Sequenced glycopolymers

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Sequence controlled multi-block glycopolymers to inhibit DC-SIGN-gp120 binding**

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Glycan-protein interactions are essential for many physiological processes including cell-cell recognition, cell adhesion, cell signalling, pathogen identification and differentiation. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN; CD209) is a C-type lectin (carbohydrate-binding protein) present on both macrophages and dendritic cell subpopulations and play a critical role in many cell interactions. DC-SIGN binds to microorganisms and host molecules by recognizing surface rich mannose containing glycans through multivalent glycan-protein interactions and serves a target for several viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV).[1] Carbohydrate binding proteins (CBP) have been suggested as potential candidate microbiocides for the prevention of HIV infection.[2] However, the isolation of natural CBPs is relatively difficult due to their hydrophilic nature and they show notably low affinities to virus.[3],[4] and thus synthetic lectins are of interest for carbohydrate recognition studies.[5] Alternatively, non-carbohydrate inhibitors of mammalian lectins can be used to prevent the interaction between DC-SIGN and gp120.[6] The architectures of the multivalent ligands have a great effect on carbohydrate binding to lectins and the use of linear polymers on effective lectin binding has been demonstrated by several research groups.[7]

Synthetic polymer chemistry has developed rapidly in recent years.[8] Currently, polymerization of functional monomers with desired chain length, architecture and composition is straightforward whereas polymers with any monomer sequence control structures remains challenging[9][10] which have implications for the controlled folding of synthetic macromolecules.[11] There are a few recent reports where sufficient control has been achieved in controlling the monomer sequence along the polymer chain.[12] To the best of our knowledge this is the first report where some control over the relative position of sugars is exhibited with a demonstration of their binding to the human lectin DC-SIGN. We have utilized a controlled polymerization technique, single electron transfer living radical polymerization (SET-LRP)[13][14][15][12c], to polymerize glycomonomers reaction prior to polymerization.

A series of glycomonomers were prepared by reaction of 3-azidopropylacrylate (APA) and alkylated mannose, glucose and fucose, *via* Fischer-Helferich glycosylation. This was performed using $CuSO_4$ and sodium ascorbate in methanol/water mixture (see supporting information). SET-LRP of glucose monomer (GluA) was performed in dimethylsulfoxide (DMSO) using a Cu(0)/Cu(II) and Me_6TREN derived catalyst. Polymerization reached over 90% monomer conversion in 6 hours whilst maintaining a narrow molecular weight distribution with increasing molecular weight. (ESI, Fig. S4) The obtained polymers were characterized by size exclusion chromatography (SEC) and matrix assisted laser desorption ionization time of flight mass spectroscopy (MALDI-ToF MS) or high-resolution electrospray ionization mass spectroscopy (HR-ESI MS) that indicated very high chain end fidelity allowing for sequential monomer addition.

We designed a polymerization reaction starting with 1 equivalent of initiator and 2 equivalents of mannose glycomonomer (ManA), (Fig 1a). ManA was fully consumed after 12 hours, 2 equivalents of GluA in DMSO were added to the reaction mixture and GluA was consumed in 16 hours and 2 equivalents of ManA in DMSO was subsequently injected into the reaction mixture. This cycle was continued until six short blocks of glycopolymers was achieved (DP=2 for each block, $(mannose)_2$-$(glucose)_2$-$(mannose)_2$-$(glucose)_2$-$(mannose)_2$-$(glucose)_2$). No purification steps were required prior to subsequent addition of the subsequent monomer. The conversion of the first four blocks, ^{1}H NMR analysis, reached 100% evidenced by complete disappearance of vinyl groups, 5.7 to 6.5 ppm. The glycomonomers were dissolved in purged DMSO prior to the addition and this resulted in further dilution of the reaction medium for each monomer addition. Traces of vinyl groups could still be detected after the fifth (conv: 99%) and sixth (conv: 97%) blocks. Moreover, the reaction was followed by SEC and, especially for the short chain length, the resolution of the columns allowed identification of the molecules with one, two and three repeating units as would be expected for a controlled polymerisation reaction.

