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Articles

Polymerization of γ-Methylglutamate *N*-Carboxyanhydride Using Al−Schiff's Base Complexes as Initiators

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ABSTRACT: The polymerization of γ-methylglutamate *N*-carboxyanhydride (MeGluNCA) using Schiff's base/aluminum methoxide or isopropoxide (HAPENAlOMe or HAPENAlO^{i}Pr) at ambient temperature is reported. A range of polymers have been synthesized by varying the nature of the solvent and initiator concentrations. The initiation is shown to proceed through complexation of the nitrogen of an NCA to the aluminum atom. The coordinated NCA then undergoes a nucleophilic attack by the alkoxide with loss of carbon dioxide. Chain propagation takes place by nucleophilic attack of the bound amido onto C(5)═O of an NCA. The presence of a methoxy or isopropoxy ester group at the initiated chain end has been confirmed by MALDI−TOF analysis.

Introduction

Synthetic polypeptides[1] have been known for a long time, and their chemical synthesis has undergone extensive developments over the past three decades. However, they have not achieved widespread applications since their very high cohesive energy and absence of melting transition make them difficult to process. Some examples of practical interest are known in the biomedical field where, for instance, copolypeptides are used as wound dressings.[2] The most efficient approach for synthesizing polypeptides is the ring-opening polymerization of α-amino acid−*N*-carboxyanhydrides (NCAs).[3−7] A large range of initiators/catalysts have been used for that purpose, and these include primary amines (RNH_2), tertiary amines (R_3N), or alkoxides (RO^-). Initiators are generally classified into two categories: initiators that function via proton abstraction at the NH (Scheme 1a) and initiators that initiate polymerization by nucleophilic attack at the C(5) carbonyl (Scheme 1b).

Other initiator systems, mostly heterogeneous in character and based on transition metals, have been used for NCA polymerization, namely tributylphosphine/metal acetate complexes (metal = Ni, Co, Cr, Cd, Mg; PR_3: $M = 40{:}1$).[8,9] In addition, compounds such as dialkylzinc and trialkylaluminum were reported by Tsuruta et al.[10−12] to give rise to NCA polymerization. However, initiation and propagation mechanisms remain unresolved. Moreover, very long polymerization times were required to achieve moderate monomer conversion, and very low molar mass polymers were obtained.

More recently, Deming[13−15] has reported the controlled polymerization of NCA using transition metal−amine complexes (M_t = Ni, Cu, Pd, Co, Fe) which are soluble in the reaction medium. The livingness of the polymerization was evidenced notably with zerovalent Ni and Co, by narrow molar mass distributions and good correlation between experimental and theoretical molar masses.

We have reported in previous articles that a new family of Al−Schiff's base complexes, denoted as HAPENAlOR, are effective initiators for the controlled polymerization of lactides.[16,17] In the present work, we discuss their use as initiating systems for the ring-opening polymerization of MeGluNCA.

Experimental Section

Materials. Anhydrous dioxane sealed under nitrogen was purchased from Aldrich and used without further purification. Dichloromethane was refluxed over calcium hydride, distilled under nitrogen, and finally stored under argon. Toluene was refluxed under nitrogen, then distilled over sodium, and finally kept under argon. MeGluNCA was offered as gift by Flamel Technologies Co. (Lyon, France) and was used without further purification. It was synthesized from L-glutamic acid 5-methyl ester.

Synthesis of Initiators. The aluminum−Schiff's base initiators HAPENAlO^{i}Pr (**I**) and HAPENAlOMe (**II**) were synthesized according to procedures described previously.[16,17]

Homopolymerization of MeGluNCA. All the polymerizations were carried out in Schlenk tubes under argon in a glovebox. The monomer was first dissolved in solvent (dichloromethane or dioxane), and the calculated amount of initiator was then added. Polymerization was allowed to proceed at ambient temperature, and after the required time, the solvent was removed under vacuum and the percentage conversion was determined by ^1H NMR. The polymer was finally isolated after several washings with water and dried under vacuum at 40 °C.

