Molecular Recognition of Carbohydrates by Artificial Receptors: Systematic Studies towards Recognition Motifs for Carbohydrates

Monika Mazik* and Willi Sicking[a]

Abstract: The synthesis and binding properties for carbohydrates of several artificial, acyclic receptors containing two or three heterocyclic recognition units covalently attached to a phenyl spacer is described. These host molecules having uncharged hydrogen-bonding sites were used in a systematic study towards the evaluation of recognition motifs for carbohydrates. A novel effective, acyclic hydrogen-bonding receptor possessing naphthyridine–amide moieties as heterocyclic recognition units has been developed.

Keywords: carbohydrates · carbohydrate receptors · hydrogen bonds · molecular recognition · supramolecular chemistry

Introduction

The design of effective and selective receptors for carbohydrates is subject of current intensive research. Although many interesting host molecules have been developed,[1–4] the recognition of carbohydrates by artificial receptors is still one of the challenging goals of supramolecular and biomimetic chemistry.[1] The difficulty in designing selective and effective hosts for these very important biomolecules[5] is inherent in the three-dimensional complexity of sugar structures. Consideration of the crystal structures of sugar–protein complexes can give some insight into how the formation of stable carbohydrate-receptor complexes might be achieved. According to the X-ray analyses of sugar–protein complexes the hydrogen bonds between sugar OH groups as well as ring oxygens and polar residues of the protein are the main factors determining the specificity and affinity of protein–carbohydrate interactions.[6] The sugar OH groups have the tendency to participate in cooperative hydrogen bonds, which result from the simultaneous participation of sugar hydroxyls as donor and acceptor of hydrogen bonds. Furthermore, stacking of sugar CH moieties with aromatic residues of amino acids influences the stability of protein–carbohydrate complexes. Moreover, numerous van der Waals contacts involving all the atoms of the bound sugar contribute to the stabilisation of complexes between protein and carbohydrates. The importance of hydrogen bonding in protein–sugar complexes has inspired the design of artificial hydrogen bonding carbohydrate receptors.[1, 2] The host molecules described so far are characterized mostly by a macrocyclic structure and are accessible only by multistep synthesis. Due to their macrocyclic structure, the binding sites for hydrogen bonds in these receptors are rarely arranged in a three-dimensional mode.[7] To our knowledge, very little has been done to study systematically the preconditions for effective hydrogen bonding recognition motifs for carbohydrates. Well-chosen systematic changes in the receptor structure may provide invaluable insights into molecular recognition phenomena. We believe that a strategy of systematic search for recognition motifs can lead to an easier development of new carbohydrate receptors with favorable binding properties.

Our interest in this area concentrates on receptors which possess a simple, acyclic structure and which are expected to complex carbohydrates through hydrogen bonding in combination with stacking interactions. Furthermore, the employed arrangement of hydrogen-bonding sites should allow the three-dimensional recognition of sugar molecules. The simple acyclic structure offers the possibility for an easy variation of the receptor structure in order to modulate the binding properties of the host molecules. Recently, we have found that receptors **1** and **2**, which incorporate three pyridine-amide or pyrimidine-amide moieties linked by a phenyl spacer, are effective host molecules for recognition of pyranosides in chloroform solution.[8] The binding properties of **1** and **2** have demonstrated the adjustability of the pyridine-amide and pyrimidine-amide subunits as hydrogen bonding motifs for carbohydrates.

Herein we report on a systematic study towards the evaluation of recognition motifs for carbohydrates. In order to explore which hydrogen-bonding motifs are essential for recognition of monosaccharides structures **1** and **2** were modified. To evaluate the recognition capabilities of the new receptors for glucopyranosides in aprotic solvents such as chloroform and compare their binding properties with properties of **1** and **2**, octyl-β-D-glucopyranoside (**3**) was

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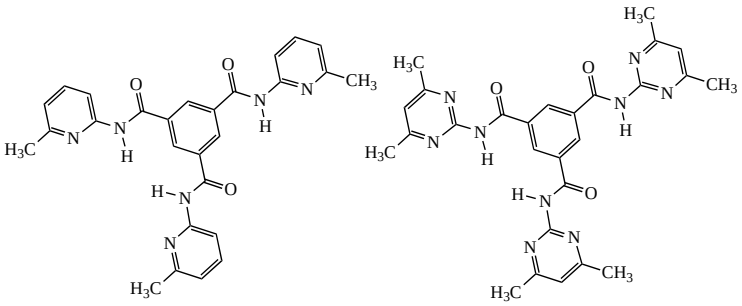
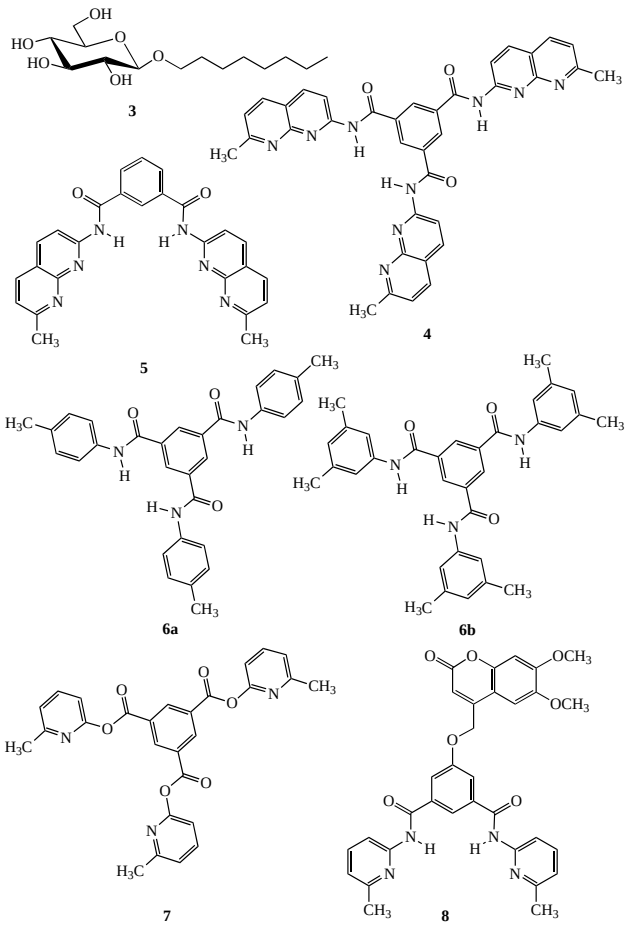


