Research Article

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Chemoenzymatic synthesis of novel *N*-(2-hydroxyethyl)-β-peptoid oligomer derivatives and application to porous polycaprolactone films

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Abstract

Poly[*N*-(2-hydroxyethyl)-β-propylamide] oligomer is synthesized using a simple enzymatic procedure involving *Candida antarctica* lipase B. This novel compound is obtained by a green and chemoselective method from economic reactants in good yield. The β-peptoid oligomer is characterized by spectroscopic methods showing low molecular weight and low dispersity. Two derivatives of the β-peptoid oligomer are prepared by acetylation and by grafting polycaprolactone by ring opening polymerization from the pendant hydroxyl groups. These products are blended with polycaprolactone to make films by solvent casting. The inclusion of the acyl derivatives of the β-peptoid to polycaprolactone affects the morphology of the film yielding microstructured and nanostructured patterns.

Supporting information may be found in the online version of this article.

Keywords: lipase; β-peptoid; polycaprolactone; films; surface properties

INTRODUCTION

The synthesis and characterization of β-peptides and β-peptoids have received considerable interest in recent years due to the ability of these compounds to resist proteases and mimic α-peptides and their potential as biomedical materials. Among these applications, oligomers with β-peptide and β-peptoid motifs served for the development of peptidomimetic γ-secretase inhibitors,[1] biocompatible polymeric micelles for drug delivery[2] and novel antimicrobial agents.[3] The tertiary amides of peptoids provide a backbone structure that is more stable to hydrolysis and less polar than typical peptide amide bonds.[4] As a result, β-peptoids would be expected to have greater metabolic stability and improved absorption properties than peptides. The combination of biocompatibility, degradability and processability makes polypeptoids potentially useful for certain biotechnological applications such as smart coatings, drug delivery, bioseparation etc.[5]

According to the literature, β-peptides and β-peptoids have been prepared by the ring opening polymerization of β-lactams[6,7] and β-amino acid-*N*-carboxyanhydrides,[8,9] the copolymerization of *N*-substituted aziridines with carbon monoxide[10,11] and solid-phase synthesis.[1,12] The first two methods are useful to provide high molecular weight polymers in a single step, whereas the latter provides a multi-step route for sequence-defined oligomers. Ring opening polymerization provides a direct route for β-peptides whereas the copolymerization method is useful for obtaining β-peptoids. Both methods require the use of either strong bases or metal complexes as catalysts.

Lipases are well known as catalysts for polymerizations that involve acyl substitution reactions. Specifically, they have been successfully employed for the preparation of many polyesters[13,14] and polyamides,[7,14,15] via either ring opening polymerization or polycondensation. Moreover, lipases are able to catalyze polymerization reactions without involving acyl substitution at all,

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e.g. acrylic polymerization[16] and aza-Michael addition.[15,17] Lipase-catalyzed polymerizations enable polymer preparation under smooth reaction conditions. Polymers obtained using this sort of catalyst have homogeneous molecular weight distributions albeit not very high molecular weight.

Polycaprolactone (PCL) is a synthetic, biodegradable and biocompatible polyester known to be useful for biomedical,[18] tissue engineering and active food packaging[19] applications. PCL is highly crystalline and hydrophobic and its biodegradation rate is slow.[20] For this reason, PCL is suitable for long-term implants and drug-release devices. However, its high hydrophobicity leads to poor compatibility with hydrophilic modifiers that may improve its properties, such as cell adhesion[21] or toughness.[22,23] For example, a third component or a covalently bonded modifier may be included for enhancing the compatibility of PCL with cellulose,[23] chitosan,[21,24] hydroxyapatite[25] and clays.[22,26] Moreover, protein-like polymers have been grafted with PCL for making amphiphilic polymers for drug delivery with tunable degradation rate.[27] The surface characteristics of polymer films are also very important with regard to their potential applications. For instance, porous surfaces usually facilitate cell adhesion and tissue growth. PCL and its copolymers have been employed for the preparation of porous films using diverse processing techniques.[21,28,29]

Considering this background we report the lipase-catalyzed synthesis of the β-peptoid oligomer poly[*N*-(2-hydroxyethyl)-β-propylamide]. This product was acetylated by chemical methods and PCL was grafted to the β-peptoid oligomer.

Considering the applications of modified PCL through its association with various polymers, PCL films modified by the β-peptoid oligomer and the copolymers were prepared and the properties of PCL and PCL-peptoid films were analyzed and compared.

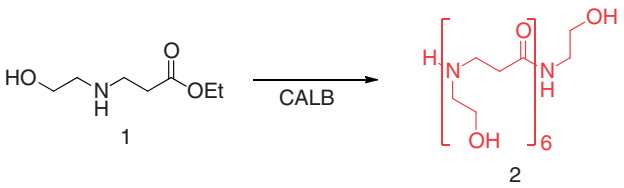
EXPERIMENTAL

Materials

All solvents were reagent grade and were used straight from the bottle. Ethyl acrylate, ethanolamine, ϵ-caprolactone, polycaprolactone (PCL-80K) (M_n = 80 000 g mol^{-1}) and tartaric acid were purchased from Sigma-Aldrich de Argentina (Bs. As., Argentina). ϵ-Caprolactone was distilled under reduced pressure prior to use. *Candida antarctica* B lipase (CAL B, Novozym 435, 7400 PLU g^{-1}) was a generous gift from Novozymes Spain. The enzyme was used straight from the bottle. Norharmane (nHo), harmaline, gentisic acid, dithranol, sinapinic acid, α-cyano-4-hydroxycinnamic acid, citric acid and β-cyclodextrin (cyclomaltoheptaose) were purchased from Sigma-Aldrich Chemical Co. Acenaphten and pyrene were purchased from Fluka and anthracene from Mallinckrodt. Water of very low conductivity (Milli Q grade) was used. Reactions were carried out in a Sontec incubator shaker (Scientifica SA) at 200 rpm.

