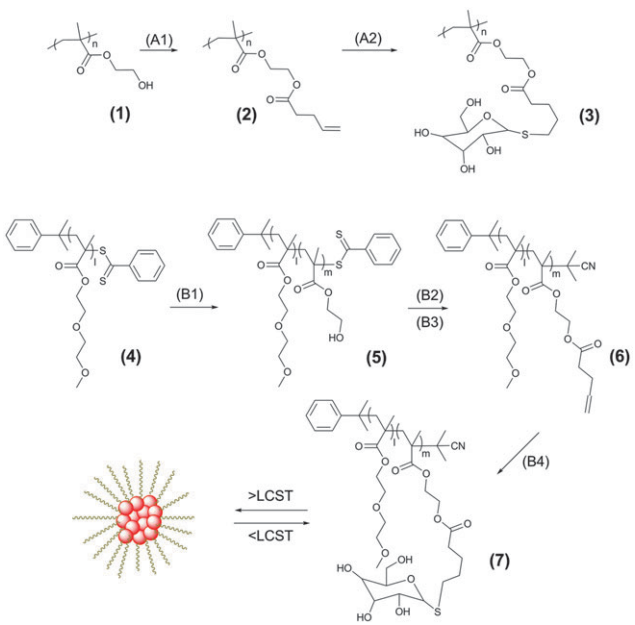
Synthesis of thiol-linked neoglycopolymers and thermo-responsive glycomicelles as potential drug carrier†

Gaojian Chen, Sadik Amajjahe and Martina H. Stenzel*

Received (in Cambridge, UK) 6th January 2009, Accepted 27th January 2009
First published as an Advance Article on the web 4th February 2009
DOI: 10.1039/b900215d

Homopolymer and block copolymer bearing carbohydrate side chain functionality were obtained by grafting glucothiose onto alkene functional scaffolds *via* a thiol–ene click reaction and the resulting copolymer was used to form thermo-responsive micelles as a potential drug carrier.

Carbohydrates are involved in a number of important biological processes involving highly specific events in cell–cell recognition, cell–protein interactions, and the targeting of hormones, antibodies and toxins.[1–4] Whereas individual protein–saccharide interactions are typically weak, the multivalent interactions employed in biological systems, known as the 'cluster glycoside effect',[5–7] can be characterised as high affinity and high specificity.[8] Due to their biomimetic properties and many potential applications, there is an increasing interest in synthetic polymers with pendent sugar moieties, which are able to interact with lectins as multivalent ligands in a similar manner to natural glycoproteins. Two different approaches have been used for the synthesis of glycopolymers: polymerisation of a sugar-containing monomer, or post-functionalisation of pre-formed polymers using sugar moieties. The former approach, especially using the Living Radical Polymerisation (LRP) technique, has proven useful for the synthesis of well-defined glycopolymers.[9,10] On the other hand, the post-functionalisation approach is convenient to produce libraries of glycopolymers with the same macromolecular features by attaching different sugar moieties to pre-formed polymer scaffolds; it also generally offers a simple procedure as some sugar-containing monomers have a tendency to self-polymerise during purification procedures.[11] A good example of using the post-functionalisation approach is the construction of glycopolymers from alkyne backbone-functional polymers *via* Cu-catalyzed azide–alkyne click (CuAAC) chemistry as reported by Haddleton and coworkers.[12–14] Recently, another reaction which has emerged as an attractive click process is the addition of thiols to alkenes, which is called thiol–ene coupling or thiol–ene click reaction.[15–19] It is highly efficient and orthogonal to a wide range of functional groups, and is compatible with water and oxygen. The biologically friendly nature of the coupling reaction (metal free) and its simple reaction process are very attractive for synthesising glycopolymers. In addition, investigation of protein–thio-oligosaccharide binding indicates that thiol linkages offer a higher degree of flexibility between glycol units and possess more conformers than *O*-linked ligands.[20] Furthermore, *S*-linked glycopeptides display enhanced chemical and enzymatic stability and *S*-linked oligosaccharides were found to have an additional benefit when used as enzyme inhibitors as the interglycosidic sulfur atom may act as a hydrogen-bond acceptor which in nature could play an important role in binding of ligand.[21] Thiol-linked glycopolymers will see advantages either as mimics of natural glycoprotein for investigating bioprocesses or as multivalent ligands in drug delivery applications. In the current study, we present the synthesis of novel glycopolymers *via* a thiol–ene reaction of unprotected glucothiose with alkene functional scaffolds. Furthermore, we synthesised a block copolymer containing di(ethylene glycol) methyl ether methacrylate (DEGMA) and 2-hydroxyethyl methacrylate (HEMA) by RAFT polymerisation; subsequent modification with glucothiose was accomplished and the resulting glycosylated block copolymer led to the formation of thermo-responsive micelles, a potential candidate for targeted drug delivery (Scheme 1).



