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A Self-Assembled Multivalent Pseudopolyrotaxane for Binding Galectin-1

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Abstract: A self-assembled pseudopolyrotaxane consisting of lactoside-displaying cyclodextrin (CD) "beads" threaded onto a linear polyviologen "string" was investigated for its ability to inhibit galectin-1-mediated T-cell agglutination. The CDs of the pseudopolyrotaxane are able to spin around the axis of the polymer chain as well as to move back and forth along its backbone to alter the presentation of its ligand. This supramolecular superstructure incorporates all the advantages of polymeric structures, such as the ability to span large distances, along with a distinctively dynamic presentation of its lactoside ligands to afford a neoglycoconjugate that can adjust to the relative stereochemistries of the lectin's binding sites. The pseudopolyrotaxane exhibited a valency-corrected 10-fold enhancement over native lactose in the agglutination assay, which was greater than the enhancements observed for lactoside-bearing trivalent glycoclusters and a lactoside-bearing chitosan polymer tested using the same assay. The experimental results indicate that supramolecular architectures, such as the pseudopolyrotaxane, provide tools for investigating protein−carbohydrate interactions.

Phenomena that control the form and function of living systems, such as self-assembly,[1] molecular recognition,[2] and multivalency,[3] have served to hasten the development of supramolecular chemistry[4] and template-directed synthesis,[5] particularly of mechanically interlocked molecular compounds.[6] Once considered chemical curiosities, complexes such as pseudorotaxanes[7] and compounds such as catenanes[8] and rotaxanes[9] are now contributing to advances in molecular electronics[10] and the construction of artificial molecular machines.[11] In addition, polymeric pseudorotaxanes[12] and mechanically interlocked polyrotaxanes[13] are enriching polymer chemistry and materials science in terms of both structure[14] and function.[15] While biology has provided much of the inspiration

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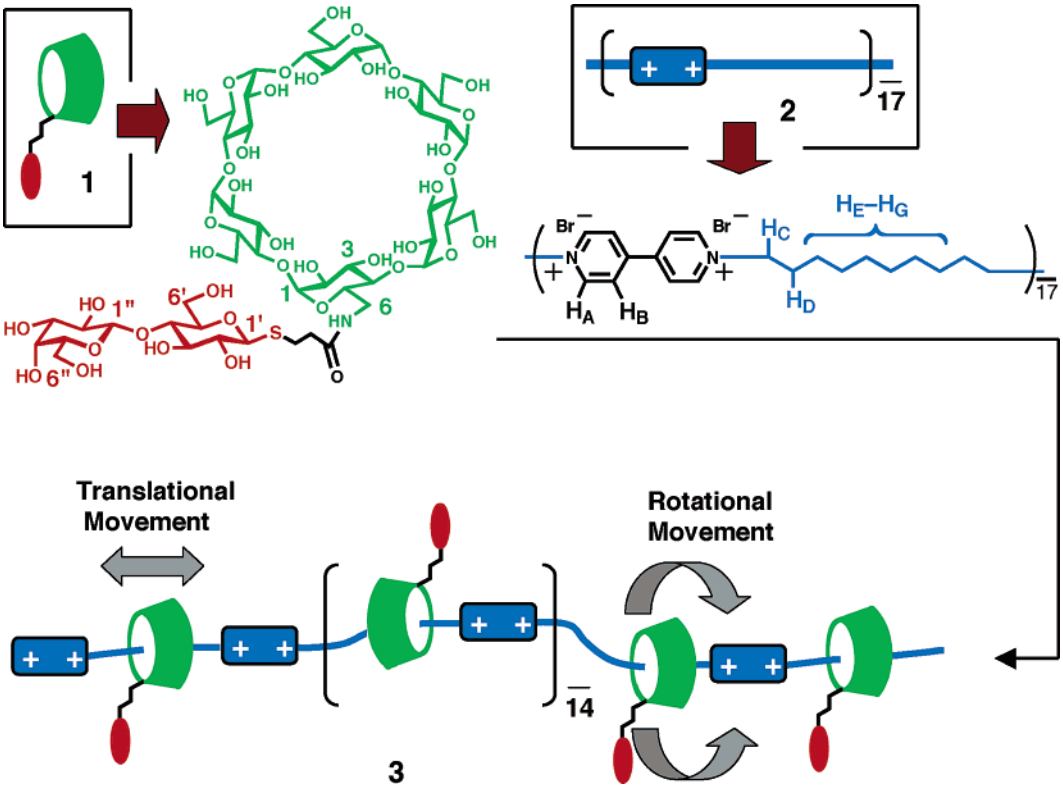
Scheme 1. Formation of Pseudopolyrotaxane **3**



and many of the conceptual tools for the growth of supramolecular chemistry[4] and template-directed synthesis,[5] the biochemistry that interfaces with wholly synthetic complexes and mechanically interlocked molecules is still in its infancy.[16]

The concept of multivalency[3] is especially important in glycobiology.[17] Nature uses multivalent interactions between multimeric and/or membrane-bound carbohydrate-binding proteins (lectins) and their matching carbohydrate ligands (epitopes) to mediate both physiological and pathological processes. Although a typical monovalent interaction between a lectin and its epitope is in the millimolar regime, Nature attains much higher avidities through multivalency.[3] Affinity enhancements over and beyond those expected on the basis of valency have been defined by Lee[18] as the glycoside cluster effect. Chemists have synthesized a variety of neoglycoconjugates using dendritic,[19] polymeric,[20] and self-assembled[21] architectures in attempts to mimic the complex multivalent scaffolds found in biological systems. There is considerable interest[22] in how the architectural features including rigidity, spacing, topology, and density of saccharides—characteristic of these synthetic multivalent structures—influence their avidity for their respective lectins. Glycodendrimers[19] generally fill some kind of three-dimensional space and usually display saccharide residues on their peripheries, while glycopolymers[20] are composed of a polymeric backbone with pendant saccharide residues. In general, the extended lengths and high valencies of glycopolymers offer particular advantages[20e] that have led to their extensive use[20] in binding lectins. Self-assembled dynamic systems, such as those provided by gold nanoparticles,[21e] micelles,[21f,g] and liposomes[21a] represent yet other avenues by which multivalency can be investigated. These systems support

