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α-Functional glycopolymers: New materials for (poly)peptide conjugation

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Abstract

The synthesis and characterization of a number of *N*-(hydroxy)succinimidyl ester-terminated glycopolymers obtained via copper(I)-catalysed living radical polymerisation have been described. Monomers employed were based on protected glucose and galactose, glucofuranoside monomer (**1**) and galactopyranoside monomer (**2**). The corresponding polymers featured a relatively narrow molecular weight distribution (PDI = 1.10–1.31) and M_n between 4.5 and 10.2 kDa. The protecting groups were removed by treatment with formic acid. Analogous fluorescent polymers have been synthesized by copolymerisation of a monomer which fluoresces in the visible, the fluorescent behaviour of these materials has been investigated. Preliminary experiments have also shown that the terminally functional sugar polymers can react with molecules containing primary amino groups and some triblock ABA copolymers have been prepared.

Keywords: Bioconjugation; Glycopolymers; Living radical polymerisation

1. Introduction

The last two decades have witnessed a growing interest by both industry and academia in (poly)peptide-based drugs resulting in a wide range of protein therapeutics that are now available on the marketplace. Despite these advances several problems still remain to be solved. One of the main problems in using (poly)peptides therapeutics arise from their low circulation half-life, due to a number of factors including rapid renal excretion and low stability in the presence of proteolytic enzymes and antibodies. Oral delivery is even more problematic, as, among other things, protein-based drugs tend to be destroyed by the digestive system. One of the more successful methods for circumventing these problems is 'PEGylation', the attachment of water-soluble synthetic polymers, mainly α-functional polyethylene glycol (PEG), to appropriate proteins and peptide therapeutics [1–3]. The main benefits of using these polymers are two-fold: Firstly, they reduce the drug elimination from the body by increasing its hydrodynamic volume and shield its surface from antibodies; and secondly, they help to maintain the drug concentration in the therapeutic window (reducing the frequency of required intakes) by increasing the solubility of highly hydrophobic drugs [4,5].

Amino groups present at the surface of (poly)peptides, especially ε-amino units of lysine residues and the N-terminal amino groups are the usual site for polymer–protein linking. The reactive polymers most commonly employed for these purposes can be classified into two main groups: alkylating agents, mainly aldehyde and epoxide moieties, and acylating groups including *N*-hydroxysuccinimidyl (NHS) esters and carbonates, chloro-triazines and benzotriazole carbonates. In general, the former is usually preferred when the retention of the overall charge in the conjugate is important for maintaining the biological activity, the latter when a high reactivity of the conjugating polymer is requested.

Recently we reported the copper-mediated synthesis of new monofunctional water-soluble methacrylates bearing reactive chain-ends, and their application in protein conjugation [6,7]. One of the advantages in using controlled radical polymerisation for these purposes, in addition to the well-established functional group tolerance, is the possibility of obtaining polymers with narrow molecular weight distribution. This parameter is important in bioconjugation chemistry, as a low PDI of the end-functional polymer employed will reflect into a narrow molecular weight distribution of the resulting polymer–(poly)peptide

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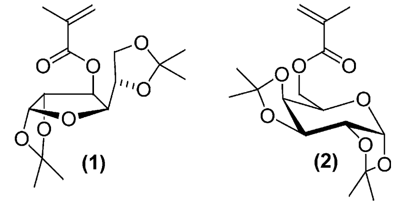

Chart 1. Glucofuranose (**1**) and galactopyranose (**2**) monomers.

conjugate [4]. Moreover, the extreme versatility of this polymerisation technique allows tailoring of both the molecular weight and the macromolecular structure. The latter factor is also extremely important, as it has been shown that the use of branched polymers instead of linear structures can, in some cases, improve a number of properties such as resistance to proteolysis, to the action of antibodies and resulted in a lower immunogenicity of the corresponding bioconjugates, due to the so-called 'umbrella-like' shape of these polymers [4,8,9]. The high biocompatibility as well as the excellent solubility in water of glycopolymers made them an ideal candidate for the synthesis of new biohybrid materials [10]. Moreover, the possibility of having additional non-covalent interactions between the synthetic glycopolymer and the (poly)peptides employed for the conjugation may be an intriguing subject for further investigation. Thus far, a relatively small number of papers described the synthesis of glycopolymers via controlled radical polymerisation [11–17]. In the present work, we report the synthesis and characterization of *N*-succinimidyl ester terminated glycopolymers obtained by copper-mediated living radical polymerization (LRP). The monomers employed are the glucofuranose (**1**) [18–20] and galactopyranose (**2**) [13,21], Chart 1. Preliminary results concerning the conjugation reaction in the presence of amino-substrates containing free amino groups are also reported.

