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High-Avidity, Low-Affinity Multivalent Interactions and the Block to Polyspermy in *Xenopus laevis*

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Abstract: The interaction of the lectin XL35 with the jelly coat protein (JCP) surrounding oocytes in *Xenopus laevis* is essential for the block to polyspermy. The molecular details of this event are poorly understood, and the present study has been undertaken with a view to delineating the mechanism of formation of the fertilization envelope. A range of JCP-derived oligosaccharides were synthesized, and all were installed with an artificial aminopropyl arm. This arm allowed the preparation of monovalent derivatives by acetylation of the amino group or the synthesis of polyvalent compounds by attachment to an activated polyacrylamide polymer. A number of analytical techniques, including enzyme-linked lectin assays and surface plasmon resonance, have been developed and utilized to study the interactions of the mono- and polyvalent compounds with XL35. The results reveal that the lectin XL35 has remarkably broad specificity for galactose-containing saccharides and the affinities are only slightly modulated by secondary features, such as anomeric configuration of the terminal sugar or the identity and linkage pattern of branching sugars. Broad specificity was also observed when the saccharides were presented in a polyvalent fashion. The glycopolymers displayed 10−20-fold increases in valency-corrected affinities compared to the corresponding monovalent counterparts. Although the synthetic polymers are not as potent as the JCP, the kinetics of their interactions mirror closely those of the native ligand, and in each case extremely long-lived interactions were observed. The results of this study indicate that, in *X. laevis*, the true *biological function* of multivalency is not to create an extremely tightly binding complex between XL35 and its natural ligand but, instead, to create a very stable protective layer that will not dissociate and is yet flexible enough to encapsulate the *developing* embryo. It is postulated that, even if these partners are unable to attain true equilibrium on the time scale of the biological event, their mode of interaction would, nevertheless, be expected to guarantee an insurmountable physical block to polyspermy. This study has also highlighted that multivalent interactions require a very long time to achieve equilibrium, and this feature may well be the origin of several of the ambiguities reported in the literature when multivalent ligands have been evaluated.

Introduction

Carbohydrates are key to many of the events leading to fertilization,[1−3] and examples include recognition of the egg by the sperm,[4] induction of the acrosome reaction, fusion of the sperm and egg, and the formation of the fertilization layer. This last process involves fusion of cortical granules with the plasma membrane and the subsequent release of the granule contents into the glycoprotein matrix enveloping the oocyte. Interaction of the cortical granule content with the egg glycoprotein matrix induces an irreversible change to the egg jelly coat, establishing a block to polyspermy.

The South African clawed toad, *Xenopus laevis*, is a useful model animal in which to study phenomena associated with fertilization and early development. In this organism, the interaction of the novel carbohydrate-binding protein XL35[5−7] with the *O*-glycans of the jelly coat protein (JCP) enrobing the egg is considered key in the prevention of polyspermy. XL35 is the first of a newly identified class of carbohydrate-binding proteins[8] that is characterized by its requirement for calcium ions to bind its ligand, despite the fact its members do not contain the calcium-binding motif found in the well-studied C-type lectins.[9] Homologues of XL35 have been found in mice,[10] rats,[6] and humans,[8] and the latter have been shown to

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display unusual patterns of cell-type expression and most likely function in microbial surveillance.[8]

Although native XL35 is an oligomeric glycoprotein with a molecular weight of approximately 450000, under reducing SDS−PAGE it has an apparent molecular weight of about 45000 and has consequently been estimated to be an oligomer of between nine and twelve carbohydrate-binding units.[6,11] Other questions relating to the structure of XL35 and to its putative ligand, however, remain only partially resolved. Limited information on the identity of the natural ligand has come from inhibition studies with simple saccharides, and these have underlined the importance of terminal D-galactopyranose moieties present in the JCP for its recognition by XL35.[7,12] The difficulties encountered in establishing the identity of the endogenous carbohydrate epitope that gives optimum affinity for XL35 stem from the fact that the jelly coat which layers the unfertilized oocyte turns out to be structurally complex. The JCP is composed predominantly of high-molecular-weight ($>2 \times 10^6$) mucins and is over 60% sugar by weight.[13] Moreover, NMR and mass spectroscopic studies have revealed the mucin to be composed of a very heterogeneous set of *O*-glycans.[14−17] Furthermore, several of the reported JCP-derived oligosaccharides are novel, such as a dimeric H antigen and a blood group A antigen terminated with a GalNAc residue, but which of these myriad of structures is important in the binding of XL35 to the JCP has, however, remained a matter of conjecture.

Any attempt to elucidate the mechanism of binding of XL35 with its natural ligand is complicated not only by the heterogeneity of the JCP, but also by the fact that its interaction with XL35 is probably "multivalent" in nature.[11,12] Many receptors of carbohydrates when multiply presented bind avidly to their respective ligands but *only* when these are presented multivalently.[18−21] This cluster of weakly binding monovalent ligands when properly orientated can sometimes generate enhancements in association constants, over and above those expected on the basis of valency. The "cluster effect", as it has been coined,[22] is now routinely proposed as the mechanism by which intrinsically weak protein−carbohydrate interactions are strengthened and are manifested in vivo. The implication is that inherently weak interactions are unlikely to be of any biological relevance. Studies with synthetic ligands and a variety of polyvalent lectins have established that these partners can enter into a tight binding relationship if the initially weakly binding monovalent carbohydrate epitope is taken and presented multivalently and in the appropriate orientation.[22−27]

In the present study, the interaction of XL35 with the JCP has been systematically investigated using a range of chemically synthesized mono-, di-, and trisaccharide derivatives—truncated analogues of JCP-derived oligosaccharides. These saccharides were also appended to a high-molecular-weight polymer to probe the importance of the cluster effect for binding of the JCP. Independent analytical techniques including enzyme-linked lectin assays (ELLA) and surface plasmon resonance (SPR) have been exploited to probe the interactions of these compounds with XL35. A comparison has been made of the binding behavior of the various synthetic glycopolymers with that of the corresponding monovalent ligands and also with that of the natural polymeric ligand, the JCP. This study has allowed important aspects of the mode of interaction of XL35 with the JCP to be delineated and provides an explanation of how a physical block to polyspermy can be mounted.

Results and Discussion

Design of Carbohydrate Epitopes and Synthesis of Monovalent Ligands. *O*-Glycans released upon mild base treatment of the mucin samples derived from the oocytes of six individual *X. laevis* toads have been found by NMR spectroscopy to comprise 23 different structural motifs, 11 of which are unique.[16] A large number of these structures are seen to carry a terminal α-Gal(1−4) moiety, and taken together with the results of the earlier inhibition studies,[5,7,28−31] these data establish a terminal α-D-galactopyranoside unit as an important structural determinant of the putative ligand of XL35. The early inhibition studies also indicate that various features of nonterminal sugar moieties might serve as recognition elements.[29] The trisaccharide D-Gal*p*-[L-Fuc*p*-(α-1−4)]*β*-D-Gal*p* and the two disaccharides D-Gal*p*-(α-1−4)*β*-D-Gal*p* and L-Fuc*p*-(α-1−2)*β*-D-Gal*p* appear as terminal sequences in a number of the reported JCP-derived *O*-glycans, and consequently, these oligosaccharide structures as well as those of simpler α- and *β*-D-galactopyranosides were selected for investigation. Thus, the monosaccharides **1** and **2**, disaccharides **3**−**5**, and trisaccharide **6** were targeted for chemical synthesis (Figure 1). All are "installed" with aminopropyl arms via which their eventual attachment to a desired scaffold for presentation in a multimeric form would be facilitated. Compounds **1**−**3** were prepared by routine procedures (see the Supporting Information). Disaccharides **4a** and **5a** and trisaccharide **6a** were assembled in a convergent manner[32] from the readily available glycosyl acceptors **9**, **11**, and **16**, respectively, by coupling with the appropriate glycosyl donor **12** or **14** (Scheme 1). Furthermore, acceptor **11** could be obtained from acceptor **9** by a simple two-step reaction sequence. Thus, regioselective monobenzylation of the C-3 hydroxyl of precursor **7** was accomplished via a stannylidene acetal, formed in situ by treatment of this tetrol with dibutyltin oxide in refluxing methanol.[33] Reaction of the thus formed tin

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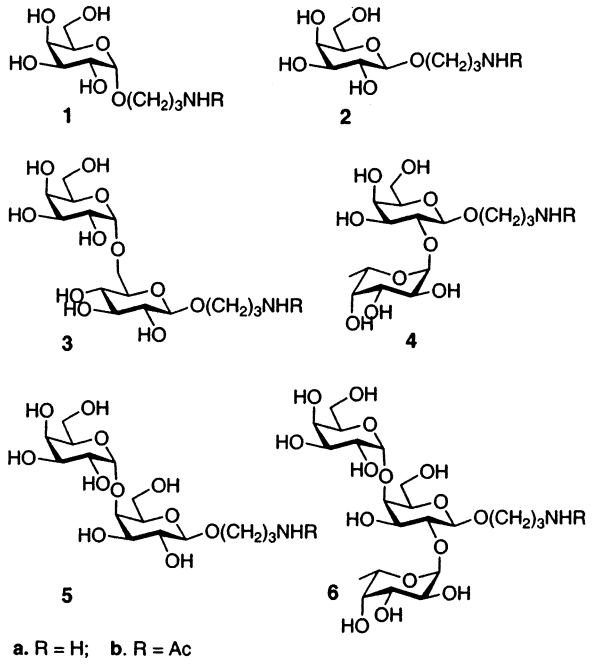
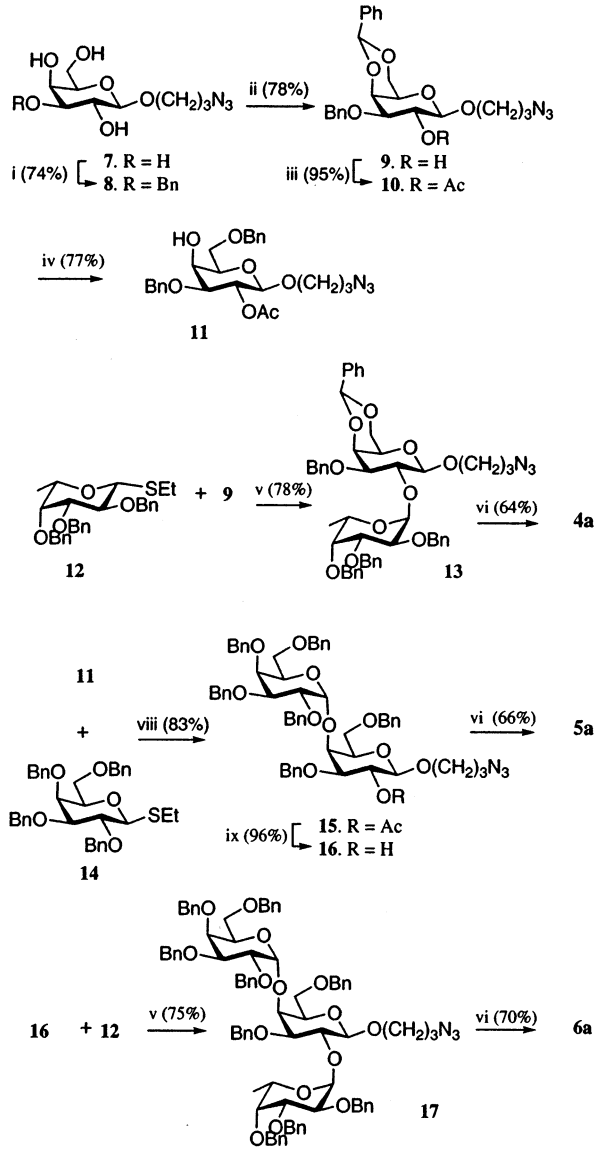


