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 plays 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 as 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 microbicides for the prevention of HIV infection.[2] However, the isolation of natural CBPs is relatively difficult because of their hydrophilic nature and low affinity for the virus.[3,4] 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 structures of these multivalent ligands have a great effect on carbohydrate binding to lectins, and the use of linear polymers to effectively inhibit 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 the desired chain length, structure, and composition is straightforward; whereas producing polymers with monomer sequence control remains challenging,[9,10] which has 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 the sugars is exhibited and their binding to the human lectin DC-SIGN is demonstrated. We have used a controlled polymerization technique, single-electron transfer living radical polymerization (SET-LRP),[12c,13–15] to polymerize glycomonomers, which are prepared by copper catalyzed azide–alkyne click (CuAAC) reaction prior to polymerization.

A series of glycomonomers were prepared by reaction of 3-azidopropylacrylate (APA) and alkylated mannose, glucose, and fucose using a Fischer–Helferich glycosylation. This was performed using $CuSO_4$ and sodium ascorbate in a methanol/water mixture (see the Supporting Information). SET-LRP of the glucose monomer (GluA) was performed in dimethylsulfoxide (DMSO) using a copper(0)/copper(II) and tris[2-(dimethylamino)ethyl]amine (Me_6TREN)-derived catalyst. Polymerization reached over 90 % monomer conversion in six hours whilst maintaining a narrow molecular weight distribution with increasing molecular weight. (Supporting Information, Figure S4). The obtained polymers were characterized by size exclusion chromatography (SEC) and MALDI-TOF mass spectroscopy (MS) or high-resolution electrospray ionization mass spectroscopy (HR-ESI MS), which indicated very high chain-end fidelity allowing for sequential monomer addition.

We designed a polymerization reaction starting with one equivalent of initiator (I) and two equivalents of mannose glycomonomer (ManA; Figure 1a). ManA was fully consumed after 12 hours; then two equivalents of GluA in DMSO were added to the reaction mixture and GluA was consumed in 16 hours. Two equivalents of ManA in DMSO were subsequently added to the reaction mixture, and this cycle was continued until six short blocks of glycopolymers were produced (the degree of polymerization (DP) = 2 for each block, $(mannose)_2$-$(glucose)_2$-$(mannose)_2$-$(glucose)_2$-$(mannose)_2$-$(glucose)_2$). No purification steps were required prior to addition of the subsequent monomer. The conversion of the first four blocks, as analyzed by ^{1}H NMR spectroscopy, reached 100 %, shown by the complete disappearance of vinyl groups at 5.7–6.5 ppm. The glycomonomers were dissolved in purged DMSO prior to their addition and this resulted in further dilution of the reaction mixture upon each monomer addition. Traces of vinyl groups could still be detected after

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Supporting information for this article (syntheses of all materials and details of the characterization methods) is available on the WWW under http://dx.doi.org/10.1002/anie.201300068.