Analysis of products

Fourier transform IR (FTIR) measurements were performed on a Shimadzu FTIR-8300 spectrophotometer in film with KBr windows and an attenuated total reflectance (ATR) cell. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC 200 NMR spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane set at 0 δ, and coupling constants are given in hertz. Solvents are indicated. Elemental analysis was carried on an Elemental Analyzer CE-440 (Exeter Analytical Inc.). Ultraviolet matrix assisted laser desorption ionization mass spectrometry (UV-MALDI MS) and ultraviolet laser desorption ionization mass spectrometry (UV-LDI MS) were performed on a Bruker Ultraflex Daltonics TOF/TOF mass spectrometer. Experimental details for mass spectrometry are presented in the supporting information. DSC measurements were performed in a Perkin Elmer Pyris-1 DSC. Samples were heated from 25 to 250 °C at a rate of 10 °C min^{-1}. SEM was performed on a Zeiss Leo 982 Gemini (Carl Zeiss SMT GmbH) and a Helios Nanolab 650 (FEI). Gel permeation chromatography (GPC) analysis was performed using a Waters 1515 isocratic HPLC pump connected to two Phenomenex 10^4 and 10^5 columns and a Waters 2489 UV detector (λ = 254 nm) and a Waters 2414 refractive index detector at room temperature. Chloroform was used as eluent with a flow rate of 1 mL min^{-1} and narrow polystyrene standards having peak molecular weights of 7600, 12 600, 28 000 and 100 000 g mol^{-1} were used for calibrating the GPC. An aqueous solution of the polymer was titrated with 0.1 mol L^{-1} HCl, measuring the pH variation with a Hanna pH 209 pH-meter (Hanna Instruments) equipped with a combined glass/AgCl electrode. The electrode was calibrated with buffer pH 10.00 and pH 7.00 standard solutions (Ciccarelli) and anhydrous sodium carbonate (Ciccarelli) was used as primary standard.



Scheme 1. Enzymatic preparation of β-peptoid oligomer **2**.

Synthesis

*Poly[N-(2-hydroxyethyl)-β-propylamide] oligomer (**2**)*

Ethyl *N*-(2-hydroxyethyl)-β-alaninate (**1**) as monomer starting material was prepared as previously reported.[30] The oligomer **2** was prepared by addition of the enzyme CAL B (1 g) to a solution of 90 mmol of **1** in acetone (75 mL). The enzymatic reaction was incubated for 72 h in an orbital shaker (30 °C, 200 rpm) and the product precipitated from the reaction medium. The solvent was removed by filtration and the precipitate (oligomer **2** and CAL B) was washed with ethanol:chloroform 2:1 (3 × 20 mL). The supernatants were combined and evaporated under reduced pressure. The residue was dried in a vacuum oven at 40 °C for 72 h to yield oligomer **2** as a white waxy solid (8.3 g, 11 mmol). The melting point M_p was 136 °C (determined by DSC). FTIR: 792, 856, 1024, 1072, 1211, 1317, 1402, 1457, 1517, 1627, 2852, 2964, 3413 cm^{-1}. The ^{1}H NMR spectrum is given in Fig. 1 and the UV-MALDI-TOF MS results are given in Fig. 2, Table S1 and Figure S1 of the supporting information; the elemental analysis is given in Table S2 of the supporting information.

*Poly[N-(2-acetoxyethyl)-β-propylamide] oligomer (**3**)*

To a solution of oligomer **2** (200 mg, 0.26 mmol) in acetic anhydride (2 mL), pyridine (0.5 mL) was added and the suspension was vigorously stirred at room temperature for 8 h. The reaction was finished by addition of 10% HCl solution (10 mL). Acetylated oligomer **3** was extracted with chloroform (3 × 10 mL). The organic phases were combined and dried. By evaporation under reduced pressure, 120 mg (0.12 mmol) of a colorless viscous liquid was

