Helically Arranged Azobenzene Chromophores along a Polypeptide Chain. 1. Synthesis and Circular Dichroism

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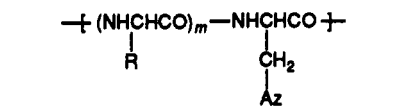
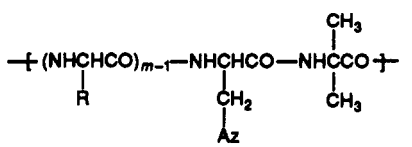
ABSTRACT: Two series of sequential polypeptides having repeating units Lys(Z)$_m$-azoAla (I; m = 1–3), azoAla-Lys(Z)-azoAla-Aib (II; m' = 1), and Lys(Z)$_{m'-1}$-azoAla-Aib (II; m' = 2 and 3) were synthesized [Lys(Z) = N^{ϵ}-[(benzyloxy)carbonyl]-L-lysine, azoAla = L-*p*-phenylazophenylalanine, Aib = α-aminoisobutyric acid]. The relation between the sequence of azoAla units and the circular dichroism (CD) was studied on these polypeptides. The six polypeptides in the trans form showed exciton couplets of the same sign (negative peak at longer wavelength), but the intensity of the CD depended markedly on the sequence. The two series of polypeptides were suggested to have different main-chain conformations. Poly[Lys(Z)-azoAla-Aib] showed the largest CD among the six polypeptides studied. The polypeptides showed a reversible change of optical rotation at 589 nm associated with trans/cis photoisomerization. A possible application of chiral photochromic compounds to chiroptical photorecording is discussed.

A helical polypeptide chain has been shown to be an excellent molecular framework that supports a variety of chromophores in a specific order with a specific spatial arrangement.[1,2] In particular, sequential polypeptides containing an aromatic amino acid as one component in the repeating unit are unique in supporting the helical one-dimensional array of the chromophores.[3–5] For example, a sequential polypeptide, poly[Lys(Z)$_2$-antAla] [Lys(Z) = N^{ϵ}-[(benzyloxy)carbonyl]-L-lysine, antAla = L-9-anthrylalanine], exhibited extremely strong circular dichroism (CD) at the absorption band of the anthryl group ($\Delta\epsilon_{261}$ = +525).[5] The strong CD indicates unequivocally that the anthryl groups are rigidly fixed on the helical polypeptide main chain, and the exciton-type interactions among the anthryl groups are very strong. In the sequential polypeptide, anthryl groups are linked to the helical main chain by a single methylene unit and their orientation is highly constrained. A helical polypeptide carrying anthryl groups linked by a long and flexible spacer [poly(β-9-anthrylmethyl L-aspartate)] showed much weaker CD at the anthryl absorption band than poly[Lyz(Z)$_2$-antAla].[6,7] Therefore, the importance of steric constraint on the side-chain orientation to induce strong chiroptical property is evident. In the present study, similar sequential polypeptides carrying azobenzene groups instead of anthryl groups (I-m, II-m) were synthesized.



I (m = 1–3)



II (m' = 2 and 3)

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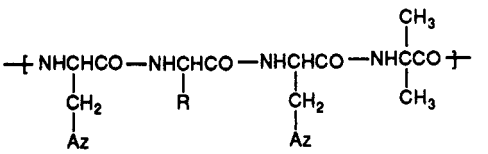
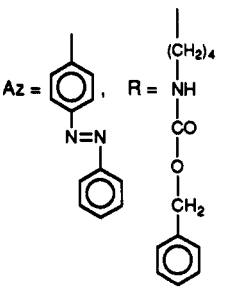


II (m' = 1)



Since the azobenzene groups are linked by a single methylene group, they may be expected to show stronger circular dichroism and optical rotation than other azobenzene-containing polypeptides reported so far.[8–27] Associated with the trans/cis reversible photoisomerization of azobenzene groups, these polypeptides will show different CD spectra or optical rotations upon photoirradiation. The photoreversible change of optical rotation may be utilized for a chiroptical photorecording, in which a digital record written as a photoisomerized state (trans/cis) may be read out by the change of optical rotation at the wavelength longer than the wavelengths used for the recording. The read-out process by optical rotation is definitely advantageous, because the process does not destroy the original record. In this study, the chiroptical properties of the polypeptides were investigated as the function of the arrangements of the azobenzene chromophores along helical polypeptide chains, and an optimal arrangement of the photochromic groups that gives the strongest CD or optical rotation was searched.