Scheme 1 Synthetic strategies for the preparation of glucose functionalised (co)polymers. (A1) 4-pentenoic anhydride, DMAP, pyridine, DMF; (A2) UV, glucothiose, DMPA, DMF; (B1) HEMA, AIBN, DMAc, 70 °C; (B2) AIBN, toluene, 80 °C; (B3) 4-pentenoic anhydride, DMAP, pyridine, DMF; (B4) UV, glucothiose, DMPA, DMF.

Centre for Advanced Macromolecular Design (CAMD), School of Chemical Science and Engineering, The University of New South Wales, Sydney, NSW 2052, Australia.
E-mail: M.Stenzel@unsw.edu.au; Tel: +61-293854344
† Electronic supplementary information (ESI) available: Synthetic procedures; GPC, NMR and FT-IR of all described polymers; experimental methods. See DOI: 10.1039/b900215d

 This journal is © The Royal Society of Chemistry 2009

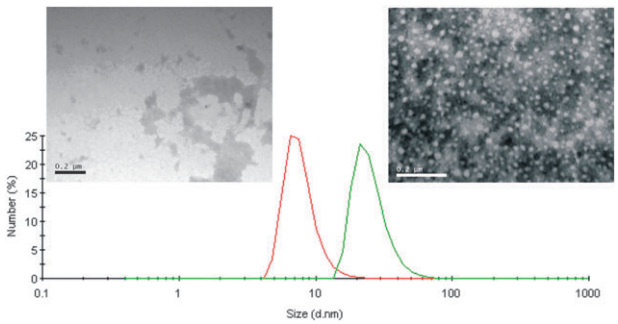


Fig. 1 TEM (above) and DLS (below) characterisation of the copolymers **(7)** at different temperatures (Left: 20 °C; Right: 40 °C).

Commercially available PolyHEMA **(1)** (M_n = 21 k, PDI = 1.68) was first used as a starting material to prepare the alkene functional polymer. The modification was carried out in DMF with 4-pentenoic anhydride at 40 °C. The successful modification is confirmed by ^{1}H NMR and FT-IR, although GPC data show only a slight increase of molecular weight to 28 k after modification that may be due to the difference of polymer to polystyrene standards (see ESI†). Polymer **(2)** was then dissolved in DMF and reacted with glucothiose sodium salt with HCl and a photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) under UV for 2 h, to afford the new thiol-linked glycopolymer **(3)** with a molecular weight of 65 k and PDI of 1.33. The disappearance of alkene signals in both NMR and FT-IR further confirms the successful modification (see ESI†). The new polymer was found—in contrast to its precursors—to be highly water-soluble.

A copolymer containing the sugar block and poly(ethylene glycol)methacrylate block was then synthesised *via* RAFT and thiol–ene click reaction to obtain thermo-responsive micelles. PEG is a cheap, neutral, water-soluble, biocompatible polymer and one of the most applied synthetic polymers in the biomedical field, and has recently raised interest in terms of its thermo-responsive water-soluble property.[22–24] Herein, the homopolymer of di(ethylene glycol) methyl ether methacrylate (DEGMA) has been obtained using the RAFT technique with cumyl dithiobenzoate (CDB) at 60 °C in toluene. The polymerisation was monitored using NIR and shows linear first order kinetics. The final polymer maintained low PDI (M_n = 20 k, PDI = 1.09) with a LCST of 29 °C (see ESI†).

