Sequential Hydrophobic Polypeptides Consisting of Leucine and Isoleucine. Synthesis, Conformational Characterization in the Solid State, and Governing Factors for the Specification in the β-Structure/α-Helix Decision

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ABSTRACT: Six new sequential hydrophobic polypeptides consisting of leucyl and isoleucyl residues were synthesized by polycondensation of peptide active esters. Solid samples of these polypeptides were obtained by fast reprecipitation from a solution in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) or HFIP/dichloroacetic acid with diethyl ether. The conformation of the solid sample was determined by IR spectroscopy as follows: the β-structure for $(Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$, a mixture of the β-structure and α-helix for $(Leu\text{-}Ile\text{-}Leu)_n$, and the α-helix for $(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$, $(D\text{-}Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$, $(Leu\text{-}Ile\text{-}Ile\text{-}Gly)_n$, and $(Leu\text{-}Ile\text{-}Ile\text{-}Aib)_n$. The factor governing the specification of the β-structure/α-helix was deduced to be the consecutiveness of the tidily aligned hydrophobic side chains of the amino acid residues to afford sterically well-regulated intermolecular aggregation of the polypeptides prior to the conformational preference of the individual amino acid residue.

Introduction

There have been numerous studies of the statistical elucidation of protein secondary structures,[1−5] mainly for the purpose of the prediction of their higher ordered structures. Nagano estimated the contribution of individual amino acid residues to the conformation of proteins based on the statistical probability of the occurrence of each conformation.[1] Chou and Fasman predicted the secondary structure of proteins, using globular protein models, and introduced the conformational parameters P_α and P_β for 20 amino acid residues.[2] This method predicted the secondary structures of some proteins with 80% accuracy. They extended the prediction method by use of the sequences of amino acids.[3] Busetta and Hospital developed a prediction method for the secondary structures of proteins based on the occurrence porbabilities of specific higher structure in relation to the amount of each amino acid residue.[4] Chang and his co-workers improved the prediction accuracy of the Chou and Fasman method by 8%.[5] Now, these prediction methods have reached a nearly satisfactory level of accuracy. In order to attain higher prediction performance, other factors for the formation of secondary structures in addition to the conformational preference of each amino acid residue should be considered based on the accumulated data on the conformational characteristics of polypeptides having various sequences of amino acids.

The solid-state conformation of sequential polypeptides, on the other hand, has scarcely been investigated, though it may be very important, because these polypeptides might be one of the potential candidates for structural organic materials which can be fully metabolized ecologically in the future, such as coating materials on a metal surface, fusible materials in the surface of the earth, or materials that support absorption of drugs into the human body. The conformational information of them in the solid state is essential for the application of the sequential polypeptides for such uses.

We embarked on research to increase our understanding of the factors for the specification in the β-structure/α-helix decision by determination of the structures of hydrophobic polypeptides with various sequences of amino acids in the solid state by IR spectroscopy.[6−8] The solid state of these polypeptides obtained by fast precipitative transformation from a solution state is considered to be affected by the polypeptide structure in solution.[9] The IR spectroscopic analysis is the most suitable conventional method for this purpose, because the amide I, II, and V bands are characteristic of the individual secondary structures of the polypeptides.[10−17]

We designed and synthesized polypeptides $(Leu\text{-}Ile\text{-}Leu)_n$ (**1**) and $(Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**2**) as leading polypeptides in this study which consist of leucine and isoleucine residues. Leucine has a strong preference for forming the α-helix (Leu: $P_\alpha = 1.34$ and $P_\beta = 1.22$) and isoleucine has a very strong preference for forming the β-structure (Ile: $P_\alpha = 1.09$ and $P_\beta = 1.67$).[5] We have introduced perturbation on the sequence of Leu-Ile-Ile-Leu by substituting an amino acid residue with another one: $(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**3**), $(D\text{-}Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**4**), $(Leu\text{-}Ile\text{-}Ile\text{-}Gly)_n$ (**5**), and $(Leu\text{-}Ile\text{-}Ile\text{-}Aib)_n$ (**6**). Alanine has a similar preference for forming the α-helix (Ala: $P_\alpha = 1.41$ and $P_\beta = 0.72$)[5] but has a smaller hydrophobic side chain in comparison with leucine. D-Leucine is the stereoisomer of leucine, and glycine is an amino acid without a side chain having a tendency to break both the α-helix and β-structure (Gly: $P_\alpha = 0.43$ and $P_\beta = 0.58$). α-Aminoisobutyric acid (Aib) is an amino acid α,α-disubstituted by hydrophobic methyl groups and is considered to strongly promote helical folding because of the steric hindrance of the dimethyl groups.[18]