Results and Discussion

Synthesis. The six sequential polypeptides were first synthesized in this study. L-*p*-Phenylazophenylalanine (azoAla) was synthesized according to the procedure

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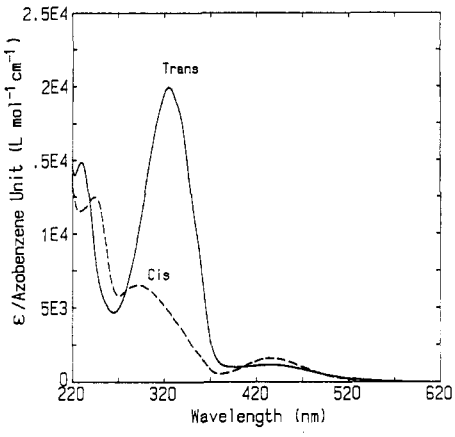


Figure 1. Absorption spectra of poly[Lys(Z)$_3$-azoAla] with *trans*-azobenzene groups (—) and with *cis*-azobenzene groups (- - -). [azoAla] = 1.2×10^{-4} M in TMP, room temperature.

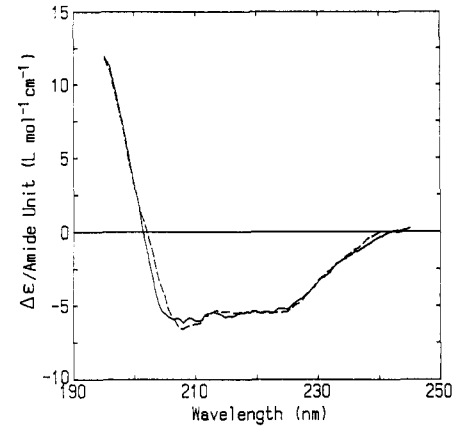


Figure 2. CD spectra of poly[Lys(Z)$_3$-azoAla] with *trans*-azobenzene groups (—) and with *cis*-azobenzene groups (- - -). [azoAla] = 1.0×10^{-4} M in TMP, room temperature. $\Delta\epsilon$ = (molar ellipticity)/3298.

reported by Goodman and Kossoy.[8] The optical purity of L-azoAla was checked by an ^{1}H NMR spectrum of its methyl ester in the presence of chiral shift reagent.[28] Under the condition where racemic azoAla methyl ester showed two methyl peaks, the L-isomer showed a single peak, indicating that the optical purity of the latter is better than ca. 98%. The polypeptides were prepared by polymerizing the activated esters of the relevant oligopeptides, i.e., azoAla-Lys(Z)-azoAla-Lys(Z)-OSu (m = 1), Lys-(Z)-azoAla-Lys(Z)-OSu (m = 2), and Lys(Z)$_2$-azoAla-Lys-(Z)-OSu (m = 3) for the poly[Lys(Z)$_m$-azoAla] series, azoAla-Lys(Z)-azoAla-Aib-OSu for the poly[azoAla-Lys-(Z)-azoAla-Aib] (II; m' = 1) series, and Lys(Z)-azoAla-Aib-OSu (m' = 2) and Lys(Z)$_2$-azoAla-Aib-OSu (m' = 3) for the poly[Lys(Z)$_{m'-1}$-azoAla-Aib] series. The average molecular weights of the polypeptides were usually not high, but the component eluted at the limiting elution volume of Sephadex LH-60 gel in trimethyl phosphate (TMP) solution (MW > 5000) was used for the spectroscopic measurements. ^{1}H NMR spectra in DMSO-d_6 solution and IR spectra in KBr pellet were compatible with the polypeptide structures I and II.

Absorption and CD Spectra of Poly[Lys(Z)$_m$-azoAla] (I-m; m = 1-3). Typical absorption spectra of poly[Lys(Z)$_m$-azoAla] in solution adapted in the dark and in the photostationary state under irradiation at 340 nm are shown in Figure 1. Absorption spectra of the three polypeptides are virtually indistinguishable to each other and to the spectrum of *N*-acetyl-L-azoAla methyl ester. Azobenzene groups are known to form J- and H-aggregates in bilayer assemblies.[29] However, the absorption spectra of the present polypeptides showed no indication for the aggregates. The azobenzene groups on the polypeptides may be moderately spaced along a helical main chain. The spectrum of the cis form also indicates no interchromophore interactions. The azobenzene groups on the polypeptide chain can photoisomerize as easy as a free azobenzene molecule in solution.

