ration of the polyalkenamer thus formed.

Conclusion

When a polymerization leads to the formation of a polyalkenamer, two situations may arise. In the first case the polymerization is stereospecific and polymer with near 100% cis or trans configuration is obtained. In the second case the polymerization is not stereospecific and the configurations of the monomer units are not totally cis or trans. The present paper shows that in the latter case a term ΔG_D ought to be introduced in the expression of ΔG_{lc}. This term takes into consideration the fact that the polymer is made of two types of distinguishable units. Moreover, it is concluded that, if the formation of the polymer is thermodynamically controlled, the composition of the polymer is unique, which is in agreement with the results obtained for the polymerization of cyclopentene. If a monomer–polymer equilibrium exists, it is also found that the amount of monomer present under equilibrium condition is dependent on the configuration of the polymer. This is observed even if the configuration of the polymer thus formed is not controlled by thermodynamics as it is the case for the conversion of cyclopentene into near 100% *cis*-polypentenamer.

Acknowledgment. We acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada.

References and Notes

(1) I. A. Oreshkin, L. I. Redkina, I. L. Kershenbaum, G. M. Chernenko, K. L. Makovetsky, E. I. Tinyakova, B. A. Dolgoplosk, *Eur. Polym. J.*, **13**, 447 (1977).
(2) E. A. Ofstead and N. Calderon, *Makromol. Chem.*, **154**, 21 (1972).
(3) K. L. Makovetskii and L. I. Red'kina, *Dokl. Akad. Nauk SSSR*, **231**, Ang. 1033 (1976).
(4) J. Leonard, *Macromolecules*, **2**, 661 (1969).
(5) K. J. Ivin and J. Leonard, *Eur. Polym. J.*, **6**, 331 (1970).
(6) H. Höcker, W. Reimann, L. Reif, and K. Riebel, *J. Mol. Catal.*, **8**, 191 (1980).
(7) P. E. Harvey and J. Leonard, *Macromolecules*, **5**, 698 (1972).
(8) A. Drapeau and J. Leonard, to be submitted for publication.
(9) L. Hocks, *Bull. Soc. Chim. Fr.*, **78**, 1894 (1975).
(10) F. S. Dainton, T. R. E. Devlin, and P. A. Small, *Trans. Faraday Soc.*, **51**, 1710 (1955).
(11) G. Pampus and G. Lehnert, *Makromol. Chem.*, **175**, 2605 (1974).
(12) G. Natta, G. Dall'Asta, I. W. Bassi, and G. Carella, *Makromol. Chem.*, **91**, 87 (1966).
(13) E. A. Ofstead, J. R. Lawrence, M. L. Senyek, and N. Calderon, *J. Mol. Catal.*, **8**, 227 (1980).
(14) Y. Chauvin, D. Commereuc, and D. Cruypelinck, *Makromol. Chem.*, **177**, 2637 (1976).
(15) V. A. Khodzhemirov, V. A. Yevdokimova, and V. M. Cherednichenko, *Polym. Sci. USSR (Engl. Transl.)*, **18**, 581 (1976).
(16) N. I. Pakuro, A. R. Gantmakher, and B. A. Dolgoplosk, *Dokl. Akad. Nauk SSSR*, **223**, Ang. 868 (1975).
(17) Cecilia M. C. De Figuêiredo and Ailton De Souza Gomes, *J. Polym. Sci.*, **17** (A1), 2845 (1979).
(18) T. J. Katz, S. J. Lee, and N. Acton, *Tetrahedron Lett.*, **47**, 4247 (1976).
(19) J. R. Arlie, Y. Chauvin, D. Commereuc, and J. P. Soufflet, *Makromol. Chem.*, **175**, 861 (1974).
(20) W. R. Lau, C. J. Clover, and J. C. Holste, *J. Appl. Polym. Sci.*, **27**, 3067 (1982).
(21) R. S. Jessup, *J. Res. Natl. Bur. Stand (U.S.)* **60**, 47 (1958).
(22) T. Morisue, K. Noda, and K. Ishida, *J. Chem. Eng. Soc. Jpn.*, **6**, 355 (1973).
(23) E. A. Ofstead, ACS Rubber Division, Spring Meeting, Detroit, MI, 1973.

Amino Acid Sequence Determination for Sequential Polypeptides Consisting of γ-Benzyl and γ-Methyl L-Glutamates by ^1H and ^{13}C NMR Methods

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ABSTRACT: A series of sequential polypeptides consisting of γ-benzyl L-glutamate (B) and γ-methyl L-glutamate (M) prepared by the fragment self-condensation method using the dicyclohexylcarbodiimide/*N*-hydroxysuccinimide (DCC/HONSu) activated ester method were measured in trifluoroacetic acid (TFA) and TFA/$CDCl_3$ (1:1) solutions by means of 100-MHz ^1H, 500-MHz ^1H, 25-MHz ^{13}C, and 125-MHz ^{13}C NMR spectrometers. It was found from these observations that the amino acid sequence determination for these copolymers is well established on the basis of a definite distribution of the hexad (or pentad) sequence of B and M in the chain. In the ^1H NMR observations, the hexad and also higher order sequence effects, probably caused by the ring current effect of side-chain phenyl groups, were revealed for the OCH_3 proton peak of the M residue in the copolymers. On the other hand, in the ^{13}C NMR, the pentad and/or higher order sequence effects were revealed for the $C{=}O_{ester}$ and C_α peaks of the M residue and for all the peaks (except for C_{phenyl}) of the B residue in the copolymers.

