^{13}C NMR Spectroscopy of Alternating Poly(γ-benzyl D–L-glutamate) in α- and Double-Helical Conformations

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ABSTRACT: Carbon-13 NMR spectra of α single-helical and $\pi\pi_{DL}$ double-helical conformations of alternating poly(γ-benzyl D–L-glutamate) are presented. In both cases, the existence of doublet for the backbone carbon atoms is consistent with D and L residues being in different conformational states when engaged in the same helix. The resonances of the C_α atoms in both helices, which are found at identical chemical shifts, and comparison with the ^1H NMR observation, favor the hypotheses that solvent molecules are located inside the helical core of the double helices.

Polypeptides built with an alternation of D and L residues offer a wide range of conformational possibilities among which are the α and π_{DL} single-stranded helices[1] and double-stranded helices.[2] For alternating poly(γ-benzyl D–L-glutamate) (PBD–LG) two single-stranded helices (α_{DL} and $\pi_{DL}{}^{4.4}$)[3] and four double helices ($\pi\pi_{DL}{}^{5.6}$, $\pi\pi_{DL}{}^{7.2}$, $\pi\pi_{DL}{}^{9.0}$, and $\pi\pi_{DL}{}^{10.8}$)[4] have been identified in the solid state. The $\pi_{DL}{}^{4.4}$ and the double helices are conformations which are specific to alternating poly(D–L-peptides). The L and D residues have conformational angles in the region of β structures of poly(L-peptides) and poly(D-peptides), respectively. NH and carbonyl groups point in both directions of the helical axis and hydrogen bonds are intrachain ($\pi_{DL}{}^{4.4}$ helix) or interchain (double helices) as illustrated on Scheme I. Except for the $\pi\pi_{DL}{}^{5.6}$ helix which appears unstable in the presence of solvent, all these conformations have been intensively studied in solution using mainly infrared, CD,[5,6] and ^1H NMR[7,8] techniques. The ^1H NMR investigations revealed that the α helix of PBD–LG is characterized by the existence of two α-C*H* peaks at 3.65 and 3.82 ppm (single resonance at 3.92 ppm for PBLG) while the $\pi_{DL}{}^{4.4}$ helix and the double helices ($\pi\pi_{DL}{}^{7.2}$ and $\pi\pi_{DL}{}^{9.0}$) show α-C*H* peaks lying at unusually low field positions (4.45 and 5.40 ppm for the π_{DL} and $\pi\pi_{DL}$ helices, respectively). The downfield shift of the α-C*H* resonance, when going from the α to $\pi\pi_{DL}$ helices, is also accompanied by modifications of the resonance of the protons of the side chains but the complexity of the β- and γ-CH_2 region makes interpretation difficult. In order to gain more information on the characteristics specific to these different helices, especially on the role of the side chains and on possible local deformations of the backbone, a ^{13}C NMR study on α- and $\pi\pi_{DL}$-helical conformations of PBD–LG was undertaken. Unfortunately, since high concentrations are needed for these experiments, the α → π_{DL} transconformation (which occurs at low polymer concentration) could not be observed as double helices are formed simultaneously.[6]

Experimental Section

The PBD–LG sample used in this study was prepared as described in Caille et al.[9] $[\eta]^{25}_{DCA}$ = 12.0 mL g^{-1}. The α → $\pi\pi_{DL}{}^{5.6}$ transconformation was achieved by heating the sample for 3 h at 200 °C and the transconformation was checked by infrared spectroscopy. All spectra were recorded on 150 mg/mL solutions at 35 ± 2 °C on a Bruker WH 90 spectrometer working in the FT mode with proton

Scheme I

Hydrogen Bonding Pattern in (a) α Helix, (b) $\pi_{DL}{}^{4.4}$ Helix, and (c) $\pi\pi_{DL}{}^{5.6}$ Helix with Antiparallel Strands

