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# Microstructure characterization and thermal analysis of hybrid block copolymer $\alpha$ -methoxy-poly(ethylene glycol)-*block*-poly[ $\epsilon$ -(benzyloxycarbonyl)-L-lysine] for biomedical applications

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# ABSTRACT

Hybrid block copolymers, which combine the economy and processibility of synthetic polymers with the functionality and highly ordered structures of polypeptides, can enhance control over structure formation at the nanoscale, as well as afford interesting materials that interface with Nature for a diversity of bio-medical applications, such as drug delivery, tissue engineering and bioimaging. Hybrid block copolymer,  $\alpha$ -methoxy-poly(ethylene glycol)-*b*-poly[ $\varepsilon$ -(benzyloxycarbonyl)-L-lysine], MPEG-*b*-PLL(*Z*), was synthesized by anionic ring-opening polymerization in excellent yield, and fully characterized using IR, <sup>1</sup>H, <sup>13</sup>C and 2-D NMR, GPC, TGA, DTGA, DSC, MDSC and polarimetry. Its precursor NCA,  $\varepsilon$ -(benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride, L-Lys(*Z*)-NCA, was prepared in 97% yield; and a double doublet observed in the 1850–1750 cm<sup>-1</sup> absorption region of its infrared spectrum, for the characteristic NCA carbonyl absorption bands, is discussed.

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# 1. Introduction

Polymers are invaluable to man, and continue to find utility in virtually all facets of human existence. Synthetic polymers are successfully replacing a number of natural materials, not only because the latter are limited and in short supply but also for their physicochemical and mechanical properties, which usually surpass those of materials available from Nature. Synthetic biodegradable polymers have found applications in tissue reconstruction and as surgical materials, in bone bonding and repairing, and in the immobilization of cells and enzymes. They also have great potentials as biosensors, artificial organs and prostheses, and as delivery systems for drugs, vaccines and gene therapy [1,2]. Not forgetting the continued relevance of synthetic polymers in photography, computing and electronics [3].

Polymers are usually chemically combined to form copolymers, which benefit from the synergistic characteristics of the component polymers and/or overcome shortcomings inherent to the constituent homopolymers. Block copolymers are composed of two or more covalently-bonded polymer blocks with diverse chemical and physical properties. They are increasingly generating great interest in academia and industry [4]. A characteristic feature of block copolymers is their tendency to form phase-separated microdomains in solid state, as well as in the presence of selective solvents. Many of the interesting and desirable properties displayed by this group of macromolecules are attributable to this trait of microphase separation. The morphology of such multiphase systems influences their behaviour and can be usually controlled by altering the ratio of block lengths, the molecular weight distribution and/or the nature of the constituent blocks [5].

Hybrid block copolymers with synthetic (homo)polymers and peptide segments as components form an interesting class of materials. They combine the economy and processibility of conventional synthetic polymers with the functionality and highly ordered structures of polypeptides [6–9]. The combination of polypeptide sequences with conventional polymers do not only enhance control over structure formation at the nanoscale, but can also afford materials that can interface with biology, for a variety of biomedical and bioanalytical applications [7].

Poly(ethylene glycol) [PEG] is a highly investigated synthetic polymer for the covalent modification of biological macromolecules and surfaces for many pharmaceutical and biotechnical applications [10]. PEG is non-toxic and non-immunogenic but non-biodegradable; it is absorbed by the digestive tract and excreted in urine and faeces. PEG is hydrophilic, soluble in some organic solvents, and very effective in solubilizing hydrophobic polymers coupled to it. PEG also inhibits protein adsorption, thereby allowing longer residence times in the body for substrates to which it is conjugated. Additionally, available data indicate that PEG is neither mutagenic





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Nomenclature			
Cbz benzyl	oxycarbonyl	L-Lys(Z)	ε-(benzyloxycarbonyl)-ι-lysine
COSY (proto	n-proton) correlation spectroscopy (NMR)	L-Lys(Z)	-NCA ε-(benzyloxycarbonyl)-L-lysine N-carboxyanhy-
dec. decom	position		dride
DEPT distort	ionless enhancement by polarisation transfer	MDSC	modulated temperature differential scanning calorime-
(NMR)			try
DMF N,N-di	methylformamide	MPEG-b	-PLL(Z) $\alpha$ -methoxy-poly(ethylene glycol)- <i>b</i> -poly[ $\varepsilon$ -(ben-
DMSO-d <sub>6</sub> dimeth	nyl sulphoxide (deuterated)		zyloxycarbonyl)-L-lysine]
DSC differe	ntial scanning calorimetry	MPEG-N	$M_2 \alpha$ -methoxy- $\omega$ -aminopoly(ethylene glycol)
DTGA deriva	tive thermogravimetric analysis	PLL	poly(L-lysine)
ESI-MS electro	ospray ionization mass spectrometry	PLL(Z)	poly[ɛ-(benzyloxycarbonyl)-L-lysine]
HMBC hetero	nuclear multiple bond coherence spectroscopy	ROP	ring-opening polymerization
(2-D N	IMR)	TGA	thermogravimetric analysis
HSQC hetero	nuclear single quantum coherence spectroscopy	THF	tetrahydrofuran
(2-D N	IMR)	TMS	tetramethylsilane
L-Lys L-lysin	e		

nor carcinogenic [11]. Other valuable properties of PEG include biostability, excellent processibility, strong metal ion interactions, and ease of chemical modification [12–14].

Poly( $\alpha$ -amino acid)s [PAAs], on the other hand, also exhibit outstanding properties, which persuade their many utilities in a myriad of medical and biological applications [15,16]. Poly(L-lysine) [PLL] is the polymer of L-lysine, which is a proteinogenic amino acid. It is biocompatible and biodegradable. It yields L-lysine quantitatively on hydrolysis [17]. PLL offers attractive utility in surface coatings [18,19] as well as electron microscopy [20]. Its microbial [21,22] and cationic salts (PLL·HCl) [13] exhibit antibacterial activities. Poly[ $\varepsilon$ -(benzyloxycarbonyl)-L-lysine] [PLL(Z)] membranes have found use in bladder reconstruction [23].

PEG and PLL have been covalently combined to form graft [24,25], diblock [26–29], triblock [30–32], and random [33] copolymers and dendrimers [34,35]; some of which have been conjugated to other moieties, such as: folates [36], acids [37,38], sugars [39,40], RGD [41,42], and amphotericin B [43], to mention a few. They have also been used in polyion complex (PIC) formation [27,44,45], and investigated for drug [46,47] and gene [48– 50] delivery, colloidal lithography [51,52], biomimetic applications [53], photodynamic therapy (PDT) [54,55], antithrombogenicity [56], and as bioimaging agents [57,58]. PEG-*b*-PLL is considered to be biodegradable [59], biocompatible and non-toxic [60].