Introduction

High-resolution NMR spectroscopy has provided very useful information about the stereochemical structure of polymers.[1] Such information sometimes permits a definite determination of possible structures to be made. For proteins, knowledge of the primary structure of the peptide chains is critical for many biochemical and biophysical studies. At present, amino acid sequence determination is well established and this is almost a routine procedure for many smaller proteins.[2] NMR proves to be a very

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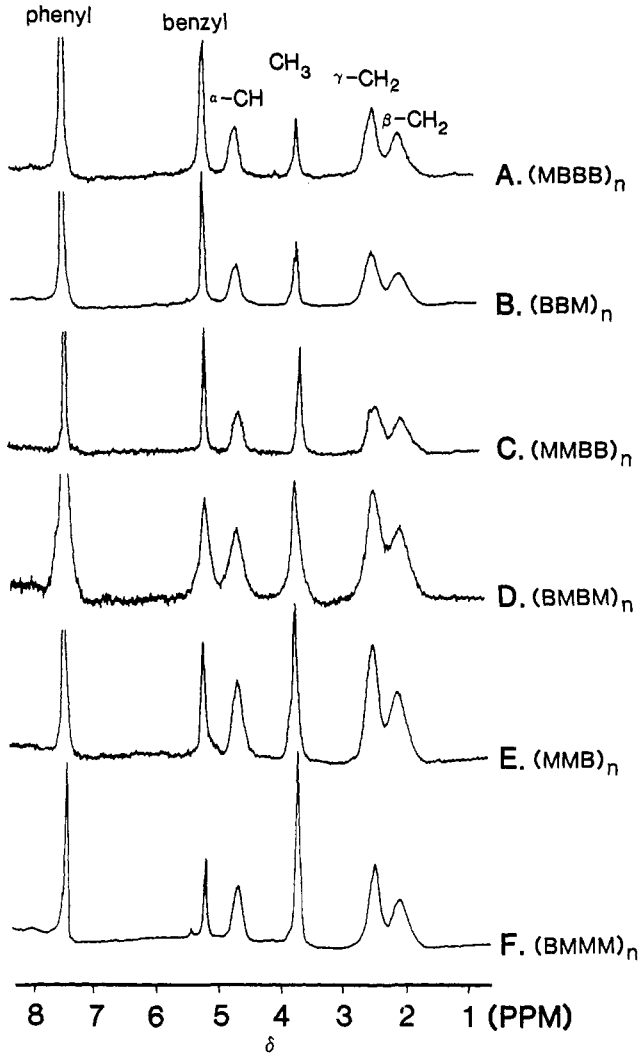

Figure 1. 100-MHz ^1H NMR spectra of various sequential copolymers consisting of B and M in a mixed solvent deuterated TFA/$CDCl_3$ (1:1); concentration = 5 wt/vol %.

useful and powerful technique for amino acid sequence determination and this is possible without any cleavage of peptides and proteins. The amino acid sequence and tacticity determinations in peptides and polypeptides have been studied by some researchers using ^1H and ^{13}C NMR in solution.[3,4]

Recently, Hiraoki et al.[5] have reported that the OCH_3 proton NMR signal of a random copolymer consisting of γ-benzyl L-glutamate (B) and γ-methyl L-glutamate (M) consists of three overlapping peaks which were assumed to be assignable to the triad sequence (*MM*M, M*M*B, B*M*B). Further, they have shown that the OCH_3 protons are shifted upfield with increasing B content. However, it seems that such chemical shift behavior is too complicated to assign the OCH_3 proton resonance to a specific triad configuration.

It is well-known[6-8] that if a polypeptide takes an ordered structure such as the α-helix or β-sheet form, there must be intra- and intermolecular hydrogen bonding, while in a disordered (random coil) conformation in a polar solvent such as trifluoroacetic acid (TFA), further hydrogen-bonding interactions occur between the polymer and the solvent. The NMR signal may show these types of interactions. Thus, if a polypeptide with a known configuration (sequence) is used, the methodology for the amino acid sequence determination through NMR will be established with certainty.

The purpose of the present work, therefore, is to examine the correlation between the ^1H and ^{13}C NMR chemical shifts and the amino acid sequences for copolymers consisting of B and M units in a random-coil supporting solvent and to establish the sequence determination analysis with NMR.

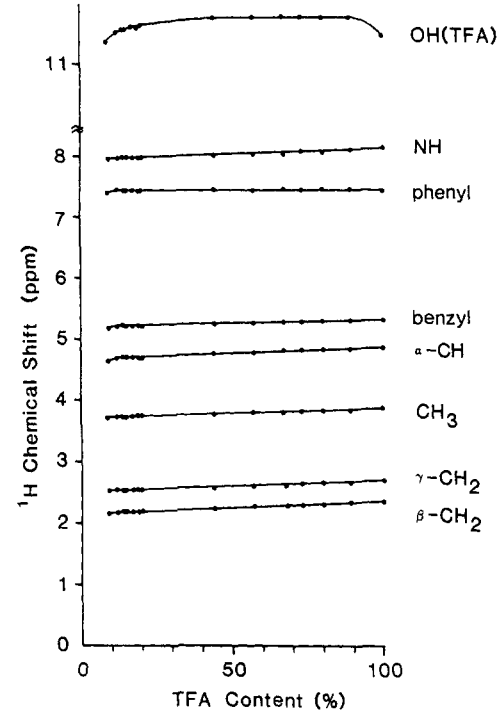

Figure 2. ^1H chemical shifts of the sequential copolymer $(BBM)_n$ as a function of TFA content (%) in the TFA/$CDCl_3$ mixtures.

Experimental Section

Materials. Sequential polypeptides consisting of γ-benzyl L-glutamate and γ-methyl L-glutamate, denoted as $(MBBB)_n$, $(BBM)_n$, $(MMBB)_n$, $(BMBM)_n$, $(MMB)_n$, and $(BMMM)_n$, were prepared by the DCC/HONSu coupling method. The synthetic procedure of the samples used in this study and their characteristics are shown elsewhere.[9]

Methods. ^1H NMR spectra were recorded on a JEOL PS-100 spectrometer (100 MHz) equipped with Fourier transform accessories and on a JEOL GX-500 spectrometer (500 MHz) at room temperature. ^{13}C NMR spectra were recorded on a JEOL PS-100 spectrometer (25 MHz) equipped with Fourier transform accessories and on a JEOL GX-500 spectrometer (125 MHz) at room temperature. Chemical shift is denoted by the δ value (ppm) from tetramethylsilane (Me_4Si). The detailed NMR measurement conditions are described in the figure captions.

Results and Discussion

^1H NMR Spectra. Figure 1 shows 100-MHz ^1H NMR spectra of a series of sequential polypeptides consisting of B and M in a mixed solvent TFA-*d*/$CDCl_3$ (1:1), where their peaks are assigned. The ^1H chemical shifts of all signals except for the OCH_3 peak (δ 3.6–3.8) are essentially unchanged (within experimental error) among the sequential copolymers used in this study, indicating that the chemical shifts are probably independent of B (or M) composition and B, M sequence. The chemical shift of the OCH_3 peak depends significantly on the M composition and B, M sequence in the copolymers. In TFA-*d* solution, the ^1H NMR spectra of the sequential copolymers give rise to spectral features very similar to those found in the mixed solvent TFA-*d*/$CDCl_3$ (1:1). This behavior will be discussed below.

