

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

Adelaide Donvito, Giancarlo Fantin, Marco Fogagnolo, Pier Paolo Giovannini & Marco Scoponi

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](https://doi.org/10.1080/15685551.2016.1187437)

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



Published online: 24 May 2016.



Submit your article to this journal [↗](#)



Article views: 6



View related articles [↗](#)



View Crossmark data [↗](#)

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

Adelaide Donvito<sup>a</sup> , Giancarlo Fantin<sup>a</sup> , Marco Fogagnolo<sup>a</sup> , Pier Paolo Giovannini<sup>a</sup>  and Marco Scoptoni<sup>b</sup> 

<sup>a</sup>Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Ferrara, Ferrara, Italy; <sup>b</sup>ISOF-CNR, sez. c/o Dip.to di Scienze Chimiche e Farmaceutiche dell'Università di Ferrara, Ferrara, Italy

### 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 <sup>1</sup>H, <sup>13</sup>C 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 <sup>1</sup>H 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.

### ARTICLE HISTORY

Received 3 December 2015  
Accepted 1 May 2016

### KEYWORDS

Star-polymers; cholic acid; ring-opening polymerization; initiator

### Introduction

3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -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( $\epsilon$ -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;

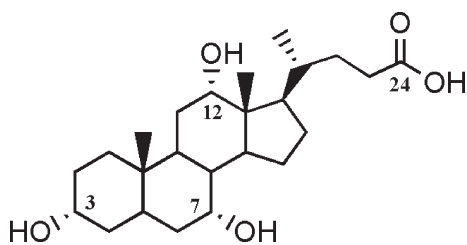


Figure 1. Cholic acid (1a).

$M_p = 4.95 \times 10^3$  Da;  $M_p = 9.86 \times 10^3$  Da;  $M_p = 2.85 \times 10^4$  Da;  $M_p = 7.22 \times 10^4$  Da;  $M_p = 1.517 \times 10^5$  Da;  $M_p = 4.266 \times 10^5$  Da;  $M_p = 1.29 \times 10^6$  Da). Reactions were monitored by TLC on precoated silica gel 60 F<sub>254</sub> (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 <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with a Varian Mercury Plus 400 or with a Varian Gemini 300 operating at 400 MHz (<sup>1</sup>H) and 100 MHz (<sup>13</sup>C) or at 300 MHz (<sup>1</sup>H) and 75 MHz (<sup>13</sup>C), respectively, and were processed using the manufacturer's software. The chemical shifts were referenced to the solvent signals (CHCl<sub>3</sub>:  $\delta_H$  7.26; CDCl<sub>3</sub>:  $\delta_C$  77.0; CH<sub>3</sub>OH:  $\delta_H$  3.34; CD<sub>3</sub>OD:  $\delta_C$  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  $5 \times 10^5$  g/mol, nominal particle size: 3  $\mu$ m) and (ii) a TSKgel G2500H<sub>HR</sub> column (TOSOH – 7.8 mm ID × 300 mm L, operating range up to  $2 \times 10^4$  g/mol, particle size: 5  $\mu$ m). The Mn and IPD values were calculated using OmniSEC 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**,  $[\alpha]_D^{20} = +16.6$  (c 0.5, CH<sub>2</sub>Cl<sub>2</sub>). FT-IR (neat,  $\nu$ , cm<sup>-1</sup>): 3180, 2942, 2865, 1722, 1704, 1222, 1162. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) selected peaks,  $\delta$ : 5.09 (br s, 1H, 12-CH), 4.99 (br m, 1H, 7-CH), 4.52 (br m, 1H, 3-CH), 0.92 (s, 3H, 19-CH<sub>3</sub>), 0.81 (d, 3H,  $J = 6.1$  Hz, 21-CH<sub>3</sub>), 0.71 (s, 3H, 18-CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>),  $\delta$ : 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<sup>+</sup>) C<sub>36</sub>H<sub>52</sub>NaO<sub>14</sub> (M + Na<sup>+</sup>), 731.32548; found, 731.32408.

**3b**,  $[\alpha]_D^{20} = +40.2$  (c 1, CH<sub>2</sub>Cl<sub>2</sub>). FT-IR (neat,  $\nu$ , cm<sup>-1</sup>): 3180, 2943, 2876, 1726, 1707, 1254, 1220, 1160. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) selected peaks,  $\delta$ : 5.07 (br s, 1H, 12-CH), 4.93 (br m, 1H, 7-CH), 4.53 (br m, 1H, 3-CH), 3.66 (s, 3H, 25-OCH<sub>3</sub>), 0.91 (s, 3H, 19-CH<sub>3</sub>), 0.77 (d, 3H,  $J = 6.1$  Hz, 21-CH<sub>3</sub>), 0.71 (s, 3H, 18-CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>),  $\delta$ : 179.25, 178.74, 177.42, 174.80, 171.69, 170.88, 170.75, 76.33, 74.71, 71.31, 51.64, 47.45, 45.29, 43.29, 40.71, 37.83, 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 C<sub>37</sub>H<sub>54</sub>NaO<sub>14</sub> (M + Na<sup>+</sup>), 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

**Table 1.** SEC, TGA and DSC characterization data collected for the star shaped polyesters obtained with cholic acid-based core as initiators.

Sample	<sup>a</sup> M <sub>n,th</sub> Da	<sup>b</sup> M <sub>n,exp</sub> Da	<sup>c</sup> IPD	<sup>d</sup> T <sub>d</sub> °C	<sup>e</sup> T <sub>g</sub> °C
CA-(O-LL) <sub>4/20</sub>	2880	3650	1.34	219.17	36.5
CA-(O-LL) <sub>4/40</sub>	5760	5695	1.26	220.54	24.5
CAMe-(O-LL) <sub>3/20</sub>	2880	3005	1.19	216.25	13.7
CAMe-(O-LL) <sub>3/40</sub>	5760	5902	1.29	220.21	31.9

Notes: <sup>a</sup>Theoretical values calculated for polyester chains, assuming a complete conversion of L-lactide monomers and equal chain length for each arm; <sup>b</sup>Experimental values obtained from SEC analysis; <sup>d,e</sup>Experimental values obtained from TGA and DSC thermal analyses, respectively.

for 7 h, then poured into 200 mL of aqueous solution of 5% HCl containing 10% of brine and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 50 mL). The organic phase was washed with brine (2 × 100 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure; the residue containing the target adducts **2a** or **2b** was purified by chromatography.