1 2

selected as a probe. In the new host molecules the phenyl spacer was kept constant and the three hydrogen bonding recognition units were varied (compounds **4**, **6**, and **7**).



3 4 5 6a 6b 7 8

Abstract in German: *Es werden die Synthesen von künstlichen, acyclischen Rezeptoren **4**–**8** und ihre Bindungseigenschaften gegenüber Kohlenhydraten beschrieben. Diese Rezeptorverbindungen enthalten zwei oder drei heterocyclische Erkennungseinheiten, die kovalent über einen Phenyl-Spacer verbunden sind. Diese Wirtmoleküle, die nicht ionische Wasserstoffbrückenbindungs-Gruppen besitzen, wurden für systematische Studien zur Bestimmung von Erkennungsmotiven für Kohlenhydrate verwendet. Der neue, effektive, über Wasserstoffbrücken bindende Rezeptor **4**, der Naphthyridin-Amid-Einheiten als heterocyclische Erkennungsstrukturen enthält, wurde entwickelt.*

Additionally, comparative complexation studies with hosts based on 1,3-benzenedicarbonyl unit (compounds **5** and **8**) were carried out. Remarkably strong uncharged hydrogen-bonding interactions were observed between glucopyranoside and the new acyclic receptor **4**.

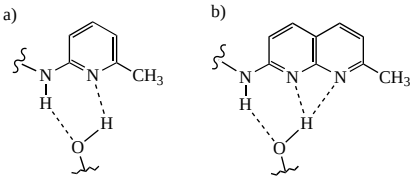
Results and Discussion

Receptors **4, 6b**, and **7** were prepared from benzene-1,3,5-tricarbonyl chloride (trimesic chloride) and with either 2-amino-7-methyl-1,8-naphthyridine,[9] or 3,5-dimethyl-aniline, or 2-hydroxy-6-methyl-pyridine. Although 2-hydroxy-6-methylpyridine exists predominantly in the keto form, the compound **7** could be obtained in high yield (see Experimental Section) under the given reaction conditions. Host **5** was prepared by reaction of isophthaloyl chloride with 2-amino-7-methyl-1,8-naphthyridine.[10] The synthesis of host **8** involved the reaction of **9** with 4-bromomethyl-6,7-dimethoxy-cumarine.

The interactions of all hosts and glucopyranoside were investigated by ^{1}H NMR spectroscopy. The binding constants were determined in chloroform at 25 °C by titration experiments and the titration data were analyzed by nonlinear regression analysis using the Hostest 5.6 program.[11] For all binding experiments the ratio method[12] indicates a 1:1 stoichiometry. The titration data were therefore fitted to a 1:1 binding model.

The analysis of the protein–carbohydrate interactions reveals that good binding constants for the receptor–sugar complexes require the involvement of recognition units with multiple, adjacent hydrogen bonding sites, that is such which participate in cooperative and bidentate hydrogen bonds with sugar hydroxyls. Such multiple adjacent hydrogen bonding sites can be realised by employing a limited range of nitrogen based donor/acceptor moieties, such as naphthyridine-amide units.[13] With the aim of increasing the affinity of the carbohydrate receptors of type **1**–**2** the polynaphthyridine host **4** was therefore synthesized and its complexation behavior was examined. Replacement of the pyridine or pyrimidine subunits in the previously studied hosts by naphthyridine groups resulted in a substantial enhancement of guest-binding properties. The formation of a complex between **4**, which is poorly soluble in chloroform, and **3** became evident when powdered **3** was added to a suspension of receptor **4** in chloroform, leading to facile dissolution to give a clear solution. The complexation of glucopyranoside **3** through receptor **4** was also evidenced by the significant downfield shifts of the receptor amide protons ($\Delta\delta = 0.83$) in the NMR spectrum of the complex, reflecting the formation of a hydrogen-bonded complex. The association constant for the complex of **4** and **3** was determined to $K_a = 26500\ \mathrm{M}^{-1}$ ($-\Delta G^0 = 25.2\ \mathrm{kJ\,mol}^{-1}$). Thus the three naphthyridine-amide moieties in **4** led to a three-fold higher binding constant with

glucopyranoside compared with **1**; this suggests that all naphthyridine nitrogens are probably involved in hydrogen-bonding complexation and demonstrate the particular suitability of the amido-naphthyridine arrangement as a hydrogen bonding recognition motif for monosaccharides. The additional stability in the glucopyranoside complex with **4** compared with that with **1** may be due to long range electrostatic interactions with hydroxyl hydrogens of glucopyranoside (secondary hydrogen bonds, Scheme 1).



Scheme 1. a) Cooperative hydrogen-bond pattern in carbohydrate complexes with **1**. b) Cooperative and secondary hydrogen-bond pattern in carbohydrate complexes with **4**.