Figure 1. Truncated JCP-derived analogues targeted for synthesis in this study.

intermediate with benzyl bromide in the presence of the activator tetrabutylammonium iodide gave the triol **8**, which, upon treatment with benzene dimethyl acetal in the presence of a catalytic amount of *p*-toluenesulfonic acid in acetonitrile, gave the desired 4,6-*cis*-benzylidene acetal **9** in a yield of 78%. Acetylation of the C-2 hydroxyl of acceptor **9** and treatment of the resulting derivative **10** with $NaCNBH_3/HCl$[34] led to regioselective opening of the benzylidene acetal function to give the glycosyl acceptor **11** in a yield of 77%. IDCP-mediated coupling[35] of acceptor **9** with the highly reactive thiofucosyl donor **12**[36] gave solely the α-disaccharide **13** in good yield. Removal of the benzyl ether and benzylidene protecting groups in **13** with concomitant reduction of the azido moiety was accomplished smoothly by catalytic hydrogenation over Pd/C in ethanol acidified with HCl, to give target disaccharide **4a**. Coupling of acceptor **11** with thioglycoside **14** occurred efficiently in the presence of the thiophilic promoter NIS/TMSOTf[35] to give the (1−4)-linked galactoside **15** in a yield of 83% as a separable mixture of anomers ($\alpha/\beta = 9/1$). Removal of the acetyl function in disaccharide **15** under standard conditions gave derivative **16**, which upon catalytic hydrogenation over Pd/C led to removal of the benzyl ether groups and reduction of the azido function to give the target disaccharide **5a**. Alternatively, derivative **16** could serve in the synthesis of the target trisaccharide **6a**. Thus, IDCP-mediated[35] coupling of donor **14** with acceptor **16** gave the α-trisaccharide **17** in a yield of 75% as the only anomer. Removal of the benzyl ether groups occurred simultaneously with reduction of the azido group under standard conditions to afford the target trisaccharide **6a**. Chemoselective *N*-acetylation of target building blocks **1a**−**6a** with acetic anhydride in methanol gave the required monovalent derivatives **1b**−**6b**, respectively. Disaccharide **3a** was also biotinylated for use in subsequent SPR experiments (see later).

Scheme 1 [a]



[a] Reagents and conditions: (i) Bu_2SnO, MeOH, Δ, then BnBr, Bu_4NI, toluene; (ii) $PhCH(OMe)_2$, CSA, CH_3CN; (iii) Ac_2O, pyridine; (iv) $NaCNBH_3$, $HCl{\cdot}Et_2O$, THF; (v) IDCP, Et_2O, CH_2Cl_2, MS, 4 Å; (vi) Pd−C, H_2, 5% HCl−EtOH; (vii) Ac_2O, MeOH; (viii) NIS/TMSOTf, DCM, MS, 4 Å; (ix) NaOMe, MeOH, then Dowex H^+.

Synthesis of Glycopolymers. It was considered that a relatively high-molecular-weight, water-soluble, linear polymer, onto which the various monovalent aminopropyl-armed precursors were appended, might constitute an adequate mucin mimic with which to probe the binding of the JCP. Glycopolymers derived from poly[*N*-(acryloyloxy)succinimide] (pNAS) and an aminoalkyl-armed oligosaccharide are well-studied[25,37−42] and were chosen for the present study. The target glycopolymers

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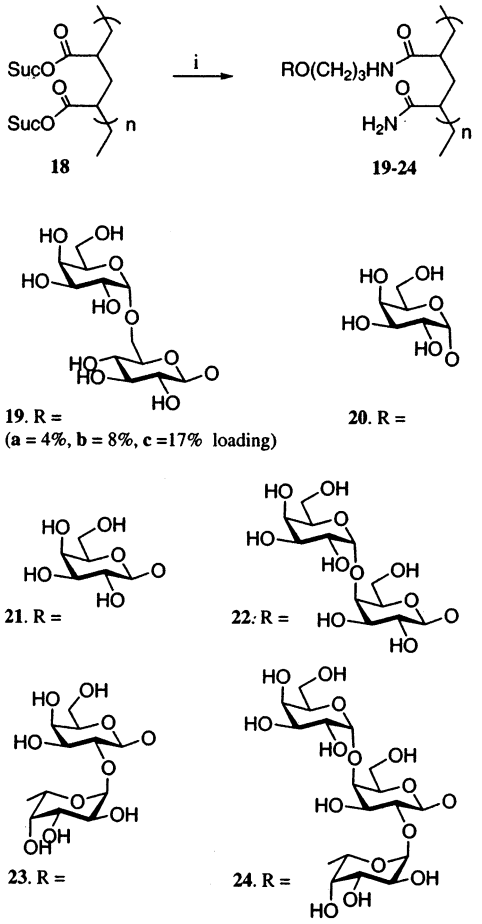
Scheme 2 [a]



[a] Reagents and conditions: (i) aminopropyl saccharide **1a**−**6a**, Et_3N, DMF, then NH_4OH.

19−**24** were prepared by condensation of pNAS **18** with each of the aminopropyl glycosides **1a**−**6a** (Scheme 2), respectively, followed by quenching of the remaining succinimate ester groups with aqueous ammonia. Purification was achieved by exaustive dialysis against water using a membrane with a ~14000 molecular weight cutoff. All glycopolymers were prepared from a single batch of pNAS, which was itself obtained by radical-mediated polymerization of *N*-(acryloyloxy)succinimide. By appropriately controlling the ratio of aminopropyl glycosides to activated esters in pNAS **18**, functionalized polymers **19c** and **20**−**24** with 17% loading were obtained from the corresponding aminopropyl glycosides **1a**−**6a**. Two additional polymers of 4% and 8% loading (**19a** and **19b**, respectively) were also obtained from aminopropyl disaccharide **3**. The proposed structures of the glycopolymers were supported by characteristic features in their ^1H NMR spectral data, but the loading of sugar on each glycopolymer was accurately determined by a phenol−sulfuric assay using the appropriate molar ratio of monosaccharides. The pNAS-derived poly(acrylic acid) sodium salt and pNAS-derived polyacrylamide "backbones" were synthesized by treatment of precursor polymer **18** with sodium hydroxide or excess aqueous ammonia, respectively. A molecular weight of 156000 was determined for the pNAS-derived polyacid by gel filtration using commercial poly(acrylic acid) and dextran standards. The pNAS-derived amide and acid were subsequently employed to determine possible biological effects of the polymer backbone.

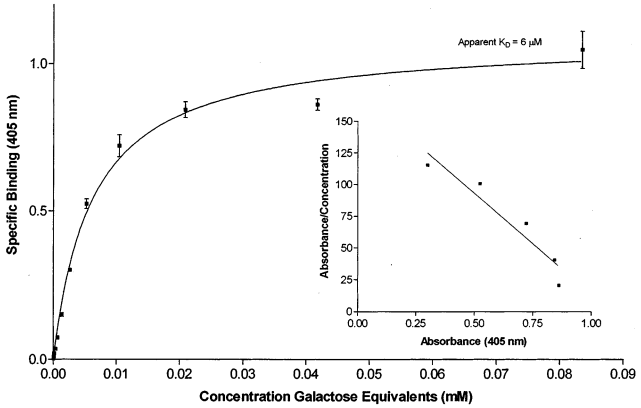


Figure 2. Saturation binding study to characterize ELLA assay. Increasing amounts of JCP−BAP conjugate were added to microtiter wells containing no protein (blank) or a fixed amount of passively immobilized XL35. The result for each concentration of JCP−BAP conjugate is expressed as the difference between the absorbance readings for the wells containing XL35 and that of the blank (total − nonspecific binding). The assay saturated reproducibly (nonlinear regression analysis), with an apparent K_D of 6 μM, on the basis of the quantity of galactose present in the JCP mucin (valence-corrected). The inset shows a Scatchard transformation of the binding isotherm. (The transformed data were not used to calculate the apparent K_D).

Assay Development and Validation. An assay format was developed wherein the JCP was conjugated directly to an enzyme and XL35 coated onto the microtiter plate. The required JCP-linked enzyme was obtained by conjugating bacterial alkaline phosphatase (BAP) to the mucin using a heterobifunctional cross-linker. The amine-reactive cross-linker *S*-acetylmercaptosuccinic anhydride (SATA) was employed, as coupling of components could be carried out at near-physiological pH, safeguarding the activity of the enzyme. Coupling of the JCP to the linker was followed by deprotection of the thiol function (0.5 M hydroxylamine), and this intermediate was reacted with BAP that had been previously functionalized with maleimide to give the required JCP−BAP conjugate. Although BAP is larger and has a lower catalytic activity than the more commonly used HRP, the former enzyme is not glycosylated and was expected to be less likely to give artifacts in the subsequent binding assay or when the sugar content of the JCP conjugate was determined. Saturation binding studies were performed in this second format, again to ensure that the conjugate JCP−BAP interacted in a specific fashion (in this case, with the microtiter-plate-bound lectin). The assay saturated reproducibly with a valence-adjusted K_D of 6 μM for the JCP based on the quantity of galactose present on the mucin (Figure 2). This result was confirmed with an inhibition assay using a JCP derivative as a homologous inhibitor. The latter inhibitor was obtained by taking the JCP derivatized with a thiol linker and alkylating it with iodoacetamide rather than conjugating it to BAP. Gratifyingly, the apparent K_I for the JCP determined from the data of this experiment (Figure 3) is very similar to the apparent K_D obtained from the original saturation binding experiments. To verify the specificity of the binding reaction, several negative controls were performed, including assays with monosaccharides, known from previous agglutination studies not to interact with XL35. For example, *N*-acetyl-D-glucosamine or D-mannose proved not to inhibit even at concentrations as high as 50 mM. These results support that coating XL35 onto the microtiter plate did not alter its binding specificity (see the inset in Figure 3). Taken together, these experiments established that the assay was

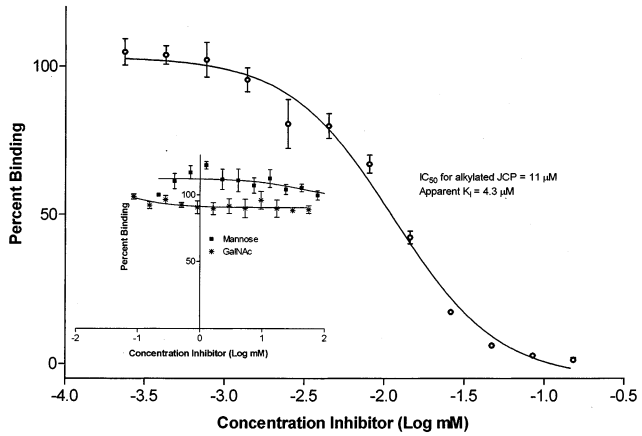


Figure 3. Homologous inhibition experiments and negative controls. To verify that the concentration of the JCP−BAP was appropriate for subsequent inhibition studies, alkylated JCP was used as a homologous inhibitor. Its apparent K_I of 4.3 μM is in good agreement with the apparent K_D as determined previously from saturation binding experiments. Glycans deemed to be irrelevant (from hemagglutination studies) were used as negative controls and indeed show no effect on binding (insets).

