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# Comparison of the Effect of Five Guest Residues on the β-Sheet Conformation of Host (L-Val)$_n$ Oligopeptides

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ABSTRACT: The synthesis, characterization, and IR absorption, $^1$H NMR, and CD properties of (L-Val)$_n$ host tetra-, penta-, and hexapeptides containing an L-Ile, L-Ala, D-Val, L-Pro, or Aib guest residue are reported, with the intention of establishing a scale of β-sheet disrupting capability of various amino acids. The results indicate that in the solid state the β-sheet structures of the (L-Val)$_n$ ($n$ = 5, 6) homopeptides are at least partially disrupted by the L-Pro and Aib residues. In $CH_2Cl_2$/$Me_2SO$ solvent mixtures the following rank order is obtained for the stability of the β-sheets: L-Val > L-Val > L-Ile ≫ L-Ala ≫ D-Val > L-Pro ≫ Aib. In $CDCl_3$ solutions the Aib hexapeptide is folded into a partially labile $3_{10}$-helical structure. Statistically disordered conformations largely prevail in more polar solvents like 2,2,2-trifluoroethanol and ethanol in all the peptides examined. Only the (L-Val)$_n$ hexapeptide is prone to adopt the β-sheet conformation upon addition of 40% (v/v) water to the trifluoroethanol solution.

## Introduction

The approach of coupling a single guest residue into selected positions of a strictly monodisperse, well characterized, linear host homooligopeptide chain was introduced in 1978 by Goodman and co-workers to gain a deeper insight into the factors influencing the conformational preferences of low molecular weight peptides.[1,2] Subsequently, this host–guest approach has been extended with the aim of (i) establishing a link between experimental studies of the parameters determining peptide conformation and theoretical efforts aimed at the prediction of secondary structure from sequence and (ii) designing the synthetic route for peptides based on conformation-dependent solubility predictions.[3-14]

Although a number of regular peptide structures (α-helix, $3_{10}$-helix, and β-sheet conformation) have already been the target of investigations, a systematic study providing a quantitative evaluation of the factors playing a role in secondary structure formation and stability is still to be performed. Consequently, we have decided as a first step in this direction to synthesize, fully characterize, and determine the solid-state and solution conformational preferences of monodisperse (L-Val)$_n$ host homooligopeptides containing a variety of amino acids as single guest residues, with the purpose of establishing a scale of β-sheet disrupting capability under different experimental conditions. We have selected –(L-Val)$_n$– sequences in view of their known tendencies to favor β-sheet structures (twisted, parallel type).[9,15-29] This paper describes the results obtained with a first set of five residues which introduce different types of stereochemical perturbations. These are (i) Aib, α-aminoisobutyric acid (α-alkylation), (ii) L-Pro (N-alkylation and N–$C^\alpha$ restriction), (iii) D-Val (inversion of configuration at $C^\alpha$), (iv) L-Ile (introduction of an additional $CH_2$ group into the β-branched side chain), and (v) L-Ala (reduction of side-chain bulk).

## Experimental Section

**Synthesis of Peptides.** The synthesis and characterization of Boc-(L-Val)$_n$-OMe ($n$ = 2, 3, 5, and 6) homopeptides have already been reported.[17] The other peptides were synthesized by the racemization-free mixed anhydride method with isobutyl chloroformate and *N*-methylmorpholine in 1,4-dioxane/*N*,*N*-dimethylformamide[30] using the appropriate Boc-protected amino acid. The Aib residue was, however, introduced by using dicyclohexylcarbodiimide-1-hydroxybenzotriazole mediated couplings in DMF. A summary of the physical properties and analytical data for the host–guest peptides is given in Table I.

**Infrared Absorption.** Infrared spectra (IR) were recorded using a Perkin-Elmer Model 580 B spectrophotometer equipped with a Model 3600 IR data station. The band positions are accurate to ±1 cm$^{-1}$. Cells with path length 0.1, 1.0, and 10 mm (with $CaF_2$ windows) were used for the solution measurements. Spectrograde chloroform (99% D), methylene chloride, dimethyl sulfoxide, and 2,2,2-trifluoroethanol were purchased from Fluka. For the solid-state measurements the KBr disk technique was used.

**Circular Dichroism.** CD spectra were recorded on a Jasco Model J-500 A spectropolarimeter equipped with a DP-501 N data processor. Cylindrical fused quartz cells of 0.01- and 0.02-cm path length were employed. The values are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg cm$^2$ dmol$^{-1}$).

**$^1$H Nuclear Magnetic Resonance.** The $^1$H NMR spectra were recorded on a Bruker AM-400 spectrometer. Measurements were carried out in $CDCl_3$ (99.96% D) and $Me_2SO$-$d_6$ (99.96% D) with tetramethylsilane as the internal standard.

