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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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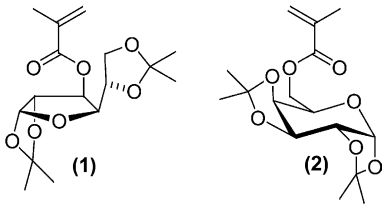


Chart 1. Glucofuranose (**1**) and galactopyranose (**2**) monomers.

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

pyridine, 32 mL of methacrylic anhydride (202 mmol) was added dropwise at ambient temperature. The mixture was heated at 65 °C for 4.5 h and for a further 1 h after the addition of 100 mL of water. The mixture was allowed to cool to ambient temperature overnight and then extracted with petroleum ether (3×100 mL). The combined extracts were washed twice with 150 mL of 5% aqueous sodium hydroxide solution and three times with 100 mL of water and dried over anhydrous magnesium sulphate. The solvent was removed under vacuum and the oily residue was purified by flash chromatography (CC, SiO_2, petroleum ether/ethyl acetate 4:1, R_f (**1**)=0.35), yielding (**1**) as a colourless oil which slowly crystallized (30.27 g, 80%). mp 47–49 °C.

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 6.03 (s, 1H, CH*H*=C(CH_3)), 5.80 (d, 1H, ^3J=3.5 Hz, CH), 5.53 (m, 1H, *CH*H=C(CH_3)), 5.20 (s, 1H, CH), 4.44 (d, 1H, ^3J=3.5 Hz, CH), 4.15 (m, 2H, CH), 4.00–3.89 (m, 2H, CH), 1.86 (s, 3H, CH_2=C(C*H*$_3$)), 1.43 (1s, 3H, CH_3), 1.31 (1s, 3H, CH_3), 1.21 (1s, 3H, CH_3), 1.20 (1s, 3H, CH_3). ^{13}C{^{1}H}NMR ($CDCl_3$, 75.4 MHz): δ (ppm) 165.96 (1C, C(O)O), 135.84 (1C, *C*(CH_3)=CH_2), 126.52 (1C, C(CH_3)=*C*H_2), 112.28 (1C, (CH_3)$_2$*C*), 109.33 (1C, (CH_3)$_2$*C*), 83.30 (1C, CH), 79.94 (1C, CH), 72.54 (1C, CH), 76.45 (1C, CH), 67.25 (1C, CH), 26.80 (1C, CH_3), 26.73 (1C, CH_3), 26.20 (1C, CH_3), 25.22 (1C, CH_3), 18.26 (C(*C*H$_3$)=CH_2). IR (solid, ATRcell) $\tilde{\nu}$ (cm^{-1}): 2980, 1724, 1638, 1454, 1375, 1317, 1293, 1262, 1207, 1147, 1068, 1020, 942, 889, 849, 814, 760, 651, 625. Anal. Calcd for $C_{16}H_{24}O_7$ C, 58.5%; H, 7.4%; O, 34.1%. Found C, 58.3%; H, 7.3%; O 34.4%. HR-FABMS (M+H$^+$) calculated for $C_{16}H_{25}O_7$: 329.1600, found 329.1597.

2.1.2. 6-O-Methacryloyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (2)

R_f (**2**)=0.35 (petroleum ether/ethyl acetate 4:1); Yield: 91%. mp 61–63 °C.

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 6.04 (s, 1H, *CH*H=C(CH_3)), 5.48 (m, 1H, CH*H*=C(CH_3)), 5.43 (d, 1H, ^3J=4.9 Hz, anomeric CH), 4.54 (dd, 1H, ^2J=7.8 Hz, ^3J=2.5 Hz, CH*H*), 4.16–4.26 (m, 4H, 1*CH*H+3CH), 4.00 (m, 1H, CH), 1.85 (s, 3H, CH_2=CH(C*H*$_3$)), 1.36 (1s, 3H, CH_3), 1.41 (1s, 3H, CH_3), 1.25 (1s, 3H, CH_3), 1.24 (1s, 3H, CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 75.4 MHz): δ (ppm) 167.23 (–*CO*–), 136.35 1C, (–*C*(CH_3)=CH_2), 125.86 (1C, C(CH_3)=*C*H_2), 108.87, 109.74 (2C, (CH_3)$_2$*C*–), 96.50, 71.35, 70.94, 70.74, 66.32, 63.87 (6C, *C*H), 26.14, 25.16, 24.63 (4C, –*CH*$_3$), 18.44 (–C(*CH*$_3$)=CH_2). IR (solid, ATRcell) $\tilde{\nu}$ (cm^{-1}): 2979, 2928, 1715, 1636, 1459, 1384, 1333, 1295, 1257, 1206, 1163, 1112, 1064, 1005, 935, 919, 894, 864, 812, 771, 687, 648. Anal. Calcd for $C_{16}H_{24}O_7$ C, 58.5%; H, 7.4%; O, 34.1%. Found C, 58.2%; H, 7.3%; O 34.5%. HR-FABMS (M+H$^+$) calculated for $C_{16}H_{25}O_7$: 329.1600, found 329.1602.