The ring-opening polymerization (ROP) of  $\alpha$ -amino acid *N*-carboxyanhydrides (NCAs), usually initiated by an  $\omega$ -amino polymer, is the most economical and expedient process for the synthesis of polypeptide-containing block copolymers [61,62]. The polymerization of the  $\varepsilon$ -benzyloxycarbonyl-L-lysine NCA is one of the best controlled [63]. Macroinitiators with primary amino end group(s) are commonly used to generate hybrid block copolymers because, among the different traditional NCA ring-opening polymerization initiators, only primary amine-containing initiators are quantitatively incorporated at the *C*-terminus of the polypeptide chain [64]. Generally, primary amine-initiated ROP of *N*-carboxyanhydrides, though not "living" in the strictest sense of the word, afford hybrid block copolymers in good to excellent yields, with degrees of polymerization (DP) in agreement with monomer–initiator ratios (M/I) [65]; allowing for a fairly accurate control of chain length.

As part of our programme on the synthesis of biodegradable and biocompatible copolymers for drug delivery and biomedical applications [66–69], we have prepared  $\alpha$ -methoxy-poly(ethylene glycol)-*b*-poly[ $\epsilon$ -(benzyloxycarbonyl)-*L*-lysine] [MPEG-*b*-PLL(Z), **5**] (Scheme 1) by the anionic ROP of  $\epsilon$ -benzyloxycarbonyl-*L*-lysine *N*-carboxyanhydride [*L*-Lys(Z)-NCA, **3**] in *N*,*N*-dimethylformamide, initiated by  $\alpha$ -methoxy- $\omega$ -aminopoly(ethylene glycol) [MPEG-

NH<sub>2</sub>, **4**]. We were surprised to note, however, that no detailed elucidation of the block copolymer's microstructure could be found in the literature, in spite of its numerous application potentials (*vide supra*). The properties of a copolymer are intrinsically linked to the chain and compositional arrangement of its constituting monomer units [70]. Polymer microstructure elucidation is therefore not only pertinent to structure–property relationship studies but to the design of desirable and application-driven polymeric materials.

Presented herein, is the synthesis and characterization of the chain microstructure of hybrid block copolymer, MPEG-*b*-PLL(*Z*) **5** and its precursor *N*-carboxyanhydride, L-Lys(*Z*)-NCA **3**, using IR, <sup>1</sup>H, <sup>13</sup>C and 2-D NMR spectroscopy, and polarimetry. Molar mass and molecular weight distribution were determined by mass spectrometry (ESI-MS) and gel permeation chromatography (GPC), respectively. The thermoanalytical measurements of the block copolymer were made using TGA, DTGA, DSC and MDSC.

# 2. Experimental

# 2.1. Materials

ε-Benzyloxycarbonyl-L-lysine, *bis*(trichloromethyl) carbonate [triphosgene], and α-methoxy-ω-aminopoly(ethylene glycol) [MPEG-NH<sub>2</sub>; 5000, *extent of labeling*:  $\ge 0.17 \text{ mmol/g NH}_2$  *loading*] were from Fluka. Poly(ε-benzyloxycarbonyl-L-lysine) [PLL(Z); 1000–4000], tetrahydrofuran [THF; anhydrous,  $\ge 99.9\%$ , inhibitor-free] and *N*,*N*-dimethylformamide [DMF; anhydrous, 99.8%] were purchased from Aldrich. All solvents were of analytical grade, and were used as received without further purification. All reactions were conducted under moisture-free nitrogen atmosphere.

# 2.2. Characterization

Melting points were determined using a Gallenkemp melting point apparatus, and are uncorrected. Optical rotation measurements were made with an Optical Activity AA-5 automatic polarimeter, using a tungsten filament lamp as light source. All measurements were made in a 2 dm (path length) sample tube at 24.0 ± 0.5 °C and 0.2% solution concentration. <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy were carried out in CDCl<sub>3</sub> (30 °C) and DMSO-*d*<sub>6</sub> (60 °C) on a Varian Inova 500 spectrometer at 499.8 MHz and 125.7 MHz, respectively, using tetramethylsilane (TMS) and deuterated solvent signals as internal standards. About 1% w/v. and 10% w/v. sample solutions were used in the <sup>1</sup>H and <sup>13</sup>C NMR measurements, respectively. Two-dimensional (2-D) NMR experiments



Scheme 1. Synthetic route to hybrid block copolymer, MPEG-b-PLL(Z) 5, starting from L-Lys(Z) 1.

were run on a Varian Inova 500 spectrometer. IR spectroscopy was performed on a Perkin Elmer System 2000 FT-IR instrument. The spectra were recorded using pressed KBr disks, scanning from  $4000 \text{ cm}^{-1}$  to  $650 \text{ cm}^{-1}$  with a resolution of  $4 \text{ cm}^{-1}$ . High-resolution electrospray ionization mass spectra were obtained using a Waters Micromass Quattro micro™ LC-MS-MS mass spectrometer. Molecular weight distribution was determined by gel permeation chromatography at a flow rate of 1.0 mL/min in THF (stabilized with 250 ppm BHT) at 40 °C, using a PLgel 5 µm MIXED-D column  $(300 \times 7.5 \text{ mm})$  in a PL-GPC 120 integrated system instrument, equipped with a PL-AS RT GPC Autosampler and PL DataStream monitor, for data acquisition, running on Cirrus™ GPC software. The system was calibrated with narrow polydispersity polystyrene standards (Polymer Laboratories, UK), and the signals were refractive-index detected. Thermogravimetric analysis was performed on a Perkin Elmer TGA 7 Thermogravimetric Analyzer, coupled to a Perkin Elmer Thermal Analysis Controller TAC7/DX, under nitrogen and oxygen atmospheres, respectively, from 30 °C to 600 °C, at a heating rate of 10 °C/min. Differential scanning calorimetry was recorded on a TA Instruments 2010 DSC instrument, calibrated with high purity indium, under a 30 mL/min flow rate of nitrogen gas. The samples (ca. 10.0 mg) were ramped at 10 °C/min from 20 °C to 110 °C to erase previous thermal history, and remove any residual volatiles and moisture. All samples were then quench-cooled using liquid nitrogen and scanned from a temperature of -100 °C to +100 °C at a heating rate of 10 °C/min. Mid-point temperature readings are reported. The measurements were conducted in crimped non-hermetic Perkin Elmer aluminium pans, with an empty crimped aluminium pan as reference cell. Modulated temperature differential scanning calorimetry (MDSC) measurements were carried out using a TA Instruments DSC 2920 Modulated DSC calorimeter, coupled with a refrigerated cooling system. Samples (ca. 10.0 mg) contained in crimped non-perforated aluminium pans, were initially ramped at 10 °C/min from 20 °C to 110 °C, air-cooled, and then scanned from -60 °C to +100 °C at a ramp rate of 1 °C/min, under an atmosphere of nitrogen gas (30 mL/min and 100 mL/min). All measurements were modulated at  $\pm 1.0$  °C/min and held isothermal for 5 min, with an empty crimped non-perforated aluminium pan as reference cell. The calorimeter was calibrated by standard methods using base-line and temperature (indium) calibrations. Mid-point temperature readings are reported.

