Comblike Alkyl Esters of Biosynthetic Poly(γ-glutamic acid). 1. Synthesis and Characterization

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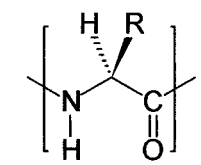
ABSTRACT: Two series of comblike poly(α-*n*-alkyl γ-glutamate)s (PAAG-*n*, *n* being the number of carbons contained in the linear alkyl side chain), one with a D:L enantiomeric ratio approximately 9:1 and the other with a nearly racemic composition, were prepared and characterized for even values of *n* ranging from 12 to 22. Esterification of bacterial poly(γ-glutamic acid) to poly(α-ethyl γ-glutamate) followed by transesterification of the latter with long linear 1-alkanols afforded pure PAAG-*n* in almost full conversions and high yields. Thermal decomposition temperatures of PAAG-*n* were above 300 °C, and they increased slightly with the length of the alkyl side group. ^{1}H NMR studies in chloroform solution revealed the presence of regularly folded conformations that were disrupted upon addition of strong acids. In the solid state, PAAG-*n* with $n \geq 14$ displayed melting of the alkyl side chains at temperatures increasing from 20 up to 80 °C for increasing values of *n*. Preliminary X-ray diffraction showed that PAAG-*n* adopt the typical layered structure of comblike polypeptides with alkyl groups crystallized in a paraffinic interlayer phase. The layer periodicity was found to steadily increase from 2.7 to 3.7 nm in agreement with a structure made of polypeptide chains in helical conformation with the alkyl side groups oriented nearly normal to the layers and extensively interdigitized. No significant differences either in structure or in thermal behavior were observed between the two investigated series.

Introduction

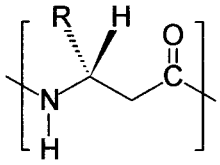
Comblike polypeptides bearing long linear alkyl side groups are of current interest for their ability to adopt supramolecular structures adequate to be used as components of functional materials.[1] These systems consist of a rodlike main chain helix and a flexible side chain that can be more or less disordered or crystallized according to temperature. Although such structures are made of only one component, at temperatures above the melting point they may be regarded as lyotropic liquid crystals since the molten paraffinic phase plays the role of a solvent. Some of these systems are able to display striking thermochromic properties characteristic of cholesteric structures.[2,3]

It was the pioneering work made by Watanabe et al.[4] which revealed for the first time that the structural behavior of poly(γ-alkyl α,L-glutamate)s is greatly determined by the size of the side chain. Whereas lower members tend to crystallize in three-dimensional structures, those with 10 or more carbon atoms in the alkyl side group form two-dimensional biphasic layered structures. These are made of sheets of side-by-side packed α-helices with the polymethylene side chains crystallized in a separate paraffinic phase. The synthesis, structure, and properties of these systems have been recently reviewed.[5] It has been shown further that stoichiometric complexes of ionized poly(α-glutamic acid) with alkylammonium cations may be arranged in the same type of structures.[6]

Formation of biphasic layered structures made of main chain rigid helices is not exclusive of poly(α-peptide)s. It has been recently shown that comblike poly(β-peptide)s, which are characterized by containing an additional methylene group in the repeating unit, form the same type of structures and display similar properties.[7] This is the logical consequence of the ability of poly(β-peptide)s to adopt helical conformations of α-helix type, a feature that was first evidenced a number of year ago[8] and that has been corroborated in these past years by results afforded in the structural analysis of oligo(β-peptide)s.[9]



poly(α-L-amino acid)s



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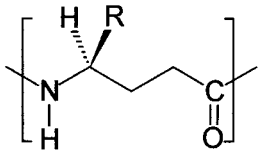

poly(γ-D-amino acid)s

Like poly(α-peptide)s and poly(β-peptide)s, poly(γ-peptide)s may also adopt regular folded conformations stabilized by intramolecular hydrogen bonds. In this case, two additional methylenes are present in the repeating unit when compared to poly(α-peptide)s. Despite the much higher flexibility of the poly(γ-peptide) backbone, a rigid 5/2 helix has been found to occur in the synthetic methyl and benzyl esters of poly(γ,L-glutamic acid)[10] and in oligo(γ,L-amino acid)s.[11] Recent studies carried out on poly(γ-glutamate)s of biosynthetic origin and with varying D:L ratio has suggested that the helical structure is common to the whole family of poly(α-alkyl γ-glutamate)s and that it seems to be compatible with a racemic composition of the polymer.[12]

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In this work we wish to report on comblike poly(γ-peptide)s, specifically on comblike poly(α-alkyl γ-glutamate)s, called thereinafter PAAG-*n*, where *n* indicates the number of carbons contained in the linear alkyl side chain. They will be prepared from bacterially produced poly(γ-glutamic acid) (PGGA), a water-soluble poly(γ-peptide) that is receiving increasing attention for its potential biodegradability.[13] This polymer is scarcely soluble in organic solvents and decomposes before melting. With the aim at rendering a polymer easier to handle, esterification of PGGA leading to poly(γ-glutamate)s with short alkyl side chains has been carried out by a number of authors using different methods.[14−16] There is not unanimity however about what is the optimum procedure, and special difficulties were found when long alkyl chains were concerned. In this work we use a two-step esterification method developed by us that was proved to be efficient for the synthesis of poly(γ-glutamate)s bearing alkyl side chains of short and medium size.[17] Now we extend this method to the synthesis of poly(γ-glutamate)s with alkyl chains containing from 12 up to 22 carbon atoms.

Whereas the structure of PAAG-*n* with $n \leq 10$ has been already examined,[12] no attention has been paid so far to poly(γ-glutamate)s bearing long alkyl side chains. The fact that both poly(α-peptide)s and poly(β-peptide)s adopt a common lamellar structure in the solid state encouraged us to explore the occurrence of similar supramolecular arrangements in comblike poly(γ-glutamate)s. In this case, the interest for the study is reinforced by the possibility of preparing PGGA by biosynthetic laboratory methods.[18−20] The enantiomeric composition of bacterially produced PGGAs is dependent on a number of processing factors. Products with D:L ratios between 9:1 and 1:1 are usually accessible, those with industrial interest being nearly racemic. Because of the relevance that polymer configuration may have to the structure and properties of the deriving esters, the present study covers two series of PAAG-*n*: one with a D:L ratio of 9, called PAA(D)G-*n*, and the other with a nearly racemic composition, called PAA(DL)G-*n*.

