

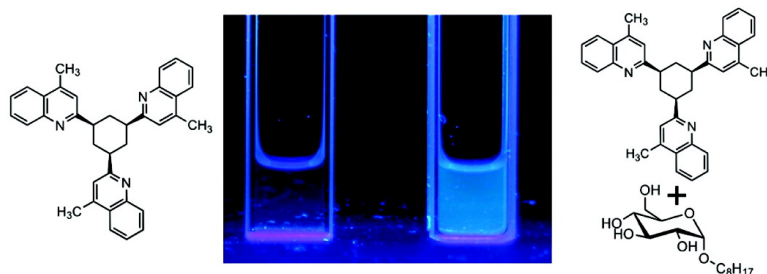
Article

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## Selective Recognition of Alkyl Pyranosides in Protic and Aprotic Solvents

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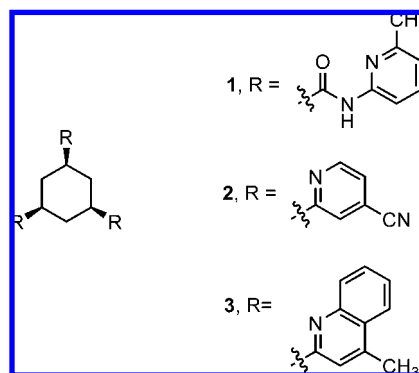
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**Abstract:** The design and synthesis of receptors capable of selective, noncovalent recognition of carbohydrates continues to be a signature challenge in bioorganic chemistry. We report a new generation of tripodal receptors incorporating three pyridine (compound **2**) or quinoline (compound **3**) rings around a central cyclohexane core for use in molecular recognition of monosaccharides in apolar and polar protic solvents. These tripodal receptors were investigated using <sup>1</sup>H NMR, UV, and fluorescence titrations in order to determine their binding abilities toward a set of octyl glycosides. Receptor **2** displayed the highest binding affinity reported to date for noncovalent 1:1 binding of an  $\alpha$ -glucopyranoside in chloroform ( $K_a = 212\,000 \pm 27\,000\text{ M}^{-1}$ ) and an approximately 8-fold selectivity for the  $\alpha$  anomer over the  $\beta$  anomer of the glucopyranoside. Most importantly, **2** retained its micromolar range of affinities toward monosaccharides in a polar and highly competitive solvent (methanol). The quinoline variant **3** also displayed micromolar binding affinities for selected monosaccharides in methanol (as measured by fluorescence) that were generally smaller than those of **2**. Compound **3** was found to follow a selectivity pattern similar to that of **2**, displaying higher affinities for glucopyranosides than for other monosaccharides. The binding stoichiometry was estimated to be 1:1 for the complexes formed by both **2** and **3** with glucopyranosides, as determined by Job plots. Nuclear Overhauser effect spectroscopy allowed for the derivation of a binding model consistent with the observed selectivities.

### Introduction

Carbohydrates represent a particularly enticing and important challenge to our understanding of molecular recognition.<sup>1</sup> Novel carbohydrate receptors play an important role in addressing the fundamental problem of understanding binding and selectivity and also have significant potential as tools in the rapidly evolving field of glycomics,<sup>2</sup> as components of sensing systems for isolated sugars<sup>3</sup> and pathogens,<sup>4</sup> and as leads for the development of new therapeutic agents.<sup>5</sup> However, as others have noted, the design of organic receptors able to bind simple, nonionic sugars via noncovalent interactions is a daunting task.<sup>6</sup> This is especially true when the goal is binding sugars in

**Chart 1.** Structures of Cyclohexane-Centered Tripodal Carbohydrate Receptors Studied in This Work



protonated solvents, since the solute looks much like the ordered solvent. As recently disclosed by the Davis group, however, binding carbohydrates in water is not altogether impossible: an elegant cage structure they synthesized<sup>7</sup> is able to bind the disaccharide cellobiose in water with a binding constant ( $K_a$ ) of  $650\text{ M}^{-1}$ , as measured by isothermal titration calorimetry (ITC).<sup>8</sup>

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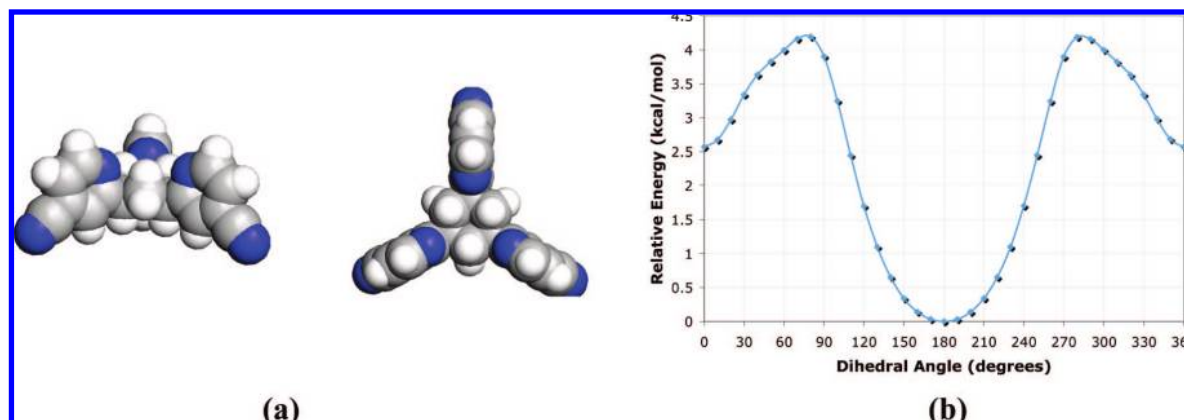
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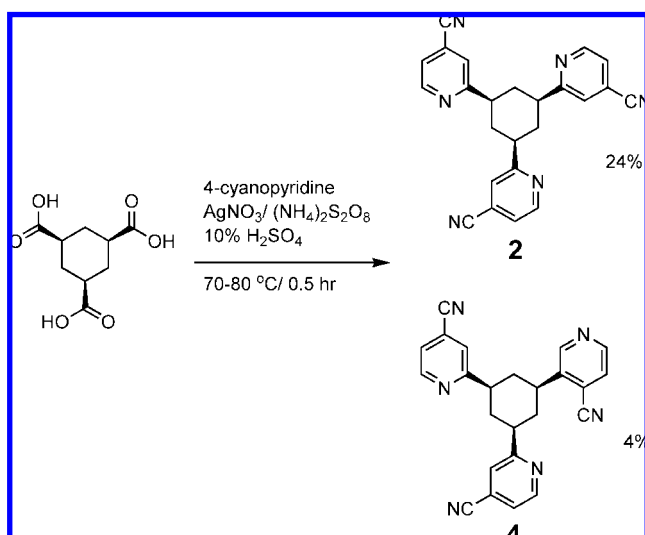
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**Figure 1.** (a) (left) Side and (right) top views of the calculated minimum-energy conformation of **2**. (b) Dihedral-angle drive calculation (using the MMFFs force field<sup>22</sup> and the GB/SA continuum water solvation model<sup>23</sup>) showing the 2.5 kcal/mol preference for the conformer shown in (a) as opposed to the one in which the pyridine ring is flipped 180° across the C–C single bond.

