Bulk Organocatalytic Synthetic Access to Statistical Copolyesters from γ-Lactide and ε-Caprolactone Using Benzoic Acid

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Introduction

As biodegradable, nontoxic, and biocompatible polymers, polylactide (PLA) and poly(ε-caprolactone) (PCL) can be attractive biosourced surrogates for petroleum-based polymers.1−7 Both PLA and PCL have been intensively investigated in applications ranging from pharmaceutics to packaging and electronics.4−7 Yet, both PLA and PCL show some limitations in these applications. For instance, PLA is brittle, exhibits poor elasticity,6 low thermal stability, and modest permeability to drugs. PCL has higher thermal stability and elasticity than PLA, with a glass transition temperature (Tg) around −60 vs 45−65 °C for PLA3,10 but suffers from poor mechanical properties. PCL has also a higher permeability to drugs11 and half time in vivo of 1 year,12 vs a few weeks for PLA.13 As a result, statistical copolyesters of lactide (LA) and ε-caprolactone (CL), i.e., P(LA-stat-CL) aliphatic copolysteres are highly sought-after materials as they combine the strengths and minimize the weaknesses of both homopolymers. P(LA-stat-CL)s have thus attracted a great deal of attention in the biomedical and pharmaceutical fields14−18 and as compatibilizers for PLA/PCL blends.19 The precision synthesis of P(LA-stat-CL) copolymers is still particularly challenging whether organometallic20 or organic21−30 catalysts are used. This is due to the highly differing reactivity of the two monomers during ring-opening copolymerization (ROcP). LA is typically incorporated first, although CL gives faster rates than LA in homopolymerization reactions.25,27,29,31−37 Aluminum-based catalysts are most efficient for the statistical and controlled ROcP of LA and CL,35,38−40 although less toxic

Supporting Information

ABSTRACT: The development of synthetic strategies to produce statistical copolymers based on γ-lactide (γ-LA) and ε-caprolactone (CL), denoted as P(LA-stat-CL), remains highly challenging in polymer chemistry. This is due to the differing reactivity of the two monomers during their ring-opening copolymerization (ROcP). Yet, P(LA-stat-CL) materials are highly sought after as they combine the properties of both polylactide (PLA) and poly(ε-caprolactone) (PCL). Here, benzoic acid (BA), a naturally occurring, cheap, readily recyclable, and thermally stable weak acid, is shown to trigger the organocatalyzed ring-opening copolymerization (ORoCP) of γ-LA and CL under solvent-free conditions at 155 °C, in presence of various alcohols as initiators, with good control over molar masses and dispersities (1.11 < D < 1.35) of the resulting copolymers. Various compositions can be achieved, and the formation of statistical compounds is shown through characterization by1H, 13C, and di

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catalysts based on zinc\textsuperscript{41} and molybdenum\textsuperscript{42} have also been successfully employed for this purpose.

Organic catalysis for polymerization is a fast developing field in polymer chemistry, offering a number of advantages over metal catalysis, such as more sustainable processes, reduced toxicity and cost, and easier catalyst synthesis, purification, handling, and storage.\textsuperscript{43,44} In this regard, the organocatalyzed ring-opening polymerization (OROP) of LA and CL has been particularly investigated.\textsuperscript{34–35} Attempts to design P(LA-stat-CL) copolymers by ORoCp, however, have met with limited success.\textsuperscript{21–29} Basic-type organocatalysts, such as phosphazenes,\textsuperscript{2,2} N-heterocyclic carbenes,\textsuperscript{2,3,7} 1,5,7-triazabicyclo[4.4.0]dec-5-ene guanidine,\textsuperscript{21} and thiourea-amine,\textsuperscript{28} only enable incorporation of LA in the polymer chain, and P(LA-stat-CL) copolymer synthesis cannot be achieved in this way. In contrast, a few Brønsted acid-type catalysts have been shown to perform the statistical ORoCP of LA and CL.\textsuperscript{24,25,29} Trifluromethanesulfonic acid (TfOH) has been used in dichloromethane at 35 °C, providing preferential insertion of LA units in copolymer chains.\textsuperscript{2,2} We have reported the use of dibenzoylmethane, a naturally occurring β-diketone, for the ORoCP of l-lactide (l-LA) and CL in bulk at 155 °C, forming a gradient to statistical-like copolymers.\textsuperscript{27} In a recent addition, we have described that benzoic acid (BA), another naturally occurring organocatalyst that is also cheap, thermally stable,\textsuperscript{51} and a readily recyclable weak carboxylic acid, can serve for the metal-free synthesis of polyesters based on PLA and PCL.\textsuperscript{52} BA thus allows achieving well-defined PLA and PCL by ORoP in bulk, in a temperature range of 155–180 °C, in presence of alcohols as initiators. A bifunctional mechanism where the catalyst would act as a proton shuttle between the monomer and the initiator/chain ends has been postulated. We have also described one example of P(LA-stat-CL) copolymer synthesis by BA-ORoCP carried out in bulk.\textsuperscript{52}