PolyDEGMA **(4)** was then used for chain extension with HEMA to afford polymer **(5)** (M_n = 52 k, PDI = 1.22). A modification approach similar to that for the synthesis of **(3)** was then used to obtain a block copolymer containing glucose moieties (**(7)**, M_n = 93 k, PDI = 1.27) and the modification is further confirmed by NMR (see ESI†). It is necessary to note that in the modification step (B2), we used an approach described by Perrier and coworkers[25] to remove the RAFT groups by dissolving the polymer **(5)** in toluene and heating it with AIBN at 80 °C for 2.5 h. By removing the thiocarbonylthio endgroup possible side reactions between the alkene functionality and the terminal RAFT groups under UV are prevented.

The final glucose-containing copolymer **(7)** was fully water-soluble up to a temperature of 29 °C, which is below the LCST of PolyDEGMA. Dynamic light scattering (DLS) analysis confirmed the presence of molecularly dissolved unimers exhibiting a hydrodynamic diameter equal to 7.8 nm at 20 °C. Above this temperature, the measured hydrodynamic diameters at 40 °C were raised to 28 nm indicating the micellisation of the block copolymer, which is further confirmed by TEM (Fig. 1). No micelle formation was found for samples prepared at room temperature. For samples prepared at 40 °C, the diameters of the micelles from TEM are slightly smaller (~25 nm), which may be due to shrinkage of the micelles in the dry state.

The rate of the ligand–lectin binding is a critical factor in biological systems. A biochemical evaluation of the glycopolymers **(3)** and **(7)** was undertaken with Concanavalin A (Con A), a mannose and glucose specific lectin.[5] The rate of the binding of Con A to the glucose-containing glycopolymer was assessed by a turbidimetric assay,[12,26] measuring changes of the absorbance at 420 nm of appropriate solutions of the lectin and polymer in HEPES buffer at pH 7.4. As shown in Fig. 2, the rate of binding for **(7)** is slower than that of the homopolymer **(3)**, due to the relatively lower epitope density of the copolymer, which is consistent with previous reports.[12,26] At higher temperature, both **(3)** and **(7)** show a quicker rate of binding and for **(7)**, the change of rate is even higher, which may be due to the formation of micelles at 40 °C, where the non-active DEGMA were hidden inside. In addition, the micellar system has a high sugar density arranged in a spherical shape, which multivalency theories deem to be favourable. DLS experiments further confirmed the clustering of Con A in the presence of **(3)** or **(7)** at both temperatures, inducing a shift of hydrodynamic diameter to the range between 660 nm and 1400 nm (Fig. 2).

In summary, metal-free thiol–ene click reaction was successfully applied for the synthesis of (co)polymers containing pending sugar moieties. Thermo-responsive micelles were then obtained based on the block copolymer. This provides a convenient and non-toxic approach to make complex glycosylated macromolecular constructs, especially in creating targeted drug delivery systems. In our ongoing research, we are investigating the synthesis of glycosylated hollow particles for carrying and localising drug to specific sites and exploring other systems based on the thiol-linked glycosides. We believe this to be a promising route for sugar related synthesis and modifications.

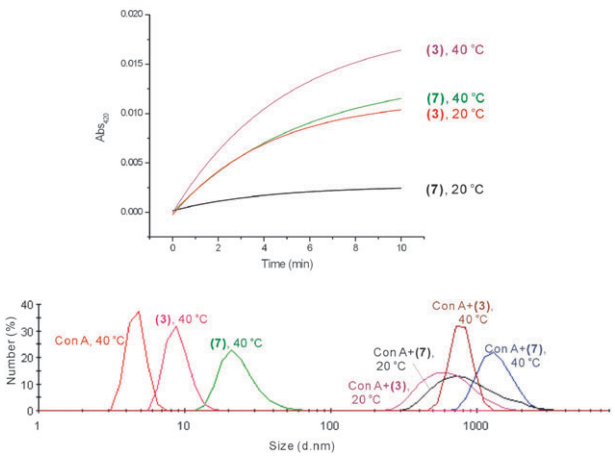


Fig. 2 Turbidimetry assay (above) and DLS (below) experiments of the glycopolymers with Con A.

