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Hydroxyl-bearing poly(α-hydroxy acid)-type aliphatic degradable polyesters prepared by ring opening (co)polymerization of dilactones based on glycolic, gluconic and L-lactic acids

Benjamin Saulnier [1], Jean Coudane *, Henri Garreau [2], Michel Vert [3]

CRBA, UMR. CNRS 5473, Faculty of Pharmacy, 15, avenue Charles Flahault, 34000 Montpellier, France

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Abstract

The synthesis of HO-protected poly(glycolic acid-*co*-gluconic acid) and poly(L-lactic acid-*co*-glycolic acid-*co*-gluconic acid) by copolymerisation of L-lactide and 3-(1,2-3,4-tetraoxobutyl-diisopropylidene)-1,4-dioxane-2,5-dione (DIPAGYL) is reported. The resulting polymers were characterized by size exclusion chromatography, FT Infrared, nuclear magnetic resonance, differential scanning calorimetry and X-ray diffractometry. The composition of reaction media and the reactivity ratios of the two cyclic monomers were determined at low conversion and indicated random distributions of lactyl and gluconoglycolyl constitutive units. X-ray diffraction data showed semi-crystalline morphology, as observed for poly (lactide) stereocopolymers containing more than 90% of L-enantiomer. Deprotection of the isopropylidene-protected side chain OH was possible under acidic conditions and yielded copolymers with various degrees of hydroxylation. Deprotection of the 5–6 OH groups was fast and complete whereas that of 3–4 ones was partial and occurred at the expenses of partial degradation of the aliphatic polyester chains. T_g increased with the number of hydroxyl functions, a feature attributed to the formation of hydrogen bonds. Comparison is made with features reported previously for analogs derived from DL-lactide.

Keywords: Poly(α-hydroxyacid); Gluconic acid; Functional polyester

1. Introduction

Poly(lactic acid) stereocopolymers (PLA_x) and poly(lactic-*co*-glycolic acids) (PLA_xGA_y) (where x and y stand, respectively, for the percentages of L-lactyl and glycolyl units in polymer chains) are the two main members of the poly (α-hydroxyacid)s family, widely used for biomedical and pharmacological applications [1–10]. They have been used for many years for osteosynthesis in bone surgery [1–4], for cell cultures supports [5,6] as well as for sustained drug delivery [7,8]. However, these polymers are hydrophobic and not functionalised except at chain ends. This feature limits further developments, especially by chemical modification. Various routes have been investigated that were aimed at introducing functional groups along lactic acid-based polymer chains to broaden the range of properties while keeping the water sensitive polymeric backbone. The formation of copolymers and terpolymers combining lactic, glycolic and glutamic acids was used to generate hydrophilicity and even hydrosolubility [11]. The copolymerisation of lactide and depsipeptide that bore reactive groups led to surface functionalised particles [12,13]. Functional copolymers based on polyhydroxylated compounds and lactic or glycolic acids have also been described [14–18] that exhibited various physicochemical properties. When containing bulky OH cyclic protections, T_g was increased as a function of the content in bulky units. In most cases, T_g shifted backward after deprotection. All these copolymers had a backbone which was only partially aliphatic polyester, e.g. poly(ester amide) or poly(ester carbonate).

In parallel, a novel functionalised poly (α-hydroxy acid) bearing isopropylidene-protected diol moieties was obtained by copolymerisation of DL-lactide with 3-(1,2,-3,4-tetraoxobutyl-diisopropylidene)-1,4-dioxane-2,5-dione (DIPAGYL). The resulting terpolymers combining D-lactyl (D-LA), L-lactyl (L-LA), glycolyl (GA) and Gluconyl (GL), (P_{DL}-LA_xGAGL_y

* Corresponding author. Tel.: +33 4 67 41 82 64.
E-mail addresses: saulnier@ics.u-strasbg.fr (B. Saulnier), jcoudane@univ-montp1.fr (J. Coudane), garreau@univ-montp1.fr (H. Garreau), mvert@univ-montp1.fr (M. Vert).
[1] Tel.: +33 3 88 36 92 56.
[2] Tel.: +33 4 67 41 82 62.
[3] Tel.: +33 4 67 41 82 60.

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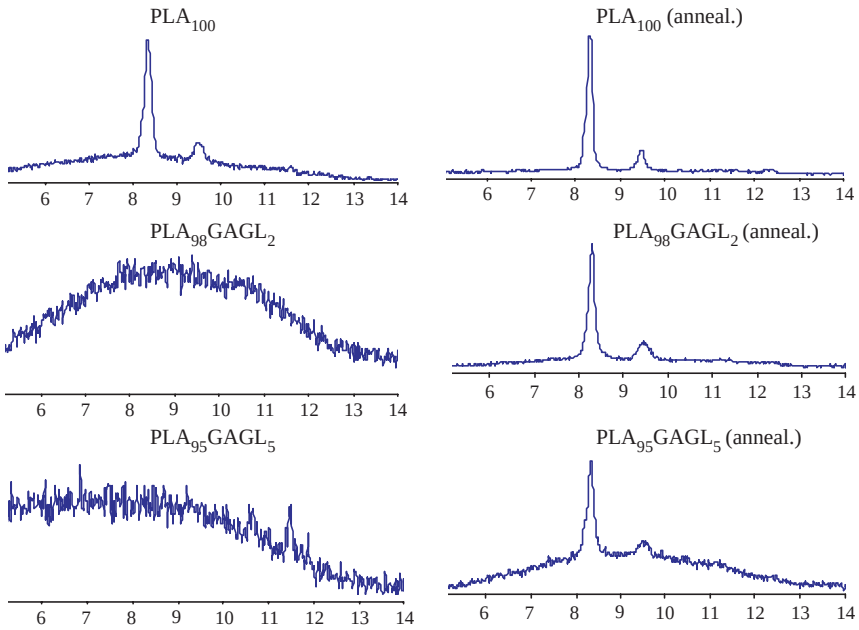
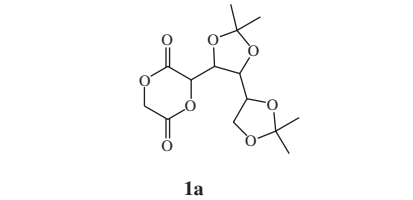


Fig. 1. Small angles X-ray diffractograms of PL-LA and PL-LAGAGL before (left column) and after 1 h annealing (right column).

were x stands for the percentage of repeating units of L-lactyl deriving from the racemic lactide monomer and y for that of gluconolglycolyl deriving from DIPAGYL monomer) showed higher glass transition temperatures T_g as compared with PLA_x or PLA_xGA_y polymers [19].