Figure 2 shows the chemical shifts for one of the sequential copolymers, $(BBM)_n$, as a function of TFA content in the TFA/$CDCl_3$ mixtures. The chemical shifts of

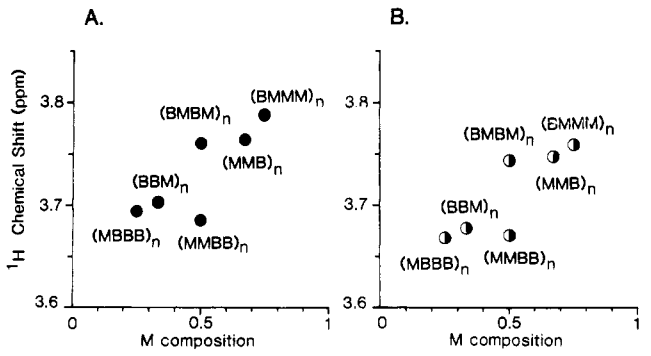

Figure 3. ^{1}H chemical shifts of the OCH_3 peak and M composition in TFA (A) and in TFA/$CDCl_3$ (1:1) (B) for various kinds of sequential copolymers.

individual signals change remarkably in the range below 20% TFA content, and such behavior is ascribed to the conformational transformation from α-helix to the random-coil form.

Figures 1 and 2 show that the chemical shifts of these copolymers show different conformational transitions compared to a homopolymer such as poly(γ-benzyl L-glutamate).[10] The results obtained here for the sequential copolymers are as follows: (a) approximately a 0.1–0.2 ppm change in the chemical shift of the α-CH; (b) no sigmoid curve and a transition that is over well before 14% TFA; (c) a small but significant change in the chemical shift of the β-CH_2, benzyl, and phenyl protons; (d) a visible NH peak in the helical region.

However, Suzuki et al.[10] have made the following observations concerning the conformational transition of poly(γ-benzyl L-glutamate): (a) 0.67 ppm overall change in the chemical shift of the α-CH; (b) sigmoid curve of the α-CH, with transition midpoint at 14.5% TFA; (c) significant change (ca. 0.2 ppm) in the chemical shift of the β-CH_2 and no change for benzyl and phenyl protons; (d) line broadening of the NH peak and its coalescence with the wing of the phenyl peak, preventing its detection in the helical region.

These two sets of data are clearly not in agreement with each other and can be explicitly explained in terms of molecular weight differences (for a, b, and d) and B, M sequences (for b and c). The homopolymer used by Suzuki et al.[10] was a high molecular weight sample synthesized by the amino acid *N*-carboxyanhydride (NCA) method. However, the sequential copolymers studied in the present publication were of lower molecular weight. In addition, Hiraoki et al.[5] have reported for random copolymers of B and M (which were synthesized by the NCA method) that the chemical shift change of the α-CH proton shows a sigmoid curve and the transition midpoint is at 9–11%

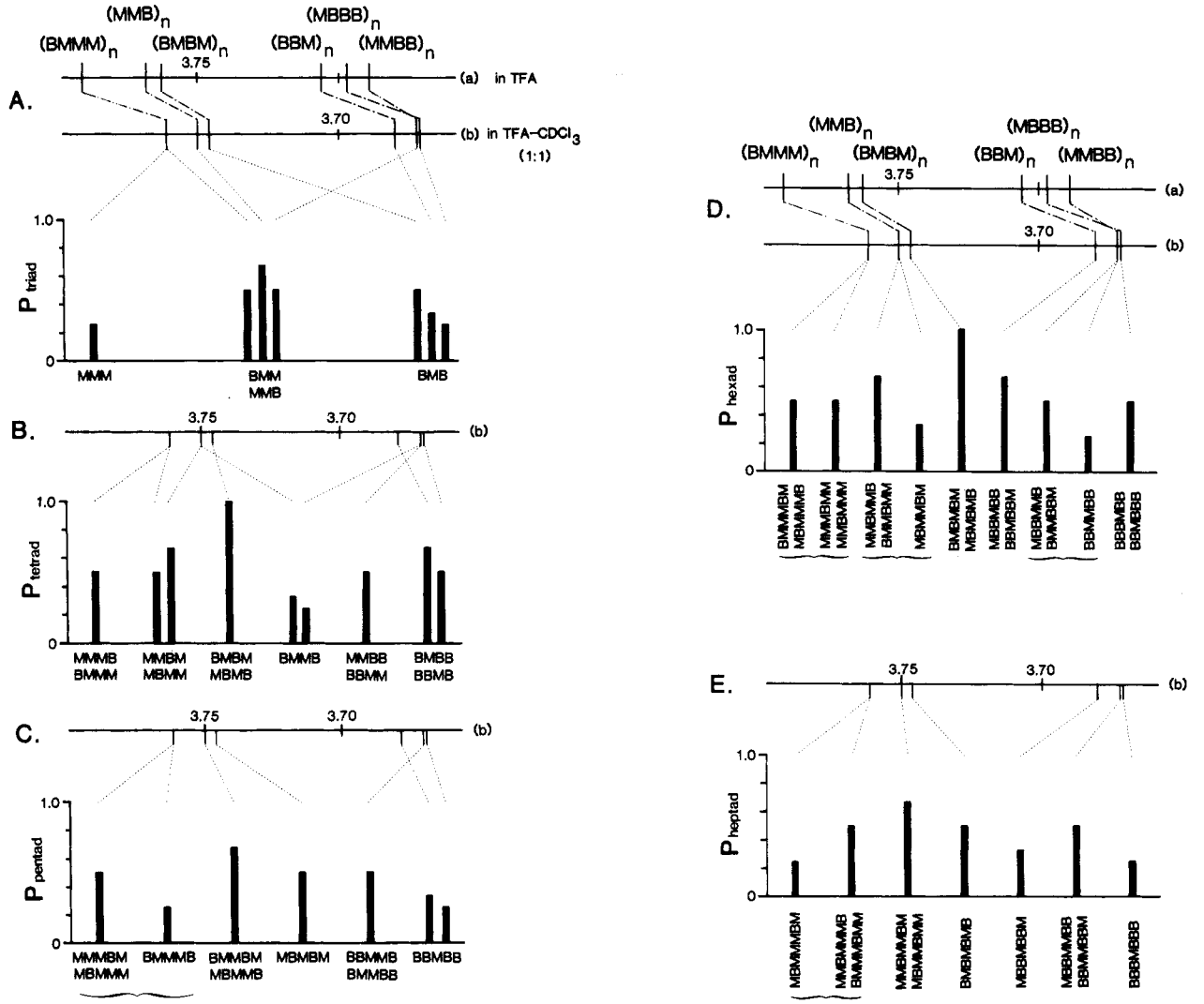

Figure 4. Diagram of the observed ^{1}H chemical shifts of the OCH_3 peak for sequential copolymers in solution (upper part), and the calculated peak intensities (sequence probability) of the B, M sequence for the sequential copolymers (bottom part): (A) triad sequence; (B) tetrad; (C) pentad; (D) hexad; (E) heptad.

