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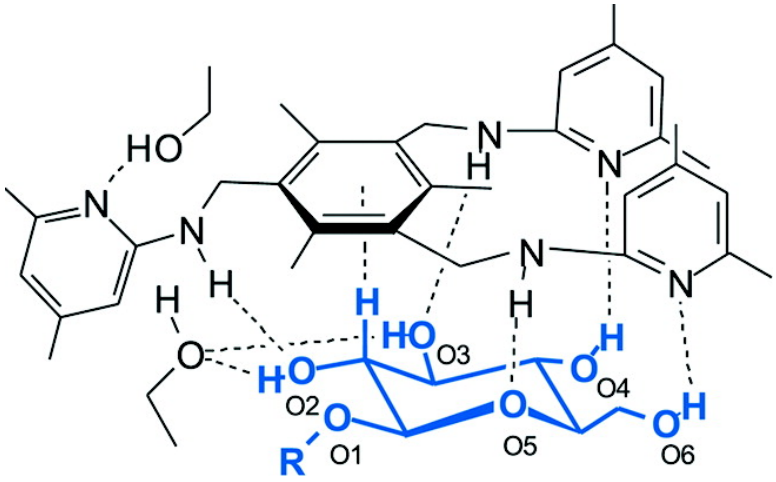

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Molecular Recognition of Carbohydrates with Artificial Receptors: Mimicking the Binding Motifs Found in the Crystal Structures of Protein−Carbohydrate Complexes

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Abstract: The binding motifs found in the crystal structures of protein−carbohydrate complexes have been successfully mimicked with simple acyclic pyridine- and pyrimidine-based receptors. A full discussion of the recognition motifs observed in the crystal structures of complexes of receptors **1** and **3** with glucopyranosides **4a** and **4b** is provided. A remarkable similarity of these motifs to those observed in the crystal structures of sugar-binding proteins and those found by molecular modeling is shown. In addition, the recognition properties of the new pyrimidine receptor **3** toward monosaccharides **4**−**6** are described. This molecule has been established as a highly effective receptor for β-glucopyranosides.

Introduction

The molecular recognition of carbohydrates with artificial receptors is a subject of intensive current research.[1] Advances in this area are likely not only to provide insight into the molecular recognition phenomena, but also to facilitate the development of new therapeutic agents or chemosensors.[1n] Because of the subtle variation in the sugar structures and the three-dimensional arrangement of their functionality, the design of selective and effective biomimetic receptors for these ubiquitous and important biomolecules still represents a significant challenge. Our systematic studies toward recognition motifs for carbohydrates showed that aminopyridines and amidopyridines provide an excellent structural motif for binding carbohydrates, associated with the ability to form cooperative and bidentate hydrogen bonds with the sugar OH groups.[1b,c,2] Aminopyridine receptors based on a 2,4,6-trimethyl- or 2,4,6-triethylbenzene frame (for example, compounds **1** and **2**) show high β versus α binding selectivity in the recognition of glucopyranosides.[1b,2c] Amidopyridine receptors, which are sterically less hindered at nitrogen (for example, *N*,*N*′,*N*″-tris-(4-methylpyridin-2-yl)benzene-1,3,5-tricarbonamide; see ref 1c), display high efficiency and an inverse selectivity since they bind the α-glucopyranoside better than the β-anomer. The design of this type of artificial receptors was inspired by consideration of the crystal structures of protein−carbohydrate complexes.[3] The possible binding modes between the acyclic receptors and monosaccharides were discussed in detail on the basis of chemical shift changes in ^{1}H NMR spectra and molecular modeling calculations.[1b,c,2c]

We can now show that the suggested binding modes are also supported by X-ray analyses of the complexes formed between the acyclic receptor system and sugar molecules. Interestingly, the binding motifs found in the crystal structures of the complexes between the receptor **1** and methyl β-glucopyranoside **4a** and also between the new receptor **3** and octyl β-glucopyranoside **4b** show remarkable similarity to the motifs observed in the crystal structures of protein−carbohydrate complexes. In this article we discuss in detail the receptor−sugar interactions influencing the stability of the crystalline complexes **1**·**4a** and **3**·**4b**. In addition, we describe the binding properties of the new pyrimidine-based receptor **3** toward monosaccharides **4**−**6** in chloroform solutions. The interactions of the receptor and

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(1) Some recent examples: (a) Benito, J. M.; Meldal, M. *QSAR Comb. Sci.* **2004**, *23*, 117−129. (b) Mazik, M.; Radunz, W.; Boese, R. *J. Org. Chem.* **2004**, *69*, 7448−7462. (c) Mazik, M.; Sicking, W. *Tetrahedron Lett.* **2004**, *45*, 3117−3121. (d) Wiskur, S. L.; Lavigne, J. J.; Metzger, A.; Tobey, S. L.; Lynch, V.; Anslyn, E. V. *Chem.−Eur. J.* **2004**, *10*, 3792−3804. (e) Gupta, G.; Lowe, Ch. R. *J. Mol. Recognit.* **2004**, *17*, 218−235. (f) Velasco, T.; Lecollinet, G.; Ryan, T.; Davis, A. P. *Org. Biol. Chem.* **2004**, *2*, 645−647. (g) Fang, J.-M.; Selvi, S.; Liao, J.-H.; Slanina, Z.; Chen, C.-T.; Chou, P.-T. *J. Am. Chem. Soc.* **2004**, *2*, 645−647. (h) Welti, R.; Abel, Y.; Gramlich, V.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 548−562. (i) Wada, K.; Mizutani, T.; Kitagawa, S. *J. Org. Chem.* **2003**, *68*, 5123−5131. (j) Segura, M.; Bricoli, B.; Casnati, A.; Muñoz, E. M.; Sansone, F.; Ungaro, R.; Vicent, C. *J. Org. Chem.* **2003**, *68*, 6296−6303. (k) Welti, R.; Diederich, F. *Helv. Chim. Acta* **2003**, *86*, 494−503. (l) Dukh, M.; Saman, D.; Lang, K.; Pouzar, V.; Cerny, I.; Drasar, P.; Král, V. *Org. Biomol. Chem.* **2003**, *1*, 3458−3463. (m) Ishi-I, T.; Mateos-Timoneda, M. A.; Timmerman, P.; Crego-Calama, M.; Reinhoudt, D. N.; Shinkai, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 2300−2305. (n) For a review, see: Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2979−2996.