We communicated preliminarily a part of the results of this study.[19] In this paper, we wish to report the synthesis of the sequential polypeptides by polycondensation of the corresponding tetra- or tripeptide active esters as a monomer, solidification of the polypeptides by precipitation from a solution state, a refined characterization of the solid-state structures of the precipitated polypeptides, and clarification of the factors governing the specificity in the β-structure/α-helix deci-

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Table 1. Results of the Syntheses of Monomers

monomer	yield (%)	mp (°C)	R_f	$[\alpha]_D$ (c 1.0, THF)	anal.[a] (%) C	H	N
Boc-Leu-Ile-Leu-ONSu	89	155–157	0.51[b]	−66.9	58.47	8.36	10.10
					56.73	8.03	9.86
Boc-Leu-Ile-Ile-Leu-ONSu	87	211–213 (dec)	0.40[b]	−83.6	59.35	8.60	10.49
					59.16	8.64	10.27
Nps-Ala-Ile-Ile-Leu-ONSu	90	178–180 (dec)	0.76[c]	−96.7	54.85	6.83	12.38
					54.09	7.00	11.87
Nps-D-Leu-Ile-Ile-Leu-ONSu	44	198–200 (dec)	0.62[d]	−17.6	56.65	7.27	11.66
					56.80	7.33	11.77
Boc-Leu-Ile-Ile-Gly-OPcp	77	211–213 (dec)	0.30[e]	−23.1[f]	48.80	5.94	7.34
					47.81	5.63	6.65
Nps-Leu-Ile-Ile-Aib-ONSu	49	157–159 (dec)	0.73[c]	−76.0	55.48	6.98	12.13
					54.24	6.86	11.72

[a] Calculated values are given in the first row and found values in the second row. [b] 1:1 EtOAc–benzene. [c] 1:1 THF–benzene. [d] 3:2 THF–benzene. [e] 8:2 THF–methanol. [f] (c 1.0, DMF).

Table 2. Results of the Syntheses of the Sequential Polypeptides

polypeptide	yield (%)	$[\eta]$ (dL/g)[a]	anal. calcd (%) C	H	N	found (%) C	H	N
$(Leu\text{-}Ile\text{-}Leu)_n$	89	0.15	63.69	9.80	12.38	61.08	9.40	11.74
$(Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$	91	0.10	63.69	9.80	12.38	59.89	9.25	11.36
$(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$	98	0.21	61.44	9.33	13.65	59.72	9.49	12.86
$(D\text{-}Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$	81	0.28	63.69	9.80	12.38	63.01	9.64	12.26
$(Leu\text{-}Ile\text{-}Ile\text{-}Gly)_n$	55	0.13	60.58	9.15	14.13	58.63	8.82	13.38
$(Leu\text{-}Ile\text{-}Ile\text{-}Aib)_n$	78	0.14	62.24	9.50	13.20	60.64	9.36	12.92

[a] Measured at 25 °C in dichloroacetic acid.

sion for these hydrophobic polypeptides in the solid state.

Results and Discussion

Synthesis and Polycondensation of Tri- and Tetrapeptides. Syntheses of N-protected tri- or tetrapeptide ethyl esters were carried out by the conventional method for peptide synthesis involving stepwise elongation of the peptide chains from C-terminal amino acid ethyl esters. The N-protected peptide ethyl esters were activated by removal of the ethyl ester by saponification followed by introduction of *N*-oxysuccinimide (ONSu) or (pentachlorophenyl)oxy (OPcp) groups. The steps of the saponification of the ethyl ester and the activation of the C-terminal carboxyl group might have a risk of racemization of the C-terminal amino acid moieties. Thus the N-protected peptide active esters as a monomer were carefully analyzed by 500 MHz ^{1}H-NMR and ^{13}C-NMR spectroscopies. These NMR spectra demonstrated the presence of little stereoisomers. Results of the syntheses of the N-protected peptide active esters as a monomer are summarized in Table 1.

The polypeptides were synthesized by polycondensation of the peptide active esters.[8] The Nps or Boc groups of the monomers were deprotected with HCl/dioxane or trifluoroacetic acid (TFA) just before the polymerization. The polymerizations were done by dissolving the monomer salts in dimethyl sulfoxide (DMSO) followed by addition of triethylamine. After the polycondensation, the resulting polymers were isolated by precipitation from the reaction system by addition of methanol. Results of the polymerizations are summarized in Table 2. The synthetic routes are illustrated by the synthesis of $(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$ in Figure 1.

