CHEMISTRY
A European Journal

A Journal of
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Accepted Article

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To be cited as: *Chem. Eur. J.* 10.1002/chem.201803317

Link to VoR: http://dx.doi.org/10.1002/chem.201803317

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10.1002/chem.201803317

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Binding Studies on a Library of Induced-Fit Carbohydrate Receptors with Mannoside Selectivity

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Abstract: Synthetic carbohydrate receptors could serve as agents for disease detection, drug delivery, or even therapeutics, however, they are rarely used for these applications because they bind weakly and with a preference towards the all-equatorial glucosides that are not prevalent on the cell surface. Herein we report the binding of 8 receptors with 5 distinct octyloxy pyranosides, which was measured by mass spectrometry and by ^{1}H NMR titrations in CD_2Cl_2 at 298 K, providing binding affinities that vary from ~10^1–10^4 M^{-1}. Although receptors are promiscuous, **1** shows selectivity for β-Man at a ratio of 103:1 β-Man:β-Gal, receptors **2** – **4** and **6** have preference for α-Man, **5** is selective for β-Gal, and **10** prefers α-Glc. A variety of 1D and 2D NMR, and computational techniques were used to determine the thermodynamic binding parameters (ΔH^o and ΔS^o) and the structure of the host-guest complex, revealing that dimeric receptor **10** binds β-Man with increased enthalpy, but a larger entropic penalty than **1**. The first-principles modelling suggests that **10**•β-Man forms an inclusion-type complex where the glycan engages both monomeric subunits of **10** through H−bonding and C−H•••π interactions. Like natural glycan binding proteins, these receptors bind pyranosides by accessing multivalent and cooperative interactions, and these studies suggest a new approach towards biomimetic synthetic carbohydrate receptors, where conformational flexibility and promiscuity are incorporated into design.

Introduction

The surface of every eukaryotic cell is coated with a layer of glycolipids, glycoproteins, and glycopolymers – termed the glycocalyx – and binding events involving these oligosaccharides mediate a wide variety of biological events, including cell-cell communication, immunological response, cell-pathogen interactions, and disease progression.[1] Cell-surface glycosylation patterns are unique and accessible identifiers of cell-type. For example, α-mannose is overexpressed on the surface of human lung[2], and prostate[3] cancer cells, whereas β-galactose is abundant on human testicular[4], brain[5], and white blood[6] cancer cells. So synthetic molecules that recognize with some preference specific mono- and oligosaccharides in the glycocalyx could be used for disease detection, drug delivery, therapeutics or even for understanding how information is transmitted in biological networks.[7] Although mannose and galactose are abundant on cell-surface glycans, their epimer, glucose, is almost entirely absent from cell surfaces because it occurs in such high concentration in the blood and cytoplasm[8], and, as such, for sugar-binding molecules to migrate from the circulatory system, they must bind non-glucosides. Despite the medicinal and biological significance of targeting the glycocalyx, cell surface glycans are generally considered as “undruggable targets” because highly specific glycan receptors are confined to natural lectins and antibodies, which have potential toxicology and immunological limitations.[9,10] In this context, small molecule receptors are of interest but their design is extremely challenging as selectivity is needed for complex molecules that differ sometimes by only the orientation of a single stereocenter.

Despite these difficulties, a significant number of synthetic carbohydrate receptors – including some that bind in water – have been developed.[11,12] These fall primarily into two classes: those that bind through the formation of boronate esters[11] and rigid scaffolds that bind entirely through noncovalent contacts.[12] The latter include rationally-designed, small molecules as well as peptide- and aptamer-based hosts, and some discovered through dynamic libraries.[13] The boronates bind monosaccharides possessing *syn*-diols with binding affinities (K_as) in water ranging between 10^3–10^4 M^{-1}, and particularly noteworthy examples are the chiral diboronic acid receptors by Shinkai that bind D-Fructose and D-Glucose with K_as of ~10^4 M^{-1} [14] and others developed by Anslyn for pattern-based saccharide sensing[15]. The noncovalent, small molecule receptors, in contrast, organize polar and nonpolar domains around a rigid scaffold, and examples include calixarenes and oligoaromatic receptors[16], cyclodextrins[17], porphyrin conjugates[18], podand receptors[19-20], encapsulating receptors[21], peptide-based receptors[13,22] and the temple receptors[23] developed by Davis that bind primarily all-equatorial glycans in organic solvents[23n] with K_a ~ 3.0 x 10^5 M^{-1} and in aqueous solvents with K_as as high as 1.2 x 10^4 M^{-1} [23a]. The applications for these glucoside-binding receptors are manifold, including the monitoring of blood glucose[8b,23a], the early detection of disease biomarkers such as sialyl Lewis X antigen and TF antigen[24], and the site-specific imaging of cancer cells[25], which is still dominated by glucose and sialic acid binders. However, for applications including cell-surface targeting, carbohydrate-based nanotechnology[7,26], or characterizing the structure of complex oligosaccharides, there remains a need to continue developing

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synthetic carbohydrate receptors that associate to non-glucosides or other all-equatorial glycans.

Generally, the synthetic receptors that bind through noncovalent interactions are designed by following the principle of preorganization developed by Cram, who demonstrated that binding affinity increases in rigid receptors because the entropic penalty of reorganization is minimized.[27] This design strategy is consistent with Fisher's "lock-and-key" model of protein binding, which assumes that both enzyme and substrate have rigid conformations that lead to an ideal fit with relatively high K_as.[28] Glycan binding proteins – like lectins or the periplasmic binding proteins – are examples of the more nuanced "induced-fit" model, where enzyme flexibility and substrate influence dictate the structure of the enzyme-substrate complex.[29] Typically, glycan binding proteins are characterized by promiscuity[1] – they will often bind several monosaccharides with weak 1:1 binding, but achieve affinity enhancement of up to 10^6 M^{-1} and increased selectivity by accessing cooperative and multivalent binding pathways, a phenomena termed the "cluster-glycoside effect".[30] Most synthetic carbohydrate receptor designs do not consider these aspects of natural systems. Thus, developing synthetic carbohydrate receptors that associate with non-glucosidic monosaccharides may require approaches towards receptor design that reconsider the role of preorganization and the meanings of selectivity and specificity in the unique context of carbohydrate recognition.

To this end, we have reported previously a highly flexible synthetic tetrapodal carbohydrate receptor **1** (Figure 1B) that possesses four aminopyrroles organized around biaryl core that binds α-mannosides preferentially in chloroform through H-bonding and C−H•••π interactions in concert with multivalent and cooperative equilibria.[31] This receptor is one of only very few synthetic receptors so far reported that are selective for mannose.[19a,c,e,f,g,m] Like natural glycan binding proteins, this receptor is promiscuous and forms 1:1 complexes in $CHCl_3$ with all monosaccharides assayed, and selectivity as high as 16.8:1 α-Man: α-Gal and 1.5:1 α-Man:β-Glc is achieved as a result of 2:1 and 1:2 receptor:substrate complexes. This receptor demonstrates the potential of flexible scaffolds for addressing the unmet challenge of creating synthetic carbohydrate receptors that possess non-glucosidic selectivities. Davis *et al.*, using an anthracene-based receptor, have subsequently confirmed the value of incorporating conformational flexibility in receptor design as a route to increasing binding affinity.[32] Building upon this result, they subsequently reported a pyrene-based synthetic carbohydrate receptor that binds some axially substituted pyranosides in water, whose negatively charged variant forms 1:2 host:guest complexes with aminosugars, with K_1 of ~3.0×10^3 M^{-1} for D-mannosamine. In turn, a positively charged variant binds α-sialyl units with K_1 of ~1.3×10^3 M^{-1}.[9a] These studies show the promise of flexible molecules as selective carbohydrate receptors, and that there remains a need to continue exploring how changes in synthetic carbohydrate receptor structure can access the binding modes common in nature – particularly cooperativity and multivalency.[30,33] Here we do so by exploring how receptor structure affects K_a and selectivity in a library of flexible synthetic carbohydrate receptors based upon the structure of previously-studied receptor **1**, and these data will guide the rational design of future carbohydrate receptors.