Such secondary hydrogen bonds were also found in other supramolecular, multiple hydrogen bonded systems described for example in ref.[10, 14, 15] The configuration where the hydrogen atom is located between three electronegative atoms, being covalently bound to one and hydrogen bonded to the other two, is also referred to as three-center hydrogen bonds. Examples of these have been well established by the neutron diffraction studies of the amino acids and the pyranose sugars.[16] Molecular modelling[17] indicated the suitable multipoint hydrogen-bonding arrangement between the hydroxyl groups of **3** and the naphthyridine units of **4** (Figure 1).

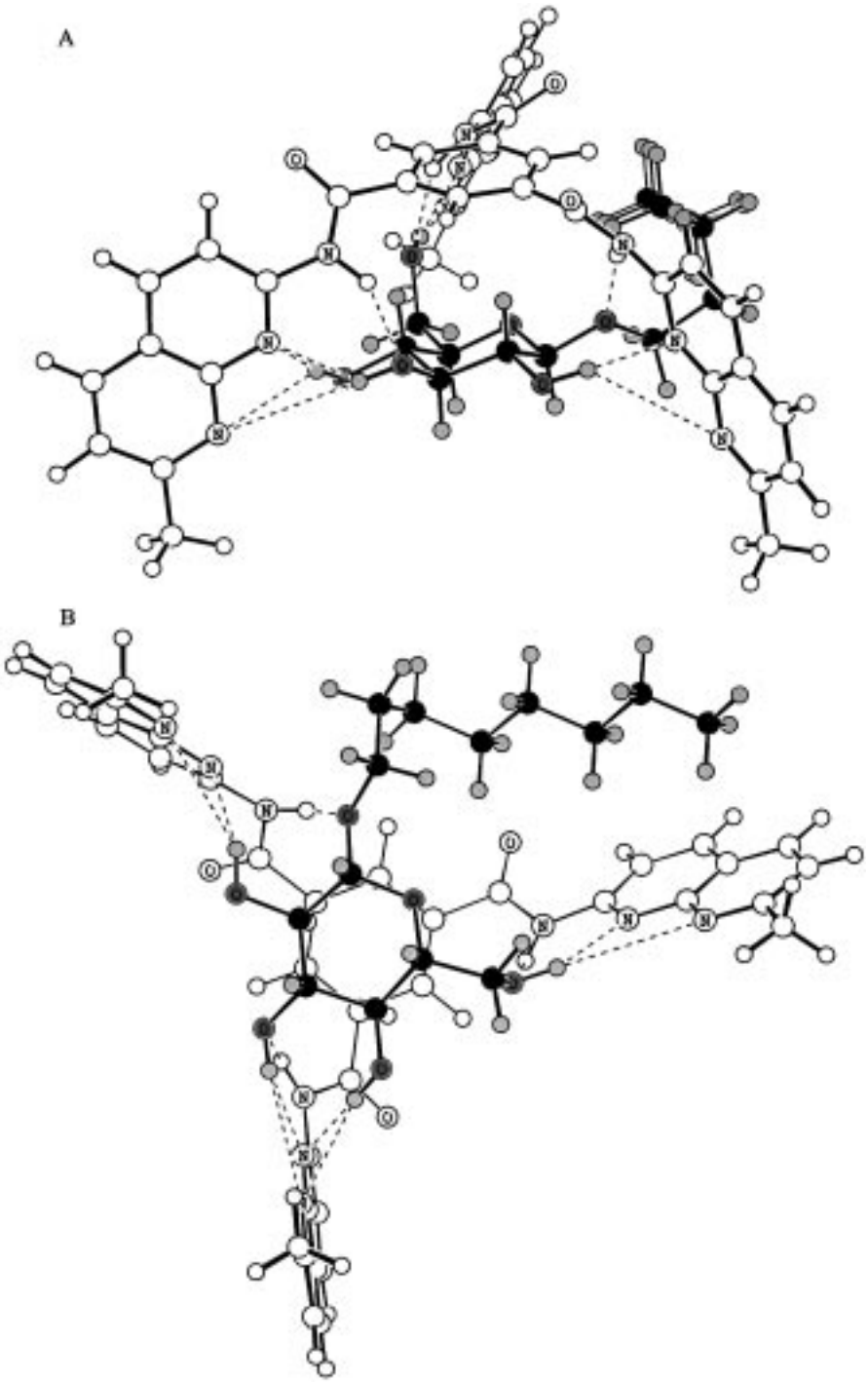


Figure 1. The energy-minimized structure of the complex formed between **4** and **3** (Monte Carlo conformational searches, 50000 steps, MacroModel V. 6.5, Amber* force field). The intermolecular amide-NH···O and naph-N···HO hydrogen bonds and stacking interactions in the complex are shown. A: side view, B: top view.

All glucopyranoside hydroxy groups and the alkoxy oxygen are involved in the formation of hydrogen bonds. A complex between **3** and **4** can potentially be stabilized by at least seven intermolecular hydrogen bonds (four O-H···N-naph and three amide-NH···O) including two cooperative hydrogen bonds. Additionally, at least three secondary hydrogen bonds may be present in the complex because the OH group can serve as a long-range donor to the second naphthyridine nitrogen. Furthermore, molecular modelling studies indicated the formation of stacking interactions between the pyranoside ring and the central phenyl ring of **4** and supported the three-dimensional recognition of the glucopyranoside.

