Varying the Size of Multivalent Ligands: The Dependence of Concanavalin A Binding on Neoglycopolymer Length

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Carbohydrate-substituted polymers have emerged as important materials for the exploration of protein−saccharide interactions, which are critical components of diverse biological processes.[1,2] Structural studies of lectins reveal that many possess multiple saccharide binding sites separated by large distances (i.e., 30−70 Å),[3] which can be spanned readily with polymeric backbones. To maximize the activities of multivalent saccharide derivatives, however, complementary arrangements of individual binding elements and binding sites are required; therefore, synthetic methods that can control the display of saccharide epitopes are needed. Here, we describe the application of the ring-opening metathesis polymerization (ROMP) to generate collections of multivalent saccharide displays in which the number of repeat units within a set is systematically varied. The ability of these materials to interfere with cell agglutination mediated by the carbohydrate-binding protein concanavalin A was evaluated. The results provide direct evidence for multipoint binding, yet they also indicate statistical mechanisms contribute to the efficacy of the neoglycopolymers. Thus, in addition to the benefits afforded from the chelate effect,[4] there are other advantages of using multivalent ligands to target specific receptors.

ROMP can be used to assemble neoglycopolymers that are efficient and selective inhibitors of carbohydrate-binding proteins.[5] A key advantage of ROMP is the polymerization can be living,[6] that is, elongation can proceed more rapidly than termination or chain transfer. A report describing living reaction conditions that produce polymers of low polydispersity with use of ruthenium carbene **2**[7] and a polar monomer[8] led to us to speculate that neoglycopolymers of different lengths could be generated by varying the ratio of monomer to catalyst. A living polymerization method that progresses by a rapid initiation and slower elongation steps offers new opportunities for oligomer synthesis. Moreover, the resulting polymers would possess alkene groups that could be functionalized to alter the conformational and solubility properties of the scaffold. Given these advantages, we explored the application of ROMP to generate neoglycopolymers of various lengths.

Monomer **1** was designed to display a single saccharide residue per repeat unit[9] and to serve as a highly reactive substrate for ROMP. Because living polymerizations rely on the propensity of the monomer to engage in elongation, the template was devised to undergo more rapid metathesis than the carbohydrate-substituted substrates previously employed.[5a−c,9] The increased reactivity was envisioned to arise from two features. First, the strain in the bicyclo[2.2.1] system is augmented by fusion to the cyclic imide, which should increase initiation and propagation rates. Second, the more electron rich norbornene derivative was selected over the 7-oxanorbornene analog to favor elongation. Target compound **1** was generated by attachment of an α-linked mannose residue to an imide-fused norbornene scaffold.[10]

Table 1. Varying the Monomer:Catalyst Ratio Alters the Average Number of Repeat Units (DP) in Neoglycopolymer Series **3**

entry	m/c	conditions[a]	av *n* (DP)	yield/%[b]
1	7/1	a	10	80 (98)
2	25/1	a	25	68 (87)
3	50/1	a	52	68 (91)
4	100/1	b	143	67 (98)

[a] For conditions, see Figure 1. [b] Isolated yield. NMR yields in parentheses were calculated from the ratio of the polymer and the starting monomer remaining by ^{1}H NMR of the crude mixture.

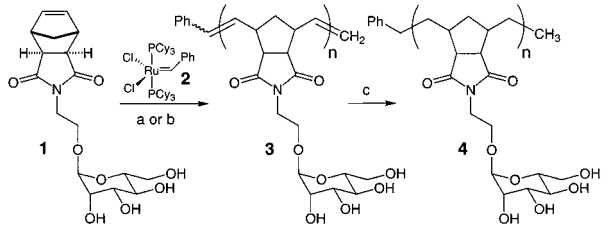

Figure 1. Scheme for the synthesis of neoglyopolymers by ROMP: (a) $MeOH/H_2O/(CH_2Cl)_2$ (6:1:5) then add H_2O, room temperature. (b) $H_2O/(CH_2Cl)_2$ (2:1), DTAB, room temperature. (c) $TsNHNH_2$, H_2O, 100 °C, 56% ($n = 10$), 91% ($n = 25$), 58% ($n = 52$), 100% ($n = 143$).

