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Synthesis of Brush-Like Glycopolymers with Monodisperse, Sequence-Defined Side Chains and Their Interactions with Plant and Animal Lectins

*Fadi Shamout, Alessandra Monaco, Gokhan Yilmaz, Caglar Remzi Becer, and Laura Hartmann**

The synthesis of brush glycopolymers mimicking the architecture of proteoglycans is achieved by grafting sequence-defined glycooligomers derived from solid-phase polymer synthesis onto a poly(active ester) scaffold. This approach gives access to a first library of brush glycopolymers with controlled variations in the degree of branching and number of carbohydrate ligands per branch. When studying lectin binding of linear and brush glycopolymers to lectins Concanavalin A (ConA), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), and mannose-binding lectin (MBL), different preferences are observed with MBL showing higher binding to linear glycopolymer and ConA and DC-SIGN favoring brush glycopolymers. This finding suggests that the architecture of polymeric glycan mimetics affects binding to lectins not only in terms of creating higher avidity but potentially also selectivity ligands.

Glycopolymers are a widely studied class of bioactive polymers presenting carbohydrate ligands in the side chains along a synthetic, polymeric scaffold.[1] They are considered glycan mimetics as they show binding to carbohydrate-recognizing protein receptors such as lectins that is comparable with their natural glycan analogs, oligo-, and polysaccharides.[2] One of the outstanding features of carbohydrate–lectin interactions is the usually weak binding of single glycan ligands or fragments to a receptor.[3] An increase in binding is achieved through multivalent interactions, for example, of a single ligand with several receptor binding sites or multiple ligands interacting with multiple receptor binding sites or receptor molecules.[4] Such avidity can also be achieved by glycopolymers through the multivalent presentation of carbohydrate ligands along the polymer chain.[5] Indeed, Nature itself uses different strategies to realize multivalent presentation: besides the all-carbohydrate oligo- and polysaccharides, glycoconjugates such as glycolipids and glycoproteins constitute major classes of carbohydrate ligands. An important example are proteoglycans consisting of a high molecular weight protein scaffold with oligosaccharide side chains. Proteoglycans are a highly diverse class of glycoconjugates, both in terms of their composition and function: they are essential structures in the cell wall of most bacteria while others play key roles in embryogenesis and regenerative processes.[6] The common feature, however, is their brush-like architecture. In this work we aim at creating polymer-based glycan mimetics that explore the brush-like structure of proteoglycans to study the effect of such polymeric architectures on their lectin binding properties. Glycopolymer brushes have previously been realized either through attachment of glycopolymer chains onto solid surfaces, for example, gold chips or nanoparticles, or through various grafting methods, for example, grafting-from,[7] grafting-through,[8] or grafting-to[9] approaches. Stenzel and co-workers have shown that when presenting glucose ligands either on a linear polymeric scaffold or branched dendritic scaffold within micellar assembled structures, the branched structure gives a higher avidity in binding to Concanavalin A (ConA), an important model lectin.[10] Similarly, studies by Müller and co-worker[11] and Ulbricht and co-worker[12] showed increased lectin binding for branched versus linear glycopolymers. More recently, Stenzel and co-workers demonstrated that high density of fructose ligands is required in brush-like micellar assemblies to achieve efficient cell uptake.[13] However, to the best of our knowledge, the effect of branching on the binding of glycopolymers toward different lectins has not been studied before.

In order to achieve high levels of control over the branched architecture, in this study we employ for the first time sequence-defined and monodisperse glyco(oligoamides) as side chains of soluble brush glycopolymers. These oligomeric glycan mimetics are obtained via the solid phase assembly of tailor-made building

F. Shamout, Prof. L. Hartmann
Department for Organic Chemistry and Macromolecular Chemistry
Heinrich Heine University Duesseldorf
Universitätsstraße 1
Düsseldorf 40225, Germany
E-mail: laura.hartmann@hhu.de
A. Monaco, Prof. C. R. Becer
Department of Chemistry
University of Warwick
Coventry CV4 7AL, UK
G. Yilmaz
School of Pharmacy
University of Nottingham
Nottingham NG2 2RD, UK
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/marc.201900459.