Table 1. Inhibition Results of Monovalent Compounds Using Inhibition ELLA (Immobilized XL35)

compd	IC_{50} ($M \times 10^{-3}$)[a]	compd	IC_{50} ($M \times 10^{-3}$)[a]	compd	IC_{50} ($M \times 10^{-3}$)[a]
1b	8 (6−10)	**3b**	14 (8−17)	**5b**	8 (6−11)
2b	26 (21−31)	**4b**	21 (16−28)	**6b**	3 (2−4)

[a] Values in parentheses represent the 95% confidence intervals for each IC_{50}.

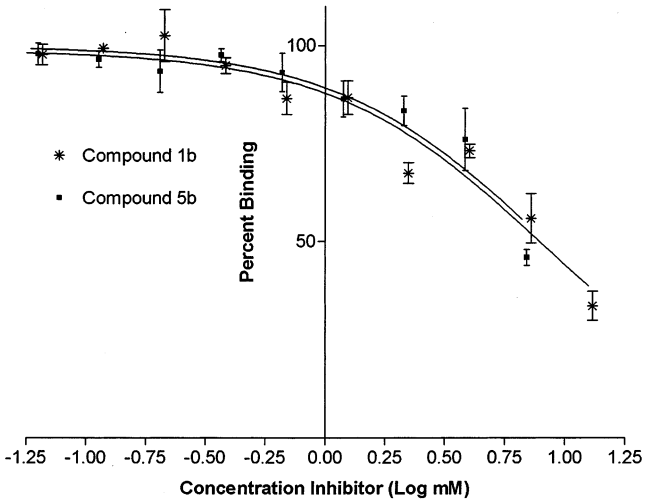


Figure 4. Representative inhibition curves for monovalent compounds using competition ELLA. XL35 was passively immobilized onto a microtiter plate, and a fixed amount of JCP−BAP conjugate was competed away using increasing concentrations of monovalent aminopropyl saccharides.

robust and that the newly synthesized JCP−BAP conjugate was at the proper dilution to provide accurate IC_{50} values for other inhibitors.

Inhibition by Monovalent Compounds. The newly developed ELLA inhibition assay was first used to evaluate the affinities of the synthetic monovalent *O*-glycosides **1b**−**6b** (Figure 1). IC_{50} values are listed in Table 1, and representative inhibition curves from which these values were obtained are shown in Figure 4. Both monosaccharides **1b** and **2b** are inhibitors, although the α-derivative is measurably preferred. The same difference was also seen with α- and β-methyl galactopyranosides (data not shown). Disaccharide **4b** (Fuc-(β1,2)Gal) is a slightly poorer inhibitor than the corresponding galactopyranoside derivative **1b**, which suggests that the L-fucoside moiety adds little to the binding affinity of the galactopyranose epitope. This latter observation is consistent with the failure to inhibit XL35 binding with high concentrations of L-fucose in this assay (data not shown). Disaccharide derivative **5b** (Galα(1,4)Gal) is again no better an inhibitor than the simpler α-galactoside derivative **1b**, although trisaccharide derivative **6b** (Galα(1,4)[Fucα(1,2]Gal), which contains a core epitope found at the terminus of many of the mucin-derived neutral sugars, is seen to be a measurably better inhibitor than the other compounds tested. Collectively, our data suggest that XL35 has a very broad specificity for galactose-containing saccharides and the affinities are only slightly modulated by secondary features, such as anomeric configuration of this terminal sugar or the identity and linkage pattern of branching sugars. Whereas earlier agglutination studies gave conflicting results as to which D-galactopyranoside anomer was inhibitory, the present assay establishes that both are recognized although the α-anomer is preferred. It is interesting to note that the inability of lectins to discriminate between anomers has only been reported in a few cases. One such example pertinent to this study relates to a family of oligosaccharide constructs terminating in either α- or- β-D-galactose.[43] Both anomers of this family were found to be effective inhibitors of sperm binding to mouse eggs.

Inhibition by Multi- and Polyvalent Ligands. The inhibition results for the glycopolymers **19**−**24** are presented in Table 2. The data take into account the molecular weight of the parent polymer backbone and are corrected for the total amount of saccharide on each glycopolymer. As can be seen, the glycopolymers display a marked enhancement in activity; however, on a valence-corrected basis the increase is only 10−20-fold. Furthermore, the data of compounds **19a**−**c** indicate that ligand density has only a marginal effect on binding. The inhibition assay also established that clustering of any of the monomeric *O*-glycosides on a polymeric backbone has no bearing on the specificity of the interaction with XL35, as the relative ranking of the polymers parallels that of their corresponding monovalent counterparts. Thus, XL35 retains its broad specificity even when the ligands are presented in a polyvalent fashion. In contrast to our results, other studies have shown that an oligosaccharide clustering can increase or even switch the selectivities of binding.[44−48] Poly(acrylic acid) sodium salt and the polyamide did not inhibit the binding, confirming that XL35 does not interact with the polymer backbone.

Binding curves for the JCP derivative (from a homogeneous inhibition experiment), the glycopolymer **19c**, and melibiose alone are presented in Figure 5. The data demonstrate that, in an equilibrium setting, the presentation of melibiose in a multivalent fashion in the form of a synthetic polymer substan-

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Table 2. Inhibition Data of Glycopolymers

compd	IC_{50} ($M \times 10^{-5}$), glycopolymer concentration	IC_{50} ($M \times 10^{-3}$), valence-adjusted[a]	compd	IC_{50} ($M \times 10^{-5}$), glycopolymer concentration	IC_{50} ($M \times 10^{-3}$), valence-adjusted[a]
19 (4%)	1.6	0.4 (0.2−0.7)	**21**	0.9	0.4 (0.2−0.6)
19 (8%)	1.6	0.7 (0.4−1.2)	**22**	1.9	1.2 (0.2−0.6)
19 (17%)	0.7	0.6 (0.4−0.9)	**23**	2.4	1.7 (1.1−2.5)
20	1.8	2.5 (1.9−3.3)	**24**	0.5	0.4 (0.3−0.5)

[a] Values in parentheses represent the 95% confidence intervals for each IC_{50}.

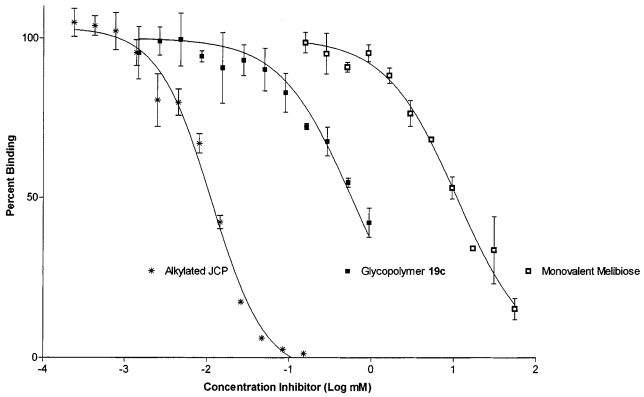

Figure 5. Comparison of monovalent and multivalent ligands using competition ELLA. The results presented are valence-corrected and serve to assess the magnitude of any cluster effect exhibited between XL35 and any multivalent ligand. Glycopolymer **19c** is approximately 16-fold more potent an inhibitor as compared to monovalent melibiose, while the JCP is approximately 60 times more potent than the glycopolymer.

tially alters its inhibition profile relative to that of monovalent melibiose. The improvement in binding is, nevertheless, not as dramatic as that observed for the native ligand. The JCP is approximately 1000 times more potent than monovalent melibiose when the data are corrected for valence. The difference in efficacy between the natural mucin and neoglycopolymers may well be related to differences in their architectures: the JCP is approximately 15 times larger in size than the melibiose glycopolymer **19c** and has a different backbone (protein vs polyacrylamide backbone). Furthermore, the native ligand is very heavily glycosylated, containing a quantity of carbohydrate approximately 2−5 times the quantity of protein.

Assessing Multivalency and Ligand Binding Using Surface Plasmon Resonance. SPR is an optical evanescent wave method that is increasingly finding use in the study of interactions of biological molecules.[49] This approach has as an advantage that it provides rate constants for both forward and reverse reactions. If the interaction of a multivalent lectin and a polyvalent ligand were to display a cluster effect, the observed SPR dissociation profile would differ from that of a monovalent ligand, since the apparent off-rate (k_d(apparent)) is the microscopic k_d raised to the valency of the interaction (k_d(apparent) = k_d^n), where n is the valency of the interaction. Qualitatively this may be thought of as the decreased probability that n sites can dissociate simultaneously, a condition that is required for the multivalent ligand to completely dissociate from the complex. Estimates of the half-lives for lectin−ligand complexes, when calculated in this fashion, are seen to increase dramatically with valency. If the effects of rebinding were also to be taken into account in such a calculation, the stability of a given complex would be expected to be further enhanced, resulting in significant increases in the estimated half-life of a given complex.

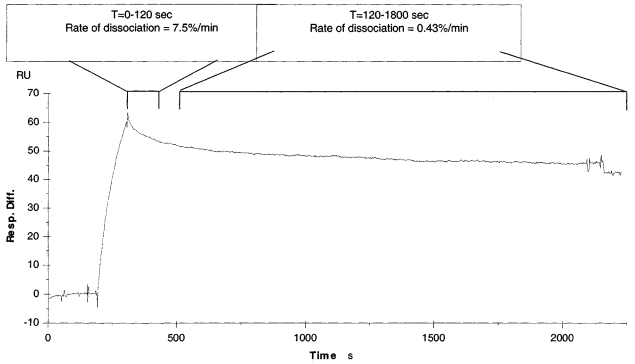

Figure 6. SPR experiments designed to assess the effect of multivalency on the kinetics of the interaction. The injection of glycopolymer **19c** shows a rapid but measurable association phase (from 200 to 300 s) that does not come to equilibrium. After the initial 120 s of dissociation, the rate begins to slow dramatically. The failure to reach equilibrium and the unusually slow dissociation rate (varying with time) are both indicative of a multivalent interaction.