### Scheme 1. Synthesis of Receptor 2



Tripodal receptors have been a mainstay of the molecular recognition field<sup>9</sup> and have found favor as scaffolds for the creation of boronic acid-based receptors for covalent carbohydrate recognition.<sup>10</sup> In the realm of noncovalent carbohydrate recognition, tripodal receptors based on arenes have been explored extensively by the Mazik group, who demonstrated that aromatic compounds incorporating aminopyridine side chains<sup>11</sup> are able to bind alkyl pyranosides as 1:1 and 2:1 complexes in chloroform with substantial affinities and selectivities.<sup>12</sup> Other notable examples of arene-based tripodal carbohydrate receptors have been described by the Roelens,<sup>13</sup> Abe,<sup>14</sup> and Schmuck<sup>15</sup> groups, who studied hexasubstituted benzene-based tripodal receptors incorporating urea, phenol, and guanidinium substituents, respectively.

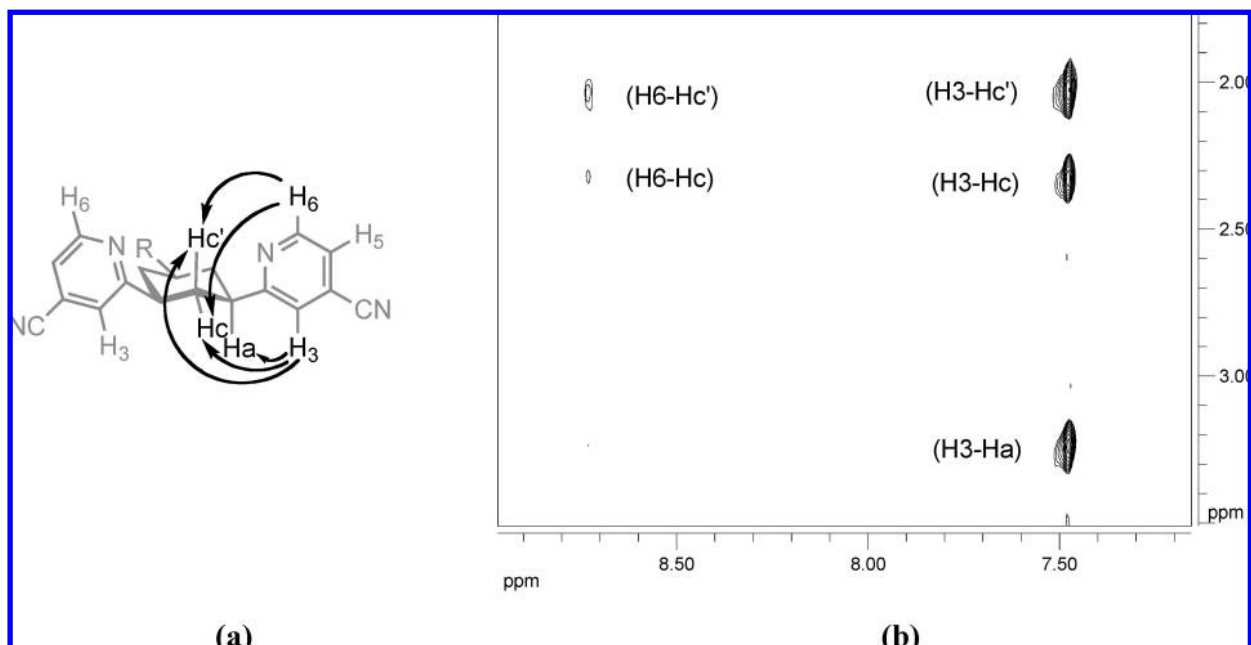
Arenes have proven to be successful core elements in the design of carbohydrate receptors for two primary reasons. First, they provide a surface variously described as complementary to the “hydrophobic patch” of target sugars<sup>8</sup> or able to provide stabilizing interactions via C–H/ $\pi$  bonding.<sup>16</sup> Second, hexasubstituted benzenes provide rigid control over the positioning of pendant functionality via a conformational “gearing” effect. In principle, highly substituted cycloalkanes should provide some of the same benefits as arenes: a hydrophobic surface and conformational control<sup>17</sup> of pendant functionality via avoidance of “anti” interactions (though this control is less stringent than that provided by hexasubstituted benzenes). Indeed, we have successfully employed cycloalkane oligomers as conformational control elements in the development of receptors for lipid A.<sup>18</sup> This led us to begin considering the possibility that other cycloalkane-based scaffolds might have utility as receptors for monosaccharides. In particular, we were intrigued by the possibility of employing *cis*-1,3,5-trisubstituted cyclohexane as a core design element. While highly substituted cyclohexanes, such as Kemp’s triacid,<sup>19c</sup> have found considerable utility as components in molecular recognition systems,<sup>19</sup> receptors based on a simple *cis*-1,3,5-trisubstituted cyclohexane core are less well-known.<sup>20</sup> We report here that two such receptors, **2** and **3** (Chart 1), display significant affinities and selectivities for simple alkyl pyranosides in both halogenated and protic solvents.