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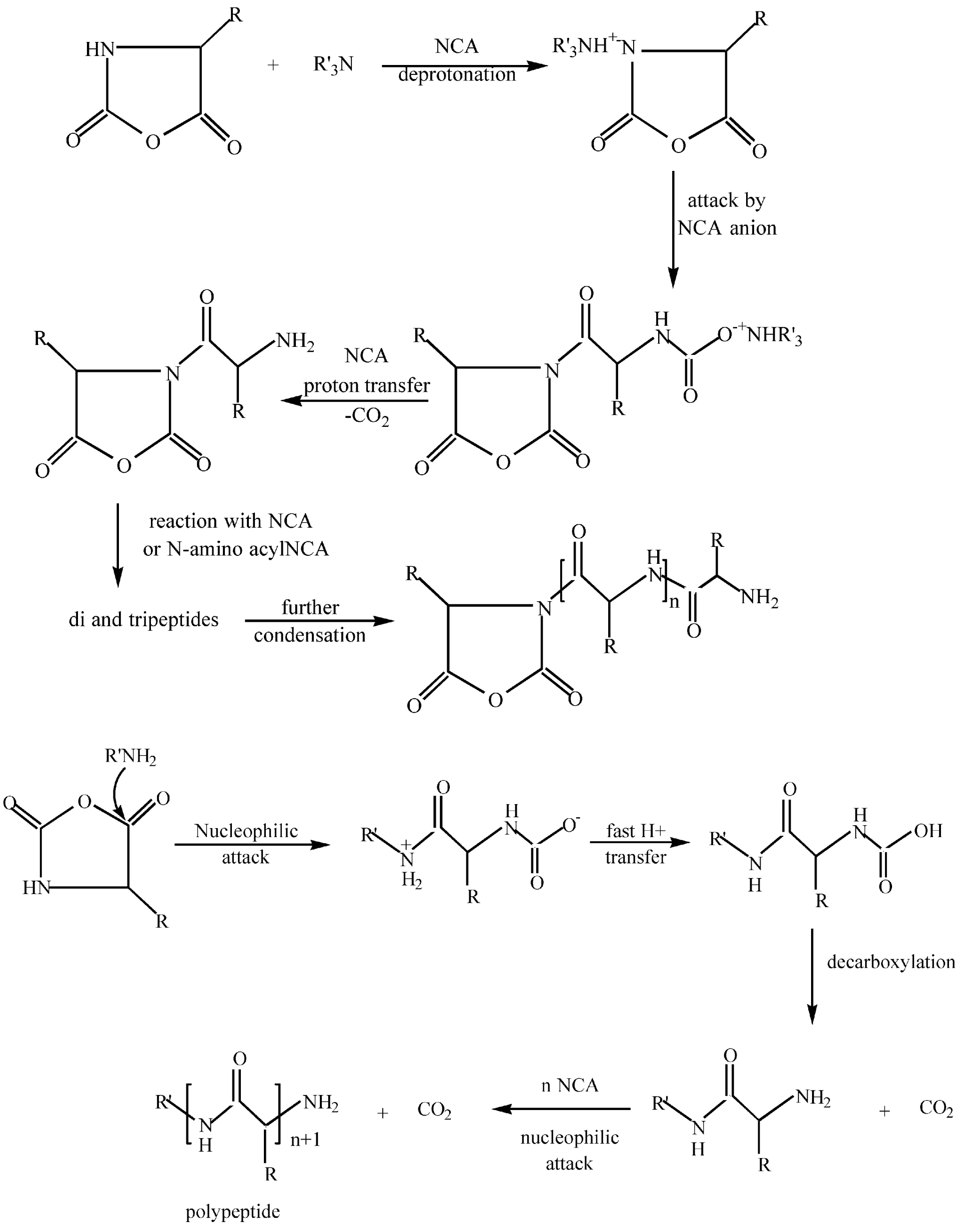
Scheme 1