# 2.3. Synthesis

# 2.3.1. ε-(Benzyloxycarbonyl)-ι-lysine N-carboxyanhydride [ι-Lys(Z)-NCA]

L-Lys(Z)-NCA 3 was synthesized by the Fuchs-Farthing method, using a slightly modified procedure [71]. In a well-ventilated fume cupboard, ε-(benzyloxycarbonyl)-L-lysine **1** (2.48 g, 8.85 mmol) was added to an oven-dried round-bottomed flask; pre-flushed with dry nitrogen gas, into which anhydrous THF (25 mL) was syringed. The resulting cloudy suspension was heated with stirring, under a dry nitrogen atmosphere, at 50 °C for 5 min. Triphosgene 2 (1.16 g, 3.91 mmol) was dissolved in anhydrous THF (5 mL) and added to the L-Lys(Z)-THF suspension. The stirring at 50 °C, under nitrogen, was continued for another 30 min, with effervescence, and the reaction mixture turning clear in less than 10 min. The clear solution was allowed to cool to ambient temperature, and precipitated with *n*-hexane (100 mL). The resulting white precipitate was then washed with more *n*-hexane (40 mL  $\times$  2) and dried at ambient temperature in vacuo to afford a white solid (2.09 g). The mother liquor was concentrated under reduced pressure, recrystallized from a THF/n-hexane solvent system, and dried in a vacuum oven, at ambient temperature to give a white flaky solid (0.54 g). The combined yield was 2.63 g.

Yield: 97%. mp: 98–99 °C (lit. [72]: 100 °C dec.).  $[\alpha]_D^{24}$ : -41.7 (*c* = 0.2, CHCl<sub>3</sub>).

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 303.1 K): δ 1.41–1.44 (m,  $\gamma$ -CH<sub>a</sub>H<sub>b</sub>, 1H), 1.45–1.50 (m,  $\gamma$ -CH<sub>a</sub>H<sub>b</sub>, 1H), 1.51–1.57 (m,  $\delta$ -CH<sub>2</sub>, 2H), 1.82– 1.94 (m,  $\beta$ -CH<sub>a</sub>H<sub>b</sub>, 1H), 1.96–2.10 (m,  $\beta$ -CH<sub>a</sub>H<sub>b</sub>, 1H,), 3.16–3.25 (m,  $\varepsilon$ -CH<sub>2</sub>, 2H), 4.26 (m,  $\alpha$ -CH, 1H), 4.91 (s,  $\varepsilon$ -NH, 1H), 5.11 (dd,  $J_1$  = 12.5 Hz;  $J_2$  = 4.1 Hz, C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>—, 2H), 6.81 (s, *ring* NH, 1H), 7.31–7.38 (m, C<sub>6</sub>H<sub>5</sub>—, 5H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 303.1 K): δ 21.2 ( $\gamma$ -CH<sub>2</sub>), 29.2 ( $\delta$ -CH<sub>2</sub>), 30.7 ( $\beta$ -CH<sub>2</sub>), 40.0 ( $\epsilon$ -CH<sub>2</sub>), 57.4 ( $\alpha$ -CH), 67.0 (C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>—), 128.1; 128.3; 128.6 (C<sub>6</sub>H<sub>5</sub>—), 136.4 (*i*-C<sub>6</sub>H<sub>5</sub>), 152.3 (*ring* NHCO<sub>2</sub>), 157.0 ( $\epsilon$ -NHCO<sub>2</sub>), 169.8 (*ring* CHCO<sub>2</sub>).

IR (KBr):  $v_{max}$  (cm<sup>-1</sup>): 3341 (N–H str.), 3092; 3063; 3032 (*ring* C–H str.), 2939; 2868 (C–H str.), 1855 (*sym.* CH(C=O)<sub>2</sub>O str.), 1813 (*sym.* NH(C=O)<sub>2</sub>O str.), 1775 (*antisym.* CH(C=O)<sub>2</sub>O str.), 1748 (*antisym.* NH(C=O)<sub>2</sub>O str.), 1689 (*amide* C=O str.), 1535 (*amide* N–H bend.), 1457 (C–H bend.), 1371 (C–N str.), 1317; 1286 (*anhydride* C–O–C str.), 1260 (N–C–O str.), 1141; 1015 (C–O–C str.), 945 (*anhydride* C–O–C str.), 750 (*ring* C–H bend.), 695 (*ring* C=C bend.).

HRMS (ESI): m/z calcd for  $C_{15}H_{18}N_2O_5Na$ , 329.1113; found, 329.1121 [M+Na]<sup>+</sup>.

# 2.3.2. α-Methoxy-poly(ethylene glycol)-b-poly[ε-(benzyloxycarbonyl)*ι*-lysine] [MPEG-b-PLL(Z)]

MPEG-*b*-PLL(Z) **5** was synthesized according to literature [27] thus: Under a dry nitrogen atmosphere, L-Lys(Z)-NCA 3 (2.40 g, 7.83 mmol) was dissolved in anhydrous DMF (25 mL), with stirring. Into the L-Lys(Z)-NCA-DMF solution was added a solution of MPEG-NH<sub>2</sub> **4** (2.00 g, 0.40 mmol) in anhydrous DMF (30 mL). The stirring was continued for 10 min, with the cloudy mixture turning into a clear pale yellow solution, and was then heated at 40 °C for 17 h; whereupon an IR spectrum of an aliquot of the reaction mixture indicated the disappearance of the starting material's *N*-carboxyanhydride peaks. Effervescence was observed during the reaction. The reaction was left running for another 3 h, and left to cool to ambient temperature. DMF was then removed under reduced pressure to leave a yellow paste, to which chloroform (50 mL) was added to give a yellow gel. The gel was precipitated into diethyl ether (150 mL  $\times$  4) and the resulting precipitate was filtered, washed with more diethyl ether (50 mL  $\times$  2), and air-dried to afford an off-white solid (3.65 g).

Yield: 91%.  $[\alpha]_D^{24}$ : +138.9 (*c* = 0.2, CHCl<sub>3</sub>), +27.8 (*c* = 0.2, DMF).

