

Syntheses of Poly(*S*-benzyl *D*- and *L*-penicillamines) and Their Secondary Structures

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ABSTRACT: Poly(*S*-benzyl *D*- and *L*-penicillamines) were prepared from the corresponding *N*-carboxyanhydrides (NCA). These polymers were soluble in alkyl halides, such as chloroform and methylene dichloride, but insoluble in dichloroacetic acid (DCA) and trifluoroacetic acid (TFA). Judging from the results obtained by a study of the infrared absorption (IR) spectra and X-ray photographs, polymers are in the ω -helical structure in the solid state. The results of the optical rotatory dispersion (ORD) and the circular dichroism (CD) in chloroform suggest that these polymers have the ω -helical structure and their helical senses are reversed. By adding TFA to the chloroform solution of these polymers, both ORD and CD curves are markedly changed from the ω -helical structure to the β -structure. The intrinsic viscosity and the flotation data indicated that the polymer exists in the intermolecular β -structure in a chloroform—TFA mixture.

KEY WORDS Poly(*S*-benzyl *D*- and *L*-penicillamines) / Synthesis / $\omega \rightleftharpoons \beta$ Transition / Infrared Absorption Spectra / X-Ray Diffraction / Optical Rotatory Dispersion / Circular Dichroism /

D-Penicillamine (β , β -dimethyl cysteine) exists in antibiotic penicillin and is an α -amino acid with a thiol group in its side chain. It is well established that many synthetic polypeptides form stably the α -helical conformation. In the case of poly(*S*-alkyl cysteine), the steric interference between the side chain and main chain makes the α -helical conformation unstable. Many kinds of poly(*S*-alkyl cysteines) have been synthesized and reported to be a β -structure in the solid state and in solution.¹⁻⁴ Furthermore, Blout⁵ reported that polyamino acids with two groups attached to the same β -carbon, such as polyvaline and polyisoleucine, do not form the α -helix due to the steric factors.

In the present study, we synthesized penicillamine,⁶ which has two branched methyl groups on the β -carbon of cysteine, and polymerized its *S*-benzyl derivative to poly(*S*-benzyl *D*- and *L*-penicillamines) *via* the NCA method. The secondary structures of these polymers were studied by means of the ORD, CD, IR, and X-ray techniques in the solid state and in solution.

EXPERIMENTAL

Materials

S-Benzyl *D*- and *L*-Penicillamines. These were synthesized as reported by Crooks.⁶

S-Benzyl *D*-Penicillamine NCA. Dry phosgene was passed through a suspension of *S*-benzyl *D*-penicillamine (2.7 g) in 54 ml of dry dioxane for 30 min at 40°C. Nitrogen was then passed through the reaction mixture for 30 min. The solvent was removed at 40°C under reduced pressure. The residue was treated with *n*-hexane, filtered, and dried. This was recrystallized from ethyl acetate—*n*-hexane; yield, 2.65 g (88%); mp 163°C.

Anal. Found: C, 59.07; H, 5.53; N, 5.16%.
Calcd for C₁₃H₁₅NO₃S:

C, 58.85; H, 5.70; N, 5.28%.

S-Benzyl *L*-Penicillamine NCA. *S*-Benzyl *L*-penicillamine NCA was prepared from *S*-benzyl *L*-penicillamine (2.0 g) and phosgene in the above mentioned manner; yield, 1.8 g (81%); mp 163°C.

Anal. Found: C, 59.05; H, 5.53; N, 5.06%.
Calcd for $C_{13}H_{15}NO_3S$:

C, 58.85; H, 5.70; N, 5.28%.

Poly(S-benzyl D-penicillamine). *S*-Benzyl *D*-penicillamine NCA (2.6 g) was dissolved in a chlorobenzene (4.5 ml)—DMF (1.5 ml) mixture and *n*-hexylamine (0.01 ml) was added to the solution. The mixture was polymerized at 50°C for two days and yielded a semisolid gel. The polymer was washed successively with ether, methanol, water, and acetone, and dried; yield, 2.1 g (97%).

Anal. Found: C, 64.91; H, 6.51; N, 6.27%.
Calcd for $(C_{12}H_{15}NOS)_n$:

C, 65.12; H, 6.38; N, 6.33%.

N-Terminal titration, MW=16000 (DP=72).