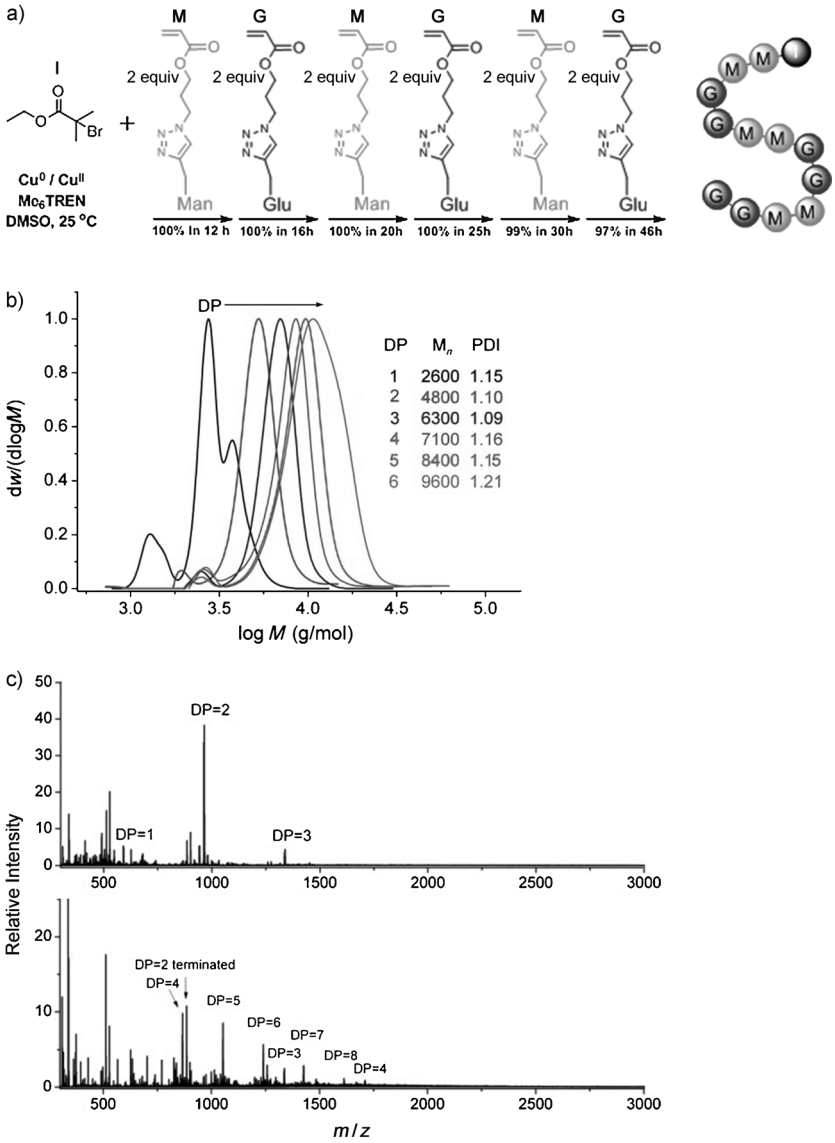

Figure 1. Sequence-controlled mannose–glucose hexablock copolymer. a) Schematic representation of the sequence-controlled multi-block copolymerization of ManA (M) and GluA (G). b) SEC traces of the glycopolymers prior to each addition of glycomonomer. DP = degree of polymerization. Arrow shows the direction of increasing DP. c) HR-ESI MS of the first poly(Man)$_2$ (top) and second block poly(Man)$_2$-*s*-(Glu)$_2$ (bottom).

the fifth (conversion = 99 %) and sixth (conversion = 97 %) blocks. Moreover, the reaction was followed by SEC and, especially for the short chain lengths, the resolution of the columns allowed identification of the molecules with one, two, or three repeating units, as would be expected for a controlled polymerization reaction. The hydrodynamic volume of the polymers increased with each monomer addition and the polydispersity indices (PDI) remained low (Figure 1 b). A more detailed structural characterization was performed using HR-ESI MS and most of the formed molecules could be identified (Figure 1 c).

Similar reaction conditions were used for the preparation of a polymer with longer block lengths. The average degree of polymerization was increased to four and the reaction time was kept at greater than 20 hours for each step to ensure full monomer conversion. The molecular weight of the first block poly(Man)$_4$ = 4.7 kDa with a PDI = 1.11. After addition of four equivalents of 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 equiv of Man) and fourth (4 equiv 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 (Figure S10). The chain-end fidelity was probed by transformation of the bromide to azide and performing a CuAAC reaction with propargyl dibromomaleimide. SEC equipped with both refractive index and UV detectors ($\lambda = 400$ nm) showed a high chain-end fidelity for the hexablock polymer (Figure S20).

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

Stimuli-responsive materials are important because they allow for solubility changes upon corresponding changes in the surrounding environment. Notably, the distance between the sugar units can be critically important for lectin binding. Therefore, we developed a thermo-responsive mannose-carrying sequence-controlled multi-block copolymer (Figure 2). SET-LRP of di(ethylene glycol) ethyl ether acrylate (DEGEEA) was initiated with a [monomer]/[initiator] ratio of six and the monomer conversion reached over 90 % in 2.5 hours, **S4** (Table 1). In the subsequent step, three equivalents of ManA were added and more than 90 % of this monomer was consumed in 4.5 hours. The cycle continued until the polymer reached a hexablock structure. SEC traces (Figure 2 b) showed an increased molecular weight where the PDI values stayed below 1.28 throughout the reaction. More detailed structural characterization was performed using MALDI-TOF MS (Figure 2 c) and all species could be identified. These examples show a level of sequence control obtained in one pot, with a relatively large scale, using cheap starting materials, and with minimal work up at the end of the polymerization.