2.1.3. N-Succinimidyl-2-bromopropionate (3)

N-Hydroxysuccinimide (4.51 g, 39.2 mmol) and 2-bromopropionic acid (2.90 ml, 32.7 mmol) were dissolved, under dinitrogen atmosphere, in anhydrous DCM (1000 ml) in a 2000 ml round-bottomed flask, equipped with a magnetic stirrer. The flask was cooled to 0 °C and a solution of *N*,*N*′-dicyclohexylcarbodiimide (6.70 g, 32.7 mmol) in DCM (50 ml) was added dropwise. After stirring at ambient temperature overnight the reaction mixture was filtered and the solvent removed under reduced pressure to give a yellow solid that was purified by flash chromatography (CC, SiO_2, Et_2O, R_f (**3**)=0.31). Obtained 7.20 g (28.9 mmol, 74%) of (**3**) as a white solid that was used for the polymerization reactions without further purification. An analytical sample was obtained by re-crystallization from hot 2-propanol. mp 69–70 °C.

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 4.61 (q, 1H, *CH*(CH_3)Br), 2.85 (s, 4H, CH_2), 1.95 (d, 3H, CH(C*H*$_3$)Br). ^{13}C{^{1}H} NMR ($CDCl_3$, 75.4 MHz): δ (ppm) 169.34 (2C, CH_2*C*(O)N), 166.72 (1C, C(O)O), 35.28 (1C, *CH*(CH_3)Br), 26.00 (2C, CH_2), 21.09 (1C, CH(*CH*$_3$)Br). IR (solid, ATRcell) $\tilde{\nu}$ (cm^{-1}) 2982, 2954, 1808, 1781, 1728, 1445, 1412, 1386, 1352, 1245, 1186, 1105, 1063, 1045, 996, 981, 863, 822, 756, 745, 644, 609, 581, 562, 507. Anal. Calcd For $C_7H_8BrNO_4$ C, 33.62%; H, 3.22%; N, 5.60%. Found: C, 33.57%; H, 3.17%; N, 5.49%. HR-FABMS (M+H$^+$) calculated for $C_7H_9BrNO_4$ 249.9714, found 249.9727.

2.2. Polymers

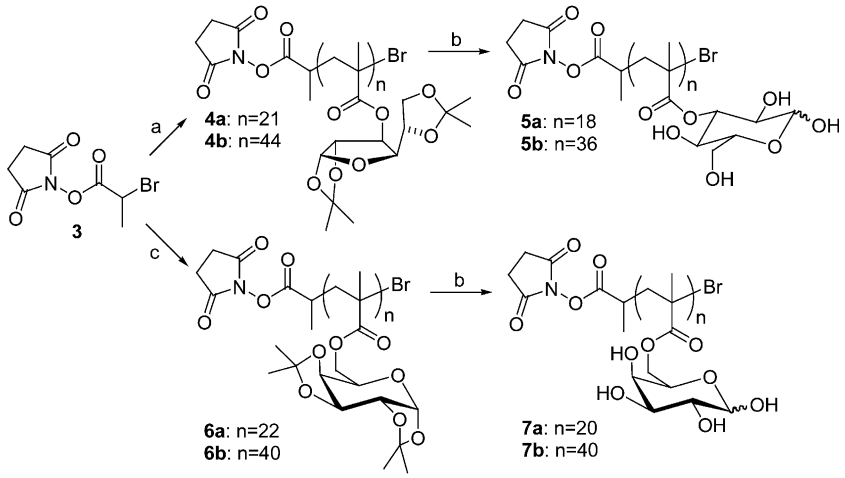
2.2.1. General procedure: synthesis of polymer (4a)

Copper(I)bromide (0.262 g, 1.82 mmol), (**3**) (0.457 g, 1.82 mmol), (**1**) (6.00 g, 18.3 mmol) along with toluene (12 mL) as the solvent and mesitylene (0.73 g) as the internal standard for kinetic study were charged to a dry Schlenk tube containing a magnetic follower. The tube was sealed with a rubber septum and subjected to three freeze-pump-thaw cycles. *N*-(*n*-Octyl)-2-pyridylmethanimine ligand (0.430 mL, 1.87 mmol) was injected in the Schlenk tube via a syringe and the tube was immerged in an oil bath heated at 70 °C (t=0). Samples were taken periodically using a degassed syringe for molecular weight and conversion analyses. At the end of the polymerisation the mixture was diluted with 20 mL of toluene and air was bubbled overnight and the resulting green mixture was passed through an acid alumina pad to remove the copper complexes. The solution was concentrated under reduced pressure and the polymer was precipitated in petroleum ether to give the polymer (**4a**) as a slightly brownish powder.