A series of SPR experiments were designed to study in detail the avidity of the multivalent interaction by determining the dissociation constants of monovalent melibiose and comparing them with those of the melibiose glycopolymers. The kinetic analyses were performed using a CM5 chip onto which was coupled 5000 resonance units (RUs) of XL35. A series of concentrations of polymer **19c** were separately injected onto a chip using the Kinject command with an association time of 120 s and a dissociation time of 1800 s. The particularly long dissociation time was chosen in an attempt to maximize the chances that bound polymer could dissociate. The data from a representative sensorgram are shown in Figure 6 and represent the signal from the XL35 surface minus the signal observed for a surface to which no XL35 had been coupled. The sensorgram shows a rapid but measurable association profile. The initial dissociation phase was relatively rapid, with 15% of the bound glycopolymer dissociating after 120 s, corresponding to a rate of dissociation of 7.5%/min (Figure 6). The population of glycopolymer capable of dissociating quickly presumably represents the fraction tethered to the lectin by a minimal number of epitopes and consequently able to dissociate. After the initial 120 s, however, the rate of dissociation is seen to drop sharply to 0.43%/min. *After a 30 min dissociation phase under flow, only 27% of the bound polymer was able to dissociate.* To verify that the multivalent interaction between XL35 and the glycopolymer was glycan-specific, a negative control experiment, utilizing a similar glycopolymer to which *N*-acetylglucosamine, rather than melibiose, had been conjugated, was performed. This glycopolymer, when injected over the XL35-derivatized surface, gave no detectable response, demonstrating that the interactions observed for glycopolymer **19c** with the XL35 coupled to the SPR chip were indeed selective.

The observed stability of the multivalent interaction renders its evaluation by an SPR analysis complex. Determination of the K_D of any multivalent interaction using SPR is not straightforward since the data cannot be fit to any simple mono- or bivalent model. An alternative approach is to take advantage of an equilibrium SPR analysis to obtain the dissociation

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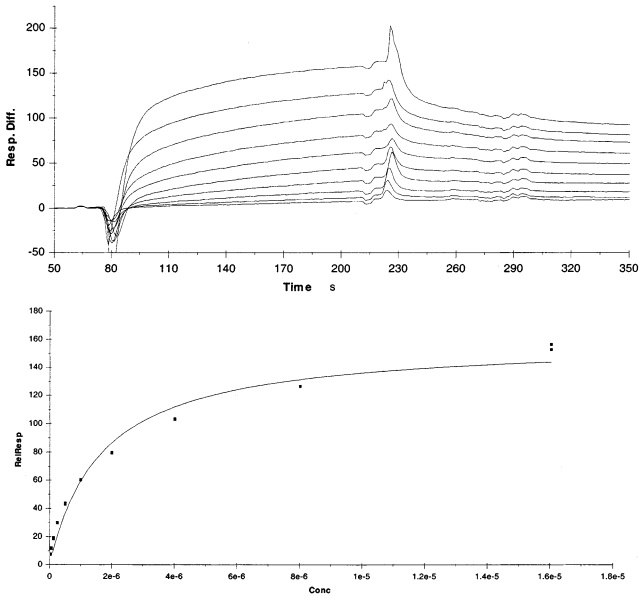

Figure 7. Equilibrium SPR binding experiments using 5000 RUs of XL35 coupled to a CM5 chip. (A, top) Sensorgrams using 2-fold serial dilutions of melibiose glycopolymer **19c** (from 1.6×10^{-5} to 3.1×10^{-8} M). Injection times were maximized to allow equilibrium to be reached (as the data obtained would be amenable to a nonlinear regression analysis). (B, bottom) A plot of the nonlinear regression analysis using the glycopolymer concentrations and the associated equilibrium responses. This approach allowed the K_D of the interaction between XL35 and glycopolymer **19c** to be determined despite the complications associated with multivalent interactions.

constant, although multivalency and the resulting stability of the bound complex again need to be contended with. If one considers the rate equation of a receptor−ligand interaction

$$d[RL]/dt = k_a[R][L] - k_d[RL]$$

where [R] = concentration of the receptor, [L] = concentration of the ligand, k_a = association constant, k_d = dissociation constant, and d[RL]/dt represents the change in the extent of binding with respect to time.

It follows that equilibrium is reached when d[RL]/dt = 0, but this is only true as t approaches infinity. After 5 half-lives, however, the measured binding is within 3% of the true equilibrium value, so this represents a practical basis for achieving equilibrium to conduct equilibrium SPR experiments. In the case of multivalent interactions, it is extremely difficult to approach equilibrium, as this requires that, for every receptor−ligand complex formed, another must dissociate, which is statistically unlikely (see above). Additionally, since a concentration of ligand 10 times that of the K_D is required for an equilibrium analysis to be properly evaluated (to saturate the receptor), this type of experiment requires not only long injections or contact times but also that large quantities of ligand be injected. The sensorgrams shown in Figure 7 are derived from experiments in which a balance has been struck between the long injection times required to reach equilibrium and the large quantities of material consumed under such conditions. Even under these relatively drastic conditions, the system is seen not to come completely to equilibrium. Nevertheless, using this approach, a non-valence-corrected K_D of 1.67×10^{-6} M can be calculated from the sensorgram for the glycopolymer **19c**, in excellent agreement with the value of 4.3×10^{-6} M calculated for the apparent K_I from the competition ELLA using an exact correction to the Cheng−Prushoff[50,51] approximation. This finding is important because studies by Toone and others[21] have shown that considerable discrepancies may be observed when different methodologies are employed to determine binding affinities.

An additional SPR experiment using a solution competition format was designed to determine the K_D for monovalent melibiose. In this experiment, melibiose was immobilized on the surface of a chip and the system calibrated using increasing concentrations of XL35. Fixed concentrations of XL35 were each mixed with increasing amounts of melibiose, and each of these mixtures was then separately injected over the surface of immobilized melibiose. As the concentration of melibiose in the solution was increased, XL35 was competed away from the melibiose surface, thereby decreasing the signal. Since the initial concentrations of both XL35 and melibiose are known, the signal obtained represents the quantity of XL35 that is free (and therefore able to bind the surface) at a given concentration of melibiose in solution. On the basis of the concentrations of free and bound XL35 at each concentration of melibiose in solution, the K_D may be determined. The format of these experiments takes advantage of XL35's large size, since SPR responds directly to the mass of material adsorbed onto the surface of the chip. Additionally, prior experiments (data not shown) demonstrated that attempts to directly measure the K_D for monovalent melibiose by injecting increasing concentrations of melibiose over an XL35-conjugated chip yielded extremely low signals due to the small mass of the disaccharide. Since these experiments were designed to measure monovalent affinities rather than multivalent interactions, a critical feature of the experiment was that the ligand density of the melibiose on the surface must be sufficiently low to prevent multivalent interactions. This condition was achieved by biotinylating melibiose derivative **3** and capturing the biotinylated disaccharide onto a CM5 chip containing immobilized streptavidin. By contrast to the strongly multivalent interaction between XL35 and the glycopolymers where a minimal amount of the complex dissociated even after 30 min of flow, under the low coupling density conditions described here nearly 90% of the bound complex dissociated after only 2.5 min of flow. The monovalent dissociation profile demonstrated that the subsequent competition experiments measured monovalent affinities. On the basis of these data, the K_D for monovalent melibiose was determined to be 1.57×10^{-3} M, which is an order of 10-fold less than the corresponding IC_{50} established by competition ELLA. This difference could be due to the fact that the SPR solution competition assay developed in this study is designed to measure binding at a single site, as opposed to evaluating the ability of a monovalent inhibitor to compete multivalent ligands away from a multivalent receptor.

The glycopolymers synthesized in this study are seen to exhibit behavior akin to that of the natural ligand and, in important respects, do serve as reasonable mimics for this mucin. Although the SPR-derived equilibrium inhibition profiles demonstrate that the synthetic polymers are not as potent as the JCP, the kinetics of their interactions mirror closely those of the native ligand. This is despite the fact that this mucin is 10

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times larger than the polymers and shows a correspondingly higher potential for interacting multivalently. It is likely that XL35 induces cross-linking of the mucin, which would result in formation of a three-dimensional matrix impenetrable to sperm. In addition, this cross-linking would be expected to result in a modest increase in valence-corrected affinity as is indeed borne out by our results. It appears that XL35 has to sample many different regions of the jelly coat matrix before forming a thermodynamically favorable complex that has minimal chance of dissociating from the jelly coat mucin. An alternative scenario would be one in which XL35 underwent a tight and irreversible multivalent binding interaction with a specific ligand on the surface of the oocyte immediately upon secretion. Under such circumstances, which are reminiscent of a classical cluster effect, the lectin would not be able to diffuse through the jelly coat enrobing the egg. As a consequence, the fertilization envelope would be only partially formed and thus would not be expected to provide an effective physical block to polyspermy.

The present study has also highlighted the requirement to establish equilibrium if an assay is to report accurately on any binding event. This condition is seen to be particularly difficult to meet when the binding partners are multivalent. This may well be the origin of several of the ambiguities reported in the literature when multivalent ligands have been evaluated.[52]

Conclusion

The lectin XL35 shows a remarkable ability to bind a wide variety of both monovalently and polyvalently presented D-galactopyranosides. Taken together, the results strongly suggest that broad lectin specificity and mucin heterogeneity are integral to the interaction of XL35 with the JCP. That the observed broad specificity is biologically relevant is supported by the observation that intraspecific *O*-glycan heterogeneity observed in *Xenopus* does not have a crucial bearing on the viability of the fertilized eggs.[14] Multivalency is certainly important in the binding observed for the synthetic glycopolymers synthesized in this study, but the interactions manifest only modest increases in their valency-corrected affinities, relative to their monomeric counterparts. However, the net interaction and the kinetics of binding displayed by the glycopolymers are seen to mirror closely those of the JCP when examined by SPR. The results suggest that in *X. laevis* the true *biological function* of multivalency is not to create an extremely tightly binding complex between XL35 and its natural ligand. It serves, instead, to create a very stable protective layer that will not dissociate and is yet flexible enough to encapsulate the *developing* embryo. In the system under scrutiny, an extremely long-lived interaction of polymeric ligand and its multimeric receptor is achieved, essentially, through multiple and successive weak binding and rebinding events and not through any one all-important and static, tight binding interaction. Through this cascade of reversible low-affinity interactions a dynamic interplay between the JCP and XL35 is established, which must manifest itself macroscopically as what is, in effect, a fluid hydrogel. These partners might not be able to attain true equilibrium on the time scale of the biological event, but their mode of interaction would, nevertheless, be expected to guarantee an insurmountable physical block to polyspermy.

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Experimental Section

General Information. Chemistry. Chemicals for synthesis were purchased from Aldrich, Acros, Sigma, and Fluka and used without further purification. Molecular sieves were activated at 350 °C in vacuo for 3 h. All solvents were distilled from the appropriate drying agents. All reactions were performed under anhydrous conditions according to routine dry techniques unless otherwise indicated. Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F_{254} (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Column chromatography was performed on silica gel (Merck, 70−230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Inova300, Inova500, and Inova600 spectrometers equipped with Sun workstations. Spectra were recorded in deuterated solvent and referenced appropriately. Assignments were made, when required, with the aid of standard gCOSY, gHSQC, TOCSY, and gHMBC experiments. Negative ion matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using either gentisic acid or dihydroxybenzoic acid as the matrix. High-resolution mass spectroscopy was performed on a Voyager delayed extraction STR using 2,5-dihydrobenzoic acid as an internal calibration matrix.