Experimental Section

Materials and Synthesis. All chemicals were obtained commercially from either Aldrich or Merck. They were analytical grade or higher and used without further purification. Solvents to be used under anhydrous conditions were dried by standard methods. Two poly(γ-glutamic acid)s differing in the enantiomeric composition were used in this work. PGG(DL)A with a D:L ratio of 59:41 and a weight-average molecular weight of 394 000 was kindly supplied by Dr. Kubota of Meiji Co. (Japan). PGG(D)A with a D:L ratio of 89:11 and a weight-average molecular weight of 1 030 000 was prepared specifically for the purpose of this work by fermentation *of B. licheniformis* as reported in detail elsewhere.[20] For a more comparable study between PGG(DL)A and PGG(D)A, the molecular weight of the latter was reduced by irradiation with microwaves according to the method developed by us for ultrasonic degradation of poly(aspartate)s and poly(glutamate)s.[21] The sample of PGG(D)A used for esterification had a weight-average molecular weight of 340 000. The main characteristics of the three PGGA samples used in this work are shown in Table 1.

Poly(α-ethyl γ-glutamate) (PAAG-2) was obtained as follows: 2 g (15.5 mmol) of PGGA suspended in *N*-methylpyrrolidone (200 mL) was left overnight under stirring at 80 °C. The mixture was cooled to 60 °C and added with $NaHCO_3$ (5.25 g, 62.5 mmol). Then ethyl bromide (6 mL, 80 mmol) was slowly added for a period of 2 h and left to react for a time between 20 and 30 h. The ethylation reaction was followed by ^{1}H NMR, and it was considered to be finished when the signal area ratio of the side chain CH_2 to the main chain CH was 2. After removing the NaBr precipitate, the reaction solution was poured into acidified cool water (1.5 L, pH 1.5) to precipitate PGGA-2, which was then separated by filtration. The resulting white powder was repeatedly washed with cool water and ethyl ether and finally dried under vacuum at 50 °C (2.1 g, yield: 88%).

Table 1. Poly(γ-glutamic acid)s Used in This Work

	GPC data[a]			[η] (dL g^{-1})[b]			
	$M_w \times 10^{-3}$	$M_n \times 10^{-3}$	PD	H_2O	DMSO	D:L[c]	source
PGG(DL)A	394	148	2.6	3.5	1.8	59:41	Meiji Co.[d]
PGG(D)A	1030	370	2.8	8.5	5.0	89:11	this work[e]
PGG(D)A	340	150	2.2	3.5	1.7	89:11	this work[f]

[a] Determined using buffered water as the mobile phase. [b] Limiting viscosity measured in the indicated solvents. [c] Enantiomeric composition determined by HPLC. [d] Kindly gifted by Dr. Kubota from Meiji Co (Japan). [e] Produced by biosynthesis as described in ref 20. [f] Produced by biosynthesis and degraded by microwave irradiation.

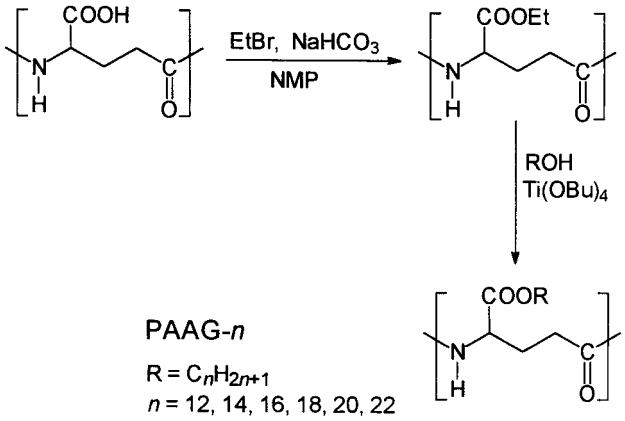

Figure 1. Scheme of the synthesis leading to PAAG-*n*.

PGGA-*n* for $n = 12-22$ were obtained according to the following general procedure: PGGA-2 (*x* mol) was suspended in (10−50)*x* mol of the alcohol of choice at a temperature between 150 and 190 °C and magnetically stirred under a nitrogen atmosphere. $Ti(OBu)_4$ was added in a proportion ranging from 10 to 25 mol % of the polymer for *n* increasing from 12 to 22. The polymer was entering the solution as the transesterification reaction proceeded. The advancement of the reaction was monitored by ^{1}H NMR by comparing the areas of appropriate signals. When conversion was total, the final solution was poured into boiling ethanol, and the mixture was left to cool to room temperature. The polymer precipitated, and it was separated from the supernatant solution by decantation. Purification was accomplished by dissolving the product either in chloroform or in a mixture of chloroform−trifluoroacetic acid and reprecipitating it with methanol or ethanol.

Measurements. Elemental analyses were performed in Servicios Científico-Técnicos de la Universitat de Barcelona. Enantiomeric compositions of PGGA and PAAG-*n* were determined according to the method developed by Cromwick and Gross[22] for PGGA. The hydrolyzed polymer was made to react with the Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, Pierce, Rockford, IL) to form the diastereoisomeric dipeptides which were then analyzed by HPLC. Hydrolysis of polymers were carried out in 6 M HCl at 110 °C for 24 h. Viscosities were measured at 25.0 ± 0.1 °C using an Ubbelohde viscometer. Phosphate buffered water at pH 7.4 and dimethyl sulfoxide (DMSO) were the solvents used for PGGA whereas tetrahydrofuran (THF) or *n*-heptane was the solvent of choice for PAAG-*n*. Size exclusion chromatography (SEC) of PGGA was performed on a Waters Associates instrument fitted with a refractive index detector and a set of two Styragel columns with exclusion limits at 10^3 and 10^2 nm. The mobile phase was buffered water at pH 7.4, and Waters Millipore polystyrene standards purchased from Waters Millipore were