The molecular weight of the final polymer was calculated by ^{1}H NMR comparing of the integrals of the initiator signal at 2.80 ppm and that of the anomeric proton of the sugar moiety, at approximately 5.8 ppm. The conversions were calculated by comparison between the integrals relative to the aromatic protons of the mesitylene (singlet, 6.65 ppm, internal standard) and one of the vinyl protons relative to the monomer (broad singlet, 6.03 ppm, decreasing with time).

Table 1
Polymers obtained by LRP of the monomers (**1**) and (**2**), in the presence of the fluorescent comonomer (**8**) and the initiator (**3**)

Polymer (code)	Conversion (%)	DP (NMR)	M_n (NMR) (kDa)	M_w/M_n (SEC)	Initiating efficiency
(**9**)	93	18	6.1	1.18	0.53
(**11**)	87	18	6.1	1.08	0.50
(**13**)	85	45	15	1.26	0.37



Scheme 1. Reagents and conditions: (a) Cu(I)Br, *N*-(*n*-octyl)-2-pyridylmethanimine, (**1**), (**3**), toluene, 70 °C; (b) 80% aqueous formic acid, ambient temperature, 2 d; (c) Cu(I)Br, *N*-(*n*-octyl)-2-pyridylmethanimine, (**2**), (**3**), toluene, 70 °C.

2.2.1.1. Fluorescent polymers. The procedure is essentially analogous to that followed for the synthesis of the non-fluorescent polymers, using a fluorescent methacrylate (**8**) as a co-monomer (Table 1).

2.2.2. Deprotection of the polymers

The polymers were deprotected following the protocol described by Fukuda and co-workers [12], with some minor modifications. Briefly, the protected polymers (0.50 g) were dissolved in 80% formic acid (500 mL) and stirred for 3 days at ambient temperature. The solvents were then removed under vacuum, water (100 mL) was then added and the solvent removed under vacuum again. This procedure was repeated three times to ensure that no formic acid was left. The residues were finally redissolved in water (50 mL) and freeze-dried to give the deprotected polymers in close to 100% yield.

2.2.2.1. Non-fluorescent polymers. Table 2.

Table 2
Polymers (**5**) and (**7**) obtained by acid deprotection of polymers (**4**) and (**6**), respectively

Polymer (code)	DP (NMR)	M_n (NMR) (kDa)	M_w/M_n (SEC)
(**5a**)	18	4.7	1.19
(**5b**)	36	9.2	1.30
(**7a**)	20	5.2	1.28
(**7b**)	40	10.2	1.19

2.2.2.2. Fluorescent polymers. Table 3.

2.3. Conjugation reaction

Polymer (**11**) (20 mg, 3.3 μmol), poly(ethylene glycol) bis-(3-aminopropyl) terminated (5 mg, 3.3 μmol) and triethylamine (5 μL, 33 μmol) were dissolved in 1.0 mL of anhydrous DMSO.

3. Results and discussion

The desired α-terminally functional glycopolymers have been obtained following the synthetic protocol depicted in Scheme 1. The initiator (**3**), obtained from 2-bromo propionic acid and *N*-hydroxy succinimide [6], was used for the polymerisation of protected sugar monomers, (**1**) and (**2**), using Cu(I)Br/pyridineimine ligand [25] as catalyst to give the ketal-protected polymers (**4**) and (**6**). The isopropylidene protective groups were subsequently removed following the protocol of Fukuda and coworkers [12], at ambient temperature in 80% aqueous formic acid, to

Table 3
Polymers (**10**), (**12**) and (**14**) obtained by acid deprotection of polymers (**9**), (**11**) and (**13**), respectively

Polymer (code)	DP (NMR)	M_n (NMR) (kDa)
(**10**)	18	6.1
(**12**)	18	6.1
(**14**)	45	15.0

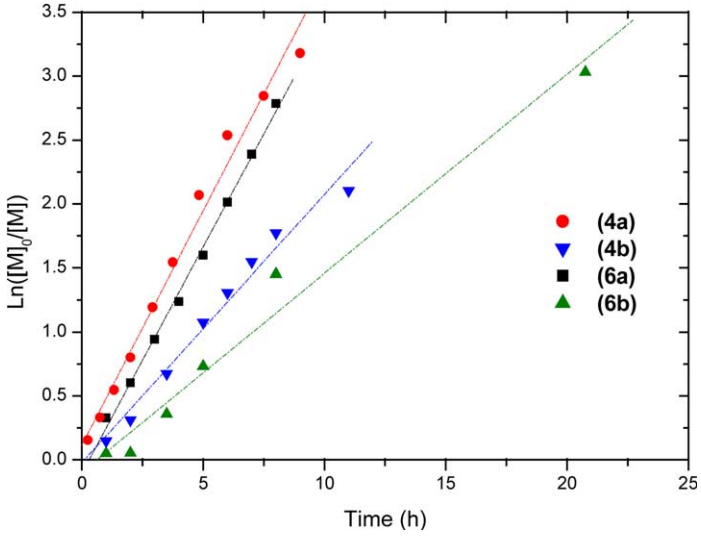


Fig. 1. First-order kinetic plots for the polymerisation of monomer (**1**) and (**2**) to give (**4a**), (**4b**) and (**6a**), (**6b**), respectively. Reaction conditions: (**4a**) and (**6a**), [monomer]:[(**3**)]:[Cu]:[ligand] = 10:1:1:2; (**4b**) and (**6b**), [monomer]:[(**3**)]:[Cu]:[ligand] = 20:1:1:2; toluene (solvent)/monomer: 1:1 (vol/vol), 70 °C. Lines represent regression fits of data points.

