Synthesis and properties of diblock copolymers of ω -pentadecalactone and α -amino acids

Ernesto Tinajero-Díaz, Antxon Martínez-de Ilarduya, Sebastián Muñoz-Guerra*

Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, ETSEIB

Diagonal 647, 08028 Barcelona, Spain

*Corresponding author: sebastian.munoz@upc.edu

Abstract

Diblock copolymers (PPDL_x-*b*-pPAA_y) were prepared from ω -pentadecalactone and L-glutamic acid or L-lysine amino acids by ring opening polymerization initiated by amino groups. Telechelic amino-ended poly(ω -pentadecalactone) with a length of 15-20 repeating units was firstly synthesized by enzymatic polymerization by means of CALB and then used as macroinitiator for the polymerization of the *N*-carboxyanhydrides of the two α -amino acids conveniently protected as benzyl and ϵ -*N*-carboboxy derivatives, respectively. The molecular weight of the polypeptide block was accurately controlled by adjusting the amino acid/macroinitiator ratio used in the feed. Copolymers with M_n ranging between ~5000 and ~40,000 g·mol⁻¹ and varying ester/peptide ratio were obtained and characterized in detail by GPC and NMR spectroscopy. The thermal properties of these copolymers were evaluated by TGA and DSC, and their structure in the solid-state including their response to heating, were examined by FTIR and XRD at variable temperature. It was shown that the polypentadecalactone segment was crystallized for all compositions and that the polypeptide counterpart adopted a two-dimensional hexagonal packing of α -helices at temperatures above melting of the polyester block. SAXS revealed the presence of a biphasic nanostructure with a repeating distance of 27 nm for the case of glutamic-based copolymers. It was demonstrated that both glutamic and lysine-based PPDL_x-*b*-pPAA_y copolymers could self-assemble in well-shaped nanospheres with a diameter in the ~200-400 nm range and a negative zeta-potential.

1. Introduction

Block copolymers containing at least one polypeptide block provide advantages over conventional synthetic polymers due to their ability to hierarchically self-assemble into stable ordered arrangements [1,2]. The polypeptide moiety in these copolymers is usually arranged in the familiar α -helix or β -sheet structure depending on the side chain of the constituent amino acid. The stiff polypeptide conformation is known to exert a decisive influence on the copolymer structure adopted in the solid state and its self-assembling properties [3,4]. In fact structures at several length-scales with uncommon properties have been observed in the solid state for these copolymers, in which one of the blocks is a rod-like polypeptide and the other one is a flexible polymer [5,6]. Formation of micelles [7], vesicles, and bilayer aggregates [8] is also known to take place in these copolymers in aqueous medium according to their composition and environment conditions. Polypeptide-based copolymers are therefore outstanding building compounds for biomaterials due to their tunable chemical architecture, biocompatibility, biodegradability, and ability to take up responsive secondary structures [9].

Among the diversity of peptide-based block copolymers today available [10,11], those derived from lactones become distinguished by both their feasible synthesis through ring opening polymerization (ROP) and their distinguishing properties. It is well known that amino acid *N*-carboxyanhydrides (NCA) are prone to undergo ROP initiated by aliphatic primary amines with the initiator remaining attached to the growing chain [9]. This approach has been applied to the synthesis of a number of polyester-*b*-polypeptide copolymers. In most of cases the polyester is generated from medium-size lactones (ϵ -caprolactone, L-lactide, etc), and the synthesis strategy consisted of preparing first the macroinitiator with an ending free-amino group to initiate then the ROP of the NCA's. Thus Caillol *et al.* synthesized a poly(L-lactide) amino-ended macroinitiator, which was then used for the ROP of γ -benzyl L-glutamate *N*-

carboxyanhydride (BLG-NCA). These PLLA-*b*-PBLG copolymers were organized in separated domains containing crystalline PLLA and liquid-crystal columnar hexagonal PBLG [6]. Likewise others amino acids (L-Ala, L-Phe, L-Leu, etc.) have been used in the preparation of a diversity of poly(ester-peptide)s with the hydrophobic block made from L-lactide or ϵ -caprolactone [12-16].

A critical factor for the successful synthesis of telechelic polymers is the end-group fidelity, which is achieved by a good control of the functionalizing reaction [17]. Ritter prepared a library of poly(L-lysine-*b*-caprolactone) block copolymers using amino-ended poly(ϵ -N-carbobenzoxy L-lysine) (PZLL_x-NH₂) to effectively initiated the ROP of ϵ -caprolactone [18]. After removal of the Z-protecting group, water-soluble copolymers were obtained which were able to spontaneously self-organize into nanometer size aggregates (core-shell particles or vesicles). In our research, we initially tried this approach using well-defined PBLG_x-NH₂ or PZLL_x-NH₂ for the ROP of ω -pentadecalactone (PDL) mediated by 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) or *Candida antarctica* lipase B (CALB) as catalysts. According to what has been reported [19,20] the use of TBD promoted transesterification reactions over the –COOBn group with release of benzyl alcohol as a by-product, and when CALB was used, the steric hindrance of the macroinitiator precluded its attack to the enzyme activated PDL so that copolymerization did not proceed. Recently we reported on the synthesis of well-defined allyl-ended telechelic poly(ω -pentadecalactone)s (PPDL) intended to be used as initiator of ROP after appropriate functionalization [21]. Deliberately, an adequate functionalized alkene was used as initiator to insert an unsaturation into the PPDL end-chain. The amine functionality was then introduced into the double-bond ended polyester via thiol-ene coupling with 2-(Boc-amino)-ethanethiol (BAET) followed by Boc removal. Such amine-functionalized polyester was claimed to be suitable for initiating ROP of either NCAs or other lactones, and this is in fact the approach we have adopted for the synthesis of the copolymers studied in this work.

In biomedical applications, and more specifically in the design of polymeric drug carriers, polymer amphiphilicity plays a crucial role. Drug carriers with significant lipophilic character have been found to be particularly effective in the stabilization of certain drugs and enlarging its circulatory retention in the blood stream. Macrolactones (MLs) have recently emerged as a family of building blocks for novel polymer biomaterials displaying properties close to those typical of long aliphatic chains but being potentially biodegradables [22]. The polyesters generated in the ROP of MLs have a hydrophobic character comparable to paraffins and display a strong tendency to crystallize producing well-developed crystallites of high thermal and chemical stability. Such features have motivated their use in the building of different block copolymers intended for drug delivery applications [23-25]. ω -Pentadecalactone is an easily accessible macrolactone that has been largely studied as ROP monomer for producing hydrophobic polyesters (PPDLs). Despite the exceptional potential of PPDL as biomaterial component, to our knowledge its marriage to polypeptides has never been reported, which prompted us to investigate the family of block copolymers made of PDL and L-glutamic or L-lysine (PPDL-*b*-pPAA). The work reported in this paper constitutes a first step in this investigation which is focused on neutral copolymers with the amino acids bearing their carboxylic or amino side groups conveniently protected. The PPDL-*b*-pPAA copolymers are exempted of organometallic catalysts and are of interest, not only by themselves due to their capacity to form stable nano-aggregates, but also as precursors of ionic copolymers potentially exploitable in highly sophisticated biomedical applications. In fact the -COOH and -NH₂ side groups of the glutamic and lysine residues are readily recoverable by acid treatment to render negatively and positively charged copolymers, respectively. These copolymers will display strong affinity for proteins and will be able to combine ionically with drugs and DNA's to form stable nanoconjugates [26,27].

