209. Discrimination between an Antiparallel and a Parallel $\beta^{5.6}$-Helix Using $^1H^\alpha$-NMR Spin-Lattice Relaxation

by **Gian Paolo Lorenzi***, **Lera Tomasic**, **Felix Bangerter** and **Peter Neuenschwander**
Institut für Polymere, ETH-Zentrum, CH-8092 Zürich

and **Benedetto Di Blasio**
Istituto Chimico, Università di Napoli, 80134 Napoli, Italy

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Summary

The ^{1}H-NMR spectrum of Boc-(L-Val-D-Val)$_4$-OH in $CDCl_3$ can be attributed either to an antiparallel or to a parallel double-stranded $\beta^{5.6}$-helical structure. To resolve this ambiguity, T_1-measurements have been carried out for the α-protons of Boc-(L-Val-D-Val)$_4$-OH and its monodeuterated analogue Boc-(L-Val-D-Val)$_2$-α-^{2}H-L-Val-D-Val-L-Val-D-Val-OH. The results show that the H^α of the fifth residue in one strand is close to the H^α of the seventh residue in the other strand, as expected for an antiparallel arrangement, a conclusive demonstration that the correct structure of Boc-(L-Val-D-Val)$_4$-OH is antiparallel.

Introduction. – All non-hybrid, antiparallel ($\uparrow\downarrow$) β-helices have a twofold symmetry axis perpendicular to the axis of the helix, and thus C_2-symmetry. Of the parallel ($\uparrow\uparrow$) β-helices, the $\uparrow\uparrow$ $\beta^{7.2}$-helices (the superscript indicates the approximate number of residues per turn) are all asymmetric, but non-hybrid $\uparrow\uparrow$ $\beta^{5.6}$-helices may have C_2-symmetry because of the presence of a twofold symmetry axis coincident with the axis of the helix [1]. Thus, ^{1}H-NMR spectra of $\uparrow\downarrow$ $\beta^{5.6}$- and $\uparrow\uparrow$ $\beta^{5.6}$-helices may not be easily distinguishable from each other. In these cases the choice between the alternative structures may require an experimental evaluation of distances between atoms or groups belonging to the two different strands. We describe here how we have solved this experimental problem for the D,L-alternating octapeptide Boc-(L-Val-D-Val)$_4$-OH (abbreviated as **I**) in $CDCl_3$. For this purpose we have selectively replaced an α-proton ($^1H^\alpha$) by ^{2}H and measured the effect on the spin-lattice relaxation times (T_1) of the remaining $^1H^\alpha$'s.

Results and Discussion. – a) *Evidence for a* $\uparrow\downarrow$ $\beta^{5.6}$- *or a* $\uparrow\uparrow$ $\beta^{5.6}$-*Helical Structure for* **I** *in* $CDCl_3$. At 25 °C, according to molar mass determinations by vapor-pressure osmometry, **I** is dimeric in $CHCl_3$, even at concentrations as low as 4 mg/ml. For the same temperature, and for the investigated concentration range between 18 and 46 mg/ml, the ^{1}H-NMR spectrum of **I** in $CDCl_3$ is characterized by a single set of resonance signals *(Fig. 1)*. The vicinal coupling constants for the backbone protons are in the

