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Synthesis and characterisation of poly (L-lactic acid) galactosyl derivatives; access to functionalised microspheres

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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 (73.000) 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. The characterisations of the new products were carried out using ^{1}H NMR, gel permeation chromatography, and acid base titration; the size of the functionalised microspheres was determined to be 210–270 μm by DLS. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: poly L-lactic acid; galactosyl antennae; covalent linkage; microspheres.

Biodegradable polymeric drug delivery systems possess several advantages compared to conventional drug therapies.[1,2] The use of poly L-lactic acid (L-PLA), a non hydrosoluble biodegradable polymer, in controlling the release of drugs has become important in pharmaceutical applications. Encapsulation of drugs in microspheres based on PLA has already been investigated by several groups.[3–7] However, the main problem in using conventional microspheres is that they are rapidly eliminated by the reticulo-endothelial system or macrophages. One method to circumvent this problem of macrophage lies in the formation of large microspheres. A second method lies in the targeting of specific bio-receptors via surface modification involving coupling the carbohydrate antennae to the vectors. Numerous biological recognition processes are regulated by protein–carbohydrate interactions.[8] To this end, Roy et al. described the synthesis and applications of neoglycoconjugates,[9] especially the synthesis of glycopolymers, by the incorporation of simple and complex oligosaccharide sequences into polymer.[10] In the case of graft polymerisation, the desired carbohydrate is directly coupled into polymers having reactive

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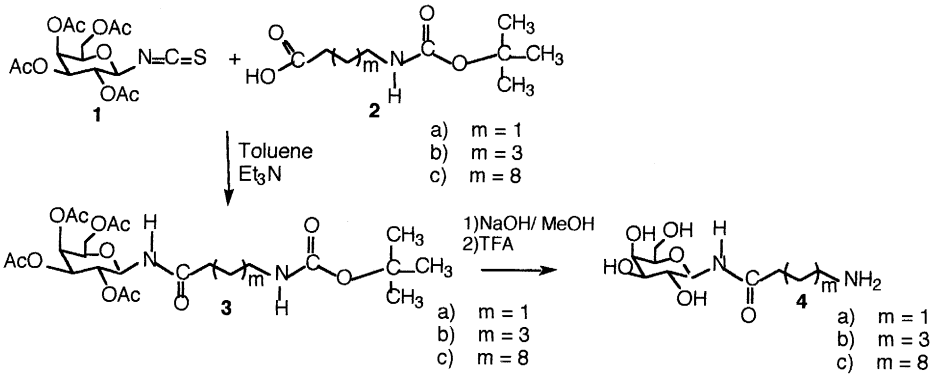
functionalities: polyacrylamides,[11] polyacrylates,[11] poly-L-glutamic acid[12] and poly (acrylamide-co-allylamine).[13]

In this context, the coupling of carbohydrate antennae to the L-PLA and this microsphere organisation provided a new class of vector for molecular recognition and controlled the time-release of the drugs, thus presenting the advantages both of large size and of biorecognition. In this paper we report the synthesis and characterisation of novel poly (L-lactic acid) galactosyl derivatives and their formation of microspheres. The synthetic strategy consists in the modification of the terminal L-PLA carboxylic group by coupling to a galactosyl antenna. We have previously demonstrated that coupling galactosyl antennae to cyclodextrins leads to good molecular recognition by the *Kluveromyces bulgaricus* cell wall lectins (Kb CWL).[14]

The galactosyl antennae **4a**–**c** were synthesised from the corresponding tetra-*O*-acetyl-β-D-galactosyl isothiocyanate[15] **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-aminohexanoic acid **2b** or *N*-Boc-11-aminoundecanoic acid **2c** (3.20 mmol) in dry toluene (14 mL) in the presence of triethylamine (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** or **3c**[16] is achieved using methanolic sodium hydroxide (1 M) and trifluoroacetic acid (TFA).