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

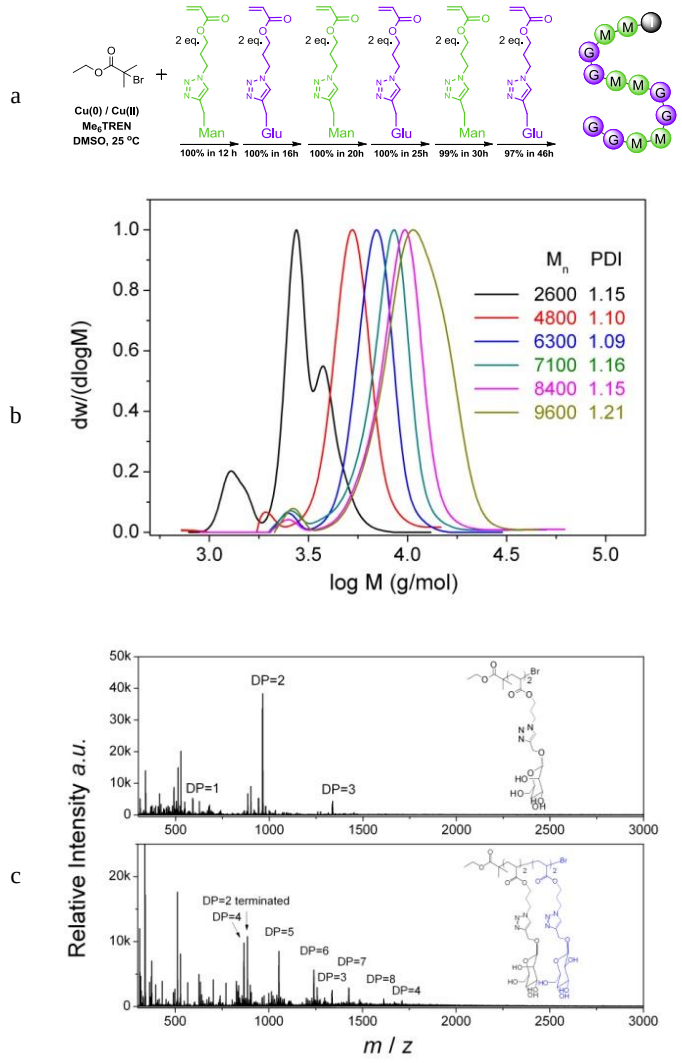

Figure 1. Sequence controlled mannose-glucose hexa block copolymer. **a,** Schematic representation of the sequence controlled multi block copolymerization of ManA and GluA. **b,** SEC traces of the glycopolymers prior to each addition of glycomonomer. **c,**HR-ESI MS of the first poly(Man)$_2$ and second block poly(Man)$_2$-*s*-(Glu)$_2$.

The hydrodynamic volume of the polymers increased with every monomer addition and polydispersity indices (PDI) remained low (Fig 1b). A more detailed structural characterization was performed using HR-ESI MS and most of the formed molecules could be identified (Fig 1c).

Similar reaction conditions were employed for the preparation of a polymer with longer block lengths. The average degree of polymerization was increased to 4 and the reaction time was kept > 20h for each step to ensure the full monomer conversion. The molecular weight of the first block (poly(Man)$_4$) =4.7 kDa with a PDI =1.11 and after addition of 4 eq. GluA the molecular weight of the polymer (poly(Man)$_4$-*s*-(Glu)$_4$) was increased to 7.7 kDa with a PDI = 1.08. However, after the third (4 eq. of Man) and fourth (4 eq. of Glu) glycomonomer additions, the PDI of the polymers increased to 1.22 and 1.37, respectively, which indicate some loss of control and a reduction in chain end fidelity. (Fig.S10) The chain end fidelity was probed by transformation of the bromide to azide and performing CuAAC with propargyl dibromomaleimide. SEC equipped with both RI and UV detectors (λ = 400 nm) demonstrated a high chain end fidelity for the hexablock polymer (FigS20).

