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Molecular Recognition of Carbohydrates with Acyclic Pyridine-Based Receptors

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Received June 17, 2004

The recognition capabilities of acyclic pyridine-based receptors toward monosaccharides were evaluated. Aminopyridine receptors based on the 2,4,6-trimethyl- or 2,4,6-triethylbenzene frame show high β vs α binding selectivity in the recognition of glucopyranosides. Amidopyridine receptors, which are sterically less hindered at nitrogen, display high efficiency and an inverse selectivity. The 2-aminopyridine group has been established as a highly effective recognition group in the binding of monosaccharides. The factors influencing the binding properties of receptors **1**−**15**, which differ in the nature and number of binding and spacer subunits used as the buildings blocks, are discussed.

Introduction

A wide range of biological processes require the interactions of proteins with carbohydrates;[1] thus, the molecular understanding of the features associated with these interactions is of particular importance. The analyses of the crystal structures of protein−carbohydrate complexes give some insight into the molecular recognition phenomena. According to these results, the protein−carbohydrate interactions include hydrogen bonding, van der Waals forces, interactions of sugar CHs with aromatic residues of the protein (often one or two aromatic residues stack on the sugar ring), and metal coordination. The sugar OHs usually participate in cooperative hydrogen bonds, which result from the simultaneous participation of OHs as donor and acceptor of hydrogen bonds. The hydrogen bonds are both direct and water mediated and are the main factors determining the specificity of protein−carbohydrate interactions. Carboxylate side chains of the protein are especially important in binding of anomers and epimers.[2] The protein−carbohydrate interactions inspire the development of artificial receptor molecules,[3−5] which in the feature may serve as therapeutics or chemosensors. On the other hand, the artificial carbohydrate receptors also provide model systems to study the basic molecular features of carbohydrate recognition.

Recently, we have developed an acyclic receptor system for the recognition of monosaccharides in chloroform solutions.[5] The effective acyclic receptors (such as **1**−**3** and **5**−**7**) are able to form both the multiple hydrogen bonds and the stacking interactions with sugar rings. An important design criterion is that the receptor structures contain both donor and acceptor groups which are able to participate in the cooperative hydrogen bonds with the carbohydrate OHs, similar to those in the protein−carbohydrate complexes. The presence of moieties which are able to take part in additional secondary hydrogen bonds with sugar hydroxyls (such as 1,8-naphthyridine groups)[5b] causes a substantial enhancement of the binding properties. In addition, structural elements forming additional van der Waals contacts with the carbohydrate molecule favor the binding process. Our systematic studies showed the suitability of the amide-NH/pyridine-

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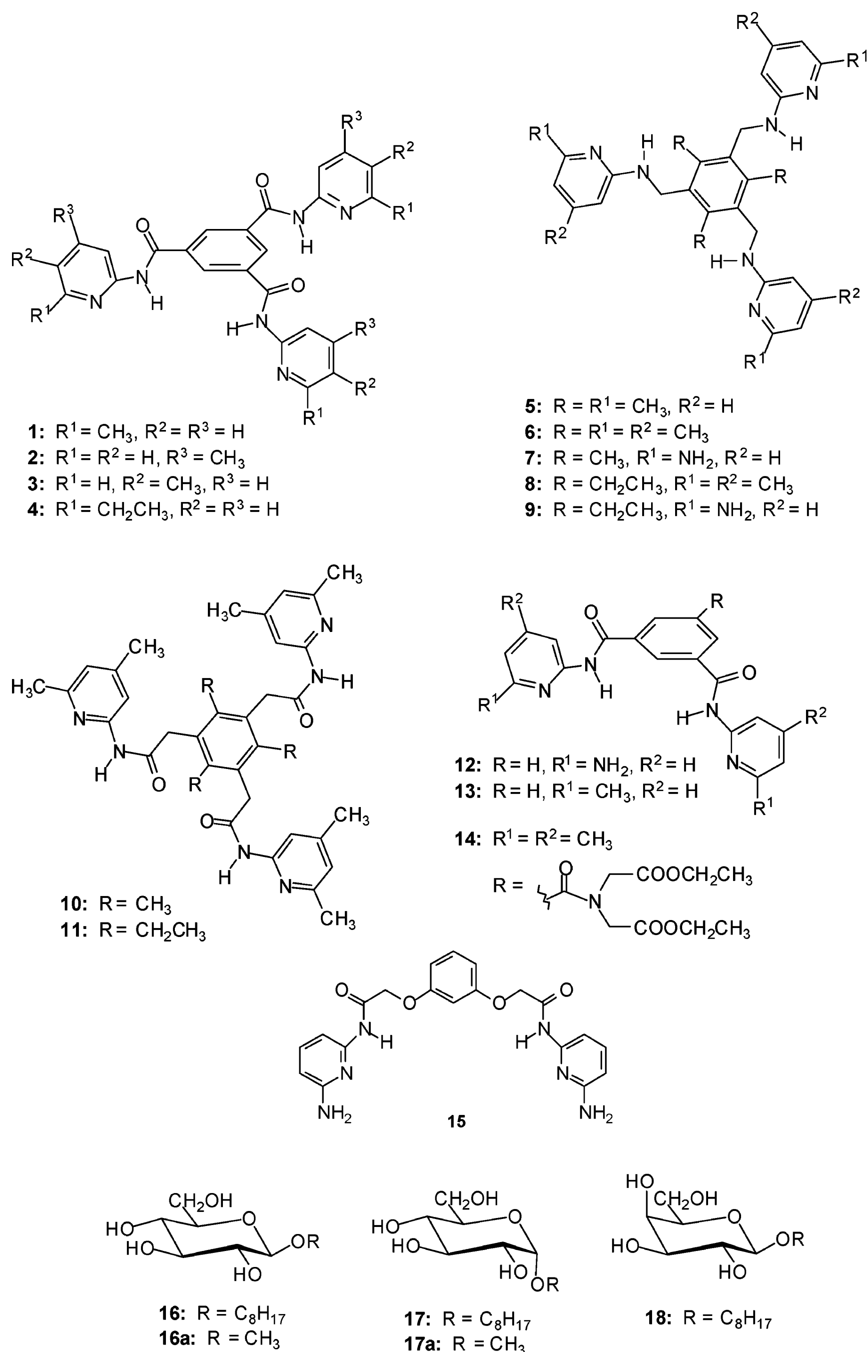
10.1021/jo048979k CCC: $27.50 © 2004 American Chemical Society

Published on Web 10/02/2004

CHART 1



N, pyrimidine-N, or naphthyridine-N recognition units as hydrogen bonding groups for monosaccharides.[5a,b,d] Furthermore, the replacement of the pyridine-N/amide-NH groups by pyridine-N/amine-NH moieties (receptors **5**−**7**) in the receptor structure leads to favorable changes of the binding affinity and selectivity.[5c] Interestingly, the addition of small amounts of water to the chloroform solutions enhances in some cases the binding affinity of the receptors, indicating the formation of water-mediated hydrogen bonds,[5c] in line with the observation in protein−carbohydrate complexes.[2] The three-dimensional arrangement of the binding sites was shown to be of great importance.

In this paper we describe the syntheses, crystal structures, and binding properties of new acyclic pyridine-based receptors **4**, **8**−**12**, **14**, and **15** toward monosaccharides. We compare their recognition properties with previously studied receptors and discuss the factors, which influence the recognition of monosaccharides. Well-chosen systematic changes in the receptor structure provide invaluable insights into molecular recognition phenomena and can lead to an easier development of new carbohydrate receptors with favorable recognition properties.

Results and Discussion

In this study, the binding properties of four series of pyridine-based receptors were explored and compared: receptors including three heterocyclic groups attached to the central phenyl ring via the −NHCO− unit (**1**-**4**), receptors based on the 2,4,6-trimethyl- or triethylbenzene

TABLE 1. Association Constants K_a[a] (M^{-1}) and Corresponding Free Energy Changes $\Delta G°$ (kJ mol^{-1}) for Receptors 2, 3, and 6–9 and Glucopyranosides 16–18

host–guest complex	K_{a1} ($\Delta G°$)	K_{a2} ($\Delta G°$)	$\Delta\delta_{max}$[d] ($\Delta\delta_{obs}$)[e] (ppm)
2·16[i]	660 (−16.1)	24200 (−25.0)[b]	NH:[f] 0.85 (0.85); CH:[g] 0.18 (0.18)
2·17[i]	3640 (−20.3)	82450 (−28.0)[b]	NH:[f] 1.10(1.09); CH:[g] 0.56 (0.55)
2·18	420 (−14.9)	50770 (−26.8)[b]	NH:[f] 0.95(0.92); CH:[g] 0.15 (0.15)
3·16[i]	440 (−15.1)	22600 (−24.8)[b]	NH:[f] 0.80(0.80); CH:[g] 0.17 (0.15)
3·17[i]	2100 (−18.9)	47600 (−26.7)[b]	NH:[f] 1.10(1.08); CH:[g] 0.50 (0.48)
3·18	300 (−14.1)	29350 (−25.5)[b]	NH:[f] 0.80(0.75); CH:[g] 0.15 (0.12)
6·16[j]	20950 (−24.7)	790 (−16.5)[c]	NH:[h] 1.28 (1.23)
6·17[j]	800 (−16.6)		NH:[h] 1.45 (1.14)
6·18	1360 (−17.9)	211 (−13.3)[c]	NH:[h] 1.20 (0.97)
7·16[j]	9500 (−22.7)	4800 (−21.0)[b]	NH:[h] 1.20 (1.10)
7·17[j]	620 (−15.9)		NH:[h] 1.50 (0.98)
8·16	48630 (−26.7)	1320 (−17.8)[c]	NH:[h] 1.35 (1.31)
8·17	1310 (−17.8)		NH:[h] 1.44 (1.16)
8·18	3070 (−19.9)	470 (−15.2)[c]	NH:[h] 1.33 (1.15)
9·16	19590 (−24.5)	14490 (−23.7)[b]	NH:[h] 1.28 (1.16)
9·17	1100 (−17.4)		NH:[h] 1.35 (1.02)
9·18	8410 (−22.4)	8680 (−22.5)[b]	NH:[h] 1.29 (1.04)

[a] Average K_a values from multiple titrations ($CDCl_3$, stored over activated molecular sieves and deacidified with Al_2O_3). The reproducibility of the K_a values was ±10–20%. Uncertainty in a single K_a estimation was ±2–10%. Dilution experiments show that receptors do not self-aggregate in the used concentration range. [b] 2:1 Receptor/pyranoside complex. [c] 1:2 Receptor/pyranoside complex. [d] Change in chemical shift at saturation binding, values provided by HOSTEST. [e] Largest change in chemical shift observed during the titration. [f] Complexation-induced shifts observed for the amide-NH of receptor (the concentration of receptor was kept constant and that of sugar varied). [g] Complexation-induced shifts observed for the anomeric CH of sugar (the concentration of sugar was kept constant and the host concentration varied). [h] Complexation-induced shifts observed for the amine-NH (CH_2*NH*) of receptor (the concentration of receptor was kept constant and that of sugar varied). [i] Results from ref 5d. [j] Results from ref 5c.

frame with three pyridine moieties attached via −NHCH$_2$− (**5**–**9**), or −NHCOCH$_2$− spacer (**10** and **11**) and receptors incorporating only two heterocyclic groups (**12**–**15**). The interactions of the receptors and monosaccharides were investigated by ^{1}H NMR spectroscopy, microcalorimetry, and extraction experiments. Octyl β-D- and α-D-glucopyranosides (**16** and **17**), octyl β-D-galactopyranoside (**18**), as well as methyl α- and β-glucopyranosides (**16a**, **17a**) were selected as substrates for the receptors. The ^{1}H NMR titration was the main method used in this work for the determination of the association constants. The titration data were analyzed by nonlinear regression analysis using the HOSTEST 5.6 program.[6a] The stoichiometry of receptor–sugar complexes was established by the curve-fitting analysis of the titration data and by mole ratio plots.[7] The binding constants[6b] are given in Tables 1 and 2.

