Nature and Relative Stability of Monomeric and Dimeric Species of the D,L-Alternating Octapeptide Boc-(L-Val-D-Val)$_4$-OMe in Cyclohexane or Chloroform Solution[1]

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Received July 10, 1981

Abstract: The conformational characteristics of the alternating stereocooligopeptide Boc-(L-Val-D-Val)$_4$-OMe in solution in cyclohexane or chloroform, at 25 °C, have been investigated by using proton magnetic resonance at 360 MHz and IR spectroscopy. Different species that interconvert slowly compared with the proton spin time scale occur in each of these two solvents. In both solvents at least one of them is a left-handed, double-stranded helical species of the type $\uparrow\downarrow \beta^{5.6}$ with 14 interstrand hydrogen bonds. In cyclohexane one such dimeric species is the most populated one even at concentrations as low as 0.0008 m. One very similar species is comparatively less stable in chloroform; in this solvent there is a monomeric species which is not detected in cyclohexane and which is predominant at low concentrations.

Numerous experimental studies of the conformational characteristics of linear peptides formed by regularly alternating D and L residues (syndiotactic cooligo- or copolypeptides) have been carried out in the last decade. A lot of attention has been paid[2-15] to one natural pentadecapeptide of this kind, Gramicidin A, in view of its peculiar property of forming specific ion-conducting channels across natural or synthetic membranes. Syndiotactic copoly(γ-benzyl glutamate) has also been investigated[16-25] extensively, and a number of other synthetic syndiotactic copolypeptides[16,18,26-30] and cooligopeptides[9,14,26] have been studied. These experimental studies have indicated that syndiotactic peptides are able to assume very specific conformations. These include, among others, some of the various kinds of single- and double-stranded β-helical structures predicted[31-34] for syndiotactic peptides on theoretical grounds. However, the experimental characterization of these conformations is rather poor. Moreover, the studies provide no, or only marginal, information about the influence that various structural factors, such as the length of the peptide chain, the nature of the lateral substituents and of the end groups, and the specific pattern of configurations (DLD... or LDL...), may have on the conformational properties of syndiotactic peptides.

With the aim to better characterize these properties and to evaluate the role played by these factors in determining them, a few years ago we initiated a systematic conformational investigation of different series of cooligo-D,L-peptides formed by alternating enantiomeric[35-38] or diastereomeric[39,40] amino acid residues (stereo-*co*-oligopeptides). One of the most significant results of this investigation so far has been achieved by the single-crystal X-ray analysis[36] of the crystal structure of the octapeptide Boc-(L-Val-D-Val)$_4$-OMe. In fact, it was found that this octapeptide exists in the crystals in the form of double-stranded β-helical dimers with antiparallel chains. In consideration of the important role attributed[14] to β helices in the formation of transmembrane channels, it was of interest to study the stability of this dimeric structure in a dispersed state, in a lipophilic environment. With this aim we have carried out a conformational investigation of Boc-(L-Val-D-Val)$_4$-OMe dissolved in cyclohexane and in chloroform by means of proton magnetic resonance measurements at 25 °C and IR spectroscopy. The results are presented and discussed in this paper. Brief accounts of preliminary spectroscopic studies of the octapeptide in cyclohexane[35] and chloroform[38] solution have already been published.

Experimental Section

Boc-(L-Val-D-Val)$_4$-OMe was prepared by a racemization-free procedure and purified as described.[37] Samples that had been exhaustively dried in high vacuum at 85-120 °C were used in all measurements. For the solid-state Ir measurements, a sample of monohydrated crystals

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(1) This is part 3 of a series on linear stereocooligopeptides with alternating D and L residues. For part 2 see ref 38.

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Table I. Hydrogen-Bonding Characteristics of the Single- and Double-Stranded β Helices of Highest Possible Pitch for Boc-(L-Val-D-Val)$_4$-OMe[a]

helix[b]	handedness	no. of H-bonded NHs per chain[c]	non-H-bonded NHs[d]	CO: urethane	CO: ester	total no. of H bonds per chain
$\beta^{4.4}$	right	2 (D), 2 (L)	NH (1), NH (3), NH (6), NH (8)	bonded	free	4
$\beta^{4.4}$	left	2 (D), 3 (L)	NH (2), NH (4), NH (7)	free	bonded	5
$\uparrow\downarrow \beta^{5.6}$	right	2 (D), 4 (L)	NH (6), NH (8)	bonded	free	6
$\uparrow\downarrow \beta^{5.6}$	right	3 (D), 3 (L)	NH (1), NH (8)	bonded	free	6
$\uparrow\downarrow \beta^{5.6}$	right	4 (D), 2 (L)	NH (1), NH (3)	bonded	free	6
$\uparrow\downarrow \beta^{5.6}$	left	3 (D), 4 (L)	NH (2)	free	bonded	7
$\uparrow\downarrow \beta^{5.6}$	left	4 (D), 3 (L)	NH (7)	free	bonded	7
$\uparrow\uparrow \beta^{5.6}$	right[e]	2 (D), 4 (L)	NH (6), NH (8)	bonded	free	6
		4 (D), 2 (L)	NH (1), NH (3)	bonded	free	
$\uparrow\uparrow \beta^{5.6}$	left[e]	2 (D), 4 (L)	NH (2), NH (4)	free	bonded	6.5
		4 (D), 3 (L)	NH (7)	bonded	free	

