharm. Bull.* **1979**, *27*, 2295. Sasaki, S.; Koga, K. *Heterocycles* **1979**, *12*, 1305. (c) Lehn, J. M.; Sirlin, C. *J. Chem. Soc., Chem. Commun.* **1978**, 949.

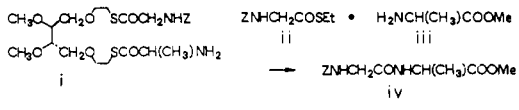
(6) Yamada, S.; Yokoyama, Y.; Shioiri, T. *Experientia* **1976**, *32*, 967.

(7) Biosynthesis of gramicidin S via thio esters of α-amino acids has been reported. Lipmann, F. *Acc. Chem. Res.* **1973**, *6*, 361.

(8) (a) Elemental analysis (C,H,N) was satisfactory. (b) Molecular ion gave the expected value in its high-mass spectrum. (c) ^{1}H NMR spectrum was reasonable.

(9) The synthesis of **6**, **7**, and **8** will be reported elsewhere. Physical data for **6**, **7**, **8**: **6**, oil, $[\alpha]^{20}_D$ +0.5° ($CHCl_3$); **7**, mp 92–94 °C, $[\alpha]^{20}_D$ −7.0° ($CHCl_3$); **8**, mp 103–105.5 °C, $[\alpha]^{20}_D$ −4.9° ($CHCl_3$).

(10) The intramolecular aminolysis of **i** was completed within 15 h (0.005



M each of TEA, pivalic acid, and **i** in benzene at room temperature, monitored by HPLC). Less than 10% of **iv** was formed from **ii** and **iii** under the same condition.

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

(12) Intracomplex thiolysis was performed by using 0.05 M each of crown ether and α-amino acid *p*-nitrophenyl ester hydrobromide in pyridine.

(13) The reaction mixtue was poured into 10% aqueous HCl, and the whole was first extracted with ether to give **10**[8a,c] (44% from **8**). Further extraction with $CHCl_3$ afforded **9**.

(14) Intramolecular aminolysis was performed by using 0.005 M crown ether and 0.15 M each of TEA and pivalic acid in benzene or benzene-ethyl acetate.

(15) Identified with the authentic sample[8a] (mp 185–187 °C, $[\alpha]^{20}_D$ −60.2° (MeOH)) prepared by the conventional method.

(16) The diastereomers were separated by HPLC (Waters, Radial Pack B, 20% Et_2O in AcOEt) and the main isomer was identified with the authentic sample[8a] (mp 106–107 °C, $[\alpha]^{20}_D$ −43° (MeOH)). Under the same methanolysis condition, optically pure (Z-L-Ala-L-Ala-$SCH_2CH_2)_2O$ prepared by the conventional method gave Z-L-Ala-L-Ala-OMe contaminated with ca. 1.5% of its diastereomer.

(17) The reaction was not completed by TLC monitoring.

(18) The reaction was completed after 6 h by TLC monitoring.