## Results and Discussion

**Design and Synthesis.** To begin to assess the utility of cyclohexane-centered tripodal carbohydrate receptors, we synthesized receptor **1** (Chart 1). We observed that **1** showed only weak binding ( $K_a \approx 90 \text{ M}^{-1}$ ) to *n*-octyl- $\beta$ -D-glucopyranoside in chloroform, as measured by <sup>1</sup>H NMR titration. Conforma-

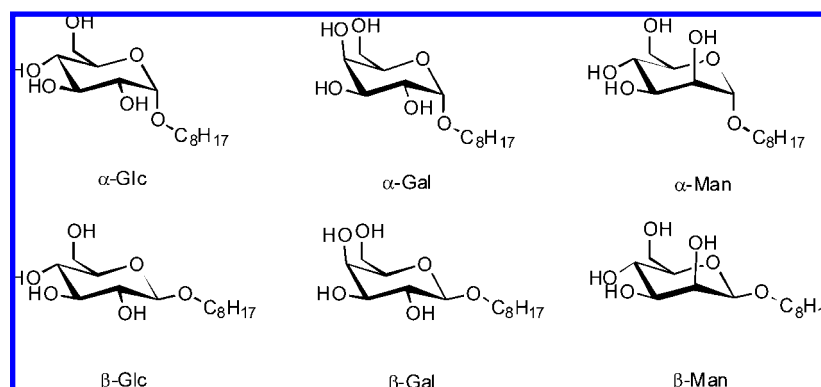
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**Figure 2.** (a) Summary of the NOE contacts observed in the  $^1\text{H}$ – $^1\text{H}$  NOESY spectrum of **2**. (b) Expansion of the  $^1\text{H}$ – $^1\text{H}$  NOESY spectrum of **2** (500 MHz, 200 ms mixing time,  $\text{CDCl}_3$ , 298 K).

#### Chart 2. Octyl Pyranosides Used in Binding Studies



tional analysis of **1**<sup>21</sup> led us to hypothesize that a receptor with fewer torsional degrees of freedom (such as **2**) might prove to be better suited to monosaccharide binding. Indeed, both Monte Carlo conformational searches and dihedral-angle drives of the cyclohexane–pyridine Ha–C–C–N torsional angle indicated that **2** would have a 2.5 kcal/mol preference for a conformation in which all of the pyridine nitrogen atoms are oriented on the same face of the molecule (Figure 1) and available for hydrogen bonding to hydroxyl groups of a target sugar.

Synthesis of **2** was readily achieved via a single-step, tridirectional Minisci reaction<sup>24</sup> between *cis*-1,3,5-cyclohexanetricarboxylic acid and 4-cyanopyridine (Scheme 1), as recently

reported by us.<sup>25</sup> A minor, regioisomeric product (**4**) was also obtained. This compound served as a useful control for the binding abilities of **2** (see below). The structure of **2** was confirmed by X-ray crystallography and further analyzed in solution by one- and two-dimensional (1D and 2D) nuclear Overhauser effect (NOE) spectroscopy. The single-crystal X-ray structures of **2** in toluene and methanol (see the Supporting Information) showed that the crystal packing forces and the recrystallization solvent strongly influence the observed conformation in the solid state, causing two of the three pyridine rings to adopt high-energy torsional angles (as indicated by molecular mechanics). However, 2D NOE studies on **2** indicated that the molecule in solution primarily adopts a conformation similar to that shown in Figure 1. In particular, we observed moderate NOE cross-peaks between the pyridyl H6 and cyclohexyl Hc and Hc' protons as well as strong NOEs between the pyridyl H3 and cyclohexyl Ha, Hc, and Hc' protons (Figure 2).

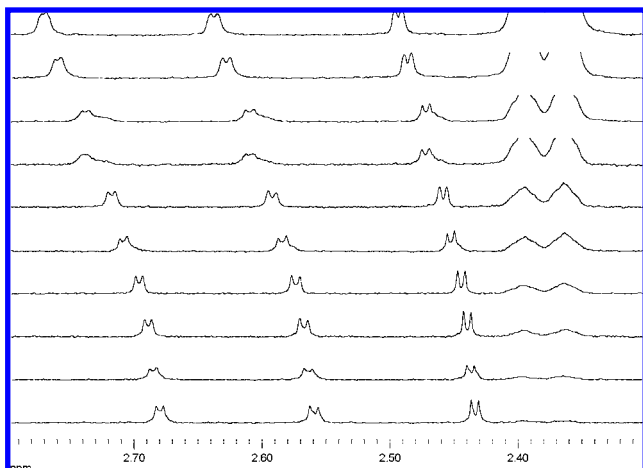
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**Figure 3.**  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 298 K) of receptor **2** into  $\beta$ -Glc (1.09 mM). The portion of the  $^1\text{H}$  NMR spectrum of  $\beta$ -Glc that includes (left to right) the 3-, 4-, and 2-OH proton resonances is shown after addition of (bottom to top) 0, 0.17, 0.35, 0.52, 0.86, 1.20, 1.59, 2.40, 3.20, 3.60, and 4.80 equivalents of receptor **2**.

Sensing of saccharides with fluorescence-active receptors is of particular interest because of the inherent sensitivity and simplicity of the fluorescence technique.<sup>3b,26</sup> We therefore also synthesized the quinoline variant **3**, anticipating that the intrinsic fluorescence of the quinoline heterocycle would yield a receptor suitable for binding analysis by fluorescence. Additionally, extending the aromatic surface from pyridine (**2**) to quinoline (**3**) allowed us to examine the effect of added  $\pi$  surface on the conformations, binding affinities, and selectivities of cyclohexane-centered tripodal receptors.

**Binding Studies.** The binding affinities of **2** toward a set of octyl glycosides (Chart 2) at 298 K were measured using  $^1\text{H}$  NMR titrations in  $\text{CDCl}_3$  and UV titrations in chloroform or dry methanol. After we confirmed that **3** exhibited intrinsic fluorescence properties, binding studies of **3** with the selected monosaccharides were primarily carried out by fluorescence spectroscopy in dry methanol.

**$^1\text{H}$  NMR Titrations.** NMR has been extensively used in studying carbohydrate recognition by synthetic receptors.<sup>27</sup> This method has been very helpful in understanding the recognition process because of its convenience for detecting noncovalent interactions, especially in nonpolar solvents such as  $\text{CDCl}_3$ . However, as reported by Vacca *et al.*,<sup>13</sup> interpreting the results of binding studies using this method can be complicated by the tendency of octyl pyranosides to aggregate and form micelles. In particular, aggregation of alkylated sugars seriously affects accurate estimation of binding constants. Therefore, we used NMR titration experiments primarily as qualitative indicators of binding. In our binding experiments with  $\beta$ -Glc in  $\text{CDCl}_3$  using NMR titration, dilution-mediated changes in the chemical shifts of sugar hydroxyl protons were observed at  $\beta$ -Glc