Results and Discussion

Here, we seek to understand how variations in the structures of flexible carbohydrate receptors affect their K_as and selectivities towards a series of carbohydrate guests. To this end, we have prepared a library of receptors based upon the biaryl core of our previously reported tetrapodal synthetic receptor **1**, and these synthetic carbohydrate receptors differ from **1** in the nature of the heterocycle, the bond between the heterocycle and the biaryl core, and whether the receptor is dimeric (Figure 1).

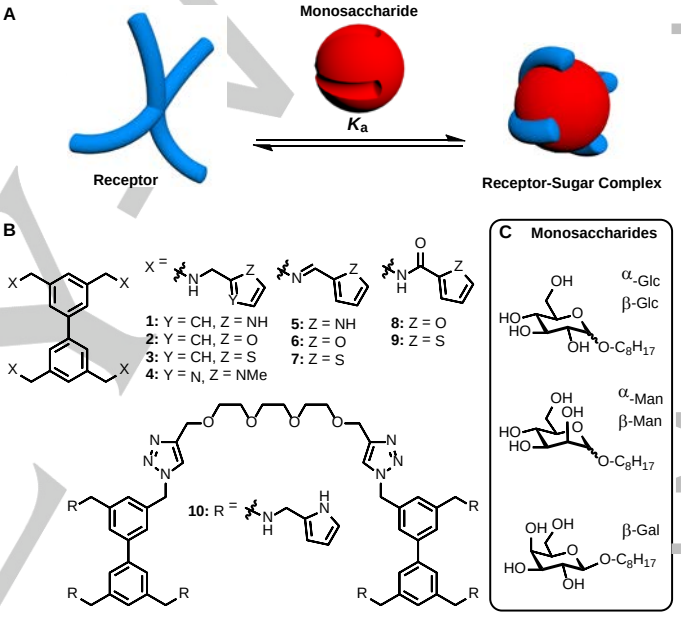

Figure 1. A) The equilibrium between flexible receptors (blue) and pyranosides (red) is governed by the equilibrium constant, K_a. B) Synthetic receptors. C) C1-Octyloxy pyranosides, whose binding with the receptors have been studied.

Subsequently, their binding to a small library of glycans functionalized with solubilizing octyloxy groups at the anomeric (C1) carbon was studied in CH_2Cl_2 by mass spectrometry and in CD_2Cl_2 by NMR spectroscopy, where the latter was used to quantify K_as. Finally, variable temperature (VT) NMR titrations, Nuclear Overhauser Effect (NOE) 2D NMR spectroscopy, and molecular modeling were used to interrogate the thermodynamic and structural details of the association between **10** and β-Man. The data from the host:guest system composed of **10** and β-Man is used as an illustrative example to describe how each of the different analyses were performed, and the data from the other 39 host:guest pairs are provided in the Supporting Information, with results summarized below. Because **8** and **9** were not soluble in CH_2Cl_2, their binding with the monosaccharides was not studied.

10.1002/chem.201803317

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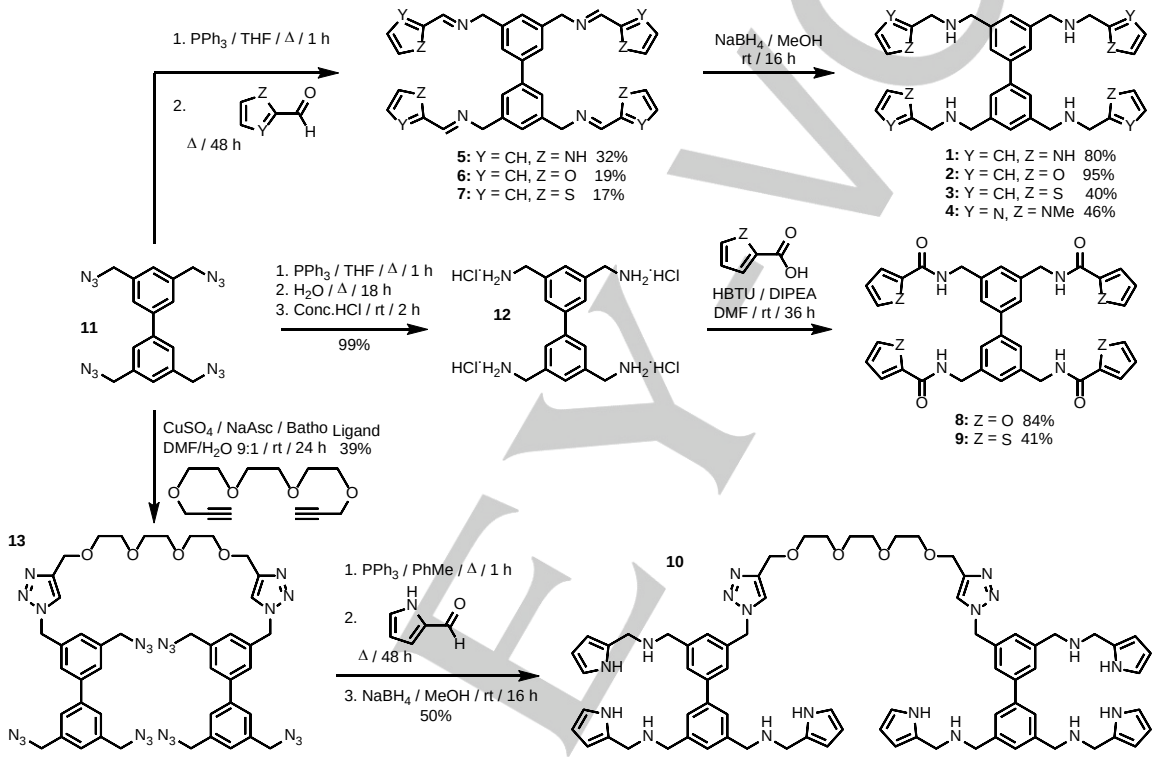
Synthesis of the receptors. Inspired by the initial results with **1**, we sought to vary the receptor structures, while maintaining the overall flexibility of the scaffold by building upon the freely rotating biphenyl core. Based on prior work by the groups of Roelens[19] and Mazik[20] , we reasoned that receptors with different H-bond donors and acceptors may differ in their specificities to the carbohydrate guests as a result of differences in noncovalent bonding with the sugars. The structural variations explored here consist of changing the heterocycles to include furan, thiophene, and *N*-methyl imidazole groups with amine, imine and amide linkages of the heterocycle to the and increasing receptor valency by linking two biaryl cores with an oligoethylene glycol chain, the latter inspired from previous studies showing **1** binds β-Glc and β-Man in a 2:1 host:guest stoichiometry[31]. All receptors were synthesized from the common tetraazide intermediate **11**[31] (Scheme 1). The amine-based receptors **1** – **4** were prepared from intermediate **11** in a one-pot procedure involving three reactions occurring on each of the four azide sites. To form **1**, a Staudinger amination of tetraazide **11** to the corresponding iminophosphorane was followed by an aza-Wittig reaction with four-fold excess of 1*H*-pyrrole-2-carbaldehyde to