2. Experimental section

2.1. Materials

Triphosgene, α -pinene, trifluoroacetic acid (TFA) and HBr/acetic acid, were purchased from Sigma-Aldrich and γ -benzyl L-glutamate (BLG) and ϵ -N-carbobenzoxy L-lysine (ZLL) were purchased from Bachem. Anhydrous dimethyl formamide (DMF), tetrahydrofuran (THF), ethyl acetate and heptane were used directly from the bottle under an inert atmosphere. Toluene and chloroform were distilled and dried on 3 Å molecular sieves. ω -Pentadecalactone (PDL) was purchased from Sigma-Aldrich and distilled under vacuum previously to use. 2-(Boc-amino)ethanethiol (BAET) was purchased from Sigma-Aldrich and used without further purification. Novozyme 435 (CALB, *Candida antarctica* Lipase B immobilized on cross-linked polyacrylate beads) was a gift of Novozymes. The received sample was subjected to drying over molecular sieves under vacuum at 50 °C for 24 h before use.

2.2. Characterization

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 spectrometer at 25 °C operating at 300.1 and 75.5 MHz, respectively. Compounds were dissolved in deuterated chloroform (CDCl_3) or a mixture of TFA and CDCl_3 , and spectra were internally referenced to tetramethylsilane (TMS). About 10 and 50 mg of sample in 1 mL of solvent were used for ^1H and ^{13}C NMR, respectively. Sixty-four scans were recorded for ^1H , and between 1000 and 10,000 scans for ^{13}C NMR.

FTIR measurements were made on a Perkin-Elmer spectrometer. Spectra in the region of 4000-450 cm^{-1} region were obtained from 8 scans with a resolution of 4 cm^{-1} . Molecular weight analysis was performed by GPC on a Waters equipment provided with RI and UV detectors and using hexafluoroisopropanol (HFIP) as eluent. 100 μL of 0.1% (w/v) sample solution in HFIP were injected and chromatographed with a flow of 0.4 $\text{mL}\cdot\text{min}^{-1}$. HR5E and HR2 Waters linear Styragel columns (7.8 mm x 300 mm, pore size 10^3 - 10^4 Å) packed with crosslinked polystyrene and protected with a pre-column

were used. Molar mass averages and their distributions were calculated against PMMA standards.

Thermogravimetric analysis (TGA) was performed on a Mettler-Toledo TGA/DSC 1 Star System under a nitrogen flow of 20 mL min⁻¹ at a heating rate of 10 °C min⁻¹ and within a temperature range of 30 to 600 °C. The reversible thermal behavior was examined by differential scanning calorimetry (DSC) using a Perkin-Elmer Pyris apparatus. Thermograms were registered from 4-6 mg samples at heating and cooling rates of 10 °C min⁻¹ under a nitrogen flow of 20 mL min⁻¹. Indium and zinc were used as standards for temperature and enthalpy calibration.

Real time X-ray diffraction (XRD) studies were carried out using synchrotron radiation at the BL11 beamline for non-crystalline diffraction (NCD), at ALBA (Cerdanyola del Vallés, Barcelona, Spain). WAXS and SAXS spectra were recorded simultaneously from powder samples subjected to heating-cooling cycles at a rate of 10 °C min⁻¹. The energy employed corresponded to a 0.10 nm wavelength, and spectra were calibrated with silver behenate (AgBh) and Cr₂O₃.

2.3. Nanoparticle formation

Nanoparticles (NPs) were fabricated using a modified oil-in-water single emulsion technique as reported elsewhere [21,28]. Specifically, 10 mg of PPDL_x-*b*-PBLG_y was dissolved in 2 mL of CHCl₃ and this solution was added to 10 mL of 1 wt-% poly(vinyl alcohol) (PVA, *M_w*~3000 g mol⁻¹) aqueous solution and the mixture was sonicated for 15 s three times. The sonicated solution was then poured into 10 mL of 0.3 wt-% PVA aqueous solution and the whole mixture was magnetically stirred in an open beaker at room temperature overnight. This process allowed NPs to be formed via gradual evaporation of the CHCl₃ solvent. The produced NPs were isolated by centrifugation and washed with de-ionized water three times. NPs made of PPDL_x-*b*-PZLL_y copolymers were prepared in a similar manner but using a mixture of CHCl₃/TFA (95:5) in order to get the copolymer completely solubilized in the organic phase.

2.4. Synthesis

2.4.1. Amino acids *N*-carboxyanhydrides (BLG-NCA and ZLL-NCA). γ -Benzyl L-glutamate and ϵ -*N*-carbobenzoxy L-lysine *N*-carboxyanhydrides were prepared using the usual procedures described in literature [29,30].

2.4.2. Amino-ended poly(ω -pentadecalactone) (PPDL_x-NH₂)

Allyl-ended poly(ω -pentadecalactone) (PPDL-All). An exact amount (82 mg) of dried Novozym 435 was weighed into a Schlenk tube provided with a magnetic stirrer and added with 2 mL of dried toluene and then with 67 μ L of a 1.33 M solution of allyl amine in toluene by means of a syringe through a rubber septum. The tube was immersed in an oil bath at 100 °C and the reaction started upon injection of a solution of PDL (414 mg, 1.66 mmol) in toluene (1.5 mL). After 4 hours of reaction under stirring, the mixture was allowed cooling down and toluene was fully removed using a rotary evaporator. The solid residue was re-dissolved in chloroform and the enzyme removed by filtration. The filtered clean solution was then poured into cold methanol to render PPDL_x-All as a precipitate that was recovered by filtration and dried overnight under vacuum. Yield: 90%.

PPDL_x-NH₂. PPDL_x-All (474 mg, 1.595 mmol) and 2-(Boc-amino)ethanethiol (BAET) (1.49 g, 8.41 mmol) were added into a Schlenk tube containing azo-*bis*-isobutyronitrile (AIBN) (138 mg, 0.841 mmol) and provided with magnetic stirring. The mixture was then dissolved in 1 mL of chlorobenzene and the reaction tube was purged with nitrogen gas. The reaction was started by immersing the tube into an oil bath at 80 °C and it was left to proceed for 4 h. For isolation and purification of the 2-(Boc-amino)ethanethiol-ended poly(ω -pentadecalactone) (PPDL_x-BAET), the reaction mass was poured into cold methanol, the precipitate recovered by centrifugation, and the operation repeated for two times. Yield: 87%.