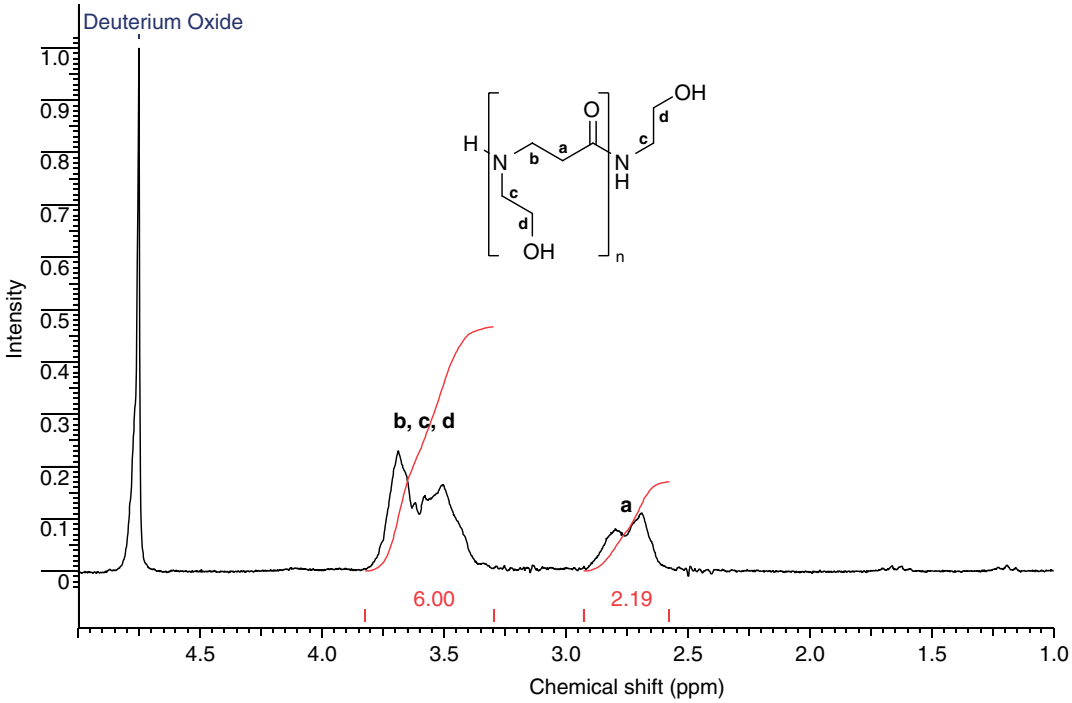


Figure 1. ^{1}H NMR spectrum of oligomer **2**.

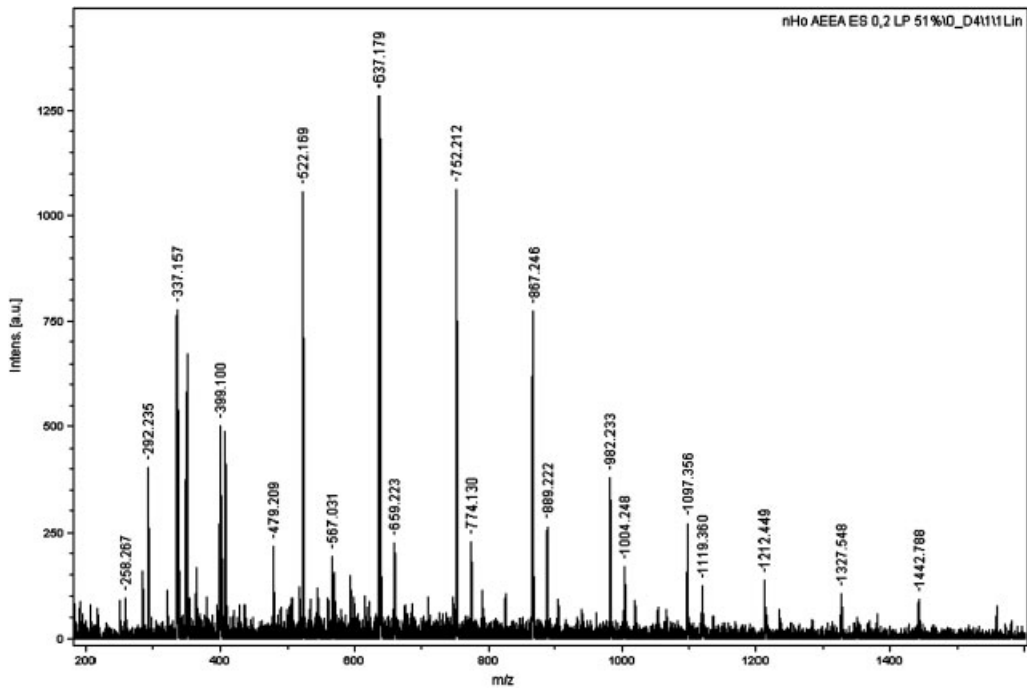


Figure 2. UV-MALDI-TOF spectrum of oligomer **2** (ion mode positive, matrix nHo).

obtained. FTIR: Fig. S2 of the supporting information. The ^{1}H NMR spectrum is given in Fig. 3(a).

Poly[N-(2-hydroxyethyl)-β-propylamide-graft-polycaprolactone] (**4**)

The preparation of **4** was carried out through ring opening polymerization by adapting a reported procedure.[31] Oligomer **2** (100 mg, 0.13 mmol) was suspended in freshly distilled ϵ-caprolactone (1 mL, 8.77 mmol) and tartaric acid (15 mg, 0.1 mmol) was added. The reaction vessel was purged with nitrogen and heated to 130 °C with magnetic stirring for 24 h. Then the reaction was cooled to room temperature and the product was dissolved in chloroform (25 mL) and precipitated by addition of excess methanol. The solvent was filtered off and the precipitate was dried, producing 775 mg of a white solid. FTIR: Fig. S3 of the supporting information. GPC: Fig. S4 and S5 of the supporting information. PCL content was determined by elemental analysis (Table S2) and ^{1}H NMR (Fig. 3(b)).