**2a:** column chromatography with 1: 1 CH<sub>2</sub>Cl<sub>2</sub>-AcOEt (containing 6% of methanol) (1.4 g, 70%, colourless syrup, failed to crystallize). [α]<sub>D</sub><sup>20</sup> = + 39.2 (c 0.5, CH<sub>2</sub>Cl<sub>2</sub>). FT-IR (neat, ν, cm<sup>-1</sup>): 3437, 2943, 2873, 1724, 1381, 1159. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) selected peaks, δ: 5.12 (br s, 1H, 12-CH), 4.92 (br m, 1H, 7-CH), 4.55 (br m, 1H, 3-CH), 4.25–4.15 (m, 8H, –CH<sub>2</sub>–OCO), 3.80–3.77 (m, 8H, –CH<sub>2</sub>–OH), 2.75–2.58 (m, 12H, OOC–CH<sub>2</sub>–CH<sub>2</sub>–COO), 0.89 (s, 3H, 19-CH<sub>3</sub>), 0.79 (d, 3H, J = 6.1 Hz, 21-CH<sub>3</sub>), 0.71 (s, 3H, 18-CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>), δ: 174.61, 172.74, 172.73, 172.68, 172.07, 171.65, 171.62, 75.72, 74.61, 71.35, 66.36, 66.27, 66.20, 65.93, 61.01, 60.81, 60.79, 60.77, 46.83, 45.07, 43.24, 40.74, 37.78, 34.64, 34.50, 34.30, 31.18, 30.56, 29.77, 29.73, 29.59, 29.27, 29.20, 29.16, 28.72, 27.22, 26.64, 25.41, 22.78, 22.45, 17.39, 12.10. HR-MS (ESI, positive ion mode): calcd. for C<sub>44</sub>H<sub>68</sub>NaO<sub>18</sub> (M + Na<sup>+</sup>), 907.43034; found, 907.42947.

**2b:** column chromatography with 1: 1 CH<sub>2</sub>Cl<sub>2</sub>-AcOEt (containing 3% of methanol) (1.6 g, 78%, colourless syrup, failed to crystallize). [α]<sub>D</sub><sup>20</sup> = + 32.2 (c 0.5, CH<sub>2</sub>Cl<sub>2</sub>). FT-IR (neat, ν, cm<sup>-1</sup>): 3443, 2948, 2873, 1725, 1381, 1158. <sup>1</sup>H-NMR (300MHz, CDCl<sub>3</sub>) selected peaks, δ: 5.11 (br s, 1H, 12-CH), 4.92 (br m, 1H, 7-CH), 4.56 (br m, 1H, 3-CH), 4.22 (m, 6H, –CH<sub>2</sub>–OCO), 3.79 (m, 6H, –CH<sub>2</sub>–OH), 3.64 (s, 3H, 25-OCH<sub>3</sub>), 2.77–2.57 (m, 12H, OOC–CH<sub>2</sub>–CH<sub>2</sub>–COO), 0.90 (s, 3H, 19-CH<sub>3</sub>), 0.79 (d, 3H, J = 6.1 Hz, 21-CH<sub>3</sub>), 0.71 (s, 3H, 18-CH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>), δ: 174.71, 172.74, 172.63, 172.50, 172.03, 171.63, 77.35, 77.03, 76.71, 75.75, 74.59, 71.35, 66.28, 66.21, 60.83, 51.56, 47.23, 45.08, 43.24, 40.75, 37.79, 34.65, 34.54, 34.31, 31.18, 30.84, 30.69, 29.76, 29.59, 29.29, 29.22, 29.17, 28.73, 27.23, 26.63, 25.38, 22.78, 22.46, 17.39, 12.11. HR-MS (ESI, positive ion mode): calcd. for C<sub>43</sub>H<sub>66</sub>NaO<sub>17</sub> (M + Na<sup>+</sup>), 877.41977; found, 877.41992.

### Preparation of star shaped-polymers

The tri- or tetrahydroxylated cholic acid-based initiators **2a** or **2b** (0.17 mmol), Sn(Oct)<sub>2</sub> (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:

**CAMe-(OLL)<sub>3/20</sub>:** <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) selected peaks, δ: 0.72 (s, 3H), 0.80 (d, 3H, J = 6.1 Hz), 0.91 (s, 3H), 1.44–1.68 (m, 144H), 2.54–2.77 (m, 12H), 3.65 (s, 3H), 4.22–4.40 (m, 15H), 4.57 (m, 1H), 4.92 (m, 1H), 5.11–5.24 (m, 38H).

**CAMe-(OLL)<sub>3/40</sub>:** <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) selected peaks, δ: 0.72 (s, 3H), 0.80 (d, 3H, J = 6.1 Hz), 0.91 (s, 3H), 1.44–1.68 (m, 231H), 2.54–2.77 (m, 12H), 3.65 (s, 3H), 4.22–4.40 (m, 15H), 4.57 (m, 1H), 4.92 (m, 1H), 5.11–5.24 (m, 78H).

**CA-(OLL)<sub>4/20</sub>:** <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) selected peaks, δ: 0.72 (s, 3H), 0.79 (d, 3H, J = 6.1 Hz), 0.91 (s, 3H), 1.45–1.70 (m, 108H), 2.53–2.75 (m, 12H), 4.21–4.41 (m, 20H), 4.57 (m, 1H), 4.92 (m, 1H), 5.10–5.24 (m, 37H).

