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Polymer 46 (2005) 11263–11272
polymer
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Synthesis of new versatile functionalized polyesters for biomedical applications

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Received 12 May 2005; received in revised form 16 September 2005; accepted 22 September 2005
Available online 24 October 2005

Abstract

A new family of branched polymers was synthesized for different biomedical applications such as the preparation of targeted nanoparticulate drug carriers. They are new copolymers of hydroxy-acids and allyl glycidyl ether. The functional groups (allyl-, hydroxyl- and carboxyl-) to which various groups will be grafted are linked to the polymer backbone. The resulting polymers were characterized by ^{1}H NMR, ^{13}C NMR, size exclusion chromatography (SEC), elemental analysis and differential scanning calorimetry (DSC). In vitro cytotoxicity assays were also conducted to ensure biocompatibility of the polymers. In order to obtain some structural evidences, different molecules have been grafted on the pendant groups. The method allows a rapid and easy synthesis of allyl-, hydroxyl- and carboxyl-branched degradable polymers for grafting various bioactive molecules.

Keywords: Branched polymer; Biocompatible; Bioadhesive

1. Introduction

Biodegradable polymers have had a remarkable impact in the field of controlled drug delivery. Over the past two decades, poly-(L-lactic acid) (PLLA) and its copolymers with D-lactic acid or glycolic acid or ε-caprolactone (CL) or polyethylene glycol (PEG) have been extensively studied as controlled drug delivery carriers. Such carriers offer various advantages such as controlled drug release rate, improved therapeutic efficiency, prolonged biological activity and decreased administration frequency [1]. With the in-depth understanding of the pathophysiology and cellular mechanisms of the disease, targeted or cell specific drug delivery is the focus of the current research. Block and graft copolymers of PLA and PEG have opened new avenues to the field of targeted drug delivery by prolonging the circulation time of the polymeric colloidal drug carriers. To target specific cell type in the body (as in case of tumour cells), the presence of specific ligands is necessary on the surface of the colloidal carriers. This would reduce the systemic side effects of the drug by improving the receptor-mediated uptake by the targeted cells. For instance, targeted doxorubicin delivery could be achieved by folate conjugated mixed micelles of the PLGA-*b*-PEG-folate polymer [2]. In other words, targeted drug delivery has generated a great need for biomaterials with bioadhesive and/or specific recognition properties. Thus, the ability to impart bioadhesivity, cell specificity or other specific characteristics to the existing biocompatible polymers represents an important synthetic challenge as well as holds the promise for better therapeutic protocols. Indeed, the availability of functional pendant groups is highly desirable for the fine tuning of the above- mentioned properties. Various efforts are directed towards achieving this goal [3,4]. However, chemistry involved in the synthesis of the functional monomers is complex and/or tedious, whereas the subsequent polymerization is generally out of control. For example, Bizzarri et al. have synthesized functionalized malolactonate polymers and copolymers, where the synthesis of monomers itself was long with low yields (12–45%) and polymerization reactions were very slow (over 4–30 days) [3]. Similarly, Ouchi et al. have reported the synthesis of PLA-grafted polysaccharides, however, their method required protection/deprotection steps [5]. Thus, there are very few reports on the efficient and easy synthesis of functionalized polyesters. Amongst them, Finne et al. have reported very

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doi:10.1016/j.polymer.2005.09.079

efficient synthesis method for functionalized PCL and PLLA with controlled molecular weight and low polydispersity [6].

The purpose of this paper is to develop a high yield and rapid synthesis method for grafted PLA, PCL and polyglycidine with allyl, hydroxy and carboxylic pendant functional groups. These are versatile copolymers of polyesters and allyl glycidyl ether. These polymers have been developed for grafting various bioactive molecules like salens [7] or ligands for E-selectin and for P-glycoprotein. Also, Methoxy PEG-*g*-PLA has been successfully used to prepare colloidal nanoparticles for sustained drug release [8].

2. Experimental section

2.1. General

All materials (reagents and solvents) were purchased from Laboratoire MAT (Montréal, Canada) and Sigma-Aldrich (St-Louis, USA) and used without further purification. The polymers were characterized by ^{1}H NMR and ^{13}C NMR (Brüker ARX 400 MHz spectrometer at Centre Regional de RMN) using tetramethylsilane (TMS) as internal standard in $CDCl_3$ or DMSO-D_6, by IR-FTIR (Perkin–Elmer spectrometer), by SEC (Water SEC coupled with refractive index detector) using THF or chloroform as mobile phase and polystyrene as standards and by measurement of melting point (electrothermal apparatus). Elemental analysis were performed by the Laboratoire d'Analyse Élémentaire of the Université de Montréal with a Fisons Instrument, model EA 1108 CHN.

2.2. Cytocompatibility studies

2.2.1. Cell lines

RAW 262.7 and J774A.1 murine macrophage cell lines (American Type Culture Collection, Rockville, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% Fetal Bovine Serum (Gibco, USA) and penicillin/streptomycin (Gibco, USA). The cells were grown and maintained in tissue culture flasks and incubated at 37 °C in a 5% carbon dioxide atmosphere.

2.2.2. Proliferation assays

Polymers dissolved in 10 μl dimethyl sulfoxide (DMSO) were added in a 96 well flat-bottomed microplate (Corning, NY, USA), in triplicate. The amounts tested were: 250, 100, 10, 1, 0.1, and 0.01 μg. DMSO was subsequently removed under vacuum. RAW 264.7 or J774A.1 cells were diluted in complete medium at a final concentration of 5×10^{5} cells/ml and plated (100 μl/well). The plates were incubated for 24 h after which cell proliferation was assessed with MTT assay [9]. Briefly, 10 μl of thiazolyl blue tetrazolium bromide (5 mg/ml) dissolved in PBS (10 mM, pH 7.4) and filtered on 0.22 μm sterile filter (Millipore, Bedford NMA, USA), was added to each well. After 3 h of incubation time at 37 °C in 5% carbon dioxide atmosphere, 50 μl of a solubilization solution (isopropanol, 10% Triton 100X, 0.1 N HCl) was added to each well to dissolve the dark blue formazan crystals. Absorbance was read at 570 nm on a microplate reader (SAFIRE, Tecan, Austria).

