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To cite this article: Adelaide Donvito, Giancarlo Fantin, Marco Fogagnolo, Pier Paolo Giovannini & Marco Scoponi (2016) Novel tri- and tetrafunctional cholic acid-based initiators for the synthesis of star-shaped poly(L-lactide)s, Designed Monomers and Polymers, 19:6, 535-544, DOI: 10.1080/15685551.2016.1187437

To link to this article: http://dx.doi.org/10.1080/15685551.2016.1187437

Published online: 24 May 2016.

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Novel tri- and tetrafunctional cholic acid-based initiators for the synthesis of star-shaped poly(L-lactide)

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ABSTRACT
In this investigation, two novel multifunctional initiators for ring-opening polymerization were synthesized in three steps starting from cholic acid. Thus, cholic acid (1a) and its methyl ester (1b) were quantitatively transformed, via solvent-free reaction with succinic anhydride, to the corresponding 3, 7, 12-tri-hemisuccinate derivatives (3a-b). The polyacidic compounds (3a-b) were treated with thionyl chloride affording the corresponding acyl chlorides (4a-b) which, in turn, were reacted with ethylene glycol to give the derivatives 2a-b having three and four primary alcohol end groups. These compounds, fully characterized by 1H, 13C NMR and mass spectrometry, have been assessed as initiators in the ring-opening polymerization of L-lactide using stannous octanoate as catalyst. The resulting three- and four-armed star-shaped poly(L-lactide)s, which were characterized by 1H NMR, SEC, DSC and TGA analysis, were amorphous, and their glass transition temperatures ranged from 13.7 to 36.5 °C. Additionally, some cholic acid-based star-shaped polylactic structures recently published have been critically reconsidered showing that these molecules, described as star polymers, were actually linear polymers.

Introduction

3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (1a), also known as cholic acid (CA) (Figure 1), one of the most common naturally occurring bile acids,[1] was largely used in the chemistry, pharmacology and material sciences areas.[2–4] This easily available and low-cost chemical, thanks to its intrinsic biodegradability, biocompatibility and renewability combined with the easy modifiability of the hydroxyl and carboxyl groups and the stability of steroid skeleton, was chosen by many researchers to design and synthesize new cholic acid-containing oligomeric or polymeric materials.[5,6] Various types of such polymers were produced in the last two decades: in several of these, the CAs were part of the main chain,[7–10] while in others, the CAs were connected as pendants [11–13] or, more recently, as the core of star-shaped polymers.[14–19]

New star-shaped polymers have attracted the attention of scientists for many years in virtue of their unique physicochemical properties. If compared with a linear polymer with similar average molecular weight, these systems show lower intrinsic viscosity and smaller hydrodynamic radius thanks to their compact structure and high density of end-functional groups.[20] Among them, the polyester family such as polylactide (PLA), polyglycolide (PGA) and poly(ε-caprolactone) (PLC) have been extensively studied and have shown great potential for use in drug delivery, biomedical devices and flexible food packaging applications thanks to their good biocompatibility and biodegradability.[20,21]

In the present paper, we propose the design and synthesis of novel polyols cholic acid-based derivatives able to act as initiators in the ring-opening polymerization of lactide to produce three- and four-armed star polymers. In addition, the chemical structures of some star-shaped polylactic cholic acid-based molecules recently published [14] have been critically reconsidered.

Experimental

Materials and instrumentation

Bile acids and other chemicals were purchased from Aldrich and used as received. All solvents used were reagent grade Aldrich and were used as received. Tetrahydrofuran (THF) used for SEC was HPLC grade, > 99.9%. Nine commercial PS samples (Polymer Laboratories) were used for calibration of universal method ($M_p = 5 \times 10^2$ Da; $M_p = 1.3 \times 10^3$ Da; $M_p = 1.3 \times 10^4$ Da).
Mₚ = 4.95 × 10^3 Da; Mᵣ = 9.86 × 10^3 Da; Mᵣ = 2.85 × 10^4 Da; Mᵣ = 7.22 × 10^4 Da; Mᵣ = 1.517 × 10^5 Da; Mᵣ = 4.266 × 10^5 Da; Mᵣ = 1.29 × 10^6 Da). Reactions were monitored by TLC on precoated silica gel 60 F254 (thickness 0.25 mm, Merck) with detection by charring with phosphomolybdic acid. Silica gel 60 (70–230 mesh) was used for preparative column chromatography.