CD spectra of the polypeptides were recorded in TMP solution. As typical examples, CD spectra of the I-3 polypeptide in TMP in the amide absorption region are shown in Figure 2. The CD profiles resemble that of a right-handed α-helix. Similar CD patterns were observed for two other polypeptides. The photoisomerization of the azobenzene groups in the side chains did not induce any change in the main-chain conformation of all three polypeptides. The $\Delta\epsilon_{222}$ values, as the measure of helix content, were −3.6 (m = 1), −3.5 (m = 2), and −5.4 (m = 3) in the trans state. These values are smaller than that of the full α-helix ($\Delta\epsilon_{222}$ = −11[30]), and the helix contents were estimated to be 34% (m = 1), 33% (m = 2), and 51% (m = 2). The reason for the small helix content is not clear at present. The helix content of poly[Lys(Z)$_2$-antAla] was close to 100% in TMP.[5] That of poly[Lys(Z)-napAla] (napAla = 1-naphthylalanine) should be near 100% in TMP, as judged from very strong CD in the absorption region of the naphthyl groups.[3] Since the steric constraint by the azobenzene group to the main-chain conformation must be smaller than that of a naphthyl or anthryl group, the steric effect alone cannot be responsible for the low helix content of poly[Lys(Z)$_m$-azoAla]. The polarity of the azobenzene would affect the main-chain conformation. However, since the helix content remained unchanged when the azobenzene group changed from nonpolar trans form (dipole moment = 0.5 D) to polar cis form (3.1 D),[31] the effect of the side-chain polarity cannot be a major reason.

CD spectra in the absorption region of an azobenzene group are collected in Figure 3. The trans azobenzene groups in the polypeptides show moderately strong exciton couplets with a negative peak at longer wavelength. The largest CD signal was observed for the m = 3 polypeptide at the $\pi\pi^*$ band ($\Delta\epsilon_{343}$ = −6.2). The CD spectrum was much weaker in the cis form of the polypeptides, but the m = 2 polypeptide shows weak CD signals at the $\pi\pi^*$ region (300 nm) and at the $n\pi^*$ region (420 nm). The weak CD signal of the cis form does not mean random orientation of the *cis*-azobenzene groups but may be attributed to a weak absorption transition moment of the *cis*-azobenzene group. The photoinduced change of the CD spectrum was reversible and could be repeated many times, upon alternate photoirradiation at 340 nm (trans → cis) and at 425 nm (cis → trans).

Absorption and CD Spectra of Poly[Lys(Z)$_{m'-1}$-azoAla-Aib] (II-m'). Since the helix contents of the poly[Lys(Z)$_m$-azoAla] series were not higher than 50%, α-aminoisobutyric acid (Aib) was introduced in the polypeptides to increase their helix content. The Aib unit is unable to take the β-sheet conformation due to a steric overlap between an α-methyl group and the neighboring carbonyl oxygen.[32,33]

Absorption spectra before and after photoirradiation showed similar changes, as shown in Figure 1. Again, no interchromophore interaction among azobenzene groups was detected in the absorption spectrum. CD spectra of the three polypeptides with *trans*- and *cis*-azobenzene groups were measured. CD spectra in the amide absorption region showed a pattern of helix conformation. However,

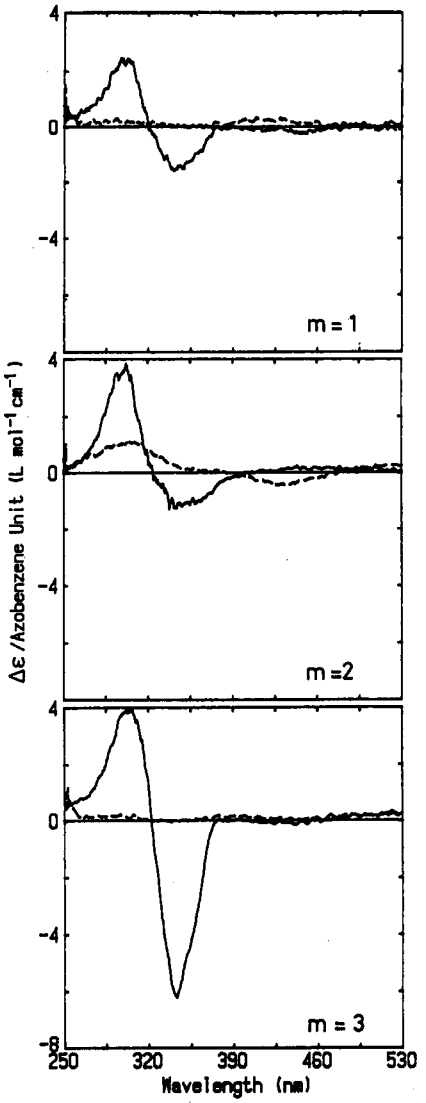


Figure 3. CD spectra of poly[Lys(Z)$_m$-azoAla] (m = 1–3) with *trans*-azobenzene groups (—) and with *cis*-azobenzene groups (- - -). In TMP, room temperature.