2.2.3. Lysis assays

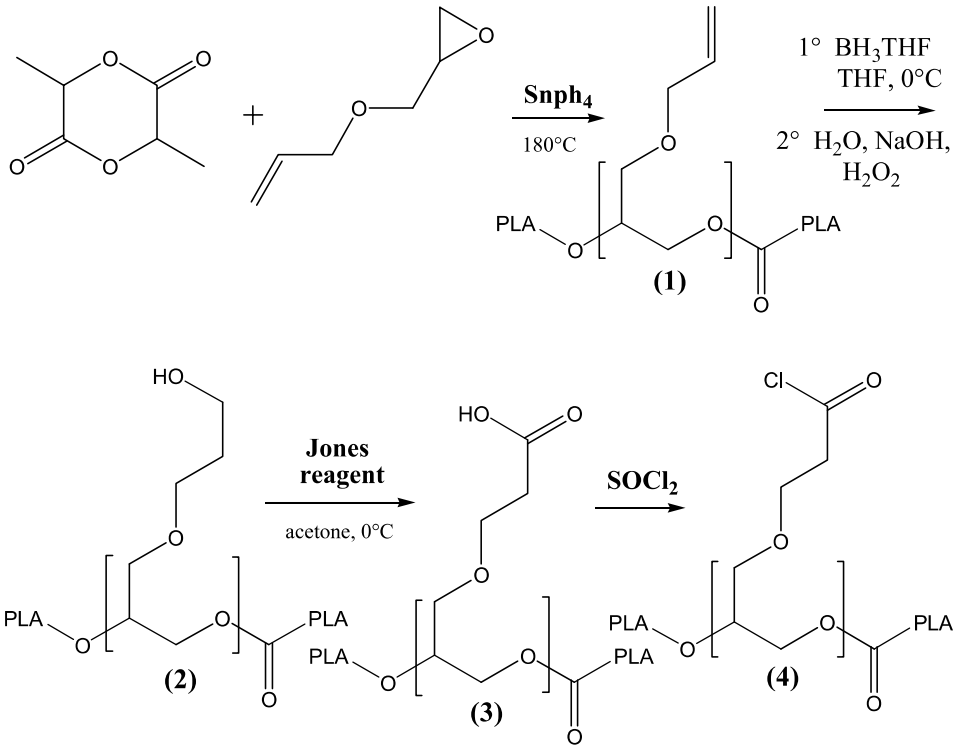
The presence of lactate dehydrogenase (LDH) in the supernatant obtained from proliferation assays was used as an indicator of cell lysis and death and determined using a commercial dosing kit (Sigma, St-Louis, MO, USA) used as directed by the manufacturer. Briefly, 5 μl of supernatant (taken up after the 24 h incubation time) were transferred to a new 96 well microplate and incubated with the reaction mixture for 30 min. The reaction was stopped with 0.1 N HCl. Microplates were read by using a microplate reader (SAFIRE, Tecan, Austria), at the wavelength of 450 nm (reference wavelength at 690 nm). Results were plotted in reference to positive control wells with 100% lysed cells.

2.3. Synthesis of PLA with 1% allyl pendant group (*1*)

To remove all trace of water from the reagents, dilactide (21.5 g, 149 mmol) and tetraphenyltin (7.7 mg, 0.02 mmol) were dissolved in toluene and then solvent was removed by rotary evaporation. The reagents were dried under vacuum in the reaction flask. The allyl glycidyl ether (0.36 mL, 3 mmol) was added and stirred at 180 °C under an inert atmosphere (argon) for 6 h. The mixture was cooled to ambient temperature. The crude reaction product was dissolved in 50 ml of ethyl acetate and then 100 ml of water was added. The organic solvent was removed by rotary evaporation, which resulted in precipitation of the polymer in water. The water was discarded to obtain the polymer. The mixture was washed three times and dried under vacuum to yield 20.8 g (95%) of white polymer (Scheme 1); IR (cm^{-1}) 2996, 2945, 1753, 1599, 1454, 1383, 1303, 1181, 1129, 1084, 1043, 694 and 569; ^{1}H NMR ($CDCl_3$) δ (ppm)1.5 (m, 3H, CH_3) and 5.2 (m, 1H, CH); ^{13}C NMR (DMSO-D_6) δ (ppm) 16 (CH_3), 69 (CH), 169 (C=O); Elemental analysis calculated: C, 50.2%; H, 5.6%, found: C, 49.7%; H, 6.0%; M_n 19,700 g/mol; M_w 35,000 g/mol.