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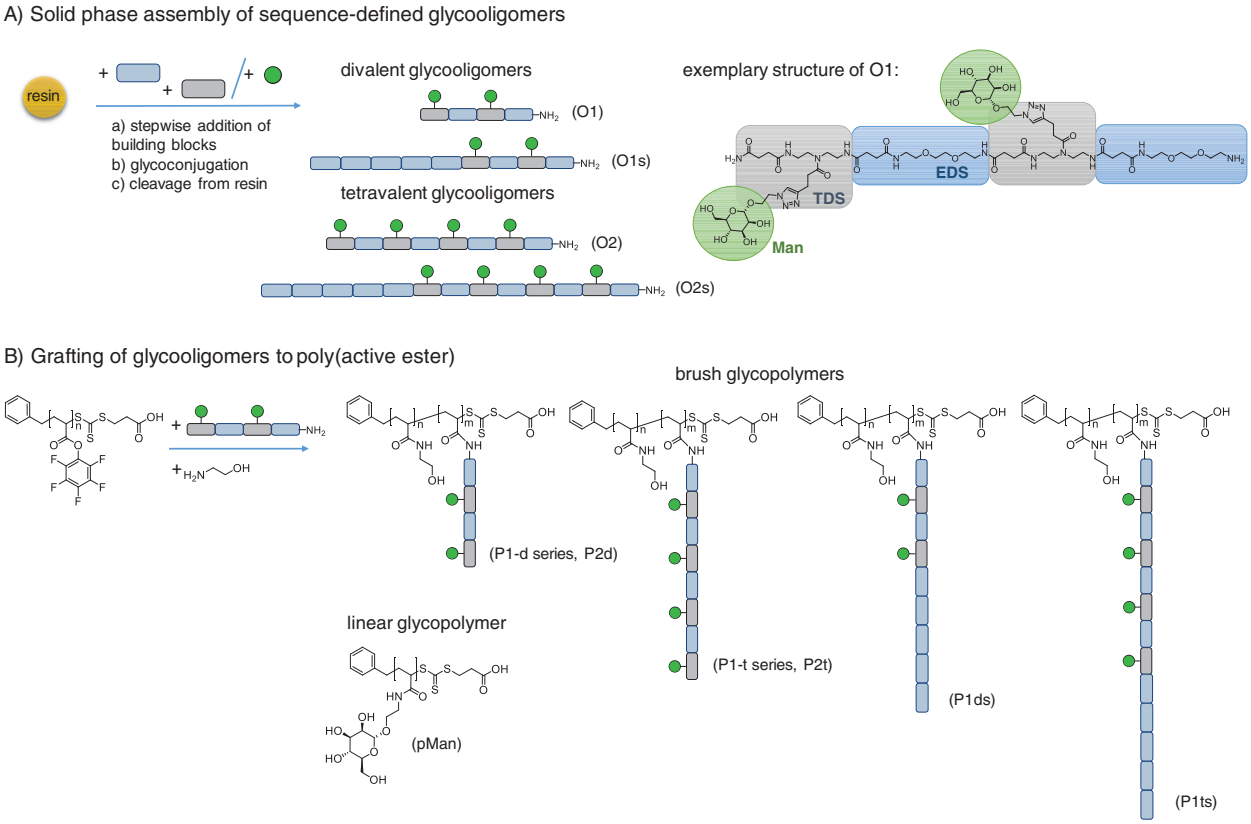

Figure 1. Schematic presentation of brush glycopolymer synthesis starting from A) solid phase assembly of sequence-defined glycooligomers followed by B) grafting of glycooligomers to poly(pentafluorophenyl acrylate) (pPFP) poly(active ester) scaffold. Nomenclature for the glycopolymers indicates use of one out of two scaffolds of different molecular weight (P1 with average DP of 40 and P2 with average DP of 100) functionalized with either divalent (d) or tetravalent (t) glycooligomers as well as glycooligomers with additional shielding block (ds or ts).