Table 2. Synthesis Data of Poly(α-alkyl γ-glutamate)s

	reaction conditions			viscosity data[a]		SEC-LS data[b]			elementary analysis[d]		
	t (h)	*T* (°C)	yield (%)	[η] (dL g^{-1})	$M_w \times 10^{-3}$	$M_w \times 10^{-3}$	PD	D:L[c]	C (%)	H (%)	N (%)
PAAG-2											
D	24	60	88	0.85	–	–	–	88:12	53.20	7.03	8.98
DL	24	60	88	0.70	–	–	–	60:40	52.10	6.89	8.90
									(53.50)	(7.00)	(8.90)
PAAG-12											
D	11	150	84	0.56	140	150	1.31	–	67.98	10.99	4.86
DL	6	150	63	0.48	125	150	1.27	–	67.72	10.63	4.81
									(68.70)	(10.44)	(4.71)
PAAG-14											
D	3	170	48	0.49	126	250	1.33	–	68.62	10.84	4.40
DL	2	190	70	0.28	66	89	1.22	–	68.94	10.77	4.41
									(70.20)	(10.78)	(4.31)
PAAG-16											
D	4	190	82	0.44	116	140	1.36	–	70.84	11.45	4.04
DL	4	190	70	0.21	95	100	1.40	–	70.90	11.52	4.06
									(71.40)	(11.05)	(3.97)
PAAG-18											
D	6	190	85	0.50	130	170	1.42	88:12	70.71	11.31	3.70
DL	22	190	69	0.14	45	32	1.52	60:40	71.73	11.74	3.77
									(72.40)	(11.29)	(3.67)
PAAG-20											
D	8	190	80	0.34	95	100	1.40	–	72.26	11.76	3.48
DL	4	190	69	0.30	86	86	1.45	–	72.78	11.94	3.52
									(73.30)	(11.49)	(3.40)
PAAG-22											
D	8	190	80	0.21	66	66	1.30	–	74.23	12.30	3.27
DL	8	190	68	0.16	52	42	1.28	–	74.23	12.33	3.32
									(74.14)	(11.67)	(3.20)

[a] Intrinsic viscosity measured in THF at 25 °C except for PAAG-2 which was measured in DMSO. M_w calculated on the basis of the viscometric equation $[\eta] = (1.29 \pm 0.35) \times 10^{-5} M_w^{1.29 \pm 0.09}$ (η in mL g^{-1}) given in ref 5. [b] Light scattering measurements in THF. [c] Enantiomeric composition determined by HPLC. [d] Calculated values in parentheses.

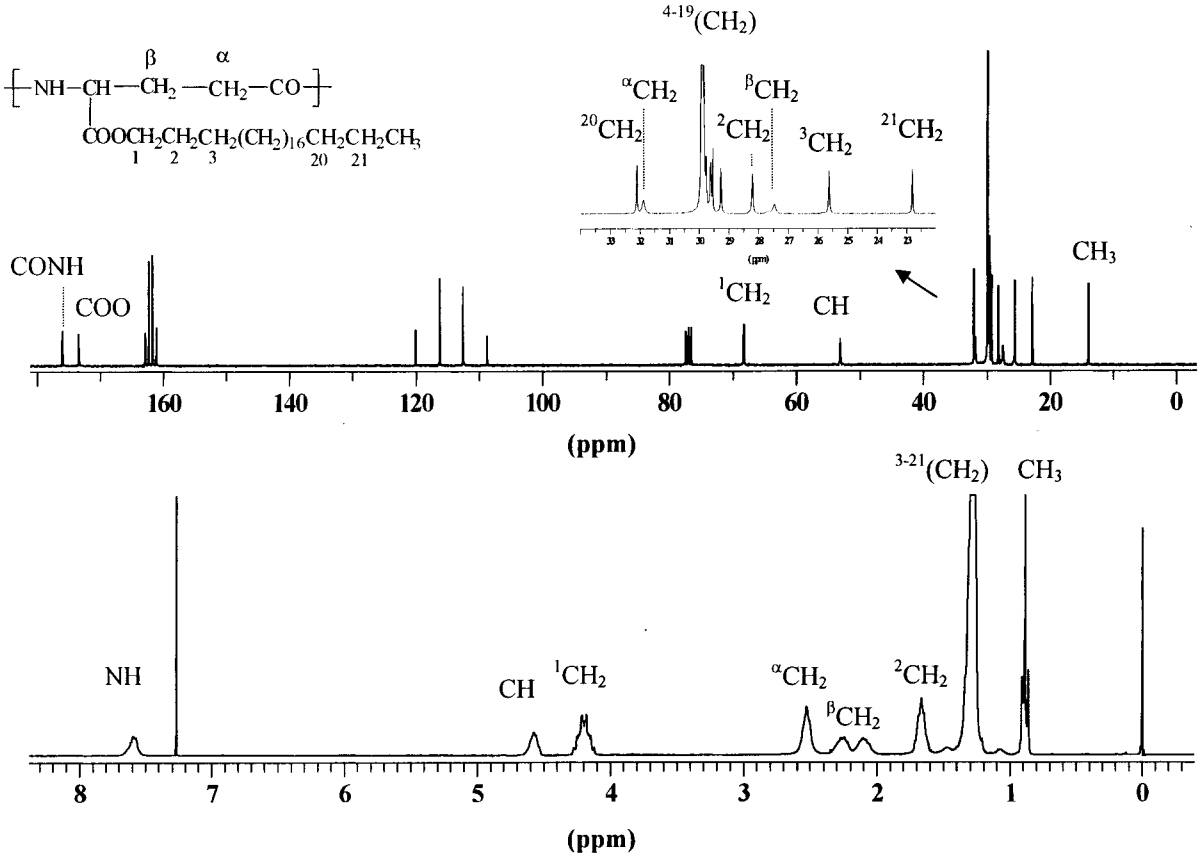

Figure 2. ^{1}H NMR and ^{13}C NMR spectra of PAA(D)G-22 in TFA/$CDCl_3$ (5% v/v) at 25 °C.

used to create a calibration curve. SEC determinations of PAAG-*n* were carried out on triplicates in THF using a Waters 510 equipment fitted with a laser and light scattering miniDAWN detector (Wyatt Tech Ins.) and a Shimadzu RID-6A refractive index detector. The refractive index was calibrated with polystyrene standards.