concentrations greater than 1.2 mM, indicative of aggregation. No detectable chemical-shift changes were observed upon further dilution of  $\beta$ -Glc to concentrations less than 1.2 mM, consistent with the observations of Vacca *et al.*<sup>13</sup> Therefore, we followed a reverse-titration format in which a fixed concentration of  $\beta$ -Glc ( $\sim 1$  mM) was titrated with an increasing concentration of **2**. It was also verified that the NMR spectrum of **2** did not show any concentration-dependent changes. Receptor-dependent changes in the hydroxyl proton chemical shifts of  $\beta$ -Glc upon titration with **2** were observed, indicating the binding event (Figure 3). Although one can use Scatchard analysis to derive a binding constant for the interaction of **2** with  $\beta$ -Glc based on the data shown in Figure 3 (and indeed, such an analysis yielded a  $K_a$  value of  $1.5 \times 10^5 \text{ M}^{-1}$ ), quantitative analysis of this type of NMR experiment is highly error-prone when the starting concentration of the sugar is more than 10-fold greater than the dissociation constant.<sup>28</sup> Therefore, as discussed below, we used other analytical techniques to derive quantitative information. Importantly, no chemical-shift changes were observed in an analogous titration of 4-cyanopyridine into  $\beta$ -Glc. This initial result confirmed that binding requires the full structure of the receptor and is not due to nonspecific interactions between the alkyl glucosides and pyridine. Job plot analysis (as modified for NMR<sup>29</sup>) verified a 1:1 association between **2** and  $\beta$ -Glc.

**UV Titrations.** In order to verify the binding observed by NMR and derive quantitative binding information, UV titrations of the monosaccharides in Chart 2 with **2** were performed. In each case, the receptor at fixed concentration was first titrated with the octyl glycoside<sup>30</sup> in dry, deacidified chloroform. An increase in the intensity of absorbance of **2** was observed as the monosaccharide concentration was increased (Figure 4a). The data were fit to a 1:1 binding model on the basis of the Job plot (NMR) results. Receptor **2** showed the highest binding affinity for  $\alpha$ -Glc ( $K_a = 212\,000 \pm 27\,000 \text{ M}^{-1}$ ) among the set of monosaccharides tested (Figure 4b and Table 1). This is the highest binding affinity reported to date for any artificial receptor toward  $\alpha$ -Glc in chloroform. There was also an 8-fold decrease in the affinity observed for  $\beta$ -Glc, indicating anomeric selectivity for the  $\alpha$  form of the pyranoside. Additionally, substantial selectivity for glucopyranosides over other sugars was observed: while  $\beta$ -Gal was bound with roughly one-third the affinity of  $\beta$ -Glc,  $\beta$ -Man did not reach saturation up to the limit of the titration (Table 1). No binding was observed between **2** and octanol, indicating that nonspecific interactions between the receptor and the octyl chain of the glycopyranosides were at best a minor contributor to binding. Likewise, the regioisomer **4** showed no affinity for  $\alpha$ -Glc. Combined with the NMR titration data described above, these data demonstrate that the ability of **2** to bind alkyl pyranosides in chloroform is highly selective and that proper orientation of the three pyridine rings is an absolute requirement for affinity.

Since **2** showed excellent binding affinities and selectivities in chloroform, we became interested in investigating its capability to function in polar protic solvents, where the competition from solvent molecules is high. Hence, we next examined

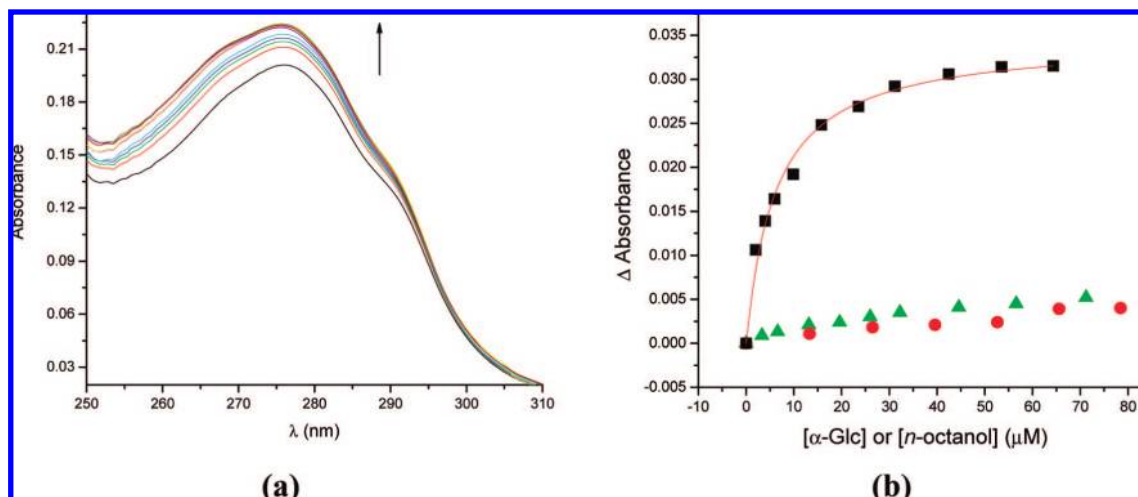
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(30) In order to avoid aggregation-induced error in the binding constants, care was taken to ensure that the concentration of sugar in the cuvette was never higher than 1.2 mM.



**Figure 4.** UV titration of  $\alpha$ -Glc into receptor **2**. (a) Absorbance spectra of **2** recorded during the course of the titration, showing the sugar-concentration-dependent increase in the absorbance intensity. (b) Binding isotherms for receptor **2** (25  $\mu$ M; black squares) and its regioisomer **4** (25  $\mu$ M; green triangles). The titration of *n*-octanol into **2** (red circles) is shown as a control.

**Table 1.** Results from UV Titrations of Selected Octyl Pyranosides with Receptor **2** in Chloroform or Dry Methanol at 298 K<sup>a</sup>

pyranoside	$K_a$ in chloroform ( $M^{-1}$ )	$K_a$ in methanol ( $M^{-1}$ )
$\alpha$ -Glc	212000 $\pm$ 27000	52000 $\pm$ 1600
$\beta$ -Glc	29000 $\pm$ 2500	9700 $\pm$ 900
$\alpha$ -Gal	17700 $\pm$ 200	9600 $\pm$ 950
$\beta$ -Gal	10900 $\pm$ 300	5200 $\pm$ 400
$\alpha$ -Man	6500 $\pm$ 700	> 1300
$\beta$ -Man	(not saturable)	(not saturable)
<i>n</i> -octanol	(no binding)	(not determined)

<sup>a</sup> *n*-Octanol was used as a control. Each titration was repeated at least twice. Each reported  $K_a$  value is the average of results from two independent titrations.

binding in dry methanol. UV titrations again demonstrated that **2** binds alkyl pyranosides with high affinities and selectivities (Table 1). While the affinities were smaller than those measured in chloroform (as would be expected because of increased competition from solvent), their rank order was maintained. This suggests that the mode of binding is not altered to any great extent by changing the solvent system from nonpolar to polar. Again, the receptor showed the highest binding affinity for  $\alpha$ -Glc ( $K_a = 51\,000 \pm 1600\,M^{-1}$ ) among the tested monosaccharides. The anomeric selectivity for the  $\alpha$  form over the  $\beta$  form for each glucopyranoside was found to be similar (nearly within experimental error) in chloroform and methanol.