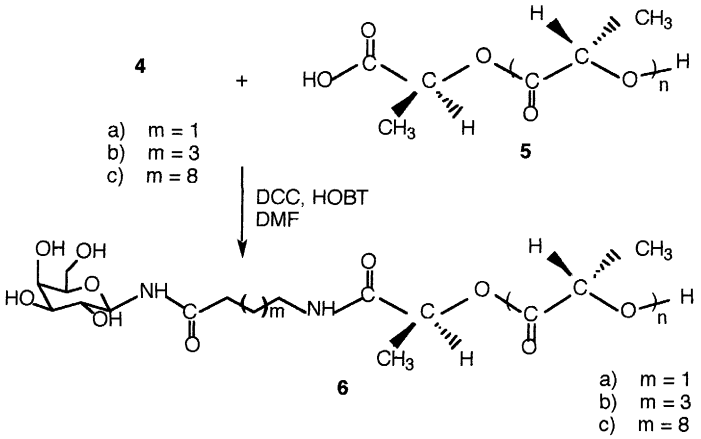
Scheme 1. Synthesis of galactosyl derivatives

The L-PLA derivatives **6a**, **6b** and **6c** were synthesised, respectively, by condensation at room temperature of β-amido-D-galactopyranosides **4a**, **4b** or **4c**[17] (2 equiv.) with L-PLA[18]**5** (Mw: 73.000) (1 g, 2.10^{-4} function COOH) in dry DMF/CH_2Cl_2 mixture (20 mL) using the standard peptide coupling reagents: dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBT 2 equiv./2 equiv.) (Scheme 2).

The characterisation of the L-PLA Gal derivatives was carried out according to three methods:

(i) *Quantification of the galactosyl antennae.* Before coupling acid–base titration using 0.01N methanolic sodium solution against bromothymol blue as indicator shows the presence of 10^{-5} carboxylic acid functions per 100 mg of L-PLA dissolved in 20 mL of mixture CH_2Cl_2/CH_3OH (1/1). Titration of the L-PLA Gal derivatives **6a**, **6b** and **6c** shows, respectively, the presence of 3.5 10^{-6}, 3.0 10^{-6} and 4.0 10^{-6} carboxylic acid functions per 100 mg. The quantity of antennae functions coupled are thus 6.5 10^{-6} moles (1.74 mg) for **6a**, 7.0 10^{-6} moles (2.04 mg) for **6b** and 6.0 10^{-6} moles (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** and **6c**, respectively.

(ii) *^1H NMR spectra.* NMR analysis at 500 MHz of functionalised PLA is difficult due to the amphiphilic nature of the polymer. Thus, in $CDCl_3$ the galactosyl antennae are folded back into



Scheme 2. Functionalisation of L-PLA

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), ^1H NMR can be used to detect the galactosyl antennae.[19]

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-d_5 (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 functionalised 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.

(iii) *Measurement of molecular weight*. The molecular weight of derivatives **6a, 6b** and **6c** was measured by gel permeation chromatography (GPC).[20] The number-average molecular weight (Mn), the weight-average molecular weight (Mw) and the polydispersity (I=Mw/Mn) of polymers were calibrated using monodisperse polystyrene as the standard. The variation in Mw may be explained by the functionalisation of the polymer. The increase in Mn is possibly due to hydrolysis during the analytical process.

Microspheres derived from these new amphiphilic polymers are fabricated by the 'solvent evaporation' method.[21] L-PLA Gal derivative (0.5 g) **6a**, **6b** or **6c** was dissolved in CH_2Cl_2 (20 mL). This organic phase was added to an aqueous phase (250 mL) containing 4 g of Tween 80 (surfactant). The resulting biphasic system was stirred for 8–9 h at 700 rpm. The microspheres were filtered, washed with water and finally dried under reduced pressure. The size of the microspheres was determined using dynamic light scattering (DLS). Comparison of the blank microspheres (204 μm diameter) with those possessing the galactosyl antenna (207 μm for **6a**, 210 μm for **6b** and 271

μm 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}H NMR 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 μm to 271 μm diameter. A preliminary study carried out on the microsphere recognition by galactose specific lectins confirms the activity of the galactosyl antennae on the surface of the microspheres and anticipates a promising future for the use of these L-PLA functionalised microspheres in the field of vectorisation of bioactive molecules.