In the present contribution, we provide a complete description of the P(LA-stat-CL) copolymer synthesis in solvent-free conditions. In particular, reactivity ratios of comonomers have been determined, using both the Kelen-Tüdös linear method and a nonlinear method referred to as “the visualization of the sum of squared residuals space” (VSSRS).\textsuperscript{53,54} The P(LA-stat-CL) statistical copolymers are characterized by combined analyses, including \(^1\)H, \(^{13}\)C and differential fusion ordered spectroscopy (DOSY) NMR, differential scanning calorimetry (DSC), and size exclusion chromatography (SEC). The controlled character of this BA-ORoCP process is further exploited to achieve PLA-b-P(LA-stat-CL)-b-PLA triblock copolymers, by sequential RoCp-mediated synthesis.

**Experimental Part**

**Materials.** l-Lactide (l-LA, 98%, TCI) was recrystallized three times from toluene and dried under vacuum for 2 days. \(\varepsilon\)-Caprolactone (CL, 99%, ACROS), butane-1,4-diol (BD, 99%, VWR), and heptan-1-ol (HeptOH, 98%, Sigma-Aldrich) were dried over CaH\(_2\) for 48 h prior to distillation under reduced pressure and were stored on molecular sieves. Methoxypoly(ethylene glycol) (mPEG\(_{1000}\), TCI, \(M_n \approx 1000\,\text{g}\,\text{mol}^{-1}\)) was dried by three azeotropic distillations using toluene. Compounds were stored in a glovebox (O\(_2\) < 6 ppm, H\(_2\)O < 0.5 ppm). THF and toluene were dried using SPS from Innovative technology, stored over sodium benzenophene and polysyllythium, respectively, and distilled prior to use.

**Methods.** NMR spectra were recorded on a Bruker Avance 400 (\(\varepsilon\), 1\(^{13}\)C, 400.2 and 100.6 MHz, respectively) in CDCl\(_3\), at 298 K. Quantitative \(^3\)C NMR was performed on the copolymer sample (60 mg in 0.6 mL) using the “INVGATE” sequence with a pulse width of 30°, an acquisition time of 0.7 s, a delay of 4 s between pulses, and 6144 scans to investigate the comonomer distribution within copolymers.\textsuperscript{55} Diffusion ordered spectroscopy (DOSY)\textsuperscript{56} measurements were performed at 298 K on a Bruker Avance III 400 spectrometer operating at 400.33 MHz and equipped with a 5 mm Bruker multinuclear z-gradient direct cryoprobe-head capable of producing gradients in the \(z\) direction with strength 53.5 G cm\(^{-1}\). The sample was dissolved in 0.4 mL of CDCl\(_3\) for internal lock and spinning was used to minimize convection effects. The sample was thermostated at 298 K for at least 5 min before data accumulation. The DOSY spectra were acquired with the ledppg2s pulse program from Bruker topspin software. The duration of the pulse gradients and the diffusion time were adjusted to obtain full attenuation of the signals at 95% of the maximum gradient strength. The values were 2.4 ms for the duration of the gradient pulses and 100 ms for the diffusion time. The gradient strength was linearly incremented in 16 steps from 5 to 95% of the maximum gradient strength. A delay of 5 s between echoes was used. The data were processed using 8192 points in the F2 dimension and 128 points in the F1 dimension with Bruker topspin software. Field gradient calibration was accomplished at 25 °C using the self-diffusion coefficient of H\(_2\)O + D\(_2\)O of 19.0 \(\times\) 10\(^{-11}\) m\(^2\) s\(^{-1}\).\textsuperscript{58}

