77. Conformational Studies on Potential β-Turn-Forming Model Peptides

by **Karsten Bode, Murray Goodman**

Department of Chemistry, University of California, San Diego, La Jolla, CA 92093, USA

and **Manfred Mutter***

Institute of Organic Chemistry, University of Basel, CH-4056 Basel

(30.I.85)

The conformational behavior of POE-bound model peptides Boc-(L-Ala)$_2$-X-Y-(L-Ala)$_2$-NHPOE (X – Y = L-Pro-Gly (**I**), Gly-L-Ile (**II**); NHPOE = aminopoly(oxyethylene)) as well as of the repetitive hexapeptide of elastin, Boc-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M (**III**) (A = photosensitive 3-nitro-4-(bromomethyl)benzoyl group; NHPOE-M = aminopoly(oxyethylane) monomethyl ether) has been studied by means of ^{1}H-NMR and CD spectroscopy. Compounds **I** and **III** form a β-turn with Pro and Gly in positions $i + 1$ and $i + 2$, respectively, while an aggregated state for **II** has been identified. The results are in good agreement with published prediction codes giving experimental evidence for the dominance of short-range interactions to establish secondary structure in solution.

Introduction. – The folding of proteins is considered to be largely determined by short-range interaction forces between the side-chain and backbone of the residue [1–3]. Medium- and long-range interactions are of minor importance. Because of the fact that only four rotational angles (ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, ψ_{i+2}) must adopt appropriate values to form a β-turn, this secondary structure represents a suitable model for the investigation of the importance of short-range interactions. Hence, β-turns are significant in the folding process of proteins by directing nucleation centers, *e.g.* α-helical segments, towards each other for a temporary stabilization [1] [4] [5].

The validity of the short-range interaction concept in the crystalline state has been supported by the establishment of prediction codes [6–9] assigning probability parameters for the adoption of an α-helix, β-sheet and β-turn conformation to each single amino-acid residue. In the event of a dominance of long-range forces, the more complex interdependent interaction would result in a more or less statistical distribution of the individual residue for each conformation.

Since prediction codes are based on the statistical analyses of X-ray data of crystalline proteins [6–9], an examination of their validity in solution is important. To this end, potential β-turn forming model hexapeptides were synthesized according to the liquid-phase method (LPS) [10]. Peptides of the type Boc-(L-Ala)$_2$-X-Y-(L-Ala)$_2$-NHPOE (X – Y = L-Pro-Gly (**I**), Gly-L-Ile (**II**))[1]) attached to solubilizing poly(oxyethylene) were conformationally investigated by means of ^{1}H-NMR and CD spectroscopy. This first study is part of a host-guest approach to the applicability of the LPS to conformational

[1]) Abbreviations are used according to the IUPAC-IUB Commission recommendations in most cases: Boc, (*tert*-butyl)oxycarbonyl; NH-POE, aminopoly (oxyethylene) monomethyl ether.

investigations in solution [11a]. A comparable work has been initiated with regard to the evaluation of parameters influencing β-sheet structure formation [11b].

The residues X-Y represent pairs of amino acids with different probabilities to occupy the relevant positions $i + 1$ (X) and $i + 2$ (Y) of a bend.

According to an investigation by *Kolaskar et al.* [8] L-Pro-Gly and Gly-L-Ile show folding tendencies of 38% and 0%, respectively. Furthermore, proline is known to have a high preference for position $i + 1$ [7–9] facilitating the chain reversal because of its rigid pyrrolidine structure. Alanine was chosen as the host amino acid because of the variety of conformational properties it can adopt under different conditions [12–17] and its relatively hydrophobic character [18] enabling it to stabilize a potential turn. These sequences are part of a series dealing with the host-guest technique for the evaluation of the preferred conformation of amino acid residues and their derivatives [19] taking prediction codes as a first basis for their conformational properties.

To extend the information to a biologically important system with similar sequential features, the repetitive hexapeptide of the connective tissue component elastin (Boc-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M; **III**; A = photosensitive 3-nitro-4-(bromomethyl)benzoyl anchoring group) and its lower analogues were investigated. The peptide also is characterized by a L-Pro-Gly unit in the central position. The stabilization of a potential turn, however, comes from a sequential rather than a homo-oligopeptide.

Amide protons involved in H-bonding were established by ^{1}H-NMR spectroscopy. The experiments carried out involved temperature dependence (in (D_6)DMSO), concentration dependence (in $CDCl_3$), and solvent composition (in (D_6)DMSO/H_2O), making use of the different values of the chemical shift for solvent-exposed and shielded NH's. The determination of δ(NH) as a function of concentration allows a distinction between intra- and intermolecularly bonded protons.

CD-spectroscopic investigations provide additional information on the conformational behavior of these peptides in solution.