2.4. Synthesis of PLA with 1% hydroxyl pendant group (*2*)

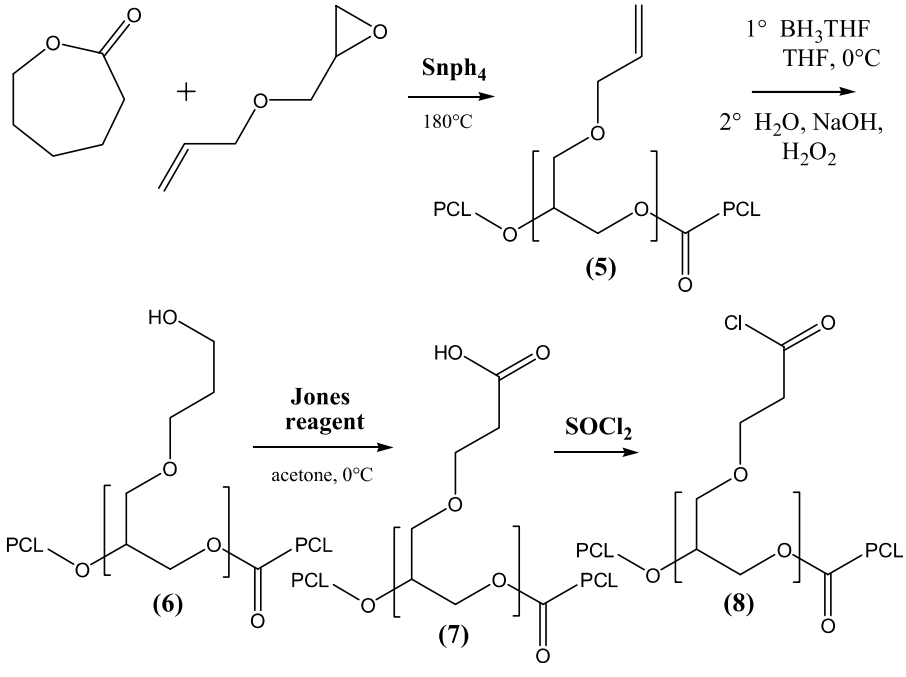
PLA with allyl pendant group (19.9 g, 2.7 mmol) was dissolved in 300 mL of THF at 0 °C, to which $BH_3\cdot$THF [1 M] (3 mL, 3 mmol) was added. The reaction was carried out for 2 h at 0 °C. To this reaction mixture, 25 ml of water, 25 ml of NaOH [3 N] and 25 ml of H_2O_2 (30%) were added. After 1 h, 300 ml of water was added. The solvent was removed by rotary evaporation. Then, the precipitated polymer (**2**) was purified with the same method used for compound (**1**). The polymer was dried under vacuum to give 14.9 g (75%) of white polymer (Scheme 1); IR (cm^{-1}) 2997, 1756, 1455, 1383, 1182, 1131, 1087, 1044, 871, 755, 689 and 569; ^{1}H NMR ($CDCl_3$) δ (ppm)1.5 (m, 3H, CH_3) and 5.2 (m, 1H, CH); ^{13}C NMR (DMSO-D_6) δ (ppm) 16 (CH_3), 69 (CH), 169 (C=O); elemental analysis calculated: C, 50.0%; H, 5.6%, found: C, 49.4%; H, 5.8%; M_n 22,000 g/mol, M_w 36,400 g/mol.



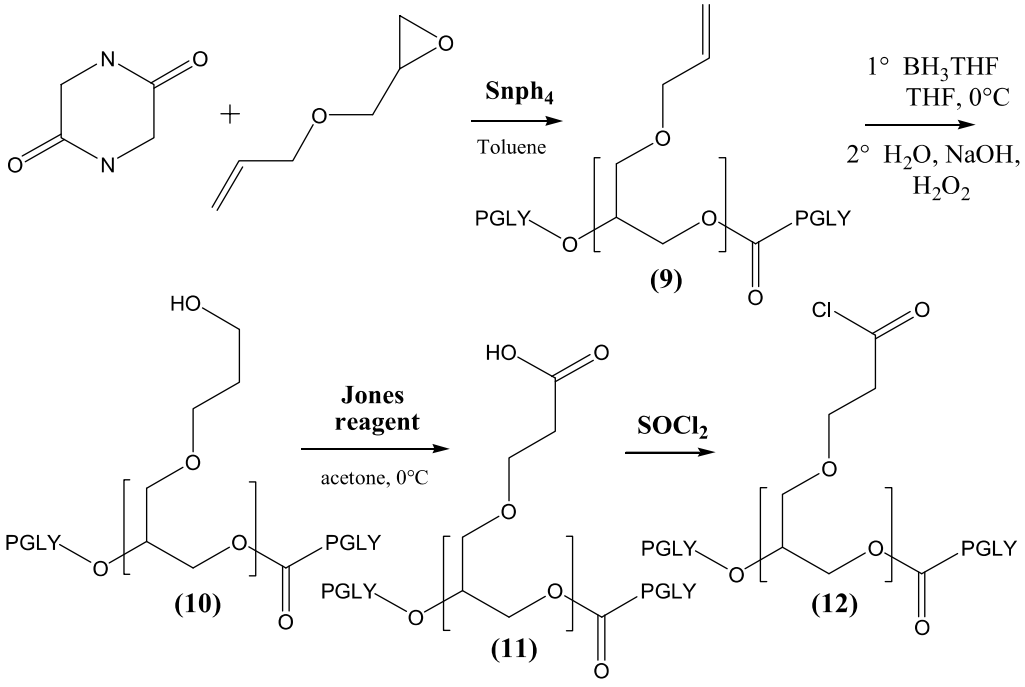
Scheme 1. Synthesis of grafted PLA.

*2.5. Synthesis of PLA with 1% carboxylic pendant group (**3**)*

PLA with hydroxyl pendant group (8.4 g, 1.15 mmol) was dissolved with 500 mL of acetone at 0 °C. Then Jones reagent was prepared; CrO_3 (0.23 g, 2.30 mmol), H_2SO_4 (0.23 mL) and water (0.70 mL) in this order and added to polymer solution. The mixture was stirred for 3 h at 0 °C and the reaction was stopped by addition of 30 mL of isopropanol. To this reaction mixture, 500 mL of HCl (1 N) was added. The solvent was removed by rotary evaporation. The precipitate was washed using the same method as for compound (**1**). The polymer was dried under vacuum to yield 7.8 g (93%) of white polymer (Scheme 1); IR (cm^{-1}) 2997, 1750, 1455, 1362, 1182, 1130, 1085, 1044, 754, 755, 688 and 570; ^1H NMR ($CDCl_3$) δ (ppm) 1.5 (m, 3H, CH_3) and 5.2 (m, 1H, CH); ^{13}C NMR (DMSO-D_6) δ (ppm) 16 (CH_3), 69 (CH), 169 (C=O); elemental analysis



Scheme 2. Synthesis of grafted PCL.



Scheme 3. Synthesis of grafted glycine polymers.

calculated: C, 50%; H, 5.6%, found: C, 49.5%; H, 6.0%; M_n 14,000 g/mol; M_w 31,200 g/mol.