This approach was extended further by the introduction of a third, and different glycomonomer, fucose acrylate (FucA) and prepared a hexablock copolymer of ManA, GluA and FucA. (ESI, Fig S11) Molecular weights of the obtained glycopolymers increased as expected and PDI values remained <1.13 throughout the reaction. This allows for the synthesis of synthetic glycans that may form the basis of an extensive synthetic glycocode.[16]

Stimuli-responsive materials attract attention, as this allows for solubility changes upon corresponding changes in the surrounding environment. It should also be noted that the distance between sugar units can have a critical importance for lectin binding. Therefore, we developed a thermo-responsive mannose carrying sequence controlled multi-block copolymer. (Fig. 2) SET-LRP of di(ethylene glycol) ethyl ether acrylate (DEGEEA) was initiated with a [monomer]/[initiator] ratio of 6 and the monomer conversion reached over 90% in 2.5 h, **S4**. In the subsequent step, 3 equivalents of ManA were added and more than 90% of this monomer was consumed in 4.5 h and the cycle continued until the polymer reached a hexablock structure. SEC traces (Fig 2b) showed an increased molecular weight where the PDI values stayed <1.28 throughout the reaction. More detailed structural characterization was performed utilizing MALDI-ToF MS (Fig 2c) and all species could be identified. These examples show a level of sequence control obtained in one pot, relatively large scale, using cheap starting materials, and with the minimum work up at the end polymerization.

Interactions between the glycopolymers and DC-SIGN were measured using surface plasmon resonance spectroscopy. ManMA$_{58}$ was used as a control for binding (Fig 3a).[17] All glycopolymers and gp120 (analytes) were measured at 6-10 different concentrations and a representative spectrum is shown in Figure 3c. The blue zones are the ones where buffer was flowed over the chip alonebefore (90 sec) and after (650 sec) injection of the analyte (900 s). Association (k_{on}) and dissociation rate constants (k_{off}) were calculated using a 2-ligand binding model using Biacore evaluation software, and K_D values were calculated from the ratio of k_{off} to k_{on}, (Table 1) HIV gp120 showed the highest affinity for DC-SIGN (0.11 nM). All of the glycopolymers bound to DC-SIGN with affinities ranging between 0.6 and 34 nM. Typically, polymers with a higher mannose content bound with higher affinities reflecting the known preference of DC-SIGN for mannose over glucose. Incorporation of fucose restored a degree of high affinity binding.[18]

It is noted that the k_{off} values were low and approaching lower limit for surface plasmon resonance measurements.

Competition experiments were performed by flowing 4 nM of DC-SIGN in buffer with varying amounts of glycopolymers over immobilized gp120. In the representative SPR sensogram (Fig 3d) the highest R_{max} is observed, as expected, when DC-SIGN is passed over gp120 in the absence of glycopolymer, thus representing maximal binding. DC-SIGN binding decreases upon addition of more glycopolymer as carbohydrate moieties of the polymer block binding sites on the lectin (ESI, Fig S33). The lowest IC50 value observed was for solution-phase gp120 (11 nM). Nevertheless, impressive inhibition was also observed for ManMA$_{58}$, ManA$_{23}$ and ManA$_{13}$-b-OEGA$_2$, only 10-30-fold weaker than for gp120. S3, S5 and S6 showed little inhibition over the measured concentration range. Favourable binding of ManA$_{23}$ over ManMA$_{58}$, despite its shorter chain length may reflect differences in acrylic and methacrylic polymer backbones and also the linker structure, which could lead to different behaviour in chain folding and lectin binding (ESI, Fig S33).