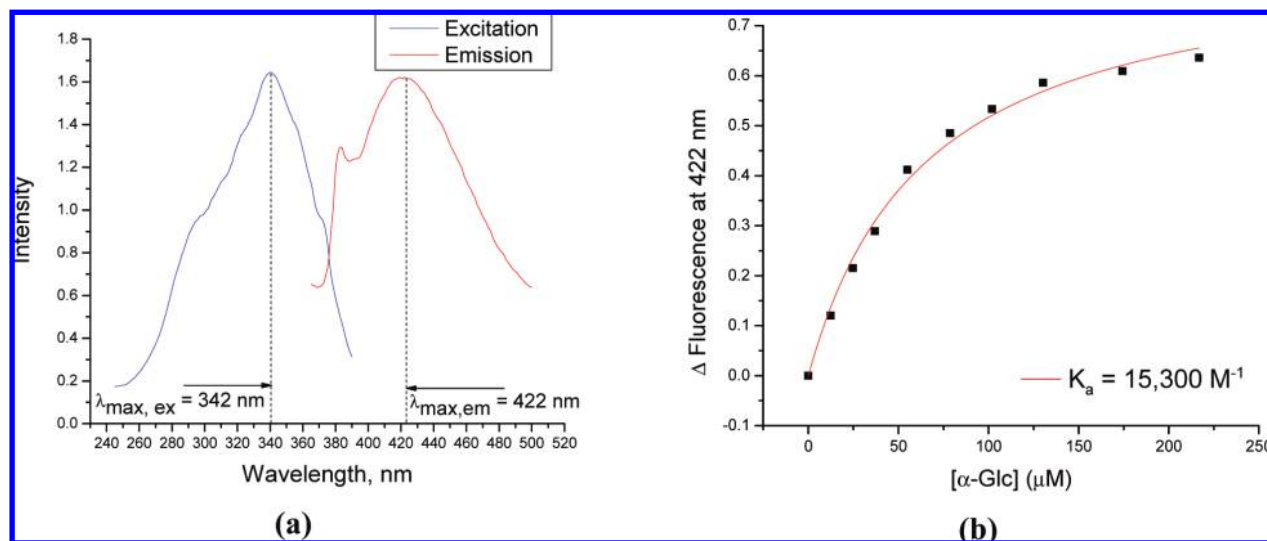
**Fluorescence Titrations.** The exceptionally strong binding constants measured for **2** led us to seek other analytical methods to confirm our observations. Unfortunately, **2** is not fluorescent, precluding the use of direct fluorescence methods. Therefore, we conceived receptor **3** as a fluorescent analogue of **2**. Receptor **3** was found to exhibit a fluorescence maximum at 422 nm when excited with a wavelength of 342 nm (Figure 5a). Initial fluorescence titrations of **3** in  $CHCl_3$  showed a high affinity for  $\alpha$ -Glc in  $CHCl_3$  ( $K_a = 42\,000\,M^{-1}$ ) and established a correspondence with the UV-vis titrations of **2** described above. Next, we measured octyl pyranoside affinities of **3** in dry methanol, again by fluorescence. As for **2**, we found that switching to methanol caused only modest decreases in the affinities. While **3** showed an overall decrease in affinity for octyl glucosides relative to **2** (Table 2), the measured binding constants were nevertheless quite large ( $K_a = 15\,300 \pm 150\,M^{-1}$  for  $\alpha$ -Glc). Compound **3** showed a selectivity pattern

similar to that displayed by **2**: glucosides were favored over other pyranosides and  $\alpha$ -Glc was favored over  $\beta$ -Glc. The fact that the change in fluorescence was of sufficient intensity to be visible to the naked eye (Figure 6) suggests that **3** might have utility as a “turn-on” carbohydrate sensor. A Job plot of the interaction of **3** with  $\alpha$ -Glc confirmed a 1:1 binding stoichiometry (Figure 7). Together, these data suggest a common binding mode for **2** and **3** with octyl pyranosides.

**Mode of Interaction.** A preliminary model for binding was derived on the basis of 1D difference NOE experiments on **2** in the presence of  $\alpha$ - and  $\beta$ -Glc in  $CDCl_3$  or  $CD_3OD$ . Initial experiments conducted at a sugar concentration below 1.2 mM did not provide usable data. Therefore, it was necessary to carry out the NOE analyses at a higher sugar concentration, where aggregation was a potential complicating factor. Nevertheless, these experiments served as a useful starting point for understanding the structural basis of sugar recognition by **2** and **3**. Selective irradiation of the frequency corresponding to the C-6 aromatic proton of **2** in the presence of  $\beta$ -Glc showed enhancement of the 2-, 3-, and 4-hydroxyl protons of the sugar (Figure 8). This suggests the possibility that the sugar hydroxyl protons are hydrogen-bonded to the pyridine nitrogens of **2** and hence are located within the NOE distance range of the C-6 aromatic protons. The key NOE contacts (summarized in Figure 9a) indicate that  $\alpha$ - and  $\beta$ -Glc bind with similar orientations. A binding model derived from applying the NOE constraints to the  $\beta$ -methylglucoside and receptor **2** is shown in Figure 9b. This model indicates that crucial hydrogen-bonding interactions can be lost due to the axial orientation of the 4- and 2-hydroxyl groups in Gal and Man, respectively, thus reducing the affinity of **2** toward these sugars; this is consistent with the observed binding selectivity of **2** for Glc over Gal and Man. The model also suggests that weak hydrophobic interactions with the octyl chain may contribute to the observed anomeric selectivity toward the  $\alpha$  forms of the pyranosides.

