10th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-10). 1-30 November 2006. http://www.usc.es/congresos/ecsoc/10/ECSOC10.htm & http://www.mdpi.org/ecsoc-10/

[f002]

Formulation of modified microspheres based on galactosylated lactic acid polymers

R.Kassab [(a)], H. Fessi [(b)], H. Parrot-Lopez [(c)].

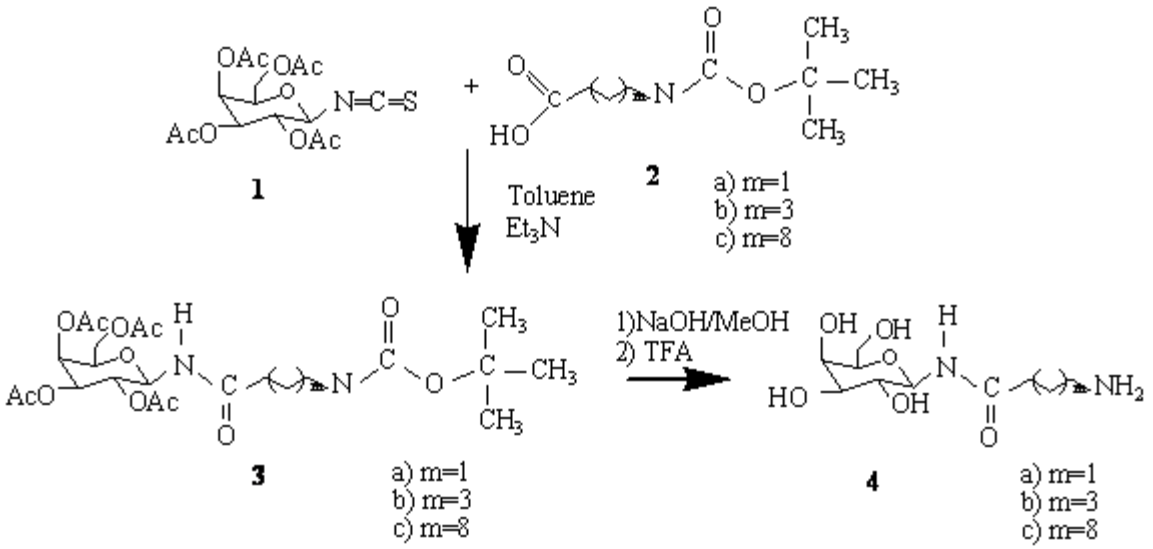
a) *University of Balamand, Faculty of Sciences, P.O.Box :100-Tripoli, Lebanon*
b) *Laboratoire de Genie pharmaceutique UMR 5007 CNRS, Faculte de pharmacie, 8 avenue Rockfeller, 69373 Lyon cedex, France*
c) *UMR 5078 CNRS, Université Claude Bernard, bat 305, 43bd du 11 Nov 1918.69622 Villeurbanne Cedex, France*

Abstract

A new series of galactosyl-derived polymers has been used for the preparation of microspheres. The strategy is based on the modification of the terminal carboxylic group L-PLA by coupling to a galactosyl antenna in the presence of the peptide coupling agents : DCC/HOBT . the degree of functionalisation varies between 60 and 70%, and antenna density between 1.74 and 2.78. In an effort to develop a new way of drug delivery, especially for polymeric antifungal molecules, we have incorporated amphotericin B (AmB) into biodegradable galactosylated poly(L-lactic acid) L-PLA . These drug carriers were prepared by solvent evaporation method using an oil/water (o/w) emulsion. The ratio of galactosylated microspheres was 7.14 mg for L-PLA (encapsulation rate 45% of mole). In our yeast model, drug release depend on three factors : i) presence of galactosylated antennae, ii) length of galactosyl antenna and iii) nature of the polymer . These novel functionalized microspheres could be required for the delivering of therapeutic agents according to their recognition to specific cells .

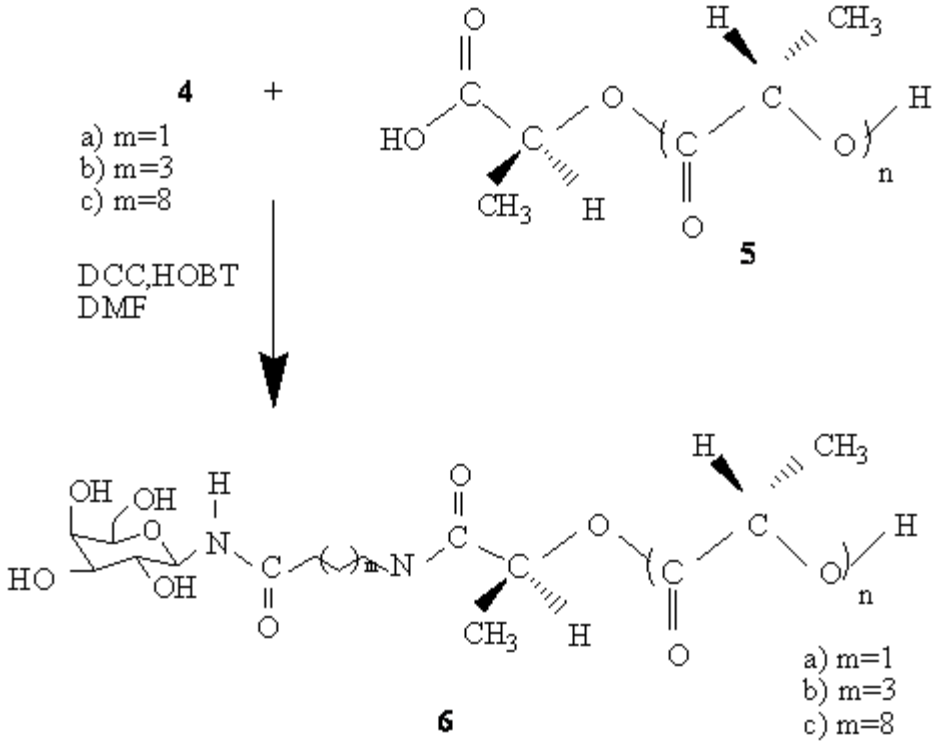
Homopolymers and copolymers based on L-lactic acid, and glycolic acid have received interest in the medical and pharmaceutical field because of their degradability and low toxicity . In order to enhance the process recognition by the lectis (KbCWL), we developed a method consisting of coupling a saccharide antennae onto lactic acid polymers . To demonstrate the utility of these novel carrier systems, we investigated the possibility of targeting drugs using specifically designed particulate carriers. Microspheres and nanospheres were envisaged as a site specific drug delivery system . In this study, a solvent evaporation technique of water/oil emulsion was adopted for microparticle preparation .