[a] Only those double-stranded helices are considered that exhibit the maximum possible number of hydrogen bonds. [b] See ref 42. [c] (D) and (L) indicate the configurations of the residues to which the NHs belong. [d] Indicated in parentheses is the sequence number of the residue to which the free NH belongs. [e] The two sets of data refer to the two differently bonded peptide chains.

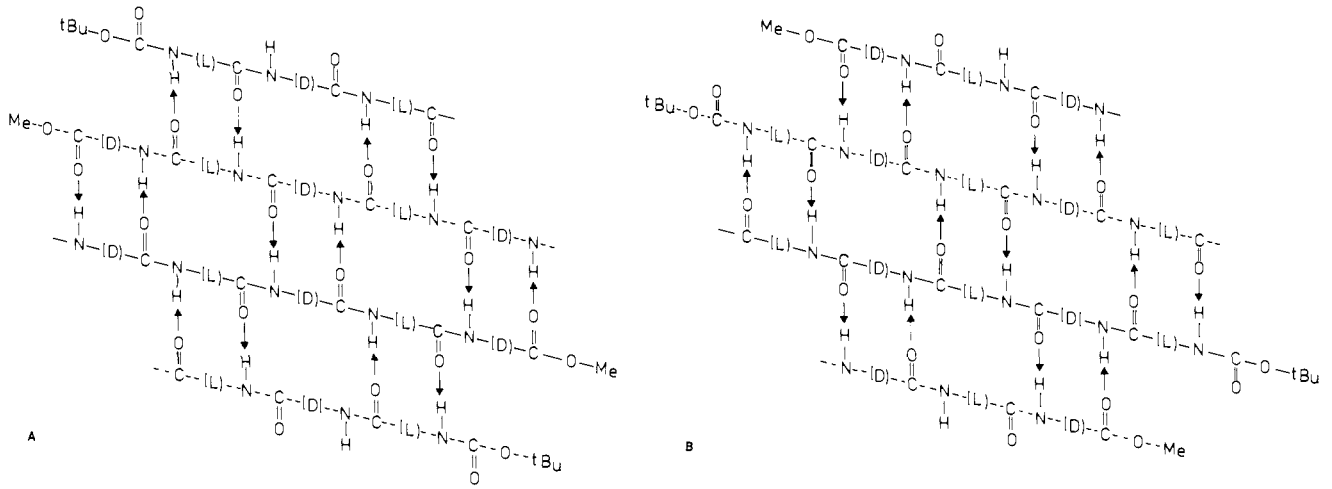

Figure 1. Hydrogen-bonding pattern of the two left-handed $\uparrow\downarrow \beta^{5.6}$ helical structures of Boc-(L-Val-D-Val)$_4$-OMe with 14 interturn hydrogen bonds. The diagrams look at helices split along the back and laid out flat. Of the two peptide chains—distinguished by a solid line and a dashed line—only the CO and NH groups and the configurations of the α-CHs (in parentheses) are indicated. (A) Helix A found for crystalline Boc-(L-Val-D-Val)$_4$-OMe; (B) helix B.

grown from nonanhydrous ethyl acetate was also used.

The solutions were prepared by adding the approximate volume of solvent to a weighed amount of peptide and then reweighing. The concentrations are indicated generally in this paper as stoichiometric molal concentrations (m) (formula mass of the octapeptide, 925.23).

^{1}H NMR spectra were recorded on a Bruker HXS-360 spectrometer operating in the Fourier transform mode. The sample temperature was 25 °C. Chemical shifts are relative to internal Me_4Si.

IR spectra were determined with a Perkin-Elmer 177 spectrophotometer. Powders dispersed in Nujol were used for the solid samples. A compensating matching cell was used to obtain the spectrum of a cyclohexane solution. The cell compartment temperature was about 31 °C. The positions of the bands indicated in the spectra reported are accurate to ±5 cm^{-1} in the amide A region and to ±2 cm^{-1} in the amide I and II region.

Vapor pressure osmometry measurements were carried out with the osmometer Mechrolab 302 at 25 °C. Benzil was used for the calibration.

Results and Discussion

The octapeptide dissolves in cyclohexane at room temperature to the extent of about 5 mg/mL. In chloroform at room temperature the solubility is over 100 mg/mL. This solubility behavior of the octapeptide is remarkable. Oligo-L-peptides of comparable length, with alkyl side chains, are very poorly soluble in nonacidic organic solvents. β-Helical structures, where the polar peptide backbone is shielded by the apolar isopropyl side chains, are plausible for the octapeptide in the lipophilic solvents used.