2.6. Synthesis of PLA with 1% chloro alkoyl pendant group (4)

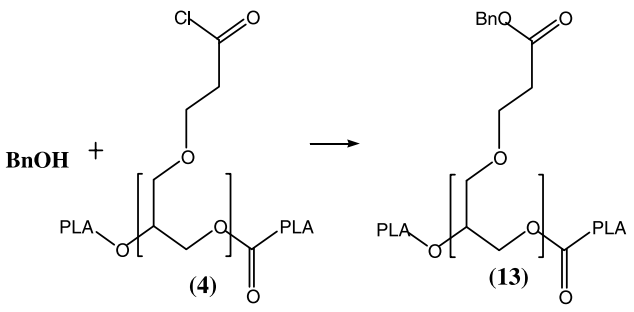
A reaction flask containing PLA with carboxylic pendant group (2 g, 0.27 mmol) in $SOCl_2$ (25 mL) was stirred at ambient temperature for 2 h. After this time, thionyl chloride was removed by rotary evaporation. The precipitate was collected to give 1.23 g (61%) of white polymer (Scheme 1); IR (KBr, cm^{-1}) 3050, 1750, 1454, 1363, 1350, 1225, 1100 and 867; ^{1}H NMR ($CDCl_3$) δ (ppm)1.5 (m, 3H, CH_3) and 5.2 (m, 1H, CH); ^{13}C NMR (DMSO-D_6) δ (ppm) 16 (CH_3), 69 (CH), 169 (C=O); elemental analysis calculated: C, 49.8%; H, 5.6%, found: C, 51.1%; H, 5.9%.

2.7. Synthesis of PCL with 20% allyl pendant group (5)

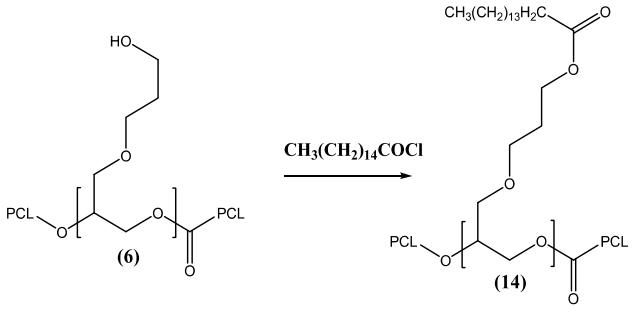
The polymer (**5**) was prepared similarly to (**1**) but caprolactone was used rather than dilactide. It is noteworthy that one mole of caprolactone gives one mole of caproic acid although one mole of dilactide gives two moles of lactic acid. The modified PCL was synthesized with 20% of grafting sites and yielded 85% of a polymer with waxy appearance (Scheme 2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.31–1.46 (m, 2H, CH_2), 1.57–1.72 (m, 4H, CH_2CH_2), 2.26–2.40 (m, 2H, CH_2CO), 4.01–4.11 (m, 2H, CH_2O); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 24.56 (CH_2), 25.51 (CH_2), 28.32 (CH_2), 34.10 (CH_2), 64.15 (CH_2O), 173.58 (CO); M_n 5,200 g/mol; M_w 12,100 g/mol.

2.8. Synthesis of PCL with hydroxyl pendant group 20% (6)

The polymer (**6**) was synthesized like (**2**) except using polymer (**5**) rather than (**1**) to yield 66% of polymer (Scheme 2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.31–1.46 (m, 2H, CH_2), 1.57–1.72 (m, 4H, CH_2CH_2), 2.26–2.40 (m, 2H, CH_2CO), 4.01–4.11 (m, 2H, CH_2O); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 24.56 (CH_2), 25.51 (CH_2), 28.32 (CH_2), 34.10 (CH_2), 64.15 (CH_2O), 173.58 (CO); M_n 4,100 g/mol; M_w 8,500 g/mol.



Scheme 4. Benzylation of polymer (**4**).



Scheme 5. Palmitoylation of polymer (**6**).

Table 1
T_g of grafted PLA

PLA grafted (%)	Allyl (°C)	Alcohol (°C)	Acid
1	27	28	31 °C
5	20	26	34 °C
20	13	16	24 °C
30	18	17	N/A

2.9. Synthesis of PCL with 20% carboxylic pendant group (7)

The polymer (**7**) was synthesized like (**3**) except using polymer (**6**) rather than (**2**) to yield 87% of polymer (Scheme 2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.31–1.46 (m, 2H, CH_2), 1.57–1.72 (m, 4H, CH_2CH_2), 2.26–2.40 (m, 2H, CH_2CO), 4.01–4.11 (m, 2H, CH_2O); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 24.56 (CH_2), 25.51 (CH_2), 28.32 (CH_2), 34.10 (CH_2), 64.15 (CH_2O), 173.58 (CO); M_n 2,500 g/mol; M_w 34,000 g/mol.

2.10. Synthesis of PCL with 20% chloro alkoyl pendant group (8)

The polymer (**8**) was synthesized like (**4**) except using polymer (**7**) rather than (**3**) to yield 60% of polymer (Scheme 2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.31–1.46 (m, 2H, CH_2), 1.57–1.72 (m, 4H, CH_2CH_2), 2.26–2.40 (m, 2H, CH_2CO), 4.01–4.11 (m, 2H, CH_2O); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 24.56 (CH_2), 25.51 (CH_2), 28.32 (CH_2), 34.10 (CH_2), 64.15 (CH_2O), 173.58 (CO).

2.11. Synthesis of PGLY with 2% allyl pendant group (9)

The polymer (**9**) was synthesized by mixing glycidine and allyl glycidyl ether in toluene. The reaction mixture was refluxed for 16 h. To wash the obtained gum, the mixture was dissolved in 50 mL of ethyl acetate and then 100 mL of water was added. The organic solvent was removed by extraction while the polymer was retrieved in the aqueous phase. The polymer mixture was washed three times and dried under vacuum. One mole of glycidine gave two moles of glycine as one mole of dilactide gave two moles of lactic acid. The modified glycine polymer with 2% substitution of pendant group was synthesized to obtain 50% yield of white polymer (Scheme 3); IR (cm^{-1}) 2987, 2876, 1749, 1682, 1507, 1469, 1436, 1338, 1250, 1215, 1074, 912, 829, 805, 669, 519 and 508; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.8 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ (ppm) 42 (CH_2), 165 (C=O); elemental analysis calculated: C, 42.9%; H, 5.4%; N, 23.6%, found: C, 42.3%; H, 5.3%; N, 24.3%.

