Glycopolymers *via* catalytic chain transfer polymerisation (CCTP), Huisgens cycloaddition and thiol–ene double click reactions†

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CCTP has been used to give alkyne-functional macromonomers which are subsequently functionalised with sugar azides and thiols, using both CuAAC and thiol–ene Michael addition reactions, to yield end-functionalised glycopolymers in a convenient manner.

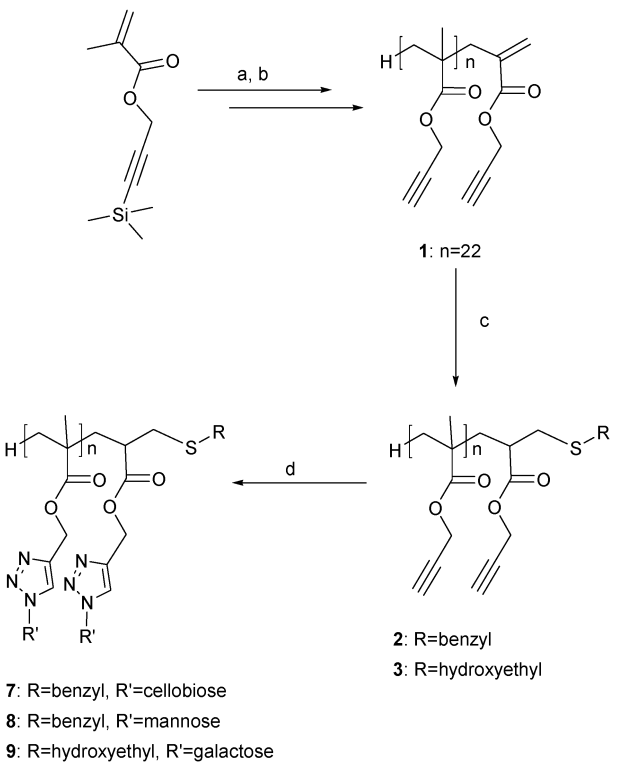
Click chemistry as a concept of simplifying synthesis is very useful in polymer science to produce complex macromolecular structures, functional polymers and protein conjugates. The Cu(I)-catalysed azide–alkyne cycloaddition (CuAAC) has been the most widely studied and employed of the available click reactions.[1–9] Recently, there has been an increasing interest in using the well-known addition of thiols to alkenes as a click process, so-called thiol–ene click chemistry.[10] Although thiol–ene click coupling has mainly been focused on a radical-mediated version to non-activated alkenes, this reaction can also proceed *via* Michael addition, especially when the vinyl group is alpha to an electron withdrawing moiety.[11–16] The use of thio-Michael addition as a click reaction was recently reported by Lowe *et al.* for the synthesis of star polymers.[17]

Synthetic glycopolymers containing pendent sugar moieties have been shown to interact multivalently with carbohydrate-binding proteins, lectins, in a similar manner to natural glycoproteins. These biomimetic properties have caused significant interest in the synthesis of glycopolymers, and a number of different strategies have been employed to obtain the required multivalent carbohydrate ligands.[18–20]

In our group, we have previously combined copper(I)-mediated living radical polymerisation (often called ATRP) and CuAAC to produce glycopolymers by post-functionalisation of well-defined "clickable" polyalkyne scaffolds with sugar azides.[21–25] An established, but dormant, method of obtaining end-functional polymers available for click reactions is catalytic chain transfer polymerisation (CCTP). This is an extremely efficient process to produce vinyl terminated methacrylic oligomers.[26–31]

In this present study we have used CCTP to give alkyne-functional oligomers available for both CuAAC and thio-Michael addition reactions. Post-functionalisation of the oligomers with these dual click reactions results in end-functionalised glycopolymers in a very convenient manner.