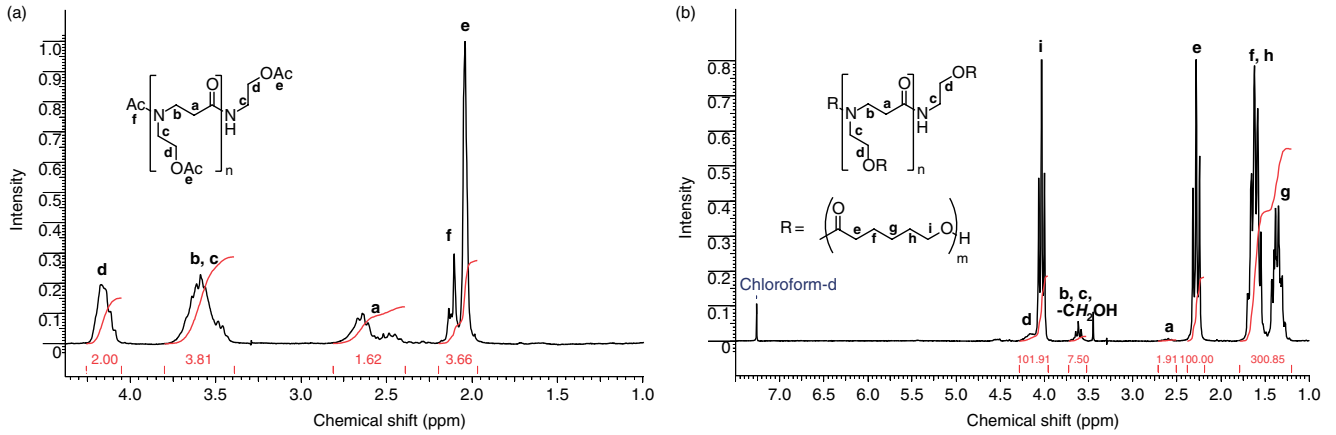


Figure 3. ^{1}H NMR spectrum of oligomer **3** (a) and copolymer **4** (b).

Film preparation

A mixture containing PCL-80K (500 mg) and 5%, 10% and 20% of oligomer (**2**, **3** or **4**) was dissolved in chloroform (10 mL). Ethanol (3 mL) was added for the dissolution of **2**. The solution was evaporated at 25 °C in 90 mm glass Petri dishes. The Petri dishes were covered in order to prevent water condensation during chloroform evaporation. The films were vacuum dried at 40 °C for 72 h. Films were characterized by SEM (Fig. 4), ATR FTIR (Fig. 5) and DSC (Fig. 6).

RESULTS

Enzymatic reaction conditions

The β-peptoid oligomer **2** was prepared by polymerization of ethyl *N*-(2-hydroxyethyl)-β-alaninate (**1**) using *Candida antarctica* lipase as catalyst (Scheme 1).

The preliminary experiments were carried out at 30 °C using diisopropyl ether (DIPE) as solvent, a substrate concentration (SC) of 1.2 mol L^{-1} and an enzyme:substrate ratio (E/S) = 1. The maximum product yield was achieved after 72 h.

Regarding the screening of the solvent, ketones (acetone, methyl isobutyl ketone), acetonitrile and *tert*-butanol were as good as DIPE for the reaction. Because of its efficiency and low cost acetone was the best choice.

The optimum E/S ratio was studied for CAL B in acetone using an SC of 1.2 mol L^{-1} and it was found that the reaction could be performed using an E/S ratio as low as 0.14 giving very good yield (83%). Control experiments with ethyl *N*-(2-hydroxyethyl)-β-alaninate (**1**) in acetone in the absence of enzyme did not afford polymeric products.

We also studied the effect of SC for the polymerization in acetone using E/S = 0.14 and varying SC from 1.2 mol L^{-1} to neat substrate. The results showed that SC higher than 1.2 mol L^{-1} afforded the oligomer in lower yield due to ester hydrolysis to give the β-amino acid. Regarding the optimal temperature it was observed that increasing the temperature from 30 °C to 55 °C did not increase the reaction rate significantly. On the contrary, an inert atmosphere had to be employed at 55 °C in order to avoid reaction darkening by oxidation.

In conclusion, the optimal conditions for the CAL B-catalyzed preparation of product **2** are an SC of 1.2 mol L^{-1}, acetone solvent, T = 30 °C and E/S = 0.14. After 72 h the product could be recovered in 83% yield.

Product characterization

The analysis of **2** by FTIR confirms the presence of a polyamide motif through the strong amide I band at 1627 cm^{-1} corresponding to C=O stretching. The spectrum also shows a broad band at 3413 cm^{-1} indicating the presence of hydroxyl groups.

^{1}H NMR of oligomer **2** did not show sharp and distinguishable signals due to restricted rotation of the substituted amide C–N bonds (Fig. 1). This behavior has been described for previously prepared β-peptoids.[11] Despite the lack of signal coalescence, the broad signals can be assigned to the protons of methylene in the repetitive unit. The peaks in the range 2.6–2.9 ppm can be assigned to the protons of methylene adjacent to the carbonyl group. The region between 3.3 and 3.9 ppm shows two partially overlapped methylene signals: one comes from the methylene adjacent to the hydroxyl group (3.69 ppm) and the other (3.50 ppm) corresponds to the two methylene groups attached to the nitrogen atom.