2.12. Synthesis of PGLY with 2% hydroxyl pendant group (10)

The polymer (**10**) was synthesized as described for (**2**) except using polymer (**10**) rather than (**1**) to yield 69% of white polymer (Scheme 3); IR (cm^{-1}) 3672, 3035, 2984, 2871, 2830, 1680, 1540, 1516, 1466, 1436, 1335, 1071, 910, 830, 803, 687 and 520; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.0 (m, 2H, CH_2); ^{13}C NMR (400 MHz, DMSO-D_6) δ (ppm) 42 (CH_2), 165 (C=O); elemental analysis calculated: C, 42.8%; H, 5.4%; N, 23.5%, found: C, 42.3%; H, 5.4%; N, 24.5%.

2.13. Synthesis of PGLY with 2% carboxylic pendant group (11)

The polymer (**11**) was synthesized as described for (**3**) except using polymer (**10**) rather than (**2**) to yield 46% of white polymer (Scheme 3); IR (cm^{-1}) 3672, 3648, 2987, 2868, 2780, 1749, 1699, 1539, 1513, 1470, 1435, 1366, 1337, 1219, 1073, 912, 838, 805, 687 and 520; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.0 (m, 2H, CH_2); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 42 (CH_2), 165 (C=O).

2.14. Synthesis of PGLY with chloro alkoyl pendant group 2% (12)

The polymer (**12**) was synthesized similar to (**4**) except using polymer (**11**) rather than (**3**) to yield 98% of white polymer (Scheme 3). This compound was used directly for benzylation; IR (cm^{-1}) 2994, 2870, 1752, 1510, 1452, 1361, 1269, 1186, 1128, 1098, 1046, 913, 867, 736 and 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.0 (m, 2H, CH_2); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 42 (CH_2), 165 (C=O); M_n 4,864 g/mol; M_w 6,762 g/mol.

2.15. Benzylation (13)

To 95 mg (0.01 mmol) of polymer (**4**) in a chloroform solution, 100 mL of benzylic alcohol, 100 mL of pyridine and 10 mg (0.08 mmol) of DMAP were added. The solution was stirred for 3 h and then neutralized by 1 N HCl. The organic layer was collected and evaporated successively to obtain 100 mg of (**13**). The same protocol was used for benzylation of

Table 2
Molecular weight of PLA grafted by SEC with standard condition

PLA grafted (%)	Allyl			Alcohol			Acid		
	M_n[a]	M_w[a]	M_w/M_n[a]	M_n[a]	M_w[a]	M_w/M_n[a]	M_n[a]	M_w[a]	M_w/M_n[a]
1	19,700	35,000	1.8	22,000	36,400	1.7	14,000	31,200	2.2
5	7200	14,900	2.1	3300	4300	1.3	5200	9200	1.7
20	5300	11,400	2.1	2800	4400	1.6	2900	4700	1.6
30	3300	5300	1.6	2200	3200	1.5	N/A		

[a] (g/mol).

Table 3
Molecular weight of PLA grafted by SEC with optimized conditions

PLA grafted (%)	Allyl			Alcohol			Acid		
	M_n[a]	M_w[a]	M_w/M_n[a]	M_n[a]	M_w[a]	M_w/M_n[a]	M_n[a]	M_w[a]	M_w/M_n[a]
1	35,800	59,800	1.7	32,600	55,700	1.7	35,400	59,900	1.7

[a] (g/mol).

polyglycine-*co*-ether (**12**) with a difference in the purification procedure. We dialysed the mixture solution, firstly with a solution of isopropanol 70% and secondly with water to remove benzylic alcohol. The solution was then freeze-dried (Scheme 4); IR (cm^{-1}) 2994, 2870, 1752, 1510, 1452, 1361, 1269, 1186, 1128, 1088, 1046, 913, 867, 736 and 700; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.8 (m, 127H, CH_2), 7.4 (m, 5H, 5(CH benzylic)); M_n 6,000 g/mol; M_w 9,200 g/mol.

2.16. Palmitoylation (*14*)

To a solution of 5 g (0.4 mmol) of polymer (**6**) in 50 mL pyridine, 4.67 g (0.017 mol) of palmitoyl chloride was added. The solution was stirred for 3h and then, 10 mL of water was added. The solvent was evaporated and the product was crystallized in diethyl ether to obtain 3.2 g (64% yield) of (**14**) (Scheme 5); M_n 8,800 g/mol; M_w 13,500 g/mol.

3. Results and discussion

3.1. General synthesis approach of grafted polylactide

The approach was to synthesize polyesters having reactive groups on branched structures. The dilactide is well described and industrially used [10]. Allyl glycidyl ether (AGE) was added to obtain a PLA backbone with pendant allyl functions (**1**). This molecule, with epoxy function, gets attached to the polymer backbone after ring opening polymerization. The allyl function does not react if the reaction is carried without an inert atmosphere. The presence of oxygen can lead to radical polymerization of allyl function. On the other side, the polymerization of PLA cannot be conducted in presence of air without significant degradation.

The polymer (**1**) is an intermediate to obtain various chemical functions because the allyl function can be easily modified. For example, by hydroboration, we have obtained a primary alcohol (**2**) and by oxidation of alcohol with Jones mixture, we have obtained the corresponding acid (**3**).

The synthesis was carried out to obtain PLA grafted with allyl, hydroxyl and carboxyl functions at 1, 5, 20 and 30% (moles/moles of respective monomers). At 30% grafting density, the obtained polymer was very viscous. The hydrophilic character of the polymer makes the purification difficult. For this reason, it was not possible to carry out the oxidation of the corresponding alcohol to carboxylic acid.

