Design of the Synthetic Route for Helical Peptides.[1] Synthesis and Solubility of Model Peptides Having a Helical Structure

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Fragment condensations between amino- and carboxyl-components of hydrophobic decapeptides, which have a β-sheet structure in the solid state and are insoluble in DMF, NMP, and DMSO, were examined in a mixture of CH_2Cl_2 and TFE (4/1, v/v) using various coupling reagents. By the use of DCC and HOBt as coupling reagents, the reaction proceeded smoothly in moderate yield to give eicosapeptides. These have a stable helical structure in the solid state and are easily soluble in a variety of organic solvents. The hydrophobic eicosapeptides obtained were subjected to a successive coupling reaction in CH_2Cl_2 alone to give helical tetracontapeptides in high yield, which also have high solubility in various organic solvents of low polarity. The solubility of hydrophobic helical peptides presents a solubility feature of helical peptides which are obtained as peptide intermediates in protein synthesis. The synthetic strategy for helical peptides and proteins is discussed in connection with the solubility prediction method.

One of the most serious obstacles in peptide and protein syntheses is the insolubility of protected peptides in organic solvents, which causes difficulty regarding successive reactions. In previous papers[2,3] we proposed the solubility prediction method for protected peptides and showed its usefulness for the design of synthetic routes for peptides and proteins. In this method, peptides which were to be made an object of the prediction were as long as or larger than an octapeptide. Solvents to be done were such as DMF, DMA, NMP, DMSO, and HMPA, which are commonly used for peptide and protein synthesis. Furthermore, the prediction method was practically based on the following four assumptions: (1) a tertiary peptide bond, such as an X-Pro, separates a peptide to peptide segments with respect to the peptide structure (peptide segment separation); (2) the insolubility of protected peptides is caused by a β-sheet aggregation consisting of peptide segments as long as or larger than an octa- or nonapeptide sequence; (3) peptides having helical and/or coiled structures have high solubility in organic solvents; and (4) the potential of peptide segments for a β-sheet formation is estimated using their average coil conformation $\langle P_c \rangle$ values when a peptide is smaller than the critical chain length for the development of a stable helix in the solid state.

In a solubility prediction for protected peptides,[2] the average coil conformation $\langle P_c \rangle$ value was practically applied to a whole peptide instead of a peptide segment, and the adequacy of the above assumptions was widely supported by the application of the solubility prediction method to various kinds of oligopeptides.[4-10] Especially, the potential of protected peptides for β-sheet formation was relevantly estimated using their $\langle P_c \rangle$ values rather than their average β-sheet conformation $\langle P_\beta \rangle$ values when they were smaller than the critical chain length for the development of a stable helix in the solid state. In agreement with the estimation, octa- to tetradecapeptides having a high potential for a helix formation actually had a β-sheet structure in the solid state and were insoluble in DMF, DMA, NMP, DMSO, and HMPA. On the other hand, eicosapeptides having a high potential for helix formation were assumed to be larger than the critical size for the development of a stable helix in the solid state,[11] and were expected to be easily soluble in various organic solvents. Therefore, it is quite important how peptide intermediates larger than the critical chain length for the development of a stable helix in the solid state are prepared.

We recently found that a mixture of CH_2Cl_2 and a strong electron-acceptor solvent such as HFIP, TFE, or phenol have a high solubilizing potential for protected octa- to tetradecapeptides to be insoluble in DMF, DMA, NMP, DMSO, and HMPA.[12,13] This fact suggests that their fragment condensation in a mixture of CH_2Cl_2 and TFE is excellent for the preparation of peptide intermediates larger than the critical chain length for the development of a stable helix in the solid state. In the present study we practically examined fragment condensation between amino and carboxyl components of hydrophobic decapeptides in a mixture of CH_2Cl_2 and TFE (4/1, v/v) using various coupling reagents and demonstrated the usefulness of the mixture for fragment condensation. Here, we also discuss the solubility of helical peptides in organic solvents, and a synthetic strategy for helical peptides and proteins is proposed.