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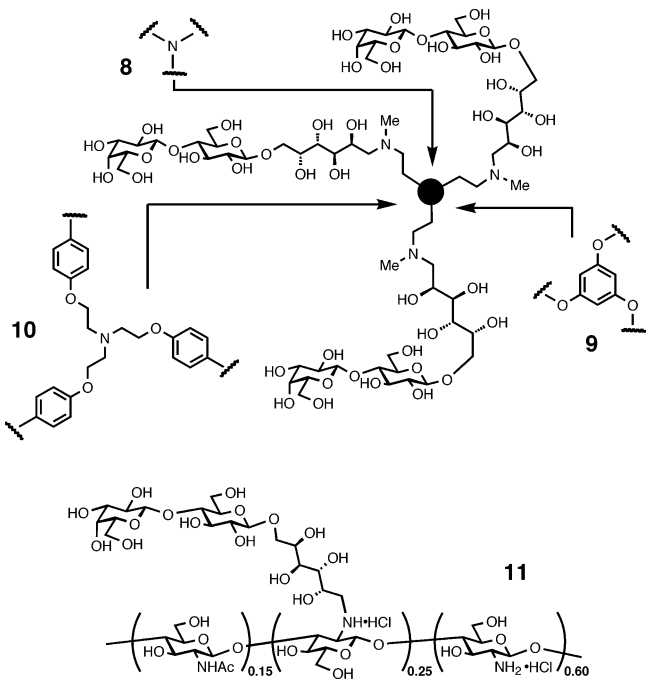
fluid surfaces, reminiscent of natural cellular membranes,[23] which allow for the arrangement of saccharide ligands coated on their surfaces to find the appropriate positions and orientations to maximize their interactions with the particular lectin binding site.

Recently, we have become interested[24] in carbohydrate-displaying pseudopolyrotaxanes and polyrotaxanes of cyclodextrins (CDs) threaded onto linear polymer chains as dynamic multivalent neoglycoconjugates. Although the supramolecular chemistry of CD-based pseudopolyrotaxanes and polyrotaxanes, which was pioneered by Harada,[25] is well-established,[26] these "beads-on-a-string" have only recently been adapted[27] for the multivalent display of biological ligands. While polyrotaxanes displaying maltosides have been synthesized by Yui and coworkers,[28] CD-based pseudopolyrotaxanes displaying lactosides have been self-assembled recently in our own laboratory.[24] The CDs are able to spin around the axes of the polymer chains as well as move back and forth along the polymers' backbones and thus alter the presentations of their saccharide ligands. In addition to the convenience of the synthesis, which relies on a self-assembly protocol, these "beads-on-a-string" offer the advantages of polymeric features such as the ability to span nanometer lengths as well as adaptable presentation of epitopes on account of the dynamic flexibility of the pseudopolyrotaxane architecture.

In this article, we describe the preparation (Scheme 1) of a stable pseudopolyrotaxane **3** using the lactoside-bearing α-CD **1** and polyviologen **2** for targeting galectin-1 (Gal-1).[29] Gal-1 is a soluble 14 kDa dimeric galactoside-binding lectin that can organize cell surface glycoproteins through binding and cross-linking of terminal or polymeric lactose or *N*-acetyllactosamine residues. Gal-1 is the prototypical member of the galectins, a ubiquitous family of galactoside-binding lectins that mediate cell adhesion, signaling, and death. Certain galectins, particularly Gal-1 and galectin-3, are overexpressed in many types of cancer.[30] Gal-1 plays multiple roles in cancer, from defense against the immune system by exploiting its natural pro-apoptotic function[31] to promoting motility and homotypic cell agglutination, which is believed[32] to be important for aggregating cancer cells into tumor emboli, thus increasing malignant cell survival in circulation. Synthetic multivalent ligands for Gal-1 have the potential to act as cancer diagnostics and therapeutics and to shed light on the supramolecular interactions of Gal-1 and its natural and oncogenic carbohydrate ligands. Chemists targeting Gal-1 have focused primarily on glycodendrimers and smaller glycoclusters with mixed results.[33] Topologically, Gal-1 is a particularly interesting challenge[34] since it is a rigid dimer with two binding sites oriented in opposite directions such that the entrance to each of the binding sites is located 6 nm apart. Herein, we describe the formation of a lactoside-displaying CD-based polyviologen pseudopolyrotaxane and the supramolecular inhibition of Gal-1-mediated T-cell agglutination in comparison with a series of trivalent glycoclusters **8**, **9**, and **10** and a rigid covalent neoglycopolymer **11** as controls.



Results

Synthesis of Trivalent Lactoside Glycoclusters. The three trifunctional core compounds, **4**, **5**, and **6**, which present methylamino residues for the attachment of dendrons by reductive amination, were prepared[35] for the synthesis (Scheme 2) of the trivalent glycoclusters **8**, **9**, and **10**. The arms of these cores differ by (a) the number of atoms that separate the methylamino groups and (b) the overall rigidity of the structures, which may alter the interaction of the resulting trivalent structure with its receptor. Both cores **4** and **6** possess a nitrogen atom at their center, and while **4** is the smaller in size, **6** contains *para*-phenylene spacers within its arms to yield a more extended conformation. Core **5**, instead of having a central nitrogen atom, uses phloroglucinol as a more rigid central core. The trisaccharide[22b] **7** was coupled (Scheme 2) with trifunctional trismethylamino core compounds **4**, **5**, and **6** to afford the lactose clusters **8**, **9**,