Poly(S-benzyl L-penicillamine). *S*-Benzyl *L*-penicillamine NCA (1.3 g) was polymerized in the same way as mentioned above; yield, 0.90 g (83%).

Anal. Found: C, 65.42; H, 6.68; N, 6.35%.
Calcd for $(C_{12}H_{15}NOS)_n$:

C, 65.12; H, 6.38; N, 6.33%.

N-Terminal titration, MW=15700 (DP=71).

Methods

Optical rotatory dispersion (ORD), circular dichroism (CD) and infrared absorption (IR) spectra measurements were made on the ORD/UV-5 instrument with a CD attachment and the IR DS-301 instrument, respectively, both made by the Japan Spectroscopic Co., Ltd. The ORD and CD were measured with 0.1–10-mm cells for the 210–600-nm wavelengths. As for CD, the measured values of $\epsilon_L - \epsilon_R$ were converted to molar ellipticity. The concentration of the samples were in the 0.1–1.0-% range. The X-ray diffraction photographs were taken with a Rigakudenki Geigerflex, using a Cu-target. The intrinsic viscosity was measured in an Ubbelohde-type viscometer at 20°C. The measurement of the flotation coefficient was made in a Hitachi Model UCA-1A ultracentrifuge at 22°C. The rotor speed used was 51200 rpm.

RESULTS AND DISCUSSION

Conformation of the Polymers in the Solid State IR Studies and X-Ray Analyses. The IR spectra of the films of *D*- and *L*-polymers cast from the chloroform solution are shown in

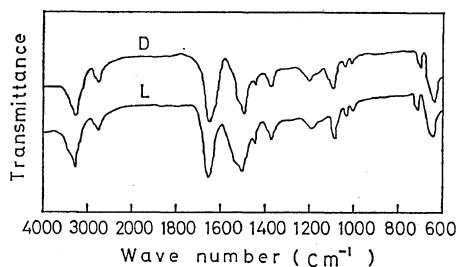


Figure 1. Infrared spectra of poly(*S*-benzyl *D*- and *L*-penicillamines) in solid films.

Table I. Characteristic absorption bands of poly(*S*-benzyl *D*- and *L*-penicillamines) in solid film

Assignment	Wave number, cm ⁻¹	
	<i>D</i> -Polymer	<i>L</i> -Polymer
Amide A	3370 (vs) ^a	3365 (vs)
Amide B	3037 (w)	3035 (w)
Amide I	1660 (vs)	1658 (vs)
Amide II	1545 (sh)	1545 (sh)

^a vs, very strong; w, weak; sh, shoulder.

Figure 1. The wave numbers of the characteristic amide bands are listed in Table I. Both the *D*- and *L*-polymers gave almost the same IR spectra. The amide I and amide II are found at 1660 and 1545 cm⁻¹. The absorption shoulder at 1545 cm⁻¹ may be due to the influence of the absorption of the *S*-benzyl group. This suggests the polymers do not form the β -conformation. The IR dichroism of the stretched film of poly(*S*-benzyl *D*-penicillamine) is shown in Figure 2. This shows that the direction of the $>C=O \cdots H-N<$ in the polypeptide is the same

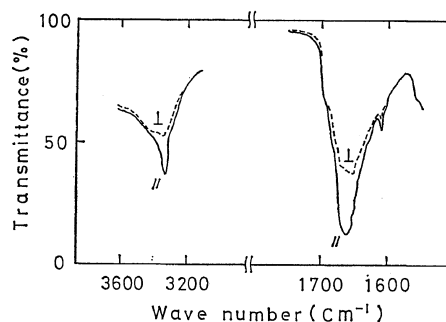


Figure 2. Infrared dichroism of stretched film of poly(*S*-benzyl *D*-penicillamine).

Table II. Spacings in X-ray diffraction patterns of poly(*S*-benzyl D- and L-penicillamines) in solid film

Order	D-Polymer		L-Polymer	
	<i>d</i> , Å	Intensity ^a	<i>d</i> , Å	Intensity ^a
R ₁	13.3	vs	13.3	vs
R ₂	5.10	vs	5.15	vs
R ₃	4.02	w	4.02	w
R ₄	3.27	w	3.30	w

^a vs, very strong; w, weak.