Receptors 1–4. The binding studies with receptors **1**–**4** showed that the steric interactions involving the pyridine substituents significantly affect the binding properties. Even a minimal structural variation can lead to remarkable changes of the receptor properties, as shown with receptors **2** and **3**. These two receptors, compared to the receptor **1**, reflect only the change from α,α- to the α,β- or α,γ-disubstituted pyridine groups; however, the binding properties of **2** and **3** change dramatically. The pyridine units in **2** and **3** are sterically less hindered at nitrogen (free pyridine α-position) and can be involved more easily and effectively in the binding interactions. Consequently, very strong 2:1 receptor/sugar complexes are formed between the receptors **2** and **3** and glucopyranosides **16** and **17**.[5d] Compared to **1**, receptors **2** and **3** show not only higher efficiency and different binding modes but also an inverse selectivity since they bind the α-glucopyranoside better than the β-anomer. These α/β-anomer selectivities differ from those observed for the hydrogen-bonding host molecules described so far, which usually show higher affinity toward the β-anomer. One reason for this tendency is the formation of different intramolecular hydrogen bonds in the two anomers.[4h,8] The axial 1-alkoxy group in α-anomer **17** can form intramolecular hydrogen bonds with the 2-OH group more easily than the equatorial 1-alkoxy substituent in the β-anomer can do. Therefore, the 2-OH in β-glucopyranoside **16** is relatively free from intramo-

TABLE 2. Association Constants K_a[a] (M^{-1}) and Corresponding Free Energy Changes $\Delta G°$ (kJ mol^{-1}) for Receptors 1, 4, 10–12, 14, and 15 and Octyl-β-D-glucopyranoside (16)

receptor	K_{a1} ($\Delta G°$)	K_{a2} ($\Delta G°$)	$\Delta\delta_{max}$[c] ($\Delta\delta_{obs}$)[d] (ppm)
1[e]	8700 (−22.5)		0.70 (0.65)
4	990 (−17.1)		0.60 (0.39)
10	650 (−16.0)		0.47 (0.14)
11	1230 (−17.6)		0.25 (0.19)
12	1420 (−18.0)	3890 (−20.5)[b]	0.69 (0.66)
14	950 (−17.0)		0.77 (0.50)
15	190 (−12.9)		0.36 (0.15)

[a] See Table 1. [b] 2:1 Receptor/glucopyranoside complex. [c] Change in chemical shift at saturation binding, values provided by HOSTEST. [d] Largest change in chemical shift observed for the amide-NH of receptor during the titration. [e] Results from ref 5a.

(6) (a) Wilcox, C. S.; Glagovich, N. M. Program HOSTEST 5.6, University of Pittsburgh. This program is designed to fit data to different binding models, which include both "pure" binding models, taking into consideration the formation of only one type of complex in solution, and "mixed" binding models containing more than one type of complex in solution. (b) K_{a1} corresponds to the 1:1 association constant. K_{a2} corresponds to the 1:2 or 2:1 receptor/sugar association constant (for details see the text and Tables 1–3).

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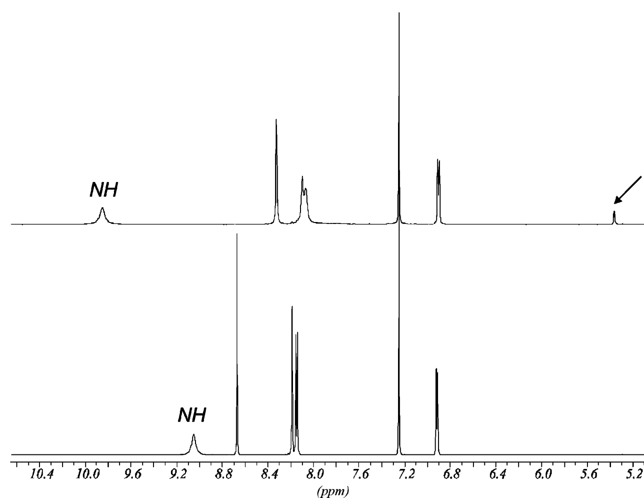

FIGURE 1. ^{1}H NMR spectra showing the NH and the aromatic protons of receptor **2** before (bottom) and after (top) the extraction of solid methyl-α-glucopyranoside (**17a**) by a $CDCl_3$ solution of receptor **2** (1.38×10^{-3} mol/l). The anomeric CH of sugar **17a** is marked (top).

lecular hydrogen bonding and can interact with receptor molecules more strongly. The particularly strong binding of α-anomer **17** with **2** or **3** indicates that the intermolecular host−guest interactions compete effectively with the intramolecular H-bonding network in carbohydrates. As indicated by molecular modeling the α-anomer is particularly favorable positioned in the cavity between the two receptors in 2:1 receptor/sugar complexes. These complexes are stabilized by hydrogen bonds between sugar OHs and the amide-NH/pyridine-N of **2** or **3** (including cooperative hydrogen bonds OH···N-pyr, HO···HN-amide), interactions of sugar CH moieties with the phenyl and pyridine groups of the receptors (both sides of the pyranoside ring are involved in the stacking interactions with aromatic residues of the two receptor molecules) as well as by weak CH···N-pyr and CH···O=C hydrogen bonds. The evidence for the particularly strong complexation of glucopyranosides with the receptors **2** and **3** was obtained by NMR spectroscopy[5d] and extraction experiments. The extraction experiments with methyl glucopyranosides **16a** and **17a** also provided an indication for the formation of 2:1 receptor/glucopyranoside complexes. For example, the receptor **2** is able to extract 0.5 equiv of methyl α-D-glucopyranoside (**17a**) from the solid state into a $CDCl_3$ solution (Figure 1), the anomeric C*H* and the OCH_3 protons were integrated with respect to the host's proton signals to give the sugar/receptor ratio.

Furthermore, the titration experiments performed with receptors **2**/**3** and octyl *β*-D-galactopyranoside (**18**) also revealed the formation of strong 2:1 receptor/sugar complexes. The ^{1}H NMR titration experiments were carried out by adding increasing amounts of the sugar **18** to a solution of the host **2** or **3**. The complexation was evidenced by the downfield shifts of the receptor amide protons ($\Delta\delta_{max}$ = 0.95 and 0.80 ppm for **2** and **3**, respectively), reflecting the formation of hydrogen-bonded complexes, and by the upfield shifts of the phenyl protons ($\Delta\delta_{max} = -0.17$ ppm for both receptors), as illustrated in Figure 2a for **2·18**. The plot of the observed and calculated downfield chemical shifts of the NH resonances of **2** as a function of added sugar **18** is shown in Figure 2b. Both the fitting the binding isotherms and the molar ratio plots indicated that hosts **2** and **3** form 2:1 receptor/sugar complexes with **18**. Moreover, additional inverse titrations were performed in which the concentration of galactopyranoside **18** was held constant and that of receptor **2** or **3** varied. The ^{1}H NMR spectra obtained during binding experiments showed large downfield shifting of the sugar hydroxyl resonances, indicating hydrogen bond formation; 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 **18**, which is shifted downfield by 0.15 ppm (Figure 3a), was

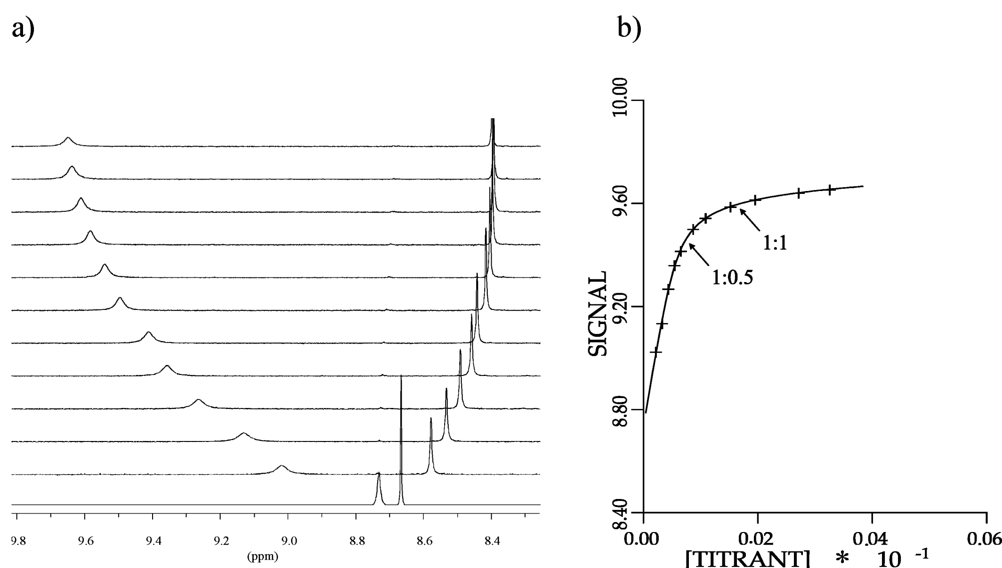

FIGURE 2. Titration of receptor **2** (1.32 mM) with octyl *β*-D-galactopyranoside (**18**). (a) ^{1}H NMR spectra ($CDCl_3$, 25 °C) of the receptor **2** (NH and CH_{Ph} resonances are shown) after addition of (from bottom to top) 0, 0.15, 0.23, 0.31, 0.39, 0.47, 0.63, 0.78, 1.10, 1.41, 1.96, and 2.35 equiv of **18**. (b) Plot of the observed (+) and calculated (−) downfield chemical shifts of the δ_{N-H} resonances of **2** as a function of added **18**. The [receptor]/[galactopyranoside] ratio is marked.

