Modular synthesis of multivalent glycoarchitectures and their unique selectin binding behavior†

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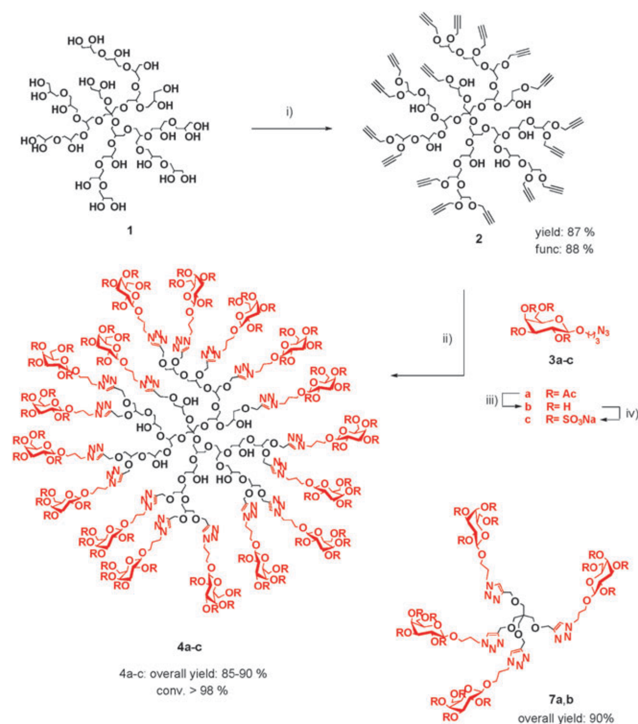
Hyperbranched polyglycerols (HPGs) are ideal scaffolds for the multivalent presentation of saccharides, due to their biocompatible, carbohydrate-like properties; here, we report the conjugation of galactose sugar moieties to HPG, and the multivalent effect of these constructs on selectin binding.

Carbohydrates play a significant role in biological systems, spreading from cellular recognition and adhesion to cell growth and differentiation.[1] Wide interest has been devoted to the interplay of cell-surface receptors with their corresponding binding carbohydrate moieties. Unfortunately, most carbohydrate–receptor interactions are weak (dissociation constants are in the *milli*molar concentration range)[2] and in order to compensate for their low binding affinity, different strategies based on multivalent interactions have been designed, including carbohydrate clusters, glycopolymers and glycopeptides.[3] Multivalent carbohydrates are attractive synthetic targets as they often bind much more strongly to the interacting protein partner than their monovalent counterparts. Inspired by other examples[4] as well as recent breakthroughs in dendrimer synthesis using click chemistry[5] we developed a new class of functional, multivalent glycoarchitectures based on HPG **1** as a biocompatible building block.

Hyperbranched polymers are highly branched, narrowly disperse, three-dimensional macromolecules, with unique structures and properties.[6] More specifically HPGs **1** are water-soluble, highly biocompatible polyether polyols, which compared to their perfect dendritic analogs possess more linear units and are more flexible.[7] However, they can be prepared in an efficient one-pot reaction, with predetermined molecular weights and narrow molecular weight distributions.[8] They show excellent biocompatible properties both *in vitro* and *in vivo*.[9] Their hyperbranched architectures and high density of peripheral functional groups have prompted us to explore multivalent glycodendritic polymers as mimics of cell-surface glycans. For this purpose we developed a synthetic strategy, which employs the Cu(I)-catalyzed azide–alkyne cycloaddition reaction previously used by Sharpless, Hawker and co-workers to prepare diverse dendritic structures.[10] These reactions are quite efficient, as they proceed in high yields, under aqueous conditions, and with complete regioselectivity. During the course of this work Riguera and co-workers and Roy and co-workers[5] have used the concept of click-chemistry in combination with carbohydrate functionalized dendrimers. Applying the click methodology, we first introduced alkyne groups into the HPG **1** by reacting with propargyl bromide in the presence of NaH. The obtained polymer **2** was highly substituted, resulting in 88% functionalization according to the ^{1}H NMR calculations, with a number average molar mass of 4.3 kDa. The sugar moiety, galactose (Gal) was easily outfitted with an azido group using a propylene spacer, based on a published procedure.[11]

Employing the click conditions polymer **2** was reacted with **3a–c** (protected, unprotected and sulfated carbohydrates) to furnish the



Scheme 1 Synthesis of multivalent HPG **4a–c** and PE **7a,b** architectures. *Reagents and conditions*: (i) propargyl bromide, NaH, DMF, 16 h, 87%, functionalization 88% (**2**); (ii) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF–water = 1 : 1, 24 h, **4a** (89%), **4b** (92%), **4c** (86%), **7a** (85%), **7b** (94%); (iii) MeONa, MeOH, RT, 4 h, 90%; (iv) $SO_3 \cdot Py$, DMF, 90%; HPG depicted as smaller partial structure (20 mer) for clarity. HPG (**1**) used was average 3 kDa molecular weight (~40 mer), M_w/M_n = 1.18, DB (degree of branching) = 0.57.

