Platform Synthetic Lectins for Divalent Carbohydrate Recognition in Water

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Abstract: Biomimetic carbohydrate receptors (“synthetic lectins”) have potential as agents for biological research and medicine. However, although effective strategies are available for “all-equatorial” carbohydrates (glucose, etc.), the recognition of other types of saccharide under natural (aqueous) conditions is less well developed. Herein we report a new approach based on a pyrene platform with polar arches extending from aryl substituents. The receptors are compatible with axially substituted carbohydrates, and also feature two identical binding sites, thus mimicking the multivalency observed for natural lectins. A variant with negative charges forms 1:2 host/guest complexes with aminosugars, with $K_1 > 3000 \text{m}^{-1}$ for axially substituted mannosamine, whereas a positively charged version binds the important $\alpha$-sialyl unit with $K_1 \approx 1300 \text{m}^{-1}$.

Carbohydrate recognition is a central biological phenomenon that mediates a range of cellular processes. Carbohydrate-binding molecules are important as research tools for investigating these processes, and potentially as diagnostic and therapeutic agents in medicine. Studies in this area most commonly use lectins, the major class of saccharide-binding proteins, but lectins often lack the desired selectivities and tend to show low affinities (generally $10^{-3} – 10^{-4} \text{m}^{-1}$ for monosaccharides). Moreover, as proteins, their therapeutic potential is limited by issues such as immunogenicity. There is consequently much interest in small-molecule receptors, which could complement lectins and perhaps be developed for new types of application. However, the design of such molecules has proved difficult, especially for biomimetic systems based on noncovalent bonding. Although a variety of structures have been shown to be active in organic solvents, there are few which can operate in the natural but challenging environment of water.

We have approached this problem by constructing symmetrical cavities with an aromatic “roof” and “floor” separated by polar spacers (e.g. 1, Figure 1a). The designs are complementary to saccharides with all-equatorial substitution patterns (e.g. 2), and have yielded encouraging results. Selectivities are good, and some affinities are above $10^4 \text{m}^{-1}$, even for uncharged substrates. However, the selectivity for all-equatorial carbohydrates is a constraint on potential applications, as many substrates of interest do not belong to this family. Herein we report an alternative design strategy which rationalizes targets carbohydrates with axial substituents and which, for the first time, mimics the multivalency exhibited by many lectins.

While all-equatorial saccharides possess two roughly similar hydrophobic patches, other carbohydrates tend to be...
facially amphiphilic. The inversion of a stereocenter, as in β-galactosyl 3, adds to the polarity of one face while decreasing the polarity of the other. A complementary binding site should therefore contain just one extended apolar surface, the remainder being mainly polar.[10] An approach to such structures might involve an aromatic platform with polar substituents that can arch over a bound carbohydrate (e.g. Figure 1b). When considering options for realizing this architecture, we noted the potential of 1,3,6,8-tetraarylpyrenes (Figure 1c). These compounds are readily prepared from tetrabromopyrene (8) by Suzuki–Miyaura methodology, and are forced to adopt nonplanar conformations owing to interactions between aryl and peri-H groups.[11] The meta positions on the aryl groups could provide anchor points for the polar “arches”. Stereoisomers would be possible in the general case, but could be avoided by using symmetrically substituted aryl groups, as shown in Figure 1c. Interestingly, this arrangement would generate two equivalent binding sites, thus mimicking the multivalency common in lectins.[1] Groups Z could be used to confer water-solubility as well as to provide polar interactions. For example, polyionic dendrimers, which are highly water solubilizing and capable of hydrogen bonding to polar carbohydrate substituents, could be readily installed.[8]

As a prototype for this design, we chose the tetracosa-carboxylate 9. Modeling[12] showed that the dendrimers in 9, though relatively small, possessed sufficient reach to interact with axial groups on a substrate. Protonated mannosamine 10·H⁺, with an axial NH₃⁺ group, was found to be an especially promising substrate (Figure 2). Receptor 9 was prepared in 23% yield over four steps from diacid 4, amine 5[13] and 1,3,6,8-tetrabromopyrene (8)[14] Scheme 1).[12] The anionic receptor 9 dissolved freely in water to give well-resolved ¹H NMR spectra, which were concentration-inde-

**Scheme 1.** Synthesis of receptor 9. a) SOCl₂, reflux; then 5, EtNIPr₂, THF, 53%; b) [Pd(dppf)Cl₂], bis(pinacolato) diboron, KOAc, dioxane, 80°C, 80%; c) 8, [Pd(dppf)Cl₂], Cs₂CO₃, dioxane, H₂O, 90°C, 53%; d) CF₃CO₂H, SiHEt₃, CH₂Cl₂; then aqueous NaOH, quantitative. dppf = 1,1’-bis(diphenylphosphino)ferrocene.