UV-MALDI-TOF (Fig. 2) allowed identification of ending functional groups as well as the molecular weight distribution. All peaks match the calculated *m/z* for oligomers having a hydrogen atom at the N-terminus and –NH(CH$_2$)$_2$OH at the C-terminus (for calculated and experimental *m/z* data see Table S1). A number-average molecular weight of 755.7 was estimated from peak intensity analysis. Thus a degree of polymerization of 6 can be derived. A dispersity of 1.12 for **2** was also calculated by comparison of UV-MALDI-TOF signal intensities. In addition, UV-MALDI-TOF experiments were performed on product **2** obtained in different reaction conditions at different reaction times and demonstrated that molecular weight and ending functional group identity were not affected by these variables.

No signals were detected in ^{1}H NMR supporting the occurrence of an ethyl ester. The amide I signal in the FTIR spectrum has a tiny shoulder that could suggest the presence to a small extent of carbonyl from acid or ester. Further UV-MALDI-TOF experiments performed in negative ionization mode (Fig. S1) showed signals that could be assigned to some oligomers having a free carboxylic acid at the C-terminus.

A 1% aqueous solution of **2** was prepared in order to investigate acid–base properties of the product. The pH of this solution was 10.2. Moreover, the solution was titrated with 0.1 mol L^{-1} HCl (titration curve: Fig. S6 of the supporting information). It was found that there were 1367 ± 9 µmol of amine groups and 27 ± 9 µmol of carboxylic acid groups per gram of product. Considering the structure of **2** a molecular mass of 732 ± 9 g mol^{-1} is estimated for

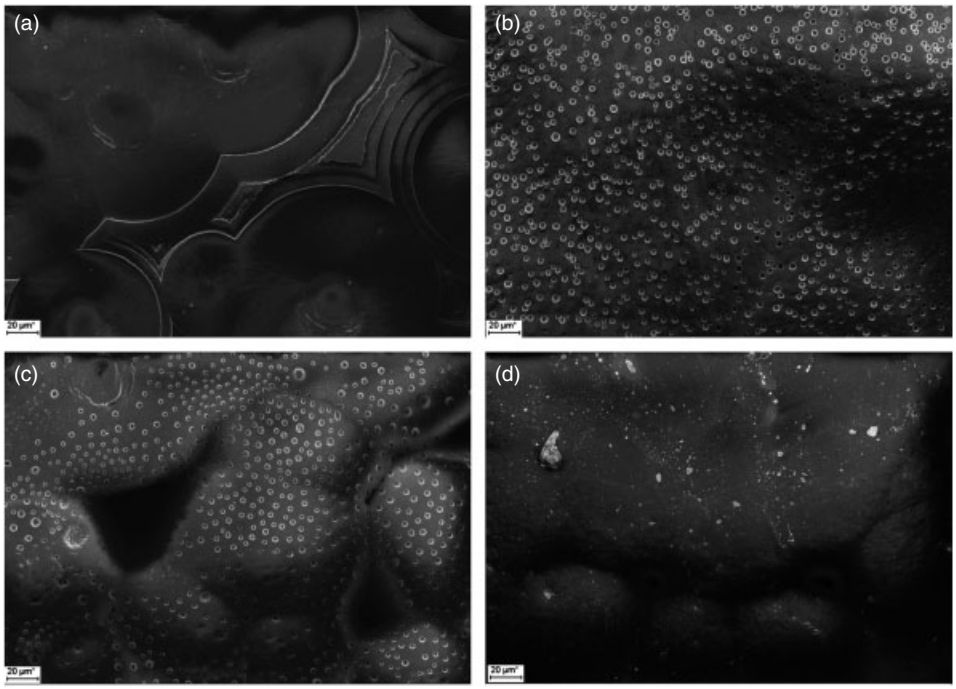


Figure 4. SEM image of PCL film alone (a), blended with 20% **3**, (b) blended with 20% **4** (c) and blended with 20% **2** (d). Films were prepared by solvent casting (the solvent was chloroform for (a), (b) and (c); chloroform with 23% v/v ethanol for (d)).

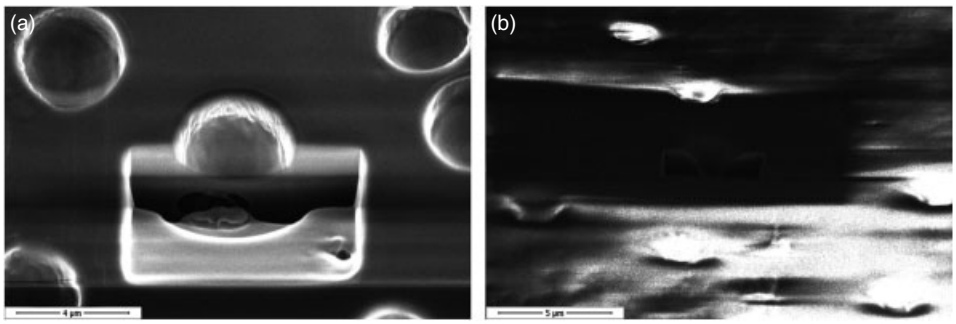


Figure 5. SEM image of PCL film blended with 20% **4** (a) and with 10% **4** (b). A pore has been cut with an ion beam in each case showing changes in the pore shape and size and an underlying pore in the sample of highest peptoid derivative concentration.