3-Azidopropyl 3-*O*-Benzyl-*β*<rsf>-D-galactopyranoside (8). A mixture of 3-azidopropyl *β*-D-galactopyranoside (1.58 g, 6.0 mmol) and dibutyltin oxide (1.65 g, 6.7 mmol) in MeOH (45 mL) was heated under reflux for 2 h, after which time the reactants had completely dissolved. The methanol was then evaporated under reduced pressure. The resulting syrup was dissolved in toluene (45 mL), and tetrabutylammonium iodide (2.44 g, 6.7 mmol) and benzyl bromide (0.78 mL, 6.7 mmol) were added. This mixture was heated under reflux for 12 h and then concentrated in vacuo. The resulting oil was purified by flash column chromatography over silica gel (eluent ethyl acetate/hexanes, 2/1, v/v) to give triol **8** as a syrup (1.56 g, 74%). $[\alpha]^{23}_D$ +1.8 (c = 0.39, CH_2Cl_2). FAB-MS (m/z): 354.2 ($M^+ + 1$). ^{1}H NMR (300 MHz, $CDCl_3$): 7.40−7.25 (m, 5H, CHAr), 4.74 (s, 2H, CH_2Ph), 4.25 (d, 1H, J = 7.7 Hz, H-1), 4.02−3.92 (m, 3H, SpOCH_2^a, H-6^a, H-4), 3.86−3.72 (m, 2H, H-2, H-6^b), 3.70−3.62 (m, 1H, SpOCH_2^b), 3.52−3.38 (m, 4H, CH_2N_3, H-3), 2.70−2.00 (br s, 3H, 3 OH), 1.87 (m, 2H, J = 6.3 Hz, Sp−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7 (C−Ar), 128.8, 128.3, 128.1 (CH−Ar), 103.4 (C-1), 80.5, 74.6 (C-5, C-3), 72.4 (CH_2-Ph), 71.2 (C-2), 67.3 (C-4), 66.9 (Sp−OCH_2), 62.7 (C-6), 48.6 (CH_2N_3), 29.5 (Sp−CH_2). HR MS (m/z): calcd for $C_{16}H_{23}N_3O_6Na$ 376.1485, found 376.1460.

3-Azidopropyl 3-*O*-Benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside (9). A solution of **8** (1.24 g, 3.5 mmol) in dry acetonitrile (20 mL) was treated with α,α-dimethoxytoluene (1.05 mL, 7 mmol) and *p*-toluenesulfonic acid (33 mg). The mixture was stirred for 22 h at room temperature and then neutralized with Et_3N. The reaction mixture was concentrated under reduced pressure and the residue purified by silica gel column chromatography (eluent ethyl acetate/hexanes, 3/1, v/v) to yield alcohol **9** as an amorphous solid (1.2 g, 77.5%). $[\alpha]^{24}_D$ +42.0 (c = 4.1, CH_2Cl_2). FAB-MS (m/z): 442.2 ($M^+ + 1$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50−7.10 (m, 10H, ArCH), 5.36 (s, 1H, OCHO), 4.72−4.60 (AB, 2H, J = 12.4 Hz, CH_2Ph), 4.28−4.14 (m, 2H, H-1, H-6^a), 4.03 (d, 1H, J = 3.1 Hz, H-4), 3.98−3.84 (m, 3H, H-2, H-6^b, Sp−OCH_2^a), 3.60−3.50 (m, 1H, Sp−OCH_2^b), 3.44−3.36 (dd, 1H, J = 9.6 Hz, J = 3.1 Hz, H-3), 3.33 (t, 2H, J = 6.6 Hz, CH_2N_3), 3.28−3.22 (m, 1H, H-5), 1.90−1.70 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.1, 137.8 (ArC), 129.0, 128.5, 128.2, 127.9, 126.4 (ArCH), 103.2 (C-1), 101.2 (OCHO), 79.4 (C-5), 73.2 (C-3), 71.7 (CH_2-Ph), 70.1 (C-2), 69.4 (C-6), 66.9 (C-4), 66.7 (Sp−OCH_2), 48.7 (CH_2N_3), 29.4 (Sp−CH_2). HR MS (m/z): calcd for $C_{25}H_{27}N_3O_6Na$ 464.1798, found 464.1794.

3-Azidopropyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside (10). A solution of **9** (1.2 g, 2.7 mmol) in pyridine (20 mL) was treated with acetic anhydride (20 mL). After being stirred

at room temperature for 12 h, the reaction mixture was concentrated under reduced pressure and the residue purified by flash silica gel column chromatography (eluent ethyl acetate/hexanes, 1/1.5, v/v) to give **10** as an oil (1.25 g, 95%). $[\alpha]^{23}_D$ +55.4 (c = 0.57, CH_2Cl_2). FAB-MS (m/z): 482.3 ($M^+ - 1$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60−7.20 (m, 10 H, ArCH), 5.48 (s, 1H, OCHO), 5.40−5.31 (dd, 1H, J = 9.9 Hz, J = 8.0 Hz, H-2), 4.75−4.58 (AB, 2H, J = 12.6 Hz, CH_2Ph), 4.39 (d, 1H, J = 8.0 Hz, H-1), 4.32−4.24 (m, 1H, H-6^a), 4.17 (d, 1H, J = 3.3 Hz, H-4), 4.07−4.00 (m, 1H, H-6^b), 4.00−3.90 (m, 1H, Sp−OCH_2^a), 3.63−3.52 (m, 2H, H-3, Sp−OCH_2^b), 3.40−3.30 (m, 3H, H-5, CH_2N_3), 2.08 (s, 3H, CH_3CO), 1.94−1.70 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.4 (C═O), 138.1, 137.7 (ArC), 129.0, 128.9, 128.5, 128.4, 128.1, 127.8, 127.6, 126.4 (ArCH), 101.2, 101.1 (C-1, OCHO), 77.4 (C-5), 73.2 (C-3), 71.2 (CH_2Ph), 70.2 (C-2), 69.2 (C-6), 66.7 (C-4), 65.6 (Sp−OCH_2), 48.2 (CH_2N_3), 29.2 (Sp−CH_2), 21.2 (CH_3). HR MS (m/z): calcd for $C_{25}H_{29}N_3O_7Na$ 506.1903, found 506.1841.

3-Azidopropyl 2-*O*-Acetyl-3,6-*O*-dibenzyl-*β*-D-galactopyranoside (11). To a mixture of **10** (0.21 g, 0.44 mmol), $NaBH_3CN$ (0.35 g, 5.6 mmol), and powdered 3 Å molecular sieves in dry THF was added a 1 M HCl/ether solution dropwise until evolution of gas had ceased. The reaction mixture was then stirred for 2 h at room temperature, diluted with THF, and filtered through a pad of Celite and the filtrate concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (eluent ethyl acetate/hexanes, 1/2, v/v) to afford **11** as an oil (0.16 g, 77%). $[\alpha]^{25}_D$ +14.5 (c = 1.8, CH_2Cl_2). FAB-MS (m/z): 508.3 (M^+ + Na). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40−7.20 (m, 10 H, ArCH), 5.10 (dd, 1H, J = 9.6 Hz, J = 8.0 Hz, H-2), 4.62 (d, 1H, J = 12.4 Hz, CH_2Ph), 4.52 (s, 2H, CH_2Ph), 4.45 (d, 1H, J = 12.4 Hz, CH_2Ph), 4.25 (d, 1H, J = 8.0 Hz, H-1), 4.00 (d, 1H, J = 3.3 Hz, H-4), 3.90−3.80 (m, 1H, Sp−OCH_2^a), 3.80−3.60 (m, 2H, H-6), 3.52 (m, 1H, H-5), 3.50−3.44 (m, 1H, Sp−OCH_2^b), 3.42 (dd, 1H, J = 9.6 Hz, J = 3.3 Hz, H-3), 3.28 (t, 2H, J = 6.6 Hz, CH_2N_3), 1.98 (s, 3H, CH_3CO), 1.90−1.80 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.6 (C═O), 138.1, 137.5 (ArC), 128.7, 128.6, 128.2, 127.9 (ArCH), 101.3 (C-1), 78.9 (C-3), 74.0 (CH_2Ph), 73.7 (C-5), 71.8 (CH_2Ph), 70.9 (C-2), 69.2 (C-6), 66.4 (C-4), 66.0 (Sp−OCH_2), 48.3 (CH_2N_3), 29.3 (Sp−CH_2), 21.2 (CH_3). HR MS (m/z): calcd for $C_{29}H_{32}N_3O_7Na$ 508.2060, found 508.1954.

3-Azidopropyl 2-*O*-Acetyl-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α/*β*-D-galactopyranosyl)-*β*-D-galactopyranoside (15). A mixture of **14** (0.118 g, 0.2 mmol), **11** (98 mg, 0.2 mmol), and freshly activated 4 Å molecular sieves in dry ether/CH_2Cl_2 (4/1, v/v; 2.5 mL) was stirred for 1 h at room temperature and then 30 min at −25 °C. NIS (68 mg, 0.3 mmol) and TMSOTf (5.5 μL, 0.03 mmol) were then added to the mixture. After 20 h of stirring at −25 °C, the molecular sieves were filtered off, the filtrate was diluted with CH_2Cl_2 (20 mL), and the organic phase was washed with aqueous $Na_2S_2O_3$ (20 mL) and water (3 × 20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent ethyl acetate/hexanes, 1/4, v/v) to obtain **15** as an α/β mixture (9/1 by ^{1}H NMR) of (0.167 g, 83%) which was separated by silica gel column chromatography (eluent ethyl acetate/toluene, 0.5/10, v/v) to give the pure α anomer. $[\alpha]^{25}_D$ +57.9 (c = 0.22, CH_2Cl_2), FAB-MS (m/z): 1030.7 (M^+ + Na). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−7.05 (m, 30 H, ArCH), 5.16 (dd, 1H, J = 10.2 Hz, J = 8.0 Hz, H-2), 4.94 (d, 1H, J = 3.0 Hz, H-1′), 4.84 (d, 1H, J = 12.1 Hz, ^{a}CH_2Ph), 4.80 (d, 1H, J = 11.3 Hz, ^{a}CH_2Ph), 4.70 (s, 2H, CH_2Ph), 4.68 (d, 1H, J = 12.9 Hz, ^{a}CH_2Ph), 4.60 (d, 1H, J = 12.1 Hz, ^{b}CH_2Ph), 4.46 (d, 1H, J = 11.3 Hz, ^{b}CH_2Ph), 4.40 (dd, 1H, J = 8.8 Hz, J = 5.2 Hz, H-6^a), 4.30 (d, 1H, J = 12.9 Hz, ^{b}CH_2Ph), 4.24 (d, 1H, J = 8.0 Hz, H-1), 4.20 (d, 1H, J = 12.0 Hz, ^{a}CH_2Ph), 4.14 (d, 1H, J = 12.0 Hz, ^{b}CH_2Ph), 4.12−4.00 (m, 6H, CH_2Ph, H-2′, H-3′, H-4′, H-4), 3.98−3.78 (m, 2H, H-5′, Sp−OCH_2), 3.52−3.40 (m, 4H, H-5, H-6′, Sp−OCH_2^b), 3.32 (dd, 1H, J = 10.4 Hz, J = 2.7 Hz, H-3), 3.26 (t, 2H, J = 6.9 Hz, CH_2N_3), 3.15 (dd, 1H, J = 8.5 Hz, J = 4.9 Hz, H-6^b), 1.96 (s, 3H, CH_3CO), 1.86−1.66 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.5 (C═O), 139.2, 139.1, 138.9, 138.4, 138.3, 138.1 (ArC), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3 (ArCH), 101.6 (C-1), 100.8 (C-1′), 79.3 (C-3), 79.2 (CH), 77.6 (CH_2Ph), 77.2 (CH_2), 76.8 (CH_2), 76.6 (CH), 75.1 (CH_2), 75.0 (CH), 74.1 (CH), 73.9 (CH_2Ph), 73.7 (CH), 73.3 (CH_2Ph), 73.2 (CH_2Ph), 72.5 (CH_2Ph), 71.8 (CH_2Ph), 71.0 (C-2), 69.4 (C-5 or C-5′), 68.2 (C-6), 67.9 (C-6′), 65.8 (Sp−OCH_2), 48.4 (CH_2N_3), 29.4 (Sp−CH_2), 21.3 (CH_3). HR MS (m/z): calcd for $C_{59}H_{65}N_3O_{12}Na$ 1030.4466, found 1030.4435.