The IR spectra were recorded with a Perkin-Elmer FT-IR Paragon 500 spectrometer. The optical rotations were measured on a Perkin-Elmer mod. 241 polarimeter. The 1H and 13C NMR spectra were recorded with a Varian Mercury Plus 400 or with a Varian Gemini 300 operating at 400 MHz (1H) and 100 MHz (13C) or at 300 MHz (1H) and 75 MHz (13C), respectively, and were processed using the manufacturer’s software. The chemical shifts were referenced to the solvent signals (CHCl₃: δC 7.26; CDCl₃: δC 77.0; CH₃OH: δC 3.34; CD₃OD: δC 49.0). ESI-HRMS were acquired on an Agilent Dual ESI Q TOF 6520, in positive ion mode, using mixtures of methanol–water. Molecular weights and polydispersity index of polymers were determined by size exclusion chromatography (SEC, WATERS) equipped with two detectors: a refraction index (Smartline 2300, KNAUER) and a viscosimeter (Viscotek, Model 2700, MALVERN). The chromatographic analyses were performed in THF at 35 °C with a flow of 1 ml/min (HPLC Pump K-501, KNAUER) using two column set: (i) ResiPore column (Polymer Lab – 7.5 mm ID × 300 mm L, operating range up to 5 × 10^5 g/mol, nominal particle size: 3 μm) and (ii) a TSKgel G2500HHR column (TOSOH – 7.8 mm ID × 300 mm L, operating range up to 2 × 10^4 g/mol, particle size: 5 μm). The Mn and IPD values were calculated using Omniscience software v.4.6.

Thermogravimetric analyses (TGA) were carried out using a TGA/SDTA Mettler Toledo mod. 851 equipped with a cryostat for the furnace cooling using cooled water. The used crucibles in alumina were heated using a method having two different heating rates: samples were heated using fast heating rate (20 °C/min) if the weight loss was less than 1% by weight and, conversely, the heating rate was 2 °C/min when the detected weight loss was above 1% by weight. The decomposition temperature was measured at 5% of the weight loss.

The thermal transition temperatures were carried out using a TA differential scanning calorimeter model DSC 2000 equipped with intracooler system and nitrogen gas with a flow of 50 ml/min. The samples were heated in aluminium pans at 10 °C/min from −20 to 200 °C, cooled with same rate and then re-heated in the same experimental conditions. The DSC calorimeter was calibrated with an indium standard. The TA software was used for determining the onset T_g values from DSC curves.

**Preparation of tri- and tetra-hydroxylated cholic acid-based initiators 2a and 2b**

A mixture of cholic acid 1a or methyl cholate 1b (2.45 mmol), succinic anhydride (2.45 g, 24.5 mmol, 10 equiv) and a catalytic amount of 4-dimethylaminopyridine (DMAP) was placed in a rotary oven preheated to 180 °C. The temperature was then lowered to 150 °C and kept for 9 h for the substrate 1a and 5 h for the substrate 1b. Vacuum sublimation of succinic anhydride from the crude material gave the expected tri-hemisuccinate derivatives 3a and 3b in almost quantitative yield as colourless foam (failed to crystallize) that were used without further purification.