Solidification of the Polypeptides from Solution. The preparation of the solid-state samples of the polypeptides was done by precipitation from a solution of the polypeptides in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP)/dichloroacetic acid (DCA) or HFIP by fast dilution with diethyl ether. As HFIP and DCA are the best solvents

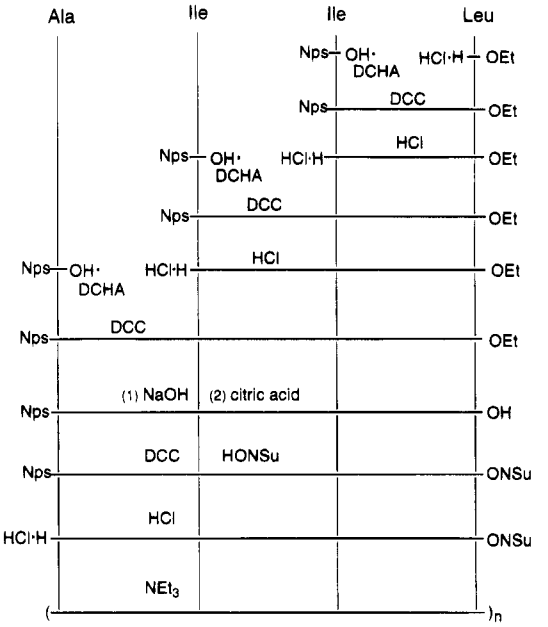

Figure 1. Representative synthetic route of $(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**3**).

for the polypeptides, the polypeptide molecules are strongly solvated with HFIP and DCA molecules in the solution.[20] When the solution of the polypeptides in HFIP/DCA was diluted with diethyl ether, a poor solvent for these polypeptides, the polypeptide molecules lose the solvating HFIP/DCA molecules, and a highly ordered structure of the polypeptides may be formed through aggregation of the molecules just before precipitation from the solution. Then, the conformation of the polypeptides in the solid state thus obtained should be strongly affected by their steric and electric (hydrophobic or hydrophilic) nature as well as the conformation in solution.

Characterization of the Conformation of the Polypeptides in the Solid State. Characterization

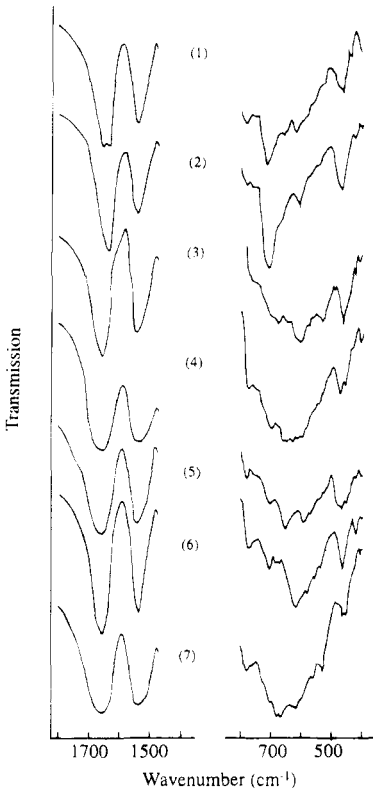

Figure 2. IR spectra in the amide I, II, and V regions of the sequential polypeptides consisting of leucyl and isoleucyl residues: (1) $(Leu\text{-}Ile\text{-}Leu)_n$; (2) $(Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$; (3) $(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$; (4) $(D\text{-}Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$; (5) $(Leu\text{-}Ile\text{-}Ile\text{-}Gly)_n$; (6) $(Leu\text{-}Ile\text{-}Ile\text{-}Aib)_n$; (7) $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$.

of the conformation of the polypeptides was done by IR spectroscopy. The IR bands in the amide I, II,[10,11,13] and V[12,14,15] regions are very sensitive to the conformational change and have been used for characterization of the conformation of many peptides.[9,16,21,22] Figure 2 shows the IR spectra in these regions of the polypeptides. The IR spectrum of polypeptide **1** shows bands at 1656, 1632, 1542, and 719 cm^{-1} and a shoulder at 1690 cm^{-1} [Figure 2(1)]. The bands at 1656 and 1542 cm^{-1} are the amide I and II bands characteristic of the α-helix, respectively.[11] The bands at 1632 cm^{-1} together with the shoulder at 1690 cm^{-1} and at 719 cm^{-1} are the amide I[11] and V[12,14] bands characteristic of the antiparallel β-structure of peptides, respectively. Thus, this spectrum suggests that polypeptide **1** assumes a mixture of α-helix and β-structure. The IR spectrum of polypeptide **2** has bands at 1638, 1545, and 712 cm^{-1} and a shoulder at 1690 cm^{-1} [Figure 2(2)]. These are characteristic of the antiparallel β-structure.[11,12,14] This spectrum suggests that polypeptide **2** assumes predominantly the β-structure in the solid state.