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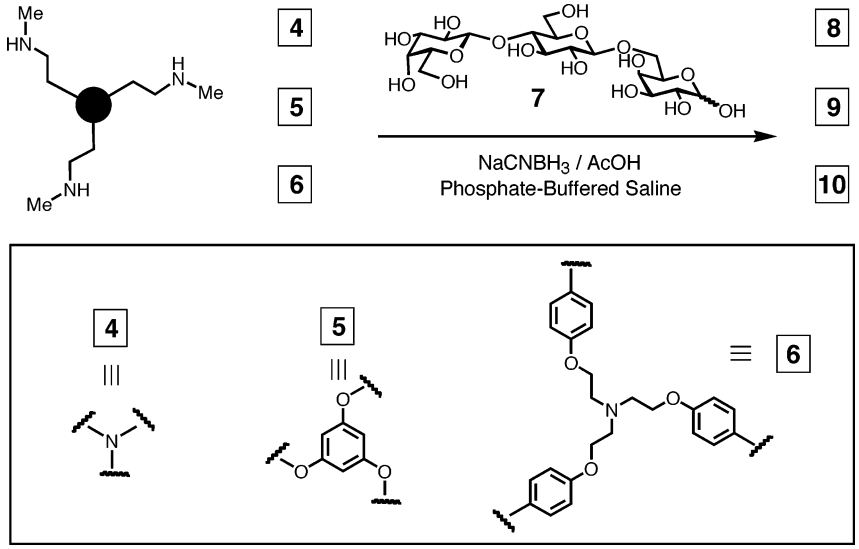
(35) The synthesis of these cores is provided in the Supporting Information.

Scheme 2. Synthesis of Trivalent Glycoclusters by Reductive Amination



and **10**, respectively, by reductive amination. All three glycocluster compounds were fully characterized using high-resolution mass spectrometry and 1D and 2D NMR spectroscopies.

Synthesis of Chitosan-Based Polymer. Chitosan is a linear polysaccharide of repeating β-1,4-linked glucosamine and *N*-acetylglucosamine, obtained by the partial hydrolysis of the *N*-acetyl groups of chitin.[36] Like cellulose, chitosan possesses a rigid linear conformation due to the presence of intramolecular hydrogen bonds in its secondary structure. Functionalized chitosan has been investigated by a number of groups for applications such as gene[37] and drug[38] delivery, as well as antimicrobial agents.[39] Roy and co-workers[40] have demonstrated that functionalization of the amino groups in chitosan can be achieved by reductive amination. Commercially available chitosan was reacted with the trisaccharide **7** under reductive amination conditions using $NaCNBH_3$. Chitosan provides a rigid rod scaffold for the multivalent display of lactose. On the basis of integrations of the ^{1}H NMR spectra, 25% of the monosaccharide residues of the chitosan polymer **11** were found to be substituted with a lactoside residue.

Synthesis of the Polyviologen Polymer. Previously, we had focused[24] our attention on dynamic systems using α- and β-CD derivatives threaded onto poly(tetrahydrofuran) and poly(propylene glycol). We had also observed that when 2,6-di-*O*-methlyated CD derivatives were used, the threading/dethreading of the CDs were fast on the ^{1}H NMR time scale to the extent that isolating the pseudopolyrotaxanes was not possible. In an effort to lower the rate of translational motion of the CD beads along the polymer chain, we decided to investigate polycationic polymers. Polyviologen AB-copolymers, comprising alternating decamethylene (A) and positively charged bipyridinium (B) segments, are known[41] to form stable, water-soluble complexes with α-CD. The positive charges, associated formally with the nitrogen atoms on the bipyridinium segments of the polymer, should act[42,43] as electronic "speed bumps" over which the CD rings must pass. Thus, the α-CDs should thread onto the polymer chain in aqueous solution, resting predominantly on its decamethylene segments, stabilized by the hydrophobic interactions inside the cavities of the CD beads. The polyviologen **2** was synthesized as reported in the literature.[41a] The number average molecular weight ($M_n = 7760$) of **2**, which was determined (end group analysis) by ^{1}H NMR spectroscopy, corresponds to its having an average of 17 repeating AB units.

Formation of the Pseudopolyrotaxane. The lactoside-bearing CD derivative **1** was synthesized as reported in the literature.[24] The self-assembly (Scheme 1) of the pseudopolyrotaxane **3** from **1** and **2** was monitored (Figure 1) by ^{1}H NMR spectroscopy. When **2** was added to a 17-fold excess of **1** (20 mM) in D_2O, changes in the proton resonances of both the polymer and CD were observed. In addition to the broadening of the CD signals, upfield shifts were observed for the H-3 protons (which are pointing into the center of the CD cavity), an observation that indicates that the CDs have threaded onto the polymer chains. We estimate[44] that >90% of the CDs are threaded after 4 days. Moreover, the signals ($\delta = 1.3-1.5$ and 2.12 ppm) arising from the protons on the decamethylene segments of free **2** (H_{E-G}(uc)) decrease in their intensities with time, while new signals, arising from decamethylene segments onto which the CDs have threaded in **3** (H_{E-G}(c)), appear at $\delta = 1.5-1.7$ and 2.22 ppm. Additional signals for the complexed H_D and H_{E-G} overlap with the original uncomplexed signals. The presence of two sets of resonances for H_D and H_{E-G} at equilibrium is attributed to the asymmetry of the two rims of the CDs, a property that renders each half of the decamethylene chain chemically nonequivalent.[43b]

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(44) The formation of the pseudopolyrotaxane was also monitored by TLC (4:3:3:2 EtOAc/MeOH/H_2O/AcOH) using 5% H_2SO_4 in EtOH as the developing solution. The free CD **1** (RF = 0.5) disappeared over time as a new spot representing complex **3** appeared at the baseline.