TFA, suggesting the transition midpoint will be affected by the B, M sequence.

In the range of 30–100% TFA content, the chemical shifts of individual signals change gradually with increasing TFA content, all of which are fully random-coil supporting solvents for the B, M copolymers used here. Thus, this behavior is attributable to the solute–solvent interaction. The same result as for (BBM) is obtained for the other sequential copolymers in the TFA/$CDCl_3$ mixtures (10–100% TFA). Further, the chemical shifts of all proton signals except for the OCH_3 do not change among the sequential copolymers in TFA and in TFA/$CDCl_3$ (1:1) (figure not shown).

Amino Acid Sequence Analysis by ^1H NMR. Figure 3 shows the chemical shift of the OCH_3 peak and M composition in TFA (A) and in TFA/$CDCl_3$ (1:1) (B) for various kinds of sequential copolymers. It appears that the influence of solute–solvent interaction on the chemical shift of the OCH_3 peak is small, whereas this peak in TFA moves slightly downfield compared with that in TFA/$CDCl_3$ (1:1). These experimental results can be summarized as follows: the ^1H chemical shifts of the OCH_3 peak in the $(MBBB)_n$, $(BBM)_n$, and $(MMBB)_n$ appear at the upper field region, and those in the $(BMBM)_n$, $(MMB)_n$, and $(BMMM)_n$ at the lower field region. This trend is found to hold without exception over a wide range of TFA contents (20–100%). These chemical shift displacements of the OCH_3 proton are therefore by no means attributable to the difference of the molecular weights of copolymers.[9] Moreover, following detailed optical characterization of the samples using ^1H and ^{13}C NMR in solution, ^{13}C CP-MAS (cross polarization–magic angle spinning) NMR in the solid state, and far-IR spectroscopy, no evidence for optical impurities was found.[9]

On the basis of the chemical shift behavior of the OCH_3 proton, the sequence analysis of the sequential copolymers in solution was examined. Figure 4 illustrates the chemical shift of the OCH_3 peak for sequential copolymers in solution, and the calculated peak intensities of the B, M sequence for the sequential copolymers. In sequence analysis, the following points are assumed: (i) the chemical shift of the OCH_3 proton at the C-side neighboring amino acid residue of the sequence is identical with that at the N-side neighboring one; for example, $P_{MMBM} = P_{MBMM}$ for the tetrad sequence, where P is the probability (fraction), $P_{BBMMB} = P_{BMMBB}$ for the pentad sequence, and so on; (ii) the sequence probability is calculated directly from the known B, M sequence of the copolymers by the equation P = (the number of any specified sequence)(fraction of M residue)/(the number of total sequences); (iii) the chemical shifts of the OCH_3 peak for the copolymers containing the BB sequence in the neighborhood of M appear at a lower field than those containing the BM (or MB) and MM sequences. From Figure 4A, the experimental results cannot be explained by considering a triad sequence, where the upper part of the figure is the observed stick spectrum and the bottom part is the calculated one. A similar difficulty is encountered when an attempt is made to interpret the data on the basis of a tetrad sequence (Figure 4B). This implies that a long-range chemical shift effect is observed for the side chains in the copolymers consisting of B and M. When a pentad sequence was used in the assignment, it was found that the chemical shift displacement observed for the copolymers (except for the $(MMBB)_n$ and $(BBM)_n$) can reasonably explain the calculated sequence probability (Figure 4C). Eventually, it was noted that the observed chemical shift agrees qualitatively with that of the calculated sequence assignment

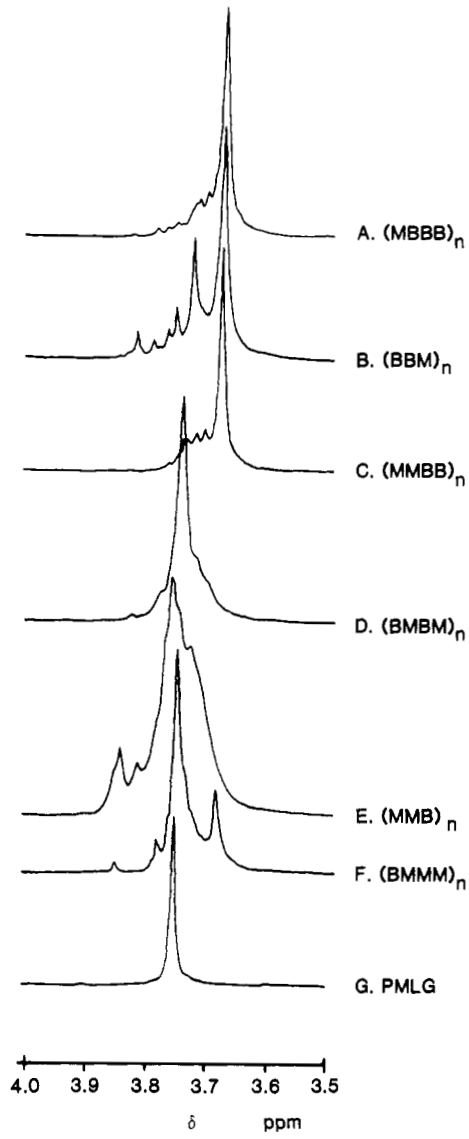

Figure 5. 500-MHz ^1H NMR spectra of the OCH_3 peak of various sequential copolymers in TFA/$CDCl_3$ (1:1); polymer concentration = 5 wt/vol %.