3-Azidopropyl 3,6-Di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-*β*-D-galactopyranoside (16). A solution of **15** (92 mg, 0.09 mmol) in MeOH (5 mL) was treated with a catalytic amount of NaOMe. After being stirred for 24 h at room temperature, the reaction mixture was neutralized with Dowex H^+ resin, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent ethyl acetate/hexanes, 1/3, v/v) to give **16** as a syrup (84.6 mg, 96% yield). $[\alpha]^{25}_D$ +44.4 (c = 0.44, CH_2Cl_2). FAB-MS (m/z): 987.9 (M^+ + Na). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−7.10 (m, 30 H, ArCH), 4.95 (d, 1H, J = 2.2 Hz, H-1′), 4.83 (d, 1H, J = 11.3 Hz, ^{a}CH_2Ph), 4.81 (d, 1H, J = 11.8 Hz, ^{a}CH_2Ph), 4.73 (d, 1H, J = 12.6 Hz, ^{a}CH_2Ph), 4.67 (s, 2H, CH_2Ph), 4.60 (d, 1H, J = 11.8 Hz, ^{b}CH_2Ph), 4.45 (d, 1H, J = 11.3 Hz, ^{b}CH_2Ph), 4.41 (d, 1H, J = 12.6 Hz, ^{b}CH_2Ph), 4.33 (dd, 1H, J = 8.8 Hz, J = 4.8 Hz, H-6^a), 4.24−4.10 (m, 5H, ^{b}CH_2Ph, ^{b}CH_2Ph, CH_2Ph, H-1), 4.04−3.82 (m, 6H, H-2′, H-3′, H-4, H-5′, H-6′^{a}, Sp−OCH_2^a), 3.73 (dd, 1H, J = 9.6 Hz, J = 7.7 Hz, H-2), 3.62−3.38 (m, 4H, H-4′, H-5, H-6′^{b}, Sp−OCH_2^b), 3.14 (t, 2H, J = 6.6 Hz, CH_2N_3), 3.24−3.16 (m, 2H, H-3, H-6^b), 1.95−1.85 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.0, 138.8, 138.7, 138.1 (ArC), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5 (ArCH), 103.5 (C-1), 100.5 (C-1′), 80.7, 79.2, 76.7 (CH), 75.1 (CH_2Ph), 75.0, 74.1 (CH), 73.9 (CH_2), 73.7 (CH), 73.4, 73.2, 72.5, 71.9 (CH_2), 70.8, 69.6 (CH), 68.2, 68.1, 66.7 (CH_2), 48.6 (CH_2N_3), 29.4 (Sp−CH_2). HR MS (m/z): calcd for $C_{57}H_{63}N_3O_{11}Na$ 988.4360, found 988.4426.

3-Azidopropyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-*β*-D-galactopyranoside (13). Compounds **9** (0.25 g, 0.56 mmol) and **12** (0.32 g, 0.68 mmol), freshly activated 4 Å molecular sieves, and IDCP (0.53 g, 1.1 mmol) were stirred in ether/CH_2Cl_2 (5/1, v/v, 18 mL) at room temperature for 3.5 h. The molecular sieves were filtered off, and the filtrate was diluted with CH_2Cl_2 (20 mL) and washed with aqueous $Na_2S_2O_3$ (20 mL) and then water (3 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent ethyl acetate/hexanes, 1/2, v/v) to yield disaccharide **13** as a white foam (0.38 g, 78% yield). $[\alpha]^{25}_D$ −28.9 (c = 5.1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76−7.20 (m, 25 H, ArCH), 5.73 (d, 1H, J = 3.6 Hz, H-1′), 5.49 (s, 1H, OCHO), 5.03 (d, 1H, J = 11.5 Hz, ^{a}CH_2Ph), 4.94 (d, 1H, J = 11.8 Hz, ^{a}CH_2Ph), 4.83 (d, 1H, J = 11.8 Hz, ^{b}CH_2Ph), 4.80 (d, 1H, J = 11.8 Hz, ^{a}CH_2Ph), 4.75 (d, 1H, J = 12.1 Hz, ^{a}CH_2Ph), 4.72 (d, 1H, J = 11.5 Hz, ^{b}CH_2Ph), 4.64 (d, 1H, J = 12.1 Hz, ^{b}CH_2Ph), 4.63 (d, 1H, J = 11.8 Hz, ^{b}CH_2Ph), 4.54 (d, 1H, J = 8.0 Hz, H-1), 4.45−4.34 (m, 2H, H-5′, H-6^a), 4.26 (dd, 1H, J = 8.0 Hz, J = 9.3 Hz, H-2), 4.22 (d, 1H, J = 3.6 Hz, H-4), 4.12−3.98 (m, 4H, H-3′, H-2′, H-6^b, Sp−OCH_2^a), 3.88 (dd, 1H, J = 9.3 Hz, J = 3.6 Hz, H-3), 3.76 (m, 1H, H-4′), 3.69−3.59 (m, 1H, Sp−OCH_2^b), 3.46−3.38 (m, 3H, H-5, Sp−CH_2N_3), 1.96−1.84 (m, 2H, Sp−CH_2), 1.22 (d, 3H, J = 6.6 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.1, 138.8, 138.6, 138.3, 137.8 (ArC), 128.9, 128.4, 128.3, 128.0, 128.2, 127.8, 127.6, 127.5, 127.4, 127.2, 126.3 (ArCH), 102.0 (C-1), 101.0 (OCHO), 97.3 (C-1′), 81.7 (C-3), 79.5 (C-3′), 78.2 (C-4′), 76.1 (C-2′), 74.9 (CH_2Ph), 73.1 (CH_2Ph), 72.9 (C-4), 72.7 (CH_2Ph), 72.0 (C-2), 70.8 (CH_2Ph), 69.4 (C-6), 66.6 (C-5), 66.4 (C-5′), 66.2 (Sp−OCH_2), 48.6 (CH_2N_3), 29.5 (Sp−CH_2), 16.9 (CH_3). HR MS (m/z): calcd for $C_{50}H_{55}N_3O_{10}Na$ 880.3785, found 880.3736.

3-Azidopropyl 3,6-Di-*O*-benzyl-2-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-*β*-D-galactopyranoside (17). A mixture of **14** (27.6, 0.058 mmol), **16** (46.6 mg,

0.048 mmol), freshly activated 4 Å molecular sieves, and IDCP (45 mg, 0.096 mmol) was stirred in dry ether/CH_2Cl_2 (4/1, v/v, 3 mL) at room temperature for 3 h. The molecular sieves were filtered off, the filtrate was diluted with CH_2Cl_2 (10 mL), and the organic phase was washed with aqueous $Na_2S_2O_3$ (10 mL) and water (3 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent ethyl acetate/hexanes, 1/3, v/v) to give **17** (50 mg, 75% yield). $[\alpha]^{25}_D$ −17.4 (c = 0.91, CH_2Cl_2). MALDI-TOF MS (m/z): 1403.7 (M^+ + Na). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−6.90 (m, 45 H, ArCH), 5.61 (d, 1H, J = 3.6 Hz, H-1″), 4.94−4.52 (m, 11H, $J_{1',2'}$ = 4.1 Hz, 5 CH_2Ph, H-1′), 4.44−4.24 (m, 7H, $J_{1,2}$ = 8.0 Hz, H-6^a, H-5″, H-1, 2 CH_2Ph), 4.09 (AX, 2H, J = 11.8 Hz, CH_2Ph), 4.06−3.75 (m, 11H, H-2′, H-3′, H-2″, H-4, H-5′, H-6′^{a}, H-2, H-3, H-4, CH_2Ph, Sp−OCH_2^a), 3.62 (br s, 1H, H-4′), 3.54−3.34 (m, 5H, H-3″, H-4″, H-6″^{b}, H-5, Sp−OCH_2^b), 3.22 (t, 2H, J = 6.9 Hz, CH_2N_3), 3.12 (dd, 1H, J = 8.0 Hz, J = 4.9 Hz, H-6^b), 1.70−1.80 (m, 2H, Sp−CH_2), 1.10 (d, 3H, J = 6.3 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.2, 139.0, 138.9, 138.5, 138.4, 138.3, 138.2 (ArC), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 126.6 (ArCH), 102.3, 100.1, 97.4 (C-1, C-1′, C-1″), 83.4, 79.7, 79.1, 78.4, 76.3 (CH), 75.1 (2 CH_2-Ph), 75.0, 73.9 (CH), 73.7 (CH_2), 73.6 (CH), 73.4, 73.1, 72.4 (CH_2), 72.3 (CH), 72.1 (CH_2), 69.5 (CH), 68.5, 68.1 (CH_2), 66.5 (CH), 66.1 (CH_2), 48.7 (CH_2N_3), 29.7 (Sp−CH_2), 17.0 (CH_3). HR MS (m/z): calcd for $C_{84}H_{91}N_3O_{15}$, 1404.6348, found 1404.6231.

3-Aminopropyl 2-*O*-(α-L-Fucopyranosyl)-*β*-D-galactopyranoside (4a). Pd/C (160 mg) was added to a suspension of disaccharide **13** (80 mg, 0.09 mmol) in a mixture of EtOH/HCl (19/0.08 mL) and the mixture stirred under an atmosphere of H_2 for 4 h. The catalyst was then filtered off and the filtrate neutralized with Dowex OH^- resin and concentrated under reduced pressure. The residue was purified using an iatrobeads column (eluent methanol/water/acetic acid, 9/3/0.9, v/v/v) to yield **4a** as its acetate salt (26 mg, 64%). $[\alpha]^{26}_D$ −78.3 (c = 0.34, H_2O). ^{1}H NMR (300 MHz, CD_3OD): δ 5.08 (d, 1H, J = 2.7 Hz, H-1′), 4.38 (d, 1H, J = 7.4 Hz, H-1), 4.18 (q, 1H, J = 6.6 Hz, H-5′), 4.05−3.95 (m, 1H, Sp−OCH_2^a), 3.87−3.50 (m, 10H, H-4, H-6, H-2, H-2′, H-3, H-3′, H-4′, H-5, Sp−OCH_2^b), 3.16−2.98 (m, 2H, CH_2N), 2.00−1.90 (m, 2H, Sp−CH_2), 1.22 (d, 3H, J = 6.6 Hz, CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 103.8 (C-1), 102.6 (C-1′), 81.2 (C-2), 76.8, 75.1, 73.7, 71.9, 70.9, 70.2 (C-5, C-2′, C-3, C-3′, C-4′, C-4), 68.7 (OCH_2), 68.5 (C-5′), 62.6 (C-6), 39.5 (CH_2N), 29.5 (CH_2), 17.1 (CH_3). HR MS (m/z): calcd for $C_{15}H_{29}NO_{10}Na$ 406.1689, found 406.1669.