FTIR spectra were recorded on a Perkin-Elmer FT-2000 instrument from films prepared by casting from chloroform

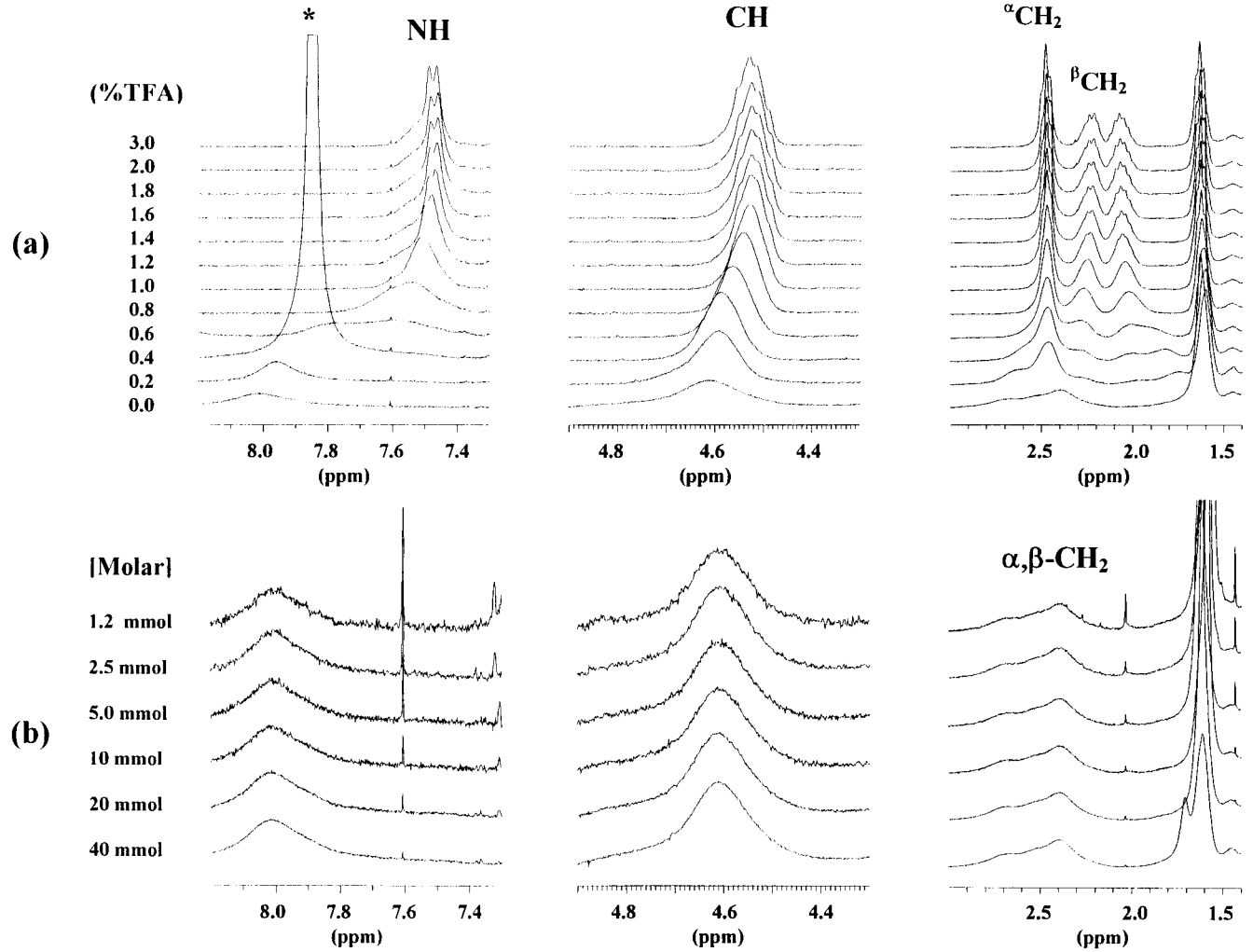

Figure 3. Changes observed in the ^{1}H NMR spectra of PAA(D)G-20 at 25 °C in $CHCl_3$ upon addition of TFA (a) and dilution (b). (*) Peak of TFA.

solution. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker AMX-300 NMR instrument with samples dissolved in $CDCl_3$ or a mixture of $CDCl_3$/TFA at the indicated concentrations and using TMS as internal reference. Quantitative ^{1}H NMR spectra were recorded with relaxation delays of 10 s. Calorimetric measurements were performed with a Perkin-Elmer Pyris I DSC instrument operating under a nitrogen atmosphere and calibrated with indium. Sample weights of about 2−5 mg were heated or cooled at rates of ±10 °C min^{-1}. Thermogravimetric analyses were performed with a Perkin-Elmer TGA6 thermobalance at a heating rate of 20 °C min^{-1} under flowing nitrogen. X-ray diffraction patterns were taken from both oriented and unoriented polymer films in a Statton-type camera using nickel-filtered Cu Kα radiation of wavelength 0.1542 nm. The patterns were recorded on flat photographic films and were calibrated with molybdenum sulfide (d_{002} = 0.6147 nm).

Results and Discussion

Synthesis of PAAG-*n*. A systematic synthesis of comblike poly(α-alkyl γ-glutamate)s has been carried out in this work. Direct alkylation with alkyl halides is the commonly reported procedure to obtain a variety of esters from PGGA.[14−16] Although this procedure is satisfactory for the synthesis of short alkyl esters, it has been proved to be less efficient when poly(γ-glutamate)s bearing alkyl side chains of medium size are desired. Thus, alkyl iodides in unusual solvents were required in order to attain conversions higher than 95% in the synthesis of poly(α-dodecyl γ,D-glutamate).[14] In this work, poly(γ-glutamic acid) esters with alkyl side chain lengths from n = 12 (dodecyl) up to n = 22 (docosyl) were prepared for two different enantiomeric polypeptide compositions, i.e., D:L ≈ 1 and 9. To circumvent foreseeable difficulties deriving from the use of long alkyl chain lengths such as that are here concerned, we have applied here a method previously developed by us[17] consisting of two well-differentiated steps. First, poly(γ-glutamic acid) was esterified to poly(α-ethyl γ-glutamate) (PAAG-2), with ethyl bromide in *N*-methylpyrrolidone according to Kubota's method.[15] Second, PAAG-2 was subjected to transesterification with the appropriate long chain linear alkanol to produce the corresponding PAAG-*n*. The chemical pathway of this synthesis is depicted in Figure 1. Reaction conditions and characteristics of the resulting poly(γ-glutamate)s are shown in Table 2.

According to previously reported results,[17] PAA(D)G-2 and PAA(DL)G-2 were obtained with almost complete conversion in near to 80% yields. No change in the enantiomeric composition was detected after esterification, indicating absence of significant racemization during reaction. On the contrary, viscosity data revealed a significant decrement in molecular weight, apparently to about one-half of the initial value, indicating that a certain scission of the main chain took place during the modification process.

