

Synthesis and structure of random and block copoly(β ,L-aspartate)s containing short and long alkyl side chains

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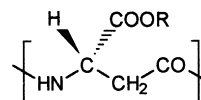
Abstract

Copoly(α -alkyl- β ,L-aspartate)s containing *n*-octadecyl and *n*-butyl side groups at different ratios were prepared by anionic ring-opening polymerization of the corresponding optically pure (*S*)-4-alkoxycarbonyl-2-azetidiones. Random copolymers were obtained by polymerization of comonomer mixtures. Diblock copolymers were achieved by sequential copolymerization using the living poly(α -*n*-octadecyl- β ,L-aspartate) block to initiate the polymerization of (*s*)-4-*n*-butoxycarbonyl-2-azetidione as a second block. Composition and sequence distribution were characterized by NMR with the help of a model copoly(β ,L-aspartate) made of α -*n*-dodecyl and α -benzyl β ,L-aspartate units. All the copolymers were found to adopt the layered structure made of 13/4 helices described previously for comb-like poly(α -*n*-alkyl- β ,L-aspartate) homopolymers. Copolymers containing at least 50% of *n*-octadecyl side groups have these groups crystallized in an interlayer microphase and aligned normal to the main helical chain. Melting of the paraffinic crystallites happened within the 40–60°C temperature range with formation of a liquid-crystal phase in which side chains are molten but retain the alignment of the low temperature phase. Different from what is known to happen to poly(α -*n*-octadecyl- β ,L-aspartate), no indications on the occurrence of a second structural transition were observed at higher temperatures. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Poly(β -aspartate)s; Copoly(β -peptide)s; Copoly(β -aspartate)s

1. Introduction

Throughout a number of papers that have been appearing in the last decade, it has become well established that β -peptide oligomers and polymers are able to adopt rod-like helical conformations with features similar to the familiar α -helix characteristic of poly(α -amino acids) [1–4]. The fascinating stereochemistry displayed by chiral peptides and polypeptides containing ω -amino acids has been recently reviewed [5]. Poly(α -alkyl- β ,L-aspartate)s are the poly(β -peptide)s deriving from the α -alkyl esters of L-aspartic acid. They can be looked at as nylon 3 derivatives with an alkoxycarbonyl group stereoregularly attached to the β -carbon of the repeating unit.



The helical arrangement usually adopted by these polymers consists of a right-handed 13/4 helix stabilized by intramolecular hydrogen bonds set between every third β -amino acid unit [6,7]. Such a helix has been demonstrated to exist both in solution and in the solid state for a number of poly(β ,L-aspartate)s including a wide variety of side groups [8]. Concentrated solutions of these polymers in halogenated solvents are known to form cholesteric mesophases [9] and oriented films having D_{∞} macroscopic symmetry were found to display piezoelectricity [10].

The structure adopted by poly(α -alkyl- β ,L-aspartate)s in the solid state is largely dependent on the length of the alkyl side chain. Those members bearing short alkyl groups

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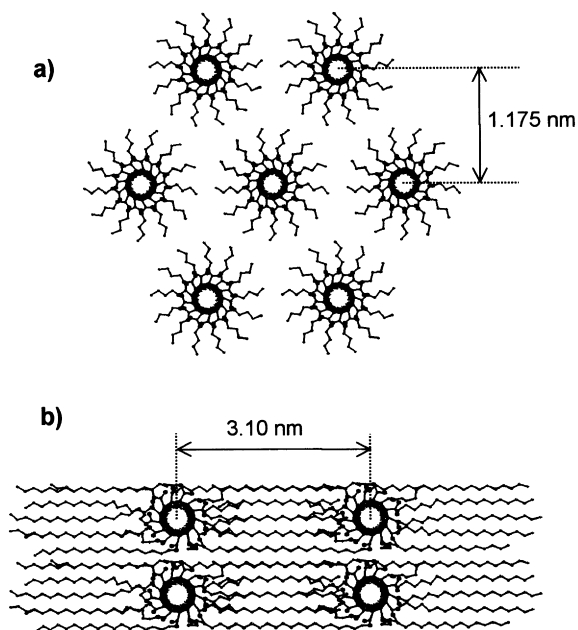


Fig. 1. Equatorial view of the packing of 13/4 helices of PAALA-*n*: (a) pseudo-hexagonal structure of PAALA-4; and (b) layered structure of PAALA-18.

(C₁–C₅) crystallize in three-dimensional ordered structures with the helices usually packed in hexagonal or tetragonal lattices [11–16]. On the other hand, those members with linear alkyl side groups consisting of 12 or more carbon atoms form two-dimensional ordered arrays with helices arranged in layers separated by the crystallized polymethylene side chains [17]. The structure and thermal behavior of these systems have been studied in detail by polarized infrared spectroscopy, CP-MAS NMR, DSC and X-ray and electron diffraction [17–19]. Three phases A, B and C are known to exist for these comb-like poly(β ,L-aspartate)s as a function of temperature. Melting of the side chains at temperatures between -15 and 75°C induces the conversion of the low-temperature phase A into a mesophase B, which presumably has a cholesteric structure. Upon heating at higher temperatures the phase B is converted into a third phase C in which the layered structure is lost but the main helical conformation is still retained. This pattern of behavior is not exclusive of poly(β ,L-aspartate)s since it had been observed before for thermotropic poly(γ -alkyl- α ,L-glutamate)s [20–22].

Copoly(α ,L-glutamate)s [23–30] and copoly(α ,L-aspartate)s [31] containing long alkyl side chains together with benzyl or methyl groups have been investigated in connection to their mesogenic properties and their capability to generate supramolecular structures. Copoly(α ,L-glutamate)s have been shown to form crystal structures and mesophases similar to those adopted by their parent homopolymers over a wide range of compositions. Phase transitions in these copolymers appear to occur at temperatures that depend on the composition. In this paper we wish to

report on poly(β ,L-aspartate) copolymers bearing both short and long alkyl side chains. Specifically, *n*-butyl and *n*-octadecyl side groups were combined in different proportions to provide a series of copoly(α -alkyl- β ,L-aspartate)s, abbreviated *co*PAALA-18,4, which is able to cover the whole range of behavior comprised between the two parent homopolymers. Poly(α -*n*-butyl- β ,L-aspartate), abbreviated PAALA-4, is known to crystallize preferentially in a pseudo-hexagonal structure of 13/4 helices with $a_0 = 1.35$ nm (Fig. 1a), although a second crystal structure made of a tetragonal packing of 4/1 helices is also known for this polymer [14]. On the other hand, poly(α -*n*-octadecyl- β ,L-aspartate), abbreviated PAALA-18, forms the typical lamellar structure of comb-like polymers with layers separated 3.1 nm and side chains partially crystallized with about eight to nine methylenes being included in the crystalline core (Fig. 1b). Two transition temperatures, T_1 ($\sim 60^\circ\text{C}$) and T_2 ($\sim 120^\circ\text{C}$), separating the three phases A, B and C, were characterized for this poly(β ,L-aspartate) [17].