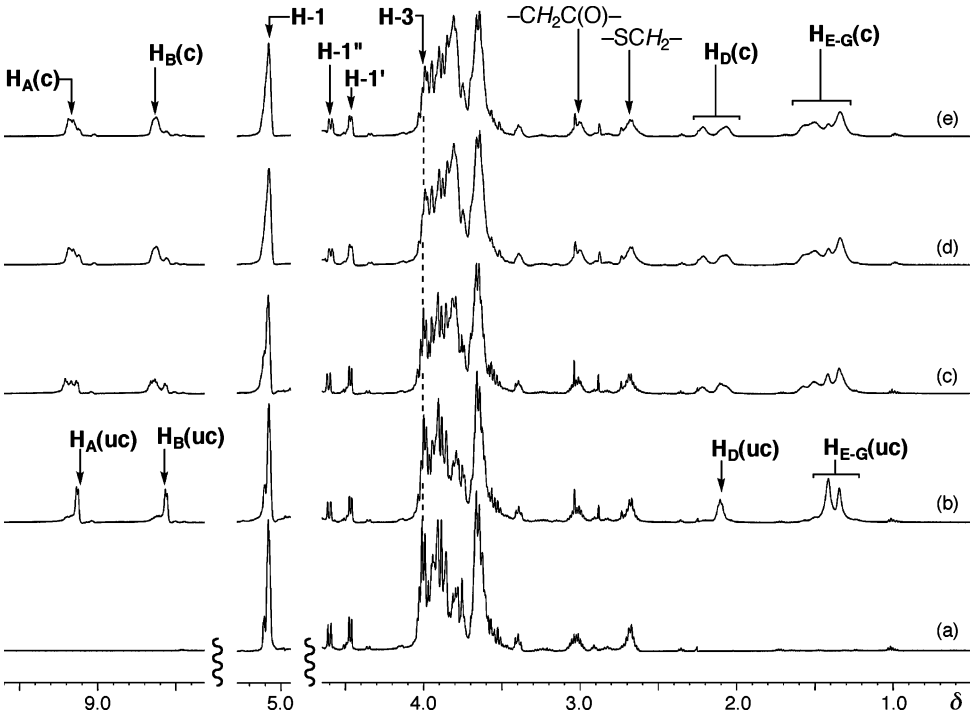

Figure 1. ^{1}H NMR spectra (500 MHz, D_2O, 298 K) of (a) CD **1**, and the mixture of CD **1** and polymer **2** at (b) 0.5 h, (c) 1 d, (d) 2 d, and (e) 4 d after mixing.

Complexation of the decamethylene segments also influences the chemical shifts of the protons on the bipyridinium segments as evidenced by the presence of new signals, H_A(c) and H_B(c), in the aromatic region of the ^{1}H NMR spectrum. The presence of these signals, arising from the CD-occupied segments, demonstrates that the equilibrium is displaced toward the formation of the pseudopolyrotaxane. Once equilibrium is attained (4 days), the spectrum does not change, and even after a 50-fold dilution of the D_2O solution, the spectrum still stays the same over the course of a week, demonstrating that the CDs and polymer chains form a stable pseudopolyrotaxane.

2D NMR spectroscopy was particularly useful in determining that the CDs were resting on the decamethylene (and not the bipyridinium) segments of the polymer. The H-3 and H-5 protons of the CD torus point into the center of its cavity and therefore serve as probes for the complexation of a guest molecule. The presence of cross-peaks in the 2D TROESY NMR of pseudopolyrotaxane **3** between the decamethylene signals of the polymer and the H-3 and H-5 resonances is consistent with the close proximity of the decamethylene protons to the H-3 and H-5 protons as a result of formation of the complex.

Gal-1-Induced T-cell Agglutination. The ability of Gal-1 to aggregate cells, used to malignant advantage in cancer, formed the basis of a straightforward assay. Treatment of CEM cells (cultured human T-leukemia cells) with 10 μM recombinant human Gal-1 for 5 min at 37 °C resulted in large aggregates that resemble tumor emboli (Figure 2i,ii). As determined by light microscopy, pretreating Gal-1 with 2 mM lactose (final concentration) for 10 min at room temperature allows the cells to remain largely dispersed (Figure 2iii), whereas a mixed state of aggregated and dispersed cells (Figure 2iv) is observed down to 1.5 mM lactose. A value of 1.5 mM for the minimum inhibitory concentration (MIC) of lactose with Gal-1 matches well with the literature values in other agglutination assays.[33b,45] The trivalent glycoclusters, the chitosan-derived polymer, the pseudopolyrotaxane, and its components were evaluated qualitatively using this T-cell agglutination assay.

Evaluation of the Trivalent Glycoclusters with Gal-1. The trivalent lactosides **8** and **9** completely inhibited Gal-1-induced aggregation (Table 1) at a concentration of 0.7 mM of the glycocluster in solution, with the trivalent lactoside **10** performing nominally better (0.6 mM). On a valency-corrected basis, taking into account three lactose residues per glycocluster, the performance of trivalent glycoclusters **8** and **9** was equivalent to native lactose, while the tris-lactoside **10** exhibited a slight enhancement to attain the dispersed state (Figure 3iv−vi). The lowest concentration of the mixed state (0.5 mM) was the same for all three trivalent lactosides, and this valency-corrected MIC (1.5 mM) is identical to lactose. Thus, the trivalent compounds, at best, demonstrated only a small improvement in inhibiting Gal-1-induced cell agglutination.

Table 1. Gal-1 Inhibition with Glycoclusters **8**, **9**, and **10**[a]

glycocluster concn [μM]	lactose- and valency-corrected concn [mM]	lactose	cluster **8**	cluster **9**	cluster **10**
800	2.4	D	D	D	D
700	2.1	D	D	D	D
600	1.8	M	M	M	D
500	1.5	M	M	M	M
400	1.2	A	A	A	A
300	0.9	A	A	A	A
200	0.6	A	A	A	A
100	0.3	A	A	A	A

[a] Series of concentrations tested for lactose and each glycocluster at which an aggregated (A), mixed (M), or dispersed (D) state of T-cells was observed in the presence of 10 μM galectin-1 in the agglutination assay. The compounds were premixed with galectin-1 for 10 min at ambient temperature before being incubated with the CEM cells for 5 min at 37 °C. Each compound was tested at least three times at each concentration.