In order to show whether replacing DL-lactide by L-lactide could provide property changes related to the configuration of lactyl units, DIPAGYL (**1a**) was copolymerised with L-lactide (**1b**) to yield copolymers (PL-LA_xGAGL_y) (**1c**) that were compared with the poly(glycolic acid-*co*-gluconic acid) copolymers, (PGAGL) (**1d**), obtained by homopolymerisation of DIPAGYL. Fig. 1(c) and (d) do not take into account probable transesterification reactions. The hydrolysis of isopropylidene protecting groups was investigated in attempts to vary the degree of deprotection while controlling chain degrading side reactions. The structure and some properties of the protected and the more or less deprotected copolymers were studied. Data were compared with those previously reported for analogous polymers and copolymers derived from DL-lactide.



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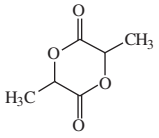

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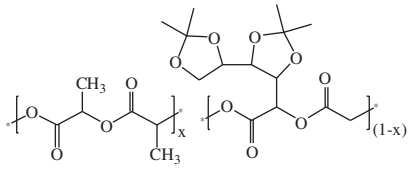

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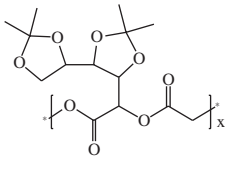


1d

PGAGL and PL-LA_xGAGL_y copolymers belong to the poly (α-hydroxy acid) family and have thus structures comparable to those of PLA_xGA_y copolymers, especially because of the presence of lactic and glycolic acid repeating units. The structure of the protected copolymer main chains was investigated by the determination of reactivity ratios of lactide and DIPAGYL from data at low conversion. The aldehyde moieties used to protect the DIPAGYL side chain OH groups were removed by treating PGAGL and PL-LA_xGAGL_y with a hydro-organic solution of acetic acid and of trifluoroacetic acid, for various reaction times. The advance of the deprotection reaction was monitored by NMR. The thermal properties of the resulting hydroxylated copolymers were investigated with a special attention to the variation of the glass transition temperature T_g.

2. Experimental

2.1. Synthesis

DIPAGYL was synthesised from δ-gluconolactone according to a previously described method [14], except for the intramolecular cyclisation and the purification stages. Typically, 30 g of 2-bromoaceto (3,4-5,6) di-isopropylidene gluconic acid (see Ref. [14]) was dissolved in DMF (300 cm^3) and the solution was added dropwise over 4 h to a suspension of sodium hydrogenocarbonate $NaHCO_3$ (10.8 g) in DMF (1.2 dm^3). The reaction was performed under argon flow. The reaction mixture was stirred during 4 more hours. Then the solvent was evaporated under vacuum (35 °C/10^{-3} mmHg). The residue was dissolved in diethyl ether. The solution was washed twice with cold water and dried using anhydrous magnesium sulfate. Purifications were performed by filtration

on a silica-filled sintered glass filter followed by two recrystallisations from 3/1/1 V/V/V heptane/chloroform/dichloromethane solutions. Immediately before use, sublimation was carried out at 80 °C under 10^{-3} mmHg.

PGAGL was obtained by bulk polymerisation of DIPAGYL at 140 °C under vacuum during two weeks. The polymerisation was initiated by tin (2-ethylhexanoate) (I/M = 1/400), the monomer being degassed 1/2 h prior to addition of the initiator solution. The initiator was diluted up to 1 cm^3 with heptane, the solution being injected under argon atmosphere via a microsyringe. Heptane was then evaporated by performing at least three vacuum/argon cycles. PGAGL was then dissolved in acetone, precipitated by addition of methyl alcohol, dried under vacuum at 40 °C during 24 h.

PL-LA_xGAGL$_y$ were synthesised as described previously for PDL-LA_xGAGL$_y$ [15] and purified like PGAGL. Briefly, the monomers purified by recrystallisation from acetone (lactide) or recrystallisation and sublimation (DIPAGYL), were introduced in polymerisation vials in the desired proportions. Initiation and degassing were conducted as for PGAGL, and the polymerisations were carried out for periods going from 3 days to one week, depending on the monomer feed. Polymerisations were stopped by decreasing temperature up to ambient at least 24 h after the total solidification.

PGAGL deprotection was carried out on 1 g using acetic acid in 15/5/10 V/V/V acetic acid/water/acetone mixture (30 mL) at reflux during 3–5 h. The mixture was partially evaporated before dialysis using a Spectra Por membrane with a 3500 dalton cut-off against 50/50 V/V water/ethanol for 24 h. The deprotection of PL-LA_xGAGL$_y$ polymers (1 g) was carried out between 0 and 5 °C with trifluoroacetic acid in 10/1,5/10 V/V/V TFA/water/dichloromethane (21.5 mL) for various reaction times ranging from 30 s to 5 min. The recovered products were purified by dialysis (PGAGL) or precipitation from dichloromethane to cold diethyl ether (PL-LA_xGAGL$_y$ copolymers). The deprotection of PL-LA_{70}GAGL$_{30}$ was carried out at room temperature during 5 min using chloroacetic acid instead of TFA.

2.2. Characterisations

NMR spectra were recorded on polymers solutions in d_6-DMSO using a Brücker 400 MHz spectrometer. Oligomers and monomers were analysed in deuterated chloroform ($CDCl_3$) solutions.

DSC analyses were performed on a Perkin Elmer DSC6 system at a 10 °C/min heating rate. T_g values were determined using the Pyris software (middle of the transition) for second heating runs.