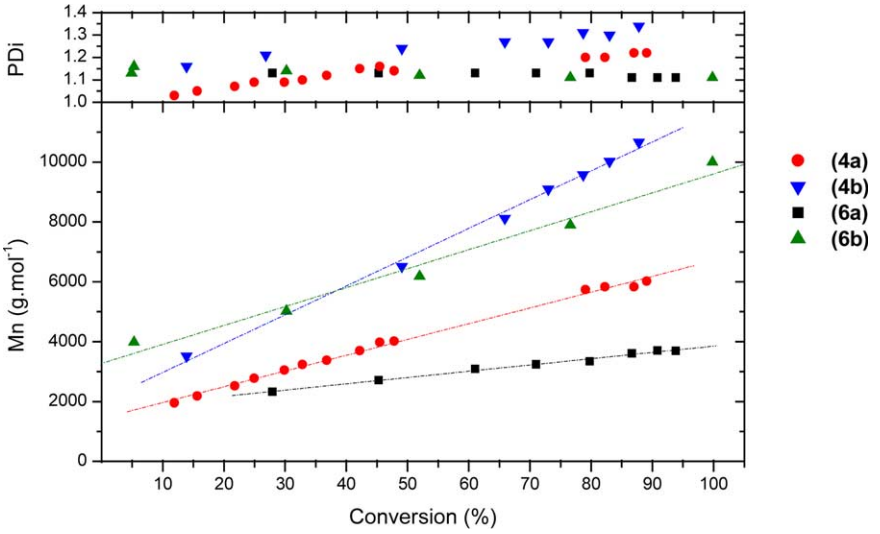


Fig. 2. Synthesis of (**4a**), (**4b**), (**6a**) and (**6b**): evolution of molecular weight and polydispersity with conversion.

give the polymers (**5**) and (**7**). *N*-Hydroxysuccinimide esters are normally not hydrolytically stable in water, especially at basic pH [6]. However, the α-terminus of polymers (**4**) and (**6**) showed good stability under these experimental conditions, with the amount of hydrolysed chain-ends $<10\%$ for all of the polymers studied. The M_n (NMR), calculated by comparison between the integrals of the NHS unit (2.8 ppm) and the peak of the anomeric proton (5.5 ppm) (or alternatively the peaks of the CH_2 and CH_3 of the polymer backbone at 2.4–0.2 ppm) are very similar to those calculated for the ketal protected polymers (**4**) and (**6**). In the case of the polymers (**4**) the deprotection step is accompanied by a structural change of the carbohydrate moieties from furanoside to pyranoside structure [12].

Table 4
Polymers obtained by LRP of the monomers (**1**) and (**2**) in the presence of the initiator (**3**)

Polymer (code)	Conversion (%)	DP (NMR)	M_n (NMR) (kDa)	M_w/M_n (SEC)	Initiating efficiency
(**4a**)	90	21	7.1	1.14	0.44
(**4b**)	90	44	14.7	1.31	0.40
(**6a**)	95	22	7.5	1.08	0.44
(**6b**)	99	40	13.4	1.10	0.50

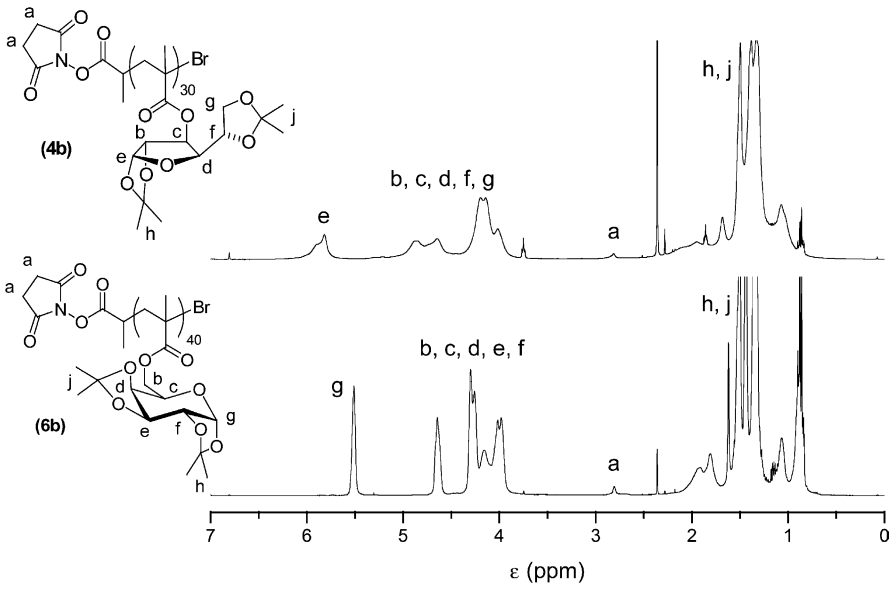


Fig. 3. ^{1}H NMR of polymers (**4b**) and (**6b**) in $CDCl_3$.