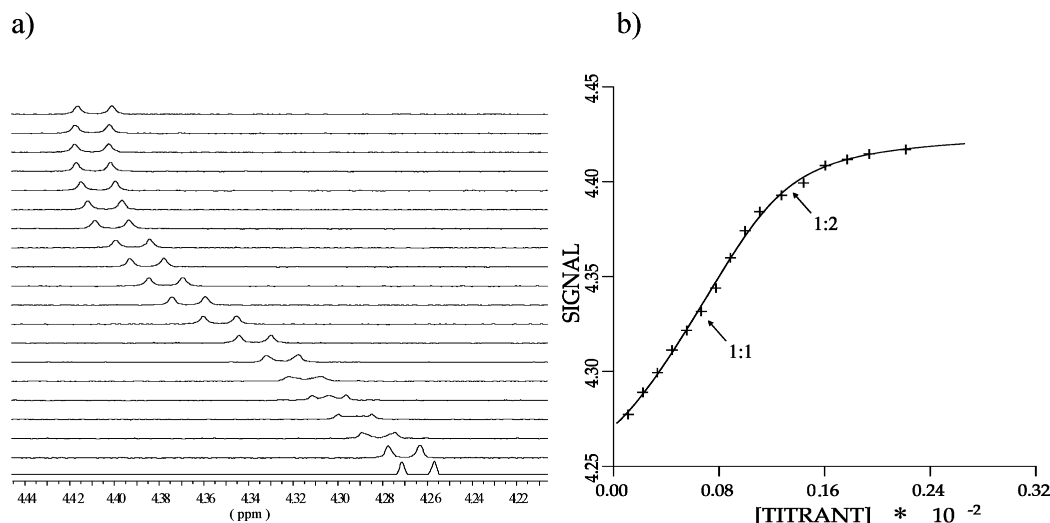

FIGURE 3. Titration of **18** (0.57 mM) with receptor **2**. (a) ^{1}H NMR spectra ($CDCl_3$, 25 °C) of **18** (anomeric CH resonances are shown) after addition of (from bottom to top) 0, 0.19, 0.38, 0.58, 0.77, 0.96, 1.16, 1.35, 1.54, 1.74, 1.93, 2.22, 2.51, 2.80, 3.09, 3.38, 3.87, 4.35, 4.84, and 5.80 equiv of **2**. (b) The corresponding binding isotherm. The [galactopyranoside]/[receptor] ratio is marked.

monitored. The chemical shift of the anomeric CH proton of β-galactopyranoside **18** during the titration with **2** or **3** is comparable to that of β-glucopyranoside **16**, which was affected significantly weakly ($\Delta\delta_{max}$ = 0.15−0.18 ppm) by complexation than the CH−(1) of α-glucopyranoside **17** ($\Delta\delta_{max}$ = 0.50−0.56 ppm). These results indicate an important contribution of the anomeric CH of **17** to the stability of the complexes **2·17** and **3·17**. The addition of 2 equiv of receptor **2** or **3** led to practically complete complexation of **18**. The typical titration curve is shown in Figure 3b. The best fit of the titration data was obtained with the "mixed" 1:1 and 2:1 receptor/sugar binding models. The association constants of 420 (K_{a1}) and 50770 M^{-1} (K_{a2}) were determined for **2·18** (Table 1), whereas the binding constants for **3·18** amount to 300 (K_{a1}) and 29340 M^{-1} (K_{a2}). Thus, among the tested sugars, the receptors **2** and **3** showed the highest affinity toward α-glucopyranoside **17**. The stronger binding of **2** in comparison to **3** can be attributed both to the different basicity of the pyridine units[9] and to different sterical effects.

When the α-methyl group in **1** was replaced by an ethyl unit (compound **4**), the expected decrease in the binding affinity toward pyranosides was observed due to the stronger steric hindrance from the α-position of the pyridine ring. Compound **4** was synthesized from benzene-1,3,5-tricarbonyl chloride and 2-amino-6-ethylpyridine. The β-glucopyranoside **16** acted as probe for the binding studies. In contrast to **1**, the amide proton of **4** showed lower displacement on complexation with the glucopyranoside (after the addition of 5 equiv of sugar the NH protons shifted downfield by only 0.39 ppm; see Table 2). The binding constant was found to be 990 M^{-1}; i.e., the receptor **4** exhibited a 9-fold lower affinity for **16** than the receptor **1**. Thus, the higher the degree of steric hindrance, the less available is the pyridine nitrogen atom for hydrogen bond formation.

Also the hydrogen-bonding motifs in the crystal structure of **4**[10] illustrate the steric hindrance from the α-position of the pyridine ring. The pyridine nitrogen atom does not participate in the typical NH··N-pyr hydrogen bonds,[11] but the molecules of **4** prefer the formation of CH···O hydrogen bonds between the 5-CH of the pyridine ring and the carbonyl oxygen of the neighboring molecule (Figure 4a). In contrast to **4**, the molecules of **2** interact via NH··N-pyr hydrogen bonds, as shown in the Figure 4b (the crystals were obtained from THF/hexane solutions). The crystals of **3** were grown from ethanol solutions, and the ethanol molecules are hydrogen bonded to the receptor molecules. As illustrated in Figure 4c, the hydroxyl groups of the ethanol molecules participate in cooperative hydrogen bonds with the amide-NH and the pyridine nitrogen atom of the receptor molecule. Between the receptor molecules the CH···O hydrogen bonds are observed.

Receptors 5−9. The replacement of the amidopyridine groups by aminopyridine moieties and the incorporation of methyl groups into the central phenyl ring provides receptors (formulas **5**−**7**) which show remarkable β/α binding selectivity in the recognition of glucopyranosides.[5c] The aminomethyl group attached to the pyridine unit as well as a second methyl group at the 4-position of the pyridine ring in host **6** or an additional α-amino group in **7** favorably increased the basicity of the pyridine moieties.[9] In addition, the incorporation of a second amino group in **7** provides an additional binding site for carbohydrates. The substituted central phenyl ring in **5**−**7** can participate in effective CH−π interactions with sugar CHs.

The variations in the determined K_a values (significantly higher affinity of **5**−**7** toward β-anomer) correspond qualitatively to expected differences in hydrogen-bonding abilities of sugar hydroxyl groups (in contrast

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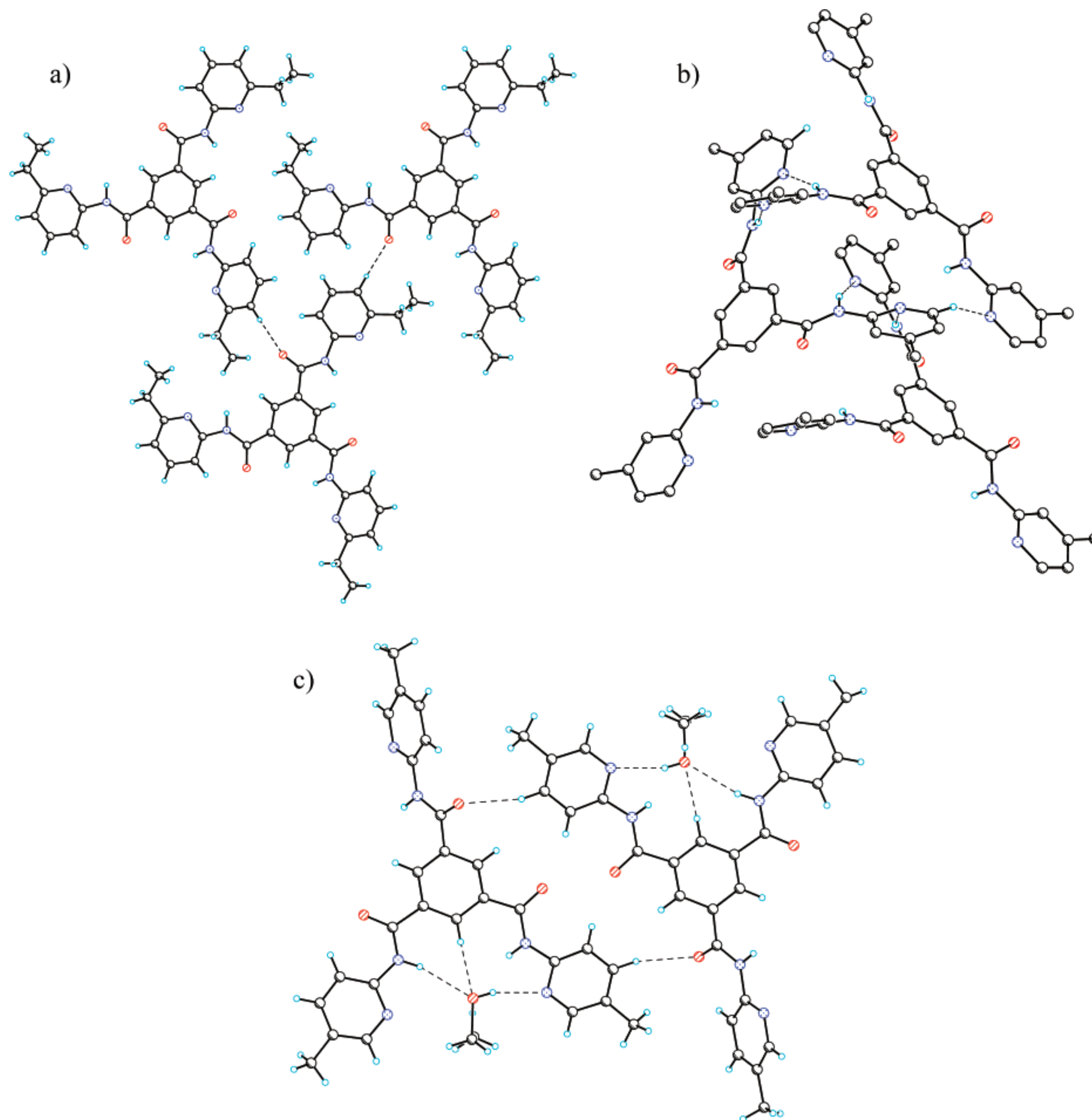

FIGURE 4. Packing of **4** (a), **2** (b, C*H* hydrogens are ommitted for clarity), and **3** (c, hydrogen-bonded ethanol molecules are shown).

TABLE 3. Thermodynamic Parameters Evaluated by ITC for 6·16 and 6·17 at 25 °C

host–guest complex	K_{a1} ($\Delta G°$)[a] (M^{-1}, kJ mol^{-1})	K_{a2} ($\Delta G°$)[b] (M^{-1}, kJ mol^{-1})	ΔH_1 (kJ mol^{-1})	ΔH_2 (kJ mol^{-1})	$T\Delta S_1$ (kJ mol^{-1})	$T\Delta S_1$ (kJ mol^{-1})
6·16	12800 (−23.4)	1300 (−17.8)	−51.3	−34.6	−27.9	−16.8
6·17	1830 (−18.6)		−20.1		−1.5	

[a] 1:1 Receptor/glucopyranoside complex. [b] 1:2 Receptor/glucopyranoside complex.

to receptors **2** and **3**, see above). The evidence for the preferred complexation of the β anomer was obtained by the NMR spectroscopy,[5c] extraction experiments,[5c] and microcalorimetry (isothermal titration calorimetry, ITC).