blocks as previously introduced and allow for control over the number and position of carbohydrate ligands along the scaffold.[14] Glycooligomers are then conjugated onto a synthetic polymer backbone giving brush glycopolymers with control over a) number of carbohydrates per side chain, b) positioning of carbohydrates in the side chains, c) number of side chains, d) overall chain length of polymer scaffold, where c) and d) are controlled within the limits of the dispersity of the polymer scaffold while a) and b) are defined by the monomer sequence of the glycooligomers (**Figure 1**).

As scaffold, a poly(pentafluorophenyl acrylate) (pPFP) active ester system as introduced by Theato and co-workers[15] was chosen and synthesized using benzylsulfanylthiocarbonylsulfany propionic acid (BSTPA) as RAFT-agent[16] for controlled radical polymerization of pentafluorophenyl acrylate following established protocols.[17] Glycofunctionalized side chains were synthesized using previously presented solid phase polymer synthesis of precision glycooligomers.[19] In short, building blocks presenting a free carboxy- and a Fmoc-protected amine group are assembled on solid support using Fmoc peptide coupling chemistry. Here, two types of building blocks were employed: A functional building block introducing an alkyne side chain (TDS) and a spacer building block introducing an ethylene glycol linker in the main chain (EDS) (Figure 1).[18] After assembly of the desired oligomer, azido functionalized acetylated Mannose (Man) was conjugated via copper(I)-catalysed alkyne azide cycloaddition (CuAAC) followed by deacetylation and cleavage from the resin (see Supporting Information for details on synthetic protocols).[18] Through variation of the number and position of TDS building blocks during solid phase assembly, glycooligomers with controlled variations of the number and positioning of Man ligands were obtained.

Polymer scaffolds of two molecular weight were synthesized and applied for grafting of glycooligomers with an average degree of polymerization (DP) of 40 (P1 series) and 92 (P2 series), respectively. In order to determine molecular weights of the polymer scaffold and to be used as negative controls in later binding studies, both polymers were first reacted with ethanolamine to obtain poly(hydroxyethylacrylamide) (pHEAA) (P1 and P2). Full conversion of pentafluorophenol groups was analyzed by ^{19}F-NMR and ^{1}H-NMR, molecular weights and dispersity were measured by aqueous gel permeation chromatography (GPC) showing molecular weights of 4.6 and 10.6 kDa, respectively with narrow dispersity of 1.01 and 1.08, respectively (Table S1, Supporting Information).

Glycooligomers with either two (divalent, O1) or four (tetravalent, O2) Man ligands were synthesized. Furthermore, both glycooligomers were also obtained as diblock structures adding a stealth block of five EDS building blocks at the N-terminus of the oligomer (O1s and O2s). All glycooligomers were obtained in high purity after cleavage from the support and precipitation from diethyl ether (see Supporting Information for details on synthetic method and analytical data).

Grafting of glycooligomers onto pPFP was carried out in DMF using the same stock solution of poly(active ester) and different amounts of glycooligomers in order to achieve brush-like glycopolymers with different numbers of side chains. pPFP and glycooligomers were stirred for 24 h at 40 °C and subsequently quenched with ethanolamine for additional 24 h to ensure full conversion (see Supporting Information for details on synthetic method and analytical data). Full conversion of all pentafluorophenol side chains was monitored by ^{19}F-NMR (see Supporting Information). Brush glycopolymers were isolated after precipitation from acetone and dialysis in water.