![Figure 2. Model of receptor 9 bound to the β anomer of protonated mannosamine 10·H⁺. Aromatic portions of the receptor are shown in space-filling mode, side chains in pale green, mannosamine carbon atoms cyan. a) View from the side, with mannosamine in space-filling mode. b) View from above, with mannosamine in stick mode. Hydrogen bonds are shown in yellow.](image)

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Fluorescence spectra showed an emission maximum at 425 nm (excitation wavelength: 380 nm), detectable down to nanomolar concentrations and with intensity directly proportional to concentration.

Carbohydrate recognition was studied by fluorescence and ¹H NMR titrations of 9 with a range of sugars.[12] Solutions were adjusted to pH 7, and the pH value was confirmed to be unchanged after each experiment. Titrations with aminosugars 10–12 (protonated at pH 7) yielded clear
evidence of complex formation. For example, in NMR titrations, the addition of mannosamine (10) was accompanied by downfield changes in the positions of the receptor aromatic signals (see Figure 3a). A Job plot[15] based on the receptor 4-H signal displacements gave a maximum at 9/10 = 1:2, thus confirming divalency (see Figure S14 in the Supporting Information). Analysis of the receptor signals, assuming the 1:2 binding model, gave stepwise binding constants $K_1 = 3120 \text{M}^{-1}$ and $K_2 = 540 \text{M}^{-1}$ (see Figure S9).[16] As $K_1 = 4K_2$, for noncooperative two-site binding,[17] it seems the two associations are almost independent. The results were supported by fluorescence titrations of 9 with 10, which showed large increases in receptor emission intensity and could be analysed to give almost identical binding constants (Figure 4 and Table 1).

Changes in the positions of carbohydrate signals during the $^1\text{H}$ NMR titration were also informative (Figure 3b). All the signals from 10 moved downfield during the titration implying that, as expected, these protons are shielded in the complex.[18] The spectra provide separate information for the 9 and 10 monomers. By focusing on the 9 anomers, the authors were able to determine close contact distances between 9 and 10 by observing signal overlap. Analyzing the $^1\text{H}$ NMR spectra, the authors identified a 1:2 binding model, confirmed by fluorescence titration and NOE data. The spectra provided evidence of complex formation.

The authors utilized a Job plot to determine the maximum ratio of 9 to 10 for divalency. By comparing the receptor signals before and after the addition of 10, they were able to determine the stepwise binding constants $K_1 = 3120 \text{M}^{-1}$ and $K_2 = 540 \text{M}^{-1}$.

Table 1: Cumulative association constants $(K_1, K_2)$ for 1:1 and 1:2 binding of receptor 9 with aminosugars in aqueous solution, as determined by $^1\text{H}$ NMR and fluorescence titrations.[14]

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>$K_1$ [M$^{-1}$]</th>
<th>$K_2$ [M$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-glucosamine (12)</td>
<td>1040[1]</td>
<td>410[1]</td>
</tr>
</tbody>
</table>

[a] $\text{pH} 7$, $T = 298 \text{K}$. [b] $^1\text{H}$ NMR titration. [c] Fluorescence titration.

Table 1 provides the cumulative association constants $(K_1, K_2)$ for 1:1 and 1:2 binding of receptor 9 with aminosugars in aqueous solution, as determined by $^1\text{H}$ NMR and fluorescence titrations.[14]

α and β anomers, present in the ratio 1:1.9[18] Signals for hydrogen atoms on the α face of the β anomer [for example, H1(β), H5(β)] showed especially large movements, consistent with the modeled structure (Figure 2). From the α anomer, the proton H5(α) signal also moved substantially. NOE data (see Figure S15) confirmed that 9 and the carbohydrate are closely associated. Binding could not be quantified reliably because of the complexity of the system (two substrates, both forming 1:1 and 1:2 complexes). However, the signal movements were consistent with $K_i \approx 3000 \text{M}^{-1}$ for both anomers.

Titrations with galactosamine (11) and glucosamine (12) gave similar changes in both $^1\text{H}$ NMR and fluorescence spectra. Analysis of the data gave the binding constants shown in Table 1. Affinities were somewhat lower, suggesting that the binding sites of 9 favor axial NH$_2^+$ substitution. Uncharged monosaccharides did not appear to bind, but titrations with the disaccharides cellobiose, lactose, and maltose, and also methylamine, gave evidence of weak-complex formation ($K_i \leq 16 \text{M}^{-1}$).[21] It thus seems that the high affinities for aminosugars result from combining carbohydrate-specific interactions (hydrophobic, CH–π, hydrogen bonding) with electrostatic attraction.