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desired glycopolymers **4a–c** in relative high yields (85–90%) (Scheme 1). In parallel, for low valency control, the commercially available pentaerythritol (PE) scaffold was used for the preparation of tetrameric galactose derivates (**7a,b**) (see ESI†). The resulting glycopolymers were isolated in relatively high yields, after purification of the reaction mixture by ultrafiltration or dialysis. The completion of the conjugation reactions, and the degree of ligand loading (galactose) on the polyglycerol were established in all cases by ^{1}H NMR spectroscopy, as well as by DLS measurements. IR data also confirmed that neither alkyne (3289 cm^{-1}; 2114 cm^{-1}) nor azide (2098 cm^{-1}) residues remain in the final glycostructures. These new multivalent glycoarchitectures were then analysed for *in vitro* inhibition of selectins. The term "selectin" is employed to designate a general class of receptor which displays a selective adhesive function and which includes a lectin-like domain responsible for such selective adhesive function. Known selectins include E-selectin, P-selectin and L-selectin. Galactose is an important binding moiety in selectin inhibition of the tetrasaccharide sialyl Lewis X (SLex) and its isomer sialyl Lewis a (SLea).

Selectins share the ability to recognize and bind the SLe^x or SLe^a and other related oligosaccharides bound to different glycoprotein scaffolds.[12]

All galactose derivatives were tested by surface plasmon resonance (SPR) in a competitive binding assay, a well established selectin-ligand interaction assay,[13] in order to analyze their potency in selectin recognition (Scheme 2).‡

These galactose functionalized HPGs **4b,c** (35 terminal galactose per molecule) showed strong inhibition of leukocytic L-selectin and platelet derived P-selectin, with IC_{50} values in the nanomolar range (nM). Only in case of **4b** a moderate inhibition of the endothelial E-selectin was observed (μM). Hyperbranched polyglycerol itself showed no inhibition to any selectins. The Gal multimer **4b** showed binding in the nanomolar range (70 and 1000 nM, respectively, for L- and P-selectin), which represents an affinity enhancement of up to 10^3-fold over the tetrameric counterpart. Furthermore, functionalizing the HPG with previously sulfated galactose moieties (88% functionalization, with a number of average molar mass of 24.4 kDa), the binding affinity to L- and especially to P-selectin was dramatically enhanced, resulting in IC_{50} values in the low nanomolar range (1 and 7 nM, respectively). (Fig. 1) This result is in accordance with prior findings that polyanionic carbohydrate ligands show a marked binding enhancement to selectins.[14,15] It is interesting to note, however, that galactose sulfation appears to hinder binding to E-selectin.

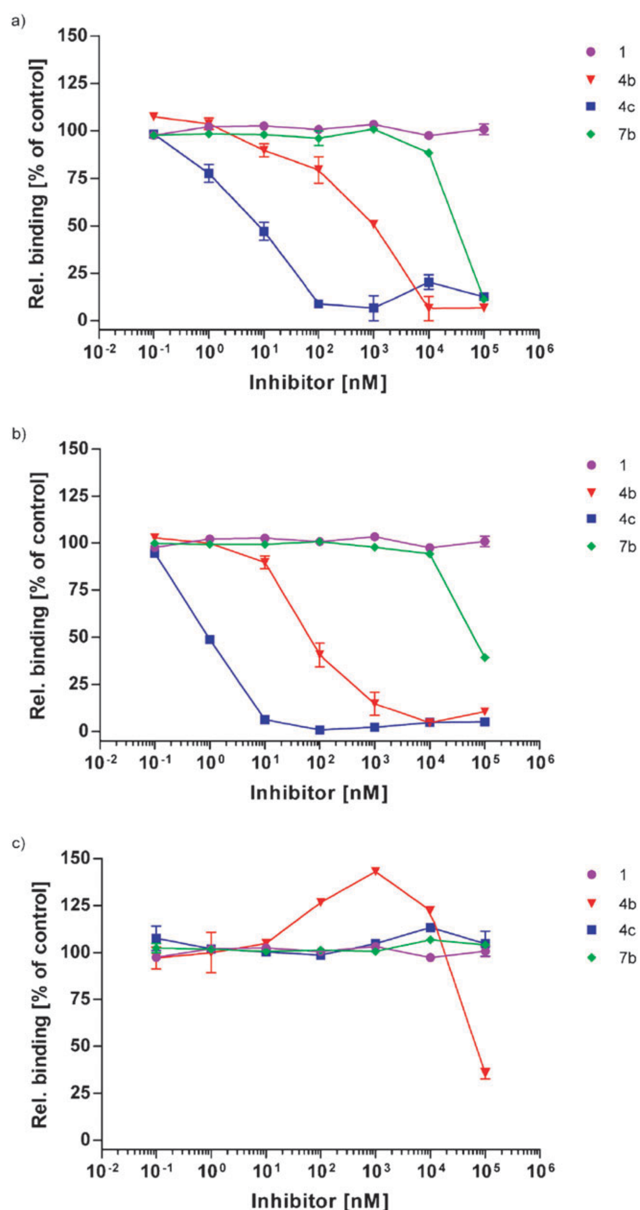
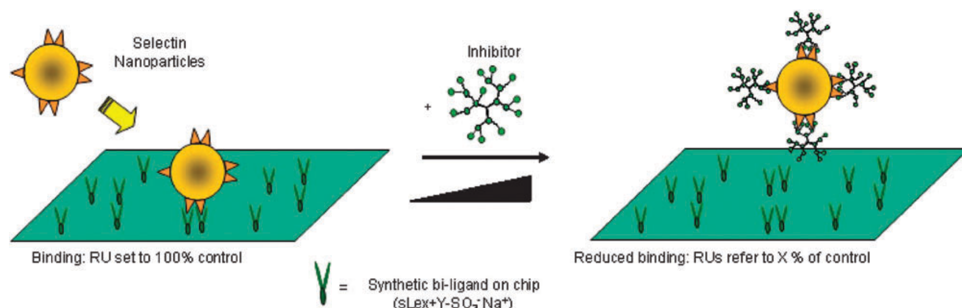