Transesterifications were made with the PAA(D)G-2 or PAA(DL)G-2 suspended in a large excess of alcohol and heated at temperatures between 150 and 190 °C in the presence of significant amounts of $Ti(BuO)_4$, a compound displaying a well-known catalyzing efficiency in ester−ester transreactions.[23] The degree of replacement was followed by comparing the areas of the side chain proton signals to that of the main chain CH signal in the ^{1}H NMR spectra. The reaction was assumed to be finished when the area ratio attained the value of $2n + 1$. Although reaction conditions were not optimized, the lowest temperature compatible with a reasonable reaction rate was usually chosen. On the basis of NMR analysis, final conversions were estimated to be 100% in all cases and yields oscillated between 48 and 85%. Elemental analysis indicated however a slightly low carbon-to-nitrogen ratio rather consistent with a conversion between 96 and 98%. The reaction effects on polymer configuration and size were qualitatively similar to those observed in the esterification step, i.e., no variation in the D:L ratio but a significant diminution in the molecular weight. The chemical structure of novel comblike PAAG-*n* was assessed by both ^{1}H NMR and ^{13}C NMR spectroscopy, as illustrated in Figure 2 for the case of PAA(D)G-22.

Solution Properties. Comblike PAAG-*n* display a solubility behavior clearly different from those bearing short side chains. They are soluble in chloroform and tetrahydrofuran but nonsoluble in fluorinated alcohols like trifluoroethanol or hexafluoro-2-propanol. Higher members can be solubilized also in *n*-heptane. ^{1}H NMR spectra in solution of PGGA-*n* showed significant differences in line broadening depending on solvent and temperature. This pattern of behavior had been previously reported for poly(α-pentyl γ,D-glutamate) and interpreted as due to the probable occurrence of definite conformations stabilized by intramolecular hydrogen bonding although the influence of aggregation effects could not be then discarded.[14]

We have looked into the structure of PGGA-*n* in solution by ^{1}H NMR with regards to formation of regular folded conformations and eventual occurrence of unspecific molecular aggregations. The evolution of the chemical shift of NH, CH, and CH_2 main chain signals of PAA(D)G-20 dissolved in $CHCl_3$ upon addition of increasing amounts of TFA is shown in Figure 3a. The beneficial influence of the hydrogen-bond breaking TFA acid on the resolution of the spectra is apparent when the serial traces are compared. The signal narrowing effect is seen to be common to the whole spectra, and at the same time, the NH and CH main chain signals are seen to move toward higher fields. On the other hand, when ^{1}H NMR spectra of PAA(D)G-20 in $CHCl_3$ were taken upon dilution from 40 mM down to 1.2 mM, no significant changes could be detected in either the shape or the position of the signals (Figure 3b). Such an invariability of the spectra within such a wide range of polymer concentrations allows us to discard the occurrence of aggregates as to be in the origin of the observed NMR changes. Some exploratory circular dichroism spectroscopy of PAA(D)G-20 was performed in order to provide further support to NMR observations. It was found that the ellipticity ($\sim 5 \times 10^2$ deg cm^2 dmol^{-1} at 240 nm) showed by the polymer in heptane was maintained upon dilution but disappeared upon addition of TFA. Although these results are in full agreement with those obtained by NMR, their supporting value is weakened by the low *Cotton* effect that is measured for PAAG-*n*. This is not surprising since it has been reported that the CD response of oligo(γ-peptide)s is highly variable depending on constitution.[11]

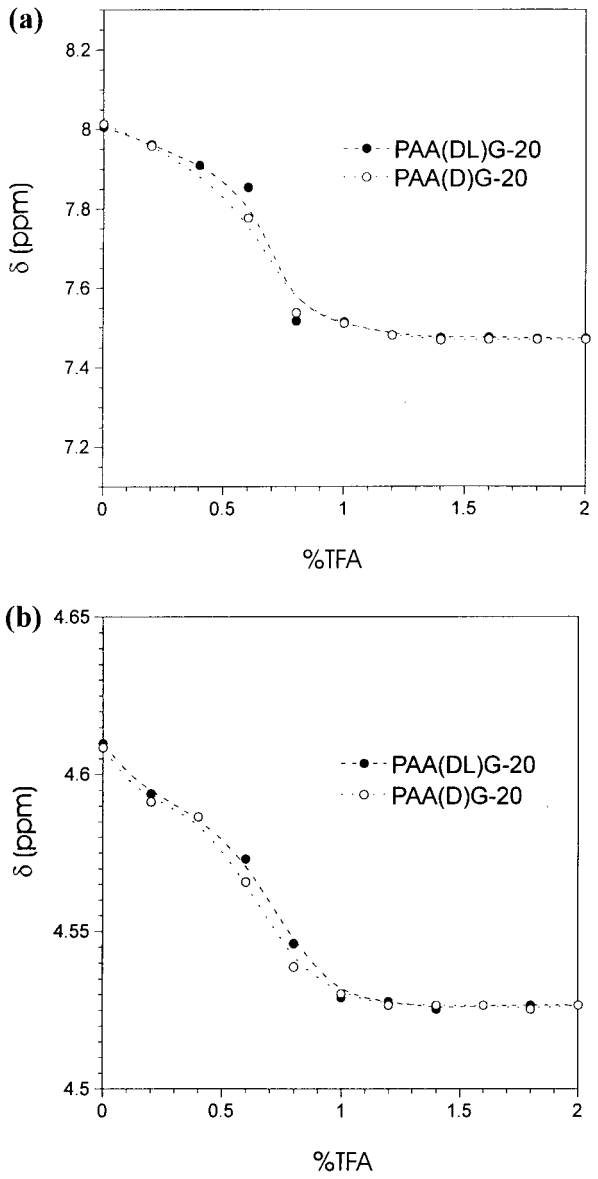

Figure 4. Variation of the chemical shifts of the NH(a) and CH (b) signals for PAA(D)G-20 and PAA(DL)G-20 upon addition of increasing amounts of TFA.