For unblocking the amino group in PPDL_x-BAET, 245 mg of this compound were dissolved in TFA (2.5 mL) and the solution stirred at room temperature for 3 min.

The solution was then poured into a large excess of diethyl ether to precipitate the free amino-ended poly(ω -pentadecalactone) (PPDL_x-NH₂) as a white powder that was recovered by centrifugation, repeatedly washed with fresh solvent, and finally with 0.5 M NaHCO₃ aqueous solution. Yield: 82%.

2.4.3. Poly[(ω -pentadecalactone)-*b*-(α -amino acid)] copolymers (PPDL_x-*b*-pPAA_y).

Poly[(ω -pentadecalactone)-*b*-(γ -benzyl-L-glutamate)] copolymers (PPDL_x-*b*-PBLG_y). In a Schlenk tube, BLG-NCA (308.1 mg, 1.170 mmol) was dissolved in dried CHCl₃ (6 mL) and immersed in a 0 °C water bath. Then a solution of PPDL_x-NH₂ (72.1 mg, 0.023 mmol) in dried CHCl₃ (3 mL) was injected into the Schlenk tube through a septum with a syringe under nitrogen atmosphere. The reaction was left under stirring until the BLG-NCA was completely consumed as monitored by FTIR spectroscopy. Then the copolymer was precipitated into an excess of diethyl ether, recovered by centrifugation and dried under vacuum. Yield: 90%.

Poly(ω -pentadecalactone)-*b*-(ϵ -N-carbobenzoxy-L-lysine)] copolymers (PPDL_x-*b*-PZLL_y). The synthesis of these copolymers was carried out using the same procedure as for PPDL_x-*b*-PLGA_y with a yield of 80%.

3. Results and discussion

3.1. Synthesis of the PPDL-NH₂ macroinitiator

The three-step pathway followed for the preparation of the PPDL_x-NH₂ macroinitiator is depicted in Scheme 1. Partial yields were between 80 and 90%

Scheme 1. Synthetic pathway leading to the PPDL-NH₂ macroinitiator.

and the ^1H NMR spectra of the three intermediate compounds respectively isolated in each step are depicted in Figure 1. The NMR analysis proved that the synthesis of the amino-ended macroinitiator was successfully achieved. The triplet b appearing at 2.2 ppm is the only signal arising from methylene neighboring to the carbonyl group apart from that due to the repeating CH_2COO unit of the polyester chain. This is taken as indicative that all polyester chains have been amino-initiated in the ROP process. The b signal is shared by the three spectra and its area is consistent with that of the f^L signal at 3.65 ppm arising from the methylene protons of the end CH_2OH . It can be hence inferred that both thiol-ene click and Boc-deprotection reactions occurred as expected, and that therefore the PPDL- NH_2 sample is practically exempted of not

Figure 1. ^1H NMR (CDCl_3) spectra of a) PPDL-All, b) PPDL-BAE and c) PPDL- NH_2 . f^L notation refers to the last repeating unit.

amino-ended chains. The ratio of the areas of the b and f signals measured in the ^1H NMR spectra of this compound was used to determine the number-average length of the PPDL- NH_2 which resulted to be in the range of 15 or 20 units depending on the batch

3.2. Synthesis of the PPDL_x-*b*-pPAA_y copolymers

Two series of PPDL_x-*b*-pPAA_y diblock copolymers with the poly(amino acid) block (pPAA_y) duly protected were prepared by ROP of the NCA of γ -benzyl L-glutamate and ϵ -N-carbobenzoxy L-lysine, respectively, initiated by PPDL- NH_2 , as it is depicted in Scheme 2. Yields, compositions and molecular weights of the resulting copolymers are given in Table 1.

Scheme 2. ROP of AA-NCA initiated by PPDL- NH_2 .

Table 1. Yields, compositions and molecular weights of PPDL_x-*b*-pPAA_y copolyesters.

Copolymer ^a	Yield (%)	Feed [Init]/[NCA]	Copolymer ^{a,b} [PDL]/[AA]	M_n^c (g·mol ⁻¹)	$L_{\text{PPDL}}/L_{\text{PAA}}^d$ (nm)/(nm)
PPDL ₁₅ - <i>b</i> -PBLG ₃₀	81	1/30	15/33	10,500	29/12
PPDL ₁₅ - <i>b</i> -PBLG ₆₀	73	1/60	15/56	15,500	29/20
PPDL ₁₅ - <i>b</i> -PBLG ₈₀	79	1/80	15/84	21,600	29/30
PPDL ₁₅ - <i>b</i> -PBLG ₁₈₀	80	1/180	15/187	44,200	29/67
PPDL ₂₀ - <i>b</i> -PZLL ₃₀	94	1/30	20/32	12,800	38/11
PPDL ₂₀ - <i>b</i> -PZLL ₇₀	81	1/70	20/68	22,300	38/28
PPDL ₂₀ - <i>b</i> -PZLL ₁₀₀	80	1/100	20/98	30,000	38/35
PPDL ₂₀ - <i>b</i> -PZLL ₁₉₀	88	1/200	20/190	54,300	38/68

^aSubscripts indicate the degrees of polymerization of the two blocks corresponding to the PPDL- NH_2 initiator to NCA molar ratio used in the feed as indicated in column 3 after having been rounded to ten.

^bCopolymers composition expressed as the number of units of each block as determined by ^1H NMR.

^cNumber-average molecular weight of copolymers determined by ^1H NMR.

^dPDL and AA-block average lengths calculated from the compositions given in column 4 for an extended chain and assuming an average projected bond length of 0.120 nm.

Copolymerization yields were in the 70-90% range with significantly higher values attained for copolymers made of Lys. The ^1H and ^{13}C NMR spectra of the $\text{PPDL}_x\text{-}b\text{-PBLG}_y$ and $\text{PPDL}_x\text{-}b\text{-PZLL}_y$ ascertained their constitution and no signals other than those assignable to the monomeric repeating units present in these copolymers were therein detected. The $\text{PPDL}_{15}\text{-}b\text{-PBLG}_{30}$ and $\text{PPDL}_{20}\text{-}b\text{-PZLL}_{30}$ ^1H NMR spectra are shown in Figure 2 as representative for their respective series. Some illustrative ^{13}C NMR spectra are shown in Figure S1 of the SI file. Compositions as well as number-

Figure 2. ^1H -NMR spectra (CDCl_3/TFA) of the $\text{PPDL}_{15}\text{-}b\text{-PBLG}_{30}$ (a) and $\text{PPDL}_{20}\text{-}b\text{-PZLL}_{30}$ (b) diblock copolymers. (b^L corresponds to the end repeating unit).