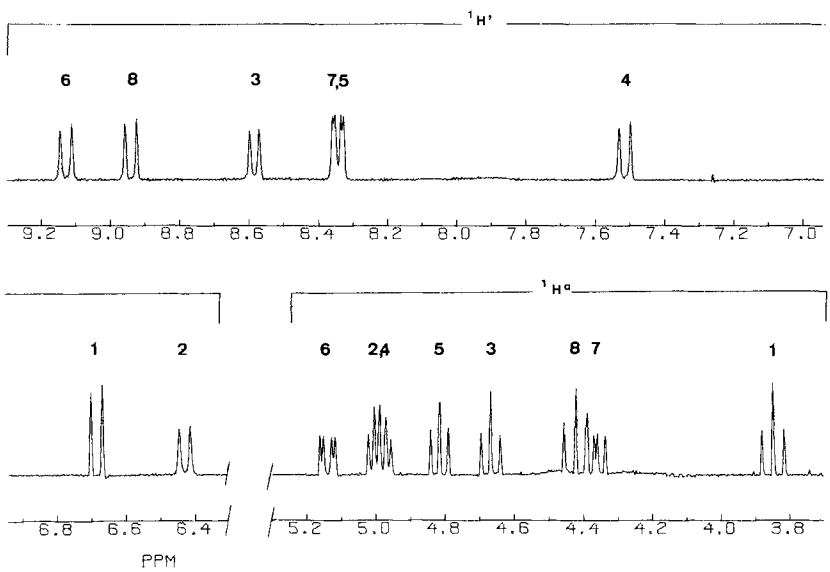

Fig. 1. *$^1H'$- and $^1H^\alpha$-regions of a ^1H-NMR spectrum (cross section of a* J*-resolved 2 D-spectrum) of Boc-(*L*-Val-*D*-Val)$_4$-OH in $CDCl_3$* (Concentration 19 mg/ml, 25°, TMS as internal reference). The sequence number of the residues responsible for the various signals is indicated.

range 7–10 Hz, indicating a β-helical structure. Six of the resonances of the NH-protons ($^1H'$) are in a frequency region (7.52–9.14 ppm) which is characteristic for H-bonded amide groups. One of the two $^1H'$-resonances below 7 ppm (at 6.43 ppm in the spectrum shown in *Fig. 1*) occurs at somewhat variable positions depending on the solution concentration, whereas the other is invariably at 6.69 ppm. On grounds formulated in our previous papers [2] [3], these resonances may be attributed to the protons of a non-H-bonded amide- and of a H-bonded urethanegroup, respectively. Thus, these results point to a double-stranded β-helical structure with C_2-symmetry, in which all NH's of each strand, except one amide-NH, participate in interstrand H-bonding.

To better characterize this structure we have made a complete assignment of the individual backbone ^1H-resonances. In a strand of a β-helix as in one of a β-sheet [4], the torsional angles ψ are such as to bring the H^α of each residue in close proximity (within 2.5 Å [5]) to the H′ of the residue immediately following. Thus, the pairs of resonances given by the backbone protons of consecutive residues can be identified by nuclear *Overhauser effects* (NOE) measurements. We have used these measurements and a two-dimensional proton-shift correlation spectrum (COSY-spectrum [6]) for the assignment. The assignment *(Fig. 1)* shows that it is the amide proton of the residue No. 2 ($^1H'(2)$; we number the residues starting from the amino terminus) that gives the highest-field doublet. Thus, the NH of this residue is not engaged in H-bonding. A careful analysis of the H-bonding characteristics of all double-stranded β-helical structures with 5.6-residues per turn conceivable for **I** indicates that there are two structures with C_2-symmetry having the NH(2) as the only free NH-group. These are the two left-handed structures A and B represented schematically in *Fig. 2*. *A* is antiparallel and *B* is parallel.