Interactions between the glycopolymers and DC-SIGN were measured using surface plasmon resonance (SPR)

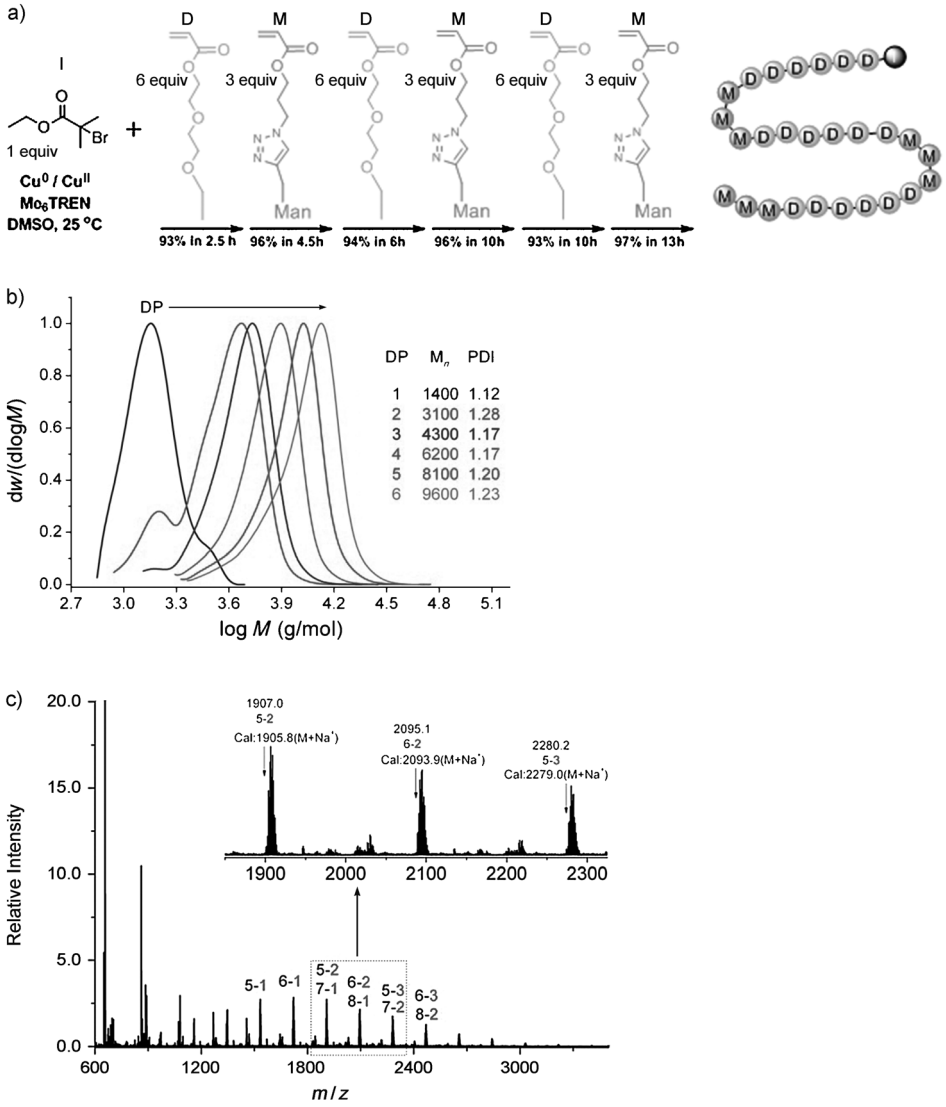

Figure 2. Sequence-controlled DEGEEA–mannose hexablock copolymer. a) Schematic representation of the sequence-controlled multi-block copolymerization of DEGEEA (D) and ManA (M). b) SEC traces of the glycopolymers before each addition of monomer. Arrow shows the direction of increasing DP. c) MALDI-TOF MS of the second block poly(DEGEEA)$_6$-*s*-(Man)$_3$. Inset shows an expanded view of the boxed region. Hyphenated numbers of the form *X*-*Y* show *X* = DP of DEEGA and *Y* = DP of ManA.

Table 1: Binding kinetics and inhibition concentration of glycopolymers.