## Results and Discussion

**Solid-State Conformational Analysis.** Preliminary information on the conformational preferences of the host–guest peptides compared to those of the (L-Val)$_n$ ($n$ = 5, 6) host peptides[18,25] was obtained by IR absorption. The most informative frequency ranges[4,8,11,18,20,25,32-34] are (i) 3500–3200 cm$^{-1}$, corresponding to the N—H stretching vibrations of the peptide (amide A) and N-protecting urethane groups, and (ii) 1800–1600 cm$^{-1}$, corresponding to the C=O stretching vibrations of the peptide (amide I), urethane, and ester groups. The results are listed in Table I. In the 3500–3200-cm$^{-1}$ region a band of low intensity is seen at a frequency >3430 cm$^{-1}$ in all the peptides examined, except Boc-(Val)$_6$-OMe, the position of which is indicative of the occurrence of free (or extremely weakly hydrogen bonded) NH groups. In addition, an intense band related to strongly hydrogen bonded NH groups is visible at 3320–3275 cm$^{-1}$. Interestingly, in the L-Pro and Aib peptides the position of this band is at significantly higher frequency than those of the other peptides.

In the 1800–1600-cm$^{-1}$ region five bands are seen: (i) the weak bands at 1746–1740 and 1693–1690 cm$^{-1}$ are assigned to the absorptions of the C=O groups of free methyl ester moieties and hydrogen-bonded urethane groups, respectively; (ii) the weak band at 1725–1717 cm$^{-1}$ is attributed to the absorptions of the C=O groups of hydrogen bonded esters and/or free urethanes; (iii) the strong bands near 1650 cm$^{-1}$ and 1640–1633 cm$^{-1}$ are as-

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**Table I**
**Summary of Physical Properties and Analytical Data for Host–Guest Peptides**

| peptide | mp,[a] °C | solvent[b] | $R_f$(A)[c] | $R_f$(B)[c] | $R_f$(C)[c] | $[\alpha]_{20}$[d] | amino acid analysis[e] |
|---|---|---|---|---|---|---|---|
| Boc-L-Ala-(L-Val)$_3$-OMe | 239–240 | DMF/$H_2O$ | 0.95 | 0.75 | 0.75 | −70.0 | Ala 1.00; Val 3.02 |
| Boc-L-Val-L-Ala-(L-Val)$_3$-OMe | >255 | hot DMF/$H_2O$ | 0.95 | 0.70 | 0.65 | −69.4 | Ala 1.07; Val 3.93 |
| Boc-L-(Val)$_2$-L-Ala-(L-Val)$_3$-OMe | >255 | hot DMF/$H_2O$ | 0.95 | 0.65 | 0.60 | −71.3 | Ala 1.10; Val 4.90 |
| Boc-D-Val-(L-Val)$_3$-OMe | 254–255 | DMF/$H_2O$ | 0.95 | 0.80 | 0.75 | −39.6 | |
| Boc-L-Val-D-Val-(L-Val)$_3$-OMe | >255 | DMF/$H_2O$ | 0.95 | 0.75 | 0.65 | −38.8 | |
| Boc-L-(Val)$_2$-D-Val-(L-Val)$_3$-OMe | >255 | DMF/$H_2O$ | 0.95 | 0.75 | 0.65 | −38.0 | |
| Boc-L-Ile-(L-Val)$_3$-OMe | 247–248 | DMF/$H_2O$ | 0.95 | 0.80 | 0.80 | −80.4 | Ile 0.95; Val 3.05 |
| Boc-L-Val-L-Ile-(L-Val)$_3$-OMe | >255 | Hot DMF/$H_2O$ | 0.95 | 0.70 | 0.65 | −75.0 | Ile 0.98; Val 4.02 |
| Boc-(L-Val)$_2$-L-Pro-(L-Val)$_3$-OMe | 183–184 | DMF/$H_2O$ | 0.95 | 0.95 | 0.60 | −152.9 | Pro 1.00; Val 5.00 |
| Boc-(L-Val)$_2$-Aib-(L-Val)$_3$-OMe | 157–158 | DMF/$H_2O$ | 0.95 | 0.95 | 0.60 | −63.4 | Aib 0.90; Val 5.10 |

[a] Melting points are uncorrected. [b] DMF, *N,N*-dimethylformamide. [c] Thin-layer chromatography (silica gel plates 60F-254, Merck) was performed in (A) 1-butanol–acetic acid–water (60:20:20, (B) chloroform–ethanol 90:10, and (C) chloroform–ethanol 96:4. Peptides were visualized by the hypochlorite–starch–iodide reaction. [d] The optical rotation measurements were made on a Perkin-Elmer Model 241 polarimeter equipped with a Haake Model L thermostat ($c$ = 0.05 in 2,2,2-trifluoroethanol). [e] Amino acid analyses were carried out on a C.Erba Model 3A 27 amino acid analyzer. Acid hydrolysis time: 72 h.[31]