Scheme 1. Synthesis of receptors **1**-**10** from **11**.

provide the respective imine intermediate, which when treated with $NaBH_4$ provided tetrapodal receptor **1** in 80% yield. In the same manner, receptors **2** – **4** were synthesized from intermediate **11** by using the respective heterocyclic aldehyde in yields ranging from 40 – 95%. From **11**, the imine-based receptors **5** – **7** were synthesized but isolated in poor yields (17 – 32%), presumably because of hydrolysis during purification by column chromatography on silica gel. The amide-based receptors **8** and **9** were synthesized via HBTU-mediated coupling of the corresponding heterocyclic carboxylic acid with the tetraamine **12**, which was obtained from **11** through a Staudinger amination in quantitative yield. For the synthesis of dimeric receptor **10**, two units of **11** were linked with the alkyne-terminated triethylene glycol chain via a Cu^I-catalyzed azide-alkyne Huisgen reaction (azide:alkyne 5:1) in the presence of $CuSO_4$, sodium ascorbate and bathocuproinedisulfonic acid disodium salt (Batho) to provide the hexaazide **13** in 39% yield. Hexaazide **13** was converted into dimeric receptor **10** by following the three-step amine-forming protocol, where 18 bond-forming steps proceed in one-pot and in 50% overall yield. Importantly, by using diferent diynes and heterocyclic precursors, this synthetic strategy can be easily diversified to create expanded libraries of carbohydrate receptors beyond those described herein.

Mass spectrometry binding studies. We first investigated binding between glycans and the synthetic receptors by electrospray ionization (ESI) mass spectrometry. Initially, solutions containing the receptors alone were subjected to mass spectrometry because understanding the fragmentation patterns of the receptors is necessary to interpret the mass spectra of the host-guest complexes. To this end, 1 mM solutions of receptors were prepared in CH_2Cl_2, diluted to 1 μM with 40% CH_2Cl_2 in CH_3CN, and then injected via direct infusion into the spectrometer

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with a syringe pump. The receptors had a distinct fragmentation pattern, where ions corresponding to the cleavage of each heterocyclic arm were prevalent because of the stability of the benzylic anions that are generated upon breaking of the N–C bond. For example, the positive mode ESI-MS spectrum of **10** possesses $[M+H]^{1+}$ peaks corresponding to the molecular ion as well as 1+ ions corresponding to the loss of one 2-methyl pyrrole group in addition to 2+ peaks corresponding to the molecular ion and loss of one 2-methyl pyrrole group (Figure 2, bottom). In addition, ionization

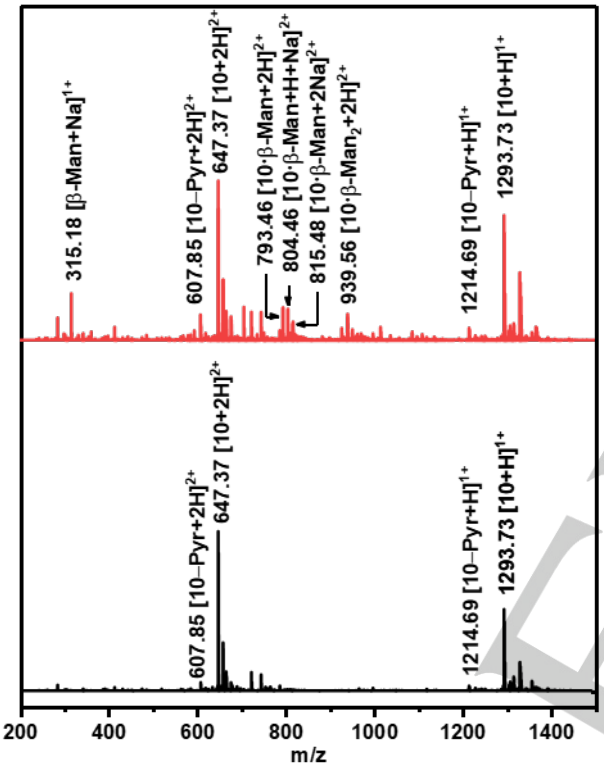

Figure 2. Top: ESI mass spectrum of a 1:1 mixture (0.5 μM, 40%:60% v/v CH_2Cl_2:CH_3CN) of **10** and β-Man. Bottom: ESI mass spectrum of **10** alone (1.0 μM, 40%:60% v/v CH_2Cl_2:CH_3CN). Peaks were assigned using Compass Data Analysis Software (Bruker).

conditions could be found so that peaks corresponding to the loss of all six pyrrole groups were observed (see Supporting Information). In the case of other receptors **1** – **7**, the positive ESI-MS spectrum possess $[M+H]^{1+}$ and 1+ ions or 2+ ions corresponding to the loss of one or more heterocyclic groups (see Supporting Information).

To study the binding of the glycans with the receptors, 1 mM solution of octyloxy glycans were prepared in CH_2Cl_2 and diluted to 1 μM with 40% CH_2Cl_2 in CH_3CN. These diluted glycan solutions were mixed in one-to-one fashion with 1 μM of receptor solution prepared as mentioned above, to create a mixture that was introduced into the spectrometer via direct infusion with a syringe pump. These same solutions were prepared for all 40 receptor:glycan combinations. Compass Data Analysis software (Bruker) was used to simulate the expected masses and isotopic distributions of the complexes and individual components to assign the ions observed in the spectra. For the **10**•β-Man mixture, various ions corresponding to the host-guest complex were observed (Figure 2, top). The most prominent receptor-glycan ions corresponded to the $[\mathbf{10}\bullet\beta\text{-Man}+2H]^{2+}$ complex, and the isotopic distributions of the peaks further confirm the formation of the **10**•β-Man complex. In addition, ions corresponding to $[\mathbf{10}\bullet\beta\text{-Man}+H+Na]^{2+}$, $[\mathbf{10}\bullet\beta\text{-Man}+2Na]^{2+}$ and $[\mathbf{10}\bullet\beta\text{-Man}_2+2H]^{2+}$ were also seen in addition to $[\mathbf{10}+2H]^{2+}$and $[\mathbf{10}–Pyr+2H]^{2+}$. Various other ions were common in the ESI spectra of **10**•glycan when other carbohydrates were added to the solutions of **10**, with the relative intensities of the ions dependent on the particular host:guest combination. While these same $[\mathbf{10}\bullet glycan+2H]^{2+}$ ions were observed in the case of **10**•α-Man and **10**•β-Glc complexes, $[\mathbf{10}\bullet glycan+3H]^{3+}$ ions were found to be prominent in the ESI-MS spectrum of **10**•α-Glc and **10**•β-Gal solutions (see Supporting Information). These ESI experiments were repeated for all receptors that had solubility in CH_2Cl_2 with all five glycans. The positive mode ESI-MS spectra revealed the presence of a 1:1 receptor-glycan complex in all 40 receptor:glycan mixtures. These mass spectrometry experiments confirm the stability of the host:guest complexes in the gas phase, and demonstrate that – like natural glycan-binding proteins – the synthetic receptors studied here are promiscuous, and that all receptors bind to all glycans to some degree.