Acknowledgements

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16. **1*N*-(*tert*-Butoxycarbonyl-4-aminobutanoyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylamine 3a**. ^{1}H NMR COSY δH (ppm) $CDCl_3$: 1.48 (s, 9H, CH_3); 1.79 (t, 2H, CH_2 βCO, J=7 Hz); 2.07; 2.14; 2.16; 2.17 (m, 12H, OAc); 2.23–2.24 (m, 2H, CH_2 α/C=O); 3.11–3.14 (q, 2H, CH_2 α/NH, J=7 Hz); 4.11–4.14 (m, H6 and H6′); 4.25 (m, 1H, H-5); 4.62 (m, 1H, NH-C=O); 5.17 (dd, 1H, $J_{2,3}$=9.5 Hz; $J_{2,1}$=9 Hz, H-2); 5.21 (dd, 1H, $J_{3,2}$=9.5 Hz; $J_{3,4}$=3.8 Hz, H-3); 5.25 (dd, 1H, $J_{H1,NH}$=10 Hz; $J_{1,2}$=9 Hz, H-1); 5.50 (dd, 1H, $J_{4,3}$=3.8 Hz; $J_{4,5}$=1 Hz, H-4); 6.62 (d, 1H, $J_{NH,H1}$=10 Hz, NHGal). R_f: 0.163; yield: 56%; mp: 77–78°C; ES-MS: *m/z*, 555.3[M+Na]$^+$, 571.4[M+K]$^+$. **1*N*-(*tert*-Butoxycarbonyl-6-aminohexanoyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylamine 3b**. ^{1}H NMR COSY δH (ppm) $CDCl_3$: 1.3 (m, 2H, CH_2); 1.48 (s, 9H, CH_3); 1.62 (t, 2H, CH_2 α/CO, J=7 Hz); 1.85 (m, 2H, CH_2); 2.10; 2.12; 2.15; 2.16 (m, 12H, 4 OAc); 2.11 (m, 2H, CH_2 β/C=O); 3.11 (q, 2H, CH_2 α/NH-CO); 4.09–4.11 (m, 2H, H6 and H6′); 4.15–4.35 (m, 1H, H-5); 4.57 (m, 1H, NH-C=O); 5.12 (dd, 1H, $J_{2,3}$=9.5 Hz; $J_{2,1}$=9 Hz, H-2); 5.19 (dd, 1H, $J_{3,2}$=9.5 Hz, $J_{3,4}$=3.7 Hz, H-3); 5.25 (dd, 1H, $J_{H1,NH}$=9.7 Hz; $J_{1,2}$=9 Hz, H-1); 5.48 (dd, 1H, $J_{4,3}$=3.7 Hz; $J_{4,5}$=1 Hz, H-4); 6.62 (d, 1H, $J_{NH,H1}$=9.7 Hz, NH Gal). R_f: 0.388; yield: 78%; ES-MS: *m/z*, 583.5[M+Na]$^+$, 599.3[M+K]$^+$. **1*N*-(*tert*-Butoxycarbonyl-11-aminoundecanoyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylamine 3c**. ^{1}H NMR COSY δ (ppm) $CDCl_3$: 1.25 (s, 12H, 6 CH_2); 1.45 (s, 9H, CH_3); 1.6 (t, 2H, CH_2 β/C=O); 2.03; 2.10; 2.15; 2.19 (m, 12H, 4 OAc); 2.21 (t, 2H, CH_2 α/C=O; J=7 Hz); 3.1 (q, 2H, CH_2 /NH, J=7 Hz); 4.09–4.11 (m, 2H, H6 and H6′); 4.12–4.14 (m, 1H, H-5); 4.61 (m, 1H, NH-C=O); 5.08 (dd, 1H, $J_{2,3}$=9 Hz, $J_{2,1}$=9.2 Hz, H-2); 5.19 (dd, 1H, $J_{3,2}$=9 Hz, $J_{3,4}$=3.5 Hz, H-3); 5.28 (dd, 1H, $J_{H1,NH}$=9.6 Hz, $J_{1,2}$=9.2 Hz, H-1); 5.5 (dd, 1H, $J_{4,3}$=3.5 Hz, $J_{4,5}$=0.8 Hz, H-4); 6.38 (d, 1H, NH-C=O, J=9.6 Hz). R_f: 0.573; yield: 63%; ES-MS: *m/z*, 653.4 [M+Na]$^+$; 597.4 [M-C$(CH_3)_3$+H+Na]$^+$.