**Table II**
**Infrared Absorption Frequencies (cm$^{-1}$) for the Host and Host–Guest Peptides in the Solid State**

| peptide | NH stretch | | C=O stretch | | | | |
|---|---|---|---|---|---|---|---|
| Boc-(L-Val)$_5$-OMe[a] | 3434[b] | *3277*[c] | 1742 | 1717 | 1692 | | *1638*[c] |
| Boc-L-Val-L-Ile-(L-Val)$_3$-OMe | 3440 | *3275* | 1744 | 1718 | 1692 | | *1637* |
| Boc-L-Val-L-Ala-(L-Val)$_3$-OMe | 3446 | *3289* | 1742 | 1720 | 1690 | | *1639* |
| Boc-L-Val-D-Val-(L-Val)$_3$-OMe | 3438 | *3284* | 1745 | 1722 | 1693 | | *1640* |
| Boc-(L-Val)$_6$-OMe[a] | | *3279* | 1746 | 1720 | 1693 | | *1634* |
| Boc-(L-Val)$_2$-L-Ala-(L-Val)$_3$-OMe | 3438 | *3285* | 1740 | 1719 | 1690 | | *1638* |
| Boc-(L-Val)$_2$-D-Val-(L-Val)$_3$-OMe | 3434 | *3299* | 1743 | 1725 | 1690 | | *1640* |
| Boc-L-(Val)$_2$-L-Pro-(L-Val)$_3$-OMe | 3422 | *3313* | 1746 | 1719 | 1691 | *1653* | 1633 |
| Boc-L-(Val)$_2$-Aib-(L-Val)$_3$-OMe | 3440 | *3320* | 1746 | | 1692 | | *1647* |

[a] See also ref 18 and 25. [b] The following symbols are used: italic, strong band; —, weak band or shoulder. [c] For all compounds bands in this spectral region are broad.

signed to the C=O groups of weakly and strongly hydrogen bonded peptide moieties, respectively.

From the data reported in Table II it appears that the spectral behavior of the L-Ile, the L-Ala, and to a lesser extent the D-Val peptides roughly parallels that of the (L-Val)$_n$ ($n$ = 5, 6) homopeptides and is typical (3290–3275-cm$^{-1}$ and 1640–1634-cm$^{-1}$ bands) of an almost fully developed $\beta$-sheet conformation. In addition, it seems reasonable to interpret the occurrence of the 3313-, 1653-, and 1633-cm$^{-1}$ bands in the spectrum of the L-Pro peptide as arising from a structural irregularity in the $\beta$-sheet. Also, the *X*-Pro tertiary amide bond is known to contribute to the 1630-cm$^{-1}$ absorption.[4,13] The spectrum of the Aib hexapeptide does not permit an unequivocal conformational assignment at this stage since it is difficult to discriminate between an irregular $\beta$-sheet and a helical conformation on the basis of IR data alone.[35,36] Interestingly, the melting points of the Aib and Pro peptides were significantly lower than those of the other host–guest peptides (Table I).

**Solution Conformational Analysis.** IR absorption is a useful technique to quantitatively titrate the extent of peptide self-association in general and of $\beta$-sheet formation in particular.[6,8,37,38] The relative stabilities of the $\beta$-sheets formed by the various host and host–guest peptides have been examined in $CH_2Cl_2$ (peptide concentration 14–19 mM), by adding increasing amounts of $Me_2SO$, a solvent known to form effective peptide–solvent (N—H···O=S) hydrogen bonds.[39] For each peptide the relative intensity was calculated from the area of the low-frequency C=O stretching band (1640–1630 cm$^{-1}$), taking the value observed in pure $CH_2Cl_2$ (or in the $CH_2Cl_2$–$Me_2SO$ mixture containing the lowest percentage of $Me_2SO$ required to dissolve the peptide if it was not completely soluble in $CH_2Cl_2$ at the concentration to be examined) as 1.0. Figure 1 illustrates a typical IR absorption pattern

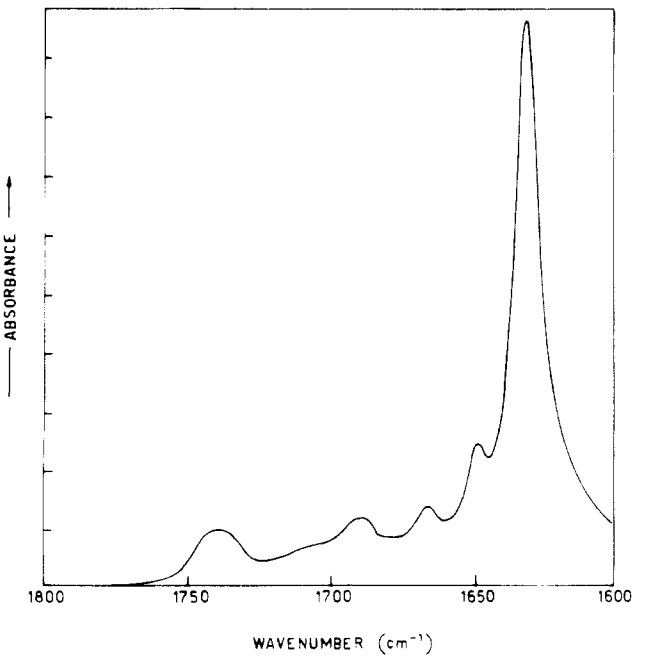


**Figure 1.** IR spectrum in the 1800–1600-cm$^{-1}$ region of Boc-(L-Val)$_2$-L-Ala-(L-Val)$_3$-OMe in $CH_2Cl_2$. Peptide concentration 14 mM.

in the 1800–1600-cm$^{-1}$) spectral region in $CH_2Cl_2$ solutions.