Code	Sequence	DC-SIGN binding k_{on} [$M^{-1}s^{-1}$]	k_{off} [s^{-1}][a]	K_D [nM][b]	IC_{50} [nM]
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 detection, so probably reflect upper limits of such values. [b] For the same reason as in [a], K_D values probably also represent the upper limits of such values. *b* = block, *r* = random, and *s* = sequence controlled.

spectroscopy. $ManMA_{58}$ (**C1**) was used as a control for binding (Figure S22).[17] All glycopolymers and gp120 (analytes) were measured at 6–10 different concentrations and a representative spectrum is shown in Figure 3a. Buffer alone was flowed over the chip before (90 s) and after (650 s) injection of the analyte (900 s). Association (k_{on}) and dissociation (k_{off}) rate constants were calculated with a two-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 somewhat higher binding affinity.[18] It is of note that the k_{off} values were low and approaching the 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 (Figure 3b) 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 (Figure S33). The lowest IC_{50} 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**, **S4**, **S5**, and **S6** showed little inhibition over the measured concentration range. Favorable binding of $ManA_{23}$ over $ManMA_{58}$, despite its shorter chain length may reflect the differences in the acrylic versus methacrylic polymer backbones and also the linker structure, which could lead to different behavior in chain folding and lectin binding (Figure S33).

This shows that inhibition of binding of DC-SIGN to gp120 is achieved by blocking carbohydrate-binding sites on

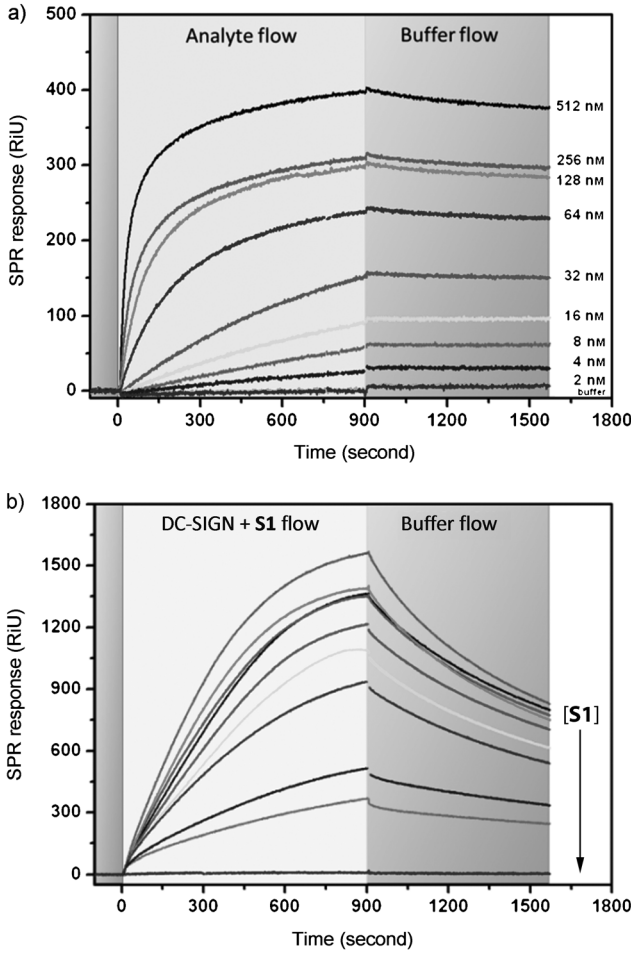

Figure 3. Glycopolymer–DC-SIGN binding and competition assays. a) typical SPR sensograms showing DC-SIGN interacting with glycopolymer **S1** at different concentrations (RiU = refractive index unit). b) Trace with asterisk (*) belongs to DC-SIGN binding to gp120 in the absence of any glycopolymer and the lower ones show binding in the presence of competitor glycopolymer **S1** at increasing concentrations in the direction of the arrow.

the lectin. Notably, however, K_D and IC_{50} values were different for each polymer. For example, K_D and IC_{50} values of $ManA_{13}$-*b*-$OEGA_2$ were 2.18 nM and 380 nM, respectively. These differences reflect the complex, multivalent nature of the interactions between the polymer and DC-SIGN, as well as between gp120 and DC-SIGN. Binding was shown to be dependent on both the sequence and structure of the polymer, highlighting the potential importance of this synthetic strategy.

In conclusion, we have prepared multi-block glycopolymers with a degree of monomer sequence control 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 gave a good degree of control over the monomer sequence along a polymer chain. The polymerization was 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 behavior 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 recognizes 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 herein show distinct binding properties to DC-SIGN and an inhibition of the DC-SIGN binding to HIV gp120 using nanomolar concentrations.

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