Results

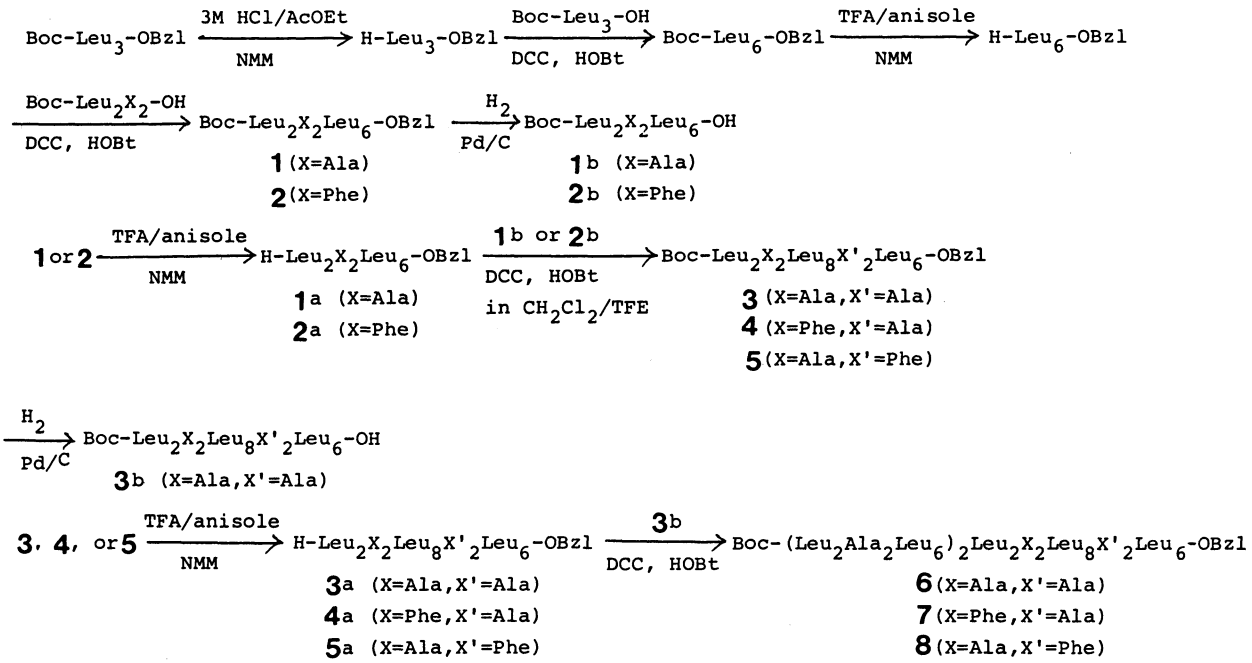
Fragment Condensations between Amino and Carboxyl Components of Decapeptides. Two hydrophobic decapeptides (Boc-$Leu_2Ala_2Leu_6$-OBzl, **1** and Boc-$Leu_2Phe_2Leu_6$-OBzl, **2**) were prepared by the usual method, as shown in Scheme 1, and were used for the present study. Since they are smaller than the

critical chain length for the development of a stable helix in the solid state, they actually have a β-sheet structure in the solid state and are insoluble in DMF, NMP, and DMSO. Due to their insolubility in a mixture of MeOH and AcOH, the removal of their Bzl group was performed by hydrogenolysis in a mixture of HFIP and AcOH using Pd/C as a catalyst to give carboxyl components **1b** and **2b**. The liberation of their amino groups was carried out by treatment with TFA/anisole (9/1, v/v), followed by treatment with NMM to give amino components **1a** and **2a**. These products were used as amino and carboxyl components of decapeptides in order to prepare three eicosapeptides, **3—5**, as shown in Scheme 1. The amino and carboxyl components of decapeptides were also insoluble in DMF, NMP, and DMSO. Their fragment condensation in these solvents was difficult. On the other hand, they were readily soluble in a mixture of CH_2Cl_2 and TFE (4/1, v/v) and their fragment condensation proceeded smoothly in moderate yield using DCC and HOBt as coupling reagents. As a coupling reagent in the reaction of **2a** with **1b**, DCC was superior to EEDQ, DEPC, and DPPA, and the combination of DCC and HOBt gave the highest yield (Table 1).