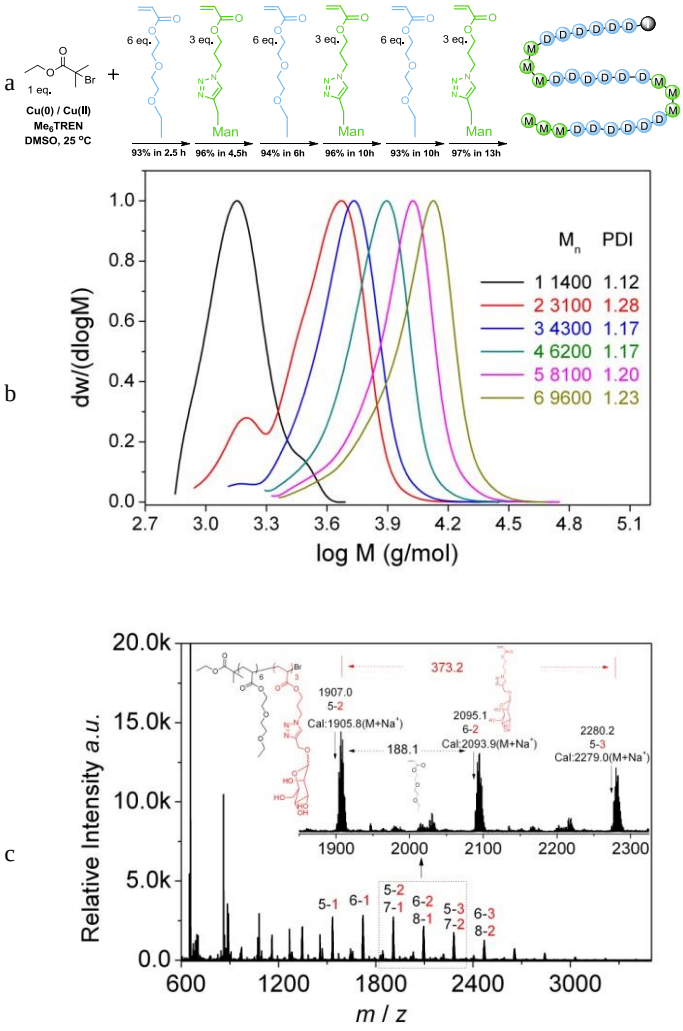

Figure 2. Sequence controlled DEGEEA-mannose hexa block copolymer. **a,** Schematic representation of the sequence controlled multi block copolymerization of DEGEEA and ManA. **b,** SEC traces of the glycopolymers before each addition of monomer. **c,** MALDI-ToF MS of the second block poly(DEGEEA)$_6$-*s*-(Man)$_3$.

Table 1.The results of binding kinetics and inhibition concentration of glycopolymers.

Code	Sequence	DC-SIGN binding			IC50 (nM)
		k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})[a]	K_D (nM)[b]	
gp120	gp120	7.3×10^{5}	7.8×10^{-5}	0.11	11
C1	$ManMA_{58}$	2.9×10^{5}	2.0×10^{-4}	0.66	230
S1	$ManA_{23}$	8.0×10^{4}	3.1×10^{-5}	0.39	153
S2	$ManA_{13}$-*b*-$OEGA_2$	3.6×10^{4}	7.6×10^{-5}	2.2	380
S3	$ManA_9$-*r*-$DEGEEA_{18}$	3.9×10^{3}	2.6×10^{-5}	6.6	>1000
S4	$ManA_9$-*s*-$DEGEEA_{18}$	4.7×10^{3}	6.7×10^{-5}	14	>1000
S5	$GluA_6$-*s*-$ManA_4$-*s*-$FucA_4$	4.0×10^{3}	6.2×10^{-5}	15	>1000
S6	$GluA_4$-*s*-$ManA_4$-*s*-$GluA_4$	9.7×10^{3}	9.7×10^{-5}	34	n/a

[a] k_{off} values are close to the limit of the SPR technique, so probably reflect upper limits. [b] For the same reason in [a] above, K_D values probably also represent upper limits. *-b-*, *-r-*, and *-s-* stand for block, random, and sequence controlled, respectively.