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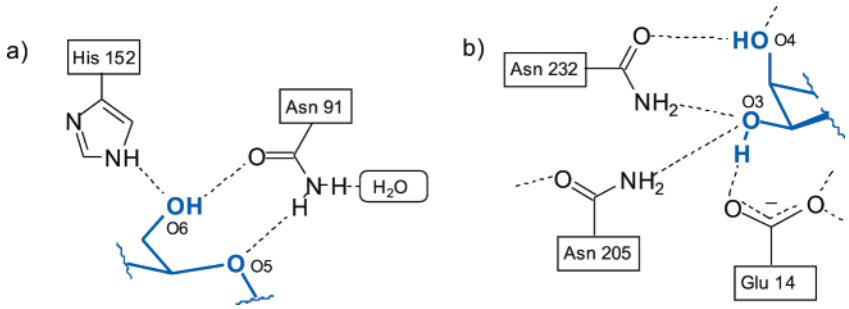


Figure 1. Examples of cooperative and bidentate hydrogen bonds in the complex of GBP with D-glucose (a) and in the complex of ABP with D-galactose or L-arabinose (b).[3a,b] As reported in ref 3a, D-galactose and D-glucose are both excellent substrates of GBP, and ABP binds D-galactose and L-arabinose with similar affinity.

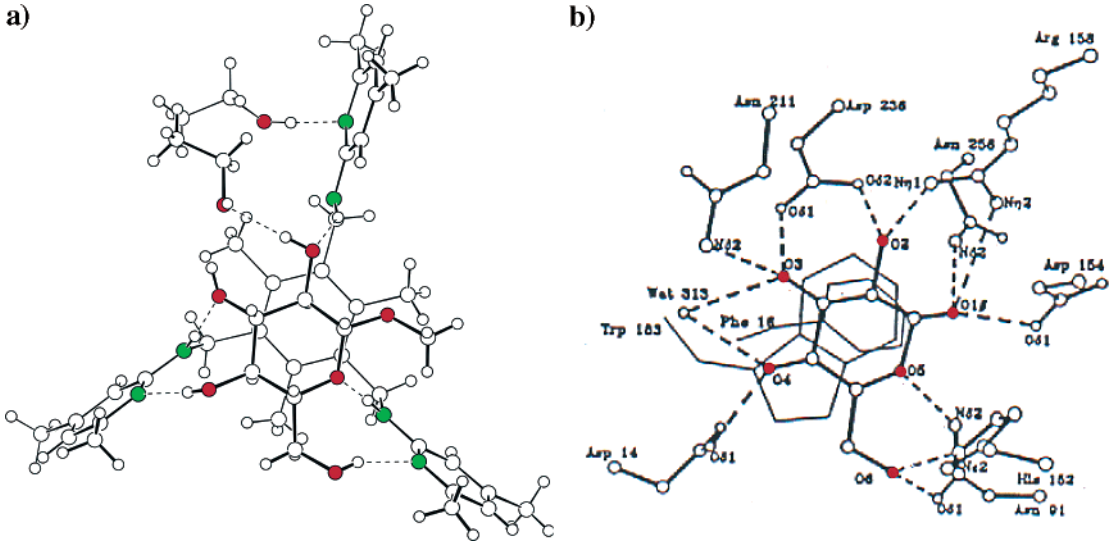
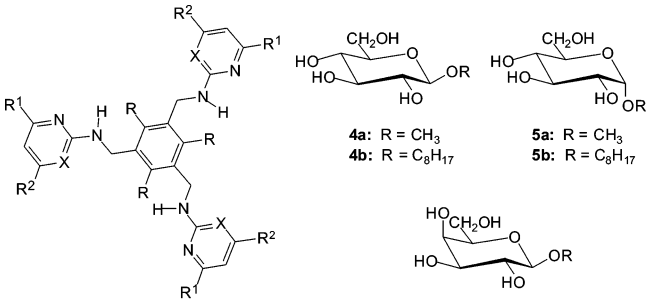


Figure 2. (a) Intermolecular hydrogen bonds and stacking interactions in the 1:1 complex between methyl β-D-glucopyranoside (**4a**) and receptor **1** in the crystal (two hydrogen-bonded ethanol molecules are shown at top left). (b) Intermolecular hydrogen bonds and stacking interactions in the complex between D-glucose and GBP. Adapted from ref 3a.

monosaccharides were investigated by ^{1}H NMR spectroscopy and extraction experiments.



1: X = CH, R = R^1 = R^2 = CH_3
2: X = CH, R = CH_2CH_3, R^1 = R^2 = CH_3
3: X = N, R = R^1 = R^2 = CH_3

4a: R = CH_3
4b: R = C_8H_{17}
5a: R = CH_3
5b: R = C_8H_{17}
6: R = C_8H_{17}

Results and Discussion

Crystal Structure of the Complexes 1·4a and 3·4b. Many of the basic molecular features associated with the interactions of proteins with saccharides are clearly recognizable in the crystal structures of the complexes of receptors **1**/**3** with glucopyranosides **4a**/**4b**. As discussed in refs 3a−e, in the complexes of sugar-binding proteins the hydrogen bonds between the NH and carbonyl groups of the protein backbone and the sugar OH groups are commonly observed. The sugar OHs are engaged in cooperative and bidentate hydrogen bonds. Cooperative hydrogen bonds are those resulting from the simultaneous participation of a sugar OH as donor and acceptor of hydrogen bonds (see, for example, the 6-OH and 3-OH in Figure 1, parts a and b, respectively).[3a,b] The bidentate hydrogen bonds are formed when two adjacent OHs of a pyranoside each interact with a different atom of the same planar polar side chain residue[3a−c] (see, for example, the interaction of 3-OH and 4-OH with the asparagine 232 in Figure 1b). The sugar ring oxygen can also participate in bidentate hydrogen bonding, as shown for example in Figure 1a (asparagine 91 hydrogen bonds to both the 6-OH group and the ring oxygen atom of glucose). It is also noteworthy that interactions of sugar CHs with aromatic residues of the protein (often one or two aromatic residues stack on the sugar ring) are commonly found in the crystal structures (see, for example, Figure 2b).

The crystal structure of the complex formed between receptor **1** and glucopyranoside **4a** (Figures 2a, 3, and 4) revealed that all OH groups and ring oxygen atom of **4a** are involved in hydrogen bonding, in line with the observation in natural complexes. The 3- and 4-OH groups, the 6-OH group, and the ring oxygen atom all participate in bidentate hydrogen bonds with the aminopyridine subunits of the receptor. These bidentate hydrogen bonds include 4-OH···N-pyridine/NH···OH-3 and 6-OH···N-pyridine/5-O···HN interactions (see Figures 2a and 4; see also the bidentate motifs in Figure 5c,d, respectively). The 2- and 3-OH groups are involved in cooperative hydrogen

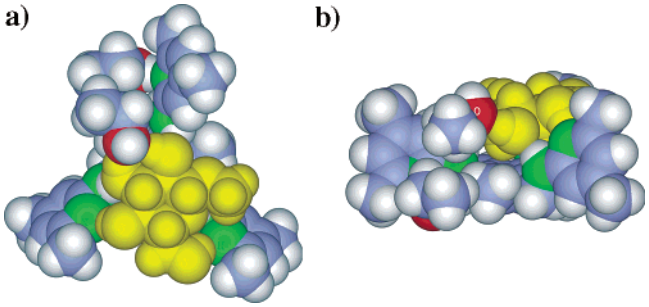


Figure 3. Crystal structure of the 1:1 complex between receptor **1** and sugar **4a** (space-filling representation, the sugar molecule is highlighted yellow), top and side views (two hydrogen-bonded ethanol molecules are shown).

bonds, which result from the simultaneous participation of the OHs as acceptor for NH groups of the receptor and as donor for ethanol oxygen atom. The 2-OH participates in 2-OH···O-ethanol and NH···OH-2 hydrogen bonds, whereas the 3-OH is involved in the formation of 3-OH···O-ethanol and NH···OH-3 hydrogen bonds (see Figures 2a and 4). Thus, one ethanol molecule participates in two hydrogen bonds with the 2-/3-OH of **4a**, and the second ethanol molecule is involved in ethanol-OH···N-pyridine hydrogen bond with the pyridine nitrogen atom of the receptor, as illustrated in Figure 4 (see also Table 1).