(a) Analysis of the Hydrogen-Bonding Characteristics of β-Helical structures for Boc-(L-Val-D-Val)$_4$-OMe. Framework molecular models have been used to inspect the hydrogen-bonding characteristics of a number of the β-helical structures that might conceivably be taken up by the octapeptide in solvents like cyclohexane or chloroform. The trans (*Z*) configuration has been assumed for a planar urethane –OCO–NH– group. The possibility of cis (*E*) configuration has not been considered since, until now, this configuration has been observed[41] only for Boc-α-amino acids

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Table II. Proton Chemical Shifts, δ,[a] and Vicinal Coupling Constants, $^3J_{NH-\alpha-CH}$,[b] Characterizing the Major Species of Boc-(L-Val-D-Val)$_4$-OMe in Cyclohexane (Species I) and in Chloroform (Species *I*, *II*, and *III*) at 25 °C

	I		*I*		*II*[d]		*III*[c,d]
proton(s)	δ	$^3J_{NH-\alpha-CH}$	δ	$^3J_{NH-\alpha-CH}$	δ	$^3J_{NH-\alpha-CH}$[e]	δ
$(CH_3)_3COCO$	1.52		1.50		1.45		1.48
CH_3OOC	3.90		3.91		3.88		3.75
urethane NH	6.61	9.8	6.55	9.5	5.99	8.6	5.81
	6.74	9.3	6.47–6.63[f]	9.2	6.75	9.3	7.16
	7.89	7.0	7.84	7.0	7.58	8.2	7.40[g]
	7.93	8.1	8.02	9.8	7.80[g]	*h*	7.40[g]
amide NHs	8.21	9.9	8.15–8.26[f]	7.5	8.22	9.0	7.50[g]
	8.45	6.3	8.41	6.4	8.30	8.2	7.60[g]
	8.49	9.7	8.61	9.5	8.35	*h*	7.70[g]
	8.88	9.9	8.87	9.5	8.69	5.5	7.80[g]

[a] In parts per million from Me_4Si (±0.02, if not otherwise indicated). [b] In hertz (±0.5, if not otherwise indicated). [c] The values of $^3J_{NH-\alpha-CH}$ for this species cannot be determined because of overlapped or broad signals. [d] The values for the NH signals are valid for a concentration of 0.0368 m or higher. [e] ±1.0. [f] The actual position depends on concentration. The values reported are for a concentration of 0.0046 m (higher value) and 0.1420 m (lower value). [g] ±0.05. [h] Cannot be determined because of overlapping.

or for Boc-oligopeptides with an N-terminal imino acid residue. Of the various types of β-helical structures, those exhibiting the maximum possible number of interturn hydrogen bonds appeared to be particularly relevant, since nonhydrogen-bonded NH and CO groups are exposed at the extremities of these structures and may act as a destabilizing factor when coming into contact with a lipophilic solvent. Therefore, only β helices of the highest possible pitch, i.e., single-stranded ($\beta^{4.4}$) helices with about 4.4 residues per turn[3] and double-stranded parallel ($\uparrow\uparrow$ $\beta^{5.6}$) and antiparallel ($\uparrow\downarrow$ $\beta^{5.6}$) helices with about 5.6 residues per turn,[33] have been considered.[42] Furthermore, in the case of the double-stranded helices, the number of interturn hydrogen bonds has been maximized by properly winding the two strands of the helix. The results are reported in Table I. The table shows that there are different ways of maximizing the number of interturn hydrogen bonds in $\uparrow\downarrow$ $\beta^{5.6}$ helices with either sense of twist. It also shows that the highest number (14) of these bonds is exhibited by two left-handed $\uparrow\downarrow$ $\beta^{5.6}$ helices. The hydrogen-bonding characteristics of these two structures are illustrated in Figure 1. In one of these helices (henceforth called helix A), the hydrogen bonds connect three pairs of D residues and four pairs of L residues (Figure 1A). This is the kind of double helix observed[36] for monohydrated crystals of the octapeptide grown from ethyl acetate. The only free NH group per chain belongs to the residue in the second position (residue no. 2). In the other left-handed $\uparrow\downarrow$ $\beta^{5.6}$ helix (henceforth referred to as helix B), the hydrogen bonds connect four pairs of D residues and three pairs of L residues, and the only free NH group per chain belongs to the residue in the seventh position (residue no. 7) (Figure 1B). Note that helix A can be converted into helix B (or vice versa) simply by transposing one strand by two residues with respect to the other. Due to the antiparallel arrangement of the chains, both helix A and helix B exhibit a twofold symmetry axis (C_2) perpendicular to the axis of the double helix.