Fragment Condensations between Amino and Carboxyl Components of Eicosapeptides. Eicosapeptides **3—5** were easily soluble in a variety of organic solvents, and the liberation of their amino groups was readily performed using the same method as described for the decapeptides to give amino components **3a—5a**. The removal of the Bzl group from the eicosapeptide **3** was also readily performed by hydrogenolysis in a mixture of MeOH and AcOH using Pd/C as a catalyst to give the carboxyl component **3b**. Successive fragment condensation between amino and carboxyl components of eicosapeptides were smoothly carried out in CH_2Cl_2 alone using DCC and HOBt as coupling reagents to produce the corresponding tetracontapeptides **6—8** in high yield (Scheme 1).

Table 1. Results of Fragment Condensation between Amino and Carboxyl Components of Decapeptides

Amino component	Carboxyl component (equivalent)	Coupling reagent (equivalent)	Product	Yield %
1a	**1b** (3)	DCC, HOBt (3)	**3**	58
1a	**2b** (3)	DCC, HOBt (3)	**4**	35
2a	**1b** (3)	DCC, HOBt (3)	**5**	48
2a	**1b** (3)	DCC, HOSu (3)	**5**	11
2a	**1b** (3)	DCC, HONB (3)	**5**	23
2a	**1b** (3)	EEDQ (3)	**5**	32
2a	**1b** (3)	DEPC (3)	**5**	below 2
2a	**1b** (3)	DPPA (3)	**5**	below 2



Scheme 1.

Table 2. Solubility Properties[a] of the Peptides (c=1.0 g dl^{-1}).

Compound ( )[c]	Solvent[b]												
	HMPA	DMSO	DMF	NMP	MeOH EtOH	DCM THF $CHCl_3$	AC	AcOEt	AN	TFE	DCM -TFE (4:1)	HFIP	DCM -HFIP (4:1)
1 (10)	A	C	D	C	D	D	D	D	D	A	A	C	A
2 (10)	C	C	D	C	D	D	D	D	D	D	A	A	A
3 (20)	A	D	A	A	A	A	A	B	B	A	A	A	A
4 (20)	B	D	A	A	C,[d] B[e]	A	B	B	B	A	A	A	A
5 (20)	A	D	A	A	B	A	A	B	B	A	A	A	A
6 (40)	A	D	D	B	D	A	B	B	D	C	A	C	A
7 (40)	A	D	D	B	D	A	B	B	D	C	A	C	A
8 (40)	A	D	D	B	D	A	B	B	D	C	A	C	A

a) Solubility: A, soluble at room temperature; B, soluble at 80 °C or refluxing temperature; C, partially soluble at 80 °C or refluxing temperature; D, practically insoluble at 80 °C or refluxing temperature. b) Abbreviations: DCM, dichloromethane; THF, tetrahydrofuran; AC, acetone; AN, acetonitrile. Others, see Ref. 1. c) Number of amino acid residues of the peptide. d) In the case of MeOH. e) In the case of EtOH.

Conformations and Solubility Properties of the Peptides 1—8. The IR absorption spectra of decapeptides **1** and **2** in the solid state showed strong bands at 3280 cm^{-1} in the amide A region and at 1630 cm^{-1} in the amide I region, indicating a β-sheet structure in the solid state.[14] On the other hand, those of eicosapeptides **3**—**5** and tetracontapeptides **6**—**8** in the solid state showed strong bands at 3330—3320 cm^{-1} in the amide A region and at 1660—1650 cm^{-1} in the amide I region, indicating a helical structure in the solid state.[14]

Table 2 shows the solubility properties of peptides **1**—**8** in a variety of organic solvents. As expected from their conformations, decapeptides **1** and **2**, having a β-sheet structure, were practically insoluble in various organic solvents, while eicosapeptides **3**—**5** and tetracontapeptides **6**—**8**, having a stable helix, were easily soluble in various organic solvents. As reported for octa- to tetradecapeptides having a β-sheet structure in the solid state,[12,13] decapeptides **1** and **2** were also easily soluble in mixtures of CH_2Cl_2 and TFE and of CH_2Cl_2 and HFIP since TFE and HFIP have a high potential for a β-sheet structure disruption due to their strong electron-accepting property. In fact, the IR absorption spectra of the amino and carboxyl components of decapeptides **1a** and **1b** in a mixture of CH_2Cl_2 and TFE showed a sharp, strong band around 1660 cm^{-1} in the amide I region, suggesting a large contribution of a helical or unordered structure and to be free from a β-sheet structure in the solution. Those of the amino and carboxyl components of eicosapeptides **3a** and **3b** in CH_2Cl_2 alone showed bands around 3320 cm^{-1} in the amide A region and around 1655 cm^{-1} in the amide I region, indicating that they have a stable helical structure in CH_2Cl_2.