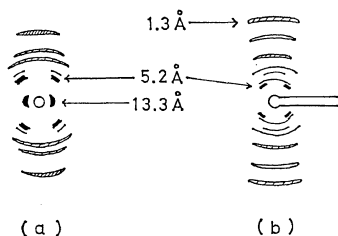


Figure 3. Schematic X-ray diffraction pattern of stretched films of poly(*S*-benzyl D-penicillamine): (a), 3.9-cm flat camera, X-ray normal to fibre axis; (b), 2.87-cm cylindrical camera, X-ray normal to fibre axis which is along camera axis.

as that of the orientation and suggests the formation of an intramolecular hydrogen-bonded helical structure similar to the α -helix.⁷

The observed spacings and intensities obtained from the X-ray diffraction patterns in the films of D- and L-polymers are listed in Table II. These results suggest both of them take the same secondary structure. Figure 3 shows the schematic X-ray diffraction pattern of an oriented poly(*S*-benzyl D-penicillamine) film. The strong spacing at about 13.3 Å, which lies along the equatorial arc, shows that the spacing corresponds to the helix-helix interval of the α -helical polypeptide (a).⁸ The layer reflection observed at 5.2 Å (a). Figure 3-b shows a meridional reflection corresponding to a spacing of about 1.3 Å, and suggests that the structure is based on the ω -helical structure like ω -poly(β -benzyl L-aspartate)⁹⁻¹¹ and poly(*S*-benzylthio L-cysteine).⁹ The conformation of poly(*S*-benzyl D- and L-penicillamines) differs from the β -structure of poly(*S*-methyl cysteine) and poly(*S*-benzyl cysteine).^{12,13}

The solid state structures of poly(*S*-benzyl D- and L-penicillamines) estimated from IR and X-ray analyses are considered to be the ω -structure.

Conformation of the Polymers in Solution

Optical Rotatory Dispersion and Circular Dichroism. The ORD curves of poly(*S*-benzyl D-penicillamine) for the 300–600-nm wavelengths in a chloroform–TFA mixture are shown in Figure 4. In 100% chloroform (curve A), the dextrorotation increases with decreasing wavelength. When 1% of TFA is added to this chloroform solution (curve B),

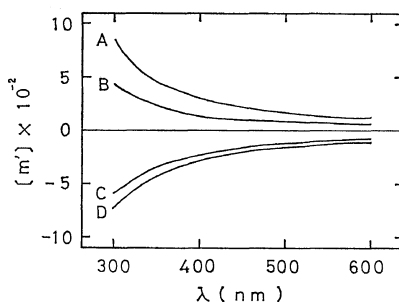


Figure 4. ORD of poly(*S*-benzyl D-penicillamine) in a chloroform–TFA mixture as a function of solvent composition at 22°C: volume % of TFA, A, 0; B, 1; C, 2; D, 5.

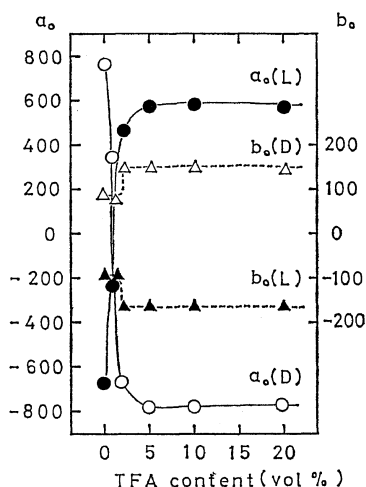


Figure 5. Dependence of the a_0 and b_0 of poly(*S*-benzyl D- and L-penicillamines) in a chloroform–TFA mixture of solvent composition at 22°C: (○) a_0 (D); (●) a_0 (L); (△) b_0 (D); (▲) b_0 (L).

the rotation decreases. 2% of TFA (curve C) inverts the rotation into levo. This means that a small amount of TFA causes a drastic conformational transition. The values of the parameters, a_0 and b_0 , derived from the Moffitt—Yang equation, were determined from the ORD curves for the 350–600-nm wavelengths and are shown in Figure 5. As seen in Figure 5, both the D- and L-polymers gave almost the same behavior. 2–3% of TFA causes the marked changes in a_0 . The a_0 value changed from +760 to –790 in the D-polymer and from –680 to +590 in the L-polymer. These results differ from the α -helix to random coil transition of the usual α -helical polypeptides¹⁴ owing to the absorption of *S*-benzyl group.