X-ray diffractograms were recorded at low angles between 2 and 20° with a horizontal CGR goniometer and resulted from five scans. Crystallinity was evaluated from diffraction peak and diffusion halo using the ratio: area of the diffraction peak/(area of the diffraction peak + area of the halo).

IR spectra were recorded on a FT-IR Perkin Elmer 1760 spectrometer. Polymer samples were cast on NaCl plates using acetone solutions.

SEC analyses were performed in tetrahydrofuran (THF at 1 cm^3/min flow rate) or chloroform (for THF insoluble polymers) with a Waters system fitted with a refractometric detector. A 60 cm PLgel (Mixed C-5 μm) column was used. Calibration was achieved with polystyrene standards.

3. Results and discussion

PGAGL and PL-LA_xGAGL$_y$ were obtained by ring opening polymerization of DIPAGYL and lactide + DIPAGYL, respectively, in the bulk at 140 °C under vacuum in the presence of tin 2-ethylhexanoate. Compositions, weight average molecular weight, and glass transition temperatures of the resulting OH-protected polymers are reported in Table 1. The PGAGL sample exhibited a relatively low molar mass (M_w = 18,000 g/mol, M_w/M_n = 1.9) compared with the PL-LA_xGAGL$_y$ copolymers (45,000 < M_w < 120,000 g/mol).

3.1. Morphology

The glass transition temperature was found to depend on composition as in the case of PDL-LA_xGAGL$_y$ racemic analogues [15,19]. The higher the content in L-LA the lower the T_g value with modulation due to the difference in molecular weight. The increase of the T_g with the proportion of GAGL units was previously observed for PDL-LA_xGAGL$_y$ copolymers [15,19]. Therefore, one can conclude that the configuration of lactyl units in PLA_xGAGL$_y$ copolymers had no significant effect on morphological properties. This is likely to be due to the predominance of the bulky isopropylidene-bearing gluconyl units.

Fig. 1 shows X-ray diffractograms of PL-LA (PLA_{100}) and of PL-LA_xGAGL$_y$ copolymers before and after 1 h annealing at 90 °C. Before annealing, none of the copolymers exhibited crystallinity, in contrast with PL-LA. However, X-ray diffraction patterns reflected the formation of crystalline domains after annealing. The higher the X value, the higher the degree of crystallisation: x_c = 14% for PLA_{95}GAGL$_5$; x_c = 48% for PLA_{98}GAGL$_2$, and x_c = 66% for PLA_{100}. The diffraction peaks of copolymers containing 2 and 5% GAGL were typical of crystalline PL-LA. When the proportion of L-lactyl units was below 90%, copolymers remained

Table 1
M_w and glass transition temperatures of PGAGL and PL-LA_xGAGL$_y$

Polymer	GAGL content (mol%)[a]	M_w[b] (g/mol)	I_p	T_g (°C)
PGAGL	100	18,000	ND	85
PL-LA_{30}GAGL$_{70}$	70	39,000	1.6	74
PL-LA_{50}GAGL$_{50}$	50	71,000	1.6	77
PL-LA_{70}GAGL$_{30}$	30	48,000	2.0	70
PL-LA_{80}GAGL$_{20}$	20	103,000	1.8	67
PL-LA_{85}GAGL$_{15}$	18	96,000	ND	64
PL-LA_{90}GAGL$_{10}$	13	115,000	2.3	65
PL-LA_{95}GAGL$_5$	6–7	70,000	1.7	62
PL-LA_{98}GAGL$_2$	2–4	120,000	1.7	61

ND, not determined.
[a] Calculated from ^{1}H NMR spectra.
[b] From GPC data in THF except for PL-LA_{98}GAGL$_2$ in $CHCl_3$.

amorphous, even after annealing, as observed for PLA_x stereocopolymers containing less than 90% L-lactyl units.

3.2. Reactivity ratios

The proportion of GAGL units in the copolymers was determined by ^1H NMR. However, it was not possible to deduce any information on the repeating unit distribution within the main chain, in contrast to what is currently done for PLA_X stereocopolymers and many lactic acid-based copolymers [20].

The reactivity ratios corresponding to the copolymerisation of DIPAGYL and L-lactide were determined. The classical Finneman–Ross method was applied. Four different monomer mixtures (10, 20, 70, 75% in DIPAGYL, namely R10, R20, R70, R75) were copolymerised for short reaction times, namely 45 min to 4 h in order to keep the degree of conversion low, i.e. $<35\%$. Long reaction times were necessary for feeds containing more than 70% DIPAGYL because the rate of polymerisation of this monomer was lower than that of L-lactide. The collected copolymers were analysed by ^1H NMR without any purification to secure significant comparison balances.

The spectrum of an almost equimolar mixture of the two monomers (Fig. 2(a)) was compared to the typical spectrum