This supports that inhibition of binding of DC-SIGN to gp120 is via blocking carbohydrate-binding sites on the lectin. Notably, however, K_D and IC50 values were different for each polymer. For example, K_D and IC50 values of ManA13-*b*-OEGA2 were 2.18 nM and 380 nM, respectively. These differences reflect the complex, multivalent nature of the interactions between polymer and DC-SIGN as well as between gp120 and DC-SIGN. Binding is shown to be dependent on both the sequence and structure of the polymer highlighting the potential importance of this synthetic strategy.

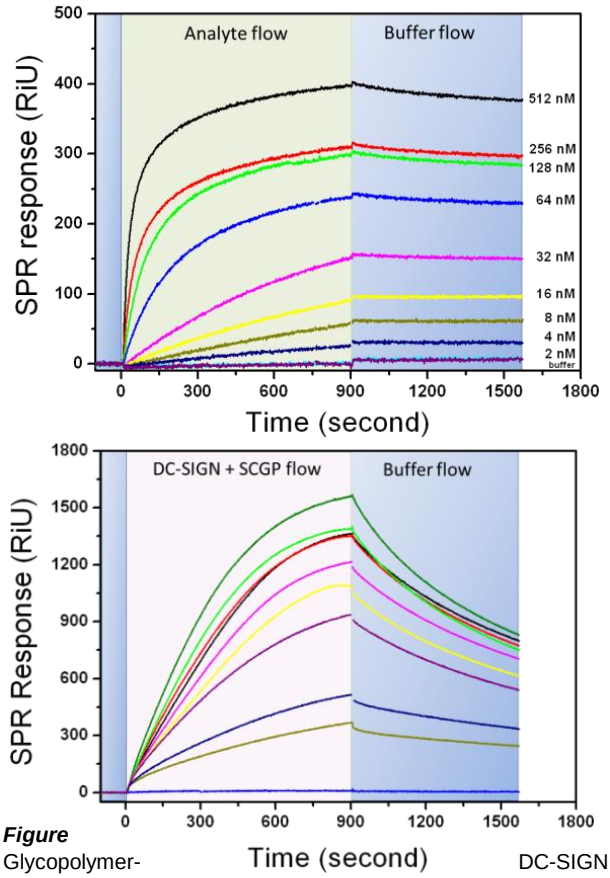

Figure 3. Glycopolymer-DC-SIGN binding and competition assays.***Top*,** typical SPR sensograms showing the DC-SIGN glycopolymer **S1** interaction at different concentrations.***Bottom*,**dark green trace with the highest intensity belongs to DC-SIGN binding to gp120 in the absence of any glycopolymer and the lower ones belong to competitive binding in the presence glycopolymer **S1** at different concentrations.

In conclusion, we have prepared multi-block glycopolymers with a degree of monomer sequence controlled in various compositions from glycomonomers containing mannose, glucose and fucose moieties. Polymerizations were followed by ^{1}H NMR, SEC and HR ESI-MS or MALDI-ToF MS to obtain information on the products. This technique gives a good degree of control over the monomer sequence along a polymer chain. The polymerization is performed in one pot by sequential addition of the subsequent monomers in a relatively a large scale. The obtained glycopolymers were examined for their binding behaviour to DC-SIGN. We observed higher affinity binding for the polymers with higher mannose content. However, we could not conclude any effect of sequence on binding in this system, most likely because DC-SIGN preferentially recognises a range of high-mannose structures whilst other DC-SIGN binding glycans of mixed monosaccharide character possess very defined glycosidic branching.[18b] Further work will be performed using lectin libraries and sequence controlled polymer

libraries to evaluate the sugar sequence or distance and their impact on specific lectin binding properties, in addition to the polymerization of small disaccharide and oligosaccharide units such as Man-α-(1,2)-Man and blood group antigens. Nevertheless, the synthesized polymers reported here show distinct binding properties to DC-SIGN and an inhibition of the DC-SIGN binding to HIV gp120 using nano molar concentrations.

Syntheses of all materials and details of characterisation methods are provided in the supporting information.

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