Whereas cationic sugars are relatively uncommon in nature, anionic carbohydrates are widespread and play important roles. Especially significant is the α-linked N-acetylenuraminic acid (α-sialyl) unit 13. This moiety commonly appears as a terminus of oligosaccharides, accessible for binding and therefore an important potential target.[20]
The substitution pattern in 13 is all-equatorial with an additional axial negatively charged substituent. This compound should be nicely complementary to our platform design (Figure 1b), provided that the polar arches are positively charged.

To test this concept, we prepared the cationic receptor 16, possessing 24 guanidinium units (see the Supporting Information). Guanidinium substituents were chosen to ensure that the receptor would be fully protonated at pH 7. Spectroscopic studies implied that receptor 16 is monomeric in water below a concentration of 1.2 mM. As expected, the $^1$H NMR spectrum of 16 was unaffected by the pH value in the range 6–8, confirming full protonation.

Studies of 16 as a receptor for the α-sialyl unit 13 required a model substrate. The parent saccharide N-acetylneuraminic acid is readily available, but exists mainly (>90%) as the β anomer 15, and is thus unrepresentative of 13. Simple α-sialosides are not commercially available, and we therefore synthesized the methyl derivative 14 through a variation of a literature procedure. The binding of 14 to 16 was studied by $^1$H NMR and fluorescence spectroscopy. NOESY cross-peaks between substrate and receptor aromatic proton signals supported complex formation (see Figure S55). On the other hand, NMR titrations yielded relatively small changes in the signal positions, implying a looser geometry than for 9 + 10. A Job plot based on a receptor aromatic signal confirmed the expected 16:14 = 1:2 binding stoichiometry (see Figure S54). For quantitative analysis, we employed a titration in which receptor 16 was added to substrate 14. The carbohydrate signals moved upfield as expected, and several could be followed throughout (see Figure S57). Simultaneous analysis of four of these signals was consistent with three successive binding events, with $K_1$, $K_2$, and $K_3$ = 1310, 570, and 30 M$^{-1}$, respectively. Given the high density of positive charge on 16, it is reasonable to suppose that a third (and possibly a fourth) molecule of 14 might bind to the receptor.

Fluorescence titration of 16 with 14 yielded a surprisingly strong effect (Figure 5); receptor emission was reduced almost to zero by addition of the carbohydrate. Analysis of the changes assuming a 1:2 binding model (see Figure 5) gave stepwise binding constants $K_1$, $K_2$ = 1300, 790 M$^{-1}$, consistent with the NMR data. The decrease in emission was found to depend on the chloride counterions; when these were replaced with trifluoroacetate, fluorescence increased on binding. We presume that the addition of 14 to 16 causes a rearrangement of the counterions, thus promoting fluorescence quenching.

Fluorescence titrations were also performed for receptor 16 with several uncharged monosaccharides. Efficient quenching was again observed, although analysis suggested that binding was much weaker than for 14. Moderate affinities were estimated for methyl β-D-glucose ($K_1$ = 43 M$^{-1}$), methyl β-D-galactoside ($K_1$ = 46 M$^{-1}$), and galactose ($K_1$ = 17 M$^{-1}$). In these cases, NMR shifts were too small for analysis, but association between the receptor and the carbohydrate was confirmed by NOE enhancements. Sodium acetate was also bound weakly ($K_1$ = 17 M$^{-1}$). The affinity for glucose was too small to be quantified implying that, as intended, the platform design can reverse the selectivity shown by our earlier synthetic lectins.

In conclusion, we have reported a rational design for synthetic lectins which mimic the multivalency shown by natural lectins and, unlike earlier systems, can accommodate substrates with axial substituents. There is scope for varying the system, for example, by altering/ extending the side chains or changing one aryl substituent. The potential for binding the β-sialyl group 13 is especially significant, and will be a focus of future efforts.

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[12] For further details, see the Supporting Information.


[16] Conditions were chosen to minimize ionic strength and maximize electrostatic interactions (see the Supporting Information). As expected, binding constants are lowered by the addition of salts.


[18] The proportion of carbohydrate bound is highest early in the titration.


[21] The corresponding tetrascosamine precursor to 16 was also studied. However, partial protonation of the amino groups resulted in broadened spectra, which could not be interpreted.

[22] As the affinities for 16–uncharged monosaccharides were quantified by just one technique, they should be seen as tentative.

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