The microcalorimetric data revealed that the receptor-glucopyranoside interactions are enthalpy driven (see, for example, the thermodynamic parameters evaluated for **6·16** and **6·17**, Table 3 and Figures 5 and 6) and the enthalpy of binding is more negative than, or equal to, the free energy of binding (similar to the lectine–carbohydrate complexes[1b]). Thus, the unfavorable loss in entropy is compensated by an advantageous change in enthalpy. The values of the binding constants obtained by the NMR spectroscopy and the microcalorimetry are of the same magnitude but differ by a factor of about 1.6–2. Such differences are typical for the results obtained by these two methods.[12]

The binding affinity obtained for receptor **6** and β-galactopyranoside **18** is comparable to that obtained with α-glucopyranoside **17**, which is significantly lower than

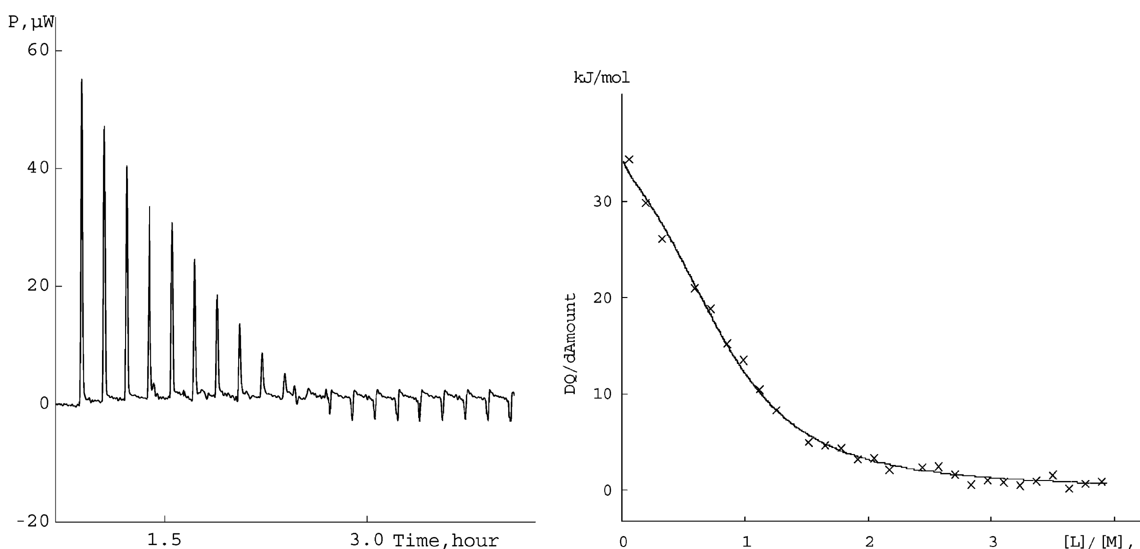

FIGURE 5. ITC-titration of receptor **6** (1.09 mM) with 10 μL aliquots of β-glucopyranoside **16** (11.62 mM) in chloroform (25 °C). Left: thermogram. Right: the integrated curve showing experimental points (×) and the best fit (—) for 1:1 and 1:2 receptor: sugar binding. The molar ratio of the sugar to the receptor is given (for the thermodynamic parameters see Table 3).

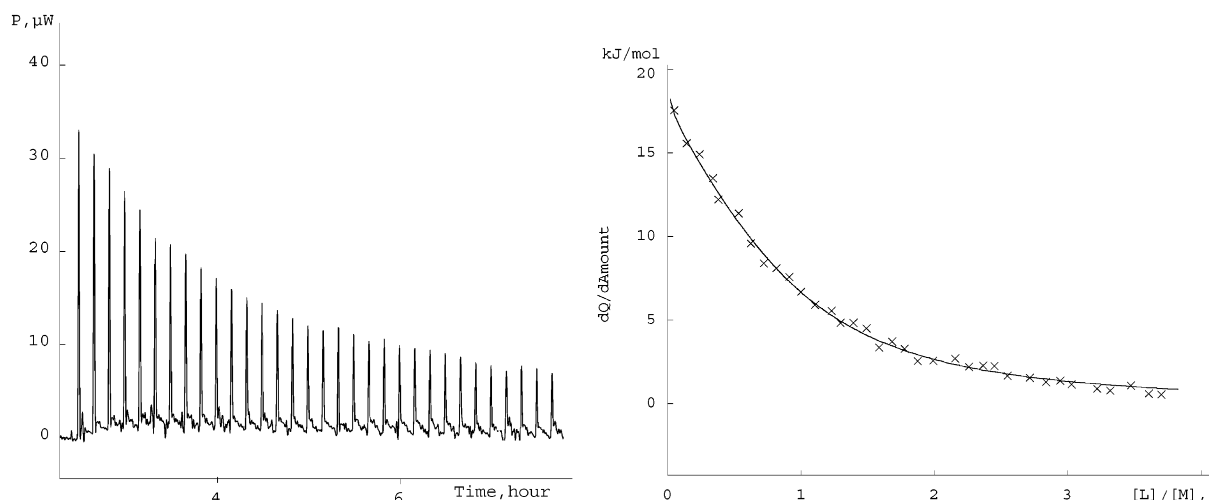

FIGURE 6. ITC-titration of receptor **6** (1.09 mM) with 12 μL aliquots α-glucopyranoside **17** (7.55 mM) in chloroform (25 °C). Left: thermogram. Right: the integrated curve showing experimental points (×) and the best fit (—) for 1:1 receptor−sugar binding. The molar ratio of the sugar to the receptor is given (for the thermodynamic parameters see Table 3).

the previously determined for β-glucopyranoside **16**. The complexation between **6** and **18** was again evidenced by several changes in the ^{1}H NMR spectra, particularly by the downfield shifts of the receptor NH protons ($\Delta\delta_{max}$ = 1.20 ppm) and upfield shifted resonances for the methylene protons of **6** ($\Delta\delta_{max} = -0.12$ ppm). The curve fitting of the titration data suggested the existence of both 1:1 and 1:2 receptor:sugar complexes, with 1:1 association constant of 1360 M^{-1} (K_{a1}) and a weaker association constant for an 1:2 receptor:sugar complex (K_{a2} = 211 M^{-1}).

Although the methylene groups in **5**−**7** give conformational mobility to the whole molecule and the receptors are far from being perfectly preorganized, the binding affinity is high (particularly for β-glucopyranoside, see Table 1). The crystal structure of **6**,[10] for example, revealed that two pyridine-based recognition units are pointing to the same face of the central aryl ring, while the third recognition unit points in the opposite direction (Figure 7). In such cases a significant conformational change of the receptor structure is required for complexation.

To achieve an improved degree of preorganization of the heterocyclic recognition units the 2,4,6-triethylbenzene frame was used for the construction of the receptor molecules **8** and **9**. Ethyl groups should stabilize a conformation in which the three recognition groups are preferably directed to one side of the benzene plane.[13] The compounds **8** and **9** were synthesized from 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene[14] and 2-amino-4,6-dimethylpyridine or 2,6-diaminopyridine, respectively. As expected, this structural variation resulted in a substantial enhancement of the binding affinity toward monosaccharides. In the crystal, however, the compound **8**[10] is

(12) Connors, K. A. In *Comprehensive Supramolecular Chemistry;* Atwood, J. L., Davis, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: Oxford, U.K., 1996; Vol. 3, p 233.

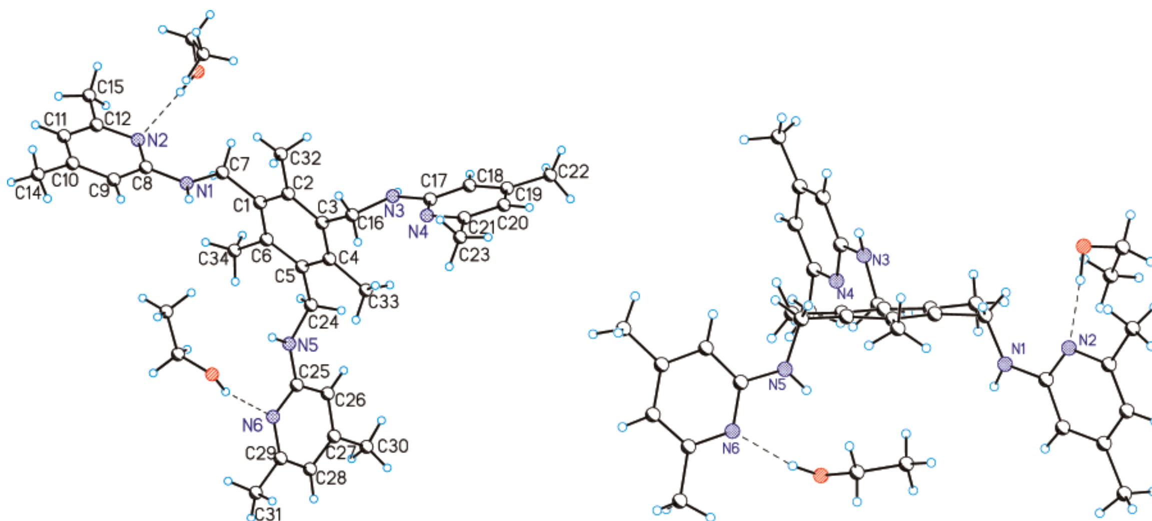

FIGURE 7. Crystal structure of **6**, top and side views (hydrogen-bonded ethanol molecules are shown).

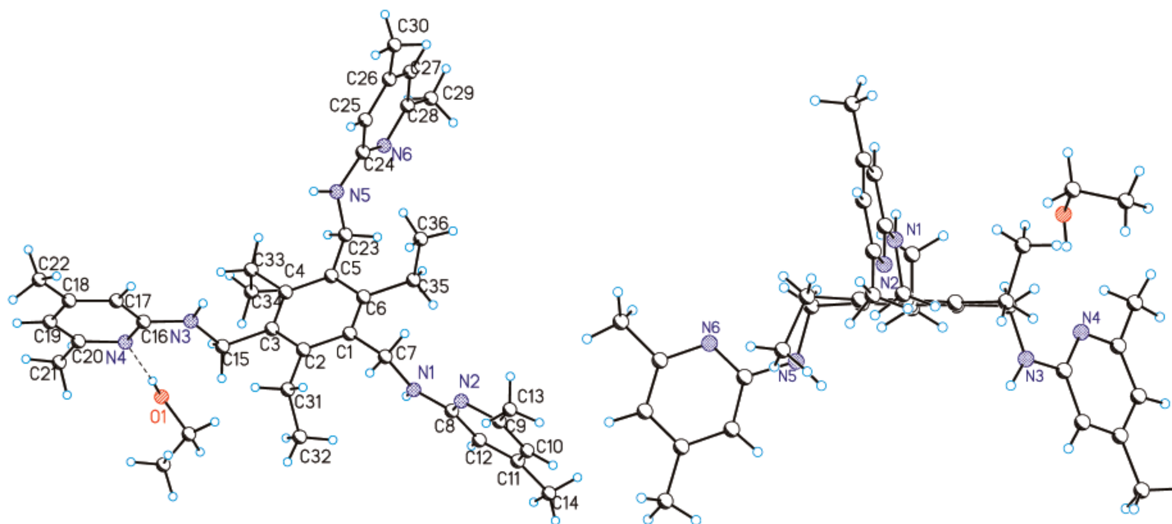

FIGURE 8. Crystal structure of **8**, top and side views (hydrogen-bonded ethanol molecule is shown).

still not perfectly preorganized and adopts a similar conformation as **6**. Two pyridine units are on one side of the central phenyl ring and one on the other (Figure 8).