The distances of the NH···O/OH···N-pyr hydrogen bonds between **1** and **4a** are in the range of 1.96−2.22 Å with angles at the H-atom of 152−176°. Altogether, eight hydrogen bonds stabilize the formation of the 1:1 receptor−sugar complex between **1** and **4a** (see Table 1). Furthermore, the complex **1·4a** is stabilized by interactions of sugar CHs with the central phenyl ring of receptor **1**. The normalized CH···Ph(centroid) distance amounts to 2.57 Å for 2-CH···Ph (see Figures 2a and 4a, and Table 1). Comparison of Figure 2a,b clearly shows remarkable similarity between the binding motifs found in the natural complex and in our synthetic complex.

The hydrogen-bonding motifs observed in the crystal structure of the complex **1·4a** are consistent with the motifs found previously by molecular modeling, as shown in Figure 5.

In comparison to **1**, the new receptor **3** possesses three additional hydrogen-bond acceptors (three additional ring nitrogen atoms). As expected, this simple structural variation

Table 1. X−H···Y Distances and Angles for Complexes **1·4a** and **3·4b**

host–guest complex	XH···Y interactions	XH···Y (Å)	X···Y[a] (Å)	X–H···Y angle (deg)
1·4a	4-OH···N-pyridine	2.01	2.85	176
	6-OH···N-pyridine	2.09	2.89	160
	NH···OH-2	2.22	3.02	152
	NH···OH-3	2.12	2.98	176
	NH···O-5	2.11	2.96	167
	2-OH···O-ethanol	1.97	2.82	175
	3-OH···O-ethanol	1.98	2.76	154
	EtOH···N-pyridine	1.96	2.79	169
	2-CH···phenyl	2.57		174
3·4b	3-OH···N-pyrimidine(II)	1.93	2.81	172
	4-OH···N-pyrimidine(I)	1.99	2.85	162
	6-OH···N-pyrimidine(I)	2.05	2.94	169
	(I)NH···OH-2	2.23	3.06	175
	(I)NH···OH-3	2.15	3.00	173
	(II)NH···OH-4	2.11	2.96	172
	(II)NH···OH-6	2.17	2.98	162
	(I)NH···O-5	2.21	3.04	168
	2-OH···O-ethanol	2.05	2.74	132
	EtOH···N-pyrimidine(I)	1.93	2.82	177
	2-CH···phenyl(I)	2.49		174
	5-CH···phenyl(II)	2.63		163

[a] Hydrogen-bond criteria used: X−H···Y angle > 130 (deg), H···Y < sum of the van der Waals radii,[4] C−H···π < 3 Å.

resulted in a substantial enhancement of the binding affinity toward monosaccharides (see below). In addition, a crystalline complex of receptor **3** with octyl β-D-glucopyranoside (**4b**) could be isolated. X-ray structure analysis revealed the formation of 2:1 receptor−sugar complexes, as shown in Figure 6. Interestingly, the sugar **4b** is encapsulated in the cavity between the two receptors in a way similar to that in the protein−sugar complexes.

All OHs and the ring oxygen atom of pyranoside **4b** participate in the formation of hydrogen bonds with the pyrimidine nitrogen atoms and the NH groups of the two receptor molecules.

Noteably, all OH groups are involved in the formation of cooperative hydrogen bonds, as illustrated in Figures 7 and 8. The 3- and 4-OH groups, the 6-OH group, and the ring oxygen atom participate in bidentate hydrogen bonds with the amino-pyrimidine subunits of the two receptor molecules (see Figure 7, parts b and c, respectively). In addition, two ethanol-mediated hydrogen bonds contribute to the stabilization of the complex

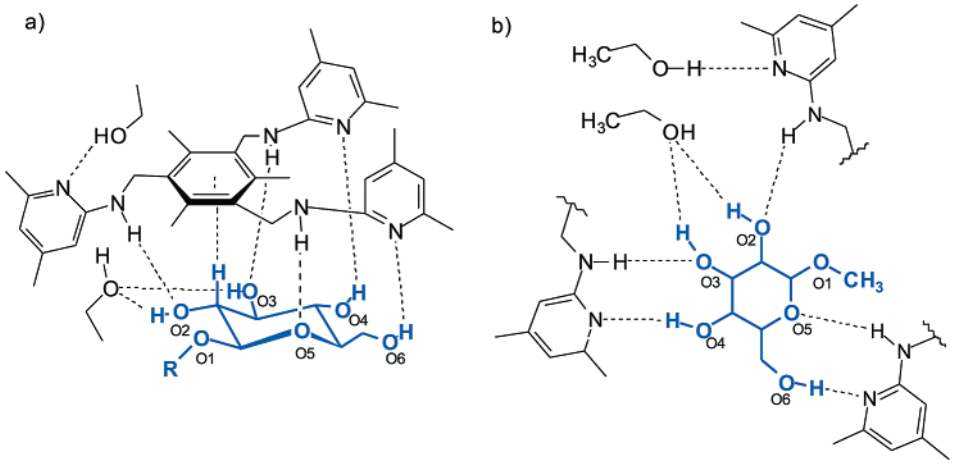


Figure 4. Schematic representation of the hydrogen bonds in the complex between methyl β-D-glucopyranoside (**4a**) and receptor **1** (two ethanol molecules are involved in the complex formation).

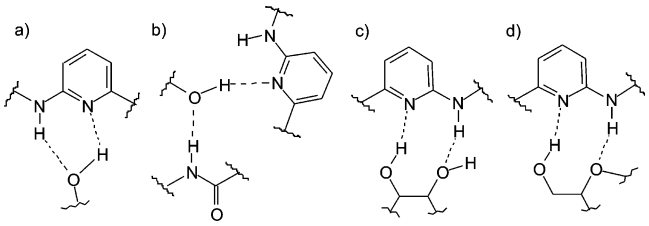


Figure 5. Hydrogen-bonding motifs found by molecular modeling studies in the complexes of aminopyridine- and amidopyridine-based receptors with pyranosides.[1b,c,2c]

3·4b. The OH group of the ethanol molecule acts as acceptor for 2-OH of sugar **4b** and as donor for pyrimidine nitrogen atom of the receptor. The distances of the NH···O/OH···N-pyr hydrogen bonds between **3** and **4b** are in the range of 1.93−2.23 Å with angles at the H-atom of 162−177° (see Table 1). In the complexes of the sugar-binding proteins many of the hydrogen bonds are water-mediated. In the artificial complex **3·4b** the ethanol molecule plays a similar role and forms ethanol-mediated hydrogen bonds. The 2:1 receptor−sugar complex between **3** and **4b** is stabilized through 10 hydrogen bonds, which exhibit almost optimal geometries.