Results and Discussion. – The *Table* summarizes the temperature ($d\delta/dT$) and relative concentration coefficients ($d\delta/dc$) (see legend to the *Table* for the definition of $d\delta/dc$) of the NH protons of peptides **I**, **II**, and **III** as well as of some selected precursor sequences in (D_6)DMSO and $CDCl_3$, respectively. The chemical shifts δ are given in ppm relative to TMS as internal reference.

For the first investigations on the formation of β-turns in oligopeptides attached to poly(oxyethylene) and the applicability of the liquid-phase method to NMR-spectroscopic conformational studies in general, (D_6)DMSO was chosen as a solvent for the evaluation of the temperature coefficients. Although it can affect the secondary structure of peptides due to its high polarity, this solvent has been used by many groups for this purpose since bends can be formed even in solvents with strong H-bonding acceptor properties. Furthermore, DMSO mostly precludes undesired aggregations at concentrations relevant for NMR studies (see *Exper. Part*). This requirement is not necessarily fulfilled by solvents such as $CDCl_3$ which are more inert in conformational respects. Moreover, measurements of temperature coefficients in $CDCl_3$ do not always result in unambiguously explicable values [20]. On the other hand, $CDCl_3$ proved to be useful for concentration-dependence studies. In the course of this work, $d\delta/dc$ was also determined in DMSO (not reported here) with results paralleling those obtained in $CDCl_3$, although the coefficient was much smaller because of the DMSO polarity. This finding indicates

Table. *360-MHz ^{1}H-NMR Temperature Coefficients $d\delta/dT$* (ppm/°C) *in (D$_6$)DMSO and Relative Concentration Coefficients* $d\delta/dc = \frac{(d\delta/dc)\ NH}{(d\delta/dc)\ CHCl_3}$ *in $CDCl_3$ of the Amide Protons of* **I**, **II**, **III**, *and Some Precursor Sequences*[a])

Peptide-POE derivatives	Parameter	Residue					
		1	2	3	4	5	6
Boc-L-Ala^1-L-Ala^2-L-Pro^3-Gly^4-L-Ala^5-L-Ala^6-NHPOE **I**	$d\delta/dT$	−0.00515	−0.00469		−0.00396	−0.00345	−0.00446
	$d\delta/dc$	1.37	0.64		2.87	1.21	1.14
Boc-L-Ala^1-L-Pro^2-Gly^3-L-Ala^4-L-Ala^5-NHPOE **IV**	$d\delta/dT$	−0.00707		−0.00405	−0.00368	−0.00507	
	$d\delta/dc$	1.26		2.55	0.90	0.96	
Boc-L-Pro^1-Gly^2-L-Ala^3-L-Ala^4-NHPOE **V**	$d\delta/dT$		−0.00436	−0.00626	−0.00586		
	$d\delta/dc$		3.53	1.11	1.19		
Boc-Gly^1-L-Ala^2-L-Ala^3-NHPOE	$d\delta/dT$	−0.00497	−0.00423	−0.00436			
	$d\delta/dc$	2.48	2.38	1.73			
Boc-L-Ala^1-L-Ala^2-Gly^3-L-Ile^4-L-Ala^5-L-Ala^6-NHPOE **II**	$d\delta/dT$	−0.00650	−0.00391	−0.00335	−0.00456*	−0.00464*	−0.00487
	$d\delta/dc$			insoluble in $CDCl_3$			
Boc-L-Ala^1-Gly^2-L-Ile^3-L-Ala^4-L-Ala^5-NHPOE	$d\delta/dT$	−0.00621	−0.00335	−0.00354*	−0.00444*	−0.00485	
	$d\delta/dc$			insoluble in $CDCl_3$			
Boc-Gly^1-L-Ile^2-L-Ala^3-L-Ala^4-NHPOE	$d\delta/dT$	−0.00456	−0.00435	−0.00475	−0.00451		
	$d\delta/dc$	4.70	7.76	2.96	2.28		
Boc-L-Ile^1-L-Ala^2-L-Ala^3-NHPOE	$d\delta/dT$	−0.00614	−0.00484*	−0.00346*			
	$d\delta/dc$	2.87	2.25	4.52			
Boc-L-Val^1-L-Ala^2-L-Pro^3-Gly^4-L-Val^5-Gly^6-A-NHPOE-M **III**	$d\delta/dT$	−0.00517	−0.00422		−0.00372	−0.00473	−0.00552
	$d\delta/dc$	1.40	2.02		4.68	1.08	4.83

[a]) * Indicates an ambiguity in signal assignments.

that no major conformational alterations occurred as a consequence of changing the solvent.