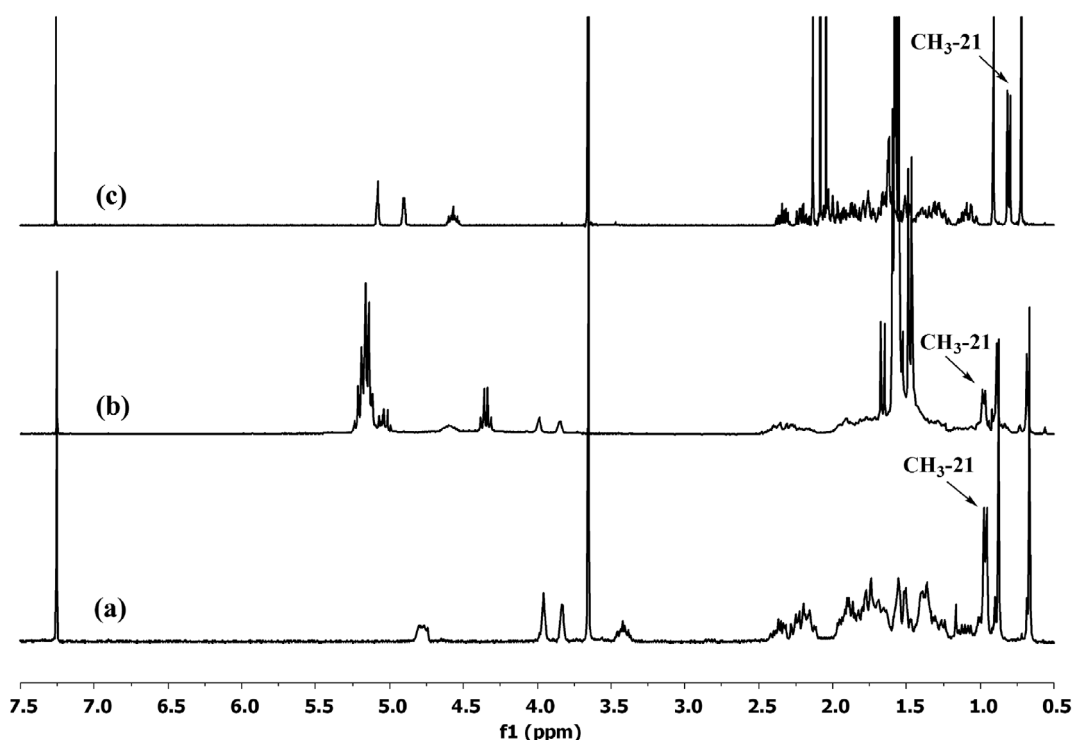
**CA-(OLL)<sub>4/40</sub>:** <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) selected peaks, δ: 0.72 (s, 3H), 0.79 (d, 3H, J = 6.1 Hz), 0.91 (s, 3H), 1.45–1.70 (m, 228H), 2.53–2.75 (m, 12H), 4.21–4.41 (m, 20H), 4.57 (m, 1H), 4.92 (m, 1H), 5.10–5.24 (m, 67H).

## Results and discussion

### 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.*[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 <sup>1</sup>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 <sup>1</sup>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



**Figure 2.**  $^1\text{H}$  NMR spectra of (a) CAME, (b) investigated polymer, (c) methyl cholate triacetate.

profile that enable the assignment of configurations to the associated hydroxyl groups.[22] These same signals move ca. 1 ppm downfield in the case of O-acylation of the respective hydroxyl groups [22] as clearly shown in the  $^1\text{H}$  NMR spectrum of methyl cholate triacetate (Figure 2(c)). However, the  $^1\text{H}$  NMR spectrum of the investigated polymer (Figure 2(b)) shows that only the 3-methine proton is moved downfield (4.59 ppm) with respect to its original position in CA (3.42 ppm), while the signals due to the 7 and 12 protons were substantially unchanged. This clearly indicated that only and exclusively the hydroxyl group at position 3 was active in the ROP of lactide, thus undergoing the acylation on itself. On the other hand, it is well known that the equatorial hydroxyl group at  $\text{C}_3$  undergoes esterification more readily than the axial hydroxyl groups at  $\text{C}_7$  and  $\text{C}_{12}$ . [23] One additional NMR spectroscopic evidence that allowed us to state that the products obtained by Cheng *et al.* (as well as by us) were CA single-end-capped polymers and not star polymers was given by the position of the 21- $\text{CH}_3$  doublet. In fact, when the 12-hydroxyl group of CA is esterified, the signal pertinent to 21- $\text{CH}_3$  must move upfield of about 0.18 ppm [22] with respect to the signal of the unesterified 12 hydroxyl group (compare Figure 2(c) with 2(a)). The  $^1\text{H}$  NMR of the polymer of Figure 2(b) does not show this shift. Furthermore, in order to verify whether CA could effectively act as a tri-hydroxylated initiator in the ROP of lactide, we tested some different experimental conditions such as higher temperatures (up

to 220 °C) or the catalyst change, replacing the most used  $\text{Sn}(\text{Oct})_2$  [24,25] with  $\text{LiCl}$ , [26]  $\text{AlMe}_3$  [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 **1a** and to the positions 3, 7 and 12 of the CA methyl ester (CAME) **1b** 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 ROP 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

succinic anhydride and a catalytic amount of DMAP under solvent-free conditions, the pure tri-hemisuccinate derivatives **3a-b** were obtained in almost quantitative yield. To the best of our knowledge, this is the first example of simple and straightforward, solvent-free preparation of tri-hemisuccinate cholic acid derivatives.