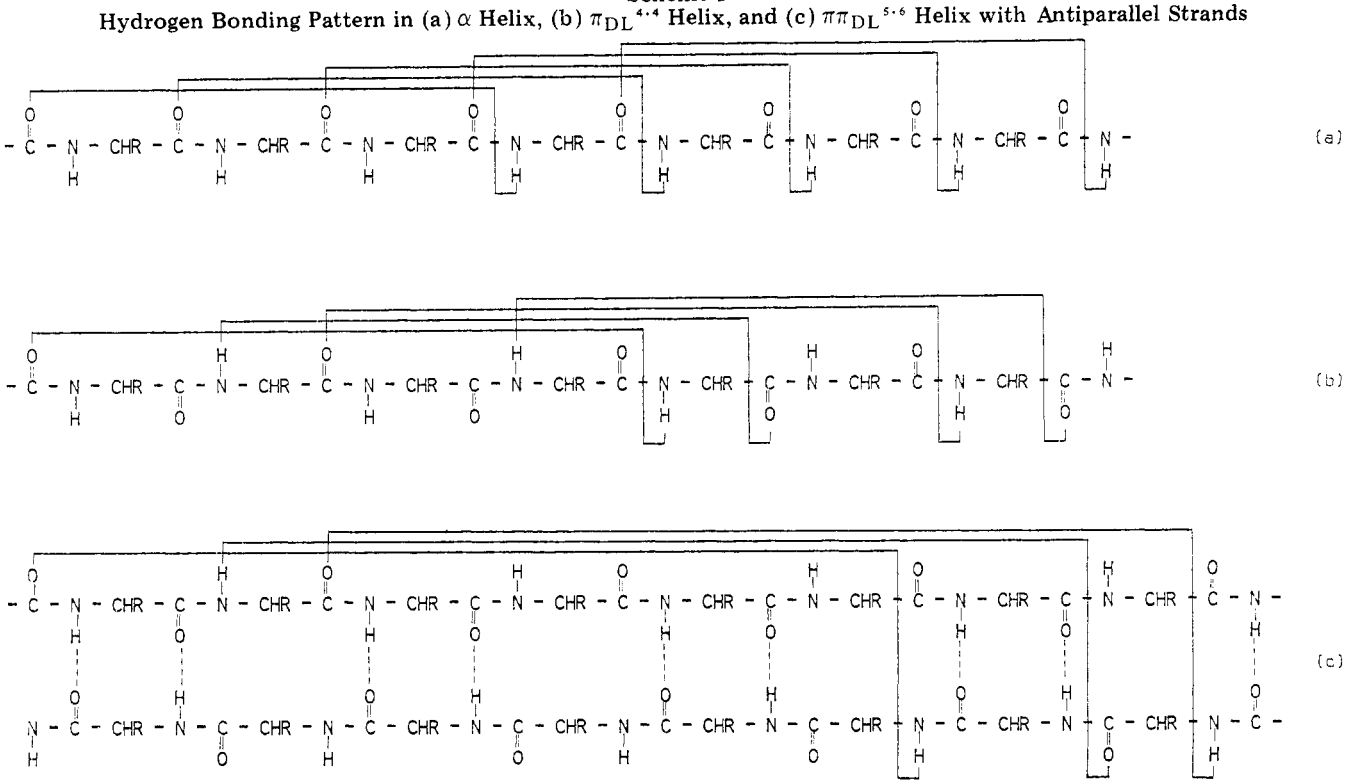

Table I
^{13}C Chemical Shifts in ppm from Internal Me_4Si of PBD-LG in Various Conformations (PBLG is Given for Comparison Purpose)

	C_β	C_γ	C_α	C_{B_z}	$C_{2,6\text{-}A_r}$	$C_{1\text{-}A_r}$	C=O ester	C=O amide
α-Helical PBLG[10] (CDCl$_3$, 3% TFA v/v)	25.9	30.7	56.3	65.9	126.6 127.1	133.6	170.5	173.1
α-Helical PBD-LG (CDCl$_3$, 1.5% TFA v/v)	22.6 25.55	30.05	53.0 56.55	66.25	127.7 128.0	135.4	171.9 172.7	172.4 174.4
α-Helical PBD-LG (dioxane-d_8)	23.6 26.7	30.1	53.0 57.4	Obscured by the solvent	128.2	136.8	172.0 172.6	173.2 175.1
Random-coil PBLG[10] (CDCl$_3$, 29% TFA v/v)	27.1	30.6	53.2	67.6	126.6 127.1	133.6	173.2	171.1
Random-coil PBD-LG (CDCl$_3$, 12% TFA v/v)	26.5	30.3	53.6	67.5	127.7 128.1	134.2	174.1	172.5
$\pi\pi_{DL}$[7,2] helix of PBD-LG (CD_2Cl_2)	(~30)	30.1	Obscured by the solvent	66.1	128.1	136.4	171.9	(~171)
$\pi\pi_{DL}$[9,0] helix of PBD-LG (dioxane-d_8)	(~30)	29.6	53.0 57.4	Obscured by the solvent	128.3	137.0	171.9	170.8 171.2