NMR titrations and determination of K_as. To confirm host:guest association and determine quantitatively how the receptor structures affect K_as and selectivities, binding was studied by performing NMR titrations at 298 K in CD_2Cl_2. NMR is widely used to study host-guest binding, and in particular for complexes whose K_as range from $1 – 10^5$ M^{-1} [34], which is a typical range for synthetic carbohydrate receptors. Also, synthetic carbohydrate receptor binding is commonly studied in non-aqueous solvents[19-20,23n] because K_as are generally higher than they would be in aqueous solvents, so changes in K_as as a result of structural variations are amplified and more easily understood. Here CD_2Cl_2 was chosen as the solvent because it does not compete for H-bonds between the glycans and the receptors. Previously, we showed that **1** undergoes dimerization with a K_d = 13.0 M^{-1} in $CDCl_3$ at 298 K. So prior to performing the receptor:glycan titrations, dilutions were performed at a concentration range of 12.5 mM – 65.6 μM with receptors **1** – **7** and **10**, and, when peak shifts occurred, they were fit to a dimerization model to determine K_d (see Supporting Information). Dimerization was only observed in receptor **1** and **5** in the receptor concentration range at which the host-guest association was studied (0.2 – 8.8 mM).

To quantify K_as, ^1H NMR titrations were subsequently performed by adding aliquots of receptor solutions (12.5 mM) to 1 mM glycan solutions in CD_2Cl_2. The receptor:glycan concentrations were varied from 1:5 to 30:1, with the glycan concentration kept at ~1 mM. All spectra obtained from these titrations are presented in the Supporting Information, and, as an example, the spectra of **10**, β-Man, and a 2:1 mixture of **10**:β-Man are shown in Figure 3A. The peaks in the ^1H NMR spectra of **10** and the octyloxy glycans were assigned with the assistance of

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^{1}H–^{1}H DQF COSY and ^{1}H–^{1}H NOESY NMR spectra (see Supporting Information). Upon association, distinct shifts occurred in the peaks corresponding to all the identifiable protons of both receptor and glycan, which indicates complexation-induced changes in chemical environments. In combination with mass spectrometry data as well as our previous studies on the binding of **1**[31], we attribute these changes in δ to the supramolecular association between the receptors and the various octyloxy glycans. In the spectra in Figure 3A, the largest shift of the β-Man proton peaks correspond to the H^5 peak, which shifts 0.13 ppm upfield. Similarly, the signals assigned to H^1 and H^2 of β-Man shifted

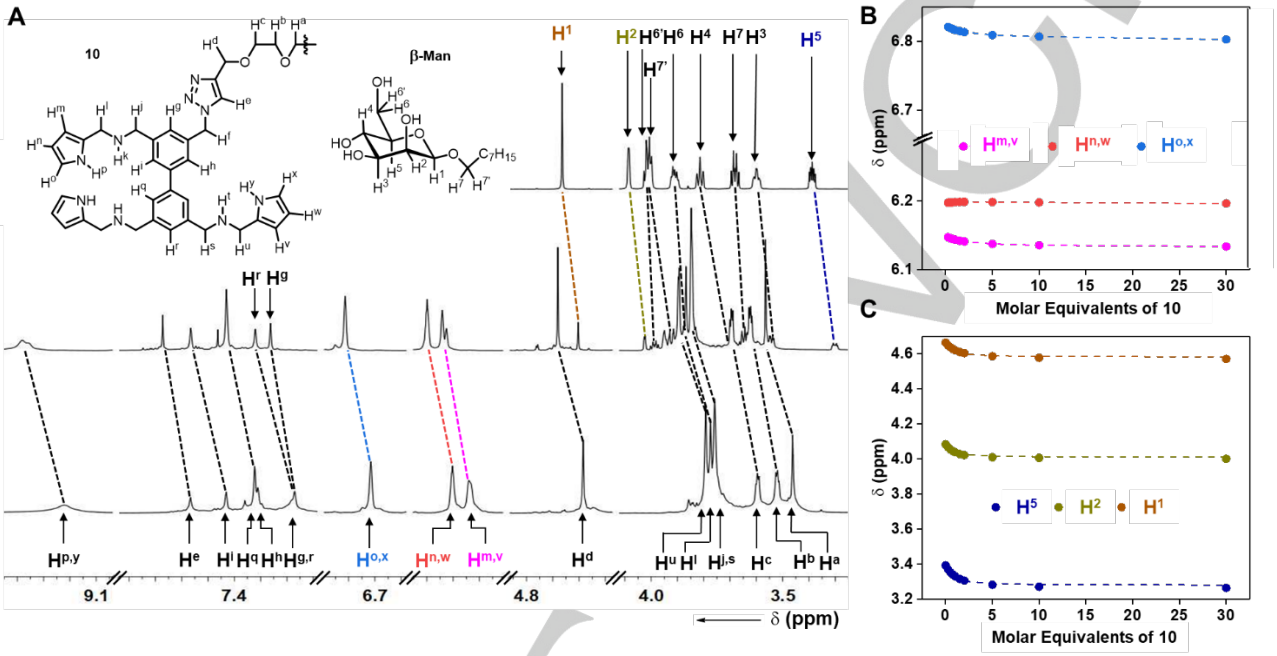

Figure 3. A) ^{1}H NMR (700 MHz, CD_2Cl_2, 298 K) of β-Man (1 mM, top), a 2:1 ratio of **10** and β-Man (middle) and **10** (0.5 mM, bottom). Dashed lines track the shifts of peaks upon mixing of **10** and β-Man. B) The shifts of the NMR peak of the $H^{m,v}$, $H^{n,w}$, and $H^{o,x}$ protons of **10** upon addition to β-Man in CD_2Cl_2 at 298 K, with bullets and lines representing the experimental data and the fit from a 1:1 binding model, respectively. C) The shift of the NMR peaks for protons H^5, H^2 and H^1 of β-Man at 298 K, with bullets and lines representing the experimental data and the fit from a 1:1 binding model, respectively.