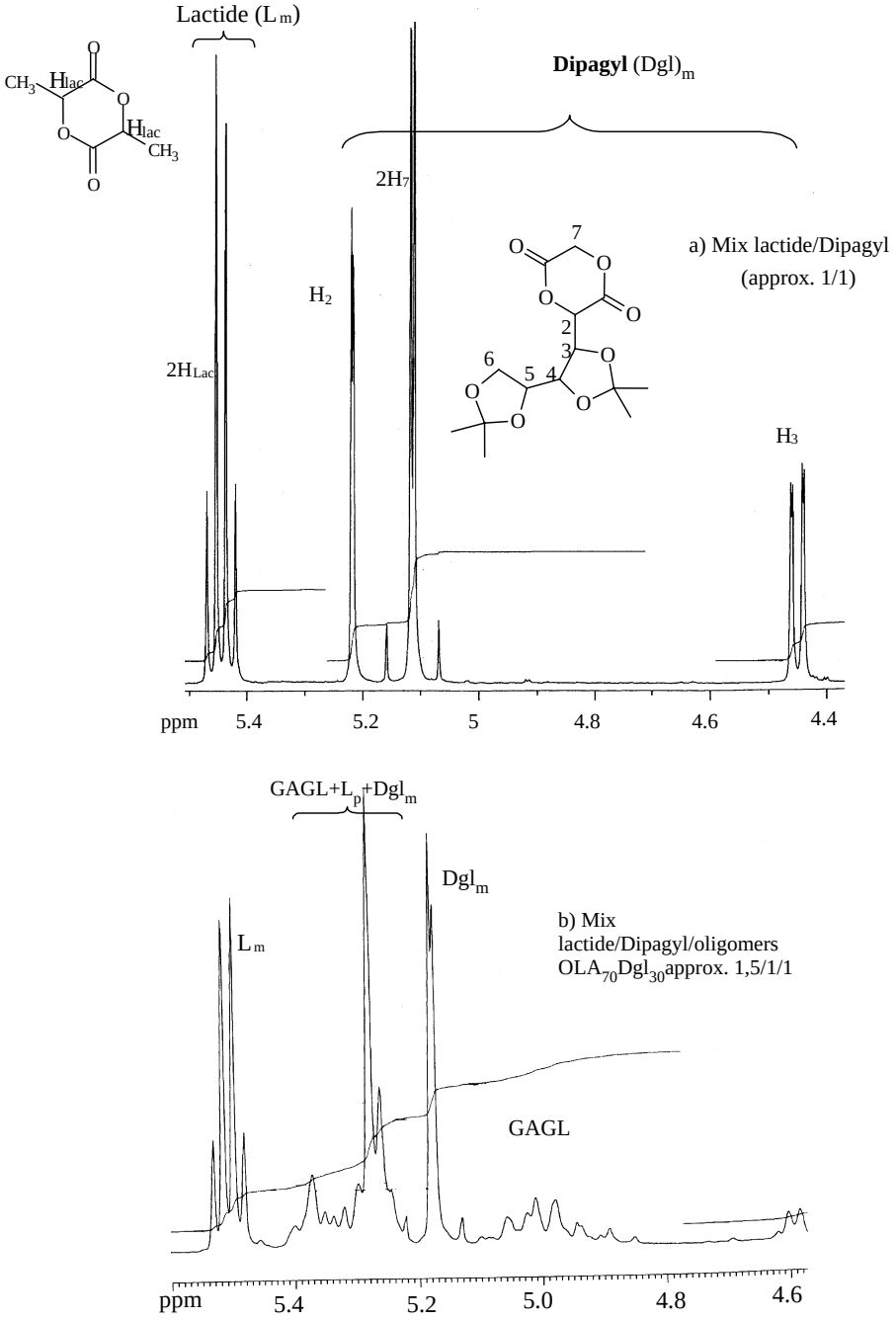


Fig. 2. (a) ^1H NMR spectra of monomers (lactide: L_m, Dipagyl: DGL_m) and (b) monomers/oligomer mixtures (lactyl unit: L_p, gluconoglycolyl unit: GAGL.

Table 2
Shape, position and assignments of NMR peaks typical of lactide (LA) and of DIPAGYL

Shape	Chemical shift (ppm)	Number of H	Name
1 Quadruplet (LA)	5.45	2	H_{lac}
1 Singlet (DIPAGYL)	1.5	6	CH_3
1 Singlet (DIPAGYL)	5.22	1	H_2
2 Doublets (DIPAGYL)	5.11	2	H_7
1 Double doublet (DIPAGYL)	4.45	1	H_3
4 Singlets (DIPAGYL)	1.15	12	CH_3

of a mixture of the two monomers and of oligomers (Fig. 2(b)) that mimic the reaction medium at low conversion. For clarity, Fig. 2 only reports the domain between 4.4 and 5.5 ppm. Shapes, chemical shifts and attributions of the peaks of the whole spectrum are listed in Table 2 (see Ref. [14] for details).

In the spectra of monomers and oligomers mixtures issued from polymerisation reactions, the peaks that appear between 5.17 and 5.35 ppm in Fig. 2(b) were not well separated. Moreover, chemical shifts depended on degree of conversion and composition. The global integration of these peaks ($I_{5.17–5.35}$) reflected the presence of DIPAGYL monomer, GAGL units and lactyl units:

$$I_{5.17–5.35} = I_{\mathrm{DIPAGYL}} + I_{\mathrm{GAGL}} + I_{\mathrm{LA}}.$$

The proportion of polymerised lactide (LA) was evaluated from the areas of the well separated peaks at 5.45 (lactide), 5.11 (DIPAGYL) and 4.9 ppm (GAGL), according to the following Eq. (1):

$$I_{\mathrm{LA}} = I_{5.17–5.35} - (I_{\mathrm{GAGL}} + I_{\mathrm{DIPAGYL}}) \quad (1)$$

Taking into account the preservation of the number of DIPAGYL protons during polymerisation, the contribution of GAGL resonances to the total area of resonances located between 5.17 and 5.35 ppm ($1H_2$) was half the area observed for the GAGL peak at 4.9 ppm ($2H_7$). Similarly, the DIPAGYL contribution to the 5.17 and 5.35 ppm zone ($1H_2$) was half the area of the resonance typical of DIPAGYL at 5.11 ppm ($2H_7$). Therefore, Eq. (1) could be rewritten as:

$$I_{\mathrm{LA}} = I_{5.17–5.35} - \left[\frac{I_{4.9} + I_{5.11}}{2}\right] \quad (2)$$

Eq. (2) was used to determine the composition of the copolymers present in the various polymerisation media. The percentage of GAGL units in copolymer (GAGL content) was defined as $I_{\mathrm{GAGL}}/(I_{\mathrm{GAGL}}+I_{\mathrm{LA}})$. The values of the degree of conversion were deduced from the ratios $I_{\mathrm{LA}}/(I_{\mathrm{lactide}}+I_{\mathrm{LA}})$ and $I_{\mathrm{GAGL}}/(I_{\mathrm{GAGL}}+I_{\mathrm{DIPAGYL}})$ for L-lactide and DIPAGYL, respectively. Initial feed compositions, conversion ratios and final compositions are listed in Table 3. Despite the rather low accuracy of the experiments and NMR analyses, the composition of the feeds and those of corresponding copolymers were rather close. The reactivity ratios deduced by using the Finneman Ross method were $r1=0.4$ (DIPAGYL) and $r2=1.24$ (L-lactide).