Molar masses were determined by size exclusion chromatography (SEC) in THF (1 mL min\(^{-1}\)) with trichlorobenzene as a flow marker at 313 K, using a refractometric (RI) detector. Analyses were performed using a three-column TSK gel TOOSO (G4000, G3000, G2000). The SEC device was calibrated using linear polystyrene (PS) standards.

Differential scanning calorimetry (DSC) measurements were carried out with a DSC Q100 LN2 apparatus from TA Instruments under a helium flow. The PCL sample was heated for the first run from −130 to 100 °C, then cooled again to −130 °C and heated again for the third run to 100 °C (heating and cooling rate, 10 °C min\(^{-1}\)), whereas PLA sample undergoes 3 runs between −40 and 200 °C and P(LA-co-CL) between −70 and 200 °C. Glass transition temperatures (\(T_g\)) and melting temperatures (\(T_m\)) were measured from the second and first heating run, respectively.

**General Procedure for Statistical Copolymerization of l-LA and CL in Presence of BA.** In a glovebox, previously flame 10 mL Schlenks were charged with the appropriate amount of l-LA and CL, the BA catalyst (2.5, 5, and 10 mol % relatively to the monomer), and a stir bar. The BD initiator (BD or HeptOH) was added via a 5 or 10 mL syringe, whereas mPEG\(_{1000}\) was charged directly in the Schlenk. The Schlenks were sealed before being introduced in an oil bath preheated at the desired temperature (155–180 °C). At specified times, one Schlenk was removed from the oil bath to monitor the reaction by \(^1\)H NMR. The as-obtained copolymers were purified by applying vacuum (0.1–0.2 mbar) to the Schlenk at 155 °C with a high stirring rate (800–1000 rpm) for 5 min. The number average molar mass (\(M_n\)) and the dispersity (\(D\)) were determined by SEC.

**General Procedure for Triblock Synthesis.** In a glovebox, a previously flame 10 mL Schlenk was charged with l-LA (0.200 g, 1.4 mmol) and CL (0.158 g, 1.4 mmol), the BA catalyst (5 mol % rel. to monomers), and a stir bar. The BD initiator (DP\(_{BD}\) = 25 for each monomer) was added via a 10 \(\mu\)L syringe. The Schlenk was then sealed before being introduced in an oil bath preheated at 155 °C. After 20 h of reaction, the Schlenk was introduced in the glovebox to estimate the monomer conversion via \(^1\)H NMR spectroscopy and to determine the average molar mass (\(M_n\)) and the dispersity (\(D\)) by SEC. The as-obtained copolymer was purified by applying vacuum at 155 °C with a high stirring rate. The Schlenk was again introduced into the glovebox to add more BA catalyst (5 mol % rel. to l-LA) and the l-LA monomer (0.200 g, 1.4 mmol) to target DP\(_{BD}\) ≈ 25. The polymerization could be restarted by immersing the Schlenk in the oil bath for 25 h. A sample was collected to estimate conversion by \(^1\)H NMR spectroscopy prior to the purification. The as-obtained triblock...
Table 1. BA-OROcP of l-LA and CL in Bulk at 155 °C in Presence of Alcohols as Initiators (I)