by assuming the hexad and/or heptad sequences, as shown in Figure 4D,E. Such significant chemical shift displacements of the OCH_3 peak of the M residue in the copolymers are probably due to the ring current effect of the phenyl groups of the B residue in the neighborhood. A large upfield shift is often observed when a resonating proton is located on the phenyl ring. It is surprising to note that the ^1H chemical shift of the OCH_3 peak for the B, M alternate copolymer $(BMBM)_n$ is in the lower field region and that of the $(MMBB)_n$ being BB, MM bialternate copolymer is in the upper field region. The above result is explained by assuming hexad sequences, as shown in Figure 4D, and it is difficult to explain the data by a triad, tetrad, or pentad sequence assignment. In particular, it is worth noting that the chemical shift of the OCH_3 peak for the copolymer with the BM (or MB) sequence in the neighborhood, $M–B_n–M_m–B_n–M$ (m = 1, 2, and 3; n = 1), appears at the lower field region, and that of the copolymer with the BB sequence in the neighborhood, $M–B_n–M_m–B_n–M$ (m = 1 and 2; n = 2 and 3), appears at the upper field region.

The above result is confirmed by the 500-MHz ^1H NMR spectra (Figure 5). Some minor peaks are observed in the 500-MHz NMR spectra although the cause is not clear at present. However, optical impurities are not likely to be

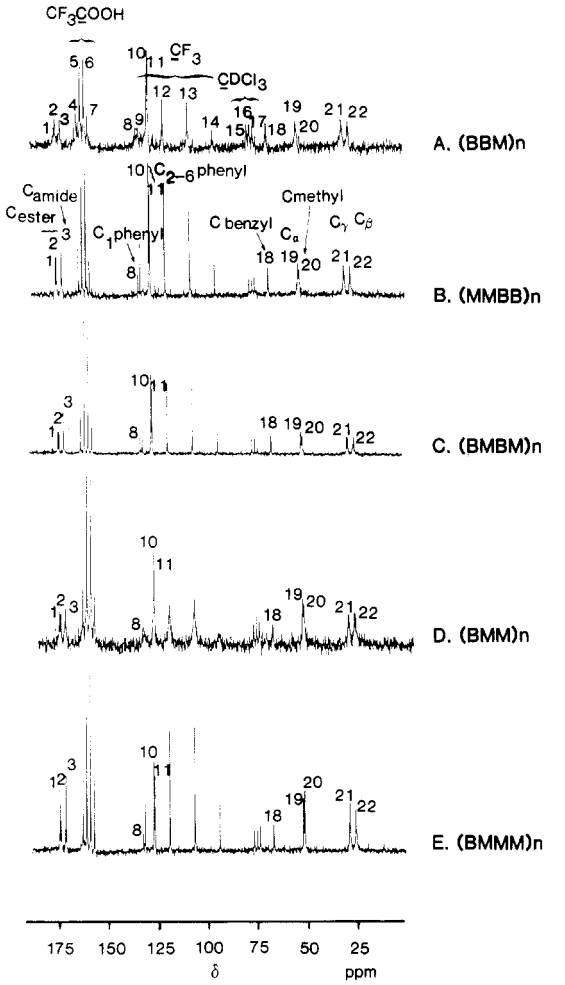

Figure 6. 25-MHz ^{13}C NMR spectra of various sequential copolymers in TFA/$CDCl_3$ (1:1); concentration = 20 wt/vol %; 2000–8000 scans. Peak assignments: 1, $C{=}O_{ester}$ (M); 2, $C{=}O_{ester}$ (B); 3, $C{=}O_{amide}$; 4–7, (quartet) C=O (TFA); 8, $C_{1\ phenyl}$; 10 and 11, $C_{2\text{-}6\ phenyl}$; 9 and 12–14, (quartet) CF_3 (TFA); 15–17, (triplet) $CDCl_3$; 18, C_{benzyl}; 19, C_α; 20, C_{methyl}; 21, C_γ; 22, C_β.

responsible for these minor peaks. Thus, it can be presumed that long-range chemical shift effects occur in the side chains in addition to the main effect of sequence or local conformation.

^{13}C NMR Spectra. Figure 6 shows 25-MHz ^{13}C NMR spectra of a series of sequential copolymers consisting of B and M in a mixed solvent (TFA/$CDCl_3$ (1:1)). Signals which come from the B and M residues are identified and assigned, based on the M composition dependence of the peak intensity in the sequential copolymers and also the reported data for the homopolymers of PBLG[10,11] and PMLG.[12]

Figure 7 shows a plot of the chemical shift for one of the sequential copolymers, $(BBM)_n$, with changes in TFA content. It is found that the ^{13}C chemical shifts of all carbon atoms are almost constant and do not change with the TFA content in the range of 20–100%, where the polymer takes a random-coil conformation.[10,11] On the other hand, the chemical shifts of some carbon peaks (C_{ester}, C_{amide}, C_{benzyl}, C_α, C_{methyl}, and C_β) change significantly in the range below 20% TFA. This is probably due to the conformational transition from a coil to a helical form, which occurs below 10% TFA. Such a trend is in good agreement with the ^1H chemical shift described previously. In addition, the magnitudes of the chemical shift differences ($\delta_{20\%\ TFA} - \delta_{5\%\ TFA}$) for the C_{amide}, C_α, C_β, and C_γ carbons in the $(BBM)_n$ are smaller by half than those in PBLG (or PMLG). However, the magnitude of the