The distinction between H-bonded and non-bonded amide protons is based on a combined interpretation of the data from NMR experiments in dependence on temperature, concentration and solvent composition. The latter investigations in (D_6)DMSO/H_2O were facilitated by both the attachment of the peptides under study to the solubilizing polymer poly(oxyethylene) and the application of the *Redfield* 2-1-4 pulse sequence [21] which overcomes some difficulties connected to the dynamic range problem. An exclusive reliance on temperature coefficients appears to be questionable in view of diverging opinions with regard to absolute $d\delta/dT$ values for exposed and shielded NH protons [22] [23]. Instead, relative coefficients should be preferred.

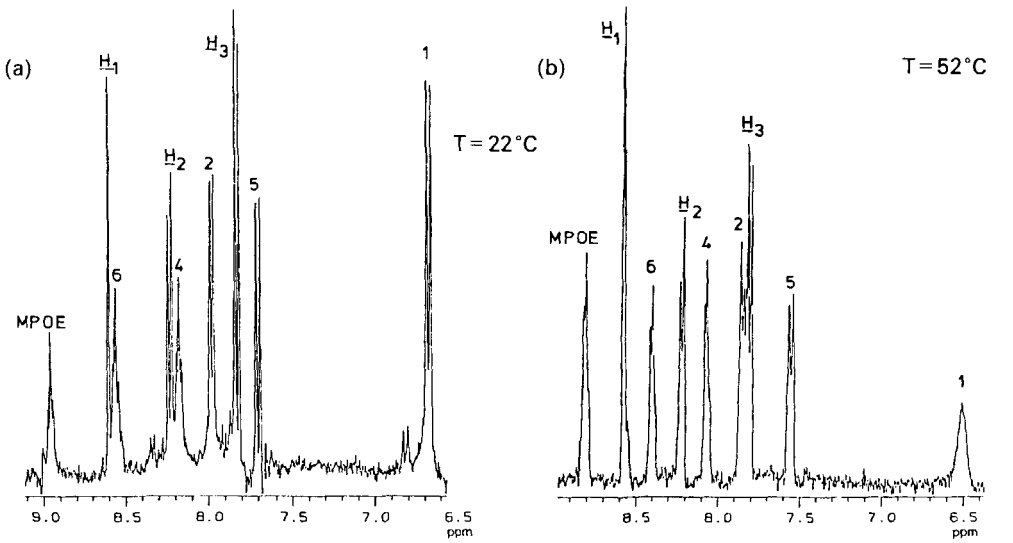

Fig. 1. *NH Region of the 360-MHz ^{1}H-NMR spectra of the repetitive hexapeptide of elastin* (Boc-L-Val^1-L-Ala^2-L-Pro^3-Gly^4-L-Val^5-Gly^6-A-NHPOE-M; **III**), *in (D$_6$)DMSO at T = 22°C (a) and T = 52°C (b)* (conc. 7.5×10^{-3} M). H_1, H_2, and H_3 refer to the aromatic protons in the anchoring group.

The 360-MHz ^{1}H-NMR spectra were mostly well-resolved as can be seen in *Fig. 1* showing the N*H* regions of peptide **III** in (D_6)DMSO at T = 22 °C(*a*) and T = 52 °C(*b*). The residues are numbered starting from the N-terminus.

The peptide **I** exhibits low temperature coefficients for Gly^4 (-3.96×10^{-3} ppm/°C) and Ala^5 (-3.45×10^{-3} ppm/°C) indicating that the amide protons of these residues are involved in H-bonding. The concentration coefficients of Gly^4 and Ala^5 reveal that the latter is involved in an intramolecular bond while the former amino acid is intermolecularly bonded *(Table)*.

Fig. 2 illustrates the results of the solvent-titration experiments in various compositions of (D_6)DMSO/H_2O demonstrating that Gly^4 and Ala^5 are shielded from the solvent, thus, supporting the results of the experiments in (D_6)DMSO and $CDCl_3$.

These findings provide evidence for a conformation in which Ala^5-N*H* forms an intramolecular H-bond most likely to Ala^2-CO effecting a chain reversal (β-turn with