The IR spectrum of polypeptide **3** has bands at 1658, 1545, and 609 cm^{-1} characteristic of the α-helix [Figure 2(3)]. The IR spectrum of polypeptide **4** has bands at 1655, 1540, and 660–610 cm^{-1} characteristic of the α-helix [Figure 2(4)]. The IR spectrum of polypeptide **5** shows bands at 1657, 1543, 651, and 592 cm^{-1} [Figure 2(5)]. The bands at 1657 and 1543 cm^{-1} are the amide I and II bands characteristic of the α-helix.[10,11] The bands at 651 and 592 cm^{-1} are assigned to the C=O bond out-of-plane bending plus N–H out-of-plane bending of the α-helix.[15] The IR spectrum of polypeptide **6** has bands at 1655, 1535,[11] and 620 cm^{-1} [12,14] characteristic of the α-helix [Figure 2(6)]. All these spectra in Figure 2(1)–(6) suggest that polypeptides **3**–**6** assume the α-helical conformation. We reported previously that $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$ **7** has the α-helix.[23] The IR spectra of the polypeptides and assigned secondary structures in the solid state are tabulated in Table 3.

Factors Governing the Specificity in the β-Structure/α-Helix Decision for the Hydrophobic Polypeptides in the Solid State. Though leucine and isoleucine are structurally isomeric hydrophobic amino acids, they are quite different in determining the secondary structure of the polypeptides in which they are included. Leucine has a strong tendency to form the α-helix (Leu: $P_\alpha = 1.34$ and $P_\beta = 1.22$)[5] and isoleucine has a very strong tendency to form the β-structure (Ile: $P_\alpha = 1.09$ and $P_\beta = 1.67$).[5] Thus the secondary structure of the hydrophobic sequential polypeptides in the solid state consisting of these two amino acid residues is expected to be determined by a critical specification.

It is plausible that the cumulated sum of the conformational probability for the component amino acids should determine the secondary structure of the polypeptides. Chou and Fasman defined $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ as the mean values of P_α and P_β of the component amino acid residues.[2] If the secondary structure is determined according to the sum of the tendencies of the amino acids, the balance of $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ should be strongly related to the observed conformation of the polypeptides. The $\langle P_\alpha \rangle$ value of 1.22 for polypeptide **2** is far smaller than the $\langle P_\beta \rangle$ value of 1.45. The $\langle P_\alpha \rangle$ value of 1.26 for polypeptide **1** is also smaller than the $\langle P_\beta \rangle$ value of 1.37, but the difference between these two values for **1** is smaller than that for **2**. The $\langle P_\alpha \rangle$ value of 1.25 for polypeptide **7** is larger than the $\langle P_\beta \rangle$ value of 1.20. As concerns the above three polypeptides, the assumption is consistent with the observed β-structure/α-helix tendency.

The $\langle P_\alpha \rangle$ value for polypeptide **4** might be smaller than that for polypeptide **2**, because the pair of the enantiomeric isomers of L-Leu and D-Leu is expected to offset the optically isotropic nature such as helix direction. The $\langle P_\beta \rangle$ value, on the other hand, may be regarded to be less affected. Thus polypeptide **4** should prefer the β-structure. Moreover, the $\langle P_\alpha \rangle$ value of 1.23 for polypeptide **3** is smaller than the $\langle P_\beta \rangle$ value of 1.32, and the $\langle P_\alpha \rangle$ value of 0.99 for polypeptide **5** is far smaller than the $\langle P_\beta \rangle$ value of 1.29. Although these polypeptides should have the β-structure, the conformation of them determined experimentally was the α-helix.