3-Aminopropyl 4-*O*-(α-D-Galactopyranosyl)-*β*-D-galactopyranoside (5a). Pd/C (360 mg) was added to a suspension of **16** (180 mg, 0.18 mmol) in EtOH/HCl (38/0.12 mL) and the mixture stirred under an atmosphere of H_2 for 3 h. The catalyst was then filtered off and the filtrate neutralized with Dowex OH^- resin and concentrated under reduced pressure. The residue was purified using an iatrobeads column (eluent methanol/water/acetic acid, 9/3/0.1, v/v/v) to yield **5a** as the acetate salt (57 mg, 66% yield). $[\alpha]^{26}_D$ +30.2 (c = 1.5, H_2O). ^{1}H NMR (300 MHz, CD_3OD): δ 4.97 (d, 1H, J = 3.6 Hz, H-1′), 4.34 (d, 1H, J = 7.1 Hz, H-1), 4.23 (t, 1H, J = 5.9 Hz, H-5′), 4.30−3.98 (m, 2H, H-4 or H-4′, Sp−OCH_2^a), 4.91 (d, 1H, J = 2.5 Hz, H-4 or H-4′), 3.88−3.44 (m, 10H, H-6, H-6′, H-2, H-2′, H-3, H-3′, H-5, Sp−OCH_2^b), 3.20−3.02 (m, 2H, CH_2N), 2.02−1.90 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, D_2O): δ 103.1 (C-1), 100.4 (C-1′), 77.5, 75.4, 72.6, 71.1, 71.0, 69.4, 69.2, 68.9 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 67.9 (CH_2), 60.8, 60.4 (CH_2), 37.8 (CH_2N), 27.1 (CH_2), 23.6 (CH_3). HR MS (m/z): calcd for $C_{15}H_{29}NO_{11}Na$ 422.1638, found 422.1601.

3-Aminopropyl 2-*O*-(α-L-Fucopyranosyl)-4-*O*-(α-D-galactopyranosyl)-*β*-D-galactopyranoside (6a). Pd/C (120 mg) was added to a solution of **17** (60 mg, 0.04 mmol) in EtOH/HCl (19/0.05 mL) and the mixture stirred under an atmosphere of H_2 for 5 h. The catalyst was then filtered off and the filtrate neutralized with Dowex OH^- resin and concentrated under reduced pressure. The residue was purified using an iatrobeads column (eluent methanol/water/acetic acid, 9/1/0.1, v/v/v) to yield target trisaccharide **6a** as its trisaccharide salt (18 mg, 70% yield). $[\alpha]^{26}_D$ −7.7 (c = 0.38, H_2O). ^{1}H NMR (600 MHz, D_2O): δ 5.10 (d, 1H, J = 3.9 Hz, H-1″), 4.81 (d, 1H, J = 3.9 Hz, H-1′), 4.45 (d, 1H, J = 7.8 Hz, H-1), 4.24 (t, 1H, J = 6.3 Hz, H-5′), 4.16 (q, 1H, J = 6.6 Hz, H-5″), 4.73−4.14 (m, 15H, H-6, H-6′, H-4, H-4′, H-4″, H-2′, H-2″, H-3, H-3′, H-3″, H-5, Sp−OCH_2), 3.68 (dd, 1H, J = 9.9 Hz, J = 7.7 Hz, H-2), 3.18 (t, 2H, J = 7.1 Hz, CH_2N), 2.14−2.02 (m, 2H, Sp−CH_2), 1.28 (d, 3H, J = 6.6 Hz, CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 103.9 (C-1), 102.6, 102.0 (C-1′, C-1″), 79.9, 79.2, 75.1, 73.7, 72.7, 71.8, 71.5, 71.2, 70.9, 70.8 (CH), 69.0 (CH_2), 68.4 (CH), 62.8 (CH_2), 61.1 (CH_2), 39.7 (CH_2NH_2), 33.3 (CH_2), 17.0 (CH_3). HR MS (m/z): calcd for $C_{21}H_{39}NO_{15}Na$ 568.2217, found 568.2182.

3-(*N*-Acetylaminopropyl) 2-*O*-(α-L-Fucopyranosyl)-*β*-D-galactopyranoside (4b). A solution of disaccharide **4a** (10 mg, 0.026 mmol) in MeOH (1 mL) was treated with acetic anhydride (0.3 mL) and Et_3N (3.7 μL) and the mixture stirred for 4 h at room temperature. The reaction was concentrated in vacuo and the residue purified by iatrobeads column chromatography (eluent chloroform/methanol/water, 12/6/0.5, v/v/v) to yield **4b** as a syrup (9.6 mg, 83% yield). $[\alpha]^{26}_D$ −87.1 (c = 0.33, H_2O). ^{1}H NMR (600 MHz, CD_3OD): δ 5.16 (br s, 1H, H-1′), 4.34 (d, 1H, J = 7.3 Hz, H-1), 4.24 (q, 1H, J = 6.3 Hz, H-5′), 3.96−3.90 (m, 1H, Sp−OCH_2^a), 3.83 (d, 1H, J = 2.9 Hz, H-4), 3.79−3.70 (m, 3H, H-6, H-2′, H-3′), 3.69 (dd, 1H, J = 9.8 Hz, J = 2.9 Hz, H-3), 3.67−3.60 (m, 3H, H-4′, H-2, Sp−OCH_2^b), 3.51 (t, 1H, J = 5.9 Hz, H-5), 3.30−3.22 (m, 2H, CH_2N), 1.94 (s, 3H, CH_3), 1.84−1.74 (m, 2H, Sp−CH_2), 1.20 (d, 3H, J = 6.3 Hz, CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 104.9 (C-1), 103.2 (C-1′), 81.0 (C-2), 77.8 (C-5), 76.8, 74.9, 73.0 (C-2′, C-3, C-4′), 71.6 (C-4), 69.5 (Sp−OCH_2), 69.3 (C-5′), 63.8 (C-6), 39.1 (CH_2N), 31.8 (CH_2), 23.8 (CH_3), 18.0 (CH_3). HR MS (m/z): calcd for $C_{17}H_{31}NO_{11}Na$ 448.1795, found 448.1847.

3-(*N*-Acetylaminopropyl) 4-*O*-(α-D-Galactopyranosyl)*β*-D-galactopyranoside (5b). A solution of **5a** (8.08 mg, 0.017 mmol) in MeOH (1 mL) was treated with acetic anhydride (1.7 μL) and Et_3N (2.4 μL) and the mixture stirred for 4.5 h at room temperature. The reaction mixture was then concentrated in vacuo and the residue purified by iatrobeads column chromatography (eluent chloroform/methanol/water, 6/3/0.5, v/v/v) to yield amide **5b** as a syrup (7.2 mg, 90% yield). $[\alpha]^{26}_D$ +72.8 (c = 0.23, H_2O). ^{1}H NMR (300 MHz, D_2O): δ 4.88 (d, 1H, J = 3.9 Hz, H-1′), 4.37 (d, 1H, J = 7.7 Hz, H-1), 4.28 (t, 1H, J = 6.6 Hz, H-5), 3.98−3.60 (m, 12H, H-6, H-6′, H-4, H-4′, H-5′, H-3, H-3′, H-2′, Sp−OCH_2), 3.46 (dd, 1H, J = 7.7 Hz, J = 10.0 Hz, H-2), 3.24−3.14 (m, 2H, CH_2N), 1.90 (s, 3H, CH_3), 1.81−1.67 (m, 2H, Sp−CH_2). ^{13}C NMR (75 MHz, D_2O): δ 170.6 (CO), 103.1 (C-10, 100.4 (C-1′), 77.4, 75.3, 72.6, 71.2, 71.1, 69.4, 69.2, 69.0 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 68.0 (CH_2), 60.8, 60.4 (CH_2), 36.7 (CH_2N), 28.7 (CH_2), 22.2 (CH_3CO). HR MS (m/z): calcd for $C_{17}H_{31}NO_{12}Na$ 464.1744, found 464.1829.

3-(*N*-Acetylaminopropyl) 2-*O*-(α-L-Fucopyranosyl)-4-*O*-(α-D-galactopyranosyl)-*β*-D-galactopyranoside (6b). A solution of **6a** (9.6 mg, 0.017 mmol) in MeOH (1 mL) was treated with acetic anhydride (1.7 μL) and Et_3N (2.4 μL) and the mixture stirred for 24 h at room temperature. The reaction was then concentrated in vacuo and the residue purified by iatrobeads column chromatography (eluent chloroform/methanol/water, 6/4/0.5, v/v/v) to yield **6b** as a syrup (8.4 mg, 81%). $[\alpha]^{26}_D$ −18.2 (c = 0.27, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 5.20 (d, 1H, J = 3.9 Hz, H-1″), 4.91 (d, 1H, J = 3.9 Hz, H-1′), 4.52 (d, 1H, J = 7.8 Hz, H-1), 4.34 (t, 1H, J = 6.3 Hz, H-5′), 4.27 (q, 1H, J = 7.0 Hz, H-5″), 4.00 (d, 1H, J = 3.0 Hz, H-4′), 3.97 (d, 1H, J = 2.6 Hz, H-4), 3.94−3.65 (m, 9H, H-3′, Sp−OCH_2, H-3, H-6, H-2′, H-4″, H-2″, H-6′), 3.57 (dd, 1H, J = 7.8 Hz, J = 10.0 Hz, H-2), 3.22 (m, 2H, CH_2N), 1.94 (s, 3H, CH_3), 1.80 (m, 2H, Sp−CH_2), 1.16 (d, 3H, J = 7.0 Hz, CH_3). ^{13}C NMR (125 MHz, D_2O): δ 104.4 (C-1), 103.2 (C-1′), 102.3 (C-1″), 80.6 (C-4), 79.9 (C-2), 77.6, 75.9, 74.5, 73.5 (C-5′), 72.1, 71.7, 71.6 (C-4′), 71.4, 71.0, 70.3 (Sp−OCH_2), 69.5 (C-5″), 63.2, 62.8 (C-6, C-6′), 39.5 (CH_2N), 31.5 (Sp−CH_2), 24.8 (CH_3), 18.4 (CH_3). HR MS (m/z): calcd for $C_{23}H_{41}NO_{16}Na$ 610.2323, found 610.2433.