(b) NMR Evidence for Different, Slowly Interconverting Species in Cyclohexane and in Chloroform. A representative ^1H NMR spectrum of Boc-(L-Val-D-Val)$_4$-OMe in cyclohexane solution is shown in Figure 2, and the ^1H NMR spectra of the octapeptide in three chloroform solutions of different concentrations are shown—divided into three parts of not comparable intensities—in Figure 3. In these figures groups of signals are assigned to the different types of protons present in the octapeptide. The assignment is based on the integrated intensities and on NMR data concerning other Boc- and MeO-protected, alternating oligo-D,L-peptides presently under investigation in our laboratory, as well as Boc- and MeO-protected oligo-L-peptides.[43,44] Some

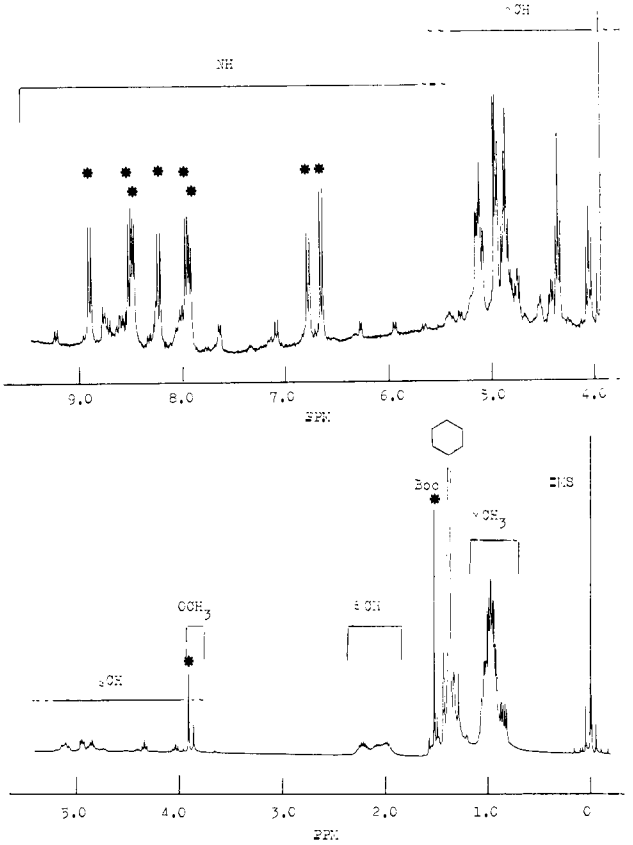

Figure 2. 360 MHz ^1H NMR spectrum of a solution of Boc-(L-Val-D-Val)$_4$-OMe in cyclohexane-d_{12} (concn 0.0061 m; *T*, = 25 °C). The low-field region—in the upper part of the figure—is expanded. Signals of species I are indicated with an asterisk.

uncertainty exists for the border regions between NH and α-CH resonances and between α-CH and $COOCH_3$ resonances, and is hinted at by dashed lines in the figures. The spectra characteristically show multiple signals for the different protons. This is especially evident for the NH protons and for the $COOCH_3$ protons. For the methyl protons of the Boc group the multiplicity of signals is also unequivocal with the chloroform solutions (Figure 3C), but it is somewhat ambiguous with the cyclohexane solution, due to the overlapping with the signals of the solvent (Figure 2). This multiplicity must reflect the coexistence in cyclohexane and in chloroform of different species, which interconvert slowly compared with the proton spin time scale.[45]

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(42) There is no general consensus on the terminology for different types of β helices. We use the letter β generally, with a superscript to indicate the approximate number of residues per turn, and, for the double helices, a pair of vertical arrows to specify the parallel or antiparallel arrangement of the chains.