Introduction of a new interpretive aspect is needed to clarify this inconsistency between the theory and the experimental fact. We suppose that in the solid state formed by such a fast process as precipitation of hydrophobic sequential polypeptides, if the β-structure is attainable, it would be more stable than the α-helix. The stabilization caused by consecutiveness of the well-regulated intermolecular interaction in the β-structure should be more effective than in the α-helix in a condensed system such as the solid state. We suppose that the β-structure in polypeptide **2** might have formed primarily by the association of the sterically regulated successive sequence of the strong hydrophobic side chain of -Leu-Ile-Ile-Leu-Leu-Ile-Ile-Leu-. The other sequential polypeptides consisting of -X-Ile-Ile-Leu- and -Leu-Ile-Ile-X- sequences, in which Ala (**3**), D-Leu (**4**), or Gly (**5**) is introduced as an alternate residue X, should destroy or weaken the intermolecular aggregation of the hydrophobic polypeptide chains. The α-helical conformation of polypeptide **5** might be caused by prohibition of formation of the associated β-structure because of the defect of the sterically regular alignment of the amino acid residues. The same argument may be possible with polypeptide **3**, having an increasing tendency to form

Table 3. Conformation of the Sequential Polypeptides Consisting of Leucyl and Isoleucyl Residues in the Solid State

polypeptide	$\langle P_\alpha \rangle^a$	$\langle P_\beta \rangle^a$	IR band observed (cm^{-1})	conformation
$(Leu\text{-}Ile\text{-}Leu)_n$ (**1**)	1.26	1.37	1656, 1642	α-helix
			1690, 1632, 719	β-structure
$(Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**2**)	1.22	1.45	1690, 1638, 1545, 712	β-structure
$(Ala\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**3**)	1.23	1.32	1658, 1545, 609	α-helix
$(D\text{-}Leu\text{-}Ile\text{-}Ile\text{-}Leu)_n$ (**4**)	<1.22	~1.45	1655, 1540, 660–610	α-helix
$(Leu\text{-}Ile\text{-}Ile\text{-}Gly)_n$ (**5**)	0.99	1.29	1657, 1543, 651, 592	α-helix
$(Leu\text{-}Ile\text{-}Ile\text{-}Aib)_n$ (**6**)			1655, 1535, 620	α-helix
$(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$ (**7**)	1.25	1.20	1655, 1540, 615	α-helix

[a] Calculated values based on ref 2.

the α-helix with displacement of leucine by alanine. The formation of the α-helix in polypeptide **4** suggests that an amino acid having a hydrophobic side chain with opposite configuration could prohibit the association of the sterically regulated successive hydrophobic side chains.

The effect of replacement of a repeating residue with another guest amino acid on the conformation of homo-oligopeptides was investigated by Toniolo and his co-workers,[24] where the β-sheet conformation of the host oligopeptides was disrupted by the guest amino acid having a tendency to disturb the formation of the β-sheet conformation. Recently, they reported that a fully extended structure of the host oligopeptides consisting of α-amino acids having two side chains on the α-carbon was forced to fold into a 3_{10} helical conformation by the guest residue having a single side chain on the α-carbon in a crystal state.[25] The results obtained in our study suggest that the formation of the conformation of polypeptides is affected by the amino acid residue introduced as a minor component. The association of the peptide chains leading to the β-structure dominates primarily in the formation of the secondary structure of the sequential hydrophobic polypeptides, even if they have an amino acid sequence with a high potency of α-helix formation. The failure of the association of peptide chains by interruption of successive hydrophobic side chains with introduction of alternative amino acid residues develops α-helical conformation in accordance with the conformational preference of the component amino acids. In such cases, isoleucine can be included stably into the α-helical conformation, though its homopolymer hardly forms this conformation.

Since the amide bonds bring rigid and polar moieties into the polymer main chains, the random coil conformation should not be a favorable one for the polypeptides in the highly condensed solid state. In such systems, hydrophobic bonding should function effectively to lower the total free energy. When the well-regulated intermolecular hydrophobic interaction occurs satisfactorily in these hydrophobic sequential polypeptide systems, the most effective energy lowering may be achieved in the planar β-structure. The intramolecular hydrogen bondings of the α-helix should be formed only when the consecutive planar alignment of the side chains could not be achieved. The α-helical conformation of polypeptide **6** implies that Aib residues introduced as an alternate amino acid particularly promote helical folding[18] which should overcome the planar alignment of the side chains. Then, the well-regulated intermolecular hydrophobic bonding specifies primarily the decision of the β-structure or the α-helix for hydrophobic sequential polypeptides. Consequently, the conformation determined experimentally for the hydrophobic sequential polypeptides consisting of Leu and Ile in this study is clearly interpreted as the tidily consecutive planar alignment of the hydrophobic side chains prior to the potential tendency for the formation of secondary structure of individual amino acids as the primary factor governing the specificity in the β-structure/α-helix decision.