3.1.1. Thermal studies of grafted PLA

The glass transition temperature (T_g) of different polymers is shown in Table 1. T_g is accompanied by long-range molecular motion, greater rotational freedom and consequently more segmental motion of the chains. The increased motion increases the space between the atoms which itself, increases the specific volume [11]. T_g is influenced by different factors such as the functional groups present, polarity and molecular weight of the polymer. Adding olefin to polymer (**1**) decreased

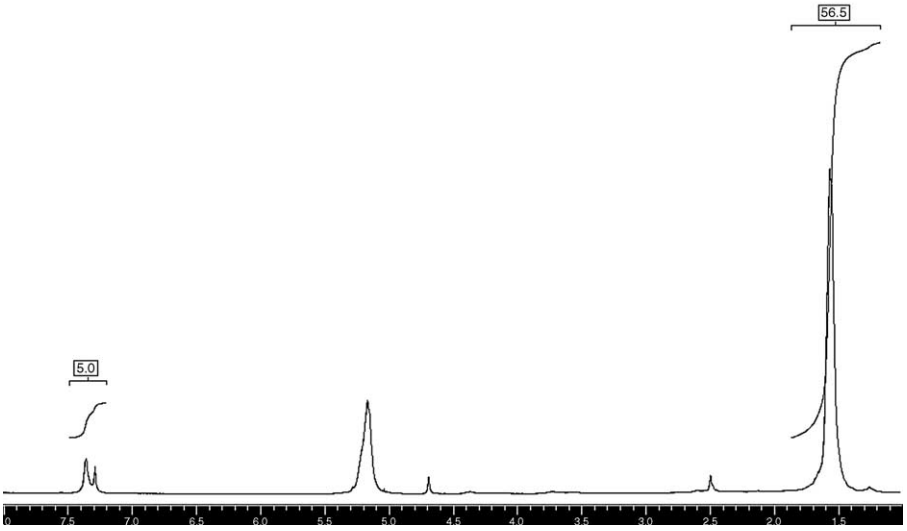


Fig. 1. ^1H NMR spectra of the polymer (**13**). The signal at 7.4 ppm represents the five aromatic protons of the benzyl function. The signal at 1.5 ppm represents the methyl protons of lactic acid. The ratio of the integration show 5.0% of benzyl grafted.

Table 4
Molecular weight by SEC of grafted PCL

PCL grafted (%)	Allyl			Alcohol			Acid		
	M_n[a]	M_w[a]	M_w/M_n[a]	M_n[a]	M_w[a]	M_w/M_n[a]	M_n[a]	M_w[a]	M_w/M_n[a]
1	5200	12,100	2.3	4100	8500	2.0	2500	3900	1.6

[a] (g/mol).

T_g because it minimizes the interaction between the chains of the polymers. Increasing grafting density of alcohol function also decreased T_g because of increased hydrophilic character of these polymers. On the other hand, the conversion of olefin to alcohol (**2**) increased T_g. This increase is probably due to the increased polarity of OH bond as compared to that of the allyl group. The more polar group led to higher T_g because of increased van der Waals interactions and hydrogen bonding [12]. The T_g increased again when the alcohol is converted into carboxylic acid (**3**) probably for the same reason. Finally, the T_g depends on the polymer molecular weight and as the molecular weight increased, T_g increased [13].

3.1.2. SEC analysis of grafted PLA

The SEC analysis results of branched polymers measured against polystyrene standard are shown in Table 2. We observed a general trend for a decrease of molecular weight in number (M_n) and in weight (M_w) during the synthesis. The increase of allyl glycidyl ether content decreased the molecular weight. Likewise, M_n and M_w decreased upon oxidation of the allylic group. This could be explained by the hard conditions in oxidation step that could lead to hydrolysis of ester links.

The residual moisture present and temperature fluctuation during the polymerization and excessive amounts of $NaOH/H_2O_2$ used during oxidation step might have decreased the molecular weight. These factors were optimized to increase the molecular weight of the polymers. Indeed, we could minimize the decrease in the molecular weight in the subsequent step of hydroboration/oxidation by decreasing the amount of $NaOH/H_2O_2$. The new optimized conditions were 1.5 equivalent of NaOH (3 N) and H_2O_2 (30%). With constant temperature throughout the polymerization, adequate drying of the starting materials and polymers at each step and milder acidic and basic oxidation conditions, we could obtain grafted PLA with M_w of 60,000 g/mol (Table 3) with no change in the polydispersity. The effect of the catalyst concentration and time of polymerization on M_w of polyesters have been investigated by different research groups [14]. It has also been shown in our laboratory that the temperature of reaction (step 1) and the time of polymerization were the most critical factors affecting the molecular weight of the polymers as compared to the other factors changing the catalyst and its concentration (data not shown).

Transesterification and hydrolysis can contribute to the M_w decrease shown in Table 2. Using milder acidic and basic oxidation conditions, we could minimize the effect of hydrolysis on M_w Table 3 shows relatively constant M_w when reaction was carried out in optimized conditions. Probably, the rate of transesterification was also minimized under these conditions.