3a, [α]20 = +16.6 (c 0.5, CH₂Cl₂), FT-IR (neat, ν, cm⁻¹): 3180, 2942, 2865, 1722, 1704, 1222, 1162. 1H-NMR (300MHz, CDCl₃) selected peaks, δ: 5.09 (br s, 1H, 12-CH), 4.99 (br s, 1H, 7-CH), 4.52 (br s, 1H, 3-CH), 0.92 (s, 3H, 19-CH₃), 0.81 (d, 3H, J = 6.1 Hz, 21-CH₃), 0.71 (s, 3H, 18-CH₃). 13C-NMR (100 MHz, CDCl₃), δ: 180.81, 179.35, 179.22, 178.79, 171.38, 171.06, 170.83, 76.15, 74.80, 71.13, 47.29, 45.12, 43.14, 40.73, 37.92, 34.82, 34.50, 34.45, 31.32, 30.99, 30.60, 30.07, 29.77, 29.67, 29.30, 29.00, 28.50, 27.21, 26.40, 25.32, 22.75, 22.48, 17.26, 11.97. HR-MS (ESI, positive ion mode): calcd. for (M + Na⁺) C₃₆H₅₂NaO₁₄ (M + Na⁺), 731.3258; found, 731.3248.

3b, [α]20 = +40.2 (c 1, CH₂Cl₂), FT-IR (neat, ν, cm⁻¹): 3180, 2943, 2876, 1726, 1707, 1254, 1220, 1160. 1H-NMR (300MHz, CDCl₃) selected peaks, δ: 5.07 (br s, 1H, 12-CH), 4.93 (br s, 1H, 7-CH), 4.53 (br s, 1H, 3-CH), 3.66 (s, 3H, 25-OCH₃), 0.91 (s, 3H, 19-CH₃), 0.77 (d, 3H, J = 6.1 Hz, 21-CH₃), 0.71 (s, 3H, 18-CH₃). 13C-NMR (100 MHz, CDCl₃), δ: 179.25, 178.74, 177.42, 174.0, 171.69, 170.88, 170.75, 76.33, 74.71, 71.31, 51.64, 47.45, 45.29, 43.29, 40.71, 31.83, 34.55, 34.49, 34.43, 31.27, 30.83, 30.74, 30.20, 29.84, 29.79, 29.27, 29.05, 28.89, 28.69, 27.13, 26.27, 25.43, 22.81, 22.53, 17.46, 12.07. HR-MS (ESI, positive ion mode): calcd. for C₃₆H₅₂NaO₁₄ (M + Na⁺), 745.34113; found, 745.34106.

A solution of the tri-hemisuccinate derivatives 3a or 3b (2.4 mmol) in freshly distilled thionyl chloride (22 mmol) was stirred at 50 °C for 2 h and then evaporated to dryness under vacuum. The crude chlorides were dissolved in 5 mL of dry THF and added dropwise to a solution of ethylene glycol (120 mmol) and triethylamine (12 mmol) at 0 °C. The reaction mixture was stirred at room temperature
for 7 h, then poured into 200 mL of aqueous solution of 5% HCl containing 10% of brine and extracted with CH₂Cl₂ (2 × 50 mL). The organic phase was washed with brine (2 × 100 mL), dried over Na₂SO₄ and concentrated (0.17 mmol), Sn(oct)₂ (0.005 mmol) and the appropriate amount of L-lactide (3.4 mmol, 20 equiv or 6.8 mmol, 40 equiv) were put into a fire-dried rotary reactor maintained under nitrogen atmosphere. The mixture was quickly heated to melt (ca. 160 °C), and then, the temperature was lowered and kept to 140 °C for 9 h. The reaction mixture was cooled, and the resulting polymer was dissolved in tetrahydrofuran and precipitated in ether and, finally, dried under vacuum. The obtained polymer stars had the characteristics listed in Table 1, whereas the spectroscopic data were the following:

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Sample & \(^{1}M_{n,\text{Da}}\) & \(^{1}M_{\text{exp, Da}}\) & \(^{1}\text{PD}\) & \(\delta_{T_{g}}\) °C & \(\delta_{T_{m}}\) °C \\
\hline
CA-(O-LL)\(_{4/20}\) & 2880 & 3650 & 1.34 & 219.17 & 36.5 \\
CA-(O-LL)\(_{4/40}\) & 5760 & 5695 & 1.26 & 220.54 & 24.5 \\
CAMe-(O-LL)\(_{4/20}\) & 2880 & 3005 & 1.19 & 216.25 & 13.7 \\
CAMe-(O-LL)\(_{4/40}\) & 5760 & 5902 & 1.29 & 220.21 & 31.9 \\
\hline
\end{tabular}
\caption{SEC, TGA and DSC characterization data collected for the star shaped polyesters obtained with cholic acid-based core as initiators.}
\end{table}

Notes: \(^{}^{1}\)Theoretical values calculated for polyester chains, assuming a complete conversion of L-lactide monomers and equal chain length for each arm; \(^{2}\)Experimental values obtained from SEC analysis; \(^{3}\)Experimental values obtained from TGA and DSC thermal analyses, respectively.