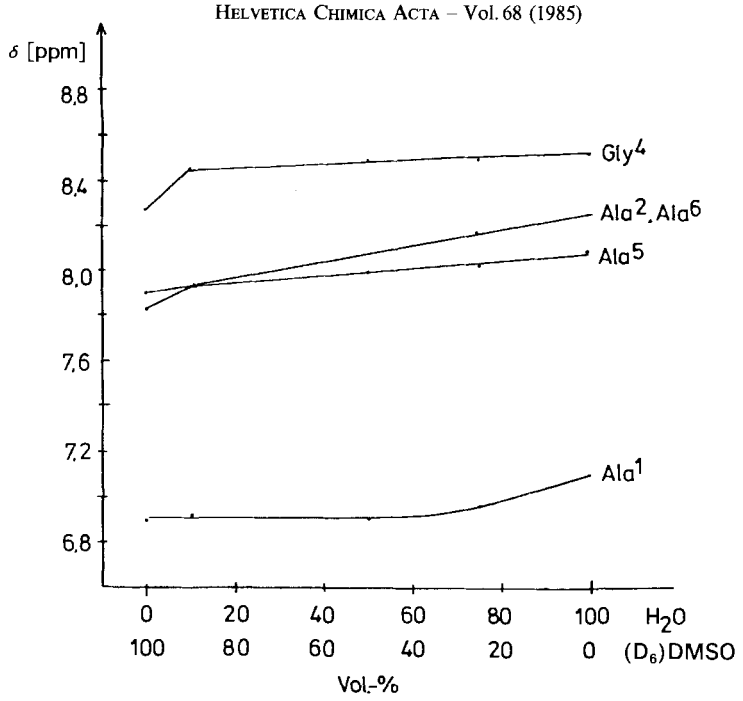

Fig. 2. *Representation of the results of the ^{1}H-NMR solvent titration (D_6)DMSO/H_2O of the* NH *protons in* **I**. T = 22 °C; conc. 7.5×10^{-3} M.

Pro^3 and Gly^4 in positions $i + 1$ and $i + 2$, respectively). A model easily illustrates that Gly^4-N*H* points away from the backbone thus being enabled to participate in an intermolecular H-bond to an adjacent chain. As seen from the model, this conformation can be stabilized by an additional H-bond resulting in a 14-membered ring system between Ala^2-N*H* (-4.69×10^{-3} ppm/°C) and Ala^5-CO *(Table)*.

The CD spectra of **I** in H_2O and 2,2,2-trifluoroethanol (TFE) are presented in *Fig. 3a* and *Fig. 4a*, respectively. These spectra reflect the problems with regard to an unambiguous identification of β-turns by means of CD. Only a few examples of CD studies on bends have been reported [24–27]. Generally, the appearance of CD curves of β-turns is similar to that of β-sheet structures [24]. There is a small difference in the position of the minima and maxima. β-Sheet spectra are characterized by a negative band at $\lambda \approx 220$ nm ($n \rightarrow \pi^*$ transition of the amide chromophore) and a maximum at about 195 nm ($\pi \rightarrow \pi^*$ transition), whereas the curves of model β-turns are shifted to longer wavelengths exhibiting a trough at $\lambda = 225$–230 nm, a positive *Cotton* effect at λ=205 nm and a second minimum at about 190 nm.

The CD spectrum of **I** in H_2O *(Fig. 3a)* clearly indicates a random coil conformation. In TFE, however, the formation of a secondary structure is obvious *(Fig. 4a)*. Negative *Cotton* effects at $\lambda \approx 220$ nm and $\lambda \approx 190$ nm and a maximum at 200–205 nm are observed. The distinction between a β-turn and a pleated-sheet structure is facilitated in the light of the results obtained in previous experiments on this series.

As shown previously, both proline and glycine are known to disrupt or destabilize ordered structures (*e.g.* β-sheet and α-helix) if inserted into the center of a peptide as in **I**