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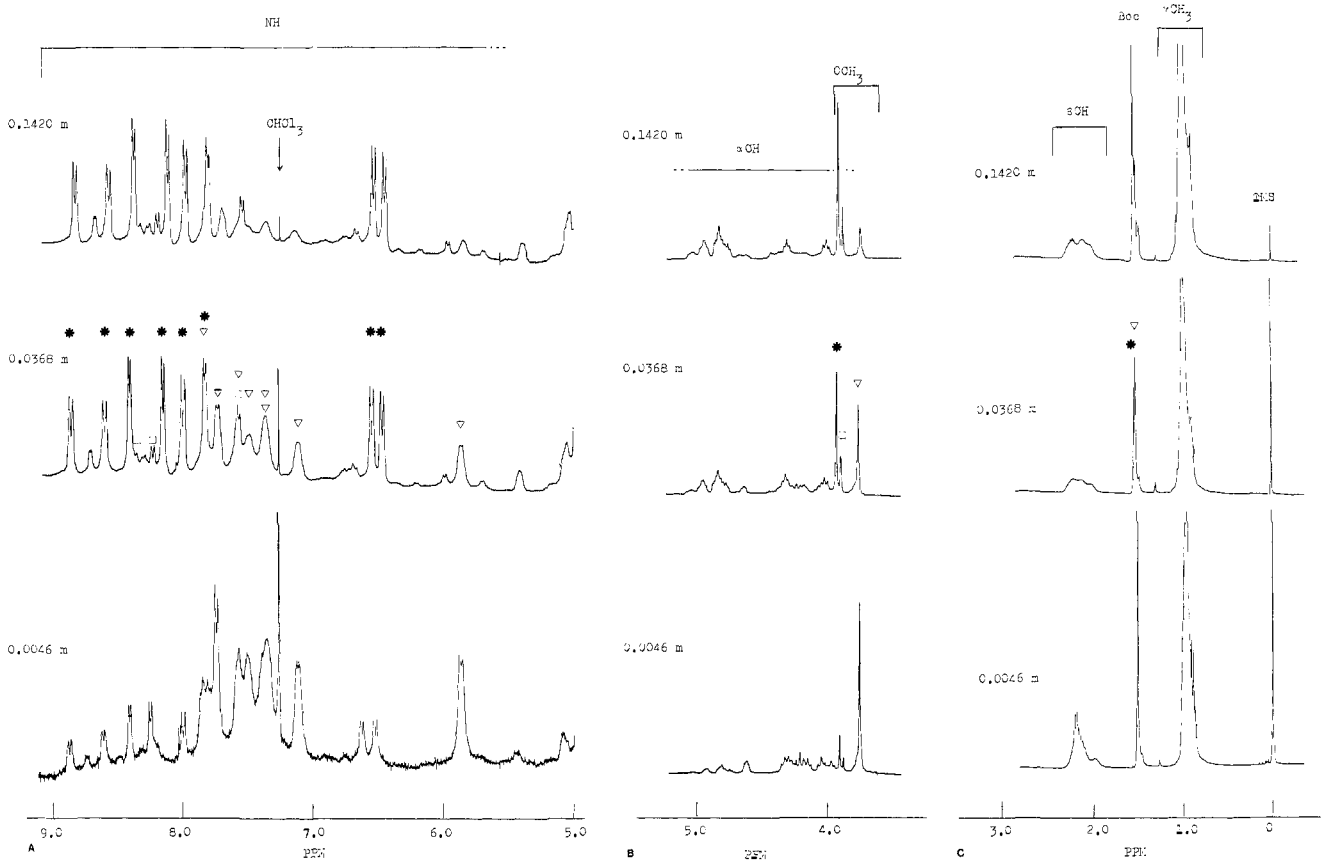

Figure 3. 360 MHz ^{1}H NMR spectra of solutions of different concentrations of Boc-(L-Val-D-Val)$_4$-OMe in $CDCl_3$ (T, = 25 °C). Signals of species *I*, *II*, and *III* are indicated with an asterisk, an open square, and an open triangle, respectively. (A) NH region; (B) α-CH and $COOCH_3$ region; (C) β-CH, Boc, and γ-CH_3 region (the signal of the γ-CH_3 protons has been truncated in order to produce this figure).

The features of the NMR spectrum shown in Figure 2 indicate that in cyclohexane there are probably three such species. One of these (species I) predominates, and of the signals associated with this species, the NH resonances and the resonances of the protons of the protecting groups can easily be identified (Figure 2). The values for the chemical shifts of these signals and for the vicinal coupling constants $^3J_{NH-\alpha-CH}$ are listed in Table II. The conclusion that there are probably two more species is based on the number and intensities of the less intense NH signals observable in Figure 2. In addition, there appears to be some indication that the less intense methyl ester signal (at 3.86 ppm; Figure 2) is formed by two overlapping singlets. Since the relative intensities of the NMR signals of the octapeptide in cyclohexane are not influenced by concentration in a measurable way in the investigated range between 0.0008 and 0.0061 m, and the effect of temperature has not been investigated, the assignment of signals to these species is difficult and has not been attempted.

The features of the NMR spectra shown in Figure 3 give clear evidence for the presence in chloroform of three species. A fourth, sparsely populated species may be present. The NH signals and the signals of the protecting groups associated with the three species have been identified. One of these (species *I*) is characterized by a series of eight nicely separated NH doublets. The other two species (species *II* and *III*) give NH signals that are in part severely overlapped. For the assignment of these signals the integrated intensities observed for solutions of different concentrations and double-resonance measurements[46] have been used. Values for the chemical shifts and for the coupling constants $^3J_{NH-\alpha-CH}$ of the three species *I*, *II*, and *III* are reported in Table II. The assignment of the Boc-NH resonance for species *I* is discussed in section d. That for species *II* and *III* is based on the indication from double-resonance experiments that the signals at 6.55, 5.99, and 5.81 ppm derive from chemically identical NH groups in the different species. Table II shows that there is a very close similarity between the NMR parameters of species I and *I*, indicating that these two species are very similar. Whether or not there is a counterpart of species *II* in cyclohexane is uncertain. Species *III* is not observable in cyclohexane.