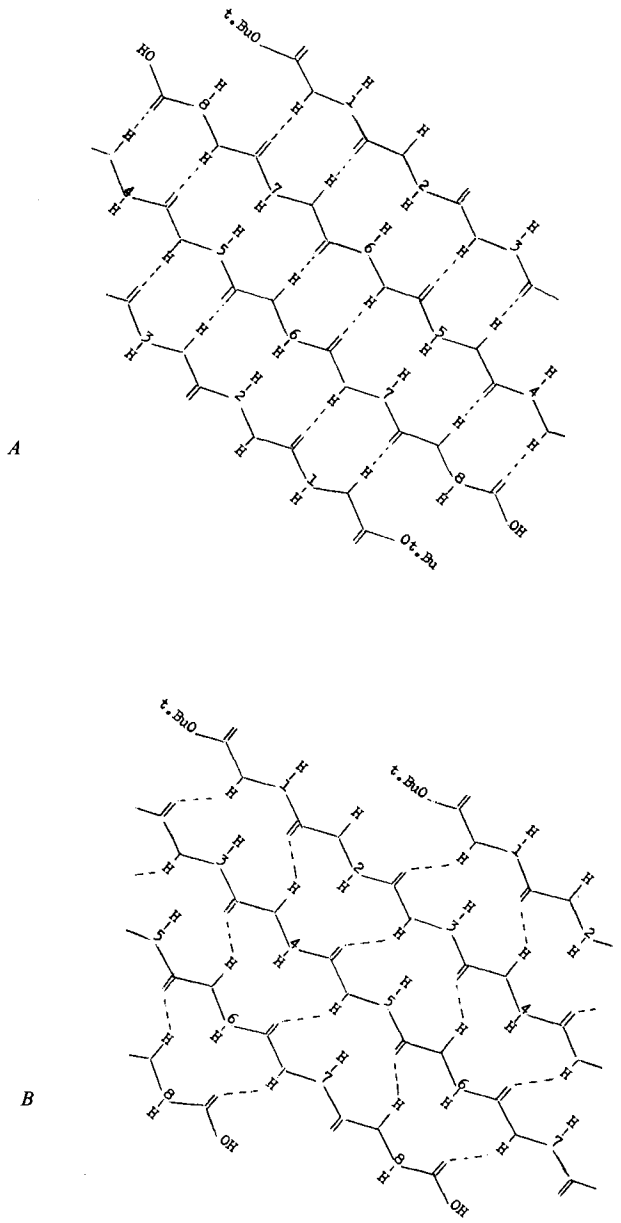

Fig. 2. *Schematic representation of the two alternative double-stranded helical structures conceivable for Boc-(*L-*Val-*D-*Val)$_4$-OH in $CHCl_3$.* The helices have been split along the back in the direction of the helix axis and flattened. Only the terminal groups, the backbone H-atoms and the sequence numbers of the residues in the two strands are indicated. The dashed lines represent interstrand H-bonds. *A* is the antiparallel, and *B* is the parallel structure. In *B* the H-bond between a CO(4) and a NH(3) is truncated.

b) *Discrimination between the Two Helical Structures.* Due to the different relative arrangement of the two strands, distances for pairs of atoms not belonging to the same strand are different in *A* and *B*. A case in point is given by the pairs of H'-atoms H'(1)–H'(7), H'(3)–H'(5), H'(4)–H'(8) and H'(6)–H'(6) and by the pairs of H^{α}-atoms $H^{\alpha}(2)$–$H^{\alpha}(6)$, $H^{\alpha}(5)$–$H^{\alpha}(7)$ and $H^{\alpha}(4)$–$H^{\alpha}(4)$ that are close in *A* and apart in *B* (*Fig. 2*). Calculations using crystallographic data give values of 3.23 Å and 2.46 Å for the distance H'(6)–H'(6) and $H^{\alpha}(5)$–$H^{\alpha}(7)$ in the $\uparrow\downarrow$ $\beta^{5,6}$-helical structure of crystalline Boc-(L-Val-D-Val)$_4$-OMe [7], and in the case of *A* the distances for the pairs of H'- and H^{α}-atoms mentioned above should be near to these. In the case of *B*, the distances for these atom pairs are much greater; for instance, the distances for H'(3)–H'(5) and $H^{\alpha}(5)$–$H^{\alpha}(7)$, that are those with the closest atoms among the pairs considered, should be not less than 5 Å [8].

NOE measurements are often used to show close proximity of protons, and we have considered their use for distinguishing between *A* and *B*. Interproton distances around 3.2 Å are expected to give maximal NOE's of only about 5% [5], and thus at the limits of detectability. As a matter of fact, our attempts to distinguish between *A* and *B* by NOE measurements in the NH-region proved inconclusive, since no evidence for interaction between NH-protons was observed within experimental error. Interproton distances around 2.5 Å would give sizable effects (near 25% [5]), but NOE experiments in the α-proton region of the ^{1}H-NMR spectrum were not carried out, since it was felt that the closeness or partial overlapping of the relevant signals in this region (*Fig. 1*) would lead to somewhat ambiguous results.

Some authors [9] [10] have used specific ^{2}H isotopic substitution in combination with relaxation data for determining the closeness of particular pairs of protons. The magnetogyric ratio of ^{2}H is roughly one sixth that of ^{1}H, and thus the replacement of ^{1}H with ^{2}H markedly reduces the spin-lattice relaxation rates of other protons in the molecule, if they are within a close distance. We have used a similar approach to circumvent the problems encountered with NOE, and we have measured the T_1-values of the α-protons for **I** and for the monodeuterated analogue Boc-(L-Val-D-Val)$_2$-α-^{2}H-L-Val-D-Val-L-Val-D-Val-OH. The replacement of $^1H^{\alpha}(5)$ with ^{2}H was expected to produce a substantial increase in the T_1-value of $^1H^{\alpha}(7)$ if *A* were the correct structure of **I**. The *Table* shows that this is indeed the case: the T_1-value of $^1H^{\alpha}(7)$ for the monodeuterated octapeptide is about 23% higher than that for **I**, while the T_1-values of the other α-protons are practically identical for the two compounds. The distance between $H^{\alpha}(5)$ and $H^{\alpha}(7)$ in *B* is too great to be consistent with this large increase in the T_1 of $H^{\alpha}(7)$. Thus, this result conclusively demonstrates that left-handed $\uparrow\downarrow$ $\beta^{5,6}$-helix *A* is the correct structure of **I** in $CHCl_3$.