Fig. 1 Inhibition curves to (a) P-, (b) L- and (c) E-selectins: percent inhibition (*y* axis) is plotted against the inhibitor concentration in (*x* axis). Inhibition is shown.

The results (Table 1) from the selectin inhibition studies indicate that the hyperbranched glycopolymers prepared in this work act as multivalent ligands. Compared on a per



Scheme 2 SPR based competitive selectin-ligand binding assay; RU = resonance unit; details in Notes section.

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Table 1 IC_{50} values (nM) of the compounds towards binding of L-, P- and E-selectins (N.I.: no inhibition below 1 mM)

Compound	7b		4b		4c	
	Total	Per Gal	Total	Per Gal	Total	Per Gal
L-Selectin	60 000	240 000	70	2450	1	35
P-Selectin	30 000	120 000	1000	35 000	7	245
E-Selectin	N.I.	N.I.	70 000	2 450 000	N.I.	N.I.

galactose basis the IC_{50} value for structure **4b** shows in case of L-selectin a 100-fold enhancement of the inhibitory power over the tetravalent counterpart **7b**. This dependence of affinity increase on the number of Gal residues per polymer clearly is a result of these Gal residues being engaged in binding at multiple binding sites. Around a four-fold increase in affinity per sugar was observed in case of P-selectin inhibition when the number of Gal moieties was increased from 4 to 35 per molecule of HPG. The identity of the binding elements, structure of the scaffold and numbers of binding groups are all parameters that influence the mechanism by which a ligand acts. These multivalent HPG-Gal architectures contain elements of structural complexity which are not present in the tetravalent counterpart.

Other studies have shown that despite the great structural complexity of many bioactive oligosaccharides, often only small portions of these molecules are actually recognized by their receptors. The remaining part seems to act as a scaffold that orients the binding determinants in the appropriate conformation.[3]

In this study we show, how even a simple carbohydrate, such as galactose, can give relatively high inhibition against selectins, when presented in a multivalent manner. Sulfated, multivalent galactose constructs gave even stronger L- and P-selectin inhibition in the lower *nano*molar range. Although it is known that the galactose unit of sLex makes contacts to selectin proteins,[15,16] it was surprising for us that polyvalent galactose without any further modification serves as a highly active selectin ligand. To the best of our knowledge this has not been described previously.

The developed protocols for multivalent glycopolymer preparation *via* click chemistry, in reproducible high yields (up to 90%), are readily adaptable to include a range of carbohydrate based binding motifs (protected, free OH and sulfated carbohydrates). Additional work is underway to further increase the inhibitory effect and to more thoroughly investigate the interaction between the peripheral carbohydrates and selectins.

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Notes and references

‡ *SPR assay description in brief*: Selectin functionalized Au-nanoparticles were passed over a standard ligand (20 mol% sLex and 5 mol% sulfotyrosine conjugated to polyacrylamide) of all selectins, immobilized on the sensor chip. The resulting binding signal was set to 100% and serves as a control. To evaluate selectin binding of potential inhibitors, a defined preincubation step with the selectin nanoparticles was performed before its passage over the sensor chip. Reduction of the binding signal with respect to the inhibitor concentration was recorded and calculated as x% of the control. The concentration of inhibitor, that causes 50% reduction in the binding of the labelled reference ligand, was referred to as IC_{50} value. Each concentration was applied at least in duplicate.

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