The variation in the chemical shifts (δ) of the HN and CH proton signals with the concentration of TFA is represented in Figure 4. The decay is observed to happen suddenly at the surroundings of 0.7% of TFA for both signals, a fact that may be taken as indicative of the occurrence of definite changes in the molecular conformation. Such changes in shape and position of peaks are known to occur also in poly(β,L-aspartate)s, and they are interpreted as a consequence of the helix-to-random coil transition taking place upon addition of TFA.[24] Thus, the existence of folded regular structures of PAA(D)G-*n* in solution can be reasonably assumed to exist provided that hydrogen-bond formation is not impeded by strong solvent interactions. This is in fact a feature common to all polypeptides able to adopt helical arrangements stabilized by intramolecular hydrogen bonding. The stability of the helix is commonly

Table 3. Thermal Data of Poly(α-alkyl-γ-glutamate)s

	T_m^1 (°C)[a]	ΔH^1 (J g^{-1})[a]	T_c (°C)[b]	T_m^2 (°C)[b]	ΔH^2 (J g^{-1})[b]	T_d^0 (°C)[c]	T_d (°C)[c]
PAAG-12							
D	−/−	−/−	-	-	-	312	341
DL	−/−	−/−	-	-	-	330	327
PAAG-14							
D	−/−	−/−	-	-	-	310	338
DL	20	14	11	21	16	317	341
PAAG-16							
D	43/40	35/36	32	36	11	321	350
DL	38/42	36/35	32	38	10	320	355, 330 sh
PAAG-18							
D	55/49	74/48	37	42	17	311	330
DL	53/54	74/50	44	51	22	311	330, 365 sh
PAAG-20							
D	61/57	96/82	49	55	48	319	360, 330 sh
DL	59/59	67/45	55	58	31	330	370, 330 sh
PAAG-22							
D	72/76	92/85	61	68	49	326	370, 330 sh
DL	71/70	96/50	64	69	34	329	370, 330 sh

[a] Melting temperature and enthalpy measured on the first heating trace for the powder of synthesis an for a film casted from chloroform. [b] Crystallization temperature and melting temperature and enthalpy measured on the second heating for a film casted from chloroform. [c] Decomposition temperatures measured for the onset of the weight loss and for the maxima of the derivative curve.

correlated with the amount of acid required for promoting the transition. Since the helix−coil transition in poly(β-peptide)s takes place at about 2% TFA, it can be concluded a lower stability for the poly(γ-peptide) helix, which correlates well with the density of hydrogen bonding present in each case.

Identical ^{1}H NMR results were obtained when racemic PAA(DL)G-20 was subjected to the same study. The extreme similarity in behavior found for the racemic and the enriched enantiomeric polymers is vividly illustrated in Figure 4, which shows the almost same trace for representing the changes in the NH and CH shifts occurring in the two enantiomorphs by the action of TFA. The independence of these results on the enantiomeric composition is certainly striking since it is known that the stability of polypeptide helices is strongly dependent on the stereorregularity of the polypeptide chain. In fact, a study made on poly(β,L-aspartate) block stereocopolymers showed that the concentration of TFA needed for transition rapidly decreased with the loss of stereorregularity and that no transition could be observed for random stereocopolymers.[24] The results obtained with racemic poly(γ,DL-glutamate)s coming from biosynthesis give support to the occurrence of a copolymer made of long stereoblocks or even to the existence a mixture of two enantiomeric enriched D- and L-homopolymers.

Thermal Properties. The results of the thermal analysis of PAA(D)G-*n* and PAA(DL)G-*n* carried out by DSC and TGA are presented in Table 3. Data for the two series are very similar, indicating that the thermal behavior is essentially unaffected by the enantiomeric composition, the observed small differences being due to uncontrolled experimental factors rather than to configurational effects.

DSC traces of PAAG-*n* for $n \geq 14$ revealed the occurrence of a melting−crystallization process taking place within the 20−80 °C range and entailing a fusion heat between 10 and 100 J g^{-1}. In Figure 5a, PAA(DL)G-14 and PAA(DL)G-22 traces corresponding to the complete heating−cooling−heating sequence are shown for illustration. Comparison of data obtained from samples coming directly from synthesis with those

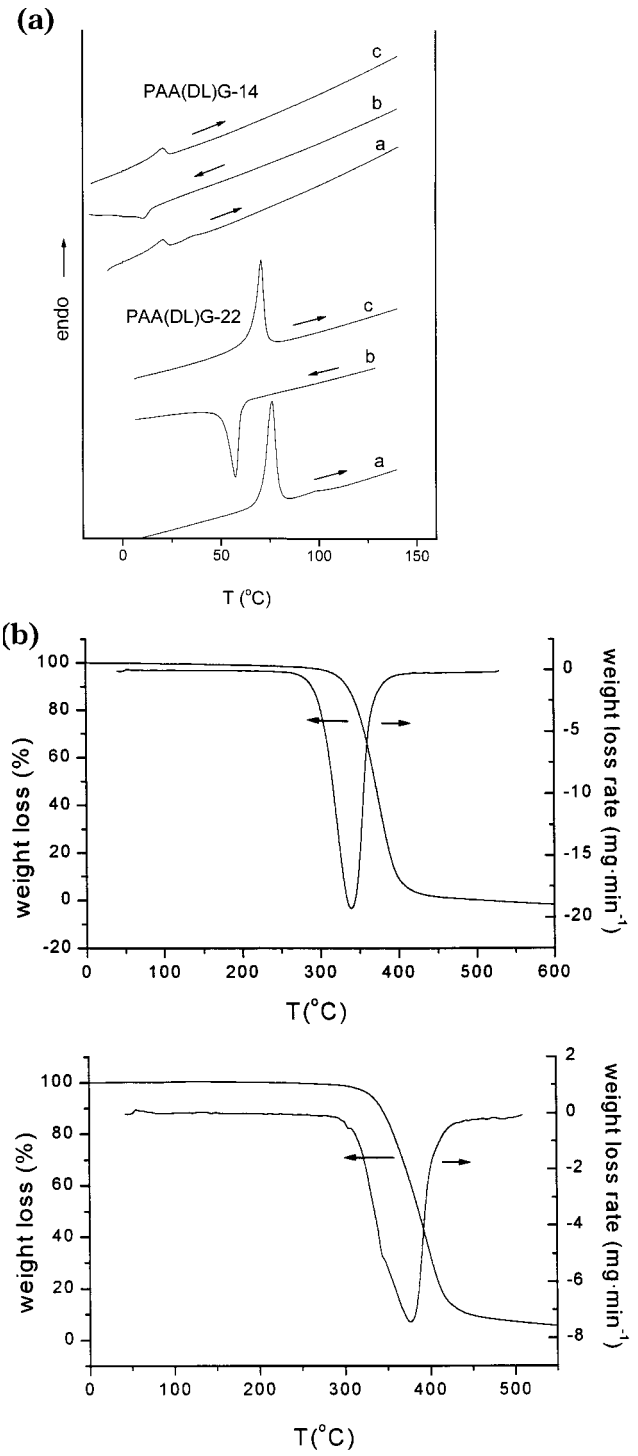

Figure 5. DSC (a) and TGA (b) traces (including derivative curves) of PAA(DL)G-14 (top) and PAA(DL)G-22 (bottom).