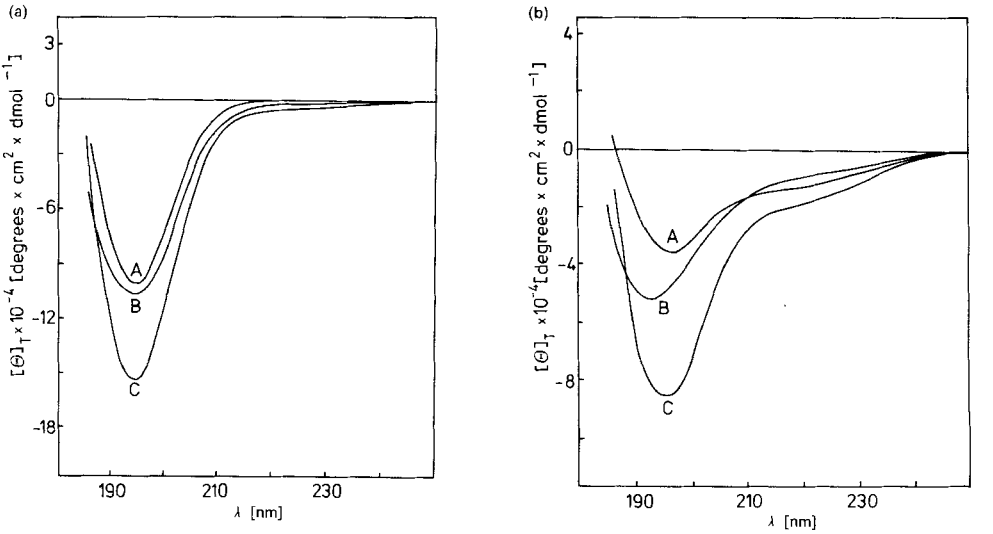

Fig. 3. *CD spectra of* **I** *and its precursor peptides (*a*)* (A: Boc-L-Pro-Gly-(L-Ala)$_2$-NHPOE; B: Boc-L-Ala-L-Pro-Gly-(L-Ala)$_2$-NHPOE; C: Boc-(L-Ala)$_2$-L-Pro-Gly-(L-Ala)$_2$-NHPOE), and **III** *and its precursor sequences (*b*)* (A: Boc-L-Pro-Gly-L-Val-Gly-A-NHPOE-M; B: Boc-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M; C: Boc-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M) *in water* (conc. 2 mg of peptide/ml solv.).

[19] [28–30]. Furthermore, the peptide investigated here has a chain length of six amino acids (n = 6), *i.e.* the critical size necessary to develop a regular β-sheet in TFE is not achieved [13] [31]. Only in the case of isoleucine, the onset of the β-sheet structure was found to occur at the hexapeptide state in this solvent [32] [33]. Considering these facts, the CD spectrum in TFE presented in *Fig. 4* indicates the existence of a chain reversal in the presence of random coil conformations causing the shift to smaller wavelengths. The

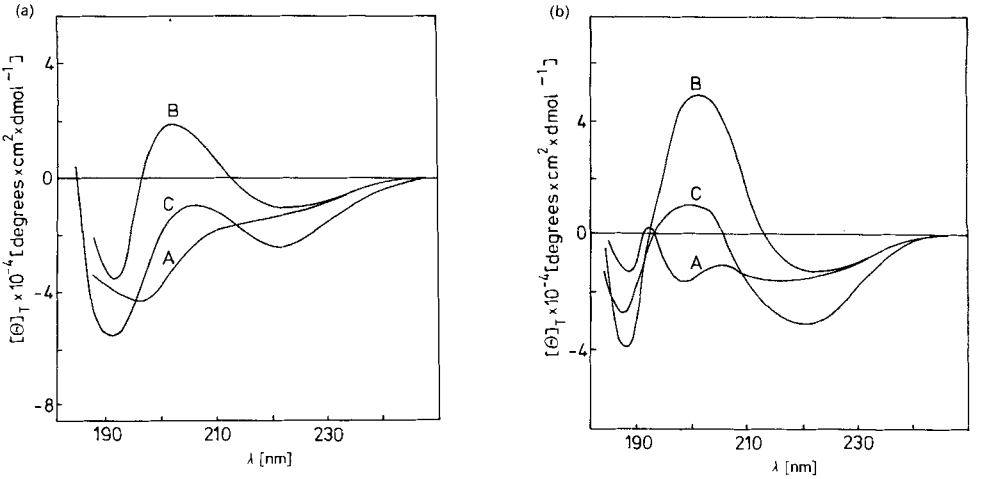

Fig. 4. *CD spectra of* **I** *and its precursor peptides (*a*)* (A: Boc-L-Pro-Gly-(L-Ala)$_2$-NHPOE; B: Boc-L-Ala-L-Pro-Gly-(L-Ala)$_2$-NHPOE; C: Boc-(L-Ala)$_2$-L-Pro-Gly-(L-Ala)$_2$-NHPOE), *and* **III** *and its precursor sequences (*b*)* (A: Boc-L-Pro-Gly-L-Val-Gly-A-NHPOE-M; B: Boc-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M; C: Boc-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M) in TFE (conc. 2 mg of peptide/ml solv.).

formation of a bend in TFE underscores its preference in favor of intramolecular interactions as already documented by the fact that this solvent is helix-supporting [13].

Interestingly, the results of the precursor peptides **IV** and **V** *(Table)* demonstrate that a β-turn does not exist in the tetrapeptide while it is evidently developed in the pentapeptide. It is worthwhile observing that the tripeptide Boc-Gly-L-Ala-L-Ala-NHPOE tends to be intermolecularly associated *(Table)*, pointing to the fact that some aggregation of the peptides occurs prior to β-structure formation. The absence of an aggregated state in the tetra- and pentapeptides **V** and **IV**, respectively, reflects the destabilizing effect of glycine and proline incorporated in the center of a sequence in a regular β-structure because of the flexibility of Gly and the rigid pyrrolidine ring in proline [19] [28] [29].

The CD spectra of the tetra- and pentapeptide in H_2O and TFE (*Fig. 3a*, curves *A* and *B*; *Fig. 4a*, curves *A* and *B*) parallel those of the hexapeptide showing a random-coil structure and a partially ordered conformation, respectively. Most notably, in TFE the pentapeptide shows a β-turn, which is even more developed compared to the hexapeptide. This decrease in the stability of the β-turn in going from the penta- to the hexapeptide might be caused by steric complications between the N-terminal Boc-protecting group and the C-terminal macromolecular amide group, which is expected to be more severe at the hexapeptide level for a β-turn-forming peptide.