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, 333.1 K): δ 1.25–1.33 (m, γ-*CH*<sub>2</sub>, 34H), 1.38 (m, δ-*CH*<sub>2</sub>, 34H), 1.51 (m, β-*CH*<sub>a</sub>H<sub>b</sub>, 17H), 1.64 (m, β-*CH*<sub>a</sub>H<sub>b</sub>, 17H), 2.96 (m, ε-*CH*<sub>2</sub>, 34H), 3.24 (s, *CH*<sub>3</sub>O—, 3H), 3.38 (m, *CH*<sub>3</sub>O*CH*<sub>2</sub>CH<sub>2</sub>—, 2H), 3.44 (m, *CH*<sub>3</sub>O*CH*<sub>2</sub>*CH*<sub>2</sub>—, 2H), 3.51 (br s,  $-(OCH_2CH_2)_n$ —, 444H), 3.57 (m,  $-CH_2CH_2NH$ —, 2H), 3.66 (m,  $-CH_2CH_2NH$ —, 2H), 4.23 (m, α-*CH*, 17H), 4.99 (s, *C*<sub>6</sub>H<sub>5</sub>*CH*<sub>2</sub>—, 34H), 6.95 (s, ε-*NH*, 17H), 7.31 (m, *C*<sub>6</sub>H<sub>5</sub>—, 85H), 7.58 (s,  $-CH_2CH_2NH$ —, 1H), 7.72 (s, amide NH, 16H), 7.91 (s, terminal *NH*<sub>2</sub>, 2H).

<sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>, 333.1 K): δ 21.8; 21.9; 23.1 (γ-CH<sub>2</sub>), 29.2; 29.4; 29.6 (δ-CH<sub>2</sub>), 31.4; 31.5; 32.2 (β-CH<sub>2</sub>), 39.0; 40.6; 40.8 (ε-CH<sub>2</sub>), 52.1; 52.3; 53.1 (α-CH), 58.5 (CH<sub>3</sub>O—), 65.6 (C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>—), 69.4 (CH<sub>3</sub>OCH<sub>2</sub>CH<sub>2</sub>—), 69.5 (CH<sub>3</sub>OCH<sub>2</sub>CH<sub>2</sub>—), 70.3 ( $-(OCH_2CH_2)_n$ —), 71.3 ( $-CH_2CH_2NH$ —), 71.8 ( $-CH_2CH_2NH$ —), 128.2; 128.8 (C<sub>6</sub>H<sub>5</sub>—), 137.7 (*i*-C<sub>6</sub>H<sub>5</sub>), 156.5 (ε-NHCO<sub>2</sub>), 169.1; 171.7; 171.9 (-CONH—).

IR (KBr):  $v_{max}$  (cm<sup>-1</sup>): 3280 (N–H str.), 3065; 3034 (*ring* C–H str.), 2889; 2741; 2695 (C–H str.), 1694; 1626 (*amide* C=O str.), 1539 (*amide* N–H bend.), 1468 (C–H bend.), 1344; 1280 (C–N str.), 1243; 1112; 962 (C–O–C str.), 843 (N–H bend.), 739 (*ring* C–H bend.), 698 (*ring* C=C bend.).

GPC (polystyrene standards): 9279 ( $M_n$ ), 9555 ( $M_w$ ), 1.03 (PDI). DSC: -51 °C ( $T_g$ ), +49 °C ( $T_m$ ), +64 °C ( $T'_g$ ).

# 3. Results and discussion

# 3.1. *L*-Lys(Z)-NCA

According to the Fuchs-Farthing method [71], stirring the  $\varepsilon$ -N-protected amino acid,  $\varepsilon$ -(benzyloxycarbonyl)-L-lysine [L-Lys(Z), **1**]

in anhydrous THF in the presence of triphosgene **2** at 50 °C for 30 min, under dry nitrogen atmosphere, afforded the *N*-carboxyanhydride **3**, as a white solid in a near-quantitative yield of 97% (*cf.* Scheme 1). The excellent yield obtained in this reaction might not be unconnected with the nitrogen gas sparging of the reaction, which is reported [71] to improve product yields by driving the hydrogen chloride evolved from the reaction medium. The HCl by-product protonates the unreacted amino group, when left in the reaction medium, reducing the amino acid's nucleophilicity and solubility.

A melting point range of 98–99 °C was obtained for L-Lys(Z)-NCA **3**. A melting point of 100 °C (with decomposition) was first reported by Bergmann et al. [72], who prepared the *N*-carboxyanhydride from its acid chloride, and this was later reproduced by Katchalski et al. [17]. L-Lys(Z)-NCA has also been prepared directly from L-Lys(Z) using phosgene [73] and triphosgene [27], with reported melting points of 101–101.5 °C and 99.2–101.8 °C, respectively. Some authors [17,72] have suggested that the melting point of the *N*-carboxyanhydride may rise above 250 °C when left standing for several months, due to (moist air-catalyzed) polymerization. This has not been observed herein. A sample of L-Lys(Z)-NCA was stored in a parafilm-sealed vial in a molecular sieve-charged desiccator for over 6 months with no apparent change in its melting point.

# 3.1.1. <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy

In the <sup>1</sup>H NMR spectrum of L-Lys(Z)-NCA **3** in CDCl<sub>3</sub> (Fig. 1), the benzyloxycarbonyl ring protons resonated between 7.31 ppm and 7.38 ppm, while the benzyl protons ( $C_6H_5CH_2$ —) gave a doublet of doublets centred at 5.11 ppm, with coupling constants (J) of 12.5 Hz and 4.1 Hz. The proton on the  $\varepsilon$ -nitrogen atom showed up as a singlet at 4.91 ppm, with the  $\varepsilon$ -methylene protons and  $\alpha$ methine proton resonating at 3.16-3.25 ppm and 4.26 ppm, respectively. The singlet at 6.81 ppm was assigned to the proton on the ring (oxazolidine-2,5-dione) nitrogen atom, with the other alkyl CH<sub>2</sub> protons coming up upfield as multiplets between 1.41 ppm and 2.10 ppm. COSY and HSQC were particularly useful in assigning the peaks of the protons on the different nitrogen atoms and the crowded upfield signals of the methylene protons. The non-equivalence of the  $\beta$ -protons of L-Lys(Z)-NCA **3** was clearly discernable in the <sup>1</sup>H NMR spectrum, with the two protons resonating as multiplets between 1.82-1.94 ppm and 1.96-2.10 ppm, respectively. A difference in chemical shift ( $\delta$ ) values was also exhibited, to a lesser extent, by the  $\gamma$ -protons (1.41–1.44 ppm; 1.45–1.50 ppm). This non-equivalence of the ( $\beta$ - and  $\gamma$ -) protons adjoining the stereogenic  $\alpha$ -carbon suggests that no amino acid racemization occurred in the course of the reaction.

The carbonyl carbon atom signal in the <sup>13</sup>C NMR spectrum (Fig. 2) at 169.8 ppm was attributed to the NCA carbonyl (*k*), while those at 157.0 ppm and 152.3 ppm were conclusively assigned to the benzyloxycarbonyl (*d*) and NCA carbamoyl (*j*), respectively, with the aid of HMBC. The *ipso*-carbon of the phenyl ring showed up at 136.4 ppm, with the other phenyl ring carbon atoms resonating around 128 ppm. The benzyl carbon ( $C_{6}H_5CH_2$ —) and  $\alpha$ -carbon ( $-HNCHCO_2$ —) peaks came up at 67.0 ppm and 57.4 ppm, respectively. The remaining carbon-13 signals for L-Lys(Z)-NCA **3** were found at 21.2 ppm ( $\gamma$ -CH<sub>2</sub>), 29.2 ppm ( $\delta$ -CH<sub>2</sub>), 30.7 ppm ( $\beta$ -CH<sub>2</sub>) and 40 ppm ( $\epsilon$ -CH<sub>2</sub>). The <sup>1</sup>H and <sup>13</sup>C NMR chemical shift values obtained for the *N*-carboxyanhydride, L-Lys(Z)-NCA **3**, (Table 1) are in agreement with predicted data [61].