Experimental Section

Measurements. The IR spectra were recorded for KBr disks with a JASCO FT/IR-5300 IR spectrometer. The inherent viscosities were determined with an Ostwald viscometer at a concentration of 0.5 g/dL in dichloroacetic acid at 25 °C. The NMR spectra were recorded for dimethyl-d_6 sulfoxide (DMSO-d_6) solutions with a JEOL α-500 NMR spectrometer (500 MHz for ^1H-NMR and 125 MHz for ^{13}C-NMR).

Representative Syntheses of Monomers. Nps-Ile-Leu-OEt. Nps-isoleucine dicyclohexylammonium salt (Nps-Ile-OH DCHA) (23.3 g, 50.0 mmol) was added to a solution of HCl H-Leu-OEt (9.08 g, 50.1 mmol) in chloroform (200 mL) at room temperature. The solution was cooled to −10 °C, and *N,N'*-dicyclohexylcarbodiimide (DCC) (11.4 g, 55.3 mmol) was added with vigorous stirring. The solution was stirred for 2 h at −10 °C and for 15 h at room temperature. Then the solution was diluted with 200 mL of ethyl acetate to precipitate *N,N'*-dicyclohexylurea (DCUrea), which was removed by filtration. The filtrate was concentrated to give an oily residue, which was dissolved in 200 mL of ethyl acetate, and unreacted Nps-Ile-OH DCHA was filtered off. The filtrate was washed with an aqueous solution of citric acid, water, an aqueous solution of $NaHCO_3$, and an aqueous solution of NaCl and dried over Na_2SO_4 overnight. The solution was concentrated under reduced pressure to give an oily residue, which was crystallized by addition of hexane. The product was recrystallized from ethyl acetate with hexane. Yield 18.3 g (86%); mp 117–119 °C; R_f 0.91 (ethyl acetate–benzene 1:1); $[\alpha]_D$ −60.8 (*c* 1.0, tetrahydrofuran); IR (KBr) 1729, 1643, 1591, 1561, 1511, 1337 cm^{-1}. Anal. Calcd for $C_{20}H_{31}N_3O_5S$: C, 56.45; H, 7.34; N, 9.87. Found: C, 56.61; H, 7.56; N, 9.86.

Nps-Ile-Ile-Leu-OEt. To a solution of Nps-Ile-Leu-OEt (11.1 g 26.1 mmol) in 30 mL of tetrahydrofuran was added 4 M HCl in dioxane (14.5 mL) at room temperature. Immediately after that, diethyl ether (200 mL) and hexane (200 mL) were added to the solution. The solvent was evaporated to give a yellow residue. The solid was washed with diethy ether until the yellow color was diminished. The residue was dried in vacuo. The dipeptide ester hydrochloride thus obtained was dissolved in ethanol (30 mL), and hexane (300 mL) was added. After insoluble material was filtered off, the filtrate was concentrated to give a crystalline product. The product was washed with diethyl ether and dried in vacuo. The purified product (5.50 g, 17.8 mmol) was dissolved in 70 mL of chloroform. To the solution was added Nps-Ile-OH DCHA (8.30 g, 17.8 mmol). The solution was cooled to −10 °C, and DCC (4.04 g, 19.6 mmol) was added with vigorous stirring. The stirring was continued for 2 h at −10 °C and for 15 h at room temperature. Then the solution was diluted with 70 mL of ethyl acetate, and crystals of DCUrea were removed by filtration. The filtrate was concentrated to give an oily residue, which was dissolved in tetrahydrofuran (100 mL) and ethyl acetate (200 mL), and undissolved material was removed by filtration. The filtrate was washed with an aqueous solution of citric acid, water, $NaHCO_3$, and water followed by drying over Na_2SO_4. The solvent was evaporated, and hexane was added to give crystals of the product. The product was purified by recrystallization from ethyl acetate with hexane. Yield 8.47 g (88%); mp 183–185 °C; R_f 0.83 (1:1 ethyl acetate–

benzene); $[\alpha]_D$ −79.6 (*c* 1.0, tetrahydrofuran); IR (KBr) 1740, 1635, 1591, 1548, 1511, 1337 cm^{-1}. Anal. Calcd for $C_{26}H_{42}N_4O_6S$: C, 57.97; H, 7.86; N, 10.40. Found: C, 58.03; H, 7.98; N, 10.39.