upfield by 0.09 and 0.08 ppm, respectively. The Mazik group[20] and the Roelens group[19] have observed upfield chemical shifts of 0.15–1.72 ppm and 0.01–1.76 ppm, respectively, for the C–H protons of sugars upon complexation in organic media, while the Davis group[23n] have reported upfield shifts of 0.2–0.3 ppm for the same. So the glycan and receptor peak shifts that occur with our receptors are consistent with these reports. These upfield shifts upon complexation suggest that shielding of these protons is likely the result of C–H•••π interactions. Shifts are also seen for the peaks corresponding to the host protons, with the largest downfield shifts of 0.11 ppm observed for the pyrrole N–H protons, suggesting that H-bonding has a role in the complexation. The Roelens group have reported a change in chemical shift of 0.70–0.96 ppm for the pyrrole N–H peak of the receptors upon complexation.[19] The relatively low shift for the peaks corresponding to the pyrrole N–H protons in **10** upon complexation compared to those of others' more preorganized receptors can be accounted for by considering that the N–H protons in **10** are already involved in H-bonding prior to complexation that is allowed by the flexibility of the structure, so changes in the chemical environment of this proton are less dramatic. This supposition is supported by the molecular modelling of **10**, which shows internal H-bonding involving the pyrroles (see Supporting Information). Chemical shift changes of 0.01–0.04 ppm were observed for the aromatic protons of **10**, while a $\Delta\delta$ of 0.01–0.80 ppm for aromatic and heteroaromatic protons is typical[19-20,23n]. Complexation of **10** and β-Man also caused a downfield shift of about 0.25–0.30 ppm for the peaks corresponding to the secondary amines N–H^k and N–H^t of **10**, however, these signals were found to overlap with peaks of the octyloxy side chain of β-Man at lower equivalents of **10**, and were therefore difficult to track and determine their $\Delta\delta$. These same titrations were repeated on all other receptor-glycan combinations. In the complexes of receptors **1**, **4**, **5**, **6** and **10** with all octyloxy glycans, and in titrations of **2**•β-Glc, **2**•α-Man, **2**•β-Man, **3**•β-Glc and **3**•α-Man, significant peak-shifting ($\Delta\delta$>0.02 ppm) was observed, whereas in the other receptor-sugar titrations, the changes in chemical shift were <0.02 ppm. The maximum

complexation-induced $\Delta\delta$ was 0.65 ppm for the peak corresponding to H^3 proton of **5**•β-Gal complex.

Determining K_a from NMR peak shift data requires choosing an appropriate model that accounts for all the equilibria present and fitting the peak shifts to these models to extract K_as.[31] We have previously shown that for the association between **1** and β-Man in $CDCl_3$, multiple equilibria, including 1:1, 1:2, and 2:1 β-Man:**1**, occur. With the exception of the binding between **1** and β-Man, we found no evidence of these higher order complexes from the peak fitting (see Supporting Information), which is consistent with the results from ESI-MS spectra. So a 1:1 binding model was considered to fit the titration data with the exception of the **1:**β-Man system, where a $\mathbf{1}_2$:β-Man equilibrium was also considered. In the case of **1**:β-Man system, the titration data did not fit well when only a 1:1 binding model was considered. Thus, the data were best fit with a 2:1 receptor-sugar binding model, and the requirement of considering a 2:1 binding was further supported by the Van't Hoff plot (*vide infra*), which did not fit the data well when only 1:1 binding was considered. To quantify K_as, the shifts ($\Delta\delta$) in the positions of glycan and receptor peaks that could be clearly resolved were plotted, and they were fit to the appropriate binding model, and K_as were determined by minimizing the sum of squared residuals between the experimental data and the modelled fit. Based on the literature reports[19f,20u] we have set a threshold that at least two peaks in each titration must have $\Delta\delta$>0.02 ppm to fit the data and to avoid overestimation of K_as. Although binding between **7** and the sugars was observed in the mass spectrometry data, as the change in the chemical shift for receptor **7** with all sugars was <0.02 ppm, the attempted fits of the titration data were not satisfactory, and, as such, we did not report a K_a. All host and guest peaks that shifted above the threshold of $\Delta\delta$>0.02 ppm were fit simultaneously to maximize the accuracy of the fit, although it should be noted that many peaks with $\Delta\delta$>0.02 ppm could not be used to calculate K_as because they overlapped with other peaks in the spectra and could not be tracked accurately. The data and fits of the guest and host protons for the titration of **10** into a solution of β-Man are provided in Figure 3B and 3C, respectively. The NMR and fits to the other 39 host:guest combinations are provided in the Supporting Information.

The K_d for all receptors, and K_a and ΔG^o values for all glycan-receptor combinations from the fits are presented in Table 1. To quantify the error in the NMR measurements, the titrations

Table 1. Association (K_a) and dimerization (K_d) constants and free energy of binding (ΔG^o) of the receptors (**1** — **7**, **10**) with the five octyloxy pyranosides as determined from NMR titrations in CD_2Cl_2 at 298 K.[a,b]

Receptor	β–Glc		α–Glc		β–Man		α–Man		β–Gal		Dilution	
	K_a (M^{-1})	ΔG^o (kcal·mol^{-1})	K_a (M^{-1})	ΔG^o (kcal·mol^{-1})	K_a (M^{-1})	ΔG^o (kcal·mol^{-1})	K_a (M^{-1})	ΔG^o (kcal·mol^{-1})	K_a (M^{-1})	ΔG^o (kcal·mol^{-1})	K_d (M^{-1})	ΔG^o (kcal·mol^{-1})
1	1.3×10^3	−4.2	3.6×10^2	−3.5	3.6×10^4 [c]	−6.1[d]	1.4×10^3	−4.3	3.5×10^2	−3.5	1.4×10^1	−1.6
2	9.6×10^1	−2.7	[e]	[e]	5.4×10^1	−2.4	1.1×10^2	−2.7	[e]	[e]	[e]	[e]
3	4.8×10^1	−2.3	[e]	[e]	[e]	[e]	1.4×10^2	−2.9	[e]	[e]	[e]	[e]
4	2.3×10^3	−4.6	6.8×10^2	−3.8	5.9×10^2	−3.8	4.4×10^3	−5.0	5.5×10^2	−3.7	[e]	[e]
5	1.7×10^3	−4.4	2.7×10^3	−4.7	2.4×10^3	−4.6	3.0×10^3	−4.7	4.2×10^3	−4.9	1.9×10^3	−4.5
6	1.1×10^2	−2.8	1.1×10^2	−2.8	3.7×10^1	−2.1	2.0×10^2	−3.1	3.0×10^1	−2.0	[e]	[e]
7	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[f]	[e]	[e]
10	2.6×10^3	−4.7	8.1×10^3	−5.3	1.7×10^3	−4.4	2.6×10^3	−4.6	1.8×10^3	−4.4	[f]	[f]

[a] Titrations were done in triplicate for **10**•β-Man, and the standard deviations of K_a and ΔG^o were 3.2×10^2 M^{-1} (15% error) and 0.1 kcal mol^{-1}, respectively. [b] K_as are based on 1:1 binding models that also consider K_d when appropriate. [c] Cumulative association constant[34] ($\beta = K_1K_2$ (M^{-2})) involving a 2:1 receptor-sugar binding model where K_1 (1.2×10^3 M^{-1}) and K_2 (3.0×10^1 M^{-1}) correspond to 1:1 and 2:1 receptor-sugar association constants, respectively. [d] Sum of free energy of binding associated with K_1 and K_2. [e] No detectable binding/dimerization above the threshold of K_a=3.0×10^1 M^{-1}. [f] No NMR peak shifts above the threshold of $\Delta\delta$>0.02 ppm

between **10** and β-Man were performed in triplicate, and the error in K_a was 15%. These data show that both binding strength and the receptor selectivity for different glycans – defined here as the ratio of K_as – are dependent sensitively on the receptor structures. Binding results reveal that receptors **1**, **4**, **5**, **6** and **10** are promiscuous and form 1:1 complexes with all monosaccharides examined, while **2** had measurable binding with only β-Glc, β-Man and α-Man, and **3** only had measurable binding with β-Glc, and α-Man. Receptor **7** did not have quantifiable binding ($\Delta\delta$<0.02 ppm) with any of the glycans.