The initiation efficiencies observed in the polymerisation reactions were between 37 and 53%, values that are very similar to the ones observed in the polymerization of (methoxyPEG)methacrylate with initiator (**3**) [6]. These results identify a situation in which the initiation step is slower than propagation. In previous work, we observed that by using an analogous initiator bearing two methyl groups in alpha position to the ester function, the initiating efficiency rose close to 100% [6]. This led us to conclude that for that particular system the efficiency was solely a function of the initiator structure. This may be the case also for the glycopolymers described in this work, although the presence of other concomitant factors cannot be ruled out at present [26]. In the present work, we focused our attention towards the use of initiators bearing only one methyl group in alpha position ((**3**)) since a previous study carried out in our group showed that the corresponding polymers are much more reactive in the conjugation reaction in the presence of proteins and peptides [6].

The polymerisation rates are quite similar for the two monomers employed, with the reaction being slightly faster, under the same experimental conditions, when the glucopyranoside monomer (**1**) was employed (Fig. 1). The molecular weights obtained by SEC analysis appeared to be higher for polymers (**4**) than for polymers (**6**), at comparable M_n (NMR). (Table 4, entry (**4a**) and (**6a**)). This behaviour may simply be explained in terms of a different hydrodynamic volume of the two different type of polymers (Fig. 2).

Interestingly, ^{1}H NMR analysis of the deprotected sugar

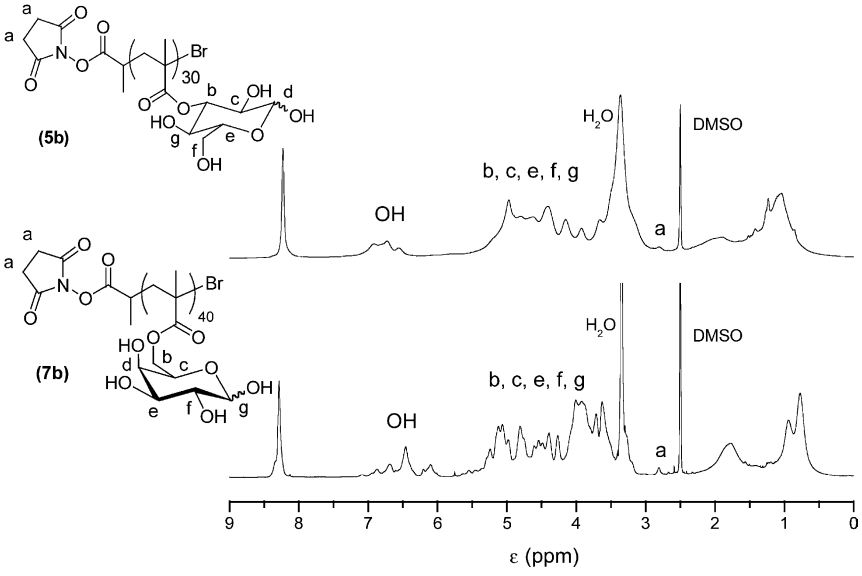


Fig. 4. ^{1}H NMR of polymers (**5b**) and (**7b**) in d_6-DMSO.

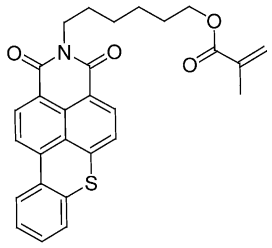


Chart 2. Fluorescent monomer (**8**).

polymers were prepared. The introduction of a visibly fluorescent tag in the conjugating backbone offers a number of potential advantages that include the easier detection and characterisation of the conjugated using fluorescence analytical techniques (confocal microscopy, UV/visible SEC, HPLC, fluorimetry, CD) and increased traceability in biological systems during biomedical assays. The synthetic strategy followed for the synthesis of the fluorescent polymers was analogous to that developed for

Table 5
Nature of the fluorescent polymers prepared in this study

Code	Monomer (M)	$[(M)]_0/[(\mathbf{8})]_0$	$[(M)]/[(\mathbf{8})]$	DP (NMR)	M_n (kDa) (NMR)
(**9**)	(**1**)	20:1	28:1	18	6.1
(**10**)		Deprotection of (**9**)		17	4.5
(**11**)	(**2**)	20:1	27:1	16	6.1
(**12**)		Deprotection of (**11**)		17	45.0
(**13**)	(**1**)	100:1	142:1	45	15.0
(**14**)		Deprotection of (**13**)		40	10.2

polymers (**5**) and (**7**) in both d_6-DMSO and D_2O revealed the presence of an unexpected singlet at ca. 8.2–8.3 ppm (Figs. 3 and 4). This may be explained in terms of a partial esterification of the free hydroxy-groups of the carbohydrate moieties caused by the large excess of formic acid employed in the deprotection step.