<table>
<thead>
<tr>
<th>run</th>
<th>I</th>
<th>fCL,0 (%)</th>
<th>BA (mol %)</th>
<th>time (h)</th>
<th>CCL/LA (mol %)</th>
<th>FCL (%)</th>
<th>MSEC (g mol⁻¹)</th>
<th>B'</th>
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Notes: Reactions were performed in bulk at 155 °C under an argon atmosphere with reaction conditions: nCL + nLA = 2.8 mmol; and [M]₀/[I]₀ = 50:1 with [M]₀ = [l-LA]₀ + [CL]₀. CL fraction in the initial feed. mol % of catalyst loading relative to the monomers. CL and l-LA conversions were determined by °H NMR analysis. °CL fraction in the pure copolymer. °Uncorrected average molar mass and dispersity (B) of crude copolymers determined by SEC chromatography (polystyrene standards) at 40 °C and THF as eluent. °Theoretical degree of polymerization DPinh = ([L-LA]₀/[CL]₀) x CCL + ([CL]₀/[CL]₀) x CCL. °Degree of polymerization calculated from the chain ends determined by °H NMR. °[M]₀/[I]₀ = 100:1. na: not available.

Results and Discussion

Investigations into the BA-OROcP of l-LA and CL in Bulk

BA was used to catalyze the ROP of l-LA and CL in bulk at 155 °C in the presence of butane-1,4-diol (BD), heptanol (HeptOH), and methoxypoly(ethylene glycol) (mPEG10000) as initiators. Scheme 1 shows the general synthesis method, and Table 1 summarizes the main results obtained. A first series of copolymerization experiments employed different BA catalyst loadings and BD as the initiator, with an initial monomer-to-initiator ratio [l-LA]₀/[CL]₀/[I]₀ of 25:25:1 (Table 1, runs 1–4). While the reaction proved sluggish in the absence of BA, with l-LA being inserted preferentially (Table 1, run 1), the copolymerization kinetics could be appreciably enhanced by increasing the BA loading from 2.5 to 10 mol % relative to the monomers, confirming the catalytic role of BA (runs 2–4). A catalyst loading of 5 mol % relative to the monomers was selected for the rest of the study, as both monomers were consumed at the same rate. The initial monomer feed ratio (fCL,0 = 0.2, 0.3, 0.5, 0.7, 0.9) was then varied, while maintaining a comonomer-to-initiator ratio of 50:50:1 (runs 3, 5–8). In all cases, well-defined and transparent α,ω-bishydroxy-P(LA-co-CL) copolymers were obtained (Figure S1, Picture S1). Experimental degrees of polymerizations (DPexp) were consistent with theoretical values (DPinh, Table 1), and molar masses (MSEC) increased linearly with the overall conversion of the monomers (CTOT, Figures 1a, S2, and S3). Monomodal and symmetrical SEC traces were observed with dispersity remaining low (1.11 < D < 1.25; Figures 1a,b and S2–S4), confirming the good control over the OROcP process. Similar results were obtained using a comonomer-to-initiator ratio equal to 100 (Table 1, run 9, Figure 1a,c), although a slight discrepancy between DPexp and DPinh was noted in this case, probably due to side initiation by traces of water.

As BA organocatalyst remained in the crude copolymers, these were purified to avoid any premature degradation, for instance, during processing. For this purpose, we exploited the capability of BA and l-LA to sublime and of CL to evaporate following a solvent-free and straightforward purification procedure that was set up in our previous study.
This also allowed us to recycle and reuse BA for further organocatalytic cycles, leading to chemically pure P(LA-co-CL) copolyesters.

Determination of Reactivity Ratios and Analysis of P(LA-co-CL) Microstructure. Analysis by 1H NMR spectroscopy evidenced that both comonomers were inserted in the copolymer chain throughout this BA-OROcP process, irrespective of the initial comonomer feed (Figures 2 and S5, Table 1).