The significance of a three-dimensional recognition was also reflected by a comparison of the binding properties of host **4** with those of host **5**. The receptor **5** revealed a K_a value of $4530\,\text{M}^{-1}$ ($-\Delta G^0 = 20.9\,\text{kJ mol}^{-1}$) for glucopyranoside **3**, that is the hydrogen-bonding interactions with host **5** having a multiple but essentially two-dimensional binding site are less

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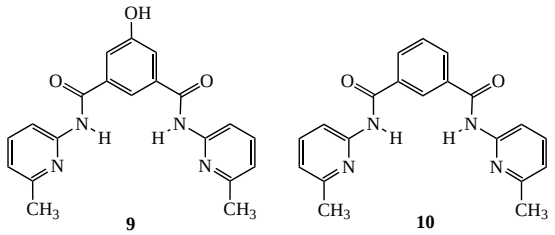
favorable than with host **4.** A possible structure for the complex of **5** and **3** revealed molecular modelling, which suggested the formation of four primary hydrogen bonds (two amide-NH···O and two naphthyridine-N···HO hydrogen bonds), at least two secondary hydrogen bonds and stacking interactions between the pyranoside ring and the phenyl ring of **5** (Figure 2). Molecular modelling studies indicated that two OH groups of **3** do not participate in hydrogen bonding with **5**, thus confirming the weaker binding with **5** compared with that with **4**.

The determined binding constant for complex of **3** and **5** was about four-fold larger than the previously estimated binding constant for complex of **3** and bipyridyl receptor **10**.[8] This fact showed once again that the hydrogen-bonding interactions with naphthyridine-amide units are more favorable than with pyridine-amide moieties and revealed the suitability of the naphthyridine-amide unit as a recognition motif for carbohydrates.

The favorable binding properties of the receptors possessing naphthyridine-amide subunits displayed the significance



of the nitrogen based donors/acceptors in the receptor structure. The importance of the presence of nitrogen based acceptors, such as pyridine nitrogen atoms, has been already determined in our recent study on host **6a** where the pyridine-amide units were replaced by phenyl-amide groups. Host **6a** could not be solubilized in chloroform even at high concentrations of octyl glucopyranoside, thus it was not possible to estimate the binding constant between **6a** and glucopyranosides in this solvent. In order to determine the binding constant for a receptor containing only phenyl-amide subunits the better soluble dimethyl-phenyl derivative **6b** was synthesized. In contrast to **1**, the amide proton of **6b** showed minimal displacement on complexation with **3** ($\Delta\delta_{max} = 0.07$ compared with 0.70 for **1**), confirming the weak binding. The K_a value of **6b** amounted to $750\,M^{-1}$ ($-\Delta G^0 = 16.4\,kJ\,mol^{-1}$); thus the binding constant was more than 11-fold lower as that for the complex between **1** and **3**. Molecular modelling studies also supported the observed weak complexation between **6b** and **3**; this indicates that the three arms of receptor **6b** do not act together in complexing of glucopyranoside. The comparison of the binding constant for host **6b** with that for **1** revealed the significant contribution of pyridine nitrogens to the stabilisation of the receptor–sugar complexes.

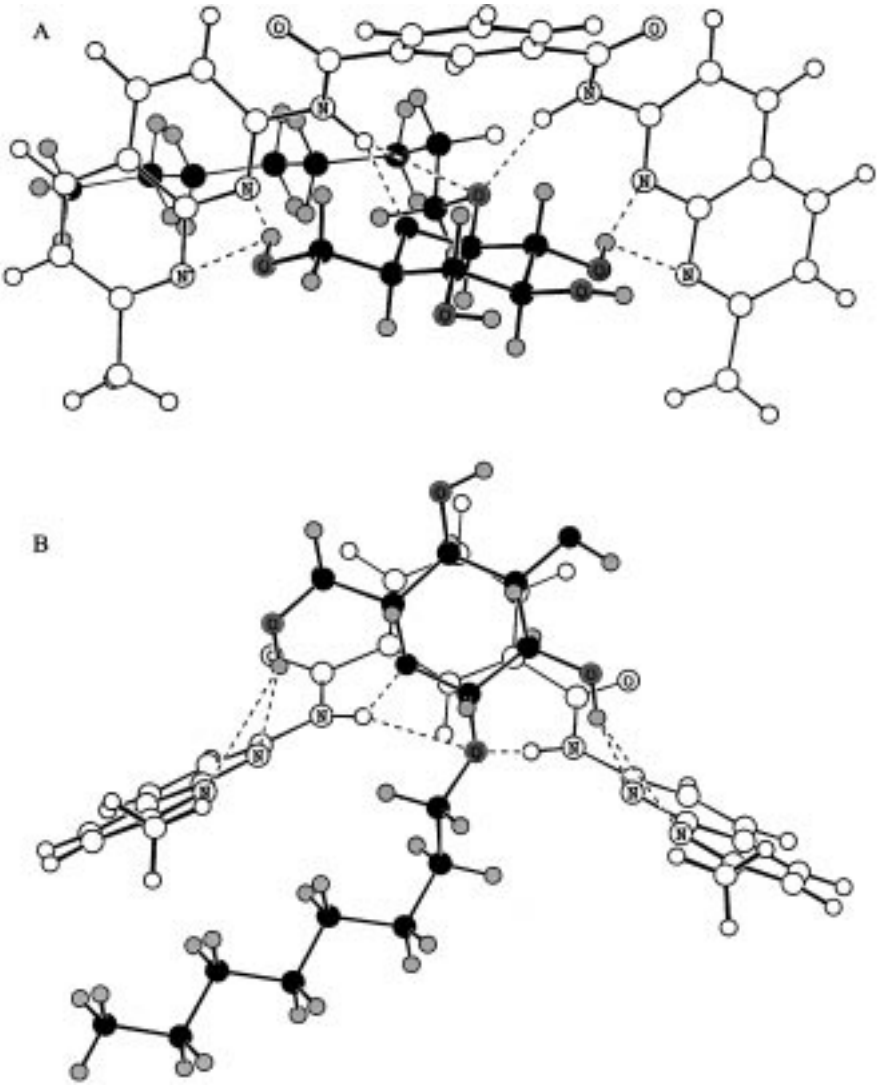


Figure 2. The energy-minimized structure of the complex formed between **5** and **3** (Monte Carlo conformational searches, 50000 steps, MacroModel V. 6.5, Amber* force field). The intermolecular amide-NH···O and naph-N···HO hydrogen bonds and stacking interactions in the complex. A: side view, B: top view.