(c) Aggregation State of the Different Species. As shown in Figure 3 the species *I* and *II* become increasingly populated relative to *III* upon increasing the concentration. In the 0.0368 m solution, species *I*, *II*, and *III* are populated by the octapeptide chains in a ratio of 1.40:0.40:1, and in the 0.1420 m solution in a ratio of 3.50:0.90:1. These figures have been calculated by using the integrated intensities of appropriate NH signals and are considered approximate to ±0.05. This concentration dependence indicates that *I* and *II* are aggregated species. These species appear to contain the same number of peptide chains, since their relative populations are not significantly different at the two concentrations indicated above. Molar mass measurements by vapor pressure osmometry at 25 °C on chloroform solutions with concentrations between 0.0037 and 0.0512 m have yielded apparent values in the range 1.2–1.5 times the formula mass of the octapeptide. On these bases species *I* and *II* can be considered to be dimeric and *III* monomeric.

In view of its similarity with *I*, the species I that predominates in cyclohexane can also be considered to be dimeric. Based on the relative heights of the $COOCH_3$ signals of Figure 2, this species is populated by about 70% of the octapeptide chains.

(d) Nature of the Species I and *I*. There is strong NMR and IR evidence that the dimeric species I and *I* have an antiparallel β-helical structure with a left-handed sense of twist and with the same hydrogen-bonding pattern as helix A or B (Figure 1). The relevant NMR data are: (i) the presence of only eight NH doublets in the NMR spectra for these species, which is consistent

(45) Attempts have been made to physically separate these species by thin-layer chromatography. Silica gel plates were used, with cyclohexane or chloroform as the eluent, but in no case was more than a single spot observed.

(46) G. P. Lorenzi, H. Jäckle, and L. Tomasic, to be published.

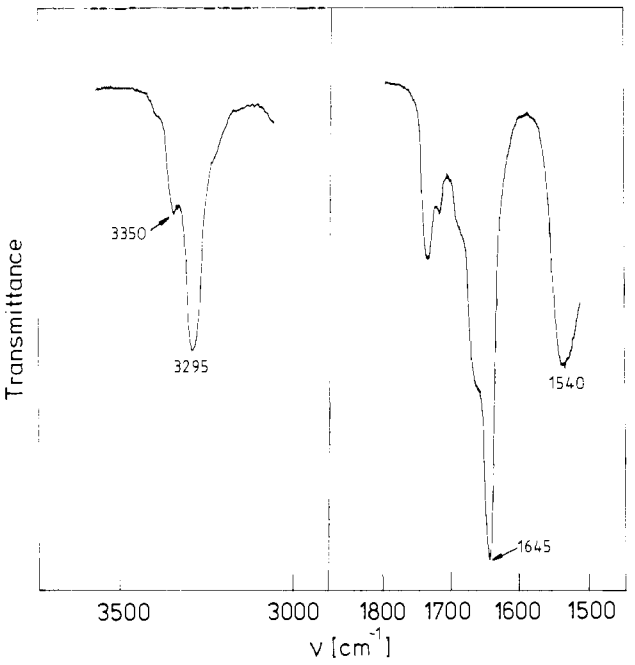

Figure 4. IR spectrum of Boc-(L-Val-D-Val)$_4$-OMe in cyclohexane solution (concn 0.0029 m).

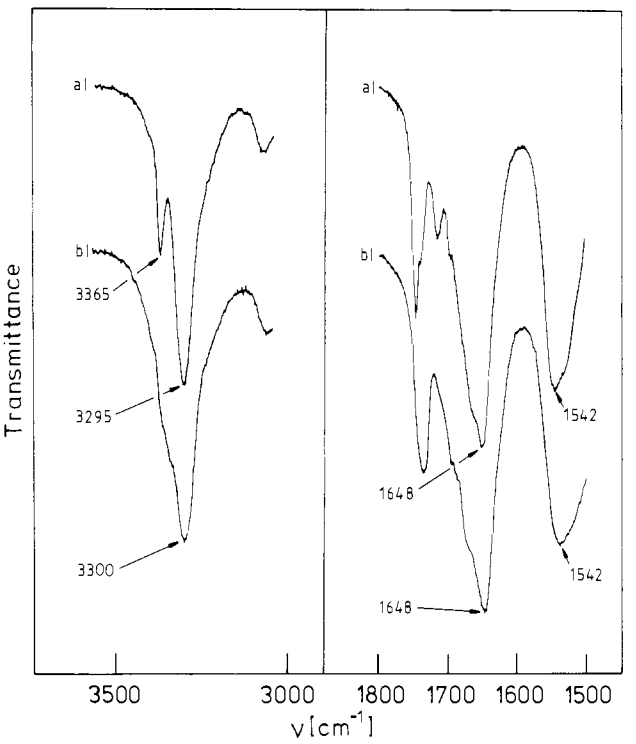

Figure 5. IR spectra of cyrstalline Boc-(L-Val-D-Val)$_4$-OMe. (a) Monohydrated crystals obtained from ethyl acetate; (b) same crystals after exhaustive drying.