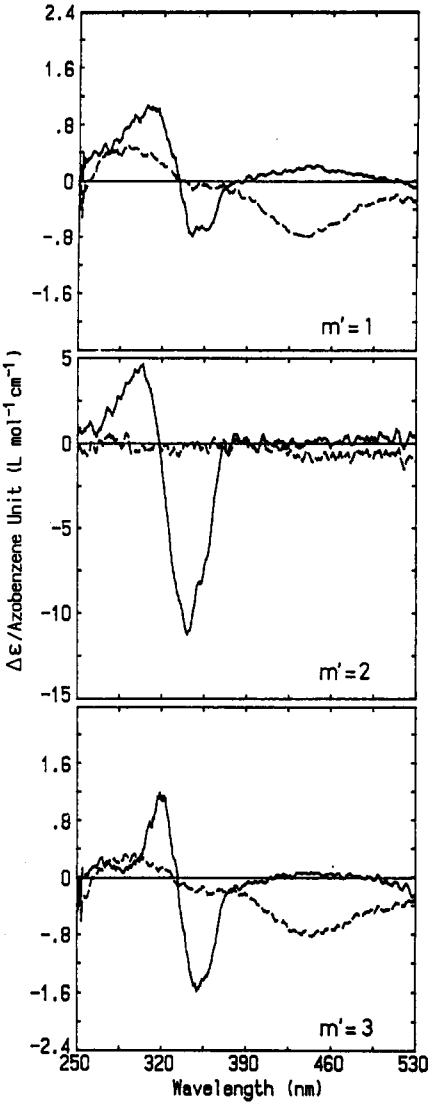


Figure 4. CD spectra of poly[azoAla-Lys(Z)-azoAla-Aib] (m' = 1), poly[Lys(Z)-azoAla-Aib] (m' = 2), and poly[Lys(Z)$_2$-azoAla-Aib] (m' = 3) with *trans*-azobenzene groups (—) and with *cis*-azobenzene (- - -) groups. In TMP, room temperature.

at this stage one cannot conclude that it is an α-helical conformation for this series of polypeptides, since the Aib unit has been known to favor both α-helix and 3_{10}-helix,[34-36] and the two helical conformations cannot be discriminated only from CD spectroscopy. The $\Delta\epsilon_{222}$ values of the II-m' polypeptides were more negative than the I-m polypeptides; −5.4 (m' = 1), −6.9 (m' = 2), −5.8 (m' = 3). If an α-helical conformation is assumed, the helix contents are 51% (m' = 1), 65% (m' = 2), and, 55% (m' = 3). The apparent helix contents are higher than those of the I-m series, indicating the helix-stabilizing effect of the Aib units. Again, the $\Delta\epsilon$ values were not influenced by the photoirradiation.

The polypeptides of the II-m' series contain one azoAla unit in every m' + 1 amino acid units, as in the case of the I-m polypeptides. Consequently, if the main-chain and the side-chain conformations were the same in the two series of polypeptides, those of the same $m(m')$ number should show similar CD patterns. CD spectra of the II-m' polypeptides in the azobenzene absorption region are collected in Figure 4. Similar to the case of the I-m series, polypeptides with *trans*-azobenzene groups showed exciton couplets with negative peaks at longer wavelengths at the $\pi\pi^*$ absorption band. It should be noted that all the polypeptides synthesized in this study showed the same patterns of the exciton couplet when the azobenzene groups are in trans form. This finding is difficult to interpret even qualitatively, since spatial arrangement of the azobenzene groups may be quite different for polypeptides of different m numbers. Theoretical CD calculation based on the conformations predicted from energy calculations will give some insight into this question.

The CD intensity of the II-2 polypeptide at 340 nm ($\Delta\epsilon$ = −11) was the largest among the sequential polypeptides examined in this study, although the density of the azobenzene groups along the polypeptide chain is not the highest of the six polypeptides. The magnitude of the CD of II-2 with respect to the molar concentration of azobenzene groups is comparable to or larger than those of polyglutamates and polyaspartates containing azobenzene groups on the ester side chains[12-24] or those of polylysine derivatives,[25-27] although the density of the azobenzene groups (33%) of II-2 is lower than the latter polypeptides. The strong CD indicates that the orientation of the azobenzene groups is highly constrained and the spatial arrangement is favorable for the exciton-type interactions.