This hypothesis seemed to be confirmed by ^{13}C NMR analysis that showed the presence of tertiary carbon atoms at ca. 166 ppm. However, the concentration of these formate groups is relatively low (≤ 1 group/sugar unit) and the solubility in water of the polymers, a crucial parameter in view of an application of these products for the synthesis of biomaterials, still remains very high.

In addition, some NHS α-functional fluorescent the polymers (**4**) and (**6**), using the (**8**) [24,27–29] as a co-monomer. The advantage of using this co-monomer is that a very small percentage ($\leq 1\%$ mol/mol) of it is sufficient for conferring a high visible fluorescence to the resulting polymers. Moreover, it can be obtained very easily from anhydride starting material, an extremely cheap starting material, usually employed as a pigment for polyolefins (Chart 2).

Polymers (**9**), (**11**) and (**13**) differ in both the sugar monomer and/or the percentage of fluorescent comonomer (**8**) employed (Table 1). Statistical copolymerisations of either (**1**) or (**2**) with (**8**) initiated with *N*-succinimidyl-2-bromopropionate (**3**) in toluene proceeded in a controlled manner with linear first order kinetic plots, Fig. 5, and with a

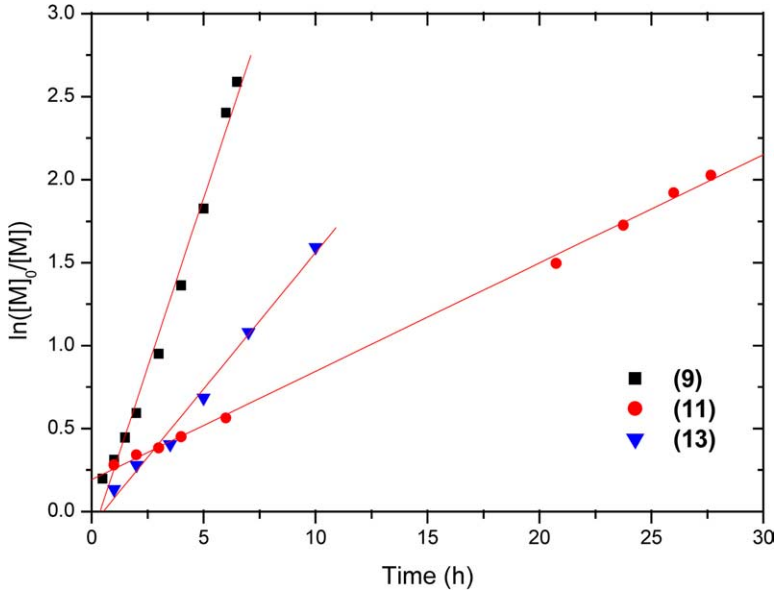


Fig. 5. First-order kinetic plots for the copolymerisation of monomer (**1**) and (**8**), (**2**) and (**8**) to give (**9**), (**11**) and (**13**), respectively. Reaction conditions: (**9**): $[M_{(1)}]:[M_{(8)}]:[I]:[Cu]:[L] = 10:0.5:1:1:2$; (**11**): $[M_{(2)}]:[M_{(8)}]:[I]:[Cu]:[L] = 10:0.5:1:1:2$, (**13**): $[M_{(1)}]:[M_{(8)}]:[I]:[Cu]:[L] = 20:0.2:1:1:2$; Toluene, 70 °C.

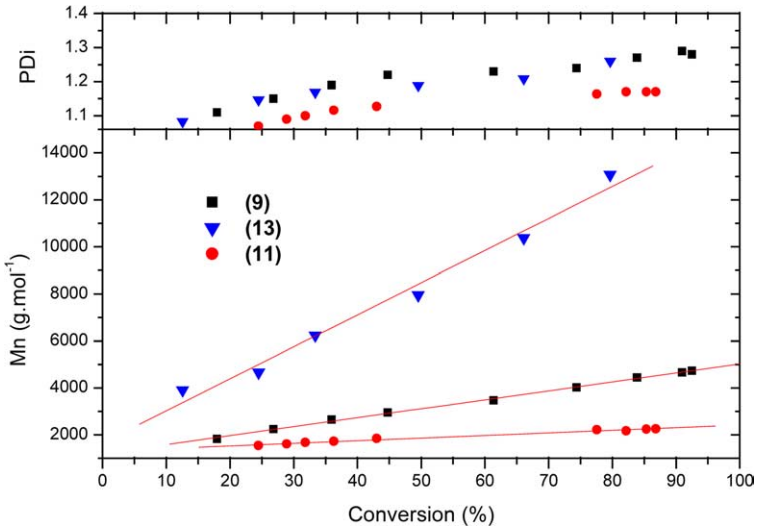


Fig. 6. Evolution of molecular weight and PDI with conversion for polymers (**9**), (**11**) and (**13**).

linear evolution of M_n with time, Fig. 6. The amount of fluorescent comonomer incorporated in the polymer backbone was evaluated by NMR, by comparing the integrals in the aromatic region from the dye to the integrals from the sugar moieties, Fig. 7 (Table 5).