## Conclusion

In the present work, we have prepared and analyzed three novel tripodal receptors for monosaccharides using a *cis*-1,3,5-trisubstituted cyclohexane as a core structural element. While compound **1** showed only weak binding ability, our findings with respect to compounds **2** and **3** confirmed that cyclohexane

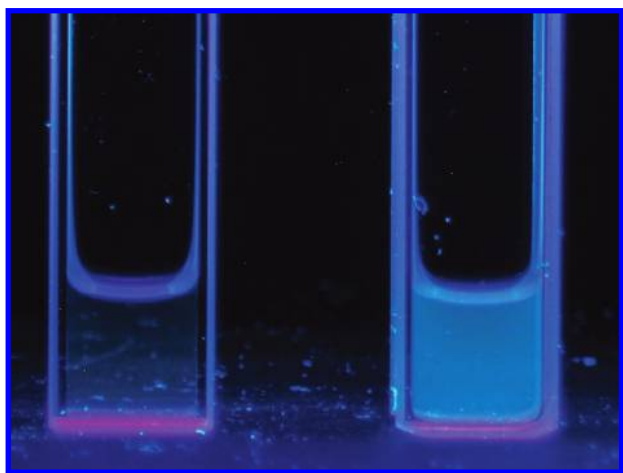


**Figure 5.** (a) Fluorescence spectrum of **3** in dry methanol. (b) Binding isotherm for the interaction between **3** (12  $\mu\text{M}$ ) and  $\alpha\text{-Glc}$ .

**Table 2.** Results of Fluorescence Titrations of the Glycopyranosides in Chart 2 with Receptor **3** in Dry Methanol at 298 K<sup>a</sup>

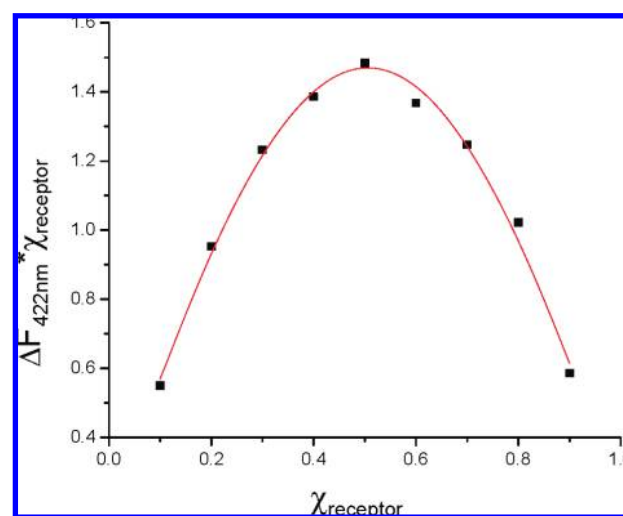
pyranoside	$K_a$ in methanol ( $\text{M}^{-1}$ )
$\alpha\text{-Glc}$	$15300 \pm 150$
$\beta\text{-Glc}$	$9200 \pm 600$
$\alpha\text{-Gal}$	$6600 \pm 200$
$\beta\text{-Gal}$	$3200 \pm 80$
$\alpha\text{-Man}$	$2600 \pm 200$
$\beta\text{-Man}$	$>400$
octanol	(no binding)

<sup>a</sup> *n*-Octanol was used as a control. Each reported  $K_a$  value is the average of results from two independent titrations.



**Figure 6.** Visible fluorescence detection of octyl  $\alpha\text{-glucopyranoside}$ : 400  $\mu\text{M}$  **3** in  $\text{CH}_3\text{OH}$  in the (left) absence and (right) presence of 1 mM  $\alpha\text{-Glc}$ , illuminated with a hand-held long-wave (365 nm) UV lamp.

can serve as an effective scaffold for the production of receptors capable of efficient carbohydrate recognition. Importantly, compound **2** showed the highest affinity recorded to date for noncovalent recognition of monosaccharides in a protic solvent (methanol) and also demonstrated significant selectivities in both solvents tested. Compound **3**, while displaying somewhat lower carbohydrate affinities than **2**, is intriguing because of its ability to function as a turn-on fluorescence sensor for monosaccharides in protic solvents. Compounds **2** and **3** should both be suitable



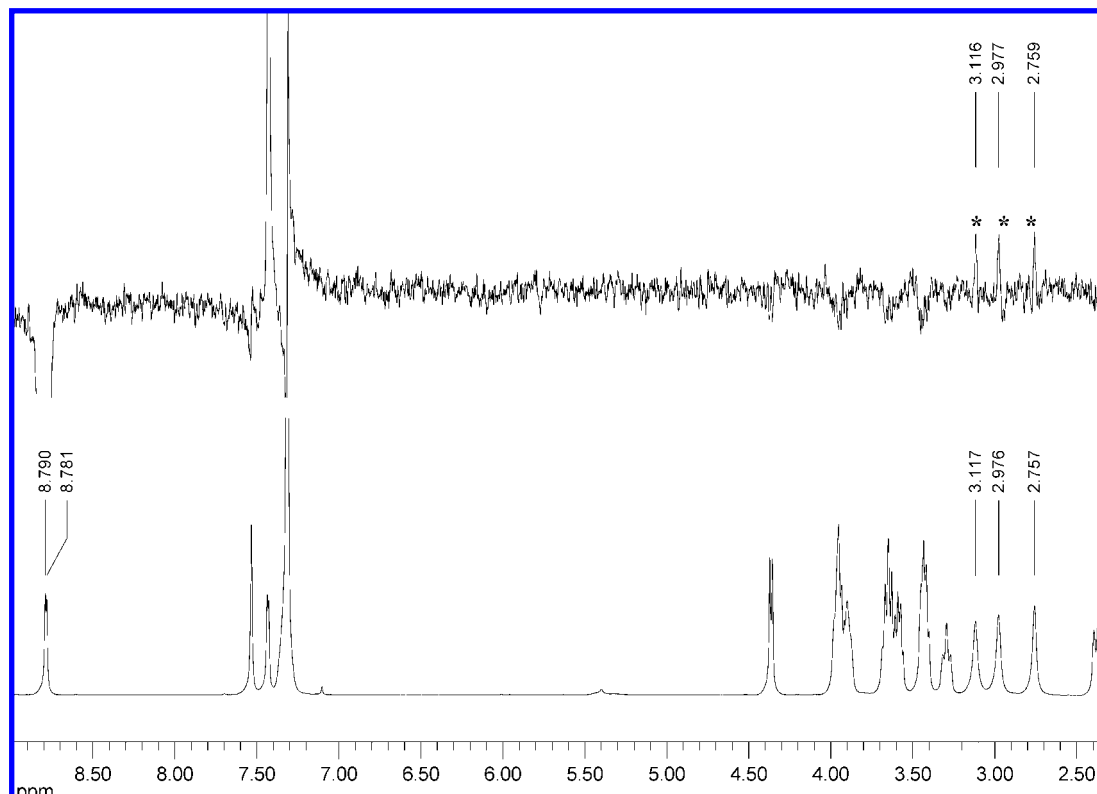
**Figure 7.** Job plot for the complex formed between **3** and  $\alpha\text{-Glc}$ , showing 1:1 binding stoichiometry in dry methanol by fluorescence.

for simple derivatization via several methods, such as hydrolysis of the nitrile groups of **2** and oxidation of the methyl groups of **3**. These experiments are currently underway. Thus, we anticipate that incorporation of analogues of **2** and **3** into more-complex structures that target complex carbohydrates and carbohydrate derivatives will enable the synthesis of novel structures targeting a broad range of carbohydrates and provide fundamental insight into the recognition of simple sugars by designed receptors.