The relative CD intensities of the II-m' series is different from those of the I-m series. In the I-m series, the m = 3 polypeptide gave the strongest CD, whereas the m' = 2 polypeptide showed the strongest CD in the II-m' series. The CD pattern of the I-2 polypeptide is significantly

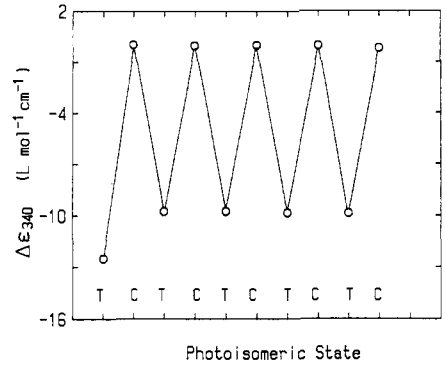
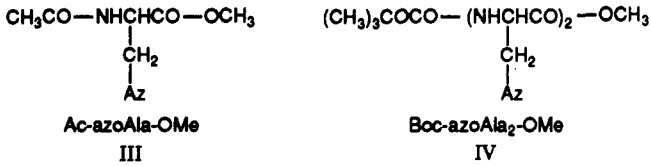


Figure 5. Reversible change of CD intensity at 340 nm of poly[Lys(Z)-azoAla-Aib] in TMP. For trans to cis photoisomerization, the solution was irradiated at 340 nm, and for cis to trans isomerization, it was irradiated at 425 nm.

different from that of the II-2 polypeptide. Furthermore, CD of the II-3 polypeptide is very weak, but that of the I-3 polypeptide is strong. These differences suggest that the main-chain conformations of the two series of polypeptides may be different. Since polypeptides containing an Aib group have been known to take a 3_{10}-helix as well as an α-helix, the 3_{10}-helical conformation is suggested for the II-*m* series.

Again, CD intensity was small in the cis form of the Aib-containing polypeptides. Only a small contribution from the nπ^* transition was observed. The change of CD pattern was again reversible in the II-*m*′ series. When the sample solution was irradiated alternately at 340 nm (trans → cis) and at 425 nm (cis → trans), alternate changes of the CD spectrum were observed for all the polypeptides. As an example, the photocycle for II-2 is shown in Figure 5.

CD Spectra of Model Peptides. In order to compare the CD spectra of helically arranged azobenzene groups with those randomly oriented ones, CD spectra of low molecular weight model compounds containing one or two azoAla units (III and IV) were measured. CD spectrum



of III in the trans form showed a small positive signal at the $\pi\pi^*$ band of azobenzene ($\Delta\epsilon_{320}$ = 1.1) without an exciton couplet. The cis form also showed a small positive signal at the nπ^* band with $\Delta\epsilon_{420}$ = 1.3. Dipeptide IV showed a weak exciton coupling at the $\pi\pi^*$ band of *trans*-azobenzene ($\Delta\epsilon_{308}$ = 1.6, $\Delta\epsilon_{342}$ = −1.3). The dipeptide in the cis form showed only marginal CD at the nπ^* band ($\Delta\epsilon_{439}$ = −0.28). These results show the advantage of a helical polypeptide structure to induce strong CD of azobenzene groups when their spatial arrangement is adequate, as in the case of the polypeptide II-2. However, if the arrangement is inadequate, the azobenzene groups do not induce strong CD even if they are arranged along a helix. This conclusion demonstrates the importance of the molecular design to synthesize compounds that show strong CD or strong optical rotation.

Optical Rotation of the Polypeptides in the Trans and Cis States. Table I lists the $[\alpha]_D$ values of the polypeptides and the model peptides in TMP solution. The optical rotation was largest in the I-2 or II-2 polypeptide. The $[\alpha]_D$ values change reversibly by the alternate photoirradiation. The polypeptides that gave a large CD signal showed large optical rotation. This is reasonable, since the CD spectrum and the optical rotatory dispersion are related to each other by the Kramers–Krönig relation.[37]

The reversible change of optical rotation by photoirradiation opens a way to use the chiral photochromic compounds as a photorecording material.

Table I
Optical Rotation at 589 nm for Polypeptides and Model Peptides (Solvent = TMP, 5-cm Cell)

polypeptide	$[\alpha]_D$(trans), deg	$[\alpha]_D$(cis), deg	concn, M
I-1[a]	<30		
I-2[a]	270	180	8.6×10^{-5}
I-3[a]	<10		
II-1	2.2	−19	3.2×10^{-3}
II-2	−206	−110	1.95×10^{-3}
II-3	−18	−48	3.58×10^{-3}
Ac-azoAla-OMe	<5	116	3.96×10^{-3}
Ac-(azoAla)$_2$-OMe	−13	−19	4.12×10^{-3}

[a] Due to a limited solubility of the I-*m* polypeptides, accurate $[\alpha]_D$ values could not be measured.

Conclusions

Six different sequential polypeptides carrying azobenzene groups linked by a single methylene spacer to the α-carbon were synthesized. The relation between the arrangement of azobenzene chromophores and the profile of CD spectrum was investigated. When an azobenzene group is in the trans form, the six polypeptides showed an exciton couplet with a negative peak at longer wavelength, but the magnitude of the CD intensity depended markedly on the spatial arrangement. The cis form showed a very small CD spectrum without the exciton couplet. The importance of a spatial arrangement of chromophores to design a photochromic compound for chiroptical photorecording was demonstrated.