The solvent-titration curves for the hexa-, penta-, and tetrapeptides are shown in Figures 2–4, respectively. It is observed that more $Me_2SO$ is needed to disrupt the $\beta$-sheets of the longest peptides. The increasing stability of the self-associated species of these small peptides with increasing main-chain length should be related to the decreasing role of the more easily solvability end groups. The following rank order is observed for the stability of

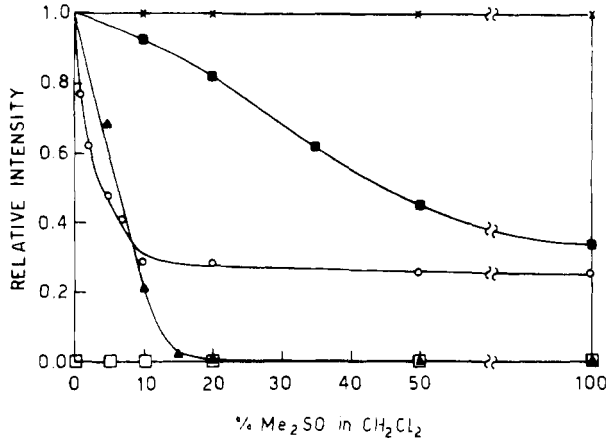


**Figure 2.** Relative intensity of the amide I C=O stretching band related to β-sheet conformations (1630–1640 $cm^{-1}$) in the IR spectra of the hexapeptides Boc-(L-Val)$_2$-Xxx-(L-Val)$_3$-OMe: Xxx = L-Val (×), L-Ala (■), D-Val (▲), L-Pro (○), and Aib (□) in $CH_2Cl_2$–$Me_2SO$ mixtures as a function of increasing concentration of $Me_2SO$. Peptide concentration 14 mM.

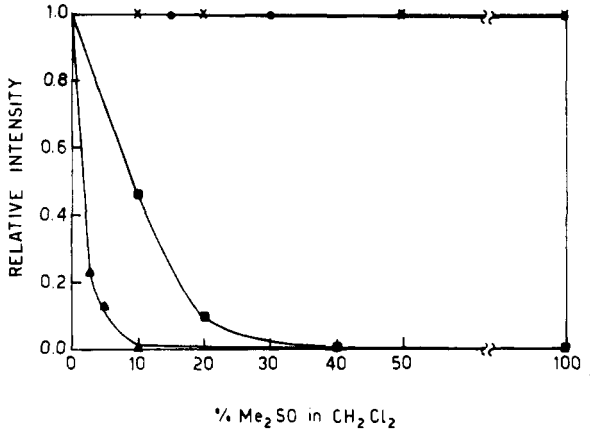


**Figure 3.** Relative intensity of the amide I C=O stretching band corresponding to β-sheet conformations in the IR spectra of the pentapeptides Boc-L-Val-Xxx-(L-Val)$_3$-OMe: Xxx = L-Val (×), L-Ile (●), L-Ala (■), and D-Val (▲) in $CH_2Cl_2$–$Me_2SO$ mixtures as a function of increasing concentration of $Me_2SO$. Peptide concentration 16 mM.

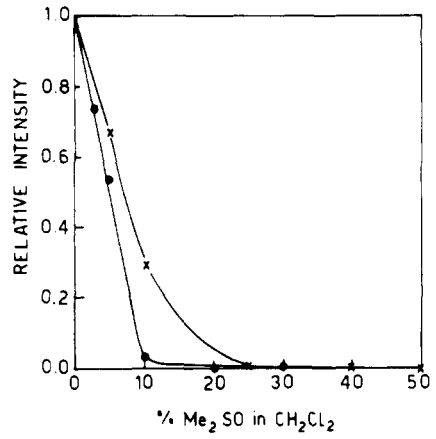


**Figure 4.** Relative intensity of the amide I C=O stretching corresponding to the β-sheet conformations in the IR spectra of the tetrapeptides Boc-Xxx-(L-Val)$_3$-OMe: Xxx = L-Val (×) and L-Ile (●) in $CH_2Cl_2$–$Me_2SO$ mixtures as a function of increasing concentration of $Me_2SO$. Peptide concentration 19 mM.