The structure of these compounds was proved by means of NMR and MS spectroscopy. In particular, the  $^1\text{H}$  NMR spectrum of compound **3b** showed that the peaks assigned to the protons at C3, C7 and C12 moved downfield of about one ppm with respect to the same peaks in the spectrum of **1b** ( $\delta = 4.53, 4.93, 5.07$  ppm for **3b** against  $\delta = 3.42, 3.83, 3.95$  ppm for **1b**). Furthermore, the  $^{13}\text{C}$  NMR spectrum of compound **3b** showed seven carbon signals between 170.7 and 179.2 ppm pertinent to seven CO groups confirming the acylation of all three hydroxyl groups of CA methyl ester by succinic anhydride. Similar considerations can be made for confirming the structure of compound **3a**.

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 final cholic acid-based initiators **2a-b** which were fully characterized by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and mass spectrometry.

### Synthesis and characterization of star-shaped poly(L-lactide)s

The ROP of L-lactide with the multifunctional initiators **2a-b** and  $\text{Sn}(\text{Oct})_2$  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 both to the initiator and to the molar ratio of the reagents: that is,  $\text{CA}(\text{-O-LL})_{4/20}$  and  $\text{CA}(\text{-O-LL})_{4/40}$  if obtained starting from **2a**,  $\text{CAME}(\text{-O-LL})_{3/20}$  and  $\text{CAME}(\text{-O-LL})_{3/40}$  if obtained starting from **2b**.

The  $^1\text{H}$ -NMR spectrum of  $\text{CA}(\text{-O-LL})_{4/20}$  with peak assignments is shown in Figure 4(b). The methyl groups of cholic acid moiety (0.72 ppm, 18- $\text{CH}_3$ ; 0.79 ppm, 21- $\text{CH}_3$ ; 0.91 ppm, 19- $\text{CH}_3$ ) 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  $-\text{CH}_2-\text{OH}$  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).

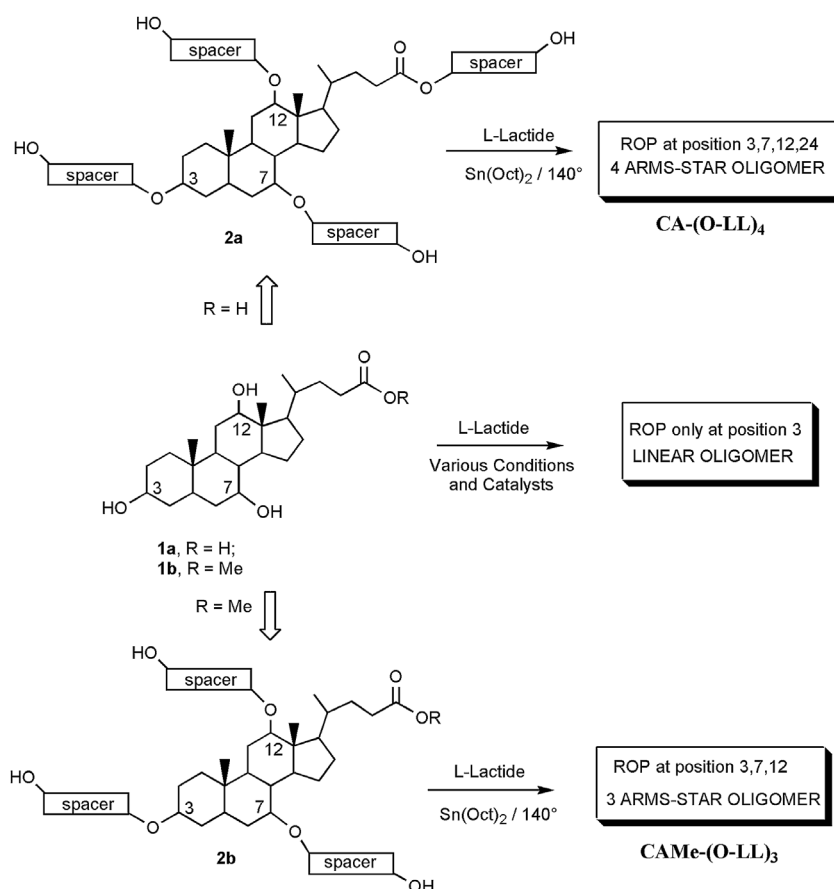
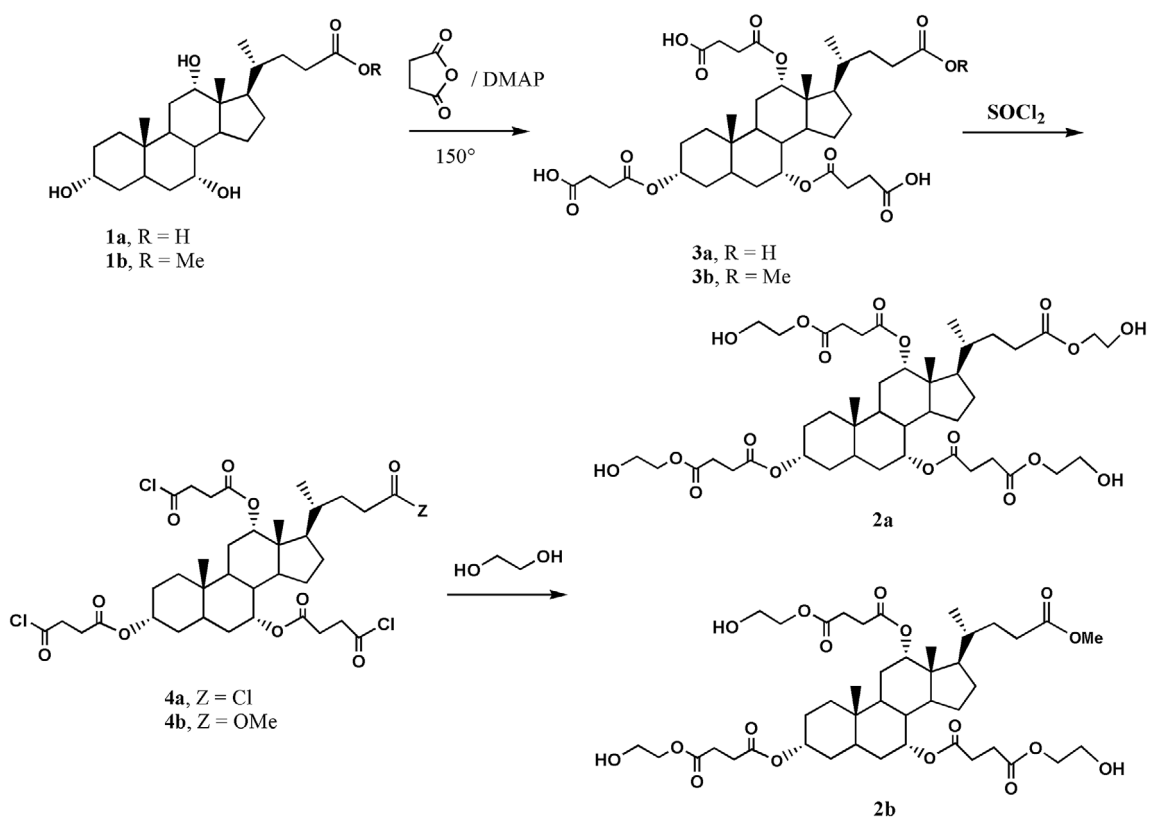
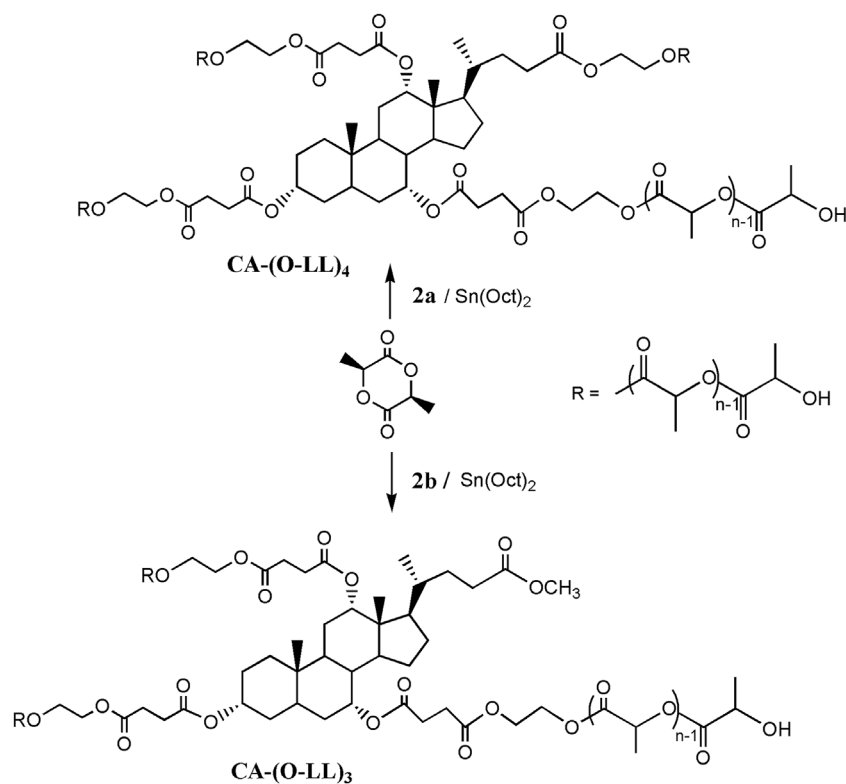