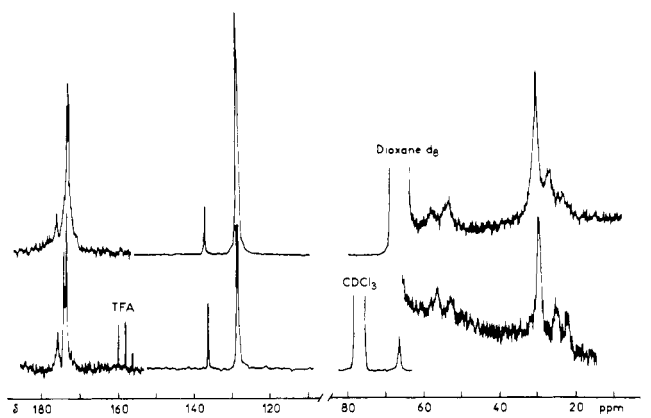

Figure 1. ^{13}C NMR spectra of α-helical PBD-LG. Lower spectrum in CDCl$_3$/1.5% TFA (v/v). Upper spectrum in dioxane-d_8. The spectra are shown at various vertical expensions.

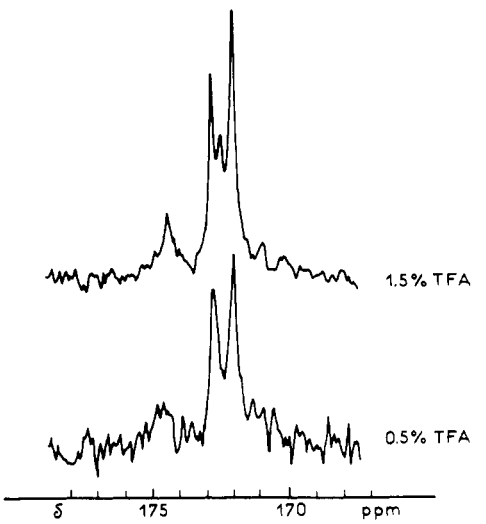

Figure 2. Expended spectra of the carbonyl region for the α-helical conformation in CDCl$_3$/0.5% TFA (v/v) and CDCl$_3$/1.5% TFA (v/v).

noise decoupling. The spectrometer was locked on the deuterium resonance of the solvent (CDCl$_3$, CD_2Cl_2, or dioxane-d_8) and Me_4Si was used as internal reference.

Results and Discussion

(I) α Helix. The ^{13}C NMR spectrum at 22.63 MHz of α-helical PBD-LG in dioxane-d_8 closely resembles that in CDCl$_3$ (1.5% TFA, v/v) (see Figure 1 and Table I) and the resonances can be attributed by analogy with PBLG.[10-12] These spectra are mainly characterized by doublets for the resonances of both the C_α and C_β atoms. Also, from the spectrum obtained at very low amounts of TFA (0.5%) the ester carbonyl resonance appears also to be a doublet (Figure 2). Similarly, a doublet for the amide carbonyl may exist but it appears less clearly owing to the overlapping of the resonances (Figure 2). By analogy with the ^1H NMR spectra of PBD-LG in the same conformational state,[7] which also revealed two α-C*H* peaks, the existence of two C_β peaks may be attributed to L (or D) residues in left (or right) handed α helices and to D (or L) residues in right (or left) handed α helices. Thus, as both helix senses are present,[5] the low-field C_β peak, which lies at nearly the same position as that of PBLG,[10-12] can be attributed to the residues in the "correct" helix sense (i.e., L residues in a right handed helix or D residues in a left handed helix) while the high field peak corresponds to residues in the "wrong" helix sense. However, the difference of chemical shifts of both C_β atoms is large compared to that of the α-CH protons.[7] This larger upfield shift of the C_β resonance attributed to the residues engaged in the "wrong" helix sense may be due to the vicinity of this atom with the amide carbonyl group of the same residue. This feature comes in addition to the fact that the environment of this atom differs from that of residues in their "correct" helix sense (see Figure 2 in ref 7). As to the two ester carbonyl resonances, they may arise from different side-chain conformations, one for the D (or L) residues and the other for the L (or D) ones. It must be noticed that all the carbon atoms of the side chains do not reflect (or are not sensitive to) these conformational differences.