The oligomerization of monomer **1** was effected with ruthenium carbene **2**, with increasing monomer-to-catalyst ratios (m/c) employed to produce polymers of increasing length (Table 1).[10,11] With homogeneous conditions (Figure 1), a linear relationship between the m/c (7/1, 25/1, 50/1) and the average degree of polymerization (DP = 10, 25, 52) was observed; this relationship is characteristic of living polymerizations. At higher m/c, however, the polymerization stalled at approximately 50% conversion, suggesting that termination processes were competing effectively with elongation. Emulsion conditions, which afford increased efficiencies with some hydrophilic monomers,[8] were investigated. Longer neoglycopolymers (DP =143) were obtained with these conditions (m/c = 100). Completing the collection of oligomeric materials, a high molecular weight polymer (DP ∼ 3000) was synthesized with ruthenium trichloride.[9] Thus, a series of neoglycopolymers (Figure 1, series **3**) of increasing length could be obtained simply by altering the reaction conditions. To provide insight into the impact of polymer backbone flexibility on activity, the components of set **3** were reduced to produce a parallel series of neoglycopolymers with saturated backbones (Figure 1, series **4**).

The effect of chain length on the functional affinity of the saturated and unsaturated neoglycopolymers (sets **3** and **4**) was assessed using the tetrameric, mannose-binding concanavalin A in a hemagglutination inhibition assay (Figure 2). Concanavalin A was selected for this study because its structure

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(11) Average polymer lengths (DP) were determined by NMR integration of polymer end groups versus internal olefin resonances.

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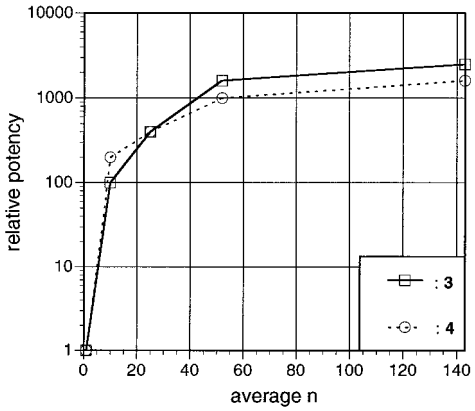

Figure 2. Dependence of hemagglutination inhibition on polymer length. Relative potencies = [inhibitory dose of polymer bound mannose]/[inhibitory dose of monovalent mannoside **1**]. Inhibitory doses were obtained by averaging the results of three independent experiments. The error associated with the dose determination is a factor of 2, as dictated by the 2-fold dilutions in the assay.

and saccharide binding properties are well characterized, thus facilitating molecular level analyses of the bioactivities of the neoglyocopolymers.[12,13] The concentration of mannose residues required to inhibit concanavalin A-promoted erythrocyte agglutination was determined for each substrate. Relative potency was calculated from the ratio of the minimum inhibitory concentrations of polymer-bound mannose and the monomer **1**.

Consistent with our previous observations,[5b] all multivalent mannoside ligands inhibited agglutination at lower mannose residue concentrations than a monovalent mannoside. Moreover, the potencies of the unsaturated polymers (series **3**, evaluated on the basis of saccharide residue concentration) increase exponentially as the average length increases linearly for polymers up to DP = 50 (Figure 2): a 100-fold enhancement relative to monomer **1** at DP = 10, 300-fold at DP = 25, and 2000-fold at DP = 52. The activities reach a plateau, with all chain lengths of 50 or greater exhibiting approximately the equivalent inhibitory potencies when analyzed on a saccharide residue basis: 2500- and 2000-fold enhancements for polymers of DP = 143 and 3000, respectively. A similar trend is observed in the saturated polymer series **4**: activity enhancements of 200-fold at DP = 10, 300-fold at DP = 25, 1000-fold at DP = 52, and 2000-fold at DP = 143 are measured. Despite the differences in backbone flexibility, the most potent ligands in each series had approximately the same efficacy.