Experimental Section

Materials. The six polypeptides were prepared by the polymerization of the corresponding oligopeptide-activated esters. The oligopeptides were synthesized by a conventional liquid-phase techniques. L-Phenylazophenylalanine was synthesized by the procedure reported by Goodman and Kossoy.[8] Its optical purity was checked by measuring ^{1}H NMR spectra of the methyl esters of the L- and DL-amino acids in the presence of a chiral shift reagent.[28]

L-*p*-Phenylazophenylalanine Methyl Ester Hydrochloride (HCl·azoAla-OMe).[8] L-Phenylalanine was nitrated and hydrogenated to prepare L-*p*-aminophenylalanine. The latter was coupled with nitrosobenzene to give L-*p*-phenylazophenylalanine. The amino acid was dissolved in methanol containing 3 N dry HCl and stirred for 2 days. After evaporating excess methanol, the product was solidified by adding ether.

Optical Purity of azoAla-OMe. The optical purity of the amino acid was checked for the methyl ester derivative. The ^{1}H NMR spectrum of the L-amino acid methyl ester was measured in deuteriochloroform in the presence of 0.7 mol equiv of chiral shift reagent, (tfc)$_3$Eu. The spectrum showed only a single peak for the *O*-methyl protons. In order to confirm the optical purity, D-azoAla-OMe was synthesized from D-phenylalanine. A racemic mixture of azoAla-OMe showed two *O*-methyl peaks in the presence of (tfc)$_3$Eu under the same conditions as above. Therefore, the optical purity of the L-isomer is better than 98%.

Boc-Lys(Z)-azoAla-OMe. Boc-Lys(Z) (Kokusan Chemical Works, Japan) (0.604 g, 1.59 mmol) and HCl·azoAla-OMe (0.50 g, 1.56 mmol) were mixed in chloroform (5 mL). To the ice-cooled solution, triethylamine (TEA); 0.293 mL, 2.15 mmol), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) (0.33 g, 1.72 mmol) and 1-hydroxybenzotriazole (HOBt) (0.24 g, 1.57 mmol) were added. Stirring was continued for 2 h at ice temperature and for 12 h at room temperature. The solvent was evaporated, and the residual was redissolved in ethyl

acetate. The organic solution was washed with 10% citric acid, 10% NaCl, 4% $NaHCO_3$, and 10% NaCl solution and then dried over $MgSO_4$. After evaporating the solvent, the crude solid was recrystallized from ethyl acetate/ether. Yield: 0.24 g (24%). Mp: 115–115.5 °C. Anal. Calcd for $C_{35}H_{43}N_5O_7$: C, 65.09; H, 6.71; N, 10.84. Found: C, 64.98; H, 6.71; N, 10.76.

Boc-Lys(Z)-azoAla-OH. Boc-Lys(Z)-azoAla-OMe (103 mg, 0.159 mmol) was dissolved in a dioxane (DOX)/ethanol mixture (2.5 mL/2.5 mL) and a 1 N NaOH solution (0.233 mL) was added. The ester hydrolysis was followed by TLC. After 2 h, the solution was neutralized with citric acid and the solvent was evaporated. The residual product was treated as above and an oily product was obtained. The removal of methyl ester was confirmed by ^1H NMR.

HCl·Lys(Z) and *N*-Hydroxysuccinimide Ester (HCl·Lys(Z)-OSu). Boc-Lys(Z)-OSu (Kokusan Chemical Works) was dissolved in 4 N HCl/DOX and left standing for 30 min at room temperature. The solvent was evaporated, and the product was solidified by the addition of excess ether. The removal of the Boc group was confirmed by ^1H NMR.

Boc-Lys(Z)-azoAla-Lys(Z)-OSu. Boc-Lys(Z)-azoAla-OH (67 mg, 0.106 mmol) was dissolved in tetrahydrofuran (THF; 0.5 mL), and the solution was cooled at −10 °C. *N*-Methylmorpholin (MM; 14 μL, 0.128 mmol) and isobutyl chloroformate (IBCF; 17 μL, 0.128 mmol) were added. After 5 min, a dimethylformamide (DMF) solution (0.1 mL) of HCl·Lys(Z)-OSu (44.2 mg, 0.107 mmol) and MM (14 μL, 0.128 mmol) was added. The mixture was stirred for 1 h at −10 °C and stored in a refrigerator overnight. The solvent was removed, and the residual oil was redissolved in ethyl acetate. The solution was washed as usual using a 1% $NaHCO_3$ solution as an alkaline solution. The product was solidified with ether. Yield: 37 mg (35%). Mp: 112–114 °C. Anal. Calcd for $C_{52}H_{62}N_8O_{12}$: C, 63.02; H, 6.31; N, 11.31. Found: C, 62.49; H, 6.40; N, 10.97.