The absorption and emission spectra were quite similar for all fluorescent polymers synthesized, with a typical Stokes shift of approximately 70 nm (Fig. 8).

The observed fluorescence was higher in polymer (**14**) than for polymers (**10**) and (**12**), despite the lower content of fluorescent comonomer in (**14**). This behaviour may be explained in terms of fluorescent quenching due to fluorophore–fluorophore interactions that normally tend to occur when high loading concentrations or labelling densities are used. In order to confirm this hypothesis we studied the dependence of the fluorescence on the polymer concentration and, as expected, a decrease in the quantum yields was observed when moving towards increased polymer/fluorophore concentrations, Fig. 9.

3.1. Reaction of NHS functional polymers with diamino-terminated poly(ethylene glycol)

Preliminary experiments have been carried out in order to evaluate the reactivity of the polymers synthesized in the presence of water-soluble molecules containing free amino groups. Commercially available poly(ethylene glycol) bis-(3-aminopropyl) terminated ($M_n \sim 1500$) was used as a model substrate and the reaction was followed by SEC analysis. The SEC analysis of the conjugation performed using polymer (**11**) is shown in Fig. 10. In this case the use of an 1:1 ratio between the two reactants allowed us to identify all the products involved in the condensation step: the adduct containing two sugar polymer chains (ABA

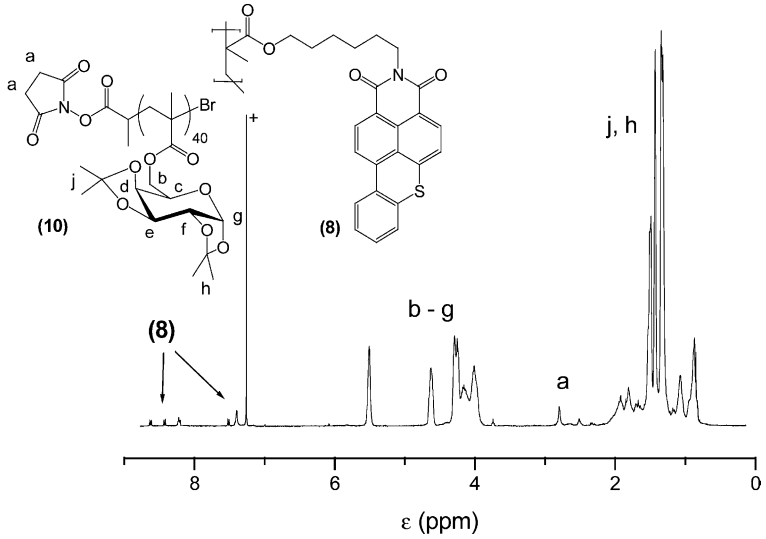


Fig. 7. ^{1}H NMR of polymer (**11**) in $CDCl_3$.

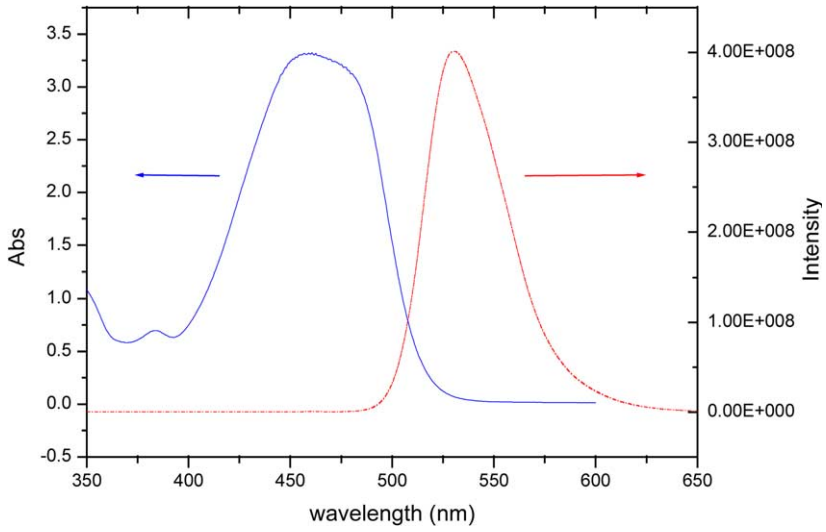


Fig. 8. Absorption (blue) and emission (red) spectra for (**12**) in DMSO (polymer concentration: 3.3 mg/mL). The absorbance maximum is at 460 nm, while the emission maximum =530 nm.

block), the monocondensation product (AB block) and the starting material (**11**) (Scheme 2).