To account for the copolymer microstructure, reactivity ratios of 1-LA ($r_{LA}$) and CL ($r_{CL}$) were evaluated using the Kelen–Túdos method, for the BA-OROcP of 1-LA and CL carried out in bulk at 155 °C (Scheme S1, Figure S6, and Table S1). These kinetic investigations led to the following values: $r_{LA} = 0.66$, $r_{CL} = 0.91$. However, linearized methods such as the Kelen–Túdos method, which derive from the Mayo–Lewis equation and require that monomer conversion should be kept very low, can distort the error structure of the data and may provide biased estimates of reactivity ratios. This prompted us to implement a less biased method, the visualization of the sum of squared residuals space (VSSRS), a nonlinear method developed by Van den Brink et al. This VSSRS method not only allows for an estimate of the reactivity ratios at high conversion but also takes into account errors both on the monomer conversion and the comonomer ratios, thus providing unbiased estimates of the reactivity ratios as well as joint confidence regions. Data related to monomer conversion, copolymer composition ($F$), and comonomer ratio ($f$) were fitted to the integrated form of the Mayo–Lewis copolymer composition in eq 1

$$C_{TOT} = 1 - \left(\frac{f_{CL}}{f_{CL,0}}\right)^{\alpha} \times \left(\frac{f_{LA}}{f_{LA,0}}\right)^{\beta} \times \left(\frac{f_{CL,0} - \delta}{f_{CL} - \delta}\right)^{\gamma}$$

(1)

Figure 1. (a) Evolution of uncorrected $M_{SEC}$ (●) and dispersity $D$ (×) with total monomer conversion ($C_{TOT}$); (b) evolution of SEC molar masses with time (conditions corresponding to run 3, Table 1); (c) SEC comparison of runs 3 and 9 (Table 1).

Figure 2. Evolution of the overall monomer conversion vs time for different contents in CL in the initial feed ($f_{CL,0}$ = 0.2, 0.3, 0.5, 0.7, 0.8; runs 3 and 5–8, respectively) and evolution of CL content in the final copolymer ($F_{CL}$) vs $f_{CL,0}$. Dashed line represents expected $F_{CL}$ for $r_{CL} = r_{LA} = 0.86$ using the Mayo–Lewis eq 2.
The reactivity ratios were found equal to $r_{CL} \approx r_{LA} \approx 0.86$. Figure 3a shows the point estimates and the confidence regions obtained using the VSSRS method. The 95% confidence intervals were almost the same for both $r_{CL}$ (0.74—1.01) and $r_{LA}$ (0.75—1.00).

Theoretical plots of $f_{CL}$ and $F_{CL}$ as a function of $C_{TOT}$ were then modeled from values of the reactivity ratios obtained by the VSSRS method (Figure 3b,c, solid lines). These plots fit well the experimental data obtained in the course of the bulk BA-ORoCP of 1-LA and CL at 155 °C (Figure 3b,c, black dots). When plotting $F_{CL,th} = g(f_{CL,d})$ from the reactivity ratios obtained by the VSSRS method (Figure 2, dashed line) using the Mayo–Lewis eq 2, we observed a total agreement with experimental data ($F_{CL,exp} = g(f_{CL,d})$, Figure 2). The overall composition $F_{CL}$ was also found in full accordance with comonomer contents used in the feed ratio throughout the whole OROcP process. The slight deviation observed at a conversion lower than 20% might be due to uncertainties in the NMR measurements. These deviations at low conversion account for the difference in estimates of reactivity ratios by the Kelen–Tudós method and the VSSRS method.

$$F_{CL,th} = \frac{r_{CL} \times f_{CL}^2 + f_{CL} \times f_{LA} + r_{LA} \times f_{LA}^2}{r_{CL} \times f_{CL}^2 + 2 \times f_{CL} \times f_{LA} + r_{LA} \times f_{LA}^2}$$

(2)

These kinetic results strongly support the formation of statistical copolymers when using BA in OROcP of 1-LA and CL. Copolymer structures were further analyzed by $^1$H and $^{13}$C NMR spectroscopy from various 1-LA/CL ratios (Table 1, runs 3, 5—8). The $^1$H NMR spectrum of the copolymer obtained from BA-ORoCP involving an equimolar ratio of 1-LA and CL (Table 1, run 3) showed all representative peaks due to homo- and heterodiads, as illustrated in Figure 4a.