The same assignment holds also for the two C_α peaks. The quite large differences of 3.55 ppm (in CDCl$_3$, 1.5% TFA) or 4.40 ppm (in dioxane-d_8) for the two α-carbon atoms may reflect local deformations of the backbone. This hypothesis is supported by energy minimization calculations which reveal that the two residues have different conformations and that the rise per repeat unit along the helical axis is unequally distributed between the two residues[4,13] (for example: $\phi_L = -60.0$, $\psi_L = -46.1$, $\phi_D = -44.4$, and $\psi_D = -60.0$; $h = 1.80$ and 1.17 Å for a right handed α helix[4]). This would also be in agreement with two amide carbonyl peaks.

(II) α-Helix-Coil Transition. Addition of 4 to 7% (v/v)

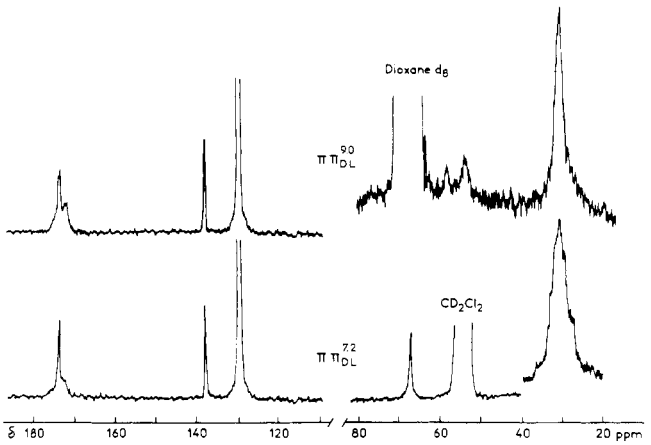

Figure 3. ^{13}C NMR spectra of double-helical PDB-LG. Lower spectum $\pi\pi_{DL}^{7.2}$ helix in CD_2Cl_2. Upper spectrum $\pi\pi_{DL}^{9.0}$ helix in dioxane-d_8. The spectra are shown at various vertical expensions.

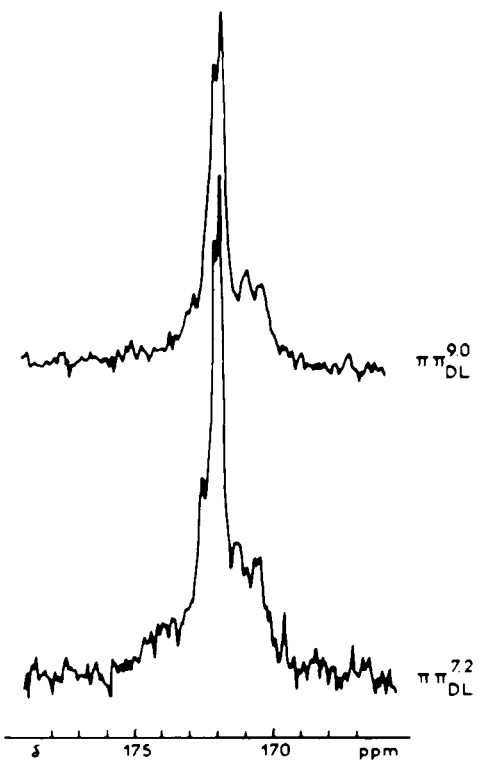

Figure 4. Expended spectra of the carbonyl region for the double-helical conformations.

TFA to a solution of PBD–LG in $CDCl_3$ leads to the α-helix–coil transition (note that the amount of TFA used to induce the transition is higher than that used for ^1H NMR observations, because the concentration of PBD–LG is about 20-fold higher). The general feature, especially for the C_α resonances, is that of a "double peak" behavior as described for the transition observed by ^1H NMR.[7] As for the ^1H NMR spectrum, for PBD–LG in the random coil form, all peaks are single and their chemical shifts are comparable to those of PBLG in the same conformation (Table I).