with a structure having a C_2 symmetry and excludes the possibility of parallel helical structures that are devoid of such a C_2 symmetry; (ii) the large values for the vicinal coupling constants $^3J_{NH\text{-}\alpha\text{-}CH}$ characterizing these doublets (Table II), which are generally in the range of values expected[13] for β helices (the two or three experimental values that seem to be significantly below this range may reflect an imperfect helical structure); and (iii) the position of the NH doublets, which is well in keeping with structures such as helices A and B, exhibiting a hydrogen-bonded urethane-NH group, six hydrogen-bonded amide-NH groups, and one free amide-NH group per peptide chain. This last statement is based on the following considerations. It seems to be well established[43,44,47] that the Boc-NH resonance is shifted to high field, as compared to the resonances of the amide-NH protons. It has also been reported[43,44,47] for various Boc-oligopeptides that the α-CH signal of the *N*-terminal residue is the highest-field signal in the α-CH region of the NMR spectrum. The NMR spectra in Figures 2 and 3B indicate a triplet at 4.02 ppm as the highest-field α-CH signal of species I and *I*. Irradiation of this α-CH signal causes collapse of the NH resonance at 6.61 and 6.55 ppm in the spectra of the cyclohexane and chloroform solutions, respectively. These are the highest-field NH resonance of species I and, depending on concentration (Table II), the second-highest- or highest-field NH resonance of species *I*. Therefore, it appears to be justified to assign these resonances to the urethane-NH protons. In light of the NMR results of Branik and Kessler,[48] the relatively high chemical shifts characterizing these Boc-NH protons can be seen to indicate that they participate in hydrogen bonding. Following this assignment of the Boc-NH resonance, there remain for species I and *I* six amide-NH resonances between 7.8 and 8.9 ppm, and one below 7 ppm (Table II). It is well known that the resonances of hydrogen-bonded amide-NH groups are shifted downfield as compared with those of similar groups that are not hydrogen bonded. Chemical shifts between 8.23 and 9.12 ppm have been reported[24] for the amide-NH groups of syndiotactic poly(γ-benzyl glutamate) in α- and β-helical structures. Thus, it seems reasonable to assign the six low-field resonances of species I and *I* to NH groups engaged in the interstrand hydrogen bonding and the resonance below 7 ppm to a NH group not engaged in this bonding. It is worth noting that on dilution—especially with chloroform solutions (Table II)—this latter resonance shifts to lower field. This effect might reflect an interaction between this exposed NH and traces of water in the solvent.

Evidence from IR data is based on the similarities between the IR spectra of the octapeptide in cyclohexane, where species I predominates (Figure 4), and in the crystalline helix-A-type structure (Figure 5). As shown, the same major amide bands are at about the same positions in these spectra.

At the moment it is not possible to choose between the two alternative structures of helix A and helix B. The similarity of IR spectra noted above does not exclude the possibility that species I and *I* have a helix-B-type structure, since IR spectra of helix-A- and helix-B-type structures should not be markedly different. Neither can the choice be made on the basis of a comparison between calculated and crystallographic[49] ϕ angles. By using Bystrov's relationship[50] one finds that the lowest positive ϕ compatible with the $^3J_{NH\text{-}\alpha\text{-}CH}$ of the highest-field amide-NH resonance of species *I* (Table II) is +98°. For residue no. 2 of the crystalline helix-A-type structure the value +80° has been reported. Within the limits of validity of this comparison, if species I and *I* have a helix-A-type structure, this structure must be of a different (average) geometry than the crystalline structure.

(e) Possible Nature of Species *II* and *III*. The number and position of the NH signals, as well as the values of the vicinal coupling constants for the dimeric species *II* (Table II), appear to indicate that this species, like species I and *I*, has a $\uparrow\downarrow$ $\beta^{5,6}$ helical structure with six hydrogen-bonded amide-NH groups. This structure may have a left-handed sense of twist. In this case, since species I and *I* have one of the two possible left-handed structures of this type (helix A or B), species *II* would have the alternative left-handed structure (helix B or A). However, right-handed structures cannot be excluded for species *II*. Specifically, the two right-handed structures with a non-hydrogen-bonded Boc-NH (Table I) are possible. In fact, the position of the Boc-NH resonance of species *II* (at 5.99 ppm, Table II) is not as downfield as that of species I and *I*, so it is not possible to exclude a free

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urethane-NH group. In addition, the differences in chemical shift of the $COOCH_3$ and Boc signals of species *I* and *II* (Table II) may reflect the different geometries of the helix-A- and helix-B-type structures, but they may also be a consequence of the different (bonded vs. free) states of the relevant carbonyl groups in the helical structures of opposite handedness (Table I).

As for the monomeric species *III*, it is probably a mixture of various interconverting conformers. This is suggested by the position of the NH resonances, most of which fall (Table II) in a range (between 7.16 and 7.8 ppm) close to that observed[46] for such alternating oligopeptides as Boc-D-Val-(L-Val-D-Val)$_2$-OMe and Boc-(L-Val-D-Val)$_3$-OMe that are presumably not long enough to form stable regular structures in solution. IR spectra measured[38] on chloroform solutions of different concentrations indicate for species *III* an amide I band centered at 1665 cm^{-1}.