In order to examine the influence of composition and microstructure of the polymer chain on properties, both random and block *co*PAALA-18,4 copolymers have been synthesized. Although several methods are available for the preparation of copoly(α -peptide)s [32,33] and nylon copolymers [34], it is the first time that the synthesis of copoly(β -peptide)s with adjusted composition and microstructure is undertaken. Non-assisted ring-opening polymerization of β -lactams catalyzed by bases has been the method of choice since it has repeatedly proven to be very efficient in the preparation of poly(β ,L-aspartate) homopolymers [35,36]. Whereas the direct application of the method as it is exactly used for the preparation of homopolymers, appears to be convenient for the copolymerization of alkoxy-carbonyl- β -lactam mixtures addressed to obtain the random copolymers, a novel procedure without precedents in the synthesis of poly(β ,L-peptide)s, has been developed for the preparation of the diblock copoly(β ,L-aspartate)s. The chemical structure of all these copolymers have been characterized in detail by NMR spectroscopy and their thermal properties evaluated by DSC and TGA. Their structure in the solid state has been investigated by polarizing infrared spectroscopy and X-ray diffraction techniques. Finally, the effect of temperature on the supramolecular structure has been also examined and the observed behavior discussed in relation to that displayed by their parent homopolymers PAALA-4 and PAALA-18.

2. Experimental

2.1. Materials and methods

All reagents and solvents used in this work were analytical grade or higher. L-Aspartic acid (98 + %, $[\alpha]_D^{25} = +25$, $c = 5$ in 2.5 M HCl) was supplied by Aldrich. All solvents were exhaustively dried according to standard procedures.

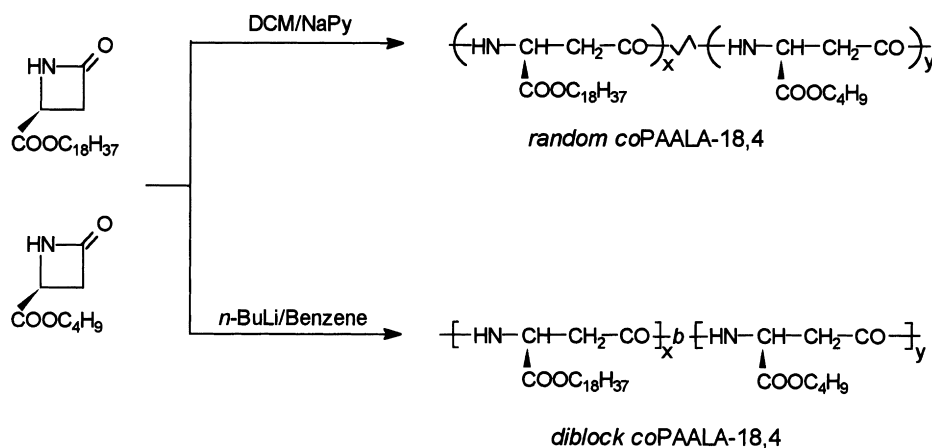


Fig. 2. Synthetic routes used in this work to prepare random and block poly(α - n -octadecyl- α - n -butyl- β -L-aspartate) copolymers.

Melting points of intermediate compounds were measured on a Gallenkamp apparatus and are uncorrected. Densities were measured by the flotation method in aqueous KBr–water mixtures. Infrared spectra were registered on a Nicolet Magna-IR spectrophotometer provided with a gold wire polarizer. Dichroic infrared spectra were recorded from thin polymer films oriented by mechanical stretching. Dichroic ratios were determined from the absorbances measured for the parallel and perpendicular orientations of the film to the infrared polarization vector. NMR spectra were recorded in solution at room temperature on an AMX-300 Bruker instrument operating at 300.1 and 75.5 MHz for ^1H and ^{13}C , respectively. Samples were dissolved in deuterated chloroform–trifluoroacetic acid mixtures added with a small amount of tetramethylsilane for internal reference. Calorimetric measurements were carried out with a Pyris I Perkin–Elmer instrument calibrated with indium. Samples of about 3–5 mg weight were heated or cooled at rates of $10^\circ\text{C min}^{-1}$ under a nitrogen atmosphere. TGA was performed on a Perkin–Elmer TGA 6 thermobalance with pieces of samples of about 5 mg placed under a nitrogen atmosphere and heated at a rate of $20^\circ\text{C min}^{-1}$ in the 20–600°C temperature range. XRD patterns were taken from both oriented and unoriented polymer films in a Statton-type camera using nickel-filtered CuK_α (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). Thermodiffractograms were recorded from unoriented polymer films in a Siemens D-500 diffractometer using CuK_α radiation and provided with a TPK-A Park heating stage and a scintillation counter. All polymer films used for this study were prepared by casting from chloroform and dried under vacuum. To induce orientation, as needed for dichroism infrared and X-ray diffraction, rectangular pieces of these films were subjected to uniaxial mechanical stretching under controlled temperature.

2.2. Synthesis of monomers and polymers

(*S*)-4- n -butoxycarbonyl- and (*S*)-4- n -octadecoxycarbonyl-2-azetidinones used in this work were prepared by transesterification of the optically pure (*S*)-4-benzyloxycarbonyl-2-azetidinone with the corresponding alcohol as described in detail elsewhere [37]. The latter compound was obtained from L-aspartic acid according to the method of Salzmann [38]. The chemical structure of the all three β -lactams was confirmed by NMR and their melting points were in accordance with previously published data [37]. The absence of occurrence of racemization reactions along the whole process of synthesis was assessed by ^{13}C NMR spectroscopy using the chiral shift reagent $\text{Eu}(\text{TFC})_3$. In fact, no splitting was observed for the β -lactam carbon atoms in the “in situ” formed $\text{Eu}(\text{TFC})_3$ - β -lactam complex. This is a method frequently used to determine the enantiomeric purity of amino compounds [39].