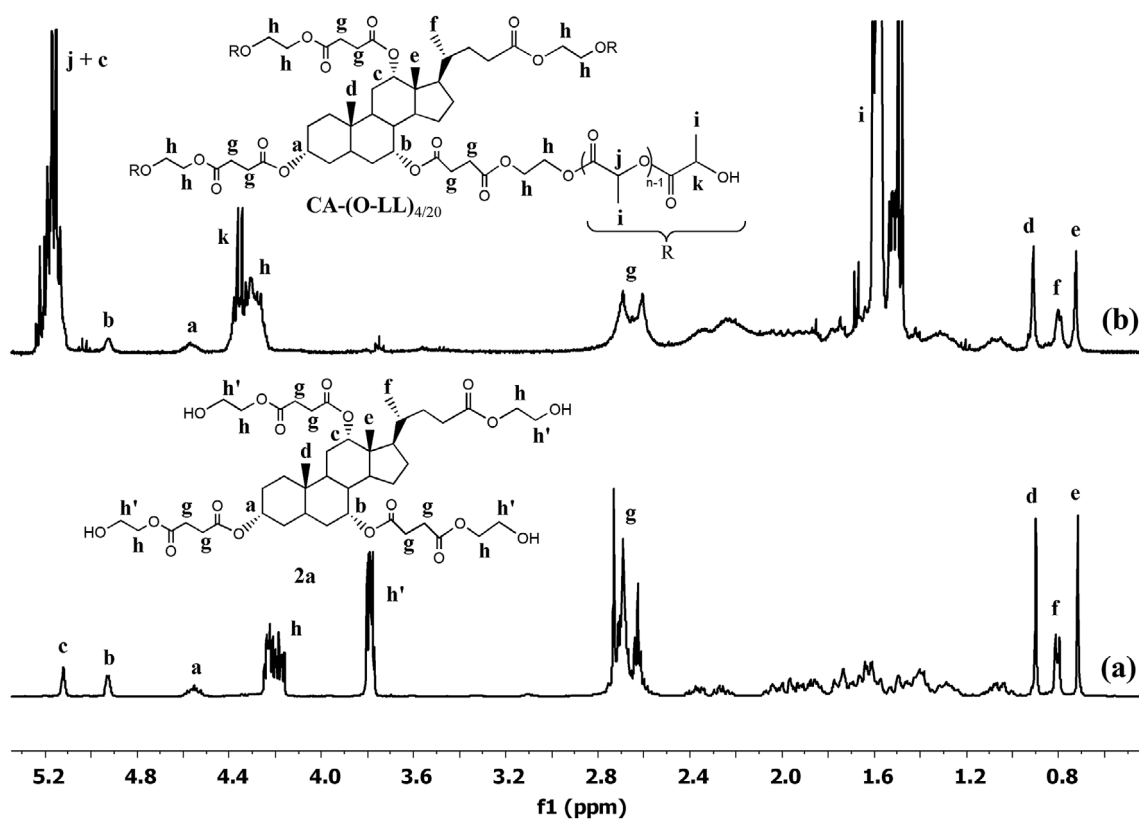
Figure 3. Tri- and tetrahydroxylated cholic acid-based initiators .