# 3.1.2. FT-IR spectroscopy

The strong infrared absorption bands observed at  $1855 \text{ cm}^{-1}$ ;  $1813 \text{ cm}^{-1}$  and  $1775 \text{ cm}^{-1}$ ;  $1748 \text{ cm}^{-1}$  were attributed to the symmetric and antisymmetric C=O stretching modes of the carboxyanhydride (O=C-O-C=O) functionality, respectively (Fig. S1). NCAs



Fig. 1. <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>; 30 °C) of *N*-carboxyanhydride, L-Lys(Z)-NCA **3**, with peak assignments.



are reported to exhibit a characteristic doublet at  $1855 \pm 5 \text{ cm}^{-1}$ and  $1780 \pm 5 \text{ cm}^{-1}$  in this region [61]. The bands at  $1855 \text{ cm}^{-1}$ and  $1775 \text{ cm}^{-1}$  were assigned herein to the carbonyl C=O stretching while those at  $1813 \text{ cm}^{-1}$  and  $1748 \text{ cm}^{-1}$  were ascribed to the carbamoyl C=O stretching vibrations. Medium C–O stretching bands at  $1286 \text{ cm}^{-1}$  and  $945 \text{ cm}^{-1}$  were also observed, while sharp bands of strong intensities were evident at  $3341 \text{ cm}^{-1}$  and  $1689 \text{ cm}^{-1}$  due to the N–H stretching and amide C=O stretching vibrations, respectively. The N–H bending vibration frequency (Amide II) was equally strong at  $1535 \text{ cm}^{-1}$ . The strong absorption band at  $1260 \text{ cm}^{-1}$  was due to the N–C–O stretching frequency of the compound, L-Lys(Z)-NCA **3** [74].

The IR spectra of acid anhydrides are characterized by a doublet in the 1850–1750 cm<sup>-1</sup> region, arising from the coupling between the in-phase and out-of-phase carbonyl vibration modes [75,76]. The resonance within the O=C-O-C=O system causes it to be coplanar, and this enhances the coupling between the carbonyl groups to an extent that the two carbonyl frequencies correspond to the symmetric and antisymmetric vibrations of the C=O groups [76]. The higher of the two frequencies is attributed to the symmetric C=O mode [75]. Cyclic acid anhydrides in conjugation with olefinic moieties are reported to exhibit a lowering of frequencies in this region [76]. Similarly, in the case of NCAs, the presence of a delocalized electron pair on the nitrogen atom of the carbamoyl C=O group ought to result in reduced frequency and increased extinction coefficient [61]. Consequently, we suggest that the absorption bands at 1813 cm<sup>-1</sup> and 1748 cm<sup>-1</sup> are due to the symmetric and antisymmetric vibrations of the N-(C=O)-O moiety.

A cursory look at Fig. S1 reveals, as expected [76], that for the *C*-5 carbonyl, the lower frequency band is the more intense of the

#### Table 1

<sup>1</sup>H and <sup>13</sup>C NMR chemical shift ( $\delta$ ) values of *N*-carboxyanhydride, L-Lys(Z)-NCA **3**.



Atom no.	<sup>1</sup> H (ppm)		<sup>13</sup> C (ppm)
	δ	Multiplicity	
1		-	152.3
2	6.81	S	-
3	4.26	m	57.4
4		-	169.8
5a	1.82-1.94	m	30.7
5b	1.96-2.10	m	
6a	1.41-1.44	m	21.2
6b	1.45-1.50	m	
7	1.51-1.57	m	29.2
8	3.16-3.25	m	40.0
9	4.91	S	-
10		-	157.0
11	5.11	dd	67.0
		J = 12.5, 4.1 Hz	
12		-	136.4
13	7.31-7.38	m	128.1
			128.3
			128.6

two. The converse is, however, the case for the carbamoyl C==0 vibration bands at 1813 cm<sup>-1</sup> and 1748 cm<sup>-1</sup>. The constraint produced by the ring in cyclic anhydrides increases the angle between the two C==0 groups; approaching as a limit a collinear arrangement. As this collinear limit is approached, an equally progressive decrease in the intensity of the in-phase motion is envisaged because of the cancellation of dipole charges which, in turn, is accompanied by an increase in the intensity of the out-of-phase motion [75]. Therefore, it is plausible to propose that, in the case of L-Lys(Z)-NCA **3**, whilst the dipole-moment change of the symmetric mode which gives rise to the band at 1855 cm<sup>-1</sup> is reduced relative to the antisymmetric mode at 1775 cm<sup>-1</sup>, an opposite scenario is operative for the bands at 1813 cm<sup>-1</sup> and 1748 cm<sup>-1</sup>; probably

as a result of the delocalized lone pair electrons [77] of the adjoining nitrogen atom, leading to the double doublet observed in its IR spectrum.

The occurrence of four anhydride C=O absorption bands is not uncommon. Hochstein et al. [78] reported a similar observation for 3-methoxy-6-methylpyromellitic acid anhydride, which consisted of two anhydride systems separated by a disubstituted aromatic ring. It is noteworthy that the anhydride bands in the infrared spectrum of L-Lys(Z)-NCA **3** are characteristically approximately the same distances apart (ca.  $60 \text{ cm}^{-1}$ ) [76]. An IR spectrum of triphosgene (in KBr), obtained in order to rule out its presence (as an impurity) in the spectrum of L-Lys(Z)-NCA 3, showed a lone, very strong C=O stretching vibration at 1823 cm<sup>-1</sup>. It is instructive to note that the analogous carbonyl doublet of glycolylurea (hydantoin) [79] absorbs around 1778  $\rm cm^{-1}$  and 1706  $\rm cm^{-1}$ , while those of succinic anhydride and maleic anhydride are observed at 1865  $cm^{-1}$  and 1782  $cm^{-1}$ ; and 1848 cm<sup>-1</sup> and 1790 cm<sup>-1</sup>, respectively [76]. Equally worth mentioning is the fact that the IR spectrum of an air-dried sample of the N-carboxyanhydride, prepared herein, showed only two C=O stretching bands at 1836  $\text{cm}^{-1}$  and 1791  $\text{cm}^{-1}$  (cf. Fig. S2).