The conformation of the elastin model peptide **III** *(Table)* is comparable to that of the model sequence **I**. The NMR data in (D_6)DMSO, $CDCl_3$, and (D_6)DMSO/H_2O reveal two intramolecular H-bonds with Ala^2-N*H* (indicating a 14-membered ring system to Val^5-CO) and Val^5-N*H* and Ala^2-CO (β-turn with Pro^3 and Gly^4 occupying positions $i + 1$ and $i + 2$, respectively) as well as a strong intermolecular interaction of Gly^4-N*H*. It is obvious that the temperature coefficient of Val^5-N*H* in **III** (-4.73×10^{-3} ppm/°C) is considerably larger than that of Ala^5-N*H* in **I** (-3.45×10^{-3} ppm/°C; *Table*) indicating a less stable turn in (D_6)DMSO. This finding may illustrate the influence of the different residues surrounding Pro^3 and Gly^4 on the stability of the bends in **I** and **III**.

The identification of a β-turn in **III** is in agreement with results obtained by *Urry* and coworkers [22] [34] on the sequence HCO-L-Ala^1-L-Pro^2-Gly^3-L-Val^4-Gly^5-L-Val^6-OMe. In our work, the sequential arrangement of the hexapeptide was slightly modified in order to compare the conformational behavior of the elastin peptide with that of the model compound **I**. This alteration along with the different protecting groups might be the reason for some deviations in the temperature coefficients of the residual N*H* groups of *Urry*'s and our peptide.

On the basis of the data at hand, it is possible to postulate a multi-layer structure for the elastin sequence with one hexapeptide unit in each plane. According to this model, the planes formed by the β-turn and the 14-membered ring are in contact *via* an intermolecular H-bond between the two groups Gly^4-N*H* and Pro^3-CO lying one over the other.

The CD studies on **III** and its precursors in water and TFE (*Fig. 3b* and *Fig. 4b*) also result in the identification of a random-coil conformation and a secondary structure, respectively. For the latter, the same rationalization as for **I** justifies the assumption of the existence of a turn rather than a β-sheet structure. As observed in **I**, the formation of a well-developed β-turn is maximal at the pentapeptide level. However, a possible effect of POE on the stability of the β-turn must be very small. For example, the CD spectra of the elastin sequence after its removal from the support by UV light (not shown here) do not

deviate significantly from those of the POE-bound compound, supporting the results of previous investigations [35–37] that the influence of POE on the preferred conformation of the attached peptide is negligible.

The NMR investigations on **III**, Boc-L-Ala[1]-L-Ala[2]-Gly[3]-L-Ile[4]-L-Ala[5]-L-Ala[6]-NHPOE, which is supposed to have a zero probability to occur in a turn [8] reveal small temperature coefficients for every residue (except the Boc-protected terminal group). This is also true for the precursor sequences *(Table)*. Concentration-dependent measurements of $\delta(NH)$ of the penta- and hexapeptides were not possible because of their very low solubility in $CDCl_3$. The *Table* illustrates the remarkably high values of $d\delta/dc$ in the tri- and tetrapeptide derivatives showing a strong tendency to form aggregations at these short chain-length (n = 3,4).

The CD spectra of **II** and its precursors (not presented) are characteristic of a random-coil structure in both H_2O and TFE.

The ^{1}H-NMR and CD data establish the preference of proline and glycine to be involved in the formation of a β-turn in position $i + 1$ and $i + 2$, respectively. They demonstrate the ability of L-Pro to initiate a bend because of its rigid ring structure. These results, supporting studies of other groups [22] [34], prove that peptides attached to the polymeric C-terminal carrier POE are able to form β-turns even with short chain lengths (n = 5). Thus, both the rapid synthetic procedure and the solubilizing effect of POE can be used for the design and spectroscopic investigation of the conformationally interesting oligopeptides.

The presence of a turn in solution in **I** and **III** containing Pro and Gly in positions $i + 1$ and $i + 2$, respectively, as well as the absence of a chain reversal in **II** with Gly and Ile as guest amino acids is in agreement with the prediction codes being based on crystallographic data [6–9]. The results obtained on **I** and **III** reflect the high preference of Pro for position $i + 1$ of a β-turn. They experimentally underscore conformational calculations by *Scheraga* and coworkers carried out on dipeptides of the type Pro-X and X-Pro (X = amino acid) [38–40] resulting in a higher probability for the occurrence of a bend in Pro-X than in X-Pro sequences. Thus, the prediction codes published in the literature [6–9] represent a good first approximation to address dipeptide pairs with a pronounced tendency to form a chain reversal in a given sequence.