Overall two series of brush glycopolymers were obtained using P1 as scaffold and varying the number of side chains from 1–35 for both the divalent (P1-d series) and tetravalent glycooligomers (P1-t series) (**Table 1**) as well as glycopolymers carrying shielding EDS-blocks in every side chain (P1-ds, P1-ts). Furthermore, based on the higher molecular weight scaffold P2, two additional brush glycopolymers presenting either O1 or O2 in the side chain were obtained (P2-d, P2-t). For comparison with the brush glycopolymers, linear Man functionalized glycopolymer (pMan) was obtained by conjugation of P1 with amine functionalized Man (Figure 1). For all glycopolymers, degree of functionalization and molecular weights were determined by ^{1}H-NMR and aqueous GPC showing molecular weights ranging from 8.4 up to 60 kDa with low dispersities of 1.01–1.15 (Table S1, Supporting Information).

Coupling efficiency decreases with increasing chain length of both, oligomers and polymers, probably due to reduced accessibility of functional groups. Accordingly, functionalization of oligomers carrying the additional EDS-block (O1s and O2s) and functionalization of higher molecular weight P2 showed lowest coupling efficiencies (see Supporting Information). Nevertheless, using different equivalents of oligomer per polymer scaffold, glycopolymers with different degrees of branching were obtained. In order to differentiate glycopolymers with different numbers of side chains, equivalents used during coupling are included in the polymer nomenclature, for example, P1-10d indicating P1 scaffold reacted with 0.1 equivalents of divalent glycooligomer targeting a maximum degree of functionalization of 10%. Table 1 gives effective number of side chains as determined by ^{1}H-NMR and GPC.

With this set of oligomers and polymers in hand, effects of brush in comparison to linear architecture on lectin binding were studied by measuring binding toward Man-recognizing lectins ConA, DC-SIGN, and MBL.

First, binding of glycooligomers (O-series) as well as brush glycopolymers (P-series) to ConA was measured using surface plasmon resonance (SPR) spectroscopy. A direct binding assay was performed following previously established protocols (see Supporting Information for SPR protocols) (**Table 2**).[18] For further discussing structure–property correlations within the binding study, we also give the K_a divided by the (average) number of Man units (K_a/Man). As expected, negative controls P1 and P2 bearing no carbohydrate side chains show no binding to ConA (see Supporting Information). pHEAA has previously been reported to exhibit high resistance to both protein adsorption and bacteria adhesion, thus making this a suitable scaffold for future biomedical applications of the brush glycopolymers.[19]

For glycooligomers as well as glycopolymers, we generally see an increase in binding with increasing valency (**Figure 2**A). Previously, we have seen a dependence in binding not only on the number of carbohydrate units but also the overall size of linear glycopolymers.[18] We have observed two regimes: at first increase in the overall size is beneficial leading to an increase in binding, however, when further increasing the molecular weight, binding decreases again. We have attributed this to a reduced

Table 1. Molecular weights and dispersities of p(HEAA), linear and brush glycopolymers.

Polymer	$Đ$[a)]	DP[b)]	DP[a)]	M_n[a)] [g mol^{-1}]	
P1	1.01	40	54	6.409	
P2	1.08	100	92	10.550	
pMan	1.04	40	35	12.000	
	$Đ$	M_n[a)] [g mol^{-1}]	N (side chains)[b)]	N (Man)[a)]	N (Man)[b)]
P1-10d	1.05	8.375	2	4	3
P1-40d	1.03	20.780	7–8	21	15
P1-80d	1.07	34.100	17–18	40	35
P2-20d	1.06	15.000	7–8	14	15
P1-40ds	1.08	52.240	13	37	26
P1-10t	1.05	8.678	1	5	4
P1-40t	1.15	38.500	12–13	46	50
P1-80t	1.05	57.980	16–17	72	65
P2-20t	1.01	22.450	3	16	12
P1-40ts	1.04	29.500	6	25	25

[a)]Determined by aqueous GPC; [b)]Determined by ^{1}H-NMR (300 MHz, D_2O).

Table 2. SPR results for glycooligomers and brush glycopolymers binding to ConA.