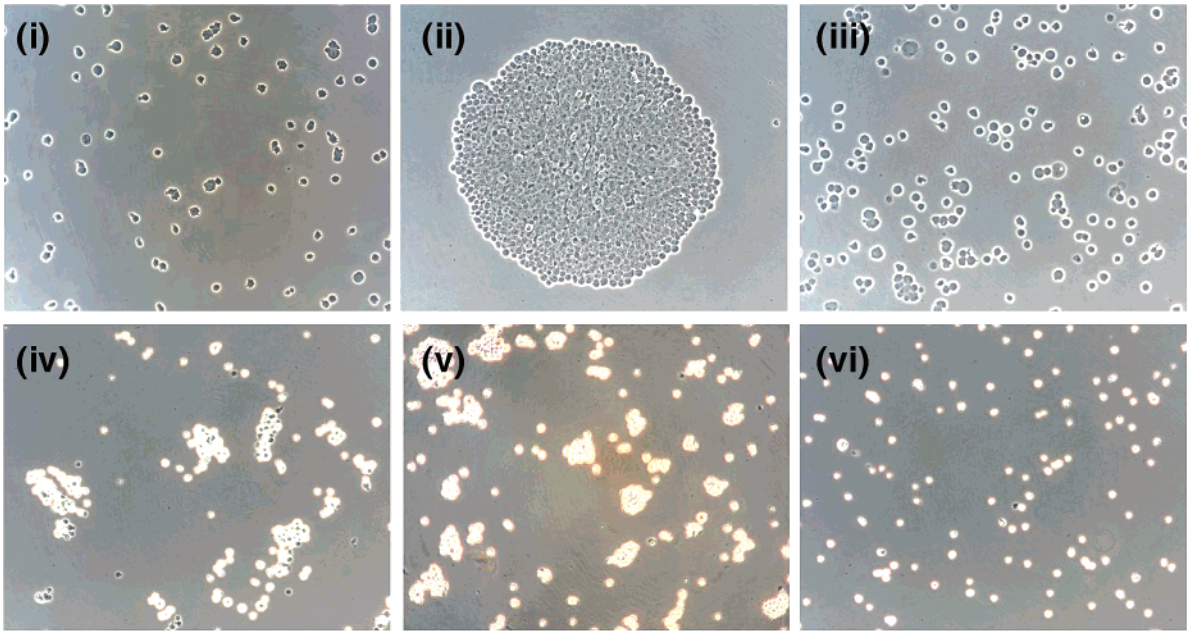

Figure 2. Light microscopy images (10×) of CEM cells in the presence or absence of Gal-1 and inhibitors. (i) In the absence of Gal-1, cells are dispersed (D). (ii) With 10 μM Gal-1 cells are aggregated (A). (iii) With 10 μM Gal-1 and 2 mM lactose, cells are dispersed (D). (iv) With 10 μM Gal-1 and 1.8 mM lactose, cells are in a mixed state (M). (v) With 10 μM Gal-1 and 1.8 mM (valency-corrected) **8**, cells are in a mixed state (D). (vi) With 10 μM Gal-1 and 1.8 mM (valency-corrected) **10**, cells are dispersed (M).

Evaluation of Chitosan-Based Polymer with Gal-1. The HCl salt of the commercially available chitosan polymer was not inhibitory at over 9 mM of the repeating unit. When 25% of the monosaccharides of this polymer were substituted with the galactityl-linked lactoside to yield neoglycopolymer **11**, an inhibitor was obtained. Cells were dispersed (Table 2) with 1.8 mM **11** and exhibited a mixed state down to 900 μM **11** (concentrations based on lactoside). This valency-corrected MIC of 900 μM compares favorably to monovalent lactose (1.5 mM), but only by 1.7-fold.

Table 2. Gal-1 Inhibition with the Chitosan **11**[a]

concn (valency-corrected)[mM]	lactose	chitosan **11**
1.8	M	D
1.7	M	M
1.5	M	M
1.3	A	M
0.9	A	M
0.75	A	A
0.6	A	A

[a] Series of valency-corrected concentrations tested for lactose and chitosan **11** at which an aggregated (A), mixed (M), or dispersed (D) state of T-cells was observed in the presence of 10 μM galectin-1 in the agglutination assay. The compounds were premixed with galectin-1 for 10 min at ambient temperature before being incubated with the CEM cells for 5 min at 37 °C. Each compound was tested at least three times at each concentration.

Evaluation of Pseudopolyrotaxanes with Gal-1. Interestingly, the lactoside-bearing α-CD had a noticeable shift in the onset of aggregation compared (Table 3) to native lactose. Whereas a mixed state was not observed below 1.5 mM of lactose or 500 μM trivalent lactoside, the cells were still dispersed at 1.5 mM of the CD **1** and the mixed state was observed at 1 mM of the CD **1** (Figure 3ii). Thus, the monovalent CD **1** performed similarly to the rigid chitosan-based polymer **11** with valency-corrected MICs of 1 mM and 0.9 mM. In fact, CD **1** attains the dispersed state at a lower concentration (1.5 vs 1.8 mM, based on lactoside concentration) than does the polymer **11**. The monovalent CD **1** also outperforms the trivalent lactosides on a valency-corrected basis. Considerably more potent inhibition was uncovered, however, when the CD **1** was presented multivalently in the pseudopolyrotaxane **3**.