As expected for statistical copolymers, integral values of the homosequences closely matched those of the heterosequences for both PLA (10.1/10.7, $\delta$ of around 5.1 ppm) and PCL (11.7/10.0, $\delta$ of around 2.35 ppm). Interestingly, the integral value of the terminal lactidyl units ($e_{LA}$) appearing at 4.36 ppm was 10 times greater than that of the terminal caproyl units ($e_{CL}$) at 3.62 ppm. This might be explained by the higher reactivity of caproyl units at chain ends, which after fast crossover gave rise to less reactive terminal lactidyl units. This can only be stated because reactivity ratios are close to 1 for...
both monomers \( (r_{CL} \approx r_{LA} \approx 0.86) \) and because the BA-OROP of CL is 20 times faster than that of L-LA \( (k_{CL-LA} \gg k_{LA-LA}; \text{Scheme } 1) \). In these conditions, one can write the following relationships: \( k_{CL-LA} \approx k_{CL-CL} \gg k_{LA-LA} \approx k_{LA-CL} \) using eq 3

\[
r_{CL} = \frac{k_{CL-CL}}{k_{CL-LA}} \quad n_{LA} = \frac{k_{LA-CL}}{k_{LA-LA}}
\]

Analysis by \(^{13}\)C NMR spectroscopy (Figure 4b) also confirmed the microstructure of the copolymer with the expected homo- and heterotriads, as previously reported. Kasperczyk and Bero described two distinct modes of transesterification reactions (Figures 5 and S13, Table S2). The same peaks of homo- and heterotriads, although of different intensities, were observed for copolymers of differing compositions (runs 3 and S–8, Table S2 and Figures S12–S14). As expected, the content of CL heterotriads, as determined by \(^1\)H NMR, decreased linearly with the initial feed in CL (Table S2 and Figure S15). The average block length of the caproyl \( (L_{CL}) \) and lactidyl \( (L_{LA}) \) units could also be assessed by \(^1\)H and quantitative \(^{13}\)C NMR analyses and compared with the theoretical values \( (L_{CL,th} \text{ and } L_{LA,th}) \) obtained from eq 4 (Figures 5 and S13, Table S2).

\[
L_{CL,th} = \frac{r_{CL} \times f_{CL} + f_{LA}}{f_{LA}} \quad L_{LA,th} = \frac{r_{LA} \times f_{LA} + f_{CL}}{f_{CL}}
\]

For the pure copolymer of \( f_{CL} = 0.5 \) (run 3, Table 1), \( L_{CL} \) and \( L_{LA} \) values determined by \(^{13}\)C NMR \( (L_{CL,13C} = 2.2 \text{ and } L_{LA,13C} = 2.1) \) were in excellent agreement with the theoretical values \( (L_{CL,th} = 1.9 \text{ and } L_{LA,th} = 1.8) \) and with those determined by \(^1\)H NMR spectroscopy \( (L_{CL,1H} = 2.1 \text{ and } L_{LA,1H} = 1.9) \). Finally, glass transition temperatures, as determined from the second run of DSC analyses \( (T_{g,exp}) \), were consistent with values expected from the Fox equation \( (T_{g,Fox}; \text{Figure 6, Table S2, and Figure S16}) \).