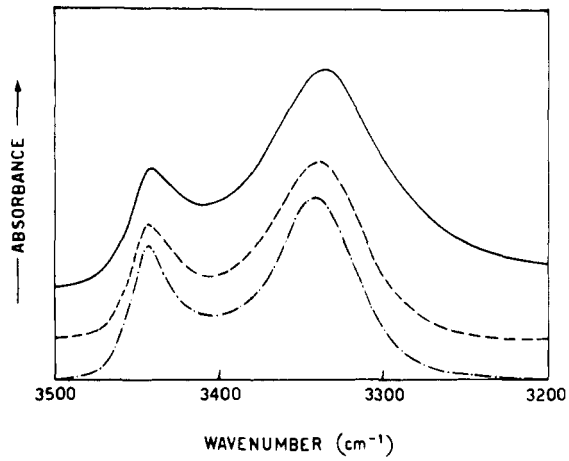


**Figure 5.** Concentration dependence in the IR spectrum of Boc-(L-Val)$_2$-Aib-(L-Val)$_3$-OMe in $CDCl_3$: (—) 14 mM, (- - -) 1.4 mM, (- · -), 0.14 mM.

the β-sheets: (i) hexapeptides Boc-(L-Val)$_2$-Xxx-(L-Val)$_3$-OMe, L-Val ≫ L-Ala ≫ D-Val > L-Pro ≫ Aib; (ii) pentapeptides Boc-L-Val-Xxx-(L-Val)$_3$-OMe, L-Val = L-Ile ≫ L-Ala > D-Val; (iii) tetrapeptides, Boc-Xxx-(L-Val)$_3$-OMe, L-Val > L-Ile.

Using Mizushima's dilution technique[40] it is seen that at a concetration of 0.14 mM N–H···O=C intermolecular hydrogen bonding is negligible for the Aib hexapeptide in $CDCl_3$, whereas weak intermolecular hydrogen bonds occur to a limited extent at a concentration of 1.4 mM or higher (Figure 5). In fact when the concentration is increased, the area of the band at 3338 ± 2 $cm^{-1}$ also increases, since the broad band typical of weakly intermolecularly hydrogen bonded N–H groups overlaps with the corresponding band due to the intramolecularly hydrogen bonded groups. The ratio of the integrated intensity of the hydrogen bonded (3338 ± 2 $cm^{-1}$) to free N–H (3444 ± 1 $cm^{-1}$) bands, $A_H/A_F$, is 2.59 at a concentration of 0.14 mM and increases to 3.42 at 1.4 mM and 4.15 at 14 mM, respectively. The area of the 3336-$cm^{-1}$ band dominates that of the 3440-$cm^{-1}$ band even in the absence of self-association (0.14 mM).

The onset of a highly folded conformation for the Aib hexapeptide in $CDCl_3$ in the absence of self-association (1.5 mM) is supported by a 400-MHz $^1H$ NMR investigation. The solvent accessibilities of the NH protons of the various oligomers have been probed by examining sensitivity of chemical shifts to solvent composition in $CDCl_3$–$Me_2SO$ mixtures[41] and by monitoring paramagnetic radical (Tempo) induced line broadening in $CDCl_3$ solutions.[42] Three types of NH protons are observed: (i) two NH protons, assigned to Val (1) and Val (2) on the basis of a comparison with a variety of model compounds[43] and labeled A and B in Figure 6, are solvent exposed; (ii) two NH protons, C and E, are solvent shielded forming stable intramolecular hydrogen bonds; (iii) two NH protons, D and F, the latter unambiguously assigned to Aib (3) because of its appearance as a singlet, form more labile intramolecular hydrogen bonds. Taken together the NMR and IR data suggest that incorporation of a single Aib guest residue is capable of converting the β-sheet conformation of the host (L-Val)$_n$ into a $3_{10}$-helical structure, characterized by four consecutive intramolecular hydrogen bonds. The two central hydrogen bonds, related to the -Val-Aib-Val- sequence, are particularly stable. In analogy with other $3_{10}$ helices self-association does take place at higher peptide concentrations, with the Val (1) NH proton and to a lesser extent the Val (2) NH group acting as the intermolecular hydrogen bonding donors.[44]

In the more polar solvent 2,2,2-trifluoroethanol (TFE), the IR spectra of the five hexapeptides in the C=O stretching region (Figure 7) are all similar and indicative of an unordered conformation, the intense band at 1659–1656 $cm^{-1}$ being related to solvated peptide C=O

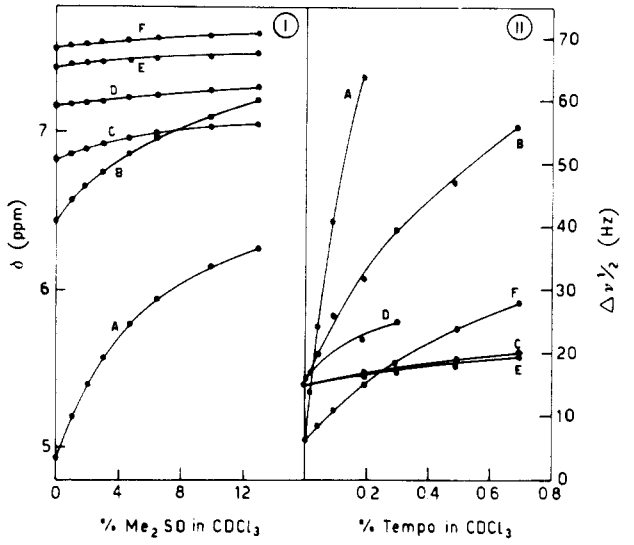


**Figure 6.** (I) Plot of NH chemical shifts of Boc-(L-Val)$_2$-Aib-(L-Val)$_3$-OMe versus solvent composition in $CDCl_3$–$Me_2SO$ mixtures (v/v %). (II) Line width of NH resonances in Boc-(L-Val)$_2$-Aib-(L-Val)$_3$-OMe as a function of Tempo concentration (w/v %) in $CDCl_3$ solutions. Peptide concentration 1.5 mM.