In order to clarify the conformation in solution, ORD and CD measurements were carried out in the ultraviolet region. Figure 6 shows the ORD curves of poly(*S*-benzyl D- and L-penicillamines) in chloroform. Poly(*S*-benzyl D-penicillamine) exhibits a peak at 248 nm with $[m']_{248} = +2900 \text{ deg cm}^2/\text{dm}$, a crossover point near 233 nm, a trough at 228 nm with $[m']_{228} = -1600 \text{ deg cm}^2/\text{dm}$, and a second crossover point near 222 nm, and a peak can be expected below 220 nm. Furthermore, the ORD shows two small aromatic (benzyl group) Cotton effects in the 273- and 265-nm regions.

The CD curves are shown in Figure 7. The ORD and the CD spectra correspond well to

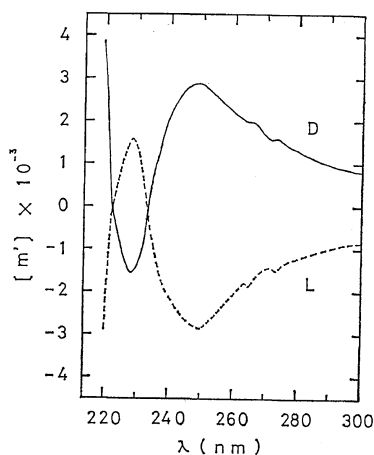


Figure 6. ORD of poly(*S*-benzyl D- and L-penicillamines) in chloroform at 22°C.

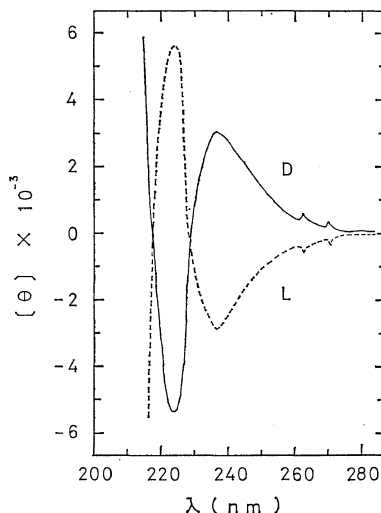


Figure 7. CD of poly(*S*-benzyl D- and L-penicillamines) in chloroform at 22°C.

each other. Two dichroism bands at 236 and 223 nm, with $[\theta]_{236} = +3000$ and $[\theta]_{223} = -5400$ were observed for D-polymer, and a positive dichroism band below 220 nm can be expected. The ORD and the CD curves of the L-polymer are found in the same positions and have the same magnitudes with an opposite sign. The positive ellipticity band at 236 nm appears to be the $\pi-\pi^*$ transition of the *S*-benzyl group and the negative band at 223 nm is to be assigned to the $n-\pi^*$ peptide electronic transition. In comparison with the usual polypeptides, this $n-\pi^*$ peptide electronic transition shows that β , β -dimethyl groups in the side chain reverse the conformational screw sense of this polypeptide. As in the case of the left-handed α -helical poly(β -benzyl L-aspartate),^{15–17} we may consider that L-polymer takes a left-handed sense and D-polymer does a right-handed one. The ellipticity band at 236 nm associated with the absorption bands of the aromatic group is close to poly(N^{ω} -carbobenzoxy L-*p*-aminophenylalanine)¹⁸ and poly($N^{\omega, \omega'}$ -dicarbobenzoxy L-arginine),¹⁹ in which the helical arrangements in solution delineate the stacking of the aromatic Cotton effect. The band at 223 nm seems close to the $n-\pi^*$ transition observed in the usual α -helical polypeptide, but the magnitude is much smaller.

Poly(*S*-benzyl D- and L-penicillamines)

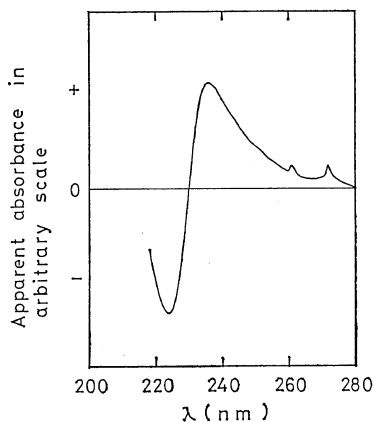


Figure 8. CD of a solid film of poly(*S*-benzyl D-penicillamine).