2. Experimental

Copper(I)bromide (Aldrich, 98%) was purified according to the method of Keller and Wycoff [22]. *N*-(*n*-Propyl)-2-pyridylmethanimine [23] and the fluorescent co-monomer (**8**) [24] were prepared as described earlier. Triethylamine (Fischer, 99%) was stored over sodium hydroxide pellets. All other reagents and solvents were obtained at the highest purity available from Aldrich Chemical Company and used without further purification unless stated.

All reactions were carried out using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated. R_f values refer to analytical TLC performed using pre-coated silica gel 60 F254 and developed in the solvent system indicated. Compounds were visualized by use of UV light (254 nm) or a basic solution (10% wt/wt K_2CO_3 in water) of $KMnO_4$. Merck 60 (230–400 mesh) silica gel was used for column chromatography. Molar mass distributions of polymers (**4**), (**6**), (**9**), (**11**) and (**13**) were measured using size exclusion chromatography (SEC), on a system equipped with two PL gel 3 μm mixed E-columns (300×7.5 mm^2) and one PL gel 3 μm guard column (50×7.5 mm^2) (Polymer Laboratories) with differential refractive index detection using THF at 1.0 mL min^{-1} as the eluent. Poly(MMA) standards (3× 10^5–200 g mol^{-1}) were used for calibration. The analysed samples contained toluene (0.2% vol/vol) as the flow marker. Molar mass distributions of polymers (**5**) and (**7**) were measured using size exclusion chromatography (SEC), on a system equipped with two PL aquagel–OH 8 μm mixed columns (300×7.5 mm^2) and one PL aquagel–OH 8 μm guard column (50×7.5 mm^2) (Polymer Laboratories) with differential refractive index detection using water (2.12% $NaNO_3$ wt/wt; 0.12% NaH_2PO_4 wt/wt) at 0.8 mL min^{-1} as the eluent. PEG standards (1×10^6–600 g mol^{-1}) were used for calibration. The analysed samples contained methanol (0.2% vol/vol) as flow marker. Conjugation of polymer (**11**) with poly(ethylene glycol) bis-(3-aminopropyl) terminated was followed by size exclusion chromatography (SEC), on a system equipped with four PL gel 3 μm mixed E-columns (300×7.5 mm^2) and one PL gel 3 μm guard column (50× 7.5 mm^2) (Polymer Laboratories) with UV detection using THF at 0.5 mL min^{-1} as the eluent. Polystyrene standards were used to calibrate the SEC. The M_n reported in the M_n vs conversion (%) plots are obtained from SEC data calibrated with PMMA standards and are uncorrected. Those plots are reported in order to show the evolution of M_n with conversion. NMR spectra were obtained on Bruker DPX300 and Bruker DPX400 spectrometers. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances (^1H and ^{13}C). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet; dd = doublet of doublets; t = triplet, q = quartet, m = multiplet. The molecular weight of the polymers M_n (NMR) are calculated by comparing the integrals of the chain-end signals and appropriate peaks related to the polymer backbone. Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell. Absorption spectra were recorded on or a Hewlett Packard 9452A spectrophotometer. An Aminco SPF-500 spectrofluorometer was used for the fluorescence measurements with emission and excitation bandpasses of 2.5 nm. All the samples were excited at 460 nm. Mass spectra were recorded using a micromass autospec apparatus. The melting points were measured on a Büchi 510 apparatus using open glass capillaries, the data are uncorrected. The yields are not optimised.

2.1. Monomers and initiator synthesis

2.1.1. 3-O-Methacryloyl-1,2:5,6-di-O-isopropylidene-D-glucofuranose (1)

To a solution of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (30.6 g, 115.2 mmol) in 150 mL of anhydrous