average molecular weights were determined by ^1H NMR using the areas of signals specifically arising from each block and from both amino and hydroxyl end groups (see details in Figure S2 of the SI file). The compositions of the resulting copolymers were very close to those expected from the initiator to amino acid ratios set for their respective feeds with deviations being lower than 10%. M_n oscillated between $\sim 10,000$ and $\sim 55,000 \text{ g}\cdot\text{mol}^{-1}$ which correspond to copolymer chain lengths between 40 and 100 nm and block lengths ratios ranging from 0.4 to 3.5 with values close to 1 in the cases of $\text{PPDL}_{15}\text{-}b\text{-PBLG}_{80}$ and $\text{PPDL}_{20}\text{-}b\text{-PZLL}_{100}$. On the other hand, the chromatograms recorded in the GPC analysis displayed monomodal distributions (peaks appearing a longer elution times do arise from salts, SI file, Figure S3) with molar-mass dispersities within the 1.2-2.2 range. However molecular weight values given by this technique were found to be much lower than those determined by NMR. As it has been reported for polypeptides carrying aromatic groups [16,31], it can be assumed that exceptional interactions taking place between the benzyl groups of the polypeptides and the aromatic matrix of the column could be responsible for the delay observed in the elution times.

Contamination of the copolymers with some minor amounts of homo-oligopeptides that might be generated by non-amino initiated ROP of the NCA cannot be discarded. Although dried CHCl_3 was the solvent used, the presence of small quantities of water may initiate NCA polymerization. Unfortunately the detection and quantification of these oligopeptides is not easy. Nevertheless, their amount must be small since GPC results do not provide indication on their existence. Their elution together with the salts is highly unlikely because their molecular weights would be much greater and expected therefore to come out at noticeable shorter times. It should be noticed anyhow that the presence of minor oligopeptide impurity in the $\text{PPDL}_x\text{-}b\text{-pPAA}_y$ copolymers would not invalidate the structural and property study carried out on them.

3.3. Thermal properties of the PPDL_x-*b*-pPAA_y copolymers

The thermal stability of the PPDL_x-*b*-pPAA_y copolymers was examined by TGA under an inert atmosphere. The thermogravimetry traces recorded in the 25-600 °C range for the two whole series including the parent homopolymers, as well as their derivative curves, are given in Figure S4 in the SI file, and decomposition parameters are listed in Table 2. The onset temperatures of copolymers vary unsteadily from 237 to 230 °C whereas the two homopolypeptides started to decompose above 285 °C and 260 °C, respectively. Decomposition of PPDL took place through one main step at a $^{max}T_d$ of 430 °C with a weight loss of about 90% followed by a second minor step at a $^{max}T_d$ of 475 °C. These two steps have been made to correspond to decarboxylation and polymethylene disintegration reactions, respectively. On the other hand the polypeptides displayed a thermal decomposition behavior consisting basically in two well-separated stages centered about 290 and 320 °C for PBLG, and 320 and 430 °C

Table 2. Thermal properties of the PPDL_x-*b*-pPAA_y diblock copolymers.

Copolymers	TGA ^a			DCS ^b								
	$^{\circ}T_d$ $^{\circ}\text{C}$	$^{\text{max}}T_d$ $^{\circ}\text{C}$	R_w %	first heating					cooling		second heating	
				T_g $^{\circ}\text{C}$	T_m $^{\circ}\text{C}$	ΔH $\text{J}\cdot\text{g}^{-1}$	T_{LC}^c $^{\circ}\text{C}$	ΔH $\text{J}\cdot\text{g}^{-1}$	T_c $^{\circ}\text{C}$	ΔH $\text{J}\cdot\text{g}^{-1}$	T_m $^{\circ}\text{C}$	ΔH $\text{J}\cdot\text{g}^{-1}$
PPDL ₁₅	285	430, 470	1	-	90	151	-	-	75	-119	90	123
PPDL ₁₅ - <i>b</i> -PBLG ₃₀	237	230-470	1	21	90	49	109	2	73	-45	89	44
PPDL ₁₅ - <i>b</i> -PBLG ₆₀	230	235-470	16	21	88	26	109	2	68	-24	86	27
PPDL ₁₅ - <i>b</i> -PBLG ₈₀	230	240-470	15	21	88	13	119	2	67	-10	85	12
PPDL ₁₅ - <i>b</i> -PBLG ₁₈₀	230	260-430	14	20	88	9	120	4	70	-6	88	6
PBLG ₅₀	280	290,320	17	20	-	-	118	9	-	-	-	-
PPDL ₂₀	285	430, 470	1	-	90	151	-	-	75	-119	90	123
PPDL ₂₀ - <i>b</i> -PZLL ₃₀	200	260-470	10	21	83	33	-	-	63	-37	84	30
PPDL ₂₀ - <i>b</i> -PZLL ₇₀	210	230-410	9	21	84	23	~140	nd	67	-32	84	23
PPDL ₂₀ - <i>b</i> -PZLL ₁₀₀	215	250-410	9	20	84	18	~140	nd	66	-22	84	15
PPDL ₂₀ - <i>b</i> -PZLL ₁₉₀	240	270-410	5	20	90	2	-	-	77	-0.7	91	0.62
PZLL ₅₀	260	290, 430	9	21	-	-	~140	nd	-	-	-	-

^aOnset temperature for 5% of weight loss ($^{\circ}T_d$), maximum rate ($^{max}T_d$) decomposition temperatures and remaining weight (R_w) after heating at 600 °C.

^bGlass transition (T_g), melting (T_m and ΔH_m) and crystallization (T_c and ΔH_c) temperatures and enthalpies measured by DSC.

^c T_{LC} is the temperature for the helical transition undergone by the polypeptide block. Values given for PPDL₂₀-*b*-PZLL₇₀ and PPDL₂₀-*b*-PZLL₁₀₀ are approximate.

for PZLL. At difference with the homopolymers, the response of the copolymers to heating was complex and rather aleatory without showing apparent correlation between decomposition temperatures and composition. All derivative curves display several peaks corresponding to partial $^{max}T_d$ located within the interval delimited by the parents homopolymers and with values approaching to the one or the other limit according to composition. The existence of thermal transitions in the $PPDL_x-b-pPAA_y$ diblock copolymers was investigated by DSC. The traces recorded at both heating and cooling for the two whole series including the parent homopolymers are depicted in Figure 3 and the characteristic parameters measured in this analysis are listed in Table 2. As expected from what is reported from literature PPDL behaved as a semicrystalline polyester with T_m around 90 °C and being able to crystallize upon cooling from the melt with high recovery of the original crystallinity [32]. The DSC traces registered from copolymers displayed typical melting and crystallization peaks corresponding to the PPDL block for the two series and for whichever composition, and the second heating traces reproduced the endothermal peak observed on the first heating traces with

Figure 3. DSC first heating and cooling traces of $PPDL_x-b-pPAA_y$ diblock copolymers a) BLG-containing copolymers and b) ZLL-containing copolymers.

acceptably close values in both melting temperature and enthalpy (see Figure S5 in the SI file). It is worth noting that in the PPDL_x-*b*-PBLG_y series, the PPDL block melting peak appeared at the same temperature than in the homopolymer, but showing a shoulder at lower temperature that may be attributed to the fraction of polyester generated by secondary crystallization. On the contrary, the PPDL block in the PPDL_x-*b*-PZLL_y series melted at a few degrees lower than the homopolymer with a shoulder around 90 °C indicating that the PZLL block exerts a greater distorting effect on PPDL crystallization than the PBLG_y block.