Concluding Remarks

The results presented in this paper convincingly demonstrate that the species of Boc-(L-Val-D-Val)$_4$-OMe that predominates in the apolar cyclohexane has a left-handed $\uparrow\downarrow$ $\beta^{5.6}$ structure with 14 interstrand hydrogen bonds similar, if not identical, to the structure formed by the octapeptide in the crystalline state. These results correct a preliminary report[35] that attributed a β-helical structure with a left-handed sense of twist to this species, but considered the structure to be monomeric and not dimeric, as has now been established. The results obtained indicate that this structure occurs also in chloroform; however, in this solvent it is markedly less stable. The difference in stability may derive, in part at least, from the fact that contacts between the polar peptide backbone and chloroform are less unfavorable, permitting monomeric structures with less shielded backbones to occur in this solvent.

There are a number of aspects in this helical structure that need further investigation. One concerns the hydrogen bonding pattern and the choice between the alternative helix-A- and helix-B-type structures. Another aspect is the interaction with water. Traces of water present in the deuterated solvents used—especially chloroform—seem to be responsible for some differences in the NMR parameters of the β-helical structure in solutions of different concentrations (Table II). We plan to comment further on these aspects.

Acknowledgment. Partial financial support for this investigation by the Swiss National Science Foundation (Projects Nos. 2.884-0.77 and 2.414-0.79) is gratefully acknowledged. The editing of the manuscript by B. Straub is deeply appreciated.

Registry No. Boc-(L-Val-D-Val)$_4$-OMe, 65519-02-0.

Communications to the Editor

Correlation of the Rate of Thermal Cis-Trans Isomerization of *p*-Nitro-*p'*-dialkylaminoazobenzenes with Solvent *Z* Value Applied To Study Polarity in Aqueous Surfactant Solutions

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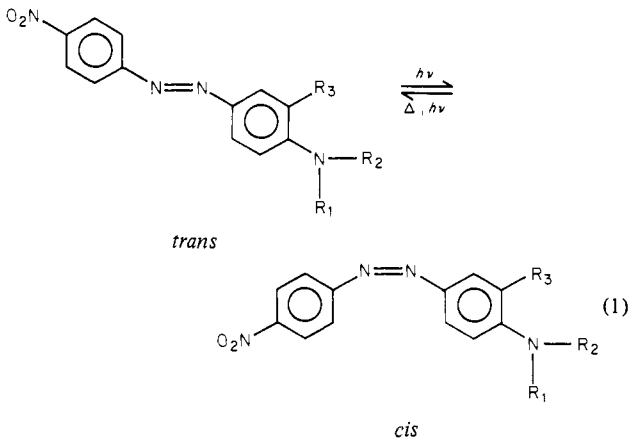
Received November 23, 1981

The site of probe solubilization and microenvironmental effects in aqueous micellar and vesicle solutions is a topic of considerable interest.[1-3] It is frequently observed that changes in chemical and physical characteristics of probe molecules accompanies their incorporation into organized media. Numerous studies have utilized chemical and spectroscopic probes to determine the micropolarity and the degree of water penetration into aqueous surfactant assemblies.[1-6]

In 1972 it was reported that the rate of cis-trans thermal isomerization (k_{ct}, eq 1) of **1** is extremely solvent sensitive; rate enhancement of 10^4 occurs on changing solvent from hexane to DMF.[7] Similar effects were also reported for **4** and **5**.[8] Dyes **1-3** also exhibit solvochromism accompanying changes in solvent polarity. In the present paper we report results of a study of k_{ct} and the absorption maxima of **1-3** in a number of organic solvents



1, $R_1 = R_2 = CH_2CH_3$; $R_3 = H$
2, $R_1 = CH_2CH_3$; $R_2 = C_6H_5$; $R_3 = H$
3, $R_1 = CH_3$; $R_2 = n\text{-}C_{12}H_{25}$; $R_3 = H$
4, $R_1 = R_2 = CH_3$; $R_3 = H$
5, $R_1 = R_2 = CH_2CH_3$; $R_3 = CH_3$

and in aqueous micellar and vesicle solutions. These results indicate that these neutral azobenzene dyes are solubilized in relatively polar sites; however, a most interesting aspect of this study is an extrapolation that suggests that water is expelled concurrent with the solubilization process.

Dyes **1** and **2** were provided by G. Irick and their purity was checked by TLC and melting point analysis. Dye **3** was prepared by alkylation of *N*-methylaniline followed by coupling of the alkylated aniline with *p*-nitrobenzenediazonium chloride. Purification of **3** was carried out by HPLC; its purity was checked by HPLC and FT NMR. Most isomerization rates were measured by the flash photolysis technique[7] in a 2.5-cm cell thermostatted at 25 °C. Rates in benzene and heptane were measured with a Perkin-Elmer 576 spectrometer at 25 °C as previously described. Dye concentrations were 5×10^{-6} to 1×10^{-5} M. SDS was electrophoresis grade, recrystallized once from ethanol; CTAB

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