Table 3
Initial feed, conversion ratios and final composition of PL-LA_xGAGL_y copolymers

Copolymer	Reaction time	M_w	I_p	Conversion ratio (%)	GAGL contents (%)
R10	45 min	8100	1.1	16	8
R20	45 min	3650	1.2	10	15
R70	3 h 30 min	3300	1.3	30	60
R75	4 h	3300	1.3	35	63

As reactivity ratios are not dramatically different, it can be said that there is a more or less random distribution of monomeric units on the polymeric backbone.

3.3. Deprotections

3.3.1. Homopoly (DIPAGYL)s: PGAGL and PGAGL–OH

The deprotection of PGAGL was carried out in a solution of 50/50 V/V acetic acid/acetone at reflux. Polymers were purified by dialysis to remove compounds with molecular weights lower than 3000 Da according to the membrane cut-off. The values of the degree of deprotection were evaluated by ^1H and ^{13}C NMR.

3.3.1.1. NMR analysis of PGAGL–OH_z. The PGAGL–OH_z acronyms (where $z=a$, b, etc. stands for the reaction time, namely $a=3$ h; $b=4$ h; $c=4.5$ h) were used to differentiate the various deprotected compounds. With respect to its parent PGAGL, the spectrum of PGAGL–OH_a ^{13}C NMR spectrum exhibited two new peaks located at 73 ppm (CH-type carbon, $C_{5'}$) and 65 ppm (CH_2-type carbon, $C_{6'}$) (Fig. 3). According to the ^{13}C–^1H NMR correlation spectrum, these two carbon atoms were correlated with a proton resonance at 3.5 ppm which thus reflected overlapped $H_{5'}$ and $H_{6'}$ signals, actually. Moreover, a small new peak appearing near the C_4 resonance was correlated to H_4 and thus attributed to a change in the magnetic environment of C_4, in agreement with deprotection in 5,6 position.

To ensure and complete these attributions, the chemical shifts and the proportions of OH-type hydrogen atoms were determined by comparing the PGAGL–OH_a ^1H NMR spectrum before and after addition of D_2O. Fig. 4 shows the hydroxyl proton resonances initially located at 4.65 and 5.15 ppm. In the presence of D_2O, the relative weight of the peaks located between 4.4 and 5.3 ppm decreased from 3.65 to 3, in agreement with $^1H \leftrightarrow {}^2H$ exchange that moved away the resonances related to free OH groups. By difference, the removed resonances weighted 0.65, i.e. 0.325 per OH proton. On the other hand, deprotection resulted in the appearance of the three new protons in the zone of H_5 and H_6 resonances, which are correlated to carbon 5 (1H named $H_{5'}$) and to carbon 6 (2H named $H_{6'}$) overlapping at 3.5 ppm. Basically, the weight of these overlapped resonances should be 1, ($\approx 3\times 0.325$ on the basis of the $^1H \leftrightarrow {}^2H$), a value which agrees well with the experimental relative weight exhibited by the 3.5 ppm resonance in Fig. 4.

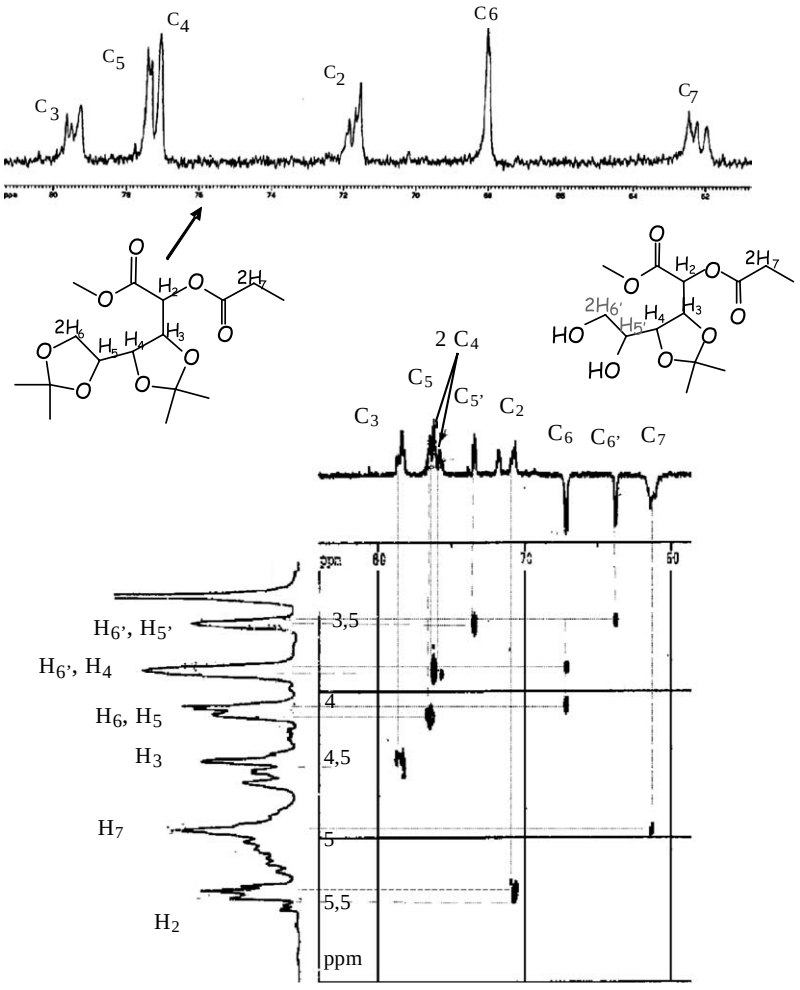


Fig. 3. Protected PGAGL ^{13}C NMR spectrum (top) and ^{13}C–^{1}H correlation NMR Spectrum of PGAGL–OH_a (bottom).