Discussion

It was shown that the synthetic strategy used for the preparation of helical eicosa- and tetracontapeptides overcame the obstacle of the insolubility of peptide intermediates having a high potential for helix formation. A mixture of CH_2Cl_2 and TFE has a high solubilizing potential for decapeptides to be insoluble in highly polar solvents which are commonly used in peptide and protein syntheses. Fragment condensations between amino- and carboxyl-components of hydrophobic decapeptides in the mixture could be smoothly carried out using DCC and HOBt as coupling reagents. Although the yield was not so high due to the high nucleophilicity of TFE, as exhibited in peptide bond formation in TFE and HFIP,[15] the result indicates that the mixture was excellent for a coupling reaction between deca- to tetradecapeptides having a high potential for a helix formation. As expected from the solubility prediction method,[2] the eicosapeptides obtained were easily soluble in a variety of organic solvents (listed in Table 2). As a result, fragment condensation between eicosapeptides could be readily performed in CH_2Cl_2 alone to give the corresponding tetracontapeptides in high yield, indicating that peptides having a stable helical structure in the solid state can be coupled in CH_2Cl_2 to achieve further peptide chain elongation without any difficulty. Concerning the critical chain length of protected peptides for the development of a stable helix in the solid state, the solubility and conformational behavior of eicosapeptides **3**—**5** indicate that it is sufficient at an eicosapeptide level when protected peptides have a high potential for the formation of a helical structure.

Here, it becomes important to know how to estimate the helix-forming potential of protected peptides. In previous papers[16-18] we showed that the potential can be pertinently estimated using their average helix conformation $\langle P_\alpha \rangle$ values. In fact, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values of protected peptides were useful for estimating their potential for the β-sheet $\rightarrow$ helix transformation with an increase in the peptide chain length or on the application of strong shear stress.

Both values were also useful for estimating the potential of protected peptides for a β-sheet structure-disruption by the adding of strong electron-acceptor solvents. The $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values of the decapeptide **1** are obtained by taking conformational parameters of P_α and P_β to be 1.36 and 1.17, respectively, and those of decapeptide **2** to be 1.30 and 1.23, respectively. Therefore, the amino and carboxyl components of decapeptides **1** and **2** are estimated to have high potentials for a helix formation and for a β-sheet-structure disruption by strong electron-acceptor solvents. In fact, the β-sheet structure of decapeptides **1a** and **1b** was disrupted in a mixture of CH_2Cl_2 and TFE, and fragment condensation between amino and carboxyl components of decapeptides proceeded smoothly in the mixture using DCC and HOBt as coupling reagents to give helical eicosapeptides in moderate yield. Although the $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values of protected peptides are useful for estimating their helix-forming potential, we should make an effort to establish how to estimate a helix-forming potential of protected peptides exactly, since the conformational behaviors of protected peptides must reflect the individuality of amino acid residues in protected peptides.[17]

With respect to the conformation and solubility of protected peptides, the results of the peptide solubility (shown in Table 2) strongly indicate the adequacy of the assumption in the solubility prediction method. The solubility behavior of the β-sheet-forming decapeptides was the same as that discussed before.[12,13] Furthermore, the solubility behavior of the helix-forming eicosapeptides was similar to that of Aib-containing oligoleucines,[8] which have a stable helix in the smaller peptide size due to a helix-promoting potential of Aib. Actually, hydrophobic eicosapeptides **3—5** were easily soluble in a variety of organic solvents. On the other hand, hydrophobic tetracontapeptides **6—8** were less soluble in highly polar solvents and easily soluble only in solvents of low polarity. Namely, the eicosapeptides were more easily soluble in DMF, NMP, EtOH, and MeOH than the tetracontapeptides. This can clearly be attributed to a large contribution of polar N- and C-termini of a helix to solvation of the eicosapeptides. The high solubilizing potential of HMPA for the hydrophobic tetracontapeptides may be due to the richness of hydrocarbon moieties in HMPA. For the same reason, the solubilizing potential of NMP is higher than that of DMF.