Table 3. Gal-1 Inhibition with CD **1** and Pseudopolyrotaxane **3**[a]

concn (valency-corrected)[mM]	lactose	CD **1**	complex **3**
2.0	D	D	D
1.5	M	D	D
1.0	A	M	D
0.75	A	A	D
0.5	A	A	M
0.25	A	A	M
0.15	A	A	M
0.1	A	A	A
0.05	A	A	A

[a] Series of valency-corrected concentrations tested for lactose, CD **1**, and pseudopolyrotaxane **3** at which an aggregated (A), mixed (M), or dispersed (D) state of T-cells was observed in the presence of 10 μM galectin-1 in the agglutination assay. The compounds were premixed with galectin-1 for 10 min at ambient temperature before being incubated with the CEM cells for 5 min at 37 °C. Each compound was tested at least three times at each concentration.

The assays revealed a dramatic effect of the self-assembled multivalent pseudopolyrotaxane upon the inhibition of Gal-1-induced cell agglutination. (It should be noted that the concentrations recorded in Figure 3 are "valency-corrected" based on lactose residues.) Controls of the polymer **2** alone and the pseudopolyrotaxane formed with native α-CD (without lactose) were not inhibitory at concentrations equivalent to the highest tested for **3**. At identical "lactose" concentrations (horizontal comparisons in Figure 3), large differences are observed in the response of the cells to Gal-1; for example, at a 1 mM "lactose" concentration, the cells (i) remain aggregated when they are treated with native lactose, (ii) assume a mixed state of

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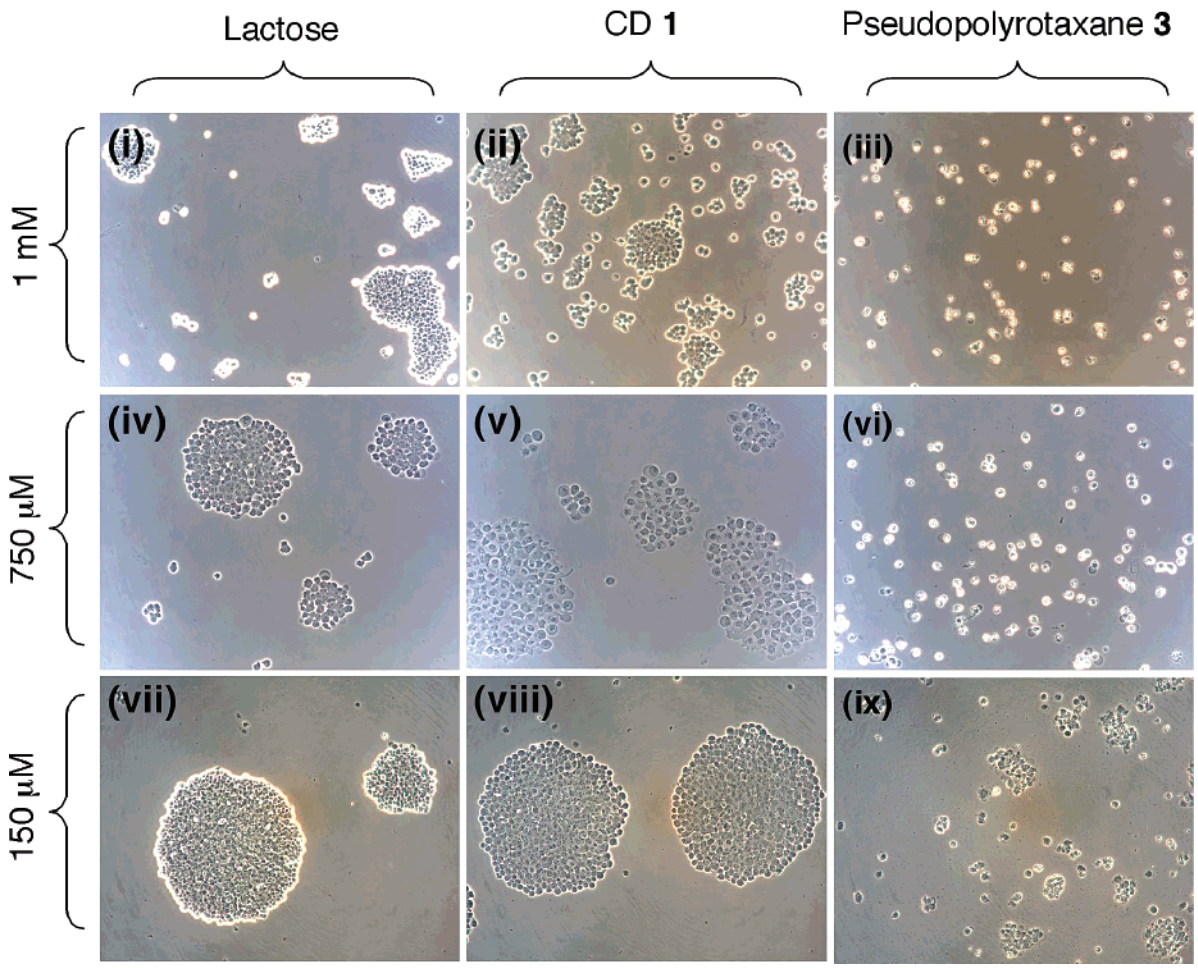

Figure 3. Pictures (i−ix) taken by light microscopy (10×) of CEM cells with 10 μM Gal-1 and varying concentration of lactose (column 1), CD **1** (column 2), and pseudopolyrotaxane **3** (column 3). Valency-corrected concentrations are shown.

aggregated and dispersed cells when treated with **1**, and (iii) become dispersed when treated with **3**. At 750 μM, the lactose and **1** treated cells are mostly aggregated (iv and v) while the cells treated with **3** remain dispersed (vi). With **3**, a mixed population (aggregated and dispersed) is observed (vii, viii, and ix) down to 150 μM of "lactose". It is of note that, at 150 μM "lactose", the concentration of individual polymer chains is 8.8 μM, a value just below the concentration of Gal-1. A comparison of the lowest concentrations at which the mixed populations are observed revealed a 6.7-fold improvement of **3** over **1** and a 10-fold improvement of **3** over lactose itself.