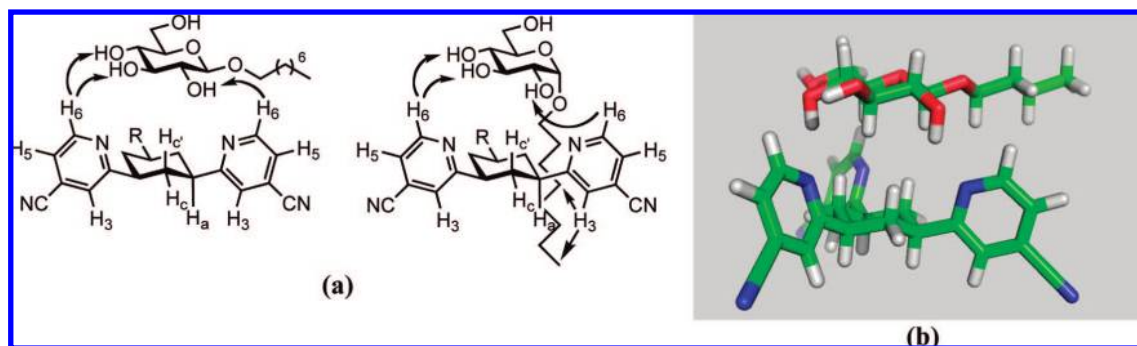
## Experimental Section

**General.** Reagents were purchased from commercial suppliers and used without purification unless otherwise stated.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance 400 instrument. Chemical shifts ( $\delta$ ) are reported in parts per million relative to tetramethylsilane. Melting points are uncorrected. High-resolution mass spectrometry (HRMS) was performed by the mass spectrometry facility at the University of California, Riverside, using electrospray ionization/atmospheric pressure chemical ionization.

**Preparation of Octyl Monosaccharides.** Octyl  $\alpha\text{-Glc}$ ,  $\beta\text{-Glc}$ ,  $\beta\text{-Gal}$ , and  $\beta\text{-Man}$  were obtained commercially. Octyl  $\alpha\text{-Gal}$  and



**Figure 8.** (bottom)  $^1\text{H}$  NMR spectrum of **2** (1 mM) complexed with  $\beta$ -Glc (3 mM) in  $\text{CDCl}_3$  at 298 K. The irradiation frequency, corresponding to C-6 ArH on the pyridines of **2** ( $\delta$  8.785), is marked, as are (left to right) the 3-, 2-, and 4-OH groups of the sugar. (top) The 1D difference NOE spectrum shows a strong positive intramolecular NOE from the irradiated C-6 ArH to C-5 ArH and weak positive intermolecular NOEs (labeled with \*) from the irradiated proton to the 3-, 2-, and 4- OH groups of the sugar.



**Figure 9.** (a) Summary of NOE interactions observed between **2** and (left)  $\beta$ - and (right)  $\alpha$ -Glc in  $\text{CDCl}_3$ . (b) Binding model derived by applying the NOE constraints from 1D difference NOE experiments to models of  $\beta$ -alkylglucoside and **2**.

$\alpha$ -Man are known compounds<sup>31</sup> and were prepared according to a literature method.<sup>32</sup> Spectral assignments were in agreement with the literature data.<sup>31</sup>

**cis-Cyclohexane-1,3,5-tricarboxylic Acid Tris[(6-methylpyridin-2-yl)amide] (1).** The *cis*-1,3,5-cyclohexanetricarboxylic acid chloride was prepared by previously described methodology.<sup>33</sup> Briefly, anhydrous *N,N*-dimethylformamide (0.12 g, 1.65 mmol) was added dropwise to a slurry of *cis*-1,3,5-cyclohexanetricarboxylic acid (0.60 g, 2.72 mmol) and thionyl chloride (1.60 g, 13.60 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$ . The slurry was allowed to reflux for 4 h and dried in vacuo to yield a yellow oil. An aliquot of the triacid chloride

(110 mg, 0.40 mmol) was diluted to 0.1 mM in  $\text{CH}_2\text{Cl}_2$ . Next, 2-amino-6-methylpyridine (0.44 g, 4.05 mmol), 4-(dimethylamino)pyridine (0.24 g, 0.20 mmol) and triethylamine (0.41 g, 4.05 mmol) were dissolved in 10 mL of anhydrous  $\text{CH}_2\text{Cl}_2$  and added dropwise to the acid chloride. After 24 h, the resulting yellow solution with precipitate was treated with 0.5 mL of  $\text{H}_2\text{O}$  and dried in vacuo to yield a whitish solid. The compound was purified by silica gel flash chromatography (49:1  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ ) followed by preparative reversed-phase high-performance liquid chromatography (RP-HPLC) (85:15  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$  + 0.1% trifluoroacetic acid) to obtain a yellow oil in 51% overall yield. FTIR (thin film from  $\text{CDCl}_3$ )  $\nu$  ( $\text{cm}^{-1}$ ): 722, 798, 1153, 1196, 1431, 1636, 2867.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) for the trifluoroacetic acid salt from preparative RP-HPLC:  $\delta$  8.39 (d,  $J$  = 8 Hz, 3H), 8.06 (t,  $J$  = 8 Hz, 3H), 7.13 (d,  $J$  = 8 Hz, 3H), 2.97 (t,  $J$  = 14 Hz, 3H), 2.71 (s, 9H), 2.39 (d,  $J$  = 12 Hz, 3H) 1.80 (q,  $J$  = 12 Hz, 3H).  $^{13}\text{C}$  NMR (100.7 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.4, 30.1, 43.2, 114.4, 119.6, 145.8, 148.9,

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150.7, 174.9. HRMS ( $m/z$ ): calculated for  $C_{29}H_{34}O_4$  ( $[M + H]^+$ ), 487.2458; found, 487.2457.