### Scheme 2. Two Modes of Transesterification Reactions

**The first mode of transesterification reaction**

\[
P\overset{-\text{O}}{\underrightarrow{\text{CL-OH}}} + P\overset{-\text{O}}{\underrightarrow{\text{CL-CL}}} \longrightarrow P\overset{-\text{O}}{\underrightarrow{\text{CL-CL}}} + P\overset{-\text{O}}{\underrightarrow{\text{CL-OH}}}
\]

**The second mode of transesterification reaction**

\[
P\overset{-\text{O}}{\underrightarrow{\text{CL-OH}}} + P\overset{-\text{O}}{\underrightarrow{\text{CL-CL}}} \longrightarrow P\overset{-\text{O}}{\underrightarrow{\text{CL-LA}}} + P\overset{-\text{O}}{\underrightarrow{\text{LA-OH}}}
\]

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**Figure 5.** Theoretical number average block lengths \( (L_{CL,th} \text{ and } L_{LA,th}) \) as a function of \( f_{CL} \) and experimental number average block lengths determined by \(^1\)H NMR spectroscopy \( (L_{CL,1H} \text{ and } L_{LA,1H}) \) for different \( f_{CL} \).

**Figure 6.** Experimental glass transition temperature of the P(LA-co-CL) copolymers as a function of the weight fraction of CL in the copolymer. The dotted line is the theoretical glass transition temperature of the copolymers calculated from the Fox equation.

**Tricblock Copolymer Synthesis and Use of Alcohol Initiators other than BD.** Controlled synthesis of P(LA-stat-CL) copolyesters prompted us to derive triblock copolymers by sequential BA-OROP, using BD as an initiator. As depicted in Scheme 3, an \( \alpha,\omega \)-bis-hydroxy P(LA-stat-CL) precursor \( (M_{n,SEC} = 5480 \text{ g mol}^{-1}, D = 1.12) \) was synthesized first, using the conditions described previously \( ([\text{L-LA}]_0/[\text{CL}]_0/[\text{BD}]_0/[\text{BA}]_0 = 25:1:2:5; \text{Table S3}) \). After 20 h, extra L-LA was added at a \( [\text{L-LA}]_0/[\text{BA}]_0/[\text{P(LA-stat-CL)]}_0 \) ratio equal to 25:1:25:1 and the reaction was stirred for 25 h at 155 °C, reaching a conversion in PLA of 50%. Formation of the PLA-b-P(LA-stat-CL)-b-PLA triblock copolymer was attested by a clear shift in SEC to a higher molar mass \( (M_{n,SEC} = 8450 \text{ g mol}^{-1}, D = 1.15, \text{Figure 7c and Table S3}) \). Analysis by \(^1\)H NMR confirmed the presence of both P(LA-stat-CL) and PLA blocks, with representative protons of heterodiads from the statistical central block and increased intensity of PLA homodiads after BA-OROP of L-LA (see Figure 7a,b). The proton signals at 3.6 ppm due to hydroxy-methylene PCL end groups of the copolymer precursor totally vanished (Figure 7a) in favor of the methine end groups of PLA block at 4.36 ppm (Figure 7b). Furthermore, the experimental degree of polymerization determined by \(^1\)H NMR was very close to the theoretical value based on the initial ratio of L-LA and P(LA-stat-CL). The increased intensity of the LL-LL-LL triads (Figure S17) in \(^{13}\)C NMR confirmed the triblock copolymer synthesis. No evidence for the occurrence of transesterification.
reactions of type II was noted, as anomalous CL-L-CL triads at 170.8 ppm were not observed (Figure S17).

To demonstrate the versatility of BA as an organocatalyst, HeptOH and mPEG1000 were evaluated as initiators for the bulk OROcP of L-LA and CL at 155 °C (Table 1, runs 10–11). Well-defined P(LA-stat-CL) could be obtained using HeptOH ([L-LA]₀/[CL]₀/[HeptOH]₀/[BA]₀ = 25:25:1:2.5), with $M_n,SEC$ increasing linearly with $C_{TOT}$, a theoretical DP in agreement with the experimental one, and monomodal SEC traces with fairly low dispersity ($Đ < 1.35$) for bulk ROCp (Figures S19, S20, and Table 1, run 10). The overall composition in the copolymer was in agreement with the initial comonomer ratio ($f_{CL,0} = 0.52, F_{CL} = 0.51$). Synthesis of mPEG-b-P(LA-co-CL) diblock copolymer could also be achieved using commercial mPEG$_{1000}$ as a macroinitiator under the same conditions mentioned above. An initial comonomer composition of $f_{CL,0} = 0.94$ was selected to obtain a semicrystalline diblock copolymer (Table 1, run 11, Figures S21–S23). Efficient crossover from mPEG$_{1000}$ to the targeted diblock was confirmed by the shift to a lower elution volume after polymerization with a monomodal SEC trace ($M_n,SEC = 8790$ g mol$^{-1}$, $D = 1.58$; Figure S22).