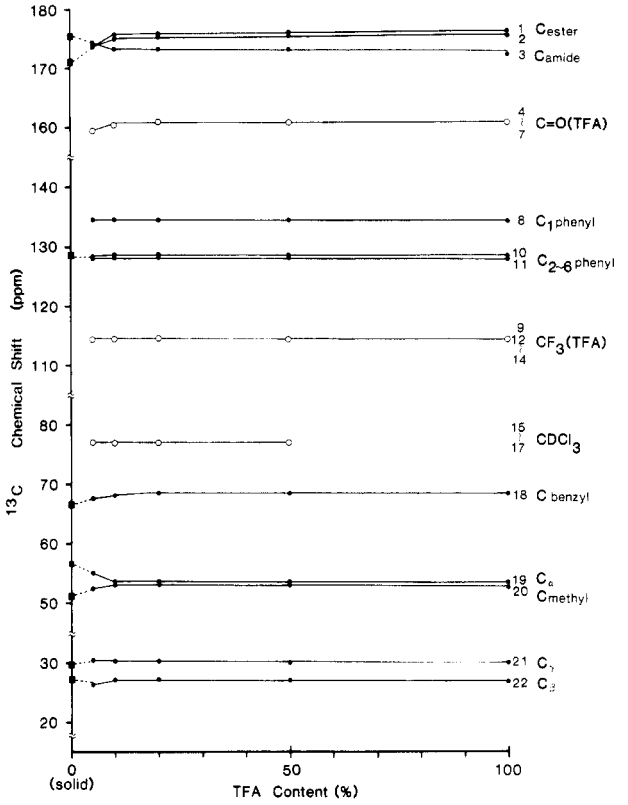

Figure 7. ^{13}C chemical shifts (25 MHz) of the sequential copolymer $(BBM)_n$ as a function of TFA content (%) in the TFA/$CDCl_3$ mixtures. The symbol ○ denotes a chemical shift of the solvent, and the symbol ■ denotes ^{13}C chemical shifts of the $(BBM)_n$ in the solid state, as determined by the 75.46-MHz ^{13}C cross polarization–magic angle spinning (CP-MAS) NMR spectra (Bruker CXP 300).[9]

chemical shift difference for the C_{ester} and C_{benzyl} (or C_{methyl}) carbons in the $(BBM)_n$ are equal to or larger than those in PBLG (or PMLG). The chemical shifts of the $(BBM)_n$ in the solid state obtained by the ^{13}C CP-MAS NMR method are shown in Figure 7.[9] These shifts, it is suggested, are comparable to the chemical shifts in $CDCl_3$ solution (in which the polymer is only slightly soluble). This means that the polymer in $CDCl_3$ solution takes the same conformation as in the solid state. Moreover, the individual magnitude of the chemical shift differences ($\delta_{20\%\ TFA} - \delta_{solid\ state}$) in Figure 7 is almost the same as that of PBLG (or PMLG), except for the side-chain C_{ester} and C_{benzyl} (or C_{methyl}) peaks mentioned above. Results similar to the $(BBM)_n$ data are obtained for other sequential copolymers in the same solvent systems. The above results suggest that the side-chain conformation (and side-chain interaction) is different for the sequential copolymer and its homopolymer PBLG (or PMLG). This implies that the electronic nature of amino acid residue can be detected by the ^{13}C chemical shifts since the ^{13}C nuclei are sensitive to electron density. This is in contrast to the ^1H chemical shifts which are very sensitive to surroundings such as ring current effects by phenyl groups. Moreover, the ^{13}C shift of C=O (TFA) is displaced upfield due to helix–coil transition, whereas the CF_3 signal of TFA and the $CDCl_3$ signal are kept constant.

Amino Acid Sequence Analysis by ^{13}C NMR. Figure 8 shows 125-MHz ^{13}C NMR spectra of a series of sequential copolymers consisting of B and M in a mixed solvent (TFA/$CDCl_3$ (1:1)). It is noted that the peaks have a much better definition compared with the 25-MHz ^{13}C spectra.

The ^{13}C chemical shifts of these signals in TFA/$CDCl_3$ (1:1) with different M compositions are shown in Figure

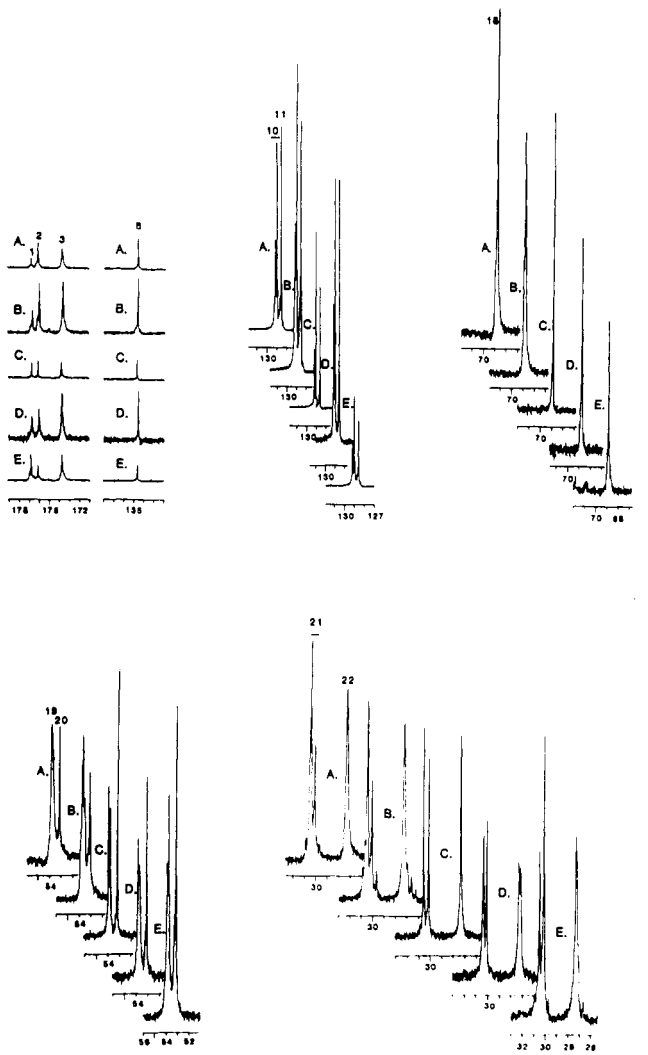

Figure 8. Partial ^{13}C NMR spectra (125 MHz) of various sequential copolymers in TFA/$CDCl_3$ (1:1); concentration = 20 wt/vol %: A, $(MBBB)_n$; B, $(BBM)_n$; C, $(MMBB)_n$; D, $(BMBM)_n$; E, $(BMMM)_n$. Peak assignments: 1, $C{=}O_{ester}$ (M); 2, $C{=}O_{ester}$ (B); 3, $C{=}O_{amide}$; 8, C_1 phenyl; 10 and 11, $C_{2\text{-}6\ phenyl}$; 18, C_{benzyl}; 19, C_α; 20, C_{methyl}; 21, C_γ; 22, C_β.