The packing of the molecules of **6** and **8** in the crystals is shown in Figure 9. The crystals were obtained from ethanol solutions, and in both crystal structures the ethanol molecules are hydrogen bonded to the receptor molecules. In the case of **6**, two ethanol molecules are placed between two receptors (Figure 9a).

The noncovalent interactions between the ethanol and receptor molecules involve OH⋯N-pyr and HO⋯HN hydrogen bonds as well as interactions between the ethyl groups and the central benzene rings of the two receptors. The hydroxyl groups of the ethanol molecules form cooperative hydrogen bonds through simultaneous participation as donor and acceptor of hydrogen bonds. Such cooperative hydrogen bonds are also present in the crystal structure of **8** (Figure 9b). The participation of the amide-NH/N-pyr units in the formation of hydrogen bonds with the hydroxy groups of the solvent molecules shows the potential of this type of recognition units in the formation of complexes with substrates involving hydroxy substituents.

The complexation between the receptors **8**/**9** and pyranosides **16**−**18** was evidenced by the downfield shift of the receptor amine protons and upfield shift of the CH_2 resonances. During the titration of **8** with β-D-glucopyranoside **16** the signal due to the amine NH moved downfield by about 1.3 ppm and the methylene CH_2 moved upfield by 0.16 ppm (Figure 10a). Similar to **6**, the curve fitting of the titration data for receptor **8** and **16** suggested the existence of both 1:1 and 1:2 receptor/sugar complexes in the chloroform solution (Figure 10b), with a strong 1:1 association constant (K_{a1} = 48630 M^{-1})

(13) For examples on the use of other systems based on the triethylbenzene frame, see: (a) Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993**, *115*, 6466−6467. (b) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533−8534. (c) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *J. Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 862−865. (d) Kim S.-G.; Ahn, K. H. *Chem. Eur. J.* **2000**, *6*, 3399−3403. (e) Chin, J.; Walsdorff C.; Stranix, B.; Oh, J.; Chung, H.-J.; Park, S.-M.; Kim, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 2756−2759.

(14) Cabell, L. A.; Best, M. D.; Lavigne, J. J.; Schneider, S. E.; Perreault, D. M.; Monahan, M.-K.; Anslyn, E. V. *J. Chem. Soc., Perkin Trans. 2* **2001**, 315−323.

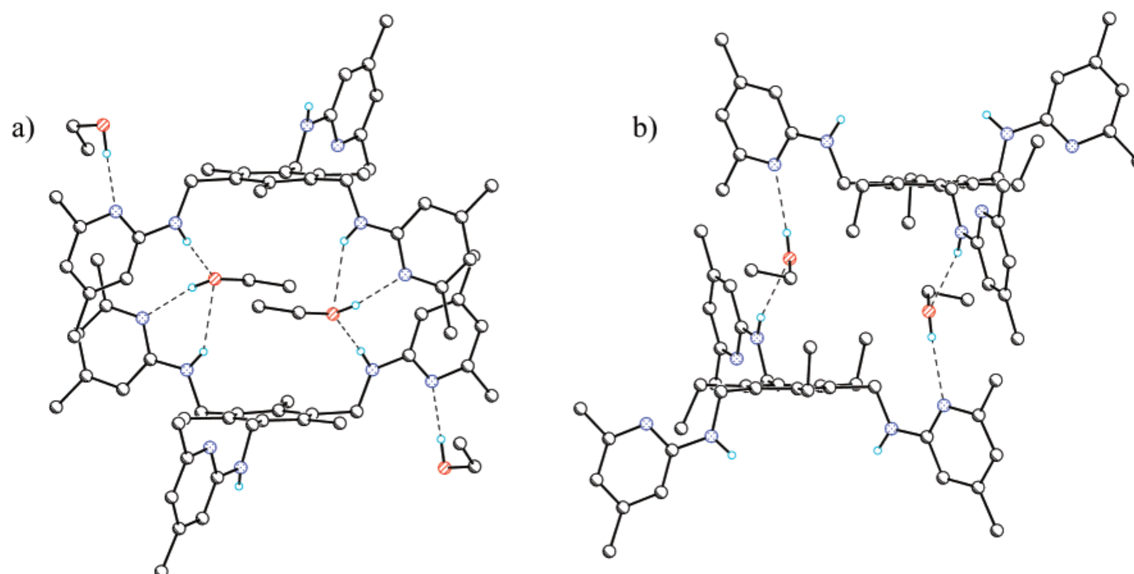

FIGURE 9. Packing of **6** (a) and **8** (b) in the crystal (hydrogen-bonded ethanol molecules are shown, C*H* hydrogens are ommitted for clarity).

and a weaker association constant for 1:2 receptor/sugar complex (K_{a2} = 1320 M^{-1}). Thus, the determined binding constants for complex of **8** and **16** were about 2-fold higher than the previously estimated binding constants for complex of **6** and **16**.[5c]

An ^{1}H NMR titration of **8** with α-glucopyranoside **17** or β-galactopyranoside **18** produced similar spectral changes. In particular, the signal due to the amine NH moved downfield by about 1.15 ppm, and the methylene CH_2 moved upfield by 0.15 ppm (Figure 10c,e). The fit of NMR shift changes of the NH as well as the CH_2 group of **8** during the titration with **17** agreed with a 1:1 binding model (Figure 10d), yielding an association constant of 1310 M^{-1}. The motions of the signals **8** observed during the titration with **18** were consistent with 1:1 and 1:2 receptor/sugar binding (Figure 10f) and could be analyzed to give association constants of 3070 M^{-1} (K_{a1}) and 470 M^{-1} (K_{a2}). Thus, among the examined monosaccharides, β-glucopyranoside **16** exhibited the highest affinity toward **8**. Comparison of Figure 10b and d/f clearly shows that the process of complexation is not reflected by these chemical shifts upon binding with these sugars in the same way. Whereas after the addition of 2 equiv of β-glucopyranoside almost no change was observed in the chemical shift of receptor signals, with the α-glucopyranoside or β-galactopyranoside chemical shift changes continue to higher sugar/receptor ratios. Thus, similar to **6**, the compound **8** displays high β/α anomer selectivity in the recognition of glucopyranosides. The receptor **8** exhibited about 2-fold higher affinity for sugars **16**-**18** than the receptor **6**. These results again demonstrate the importance of preorganization in the molecular recognition process.

In the case of receptor **9**, the interactions with the additional α-amino group in the pyridine ring play a significant role and influence the binding model (similar to the receptor **7**). The α-amino group increases the basicity and provides additional hydrogen bonding sites. The titration data revealed that with β-glucopyranoside **16** and β-galactopyranoside **18** the 1:1 and 2:1 receptor/sugar complexes are formed. According to molecular modeling the 2:1 complexes display hydrogen-bonding between receptor and sugar, as well as between the two receptor molecules. The titration data with α-glucopyranoside **17** showed that 1:1 binding is taking place. Several changes in the NMR spectra of **9** after the addition of pyranosides **16**−**18** were observed. The NMR signals of the NH and NH_2 groups of **9** were substantially shifted downfield (NH: $\Delta\delta_{max}$ = 1.20−1.30 ppm, NH_2: $\Delta\delta_{max}$ = 0.20−0.30 ppm) and the signal of the CH_2 group moved upfield ($\Delta\delta_{max}$ = −0.10−0.20 ppm). The binding constants of β-glucopyranoside **16** and receptor **9** were found to be 19590 M^{-1} (K_{a1}) and 14490 (K_{a2}), the one for α-glucopyranoside **17** and **9** amounts to 1100 M^{-1}, and the binding constants for the complex with β-galactopyranoside **18** were determined to 8410 M^{-1} (K_{a1}) and 8680 (K_{a2}). Thus, significantly weaker binding for the α-anomer was determinated (similar to the previously described receptor **7**[5c]).

Receptors 10 and 11. The receptors **10** and **11**, bearing three α,γ-dimethyl-substituted pyridine groups covalently attached to the 2,4,6-trimethyl- or -triethyl-substituted phenyl ring via −$NHCOCH_2$− units, show significantly lower affinity toward glucopyranosides in comparison to both groups of receptors, **1**−**3** and **5**−**9**. According to the molecular modeling, the receptors **10** and **11** should be able to fully encapsulate a pyranoside molecule (better than the more rigid **1**) in 1:1 receptor/sugar complexes. On the other hand, these receptors have a higher conformational freedom and the unfavorable entropy hinders the effective recognition process. On titration of **10** or **11** with β-glucopyranoside **16** no substantial shifts for receptor protons were observed (see Table 2), indicating a weak binding. The pattern of shifts was consistent with 1:1 stoichiometry. Whereas the binding constant of **10** and **16** was calculated to be 650 M^{-1}, the value for **11** and **16** with K_a = 1230 M^{-1} is about 2-fold higher (receptor based on 2,4,6-triethylbenzene frame). Thus, the unfavorable loss in entropy is not compensated by an advantageous change in enthalpy.