# 3.2. MPEG-b-PLL(Z)

The anionic ROP of L-Lys(Z)-NCA **3** to hybrid block copolymer, MPEG-*b*-PLL(Z) **5** was carried out in anhydrous DMF, under dry nitrogen atmosphere, with MPEG-NH<sub>2</sub> **4** as macroinitiator. The polymerization was monitored *via* infrared spectroscopy; stopping the reaction when the NCA carbonyl absorption bands were no longer visible in the reaction mixture's spectrum.

# 3.2.1. NMR and FT-IR spectroscopy

The protons on the nitrogen atoms of MPEG-*b*-PLL(*Z*) **5** resonated as singlets at 6.95 ppm, 7.58 ppm, 7.72 ppm and 7.91 ppm, while the singlets of  $CH_3O$ — and  $-(OCH_2CH_2)_n$ — came up at 3.24 ppm and 3.51 ppm, respectively (*cf.* Fig. 3). The methoxy (*CH*<sub>3</sub>O—) protons' singlet was small and partially overlapped by the much-bigger dissolved water (HDO) peak [80], but was successfully assigned with the aid of HSQC, which gave a cross peak between it (*CH*<sub>3</sub>O— signal) and the carbon signal at 58.5 ppm – depicted in DEPT as a primary carbon.



Fig. 3. <sup>1</sup>H NMR spectrum (DMSO-d<sub>6</sub>; 60 °C) of hybrid block copolymer, MPEG-b-PLL(Z) 5, with peak assignments.



Fig. 4. <sup>13</sup>C NMR spectrum (DMSO-*d*<sub>6</sub>; 60 °C) of hybrid block copolymer, MPEG-*b*-PLL(Z) 5, with peak assignments.

In the <sup>13</sup>C NMR spectrum (Fig. 4), the peaks at 156.5 ppm and 171.9 ppm were assigned to the carbamoyl and amide carbonyl carbon atoms, respectively. The carbonyl carbon signals were differentiated using HMBC. The <sup>13</sup>C resonance signal at 156.5 ppm showed (long range) cross peaks with the <sup>1</sup>H signals at 2.96 ppm  $(\varepsilon$ -CH<sub>2</sub>) and 4.99 ppm (C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>-). It is notable that the carbamoyl C=O peak of the oxazolidine-2,5-dione ring at 152.3 ppm (in the <sup>13</sup>C NMR spectrum of the precursor NCA) has disappeared, with the benzyloxycarbonyl C=O signal remaining unchanged (cf. Fig. 2). The <sup>13</sup>C signals at 128.2 ppm, 128.8 ppm and 137.7 ppm were due to the aromatic carbon atoms, while peaks for the CH<sub>3</sub>Oand  $-(OCH_2CH_2)_n$  – carbon atoms were observed at 58.5 ppm and 70.3 ppm, respectively. The  $\varepsilon$ -CH<sub>2</sub> signals at 40.6 ppm and 40.8 ppm, obscured by the solvent (DMSO- $d_6$ ) septet centred at 40.2 ppm, was solved by 2-D NMR. DEPT separated the overlapping carbon atom signals by depicting the solvent and  $\varepsilon$ -CH<sub>2</sub> peaks as primary and secondary carbon signals, respectively. Additionally, HSQC showed that the secondary carbon atoms resonating at 40.6 ppm and 40.8 ppm ( $\varepsilon$ -CH<sub>2</sub>) were attached to the proton signals around 2.96 ppm.

The chain microstructure of the hybrid block copolymer, MPEG*b*-PLL(Z) **5** is reflected in the observed symmetry and fine structures of its <sup>1</sup>H and <sup>13</sup>C NMR spectra; the chemical shift ( $\delta$ ) data is compiled in Table 2. The non-appearance of multiple resonance signals for the same proton and/or carbon atom, for example, is attributable to the presence of identical constituent monomer chain sequences in the copolymer, resulting in a similar chemical environment. It is known that the NMR spectra of random copolymers show broad or multiple resonance signals per atom and less symmetry [81]. Herein, the spectra indicate that the monomer chain sequence is the same within the constituting blocks.

Considering the infrared spectrum of MPEG-*b*-PLL(Z) **5** (Fig. S3), the disappearance of the distinctive O=C-O-C=O stretching bands of the precursor NCA **3** was indicative of a successful polymerization. The N–H stretching vibration frequency was centred at 3280 cm<sup>-1</sup>, indicating a *trans* (bonded N–H) secondary amide [76]. The amide C=O stretching bands absorbed at 1694 cm<sup>-1</sup> and 1626 cm<sup>-1</sup> due to the side chain (Cbz) and main chain C=O

vibrations, respectively. The strong Amide I band observed at 1626 cm<sup>-1</sup> alluded to the formation of new amide bonds, and possible presence of hydrogen bonding. The broad, strong absorption bands at 2889 cm<sup>-1</sup> and 1112 cm<sup>-1</sup> were assigned to the aliphatic C–H stretching and C–O–C stretching frequencies, respectively. Characteristic polymer bands due to C–H deformation at 1468 cm<sup>-1</sup> and N–H wagging at 843 cm<sup>-1</sup> were also observed [82].

The IR spectra of the NCA **3**, macroinitiator **4** and block copolymer **5** are stacked in Fig. 5, illustrating the disappearance of the *N*-carboxyanhydride C=O stretching bands (seen in **a**), and the emergence of a new amide C=O stretching frequency (in **c**). The contributions of the C-H stretching and C-O-C stretching absorption bands (in **b**) are also prominent. The variations in the intensity, shape and frequency (3341 cm<sup>-1</sup> (**a**)  $\rightarrow$  3439 cm<sup>-1</sup> (**b**)  $\rightarrow$  3280 cm<sup>-1</sup> (**c**)) of the N-H stretching band are equally interesting.

The Amide I, Amide II and N—H stretching vibration frequencies observed at 1626 cm<sup>-1</sup>, 1539 cm<sup>-1</sup> and 3280 cm<sup>-1</sup>, respectively, in the IR spectrum of MPEG-*b*-PLL(Z) **5** indicate that its constituent PLL(Z) block adopts a  $\beta$ -sheet secondary structure. Polypeptides possessing the  $\beta$ -sheet conformation (in the solid state) are reported to exhibit characteristic Amide I, Amide II and N—H stretching frequencies around 1630 cm<sup>-1</sup>, 1530 cm<sup>-1</sup> and 3290 cm<sup>-1</sup>, respectively [83].

# 3.2.2. GPC and polarimetry

Gel permeation chromatography (GPC) experiments in THF, using narrow polydispersity polystyrene standards as calibrant, revealed a monomodal molecular weight distribution for MPEG-*b*-PLL(Z) **5** (*cf.* Fig. 6); with a number-average molecular weight ( $M_n$ ) and weight-average molecular weight ( $M_w$ ) of 9279 g/mol and 9555 g/mol, respectively, as well as a polydispersity of 1.03. <sup>1</sup>H NMR spectroscopy was also employed in the estimation of the degree of polymerization (DP) of the PLL(Z) block and  $M_n$  of the block copolymer, MPEG-*b*-PLL(Z) **5**. The <sup>1</sup>H NMR and GPC results are summarized in Table 3. A comparison of the relative peak intensities of the methylene ( $-(OCH_2CH_2)_n-; \delta$  3.51) and benzyl ( $C_6H_5CH_2-; \delta$  4.99) protons of the MPEG and PLL(Z) blocks, respectively, gave the degree of polymerization of PLL(Z) as 17.