Sample	N (Man)	K_a [M^{-1}] × 10^4	K_a/Man [M^{-1}] × 10^2	
O1	2	1.6 ± 0.4	80 ± 20	
O2	4	2.7 ± 0.3	68 ± 8	
O1s	2	1.6 ± 0.3	80 ± 20	
O2s	4	2.45 ± 0.04	61 ± 1	
	N (side chains)[a)]	N (Man)[a)]	K_a [M^{-1}] × 10^4	K_a/Man [M^{-1}] × 10^2
pMan	–	40	14 ± 0,1	35 ± 0,8
P1-10d	2	4	10.8 ± 0.2	269 ± 5
P1-40d	7–8	15	37 ± 1	248 ± 7
P1-80d	17–18	35	70 ± 2	199 ± 6
P2-20d	7–8	15	8.4 ± 0.1	56 ± 0,9
P1-40ds	12	25	2.7 ± 0.2	10.7 ± 0.9
P1-10t	1	4	6.8 ± 0.8	170 ± 20
P1-40t	12–13	50	49 ± 1	102 ± 2
P1-80t	16–17	65	82 ± 20	130 ± 30
P2-20t	3	12	23 ± 1	180 ± 10
P1-40ts	6	25	26 ± 2	105.4 ± 0.8

[a)]As determined by ^{1}H-NMR (300 MHz, D_2O).