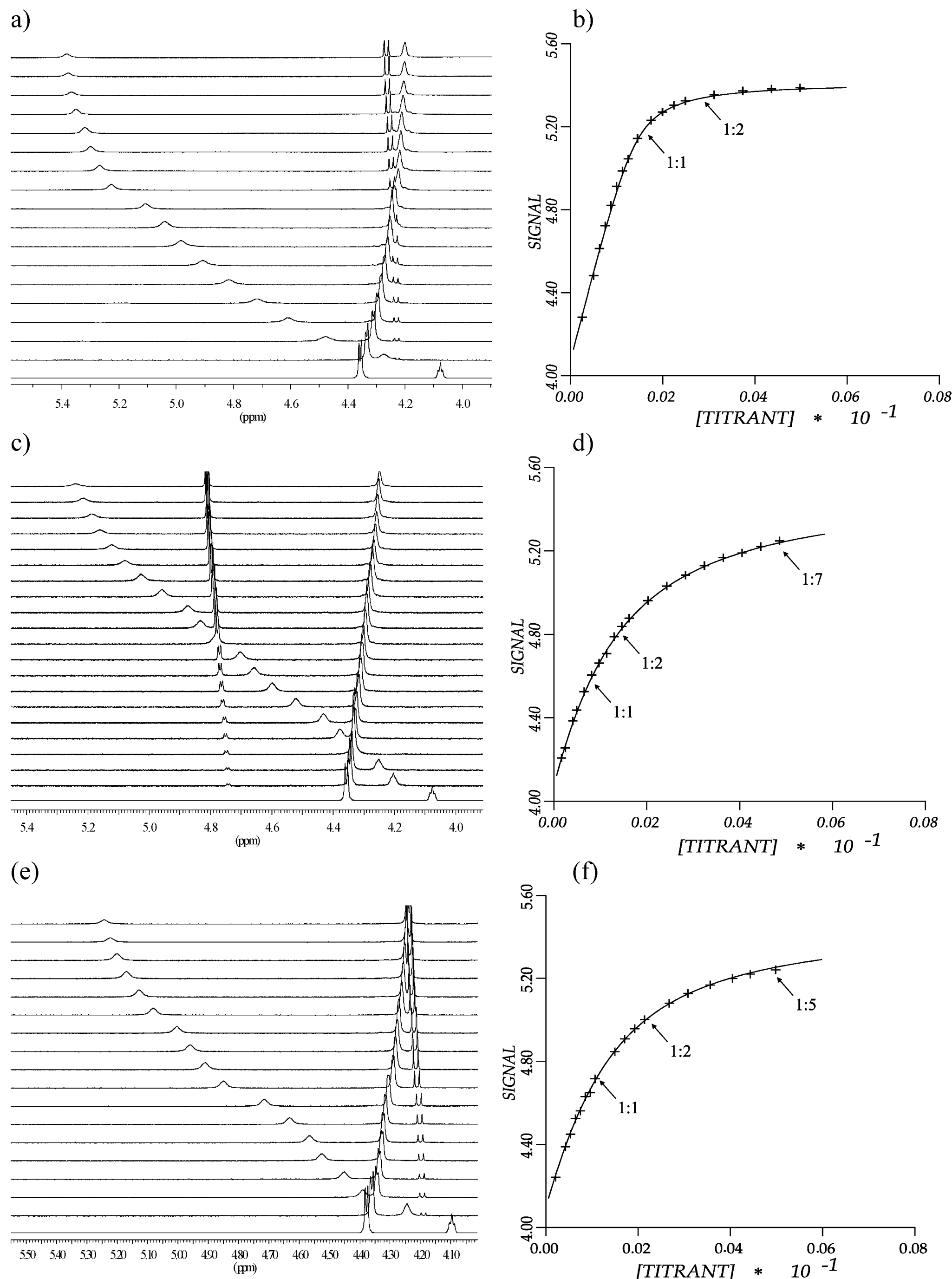

FIGURE 10. Titration of receptor **8** with β-glucopyranoside **16**, α-glucopyranoside **17**, and β-galactopyranoside **18** ([**8**] = 1.23 or 0.93 mM). ^{1}H NMR spectra ($CDCl_3$, 25 °C) of the receptor **8** (NH and CH_2 resonances of **8** and the anomeric CH of sugar are shown) after addition of 0−3.50 equiv (from bottom to top) of **16** (a), 0−7.20 equiv of **17** (c), and 0−5.05 equiv of **18** (e). Plot of the observed (+) and calculated (−) downfield chemical shifts of the δ_{N-H} resonances of **8** as a function of added **16** (b), **17** (d), and **18** (f). The [receptor]/[pyranoside] ratio is marked.

The results indicate the difficulty of stable complex formation when the arms of the amidopyridine receptor are longer (in comparison to **1**).

The compounds **10** and **11** were synthesized from 2-amino-4,6-dimethylpyridine and acyl chloride **23a** or **23b**, respectively. The synthesis of **23a**,**b** is shown in Scheme 1 (the preparation of the acids **22a** and **22b** is described in refs 16 and 17, respectively).

Receptors 12−15. The favorable properties of the amidopyridine receptors possessing an additional α-amino group are reflected by receptor **12**, including only two heterocyclic subunits interconnected by a phenyl spacer (**12** was prepared by the reaction of 2,6-diaminopyridine with isophthaloyl dichloride[11,15]). The strong complexation

(15) Chang, S.-K.; Van Engen, D.; Fan, E.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 7640−7645.

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(17) (a) Jeong, K.-S.; Shin, K. H.; Kim, S.-H. *Chem. Lett.* **2002**, 1166−1167. (b) O'Leary, B. M.; Szabo, T.; Schalley, C. A.; Luezen, A.; Schaeer, M.; Rebek, J. *J. Am. Chem. Soc.* **2001**, *123*, 11519−11533.

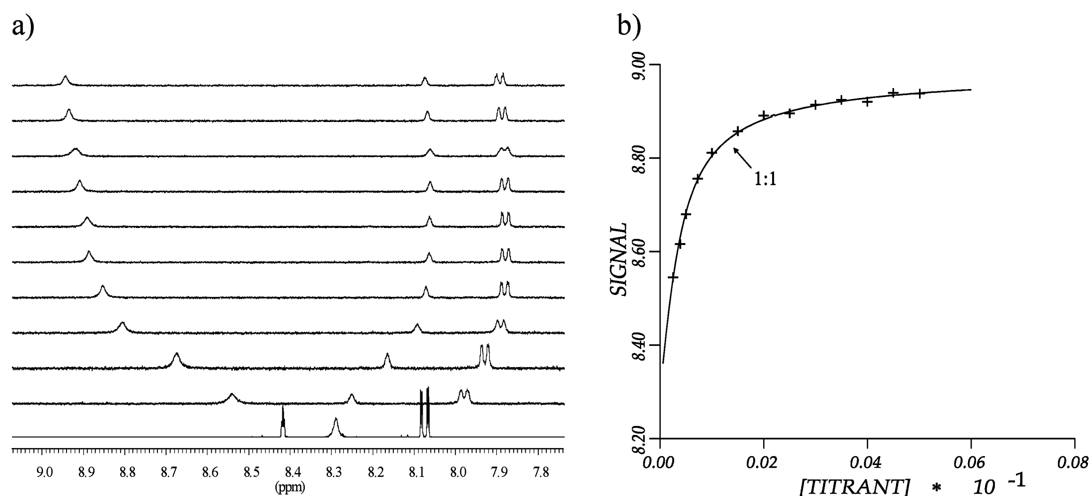


FIGURE 11. Titration of receptor **12** (0.92 mM) with octyl β-D-glucopyranoside (**16**). (a) ^{1}H NMR spectra ($CDCl_3$, 25 °C) of the receptor **12** (NH, 2-CH_{Ph} and CH_{pyr} resonances are shown) after addition of 0.00–3.95 equiv (from bottom to top) of **16**. (b) Plot of the observed (+) and calculated (–) downfield chemical shifts of the δ_{N-H} resonances of **12** as a function of added **16**. The [receptor]/[glucopyranoside] ratio is marked.

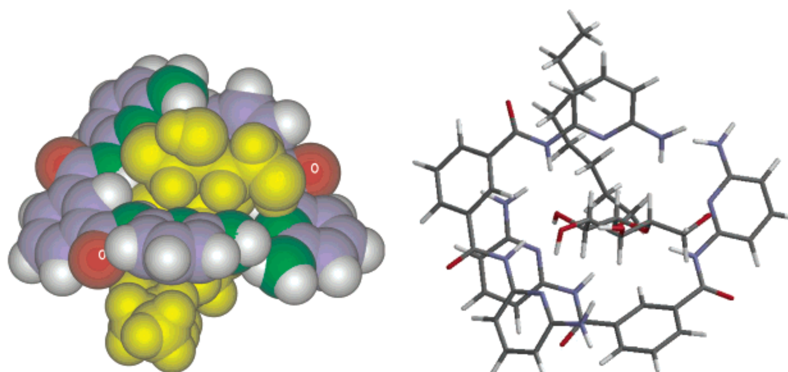
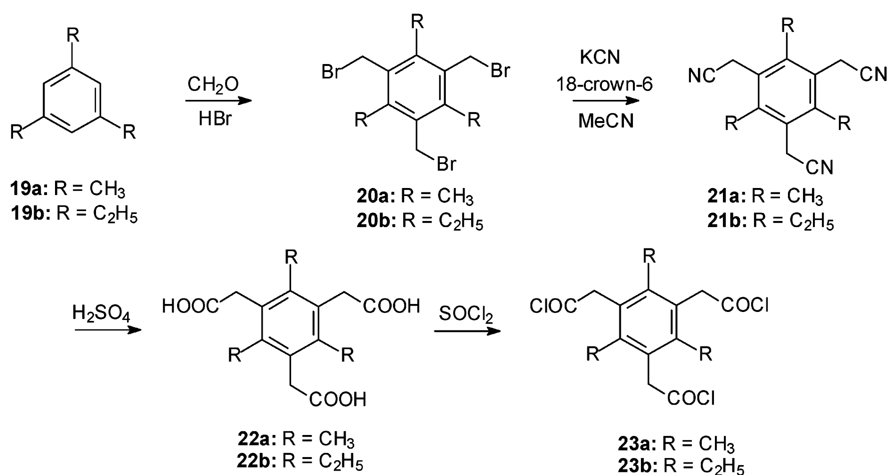


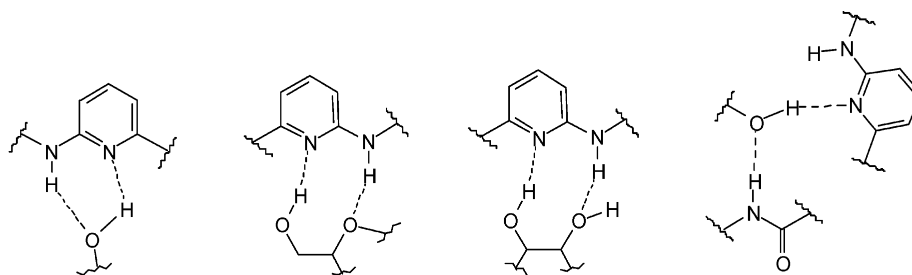
FIGURE 12. Energy-minimized structure of the 2:1 complex formed between receptor **12** and β-glucopyranoside **16** (MacroModel V.6.5, Amber* force field, Monte Carlo conformational searches, 50000 steps).

SCHEME 1

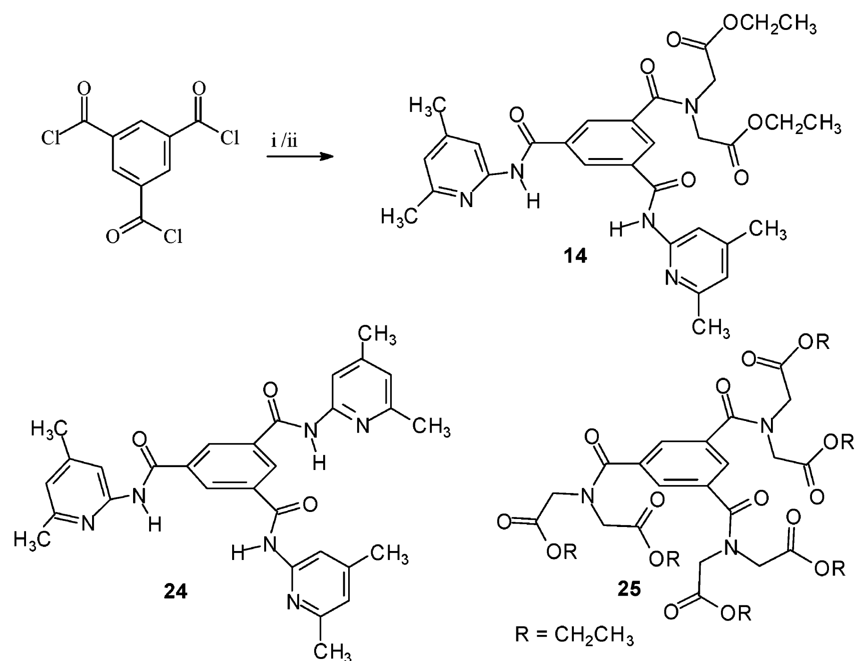


of sugars was clearly indicated by ^{1}H NMR spectroscopy. The molecules of **12** possess four potential groups of proton donors (two amide-NH and two NH_2 groups) available for hydrogen bonding. During the titration of **12** by **16** substantial downfield motions were observed for both the amide-NH (Figure 11a) and NH_2 signals ($\Delta\delta_{max}$ = 0.66 and 0.43 ppm for NH and NH_2, respectively), indicating that these groups are engaged in hydrogen bonds. Furthermore, the aromatic CH resonances (both the CH_{Ph} and CH_{pyr}) shifted upfield by 0.18–0.35 ppm. The addition of only 1 equiv of sugar **16** led to practically complete complexation of the hosts **12**. The