Furthermore, the central phenyl rings of the two receptors stack on the sugar ring. Both sides of the pyranose ring are involved in the CH···π interactions. The normalized CH···Ph-(centroid) distances amount to 2.49 and 2.63 Å for 2-CH···Ph and 5-CH···Ph, respectively. The 2-CH interacts with the phenyl ring of one receptor molecule, whereas the 5-CH interacts with the central phenyl ring of the other receptor, as shown in Figures 7a,c,d and 8.

Crystal Structure of Receptor 3. The X-ray analysis of a single crystal of **3** established that the molecules of **3** are not perfectly preorganized and adopt a similar conformation to **1**. Two pyrimidine units of **3** point to the same face of the central phenyl ring, while the third unit points in the opposite direction (Figure 9).

The packing of the molecules of **3** in the crystal is shown in Figure 10a. The crystals were obtained from ethanol solution, and the ethanol molecules are hydrogen-bonded to the receptor molecules. The hydroxyl groups of the ethanol molecules act as donor to the pyrimidine nitrogen atom (OH···N distance, 1.88 Å; O···N distance, 2.89 Å; OH···N angle, 172°) and as acceptor for the methyl hydrogen (methyl group from the 3- or 5-position of the pyrimidine ring; CH···O distances, 2.67−2.73 Å; C···O distances, 3.50 Å; CH···O angles, 136−144°). The interactions between the two receptor molecules involve NH···N and HCH···N hydrogen bonds with distances of 2.45 and 2.69 Å, respectively (N···N distance, 3.23 Å; NH···N angle, 164°; C···N distance, 3.55 Å; CH···N angle, 146°). The NH group and ring nitrogen of one receptor molecule interact with the ring nitrogen and methylene group of the neighbor molecule, corresponding to the motif in Figure 10b.

Binding Studies. Although the receptor **3** is far from being perfectly preorganized and the methylene groups give conformational mobility to the whole molecule, the binding affinity toward monosaccharides is high (particularly for β-glucopyranoside). The interactions of the receptor and monosaccharides were investigated by ^{1}H NMR spectroscopy and extraction experiments. Octyl β-D- and α-D-glucopyranosides (**4b** and **5b**),

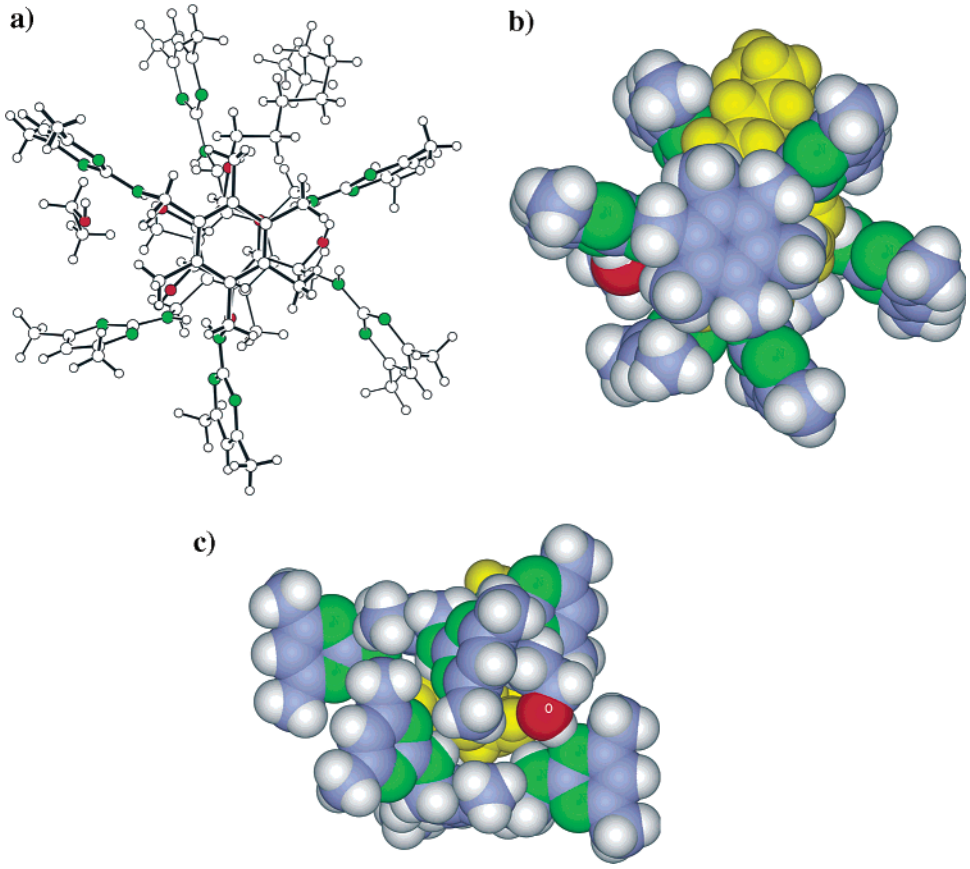


Figure 6. Crystal structure of the 2:1 complex between receptor **3** and octyl β-D-glucopyranoside (**4b**) (the sugar molecule is encapsulated in the cavity between the two receptors, one ethanol molecule is involved in the complex formation). Top (a,b) and side (c) views (in the space-filling representation the sugar molecule is highlighted yellow).