Poly[Lys(Z)$_2$-azoAla]. The above compound (25 mg, 0.025 mmol) was dissolved in 4 N HCl/DOX (0.5 mL) and left standing for 30 min. The solvent was removed, and the residue was solidified and washed with ether. Yield: 17 mg (0.018 mmol). The N-deprotected tripeptide-activated ester was dissolved in DMF (3 drops), and TEA (10 μL) was added. The polymerization was continued for 1 week at room temperature. The polymer was precipitated with methanol, and the precipitate was washed with methanol/water (2/1) and finally with ether.

The polymer was analyzed with a Sephadex LH-60 gel (equilibrated with trimethyl phosphate (TMP)). The elution pattern showed a sharp peak at the elution limit of the gel (MW = 5×10^3–1×10^4). Therefore, the polypeptide has an average molecular weight larger than 5000, or the average degree of polymerization is larger than 7. For this polypeptide, spectroscopic measurements were made on an unfractionated sample.

Poly[Lys(Z)-azoAla]. Since a condensation of azoAla-Lys(Z)-OSu was expected to give a cyclic dipeptide, the polypeptide was prepared by the polymerization of azoAla-Lys(Z)-azoAla-Lys(Z)-OSu. First, HCl·Lys(Z)-azoAla-OMe and Boc-azoAla were coupled to obtain Boc-azoAla-Lys(Z)-azoAla-OMe. The terminal methyl ester was then removed by alkaline hydrolysis, and the tripeptide free acid was coupled with Lys(Z)-OSu. The Boc group of the tetrapeptide was removed, and the resulting tetrapeptide-activated ester was polymerized. The gel chromatographic analysis showed a sharp peak at the elution limit of the gel together with a small amount of low molecular weight components. The fraction eluted at the elution limit (MW > 5000) was collected and used for spectroscopic study.

Poly[Lys(Z)$_3$-azoAla]. The polypeptide was prepared by the polymerization of Lys(Z)$_2$-azoAla-Lys(Z)-OSu. The tetrapeptide-activated ester was synthesized by the coupling of Boc-Lys(Z)$_2$-azoAla-OH with Lys(Z)-OSu. The former tripeptide was synthesized by a coupling of Boc-Lys(Z) with Lys(Z)-azoAla-OMe. The polypeptide was fractionated with the gel chromatography, and the major fraction at the elution limit was used for spectroscopic study.

Boc-azoAla. AzoAla (1.97 g, 7.32 mmol) was dissolved in DOX/water (2/1, 21 mL) and cooled with ice. NaOH (1 N, 7.43 mL) and di-*tert*-butyl dicarbonate (Boc_2O) (1.78 g, 8.16 mmol) were added simultaneously. The mixture was stirred at the ice temperature for 1 h and at room temperature overnight. The mixture was concentrated, and ethyl acetate and a 5% $KHSO_4$ solution were added. The organic layer was washed with a 10% NaCl solution and dried over $MgSO_4$. The solvent was evaporated, and the oil that remained was collected. Yield: 2.01 g (73%).

Boc-azoAla-Aib-OMe. Boc-azoAla (1.52 g, 4.11 mmol) and HCl·Aib-OMe (0.665 g, 4.33 mmol) were coupled as described for Boc-Lys(Z)-azoAla-OMe. An oily product was obtained. Yield: 0.865 g (45%).

Boc-Lys(Z)-azoAla-AIb-OMe. The above dipeptide was dissolved in 4 N HCl/DOX and left standing for 30 min. The solvent was removed, and the N-deprotected dipeptide hydrochloride was collected as a solid product. Boc-Lys(Z) (0.534 g, 1.40 mmol) and the dipeptide hydrochloride (0.516 g, 1.28 mmol) were coupled as described for Boc-Lys(Z)-azoAla-OMe. The solid product was recrystallized from ethyl acetate/ether. Yield: 0.564 g (77%). Mp: 159–160 °C. Anal. Calcd for $C_{39}H_{50}N_6O_8$: C, 64.09; H, 6.90; N, 11.50. Found: C, 64.16; H, 6.99; N, 11.35.