SCHEME 2



SCHEME 3 [a]



[a] Key: (i) 2 equiv of 2-amino-4,6-dimethylpyridine, 2 equiv of NEt_3, CH_2Cl_2, 25 °C, 40 min; (ii) 1 equiv of $HN(CH_2COOCH_2CH_3)_2$, 1 equiv of NEt_3, CH_2Cl_2, 25 °C, 24 h.

measurements indicated the formation of complexes with receptor:sugar stoichiometry of 2:1 (similar to receptors **7** and **9** having also an α-NH_2 group). The motions of the NH of **12** were consistent with 1:1 and 2:1 receptor/sugar binding (Figure 11b) and could be analyzed to give association constants of 1420 M^{-1} (K_{a1}) and 3890 M^{-1} (K_{a2}). Thus, the receptor **12** exhibits more favorable binding properties than the previously described bipyridyl host **13**[5a] as well as the triarmed receptors **4**, **10,** and **11**. These results reflect clearly the particular suitability of the 2-aminopyridine group for the recognition of carbohydrates. The potential of the 2-aminopyridine group in the recognition processes has been already discussed by other groups. For example, Anslyn and coworkers have noted the potential of this motif for binding vicinal diols.[18] Anslyn has exploited the 2-aminopyridine unit as a heterocyclic analogue of the asparagine/glutamine primary amide side chain.[3a,18]

Molecular modeling indicated the suitable multipoint hydrogen bonding arrangement between **12** and **16**, as shown in Figure 12. A 2:1 receptor/sugar complex can potentially be stabilized by several hydrogen bonds between the OHs of sugar and the amide-NH, amine-NH, and pyr-N of the two receptors (pyr-N···HO, amide-NH···OH, amine-NH···OH) as well as by O···HN hydrogen bond between the ring oxygen and the amide-NH. Typical hydrogen-bonding motifs found by molecular modeling studies are shown in Scheme 2. Furthermore, the complex is stabilized by interactions of sugar CHs with the aromatic groups of the two receptor molecules and weak CH···N-pyr hydrogen bonds. Moreover, the interactions between the two receptor molecules contribute to the stabilization of the 2:1 receptor/sugar complex.

Receptor **12** shows also significantly higer affinity than the host **14** incorporating not only two heterocyclic units, but also two ester groups. ^{1}H NMR binding studies with *β*-glucoside **16** as substrate yielded rather low association constant of 950 M^{-1} for **14·16** (Table 2). The ^{1}H NMR spectra obtained during binding experiments showed only moderate downfield shifting of the NH resonances. Even after the addition of 5 equiv of **16** the saturation has not been achieved. However, further improvement of the complexation properties of **14** can be expected after the hydrolysis of the ester groups. This structural change will provide compound including both the carboxylate groups

(18) Huang, C.-Y.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778–2792.

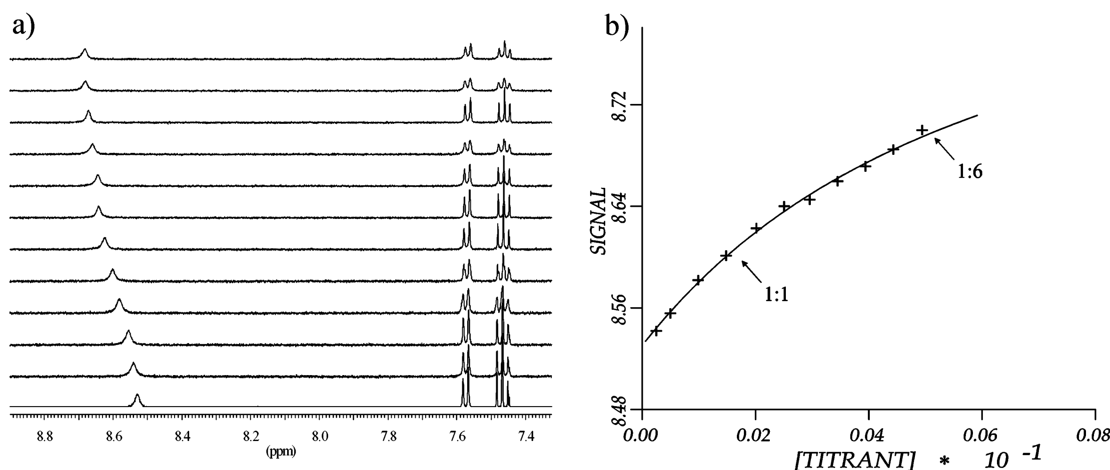


FIGURE 13. Titration of **15** (0.88 mM) with octyl β-D-glucopyranoside (**16**). (a) ^{1}H NMR spectra ($CDCl_3$, 25 °C) of the receptor **12** (NH, CH_{Ph} and CH_{pyr} resonances are shown) after addition of 0−5.80 (from bottom to top) equivalents of **16**. (b) Plot of the observed (+) and calculated (−) downfield chemical shifts of the δ_{N-H} resonances of **15** as a function of added **16**. The [receptor]/[glucopyranoside] ratio is marked.

and the heterocyclic units and may lead to favorable binding properties both in water and organic solution. The synthesis of **14** involves the reaction of benzene-1,3,5-tricarbonyl chloride with 2 equiv of 2-amino-4,6-dimethylpyridine, followed by the reaction with 1 equiv of iminodiacetic acid diethyl ester. The separation of the product **14** and the byproducts **24** and **25** (Scheme 3) was carried out by crystallization (see the Experimental Section).

Additionally, comparative complexation studies with host **15** were carried out (**15** was prepared by the reaction of 2,6-diaminopyridine with *m*-phenylenedioxydiacetyl chloride[11]). Receptors **12** and **15** have the same recognition groups, but differ in the flexibility, since the OCH_2-unit in **15** gives high conformational mobility to the whole molecule. As expected, the binding studies with β-glucopyranoside **16** as probe verified that the interactions with host **15** are less favorable than with host **12**. The lower binding affinity of **15** can be also explained by the formation of the intramolecular hydrogen bonding between amide-NH and oxygen atom of the OCH_2 group. Such intramolecular hydrogen bonds have been previously observed in the crystal structure of **15**.[11] In contrast to **12**, the amide proton of **15** showed minimal displacement on complexation with **16** ($\Delta\delta_{max} = 0.15$ ppm, see Table 2), confirming the weak binding. Figure 13 shows the small chemical shifts of the NH and aromatic resonances during the titration with β-glucopyranoside **16**. The pattern of shifts was consistent with 1:1 stoichiometry. The receptor **15** revealed a K_a value of only 190 M^{-1} for glucopyranoside **16**. Comparison of Figures 11 and 13 clearly reflects the strong differences in the complexation ability of **12** and **15**. These studies showed, that the flexibility and the formation of intramolecular hydrogen bonding can partially or wholly cancel the increase in the affinity caused by α-amino group in the pyridine ring.

Conclusion

The results showed that acyclic receptors containing amino- or amidopyridine binding subunits perform effective recognition of carbohydrates through multiple interactions. Depending on the nature and number of recognition subunits and connecting bridges used as the buildings blocks, a variety of structures with different binding properties could be obtained.

The 2-aminopyridine group has been established as a highly effective functional group in the binding of monosaccharides. The aminopyridine receptors **5**−**9** show high affinity for β-D-glucopyranoside and marked β vs α selectivity. Receptors based on 2,4,6-triethylbenzene frame (**8** and **9**) display about 2-fold higher binding affinity than those based on 2,4,6-trimethylbenzene unit (**6** and **7**), indicating more favorable preorganization of **8** and **9**. The binding constants for receptors **6**−**9** decrease in the sequence β-glucoside > β-galactoside > α-glucoside.

The comparison of the properties of **1**−**4** shows that the steric interactions involving the pyridine substituents in pyridine-based receptors significantly affect the binding properties. Remarkable changes in the binding affinity and selectivity have been observed when the degree of steric hindrance at the pyridine nitrogen atom decreases. Receptors **2** and **3**, which are sterically less hindered at nitrogen, show high efficiency and an inverse selectivity (in contrast to receptors **5**−**9** and **1**) since they bind the α-glucopyranoside better than the β-anomer. Compounds **2** and **3** have a tendency to form strong 2:1 receptor/sugar complexes; the binding constants K_{a2} ranged between 22600 and 82450 M^{-1} in the sequence α-glucoside > β-galactoside ≥ β-glucoside. Similar tendencies to the formation of 2:1 receptor/sugar complexes show the effective receptors possessing α-NH_2 group as a building block (hosts **7**, **9**, and **12**).

High conformational mobility of the receptor structure and the formation of intramolecular hydrogen bonding drastically decrease the binding affinity of receptors possessing effective recognition units, as reflected by the properties of **10**, **11**, and **15**.

Experimental Section

^{1}H and ^{13}C NMR spectra were measured using a 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.

General Procedure for the Synthesis of 2−4. To a solution of 2-aminopyridine derivative (2-amino-4-methylpyridine, 2-amino-5-methylpyridine, or 2-amino-4-ethylpyridine; 0.033 mol) and triethylamine (2.3 mL) in dry CH_2Cl_2 (50 mL) was added dropwise a CH_2Cl_2 (25 mL) solution of benzene-1,3,5-tricarbonyl chloride (1.35 g, 0.005 mol). The reaction mixture was stirred at room temperature for 24 h, and then 100 mL of water was added. The suspension was stirred for 30 min, and then CH_2Cl_2 was removed under reduced pressure. The resulting precipitate was filtered, washed several times with water, and dried. The crude product was crystallized from THF/hexane and ethanol (compounds **2** and **3** were crystallized two to three times from ethanol).

***N,N,N′*-Tris(4-methylpyridin-2-yl)benzene-1,3,5-tricarbonamide (2).** Yield: 30%. Mp: 260−261 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.40$ (s, 9H), 6.93 (d, 3H, $J = 5.2$ Hz), 8.17 (d, 3H, $J = 5.2$ Hz), 8.20 (s, 3H), 8.68 (s, 3H), 8.82 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 21.4$, 114.9, 121.5, 129.4, 135.7, 147.6, 150.1, 151.3, 163.9. HR-MS: calcd for $C_{27}H_{24}N_6O_3$ 480.1910, found 480.1903. Anal. Calcd for $C_{27}H_{24}N_6O_3$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.24; H, 5.15; N, 17.66.