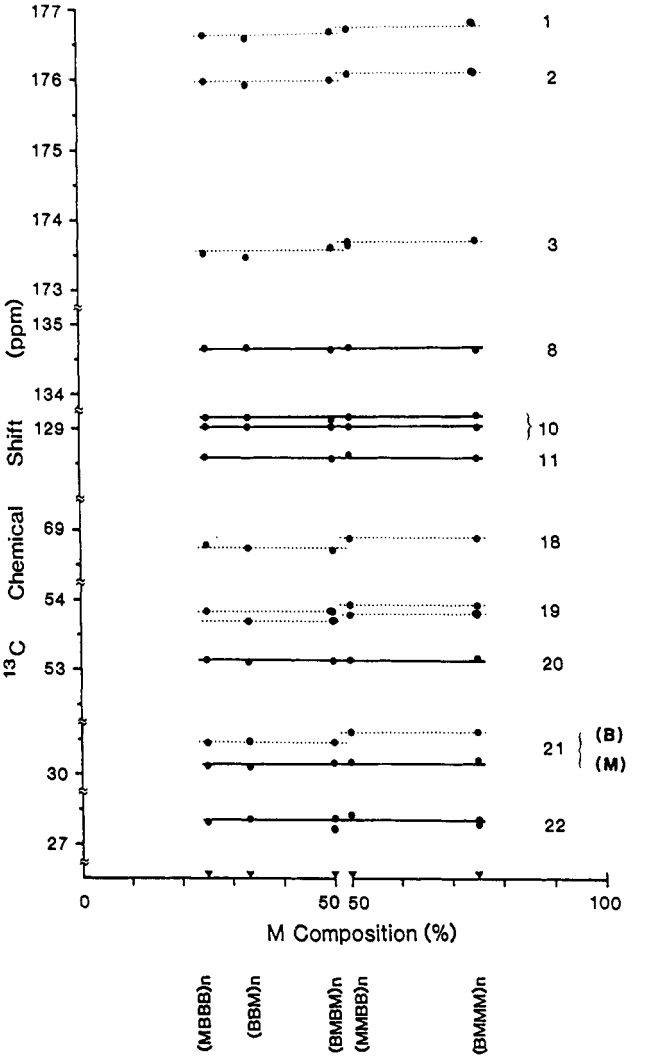

Figure 9. ^{13}C chemical shifts (125 MHz) of the various sequential copolymers as a function of M composition (%) in TFA/$CDCl_3$ (1:1).

9. It is found that some chemical shifts for various copolymers, especially for most of the ^{13}C peaks of the M residue and the C_{phenyl} peak of the B residue, are nearly constant and these peaks seem to be independent of M composition and B, M sequence. On the contrary, the C=O, C_{benzyl}, C_α, and C_γ peaks (depicted by dashed lines in Figure 9) are very sensitive to M composition and B, M sequence, and they seem to be separated into two classes. Thus, it is concluded from the chemical shift of the C_{benzyl} peak that the copolymers having MM sequence, $(MMBB)_n$ and $(BMMM)_n$, are in the lower field region, and those having BM (or MB) and BB sequences, $(MBBB)_n$, $(BBM)_n$, and $(BMBM)_n$, are in the upper field region. In view of this, a chemical shift displacement similar to that for the C_{benzyl} peak is observed for all the peaks except for $C{=}O_{amide}$ and C_α mentioned above. However, the $C{=}O_{amide}$ and C_α peaks of $(MBBB)_n$ are in a lower field than those of $(BBM)_n$. These results indicate that the ^{13}C chemical shift behavior can be explained by assuming a pentad or higher order sequence. That is, for all the ^{13}C peaks (except for C_{phenyl}) of the B residue, the copolymer $B\text{-}M_m\text{-}B_n\text{-}M_m\text{-}B$ (m = 2 and 3; n = 1 and 2) appears in the lower field region, and that of the copolymer $B\text{-}M_m\text{-}B_n\text{-}M_m\text{-}B$ (m = 1; n = 1–3) appears in the upper field region. It is, as a result, particularly interesting to note that the ^{13}C NMR has provided significant information regarding B, M sequence determination from the ^{13}C chemical shifts of the B residue. ^1H NMR has provided information regarding B, M sequence from OCH_3 proton peaks of the M residue while ^{13}C NMR has provided similar information but from the carbon peaks of the B residue. Moreover, it is notable that there is a significant chemical shift displacement of $C{=}O_{ester}$, $C{=}O_{amide}$, C_{benzyl}, C_α, C_β, and C_γ peaks between $(BMBM)_n$ and $(MMBB)_n$, which is strong evidence in support of B, M sequence determination in the copolymers by ^{13}C NMR.

In conclusion, as ^{13}C chemical shifts are closely related to the interaction between the polymer and solvent molecules as well as the local conformations and electronic nature of the amino acid residue, it is very important to select an appropriate sequential copolymer as a model to establish sequence determination analysis of polypeptides and proteins with the ^{13}C NMR method.

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Registry No. $(MBBB)_n$ (homopolymer), 90696-46-1; $(MBBB)_n$ (SRU), 90696-58-5; $(BBM)_n$ (homopolymer), 90696-48-3; $(BBM)_n$ (SRU), 90696-59-6; $(MMBB)_n$ (homopolymer), 90696-50-7; $(MMBB)_n$ (SRU), 90696-60-9; $(BMBM)_n$ (homopolymer), 90696-52-9; $(BMBM)_n$ (SRU), 90696-61-0; $(MMB)_n$ (homo-

polymer), 90696-54-1; $(MMB)_n$ (SRU), 90696-62-1; $(BMMM)_n$ (homopolymer), 90696-56-3; $(BMMM)_n$ (SRU), 90696-63-2.