The solubility data of hydrophobic helical peptides illustrate a solubility feature of helical peptides which are obtained as peptide intermediates in protein synthesis. Especially, the solubility of the hydrophobic tetracontapeptides indicates that the solubility depends on the side chain moieties of the helix and that interactions between intermolecular side chain moieties are not strong, even at a tetracontapeptide level. The helical peptides obtained as peptide intermediates in protein synthesis contain various kinds of amino acid residues. Thus, they are probably easily soluble in a variety of organic solvents. The results in the present study strongly suggest that the synthetic strategy for helical peptides **3—8** is applicable to the synthesis of proteins which consist of a few long helical structures. *E. coli* acidic ribosomal protein L12[19] is presumed to be an example.

Experimental

General. Uncorrected capillary melting points will be reported. The optical rotations were taken in a 1 cm-path length cell on a JASCO model ORD/UV-5 optical rotatory dispersion recorder. The amino acid compositions of acid hydrolysates were determined with a Shimadzu HPLC LC-3A all amino acid analysis system. The acid hydrolyses of the peptides were carried out with propionic acid/12 M† HCl (2/1, v/v) for 2 days at 115 °C in evacuated sealed tubes. The IR spectra in the solid state were measured on KBr disks using a JASCO DS 403 G IR spectrophotometer. It was observed that shear stress on peptides examined here scarcely affected the IR spectra. The IR spectra in solution were recorded with a JEOL JIR-100 FTIR spectrophotometer by employing 0.1 mm path length cells with potassium bromide windows.

Preparation of Boc-Leu$_2$Ala$_2$Leu$_6$-OBzl 1 and Boc-Leu$_2$Phe$_2$Leu$_6$-OBzl 2. Boc-Leu$_2$Ala$_2$-OBzl, Boc-Leu$_2$-Phe$_2$-OBzl, and Boc-Leu$_3$-OBzl were prepared by the usual stepwise elongation under the conditions previously described.[4] The Bzl group of these peptides was removed by the usual hydrogenolysis using Pd/C as a catalyst to obtain Boc-Leu$_2$Ala$_2$-OH, Boc-Leu$_2$Phe$_2$-OH, and Boc-Leu$_3$-OH, respectively. Boc-Leu$_6$-OBzl was obtained by fragment condensation of H-Leu$_3$-OBzl (obtained from Boc-Leu$_3$-OBzl by treatment with 3 M HCl/AcOEt, followed by treatment with NMM) with Boc-Leu$_3$-OH in CH_2Cl_2 using DCC and HOBt as coupling reagents.

Boc-Leu$_6$-OBzl 15 g (16.9 mmol) was treated with TFA/anisole (4/1, v/v) (30 ml) at 0 °C for 2 h. Then, the mixture was concentrated in vacuo. The addition of a small portion of hexane to the mixture and its removal were repeated several times. DMF (65 ml) was added to the residue, and the mixture was poured into water (400 ml). The resulting precipitate was filtered, washed with water, and dried. Yield of TFA·H-Leu$_6$-OBzl was 14 g (91%). To NMP (200 ml) solution of TFA·H-Leu$_6$-OBzl, NMM (5 equiv) was added and the mixture was stirred for 10 min at room temperature. Then, the mixture was poured into water (700 ml), and the precipitate was filtered, washed with water, and dried. Yield of H-Leu$_6$-OBzl was 13 g (97%).

To the ice-chilled NMP (50 ml) solution of H-Leu$_6$-OBzl 4 g obtained above, Boc-Leu$_2$Ala$_2$-OH (1.5 equiv) and HOBt (1.5 equiv), was added DCC (1.5 equiv) and the mixture was stirred at 0 °C for 3 h and then at room temperature overnight. The mixture was poured into water and the precipitate was filtered, washed successively with 5% $NaHCO_3$ and water, and dried. The crude product was treated twice with refluxing methanol. Yield of **1**; 4.9 g (76%). mp 273.5—274.5 °C, $[\alpha]_D^{20}=-170°$ (c=1, in CH_2Cl_2-

† 1 M=1 mol dm^{-3}.