4. Conclusions

The synthesis and characterization of a number of *N*-(hydroxy)succinimidyl ester-terminated glycopolymers obtained via copper(I)-catalysed living radical polymerisation has been described. The monomers employed have been obtained from glucose and galactose derivatives and the corresponding polymers featured a relatively narrow molecular weight distribution (PDI=1.10–1.31). Fluorescent polymers have been synthesized by copolymerisation of the sugar monomers with a fluorescent comonomer and the fluorescent behaviour of these materials have been investigated. Preliminary experiments have shown that the terminally functional sugar polymers can react with molecules containing primary amino groups and some triblock ABA copolymers have been prepared.

Functional polymers bearing NHS reactive chain ends have been widely employed in the synthesis of bioconjugates, however, only very recently polymers other than poly(ethylene glycol) (PEG) have been employed for these purposes. Sugar polymers have a number of characteristics, such as high biocompatibility and water solubility, that make them a very attractive materials for the synthesis of biohybrid macromolecules. Moreover, they may also interact with the proteins via additional non-covalent interactions and this could have an influence on the bioactivity of the corresponding polymer–polypeptide conjugates. The synthetic strategy presented in this work allows to obtain pure mono-functionalised polymers, avoiding the possibility of protein–protein cross-linking

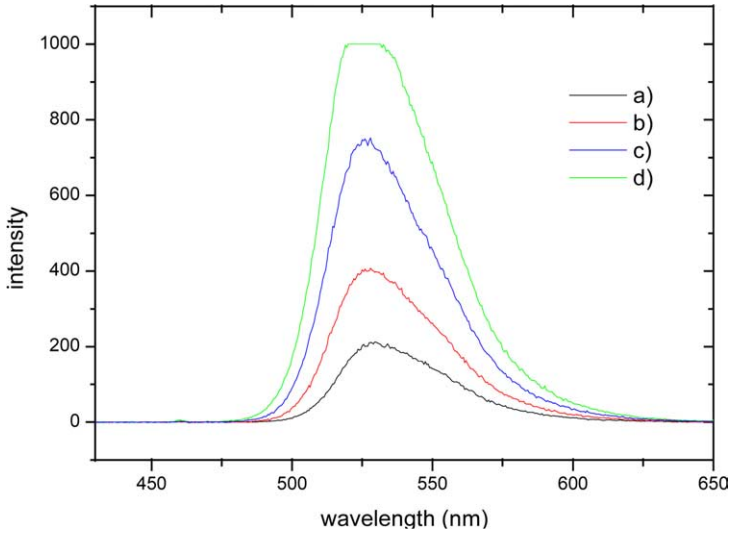


Fig. 9. Emission spectra of polymer (**14**) at different concentrations in DMSO. (a) 10, (b) 7.5, (c) 6.75, (d) 5.0 mg/mL.

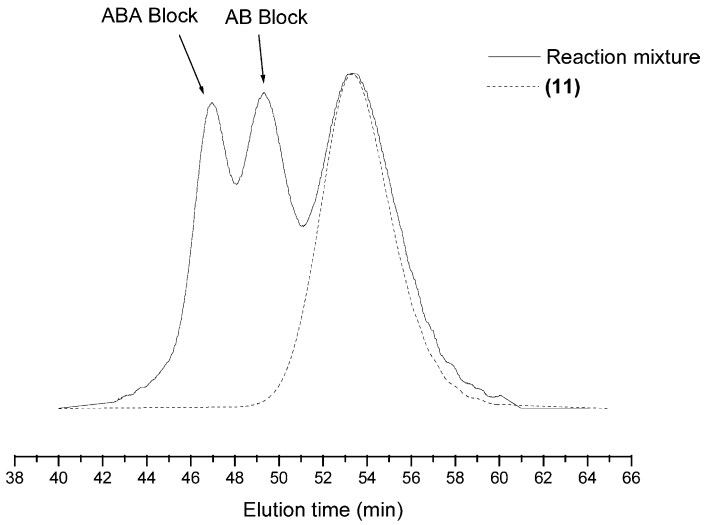
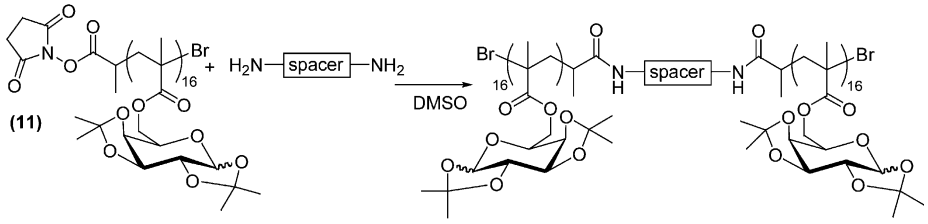


Fig. 10. Reaction between (**11**) and bis-amino terminated PEG (1:1 mol/mol): SEC analysis of the reaction mixture.



Scheme 2. Conjugation of polymer (**11**) with bifunctional amines.

that is sometimes observed using conventional PEGylation chemistry, due to the presence of impurities in the functional polymers.

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