Analysis of the data revealed that receptors with pyrrole and imidazole heterocycles bind to all five glycans tested (Figure 4). Furan and thiophene-based receptors showed either weak, negligible, or no binding. The promiscuous and strong binding of pyrrole-based receptors underscore the importance of H-bond donors for supramolecular association with the glycan guests. This hypothesis is supported by the downfield shifts of the NMR peaks corresponding to the receptor N–H groups. Generally, receptors with furan or thiophene heterocyclic groups that lack heterocyclic H-bond donors bound the glycans weakly, which may account for their weak binding.

For many applications, selectivity may be more important than K_a, and the changes in receptor structure explored here have significant consequences on receptor selectivity (Figure 4 top). Among all the receptors tested, **1** has selectivity for β-Man with K_1 of 1.2×10^3 M^{-1} and K_2 of 3.0×10^1 M^{-1}, and a cumulative ΔG^o of –6.1 kcal mol^{-1}. While **1** shows selectivity for β-Man as high as 103:1 β-Man:β-Gal, **2** – **6** show selectivity for α-Man. Receptors **4** and **5** show selectivity as high as α-Man:β-Gal 8:1 and α-Man:β-Glc 4:1, respectively. Receptor **2** prefers α-Man:β-Man at a ratio of 2:1, **3** binds α-Man:β-Glc at a ratio of 2.8:1, and **6** shows selectivity as high as 4.8:1 α-Man:β-Gal. Dimeric receptor **10** prefers α-Glc with selectivity as high as α-Glc:β-Man 4.9:1. We have set a threshold of 3.0×10^1 M^{-1}, below which we did not

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report K_as, as shown in the cases of **2**•α-Glc, **2**•β-Gal, **3**•α-Glc, **3**•β-Man and **3**•β-Gal. Alternatively, selectivity can also be analyzed from the perspective of the glycans (Figure 4, bottom). β-Man has a selectivity for **1** over **6** at a ratio of 973:1. β-Glc binds preferentially **10:3** at a ratio of 55:1, and α-Glc prefers **10**:**6** at a ratio of 74:1. α-Man and β-Gal are selective towards **5**. α-Man prefers **5:2** at a ratio of 63:1, and for β-Gal the preference is **5:6** at 140:1. The K_as provided in **Table 1** reveal the importance of H-bonding motifs, like N–H groups, in the receptor for strong glycan binding. This could account for the observation that receptor **3** with N–H groups in the linker shows

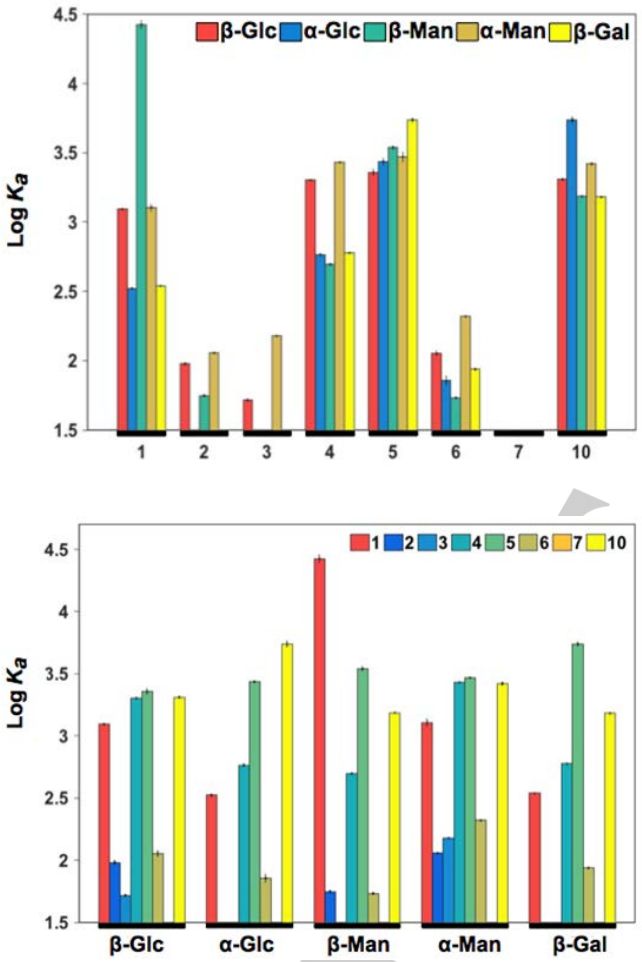

Figure 4. Top: Relative affinities of the receptors towards different glycans. Bottom: Relative affinities of the glycans towards different receptors. In both graphs, the baseline is set to log K_a of 1.5 (K_a=3.0 x 10^1 M^{-1}) as a threshold below which binding is not reported.

moderate binding, whereas **1, 5,** and **10**, which possess H-bonding donors in the heterocycles, bind the strongest. Further, although receptor **10** has more N–H groups compared to **1**, entropy plays a major role in attenuating binding. Moreover, further experiments are necessary to understand why furan-functionalized receptors bind the glycans more strongly than the thiophene-functionalized receptors. To summarize, **1** is selective for β-Man, receptors **2**–**4** and **6** are selective for α-Man, **5** is selective for β-Gal (receptor **5** shows a nominal preference for β-Gal over α-Man, but the difference in these two K_as is close to the reported error of our measurements), and **10** is selective for α-Glc. Although there are several factors like electronegativity, polarizability and atomic radius that may affect both K_a and selectivity, we do not yet understand what causes the differences in selectivity. While these empirical data will guide the design of future synthetic carbohydrate receptors, further experimental and theoretical investigations are needed to explain why these structural differences manifest as differences in K_a and selectivity.

Thermodynamic study on the binding of 1 and 10 with β-Man. To determine how the dimeric structure affected ΔH^o and ΔS^o, variable temperature titrations between **10** and β-Man and **1** and β-Man were performed. The titrations and determinations of K_d and K_a were repeated at 273, 278, 283, and 288 K following the same procedures described above (see Supporting Information). These titration data were fit to the same binding model involving K_d and 1:1 equilibria to determine the K_as at each temperature between **10** and β-Man. The K_as increase with decreasing temperature, suggesting that the binding is entropically disfavored, which is consistent with previous studies of the binding of **1** with β-Man.[31] The obtained K_as were subjected to a van't Hoff analysis to determine ΔH^o and ΔS^o for the binding of **10** to β-Man, and values of –28.5 kcal mol^{-1} and –81.3 e.u. were determined, respectively (see Supporting Information). Similarly, the variable temperature titration data of the **1** and β-Man system were fit using a model involving K_d, 1:1 and 2:1 receptor-sugar equilibria, and the determined K_as also increased with decreasing temperature. A van't Hoff plot was generated from the K_1s, and ΔH^o and ΔS^o were determined to be –21.6 kcal mol^{-1} and –58.5 e.u., respectively (see Supporting Information). Similarly, a van't Hoff plot generated from the K_2s revealed ΔH^o and ΔS^o of –4.8 kcal mol^{-1} and –9.4 e.u., respectively (see Supporting Information). To understand how dimerizing the receptor structure affects the thermodynamics of binding, the enthalpy and entropy of the binding of **10**•β-Man should be compared to the sum of the enthalpy and entropy from both binding events of **1**:β-Man. In doing so, the decrease in unfavorable ΔS^o for **1**•β-Man compared to **10**•β-Man reveals that **1** binds β-Man with less entropic penalty compared to **10**, which likely reflects the substantial reorganizational penalty of the larger, flexible molecule. The increase in ΔH^o for **10**•β-Man compared to **1**•β-Man indicates that **10** likely forms more noncovalent interactions with β-Man compared to **1**, which may occur between the glycan and the ethylene glycol chain. These thermodynamic studies suggest that dimerizing the receptor imbues **10** with multivalency that manifests as an overall increase in binding enthalpy compared to **1**.