Notes and references

1 A. Dove, *Nat. Biotechnol.*, 2001, **19**, 913–917.
2 L. L. Kiessling and C. W. Cairo, *Nat. Biotechnol.*, 2002, **20**, 234–235.
3 D. A. Tirrell, *Nature*, 2004, **430**, 837.
4 R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683–720.
5 M. Ambrosi, N. R. Cameron and B. G. Davis, *Org. Biomol. Chem.*, 2005, **3**, 1593–1608.
6 Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321–327.
7 J. J. Lundquist and E. J. Toone, *Chem. Rev.*, 2002, **102**, 555–578.
8 M. Mammen, S.-K. Chio and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2755–2794.
9 V. Ladmiral, E. Melia and D. M. Haddleton, *Eur. Polym. J.*, 2004, **40**, 431–449.
10 M. H. Stenzel, *Chem. Commun.*, 2008, 3486–3503.
11 S. G. Spain, M. I. Gibson and N. R. Cameron, *J. Polym. Sci., Part A: Polym. Chem.*, 2007, **45**, 2059–2072.
12 V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin and D. M. Haddleton, *J. Am. Chem. Soc.*, 2006, **128**, 4823–4830.
13 J. Geng, G. Mantovani, L. Tao, J. Nicolas, G. Chen, R. Wallis, D. A. Mitchell, B. R. G. Johnson, S. D. Evans and D. M. Haddleton, *J. Am. Chem. Soc.*, 2007, **129**, 15156–15163.
14 J. Geng, J. Lindqvist, G. Mantovani, G. Chen, C. T. Sayers, G. J. Clarkson and D. M. Haddleton, *QSAR Comb. Sci.*, 2007, **26**, 1220–1228.
15 A. Dondoni, *Angew. Chem., Int. Ed.*, 2008, **47**, 8995–8997.
16 A. Gress, A. Voelkel and H. Schlaad, *Macromolecules*, 2007, **40**, 7928–7933.
17 K. L. Killops, L. M. Campos and C. J. Hawker, *J. Am. Chem. Soc.*, 2008, **130**, 5062–5064.
18 L. M. Campos, K. L. Killops, R. Sakai, J. M. J. Paulusse, D. Damiron, E. Drockenmuller, B. W. Messmore and C. J. Hawker, *Macromolecules*, 2008, **41**, 7063–7070.
19 J. W. Chan, B. Yu, C. E. Hoyle and A. B. Lowe, *Chem. Commun.*, 2008, 4959–4961.
20 Z. Pei, H. Dong, R. Caraballo and O. Ramstrom, *Eur. J. Org. Chem.*, 2007, 4927–4934.
21 H. Driguez, *ChemBioChem*, 2001, **2**, 311–318.
22 S. Han, M. Hagiwara and T. Ishizone, *Macromolecules*, 2003, **36**, 8312–8319.
23 J. F. Lutz, O. Akdemir and A. Hoth, *J. Am. Chem. Soc.*, 2006, **128**, 13046–13047.
24 G. Chen, P. M. Wright, J. Geng, G. Mantovani and D. M. Haddleton, *Chem. Commun.*, 2008, 1097.
25 S. Perrier, P. Takolpuckdee and C. A. Mars, *Macromolecules*, 2005, **38**, 2033–2036.
26 C. W. Cairo, J. E. Gestwicki, M. Kanai and L. L. Kiessling, *J. Am. Chem. Soc.*, 2002, **124**, 1615–1619.

This journal is © The Royal Society of Chemistry 2009