Nps-Ala-Ile-Ile-Leu-OEt. To a solution of Nps-Ile-Ile-Leu-OEt (13.2 g, 24.5 mmol) in 40 mL of tetrahydrofuran was added 4 M HCl in dioxane (15 mL). After 5 min, hexane was added to the resulting gel to give a crystalline product. The product was washed with diethyl ether and recrystallized from ethanol with diethyl ether. The resulting tripeptide ester hydrochloride (6.83 g, 16.2 mmol) was dissolved in 200 mL of chloroform, and Nps-Ala-OH DCHA (7.54 g, 17.8 mmol) was added. The solution was cooled to −10 °C, and DCC (4.05 g, 19.6 mmol) was added. The solution was treated as above to give crystals of a crude Nps-tetrapeptide ester. The product was recrystallized from a mixture of tetrahydrofuran and ethyl acetate with hexane. Yield 7.97 g (81%); mp 232−234 °C; R_f 0.72 (1:1 ethyl acetate−benzene); $[\alpha]_D$ −102.0 (*c* 1.0, tetrahydrofuran); ^1H NMR in DMSO-d_6 δ 0.73−0.88 (18H, m), 1.15 (3H, t, J = 7.0 Hz), 1.25 (3H, d, J = 7.0 Hz), 1.34−1.48 (4H, m), 4.00−4.08 (2H, m), 4.19 (1H, t, J = 8.0 Hz), 4.24 (2H, t, J = 8.0 Hz), 4.26−4.29 (1H, m), 5.10 (1H, d, J = 7.0 Hz), 7.37 (1H, t, J = 8.0 Hz), 7.78 (1H, t, J = 8.0 Hz), 7.85 (1H, d, J = 9.0 Hz), 7.90 (1H, d, J = 9.0 Hz), 8.01 (1H, d, J = 9.0 Hz), 8.25 (1H, d, J = 9.0 Hz), 8.26 (1H, d, J = 8.0 Hz) ppm; ^{13}C NMR in DMSO-d_6 δ 10.74, 10.82, 13.99, 15.09, 19.26, 20.98, 22.85, 24.05, 24.26, 36.50, 36.55, 39.50, 50.03, 56.40, 56.59, 58.92, 60.37, 124.83, 125.05, 125.66, 134.28, 142.02, 145.66, 170.56, 170.96, 172.15, 173.21 ppm; IR (KBr) 1739, 1635, 1591, 1550, 1511, 1338 cm^{-1}. Anal. Calcd for $C_{29}H_{47}N_5O_7S$: C, 57.12; H, 7.77; N, 11.48. Found: C, 57.10; H, 8.08; N, 11.20.

Nps-Ala-Ile-Ile-Leu-OH. To a solution of the tetrapeptide ester (7.34 g, 12.0 mmol) in 80 mL of tetrahydrofuran was added an aqueous 1 M solution of NaOH (12.5 mmol). The reaction was monitored by thin-layer chromatography (TLC: Merck 5735, 1:1 ethyl acetate−benzene). After 14 h, the saponification was completed. Addition of methanol (20 mL) to the reaction system could accelerate the saponification reaction, and the reaction time was reduced to 1.5 h. The aqueous solution was diluted with water (50 mL) and extracted with diethyl ether to remove any unreacted materials. Then the aqueous solution was acidified with 8% citric acid to give a precipitate. The precipitate was collected on a glass filter, washed with water and diethyl ether, and dried. The product was purified by reprecipitation from tetrahydrofuran with hexane. Yield 6.00 g (86%); mp 236−238 °C; R_f 0.78 (1:1:1 ethyl acetate−tetrahydrofuran−benzene); $[\alpha]_D$ −21.6 (*c* 0.1, DMF); ^1H NMR in DMSO-d_6 δ 0.73−0.87 (18H, m), 1.25 (3H, d, J = 7.0 Hz), 1.34−1.56 (6H, m), 1.58−1.71 (3H, m), 4.17−4.25 (4H, m), 5.09 (1H, d, J = 7.0 Hz), 7.37 (1H, t, J = 8.0 Hz), 7.78 (1H, t, J = 8.0 Hz), 7.83 (1H, d, J = 9.0 Hz), 7.90 (1H, d, J = 9.0 Hz), 8.01 (1H, d, J = 7.0 Hz), 8.11 (1H, d, J = 8.0 Hz), 8.25 (1H, d, J = 9.0 Hz) ppm; ^{13}C NMR in DMSO-d_6 δ 10.76, 10.83, 15.10, 15.18, 19.27, 21.04, 22.95, 24.14, 24.27, 36.47, 36.60, 39.50, 49.98, 56.48, 56.64, 58.93, 124.84, 125.05, 125.66, 134.29, 142.02, 145.67, 170.53, 170.87, 173.22, 173.85 ppm; IR (KBr) 1721, 1635, 1591, 1545, 1509, 1335 cm^{-1}. Anal. Calcd for $C_{27}H_{43}N_5O_7S$: C, 55.75; H, 7.45; N, 12.04. Found: C, 54.83; H, 7.87; N, 11.40.