Structure of the 10•β-Man complex. We sought to investigate the structure of the **10**•β-Man complex to determine how the flexibility of the receptor enabled "induced-fit" binding, in other words, how the host reorganizes from its lowest energy conformation to form a more stable complex with the glycan. The host:guest structure was determined by ^1H–^1H NOESY

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measurements in CD_2Cl_2 and by computational modelling. ^1H–^1H NOESY spectra provide through-space contacts between the host and guest, and were taken at 700 MHz on a 1:1 mixture of **10** and β-Man so that the peaks of host and guest could both be resolved (Figure 5). Because binding is entropically disfavored, the measurements were performed at 268 K to drive the mixture towards complexation. In addition, the ^1H–^1H NOESY and DQF COSY spectra were also recorded at 268 K and 298 K to assign the peaks of the individual components and to determine if **10** rearranges upon complexation (see Supporting Information).

In the NOESY experiment, the ratio of complexed over uncomplexed host in equilibrium was estimated to be 1:1.2 based on the K_a at 268 K. Several cross-peaks corresponding to host-guest contacts were observed in the NOESY spectrum. As shown in Figure 5, the intermolecular NOE contours between H^4, H^6 and $H^{6'}$ of β-Man with H^e of **10** show the interaction of receptor with the β-face of β-Man. The NOE contacts between H^1, H^2 and H^5 of β-Man with H^i, H^q and H^r of **10** show evidence for the interaction of **10** with the α-face of sugar. Observing cross-peaks with **10** on both faces of β-Man suggest an inclusion complex where the glycan rests within a pocket formed by the receptor (Figure 6).

Theoretical techniques have been employed to elucidate the structural details of the **10**•β-Man complex. First, an initial screening of the guest:host conformational space at the force-field level was performed using mixed torsional/low-mode sampling algorithm available in Maestro software[35] (for details, see Supporting Information). The screening consisted of several constrained, using through-space contacts derived from

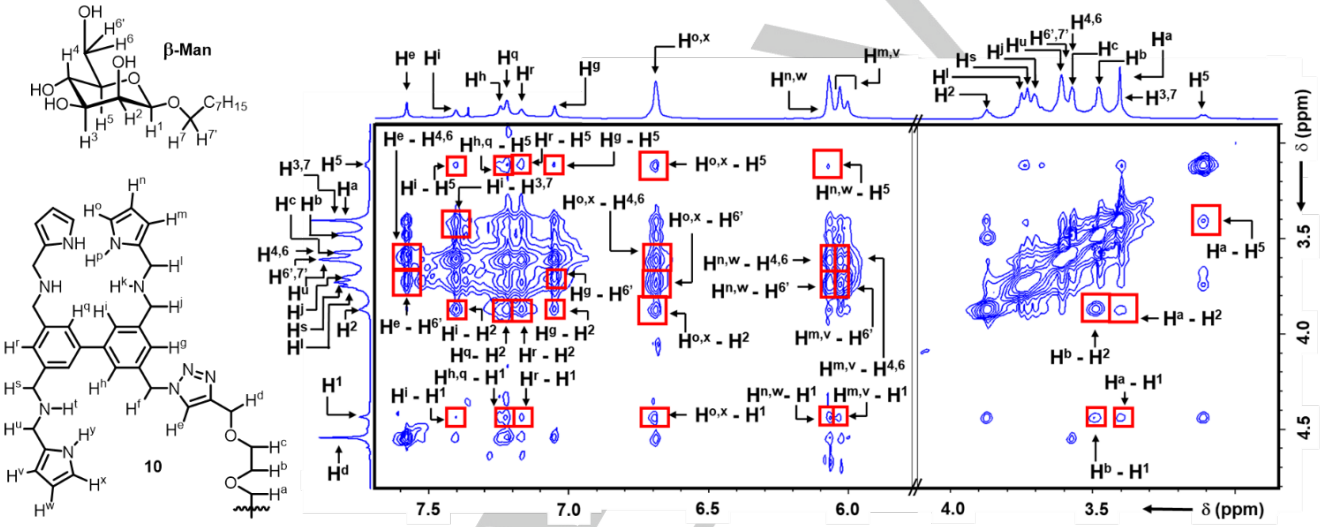

Figure 5. ^1H–^1H 2D NOESY spectrum (700 MHz, CD_2Cl_2, 268 K) of a 1:1 mixture of **10** (5.6 mM) and β-Man (5.6 mM) showing the intermolecular correlations between host and guest protons.

the NOESY spectrum, and unconstrained runs which were initiated from different starting structures. The search yielded more than 1500 conformations featuring different host:guest arrangements. Subsequent clustering of these structures, using loose geometric criterion (RMSD ≤ 0.2 Å), yielded a set of 117 representative structures. The set was further augmented by 40 conformations, generated from two additional conformational search runs, which featured H–H contacts closest to those provided by the NOESY experiment. The resulting 157 structures were next optimized using PBE[36] exchange-correlation density functional augmented with long-range dispersion correction (PBE+vdW^{TS}[37]) using the FHI-aims code[38]. Accurate energetics of different complex binding modes derived from density-functional calculations can add extra dimension to the structural analysis to pinpoint and validate the structure of the complex.[39] The geometry-optimizations rendered one exceptionally stable conformation that surpassed the next low-energy structural candidate by 8.6 kcal mol^{-1}. Although no restraints derived from NOE data were applied to generate this model, all H–H contacts observed in the NOE spectrum are within 10 Å (see table in Supporting Information). This structure (Figure 6A) features the receptor wrapping around the guest molecule in an inclusion-type complex. An aryl ring of one of the two biaryl subunits participates in C–H•••π interaction with the α-face of the sugar whereas four H-bonds in an equatorial arrangement around the sugar ring are formed by two aminomethylpyrroles and a triazole groups of the same biaryl subunit. Furthermore, the glycol linker wraps the second subunit around the cavity to from four axial H-bonds with the guest. These H-bonding motifs are also consistent with the 2D NOE data. This conformation is further boosted by several additional intramolecular H-bonds, which provides some additional structural stability. While the proposed structure is in satisfactory agreement with the experimental data, we recognize that this single-molecule model does not fully explain all H-H

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contacts and for such conformationally flexible receptors other low-energy structures could coexist. Nevertheless, the predicted structure of the complex validates the premise of the design: first, it provides multiple bonding groups that adapt to the guest molecule, which render binding to different monosaccharides promiscuous. Second, the guest binding engages both subunits of the dimeric host molecule, the structural design that was postulated based on study on monomeric receptors[31].