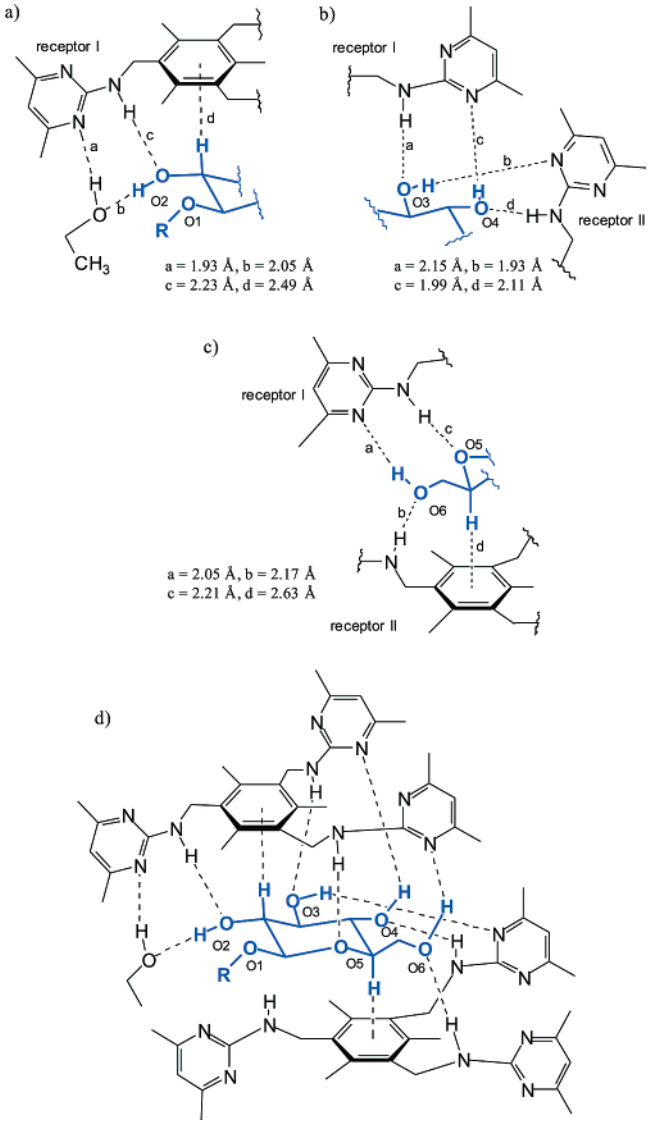


Figure 7. Schematic representation of the binding motifs in the 2:1 complex between receptor **3** and octyl β-D-glucopyranoside (**4b**).

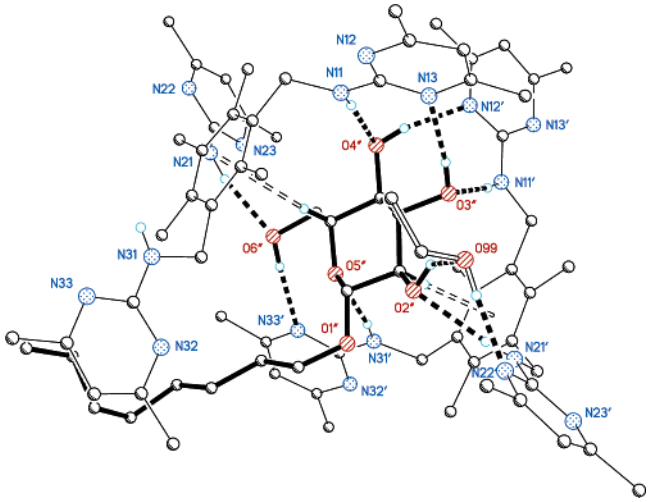


Figure 8. Hydrogen bonding and CH···π interactions in the 2:1 complex between receptor **3** and octyl β-D-glucopyranoside (**4b**).

octyl β-D-galactopyranoside (**6**), and methyl β- and α-glucopyranosides (**4a** and **5a**) were selected as substrates for the receptor.

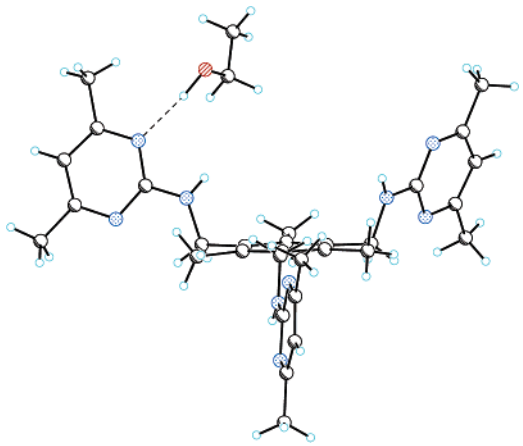


Figure 9. Crystal structure of **3**, top and side views (hydrogen-bonded ethanol molecule is shown; OH···N distance, 1.88 Å; O···N distance, 2.89 Å; OH···N angle, 172°).

The complexation between the receptor **3** and pyranosides **4**−**6** was evidenced by several changes in the NMR spectra. The ^{1}H NMR titration experiments were carried out by adding increasing amounts of the corresponding sugar to a solution of the receptor **3**.[5] In addition, inverse titrations were performed in which the concentration of pyranoside was held constant and that of receptor **3** was varied. During the titration of **3** with β-glucopyranoside **4b** the signal due to the amine NH moved downfield by about 1.2 ppm (see Figure 11a). The addition of 2 equiv of sugar **4b** led to practically complete complexation of **3**. The typical titration curve is shown in Figure 11b. Furthermore, the ^{1}H NMR spectra showed significant changes in the chemical shift of the CH_2 resonances, as illustrated in Figure 11a. The improvement of spectral resolution in the **3**·**4b** complex as compared with that of the unbound host **3** is consistent with anchoring of the receptor conformation and a formation of relatively rigid complex architecture.

The ^{1}H NMR spectra obtained through inverse titrations of sugar **4b** with receptor **3** showed large downfield shifting of the sugar hydroxyl resonances; however, the strong broadening of these resonances prevented their use in the estimation of the binding constants. For this reason, the motion of the signal due to anomeric CH proton of **4b**, which is shifted upfield by 0.13 ppm, was monitored (see Supporting Information). The best fit of the titration data for receptor **3** and sugar **4b** was obtained with the "mixed" 1:1 and 2:1 receptor−sugar binding models.[6,7] Both the curve fitting of the titration data and the mole ratio plots[8,9] suggested the existence of 1:1 and 2:1 receptor−sugar complexes in the chloroform solution, in line with the observation in the crystal structure (see above). The binding constants for sugar **4b** and receptor **3** were found to be 125 800 M^{-1} (K_{a1}) and 6980 (K_{a2}).[10] These results clearly show that the interactions

(4) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−451.
(5) Dilution experiments show that receptor **3** does not self-aggregate in the used concentration range.
(6) The titration data were analyzed by nonlinear regression analysis using the Hostest 5.6 program (see ref 7). The stoichiometry of receptor−sugar complexes was established by the curve-fitting analysis of the titration data and by mole ratio plots (see refs 8 and 9).
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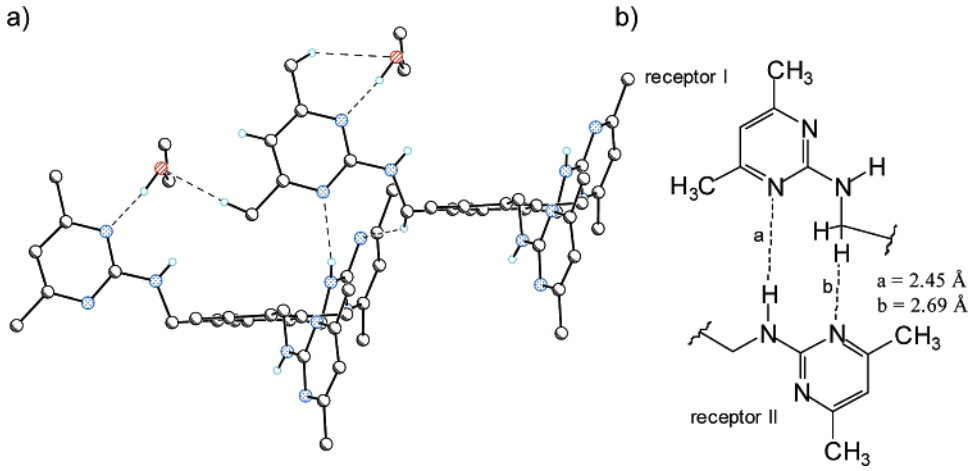

Figure 10. Packing of **3** in the crystal (hydrogen-bonded ethanol molecules are shown; non-hydrogen-bonded C*H* hydrogens are omitted for clarity).