Nps-Ala-Ile-Ile-Leu-ONSu. Nps-Ala-Ile-Ile-Leu-OH (10.9 g, 18.7 mmol) and HONSu (4.37 g, 38.0 mmol) were dissolved in 150 mL of tetrahydrofuran. The solution was cooled to −10 °C, and DCC (5.60 g, 27.1 mmol) was added to the solution. The solution was stirred for 8 h at −2 °C. Then the solution was diluted with ethyl acetate, and the resulting crystals of DCUrea were removed by filtration. The filtrate was washed rapidly with aqueous 2% $NaHCO_3$ and aqueous saturated NaCl. The solvent was evaporated under reduced pressure to give an oily residue. Addition of hexane gave a crystalline product. The product was purified by recrystallization from a mixture of tetrahydrofuran (200 mL) and methanol (100 mL) with hexane. ^1H NMR in DMSO-d_6 δ 0.74−0.92 (18H, m), 1.26 (3H, t, J = 7.0 Hz), 1.34−1.51 (2H, m), 1.60−1.78 (7H, m), 2.80 (4H, s), 4.19 (1H, t, J = 9.0 Hz), 4.25 (1H, t, J = 9.0 Hz), 4.62−4.67 (1H, m), 5.10 (1H, d, J = 7.0 Hz), 7.37 (1H, t, J = 8.0 Hz), 7.79 (1H, t, J = 8.0 Hz), 7.88 (1H, d, J = 9.0 Hz), 7.95 (1H, d, J = 9.0 Hz), 8.01 (1H, d, J = 9.0 Hz), 8.25 (1H, d, J = 9.0 Hz), 8.65 (1H, d, J = 7.0 Hz) ppm; ^{13}C NMR in DMSO-d_6 δ 10.65, 10.83, 15.07, 19.27, 20.74, 22.73, 24.00, 24.27, 24.33, 25.21, 25.43, 36.34, 36.63, 39.50, 48.06, 56.39, 56.50, 58.93, 124.84, 125.05, 125.66, 134.29, 142.02, 145.66, 168.44, 169.93, 170.67, 171.36, 173.18 ppm; IR (KBr) 1820, 1785, 1741, 1635, 1594, 1548, 1511, 1339 cm^{-1}.

Polycondensation of Monomers. Representative Synthesis of (Ala-Ile-Ile-Leu)$_n$. Nps-Ala-Ile-Ile-Leu-ONSu (11.4 g, 16.8 mmol) was dissolved in 80 mL of tetrahydrofuran. To the solution was added 4 M HCl in dioxane (10 mL). The resulting gel was diluted with hexane (100 mL) to give a precipitate, which was collected on a glass filter, washed with diethyl ether, and dried. The crude product was purified by reprecipitation from tetrahydrofuran with hexane. The monomer hydrochloride thus obtained (8.94 g, 15.9 mmol) was dissolved in DMSO (44.7 mL), and triethylamine 2.45 mL (17.5 mmol) was added with vigorous stirring. The stirring was continued for 9 days at 20 °C. The polymerization system was diluted with methanol (100 mL) to precipitate the polypeptide, which was collected on a glass filter, washed with methanol and diethyl ether, and dried in vacuo.

Reprecipitation of the Polypeptides. Polypeptide **3** (3.0 g) was dissolved in HFIP (10 mL). After insoluble material was removed by filtration, the solution was diluted with diethyl ether. The resulting precipitate was collected on a glass filter, washed with diethyl ether, and dried in vacuo for several days.

Polypeptides **4** and **6** were treated as above, and polypeptides **1**, **2**, **5**, and **7** were dissolved in a mixture of HFIP and dichloroacetic acid and reprecipitated by addition of diethyl ether.

Although polypeptides **3**, **4**, and **6** were treated with a mixture of HFIP and dichloroacetic acid, the conformations of the polypeptides in the solid state were independent of the solvents.

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