Characterization. ^{1}H and ^{13}C NMR spectra were recorded in 5 mm tubes in $CDCl_3$, CD_2Cl_2, or deuterated trifluoroacetic acid at 25 °C on a FT Bruker 250 MHz spectrometer. IR spectra were recorded on a Thermo Nicolet Avatar spectrometer. Reduced viscosity measurements were carried out using a Fisons Scientific viscometer with a Ubbelohde type capillary tube (type O) at 25 °C in trifluoroacetic acid at 0.5 g dL^{-1}. MALDI−TOF spectrometry was performed at the Université Pierre et Marie Curie (Paris), Laboratoire de Chimie Macromoléculaire, using a PerSeptive Biosystems Voyager Elite (Framingham, MA) time-of-flight mass spectrometer. This instrument is equipped with a nitrogen laser (337 nm−3 ns pulse), a delayed extraction, and a reflector. It was operated at an accelerating potential of 25 kV in linear mode. The MALDI mass spectra represent averages over 2048 consecutive laser shots (3 Hz repetition rate). The polymer solutions (2−5 g L^{-1}) were prepared in hexafluoropropanol. The matrix, 2,5-dihydroxybenzoic acid, was also dissolved in THF (25 g L^{-1}). A 10 μL portion of the polymer solution were mixed with 20 μL of the matrix solution. A sodium iodide solution (10 μL of a solution at 20 g L^{-1} in THF) was finally added to favor ionization by cation attachment. 1 μL of the final solution was deposited onto the sample target and allowed to dry in air at room temperature. Standards (polystyrene of known structure, M_n = 1620 and 3420 g mol^{-1} purchased from Polymer Standards Service) were used to calibrate the mass scale using the two-point calibration software 3.07.1 from PerSeptive Biosystems.

Results and Discussion

Amine-Initiated NCA Polymerization. As a basis for comparison, MeGluNCA polymerization was performed using primary and tertiary amines. With primary alkylamines such as allylamine, polymerization occurs by nucleophilic attack of the amine group onto an NCA, and a better correlation between experimental and calculated molar masses is usually observed. In the case of triethylamine, it is known that polymerization proceeds by the monomer-activated mechanism. These polymerizations are not living, and high molar masses are obtained that cannot be predicted from [M]/[I] ratios.

Al−Schiff's Base-Initiated NCA Polymerization. The polymerization of MeGluNCA using aluminum−Schiff's base complexes **I** and **II** (Figure 1) as initiators has been studied in dioxane and dichloromethane at ambient temperature. The monomer has also been

Table 1. Polymerization of γ-MeGluNCA in DCM and Dioxane Using Complexes I and II at Ambient Temperature[a]

reference	initiator	solvent	M/I	M_n^{MALDI}	M_w^{MALDI}	MWD	η^b	random coils: α^c (%)
P1	TEA	dioxane	50	2150	2480	1.15	0.21	0:100
P2			100				0.21	0:100
P3	allylamine	dioxane	50	2100	2350	1.13		63:37
P4			100					41:59
P5	complex **II**	DCM	50	2600	2870	1.10	0.26	100:0
P6			100				0.18	62:38
P7		dioxane	50	2390	2630	1.10	0.21	100:0
P8			100	2690	2935	1.09	0.17	80:20
P9	complex **I**	DCM	50	2660	2920	1.09	0.19	100:0
P10			75	2620	2800	1.07		100:0
P11			100	2675	3000	1.12	0.12	56:44
P12		dioxane	25	1925	2175	1.13		100:0
P13			50	2083	2291	1.10	0.19	100:0
P14			75	2450	2645	1.08		100:0
P15			100	2520	2850	1.13	0.20	62:38

[a] [M] = 0.5 mol/L; polymerization time = 2 h; conversion = 100%. [b] Reduced viscosity measured in TFA at 26 °C. $c = 0.5$ g dL^{-1}. [c] Ratio of random coils:α−helix, determined by ^{13}C NMR.

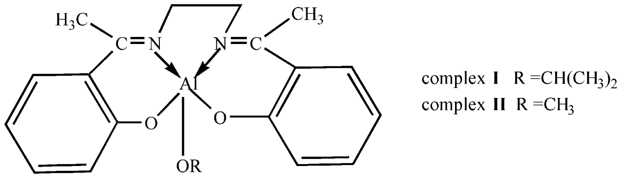


Figure 1. Structure of complexes **I** and **II** as determined from ^{1}H NMR.

polymerized with triethylamine and allylamine for comparison purposes and also because the mechanisms of polymerization are well established in both these latter cases. All reactions were allowed to go to completion, and the absence of residual NCA was confirmed by ^{1}H and ^{13}C NMR (see Supporting Information). The polymers were characterized by viscometry as shown in Table 1. Contrary to the normal expected trend, the reduced viscosity values were found to increase with decreasing [M]/[I] ratios irrespective of the solvent. The molar masses of the polymers could not be determined by SEC due to their limited solubility in commonly used solvents. To better understand the polymerization in the presence of the Al complexes, it was important to further investigate its mechanism.