TFE(4/1, v/v)). Found: C, 62.18; H, 9.20; N, 11.02%. Calcd for $C_{66}H_{114}O_{13}N_{10}\cdot H_2O$: C, 62.24; H, 9.18; N, 11.00%. Amino acid analysis; Ala 2.00; Leu 8.13.

The peptide **2** was obtained by the same procedure as described above using Boc-Leu_2Phe_2-OH in place of Boc-Leu_2Ala_2-OH. Yield of **2**: 82%. mp>300 °C, $[\alpha]_D^{20}=-70°$ (c=1, in CH_2Cl_2-TFE(4/1, v/v)). Found: C, 65.70; H, 8.73; N, 9.86%. Calcd for $C_{78}H_{122}O_{13}N_{10}\cdot H_2O$: C, 65.70; H, 8.77; N, 9.82%. Amino acid analysis: Leu, 7.63; Phe, 2.00.

Preparation of H-$Leu_2Ala_2Leu_6$-OBzl 1a and H-$Leu_2Phe_2Leu_6$-OBzl 2a. The peptide **1a** and **2a** were obtained from **1** and **2**, respectively, by their treatment with TFA/anisole (4/1, v/v) as described for preparation of H-Leu_6-OBzl. Yield of **1a**: 94%. Yield of **2a**: 94%.

Preparation of Boc-$Leu_2Ala_2Leu_6$-OH 1b and Boc-$Leu_2Phe_2Leu_6$-OH 2b. To the HFIP (5 ml) solution of **1** (1.5 g, 1.2 mmol) was added 10% Pd/C 0.15 g with acetic acid 3 ml and the mixture was stirred under hydrogen at atmospheric pressure for 3 days. After removing Pd/C by filtration and washing with a small amount of a mixture of HFIP and CH_2Cl_2, the joined filtrate and washings were poured into water (100 ml). The resulting precipitate was filtered, washed with water, and dried. Yield of **1b**: 1.35 g (97%). The peptide **2b** was prepared from **2** by the same procedure. Yield of **2b**: 99%.

Fragment Condensation between Amino and Carboxyl Components of Decapeptides. To the ice-chilled solution of an amino component decapeptide (**1a** or **2a**) 100 mg, a carboxyl component decapeptide (**1b** or **2b**) (3 equiv), and an additive (HOBt, HOSu, or HONB) (3 equiv) in CH_2Cl_2-TFE (4/1, v/v) (5 ml) was added DCC (3 equiv) with CH_2Cl_2-TFE (4/1, v/v) (5 ml). The mixture was stirred at 0 °C for 3 h and then at room temperature for 45 h. After removing *N,N*-dicyclohexylurea by filtration, the filtrate was concentrated in vacuo, and water (25 ml) was added to the residue. The resulting precipitate was filtered, washed successively with water, 10% citric acid, water, 5 % $NaHCO_3$, and water, and dried. The crude product was treated with refluxing dichloromethane (15 ml). After allowing the mixture to stand and cool to room temperature, the resulting precipitate was filtered off, and the filtrate was concentrated. The residue was treated with refluxing ethyl acetate (20 ml) in the same manner, and the product was obtained as a residue. A coupling reaction using EEDQ, DEPC, or DPPA was carried out essentially in the same manner as described above. Yields of the peptides **3**—**6** are listed in Table 1. Peptide **3**: mp>300 °C, $[\alpha]_D^{20}=-80°$ (c=1, in CH_2Cl_2). Found: C, 62.06; H, 9.02; N, 12.13%. Calcd for $C_{120}H_{212}O_{23}N_{20}\cdot H_2O$: C, 62.10; H, 9.29; N, 12.07%. Amino acid analysis: Ala, 4.00; Leu 15.70. Peptide **4**: mp>300 °C, $[\alpha]_D^{20}=0°$ (c=1, in CH_2Cl_2). Found: C, 62.89; H, 8.98; N, 11.30%. Calcd for $C_{132}H_{220}O_{23}N_{20}\cdot 3H_2O$: C, 63.18; H, 9.08; N, 11.16%. Amino acid analysis: Ala, 2.19; Leu 15.98; Phe, 2.00. Peptide **5**: mp>300 °C, $[\alpha]_D^{20}=0°$ (c=1, in CH_2Cl_2). Found: C, 64.37; H, 9.08; N, 11.30%. Calcd for $C_{132}H_{220}O_{23}N_{20}$: C, 64.10; H, 9.05; N, 11.33%. Amino acid analysis: Ala, 1.86; Leu, 16.01; Phe, 2.00.