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\textbf{Results and discussion}

\textbf{Prodrome}

In the attempt to synthesize a star-shaped poly(D,L-lactide) with a CA core according to the procedure proposed by Cheng et al.\cite{14}, we surprisingly obtained a linear polymer. In fact, after isolation of the polymer, we confirmed that the product obtained by us showed the same \(^{1}\)H NMR spectrum of that obtained by Cheng et al. but, we noted, however, that these spectra were not consistent with the expected three-armed star-shaped polymer but with a linear polymer developed exclusively at position 3 of the CA unit, namely a CA single-end-capped polymer.

In fact, the \(^{1}\)H NMR spectrum of CA (Figure 2(a)) shows, among others, the three signals of the methine protons at position 3, 7 and 12, having a characteristic shape and
to 220 °C) or the catalyst change, replacing the most used Sn(Oct)$_2$ [24,25] with LiCl [26], AlMe$_3$ [27] or DMAP [28], but in all these experiments, we obtained exclusively the linear polymer generated by 3-OH group of the bile acid. These results prompted us to design new polyhydroxylated cholic acid-based initiators suitable for the ‘core first’ synthesis of star oligo/poly(L-lactide)s.

Our approach relied on the insertion of suitable spacer groups to the positions 3, 7, 12 and 24 of the CA and to the positions 3, 7 and 12 of the CA methyl ester (CAMe) so as to obtain the derivatives 2a and 2b bearing four or three primary hydroxyl groups, respectively. Now, the comparable nucleophilic reactivity of the hydroxyl groups towards the R oP of lactide should allow the production of the corresponding star-shaped oligomers coded CA-(o-LL)$_4$ and CAMe-(o-LL)$_3$ (Figure 3).

Synthesis and characterization of polyhydroxylated cholic acid-based initiators, 2a-b

We decided to follow the synthetic route shown in Scheme 1. The most challenging step of the whole synthesis was the triple esterification of CA with succinic anhydride. In fact, as evidenced by Heusser and Wuthier [29], even using drastic conditions (dry pyridine at 100 °C), the reaction of succinic anhydride with methyl cholate afforded only an adduct at position 3. Nevertheless, heating to 150 °C the bile acids 1a-b with an excess of

Figure 2. $^1$H NMR spectra of (a) CAMe, (b) investigated polymer, (c) methyl cholate triacetate.
Synthesis and characterization of star-shaped poly(L-lactide)

The ROP of L-lactide with the multifunctional initiators 2a-b and Sn(Oct)$_2$ as a catalyst was carried out in bulk (Scheme 2). To prepare polymers with different molecular weights, two molar ratios initiator/lactide, 1/20 and 1/40, were chosen; the best ROP reaction conditions (conversions around 95%) were identified to be 9 h at 140 °C. The obtained polymers were labelled according to both the initiator and to the molar ratio of the reagents: that is, CA-(O-LL)$_{4/20}$ and CA-(O-LL)$_{4/40}$ if obtained starting from 2a, CAMe-(O-LL)$_{3/20}$ and CAMe-(O-LL)$_{3/40}$ if obtained starting from 2b.

The 1H-NMR spectrum of CA-(O-LL)$_{4/20}$ with peak assignments is shown in Figure 4(b). The methyl groups of cholic acid moiety (0.72 ppm, 18-CH$_3$; 0.79 ppm, 21-CH$_3$; 0.91 ppm, 19-CH$_3$) are evident as well as the methylene signals of the succinic units (2.69–2.60 ppm).