Discussion

The topology of Gal-1, where the binding sites are located on opposite faces of the protein, provides a lectin that is challenging to target multivalently. A number of glycoclusters have been investigated,[33] with variable results, ranging from negligible[33d] to potent[33b] in vitro assays and up to 14.5-fold valency-corrected enhancement in cellular assays.[33b] Glycoclusters with selectivities for galectin-3 over Gal-1 have been reported in the literature.[33c,d] Recently, Gabius and co-workers[33e] have described 2.8-fold and 5.9-fold valency-corrected enhancements for per-substituted lactose and *N*-acetyllactosamine β-CD glycoclusters, respectively, in a Gal-1-mediated hemagglutination assay. These β-CD glycoclusters displayed a higher activity toward galectin-3 by up to 28-fold. Starburst glycodendrimers synthesized by Roy and co-workers[33a] displayed excellent multivalent enhancements (on a per-lactoside basis) for binding Gal-1 in solid-phase competition assays. However, these assays yield highly variable results, depending on the nature of the competitor and the surface-immobilized species.[33] To our knowledge, multivalent enhancements for the inhibition of Gal-1 in cell-based assays of over 16-fold have not been achieved.[33e]

For the purpose of comparing the pseudopolyrotaxane with other multivalent structures, the trivalent lactosides **8**, **9**, and **10** and chitosan-based polymer **11** were synthesized and evaluated in the agglutination assay. In our hands, the valency-corrected MIC for the trivalent lactosides **8**, **9**, and **10** showed no improvement over native lactose as inhibitors of Gal-1-induced T-cell agglutination. These three glycoclusters differ in their size, shape, and flexibility, yet behave similarly in the assays. Although all three glycoclusters had identical valency-corrected MICs compared to that of lactose, the dispersed state for the largest glycocluster **10** was observed at a lower concentration than those for the other two glycoclusters.

The rigid chitosan polymer **11** bearing lactose epitopes showed only a slight enhancement over native lactose in interacting with Gal-1 in the assay. While chitosan-based **11** and the pseudopolyrotaxane **3** are both polymeric structures, we observed dramatic differences in their interactions with Gal-1. The relatively small enhancement in the valency-corrected MIC (1.7-fold over native lactose) in the binding of Gal-1 by the

polymer **11** may be attributed to the rigidity of the polymer backbone, which could prevent binding to the lectins in the same manner as the pseudopolyrotaxane **3** where a 10-fold enhancement was observed. It is of note that Roy and co-workers[40b] have successfully targeted plant lectins using α-gal and sialic acid epitopes appended to chitosan by reductive amination. While neogylcopolymers have not been widely explored[46] for binding Gal-1, lactoside-functionalized polymers with *N*-(2-hydroxypropyl)methacrylamide[47] and poly(norbornene-imide)[48] backbones, both of which are more flexible than that of chitosan, have been used to target Gal-3.

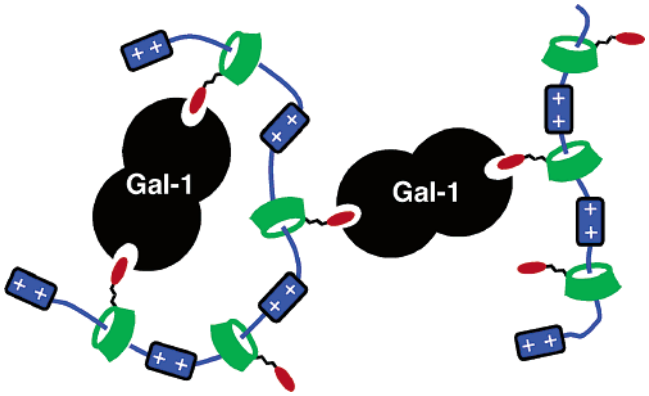

Figure 4. Possible binding modes for Gal-1 and pseudopolyrotaxane **3**.

The pseudopolyrotaxane **3**, the product of numerous self-assembling components, contains mobile ligands bound by noncovalent interactions, with the ability to rotate and translate on the polymer backbone, which may be important factors in its exhibiting enhanced avidity for Gal-1. In contrast to chitosan, the polyviologen backbone of the pseudopolyrotaxane **3** possesses flexible decamethylene units. The flexibility afforded by the polymer backbone, in conjunction with the dynamic property of its lactoside ligands, affords a supramolecular structure with the fluidity and adaptability of cellular membranes, the biological target of Gal-1.[29a,e] Since the lectin is itself bivalent and the pseudopolyrotaxane **3** is up to 17-valent (on the basis of the average number of repeating units in the polyviologen), many binding modes between the protein and pseudopolyrotaxane are possible (Figure 4). The 10-fold enhancement in the valency-corrected MIC is on the same order of magnitude as that observed by Yui and co-workers[28] for their CD-based poly(ethylene glycol) polyrotaxanes displaying variable amounts of maltose residues in a concanavalin A hemagglutination assay. These authors found that the degree of inhibition depended on both the number of carbohydrate ligands and the dynamics of the CDs to which they were attached. The 10-fold enhancement observed in our assay is also on the same order of magnitude as has been observed in cellular assays for Gal-1 inhibition[33b] with wedge-like multivalent lactoside dendrons and the glycoprotein asialofetuin. This enhancement is higher than was observed in a hemagglutination assay with β-CD bearing seven lactosides.[33e] While monosubstituted α-CD has been employed in this present study, many research groups are utilizing per-substituted CDs as glycoclusters for multivalent display.[49] Pseudopolyrotaxanes formed with per-substituted CDs will have a greater local concentration of ligand, which may increase the potency of the complex.