Table 2. Association Constants K_a [a] (M^{-1}) and Corresponding Free Energy Changes $\Delta G°$ (kJ mol^{-1}) for Receptors **3** and **1** and Glucopyranosides **4b**, **5b**, and **6**

host–guest complex	K_{a1} ($\Delta G°$)	K_{a2} ($\Delta G°$)	$\Delta\delta_{max}$ [d] ($\Delta\delta_{obs}$)[e] [ppm]
3·4b	125800 (−29.1)	6980 (−21.9)[b]	NH[f]: 1.20 (1.19); CH[g]: −0.13 (−0.12)
3·5b	5640 (−21.4)	400 (−14.8)[c]	NH[f]: 1.50 (1.24)
3·6	13060 (−23.5)	640 (−16.0)[c]	NH[f]: 1.12(0.96)
1·4b[h]	20950 (−24.7)	790 (−16.5)[c]	NH[f]: 1.28 (1.23)
1·5b[h]	800 (−16.6)		NH[f] 1.45 (1.14)
1·6[h]	1360 (−17.9)	211 (−13.3)[c]	NH[f]: 1.20 (0.97)

[a] In $CDCl_3$ (stored over activated molecular sieves and deacidified with Al_2O_3). For each system at least three titrations were carried out. Uncertainty in a single K_a estimation was ±5−10%. [b] 2:1 Receptor/pyranoside complex. [c] 1:2 Receptor/pyranoside complex. [d] Change in chemical shift at saturation binding, values provided by HOSTEST.[7] [e] Largest change in chemical shift observed during the titration. [f] Complexation-induced shifts observed for the amine-NH (CH_2*NH*) of receptor (the concentration of receptor was kept constant and that of sugar was varied). [g] Upfield complexation-induced shifts observed for the anomeric CH of sugar (the concentration of sugar was kept constant and the host concentration was varied). [h] Results from ref 1b.

with the second ring nitrogen of **3** play a significant role and influence both the binding model and the receptor affinity (for comparison of the binding constants for **3·4b** and **1·4b**, see Table 2).

The ^{1}H NMR titrations of **3** with α-glucopyranoside **5b** or β-galactopyranoside **6** produced also several spectral changes. Particularly, the signal due to the amine NH of **3** moved substantially downfield ($\Delta\delta_{max}$ = 1.50 and 1.12 ppm for the titration with **5b** and **6**, respectively). In addition, notable changes of the chemical shift of the methylene protons were also established (see Figure 11c,e). However, whereas after the addition of 2 equiv of β-glucopyranoside **4b** almost no change was observed in the chemical shift of receptor signals (Figure 11b), with α-glucopyranoside **5b** or β-galactopyranoside **6** (Figure 11d,f, respectively) chemical shift changes continued to higher sugar−receptor ratios, indicating lower affinity of **3** toward **5b** and **6**. The fit of NMR shift changes of the NH of **3** during the titration with **5b** and **6** agreed with the mixed 1:1 and 1:2 receptor−sugar binding model. The association constants of 5640 (K_{a1}) and 400 M^{-1} (K_{a2}) were determined for **3·5b** (Table 2), whereas the binding constants for **3·6** amount to 13 060 (K_{a1}) and 640 M^{-1} (K_{a2}).[10] Thus, among the examined monosaccharides, β-glucopyranoside **4b** exhibited the highest affinity toward **3** (similar to the previously described receptor **1**[1b]).

Additional evidence for the preferred complexation of β-glucopyranoside was obtained from extraction experiments, where β- and α-methyl-glucopyranoside (**4a** and **5a**) were extracted from the solid state into a $CDCl_3$ solution of receptor **3**. The changes in the NMR spectrum of **3** after the extraction of **4a** or **5a** are shown in Figure 12c,b, respectively.

Interestingly, the addition of small quantities of water (0.03−0.07 mol/L) into the chloroform solutions of both **1** and **3** enhanced the binding affinity of these receptors, indicating the formation of water-mediated hydrogen bonds, in line with the observation in protein−carbohydrate complexes,[3] where the hydrogen bonds are both direct and water-mediated (see Figure 2b). For example, β-glucopyranoside **4b** is bound to receptor **1** in water-containing $CDCl_3$ (0.07 mol/L) at least 5 times more efficiently than in pure $CDCl_3$. Both the stoichiometries observed by fitting the binding isotherms (see Supporting Information) and the molar ratio plots verified the mixed 1:1 and 1:2 receptor−sugar binding model (K_{a1} = 103 190, and K_{a2} = 8650 M^{-1}). Receptor **3** was found to have particularly favorable binding properties in water-containing chloroform solutions. For the system **3·4b** the measurements implied receptor−sugar stoichiometries of 1:1 (binding model different from that observed in pure $CDCl_3$; see Table 2). The plot of the observed and calculated downfield chemical shifts of the NH resonances of **3** as a function of added sugar **4b** is illustrated in Figure 13. The graph rises linearly with increasing sugar concentration until $\Delta\delta_{max}$ is reached at the 1:1 receptor−sugar stoichiometry. The linearity of the shift changes shows that in the presence of water the association constant is very large and could not be precisely determined from the NMR titrations data.[12] The binding constant was estimated to be $K_a > 10^6$ M^{-1}. A clear break in the molar ratio plot[9] (Supporting Information) indicates likewise the formation of a complex with high association constant. In the case of α-glucopyranoside **5b** and β-galactopyranoside **6**, the addition of water also raised the binding affinity of receptor **3**

(10) K_{a1} corresponds to the 1:1 association constant. K_{a2} corresponds to the 2:1 or 1:2 receptor/sugar association constant (for details see Table 2).

(11) The best fit was obtained with the "mixed" 1:1 and 2:1 receptor/sugar binding models (Figure 11b) or the "mixed" 1:1 and 1:2 receptor/sugar binding models (Figure 11d,f) incorporated in the Hostest 5.6 program (see ref 7).

(12) For a review discussing the limitations of the NMR method, see: Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.