#### Table 2

<sup>1</sup>H and <sup>13</sup>C NMR chemical shift ( $\delta$ ) values of hybrid block copolymer, MPEG-*b*-PLL(Z) **5**.



Atom no.	<sup>1</sup> H (ppm)		<sup>13</sup> C (ppm)	Atom no.	<sup>1</sup> H (ppm)		<sup>13</sup> C (ppm)	
	δ	Multiplicity			δ	Multiplicity		
1	3.24	S	58.5	18	1.25-1.33	m	21.8	
2	3.38	m	69.4	19	1.38	m	29.2	
3	3.44	m	69.5	20	2.96	m	39.0	
4	3.51	br s	70.3	21	6.95	S	-	
5	3.57	m	71.3	22		_		
6	3.66	m	71.8	23	4.99	S	65.6	
7	7.58	S	-	24		-	137.7	
8		-	169.1	25	7.31	m	128.0	
9	4.23	m	52.3				128.7	
10	7.72	S	-	26a	1.51	m	32.2	
11		-	171.9	26b	1.64	m		
12	4.23	m	53.1	27	1.25-1.33	m	23.1	
13	7.72	S	-	28	1.38	m	29.6	
14		-	171.7	29	2.96	m	40.8	
15	4.23	m	52.1	30a	1.51	m	31.5	
16	7.91	S	-	30b	1.64	m		
17a	1.51	m	31.4	31	1.25–1.33 m		21.9	
17b	1.64	m		32	1.38	m	29.4	
				33	2.96	m	40.6	

Optical rotation measurements of the hybrid block copolymer MPEG-*b*-PLL(*Z*) **5** in CHCl<sub>3</sub> and DMF at 24 °C gave a specific optical rotation of +138.9° and +27.8°, respectively. The dissolution of MPEG-*b*-PLL(*Z*) **5** in chloroform, as well as in dichloromethane, was slow – even at low concentrations. The optical rotatory dispersion (ORD) of poly(*ɛ*-benzyloxycarbonyl-*L*-lysine) has been measured in DCA-CHCl<sub>3</sub> solution [73] and DMF [84]. Fasman et al. [73] determined the optical rotation  $([\alpha]_{25}^{546})$  of high molecular weight PLL(*Z*) in CHCl<sub>3</sub> to be +5°.

# 3.2.3. Thermal properties

The thermal stability of MPEG-*b*-PLL(Z) **5** under nitrogen and oxygen atmospheres was evaluated by thermogravimetric analysis (TGA). Fig. 7 shows the TGA and Derivative TGA plots of the block copolymer with the macroinitiator, MPEG-NH<sub>2</sub> **4**. Under oxygen atmosphere, the TGA curve of MPEG-*b*-PLL(Z) **5** showed about four inadequately resolved steps, but the initial onset of weight loss and final thermal decomposition occurred around 204 °C and 568 °C, respectively. These were, however, respectively higher (at 260 °C) and lower (at 426 °C) under nitrogen atmosphere, where a two-stage decomposition (260–299 °C and 390–426 °C) was easily discernable. In relation to the homopolymer, MPEG-NH<sub>2</sub> **4**, higher degradation temperatures were observed for the copolymer in

both atmospheres; 328–568 °C in oxygen and 400–426 °C in nitrogen, implying enhanced thermal stability for MPEG-*b*-PLL(Z) **5**.

The DSC thermogram (Fig. 8) of MPEG-*b*-PLL(Z) **5** showed transition events at  $-51 \degree C(T_g)$ ,  $+49 \degree C(T_m)$  and  $+64 \degree C(T'_g)$ . The glass transition and crystalline melting temperatures of the macroinitiator MPEG-NH<sub>2</sub> **4** were similarly determined as  $-49 \degree C(T_g)$  and  $+60 \degree C(T_m)$ , respectively. The prominence of the melting event ( $T_m$ ) dwarfed the  $T_g$  event, making it difficult to detect. The portion of the curve labelled *i* is instrument-related, while *ii* is possibly a relaxation phenomenon [13] (*cf.* Fig. 8). The thermal event at  $+64 \degree C$  was interesting for a number of reasons. Not only was it proximal to the prominent  $T_m$  centred at  $+49 \degree C$ , but it was about 30  $\degree C$  higher than reported [85,86]  $T_g$  values for homopolymer PLL(Z). Some authors have also reported on their inability to detect a  $T_g$  for the PLL(Z) block [87]. Thermal analysis results of poly[ $\varepsilon$ -(benzyloxycarbonyl)-L-lysine] [PLL(Z), *MW* 1000–4000], obtained for reference purposes, are shown in Fig. 9 and Table 4.

In order to further investigate the thermal event at +64 °C in the DSC thermogram of MPEG-*b*-PLL(Z) **5**, modulated temperature differential scanning calorimetry (MDSC) measurements were conducted. MDSC deconvolutes total heat flow signals into rapidly reversible (heat capacity-related) and non-rapidly reversible (kinetic) components [88]. The reversible heat flow component of



Fig. 5. IR spectra (KBr) of: (a) N-carboxyanhydride, L-Lys(Z)-NCA 3 vs. (b) macroinitiator, MPEG-NH<sub>2</sub> 4 vs. (c) hybrid block copolymer, MPEG-b-PLL(Z) 5.



Fig. 6. Molecular weight distribution plot (GPC) of macroinitiator, MPEG-NH<sub>2</sub> 4 vs. hybrid block copolymer, MPEG-b-PLL(Z) 5.

MPEG-*b*-PLL(Z) **5** and MPEG-NH<sub>2</sub> **4** are shown in Fig. 10, and the clear step change at +58 °C corroborates the earlier assignment. The observation of slight differences in glass transition temperature values obtained from conventional DSC and the reversible heat flow component of MDSC is not uncommon. This is because the position of  $T_g$  is strongly dependent on frequency and the rate of cooling, and the underlying measurements for the average and

reversing signals in MDSC are on different time scales [89]. Additionally, the notion that the event observed at +64 °C in the DSC thermogram was due to enthalpic relaxation was dispelled by its non-observance in the non-reversible heat flow component signal of the MDSC thermogram. The sub-zero events in the MDSC scans were ignored because of their proximity to the calorimeter's minimum working temperature (-60 °C).

### Table 3 Molecular weight distribution of macroinitiator, MPEG-NH<sub>2</sub> 4 and hybrid block copolymer, MPEG-*b*-PLL(Z) 5.