On the contrary the DSC traces of PBLG and PZLL, which are polypeptides known to be unable to crystallize, displayed an endotherm in the 100-150 °C range. According to literature [16, 33] such peak is attributed to a conformational transition involving the conversion of the 7/2 helix to the 18/5 α -helix characteristic of poly(α -amino acid)s. The transition was clearly observed for PBLG as a sharp peak at 120 °C whereas it was much less noticeable in PZLL where only a smooth wide hill was shown in its heating trace. The occurrence of such transition in the PPDL_x-*b*-PBLG_y series was evidenced for both 15/80 and 15/180 compositions by the presence of an endothermal peak appearing at 110-120 °C. Heat exchanges with similar meaning were observed nearby 140-150 °C for the PPDL_x-*b*-PZLL_y copolymers with x/y values of 20/70 and 20/100 but not for 20/190, which is a highly striking result without apparent explanation.

3.4. Solid-state structure of the PPDL_x-*b*-pPAA_y diblock copolymers

The occurrence of regular arrangements in the polypeptide chain of the PPDL_x-*b*-pPAA_y copolymers and its dependence on the amino acid constitution was clearly demonstrated by FTIR analysis. The spectra of all the polymers studied in this work are comparatively represented in Figure 4. Bands at 1650 and 1545 cm⁻¹ characteristic of α -helix type were conspicuous in the spectra recorded from all the PPDL_x-*b*-PBLG_y. In the case of the PPDL_x-*b*-PZLL_y, the spectra showed the absorption characteristic of the β -sheet at 1625 cm⁻¹ in addition to the α -helix bands indicating that both forms coexist

in this series. It should be noted that cannot be disregarded that the observed β -sheet structure may arise, at least in part, from homo-oligopeptides that could be present in the copolymer as a minor impurity. Nevertheless, in the two series, the relative amount of helical arrangement present in the copolymer is largely predominant and it increased with the length of the polypeptide block.

Figure 4. 1800-1500 cm^{-1} region of FTIR spectra of $\text{PPDL}_x\text{-}b\text{-pPAA}_y$ diblock copolymers and the PBLG and PZLL homopolymers showing bands characteristic of α -helix and β -sheet secondary structures

The large difference between the PPDL and pPAA blocks in chemical constitution leads to expect that the $\text{PPDL}_x\text{-}b\text{-pPAA}_y$ copolymers are able to self-assemble in the solid state in a biphasic structure at the nanometric scale. To get insight into this structure, a real-time X-ray diffraction study was carried out by using synchrotron radiation with samples subjected to variable temperature. Both wide and small angle scattering were simultaneously recorded at either heating or cooling within the 10-150 $^{\circ}\text{C}$ temperature range. The evolution followed by the scattering profile of a pristine sample of $\text{PPDL}_{15}\text{-}b\text{-PBLG}_{80}$ with temperature changes is displayed in Figure 5.

Figure 5. Evolution of the X-ray diffraction profiles of PPDL₁₅-*b*-PBLG₈₀ recorded at heating and cooling over the 10-150 °C range. a, a') WAXS and b,b') SAXS (details may be clearly seen in the enlarged Figure S6 of the SI file).

The WAXS recorded at 10 °C shows exclusively the 0.41 nm and 0.37 nm reflections arising from the 110 and 200 planes of the pseudo-rhombic unit cell of PPDL with approximate dimensions $a = 0.75$ nm, $b = 5.0$ nm, and $c = 20.0$ nm and $\alpha = 90.0^\circ$ [34] indicating that the polyester block is the only one crystallized in this sample. This profile remained essentially unchanged under heating until temperature reached approximately 90 °C where both peaks disappeared and new reflections with Bragg spacings of 1.36 nm, 0.79 nm and 0.67 nm started to emerge and became more pronounced as temperature increased. These spacing values are related as $1:3^{1/2}:2$ and according to literature, they must arise from a two-dimensional columnar hexagonal packing of PBLG α -helices of 1.55 nm diameter. After cooling from 150 °C these peaks decreased in intensity as soon as crystallization of the PPDL block started, a fact that happened around 50 °C, so that the 1.36 nm peak was the only one

remained at 10 °C. The presence of the crystalline and liquid crystal structures could be further evidenced by polarizing optical microscopy (Figure S7 in the SI file).

The SAXS profiles recorded from PPDL₁₅-*b*-PBLG₈₀ at heating up to 150 °C revealed the appearing of discrete reflections at 27 nm (main peak) and 13.5 nm simultaneously to the development of the columnar phase evidenced by WAXS. According to what has been reported for copolymers composed of PBLG and PLA blocks [6], such spacings are interpreted to arise from a nano-structure of alternating layers made of PBLG helices and liquid PPDL. After cooling from 150 °C the nanostructure remained essentially unchangeable until crystallization of the PPDL block was initiated. It seems therefore that the occurrence of the nanometric structure is concomitant with the two-dimensional packing of the PBLG helices, and that the adoption of these ordered arrangements is disfavored by the presence of PPDL in the crystallized state.

The XRD analysis of PPDL₂₀-*b*-PZLL₁₀₀ was then carried out to get information from the copolymers containing lysine. The cumulative graphs showing the evolution of the WAXS profile of such copolymer with temperature changes along the 10-130 °C range are provided in Figure 6. The response of this copolymer to the thermal treatment was significantly different to that observed for PPDL₁₅-*b*-PBLG₈₀. In this case, both the monoclinic crystal phase of PPDL and the 2D-hexagonal columnar phase of PZLL (with spacings at 1.5 nm, 0.86 nm and 0.74 nm [3,5,35]) are coexisting in the original sample, and peaks arising from the latter remained unchanged over the whole range of temperatures. The broad scattering observed around 0.47 nm at high temperature is attributed not only to the amorphous state of PPDL but also to PZLL in β -sheet form that remains unaffected by temperature along the range of the treatment. It is concluded therefore that, at difference with that happens in the PBLG containing copolymers, the formation and stability of the columnar structure made of the polypeptide block based on lysine is essentially independent on the state adopted by the PPDL phase. The weak conformational response to temperature given by PPDL₁₅-

b-PBLG₈₀ and PPDL₂₀-*b*-PZLL₁₀₀ copolymers was supported by FTIR evidences (Figure S8 of SI).