**Scheme 1.** Synthetic route of polyhydroxylated cholic acid-based initiators.



**Scheme 2.** The ROP of L-lactide with the multifunctional initiators **2a-b**.



**Figure 4.**  $^1\text{H}$  NMR spectra of (a) tetrahydroxylated initiator **2a**, (b)  $\text{CA}-(\text{O-LL})_{4/20}$ .

Methine and methyl groups of the repeat lactic units at 5.24–5.11 ppm and at 1.60–1.48 ppm, respectively, were also detected. The number average molecular weight could be calculated from the integration ratio between the methine protons in the lactic repeat units and those in the terminal unit (**k**). However, we were unable to estimate this proportion because the diagnostic **k** signal was partly overlapped to the **h** resonance due to the glycol moieties. Nevertheless, from the just mentioned spectrum, it was possible to get some crucial values of integrals useful to confirm the star shape of  $\text{CA}-(\text{O-LL})_{4/20}$ . In fact, the integral ratio of the peaks [(**j + c**)/**b/a**]/(**k + h**)/**g/f** = 38.8/1/1.2/19.8/13.9/3.1] was in good agreement with the theoretical value [37/1/1/20/12/3] deduced assuming that  $\text{CA}-(\text{O-LL})_{4/20}$  to be a four-arms molecule. Moreover, experimental values so close to theoretical ones also ensure that there were no side reactions during polymerization (i.e. transesterifications).[30] The  $^1\text{H}$ -NMR spectra of the  $\text{CAME}-(\text{O-LL})_{3/40}$  and its initiator **2b** with the relative peak assignments are displayed in Figure 5(a) and (b). These spectra can be explained and interpreted adopting the same considerations just done above, taking into account that the singlet resonating at 3.64 ppm is clearly due to the methyl ester on cholic acid moiety (not present in the  $\text{CA}-(\text{O-LL})_{4/20}$ ).

As stated before, we were unable to estimate the number average molecular weight ( $M_n$ ) from the  $^1\text{H}$

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  $M_n$  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  $M_n$  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



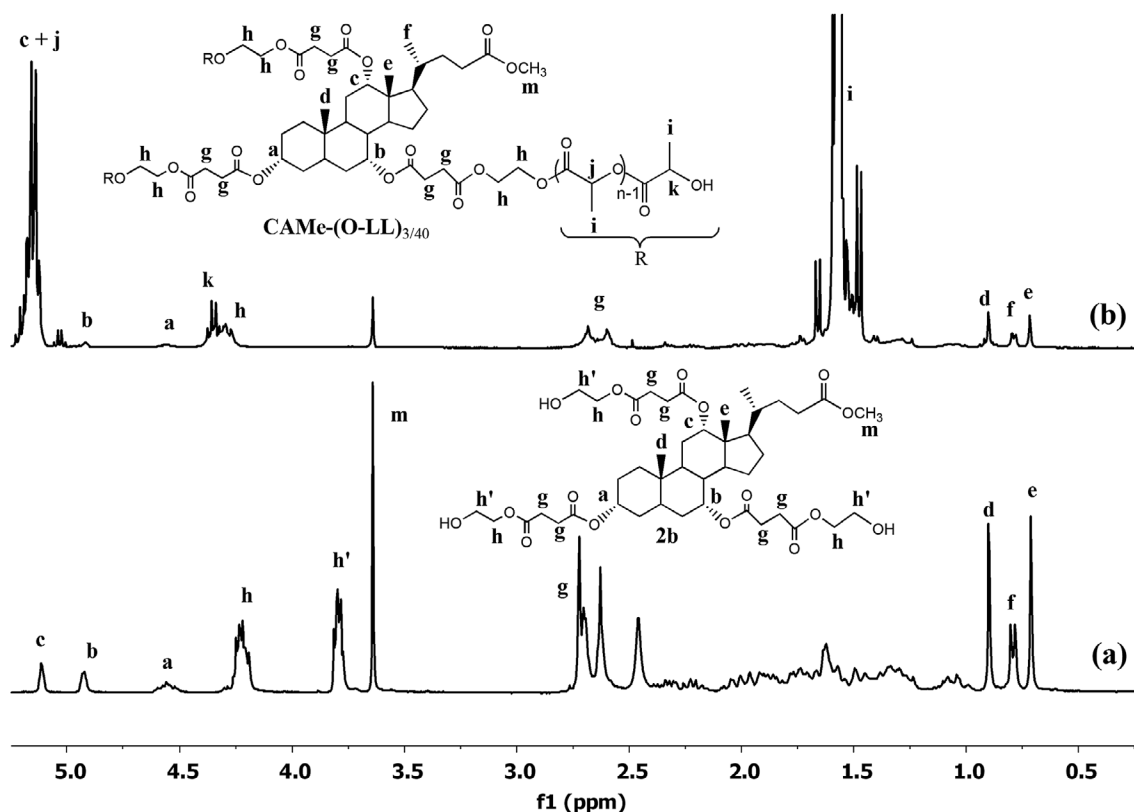


Figure 5.  $^1\text{H}$  NMR spectra of (a) trihydroxylated initiator **2b**, (b)  $\text{CAME}-(\text{O-LL})_{3/40}$ .