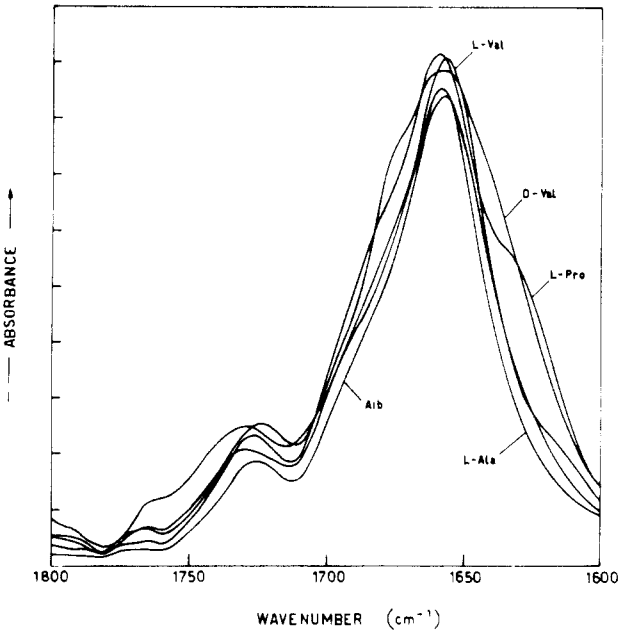


**Figure 7.** Partial IR spectra of the five Boc-(L-Val)$_2$-Xxx-(L-Val)$_3$-OMe hexapeptides in TFE. Peptide concentration 1 mM.

groups.[38] This assignment is corroborated by the observation that a band in this spectral region is also observed for the peptides Boc-(L-Val)$_n$-OMe ($n$ = 2, 3, and 5), which are too small to form helical structures in this solvent (data not shown). Again, the shoulder near 1630 $cm^{-1}$ visible in the spectrum of the L-Pro peptide should be attributed to the X-Pro tertiary amide vibrator. No concentration effects were observed in this solvent.

Circular dichroism (CD)[45,46] studies were also undertaken to complement the information obtained from IR studies. The CD patterns of the hexa- and pentapeptides in TFE (1 mM) shown in Figure 8 favor the conclusion that the L-Val, L-Ala, and Aib hexapeptides and the L-Val, L-Ala, and L-Ile pentapeptides predominantly adopt a statistically unordered conformation in this solvent, characterized by a relatively intense negative maximum near 195 nm ($\pi \rightarrow \pi^*$), accompanied by a weak negative maximum located at about 220 nm ($n \rightarrow \pi^*$). The more pronounced negative maximum at 218 nm in the L-Pro hexapeptide and the

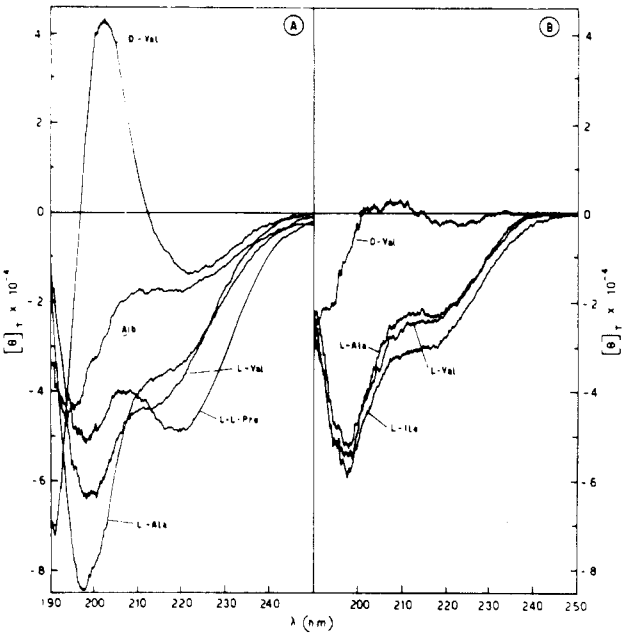


**Figure 8.** CD spectra of peptides in TFE (concentration 1 mM). (A) Hexapeptides Boc-(L-Val)$_2$-Xxx-(L-Val)$_3$-OMe and (B) pentapeptides Boc-L-Val-Xxx-(L-Val)$_3$-OMe.