The observed dependence of the potency of these multivalent ligands on length suggests the functional affinity increases can be attributed to the chelate effect.[4] In this model, a single polymer chain of the proper length interacts simultaneously with two binding sites at least; only a single translational entropy penalty is paid, while favorable ethalpic contributions from both sites (and perhaps subsites) will contribute to binding. The saccharide binding sites of tetrameric concanavalin A are separated by approximately 65 Å.[12,13] Molecular modeling of the unsaturated polymers indicates that those composed of 35 repeat units can span the distance separating the saccharide binding sites.[14] Between average lengths of DP = 25 and 50, polymer activities reach a plateau. The exponential rise in potencies indicates the chelate effect operates to yield major increases in neoglycopolymer activities. Specifically, as the average polymer length increases, a larger fraction of the population can span the distance between two sites. With this mechanism, the inhibitory potency of polymer-bound mannose residues is expected to reach a maximum at the optimum length for chelation, as is observed (Figure 2). A comparison of the activities of polymers in series **3** with those in **4** offers insight into the importance of conformational entropy on binding. Multisite binding will result in a loss of rotational degrees of freedom, and the associated energetic cost should be more severe for series **4**. Given that the unsaturated polymers **3** and the more flexible polymers **4** are equivalently potent, conformational entropy appears to play only a modest role with these ligands.[16]

Despite its contributions to neoglycopolymer activity, the chelate effect alone cannot explain the data. Oligomers composed of more residues than is necessary to span the sites should display decreased activities when evaluated on a saccharide residue basis, but they do not. Consequently, the potency of the longer polymers is greater than chelation predicts. The efficacies of these longer polymers and of oligomers too short to span the protein binding sites can be attributed to a high local concentration of mannose residues, which would perturb the rate of dissociation of multivalent ligands.[17,18] Neoglycopolymers that contain many saccharide recognition elements will exhibit slower dissociation rates, with rebinding becoming more favorable and dissociation less so. Although other mechanisms may make some contributions,[19] the observed dependence of the inhibition activity on the polymer length is largely due to a combination of statistical and chelation effects.[20]

An increase in functional affinity upon multivalent presentation of carbohydrate ligands has been observed in a variety of systems.[2] Despite the diversity of reported multivalent scaffolds,[2] few studies have addressed the mechanisms that underlie observed functional affinity increases.[21] The strategy described here can be applied to explore diverse multivalent binding events. For example, neoglycopolymers can be used to investigate multivalent recognition, to elicit or suppress an immune response, and to modulate protein−saccharide interactions. The ability to control and systematically alter the features of materials with ROMP provides new opportunities to investigate the widespread role of multivalent binding in biological systems.

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Supporting Information Available: Experimental procedures and spectral data for compounds **1**, **3**, and **4** (3 pages). See any current masthead page for ordering and Internet access instructions.

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(14) Series **3** polymers are atactic and possess both cis and trans alkene geometries. Oligomers representing the four possible backbone configurations (*cis*-syndiotactic, *cis*-isotactic, *trans*-syndiotactic, and *trans*-isotactic) were modeled in the Macromodel modeling package[15] (MM2 force field). From the most compact structure obtained, the *cis*-syndiotactic isomer, the chain length required to span the binding sites was calculated to be 35. The minimum number of residues needed to span the binding sites for the most extended structure (*trans*-syndiotactic) is 15.

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(16) Half widths of the allylic carbon signals in the ^{13}C NMR spectrum of *trans*-**3**, *cis*-**3**, and the corresponding carbon signal of **4** are 30, 51, and 19 Hz, respectively, suggesting that series **4** are more flexible than **3**. More complex mechanisms for their activities, such as those involving compensating entropic and enthalpic effects, are also possible.

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