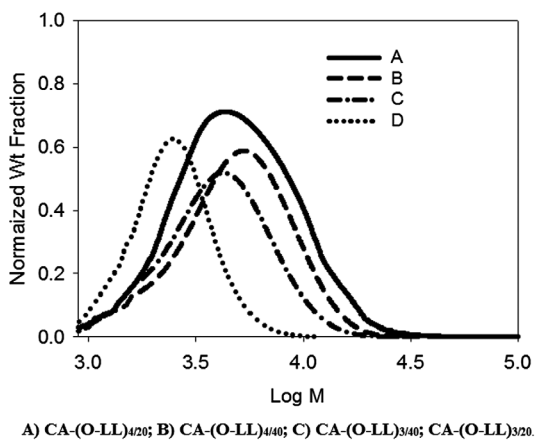


Figure 6. The molecular weight distributions of the branches of the star shaped PLA polymers obtained by using SEC technique by using universal calibration method: (A)  $\text{CA}-(\text{O-LL})_{4/20}$ ; (B)  $\text{CA}-(\text{O-LL})_{4/40}$ ; (C)  $\text{CAME}-(\text{O-LL})_{3/40}$ ; (D)  $\text{CAME}-(\text{O-LL})_{3/20}$ .

with those theoretical values ( $M_{n,\text{th}}$ ). Furthermore, the observed polydispersity index (PDI) for all star-shaped PLA polymers ranging from 1.19 to 1.34, for  $\text{CAME}-(\text{O-LL})_{3/20}$  and  $\text{CA}-(\text{O-LL})_{4/20}$ , 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]

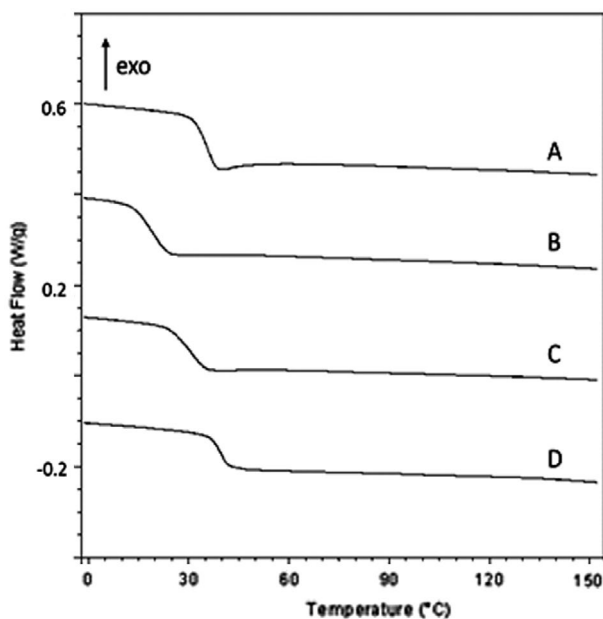


Figure 7. The second heating DSC curves of all star-shaped polyesters. (A)  $\text{CAME}-(\text{O-LL})_{3/40}$ ; (B)  $\text{CAME}-(\text{O-LL})_{3/20}$ ; (C)  $\text{CA}-(\text{O-LL})_{4/40}$ ; (D)  $\text{CA}-(\text{O-LL})_{4/20}$ .

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)<sub>3/20</sub> and CAME-(O-LL)<sub>3/40</sub>. 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).

## ORCID

Adelaide Donvito  <http://orcid.org/0000-0003-1193-491X>  
 Giancarlo Fantin  <http://orcid.org/0000-0002-6931-7976>  
 Marco Fogagnolo  <http://orcid.org/0000-0002-3261-5355>  
 Pier Paolo Giovannini  <http://orcid.org/0000-0002-7089-5466>  
 Marco Scoponi  <http://orcid.org/0000-0003-0881-3084>