positive maximum at approximately 205 nm in the D-Val peptides (particularly evident in the higher oligomers) might be associated, at least in part, with the onset of cis → trans isomerism in the former and the presence of a population of folded conformers due to the presence of a D-chiral center in the latter. The -L-Val-D-Val-L-Val- segment in the D-Val containing hexapeptide may be expected to promote formation of a central type II or II′ β-turn. Indeed, the strong positive CD band observed at ~205 nm and the less intense negative band at 218 nm, observed in this peptide (Figures 8 and 9), are reminiscent of the "Class B" CD spectrum predicted for type II β-turns and observed in some model peptides.[29] The conformational assignments agree well with those proposed on the basis of IR results. Strictly comparable results were obtained in absolute ethanol (not shown). It is of interest to examine the peptide models in water. Unfortunately, the present peptides proved to be insoluble in water. However, CD spectra could be obtained in TFE/$H_2O$ mixtures. The addition of water (up to 40% v/v) to solutions of hexapeptides in TFE causes virtually no change in the overall spectral shapes of the L-Ala, L-Pro, Aib, and D-Val peptides (Figure 9). However, the CD spectrum of the L-Val peptide is dramatically different from that in 100% TFE, the presence of a negative maximum at 233 nm and a strong positive maximum at 204 nm suggesting the onset of a β-sheet structure in this solvent mixture.

The rank order of disrupting capability of the β-sheet structure of the (L-Val)$_n$ host oligopeptides by five selected guest residues is Aib ≫ L-Pro > D-Val ≫ L-Ala ≫ L-Ile. The β-sheet structure of the homopeptide (L-Val)$_6$ is the most stable among those investigated in this study. This hierarchy might be explained on the following grounds: (i) host–guest peptides generally adopt a less stable β-sheet conformation as compared to the host homooligopeptides. This phenomenon is presumably independent of the intrinsic β-sheet potential of the host and guest amino acids and is related to the disruption of the uniformity of the side-chain structure. Most notably the relative size of neighboring residues in host–guest peptides seems to be an important factor for the stability of β-sheets.[7-9] These considerations adequately rationalize the observed L-Val > L-Ile ≫ L-Ala β-sheet stability scale. (ii) Incorporation

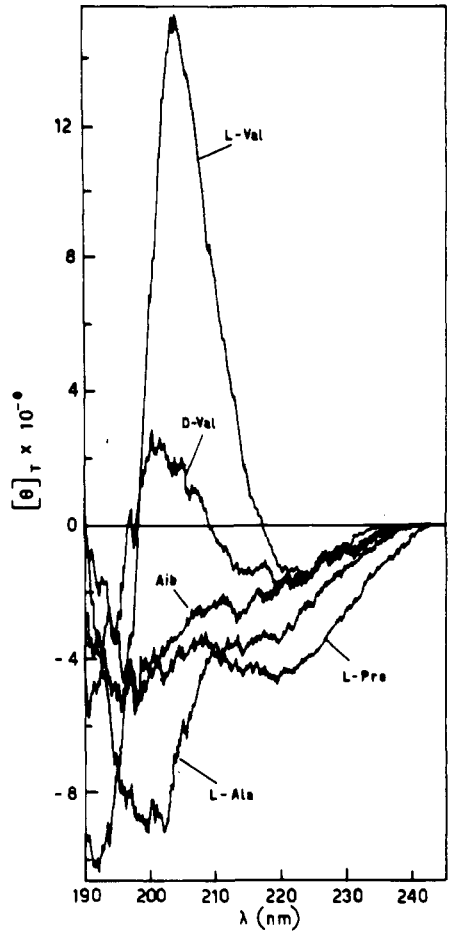


**Figure 9.** CD spectra of the five hexapeptides Boc-(L-Val)$_2$-Xxx-(L-Val)$_3$-OMe in a TFE–$H_2O$ mixture (60:40 v/v). Peptide concentration 0.3 mM.

of the conformationally restricted Aib and L-Pro residues as guests tends to disrupt the (L-Val)$_n$ $\beta$-sheet conformation owing to their well-known tendency to disfavor $\beta$-sheets and to support folded and helical conformations.[3-5,10-14,35,36,47-55] The present results suggest that an Aib residue is significantly more effective than an L-Pro residue in causing $\beta$-sheet disruption. (iii) Peptides with L-D-L sequences and specifically L-Val-D-Val-L-Val peptides do not adopt $\beta$-sheet conformations, characteristic of their all-L diastereoisomers[9,15-29], in view of their tendency to fold into turn or novel helical conformations.[15,53,56-60] However, the insertion of a D-Val residue appears to be less liable to cause $\beta$-sheet disruption than insertion of an L-Pro residue in (L-Val)$_n$ host oligopeptides.

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**Registry No.** BOC-Ala-(Val)$_3$-OMe, 118832-60-3; BOC-Val-Ala-(Val)$_3$-OMe, 118832-61-4; BOC-(Val)$_2$-Ala-(Val)$_3$-OMe, 116787-39-4; BOC-D-Val-(Val)$_3$-OMe, 118832-62-5; BOC-Val-D-Val-(Val)$_3$-OMe, 118832-63-6; BOC-(Val)$_2$-D-Val-(Val)$_3$-OMe, 118832-64-7; BOC-Ile-(Val)$_3$-OMe, 118832-65-8; BOC-Val-Ile-(Val)$_3$-OMe, 118832-66-9; BOC-(Val)$_2$-Pro-(Val)$_3$-OMe, 118832-67-0; BOC-(Val)$_2$-Aib-(Val)$_3$-OMe, 118832-68-1; BOC-(Val)$_5$-OMe, 19963-34-9; BOC-(Val)$_6$-OMe, 53197-50-5.