On the other hand, the results obtained by SAXS were radically different since no discrete reflections were detected in these profiles (Figure S6 in SI file) within the recording limits (up to approximately 40 nm). It may be concluded therefore that no segregation in ordered domains takes place in the PPDL₂₀-*b*-PZLL₁₀₀ copolymer in spite of that the liquid crystal structure was in this case readily formed.

Figure 6. Evolution of the WAXS profiles of PPDL₂₀-*b*-PZLL₁₀₀ recorded at heating (a) and cooling (b) over the 10-130 °C range.

A crystallization kinetics analysis was carried out in order to investigate the possible influence of both the polypeptide block length and the thermal history of the sample on the crystallizability of the PPDL block. For such a purpose isothermal crystallizations of PPDL₁₅-*b*-PBLG_y copolymers for $y = 30, 80$ and 180 and PPDL₂₀-*b*-PZLL_y for $y = 30, 100$ and 190 were carried out at $77\text{ }^{\circ}\text{C}$. Samples were previously heated at $150\text{ }^{\circ}\text{C}$ to ensure that both PPDL melting and PAA helical transition had taken place. As it is shown in Figure 7, where relative crystallinity vs. time is comparatively represented for the two series and compared with their respective PPDL homopolymer, the crystallization rate of the PPDL block is notably enhanced by the presence of the polypeptidic counterpart. Furthermore the enhancing effect appeared to be independent from the polypeptide block length. It is a striking result that is

however in agreement with both DSC and thermal XRD observations. Similar results were obtained when the sample was previously heated at 93 °C, *i.e.* between the PPDL melting and helical transition temperatures (see Figure S9 in SI file) indicating that the polypeptide block conformation does not exert appreciable effect on the crystallizability of the PPDL block. These results allow extending the conclusions drawn in the XRD analysis of PPDL₁₅-*b*-PBLG₈₀ and PPDL₂₀-*b*-PZLL₁₀₀ (Figures 5 and 6) to other compositions.

Figure 7. Evolution of the relative crystallinity as a function of time in the isothermal crystallization at 77 °C of PPDL_x-*b*-PBLG_y (a) and PPDL_x-*b*-PZLL_y (b) copolymers from samples melted at 150 °C.

3.5. Nanoparticles made of PPDL_x-*b*-pPAA_y copolymers.

As the last stage in our examination of the PPDL_x-*b*-pPAA_y copolymers, their capacity to form stable nano-aggregates was preliminary explored for one pair of selected copolyesters of each series differing in the length of the polypeptide segment. Since these copolymers are non-soluble in water but soluble in volatile organic solvents as CHCl₃, the well-settled emulsion-evaporation technique was applied for creating the particles. The sizes and surface charges of the entities obtained by such method were determined by DLS measurements and the resulting values are compared in Table 3. Monomodal DLS curves were recorded for every case (Figure 8) with average diameters (*D*) in the 200-340 nm range with values being very close similar for the two

series when copolymers with similar PDL/AA ratio are compared. All particles displayed small negative zeta potentials (ζ) with insignificant differences between the two copolymers integrating each pair. The morphology of these NPs was examined by SEM and illustrative pictures from selected copolymers are shown in Figure 9. A well-defined spherical shape is displayed in all cases with sizes in acceptable consistency with values measured by DLS.

Table 3. Nanoparticles made of PPDL_x-*b*-pPAA_y copolymers.

Polymer	D (nm)	PDI	ζ (mV)
PPDL ₁₅ - <i>b</i> -PBLG ₃₀	340	0.40	-8.6
PPDL ₁₅ - <i>b</i> -PBLG ₈₀	220	0.12	-8.4
PPDL ₂₀ - <i>b</i> -PZLL ₃₀	300	0.18	-1.4
PPDL ₂₀ - <i>b</i> -PZLL ₁₉₀	200	0.14	-1.7

Figure 8. DLS curves of PPDL-*b*-pPAA nanoparticles.

The chemical modification of the -COOH group of glutamic acid as benzyl ester and the -NH_2 group of lysine as carbobenzoxy amide greatly decreased the genuine hydrophilic character of the amino acids and consequently the amphiphilic nature of the PPDL_x-*b*-pPAA_y copolymers too. Nevertheless, clear evidences have been provided by DSC and XRD to demonstrate that the polyester and the polypeptide phases must be segregated in the PPDL_x-*b*-pPAA_y copolymers when they are in the solid state, at least in the BLG-based copolymers. It should be expected therefore that these nanoparticles

have a certain degree of heterogeneity with the polypeptide block being preferentially located at the outer region. It is however striking that the particle size notably decreased in each series with the increasing length of the polypeptide block. No simple explanation can be given at this moment for this apparent inconsistency.

Figure 9. SEM images of nanoparticles made of PPDL-*b*-pPAA: A) PPDL₁₅-*b*-PBLG₃₀, B) PPDL₁₅-*b*-PBLG₈₀, C) PPDL₂₀-*b*-PZLL₃₀ and D) PPDL₂₀-*b*-PZLL₁₉₀.

4. Conclusions

Two series of diblock copolymers (PPDL-*b*-pPAA) made of ω -pentadecalactone (PDL) and γ -benzyl L-glutamate (BLG) or ϵ -N-carbobenzoxy-L-lysine (ZLL) were successfully synthesized by using a copolymerization approach based on the amino-initiated ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCA) that avoided the use of organometallic compounds. CALB has been proved to be an efficient catalyst for the ROP of PDL leading to polyester blocks of well-defined lengths

and exempted of undesirable side reaction products. The amino mediated opening of NCA allowed polypeptide segments of predetermined length according to the relative amount of macroinitiator that was used. PPDL-*b*-pPAA copolymers started to decompose noticeably at temperatures above 200 °C to undergo major weight losses at much higher values. Despite that the genuine hydrophilicity was significantly diminished in the protected amino acids, the copolymers showed DSC characteristic of biphasic material indicating that even so, the PDL and AA blocks are still incompatible. Both phases were found to be organized in ordered arrangements, the polyester in the typical monoclinic crystal lattice of PPDL with chains in extended conformation, and the polypeptides in a 2D columnar pseudo-hexagonal liquid-crystal phase made of α -helices. The two phases are strongly interactive as it is revealed by the enhancing influence of the polypeptide on the crystallizability of the PPDL block and the occurrence of a nanometric periodical structure at temperatures above melting of the PPDL phase. These copolyesters are able to form well-defined quasi-spherical shape nanoparticles with diameters in the ~200-350 nm range and slight negative zeta-potential. It should be remarked that these PPDL-*b*-pPAA diblock copolymers are exempted of metallic contamination and they are therefore well-suited to build drug nanocarriers for biomedical applications. Furthermore, as the protected amino acids may be easily liberated, they stand as immediate precursors of electrostatic charged copolymers suitable for efficient loading and controlled release of ionic drugs and nucleic acids.