We emphasize here that the pseudopolyrotaxane **3** is not a single molecule, but rather a *supramolecular species*. On average, it is the confluence of ca. 18 different molecules (the polyviologen "string" plus ca. 17 CD "beads") that produce the multivalent effect. There are many examples[50] in biology where individual proteins self-assemble into superstructures wherein the complexes gain or enhance functions relative to those of the individual components. Although the time scale of assembly (4 days) is slower as a result of the viologen units on the polymer backbone, the pseudopolyrotaxane **3** is a rudimentary example of this phenomenon. The ligands, bound by noncovalent interactions, are able to rotate freely and independently around the polymer backbone. The CDs can translate within the confines of the decamethylene units, which have a length that is twice the depth of a CD cavity. Not only do the lactoside ligands have the ability to "fine tune" their positions without enthalpic penalties having to be paid on account of inducing strain, but the unbound ligands may also reside away from the bulky protein in order to reduce steric clashes. The polymer backbone influences directly the dynamics of the translational movement of the CDs. Whereas the polyviologen contains electrostatic "speed bumps" that reduce the translational motion of the CD beads, other polymers, such as poly(ethylene glycol) and poly(tetrahydrofuran), allow the free movement of the CDs along the polymer. As the biochemistry involving unnatural supramolecular species and mechanically interlocked molecules evolves and matures, the dynamics of ligand display will shed light on the supramolecular chemistry of natural systems.

Conclusion

A supramolecular species formed from almost 20 self-assembling components has been used successfully to inhibit Gal-1 in a T-cell agglutination assay. While other multivalent ligands tested displayed little to no enhancement in the valency-corrected MIC, a 6.7-fold improvement over the lactoside-CD **1** and a 10-fold improvement over lactose itself was observed for the lactoside-displaying CD-based polyviologen pseudopolyrotaxane **3**. We are pursuing actively a more detailed characterization of the structural and thermodynamic nature of the multivalent interactions between **3** and Gal-1. Self-assembled pseudopolyrotaxanes, such as **3**, display highly mobile ligands as a result of the CD rotating about the polymer chain while exhibiting limited translation along them. This flexible and dynamic ligand presentation adds a new dimension to the study of protein−carbohydrate interactions and the exploitation of multivalency for targeting therapeutically relevant lectins.

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Experimental Section

General Methods. Tris-methylamino core compound **4**,[22b] trisaccharide **7**,[22b] and CD 124 were prepared as described in the literature. CD **1** was prepared as described in the literature.[24] All other chemicals were purchased from Aldrich and used as received. Solvents were used as purchased, except for CH_2Cl_2 (distilled from CaH_2) and MeOH (distilled from Mg turnings). Analytical TLC was performed on silica gel 60-F_{254} (Merck) with detection by fluorescence and/or by charring following immersion in a 5% H_2SO_4/EtOH. Flash chromatography was performed with silica gel 60 (Silicycle). Preparative gel permeation chromatography was performed using (1) a 25 × 900 mm^2 column of Sephadex LH20 resin (Sigma), eluting with MeOH or (2) a 5 × 100 cm^2 column of Sephadex G-25 resin (Amersham Pharmacia Biotech), eluting with 5% *n*BuOH in water. High-resolution matrix-assisted laser desorption ionization (HR-MALDI) mass spectra were recorded using dihydroxybenzoic acid as a matrix and external calibration using substance P on an IonSpec Ultima 7.4 T FTMS instrument. ^1H and ^{13}C NMR spectra were recorded on a Brüker Avance 500 spectrometer (at 500 and 125 MHz, respectively) or Brüker Avance 600 spectrometer (at 600 and 150 MHz, respectively) at ambient temperature using D_2O as the solvent.

Polyviologen 2. 1,10-Dibromodecane (2.6 g, 16.7 mmol) and 4,4′-bipyridine (5 g, 16.7 mmol) were dissolved in 1:1 MeOH/DMF (16 mL), and the reaction mixture was stirred for 20 h at 75 °C. It was diluted with H_2O (50 mL) and then extracted with $CHCl_3$ (50 mL). The aqueous layer was concentrated under vacuum to afford a dark yellow solid (6.5 g). ^1H NMR (500 MHz, D_2O) δ = 1.13−1.5 (m, H_{E-G}), 2.12 (br s, H_D), 4.68 (br t, H_C, J = 7.4 Hz), 7.98 (br d, H_B from end groups), 8.34 (br d, $H_{B'}$, from end groups), 8.51 (d, H_B, J = 6.4 Hz), 8.78 (br s, H_A from end groups), 8.94 (d, $H_{A'}$, from end groups), 9.08 (d, H_A, J = 6.4 Hz).

Pseudopolyrotaxane 3. The polyviologen **2** (4.5 mg, 0.58 μmol) was added to 0.5 mL of a 20 mM solution of the CD **1** in H_2O. This aqueous solution was allowed to stand 4 days at ambient temperature. The threading process was monitored by TLC using 4:3:3:2 EtOAc/MeOH/H_2O/AcOH and 5% H_2SO_4 in EtOH as the developing agent. This stock solution (20 mM per molar lactose) was diluted accordingly to provide the appropriate per molar lactose concentrations for the agglutination assay.

Agglutination Assay. Lactoside (6.26 × final concentration) and recombinant human[51] Gal-1 (62.6 μM) were mixed in phosphate-buffered saline (8 μL) at room temperature for 10 min. A solution (42 μL) of 7.5 × 10^6 CEM cells/mL in serum-free RPMI 1640 media was added. The mixture (10 μM galectin-1, 3.15 × 10^5 cells) was allowed to stand for 5 min at 37 °C. The mixture was vortexed briefly, and an aliquot (10 μL) was taken for imaging by light microscopy. At least three independent experiments were performed at each lactoside concentration. CEM cells (subclone of ATCC no. CCL-119) were grown in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 10 mM HEPES, and 2 mM L-glutamine at 37 °C and 5% CO_2.

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Supporting Information Available: Experimental procedures, data from the agglutination assays, and NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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