***N,N,N′*-Tris(5-methylpyridin-2-yl)benzene-1,3,5-tricarbonamide (3).** Yield: 38%. Mp: 225−226 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.32$ (s, 9H), 7.58 (dd, 3H, $J = 8.6/2.2$ Hz), 8.13 (d, 3H, $J = 2.2$ Hz), 8.25 (d, 3H, $J = 8.6$ Hz), 8.67 (s, 3H), 8.73 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 17.9$, 113.8, 129.2, 129.8, 135.7, 139.1, 147.9, 148.9, 163.4. HR-MS: calcd for $C_{27}H_{24}N_6O_3$ 480.1910, found 480.1907. Anal. Calcd for $C_{27}H_{24}N_6O_3$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.36; H, 5.08; N, 17.58.

***N,N,N′*-Tris(6-ethylpyridin-2-yl)benzene-1,3,5-tricarbonamide (4).** Yield: 70%. Mp: 215−216 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.27$ (t, 9H, $J = 7.6$ Hz), 2.72 (q, 6H, $J = 7.6$ Hz), 6.95 (d, 3H, $J = 7.6$ Hz), 7.66 (t, 3H, $J = 7.9$ Hz), 8.15 (d, 3H, $J = 8.2$ Hz), 8.69 (s, 3H), 8.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 13.7$, 30.9, 111.3, 118.6, 129.2, 135.8, 138.9, 150.4, 162.3, 163.4. HR-MS: calcd for $C_{30}H_{30}N_6O_3$ 522.2379, found 522.2360.

General Procedure for the Synthesis of 6, 8, and 9. A mixture of 1,3,5-tris(bromomethyl)-2,4,6-triethyl-[14] or -2,4,6-trimethylbenzene[19] (3.50 mmol), 2-amino-4,6-dimethylpyridine (2.40 g, 19.67 mmol), or 2,6-diaminopyridine (2.40 g, 22.02 mmol) and K_2CO_3 (1.50 g) in CH_3CN (120 mL) was stirred at room temperature for 24 h (in the case of **6**) or heated under reflux for 6 h and then stirred at room temperature for 34 h (in the case of **8** and **9**). After filtration of the reaction mixture and evaporation of CH_3CN, the obtained powder was suspended in $CHCl_3$. The suspension was filtrated (in the case of **9** the chloroform solution was first washed several times with water and then dried), and $CHCl_3$ was removed under reduced pressure. The crude product was crystallized from THF/hexane, ethanol, or diethyl ether.

1,3,5-Tris[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-trimethylbenzene (6): Yield: 60%. Mp: 193−195 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.21$ (s, 9H), 2.33 (s, 9H), 2.38 (s, 9H), 4.11 (t, 3H, $J = 4.2$ Hz), 4.37 (d, 6H, $J = 4.2$ Hz), 6.08 (s, 3H), 6.32 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 15.9$, 21.1, 24.2, 41.8, 103.4, 113.9, 133.7, 136.8, 148.8, 156.7, 158.4. HR-MS: calcd for $C_{33}H_{42}N_6$ 522.3471, found 522.3477. Anal. Calcd for $C_{33}H_{42}N_6$: C, 75.82; H, 8.10; N, 16.08. Found: C, 76.01; H, 7.97; N, 15.89.

1,3,5-Tris[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (8): Yield: 57%. Mp: 183−185 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.21$ (t, 9H, $J = 7.6$ Hz), 2.21 (s, 9H) 2.33 (s, 9H), 2.72 (q, 6H, $J = 7.6$ Hz), 4.09 (t, 3H, $J = 4.3$ Hz), 4.36 (d, 6H, $J = 4.3$ Hz), 6.06 (s, 3H), 6.32 (d, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 17.0$, 21.2, 23.0, 24.3, 40.8, 103.7, 114.0, 133.3, 143.7, 148.8, 156.8, 158.3. HR-MS: calcd for $C_{36}H_{48}N_6$ 564.3940, found 564.3931. Anal. Calcd for $C_{36}H_{48}N_6$: C, 76.55; H, 8.57; N,14.88. Found: C, 76.38; H, 8.64; N, 14.99.

1,3,5-Tris[(6-aminopyridin-2-yl)aminomethyl]-2,4,6-triethylbenzene (9). Yield: 40%. Mp: 163−165 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.22$ (t, 9H, $J = 7.5$ Hz), 2.75 (q, 6H, $J = 7.5$ Hz), 4.05 (t, 3H, $J = 4.2$ Hz), 4.20 (s, 6H), 4.35 (d, 6H, $J = 4.2$ Hz), 5.83−5.86 (m, 6H), 7.24 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 16.9$, 23.0, 40.6, 95.9, 96.9, 133.3, 139.4, 143.8, 157.8, 157.9. HR-MS: calcd for $C_{30}H_{39}N_9$ 525.3328, found 525.3322.

General Procedure for the Synthesis of 10 and 11. A solution of acyl chloride **23a** or **23b** (0.61 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to a solution of 2-amino-4,6-dimethylpyridine (0.34 g, 2.80 mmol) and triethylamine (0.3 mL) in dry CH_2Cl_2 (5 mL). After complete addition, the mixture was stirred at room temperature for 24 h. The resulting precipitate was filtered and crystallized from THF/hexane or ethanol.

2-[3,5-Bis[(4,6-dimethylpyridin-2-ylcarbamoyl)methyl]-2,4,6-trimethylphenyl]-*N*-(4,6-dimethylpyridin-2-yl)acetamide (10). Yield: 65%. Mp: 273−275 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.26$ (s, 9H), 2.29 (s, 9H), 2.34 (s, 9H), 3.89 (s, 6H), 6.67 (s, 3H), 7.83 (s, 3H), 8.30 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 17.4$, 21.1, 23.6, 40.1, 111.7, 120.5, 130.6, 136.9, 149.9, 150.5, 156.1, 169.1. HR-MS: calcd for $C_{36}H_{42}N_6O_3$ 606.3318, found 606.3309. Anal. Calcd for $C_{36}H_{42}N_6O_3$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.09; H, 7.14; N, 14.03.

2-[3,5-Bis[(4,6-dimethylpyridin-2-ylcarbamoyl)methyl]-2,4,6-triethylphenyl]-*N*-(4,6-dimethylpyridin-2-yl)acetamide (11). Yield: 60%. Mp: 235−237 °C. ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 1.17$ (t, 9H, $J = 7.6$ Hz), 2.20 (s, 9H), 2.24 (s, 9H), 2.70 (q, 6H, $J = 7.6$ Hz), 3.89 (s, 6H), 6.65 (s, 3H), 7.77 (s, 3H), 8.38 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 14.2$, 21.2, 23.6, 24.1, 38.8, 112.5, 120.5, 129.6, 143.6, 149.9, 150.5, 159.9, 170.1. HR-MS: calcd for $C_{39}H_{48}N_6O_3$ 648.3788, found 648.3785. Anal. Calcd for $C_{39}H_{48}N_6O_3$: C, 72.19; H, 7.46; N, 12.95. Found: C, 71.98; H, 7.41; N, 13.13.

***N*-Di(ethoxycarbonylmethyl)-*N,N′*-bis(4,6-dimethylpyridin-2-yl)benzene-1,3,5-tricarbonamide (14).** A solution of 2-amino-4,6-dimethylpyridine (1.38 g, 11.24 mmol) and triethylamine (1.6 mL) in dry CH_2Cl_2 (30 mL) was added dropwise (during 10−15 min) to a solution of 1,3,5-benzenetricarbonyl trichloride (1.49 g, 5.62 mmol) in dry CH_2Cl_2 (20 mL). After complete addition, the mixture was stirred at room temperature for 40 min, and then a solution of iminodiacetic acid diethyl ester (1.06 g, 5.62 mmol) and triethylamine (0.8 mL) in dry CH_2Cl_2 (20 mL) was added dropwise. After complete addition, the mixture was stirred at room temperature for 24 h, the CH_2Cl_2 was removed under reduced pressure, and the crude product was suspended in Et_2O. After filtration and evaporation of Et_2O, the obtained powder was crystallized from ethanol and diethyl ether (the repeated crystallization is necessary for the complete separation of the byproducts **24** and **25**). Yield: 30%. Mp: 165−166 °C. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.22$−1.32 (m, 6H),), 2.33 (s, 6H), 2.37 (s, 6H), 4.08−4.33 (m, 8H), 6.76 (s, 2H), 7.96 (s, 2H), 8.18 (s, 2H), 8.53 (s, 1H), 8.58 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 14.1$, 21.3, 23.7, 47.8, 51.8, 61.5, 62.1, 111.6, 120.9, 127.6, 128.9, 135.8, 136.1, 150.1, 150.3, 156.6, 163.4, 168.6, 168.8, 170.2. HR-MS: calcd for $C_{31}H_{35}N_5O_7$ 589.2536, found 589.2545. $R_f = 0.49$ (ethyl acetate/toluene 3:1 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 12−21 samples were prepared). The titration data were analyzed by nonlinear regression analysis using the HOSTEST 5.6 program.[6] Examples: (a) [**2**] = 1.32 mM, [**18**] = 0.15−3.26 mM; (b) [**4**] = 1.01 mM, [**16**] = 0.22−5.47 mM; (c) [**8**] = 1.23 mM, [**16**] = 0.25−5.11 mM; (d) [**8**] = 0.67 mM, [**17**] = 0.16−4.85 mM; (e) [**8**] = 0.93 mM, [**18**] = 0.18−4.73 mM; (f) [**12**] = 0.94 mM, [**16**] = 0.12−3.73 mM; (g) [**14**] = 1.05 mM, [**16**] = 0.35−7.02 mM; (h) [**18**] = 0.57 mM, [**2**] = 0.11−2.49 mM. For each system at least three titrations were carried out.

The microcalorimetric titrations were carried out at 298 K using a Thermometric titration calorimetric system. The

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reproducibility of the calorimeter was checked using the complexation of Ba^{2+} by 18-crown-6. A solution of octyl-α-D-glucopyranoside (6.33−7.55 mM) or octyl-β-D-glucopyranoside (7.78−11.62 mM) in chloroform was titrated into a 1.01−1.09 mM solution of receptor in chloroform. The data obtained were analyzed using the Digitam 4.1 software provided by Thermometric (heat of dilution was corrected). Examples: (a) [**6**] = 1.07 mM, [**16**] = 11.62 mM, 30 automatic injections, 10 μL each; (b) [**6**] = 1.09 mM, [**17**] = 6.33 mM, 40 injections, 12 μL each; (c) [**8**] = 0.97 mM, [**16**] = 7.78 mM, 40 injections, 12 μL each; (d) [**8**] = 1.03 mM, [**17**] = 7.78 mM, 40 injections, 12 μL each.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 452). We thank Dipl.-Ing. Dieter Bläser (Institut für Anorganische Chemie der Universität Duisburg-Essen) for performing the X-ray measurements and Kerstin Antepoth for technical assistance. We thank also Prof. Craig Wilcox for giving access to the HOSTEST program.

Supporting Information Available: X-ray data for compounds **2**−**4**, **6,** and **8**. ^{1}H and ^{13}C NMR spectra of compounds **2**−**4**, **6**, **8**, **9**−**11**, and **14**. ^{1}H NMR Titration of receptor **11** with octyl β-D-glucopyranoside **(16)**. Representative mole ratio plot (complexation of receptor **3** with α-glucopyranoside **17**). This material is available free of charge via the Internet at http://pubs.acs.org.

JO048979K