The good agreement between predicted and experimental results supports the thesis that short-range interactions are the driving force for the formation of chain reversals in solution. Hexapeptides are not able to build up long-range interactions. The ability of peptides to fold back even in a tetrapeptide state as shown here and in similar model compounds [41] [42] gives support to the hypothesis that turns are an important conformational feature during the folding process of proteins by directing nucleation centers towards each other.

K.B. and *M.M.* were supported by the *Deutsche Forschungsgemeinschaft* through the *Sonderforschungsbereich 41 'Chemie und Physik der Makromoleküle'*. *M.G.* was supported by the *National Science Foundation* (grant No. 80-23002).

Experimental Part

The syntheses of the monodispersed, chemically and optically pure model peptides Boc-(L-Ala)$_2$-X-Y-(L-Ala)$_2$-NHPOE (X – Y = L-Pro-Gly (**I**), Gly-L-Ile (**II**)) and the repetitive hexapeptide of elastin (Boc-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M (**III**)) were carried out according to the liquid-phase method [40]. The procedure has been described in [10] [43] [44]. All amino acids were purchased as Boc-protected derivatives from *Bachem*, Bubendorf, Switzerland. As coupling reagents, *N,N'*-dicyclohexylcarbodiimide/1-hydroxybenzotriazole were used. The purity of the peptides was confirmed by standard techniques. The degree of racemization was tested by means of a GC method by *Bayer et al.* [45]. The polymeric supports H_2NPOE and H_2NPOE-M were prepared from bifunctional POE, mol.wt. 6000 (*Fluka*, Buchs), and POE monomethyl ether, mol.wt. 5000 (*Fluka*, Buchs), respectively [46]. An overall conversion of 75% of the terminal OH groups was achieved. The photosensitive anchoring group 3-nitro-4-(bromomethyl)benzoic acid abbreviated by A was synthesized and incorporated as published [46] [47].

The ^{1}H-NMR spectra were recorded using a 360-MHz instrument *(Varian)* equipped with a *NIC-1180 E* data processor *(Nicolet)*. The intense POE methylene signal at about 3.6 ppm was selectively presaturated. To overcome the dynamic range problem the H_2O signal was suppressed by means of the *Redfield* pulse sequence [21] modified by *Nicolet*. TMS (*Aldrich*, Milwaukee, Wisconsin) was added as internal reference for measurements in the purely org. solvents. Sodium 3-(trimethylsilyl)tetradeuteropropionate (TSP) (*Wilmad*, Buena, New Jersey) was used for aq. solns. The range of temp. was 20–50° (conc. 7.5×10^{-3}M), the concentration was varied between 0.5×10^{-2} and 4.1×10^{-2}M (T = 22°). The temp. was accurate to ±1°. All signal assignments were made by measuring every precursor peptide under the respective conditions.

CD experiments were carried out with *CARY 61* and *JASCO J 41-C* instruments.

The spectrograde solvents (D_6)DMSO (min. 99.5% D) and $CDCl_3$ (min. 99.96% D) were purchased in ampules to protect them from water (*Merck Sharp & Dohme*, Montreal, Canada). TFE was acquired from *Aldrich*, Milwaukee, Wisconsin, and *Merck-Schuchart*, Hohenbrunn.