Mechanism of Polymerization. A model reaction was performed by allowing a stoichiometric amount of

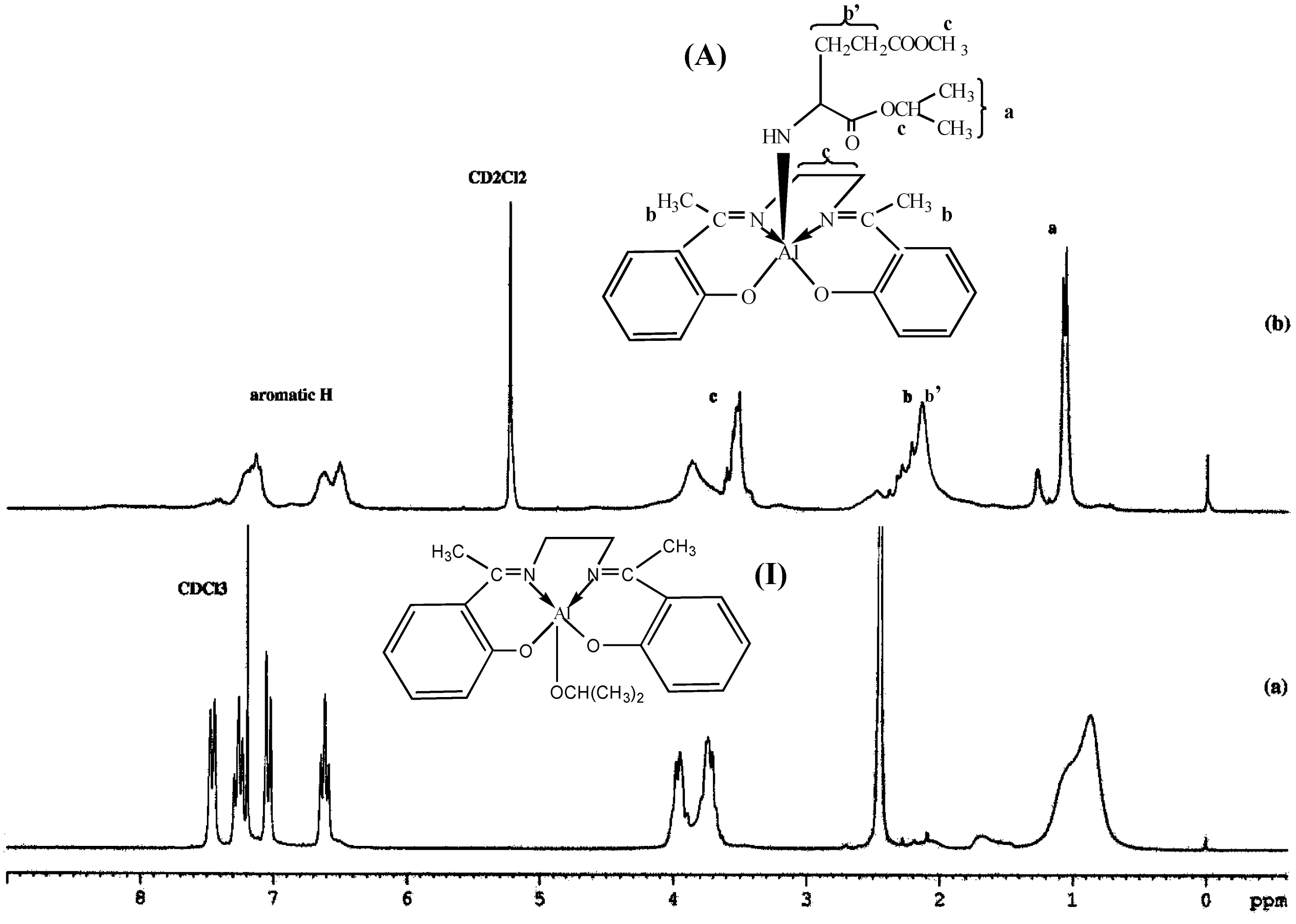
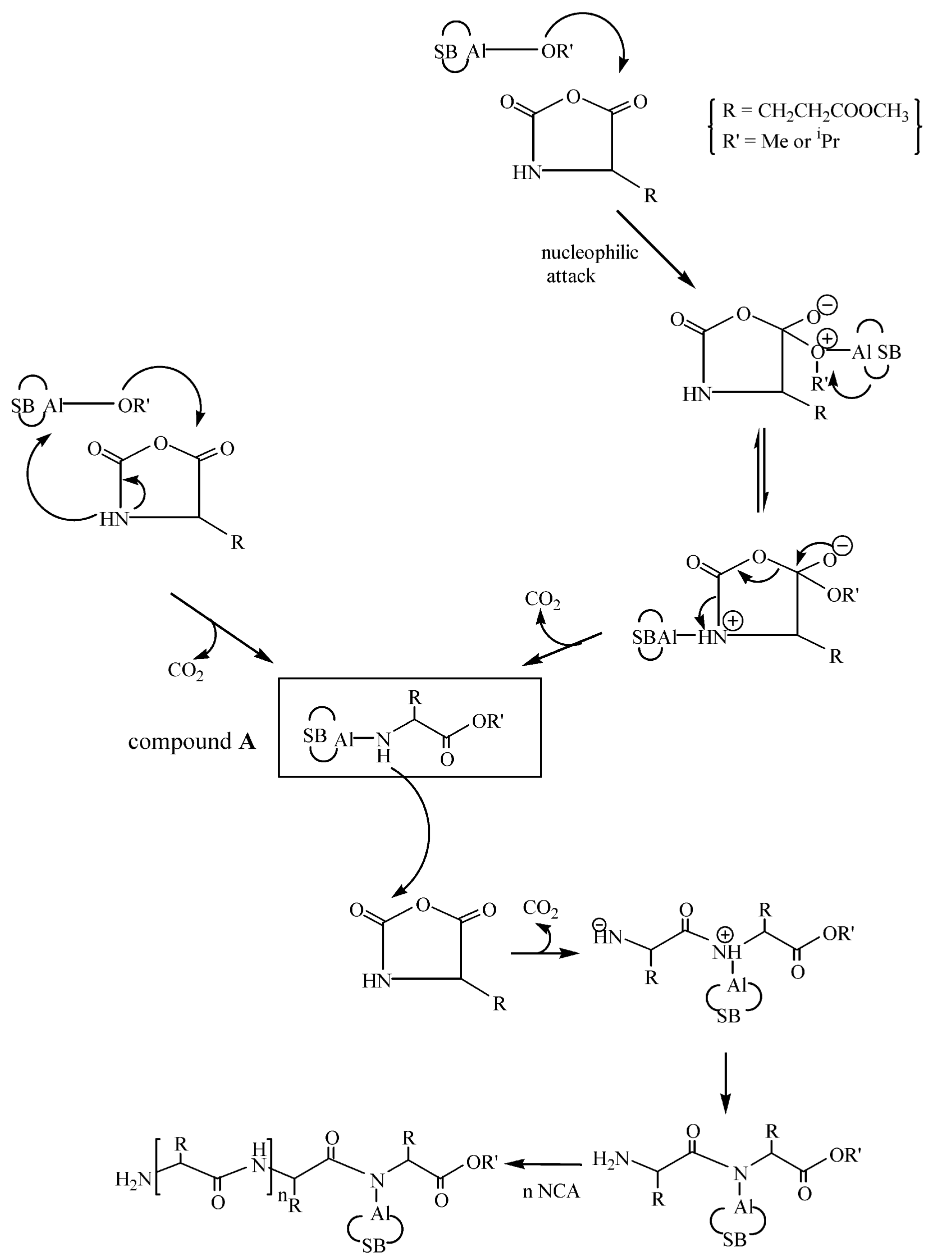