The galactosyl antennae **4a-c** were synthesized from the corresponding terta-O-acetyl-b-D-galactosyl isothiocyanate **1** [(1)] in two steps (Scheme 1). Compound **1** (2.28 mmol) was condensed with the N-protected amino acid derivative N-Boc-4-aminobutyric acid **2a**, N-Boc-6-amino hexanoic acid **2b** or N-Boc aminoundecanoic acid **2c** (3.20 mmol) in dry toluene (14 mL) in the presence of triethyl amine (0.23 mmol) for 2 days at 20 C . After purification by flash chromatography on silica gel (toluene : acetone 8:2), complete deprotection of the derivatives **3a, 3b, 3c** is achieved using methanolic sodium hydroxide (1M) and trifluoroacetic acid (TFA) .



Scheme 1. Synthesis of galactosyl derivatives

The synthetic strategy consists of the modification of the terminal L-PLA (Mw = 73000)[(2)] carboxylic group by coupling to galactosylated antennae (Scheme 2). the quantity of antennae coupled is 1.74 mg for **6a**, 2.04 mg for **6b,** 2.78 mg for **6c,** the figures in parentheses being weights per 100 mg . The degree of functionalisation thus varies between 60 and 70%, with density of antennae being 1.74, 2.04, and 2.78 for **6a, 6b, 6c** respectively .



Scheme 2. Functionalisation of L-PLA

NMR analysis at 500 MHz of functionalized PLA is difficult due to the amphiphilic nature of the polymer . Thus, in $CDCl_3$ the galactosyl antennae are folded back into the interior of the polymer and are not observed in the NMR experiment. By modifying the hydrophobic/hydrophilic balance of the solvent mixture, for example in $CDCl_3/CD_3OD$ (70/30), ^{1}HNMR can be used to detect the galactosyl antennae .

The signals arising from the polymers occur as broad doublet at 1.4 ppm (CH_3) and a quadruplet at 5.1 ppm (CH) .

But the best results are obtained in pyridine-d5 (Table 1) , and a COSY experiment gives the proof that the antennae are linked covalently with the L-PLA. For **6c**, the NH amide proton of gal detected at 9.43 ppm (d, J=9.6 Hz) is correlated to the H-1 gal proton at 5.9 ppm (t, J= 9.5 Hz) .

Table 1
1H NMR of the L-PLA functionalized in C_5D_5N

Products	H-1	H-2	H-3	H-4	H-5	H-6,6'	NH-gal	NH-PLA
6a	5.6	4.3	4.2	4.7	4.1	4.5	9.3	7.9
6b	5.6	3.95	4.3	4.4	4.25	4.2	9.1	8.3
6c	5.9	4.5	4.16	4.58	4.11	4.36	9.43	8.2

The NH amide signal characteristic for covalent linkage onto Gal antenna and L-PLA is observed at 8.2 ppm. This signal is correlated to the CH_2 terminal of the spacer at 3.4 ppm.

Microspheres derived from these new amphiphilic polymers are fabricated by the solvent evaporation method [(3)]. L-PLA gal derivatives (0.5g) **6a,6b,6c** was dissolved in CH_2Cl_2 (20 mL) . This organic phase was added to an aqueous phase (250 mL) containing 4g of Tween 80 (surfactant) . The resulting biphasic system was stirred for 8-9h at 700 rpm . The microspheres were filtered, washed with water and finally dried under reduced pressure . Comparison of the blank microspheres (204 mm diameter) with those possessing the galactosyl antenna (207 mm for **6a**, 210 mm for **6b** and 271 mm for **6c**) shows clearly that the antennae do not strongly modify the properties of L-PLA for the formation of such structures .

In conclusion, by ^{1}HNMR spectroscopy the functionalisation of the terminal carboxylic group of the L-PLA by covalent linkage of galactosylated antennae has been confirmed. Given the amphiphilic character of these modified polymers, it was possible to formulate microspheres having 207 mm to 271 mm diameter .

References

1) kassab, R.; Felix, C.; Parrot-Lopez, H; Bonaly, R.; Tetrahedron lett. 1997, 38, 7555-7558
2) L-PLA 100 (Mw = 73000) was obtained from PHUSIS (Grenoble, France) Ring-opening polymerization of L-Lactide was conducted using metallic Zn as catalyst (0.05%) at 140 C
3) Kwong, A.K.; Chou, S.; Sun, A.M.; Sefton, M.V. J.Controlled Release 1986, 4, 47-62