The "double clickable" alkyne-functional macromonomer **1** was prepared by CCTP of trimethylsilane-protected propargyl methacrylate using bis(boron difluorodimethylglyoximate) cobalt(II) (CoBF) as catalyst, followed by deprotection of the TMS groups (Scheme 1). The vinyl end group of propargyl methacrylate macromonomer **1** was reacted with benzyl mercaptan *via* a thio-Michael addition using dimethylphenylphosphine (DMPP) as catalyst. After conversion of the vinyl groups was achieved, the benzyl end-functionalised product, **2**, was isolated by precipitation. The success of the thio-Michael addition was confirmed by ^{1}H NMR with the disappearance of vinyl peaks at 5.7 ppm and 6.2 ppm and the appearance of a terminal benzyl at 7.2–7.4 ppm, Fig. 1a and 1b. To investigate the versatility of the thio-Michael addition to these oligomers, the vinyl end group of **1** was also reacted with



Scheme 1 Synthetic approach to end-functionalised glycopolymers. *Conditions*: (a) TMS-protected propargyl methacrylate, AIBN, CoBF, toluene, 60 °C; (b) TBAF, acetic acid, THF, ambient temperature (AT); (c) benzyl mercaptan or mercaptoethanol, **1**, DMPP, acetone, AT; (d) sugar azide **4**, **5** or **6**, polymer **2** or **3**, CuBr, bipy, TEA, DMSO, 60 °C.

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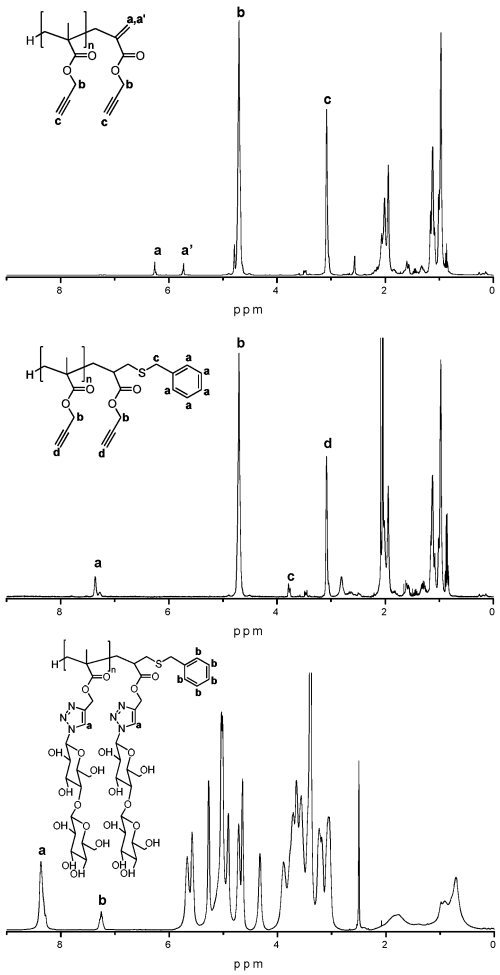

Fig. 1 ^{1}H NMR spectra of (a) **1** in acetone-d_6, (b) benzyl end-functionalised polymer **2** in acetone-d_6, and (c) benzyl end-functionalised cellobiose-functional glycopolymer **7** in DMSO-d_6.

mercaptoethanol (Scheme 1). The ^{1}H NMR spectrum of the purified hydroxyethyl-functional product **3** shows the disappearance of vinyl peaks and the appearance of peaks corresponding to the hydroxyethyl end group, indicating a successful reaction (see ESI†). MALDI-TOF analysis confirms the formation of the desired product (see ESI†). In addition, a by-product resulting from the conjugation of the DMPP to the polymer was visible in the MALDI-TOF spectrum. However, this by-product is cationic and is therefore expected to give disproportionately large peaks in this spectrum. It is noted that no peaks could be detected in the aromatic region of the ^{1}H NMR spectrum, or indeed no peaks could be detected in the ^{31}P NMR spectrum, from this by-product and thus the amount is assumed to be low.