To quantify the influence of the amide NH protons on the formation of stable complexes, the binding properties of host **1** were compared with those of host **7** containing instead of amide groups the ester groups. Such a receptor can only act as an acceptor for hydrogen bonding thereby prohibiting a cooperative hydrogen bond sequence. On titration of host **7** with **3** no significant shifts for aromatic receptor protons were observed, thus indicating weaker binding than between **1** and **3**. For this reason, the complexation was further investi-

gated by titration of **3** with host **7**. The ^{1}H NMR spectra obtained during binding experiments showed downfield shifting of the guest hydroxyl resonances, which indicated hydrogen bond formation; however, the broadening of these resonances complicated their use in the estimation of the binding constant. The C*H*OH protons of the guest shifted upfield and remained sharp, but the shifts were small. The binding constant was determined to $800\,\text{M}^{-1}$ ($-\Delta G^0 = 16.6\,\text{kJ mol}^{-1}$). Molecular modelling suggested that the glucopyranoside tends to associate with only two arms of the receptor forming hydrogen bonding with pyridine nitrogens. The comparison of the binding properties of hosts **1**, **6b**, and **7** implied the importance of cooperative hydrogen bonding in receptor–carbohydrate recognition. The determined binding constants indicated, that both the pyridine nitrogen atoms and the amide NH protons contribute significantly to the stabilisation of carbohydrate–receptor complexes.

The results of the complexation experiments of glucopyranoside with the previously studied hosts **1**–**2** and with the new hosts **4**–**7** show clearly that hydrogen bonding between amide-NH/pyridine-N, amide-NH/pyrimidine-N, or amide-NH/naphthyridine-N of receptors and OH groups of glucopyranoside is primarily responsible for the complexation in chloroform.

The importance of additional van der Waals interactions in determining the energy of complex formation was reflected by the comparison of the binding properties of host **8** with those of the previously studied host **10**. The molecular modelling studies indicated the participation of two sugar hydroxyls and alkoxy oxygen in hydrogen bonding with two pyridine-amide units of **8** (similar as in complex with **10**) and the formation of additional van der Waals interactions between **3** and the cumarine unit of **8**, giving rise to the placing of the sugar molecule in the cavity of the receptor (Figure 3). According to molecular modelling the cumarine oxygens are not suitably positioned to bridge the hydroxyl protons of **3** thereby terminating the participation of OH···O hydrogen bonds in complex stabilisation. The binding constant of **3** and **8** was found to be $4270\,\text{M}^{-1}$ ($-\Delta G^0 = 20.7\,\text{kJ mol}^{-1}$), that is almost four-fold better than that of host **10**. Thus, similar as in protein–sugar complexes, the van der Waals interactions favor the binding between artificial receptor and carbohydrate.

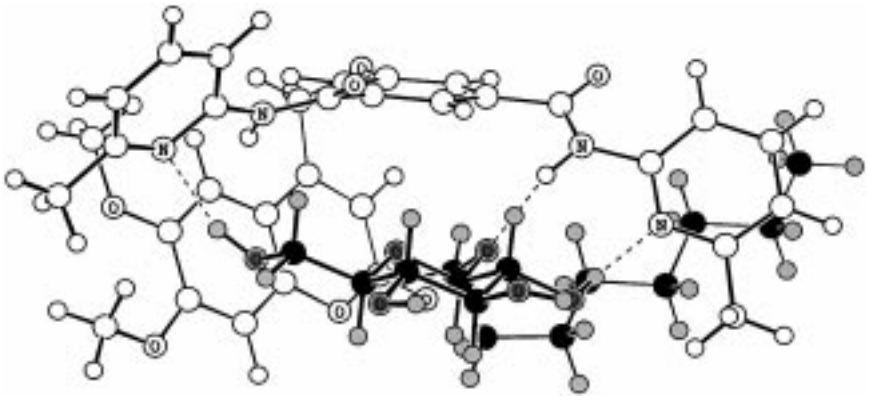


Figure 3. The energy-minimized structure of the complex formed between receptor **8** and **3** (Monte Carlo conformational searches, 50000 steps, MacroModel V. 6.5, Amber* force field).

The affinities of octyl-β-D-glucopyranoside (**3**) to the receptors explored in this study decreased in the following order **4** > **2** > **1** > **5** > **8** > **10** > **7** ≥ **6b**. Table 1 lists all binding constants and corresponding free energy changes for the various receptors and glucopyranoside **3**.

Conclusion

We described herein a study towards the evaluation of efficient recognition motifs for carbohydrates. The receptor structures investigated were found to have the sufficient simplicity for a systematic study. The binding properties of the receptors described herein demonstrated the adaptability of the pyridine-amide, pyrimidine-amide, and particularly naphthyridine-amide recognition units as a hydrogen-bonding motif for glucopyranosides. This study showed, that in the case of uncharged hydrogen-bonding interactions only recognition units containing both donors and acceptors for hydrogen bonding are effective for the recognition of monosaccharides. Thus, an important design criterion is that the hosts contain donor/acceptor groups which are able to participate in the cooperative and bidentate hydrogen bonds with the carbohydrate OH groups, similar as in the protein–carbohydrate complexes. The highly variable stability of the hydrogen-bonded complexes examined in this work do correlate with the number of hydrogen bonds. The three-dimensional arrangement of the binding sites was shown to be of great importance. Furthermore, the presence of moieties which are able to take part in additional secondary hydrogen bonds with sugar hydroxyl groups causes a substantial enhancement of guest binding properties. Moreover, structural elements forming additional van der Waals contacts with the carbohydrate molecule favor the binding process.