Fragment Condensations between Amino and Carboxyl Components of Eicosapeptides. H-($Leu_2Ala_2Leu_6$)$_2$-OBzl, H-$Leu_2Phe_2Leu_8Ala_2Leu_6$-OBzl, and H-$Leu_2Ala_2Leu_8Phe_2Leu_6$-OBzl were prepared by the same procedure used for preparation of **1a** and **2a**, from the peptides **3**—**5** in 86, 80, and 89% yield, respectively. Boc-($Leu_2Ala_2Leu_6$)$_2$-OH was also prepared from **3** by the usual hydrogenolysis in MeOH-AcOH in 93% yield.

To an ice-chilled solution of an amino component peptide 120 mg, Boc-($Leu_2Ala_2Leu_6$)$_2$-OH (1.2 equiv), and HOBt (1.2 equiv) in CH_2Cl_2 (5 ml), was added DCC (1.2 equiv) with CH_2Cl_2 (3 ml), and the mixture was stirred at 0 °C for 3 h, and then at room temperature overnight. After the addition of a small amount of CH_2Cl_2, the mixture was filtered and the filtrate was concentrated in vacuo. The residue was washed successively with water, 10% citric acid, water, 5% $NaHCO_3$, and water, and dried. The crude product was treated with refluxing MeOH (20 ml). After standing, the mixture cooled to room temperature and the precipitate was filtered and recrystallized with ethyl acetate. Yields of the peptides **6**—**8** were 48, 60, and 54%, respectively. Peptide **6**: mp>300 °C, $[\alpha]_D^{20}=-40°$ (c=1, in CH_2Cl_2). Found: C, 61.07; H, 9.14; N, 12.02%. Calcd for $C_{228}H_{408}O_{43}N_{40}\cdot 5H_2O$: C, 61.02; H, 9.39; N, 12.48%. Amino acid analysis: Ala, 8.19; Leu, 32.00. Peptide **7**: mp>300 °C, $[\alpha]_D^{20}=0°$ (c=1, in CH_2Cl_2). Found: C, 62.41; H, 8.97; N, 11.71%. Calcd for $C_{240}H_{416}O_{43}N_{40}\cdot 5H_2O$: C, 62.12; H, 9.25; N, 12.07%. Amino acid analysis: Ala, 6.15; Leu 32.00; Phe, 1.83. Peptide **8**: mp>300 °C, $[\alpha]_D^{20}=0°$ (c=1, in CH_2Cl_2). Found: C, 62.02; H, 8.91; N, 11.62%. Calcd for $C_{240}H_{416}O_{43}N_{40}\cdot 5H_2O$: C, 62.12; H, 9.25; N, 12.07%. Amino acid analysis: Ala, 6.11; Leu 32.00, Phe, 1.86.

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1) The abbreviations for amino acids are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). Amino acid symbols except for Gly denote the L-configuration. Additional abbreviations used are the following: DMF, *N,N*-dimethylformamide; NMP, *N*-methylpyrrolidone; DMSO, dimethyl sulfoxide; TFE, 2,2,2-trifluoroethanol; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxy-1*H*-benzotriazole; DMA, *N,N*-dimethylacetamide; HMPA, hexamethylphosphoric triamide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; Boc, *t*-butoxycarbonyl; OBzl, benzyl ester; MeOH, methanol; AcOH, acetic acid; TFA, trifluoroacetic acid; NMM, *N*-methylmorpholine; AcOEt, ethyl acetate; HOSu, *N*-hydroxysuccinimide; HONB, *N*-hydroxy-5-norbornene-2,3-dicarboximide; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; DEPC, diethoxyphosphoryl cyanide; DPPA, diphenylphosphoryl azide; IR, infrared; EtOH, ethanol.

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