For the preparation of the random copolymers the following recipe was followed: under a stringent nitrogen atmosphere and vigorous stirring, a mixture of the two β -lactam comonomers at the chosen ratio was dissolved in dichloromethane to have a final monomer concentration of 8% and sodium pyrrolidone was then added to have a molar ratio of catalyst to monomer of 4%. Molar ratios of n -octadecoxycarbonyl to n -butoxycarbonyl β -lactams of 90:10, 70:30, 50:50 and 30:70 were used. Polymerizations were left to proceed at room temperature for 24 h after which the reaction mixture was diluted with 1 volume of solvent and precipitated with 3 volumes of methanol. For purification, crude products were dissolved in pure chloroform or chloroform containing a small amount of TFA, and precipitated by adding 1.5 volumes of methanol dropwise. The precipitated copolymers were recovered by centrifugation and stored under vacuum.

Block copolymers were prepared by sequential copolymerization in benzene under an argon atmosphere. The required amounts of the two β -lactams were separately

Table 1
Polymerization results of *co*PAALA-18, 4 (n.d: not determined)

Polymer (C ₁₈ :C ₄) ^a	Yield (%)	[η] ^b (dl g ⁻¹)	(C ₁₈ :C ₄) ^c	ρ ^d (g ml ⁻¹)
<i>Random</i>				
100:0	84	3.35	100.0	1.03
90:10	76	3.95	92.0:8.0	1.04
70:30	71	4.91	71.0:29.0	1.02
50:50	74	3.28	50.0:50.0	1.06
30:70	95	4.95	33.5:67.5	1.06
0:100	55	2.00	0:100	1.17
<i>Diblock</i>				
100:0	85	2.67	100:0	n.d
90:10	79	0.97	78.5:21.5	1.03
70:30	77	0.97	63.0:37.0	1.05
50:50	74	0.90	54.0:46.0	1.06
0:100	45	n.d	0:100	n.d

^a Molar ratio of (*S*)-4-*n*-octadecoxycarbonyl to (*S*)-4-*n*-butoxycarbonyl-2-azetidinone in the feed.

^b Intrinsic viscosity measured in CHCl₃:DCA (2:1) mixture except for the 0:100 ratio which was measured in pure DCA.

^c Molar ratio of α-*n*-octadecyl to α-*n*-butyl β-L-aspartate unit in the copolymer calculated on the basis of ¹H NMR data with an assumable error of ±0.5.

^d Density measured by the flotation method in KBr aqueous solution.

placed in dried flasks under a vacuum of 10⁻⁴ mmHg and fused by gently heating. Benzene was distilled onto the molten monomers to have a solution of 5% (w/w) of each. First, polymerization of the (*S*)-4-*n*-octadecoxycarbonyl-2-azetidinone was performed by adding *n*-butyllithium under stirring and the mixture left to react for 1 h at room temperature. The appropriate volume of benzene solution of the (*S*)-*n*-butoxycarbonyl-2-azetidinone was then added and the mixture left to react for 1 h under the same conditions that

were used in the first step. Copolymers with *n*-octadecyl to *n*-butyl side chain nominal molar ratios of 90:10, 70:30 and 50:50 in addition to the two homopolymers PAALA-4 and PAALA-18 were prepared by this procedure.

3. Results and discussion

3.1. Synthesis

Two series of copoly(β-L-aspartate)s, one with a random microstructure and the other in blocks, were prepared from optically pure (*S*)-4-butoxycarbonyl and (*S*)-4-octadecoxycarbonyl-2-azetidinones covering a range of compositions including from homopolymers up to copolymers with 50:50 molar ratios of the two comonomers. The two procedures used in the preparation of these copolymers are shown in the reaction scheme depicted in Fig. 2 and results obtained therefrom are collected in Table 1. Although in both cases polymerization proceeded by an anionic ring-opening mechanism, the methodology applied was a function of the type of microstructure desired for the copolymer. For the preparation of random copolymers, mixtures of the two β-lactam comonomers with the selected compositions were copolymerized using exactly the same methodology developed earlier by us for the synthesis of the homopolymers. By this means polymers with intrinsic viscosities above 2.0 and yields between 55 and 95% were achieved. For the preparation of the diblock copolymers, polymerization was performed in two steps corresponding to the generation of the two blocks. Firstly, the (*S*)-4-octadecoxycarbonyl-2-azetidinone was polymerized to create the living poly(α-*n*-octadecyl-β-L-aspartate) block, which was then used to

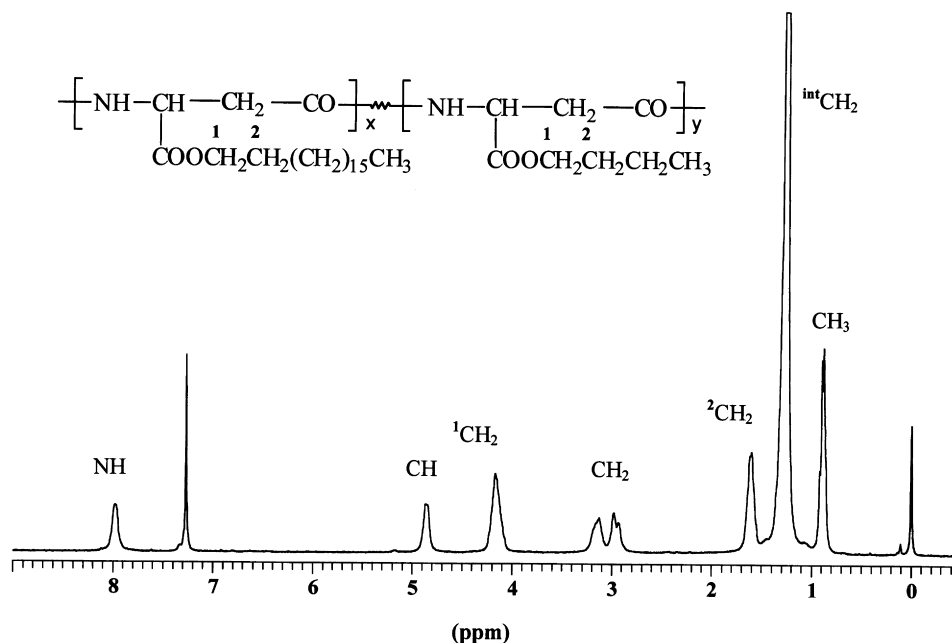


Fig. 3. ¹H NMR spectrum of random *co*PAALA-18,4 (70:30).