It is worth noting that the T_1-values of $^1H^{\alpha}(1)$, $^1H^{\alpha}(3)$ and $^1H^{\alpha}(8)$ are markedly higher than those of the other α-protons and that the latter have very close T_1-values (*Table*). In *A*, as *Fig. 2* shows, the H^{α}'s of the residues No. 1, 3 and 8 of each strand protrude at the extremities of the structure and have no close partner; therefore the $^1H^{\alpha}$'s of these residues cannot relax *via* dipolar interaction as efficiently as the $^1H^{\alpha}$'s of the other residues do. Moreover, for the $^1H^{\alpha}(8)$, which has the highest T_1-value, there is no possibility for relaxation with the amide proton of a following residue.

Table. *$^1H^\alpha$-NMR Spin-Lattice Relaxation Times for Boc-(L-Val-D-Val)$_4$-OH* (**I**) *and Boc-(L-Val-D-Val)$_2$-α-^{2}H-L-Val-D-Val-L-Val-D-Val-OH ($^2H^\alpha(5)$-**I**) in $CDCl_3$ at 25°C and 360 MHz*

Proton	δ[a])	Peak(s) used for the measurement	T_1(s)[b])	
			I	$^2H^\alpha(5)$-**I**
$^1H^\alpha(1)$	3.85	3.85	0.54	0.57
$^1H^\alpha(2)$	5.00	5.01, 5.00	0.35	0.36
$^1H^\alpha(3)$	4.67	4.67	0.45	0.46
$^1H^\alpha(4)$	4.97	4.97	0.37	0.38
$^1H^\alpha(5)$	4.81	4.81	0.37	0.39[c])
$^1H^\alpha(6)$	5.13	5.15, 5.12	0.36	0.37
$^1H^\alpha(7)$	4.36	4.365, 4.355	0.39	0.48
$^1H^\alpha(8)$	4.42	4.42	0.65	0.66

[a]) In ppm, with TMS as internal reference.
[b]) Where two peaks were measured, the figures represent average values. The two values were within 0.02 s.
[c]) The measurement was possible, since the degree of isotopic α-substitution was only 85%.

Concluding Remarks. – This work provides an example of the applicability of $^1H^\alpha$-T_1-measurements to the determination of the relative arrangement of peptide chains in a β-structure. For our continuing studies on D, L-alternating stereo-co-oligopeptides the results presented here are of particular value, since they provide an unequivocal set of ^{1}H-NMR reference data for a ↑↓ $\beta^{5,6}$-helix. A forthcoming paper [1] will show how these data confirm the correctness of our earlier conclusions of an antiparallel arrangement of the strands for some of the $\beta^{5,6}$-helices found [2] [3] for other oligovalines in $CDCl_3$.

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Experimental Part

Boc-(L-Val-D-Val)$_4$-OH was synthesized as described earlier [12] and purified by recrystallization from EtOAc.

Boc-(L-Val-D-Val)$_2$-α-^{2}H-L-Val-D-Val-L-Val-D-Val-OH with an isotopic purity of *ca.* 85% was prepared by saponification of the corresponding methylester [11] under conditions similar to those used for the non-deuterated octapeptide acid, and was purified analogously.

The vapor pressure osmometry measurements were carried out at 25° with the osmometer *Model 232* of *Wescan Instruments, Inc.* Benzil was used for the calibration. $CHCl_3$ used as solvent was passed through alumina before use.

All ^{1}H-NMR measurements were made on solutions degassed *via* freeze-pump-thaw cycles and sealed in standard tubes. The spectrum shown in *Fig. 1* and the COSY-spectrum were recorded with a *Bruker HM-300* spectrometer. The NOE's were observed in difference spectra obtained by subtracting reference spectra from spectra with NOE's using a *Bruker WH-90* spectrometer. The spin-lattice relaxation times were determined with a *Bruker HXS-360* spectrometer by using the conventional inversion recovery pulse sequence (180°–τ–90°–t). The value of t was 5 s. T_1-values were calculated *via* a regression analysis using a single exponential function to describe the approach of the inverted magnetization to the equilibrium value. For the analysis the program DISNMRP, 1982, Version 820601 by *Bruker Instruments*, Inc., was used.

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