The conformational search was repeated for the receptor itself, following the same procedure as for the host:guest complex. The density-functional optimization yielded the most stable structure in which importantly, the receptor alone does not bear any cavity suitable for binding the guest (Figure 6B). Instead, the binding must proceed by inducing a major conformational change within the receptor to accommodate the guest molecule (Figure 6B). As such, the receptor itself maintains a large degree of flexibility which enables desired promiscuous binding of sugars with different hydroxyl group orientations, displaying a behaviour similar to many natural glycan binding proteins.[1] Importantly, the pyrroles in this structure are involved in internal H–bonding in the absence of the receptor, which explains why relatively small shifts

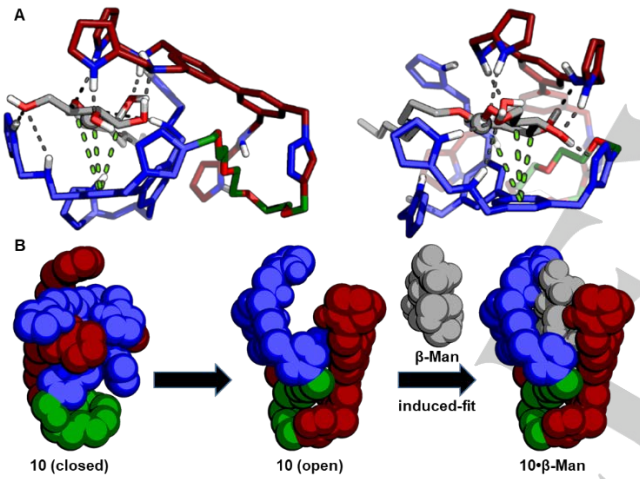

Figure 6. A) Two different views of global energy-minimum structure of the 1:1 **10**•β-Man inclusion complex. The grey dashed lines denote intermolecular H–bonds, and the green dashed lines show the C–H•••π interactions. The C1 carbon of β-Man is represented as a sphere and non-polar hydrogens are omitted for clarity. The carbons of the segment of **10** binding the α-face of β-Man are colored blue, and the carbons of the segment of **10** bound to the β-face are colored red. B) An induced-fit model shows that the structure of **10** changes substantially to accommodate the monosaccharide guest.

are observed in the peaks corresponding to the pyrrole N–H protons as their chemical environment does not change substantially upon binding the carbohydrate guests.

Conclusions

Inspired by our earlier work on tetrapodal receptor **1**, which showed selectivity towards mannosides, we designed an additional 9 flexible receptors to understand relationships between receptor structure and K_a. These receptors were all synthesized from common intermediate **11** in moderate to excellent yields, demonstrating a modular synthesis that is appropriate for making a broad range of glycan-binding molecules. The binding of these receptors was studied against five octyloxy pyranosides by ESI mass spectrometry in CH_2Cl_2 and ^{1}H NMR titrations in CD_2Cl_2 at 298 K to quantify K_as. Binding studies were not carried out on **8** and **9** because the amides rendered these molecules insoluble in CH_2Cl_2. ESI-MS spectra of all receptor-sugar complexes showed the presence of the 1:1 receptor-sugar complex in all cases, revealing that, similar to many natural glycan binding proteins, the receptors are promiscuous and bind all glycans in a 1:1 stoichiometry. NMR titrations further confirmed binding was driven by H-bonding and C–H•••π interactions between the glycan protons and the aromatic groups of the receptors. Curve fitting of the titration data was carried out to quantify association for all 40 sugar-receptor combinations, and showed that, with the exception of **5** and **10**, all receptors were selective for mannosides, a compelling biological target. The binding studies also reveal that amine- and imine-based receptors with pyrrole and *N*-methyl imidazole heterocycles are particularly important. Receptor **1** shows the greatest binding with β-Man with K_1 = 1.2 x 10^3 M^{-1} and K_2 = 3.0 x 10^1 M^{-1}, and selectivity of β-Man:β-Gal of 103:1. Moreover, the change of solvent from $CDCl_3$ to CD_2Cl_2 does not modify significantly the affinity and specificity of **1** other than the increased selectivity towards β-Man. While **1**,**4**,**5**,**6** and **10** bind all five glycans, **2** binds only β-Glc, β-Man, and α-Man, and **3** binds only β-Glc and α-Man.

The table of K_as revealed the importance of H-bonding motifs for the strong binding of glycans. By changing the number of H-bonding donors, acceptors, and receptor valency, the selectivity towards the carbohydrates we assayed could be altered. Although we cannot yet fully rationalize the affinities the different receptors display towards the different monosaccharides, which is probably rooted in the subtle interplay of van der Waals and H-bonding interactions, the data provides empirical guidance for designing this class of synthetic carbohydrate receptors. The structure and binding thermodynamics of the **10**•β-Man complex was explored to determine how the dimerization affected binding, which indicate that **10** binds β-Man with larger entropic penalty but forms more intermolecular H−bonds compared to **1** with β-Man. The intermolecular NOE contacts of the receptor with both faces of the sugar suggest an inclusion complex where the glycan rests within a pocket formed by the receptor. Formation of the 1:1 receptor-sugar complex and the intermolecular interactions were further supported by molecular-modelling studies. Importantly, the host rearranges to accommodate the guest, confirming that the "induced-fit" model accurately describes this complex. Upon rearrangement, **10** forms multiple noncovalent interactions with β-Man, but none of the specific supramolecular contacts were designed, rather, our approach involved adding sufficient flexibility into the host and retroactively determining the structure.

The majority of synthetic receptors for carbohydrates are specific for all-equatorial monosaccharides, while other

monosaccharides are desirable targets for drug delivery or therapeutics because they are over-expressed on the surfaces of many diseased cells. Glycan binding proteins are generally flexible and promiscuous, and achieve selectivity through cooperative and multivalent binding modes. Here, with a series of conformationally-flexible hosts we demonstrate the value of considering and incorporating biomimetic binding modes into the design of synthetic carbohydrate receptors that bind mannosides.

Experimental Section

For full experimental procedures, synthetic protocols, analytical data, and copies of NMR spectra for all new compounds, ESI mass spectrometry data, NMR titrations and peak shift fittings, variable temperature NMR, complete table of binding constants, Van't Hoff analysis, 2D NMR experiments, and computational modelling data, see the Supporting Information.

Acknowledgements

We thank the Air Force Office of Scientific Research (FA9550-17-1-0356) for financial support. The NMR data (Bruker Avance 600, 700 and 800 MHz) presented herein were collected in part at the CUNY ASRC Biomolecular NMR facility and the NMR facility of the City College of the CUNY. Mass spectra were collected at the CUNY ASRC Biomolecular Mass Spectrometry Facility and the Hunter College Mass Spectrometry Facility of the CUNY. We thank Dr. James Aramini (Biomolecular NMR Facility at ASRC), Dr. Rinat Abzalimov (Biomolecular Mass Spectrometry Facility at ASRC) and Dr. Barney Yoo (Hunter College Mass Spectrometry Facility) for their help in acquiring the spectral data. Special thanks to Nikita Moiseyev for his help in programming the solver used to fit the titration data.

Keywords: synthetic lectins • host-guest • carbohydrates • glycobiology • supramolecular chemistry

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10.1002/chem.201803317

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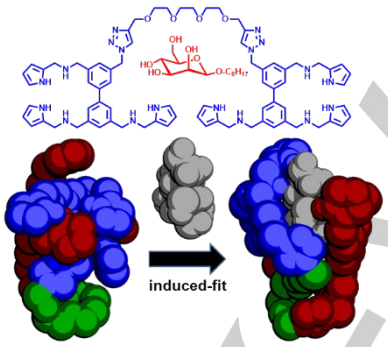

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