Figure 2. ^{1}H NMR spectra of (a) complex **I** and (b) model reaction product **A**.

Scheme 2

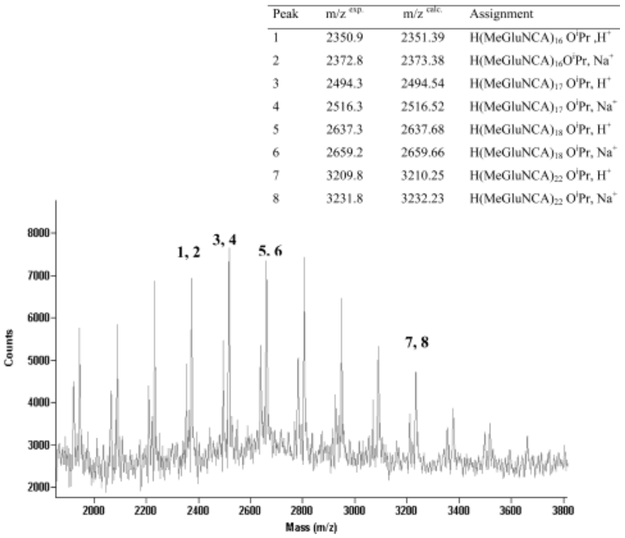


MeGluNCA to react with complex **I** in dichloromethane under conditions similar to those of the polymerization. The reaction was followed by ^{1}H NMR. Figure 2a,b shows the spectra of the aluminum complex **I** before and after reaction with the NCA molecule. For complex **I** alone, a broad band (0.85−1.48 ppm) is observed, which is assigned to the two methyl groups of the Al−isopropoxide moiety (Al−OCH(CH_3)$_2$). The OC*H* proton is in the region 3.39−3.98 ppm and superposed with other protons of the complex. In the aromatic region, four distinct signals (t, d, t, d) are present (see Supporting Information). After reaction with the NCA molecule, the ^{1}H NMR spectrum depicts important changes in the chemical shifts and multiplicities of signals in both regions, indicative of new chemical environments. A doublet is now observed at 1.06−1.08 ppm characteristic of CH_3 signals of the isopropyl ester no longer linked to Al. The aromatic signals are broadened and consist of only two series of multiplets. The spectrum pattern and integration intensity seem to be concordant with the structure proposed in Figure 2b. The mechanism leading to the formation of this compound (**A**) may be a concerted one involving coordination and insertion of the NCA into the Al−isopropoxide bond and subsequent loss of carbon dioxide (Scheme 2). It is difficult, however, to rule out a two-step mechanism involving, first, attack by the isopropoxide, followed by coordination of the Al to the nitrogen. Both mechanisms are here envisaged.

The propagation step is then quite similar to that of polymerization with a primary amine which occurs by nucleophilic attack onto C(5)=O of an NCA to generate an active amino chain end.

The presence of the methoxy or isopropoxy ester group at the chain terminus has been confirmed by MALDI−TOF analysis (Figure 3). The MALDI spectra show monomodal distributions (see Supporting Information for spectra of **P10** and **P14**). As can be seen in Table 1, the molar mass distribution of polymers obtained with Al complexes is quite narrow.

Characterization of Homopolymers. The influence of reaction conditions on the secondary structure



Peak	m/z $^{exp.}$	m/z $^{calc.}$	Assignment
1	2350.9	2351.39	H(MeGluNCA)$_{16}$ O^{i}Pr ,H$^+$
2	2372.8	2373.38	H(MeGluNCA)$_{16}$O^{i}Pr, Na$^+$
3	2494.3	2494.54	H(MeGluNCA)$_{17}$ O^{i}Pr, H$^+$
4	2516.3	2516.52	H(MeGluNCA)$_{17}$ O^{i}Pr, Na$^+$
5	2637.3	2637.68	H(MeGluNCA)$_{18}$ O^{i}Pr, H$^+$
6	2659.2	2659.66	H(MeGluNCA)$_{18}$ O^{i}Pr, Na$^+$
7	3209.8	3210.25	H(MeGluNCA)$_{22}$ O^{i}Pr, H$^+$
8	3231.8	3232.23	H(MeGluNCA)$_{22}$ O^{i}Pr, Na$^+$

Figure 3. MALDI−TOF spectrum of **P13** and the corresponding assignments of peaks.