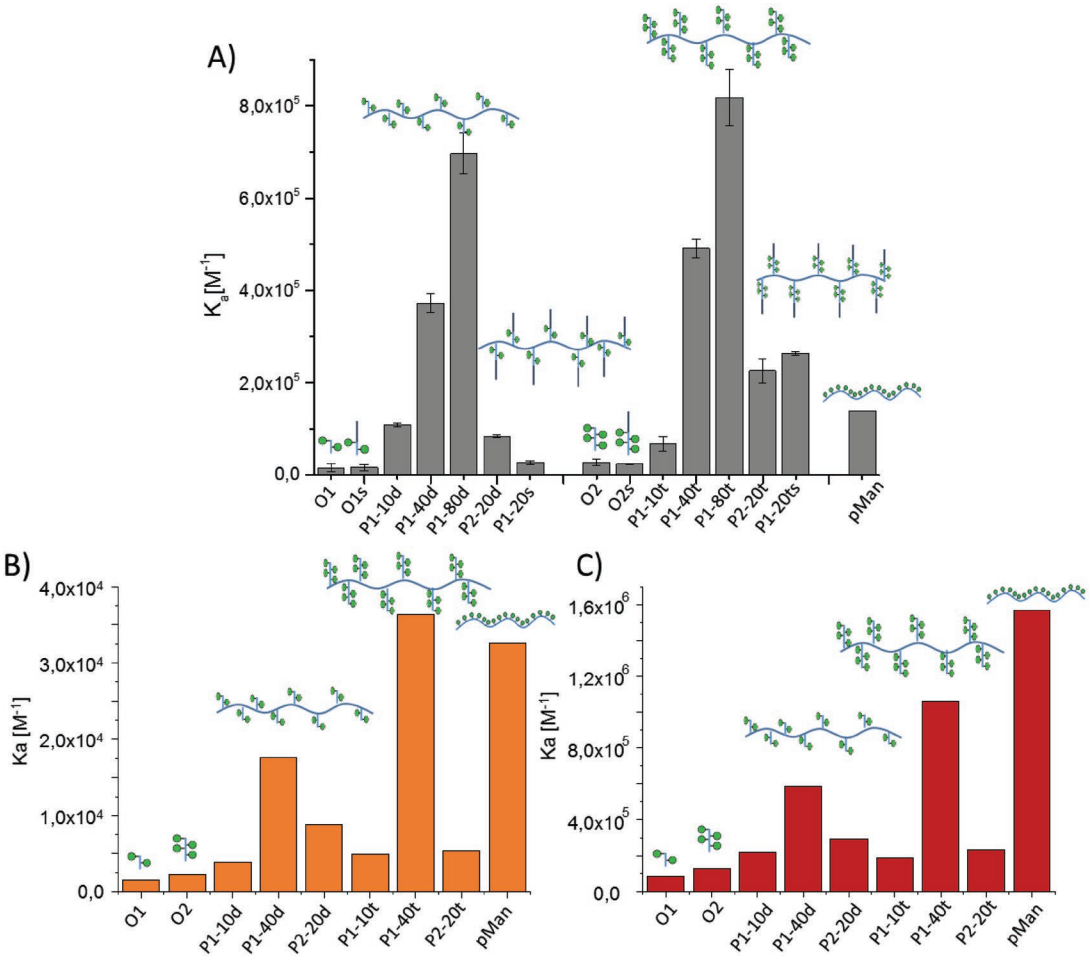

Figure 2. Binding of brush and linear glycopolymers to A) ConA, B) DC-SIGN, and C) MBL as determined by SPR direct binding assay (see Supporting Information).

accessibility of carbohydrate ligands within the polymer coil. Indeed, we observe a similar trend for the brush glycopolymers. Increase in molecular weight, for example, comparing O2 with P1-10d and P1-10t all presenting an average of four Man units, the polymeric structures show higher affinity in comparison to the oligomeric structure. However, when comparing P1-40d and P2-20d, both presenting an average of 15 Man units and 7–8 side chains, the glycopolymer based on the lower molecular weight polymeric scaffold shows higher affinity.

Looking at the effects of branching, brush glycopolymers show similar trends as were observed previously for branched glycooligomers.[14c] For glycopolymers with a similar degree of branching but differing in the number of carbohydrate units (P1-80d and P1-80t), an increase in valency only leads to a small increase in binding. We attribute this to Man units positioned closer to the polymer backbone in the longer side chains of the P1-t series being less accessible for binding with the protein.

In order to further test for this hypothesis, glycooligomers with non-carbohydrate functionalized block attached to the carbohydrate carrying segment were synthesized. Upon grafting onto the polymer scaffold the non-carbohydrate carrying block sits on the outside of the brush structure. We expect that this would lead to a shielding of the carbohydrate units positioned closer to the polymer backbone and therefore lead to a decrease in binding. Indeed, when comparing P1-ds and P1-ts with P1-40d, introduction of the shielding block leads to a clear decrease in binding. While in this study introduction of the shielding block was used to investigate the potential binding mechanisms of the brush glycopolymers, in the future this opens the opportunity to create glycopolymers with a tunable lectin avidity, for example, by introducing selectively cleavable moieties in between the carbohydrate-carrying segment and shielding block that would release the high affinity polymer upon a stimulus such as light or pH. Interestingly, for longer polymer scaffolds, P2-20t with lower degree of branching but higher valency per side chain shows higher affinity in comparison to P2-20d with a similar overall valency but higher degree of branching.

Overall, the glycopolymer with the highest avidity for ConA is P1-80t. However, when looking at the increase in overall avidity per number of Man units (K_a/Man), P1-10d and P1-40d show the largest gain in binding per Man. Thus future studies will explore the possibility to further increase avidity of these polymers, for example, through conjugation of brush glycopolymers onto liposomes[20] or attachment onto gold nanoparticles.[21]

While ConA is an important model lectin due to its well-characterized structure and binding behavior[22] as well as

widespread use in studies investigating structure–property correlations of glycan mimetics, it has only limited relevance in terms of potential biotechnological and biomedical applications of glycopolymers. Therefore, selected glycooligomers and brush glycopolymers were tested for their binding toward two additional Man-recognizing lectins, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and mannose-binding lectin (MBL). Both of these lectins are found in the human body and are well characterized for their carbohydrate recognition and known to mediate contacts with pathogens such as viruses as part of the immune system.