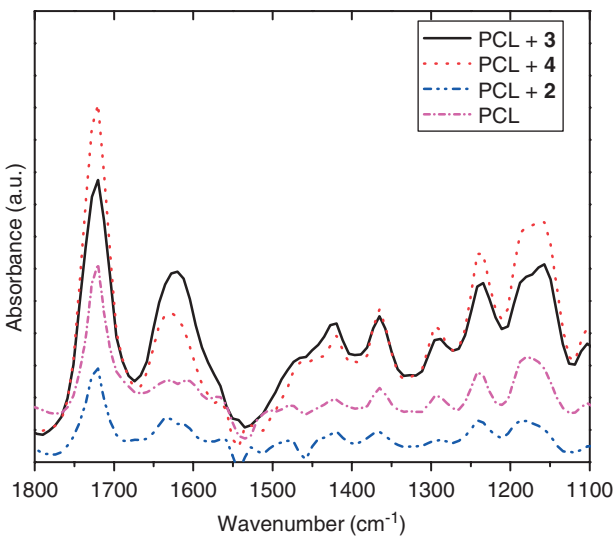


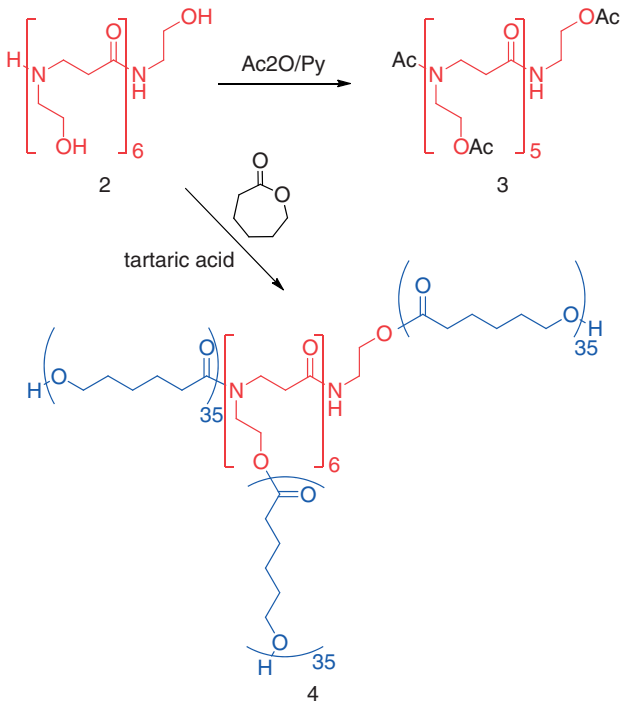
Figure 6. Stacked ATR spectra of PCL films.

the oligomer with 1.9% $\pm$ 0.7% of carboxyl terminated oligomer content.

Synthesis of acyl derivatives of 2

The acetyl oligomer **3** was prepared by treatment of **2** with acetic anhydride and pyridine at room temperature for 8 h. Copolymer **4** was synthesized by the ring opening polymerization of ϵ-caprolactone using the β-peptoid oligomer **2** as initiator and tartaric acid as catalyst.[32] Both reaction schemes are shown in Scheme 2.

The ^{1}H NMR spectrum of **3** (Fig. 3(a)) also showed broad signals due to the restricted rotation about substituted amide C—N bonds. The signals for acetamide and α-carbonyl methylene are clearly split due to the presence of at least two stable conformers. The acetylation of **2** on the hydroxyl groups was confirmed by observing the shift in the ^{1}H NMR signal of methylene groups adjacent to carbonyl from 3.69 ppm in **2** to 4.17 ppm in the acetyl derivative **3**. A singlet can also be observed at 2.04 ppm corresponding to *O*-acetyl groups. *N*-acetylation of terminal nitrogen was confirmed by the presence of a small singlet at 2.10



Scheme 2. Acetylation of peptoid oligomer **2** and synthesis of β-peptoid oligomer **2**-PCL copolymer **4**.

ppm. By comparing the relative areas of the ^{1}H NMR signals, a polymerization degree (*n*) of about 5 was estimated.

On the other hand ^{1}H NMR of **4** (Fig. 3(b)) showed the characteristic signals of the repeating units of PCL (1.35, 1.60, 2.28 and 4.03 ppm). The signals of the acylated peptoid backbone were also present (2.60, 3.62 and 4.16 ppm) and their chemical shifts were similar to the corresponding signals of product **3**. The triplet of the α-hydroxyl methylene at the end of the PCL chains overlapped the signals of the α-nitrogen methylenes.

The spectrum of Fig. 3(b) could be used for calculation of the amount of PCL grafted in **4** and the degree of polymerization of the pendant chains (details on the calculations are given in the supporting information). Assuming that *n* is equal to 6 the degree of polymerization of the PCL chains (*m*) is 35.

The amount of PCL grafted to the oligomer could also be estimated by elemental analysis. The PCL content (% w/w) was consistent by both methods. Estimation of PCL content from elemental analysis ranged from 97.54% (from %C) to 98.11% (from %N) whereas estimation from ^{1}H NMR and UV-MALDI-TOF was between these values (97.69%).