17. The products are hygroscopic and should be manipulated directly. **1-*N*-(4-Aminobutanoyl)-β-D-galactopyranosylamine 4a**. ^{1}H NMR δH (ppm) DMSO-d_6: 1.7 (m, 2H, CH_2); 2.2 (t, 2H, CH_2-C=O); 2.8 (t, 2H, CH_2-NH_2); 2.9–3.1 (m, 1H, H-2); 3.6 (m, 4 OH); 3.6–3.7 (m, 1H, H-3); 4.7 (t, 1H, H-1, J=10 Hz); 8.5 (d, 1H, NH-C=O, J=10 Hz). Yield: 58%. **1-*N*-(6-Aminohexanoyl)-β-D-galactopyranosylamine 4b**. ^{1}H NMR δH (ppm) DMSO-d_6: 1.3 (m, 2H, CH_2); 1.5 (m, 2H, CH_2); 2.1 (t, 2H, CH_2-C=O); 2.65 (t, 2H, CH_2-NH_2); 2.9–3.1 (m, 1H, H-2); 3.6 (m, 4 OH); 3.7–3.8 (m, 1H, H-3); 4.7 (t, 1H, H-1, J=9.4 Hz); 8.4 (d, 1H, NH-C=O, J=9.5 Hz). Yield: 52%. **1*N*-(11-Aminoundecanoyl-β-D-galactopyranosylamine 4c**. ^{1}H NMR δH (ppm) DMSO-d_6: 1.3 (s, 12H, 6CH_2); 1.5 (m, 4H, 2CH_2); 2.1 (t, 2H, CH_2-C=O); 2.75 (m, 2H, CH_2-NH_2); 3.1–3.2 (m, 1H, H-2); 3.6 (m, 4 OH); 3.7–3.8 (m, 1H, H-3); 4.7 (t, 1H, H-1, J=9 Hz); 8.4 (d, 1H, NH-C=O, J=9 Hz). Yield: 55%.
18. L-PLA 100 (Mw=73000) was obtained from PHUSIS (Grenoble, France). Ring-opening polymerisation of L-lactide was conducted using metallic Zn as catalyst (0.05%) at 140°C.
19. L-PLA Gal **6a**. ^{1}H NMR COSY δH (ppm) ($CDCl_3$:CD_3OD 7:3): 1.4 (d, CH_3 L-PLA); 1.8 (m, 2H, CH_2); 2.2 (t, 2H, CH_2-C=O); 3.15 (m, 2H, CH_2-NH); 3.4 (dd, 1H, H-2 Gal); 3.5 (d, 1H, H-4 Gal); 3.6–3.65 (dd, 2H H6,6′ Gal); 3.7 (m, 1H, H-5 Gal); 3.8 (dd, 1H, H-3 Gal); 4.8 (d, 1H, H-1 Gal); 5.1 (q, CH L-PLA). L-PLA Gal **6b**. ^{1}H NMR COSY δH (ppm) ($CDCl_3$:CD_3OD 7:3): 1.1 (m, 4H, 2CH_2 βC=O); 1.6 (d, CH_3 L-PLA); 2.0 (m, 2H, CH_2δC=O); 2.2 (t, 2H, CH_2αC=O); 3.2 (m, 2H, CH_2-NH); 3.5 (dd, 1H, H-2 Gal); 3.6 (d, 1H, H-4 Gal); 3.62–3.75 (dd, 2H, H-66′ Gal); 3.8 (m, 1H, H-5 Gal); 3.9 (dd, 1H, H-3 Gal); 4.9 (d, 1H, H-1 Gal); 5.2 (q, CH L-PLA). L-PLA Gal **6c**. ^{1}H NMR COSY δH (ppm) ($CDCl_3$:CD_3OD 7:3): 1.05 (m, 16H, 8 CH_2); 1.3 (d, 3H, CH_3 L-PLA); 1.9 (t, 2H, CH_2-C=O); 3.2 (d, 1H, H-2 Gal); 3.15 (d, 1H, H-3 Gal); 3.3 (d, 1H, H-5 Gal); 3.5 (dd, 2H, H-6,6′ Gal); 3.6 (d, 1H, H-4 Gal); 3.9 (t, 2H, CH_2-NH); 4.6 (d, 1H, H-1 Gal); 5.0 (q, 1H, CH L-PLA).
20. GPC measurements were carried out using a Waters chromatograph system equiped with trible columns using a differential refractometer for detection. Tetrahydrofuran (THF) was used as solvent. Mw is 73.000 for L-PLA **5**, 76.200 for **6a**, 77.800 for **6b** and 76.600 for **6c**; Mn is 32.000 for L-PLA **5**, 48.600 for **6a**, 44.300 for **6b** and 42.500 for **6c**; **I** 2.4 for L-PLA **5**, 1.56 for **6a**, 1.75 for **6b** and 1.80 for **6c**.
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