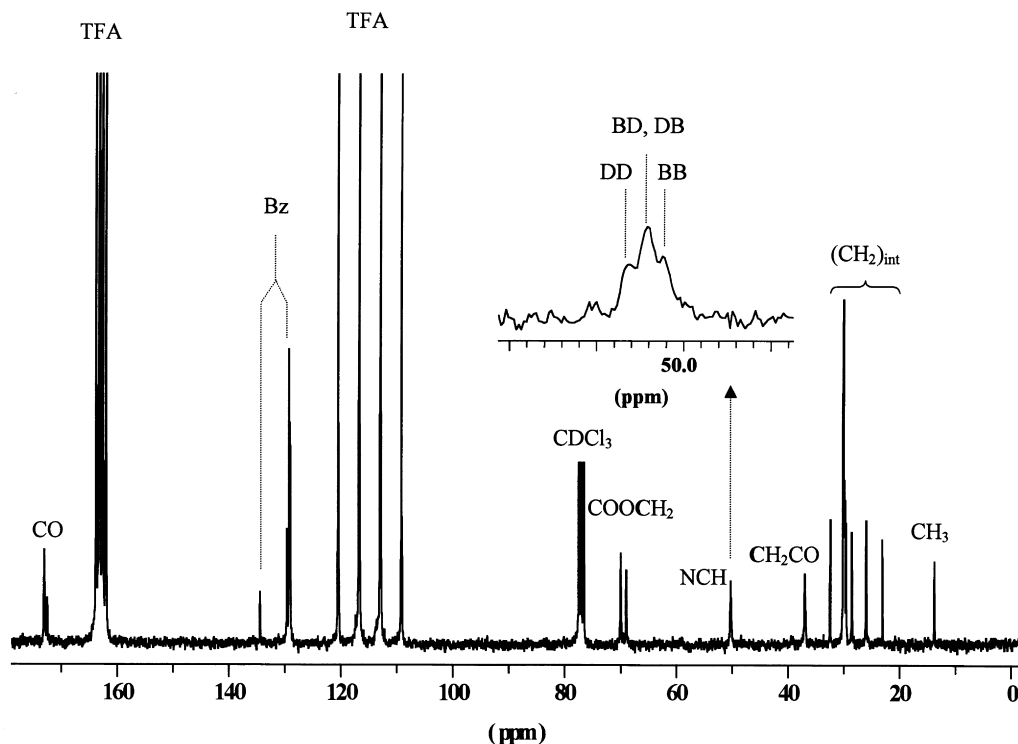


Fig. 4. ^{13}C NMR spectrum of *coPAALA-Bz,12* (50:50); inset: the main chain methine carbon signal used for the determination of diad contents.

initiate the polymerization of the (*S*)-4-butoxycarbonyl-2-azetidinone. Copolymers with intrinsic viscosities above 0.90 at yields between 45 and 85% were thus produced. It appears therefore that significantly lower polymer molecular weights are obtained by this procedure when compared to simultaneous polymerization of the two β -lactams leading to random copolymers. It should be noted however that no direct measurement of the molecular weights of the copolymers has been carried out in this work and only a rough estimation of the polymer size can be made on the basis of viscosity data given in Table 1. Previous determinations made with PAALA homopolymers using the viscosimetric equations reported for related poly(α ,L-amino acid)s [40,41] indicated a molecular weight over 10^5 for a sample of PAALA-18 having an intrinsic viscosity of 1.6 in chloroform:DCA (2:1). By comparison, molecular weights of the *coPAALA-18,4* copolymers synthesized in this work may be assumed to span within the broad range $0.5\text{--}5 \times 10^5$.

Both ^1H and ^{13}C NMR spectra were in accordance with the chemical constitution expected for the copolymers. The ^1H NMR spectrum of random *coPAALA-18,4* (70:30) is shown in Fig. 3 for illustration. Signals arising from protons attached to the main chain appear clearly separated from those due to alkyl side protons and are in agreement with the regular stereochemistry expected for the polymer. Copolymer compositions were determined by relating the integrated areas of the peaks arising from the methylene protons in the alkyl side chain to that of the methine proton in the

main chain. Results from this analysis are shown in Table 1 indicating that the correspondence between the experimentally determined values and those anticipated from the compositions used in the respective feeds is overall satisfactory. Nevertheless, some discrepancies worth of mention were detected in certain cases. Whereas a very good agreement is observed for random copolymers for any composition, significant divergences were found in the case of diblock copolymers that increased with the ratio of octadecyl to butyl comonomer used in the feed. Furthermore, considerable deviations in the compositions, which may arrive up to 40% in the case of diblock copolymers and up to 20% in the case of random copolymers, were found for different batches. Such a lack of reproducibility appears to be a serious drawback of this synthesis, which requires additional investigation. The *coPAALA-18,4* copolymers examined in this work are those with the compositions indicated in Table 1, which, for simplicity, have been labeled in the text with the composition ratios used in their respective reaction feeds.

Unfortunately, differences in the microstructure of these copolymers cannot be distinguished in the NMR spectra at the magnetic field applied in our analysis. With the aim to assess the random nature of *coPAALA-18,4* copolymers, a model copoly(β -L-aspartate) was prepared from an equimolar mixture of (*S*)-4-dodecoxy and (*S*)-4-benzyloxycarbonyl-2-azetidinones. The obtained copolymer, coded as *coPAALA-Bz,12*, had an intrinsic viscosity of 3.6 ml g^{-1} and a composition ratio of 51:49 as determined by ^1H

Table 2
Composition and microstructure of *co*PAALA-Bz, 12 (50:50)

	Composition		Diads (%)			Average sequence length		Randomness
	x_B	x_D	BB	BD,DB	DD	n_B	n_D	
Exp ^a	51	49	16.8	61.5	21.7	1.55	1.70	1.23
Theor ^b	50	50	25	50	25	2.0	2.0	1.00

^a Experimentally determined by ¹H NMR.

^b Calculated for a Bernoullian statistical model for a copolymer with 50:50 composition.