(III) Double Helices. As shown in a previous paper,[6] double-helical PBD–LG adopts the $\pi\pi_{DL}^{7.2}$ or $\pi\pi_{DL}^{9.0}$ helical conformations when dissolved in methylene chloride or dioxane, respectively. These double helices belong to the same family and have closely similar torsional angles.[4] It is not surprizing therefore that the ^{13}C spectra of double-helical PBD–LG in CD_2Cl_2 and in dioxane-d_8 are almost identical (Figure 3, Table I). Comparison with the spectra of α-helical PBD–LG reveals drastic changes in the carbonyl and C_β regions. Indeed, the C_β resonances of double-helical PBD–LG are below those of the C_γ atoms. Thus, the C_β resonances lie far downfield from that of the α helix (~3 ppm), which indicates widely different conformations of the side chains in α and $\pi\pi_{DL}$ helices. The situation in the carbonyl region is not clear enough to allow any firm conclusion. However, a single ester carbonyl peak (Figure 4) seems to indicate that, in $\pi\pi_{DL}$ helices, all side chains have the same or at least similar conformations. The amide carbonyl resonance is, again, a doublet (clearly seen for the $\pi\pi_{DL}^{9.0}$ helix (cf. Figure 4)) which lies upfield from the α-helix position, in agreement with a different backbone conformation in the two helices. The resonance of the C_α carbon atoms of the backbone can unfortunately be seen only for the $\pi\pi_{DL}^{9.0}$ helix; it is also a doublet which has the same chemical shift as that of the α helix. The existence of doublets for the C_α and amide carbonyl carbon atoms can again be interpreted as indicating that the conformations of the two residues of the dipeptide unit differ. It is surprizing, however, that the C_α resonances of the α and $\pi\pi_{DL}^{9.0}$ helices have the same chemical shifts. By ^1H NMR it was indeed shown that, when going from α to $\pi\pi_{DL}$ helices, the α-C*H* peaks are shifted downfield (from 3.65 and 3.82 to 5.40 ppm, respectively[7,8]), with an intermediate position (4.45 ppm) for the π_{DL} helix.

These downfield shifts for the double helices were first attributed to the existence of a β-type structure and to side-chain effects.[8] There are however strong similarities between π_{DL} and $\pi\pi_{DL}$ helices, especially with respect to the position of the α-C*H* atoms, and the differences cannot be accounted on this basis only. In the light of the present results, the downfield shift of the α-C*H* resonance observed by ^1H NMR from π_{DL} to $\pi\pi_{DL}$ helices may therefore more probably be due to different solvation states of these protons. The π_{DL} helix found for PBD–LG in solution is most probably the $\pi_{DL}^{4.4}$ helix which cannot incorporate solvent molecules in its core, while such solvent molecules can indeed be located inside the larger helix core of some $\pi\pi_{DL}$ helices.[4] The proximity of hydrogen bonds between antiparallel chains (see Scheme I) and the presence of these solvent molecules are sufficient to drastically modify the magnetic environment of the α-C*H* atoms in the $\pi\pi_{DL}$ helices as compared to the π_{DL} one. Besides the obvious fact that the observations are made on different atoms, the proposed explanation would account, at least in part, for the difference between the ^{13}C and ^1H NMR behaviors of PBD–LG.

Conclusion

^{13}C NMR investigations on alternating PBD–LG show that, when this polypeptide is in the α-helical conformation, the two enantiomers are distinguishable on the basis of their "correct" and "wrong" screw sense. Observation of doublets for the C_α, C_β, and carbonyl resonances is in line with energy analysis results which indicate that, for a given screw sense, the conformations (backbone and also side chain) of the L and the D residues are different. When PBD–LG is in a double-helical conformation, the side-chain spectrum reveals that the side-chain conformations differ from that of the α helix. The backbone resonances are interpretable on the same basis as for the α helix and the absence of variation of the chemical shift, from α to $\pi\pi_{DL}$ helices, for the C_α resonance is consistent with the presence of solvent molecules located inside the double-helix core.

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Conformational Studies of Poly(alanine)s in Dichloroacetic Acid by Nuclear Magnetic Resonance

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ABSTRACT: Proton nuclear magnetic resonance spectra were studied for monodisperse L-alanine oligopeptides (the dimer, trimer, tetramer, and nonamer) containing an *n*-butylamide group at the C-terminal residue and for the random and block copolymers of D- and L-alanines having sharp molecular weight distributions in dichloroacetic acid (DCA). It was found that the NH signals of poly(L-alanine)s and some copoly(D,L-alanine)s were split into three peaks in DCA, suggesting that these NH peaks reflect the conformation of the polymers. These NH peaks were assigned to the terminal helix (helix–coil junction) and the random-coil and the inner helix, respectively, from the lowest field. On the basis of the above assignment, the microconformations of poly(alanine)s in DCA were examined quantitatively. Moreover, it was concluded from the specific nature of the solvent that both the effects of the hydrophobic side chains of the polymers and the acidity of the solvent are most important for the formation of the helical conformation of poly(L-alanine).