PCL copolymer **4** was also characterized by GPC (Fig. S4). The molecular weight parameters for this copolymer were M_n 5068 g mol^{-1}, M_w 6740 g mol^{-1} and polydispersity index 1.33.

Preparation and characterization of modified PCL films

All films were prepared by solvent casting. As β-peptoid oligomer **2** is insoluble in chloroform, a chloroform:ethanol mixture was used for dissolving both PCL and **2**. The content of **2** in the film was 20% by weight.

Oligomer **2** and its derivatives **3** and **4** were mixed with PCL-80K for film preparation. The amount of **3** and **4** added to the films varied from 20% to 5%.

Images of films prepared by solvent casting of PCL and products **2**, **3** and **4** were acquired by SEM characterization and are shown in Fig. 4. The film made only with PCL shows a microstructure made of spherulites 60–100 µm in diameter surrounded by lamellae (Fig. 4(a)). However, the SEM image of the film prepared with **2** showed that the β-peptoid crystallized separately and had an uneven distribution in the PCL matrix.

Interestingly, we found that the surface of the films prepared with **3** and **4** had spherical pores (average diameter 5 µm) evenly distributed. Some pores were also present beneath the film surface (Fig. 5). With the aim of testing the influence of the concentrations of **3** and **4** on the microstructure of the film surface, these concentrations were decreased from 20% to 5%. We observed that smaller wells of different shape were formed as the concentration decreased. At 10% of **3** and **4** the pore size decreased from 5 µm to 800 nm and turned from spherical to cone-shaped. At 5% of **3** and **4** pore abundance decreased dramatically. Moreover, no pores at all were detected in films prepared with PCL and 5% of **4** (Fig. S7 of the supporting information).

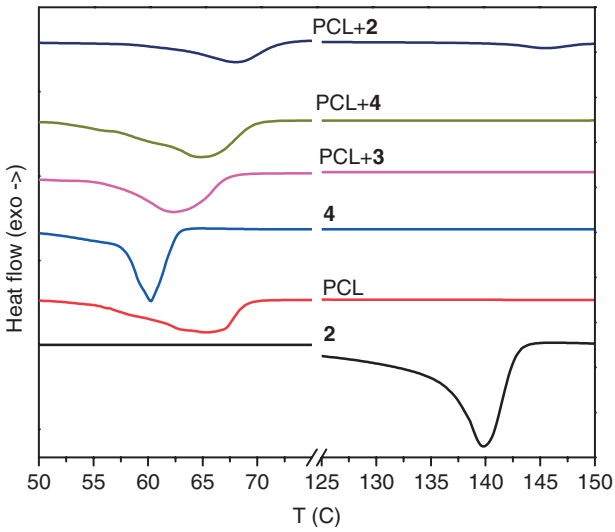


Figure 7. DSC curves of PCL, compounds **2**, **4** and blended films prepared by solvent casting. The PCL fusion peak appears between 50 and 75 °C. The fusion peak for peptoid **2** appears between 125 and 150 °C.

ATR FTIR spectra of the films were recorded in order to estimate the qualitative distribution of the β-peptoid oligomer derivative in the film. This technique has been used for estimating the migration of compounds to the surface of polymer films.[33] The occurrence and intensity of carbonyl bands for each case were analyzed and Fig. 6 shows stacked spectra of the films between 1100 and 1800 cm^{-1}.

All ATR spectra showed an intense band at 1720 cm^{-1} corresponding to the ester carbonyl stretching in PCL. Films prepared with **3** and **4** showed an additional band at 1627 cm^{-1} that could be attributed to amide carbonyls on the surface of the film. It was also observed that ATR spectra of these films have a different pattern in the region between 1150 and 1200 cm^{-1}.

DSC analysis of films was useful for evaluating the compatibility of PCL with peptoids **2** and **3** and copolymer **4**. The DSC curves are shown in Fig. 7. PCL has a melting point of 60 °C. All films and compound **4** showed a peak at a temperature close to the PCL melting transition. On the other hand, compound **2** melts at 136 °C. Compound **3** did not show any DSC peak (data not shown) and **4** showed a single peak corresponding to PCL melting. The DSC analysis of films prepared by blending **3** and **4** with PCL also showed only melting of PCL.

DISCUSSION

Absence of ester moieties in product **2** confirms the chemoselective behavior of CAL B. It is known that lipases can catalyze either transesterification or aminolysis reactions. For instance, substrate **1** is an *N*-hydroxyalkyl-amino ester and polymerization reaction could have taken place by acylation of hydroxyl groups. However, transesterification did not occur. We could confirm the absence of ester moieties in **2** by spectroscopic methods, concluding that the amino groups were selectively acylated by CAL B. Hence, the β-peptoid structure of **2** was demonstrated. Moreover, molecular weight parameters and ending functional group identity were not significantly affected by different reaction conditions. We assume that this effect is due to precipitation of product from the reaction medium and lipase catalysis. The low dispersity is also a characteristic of enzymatic polymerizations. In previous work we obtained nearly monodisperse products by CAL B-catalyzed polymerization reactions.[15,16]

The molecular weight of **2** was estimated by UV-MALDI-TOF experiments and titration of amine groups. Both results were in good agreement with each other.

The occurrence of some free carboxylic acid at the C-terminus could be attributed to enzyme-catalyzed hydrolysis of ester **1**. We found that the occurrence of free carboxylic acid at the C-terminus was very low, based on titration of the carboxylic acid of oligomer **2**.