NMR. The larger constitutional difference between the benzyl and dodecyl groups makes feasible the analysis of the microstructure of this copolymer by NMR. The ¹³C NMR spectrum of *co*PAALA-Bz,12 is shown in Fig. 4. A close inspection of this spectrum revealed that the signal at 50.2 ppm arising from the main chain methine carbon is sensitive to comonomer sequences and can be used therefore to characterize the microstructure of the copolymer. The signal is resolved in three peaks attributable to diads DD, DB/BD and BB present in the copolymer. An estimation of the content in such diads was performed by quantification of the areas of the three peaks resulting from the Lorentzian deconvolution of the merged signal and the obtained results are shown in Table 2. It can be concluded from such data that the structure of *co*PAALA-Bz,12 copolymer is at random and that the copolymerization nearly

follows a Bernoullian statistics. This result can be readily extrapolated to *co*PAALA-18,4 since the chemical difference between the two comonomers is smaller in this case and the two respective homopolymers (PAALA-18 and PAALA-4) are known to adopt the same kind of secondary structure. It should be remarked that the generation of a random copolymer in the polymerization of optically pure β -lactam mixtures is not obvious. In fact, stereoselective copolymerization is known to occur in the copolymerization in solution of racemic mixtures of 4-isobutyl-2-azetidiones due to the formation of helical secondary structures with opposite handedness [42].

3.2. Thermal behavior

It is known that poly(α -alkyl- β ,L-aspartate)s with short alkyl side groups ($n < 10$) do not show any thermal transition other than a melting-decomposition temperature in the proximity of 300°C [36]. Conversely, members bearing linear alkyl side groups with 12 or more carbon atoms display an endotherm in the -20 to 80°C temperature region, depending on the alkyl chain length, which is due to melting of the paraffinic crystallites made of such side chains. Specifically, PAALA-18 shows a melting peak at ~50–60°C with an associated enthalpy of about 13 cal g⁻¹. The heating traces produced by *co*PAALA-18,4 copolymers over the 20–100°C temperature region are depicted in Fig. 5. A broad bimodal endotherm located around 40–60°C is seen on the traces of all these copolymers except for random *co*PAALA-18,4 (30:70) whose thermogram appears thoroughly flat. Both temperatures and enthalpies for the two sets of *co*PAALA-18,4 are given in Table 3. Similar data corresponding to PAALA-18 and PAALA-4 homopolymers obtained by the two methods of synthesis used in this work are included for comparison. The enthalpy associated to such transition was found to steadily increase with the content in α -*n*-octadecyl- β ,L-aspartate units in both random and block copolymers with values being higher for the second series. According to preceding work made on comb-like PAALA homopolymers, such endotherm is attributed to melting of the crystallites made up of *n*-octadecyl chains. The bimodal nature of the melting endotherm is interpreted as due to the existence of diverse populations of crystallites differing in size as it was reported to occur also for PAALA-18 [17]. In fact, annealing of block

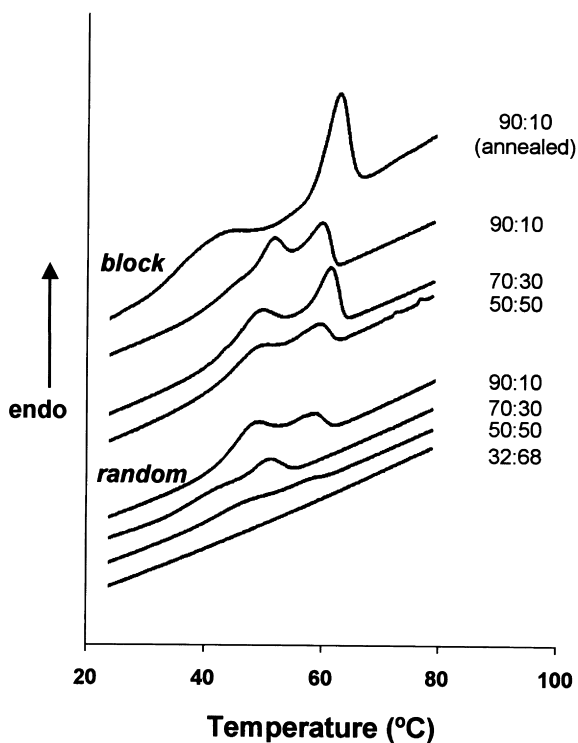


Fig. 5. Compared heating traces for random and diblock *co*PAALA-18,4 polymers with the indicated compositions. The top trace illustrates the effect of annealing on melting of diblock copolymer *co*PAALA-18,4 (90:10).

Table 3
Thermal properties of *coPAALA-18,4* (n.o: not observed)

Polymer (C ₁₈ :C ₄)	T _m ^a (°C)	ΔH ^b (cal g ⁻¹)	ΔH ^c (kcal mol ⁻¹ C ₁₈ ⁻¹)	T _d ^d (°C)
<i>Random</i>				
100:0	54–64	13.2	4.8	330
90:10	48–58	6.2	2.4	340
70:30	40–51	3.0	1.3	340, 385sh
50:50	46–58	1.1	0.6	340, 385sh
32:68	n.o	n.o	n.o	330, 385sh
0:100	n.o	n.o	n.o	295, 385
<i>Diblock</i>				
100:0	48–60	15.3	5.6	340
90:10	51–61	10.5	4.5	340
70:30	48–61	9.8	4.7	330, 390
50:50	46–59	5.0	2.7	305, 390
0:100	n.o	n.o	n.o	305, 390

^a Melting temperatures measured on pristine samples at a heating of 10°C min⁻¹.

^b Melting enthalpies measured by DSC at the conditions indicated above.

^c Molar melting enthalpies calculated for the compositions experimentally determined for each copolymer.

^d Decomposition temperatures given by the peaks of the derivative curve of the TGA trace; (sh, seen as a shoulder).