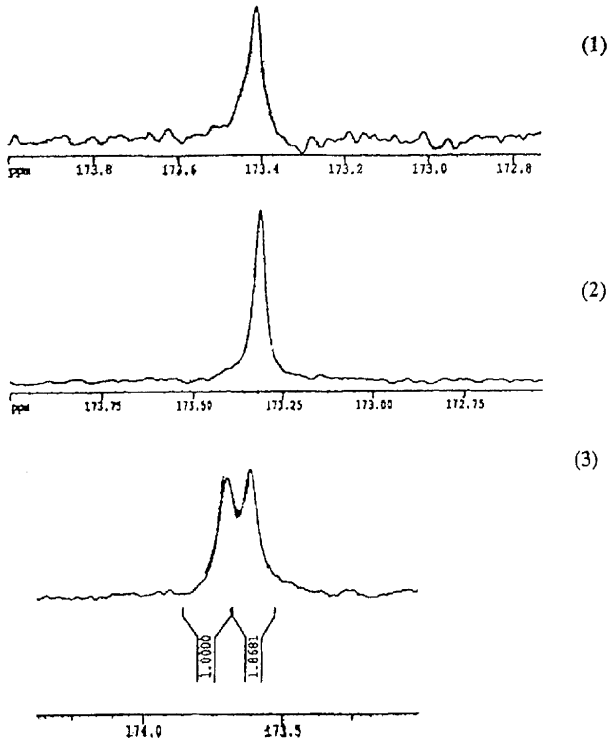


Figure 4. Carbonyl region of ^{13}C NMR spectrum of poly(MeGluNCA) for (1) α-helix (**P1**), (2) random coils (**P5**), and (3) α-helix and random coils (**P15**).

of the polypeptides was investigated by ^{13}C NMR in D-trifluoroacetic acid. In this solvent, the oligopeptides which generally adopt a β-sheet structure are transformed into random coils while, depending on the molar masses, the α-helix conformation may be maintained with longer chain lengths favoring the α-helix.[7] The relative proportions of the random coil and α-helix can be determined by examining the carbonyl region of the ^{13}C NMR spectra, as summarized in Table 1. A slight downfield shift is observed for the α-helix (δ = 173.4 ppm) compared with the random coils (δ = 173.3 ppm) while a polymer containing both structures contain two signals at 173.3 and 173.4 ppm (Figure 4).

It is well established from previous studies that polymerization of MeGluNCA with triethylamine leads to high molar masses, thereby favoring the α-helix conformation. As can be seen in Table 1, this is indeed confirmed in dioxane irrespective of the initiator concentration. A high α-helix content is generally the result of a slow initiation followed by rapid propagation, in accordance with the activated monomer mechanism.

From our ^{13}C NMR spectra in TFA, it appears that polymers obtained in 1,4-dioxane or DCM with complexes **I** and **II** exhibit the presence of both α-helix and random coils and the α:random coil ratio depends on [M]/[I]. The presence of the α-helical conformation observed in pure TFA (**P1**, **P3**, and **P8** for example) contrasts with previously reported results of Poché et al.,[18] who showed by ^{1}H NMR that 5.6−6.5% TFA was required to completely disrupt the helix conformation of a 10% poly(γ-stearyl α-L-glutamate) in $CDCl_3$. More strangely however in our study, we found that in both DCM and dioxane complexes **I** and **II** ([M]/[I] = 50) seem to give rise to the exclusive formation of random coils or β-sheets at quantitative polymer conversions (**P5**, **P7**, **P9**, and **P13**).

To confirm the NMR findings, the polymers were further analyzed in the solid state by FTIR spectroscopy. The α-helical conformation is characterized by the presence of CONH bands at 1660 and 1550 cm^{-1} while the β-sheet form gives rise to bands at 1700, 1635, and 1535 cm^{-1}.[18] FTIR analysis of all polymers (see Supporting Information) reveals the presence of bands typical of both conformations as opposed to ^{13}C NMR analysis in solution where all the α-helices are disrupted by TFA. From these results, we can deduce that the polymers obtained in the presence of the Al−Schiff's base complexes at high initiator concentration contain a lower proportion of α-helical conformation and probably having lower molar masses as compared to the amine-initiated polymerizations under similar experimental conditions.

Conclusions

In this article, we have shown that Al−Schiff's base complexes **I** and **II** are effective initiators for the polymerization of γ-methylglutamate *N*-carboxyanhydride. A plausible mechanism of polymerization based on model reactions, NMR, and MALDI−TOF analyses has been proposed which involves insertions of NCA molecules into a newly formed aluminum−nitrogen bond. Analyses also show that low-molar-mass polymers are obtained.

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Supporting Information Available: Text and figures (NMR and IR spectra) giving characterization details of polymers and compound **A**. This material is available free of charge via the Internet at http://pubs.acs.org.

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