3.1.3. Structural analysis by NMR of PLA grafted

The allyl glycidyl ether has one chiral carbon, which made NMR analysis difficult because each proton is diastereotopic. The signals for these protons are numerous and with a relatively low intensity compared to protons located on the PLA chain. Even when there is 20% substitution, it was not possible to distinguish correctly these protons. In ^{13}C NMR

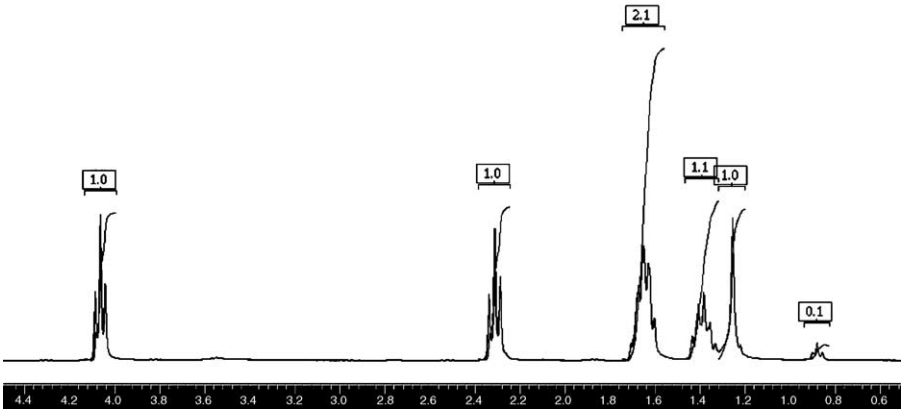


Fig. 2. ^1H NMR spectra of the polymer (**14**). The characteristic signal of PCL can be observed with the characteristic signal of palmitate. The signal at 1.2–1.3 ppm represents the twelve CH_2 of palmitate and the signal at 0.8–0.9 ppm is due to the CH_3 of palmitate.

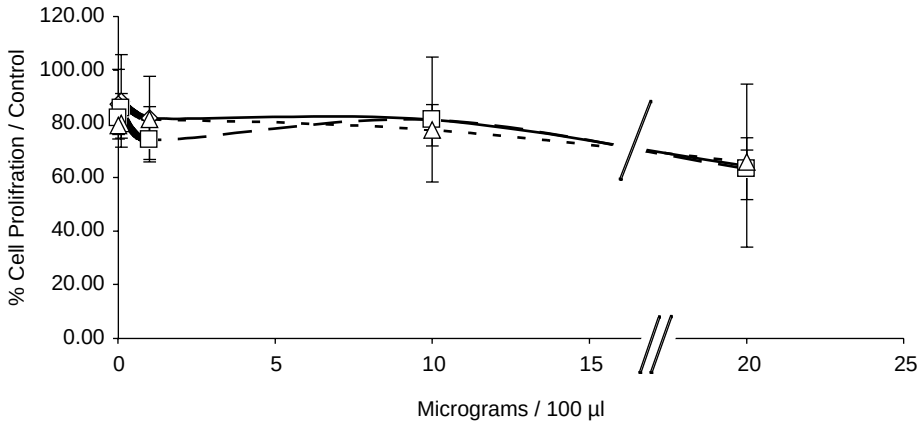


Fig. 3. MTT cell proliferation assay. PLA with 1% allyl pendant group (◇), PLA with 1% hydroxyl pendant group (□) and PLA with 1% carboxylic pendant group (△).

also, no signals were observed. However, the signals of ^{1}H and ^{13}C were clear for the lactide part of the polymer.

An experiment was conducted to confirm the possibility to graft other molecules by ester bond on this polymer backbone. The benzyl alcohol was selected to be grafted on the polymer (**4**). Benzyl alcohol gives characteristic signals in NMR for the aromatic protons that are not present in the spectrum of polymeric backbone (**3**). Other advantage of benzyl alcohol is that the excess can be easily washed out because it is miscible with water. The Scheme 4 shows the benzylation and the Fig. 1 shows the NMR of PLA grafted with 5% benzyl group.

The benzylation of the grafted domain confirms the percentage of pendant groups on the polymer. This study also proved that the reactivity of dilactide was as good as that of the epoxy. The branched parts are not only present at the end but all along the polymer backbone. This also means, a versatile branched polymers based on PLA could be successfully synthesized.

3.2. General synthetic approach of grafted polycaprolactone

The previous synthesis with dilactide could be repeated with other starting monomer to obtain copolymers of pharmaceutical interest. In order to synthesize grafted PCL, we used caprolactone in place of dilactide. The same synthetic procedure was used. The synthesis of grafted PCL was done with 20% substitution.

3.2.1. Molecular weight of PCL

w?>The molecular weights of polymer (**5**), (**6**) and (**7**) substituted with 20% pendant groups as determined by SEC are shown in Table 4. As in case of grafted PLA, we observed that the molecular weight of grafted PCL also decreased at each step of the synthesis. Polydispersity showed the same trend. As explained earlier, it may be due to the harsh conditions of oxidation. Hydrolysis could be minimized by changing conditions.

3.2.2. Structural analysis of grafted PCL by NMR

Similar to PLA with pendant carboxylic function (**3**), a coupling experiment was performed on (**6**). Palmitoyl chloride was grafted on PCL having pendant alcoholic function (**6**) (Scheme 5). The polymer obtained (**14**) was insoluble in ether but the palmitic acid is soluble, therefore, the excess of reagent could be removed.

The interpretation of NMR spectrum (Fig. 2) of polymer (**14**) is less straight forward than for the polymer (**13**) because, with grafted benzyl group, the aromatic signal is strong. For polymer (**14**), the signal observed at 1.2–1.3 ppm corresponds to the twelve hydrogens of the palmitate CH_2. The signal at 0.8–0.9 ppm is due to the palmitate CH_3. The palmitate CH_2COO protons signal is mixed with that from CH_2 COO protons in PCL. As there is peaks overlapping between 1.2 and 1.4 ppm, integration of the signal is not possible. It is however possible to evaluate a ratio of 1 part of palmitate for twelve part of caproic, for a substitution of 8%. This value is less than the expected 20%. Two reasons can explain this difference: either the esterification reaction was not complete or the incorporation of AGE was not quantitative. The palmitoyl chloride was added in excess. This suggests that the AGE was not regularly inserted in the polymeric backbone due to the high reactivity of the ring with seven atoms of caprolactone. Opening of the cyclic ring of caprolactone needs less energy ($\Delta_f H_{ion} = 629$ kJ/mol) than the ring opening of AGE ($\Delta_f H_{ion} = 966$ kJ/mol) [15]. Less reactive AGE has to compete with caprolactone for polymerization. Therefore, AGE has been

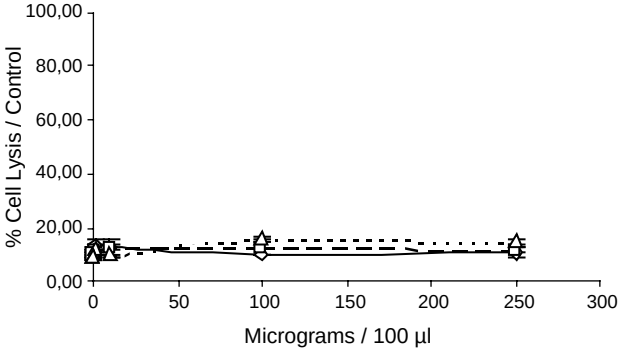


Fig. 4. LDH cell lysis assay. PLA with 1% allyl pendant group (◇), PLA with 1% hydroxyl pendant group (□) and PLA with 1% carboxylic pendant group (△).