Boc-Lys(Z)-azoAla-Aib-OSu. The tripeptide methyl ester (0.401 g, 0.549 mmol) was hydrolyzed in DOX/ethanol (4 mL/4 mL) containing 1 N NaOH (0.821 mmol). The mixture was treated as in the case of Boc-Lys(Z)-azoAla-OH, and an oily product was obtained. Yield: 0.341 g (87%). The latter was dissolved in DMF (2 mL) containing *N*-hydroxysuccinimide (HOSu) (115 mg, 1.0 mmol) and cooled with ice. EDC (0.144 g, 0.75 mmol) was added, and the mixture was stirred for 2 h at the ice temperature and for 2 days at room temperature. The solvent was evaporated, and the oil was redissolved in ethyl acetate. The solution was washed as usual and dried over $MgSO_4$. Evaporation gave an oily product. Yield: 0.28 g (73%).

Poly[Lys(Z)-azoAla-Aib]. The above tripeptide *N*-hydroxysuccinimide ester was treated with 4 N HCl/DOX to remove the Boc group. The product was an oil. The N-deprotected tripeptide-activated ester (88 mg, 0.118 mmol) was dissolved in 8 drops of DMF, and TEA (20 μL, 0.144 mmol) was added. The mixture was left standing at room temperature for 15 days. The polypeptide obtained was fractionated with a Sephadex LH-60 column. The elution curve showed a broad peak, and the average molecular weight was less than 5000. For spectroscopic study, the fraction that appeared at the elution limit was used.

Poly[azoAla-Lys(Z)-azoAla-Aib]. This polypeptide was prepared by the polymerization of the corresponding tetrapeptide *N*-hydroxysuccinimide ester. Boc-azoAla-Lys(Z)-azoAla-Aib-OMe was synthesized by a coupling of Lys(Z)-azoAla-Aib-OMe with Boc-azoAla. The methyl ester of the tetrapeptide was hydrolyzed, and the free acid was coupled with *N*-hydroxysuccinimide to synthesize the tetrapeptide-activated ester. The polypeptide showed a broad elution pattern in the gel chromatogram, and the average molecular weight is less than 5000. The fraction of the highest molecular weight (molecular weight = ca. 5000) was used for the spectroscopic study.

Poly[Lys(Z)$_2$-azoAla-Aib]. The polypeptide was prepared by the polymerization of Lys(Z)$_2$-azoAla-Aib-OSu. The tetrapeptide was synthesized by a coupling of Lys(Z)-azoAla-Aib-OMe with Boc-Lys(Z). The methyl ester was then exchanged to hydroxysuccinimide ester. The elution diagram of the polypeptide showed a sharp rise at the elution limit, but a significant amount of low molecular weight component was also present. The fraction of the highest molecular weight was used.

Ac-azoAla-OMe. HCl·azoAla-OMe (199 mg, 0.662 mmol) was dissolved in chloroform (5 mL) containing TEA (0.172 mL, 1.24 mmol). Acetic anhydride (0.294 mL, 3.12 mmol) was added, and the mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. The solvent was evaporated, and the oil that remained was redissolved in ethyl acetate. The organic solution was washed as usual and dried over $MgSO_4$. Evaporation gave a solid product, and the latter was recrystallized from ethyl acetate/ether. Yield: 77 mg (38%). Mp: 159–161 °C. Anal. Calcd for $C_{18}H_{18}N_3O_5$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.29; H, 5.93; N, 12.99.

Boc-azoAla-azoAla-OMe. Boc-azoAla (86.2 mg, 0.233 mmol) and HCl·azoAla-OMe (70.4 mg, 0.220 mmol) were dissolved in chloroform (2 mL) containing TEA (38 μL, 0.274 mmol), EDC (46.2 mg, 0.241 mmol), and HOBt (36.9 mg, 0.241 mmol). The mixture was stirred under cooling with ice for 2 h and then 12 h at room temperature. The reaction mixture was treated as usual to obtain a solid product, which was recrystallized from

ethyl acetate/hexane. Yield: 68.1 mg (47%). Mp: 186–189 °C. Anal. Calcd for $C_{36}H_{38}N_6O_5$: C, 68.12; H, 6.03; N, 13.24. Found: C, 68.01; H, 5.81; N, 13.03.

Measurements. Trimethyl phosphate (TMP) was used as solvent throughout this study, because of the transparency down to 190 nm. The concentration of the polypeptide was determined by the absorbance of dark-adapted solution (trans form). The molar absorption coefficient of Ac-azoAla-OMe ($\epsilon_{325} = 2.03 \times 10^4$) was used. The polypeptide solutions were irradiated by using a Jasco CRM-FA monochromatic irradiator equipped with a 2-kW xenon light source. The following instruments were used: absorption, Hitachi 320; CD, Jasco J-500; optical rotation, Jasco DIP370; ^{1}H NMR, JEOL FX90Q. The absorption and CD spectrometers were interfaced to an NEC PC9801 computer.

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