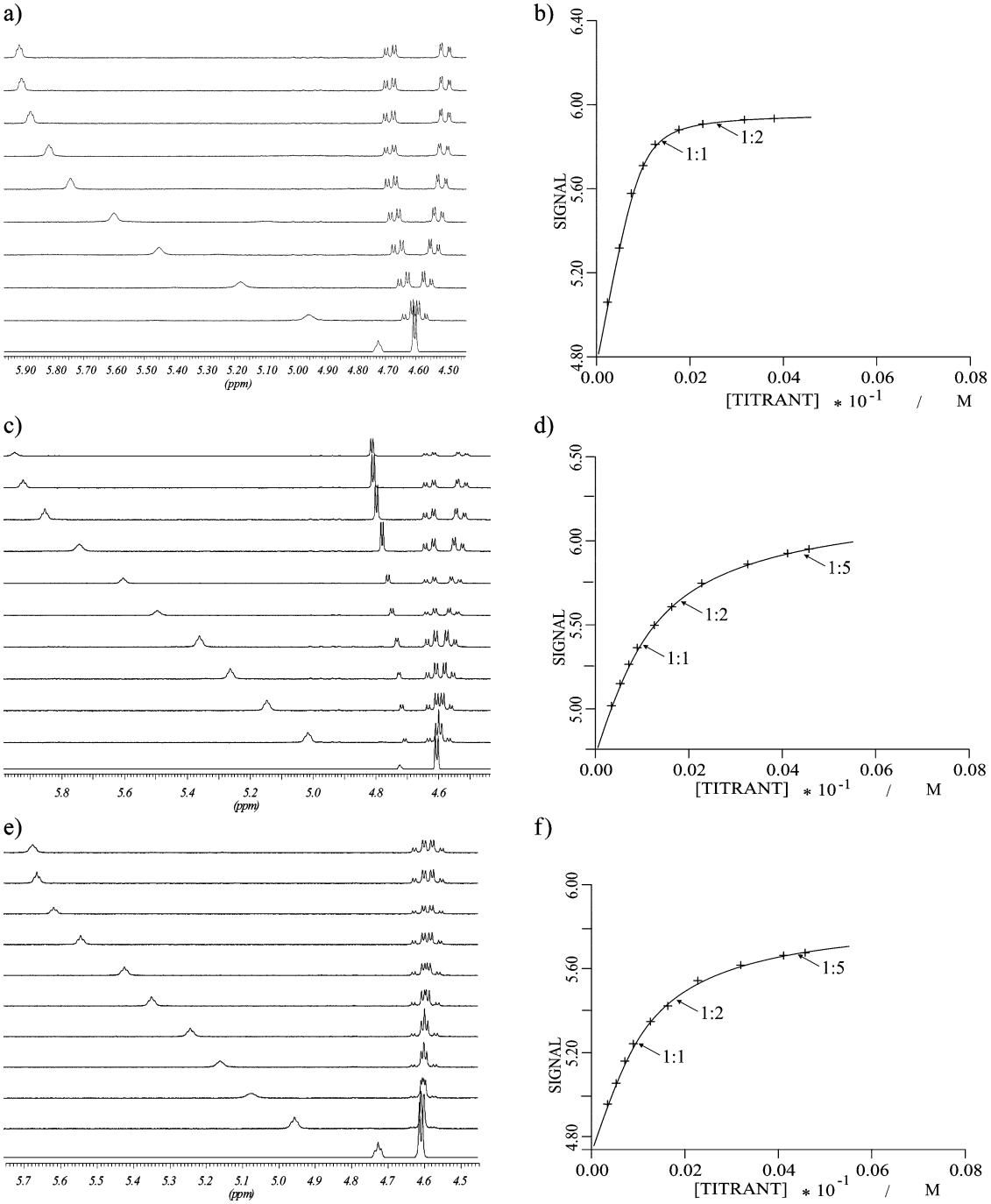

Figure 11. Titration of receptor **3** with β-glucopyranoside **4b**, α-glucopyranoside **5b**, and β-galactopyranoside **6** ([**3**] = 1.22 or 0.90 mM). ^{1}H NMR spectra ($CDCl_3$, 25 °C) of **3** (NH and CH_2 resonances are shown) after addition of (from bottom to top) 0, 0.19, 0.39, 0.59, 0.78, 0.98, 1.77, 2.46, and 2.96 equiv of **4b** (a); 0, 0.42, 0.63, 0.84, 1.05, 1.47, 1.89, 2.63, 3.68, 4.74, and 5.26 equiv of **5b** (c); and 0, 0.40, 0.60, 0.81, 1.01, 1.42, 1.82, 2.53, 3.55, 4.56, and 5.07 equiv of **6** (e). Plot of the observed (+) and calculated (−) downfield chemical shifts of the δ_{N-H} resonances of **3** as a function of added **4b** (b), **5b** (d), and **6** (f).[11] The [receptor]:[pyranoside] ratio is marked.

(by a factor of 2−3; mixed 1:1 and 1:2 receptor−sugar binding model).

An enhancement of the receptor affinity on addition of small quantities of water into the chloroform solutions has also been observed for porphyrine-based receptors.[13] Bonar-Law and Sanders pointed out that the addition of water increases pyranoside binding "by filling in the gaps between the receptor and ligand".

The formation of water-mediated hydrogen bonds in water-containing chloroform solutions and ethanol-mediated hydrogen bonds in the crystals of complexes **1**·**4a** and **3**·**4b** (see Figures 4 and 7) reflects the tendency of our synthetic systems to adopt the strategy widely used by Nature (plugging the gap between receptor and substrate with solvents involving hydroxy groups).

Conclusion

The complexes of receptors **1** and **3** with glucopyranosides **4a** and **4b** have proved to contain many of the molecular features associated with protein−carbohydrate interactions. All OH

(13) (a) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259−271. (b) Mizutami, T.; Kurahashi, T.; Murakami, T.; Matsumi, N.; Ogoshi, H. *J. Am. Chem. Soc.* **1997**, *119*, 8991−9001.

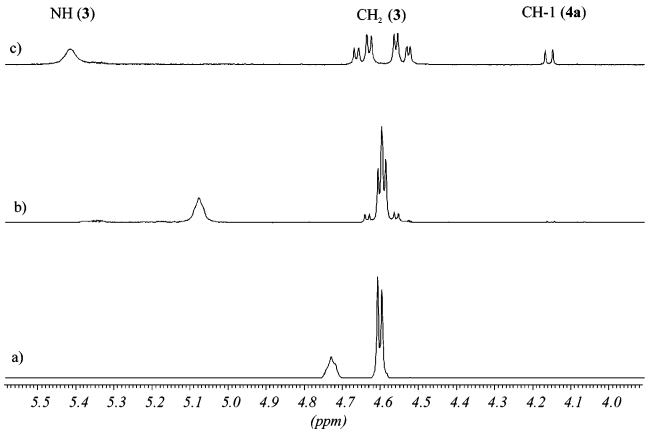

Figure 12. ^{1}H NMR spectra showing the NH and the CH_2 protons of receptor **3** before (a) and after (b) the extraction of solid methyl-α-glucopyranoside **5a** or methyl-β-glucopyranoside **4a** (c) by a $CDCl_3$-solution of receptor **3** (1.00 mM).