**6-[(3R,5S)-3,5-Bis(4-cyanopyridin-2-yl)cyclohexyl]nicotinonitrile (2).** To a solution of 1,3,5-cyclohexanetricarboxylic acid (1.50 g, 6.93 mmol) in 10%  $H_2SO_4$  (40 mL) were added silver nitrate (0.43 g, 2.49 mmol) and 4-cyanopyridine (8.64 g, 83.16 mmol). The reaction mixture was heated to 80 °C. A saturated solution of ammonium persulfate (19.00 g, 83.16 mmol) in water (10 mL) was added to the reaction mixture dropwise over 10 min with evolution of carbon dioxide (indicated by bubbling in the solution). After emission of carbon dioxide ceased, the reaction mixture was allowed to stir for an additional 20 min at 80 °C. The mixture was then poured into ice and neutralized using a sufficient quantity of saturated ammonium hydroxide solution. The resulting suspension was then extracted with chloroform ( $3 \times 30$  mL), and the organic extract was washed with brine solution ( $3 \times 20$  mL). Finally, the organic layer was dried over  $Na_2SO_4$  and concentrated to yield a yellow oil. The crude product was then purified using flash chromatography (0–3%  $CH_3OH$  in  $CHCl_3$ ) to obtain compound **2** as the major product in 24% yield (0.65 g) and its regioisomer **4** as a minor product in 4% yield (95 mg). Mp of **2**: 163–165 °C. IR (thin film from  $CHCl_3$ )  $\nu$  ( $cm^{-1}$ ): 2925, 2237, 1594, 1550, 1472, 1397, 1260, 842, 754.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  8.73 (d,  $J = 4.8$  Hz, 3H), 7.48 (s, 3H), 7.38 (dd,  $J = 5$  Hz, 1.4 Hz, 3H), 3.27–3.21 (m, 3H), 2.33 (d,  $J = 12$  Hz, 3H), 2.02 (q,  $J = 12.4$  Hz, 3H).  $^{13}C$  NMR (100.7 MHz,  $CDCl_3$ ):  $\delta$  165.8, 150.3, 123.2, 121.1, 116.7, 45.7, 37.4. HRMS ( $m/z$ ): calculated for  $C_{24}H_{18}N_6$  ( $M^+$ ), 390.1600; found, 390.1601.

**2,2'-[(1R,3S)-5-(4-Cyanopyridin-3-yl)cyclohexane-1,3-diyl]di-isonicotinonitrile (4).** Mp 140–142 °C. IR (thin film from  $CHCl_3$ )  $\nu$  ( $cm^{-1}$ ): 2926, 2856, 2236, 1694, 1594, 1550, 1473, 1412, 840, 754.  $^1H$  NMR (400 MHz,  $CHCl_3$ ):  $\delta$  8.85 (s, 1H), 8.74 (d,  $J = 4.8$  Hz, 2H), 8.65 (d,  $J = 4.4$  Hz, 1H), 7.50 (d,  $J = 4.4$  Hz, 1H), 7.48 (s, 2H), 7.39 (dd,  $J = 4.8$  Hz, 1.4 Hz, 2H), 3.43 (t,  $J = 12.2$  Hz, 1H), 3.31–3.24 (m, 2H), 2.34–2.28 (m, 3H), 2.17–2.01 (m, 3H).  $^{13}C$  NMR (100.7 MHz,  $CDCl_3$ ):  $\delta$  164.5, 149.7, 148.5, 147.7, 122.4, 120.3, 119.1, 115.8, 114.9, 44.9, 39.8, 36.9, 36.5. HRMS ( $m/z$ ): calculated for  $C_{24}H_{19}N_6$  ( $[M + H]^+$ ), 391.1671; found, 391.1671.

**NMR Studies.** The solvent used in NMR studies ( $CDCl_3$ ) was passed through a basic alumina column and stored on 4 Å molecular sieves. The monosaccharides were dried thoroughly for 12 h under high vacuum at 61 °C in a drying pistol using  $P_2O_5$  as the desiccant. NMR titrations were performed in 5 mm NMR tubes using a Bruker Avance 400 instrument. For the NOE experiments, the samples were degassed using nitrogen, and the experiments were carried out on a Bruker Avance 500 instrument. NMR spectral data were processed and analyzed using MestReC version 4.4.1.0 (Mestrelab Research).

**UV Titrations.** UV titrations were performed on a Shimadzu UV-1601PC UV–vis spectrophotometer using 1 cm path-length cuvettes. The chloroform solvent was passed through a short basic alumina column to remove traces of acid and stored over 4 Å molecular sieves. Methanol used in titrations was freshly distilled over magnesium turnings and iodine (a procedure for making superdry methanol). Titrations were performed by placing the receptor (17–40  $\mu M$ , 0.6 mL) into the cuvette and adding increasing amounts of the octyl pyranoside. Hamilton microsyringes were used for the addition of monosaccharides. After each addition, an equilibration time of 10–15 min was allowed before the absorbance was recorded. Statistical analysis of the data was carried out using Origin 7 (OriginLab Corporation). Binding constants were determined by fitting the data to a one-site binding model equation.

**Fluorescence Titrations.** Fluorescence titrations were performed on an Aminco-Bowman Series 2 luminescence spectrometer fluorometer using a 1 cm path-length quartz fluorescence cell. The titrations with  $\alpha$ - and  $\beta$ -Glc were also repeated on a Horiba Jobin Yvon Fluoromax spectrofluorometer. Chloroform was passed through a short basic alumina column to remove traces of acid and stored over molecular sieves. Superdry methanol used in the titrations was freshly distilled over magnesium turnings and iodine. Titrations were performed by placing the receptor (12–30  $\mu M$ , 1 mL) into the 1 mL cuvette and adding increasing amounts of the octyl pyranoside using a Hamilton microsyringe. The receptor solution was excited at 342 nm, and the emission spectrum was recorded from 365 to 500 nm. After each addition, an equilibration time of 10–15 min was allowed before the fluorescence was recorded. A PMT voltage of 955 V was used for collecting the fluorescence data. Statistical analysis of the data was carried out using Origin 7 (OriginLab Corporation). Binding constants were determined by fitting the data to a one-site binding model equation.

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**Supporting Information Available:** Detailed  $^1H$  NMR, UV, and fluorescence titration data; details of 1D and 2D NOE experiments and spectra; and single-crystal X-ray data in CIF format. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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