The CD spectrum of the film of poly(*S*-benzyl D-penicillamine) cast from the chloroform solution is shown in Figure 8. The positions of the CD curve of the film are very similar to that of the polypeptide in chloroform. Supposing that the solid conformation of the polypeptide is maintained in solution, this suggests that the polypeptide has an ω -structure in chloroform solution. From these results we consider that poly(*S*-benzyl D-penicillamine) forms a right-handed ω -helical structure and poly(*S*-benzyl L-penicillamine) forms a left-

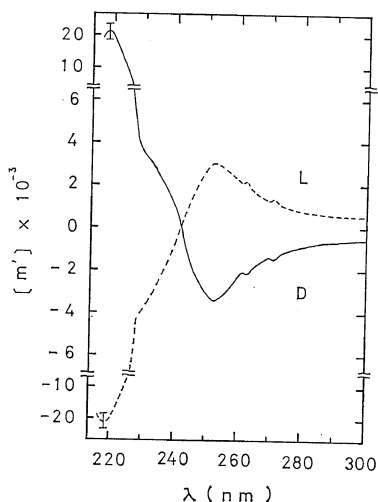


Figure 9. ORD of poly(*S*-benzyl D- and L-penicillamines) in a 95-% chloroform—5-% TFA mixture at 22°C.

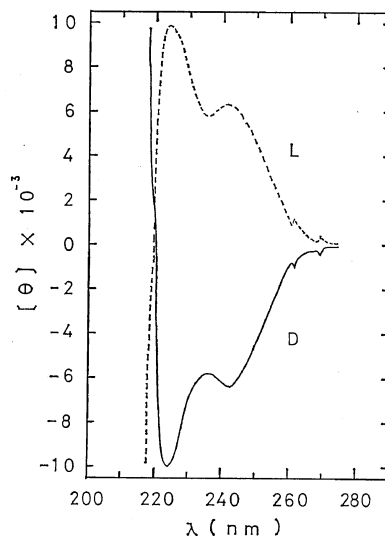


Figure 10. CD of poly(*S*-benzyl D- and L-penicillamines) in a 95-% chloroform—5-% TFA mixture at 22°C.

handed one.

The ORD and the CD curves in a chloroform—TFA (95 : 5) mixture are shown in Figures 9 and 10. In this solvent system, the behaviors of both D- and L-polymers differ greatly from the results shown in Figures 6 and 7. The absorbency of the side chain shows a sudden reversal. Poly(*S*-benzyl D-penicillamine) has a negative trough at 252 nm and L-polymer has a positive one. The D-polymer exhibits two negative Cotton effects at 272 and 263 nm due to the side chain, a trough at 252 nm with $[m']_{252} = -3400 \text{ deg cm}^2/\text{dm}$, a crossover point at 242 nm, a shoulder at 230 nm with $[m']_{230} = +4000 \text{ deg cm}^2/\text{dm}$, and a peak at 219 nm with $[m']_{219} = +20000 \text{ deg cm}^2/\text{dm}$. As shown in Figure 10, the CD curves correspond well to the ORD curves and exhibit two negative dichroism bands at 242 and 224 nm with $[\theta]_{242} = -6400$ and $[\theta]_{224} = -10000$, in the D-polymer. The L-polymer yielded an almost identical curve with an opposite sign. In a chloroform—TFA (95 : 5) mixture the D- and L-polymers have a reverse-type polypeptide conformation from that in 100-% chloroform. Comparing the conformation in a chloroform—TFA mixture with that in 100-% chloroform, the $n-\pi^*$ peptide transition is found in the same position and has

the same sign and a larger magnitude. However, the $\pi-\pi^*$ transition due to the benzyl group has the opposite sign showing a change in the secondary structure.

Infrared Spectra of Poly(*S*-benzyl *D*-penicillamine) in Solution. In order to determine the secondary structure in a chloroform—TFA mixture, IR solution measurements were carried out. The results are shown in Figure 11. In 100% chloroform the amide I band at 1660 cm^{-1} shows the ω -structure and in a chloroform—TFA (97 : 3) mixture the amide I at 1625 cm^{-1} shows the β -structure. The latter is very close to the conformation of poly(L-cysteine) deriva-

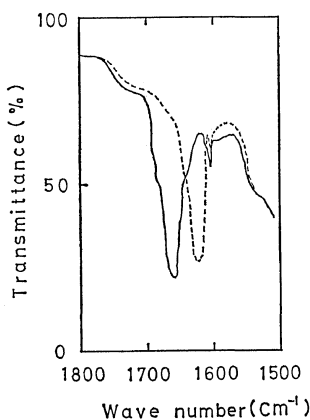


Figure 11. Infrared spectra of poly(*S*-benzyl *D*-penicillamine) in solution at 22°C : (—) in chloroform; (-----) in 97% chloroform—3% TFA.