Polymer	Theoretical M <sub>n</sub> (g/mol)	<sup>1</sup> H NMR			GPC					
		M/I	DP	M <sub>n</sub> (g/mol)	M <sub>n</sub> (g/mol)	M <sub>w</sub> (g/mol)	PDI $(M_w/M_n)$			
MPEG-NH <sub>2</sub>	5010 ( <i>n</i> = 112)	-	-	5006	4996	5505	1.10			
MPEG- <i>b</i> -PLL(Z)	9470 ( <i>n</i> = 112, <i>m</i> = 17)	19	17	9416	9279	9555	1.03			

M/I: monomer to initiator molar ratio, DP: degree of polymerization,  $M_n$ : number-average molecular weight,  $M_w$ : weight-average molecular weight, PDI: polydispersity index.



Fig. 7. (a) TGA curves of MPEG-NH<sub>2</sub> 4 and MPEG-b-PLL(Z) 5 in oxygen and nitrogen atmospheres. (b) Derivative TGA plot of MPEG-NH<sub>2</sub> 4. (c) Derivative TGA plot of MPEG-b-PLL(Z) 5.



Fig. 8. DSC thermograms of macroinitiator,  $MPEG-NH_2$  4 and hybrid block copolymer, MPEG-b-PLL(Z) 5.

The thermal transitions at -51 °C and +49 °C, and +64 °C were therefore attributed to the MPEG and PLL(Z) blocks, respectively,

because of their proximity to analogous transitions in MPEG and PLL(Z) homopolymers. The two  $T_g$  values (-51 °C and +64 °C) observed in the DSC thermogram of block copolymer MPEG-b-PLL(Z) 5 are a good indication of the existence of phase-separated microdomains [90]. The data from DTGA alluded to a more thermally stable MPEG block in MPEG-b-PLL(Z) 5 (relative to the homopolymer, MPEG-NH<sub>2</sub> **4**) in nitrogen atmosphere;  $T_s$  peaks of 413 °C and 387 °C were observed for the MPEG block of the copolymer and homopolymer, respectively. The PLL(Z) block, on the other hand, experienced a lower  $T_s$  (at 278 °C). Chain mobility in the PLL(Z) block appears to have been reduced by the MPEG block, while the lowering of the block copolymer's  $T_{\rm m}$ , relative to that of the homopolymer MPEG, can be interpreted as a decrease in its degree of crystallinity. This was similarly observed by Thünemann et al. [38], and it is probable that the copolymerization with L-lysine led to a disturbance in the extended chain conformation of the crystalline MPEG homopolymer, resulting in the lower  $T_m$  for the block copolymer, MPEG-b-PLL(Z) 5. This is also reflected in the heat of fusion, where the observed  $\Delta H_{\rm m}$  for MPEG-NH<sub>2</sub> **4** is more than twice that of the copolymer (cf. Table 4).

# 4. Conclusion

The preparation of the hybrid block copolymer, MPEG-*b*-PLL(Z) **5** by anionic ROP, and its precursor *N*-carboxyanhydride, L-Lys(Z)-NCA **3**, in excellent yields and their characterization have been de-



Fig. 9. (a) TGA and DTGA curves and (b) DSC thermograms of poly[&-(benzyloxycarbonyl)-L-lysine] [PLL(Z), mol. wt. 1000-4000].

Table 4										
Thermal	l analysis da	ata for h	omopolymer	s MPEG-NH	and PLL(Z),	and hybrid	block cop	olymer I	MPEG-b-	PLL(Z).

Polymer	TGA							DSC					MDSC			
	Nitrogen atmosphere				Oxygen atmosphere											
	Decomposition temperature (K)		DTGA (K)	Mass loss (%)	Decomposition temperature (K)		Mass loss (%)	Transition temperature $T_g$ $T'_g$ (K) (K)		T <sub>m</sub> (K)	Heat ca enthalp $\Delta C_{ m p}$ (J/g K)	pacity/ by $\Delta C'_p$ (I/g K)	$\Delta H_{\rm m}$ (J/g)	Trans temp T <sub>g</sub> (K)	sition erature T'g (K)	T <sub>m</sub> (K)
	Initial (T <sub>i</sub> )	Final (T <sub>f</sub> )	Peak (Ts)		Initial (T <sub>i</sub> )	Final (T <sub>f</sub> )			()			0187			()	
MPEG-NH <sub>2</sub>	633	673	660	89.6	442	601	94.9	224	-	333	0.059	-	129.9	ND	-	327 <sup>a</sup> 333 <sup>b</sup>
MPEG-b-PLL(Z)	533 663	572 699	551 686	19.7 61.2	477 523 651 781	488 587 687 841	12.4 37.9 12.2 8.6	222	337	322	0.127	0.236	47.8	ND	331 <sup>a</sup> _ <sup>c</sup>	315 <sup>a</sup> 320 <sup>b</sup>
PLL(Z) (Mol. wt. 1–4 kDa)	524 616	583 680	562 646	44.6 25.4	516 756	599 835	64.3 15.5	-	298 <sup>d</sup> 305 <sup>e</sup>	-	-	0.058 <sup>d</sup> 0.291 <sup>e</sup>	-	-	ND	-

ND: not determined.

<sup>a</sup> Reversible heat flow.

<sup>b</sup> Total heat flow.

<sup>c</sup> Obscured by *T*<sub>m</sub> curve.

<sup>d</sup> Quench-cooled to -100 °C.

<sup>e</sup> Quench-cooled to 0 °C.



Fig. 10. MDSC thermograms (reversible heat flow component only) of macroinitiator, MPEG-NH<sub>2</sub>  $\bf 4$  and hybrid block copolymer, MPEG-*b*-PLL(Z)  $\bf 5$ .

scribed. L-Lys(Z)-NCA **3** gave a double doublet in the carbonyl absorption region of its IR spectrum for the *N*-carboxyanhydride (O=C-O-C=O) stretching vibration modes at 1855 cm<sup>-1</sup>, 1813 cm<sup>-1</sup>, 1775 cm<sup>-1</sup> and 1748 cm<sup>-1</sup> – as opposed to the previously reported characteristic NCA doublets at 1855 ± 5 cm<sup>-1</sup> and 1780 ± 5 cm<sup>-1</sup>. An attempt has been made to rationalize this observation. Furthermore, the IR spectrum of MPEG-*b*-PLL(*Z*) **5** indicates that the constituting PLL(*Z*) block assumes a  $\beta$ -sheet conformation in the solid state. NMR spectroscopy established the block sequence in the chain structure of MPEG-*b*-PLL(*Z*) **5** while thermal analysis data signified the occurrence of microphase separation in the bulk.

Although the synthesis of MPEG-*b*-PLL(*Z*) **5** has been previously reported, this is its first complete chain microstructure characterization; which has become pertinent especially in view of the block copolymer's burgeoning application potentials, and the significance of structural elucidation to application-driven materials' design as well as structure–property relationship studies. Spectroscopic, molecular weight distribution and thermal analysis data have been presented.

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# Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2010.05.026.

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