Study of the Cytotoxicity of Benzoic Acid. Residues of some organocatalysts, such as thioureas$^{43}$ and phosphazenium salt,$^{64}$ have been found in synthetic (co)polymers, and these catalysts induce significant cytotoxicity. As benzoic acid remained in our copolymers (<0.125 mol %),$^{52}$ it was crucial to study its toxicity. We assessed the cytotoxicity of BA using the human HepaRG hepatoma cells in two culture conditions. These cells are bipotent hepatic progenitors actively proliferating at low cell density, which provides a first experimental condition to assess cytotoxicity in the process of cell division since these cells kept the major cell cycle checkpoints and express wild-type P53, Retinoblastoma, and β-catenin genes.$^{65}$ When these cells are cultured at high cell density, they become quiescent and differentiate to generate a coculture cell model combining cholangiocyte- and hepatocyte-like cells,$^{66}$ which is recognized as a suitable alternative model for primary culture of human hepatocytes to study hepatic metabolism$^{67,68}$ and (geno)toxicity$^{69,70}$ of xenobiotics because differentiated HepaRG cells express all transporters and drug-metabolizing enzymes found in vivo in the liver. Both progenitor and differentiated cells were incubated in culture media containing BA in a wide range of concentrations from 1
to 300 μm to assess the effect(s) of BA on proliferating hepatic cells as well as cholangiocyte- and hepatocyte-like cells. Benzoic acid was found to be nontoxic at these concentrations (Figure 8) in these different in vitro models of human hepatic cells, demonstrating that BA did not trigger adverse effects on cell proliferation and cytotoxicity in metabolically competent hepatic cells.

CONCLUSIONS

This work addresses a difficult challenge in polymer chemistry, namely, statistical copolymer synthesis based on poly(ε-caprolactone) and poly(1-lactide). Benzoic acid (BA) proves very versatile to this end as it can catalyze the metal-free and statistical ring-opening copolymerization (ROcP) of 1-lactide (1-LA) and ε-caprolactone (CL). A library of statistical copolymers of varying 1-LA/CL compositions can thus be synthesized in bulk at 155 °C, in presence of various alcohols as initiators, with a relatively good control over molar masses and dispersities. The statistical character of the copolymers is supported by 1H and 13C NMR analyses showing homo- and heterosequences and by the glass transition temperatures of the copolymers, which are in good agreement with values calculated from the Fox equation. Moreover, reliable reactivity ratio values of 1-LA (r1LA = 0.86) and CL (rCL = 0.86) have been calculated using the visualization of the sum of squared residuals space (VSSRS) method, with a narrow 95% confidence interval for 1-LA (0.75–1.01) and CL (0.74–1.0). The average block lengths of lactidyl and caproyl units, as determined by 1H NMR and by quantitative 13C NMR spectroscopies, closely match theoretical values. The “controlled/living” character of this BA-catalyzed process is further demonstrated through the synthesis of PLA-b-P(LA-stat-CL)-b-PLA triblock copolymers, using butane-1,4-diol as an initiator. This work thus expands the scope of organocatalyzed polymerization reactions, by providing a straightforward and metal-free synthetic alternative to biodegradable, biocompatible, and aliphatic statistical copolysters based on PLA and PCL, thanks to the use of BA as a weakly acidic and nontoxic organocatalyst.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bio-mac.9b00190.

REFERENCES