obtained from films prepared by casting showed that a greater crystallinity is always present in the pristine samples. Both melting temperature and enthalpy were found to increase steadily with the value of *n*. By analogy with comblike poly(α-glutamate)s[4] and poly(β-aspartate)s,[7] this transition should be associated with the presence of a crystalline paraffinic phase made of alkyl side chains. At difference with these two other related families of polymers, the dodecyl side chains of PAAG-12 were found to be unable to crystallize. On the other hand, very small endotherms with enthalpies less than 1 J g^{-1} could be detected at temperatures above the main melting peak in certain traces. The presence

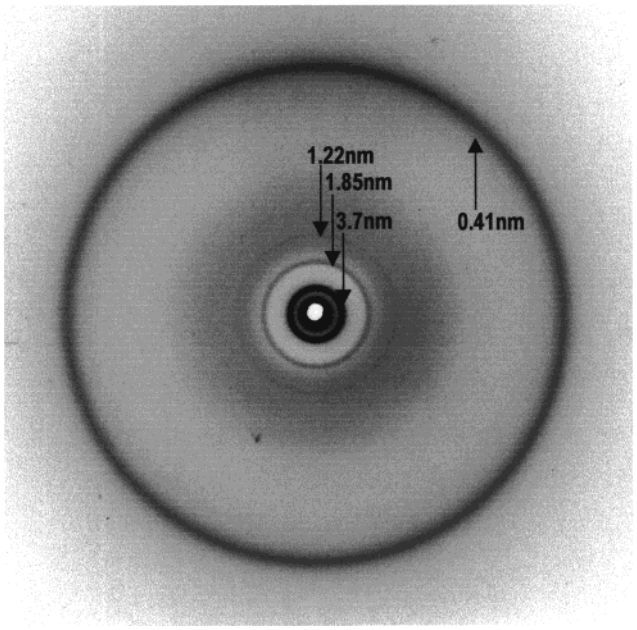

Figure 6. Powder X-ray diffraction pattern of PAA(DL)G-22.

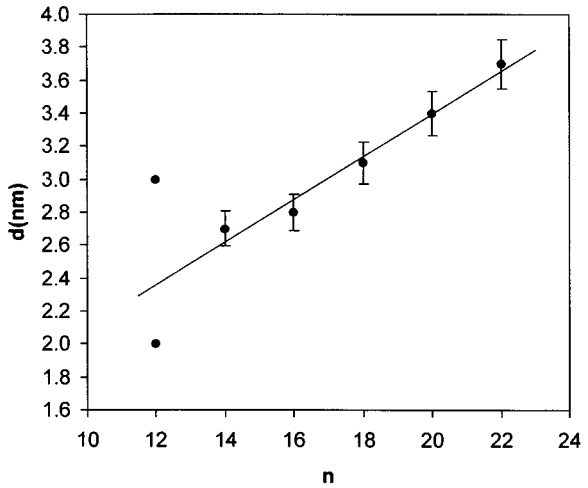

Figure 7. Long *d*-spacing observed by X-ray diffraction vs the number of carbons contained in the alkyl side chain for PAA(DL)G-*n*. Exactly the same values were obtained for the D-series.

of minor peaks in both poly(α-glutamate)s[4] and poly(*β*-aspartate)s[7] was interpreted as due to the occurrence of a second transition involving the interconversion between two crystal−liquid phases. The investigation of such peaks in PGGA-*n* is currently under way, and results will be accounted as a second part of this paper exclusively devoted to describe the solid-state structure of these polymers.

The TGA analysis of PAAG-*n* showed that decomposition of the polymers started to be appreciable above 300 °C and that the onset decomposition temperature is practically independent of the length of the alkyl side chain. A more uneven behavior was displayed at higher temperatures, specifically at those values at which the degradation process reaches the highest rate. Decomposition was found to take place in one or two steps at temperatures slightly increasing with *n*. The sample weight remained constant above 500 °C, and the weight loss at this temperature was proportional to the size of the alkyl side chain size. A recent study on the thermal degradation of PGGA and their alkyl esters has revealed that decomposition of these polypeptides implies the release of the alkyl side chain as a process concomitant to the scission of the main chain.[17] TGA traces of PAA(DL)G-14 and PAA(DL)G-22 are shown in Figure 5b for illustration.

X-ray Diffraction. Results obtained from powder X-ray diffraction of PAAG-*n* at room temperature were fully consistent with DSC results. The diffraction pattern of PAA(DL)G-22 is shown in Figure 6, and a comparative account of all the reflections observed for every polymer is given in Table 4. Data are only given for the DL-series since those obtained for the D-series are practically indistinguishable. These data can be interpreted on the basis of available knowledge on the structure of comblike poly(α-glutamate)s[4] and poly(*β*-aspartate)s.[7] A strong sharp ring with an associated spacing of 0.41−0.42 nm was present in the wide angle region of patterns obtained from PAAG-*n* for $n \geq 14$. Such reflection is thought to arise from the crystallized paraffinic phase made of hexagonal packing of alkyl side chains with an interchain distance of 0.48 nm. Conversely, a diffuse ring with an associated spacing of 0.450 nm characteristic of a disordered paraffinic phase was observed for PAAG-12. On the other hand, reflections with associated spacings of the order a few nanometers appeared in the medium angle region of the diffraction patterns. These reflections are interpreted to arise from a two-dimensional ordered supramolecular structure consisting of a layered arrangement of alternating paraffinic and polypeptide phases with the alkyl side chains partially interdigitized. Diffraction of stretched samples revealed that the alkyl chains are oriented at a right angle to the main chain.