Compared to the spectrum of the initiator 2a (Figure 4(a)), the multiplet peak assigned to the four terminal −CH$_2$−OH protons (h' signals, 3.80–3.77 ppm) disappeared, or better shifted downfield to 4.40–4.22 ppm, as a consequence of the polymerization (esterification).
Scheme 1. Synthetic route of polyhydroxylated cholic acid-based initiators.

Scheme 2. The ROP of L-lactide with the multifunctional initiators 2a-b.
NMR, so we used the size exclusion chromatography (SEC) technique to obtain these data. Considering the lack of star-shaped polymer standards, the conventional calibration method is not conveniently used for determining the Mn of this kind of polymers.[31] The best approach for the determination of molecular weight distribution is the universal calibration method using a SEC technique equipped with concentration detector (i.e. refractive index detector) combined with differential viscosimeter. In fact, the unique separation factor in SEC is the hydrodynamic volume \( V_h \) of the solute molecules which is proportional to the product of intrinsic viscosity (IV) and molecular weight (M). Moreover, some previous works focused on polymer characterization have demonstrated that universal calibration is the best solution for the experimental determination of the molecular weight distribution for star-shaped polymers.[32–34] The SEC chromatograms of all star-shaped polyesters are reported in Figure 6. The Mn experimental values for the branched polyester chains (900–6000 Da) are compared with the theoretical values calculated by the ratios between the L-lactide monomer and initiator concentrations for each star-shaped polyester, assuming a complete conversion and reasonably the equal chain length of each arm.

Table 1 shows that experimental values \( M_n, \text{exp} \) obtained by SEC technique are in a good agreement
The thermal properties were obtained by TGA and DSC for determining the thermal stability and thermal transition temperatures, respectively, of the four star-shaped poly(L-lactide)s. The observed DSC thermograms for all with those theoretical values (M_n,th). Furthermore, the observed polydispersity index (PDI) for all star-shaped PLA polymers ranging from 1.19 to 1.34, for CA-Me-(O-LL)_{3/20} and CA-(O-LL)_{4/20}, respectively. The narrow molecular weight distributions are expected in case of quasi-living ring-opening polymerization (ROP) mechanism, which is generally observed during L-lactide monomer polymerization under similar experimental conditions.[35]

![Figure 7.](image)

The thermal properties were obtained by TGA and DSC for determining the thermal stability and thermal transition temperatures, respectively, of the four star-shaped poly(L-lactide)s. The observed DSC thermograms for all...
star-shaped polyesters during the second heating cycle are reported in Figure 7. The figure shows that the star-shaped PLA polymers are amorphous, and their glass transition temperatures range from 13.7 to 36.5 °C (see Table 1).

These results are in agreement with SEC data indicating low average polymerization degrees of the branched polyesters ranging from 13 and 26, respectively, for CAMe-(OLL)_{3/20} and CAMe-(O-LL)_{3/40}. Under pyrolysis conditions, the measured decomposition temperatures by TGA analysis show similar values for all star-shaped polyesters (see Table 1). It is worth noting that the branched polyesters, having similar polymerization degrees and architectural chains, show the same thermal stability.[35]

Conclusions

In summary, herein we described the highly efficient solvent-free synthesis of the 3, 7, 12-tri-hemisuccinate derivatives of either cholic acid or its methyl ester. These key intermediates allowed the synthesis of two novel cholic acid-based tri- and tetra-functional primary alcohols which resulted effective initiators in the ring-opening polymerization of L-lactide. Thus three- and four-armed poly(L-lactide)s with cholic acid core were synthesized using stannous octanoate as catalyst. The molecular weights of the star polymers have been adjusted by controlling the feed ratio of the initiators to the monomer L-lactide. Furthermore, the structures of some cholic acid-based star-shaped polylactic molecules recently published were critically reconsidered.

We believe that these branched amorphous polyesters, derived from renewable resources, could be used as drug carriers and as plasticizer agents for high molecular weight polylactic acid for food packaging application.

Acknowledgements

Thanks are also given to Dr. Paolo Formaglio for NMR experiments and to Dr. Tatiana Bernardi for ESI-MS.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the University of Ferrara (FAR 2015).

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