The average degree of deprotection was evaluated using Eq. (3) [15].

$$\%\mathrm{OH} = 100\left[1 - \frac{I_{\mathrm{CH3}} \text{ at } 1.4 \text{ ppm}}{12 I_{\mathrm{H2}} \text{ at } 5.4 \text{ ppm}}\right] \quad (3)$$

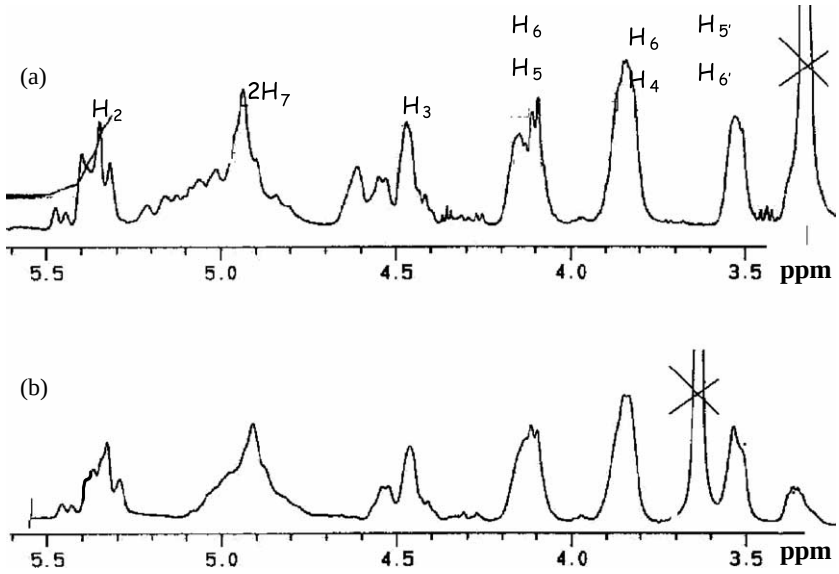
The obtained value (18%) was rather accurate because it was based of the large methyl resonance due to the 12 isopropylidene H at 1.2–1.5 ppm (not shown). The comparison of the spectra recorded with and without D_2O offered another means to evaluate the degree of deprotection. As two isopropylidiene groups were initially present per units, the deprotection degree was calculated using $\%\mathrm{OH} = 100 I_{\mathrm{OH}}/(2 I_{\mathrm{H2}})$ (i.e. $100 \times 0.325/2 = 16.25\%$) and well agreed with the result given by previous calculation method. This second method is of particular interest when the positions of OH resonances are not well identified, e.g. when the position of OH peaks varies. The good correlation between the two methods showed that there was no OH contribution overlapping the reference peak H_2.

Table 4 shows the characteristics of the three partially deprotected PGAGL according to reaction time. The percentage of deprotection obtained from ^{1}H NMR increased with reaction time but did not reach the 50% level corresponding to complete hydrolysis of the 5,6-isopropylidene groups. Molecular masses were not affected by the acidic conditions, at least during the four first hours. In order to determine whether one or the two isopropylidene protecting groups were involved in the deprotection process, the relative weights of each pair of CH_3 resonances were evaluated, namely pair 1 (located at 1.35 ppm) and pair 2 (located at 1.25 ppm). Table 5 shows that only pair 2 was affected. Based on the fact it is the most accessible and the most reactive with the presence of a primary alcohol group, pair 2 was assigned to the 5,6 position.

3.3.2. PL-LA$_x$GAGL$_y$ Copolymers

The deprotection of PL-LA$_x$GAGL$_y$ copolymers was carried out in the presence of TFA i.e. under conditions known to partially degrade the main chains, according to previous work on the racemic PDL-LA$_x$GAGL$_y$ analogues [20]. Deprotection kinetics and chain breaking were investigated by ^{1}H NMR and SEC. The percentage of deprotected OH (%OH) was deduced from NMR spectra taking into account the methyl protection normalised to one GAGL main chain proton, as for PGAGL (Eq. (3))

Fig. 5 shows the increase of OH% with the reaction time in the case of $PLA_{80}GAGL_{20}$. Data shows that 50–60% deprotection



Name :	H2	2H7	H3	H5-6	H4-6	H5'-6'
δ (ppm) :	5.45	5	4.5	4.1	3.8	3.5
Integ. Val.(a):	1	2.35	1.3	1.33	1.66	1
Integ. Val.(b):	1	2	1	1.33	1.66	1

Fig. 4. ^{1}H NMR spectra of PGAGL–OH$_a$ before (a) and after (b) addition of D_2O. Chemical shifts and integration values are quoted under the spectra.

Table 4
Molecular weights and deprotection ratios of PGAGL–OH$_z$

Copolymer	Deprotection time (h)	M_w (g/mol)[a]	Deprotection ratio[b] (%)
PGAGL–OH$_a$	3	18,000	18
PGAGL–OH$_b$	4	18,000	31
PGAGL–OH$_c$	4.5	ND	35

ND, not determined.
[a] Initial M_w = 18,000.
[b] According to Eq. (3).

was achieved in 30 s. According to the findings collected from the study of PL-LAGAGL and its racemic LA analogues, the fast reaction was assigned to the removal of the more accessible 5,6-isopropylidene groups as observed for the homopolymer PGAGL. Deprotecting the 3,4-isopropylidene group was feasible but the reaction required more time, presumably because of steric hindrance and lower reactivity of the 3,4-secondary alcohol groups. Furthermore, beyond 30 s, deprotection occurred at the expenses of the molecular weights as shown Fig. 6. Data clearly show that chain breaking became dramatic when the degradation of the 3,4-isopropylidene group started. Deprotection up to 90% was feasible but only oligomers were collected. On the other hand, the deprotection of the 5,6-OH groups was so fast that deprotection extents lower than 50% could hardly be obtained. In order to minimise the degradation, chloroacetic acid was used instead of trifluoroacetic acid. Under these conditions, 25% deprotection was obtained in 5 min without affecting chain lengths significantly. This is thus a method to obtain the deprotection extents smaller than 50% that were not accessible when using TFA.