Acyl derivatives of compound **2** were prepared in order to increase the compatibility of β-peptoid oligomer with PCL. In particular, copolymer **4** has PCL pendant chains and thus compatibility of this compound with PCL is expected.

Regarding compound **3**, a slight decrease in polymerization degree between **2** and **3** was observed. This could be attributed to the purification step performed after derivatization. The structure of acetyl peptoid **3** confirmed that $-NH(CH_2)_2OH$ is the main ending group in the β-peptoid oligomer **2**. This is an interesting result regarding the lipase-catalyzed behavior. As we mentioned before, lipases proved to be efficient catalysts for aza-Michael additions. The high occurrence of $-NH(CH_2)_2OH$ at the C-terminus indicates that another reaction occurred besides polymerization. The aminoester **1** used as monomer was obtained in high purity, free of ethanolamine, so the effect could not arise from the alkanolamine. On the other hand, no ester bonds were detected in **2**. Therefore it could be assumed that CAL B catalyzes a retro aza-Michael reaction to produce this ending group. As enzyme catalysis allows reactions to proceed under thermodynamic control CAL B could catalyze reverse aza-Michael additions. In the recent literature we found examples of organic catalysts, which catalyze the reaction forward and backward, that support this assumption.[34] However, we were not able to determine whether the retro aza-Michael occurred before or after polymerization. The ending group could be generated by either the presence of ethanolamine released from the retro aza-Michael reaction on monomer **1** or detachment of an ethyl acrylate molecule from the polymer.

Regarding compound **4**, ring opening polymerization catalyzed by tartaric acid[32] has proven to be an efficient method to synthesize this PCL copolymer. A brush copolymer structure was confirmed and PCL chain length was estimated by ^{1}H NMR. GPC analysis of compound **4** also confirmed the presence of amide bonds due to increased UV absorption (Fig. S5) and showed only a slight increase in polydispersity index compared with product **2**.

Literature data for films prepared by solvent casting of PCL of similar molecular weight at room temperature describe spherulites of about the same size as those we found.[35] On the other hand, aggregates of smaller spherulites were observed in the films prepared with **3** and **4** (Figs 4(b) and 4(c) respectively). We assume that these pores were generated by migration of the oligomer derivative to the surface of the PCL film and phase separation during solvent evaporation. The presence of both smaller spherulites and pores of different shapes in peptoid-modified PCL films is an interesting feature provided that greater film roughness promotes better cell proliferation.[36]

From the SEM images of the films prepared with different concentrations of peptoid derivative we assume that critical concentrations of **3** and **4** for microstructure and nanostructure formation on the film surface are between 5% and 10%.

Changes in the ATR spectra of films prepared with **3** and **4** between 1150 and 1200 cm^{-1} could be attributed to a different intermolecular association around C—O—C bonds because of the presence of acetyl β-peptoid **3** and the brush copolymer **4** on the surface of the film. Moreover, in the ATR spectrum of the film prepared with **3** the intensity of the amide band is more than half the intensity of the ester band. This seems to indicate that compound **3** is rather abundant on the surface of the film.

DSC analysis of oligomer **2** suggests that this β-peptoid has similar thermal behavior to α-peptoids.[37] In particular, the melting point of **2** is similar to α-peptoids having long linear *N*-alkyl substitutions. Therefore, there is no important structural contribution from hydrogen bonding of hydroxyl groups. This also confirms that β-peptoids have promising characteristics for materials applications where processability and stability are necessary.

Incompatibility between **2** and PCL could be confirmed by DSC, as both peaks for PCL and peptoid fusion could be detected separately in the thermogram of a film containing these two compounds. However, the derivatized peptoids proved to be compatible with PCL. For these two films the PCL melting point was slightly depressed, which demonstrates that PCL was miscible with **3** and **4**.

In conclusion, the acylation of oligomer **2** increased the compatibility with PCL and allowed a better integration and a more even distribution of the peptoid moieties in the PCL films.

CONCLUSIONS

A β-peptoid oligomer with pendant hydroxyalkyl groups could be synthesized by an enzyme-catalyzed polymerization. The oligomer obtained shows low dispersity. It is interesting to point out that enzymatic catalysis is highly selective. Under these conditions, hydroxyl groups remained unreactive and no protective groups were necessary to obtain the β-peptoid. The lipase was able to catalyze aminolysis reactions selectively for polymerization. In addition, the identity of the polymer C-terminus suggests that a lipase-catalyzed retro aza-Michael has occurred.

The acetyl derivative of the β-peptoid oligomer and a β-peptoid-PCL copolymer were also synthesized. These derivatives were useful for oligomer characterization and for increasing compatibility of the oligomer with a hydrophobic matrix such as PCL. PCL and **2** were not compatible but the acetyl derivative **3** and **4** showed better compatibility with PCL and actually changed the surface characteristics of PCL films. This is an important feature because the occurrence of microstructures and nanostructures on the film surface was found to be important for tuning biological properties such as cell adhesion.[38,39]

In conclusion this work reports the synthesis by enzymatic and chemical methods of a β-peptoid and copolymers peptoid-PCL and their application in the preparation of porous PCL films. It provides a starting point for further research in their potential

applications, such as films with enhanced cell adhesion or patterned biocatalytic films.[40]

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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