Acknowledgements

Financial support for this research was afforded by MINECO with grants MAT-2016-77345-CO3-01. E. Tinajero-Díaz thanks CONACYT (México) for the Ph.D. grant and to L'Obra Social la Caixa (Spain) for the Beca de Mobilitat Internacional in 2016. Authors are indebted to Dr. Ana Gamarra and Dr. Lourdes Urpi for their assistance with

XRD experiments carried out in the B11 line of ALBA synchrotron in Cerdanyola del Vallès (Barcelona).

Supporting Information

Details of calculations made for copolymer composition. GPC chromatograms of representative PPDL_x-*b*-pPAA_y copolymers. TGA plots of PPDL_x-*b*-PBLG_y and PPDL_x-*b*-PZLL_y copolymers. Second heating DSC traces of PPDL_x-*b*-pPAA_y copolymers. SAXS of PPDL_x-*b*-pPAA_y copolymers. Polarizing optical micrographs of selected PPDL_x-*b*-pPAA_y copolymers. Relative crystallinity vs crystallization time for the isothermal crystallization of PPDL_x-*b*-pPAA_y copolymers previously heated at 93 °C, FTIR spectra at variable temperature.

Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to time limitations.

References

- [1] A. Carlsen, S. Lecommandoux, Self-assembly of polypeptide-based block copolymer amphiphiles, *Curr. Opin. Colloid Interface Sci.* 14 (2009) 329–339. <https://doi:10.1016/j.cocis.2009.04.007>.
- [2] H.A. Klok, S. Lecommandoux, Solid-state structure, organization and properties of peptide-synthetic hybrid block copolymers, *Adv. Polym. Sci.* 202 (2006) 75–111. https://doi:10.1007/12_083.
- [3] J. Babin, J. Rodriguez-Hernandez, S. Lecommandoux, H.-A. Klok, M.-F. Achard, Self-assembled nanostructures from peptide–synthetic hybrid block copolymers: complex, stimuli-responsive rod–coil architectures, *Faraday Discuss.* 128 (2005) 179–192. <https://doi:10.1039/B403203A>.
- [4] B.D. Olsen, R.A. Segalman, Self-assembly of rod-coil block copolymers, *Mater.*

- Sci. Eng. R Reports. 62 (2008) 37–66. <https://doi:10.1016/j.mser.2008.04.001>.
- [5] C.-J. Huang, F.-C. Chang, Polypeptide diblock copolymers: syntheses and properties of poly(*N*-isopropylacrylamide)-*b*-polylysine, *Macromolecules* 41 (2008) 7041–7052. <https://doi:10.1021/ma801221m>.
- [6] S. Caillol, S. Lecommandoux, A.-F. Mingotaud, M. Schappacher, A. Soum, N. Bryson, R. Meyrueix, Synthesis and self-assembly properties of peptide-poly(lactide) block copolymers, *Macromolecules* 36 (2003) 1118–1124. <https://doi:10.1021/Ma021187c>.
- [7] F. Chécot, A. Brûlet, J. Oberdisse, Y. Gnanou, O. Mondain-Monval, S. Lecommandoux, Structure of polypeptide-based diblock copolymers in solution: stimuli-responsive vesicles and micelles, *Langmuir* 21 (2005) 4308–4315. <https://doi:10.1021/la0468500>.
- [8] J.J. Cornelissen, Helical superstructures from charged poly(styrene)-poly(isocyanodipeptide) block copolymers, *Science* 280 (1998) 1427–1430. <https://doi:10.1126/science.280.5368.1427>.
- [9] S. Hehir, N.R. Cameron, Recent advances in drug delivery systems based on polypeptides prepared from *N*-carboxyanhydrides, *Polym. Int.* 63 (2014) 943–954. <https://doi:10.1002/pi.4710>.
- [10] A. Shah, M.S. Malik, G.S. Khan, E. Nosheen, F.J. Iftikhar, F.A. Khan, S.S. Shukla, M.S. Akhter, H.B. Kraatz, T.M. Aminabhavi, Stimuli-responsive peptide-based biomaterials as drug delivery systems, *Chem. Eng. J.* 353 (2018) 559–583. <https://doi:10.1016/j.cej.2018.07.126>.
- [11] K. Bauri, M. Nandi, P. De, Amino acid-derived stimuli-responsive polymers and their applications, *Polym. Chem.* 9 (2018) 1257–1287. <https://doi:10.1039/C7PY02014G>.
- [12] M. Gotsche, Amino-terminated poly(L-lactide)s as initiators for the polymerization of *N*-carboxyanhydrides: synthesis of poly(L-lactide)-block-poly(α -amino acid)s, *Macromol. Chem. Phys.* 196 (1995) 3891–3903.

- <https://doi.org/10.1002/macp.1995.021961203>
- [13] H.R. Kricheldorf, K. Hauser, Polylactones. 55. A-B-A triblock copolymers of various polypeptides. Syntheses involving 4-aminobezoyl-terminated poly(ϵ -caprolactone) as B block, *Biomacromolecules* 2 (2001) 1110–1115. <https://doi.org/10.1021/bm0100561>.
- [14] Y.F. Fan, G. Chen, J. Tanaka, T. Tateishi, L-Phe end-capped poly(L-lactide) as macroinitiator for the synthesis of poly(L-lactide)-*b*-poly(L-lysine) block copolymer, *Biomacromolecules* 6 (2005) 3051–3056. <https://doi.org/10.1021/bm050368u>.
- [15] Y. Li, Q. Li, F. Li, H. Zhang, L. Jia, J. Yu, Q. Fang, A. Cao, Amphiphilic poly(L-lactide)-*b*-dendritic poly(L-lysine)s synthesized with a metal-free catalyst and new dendron initiators: chemical preparation and characterization, *Biomacromolecules* 7 (2006) 224–231. <https://doi.org/10.1021/bm050602g>.
- [16] M. Schappacher, A. Soum, S.M. Guillaume, Synthesis of polyester-polypeptide diblock and triblock copolymers using amino poly(ϵ -caprolactone) macroinitiators., *Biomacromolecules* 7 (2006) 1373–9. <https://doi.org/10.1021/bm060008m>.
- [17] Y. Tsukahara, K. Adachi, Telechelic polymers: preparation and application, in: S. Kobayashi, K. Mullen (Eds.), *Encyclopedia of Polymeric Nanomaterials*, Springer, Berlin, Heidelberg, 2014, pp. 1–8. <https://doi.org/10.1007/978-3-642-36199-9>.
- [18] S. Motala-timol, D. Jhurry, J. Zhou, A. Bhaw-luximon, G. Mohun, H. Ritter, Amphiphilic Poly(L-lysine)-*b*-caprolactone) block copolymers, synthesis, characterization, and solution properties, *Macromolecules* 18 (2008) 5571–5576. <https://doi.org/10.1021/ma800917c>
- [19] L. Simón, J.M. Goodman, The mechanism of TBD-catalyzed ring-opening polymerization of cyclic esters, *J. Org. Chem.* 72 (2007) 9656–9662. <https://doi.org/10.1021/jo702088c>.