Table 5
Evolution of ^{1}H NMR isopropylidene CH_3 resonances with degradation time, PGAGL being taken as 100% reference

Sample	PGAGL (%)	PGAGL–OH$_a$ (%)	PGAGL–OH$_b$ (%)	PGAGL–OH$_c$ (%)
% Pair 1	100	100	95	98
% Pair 2	100	74	43	34

3.4. Calorimetric analysis

The glass transition temperature of partially deprotected PGAGL–OH and PL-LA$_x$GAGL$_y$–OH copolymers depended

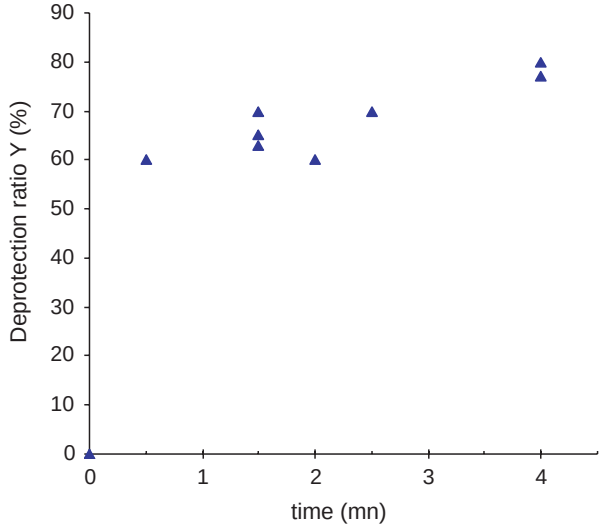


Fig. 5. Evolution of deprotection ratio (▲:Y) with reaction time.

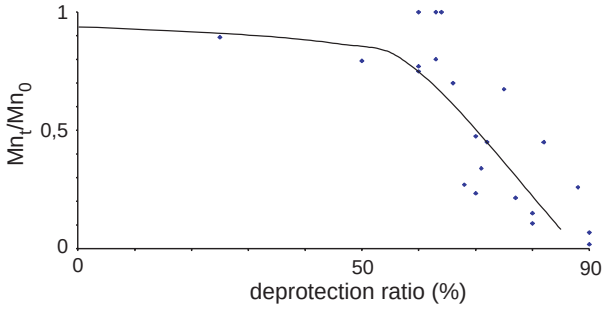


Fig. 6. relative variations of molar masses vs deprotection ratios for various deprotected copolymers.

Table 6
Glass transition temperatures for protected and deprotected PGAGL

Acronyme	T_g (°C)	Deprotection ratio (%)	OH number
PGAGL	85	0	0
PGAGL–OH_a	ND	18	72
PGAGL–OH_b	74	31	124
PGAGL–OH_c	86	35	140
PGAGL–OH_d[a]	93	44[a]	180

[a] Deprotected during 5 h, for this compound, deprotection ratio was evaluated by Raman spectroscopy following a method described elsewhere [21].

on the percentage of deprotection and of GAGL units (Tables 6 and 7). Because the presence of OH groups along the chain depended both on the degree of deprotection and on the proportion of gluconyl units for PL-$LA_xGAGL_yOH_z$ copolymers, the OH number parameter β was introduced as the number of free OH groups for 100 units regardless of the substituent (H, CH_3 or gluconyl). $\beta = 4\times10^{-2}\times y\times\%OH$ (y: GAGL content, $0\leq y\leq100$; $0\leq\%OH\leq90$) where $y=100$ in the case of PGAGL–OH_z derivatives. Although there was no simple correlation between T_g values and OH% or β values, one can conclude that the presence of OH groups tended to increase T_g, a trend that can be assigned to the formation of hydrogen bonds as already proposed by Kumar et al. in the case of copolymers containing sugar moieties, namely poly (L-lactide-*co*-pentofuranose) [16] and also as described in the case of poly(vinyl alcohol-*co*-vinyl acetate) copolymers [22,23].

IR spectra of PGAGL (not shown) exhibited two different contributions in the OH domain, between 3200 and 3600 cm^{-1}. The contribution with the lowest wavenumber (3300–3400 cm^{-1}) is generally assigned to H-bonded OH stretching vibrations. This contribution increased with the OH number (as T_g did) and became higher than the contribution attributed to free OH at $\approx$3500 cm^{-1}. The comparison of T_g value evolution between PL-LA_xGAGL_y and PL-LA_xGAGL_y–OH_{50} as a function of y only is shown on Fig. 7.

Table 7
Protected and deprotected polymers T_g

Copolymer	T_g (°C)	Copolymer	T_g (°C)	Deprotection ratio (%OH)	OH number (β)
$PLA_{30}GAGL_{70}$	74	$PLA_{30}GAGL_{70}$–OH_{25}	82	25	70
$PLA_{50}GAGL_{50}$	77	$PLA_{50}GAGL_{50}$–OH_{50}	78	50–60	100–120
$PLA_{70}GAGL_{30}$	70	$PLA_{70}GAGL_{30}$–OH_{50}	69	50–60	60–75
$PLA_{80}GAGL_{20}$	67	$PLA_{80}GAGL_{20}$–OH_{50}		50–60	40–50
$PLA_{85}GAGL_{15}$	64	$PLA_{85}GAGL_{15}$–OH	49	>70	>40
$PLA_{90}GAGL_{10}$	65	$PLA_{90}GAGL_{10}$–OH_{50}	53	50–60	20–25
$PLA_{95}GAGL_5$	62	$PLA_{95}GAGL_5$–OH_{50}	55	Id	10–12
$PLA_{98}GAGL_2$	61	$PLA_{98}GAGL_2$–OH_{50}	58	Id	4–5

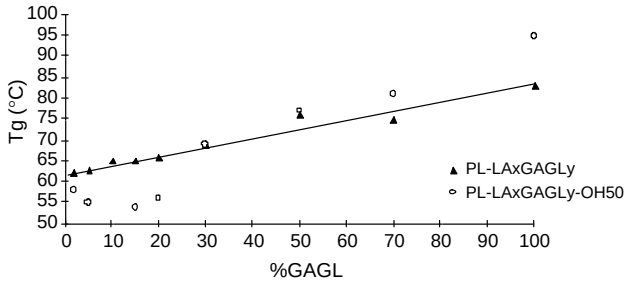


Fig. 7. T_g of PL-LAGAGLs and PL-LAGAGL–OH_{50}s as a function of GAGL proportion.