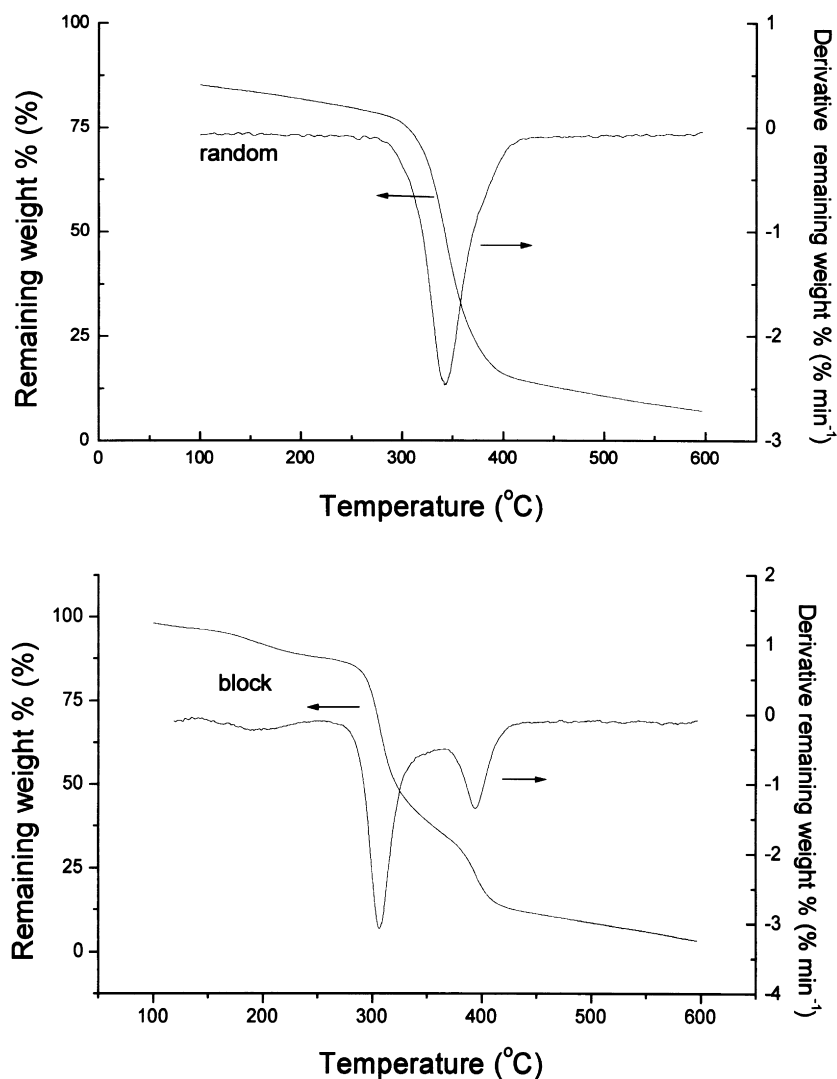


Fig. 6. TGA traces of: (a) random; and (b) diblock copolymers *coPAALA-18,4* (50:50) and their corresponding derivative curves.

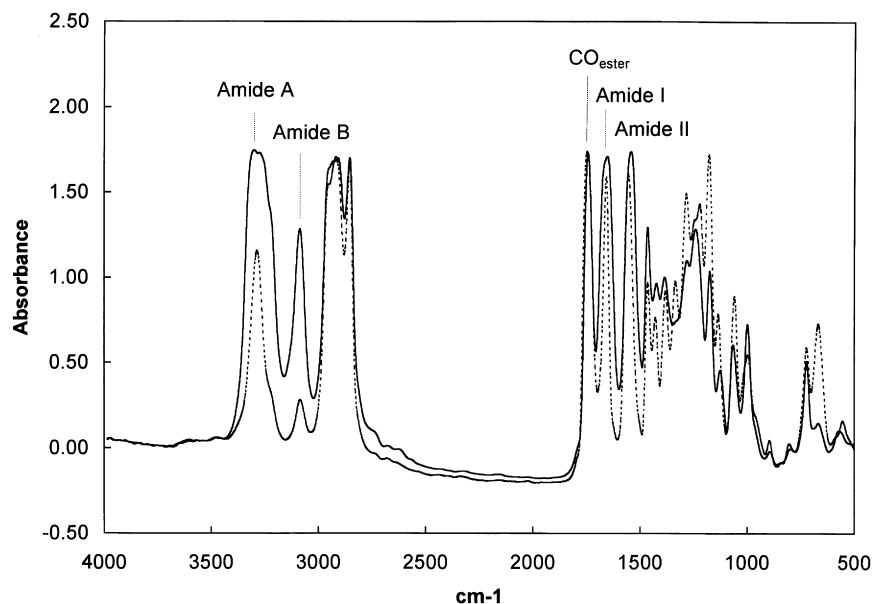


Fig. 7. Polarized infrared spectra of *coPAALA-18,4* (70:30) recorded with the electric vector of the polarized radiation parallel (solid line) and perpendicular (dotted line) to the orientation axis of the copolymer film.

coPAALA-18,4 copolymers at temperatures just below the low-temperature peak induced the removal of such peak as well as the shift of the higher temperature one towards higher values (see Fig. 5, top trace). The broad endotherm appearing at about 45°C after the treatment is thought to arise from melting of crystallites that were deficiently formed upon cooling. It can be concluded from these results therefore that crystallization of the octadecyl side groups in poly(β ,L-aspartate)s is feasible even in the presence of large amounts of butyl groups. Whereas melting temperature remains nearly constant for all compositions, the degree of crystallinity appears to be notably reduced in the case of copolymers with a random distribution of the two comonomers.

The thermal stability of *coPAALA-18,4* copolymers under inert atmosphere was evaluated by TGA. Representative curves are shown in Fig. 6 for the specific cases of random and block *coPAALA-18,4* (50:50). Decomposition temperatures are given in Table 3 for the two copolymer series. A small weight loss around 200°C of unknown origin and not included in Table 3, was incidentally observed for some block copolymers. As reported for homopolymers, decomposition of copolymers was found to take place above 300°C in a two-step process. Although the presence of the two peaks in the derivative curve indicative of the two steps becomes detectable in all cases, the separation between them is clearer for block copolymers and the relative importance of the second step increases with the content in butyl side groups. The first decomposition step of poly(β ,L-aspartate)s is known to consist of an intramolecular imidation reaction implying the scission of the alkyl side group and volatilization of the alcohol then produced. This mechanism is also believed to operate in the *coPAALA-*

18,4 copolymers, although the separation between the two steps becomes less apparent for the random copolymers due to the simultaneous decomposition of the α -*n*-octadecyl and α -*n*-butyl-L-aspartate units and subsequent simultaneous release of 1-butanol and 1-octadecanol. The process responsible for the second step has been related to unspecific main chain scissions. Along the poly(α -alkyl- β ,L-aspartate) homopolymer series, the relative importance of the second decomposition step with regards to the first one is known to decrease with the length of the alkyl side group. Such a trend is shared by *coPAALA-18,4* copolymers provided that the apparent side chain length is taken as the average resulting from the copolymer composition.