The principal advantages of the acyclic receptor systems developed herein are the simple and straightforward synthesis, and therefore an easy variation of the structures, the presence of both hydrogen bonding sites, π bonds for facilitating stacking interactions, and the capability of the three-dimensional recognition of guest molecules. The presented type of studies supplies important information about the quality of the hydrogen bonding units which can be used for recognition and binding of carbohydrates. As such, they provide an important screening platform for candidates to be incorporated into receptor structures. The manipulation of binding sites and structural units leads to a better understanding of energetic and geometric factors which control molecular association and recognition of carbohydrates. The obtained results serve as a basis for the construction of new effective artificial receptors.

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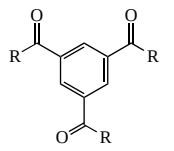
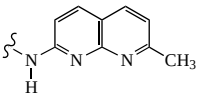
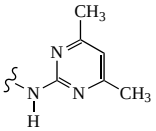
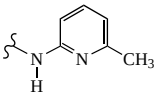
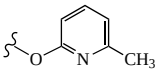
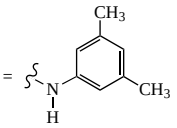
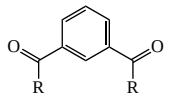
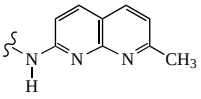
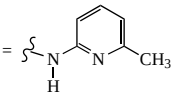
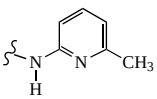
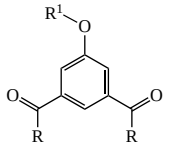
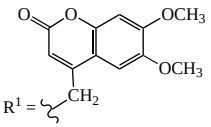
Table 1. Binding constants K_a[a] and corresponding free energy changes ΔG^0 (298 K) for various receptors and glucopyranoside **3**. Also shown are the maximum complexation-induced shifts $\Delta\delta_{max}$ (K_a [M^{-1}], $-\Delta G^0$ [kJ mol^{-1}], $\Delta\delta_{max}$ [ppm]).

R	K_a	$-\Delta G^0$	$\Delta\delta_{max}$
 **4**: R = 	26500	25.2	0.83[b]
2: R = 	13700	23.6	0.68[b]
1: R = 	8700	22.5	0.70[b]
7: R = 	800[d]	16.6	0.03[c]
6b: R = 	750	16.4	0.07[b]
 **5**: R = 	4530	20.9	0.81[b]
10: R = 	1100	17.3	0.25[b]
8: R = 			
 	4270	20.7	0.32[b]

[a] Average K_a values from multiple titrations ($CDCl_3$). The reproducibility of the K_a values was ±10–20%. Uncertainty in a single K_a estimation was ±2–10%. [b] The shifts were observed for the amide NH of receptor, values provided by HOSTEST. [c] The concentration of **3** was kept constant and the host concentration varied, the shifts were observed for the CH of glucopyranoside **3.** [d] Higher uncertainty in K_a due to the small $\Delta\delta_{max}$.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer; chemical shifts are reported in ppm downfield to TMS as internal standard. Mass spectra were measured with a Fisons VG Prospec 3000 and a Hewlett Packard HP 5971A MSD spectrometer. Analytical TLC was carried out on Merck Kieselgel 60 F_{254} plates employing a methanol/chloroform 1:7 *v*/*v* as the mobile phase.

***N,N′,N″*-Tris(7-methyl-1,8-naphthyridin-2-yl)-benzene-1,3,5-tricarbonamide (4)**: A solution of 1,3,5-benzenetricarbonyl trichloride (1.35 g, 5.09 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a solution of 2-amino-7-methyl-naphthyridine[9] (2.47 g, 15.53 mmol) and triethylamine (2.1 mL) in dry CH_2Cl_2 (100 mL). After complete addition, the mixture was stirred at room temperature for 24 h. The reaction mixture was treated with water (100 mL), stirred for 20 min and CH_2Cl_2 was removed under reduced pressure. The resulting precipitate was filtered, washed several times with water, dried at room temperature and suspended in CH_2Cl_2. After filtration and drying of the precipitate, **4** was obtained as a yellow powder (2.41 g, 75%). M.p. 220 °C (decomp.); $R_f = 0.53$; ^{1}H NMR ($CDCl_3$): $\delta = 2.76$ (s, 9H, 3 × CH_3), 7.30–7.31 (3H$_{naph}$), 8.05–8.07 (3H$_{naph}$), 8.20–8.22 (3H$_{naph}$), 8.63–8.64 (3H$_{naph}$), 8.97 (s, 3H$_{Ph}$), 9.60 (s, 3H, 3 × NH); ^{1}H NMR ([D$_6$]DMSO): $\delta = 2.69$ (s, 9H, 3 × CH_3), 7.45–7.46 (3H$_{naph}$), 8.30–8.32 (3H$_{naph}$), 8.48–8.53 (6H$_{naph}$), 8.89 (s, 3H$_{Ph}$), 11.62 (s, 3H, 3 × NH); ^{13}C NMR ([D$_6$]DMSO): $\delta = 25.37$, 114.37, 118.33, 121.65, 131.46, 134.34, 136.88, 139.47, 153.99, 154.46, 162.72, 165.37; FAB-MS (3-nitrobenzylalcohol) *m*/*z* (%): 634 (100) $[M+H]^+$, 656 (88) $[M+Na]^+$; elemental analysis calcd (%) for $C_{36}H_{27}N_9O_3$ (633.6): C 68.25, H 4.26, N 19.90; found C 68.15, H 4.40, N 19.85.