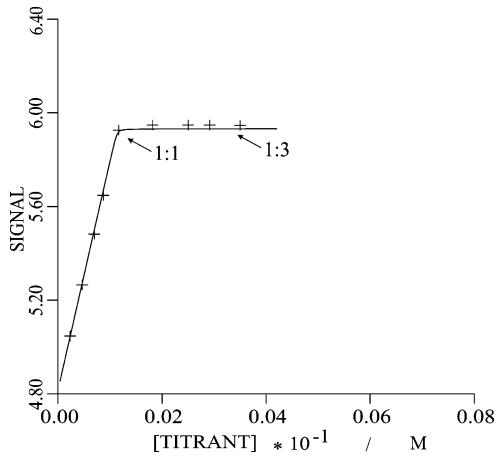

Figure 13. Titration of receptor **3** with β-glucopyranoside **4b** ($CDCl_3$ + H_2O, 0.03 mol/L H_2O; [**3**] = 1.14 mM; 25 °C). Plot of the observed (+) and calculated (−) downfield chemical shifts of the δ_{N-H} resonances of **3** as a function of added **4b**. The [receptor]:[pyranoside] ratio is marked.

groups and the ring oxygen atom of the bound sugar **4a** or **4b** are involved in the formation of hydrogen bonds, including cooperative and bidentate hydrogen bonds. Eight hydrogen bonds contribute to the stabilization of the complex **1·4a**, whereas 10 hydrogen bonds stabilize the complex **3·4b**. Most of the hydrogen bonds exhibit nearly optimal geometries. The typical hydrogen-bonding scheme involving sugar OHs is NH → OH → N, where NH is the amine group and N is the pyridine or pyrimidine nitrogen atom of the receptor **1** or **3**. The CHs of the sugar molecule participate in the formation of the CH···π interactions[14] with the central phenyl ring of the receptor molecule. One (as in **1·4a**) or two phenyl rings (as in **3·4b**) stack on the sugar ring. Thus, the binding motifs found in the complexes **1·4a** and **3·4b** show remarkable similarity to the motifs observed in the natural complexes. Indeed, in the complexes of sugar-binding proteins, all the polar groups (OHs and ring oxygen) of the bound monopyranosides are involved in the formation of hydrogen bonds. For example, the arabinose-binding protein (ABP)−arabinose and the galactose-binding protein (GBP)−glucose complex are stabilized through 10 and 12 hydrogen bonds,[3a] respectively. The hydroxyl groups at positions 2, 3, and 4 of L-arabinose bound to ABP and at all positions of D-glucose bound to GBP are engaged in cooperative hydrogen bonds. The most common hydrogen-bonding scheme involving sugar OHs is (NH)n → OH → O═C, where NH is a hydrogen-bond donor group and O═C is a carbonyl or carboxylate acceptor.[3a−c] Furthermore, often one or two aromatic residues stack on the sugar ring (see Figure 2b).[3a]

The crystal structures of the complexes **1·4a** and **3·4b** provide valuable model systems to study the basic molecular features of carbohydrate recognition. The results showed that acyclic receptors containing aminopyridine or aminopyrimidine binding subunits perform effective recognition of carbohydrates through multiple interactions. The stronger binding of **3** in comparison to that of **1** can be attributed to the possible participation of both pyrimidine nitrogen atoms in the complexation process. The results obtained with the receptors of types **1** and **3** serve as a basis for the construction of new effective and selective artificial receptors, including water-soluble receptor molecules. Synthesis of new receptors of this type and complexation studies with numerous sugar molecules in organic and aqueous solutions[15] are in progress.

Experimental Section

^{1}H and ^{13}C NMR spectra were measured using a Bruker DRX 500 MHz spectrometer; chemical shifts are reported in ppm downfield to TMS as internal standard. Analytical TLC was carried out on Kieselgel 60 F_{254} plates employing a methanol−chloroform 1:7 (v/v) or ethyl acetate−toluene 3:1 (v/v) as the mobile phase. Melting points are uncorrected.

1,3,5-Tris[(4,6-dimethyl-pyrimidin-2-yl)aminomethyl]-2,4,6-trimethyl-benzene (3). A mixture of 1,3,5-tris(bromomethyl)-2,4,6-trimethyl-benzene[16] (1.00 g, 2.50 mmol), 2-amino-4,6-dimethylpyrimidine (0.93 g, 7.50 mmol), and K_2CO_3 (1.04 g, 7.50 mmol) in CH_3CN (120 mL) was stirred at room temperature for 72 h. After filtration of the reaction mixture and partial evaporation of CH_3CN, the obtained suspension was filtrated. The crude product was crystallized from ethanol. Yield 30%. mp 123−125 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.29 (s, 18H), 2.42 (s, 9H), 4.60 (d, 6H, J = 4.3 Hz), 4.81 (t, 3H, J = 4.3 Hz), 6.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.0, 23.9, 41.1, 109.8, 133.7, 136.8, 161.9, 167.5; HR-MS calcd for $C_{30}H_{39}N_9$: 525.3328; found: 525.3333. R_f = 0.51 (methanol−chloroform 1:7 v/v)

Binding Studies. ^{1}H NMR titrations were performed at 298 K in $CDCl_3$ stored over activated molecular sieves and deacidified with Al_2O_3 (for each titration 11−20 samples were prepared). The titration data were analyzed by nonlinear regression analysis using the Hostest 5.6 program.[7] For each system at least three titrations were carried out. The used concentration ranges are given in the legend of Figure 11 (titration of receptor **3** with sugars **4b**, **5b**, or **6)** and in the Supporting Information (titration of sugar **4b** with receptor **3**, inverse titration).

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Supporting Information Available: X-ray data for compound **3**, complex **1·4a** and complex **3·4b**. ^{1}H- and ^{13}C NMR spectra of compound **3**. ^{1}H NMR titration of sugar **4b** with receptor **3**. ^{1}H NMR titration of receptor **1** and **3** with sugar **4b** ($CDCl_3$ + H_2O). Representative mole ratio plot (complexation of **3** with **4b** in water-containing $CDCl_3$). This material is available free of charge via the Internet at http://pubs.acs.org.

JA043037I

(14) The observed geometries for C−H···π interactions compare well with those of similar interactions found in the proteins. See: Steiner, T.; Koellner, G. *J. Mol. Biol.* **2001**, *305*, 535−557.

(15) Carbohydrate recognition in aqueous solution remains an important challenge in supramolecular chemistry. See: (a) Klein, E.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2005**, *44*, 298−302 and references therein.

(16) Van der Made, A. W.; van der Made, R. H. *J. Org. Chem.* **1993**, *58*, 1262−1263.