General Procedure for the Preparation of Glycopolymers 19−24. pNAS was synthesized by an AIBN-initiated radical polymerization of *N*-(acryloyloxy)succinimide dissolved in dry benzene as previously described,[39] and the same batch of material was used as the precursor for the synthesis of all sugar-bearing polymers. For example, the polymer **23c** containing a 17% loading of saccharide was prepared by suspending the 3-aminopropyl saccharide **3 (**2.2 mg), pNAS (7.3 mg), and Et_3N (5 μL) in dry DMF (1 mL) and stirring the mixture for 22 h at room temperature under argon and for a further 6 h at 65 °C. The reaction was allowed to cool to room temperature and quenched with concentrated aqueous ammonia (0.1 mL). Exhaustive dialysis of the reaction mixture against distilled water with a molecular weight cutoff of ∼14000 followed by freeze-drying gave the saccharide-bearing polymer **19c** of 17% loading as a white, fluffy solid. Polymers **19a** and **19b** (with 5% and 10% sugar content, respectively) were obtained from glycoside **3** in the same manner except that the amounts of pNAS were adjusted accordingly to give the desired final loading in each case. The following are selected NMR spectroscopic data. (Polymer **19a**) ^{1}H NMR (600 MHz, D_2O): δ 4.90 (br s, H-1′), 4.40 (br s, H-1). (Polymer **19b**) ^{1}H NMR (600 MHz, D_2O): δ 4.96 (br s, H-1′), 4.46 (br s, H-1). (Polymer **19c**) ^{1}H NMR (600 MHz, D_2O): δ 4.97 (br s, H-1′), 4.45 (br s, H-1). ^{13}C NMR (125 MHz, D_2O): δ 98.3 (C-1′), 102.5 (C-1). (Polymer **20**) ^{1}H NMR (600 MHz, D_2O): δ 4.40 (br s, H-1), 4.00−3.85 (m), 3.80−3.45 (m). (Polymer **21**) ^{1}H NMR (600 MHz, D_2O): δ 4.89 (br s, H-1). (Polymer **22**) ^{1}H NMR (600 MHz, D_2O): δ 5.22 (br s, H-1′), 4.5 (br s), 4.3 (br s, H-5′). ^{13}C NMR (150 MHz, D_2O): δ 104.5 (C-1), 102.4 (C-1′), 102.9. (Polymer **23**) ^{1}H NMR (600 MHz, D_2O): δ 4.9 (br s, H-1′), 4.45 (br s, H-5′), 4.37 (br s, H-1). ^{13}C NMR (150 MHz, D_2O): δ 105.2 (C-1), 102.9 (C-1′). (Polymer **24**) ^{1}H NMR (600 MHz, D_2O): δ 5.25 (br s, H-1″), 4.95 (br s, H-1′), 4.52 (br s, H-1), 4.52 (br s, H-1), 4.37 (br s, H-5′), 4.27, (br s, H-5″).

pNAS-Derived Polyacid and Polyamide Controls. These compounds were obtained as previously described, from the same batch of pNAS as the glycopolymers, purified by exhaustive dialysis and characterized by ^{1}H NMR spectroscopy (data not shown).[38−40]

General Information. **Biochemistry**. SATA, 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), *N*-Hydroxysuccinimide−biotin (NHS−biotin), 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC), NHS, sulfo-NHS−LC−biotin, and Streptavidin−HRP were purchased from Pierce and used without further purification. All chromatography solvents were HPLC grade and were filtered using a 0.45 μm filter prior to use. Commercially available saccharides were obtained from Sigma, and poly(acrylic acid) and dextran standards were purchased from Fluka. BAP was purchased from Worthington and dialyzed into phosphate-buffered saline (PBS) to which 5 mM EDTA had been added.

Purification of Lectin. XL35 was purified from oocytes as previously described[53] except that an anion-exchange step was added prior to melibiose affinity chromatography. Briefly, mature female xenopi were anesthetized using 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222, Sigma), and the oocytes were obtained surgically and homogenized in TCS prechilled to 4 °C. Cold acetone (9 volumes) was added to the initial homogenate, and the pellet was captured by filtration and then washed three times with cold acetone to remove residual lipids. The delipidated pellet was resuspended in 0.3 M galactose (in TCS) and centrifuged at 10000*g* for 20 min. The clarified supernatant was dialyzed against 50 mM Tris, pH 8.5, prior to being applied to a 2.6 × 10 cm DEAE-650M column (TosoHaas) equilibrated in the same buffer. XL35 was eluted using 50 mM Tris, 1.2 M NaCl, pH 8.5, and the protein fractions were pooled and applied to a 3 mL melibiose affinity column equilibrated in TCS. The melibiose column was washed extensively with TCS, and purified XL35 eluted with TBS + 50 mM EDTA.

Purification of the JCP. Total JCP was obtained as previously described[54] by treating oviposited eggs with TBS + 50 mM β-mercaptoethanol, pH 8.9 (three 5 min washes) until the egg jelly was solubilized. The egg jelly was then decanted, dialyzed against TCS, and lyophilized prior to conjugation with BAP.

Thiolation of the JCP. The JCP (∼10 mg) was dissolved in 4 M guanidine hydrochloride, 0.1 M Tris, pH 8, and treated for 1 h at 37 °C using 10 mM DTT. Iodoacetamide was then added to the reduced JCP at a final concentration of 50 mM and the resulting mixture left for 1 h at 37 °C. The thus formed carboxymethylated JCP (RCM-JCP) was centrifuged at 15000*g* for 1 h to remove particulates and then desalted twice against PBS with 5 mM EDTA, pH 8.0, in preparation for thiolation. SATA (3.1 mg) was dissolved in DMSO (100 μL), and 30 μL of this stock was added to RCM-JCP in PBS (3 mL) containing 5 mM EDTA, pH 8 (final concentration of SATA 1.3 mM). After 2 h at 25 °C, excess SATA was removed by dialyzing the reaction mixture three times against PBS with 5 mM EDTA. The JCP−SATA conjugate (1 mL, 2.6 mg of protein) thus obtained was treated with 0.5 M hydroxylamine in PBS with 25 mM EDTA, pH 7.5, for 2 h and then desalted against PBS with 25 mM EDTA, pH 7.2, to prepare it for conjugation to derivatized BAP.

Conjugation of the JCP to BAP. BAP (5 mg) was desalted three times against PBS with 5 mM EDTA, pH 8. To this BAP solution was added SMCC in DMSO (60 mM; 50 μL), and the resulting solution was left for 1 h at 25 °C. The mixture was then desalted twice against PBS with 5 mM EDTA, pH 7.2, and the resulting BAP−SMCC added to the deacetylated JCP−SATA conjugate. The reaction was left for 2 h at 25 °C and overnight at 4 °C and then dialyzed against two buffer changes of TBS with 1 mM $MgCl_2$. The activity of the final conjugate was confirmed using saturation binding assays.

Competition ELLA (Lectin on Plates). Purified XL35 (20 μg) in 50 mM sodium bicarbonate, pH 9.4, was coated onto Corning half-area ELISA plates for 1 h at 25 °C, and the plates were blocked using TCS with 1% BSA and 0.1% Tween 20 as described above. The blocking buffer was discarded and replaced with fresh blocking buffer (20 μL). Inhibitors dissolved in blocking buffer were added to the first column and serially diluted across the microtiter plate. The JCP−BAP conjugate (20 μL of a 1/2500 stock in blocking buffer) was added to each well and allowed to incubate overnight at 4 °C. The plates were washed three times using TCS and developed at 37 °C using *p*-nitrophenol in 0.2 M Tris, pH 10.2 (120 μL), before being read at 405 nm.

Saturation Binding Assays. Assays were performed in a fashion similar to that of the corresponding inhibition assays with the exception that no inhibitor was added. Instead, saturating concentrations of JCP−BAP were added to the first column of wells and serially diluted across the microtiter plate. Following incubation of the primary conjugate, the plates were washed three times and developed.

SPR Analyses. SPR experiments were conducted on a BIAcore 3000 apparatus (BIACORE AB, Uppsala, Sweden). Lectin was coupled to a CM5 chip following the manufacturer's instructions. Purified XL35 (100 μg) was dialyzed against 50 mM sodium acetate, pH 5, for 2 h. Immediately prior to coupling, the dialyzed XL35 was diluted 10-fold in 10 mM sodium acetate, pH 4. EDC and NHS were weighed out and suspended in water immediately before use at final concentrations of 400 and 100 mM, respectively. The coupling reaction was carried out using the BIAcore control software, and 15000 RUs was selected as the target level using the "Aim for Immobilization Level" program. Glycopolymer **19c** and a glycopolymer loaded with 17% *N*-acetylglucosamine (negative control) were separately dissolved in running buffer (10 mM Hepes, 10 mM $CaCl_2$, 150 mM NaCl, with 0.005% Tween 20, pH 7.2) at a concentration of 0.625 mg/mL. The flow rate was set to 15 μL/min, and each sample (50 μL) was injected using the "Kinject" command.

Kinetic and Equilibrium Analyses. Serial dilutions of the glycopolymer **19c** were injected over a chip surface containing 5000 RUs of XL35 prepared as described above. Kinetic analyses were conducted at a flow rate of 15 μL/min to minimize mass transport effects although

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mass transport limitations were not observed even at lower flow rates. Equilibrium analyses were performed using the same surface but at a flow rate of 4 μL/min.

SPR Analysis of Monovalent Ligands. Disaccharide **3** was biotinylated, using sulfo-NHS−LC−biotin and the product purified by preparative reversed-phase chromatography using a linear gradient of water/methanol (0−100% in 50 min). The absorbance was monitored at 206 nm, and fractions were initially screened using MALDI-TOF prior to confirmation by NMR spectroscopy (data not shown). Streptavidin (10000 RU) was coupled to a CM5 chip using the NHS and EDC as described above, but the coupling was carried out by manually injecting the NHS/EDC mixture for 40 min followed by a 40 min injection of streptavidin (25 μg/mL in 10 mM sodium acetate, pH 4.0). Any unreacted NHS esters on the chip were then blocked using an injection of 1 M ethanolamine, pH 8. Biotinylated **3** was then injected over the chip surface and was immobilized by the coupled streptavidin. A calibration curve was generated for XL35 by diluting it serially (200 μg/mL starting concentration), and a concentration of 75 μg/mL was used for solution competition experiments. Increasing concentrations of melibiose (up to 10 mM final concentration) were used to compete XL35 from the streptavidin−melibiose surface.

Molecular Weight Determination of the Glycopolymers. The size of the glycopolymers was determined using gel filtration on a Sephacryl S-400 column (1.5 × 50 cm) equilibrated in 200 mM NaCl with 10 mM H_3PO_4 titrated to pH 6.0 using NaOH. The flow rate for all analyses was 2.5 mL/min, and detection was performed at 206 nm. The system was calibrated using a combination of commercially available polyacid and dextran standards.

Phenol−Sulfuric Assay for Determination of the Sugar Content of the JCP and the Glycopolymers. Using the molar composition of the JCP obtained by compositional analysis,[55] standards were prepared by using the appropriate molar combination of monosaccharides suspended in blocking buffer (TCS with 1% BSA and 0.1% Tween 20) as previously described.[56] Briefly, a 5% phenol (w/w) (10 μL) solution in deionized water was added to individual wells of a microtiter plate followed by the addition of sample (10 μL) or standard. Concentrated H_2SO_4 (150 μL) was added to each of the wells, and the contents were immediately mixed (five times). Microtiter plates were then placed on a shaker for 10 min prior to the absorbance being read at 495 nm. The assays were performed in duplicate, and a linear regression was used to obtain the sugar concentration. The same procedure was used to determine the sugar content of the glycopolymers.

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Supporting Information Available: Procedures for the preparation of compounds **1**−**3**, ^{1}H NMR spectra of compounds **1**−**17**, and Cheng−Prusoff correction for calculating K_I (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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