***N,N′*-Bis(7-methyl-1,8-naphthyridin-2-yl)benzene-1,3-dicarbonamide (5)**: A solution of 1,3,5-benzenetricarbonyl trichloride (1.01 g, 4.97 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a solution of 2-amino-7-methyl-naphthyridine (1.60 g, 10.06 mmol) and triethylamine (1.4 mL) in dry CH_2Cl_2 (80 mL). After complete addition, the mixture was stirred at room temperature for 24 h. The reaction mixture was treated with water, stirred for 20 min and CH_2Cl_2 was removed under reduced pressure. The resulting precipitate was filtered, washed several times with water, dried at room temperature. The crude product was recrystallized from CH_2Cl_2 yielding **5** (1.95 g, 88%). M.p. 190 °C (decomp.); $R_f = 0.50$; ^{1}H NMR ($CDCl_3$): $\delta = 2.78$ (s, 6H, 2 × CH_3), 7.31 (d, 2H$_{naph}$, $J = 8.2$ Hz), 7.70 (t, 1H$_{Ph}$, $J = 7.5$ Hz), 8.06 (d, 2H$_{naph}$, $J = 8.2$ Hz), 8.18 (d, 2H$_{Ph}$, $J = 7.5$ Hz), 8.23 (d, 2H$_{naph}$, $J = 8.5$ Hz), 8.64 (br s, 2H$_{naph}$), 8.72 (s, 1H$_{Ph}$), 9.30 (s, 2H, 2 × NH); ^{13}C NMR ($CDCl_3$): $\delta = 25.11$, 114.18, 118.31, 121.41, 126.69, 129.20, 131.37, 134.40, 136.26, 138.55, 153.71, 154.06, 162.90, 165.19; MS (70 eV) *m*/*z* (%): 448 (58) $[M]^+$, 419 (29), 289 (60), 263 (78), 260 (54), 234 (25), 186 (100), 160 (88), 143 (33), 132 (12), 116 (12), 104 (14), 76 (18); HR-MS: calcd for $C_{26}H_{20}N_6O_2$: 448.1648; found: 448.1655; elemental analysis calcd (%) for $C_{26}H_{20}N_6O_2$: C 69.64, H 4.46, N 18.75; found C 69.48, H 4.61, N 18.59.

***N,N′,N″*-Tris(-(3′,5′-dimethylphenyl)benzene-1,3,5-tricarbonamide (6b)**: A solution of 1,3,5-benzenetricarbonyl trichloride (0.99 g, 3.71 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a solution of 3,5-dimethylaniline (1.35 g, 11.16 mmol) and triethylamine (2.5 mL) in dry CH_2Cl_2 (70 mL). After complete addition, the mixture was stirred at room temperature for 24 h. The reaction mixture was treated with water (100 mL), stirred for 20 min and the resulting precipitate was filtered. The crude product was washed several times with water, dried at room temperature and suspended in CH_2Cl_2. After filtration and drying of the precipitate, **6b** was obtained as a white powder (1.60 g, 83%). M.p. 300 °C; $R_f = 0.75$; ^{1}H NMR ($CDCl_3$): $\delta = 2.32$ (s, 18H, 6 × CH_3), 6.82 (s, 3H$_{Ph}$), 7.82 (s, 6H$_{Ph}$), 8.10 (s, 3H, 3 × NH), 8.49 (s, 3H$_{Ph\text{-}spacer}$); ^{1}H NMR ([D$_8$]THF): $\delta = 2.28$ (s, 18H, 6 × CH_3), 6.74 (s, 3H$_{Ph}$), 7.44 (s, 6H$_{Ph}$), 8.58 (s, 3H, H$_{Ph\text{-}spacer}$), 9.61 (s, 3H, 3 × NH); ^{13}C NMR ([D$_8$]THF): $\delta = 21.59$, 118.78, 126.21, 129.76, 137.31, 138.80, 140.16, 165.11; MS (70 eV): *m*/*z* (%): 519 (84) $[M]^+$, 399 (100), 278 (6), 250 (26), 222 (15), 210 (7), 121 (20), 75 (13); HR-MS: calcd for $C_{33}H_{33}N_3O_3$: 519.2522; found: 519.2541; elemental analysis calcd (%) for $C_{33}H_{33}N_3O_3$: C 76.30, H 6.36, N 8.09; found C 76.17, H 6.26, N 8.24.

Benzene-1,3,5-tricarboxylic acid tris(6-methyl-2-pyridinyl) ester (7): A solution of 1,3,5-benzenetricarbonyl trichloride (0.30 g, 1.13 mmol) in dry CH_2Cl_2 (15 mL) was added to a solution 2-hydroxy-6-methylpyridine (0.36 g, 3.40 mmol) and triethylamine (0.5 mL) in CH_2Cl_2. Then, the reaction mixture was heated under reflux for 6 h, stirred at room temperature for 18 h, treated with water (30 mL), stirred for further 20 min and CH_2Cl_2 was removed in vacuum. The precipitate was filtred and washed with diethyl ether. The crude product was obtained as a white powder with a purity of about 95% (0.45 g, 83%). Further purification by preparative TLC chromatography gave pure **7**. M.p. 195–197 °C; $R_f = 0.70$; ^{1}H NMR ($CDCl_3$): $\delta = 2.54$ (s, 3H, CH_3), 7.01 (d, 3H$_{pyr}$, $J = 8$ Hz), 7.13 (d, 3H$_{pyr}$, $J = 7.5$ Hz), 7.72 (t, 3H$_{pyr}$, $J = 8$ Hz), 9.26 (s, 3H$_{Ph}$); ^{13}C NMR ($CDCl_3$): $\delta = 23.99$, 113.14, 121.99, 130.86, 136.56, 139.85, 156.94, 158.42, 162.96; MS (70 eV): *m*/*z* (%): 483 (15) $[M]^+$, 375 (100), 346 (9), 314 (7), 210 (9), 194 (7), 154 (4), 109 (7), 75 (5); HR-MS: calcd for $C_{27}H_{21}N_3O_6$: 483.1430; found: 483.1420; elemental analysis calcd (%) for $C_{27}H_{21}N_3O_6$: C 67.08, H 4.35, N 8.69; found C 67.20, H 4.43, N 8.60.