- [20] M. de Geus, R. Peters, C.E. Koning, A. Heise, Insights into the initiation process of enzymatic ring-opening polymerization from monofunctional alcohols using liquid chromatography under critical conditions, *Biomacromolecules*. 9 (2008) 752–757. <https://doi:10.1021/bm701158y>.
- [21] E. Tinajero-Díaz, A. Martínez de Ilarduya, S. Muñoz-Guerra, M. V. de Paz, E. Galbis, Metal-free catalyzed ring-opening polymerization and block copolymerization of ω -pentadecalactone using amino-ended initiators, *Eur. Polym. J.* 108 (2018) 380–389. <https://doi:10.1016/j.eurpolymj.2018.09.006>.
- [22] J. A. Wilson, Z. Ates, R.L. Pflughaupt, A.P. Dove, A. Heise, Polymers from macrolactones: from pheromones to functional materials, *Progr. Polym. Sc.* 91 (2019) 29–50. <https://doi.org/10.1016/j.progpolymsci.2019.02.005>
- [23] X. Zhang, B. Liu, Z. Yang, C. Zhang, H. Li, X. Luo, H. Luo, D. Gao, Q. Jiang, J. Liu, Z. Jiang, Micelles of enzymatically synthesized PEG-poly(amine-co-ester) block copolymers as pH-responsive nanocarriers for docetaxel delivery, *Colloids Surfaces B Biointerfaces* 115 (2014) 349–358. <https://doi:10.1016/j.colsurfb.2013.12.029>.
- [24] E.M. Chen, A.R. Quijano, Y.E. Seo, C. Jackson, A.D. Josowitz, S. Noorbakhsh, A. Merlettini, R.K. Sundaram, M.L. Focarete, Z. Jiang, R.S. Bindra, W.M. Saltzman, Biodegradable PEG-poly(ω -pentadecalactone-co-p-dioxanone) nanoparticles for enhanced and sustained drug delivery to treat brain tumors, *Biomaterials* 178 (2018) 193–203. <https://doi:10.1016/j.biomaterials.2018.06.024>.
- [25] B. Liu, X. Zhang, Y. Chen, Z. Yao, Z. Yang, D. Gao, Q. Jiang, J. Liu, Z. Jiang, Enzymatic synthesis of poly(ω -pentadecalactone-co-butylene-co-3,3'-dithiodipropionate) copolyesters and self-assembly of the PEGylated copolymer micelles as redox-responsive nanocarriers for doxorubicin delivery, *Polym. Chem.* 6 (2015) 1997–2010. <https://doi:10.1039/c4py01321b>.
- [26] Y. Huang, Z. Tang, X. Zhang, H. Yu, H. Sun, X. Pang, X. Chen, pH-triggered

- charge-reversal polypeptide nanoparticles for cisplatin delivery: Preparation and in vitro evaluation, *Biomacromolecules*. 14 (2013) 2023–2032. <https://doi.org/10.1021/bm400358z>.
- [27] X. Guan, Y. Li, Z. Jiao, L. Lin, J. Chen, Z. Guo, H. Tian, X. Chen, Codelivery of antitumor drug and gene by a pH-sensitive charge-conversion system, *ACS Appl. Mater. Interfaces*. 7 (2015) 3207–3215. <https://doi.org/10.1021/am5078123>.
- [28] J. Liu, Z. Jiang, S. Zhang, W.M. Saltzman, Poly(ω -pentadecalactone-co-butylene-co-succinate) nanoparticles as biodegradable carriers for camptothecin delivery, *Biomaterials*. 30 (2009) 5707–5719. <https://doi.org/10.1016/j.biomaterials.2009.06.061>.
- [29] G.J.M. Habraken, M. Peeters, C.H.J.T. Dietz, C.E. Koning, A. Heise, How controlled and versatile is *N*-carboxyanhydride (NCA) polymerization at 0 °C? Effect of temperature on homo-, block- and graft (co)polymerization. *Polym. Chem*. 1 (2010) 514–524. <http://doi.org/10.1039/b9py00337a>.
- [30] W.H. Daly, D. Poché, The preparation of *N*-carboxyanhydrides of α -amino acids using bis(trichloromethyl)carbonate, *Tetrahedron Lett*. 29 (1988) 5859–5862.
- [31] Ph. Degée, Ph. Dubois, R. Jérôme, Ph. Teyssie, Synthesis and characterization of biocompatible and degradable poly(ϵ -caprolactone-*b*- γ -benzyl-L-glutamate) diblock copolymers. *J. Polym. Sci., Part A: Polym. Chem*. 31 (1993), 275–278. <https://doi.org/10.1002/pola.1993.080310132>
- [32] M.L. Focarete, M. Scandola, A. Kumar, R. Gross, Physical characterization of poly(pentadecalactone) synthesized by lipase-catalyzed ring-opening polymerization, *J. Polym. Sci. Part B Polym. Phys*. 39 (2001) 1721–1729. <https://doi.org/10.1002/polb.1145>.
- [33] G. Rong, M. Deng, C. Deng, Z. Tang, L. Piao, X. Chen, X. Jing, Synthesis of poly(ϵ -caprolactone)-*b*-poly(γ -benzyl-L-glutamic acid) block copolymer using amino organic calcium catalyst, *Biomacromolecules*. 4 (2003) 1800–1804.

<https://doi:10.1021/bm034208z>.

- [34] M. Gazzano, M.L. Focarete, M. Scandola, R. a Gross, Crystal structure of poly (ω -pentadecalactone), J. Polym. Sci. Part B Polym. Phys. 41 (2003) 1009–1013. <https://doi:10.1002/polb.10419>.
- [35] S. Lecommandoux, M.-F. Achard, J.F. Langenwalter, H.-A. Klok, Self-assembly of rod-coil diblock oligomers based on α -helical peptides, Macromolecules 34 (2001) 9100–9111. <https://doi:10.1021/ma010940j>.

Highlights

- Polyester-polypeptide diblock copolymers are synthesized avoiding organo-metals.
- Both copolymer molecular weight and composition are well-controlled in the synthesis.
- In the solid state the copolymers have the two blocks separated and orderly arranged.
- The copolymers become self-assembled in water as spheres of 200-400 nm diameter.
- They are precursors for charged copolymers suited for stimuli-responsive biomaterials.

Diblock copolymers of ω -pentadecalactone and α -amino acids prepared by metal-free synthesis

Ernesto Tinajero-Díaz, Antxon Martínez-de Ilarduya, Sebastián Muñoz-Guerra