Glycopolymers were synthesised by reacting the alkyne groups in the thiol-conjugated oligomers with sugar azides **4–6** (Chart 1) using CuAAC. Mannose azide and galactose azide were synthesised as described previously,[21] and cellobiose azide was synthesised using the same methodology, starting from the corresponding peracetylated sugar (see ESI†). The CuAAC reactions were catalysed by CuBr–bipyridine and performed in DMSO to ensure complete solubility of all reactants. Benzyl-functionalised oligomer **2**

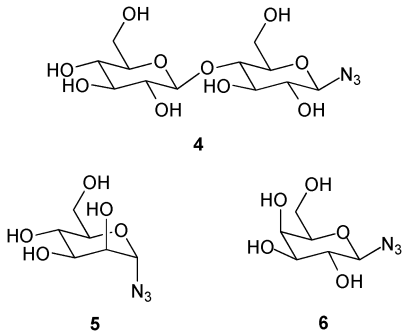

Chart 1 Sugar azides employed in the study: cellobiose azide (**4**), mannose azide (**5**) and galactose azide (**6**).

was reacted with cellobiose azide **4** to yield **7** (Scheme 1). The ^{1}H NMR spectrum of **7** shows the appearance of the triazole peak at 8.3 ppm, confirming the successful CuAAC reaction (Fig. 1c). It can also be seen that the benzyl end group is not affected by this reaction.

Oligomer **2** was reacted with mannose azide **5**, and hydroxyethyl-functionalised macromer **6** was reacted with galactose azide **7**, to yield end-functionalised glycopolymers **8** and **9**, respectively (Scheme 1). The CuAAC reactions with mannose azide and galactose azide required less catalyst and shorter reaction times than for cellobiose azide. This is in accordance with our previous results for the CuAAC reaction using lactose azide,[21] and may be explained by increased steric demands of the disaccharide cellobiose rendering the unreacted alkyne functionalities in the partly clicked polymer less accessible to further functionalisation.

Although in this study we have chosen to functionalise the oligomer end group prior to reacting the alkyne groups, it may for some applications be preferable to change the order of the reactions. To ensure that the vinyl end groups in the oligomer would not be affected by the CuAAC reaction of the alkyne side groups, unfunctionalised oligomer was also reacted with cellobiose azide. The click reaction could be confirmed by ^{1}H NMR by the appearance of a triazole peak at 8.3 ppm, and it could further be seen that the vinyl end group was retained after this reaction (see ESI†).

The potential of the end-functionalised mannose- and galactose-functional glycopolymers **8** and **9** to be recognised by different lectins was investigated. For the mannose-functional glycopolymer **8**, turbidimetry was used to study the rate of the binding of the sugar epitopes to the mannose-specific lectin concanavalin A (con A). Absorbance changes at 420 nm were measured over time in a solution of con A and **8** in HEPES buffer at pH 7.4. When the glycopolymer binds to the lectin, the formation of precipitating clusters changes the absorbance in the solution.[32] It could be seen that upon mixing **8** and the lectin con A, the absorbance initially quickly increased, then reached a plateau, indicating that most of the polymer was able to rapidly interact with the lectin, forming stable clusters (see ESI†).

For the polymer **9**, the interaction with the galactose selective lectin *Ricinus communis* agglutinin I (RCA I) was studied by affinity chromatography analysed by HPLC. A solution of **9** was injected into a column packed with immobilised RCA I. The glycopolymer is retained by the column as the sugar

moieties are recognised by the lectin. Using a mobile phase containing galactose as a competing ligand, the polymer is released from the column and can be detected by UV absorbance.[33] By increasing the concentration of galactose in the mobile phase, the amount of eluted glycopolymer increased, indicating that the retention of the polymer in the column was indeed due to the sugar–lectin interaction.

In summary, we have used CCTP to synthesise polymers containing two different clickable groups, which can be functionalised using two different click reactions. The polymer end group has been reacted using Michael addition with different thiols to obtain end-funtionalised polymers, and glycopolymers have been produced by clicking sugar moieties to the side chain of these polymers by CuAAC. The ability of the resulting end-functionalised glycopolymers to be recognised by lectins has been investigated. The results from this study indicate that the mannose- and galactose-containing glycopolymers prepared with the method described herein are able to function as multivalent ligands for the recognition of the lectins con A and RCA I, respectively.

Thus, CCTP in combination with thio-Michael addition and CuAAC is a useful synthetic approach not only to glycopolymers but also to many different types of functional polymers and conjugates.

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