DC-SIGN has been found to be targeted by gp120, which is a glycoprotein expressed by the HIV-1 virus. Using glycopolymers that show stronger interactions with DC-SIGN could prevent the transmission of HIV-1 as well as other viruses and infections.[23] The tetrameric structure of DC-SIGN leads to great selectivity toward glycopolymers that contain mannose and/or fucose units that are preferably closely spaced.[24] Previous studies have shown that DC-SIGN typically recognizes structures that possess at least three units of sugars with higher sugar density giving stronger binding.[25]

MBL plays a key role in the regulation of inflammations and has been observed to bind to numerous parasites, viruses, and bacteria, such as N. meningitis, Ebola, and influenza A.[26] MBL presents different structural forms ranging from dimers to hexamers based on oligomers exhibiting three peptide chains with a carbohydrate-recognition domain each which allows MBL to bind multiple sugars and results in very strong interactions with glycopolymers.[26a]

In general, binding to DC-SIGN and MBL shows similar trends as previously observed for ConA (Figure 2): We see an increase in binding with increasing valency as well as increasing branching. However, a large increase in the molecular weight decreases the binding.

Interestingly, when comparing the linear glycopolymer pMan with an average of 40 Man moieties and different brush glycopolymers, the linear construct shows a lower affinity to ConA than brush structures of similar or even lower valency, for example, P1-40d. For DC-SIGN, linear and brush glycopolymers of similar valency show similar avidity, for example, when comparing pMan and P1-40t (Figure 2C, Supporting Information). In contrast, MBL clearly shows stronger interactions with the linear sample giving the highest K_a for pMan measured in this series (Figure 2B, Supporting Information).

When comparing the effect of increasing either the valency per branch or the degree of branching, we see that these structural parameters affect binding to different extents depending on the lectin. Comparing P1-40d and P1-40t, brush glycopolymers with similar number of side chains but carrying either two or four Man unit per side chain, thus differing in the overall valency by more than a factor of two, ConA only shows a slight increase in avidity by a factor of 1.3 for the higher valent ligand. For DC-SIGN and MBL on the other hand, binding is increased by a factor of ≈2.

Previous studies have shown that the length of the branching arms can also have an influence in the bindings between lectins and complex glycopolymers.[27] For instance, it is important that the glycopolymers possess arms that are not too short or too long so that they are able to reach the binding sites of the lectins more effectively. Surprisingly, even though P1-40d, P1-40t, and pMan exhibited very similar binding trends for DC-SIGN according to their binding values at 80 μM (Figure 2B), P1-40d demonstrates the highest K_a/Man value (see Supporting Information). This points toward the interacting mannose units in the side chains of P1-40d tending to remain bound or that rebinding of released mannose units occurs rather than the dissociation of the complex. Thus the sugar number in the side chains of the glycopolymer effects binding properties significantly, even though it does not enhance binding further comparing tetravalent and divalent side chains.

Therefore, these results support the finding that large numbers of sugar moieties are not always necessary to achieve high binding and that the design of the polymer architecture can influence the interactions with the lectins significantly.

Finally, even though all polymers showed stronger interactions with MBL and DC-SIGN than the oligomers, they were notably weaker than those obtained with P1-40d and P1-40t as well as those obtained with the linear pMan. However, these results are expected since these glycopolymers present less branching and contain lower quantities of sugar moieties.

In conclusion, we present the synthesis of brush glycopolymers with sequence-defined side chains based on combining the solid phase synthesis of precision glycomacromolecules and grafting-to a poly(active ester) scaffold. Variations of structural parameters such as degree of branching, valency per branch as well as overall valency and length of polymeric scaffold impact binding of brush glycopolymers to model lectin ConA. Interestingly, binding to other lectins such as DC-SIGN and MBL is affected differently, with MBL favoring a linear presentation of carbohydrate ligands while ConA and DC-SIGN show higher binding to brush glycopolymers. Additionally, the obtained SPR results indicate that above a critical number of carbohydrates in the side chains, more increment in the side chains does not contribute to the binding and avidity anymore due to a low ability of the sugars being too close to the backbone reaching lectin's binding domains. Future studies will further explore the potential selectivity of different lectins to glycopolymers of different architecture.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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