4. Conclusions

Protected and deprotected copolymers PL-LA_xGAGL_y with rather high molecular weights were synthesised by bulk ring opening copolymerization using stannous octoate as initiator. Contrary to PDL-LA_xGAGL_y copolymers, PL-LA_xGAGL_y copolymers could crystallize when the L-lactide content is higher than 90% but only after annealing. When crystallinity was observed, PLA_{100}-type crystalline domains were formed. The glass transition temperature of the protected copolymers did not depend on the configuration of the lactyl units. Deprotection of PGAGL homopolymers led to OH bearing macromolecules whose T_g increased with the proportion of OH groups. In the case of PL-LA_xGAGL_y copolymers, the degree of deprotection could be modulated between 25 and 90%, chain breakings occurring for deprotection affecting more than 60–70% of the protected OH. As in the case of the DL-lactide analogs, deprotection was easier at the 5–6 position than at the 3–4 one, the former reacting within a very short period of time in the presence of trifluoroacetic acid. The hydroxyl groups generated along the poly (α-hydroxy acid) backbone offer possibilities for chemical modifications to tailor the macromolecular physicochemical or biofunctional properties especially for biomedical uses.

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References

[1] Vert M, Christel P, Chabot F, Leray J. Bioresorbable plastic materials for bone surgery. In: Hasting DW, Ducheyne P, editors. Macromolecular materials. Boca Raton: CRC Press; 1984. p. 119.
[2] Vert M, Chabot F. Stereoregular bioresorbable polyesters for orthopedic surgery. Makromolekulare Chemie 1981;(suppl 5):30.
[3] Chanavaz M, Chabot F, Donazzan M, Vert M. Further clinical applications of bioresorbable PLA 37.5 GA 25 and PLA 50 polymers

for limited bone augmentation and bone replacement. In: Christel P, Meunier A, Lee AJC, editors. Biological and biomechanical performance of biomaterials. Amsterdam: Elsevier; 1986. p. 233–8.

[4] Stancari F, Zanni B, Bernardi F, Calandriello M, Salvatorelli G. Use of PLA–PGA (copolymerised polylactic/polyglycolic acids) as a bone filler: clinical experience and histologic study of a case. Quintessenz 2000; 41(1):47.

[5] Ishaug-Riley SL, Okun LE, Prado G, Applegate MA, Ratcliffe A. Human articular chondrocytes adhesion and proliferation on synthetic biodegradable polymer films. Biomaterials 1999;20:2245.

[6] El-Amin SF, Attawia M, Lu HH, Shah AK, Chang R, Hickok NJ, et al. Integrin expression by human osteoblasts cultured on degradable polymeric materials applicable for tissue engineered bone. J Orthop Res 2002;20(1):20.

[7] Li SM, Vert M. Biodegradable polymers: polyesters. In: Mathiowitz E, editor. The encyclopedia of controlled drug delivery. New York: Wiley; 1999. p. 71.

[8] Brannon-Pepas L, Vert M. Polylactic and polyglycolic acids as drug delivery carriers. Handbook of pharmaceutical controlled release technology, New York: Marcel Dekker; 2000. p. 93–130.

[9] Kulkarni RK, Pani K, Neuman C, Leonard F. Polylactic acid for surgical implants. Arch Surg 1966;93:839.

[10] Williams DF. Review biodegradation of surgical polymers. J Mater Sci 1982;17:1233.

[11] Deng X, Yao J, Yuan M, Li X, Xiong C. Polymerization of lactides and lactones, 12a: synthesis of poly[(glycolic acid)-alt-(L-glutamic acid)] and poly{(lactic acid)-*co*-[(glycolic acid)-alt-(L-glutamic acid)]}. Macromol Chem Phys 2000;201:2371.

[12] Ouchi T, Hamada A, Ohya Y. Biodegradable microspheres having reactive groups prepared from L-lactic acid-depsipeptide copolymers. Macromol Chem Phys 1999;200:436.

[13] Ouchi T, Miyazaki H, Arimura H, Tasaka F, Hamada A, Ohya Y. Synthesis of biodegradable amphiphilic AB-type diblock copolymers of lactide and depsipeptide with pendant reactive groups. J Polym Sci, Part A: Polym Chem 2002;40:1218.

[14] Marcincinova BK, Coudane J, Boustta M, Engel R, Vert M. Synthesis and characterization of novel degradable polyesters derived from d-gluconic and glycolic acids. Macromolecules 1999;32(26):8774.

[15] Marcincinova BK, Boustta M, Coudane J, Vert M. Novel degradable polymers containing D-gluconic acid, a sugar of vegetal origin, with lactic and glycolic acids. Biomacromolecules 2001;2:1279.

[16] Kumar R, Gao W, Gross RA. Functionnalized polylactides: synthesis and characterization of poly ([L]-lactide-*co*-pentofuranose). Macromolecules 2002;35:6835.

[17] Chen X, Gross RA. Versatile copolymers from [L]-lactide and [D]-xylofuranose. Macromolecules 1999;32:308.

[18] Ray III WC, Grinstaff MW. Polycarbonate and poly(carbonate-ester)s synthesized from biocompatible blocks of glycerol and lactic acid. Macromolecules 2003;36:3557.

[19] Marcincinova BK, Boustta M, Coudane J, Vert M. Can the glass transition of PLA be increased?. In: Scholz C, Gross RA, editors. Polymers from renewable resources: biopolymers and catalysts. Washington: ACS Review; 2000. p. 200.

[20] Marcincinova BK. Synthesis and study of novel artificial degradable polymers based on a vegetal metabolite, the gluconic acid, Thesis, Faculty of Pharmacy, Montpellier, France, Jul 1st, 1997.

[21] Saulnier, B., Properties and chemical coupling of functionnalized degradable copolymers of poly(L-lactic-*co*-gluconic-*co*-glycolic acid)s, Thesis, Faculty of Pharmacy, Montpellier, France, Dec. 8th, 2003.

[22] Hess VK, Steinmann R, Kissig H. Avisiers I Kolloid-Z-Z Polym 1957; 153:128.

[23] Staudinger H, Stark W, Frey K. Ber 1927;60.