3.3. Chain conformation and supramolecular structure

Dichroic infrared spectra revealed that the α -helix-like conformation characteristic of poly(α -alkyl- β ,L-aspartate)s is retained in the *coPAALA(18,4)* polymers. The spectrum shown in Fig. 7 was recorded from a film of diblock *coPAALA-18,4* (70:30) that was uniaxially oriented by mechanical stretching. Characteristic absorption bands arising from amide main chain and carboxylate side chain groups are the prominent features of such spectrum. Amide bands known as amide A, amide B, amide I and amide II appear displaying parallel dichroism consistent with the presence of a right-handed 13/4 helix oriented with the helical axis parallel to the orientation axis of the film [18]. A similar dichroic behavior was observed for the random copolymer *coPAALA-Bz,12* (50:50). It should be noticed that the parallel dichroism shown by the amide II band, which is a feature characteristic of all poly(β ,L-aspartate)s, is contrary to the perpendicular dichroism that should

Table 4
Polarized infrared of *co*PAALA-18,4

Polymer	Dichroic ratio, D (parallel to perpendicular)					F_{\min}^a
	Amide A (3280–3290 cm^{-1})	Amide B (3085–3087 cm^{-1})	Amide I (1655–1660 cm^{-1})	Amide II (1545–1560 cm^{-1})	Ester side group (1740–1750 cm^{-1})	
<i>Random</i>						
90:10	1.31	3.28	1.05	1.07	1.03	0.43
70:30	1.24	2.99	1.01	1.03	0.94	0.40
50:50	1.50	3.87	1.06	1.10	0.94	0.49
30:70	1.07	2.48	1.01	1.02	0.97	0.33
<i>Diblock</i>						
90:10	1.57	3.06	1.13	1.20	0.97	0.41
70:30	1.49	4.27	1.04	1.06	0.98	0.52
50:50	2.73	4.77	1.49	1.57	0.95	0.56

^a Calculated using dichroic ratios for amide B band.

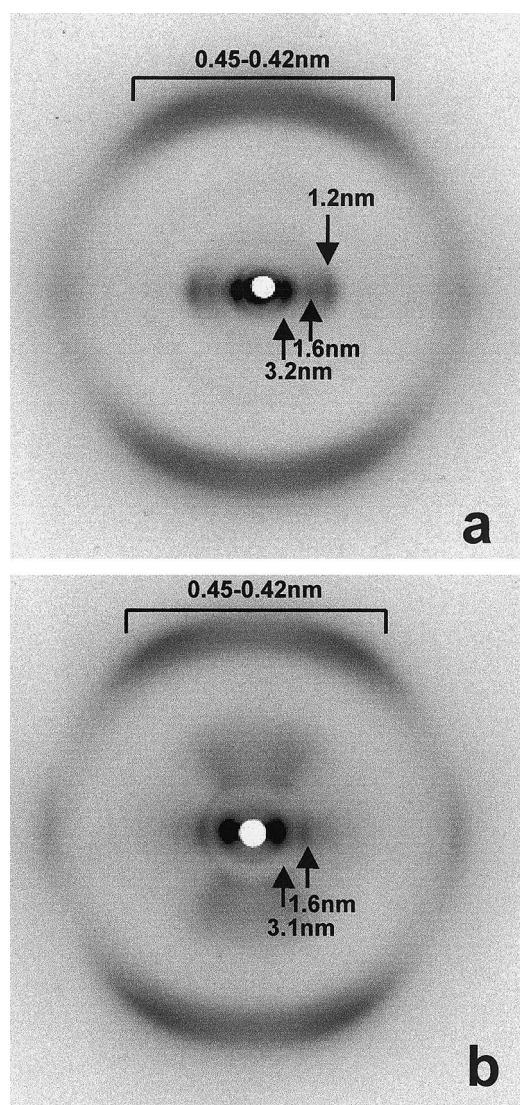


Fig. 8. X-ray diffraction patterns of oriented films of: (a) diblock *co*PAALA-18,4 (50:50); and (b) random *co*PAALA-18,4 (90:10).

be expected for the α -helix of poly(α -peptide)s. Modeling simulations studies carried out by us on several poly(β ,L-aspartate)s with different side chain groups revealed that such discrepancy is due to the particular topology of the 13/4 helical conformation. On the other hand, the dichroic ratio shown by the C=O stretching of the ester group is very small indicating that such group adopts essentially a random orientation in the film. This is contrary to what is usually observed for homopolymers in which a perpendicular orientation with respect to the helix axis is clearly preferred by the planar carboxylate group. Polarized infrared data for all *co*PAALA-18,4 and *co*PAALA-Bz,12 (50:50) are given in Table 4, which indicates similar dichroic properties for all the examined copolymers; the slight differences observed in the dichroic ratios may be attributable to differences in the degree of orientation of the films.

X-ray diffraction of stretched films of *co*PAALA-18,4 copolymers produced oriented diffraction patterns as those shown in Fig. 8. Spacings and visually estimated intensities of the reflections appearing in such patterns are compared in Table 5 for the whole *co*PAALA-18,4 series examined in this work, as well as for the PAALA-4 and PAALA-18 homopolymers. The equatorial reflections contained in the low-angle region, which have basic spacings of about ~ 3.1 nm and ~ 1.2 nm for homopolymers PAALA-18 and PAALA-4, respectively, are related to the repeating interlayer distance of the lamellar structure and to the 100 interplanar distance of a pseudohexagonal structure with helices separated 1.35 nm. The meridional and off-meridional wide-angle region of the pattern includes high-intensity scattering with spacings between 0.4 and 0.5 nm. Discrete reflections with spacings around 0.45–0.5 nm are thought to arise from the pitch of the 13/4 helix with a period of ~ 2.0 nm and their presence provides further evidence on the existence of the helical conformation. Additionally ~ 0.42 nm reflections are also present in the diffraction patterns of poly(β ,L-aspartate)s bearing crystallizable alkyl side groups. They are assigned to the 100, 010 and

Table 5

Observed X-ray spacings (nm) for *coPAALA-18,4* (visual estimates of intensities denoted as vs (very strong), s (strong), m (medium), w (weak), vw (very weak) and d (diffuse))

	Homopolymers C ₁₈ :C ₄		Random copolymers C ₁₈ :C ₄				Block copolymers C ₁₈ :C ₄		
	100:0	0:100	90:10	70:30	50:50	30:70	90:10	70:30	50:50
Low-angle region	3.1 vs 1.6 s 1.0 vw	1.2 s	3.1 vs 1.6 s	3.0 vs 1.5 s	3.0 s 1.5 w	2.3–2.4 vs	3.1 vs 1.6 m	3.2 vs 1.6 m 1.2 m	3.1 vs 1.6 m 1.2 s
Wide-angle region	0.45 m,d 0.42 s	0.45–0.49 s	0.45 m,d 0.42 s	0.45 m,d 0.42 m	0.45 m,d 0.42 w	0.45 m,d	0.45 m,d 0.42 s	0.45 m,d 0.42 s	0.45 m,d 0.42 m

–110 interplanar distances of a paraffinic hexagonal lattice made of polymethylene chains lying with an orientation perpendicular to the main chain helices. On the basis of these antecedents and the comparative inspection of data contained in Table 5, the following conclusions concerning the structure *coPAALA-18,4* copolymers may be drawn:

1. The interlayer distance characteristic of PAALA-18 is retained in copolymers with contents in octadecyl- β ,L-aspartate units of 50% or higher, regardless the distribution of the comonomers along the chain. According to DSC results, the amount of crystallized octadecyl side chain in random *coPAALA-18,4* (50:50) is quite small. The fact that this copolymer is able to retain the 3.1 nm

distance reveals the critical effect that crystallization of the side chain has on the determination of the lamellar structure periodicity. On the other hand, the 2.2–2.3 nm spacing found for *coPAALA-18,4* (30:70), for which no trace of crystallization was observed, is interpreted as the interlayer distance of a lamellar structure with side chains in a disordered state.