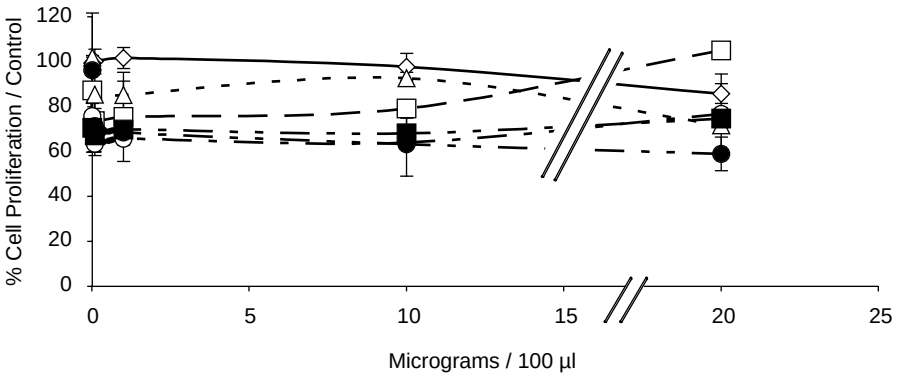


Fig. 5. Cytocompatibility of carboxyl PLA, PCL and PGLY as measured with MTT cell proliferation assay. PLA with 1% carboxy pendant group (◇), PLA with 5% carboxyl pendant group (□) and PLA with 10% carboxylic pendant group (△). PLA with 20% carboxyl pendant groups (○); PCL with 20% carboxyl group (■); PGLY with 2% carboxyl pendant group (●).

inserted in the chain but with low efficacy or it has been inserted at the end of the chain and stopped the condensation.

3.3. General synthesis approach for grafted PGLY

Polyglycine-*co*-ether was synthesized as described in Section 2 above. As the decomposition of glycidine occurred before its fusion over 300 °C, it was not possible to proceed with the polymerization by fusion as for PLA and PCL. Therefore, the polymerization was carried out in toluene solution. This is the only difference between synthesis of grafted PLA, PCL and PGLY. PGLY was grafted with 2% pendant groups.

3.3.1. Molecular weight of PGLY

Due to the poor solubility of polyglycine in chloroform and in most solvents, the measurement of molecular weight by SEC could be done only on PGLY (**12**) and PGLY-grafted with benzyl group. M_n and M_w of 4900 and 6800 g/mol, respectively, were observed for PGLY (**12**). The benzyl grafted PGLY showed M_n and M_w of 6100 and 9200 g/mol, respectively.

3.3.2. Structural analysis of grafted PGLY by NMR

In the same way as benzyl grafted on PLA, the calculated percentage of grafted blocks was found to be 1.6% (for an expected 2% grafting density). The NMR spectra confirmed the proposed structure. The benzylation of grafted domain showed that the reactivity of glycidine is as good as the reactivity of epoxy of allyl glycidyle ether.

3.3.3. Cytocompatibility

Cytocompatibility of polymers (**1**), (**2**) and (**3**) was determined as described in the experimental section. MTT colorimetric assay was used for the assessment of cell proliferation since it measures tetrazolium ring cleavage by active mitochondria of the living cells [9], whereas, level of lactate dehydrogenase (LDH) in cell supernatant indicates level of cells lysis. The MTT assay showed no effect on cell proliferation for all the polymers at concentration lower than 1 mg/mL (Figs. 3 and 4). The presence of the pendant groups with allyl, alcohol or carboxylic functions did not affect the compatibility of PLA as seen for PLA with 1% pendant groups (Fig. 3). Moreover, the LDH assay showed no significant cell death in the complete range of the concentrations studied for PLA with 1% pendant group (Fig. 4). Similar results for MTT and LDH assays were obtained with 5, 10 and 20% PLA pendant groups (data not shown). Fig. 5 shows the results of cell proliferation for different polymers (PLA, PCL and PGLY) with carboxylic pendant group at various grafting densities. The results showed the constant proliferation for all the polymers tested. The slight increase at the highest polymer concentration may be due to polymer absorbance. These in vitro results are preliminary but are encouraging for further toxicology studies.

4. Conclusion

A large variety of new biopolymers having pendant groups were synthesized by a versatile method. Moreover, the ester linkage of the pendant group is degradable by hydrolysis. The PLA has been chosen as starting polymeric backbone; however, it was possible to use other polymeric backbones as proved successfully by use of PCL and PGLY. The use of allyl glycidyl ether for grafting part allows easy modifications. The inert character of alkene group under the polymerization conditions is the principal advantage. So, the ring opening polymerization of lactide, caprolactone or glycidine and epoxy using tin as a catalyst provided a family of new polymers which could be grafted with various bioactive ligands through an ester link and could be used for drug targeting. We recently showed that DNA can form complexes with PLA with grafted salen groups [7]. Methoxy-PEG grafted on PLA has been already used successfully in our laboratory to prepare stealth nanoparticles [8]. Similarly, bioadhesive polymer grafted with a ligand specific to E-selectin has been synthesized in the same manner and its application will be discussed in an upcoming paper.

Acknowledgements

The authors wish to thank NSERC for the financial support.

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