The proton nuclear magnetic resonance (^{1}H-NMR) study of poly(amino acid)s in solution was reported for the first time by Bovey et al.[2] in 1959. Later, the NMR spectra of synthetic polypeptides and biological peptides were extensively applied to a study of their conformation.[3]

Recently, the conformational transitions of many polypeptides were studied in solution by ^{1}H-NMR spectroscopy, and the mechanisms of the helix–coil and β-coil transitions have been clarified to a considerable extent. However, microconformational studies by ^{1}H NMR, such as the studies of the tacticities of the synthetic vinyl polymers (i.e., poly(methyl methacrylate) and poly(vinyl acetate)), have been very few.

More recently, Paolillo et al.[4] measured the ^{1}H-NMR spectra of the block copolymers of benzyl L-glutamate and benzyl L-aspartate and observed double peaks for the helical α-CH signals. They assigned one of the double peaks to the right-handed helix of the benzyl L-glutamate block and the other to the left-handed helix of the benzyl L-aspartate block. We found that the cis and trans conformations of the amide bond [–N(CH_3)-CO–] of copoly(L-alanine, *N*-methyl-L-alanine)s are closely related to the second structures of the copolymers.[5]

In this report, keeping these results in mind, we attempted to clarify the microconformation of polypeptides in solution by the NMR method, using monodisperse oligo-L-alanines (the dimer, trimer, tetramer and nonamer) containing an *n*-butylamide group at the C-terminal residue, poly(L-alanine)s having sharp molecular weight distributions, and a series of random and block copolymers of D- and L-alanines.

It is well known that the poly(L-alanine) takes partially helical conformations in dichloroacetic acid (DCA)[6] and that its helix–coil transition occurs gradually over a wide range of temperatures or solvent compositions. Therefore, poly(L-alanine) is an ideal polymer for investigation of microconformations of the helix and random-coil parts of partially helical polymers.

Further, it is of interest in relation to the mechanism of the helix–coil transition why DCA is a helix-supporting solvent for poly(L-alanine), whereas this is a coil-supporting solvent for various other poly(amino acid)s.

From the above standpoints, we have studied the microconformation of various poly(alanine)s in DCA using the ^{1}H-NMR technique.

Experimental Section

Materials. L-Alanine (commercial), oligo-L-alanines, poly(L-alanine)s (PLA), poly(D-alanine) (PDA), and the random copolymers (PDLA-R) and block copolymers (PDLA-B) of D- and L-alanines were used.

A series of oligo-L-alanines containing an *n*-butylamide group at the C-terminal residue were synthesized as described earlier.[7] They are shown as follows: H-[NH-CH(CH_3)-CO]$_n$-NHCH$_2$CH$_2$CH$_2$CH$_3$ where n = 2, 3, 4, and 9, respectively.

Poly(L-alanine)s, poly(D-alanine) and the D,L-copolymers having various molecular weights and sharp molecular weight distributions[8] were obtained by the heterogeneous polymerization[9] of L- or D-alanine *N*-carboxyanhydride (NCA) and copolymerization of the NCAs in acetonitrile using *n*-butylamine as initiator, respectively. Table I shows the samples used in this experiment, the degree of polymerization, and the compositions of the copolymers.

Measurement of ^{1}H-NMR Spectra. We obtained high-resolution ^{1}H-NMR measurements over the temperature range from 25 to 100 °C with a concentration of 5 w/v % in DCA. We used a 100-MHz NMR apparatus (JNM-PS100) manufactured by JEOL.

Chemical shift is denoted by the δ value (ppm) from tetramethylsilane (TMS), which is the external standard and is corrected with regard to bulk magnetic susceptibility. Spin decoupling was applied at 25 °C, and a du Pont 310 analyzer was used for peak separation of the NMR spectra by assuming Lorentzian peaks.

Correction with Regard to Bulk Magnetic Susceptibility. By