REFERENCES

[1] H.A. Scheraga, *Pure Appl. Chem.* **1973,** *36,* 1.
[2] D. Kotelchuck, H.A. Scheraga, *Proc. Natl. Acad. Sci. USA* **1968,** *61,* 1163.
[3] D. Kotelchuck, H.A. Scheraga, *Proc. Natl. Acad. Sci. USA* **1969,** *62,* 14.
[4] C.B. Anfinsen, H.A. Scheraga, *Adv. Protein Chem.* **1975,** *29,* 205.
[5] P.N. Lewis, F.A. Momany, H.A. Scheraga, *Proc. Natl. Acad. Sci. USA* **1971,** *68,* 2293.
[6] P.Y. Chou, G.D. Fasman, *Biochemistry* **1974,** *13,* 222.
[7] P.Y. Chou, G.D. Fasman, *J. Mol. Biol.* **1977,** *115,* 135.
[8] A.S. Kolaskar, V. Ramabrahman, K.V. Soman, *Int. J. Pept. Protein Res.* **1980,** *16,* 1.
[9] P.Y. Chou, G.D. Fasman, *Biophys. J.* **1979,** *26,* 367.
[10] M. Mutter, E. Bayer, in 'The Peptides: Analysis, Synthesis, Biology', Eds. Meienhofer and E. Gross, Academic Press, New York, 1980, Vol. II, p. 285.
[11] a) M. Mutter, F. Moser, K.-H. Altmann, C. Toniolo, G.M. Bonora, *Biopolymers,* in press; b) F. Maser, Ph.D. Thesis, University of Mainz, 1983.
[12] C. Toniolo, G.M. Bonora, M. Mutter, *J. Am. Chem. Soc.* **1979,** *101,* 450.
[13] G.M. Bonora, C. Toniolo, M. Mutter, *Polymer* **1979,** *19,* 1382.
[14] M. Mutter, H. Mutter, R. Uhmann, E. Bayer, *Biopolymers* **1976,** *15,* 917.
[15] C. Toniolo, M. Palumbo, *Biopolymers* **1977,** *16,* 219.
[16] M. Palumbo, S. Da Rin, G.M. Bonora, C. Toniolo, *Makromol. Chem.* **1976,** *177,* 1477.
[17] G.M. Bonora, M. Palumbo, C. Toniolo, *Makromol. Chem.* **1979,** *180,* 1293.
[18] S. Lifson, C. Sander, in 'Protein Folding', Ed. R. Jaenicke, Elsevier – North Holland, Amsterdam, 1980, p. 289.
[19] M. Mutter, V.N.R. Pillai, H. Anzinger, E. Bayer, C. Toniolo, in Proc. 16th Europ. Pept. Symp., Helsingør, 1980, 'Peptides 1980', Ed. Brunfeld, Scriptor, Copenhagen, 1981, p. 660.
[20] E.S. Stevens, N. Sugawara, G.M. Bonara, C. Toniolo, *J. Am. Chem. Soc.* **1980,** *102,* 7048.
[21] A.G. Redfield, S.D. Kunz, E.K. Ralph, *J. Magn. Reson.* **1975,** *19,* 114.

[22] D.W. Urry, M.A. Khaled, V. Renugopalakrishnan, R.S. Rapaka, *J. Am. Chem. Soc.* **1978,** *100,* 696.
[23] Y.V. Venkatachalapathi, P. Balaram, *Biopolymers* **1981,** *20,* 625.
[24] S. Brahms, J. Brahms, *J. Mol. Biol.* **1980,** *138,* 149.
[25] S. Brahms, J. Brahms, G. Spach, A. Brack, *Proc. Natl. Acad. Sci. USA* **1977,** *74,* 3208.
[26] M. Kawai, G.D. Fasman, *J. Am. Chem. Soc.* **1978,** *100,* 3630.
[27] R. Deslauriers, D.J. Evans, S.J. Leach, Y.C. Meinwald, E. Misasian, G. Némethy, I.D. Rae, H.A. Scheraga, R.L. Somorjai, E.R. Stimson, J.W. Van Nispen, R.W. Woody, *Macromolecules* **1981,** *14,* 985.
[28] G.N. Ramachandran, C.M. Venkatachalam, S. Krimm, *Biophys. J.* **1966,** *6,* 849.
[29] F. Naider, J.M. Becker, A. Ribeiro, M. Goodmann, *Biopolymers* **1978,** *17,* 2213.
[30] F. Maser, B. Klein, M. Mutter, C. Toniolo, G.M. Bonora, *Biopolymers* **1983,** *22,* 233.
[31] C. Toniolo, G.M. Bonora, S. Salardi, M. Mutter, *Macromolecules* **1979,** *12,* 620.
[32] H. Mutter, Ph.D. Thesis, University of Tübingen, 1978.
[33] M. Goodman, F. Naider, C. Toniolo, *Biopolymers* **1971,** *10,* 1719.
[34] D.W. Urry, L.W. Mitchell, T. Ohnishi, *Biochim. Biophys. Acta* **1975,** *393,* 296.
[35] M. Mutter, *Macromolecules* **1977,** *10,* 1413.
[36] A.A. Ribeiro, R.P. Saltman, M. Goodman, M. Mutter, *Biopolymers* **1982,** *21,* 2225.
[37] W. Mayr, R. Oekonomopulos, G. Jung, *Biopolymers* **1979,** *18,* 425.
[38] S.S. Zimmerman, H.A. Scheraga, Proc. 4th Am. Pept. Symp., in: 'Chemistry and Biology of Peptides', Eds. J. Meienhofer and R. Walter, New York, 1975, p. 263.
[39] S.S. Zimmerman, H.A. Scheraga, *Biopolymers* **1977,** *16,* 811.
[40] E.R. Stimson, S.S. Zimmerman, H.A. Scheraga, *Macromolecules* **1977,** *10,* 1049.
[41] K.D. Kopple, A. Go, *Biopolymers* **1976,** *15,* 1701.
[42] F. Toma, H. Lam-Thanh, F. Piriou, M.Ch. Heindl, K. Lintner, A. Fermandjian, *Biopolymers* **1980,** *19,* 781.