2. The presence of the 1.2 nm reflection with strong intensity in the diffraction patterns of block *coPAALA-18,4* with butyl group contents of both 30 and 50% reveals the occurrence of a separated crystalline phase made up exclusively by *n*-butyl- β ,L-aspartate units in addition to the lamellar structure constituted by the poly(α -*n*-octadecyl- β ,L-aspartate) blocks. The compatibility of these two ordered phases for the same copolymer is not topologically understandable since not only is the arrangement of side chains different in each one but the density of helices is notably higher for the counterpart bearing the short *n*-butyl side groups. Nevertheless, a reasonable description may be offered if the interphase connecting the two structures is assumed to be in a disordered state. This is consistent with DSC results which indicated that a considerable amount of material remains uncrystallized state in such copolymers.

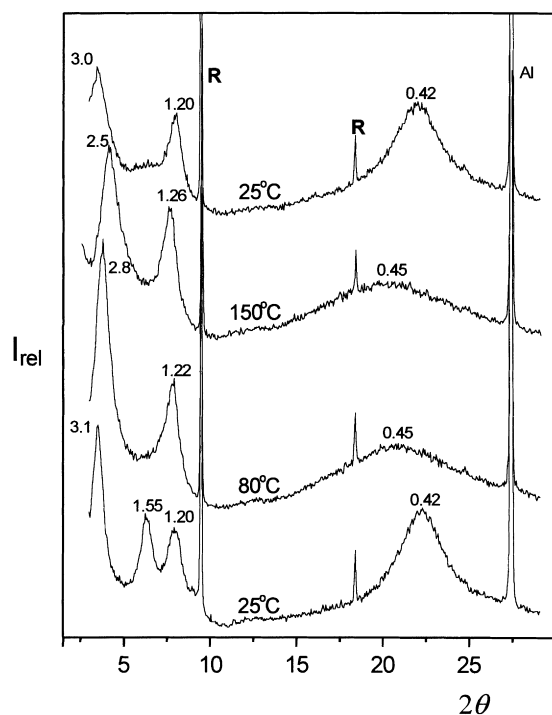


Fig. 9. Powder X-ray thermodiffractograms of diblock *coPAALA-18,4* (70:30) recorded at the indicated temperatures. Spacings are indicated in nm. R labeled peaks come from synthetic fluorophlogopite mica ($d_{001} = 0.998$ nm) used for reference. The peak at $2\theta \cong 27^\circ$ arises from the aluminum foil used for support.

In order to investigate the changes that temperature induces in the structure of *coPAALA-18,4* copolymers, X-ray diffractograms were recorded as a function of temperature within the range 25–150°C. A selection of representative traces obtained in the analysis of block *coPAALA-18,4* (70:30) are compared in Fig. 9. The profile recorded at 25°C shows the peaks with the associated spacings listed in Table 5 which are used to characterize the low-temperature phase A. Upon heating at 80°C, the 0.41 nm peak arising from the paraffinic crystalline phase broadened and shifted to 0.46 nm as it should be expected for the molten state. At the same time, a shortening of the interlayer long spacing, initially being 3.1 nm, down to 2.8 nm was observed to happen. Such changes are almost identical to those occurring in PAALA-18, which were interpreted as due to the transition of the crystalline phase A into a mesophase B with cholesteric structure. In accordance with this, striking changes in color were observed under the polarizing optical

microscope when oriented *co*PAALA-18,4 films were progressively heated at temperatures above the melting of the side chain. However, the low-angle spacing change encompassing this heating is much smaller (~ 0.25 nm) than that reported to occur in the B \rightarrow C transition (~ 0.5 nm) taking place in PAALA-18. It should be added that, different from what is known to happen in the homopolymer, no sign of heat absorption is detected in the DSC traces of *co*PAALA-18,4 obtained at heating above the side chain melting peak. Such differences in behavior throw serious doubts upon the occurrence of the B \rightarrow C transition in these copolymers and demand further detailed investigation to characterize the thermal stability of mesophase B. Finally it can be noted that, according to expectations, no significant shift upon heating is observed for the 1.2 nm peak arising from the pseudohexagonal phase made up of poly(α -*n*-butyl- β ,L-aspartate) blocks.

4. Conclusions

The method of synthesis developed earlier by us for the preparation of poly(α -alkyl- β ,L-aspartate)s, which is based on non-assisted anionic ring opening polymerization of β -lactams, has proven to be useful also for the preparation of copolymers made up of α -*n*-butyl and α -*n*-octadecyl- β ,L-aspartate units. Random and diblock copolymers have been obtained by applying the appropriate procedure of insertion of the two comonomers. The composition of the copolymer may be approximately defined by adjusting the composition of the feed although considerable divergences between expected and obtained results were found in the preparation of block copolymers. Polymerization of β -lactam mixtures proceeded with the two comonomers entering in the growing chain at random and effective living homopolymers useful for block copolymerization could be readily obtained under special conditions. All the copolymers were found to adopt layered structures made of 13/4 helices and copolymers containing at least 50% of α -*n*-octadecyl β ,L-aspartate units displayed crystallization of the long alkyl side groups. Melting of the paraffinic crystallites at 40–60°C led to the formation of a liquid-crystal phase with side chains in the molten state, but retaining the typical layered arrangement. Different from what is known to happen in comb-like poly(α -alkyl- β ,L-aspartate)s no clear evidence of a second transition occurring at higher temperatures could be obtained in this case. On the other hand, block copolymers containing 30 and 50% of *n*-butyl side groups showed, in addition to the lamellar structure, a separated second phase consisting of the pseudohexagonal arrangement of helices characteristic of poly(α -*n*-butyl- β ,L-aspartate).

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