5-[(6,7-Dimethoxy-2-oxo-2*H*-chromen-4-yl)methyl]oxy-*N*,*N*′-bis(6-methyl-2-pyridinyl)benzene-1,3-dicarbonamide (8): A mixture of **9** (0.10 g, 0.28 mmol), 4-bromomethyl-6,7-dimethoxycumarine (0.082 g, 0.28 mmol), and K_2CO_3 (0.09 g) in acetonitrile (30 mL) was heated under reflux for 4 h. After the reaction mixture had been cooled to the room temperature, the precipitate was filtered. The pure product was isolated from the precipitate by washing with chloroform. After removing the solvent in vacuum compound **9** was obtained as a yellow powder (0.13 g, 81 %). M.p. 200 °C (decomp.); $R_f = 0.72$; ^{1}H NMR ($CDCl_3$): $\delta = 2.47$ (s, 6H, $2 \times CH_3$), 3.93 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 5.31 (s, 2H, OCH_2), 6.54 (s, $1H_{cum}$), 6.88, 6.89 (2s, $2H_{cum}$), 6.94 (d, $2H_{pyr}$, $J = 7.8$ Hz), 7.65 (t, $2H_{pyr}$, $J = 8$ Hz), 7.79 ($2H_{Ph}$), 8.09 (s, $1H_{Ph}$), 8.13 (d, $2H_{pyr}$, $J = 8$ Hz), 8.63 (s, 2H, $2 \times$ NH); ^{13}C NMR ($CDCl_3$): $\delta = 24.01$, 56.39, 56.68, 66.25, 100.47, 104.00, 109.46, 110.96, 117.44, 118.43, 119.87, 136.80, 138.84, 146.51, 148.78, 149.88, 150.34, 153.13, 157.16, 158.61, 160.96, 163.87; MS (70 eV): *m*/*z* (%): 580 (7) $[M]^+$, 434 (100), 362 (21), 333 (13), 255 (20), 220 (26), 177 (8), 135 (26), 92 (17), 57 (14); HR-MS: calcd for $C_{32}H_{28}N_4O_7$: 580.1958; found: 580.1950; elemental analysis calcd (%) for $C_{32}H_{28}N_4O_7$: C 66.21, H 4.83, N 9.66; found C 66.39, H 4.88, N 9.60.

5-Hydroxy-*N*,*N*′-(6-methyl-pyridin-2-yl)-benzene-1,3-dicarbonamide (9)

a) Synthesis of 5-hydroxy-benzene-1,3-dicarbonyl chloride: A mixture of 5-hydroxy-isophthalic acid (3.0 g, 0.016 mol) (protection of OH group was not necessary) and thionyl chloride (5 mL, 0.069 mol) in THF (50 mL) was heated under reflux for 3 h. The solvent was removed in vacuum. Then, THF (50 mL) was added and again the solvent was removed in vacuum. The crude product was used directly for further reaction.

b) Synthesis of **9**: A solution of the above 5-hydroxy-benzene-1,3-dicarbonyl chloride (0.016 mol) in dry THF (20 mL) was added dropwise to a solution of 2-amino-6-methyl-pyridine (5.3 g, 0.05 mol) and triethylamine (4.6 mL) in dry THF (80 mL). After complete addition, the mixture was stirred at room temperature for 24 h. The reaction mixture was treated with water (100 mL), stirred for 20 min and THF was removed under reduced pressure. The resulting precipitate was filtered, washed several times with water, dried at room temperature, and recrystallised from CH_3CN yielding **9** as a white powder (4.3 g, 75 %). M.p. 235 °C; ^{1}H NMR ([D_8]THF): $\delta =$ 2.43 (s, 6H, $2 \times CH_3$), 6.91 – 6.93 (d, $2H_{pyr}$), 7.59 – 7.64 (m, $2H_{Ph}$, $2H_{pyr}$), 8.13 – 8.25 (m, $1H_{Ph}$, $2H_{pyr}$), 9.86 (s, 2H, $2 \times$ NH); ^{13}C NMR ([D_8]THF): $\delta = 24.13$, 111.73, 117.35, 119.25, 119.32, 137.25, 138.86, 152.90, 157.56, 159.19, 165.60; MS (70 eV): *m*/*z* (%): 362 (88) $[M]^+$, 333 (63), 255 (100), 228 (42), 186 (24), 162 (11), 135 (71), 109 (18), 92 (33), 70 (25), 41 (17); HR-MS: calcd for $C_{20}H_{18}N_4O_3$: 362.1379; found: 362.1366.

Binding studies: ^{1}H NMR titrations were performed at 298 K in $CDCl_3$ stored over activated molecular sieves (4 Å). Examples: [**4**] = 0.75 mM ([**3**] = 0.15 – 3.75 mM); [**6b**] = 0.55 mM ([**3**] = 0.06 – 2.45 mM); [**5**], [**7**], or [**8**] = 1.00 – 2.70 mM ([**3**] = 0.20 – 13.70 mM). Dilution experiments show that receptors **4** – **8** do not self-aggregate in the used concentration range. The titration data were analyzed by nonlinear regression analysis using the Hostest 5.6 program.[11]

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