References and Notes

(1) Bovey, F. A. "High-Resolution NMR of Macromolecules"; Academic Press: New York, 1972.
(2) Croft, L. R. "Handbook of Protein Sequence Analysis", 2nd ed.; Wiley: New York, 1980; p 1-152.
(3) (a) Bradbury, J. H.; Crompton, M. W.; Warren, B. *Anal. Biochem.* **1974**, *62*, 310. (b) Warren, B.; Bradbury, J. H. *J. Polym. Sci., Symp.* **1975**, *No. 49*, 65.
(4) (a) Schilling, G.; Kricheldorf, H. R. *Makromol. Chem.* **1977**, *178*, 885. (b) Hull, W. E.; Kricheldorf, H. R. *J. Polym. Sci., Lett. Ed.* **1978**, *16*, 215. (c) Kricheldorf, H. R.; Mang, T., *Makromol. Chem.* **1981**, *182*, 3077. (d) Stulz, J.; Müller, D.; Hull, W. E.; Kricheldorf, H. R. *Makromol. Chem.* **1983**, *184*, 1311.
(5) Hiraoki, T.; Tsutsumi, A.; Hikichi, K.; Kaneko, M. *Polym. J.* **1976**, *8*, 429.
(6) Bamford, C. H.; Elliott, A.; Hanby, W. E. "Synthetic Polypeptides"; Academic Press: New York, 1956.
(7) Wüthlich, K. "NMR in Biological Research: Peptides and Proteins"; North-Holland Publishing Co.: 1976; Chapters 3 and 5.
(8) (a) Shoji, A.; Kawai, T.; Nishioka, A. *Macromolecules* **1977**, *10*, 1292. (b) Shoji, A.; Kawai, T.; Nishioka, A. *Makromol. Chem.* **1978**, *179*, 611.
(9) Shoji, A.; Ozaki, T.; Saitô, H.; Tabeta, R.; Ando, I. *Macromolecules* **1984**, *17*, 1472.
(10) Suzuki, Y.; Inoue, Y.; Chûjô, R. *Biopolymers* **1975**, *14*, 1223.
(11) Paolillo, L.; Tancredi, T.; Temussi, P. A.; Trivellone, E.; Bradbury, E. M.; Crane-Robinson, C., *J. Chem. Soc., Chem. Commun.* **1972**, 335.
(12) Suzuki, Y.; Inoue, Y.; Chûjô, R., *Makromol. Chem.* **1980**, *181*, 165.

β-Helical Structure of D,L-Alternating Oligophenylalanines with Terminal Butyloxycarbonyl and Methoxy Groups in Chloroform: Comparison with Oligovalines

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ABSTRACT: This paper reports on a ^1H NMR study of the members II-X (n = 2-10) and XV (n = 15) of the series Boc-(L-Phe)$_m$-(D-Phe-L-Phe)$_{(n-m)/2}$-OMe (n = number of residues in the oligopeptide; m = 0 or 1) in chloroform solution at 25 °C. It is shown that there is a species that is strongly preferred by the oligophenylalanines with seven or more residues and that this species is a dimer with the structure of a right-handed $\uparrow\downarrow\beta^{5.6}$-helix with $2(n-1)$ interstrand H bonds. In the case of XV this species is virtually the only one occurring. The preference of these D,L-alternating oligophenylalanines for a $\uparrow\downarrow\beta^{5.6}$-helix contrasts with the behavior of the corresponding oligovalines, which, as it has been observed in earlier studies, form preferentially $\beta^{4.4}$-helices in chloroform. The role of the nature of the side chains in determining this different behavior is discussed.

Introduction

There is very little in the literature[1,2] regarding the influence of the nature of the side chains on the conformational tendencies of D,L-alternating peptides. In particular, there are only hints at the possibility that the nature of the side chains may determine preferences for specific β-helical structures. We are investigating this influence by systematically studying different series of D,L-alternating cooligopeptides formed by enantiomeric or diastereomeric amino acid residues (stereocooligopeptides[3]). Here we report on a ^1H NMR study of the members II-X (n = 2-10) and XV (n = 15) of the series Boc-(L-Phe)$_m$-(D-Phe-L-Phe)$_{(n-m)/2}$-OMe (n = number of residues in the oligopeptide; m = 0 or 1) in chloroform solution at 25 °C and compare the results obtained with the behavior observed in earlier studies[4-7] for members of a similar series derived from valine.

Experimental Section

Syntheses. II-X and XV. The oligophenylalanines II-X were synthesized following the stepwise procedure outlined in Chart I. HCl·H-L-Phe-OMe was prepared by the method of Boissonnas et al.[8] and was purified by recrystallization from MeOH/Et_2O: mp 160-162 °C, $[\alpha]^{20}_D$ +38.7° (c 2, EtOH) (lit.:[9] mp 159-161 °C, $[\alpha]_D$ +38.7° (c 2.9, EtOH)). Boc-D-Phe-OH, mp 85-87 °C, $[\alpha]^{20}_D$ -24.4° (c 1, EtOH), and Boc-L-Phe-OH, mp 84-86 °C, $[\alpha]^{20}_D$ +24.8° (c 1, EtOH), $[\alpha]^{20}_D$ -4.2° (c 1, AcOH) (lit.:[10] mp 84-86 °C, $[\alpha]^{18-25}_D$ -4.0° (c 1, AcOH)) were synthesized by using the di-*tert*-butyl dicarbonate method (method A)[11] and were purified by recrystallization from EtOAc/*n*-hexane. The Boc group was removed in all cases by treatment with trifluoroacetic acid. The mixed-anhydride method with isobutyl chloroformate as the mixed-anhydride-forming reagent[12] was used in each coupling step. The general procedure for the coupling reactions was the same

Table I
Crystallization Solvents, Melting Points, and Elemental Analysis Data for the Oligophenylalanines II-X and XV

oligopeptide	recrystn solvents	mp, °C	elemental analysis[a] % C	% H	% N
II	$CHCl_3$/petroleum ether	131-132[b]	67.59	7.09	6.57
			67.56	6.98	6.53
III	MeOH	175-176	69.09	6.85	7.33
			69.00	6.84	7.30
IV	MeOH/H_2O	183-184	69.98	6.71	7.77
			69.80	6.81	7.66
V	MeOH	188-190	70.57	6.62	8.07
			70.48	6.64	8.03
VI	MeOH/H_2O	175-180	70.99	6.55	8.28
			70.42	6.43	8.15
VII	MeOH/H_2O	190-192[c]	71.30	6.50	8.43[d]
			70.00	6.49	8.27
VIII	$CHCl_3$/MeOH	150-165[c]	71.54	6.47	8.56
			71.04	6.47	8.48
IX	$CHCl_3$/MeOH/H_2O[e]	>240[c]	71.73	6.43	8.65[f]
			70.13	6.30	8.41
X	$CHCl_3$/MeOH	>240	71.89	6.41	8.73
			71.65	6.50	8.78
XV	$CHCl_3$/MeOH	>240	72.38	6.33	8.98
			72.20	6.32	8.98

[a] First line, calcd; second line, found. [b] Lit.[14] 127-130 °C. [c] Efflorescent crystals. [d] C/N, calcd, 8.46; found, 8.46. [e] The amount of water relative to that of the other two solvents was very small. [f] C/N, calcd, 8.24; found, 8.34.

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