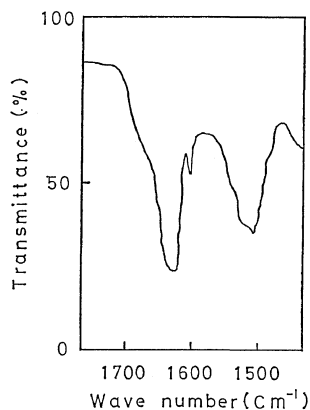


Figure 12. Infrared spectrum of poly(*S*-benzyl *D*-penicillamine) which was precipitated by the addition of excess TFA (KBr disk).

tives in solution.¹

Poly(*S*-benzyl *D*- and *L*-penicillamines) are soluble in a chloroform—TFA mixture containing up to 20% TFA. The white polymer precipitates at TFA percentages greater than 20. The polymer was precipitated by the excess addition of TFA, washed with ether, and dried. The IR spectrum of the poly(*S*-benzyl *D*-penicillamine) thus prepared is shown in Figure 12. This shows the absorptions at 1625 and 1520 cm^{-1} , and suggests the polymer has the β -structure. X-ray photograph of this sample shows a backbone spacing of about 4.6 \AA (β -structure) and coincides well with the results of the IR spectrum.

Intrinsic Viscosity and Flotation Coefficient of Poly(*S*-benzyl *D*-penicillamine) in Solution. To elucidate the β -structure in a chloroform—TFA mixture, intrinsic viscosity, $[\eta]$, and flotation coefficient, S_f , measurements were carried out. Figure 13 shows the viscosity of poly(*S*-benzyl *D*-penicillamine) in solution. The plot gave a straight line in chloroform, and the ordinate intercept, $[\eta]$, is 0.265 dl/g . In a chloroform—TFA (95 : 5) mixture it gave a smooth curve at low concentration (within 0.8 g/dl) and $[\eta]$ is about 0.420 dl/g . In comparison with the former, the $[\eta]$ value is much larger. By combining the magnitude of $[\eta]$ with the marked increase in the reduced viscosity at higher

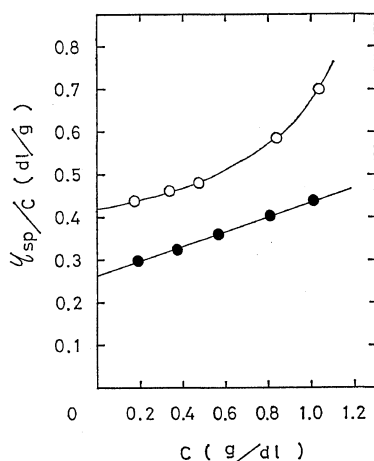


Figure 13. The reduced viscosities of poly(*S*-benzyl *D*-penicillamine) in solutions at 20°C : (●) in chloroform; (○) in a 95% chloroform—5% TFA.

concentration (above 0.8 g/dl), it is suggested that the polymer exists in an intermolecular β -structure. Further, the same behavior was observed in the measurement of the flotation coefficient, S_f , in solution. The S_f value is 1.28 (svedberg unit) in chloroform and 2.56 in a chloroform—TFA (95:5) mixture for a polymer concentration of 0.510 g/dl at 22°C. This result shows that the conformation of the polymer in a chloroform—TFA mixture is the same as the viscometry and supports the formation of an intermolecular β -structure.

As described above, the ORD, CD, IR, viscometry, and flotation results suggest that poly(S-benzyl D- and L-penicillamines) in chloroform take the ω -helical conformation, and their screw senses are reversed for the usual polypeptides. Both D- and L-polymers take the intermolecular β -structure in a chloroform—TFA mixture. These polymers cause an ω -helical conformation \rightleftharpoons β -structure transition, which differs from the usual α -helix \rightleftharpoons coil or β -structure \rightleftharpoons coil transitions. We propose here an assumption that a polypeptide with branching on the β -carbon in its side chain causes an $\omega \rightleftharpoons \beta$ transition owing to the steric hindrance between the side chain and main chain.

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