## References

- [1] Mukhopadhyay S, Maitra U. Chemistry and biology of bile acids. *Curr. Sci.* **2004**;87:1666–1683.
- [2] Virtanen E, Kolehmainen E. Use of bile acids in pharmacological and supramolecular applications. *Eur. J. Org. Chem.* **2004**;16:3385–3399.
- [3] Bortolini O, Fantin G, Fogagnolo M. Bile acid derivatives as enantiodifferentiating host molecules in inclusion processes. *Chirality.* **2005**;17:121–130.
- [4] Nonappa Maitra U. Unlocking the potential of bile acids in synthesis, supramolecular/materials chemistry and nanoscience. *Org. Biomol. Chem.* **2008**;6:657–669.
- [5] Zhu XX, Nichifor M. Polymeric materials containing bile acids. *Acc. Chem. Res.* **2002**;35:539–546.
- [6] Zhang JW, Zhu XX. Biomaterials made of bile acids. *Sci. China. Ser. B-Chem.* **2009**;52:849–861.
- [7] Gouin S, Zhu XX, Lehnert S. New polyanhydrides made from a bile acid dimer and sebacic acid: synthesis, characterization, and degradation. *Macromolecules.* **2000**;33:5379–5383.
- [8] Gautrot JE, Zhu XX. Main-chain bile acid based degradable elastomers synthesized by entropy-driven ring-opening metathesis polymerization. *Angew. Chem. Int. Ed.* **2006**;45:6872–6874.
- [9] Gautrot JE, Zhu XX. High molecular weight bile acid and ricinoleic acid-based copolyesters via entropy-driven ring-opening metathesis polymerisation. *Chem. Commun.* **2008**;14:1674–1676.
- [10] Stanciu MC, Nichifor M. New degradable polyesters from deoxycholic acid and oligo(ethylene glycol)s. *Polym. Int.* **2013**;62:1236–1242.
- [11] Liu HY, Avoce D, Song ZJ, et al. N-isopropylacrylamide copolymers with acrylamide and methacrylamide derivatives of cholic acid: synthesis and characterization. *Macromol. Rapid Commun.* **2001**;22:675–680.
- [12] Avoce D, Liu HY, Zhu XX. N-alkylacrylamide copolymers with (meth)acrylamide derivatives of cholic acid: synthesis and thermosensitivity. *Polymer.* **2003**;44:1081–1087.
- [13] Zhang JH, Bazuin CG, Freiberg S, et al. Effect of side chain structure on the liquid crystalline properties of polymers bearing cholesterol, dihydrocholesterol and bile acid pendant groups. *Polymer.* **2005**;46:7266–7272.
- [14] Zou T, Cheng SX, Zhang XZ, et al. Novel cholic acid functionalized star oligo/poly(DL-lactide)s for biomedical applications. *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2007**;82B:400–407.
- [15] Zou T, Li SL, Hu ZY, et al. Synthesis and properties of star oligo/poly(trimethylene carbonate)s with cholic acid moieties as cores. *J. Biomater. Sci., Polym. Ed.* **2007**;18:519–530.
- [16] Wan T, Liu Y, Yu JQ, et al. Synthesis and characterization of star oligo/poly(2,2-dimethyltrimethylene carbonate)s containing cholic acid moieties. *J. Polym. Sci., Part A: Polym. Chem.* **2006**;44:6688–6696.
- [17] Fu HL, Yu L, Zhang H, et al. Synthesis of novel cholic acid functionalized branched oligo/poly(ε-caprolactone)s for biomedical applications. *J. Biomed. Mater. Res. Part A.* **2007**;81A:186–194.
- [18] Giguère G, Zhu XX. Synthesis and aggregation properties of anionic star-shaped polymers with cholic acid cores and polyacrylate arms. *J. Polym. Sci., Part A: Polym. Chem.* **2007**;45:4173–4178.

- [19] Luo JT, Giguère G, Zhu XX. Asymmetric poly(ethylene glycol) star polymers with a cholic acid core and their aggregation properties. *Biomacromolecules*. 2009;10:900–906.
- [20] Kadam PG, Mhaske S. Synthesis of star-shaped polymers. *Des. Monomers Polym.* 2011;14:515–540.
- [21] Cameron DJA, Shaver MP. Aliphatic polyester polymer stars: synthesis, properties and applications in biomedicine and nanotechnology. *Chem. Soc. Rev.* 2011;40:1761–1776.
- [22] Barnes S, Kirk D. In: Setchell KDR, et al., editors. *The bile acids – chemistry, physiology and metabolism*. Vol. 4, Methods and applications. New York (NY): Plenum Press; 1988. Chapter 3, Nuclear magnetic resonance spectroscopy of bile acids; p. 65–133.
- [23] Plattner A, Heusser H. Über Steroide und Sexualhormone. (97. Mitteilung). Über Beziehungen zwischen Konstitution und optischer Drehung in der Cholsäure-Reihe [About steroids and sex hormones. About relations between constitution and optical rotation in the cholic acid-series]. *Helv. Chim. Acta.* 1944;27:748–757.
- [24] Gupta AP, Kumar V. New emerging trends in synthetic biodegradable polymers – polylactide: a critique. *Eur. Polym. J.* 2007;43:4053–4074.
- [25] Kohn FE, Omen JGA, Feije J. The mechanism of the ring-opening polymerization of lactide and glycolide. *Eur. Polym. J.* 1983;19:1081–1088.
- [26] Xie W, Chen D, Fan X, et al. Lithium chloride as catalyst for the ring-opening polymerization of lactide in the presence of hydroxyl-containing compounds. *J. Polym. Sci., Part A: Polym. Chem.* 1999;37:3486–3491.
- [27] Klok HA, Hwang JJ, Iyer SN, et al. Cholesteryl-(l-lactic acid)*n* building blocks for self-assembling biomaterials. *Macromolecules*. 2002;35:746–759.
- [28] Nederberg F, Connor EF, Glausser T, et al. Organocatalytic chain scission of poly(lactides): a general route to controlled molecular weight, functionality and macromolecular architecture. *Chem. Comm.* 2001;20:2066–2067.
- [29] Heusser H, Wuthier H. Helv. Über Steroide und Sexualhormone. Zur Überführung von Cholsäure in Lithocholsäure [About steroids and sex hormones. Conversion of cholic acid in lithocholic acid]. *Helv. Chim. Acta.* 1947;30:2165–2167.
- [30] Nampoothiri KM, Nair NR, John RP. An overview of the recent developments in polylactide (PLA) research. *Bioresour. Technol.* 2010;101:8493–8501.
- [31] Tsitsilianis C, Ktoridis A. Determination of branching of star-shaped macromolecules by gel-permeation chromatography. *Macromol. Rapid Commun.* 1994;15:845–850.
- [32] Gallot-Grubisic Z, Rempp P, Benoit H. A universal calibration for gel permeation chromatography. *J. Polym. Sci., Part C: Polym. Lett.* 1967;5:753–759.
- [33] Comanita B, Noren B, Roovers J. Star poly(ethylene oxide)s from carbosilane dendrimers. *Macromolecules*. 1999;32:1069–1072.
- [34] Lescq J, Millequant M, Patin M, et al. In: Provder T, Barth HG, Urban MW, editors. *Chromatographic characterization of polymers. Star-branched polymers in multidetection gel permeation chromatography*. *Am. Chem. Soc.* 1995;247:167–179.
- [35] Masutani K, Kimura Y. In: Jiménez A, Peltzer M, Ruseckaite R, editor. *PLA synthesis. From the monomer to the polymer, poly (lactic acid) science and technology: processing, properties, additives and applications*. RSC Polymer Chemistry Series; London: Royal Society of Chemistry; 2014. Chapter 1; p. 1–36.