Melting peak enthalpies of comblike polymers with crystallizable side chains are indicative of the number of methylenes participating in the crystalline paraffinic phase. We observed that the intensity of the melting peak was greatly depending on the method of preparation and on the thermal history of the sample. This means that correlation between *n* and the crystallized methylene fraction requires a systematic experimental study that has not been included in this work. Nevertheless, as can be seen in the plot of Figure 7, the periodicity of the layered structure increases almost linearly with *n* within the range 14−22 with a slope near 0.13 nm per methylene. Since the addition of one methylene in the all-trans conformation would entail an enlargement of the layer periodicity in ∼0.24 nm, there is little doubt that interdigitation, and very likely

Table 4. Powder X-ray Spacings (nm) of Poly(α-alkyl *γ*,DL-glutamate)s[a]

PAA(DL)G-12	PAA(DL)G-14	PAA(DL)G-16	PAA(DL)G-18	PAA(DL)G-20	PAA(DL)G-22
3.0 vs	2.7 vs	2.8 vs	3.1 vs	3.4 vs	3.7 vs
2.00 m	1.35 w	1.40 w	1.55 w	1.70 m	1.85 m
1.50 w					1.22 w
0.45 dif	0.42 s	0.42 s	0.42 s	0.42 s	0.41 s

[a] Visually estimated intensities denoted as vs (very strong), s (strong), m (medium), w (weak), dif (diffuse).

crystallization, of the alkyl side chain increases with *n*. In fact, a preliminary estimation based on the highest measured enthalpies shown in Table 3 affords a number of around 15 and 5 crystallized methylenes for PAAG-(DL)-22 and PAAG(DL)-16, respectively.

The deviation from linearity found for PAAG-12 is out of the margin of acceptable experimental error and could be attributable in principle to the uncrystallized state of the dodecyl side chain. It is noticeable however that, at difference with the all other homologues, the spacings of the three medium angle reflections observed for this compound (3.0, 2.0, and 1.5 nm) are not related by integers as should be expected for a set of reflections arising from a unique basic spacing. Accordingly, a supramolecular structure with a different type of organization should be adopted in this case. This is not surprising since poly(α-decyl γ,DL-glutamate)[12] has been found to crystallize in a three-dimensional array characteristic of PGGA-*n* bearing short alkyl side chains.

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References and Notes

(1) Loos, K.; Muñoz-Guerra, S. Microstructure and Crystallization of Comb-Like and Block Rigid-Coil Copolymers. In *Supramolecular Polymers;* Ciferri, A., Ed.; Marcel Dekker: New York, 2000; Vol. 7, pp 269−321.
(2) Watanabe, J.; Goto, M.; Nagase, T. *Macromolecules* **1987**, *20*, 298.
(3) López-Carrasquero, F. Synthesis and Structure of Poly(α-alkyl β,L-aspartate)s. Ph. D. Universitat Politècnica de Catalunya, 1995.
(4) Watanabe, J.; Ono, H.; Uematsu, I.; Abe, A. *Macromolecules* **1985**, *18*, 2141.
(5) Daly, W. H.; Poché, D.; Negulescu, I. *Prog. Polym. Sci.* **1994**, *19*, 79.
(6) (a) Ponomarenko, E. K.; Waddon, A. J.; Bakeev, K. N.; Tirrell, D. A.; MacKnight, W. J. *Macromolecules* **1996**, *29*, 4340. (b) Ponomarenko, E. K.; Waddon, A. J.; Tirrell, D. A.; MacKnight, W. J. *Langmuir* **1996**, *12*, 2169.
(7) López-Carrasquero, F.; Montserrat, S.; Martínez de Ilarduya, A.; Muñoz-Guerra, S. *Macromolecules* **1995**, *28*, 5535.
(8) Muñoz-Guerra, S.; López-Carrasquero, F.; Fernández-Santín, J. M.; Subirana, J. A. In *Polymeric Materials Encyclopedia*; Salamone, J. C., Ed.; CRC Press: Boca Raton, FL, 1996; Vol. 6, p 4694.
(9) (a) Seebach, D.; Overhand, M.; Kühnle F. N. M.; Martinoni, B. *Helv. Chim. Acta* **1996**, *79*, 913. (b) Appella, D. H.; Cristianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (c) Hanessian, S.; Luo, X.-H.; Schaum, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569.
(10) Puiggalí, J.; Muñoz-Guerra, S.; Rodríguez Galán, A.; Alegre, C.; Subirana, J. A. *Makromol. Chem., Macromol. Symp.* **1988**, *20/21*, 167.
(11) (a) Hintermann, T.; Gademann, K.; Jaun, B.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983. (b) Bremmer, M.; Seebach, D. *Helv. Chim. Acta* **2001**, *84*, 1181.
(12) (a) Muñoz-Guerra, S.; Melis, J.; Pérez-Camero, G.; Bou, J. J.; Congregado, F. *ACS Polym. Prepr.* **1998**, *39*, 138. (b) Melis, J. Synthesis and Structure of Poly(α-alkyl γ,L-glutamato)s. Ph.D. Universitat Politécnica de Catalunya, 1999.
(13) Gross, R. A. In *Bacterial Poly-γ-(glutamic acid)* in *Biopolymers from Renewable Resources*; Kaplan, D. L., Ed.; Springer: Berlin, 1998; p 195.
(14) Shah, D. T.; McCarthy, S. P.; Gross, R. A. *ACS Polym. Prepr.* **1993**, 488.
(15) Kubota, H.; Nambu, Y.; Endo, T. *J. Polym. Sci., Polym. Chem.* **1993**, *31*, 2877; **1995**, *33*, 85.
(16) (a) Borbély, M.; Nagasaki, J.; Borbély, J.; Fan, K.; Bhogle, A.; Sevoian, M. *Polym. Bull.* **1994**, *32*, 127. (b) Gonzales, D.; Fan, K.; Sevoian, M. *J. Polym. Sci., Polym. Chem.* **1996**, *34*, 2019.
(17) Melis, J. I.; Morillo, M.; Martínez de Ilarduya, A.; Muñoz-Guerra, S., submitted.
(18) Kubota, H.; Fukuda, Y.; Takebe, H.; Endo, T. USA Patent 5,118,784, 1994.
(19) Gross, R. A.; McCarthy, S. P.; Shah, D. T. USA Patent 5,378,807, 1995.
(20) Pérez-Camero, G.; Congregado, F.; Bou, J. J.; Muñoz-Guerra, S. *Biotechnol. Bioeng.* **1999**, *63*, 109.
(21) García-Alvarez, M.; López-Carrasquero, F.; Morillo, M.; Muñoz-Guerra, S. *J. Polym. Sci., Polym. Phys.* **1997**, *35*, 2379.
(22) Cromwick, A.-M.; Gross, R. A. *Int. J. Biol. Macromol.* **1995**, *17*, 259.
(23) Seebach, D.; Hungherbuhler, E.; Naef, R.; Schunurrenberger, P.; Weidmann, B.; Züger, M. *Synthesis* **1982**, 138.
(24) Martínez de Ilarduya, A.; Alemán, C.; García-Alvarez, M.; López-Carrasquero, F.; Muñoz-Guerra, S. *Macromolecules* **1999**, *32*, 3257.

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