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# Vapor Deposition Polymerization: A Study on Film Formation in Reaction of Pyromellitic Anhydride and Bis(4-aminophenyl) Ether

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ABSTRACT: A course of the vapor deposition polymerization between pyromellitic dianhydride (PMDA) and bis(4-aminophenyl) ether (ODA) was determined. The surface of deposited film of two monomers was rough due to the high crystallinity, whereas the film prepared by coevaporation of PMDA and ODA had a smooth surface. A collision of the functions of an acid anhydride and an amine occurred by the migration of the monomers and the oligomers on the substrate. The formation of a charge transfer (CT) complex was newly observed in the first step of the polycondensation by using absorption spectra and ESR spectra. The CT complex disappeared as the polymerization proceeded.

## Introduction

Demands of polyimides especially in electronics have been growing because of their excellent heat resistance, chemical stability, and dielectric property. Preparation of thin films by a wholly dry system is one of the most important technologies in this field.

Iijima et al.[1,2] and Salen et al.[3] independently reported the new method for the preparation of polyimide films via vapor deposition polymerization.[1-3] In this method, a stoichiometric amount of both monomers, a tetracarboxylic dianhydride and a diamine, was evaporated simultaneously to form a poly(amic acid) thin film, and subsequent heating of the film afforded a polyimide thin film. Thus, this technology readily offers polyimide thin films 0.1–10 $\mu$m thick.

Since the polycondensation, in the solution polymerization, always proceeds via a collision of functional groups, a carboxylic anhydride and an amine, we generally choose a solution medium to accelerate the efficient collision of these groups. In the vapor deposition polymerization, although the collision should be limited, because of the restriction of a free movement of the functional groups, we actually observed the fairly fast polycondensation in this method. In this paper, we describe our observations of the course of polycondensation between pyromellitic dianhydride and bis(4-aminophenyl) ether to clarify the nature of this new class of polymerization.

## Experimental Section

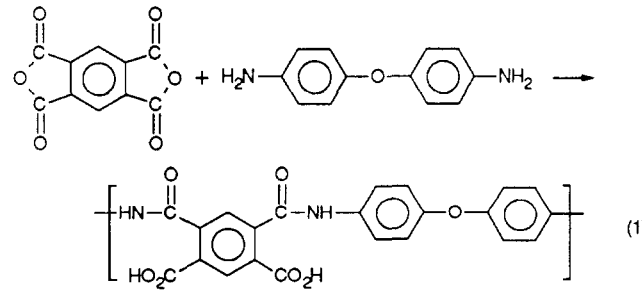
**Deposition of Pyromellitic Dianhydride and Bis(4-aminophenyl) Ether.** Both pyromellitic dianhydride (PMDA) and bis(4-aminophenyl) ether (ODA) were purified by sublimation under reduced pressure before use. These monomers were deposited on a substrate by heating at 180 ± 2.5 and 160 ± 2.5 °C, respectively, under a pressure of $2 \times 10^{-6}$ Torr. Since the deposition rate of each monomer under these conditions was 5 Å/s, the rate of coevaporation of these two monomers to prepare the polymeric film was 10 Å/s.

The migration behavior of the monomers on a substrate was observed by scanning electron microscopy (SEM) reported by Takagi et al.[4] A silicon wafer and a glass plate were used as a substrate and a mask, respectively, and placed as illustrated in Figure 1, where the vapor of the monomers comes from the normal direction of the substrate.

**Measurement.** Infrared (IR) spectra were measured on a Nicolet FT-IR 5DXB spectrometer by the ATR method, where the sample films were deposited on an aluminum foil. Ultraviolet–visible (UV–vis) spectra were recorded on a Hitachi UV-VIS 323 spectrometer. SEM photographs were taken on an Akashi DS-130C. Electron spin resonance (ESR) spectra were measured by JES-ME3X. The sample films were deposited on a poly(ethylene terephthalate) (PET) film, and they were packed with rolling in a glass tube to measure ESR spectra. The $g$ value was calibrated by using 2,2-diphenyl-1-picrylhydrazyl as the standard ($g$ = 2.0036).

## Results and Discussions

Poly(amic acid) is produced by the vapor deposition polymerization of PMDA and ODA as shown in eq 1.




(1)

We have tried to determine the migration of each monomer, PMDA and ODA, using the partially masked substrate as shown in Figure 1.[4] Rough surfaces with tiny islands, probably indicating their high crystallinity, were observed in the uncovered area of the film. The growing patterns of the island were also observed for the range of a few micrometers from the edge in the masked area in both cases. These facts indicate that a fair amount of monomer molecules apparently migrated from the uncovered area to the masked area. When PMDA and ODA

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